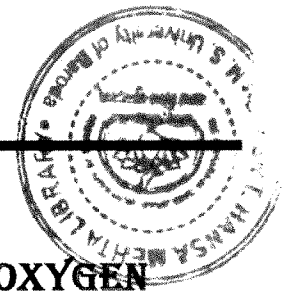

Thesis Entitled

SYNTHESIS AND CHARACTERIZATION OF NEW OXYGEN AND NITROGEN HETEROCYCLIC COMPOUNDS WITH ITS BIOLOGICAL AND GROWTH PROMOTING PROPERTIES

SUBMITTED TO

THE MAHARAJA SAYAJIRAO UNIVERSITY OF BARODA

FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

IN CHEMISTRY

BY

POONAM YADAV

DEPARTMENT OF CHEMISTRY, FACULTY OF SCIENCE

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CHL/ 33 / Ph.D.

18 APR 2011

CERTIFICATE

This is to certify that the work presented in the thesis entitled **"Synthesis and Characterization of New Oxygen and Nitrogen Heterocyclic Compounds with its Biological and Growth Promoting Properties"**, submitted to the Department of Chemistry, Faculty of Science, The Maharaja Sayajirao University of Baroda, for the award of **Ph.D.** degree by **Ms. Poonam Yadav**, is the original research work carried out by her under my guidance and supervision.

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Vadodara – 390002.

|| om shree ganeshaya namaha ||

|| anant koti brahmand nayak rajadhiraj yogiraj parbrahma shree

sachidanand sadguru shree sai nath maharaj ki jai ||

|| anant koti brahmand nayak rajadhiraj yogiraj parbrahma shree

sachidanand sadguru shree swami samarth maharaj ki jai ||

DECLARATION

I state that the work presented in this entitled, **"Synthesis and Characterization of New Oxygen and Nitrogen Heterocyclic Compounds with its Biological and Growth Promoting Properties"**, comprises of independent investigations carried out by me under the guidance of Dr. Nalini V. Purohit, Department of Chemistry, Faculty of Science, The Maharaja Sayajirao University of Baroda, and are true to the best of my knowledge. Wherever references have been made to the work of others, it has been clearly indicated with the source of information under the reference section. The work presented in this thesis has not been submitted elsewhere for the award of any other degree.

POONAM YADAV

I express my deep sense of gratitude to my research guide, Dr. Nalini V. Purohit, Department of Chemistry, Faculty of Science, The Maharaja Sayajirao University of Baroda, for giving me the opportunity to do work under her guidance. Her excellent guidance throughout the progress of this work, her ingenuity, constant encouragement, keen knowledge and interest in the subject proved to be a constant source of inspiration for me. She provided encouragement, sound advice, good teaching, good company, and lots of good ideas in scientific field during my entire course of investigation. I thank her for this contribution towards my academic career.

POONAM YADAV

ACKNOWLEDGMENT

It is always a pleasure to write an 'acknowledgement', for, more than anything else it marks an end of hard work with the accompanying joys and frustrations. I would like to put on record my sincere thanks to the people who have contributed to this study in many ways.

At the outset, I avail this opportunity to render my deep gratitude to The Maharaja Sayajirao University of Baroda, for its encouragement and full support for my research work in the field of Chemistry.

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Finally, this thesis would not have been possible without the confidence, endurance and support of my family. My family has always been a source of inspiration and encouragement. I wish to thank my parents, Mr. L. S. Yadav & Mrs. Sheela Yadav whose love, teachings and support have brought me this far. I wish to thank my brother, Pankaj Yadav for his affection and lovable support.

POONAM YADAV

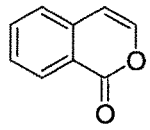
DEDICATED TO MY PARENTS

INDEX

SUMMARY

Introduction

A cyclic organic compound containing all carbon atoms in ring formation is referred as a carbocyclic compound. If at least one atom other than the carbon forms a part of the ring system then it is designated as a heterocyclic compound. Their study is of great interest both from the theoretical as well as practical point of view. Of the large family of the heterocycles, isocoumarins (*1H*-2-benzopyran-1-one) are an important class of naturally occurring lactones that exhibit a wide range of biological activities.



In addition the isocoumarin ring system is a useful intermediate for the synthesis of hetero and carbocyclic compounds. The development of methods for the formation of several carbon-carbon and/or carbon-heteroatom bonds in one reaction is one of the most important goals of the synthetic chemist, because such processes allow the assembly of complex molecular structures from relatively simple precursors.

So, the first step was to synthesize simple isocoumarins and few phthalides from easily available starting materials and then using them as starting /building blocks in subsequent chapters.

Objectives of the work:

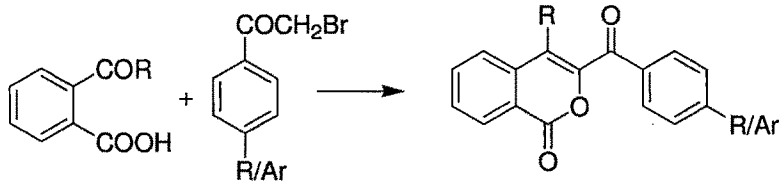
- To synthesise new isocoumarins and phthalides with different substituent's from simple precursors.
- Introduction of different heterocyclic ring systems into isocoumarins.
- Biological screening of the synthesized compounds against various disease models.

The structures of all the synthesized compounds have been established on the basis of their elemental analysis and spectral (IR, NMR, Mass) data for all the chapters.

Chapter 2: Synthesis of some new isocoumarins and phthalides*

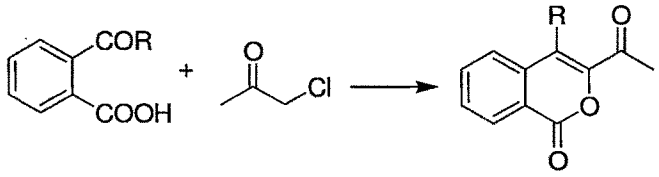
Isocoumarins and phthalides have exhibited varied biological and physiological activities. o-acyl benzoic acids and o- aroyl benzoic acids are important starting materials in the synthesis of isocoumarins and phthalides. The synthetic pathway followed by J. N. Chatterjea et al has been employed to prepare the title compounds.

Scheme I

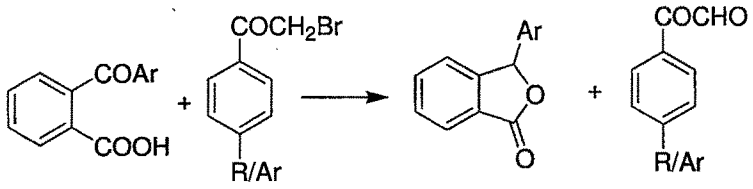


R = CH_3, C_2H_5, C_3H_7

Scheme II



Scheme III



4-alkyl-3-aroyl disubstituted isocoumarins were prepared by condensing different o-acyl benzoic acids with different bromo derivatives of substituted acetophenone in presence of anhy. K_2CO_3 and ethyl methyl ketone as solvent **(Scheme I).**

Similarly 4-alkyl-3-acetyl isocoumarins were formed when chloroacetone was taken in place of bromo acetophenone in presence of base **(Scheme II).**

Having taken o-aroyl benzoic acid instead of o-acyl benzoic acid and condensing it with different bromo acetophenone derivatives resulted in ring contraction and a mixture of phthalide and phenyl glyoxal were obtained rather than isocoumarins **(Scheme III).**

* Purohit N V, Yadav Poonam & Rajput Santosh, Ind. Journal of Heterocyclic Chemistry, 2008, 18, 169.

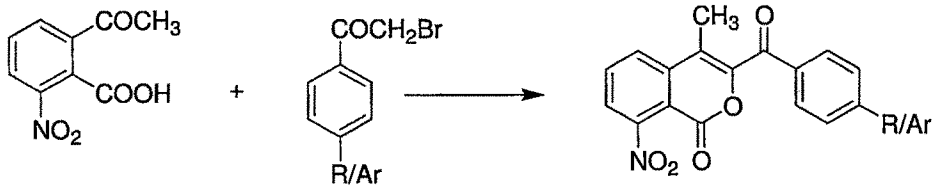
Some of the isocoumarins synthesized have been used as starting material for the synthesis of various heterocyclic compounds in subsequent chapters.

Chapter 3: Section A: Synthesis of 3, 4-disubstituted nitro, aminyl benzoyl and amino carbonyl isocoumarins

High throughput screening of the selected chemical libraries having a heterocyclic or carbocyclic ring at their core is one of the most expeditious ways to search for useful medicinal activity. The heteroatom improves binding and the rigid cyclic frame work imparts rigidity, enhancing the selectivity and further improving the binding. Hence, attempts were made to synthesize new oxygen-nitrogen containing heterocycles using different and simple pathways.

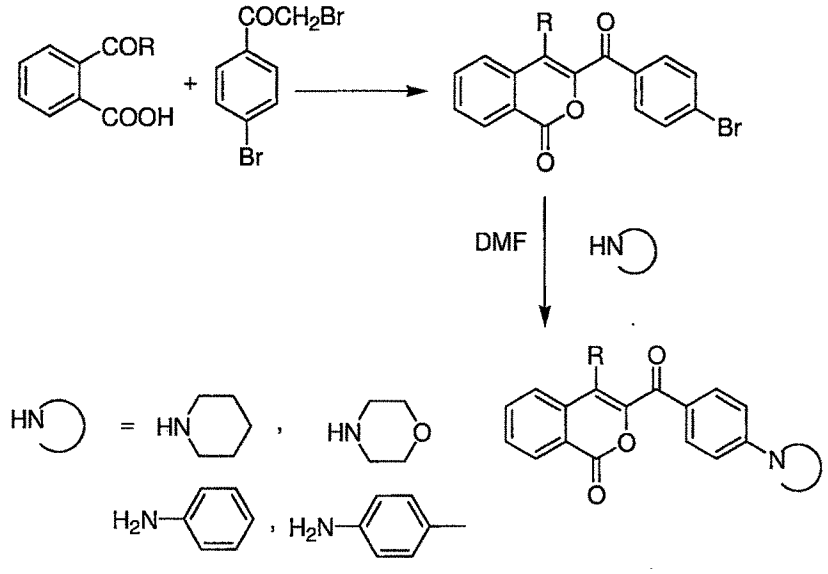
Electron density plays an important role in biological activity. Here, purposely we selected the nitro group to see the effect of electron withdrawing group on biological activity and therefore, synthesized nitro substituted isocoumarins (**Scheme I**). They were obtained by refluxing 2-acetyl-6-nitro benzoic acid instead of o-acetyl benzoic acid and following the same route (as in chapter 2) to get the final nitro substituted isocoumarins.

Scheme I



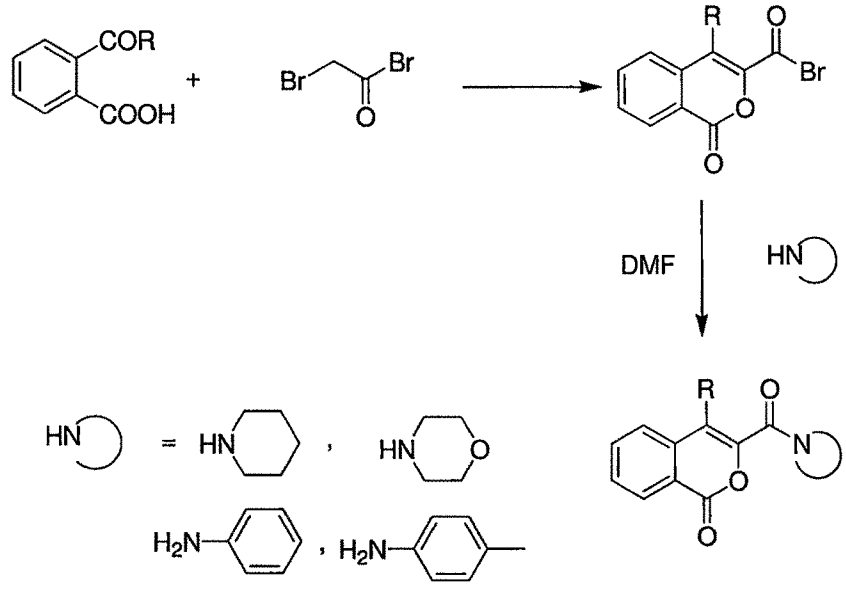
To obtain 4-alkyl-3-aminyl benzoyl isocoumarins, first 4-alkyl-3-(4'-bromo benzoyl) isocoumarin was prepared by condensing different o-acyl benzoic acids with bromo phenacyl bromide in presence of base. Then the ring was extended using -Br group substituted at para position of aroyl group and condensing it with various primary and secondary amines in DMF (**Scheme II**).

Scheme II



4-alkyl-3-aminocarbonyl isocoumarins were obtained from 4-alkyl-3-bromo carbonyl isocoumarins which were prepared by reaction between different o-acyl benzoic acids with bromo acetyl bromide following the same procedure as in chapter 2. The 4-alkyl-3-bromo carbonyl isocoumarins obtained were then condensed with primary and secondary amines to get the target compounds **(Scheme III)**.

Scheme III

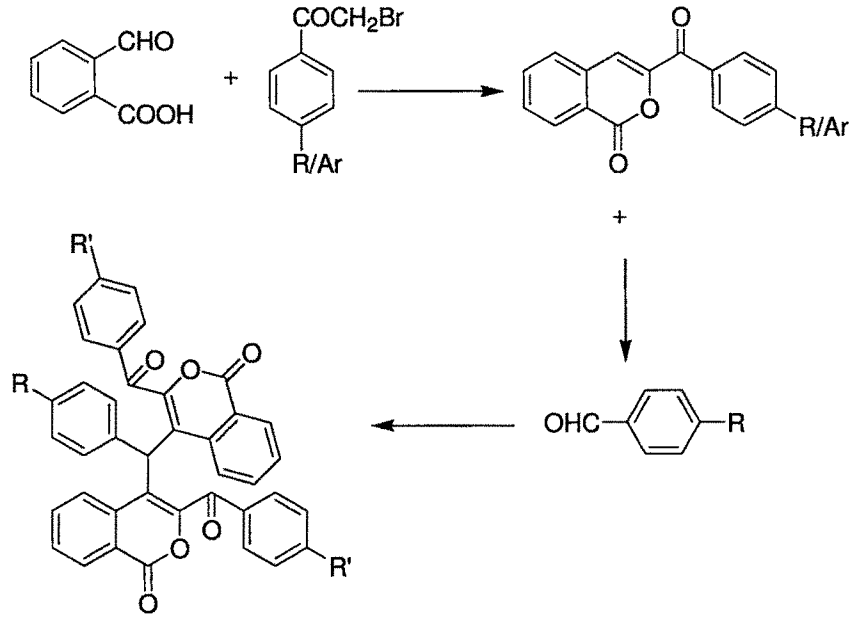


Chapter 3: Section B: Introduction of six membered lactone ring in isocoumarin moiety

Isocoumarins and coumarins both are known for their biological activities. So, the aim was to introduce one more six membered lactone ring into the already existing isocoumarin moiety and see its effect on the biological activity.

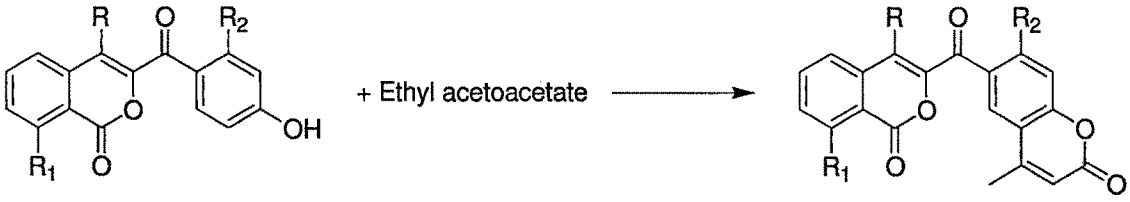
Two schemes were followed here. In **(scheme I)**, first 3-aroyl carbonyl isocoumarins were prepared by reacting 2- carboxy bezaldehyde and bromo acetophenone derivatives following the same pathway. The 3-aroyl isocoumarins formed, having no substitution at 4^{th} position were then condensed with different aromatic aldehydes in the ratio of 2:1, taking ethanol as solvent to get the bis isocoumarins.

Scheme I



In **(Scheme II)**, isocoumarins already prepared in previous chapters, having –OH group at 4' position of benzoyl group were reacted with ethyl acetoacetate in presence of conc. sulphuric acid (Pechmann condensation) to form coumarin moiety at the 4' position.

