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## M.Sc. Chemistry – IV Sem.
## Project List 2023-24

| Program Name | Program code | Name of the course that include experimental learning through project work/field work/internship | Course code | Year of offering | Name of the student studied course on experimental learning through project work/field work/internship | Register No | Guide | Link to the relevant document |
|---|---|---|---|---|---|---|---|---|
| M Sc General Chemistry | MSCH5 | Fluorescence " Turn Off" Sensing of Iron (III) Ions Utilising Coumarin Based Schiff base Sensor: Experimental and Computational Study | MSCH5 | 2024 | Rahul Siddappa Havaldar | P15KM22S032011 | Dr. S.N. Unki | |
| | | Synthesis and characterisation of some benzofuran derivatives | | 2024 | Akshata A Maranur | P15KM22S032026 | Dr. Manjunatha H.M. | |
| | | Uncatalysed oxidation and determination of thermodynamic parameters of amitriptyline by permanganate in acidic medium | | 2024 | Apeksha Shirashyad | P15KM22S032027 | Dr. S.D. Lamani | |
| | | Green Synthesis of Silver Nanoparticles by aqueous leaf extract of Spinacin oleracea | | 2024 | Sahebagoud Kumatagi | P15KM22S032003 | Dr. AmitTeradale | |
| | | Synthesis and characterisation of some benzofuran derivatives | | 2024 | Danamma Shiragoor | P15KM22S032006 | Dr. Manjunatha H.M. | |
| | | Uncatalysed oxidation and determination of thermodynamic parameters of amitriptyline by permanganate in acidic medium | | 2024 | Goudappagouda Basanagouda Angadageri | P15KM22S032008 | Dr. S.D. Lamani | |
| | | Uncatalysed oxidation and | | 2024 | Kaveri Ogeppa Utagi | P15KM22S032004 | Dr. S.D. Lamani | |

| | | | | | | | | | |
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| | | determination of thermodynamic parameters of amitriptyline by permanganate in acidic medium | | | | | | | |
| | | Fluorescence " Turn Off" Sensing of Iron (III) Ions Utilising Coumarin Based Schiff base Sensor; Experimental and Computational Study | | 2024 | Saraswati Mathapati | P15KM22S032012 | Dr. S.N. Unki | | |
| | | Synthesis and characterisation of Coumarin-4-Carbohydrazide Schiff bases | | 2024 | Rashmi Gurupad Dashyal | P15KM22S032013 | Dr. K. Mahesh Kumar | | |
| | | Green Synthesis of Silver Nanoparticles using Spinacin oleracea aqueous leaf extract | | 2024 | Mahmad Zuber Teradal | P15KM22S032014 | Dr. AmitTeradale | | |
| | | Fluorescence " Turn Off" Sensing of Iron (III) Ions Utilising Coumarin Based Schiff base Sensor: Experimental and Computational Study | | 2024 | Ketan Chinchakhandi | P15KM22S032015 | Dr. S.N. Unki | | |
| | | Synthesis and characterisation of Coumarin-4-Carbohydrazide Schiff bases | | 2024 | Pooja Nikkam | P15KM22S032018 | Dr. K. Mahesh Kumar | | |
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| | | Green Synthesis and Characterisation of Cobalt Oxide Nano paricles by aqueous leaf extract of amaranthus cruentus | | 2024 | Pratiksha Managuli | P15KM22S032022 | Dr. AmitTeradale | | |
| | | Fluorescence " Turn Off" Sensing of Iron (III) Ions Utilising Coumarin Based Schiff base Sensor: Experimental and Computational Study | | 2024 | Somanath Banagar | P15KM22S032028 | Dr. S.N. Unki | | |
| | | Synthesis and characterisation of some benzofuran derivatives | | 2024 | Keshav Chatter | P15KM22S032005 | Dr. Manjunatha H.M. | | |
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| | | by aqueous leaf extract of Spinacin oleracea | | | | | | | |
| | | Synthesis and characterisation of Coumarin-4-Carbohydrazide Schiff bases | | 2024 | Chandrashekhar N Konnurmath | P15KM22S032016 | Dr. K. Mahesh Kumar | |
| | | Synthesis and characterisation of some benzofuran derivatives | | 2024 | Rachappa Siddappa Angadi | P15KM22S032017 | Dr. Manjunatha H.M. | |
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| | | Synthesis and characterisation of Coumarin-4-Carbohydrazide Schiff bases | | 2024 | Swatishree | P15KM22S032030 | Dr. K. Mahesh Kumar | |
| | | Green Synthesis and Characterisation of Cobalt Oxide Nano paricles by aqueous leaf extract of amaranthus cruentus | | 2024 | Lakshmi Mallikarjun Patil | P15KM22S032031 | Dr. AmitTeradale | |

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VIDYASANGAMA

# SYNTHESIS AND CHARACTERIZATION OF COUMARIN-4-CARBOHYDRAZIDE SCHIFF BASES

*A DISSERTATION SUBMITTED TO THE RANI CHANNAMMA UNIVERSITY, BELAGVI IN PARTIAL FULFILLMENT OF THE REQUIREMENT FOR THE DEGREE OF*

## MASTER OF SCIENCE

IN

## GENERAL CHEMISTRY


By

SWATISHREE

(Reg. No. P15KM22S032030)

Under the Guidance of

**Dr. Mahesh Kumar** M. Sc., Ph. D.

Assistant Professor

Department of Chemistry

**B. L. D. E. A.'s SB ARTS & KCP SCIENCE COLLEGE, VIJAYAPUR**


**October-2024**

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# CERTIFICATE

This is to certify that the dissertation entitled **"Synthesis and Characterization of Coumarin-4-carbohydrazide Schiff Bases"** is a record of bonafide research carried out by **Ms. Swatishree (Reg. No. P15KM22S032030 )** under my guidance and supervision during the period 2023-2024 at the Department of P.G studies in Chemistry, **B. L. D. E. A's S. B. Arts & K. C. P. Science college, Vijayapur** and submitted to Rani Channamma University, Belagavi in partial fulfillment of the requirements for the award of **Master of Science in General Chemistry.**

**Place: VIJAYAPUR** **Signature of the Supervisor**

**Date: 26-10-2024**

**Signature of Co-ordinator** **Signature of Principal**

**Examiners:** 1)

# DECLARATION

*I, **Ms. Swatishree** (Reg. No. P15KM22S032030) declare that the thesis **"Synthesis and Characterization of Coumarin-4-carbohydrazide Schiff Bases"** prepared by me under the guidance and supervision of **Dr. K. Mahesh Kumar**, Assistant Professor, during this period of my Project Work from 2023-2024 at P. G. Department of Studies in Chemistry, B.L.D.E.A's S.B. Arts and K.C.P. Science College, Vijayapur contains no material which has been accepted for the award of any other degree or diploma in any University and that to the best of my knowledge and belief, contains no material previously published or written by any person, except when due reference is made is in the text of the thesis.*

**Place: VIJAYAPUR**

**Date: 26-10-2024**

**Signature of the Candidate**

**(Ms. Swatishree)**

*Dedicated*

*to*

*My Beloved Parents*

*&*

*My Teachers and Friends*

# Acknowledgement

*I avail this opportunity to express my heartfelt sincere gratitude and humble thanks to all who knowingly or unknowingly helped me during my project work.*

*The first most important personality, who is the care of my work, is my esteemed guru and guide,* ***Dr. K. Mahesh Kumar,*** *Department of Chemistry, S.B. Arts and K.C.P. Science College, Bijapur under Rani Channamma University, Belegavi, for introducing me to the research field which made my research study a pleasant and memorable experience.*

*I am extremely thankful to* ***Principal Dr. R. M. Mirdhe, Vice-principal Shri. Dr. Anil Naik., P.G Department of Studies in Chemistry Co-ordinater Dr. S. N. Unki and Staff Dr. Manjunath Hariharmath, Dr. Amit Teradale, Dr. S. D. Lamani, Dr. Anilkumar Patil, Department of studies in Chemistry, S.B. Arts and K.C.P. Science College, Vijayapur*** *for providing the necessary facilities. I am grateful to all the teaching faculty of the Chemistry department for their valuable discussion and suggestions during my project work.*

*Friends are the integral part of my one's life. How can I be exception? I experienced true friendship from Chandrashekhar, Shreepadraj, Rashmi, Pooja and my all friends for their affection, inspiration and constant encouragement throughout my project work.*

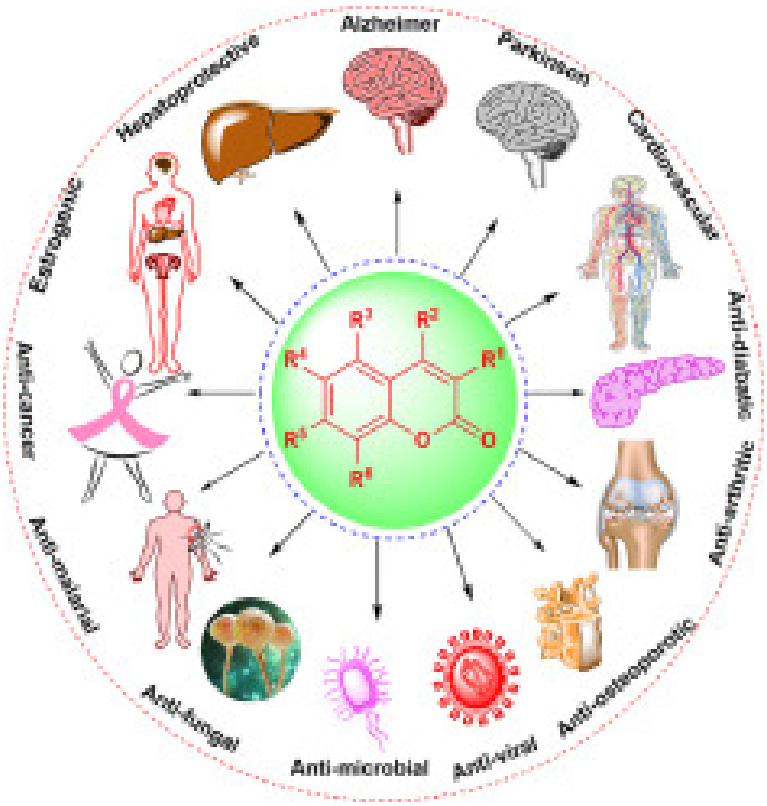
*Finally, I dedicated my work to my dearest and beloved mother and father. I am fortunate to have such lovable parents, whose dreams are yet to be fulfilled by me. I must remember my loving parents, also my brothers for their constant support, love and affection throughout my career.*

*Last but not least, I wish to thank all those friends, colleagues and well-wishers who helped me directly or indirectly in my whole educational.*

**Ms. *Swatishree***

# CHAPTER-1:

# INTRODUCTION TO COUMARINS



## 1. Exposure Data

Coumarins are very much present in nature and find their applications as fragrances, pharmaceuticals and agrochemicals. They are originally discovered in plants from coumarouna odorata. Coumarin was isolated by Vogel[1] from Tonka bean (Dipteryx odorata wild) in 1820.

### 1.1 Chemical and physical data

#### 1.1.1 *Nomenclature*

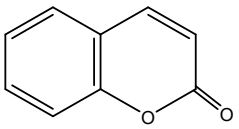
***Chem. Abstr. Serv. Reg. No.*:** 91-64-5

***Chem. Abstr. Name*:** 2*H*-1-Benzopyran-2-one

***IUPAC Systematic Name*:** Coumarin

***Synonyms*:** 1,2-Benzopyrone; 5,6-benzo-2-pyrone; benzo-α-pyrone; *cis-ortho*-coumarinic acid lactone; coumarinic anhydride; *ortho*-hydroxycinnamic acid lactone

#### 1.1.2 *Structural and molecular formulae and relative molecular mass*



$C_9H_6O_2$ **1** Relative molecular mass: 146.15

#### 1.1.3 *Chemical and physical properties of the pure substance*

**(*a*) *Description*:** Orthorhombic, rectangular plates; pleasant, fragrant odour resembling that of vanilla beans; burning taste[2].

