SCHOOL OF MATHEMATICAL AND NATURAL SCIENCES

DEPARTMENT OF CHEMISTRY

Synthetic studies and biological evaluation of chromone-3-carbaldehydes

Dissertation submitted in fulfilment of the requirement for the degree

Master of Science

by

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BSc. Hon (UNIVEN)

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Declaration

I declare that this dissertation "Synthetic studies and biological evaluation of chromone-3-carbaldehydes", has not been submitted previously for any degree at this institution or any other, that this is my own work in design and execution, and all the references contained therein have been duly acknowledged.

Name: Allen Tauya Gordon

Signature Date: ___/___/2018

Abstract

Chromones are well known naturally occurring heterocyclic compounds with oxygen as a heteroatom. Chromones are also one of the major classes of naturally occurring compounds, and the interest in their chemistry is due to their wide range of their biological activity.

In this study, three classes of target compounds were synthesized through three different pathways. The first pathway, chromone-3-carbaldehyde analogues were afforded in good to excellent yield followed by the oxidation thereof to 4-oxo-4H-chromene-3-carboxylic acids. A series of chromone-3-carboxamides was obtained from corresponding 4-oxo-4H-chromene-3-carboxylic acid via the *in situ* generation of the corresponding acid chloride in good yield. The second class of compounds were achieved by reacting corresponding chromone-3-carbaldehyde analogues with thiazolidine-2,4-dione to afford 5-((4-oxo-4H-chromen-3-yl)methylene)thiazolidine-2,4-dione analogues.

The third class of compounds followed the same reaction pathway as the second class of compounds from corresponding 8-allyl-chromone-3-carbaldehyde analogues to afford 5-((8-allyl-4-oxo-4H-chromen-3-yl)methylene)thiazolidine-2,4-dione analogues in good yield. Compounds were characterized by 1D NMR (^{1}H, ^{13}C and DEPT), 2D NMR (COSY, HSQC and HMBC), IR and elemental analysis (CHN analysis).

Selected synthesized chromone derivatives were evaluated *in vitro* for two biological assays; namely trypanocidal activity and cytotoxicity. Among all tested compounds, **41A**, **55B** and **63D** displayed promising trypanocidal activity by reducing the percentage parasite viability to 0.61, 0.15 and 0.21 respectively. These results were further substantiated by their IC_{50} values 4.3, 1.3 and 1.9 µg/mL respectively. Compounds **41B** and **59A** also showed significant trypanocidal activity, however it was below the positive control. Compounds **41A** and **55B** displayed cytotoxicity against the HeLa cells whilst compound **63D** displayed no toxicity against the HeLa cells.

Acknowledgements

I would first like to take the opportunity to thank God for being with me on this journey of my life.

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List of Abbreviations

AcOH	**acetic Acid**
Ar	**aromatic**
$CDCl_3$	**deuterochloroform**
$CHCl_3$	**chloroform**
$(CH_3)_2NH_2{\cdot}HCl$	**dimethylamine hydrochloride**
CH_2O	**formaldehyde**
COSY	**correlation spectroscopy**
DABCO	**1,4-diazabicyclo[2.2.2]octan**
DBU	**1,8-diazabicyclo.4.0]undec-7-ene**
DCM	**dichloromethane**
DEPT	**Distortionless Enhancement by Polarization Transfer**
DMF	**N,N-dimethylformamide**
DMF-DMA	**N,N-dimethylformamide dimethyl acetal**
DMSO	**dimethyl sulfoxide**
DMSO-d_6	**deuterodimethyl sulfoxide**
EtOAc	**ethyl acetate**
Et_3N	**triethylamine**
FTIR	**Fourier-transform infrared**
HCl	**hydrochloric acid**
HIV	**human Immunodeficiency Virus**

HMBC	**hetero multiple bond correlation**
HSV	**herpes simplex virus**
IR	**infrared**
KOH	**potassium hydroxide**
MAO	**monoamine oxidase**
$MgSO_4$	**magnesium sulfate**
Na_2SO_4	**sodium sulfate**
NBS	**N-bromosuccinimide**
$NH_2OH{\cdot}HCl$	**hydroxylamine hydrochloride**
NMR	**nuclear magnetic resonance**
NND	**N,N-diethylaniline**
$POCl_3$	**phosphorus oxychloride**
PTS	**polythiophene**
Py-d_5	**deuteropyridine**
$SOCl_2$	**thionyl chloride**
TMS	**tetramethylsilane**
TOSMIC	**tosylmethylisocyanide**

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Chapter 1

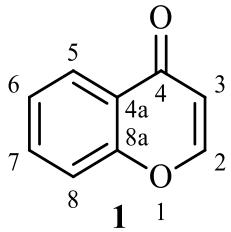
1 Introduction

Driven by chemistry and the clinical sciences, drug research has contributed more to the progress of medicine during the past century than any other scientific factor.[1] The interest of organic chemists in heterocyclic compounds in terms of synthesis, isolation, and biological study is due to their pharmaceutical and commercial use.[2] Many drugs in the pharmaceutical industry use heterocyclic compounds such as antiviral drugs, anti-tuberculosis drugs and many others.

Organic synthesis is a compound-creating activity often focused on biologically active small molecules. This study on the chemistry of chromone-3-carbaldehyde derivatives makes use of various types of organic reactions such as the Vilsmeier-Haack and Claisen rearrangement reactions to synthesize potentially biologically active compounds.

1.1 Chromones

Chromone (4H-1-benzopyran-4-one) **1** is one of the most abundant classes of compounds belonging to the flavonoid group with oxygen as a heteroatom in their framework and they occur naturally.[3] Chromones can also be found in a wide variety of synthetic products as well. The significance of these widely spread and highly diverse compounds is far beyond the important biological functions they assume in nature.[4]



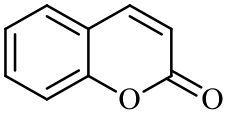
1

Chromones are considered safe and are associated with low mammalian toxicity, making them excellent chemopreventive agents,[5] and may be present in large amounts in the human diet.[6] The array of biological properties chromones possess include cytotoxic (anticancer), neuroprotective, HIV-inhibitory, antimicrobial,[7] antifungal, antioxidant activity and protein kinase C inhibitors.[8]

The study of chromone **1** chemistry is of great interest to organic and medical chemist alike.[9] This is due to their reactivity towards nucleophiles which provides a useful route to the

preparation of a variety of new heterocyclic systems. Chromones have also been found to be active in a number of plant cycles including growth regulation, dormancy inhibition and stimulating oxygen uptake in plant tissues.[10]

The chromone moiety **1** is isomeric to the coumarin moiety **2**.[11] Coumarin **2** (2-H -1-benzopyran-2-one) is the simplest naturally occurring phenolic substance possessing fused benzene and α pyrone rings and is also widely distributed in nature.[12] Coumarins also are reported to have various biological activities such as anticoagulant, insecticidal, anthelminthic hypnotic, antifungal, phytoalexin and HIV protease inhibition.[13]



2

1.2 Sources of chromones

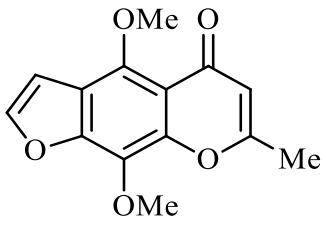
The human body is exposed to millions of pathogens on a daily basis.[14] The air we breathe, the water we drink and the food we eat all contain microbes.[15] Some which can cause serious harm to the host and even death.[16] The fight to eradicate diseases, such as AIDS, the plague, and flu have prompted chemists all over the world to work on new chemical pathways to synthesize biologically active molecules.[17] Chromone **1** can be obtained from two major sources, nature (plants and animals) and as products from synthetic chemistry. In the following discussion we briefly review two sources from which chromones are derived, namely; natural occurring and synthetic chromones. The biological activity of selected compounds will also be briefly reviewed.

Natural and synthetic chromone derivatives have been assigned as lead structures in drug development with some already being marketed as drugs.[3]

1.2.1. Naturally occurring chromones and their biological activity

For millennia, natural products, mostly plants, were the only source of drugs.[18] Many naturally occurring bioactive compounds and/or their derivatives have become drugs of central importance and represent a high percentage of the drugs used today. Antibiotics, hormones, and statins are well-known examples.[19]

Khellin **3** was extracted from the seeds of *Ammi visnaga*, which belongs to the family Apiaceae and it is a herbaceous medicinal plant. It was the first chromone to be used in its pure form for clinical practice.[20] *Ammi visnaga* grows wild in many eastern Mediterranean countries and also distributed abundantly throughout the world as introduced species. For centuries, in the Mediterranean region, Khellin was used to relieve renal colic. Khellin is a furanochromone and also used as a smooth muscle relaxant in the treatment of angina pectoris and asthma.[21] Khellin has also been used to treat vitiligo because it has phototherapeutic properties that are similar to those of the psoralens (furocoumarin), but it has substantially lower phototoxic and mutagenic effects.[22]



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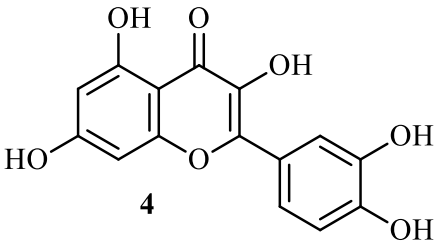
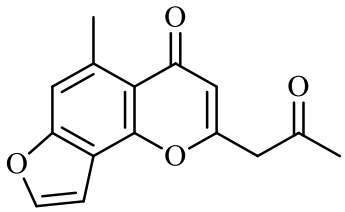
Quercetin **4** is one of the most abundant bioflavonoids commonly found in *Herba lysimachiae*.[23] It is a naturally occurring polar auxin transport inhibitor, which means compound **4** prevents the development of the bilateral growth of the plant embryo during the globular stage. Quercetin can be found in nutritional supplements and as a phytochemical remedy for a variety of diseases like diabetes/obesity and circulatory dysfunction, including inflammation as well as mood disorders. Quercetin has a strong antioxidant activity which potentially enables it to quench free radicals from forming resonance-stabilized phenoxyl radicals.[24]



4

Sperenza and co-workers extracted a methylchromone, Furoaloesone **5** from a commercial sample of the bitter aloe plant which is indigenous to South Africa. Furoaloesone is believed to have cytotoxic activity against the growth of cancer cells of the *Ehrlich ascitic* carcinoma type.[25] The bitter aloe plant has been used since ancient times as a generic chemopreventive and anti-tumour remedy in folk medicine and it has a well-documented history of use as a laxative.[26]



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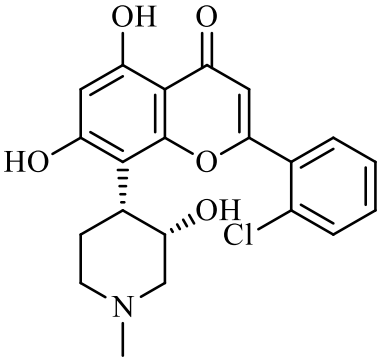
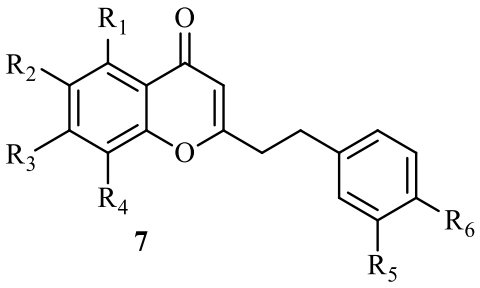
Flavopiridol **6** is a semi-synthetic flavone analogue of the alkaloid, rohitukine, a compound isolated from an Indian tree, *Dysoxylum binectariferum*. It has been shown to inhibit cyclin-dependent kinases causing cell cycle arrest and growth inhibition.[27] Furthermore, Flavopiridol has been reported to have cytotoxic activity against a wide range of cancer cell lines and has demonstrated its efficacy in several clinical trials.[28]



6

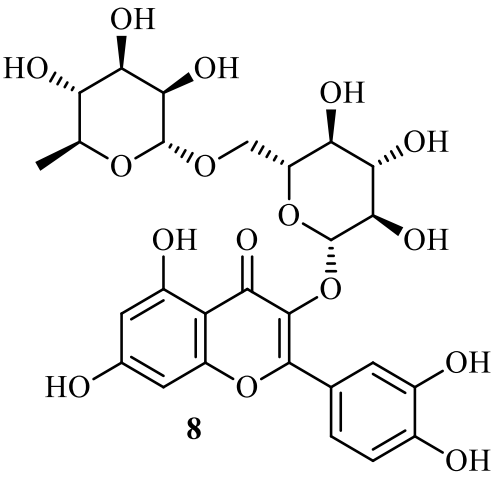
Wei Li and co-workers reported 16 compounds, 2-(2-phenylethyl) chromone derivatives **7** extracted from the agarwood plant. The four compounds **7a-d** showed antibacterial activities against two strains of bacteria namely *Staphylococcus aureus* and *Ralstonia solanacearum*.[29]



7

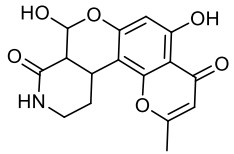
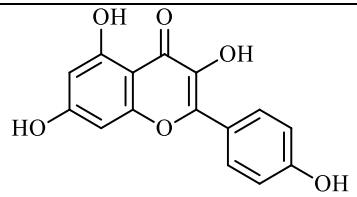
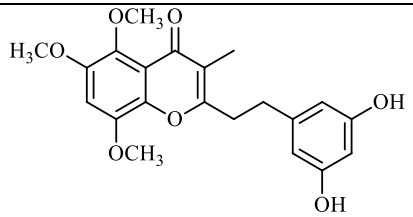
Compound	R_1	R_2	R_3	R_4	R_5	R_6
7						
a	H	OMe	H	H	OH	OMe
b	OH	OMe	H	H	OH	OMe
c	H	OH	H	H	H	OMe
d	H	OH	H	OH	H	H

Rutin **8** is a common dietary flavonoid that is widely consumed from citric plant-derived beverages and foods. Rutin **8** exhibits significant pharmacological activities, including anti-oxidation, anti-inflammation, anti-diabetic, anti-adipogenic, neuroprotective and hormone therapy.[30] Rutin **8** has been isolated from several types of plants, such as bushwheat[31] and tobacco.[32]



8

Naturally occurring compounds

Compound	Biological Properties	Structure	Source	Ref
Schumannificin **9**	antiviral activity (HIV and HSV)		*Schumanniophyton magnificum*	[33]
Kaempferol **10**	anti-inflammatory, antimicrobial, anticancer and cardioprotective		*Hippophae rhamnoides L*	[34], [35]
6-desmethoxyhormothamnion **11**	antitumor activity		*Chrysophaeum taylori* (marine crytophyte)	[12]

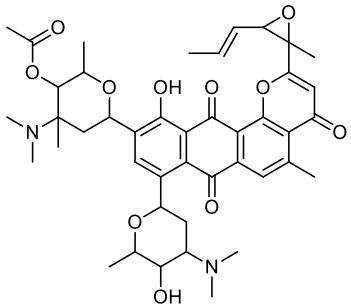
Puromycin **12**	antitumor, antibacterial and enzyme inhibitory activities		*Bacterium streptomyces*	36

Table 1: Naturally occurring compounds

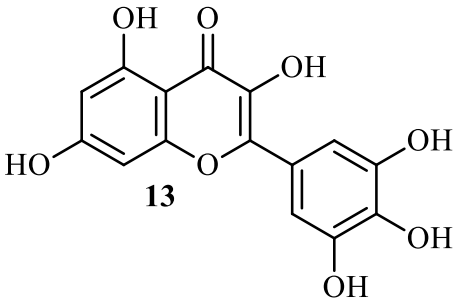
1.2.2 Synthetic chromones and their biological activity

Drug discovery efforts from plants have been evolving continuously in response to a number of recent technological advances, such as the development of chromatographic methods that allow reproducible and fast purification steps for diverse compound classes; the availability of sensitive spectroscopic methods permitting the structural characterization of samples in microgram quantities; efficient chemical methods that permit the synthesis, derivatization, and optimization of bioactive lead compounds.[37]

The vast range of biological properties associated with chromone derivatives has led to substantial research devoted to the synthesis of chromone derivatives and biological evaluation with emphasis on their potential medicinal applications.[3]

1.2.2.1 Antioxidant Activity

Antioxidant compounds in food play an important role as health-protecting factors. Scientific evidence suggests that antioxidants can reduce the risk for chronic diseases including cancer and heart disease. The main characteristic of an antioxidant is its ability to trap free radicals. Antioxidant compounds scavenge free radicals such as peroxides, hydroperoxides or lipid peroxyl and thus inhibit the oxidative mechanisms that lead to degenerative diseases.[38] Bennett and co-workers reported the synthesis of potentially therapeutic antioxidants compounds with the radical scavenging ability of myricetin **13** to effectively inhibit microsomal lipid peroxidation (Scheme 1). Myricetin **13** is a natural product with antioxidant properties.



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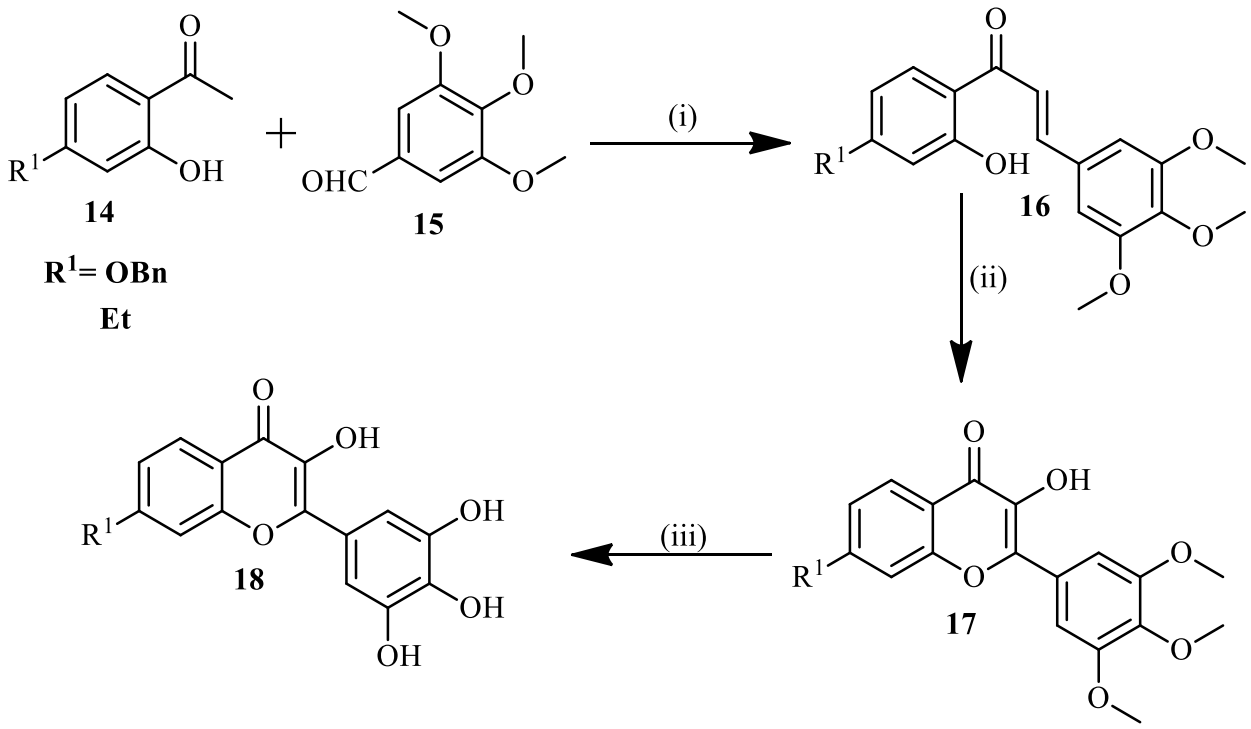
The 2-hydroxy-acetophenones **14** were condensed with trimethoxybenzaldehyde **15** to give a chalcone product **16**, which was subjected to Algar–Flynn–Oyamada (AFO) oxidation to give **17**

and finally, deprotection gave the compounds **18** with the radical scavenging ability of myricetin **13**.[39]



Reagents: (i) KOH, EtOH (ii) H_2O_2, NaOH, MeOH (iii) BBr_3, DCM

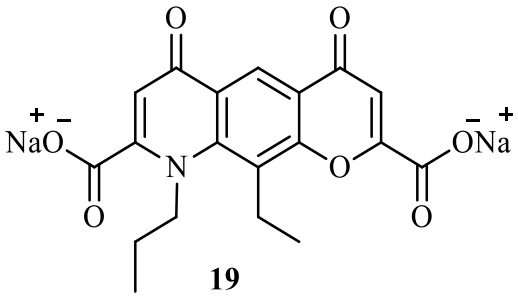
Scheme 1: Synthesis of antioxidants compounds

1.1.2.2 Anti-inflammatory activity

Inflammation is a biological response of tissue in attempting self-protection against harmful stimuli, caused by a mechanical or biological agent or by an aberrant autoimmune response.[40] In general, normal inflammation is rapid and self-limiting, but aberrant resolution and prolonged inflammation cause various chronic disorders,[41] such as atherosclerosis, diabetes, cardiovascular, Alzheimer's and Parkinson's diseases, and cancer.[42]

The search for drugs capable of disrupting the inflammatory process has become an important issue in scientific research, especially with reference to the use of natural substances and the reduction of undesirable side effects.[43] Flavonoids, particularly the chromone motif; due to their low toxicity, they represent an important source of such substances.[44]

Nedocromil **19** is a synthetic medical drug on the market which acts to prevent wheezing, shortness of breath and other breathing problems caused by asthma.[45] Nedocromil **19** acts by inhibiting the activation of inflammatory cells including neutrophils, eosinophils, macrophages, monocytes, and platelets.[45]



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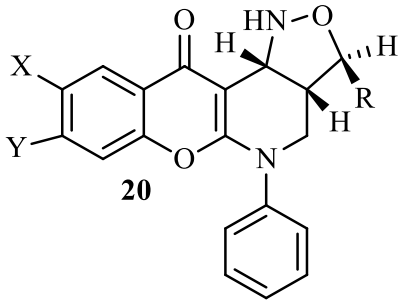
1.1.2.3 Anticancer activity

Cancer is the world's top killer each year, and the trend is set to increase in the coming years if nothing is done to curb this threat.[7] Anticancer compounds/drugs work on the affected cells thereby eradicating the disease (chemotherapy), however not all types of cancer are curable.[46]

Some chromones were reported to inhibit retroviral transcriptases, protein tyrosine kinases, reverse transcriptase and DNA topoisomerase. It has been reported that compounds that inhibit the above-mentioned enzymes have application in cancer therapy.[47]

Singh and co-workers synthesized compound **20c** and found it to be most active against neuroblastoma revealed by IC_{50} of 10.07 μM whereas compounds **20a** and **20b** were found to potent against colon cancer cells having IC_{50} of 12.6 and 29.7 μM respectively.[48]



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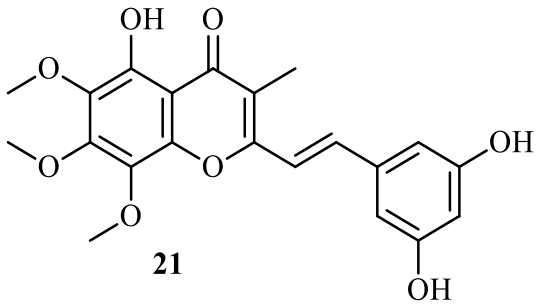
Compound	**X**	**Y**	**R**
a	H	H	Ph
b	H	Cl	Ph
c	H	Cl	H

Hormothamnione **21** is exceptionally cytotoxic against P388 lymphocytic leukaemia and HL-60 human promyelocytic leukaemia cell lines.[105]

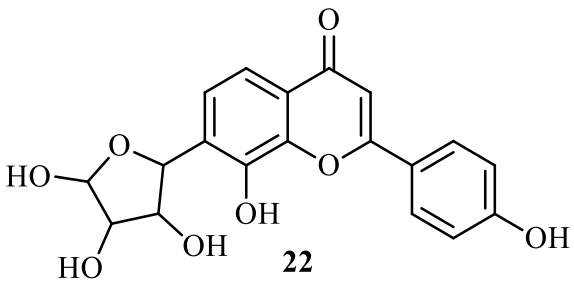


21

1.1.2.4 Anti-viral activity

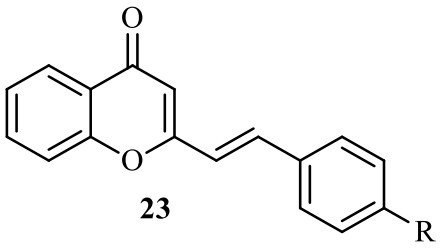
A virus is a sub-microscopic, non-cellular infectious agent that is unable to grow or reproduce outside a host cell. Viruses are obligate intracellular parasites which are a major cause of death and disease.[50] Like antibiotics for bacteria, antiviral drugs are a class of antimicrobials used specifically for treating viral infections.[51] They are relatively harmless to host because they inhibit the development of pathogens instead of destroying them. Most of the antiviral agents need to be activated by viral and cellular enzymes before exerting antiviral effect.[50]

8-Hydroxy-2-(4-hydroxyphenyl)-7(3, 4, 5-trihydroxytetrahydrofuran-2-yl)-4H-1-benzopyran-4-one **22** is active against HIV.[52] Compound **22** was synthesized by condensing 8-formyl-7-hydroxyflavone and 8-formyl-7-hydroxy-2-(2′-furyl)-3-methylchromone with acrylonitrile in the presence of DABCO using Baylis–Hillman reaction conditions.[53]



22

Desideri and co-workers reported 2-styrylchromones derivatives **23** that active against rhinovirus. These compounds **23a-d** possess anti-rhinovirus activity against human rhino virus.[49]

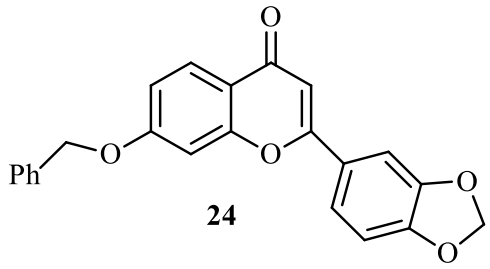


23

Compound	R
a	H
b	Cl
c	CH_3
d	OCH

1.1.2.5 Antimicrobial activity

Fungal and bacterial infections affect millions of people worldwide, and are associated with high rates of mortality and morbidity.[54] An antimicrobial is an agent that kills microorganisms or inhibits their growth. There is a large library of chromone derivatives that have been screened and have successfully shown activities against fungi and bacteria (gram negative and gram positive). 2-benzo [1, 3] dioxol-5-yl-7-benzyloxy-chromen-4-one **24** and N-isobutyl-2-(4-oxo-2-phenyl-4Hchromen-6-yl)-1 H-benzoimidazole-5-carboxamidine **25** display valuable antimycotic activity against various pathogenic fungal strains.[55]



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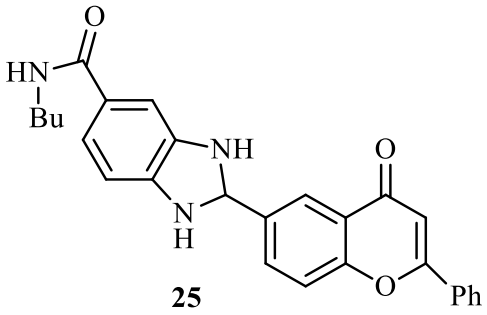


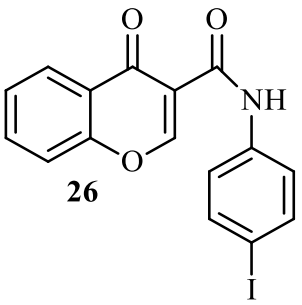
25

1.1.2.6 Enzyme inhibition activity

The chromone moiety has been reported to exhibit enzymatic inhibition properties toward different systems such as oxidoreductases, kinases, tyrosinases, and cyclooxygenases. One such enzyme is

Monoamine oxidase (MAO). MAO is an enzyme present in mammals in two isoforms MAO-A and MAO-B, located in the outer membrane of the mitochondria.[56]

Gaspar and co-workers synthesized and evaluated chromone with a carboxamide functional group located in position 2 and 3 of the chromone core. The chromone-3-carboxamide **26** synthesized condensing 4-oxo-4H-chromene-3-carboxylic acids with a substituted aniline, was tested for human MAO. The evaluation proved its potency and selectivity was positive.[9] Compound **27** was evaluated as plasmepsin (PM) II inhibitor. Compound **27** can be synthesized by condensing 2, 4, 5-trimethoxy-acetophenone with chlorobenzene using the Baker-Venkataraman rearrangement reaction conditions.[57]



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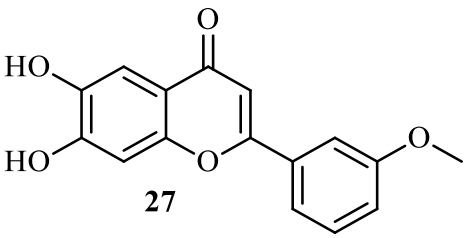
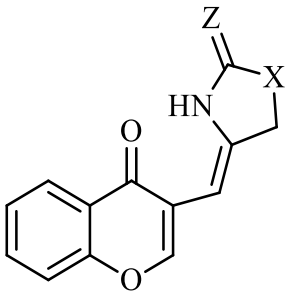


27

1.1.2.7 Other biological properties

Certain chromone carboxamides have been shown to exhibit some antiallergic activity.[58] 2, 4 thiazolidinedione compounds **28** are used as an antispasmodic agent, in the treatment of angina pectoris and as an anti-diabetic agent that improves peripheral insulin resistance in type 2 diabetic patients respectively.



28

X= S, NH, NMe, NEt
Z= O, S

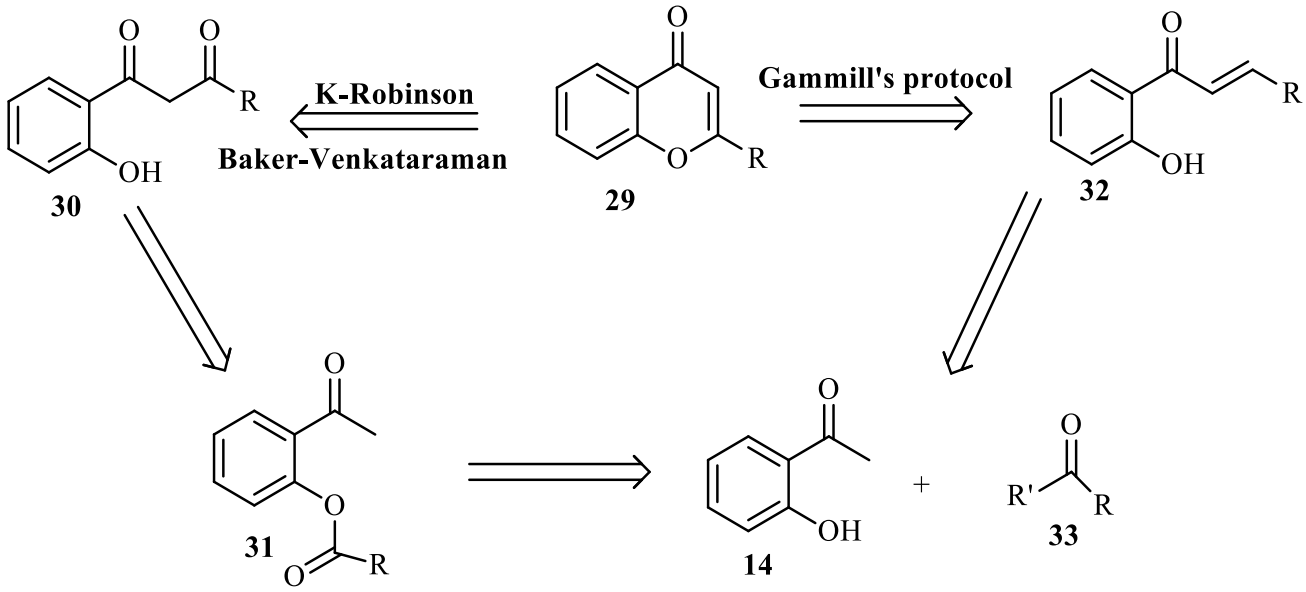
Furthermore, chromone derivatives can also be useful for a variety of other applications in medicinal chemistry, such as preparation of fluorescence probes, due to the photochemical.[59] Chromones upon exposure to light undergo various transformations such as photocycloaddition, photodimerization, photoisomerization, phototautomerization, photorearrangements, photo-oxidation and photoreduction reactions.[60]

1.3 Synthesis of chromones

The retrosynthetic strategy (Scheme 2) illustrates the disconnection approach to chromones. Several methods have been used for the synthesis of chromones such as the Baker-Venkataraman rearrangement reaction, Gammill's protocol, Claisen condensation reaction and many other methodologies. Most of these methodologies use 2-hydroxyacetophenones as the starting material.[61]

In the following discussion, we briefly review four methods for chromone synthesis, namely Kostanecki-Robinson reaction, Baker-Venkataraman rearrangement reaction, Claisen condensation reaction and the Gammill's protocol.

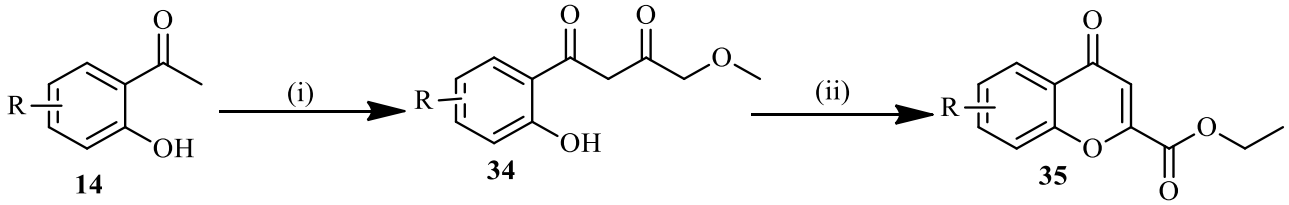
1.3.1 Retrosynthetic strategy to chromone



Scheme 2: Retrosynthetic strategy to chromone

1.3.2 Synthesis of chromone by the Kostanecki-Robinson reaction

The synthetic strategy to the chromone by Kostanecki-Robinson reaction involves the condensation of 2-hydroxy-acetophenone **14** with diethyl oxalate in the presence of sodium ethoxide in ethanol to afford the reactive intermediate **34**, which cyclizes in the presence of a strong acid to give a chromone **35** (Scheme 3).[62]



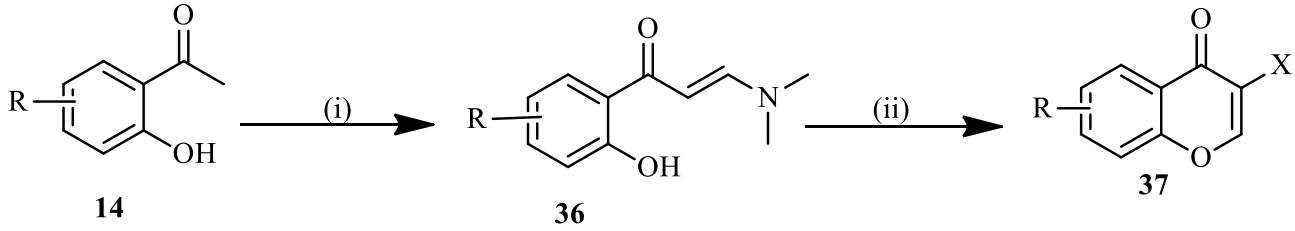
14 **34** **35**

Reagents: (i) Diethyl oxalate, NaOEt, EtOH (ii) H_2SO_4

Scheme 3: Kostanecki-Robinson reaction

1.3.3 Synthesis of chromone by Gammill's Protocol methodology

The Gammill's protocol involves condensation of appropriately substituted 2-hydroxyacetophenones **14** with DMF-DMA to form the intermediate **36**. Halogen-mediated ring closure of **36** gives the corresponding 3-halochromones **37** (Scheme 4). Compound 37 serves as a precursor in the synthesis of isoflavones.[63]



14 **36** **37**

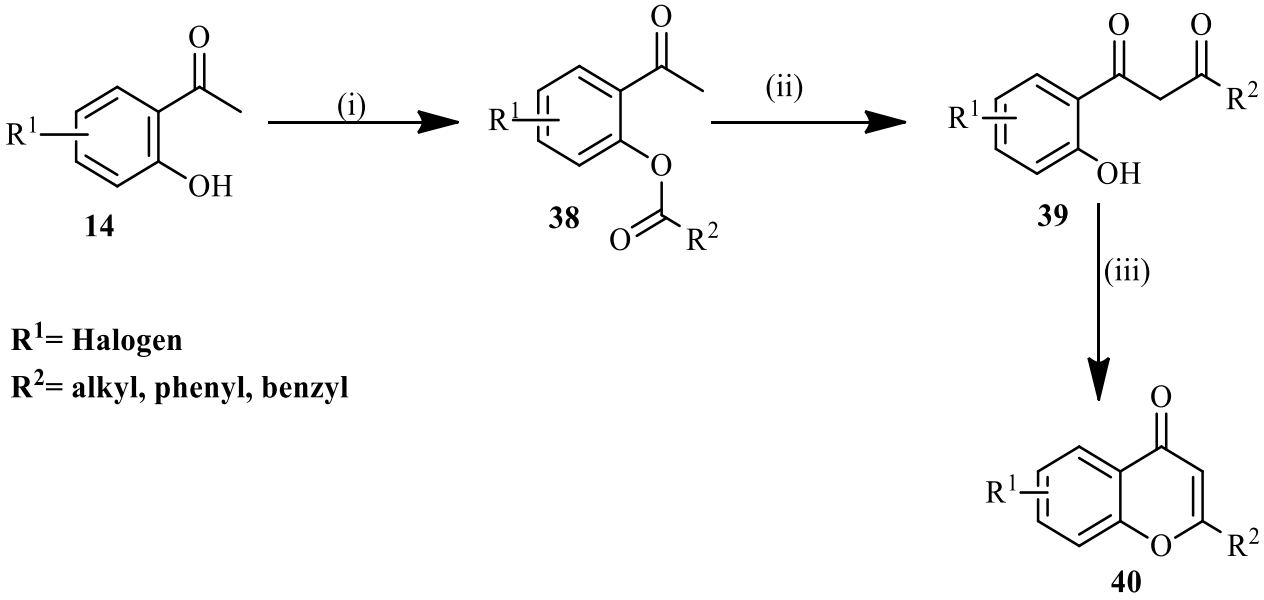
R= Halogen, alkyl, aryl
X= Halogen

Reagents: (i) DMF-DMA (ii) X_2 and $CHCl_3$

Scheme 4: Gammill's protocol

1.3.4 Synthesis of chromones by Baker-Venkataraman reaction

Baker-Venkataraman rearrangement reaction involves the acylation of substituted 2-hydroxy-acetophenones **14** with acyl chlorides to generate 2-acetylated acetophenone **38**. Heat and strongly basic conditions mediates acyl transfer to form *ortho*-hydroxy 1, 3-diketones **39**, which readily cyclize under acidic conditions to **40** (Scheme 5).[4]



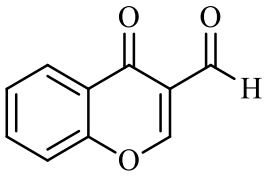
Reagents: (i) Acyl chlorides, (ii) DBU, heat (iii) H_2SO_4

Scheme 5: Baker-Venkataraman

1.4 Chromone-3-carbaldehyde and chemistry

1.4.1 Introduction

Chromone-3-carbaldehydes (4-oxo-4H-chromene-3-carbaldehyde) **41** are a group of chromone (1-benzopyran-4-one; 1, 4-benzopyrone; 4-oxo-4H-1-benzopyran) **1** derivatives characterized by the attachment of a CHO functional group to the C-3 position of the chromone structure **1**. Amongst the 3-substituted chromones, chromone-3-carbaldehyde derivatives **41** are the most widely used synthons in the synthesis of compounds with diverse biological activities.[64] Chromone-3-carbaldehyde **41** is an important biologically active compound and can serve as a precursor in the synthesis of a wide range of heterocyclic systems with the chromone moiety, many of which exhibit a broad spectrum of biological activities such as anti-mutagenisity, cytotoxicity, thymidine phosphorylase inhibition, anti-HIV activity and so on.[65]

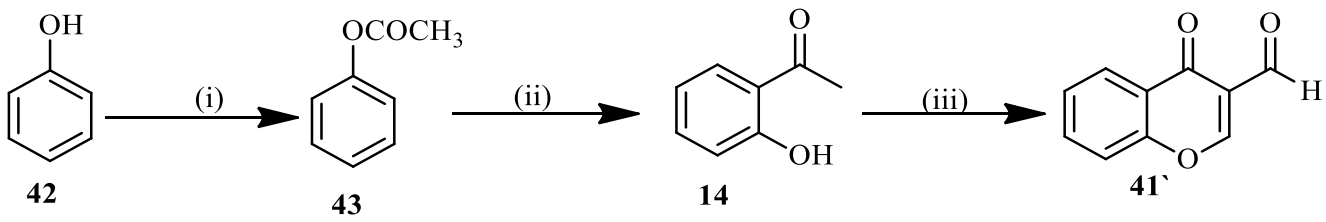


41

1.4.2 Preparation of chromone-3-carbaldehyde

The chemistry of 3-substituted chromones developed since a simple and convenient procedure for the synthesis of chromone-3-carbaldehydes **41** according to Vilsmeier–Haack was devised.

The synthetic strategy to prepare chromone-3-carbaldehyde **41** involves the reaction of phenol/substituted phenol **42** with acetyl chloride to form compound **43**, followed by Fries rearrangement with $AlCl_3$ to obtain desired 2-hydroxyacetophenones **14**. The 2-hydroxyacetophenone **14** is then condensed with DMF and $POCl_3$ in the Vilsmeier-Haack reaction to afford chromone-3-carbaldehyde **41** (Scheme 6).[66]



Reagents: (i) Acetyl chloride, HCl (ii) $AlCl_3$, heat, H_2O (iii) DMF, $POCl_3$, H_2O

Scheme 6: Synthesis of chromone-3-carbaldehyde

1.4.3 Physical properties of chromone-3-carbaldehydes

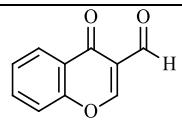
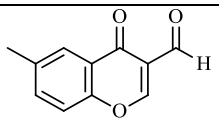
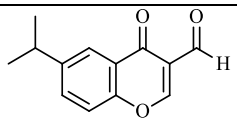
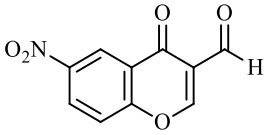
Chromone-3-carbaldehydes **41** that contain a hydroxyl, methoxy, nitro and amino groups are pale yellow to yellow compounds and have fairly high melting points. The properties of the α-pyrone ring are essentially aliphatic, even though it exhibits the reactions characteristic of an aromatic pyrylium betaine rather than α-pyrone. The properties of the heterocyclic ring are not significantly modified as a consequence of benzannulation.

The (HC=O) absorbs at 1692 ± 8 cm^{-1} and the other carbonyl on the chromone moiety (C-4) absorbs at 1665-1645 cm^{-1}. The Nuclear Magnetic resonance (NMR) spectra show that the aldehyde hydrogen resonates at δ_H: 10.35± 0.10 in $CDCl_3$ and slightly higher field 10.05 ± 0.12 in DMSO-d_6. In a mass spectrometer, the base peak of the aldehyde functional group results from the loss of carbon monoxide (CO). Further fragmentation gives another prominent peak at (M-54).[67]

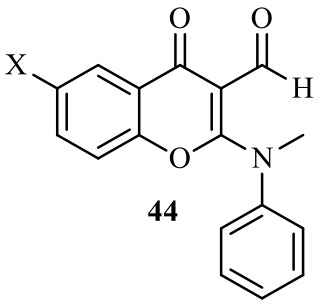
1.4.4 Biological properties of chromone-3-carbaldehydes

Kawase and co-workers examined several chromone-3-carbaldehyde analogues **41a-d** for their tumour cell-cytotoxicity, anti-*Helicobacter pylori*, urease inhibitory and anti-HIV activity. The following table (Table 2) briefly highlight selected chromone-3-carbaldehydes **41** and their reported activities. The chromone-3-carbaldehydes **41a-d** displayed mild activity against HIV, no activity against anti-*H-pylori* and were not toxic to the carcinoma cells.[68]

Table 2: Biological properties of selected chromone-3-carbaldehyde analogues **41**

Compound **41**		Anti-HIV CC_{50} µg/mL	Anti-H-pylori MIC (µg/mL)	Cytotoxic carcinoma cell CC_{50} µg/mL
41a		30.8	>100	89
41b		39.8	>100	47
41c		10.6	41	42
41d		35.4	100	84

2-(N-methylanilino)-chromone-3-carbaldehyde **44** has been reported by Gurmit Singh and co-workers for its activities against breast cancer, leukaemia, and prostate cancer.