Scheme II

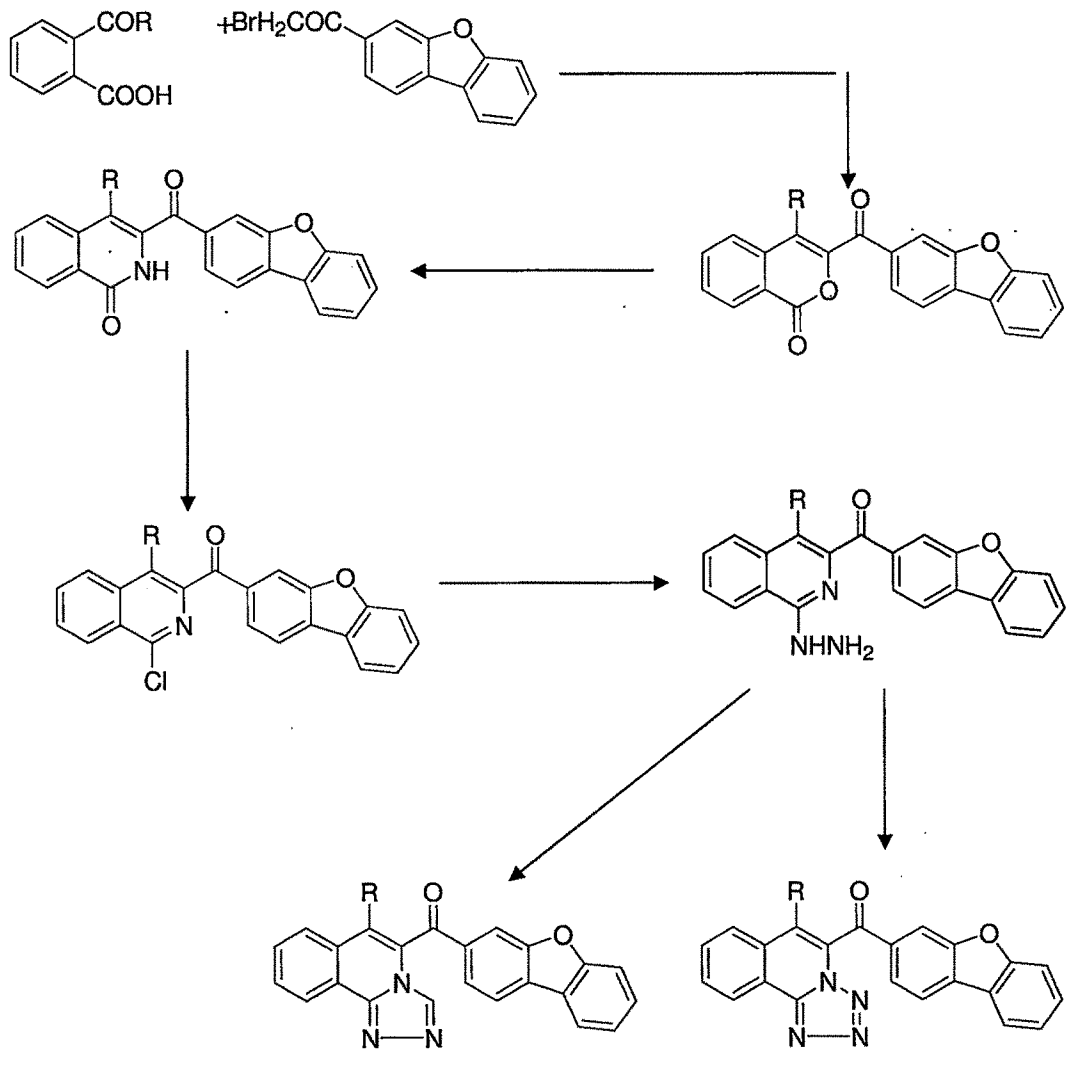


Chapter 4: Section A: Synthesis of 3, 4- disubstituted triazole and tetrazole isoquinolines

Triazolo and tetrazolo isoquinolines are known to posses good pharmacological properties. Large numbers of such compounds have been synthesized by different routes which involve 1- hydrazoisoquinolines as intermediate. Synthesis of this intermediate itself is quite difficult and comprises large number of steps. For the

Synthesis of triazole and tetrazole isoquinoline first 4-alkyl-3-dibenzofuryl isocoumarin was prepared from o-acyl benzoic acids and 2-bromo acetyl dibenzofuran following the same synthetic route as in chapter 2. The isocoumarins were converted to isoquinolones on reaction with liquor ammonia in ethanol. The resultant isoquinolones were then converted to 1-chloro isoquinolines by treatment with a mixture $POCl_3$ - PCl_5. The later on treatment with hydrazine afforded the required 1- hydrazinoisoquinolines which were finally converted to the corresponding tetrazolo isoquinoline and triazolo isoquinoline by treatment with $NaNO_2$/HCl and HCOOH respectively (**Scheme I**).

Scheme I

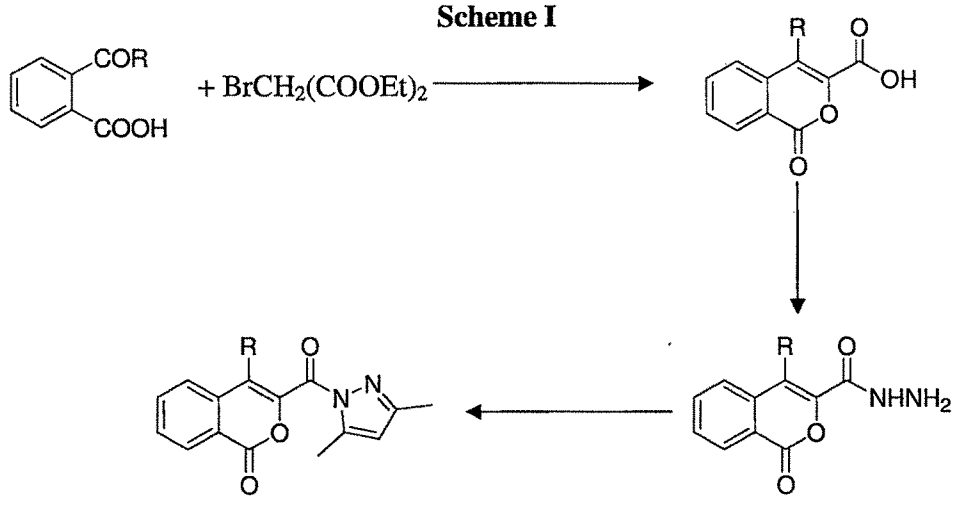


Chapter 4: Section B: Introduction of nitrogen hetrocyclic moiety in isocoumarin-3-carboxylic acid

Chemical and biological properties of hydrazides and the products of their heterocyclization have been widely studied. In particular some pyrazole derivatives exhibit convincing biological activity. Therefore, some pyrazole carbonyl derivatives were synthesized from isocoumarins (Paal knorr synthesis) as shown in **(Scheme I)**.

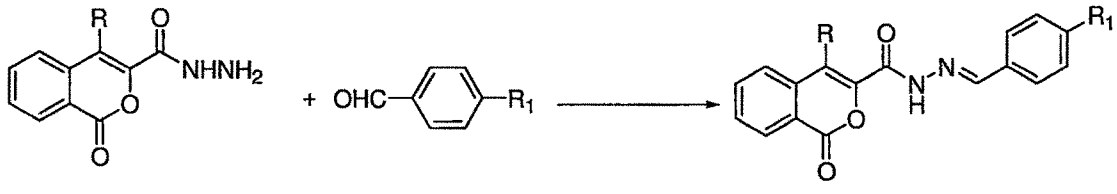
Different o-acyl benzoic acids were refluxed with bromodiethyl malonate to give 3, 3 dicarboxylic acid diethyl ester as intermediate which on treatment with glacial acetic acid and conc. hydrochloric acid gave 4-alkyl-isocoumarin-3- carboxylic acid. The later when refluxed with hydrazine in absolute alcohol provided 4-alkyl-isocoumarin-3- carboxylic acid hydrazide which on reaction with acetyl acetone and 1M HCl in methanol yielded 3-(3',5'-dimethyl-pyrazole-1'-carbonyl)- 4- alkyl isocoumarin.

Scheme I



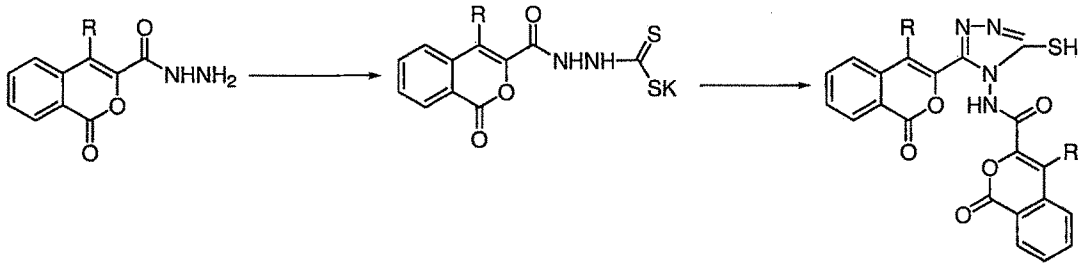
Schiff bases and their derivatives are easy to prepare and are known for their antimicrobial efficacy. So, 4-alkyl-isocoumarin-3-carboxylic acid hydrazides were converted to their respective Schiff bases by reacting them with different aromatic aldehydes in ethanol **(Scheme II)**.

Scheme II



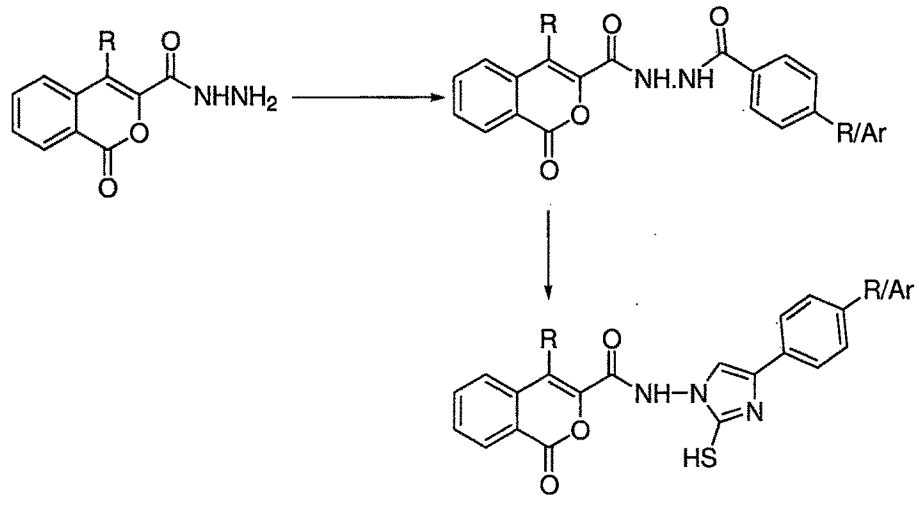
Presence of sulfur atom along with the nitrogen in the molecule is also known to show a wide spectrum of biological activities. This is mainly due to the presence of N-C-S linkage. Therefore, the 4-alkyl-isocoumarin-3- carboxylic acid hydrazides formed were extended to get mercapto triazole derivatives as they are easy to prepare. Isocoumarin-3-carboxylic acid hydrazides were stirred with carbon disulfide and potassium hydroxide for 16 hrs and then second mole of isocoumarin-3- carboxylic acid hydrazide was added to the reaction mixture and refluxed for 5 hrs in ethanol to get 4-alkyl-isocoumarin-3-carboxylic acid (3'-mercapto-5-(4''-alkyl-isocoumarin-3'-yl)- (1',2',4') triazole-4'-yl) amide **(Scheme III)**.

Scheme III



Isocoumarin -3-carboxylic acid hydrazides can also be converted to mercapto imidazole derivatives as they are potential antagonists and can be used as starting material in preparation of other potential therapeutic compounds. For this purpose, the acid hydrazides were first condensed with different bromo acetophenone derivatives to give Benzoic acid N'- (4-alkyl-isocoumarin-3-carbonyl)- hydrazide derivatives which on refluxing with potassium thiocyanate in glacial acetic acid resulted in cyclization to yield 4-alkyl-isocoumarin-3-carboxylic acid (2'-mercapto-4'-phenyl-imidazol-1-yl) amide derivatives **(Scheme IV)**.

Scheme IV



Chapter 5: Biological applications of the synthesized molecules.

The compounds synthesized in previous chapters were tested for their cyto toxicity, *in vitro*, against mosquito larvae, Culex Pipens. 500, 1000 and 1500 ppm solutions of the compounds were prepared and DMF and acetone used as control. 10 larvae were added in each solution and their survival was monitored over a period of 48 hrs. The LC values were calculated using Probit Analysis method. Most of the compounds were non toxic towards larvae till 1000 ppm and some even at 1500 ppm values except the molecules having dibenzofuran and biphenyl rings. The survival rate of larvae was 100 % in control.

Antibacterial and antifungal activity of new compounds was tested *in vitro* in the bacterial strains, *Staphylococcus Aureus* and *Escherichia Coli* and fungal strains of *Thielaviopsis Paradoxa, Phomopsis Mangiferae, Fusarium Pallidoroseum, Chaetonium & Colletotrichum Capsici* using serial agar dilution (Cup Plate method) and Potato Dextrose Agar medium (Poisoned Food technique) respectively. All the compounds showed from moderate to excellent activity against the tested organisms. Ampicillin is

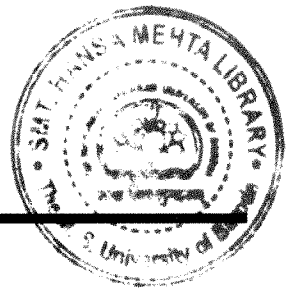
the standard drug used for antibacterial activity and DMF and DMSO used as controls for both the studies.

Analgesic activity of the synthesized compounds is also being done. The method employed is tail flick method and the reaction time of the mice towards the pain was monitored from 0-90 mins. Activity of the synthesized compounds has been compared with the standard drug Analgin. Among the tested compounds, only few exhibited good analgesic activity when compared to the standard drug and rest were showing moderate to weak activity.

Synthesized compounds were also tested for antioxidant activity by estimating scavenging activity for nitrous oxide using Griess reagent by test compounds. The % NO scavenging was different with different set of compounds, some showed poor activity and some showed more than 100% activity.

Anti inflammatory activity of the title compounds was determined by measuring the concentration of nitrite in blood plasma, a NO metabolite by Griess reagent. Lipo polysaccharide from *E. Coli* was used to induce inflammation and the NO concentration was compared with that of NO level in untreated blood plasma. Maximum of the synthesized compounds were able to decrease the inflammation as compared with the control.

Phthalides synthesized in chapter 2, were tested for their growth promoting properties on moong seeds, gram seeds and wheat seeds. A comparison with control showed good result for moong seeds, while on wheat seeds it showed average results. Maximum compounds showed 50% germination after 24hrs and almost 100% after 48 hrs except few. Radicle size was also good.



Chapter: 1

Introduction

A strict definition of a heterocyclic compound is one which possesses a cyclic structure with at least two different kinds of atoms in the ring, one of which is carbon, and can be aliphatic or aromatic. A heterocyclic compound usually possesses a stable ring structure which does not readily hydrolyze or depolymerise.

Heterocycles make up an exceedingly important class of compounds. In fact more than half of all known organic compounds are heterocycles. Many natural drugs such as quinine, papaverine, emetine, theophylline, atropine, procaine, codeine, morphine and reserpine are heterocycles. Almost all the compounds we know as synthetic drugs such as diazepam, chlorpromazine, isoniazid, metronidazole, azidothymidine, barbiturates, antipyrine, captopril and methotrexate are also heterocycles. Some dyes (e.g. mauveine), luminophores, (e.g. acridine orange), pesticides (e.g. diazinon) and herbicides (e.g. paraquat) are also heterocyclic in nature.

All these natural and synthetic heterocyclic compounds can and do participate in chemical reactions in the human body. Furthermore, all biological processes are chemical in nature. Why does nature utilize heterocycles? The answer to this question is provided by the fact that heterocycles are able to get involved in an extraordinarily wide range of reaction types. Depending on the pH of the medium, they may behave as acids or bases, forming anions or cations. Some interact readily with electrophilic reagents, others with nucleophiles, yet others with both. Some are easily oxidized, but resist reduction, while others can be readily hydrogenated but are stable toward the action of oxidizing agents. Certain amphoteric heterocyclic systems simultaneously demonstrate all of the above-mentioned properties. The ability of many heterocycles to produce stable complexes with metal ions has great biochemical significance. The presence of different heteroatoms makes tautomerism ubiquitous in the heterocyclic series. Such versatile reactivity is linked to the electronic distributions in heterocyclic molecules.