**(*b*) *Boiling-point*[3]:** 301.7 °C

**(*c*) *Melting-point*[3]:** 71 °C

**(*d*) *Density*[3]:** 0.935 g/cm3 at 20 °C

**(*e*) *Spectroscopy*:**

**IR-Spectra:**

The IR spectrum of coumarin was reported by Murthi and Sheshadri[4]. The parent coumarin shows lactone carbonyl at 1705 $cm^{-1}$, $\nu_{C=C}$ at 1608 $cm^{-1}$, 1450 $cm^{-1}$ and $\nu_{C-O-C}$ at 1254 $cm^{-1}$.

**UV-Spectra**:

The UV spectra of coumarins and their methyl derivatives were reported by Ganguly and Bagchi.[5] The introduction of methyl group in various positions does not change the nature of the spectrum to a greater extant. The λ max and ε values of coumarins are 273 nm (40,368) & 309 nm (37,449).

**PMR-Spectra:**

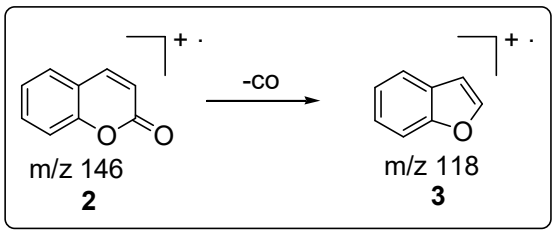
In the $^{1}H$ NMR spectrum of coumarin[6], the signal due to protons of C3 and C4 appears in the region of 6.45 δ ppm and 7.80 δ ppm with coupling constants of 9.8Hz. These values are typical of *cis* alkene rather than an aryl ring[4].

Finally the $^{13}C$ NMR spectra of coumarins[7] are consistent with an essentially aliphatic heterocyclic ring. The chemical shifts of C2, C3 and C4 in coumarin remarkably close to the values for the corresponding carbons in α-pyrone and are given below

| Compound | C2 | C3 | C4 |
|---|---|---|---|
| α-Pyrone | 162.0 | 116.7 | 144.3 |
| Coumarin | 160.4 | 116.4 | 143.4 |

**Mass spectra:**

The electron impact on coumarins has been studied by Barnes et al.[8] The molecular ion peak and fragmentation shows transient formation of Benz furan **3.**



**Crystal structure:**

The Crystal structure of coumarin was first reported by S.Ramswamy[9] in 1941. Coumarin crystals are in orthorhombic system, it has space group $Pca^z$ with Z=4.

**(*f*) *Solubility*[2,10,11]:** Slightly soluble in water (100 mg/L at 25 °C) and ethanol; very soluble in chloroform, diethyl ether and pyridine

**(*g*) *Volatility*[11]:** Vapour pressure, 0.13 kPa at 106 °C

**(*h*) *Octanol/water partition coefficient (P)*[11]:** log P, 1.39.

**(*i*) *Conversion factor*[11]:** $mg/m^3 = 5.98 \times ppm$

### 1.1.4 *Technical products and impurities*

Coumarin is commercially available with a minimum purity[12] of 99%. Coumarin is usually sold in the form of colourless shiny leaflets or rhombic crystals[13].

### 1.1.5 *Analysis*

Coumarin can be determined in vanilla extract by a photometric method, reading the absorbance or transmittance at 490 nm, and comparing against a standard[14].

## 1.2 Occurrence

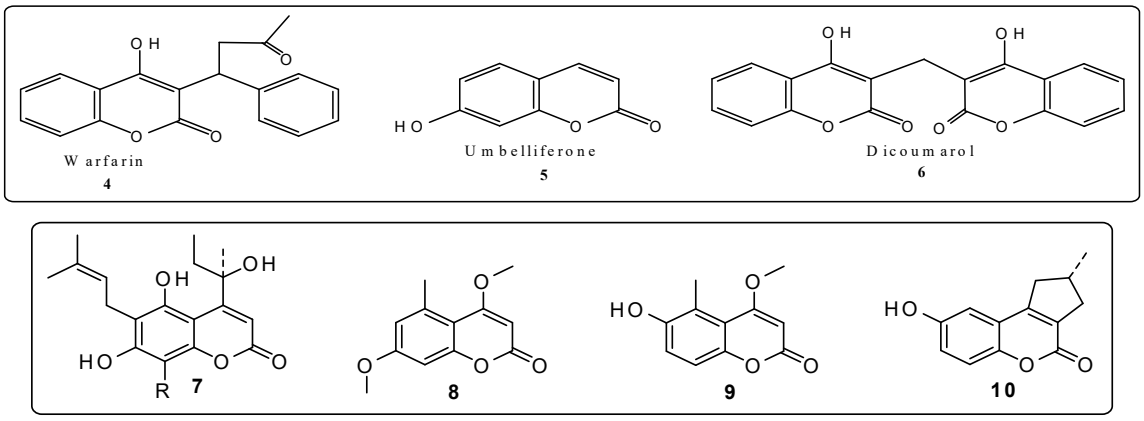
### 1.2.1 *Natural occurrence*

Coumarin was first isolated by Vogel in 1820 by extraction from tonka beans[15] (*Dipteryx odorata*)]. It was subsequently identified in a large number of plants belonging to many different families. Its better known occurrences are in sweet clover (*Melilotus alba* and *M. officinalis*), sweet woodruff (*Asperula odorata*), vanilla leaf (*Trilisa odoratissima*), vanilla beans *(Vanilla planifolia)*, cassia (*Cinnamorum cassia*), lavender (*Lavendula officinalis*) and balsam of Peru (*Myroxylon pereirae*)[2,13,16,17]. Coumarin has been isolated from legumes, orchids, grasses and citrus fruits[16]. It is found at particularly high levels in some essential oils, such as cinnamon leaf and bark oil, cassia leaf oil and lavender oil[18]. A broad spectrum of coumarin derivatives (present both in the free state and as glucosides) have also been found

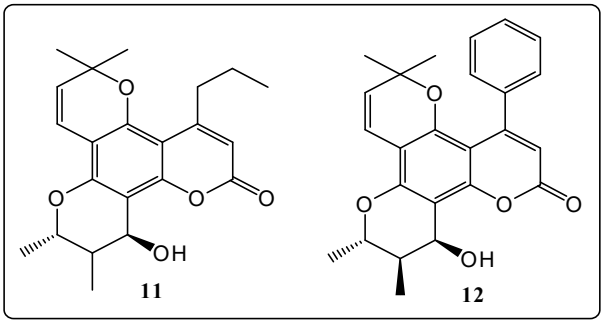
in many plants; to date at least 1300 have been identified, principally as secondary metabolites in green plants[19].

Warfarin **4** is a naturally occurring compound containing the 4-hydroxy coumarin moiety. It has been isolated from woodruff as well as from lavender and is used to prevent clotting of blood in the veins, lungs or heart.[20,21] Another well-known natural compound containing the coumarin nucleus is 7-hydroxycoumarin, also known as Umbelliferone **5**,[22] which is found in a variety of plants, such as carrots, coriander and garden angelica. It has been used as a sunscreen, a fluorescence indicator and as a dye indicator.[22,23,24] Dicoumarol **6** is another natural occurring compound containing the coumarin nucleus,

and is known for causing sweet clover disease in cattle.[25,26] It has been isolated from sweet clover hay and used as an anticoagulant.[27]

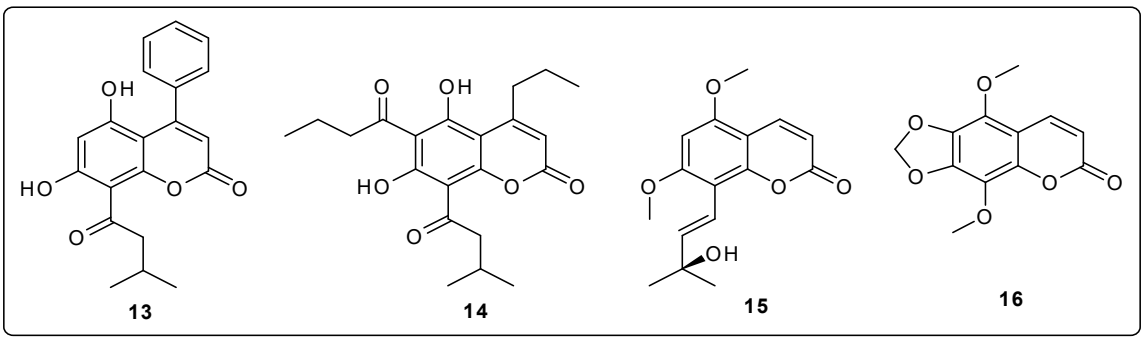



Compound 7 is isolated from the bark of *Kayea assamica* (Myanmar) and found to exhibit cytotoxic-activity against a panel of human cancer lines and Anti-malarial activity against chloroquine resistant *plasmodium falciparium*[28]. Compound **8**, a Siderin containing extracts of *Toona ciliate* has shown the promising Anti-bacterial and moderate Anti-fungal activity[29]. Compounds **9** and **10** are the methanol extracts obtained from the rhizomes of Japanese plant *Glaucidium Palmatum*. Amongst these compounds, compound **10** was identified as a microtubule stabilizing agent with a potent Anti-mitotic activity inhibiting KB cell proliferation [30].




A number of Brazilian medicinal plants containing coumarin **1** as the major constituent have been screened for Anti-nociceptive, Anti-inflammatory and Bronchodilator activities. The results have shown that the hydrochloric extracts of *J. pectoralis* and *P.polygaliflours* were found to be most promising bronchodilators in isolated *guineapig tracea* [31]. Recently the production of coumarin **1** and Umbelliferon **5** was induced by cupric chloride treated leaves of Conium macaluatam and these phytoalexins inhibited the growth of pathogenic fungi, viz *alternaria spp. Bipolaris spp.* And *fusarium*[32] over a hundred of chinese medicinal plants containing coumarins and related oxygen heterocycles have been screened for their anti-oxidant activity, in view of their medicinal value as anti-cancer agents[33].

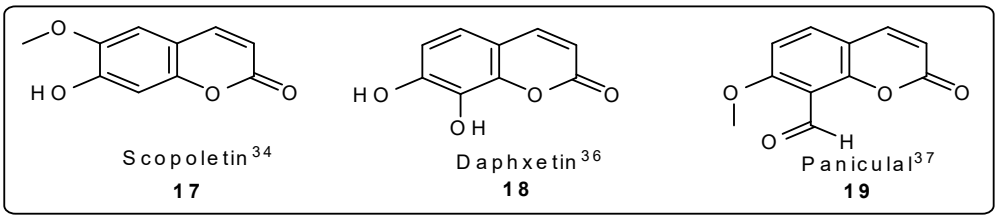
The title compounds **11** and **12** were isolated from the seeds of *calophyllum cerasiferaum* and *calophyllum ionophyllum* and were found to exhibit anti-HIV activity [34]

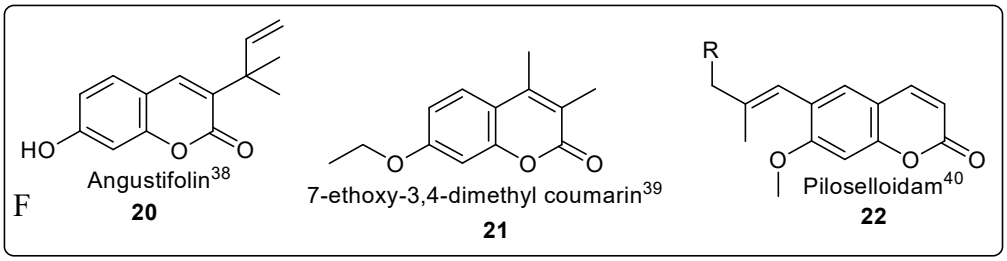


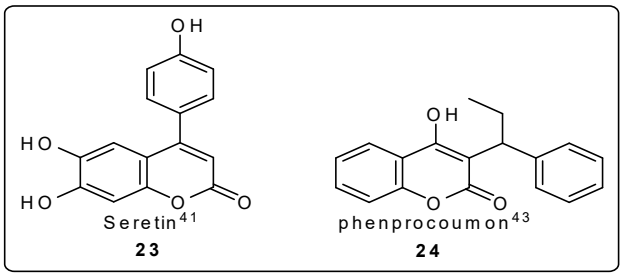

The compounds **13** and **14** isolated from ethyl acetate extracts of fruits and stem bark of *calophyllum dispar* have been reported to exhibit significant cytotoxicity against KB cell lines[35] the 5.7-dimethoxy coumarin **15** was extracted from the roots of the Kenyan plant *toddalia asiatica* was traditionally used for the treatment of malarial disorders and as a novel

antispasmodic compound[36] the furano coumarin **16** isolated from the ethanol extracts from fruits of *cnidium monneri* (china) was found to exhibit anti-oxidative activity in both lipid peroxidation and haemolysis assays[37].