44

X = H, Cl

Kumar and Koh reported chitosan-chromone derivatives with antimicrobial properties against *E. coli*. The chitosan-chromones were synthesized from chromone-3-carbaldehyde **41** and chitosan. They believed that the plausible reason for the antimicrobial character of the chitosan-chromone derivative is due to the fact that it has a positively charged amino group which can interact with the negatively charged microbial cell membranes to cause the leakage of intracellular constituents of the microorganisms, thereby resulting in microbial death.[59]

1.4.5 Reactions of chromone-3-carbaldehydes

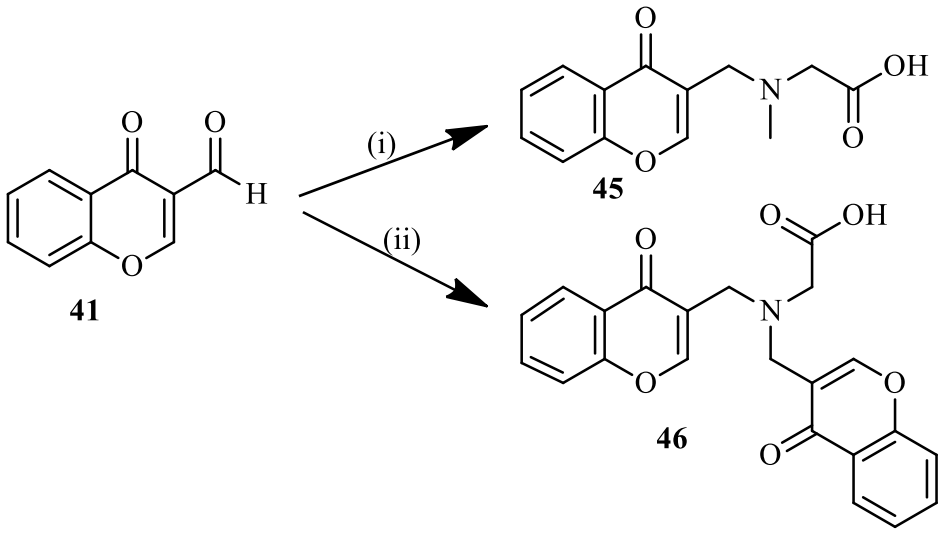
The enone moiety of the parent chromone **1** does not act as a dienophile in cycloaddition reactions. However, in chromone-3-carbaldehydes **41**, the presence of the electron withdrawing group at C-3 enhances the dienophilicity of 2, 3 double bond. These unique features make the compound **41** amenable to various reactions such as oxidation and reduction, radical, nucleophilic addition, and many other types of annulations and cycloaddition reactions.[59]

Chromone-3-carbaldehyde **41** together with its 3-substituted derivatives are very versatile molecules, reacting as Michael acceptors, with the concomitant opening of the pyrone ring; some of them can react as heterodienes and dienophiles.[69] The majority of reactions compound **41** undergoes are nucleophilic addition reactions, leading to a wide range of new heterocyclic compounds as condensation products.[70] Nucleophilic attack on compound **41** and its 3-substituted derivatives take place by two possible pathways: 1,4-addition at the C-2 atom and 1,2-addition at the electrophilic carbon atom in the 3-substituent.[71] In the following discussion, we review some

reactions in which chromone-3-carbaldehyde **41** undergoes and they serve as valuable intermediates for the synthesis of potentially biologically active novel compounds.

1.4.5.1 Mannich Reaction

Chromone-3-carbaldehyde **41** reacts with N-methylglycine or glycine in the presence of excess CH_2O to produce N-(chromone-3-ylmethyl)-N-methylglycine **45** or N,N-di(chromone-3-ylmethyl)glycine **46**, respectively, by a deformylative Mannich type reaction (Scheme 7).[72]

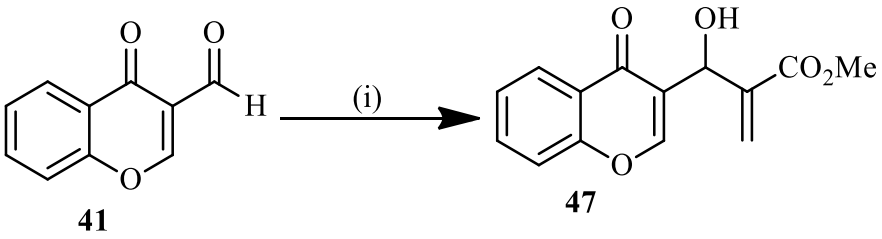


Reagents:(i) N-methylglycine, MeOH, CH_2O (ii) glycine, MeOH and CH_2O

Scheme 7: Mannich Reaction

1.4.5.2 Baylis–Hillman reaction

Kaye and co-workers reported a Baylis-Hillman reaction of chromone-3-carbaldehyde **41** and methyl acrylate using DABCO as a catalyst to afford **47** (Scheme 8).[73] Compound **47** can be reacted with various substrates to afford different chromone derivatives since it has the reactive ester group and a hydroxyl group.

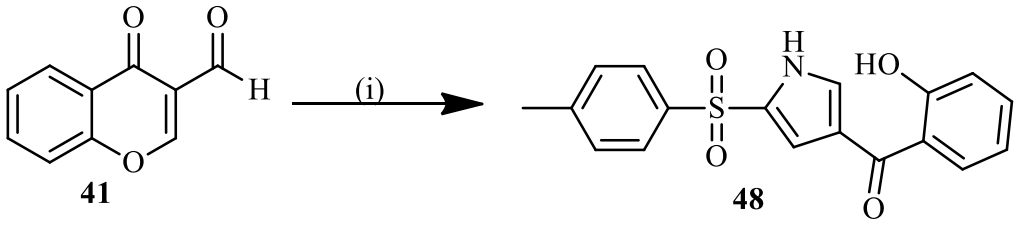


Reagents: (i) Methyl acrylate, DABCO and $CHCl_3$

Scheme 8: Baylis–Hillman reaction

1.4.5.3 Michael addition

Chromone-3-carbaldehyde **41** is a good Michael acceptor towards most nucleophiles.[74] Terzidis and co-workers reported a Michael type reaction, whereby **41** is condensed with TOSMIC in the presence of DBU in THF at room temperature to afford 2-tosyl-4-(2-hydroxybenzoyl)pyrrole **48** (Scheme 9).[75]

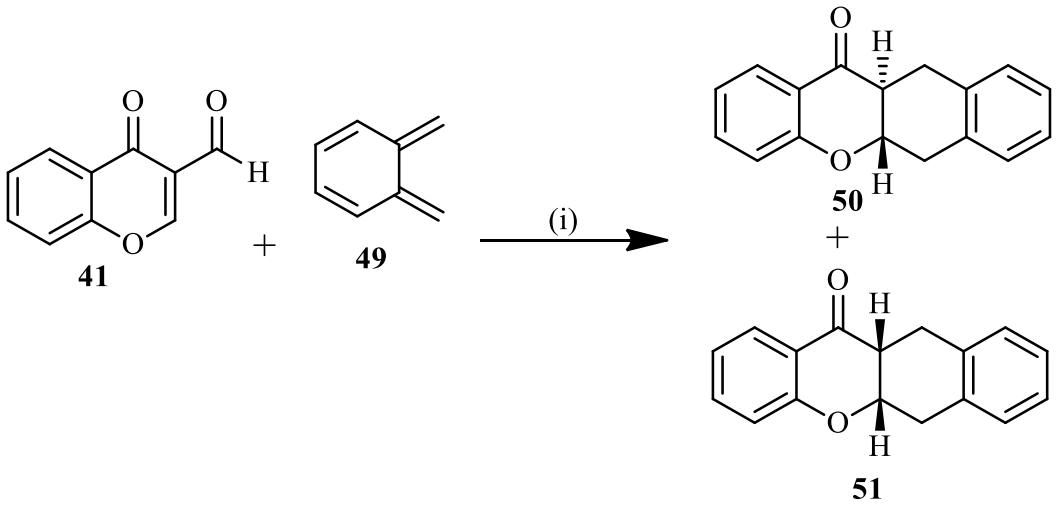


Reagents: (i) DBU, TOSMIC and THF

Scheme 9: Michael addition reaction

1.4.5.4 Diels-Alder reaction of chromone-3-carbaldehyde

Sandulache and co-workers reported a Diels-Alder reaction, which occurs when **41** is condensed with ortho-benzoquinodimethane **49** to give a diastereomeric mixture of cycloadducts **50** and **51** (Scheme 10), **41** acts as a dienophile in the reaction.



Reagents: (i) Trichlorobenzene

Scheme 10: Diels-Alder reaction of chromone-3-carbaldehyde

Chapter 2

2 Problem Statement

The chromone motif **1** is well-represented in structures of natural products, many of which exhibit pharmacological activities.[76] Over the years, several modified approaches have been adopted for the synthesis and isolation of chromones and the compounds were then screened for a wide range of biological activities. However, chromone-3-carbaldehydes **41** and derivatives are isolated in limited amounts from natural products and methods to synthesize them and other compounds are in great demand. The search for new methodologies for the synthesis of novel chromone derivatives continues to be of great interest to organic chemists. In recent years, chromone-3-carbaldehydes **41** have attracted considerable attention as highly reactive compounds which serve as the valuable moiety for incorporation in heterocycles.[77] Heterocycles play an important role in the design of novel physiological and pharmacologically active compounds. However, there are few methods that make use of mild reaction conditions, short reaction times and cheaper reagents.[78]

There is a vast library of chromone-3-carbaldehydes derivatives **41** which have medical properties and several drugs on the market that carry the chromone moiety. These chromone-3-carbaldehydes derivatives **41** are benzoyl chromones, xanthones,[79] styrylchromones etc.[80] The growing mortality rates of incurable diseases such as AIDS, diabetes, and cancer have been concerning organic and medical chemists around the world. Resistance to antimicrobial agents is becoming a major problem; microbes acquire the ability to resist antimicrobial drugs by undergoing genetic changes either by mutation or gene transfer within or between species that allow microbes to defend themselves against the antimicrobial agents. Therefore, the discovery of new antimicrobials has assumed critical importance to combat the fungal and bacterial infections. This investigation will focus on the synthesis, transformation of various chromone-3-carbaldehydes **41** and their biological evaluation as potential trypanosomiasis inhibitors.

Chapter 3

3 Aim and Objectives

The general aim of the study was the synthesis of the various chromone-3-carbaldehydes, subsequent transformation to novel derivatives and biological evaluation of selected compounds.

The specific objectives of the study were:

- To synthesis various substituted chromone-3-carbaldehydes from substituted 2-hydroxyacetophenones.
- To synthesize substituted 4-oxo-4H-chromene-3-carboxylic acid from chromone-3-carbaldehydes.
- To transform 4-oxo-4H-chromene-3-carboxylic acids to chromone-3-carboxamides.
- To synthesize substituted 5-((4-oxo-4H-chromen-3-yl)methylene)thiazolidine-2,4-dione from chromone-3-carbaldehydes.
- To transform5-((4-oxo-4H-chromen-3-yl)methylene)thiazolidine-2,4-dione to 5-((4-(2-hydroxyphenyl)-2-oxo-1,2-dihydropyrimidin-5-yl)methylene)thiazolidine-2,4-dione.
- To synthesize 8-allyl-chromone-3-carbaldehydes from substituted 1-(3-allylphenyl)ethanones
- To transform 8-allyl-chromone-3-carbaldehydes to substituted 5-((8-allyl-4-oxo-4H-chromen-3-yl)methylene)thiazolidine-2,4-dione
- Biological evaluation of selected novel target compounds as potential anti-inflammatory, antitrypanosoma and antimalarial inhibitors.

Chapter 4

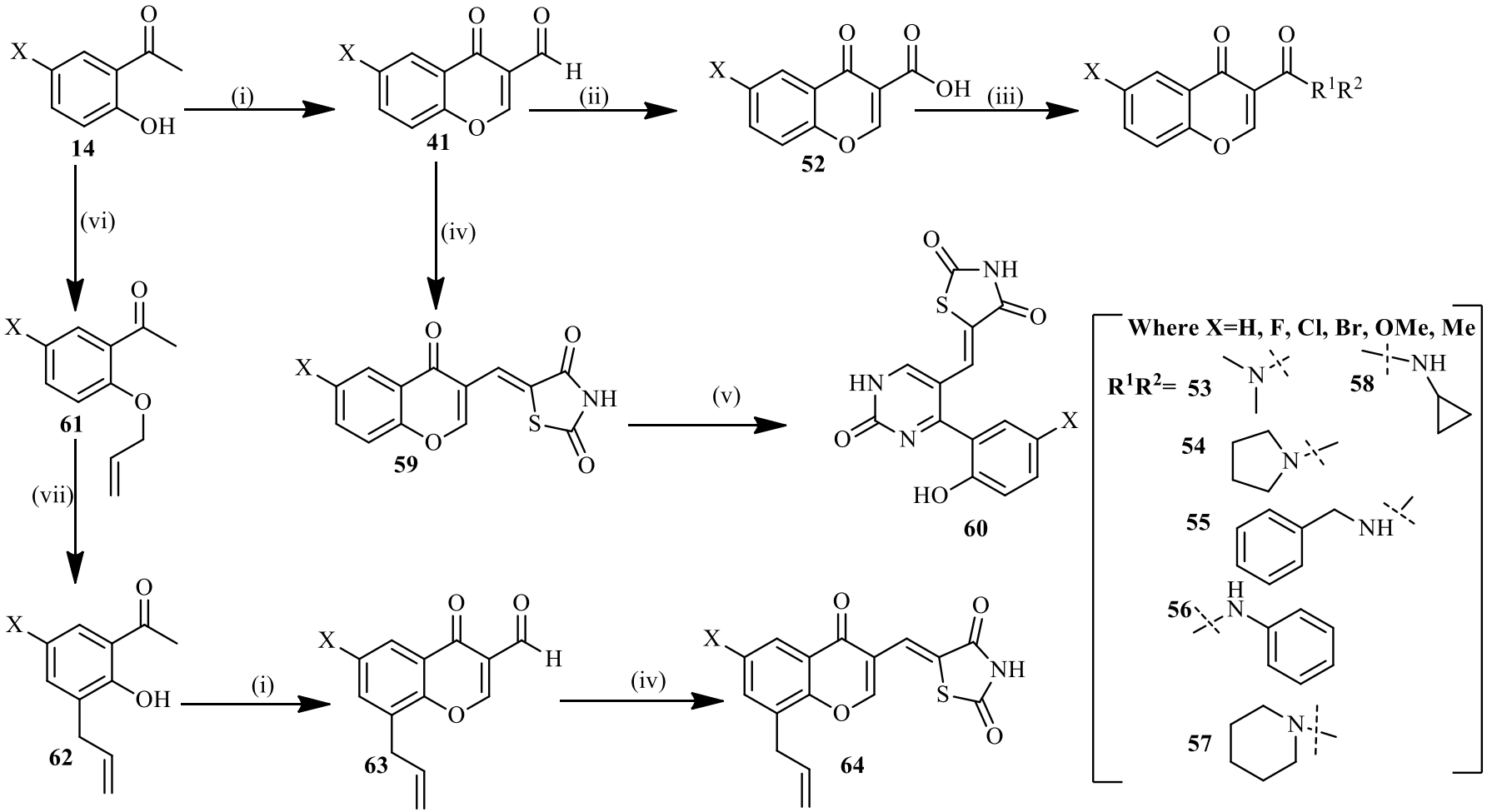
4 Results and Discussion

A general scheme for all the work carried in this study is summarized in Scheme 11, which outlines the synthesis of three categories of target compounds namely 4-oxo-4H-chromene-3-carboxamide analogues (**53-58**), 5-((4-oxo-4H-chromen-3-yl)methylene)thiazolidine-2,4-dione analogues **59** and 5-((8-allyl-4-oxo-4H-chromen-3-yl)methylene)thiazolidine-2,4-dione analogues **64**. The first series of compounds **53-58** were afforded through the precursor 4-oxo-4H-chromene-3-carboxylic acid **52** as outlined in schemes 19-23. The synthesis of 4-oxo-4H-chromene-3-carboxylic acid **52** is outlined in scheme 16.

The second series, compound **59** was synthesized by applying Knoevenagel condensation reaction of corresponding 4-oxo-4H-chromene-3-carbaldehydes **41** with thiazolidine-2,4-dione as outlined in scheme 24-25. Compound **59** was further transformed to 5-((4-(2-hydroxyphenyl)-2-oxo-1,2-dihydropyrimidin-5-yl)methylene)thiazolidine-2,4-dione analogues as outlined in scheme 26. The precursor to compound **59** namely 4-oxo-4H-chromene-3-carbaldehyde **41**, was synthesized by application of the Vilsmeier–Haack reaction to the corresponding 2-hydroxyacetophenones **14** as outlined in scheme 13.

The third series, the target compounds **64** was afforded through various precursors viz., synthesis of 1-(2-(allyloxy)phenyl)ethanone analogues **61** outlined in scheme 28. The transformation of **61** to 1-(3-allylphenyl)ethanone analogues **62** outlined in scheme 29 and 30. Compounds **62** was subjected to the Vilsmeier-Haack reaction to afford 8-allyl-4-oxo-4H-chromene-3-carbaldehyde **63** as outlined in scheme 31. Compounds **63** were subjected to Knoevenagel condensation with thiazolidine-2,4-dione to afford 5-((8-allyl-4-oxo-4H-chromen-3-yl)methylene)thiazolidine-2,4-dione analogues **64** (Scheme 32).

The synthesized compounds were characterized by the combination of various spectroscopic techniques namely 1D NMR (^{1}H, ^{13}C and DEPT), IR spectroscopic techniques and elemental analysis (CHNS analysis).

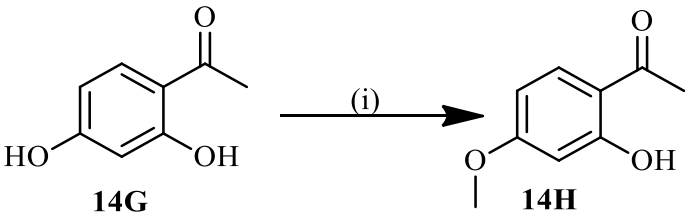


Reagents and conditions: (i) $POCl_3$, DMF, H_2O, 0-25 °C , 12 h (ii) $NaOCl_2$, sulfamic acid, H_2O, DCM, 0-25 °C, 12 h (iii) $SOCl_2$, Et_3N, DCM, R^1R^2, 0-25 °C , 12 h (iv) AcOH, NaOAc, thiazolidine-2,4-dione, 100-110 °C, reflux, 2 h (v) KOH, urea, EtOH, reflux (vi) K_2CO_3, allyl bromide, acetone, 100-110 °C, 20 h (vi) NND, 260 °C, 3-4 h

Scheme 11: General scheme for the synthesis of 4-oxo-4H-chromene-3-carbaldehyde derivatives.

4.1 Synthesis of 2-hydroxy-4-methoxy-acetophenone 14H

The synthesis of 2-hydroxy-4-methoxyacetophenone **14H** was performed following the best literature method at our convenience[81] (Scheme 12). 2,4-dihydroxyacetophenone **14G** was methylated using Me_2SO_4. The reaction mixture was cooled to room temperature, K_2CO_3 filtered off, and the filtrate was concentrated under reduced pressure to remove acetone. The excess Me_2SO_4 was destroyed with a 25 % ammonia-ice water solution and the resulting solution extracted with EtOAc. The combined organic layers were dried over $MgSO_4$ and concentrated under reduced pressure to afford 2-hydroxy-4-methoxy-acetophenone **14H** with yield of 64 %.



14G **14H**

Reagents and conditions: Acetone, Me_2SO_4, K_2CO_3, 100-110 °C, 6 h

Scheme 12: Synthesis of 2-hydroxy-4-methoxyacetophenone **14H**

^{1}H NMR spectrum of 2-hydroxy-4-methoxy-acetophenone revealed (Figure 1) the presence of two singlets in the aliphatic region corresponding to methyl protons on the methoxy protons and the acetyl group. The singlet resonating at 2.55 ppm corresponding to the methyl protons and the singlet resonating at 3.84 ppm corresponds to the methoxy protons which is deshielded due to the oxygen to carbon bond. The presence of a singlet further downfield at 12.76 ppm showed a characteristic OH proton. A doublet at 7.61 which correspond to the aromatic proton 6-H and a multiplet resonating at 6.41-7.64 ppm which correspond to the protons 3-H and 5-H were observed.

Spectroscopic data obtained from ^{13}C NMR and DEPT 135 was used to confirm the presence of four quaternary carbons, three (CH) carbons on the benzene framework, a methoxy carbon and a methyl carbon. The quaternary carbon at 202.60 ppm corresponds to the carbonyl carbon, the other three quaternary carbons correspond to the aromatic carbons C-2, C-4 and C-1 resonating at 165.26, 166.12 and 113.90 ppm respectively (Figure 2).

FT-IR revealed a C=C aromatic stretch at 1615 cm^{-1}, an alkoxy C-O stretch at 1249.71 cm^{-1} corresponding to the methoxy C-O bond and at 3020.94 cm^{-1}, a band corresponding to the OH.

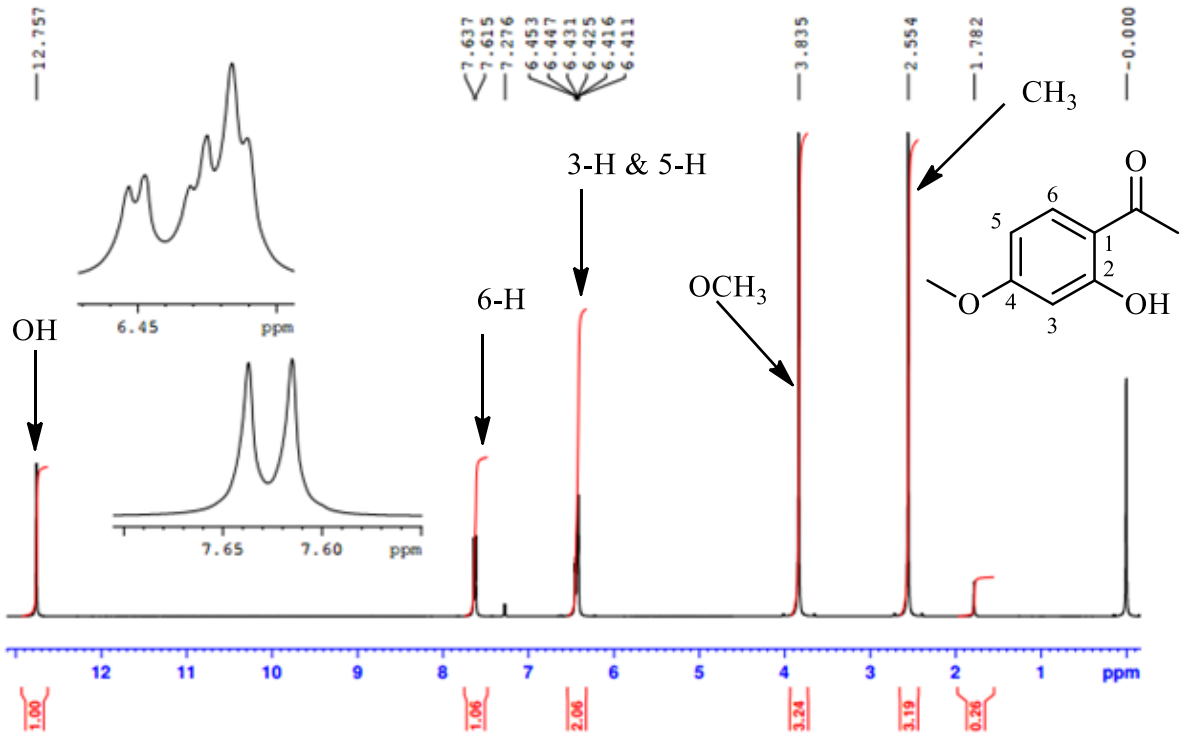


Figure 1: 400 MHz ^{1}H NMR spectrum of 2-hydroxy-4-methoxy-acetophenone **14H** in $CDCl_3$

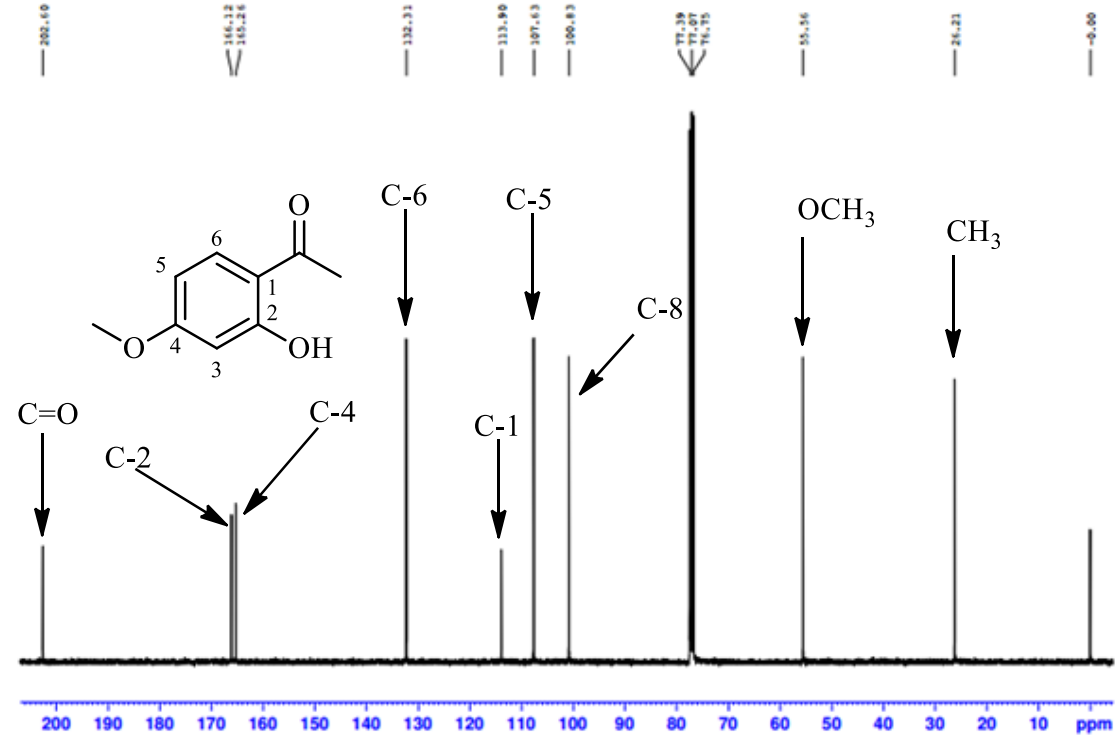
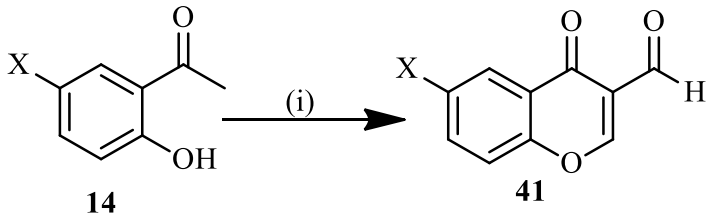


Figure 2: 100 MHz ^{13}C NMR spectrum of 2-hydroxy-4-methoxy-acetophenone **14H** in $CDCl_3$

4.2 Synthesis of 4-oxo-4H-chromene-3-carbaldehyde analogues 41A-F

The synthesis of 4-oxo-4H-chromene-3-carbaldehydes **41** was conducted following a slightly modified literature procedure (Scheme 13).[82] In our investigation, commercially available substituted 2-hydroxyacetophenones **14** were subjected to the Vilsmeier–Haack reaction by mixing the corresponding 2-hydroxyacetophenones **14** with DMF and $POCl_3$ at 0-5 °C for 0.5 h. The temperature was maintained in an ice-acetone water bath, the resulting mixture was then stirred for 12 h at room temperature and poured over crushed ice with stirring. The resulting solid was filtered, dried and recrystallized from ethanol to afford corresponding 4-oxo-4H-chromene-3-carbaldehyde analogues (**41A-F**).



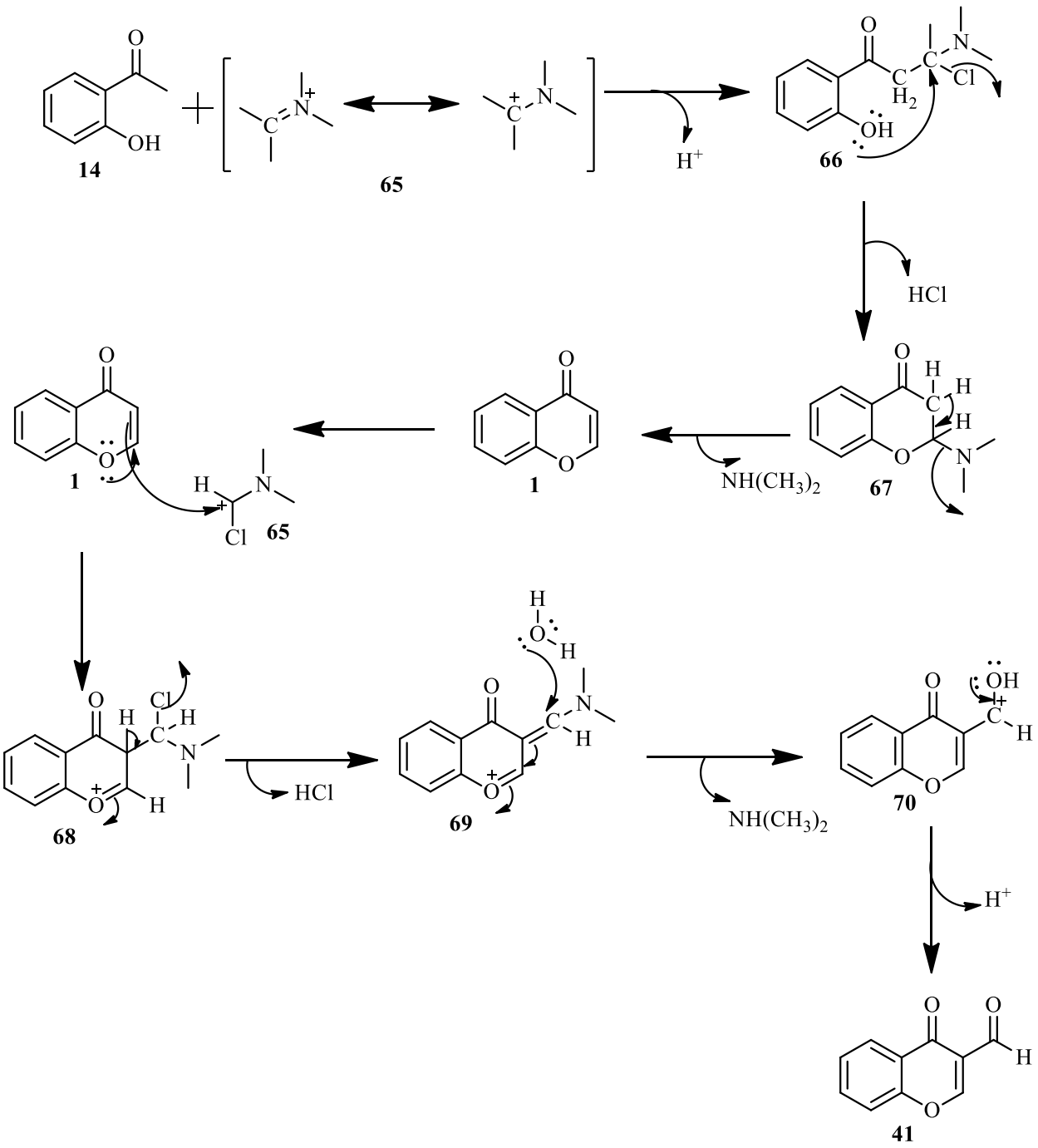
14 **41**

X	A	B	C	D	E	F
	H	F	Cl	Br	OMe	Me

Reagents and conditions: (i) $POCl_3$, DMF, H_2O, 0-25 °C, 12 h

Scheme 13: Synthesis of 4-oxo-4H-chromene-3-carbaldehyde analogues **41A-F**

The Vilsmeier-Haack reagent **65** is generated *in situ* by the reaction of dimethylformamide (DMF) with phosphorus oxychloride ($POCl_3$). The proposed reaction mechanism (Scheme 14) encompasses the formation of an enolate intermediate **66**, generated from the *in situ* reaction of 2-hydroxyacetophenone **14** and the Vilsmeier-Haack reagent **65**. The obtained intermediate undergoes an intramolecular annulation, with subsequent formation of a chromone ring **1**. The chromone ring **1** reacts once again with the Vilsmeier-Haack reagent and reactive intermediate **68** is obtained. Finally, a rapid hydrolysis of **69** occurs, giving rise to 4-oxo-4H-chromene-3-carbaldehyde **41**.



Scheme 14: Reaction mechanism for the synthesis of 4-oxo-4H-chromene-3-carbaldehyde **41**

NMR confirmed the purity of all the 4-oxo-4H-chromene-3-carbaldehyde **41A-F**. The afforded yield was good to excellent (46-94 %) and was comparable with the data reported in the literature.[82] The experimental melting point of all compounds was consistent with those reported in the literature. The melting point and percentage yields are reported in table 3.

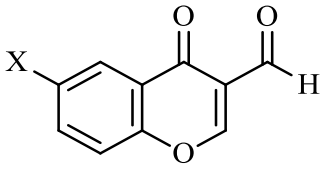


Table 3: Synthesized 4-oxo-4H-chromene-3-carbaldehyde analogues **41A-F**

Compound **41**	X	% Yield*	Melting point (°C)	Lit. Melting (°C)
A	H	55	150-152	149-151[82]
B	6-F	54	155-157	155-160[82]
C	6-Cl	93.8	165-169	166-169[82]
D	6-Br	76.9	195-197	190-193[82]
E	6-OMe	46	158-160	163-165[83]
F	6-Me	94	170-176	173-174[83]

** isolated yields after recrystallization*

These compounds were fully characterized using 1D NMR (^{1}H, ^{13}C NMR, DEPT), IR spectrum and their physical properties (melting point and colour).

The assignment of the protons for compound **41A-F** was done after considering of the chemical shifts and coupling constants observed for signals in the ^{1}H NMR spectra.

The ^{1}H NMR spectra of 4-oxo-4H-chromene-3-carbaldehydes **41A-F** were characterized by the presence of two singlets corresponding to the formyl group and methine proton at C-2. Compounds containing the methoxy (OCH_3) and methyl (CH_3) substituents were characterized by singlets in the aliphatic region at 3.89 ppm for the methoxy and at 2.49 ppm for the methyl compound.

Spectroscopic data obtained from both DEPT 135 and ^{13}C NMR was used to identify the types of carbon present in each of the 4-oxo-4H-chromene-3-carbaldehydes. The characteristic aldehyde carbon (CHO) was observed around 188.57-188.94 ppm and the methine carbon (C-2) around 163.48-163.92 ppm. For the halogen-substituted **41B-D** the quaternary carbon (C-6) was

observed around 126 ppm, for methyl substituted compound **41F** the same carbon at position 6 was observed at 137.60 ppm and the 6-methoxy substituted compound **41E** at 150.77 ppm. The presence of four quaternary carbons 4-oxo-4H-chromene-3-carbaldehyde **41A** and for monosubstituted 4-oxo-4H-chromene-3-carbaldehydes **41B-F**, five quaternary carbons were observed, thus confirming the chromone framework was successfully synthesized. The ^{1}H and ^{13}C NMR spectra for the compounds were in agreement with the data reported in the literature.[82] The chemical structures of 4-oxo-4H-chromene-3-carbaldehydes **41** were also supported by the IR spectrometry. The carbonyl stretch (C=O) on the pyrone ring, corresponding to (C-4) was observed around 1630-1650 cm^{-1} and the other carbonyl stretch observed from 1688-1735 cm^{-1} for the HC=O functionality.

The ^{1}H NMR spectrum of 6-methyl-4-oxo-4H-chromene-3-carbaldehyde **41F** (Figure 3) revealed three singlet signals corresponding to the CHO proton at 10.38 ppm the methine proton (2-H) at 8.52 ppm and the singlet in the aliphatic region at 2.48 ppm a singlet integrating for three protons corresponds to the methyl carbon. The aromatic protons were observed between 7.42 and 8.07 ppm.

Figure 4 shows the ^{13}C spectrum of 6-methyl-4-oxo-4H-chromene-3-carbaldehyde **41F** which revealed a total of 11 carbons, consistent with the compound.

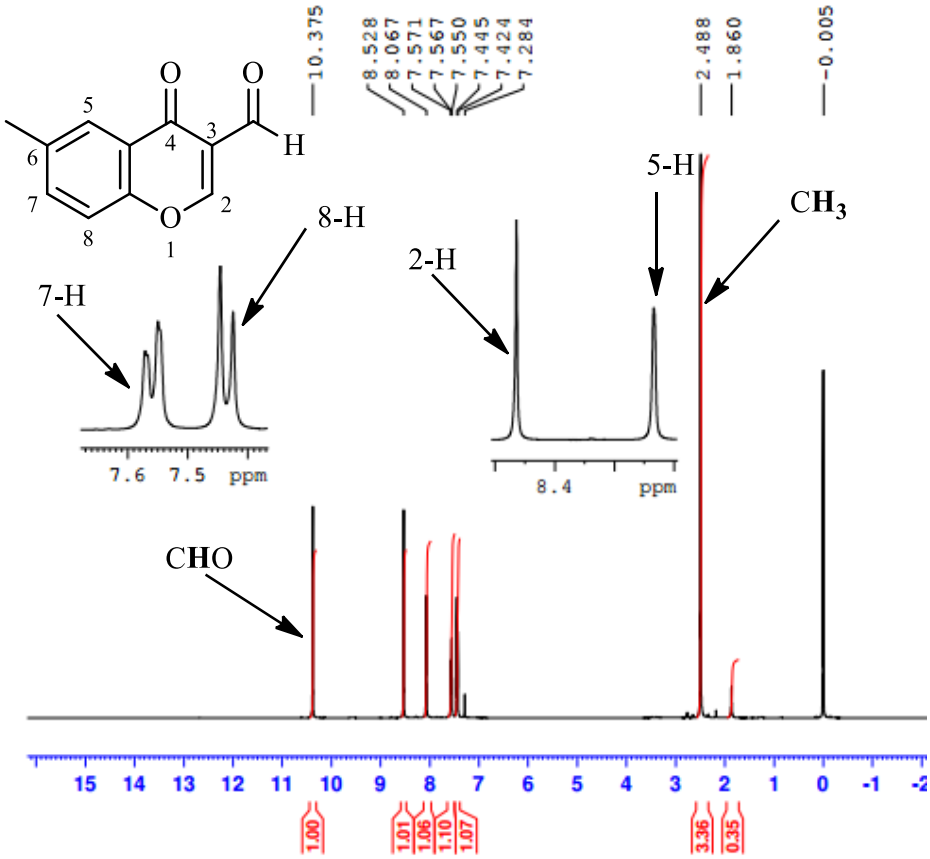


Figure 3: 400 MHz ^{1}H NMR spectrum of 6-methyl-4-oxo-4H-chromene-3-carbaldehyde **41F** in $CDCl_3$

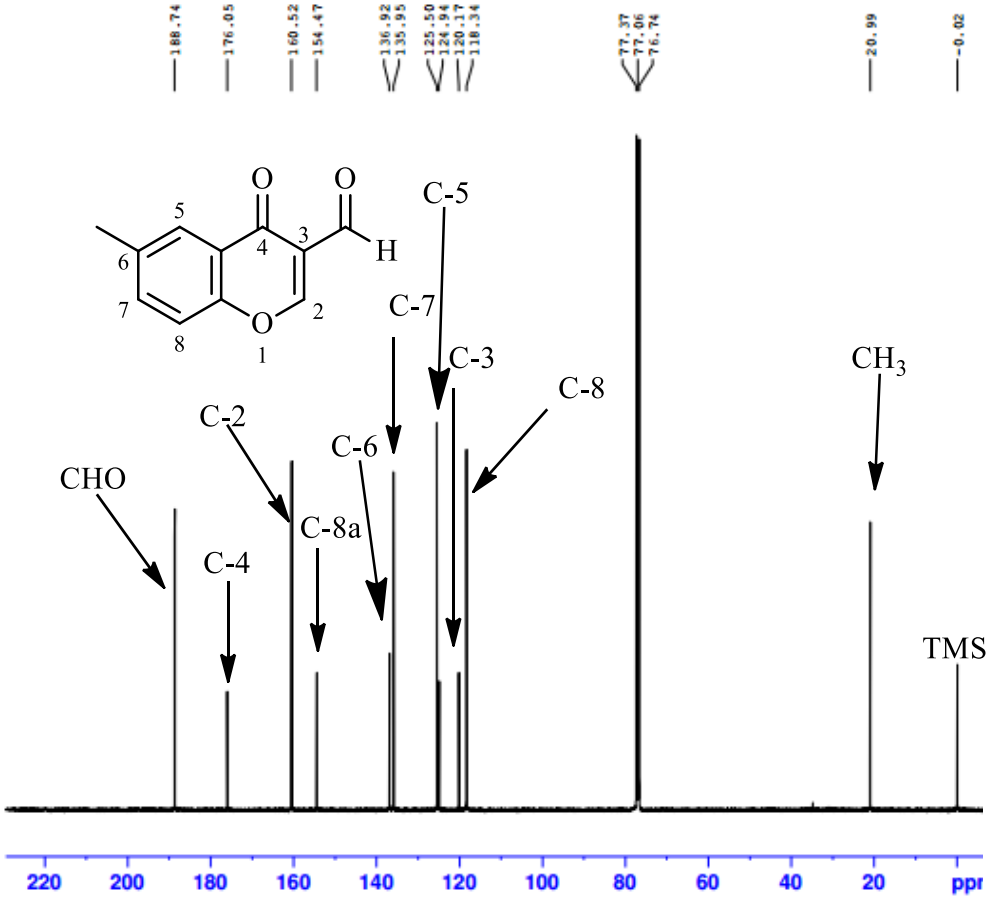
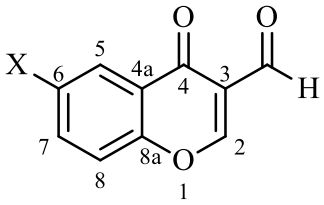


Figure 4: 100 MHz ^{13}C NMR spectrum of 6-methyl-4-oxo-4H-chromene-3-carbaldehyde **41F** in $CDCl_3$



41

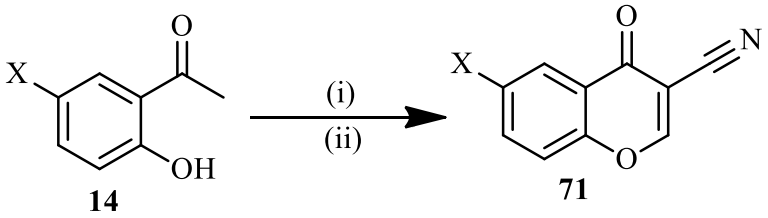
Table 4: 100 MHz ^{13}C NMR chemical shift values (ppm) of selected 4-oxo-4H-chromene-3-carbaldehydes (**41A**, **D**, **E** and **F**) in DMSO-d_6

Nucleus	**41A** **X=H**	**41D** **X=Br**	**41E** **X=OMe**	**41F** **X=Me**
C2	163.92	164.67	163.48	163.70
C3	120.44	119.67	119.73	120.29
C4	175.37	174.21	175.12	175.31
C4a	125.11	120.51	125.99	124.77
C5	127.20	127.83	121.01	125.00
C6	125.76	126.72	150.77	137.60
C7	135.69	138.21	124.30	136.60
C-8	119.38	122.04	105.81	119.13
C-8a	156.07	155.08	157.86	154.34
CHO	188.87	188.57	188.94	188.91
OCH_3	-	-	56.38	-
CH_3	-	-		20.88

NB: Chemical shift of compounds analysed in DMSO-d_6 solvent

4.3 Synthesis of 4-oxo-4H-chromene-3-carbonitrile analogues 71A-E

4-oxo-4H-chromene-3-carbonitriles **71** were synthesized as precursors to the 4-oxo-4H-chromene-3-carboxylic acid **52** and the synthetic strategy followed a modified one-pot method (Scheme 15).[84] In our study the 2-hydroxyacetophenones **14** were condensed under Vilsmeier-Haack conditions using DMF and $POCl_3$ and stirred at room temperature for 4 h. The reaction mixture was then treated with a solution of hydroxylamine hydrochloride in DMF and DCM and stirred for an additional 4 h. The reaction mixture was quenched with cold water and then extracted with DCM. The combined organic phases were washed with water, saturated $NaHCO_3$ solution and finally with water. The combined extracts were dried over anhydrous Na_2SO_4. The solvent was removed under reduced pressure and the residual solid was directly crystallized from methanol to give 4-oxo-4H-chromene-3-carbonitrile analogues **71** with an average percentage yield of 61.2 %. The melting point data and percentage yields for the afforded 4-oxo-4H-chromene-3-carbonitrile analogues **71** are reported in table 5. The experimental melting points data were consistent with the reported values and elemental analysis of the compounds revealed that the desired compounds were synthesized.



