SYNTHETIC STUDIES OF NOVEL CHROMONE-2-CARBOXYLATE DERIVATIVES AND THEIR BIOLOGICAL EVALUATIONS

By

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Abstract

Chromones are a group of naturally occurring heterocyclic compounds, with oxygen as a heteroatom. However, chromone-2-carboxylates are scarce in nature. These compounds are significant in organic chemistry and medicinal chemistry due to their wide range of biological activities which include anti-HIV, anti-tuberculosis, anti-malaria, etc. The present study focus on the synthesis of chromone-2-carboxylate derivatives and their biological evaluations. Three pathways were followed to synthesize different target compounds.

In the first pathway, chromone-2-carboxylates (**51A-F**) were successfully synthesized from 2-hydroxyacetophenones, followed by the treatment with the hydrazine, to form chromone-2-carbohydrazide derivatives (**52A-F)**. Subsequently, the carbohydrazide cyclised to form 2-(4-amino-5-mercapto-4H-1,2,4-triazol-3-yl) chromone derivatives (**53A-E**). The second pathway started with the brominating of 2-hydroxyacetophenones at position 3, there-after it followed the same pathway as the first pathways to 2-(4-amino-5-mercapto-4*H*-1,2,4-triazol-3-yl) chromone derivatives (**57A-C**).

The third route involved the ring opening of the chromone-2-carboxylate, to achieve ethyl 4-(3-bromo-5-chloro-2-hydroxyphenyl)-2-morpholino-4-oxobut-2-enoate **58**. The ethoxy moiety of ethyl 4-(3-bromo-5-chloro-2-hydroxyphenyl)-2-morpholino-4-oxobut-2-enoate **58** was substituted with the hydrazine moiety to form 6-(3-bromo-5-chloro-2-hydroxyphenyl)-4-morpholinopyridazin-3(2H)-one **59**. Compounds were characterized by 1D & 2D NMR, IR spectroscopy, HRMS, elemental analysis (CHN analysis) and the physical data of the compounds.

15 compounds were tested for biological activity against malaria. These synthesized chromone-2-carboxylate derivatives samples were tested in triplicate on two occasions over 72 hours against both the wild-type drug-sensitive strain of the human malaria parasite *Plasmodium falciparum NF54* and a multidrug-resistant strain *Plasmodium falciparum K1*. Amongst all tested compounds, **51D**, **55B**, **52A**, **52C** and **59** displayed activities against chloroquine sensitive (NF54) stains of *Plasmodium falciparum*, with 78.33 %, 88.66 %, 72.16 %, 69.5 %, and 0.195 % viability respectively which were all below the positive control. Compounds **51E**, **55D**, and **59** displayed activities against chloroquine resistant (K1) strains of *Plasmodium falciparum*, with 57.5 %, 84.33 %, and 19,5 % viability respectively which were also below that of the positive control.

Keywords: Synthesis, chromones, chromone-2-carboxylates, biological activities, antimalaria, *Plasmodium falciparum*.

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Declaration

I, MANGANYI LEMUKANI ERRON, hereby declare that the dissertation for the Master of Science (Chemistry) degree at the University of Venda, hereby submitted by me, has not been submitted previously for a degree at this or any other university, that it is my own work in design and in execution, and that all reference material contained therein has been duly acknowledged.

Signature  ……. Date: 19 September 2021

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List of abbreviations

^{13}C NMR	Carbon nuclear magnetic resonance
^{1}H NMR	Proton nuclear magnetic resonance
AcOH	Acetic Acid
AIDS	Acquired immune deficiency syndrome
ARV	Antiretroviral drugs
BCA	Bovine carbonic anhydrase
$CDCl_3$	Deuterochloroform
CH_3CN	Acetonitrile
CS_2	Carbon disulfide
d	Doublet
DCM	Dichloromethane
dd	Doublet of a doublet
DTC	Dithiocarbamates
DMF	Dimethylformamide
DMSO	Dimethyl sulphoxide
DNA	Deoxyribonucleic acid
EtOH	Ethanol
FTIR	Fourie transform Infrared
H_2SO_4	Sulphuric acid
HCl	Hydrochloric acid
HIV	Human Immunodeficiency Virus
HPV	Human papillomavirus
HRMS	High-Resolution Mass Spectroscopy
HSV	Herpes simplex virus
Hz	Hertz

J	Coupling constant
K_2CO_3	Potassium carbonate
KOH	Potassium Hydroxide
LDBBA	Lithium diisobutyl-t-butoxyaluminum hydride
mCPBA	meta-Chloroperbenzoic acid
MHz	Megahertz
MS	Mass spectroscopy
$NaBH_4$	Sodium borohydride
Na_2CO_3	Sodium carbonate
NaOEt	Sodium Ethoxide
NBS	N-Bromo succinimide
$NH_2OH{\cdot}HCl$	Hydroxylamine Hydrochloride
NMR	Nuclear magnetic resonance
NNRIs	Non-nucleoside reverse transcriptase inhibitors
NRTIs	Nucleoside reverse transcriptase inhibitors
$POCl_3$	Phosphorus Oxychloride
ppm	Parts per million
RNA	Ribonucleic acid
rt	Room temperature
RTI	Reverse-Transcriptase Inhibitors
s	Singlet
SeO_2	Selenium dioxide
TB	Tuberculosis
td	Triplet of a doublet
THF	Tetrahydrofuran
TLC	Thin layer chromatography
WHO	World Health Organisation

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CHAPTER 1

1. Introduction

1.1. General description of various diseases

Different types of antiviral drugs have been developed and are still being developed to be used for the treatment and prevention of viral infections. The use of antiviral drugs has gradually increased globally during the past 10 years in the treatment of viral diseases. This resulted in the development of some drugs which were unable to yield the anticipated results against the target viruses. Almost all the antiviral drugs have their potential side effects. That is, it is advisable to understand more about the target viruses before designing antiviral drugs. A virus is an ultramicroscopic infectious agent that grows and replicates itself only within cells of living hosts; many are pathogenic; a piece of nucleic acid (DNA or RNA) wrapped in a thin coat of protein. There are two types of viruses: enveloped viruses and non-enveloped viruses. Enveloped viruses have got an outer envelope of fats, proteins, and carbohydrates which are the same as those of the cell membrane of the host cell. The non-enveloped viruses do not have an envelope.[1] An example of enveloped viruses is indicated in **Figure 1**.

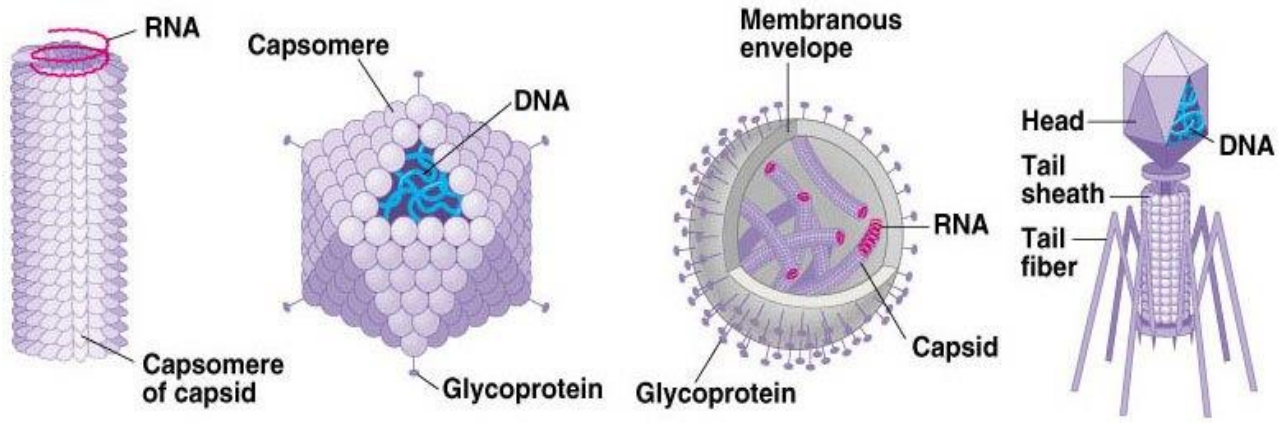


Figure 1: Structure of a Virus

The epidemic human immunodeficiency virus (HIV) remains a major problematic virus for several reasons. This is because the virus has an extraordinarily high mutation rate, such that an infected individual often harbours many variations. When HIV enters a healthy cell, it attempts to make copies of itself. It does this by using an enzyme called reverse transcriptase. If people are immunodeficient their bodies become vulnerable towards infectious diseases. Reverse-transcriptase inhibitors (RIT) or common anti-HIV drugs which are available in the market so far block some key steps in viral reproduction and uptake. The reverse-transcriptase inhibitors (RTI) work by interfering with the activity of reverse transcriptase, a viral DNA polymerase that is responsible for the replication of HIV. The key role of retroviruses drugs is to act when the cells first become infected. Without reverse transcriptase, HIV cannot make new virus copies of itself. The lifecycle and the inhibition processes in the immune system is outlined in **Figure 2**.[1-3]

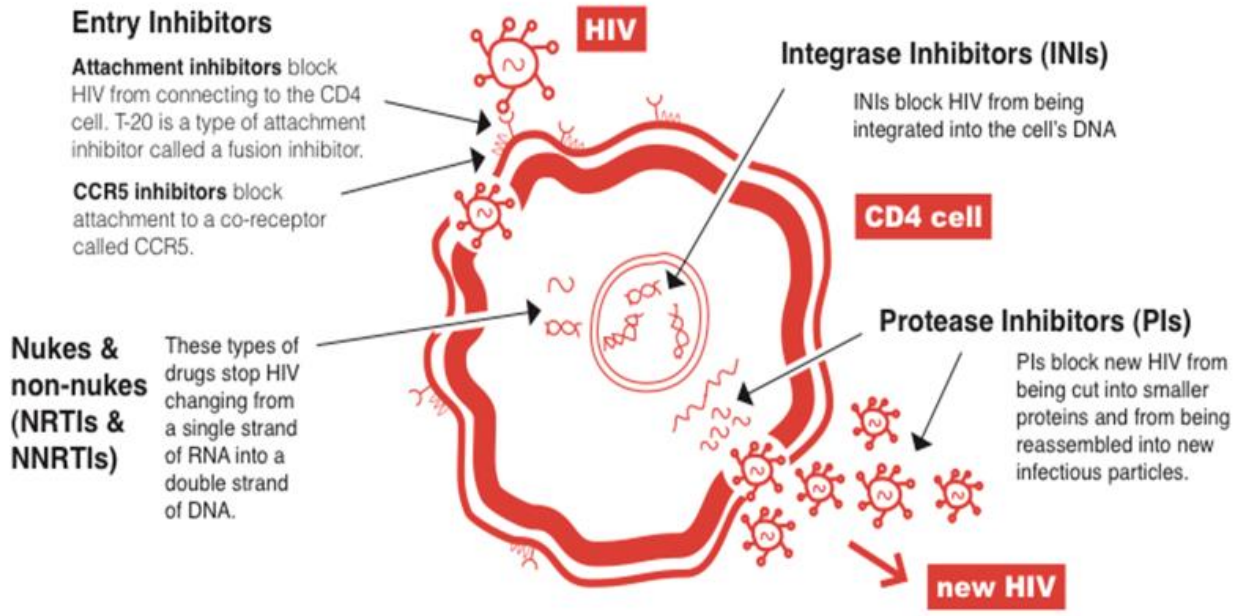


Figure 2: The lifecycle of Human Immunodeficiency Virus (HIV) in the immune system.[1]

Reverse-transcriptase inhibitors do not cure HIV, but they interfere with the virus's ability to make copies of itself and its ability to weaken the immune system. However, if HIV-infected people do not use antiretroviral drugs (ARV's) or reverse-transcriptase inhibitors, their immune systems become weaker and more vulnerable towards other infectious diseases, and consequently, HIV advances into a group of diseases called Acquired immune deficiency syndrome (AIDS).[3]

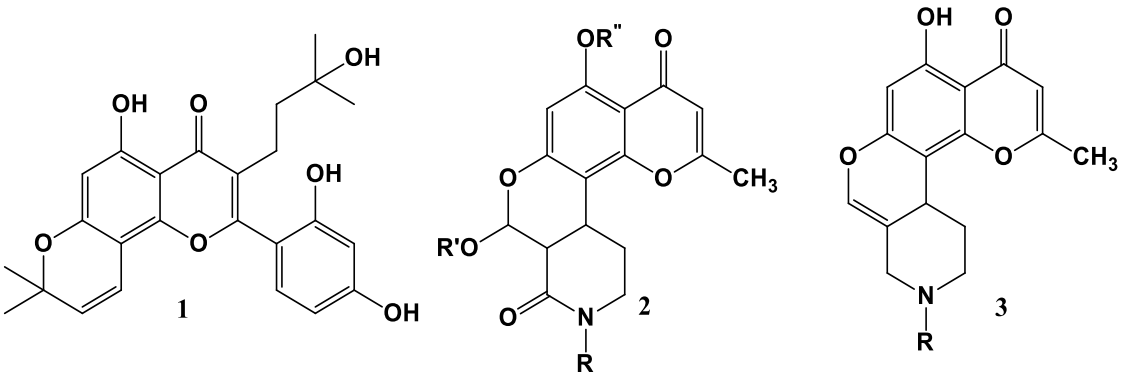
The world is surrounded by many infectious diseases, and amongst these diseases TB is now ranked alongside HIV as a leading cause of death worldwide. TB is caused by *Mycobacterium tuberculosis* which occurs in the lungs of a human being. According to the World Health Organisation, there are 9.4 million new cases of TB and 1.65 million people lose their lives every year. A 1.2 million of HIV's death toll in 2014 was estimated, which included the 0.4 million TB deaths among HIV positive people. There are also some positive advances against TB, its mortality has decreased by 47% since 1990, with almost all this improvement taking place since the Millennium Development Goals (MDG's) were set in 2000. The diagnosis and treatment of TB saved approximately 43 million lives worldwide between 2000 and 2014. Despite these advances and even though all cases of TB can be cured, TB remains one of the world's biggest infectious diseases. That is, a crucial development of new drugs which lack side effects with high efficiency is needed.[4]

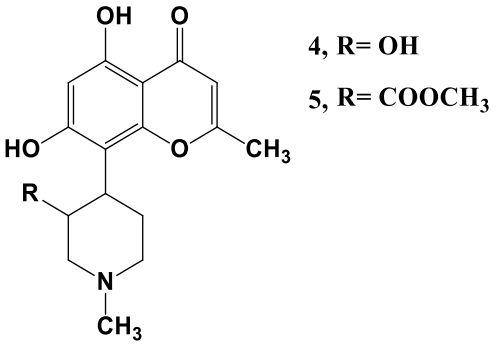
Malaria is a chronic disease caused by protozoa of the genus plasmodium transmitted to humans by female mosquitoes. Malaria is still one of the major causes of ailment and mortality, threatening and killing millions of people annually. Malaria may be caused by four different species of Plasmodium; namely: *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium*

malaria, and *Plasmodium ovale*. *Plasmodium falciparum* causes mobility and mortality on a large scale in tropical countries. Most of the malaria cases in 2017 were in the WHO African Region (200 million or 92 %), followed by the WHO South-East Asia Region (5 %) and the WHO Eastern Mediterranean Region (2 %).[5,7]

Fifteen countries in Sub-Saharan Africa and India carry almost 80 % of the global malaria burden. Furthermore, five countries account for nearly half of all malaria cases worldwide: Nigeria (25 %), the Democratic Republic of the Congo (11 %), Mozambique (5 %), India (4 %) and Uganda (4 %). In 2017 there were an estimated 435 000 deaths from malaria globally, compared with 451 000 estimated deaths in 2016, and 607 00 in 2010. Children aged 5 years and younger are the most vulnerable group affected by malaria. In 2017, this age group accounted for 61 % (266 000) of all malaria deaths worldwide.[5,6]

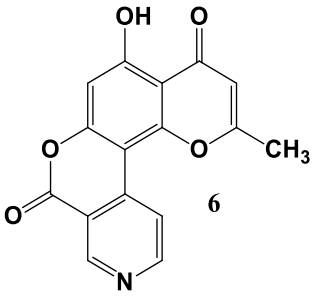
Chromone and its derivatives exhibit many biological activities such as anti-malarial, anti-TB and anti-HIV activity. Chromone alkaloid **1** extracted from the root bark of *Schumannificine magnificum* was found to be active against the Human Immunodeficiency Virus (HIV). Other chromone alkaloids and their derivatives **2-6** which were found to have a large activity against HIV.[1] Chromones also exhibit an antimycobacterial activity, the 2,10-dihydro-4[*H*]-chromeno[3,2-*c*]pyridine-3-yl derivatives such as *N*-[(4a*S*)-2-(3-methyl-2-pyridinyl)-10-oxo-2,10-dihydro-4a*H*-chromeno[3,2-*c*]pyridine-3yl]methyl-4-ethylbenzenecarboxamide **7** and N-((2-(3,5-dibromopyridin-2-yl)-10-oxo-4a,10-dihydro-2H-chromeno[3,2-c]pyridine-3-yl)methyl)-7-methyl-2-naphthamide **8** and the 3-formylchromones derivatives, compounds **9** and **10** were found to be active compounds as an antimycobacterial agent.[11] Flavonoid derivatives containing a piperazinyl chain compounds **11** and **12** exhibited antimalarial activity. The chromone derivatives **13** and **14**, extracted from the wood-decay of the fungus Rhizine, were also found to be active against malaria.[8]





4, R= OH

5, R= $COOCH_3$



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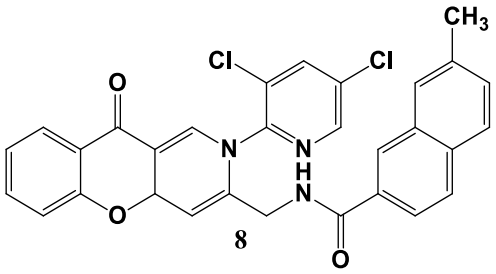

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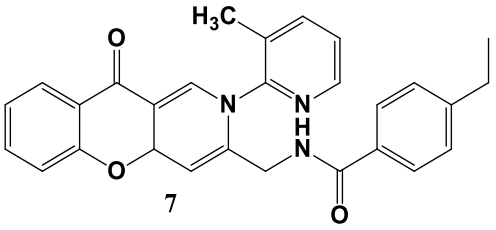

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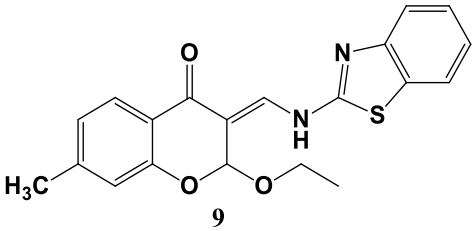

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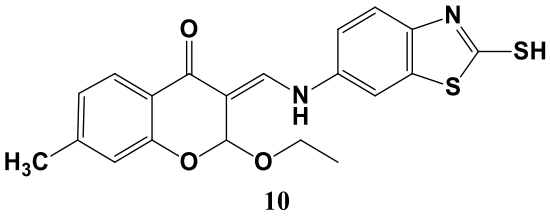

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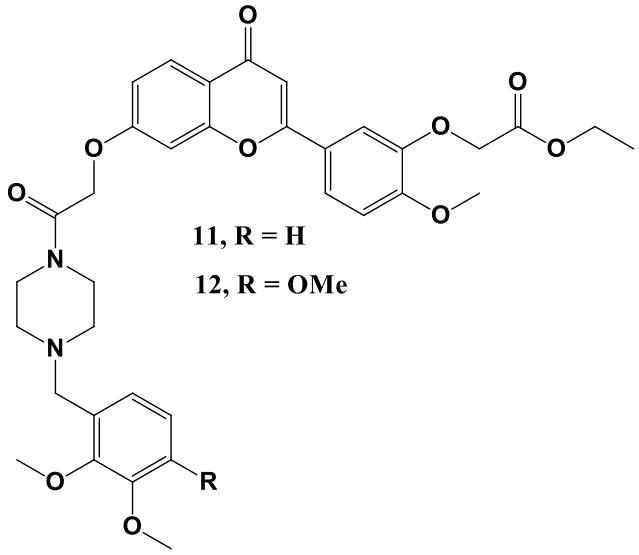
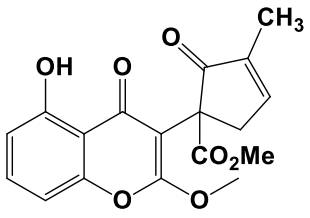


11, R = H

12, R = OMe



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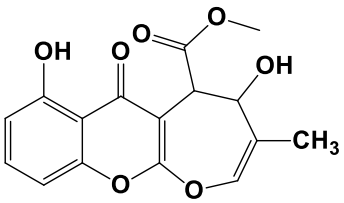

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Synthesized chromones have also displayed great activities against HIV. For example, R-di-O-(-)- camphanoyl-2',2'-dimethyldihydropyrano[2,3-*F*] chromone (DCP) compounds, have exhibited potent anti-HIV activity. The present study focuses on the synthesis of chromone-2-carboxylate derivatives which have three electron-deficient sites, carbon C-3, the ester carbonyl and the C-4 carbon of the carbonyl group in its structure. These compounds have many biological activities against various diseases which involves HIV.[10] A brief literature review of these compounds is presented below.

1.2. General description of chromones

Heterocyclic compounds play an important role in the synthesis and discovery of new physiological/pharmacologically active compounds. This is justified by the fact that more than half of the currently registered chemical compounds which are biological active contains heterocyclic compounds. The chemical structures of some of the biologically relevant structures are shown in **Figure 3** below.[1]

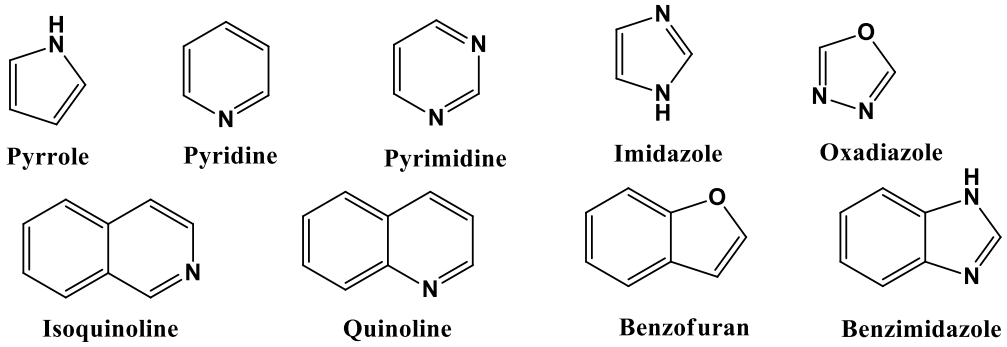
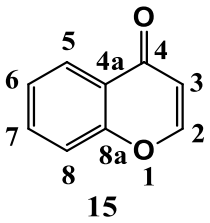


Figure 3: Chemical structures of some heterocyclic compounds

Chromones (4*H*-chromen-4-ones or 4*H*-1-benzopyran-4-ones) **15** are an important class of oxygenated heterocyclic compounds that commonly occur in nature. These compounds are widely spread in the plant kingdom from algae, wild clove, Javan plant, carrot roots, lichens to conifers.[10-14]



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15. Chromone structure

The chemistry of chromones is well known and these compounds are referred to as a privileged structure because of their diverse biological activities. Chromones are widely used in organic and medicinal chemistry as intermediate compounds used for the synthesis of various biologically active, oxygen-containing heterocyclic compounds.[15] Molecules containing a chromone moiety are easily transformed into various heterocyclic compounds due to their reactive carbonyl group. Chromone compounds are very reactive towards the nucleophiles, but they do not react when treated with some electrophiles, such as aromatic aldehyde. When chromones are treated with a carbonyl reagent, instead of condensing with the carbonyl group, they turn to open the pyrone ring. Chromones are generally stable when they are reacted with acid. Some of the biological activities of the chromone moiety are summarized in **Figure 4** below.[16]

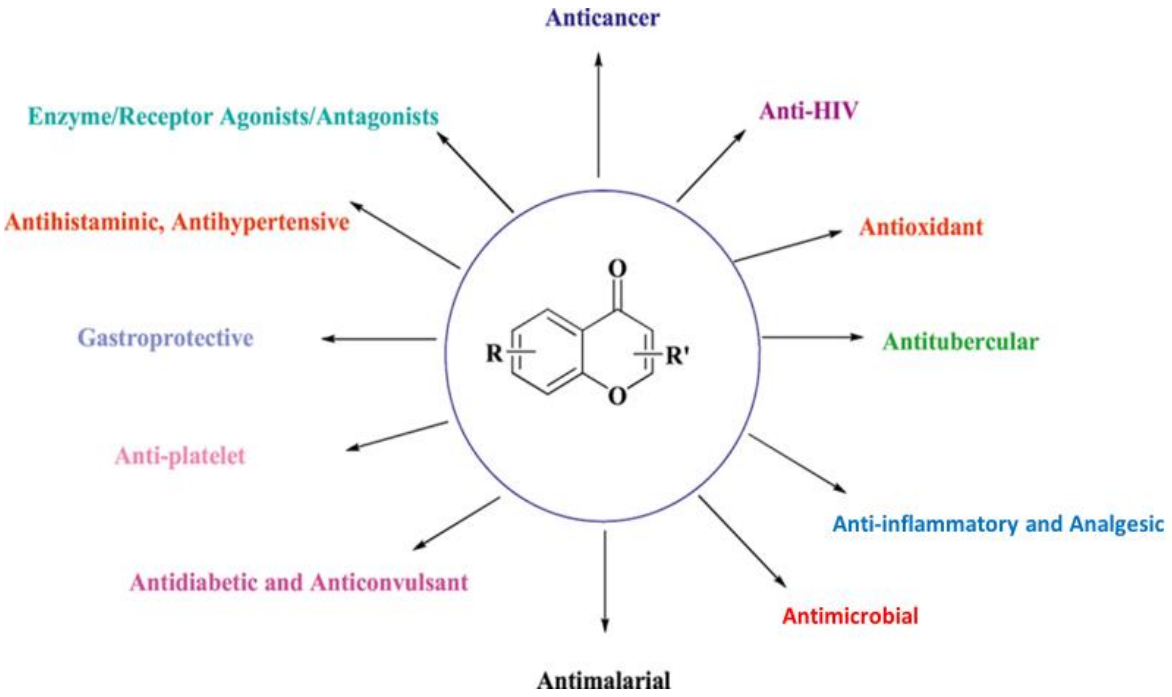
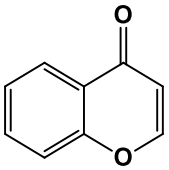
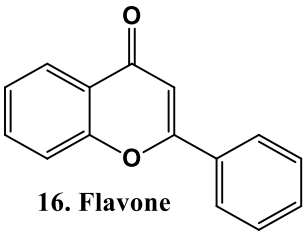


Figure 4: Biological activities of the chromones.[16]

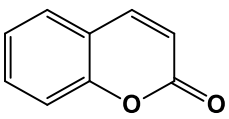
Chromones are important naturally occurring compounds possessing a wide range of valuable biological activities. The majority of chromones that occur in nature contain either the hydroxyl or alkoxyl group. Some of the chromone derivatives with different substituents have been designed to be used as mimetics of short peptides. On the other hand, chromones were extensively studied as anti-microbial, anti-allergic, anti-viral, anti-diabetes, anti-cancer, anti-inflammatory, anti-HIV, and antioxidant, etc.[17] In recent years, due to gradual increases in public interest in health care, scientists around the world have attempted to design and are currently still trying to come up with improved treatment or remedies against diseases such as HIV/AIDS, malaria, Tuberculosis (TB) and some cancers. Some of the drugs or remedies which contain the chromone moiety are sodium cromoglycate **18**, diosmin **19**, flavoxate **20**, nabilone **21**, and alvocidib **22**, among others. These drugs were found to contain high doses of chromones **15**, flavones **16**, and coumarins **17**.[23]



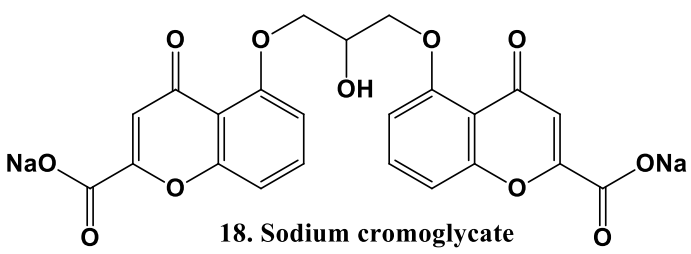
15. Chromone



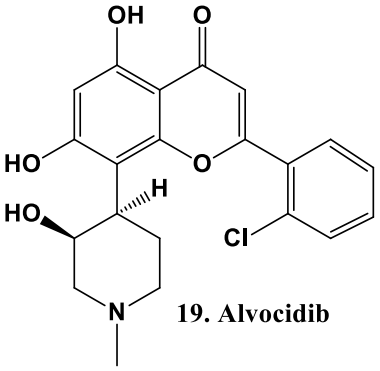
16. Flavone



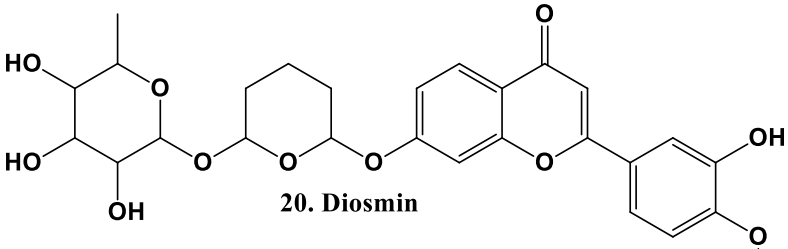
17. Coumarin



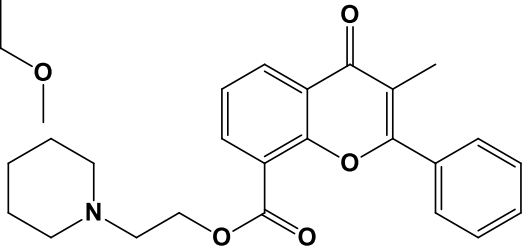
18. Sodium cromoglycate



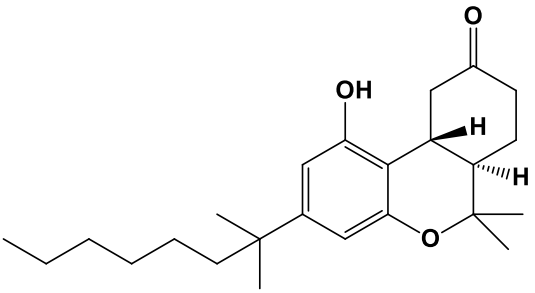
19. Alvocidib



20. Diosmin



22. Flavoxate

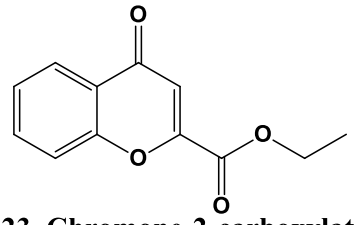


21. Nabilone

Chromones, flavones, and coumarin all exhibit a range of biological activities such as anti-tumour, anti-TB, and anti-HIV/AIDS activities. Flavone **16** is a chromone-related compound with oxygen as a hetero atom and a phenyl group in position 2 or 3. Flavone and its derivatives have an anti-bacterial, anti-fungal, anti-cancer, anti-HIV, and anti-viral properties. Coumarins **17** and chromones are structural isomers. Coumarins possess anti-inflammatory, antioxidant, anti-allergic, hepatoprotective, anti-thrombotic, anti-viral, anti-microbial, and anti-carcinogenic properties.[24-27]

1.3. Chromone-2-carboxylate

A large variety of biological pharmacological effects depend on the structural features of the compound such as the type, number, and position of substituents attached to the chromone structure. The chromone-2-carboxylates **23** are some of the scarcest classes of naturally occurring compounds. Although chromone-2-carboxylate constitutes a small family of naturally occurring chromones, their synthesis should be extensively studied. Chromone-2-carboxylate has been reported to have led to the appearance of some drugs on the market. Hence, chromone-2-carboxylate derivatives should be further investigated as part of our efforts to overcome challenges or burden facing the world of various diseases.[27-33]



23. Chromone-2-carboxylate

1.4 Biological activities of chromone-2-carboxylates

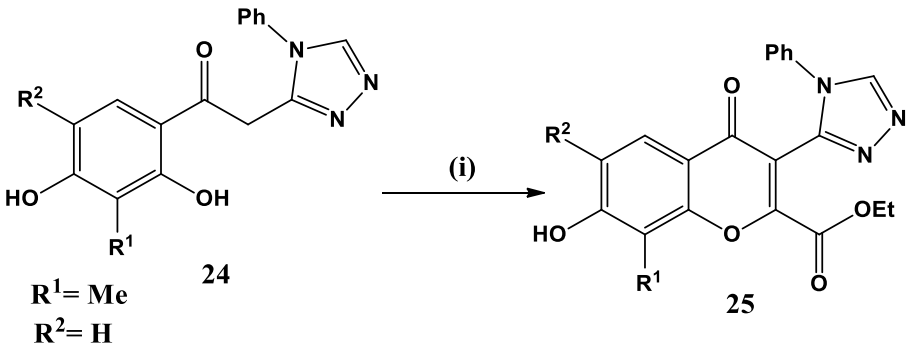
Chromones having a heterocyclic substituent in the 2-position have been reported to possess antibacterial, antifungal activities. They have also been found to exhibit good phosphodiesterase-IV inhibition activities. Some chromones have potential HIV-Integrase inhibition (e.g. compounds **1-6)**, anti-TB (e.g. compounds **7-10)**, and anti-malaria activities (e.g. compound **11-14)**.[34-36]

1.5. Synthesis of chromone-2-carboxylates

Several methods for the synthesis of chromone-2-carboxylates and their analogues have been reported in the literature. There are two common approaches for the preparation of substituted chromones: the first one is by functionalization of existing chromone-containing precursors, by introducing new substituents and cyclization of suitable substrates such as the 2-hydroxy acetophenone and 2-hydroxyphenylbutane-1,3-dione etc.[37] Several other routes with higher yields and less drastic experimental conditions have also been developed. A brief review of the synthesis of chromone-2-carboxylate is presented below.

1.5.1. Venkataraman reaction

The Venkataraman rearrangement is the chemical reaction of 2-acetoxyacetopheones with base to form 1,3-diketones. This reaction proceeds via enolate formation followed by acyl transfer. In this case α-(4-phenyl-1,2,4-triazol-3-yl)-2,4-dihydroxyacetophenones **24** with different substituents on the phenyl ring, react with ethoxalyl chloride in pyridine in the cold and cyclize to 8-methyl-7-hydroxy-3-(4-phenyl-1,2,4-triazol-3-yl) chromone **25** with carboethoxy substituents at position 2 of the molecule as show in **Scheme 1**.[35]

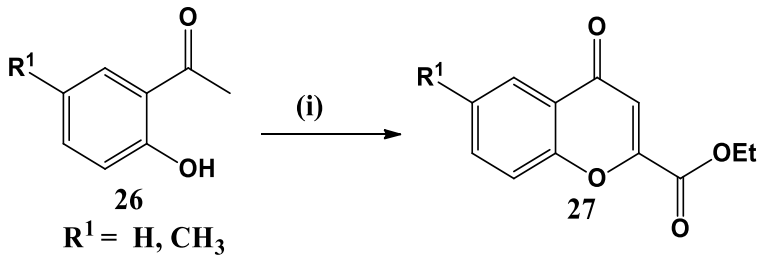


Reagents: (i) Ethoxalyl chloride, pyridine.

SCHEME 1: Venkataraman reaction.[35]

1.5.2. Synthesis of chromone-2-carboxylate using the condensation reaction.

In this reaction a solution of 2-hydroxyacetophenone **26** in THF was added to NaOEt in EtOH under inert condition, followed by diethyl oxalate, which was stirred to form ethyl chromone-2-carboxylate **27** in good yield as shown in **Scheme 2**.[36]

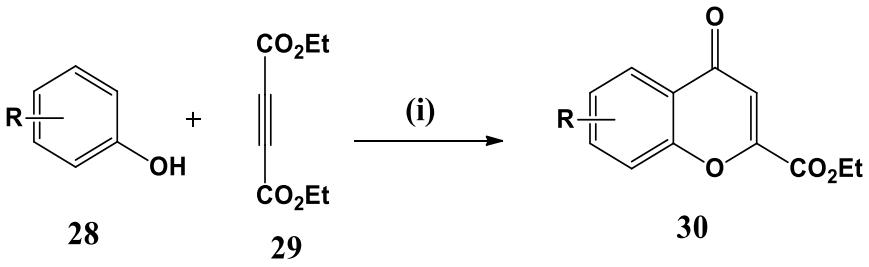


Reagents: (i) Diethyl oxalate, 21% NaOEt, EtOH, THF, 60 °C, conc. HCl

SCHEME 2: Synthesis of chromone-2-carboxylate using the condensation reaction.[36]

1.5.3. One-pot cascade reaction

An efficient synthetic strategy to chromone derivatives from commercially available diethyl acetylene dicarboxylate **29** and phenols **28** involves a one-pot cascade reaction. This reaction involves using pyridine and polyphosphoric acid as a catalyst at room temperature and at 90 °C for some derivatives without any solvent, to give the chromone-2carboxylate derivatives **30** in high yields as show in **Scheme 3**.[37]



28 29 30

Reagents: (i) PPA, 90°C, Pyridine, rt

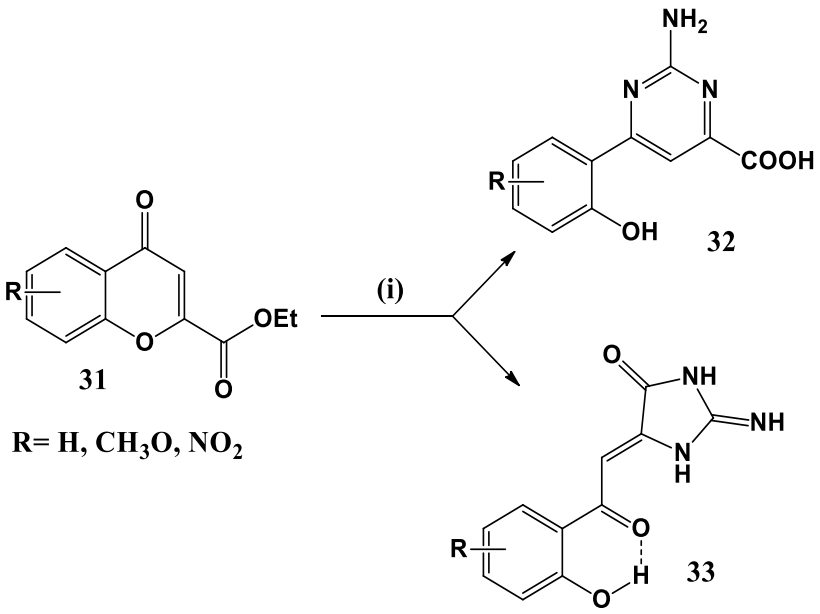
SCHEME 3: One-pot cascade reaction to synthesise chromone-2-carboxylate.[37]

1.6. Reactivity of chromone-2-carboxylates

Chromone-2-carboxylates occupy an important position in the synthesis of various heterocyclic systems. Due to the availability of three electron-deficient sites, carbon C-3, the ester carbonyl and the C-4 carbon of the carbonyl group, chromone-2-carboxylate provide access to different possible reactions in which the chromone ring is retained or the 2-hydroxybenzoyl derivatives can result from the opening of the pyran-4-one ring. The electrophilic attack takes place at the deactivated pyran-4-one ring in the C-3 position of chromone. The chromone system acts as a Michael accepter on nucleophiles.[41] The reactions of chromone-2-carboxylates are briefly described below with the aid of the reaction schemes.