**List of some of the biologically active and naturally occurring coumarins**



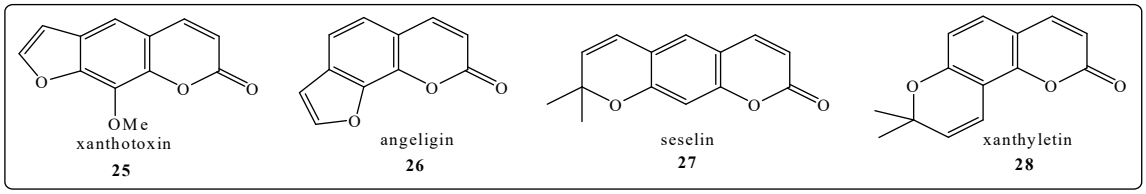




Further investigation led to the discovery of compounds containing a 3-substituted-4-hydroxycoumarin moiety, such as warfarin **4**[42] and phenprocoumon **24**[43]. However, compound **4** is weakly active against HIV-PR. The phenprocoumon **24**, however, is more active against the protease enzyme with an inhibition potential of 1 μM. The hydroxyl group on phenprocoumon **24** has the potential to hydrogen-bond with the catalytic aspartic residues, while the carbonyl group of the coumarin nucleus hydrogen-bonds with the Ile- 50 residue of protease enzyme.

Coumarins can be divided into four sub-types: i) simple coumarins which are hydroxylated, alkoxylated or alkylated on the benzene ring (*e.g.* umbelliferone **5**);[44,45] ii) Furanocoumarins, which contain a five-membered furan ring attached to the coumarin moiety

and which are sub-divided into the linear furanocoumarins (*e.g.* Xanthotoxin **25**) and the angular furanocoumarins (*e.g.* Angeligin **26**);[44,46] iii) pyranocoumarins, containing a six-membered ring attached to the coumarin moiety (*e.g.* Seselin **27** and Xanthyletin **28**);[44,47] and iv) coumarins with substituents in the pyrone ring (*e.g.* warfarin **4**).[48]



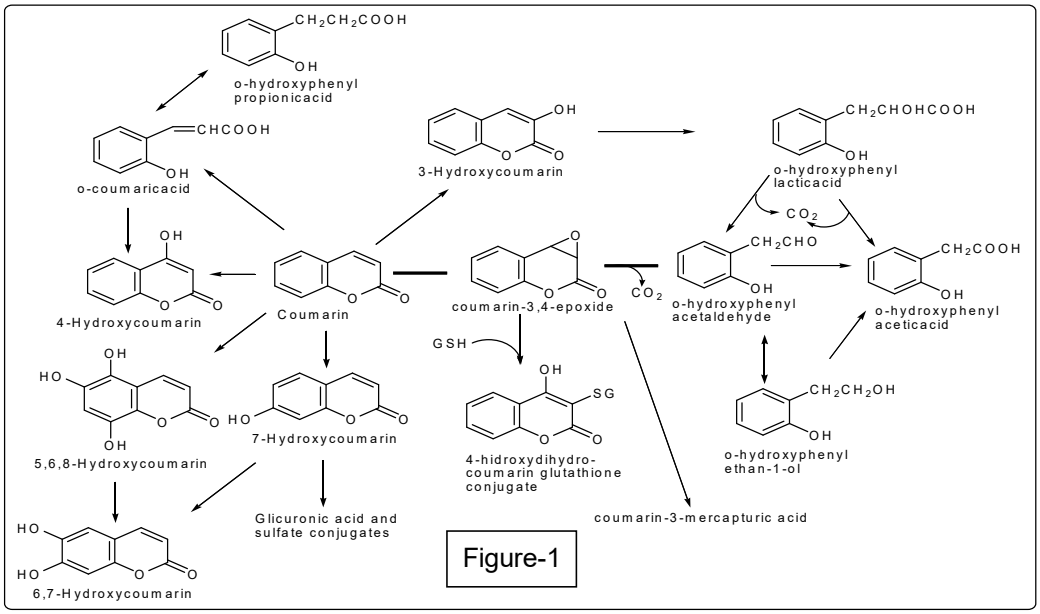

## 1.2.2 METABOLISM OF COUMARINS IN THE BIOLOGICAL SYSTEMS:

Coumarin is rapidly and extensively absorbed after topical or oral administration to human subjects. It undergoes very extensive metabolism along two major pathways, 7-hydroxylation and ring-opening to *ortho*-hydroxyphenylacetaldehyde. There are numerous minor metabolites, many of which are secondary products from the primary metabolites. The relative extent of these two major pathways is highly variable between species. Ring-opening predominates in rodents, while 7-hydroxylation is particularly evident in humans.

The absorption, distribution, metabolism and excretion of coumarin in humans have been reviewed[18,49,50]. Toxicokinetic studies in humans have demonstrated that coumarin is rapidly absorbed from the gastrointestinal tract after oral administration and extensively metabolized by the liver in the first pass, with only 2–6% reaching the systemic circulation intact[51,52,53]. The elimination of coumarin from the systemic circulation is rapid, the half-lives following intravenous doses of 0.125, 0.2 and 0.25 mg/kg bw being 1.82, 1.46 and 1.49 h [109, 88 and 89 min], respectively[54]. Coumarin is also extensively absorbed after dermal application. In one study with human subjects, some 60% of a 2.0-mg dose applied for 6 h was absorbed[19]. The percutaneous absorption of coumarin has also been demonstrated *in vitro* with human skin[55,56].

The rapid excretion of coumarin, primarily as 7-hydroxycoumarin conjugates, in the urine of human subjects given coumarin orally suggests that there is little or no biliary excretion of coumarin metabolites in humans[57,58,59,60,61]. Coumarin exhibits marked species differences in its metabolism[18,49]. The major primary pathways of coumarin metabolism are 7-hydroxylation or metabolism of the lactone ring by ring opening and cleavage at carbon atom 2 to yield carbon dioxide. The first step in the latter pathway is the formation of the unstable coumarin 3,4-epoxide which degrades spontaneously to form *ortho*-hydroxyphenylacetaldehyde and may be subsequently converted to *ortho*-hydroxyphenylethanol and *ortho*-hydroxyphenylacetic acid. Coumarin may also be metabolized by hydroxylation to yield 3-, 4-, 5-, 6- or 8-hydroxycoumarin and 6,7-dihydroxycoumarin and, by opening of the lactone ring, to yield *ortho*-coumaric acid (*ortho*-hydroxyphenylcinnamic acid) and *ortho*-hydroxyphenylpropionic acid[62,63,64,65,66]. The pathways of coumarin metabolism are shown in Figure 1.

**Metabolic pathways of Coumarin in animals and humans**




The major pathway of coumarin metabolism in most human subjects is 7-hydroxylation to form 7-hydroxycoumarin, which is excreted in the urine as both glucuronic

acid and sulfate conjugates. Coumarin 7-hydroxylation activity exhibits a Gaussian distribution in Caucasian populations[58,60], but some individuals are deficient in this activity. Hadidi *et al.*[60] gave members of a family 2 mg coumarin orally and collected their urine for 8 h. One subject excreted < 0.03% of the dose as 7-hydroxycoumarin and 50% as *ortho*-hydroxyphenylacetic acid, but three others excreted mainly 7-hydroxycoumarin (> 41% of dose) and 4–10% as *ortho*-hydroxyphenylacetic acid. Oscarson *et al.*[68] refer to two individuals (among a population of two hundred) who were totally deficient in 7-hydroxycoumarin excretion after an oral dose of 5 mg coumarin. CYP2A6 (cytochrome P450 2A6) has been purified from human liver and CYP2A6 cDNA expression systems are available. Many studies have demonstrated marked interindividual variation in the levels of hepatic CYP2A6 protein, mRNA and associated microsomal coumarin 7-hydroxylase activity[18,50]. The role of CYP2A6 in the metabolism of coumarin by human liver microsomes has been confirmed by Sai *et al.*[69], who found that a monoclonal antibody to CYP2A6 inhibited coumarin 7-hydroxylation by more than 94%. The marked interindividual variation in coumarin metabolism to 7-hydroxycoumarin has led to studies to evaluate whether a genetic polymorphism exists in human CYP2A6.

The occurrence of variant alleles in the human *CYP2A6* gene was shown by Fernandez-Salguero *et al.*[70]; these were designated *CYP2A6*1* (wild type), *CYP2A6*2* and *CYP2A6*3*. *CYP2A6*2* has a point mutation in codon 160 and the resulting protein product is unable to 7-hydroxylate coumarin[67,70]. The functional significance of the rare *CYP2A6*3* allele is uncertain. The population frequency of these mutant alleles is uncertain at present; initial claims that the incidence of the *CYP2A6*2* allele is 4–17% of European populations[70] have been challenged by Oscarson *et al.*[68], who found the incidence to be 1–3%. These authors highlighted methodological uncertainties in polymerase chain reaction based genotyping procedures. Establishment of the significance of the genetic polymorphism in

*CYP2A6* must await definitive genotyping and phenotyping procedures. While 7-hydroxylation is the major metabolic pathway of coumarin in most subjects, humans also convert coumarin to *ortho*-hydroxyphenylacetic acid. Shilling *et al*. (1969) reported that after an oral dose of 200 mg coumarin per subject, while 7-hydroxycoumarin accounted for 79% of the excreted dose (range, 68–92%), a further 4% of the dose (range, 1–6%) was present in the first 24-h urine as *ortho*-hydroxyphenylacetic acid.

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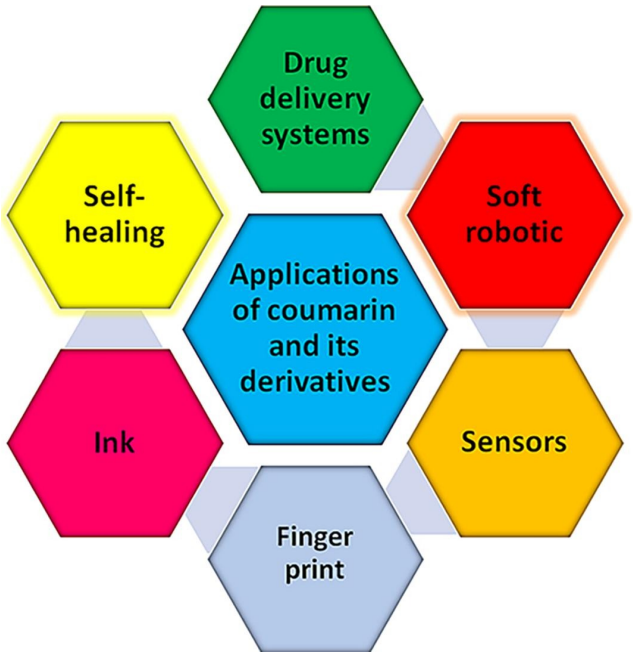
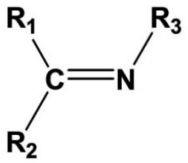
# Chapter-2:

# SYNTHESIS AND CHARACTERIZATION OF COUMARIN-4-CARBOHYDRAZIDE SCHIFF BASES



## 2.1 INTRODUCTION

Schiff bases are generally excellent chelating agents, especially when a functional group like OH or SH is present close to the azomethine group so as to form a five or six membered ring with the metal ion verstality of Schiff base ligands and biological, analytical and industrial applications of their complexes make further investigations in this area highly desirable. Nowadays, the research field dealing with Schiff base coordination chemistry has expanded enormously. The importance of Schiff base complexes for bioinorganic chemistry, catalysis and material science, separation and encapsulation processes, and formation of compounds with unusual properties and structures has been recognized and reviewed. Schiff bases resulted from aromatic aldehydes ortho-substituted with a hydroxyl group have initially arouse the researchers interest because of their ability to act as bidentante ligands for transitional metal ions [1]. Later, in studies concerning quantitative structure antitumor activity relationship of a series of Schiff bases derived from variously substituted aromatic amines and aldehydes, it has been shown that azomethines from salicylaldehydes gave the best correlation. Schiff bases of salicylaldehydes have also been reported as plant growth regulators and antimicrobian or antimycotic activity. Schiff bases also show some analytical applications. Schiff Bases are characterized by the N=CH-(imine) which imports in elucidating the mechanism of transamination and rasemination reaction in biological system. Schiff bases are active against a wide range of organisms for example: Candida albicans, Esherichia coli, Stapylococas aureus, Baccillus polmxa, Trychophyton gypseum, Mycobacteria, Erysiphe gramminis and Plasmopora viticola. A large number of different [2].




