Studies in the thiophenol mediated substitution and reductive dehalogenation of 3-bromoacetylcoumarins

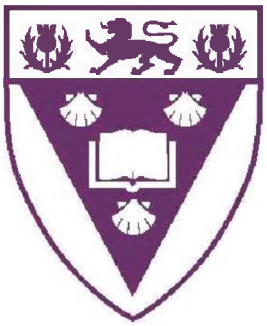
A thesis submitted in fulfilment of the

requirements for the degree of

Master of Science (Pharmacy)

Of

Rhodes University



by

Faith Magwenzi

February 2017

Abstract

A previous study conducted by our group identified indolyl-3-ethanone-α-thioethers (**2.1a** and **2.1b)** as non-toxic, nanomolar, *in vitro* inhibitors of *Plasmodium falciparum*. Since the coumarin scaffold is associated with numerous biologically active compounds including anti-protozoal, anti-viral, anti-bacterial, and anti-inflammatory agents we were prompted to investigate coumaryl-3-ethanone-α-thioethers (**2.1c**) inspired by the activity of **2.1a** and **2.1b** against *P. falciparum*. We proposed a three-step synthesis of our target compounds **2.1c.** The first step involved the Knoevenagel synthesis of 3-acetyl coumarins (**2.3.1a – e)** followed by a selective α-bromination to yield 3-bromoacetyl coumarin (**2.2a)**. The final proposed step involved the nucleophilic displacement of the bromine by appropriately substituted thiophenols in either the presence or absence of base (K_2CO_3). Our initial findings revealed an unexpected major reductive dehalogenation of **2.2a** into **2.3.1a**. Further investigation revealed a close relationship between the electron withdrawing or donating nature of the thiophenol substituents and the relative formation of nucleophilic substitution or reductive dehalogenation products. Desired thioether products were obtained in higher yields when thiophenol was substituted with electron donating groups i.e. more nucleophilic thiophenols, while conversely, electron withdrawing substituents (i.e. lowered nucleophilicity) resulted in an increase of reductive dehalogenation. Furthermore, these results were consistent when experiments were conducted using either 2 or 1.2 equivalents of thiophenols which was an important observation in the context of two previous studies, by Ōki *et. al.* and Israel *et. al.* Ōki proposed that dehalogenation of α-chloro carbonyls occurs *via* sequential nucleophilic displacement of α-thioethers, while the study of Israel concluded that the dehalogenation of α-iodo carbonyls occurred in a single discreet step. Finally, in an effort to enhance nucleophilic substitution through the addition of K_2CO_3, we observed a Robinson annulation resulting in

previously undescribed C-8 thiophenol functionalised dibenzo[*b,d*]pyran-6-ones (**3.4a – e)**. In the introduction to this thesis, we briefly summarise the utility of coumarins in medicinal chemistry and related fields. Chapter two describes the rationalisation of our original research question and a retrosynthetic analysis of our desired compounds, followed by an initial description of the unexpected reductive dehalogenation. Chapter 3, begins with a brief review of reductive dehalogenation of α-halocarbonyls, and is followed by an analysis and discussion of our results in the context of the studies by Israel *et. al*. and Ōki *et. al.*

Contents

"If *it had* not *been* the LORD who was on our side, when men rose up against us, then they would have swallowed us up quick, when their wrath was kindled against us:"

-Psalms 124 v 2-3-

Dedicated to Pamela Machidza, I Thank God for you!

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^{13}C NMR Carbon-13 Nuclear Magnetic Resonance

^{1}H NMR Hydrogen-1 Nuclear Magnetic Resonance

EtOH Ethanol

HIV Human immunodeficiency virus

°C Degrees Celsius

Calcd Calculated

δ Chemical Shift

d Doublet

dd Doublet of doublets

DDT Dichloro-diphenyl-trichloroethane

g Gram

hr Hour

HMBC Heteronuclear Multiple Bond Correlation

HRESMS High resolution electrospray mass spectrometry

HSQC Heteronuclear Single Quantum Correlation

IC_{50} 50% Inhibitory Concentration

J Coupling Constant

m Multiplet

Me	Methyl
mg	Milligram
MHz	Megahertz
min	Minute
ml	Millilitre
mmol	Millimolar
mol	Moles
NMR	Nuclear Magnetic Resonance
ppm	Parts per million
rt	Room temperature
s	Singlet
SAR	Structure activity relationship
t	Triplet
TLC	Thin layer chromatography
TEA	Triethylamine
WHO	World Health Organization

Publications and conference participation

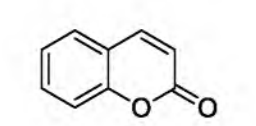
1. Unexpected transformations of 3-(bromoacetyl)coumarin provides new evidence for the mechanism of thiol mediated dehalogenation of α-halocarbonyls**. Faith N. Magwenzi, Setshaba D. Khanye, Clinton G. L. Veale**. Tetrahedron Lett., **2017**, 58, 968 – 972

2. Synthesis of substituted-3-acetyl-coumarins as potential anti-malarial agents: **36TH ANNUAL APSSA/SAAPI** Conference 17 – 19 September 2015

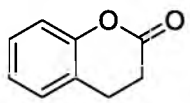
Chapter One
Introduction and literature review

1.1 General introduction

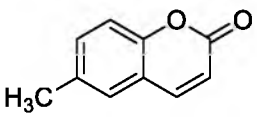
Coumarins occur naturally in plants, microorganisms and animals and have their name derived from *Coumarouna odorata Aube*.[1,2] Structurally, coumarins comprise of a fused benzene ring and an α- pyrone with **1.1** being the simplest member of this class. The conjugated π electron system is understood to have good charge-transport properties, while their diverse biological and non-biological applications have rendered them the subject of numerous synthetic organic chemical investigations.[3–6]



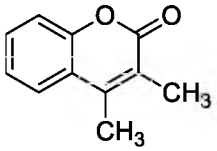
1.1



1.1a



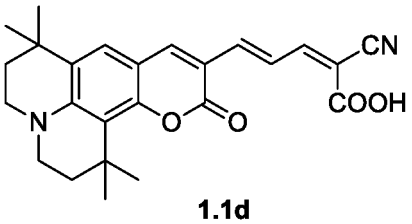
1.1b



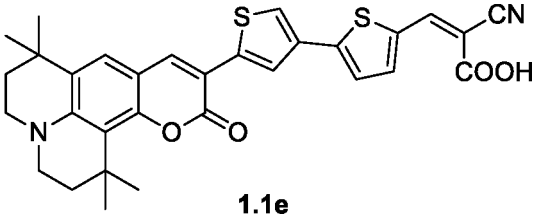
1.1c

The coumarin **1.1** has a vanilla smell and is found in lavender, sweetgrass, Tonka bean and other aromatic plants. It is due to this characteristic that coumarins and the related dihydrocoumarin **1.1a**, 6-methylcoumarin **1.1b** and 3,4-dimethylcoumarin **1.1c** have been valuable as fragrances in perfumery, soaps, detergents and cosmetics.[7–11] Furthermore, the unique electronic environment of coumarins and their interaction with the visible light spectrum, has resulted in numerous applications as dyes;[12–15] examples include **1.1d,**[16] the coumarin thiophene NKX-2677 **1.1e,**[12] its vinyl analogue **1.1f**[17] and 7-aminocoumarin **1.1g.**[18,19] Importantly, these fluorescent dyes have found application as microenvironment

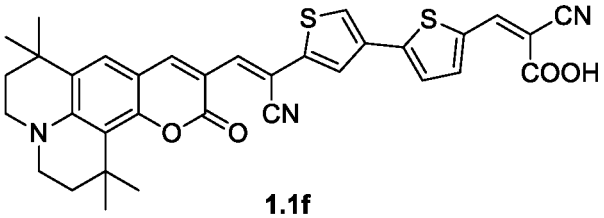
fluoroprobes for systems such as proteins, polymers and thin films of biological and commercial importance.[18–20]



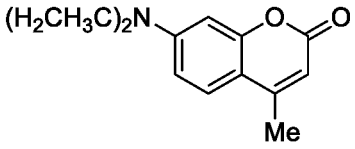
1.1d



1.1e

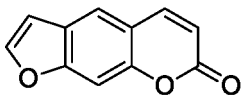


1.1f

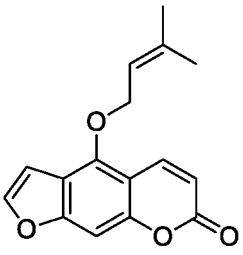


1.1g

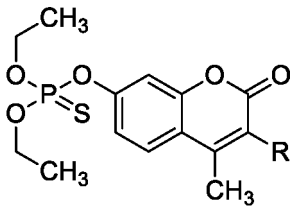
Regarding use as pesticides and insecticides compound **1.1** has exhibited ovicidal properties.[21–25] Of particular interest are the furocoumarins psoralen **1.1h** and its analogue imperatorin **1.1i** which are alellopathic coumarins that have potential to make new herbicides and pesticides that are more ecologically friendly. In addition, thiophosphates, potasan **1.1j** and coumaphos **1.1k** are coumarin derivatives that are used as pesticides.[21]



1.1h



1.1i



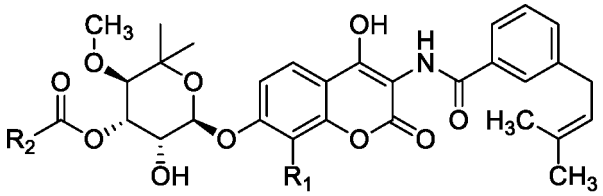
1.1j R= H
1.1lk R= Cl

1.2 Coumarins in medicinal chemistry

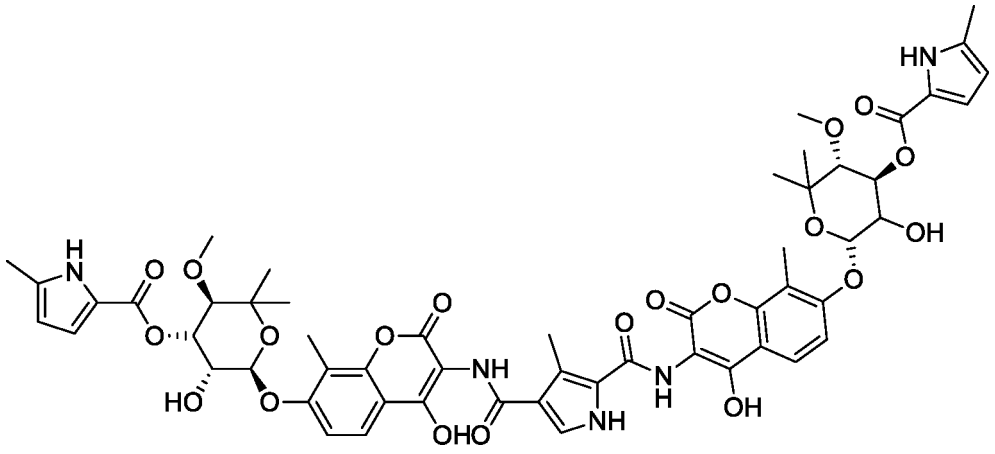
The biological malleability of coumarins has also meant that these compounds have found wide applicability in medicinal chemistry programmes, including antimicrobial, anti-cancer, and anti-viral drug discovery programmes, some highlights of which are reviewed below.

1.2.1 Coumarin as antibiotics

Coumarins have been shown to possess a great variety of activity against numerous pathogenic bacterial strains.[26–30] Possibly the most prominent of these compounds is Novobiocin **1.2.1a,** which along with chlorobiocin **1.2.1b** and coumermycin **1.2.1c** are secondary metabolites found in *Streptomyces* species of actinobacteria and work primarily against gram-positive bacteria through the inhibition of DNA gyrase. The structure of these antibiotics consists of a 3-amino-4-hydroxycoumarin and a substituted oxysugar noviose which has been found to be essential for anti-bacterial activity.[30–34]

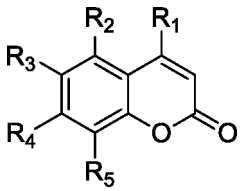


1.2.1 a R_1=CH_3 R_2=NH_2
1.2.1 b R_1=Cl R_2=$_2$-(5-CH_3-pyrrolyl)



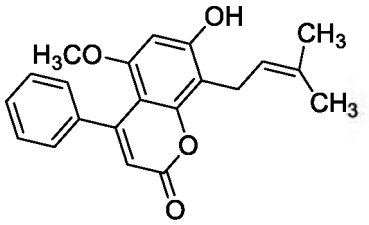
1.2.1c

Synthetic analogues of naturally occurring *O*-prenylcoumarins were tested against *Porhymonas gingivalis,* a bacterium responsible for periodontal disease. Two of these coumarins **1.2.1d, 1.2.1e,** displayed moderate activity against the bacteria, which was attributed in part to their reduced ability to permeate the bacterial cell membranes. The authors speculated further that potential anti-inflammatory activity of these compounds could be very promising as the dual action as antibacterial and anti-inflammatory agents is beneficial against periodontal disease.[35]



1.2.1d $R_1=R_2=R_3=$ isopentenyloxy R_4=OMe R_5=H

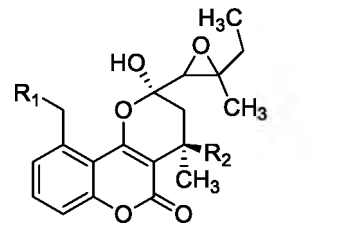
1.2.1e $R_1=R_2=R_3$=H R_4=OMe R_5=isopentenyloxy



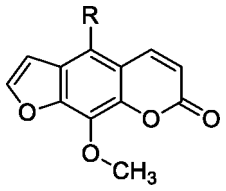
1.2.1f

The naturally occurring coumarin, cajanuslactone **1.2.1f** was isolated from pigeon pea leaves, and was seen to possess activity against *Staphylococcus aureus.* Structure activity relationship (SAR) analysis revealed that the activity shown by **1.2.1f** was attributed to the phenyl substitution at position 4 and hydroxyl substitution at position 7.[36] *Ethulia conyzoides* is an Egyptian plant that has traditionally been used to treat abdominal pain and as a deworming agent. From this plant, two terpenated coumarin compounds were extracted and evaluated for their activity against gram positive and gram negative bacteria. The two coumarins **1.2.1g** and **1.2.1h**, showed a greater activity against gram negative and gram positive bacteria compared to ampicillin and amoxicillin.[1] *Heracleum mantegazzium*, is a plant

originally from Asia but also found in some parts of Europe and North America and is a rich source of coumarins. The furanocoumarins isolated from this plant, xanthotoxin **1.2.1i** and isopimpinellin **1.2.1j** displayed good activity against gram positive bacteria especially *B. cereus.*[37]



1.2.1g R_1=OH R_2=CH=CH_2
1.2.1h R_1=H R_2=

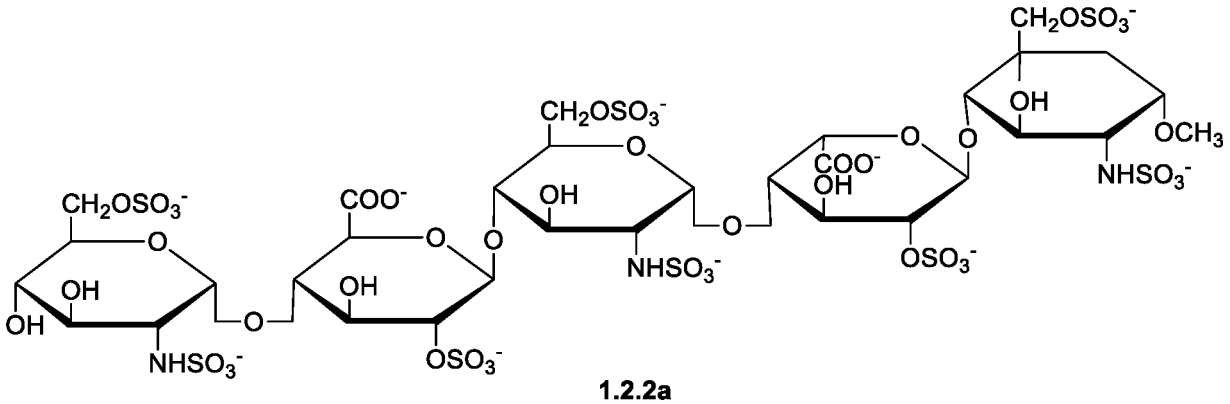


1.2.1i R= H
1.2.1j R= OCH_3

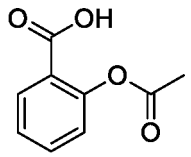
1.2.2 Coumarins as anticoagulants

Deep vein thrombosis, intravascular emboli, strokes and phlebitis are examples of life threatening thrombotic disorders. The prevalence of these disorders is increasing with the increase in the incidences of non-communicable diseases, following increasingly sedentary lifestyles.[38,39] Current drugs to manage such disorders are anti-thrombin drugs such as heparin **1.2.2a** antiplatelet aggregation drugs such as aspirin **1.2.2b**, and blood thinning agents such as coumarin drugs i.e. warfarin **1.2.2c**, acenocoumarol **1.2.2d**, cyclocoumarol **1.2.2e** and dicoumarol **1.2.2f**.[40] However, a major limitation of these drugs and warfarin in particular are their interactions with many other classes of drugs as well as food leading to increased risk of adverse effects such as bleeding. [38–40] Accordingly, the coumarin scaffold has been exploited to find new chemotherapy with reduced risk of injury related haemorrhages. A study revealed that acetamido-substituted coumarins were suitable substrates for platelet calreticulin transacetylase, an enzyme related to nitric oxide levels in platelets. SAR showed that acetyl amino at position 7 of the compounds improved specificity of the compound to

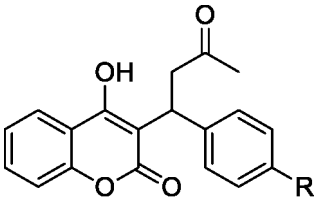
the target site with compound **1.2.2g** being the most effective in the series.[41] Another study demonstrated that acetoxy- analogues inhibited platelet aggregation. SAR of this series showed that substituting position 3 with bulky groups decreased anti platelet activity due to steric hindrance that affected important interactions within the enzyme binding site. Compound **1.2.2h** was the most active in the series and further exploration of this can prove fruitful.[42] The carboxy-coumarin **1.2.2i** also showed good anticoagulant activity with low toxicity.[4]



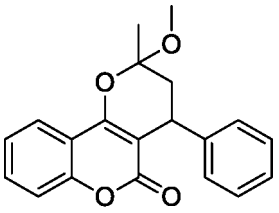
1.2.2a



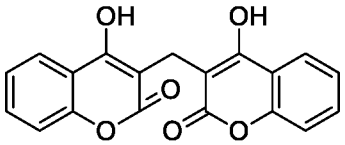
1.2.2b



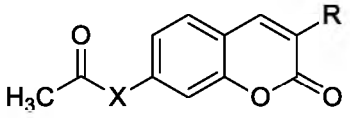
1.2.2c R=H
1.2.2d R=NO_2



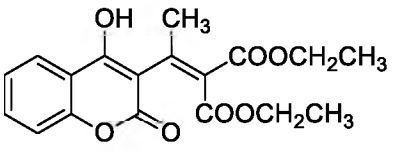
1.2.2e



1.2.2f



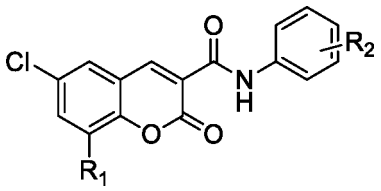
1.2.2g X=NH R=H
1.2.2h X=O R=n-C_3H_7



1.2.2i

Efforts to reduce the risk of anti-coagulant associated bleeding has focused on finding new targets on the clotting cascade. Clotting factor X11a is one such target of interest as inhibiting

this does not lead to excessive bleeding. FX11a is a trypsin-like serine protease that triggers the intrinsic coagulation pathway through contact activation. Synthetic 3-carboxamide coumarins **1.2.2j**, **1.2.2k** were found to inhibit FX11a. Furthermore, it was shown that the carboxamide coumarin core was necessary for activity and selectivity.[43,44]