1.6.1. The reaction of chromone-2-carboxylate with guanidine

Treatment of guanidine with chromone-2-carboxylate leads to the opening of the pyrone ring and to the formation of hydantoin derivatives-2-imino-5-(2-hydroxybenzoyl-methylene) tetrahydroimidazole-4-ones **33** in addition to other reaction products. In this reaction, the pyrone ring of the chromones behaves like the corresponding β-dicarbonyl compound. It was found that guanidine carbonate in boiling ethanol with ethyl chromone-2-carboxylate, forms a certain amount of hydantoin derivative-2-imino-5-(2-hydroxybenzoylmethylene) tetrahydroimidazol-4-one **33**. In addition to the normal reaction product, 2-amino-4-(2-hydroxyphenyl) pyrimidine-6-carboxylic acid **32** is formed as show in **Scheme 4**.[39]

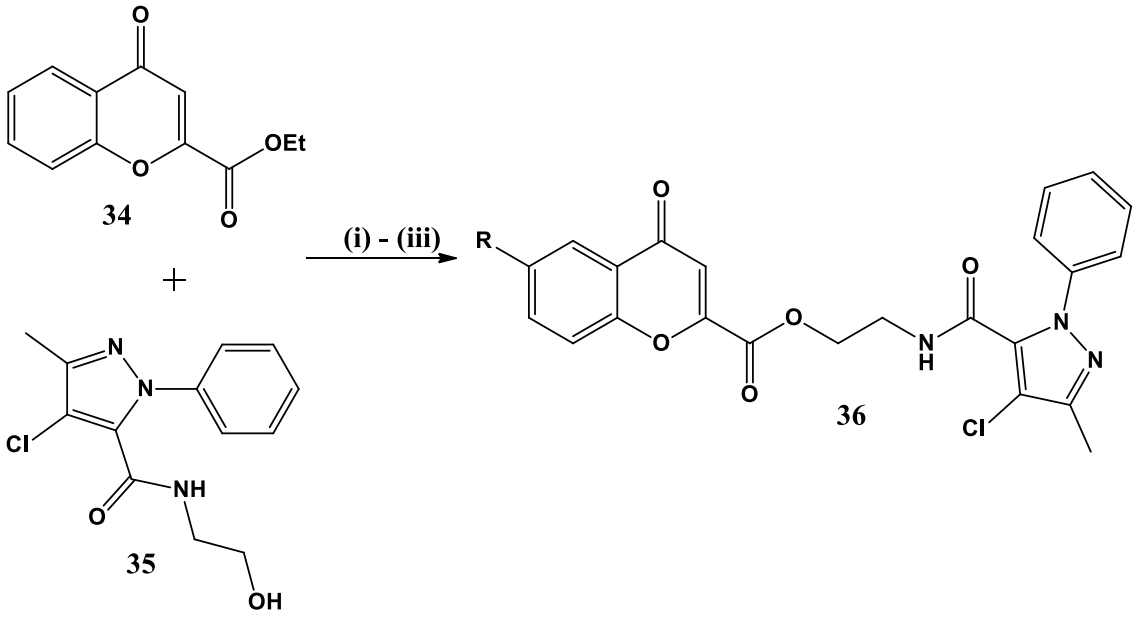


Reagents: (i) $HN=C(NH_2)_2$

SCHEME 4: The reaction of chromone-2-carboxylate with guanidine.[39]

1.6.2. Synthesis of chromone-containing substituted pyrazole from chromone-2-carboxylates

A series of chromone derivatives containing substituted pyrazole were designed and synthesized from chromone-2-carboxylate. In this synthesis, a chromone-2-carboxylate was converted into chromone-2-carboxylic acid by introducing $NaHCO_3$ in water. The carboxylic acid was then converted to chromone-2-carbonyl chloride using oxalyl dichloride and DMF in DCM. The chromone-2-carbonyl chloride was reacted with pyrazole derivatives and Et_3N in DCM to form the target compound as shown in **Scheme 5**.[59]

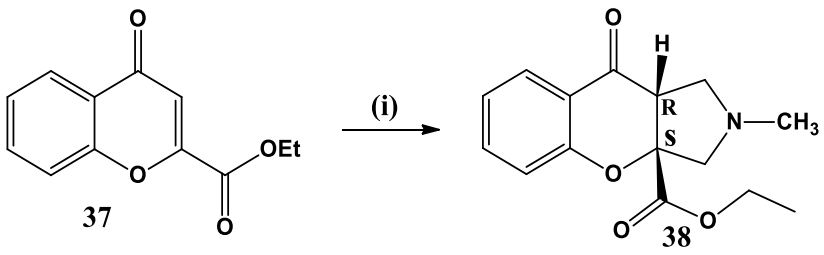


Reagent: (i) $NaHCO_3$, H_2O, 3h, 80°C (ii) Oxalyl dichloride, DMF, CH_2Cl_2, 1h, rt (iii) Et_3N, CH_2Cl_2, 0°C, Overnight, rt

SCHEME 5: Synthesis of chromone-containing substituted pyrazole from chromone-2-carboxylates.[59]

1.6.3. Cycloaddition of chromone-2-carboxylate at position 2 & 3 with nonstabilized azomethine ylides.

Chromones bearing electron-withdrawing substituents at the 2- or 3-position react with nonstabilized azomethine ylides to produce 1-benzopyrano[2,3-*c*]pyrrolidines in good yields. These reactions proceed diastereoselectively to give 1-benzopyrano[2,3-*c*]pyrrolidines and tetrahydro-1H-spiro[chromeno[2,3-*c*]pyrrol-9,50 -oxazolidine]-9a-carbonitriles, depending on the reactant ratio, as a result of 1,3-dipolar cycloaddition of the azomethine ylide at the double bond and the carbonyl group of the chromone system as outlined in **Scheme 6**. [26]

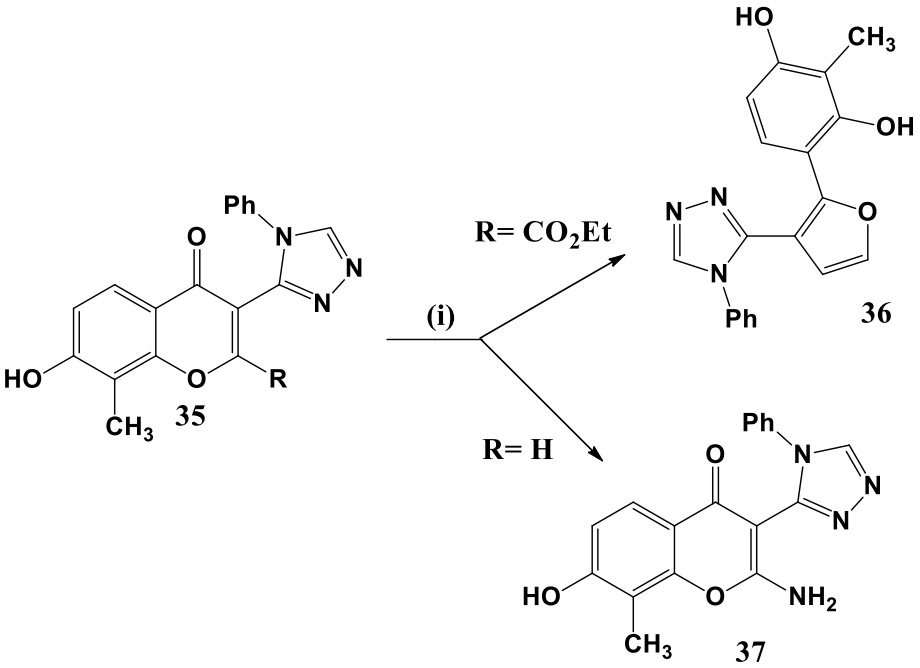


Reagent: (i) Formaldehyde, CH_3NHCH_2COOH, C_6H_6, 4h, reflux.

SCHEME 6: Cycloaddition of chromone-2-carboxylate at position 2 & 3 with nonstabilized azomethine ylides.[26]

1.6.4. Recyclization of 7-hydroxy-3-(4-phenyl-1,2,4-triazol-3yl) chromone-2-carboxylate using binucleophiles.

The reaction of 2,8-substituted 7-hydroxy-3-(4-phenyl-1,2,4-triazol-3-yl) chromone **35**, with hydroxylamine forms isoxazoles **36**, while the recyclization of chromones, unsubstituted at position 2, can proceed further with a subsequent recyclization of the isoxazole ring to 2-aminochromone **37**. These reactions occur by refluxing ethyl 7-hydroxy-8-methyl-4-oxo-3-(4-phenyl-4*H*-1,2,4-triazol-3-yl)-4*H*-chromene-2-carboxylate in pyridine with an excess of hydroxylamine hydrochloride to give the isoxazoles **36** as outlined in **Scheme 7** below.[40]



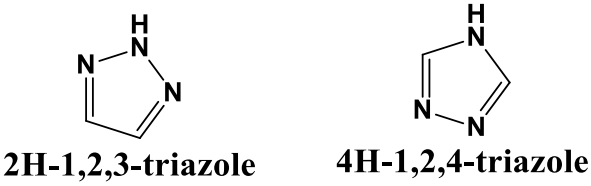
Reagent: (i) $NH_2OH{\cdot}HCl$

SCHEME 7: Recyclization of 7-hydroxy-3-(4-phenyl-1,2,4-triazol-3yl) chromone-2-carboxylate using binucleophiles.[40]

1.7. Triazole thiol

Nowadays research is concentrated towards the introduction of new and safe therapeutic agents of clinical importance. Nitrogen-containing heterocycles are found in abundance in most medicinal compounds. Triazoles are five-membered rings which contain two carbon and three nitrogen atoms. The triazole occurs in two different isomeric forms according to the position of the nitrogen atoms. They occur in two tautomeric forms: namely, 1,2,3-triazoles **42** and 1,2,4-triazole **43**. The sulphur-containing heterocycles represent important compounds that are promising in the use of practical applications in medicinal chemistry. Among these heterocycles, the mercapto and thione- substituted 1,2,4-triazole ring systems have been

extensively studied. So far, a variety of biological activities have been reported for many of their derivatives, such as antibacterial, anti-malarial, anti-fungal, anti-tubercular, anti-mycobacterial, anti-cancer, diuretic and hypoglycaemic properties.[46-48]



In recent years the chemistry of triazoles and their fused heterocyclic derivatives have received considerable attention, owing to their synthetic and effective biological importance. Known drugs containing the 1,2,4- triazole group include Triazolam **44**, and estazolam **45**, an anticonvulsant drug which contains a trizolebenzodiazepine ring. Ribavirin **46** (1-β-Þ-ribofuranosyl-1H-1,2,4-triazole-3-carboxamide) is a broad-spectrum antiviral agent containing a 1,2,4-triazole ring. The compound is active against both RNA and DNA viruses and viral diseases. Among the compounds with anti-HIV activity 2-(4H-1,2,4-triazole-3-ylthiol) acetamide derivatives **47** have also been reported. Many 1,2,4-triazole-containing ring systems; for example, fluconazole **48** and voriconazole **49**, have been incorporated into a wide variety of therapeutically interesting drugs with anti-inflammatory, CNS stimulant, sedative, antianxiety, antimicrobial, and antimycotic activity. The chemical structures of all these compounds are outlined in **Figure 5**.[48-50]

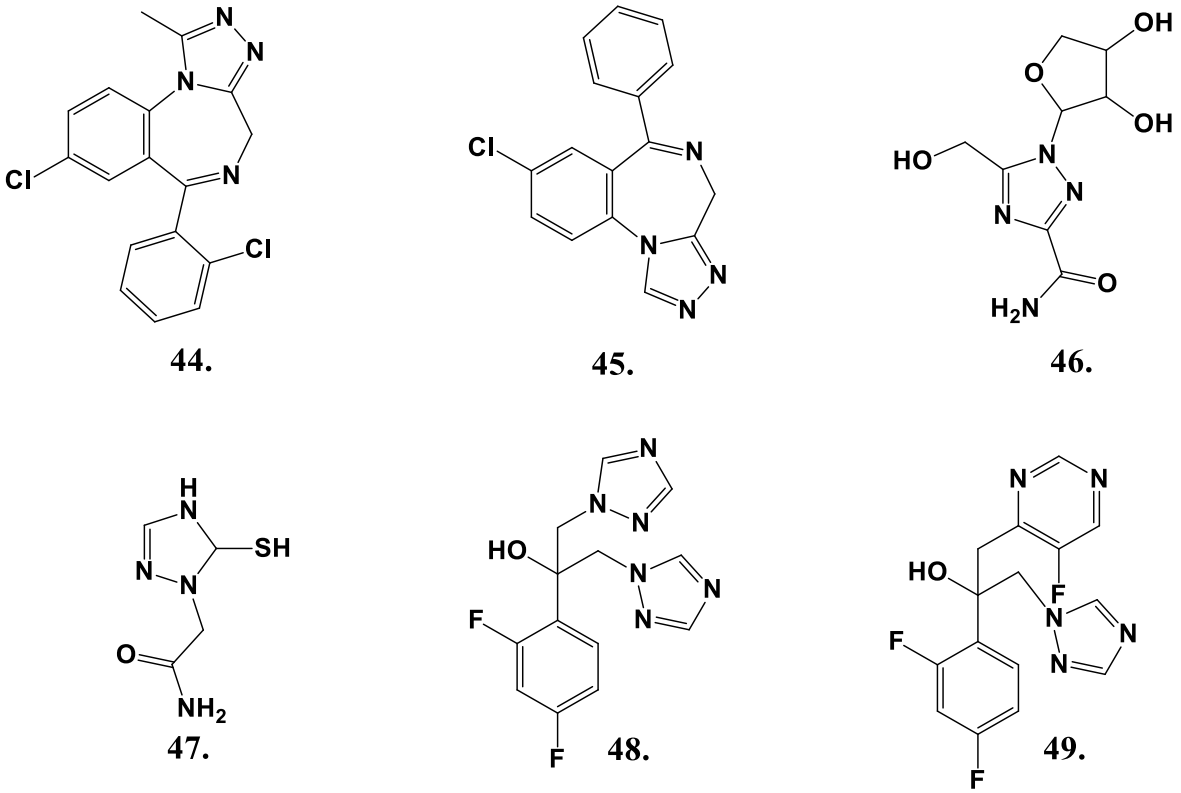


Figure 5: Chemical structures of drugs containing a 1,2,4-triazole group.[48]

Triazoles are not only transition compounds but are also very effective organic compounds. Besides, triazoles can undergo different types of reactions to yield other heterocyclic compounds such as Mannich bases, Thioureas, Thioethers, Schiff bases, Triazolothiadiazoles, Tri-zolothiazines, Thiazolothiazipine, and Tri-azolothiadiazines.[51, 52]

CHAPTER 2

Problem statement

The chromone-2-carboxylates are part of an important class of oxygen-containing heterocyclic compounds, which are scarce in nature. These compounds are significant in organic chemistry and medicinal chemistry.[27] This is due to the availability of three electron-deficient sites, carbon C-3, the ester carbonyl and the C-4 carbon of the carbonyl group in its structure. The chromone-2-carboxylate occupies an important position in the synthesis of various heterocyclic systems.[36] In addition their derivatives exhibit important biological activities, such as anti-cancer agent, anti-TB agent, anti-malaria agent, antioxidant agent, anti-HIV agent etc.[11]

The world is still facing a significant challenge regarding epidemic diseases such as TB, Malaria, cancer and HIV/AIDS. The health system of the governments globally is already strained to provide basic health care for all diseases. Over half of the hospital beds in South Africa are occupied by people suffering from TB, malaria, and HIV/AIDS. South Africa represents 0.7 % of the global population but carries 17 % of the global burden of HIV/AIDS (approximately 5.7 million people living with HIV). An estimated 31 % of all HIV cases in Africa occur within South Africa. Among the HIV-infected people with a weakened immune system, TB is the leading killer epidemic. Every year about 2 million people living with HIV die because of TB.[4]

According to the World Health Organisation, there are 9.4 million new cases of TB and 1.65 million deaths due to TB every year. Most malaria cases in 2017 were in the WHO African Region (200 million or 92 %). Furthermore, in 2017 there were an estimated 435 000 deaths from malaria globally. Children under the age of 5 years are the most vulnerable group affected by malaria. Therefore, this led the South African government to prioritize all these diseases which resulted in vision 2030, for eradicating or reducing all these diseases. Thus, further research in the field of chromone-2-carboxylate chemistry will contribute to the discovery of new leads for combating some of these diseases.[4,7]

Chromone-2-carboxylate derivatives have been reported to have contributed to the introduction of some new drugs on the market. In the present study condensation reactions were used for the preparation of novel chromone derivatives containing various fragments, of which some fragments were active in some currently used HIV drugs and found to delay (but not cure) the progression of HIV to AIDS. The scarcity of chromone-2-carboxylates in nature, as well as its limitations on the reported pharmacological studies of the synthetic research have attracted our attention and interest in investigating these compounds.

CHAPTER 3

3. Aim and objectives

Aim:

To synthesize and biologically evaluate novel chromone-2-carboxylate derivatives.

Objectives:

- Synthesis of chromone-2-carboxylate from halogenated 2-hydroxyacetophenones.
- Synthesis of ethyl 4-(3-bromo-5-chloro-2-hydroxyphenyl)-2-morpholino-4-oxobut-2-enoate
- Synthesis of chromone-2-carbohydrazide derivatives
- Conversion of chromone-2-carbohydrazide to 2-(4-amino-5-mercapto-4*H*-1,2,4-triazol-3-yl) chromone
- Use of 1D & 2D NMR, HRMS, CHN analysis (Elementary analysis) and IR spectroscopy to characterize synthesized compounds.
- Biological evaluation of the selected target compounds as potential anti-malaria, anti-TB, and anti-HIV agents

CHAPTER 4

4. Results and Discussion

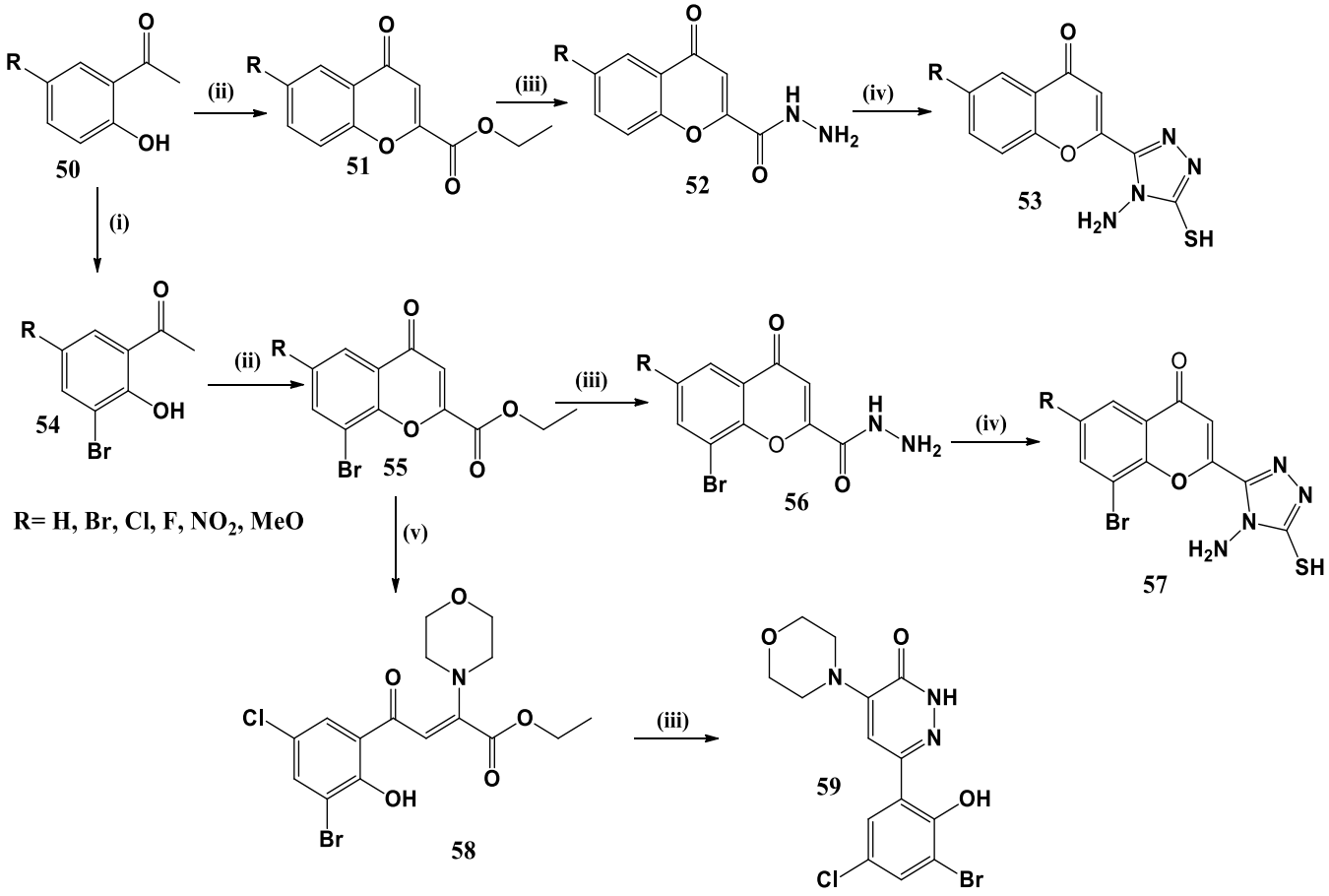
4.1. Research methodology

A general reaction scheme for all the work conducted in this research project is summarised in **Scheme 8**, which outlines the synthesis of various categories of target compounds where we have 2-(4-amino-5-mercapto-4*H*-1,2,4-triazol-3-yl) chromone **57**, which is substituted at position 8 and the non-substituted 2-(4-amino-5-mercapto-4*H*-1,2,4-triazol-3-yl) chromone **53** at position 8. The synthetic route of the target compounds substituted at position 8, started with brominating various 5-substituted-2-hydroxyacetophenones **50** at position 3, using NBS in acetonitrile, as outlined in **Scheme 9.**

The synthetic route for converting both brominated and non-brominated-2-hydroxyacetophenones at position 3 to various chromone-2-carboxylates **51 & 55** was achieved by the condensation reaction of various 5-substituted-2-hydroxyacetophenones with diethyl oxalate, in the presence of sodium ethoxide, followed by acidic cyclization, as outlined in **Scheme 10**. Attempted substitution of bromine by dithiocarbamates at position 8 of the chromone-2-carboxylates was unsuccessful, instead it resulted in ring opening. This reaction was carried out by reacting morpholine in DMF and carbon disulphide in the presence of sodium methoxide which was later reacted with 8-bromochromone-2-carboxylates **55** to achieve ethyl 4-(3-bromo-5-chloro-2-hydroxyphenyl)-2-morpholino-4-oxobut-2-enoate **58**, as outlined in **Scheme 11**.

The chromone-2-carbohydrazide derivatives **52 & 56** were synthesized by the condensation reaction of carboxylates with hydrazine hydrates in ethanol, as outlined in **Scheme 12** and **Scheme 14**. The target compounds were achieved by the treatment of chromone-2-carbohydrazide **52 & 56** with carbon disulphide in the presence of ethanolic potassium hydroxide to form an intermediate acid hydrazide. This was followed by a condensation reaction with hydrazine hydrate to achieve the target compounds as outlined in **Scheme 15**.

All reactions were monitored by thin-layer chromatography plates using UV light. The synthesized compounds were characterized by the combination of 1D & 2D NMR, HRMS, CHN analysis (Elementary analysis) and IR spectroscopic techniques, as well as the physical data of the compounds.

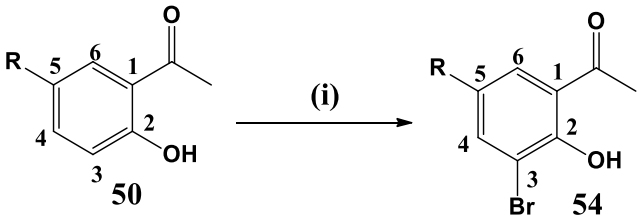


Reagent: (i) NBS, CH_3CN, rt, 12h; (ii) NaOEt, Diethyl oxalate, reflux, 1h; (iii) $NH_2NH_2.H_2O$, EtOH, Reflux, 8h; (iv) CS_2, $NH_2NH_2.H_2O$, EtOH, KOH, Reflux, 16h; (v) Amine, CS_2, DMF, rt, 30 min.

GENERAL REACTION SCHEME 8

4.2. Synthesis of 5-substituted-3-bromo-2-hydroxyacetophenones (54A-D)

The 5-substituted-2-hydroxyacetophenones were treated with NBS in acetonitrile to give a yellow solution which was stirred overnight, diluted with ethyl acetate and washed with water and brine. The organic solutions were then collected and dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was dissolved in hot MeOH and then treated with water. The precipitates which were formed were collected by filtration and dried to give 5-substituted-3-bromo-2-hydroxyacetophenones (**54A-D**). The synthesis of 5-substituted-3-bromo-2-hydroxyacetophenones (**54A-D**) was achieved with moderate to excellent (42-87%) yield.[54] An attempt to brominate the parent 2-hydroxyacetophenone and the 5-methoxy-2-hydroxyacetophenone at position 3 were unsuccessful. The chemical reaction scheme is outlined in **Scheme 9** below.



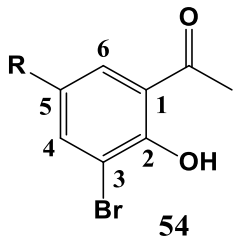
R = Br, Cl, F, NO_2

R ≠ H, MeO

Reagent: (i) NBS, CH_3CN, rt, 12h

SCHEME 9: Synthesis of 5-substituted-3-bromo-2-hydroxyacetophenone derivatives **54.**

NMR, IR spectroscopy and melting point data were used to confirm the structures of all the 3-bromo-2-hydroxyacetophenone derivatives **(54 A-D)**. The experimental melting point of all compounds was consistent with those reported in the literature. The melting points and the percentage yields of the synthesized 3-bromo-2-hydroxyacetophenone derivatives are summarised in **Table 1** below.



R= Br, F, Cl, NO_2

Table 1: Synthesised 3-bromo-2-hydroxyacetophenone derivatives **54**

Compounds 54	R	% Yield	Melting point (°C)	Lit. Melting point (°C)
A	F	42	98- 101	97[63]
B	Cl	71	108- 115	112[56]
C	Br	87	107- 112	111[56]
D	NO_2	85	130- 135	132[64]

^{1}H NMR spectra of 5-substituted-3-bromo-2-hydroxyacetophenones (**54A-D**) were characterized by a singlet at around 2.72 ppm, which corresponds to three methyl protons; a doublet at around 8.55 ppm and another doublet at around 8.61 ppm, which corresponds to the protons at position 4 and 6, respectively. The J-coupling for these two protons was calculated to be 2.8 & 2.4 Hz, respectively, thus confirming that the two protons are meta coupling to each other. The compounds also contain aromatic hydroxyl groups which were characterized by a singlet peak at around 13.55 ppm.

The ^{1}H NMR spectrum of 3-bromo-5-fluoro-2-hydroxyacetophenone **54A** was characterised by a doublet of a doublets at 7.46 ppm with a coupling constant of 4.8 Hz and 2.8 Hz, corresponding to the proton at position 6; another doublet of a doublets at 7.38 ppm with a coupling constants of 5.2 Hz and 3.2 Hz, corresponding to the proton at position 4. All this uniqueness of coupling constant was caused by the H-F coupling. The ^{1}H NMR spectrum of 5-nitro-3-bromo-2-hydroxyacetophenones **54D** is indicated in **Figure 6.**

Spectroscopic data obtained from ^{13}C NMR and DEPT 135 experiment was used to confirm the presence of five quaternary carbons, two C-H carbons, and the methyl carbon. The quaternary carbons at around 198.6 - 203.8 ppm corresponds to the carbonyl carbon, the other three quaternary carbons are the aromatic carbons C-3, C-1, C-2 resonating at around 112.3 - 115.1 ppm, 116.3 – 124.1 ppm, 153.2 - 163.9 ppm, respectively; While the quaternary carbon C-5 which is bonded to the functional groups Br, Cl, NO_2, and F was influenced by the electronegativity of the functional groups attached to it. The higher the electronegativity the more the chemical shift was shifted to the low field of the spectrum. Ranging from the quaternary carbon C-5 attached to: Br < Cl < NO_2 < F resonating at 108.4 ppm, 126.1 ppm, 139.3 ppm, and 158.5 ppm, respectively. the two C-H Carbons C-4 and C-6 resonated at around 127.4 -138.8 ppm and 115 – 129.2 ppm, respectively.

Amongst these compounds there is a fluorine containing compound which can do carbon-fluorine coupling. A 3-bromo-5-fluoro-2-hydroxyacetophenone **54A** was characterised by a carbonyl carbon peak at 203.6 ppm with a C-F coupling constant of 3.2 Hz, a C-5 peak at 158.5 ppm with a C-F coupling constant of 2 Hz and C-6 peak at 115.3 ppm with a C-F coupling constant of 3 Hz. The ^{13}C NMR spectrum of 5-nitro-3-bromo-2-hydroxyacetophenones **54D** is provided in **Figure 7. Table 2** outlines the observed chemical shifts of carbons for all 5-substituted-3-bromo-2-hydroxyacetophenones (**54A-D**) synthesized.

FT-IR spectroscopy was used to confirm the presence of functional groups in the chemical structures of 5-substituted-3-bromo-2-hydroxyacetophenones (**54A-D**). FT-IR spectrum revealed aromatic (C-H) at 3072 cm^{-1}, carbonyl (C=O) stretches at 1615 cm^{-1}, and aromatic carbon-carbon double bond (C=C) stretches at 1599 cm^{-1} as indicated in **Figure 8**.

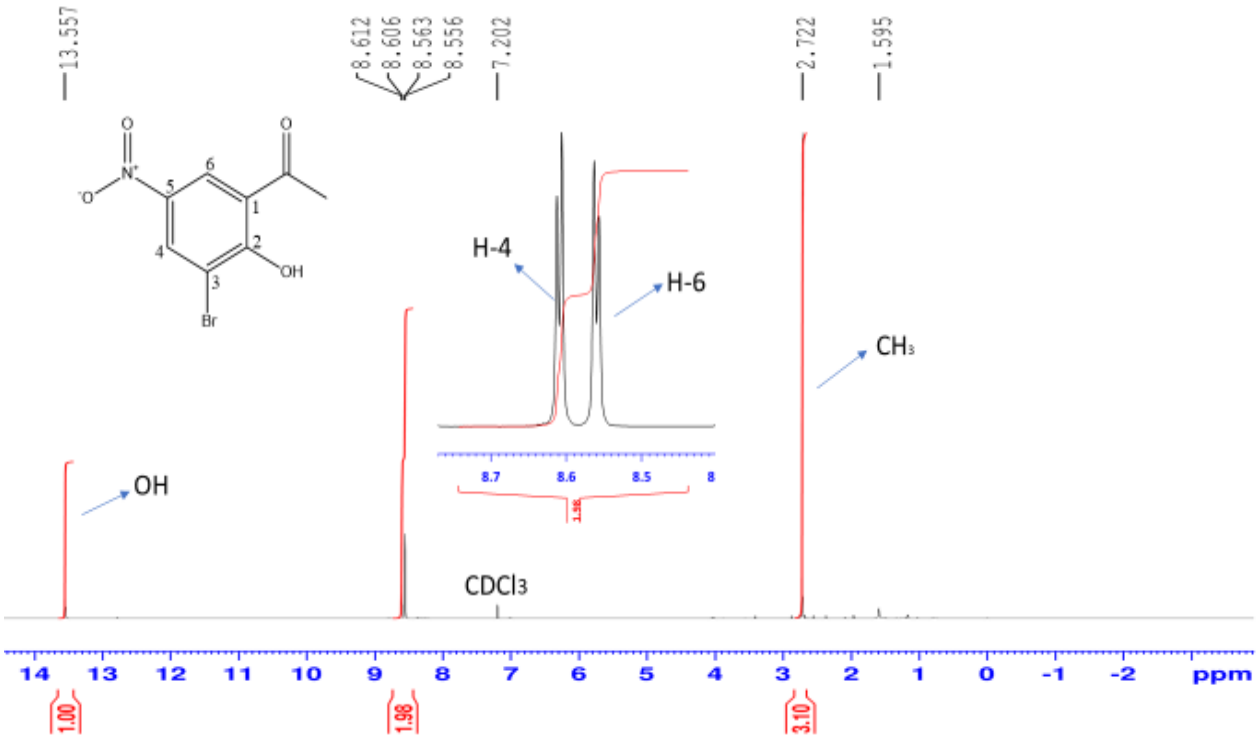


Figure 6: ^{1}H NMR spectrum of 3-bromo-2-hydroxyacetophenone **54D** in $CDCl_3$ (at 400 MHz)

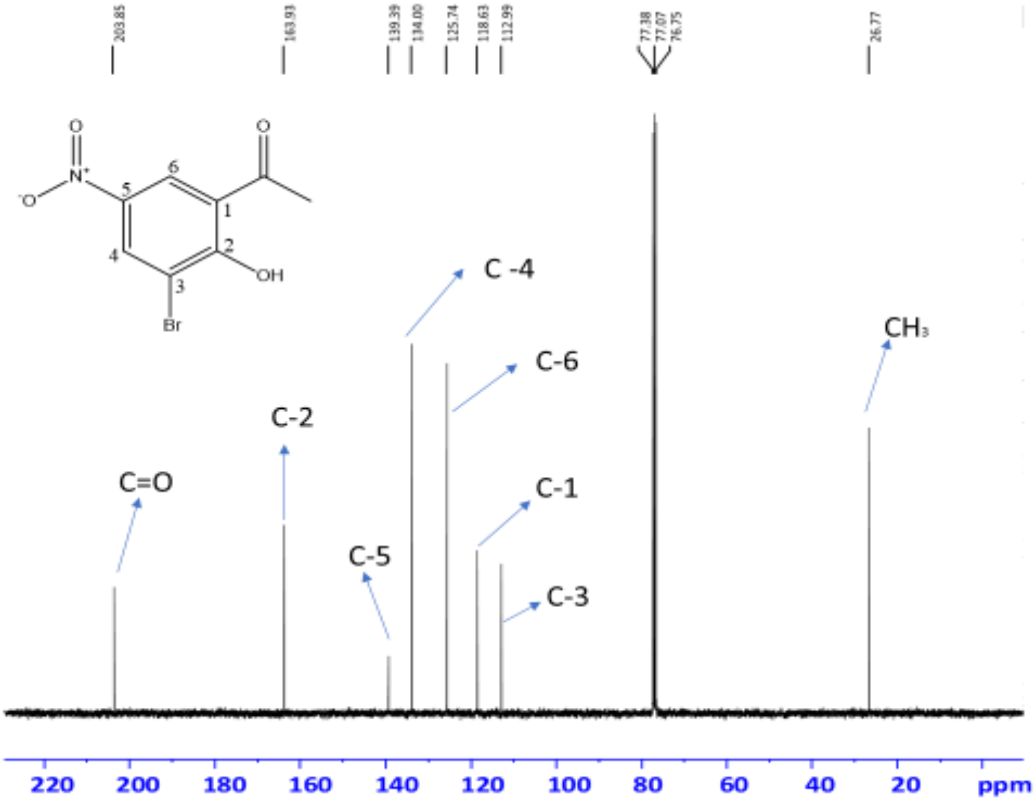


Figure 7: ^{13}C NMR spectrum of 3-bromo-2-hydroxyacetophenone **54D** in $CDCl_3$ (at 100 MHz)

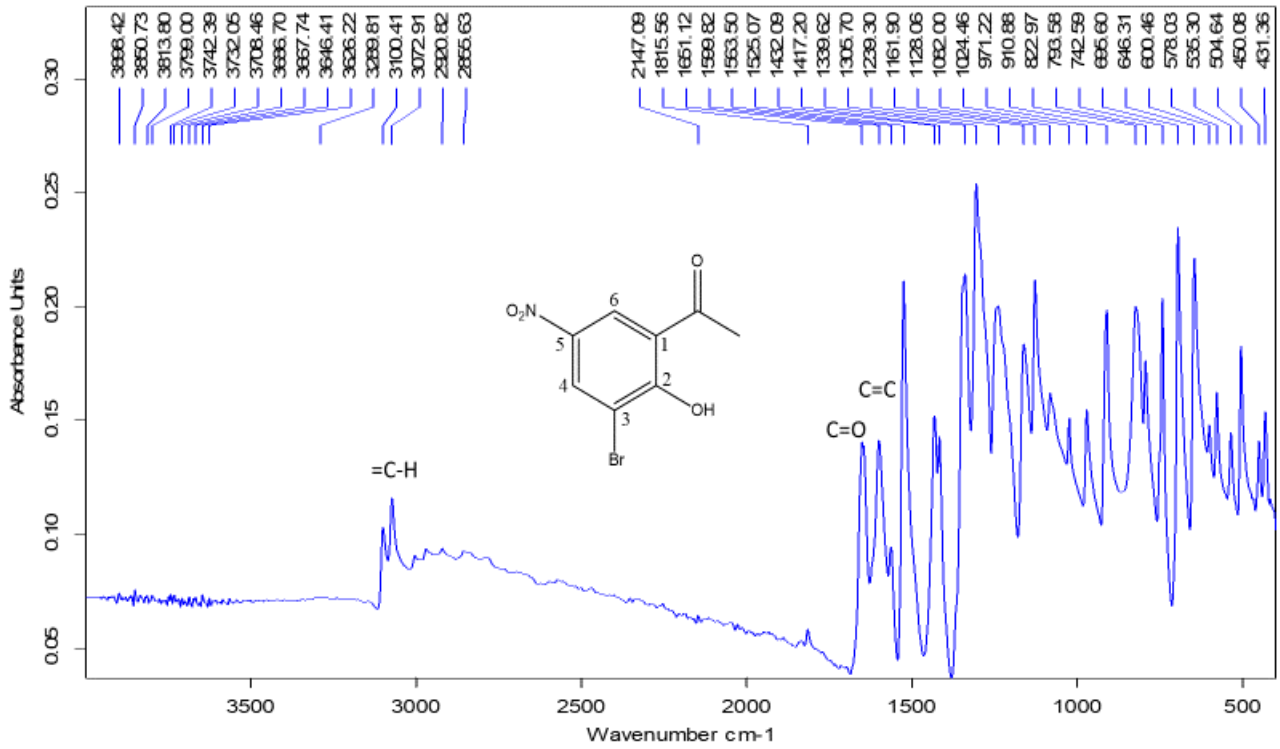
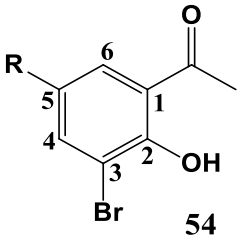


Figure 8: FTIR spectrum of 3-bromo-2-hydroxyacetophenone **54D**



54

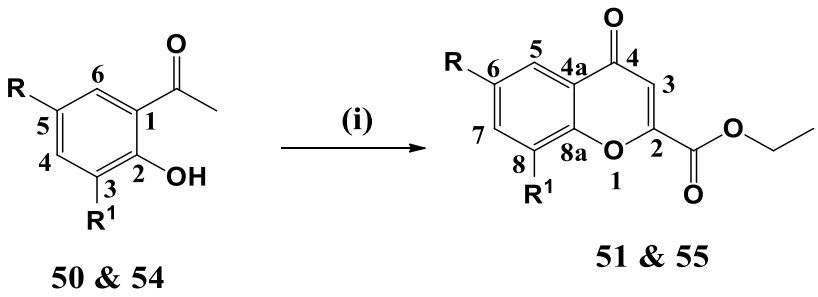
R= Br, F, Cl, NO_2

Table 2: ^{13}C NMR chemical shift values (ppm) of compound **54** in $CDCl_3$ (at 100 MHz)

Carbons	Br	Cl	F	NO_2
C-1	116.3	123.8	124.1	125.7
C-2	153.2	157.6	155.5	163.9
C-3	105.6	112.8	112.3	112.9
C-4	136.7	138.8	127.4	134.0
C-5	108.4	126.1	158.5	139.3
C-6	127.4	129.2	115.3	118.6
C=O	198.61	203.6	203.6	203.8
CH_3	22.0	26.7	26.7	26.7

4.3. Synthesis of chromone-2-carboxylate derivatives (51A-F & 55A-D)

The synthetic strategy chosen for the preparation of the 2-(4-amino-5-mercapto-4H-1,2,4-triazol-3-yl) chromone compounds (**53A-E & 57A-C**) required the preparation of chromone-2-carboxylates. Condensation of 2-hydroxyacetophenone with diethyl oxalate in the presence of sodium ethoxide in ethanol and followed by acidic cyclization afforded the white precipitate which was filtered off and the yellow filtrate was concentrated into a slurry. Water was added into a slurry and the solution was extracted with ethyl acetate, dried over sodium sulphate and evaporated, to give a light-yellow solid of the crude product. The crude product was recrystallized from methanol/ diisopropyl ether (4:1) to give chromone-2-carboxylates **(51A-F & 55A-D)**.[34] The chemical reaction scheme is outlined in **Scheme 10** below.