Also, we know that the introduction of a heteroatom into a cyclic compound imparts new properties. Heterocycles are chemically more flexible and better able to respond to the many demands of biochemical systems.

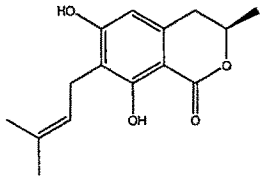
The compounds that will be treated in this work essentially fit this description.
Of the large family of heterocycles, pyrones are of utmost importance. Pyrones are six membered heterocyclic compounds containing one oxygen atom in the ring and five sp2 hybridized carbons. Two isomeric pyrones namely α – pyrone and γ- pyrones are possible.

Benzannulated pyrone derivatives of α- pyrones are Coumarins and Isocoumarins. The α-pyrones can be used as conjugated enol-lactoned, however, the extent to which they are additionally stabilized by resonance is not clear.

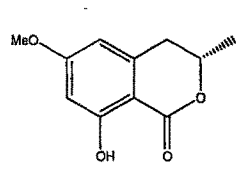
Isocoumarins are the secondary metabolites of a wide variety of microbial, plant and insect sources and encompass many interesting substances from the natural kingdom as well as useful synthetic intermediates in the synthesis of other classes of compounds. Owing to their borderline position between aromatic and aliphatic compounds, their chemistry is particularly rich and fascinating. Also, isocoumarins have shown an impressive array of biological activities. Number of known naturally occurring isocoumarins has increased dramatically mainly due to great improvements in isolation and purification techniques and availability of spectroscopic and crystallographic facilities for structure elucidation.

Few recent examples of naturally occurring isocoumarins are:

- Angelicoins A & B[1]

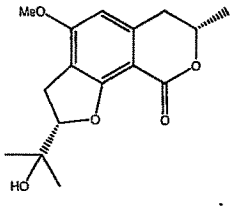


Angelicoin A

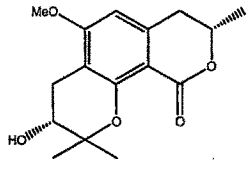


Angelicoin B

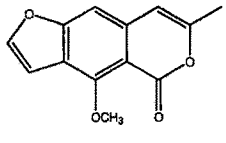
- Coriandrone A & B, Coriandrin & Dihydrocoriandrin together were obtained from the aerial parts of *Coriandrum sativum L*, an umbelliferous plants.[2-3]



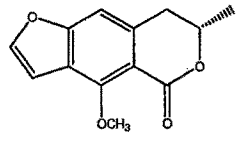
Coriandrone A



Coriandrone B

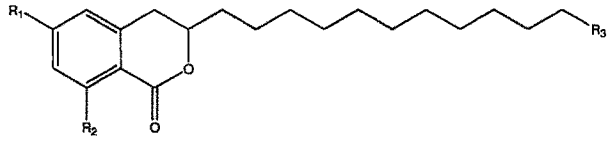


Coriandrin

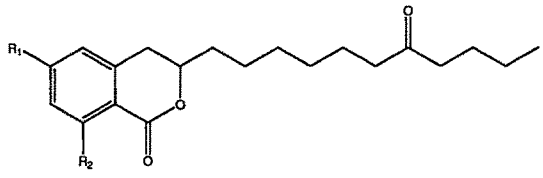


Dihydrocoriandrin

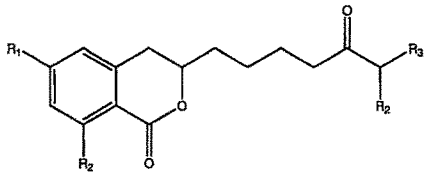
- The following alkyl isocoumarins had been obtained from the chloroform extract of aerial parts of O. *uiscosa* subsp. *breuijora* along with the other natural products.[4]



R_1 = OMe, R_2 = OH, R_3 = H
R_1 = OMe, R_2 = R_3 = H
R_1 = OMe, R_2 = R_3 = OAc

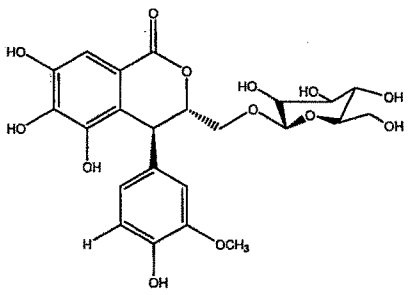


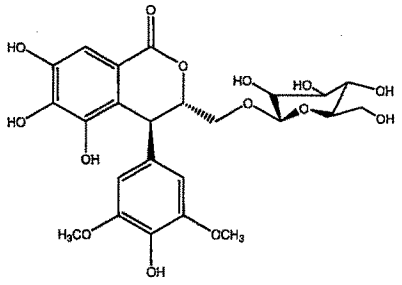
R_1 = OMe, R_2 = OH
R_1 = OMe, R_2 = OAc



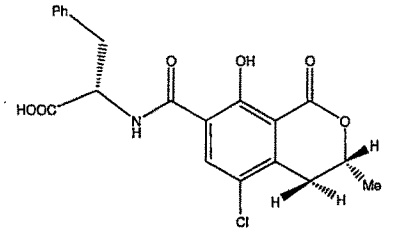
R_1 = OMe, R_2 = OH, R_3 = pentyl
R_1 = OMe, R_2 = OH, R_3 = heptyl

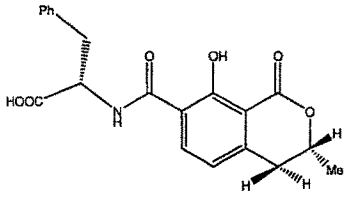
- Compounds representing the first members of a new biogenetic pathway for the isocoumarins nucleus involving shikimate derived A-ring (gallic acid) coupling with a phenyl propanoid derivative. They were isolated from the stem bark of *C. glabrum*.[5]





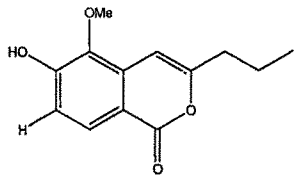
- Dihydroisocoumarin coupled with *beta* phenylalanine, known as Ochratoxin is produced by *Aspergillus ochraceus* and several related *Aspergillus* species, by a single *Penicillium* species (*P. Verrucosum*), and by *A. carbonarius* with a small percentage of isolates of the closely related *A. niger*.[6-8]



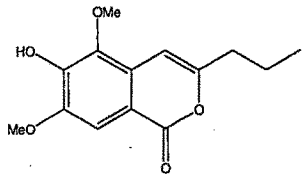


Ochratoxin A Ochratoxin B

- Scoparine A & B have been obtained from the root of *P. scoparius*, a toxic and endemic plant from North Africa.[9]

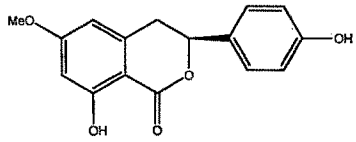


Scoparine A

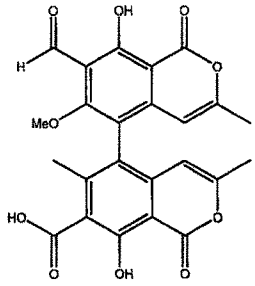


Scoparine B

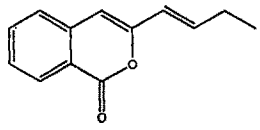
- 6-Methoxy-8-hydroxy -3-(4-hydroxyphenyl) isochroman-1-one, named as hongkongenin, have been isolated and identified from the whole plant of *P. hongkongensis.*[10]

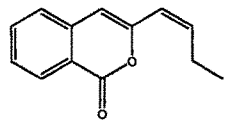


- Tithoniamarin, an isocoumarin dimer, was extracted from the flowers of *T. diversifolia.*[11]

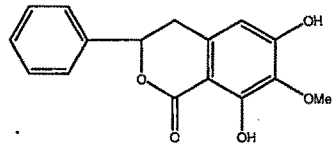


- Analysis of Tarragon (*Artemisia dracunculus L., Asteraceae* family) showed the presence of 3-(1Z-butenyl) isocoumarinc and 3-(1E-butenyl) isocoumarin in it.[12]

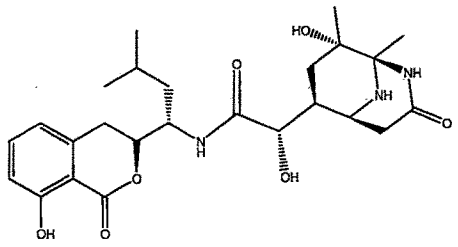




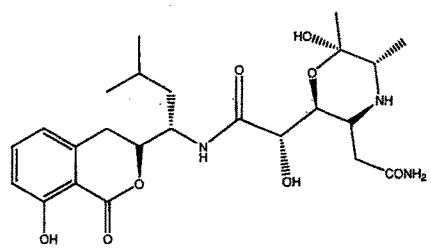
- Nedialkov et al. reported the isolation and structural elucidation of a new natural isocoumarin named as annulatomarin.[13-14]



- Cultivation of a Bacillus subtilis strain from the intestines of the sardine Sardinops melanosticta (Toyama Bay, Japan) yielded bacilosarcins A 38 and B 39.[15]

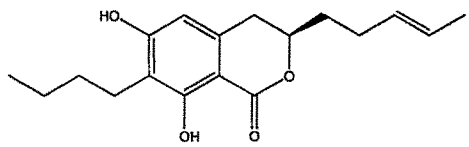


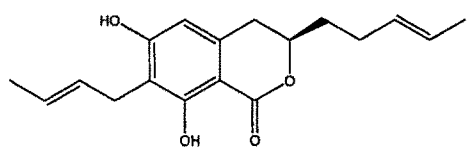
A 38

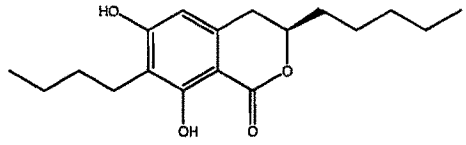


B 39

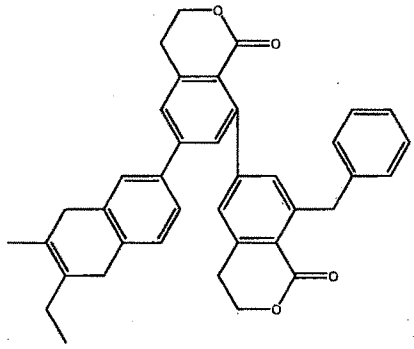
- Over the period 2001–2005, only three novel isocoumarin derivatives, have been identified from endophytic sources. These metabolites were isolated from *Geotrichum* sp., an endophyte of *Crassocephalum crepidioides.*[16-17]







- Salvadorin, a new dimeric dihydroisocoumarin, was isolated from the chloroform fraction of *Salvadora oleoides*. Its chemical structure was established as 8-benzyl-6-[6-(6-ethyl-7-methyl-5,8-dihydro-2-naphthalenyl)-1-oxo-3,4-dihydro-1*H*-isochromen-8yl]-3, 4-dihyro-1*H*-isochromen-1-one, through spectroscopic techniques and chemical analysis.[18]

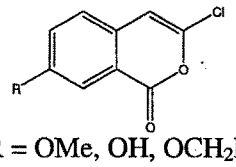


As in many areas of science, the past decennium has seen great strides made toward increasing our understanding in the various fields of chemistry. In many instances, borderlines between the various areas of science are very difficult to define. Chemistry, too, is becoming more diffuse, therefore this introduction would have been incomplete without the biogenesis and biological activity of isocoumarins.

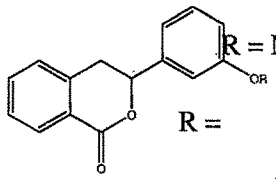
Isocoumarins are an important class of naturally occurring biologically active lactones, originating from a variety of natural sources, with multiple biological activities described ranging from antibacterial, antimalarial, antifungal, and anticancer activity etc. in addition to their use as sweetening agents.

Following are the reported examples of isocoumarins showing their diverse biological properties:-

Antibacterial Isocoumarins[19-24]

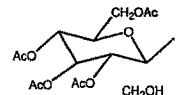


R = OMe, OH, OCH_2Ph

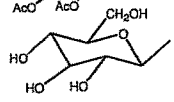


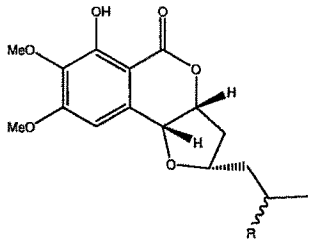
R = Me, H, Ac,

R =

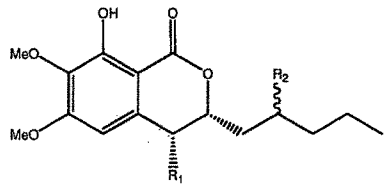


R =

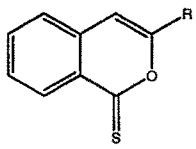




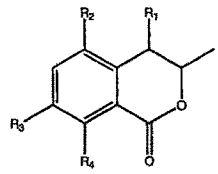
R = H, ά – OH, β- OH



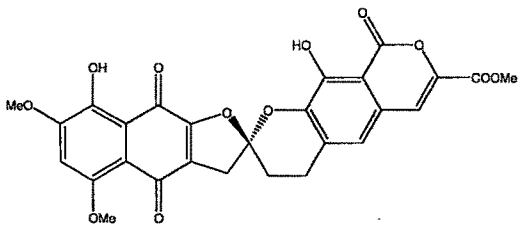
R_1 = OH, H
R_2 = ά – OH, β- OH



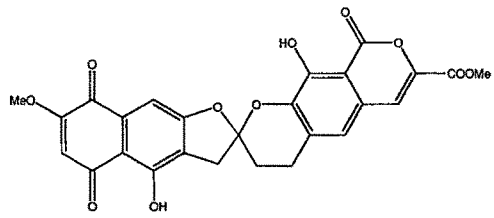
R = Alkyl, Aryl. Aralkyl, Cycloalkyl etc
$R_1 = R_3 = H$, $R_2 = CH_3$, R_4= OH



R_1 = H, $R_2 = CH_3$, $R_3 = OCH_3$,R_4= OH
$R_1 = R_3 = H$, $R_2 = CH_2OH$, R_4= OH

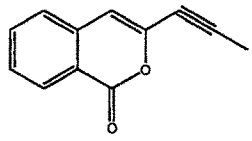


β – Rubromycin

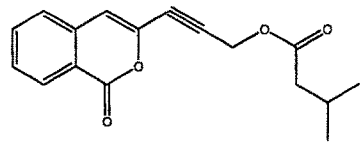


ϒ – Rubromycin

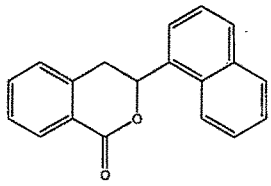
Antifungal Isocoumarins[25-27]

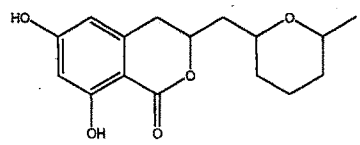


Capillarin



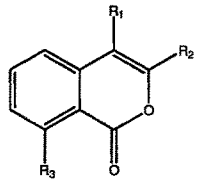
Capillarin isovalerate





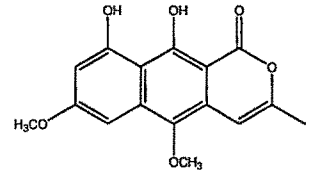
Dihydroisocoumarin Derivatives

Anti-inflammatory Isocoumarins[28-31]