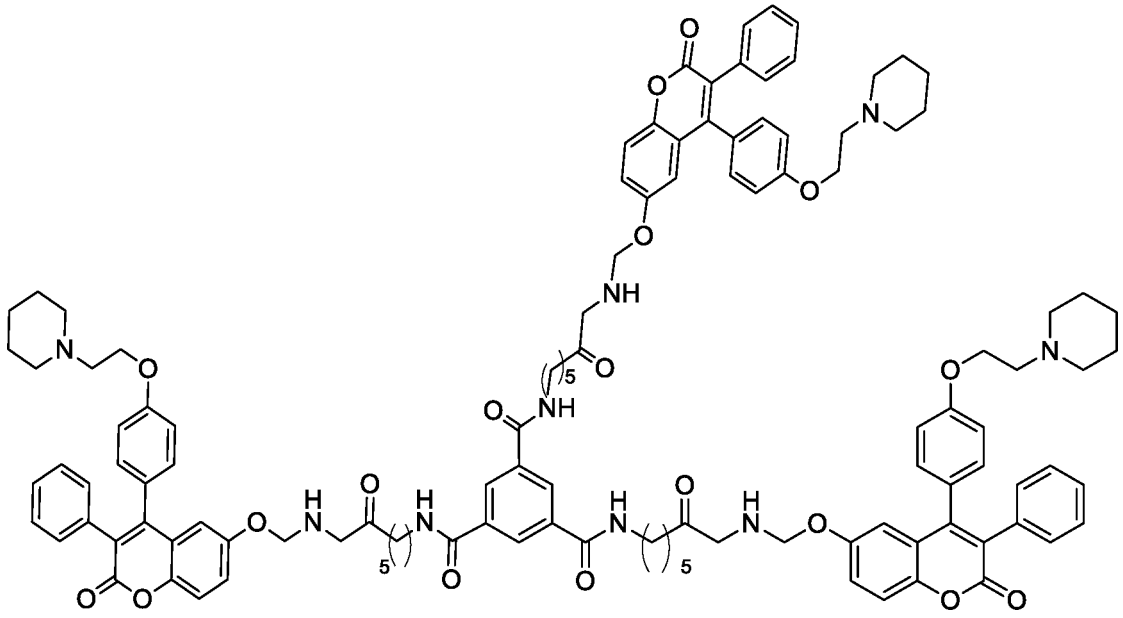


1.2.2j R_1=Cl R_2=3,5-CH3
1.2.2k R_1=Br R_2=H

1.2.3 Coumarins as anti-cancer agents

Cancer is a leading cause of death in people of all age groups. A significant challenge associated with existing chemotherapy is attributed to the severe side effects and immunosuppression in addition to resistance of tumours. Several studies have shown that coumarin derivatives may be a source of new chemotherapeutic drugs. However, further work must be done to improve the activity and reduce side effects.[33,45–54]

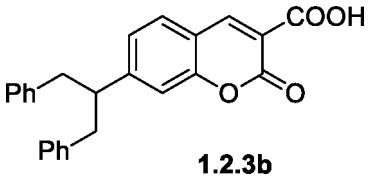
Triphenylethylene-coumarin hybrids were synthesized and evaluated for activity against MCF-7 (human breast), A549 (human lung) and HeLa (human cervical) cancer cell lines. Encouragingly, this series exhibited significant anti-proliferative activity against all three cancer cell lines. These compounds were believed to exert their affect *via* DNA intercalation, and SAR suggested that a linker of 6-carbons in length was important for activity and DNA intercalation. Compound **1.2.3a** was the most active in the series with good activity against all three cell lines, outperforming the reference cisplatin against A549.[55]



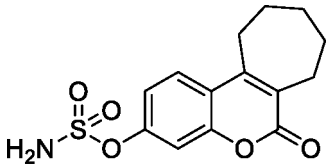
1.2.3a

Glycolysis is an important biochemical process for many organisms' cellular survival including cancer cells. However, it is critical for cells to avoid apoptosis brought on by the build-up of pyruvate and lactate by-products of glycolysis. Proton-coupled membranes transporters called monocarboxylase transporters are responsible for the removals of lactate, pyruvate and other small carboxylates and serve as a promising target to induce cancer cell apoptosis. Coumarin carboxylic acids exhibited inhibitory activity against these membranes in GL261-luc 2 glioblastoma and MDA-MB231 triple negative breast cancer cells. Of the synthetic series, **1.2.3b** showed good absorption, good metabolic stability, decreased drug efflux ratio and reduced systemic toxicity. Encouragingly, growth inhibition of **1.2.3b** was determined to be 77% in comparison to 81% for temozolomide a clinically used drug for glioblastoma. Structure activity relationships showed that *N,N*-diakyl or diaryl groups at position 4 enhanced inhibitory activity of the compounds.[53] Breast cancer is the leading cause of carcinoma in women of all age groups and coumarins have been investigated for their activity against this

form of cancer. 667-Coumate **1.2.3c** is a coumarin-based sulfatase inhibitor, and is a phase 1 candidate for the treatment of hormone-dependent breast cancer in menopausal women.[56]

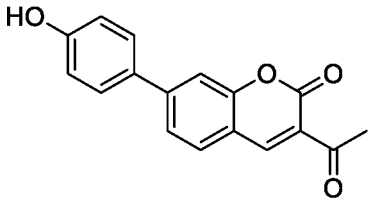


1.2.3b

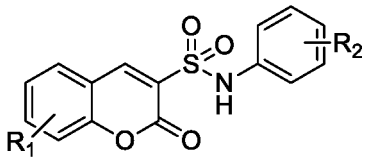


1.2.3c

Starcevic and colleagues showed that **1.2.3d** exhibited reversible and competitive inhibitory activity against 17β-HSD type 1. This led to the reduced efficacy of endogenous 17β-HSD in human T-470 breast cancer and decreased growth of oestrone dependent T-470 cells within 48 hours.[54] Arylsuphonamides had been previously shown to inhibit cancer cell lines. Accordingly, Reddy *et al.* reasoned that hybridisation of this scaffold with coumarin may result in enhanced activity. Of the compounds synthesized, **1.2.3e** and **1.2.3f** were the most active against oestrogen receptive breast cancer, myeloid leukaemia, colorectal tumours, non-small cell lung carcinoma and androgen negative prostate cancer. Moreover, the series activated the Jun kinase pathway which is immunosuppressed by many cancer cells.[46]



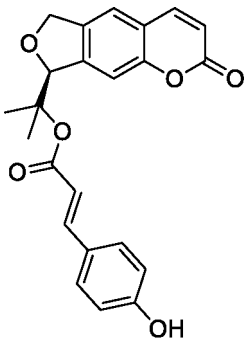
1.2.3d



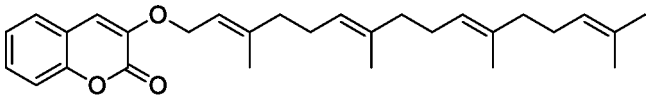
1.2.3e R_1= 8-Br R_2= 4-OMe
1.2.3f R_1= 6-Cl R_2= 4-OMe

Angelmarin **1.2.3g** a natural coumarin isolated from *Angelica pubescens*, a plant from Japan was found to exhibit cytotoxic activity. This study culminated in a series of hydroxycoumarins which were found to inhibit pancreatic cancer cells the most active in this series, **1.2.3h** had the highest cytotoxic activity was also active against pancreatic cancer cells under nutrient

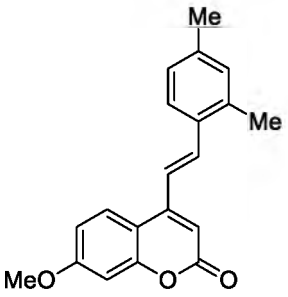
deprived conditions. SAR studies revealed that longer ether chains and the 5-carbon cyclic alkyl chain were paramount for cytotoxicity under nutrient deprived conditions.[47] Resveratrol-coumarin hybrids displayed activity against H460 lung carcinoma cells. Compounds **1.2.3i** in particular showed activity that was comparable to that of cisplatin, with excellent antiproliferative potency and apoptosis-inducing activity.[49]



1.2.3g



1.2.3h

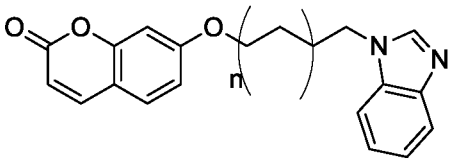


1.2.3i

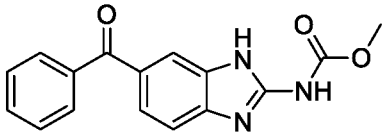
1.2.4 Coumarins as anti-parasitic agents

Parasitosis is an infectious disease that is transmitted and/or caused by a parasite. Protozoa, helminths and insects are some of the known disease causing parasites. Like the majority of infectious disease, the challenges facing treatment of parasitic infections is increasing resistance to existing therapy. Although, parasitic infections affect people and animals from all over the world, it is a particularly harsh burden amongst poverty stricken communities.

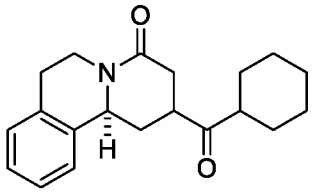
Coumarin-imidazole hybrid **1.2.4a** displayed good activity as anti-helminths. This activity was optimal with a 6-carbon linker chain between the two pharmacophores. However, these compounds were roughly 3 to 5 times more toxic than mebendazole **1.2.4b** or praziquantel **1.2.4c** which are available drugs of choice for helminthic infections.[57]



1.2.4a

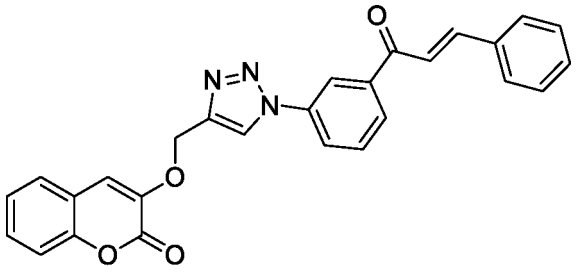


1.2.4b

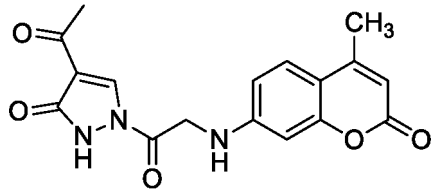


1.2.4c

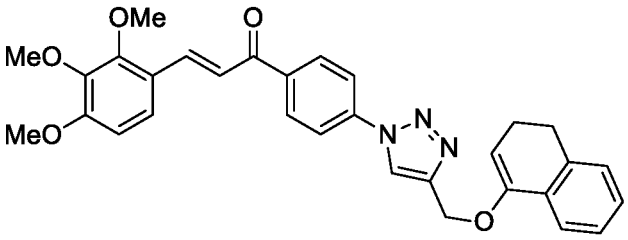
Possibly the most important parasitic infection in humans, is malaria. Malaria drug discovery remains an intense subject of study due mainly to the problem of persistent drug resistance and thus the need for new drugs, which inhibit new targets. One such study led to discovery of falcipains as a new target for malaria. Falcipains are a group of cysteine proteases which are responsible for cleavage of small peptides mediated by the nucleophilic thiol group of the cysteine residue. Inhibition of these proteases stops the erythrocytic stage of the *Plasmodium falciparum* life cycle by inhibiting the hydrolysis of human haemoglobin. Coumarin **1.2.4d** and aminocoumarin **1.2.4e** were found to inhibit falcipain-3.[58] A chalcone-coumarin, **1.2.4f** was seen to be active against K1 multidrug resistant *P.falciparum* strain by inhibiting falcipain-2 at an IC_{50} value of 1.50µM.[59] 4-arylcoumarins **1.2.4g** were synthesized and evaluated for their activity against the multi-drug resistant W2 strain of *P.Falciparum* .[60] 3-cinnamoylcoumarins were also synthesized and were evaluated for antimalarial activity by testing their activity against chloroquine sensitive and chloroquine resistant *P.Falciparum* strains. The most active compound **1.2.4h** showed greater activity against both strains compared to the chloroquine the reference.[61]



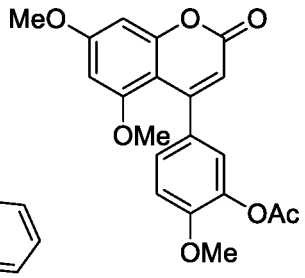
1.2.4d



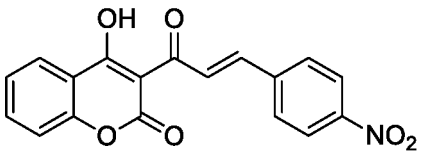
1.2.4e



1.2.4f

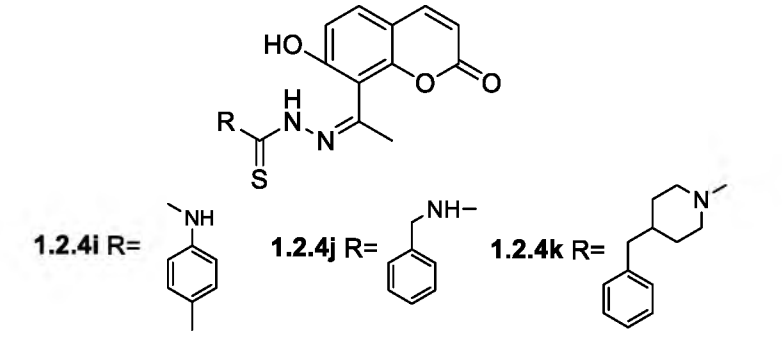


1.2.4g

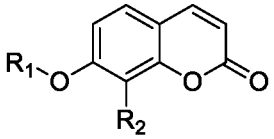


1.2.4h

Amoebiasis is a parasitic infection that is caused by consuming food contaminated with *Entamoeba hystolytica*. The current first line treatment is the antibiotic metronidazole. An extract from the bark of *Adina cordifolia*, resulted in the identification of moderately active coumarins. After an SAR investigation, three thiosemicarbazone derivative, **1.2.4i, 1.2.4j** and **1.2.4k** were found to be more potent than metronidazole against amoebasis.[62]

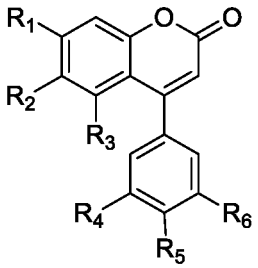


Leishmaniasis is a disease which occurs mainly in tropical and subtropical areas. The causative agent leishmania is transmitted by the sand-fly. Natural coumarins **1.2.4l and 1.2.4m** were extracted from *Galipea panamensis* a Brazilian tree. The two coumarins were seen to be active against cutaneous leishmaniasis and SAR showed substitution at position 7 and 8 improved anti-leishmaniasis activity.[63] 4-aryl coumarins were also active against anti-leishmaniasis amastigotes. **1.2.4n** was more active against *L. donovanii* as compared to amphotericin B the drug of choice for *L.donovanii* leishmaniasis and **1.2.4o** was the most active compound synthesized.[60]



1.2.4l $R_1=CH_3$ $R_2=$

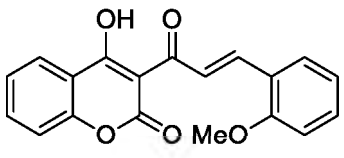
1.2.4m $R_1=$ $R_2=$



1.2.4n $R_1=R_2=R_3=H$ $R_4=R_5=OMe$ $R_6=H$

1.2.4o $R_1=R_4=R_5=OMe$ $R_2=R_3=R_6=H$

A coumarin-chalcone hybrid **1.2.4p** was designed and found to be active against American trypanosomiasis or Chagas disease. The compound **1.2.4p** was 4 and 6 more active than nirfurtimox the drug used to treat Chagas disease on both trypomastigote and amastigote stages, respectively.[64]

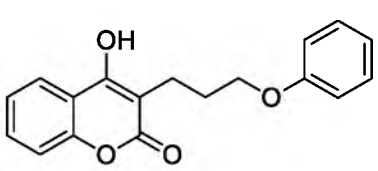


1.2.4p

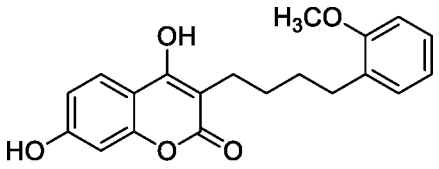
1.2.5 Coumarins as antiviral agents

In 2015 there were roughly 36.7 million people living with HIV with 1.1 million associated deaths. [65] Therefore there is a need to get new drugs to tackle this disease as well as ever present issues of cross resistance and toxicity.[2,65–71]

A validated strategy to inhibit the replication of HIV-1 is through the inhibition of the HIV-protease enzyme. Large scale screening identified the coumarin 4-hydroxy-3-(3-phenoxypropyl)-2H-1-benzopyran-2-one **1.2.5a** as a non-peptide inhibitor of HIV protease. Monte Carlo-based docking and X-ray co-crystallography was used to establish how the coumarin bound and interacted with the binding site. This structural information led to the design and synthesis of a series of analogues with improved binding interactions and binding affinity. Compound **1.2.5b** was seen to be the most active with highest affinity to protease HIV-1 enzyme and a potential anti HIV candidate.[70]



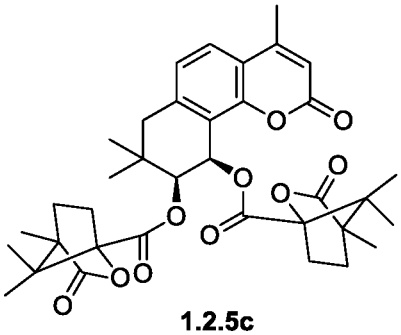
1.2.5a



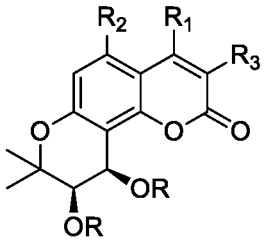
1.2.5b

A Study conducted by Xie *et al*. found that dicamphanoyl-khellactone (DCK) **1.2.5c** was more potent than zidovudine against HIV replication in H9 lymphocytes. In their study, they found out that DCK inhibited the production of double stranded DNA from a single stranded DNA intermediate, which differs from the mechanism of action of current reverse transcriptase inhibitors which exert their action by inhibiting production of single stranded DNA from an RNA template. Of the modified analogues, 4-methyl-DCK had the highest potency but it exhibited low bioavailability and solubility. This led to the design of an additional series of

DCK analogues for improved physico-chemical properties. Seven analogues **1.2.5d-h** displayed improved physico-chemical properties, had activity against HIV-1, and are potential drug candidates against HIV-1 replication.[67]