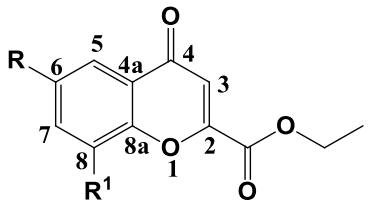


50 & 54 **51 & 55**

Reagent: (i) NaOEt-EtOH, Diethyl oxalate, reflux, 1h

SCHEME 10: Synthesis of chromone-2-carboxylate derivatives **51 & 55**

The chromone-2-carboxylate derivatives (**51A-F & 55A-D**) were fully characterized using 1D NMR (^{1}H, ^{13}C NMR, DEPT), IR spectrum, CHN analysis, and their physical properties. The assignment of the protons for chromone-2-carboxylate compounds **(51 & 55)** was done after considering the chemical shifts and coupling constants observed for signals in the ^{1}H NMR spectra for all compounds. The afforded yield was from low to excellent (32-95 %) and comparable with the data reported in the literature for some of the synthesized compounds. The experimental melting point of some compounds was consistent with those reported in the literature, except for **(51E & 55D)**, which are novel compounds and **(51B, D & 55A)**, whose melting points data were not reported in the literature. The melting point and percentage yield of the synthesized chromone-2-carboxylate derivatives are reported in **Table 3**.



51 & 55

Compounds	51 A	51 B	51 C	51 D	51 E	51 F	55 A	55 B	55 C	55 D
R	H	Br	F	MeO	NO_2	Cl	Br	Cl	F	NO_2
R^1	H	H	H	H	H	H	Br	Br	Br	Br

Table 3: Synthesised chromone-2-carboxylate derivatives **51 & 55**

Compounds	R	R^1	% Yield	Melting point (°C)	Lit. Melting point (°C)
51A	H	H	55	72-74	71-72[58]
51B	Br	H	49	151-156	142-144[59]
51C	F	H	73	136-141	-
51D	MeO	H	32	104-109	100.5-101[60]
51E	NO_2	H	38	183-189	-
51F	Cl	H	47	141-148	-
55A	Br	Br	82	138-140	134.5-135[60]
55B	Cl	Br	60	127-129	-
55C	F	Br	68	128-130	130-131[55]
55D	NO_2	Br	95	179-188	-

^{1}H NMR spectrum of chromone-2-carboxylate derivatives (**51A-F & 55A-D**) were characterized by a triplet and a quartet in the aliphatic region, triplet at around 1.36 ppm, with a coupling constant of 7.2 Hz, which corresponds to three methyl protons, a quartet at around 4.41 ppm, with a coupling constant of 7.2 Hz, which corresponds to CH_2 protons. In the aromatic region, the compound was characterized by a singlet at around 6.99 ppm, which corresponds to the proton at position 3; a doublet at around 7.92 ppm, with a coupling constant

2.4 Hz, which corresponds to the proton at position 7 and a doublet at around 8.33 ppm, with a coupling constant 2.4 Hz, which corresponds to the proton at position 5. For the chromone-2-carboxylate derivatives (**51A-F)** which are not substituted at position 8 are characterized by a doublet at around 7.81 ppm, with a coupling constant 8.8 Hz, which corresponds to the proton at position 8; a doublet of a doublet at around 7.92 ppm, with a coupling constant of 6.4 Hz and 2.8 Hz, which corresponds to the proton at position 7, and a doublet at around 7.94 ppm with a coupling constant of 2.4 Hz, which corresponds to the proton at position 5. Amongst these compounds, the fluorine containing compounds have shown some uniqueness of coupling constant in an aromatic region of the proton NMR spectrum, this is due to the H-F coupling because of high electronegativity of the fluorine and its ability to draw the bonding electron to itself whereas it leaves other proton exposed to one another and the NMR magnetic field.

Therefore, ^{1}H NMR spectrum of 6-fluorochromone-2-carboxylate **51C** was characterised by a doublet of a doublet at 7.72 ppm with a coupling constant of 4.8 Hz and 3.2 Hz, corresponding to the proton at position 5; a doublet of a doublet at 7.53 ppm with a coupling constant of a 5.2 Hz and 4 Hz, corresponding to the proton at position 8 and another doublet of a double at 7.40 ppm with a coupling constant of 7.6 Hz and 3.2 Hz, corresponding to the proton at position 7. The 8-bromo-6-fluorochromone-2-carboxylate **55C** was also characterised by of a doublet of a doublet at 8.25 ppm and 7.77 ppm with a coupling constant of 4.8 Hz & 3.2 Hz and 5.2 Hz & 2.8 Hz corresponding to the proton at positions 5 and 7, respectively. The ^{1}H NMR spectrum of 8-bromo-6-chlorochromone-2-carboxylate **55B** as shown in **Figure 9**.

Spectroscopic data obtained from ^{13}C NMR and DEPT 135 experiments were used to confirm the presence of six or seven quaternary carbons, and four or three (C-H)'s for derivatives which are not substituted **(51A-F)** and for those that are substituted (**55A-D)** at position 8 respectively, CH_2, and CH_3 carbons. The quaternary carbon at around 176.4 ppm corresponds to the carbonyl quaternary carbon at the pyrone ring and the one at 159.68 ppm corresponds to the carbonyl in the aliphatic chain, and the other four quaternary carbons in the aromatic region C-2, C-8a, C-8, C-6, C-4a resonating at 154,4 ppm, 152.9 ppm, 114.0 ppm, 131.1 ppm, and 125.2 ppm, respectively. The C-H aromatic carbons, C-3, C-7, C-5 resonating at 121.8 ppm, 124.3 ppm, 135.6 ppm, respectively. In the aliphatic region, the compound is characterized by CH_2 carbon at 63.3 ppm, and CH_3 carbon at 14.3 ppm. The ^{13}C NMR spectrum of 8-bromo-6-chlorochromone-2-carboxylate **55B** as shown in **Figure 10. Table 4** summarizes the observed chemical shifts of carbons for all chromone-2-carboxylate derivatives (**51A-F & 55A-D**) synthesized.

FT-IR spectroscopy was used to confirm the presence of functional groups in the chemical structures of chromone-2-carboxylate derivatives (**51A-F & 55A-D**). FT-IR revealed an aromatic (C-H) group at 3057 cm^{-1}, carbonyl (C=O) group stretch at 1735 cm^{-1}, and (C=C) aromatic group stretch at 1618 cm^{-1} as indicated in **Figure 11**. The CHN (Elementary) analysis was used to confirm the chemical element present in the compounds synthesized. The CHN analysis results of chromone-2-carboxylate derivatives (**51A-F & 55A-D**) were in complete agreement with the theoretical data calculated.

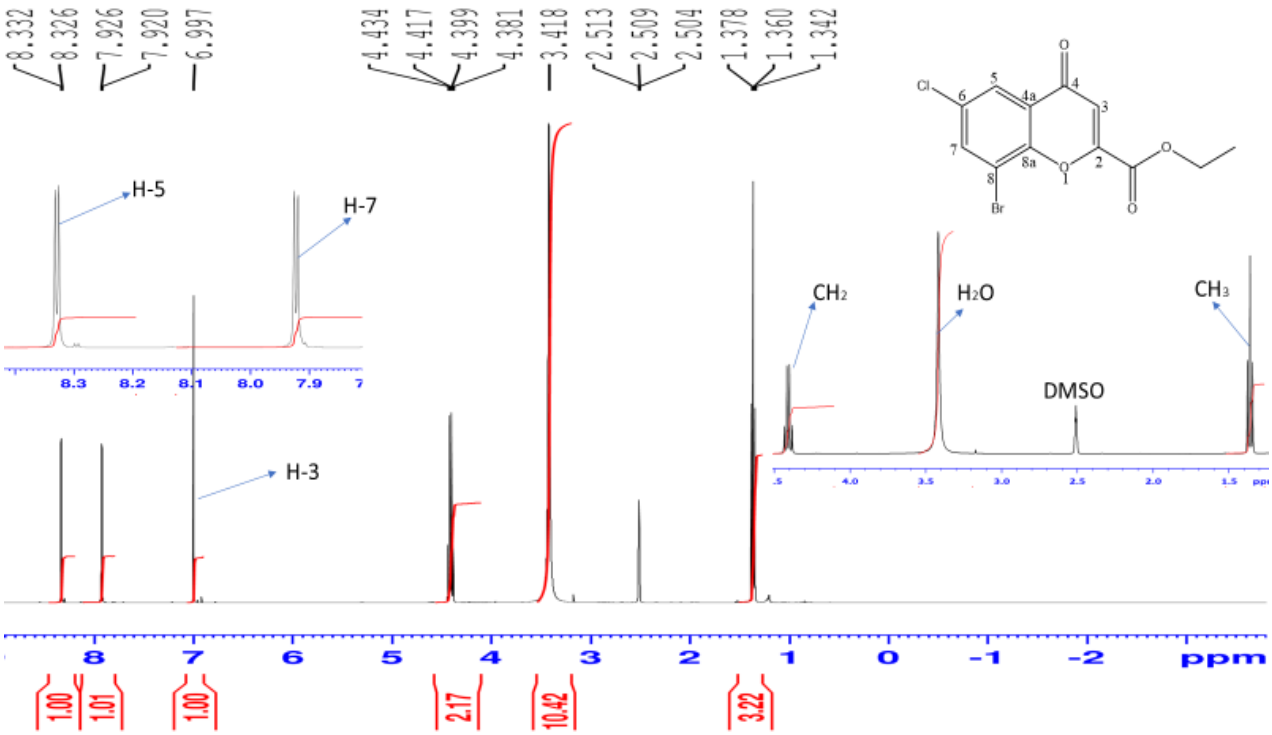


Figure 9: ^{1}H NMR (Full spectrum) of 6-chlorochromone-2-carboxylate **55B** in DMSO-d_6 (at 400 MHz)

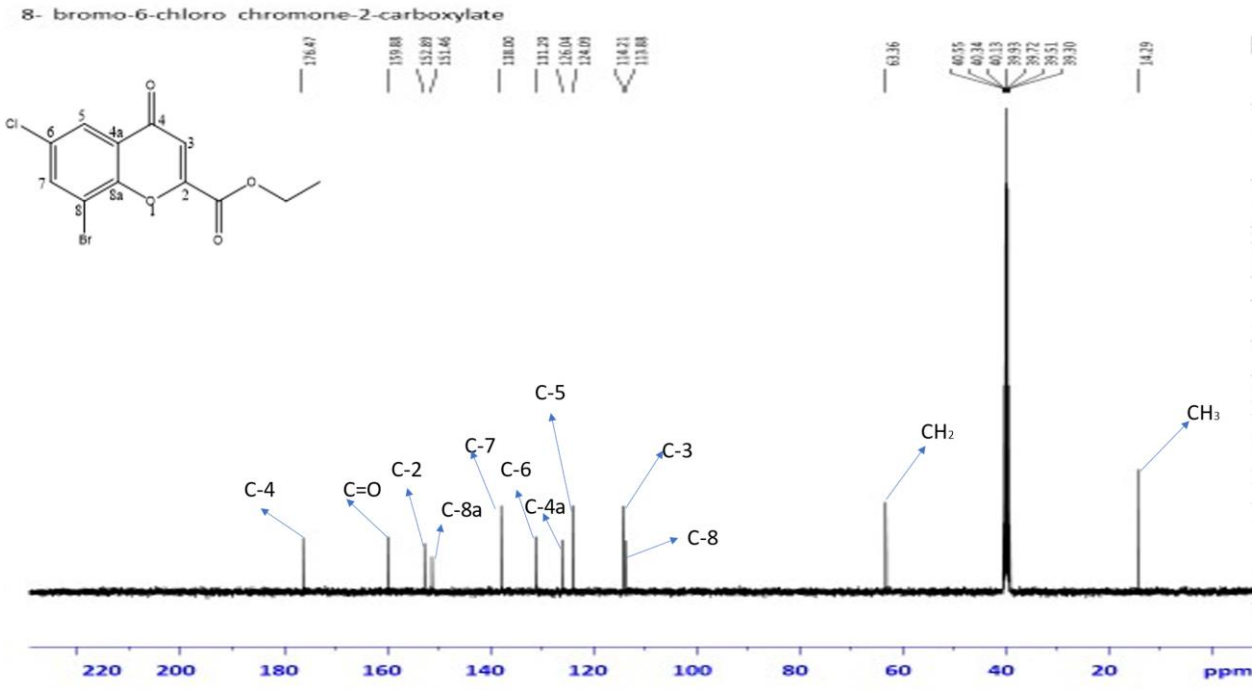


Figure 10: ^{13}C NMR spectrum of 6-chlorochromone-2-carboxylate **55B** in DMSO-d_6 (at 100 MHz)

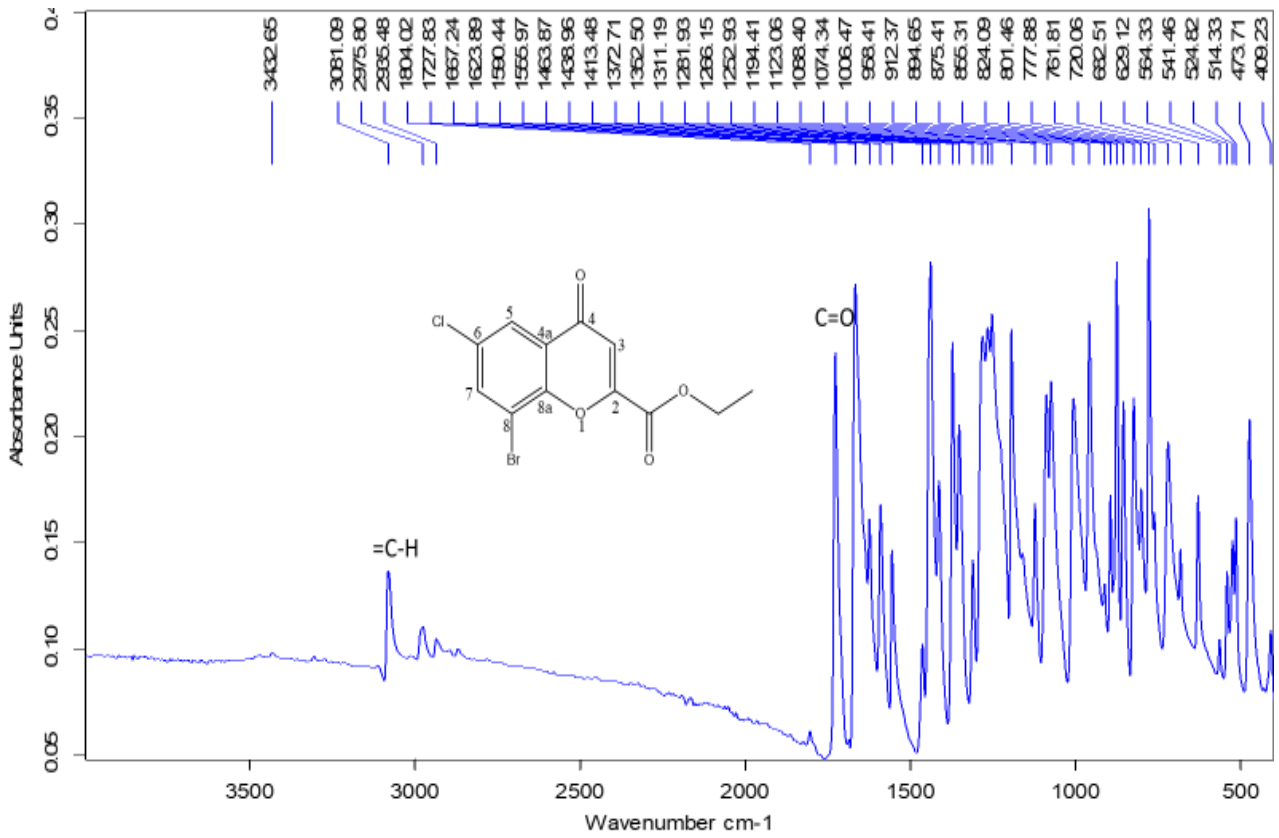
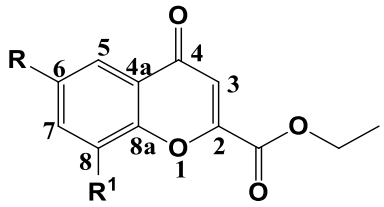


Figure 11: FTIR spectrum of 6-chlorochromone-2-carboxylate **55B**



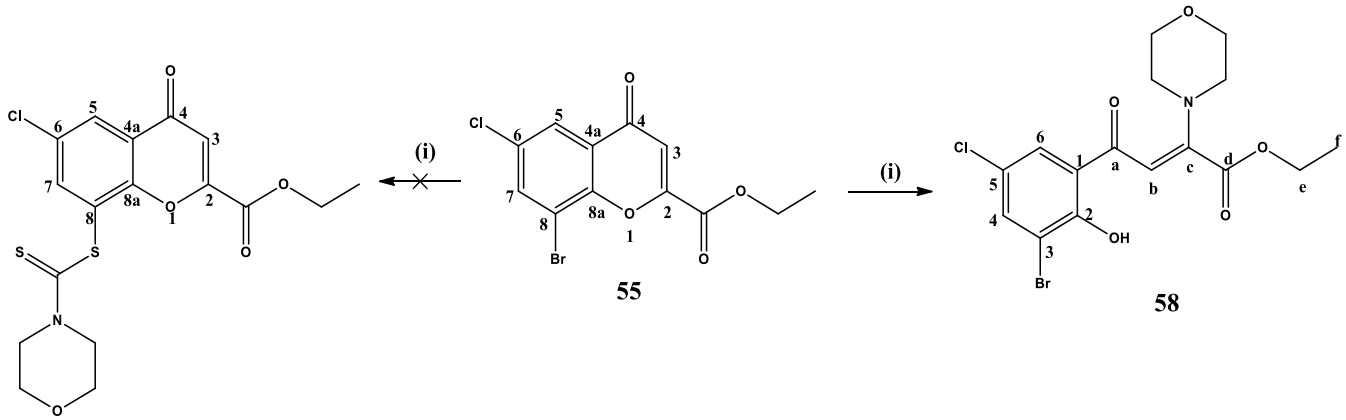
Compounds	51 A	51 B	51 C	51 D	51 E	51 F	55 A	55 B	55 C	55 D
R	H	Br	F	MeO	NO_2	Cl	Br	Cl	F	NO_2
R^1	H	H	H	H	H	H	Br	Br	Br	Br

Table 4: ^{13}C NMR chemical shift values (ppm) of compounds **51 & 55** in $CDCl_3$ and DMSO-d_6 (at 100 MHz)

Carbons	51 A	51 B	51 C	51 D	51 E	51 F	55 A	55 B	55 C	55 D
C-2	155.8	154.7	158.6	157.43	158.6	154.4	154.7	152.8	158.64	158.67
C-3	119.3	119.5	113.7	104.9	120.6	121.8	119.5	113.8	113.8	120.6
C-4	177.8	177.1	177.6	177.4	176.8	176.7	177.1	176.4	177.6	176.8
C-4a	124.2	120.7	120.9	124.8	124.3	124.3	120.7	124.3	120.9	124.3
C-5	125.4	128.3	125.5	120.9	128.9	125.2	128.3	124.0	125.5	128.9
C-6	126.6	125.6	123.1	152.3	122.4	131.1	125.6	131.2	123.1	122.4
C-7	135.7	137.7	125.6	124.9	145.1	135.6	137.7	138.0	125.6	145.1
C-8	114.1	114.7	110.4	113.1	115.0	114.0	114.7	114.0	110.4	115.0
C-8a	152.2	152.4	152.1	150.4	152.7	152.9	152.4	151.4	152.4	152.7
C=O	160.4	160.2	160.3	160.4	159.7	160.2	160.2	159.8	160.3	159.7
CH_2	63.1	63.0	63.1	63.1	63.5	63.3	63.0	63.3	63.1	63.5
CH_3	14.3	14.0	14.0	14.2	14.0	14.3	14.0	14.3	14.0	14.0
OMe	-	-	-	56	-	-	-	-	-	-

4.4. Synthesis of ethyl 4-(3-bromo-5-chloro-2-hydroxyphenyl)-2-morpholino-4-oxobut-2-enoate (58)

The substitution of the bromine at position 8 of the chromone with dithiocarbamates was unsuccessfully attempted. Instead, an opening of the pyrone ring occurred to form ethyl 4-(3-bromo-5-chloro-2-hydroxyphenyl)-2-morpholino-4-oxobut-2-enoate **58**. The ethyl 4-(3-bromo-5-chloro-2-hydroxyphenyl)-2-morpholino-4-oxobut-2-enoate **58** was achieved from corresponding brominated chromone-2-carboxylate **55B** which was treated *in-situ* with reactants prepared from morpholine in DMF and CS_2 and sodium methoxide. The yellow crystals of ethyl 4-(3-bromo-5-chloro-2-hydroxyphenyl)-2-morpholino-4-oxobut-2-enoate **58** was achieved in a high yield.[57] The chemical reaction scheme is outlined in **SCHEME 11** below.



Reagent: (i) Amine, CS_2, DMF, rt, 30 min

SCHEME 11: Synthesis of ethyl 4-(3-bromo-5-chloro-2-hydroxyphenyl)-2-morpholino-4-oxobut-2-enoate **58**

The ethyl 4-(3-bromo-5-chloro-2-hydroxyphenyl)-2-morpholino-4-oxobut-2-enoate **58** was fully characterized using 1D & 2D NMR, IR spectrum, HRMS and the physical properties. The assignment of the protons for ethyl 4-(3-bromo-5-chloro-2-hydroxyphenyl)-2-morpholino-4-oxobut-2-enoate **58** was done after considering the chemical shifts and coupling constants observed for signals**.** The afforded yield was excellent (97 %), while the experimental melting point of the compound was 179-181 °C.

The appearance of four triplet peaks in the aliphatic region, around 3.11 ppm to 4.33 ppm, confirmed the presence of morpholine moiety in the compound. Therefore, the ^{1}H NMR spectrum of the ethyl 4-(3-bromo-5-chloro-2-hydroxyphenyl)-2-morpholino-4-oxobut-2-enoate was characterized by a triplet peak at 1.29 ppm, which corresponds to the CH_3 at position f, the triplet peaks at 3.11 ppm and 3.52 ppm which, corresponds to the two CH_2-N's protons; the triplet peaks at 3.76 ppm and 4.29 ppm, which corresponds to the two CH_2-O's protons; the quartet peak at 4.33 ppm, which corresponds to the CH_2 at position e; a singlet peak at 6.25 ppm, which corresponds to the proton at position b; a doublet at 7.83 ppm, which corresponds

to the proton at position 6 and a doublet at 8.35 ppm, which corresponds to the proton at position 4. The ^{1}H NMR spectrum of the ethyl 4-(3-bromo-5-chloro-2-hydroxyphenyl)-2-morpholino-4-oxobut-2-enoate compound **58** is shown in **Figure 12**.

The spectroscopic data obtained from ^{13}C NMR and DEPT 135 experiments was used to confirm the presence of seven quaternary carbons, three C-H carbons, five CH_2 carbons, and the CH_3. The appearance of C-H (C-b) peak at 89 ppm confirm that indeed the ring was opened. The quaternary carbon at 188.1 ppm, corresponds to the C-a carbon, the quaternary carbon at 164.4 ppm, corresponds to the carbonyl carbon C-d, the quaternary carbon at 158, 2 ppm, corresponds to the carbon C-2 and the quaternary carbon C-c, C-4, C-1, C-5, C-3 resonating at 156.9 ppm, 136.6 ppm, 121.5 ppm, 127.3 ppm and 111.7 ppm respectively. The C-H aromatic carbons C-6, C-4, C-b, resonating at 128.3 ppm, 122.8 ppm, and 89.0 ppm, respectively. In the aliphatic region, three CH_2 carbon peaks were observed OCH_2, NCH_2, CH_2 at position 3 resonating at 66.5 ppm, 63.9 ppm and 62.5 ppm respectively. The CH_3 peaks at position f were also observed at 14.0 ppm. The ^{13}C NMR spectrum of ethyl 4-(3-bromo-5-chloro-2-hydroxyphenyl)-2-morpholino-4-oxobut-2-enoate compound **58** is indicated in **Figure 13**.

FT-IR spectroscopy was used to confirm the presence of functional groups in the chemical structure of ethyl 4-(3-bromo-5-chloro-2-hydroxyphenyl)-2-morpholino-4-oxobut-2-enoate compound **58**. FT-IR spectrum of an ethyl 4-(3-bromo-5-chloro-2-hydroxyphenyl)-2-morpholino-4-oxobut-2-enoate compound **58** revealed a C=O group at 1721 cm^{-1}, and =C-H group at 2968 cm^{-1} as indicated in **Figure 14**. The HRMS was also used to provide the molecular weight of ethyl 4-(3-bromo-5-chloro-2-hydroxyphenyl)-2-morpholino-4-oxobut-2-enoate compound **58**. The HRMS spectrum revealed a peak at m/z 417 which is very close to the molecular mass m/z 418 of ethyl 4-(3-bromo-5-chloro-2-hydroxyphenyl)-2-morpholino-4-oxobut-2-enoate compound **58**. The HRMS results were in complete agreement with the calculated data where a 415, 417 and 419 peaks confirming the bromine and chlorine containing compound where you are supposed to get 3 peaks in mass spectrum M, M+2 and M+4.

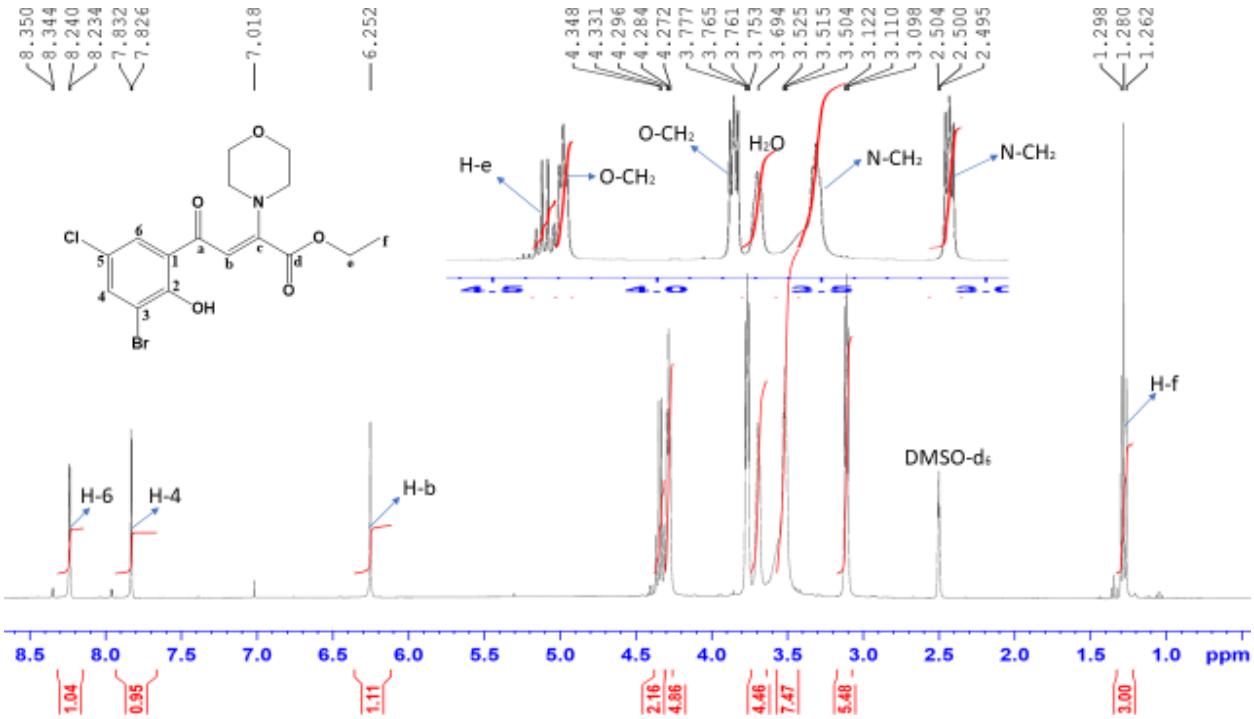


Figure 12: 1H NMR of ethyl 4-(3-bromo-5-chloro-2-hydroxyphenyl)-2-morpholino-4-oxobut-2-enoate **58** in DMSO-d_6 (at 400 MHz)

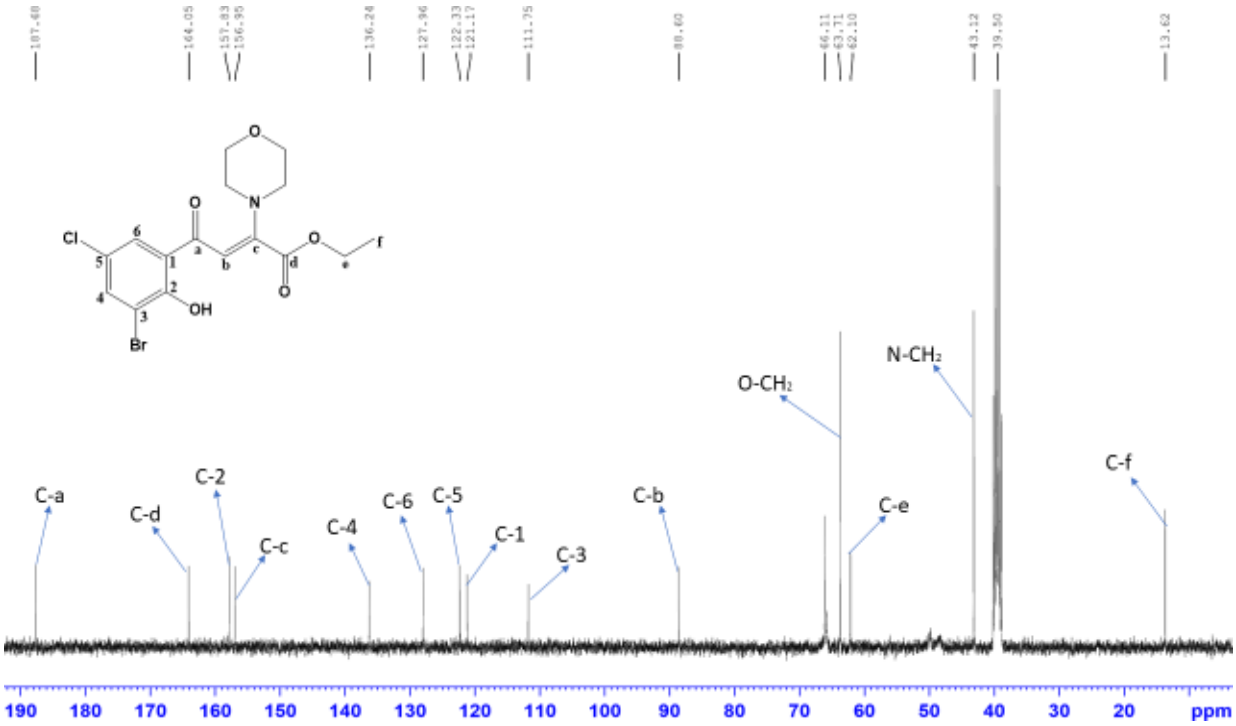


Figure 13: ^{13}C NMR spectrum of ethyl 4-(3-bromo-5-chloro-2-hydroxyphenyl)-2-morpholino-4-oxobut-2-enoate **58** in DMSO-d_6 (at 100 MHz)

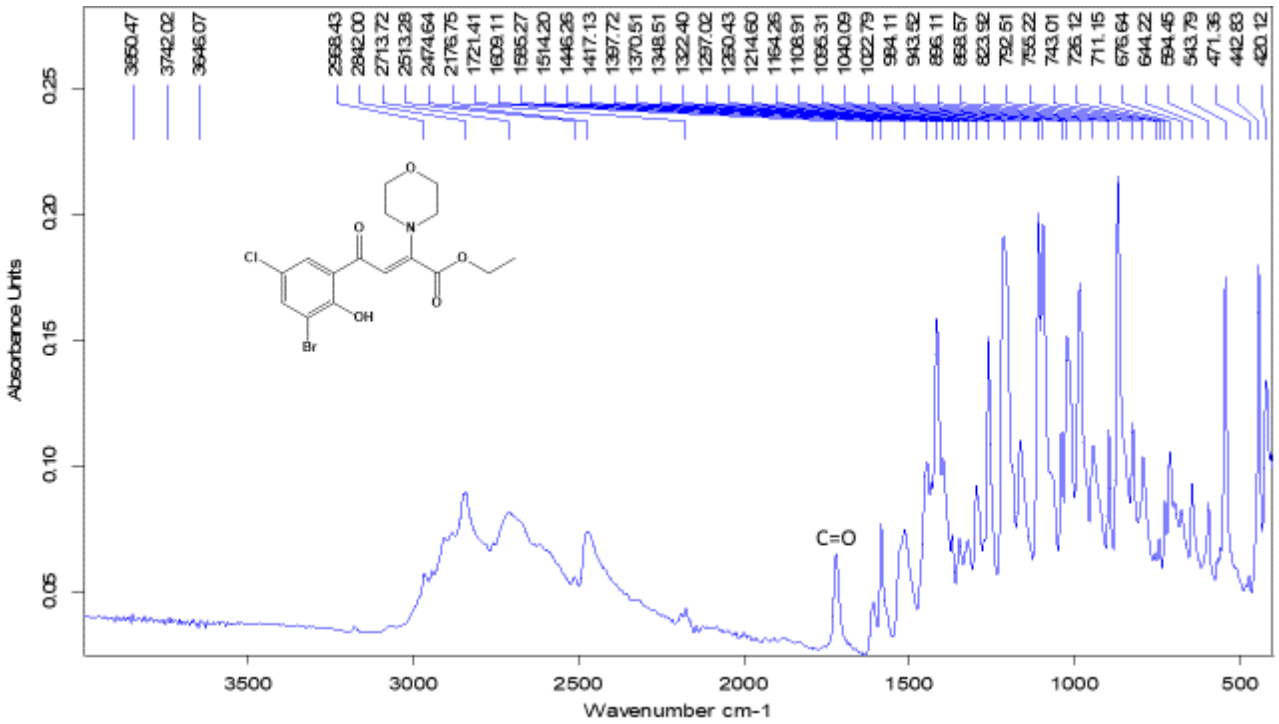


Figure 14: FTIR spectrum of ethyl 4-(3-bromo-5-chloro-2-hydroxyphenyl)-2-morpholino-4-oxobut-2-enoate **58**

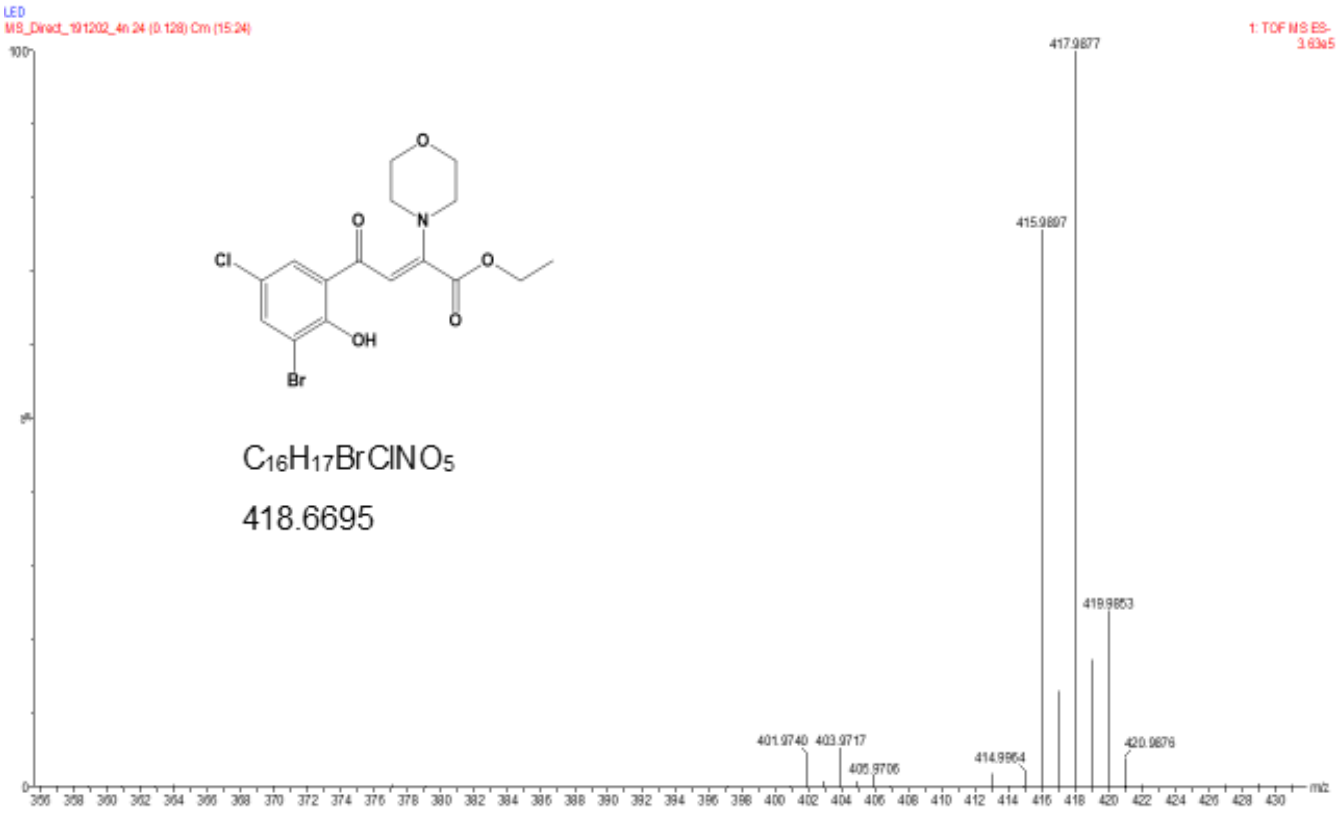
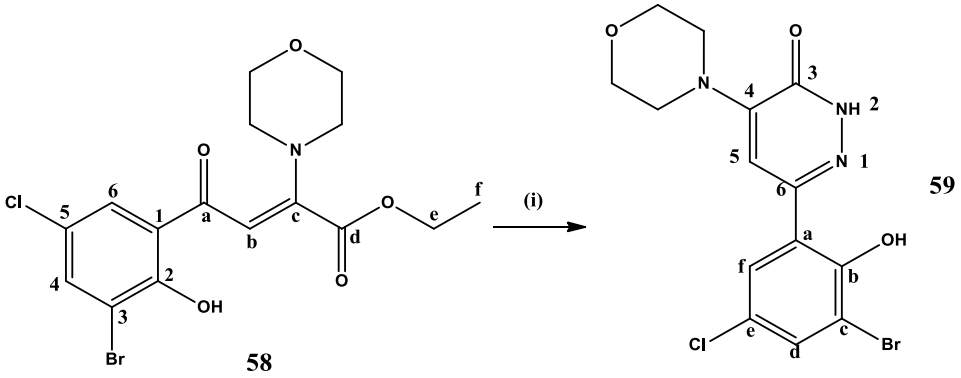


Figure 15: HRMS spectrum of ethyl 4-(3-bromo-5-chloro-2-hydroxyphenyl)-2-morpholino-4-oxobut-2-enoate **58**

4.5. Synthesis of 6-(3-bromo-5-chloro-2-hydroxyphenyl)-4-morpholinopyridazin-3(2*H*)-one 59

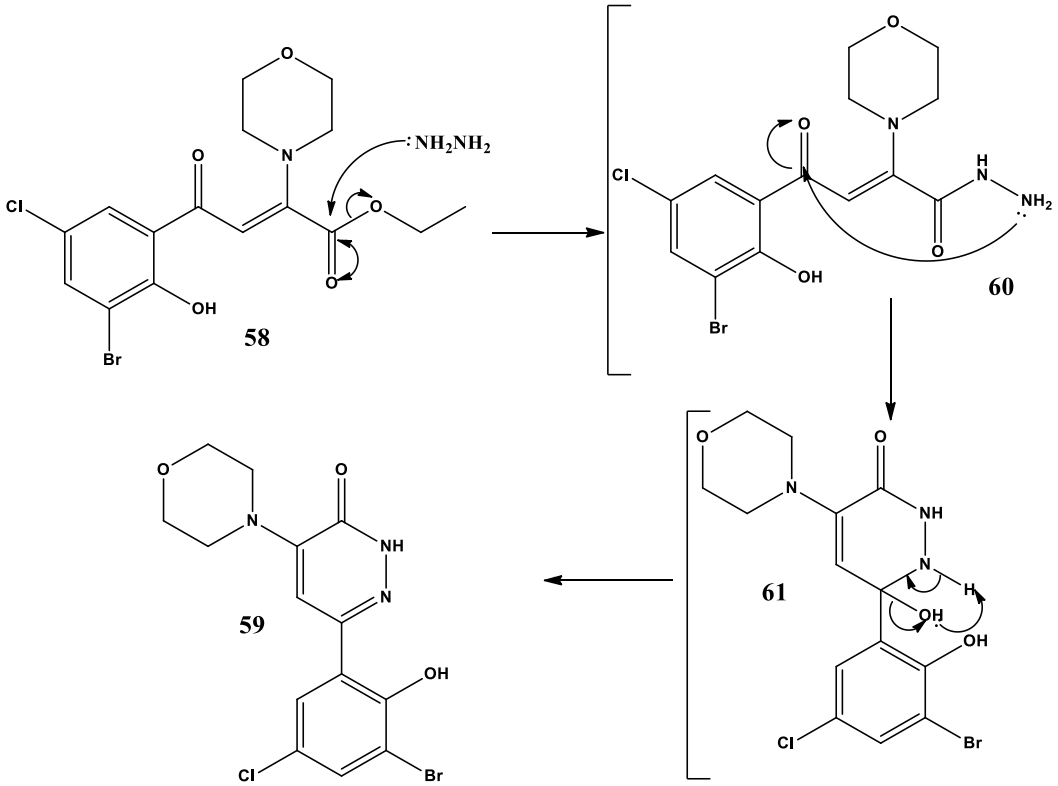
The substitution of the ethoxy group from the chromone-2-carboxylate by hydrazine which consequently cyclised to form 6-(3-bromo-5-chloro-2-hydroxyphenyl)-4-morpholinopyridazin-3(2*H*)-one **59** was executed following the method in the literature. In our case, a solution of ethyl 4-(3-bromo-5-chloro-2-hydroxyphenyl)-2-morpholino-4-oxobut-2-enoate **58** in ethanol, hydrazine hydrate, was added and heated under reflux for 8h. The solution was allowed to cool after completion. The formed precipitate was filtered off, washed with water, dried and recrystallized in ethanol, to achieve the pure 6-(3-bromo-5-chloro-2-hydroxyphenyl)-4-morpholinopyridazin-3(2*H*)-one **59** in low yield.[46] The chemical reaction scheme is outlined in **Scheme 12** below.