**$R_1$, $R_2$ and / or $R_3$=alkyl or aryl**

**Figure 1. Schiff base**

Schiff base ligands have been used as cation carriers in potentiometric sensors as they have shown excellent selectivity, sensitivity, and stability for specific metal ions such as Ag(2), Al(3), Co(2), Cu(2), Gd(3), Hg(2), Ni(2), Pb(2), Y(3), and Zn. Schiff bases have been studied for their important properties in catalysis. They show catalytic activity in hydrogenation of olefins.

They find applications in biomimetic catalytic reactions. An interesting application of Schiff bases is their use as an effective corrosion inhibitor, which is based on their ability to spontaneously form a mono layer on the surface to be protected. Many commercial inhibitors include aldehydes or amines, but presumably due to the C=N bond the Schiff bases function more efficiently in many cases. The principal interaction between the inhibitor and the metal surface is chemisorptions. The inhibitor molecule showed have centers capable of forming bonds with the metal surface by election transfer. In such cases the metal acts as an electrophile and the inhibitor acts as a lewis base. Nucleophilic centers, such as oxygen and nitrogen atoms, of the protective compound have free electron pairs which are readily available for sharing. Together with the atoms of the benzene rings they create multiple absorption sites for the inhibitor thus enabling stable monolayer formation. Imines also have biological importance. An imine linkage between the chemistry of vision [3]. Vitamins are also called coenzymes, meaning that they are to the functioning of many enzymes, which are large proteins that catalyze chemical changes in cell. An example of a biologically important aldehyde is pyridoxal phosphate, which is the active form of the vitamin B6. Vitamin B6 serves as a coenzyme, by forming an imine with an amino acid grouping an enzyme. The coenzyme, bound to the enzyme, is involved in transamination reaction, the transfer of the amino group from one amino acid to another, which is important in the metabolism and the biosynthesis of amino acids. In the last step, enzyme-catalyzed hydrolysis cleaves the imine to pyridoxol and the modified amino acid. Schiff bases have been reported in their biological properties, such as antibacterial, antifungal activities. Their metal complexes have been widely studied because they have anticancer and herbicidial applications. They serve as models for biologically important species.

**Synthesis of Schiff bases:**

The first preparation of imines was reported in the 19th century by Schiff (1864). Since then a variety of methods for the synthesis of imines have been described [3]. The classical synthesis reported by Schiff involves the condensation of a carbonyl compound with an amine under azeotropic distillation [4]. Molecular sieves are then used to completely remove water formed in the system [5]. In the 1990s an in situ method for water elimination was developed, using dehydrating solvents such as tetramethyl orthosilicate or trimethyl orthoformate [6,7]. In 2004, Chakraborti et al. [8] demonstrated that the efficiency of these methods is dependent on the use of highly electrophilic carbonyl compounds and strongly nucleophilic amines. They proposed as

an alternative the use of substances that function as Bronsted-Lowry or Lewis acids to activate the carbonyl group of aldehydes, catalyze the nucleophilic attack by amines, and dehydrate the system, eliminating water as the final step [8]. Examples of Bronsted-Lowry or lewis acids used for the synthesis of Schiff bases include $ZnCl_2$, $TiCl_4$, $MgSO_4$-PPTS, $Ti(OR)_4$, alumina, $H_2SO_4$, $NaHCO_3$, $MgSO_4$, $Mg(ClO_4)_2$, $H_3CCOOH$, $Er(OTf)_3$, $P_2O_5/Al_2O_3$, HCl [8–20]. In the past 12 years a number of innovations and new techniques have been reported, including solvent-free/clay/microwave irradiation, solid-state synthesis, K-10/microwave, water suspension medium, [bmim]$BF_4$/molecular sieves, infrared irradiation/no solvent, $NaHSO_4.SiO_2$/microwave/solventfree, solvent-free/CaO/microwave, and silica/ultrasound irradiation [21–29]. Among these innovations, microwave irradiation has been extensively used due to its operational simplicity, enhanced reaction rates, and great selectivity [28]. The use of microwave irradiation commenced with the independent studies of Rousell and Majetich groups [30,31]. Microwave irradiation is less environmentally problematic than other methods because it abolishes the excessive use of aromatic solvents and the Dean-Stark apparatus for azeotropic removal of water. Another feature of this technique is that the reactions achieve high efficiency in a shorter period of time.

**2.2 Synthesis of Coumarin Schiff Bases:**

Coumarins are therapeutically active members of the benzopyran-2-one family. Coumarins are extensively dispersed in nature and can be found in both naturally occurring and synthetic medicinally active compounds. In recent years there has been considerable growth in the chemistry of coumarins as a keystone for the design and development of a considerable number of compounds [32, 33]. Now a days, coumarin and its derivatives, especially Schiff bases derived from coumarins, belong to the most active classes of compounds and possess a wide spectrum of biological activity [34, 35]. On the other hand, metal complexes derived from Schiff base ligands of coumarin show tremendous potential in numerous fields such as fluorescent probes, optical brighteners, antioxidants, antimicrobials, anthelmintics, hypotensive, and inhibitors of platelet aggregation and cytotoxic activity [36-43].

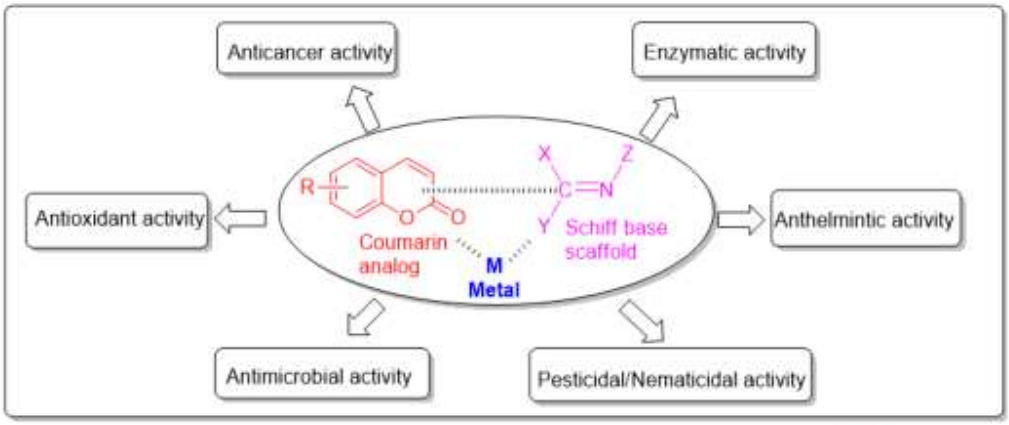
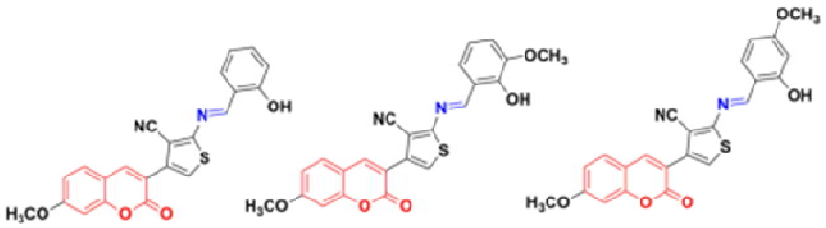


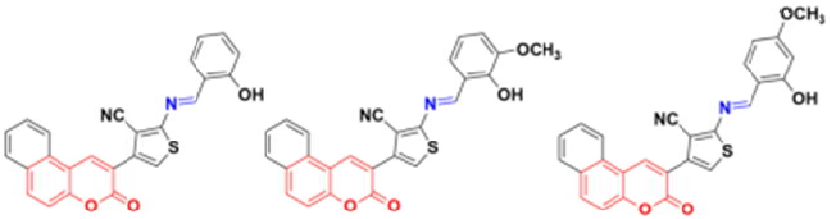

**Figure 2.** Important biological activities of the coumarin-derived imine–metal complexes.

A similar series of nine coumarin-imine hybrids (i–ix) (**Fig. 3**) was synthesized and their antiproliferative profile evaluated against fibroblast cell lines and A549 cancer cell line [44]. The percentage of viable cells was determined at different concentrations in the range of 12.5 to 200 μg $mL^{-1}$. In terms of the WST-1 results, the concentrations of the compounds did not have a prominent effect on cell mortality in the cell line. For the fibroblast cells, the results were significant for only i and ii. However, for the other samples (iii–ix), increasing the concentrations of the compounds caused an increase in cell death.

i ii iii



iv v vi




vii viii ix

**Fig. 3** Chemical structures of coumarin-imine hybrids i-ix.

A series of coumarin–hydrazone hybrids was designed and evaluated for their anticancer activities against four cancer cell lines [45]. Among them, compound x (**Fig. 4**) showed the most potency with IC50 = 2.9 ± 0.4, 5.3 ± 1.1, 7.2 ± 0.9, and 9.1 ± 1.2 μM against the HL-60, KE-37, K-562, and MDAMB-231 cell lines, respectively.

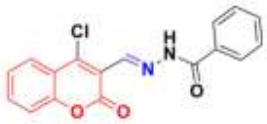
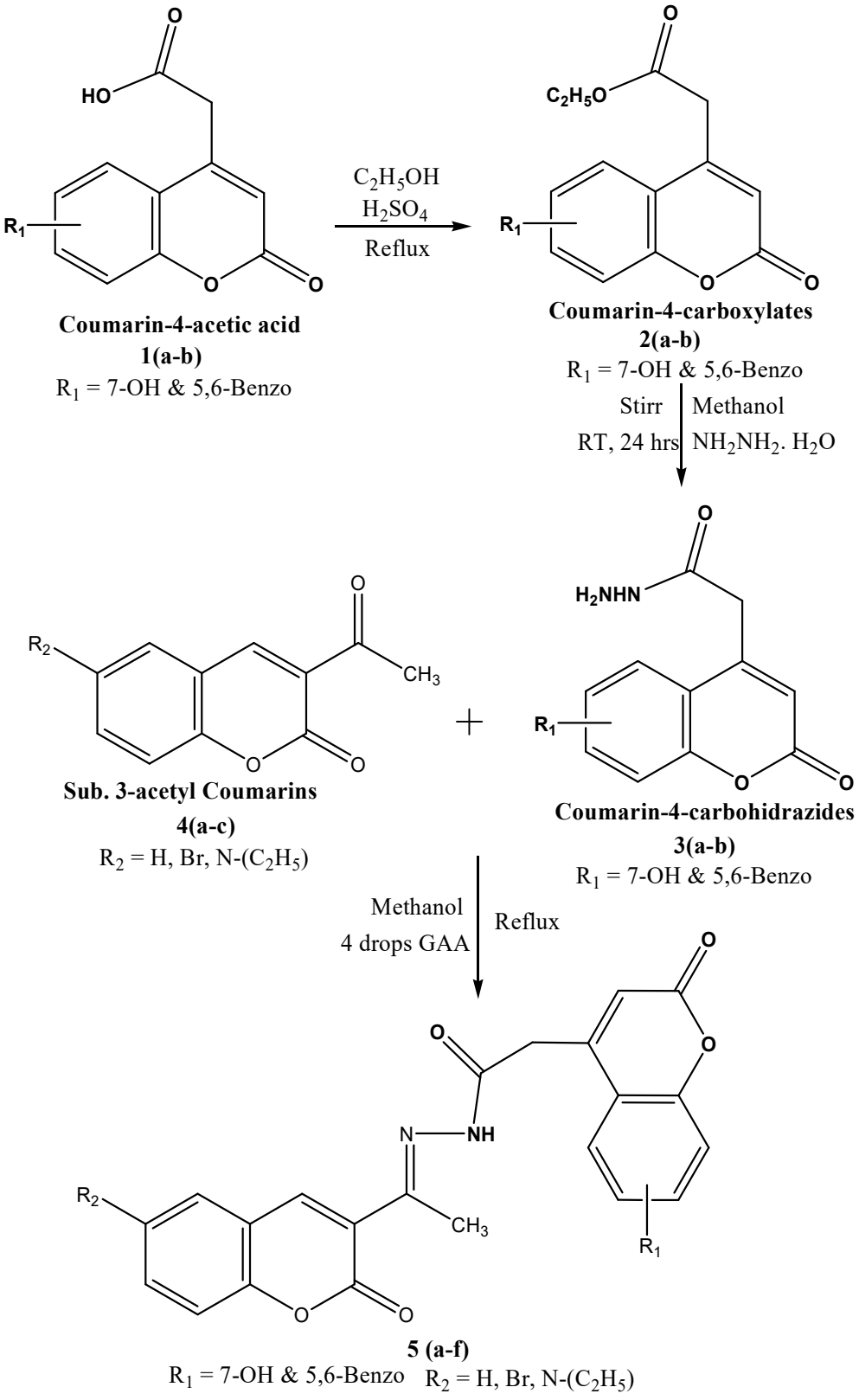



**Fig. 4** Compound x

Hence the synthesis, structural identification, and biological evaluation of new derivatives of coumarin derived Schiff bases continually pique research interest across the world.