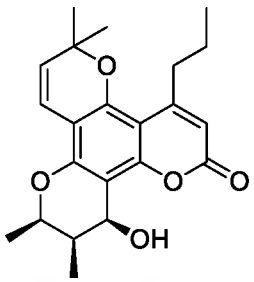


1.2.5c

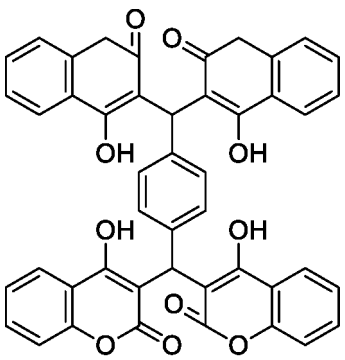


1.2.5d R_1= Me R_2= H R_3= -NO_2
1.2.5e R_1= H R_2= H R_3= -NO_2
1.2.5f R_1= Me R_2= MeO R_3= -OH
1.2.5g R_1= H R_2= H R_3= -NH_2O
1.2.5h R_1= Me R_2= MeO R_3= F

Anti-HIV bioassay guided fractionation of *Calophyllum lanigerum* extracts yielded several different compounds with anti HIV activity. Of the extracts obtained, the coumarin (+)-calanolide **1.2.5e** was found to be active against HIV-1 replication and inactive against HIV-2.[69] Safety and toxicity studies conducted on (+)-calanolide revealed that the compound is relatively safe and non-toxic.[72] Finally, 4-hydroxycoumarins analogues were synthesized and compound **1.2.5f** displayed potency against HIV-1 integrase. [66]

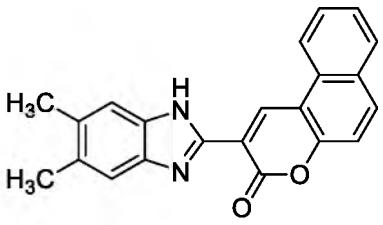


1.2.5e

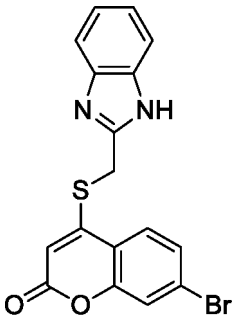


1.2.5f

Hepatitis C virus affects about 3.5% of the world's population. Infection can progress to cirrhosis, chronic liver disease or liver cancer. Coumarins have shown application as anti-hepatitis virus agents. Benzimidazole-coumarin hybrids **1.2.5g** displayed anti hepatitis C activity,[2,68] An SAR analysis of a series of heterobicycle-coumarin conjugates uncovered compound **1.2.5h** which displayed the most activity against hepatitis C virus. [71]



1.2.5g



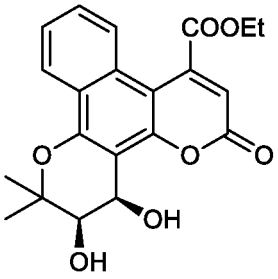
1.2.5h

1.2.6 Coumarins as antioxidants and anti-inflammatory agents

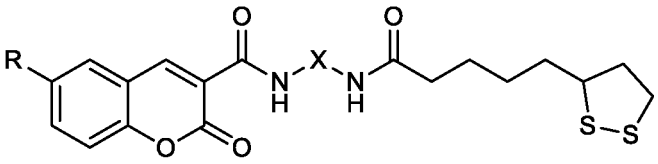
Oxidation is a normal process that occur in our bodies daily and may lead to accumulation of disruptive reactive oxygen species (ROS) causing oxidative stress.[73] ROS are found in higher concentrations during infections and inflammation where, free radicals are produced by macrophages. Moreover, ROS are involved in the cyclooxygenase and lipoxygenase mediated conversion of arachidonic acid in pro-inflammatory intermediates. [74,75]

Benzo[1]khellactone derivatives and analogues were synthesized and were evaluated for anti-inflammatory and antioxidant activities. Antioxidant activity was evaluated by the interaction between the synthesized analogues and a stable free radical, 1,1-diphenyl-2-

pioryl-hydrazyl (DPPH) while reducing abilities were measured by conversion of DPPH to 1,1-diphenyl-2-pioryl-hydrazine at a variety of concentrations. Furthermore, anti-inflammatory activity was measured by the relative ability to inhibit soybean lipoxygenase and trypsin. Compound **1.2.6a** was seen to be highly interactive with DPPH in a time dependant manner as well as being an inhibitor of lipoxygenase. From a series of coumarin-3-aminoamides Compounds **1.2.6b**, **1.2.6c** displayed superior anti-inflammatory activity than reference indomethacin, in a carrageenan-induced rat paw oedema assay. The two were also found to be potent antioxidants as demonstrated by their interaction with DPPH.[76] **1.2.6d** and **1.2.6e** demonstrated reducing activity against DPPH.[73,75–80]

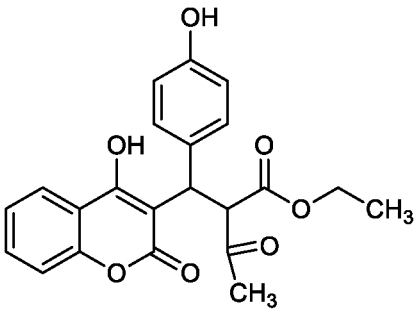


1.2.6a

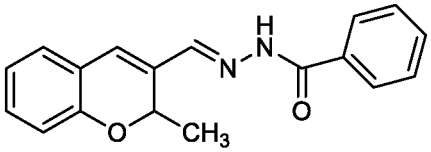


1.2.5b R= H X= $(CH_2)_6$

1.2.6c R= CH_3 X= $(CH_2)_8$



1.2.6d



1.2.6e

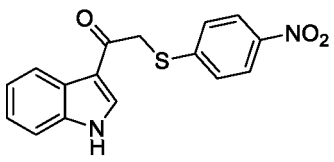
1.2.7 Summary

To summarise, coumarins have displayed significant utility in medicinal chemistry programmes whether inspired from natural products or purely synthetic libraries. Their ubiquity in biologically active molecules also offers opportunities for multi-target inhibition of disease states. Accordingly, we aimed to incorporate this scaffold into our drug discovery research, which will be detailed in the following chapters.

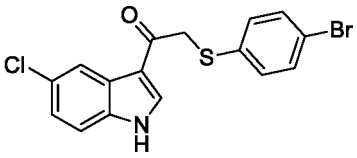
Chapter Two

2.1 Rationale of this project

A previous study conducted by our group led to the discovery of a series of indolyl-3-ethanone-α-thioethers as a new class of antimalarial agents. Of the series, compounds **2.1a** and **2.1b** exhibited antimalarial activity in the nanomolar range with IC_{50} values of 0.24 and 0.09 respectively. The indolyl-3-ethanone-α-thioether SARs revealed that one key element of the pharmacophore was a *para*-substituent on the thiophenol portion of the compound. Furthermore, activity was heavily dependent on the nature of the para substituent, with a nitro substituent possessing the greatest activity. Modifications on the indole portion showed that both size and position of substituent were important for activity, with the greatest activity observed by C-5 substitution of the indole with a chlorine.[81] Moreover, the synthesized compounds were nontoxic to HeLa cells.[81,82]

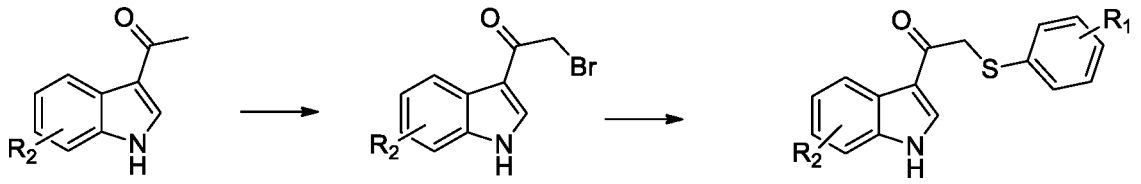


2.1a



2.1b

These compounds were synthesized *via* a three-step process (**Scheme 2.1a**), the first step involved a Friedel-Crafts acetylation of an appropriate indole, followed by selective bromination to yield an α-bromoketone. Finally, nucleophilic substitution with various thiophenols resulted in the active compounds as shown in the scheme below.



Scheme 2.1a: General synthesis of indolyl-3-ethanone-thioethers

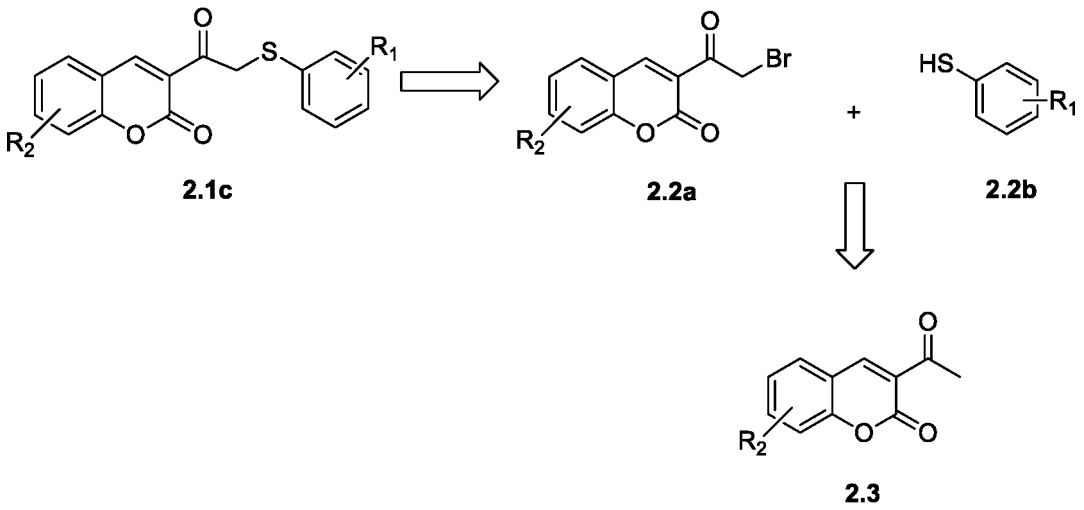
As discussed above, both natural and synthetic coumarin analogues have displayed remarkable utility in medicinal chemistry due to their privileged nature. Accordingly, we were interested in investigating the effect this moiety would impact activity against *P. falciparum* when substituting the indole scaffold.

Proposed modifications to the structure would involve R_1 and R_2 substitution with halogens and/or alkyl groups on any position of the coumarin scaffold and thiophenol portion respectively. The modifications on both the coumarin scaffold and thiophenyl ring would then be assessed and SARs determined. Structural modifications on the coumarin and thiophenol portions of our compounds and SAR studies would enable us to compare the two systems and determine whether similar patterns would be observed. Our target compound coumaryl-3-ethanone-α-thioether **2.1c** is shown below.

2.2 Retrosynthesis

In our retrosynthetic analysis (**Scheme 2.1b**) we proposed that **2.1c** would be synthesized from the nucleophilic displacement reaction between α-bromoketone **2.2a** and thiophenol derivatives **2.2b** as per the method used in the synthesis of indoyl-3-ethanone-α-thioethers.[81,82] Compound **2.2a** could be accessed through the selective bromination of 3-acetyl coumarin **2.3.** While we had general expertise in both the selective bromination of

methyl ketones as well as their subsequent nucleophilic substitution, we had limited knowledge of appropriate methods to access 3-acetyl coumarins (**2.3**).

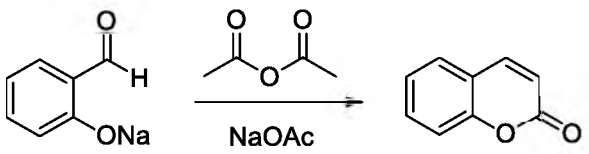


Scheme 2.1b: proposed general retrosynthetic scheme

Numerous synthetic procedures have been developed to synthesize 3-acetylcoumarin.[83–89] Perkin, Knoevenagel and Pechmann reactions respectively, are the most common methods of synthesizing coumarins although they have the disadvantages of using expensive catalysts, and/or harsh conditions. This has led to modifications and slight alterations in the methods in order to counter these undesirable effects.

2.2.1 The Perkin reaction

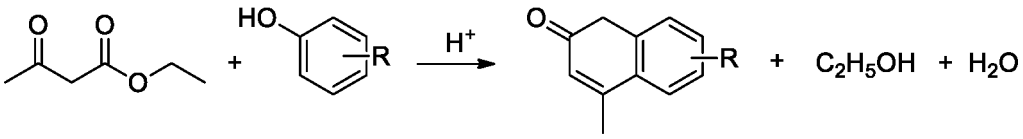
In the mid nineteenth century, Perkin discovered a method to synthesise coumarins through an aldol condensation of an aromatic *ortho*-hydroxybenzaldehyde and an acid anhydride in the presence of alkali salt (**Scheme 2.2.1**).[1,90–94]



Scheme 2.2.1. Perkin reaction scheme

2.2.2 The Pechmann reaction

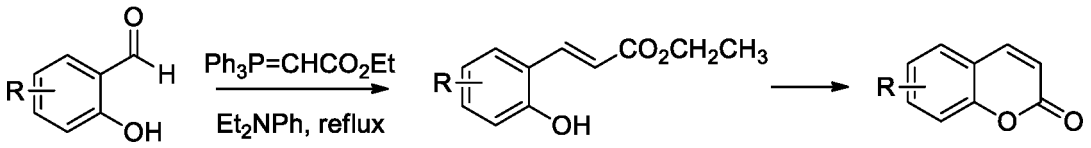
This reaction involves the synthesis of coumarins by condensation of phenols with β-ketoesters in the presence of an acid catalyst under high temperatures as shown in **Scheme 2.2.2**.[95] When acetoacetic esters or their derivatives are used, it is called the Pechmann-Duisberg reaction.[1] The conventional acid used is usually sulphuric acid, although other acids such as $HClO_4$, H_3PO_4 and HCl may be used as well as metal salts including indium chloride,[96] ionic liquids and microwave irradiation reduce reaction times and improve yields.[77]



Scheme 2.2.2. Pechmann reaction scheme

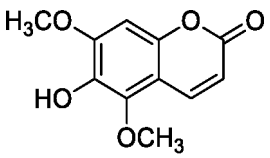
2.2.3 Wittig Reaction

The Wittig reaction or Wittig olefination involves the reaction of an aldehyde or ketone with a triphylphosphonium ylide to give an alkene and triphenyl phosphine oxide (**Scheme 2.2.3**).[1] the wittig reaction is used to synthesize O-hydroxyenzaldehydes that will then be converted to corresponding acetylcoumarins.[97]



Scheme 2.2.3 Wittig general reaction scheme

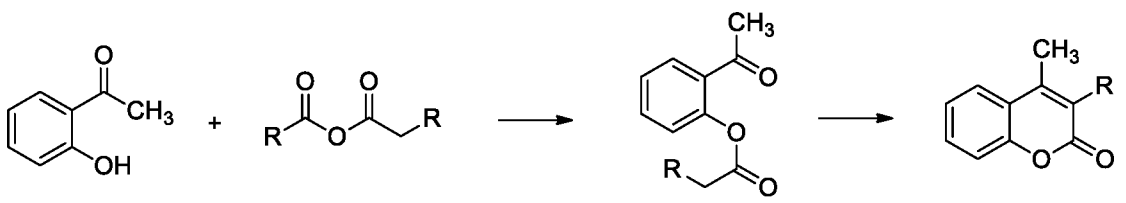
The Wittig reaction finds utility in the synthesis of 3,4-unsubstituted coumarins such as the naturally occurring **2.2.3a**,[97] which are difficult to synthesize using the traditional Pechmann and Knoevenagel reactions. However, this reaction was not suitable for our purposes, since we were looking specifically to make 3-acetylcoumarins.



2.2.3a

2.2.4 Kostanecki-Robinson Reaction

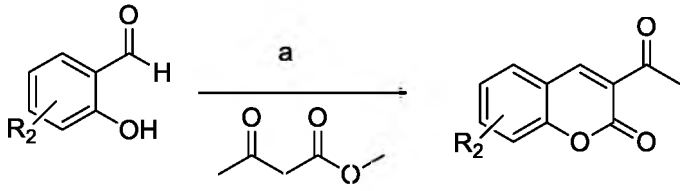
Related to the Perkin reaction, this reaction pathway involves the condensation of *ortho*-hydroxyketones with aliphatic acid anhydrides to yield either coumarins or chromones (**Scheme 2.2.4**).[1] The Kostanecki reaction has also been used in the acylation of coumarins. [98]



Scheme 2.2.4. Kostanecki-Robinson reaction scheme

2.2.5 The Knoevenagel condensation

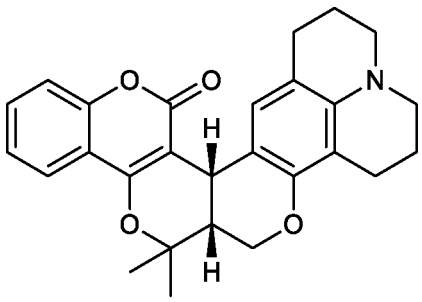
The Knoevenagel condensation (**Scheme 2.3a**) involves the aldol condensation of aldehydes with activated enolate in the presence of amines or ammonia, weak bases or Lewis bases under homogenous conditions. The Knoevenagel reaction can be modified by using malonic acid and pyridine in either the presence or absence of piperidine to be called the Doebner modification.[1,99–102]



2.3a **2.3b** **2.3**

Scheme 2.3a. Synthsis of substituted 3-acetylcoumarins, a) EtOH, piperidine, rt, 16h

Julolidine hybrid analogues **2.2.5a** were synthesized using a Knoevenagel intramolecular hetero diels-alder strategy. Various reaction conditions were explored revealing piperidine to be the catalyst of choice for this reaction.[103]



2.2.5a

For the purposes of our synthesis, we resolved to utilise the Knoevenagel reaction, since it has found the greatest utility in the synthesis of our desired 3-acetylcoumarins from salicylaldehyde's which was commercially available from Sigma Aldrich. [83–89,104]

2.3 Results and discussion

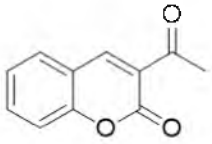
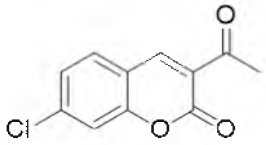
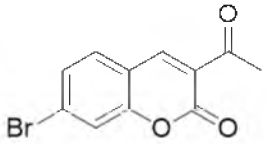
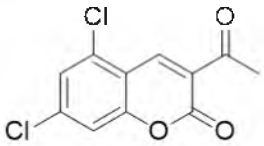
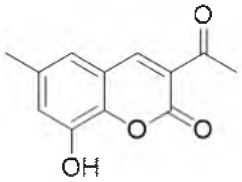
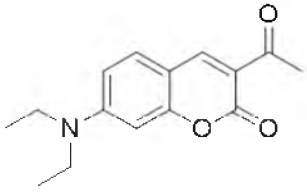
2.3.1 Synthesis of 3-acetylcoumarin and derivatives (2.3.1 – 2.3.6)

Interrogation of the relevant Knoevenagel related literature revealed that **2.3** had been synthesized from appropriately substituted salicylaldehyde **2.3a**, ethylacetoacetate **2.3b** in the presence of piperidine under several variable conditions (**Scheme 2.3a**). While generally this reaction can proceed smoothly at room temperature, followed by recrystallization from ethanol or water and ethanol,[84,105,106] several modifications to this overall method have been reported including reactions at reduced temperature,[107] recrystallization from chloroform,[108] or glacial acetic acid[83] as well as extended reaction times.[104] Furthermore, some have sought to accelerate reaction rates and yields either through traditional methods of increasing temperature,[62,85,109] or through microwave irradiation[110,111]