Reagent: (i) $NH_2NH_2.H_2O$, EtOH, Reflux, 8h

SCHEME 12: Synthesis of 6-(3-bromo-5-chloro-2-hydroxyphenyl)-4-morpholinopyridazin-3(2*H*)-one **59**

The 6-(3-bromo-5-chloro-2-hydroxyphenyl)-4-morpholinopyridazin-3(2*H*)-one **59** was prepared in situ by the reaction of ethyl 4-(3-bromo-5-chloro-2-hydroxyphenyl)-2-morpholino-4-oxobut-2-enoate **58** in ethanol with hydrazine hydrate which resulted in the substitution of ethoxy moiety by the hydrazine moiety to form the intermediate **60** which consequently cyclised through the nucleophilic attack at the carbonyl carbon (electrophile) next to the benzene ring with the NH_2 (nucleophile) of the hydrazine moiety to form 6-(3-bromo-5-chloro-2-hydroxyphenyl)-4-morpholinopyridazin-3(2*H*)-one **59** via the loss of H_2O (water). The chemical reaction mechanism is outlined in **Scheme 13** below.



SCHEME 13: Proposed reaction mechanism of the 6-(3-bromo-5-chloro-2-hydroxyphenyl)-4-morpholinopyridazin-3(2*H*)-one **59**

The NMR spectroscopic results confirmed the structure of the 6-(3-bromo-5-chloro-2-hydroxyphenyl)-4-morpholinopyridazin-3(2*H*)-one **59**. The afforded yield was low at 33 %, while the experimental melting point of the compound was 326- 329 °C.

The ^{1}H NMR spectrum was characterized by the disappearance of the methyl signal (CH_3) at around 1.09 ppm and the quartet signal (CH_2) at around 3.55ppm, which corresponds to the CH_2 and CH_3 protons confirming the complete substitution of the ethoxy by the hydrazide. Thus, the appearance of the broad signal at 5.69 ppm and around 8.66 ppm, which corresponds to the OH and NH respectively. Therefore, the ^{1}H NMR spectrum of the 6-(3-bromo-5-chloro-2-hydroxyphenyl)-4-morpholinopyridazin-3(2*H*)-one **59** in the aromatic region was characterized by a singlet at 7.22 ppm, which corresponds to the proton at position 5; a doublet at 7.47 ppm and 7.57 ppm both with a coupling constant of 2,8 Hz, which corresponds to the protons at position f and d respectively. The ^{1}H NMR spectrum of the 6-(3-bromo-5-chloro-2-hydroxyphenyl)-4-morpholinopyridazin-3(2*H*)-one **59** is indicated in **Figure 15**.

Spectroscopic data obtained from ^{13}C NMR and DEPT 135 was used to confirm the presence of seven quaternary carbons, four CH_2 carbons and three (C-H) carbons. The quaternary carbon at the 160.5 ppm corresponds to the carbonyl carbon C-b, while the one at 157.2 ppm corresponds to C-3 and the other five quaternary carbons, which corresponds to carbons C-4, C-6, C-e, C-a, C-c resonating at 145.6 ppm, 142.3 ppm, 118.3 ppm, 117.5 ppm, and 114.2 ppm respectively. The (C-H) aromatic carbons C-5, C-f, C-d, resonating at 101.2 ppm, 125.1 ppm,

130.5 ppm, respectively. The carbon peak at 181 ppm could be the impurities or something which could be determined by the X-ray analysis as stated in the future work. The ^{13}C NMR spectrum of 6-(3-bromo-5-chloro-2-hydroxyphenyl)-4-morpholinopyridazin-3(2*H*)-one compound **59** is indicated in **Figure 16**.

FT-IR spectroscopy was used to confirm the presence of functional groups. The FT-IR spectrum of the 6-(3-bromo-5-chloro-2-hydroxyphenyl)-4-morpholinopyridazin-3(2*H*)-one **59** revealed an aromatic C-H at 2969 cm^{-1}, (O-H) at 3323 cm^{-1}, and the carbonyl group (C=O) stretching at 1671 cm^{-1} as indicated in **Figure 17**. The HRMS was also used to provide the molecular weight of 6-(3-bromo-5-chloro-2-hydroxyphenyl)-4-morpholinopyridazin-3(2H)-one **59**. The HRMS spectrum revealed a peak at m/z 385 which is very close to the molecular mass m/z 386 of 6-(3-bromo-5-chloro-2-hydroxyphenyl)-4-morpholinopyridazin-3(2*H*)-one compound **59**.

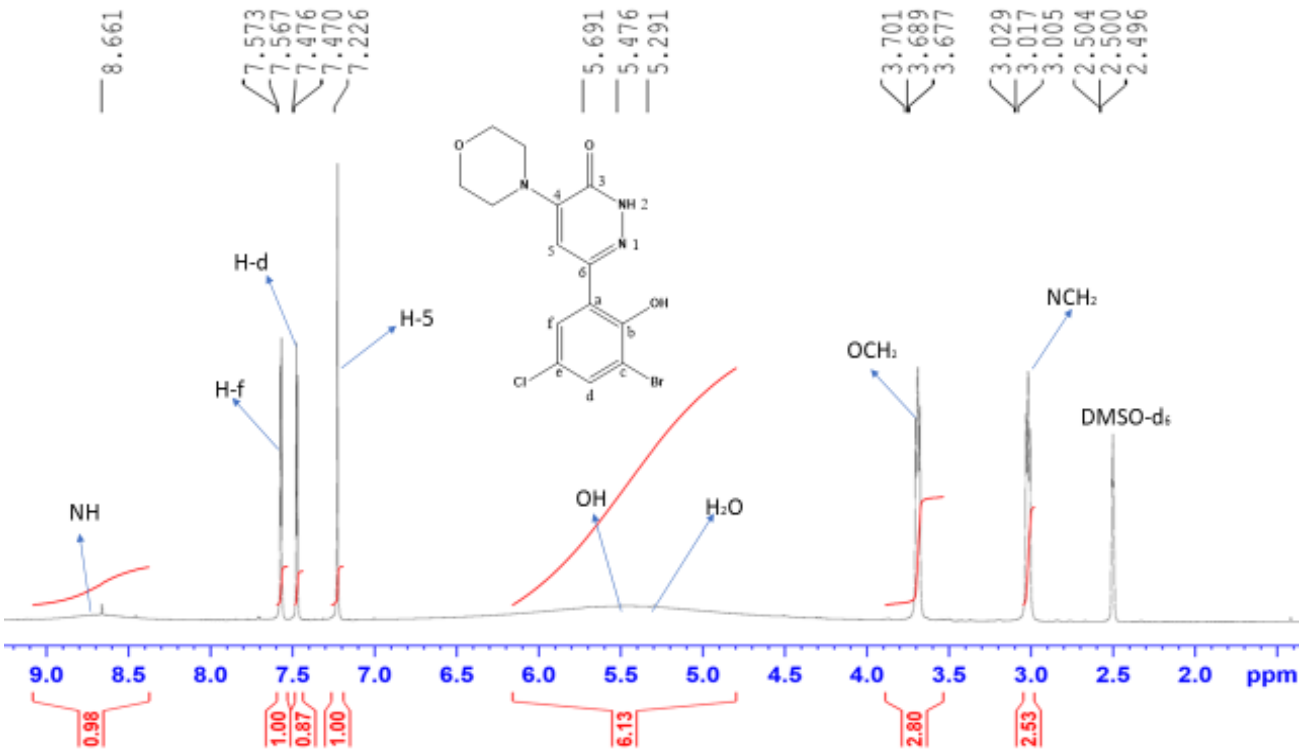


Figure 16: ^{1}H NMR (full spectrum) of 6-(3-bromo-5-chloro-2-hydroxyphenyl)-4-morpholinopyridazin-3(2*H*)-one **59** in DMSO-d_6 (at 400 MHz)

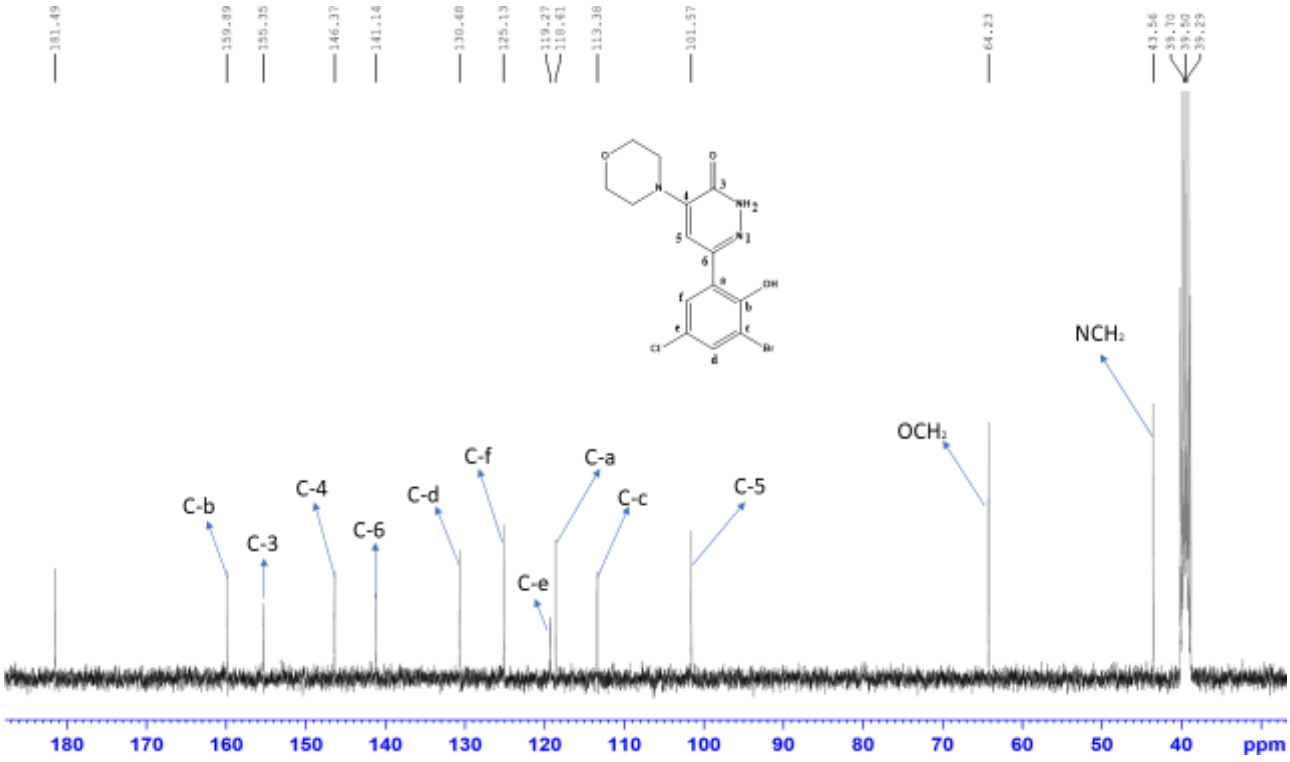


Figure 17: ^{13}C NMR spectrum of 6-(3-bromo-5-chloro-2-hydroxyphenyl)-4-morpholinopyridazin-3(2*H*)-one **59** in DMSO-d_6 (at 100 MHz)

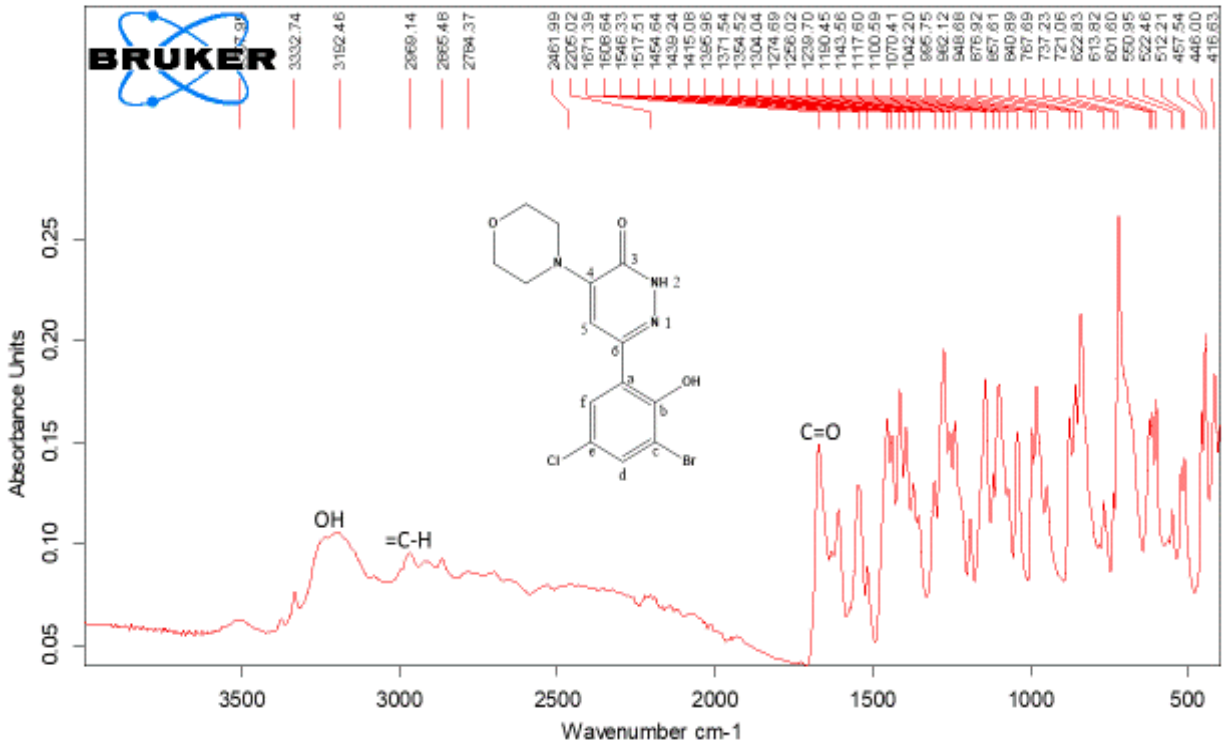


Figure 18: FTIR spectrum of 6-(3-bromo-5-chloro-2-hydroxyphenyl)-4-morpholinopyridazin-3(2*H*)-one **59**

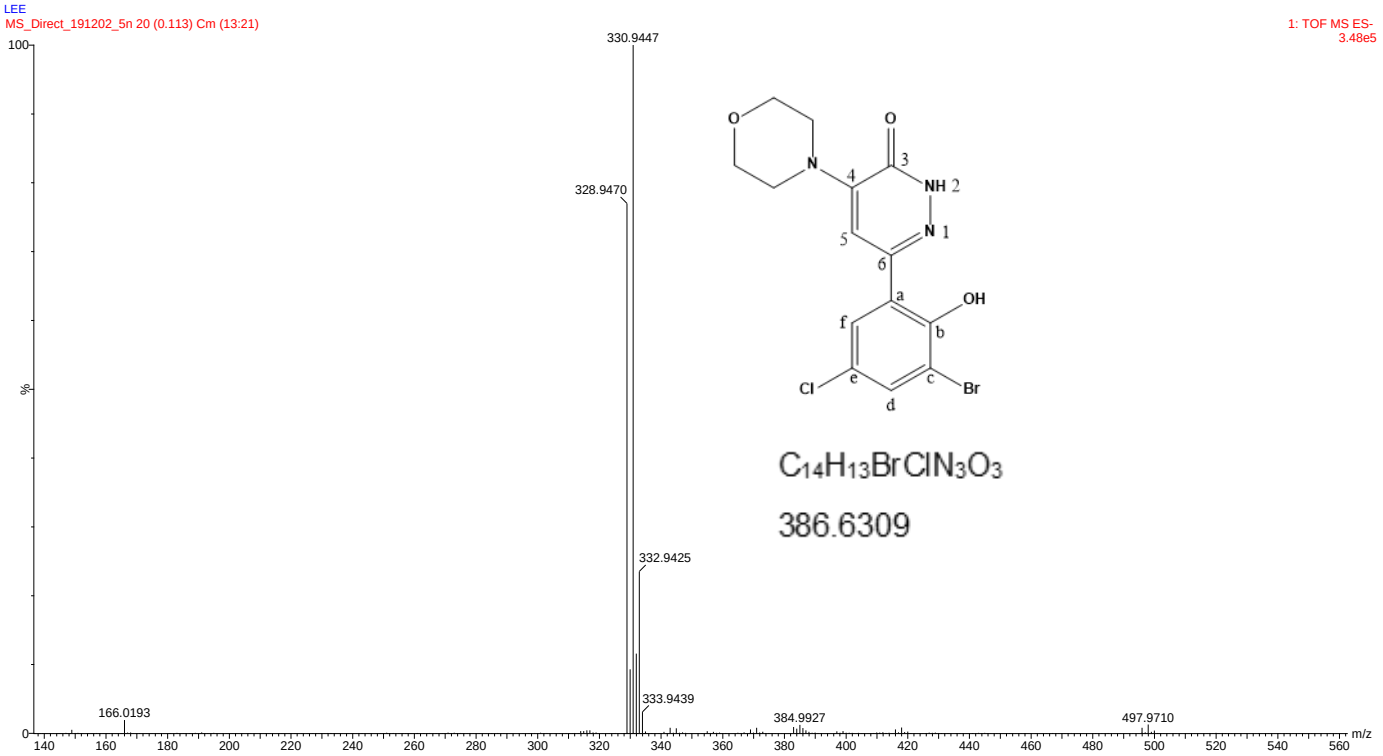


Figure 19: HRMS spectrum of 6-(3-bromo-5-chloro-2-hydroxyphenyl)-4-morpholinopyridazin-3(2*H*)-one **59**

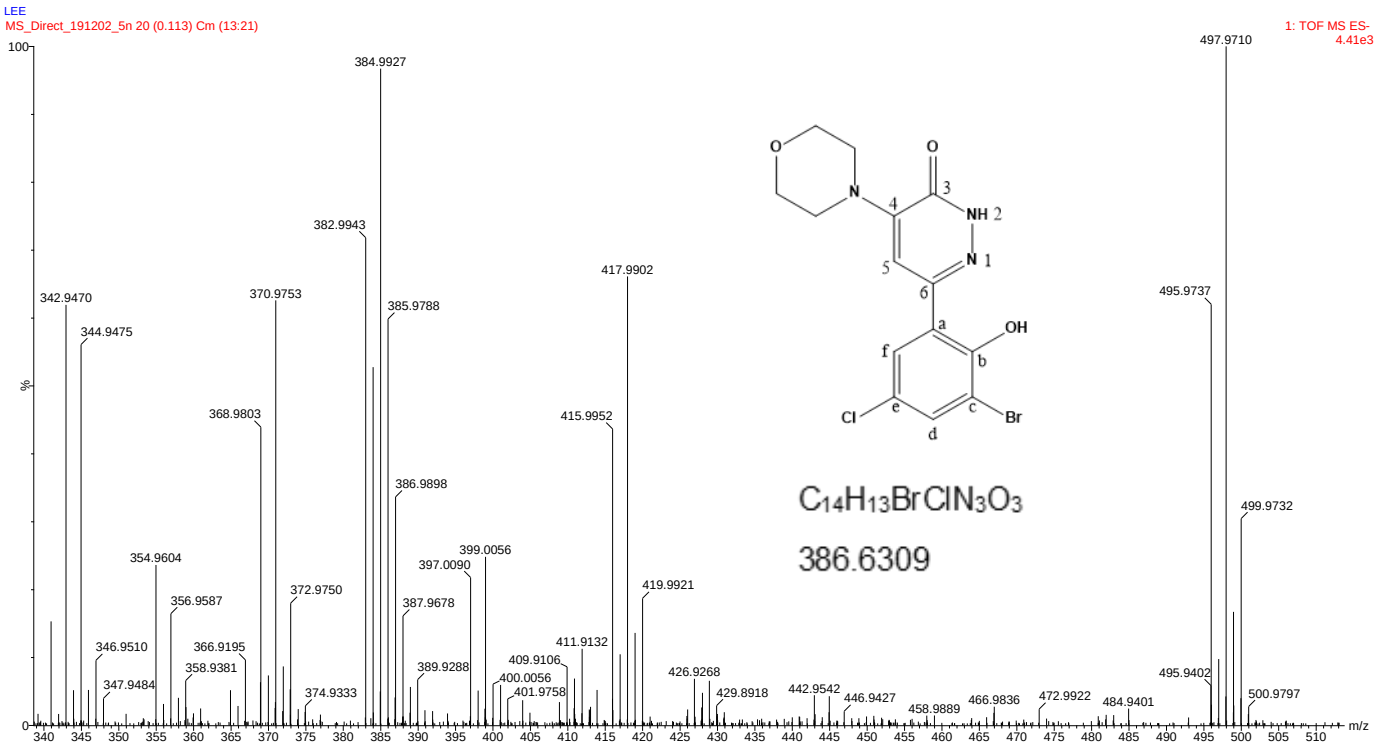
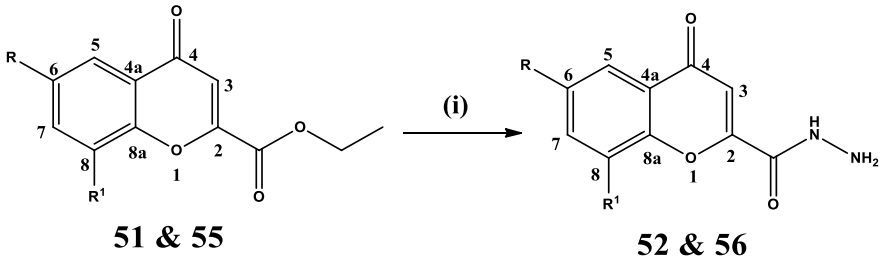


Figure 20: Expanded HRMS spectrum of 6-(3-bromo-5-chloro-2-hydroxyphenyl)-4-morpholinopyridazin-3(2*H*)-one **59**

4.6. Synthesis of chromone-2-carbohydrazide (52A-F & 56A-D)

The conversion of chromone-2-carboxylates **51 & 55** to chromone-2-carbohydrazide derivatives **52 & 56** using hydrazine hydrate in ethanol was executed following the literature method. [46] In this case, a solution of chromone-2-carboxylate in ethanol, hydrazine hydrate was added and heated under reflux for 8h. The solution was allowed to cool after the reaction had reached completion, the formed precipitate was filtered off, washed with water, dried and recrystallized in ethanol, to achieve the pure chromone-2-carbohydrazide derivatives **52 & 56**.[46] The chemical reaction scheme is outlined in **Scheme 14** below.

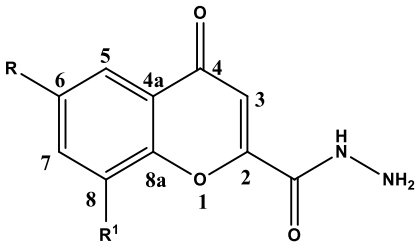


R= H, MeO, NO_2, Cl, Br, F
R^1= H, Br

Reagent: (i) $NH_2NH_2.H_2O$, EtOH, Reflux, 8h

Scheme 14: synthesis of chromone-2-carbohydrazide derivatives **56**

These compounds were fully characterized using 1D & 2D NMR, IR, HRMS spectrum and their physical properties. The assignment of the protons for chromone-2-carbohydrazide derivatives **(52 & 56)** was done after considering the chemical shifts and coupling constants observed for signals in the ^{1}H NMR spectra of all the chromone-2-carbohydrazide derivatives **(52A-F & 56A-D).** The afforded yield was moderate to excellent yield (40.64 - 95.29%). The melting point and the percentage yield of the synthesized chromone-2-carbohydrazide derivatives **52 & 56** are reported in **Table 5**.



52 & 56

Table 5: Synthesised chromone-2-carbohydrazide derivatives **52 & 56**

Compound	R	R^1	% Yield	Melting point (°C)
52A	H	H	78	405-408
52B	MeO	H	80	386-390
52C	NO_2	H	52	274-280
52D	Cl	H	82	301-305
52E	Br	H	40	321-326
52F	F	H	46	360
56A	Br	Br	93	331-334
56B	NO_2	Br	95	277-286
56C	Cl	Br	82	336-339
56D	F	Br	45	255-262

The disappearance of the methyl signal (CH_3) at around 1.09 ppm and the quartet signal (CH_2) at around 3.5ppm, which corresponds to the CH_2 and CH_3 protons confirmed the complete conversion of chromone-2-carboxylate to chromonyl-2-carbohydrazide. Thus, the appearance of the broad signal at 4.4 ppm and around 9.7 ppm, which corresponds to the NH_2 and NH respectively. The ^{1}H NMR spectrum of the chromone-2-carbohydrazide derivatives (**52A-F & 56A-D**) in the aromatic region was characterised by a triplet of a doublet at 6.89 ppm, with a coupling constant of 7 Hz and 1.2 Hz which corresponds to the proton at position 7; doublet at 6.98 with a coupling constant of 7.6 Hz, which corresponds to the proton at position 8; triplet of a doublet at 7.1 ppm, with a coupling constant of 6.8 Hz and 1.6 Hz, which corresponds to the proton at position 6; singlet at 7.2 ppm, which correspond to the proton at position 3 and the doublet of a doublet at 7.6 ppm with a coupling constant of 6.4 Hz and 1.6 Hz, which

corresponds to the proton at position 5. But for other chromone-2-carbohydrazide derivatives (**56A-D)** which are substituted at positions 6 and 8 was characterized by the appearance of three protons in the aromatic region.

Amongst these compounds we also had a fluorine containing compounds 6-fluorochromone-2-carbohydrazide **52F** which was characterised by a doublet of a doublet at 7.52 ppm with a coupling constant of 6.8 Hz and 2.8 Hz, corresponding to the proton at position 5; a doublet of a doublet at 7.01 ppm with a coupling constant of 5.6 Hz and 3.2 Hz, corresponding to the proton at position 7 and another doublet of a doublet at 6.96 ppm with a coupling constant of 5.2 Hz and 3.2 Hz, corresponding to the proton at position 8. This uniqueness of coupling in the aromatic region of the proton NMR spectrum is due to the high electronegativity of the fluorine atom. The ^{1}H NMR spectrum of the chromone-2-carbohydrazide compound **52A** is indicated in **Figure 18**.

Spectroscopic data obtained from ^{13}C NMR and DEPT 135 experiments was used to confirm the presence of five or six or seven quaternary carbons and five or four or three (C-H)'s for chromone-2-carbohydrazide derivatives which are not substituted **52A**, substituted at position 6 (**52B-F)** and those that are substituted at both position 6 and 8 (**56G-J)** respectively. The quaternary carbon at 175.3 ppm corresponds to the carbonyl in the aromatic region and the one at the 161.8 ppm corresponds to the quaternary carbon of the carbohydrazide group and the other three quaternary carbons which correspond to the aromatic carbons C-2, C-8a, C-4a, resonating at 144.8 ppm, 143.7 ppm, and 123.9 ppm, respectively. The (C-H) aromatic carbons C-3, C-5, C-6, C-7, C-8, resonating at 100.5 ppm, 125.9 ppm, 120.9 ppm, 130.9 ppm, 143.9 ppm, respectively. The ^{13}C NMR spectrum of chromone-2-carbohydrazide compound **52A** is indicated in **Figure 19**. **Table 6** outlines the observed chemical shifts of carbons for all chromone-2-carbohydrazide derivatives (**52A-F & 56G-J**) synthesized.

FT-IR spectroscopy was used to confirm the presence of functional groups. The FT-IR spectrum of chromone-2-carbohydrazide compound **52A** revealed an aromatic (C-H) at 2990 cm^{-1}, NH group at 3328 cm^{-1}, and the carbonyl group (C=O) stretching at 1639 cm^{-1} as indicated in **Figure 20**. The HRMS was used to provide the molecular weight of chromone-2-carbohydrazide derivatives (**52A-F & 56G-J**). The HRMS results were in complete agreement, where there was a parent peak on the HRMS results spectrum at m/z 203 and the m/z of chromone-2-carbohydrazide compound **52A** is at 204 as indicated in **Figure 21**.

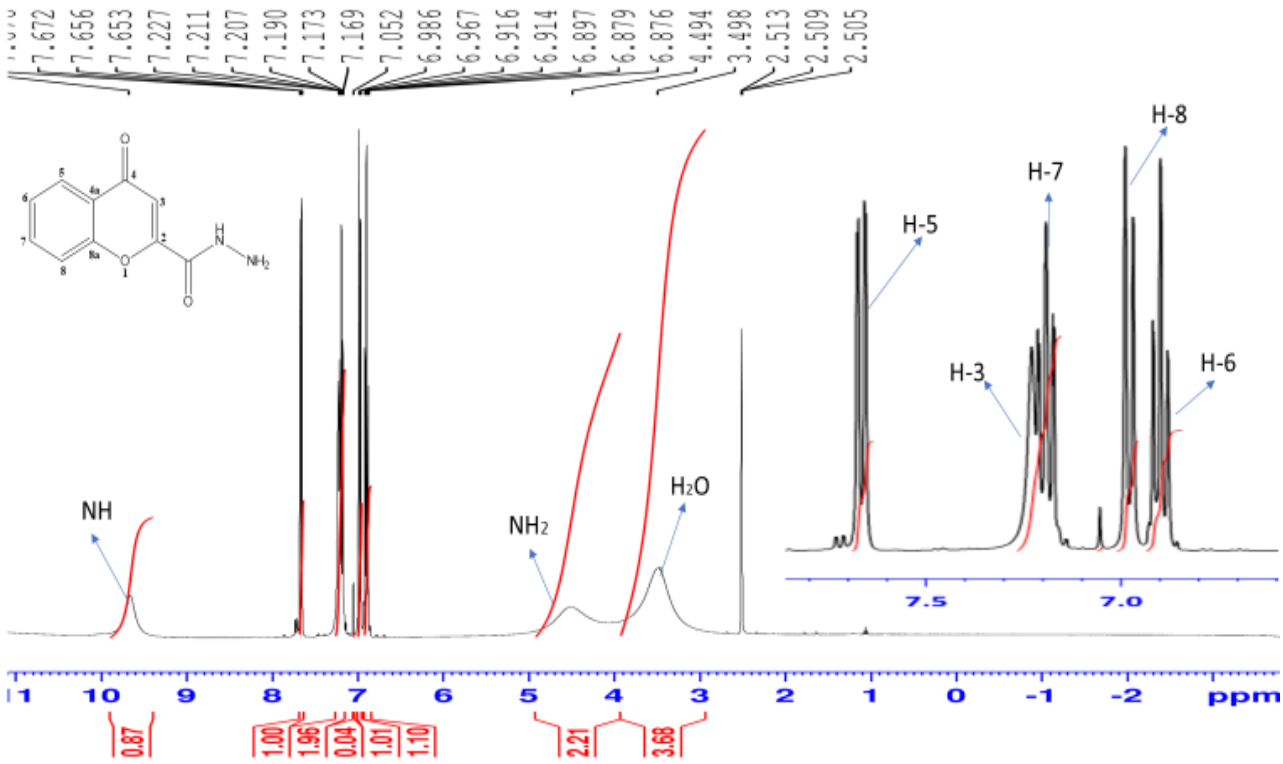


Figure 18: ^{1}H NMR of chromone-2-carbohydrazide **52A** in DMSO-d_6 (at 400 MHz)

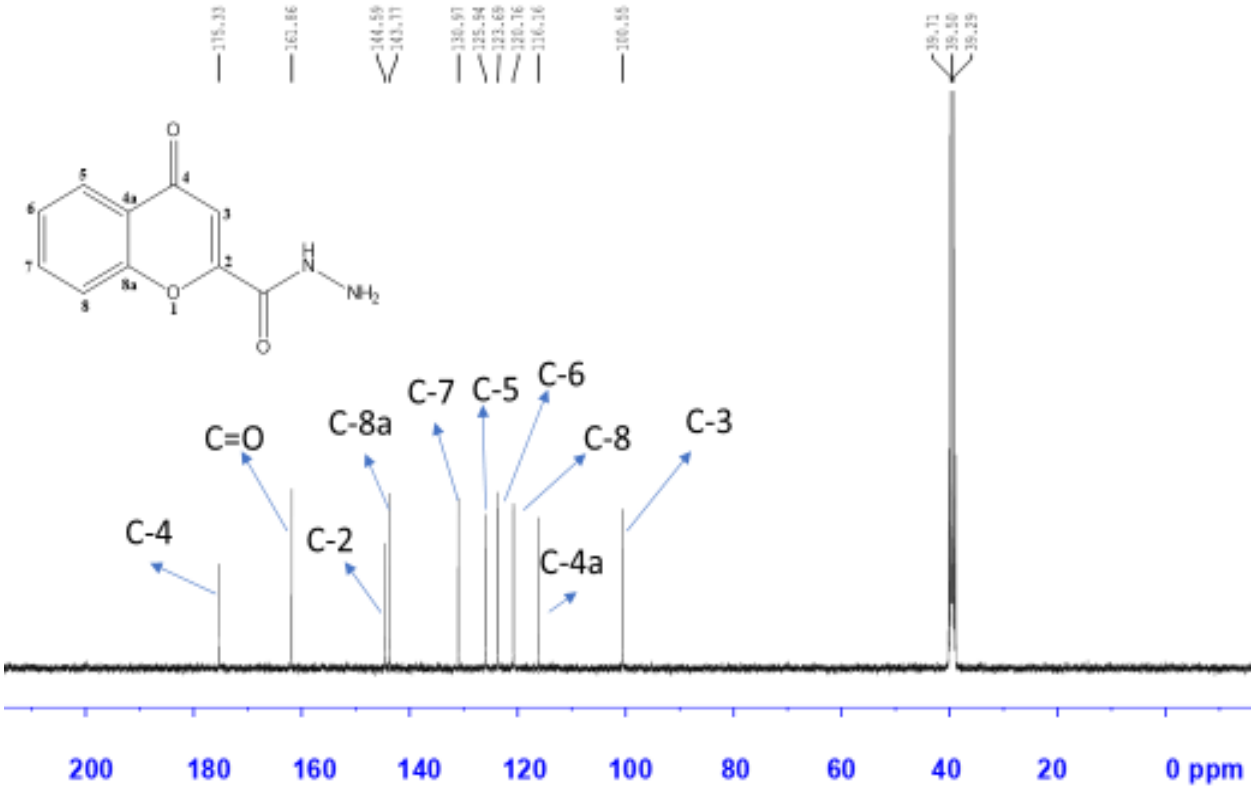


Figure 19: ^{13}C NMR spectrum of chromone-2-carbohydrazide derivatives **52A** in DMSO-d_6 (at 100 MHz)