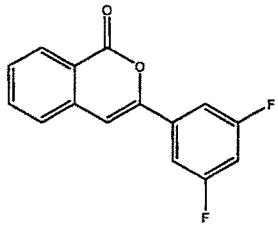


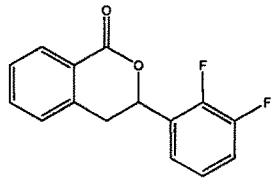
Oosponol

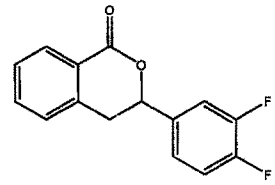
$R_1 = COCH_2OH$, $R_2 = OH$, $R_3 = H$



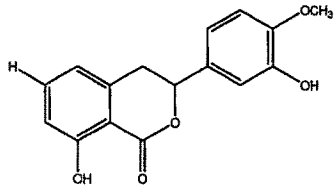
Paepalantine



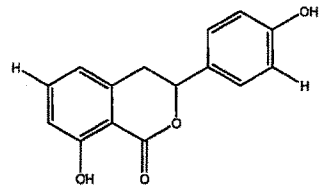




Fluorinated isocoumarins

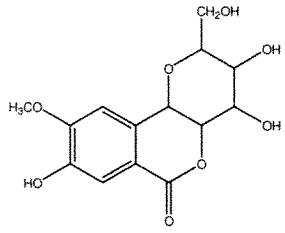


Phyllodulcin

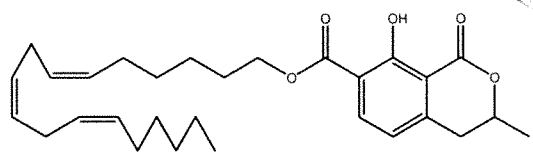


Hydrangenol

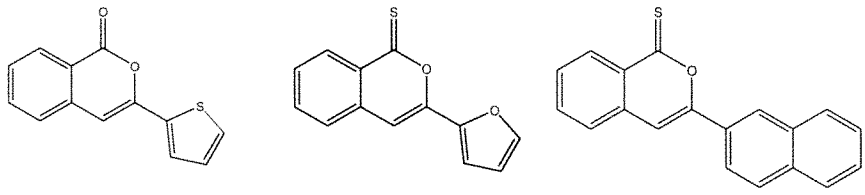
Anti cancer Isocoumarins[32-37]



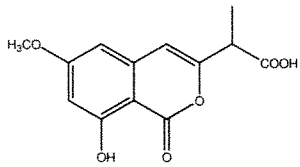
Berginin



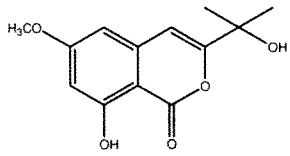
γ-Linolenyl Ester Linked Compounds



Thioisocoumarin derivatives



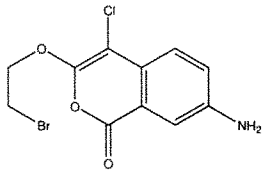
NM – 3

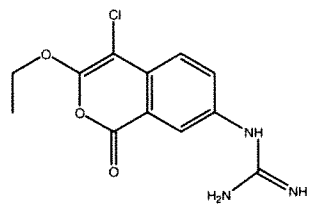


Cytogenin

Enzyme Inhibiting Isocoumarins[38-45]

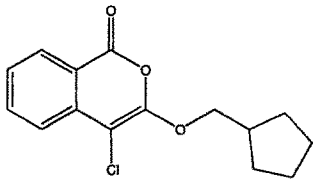
Irreversible inhibitors of serine protease

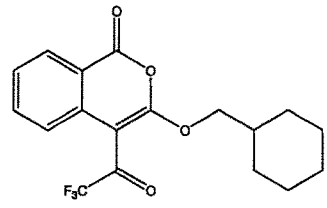




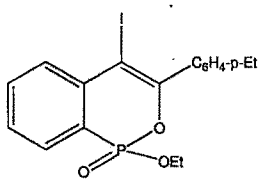
Amino isocoumarins

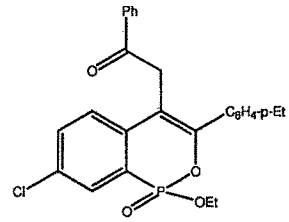
Inhibitors of pancreatic cholesterol esterase





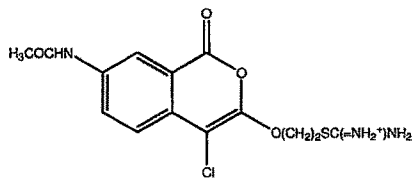
3-alkoxyhaloisocoumarins

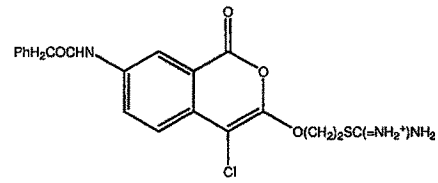




Phosphaisocoumarins

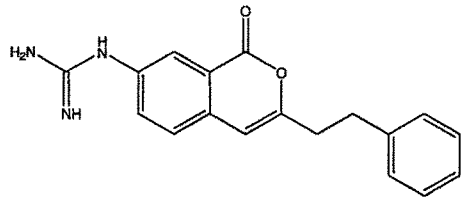
Inhibitors for Blood Coagulation Serine Proteases



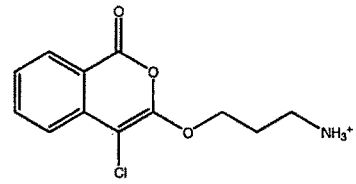


3-(Isothioureidoalkoxy) isocoumarins

Inhibitors of trypsin- like enzymes

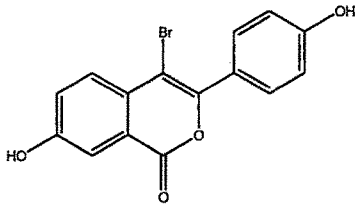


Guandino isocoumarin

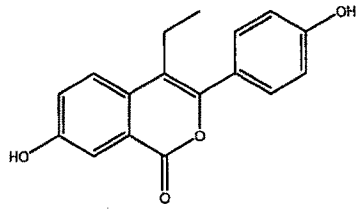


4- Chloro – 3 – propyloxy ammonium isocoumarin

Estrogen receptor binders

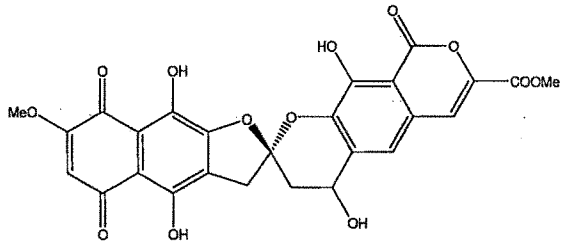


4-Bromo-7-hydroxy-
3-(4'-hydroxyphenyl)isocoumarin



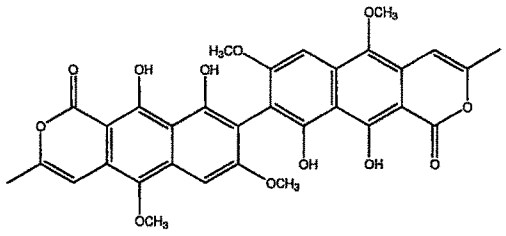
4-Ethyl-7-hydroxy-3-(4'-
hydroxyphenyl)isocoumarin

Human telomerase and mammalian DNA-polymerase inhibitor

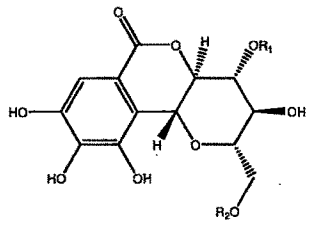


Purpuromycin

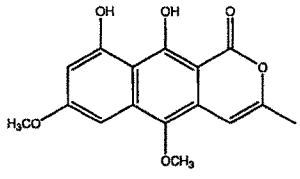
Cytotoxic Isocoumarins[46-49]



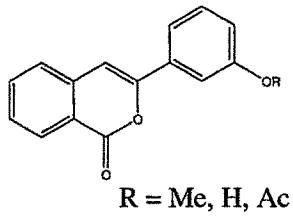
8, 8'-Paepalantine dimer



Norbergenin derivative

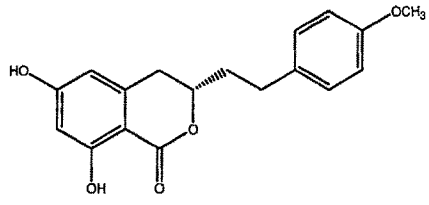


Paepalantine

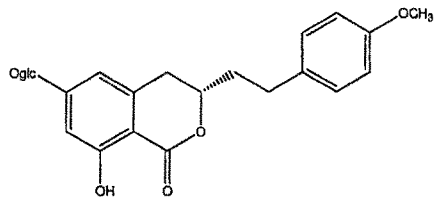


R = Me, H, Ac
3- substituted isocoumarin Derivatives

Hepatoprotective Isocoumarins[50]

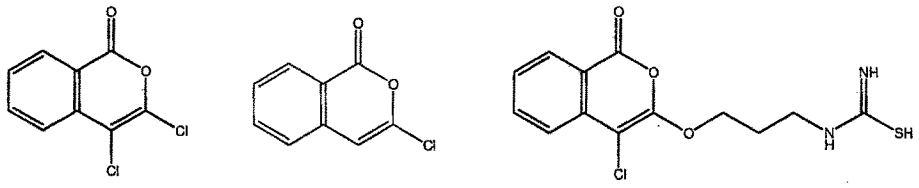


Agrimonolide



Agrimonolide 6-*O*-β-D-glucoside

Anti influenza Isocoumarins[51]

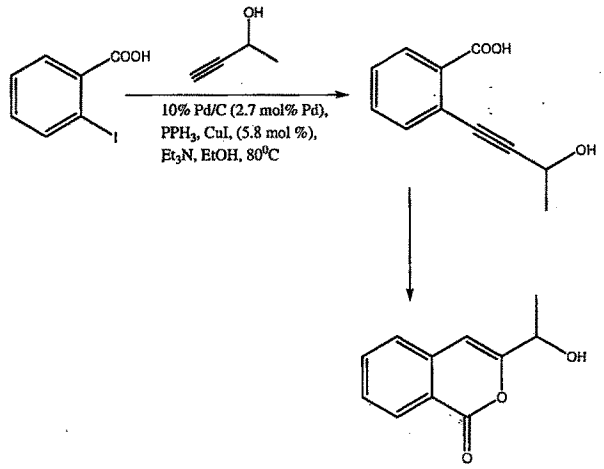


Chloroisocoumarin Derivatives

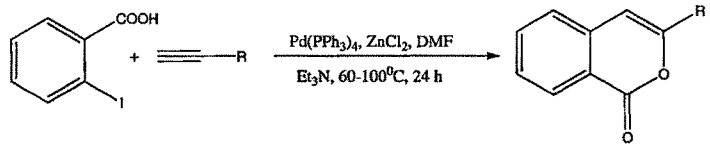
Isocoumarins were reviewed comprehensively by Barry in 1964 and Elio Napolitano in 1997. The reasons for interest in this class of compounds have not changed since then. In the more than thirty years elapsed, a conspicuous and still increasing number of new isocoumarins have been found in nature (in prevalence among the products of secondary metabolism of plants and lower microorganisms but also among insect pheromones and venoms) exhibiting a wide structural diversity in dependence of their natural source and their biosynthetic pathway; these findings have been a constant stimulus for synthetic work, which has been undertaken either to confirm novel structures or to provide substantial amounts of material for biochemical and pharmaceutical studies in those cases in which an isocoumarin exhibited interesting properties or was suspected of being responsible for the significant properties associated with its natural source.

Several methods have been known for the synthesis of isocoumarins. These include:

- Isocoumarins have also been prepared via cyclization from suitable *o*-(1-alkynyl) benzoic acids or their esters, obtained by Sonogashira coupling.[52-53]

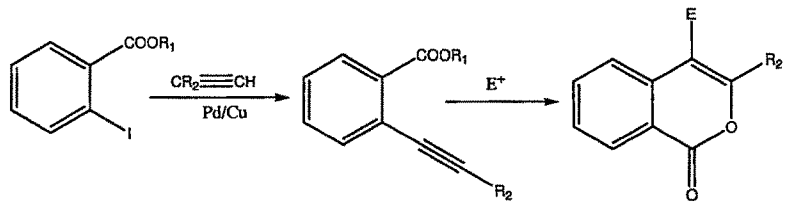


- Iodobenzoic acid reacts with various terminal alkynes in the presence of Pd $(PPh_3)_4$, Et_3N, and $ZnCl_2$ in DMF to give the corresponding 3-substituted isocoumarins.[54]



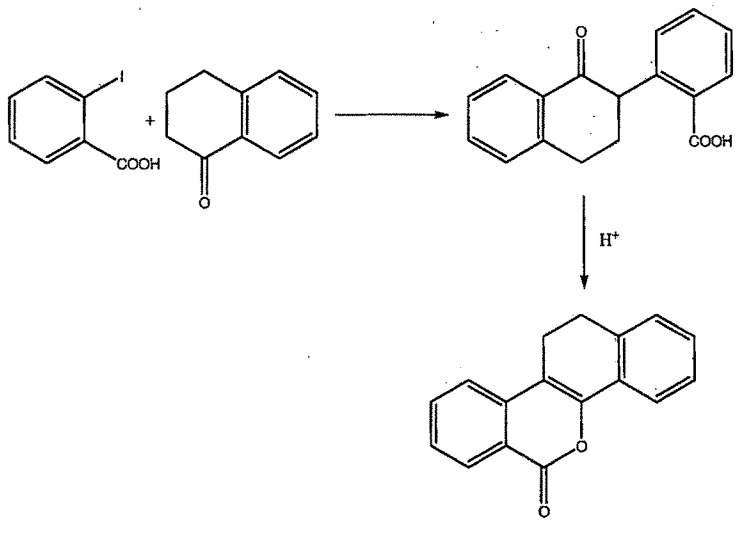
R= H, *n*-C_4H_9, *n*-C_3H_7, *o*-$C_6H_{11}CH2OH$, CH_2OCH_3, $C(CH_3)_2OH$, Ph, *p*-CH_3O-Ph, *o*-NH_2-Ph

- Synthesis of isocoumarins have also been achieved by electrophilic cyclization of analogous esters like *o*-(1- alkynyl) benzoates and (Z)-2-alken-4-ynoates.[55-57]

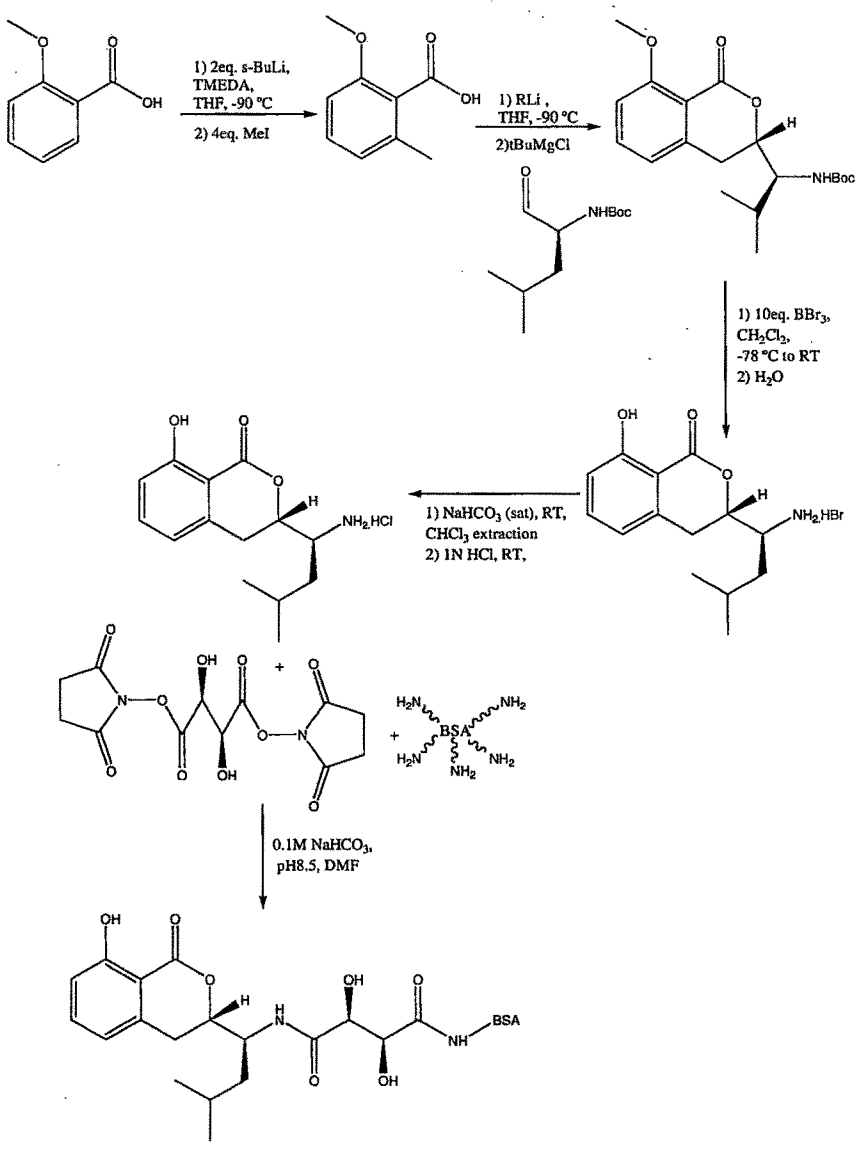


E^+ = ICl, I_2, HI, *p*-O_2N-Ph-SCl, PhSeCl

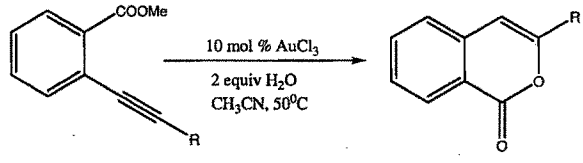
- The photochemical coupling of ortho-iodobenzoic acids with alkali metal enolates ($S_{NR}1$ reaction) is a long known and quite direct method for the preparation of isocoumarins, which has most recently been applied to the preparation of an intermediate *en* route to benzophenantridine alkaloids.[58]