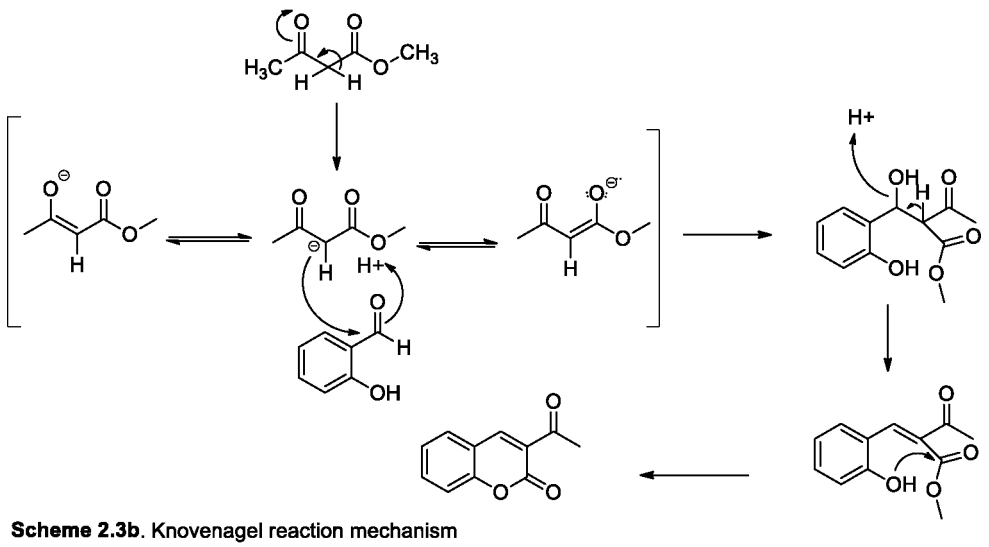
We initially attempted to conduct this reaction at reduced temperature, accordingly we mixed 0.8 molar equivalents of salicylaldehyde, 1 molar equivalents ethyl acetoacetate and maintained the reaction temperature between 0 – 5 ^{o}C. 2 Equivalents of piperidine was added dropwise to the stirring mixture and the reaction was left to run overnight, resulting in the formation of a yellow solid. We initially attempted to purify the crude mixture by recrystallizing from EtOH,[107] however, the solid mixture completely dissolved in cold EtOH. Following removal of EtOH under reduced pressure, we attempted to recrystallize from a 50:50 mixture of water and EtOH.[84] While this appeared successful, ^{1}H NMR data did not correspond with reported data for compound **2.3.1**. Reducing the equivalents of piperidine to 1 eq. was also unsuccessful. We were finally successful after adding catalytic amounts of piperidine into a stirred solution of **2.3a** and **2.3b** in EtOH.[104] After stirring for 5 hours at room temperature the resulting ppt was filtered off and recrystallized from ethanol resulting in our

desired product at a yield of 30%. Increasing the reaction time to run overnight[84] improved the yields to 81%. This optimised method was employed in the synthesis of substituted 3-acetylcoumarins (**2.3.2 – 2.3.6, Table 2.3a.**) shown below.

Table 2.3a. Substituted 3-acetylcoumarins synthesized and their percentage yields.

Compound no.	Structure	Yields (%)
2.3.1		81
2.3.2		67
2.3.3		52
2.3.4		7.8
2.3.5		53
2.3.6		29

Following the reaction mechanism (**Scheme 2.3b**), the nitrogen abstracts a proton from ethyl acetoacetate, resulting in the formation of a resonance-stabilised enolate intermediate. This is followed by an aldol condensation with salicylaldehyde and dehydration to give the α,β-unsaturated product. Finally, the phenol nucleophilically displaces the ester resulting in lactone formation.[107,112–116] The successful synthesis of compounds **2.3.1 – 2.3.6** was confirmed by ^{1}H and ^{13}C NMR analysis and HRMS which correlated well with reported data. Given the structure of our methyl ketones, we expected aromatic signals between 7 – 8ppm region on the ^{1}H NMR spectra. We also expected a singlet further downfield corresponding to H-4 (8.49 ppm) as well as the CH_3 (H-2') singlet signal at 2.72 ppm (**Figure 2.3a**). These two signals were the most diagnostic as they are the ones that distinguish our methyl ketones from the starting material **2.3a** .The ^{13}C NMR was expected to correspond to the number of carbons on each methyl ketone. The chemical shifts of carbon 1' representing the ketone carbon and the carbon of the methyl C-2' were 195.5 and 30.5 ppm, respectively were the diagnostic signals confirming that our desired methyl ketone was synthesized. Moreover. The mass spectrum showed the mass of 189.0549 and our calculated mass was 188.0552 which is within the acceptable deviation range.



Scheme 2.3b. Knovenagel reaction mechanism

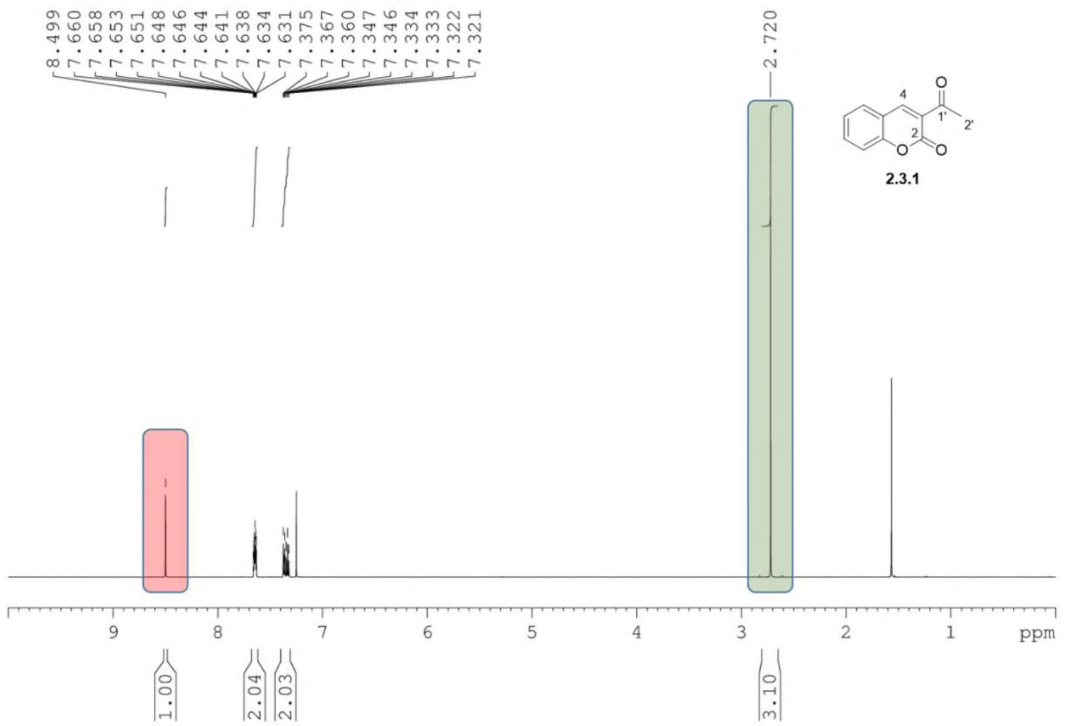
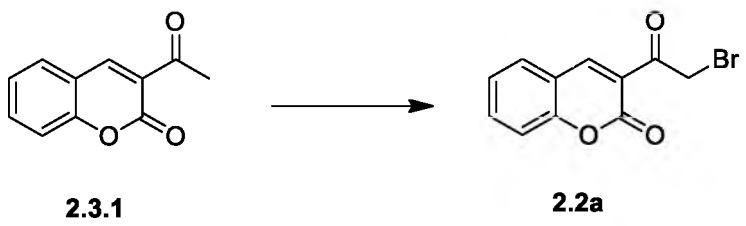


Figure 2.3a 1H NMR of compound **2.3.1** in $CDCl_3$. The aromatic signals are in the expected region corresponding to the expected four protons highlighted in back, as well as proton H-4 and H-2' in red and green, respectively.

2.3.2 Synthesis of 3-(bromoacetyl) coumarin 2.2a

Following the successful synthesis of methyl ketone **2.3.1**, we endeavoured to generate our α-bromo ketone **2.2a**. Several methods for the selective bromination of methyl ketones have been reported, including bromine in chloroform[87,117,118] or glacial acetic acid[119] under a variety of conditions. Furthermore, copper(II)bromide has been used as a bromine source.[104] Owing to our previous success in brominating 3-acetylindole with copper(II)bromide[81,120] we attempted this method first.

This involved dissolving **2.3.1** it in chloroform followed by the addition of a suspension of copper (II) bromide in ethyl acetate. The mixture was heated to reflux with constant stirring. The reflux was halted after a colour change from green to amber was observed, after roughly 5 hours. Following work-up of the reaction, resulting solid was recrystallized from EtOH. However, after several repeats, this method was not successful.



2.3.1 **2.2a**

Scheme 2.3.2. Synthesis of 3-(bromoacetyl) coumarin
$CHCl_3$, Br_2, reflux, 15 min

Our next attempt successfully involved the addition of bromine in chloroform in a chloroform solution of **2.3.1** as per several reported methods (**Scheme 2.3.2**).[87,118] Several attempts to purify the crude mixture by silica gel column chromatography using different solvent systems was unsuccessful. In addition, washing the crude solid with diethyl ether or recrystallization from glacial acetic acid as per reported methods was only partially successful. Eventually repeated recrystallizations from EtOH yielded a sufficiently pure product with a yield of about 30%. Reaction optimisation studies revealed that exceeding a refluxing time of 15 minutes led to the formation of an additional, unknown compound, with a higher retention factor in comparison to **2.2a**. While this compound was not isolated on silica and therefore remained uncharacterised, it is likely the undesired dibrominated analogue, since this is common in similar reactions. The successful synthesis of **2.2a** was confirmed by NMR spectroscopy (**Figure 2.3b**) and MS spectroscopy (**Figure 2.3c**).

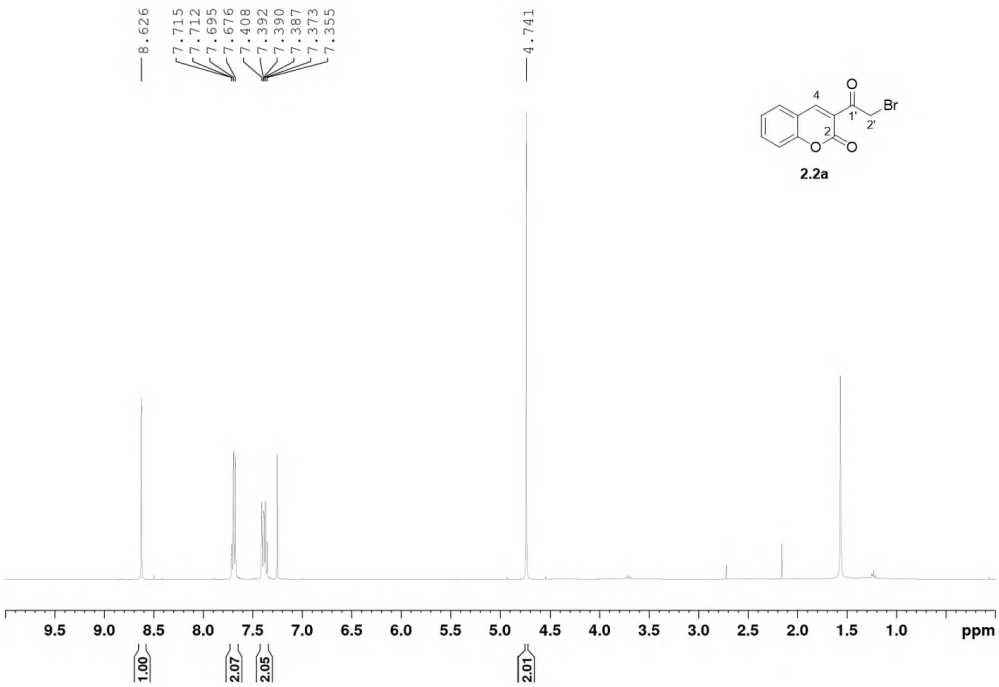


Figure 2.3b. 1H NMR of compound **2.2a** in $CDCl_3$ illustrating the appearance of a CH2 signal at 4.74ppm in place of the original methyl signal. Furthermore, the singlet at 8.62 corresponds with the H-4 singlet

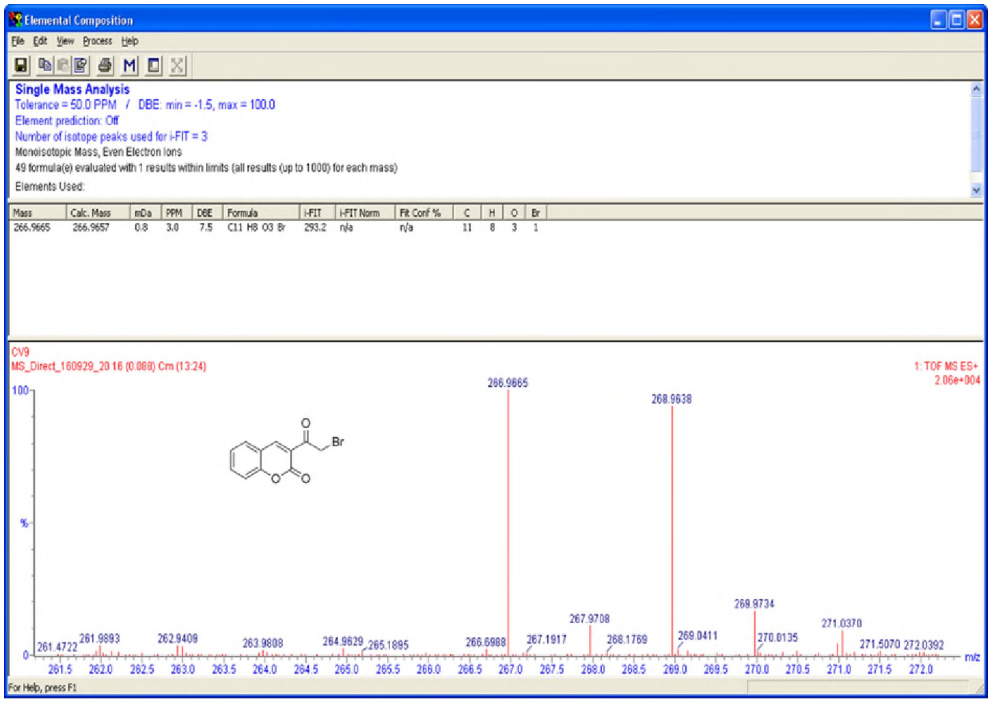
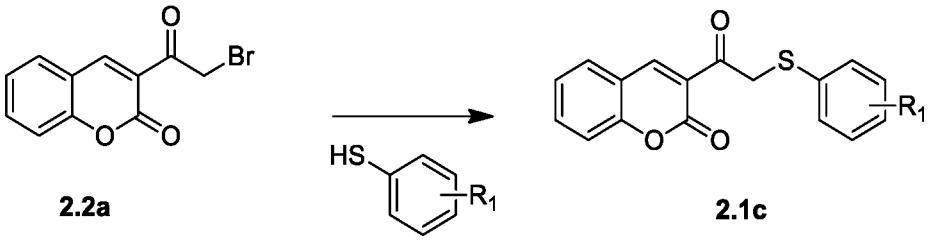


Figure 2.3c. Mass spectrum of compound **2.2a** illustrating the diagnostic presence of bromine seen by the two isotopic peaks with a gap of 2 m/z units at 266.9665 and 268.963 of equal abundance

2.3.3 Synthesis of coumaryl-3-ethanone-α-thioethers

As discussed in the retrosynthetic analysis, we reasoned that our desired coumaryl-3-ethanone-α-thioethers **2.1c** could be synthesized through nucleophilic displacement of 3-bromoacetyl coumarin **2.2a** with variably substituted thiophenols **2.2b.** Furthermore, similar reactions have been reported in ethanol[121] or acetone either in the presence or absence of a base.[81] We opted to use acetone in our system owing to previous experience where ethanol has a tendency to compete with the nucleophiles. We proposed our reaction pathway to proceed as shown below (**Scheme 2.3.3**).



Scheme 2.3.3. Synthesis of targeted compounds **2.1c**
Acetone,reflux,5 h

For our first attempt, we opted to react unsubstituted thiophenol with **2.2a** in acetone under reflux for 5 hours in order to generate the simplest member of our proposed cohort **2.1c.** Contrary to what we had anticipated, inspection of the crude ^{1}H NMR revealed that lower quantities of the expected thioether and unreacted starting material were present (**Figure 2.3d**). However, to our astonishment methyl ketone **2.3.1** was present in abundance, suggesting that under our reaction conditions, compound **2.2a** had undergone reductive dehalogenation. Our first instinct however, was to assume that our sample of **2.2a** had been contaminated with **2.3.1** or had not been sufficiently purified. However, the same reductive dehalogenation was observed at similar abundancies after several repeats. The consistency

of the results piqued our interests and prompted us to deepen our investigation into this unexpected reaction.

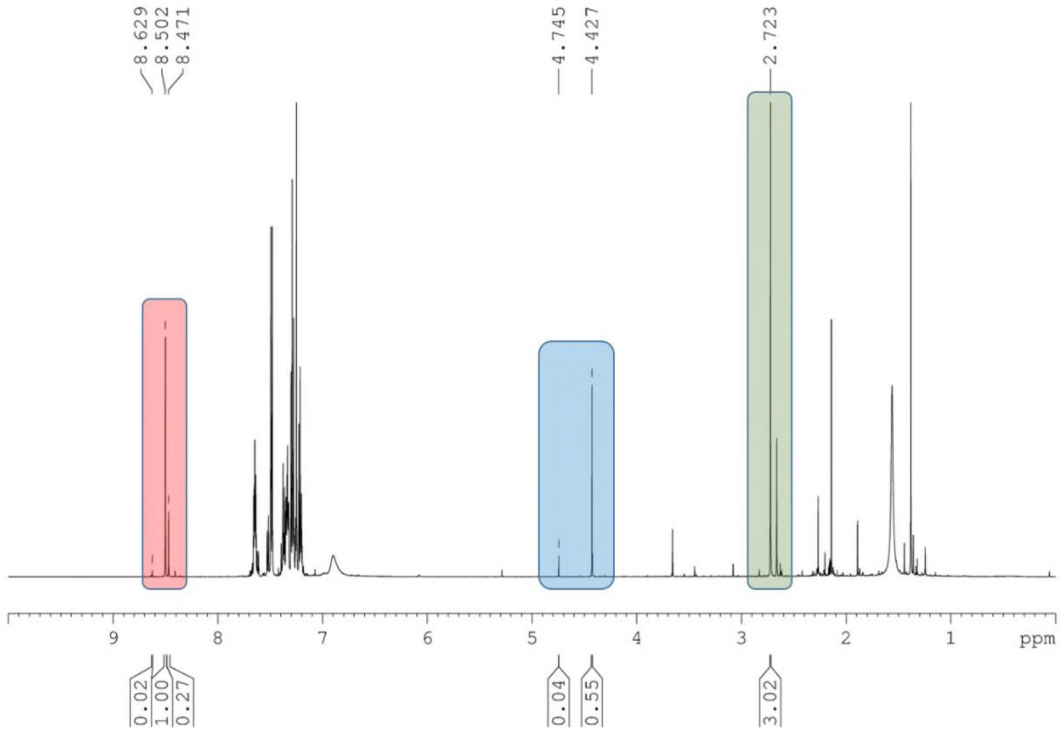


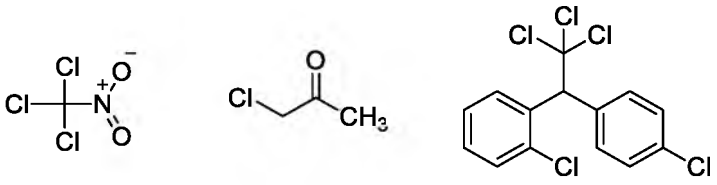
Figure 2.3d. Crude ^{1}H NMR of the reaction between **2.2a** and 2 eq. thiophenol in $CDCl_3$ highlighting important regions in the spectrum. The highlighted signals at 2.72 and 8.50 ppm respectively, correspond with the unexpected formation of methyl ketone **2.3.1**. The small signals at 4.74 and 8.62 ppm correspond with starting material **2.2a.** Furthermore, signals appearing at 4.42 and 8.47 ppm correspond to the formation of the desired thioether product.