## 2.3 PRESENT WORK

The research work carried out during the present investigation has been described in **Scheme-1.** They were synthesized the compounds in methanol media using catalytic amount of glacial acetic acid.




**Scheme-1: Synthesis of Coumarin-4-carbohydrazide Schiff Bases**

## 2.4 EXPERIMENTAL SECTION:

This section deals with the preparation of following compounds.

**1. Preparation of coumarin-4-carbohydrazides 3(a-b):**

**i. Preparation of coumarin-4-acetic acids 1(a-b):**

On heating anhydrous citric acid and conc. $H_2SO_4$ in a water bath followed by cooling gave clear solution. Appropriate phenol or naphthol was added to it along with $H_2SO_4$. The reaction mixture was then kept for 48 hrs at room temperature and then poured into ice-water due to which solid compound got separated and which was then crystallized from appropriate solvent to get coumarin-4-acetic acids [46].

**ii. Preparation of coumarin-4- carboxylates 2(a-b):**

Coumarin-4-acetic acids [47] (50 mmol) were converted into their methyl/ethyl esters by refluxing in dry methanol/ethanol (100 mL) with catalytic quantity of sulphuric acid by Fischer esterification. The reaction mixture was refluxed for 12 h, poured in ice cold water thus the precipitate formed was filtered off, washed with 5% NaHC03 and cold water. The products were dried and recrystallised from ethanol or acetic acid.

**iii. Preparation of coumarin-4-carbohydrazides 3(a-b):**

The coumarin-4-carboxylates (50 mmol) were dissolved in a solution containing methanol (120 mL) and 100 % hydrazine hydrate (12 mL) the and the mixture was left standing overnight at 25 °C. The product was separated, collected by suction filtration, washed with methanol and light petroleum, and recrystallized from diluted acetic acid or water to give coumarin-4-carbohydrazides [48].

**2. Preparation of 3-acetyl coumarins 4(a-c)**:

Thus, 3-acetyl coumarins [49] are obtained in 88% yield in the reaction between substituted salicylaldehydes and ethyl acetoacetate in the presence of piperidine under solvent-free conditions.

**3. Synthesis of Coumarin-4-carbohydrazide Schiff Bases 5 (a-f):**

The compound 5 (a-f) was synthesized by the condensation of 3-acetyl salicylaldehydes [4 (a-c)] (0.01 M) with coumarin-4-carbohydrazides [3(a-b)] (0.01M) in methanol by refluxing on water bath for 5 h in acidic (3 drops GAA) condition. The completion of the reaction was monitored by thin-layer chromatography (TLC) with a mobile phase of 25% ethyl acetate in pet

ether. The contents of the reaction mixture were then transferred to crushed ice to obtain a colored Schiff bases [50].

## 2.5 MATERIALS AND METHODS

### 1. General Information:

Melting points of all the synthesized compounds were determined in open capillaries and are uncorrected. Infrared spectra were recorded using Shimadzu FT-IR instrument. $^{1}H$, $^{13}C$ Spectra were recorded on a JEOL ECZ 500R FT NMR spectrometer ($^{1}H$ NMR at 500 MHz, $^{13}C$ NMR at 126 MHz). Chemical shifts for protons and carbons are reported in parts per million downfield from tetramethylsilane and are referenced to the residual deuterium in the solvent. NMR data are represented as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, brs = broad singlet, and m = multiplet), coupling constant (J) (Hz), and integration. The TLC was performed on neutral alumina silica gel 60 $F_{254}$ (Merck). The mobile phase was ethyl acetate and n-hexane (1:1) and Visualization of TLC was performed with a 254 nm UV lamp, and by staining in $I_2$ chamber. Organic solutions were concentrated under reduced pressure using rotary evaporator. The resulting compounds were purified by recrystalization. All the reactions were carried out in oven-dried open glass vessels. Yield refers to the isolated analytically pure material.

### 2. Materials:

All the chemicals used were of analytical reagent grade and were used directly without further purification. **Salicyladehydes**, phenols and solvents were purchased from Avra Chemicals Ind. Pvt. Ltd. The chemicals were used as such without any further purification, whereas the solvents were purified by standard methods.

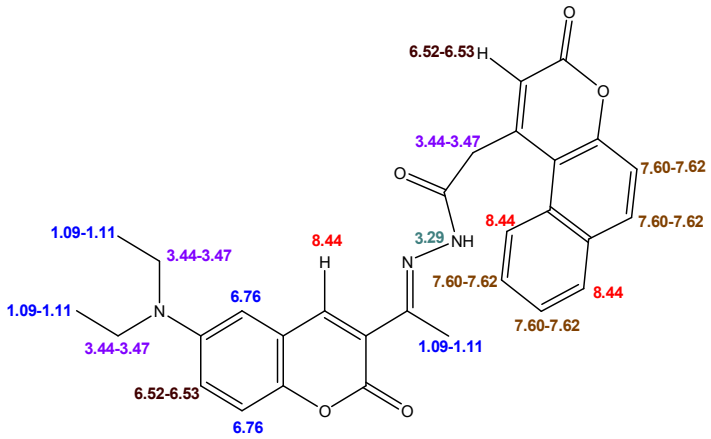
## 2.6 RESULTS AND DISCUSSION

### 2.6.1 Infrared spectral Studies

The IR spectrum of compound **5b (Spectrum No. 1)** exhibited the carbonyl stretching frequency of acetyl coumarin at 1722 $cm^{-1}$ and another carbonyl stretching frequency of carbohydrazide coumarin at 1714 $cm^{-1}$. The amide C=O stretching frequency shows the band at 1660 $cm^{-1}$. The imine C=N stretching frequency was observed at 1610 $cm^{-1}$. The NH stretching frequency exhibited at 2966 $cm^{-1}$. The IR spectrum of all the compounds **5(a-f)** had the Coumarin Carbonyl (C=O) stretching frequency at 1700-1750 $cm^{-1}$, the Amide Carbonyl (C=O)

stretching frequency at 1650-1700 cm-1 and characteristic C=N stretching band at 1550-1620 $cm^{-1}$ in all the compounds respectively.

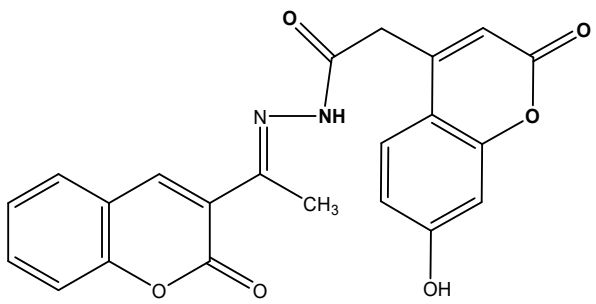
### 2.6.2 $^{1}$H-NMR spectral studies




The $^{1}$H NMR spectra of compound 5f shows a multiplet at $\delta$ 8.44 ppm for 3 aromatic protons. Another multiplet was resonated at $\delta$ 7.62-7.60 ppm for 4 aromatic protons. The multiplet at $\delta$ 6.75 -6.76 ppm was observed for another 2 aromatic protons and a multiplet at $\delta$ 6.52-6.53 ppm observed for another 2 aromatic protonsof coumarins respectively. The spectrum shows a singlet for NH proton at δ 3.29 ppm. A multiplet at δ 3.43-3.47 was observed for methylene protons (6H) and another multiplet at δ 1.09-1.11 ppm was observed for methyl protons (9H).The $^{1}$H NMR spectra of all the compounds 5(a-f) exhibited structure revealing proton signals at $\delta$ 7.00-8.50 ppm (m, t, dd, d for C=CH proton), $\delta$ 3.95-1.73 ppm (methylene protons).

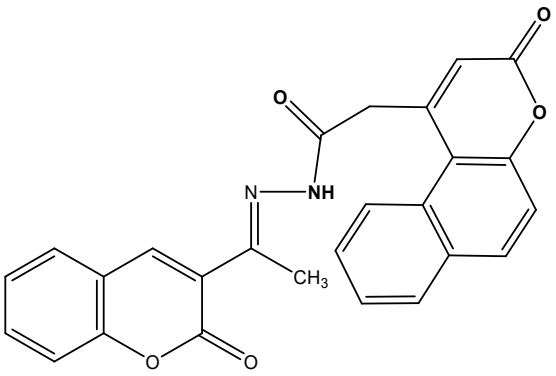
**Data of the Compounds:**

**(13E)-2-(7-hydroxy-2-oxo-2H-chromen-4-yl)-N'-(1-(2-oxo-2H-chromen-3-yl)ethylidene) acetohydrazide 5a:**



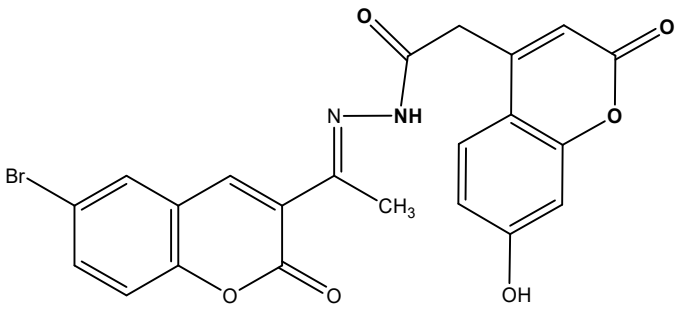
Mol. Formula: $C_{22}H_{16}N_2O_6$, Recrystalized from Ethanol. Light yellow solid (72 % yield); m.p.: 138-140 °C. IR: 2964 $cm^{-1}$ for NH, 1722 $cm^{-1}$, 1714 $cm^{-1}$ for Coumarin (C=O), 1660 $cm^{-1}$ for NHC=O,1612 for C=N. $^1$H NMR (500 MHz, DMSO): δ 10.21 (s, OH), 7.48 (s, 1H), 7.17-7.23 (m, 5H), 6.48-6.52 (d, 2H), 6.40 (s, 1H), 4.28 (s, NH), 3.40-3.46 (m, 6H), 1.08-1.12 (m, 9H). MS Calcd. for $C_{22}H_{16}N_2O_6$: 404; Found: M+1=405. CHN analysis: Calculated-C, 65.34; H, 3.99; N, 6.93; Found- C, 65.30; H, 3.95; N, 6.97.