14 **71**

X	A	B	C	D	E
	H	F	Cl	Br	OMe

Reagents and conditions: (i) $POCl_3$, DMF, 0 ◦C, 4 h (ii) $NH_2OH{\cdot}HCl$, DCM, DMF, 0-25 °C, 4 h

Scheme 15: Synthesis of chromone-3-carbonitrile analogues **71A-E**

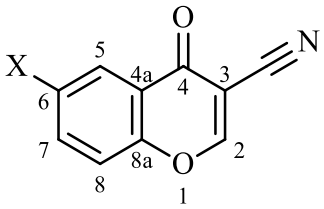


Table 5: Synthesized 4-oxo-4H-chromene-3-carbonitrile analogues **71A-E**

Compound 71	X	% Yield*	Melting point (°C)	Lit. Melting (°C)
A	H	78	174-176	177-179[85]
B	F	60	177-178	172-174[84]
C	Cl	52.4	210-211	211-213[84]
D	Br	56.7	216-219	218-220[84]
E	OMe	57.7	190-192	194-195[84]

** isolated yields after recrystallization*

All synthesized 4-oxo-4H-chromene-3-carbonitrile analogues **71** were fully characterized by spectroscopic techniques (^{1}H and ^{13}C NMR, IR spectra and elemental analysis). ^{1}H NMR spectra for synthesized compounds were characterized by a singlet around 9.26-9.28 ppm corresponding to a methine proton (2-H). The aromatic protons were observed between 7.6-8.10 ppm and a singlet at 3.87 ppm integrated for three protons corresponding to the methoxy protons for compound **71E**.

The ^{13}C NMR spectra for all the synthesized 4-oxo-4H-chromene-3-carbonitriles analogues **71**, characteristic quaternary carbon (C-4) were observed at 172.05-172.99 ppm and methine carbons (C-2) were observed at 165.40-166.17 ppm. For 4-oxo-4H-chromene-3-carbonitrile **71A**, and halogen substituted compounds, a total of 10 carbon signals were observed, and in compound **71E** the methoxy carbon was observed at 56.42 ppm. Table 6 outlines the observed chemical shifts for the carbons on the chromone-3-carbonitrile nucleus.

The ^{1}H NMR spectrum of 6-bromo-4-oxo-4H-chromene-3-carbonitrile **71D (**Figure 5) reveals the methine proton at 9.27 ppm. A doublet of doublet at 8.06 ppm corresponding to the proton 7-H was obtaining its splitting pattern from proton 8-H and 5-H, $J_{H\text{-}H}$ ortho = 8.8 Hz and $J_{H\text{-}H}$ meta =2.4 Hz respectively.

The ^{13}C NMR spectrum (Figure 6) revealed a total of 10 carbons, six quaternary carbons, methine carbon on the pyrone framework and three (CH) carbon on the benzo-framework.

IR spectrum of chromene-3-carbonitrile analogues **71A-E** revealed a carbonitrile (CN) stretch around 2239 cm^{-1} and C=O stretch around 1658 cm^{-1} corresponding to the carbonyl carbon (C-4).

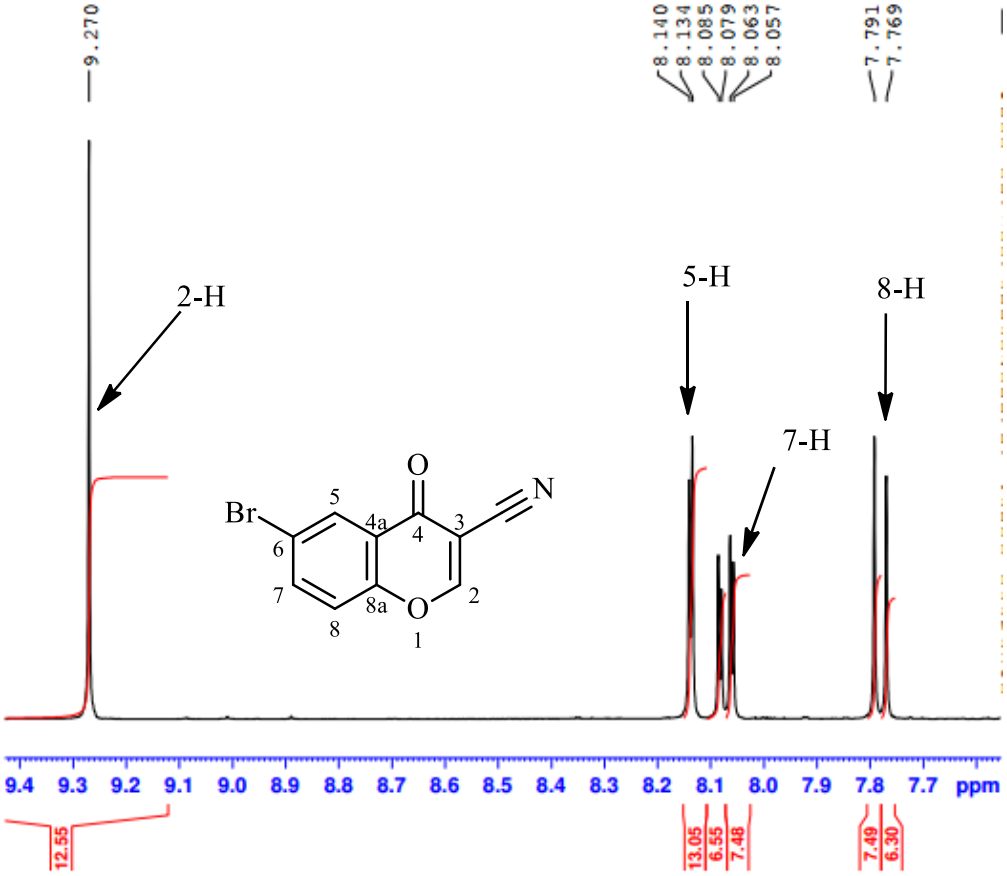


Figure 5: 400 MHz ^{1}H NMR spectrum of 6-bromo-4-oxo-4H-chromene-3-carbonitrile **71D** in DMSO-d_6

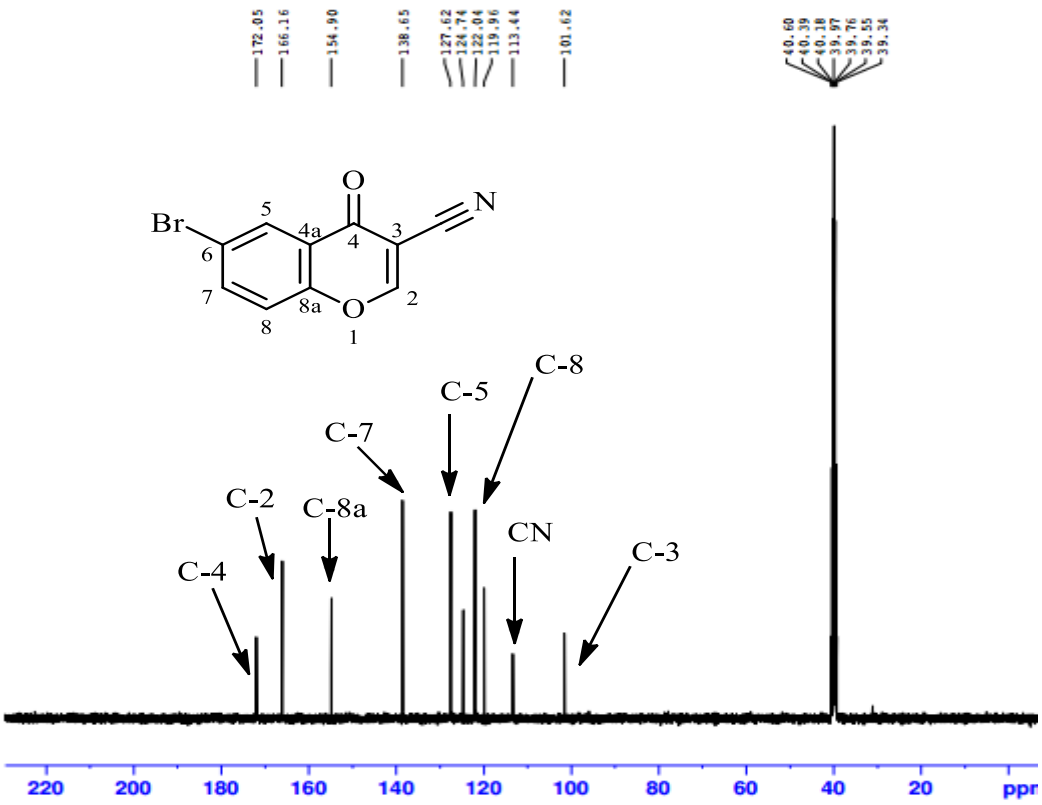


Figure 6: 100 MHz ^{13}C NMR spectrum of 6-bromo-4-oxo-4H-chromene-3-carbonitrile **71D** in DMSO-d_6

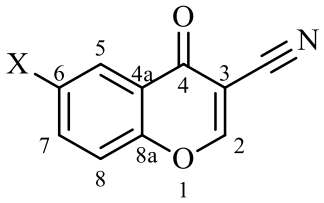


Table 6: 100 MHz ^{13}C NMR spectrum of 4-oxo-4H-chromene-3-carbonitrile **71A-E** in DMSO-d_6

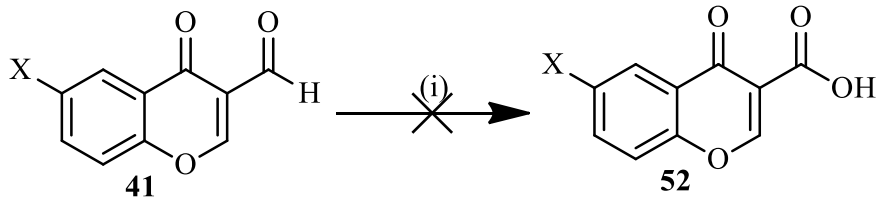
Nucleus	**71A** **X=H**	**71B** **X=F**	**71C** **X=Cl**	**71D** **X=Br**	**71E** **X=OMe**
C2	165.86	166.07	166.16	166.17	165.40
C3	101.49	100.91	101.53	101.62	100.56
C4	172.99	172.59	172.20	172.05	172.85
C4a	123.12	124.24	124.41	119.96	123.96

C5	127.54	124.53	124.55	127.62	121.08
C6	125.56	152.42	131.96	124.74	150.59
C7	136.09	124.60	135.91	138.65	124.87
C8	119.33	122.35	121.94	122.05	105.41
C-8a	155.82	158.87	154.57	154.90	158.00
CN	113.66	113.54	113.45	113.44	113.87
OCH_3	-	-	-	-	56.42

4.4 Synthesis of 4-oxo-4H-chromene-3-carboxylic acid analogues 52A-E

Various methods were attempted for the synthesis of 4-oxo-4H-chromene-3-carboxylic acids **52**, however all attempted methods but one failed to afford the desired target compounds. A brief overview of the various methods used in our attempt to afford 4-oxo-4H-chromene-3-carboxylic acid **52** follows below.

The attempt to afford 4-oxo-4H-chromene-3-carboxylic acid **52** began with a well-known literature method, which involved the reaction of 4-oxo-4H-chromene-3-carbaldehyde analogues **41** with Jones' reagent under Jones' oxidation reaction conditions. Work up afforded a solid which was recrystallized from acetone to give a white solid. TLC showed that the starting material was completely transferred to products. However, the melting point of the synthesized material was not in the same range as that reported in the literature. For the parent compound, we recorded a melting point of 166-169 °C whereas the literature value was 204-205 °C. Close inspection of the ^{1}H NMR data revealed that the desired 4-oxo-4H-chromene-3-carboxylic acid **52** was not formed, this is due to an addition signal observed in the aromatic region, accounting for two protons (Figure 7). This trend was observed in all the synthesized analogues and since our primary objective for this study was the synthesis of 4-oxo-4H-chromene-3-carboxylic acids **52**, no further structural elucidation was done on the synthesized compounds.



Reagents and conditions: (i) Jones' reagent, acetone, 10-15°C, 0.5 h,

Scheme 16: Synthesis of 4-oxo-4H-chromene-3-carboxylic acids using Jones' reagent

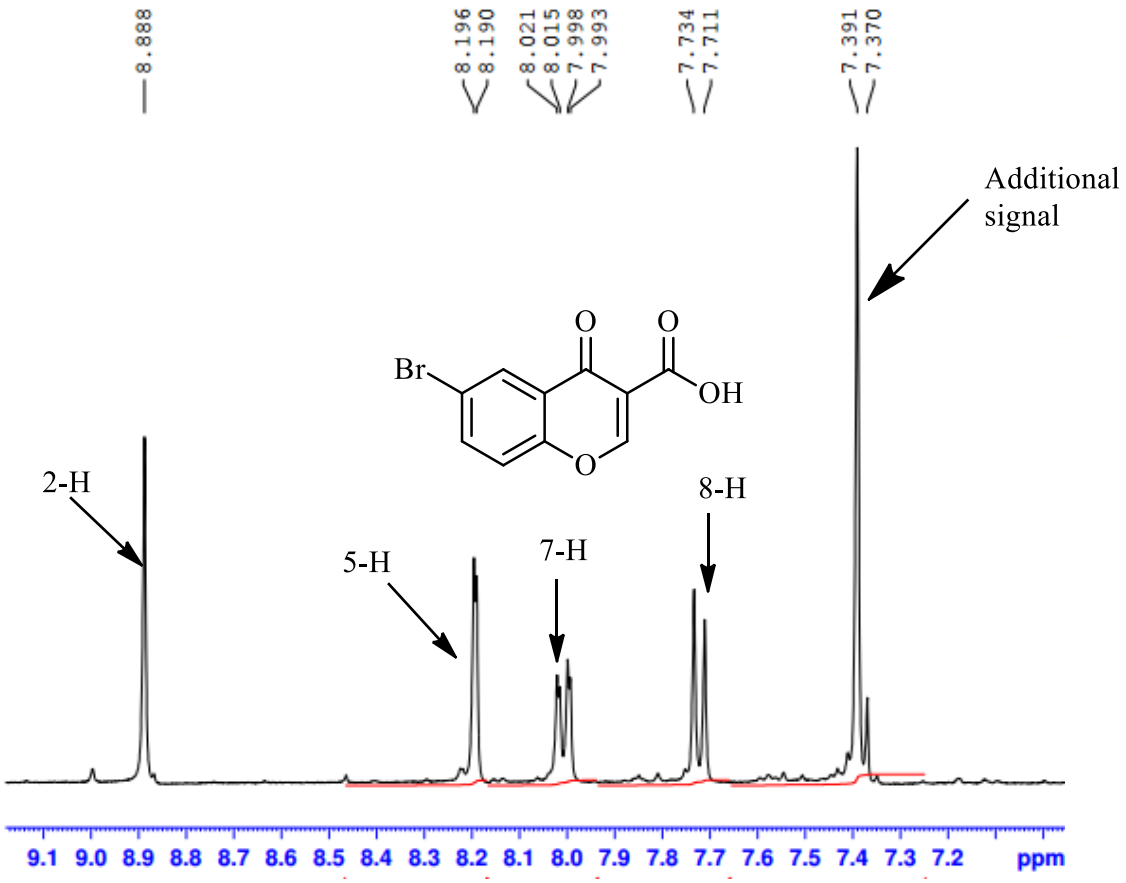
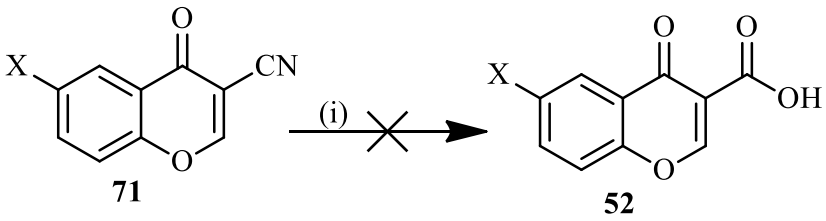


Figure 7: 400 MHz ^{1}H NMR 6-bromo-4-oxo-4H-chromene-3-carboxylic acid using Jones' reagent in DMSO-d_6

The second attempt in the synthesis of 4-oxo-4H-chromene-3-carboxylic acid **52** used 4-oxo-4H-chromene-3-carbaldehyde **41** as its precursor.[86] 4-oxo-4H-chromene-3-carbaldehyde **41** was dissolved in formic acid and cooled to 0 °C and 30 % hydrogen peroxide was gradually added to the solution maintaining the temperature at 0-5 °C as per literature method guideline. The resultant mixture was stirred for 8 h at 0-5 °C and the progress monitored by TLC which showed that the starting material was not completely transformed into products. On completion 4-oxo-

4H-chromene-3-carbaldehyde **41** was isolated. Several modifications to the literature method were made, however we recovered the starting material after each attempt. The hydrogen peroxide mixture was not a suitable oxidant, to transform 4-oxo-4H-chromene-3-carbaldehyde **41** to 4-oxo-4H-chromene-3-carboxylic acid **52**.

In another attempt[87] (Scheme 17), 4-oxo-4H-chromene-3-carbonitrile **65** was refluxed in concentrated HCl for 1 h and the resulting solution was cooled to room temperature and poured over crushed ice with stirring and allowed to stand for a while. To our disappointment, TLC analysis and the ^{1}H NMR spectrum of the crude material revealed no product formation. The starting was recovered. This reaction procedure was repeated using other concentrated acids (HNO_3 and H_2SO_4) under the same reaction conditions, however the result was the same. After numerous attempts and modifications to this method, no product was obtained.



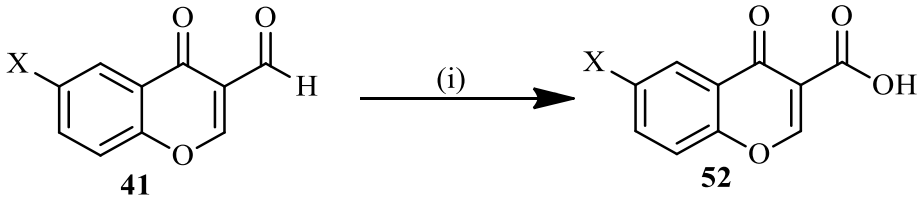
X	A	B	C	D	E
	H	F	Cl	Br	OMe

Reagents and conditions: (i) HCl, 100 °C, 1 h

Scheme 17: Acid hydrolysis of 4-oxo-4H-chromene-3-carbonitrile to 4-oxo-4H-chromene-3-carboxylic acid

The synthesis of 4-oxo-4H-chromene-3-carboxylic acid analogues **52** was done following a modified literature method[88] which involve the reaction of 4-oxo-4H-chromene-3-carbaldehyde **41**, sodium chlorite as the oxidising reagent. Oxidation of aldehyde to carboxylic acid by sodium chlorite under mildly acidic conditions was first reported by Lindgren and Nilsson in 1973. This method however is now referred to as Pinnick oxidation because the generality of this oxidation was shown by Pinnick and co-workers in 1981.[89] In our study (Scheme 18), Pinnick oxidation methodology was applied by first dissolving sodium chlorite in water and this solution was cooled to 0 °C. A mixture of the corresponding 4-oxo-4H-chromene-3-carbaldehyde **41** and sulfamic acid was added to the chilled solution, and DCM was gradually added to the mixture using a dropping funnel. The reaction mixture was allowed to warm up to room temperature and stirred for 3 h. On completion TLC monitoring displayed formation of the intended product, then

the reaction mixture was quenched with water, extracted with DCM and the combined organic layers were dried using anhydrous Na_2SO_4. The combined organic layers were concentrated under reduced pressure to afford a crude solid. The solid obtained was recrystallized from 80:20 MeOH/H_2O to afford corresponding 4-oxo-4H-chromene-3-carboxylic acids **52** with an average percentage yield of 58.4 %. The melting point for the compounds synthesized using Pinnick oxidation was consistent with the reported melting points elsewhere in the literature (Table 7).[90]



X	A	B	C	D	E	F
	H	F	Cl	Br	OMe	Me

Reagents and conditions: $NaOCl_2$, sulfamic acid, H_2O, DCM, 0-25 °C, 12 h

Scheme 18: Synthesis of 4-oxo-4H-chromene-3-carboxylic acid analogues **52A-E**

Table 7: Synthesized 4-oxo-4H-chromene-3-carboxylic acid analogues **52A-E**

Compound **52**	R_1	% Yield*	Melting point (°C)	Lit. Melting (°C)
A	H	53	200-203	204-205[90]
B	F	57	210-213	-
C	Cl	68.5	230-231	231-232[90]
D	Br	55.2	236-240	245-249[90]
E	OMe	55.6	172-173	173-174[91]
F	Me	61.3	240-247	-

** isolated yields after recrystallization*

4-oxo-4H-chromene-3-carboxylic acid **52** is characterized in the ^1H NMR by the presence of a broad signal downfield corresponding to the (OH) at 13.28 ppm. The disappearance of the

aldehyde proton that resonates around 10.14 ppm in the corresponding 4-oxo-4H-chromene-3-carbaldehyde **41** starting material primarily showed the 4-oxo-4H-chromene-3-carboxylic acid **52** were synthesized. A singlet was observed at 9.15 ppm corresponding to the proton 2-H and all the aromatic protons were accounted for, resonating from 7.63-8.21 ppm. The ^{1}H NMR spectrum of 4-oxo-4H-chromene-3-carboxylic acid **52A** revealing all six protons is outlined in figure 8.

The expected ten carbons for the 4-oxo-4H-chromene-3-carboxylic acid **52A** were observed in the ^{13}C NMR spectrum (Figure 9) with two carbonyl carbons appearing at 176 ppm and 164 ppm corresponding to C-4 and carbonyl carbon bonded to the OH. The methine carbon (C-2) was observed at around 164.20 ppm in all the synthesized carboxylic acids **52**. For the 6-substituted analogues with high electronegativity substituents (fluorine and methoxy), in cases of compounds **52B** and **52E**, (C-6) were more desheilded than the other analogues, resonating around 152.55 ppm. Compound **54A**, carbon (C-6) was observed at 125.87 ppm as outlined in table 9.

The assignments of carbons were done using DEPT 135 and ^{13}C NMR. The aromatic (CH) carbons were observed at the following chemical shifts, 119.26 ppm (C-8), 125.87 ppm (C-6), 127.28 ppm (C-5), 135.99 ppm (C-7) and the methine carbon (C-2) was observed at 164.25 ppm as shown in figure 9.

The IR spectra of 4-oxo-4H-chromene-3-carboxylic acid **52A** revealed the OH stretch at 3071.64 cm^{-1} and the two carbonyls (C=O) stretch at 1691.39 and 1651.72 cm^{-1}.

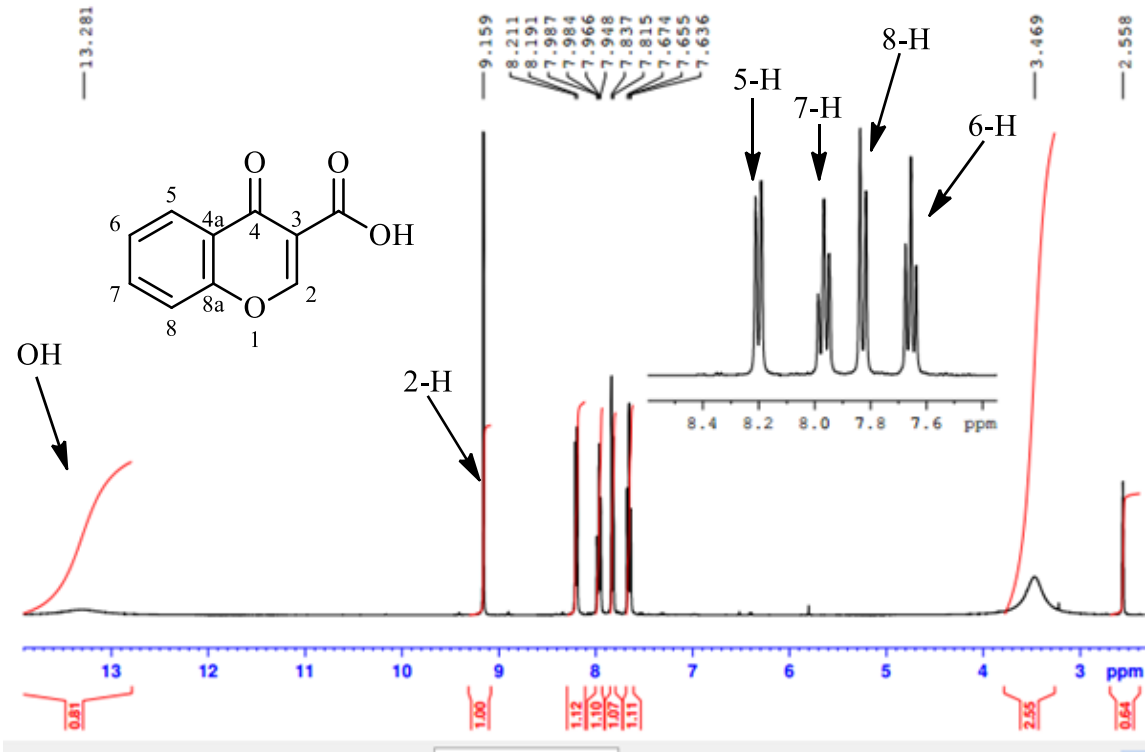


Figure 8: 400 MHz ^{1}H NMR spectrum of 4-oxo-4H-chromene-3-carboxylic acid **52A** in DMSO-d_6

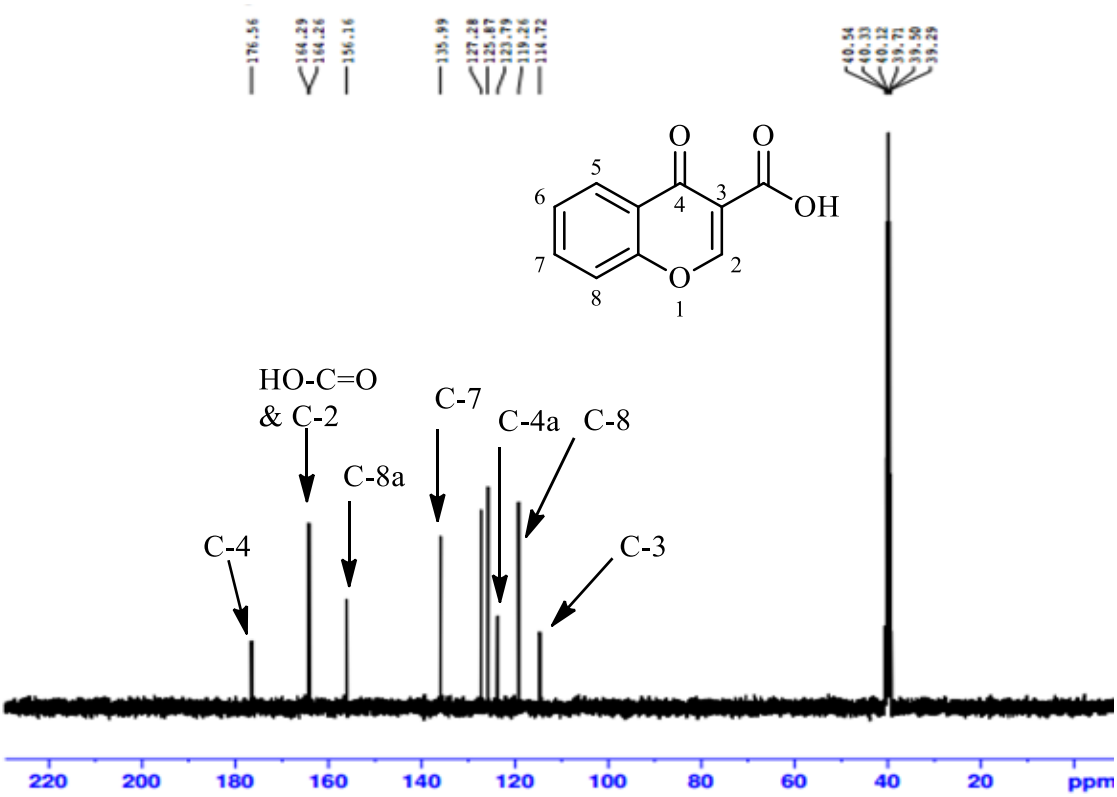


Figure 9: 100 MHz ^{13}C NMR spectrum of 4-oxo-4H-chromene-3-carboxylic acid **52A** in DMSO-d_6

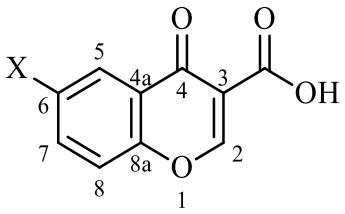


Table 8: ^{13}C NMR of selected 4-oxo-4H-chromene-3-carboxylic acid analogues (**52A, B, E, F**) in DMSO-d_6

Nucleus	**52A** **X=H**	**52B** **X=F**	**52E** **X=OMe**	**52F** **X=Me**
C2	164.25	164.17	163.94	164.19
C3	114.72	114.68	113.73	114.42
C4	176.56	175.09	176.53	176.77
C4a	123.79	123.68	124.53	123.43
C5	127.28	122.21	120.93	125.05
C6	125.87	152.55	151.02	136.63
C7	135.99	123.93	125.06	137.13
C8	119.26	110.19	105.53	119.09
C8a	156.16	158.76	157.90	154.57
C=O	164.29	164.07	164.37	164.36
OCH_3	-	-	56.39	-
CH_3	-	-	-	20.00

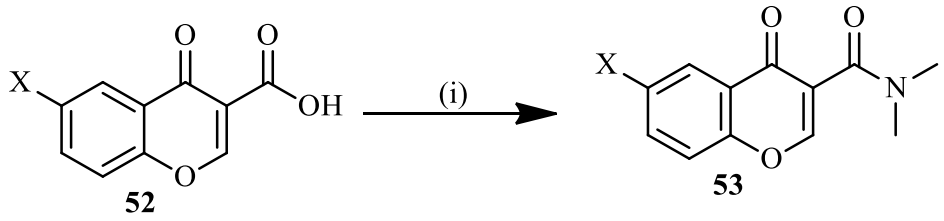
NB: Chemical shift of compounds analysed in DMSO-d_6 solvent.

4.5 General synthesis of chromone-3-carboxamide analogues

The synthetic strategy to chromone-3-carboxamide[92] was implemented by adding to a suspension of corresponding 4-oxo-4H-chromene-3-carboxylic acid analogues **52** (1.0 equivalent), $SOCl_2$ (1.5 equivalent) and dry DCM as the solvent, followed by stirring for 1 h under N_2 gas to get *in situ* formation of the corresponding acyl chloride (4-oxo-4H-chromene-3-carbonyl chloride). The solution with generated acid chloride intermediate was cooled to 0 °C, followed by the addition of Et_3N (3 equivalent) and appropriate amine (1.2 equivalent) then stirred overnight. The resultant mixture was quenched with water and extracted with DCM. The combined organic layers were washed extensively with saturated 25 % ammonia solution, washed with water then dried over anhydrous $MgSO_4$ and concentrated under reduced pressure to afford solid products. The crude products were directly crystallized from MeOH, EtOH and acetone to afford the corresponding 4-oxo-4H-chromene-3-carboxamide **53A-F**.

4.5.1 Synthesis of N,N-dimethyl-4-oxo-4H-chromene-3-carboxamide analogues 53A-F

The general method (Scheme 19) for the synthesis of chromone-3-carboxamides was followed for the synthesis of N,N-dimethyl-4-oxo-4H-chromene-3-carboxamides analogues **53** using dimethylamine hydrochloride $(CH_3)_2NH_2{\cdot}HCl$ as the appropriate amine. The solid obtained after work-up was directly crystallized from methanol to obtain N,N-dimethyl-4-oxo-4H-chromene-3-carboxamides **53** with yield ranging from 45.7-60 % (Table 9). In cases where lower yields were obtained, for instance with compounds **53B** and **53C** it is possible that some of the product was lost during the work-up process. The literature yield of N,N-dimethyl-4-oxo-4H-chromene-3-carboxamide analogues **53** was higher than the reported values on the basis that the literature source[93] obtained compounds **53A, 53E** and **53F** as by-products in the synthesis of 3,3′-carbonyl-bis(chromones). The observed melting point data was consistent with the reported data[93] with the exception of **53F**, the deviation from the reported data could be the presence the solvent used for recrystallization.



X	A	B	C	D	E	F
	H	F	Cl	Br	OMe	Me

Reagents and conditions: (i) $SOCl_2$, Et_3N, DCM, $(CH_3)_2NH_2{\cdot}HCl$, 0-25 °C, 12 h

Scheme 19: Synthesis of N,N-dimethyl-4-oxo-4H-chromene-3-carboxamide analogues **53A-E**

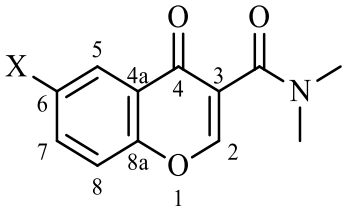


Table 9: Synthesized N,N-dimethyl-4-oxo-4H-chromene-3-carboxamide analogues **53A-E**

Compound **53**	X	% Yield*	Melting point (°C)	Lit. Melting (°C)
A	H	53.8	190-192	195-196[93]
B	F	45.7	170-175	-
C	Cl	48.9	155-157	-
D	Br	50.5	165-170	-
E	OMe	60	158-160	127-128[93]
F	Me	50.3	121-124	118-119[93]

** isolated yields after recrystallization*

Most of the synthesized N,N-dimethyl-4-oxo-4H-chromene-3-carboxamide analogues **53** are novel, as such 1D NMR (^1H, ^{13}C, DEPT), 2D NMR (COSY, HSQC, HMBC), elemental analysis and IR spectroscopic methods were used to confirm the structures of these compounds.

The ^{1}H NMR spectra of N,N-dimethyl-4-oxo-4H-chromene-3-carboxamide analogues **53** were characterized by the presence of two singlets in the aliphatic region observed around 3.00 ppm corresponding to the two amine groups (NCH_3) and each singlet integrating to three protons. The methoxy protons **53E** were observed at 3.93 ppm and for compound **53F**, the methyl protons were observed at 2.44 ppm. The assignment of protons in the ^{1}H NMR was done after considering the chemical shift, literature spectra and multiplicities.

DEPT and ^{13}C spectra were used in assignment of carbons for N,N-dimethyl-4-oxo-4H-chromene-3-carboxamide analogues **53A-F**.

The ^{1}H NMR spectrum of N,N-dimethyl-4-oxo-4H-chromene-3-carboxamide **53A** (Figure 10) reveals two singlet peaks at 3.01 and 3.13 ppm corresponding the two amine groups (NCH_3). The methine proton (2-H) was observed at 8.17 ppm in compound **53A** which in its precursor 4-oxo-4H-chromene-3-carboxylic acid **52** was observed around 9 ppm showing the amide substituent successfully displaced the OH functionality.

The ^{13}C NMR spectrum of N,N-dimethyl-4-oxo-4H-chromene-3-carboxamide **53A** (Figure 11) was characterized by a total of 12 carbons including 5 quaternary and 2 methyl carbons. The methyl carbons correspond to the NCH_3 functionality resonating at 35.40 ppm and 38.58 ppm. The carbonyl carbon corresponding to C-4 was observed at 173.76 ppm and the other carbonyl carbon (C-1') at 164.45 correspond to the amide carbonyl carbon. The coupling pattern was determined by COSY spectrum (Figure 33) and carbon peaks were assigned using HSQC, DEPT 135 (Figure 12) and HMBC correlations.

IR spectra for synthesized N,N-dimethyl-4-oxo-4H-chromene-3-carboxamide analogues **53** revealed a C=O stretching around 1638 cm^{-1} corresponding to the amide ($RCONCH_3$) functional group in the synthesized compounds.

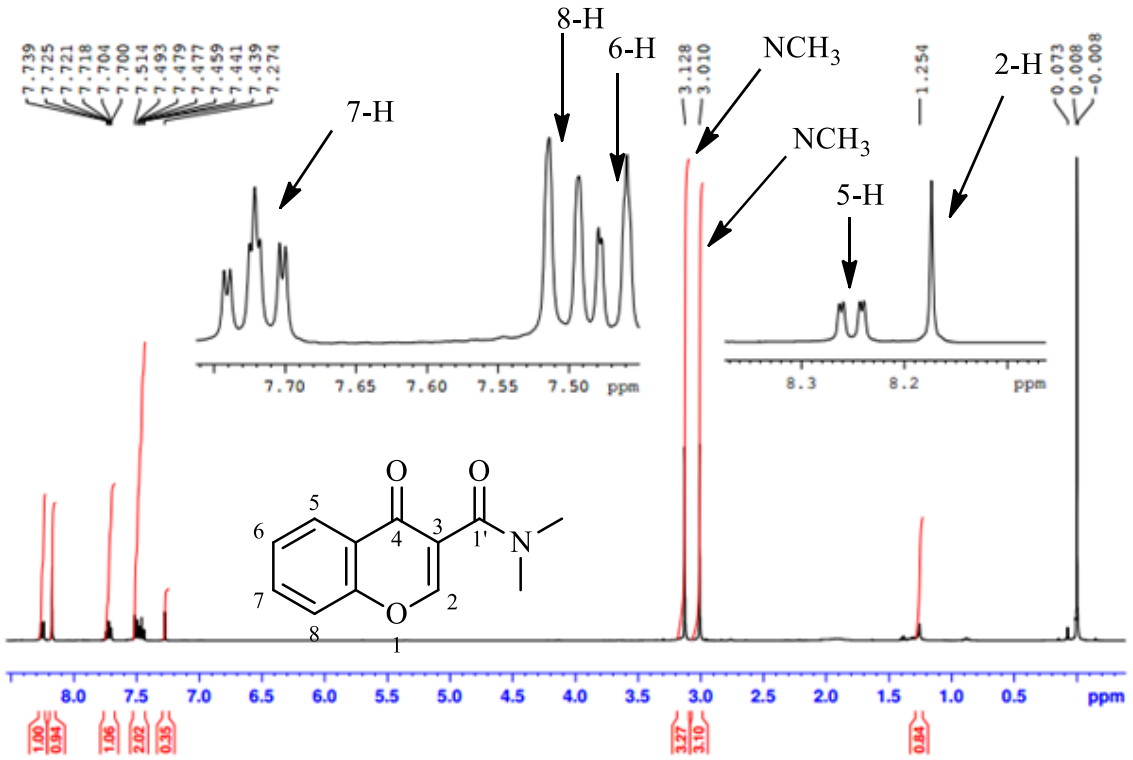


Figure 10: 400 MHz ^{1}H NMR of N,N-dimethyl-4-oxo-4H-chromene-3-carboxamide **53A** in $CDCl_3$

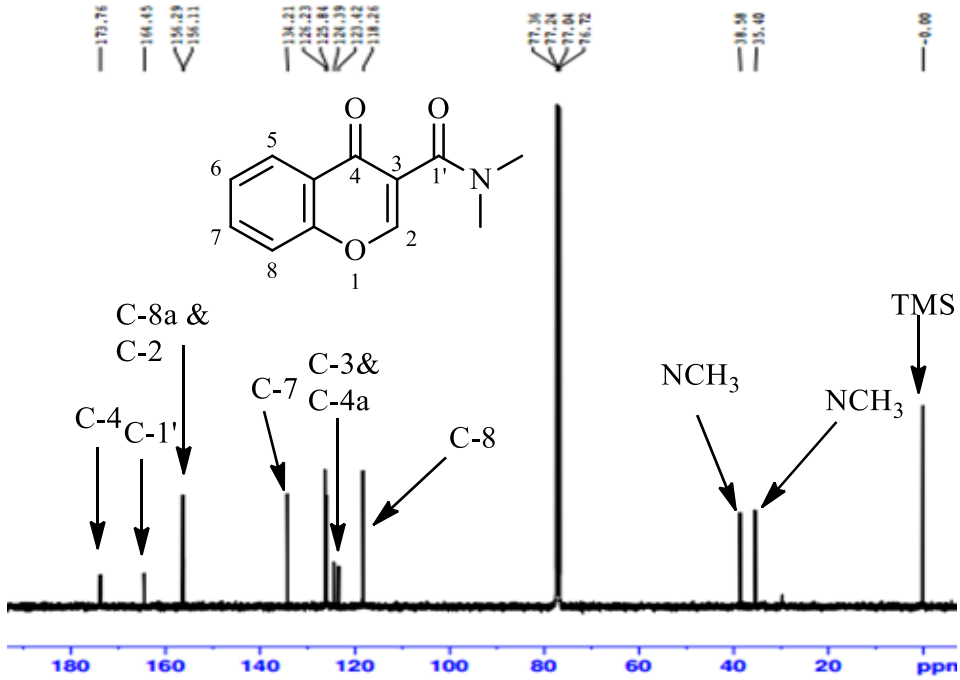


Figure 11: 100 MHz ^{13}C NMR spectrum of N,N-dimethyl-4-oxo-4H-chromene-3-carboxamide **53A** in $CDCl_3$

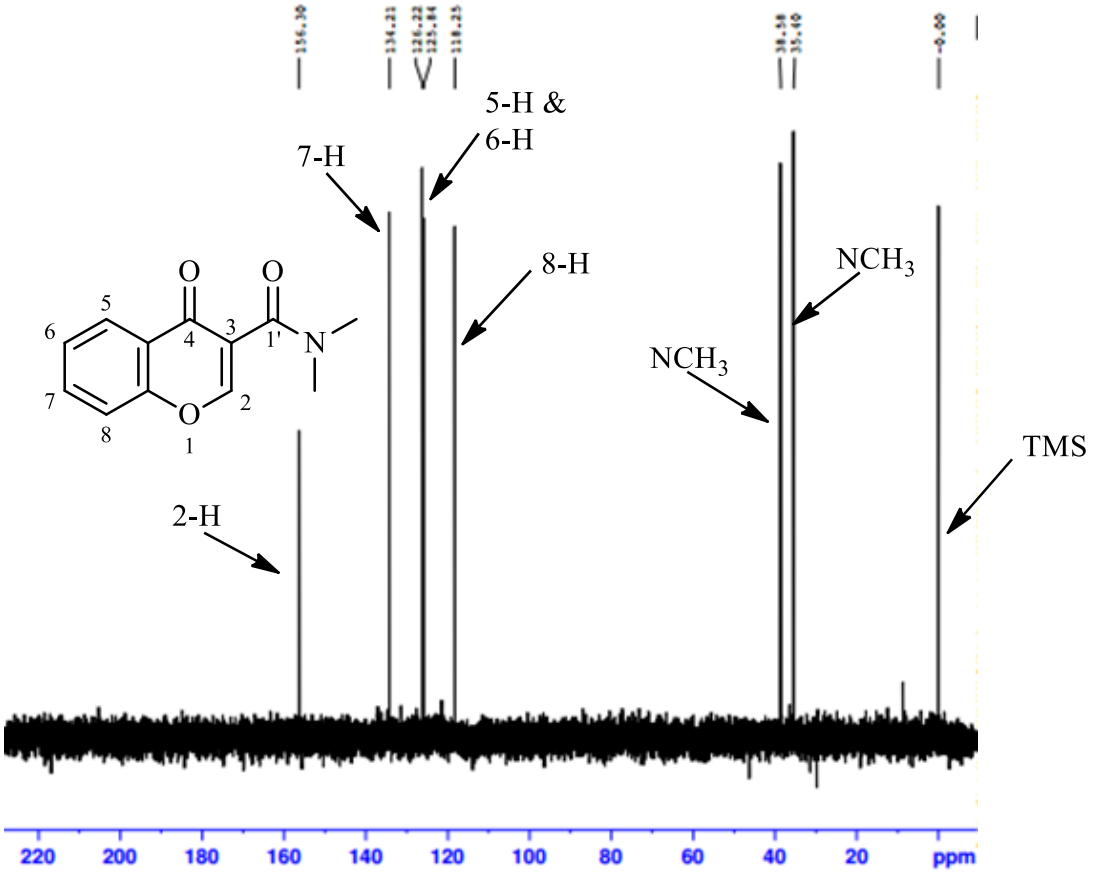
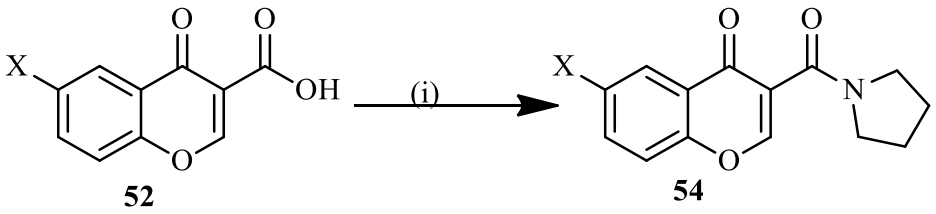


Figure 12: DEPT 135 spectrum of N,N-dimethyl-4-oxo-4H-chromene-3-carboxamide **53A** in $CDCl_3$

4.5.2 Synthesis of 3-(pyrrolidine-1-carbonyl)-4H-chromen-4-one analogues

The general method for the synthesis of chromone-3-carboxamides was followed for the synthesis of 3-(pyrrolidine-1-carbonyl)-4H-chromen-4-one (Scheme 20) using pyrrolidine as the appropriate amine. The crude material after work-up was directly crystallized from methanol to obtain a yellow solid for 6-bromo-3-(pyrrolidine-1-carbonyl)-4H-chromen-4-one and 3-(pyrrolidine-1-carbonyl)-4H-chromen-4-one was recovered as a semi-solid.