In summary, after retrosynthetic analysis, we proposed a reasonable synthesis of our desired compounds, based on sound literature precedence. However, following the successful synthesis of 3-acetyl coumarins **2.3.1 – 2.3.6** as well as selective bromination to yield 2.2a we observed an unexpected reductive dehalogenation reaction, which was confirmed through NMR and MS. The reminder of this thesis will involve deeper investigation into this transformation using **2.2a** as a consistent control.

Chapter 3

3.1 Reductive dehalogenation of α-halocarbonyls

Organic halides find utility in numerous different applications. The uses range from toxic gases that were used during the first world war like chloropicrin, [122–126] lacrimatory agents such as chloroacetone [127,128] to pesticides such as DDT, polymers such as polyvinyl, and as functional groups on innumerable drugs.[129,130]

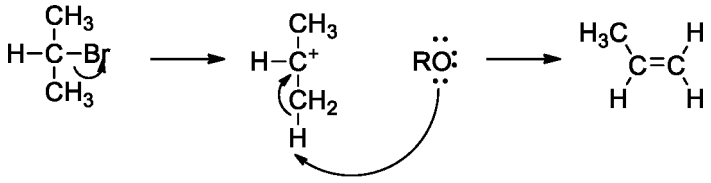


chloropicrin chloroacetone DDT

Of late preservation of the environment has been one of the major focus of governments and research institutions. Some organohalides such as CFCs are known to cause the depletion of the ozone layer, whilst other halides are regarded as environmental contaminants that can also cause organ damage, sterility, birth defects, cancer, neurological damage and skin conditions even in very low exposure concentrations. Some Polychlorinated biphenyls and polybrominated biphenyls are also serious environmental contaminants that have persisting health effects as well. [86,131–136] For these reasons, regulation of waste disposal, emissions and the disposal of organohalogenated materials is very important. Some halogenated compounds are difficult to dispose of as they do not degrade readily and incineration of these compounds usually results in noxious fumes that are still environmentally problematic. Therefore, controlled reductive dehalogenation is an important contribution to the destruction of organic halogenated materials. Cheaper, easier and less time-consuming methods of reductive dehalogenation would have vast impacts both economically and environmentally.[131,134,137,138] Many existing methods of reductive dehalogenation involve use

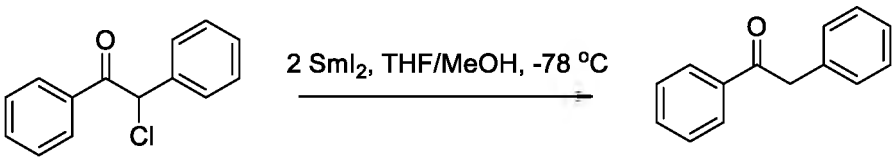
of metals including zinc, chrome, iron, tin,[134–136,138,139] photo catalysis and phosphorous silicon based reducing agents.[140]

In addition, dehalogenation is an important synthetic transformation that maybe used to synthesize starting materials or intermediates of important reactions. Alkenes for example may be obtained from dehalogenation of alkyl halides (**Scheme 3.1.1**).[141]



Scheme 3.1.1. Dehalogenation of alkyl halides to give alkenes

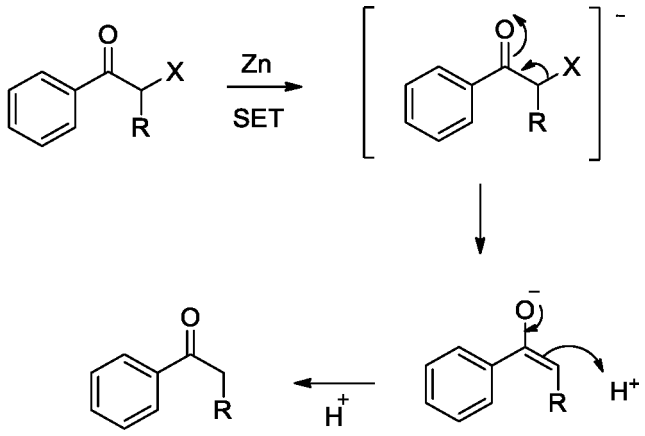
Regarding the dehalogenation of α-halo carbonyls specifically, several methods have been investigated for their utility. Samarium diiodide (SmI_2) was investigated for its scope as a reducing agent on α-heterosubstituted ketones. SmI_2 proved to be a good reductant as it prompted reduction under neutral conditions with good chemoselectivity. [142]



Scheme 3.1.2. SmI_2 assisted reduction

A similar transformation was observed in the presence of zinc and ammonium chloride in ethanol under microwave irradiation (**Scheme 3.1.3**). This method led to reduction of even dibrominated acetophenones. They proposed that the cascade begins with a single electron

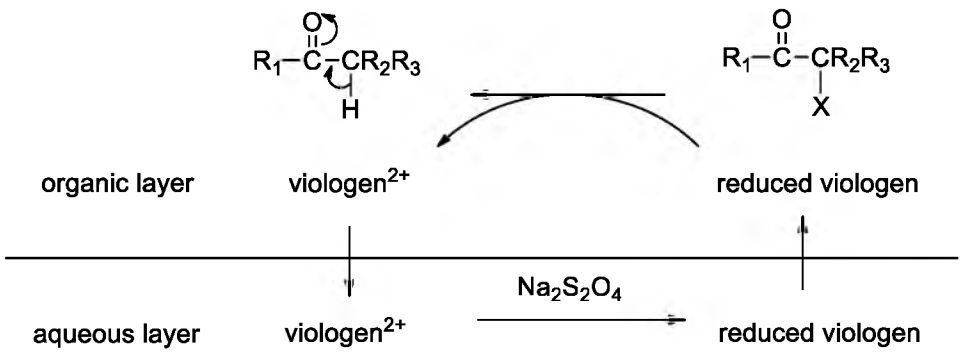
transfer (SET) from zinc to the halo ketone, thereby forming a radical anionic spices. A second SET results in enolate formation, which rearranges to the dehalogenated carbonyl species. [143]



Scheme 3.1.3. Zn assisted reductive dehalogenation

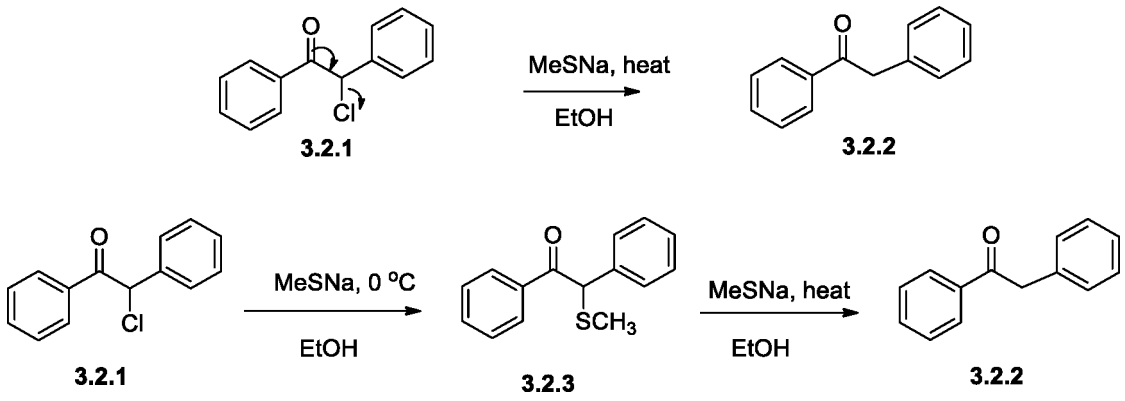
3.2 Reductive dehalogenation of α-halocarbonyls by thiols and other group 16 elements

Group 16 elements have also displayed the ability to induce reductive dehalogenation of α-haloketones. Sodium dithionite in conjunction with viologens have been reported to reductively dehalogenate α-haloketones *via* a two phase free radical mechanism **(scheme 3.2.1)**.[133]



Scheme 3.2.1 viologen mediated reductive dehalogenation

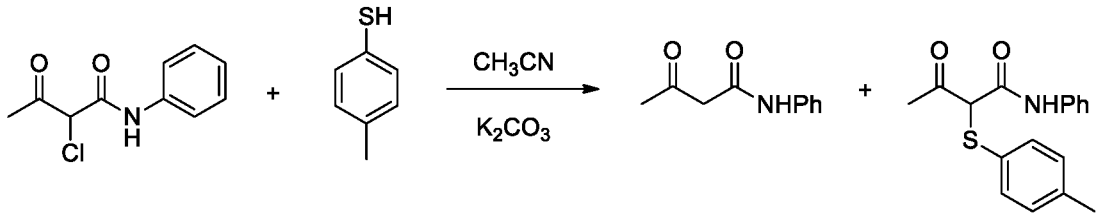
In 1970, Ōki *et al*. noted that heating α-chlorodeoxybenzoin **3.2.1** with excess sodium methanethiolate in ethanol unexpectedly formed the dechlorinated deoxybenzoin **3.2.2** in near quantitative yield instead of α-(methylthio)deoxybenzoin **3.2.3** (**Scheme 3.2.2**). However, repeating the reaction at 0 °C with one equivalence of sodium methanethiolate gave rise to **3.2.3** through a second nucleophilic displacement. Further heating of **3.2.3** with additional methanethiolate resulted in the formation of **3.2.2**. They further observed that the rate of the second displacement of methanethiolate was slower when attempted with thiophenol, which the authors reasoned was due to the comparatively lower nucleophilicity of thiophenol. They concluded from their study that the net reduction occurs as sequential nucleophilic displacement steps. [144]



Scheme 3.2.2. Thiol mediated reductive dehalogenation scheme as proposed by Oki *et al.*

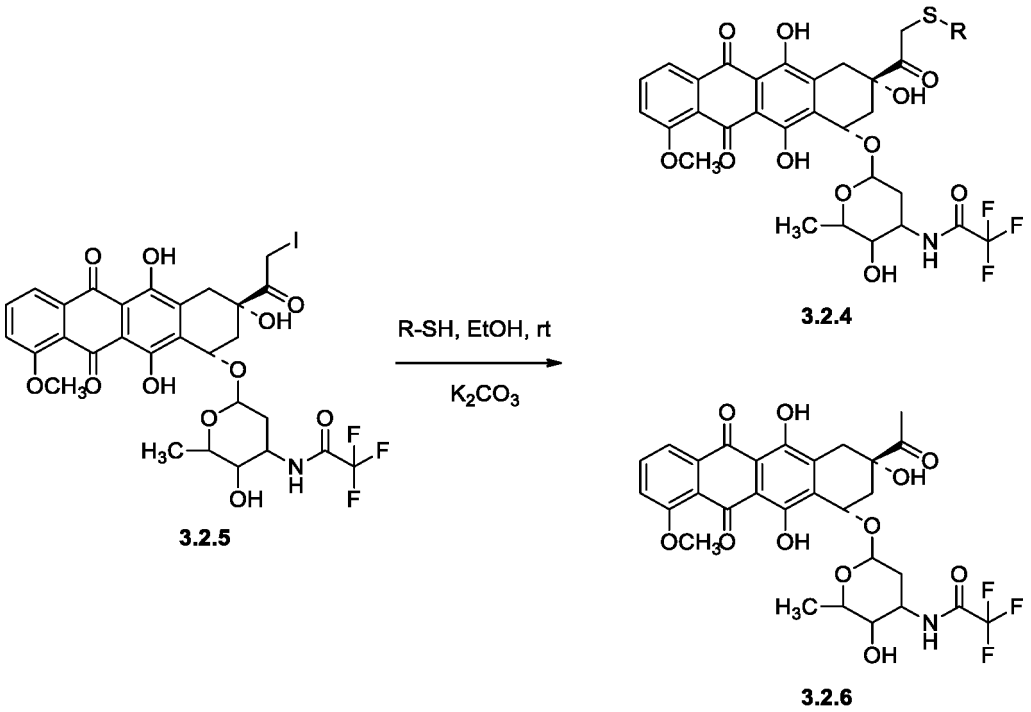
These observations were supported by a recent study conducted by Wei-Li Dong and co-workers using thiophenol and K_2CO_3 in acetonitrile (**Scheme 3.2.3**). They observed that the yields of dechlorinated ketones were dependent on the time of reaction and the specific thiol. Furthermore, they noted that when 1.2 equivalents of thiophenol were used, a higher

proportion of nucleophilic displacement product was observed, whereas excess thiophenol resulted in increased dehalogenation.[132]



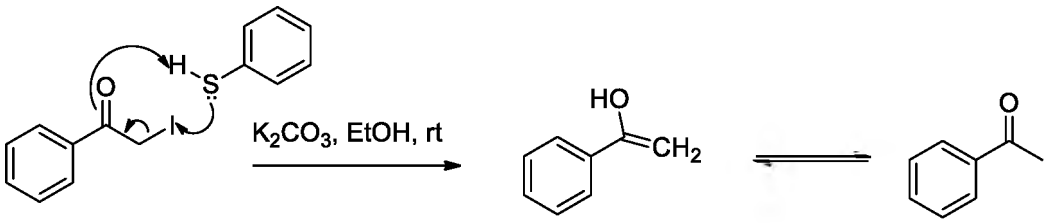
Scheme 3.2.3. Reductive dehalogenation as observed by Dong et al.

As part of an anti-tumour drug discovery project, Israel *et al.* attempted to prepare semisynthetic 14-thia derivatives of *N*-(trufluoroacetyl)adriamycin **3.2.4** through the treatment of 14-iodo-N-(trifluoroacetyl)-daunorubicin **3.2.5** with alkane or benzenethiols in the presence of K_2CO_3 in ethanol. However, this reaction resulted in an unexpected deiodination, resulting in the formation of **3.2.6**[145].



Scheme 3.2.4. Reductive dehalogenation as observed by Israel et al.

This phenomenon was again observed with the reaction of α-iodobenzophenone (**3.2.7, Scheme 3.2.5**), while repeating this reaction with α-chloro and bromoacetophenones, formed the corresponding sulfides in high yield. When the reactions were repeated with selenols instead of thiols, the same trend was observed. [145] Their findings suggested that the reactivity of the halogen on the halomethylketone played a determining role in whether reduction or substitution would take place. Furthermore, the rapid rate of dehalogenation led them to postulate that their dehalogenation occurred in a single discreet step (**Scheme 3.2.5**), rather than a sequential nucleophilic displacement as postulated by Ōki *et al*.



3.2.7

Scheme 3.2.5. Thiol mediated reductive dehalogenation mechanism as proposed by Israel *et al*.

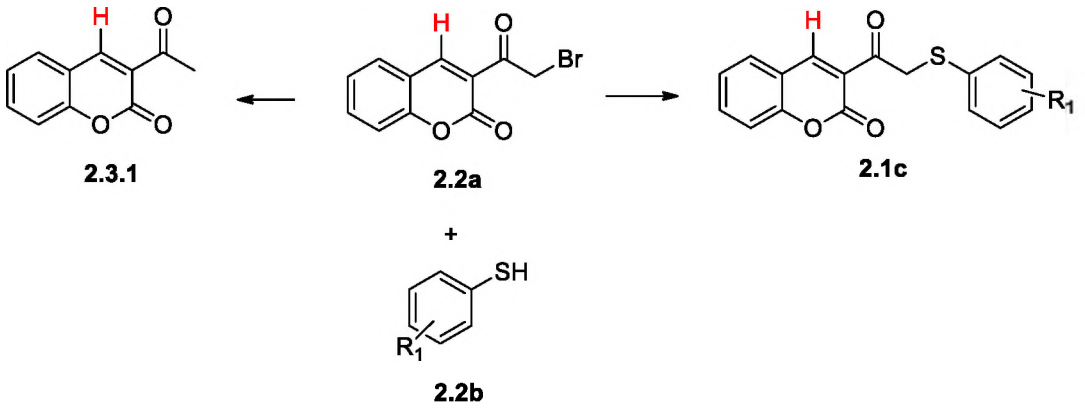
To summarise, the proposed pathway of Ōki *et al*, suggests that a reductive dehalogenation relies on 2 equivalents of strongly nucleophilic thiophenol, while Israel *et al's* mechanism requires the thiol to be able to donate a proton, resulting in enolization.

These mechanisms are seemingly conflicting. Accordingly, we were eager to gain more insight into this mechanism and were curious as to the effect that increased or decreased nucleophilicity of the thiol would have on the extent of reductive dehalogenation, as well as if changes in equivalents may impact degree of dehalogenation. Accordingly, we performed a series of reactions, whereby **2.2a** was reacted with a series of thiophenols substituted with

various electron withdrawing and donating groups in order to influence the electronic environment of the sulfur atom.

3.3 Results and Discussion

In order to monitor the relative conversion between the thioether **2.1c** and methyl ketone **2.3.1** as well as remaining α-bromoketone **2.2a**, we inspected the relative integrals of the H-4 proton of each of the three chemical species (**Scheme 3.3.1**). Referring back to **Figure 2.3d** three singlets between 8.3 and 8.7 ppm were observed. The signals at 8.50 and 8.62 corresponded to the known chemical shifts of the H-4 protons of **2.3** (**Figure 2.3a**) and **2.2a** (**Figure 2.3b**). In addition, the methyl signal of **2.3.1** (2.72 ppm) and the methylene signal of **2.2a** (4.72 ppm) both integrated at the appropriate ratios, i.e. 1:3 and 1:2, respectively.