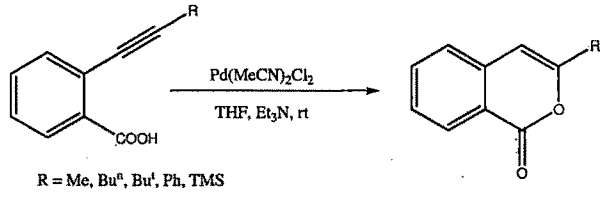
- Amicoumacin members represent a unique class of drugs because of their potent anti-ulcerogenic action without any anti-cholinergic and anti-histaminergic effects. Amicoumacins belong to a family of 3,4-dihydroisocoumarin derivatives bearing hydroxylated amino acid side chains.[59]



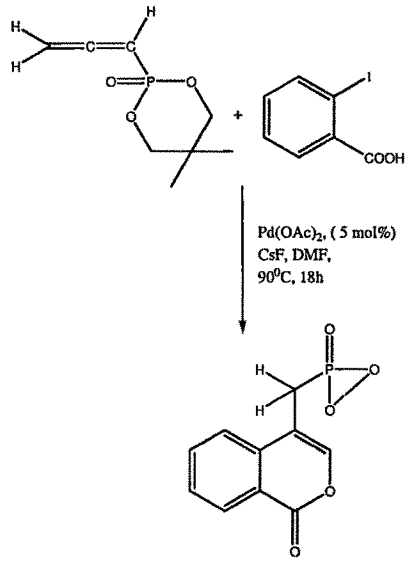
- The cyclization of γ-acetylenic esters in the presence of $AuCl_3$ yields exclusively the isocoumarins.[60]



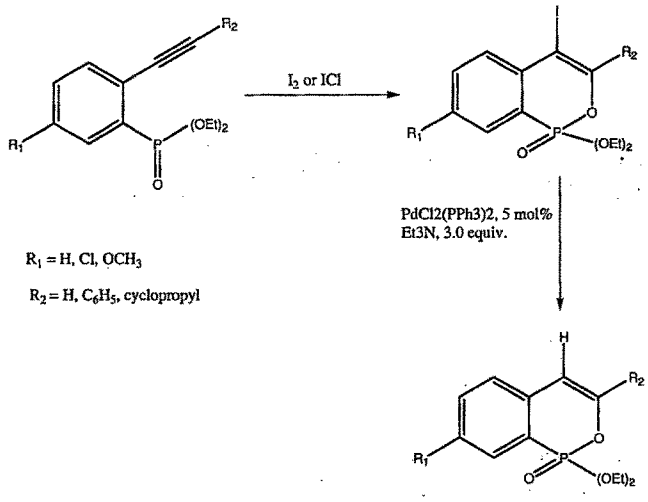
- Isocoumarins could be prepared by the palladium-catalyzed cyclization of *o*-ethynylbenzoic acids in the presence of triethylamine.[61]



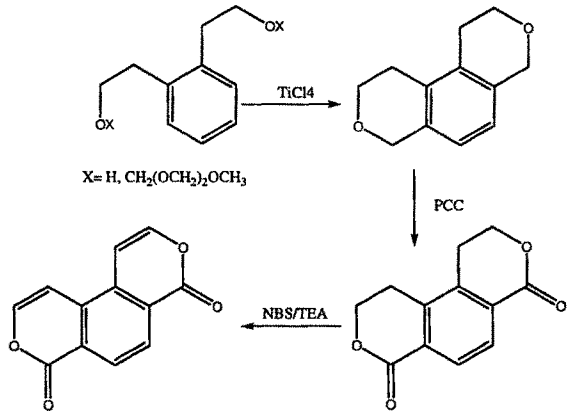
- Allenylphosphonates reacts with 2-iodobenzoic acid to give different isocoumarins.[62-63]



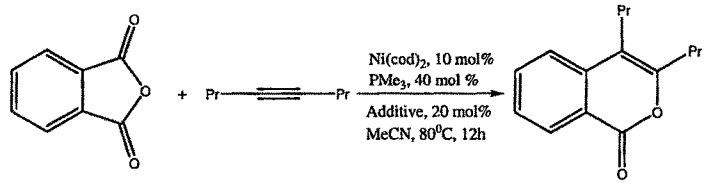
- Iodocyclization of *o*-(1-alkynyl)phenylphosphonates using I_2 or ICl as the electrophile leads to iodophosphaisocoumarins in moderate to excellent yield.[64-65]



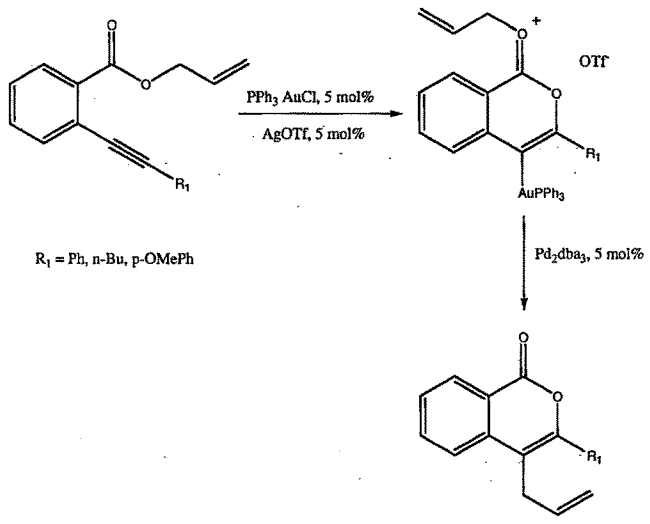
- Michael A. Kinder et al have synthesized 2-fold isocoumarin from benzo[1,2-*c*:4,3-*c*']dipyran.[66]



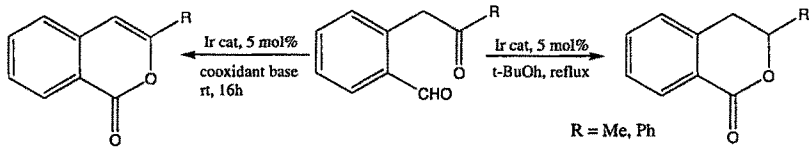
- Nickel-catalyzed decarbonylative addition of phthalic anhydride to 4-Octyne leads to 3,4-disubstituted isocoumarins.[67]



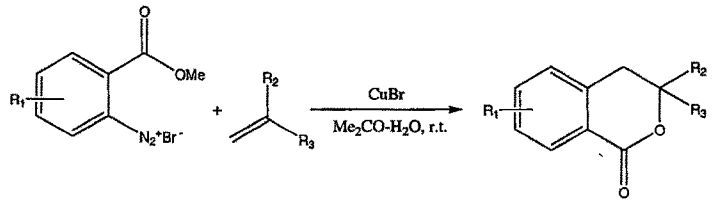
- Substituted isocoumarins can also be synthesized by using carbophilic Lewis acidic gold and Lewis basic palladium at the same time i.e dual catalyzed reactions.[68]



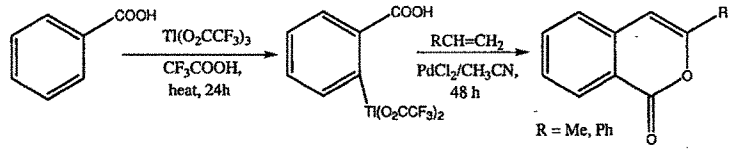
- Ir-catalyzed cyclizations of ketoaldehydes, efficiently afford isocoumarins and 3,4- dihydroisocoumarins.[69]



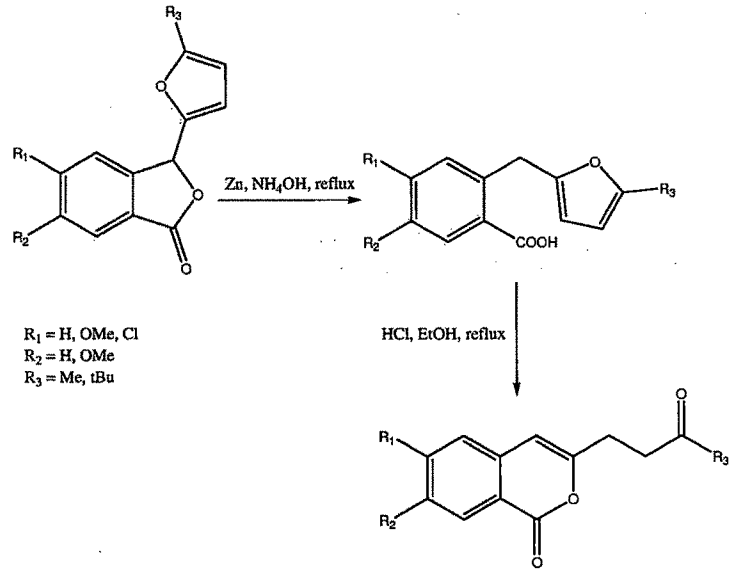
- One-pot synthesis of 3,4-dihydroisocoumarin derivatives via the CuBr-catalyzed reaction of o-methoxycarbonyl benzenediazonium bromides with unsaturated compounds have been reported by Mykola D. Obushak et al.[70]



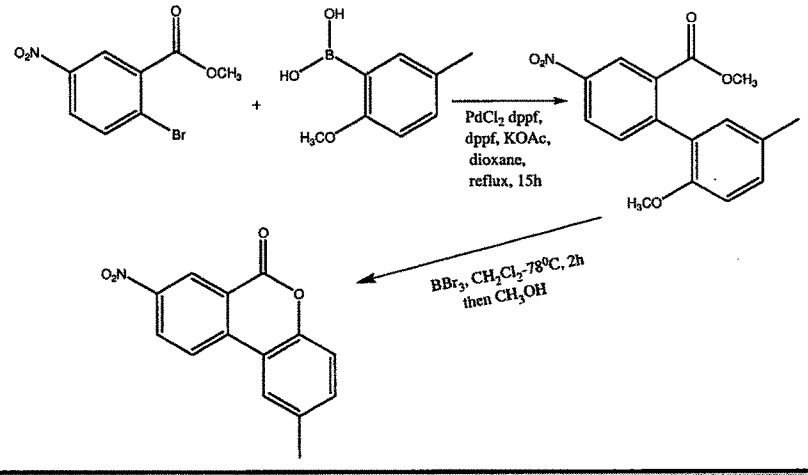
- Thallation-olefination of benzoic acids produces isocoumarins in one pot from readily available benzoic acids and a variety of simple olefins and dienes.[71]



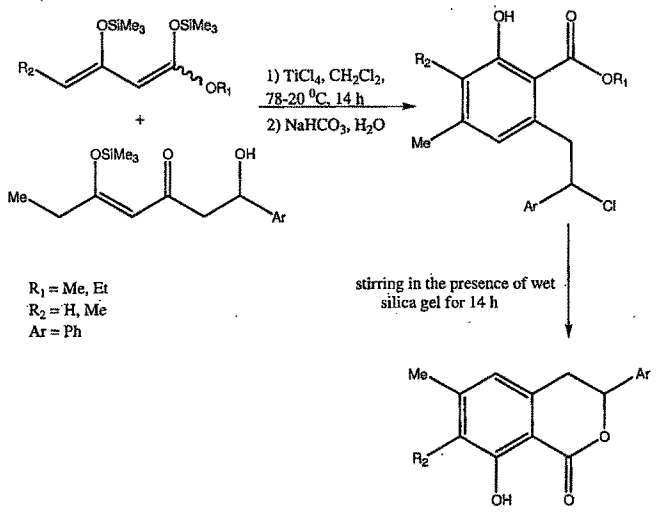
- 3-(3-Oxoalkyl) isocoumarins were prepared by Alexander V. Butin et al using 3-(2-furyl) phthalides.[72]



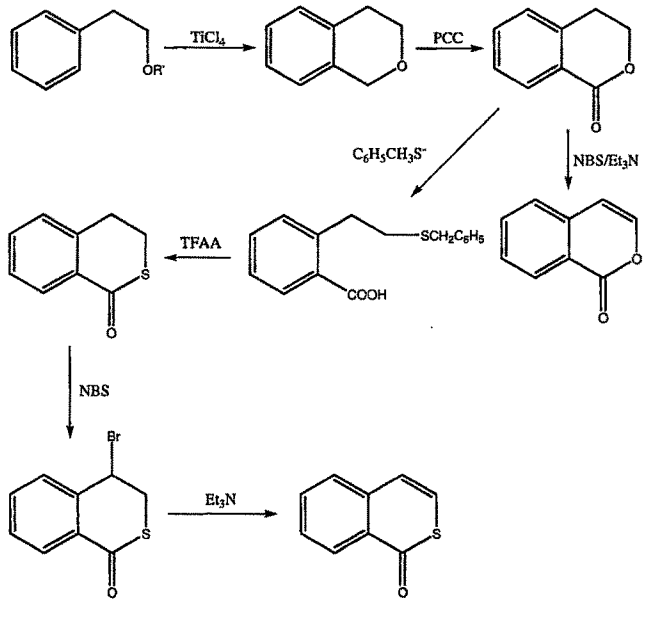
- Ce'drik Garino reported the design, synthesis and bioactive properties of benzo[c]chromen-6-one analogues from Suzuki type coupling reaction between a common bromoaryl substrate and various aryl boronic acids.[73]



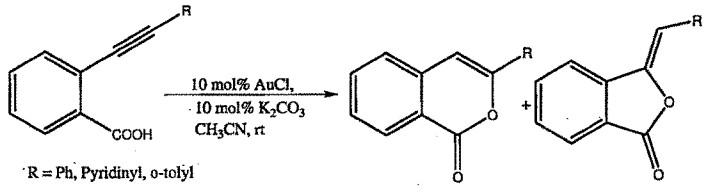
- The [3+3] cyclization of 1-aryl-1-hydroxy-5-silyloxy-hex-4-en-3-ones with 1,3-bis(silyloxy)-1,3-butadiene afforded 6-(2-phenyl-2-chloroethyl)salicylate, which was transformed into lactone by stirring in the presence of wet silica gel for 14 h . The [3+3] cyclization of 1-aryl-1-hydroxy-5-silyloxy-hex-4-en-3-ones with 1,3-bis(silyloxy)-1,3-butadienes, containing an alkyl group attached to carbon atom C_4, directly afforded the 3-phenyl-3,4-dihydroisocoumarins.[74]



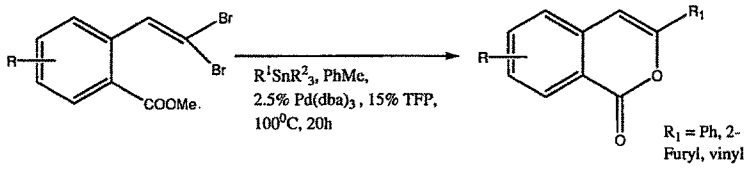
- Synthesis of novel isocoumarins and isothiocoumarins and their comparative behavior on solid state irradiations was studied by Michael A. Kinder et al.[75]