52 **54**

54	A	B
X	H	Br

Reagents and conditions: (i) $SOCl_2$, Et_3N, DCM, pyrrolidine, 0-25 °C, 12 h

Scheme 20: Synthesis of 3-(pyrrolidine-1-carbonyl)-4H-chromen-4-one analogues **54A-B**

Two 3-(pyrrolidine-1-carbonyl)-4H-chromen-4-one analogues **54A** and **54B** were successfully synthesized from the corresponding 4-oxo-4H-chromene-3-carboxylic acid **52**. The ^{1}H NMR spectra of 6-bromo-3-(pyrrolidine-1-carbonyl)-4H-chromen-4-one **54B** figure 13 reveals a total of ten protons. In the aliphatic region, at 1.78 ppm a multiplet integrated to four protons arising from the neighbouring 3'-H protons were observed corresponding to protons on carbon 2' (2'-H). Two signals (each one a triplet) arising from the C-2' protons were observed at 3.34 ppm and 3.48 ppm. The three aromatic protons were observed from 7.38-8.28 ppm corresponding to protons 5-H, 7-H and 8-H. The methine proton, 2-H was observed at 8.33 ppm.

The data obtained from DEPT 135 of **54B** (Figure 13) revealed the total of four methylene carbons, consistent with the total methylene carbons in the target molecule. The carbons observed at 47.87 ppm and 46.53 ppm correspond to the carbons next to the heteroatom nitrogen C-3', they are deshielded since they are directly attached to a nitrogen atom. The carbons C-2' resonates at 25.42 ppm and 24.01 ppm and are more shielded since they further away from the nitrogen atom. The other four carbons correspond to three aromatic (CH) carbons and one methine carbon on position 2 (C2) resonating at 158.25 ppm. For compound **54A** four aromatic (CH) carbons were observed.

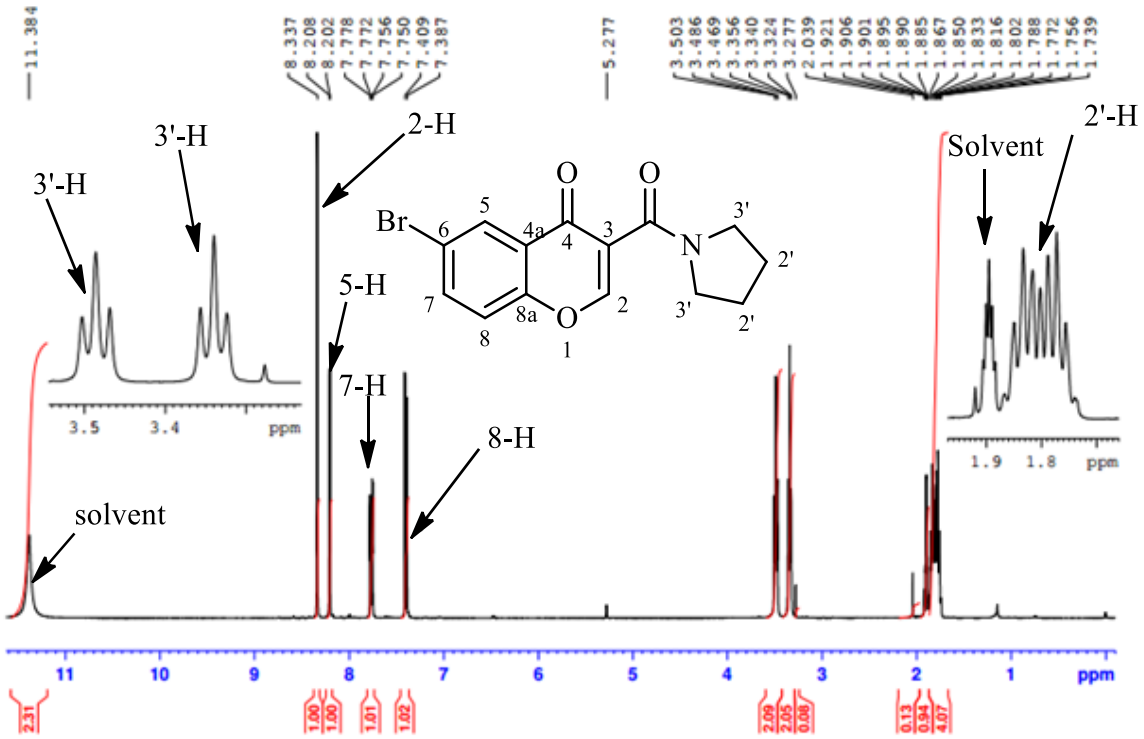


Figure 13: 400 MHz ^{1}H NMR spectra of 6-bromo-3-(pyrrolidine-1-carbonyl)-4H-chromen-4-one **54B** in acetic acid-d_4

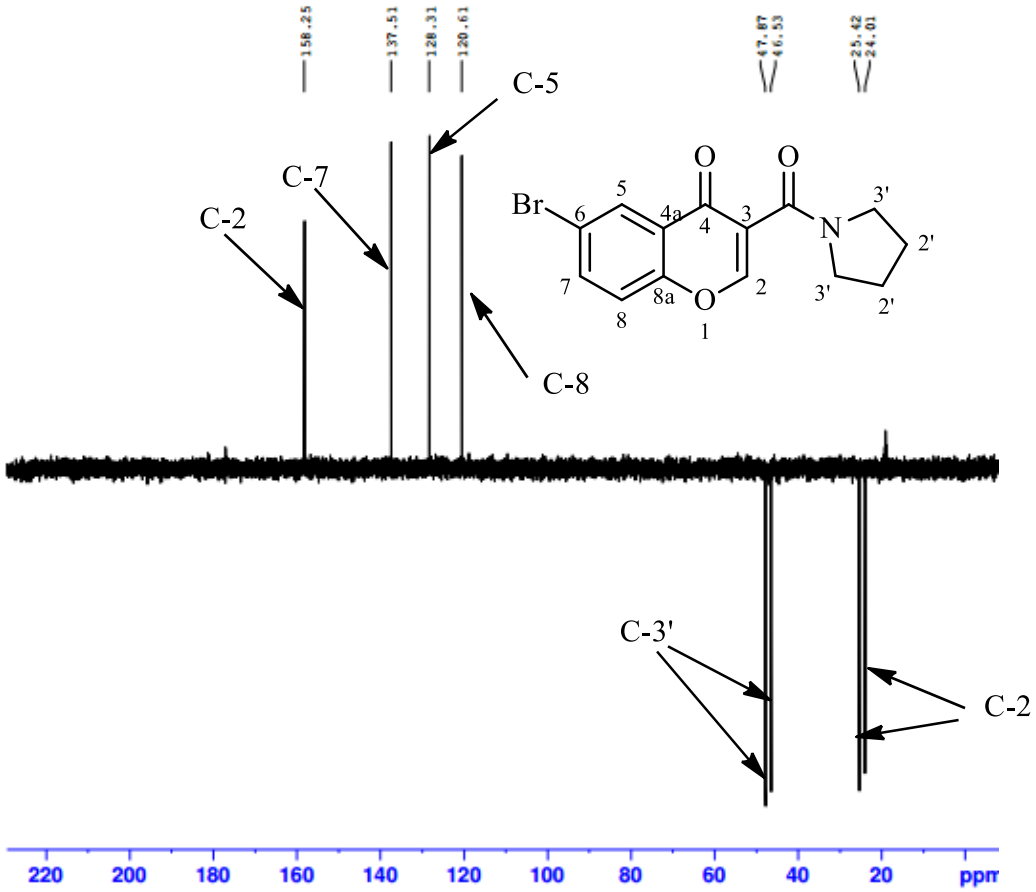
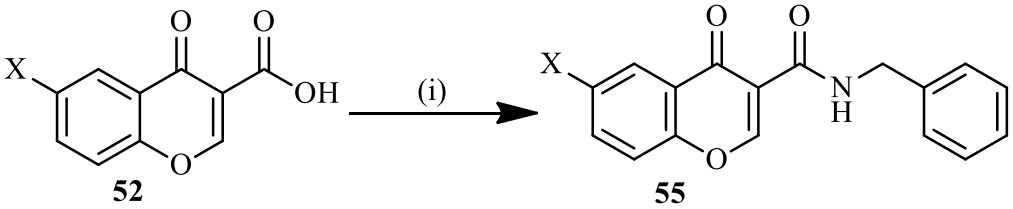


Figure 14: 100 MHz DEPT 135 spectrum of 6-bromo-3-(pyrrolidine-1-carbonyl)-4H-chromen-4-one **54B** in acetic acid-d_4

4.5.3 Synthesis of N-benzyl-4-oxo-4H-chromene-3-carboxamide) analogues 55A-B

The general method for the synthesis of chromone-3-carboxamides was followed for the preparation of N,N-dimethyl-4-oxo-4H-chromene-3-carboxamides using benzylamine as the appropriate amine. The solid obtained after work-up was directly crystallized from acetone to obtain N-benzyl-4-oxo-4H-chromene-3-carboxamide **55A** and **55B** as white crystals.



55	A	B
X	H	Br

Reagents and conditions: (i) $SOCl_2$, Et_3N, DCM, benzylamine, 0-25 °C, 12 h

Scheme 21: Synthesis of N-benzyl-4-oxo-4H-chromene-3-carboxamide analogues **55A-B**

The ^1H NMR spectrum of 6-bromo-N-benzyl-4-oxo-4H-chromene-3-carboxamide **55B** revealed a doublet at 4.65 ppm corresponding to the two protons on (C-2'), a doublet of doublet at 7.82 ppm corresponding to the proton 7-H, a doublet at 8.37 ppm corresponding to 5-H with J-value 2.4 Hz, showing that proton 7-H and 5-H are coupled together. The methine proton 2-H was observed at 9.00 ppm and the aromatic protons on the benzylamine substituent resonating from 7.29-7.47 ppm. The NH peak was observed at 9.56 ppm.

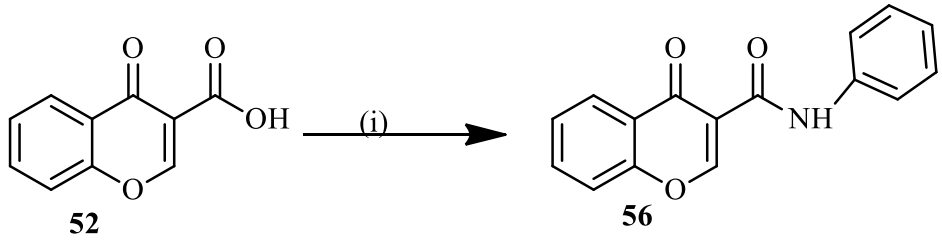
^{l3}C NMR spectrum revealed two quaternary carbons corresponding to the carbonyls C-4 and C-1' resonating at 175.93 and 154.86 ppm respectively. The methine carbon was observed at 162.58 ppm. The methylene carbon was observed at 43.36 ppm.

IR spectrum of compound **55** revealed the absence of the hydroxyl band corresponding to the OH functionality in 4-oxo-4H-chromene-3-carboxylic acid, however the presence of an NH band at 3296 cm^{-1}. The carbonyls bands were observed at 1714 and 1668 cm^{-1}.

4.5.4 Synthesis of a 4-oxo-N-phenyl-4H-chromene-3-carboxamide analogue 56

The general method for the synthesis of chromone-3-carboxamides was followed for the preparation of 4-oxo-N-phenyl-4H-chromene-3-carboxamide **56** using aniline as the appropriate amine (Scheme 22).

The solid obtained after work-up was directly crystallized from acetone to obtain 4-oxo-N-phenyl-4H-chromene-3-carboxamide **56** as a pale-yellow solid.



52 **56**

Reagents and conditions: (i) $SOCl_2$, Et_3N, DCM, aniline, 0-25 °C, 12 h

Scheme 22: Synthesis of 4-oxo-N-phenyl-4H-chromene-3-carboxamide analogue **56**

The synthesized 4-oxo-N-phenyl-4H-chromene-3-carboxamide analogue **56** was fully characterised by spectroscopic techniques (^1H, ^{13}C and IR). The ^1H NMR spectrum of 4-oxo-N-

phenyl-4H-chromene-3-carboxamide **56** (Figure 15) clearly revealed a total of ten protons. The characteristic methine proton (2-H) was observed at 9.08 ppm, a doublet of doublet at 8.33 ppm corresponding to proton 5-H and the protons on the phenyl substituent were observed from 7.12-7.39 ppm.

The ^{13}C NMR spectrum of 4-oxo-N-phenyl-4H-chromene-3-carboxamide **56** revealed (Figure 16) 14 signals where two of the 14 signals accounts for two carbons. The carbonyls C-4 and C-1' resonate at 177.44 ppm and 160.71 ppm respectively. The combination of ^{13}C and DEPT 135 data reveals a total of six quaternary carbons which correspond to the synthesised 4-oxo-N-phenyl-4H-chromene-3-carboxamide **56**.

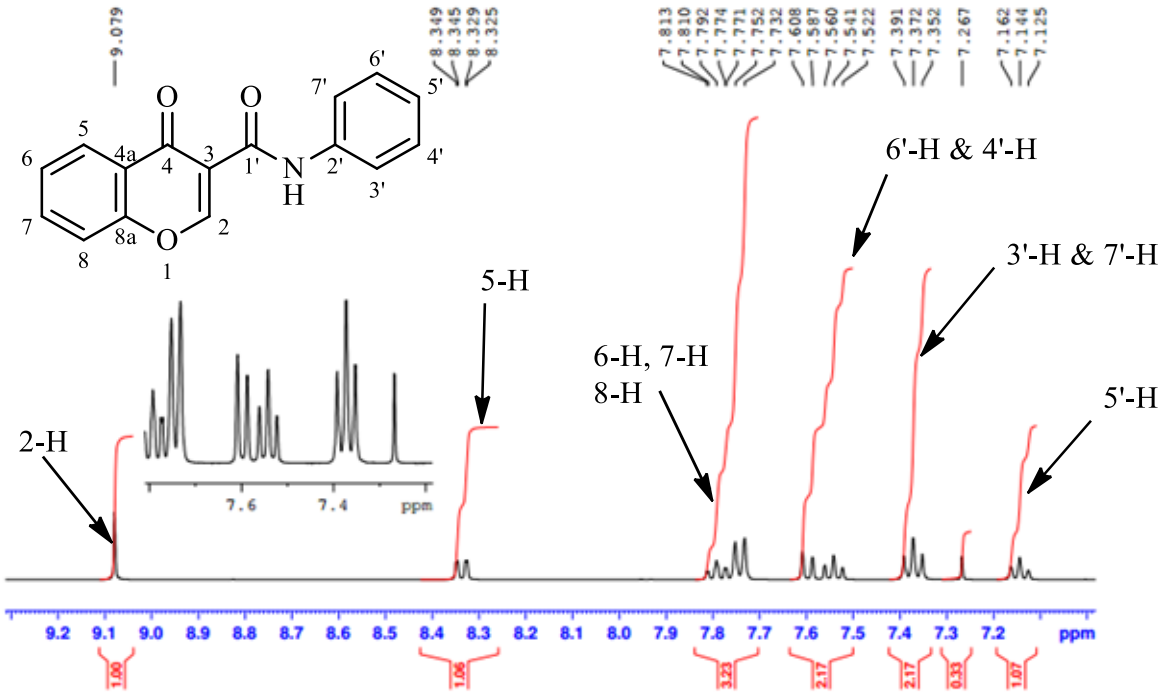


Figure 15: 400 MHz ^{1}H NMR spectrum of 4-oxo-N-phenyl-4H-chromene-3-carboxamide **56** in $CDCl_3$

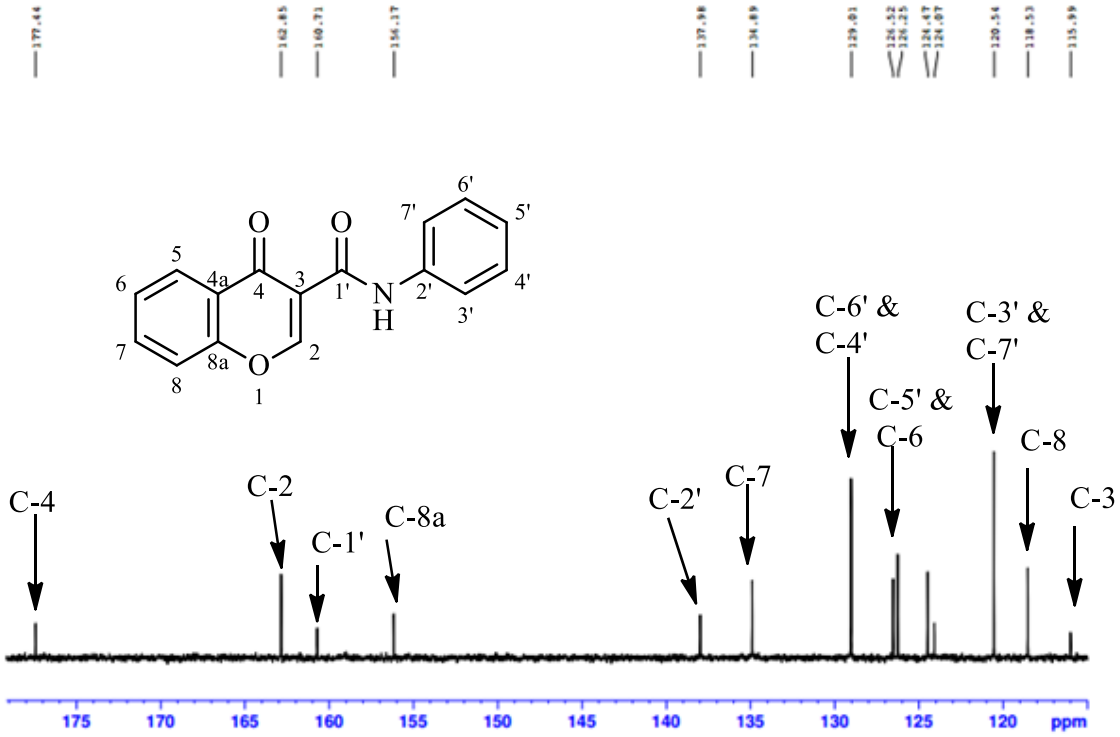
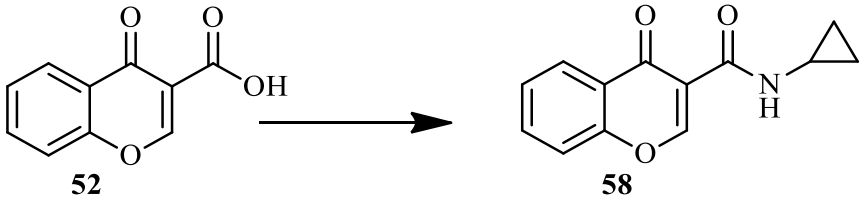


Figure 16: 100 MHz ^{13}C NMR spectrum of 4-oxo-N-phenyl-4H-chromene-3-carboxamide **56** in $CDCl_3$

4.5.5 Synthesis of N-cyclopropyl-4-oxo-4H-chromene-3-carboxamide 58

The general method for the synthesis of chromone-3-carboxamides was followed for the preparation of N-cyclopropyl-4-oxo-4H-chromene-3-carboxamide **58** using cyclopropylamine as the appropriate amine (Scheme 23). The crude solid obtained after work-up was indicated on TLC, which showed the presence of two products. A side reaction may have occurred in the synthesis of N-cyclopropyl-4-oxo-4H-chromene-3-carboxamide **58**. Due to the time constraint, the isolation of compound **58** using column chromatography was not performed. NMR spectra of the crude material and IR spectra revealed compound **58** was synthesized.

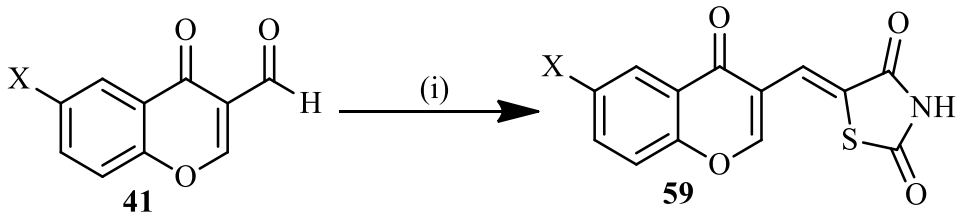


Reagents and conditions: (i) $SOCl_2$, Et_3N, DCM, cyclopropylamine, 0-25 °C, 12 h

Scheme 23: Synthesis of N-cyclopropyl-4-oxo-4H-chromene-3-carboxamide analogues **58**

4.6 Synthesis of 5-((4-oxo-4H-chromen-3-yl)methylene)thiazolidine-2,4-dione analogues 59A-F

The synthesis of 5-((4-oxo-4H-chromen-3-yl)methylene)thiazolidine-2,4-dione **59** was based on available literature methods for related compounds. In our first attempt (Scheme 24),[94] a solution of the corresponding 4-oxo-4H-chromene-3-carbaldehyde **41**, thiazolidine-2,4-dione and sodium acetate in acetic acid was subjected to a Knoevenagel condensation reaction by refluxing the solution for 2 h. The solid obtained after cooling was filtered, washed extensively with water to remove acetic acid and crystallized from EtOH to give yellow crystals. Compounds **59A**, **59E** and **59F** were successfully synthesized, evidenced by their NMR spectra. TLC and NMR data revealed that the precursors 6-fluoro-4-oxo-4H-chromene-3-carbaldehyde **41B** and 6-chloro-4-oxo-4H-chromene-3-carbaldehyde **41C** were not transformed into the product. Several adjustments to the reaction method were attempted to transform compounds **41B** and **41C** to the desired products but to our disappointment they were unsuccessful when.



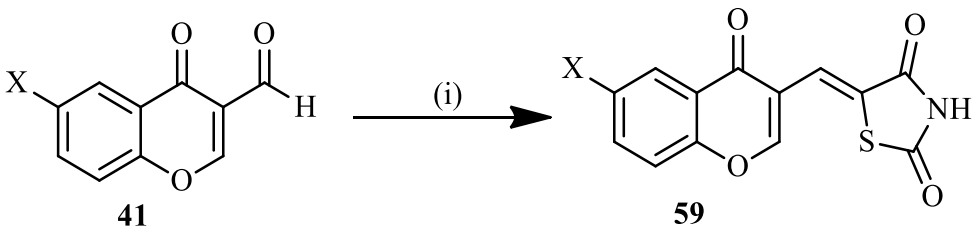
X	A	B	C	D	E	F
	H	F	Cl	Br	OMe	Me

Reagents and conditions: (i) AcOH, NaOAc, thiazolidine-2,4-dione, 100-110 °C, reflux, 2 h

Scheme 24: Synthesis of 5-((4-oxo-4H-chromen-3-yl)methylene)thiazolidine-2,4-dione analogues **59A-F** using acetic acid.

An alternative approach was investigated[95] (Scheme 25) to transform **41B** and **41C** to the corresponding 5-((4-oxo-4H-chromen-3-yl)methylene)thiazolidine-2,4-dione **59B and 59C** respectively. At this point compounds **41B** and **41C** were subjected to Knoevenagel reaction by introducing thiazolidine-2,4-dione, a catalytic amount of piperidine in ethanol to the reaction vessel and then refluxing the mixture for 16 h. TLC analysis showed the presence of spots corresponding to the product, and the starting material was completely transformed into products. The solid formed after cooling was filtered, washed with water and crystallized from acetone to

corresponding 5-((4-oxo-4H-chromen-3-yl)methylene)thiazolidine-2,4-dione **59** as white crystals with average percentage yield of 70.42 %.



X	A	B	C
	H	F	Cl

Reagents and conditions: EtOH, piperidine, thiazolidine-2,4-dione, 80-100 °C, reflux, 16 h

Scheme 25: Synthesis of 5-((4-oxo-4H-chromen-3-yl)methylene)thiazolidine-2,4-dione analogues **59** using EtOH

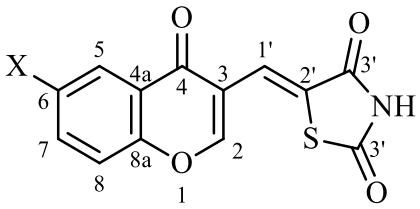


Table 10: Synthesized 5-((4-oxo-4H-chromen-3-yl)methylene)thiazolidine-2,4-dione analogues **(59A-F**)

Compound **59**	X	% Yield*	Melting point (°C)	Lit. Melting (°C)
A	H	68.8	320-322	319[94] 271[5]
B	F	68.7	304-309	-
C	Cl	72.15	310-316	-
D	Br	73.8	300-302	213-14[5]
E	OMe	86.39	290-293	204-205[5]
F	Me	64.3	255-260	185-187[5]

** isolated yields after recrystallization*

The solubility of all synthesized 5-((4-oxo-4H-chromen-3-yl)methylene)thiazolidine-2,4-dione analogues (**59A-F**) in deuterated solvents was very poor, this made it difficult to conduct the NMR analysis. Compounds **59B** and **59D** had the poorest solubility in NMR solvents, as such **59B** and **59D** were characterized by data obtained from elemental analysis and TLC.

Several techniques were used namely 1D NMR (^{1}H, ^{13}C, DEPT), elemental analysis, IR spectroscopic methods and correlation with the NMR spectra reported in the literature in the determination of structures of the synthesized compounds. The obtained melting points for the synthesized 5-((4-oxo-4H-chromen-3-yl)methylene)thiazolidine-2,4-dione analogues **59** were not consistent with literature data, with the exception of the parent compound **59A** synthesized by Ibrahim and co-workers.[94] Ibrahim and co-workers reported an error in the reported[5] melting point of **59A** to be 319 °C instead of 271 °C. Herein we also report a deviation in the reported literature data in Table 9 versus the experimental data (Table 10).

The ^{1}H NMR spectra of 5-((4-oxo-4H-chromen-3-yl)methylene)thiazolidine-2,4-dione analogues showed the desired product, with the distinct singlet peak at 7.54 ppm corresponding to the methine proton (1'-H) and the other methine proton (2-H) was observed at 8.77 ppm. The absence of the aldehyde signal further downfield revealed that the condensation reaction of 4-oxo-4H-chromene-3-carbaldehyde **41** had successfully occurred to give **59**. The aromatic protons were observed from 7.50-8.08 ppm. The ^{1}H NMR spectrum of compound **59E** is illustrated in figure 17.

The ^{13}C NMR spectra revealed 13 carbons for compounds **59A** and **59C**, compound **59E and 59F** revealed a total of 14 on the account of the methoxy and methyl groups on their respective structures. Combination of DEPT 135 and ^{13}C NMR spectra were used to assign the carbon atoms. The two-quaternary carbons resonating around 169 and 167 ppm were assigned to the carbonyl carbons on the thiazolidine-2,4-dione framework.

The carbonyl carbon (C-4) was observed at 174.79 ppm (Figure 18). The calculated elemental analysis data for compound **59E** was consistent with the experimental data.

DEPT 135 spectrum of **59E** (Figure 19) reveals a total of 6 carbons including two methine carbons (C-2 and C-1') and one methyl carbon at 56.27 ppm corresponding to the methoxy carbon.

The FT-IR spectra revealed characteristic bands of NH around 3250 cm^{-1}, C=O stretching around 1743 cm^{-1} corresponding to carbonyl carbon (C-4) and two other carbonyl bands 1669 and 1644

cm^{-1} corresponding to the carbonyl carbons on the thiazolidine-2,4-dione framework (C-3') and (C-4').

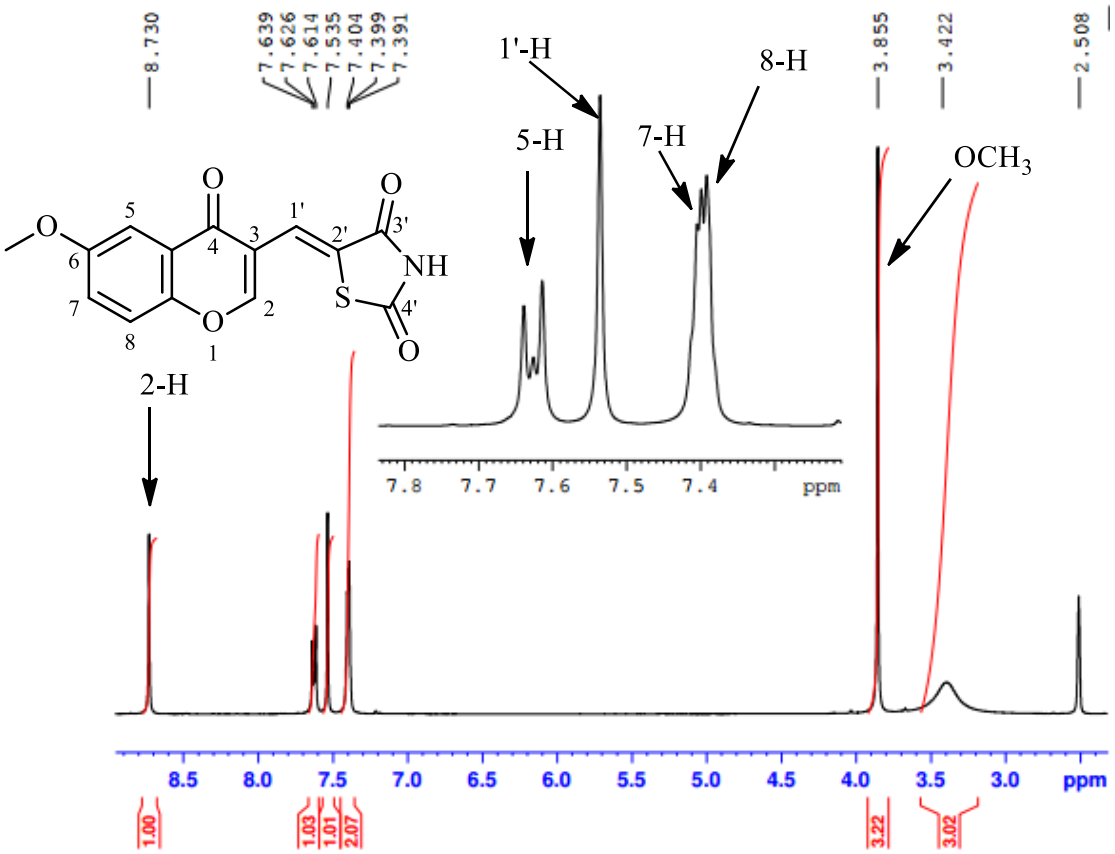


Figure 17: 400 MHz ^{1}H NMR spectrum of 5-((6-methoxy-4-oxo-4H-chromen-3-yl)methylene)thiazolidine-2,4-dione **59E** in DMSO-d_6

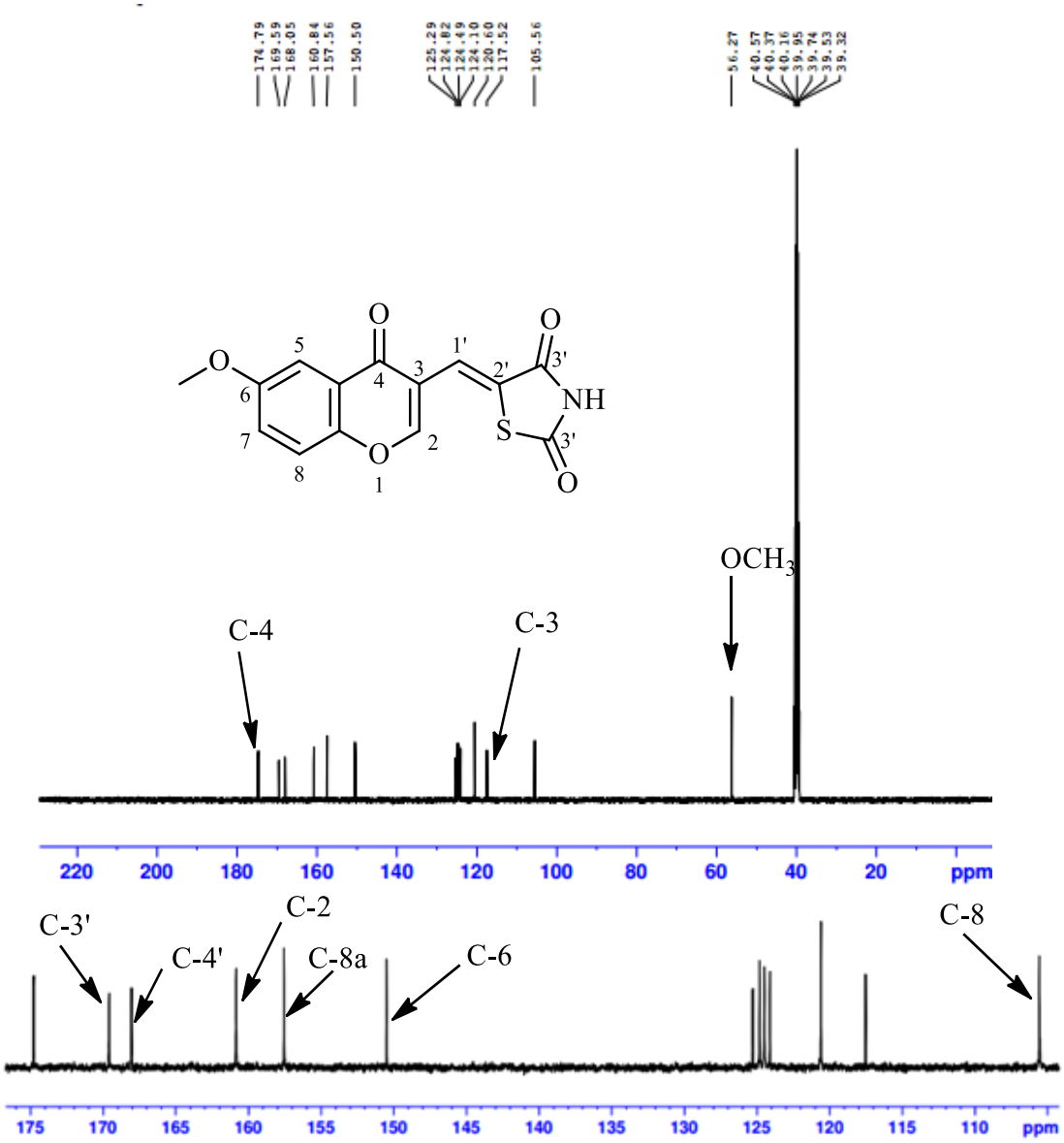


Figure 18: 100 MHz ^{1}H NMR spectrum of 5-((6-methoxy-4-oxo-4H-chromen-3-yl)methylene)thiazolidine-2,4-dione **59E** in DMSO-d_6

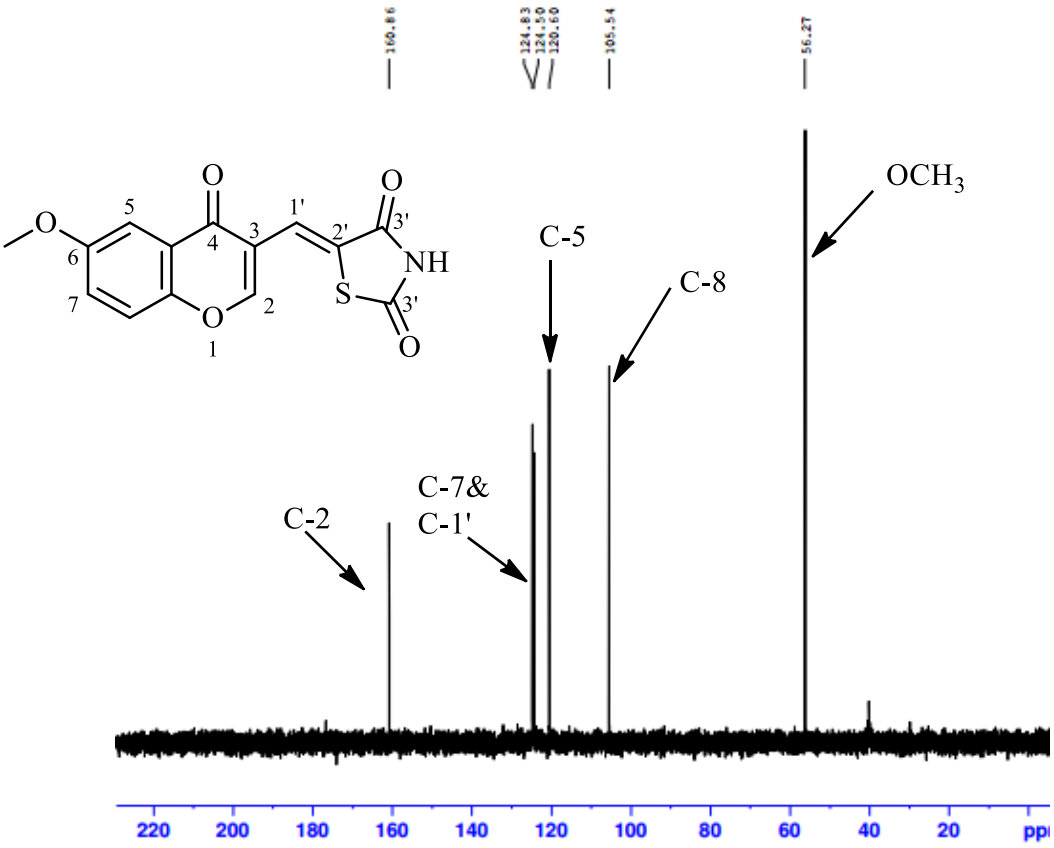
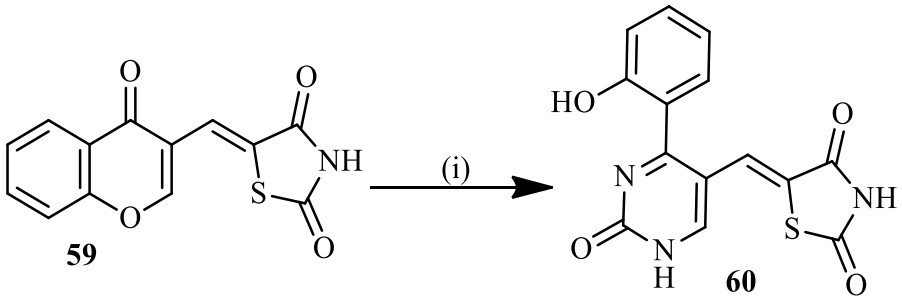


Figure 19: DEPT 135 spectrum of 5-((6-methoxy-4-oxo-4H-chromen-3-yl)methylene)thiazolidine-2,4-dione **59E** in DMSO-d_6

4.7 Synthesis of 5-((4-(2-hydroxyphenyl)-2-oxo-1,2-dihydropyrimidin-5-yl)methylene)thiazolidine-2,4-dione

The synthesis of the target compound, 5-((4-(2-hydroxyphenyl)-2-oxo-1,2-dihydropyrimidin-5-yl)methylene)thiazolidine-2,4-dione **60** was done in order to evaluate its antimalarial properties. The synthetic strategy (Scheme 26),[94] conducted by condensing 5-((4-oxo-4H-chromen-3-yl)methylene)thiazolidine-2,4-dione **59** and urea in a 5 % KOH ethanolic solution . The mixture was stirred under reflux for 6 h, quenched with water, and neutralized with dilute HCl. The formed solid was filtered, washed with water and crystallized from EtOH to give 5-((4-(2-hydroxyphenyl)-2-oxo-1,2-dihydropyrimidin-5-yl)methylene)thiazolidine-2,4-dione **60** with (54 %) yield as yellowish solid. The product yield was 54 %.

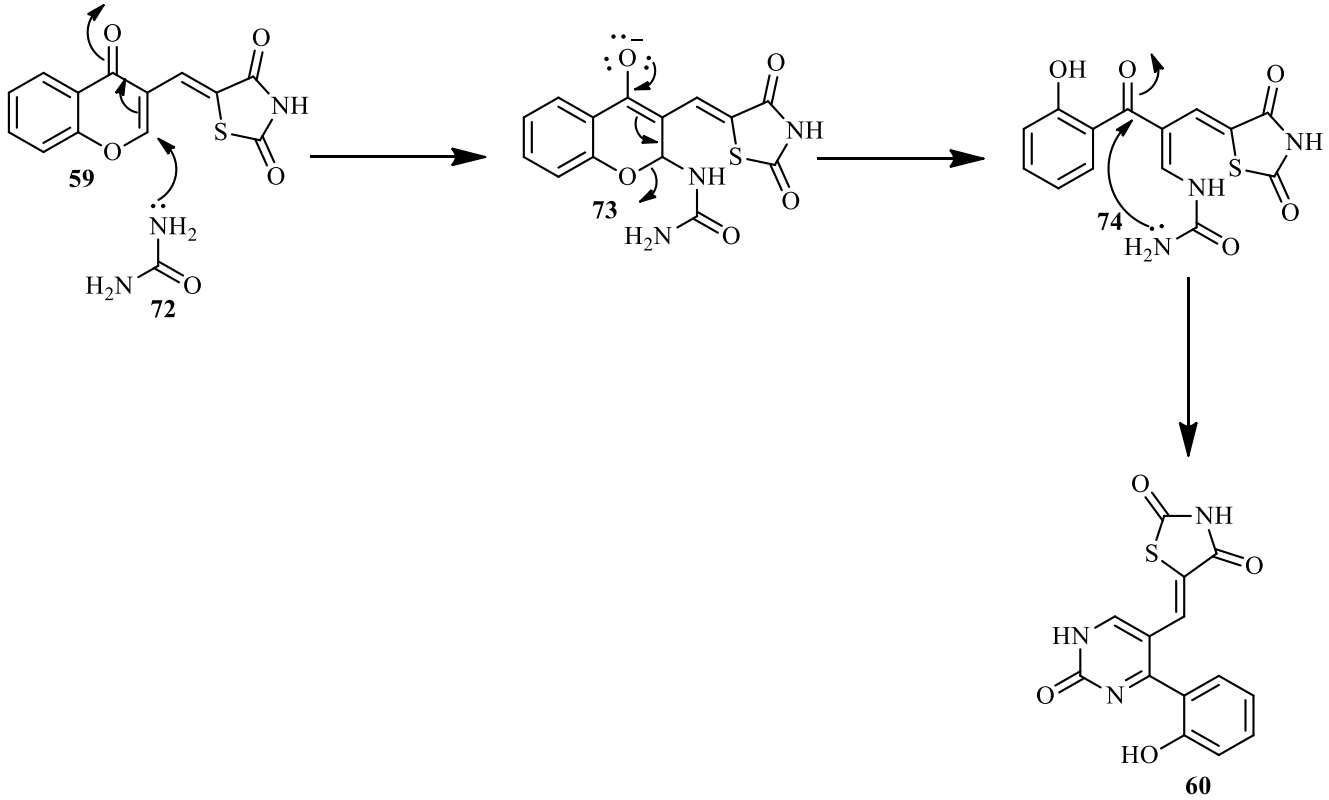


Reagents and conditions: (i) Urea, KOH and EtOH

Scheme 26: Synthesis of 5-((4-(2-hydroxyphenyl)-2-oxo-1,2-dihydropyrimidin-5-yl)methylene)thiazolidine-2,4-dione **60**

Reaction mechanism (Michael addition) for the synthesis of 5-((4-(2-hydroxyphenyl)-2-oxo-1,2-dihydropyrimidin-5-yl)methylene)thiazolidine-2,4-dione 60.

First, a lone pair of electrons on the amine group of urea **72** attacks the electron deficient carbon (C-2) of compound **59**, giving the reactive enolate **73**. The enolate **73** rearranges resulting in the pyrone ring opening to afford **74**. Compound **60** is obtained when the other amine group reacts with the carbonyl carbon to give the pyrimidine derivative **60** (Scheme 27).[94]



Scheme 27: Reaction mechanism for the synthesis of compound **60**

^{1}H NMR revealed two singlets, at 8.74 and 7.53 ppm corresponding to the pyrimidine proton (9-H) and methine proton (11-H) respectively. A doublet corresponding to the proton 3-H was observed at 8.03 ppm and the other aromatic protons were observed from 7.47-7.82 ppm (Figure 20).

The FT-IR reveals absorption bands at 3143.18 (NH), 3049.16 (OH), 1732.72 (C=O) on the thiazolidinedione and a C=O stretch at 1677.20 cm^{-1} corresponding to the pyrimidine carbonyl.