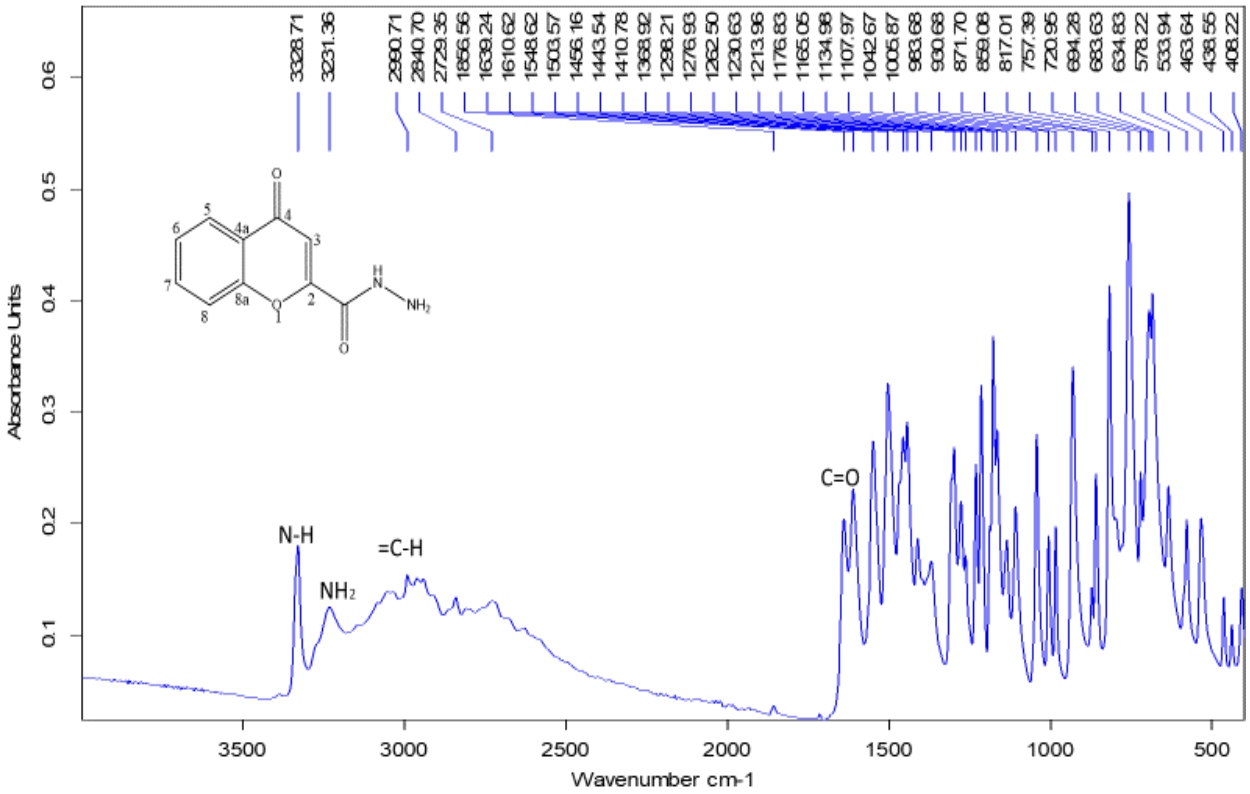


Figure 20: FTIR spectrum of chromone-2-carbohydrazide **52A**

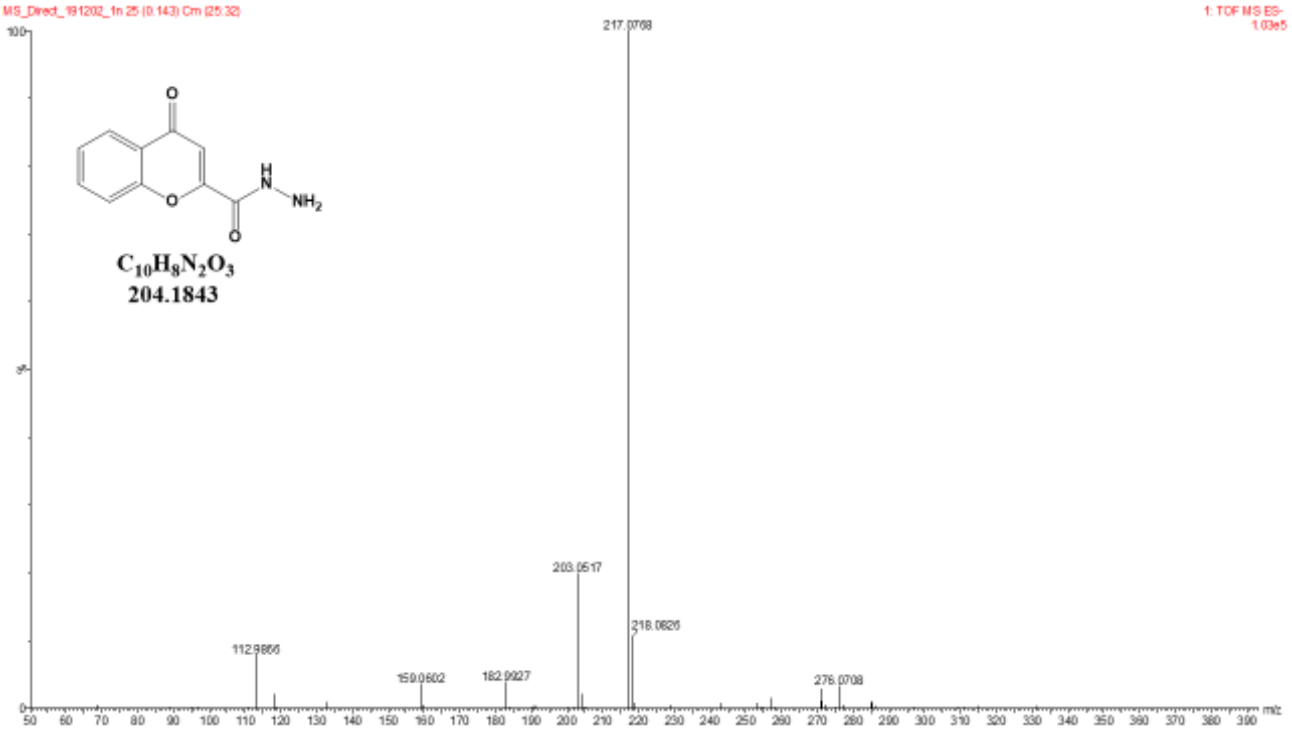
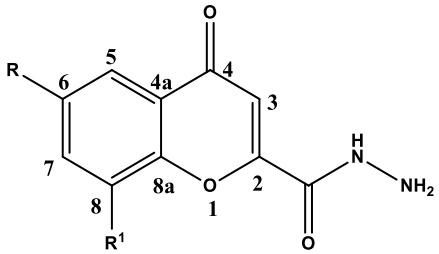


Figure 21: HRMS spectrum of chromone-2-carbohydrazide **52A**



52 & 56

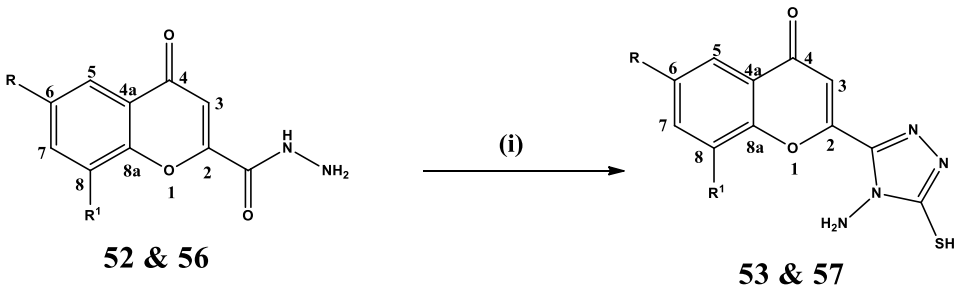
Compounds	52 A	52 B	52 C	52 D	52 E	52 F	56 A	56 B	56 C	56 D
R	H	MeO	NO_2	Cl	Br	F	Br	NO_2	Cl	F
R^1	H	H	H	H	H	H	Br	Br	Br	Br

Table 6: ^{13}C NMR chemical shift values (ppm) of compounds **52 & 56** in $CDCl_3$ and DMSO-d_6 (at 100 MHz)

Carbons	52 A	52 B	52 C	52 D	52 E	52 F	56 A	56 B	56 C	56 D
C-3	161.8	164.6	165.9	150.9	163.8	157.0	151.2	161.3	150.9	156.2
C-4	143.7	149.5	155.0	138.5	154.2	154.7	133.8	143.7	138.5	138.6
C-5	100.5	115.6	103.9	102.5	104.9	104.8	102.5	101.3	102.5	102.7
C-6	144.8	160.3	163.8	147.7	159.4	158.8	147.2	143.9	147.7	153.8
C-a	116.1	117.6	116.8	111.5	110.8	113.0	105.0	115.6	111.5	110.9
C-b	175.3	171.4	178.5	158.4	167.2	165.0	158.8	168.9	158.4	158.5
C-c	123.9	123.0	119.8	119.3	119.0	117.9	111.9	115.9	119.3	119.2
C-d	125.9	124.7	135.2	131.2	141.1	115.9	128.1	127.5	131.2	118.6
C-e	130.9	152.6	127.5	125.3	129.5	151.2	110.9	131.0	125.3	148.5
C-f	120.7	105.6	129.6	123.5	131.8	113.3	119.9	122.6	123.5	112.3
OMe	-	55.9	-	-	-	-	-	-	-	-

4.7. Synthesis of 2-(4-amino-5-mercapto-4*H*-1,2,4-triazol-3-yl) chromone derivatives (53A-E & 57A-C)

The target compound 2-(4-amino-5-mercapto-4*H*-1,2,4-triazol-3-yl) chromone derivatives **(53A-E & 57A-C)** were prepared in good yield from a corresponding chromone-2-carbohydrazide (**52 & 56)** treated in-situ with carbon disulphide and potassium hydroxide in ethanol. The obtained intermediate was subjected to a condensation reaction without further purification, with hydrazine hydrate and water. Different coloured crystals of 2-(4-amino-5-mercapto-4*H*-1,2,4-triazol-3-yl) chromone derivatives **(53A-E & 57A-C)** were obtained after recrystallisation from ethanol / di-isopropylether (4:1).[58] The chemical reaction scheme is outlined in **Scheme 16** below.



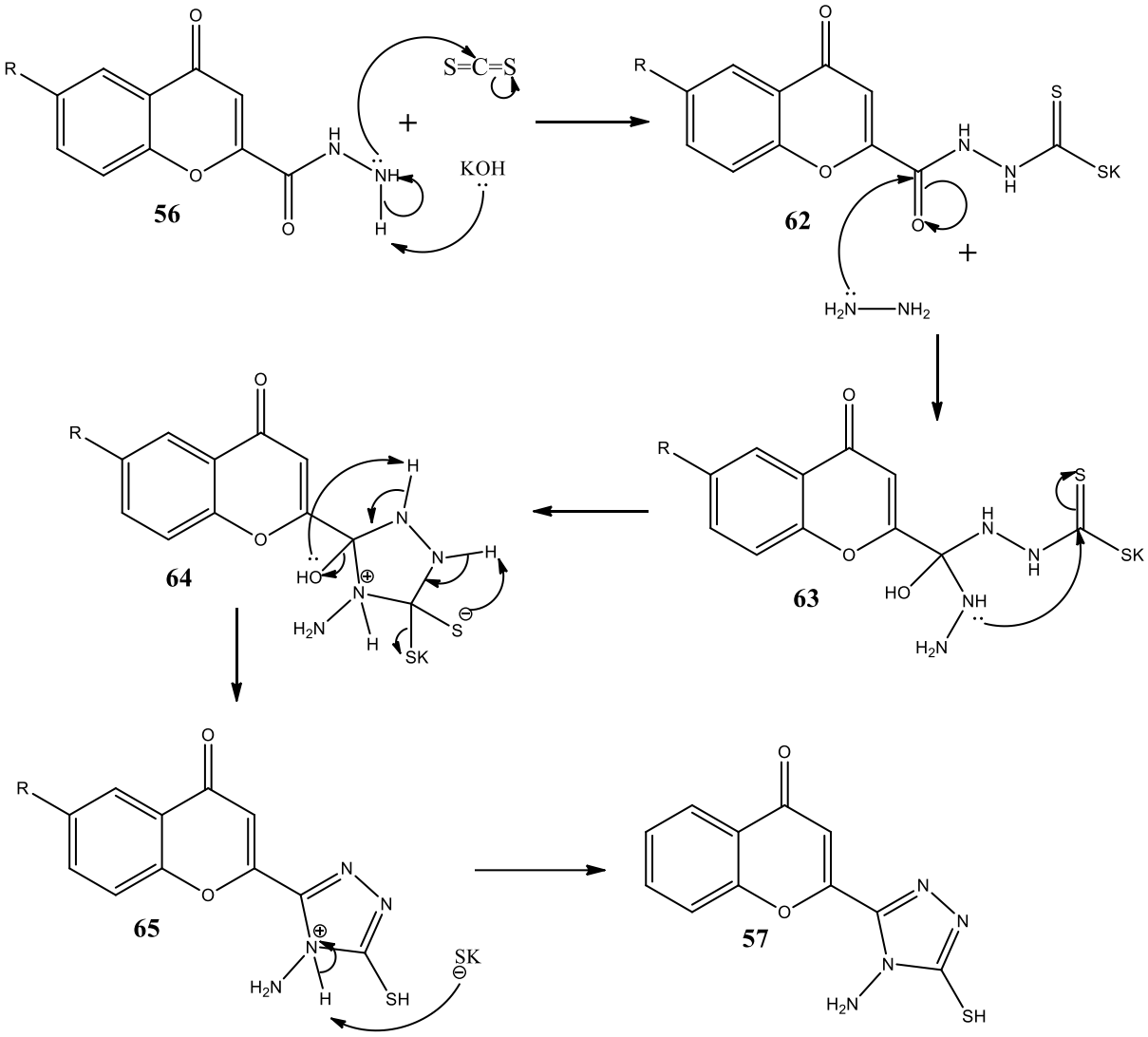
52 & 56 **53 & 57**

R= H, MeO, NO_2, Cl, Br, F
R^1= H, Br

Reagent: KOH, EtOH, CS_2, rt, 15h; $NH_2NH_2.H_2O$, H_2O, reflux, 30 min

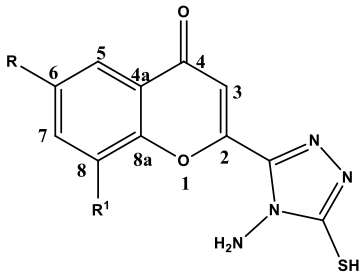
SCHEME 15: synthesis of 2-(4-amino-5-mercapto-4*H*-1,2,4-triazol-3-yl) chromone derivatives **57**

The 2-(4-amino-5-mercapto-4*H*-1,2,4-triazol-3-yl) chromone derivatives **(52A-E & 56A-C)** were prepared in situ by the reaction of chromone-2-carbohydrazide (**52 & 56)** with KOH in ethanol and carbon disulphide. The obtained intermediate of potassium 2-(4-oxo-4*H*-chromene-2-carbonyl)hydrazinecarbodithioate **62** was treated with hydrazine hydrate to form the target compounds, 2-(4-amino-5-mercapto-4*H*-1,2,4-triazol-3-yl) chromone derivatives **(52A-E & 56A-C)**. The proposed reaction mechanism involve the cyclization of potassium 2-(4-oxo-4*H*-chromene-2-carbonyl)hydrazinecarbodithioate **62** at the potassium dithiocarbazinate moiety in to the 4-amino-1,2,4-triazol-3-thiol to form the target comounds 2-(4-amino-5-mercapto-4*H*-1,2,4-triazol-3-yl) chromone derivatives **(52A-E & 56A-C)**. The chemical reaction mechanism is outlined in **Scheme 17** below.



SCHEME 16: Reaction mechanism of the 2-(4-amino-5-mercapto-4*H*-1,2,4-triazol-3-yl) chromone derivatives **53 & 57**

The 2-(4-amino-5-mercapto-4*H*-1,2,4-triazol-3-yl) chromone derivatives **(53A-E & 57A-C)** were fully characterized using 1D & 2D NMR, IR spectrum, HRMS and their physical properties. The assignment of the protons for 2-(4-amino-5-mercapto-4*H*-1,2,4-triazol-3-yl) chromone compounds **(53A-E & 57A-C)** was done after considering the chemical shifts and coupling constants observed for signals in the ^{1}H NMR spectra of all the compounds. The afforded yield was very low to excellent yield (28.75 – 84.51 %). The 2-(4-amino-5-mercapto-4*H*-1,2,4-triazol-3-yl) chromone derivatives **(53A-E & 57A-C)** synthesised are novel compounds. The melting point and the percentage yields of the synthesized 2-(4-amino-5-mercapto-4*H*-1,2,4-triazol-3-yl) chromone derivatives **(53A-E & 57A-C)** are reported in **Table 7**.



53 & 57

Table 7: Synthesised 2-(4-amino-5-mercapto-4*H*-1,2,4-triazol-3-yl) chromone derivatives **53 & 57**

Compounds	R	R^1	% Yield	Melting point (°C)
53A	H	H	28	301-308
53B	MeO	H	30	294-298
53C	Cl	H	54	277-283
53D	Br	H	63	280-286
53E	F	H	50	160-162
57A	Br	Br	84	200-205
57B	Cl	Br	46	205-212
57C	NO_2	Br	34	332-335

The shifting of the NH_2 group singlet peak at 4.49 ppm to 5.99 ppm and the appearance of the SH group singlet peak at 13.92 ppm confirmed the cyclization of the hydrazine moiety into an aromatic ring. Thus, the shifting of the NH_2 group singlet peak from 4.49 ppm to 5.99 ppm is due to the aromaticity of the 1,2,4-triazole thiol moiety. The ^{1}H NMR spectrum of the 2-(4-amino-5-mercapto-4*H*-1,2,4-triazol-3-yl) chromone (**53A-E & 57A-C**) was characterized by a singlet peak at 5.99 ppm, which corresponds to the NH_2 protons; an apparent triplet peak at 6.90 ppm, which corresponds to the proton at position 7; a doublet peak at 7.00 ppm, which corresponds to the proton at position 5; an apparent triplet peak at 7.21 ppm, which corresponds to the proton of position 6; a singlet peak at 7.38 ppm, which corresponds to the proton at position 3; a doublet peak at 7.70 ppm, which corresponds to the proton at position 8 and a broad singlet peak at 13.92 ppm, which correspond to the SH proton.

Amongst these compounds we also had a fluorine containing compound 2-(4-amino-5-mercapto-4*H*-1,2,4-triazol-3-yl) chromone **53E**. This compound has shown some uniqueness of coupling in an aromatic region of the proton NMR spectrum due to the high electronegativity of the fluorine atom and its ability to draw the bonding electron to itself. An electronegative

atoms deshield electron clouds of protons leaving them exposed to the NMR magnetic field and to other protons. A 2-(4-amino-5-mercapto-4*H*-1,2,4-triazol-3-yl)-6-fluorochromone **53E** NMR spectrum in the aromatic region was characterised by a doublet of a doublet at 7.60 ppm with a coupling constant of 6.83 Hz and 2.8 Hz, corresponding to the proton at position 7; a multiplet at 7.12 ppm corresponding to the proton at position 8 and another multiplet at 7.00 ppm corresponding to the proton at position 5. The ^{1}H NMR spectrum of the 2-(4-amino-5-mercapto-4*H*-1,2,4-triazol-3-yl) chromone compound **53A** is indicated in **Figure 22**.

The spectroscopic data obtained from ^{13}C NMR and DEPT 135 was used to confirm the presence of six or seven or eight quaternary carbons and five or four or three (C-H)'s for 2-(4-amino-5-mercapto-4*H*-1,2,4-triazol-3-yl) chromone derivatives which are (not substituted **53A**, substituted at position d (**53B-E)** and those that are substituted at both position 6 and 8 (**57A-C)**) respectively. The quaternary carbons at 165.49 ppm corresponds to the carbonyl carbon and the one at 154.86 ppm corresponds to the (C-SH) carbon while the other four quaternary carbons correspond to the aromatic carbons, N-C=N, C-8a, C-2, C-4a, resonating at 138.95 ppm, 140.92 ppm, 145.47 ppm, and 114.04 ppm respectively. The (C-H) aromatic carbons C-3, C-8, C-6, C-5, C-7 resonating at 105.62 ppm, 116.04 ppm, 120.03 ppm, 128.00 ppm, 130.14 ppm respectively. The ^{13}C NMR spectrum of 2-(4-amino-5-mercapto-4*H*-1,2,4-triazol-3-yl) chromone compound **53A** as indicated in **Figure 22**. **Table 8** outlines the observed chemical shifts of carbons for all 2-(4-amino-5-mercapto-4*H*-1,2,4-triazol-3-yl) chromone derivatives (**53A-E & 57A-C**) synthesised.

FT-IR spectroscopy was used to confirm the presence of functional groups. FT-IR spectrum of the 2-(4-amino-5-mercapto-4*H*-1,2,4-triazol-3-yl) chromone compound **57** revealed an NH group at 3320 cm^{-1}, an aromatic (C-H) at 2928 cm^{-1} and a carbonyl group (C=O) at 1751. 95 cm^{-1} as indicated in **Figure 23**. The HRMS was also used to provide the molecular weight of 2-(4-amino-5-mercapto-4*H*-1,2,4-triazol-3-yl) chromone derivatives (**53A-E & 57A-C**). The HRMS results were in complete agreement with the theoretical data where there was a peak at m/z 260 and the m/z of 2-(4-amino-5-mercapto-4*H*-1,2,4-triazol-3-yl) chromone compound **53A** is at 260 as indicated in **Figure 24**.

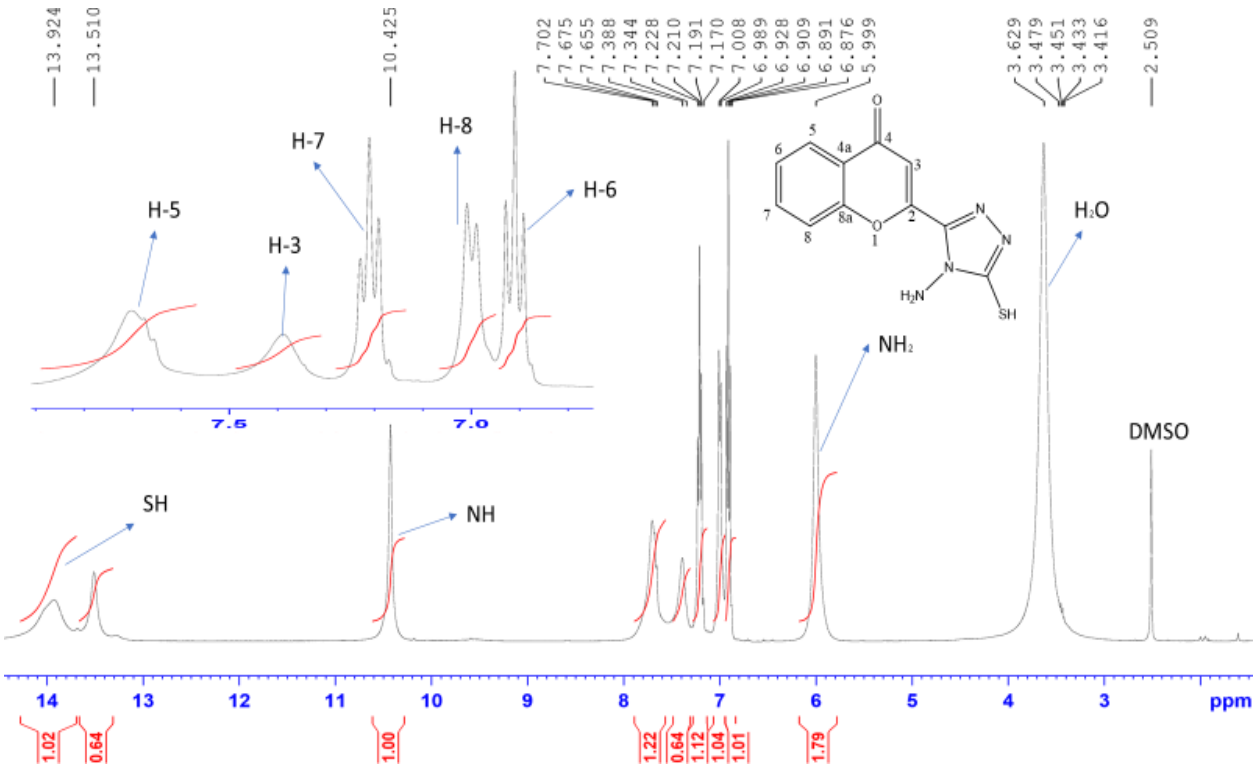


Figure 22: ^{1}H NMR (full spectrum) of 2-(4-amino-5-mercapto-4*H*-1,2,4-triazol-3-yl) chromone derivatives **53A** in DMSO-d_6 (at 400 MHz)

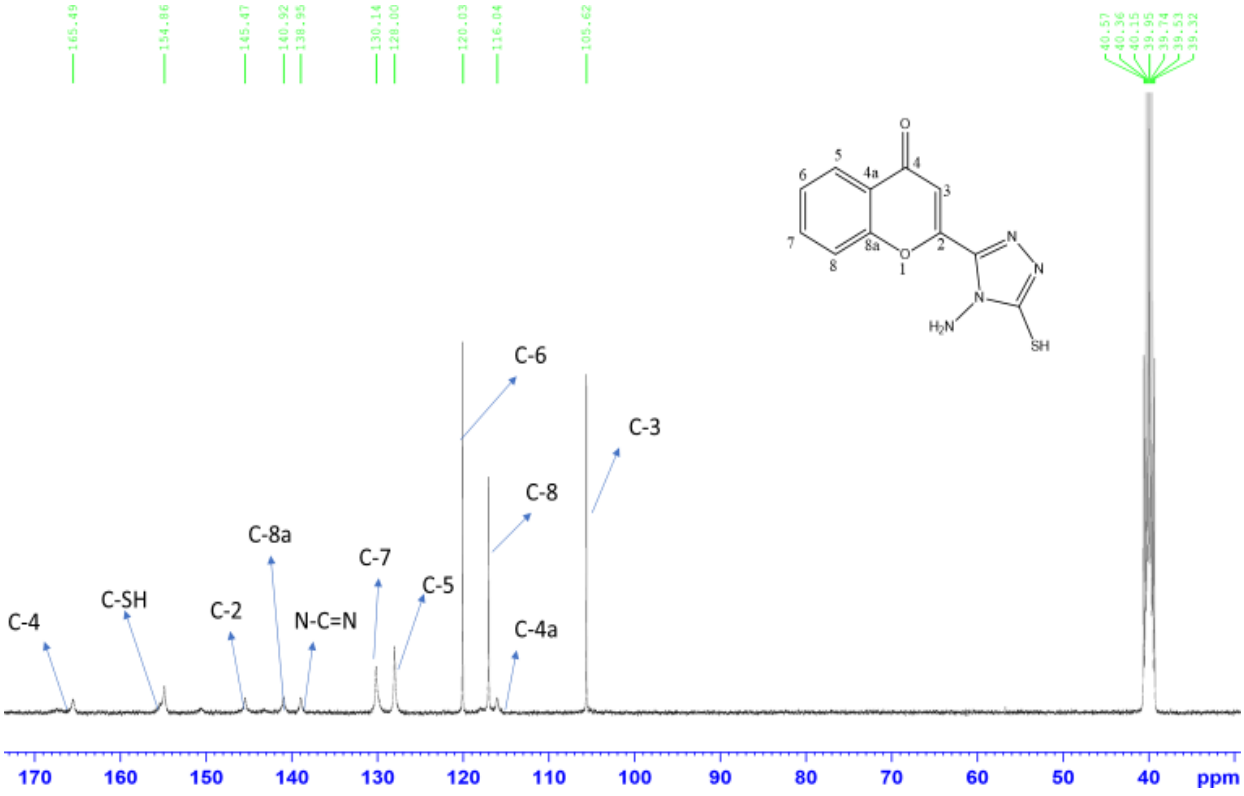


Figure 23: ^{13}C NMR spectrum of 2-(4-amino-5-mercapto-4*H*-1,2,4-triazol-3-yl) chromone derivatives **53A** in DMSO-d_6 (at 100 MHz)

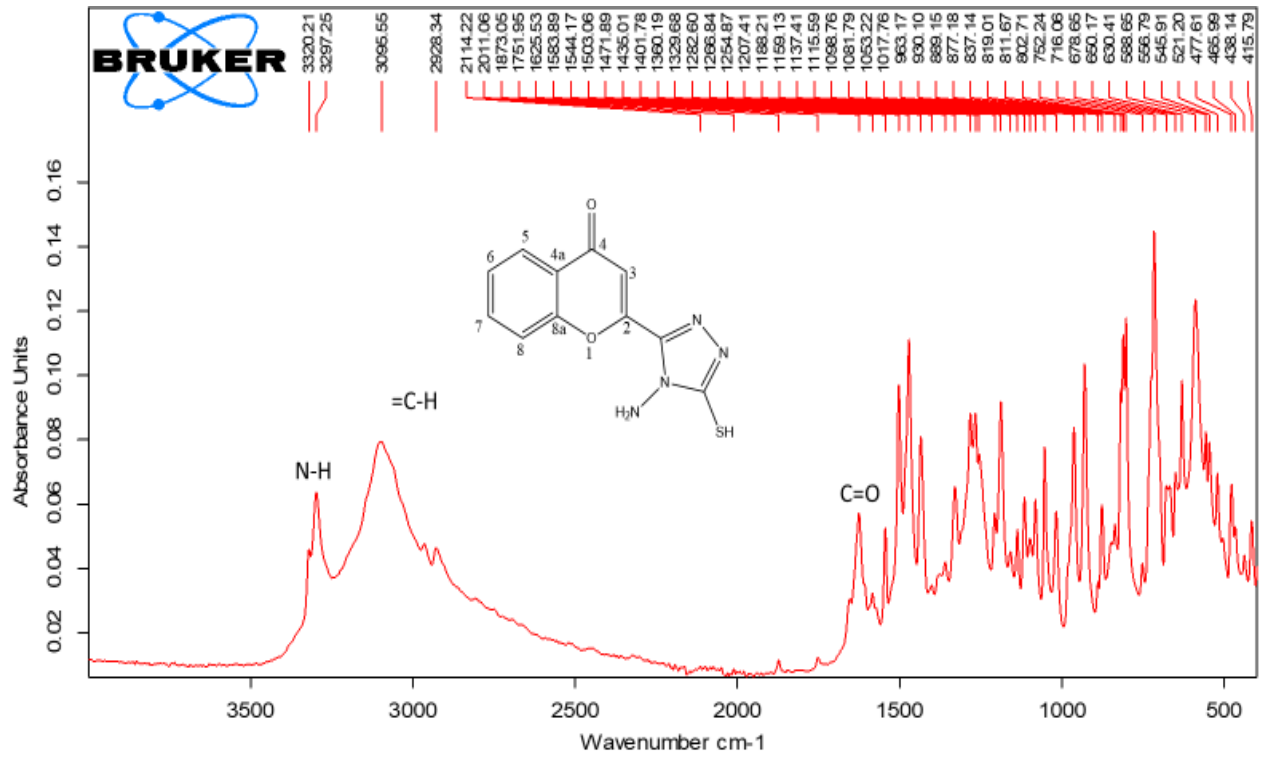


Figure 24: FTIR spectrum of 2-(4-amino-5-mercapto-4*H*-1,2,4-triazol-3-yl) chromone derivatives **53A**

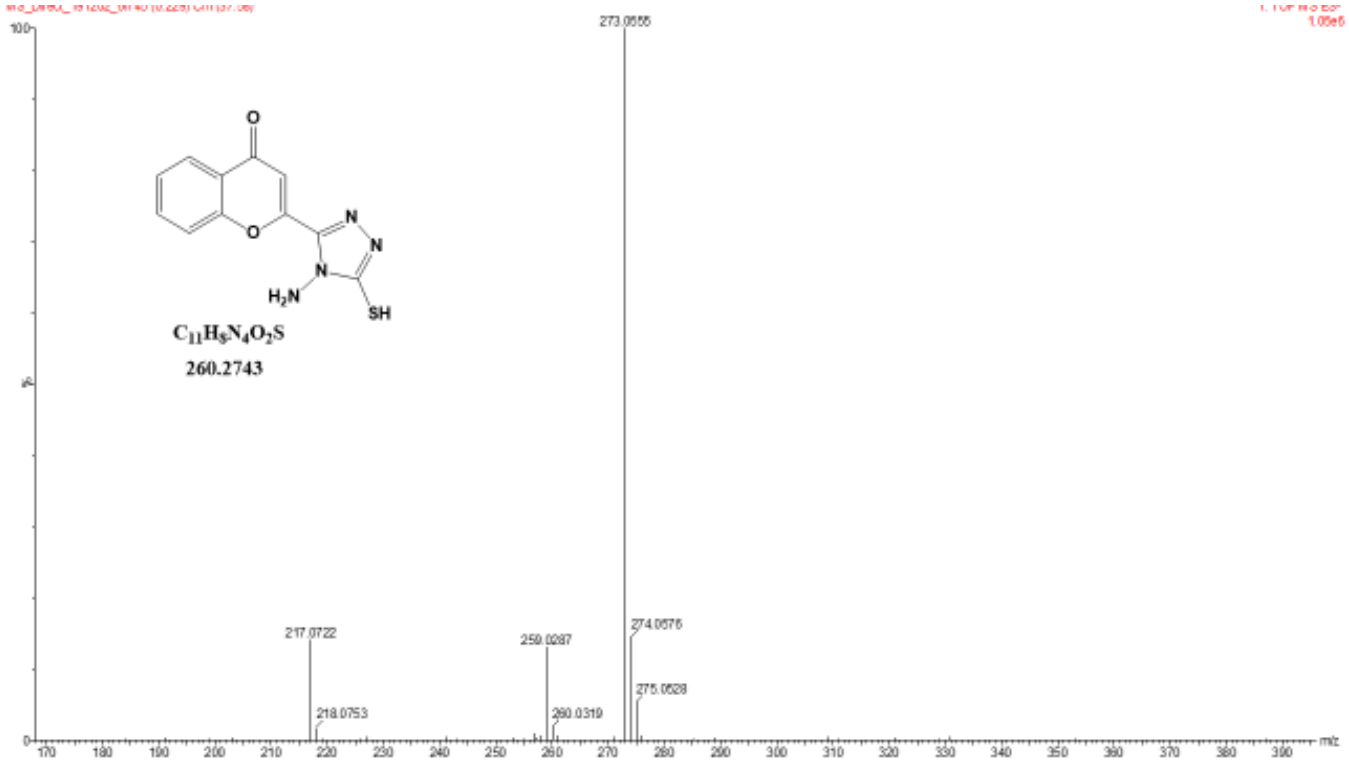
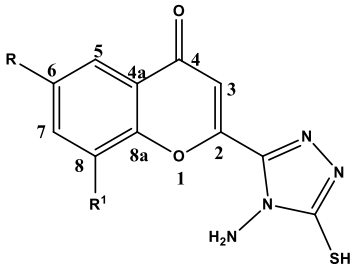


Figure 25: HRMS spectrum of 2-(4-amino-5-mercapto-4*H*-1,2,4-triazol-3-yl) chromone derivatives **53A**



53 & 57

Compounds	53 A	53 B	53 C	53 D	53 E	57 A	57 B	57 C
R	H	MeO	Cl	Br	F	Br	Cl	NO_2
R^1	H	H	H	H	H	Br	Br	Br

Table 8: ^{13}C NMR chemical shift values (ppm) of compounds **53 & 57** in $CDCl_3$ and DMSO-d_6 (at 100 MHz)

Carbons	53A	53B	53C	53D	53E	57A	57B	57C
C-3	145.2	146.6	74.3	74.2	151.5	148.4	148.6	145.5
C-5	140.7	142.1	146.9	146.7	145.7	137.0	137.0	140.3
C-8	165.2	152.6	159.9	159.9	157.1	160.6	160.6	162.3
C-9	105.4	107.0	107.0	107.0	107.6	106.9	106.9	102.9
C-10	138.8	134.5	133.5	133.4	141.4	134.1	131.6	136.1
C-a	154.6	148.9	154.2	154.6	154.7	151.3	151.0	159.6
C-b	115.8	116.5	120.4	119.2	118.3	120.0	113.4	122.0
C-c	130.0	117.8	129.0	131.9	117.1	129.0	124.2	127.5
C-d	119.8	145.5	127.0	129.8	146.7	112.0	119.5	136.5
C-e	127.9	112.3	123.5	121.1	113.8	128.9	119.4	116.9
C-f	116.8	115.9	118.7	111.0	116.1	111.4	111.6	113.0
OMe	-	56.0	-	-	-	-	-	-

4.8. Biological studies

4.8.1. Anti-malaria assay

Malaria is a chronic disease caused by protozoa of the genus plasmodium, which are transmitted to humans by female mosquitoes. *Plasmodium falciparum* is one of the protozoa that causes mortality on a large scale in tropical countries. Most malaria cases occur in the WHO African region 92 %, WHO South-East Asia region with 5%, and the WHO Eastern Mediterranean region with 2 %. Once plasmodium enters the bloodstream of a human it infects and destroys mainly liver cells and red blood cells. There are 300 – 500 million cases of malaria each year and 2-3 million deaths annually, mainly children under the age of five years[4,7]. In this study, continuous cultures of asexual erythrocyte stages of *Plasmodium falciparum* were maintained using the method described by Trager and Jensen with minor modifications.[64] Quantitative assessment of anti-plasmodial activity in vitro was determined via the parasite lactate dehydrogenase assay using the method described by Makler et al, in which parasite viability is determined calorimetrically .[63]

Synthesized chromone-2-carboxylate derivatives samples were tested in triplicate on two occasions over 72 hours against both the wild-type drug-sensitive strain of the human malaria parasite *Plasmodium falciparum NF54* and a multidrug-resistant strain *Plasmodium falciparum K1*.

The results of anti-malarial activities for selected compounds are presented in **Figure 24**. Compounds, **51D**, **55B**, **52A**, **52C** and **59** displayed activities against chloroquine sensitive (NF54) stains of *plasmodium falciparum*, with 78.33 %, 88.66 %, 72.16 %, 69.5 %, and 0.195 % viability respectively which was, however, below the positive control. Most active and moderate active compounds structures are presented in **Table 9**. Compounds **51E**, **55D**, and **59** displayed activities against chloroquine resistant (K1) stains of *plasmodium falciparum*, with 57.5 %, 84.33 %, and 19,5 % viability respectively which was also below that of the positive control. Most active and moderate active compounds structures are presented in **Table 10**.