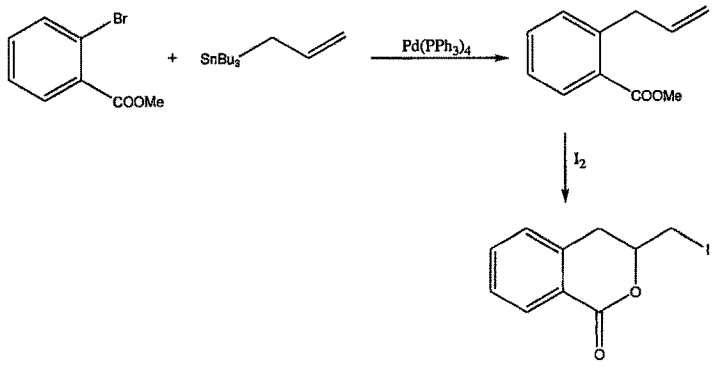
- Catalytic amount of gold (I) chloride and potassium carbonate in acetonitrile have been used to prepare some γ-alkylidene phthalides and isocoumarins from γ- and α-acetylenic acids, respectively.[76]



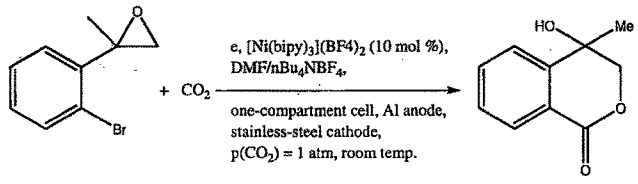
- Le Wang et al has shown that heating methyl 2-(2',2'-dibromovinyl)benzoate, an organostannane, tris(dibenzylideneacetone)dipalladium ($Pd_2[dba]_3$), and a weak ligand in a solvent of low polarity gave the corresponding 3- substituted isocoumarin in good yield . Further investigation established that best results were obtained when the reactions are run with tris (2-furyl) phosphine (TFP) in toluene.[77]



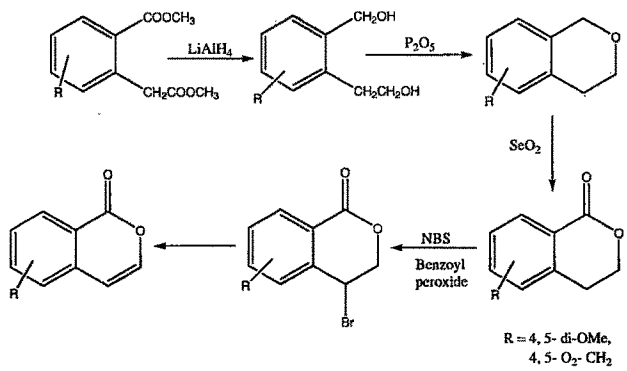
- The Pd (0) catalyzed cross coupling of alkyl ortho-iodo or ortho-bromobenzoate with unsaturated tin derivatives is an increasingly important reaction to synthesize isocoumarins and 3,4-dihydroisocoumarins.[78]



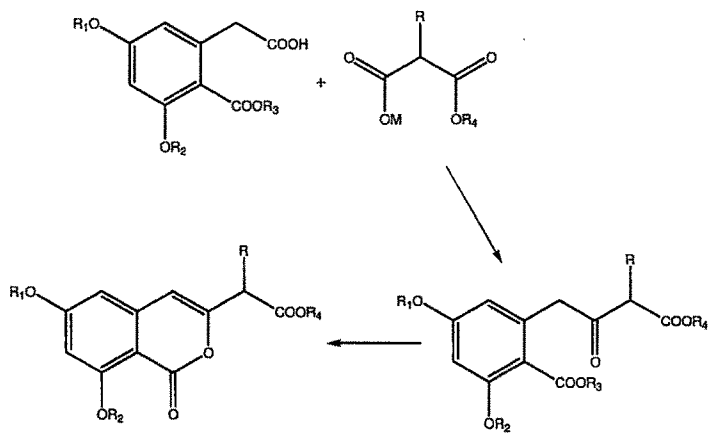
- The Ni-catalysed electrocarboxylation of aryl halides has been applied to the synthesis of benzolactones from epoxide- functionalised aromatic halides, using one-compartment cells with an Mg anode. Thus, CO_2 incorporation into 2-haloaryl epoxides led chemoselectively to carboxylated products that varied according to the nature of the substrate and of the catalytic system.[79]



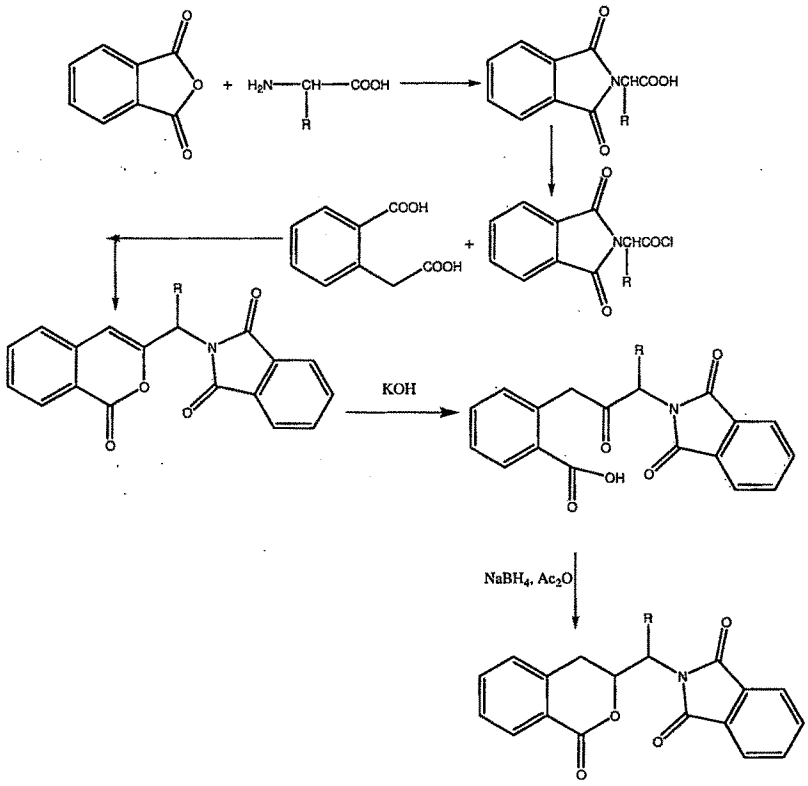
- Jagdish N Srivastava prepared isocoumarins from homophthalates.[80]



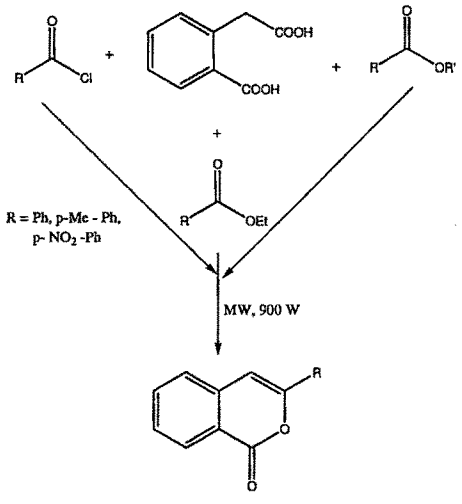
- Isocoumarin -3-yl acetic acid derivatives can be prepared by reaction between a homophthalate monoester derivative and a malonic acid monoester salt in the presence of base.[81]



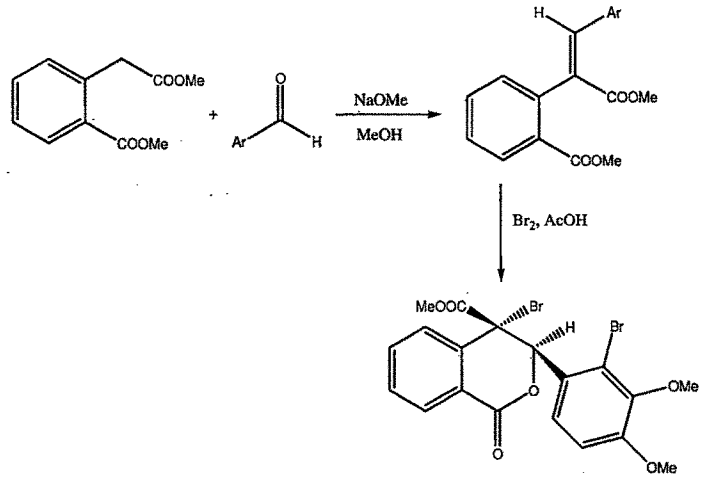
- Isocoumarins and 3,4-Dihydroisocoumarins containing L-valine and L-leucine moieties were synthesized by Khosrow Zamani et al by using the following procedure.[82]



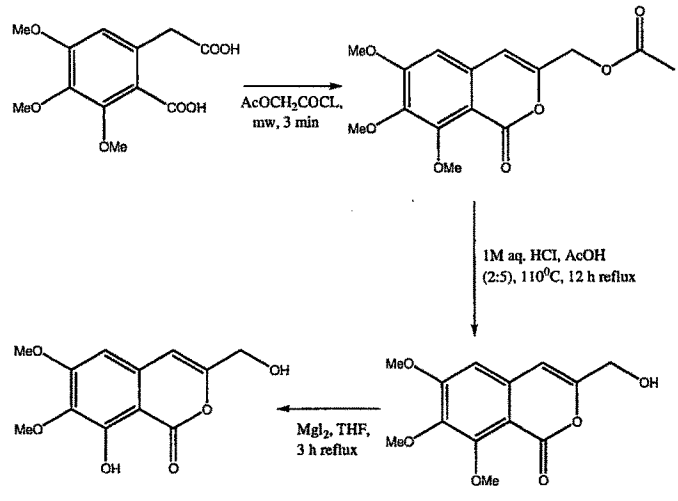
- Synthesis of 3-substitued isocoumarins, by the reaction of homophthalic acid and simpler ester derivatives, using microwave irradiation method have also been reported.[83-84]



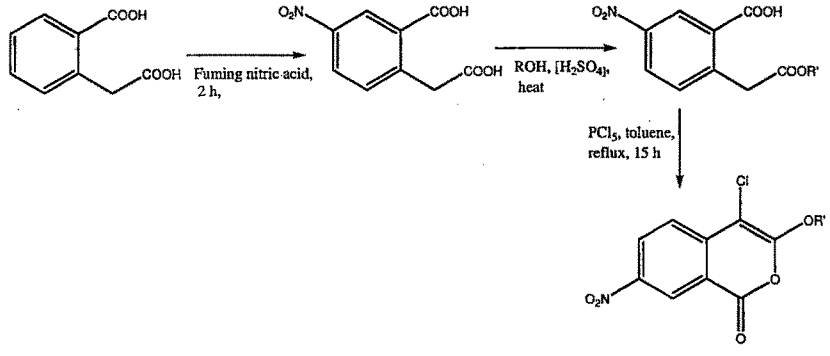
- (3R, 4S) dihydro isocoumarin derivatives have also been synthesized by using homophthalic acid derivatives and a mixture of bromine and acetic acid.[85]



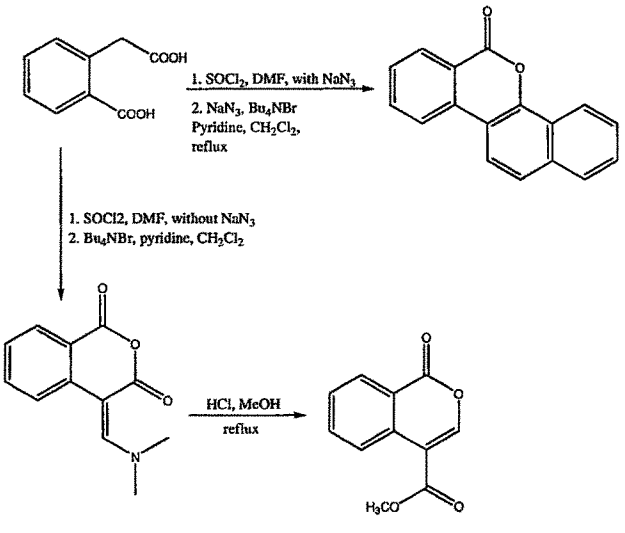
- Synthesis of cAMP Phosphodiesterase Inhibitor 8-Hydroxy-6,7- dimethoxy-3-hydroxymethylisocoumarin have been established using microwave irradiation.[86]



- 3-Alkoxy-7-nitro-4-chloro-isocoumarin derivatives have been prepared as new β-Amyloid peptide production inhibitors and their activities on various classes of protease investigated.[87]

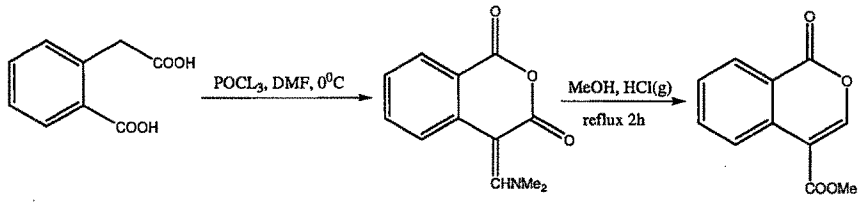


- Curtius reaction has proved to be a very good synthetic strategy for the synthesis of different isocoumarin derivatives using homophthalic acid as a starting material.[88-89]

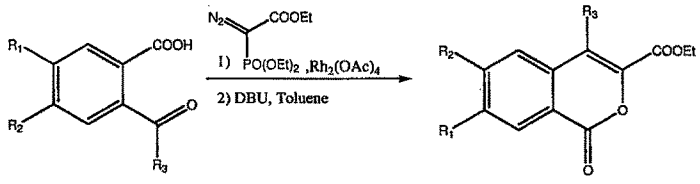


- Some time ago a novel synthesis of isocoumarin-4-carboxylic acid esters was reported, arising from initial reaction of homophthalic acid (or the anhydride

or ester derivatives) with Vilsmeier reagent (dimethyl formamide/phosphoryl chloride).[90]

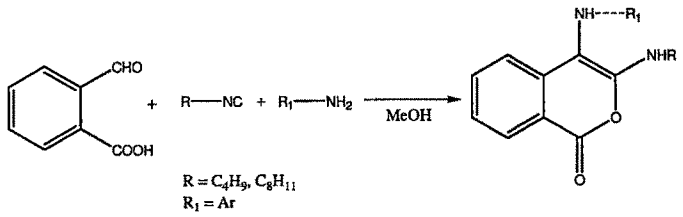


- Yoshinori Nakamura et al reported the one-pot synthesis of isocoumarins by the reaction of α-diazophosphonates with carboxylic acids.[91]



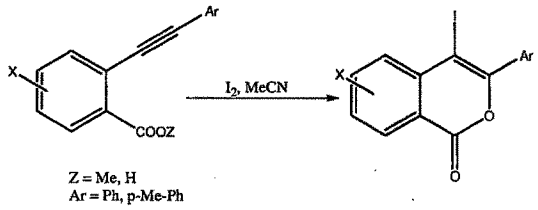
R_1 = Ph, H, Me
R_2 = H, Cl, NO_2
R_3 = H, OMe

- Ugi four-component condensation (Ugi-4CC) between 2-formylbenzoic acid, phenacylamine dimethyl acetal, and isocyanides afforded isocoumarins. These isocoumarins, where structure corresponds to the tautomeric enediamine form of the Ugi-4CC primary adducts, were stable enough to allow their isolation and characterization.[92]

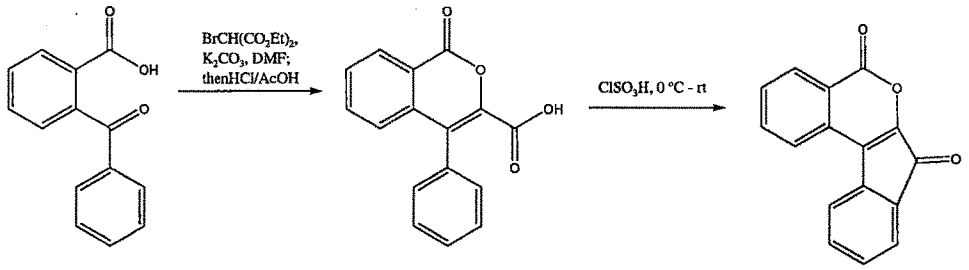


$R = C_4H_9, C_8H_{11}$
R_1 = Ar

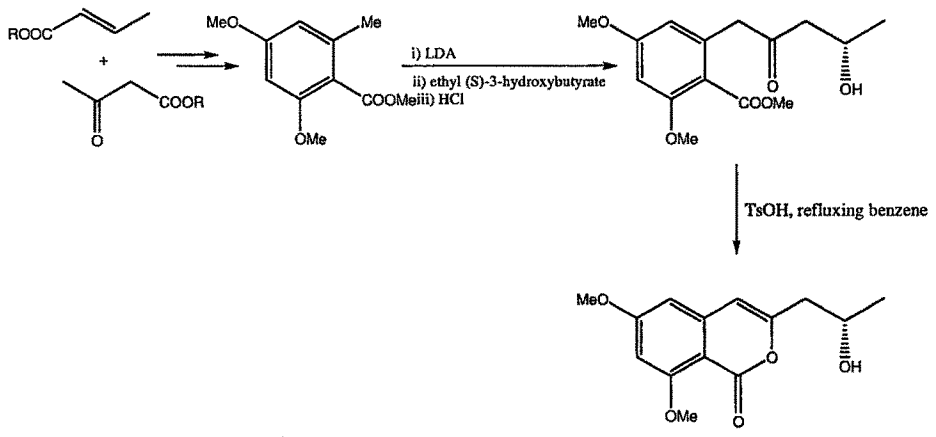
- 3-Aryl-4-iodoisocoumarins have been synthesized from corresponding methyl 2-(arylethynyl)-benzoates by iodolactonization using ICl or Iodine in MeCN, CH_2CL_2 or C_6H_6 at room temperature.[93]