Scheme 3.3.1. Observed reaction pathway

We were able to identify the signals at 8.47 ppm and 4.42 ppm as the H-4 and methylene CH_2 of compound **2.1c**, which appropriately integrated at 1:2 and added together to give a total integral. Each individual integral is then divided by the total integral and multiplied by 100 to

get the abundance of each species in percent (**Table 3.3a, entry 1a**). Unfortunately, after exhaustive chromatography, we were unable to obtain sufficiently pure samples of each of the thioethers to determine isolated yields. However, we were able to obtain samples of sufficient purity to resolve chemical structures using 2D NMR as well as HRMS (**Figure 3.3a**)

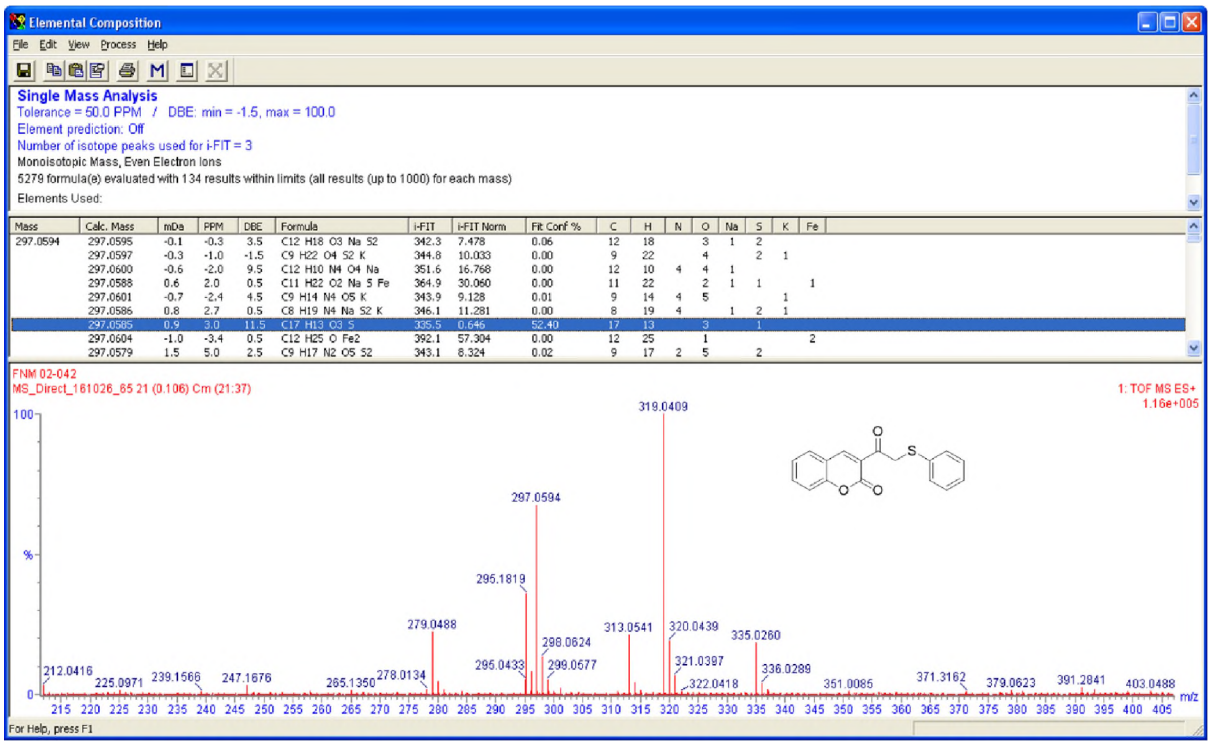
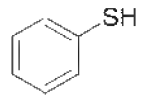
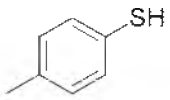
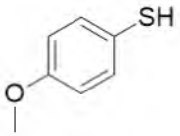
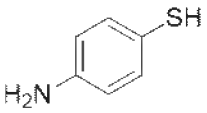
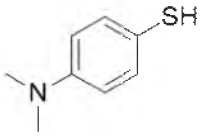


Figure 3.3a. Representative HRMS confirming the structures of the thioethers **2.1c**

Our first series of experiments involved the introduction of electron donating substituents in order to increase nucleophilicity. Entries 1a – 5a incorporated 2 equivalents of thiophenol, which donate electron density either by resonance or induction. The weakly electron donating *para*-methyl group (**entry 1a**) had a negligible influence on relative conversation. The resonance electron donating *para*-methoxy resulted in an increase in thioether production, with a reduction in the formation of 2.3.1. This trend was further enhanced with

the increased nucleophilicity of *para*-amino and dimethyl amino thiophenols which resulted in a significant conversion into the respective thioethers coupled to low levels of dehalogenation. Repeating the experiments with a lowered number of equivalents of thiophenol (1.2 eq. **entries 1b – 4b**) showed similar levels of reductive dehalogenation save for **4b** which showed greater conversion to **2.3.1** than when using 2 equivalents. Generally, in cases where lower reduction was observed the amount of unreacted **2.2a** was increased, suggesting that reductive dehalogenation of **2.2a** is not related to an initial nucleophilic displacement as proposed by Ōki *et al.*

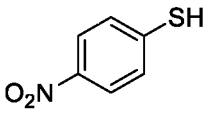
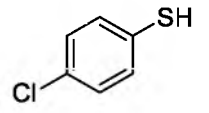
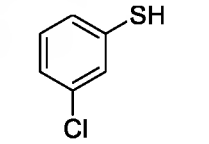
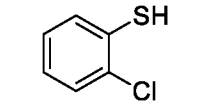
Table 3.3a.

Experiment entry	Thiophenol	2.1c	2.2a	2.3.1
1a		21	2	77
1b		3	23	74
2a		17	3	80
2b		15	9	76
3a		46	0	54
3b		9	35	56
4a		88	0	12
4b		48	0	52
5a		85	5	10
5b		NA	NA	NA

To complement our first cohort of experiments, we moved on to decreasing nucleophilicity of the thiophenols through electron withdrawing substituents (**Table 3.3b**). **Entries 6a – 9a**

were performed using 2 equivalents of *para*-nitro, *para*-chloro, *meta*-chloro and *ortho*-chloro thiophenol. Interestingly, the presence of a strongly resonant electron withdrawing *para*-nitro group (**entry 6a**) completely consumed the starting α-bromoketone **2.2a** and resulted in a significant formation of the methyl ketone **2.3.1** with a small presence thioether.

Table 3.3b. percentage conversions observed with reducing nucleophilicity

Experiment entry	Thiophenol	2.1c	2.2a	2.3.1
6a		6	0	94
6b		5	22	73
7a		0	12	88
7b		NA	NA	NA
8a		14	1	85
8b		17	7	76
9a		0	0	100
9b		NA	NA	NA
10	-	0	100	0

Similarly, *para*-chloro thiophenol (**entry 7a**) resulted in major dehalogenation, however, a small portion of **2.2a** remained with no detectable thioether indicating that thiol induced reductive dehalogenation of α-bromoketones is not necessarily related to nucleophilicity. Reaction with *meta*-chloro thiophenol (**entry 8a**) resulted in a similar degree of dehalogenation, this time with a small portion of **2.1c** forming, while *ortho*-chloro thiophenol **9a** also resulted in complete dehalogenation. As was observed with electron donating group-substituted thiophenols, repeating the experiments with 1.2 thiophenol equivalents resulted

in a similar trend to that of using 2 equivalents **6b – 9b**. As a control the experiment was repeated under the same conditions but in the absence of any thiophenol (**entry 10**) which showed no change to the starting material **2.2a.**

The general pattern observed throughout these experiments was that strongly electron-withdrawing substituents resulted in increased reductive dehalogenation, while the converse was true for strongly electron donating substituents. As discussed previously, Ōki cited the lower nucleophilicity of thiophenol as a factor contributing to lower dehalogenation. If this conclusion was applied in our system, one would expect the stronger nucleophiles such as *para*-amino thiophenol (**entry4a**) and *para*-diethylamino thiophenol (**entry5a**) to have displayed a greater degree of dehalogenation, while, the *para*-nitro thiophenol (**entry 6a**), should not result in such significant dehalogenation. A further point is that, the mechanism of Ōki requires two equivalents of thiophenol. However, we observed consistency in our results in experiments conducted with 1.2 eq. of thiophenol, instead of a greater proportion of thioether present in the reaction mixture. This result is more closely associated with the proposed mechanism of Israel *et al.* Finally, **entry 4b** displayed an increase in dehalogenation when lowered equivalents of a demonstrably good nucleophile were utilised providing more evidence that the mechanisms of dehalogenation and substitution mechanisms are unrelated in this system.

3.4 Increasing nucleophilicity through the addition of base

Having gained more insight into the unexpected reductive dehalogenation, we were still eager to synthesise our desired products. We were interested in determining whether the introduction of stoichiometric quantities of K_2CO_3 would promote nucleophilic displacement

over dehalogenation, under the same reaction conditions. K_2CO_3 is been used ubiquitously to enhance nucleophilic substitution of α-halogens.

Our first experiment in this series involved *para*-amino thiophenol since results obtained in earlier experiments in the absence of base had shown that *para*-amino thiophenol had been the best performing nucleophile. This was followed by unsubstituted thiophenol as well as para-nitro, *para*-chloro, *meta*-chloro, *ortho*-chloro thiophenol, which were the best performing reducing agents.

As was the case with all previous experiments, crude ^{1}H NMR spectra were analysed after work up, in order to identify evidence of **2.3.1**, **2.2a** and **2.1c**. Addition of K_2CO_3 to reaction between **2.2a** and *para*-amino thiophenol resulted in complete conversion into the desired thioether, representing an increase from the 88% conversion obtained in the absence of base. Furthermore, there was no evidence of **2.2a** or reduced **2.3.1**.

Analysis of the crude ^{1}H NMR spectra resulting from reaction with thiophenol, *para*-nitro thiophenol, *para*-chloro thiophenol, *meta*-chloro thiophenol, *ortho*-chloro thiophenol, with **2.2a** in the presence of K_2CO_3 showed no evidence of starting bromoketone, displacement product nor dehalogenation product. Subsequent silica purification using 95:5 hexane: ethyl acetate as a solvent system, allowed us to isolate unknown non-polar reaction products (**3.4a – e**). Analysis of the ^{1}H NMR spectrum of **3.4a** (**Figure 3.4a**) revealed the presence of a para substituted phenyl ring, suggesting that the *para*-nitro thiophenol had been incorporated into the molecule. Intriguingly, this spectrum contained a methyl signal (2.62 ppm) as well as a down field proton signal (12.18 ppm).

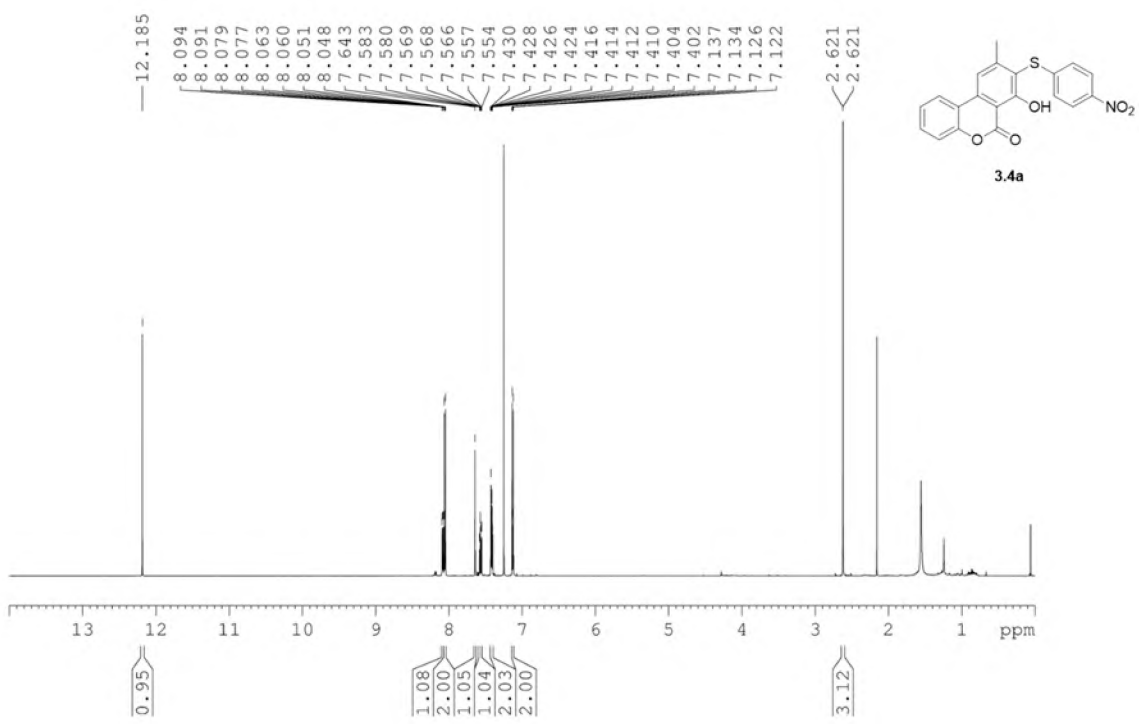


Figure 3.4a. 1H NMR for compound 3.4a

Furthermore, the diagnostic coumarin H-4 aromatic singlet had been replaced by a deshielded signal at 7.64 ppm. HMBC analysis also showed a strong correlation between the singlet at 7.64 ppm and the methyl carbon at 22.8 ppm. This combined evidence suggested that the methyl signal was not associated to a ketone and that alterations to the coumarin scaffold had occurred around position 4. The ^{13}C NMR spectrum (**Figure 3.4b**) confirmed that this compound contained 20 carbons. Which suggested the presence of three carbons in addition to the 17 carbons attributed to the acetyl coumarin and thiophenol. We surmised that these three carbons were likely from the acetone solvent. Finally, HRMS analysis confirmed the molecular formula to be $C_{20}H_{12}NO_5S$ $[M-H]^-$ **(Figure 3.4c)**

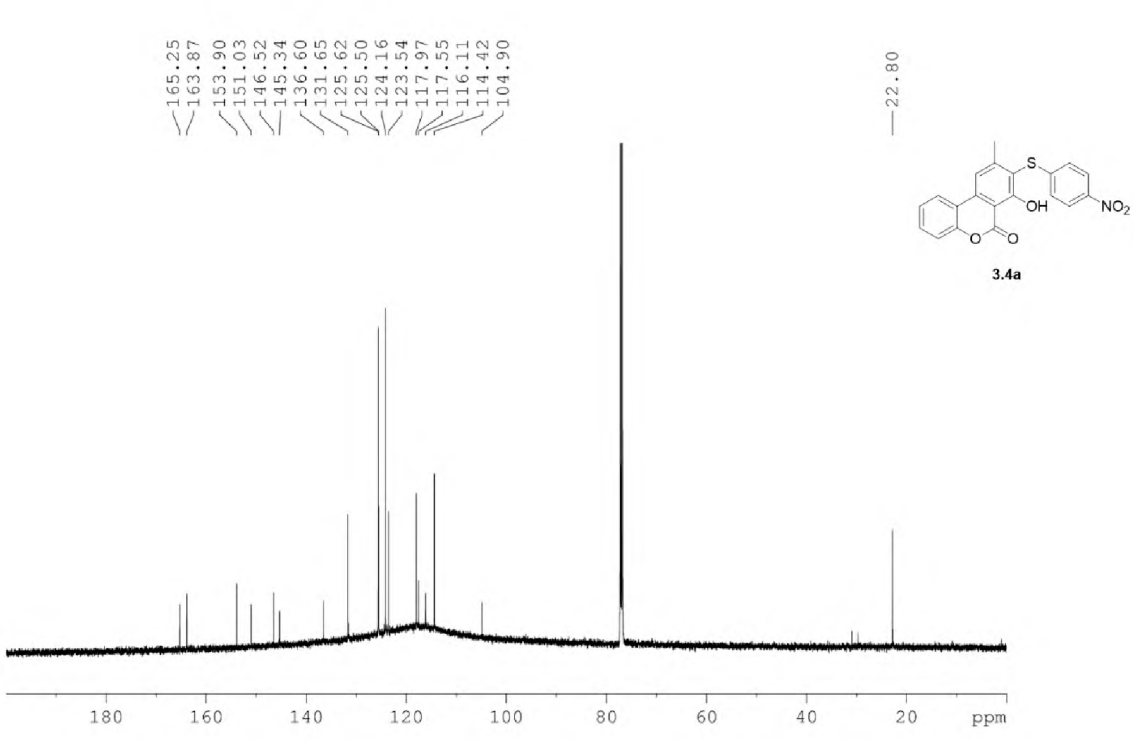


Figure 3.4b. 13C NMR for compound 3.4a

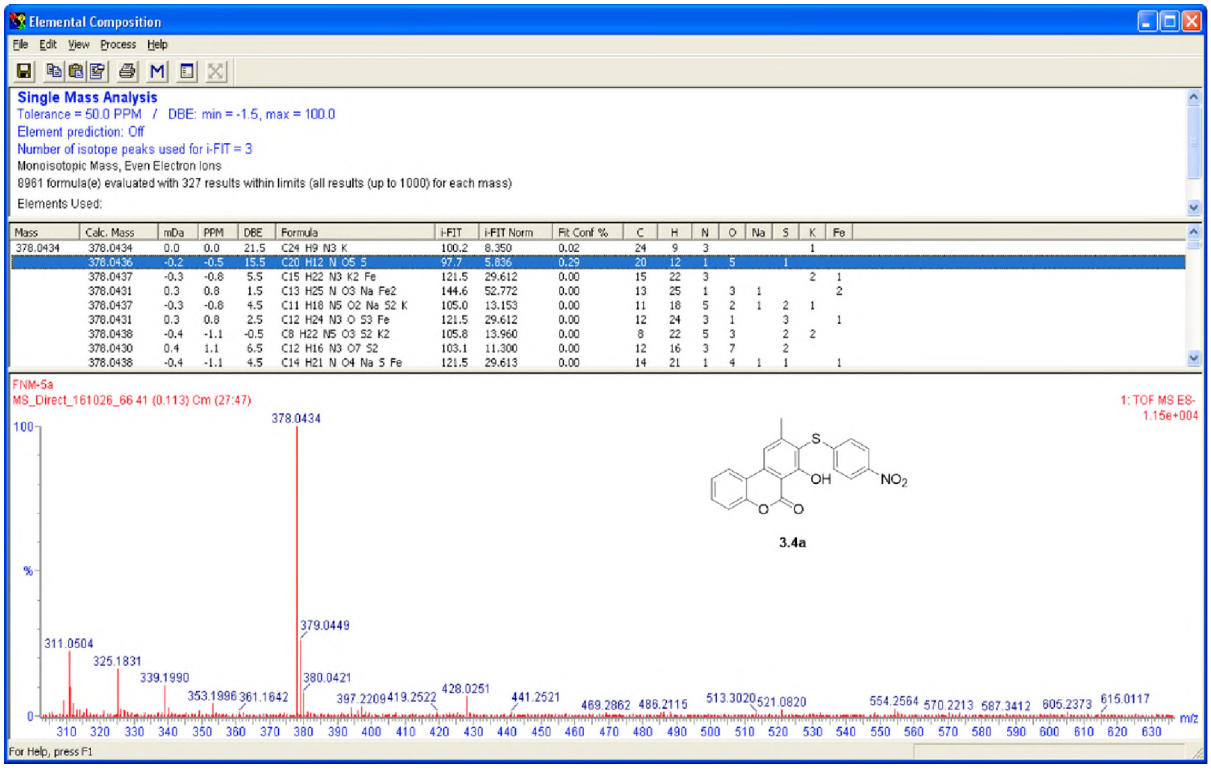
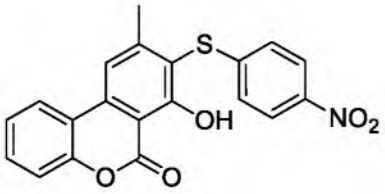
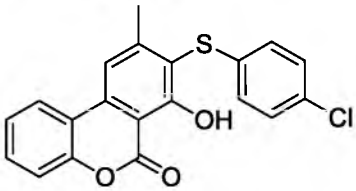
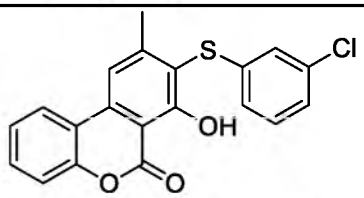
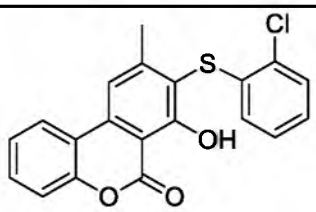
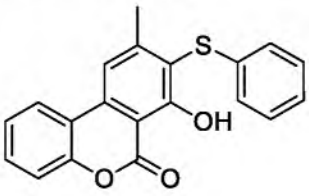


Figure 3.4c. HRMS for compound 3.4a

Considering this evidence, as well as thorough 2D NMR analysis, we were able to unambiguously assign the structure of these compounds as previously unreported 8-thiolated dibenzo[*b,d*]pyran-6-ones (**3.4a – e, Table 3.4**)

Table 3.4 novel compounds 4a-e and their percentage yields

Compound number	Structure	Yields (%)
3.4a		10
3.4b		4
3.4c		13
3.4d		12
3.4e		13

For greater insight, we then further conducted three control experiments. In the first control, we performed the experiments under the same conditions but without any thiophenol. In the

second experiment TEA was used as a base used instead of K_2CO_3 and thirdly the reaction was performed with methyl ketone **2.3.1** instead of the bromoketone **2.2a**. We did not observe the formation of the corresponding dibenzo[*b,d*]pyran-6-one in any of these three scenarios, suggesting that specific elements of the reaction conditions were important in the synthesis of these compounds.