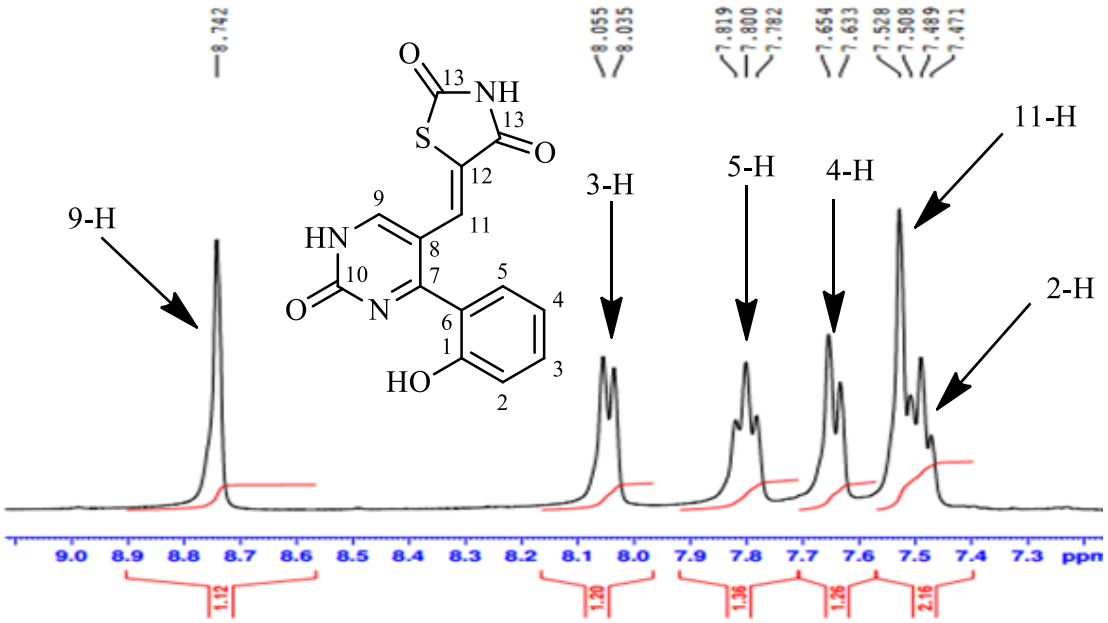
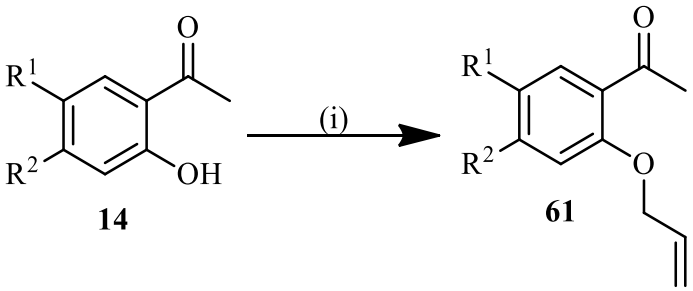


Figure 20: 400 MHz ^{1}H NMR spectrum of 5-((4-(2-hydroxyphenyl)-2-oxo-1,2-dihydropyrimidin-5-yl)methylene)thiazolidine-2,4-dione **60**

4.8 Synthesis of 1-(2-(allyloxy)phenyl)ethanone analogues 61A-E

Allyl bromide, anhydrous K_2CO_3 and the corresponding 2-hydroxyacetophenones **14** were dissolved in dry acetone (Scheme 28).[39] The solution was refluxed for 20 h. The resultant mixture was cooled to room temperature, excess K_2CO_3 filtered off and then the filtrate was concentrated under reduced pressure to remove acetone, taken up in water and finally extracted with EtOAc. The combined organic layers were dried over anhydrous $MgSO_4$ and evaporated under reduced pressure to afford a yellow oil product which was taken up in Et_2O and washed with 1M KOH solution. The resultant organic layer was washed with water, dried over anhydrous $MgSO_4$ and

concentrated under reduced pressure to afford corresponding 1-(2-(allyloxy)phenyl)ethanone analogues **61** as a colourless oil with an average percentage yield of 65.20 % (Table 11).



14 **61**

Compound **61**	R^1	R^2
A	H	H
B	Cl	H
C	Br	H
D	OMe	H
E	H	OMe

Reagents and conditions: (i) K_2CO_3, allyl bromide, acetone, 100-110 °C, 20 h

Scheme 28: Synthesis of 1-(2-(allyloxy)phenyl)ethanone analogues **61A-E**

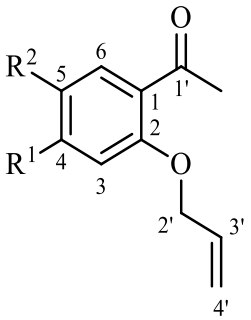


Table 11: 1-(2-(allyloxy)phenyl)ethanone analogues **61A-E**

Compound **61**	R^1	R^2	% Yield*
A	H	H	48
B	Cl	H	64.6
C	Br	H	63.7
D	OMe	H	79.72
E	H	OMe	70

** isolated yields after distillation*

The synthesized compounds were characterized by 1D NMR (^{1}H, ^{13}C and DEPT), IR spectroscopic technique.

In the ^{1}H NMR spectra, the allyl substituent was observed in the aliphatic region as three signals. The first signal observed around 4.55-4.62 ppm with a multiplicity of a doublet corresponding to the protons 2'-H, the second signal a doublet of doublet resonating at 5.24-5.37 ppm integrating for two protons revealed a geminal coupling corresponding to the protons 4'-H. The third signal (3'-H) around 5.92 ppm shows as a multiplet due to the coupling of protons 2'-H and 4'-H.

The ^{13}C NMR and DEPT 135 shows two methylene carbons at 69.00 and 118.12 ppm corresponding to the allylic carbons (C-2') and (C-4') respectively were observed. The methyl carbon was observed at 31 ppm. The methoxy carbon of **61D** was observed around 55.63 ppm. The chemical shift of the carbons in the 1-(2-(allyloxy)phenyl)ethanone analogues are represented in table 12 and the most notable differences in the chemical shifts were observed in compound **61C** and **61D** the compounds with higher electronegative substituents.

Figure 21 shows the ^{1}H NMR spectrum of 1-(2-(allyloxy)-5-chlorophenyl)ethanone **61B** revealed a quartet at 4.55 ppm integrated for two protons corresponding to the protons on carbon 2'. The protons on carbon 3' (3'-H) were observed around 5.35 ppm. The aromatic protons were observed from 6.80-7.61 ppm. The same pattern was observed in all the analogues **61A-E**.

The ^{1}H NMR spectra of the synthesized **61** revealed no presence of the OH signal downfield of the aromatic protons, which shows that the desired compound **61** was successfully synthesized.

DEPT 135 was used to assign the methylene carbons and the methine carbons. The ^{13}C NMR of 1-(2-(allyloxy)-5-chlorophenyl)ethanone (Figure 22) revealed a total of 11 carbons and each carbon atom duly assigned.

IR spectra revealed a band consistent with the carbonyl stretch around 1663 cm^{-1} and the aromatic alkoxy stretch around 1214 cm^{-1}. Furthermore, the IR spectra show the absence of the OH band, which further substantiate the corresponding 1-(2-(allyloxy)phenyl)ethanone analogues **61** were successfully synthesized.

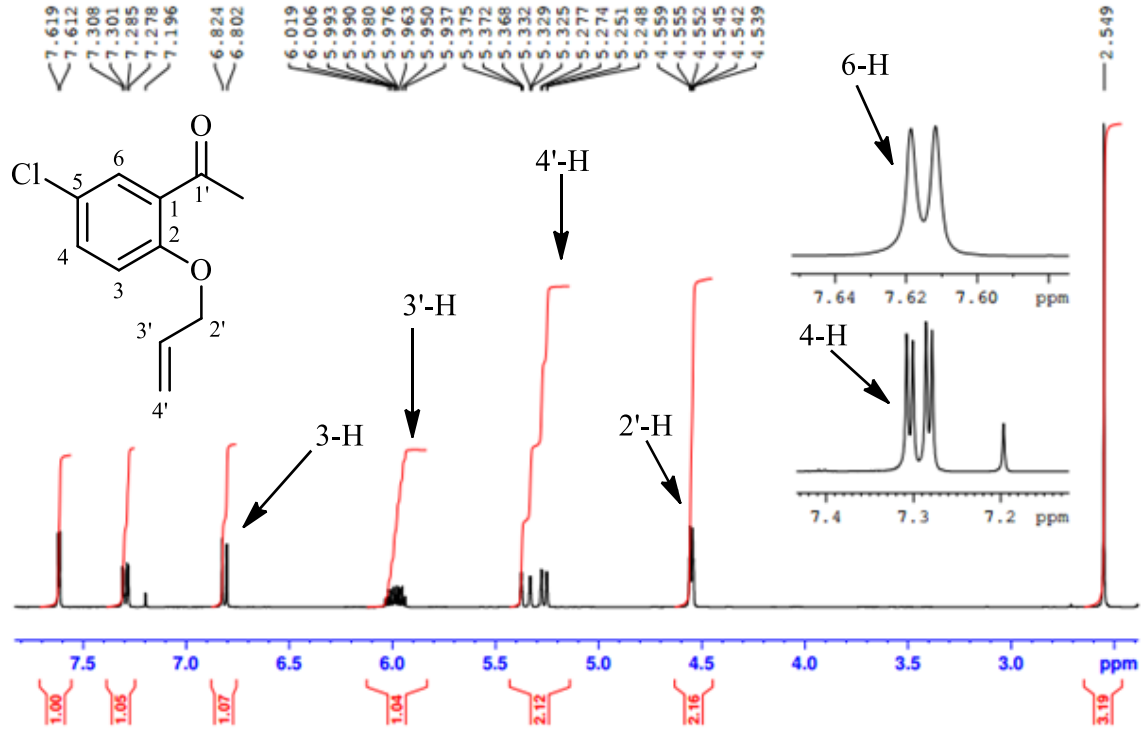


Figure 21: 400 MHz ^{1}H NMR spectrum of 1-(2-(allyloxy)-5-chlorophenyl)ethanone **61B** in $CDCl_3$

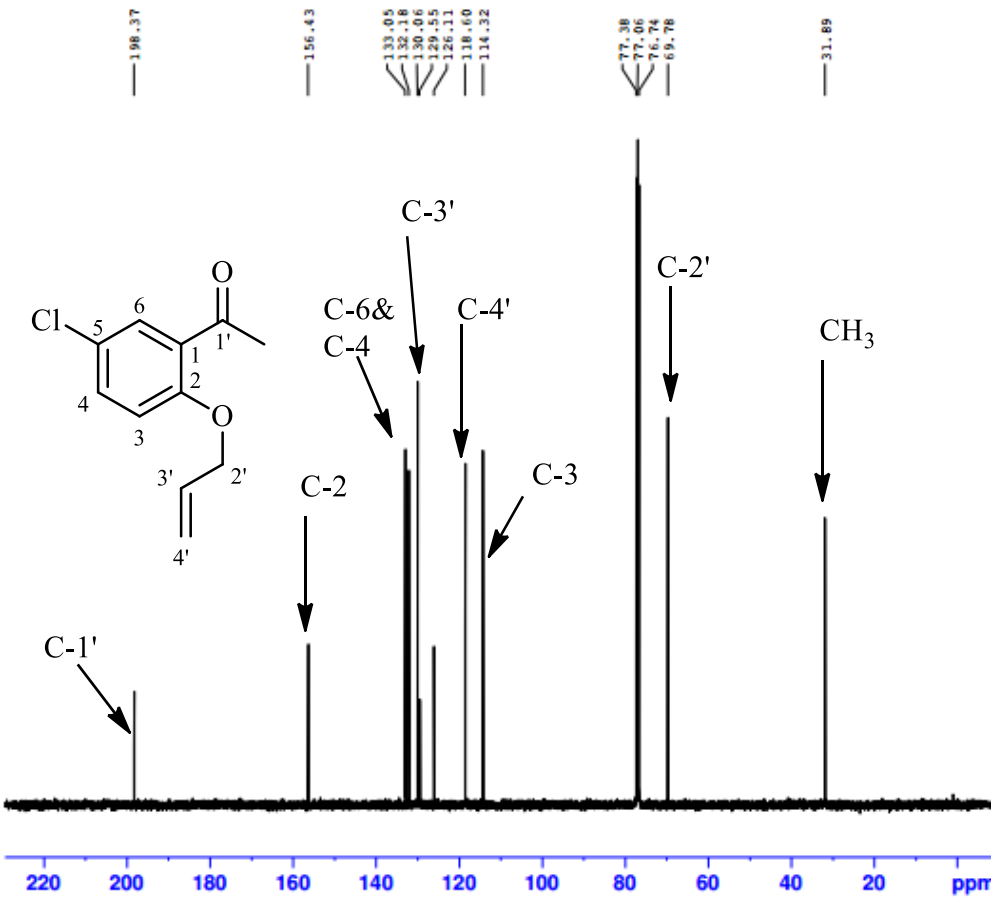


Figure 22: 100 MHz ^{13}C NMR spectrum of 1-(2-(allyloxy)-5-chlorophenyl)ethanone **61B** in $CDCl_3$

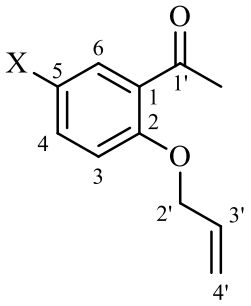


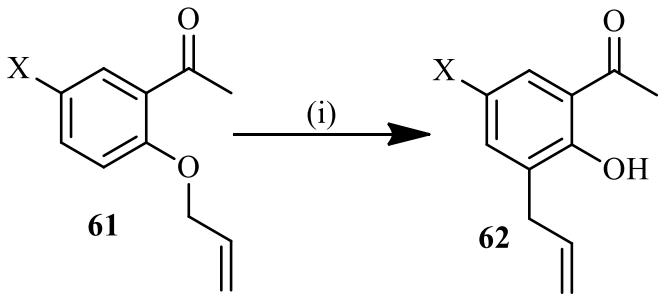
Table 12: 100 MHz ^{13}C NMR chemical shift values (ppm) of compounds **61A-D** in $CDCl_3$

Nucleus	**61A** **X=H**	**61B** **X=Cl**	**61C** **X=Br**	**61D** **X=OMe**
C1	128.35	129.55	129.90	128.67
C2	157.83	156.43	156.91	152.40
C3	112.74	114.31	114.75	113.69
C4	133.49	133.07	135.98	120.14
C5	120.56	126.11	113.25	153.45
C6	130.20	130.07	132.11	114.54
C1’	199.28	198.37	198.24	199.20
C2’	69.18	69.77	69.70	69.99
C3’	132.59	132.17	132.94	132.90
C4’	117.92	118.62	118.62	117.91
OCH_3	-	-	-	55.63
CH_3	31.84	31.89	31.88	31.97

NB: Chemical shift in $CDCl_3$ solvent

4.9 Synthesis of 1-(3-allyl-2-hydroxyphenyl)ethanone analogues 62A-D

1-(2-(allyloxy)phenyl)ethanone **61** was dissolved in N,N-diethylaniline (NND) and heated under reflux for 3 h.[96] The mixture was poured over ice water and concentrated HCl was poured over to the solution and extracted with Et_2O, dried over $MgSO_4$. The resultant mixture was concentrated under reduced pressure to afford 1-(3-allyl-2-hydroxyphenyl)ethanone **62** (Scheme 29). TLC and NMR data were used to determine the purity of the synthesized compounds.

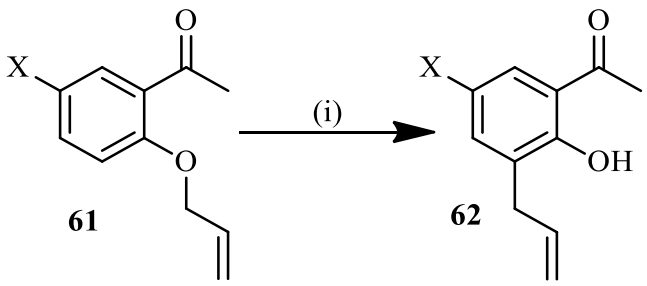


X	A	B
	H	Cl

Reagents and conditions: NND, 190-200 °C, 3 h

Scheme 29: Synthesis of 1-(3-allyl-2-hydroxyphenyl)ethanone analogues **62A-D** using NND

Upon extensive, literature search, we came across an alternative method (Scheme 30) for the synthesis of 1-(3-allyl-2-hydroxyphenyl)ethanone analogues **62** which made shorter reaction times and solvent-free conditions.[97] The methodology was applied in our study by heating all corresponding 1-(2-(allyloxy)phenyl)ethanones **61** under a nitrogen atmosphere at 260-270 °C for 0.5 h. The resultant mixture cooled to room temperature before work-up using Et_2O. This method gave higher yields 61-92 % (Table 13). Compound **62E** was lost during purification by distillation.



X	A	B	C	D
	H	Cl	Br	OMe

Reagents and conditions: Heat, 260-270 °C, 0.5 h

Scheme 30: Synthesis of 1-(3-allyl-2-hydroxyphenyl)ethanone analogues **62A-D** (solvent-free method)

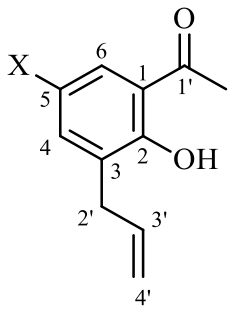


Table 13: Synthesized of 1-(3-allyl-2-hydroxyphenyl)ethanone analogues **62A-D**

Compound **62**	X	% Yield*
A	H	61
B	Cl	92
C	Br	90
D	OMe	85

** isolated yields after distillation*

The low yield recorded for compound **62A** may have been as a result of some material being lost during the work-up step.

1-(3-allyl-2-hydroxyphenyl)ethanone analogues **62** were characterized in ^{1}H NMR spectra by the presence of a signal further downfield corresponding to the OH proton which was observed as a singlet, this was evidence that the Claisen rearrangement reaction was successful. A multiplet at 5.93 ppm corresponding to the methine proton 3-H was observed which was similar to the synthesized compound precursor. A close look at the ^{1}H NMR spectra showed a reduction in the number of aromatic protons, thus the allyl group successfully transferred to the benzene ring thereby replacing one proton. The NMR spectrum of **62D** is illustrated in figure 23.

In ^{13}C NMR spectra, the occurrence of an additional quaternary carbon is evident that the allyl group migrated from the alkoxy group to carbon on position 3 (C-3). The ^{13}C NMR spectrum of **62D** is illustrated in figure 24.

The IR data revealed the presence of aromatic C-H stretch around 3000 cm^{-1} and the presence of the carbonyl carbon at 1640 cm^{-1}. Furthermore, IR revealed the absence of alkoxy stretch found in the corresponding precursor.

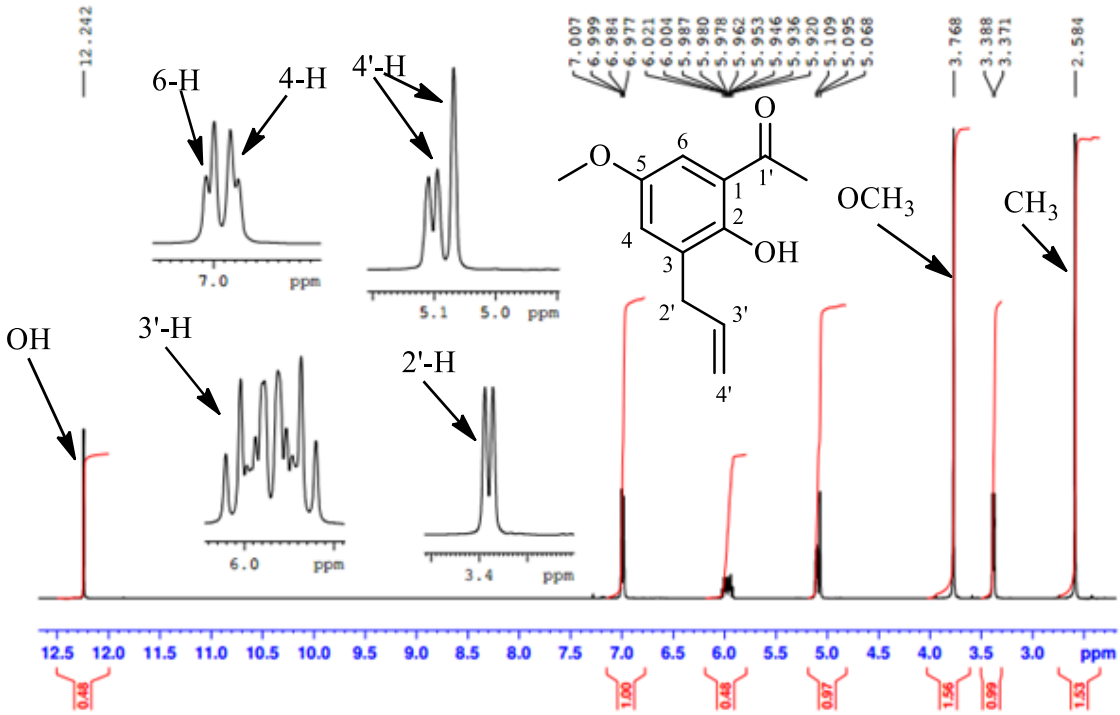


Figure 23: 400 MHz ^{1}H NMR spectrum of 1-(3-allyl-2-hydroxy-5-methoxyphenyl)ethanone **62D** in $CDCl_3$

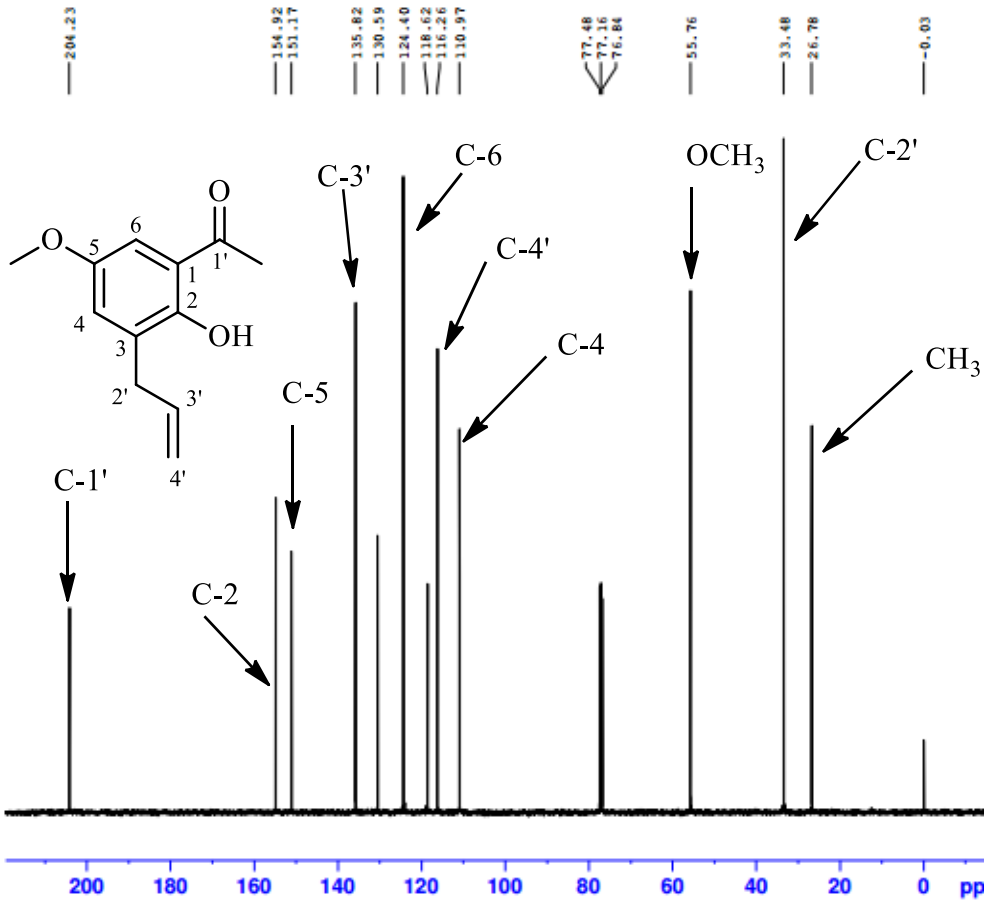
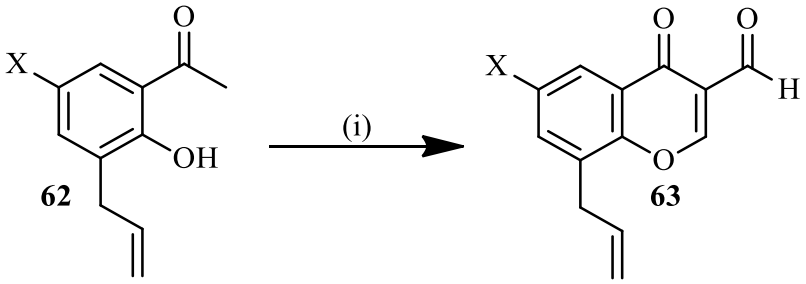


Figure 24: 100 MHz ^{1}H NMR spectrum of 1-(3-allyl-2-hydroxy-5-methoxyphenyl)ethanone **62D** in $CDCl_3$

4.10 Synthesis of 8-allyl-4-oxo-4H-chromene-3-carbaldehyde analogues 63A-D

The Vilsmeier-Haack reaction methodology was applied for the synthesis of 8-allyl-4-oxo-4H-chromene-3-carbaldehyde analogues **63** by adding a solution of corresponding 1-(3-allyl-2-hydroxyphenyl)ethanone analogues **62** in DMF to $POCl_3$ at 0 °C (Scheme 31). The solution was stirred overnight, thereafter TLC analysis indicated the formation of a product and hence the mixture was poured over crushed ice with stirring and allowed to stand for 0.5 h. The solid obtained was filtered, dried and crystallized from petroleum ether (60-80) to give the corresponding 8-allyl-4-oxo-4H-chromene-3-carbaldehyde **63** with an average yield of 36.9 % (Table 14).



X	A	B	C	D
	H	Cl	Br	OMe

Reagents and conditions: (i) $POCl_3$, DMF, H_2O, 0-25 °C, 12 h

Scheme 31: Synthesis of 8-allyl-4-oxo-4H-chromene-3-carbaldehyde analogues **63A-D**

Table 14: Synthesized 8-allyl-4-oxo-4H-chromene-3-carbaldehyde analogues **63A-D**

Compound **63**	X	% Yield*	Melting point (°C)	Lit. Melting (°C)
A	H	40.2	70-75	73-74[98]
B	Cl	37.5	90-94	-
C	Br	36.3	110-111	-
D	OMe	33.6	142-148	-

** isolated yields after recrystallization*

The ^1H and ^{13}C NMR, elemental analysis and IR data were used to characterize the synthesized compounds. The ^1H NMR spectra of chromone-3-carbaldehyde **41** and of the newly synthesized 8-allyl-4-oxo-4H-chromene-3-carbaldehyde analogue **63** were compared. The characteristic peaks for carbaldehyde compounds were observed, the only differences we noticed in compound **63** which was not in **41** is the allylic group. A singlet around 10.41 ppm corresponding to the proton on the CHO carbon and the methine proton (2-H) 8.56 ppm were observed. The aliphatic region showed the proton at 5.96 ppm as a multiplet as previously observed in the precursors.

In the ^1H NMR spectrum of 6-methoxy-8-allyl-4-oxo-4H-chromene-3-carbaldehyde **63D** a total of 12 protons were observed consistent with the intended number of protons. The methoxy protons were observed at 3.91 ppm (Figure 25)

The ^{13}C NMR spectrum of 6-methoxy-8-allyl4-oxo-4H-chromene-3-carbaldehyde **63D** show a peak at 176.10 ppm corresponding to the carbonyl carbon (C-4), the aldehyde carbon at 188.87 ppm (Figure 26). The methine (C-2) was observed at 159.84 ppm, carbon at position C-8 at 103.55 ppm and the methoxy carbon was observed at 55.95 ppm.

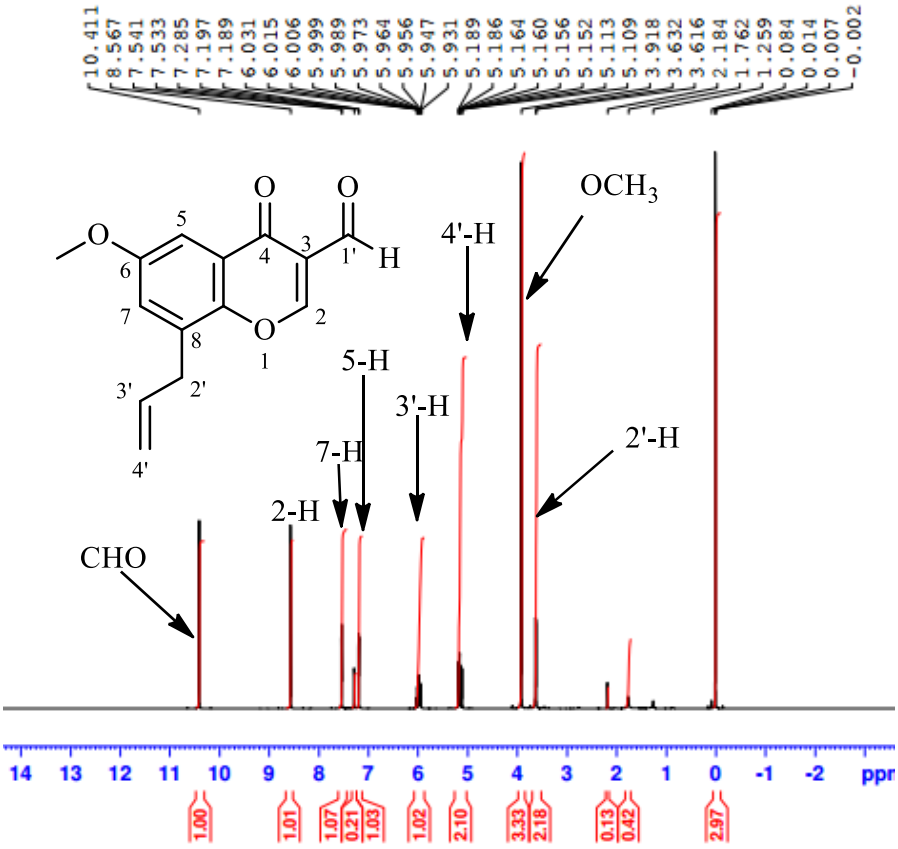


Figure 25: 400 MHz ^{1}HNMR spectrum of 6-methoxy-8-allyl4-oxo-4H-chromene-3-carbaldehyde **63D** in $CDCl_3$

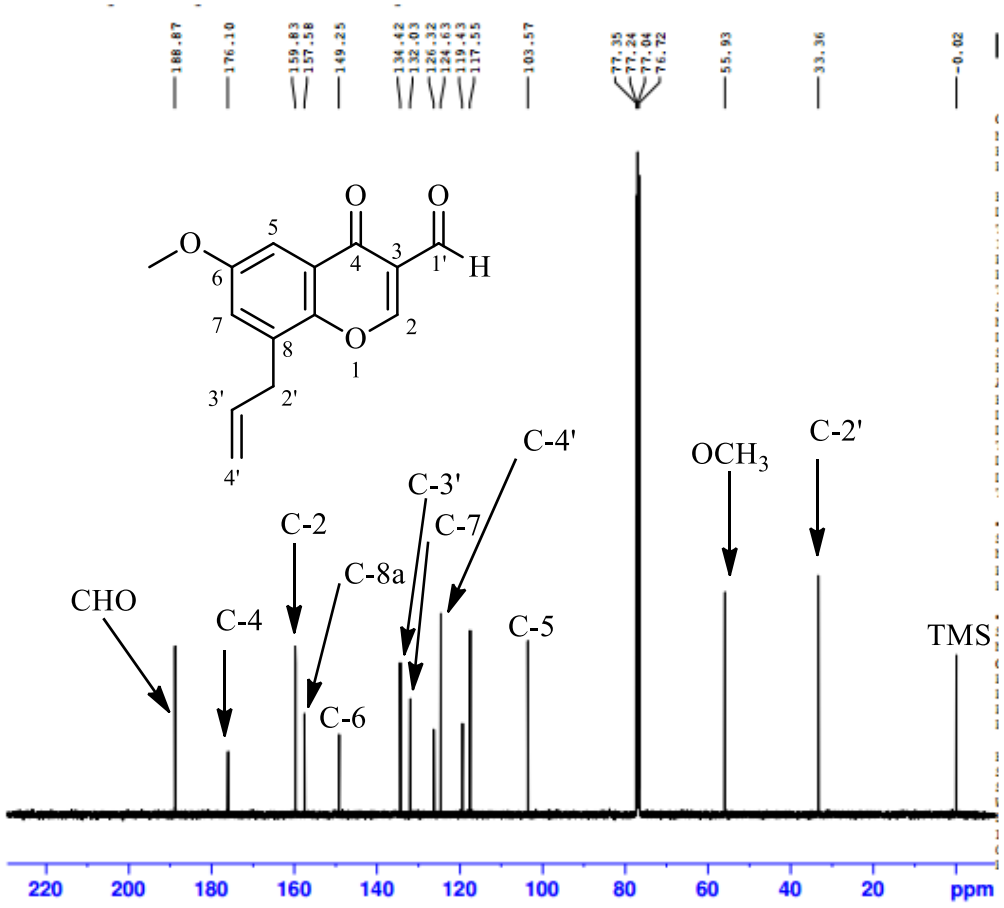
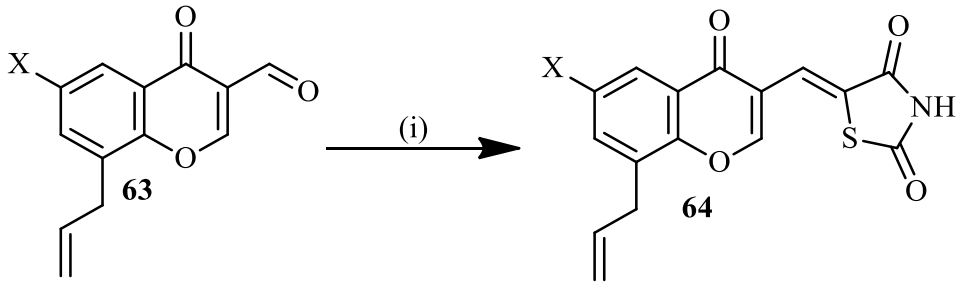


Figure 26: 100 MHz ^{13}C NMR spectrum of 6-methoxy-8-allyl4-oxo-4H-chromene-3-carbaldehyde **63D** in $CDCl_3$

4.11 Synthesis of 5-((8-allyl-4-oxo-4H-chromen-3-yl)methylene)thiazolidine-2,4-dione analogues 64A-D

Corresponding 8-allyl-4-oxo-4H-chromene-3-carbaldehydes **64**, thiazolidine-2,4-dione and sodium acetate were subjected to a Knoevenagel condensation reaction by refluxing the resulting solution for 2 h (Scheme 32). The progress of the reaction was monitored by TLC, which showed the formation of the product after 1 h with the exception of 6-chloro-8-allyl-4-oxo-4H-chromene-3-carbaldehyde **63B**. The solid material obtained in the successful reactions was recrystallized from acetone to afford corresponding target compounds (Table 15) with an average percentage yield of 60 %. In an attempt to afford **64B**, compound **63B** was refluxed for 4 h and the starting material **63B** was recovered on completion.



X	A	B	C	D
	H	Cl	Br	OMe

Reagents and conditions: (i) AcOH, NaOAc, thiazolidine-2,4-dione, 100-110 °C, reflux, 2 h

Scheme 32: Synthesis of 5-((8-allyl-4-oxo-4H-chromen-3-yl)methylene)thiazolidine-2,4-dione **64A-D** analogues

Table 15: Synthesized 5-((8-allyl-4-oxo-4H-chromen-3-yl)methylene)thiazolidine-2,4-dione **64A-D** analogues.

Compound **64**	X	% Yield*	Melting point (°C)	Lit. Melting (°C)
A	H	56.23	265-267	-
B	6-Cl	-	-	-
C	6-Br	60	290-292	-
D	6-OMe	64.3	154-157	-

** isolated yields after recrystallization*

The aromatic protons are as expected at 7.45-7.94 ppm as a multiplet integrated to three protons where one of the protons is the methine proton on carbon at position 1' (C-1'). The methine proton on carbon at position 2 (C-2) resonates as a singlet at 8.79 ppm. The methoxy protons appear at 3.39 ppm and the NH proton which was observed as a broad signal at 12.44 ppm. The proton at position C-9 is a doublet arising from the proton on position C-10. The ^{1}H NMR spectrum of compound **64D** is illustrated in figure 27 and ^{13}C NMR in figure 28.

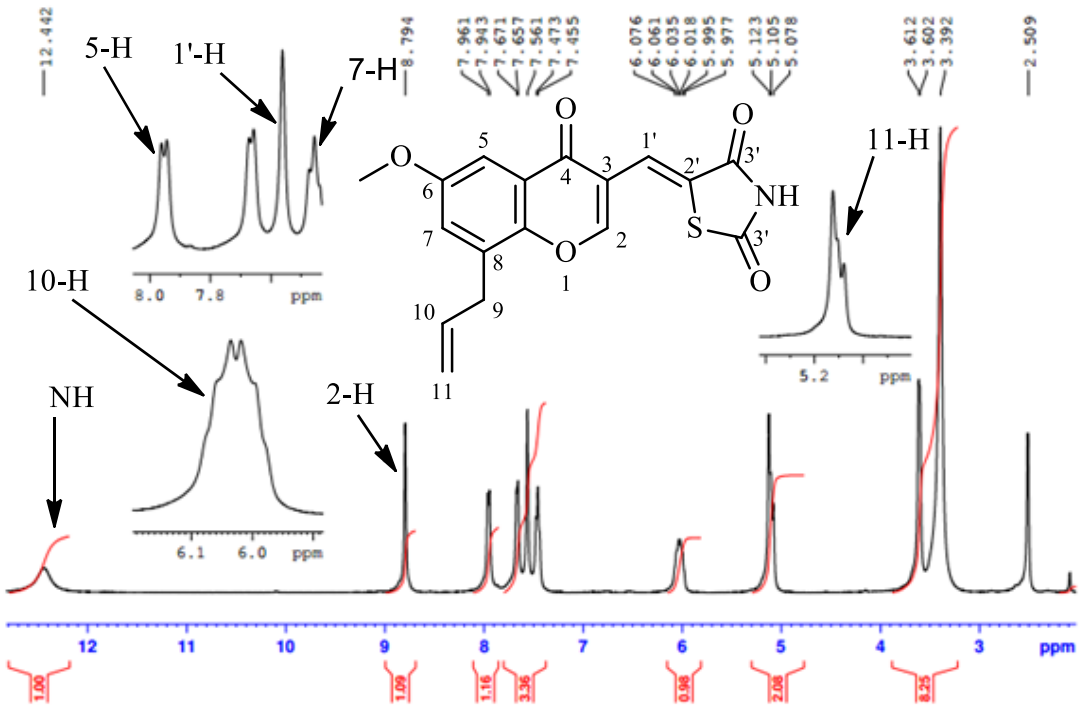


Figure 27: ^{1}H NMR of 5-((8-allyl-6-methoxy-4-oxo-4H-chromen-3-yl)methylene)thiazolidine-2,4-dione **64D** in DMSO-d_6

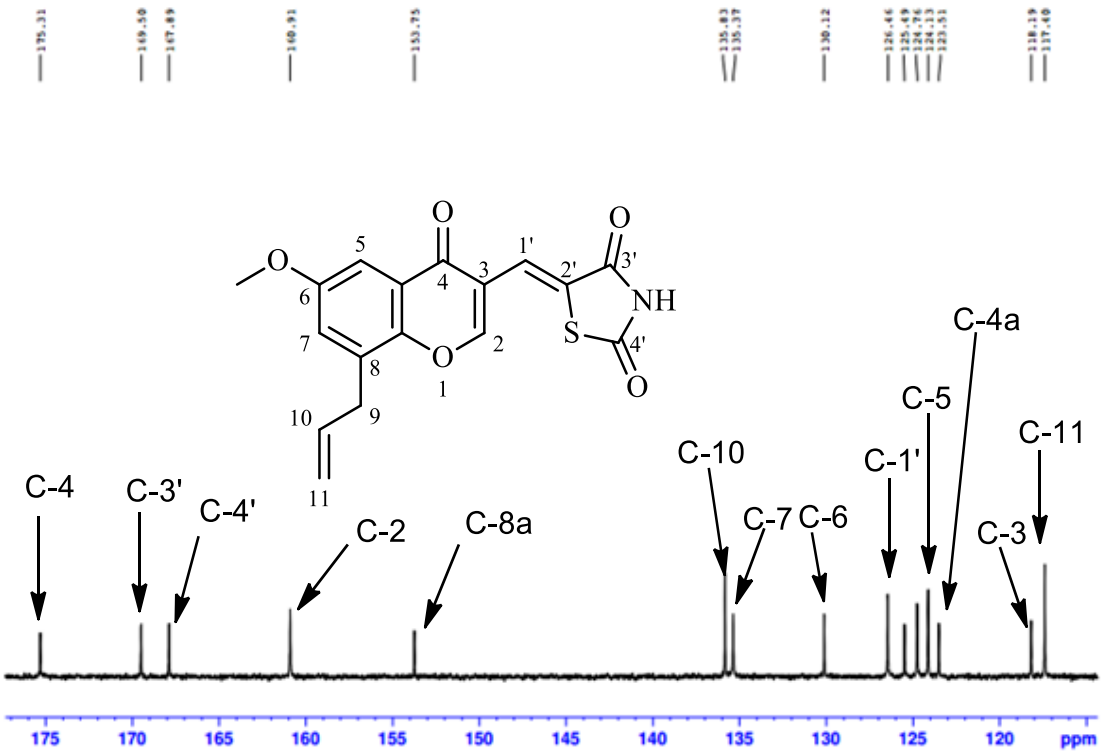


Figure 28: ^{13}C NMR spectrum of 5-((8-allyl-6-methoxy-4-oxo-4H-chromen-3-yl)methylene)thiazolidine-2,4-dione **64D** in DMSO-d_6

4.6 Biological Activities

4.6.1 Trypanosoma brucei Assay

Neglected tropical diseases (NTD) are a cause of mortality in various developing countries of tropical and subtropical regions, trypanosomiasis is amongst these class of diseases.[99] This situation is intensified by increasing treatment failures with available drugs.[100] *Trypanosomiasis* is an infection that causes a chronic immune response associated with severe neurological disorders believed to lead to coma and death. The need to alleviate this problem, is through a new class of compounds which are active against *Trypanosoma* and display minimum cytotoxicity.

Pentamidine (an existing drug treatment for trypanosomiasis) was used as a control drug standard. The concentration used was 20 µg/mL.

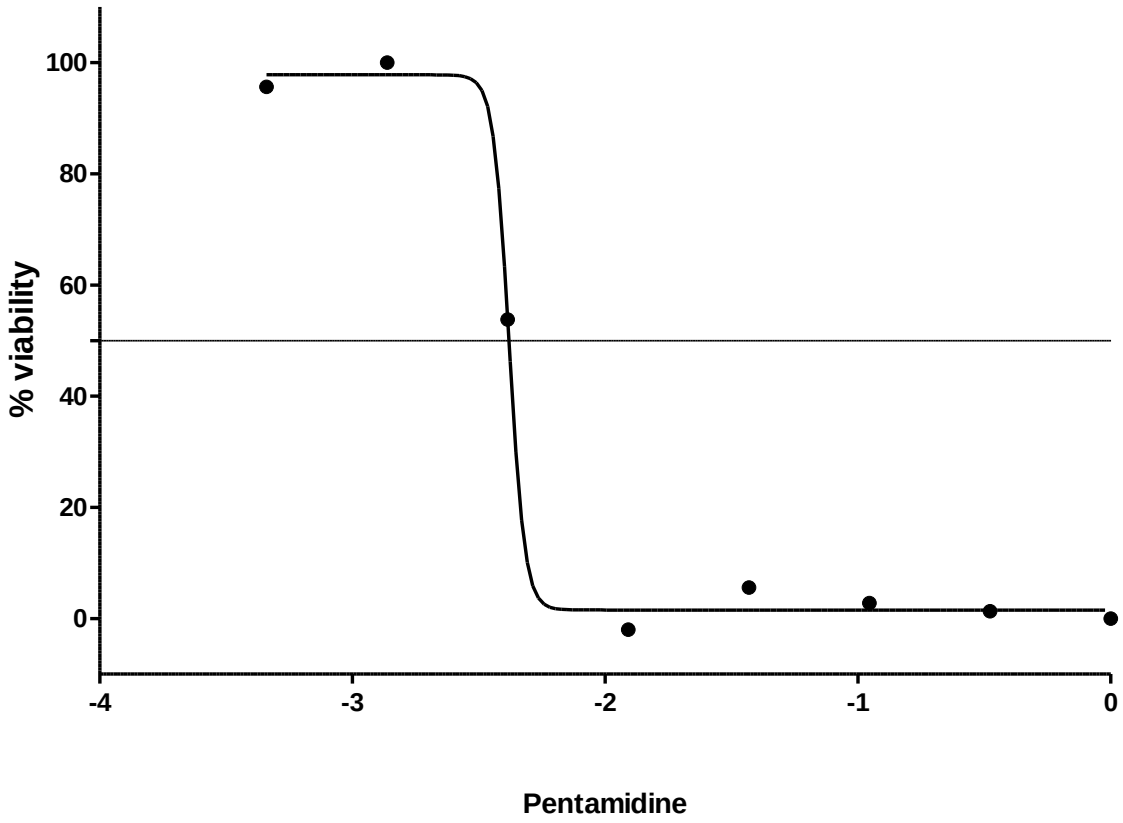


Figure 29: Pentamidine standard at 0.004 µM

A selection of 17 compounds containing the chromone functionality and one pyrimidine derivative were tested for antitryponosomal activity.