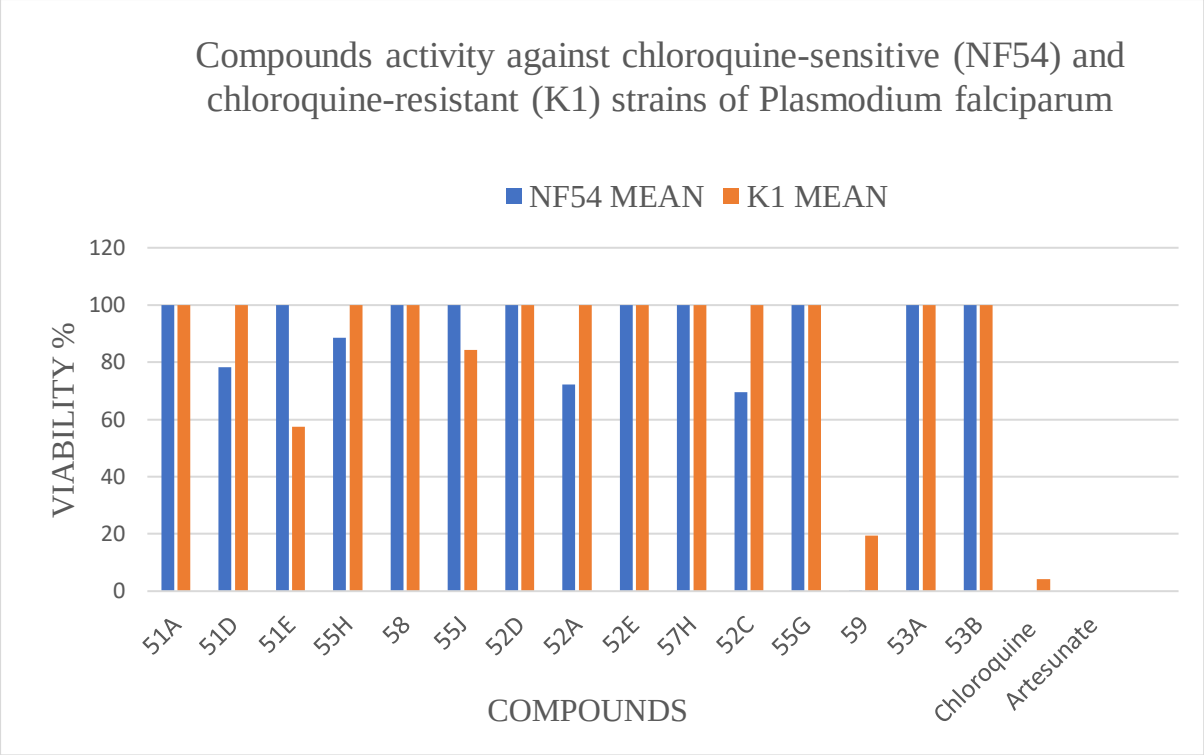
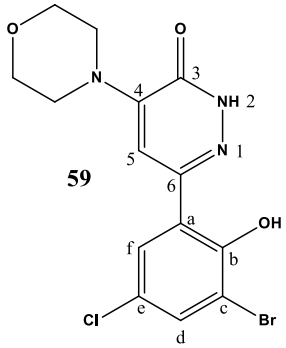
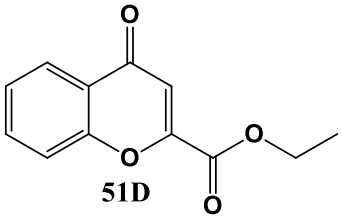
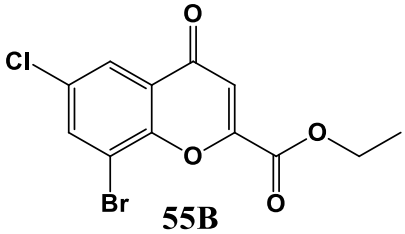


Figure 29: Compounds activity against chloroquine-sensitive (NF54) and chloroquine-resistant (K1) strains of malarial parasite *Plasmodium falciparum*

Table 9: Active compounds against chloroquine sensitive (NF54) strains of malarial parasite *Plasmodium falciparum*

Most active compounds	Moderate active compounds
 **59**	 **51D**
	 **55B**

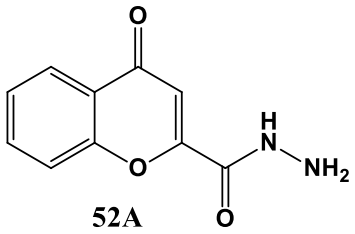
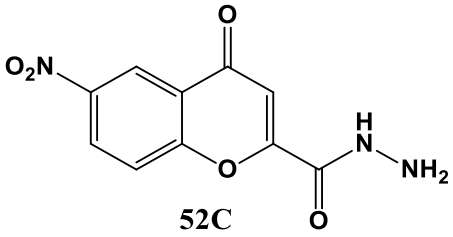
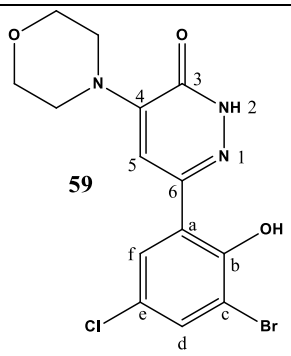
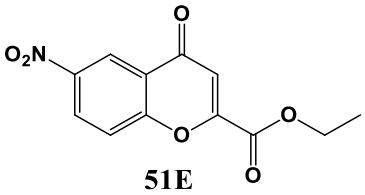
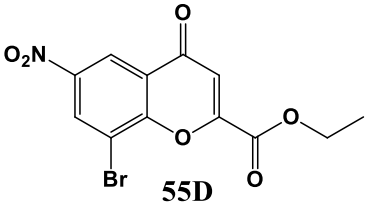
	 52A
	 52C

Table 10: Active compounds against chloroquine resistant (K1) strains of malarial parasite *Plasmodium falciparum*

Most active compounds	**Moderate active compounds**
 59	 51E
	 55D

Compounds **51 D**, **51 E**, **52 A**, **52 C**, **55 B**, and **55 D** were active to a small extent, with IC_{50} ranging from 4-6µM in one of the isolates. Compound **59** was highly active against the sensitive strain and moderately active against the resistant isolate. This may indicate some cross-resistance with the quinoline family of compounds since K1 is heavily chloroquine resistant. In contrast, the standard 5 antimalarial compounds CQ and Arts showed significant efficacy against the parasite, with both compounds giving IC_{50} values in the expected range for this laboratory and showing the distinct difference for the chloroquine result between the resistant and sensitive isolates. IC_{50} values displayed by chromone-2-carboxylate derivatives against chloroquine-sensitive (NF54) strains and against chloroquine-resistant (K1) strains of malarial parasite *Plasmodium falciparum* is presented in **Table 11** and **Table 12** respectively.

Table 11: IC_{50} values of chromone-2-carboxylate derivatives against chloroquine-sensitive (NF54) strains of malarial parasite *Plasmodium falciparum*

Compounds	IC50 (µg/mL)
51 D	4.7
52 A	4.33
52 C	4.17
55 B	5.32
59	0.0117

Table 12: IC_{50} values of chromone-2-carboxylate derivatives against chloroquine resistant (K1) strains of malarial parasite *Plasmodium falciparum*

Compounds	IC50 (µg/mL)
51 E	3.45
55D	5.06
59	1.17

CHAPTER 5

5.1. Conclusion

The study of chromone-2-carboxylate was conducted due to the broad range of biological activities the chromone scaffold has. The brominating of 2-hydroxyacetophenone at position 3, which is a precursor to the synthesis of chromone-2-carboxylate derivatives, was successfully synthesized with a moderate to excellent yield (42-87 %). Chromone-2-carboxylate derivatives (**51A-F, 55A-D & 58**) were successfully synthesized with a moderate to excellent yield (32-95 %). Chromone-2-carboxylate derivatives (**51A-F, 55A-D & 58**) were successfully converted into chromone-2-carbohydrazide derivatives (**52A-F, 56A-D & 59**), with a moderate to excellent yield (33-95 %), which some were transformed into 2-(4-amino-5-mercapto-4*H*-1,2,4-triazol-3-yl) chromone derivatives (**53A-E & 57A-C**) with a low to high yield (28-84 %). All the synthesized compounds were purified by recrystallization, characterized by the combination of 1D & 2D NMR and IR spectroscopic techniques, HRMS, CHN elementary analysis, and the physical data of the compounds.

Several synthetic methods were attempted to brominate the unsubstituted 2-hydroxyacetophenone and the 5-methoxy-2-hydroxyacetophenone. However, the reaction was unsuccessful.

Fifteen (15) compounds were tested for biological evaluations against malaria. Selected synthesized chromone-2-carboxylate derivatives samples were tested in triplicate on two occasions over 72 hours against both the wild-type drug-sensitive strain of the human malaria parasite *Plasmodium falciparum NF54* and a multidrug-resistant strain *Plasmodium falciparum K1*. Amongst all tested compounds, **51D**, **55B**, **52A**, **52C** and **59** displayed activities against chloroquine sensitive (NF54) stains of malarial parasite *plasmodium falciparum*, with 78.33 %, 88.66 %, 72.16 %, 69.5 %, and 0.195 % viability respectively which was, however, bellow the positive control. Compounds **51E**, **55D**, and **59** displayed activities against chloroquine resistant (K1) stains of malarial parasite *plasmodium falciparum*, with 57.5 %, 84.33 %, and 19,5 % viability respectively which was also below that of the positive control.

5.2. Future work

- Further transformation of chromone-2-carboxylate derivatives to various substituted 2-(4-amino-5-mercapto-4*H*-1,2,4-triazol-3-yl) chromone derivatives.
- X-ray analysis of compounds **58 & 59** to further confirm their structures.
- Further biological evaluation of the synthesized derivatives against HIV and TB
- Structure-activity relationship (SAR) studies on the synthesized compounds

CHAPTER 6

6. Experimental Procedures

6.1. General method

The various commercially available 2-hydroxyacetophenone, absolute ethanol, diethyl oxalate, metallic sodium, other reagents, and solvents used were all purchased from Sigma Aldrich were used without any purification. All compounds were synthesized in batch reactors (glass flasks) and all the reactions were monitored by thin-layer chromatography (TLC) plates. These were visualized under UV lamp/ light (λ= 254-365 nm). The synthesized compounds were purified either by silica gel column chromatography or recrystallization.

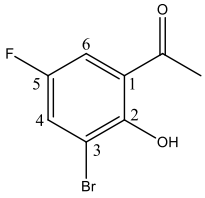
^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra were recorded on a Bruker 400 MHz spectrometer using either $CDCl_3$ or DMSO-d_6 as sample solvents and TMS as internal standard and values for the chemical shifts were expressed in parts per million (ppm): s for singlet, d for doublet, t for triplet, q for quartet, and m for multiplet; coupling constants (J) in hertz (Hz). The infrared (IR) spectra were recorded on a Perkin-Elmer 1420 spectrometer and were reported in cm^{-1}. All melting points were determined on a Buchi melting point B-540 apparatus using capillary tubes and were uncorrected.

6.2. Synthesis

Synthesis of 3-bromo-2-hydroxyacetophenone derivatives **54**

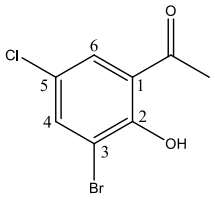
The 2-hydroxyacetophenone (1.8 g,10 mmol) was dissolved in CH_3CN (50 ml) and treated with NBS (1.78 g, 10 mmol). The resulting solution was extracted with EtOAc (200 ml), water (2x 200 ml), and brine (2x 200 ml). The combined organic extracts were then dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residues were dissolved in hot MeOH and then treated with water. The solids precipitate that formed was collected by filtration and dried to give 3-bromo-2-hydroxyacetophenones.[53]

3-Bromo-5-fluoro-2-hydroxyacetophenone (**54A**, 0.99 g, 42,31 %) cream white solid; m.p 98-

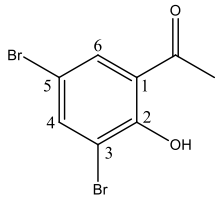


101 °C (Lit.,[53] 97); ^{1}H NMR (400 MHz, $CDCl_3$) δ_H= 12.58 (s, 1H, OH), 7.46 (dd, 1H, J= 4.8 Hz, 2.8 Hz, 6-H), 7.38 (dd,1H, J= 5.2 Hz, 3.2 Hz, 4-H), 2.57 (s, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$), δ_c= 203.6 (q, $J_{C,F}$ = 3 Hz, C=O), 158.5 (s, $J_{C,F}$ = 2 Hz, C-5), 155.5 (C-2), 127.4 (C-4), 124.1 (C-1), 115.3 (m, $J_{C,F}$ = 3Hz, C-6), 112.3 (C-3), 26.7 (CH_3); IR $\nu_{max/}$ cm^{-1}= 3080 (=C-H), 1650 (C=O) 1580 (C=C).

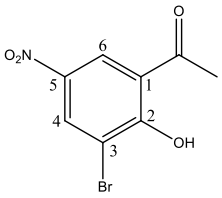
3-Bromo-5-chloro-2-hydroxyacetophenone (**54B**, 1,79 g, 71,61 %) light green solid; m.p 98-101 °C (Lit., [52] 112); ^{1}H NMR (400 MHz, $CDCl_3$) δ_H= 12.74 (s, 1H, OH), 7.62 (d, 1H, J= 2.4 Hz, 6-H), 7.59 (d, 1H, J= 2.4 Hz, 4-H), 2.54 (s, 3H, CH_3); ^{13}C NMR (100, $CDCl_3$) δ_C= 203.6 (C=O), 157.6 (C-2), 129.2 (C-6), 138.8 (C-4), 126.1 (C-5), 123.8 (C-1), 112.8 (C-3), 26.7 (CH_3); IR ν_{max}/ cm^{-1}= (=C-H), 1643 (C=O), 16OO (C=C).



3,5-Dibromo-2-hydroxyacetophenone (**54C**, 2.59 g, 87.79 %) cream white solid; m.p 107-112 °C (Lit. m.p,[52] 111 °C); ^{1}H NMR (400 MHz, $CDCl_3$) δ_H= 12.85 (s, 1H, OH), 7.84 (d, 1H, J= 2.4 Hz, 6-H), 7.81 (d, 1H, J= 2 Hz, 4-H), 2.63 (s, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ_c= 198.6 (C=O), 153.2 (C-2), 127.4 (C-6), 136.7 (C-4), 116.3 (C-1), 105.6 (C-3), 108.4 (C-5), 22.0 (CH_3); IR ν_{max}/cm^{-1}= 3060 (=C-H), 1650 (C=O), 1547 (C=C).



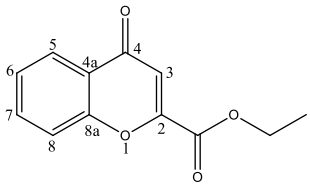
3-bromo-2-hydoxy-5-nitroacetophenone (**54D**, 2.23 g, 85.44 %) white solid; m.p 130-135 °C (Lit.,[53] 132); ^{1}H NMR (400 MHz, $CDCl_3$) δ_H= 13.55 (s, 1H, OH), 8.62 (d, 1H, J= 2.4 Hz, 6-H), 8.54 (d, 1H, J= 2.8 Hz, 4-H), 2.72 (s, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C= 203.5 (C=O), 163.93 (C-2), 139.3 (C-5), 125.7 (C-6), 134.0 (C-4), 118.6 (C-1), 112.9 (C-3), 26.7 (CH_3), IR ν_{max}/ cm^{-1}= 3072 (=C-H), 1651 (C=O), 1599 (C=C).



*Synthesis of chromone-2-carboxylate derivatives (***51 & 55***)*

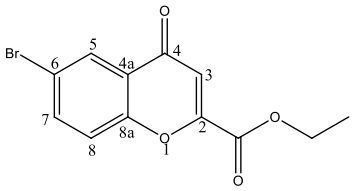
Sodium ethoxide was prepared *in situ* using sodium metal (1.49 g, 65 mmol) which was reacted with excess absolute ethanol (100 ml). Diethyl oxalate (5.12 g, 35 mmol) and 2-hydroxyacetophenone (2.04 g, 15 mmol) were dissolved in absolute ethanol (10 ml) and added to the sodium ethoxide solution. The solution was refluxed for 1 h, allowed to cool and concentrated HCl was added dropwise until the reaction was acidic and a white precipitate was formed. The white precipitate was filtered, discarded and the yellow solution was concentrated into a slurry. The slurry was extracted with ethyl acetate, dried over Na_2SO_4 and evaporated to give a light-yellow solid. The solid was recrystalised from methanol/ di-isopropylether (4: 1) to give compounds **55**.[33.]

Chromone-2-carboxylate (**51A**, 2.99 g, 91.43 %) light brown; m.p 72-74 °C (Lit.,[54] 71-72); ^{1}H NMR (400 MHz, DMSO-d_6) δ_H= 8.05 (d, 1H, J= 7.6 Hz, 5-H), 7.81 (t, 1H, J= 8 Hz, 6-H), 7.7 (d, 1H, J= 8.4 Hz, 8-H), 7.5 (t, 1H, J= 7.6 Hz, 7-H), 6.93 (s, 1H, 3-H), 4.44 (q, 2H, J= 6.8Hz, CH_2), 1.34 (t, 3H, J= 7.2 Hz, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ_C= 177.8 (C-4), 160.4 (C=O), 155.8 (C-2), 152.6 (C-8a), 135.7 (C-5), 126.6 (C-6), 125.4 (C-7), 124.2 (C-4a), 119.3 (C-3), 114.1 (C-8), 63.1 (CH_2), 14.3 (CH_3); IR ν_{max}/

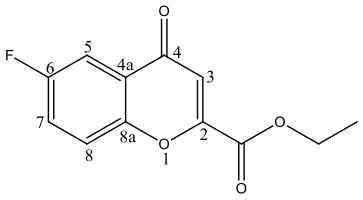


cm^{-1}= 3076 (=C-H), 1732 (C=O), 1628 (C=C); Anal. Calc. for $C_{12}H_{10}O_4$: C 66.05; H 4.62. Found: C 62.9; H 3.90.

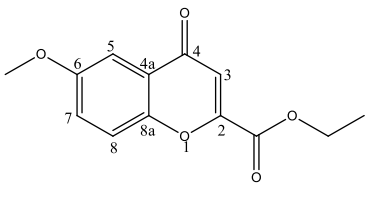
6-Bromochromone-2-carboxylate (**51B**, 2.224 g, 49.87 %) brown solid; m.p 145-148 °C (Lit.,[55] 143.5-145); ^{1}H NMR (400 MHz, $CDCl_3$) δ_H= 8.21 (d, 1H, J= 2.4 Hz, 5-H), 7.77 (dd, 1H, J= 6.4 Hz, 2.4 Hz, 7-H), 7.45 (d, 1H, J= 8.8 Hz, 8-H), 7.04 (s, 1H, 3-H), 4.45 (q, 2H, J= 7.2 Hz, CH_2), 1.38 (s, 3H, CH_3), ^{13}C NMR (100 MHz, $CDCl_3$) δ_C= 177.0 (C-4), 160.2 (C=O), 154.7 (C-2), 152.4 (C-8a), 137.7 (C-5), 128.3 (C-7), 125.6 (C-4a), 120.7 (C-3), 119.5 (C-6), 114.7 (C-8), 63.2 (CH_2), 14.0 (CH_3); IR ν_{max}/cm^{-1}= 3057 (=C-H), 1723 (C=O), 1598 (C=C).



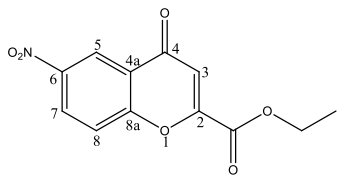
6-Fluorochromone-2-carboxylate (**51C**, 2.615 g, 73.87 %) light purple; m.p 136-140 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H= 7.72 (dd, 1H, J= 4.8 Hz, 3.2 Hz, 5-H), 7.53 (dd, 1H, J= 5.2 Hz, 4 Hz, 8-H), 7.40 (dd, 1H, J= 7.6 Hz, 3.2 Hz, 7-H), 7.01 (s, 1H, 3-H), 4.46 (q, 2H, J= 7.2 Hz, CH_2), 1.36 (t, 3H, J=14.4 Hz , CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C= 177.6 (d, $J_{C,F}$ = 3 Hz, C-4), 161.1 (C=O), 160.3 (C-6), 158.6 (C-2), 152.1 (t, $J_{C,F}$ = 1 Hz, C-8a), 125.6 (d, $J_{C,F}$ = 8 Hz, C-4a), 123.1 (C-5), 121.0 (d, $J_{C,F}$ = 8 Hz, C-7), 113.8 (C-3), 110.7 (d, $J_{C,F}$ = 23 Hz, C-8), 63.1 (CH_2), 14.0 (CH_3); IR ν_{max}/cm^{-1}= 3053 (=C-H), 1738 (C=O), 1615 (C=C).



6- Methoxychromone-2-carboxylate (**51D**, 1.2 g, 32.25 %) cream white solid; m.p 103-106 °C (Lit.,[56] 100.5-101 °C) ^{1}H NMR (400 MHz, DMSO-d_6) δ_H= 7.73 (d, 1H, J= 8.8 Hz, 8-H), 7.41 (dd, 1H, J= 6.8 Hz, 2.4 Hz, 7-H), 7.30 (d, 1H, J= 2.8 Hz, 5-H), 6.92 (s, 1H, 3-H), 4.47 (q, 2H, J= 7.2 Hz, CH_2), 3.85 (s, 3H, OCH_3), 1.34 (t, 3H, J= 7.2, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ_C= 177.4 (C-4), 160.4 (C=O), 157.4 (C-2), 152.3 (C-6), 150.4 (C-8a), 124.9 (C-4a), 124.9 (C-7), 120.9 (C-5), 113.2 (C-8), 104.9 (C-3), 63.1 (CH_2), 56.2 (0CH_3), 14.3 (CH_3); IR ν_{max}/cm^{-1}= 3079 (=C-H), 1737 (C=O), 1651 (C=C).

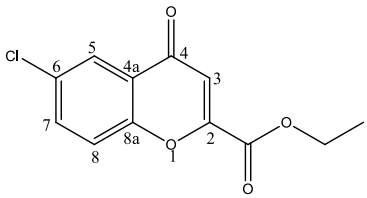


6-Nitrochromone-2-carboxylate (**51E**, 1.52 g, 38.97 %) gold solid; m.p 183-189 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H= 8.91 (d, 1H, J= 2.8 Hz, 5-H), 8.55 (dd, 1H, J= 6.4 Hz, 2.8 Hz, 7-H), 7.72 (d, 1H, J= 4.8, 8-H), 4.45 (q, 2H, J=7.2 Hz, CH_2), 1.33 (t, 3H, J= 7.2Hz, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C= 176.8 (C-4), 159.7 (C=O), 158.6 (C-2), 152.7 (C-8a), 145.1 (C-6), 128.9 (C-5), 124.3 (C-4a), 122.4 (C-7),



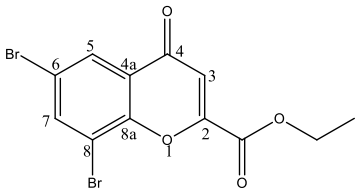
120.6 (C-3), 115.0 (C-8), 63.5 (CH_2), 14.0 (CH_3); IR ν_{max}/cm^{-1}= 3086 (C-H), 1733 (C=O), 1650 (C=C), 1625 (RNO_2); Anal. Calc. for $C_{12}H_9NO_6$: C 54.76; H 3.45; N 5.32. Found: C 54.58; H 3.03; N 5.14.

6-Chlorochromone-2-carboxylate (**51F**, 1.805 g, 47.5 %) light brown solids; m.p 141-143 °C;



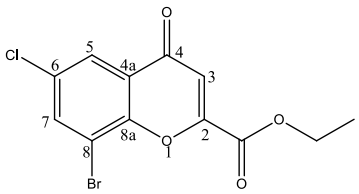
^{1}H NMR (400MHz, DMSO-d_6) δ_H= 7.94 (d, 1H, J= 2.4 Hz, 5-H), 7.90 (dd, 1H, J= 6.4 Hz, 2.8 Hz, 7-H), 7.81 (d, 1H, J= 8.8 Hz, 8-H), 6.96 (s, 1H, 3-H), 4.47 (q, 2H, J= 7.2 Hz, CH_2), 1.30 (t, 3H, J= 14.4 Hz, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ_C= 176.7 (C-4), 160.2 (C=O), 154.4 (C-2), 152.9 (C-8a), 135.6 (C-5), 131.1 (C-6), 125.2 (C-4a), 124.3 (C-7), 121.8 (C-3), 114.0 (C-8), 63.3 (CH_2), 14.3 (CH_3); IR ν_{max}/cm^{-1}= 3057 (=C-H), 1735 (C=O), 1618 (C=C); Anal. Calc. for $C_{12}H_9ClO_4$: C 57.05; H 3.59. Found: C 57.31; H 3.069.

6,8-Dibromochromone-2-carboxylate (**55A**, 2.34 g, 82.83 %) cream white solid; m.p 135-137



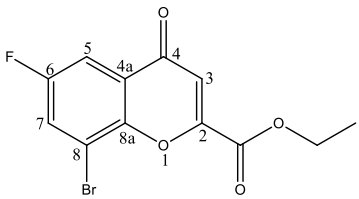
°C (Lit.,[56] 134.5-135 °C); ^{1}H NMR (400 MHz, DMSO-d_6) δ_H= 8.41 (d, 1H, J= 2 Hz, 5-H), 8.00 (d, 1H, J= 2.4 Hz, 7-H), 7.02 (s, 1H, 3-H), 4.48 (q, 2H, J= 6.8 Hz, CH_2), 1.32 (t, 3H, J= 7.2 Hz, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ_C= 206.9 (C-4), 176.3 (C=O), 159.8 (C-2), 152.9 (C-8a), 151.8 (C-5), 140.4 (C-7), 127.1 (C-4a), 126.4 (C-6), 119.0 (C-3), 114.3 (C-8), 63.3 (CH_2), 14.2 (CH_3); IR ν_{max}/cm^{-1}= 3056 (=C-H), 1735 (C=O), 1657 (C=C); Anal. Calc. for $C_{12}H_8Br_2O_4$: C 38.33; H 2.14. Found: C 38.06; H 1.903.

8-Bromo-6-chlorochromone-2-carboxylate (**55B**, 1.01 g, 60.58 %) white solid; m.p 127-129



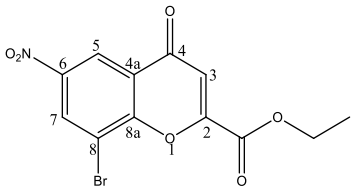
°C; ^{1}H NMR (400 MHz, DMSO-d_6) δ_H= 8.32 (d, 1H, J= 2.4 Hz, 5-H), 7.93 (d, 1H, J= 2.4 Hz, 7-H), 6.95 (s, 1H, 3-H), 4.40 (q, 2H, J= 6.8 Hz, CH_2), 1.39 (t, 3H, J= 7.2 Hz, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ_C= 176.4 (C-4), 159.8 (C=O), 152 (C-2), 151.4 (C-8a), 138.0 (C-5), 131.2 (C-6), 126.0 (C-4a), 124.0 (C-7), 114.2 (C-3), 113.8 (C-8), 63.3 (CH_2), 14.2 (CH_3); IR ν_{max}/cm^{-1}= 3089 (=C-H), 1727 (C=O), 1623 (C=C); Anal. Calc. for $C_{12}H_8BrClO_4$: C 43.47; H 2.43. Found: C 43.36; H 1.819.

8-Bromo-6-fluorochromone-2-carboxylate (**55C**, 0.81 g, 68.35 %) shiny brown solid; m.p



128-130 °C (Lit.,[56] 130-131 °C); ^{1}H NMR (400 MHz, DMSO-d_6) δ_H= 8.25 (dd, 1H, J= 4.8 Hz, 3.2 Hz, 5-H), 7.77 (dd, 1H, J= 5.2 Hz, 2.8Hz, 7-H), 6.91 (s, 1H, 3-H), 4.41 (q, 2H, J= 6.8 Hz, CH_2), 1.34 (t, 3H, J= 7.2 Hz, CH_3); ^{13}C NMR (100 MHz, DMSO-

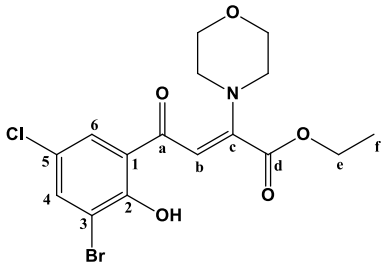
d_6) δ_C= 177.6 (d, $J_{C,F}$ = 2Hz, C-4), 160.3 (C=O), 158.6 (C-2), 125.5 (d, $J_{C,F}$ = 8 Hz, C-7), 152.4 (s, $J_{C,F}$ = 2Hz, C-8a), 125.6 (d, $J_{C,F}$ = 8 Hz, C-5), 120.9 (C-4a), 113.8 (t, $J_{C,F}$ = 10 Hz, C-3), 110.4 (d, $J_{C,F}$ = 24 Hz, C-8), 63.1 (CH_2), 14.0 (CH_3); IR ν_{max}/cm^{-1}= 2996 (=C-H), 1715 (C=O), 1654 (C=C).

8-Bromo-6-nitrochromone-2-carboxylate (**55D**, 0.12 g, 95 %) light brown solid; m.p 183-185 

°C; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H= 9.02 (d, 1H, J= 1.2 Hz, 5-H), 8.84 (d,1H, J= 1.2 Hz, 7-H), 7.22 (s, 1H, H-3), 4.50 (q, 2H, J= 7.2 Hz, CH_2), 1.54 (t, 3H, J= 14.4 Hz, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C= 176.3 (C-4), 159.3 (C=O), 155.8 (C-2), 153.0 (C-8a), 144.8 (C-6), 132.2 (C-5), 125.1 (C-4a), 121.2 (C-7), 114.9 (C-3), 114.0 (C-8), 63.6 (CH_2), 14.0 (CH_3); IR ν_{max}/cm^{-1}= 3062 (=C-H), 1740 (C=O), 1621 (RNO_2), 1659 (C=C); Anal. Calc. for $C_{12}H_8BrNO_6$: C 42.13; H 2.36; N 4.09. Found: C 42.65; H 1.769; N 3.9.

Synthesis of ethyl 4-(3-bromo-5-chloro-2-hydroxyphenyl)-2-morpholino-4-oxobut-2-enoate derivatives **58**

To a solution of amine (1 mmol) in DMF (2 ml) was added dropwise, carbon disulfide (2 mmol) and anhydrous sodium methoxide (1 mmol). The resulting mixture was stirred at room temperature for 30 min and then brominated chromone (1 mmol) was added by one-portion and stirring was continued for 60 min. After completion of the reaction (monitored by the TLC) the mixture was diluted with ice-cold water (20 ml), filtered, dried and recrystallized from ethanol to give the ethyl 4-(3-bromo-5-chloro-2-hydroxyphenyl)-2-morpholino-4-oxobut-2-enoate **58**.[57]

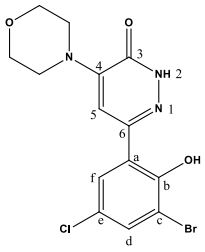
ethyl 4-(3-bromo-5-chloro-2-hydroxyphenyl)-2-morpholino-4-oxobut-2-enoate (**58**, 1.25 g, 97.35 %) yellow light solid; m.p 179-181 

°C; ^{1}H NMR (400 MHz, DMSO-d_6) δ_H= 8.23 (d, 1H, J= 2.4 Hz, 6-H), 7.83 (d, 1H, J= 2.4 Hz, 4-H), 6.25 (s, 1H, b-H), 4,34 (q, 2H, J= 7.2 Hz, e-H), 4.28 (t, 2H, J= 4.8 Hz, OCH_2), 3.76 (t, 2H, J= 4.8 Hz, OCH_2), 3.51 (t, 2H, J= 4.8 Hz, NCH_2), 3.11 (t, 2H, J= 3.6 Hz, NCH_2), 1.28 (t, 3H, J= 7.2 Hz, f-H); ^{13}C NMR (100 MHz, DMSO-d_6) δ_C= 188.1 (C-a), 164.4 (C-d), 158.2 (C-2), 157.4 (C-c), 136.6 (C-4), 128.3 (C-6), 122.8 (C-1), 121.5 (C-5), 112.1 (C-3), 89.0 (C-b), 66.2 (O-CH_2), 63.9 (N-CH_2), 62.5 (C-e), 14.0 (C-f); IR ν_{max}/cm^{-1}= 2968 (=C-H), 1721 (C=O), 1565 (C=C); HRMS *m/z*: $[M-H]^+$. Calc. for $C_{16}H_{17}BrClNO_5$-H: 418.6695, Found: 417.9877

Synthesis of 6-(3-bromo-5-chloro-2-hydroxyphenyl)-4-morpholinopyridazin-3(2H)-one **59**

A solution of ethyl 4-(3-bromo-5-chloro-2-hydroxyphenyl)-2-morpholino-4-oxobut-2-enoate **58** (10 mmol) in ethanol (20 ml), hydrazine hydrate (3 ml, 93 mmol) was added and heated under reflux for 5- 8h. After cooling, the resulting precipitate was filtered off, washed with water, dried and crystallized in ethanol.[46]

6-(3-bromo-5-chloro-2-hydroxyphenyl)-4-morpholinopyridazin-3(2H)-one (**59**, 0.34 g, 33

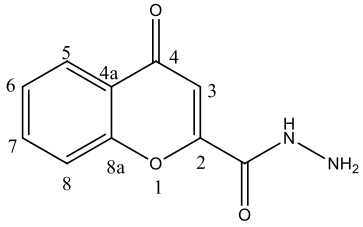


%). White solid; m.p 326-329 °C; ^{1}H NMR (400 MHz, DMSO-d$_6$) δ_H= 8.66 (s, 1H, N-H), 7.57 (d, 1H, J= 2.8 Hz, f-H), 7.47 (d, 1H, J= 2.8 Hz, d-H), 7.19 (s, 1H, 5-H), 3.68 (t, 3H, J= 4.4 Hz, OCH_2), 3.03 (t, 3H, J= 4.4 Hz, NCH_2), 5.69 (s, 2H, NH_2), 5.47 (s, 1H, OH) ^{13}C NMR (100 MHz, DMSO-d$_6$) δ_C= 160.5 (C-b), 157.2 (C-3), 145.6 (C-4), 142.3 (C-6), 130.5 (C-d), 125.1 (C-f), 118.3 (C-a), 117.5 (C-e), 114.2 (C-c), 101.2 (C-5), 64.1 (OCH_2), 54.5 (NCH_2); IR ν_{max}/cm^{-1}= 3507 (N-H), 3192 (OH), 2969 (=C-H), 1671 (C=O); HRMS *m/z*: [M-H]$^+$. Calc. for $C_{14}H_{13}BrClN_3O_3$-H: 386.6309, Found: 386.9898.

*Synthesis of chromone-2-carbohydrazide derivatives (***52 & 56)**

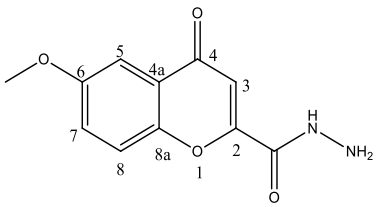
A solution of chromone-2-carboxylates (10 mmol) in ethanol (20 ml), hydrazine hydrate (3 ml, 93 mmol) was added and heated under reflux for 5- 8h. After cooling, the formed precipitate was filtered off, washed with water, dried and recrystallized in ethanol.[46]

Chromone-2-carbohydrazide (**52A**, 1.6 g, 78.5 %). Cream white solid; m.p 405 – 408 °C; ^{1}H



NMR (400 MHz, DMSO-d$_6$) δ_H= 9.6 (s, 1H, NH), 7.6 (dd, 1H, J= 6.4, 1.2 Hz, 5-H), 7.2 (s, 1H, 3-H), 7.1 (td, 1H, J= 6.8, 1.6 Hz, 6-H), 6.9 (d, 1H, J= 7.6 Hz, 8-H), 6.8 (td, 1H, J= 6.8, 1.2 Hz, 7-H), 4.4 (s, 2H, NH_2); ^{13}C NMR (100 MHz, DMSO-d$_6$) δ_c= 163.86 (C-2), 103.98 (C-3), 178.55 (C-4), 119.80 (C-4a), 129.63 (C-5), 127.53 (C-6), 135.27 (C-7), 116.88 (C-8), 155.01 (C-8a), 165.93 (C=O); IR $\mathbf{V}_{max}$/cm^{-1}= 3328.71 (NH), 3231.36 (NH_2), 2990.36 (=C-H), 1639.24 (C=O); HRMS *m/z*: [M-H]$^+$. Calc. for $C_{10}H_8N_2O_3$-H: 204.1842, Found: 203.0517.

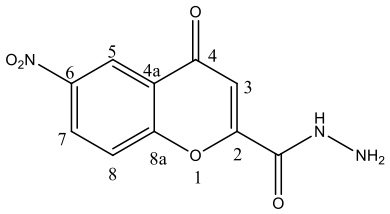
6-methoxychromone-2-carbohydrazide (**52B**, 1.89 g, 80.7 %). White solid; m.p 386 -390 °C;



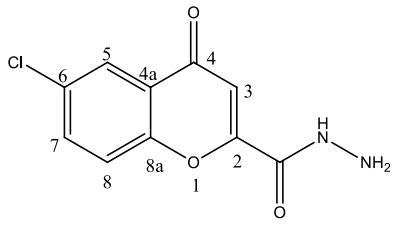
^{1}H NMR (400 MHz, DMSO-d$_6$) δ_H= 9.9 (s, 1H, NH), 7.27 (s, 1H, 3-H), 7.24 (d, 1H, J= 2.8 Hz, 5-H), 6.8 (d, 1H, J= 8.8 Hz, 8-H), 6.7 (dd, 1H, J= 5.6, 3.2 Hz, 7-H), 3.5 (s, 2H, NH_2); ^{13}C NMR (100 MHz, DMSO-d$_6$) δ_c= 160.34 (C-2), 115.63 (C-3), 171.48 (C-4), 123.08 (C-4a), 105.64 (C-5), 152.64 (C-6), 124.79 (C-7), 117.63 (C-8), 149.54 (C-8a), 164.61 (C=O),

55.91 (OCH_3); IR $\mathbf{V}_{max}/cm^{-1}$= 3328.71 (NH), 3231.36 (NH_2), 2990.71 (=C-H), 1639.24 (C=O); HRMS *m/z*: $[M-H]^+$. Calc. for $C_{11}H_{10}N_2O_4$-H: 234.2103, Found: 233.2262.

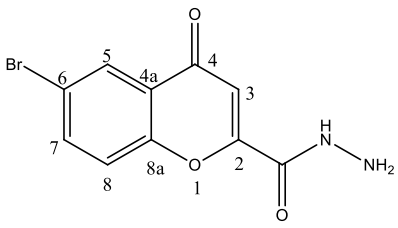
6-Nitrochromone-2-carbohydrazide (**52C**, 1.3 g, 52.2 %). Orange solid; m.p 274 -278 °C; ^1H NMR (400 MHz, DMSO-d_6) δ_H= 9.6 (s, 1H, NH), 8.3 (d, 1H, J= 3.2 Hz, 5-H), 7.8 (dd, 1H, J= 6, 2.8 Hz, 7-H), 7.0 (s, 1H, 3-H), 6.4 (d, 1H, J= 4.4 Hz, 8-H), 5.5 (s, 2H, NH_2); ^{13}C NMR (100 MHz, DMSO-d_6) δ_c= 144.86 (C-2), 100.55 (C-3), 175.33 (C-4), 123.96 (C-4a), 120.76 (C-5), 130.97 (C-6), 125.94 (C-7), 116.16 (C-8), 143.77 (C-8a), 161.86 (C=O); IR $\mathbf{V}_{max}/cm^{-1}$= 3338.42 (NH), 3193.02 (NH_2), 3130.07 (=C-H), 1631.19 (C=O).