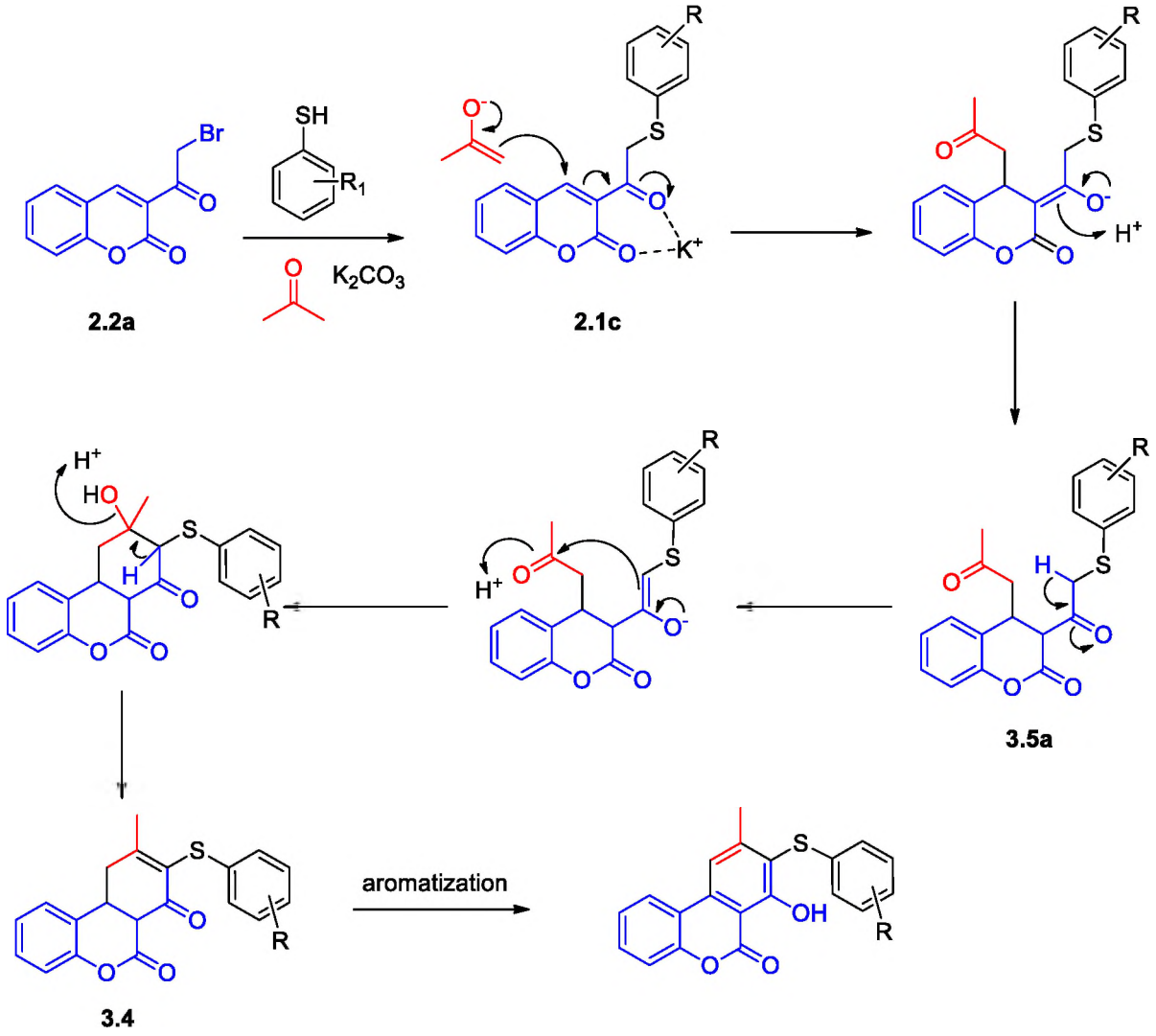
3.5 Mechanism of the Synthesis of dibenzo[*b,d*]pyran-6-ones

A number of synthetic approaches for the synthesis of dibenzo[*b,d*]pyran-6-ones have been reported including palladium catalysed Suzuki methodology, silver catalysed reactions, and Rh (III) catalysed reactions.[146–154]

Of greatest relevance to us was a method reported by C. F. Koelschan and S. A. Sundet who found that 7-hydroxy-9-coumarinyl-6*H*-benzo[*c*]chromen-6-one formed through an intramolecular Michael type condensation between two subunits of **2.3.1**, followed by spontaneous aromatising when boiled in an ethanolic solution containing piperidine. [151]

This allowed us to propose a mechanism for the formation of **3.4a – e** as follows; firstly, the addition of base facilitates nucleophilic displacement of the bromine as hypothesised, leading to the formation of an α-thiocarbonyl (**2.1c**). This is followed by a Michael addition between the α-β unsaturated system of the 3-acylcoumarin and an enolate formed from acetone follows this to form **3.5a**. The electrophilicity of this α-β unsaturated system is enhanced through chelation between potassium cations and the 1,3-dicarbonyl. The strong electron withdrawing nature of the thiol substituent, renders the methine proton slightly acidic. This results in enolization, with the subsequent nucleophile attacking the acetone carbonyl. Dehydration of the secondary alcohol completed the Robinson annulation. Aromatisation as

reported by Koelschan and Sundet, completes the multicomponent synthesis resulting in compound **3.4a – e**



Scheme 3.5.1. Our proposed mechanism for the formation of compounds **3.4a - e**

3.6 Biological Assay

Compounds **3.4a – e** were submitted for whole cell phenotypic screen against chloroquine-sensitive *P. falciparum* (3D7) strain. We also tested for cytotoxicity on the human derived HeLa cell lines. Although all the compounds were found to be non-toxic to HeLa cell lines, none of the compounds were active against this strain.

3.7 Conclusion

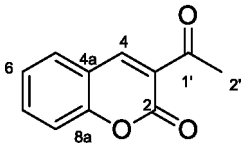
In this study, we were interested in determining the influence that substituting the indole scaffold with an equally biologically interesting coumarin scaffold might have on biological activity. Our synthetic scheme pivoted on the successful nucleophilic substitution of an α-bromoketone with a thiophenol. Unexpectedly, we observed that thiophenol results in a large proportion of the starting material undergoing reductive dehalogenation. Owing to the lack of understanding of this transformation as well as two seemingly conflicting mechanisms in the literature, we compared the effect of variably substituted thiophenol of reductive dehalogenation of a constant halogenated species. Our results point to a strong relationship between the rate of dehalogenation and the electronic environment of the thiophenol. Furthermore, we deduced that the number of equivalents have little effect on the rate of debromination. Our results support the observation of Israel, but do not provide a reason for the observation of Ōki *et. al.* This is possibly explained in the context of the Hard-Soft acid base theory, which designates sulfur as a soft base. Per this theory, sulfur preferably interact with a soft halogen such as iodine or bromine. Yet in the case of chlorine, nucleophilic substitution may be preferred. However, a more detailed study would be required to answer this question. Finally, the addition of stoichiometric amounts of K_2CO_3 assisted nucleophilic displacement as well as a multicomponent synthesis of unreported unreported 8-thiolated dibenzo[*b,d*]pyran-6-ones, which are scaffolds of general interest to the synthetic organic chemistry community.

Chapter 4
Experimental data

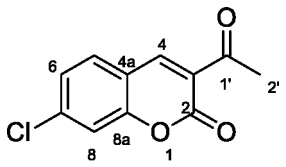
1D and 2D NMR spectra were acquired on either a Bruker Fourier 300 MHz, or a 600 MHz Avance II spectrometer. Chemical shifts are reported in ppm. Reference residual solvent resonances are as follows: $CDCl_3$ δ_H 7.25, δ_C 77.0; DMSO-d_6 δ_H 2.50, δ_C 39.50 ppm. High resolution mass spectrometry was performed on a Waters Synapt G2 TOF instrument with an ESI source. Flash chromatography was performed using Kieselgel 60 (230–400 mesh) silica gel. All solvents were distilled prior to use.

4.1 General procedure for the synthesis of methyl ketones (2.3.1 – 2.3.6)

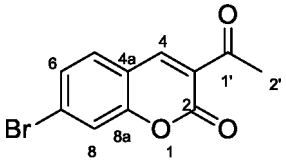
Salicylaldehyde (1 ml, 9 mmol) and ethyl acetoacetate (1.2 ml, 9 mmol) were dissolved in 22.5 ml EtOH with continuous stirring. 3 – 5 Drops of piperidine were added and the reaction mixture was left to run overnight (16 hr) at room temperature. Thereafter the mixture was cooled and the resulting ppt was collected. The purified product was obtained by recrystallization from EtOH. This representative method was adopted in the synthesis of compounds **2.3.1 – 2.3.6**.



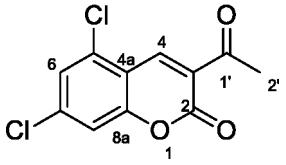
3-acetyl-2H-chromen-2-one. (2.3.1)[84,104] White solid, 81.3 % yield, ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.49 (1H, s, H-4), 7.67-7.63 (2H, m, H-5, H-7), 7.61-7.33 (2H, m, H-6, H-8), 2.73 (3H, s, H-2') ^{13}C NMR δ: (75 MHz, $CDCl_3$) 195.5 (q_c, C-1'), 159.2 (q_c, C-2), 155.3 (q_c, C-8a), 147.4 (CH, C-4), 134.4 (CH, C-7), 130.2 (CH, C-5), 125.0 (CH, C-5), 124.5 (q_c, C-3), 118.2 (q_c, C-4a), 116.7 (CH, C-8), 30.5 (CH^3, C-2') ppm; HRESMS *m/z* 189.0549 (calculated for $C_{11}H_9O_3$ $[M+H]^+$ 189.0552)



3-acetyl-7-chloro-2H-chromen-2-one.(2.3.2)[84] Off white solid, 67.4% yield, ^{1}H NMR (300 MHz, DMSO) δ: 8.61 (1H, s, H-4), 8.09 (1H, d, *J* = 3 Hz, H-8), 7.80 (1H, dd, *J* = 3.0, 9.0 Hz, H-6) 7.53-7.50 (1H, d, *J* = 9.0 Hz, H-5) 2.58 (3H, s, H-2′) ^{13}C NMR (75 MHz, DMSO) δ: 195.6 (q_c, C-1′), 158.2 (q_c, C-2), 153.8 (q_c, C-8a), 146.2 (CH, C-4), 134.3 (q_c, C-7) 129.9 (CH, C-5), 129.1 (CH, C-6), 125.9 (q_c, C-3), 120.0 (q_c, C-4a), 118.8 (CH, C-8), 30.5 (CH_3, C-2′) ppm

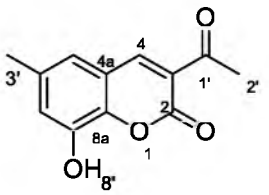


3-acetyl-7-bromo-2H-chromen-2-one.(2.3.3)[108] Off white solid, 51.7% yield, ^{1}H NMR (300 MHz, DMSO) δ: 8.61 (1H, s, H-4), 8.22 (1H, s, H-8) 7.91 (1H, dd, *J* = 9.0, 3 Hz, H-6), 7.46 (1H, d, *J* = 9 Hz, H-5) 2.58 (3H, s, H-2′), ^{13}C NMR (75 MHz, DMSO) δ: 195.2 (q_c, C-1′), 157.7 (q_c, C-2), 149.5 (q_c, 8a), 145.9 (CH, C-4), 133.6 (q_c, C-7), 129.1 (CH, C-5), 128.9 (CH, C-6), 126.6 (q_c, C-3), 121.3 (q_c, C-4a), 121.1 (CH, C-8), 30.6 (CH_3, C-2′) ppm

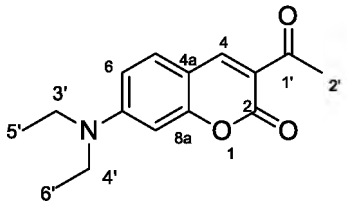


3-acetyl-5,7-dichloro-2H-chromen-2-one. (2.3.4)[104,108] Pale yellow solid, 7.8% yield, ^{1}H NMR (300 MHz, DMSO) δ: 8.61 (1H, s, H-4), 8.10 (2H, m, H-6, H-8), 2.58 (3H, s, H-2′) ^{13}CNMR (75

MHz, DMSO) δ: 195.3 (q_c, C-1'), 149.4 (q_c, C-2), 149.8 (q_c, 8a), 133.6 (CH, C-4), 129.1 (q_c, C-7), 128.9 (q_c, C-5), 126.6 (CH, C-6), 121.2 (q_c, C-3), 121.1 (q_c, C-4a), 116.3 (CH, C-8), 30.6 (CH_3, C-2') ppm



3-acetyl-8-hydroxy-6-methyl-2H-chromen-2-one. (2.3.5) Yellow solid, 53% yield, ^{1}H NMR (300 MHz, DMSO) δ: 8.61 (1H, s, H-4), 8.09 (1H, d, *J* = 3 Hz, H-8), 7.80 (1H, d, *J* = 3 Hz, H-7), 7.53-7.50 (1H, d, *J* = 3 Hz, H-5), 2.58 (3H, s, H-2'), 2.08 (3H, s, H-3') ^{13}C NMR (75 MHz, DMSO) δ: 195.7 (q_c, C-1'), 159.1 (q_c, C-2), 153.3 (q_c, C-8a), 147.5 (CH, C-4), 136.0 (CH, C-7), 134.8 (CH, C-5), 130.7 (q_c, C-6), 124.9 (q_c, C-3), 118.4 (q_c, 4a), 116.4 (q_c, C-8), 30.6 (CH, C-2'), 20.7 (CH, C-3') ppm

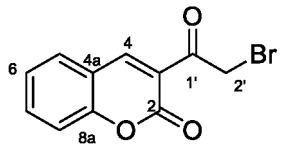


3-acetyl-7-(diethylamino)-2H-chromen-2-one. (2.3.6) Bright yellow solid, 28.8% yield, ^{1}H NMR (300 MHz, DMSO) δ: 8.46 (1H, s, H-4), 7.65 (1H, d, *J* = 9, H-4), 6.79 (1H, dd, *J* = 9, 3, H-6), 6.55 (1H, d, *J* = 3, H-8), 3.51 (4H, m, H-3', H-4'), 2.5 (3H, s, H-2'), 1.16-1.11 (6H, t, H-5', H-6'), ^{13}C NMR (75 MHz, DMSO) δ: 195.2 (q_c, C-1'), 159.9 (q_c, C-2), 158.2 (q_c, C-8a), 152.9 (CH, C-6),

147.6 (CH, C-4), 132.4 (CH, C-5), 115.0 (qc, C-7), 110 (CH, C-6), 107.5 (q_c, 4a), 95.8 (CH, C-8), 44.4 (2 CH, C-3', C-4'), 30.1 (CH, C-2'), 12.3 (2 CH, C-5', C-6') ppm

4.2 Synthesis of 3-(2-bromoacetyl)-2H-chromen-2-one. (2.2a)

3-acetyl coumarin (300 mg, 1.6 mmol) was dissolved in 2 ml of chloroform. 0.2 ml of a bromine stock solution (285 ml bromine: 500 ml chloroform) was added to the solution dropwise with vigorous stirring. The mixture was heated to reflux for 15 mins and cooled. The resulting ppt was collected and washed with diethyl ether to afford desired compound. Purification was done by multiple recrystallizations from EtOH.

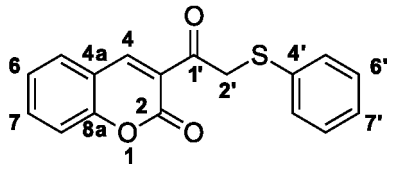


3-(2-bromoacetyl)-2H-chromen-2-one. (2.2a)[87,118] White solid, 30% yield, ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.65 (1H, s, H-4), 7.75-7.70 (2H, m, H-5, H-7), 7.43-7.38 (2H, m, H-6, H-8), 4.77 (2H, s, H-2') ^{13}C NMR (75 MHz, $CDCl_3$) δ: 188.9 (q_c, C-1'), 158.9 (q_c, C-2), 155.4 (q_c, C-8a), 149.5 (CH, C-4), 135.1 (CH, C-7), 130.4 (CH, C-5), 125.3 (CH, C-6), 122.2 (q_c, C-3), 118.1 (q_c, C-4a), 116.9 (CH, C-8), 35.6 (CH, C-2') ppm; HRESMS *m/z* 266.9665 (calculated for $C_{11}H_8{}^{79}BrO_3$ $[M+H]^+$ 266.9657)

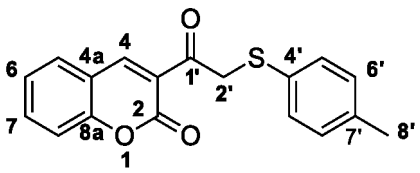
4.3 General procedure for the synthesis of compounds 2.3.1 (1 – 9)

3-(Bromoacetyl)coumarin (**2.2a**, 100 mg, 0.374 mmol, 1 equiv.) and *para*-nitro thiophenol (**6a**,116 mg, 0.749 mmol, 2 equiv.) were dissolved in 10ml of distilled acetone and heated under reflux for 5 hours. After allowing the reaction to cool, the reaction mixture was

extracted with EtOAc (3 x 10 ml) and the organic phase washed with water (2 x 10 ml). Solvent was removed *in vacuo* and the resultant crude mixture was analysed through NMR spectroscopy without purification. This representative method was applied to **Table 3.3b entries 1a-9a** using the same proportions of solvent and reagents as detailed above. This representative method was employed but thiophenol equivalents altered to 1.2 for entries **1b – 6b, 8b**, with all other parameters remaining the same.