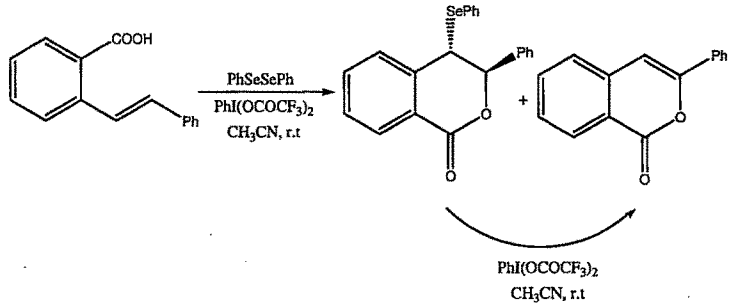
- An efficient method for the rapid construction of 3-oxoindeno [2, 1-c] isocoumarins from 4-phenyl-3-isocoumarincarboxylic acids using chlorosulfonic acid was demonstrated by Prakash G. Jagtap et al.[94]



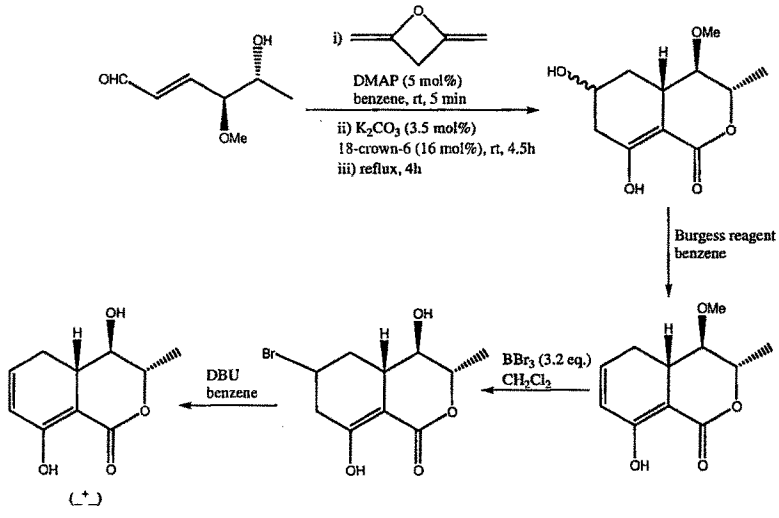
- An example of modern efficiency in isocoumarin construction *via* laterally lithiated alkylbenzoic acids is the synthesis of dimethylorthosporin.[95]



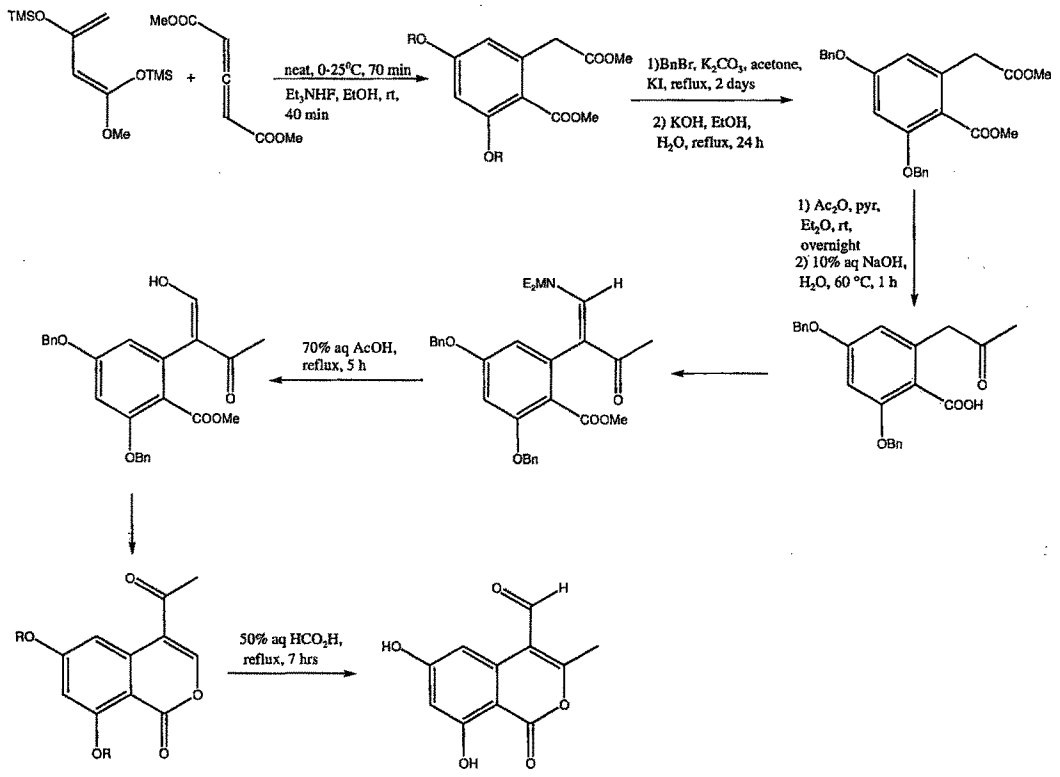
- Diphenyl disulphide has been used in the selective synthesis of dihydroisocoumarins and isocoumarins by Sohail A. Shahzad et al.[96]



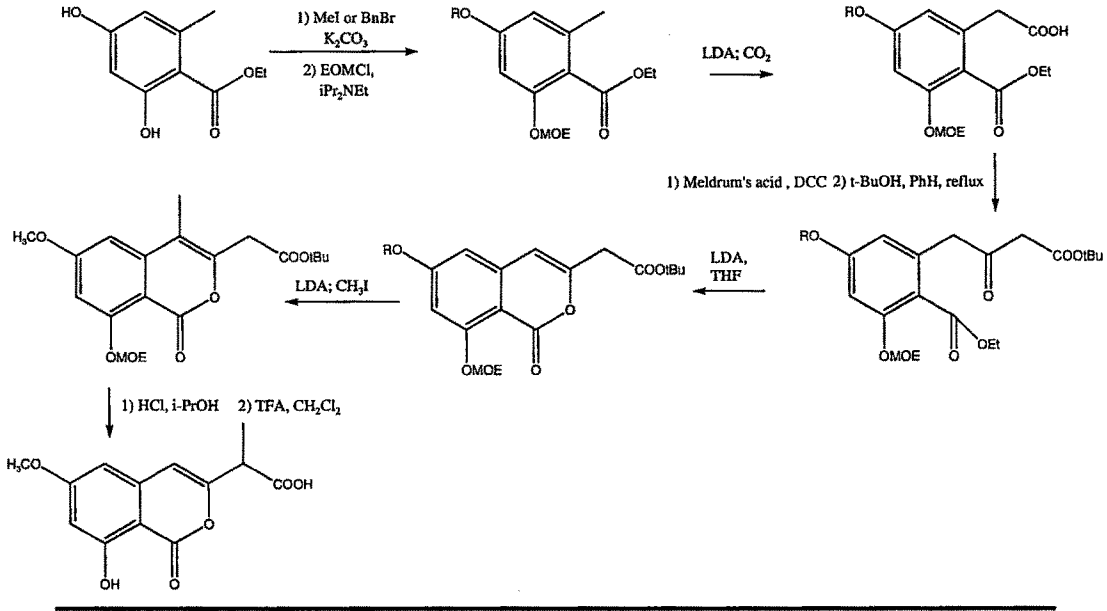
- An insecticidal tetrahydroisocoumarin, (3R,4S,4aR)-4,8-dihydroxy-3-methyl-3,4,4a,5-tetrahydro-1H-2-benzopyran-1-one, was synthesized as a racemate and as an optically active form using one-pot esterification–Michael addition–aldol reaction of δ-hydroxy-α,β-unsaturated aldehyde and diketene as a key step.[97]



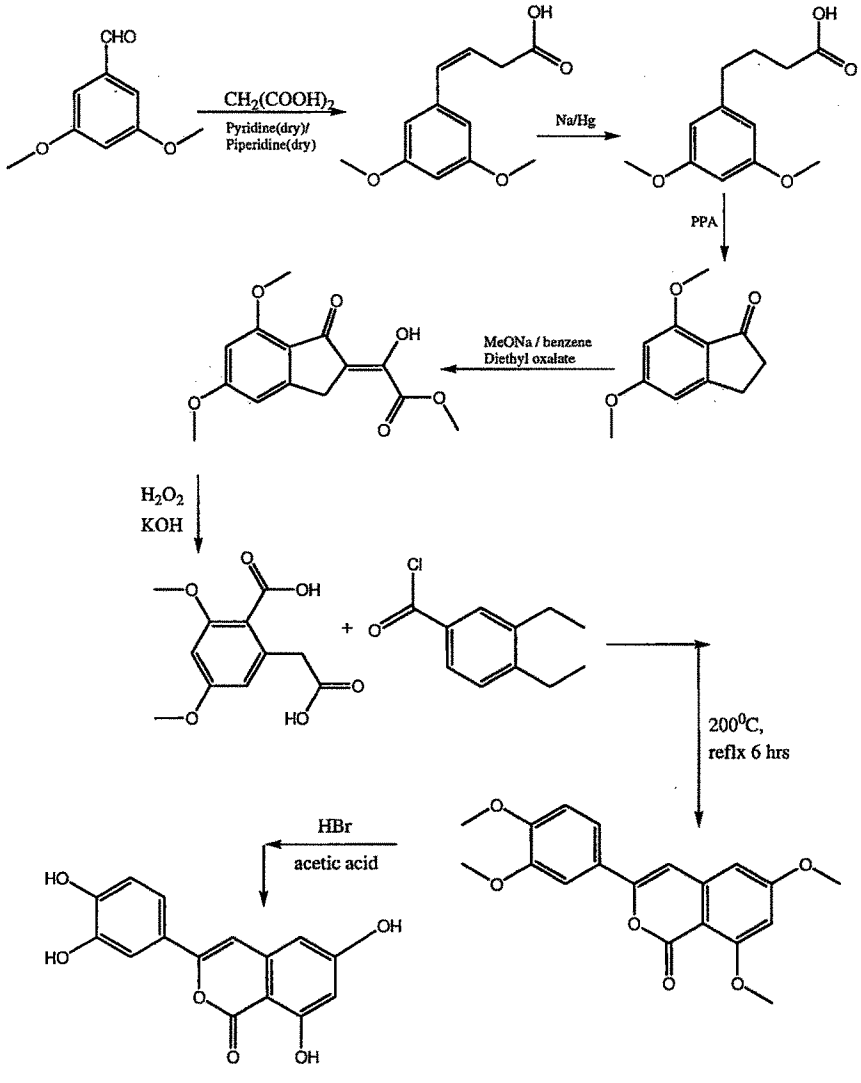
- Sanghee Kim et al recently presented the synthesis of naturally occurring isocoumarin Sescandelin.[98]



- Naturally occurring isocoumarin NM (3) was synthesized by Haiqing Yuan et al in 2004 from readily available ethyl orsellinate.[99]

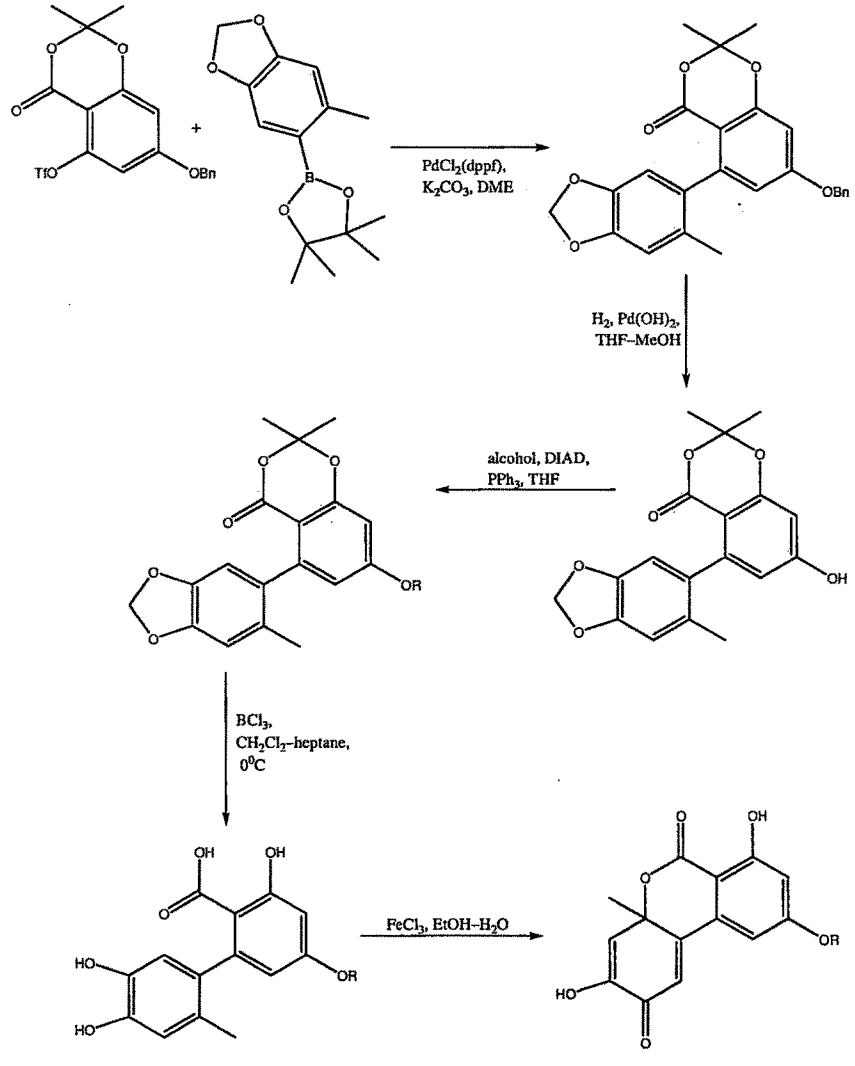


- Ghulam Qadeer et al synthesized naturally occurring isocoumarin thunberginol B in 2007 by using 3,5-dimethoxybenzaldehyde as a starting material.[100]

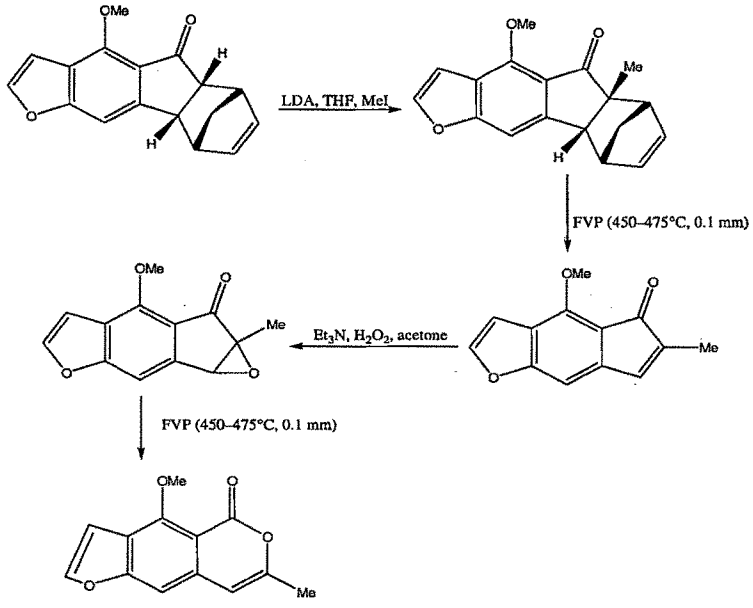


- Total synthesis of Dehydroaltenusin, first isolated from mycelium extracts of Alternaria tennuis and Alternaria kikuchiana, was reported by Kouji