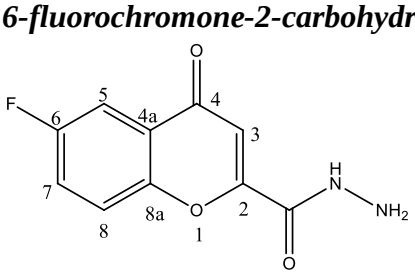
6-chlorochromone-2-carbohydrazide (**52D**, 1.98 g, 82.8 %). White solid; m.p 354 -357 °C; ^1H NMR (400 MHz, DMSO-d_6) δ_H= 9.6 (s, 1H, NH), 7.7 (d, 1H, J= 2.4 Hz, 5-H), 7.28 (s, 1H, 3-H), 7.20 (dd, 1H, J= 6, 2.8 Hz, 7-H), 6.9 (d, 1H, J= 8 Hz, 8-H), 4.5 (s, 2H, NH_2); ^{13}C NMR (100 MHz, DMSO-d_6) δ_c= 147.71 (C-2), 102.54 (C-3), 158.43 (C-4), 119.30 (C-4a), 123.58 (C-6), 131.21 (C-7), 111.58 (C-8), 138.50 (C-8a), 150.97 (C=O); IR $\mathbf{V}_{max}/cm^{-1}$= 3352.19 (NH), 3241.42 (NH_2), 3092.05 (=C-H), 1650.24 (C=O); HRMS *m/z*: $[M-H]^+$. Calc. for $C_{10}H_7ClN_2O_3$-H: 238.6292, Found: 239.0031.



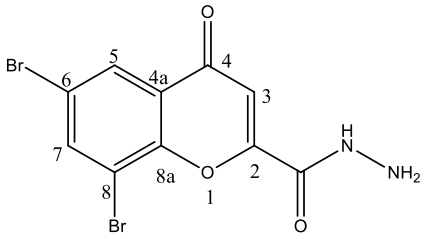
6-bromochromone-2-carbohydrazide (**52E**, 1.15g, 40.6 %). Yellow solid, m.p 291 – 294 °C; ^1H NMR (400 MHz, DMSO-d_6) δ_H= 9.6 (s, 1H, NH), 7.8 (d, 1H, J= 2.4 Hz, 5-H), 7.3 (dd, 1H, J= 6, 2.4 Hz, 7-H), 7.2 (s, 1H, 3-H), 6.9 (d, 1H, J= 8.8 Hz, 8-H), 4.5 (s, 2H, NH_2); ^{13}C NMR (100 MHz, DMSO-d_6) δ_c= 159.46 (C-2), 104.92 (C-3), 167.22 (C-4), 119.03 (C-4a), 131.85 (C-5), 129.57 (C-6), 141.15 (C-7), 110.86 (C-8), 154.29 (C-8a), 163.85 (C=O); $\mathbf{V}_{max}/cm^{-1}$= 3350.34 (NH), 3239.43 (NH_2), 3058.23 (=C-H), 1650.00 (C=O); HRMS *m/z*: $[M-H]^+$. Calc. for $C_{10}H_7BrN_2O_3$-H: 283.2103, Found: 283.9529.



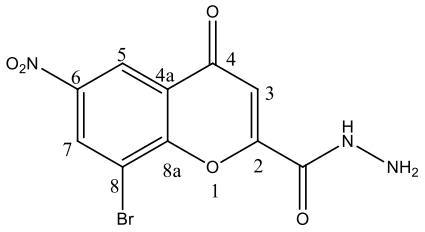
6-fluorochromone-2-carbohydrazide (**52F**, 1.03 g, 46.4 %). Yellow solid; m.p 360 °C; ^1H NMR (400 MHz, DMSO-d_6) δ_H= 9.6 (s, 1H, NH), 7.5 (dd, 1H, J= 6.8, 2.8 Hz, 5-H), 7.2 (s, 1H, 3-H), 7.0 (td, 1H, 5.6, 3.2 Hz, 7-H), 6.9 (dd, 1H, J= 5.2, 3.2 Hz, 8-H), 4.5 (s, 2H, NH_2); ^{13}C NMR (100 MHz, DMSO-d_6) δ_c= 158.83 (C-2), 104.83 (C-3), 165.09 (C-4), 111.92 (C-4a), 113.32 (C-5), 151.27 (C-6), 115.98 (C-7), 113.08 (C-8), 154.73 (C-8a), 157.05 (C=O); $\mathbf{V}_{max}/cm^{-1}$= 3352.65 (NH), 3238.53 (NH_2), 3142.52 (=C-H), 1650.55 (C=O)



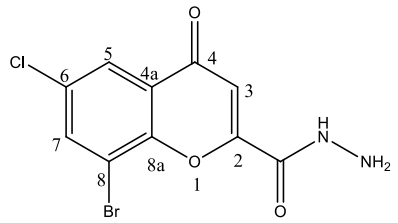
6.8-dibromochromone-2-carbohydrazide (**56A**, 1.18 g, 93.65 %). Cream white solid; m.p 331 – 334 °C; ^{1}H NMR (400 MHz, DMSO-d$_6$) δ_H= 9.8 (s, 1H, NH), 7.75 (d, 1H, J= 2.4 Hz, 7-H), 7.73 (d, 1H, J= 2.4 Hz, 7-H), 7.3 (s, 1H, 3-H), 4.5 (s, 2H, NH$_2$); ^{13}C NMR (100 MHz, DMSO-d$_6$) δ_c= 147.23 (C-2), 102.58 (C-3), 158.89 (C-4), 111.93 (C-4a), 119.92 (C-5), 110.91 (C-6), 128.18 (C-7), 105.06 (C-8), 133.83 (C-8a), 151.24 (C=O); **V**$_{max}$/cm^{-1}= 3373.40 (NH), 3248.38 (NH$_2$), 3073.38 (=C-H), 1722.94 (C=O)



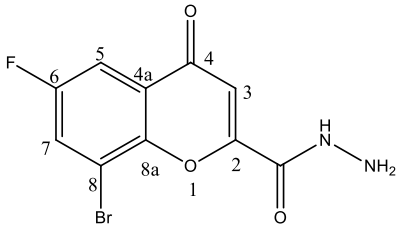
8-bromo-6-nitrochromone-2-carbohydrazide (**56B**, 1.62 g, 95.29 %). Orange solid; m.p 277 – 286 °C; ^{1}H NMR (400 MHz, DMSO-d$_6$) δ_H= 9.6 (s, 1H, NH), 8.3 (d, 1H, J= 2.8 Hz, 5-H), 8.2 (d, 1H, J= 2.8 Hz, 7-H), 7.1 (s, 1H, 3-H), 4.5 (s, 2H, NH$_2$); ^{13}C NMR (100 MHz, DMSO-d$_6$) δ_c= 143.99 (C-2), 101.38 (C-3), 168.99 (C-4), 115.92 (C-4a), 122.60 (C-5), 131.06 (C-6), 127.54 (C-7), 115.69 (C-8), 143.71 (C-8a), 161.31 (C=O); **V**$_{max}$/cm^{-1}= 3325.09 (NH), 3249.33 (NH$_2$), 2999.85 (=C-H), 1624.16 (C=O



8-bromo-6-chlorochromone-2-carbohydrazide (**56C**, 1.95g, 82.28 %). White solid; m.p. 336 – 339 °C; ^{1}H NMR (400 MHz, DMSO-d$_6$) δ_H= 9.8 (s, 1H NH), 7.63 (d, 1H, J= 2.4 Hz, 5-H), 7.60 (d, 1H, J= 2.4 Hz, 7-H), 7.38 (s, 1H, 3-H), 4.5 (s, 2H, NH$_2$); ^{13}C NMR (100 MHz, DMSO-d$_6$) δ_c= 147.71 (C-2), 102.54 (C-3), 158.43 (C-4), 119.30 (C-4a), 123.58 (C-5), 125.34 (C-6), 131.21 (C-7), 111.58 (C-8), 138.50 (C-8a), 150.97 (C=O) **V**$_{max}$/cm^{-1}= 3364.45 (NH), 3238.18 (NH$_2$), 3137.88 (=C-H), 1655.08 (C=O)



8-bromo-fluorochromone-2-carbohydrazide (**56D**, 0.36g, 45.5 %). Grey solid; m.p 255 - 258 °C; ^{1}H NMR (400 MHz, DMSO-d$_6$) δ_H= 9.9 (s, 1H, NH), 7.48 (d, 1H, J= 2 Hz, 7-H), 7.45 (d, 1H, J= 2 Hz, 5-H), 7.3 (s, 1H, 3-H), 4.5 (s, 2H, NH$_2$); ^{13}C NMR (100 MHz, DMSO-d$_6$) δ_c= 153.83 (C-2), 102.74 (C-3), 158.56 (C-4), 119.25 (C-4a), 112.32 (C-5), 148.59 (C-6), 118.61 (C-7), 110.92 (C-8), 138.62 (C-8a), 156.20 (C=O); **V**$_{max}$/cm^{-1}= 3333.28 (NH), 3287.52 (NH$_2$), 3144.21 (=C-H), 1657.71 (C=O)

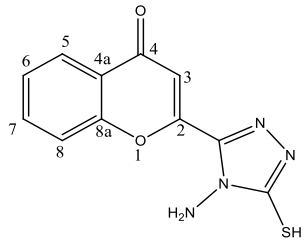


Synthesis of 2-(4-amino-5-mercapto-4H-1,2,4-triazol-3-yl) chromone derivatives (**53 & 57**)

A KOH (30 mmol) was dissolved in absolute ethanol (200 ml). To the above solution, an aryl acid hydrazide (4-hydrazinyl-6-(2-hydroxyphenyl)pyridazin-3(2*H*)-one derivatives) (20 mmol) was added and the solution cooled on ice. Carbon disulphide (20 mmol) was then added in small portions with constant stirring. The reaction mixture was agitated continuously for a period of 15 h. The resulting mixture was diluted with anhydrous ether (100 ml) and dried under vacuum. The potassium salt thus obtained was in quantitative yield and used in the next step without further purification.

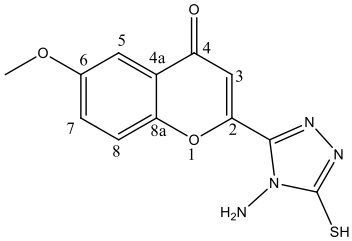
A suspension of potassium dithiocarbazinate (2 mmol) in water (5 ml) and hydrazine hydrate (15 ml, 6 mmol) was refluxed for 30 min with occasional shaking. The colour of the reaction mixture changed to green with the evolution of hydrogen sulphide gas. A homogeneous reaction mixture was obtained during the reaction process. The reaction mixture was cooled to room temperature and diluted with water (100 ml). On acidification with concentrated HCl, the required product was precipitated out, was filtered, washed thoroughly with cold water and recrystallized from ethanol to afford compounds **53 & 57**. The completion of the reaction was monitored with TLC by using ethyl acetate and petroleum ether (1:1) as eluent.[58]

2-(4-amino-5-merrcapto-4H-1,2,4-triazol-3-yl)chromone (**53A**, 0.46 g, 37 %). White solid,



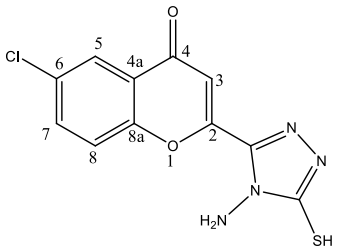
m.p 301- 308 °C; ^{1}H NMR (400 MHz, DMSO-d$_6$) δ_H= 13.92 (s, 1H, SH), 7.7 (d, 1H, J= 8 Hz, 5-H), 7.3 (s, 2H, 3-H), 7.2 (t, 1H, J= 7.2 Hz, 7-H), 7.00 (d, 1H, J= 7.6 Hz, 8-H), 6.9 (t, 1H, J= 7.6 Hz, 6-H), 5.9 (s, 2H, NH_2); ^{13}C NMR (100 MHz, DMSO-d$_6$) δ_c= 165.27 (C-4), 154.86 (C-2), 145.47 (C-SH), 140.92 (C-8a), 138.95 (C=N), 130.14 (C-7), 128.00 (C-5), 120.03 (C-6), 116.04 (C-8), 115.86 (C-4a), 105.62 (C-3); IR ν_{max}/ cm^{-1} = 3320.21 (N-H), 3095.55 (=C-H), 1751.95 (C=O); HRMS *m/z*: [M-H]$^+$. Calc. for $C_{11}H_8N_4O_2S$-H: 260.2743, Found: 260.0319.

2-(4-amino-5-mercapto-4H-1,2,4-triazol-3-yl)-6-methoxychromone (**53B**, 0.52g, 35.14 %).



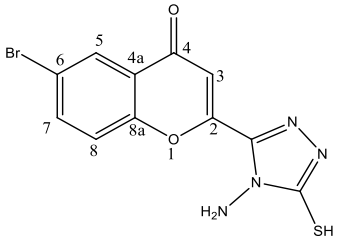
White solid; m.p 294-298 °C; ^{1}H NMR (400 MHz, DMSO-d$_6$) δ_H= 13.95 (s, 1H, SH), 7.48 (s, 1H, 3-H), 7.3 (d, 1H, J= 2.8 Hz, 5-H), 6.9 (d, 1H, J=8.8 Hz, 8-H), 6.8 (dd, 1H, J=6 Hz, 3.2 Hz, 7-H), 5.8 (s, 2H, NH_2), 3.7 (s, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO-d$_6$) δ_C= 152.68 (C-4), 148.92 (C-2), 146.61 (C-SH), 142.19 (C-8a), 134.55 (C=N), 145.59 (C-6), 117.84 (C-7), 115.99 (C-8), 116.53 (C-4a), 112.53 (C-5), 107.02 (C-3); IR ν_{max}/cm^{-1}= 3314.14 (N-H), 3071.15 (=C-H), 1614.83 (C=O); HRMS *m/z*: [M-H]$^+$. Calc. for $C_{12}H_{10}N_4O_3S$-H: 290.3004, Found: 290.0422.

2-(4-amino-5-mercapto-4H-1,2,4-triazol-3-yl)-6-chlorochromone (**53C**, 0.97 g, 63.82%).



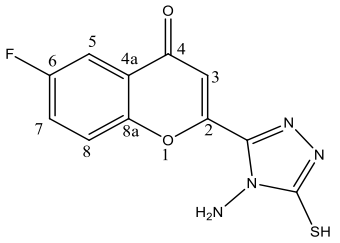
White/ grey solid; m.p 277- 285 °C; ^{1}H NMR (400MHz, DMSO-d_6) δ_H= 13.9 (s, 1H, SH), 7.9 (d, 1H, J= 2.8 Hz, 5-H), 7.8 (s, 1H, 3-H), 7.2 (dd, 1H, J= 6 Hz, 2,4 Hz, 7-H), 6.9 (d, 1H, J=8.4 Hz, 8-H), 5.9 (s, 2H, NH_2); ^{13}C NMR (100 MHz, DMSO-d_6) δ_C= 159.97 (C-4), 154.20 (C-2), 146.97 (C-8a), 133.51 (C=N), 129.08 (C-7), 127.06 (C-6), 123.58 (C-5), 120.49 (C-4a), 118.75 (C-8), 107.00 (C-3), 74.33 (C-SH); IR $\boldsymbol{V}_{max}$/ cm^{-1}= 3320.21 (N-H), 3095.55 (=C-H), 1625.53 (C=O); HRMS *m/z*: [M-H]$^{+}$. Calc. for $C_{11}H_7ClN_4O_2S$-H: 294.7193, Found: 293.0632.

2-(4-amino-5-mercapto-4H-1,2,4-triazol-3-yl)-6-bromochromone (**53D**, 8.8 g, 73.95 %).



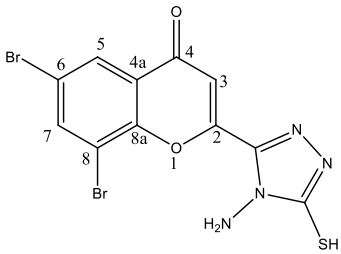
Grey solid; m.p 280- 291 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ_H= 13.9 (s, 1H, SH), 8.0 (d, 1H, J= 6 Hz, 2.8 Hz, 7-H), 6.9 (d, 1H, J= 8.4 Hz, 8-H), 5.9 (s, 2H, NH_2); ^{13}C NMR (100 MHz, DMSO-d_6) δ_C= 159.94 (C-4), 154.61 (C-2), 146.74 (C-8a), 133.47 (C=N), 131.90 (C-7), 129.86 (C-6), 121.11 (C-5), 119.21 (C-4a), 111.07 (C-8), 107.05 (C-3); IR $\boldsymbol{V}_{max}$/ cm^{-1}= 3176.17 (N-H), 3025.53 (=C-N), 1655.94 (C=O); HRMS *m/z*: [M-H]$^{+}$. Calc. for $C_{11}H_7BrN_4O_2S$-H: 339.1705, Found: 339.9399

2-(4-amino-5-mercapto-4H-1,2,4-triazol-3-yl)-6-flourochromone (**53E**, 0.51 g, 61.45 %).



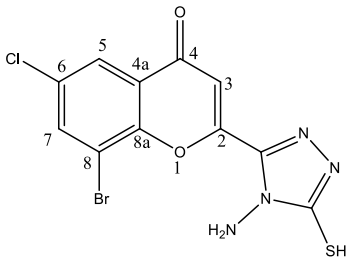
Light yellow solid; m.p 160- 162 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δH= 12.01 (s, 1H, SH), 7.7 (s, 1H, 3-H), 7.6 (dd, 1H, J= 6.8 Hz, 2.8 Hz, 7-H), 7.1 (m, 1H, 8-H), 7.0 (m, 1H, 5-H), 5.4 (s, 2H, NH_2); ^{13}C NMR (100 MHz, DMSO-d_6) δ_C= 157.11 (C-4), 154.79 (C-2), 145.77 (C-8a), 141.44 (C=N), 146 (C-6), 118.32 (C-4a), 117.16 (C-7), 116.15 (C-8), 113.87 (C-5), 107.65 (C-3), IR $\boldsymbol{V}_{max}$/cm-^{1}= 3242.22 (N-H), 2939.09 (=C-H), 1605.43 (C=O).

2-(4-amino-5-mercapto-4H-1,2,4-triazol-3-yl)-6,8-dibromochromone (**57A**, 0.6 g, 84.51 %).

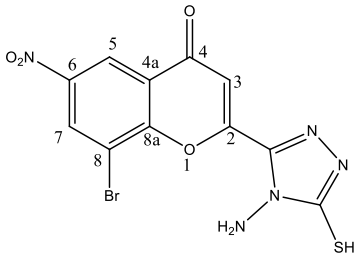


White solid; m.p 200- 205 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δH= 10.2 (s, 1H, SH), 7.99 (d, 1H, J= 2.4 Hz, 5-H), 7.75 (s, 2H, NH_2,), 7.46 (d, 1H, J= 2.4 Hz, 7-H), 7.30 (s, 1H, 3-H); ^{13}C NMR (100 MHz, DMSO-d_6) δ_C= 151.39 (C-4), 106.98 (C-2), 160.60 (C-8a), 120.06 (C=N), 128.97 (C-6), 112.03 (C-4a), 129.06 (C-7), 111.40 (C-8), 137.03 (C-5), 134.15 (C-3), IR $\boldsymbol{V}_{max}$/cm-^{1}= 3153.11 (N-H), 3058.36 (=C-N), 1665.12 (C=O).

2-(4-amino-5-mercapto-4H-1,2,4-triazol-3-yl)-8-bromo-6-chlorochromone (**57B**, 0.2 g, 46.5 %). White solid; m.p 205- 212°C; ^{1}H NMR (400 MHz, DMSO-d_6) δH= 14.31 (s, 1H, SH), 7.95 (d, 1H, J= 2.4 Hz, 5-H), 7.62 (d, 1H, J= 2.4 Hz, 7-H), 7.53 (s, 1H, 3-H), 6.46 (s, 2H, NH_2,); ^{13}C NMR (100 MHz, DMSO-d_6) δ_C= 160.62 (C-4), 151.07 (C-2), 137.05 (C-8a), 131.66 (C=N), 119.57 (C-6), 113.45 (C-4a), 124.25 (C-7), 111.64 (C-8), 119.48 (C-5), 106.99 (C-3), IR ν_{max}/cm-^{1}= 3343.27 (N-H), 3088.86 (=C-H), 1635.79 (C=O).



2-(4-amino-5-mercapto-4H-1,2,4-triazol-3-yl)-8-bromo-6-nitrochromone (**57C**, 0.15 g, 34.09 %). Brown dark; m.p 332- 335°C; ^{1}H NMR (400 MHz, DMSO-d_6) δ_H= 10.78 (s, 1H, SH), 8.65 (d, 1H, J= 2.8 Hz, 5-H), 8.42 (d, 1H, J= 2.8 Hz, 7-H), 7.58 (s, 1H, 3-H), 7.45 (s, 2H, NH_2); ^{13}C NMR (100 MHz, DMSO-d_6) δ_C= 159.60 (C-2), 102.93 (C-3), 162.39 (C-4), 122.07 (C-4a), 116.95 (C-5), 136.55 (C-6), 127.59 (C-7), 113.03 (C-8), 140.30 (C-8a), 136.10 (-C=N), 145.55 (C-SH); ν_{max}/cm-^{1}= 3370.41 (N-H), 3045.56 (=C-H), 1655.90 (C=O).



6.3. Biological studies of the synthesized chromone-2-carboxylate derivatives

6.3.1 Anti-malaria screening method

The test samples of chromone-2-carboxylate derivatives were prepared to a 10 mM stock solution in 100 % DMSO. Samples were tested as a suspension if not completely dissolved. Further dilutions were prepared in growth media on the day of the experiment. The standard antimalarial drugs chloroquine (CQ) and artesunate (Arts) were used as the reference drug in all experiments. A full dose-response was performed for all compounds in a 96-well plate to determine the concentration inhibiting 50 % of parasite growth (IC_{50}–value). Test samples were tested at a starting concentration of 6 µM, which was then serially diluted 2-fold in growth medium to generate the tested concentration range. The same dilution technique was used for all samples. CQ and Arts were tested from a starting concentration of 1 µg/mL. The highest concentration of solvent to which the parasites were exposed was <0.1 % and has no measurable effect on the parasite viability (data not shown). The assay plate was incubated at 37 °C for 72 h in a sealed gas chamber under 3 % O_2 and 4 % CO_2 with the balance being N2. After 72 h, the wells in the assay plate were gently resuspended, and 15 µL from each well was transferred to a duplicate plate containing 100 µL of Malstat reagent and 25 µL of nitroblue tetrazolium solution in each well. Plates were left to develop for 20 minutes in the dark and then absorbance of each well was quantified using a spectrophotometer at 620 nM wavelength.

The remaining population of parasites at each concentration of the test compound was determined by comparing the absorbance of each well to the absorbance of a well containing

the drug-free control. Survival was plotted against concentration and the IC-values were obtained using a nonlinear dose-response curve fitting analysis via the Dotmatics software platform.[62,63]

CHAPTER 7

7. References

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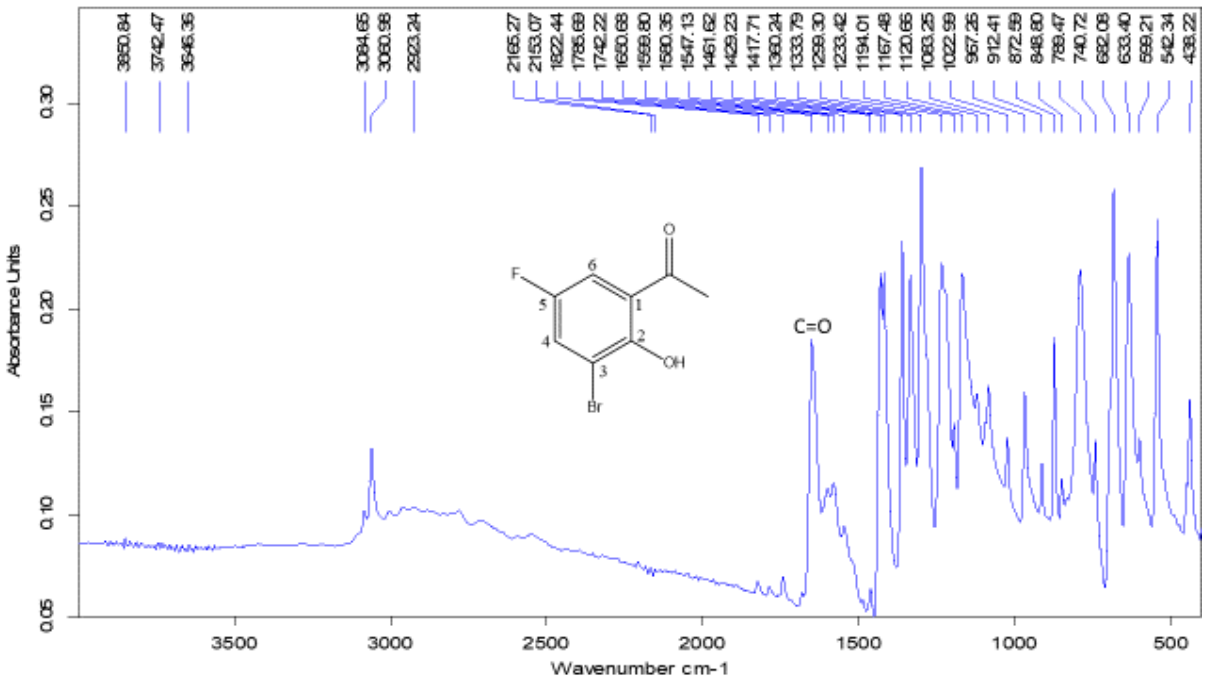
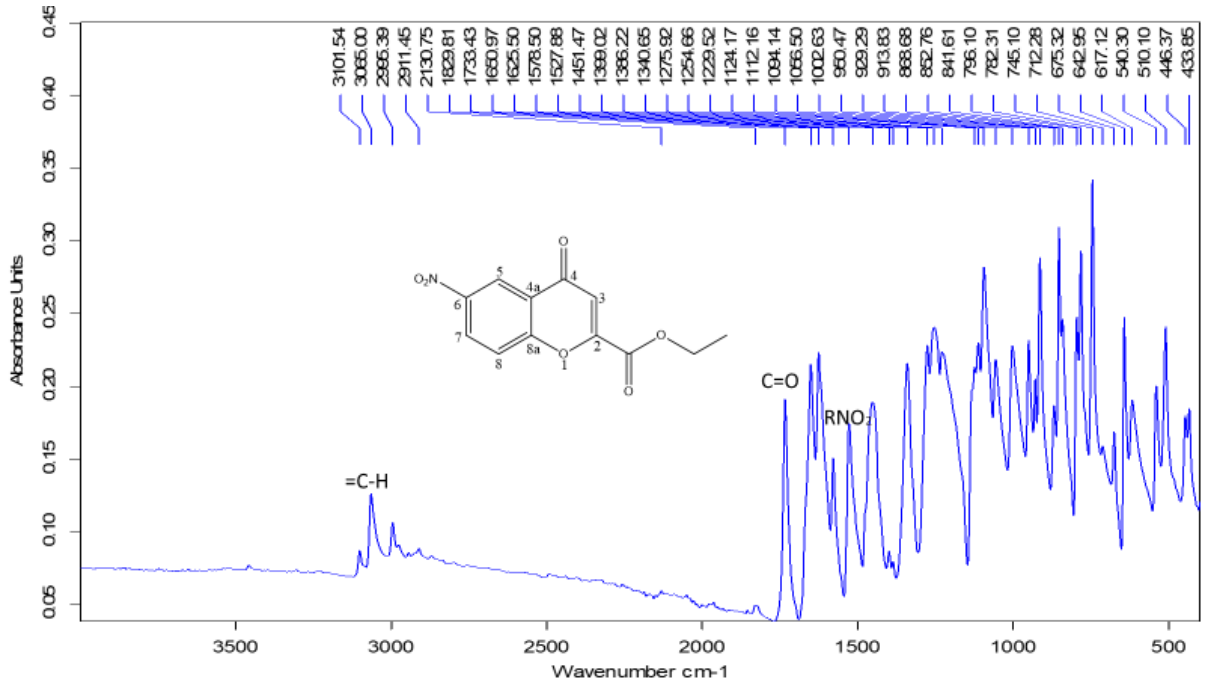
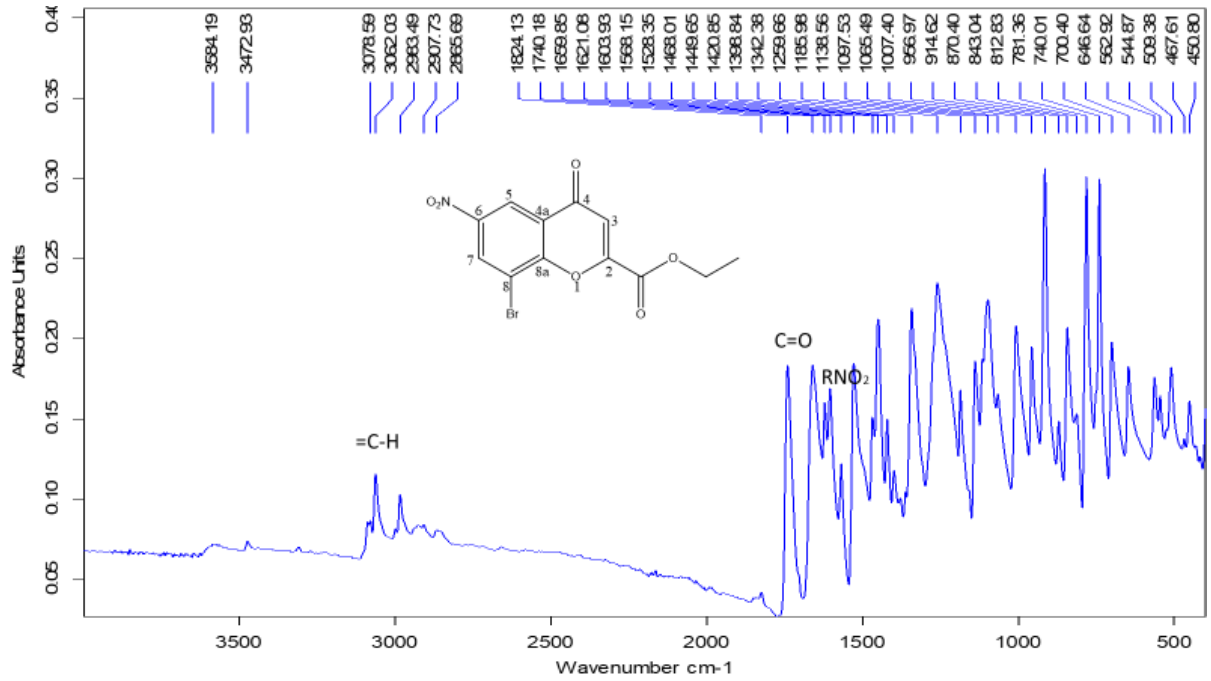
Appendix



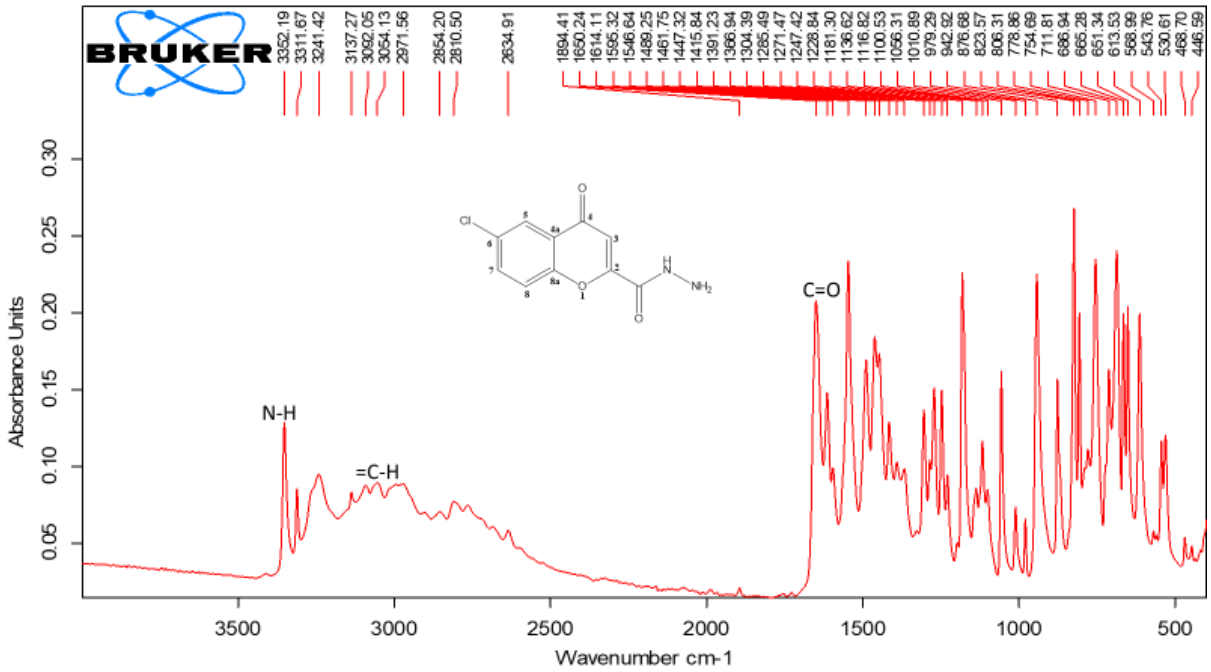
Appendix 1: FTIR spectrum of 3-bromo-5-flouro-2-hydroxyacetophenone **54A**



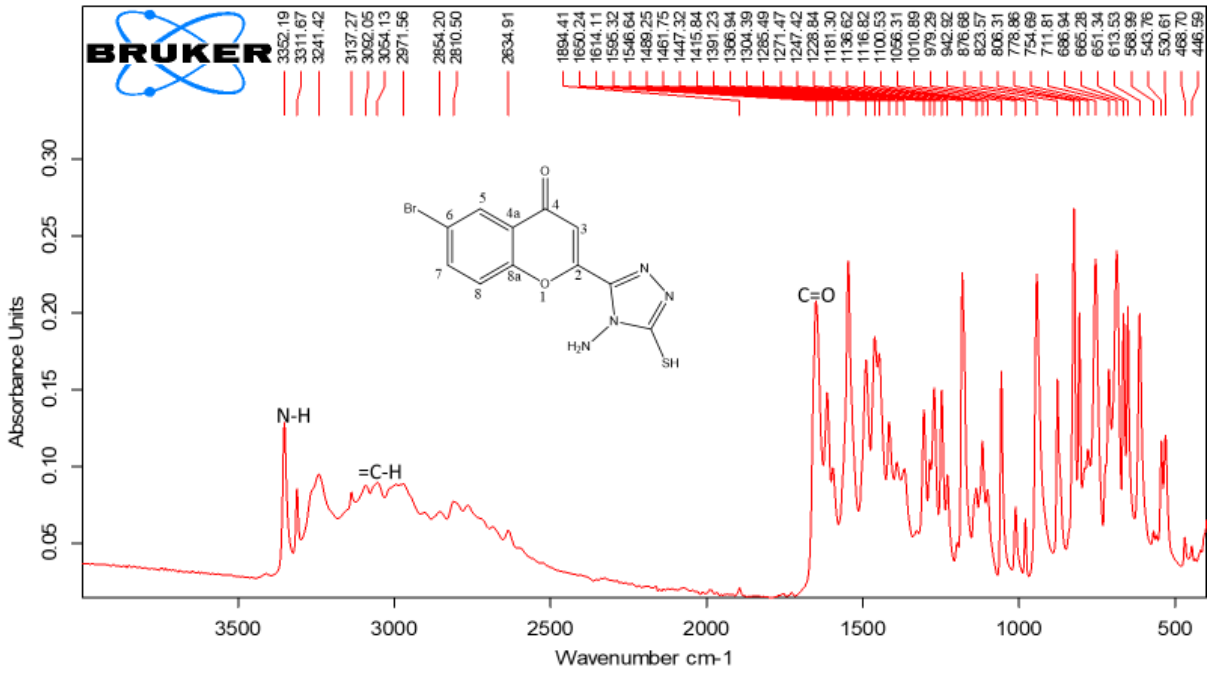
Appendix 2: FTIR spectrum of 6-nitrochromone-2-carboxylate **51E**



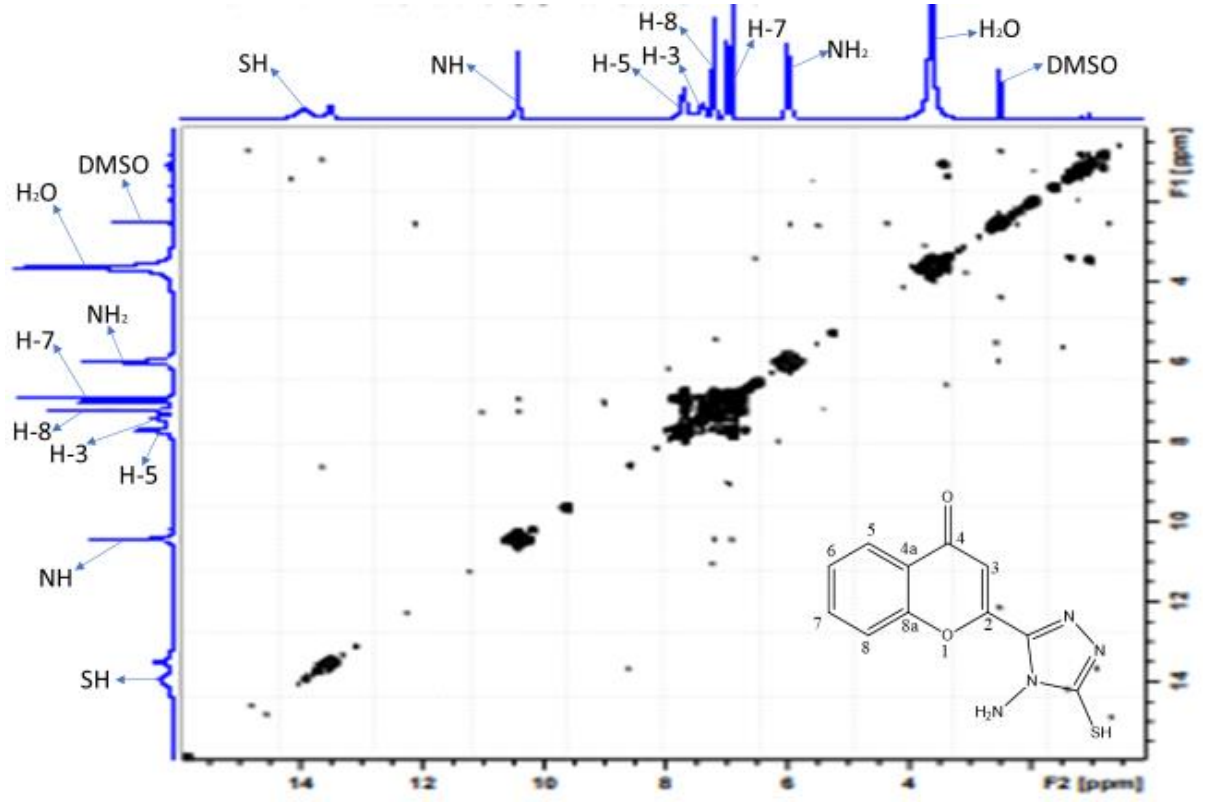
Appendix 3: FTIR spectrum of 6-chlorochromone-2-carboxylate **55D**