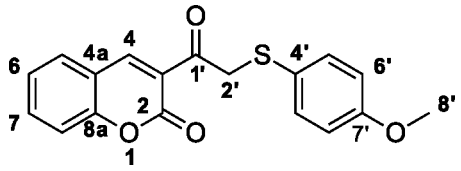


3-[2-(Phenylthio)acetyl]-2*H*-1-benzopyran-2-one, (1a). white solid, ^{1}H NMR (300 MHz, $CDCl_3$,) δ: 8.47 (1H, s, H-4), 7.66 – 7.61 (2H, m, H-5, H-7), 7.38 – 7.30 (4H, m, H-6, H-8, H-5'), 7.28 – 7.18 (3H, m, H-6', H-7'), 4.42 (2H, s, H-2'); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 191.2 (q_c, C-1'), 159.0 (q_c, C-2), 155.4 (q_c, C-8a), 148.6 (CH, C-4), 134.5 (q_c, C-4'), 134.4 (CH, C-7), 130.1 (CH, C-5), 130.2 (CH, C-5'), 129.0 (CH, C-6'), 126.9 (CH, C-7'), 124.9 (CH, C-6), 123.6 (q_c, C-3), 118.2 (q_c, C-4a), 116.7 (CH, C-8), 43.6 (CH_2, C-2') ppm; HRESMS *m/z* 297.0594 (calcd for $C_{17}H_{13}O_3S$ $[M+H]^+$ 297.0585)

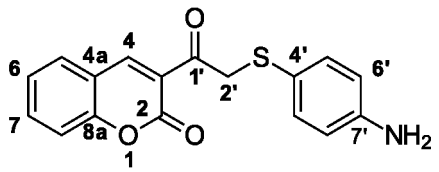


3-{[2-(4-Methylphenyl)thio]acetyl}-2*H*-1-benzopyran-2-one, (2a). White solid, ^{1}H NMR (300 MHz, $CDCl_3$,) δ: 8.46 (1H, s, H-4), 7.68 – 7.60 (2H, m, H-5, H-7), 7.39 – 7.31 (2H, m, H-6, H-8),

7.25 – 7.22 (2H, m, H-5′), 7.08 – 7.04 (2H, m, H-6′), 4.36 (2H, s, H-2′), 2.29 (3H, s, H-8′) ^{13}C NMR (75 MHz, $CDCl_3$) δ: 191.1 (q_c, C-1′), 158.9 (q_c, C-2), 155.3 (q_c, C-8a), 148.4 (CH, C-4), 137.4 (CH, C-7′), 134.4 (CH, C-7), 131.0 (CH, C-5′), 130.5 (q_c, C-4′), 129.9 (CH, C-5), 129.8 (CH, C-6′), 124.9 (CH, C-6), 123.7 (q_c, C-3), 118.3 (q_c, C-4a), 116.7 (CH, C-8), 44.1 (CH_2 C-2′), 20.9 (CH_3, C-8′) ppm; HRESMS *m/z* 311.0730 (calcd for $C_{18}H_{15}O_3S$ $[M+H]^+$ 311.0742)

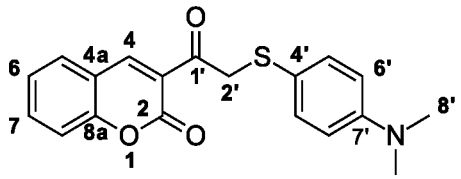


3-{[2-(4-Methoxyphenyl)thio]acetyl}-2*H*-1-benzopyran-2-one, (3a). white solid, ^{1}H NMR (300 MHz, $CDCl_3$,) δ: 8.46 (1H, s, H-4), 7.67 – 7.61 (2H, m, H-5, H-7), 7.40 – 7.34 (2H, m, H-6, H-8), 7.32 – 7.22 (2H, m, H-5′), 6.82 – 6.77 (2H, m, H-6′), 4.29 (2H, s, H-2′), 3.76 (3H, s, H-8′) ^{13}C NMR (75 MHz, $CDCl_3$) δ: 191.0 (q_c, C-1′), 159.6 (q_c, C-7′), 158.9 (q_c, C-2), 155.2 (q_c, C-8a), 148.3 (CH, C-4), 134.4 (CH, C-7), 134.1 (CH, C-5′), 129.9 (CH, C-5), 124.9 (CH, C-6), 124.3 (q_c, C-4′), 123.8 (q_c, C-3), 118.3 (q_c, C-4a), 116.7 (CH, C-8), 114.7 (CH, C-6′), 55.3 (CH_3, C-8′), 45.3 (CH_2, C-2′) ppm; HRESMS *m/z* 327.0677 (calcd for $C_{18}H_{15}O_4S$ $[M+H]^+$ 327.0691)

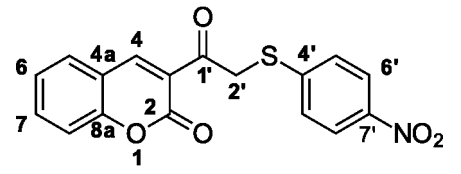


3-{[2-(4-Aminophenyl)thio]acetyl}-2*H*-1-benzopyran-2-one, (4a). White solid, ^{1}H NMR (300 MHz, $CDCl_3$,) δ: 8.47 (1H, s, H-4), 7.68 – 7.62 (2H, m, H-5, H-7), 7.39 – 7.31 (2H, m, H-6, H-8), 7.18 – 7.13 (2H, m, H-5′), 6.58 – 6.53 (2H, m, H-6′), 4.23 (2H, s, H-2′) ^{13}C NMR (75 MHz, $CDCl_3$)

δ: 190.9 (q_c, C-1′), 158.8 (q_c, C-2), 155.6 (q_c, C-8a), 148.2 (CH, C-4), 146.6 (q_c, C-7′), 134.7 (CH, C-5′), 134.3 (CH, C-7), 129.9 (CH, C-5), 124.9 (CH, C-6), 121.1 (q_c, C-3), 120.4 (q_c, C-4′), 117.2 (q_c, C-4a), 116.8 (CH, C-8), 115.6 (CH, C-6′), 45.8 (CH_2, C-2′) ppm; HRESMS *m/z* 312.0683 (calcd for $C_{17}H_{14}NO_3S$ $[M+H]^+$ 312.0694)

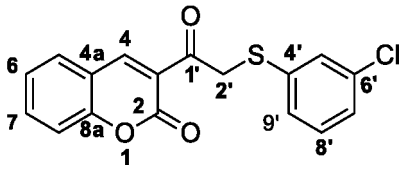


3-{[2-(4-Dimethylaminophenyl)thio]acetyl}-2*H*-1-benzopyran-2-one, (5a). White solid, ^1H NMR (300 MHz, $CDCl_3$,) δ: 8.45 (1H, s, H-4), 7.66 – 7.62 (2H, m, H-5, H-7), 7.37 (1H, d, *J* = 8.4 Hz, H-8), 7.33 (1H, d, *J* = 7.6 Hz, H-6), 7.24 – 7.22 (2H, m, H-5′), 6.57 – 6.55 (2H, m, H-6′), 4.22 (2H, s, H-2′), 2.91 (6H, s, H-8′) ^{13}C NMR (75 MHz, $CDCl_3$) δ: 191.2 (q_c, C-1′), 158.7 (q_c, C-2), 155.0 (q_c, C-8a), 150.3 (q_c, C-7′), 148.0 (CH, C-4), 134.9 (CH, C-5′), 134.2 (CH, C-7), 129.9 (CH, C-5), 124.9 (CH, C-6), 123.8 (q_c, C-3), 118.3 (q_c, C-4a), 118.0 (q_c, C-4′), 116.7 (CH, C-8), 112.7 (CH, C-6′), 45.9 (CH_2, C-2′), 40.2 (2 x CH_3, C-8′) ppm; HRESMS *m/z* 340.1005 (calcd for $C_{19}H_{18}NO_3S$ $[M+H]^+$ 340.1007)



3-{[2-(4-Nitrophenyl)thio]acetyl}-2*H*-1-benzopyran-2-one, (6a). white solid, ^1H NMR (300 MHz, $CDCl_3$,) δ: 8.56 (1H, s, H-4), 8.13 – 8.09 (2H, m, H-6′), 7.72 – 7.63 (2H, m, H-5, H-7), 7.43 – 7.33 (4H, m, H-6, H-8, H-6′), 4.59 (2H, s, H-2′) ^{13}C NMR (75 MHz, $CDCl_3$) δ: 190.2 (q_c, C-1′), 159.2 (q_c, C-2), 155.5 (q_c, C-8a), 149.6 (CH, C-4), 145.6 (q_c, C-7′), 145.5 (q_c, C-4′), 135.1 (CH, C-

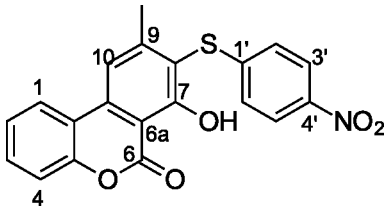
7), 130.4 (CH, C-5), 126.9 (CH, C-5′), 125.3 (CH, C-6), 124.0 (CH, C-6′), 122.8 (q_c, C-3), 118.3 (q_c, C-4a), 116.9 (CH, C-8), 41.8 (CH_2, C-2′) ppm; HRESMS *m/z* 342.0430 (calcd for $C_{17}H_{12}NO_5S$ $[M+H]^+$ 342.0436)



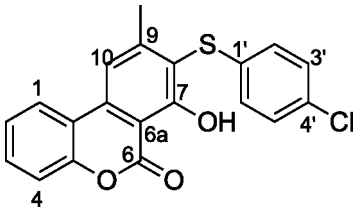
3-{[2-(3-chlorophenyl)thio]acetyl}-2*H*-1-benzopyran-2-one, (8a). white solid, ^1H NMR (300 MHz, $CDCl_3$,) δ: 8.51 (1H, s, H-4), 7.67 – 7.61 (3H, m, H-5, H-7, H-5′), 7.40 – 7.30 (4H, m, H-6, H-8, H-8′, H-9′), 7.21 – 7.15 (1H, m, H-7′), 4.44 (2H, s, H-2′) ^{13}C NMR (75 MHz, $CDCl_3$) δ: 190.7 (q_c, C-1′), 159.2 (q_c, C-2), 155.3 (q_c, C-8a), 148.9 (CH, C-4), 136.5 (q_c, C-4′), 134.8 (CH, C-7), 134.2 (CH, C-8′), 130.2 (CH, C-5), 127.8 (q_c, C-6′), 126.9 (CH, C-7′), 126.5 (CH, C-9′), 125.1 (CH, C-5′), 124.9 (CH, C-6), 124.6 (q_c, C-3), 118.3 (q_c, C-4a), 116.3 (CH, C-8), 44.2 (CH_2 C-2′) ppm; HRESMS *m/z* 331.0202 (calcd for $C_{17}H_{12}{}^{35}ClO_3S$ $[M+H]^+$ 331.0196)

4.4 General procedure for the synthesis of novel compounds 3.4a – e

3-(Bromoacetyl)coumarin (**2.2a**, 100 mg, 0.374 mmol, 1 equiv.), *para*-nitro thiophenol (116 mg, 0.749 mmol, 2 equiv.) and K_2CO_3 (103 mg, 0.749 mmol, 2 equiv) were dissolved in 10ml of distilled acetone and heated under reflux for 5 hours. After allowing the reaction to cool, the reaction mixture was extracted with EtOAc, (3 x 10 ml) and the organic phase washed with water (2 x 10 ml). Following solvent was removed *in vacuo*, the resultant crude mixture was purified through silica chromatography (9.5:0.5 Hexane, EtOAc) to afford compound **3.4a** (14.2 mg, 0.0319 mmol, 10% yield). This representative method was applied to the synthesis of compounds **3.4b – e** using the same proportions of solvent and reagents as detailed above.

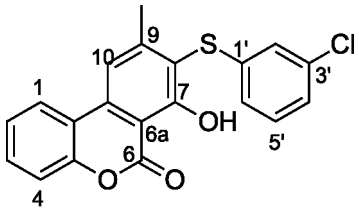


7-Hydroxy-8-[(4-nitrophenyl)thio]-9-methyl-6*H*-dibenzo[*b*,*d*]pyran-6-one, (3.4a) White solid; ^{1}H NMR (600 MHz, $CDCl_3$) δ: 12.18 (1H, s, OH-7), 8.08 (1H, dd, *J* = 8.1, 1.7 Hz, H-1), 8.07 – 8.04 (2H, m, H-2′), 7.64 (1H, s, H-10), 7.58 – 7.55 (1H, m, H-3), 7.43 – 7.40 (2H, m, H-2, H-4), 7.14 – 7.11 (2H, m, H-3′), 2.62 (3H, s, Me-9) ^{13}C NMR (150 MHz, $CDCl_3$) δ: 165.3 (q_c, C-6), 163.9 (q_c, C-10a), 153.9 (q_c, C-7), 151.0 (q_c, C-4a), 146.5 (q_c, C-4′), 145.3 (q_c, C-1′), 136.6 (q_c, C-10b), 131.7 (CH, C-3), 125.6 (2 x CH, C-3′), 125.5 (CH, C-2), 124.2 (2 x CH, C-2′), 123.5 (CH, C-1), 117.9 (CH, C-4), 117.5 (q_c, C-9), 116.1 (q_c, C-8), 114.4 (CH, C-10), 104.9 (q_c, C-6a), 22.8 (CH_3, Me-9) ppm; HRESMS *m/z* 378.0434 (calcd for $C_{20}H_{12}NO_5S$ $[M-H]^-$ 378.0436)

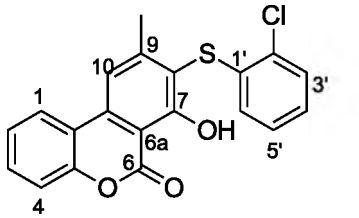


7-Hydroxy-8-[(4-chlorophenyl)thio]-9-methyl-6*H*-dibenzo[*b*,*d*]pyran-6-one, (3.4b) White solid; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 12.13 (1H, s, OH-7), 8.06 (1H, dd, *J* = 8.0, 1.9 Hz, H-1), 7.59 (1H, s, H-10), 7.57 – 7.51 (1H, m, H-3), 7.41 – 7.36 (2H, m, H-2, H-4), 7.18 – 7.15 (2H, m, H-2′), 7.06 – 7.02 (2H, m, H-3′), 2.62 (3H, s, Me-9) ^{13}C NMR (75 MHz, $CDCl_3$) δ: 165.3 (q_c, C-6), 163.7 (q_c, C-10a), 153.8 (q_c, C-7), 150.9 (q_c, C-4a), 135.7 (q_c, C-10b), 135.0 (q_c, C-1′), 131.5 (CH, C-3), 131.3 (q_c, C-4′), 129.0 (2 x CH, C-2′), 128. (2 x CH, C-3′), 125.3 (CH, C-2), 123.4 (CH, C-1), 118.7

(CH, C-4), 117.9 (q_c, C-9), 117.7 (q_c, C-8), 114.2 (CH, C-10), 104.7 (q_c, C-6a), 22.9 (CH_3, Me-9) ppm; HRESMS *m/z* 369.0343 (calcd for $C_{20}H_{14}{}^{35}ClO_3S$ $[M+H]^+$ 369.0352)

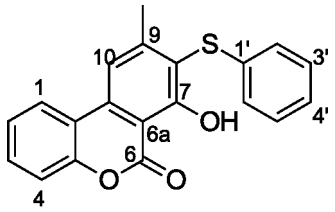


7-Hydroxy-8-[(3-chlorophenyl)thio]-9-methyl-6*H*-dibenzo[*b*,*d*]pyran-6-one, (3.4c) White solid; ^1H NMR (300 MHz, $CDCl_3$) δ: 12.13 (1H, s, OH-7), 8.06 (1H, dd, *J* = 8.0, 1.8 Hz, H-1), 7.59 (1H, s, H-10), 7.56 – 7.51 (1H, m, H-3), 7.42 – 7.36 (2H, m, H-2, H-4), 7.16 – 7.06 (2H, m, H-2', H-5'), 7.03 – 6.97 (2H, m, H-4', H-6'), 2.62 (3H, s, Me-9) ^{13}C NMR (75 MHz, $CDCl_3$) δ: 165.3 (q_c, C-6), 163.8 (q_c, C-10a), 153.9 (q_c, C-7), 150.9 (q_c, C-4a), 138.6 (q_c, C-1'), 135.9 (q_c, C-10b), 134.9 (q_c, C-3'), 131.3 (CH, C-3), 129.9 (CH, C-5'), 126.2 (CH, C-6'), 125.7 (CH, C-2'), 125.4 (CH, C-2), 124.8 (CH, C-4'), 123.5 (CH, C-1), 118.0 (CH, C-4), 117.9 (q_c, C-9), 117.8 (q_c, C-8), 114.2 (CH, C-10), 104.7 (q_c, C-6a), 22.9 (CH_3, Me-9) ppm; HRESMS *m/z* 369.0360 (calcd for $C_{20}H_{14}{}^{35}ClO_3S$ $[M+H]^+$ 369.0352)



7-Hydroxy-8-[(2-chlorophenyl)thio]-9-methyl-6*H*-dibenzo[*b*,*d*]pyran-6-one, (3.4d) White solid; ^1H NMR (300 MHz, $CDCl_3$) δ: 12.11 (1H, s, OH-7), 8.07 (1H, dd, *J* = 8.0, 1.8 Hz, H-1), 7.61 (1H, s, H-10), 7.57 – 7.51 (1H, m, H-3), 7.42 – 7.34 (3H, m, H-2, H-4, H-3'), 7.07 – 6.97 (2H, m,

H-4', H-5'), 6.62 – 6.59 (1H, m, H-6'), 2.61 (3H, s, Me-9) ^{13}C NMR (75 MHz, $CDCl_3$) δ: 165.3 (q_c, C-6), 163.9 (q_c, C-10a), 154.3 (q_c, C-7), 150.9 (q_c, C-4a), 135.9 (q_c, C-10b), 135.5 (q_c, C-1'), 131.5 (q_c, C-2'), 131.4 (CH, C-3), 129.8 (CH, C-3'), 127.1 (CH, C-4'), 126.1 (2 x CH, C-4', C-5'), 125.4 (CH, C-2), 123.5 (CH, C-1), 117.9 (q_c, C-9), 117.7 (CH, C-4), 117.4 (q_c, C-8), 114.3 (CH, C-10), 104.7 (q_c, C-6a), 22.9 (CH_3, Me-9) ppm; HRESMS *m/z* 369.0344 (calcd for $C_{20}H_{14}{}^{35}ClO_3S$ $[M+H]^+$ 369.0352)



7-Hydroxy-8-(phenylthio)-9-methyl-6*H*-dibenzo[*b*,*d*]pyran-6-one, (3.4e) White solid; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 12.11 (1H, s, OH-7), 8.06 (1H, dd, *J* = 8.0, 1.8 Hz, H-1), 7.58 (1H, s, H-10), 7.55 – 7.56 (1H, m, H-3), 7.40 – 7.35 (2H, m, H-2, H-4), 7.23 – 1.17 (2H, m, H-2'), 7.13 – 7.08 (3H, m, H-3', H-4'), 2.62 (3H, s, Me-9) ^{13}C NMR (75 MHz, $CDCl_3$) δ: 165.4 (q_c, C-6), 163.8 (q_c, C-10a), 154.1 (q_c, C-7), 150.9 (q_c, C-4a), 136.4 (q_c, C-1'), 135.5 (q_c, C-10b), 131.2 (CH, C-3), 129.0 (2 x CH, C-2'), 126.8 (2 x CH, C-3'), 125.6 (CH, C-4'), 125.3 (CH, C-2), 123.4 (CH, C-1), 119.0 (q_c, C-8), 117.9 (CH, C-4), 117.8 (q_c, C-9), 114.1 (CH, C-10), 104.6 (q_c, C-6a), 23.0 (CH_3, Me-9) ppm; HRESMS *m/z* 335.0746 (calcd for $C_{20}H_{15}O_3S$ $[M+H]^+$ 335.0742)

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