The results of the antitrypanosoma activities of selected compounds are presented in figure 30 Compounds **41A**, **55B** and **63D** displayed the highest activity against *Trypanosoma brucei* at 0.61, 0.15 and 0.21 % viability respectively. Compounds **41B** and **59A** displayed moderate antitrypanosomal activities at 28.99 and 39.98 % viability respectively which was, however, below that of the positive control. The compounds which displayed % viability above 40 % were not active against the parasite *Trypanosoma brucei*.

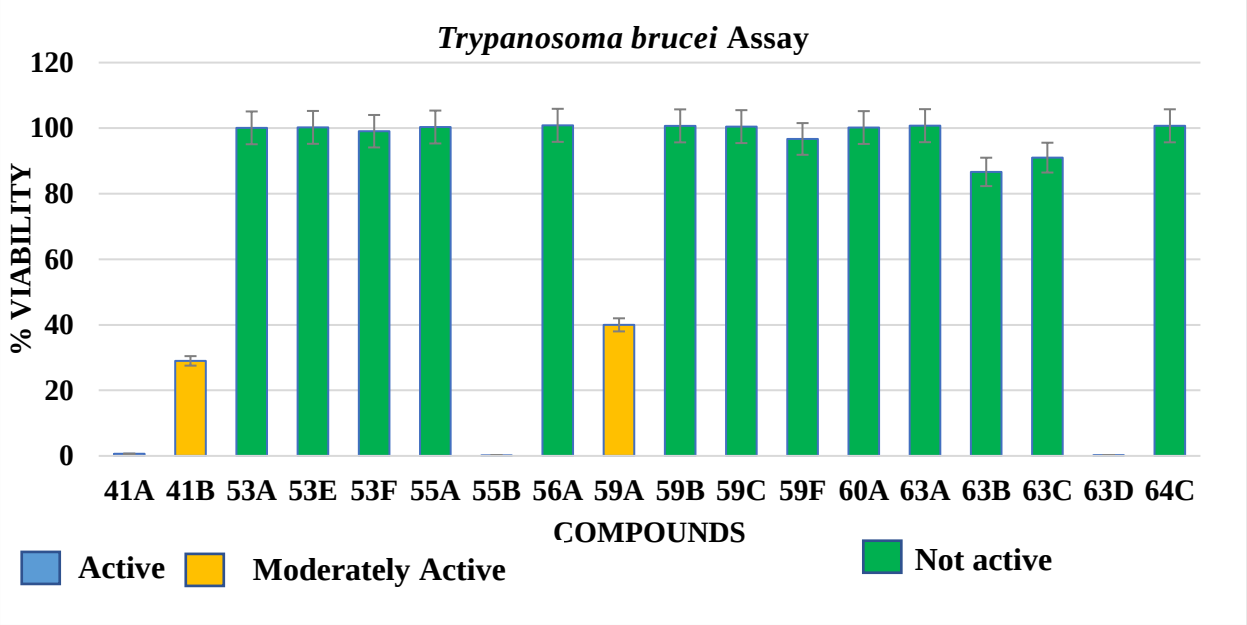
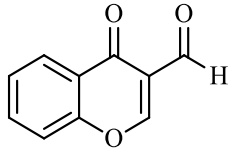
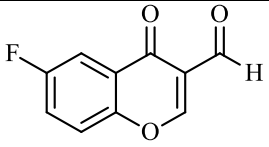
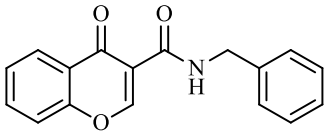
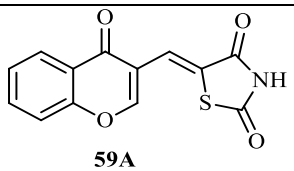
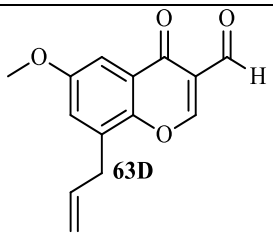


Figure 30: Activity of synthesized compounds against *Trypanosoma brucei*

Table 16: Active and moderately active compounds

Active compounds	Moderately Active compounds
 **41A**	 **41B**
 **55B**	 **59A**
 **63D**	

Trypanosoma brucei susceptibility to compounds **41A**, **55B** and **63D** was evaluated by determining the half maximal inhibitory concentration (IC_{50}) values (Table 17). The IC_{50} value was taken as the concentration of compound (**41A**, **55B** and **63D**) needed to reduce the absorbance ratio to 50 %, hence the lower the concentration displayed in IC_{50}, the more active the compound will be to the parasite *Trypanosoma brucei*. Compound **55B** displayed the highest activity against *Trypanosoma brucei* with an IC_{50} value of 1.3 µg/mL.

Table 17: IC_{50} values displayed by chromone compounds

Compound	IC_{50} (µg/mL)
41A	4.3
55B	1.3
63D	1.9

Compounds **41B** and **59A** showed moderate antitrypanosomal activity and were not further tested for IC_{50} values. There were no obvious structural correlations with antitrypanosomal activity.

4.6.2 Cytotoxicity Assay

The compounds evaluated for Trypanosoma brucei Assay were also screened for cytotoxicity activity. The results of the cytotoxicity activities of compounds are presented in Figure 31 Cytotoxicity was determined according to the percentages (%) of viability where compounds displaying % viability below 40 % are toxic, the smaller the value in % viability the more toxic the substance is. Compounds displaying cytotoxicity above 60 % are classified as not toxic.

Emetine was used as a control drug standard. Concentration of drug used was 20 µg/mL.

Compounds **63C**, **41A**, **55B and 53A** displayed the highest cytotoxicity activity against HeLa (human cervix adenocarcinoma) cells showing percentage viability of 7.39, 14.20, 24.64 and 39.90 % respectively. Compounds **41B** and **53E** displayed moderate cytotoxicity activity at 52.70 and 58.32 % viability.

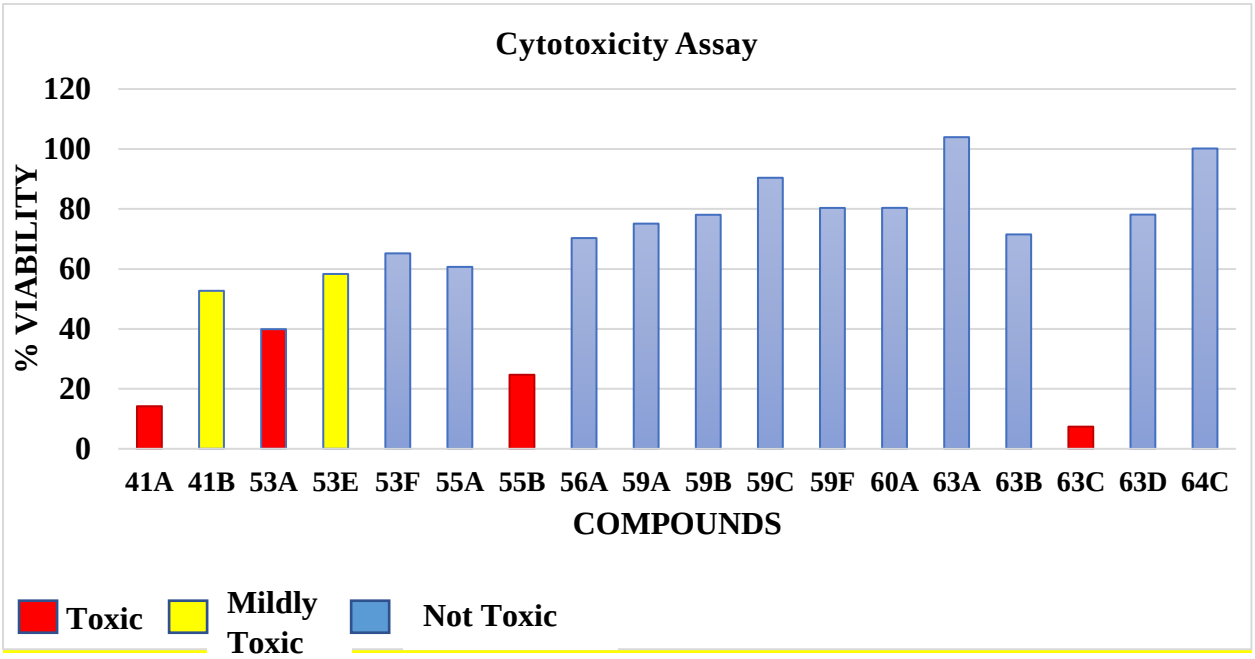
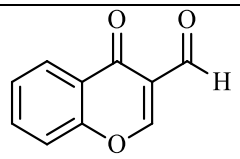
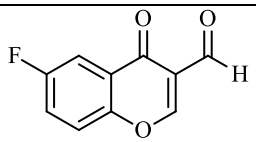
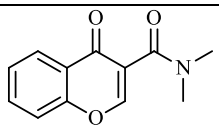
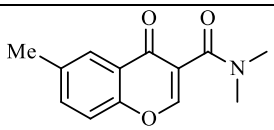
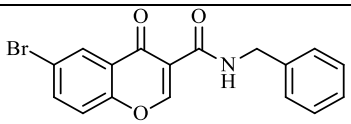
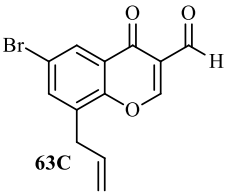


Figure 31: Cytotoxicity activity of synthesized compounds against HeLa cells

Table 18: Toxic and moderately toxic compounds

Toxic compounds	Moderately toxic compounds
 **41A**	 **41B**
 **53A**	 **53E**
 **55B**	
 **63C**	

Compound **63D** showed good trypanocidal activity and its cytotoxicity was very low displaying viability of 78.12 % making it a suitable drug candidate for *Trypanosoma brucei*. Compounds **41A** and **55B**, although very active against *Trypanosoma brucei*, they are toxic.

There were no obvious structural correlations with cytotoxicity activity.

Chapter 5

5.1 Conclusion

The investigation into 4-oxo-4H-chromene-3-carbaldehydes derivatives was conducted in the light of the various biological activities the chromone scaffold possessess. A series of 4-oxo-4H-chromene-3-carbaldehyde analogues was successfully synthesized and transformed into 5-((4-oxo-4H-chromen-3-yl)methylene)thiazolidine-2,4-dione analogues **59** in excellent yield of 72 %. 1-(2-(allyloxy)phenyl)ethanone analogues **61** were successfully converted to 1-(3-allyl-2-hydroxyphenyl)ethanone analogues **62**. Compound **62** was subjected to Vilsmeier–Haack reaction to afford 8-allyl-4-oxo-4H-chromene-3-carbaldehyde analogues **63** which served as precursors in the synthesis of 5-((8-allyl-4-oxo-4H-chromen-3-yl)methylene)thiazolidine-2,4-dione analogues **64** in good yield.

Several methodologies and attempts to synthesize 4-oxo-4H-chromene-3-carboxylic acid **52** or a suitable precursor to the chromone-3-carboxamide were investigated, however a negative result was feasible, with the exception of the Pinnick oxidation methodology which afforded 4-oxo-4H-chromene-3-carboxylic acids in good yield 53-68.5 %. Chromone-3-carboxamides (**53-58**) were successfully synthesized from corresponding 4-oxo-4H-chromene-3-carboxylic acids **52** in good yield.

Eighteen synthesized compounds were screened *in vitro* for cytotoxicity activity against human cervix adenocarcinoma (HeLa) cells and trypanocidal activity for the *Tryponosoma* species called Trypanosoma *brucei brucei* (T.b.b). Compounds **41A**, **55B** and **63D** displayed the highest activity against *Trypanosoma brucei* at 0.61, 0.15 and 0.21 % viability respectively. Compounds **41A**, **55B** and **63D** further displayed IC_{50} values of 4.3, 1.3 and 1.9 µg/mL respectively. Compound **55B** displayed the highest activity against *Trypanosoma brucei*. Compounds **63C**, **41A**, **55B** and **53A** displayed the highest cytotoxic activity against HeLa (human cervix adenocarcinoma) cells showing percentage viability of 7.39, 14.20, 24.64 and 39.90 % respectively. Furthermore, due to the low toxicity and high antitrypanosomal activity observed for compound **63D**, makes it a potential drug candidate for *Trypanosoma brucei brucei.*

5.2 Future work

In our future work, we will explore different biological assays for the synthesized compounds, such antiinflammation, antimalarial and antitumor activities. Other activities in our future work will entail, but not limited to the following:

- Structure-activity relationship (SAR) studies on the synthesized compounds.
- The transformation of 8-allyl-4-oxo-4H-chromene-3-carbaldehyde analogues to 8-(oxiran-2-ylmethyl)-4-oxo-4H-chromene-3-carbaldehyde analogues and biological studies of thereof.
- The reaction of synthesized compounds with various metals to enhance the biological activity of compounds.

Chapter 6

6. Experimental

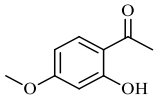
6.1 General remarks

Commercially available 2-hydroxyacetophenones, N, N-dimethylformamide (DMF), N, N-diethylaniline (NND), sulfamic acid, sodium chlorite, thiazolidine-2,4-dione, allyl bromide and other reagents and solvents used were purchased from Sigma Aldrich and Merck. All reagents were analytically pure and were used without any further purification. All reactions were carried out using oven-dried glassware and the reactions were monitored by thin layer chromatography (TLC). TLC plates were visualized under UV light (λ = 254-365 nm). Synthesized compounds were purified by distillation or recrystallization.

Bruker 400 MHz NMR spectrometer was used for determination of (1D and 2D) NMR spectra. Chemical shifts (δ) are reported in parts per million (ppm). ^{1}H shifts are referenced to $CDCl_3$ (δ = 7.26 ppm), acetone-d_6 (δ = 2.05 ppm), acetic acid-d_4 (δ = 2.03, 11.53 ppm), DMSO-d_6 (δ = 2.5 ppm), residual water in DMSO-d_6 (δ = 3.44 ppm), Py-d_5 (δ = 7.19, 7.55, 8.71 ppm). ^{13}C shifts are referenced to $CDCl_3$ (δ = 77.16 ppm), acetone-d_6 (δ = 19.84 and 206.26 ppm), acetic acid (δ = 20.0 and 178.4 ppm), DMSO-d_6 (δ = 39.52 ppm) and Py-d_5 (δ = 123.5, 135.5 and 149.5 ppm). Splitting patterns were represented as follows: s for singlet; d for doublet; t for triplet; q for the quartet, bs for broad singlet and m for multiplet. The coupling constants (J-values) were reported in hertz (Hz). IR spectra were determined on a Perkin-Elmer 1420 spectrophotometer and were reported in wave number (cm^{-1}). Elemental analysis (CHN analysis) was done at the University of Johannesburg and Stellenbosch University.

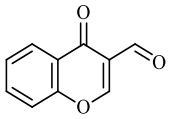
All melting points were determined on a Buchi Melting Point B-540 apparatus using open capillary tubes and were uncorrected.

Synthesis of 2-Hydroxy-4-methoxyacetophenone[81] 14H



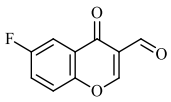
A mixture of 2,4 dihydroxyacetophenone **14G (**10 g, 65.8 mmol), Me_2SO_4 (4.80 mL, 50.7 mmol), dry acetone (100 mL) and K_2CO_3 (10.2 g, 73.8 mmol) was refluxed for 6 h. The resulting solution was cooled and the acetone removed under reduced pressure. The excess Me_2SO_4 was removed using 25 % ammonia-ice mixture (2 x 50 mL). The resulting solution was then extracted with EtOAc (3 x 60 mL); the combined organic solutions were dried over anhydrous $MgSO_4$, evaporated *in vacuo* to afford 2-hydroxy-4-methoxyacetophenone **14H** (7 g, 64 %) as a white solid, m.p. 46-47 °C (lit.,[102] 50 °C);); IR ν_{max}/cm^{-1} 2974.45 (OH), 1615 (C=O); δ_H (400 MHz, $CDCl_3$) 2.55 (3H, s, CH_3), 3.84 (3H, s, OCH_3), 6.42 (2H, m, 3-H and 5-H), 7.62 (1H, d, J = 8.8 Hz, 6-H), 12.76 (1H, s, OH); δ_C (100 MHz, $CDCl_3$) 26.21 (CH_3), 55.56 (OCH_3), 100.83 (C-3), 113.90 (C-1), 107.63 (C-5), 132.31 (C-6), 202.60 (C=O), 166.12 (C-2), 165.26 (C-4).

Synthesis of 4-oxo-4H-chromene-3-carbaldehyde analogues[82] 41A-F



4-oxo-4H-chromene-3-carbaldehyde 41A

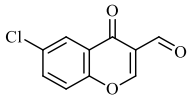
A solution of 2-hydroxyacetophenone **14A** (4.82 mL, 40 mmol) in DMF (24 mL) was cooled to 0 °C and $POCl_3$ (13.8 mL, 150 mmol) was gradually added to the solution. The reaction was stirred at room temperature for 12 h and then quenched with ice water (50 mL). The solid formed was filtered and then dried to afford 4-oxo-4H-chromene-3-carbaldehyde **41A** (3.64 g, 55 %) as a yellow solid, m.p. 150-152 °C (lit.,[82] 151-152); IR ν_{max}/cm^{-1} 1635.18 (C=O), 1691.48 (HC=O), and 3054.82 (C-H aromatic); δ_H (400 MHz, DMSO-d_6) 7.58 (1H, ddd, J = 7.6, 7.2 and 2.4 Hz, 6-H), 7.75 (1H, d, J = 8.4 Hz, 7-H), 7.88 (1H, dd, J = 1.6 and 8.4 Hz, 8-H) 8.14 (1H, dd, 1.6 and 8.4 Hz, 5-H), 8.94 (1H, s, 2-H), 10.14 (1H, s, CHO); δ_C (100 MHz, DMSO-d_6) 163.9 (C-2), 120.4 (C-3), 175.4 (C-4), 127.2 (C-5), 125.1 (C-6), 135.7 (C-7), 119.4 (C-8), 156.07 (C-8a), 125.8 (C-4a) and 188.9 (CHO).



6-Fluoro-4-oxo-4H-chromene-3-carbaldehyde 41B

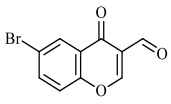
The experimental procedure employed for the synthesis of **41A** was followed using 5-fluoro-2-hydroxyacetophenone **14B** (1.37 g, 7.1 mmol), $POCl_3$ (3.9 mL, 42.39 mmol) and DMF (6.63

mL). Work-up afforded a crude solid, which was recrystallized from ethanol to give 6-fluoro-4-oxo-4H-chromene-3-carbaldehyde (0.74 g, 54 %) as a yellowish-whitish solid, m.p. 155-157 °C (lit.,[103] 155-160 °C); IR ν_{max}/cm^{-1} 1688.79 (HC=O), 1651.21 (C=O); δ_H (400 MHz, $CDCl_3$) 7.51 (1H, m, 8-H), 7.53 (1H, m, 7-H), 7.96 (1H, dd, J = 2.8 and 5.2 Hz, 5-H), 8.59 (1H, s, 2-H), 10.41 (1H, s, CHO); δ_C (100 MHz, $CDCl_3$) 160.69 (C-2), 119.68 (C-3), 175.26 (C-4), 120.90 (C-4a), 111.25 (C-5), 159.04 (C-6), 126.81 (C-7), 111.48 (C-8), 160.69 (C-8a) and 188.27 (CHO).



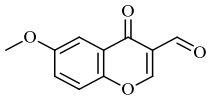
6-chloro-4-oxo-4H-chromene-3-carbaldehyde 41C

The experimental procedure employed for the synthesis of **41A** was followed using 5-chloro-2-hydroxyacetophenone **14C** (2 g, 11.7 mmol), $POCl_3$ (6.5 mL, 70.65 mmol) and DMF (10.8 mL). Work-up afforded 6-chloro-4-oxo-4H-chromene-3-carbaldehyde **41C** (2.44 g, 93.8 %) as yellow solid, m.p. 165-169 °C (lit.,[82] 166-168 °C); IR ν_{max}/cm^{-1} 1623 (C=O), 1714 (HC=O); δ_H (400 MHz, $CDCl_3$) 7.53 (1H, d, J = 9.2 Hz, 8-H), 7.71 (1H, dd, J = 2.4 and 9.2 Hz, 7-H), 8.26 (1H, d, J = 2.4 Hz, 5-H), 8.57 (1H, s, 2-H), 10.38 (1H, s, CHO); δ_C (100 MHz, $CDCl_3$) 160.77 (C-2), 120.34 (C-3), 174.85 (C-4), 125.62 (C-4a), 126.31 (C-5), 132.82 (C-6), 135.03 (C-7), 120.25 (C-8), 154.51 (C-8a) and 188.13 (CHO).



6-bromo-4-oxo-4H-chromene-3-carbaldehyde 41D

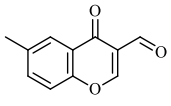
The experimental procedure employed for the synthesis of compound **41A** was followed using 5-bromo-2-hydroxyacetophenone **14D** (4.3 g, 20 mmol), $POCl_3$ (7 mL, 76 mmol) and DMF (11.7 mL). Work-up afforded 6-bromo-4-oxo-4H-chromene-3-carbaldehyde **41D** (3.89 g, 76.9 %) as a yellowish-whitish solid, m.p. 195-197 °C (lit.,[82] 190-193 °C); IR ν_{max}/cm^{-1} 1647 (C=O), 1697.68 (C=O); δ_H (400 MHz, $CDCl_3$) 7.47 (1H, d, J = 8.8 Hz, 8-H), 7.86 (1H, dd, J = 2.4 and 8.8 Hz, 7-H), 8.44 (1H, d, J = 2.4 Hz, 5-H), 8.56 (1H, s, 2-H), 10.39 (1H, s, CHO); δ_C (100 MHz, DMSO-d_6) 160.66 (C-2), 126.64 (C-3), 174.73 (C-4), 120.52 (C-4a), 128.85 (C-5), 120.36 (C-6), 137.83 (C-7), 154.98 (C-8a) and 188.12 (CHO).



6-methoxy-4-oxo-4H-chromene-3-carbaldehyde 41E

The experimental procedure employed for the synthesis of 4-oxo-4H-chromene-3-carbaldehyde **41A** was followed using 5-methoxy-2-hydroxyacetophenone **14E** (2 g, 12.04 mmol), $POCl_3$ (6.63 mL, 70.91 mmol) and DMF (15 mL). Work-up gave a solid, which was directly

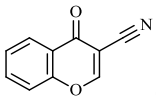
recrystallized from methanol to give 6-methoxy-4-oxo-4H-chromene-3-carbaldehyde (1.13 g, 46 %) as a brown solid; m.p. 158-160 °C (lit.,[83] 163-165°C); IR ν_{max}/cm^{-1} 1651.56 (C=O), 1727.77 (C=O); δ_H (400 MHz, DMSO-d_6): 3.89 (3H, s, OCH_3), 7.46 (1H, d, J = 9.2 Hz, 5-H), 8.91 (1H, s, 2-H), 10.14 (1H, s, CHO): δ_C (100 MHz, DMSO-d_6) 56.4 (OCH_3), 163.5 (C-2), 119.7 (C-3), 175.15 (C-4), 157.9 (C-4a), 105.9 (C-5), 126 (C-6), 124.3 (C-7), 121 (C-8), 150.8 (C-8a), 189.9 (CHO).



6-methyl-4-oxo-4H-chromene-3-carbaldehyde 41F

The experimental procedure employed for the synthesis of 4-oxo-4H-chromene-3-carbaldehyde **41A** was followed using 5-methyl-2-hydroxyacetophenone **14F** (3.39 g, 22.6 mmol), $POCl_3$ (13.8 mL, 144 mmol), DMF (25 mL). Work-up afforded 6-methyl-4-oxo-4H-chromene-3-carbaldehyde **41E** (4.0 g, 94 %) as yellow solid; m.p. 170-176 °C (lit.,[83] 173-174); IR ν_{max}/cm^{-1} 1735.83 (C=O), 1691.48 (C=O); δ_H (400 MHz, $CDCl_3$) 2.49 (3H, s, CH_3), 7.42 (1H, d, J = 8.4 Hz, 8-H), 7.56 (1H, dd, J = 1.6 and 8.4 Hz, 7-H), 8.07 (1H, s, 5-H), 8.53 (1H, s, 2-H), 10.38 (1H, s, CHO); δ_C (100 MHz, DMSO-d_6) 20.99 (CH_3), 160.52 (C-2), 120.17 (C-3), 176.05 (C-4), 124.94 (C-4a), 125.50 (C-5), 136.92 (C-6), 135.96 (C-7), 118.34 (C-8), 154.47 (C-8a), 188.74 (HC=O).

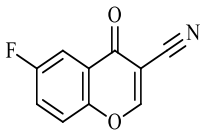
Synthesis of 4-oxo-4H-chromene-3-carbonitrile[84] **(71A-E)**



4-oxo-4H-chromene-3-carbonitrile 71A

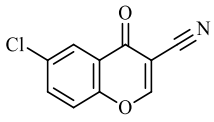
A solution 2-hydroxyacetophenone **14A** (3.4 mL, 28.24 mmol) in DMF (15.5 mL, 200 mmol) was cooled to 0 °C and $POCl_3$ (9.3 mL, 100 mmol) was gradually added to the solution. The mixture was stirred at 0-5 °C for 0.5 h. The resultant mixture was stirred at room temperature for 4 h and then a solution of $NH_2OH{\cdot}HCl$ (5.22 g, 75 mmol) in DCM (34 mL) was added to the reaction mixture at 5 °C. The resulting mixture was stirred for 6 h at room temperature and quenched with water (30 mL); extracted with DCM (3 x 25 mL), washed with water (1 x 10 mL), washed with saturated $NaHCO_3$ solution (1 x 10 mL) and dried over Na_2SO_4. The solution was concentrated under reduced pressure to give a crude solid, which was directly recrystallized from methanol to afford 4-oxo-4H-chromene-3-carbonitrile **71A** (3.8 g, 78 %) as pale yellow solid; m.p. 174-176°C (lit.,[85] 175-177 °C); IR ν_{max}/cm^{-1} 3083.13 (CH), 2239.30 (CN), 1658.96 (C=O); ^1H NMR δ_H (400 MHz, DMSO-d_6) 7.60 (1H, dd, J = 7.2 and 8.8 Hz, 6-H), 7.76 (1H, d, J = 8.8 Hz, H-8), 7.91 (1H, m, 7-H), 8.08 (1H, dd, J = 1.2 and 7.2 Hz, 5-H), 9.26 (1H,s, 2-H); δ_C (100

MHz, DMSO-d_6) 165.86 (C-2), 101.49 (C-3), 172.99 (C-4), 123.12 (C-4a), 127.54 (C-5), 125.56 (C-6), 136.09 (C-7), 119.33 (C-8), 155.82 (C-8a) and 113.66 (CN); Anal. Calc. for $C_{10}H_5NO_2$; C 70.18; H 2.94; N 8.18. Found: C 69.09; H 2.75: N 7.95;



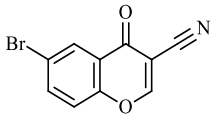
6-fluoro-4-oxo-4H-chromene-3-carbonitrile 71B

The experimental procedure employed for the synthesis of **65A** was followed, using 5-fluoro-2-hydroxyacetophenone **14B** (1 g, 6.48 mmol), $POCl_3$ (2.16 mL, 23.67 mmol), DMF (12.35 mL), DCM (13 mL) and $NH_2OH{\cdot}HCl$ (1.23 g, 17.81 mmol). Work-up afforded a crude solid, which was directly crystallized from methanol to give 6-fluoro-4-oxo-4H-chromene-3-carbonitrile **71B** (0.69 g, 60 %) as a white solid; m.p. 166 °C (lit.,[84] 172–174); IR ν_{max}/cm^{-1} 2243.43 (CN), 1648.61 (C=O) ; δ_H (400 MHz, DMSO-d_6) 9.28 (1H, s, 2-H), 7.90 (1H, dd, J = 4 and 4.8 Hz, 5-H), 7.82 (1H, m, 7-H), 7.80 (1H, dd, 2.8 and 2.4 Hz, 8-H); δ_C (100 MHz, DMSO-d_6) 166.07 (C-2), 100.91 (C-3), 172.59 (C-4), 124.24 (C-4a), 124.53 (C-5), 152.42 (C-6), 124.60 (C-7), 122.35 (C-8), 154.57 (C-8a), 113.45 (CN); Anal. Calc. for $C_{10}H_4FNO_2$: C 63.50; H 2.13; N 7.41. Found: C 63.27; H 2.05: N 7.23.



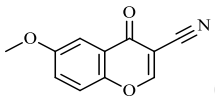
6-chloro-4-oxo-4H-chromene-3-carbonitrile 71C

The experimental procedure employed for the synthesis of **71A** was followed, using 5-chloro-2-hydroxyacetophenone **14C** (3 g, 17.59 mmol), $POCl_3$ (5.85 mL, 64.18 mmol), DMF (22.35 mL), DCM (23 mL) and $NH_2OH{\cdot}HCl$ (3.35 g, 48.30 mmol). Work-up afforded a crude solid, which was directly crystallized from methanol to give 6-chloro-4-oxo-4H-chromene-3-carbonitrile **71C** (3 g, 52.4 %) as a yellow solid; m.p. 210-211 °C (lit.,[84] 211-213); IR ν_{max}/cm^{-1} 2233.30 (CN), 1658.66 (C=O); δ_H (400 MHz, DMSO-d_6) 9.27 (1H, s, 2-H), 7.84 (1H, d, J = 8.8 Hz, 8-H), 7.95 (1H, dd, J = 2.4 and 8.8 Hz, 7-H), 8.01 (1H, d, J = 2.4 Hz, 5-H); δ_C (100 MHz, DMSO-d_6) δ_C 166.17 (C-2), 135.90 (C-7), 124.55 (C-5), 113.45 (CN), 101.53 (C-3), 121.92 (C-8), 124.41 (C-4a), 131.96 (C-6), 154.51 (C-8a), 166.16 (C-2), 172.20 (C-4); Anal. Calc. for $C_{10}H_4ClNO_2$: C 58.42; H 1.96; N 6.81. Found: C 57.30; H 1.93; N 6.67.



6-bromo-4-oxo-4H-chromene-3-carbonitrile 71D

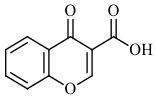
The experimental procedure employed for the synthesis of **71A** was followed using 5-bromo-2-hydroxyacetophenone **14D** (2 g, 9.3 mmol), $POCl_3$ (3.5 mL, 36.36 mmol), DMF (10 mL) and $NH_2OH{\cdot}HCl$ (1.93 g, 27.76 mmol). Work-up afforded a crude material, which was directly crystallized from methanol to afford 6-bromo-4-oxo-4H-chromene-3-carbonitrile **71D** (1.22 g, 52.4%), m.p. 216–219 °C (lit.,[84] 218-220); 3073.14 (CH), 2235.30 (CN), 1668.46 (C=O); δ_H (400 MHz, DMSO-d_6) 7.77 (1H, d, J = 8.8 Hz, 8-H), 8.06 (1H, dd, J = 2.4 Hz and 8.8 Hz, 7-H), 8.13 (1H, d, J = 2.4 Hz, 5-H), 9.27 (1H, s, 2-H); ^{13}C NMR (100 MHz, DMSO-d_6) δ_C; 166.17 (C-2), 101.62 (C-3), 172.05 (C-4), 119.96 (C-4a), 127.62 (C-5), 124.74 (C-6), 138.65 (C-7), 122.05 (C-8), 154.90 (C-8a), 113.44 (CN).



6-methoxy-4-oxo-4H-chromene-3-carbonitrile 71E

The experimental procedure employed for the synthesis of **71A** was followed, using 5-methoxy-2-hydroxyacetophenone **14E** (4 g, 24.07 mmol), $POCl_3$ (13.26 mL, 141.83 mmol), DMF (22.35 mL), DCM (30 mL) and $NH_2OH{\cdot}HCl$ (5.2 g, 74.83 mmol). Work-up afforded a crude solid, which was recrystallized from methanol to afford 6-methoxy-4-oxo-4H-chromene-3-carbonitrile **71E** (3.19 g, 66 %) as a brown solid; m.p. 190-192°C (lit.,[84] 194-195); IR ν_{max}/cm^{-1} 3070.76 (CH aromatic), 2236.13 (CN), 1656.94 (C=O); δ_H (400 MHz, DMSO-d_6) 3.87 (3H, s, OCH_3), 7.77 (1H, d, J = 8.8 Hz, 8-H), 8.06 (1H, dd, 8.8 and 2.4 Hz, 7-H), 8.13 (1H, d, J = 2.4 Hz, 5-H), 9.27 (1H, s, 2-H); δ_C (100 MHz, DMSO-d_6) 56.42 (OCH_3), 165.40 (C-2), 100.56 (C-3), 172.85 (C-4), 123.96 (C-4a), 121.08 (C-5), 150.59 (C-6), 124.87 (C-7), 105.41 (C-8), 158.00 (C-8a), 113.87 (CN); Anal. Calc. for $C_{11}H_7NO_3$ C 65.67; H 3.51; N 6.96. Found: C 65.23; H 3.22: N 6.79.

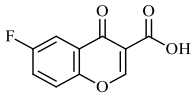
Synthesis of 4-oxo-4H-chromene-3-carboxylic acid[88] 52A-F



4-oxo-4H-chromene-3-carboxylic acid 52A

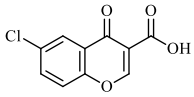
A solution of sodium chlorite (4.33 g, 47.87 mmol) in water (8 mL) was stirred at 0 °C. To this solution 4-oxo-4H-chromene-3-carbaldehyde **41A** (2.6 g, 13.67 mmol) and sulfamic acid (5.31 g, 54.69 mmol) were added, followed by gradual addition of DCM (15 mL). The resulting mixture was stirred for 3 h at room temperature and then quenched with water (25 mL), extracted with DCM (3 x 25 mL). The combined organic phases were dried over Na_2SO_4 and concentrated under reduced pressure, recrystallized from 80:20 methanol-water to give 4-oxo-4H-chromene-3-carboxylic acid **52A** (1.50 g, 53 %) as a white solid; m.p. 200-203°C (lit.,[82] 204-205°C); IR ν_{max}/cm^{-1} 3078.82 (OH), 1737.79 (C=O); δ_H (400 MHz, DMSO-d_6), 7.64 (1H, t, J = 7.6 Hz, 6-

H), 7.82 (1H, d, J = 8.8 Hz, 8-H), 7.95 (1H, dd, J = 8.8 and 1.2 Hz, 7-H), 8.19 (1H, d, J = 8 Hz, 5-H), 9.16 (1H, s, 2-H) 13.28 (1H, s, OH) ; δ_C (100 MHz, DMSO-d_6) 164.2 (C-2), 114.7 (C-3), 176. 6 (C-4), 123.8 (C-4a), 127.3 (C-5), 125.9 (C-6), 135.9 (C-7), 119.3 (C-8), 156.2 (C-8a), 164.3 (C-OH).



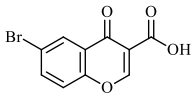
6-fluoro-4-oxo-4H-chromene-3-carboxylic acid 52B

The experimental procedure employed for the synthesis of **52A** was followed, using 6-fluoro-4-oxo-4H-chromene-3-carbaldehyde **41B** (1.70 g, 8.85 mmol), sulfamic acid (3.40 g, 35.05), sodium chlorite (2.77 g, 30.70 mmol) and DCM (9 mL). The obtained crude product was recrystallized from 80:20 methanol-water to give 6-fluoro-4-oxo-4H-chromene-3-carboxylic acid **52B** (1.04 g, 57 %) as a white solid; m.p. 210-213 °C, IR ν_{max}/cm^{-1} 1694 and 1634 (C=O), δ_H (400 MHz, DMSO-d_6) 7.80-8.34 (2H, m, 7-H and 5-H), 7.64 (1H, d, J = 8.4 Hz, 8-H), 9.10 (1H, s, 2-H), 13.15 (1H, bs, OH); δ_C (100 MHz, DMSO-d_6) 164.17 (C-2), 114.68 (C-3), 175.09 (C-4), 123.68 (C-4a), 122.21 (C-5), 152.55 (C-6), 123.93 (C-7), 110.19 (C-8), 158.76 (C-8a), 164.07 (C-OH).



6-chloro-4-oxo-4H-chromene-3-carboxylic acid 52C

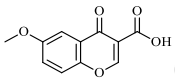
The experimental procedure employed for the synthesis of 4-oxo-4H-chromene-3-carboxylic acid was followed, using 6-chloro-4-oxo-4H-chromene-3-carbaldehyde **41C** (5.54 g, 26.56 mmol), sulfamic acid (10.21 g, 105.16 mmol), sodium chlorite (8.33 g, 92.11 mmol) and DCM (25 mL). Work-up employed gave a crude product, which was purified by recrystallization from 80:20 methanol-water to give 6-chloro-4-oxo-4H-chromene-3-carboxylic acid **52C** (3.7 g, 61.3 %). m.p. 230-231°C (lit.,[90] 231-232°C); IR ν_{max}/cm^{-1} 3087.82 (OH); δ_H (400 MHz, DMSO-d_6) δ_C 8.89 (1H, s, 2-H), 7.39 (1H, d, J= 7.2 Hz, 8-H), 7.80 (1H, d, J = 8.8 Hz, 7-H), 8.03 (1H, s, 5-H); (100 MHz, DMSO-d_6) 160.69 (C-2) 118.72 (C-3), 174.68 (C-4), 124.82 (C-4a), 129.87 (C-5), 130.70 (C-6) 134.92 (C-7), 121.57 (C-8), 154.17 (C-8a), 162.2 (C-OH).



6-bromo-4-oxo-4H-chromene-3-carboxylic acid 52D

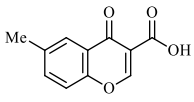
The experimental procedure employed for the synthesis of **52A** was followed, using 6-bromo-4-oxo-4H-chromene-3-carbaldehyde **41D** (2 g, 7.90 mmol), sulfamic acid (4.084 g, 42.06 mmol), sodium chlorite (3.33 g, 36.81 mmol) and DCM (10 mL). Work-up afforded a crude product,

which was recrystallized from 80:20 methanol-water to give 6-bromo-4-oxo-4H-chromene-3-carboxylic acid **52D** (1.17 g, 55.2 %) as a pale yellow solid; m.p. 236-240°C (lit.,[82] 245-249°C); IR ν_{max}/cm^{-1} 3071.64 (OH), 1691.39 (C=O), 1651.72 (C=O) δ_H (400 MHz, DMSO-d_6) 7.71 (1H, d, J = 9.2 Hz, 8-H), 8.00 (1H, dd, J= 2.4 and 9.2 Hz, 7-H), 8.20 (1H, d, J= 2.4 Hz, 5-H); δ_C (100 MHz, DMSO-d_6) 160.67 (C-2), 119.02 (C-3), 174.59 (C-4), 121.73 (C-4a), 134.95 (C-5), 129.90 (C-6), 137.65 (C-7), 118.84 (C-8) 154.59 (C-8a), 162.12 (C-OH)



6-Methoxy-4-oxo-4H-chromene-3-carboxylic acid 52E

The experimental procedure employed for the synthesis of **52A** was followed, using 6-methoxy-4-oxo-4H-chromene-3-carbaldehyde (5.43 g, 26.57 mmol), sulfamic acid (10.21 g, 105.16 mmol), sodium chlorite (8.33 g, 92.11 mmol) and DCM (25 mL). Work-up employed gave a crude product, which was recrystallized from 80:20 methanol-water to give 6-methoxy-4-oxo-4H-chromene-3-carboxylic acid **52E**; m.p. 172-173°C (lit.,[91] 173-174°C); IR ν_{max}/cm^{-1} 3068.64 (OH), 1694.71 (C=O), δ_H (400 MHz, DMSO-d_6) 3.88 (3H, s, CH_3), 7.47 (1H, d, J = 3.2 Hz, 8-H), 7.51 (1H, d, J = 3.2 Hz, 7-H), 7.74 (1H, d, J = 9.2 Hz, 5-H), 9.10 (1H, s, 2-H), 13.32 (1H, bs, OH); δ_C (100 MHz, DMSO-d_6) 56.39 (OCH_3), 163.94 (C-2), 113.73 (C-3), 176.53 (C-4), 124.53 (C-4a), 120.93 (C-5), 151.02 (C-6), 125.06 (C-7), 105.53 (C-8), 157.90 (C-8a), 164.37 (C-OH)



6-methyl-4-oxo-4H-chromene-3-carboxylic acid 52F

The experimental procedure employed for the synthesis of **52A** was followed, using 6-methyl-4-oxo-4H-chromene-3-carbaldehyde **41F** (5 g, 26.57 mmol), sulfamic acid (10.21 g, 105.16 mmol), sodium chlorite (8.33 g, 92.11 mmol) and DCM (25 mL). Workup afforded a crude product, which was recrystallized from 70:30 methanol-water to give 6-methyl-4-oxo-4H-chromene-3-carboxylic acid **52A** (3.1 g, 57.2 %) as a yellow solid; m.p. 240-247 °C; IR ν_{max}/cm^{-1} 3069.37 (OH), 1745.29 (C=O)); δ_H (400 MHz, DMSO-d_6) 7.67 (1H, d, J = 8.8 Hz, 8-H), 7.74 (1H, d, J = 8.8 Hz, 7-H), 7.94 (1H, d, J = 8 Hz, 5-H), 9.11 (1H, s, 2-H); δ_C (100 MHz, DMSO-d_6) 20.00 (CH_3) 164.19 (C-2), 114.42 (C-3), 176.77 (C-4), 123.43 (C-4a), 125.05 (C-5), 136.63 (C-6), 137.13 (C-7), 119.09 (C-8), 154.57 (C-8a), 164.36 (C-OH).

Attempted methods for the synthesis of chromene-3-carboxylic acid

Method 1:

Jones' reagent (5.53 mL) was cooled at 10-15 °C and a mixture of dry acetone (120 mL) and 4-oxo-4H-chromene-3-carbaldehyde **41A** (1.5 g, 8.6 mmol) was added gradually with stirring maintaining the temperature around 10-15 °C for 0.5 h. The mixture was then concentrated under reduced pressure to 1/3 of the initial volume. The resulting mixture was poured into water (250 mL) with stirring and allowed to stand for ~2 hrs. The resulting precipitate formed was filtered off, washed with water, dried and recrystallized from acetone.[82]

Method 2*:*

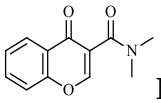
A solution 4-oxo-4H-chromene-3-carbaldehyde **41A** (1.5 g, 8.6 mmol) in formic acid (15 mL) was cooled to 0 °C. To the solution, hydrogen peroxide (20 mL) was added gradually with constant stirring for 8 h. The progress of the reaction was monitored with TLC.[86] The product recovered was 4-oxo-4H-chromene-3-carbaldehyde **41A**.[86]

Method 3

A solution 4-oxo-4H-chromene-3-carbaldehyde **41A** (1.5 g, 8.6 mmol) in HCl (15 mL) was refluxed for 2 h, and reaction progress was monitored by TLC.[87] The isolated product was 4-oxo-4H-chromene-3-carbaldehyde **41A**.

Synthesis of chromone-3-carboxamides[92] 53-58

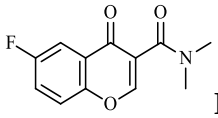
N, N-dimethyl-4-oxo-4H-chromene-3-carboxamide 53A-F



N, N-dimethyl-4-oxo-4H-chromene-3-carboxamide 53A

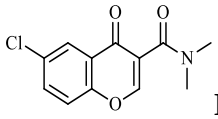
A mixture of 4-oxo-4H-chromene-3-carboxylic acid **52A** (0.57 g, 3 mmol) and thionyl chloride (0.356 g, 3 mmol) in DCM (30 mL) was stirred at room temperature for 1 h. The resulting mixture was cooled to 0 °C followed by the dropwise addition of triethylamine (0.91 g, 9 mmol) and finally dimethylamine hydrochloride (0.24 g, 3 mmol). The mixture was stirred at room temperature for 16 h. The reaction mixture was diluted with water (30 mL) and taken up into a separatory funnel and extracted with DCM (2 x 25 mL). The combined organic layers were washed with 1M NaOH (2 x 10 mL) and finally with water 10 mL. The organic phase was dried over Na_2SO_4 and concentrated in vacuo and the residual solid was directly crystallized from methanol to give N, N-dimethyl-4-oxo-4H-chromene-3-carboxamide **53A** (0.35 g, 53.8 %) as a white solid; m.p. 190-192 °C (lit.,[93] 195-196 °C); IR ν_{max}/cm^{-1} 2930.93 (CH), 1721.54 (C=O), 1625 (C=O), δ_H (400 MHz, $CDCl_3$) 3.01 (3H, s, NCH_3), 3.13 (3H, s, NCH_3), 7.44-7.51 (2H, m, 6-H and 8-H), 7.72 (1H, m, 7-H), 8.25 (1H, dd, J = 8 and 1.2 Hz, 5-H), 8.17 (1H, s, 2-H); δ_C (100

MHz, DMSO-d_6) 35.40 (NCH_3), 38.58 (NCH_3), 118.25 (C-8), 125.84 (C-6), 126.22 (C-5), 134.21 (C-7), 156.30 (C-2), 123.42 (C-3), 124.39 (C-4a), 156.11 (C-8a), 164.45 (C-N), 173.76 (C-4).