**(13E)-N'-(1-(2-oxo-2H-chromen-3-yl)ethylidene)-2-(3-oxo-3H-benzo[f]chromen-1-yl) acetohydrazide 5b:**



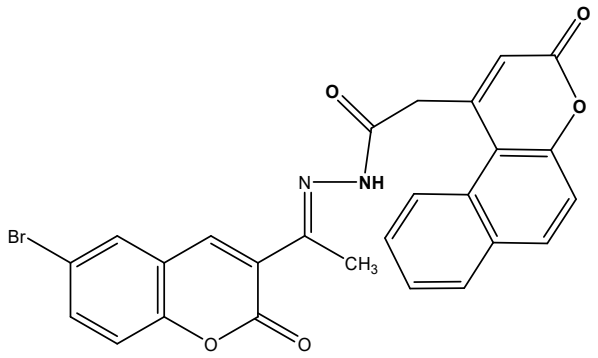

Mol. Formula: $C_{26}H_{18}N_2O_5$, Recrystalized from Ethanol. Light yellow solid (68% yield); m.p.: 131-133°C. IR: 2966 $cm^{-1}$ for NH, 1722 $cm^{-1}$, 1714 $cm^{-1}$ for Coumarin (C=O), 1660 $cm^{-1}$ for NHC=O,1610 for C=N. $^1$H NMR (500 MHz, DMSO): δ 7.44-7.50 (m, 4H), 7.18-7.25 (m, 7H), 6.51 (s, 1H), 4.24 (s, NH), 3.47-3.51 (m, 6H), 1.08-1.12 (m, 9H). MS Calcd. for $C_{26}H_{18}N_2O_5$: 438; Found: M+1=439. CHN analysis: Calculated- C, 71.23; H, 4.14; N, 6.39; Found- C, 71.18; H, 4.10; N, 6.34.

**(13E)-N'-(1-(6-bromo-2-oxo-2H-chromen-3-yl)ethylidene)-2-(7-hydroxy-2-oxo-2H-chromen-4-yl)acetohydrazide 5c:**



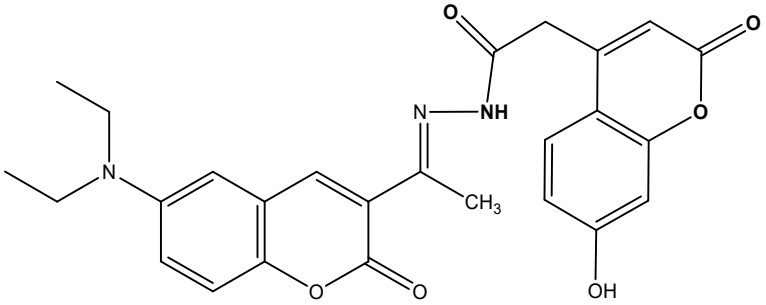
Mol. Formula: $C_{22}H_{15}BrN_2O_6$, Recrystalized from Ethanol. yellow solid (75% yield); m.p.: 116-118 °C. IR: 2964 $cm^{-1}$ for NH, 1722 $cm^{-1}$, 1714 $cm^{-1}$ for Coumarin (C=O), 1660 $cm^{-1}$ for NHC=O,1608 for C=N. $^1$H NMR (500 MHz, DMSO): δ 10.18 (s, OH), 7.68 (s, 1H), 7.18-7.25 (m, 3H), 6.47-6.51 (m, 3H), 6.35 (s, 1H), 4.21 (s, NH), 3.44-3.48 (m, 6H), 1.05-1.09 (m, 9H). MS Calcd. for $C_{22}H_{15}BrN_2O_6$: 483; Found: M+2=485 & M+1= 484. CHN analysis: Calculated- C, 54.68; H, 3.13; N, 5.80; Found- C, 54.64; H, 3.09; N, 5.75.

**(13E)-N'-(1-(6-bromo-2-oxo-2H-chromen-3-yl)ethylidene)-2-(3-oxo-3H-benzo[f]chromen-1-yl) acetohydrazide 5d:**




Mol. Formula: $C_{26}H_{17}BrN_2O_5$, Recrystalized from Ethanol. Light brownish solid (68% yield); m.p.: 198-200 °C. IR: 2966 cm-1 for NH, 1722 $cm^{-1}$, 1714 $cm^{-1}$ for Coumarin (C=O), 1660 $cm^{-1}$ for NHC=O,1608 for C=N, 758 $cm^{-1}$ for C-Br. $^1$H NMR (500 MHz, DMSO): δ 7.98 (s, 1H), 7.48-7.52 (m, 4H), 7.24-7.28 (m, 3H), 6.88-6.92 (d, 2H), 6.38 (s, 1H), 4.27 (s, NH), 3.44-3.48 (m, 6H), 1.05-1.09 (m, 9H). MS Calcd. for $C_{26}H_{17}BrN_2O_5$: 517; Found: M+2=519 & M+1= 518. CHN analysis: Calculated- C, 60.36; H, 3.31; N, 5.42; Found- C, 60.31; H, 3.27; N, 5.38.

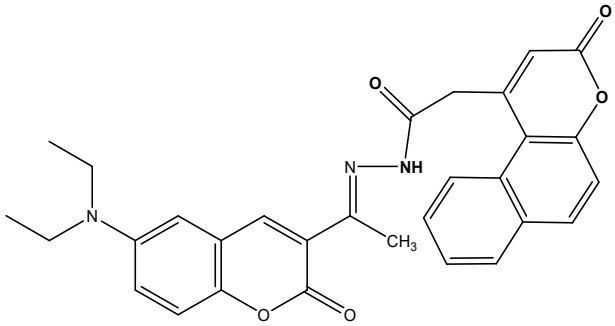
**(13E)-N'-(1-(6-(diethylamino)-2-oxo-2H-chromen-3-yl)ethylidene)-2-(7-hydroxy-2-oxo-2H-chromen-4-yl)acetohydrazide 5e:**




Mol. Formula: $C_{26}H_{25}N_3O_6$, Recrystalized from Ethanol. yellow solid (68% yield); m.p.: 208-210 °C. IR: 2966 $cm^{-1}$ for NH, 1722 $cm^{-1}$, 1714 $cm^{-1}$ for Coumarin (C=O), 1660 $cm^{-1}$ for

NHC=O,1610 for C=N. $^1$H NMR (500 MHz, DMSO): δ 10.12 (s, OH), 7.94 (s, 1H), 7.10-7.22 (dd, 1H), 6.88-6.92 (m, 2H), 6.48-6.52 (d, 3H), 6.40 (s, 1H), 4.22(s, NH), 3.42-3.45 (m, 6H), 1.09-1.13 (m, 9H). MS Calcd. for $C_{26}H_{25}N_3O_6$: 475; Found: M+1= 476. CHN analysis: Calculated- C, 65.67; H, 5.30; N, 8.84; Found- C, 65.63; H, 5.25; N, 8.80.

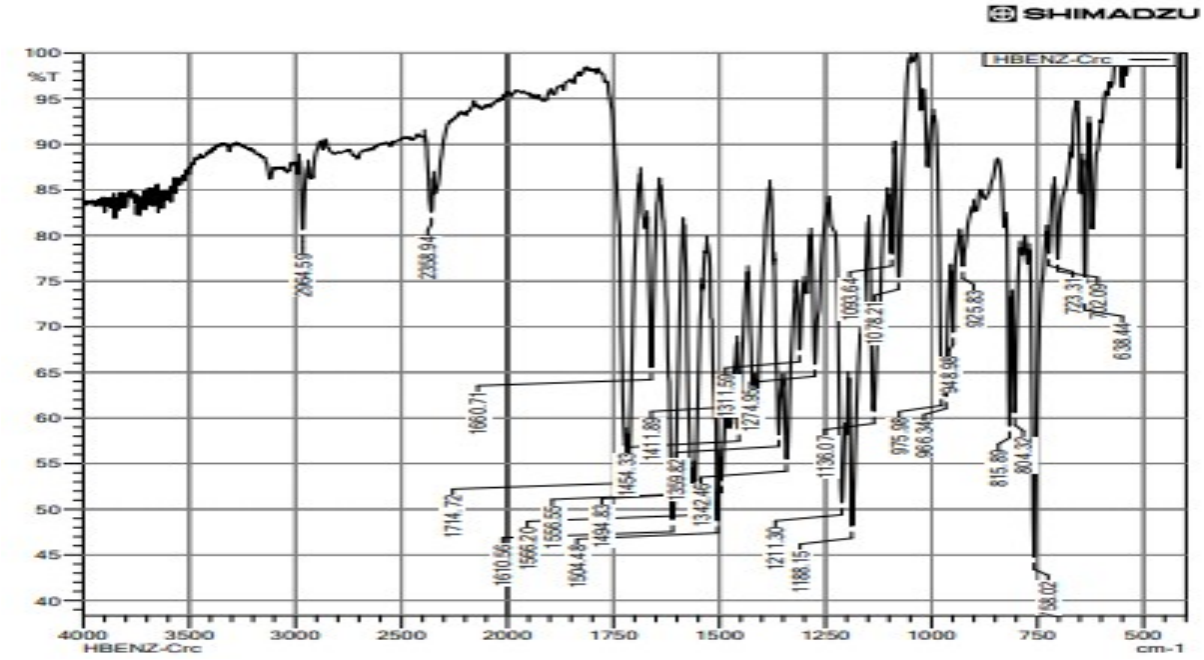
**(13E)-N'-(1-(6-(diethylamino)-2-oxo-2H-chromen-3-yl)ethylidene)-2-(3-oxo-3H-benzo[f]chromen-1-yl)acetohydrazide 5f:**



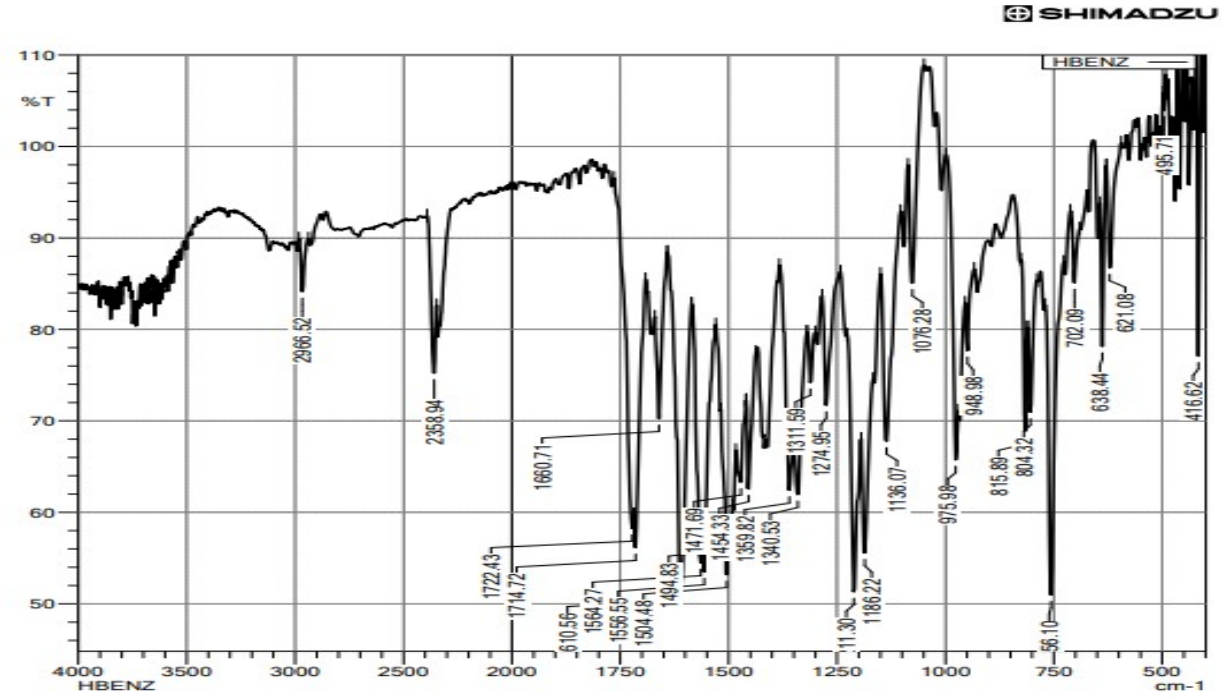

Mol. Formula: $C_{26}H_{25}N_3O_6$, Recrystalized from Ethanol. Grayish solid (68% yield); m.p.: 166-168 $^o$C. IR: 2966 cm$^-$1 for NH, 1722 cm$^{-1}$, 1714 cm$^{-1}$ for Coumarin (C=O), 1660 cm$^{-1}$ for NHC=O,1612 for C=N. $^1$H NMR (500 MHz, DMSO): δ 8.44 (m, 3H), 7.60-7.62 (m, 4H), 6.76 (m, 2H), 6.52-6.53 (m, 2H), 3.44-3.47 (m, 6H), 1.09-1.11 (m, 9H). MS Calcd. for $C_{26}H_{25}N_3O_6$: 509; Found: M+1=510. CHN analysis: Calculated- C, 70.71; H, 5.34; N, 8.25; Found- C, 70.67; H, 5.30; N, 8.20.