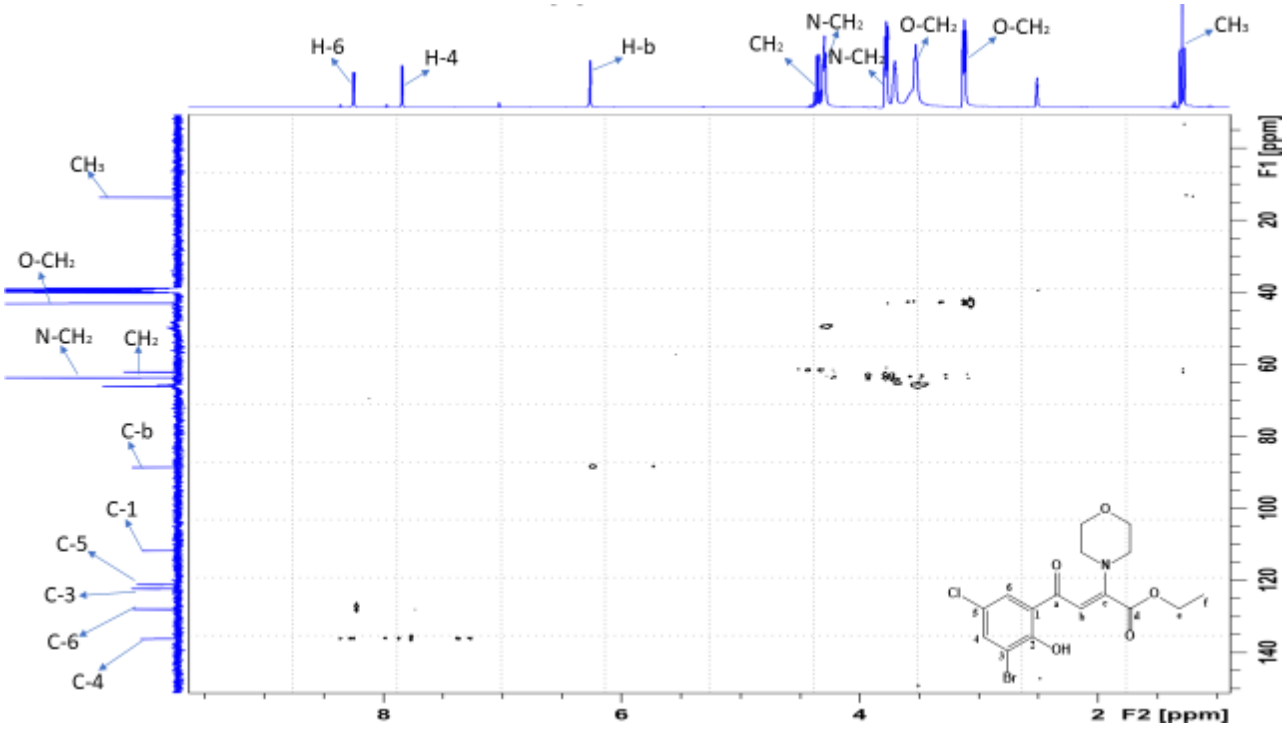
Appendix 4: FTIR spectrum of 6-chlorochromone-2-carbohydrazide **52D**



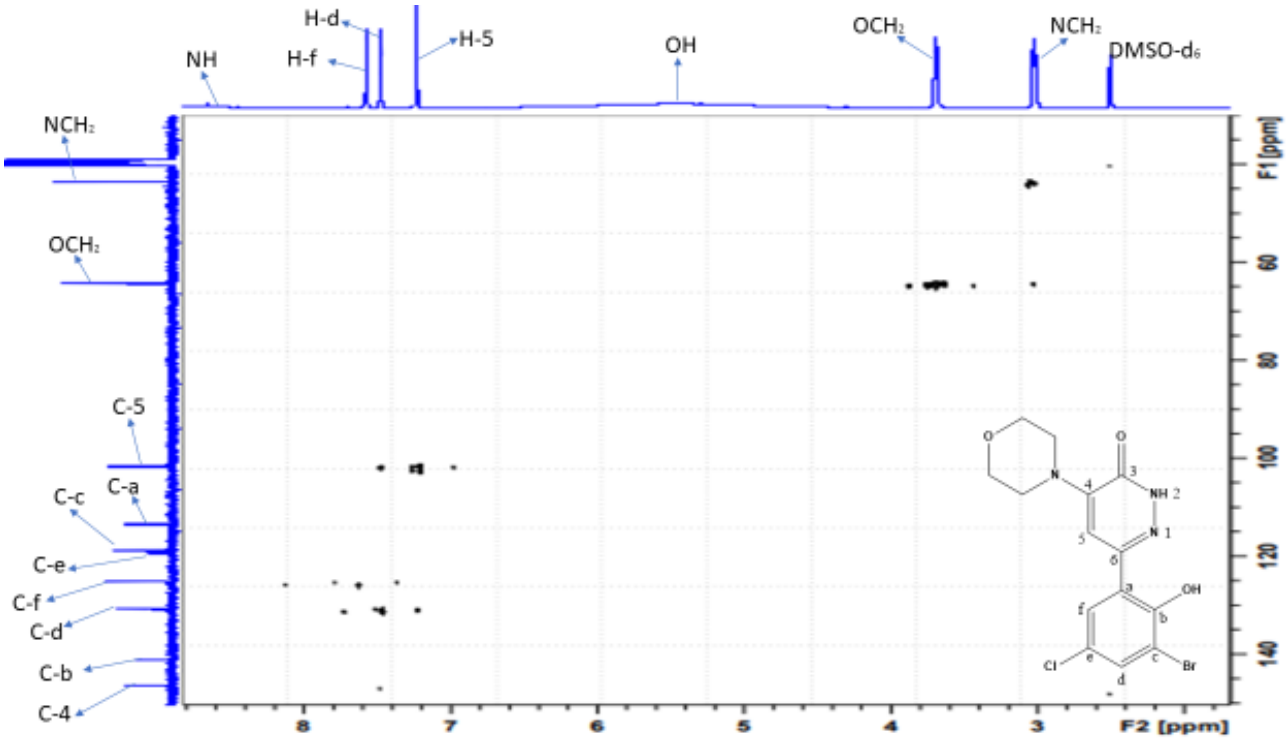
Appendix 5: FTIR spectrum of 2-(4-amino-5-mercapto-4*H*-1,2,4-triazol-3-yl) 6-bromochromone derivatives **53D**



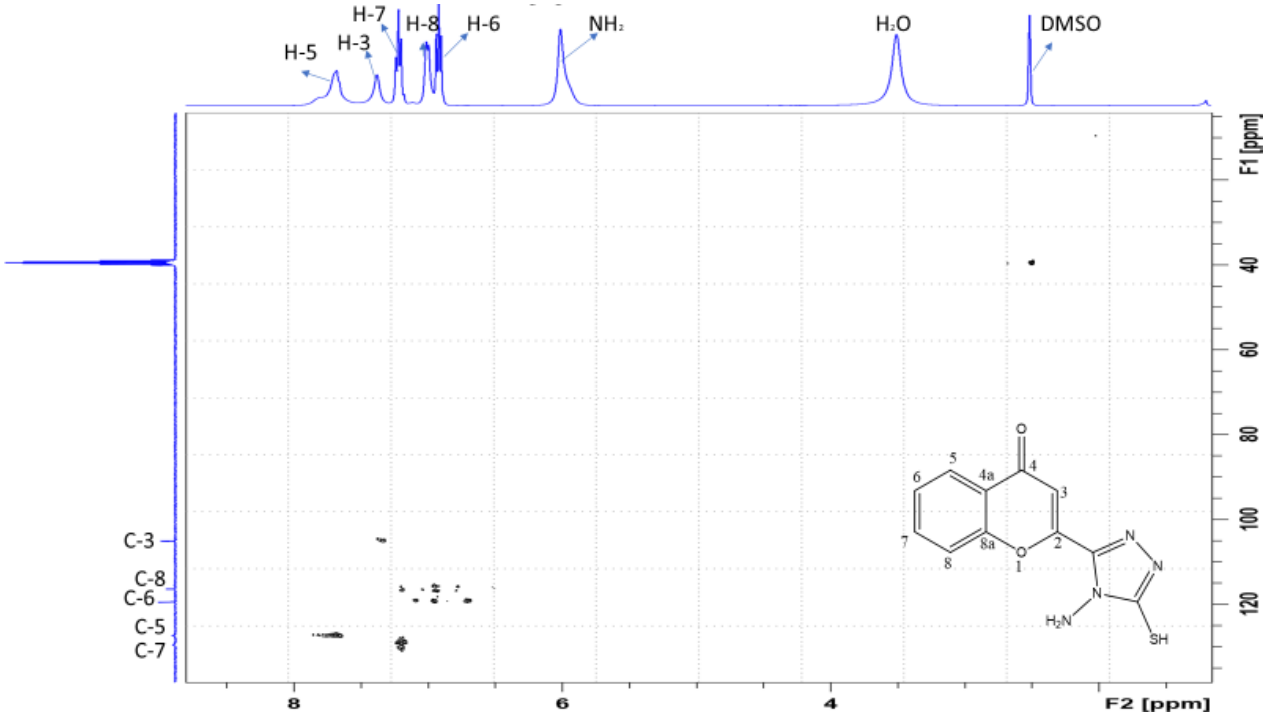
Appendix 6: COSY spectrum of 2-(4-amino-5-mercapto-4*H*-1,2,4-triazol-3-yl) chromone **53A**



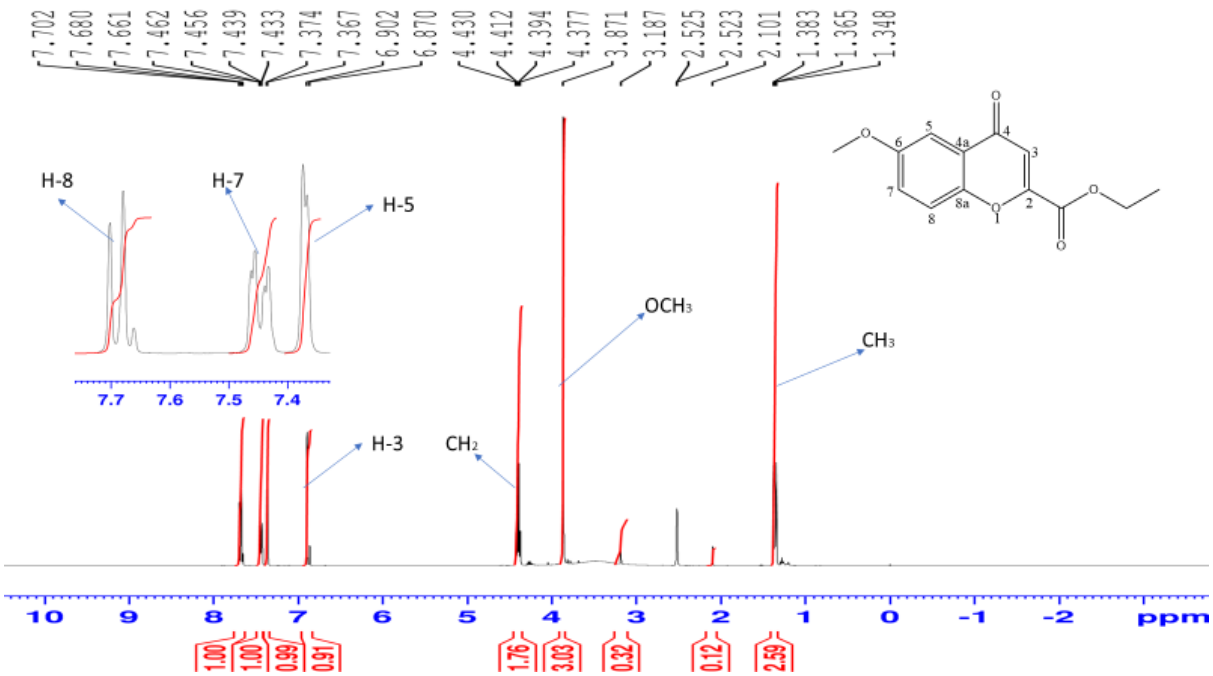
Appendix 7: HSQC spectrum of ethyl 4-(3-bromo-5-chloro-2-hydroxyphenyl)-2-morpholino-4-oxobut-2-enoate **58**



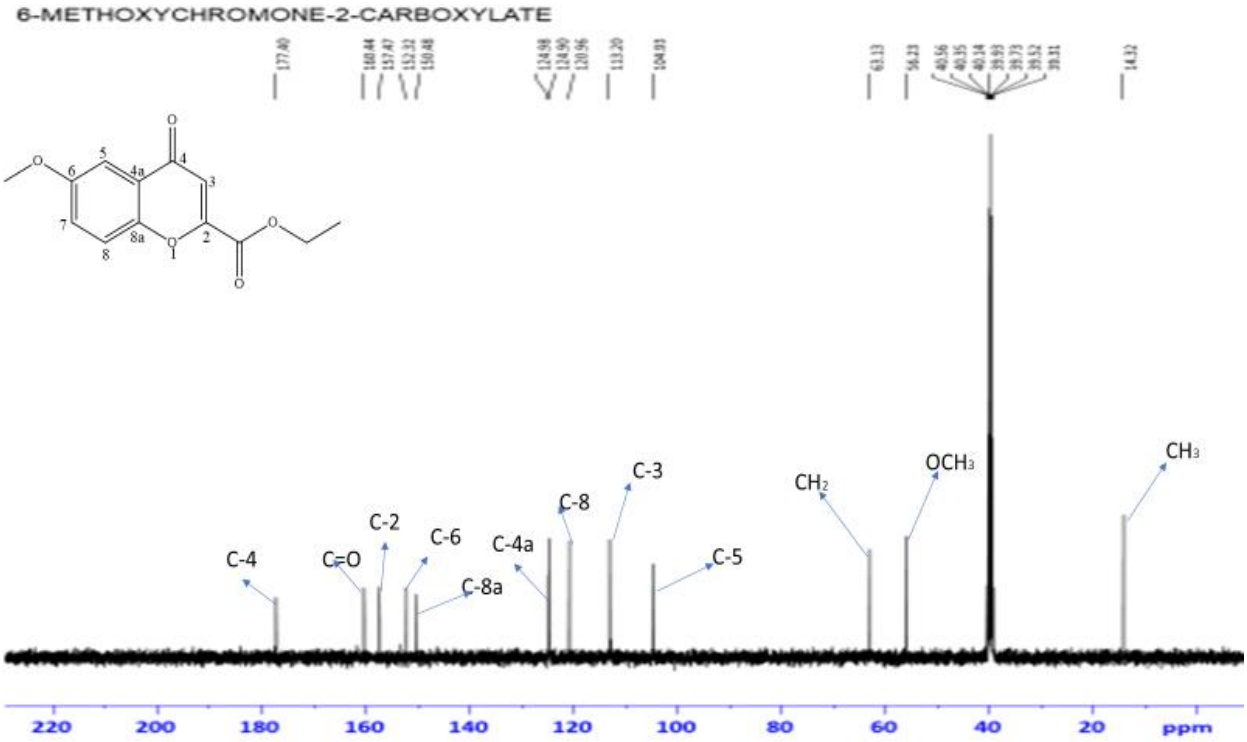
Appendix 8: HSQC spectrum of 6-(3-bromo-5-chloro-2-hydroxyphenyl)-4-morpholinopyridazin-3(2*H*)-one **59** in DMSO-d_6 (at 100 MHz)



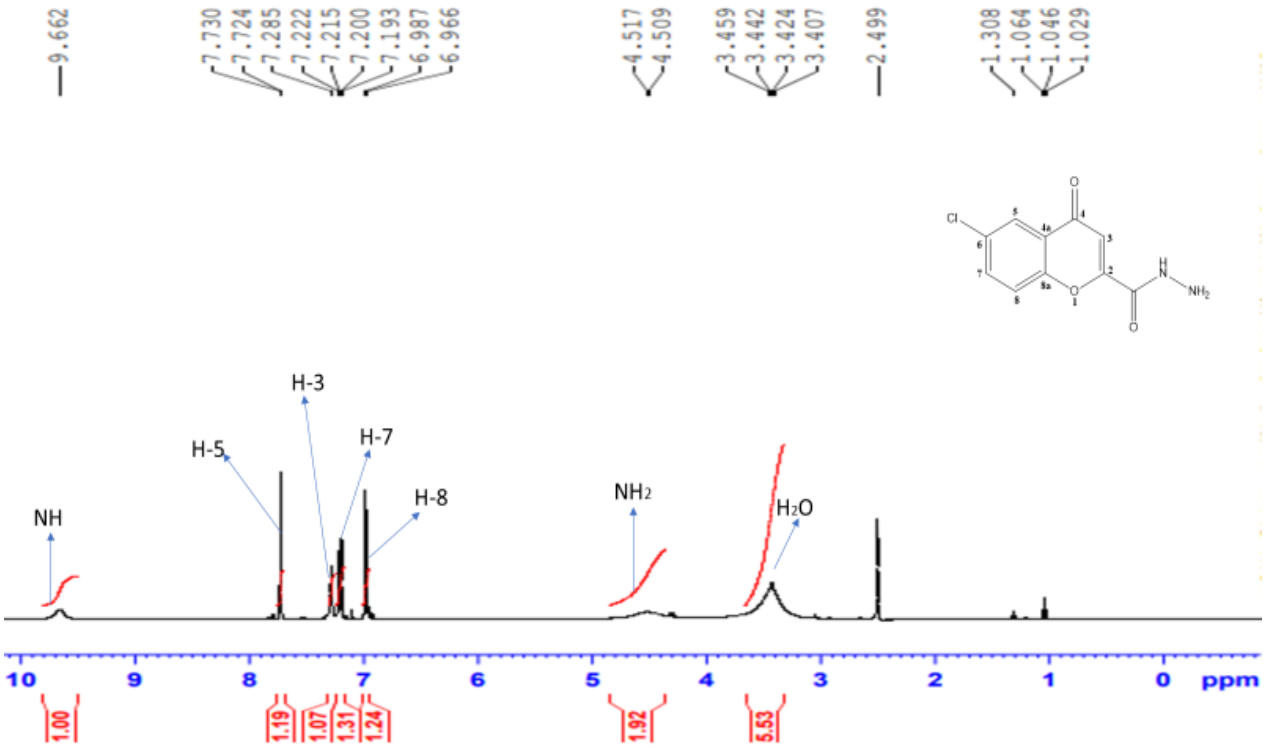
Appendix 9: HSQC spectrum of 2-(4-amino-5-mercapto-4*H*-1,2,4-triazol-3-yl) chromone **53A** in DMSO-d_6 (at 100 MHz) in DMSO



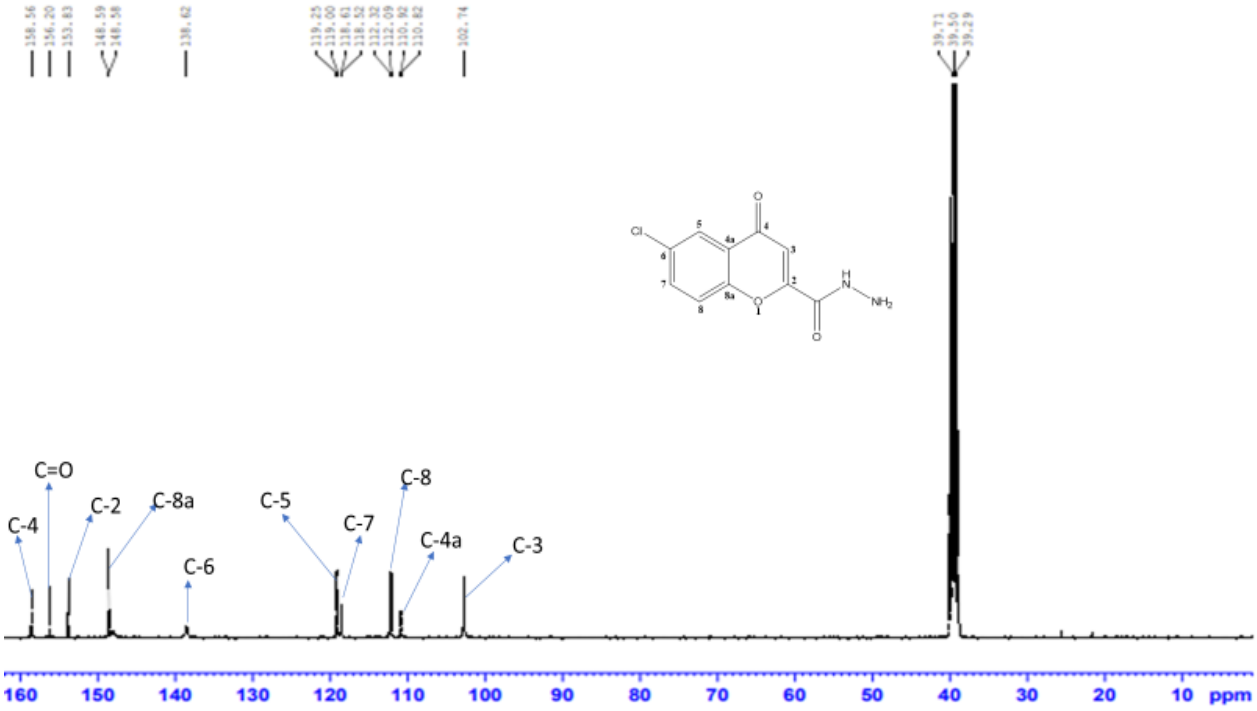
Appendix 10: ^{1}H NMR spectrum of 6-methoxychromone-2-carboxylate **51D** in DMSO-d_6 (at 400 MHz) in DMSO



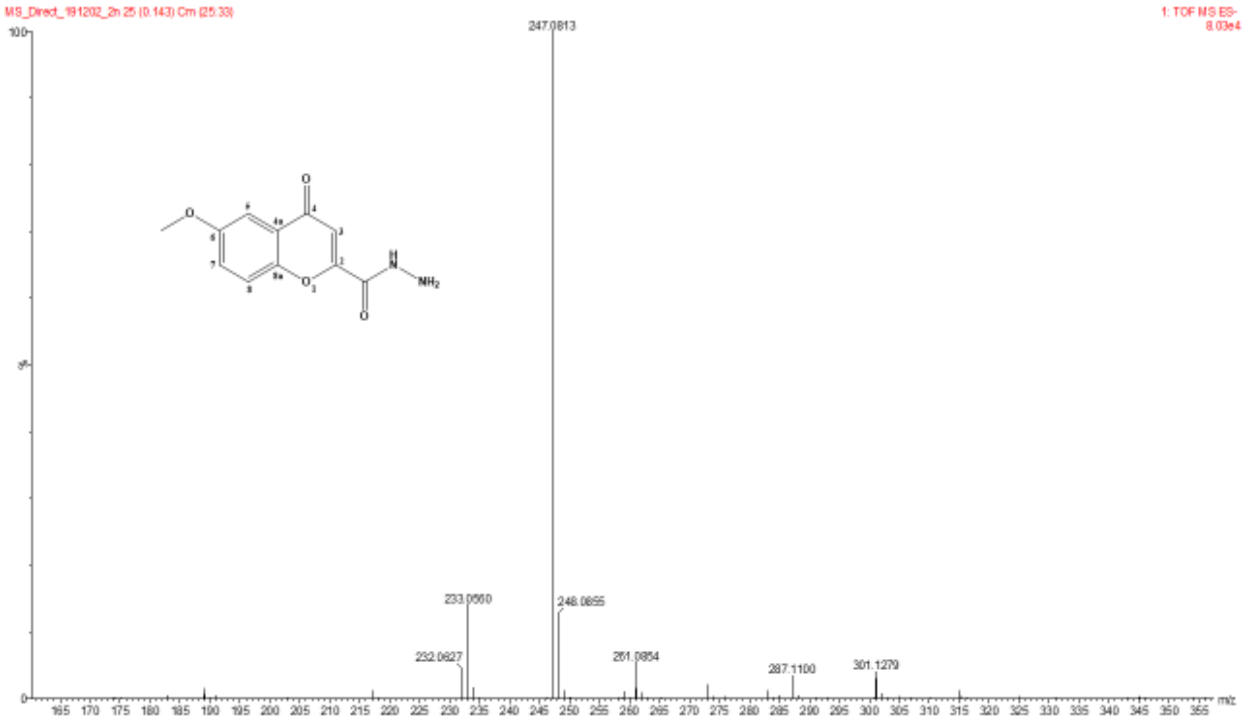
Appendix 11: ^{13}C NMR spectrum of 6-methoxychromone-2-carboxylate **51D** in DMSO-d_6 (at 400 MHz)



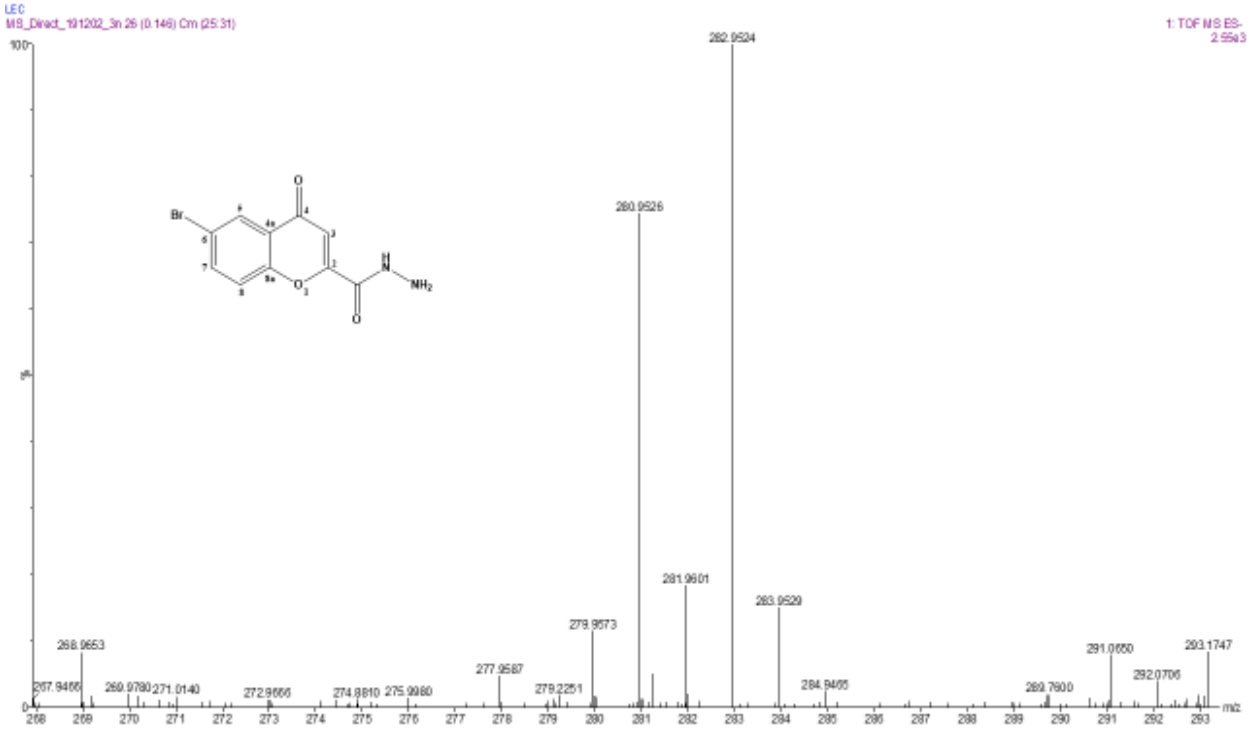
Appendix 12: ^{1}H NMR spectrum of 6-chlorochromone-2-carbohydrazide **52D** in DMSO-d_6 (at 400 MHz)



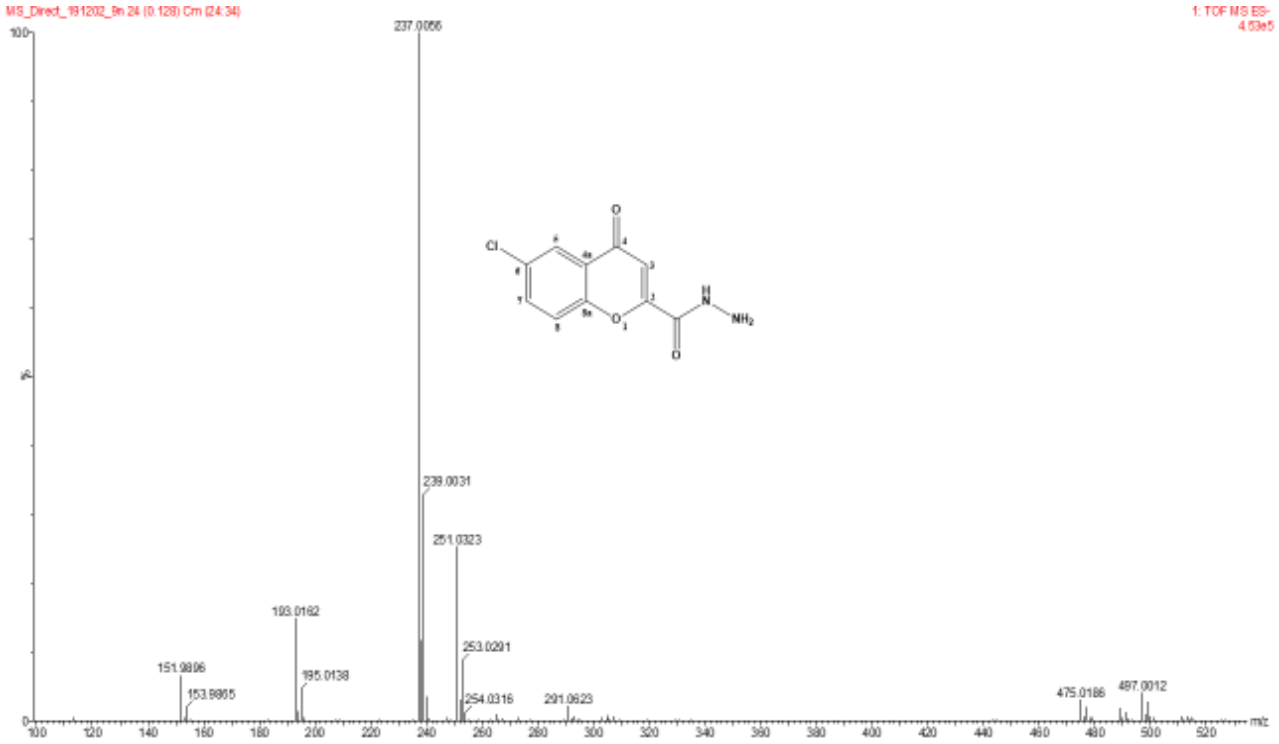
Appendix 13: ^{13}C NMR spectrum of 6-chlorochromone-2-carbohydrazide **52D** in DMSO-d_6 (at 400 MHz)



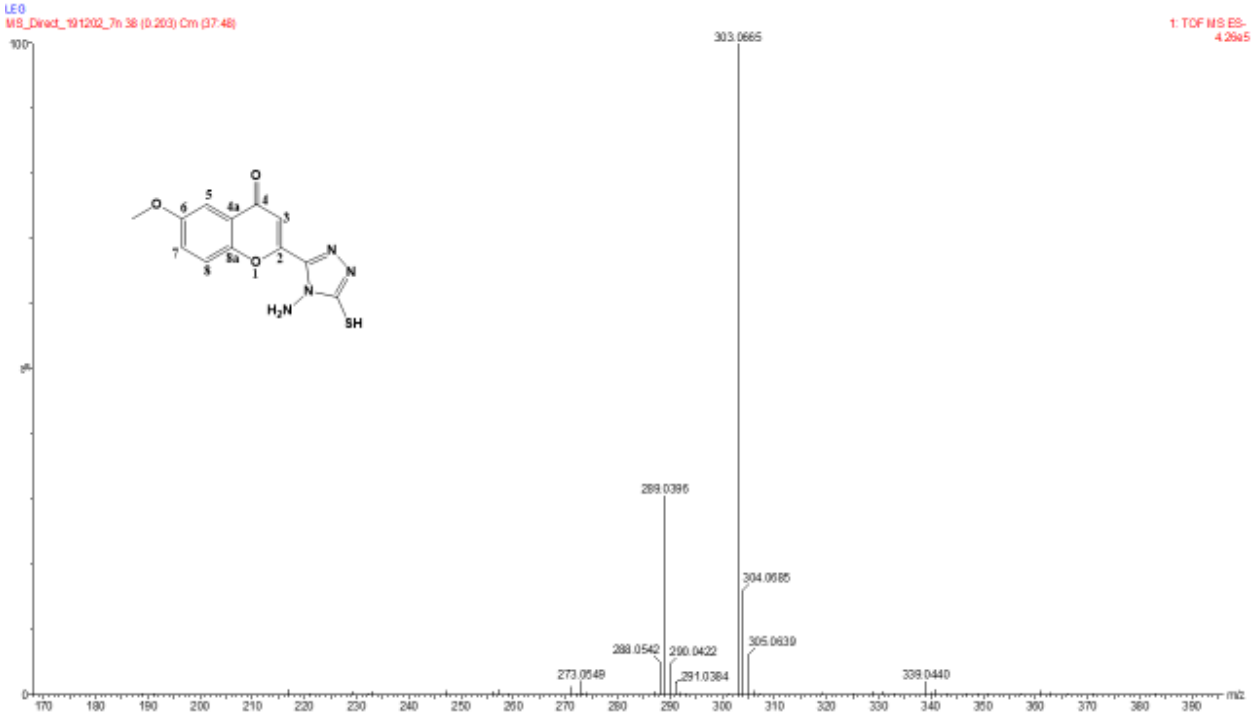
Appendix 14: HRMS spectrum of 6-methoxychromone-2-carbohydrazide **52B**



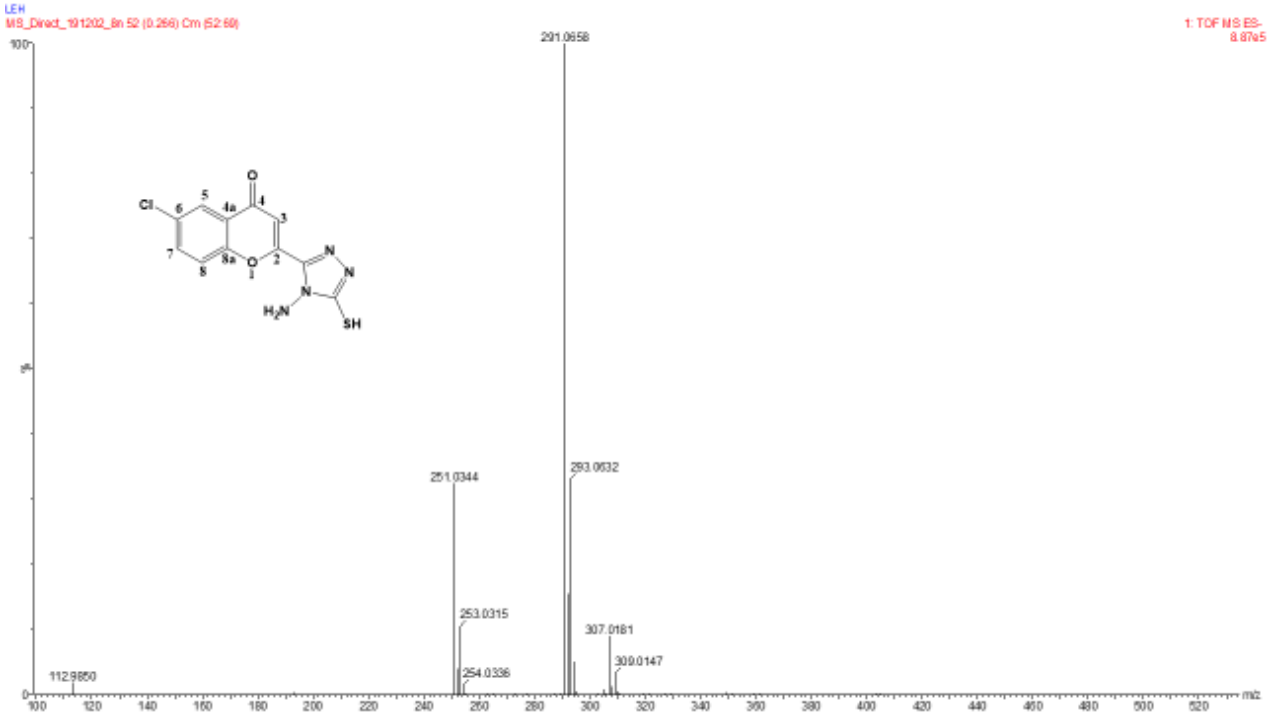
Appendix 15: HRMS spectrum of 6-bromochromone-2-carbohydrazide **52E**



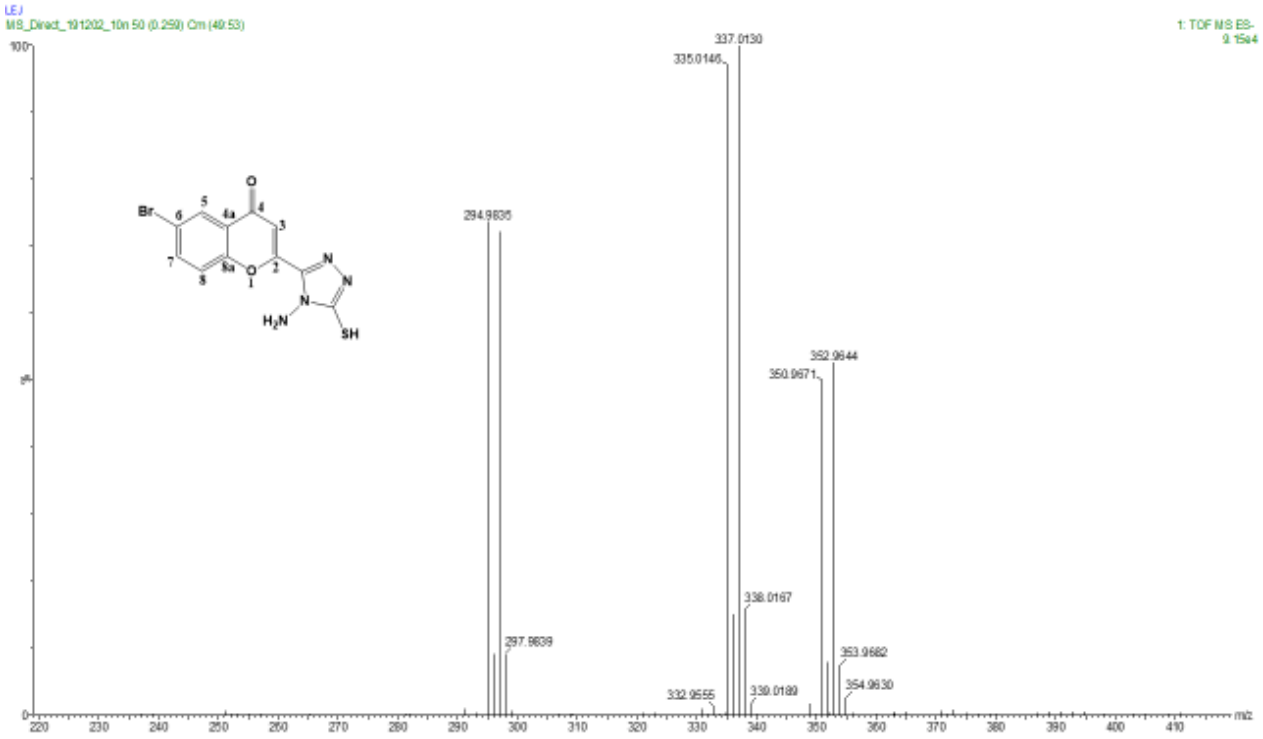
Appendix 16: HRMS spectrum of 6-chlorochromone-2-carbohydrazide **52D**



Appendix 17: HRMS spectrum of 2-(4-amino-5-mercapto-4*H*-1,2,4-triazol-3-yl)-6-methoxychromone **53B**



Appendix 18: HRMS spectrum of 2-(4-amino-5-mercapto-4*H*-1,2,4-triazol-3-yl)-6-chlorochromone **53C**



Appendix 19: HRMS spectrum of 2-(4-amino-5-mercapto-4*H*-1,2,4-triazol-3-yl) 6-bromochromone **53D**