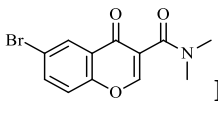
N, N-dimethyl-6-fluoro-chromone-3-carboxamide 53B

The experimental procedure employed for the synthesis of **53A**was followed using 6-fluoro-4-oxo-4H-chromene-3-carboxylic acid **52B** (0.31 g, 1.5 mmol), thionyl chloride (0.18 g, 1.5 mmol), triethylamine (0.46 g, 4.5 mmol), dimethylamine hydrochloride (0.24 g, 3 mmol). Work-up gave N, N-dimethyl-6-fluoro-chromone-3-carboxamide **53B** (0.16 g, 45.7 %) as a yellow solid, m.p. 170-175 °C ; IR ν_{max}/cm^{-1} 1694 (C=O), δ_H (400 MHz, DMSO-d_6) 2.89 (3H, s, NCH_3), 2.98 (3H, s, NCH_3), (1H, dd, J = 4.4 and 2.8 Hz, 8-H), 7.78 (1H, d, J = 2.4 Hz, 7-H), 7.83 (1H, dd, J = 4.4 and 2.8 Hz, 5-H), 8.57 (1H, s, 2-H); δ_C (100 MHz, DMSO-d_6) 35.94 (CH_3), 38.26 (CH_3), 110.45 (C-8), 121.93 (C-5), 123.36 (C-7), 156.89 (C-2), 161.74 (C-6), 125.40 (C-3), 158.40 (C-8a), 163.75 (C-N), 173.20 (C-4).



N, N-dimethyl-6-chloro-chromone-3-carboxamide 53C

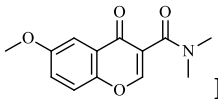
The experimental procedure employed for the synthesis of **53A** was followed using 6-chloro-4-oxo-4H-chromene-3-carboxylic acid (0.67 g, 3 mmol), thionyl chloride (0.356 g, 3 mmol), triethylamine (0.91 g, 9 mmol), dimethylamine hydrochloride (0.24 g, 3 mmol). Work-up gave (0.36 g, 48.9 %) m.p. 155-157°C IR ν_{max}/cm^{-1} 2929.65 (CH), 1705.69 (C=O), 1624.25 (C=O) δ_H (400 MHz, DMSO-d_6) 2.89 (3H, s, NCH_3), 2.97 (3H, s, NCH_3), 7.35(1H, d, 2.4 Hz, 8-H), 7.38-8.03 (2H, m, 5-H and 7-H), 8.35 (1H, s, 2-H); δ_C (100 MHz, DMSO-d_6) 34.75 (NCH_3), 38.14 (NCH_3), 112.67 (C-3), 156.91 (C-8a), 156.12 (C-2), 172.78 (C-4), 124.62 (C-4a), 127.64 (C-5), 129.33 (C-6), 134.22 (C-7), 119.33 (C-8), 154.77 (C-8a), 163.30 (C-N); Anal. Calc. for $C_{12}H_{10}ClNO_3$: C 57.27; H 4.01; N 5.57. Found: C 55.66; H 4.02: N 4.99.



N, N-dimethyl-6-bromo-chromone-3-carboxamide 53D

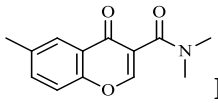
The experimental procedure employed for the synthesis of **53A** was followed using 6-bromo-4-oxo-4H-chromene-3-carboxylic acid **52D** (0.81 g, 3 mmol), thionyl chloride (0.356 g, 3 mmol), triethylamine (0.91 g, 9 mmol), dimethylamine hydrochloride (0.24 g, 3 mmol). Work-up gave

N, N-dimethyl-6-bromo-chromone-3-carboxamide **53D** (0.44 g, 50.5 %) as a yellow solid, m.p. 165-170 °C; IR ν_{max}/cm^{-1} 2927.98 (CH aromatic), 1643.03 (C=O) ; δ_H (400 MHz, acetic acid-d_4) 2.89 (3H, s, NCH_3), 2.95 (3H, s, NCH_3), 7.45 (1H, d, J = 8.8 Hz, 8-H), 7.79 (1H, dd, J = 2 Hz and 8.8 Hz, 7-H), 8.14 (1H, d, J = 2.4 Hz, 5-H), 8.29 (1H, s, 2-H); δ_C (100 MHz, acetic acid-d_4) 34.61 (NCH_3), 37.86 (NCH_3), 120.83 (C-8), 127.97 (C-5), 137.28 (C-7), 157.28 (C-2), 118.71 (C-3), 122.23 (C-4a), 125.45 (C-6), 164.98 (C-N), 172.55 (C-4); Anal. Calc. for $C_{12}H_{10}BrNO_3$: C 48.67; H 3.40; N 4.73. Found: C 48.57; H 3.71: N 4.87.



N, N-dimethyl-6-Methoxy-chromone-3-carboxamide 53E

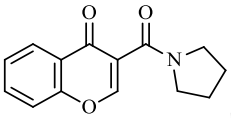
The experimental procedure employed for the synthesis of **53A** was followed using 6-methoxy-4-oxo-4H-chromene-3-carboxylic acid **52E** (0.66 g, 3 mmol), thionyl chloride (0.356 g, 3 mmol), triethylamine (0.91 g, 9 mmol), dimethylamine hydrochloride (0.24 g, 3 mmol). Work-up gave N, N-dimethyl-6-Methoxy-chromone-3-carboxamide (0.4 g, 53.93 %) as a yellow solid; m.p. 158-160°C (lit., 127-128°C); IR ν_{max}/cm^{-1} 2925.94 (CH aromatic), 1638.55 (C=O); δ_H (400 MHz, DMSO-d_6) 2.95 (3H, s, NCH_3), 3.04 (3H, s, NCH_3), 3.94 (3H, s, OCH_3), 7.50-7.52 (2H, dd, J = m, 8-H and 7-H), 7.73 (1H, d, J = 9.6 Hz, 5-H), 8.57 (1H, s, 2-H); δ_C 100 MHz, DMSO-d_6) 34.94 (NCH_3), 38.27 (NCH_3), 56.25 (OCH_3), 105.33 (C-8), 120.70 (C-5), 124.24 (C-7), 156.32 (C-2), 122.47 (C-3), 124.85 (C-4a), 150.97 (C-6), 157.30 (C-8a), 164.15 (C-N), 173.44 (C-4); Anal. Calc. for $C_{14}H_{13}NO_3$: C 63.15; H 5.30; N 5.67. Found: C 63.77; H 5.40: N 5.40;



N, N-6-trimethyl-4-oxo-4H-chromene-3-carboxamide 53F

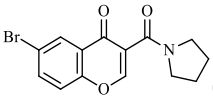
The experimental procedure employed for the synthesis of **53A** was followed using 6-methyl-4-oxo-4H-chromene-3-carboxylic acid **52F** (0.56 g, 3 mmol), thionyl chloride (0.356 g, 3 mmol), triethylamine (0.91 g, 9 mmol), dimethylamine hydrochloride (0.24 g, 3 mmol). Work-up gave N, N-6-trimethyl-4-oxo-4H-chromene-3-carboxamide **53F** (0.35 g, 50.3 %) as a yellowish-orange solid m.p. 121-124 °C (lit.,[93] 118-119 °C); IR ν_{max}/cm^{-1} 2924.36 (CH aromatic), 1643.59 (C=O); δ_H (400 MHz, DMSO-d_6); 2.44 (3H, s, CH_3), 2.89 (3H, s, NCH_3), 2.98 (3H, s, NCH_3), 7.59 (1H, d, J = 8.4 Hz, 8-H), 7.66 (1H, d, J = 8.4 Hz, 7-H), 7.88 (1H, s, 2-H) 8.49 (1H, d, J = 2.4 Hz); δ_C (100 MHz, DMSO-d_6); 20.88 (CH_3), 34.92 (NCH_3), 38.25 (NCH_3), 118.84 (C-8), 124.92 (C-5), 136.13 (C-7), 156.41 (C-2), 123.13 (C-3), 123.82 (C-4a), 136.04 (C-6), 154.49 (C-8a), 164.11 (C-N), 173.70 (C-4); Anal. Calc. for $C_{13}H_{13}NO_3$: C 67.52; H 5.67; N 6.06. Found: C 65.97; H 5.936: N 6.07.

Synthesis of 3-(pyrrolidine-1-carbonyl)-4H-chromen-4-one analogues 54A-B



3-(pyrrolidine-1-carbonyl)-4H-chromen-4-one 54A

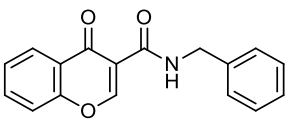
The experimental procedure employed for the synthesis of **53A** was followed using 4-oxo-4H-chromene-3-carboxylic acid **52A** (0.56 g, 3 mmol), thionyl chloride (0.356 g, 3 mmol), triethylamine (0.91 g, 9 mmol), pyrrolidine (0.21 g, 3 mmol). Work-up afforded 3-(pyrrolidine-1-carbonyl)-4H-chromen-4-one **54A** (0.32 g, 44 %), as a jelly solid. IR ν_{max}/cm^{-1} 1641.41 (C=O), 1605.44 (C=O); δ_H (400 MHz, $CDCl_3$) 1.89 (4H, m, 2'-H), 3.46 (2H, dd, J = 6.8 and 6.4 Hz, 3'-H). 3.64 (2H, m, 3'-H), 7.45 (1H, m, 6-H), 7.49 (1H, d, J = 8.4 Hz, 8-H), 7.70 (1H, m, 7-H), 8.22 (1H, s, 2-H), 8.23 (1H, dd, J = 1.6 and 8 Hz, 5-H); δ_C (400 MHz, $CDCl_3$) 24.40 and 25.93 (C-2'), 156.53 (C-2), 46.29 and 47.64 (C-3'), 123.99 (C-3), 173.65 (C-4), 125.53 (C-4a), 134.18 (C-5), 125.79 (C-6), 126.10 (C-7), 118.24 (C-8), 156.55 (C-8a), 162.75 (C-1'); Anal. Calc. for $C_{14}H_{13}NO_3$: C 69.12; H 5.39; N 5.76. Found: C 66.78; H 5.57: N 6.13.



6-bromo-3-(pyrrolidine-1-carbonyl)-4H-chromen-4-one 54B

The experimental procedure employed for the synthesis of **53A** was followed using 6-bromo-4-oxo-4H-chromene-3-carboxylic acid **52D** (0.81 g, 3 mmol), thionyl chloride (0.356 g, 3 mmol), triethylamine (0.91 g, 9 mmol), pyrrolidine (0.21 g, 3 mmol). Work-up gave 6-bromo-3-(pyrrolidine-1-carbonyl)-4H-chromen-4-one **54B** (0.62 g, 64.15 %), m.p. 234-236 °C) as a yellow solid; IR ν_{max}/cm^{-1} 2962.16 (CH aromatic), 1625.14 (C=O) δ_H 1.78 (4H, m, 2'-H), 3.32 (2H, t, J = 6.4 Hz, 3'-H), 3.47 (2H, t, 6.8 Hz, 3'-H), 7.39 (1H, d, J = 8.8 Hz, 8-H), 7.75 (1H, dd, J = 2.4 and 8.8 Hz, 7-H), 8.20 (1H, d, J = 2.4 Hz), 8.34 (1H, s, 2-H); δ_C (100 MHz, acetic acid-d_4) 24.01 and 25.42 (C-2'), 158.25 (C-2), 46.54 and 47.88 (C-3'), 119.19 (C-3), 172.92 (C-4), 122.43 (C-4a), 128.31 (C-5), 125.40 (C-6), 137.51 (C-7), 120.61 (C-8), 155.12 (C-8a), 163.53 (C-1'),; Anal. Calc. for $C_{14}H_{12}BrNO_3$: C 52.20; H 3.75; N 4.35. Found: C 52.54; H 3.80: N 4.50.

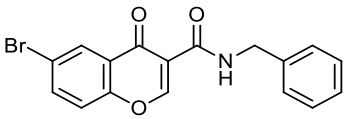
Synthesis of N-benzyl-4-oxo-4H-chromene-3-carboxamide analogues 55A-B



N-benzyl-4-oxo-4H-chromene-3-carboxamide 55A

The experimental procedure employed for the synthesis of **53A** was followed using 4-oxo-4H-chromene-3-carboxylic acid **52A** (0.56 g, 3 mmol), thionyl chloride (0.356 g, 3 mmol),

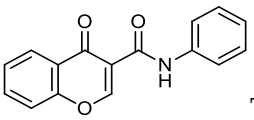
triethylamine (0.91 g, 9 mmol), benzylamine (0.38 g, 3 mmol). Work-up gave N-benzyl-4-oxo-4H-chromene-3-carboxamide **55A** (0.49 g, 60 %) as a white solid; δ_H m.p. 170-172 °C; IR ν_{max}/cm^{-1} 3262.09 (NH), 1716.57 (C=O), 1652.18 (C=O) δ_H (400 MHz, $CDCl_3$) 4.64 (2H, d, J = 7.6 Hz), 7.39 (4H, m, benzyl-H), 7.45 (1H, dd, J = 7.2 and 8 Hz, 6-H), 7.53 (1H, d, J = 4.4 Hz, 8-H), 7.70 (1H, dd, J = 1.2 and 7.2 Hz, 7-H), 8.22 (1H, dd, J = 1.2 and 6.8 Hz, 5-H), 9.00 (1H, s, 2-H), 9.69 (1H, s, NH); (100 MHz, $CDCl_3$) 43.27 (C-2'), 162.41 (C-2), 118.39 (C-3), 124.17 (C-4a), 177.00 (C-4), 134.62 (C-7), 127.30 (C-5'and C-7'), 126.35 (C-4' and C-8'),



N-benzyl-6-bromo-4-oxo-4H-chromene-3-carboxamide 55B

The experimental procedure employed for the synthesis of N, N-dimethyl-4-oxo-4H-chromene-3-carboxamide was followed using 6-bromo-4-oxo-4H-chromene-3-carboxylic acid **52D** (0.81 g, 3 mmol), thionyl chloride (0.356 g, 3 mmol), triethylamine (0.91 g, 9 mmol) and benzylamine (0.38, 3 mmol). Work-up gave N-benzyl-6-bromo-4-oxo-4H-chromene-3-carboxamide **55B** (0.68 g, 64 %) as a white solid; m.p. 179-182 °C IR ν_{max}/cm^{-1} 3296.39 (NH), 1668.80 (C=O); δ_H (400 MHz, $CDCl_3$) 4.64 (2H, d, J = 5.6 Hz, 2'-H), 7.29 (1H, d, J = 11.6 Hz, 6'-H), 7.34 (4H, ddd, J = 4, 2 and 6 Hz, 4'-H, 8'-H, 7'-H and 5'-H), 7.45 (1H, d, J = 8.8 Hz, 8-H), 7.82 (1H, dd, J = 2.4 Hz and 6.4 Hz, 7-H), 8.37 (1H, d, J = 2.4 Hz, 5-H), 9.00 (1H, s, 2-H), 9.55 (1H, bs, NH); δ_C (100 MHz, $CDCl_3$) 43.37 (C-2'), 160.82 (C-2), 115.98 (C-3), 138.02 (C-3'), 175.93 (C-4), 125.55 (C-4a), 128.72 (C-5' and C-7'), 128.82 (C-6'), 120.33 (C-6), 137.67 (C-7), (C-4' and C-8'), 154.86 (C-8a), 162.58 (C-N).

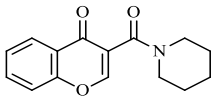
Synthesis of 4-oxo-N-phenyl-4H-chromene-3-carboxamide 56



The experimental procedure employed for the synthesis of **53A** was followed using 4-oxo-4H-chromene-3-carboxylic acid **52A** (0.56 g, 3 mmol), thionyl chloride (0.356 g, 3 mmol), triethylamine (0.91 g, 9 mmol) and aniline (0.33 g, 3 mmol). This solution was stirred at room temperature for 8 h. Work-up gave a solid, which was recrystallized from methanol to give 4-oxo-4-oxo-N-phenyl-4H-chromene-3-carboxamide **56** (0.49 g, 61 %) as a pale yellow solid; δ_H m.p. 224-226 °C (lit.,[104] 218-220 °C); IR ν_{max}/cm^{-1} 3081.17 (NH), 1672.62 (C=O), δ_H (400 MHz, $CDCl_3$) 7.14 (1H, t, J = 7.2 Hz, 8-H), 7.32 (2H, t, J = 8 Hz, phenyl-H), 7.77-7.81 (2H, m, 6-H), 7.75 (1H, d, J = 8 Hz, phenyl-H), 8.33 (1H, dd, J = 1.6 and 6.4 Hz, 5-H), 9.08 (1H, s, 2-H), 11.40 (1H, bs, NH); δ_C (100; MHz, $CDCl_3$) 160.71 (C-1'), 162.85(C-2), 137.98 (C-2') 120.54

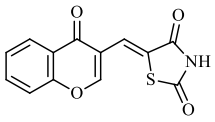
(C-3'), 115.99 (C-3), 129.01 (C-4'), 174.44 (C-4); 126.25 (C-4a), 126.52 (C-5), 124.47 (C-5'), 129.01 (C-6'), (C-6) 124.07 120.54 (C-7'), 134.89 (C-7), 118.53 (C-8), 156.17 (C-8a), , Anal. Calc. for $C_{16}H_{11}NO_3$: C 72.45; H 4.18; N 5.28. Found: C, 73.69; H, 4.31: N, 5.55

Synthesis of 3-(piperidine-1-carbonyl)-4H-chromen-4-one 57



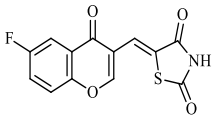
The experimental procedure employed for the synthesis of **53A** was followed using 4-oxo-4H-chromene-3-carboxylic acid (0.56 g, 3 mmol), thionyl chloride (0.356 g, 3 mmol), triethylamine (0.91 g, 9 mmol), piperidine (0.26 g, 3 mmol). Work-up gave 3-(piperidine-1-carbonyl)-4H-chromen-4-one **57** (0.44 g, 57 %) as a brown solid, m.p. 136-140 °C; IR ν_{max}/cm^{-1} 1716.31 (C=O), 1698.33 (C=O) δ_H (400 MHz, DMSO-d_6) 1.47 (6H, m, CH_2), 3.24 (2H, s, CH_2), 3.43 (2H, s, CH_2), 7.50 (1H, t, J = 7.2 Hz, 6-H), 7.66 (1H, d, J = 8 Hz, 8-H), 7.82 (1H, dd, J = 7.2 and 6.8 Hz, 7-H), 8.07 (1H, d, J = 7.6 Hz, 5-H), 8.51 (1H, s, 2-H); δ_C (100 MHz, DMSO-d_6); 24.39 (CH_2), 25.73 (CH_2), 26.45 (CH_2), 42.59 (CH_2), 48.10 (CH_2), 118.99 (CH), 125.69 (CH), 126.30 (CH), 135 (CH), 156.06 (C-2), 123.20 (C-3), 124.04 (C-4a), 156.19 (C-8a), 162.14 (C-N), 173.86 (C-4); Anal. Calc. for $C_{15}H_{15}NO_3$: C 70.02; H 5.88; N 5.44. Found: C 69.02; H 5.85: N 5.64.

Synthesis of 5-((4-oxo-4H-chromen-3-yl)methylene)thiazolidine-2,4-dione analogues[94] **59A-F**



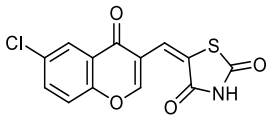
5-((4-oxo-4H-chromen-3-yl)methylene)thiazolidine-2,4-dione 59A

A mixture of 4-oxo-4H-chromene-3-carbaldehyde **41A** (1.74 g, 10 mmol) and thiazolidine-2,4-dione (1.17 g, 10 mmol), in glacial acetic acid (25 mL) and sodium acetate (0.2 g, 2.43 mmol), was heated under reflux for 2 h. The solid obtained after cooling was filtered, washed several times with water to give 5-((4-oxo-4H-chromen-3-yl)methylene)thiazolidine-2,4-dione **59A** (1.88 g, 68.8 %) as a yellow solid m.p. 320-322°C (lit.,[94] 319 °C and lit.,[5] 271); IR ν_{max}/cm^{-1} 1692.48 (C=O), 1636.18 (C=O), 1744.53 C=O); (400 MHz, DMSO-d_6) 7.49 (1H, t, J = 7.6 Hz , 6-H), 7.55 (1H, s, 1'-H), 7.65 (1H, d, J = 8.4, 8-H), 7.81 (1H, t, J = 7.6 Hz, 7-H), 8.06 (1H, d, J = 7.6 Hz), 8.76 (1H, s, 2-H), 12.53 (1H, bs, NH); δ_C (100 MHz, DMSO-d_6) 161.21 (C-2), 118.36 (C-3), 125.41 (C-2') 175.15 (C-4),), 169.49 (C-3') and 167.53 (C-3'), 123.37 (C-4a), 126.79 (C-5), 124.77 (C-6), 135.45 (C-7), 118.98 (C-8), 155.73 (C-8a; Anal. Calc. for $C_{13}H_7NO_4S$: C 57.14; H 2.58; N 5.13; S 11.73. Found: C 58.76; H 2.35: N 5.26; S 11.72.



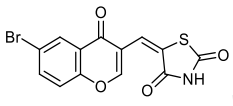
5-((6-fluoro-4-oxo-4H-chromen-3-yl)methylene)thiazolidine-2,4-dione 59B

A solution of thiazolidine-2,4-dione (0.59 g, 5 mmol), 6-fluoro-4-oxo-4H-chromene-3-carbaldehyde (0.96 g, 5 mmol) and a catalytic amount of piperidine in ethanol (15 mL) were refluxed for 16 h. The mixture was cooled to room temperature and the solid formed was filtered and washed with water to afford 5-((6-fluoro-4-oxo-4H-chromen-3-yl)methylene)thiazolidine-2,4-dione **59B** (1 g, 68.7 %); m.p. 304-309 °C (lit.,[5] 212-214°C) IR ν_{max}/cm^{-1} 3157 (NH), 3051.57 (CH aromatic), 1742.45 (C=O), 1683.07 (C=O) 1645.17 (C=O) *Did not dissolve in DMSO-d_6, $CDCl_3$ and acetic acid-d_4*; Anal. Calc. for $C_{13}H_6FNO_4S$; C 53.61; H 2.08; N 4.81; S 11.01. Found: C 55.65; H 2.25: N 4.79; S 9.46.



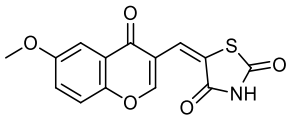
5-((6-chloro-4-oxo-4H-chromen-3-yl)methylene)thiazolidine-2,4-dione 59C

The experimental procedure employed for the synthesis of **59B** using 6-chloro-4-oxo-4H-chromene-3-carbaldehyde (1.04 g, 5 mmol), thiazolidine-2,4-dione (0.59 g, 5 mmol) and ethanol (15 mL) to afford 5-((6-chloro-4-oxo-4H-chromen-3-yl)methylene)thiazolidine-2,4-dione **59C** (1.11 g, 72.15 %). m.p 310-316 °C ,IR ν_{max}/cm^{-1} 3250.13 (NH), 3074.83 (CH, aromatic), 1743.12 (C=O), 1669.33 (C=O), 1644.19 (C=O) δ_H (400 MHz, DMSO-d_6) 7.55 (1H, s, 1'-H), 7.77-8.00 (3H, t, Ar-H), 12.51(1H, bs, NH); δ_C (100 MHz, DMSO-d_6) 124.92 (C-1'), 161.39 (C-2), 135.32 (C-2'), 118.46 (C-3), 169.36 (C-3'), 174.22 (C-4), 167.88 (C-3'), 124.60 (C-4a), 135.33 (C-5), 125.99 (C-6), 124.32 (C-7), 121.59 (C-8), 154.41 (C-8a).



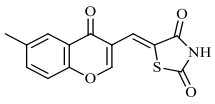
5-((6-bromo-4-oxo-4H-chromen-3-yl)methylene)thiazolidine-2,4-dione 59D

The experimental procedure employed for the synthesis of **59A** using 6-bromo-4-oxo-4H-chromene-3-carbaldehyde **41D** (1.26 g, 5 mmol), thiazolidine-2,4-dione (0.59 g, 5 mmol), sodium acetate (0.2 g, 2.43 mmol and glacial acetic acid (13 mL) to obtain 5-((6-bromo-4-oxo-4H-chromen-3-yl)methylene)thiazolidine-2,4-dione **59D** (1.3 g, 73.8 %). m.p. 300-302 °C (lit.,[5] 213-214 °C); IR ν_{max}/cm^{-1} 1728.24 (C=O), 1671.99 (C=O) and 1644.21 (C=O) *Did not dissolve in DMSO-d_6, pyridine d-$_5$ $CDCl_3$ and acetic acid-d_4*; Anal. Calc. for $C_{13}H_6BrNO_4S$: C 44.34; H 1.72; N 3.98; S 9.11. Found: C 46.58; H 1.74: N 4.22; S 9.21.



5-((6-methoxy-4-oxo-4H-chromen-3-yl)methylene)thiazolidine-2,4-dione 59E

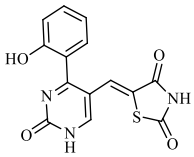
The experimental procedure employed for the synthesis of **59A** using 6-methoxy-4-oxo-4H-chromene-3-carbaldehyde **41E** (1.04 g, 5 mmol), thiazolidine-2,4-dione (0.59 g, 5 mmol), sodium acetate (0.2 g, 2.43 mmol) and glacial acetic acid (13 mL) to give 5-((6-methoxy-4-oxo-4H-chromen-3-yl)methylene)thiazolidine-2,4-dione (1.31 g, 86.39 %), m.p. 290-293 °C (lit.,[5] 204-205°C); IR ν_{max}/cm^{-1} 2970.62 (NH), 1693.40 (C=O), 1613.79 (C=O) δ_H (400 MHz, DMSO-d_6) 3.86 (3H, s, OCH_3), 7.39 (2H, m, 8-H and 7-H), 7.53 (1H, s, 1'-H), 7.62 (1H, dd, J = 4.8 and 5.2 Hz, 5-H), 8.73 (1H, s, 2-H), 12.40 (1H, bs, NH); δ_C (100 MHz, DMSO-d_6) 56.27 (OCH_3), 124.83 (C-1'), 160.86 (C-2), 125.29 (C-2'), 117.52 (C-3), 168.05 (C-3'), 174.79 (C-4), 124.10 (C-4a), 169.59 (C-3'), 120.60 (C-5), 150.50 (C-6), 124.50 (C-7), 105.54 (C-8), 157.56 (C-8a); Anal. Calc. for $C_{14}H_9NO_5S$; C 55.44; H 2.99; N 4.62; S 10.57. Found: C 55.39; H 3.11: N 4.79; S 10.53.



5-((6-methyl-4-oxo-4H-chromen-3-yl)methylene)thiazolidine-2,4-dione 59F

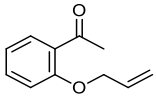
The experimental procedure employed for the synthesis of 5-((4-oxo-4H-chromen-3-yl)methylene)thiazolidine-2,4-dione using 6-methyl-4-oxo-4H-chromene-3-carbaldehyde (0.94 g, 5 mmol), thiazolidine-2,4-dione (0.59 g, 5 mmol), sodium acetate (0.2 g, 2.43 mmol and glacial acetic acid (13 mL) to attain 5-((6-methyl-4-oxo-4H-chromen-3-yl)methylene)thiazolidine-2,4-dione (0.92 g, 64.3 %) m.p. 255-260 °C (lit.,[5] 255-260 °C); IR ν_{max}/cm^{-1} 2924.36 (NH), 1729.33 (C=O), 1682.02 (C=O), 1651.48 (C=O); δ_H (400 MHz, DMSO-d_6) 2.42 (3H, s, CH_3), 7.56 (1H, d, J = 4.8 Hz, 8-H), 7.59 (1H, s, 2'- H), 7.64 (1H, m, 7-H), 7.86 (1H, s, 5-H), 8.75 (1H, s, 2-H) δ_C (100 MHz, DMSO-d_6) 20.90 (CH_3), 125.18 (C-1'), 161.02 (C-2), 118.19 (C-2'), 123.07 (C-3), 169.59 (C-3'), 125.35 (C-4a), 168.05 (C-3'), 175.08 (C-4), 136.4 C-5), 136.55 (C-6), 124.79 (C-7), 118.78 (C-8), 154.06 (C-8a); Anal. Calc. for $C_{14}H_9NO_4S$; C 58.53; H 3.16; N 4.88; S 11.16. Found: C 58.75; H 3.43: N 4.8; S 9.92.

Synthesis of 5-((4-(2-hydroxyphenyl)-2-oxo-1,2-dihydropyrimidin-5-yl)methylene)thiazolidine-2,4-dione analogue 60[94]



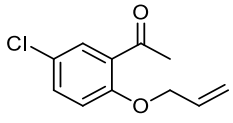
A solution of 5-((4-oxo-4H-chromen-3-yl)methylene)thiazolidine-2,4-dione **59A** (0.546 g, 2 mmol) and urea (0.12 g, 2 mmol) in ethanolic KOH (5 %, 30 mL) was refluxed for 6 h. After cooling the reaction mixture was poured onto ice/water and neutralized with dilute HCl. The formed precipitate was filtered, washed with water and crystallized from methanol to give5-((4-(2-hydroxyphenyl)-2-oxo-1,2-dihydropyrimidin-5-yl)methylene)thiazolidine-2,4-dione **60** m.p. 330-335 °C IR ν_{max}/cm^{-1} 3143 (NH), 3049.16 (OH), 2798.95 (NH), 1677.20 (C=O), 1732.72 (C=O); δ_H (400 MHz, DMSO-d_6) 7.47 (2H, m, 2-H and 11-H), 7.63 (1H, d, J = 8.4 Hz, 4-H), 7.78 (1H, m, 5-H), 8.04 (1H, d, J = 8 Hz, 3-H), 8.74 (1H, s, H pyrimidine), 12.39 (1H, s, N-H); δ_C (100 MHz, DMSO-d_6) 161.30 (CH), 135.53 (CH), 126.86 (CH), 125.97 (CH), 124.87 (CH), 119.04 (CH), 175.22 (C=O), 169.47 (C=O), 167.86 (C=O).

Synthesis of 1-(2-(allyloxy)phenyl)ethanone analogues 61A-E[39]



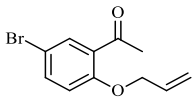
1-(2-(allyloxy)phenyl)ethanone 61A

A solution of 2-hydroxyacetophenone **14A** (10 mL, 82.86 mmol), K_2CO_3 (13.03 g, 94.27 mmol) and allyl bromide (8 mL, 92.56 mmol) in 60 mL anhydrous acetone was refluxed for 20 h. The resulting mixture was cooled to room temperature and the solid material was filtered out and the filtrate was concentrated under reduced pressure to remove acetone. The resultant was taken in water and extracted by EtOAc (2 x 25 mL); the combined organic layers were dried over anhydrous $MgSO_4$ then concentrated under reduced pressure. The product was taken in diethyl ether and washed with 1M KOH (2 x 15 mL), the combined organic layers dried over Na_2SO_4 and concentrated under reduced pressure to afford 1-(2-(allyloxy)phenyl)ethanone **61A** (7.11 g, 48 %) as a as a pale yellow oil; IR ν_{max}/cm^{-1} 1727.77 (C=O); δ_H (400 MHz, $CDCl_3$) 2.47 (3H, s, CH_3), 4.43 (2H, d, J = 5.2 Hz, 2'-H), 5.13 (1H, d, J = 10.4 Hz, 4'-H), 5.25 (1H, d, J = 17.2, 4'-H), 5.86 (1H, m, 3'-H), 6.76 (2H, m, 3-H and 5-H), 7.23 (1H, dd, J = 8.8, and 7.6 Hz, 6-H), 7.57 (1H, d, J = 7.6 Hz, 4-H): δ_C (100 MHz, $CDCl_3$) 31.84 (CH_3), 128.35 (C-1), 199.28 (C-1'), 157.83 (C-2), 69.18 (C-2'), 112.74 (C-3), 132.59 (C-3'), 133.49 (C-4), 117.92 (C-4'), 120.56 (C-5), 130.20 (C-6).



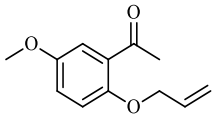
1-(2-(allyloxy)-5-chlorophenyl)ethanone 61B

The experimental procedure employed for the synthesis of **61A** was followed using 5-chloro-2-hydroxyacetophenone **14C** (4.78 g, 28 mmol), dry acetone (30 mL), potassium carbonate (6.516 g, 47.15 mmol) and allyl bromide (4 mL, 46.28 mmol). Work-up afforded 1-(2-(allyloxy)-5-chlorophenyl)ethanone **61B** (3.81 g, 64.6 %) as a colourless oil; IR ν_{max}/cm^{-1} 2931.66 (CH aromatic), 1663.51 (C=O), 1148.84 (C-O, alkoxy stretch); δ_H (400 MHz, $CDCl_3$) 2.5 (3H, s, CH_3), 4.55 (2H, m, 2'-H), 5.24 (2H, m, 4'-H), 6.80 (1H, d, J = 8.8 Hz, 3-H), 7.28 (1H, dd, J = 2.8 and 9.2 Hz, 4-H), 7.61 (1H, d, J = 2.8 Hz, 6-H); δ_C (100 MHz, $CDCl_3$) 31.89 (CH_3), 129.55 (C-1), 198.37 (C-1'), 156.43 (C-2), 69.77 (C-2'), 114.31 (C-3), 132.17 (C-3'), 133.07 (C-4), 118.62 (C-4'), 126.11 (C-5), 130.07 (C-6).



1-(2-(allyloxy)-5-bromophenyl)ethanone 61C

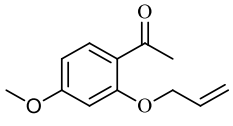
The experimental procedure employed for the synthesis of **61A** was followed using 5-bromo-2-hydroxyacetophenone **14D** (6.02 g, 28 mmol), dry acetone (30 mL), potassium carbonate (6.516 g, 47.15 mmol) and allyl bromide (4 mL, 46.28 mmol). Work-up afforded 1-(2-(allyloxy)-5-bromophenyl)ethanone **61C** (4.55 g, 63.7 %); IR ν_{max}/cm^{-1} 2929.78 (CH aromatic), 1659.25 (C=O), 1150.84 (C-O, alkoxy stretch); δ_H (400 MHz, $CDCl_3$) 2.62 (3H, s, CH_3), 4.61 (2H, d, J = 5.6 Hz, 2'-H), 5.32 (1H, dd, J = 1.2 and 10.4 Hz, 4'-H), 5.40 (1H, m, 4'-H), 6.03 (1H, m, 3'-H), 6.82 (1H, d, J = 8.8 Hz, 3-H), 7.49 (1H, dd, 2.4 and 8.8 Hz, 4-H), 7.82 (1H, d, J = 2.4 Hz, 6-H); δ_C (100 MHz, $CDCl_3$) 31.88 (CH_3), 129.90 (C-1), 198.24 (C-1'), 156.91 (C-2), 69.70 (C-2'), 114.75 (C-3), 132.94 (C-3'), 135.98 (C-4), 118.62 (C-4') 113.25 (C-5), 132.11 (C-6).



1-(2-(allyloxy)-5-methoxyphenyl)ethanone 61D

The experimental procedure employed for the synthesis of **61A** was followed using 5-methoxy-2-hydroxyacetophenone (6.02 g, 28 mmol), dry acetone (30 mL), potassium carbonate (6.516 g, 47.15 mmol) and allyl bromide (4 mL, 46.28 mmol). Work-up afforded 1-(2-(allyloxy)-5-methoxyphenyl)ethenone **61D** (4.6 g, 79.72 %) as a colourless oil; IR ν_{max}/cm^{-1} 2999.08 (CH aromatic), 1668.25 (C=O); δ_H (400 MHz, $CDCl_3$) 2.52 (3H, s, CH_3), 3.65 (3H, s, OCH_3), 4.45 (2H, d, J = 5.2 Hz, 2'-H), 5.17 (1H, d, J = 10.4 Hz, 4'-H), 5.28 (1H, d, J = 17.2 Hz, 4'-H), 5.93 (1H, m, 3'-H), 6.76 (1H, d, 8.8 Hz, 3-H), 6.87 (1H, dd, J = 3.2 and 8.8 Hz, 4-H), 7.16 (1H, d, J = 3.2 Hz, 6-H); δ_C (100 MHz, $CDCl_3$) 31.97 (CH_3), 55.63 (OCH_3), 128.67 (C-1), 199.20 (C-1'),

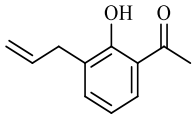
152.40 (C-2), 69.99 (C-2'), 113.69 (C-3), 132.90 (C-3'), 120.14 (C-4), 117.91 (C-4'), 153.45 (C-5), 114.54 (C-6).



1-(2-(allyloxy)-4-methoxyphenyl)ethanone 61E

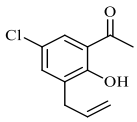
The experimental procedure employed for the synthesis of **61A** was followed using. 5-methoxy-2-hydroxyacetophenone (6.02 g, 28 mmol), dry acetone (30 mL), potassium carbonate (6.516 g, 47.15 mmol) and allyl bromide (4 mL, 46.28 mmol). Work-up afforded 1-(2-(allyloxy)-5-methoxyphenyl)ethenone **61E** (4.04 g, 70 %) as a yellow oil; IR ν_{max}/cm^{-1} 1664.44 (C=O); δ_H (400 MHz, Py-d_5) 2.47 (3H, s, CH_3), 4.56 (2H, d, J = 5.2 Hz, 2'-H), 5.26 (2H, m, 4'-H), 6.09 (1H, m, 3'-H), 6.61 (2H, m, 3-H and 5-H), 8.05 (1H, d, J = 8 Hz, 6-H): δ_C (100 MHz, Py-d_5) 32.02 (CH_3), 55.32 (OCH_3), 69.39 (C-2'), 117.87 (C-4'), 196.35 (C=O).

Synthesis of 1-(3-allyl-2-hydroxyphenyl)ethanone analogues 62A-D



1-(3-allyl-2-hydroxyphenyl)ethanone 62A

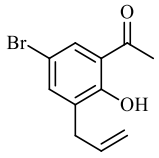
1-(2-(allyloxy)phenyl)ethanone **61A** (7.11 g , 40.34 mmol) was heated at 260 °C for 0.5 h. After cooling, 50 mL of ether was added and the product was extracted with 10 % KOH (3 x 10 mL). The organic phase was acidified with concentrated HCl (7 mL), extracted with ether (3 x 25 mL) and the combined organic phases were dried over Na_2SO_4. The combined organic layers were concentrated under reduced pressure to afford 1-(3-allyl-2-hydroxyphenyl)ethenone **62A** (4.34 g, 61 %); δ_H (400MHz, $CDCl_3$) 2.55 (3H, s, CH_3), 3.34 (2H, d, J = 6.4 Hz, 2'-H), 4.9 (2H, m, 4'-H), 5.87 (1H, m, 3'-H), 6.75 (1H, t, 7.6 Hz, 5-H), 7.27 (1H, d, J = 7.2 Hz, 6-H), 7.52 (1H, t, 7.6 Hz, 4-H), 12.53 (1H, s, OH); δ_C (100 MHz, $CDCl_3$) 26.84 (CH_3), 204.79 (C-1'), 115.99 (C-1), 160.38 (C-2), 33.4 (C-2'), 119.22 (C-3), 136.47 (C-3'), 136.09 (C-4), 116 (C-4'), 128.83 (C-5), 132.88 (C-6).



1-(3-allyl-5-chloro-2-hydroxyphenyl)ethenone 62B

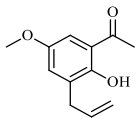
The experimental procedure employed for the synthesis of **62A** was followed using 1-(2-(allyloxy)-5-chlorophenyl)ethenone **61B** (3 g, 14.24 mmol). Work-up afforded 1-(3-allyl-5-chloro-2-hydroxyphenyl)ethenone **62B** (2.77 g, 92 %); δ_H (400 MHz, Py-d_5) 2.54 (3H, s, CH_3),

3.40 (2H, d, J = 6.8 Hz, 2'-H), 5.11 (2H, m, 4'-H), 5.98 (1H, m, 3'-H), 7.38 (1H, d, J = 2.4 Hz, 4-H), 7.73 (1H, d, J = 2.4 Hz, 6-H); δ_C (100 MHz, Py-d_5) 26.56 (CH_3), 204.57 (C-1'), 122.98 (C-1), 159.88 (C-2), 33.25 (C-2'), 131.54 (C-3), 135.83 (C-3'), 135.46 (C-4), 116.71 (C-4'), 120.01 (C-5), 128.37 (C-6).



1-(3-allyl-5-bromo-2-hydroxyphenyl)ethenone 62C

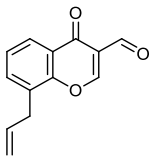
The experimental procedure employed for the synthesis of **62A** was followed using 1-(2-(allyloxy)-5-bromophenyl)ethanone **61C** (4 g, 15.68 mmol). Work-up afforded 1-(3-allyl-5-bromo-2-hydroxyphenyl)ethenone **62C** (3.6 g, 90 %);) as yellow oil ; δ_H (400 MHz, Py-d_5) 2.54 (3H, s, CH_3), 3.40 (2H, m, 2'-H), 5.09 (2H, m, 4'-H), 5.95 (1H, m, 3'-H), 7.52 (1H, d, J = 2.4 Hz, 4-H), 7.87 (1H, d, J = 2.4 Hz, 6-H), 12.87 (1H, s, OH); δ_C (100 MHz, Py-d_5); 26.56 (CH_3), 204.53 (C-1'), 120.65 (C-1), 159.30 (C-2), 33.25 (C-2'), 123.51 (C-3), 138.60 (C-3'), 135.48 (C-4), 116.71 (C-4'), 135.48 (C-4), 115.53 (C-5), 131.41 (C-6).



1-(3-allyl-2-hydroxy-5-methoxyphenyl)ethanone 62D

The experimental procedure employed for the synthesis of **62A**was followed using 1-(2-(allyloxy)-5-methoxyphenyl)ethenone **61D** (4 g, 19.39 mmol). Work-up gave 1-(3-allyl-2-hydroxy-5-methoxyphenyl)ethanone **62D** (3.4 g, 85 %) as a yellow oil; δ_H (400 MHz, $CDCl_3$) 2.58 (3H, s, CH_3), 3.77 (3H, s, OCH_3), 3.37 (2H, d, J = 6.8 Hz, 2'-H), 5.09 (2H, m, 4'-H), 5.96 (1H, m, 3'-H), 6.98 (1H, d, J = 2.8, 4-H), 7.00 (1H, d, J = 3.2 Hz, 6-H), 12.24 (1H, s, OH) δ_C (100 MHz, $CDCl_3$) 26.80 (CH_3), 55.76 (OCH_3), 130.59 (C-1), 204.23 (C-1') 151.17 (C-2), 33.51 (C-2'), 118.62 (C-3), 124.40 (C-3'), 116.30 (C-4'), 110.95 (C-4), 154.92 (C-5), 135.82 (C-6).