**Spectral Data of the Compounds:**

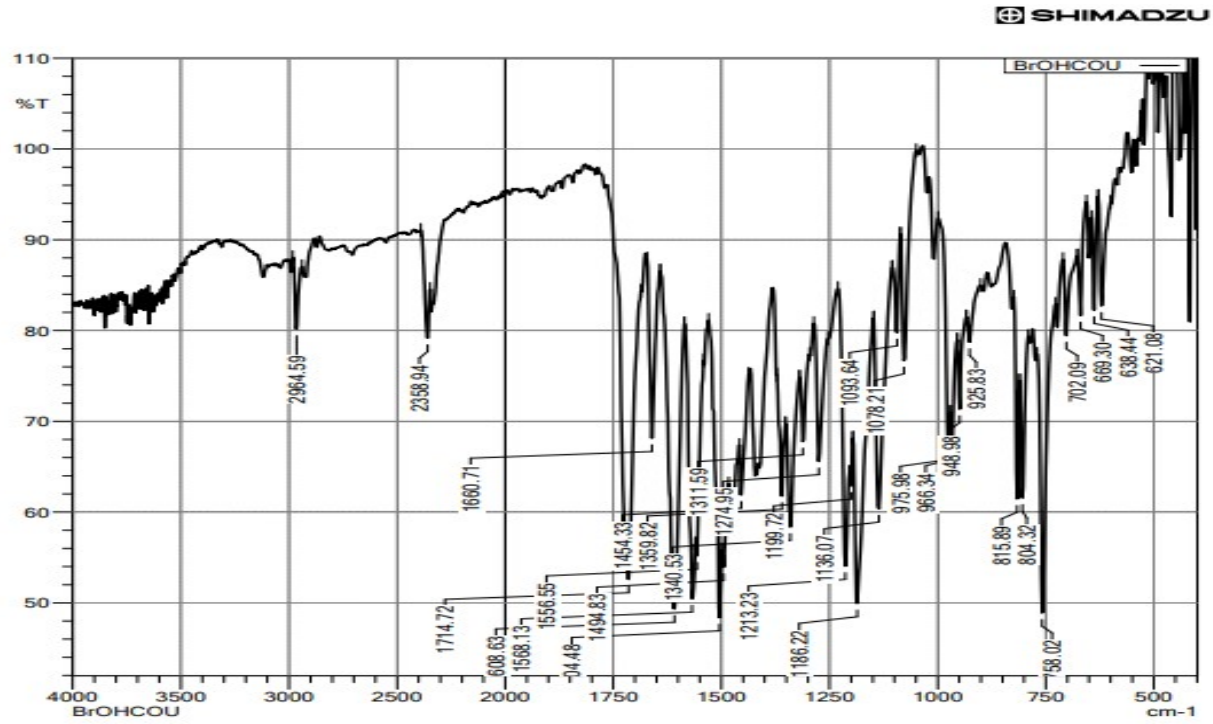
**IR Spectra of Compounds:**



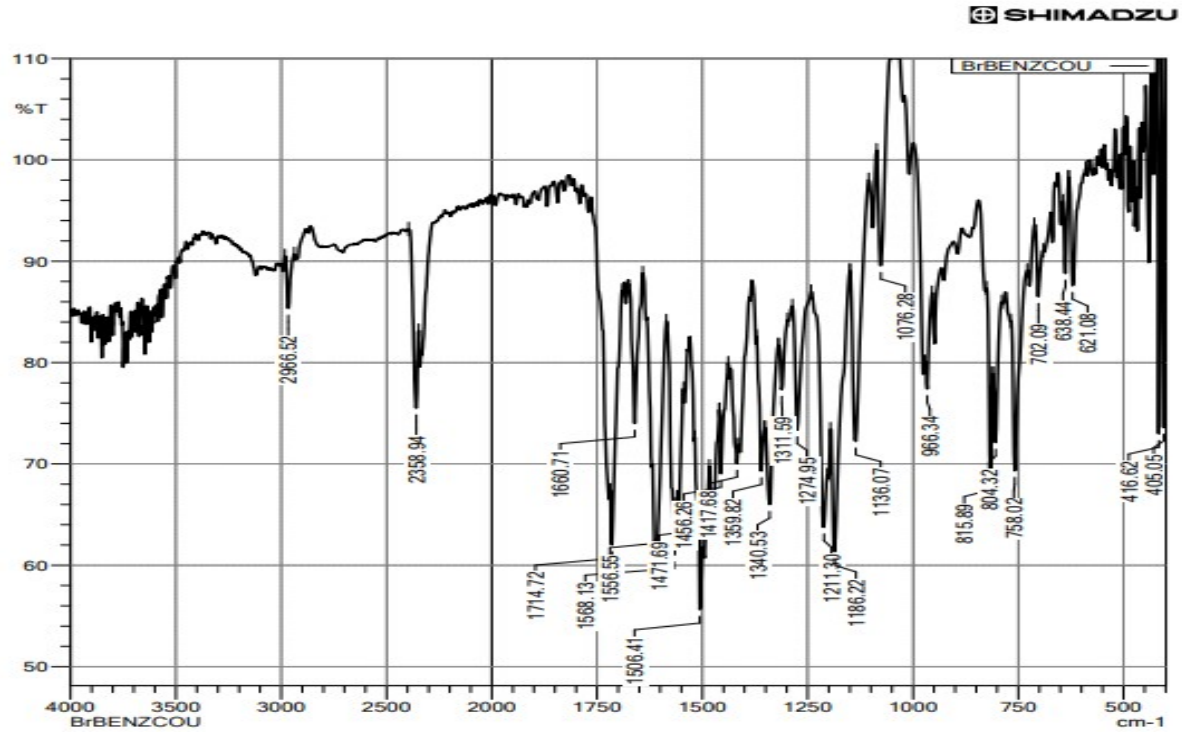

**Spectrum-1: IR Spectrum of (13E)-2-(7-hydroxy-2-oxo-2H-chromen-4-yl)-N'-(1-(2-oxo-2H-chromen-3-yl)ethylidene) acetohydrazide 5a**



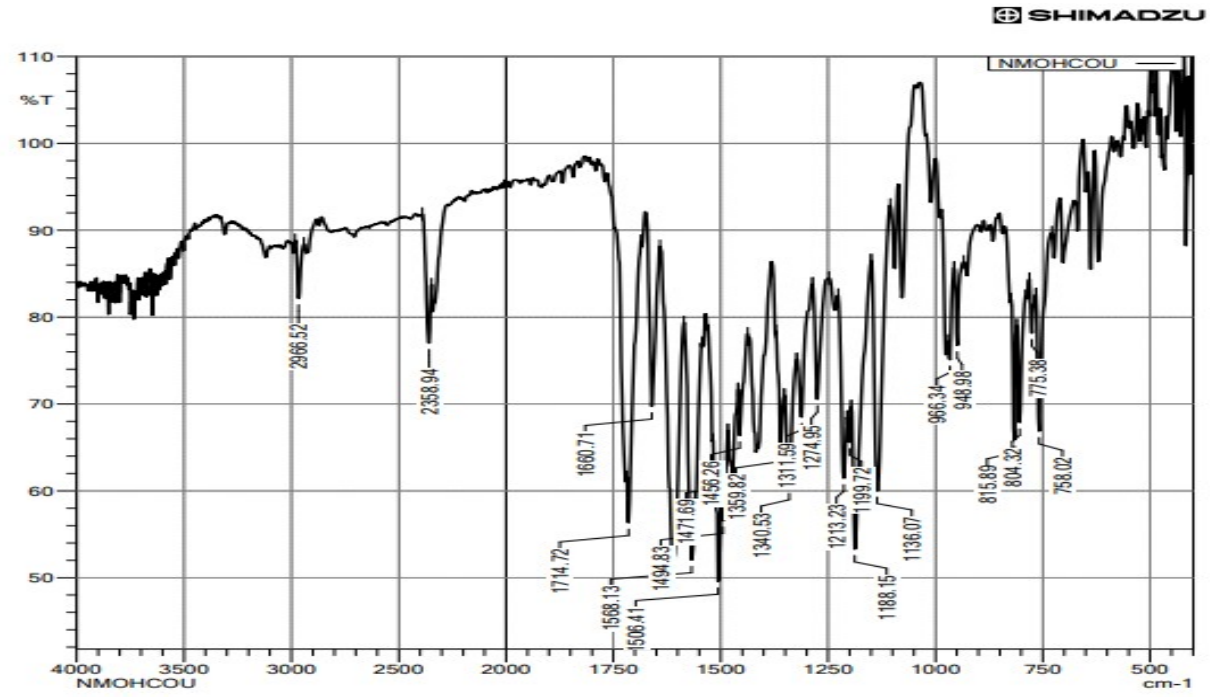

**Spectrum-2: IR Spectrum of (13E)-N'-(1-(2-oxo-2H-chromen-3-yl)ethylidene)-2-(3-oxo-3H-benzo[f]chromen-1-yl) acetohydrazide 5b**



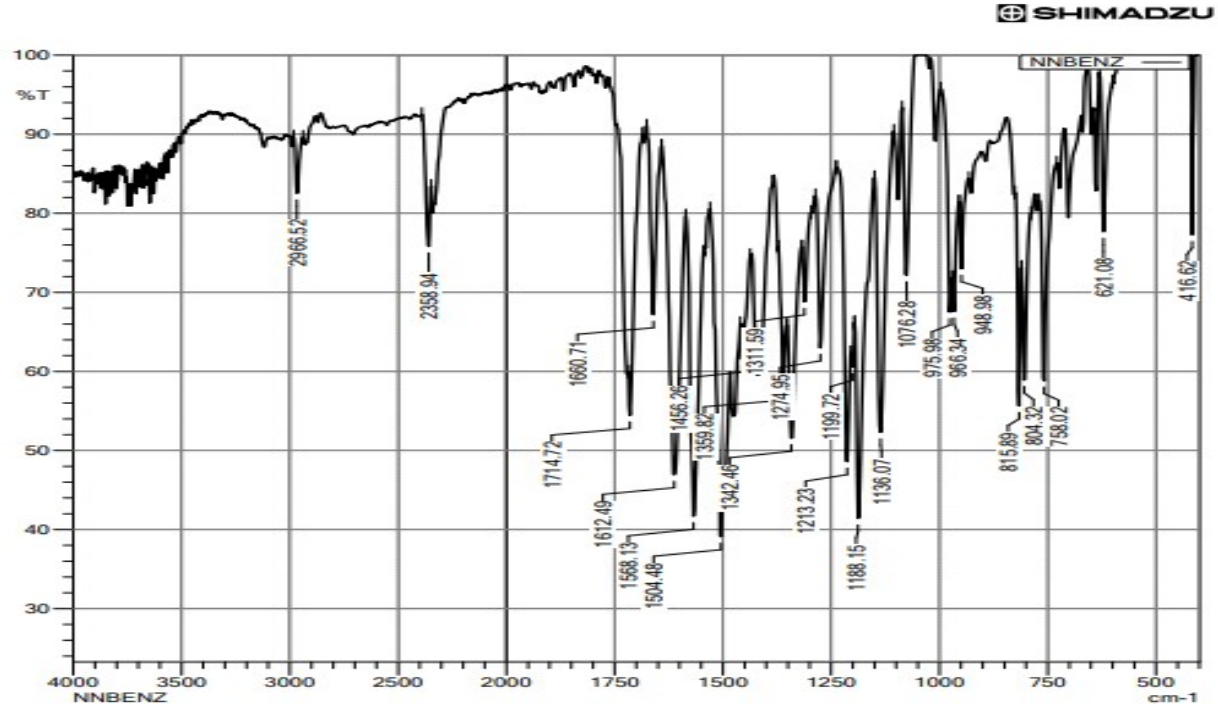

**Spectrum-3: IR Spectrum of (13E)-N'-(1-(6-bromo-2-oxo-2H-chromen-3-yl)ethylidene)-2-(7-hydroxy-2-oxo-2H-chromen-4-yl)acetohydrazide 5c**




**Spectrum-4: IR Spectrum of (13E)-N'-(1-(6-bromo-2-oxo-2H-chromen-3-yl)ethylidene)-2-(3-oxo-3H-benzo[f]chromen-1-yl) acetohydrazide 5d**