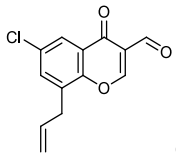
Synthesis of 8-allyl-4-oxo-4H-chromene-3-carbaldehyde analogues 63A-D



8-allyl-4-oxo-4H-chromene-3-carbaldehyde 63A

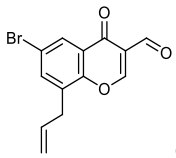
The experimental procedure employed for the synthesis of 4-oxo-4H-chromene-3-carbaldehyde was followed using 1-(3-allyl-5-bromo-2-hydroxyphenyl)ethanone (3.39 g, 22.6 mmol), $POCl_3$ (13.8 mL, 144 mmol), DMF (25 mL). Work-up afforded 6-methyl-4-oxo-4H-chromene-3-

carbaldehyde m.p. 70-75 °C (lit.,[98] 73-74 °C); IR ν_{max}/cm^{-1} 1651.67(C=O), 1744.54 (C=O); δ_H (400 MHz, Py-d_5) 10.57 (1H, s, CHO), 8.76 (1H, s, 2-H), 8.22 (1H, d, J = 7.6 Hz, 5-H), 7.48 (1H, d, J = 7.2 Hz, 6-H), 7.32 (1H, t, J = 7.6 Hz, 7-H), 5.93 (1H, m, 3'-H), 5.12 (2H, m, 4'-H), 3.50 (2H, d, J = 6.4 Hz, 2'-H); δ_C (100 MHz, Py-d_5) 33.25 (C-2'), 161.18 (C-2), 135.25 (C-3'), 116.97 (C-4'), 175.70 (C-4), 135 (C-5), 123.91 (C-6), 126.21 (C-7), 188.27 (CHO),



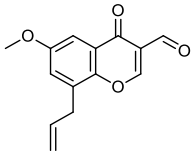
8-allyl-6-chloro-4-oxo-4H-chromene-3-carbaldehyde 63B

The experimental procedure employed for the synthesis of **41A** was followed using 1-(3-allyl-5-chloro-2-hydroxyphenyl)ethanone **62B** (3.39 g, 22.6 mmol), $POCl_3$ (13.8 mL, 144 mmol) and DMF (25 mL). Work-up afforded 6-methyl-4-oxo-4H-chromene-3-carbaldehyde m.p. 90-94 °C ; IR ν_{max}/cm^{-1} 1631.34 (C=O), 1722 (C=O); δ_H (400 MHz, $CDCl_3$) 3.55 (2H, d, J = 6.8 Hz, 2'-H), 5.05 (1H, dd, J = 1.2, 15.6 Hz, 4'-H), 5.12 (1H, dd, 1.2 and 9.2 Hz, 4'-H), 5.83 (1H, m, 3'-H), 8.49 (1H, s, 2-H); δ_C (100 MHz, $CDCl_3$) 33.18 (C-2'), 160.30 (C-2), 135.10 (C-3'), 120.07 (C-3), 126.40 (C-4a), 123.59 (C-5), 132.57 (C-6), 133.75 (C-7), 132.50 (C-8), 152.64 (C-8a), 188.21 (CHO); Anal. Calc. for $C_{13}H_9ClO_3$; C 62.79; H 3.65. Found: C 58.30; H 3.81.



8-allyl-6-bromo-4-oxo-4H-chromene-3-carbaldehyde 63C

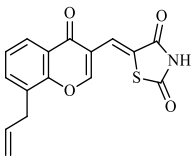
The experimental procedure employed for the synthesis of 4-oxo-4H-chromene-3-carbaldehyde was followed using 1-(3-allyl-5-bromo-2-hydroxyphenyl)ethanone **62C** (3.39 g, 22.6 mmol), $POCl_3$ (13.8 mL, 144 mmol), DMF (25 mL). Work-up afforded 6-methyl-4-oxo-4H-chromene-3-carbaldehyde m.p. 110-111 °C IR ν_{max}/cm^{-1} 1697.66 (C=O), 1734.11 (C=O); δ_H (400 MHz, $CDCl_3$) 3.54 (2H, d, J = 6.8 Hz, 2'-H), 5.01 (1H, dd, J = 1.2 and 9.6 Hz, 4'-H), 5.08 (1H, dd, J = 1.2 Hz and 9.6 Hz, 4'-H), 5.83 (1H, m, 3'-H), 7.21-8.15 (2H, m, 5-H and 7-H), 8.48 (1H, s, 2-H), 10.25 (1H, s, CHO) δ_C (100 MHz, $CDCl_3$) 33.12 (C-2'), 160.36 (C-2), 120.11 (C-3), 137.84 (C-3'), 118.23 (C-4'), 174.87 (C-4), 126.60 (C-4a), 126.70 (C-5), 116.90 (C-6), 135.08 (C-7), 130.83 (C-8), 153.05 (C-8a), 188.15 (CHO); Anal. Calc. for $C_{13}H_9BrO_3$ C 53.27; H 3.09. Found: C 52.46; H 3.59.



8-allyl-6-methoxy-4-oxo-4H-chromene-3-carbaldehyde 63D

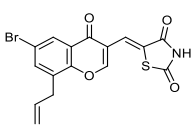
The experimental procedure employed for the synthesis of **41A** was followed using 1-(3-allyl-5-methoxy-2-hydroxyphenyl)ethanone **62D** (3.39 g, 22.6 mmol), $POCl_3$ (13.8 mL, 144 mmol), DMF (25 mL). Work-up afforded 6-methyl-4-oxo-4H-chromene-3-carbaldehyde m.p. 142-148 °C IR ν_{max}/cm^{-1} 3060.97 (CH), 1693.40 (C=O), 1649.12 (C=O) δ_H (400 MHz, $CDCl_3$) 3.62 (2H, d, J = 6.4 Hz), 3.92 (3H, s, OCH_3), 5.11 (2H, m, 4'-H), 5.94 (1H, m, 3'-H), 7.19 (1H, d, J = 3.2, 5-H), 7.53 (1H, d, J = 3.2 Hz, 7-H); δ_C (100 MHz, $CDCl_3$) 55.95 (OCH_3), 159.84 (C-2), 33.37 (C-2'), 119.43 (C-3), 134.42 (C-3'), 176.10 (C-4), 117.56 (C-4'), 126.32 (C-4a), 103.55 (C-5), 149.25 (C-6), 124.64 (C-7), 132.03 (C-8), 157.58 (C-8a), 188.87 (CHO); Anal. Calc. for $C_{14}H_{12}O_4$; C 68.85; H 4.95. Found: C 67.1; H 5.17.

Synthesis of 5-((8-allyl-4-oxo-4H-chromen-3-yl)methylene)thiazolidine-2,4-dione analogues 64A-D



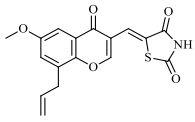
5-((8-allyl-4-oxo-4H-chromen-3-yl)methylene)thiazolidine-2,4-dione 64A

The experimental procedure employed for the synthesis of 5-((4-oxo-4H-chromen-3-yl)methylene)thiazolidine-2,4-dione **59A** using 8-allyl-4-oxo-4H-chromene-3-carbaldehyde **63A** (0.54 g, 2.5 mmol), thiazolidine-2,4-dione (0.3 g, 2.5 mmol), sodium acetate (0.1 g, 1.20 mmol and glacial acetic acid (13 mL) to give 5-((8-allyl-4-oxo-4H-chromen-3-yl)methylene)thiazolidine-2,4-dione **64A** (0.26 g, 56.23 %) m.p. 265-267 °C; IR ν_{max}/cm^{-1} 3057.82 (NH), 2808.22 (CH), 1730.77 (C=O), 1675.31(C=O), 1650.23 (C=O); δ_H (400 MHz, DMSO-d_6) 12.53 (1H, s, NH) 8.83 (1H, s, 2-H), 8.03 (1H, m, 5-H), 7.75 (1H, d, J = 7.2 Hz, 7-H), 7.65 (1H, s, 1'-H), 7.52 (1H, t, J = 7.6 Hz, 6-H), 6.05 (1H, m, 11'-H), 5.14 (2H, dt, J = 8.4 and 9.6 Hz), 3.68 (2H, d, J = 6.4 Hz), δ_C (100 MHz, DMSO-d_6) 160.90 (C-2), 135.89 (C-10), 135.44 (C-7), 126.50 (CH), 124.55 (CH), 124.16 (C), 117.39 (C-11), 33.25 (C-9), 153.80 (C-8a), 169.63 (C-3'), 168.25 (C-4'), 175.38 (C-4); Anal. Calc. for $C_{16}H_{11}NO_4S$: C, 61.33; H, 3.54; N, 4.47; S, 10.23. Found: C, 61.57; H, 3.37: N, 4.57; S, 10.21.



5-((8-allyl-6-bromo-4-oxo-4H-chromen-3-yl)methylene)thiazolidine-2,4-dione 64C

The experimental procedure employed for the synthesis of **59A** using 8-allyl-6-bromo-4-oxo-4H-chromene-3-carbaldehyde **63C** (0.73 g, 2.5 mmol), thiazolidine-2,4-dione (0.3 g, 2.5 mmol), sodium acetate (0.1 g, 1.20 mmol and glacial acetic acid (13 mL) to afford 5-((8-allyl-6-bromo-4-oxo-4H-chromen-3-yl)methylene)thiazolidine-2,4-dione **64C** (0.26 g, 56.23 %) as a pale yellow solid m.p. 265-267 °C IR ν_{max}/cm^{-1} 3118.42 (NH), 1728.08 (C=O), 1681.92 (C=O); *(Did not dissolve in deuterated solvent).* Anal. Calc. for $C_{16}H_{10}BrNO_4S$: C 49.00; H 2.57; N 3.57; S 8.18. Found: C, 49.92; H, 2.77: N, 5.55; S, 6.57



5-((8-allyl-6-methoxy-4-oxo-4H-chromen-3-yl)methylene)thiazolidine-2,4-dione 64D

The experimental procedure for the synthesis of **59A** was employed by using 8-allyl-6-methoxy-4-oxo-4H-chromene-3-carbaldehyde **63C** (0.94 g, 5 mmol), thiazolidine-2,4-dione (0.59 g, 5 mmol), sodium acetate (0.2 g, 2.43 mmol and glacial acetic acid (13 mL) to give 5-((8-allyl-6-methoxy-4-oxo-4H-chromen-3-yl)methylene)thiazolidine-2,4-dione **64D** (0.92 g, 64.3 %), m.p. 154-157 °C; IR ν_{max}/cm^{-1} 1651 3061.97 (CH), 1663.40 (C=O), 1649.12 (C=O); δ_H (400 MHz, DMSO-d_6) 3.39 (3H, s, OCH_3), 3.60 (2H, m, 9-H), 5.10 (2H, d, m, 11-H), 6.02 (1H, d, J= 6.8 Hz, 10-H), 7.56 (1H, s, 1'-H), 7.45 (1H, d, J =7.2 Hz, Ar-H), 7.66 (1H, d, J = 5.6 Hz, Ar-H), 7.94 (1H, d, J = 7.2 Hz, Ar-H), 8.79 (1H, s, 2-H), 12.44 (1H, bs, NH) δ_C (100 MHz, DMSO-d_6) 55.3 (OCH_3), 33.23 (C-9), 117.42 (C-11), 124.13 (C-3), 124.77 (CH), 124.46 (CH), 135.83 (CH), 135.37 (CH), 126.93 (C-2), 169.50 (C-3'), 167.89 (C-4'), 175.31 (C-4) 153.75 (C-8a).

Biological activities

Trypanosoma brucei Assay – Single Concentration Screen

To assess trypanocidal activity, compounds were added to cultures of *T.b. brucei* in 96-well plates at a fixed concentration of 20 µM. After a 48-hour incubation, parasites surviving drug treatment were enumerated by adding a resazurin-based reagent. Resazurin is reduced to resorufin (a fluorophore (Exc_{560}/Em_{590})) in viable cells and was thus quantified in a Spectramax M3 microplate. Results were expressed as % parasite viability.

IC_{50} results were expressed in µg/mL.

Cytotoxicity Assay – Single Concentration Screen

To assess the overall cytotoxicity, compounds were incubated at a fixed concentration of 20 µM in 96-well plates containing Hela (human cervix adenocarcinoma) cells for 48 hours. The numbers of cells surviving drug treatment were determined using the resazurin-based reagent and reading resorufin fluorescence in a Spectramax M3 microplate reader. Results were expressed as % viability.

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Appendix

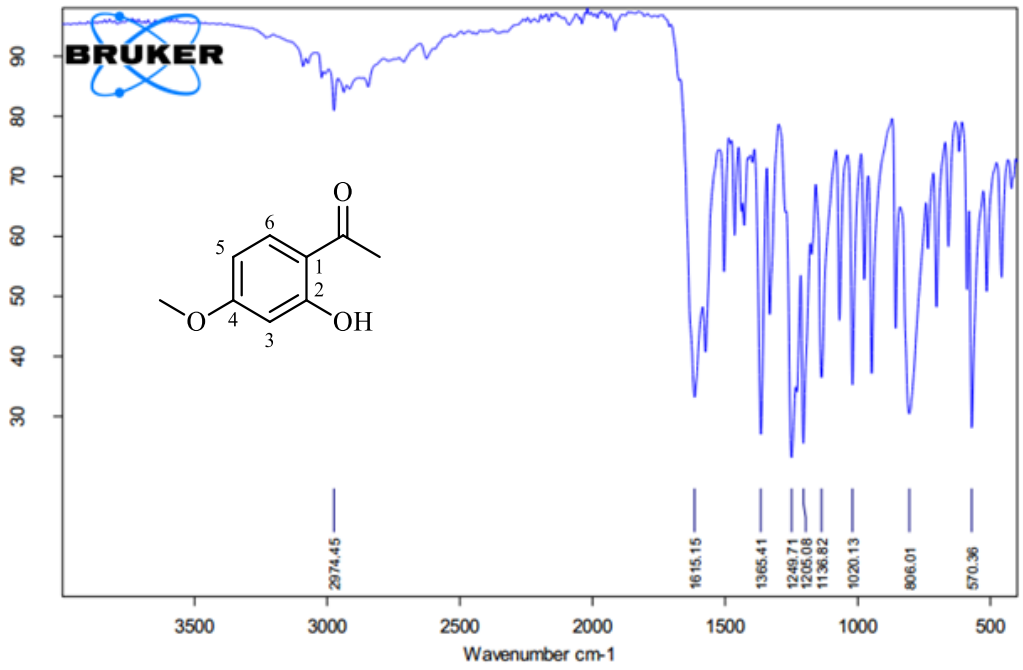

Figure 32: IR spectrum of 2-hydroxy-4-methoxy-acetophenone **14H**

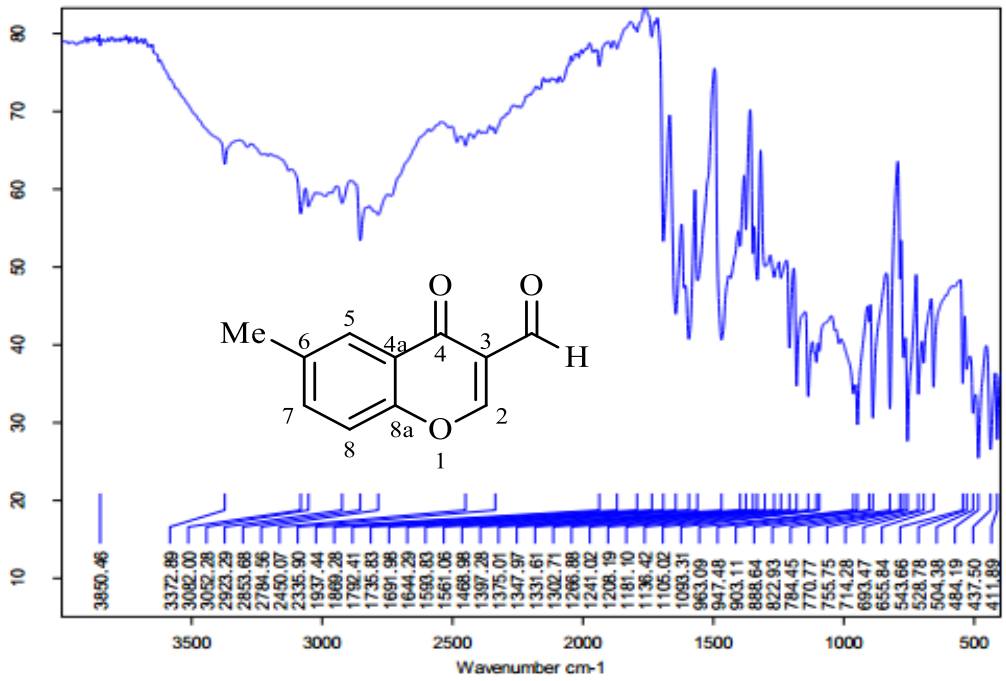

Figure 33: IR spectrum of 6-methyl-4-oxo-4H-chromene-3-carbaldehyde **41F**

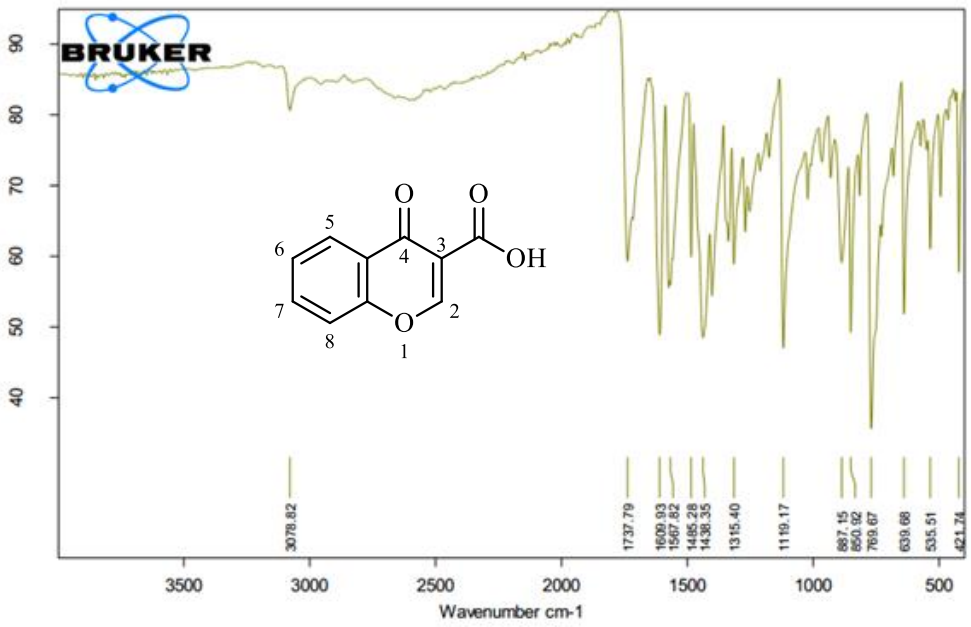

Figure 34: IR spectrum of 4-oxo-4H-chromene-3-carboxylic acid **52A**

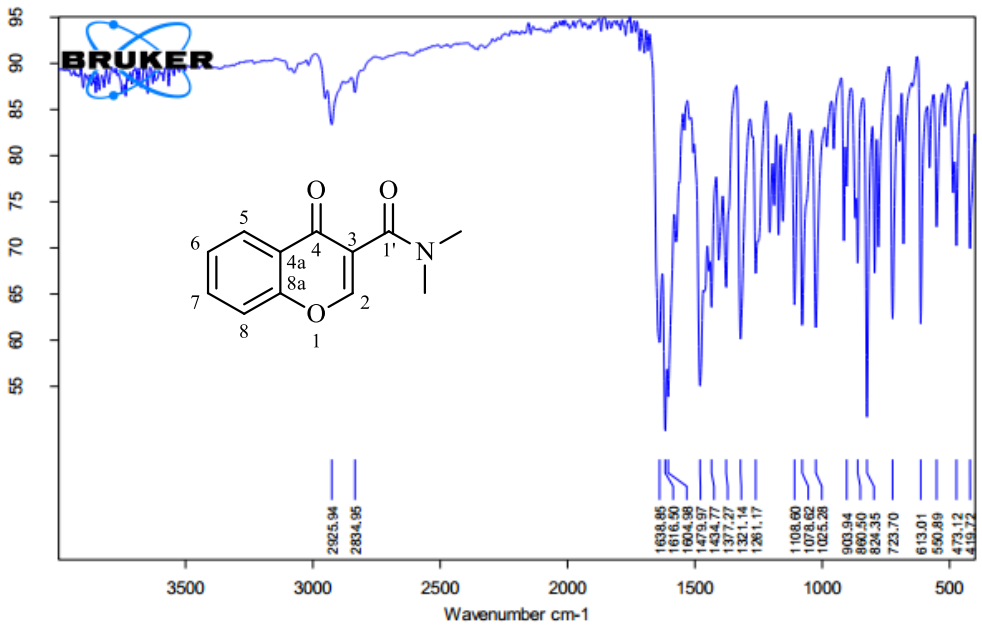

Figure 35: IR spectrum of N, N-dimethyl-4-oxo-4H-chromene-3-carboxamide **53E**

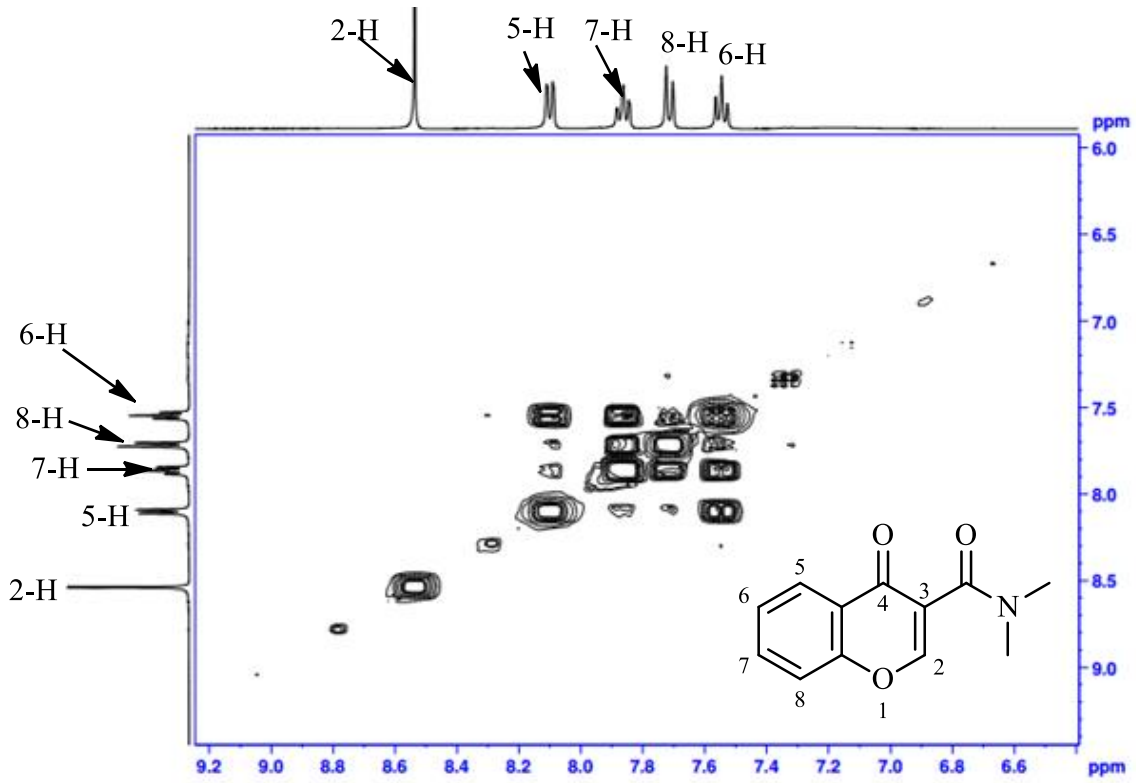

Figure 36: COSY spectrum (aromatic region) of N, N-dimethyl-4-oxo-4H-chromene-3-carboxamide **53A** in $CDCl_3$

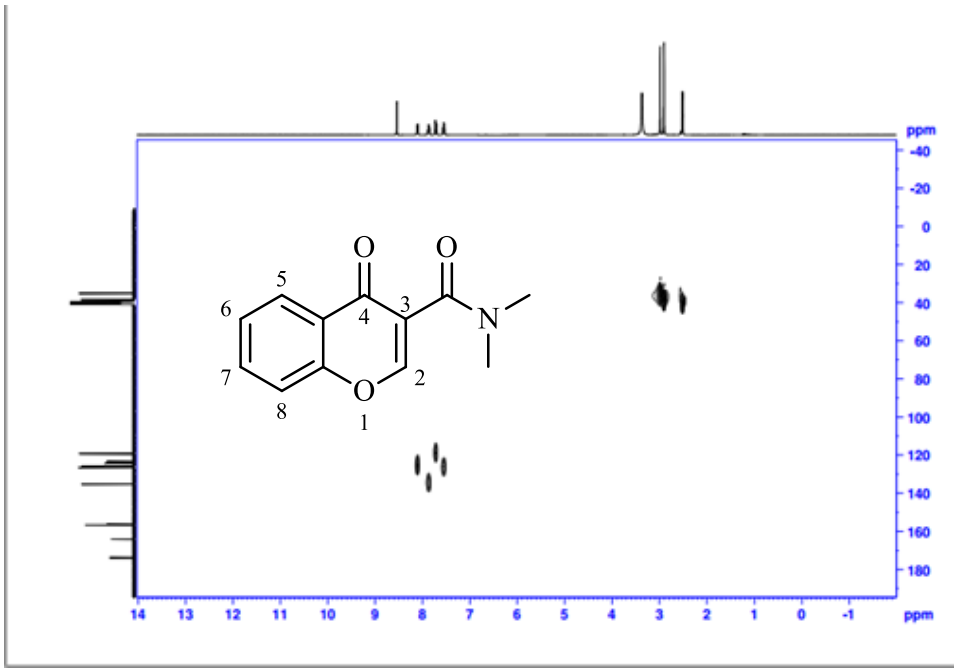

Figure 37: HSQC spectrum of N, N-dimethyl-4-oxo-4H-chromene-3-carboxamide **53A** in $CDCl_3$

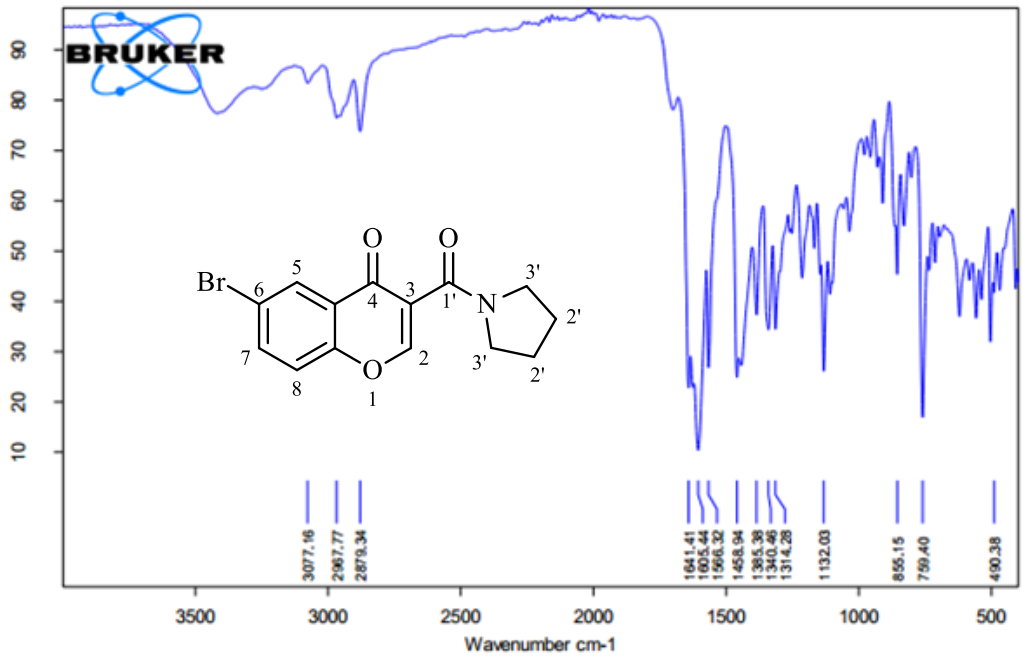

Figure 38: IR spectrum of 6-bromo-3-(pyrrolidine-1-carbonyl)-4H-chromen-4-one **54B**

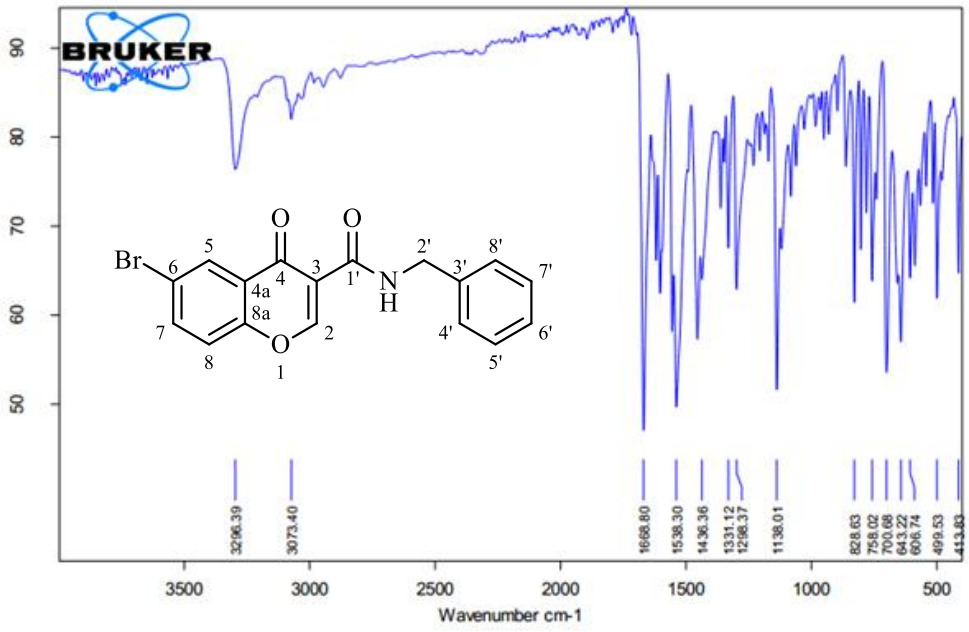

Figure 39: IR spectrum of N-benzyl-6-bromo-4-oxo-4H-chromene-3-carboxamide **55B**

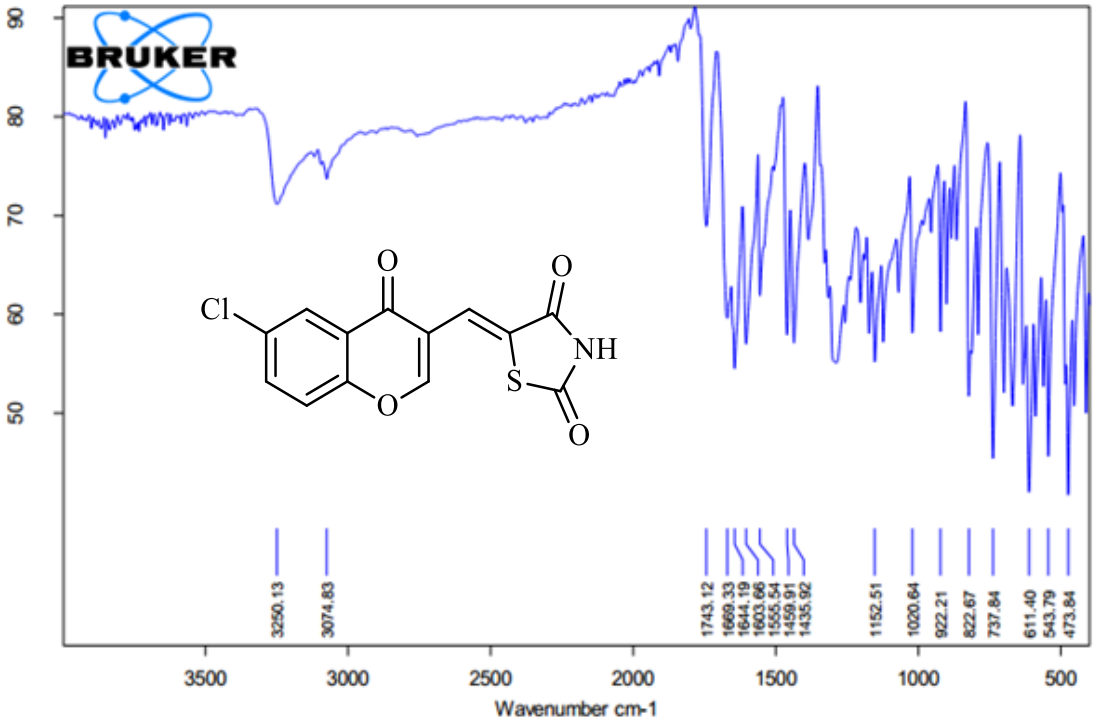

Figure 40: IR spectrum of 5-((6-chloro-4-oxo-4H-chromen-3-yl)methylene)thiazolidine-2,4-dione **59C**

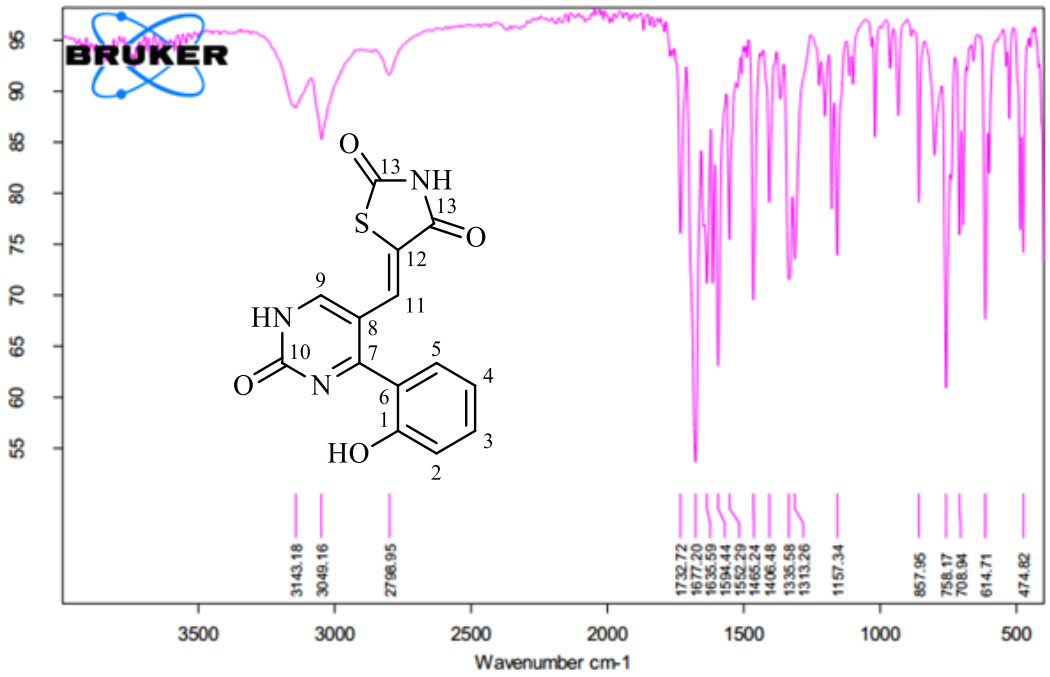

Figure 41: IR spectrum of 5-((4-(2-hydroxyphenyl)-2-oxo-1,2-dihydropyrimidin-5-yl)methylene)thiazolidine-2,4-dione **60**

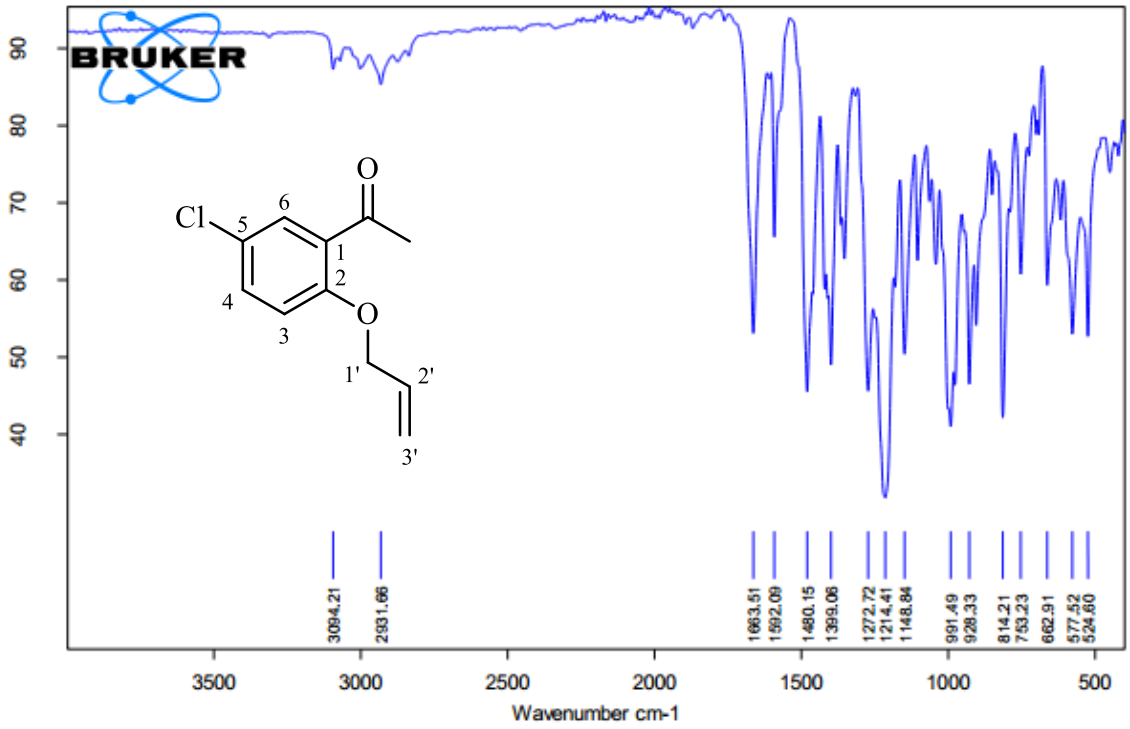

Figure 42: IR spectrum of 1-(2-(allyloxy)-5-chlorophenyl)ethanone **61B**

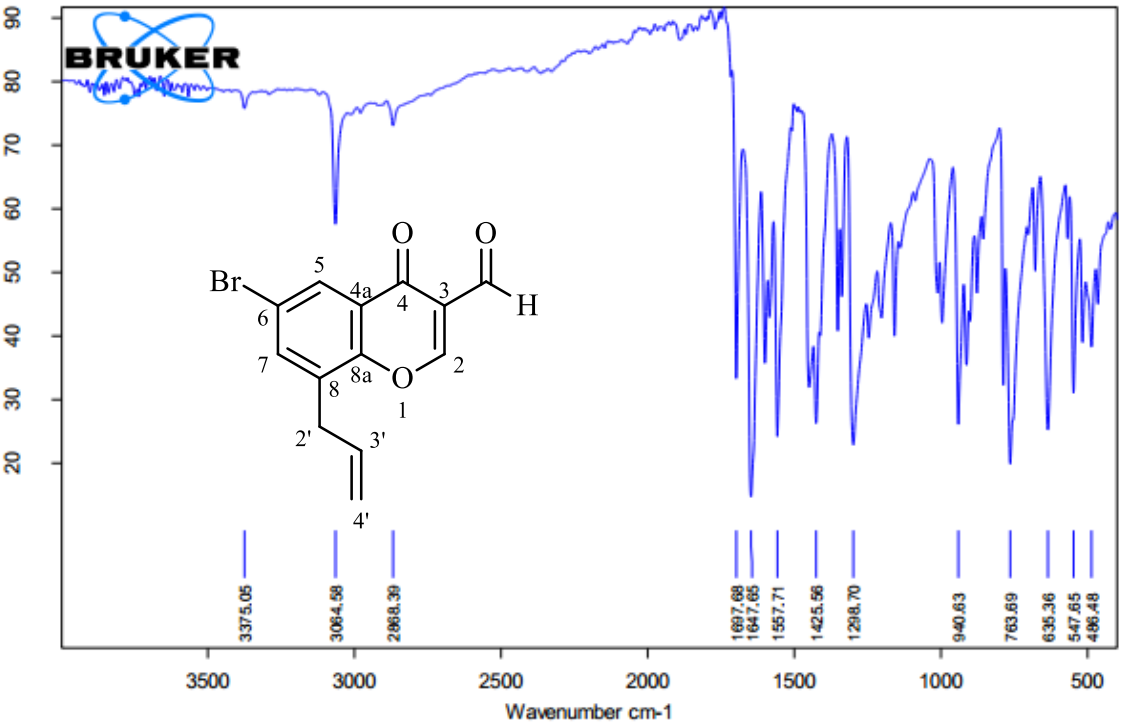

Figure 43: IR spectrum of 8-allyl-6-bromo-4-oxo-4H-chromene-3-carbaldehyde **63C**

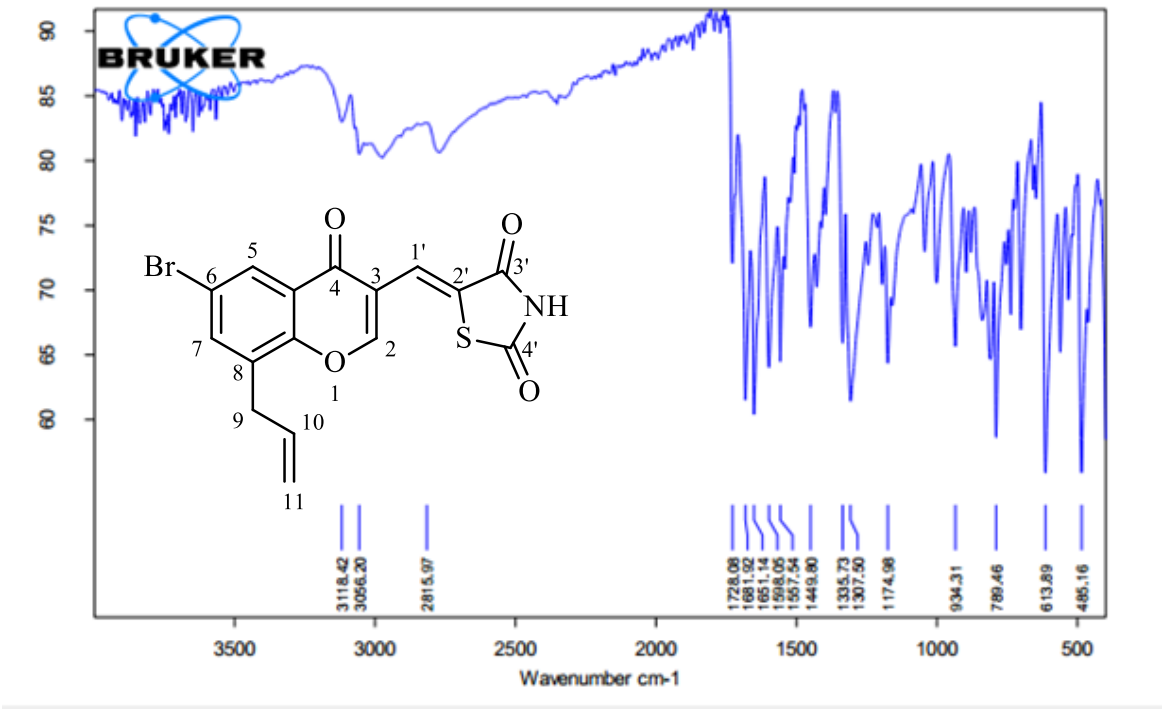

Figure 44: IR spectrum of 5-((8-allyl-6-bromo-4-oxo-4H-chromen-3-yl)methylene)thiazolidine-2,4-dione **64C**

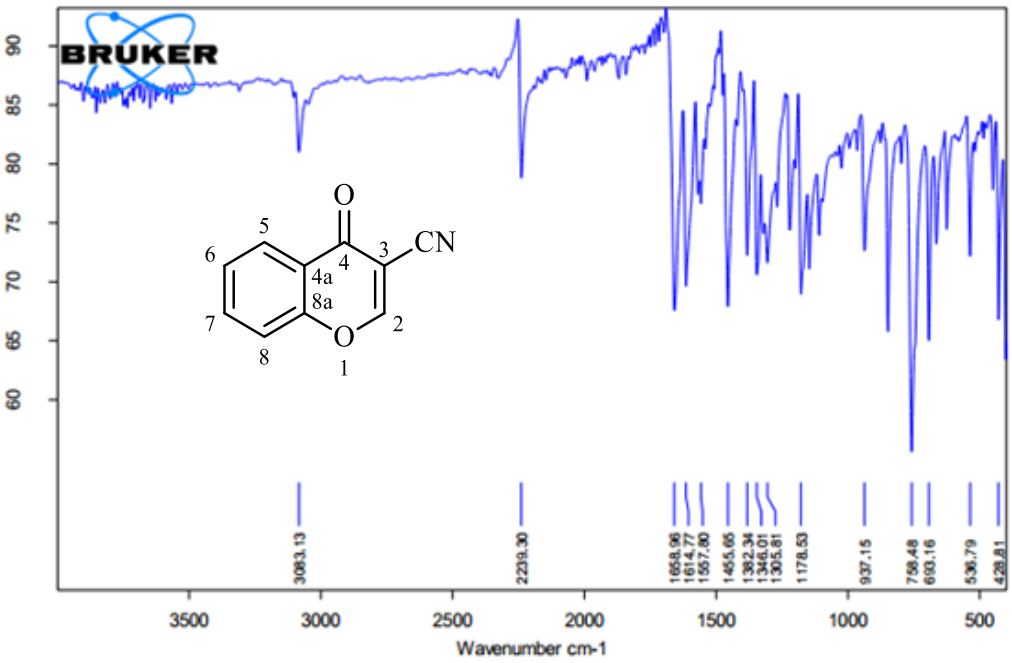

Figure 45: IR spectrum of 4-oxo-4H-chromene-3-carbonitrile **71A**