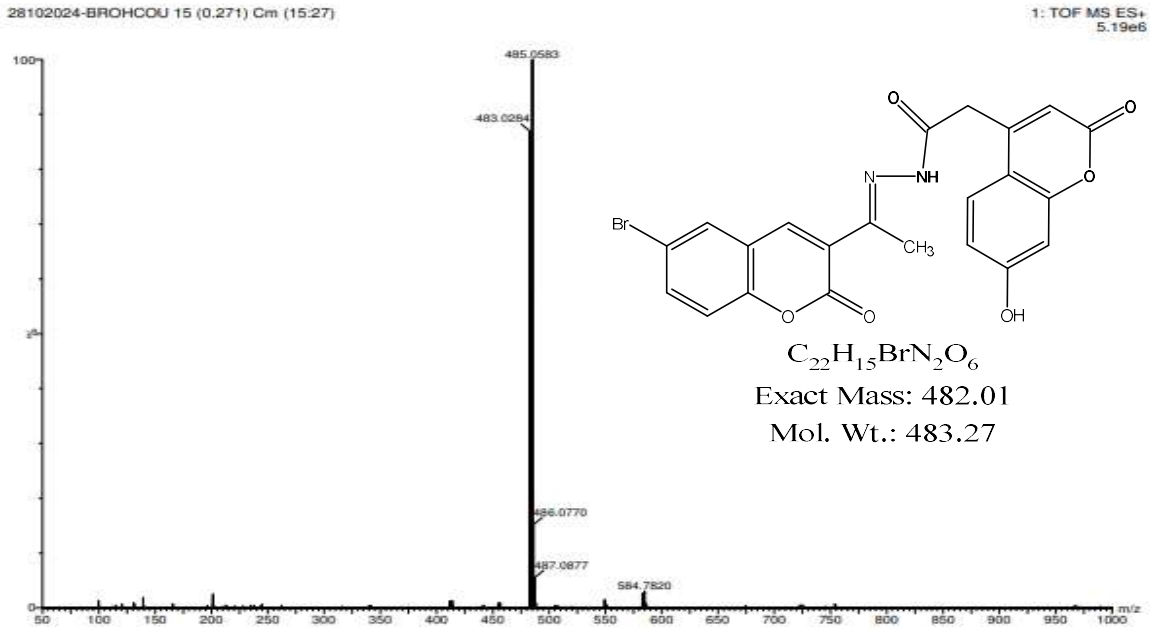



**Spectrum-5: IR Spectrum of (13E)-N'-(1-(6-(diethylamino)-2-oxo-2H-chromen-3-yl)ethylidene)-2-(7-hydroxy-2-oxo-2H-chromen-4-yl)acetohydrazide 5e**

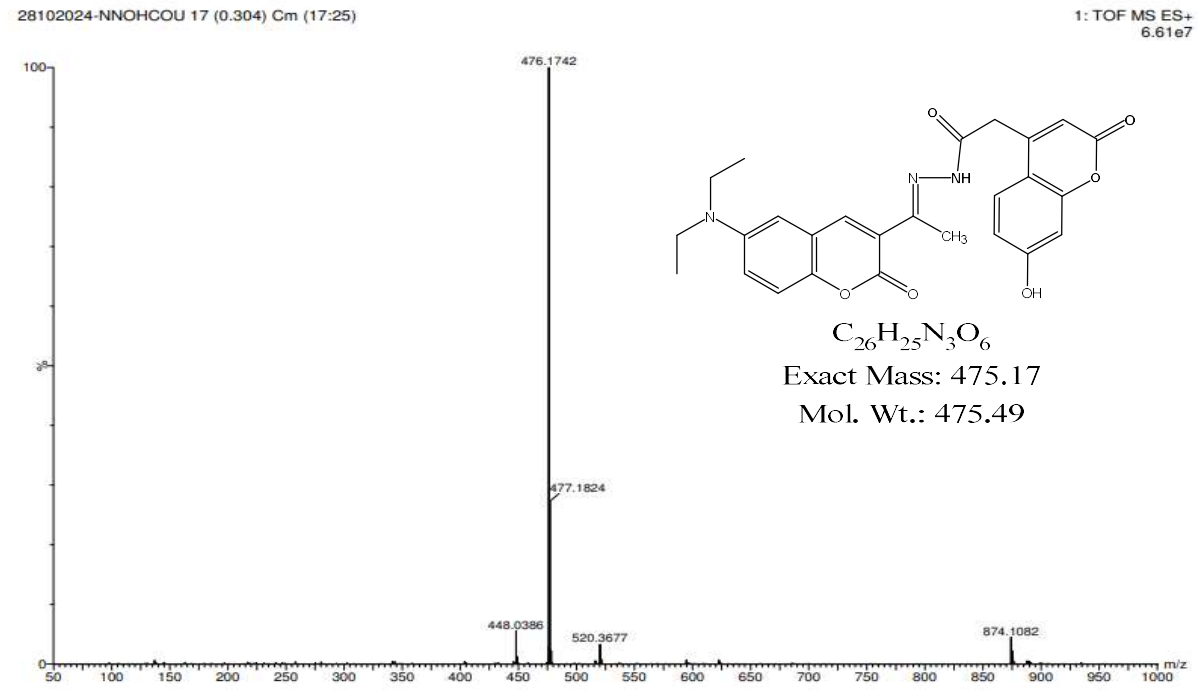



**Spectrum-6: IR Spectrum of (13E)-N'-(1-(6-(diethylamino)-2-oxo-2H-chromen-3-yl)ethylidene)-2-(3-oxo-3H-benzo[f]chromen-1-yl)acetohydrazide 5f**

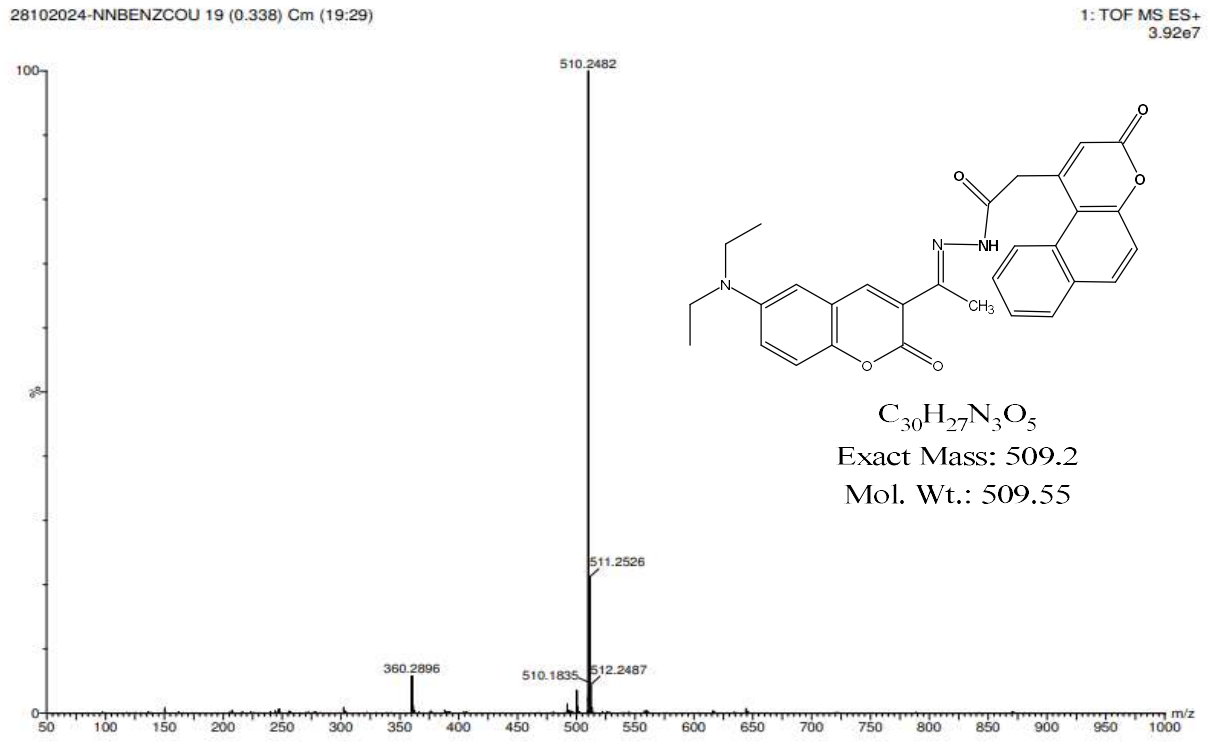
## LC Mass Spectra of Compounds:




**Spectrum-7: LCMS of (13E)-N'-(1-(6-bromo-2-oxo-2H-chromen-3-yl)ethylidene)-2-(7-hydroxy-2-oxo-2H-chromen-4-yl)acetohydrazide 5c**

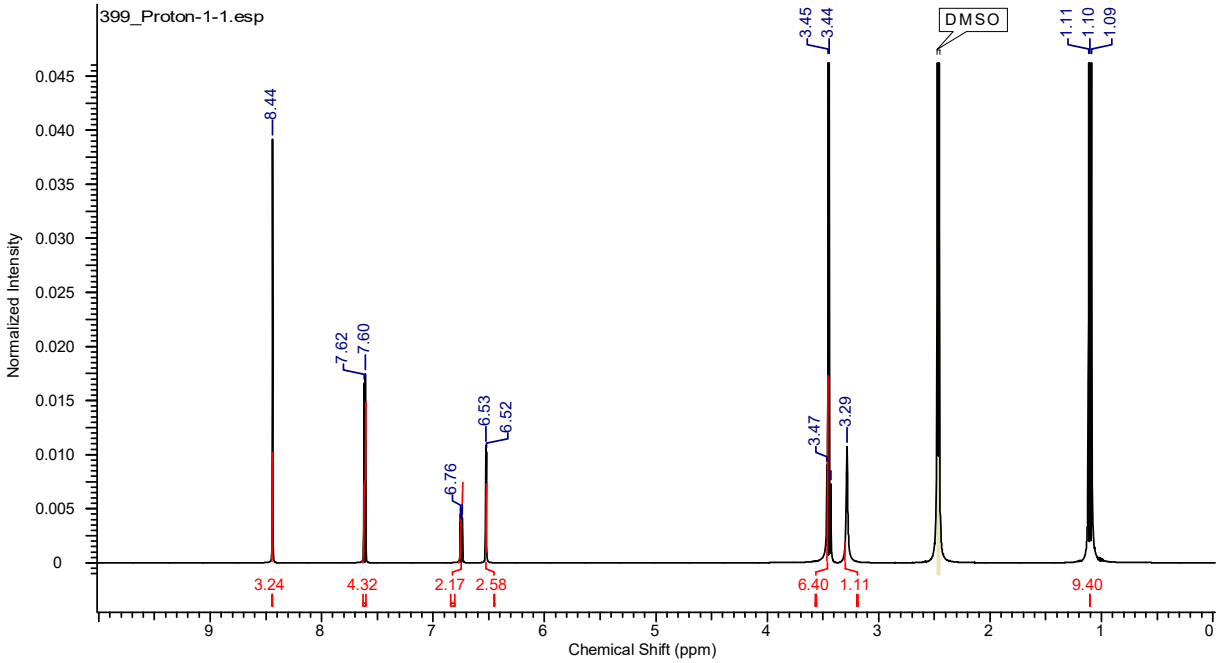



**Spectrum-8: LCMS of (13E)-N'-(1-(6-(diethylamino)-2-oxo-2H-chromen-3-yl)ethylidene)-2-(7-hydroxy-2-oxo-2H-chromen-4-yl)acetohydrazide 5e**




**Spectrum-9: LCMS of (13E)-N'-(1-(6-(diethylamino)-2-oxo-2H-chromen-3-yl)ethylidene)-2-(3-oxo-3H-benzo[f]chromen-1-yl)acetohydrazide 5f**

**NMR Spectrum:**




**Spectrum-10: $^1$H NMR Spectrum of (13E)-N'-(1-(6-(diethylamino)-2-oxo-2H-chromen-3-yl)ethylidene)-2-(3-oxo-3H-benzo[f]chromen-1-yl)acetohydrazide 5f**

**Reference:**

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