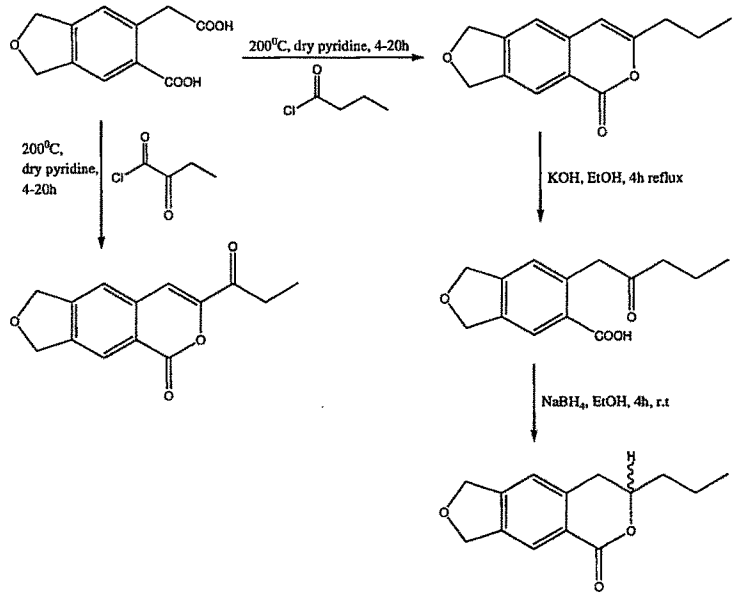
Kuramochi et al with a palladium- catalyzed Suzuki coupling as a key reaction.[101]



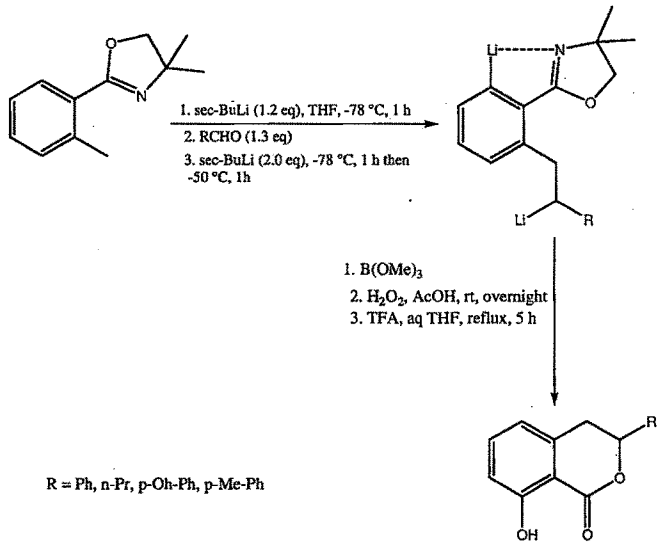
- Total synthesis of Coriandrin (**1**), one of the two naturally occurring furoisocoumarins known to date, was reported by Kraus and Ridgeway starting from 5-methylcyclohexane-1,3- dione. A Stille coupling and a Pd(II) catalyzed pyrone formation were used as the key steps. [102]



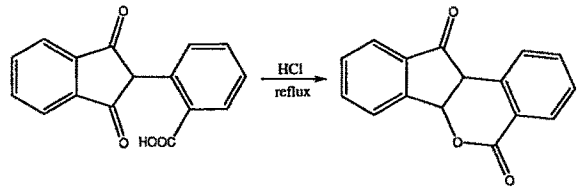
- A facile synthesis of the Xyridin A & B isolated from *Xyris indica* was accomplished by Aamer Saeed. Condensation of butanoyl chloride and 2-oxo-butanoyl chloride with 3,4 methylenedioxyhomophthalic acid afforded xyridin A and xyridin B respectively. Xyridin A was saponified to the corresponding keto acid which on reduction furnished the (±)-3,4-dihydro-6,7-methylenedioxy-3-propylisocoumarin.[103]



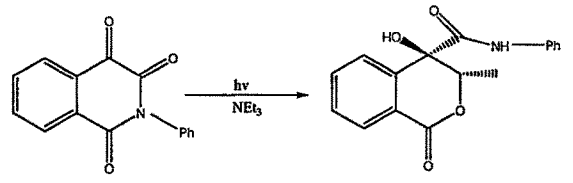
- 3-substituted 8-hydroxy-3,4-dihydroisocoumarins have been synthesized via the initial lateral lithiation of 4,4 dimethyl-2-(o-tolyl)oxazoline followed by addition to an aldehyde, the second ortholithiation, and oxidation.[104]



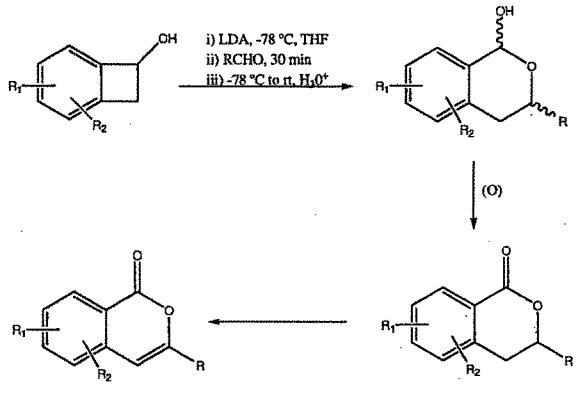
- ll-Ketoindeno[l,2-c]isocoumarin were synthesized from 2-(2-carboxypheny1)-1,3-indandion.[105]



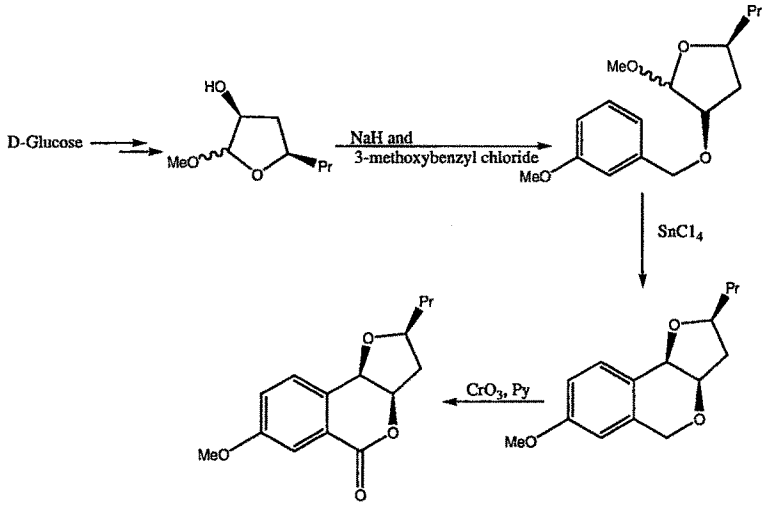
- Photochemistry of N-phenyl phthalonimide in the presence of tertiary amines leads to the competitive uptake of one alkyl radical of the amine by the α - ketoimide and a δ -1actone is obtained after oxidation.[106]



- Isocoumarin derivatives have also been synthesized by the facile conversion of a variety of sparingly/ difficultly soluble lactols into lactones using IBX under modified conditions.[107]

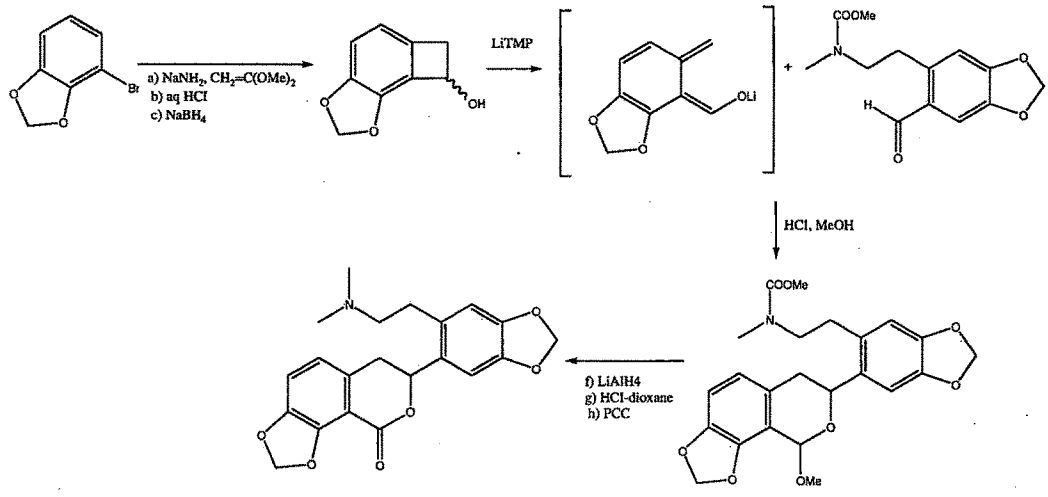


- The intramolecular electrophilic substitution of benzoylated or benzylated a-hydroxy carbonyl compounds is another traditional method to elaborate the heterocyclic portion of either isocoumarins and 3,4-dihydroisocoumarins, which have been recently adopted for the synthesis of monocerine analogues.[108]

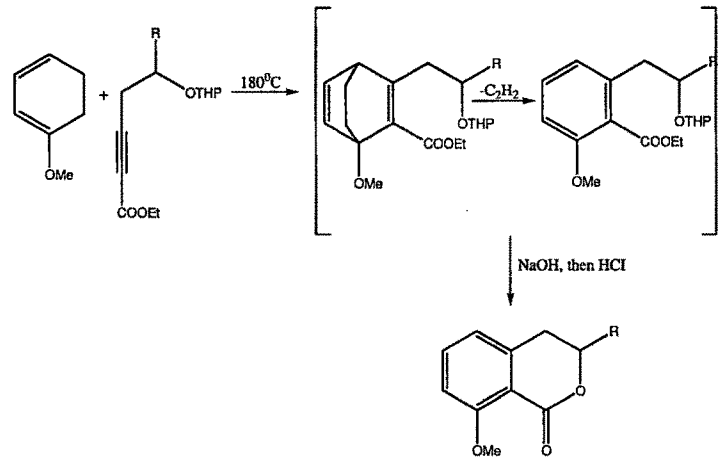


- Ortho-tolualdehyde anions, which can be generated by ring opening of benzocyclobuteneoxides; can add efficiently to aromatic aldehydes giving

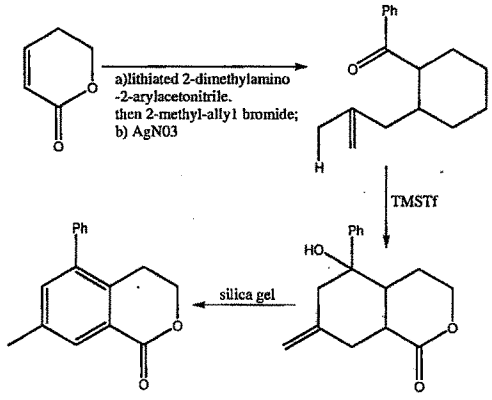
benzopyranols which are easily oxidized to 3 substituted 3, 4-dihydroisocoumarins. Such an approach is exemplified in the synthesis of peshawarine.[109]



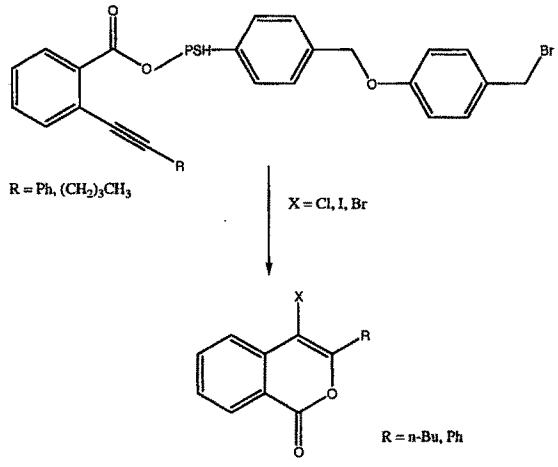
- The homocyclic portion of isocoumarins can also be constructed by means of a variety of pericyclic reactions. Mellein, methoxymellein and phyllodulcin have been obtained by Diels- Alder reaction of 1 -methoxy- 1,3-cyclohexadiene with the appropriate 5-hydroxy-2-alkynoic acid derivatives.[110-111]



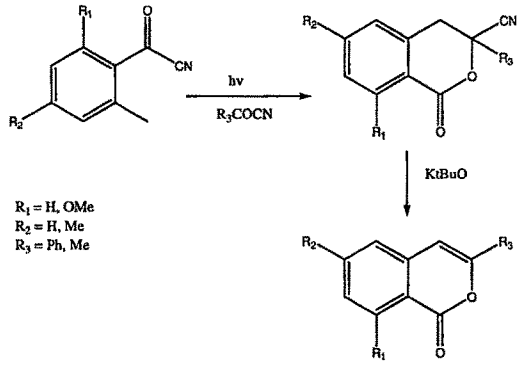
- An isocoumarin has been obtained by aromatization of a bicyclic lactone resulting from an intramolecular carbonyl-ene reaction.[112]



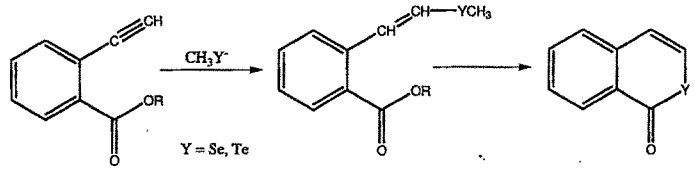
- 3-substituted 4-haloisocoumarins have been synthesized by solid-phase synthesis based on an electrophile-promoted traceless halocyclization of supported 2-(alk-1-ynyl) benzoates.[113]



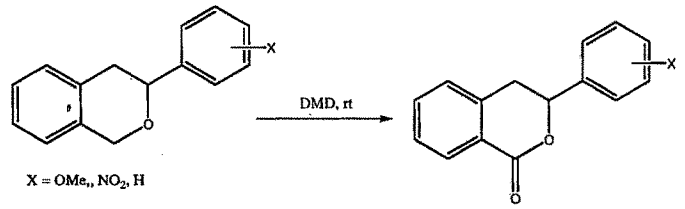
- Aryl cyanides have been utilized in the synthesis of isocoumarins.[114]



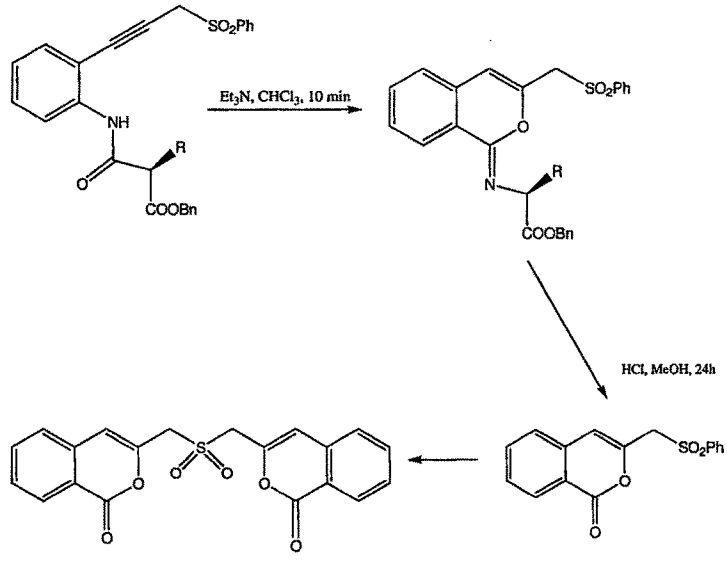
- Seleno and Telluro Isocoumarins have been synthesized from Ethyl o-ethynylbenzoate.[115]



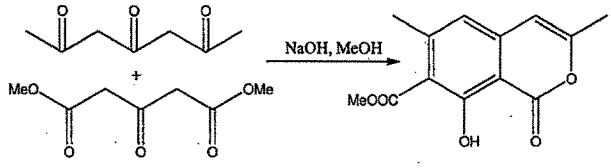
- 3-aryl isochromans on oxidation with Dimethyl dioxirane gives 3-aryl isocoumarins.[116]



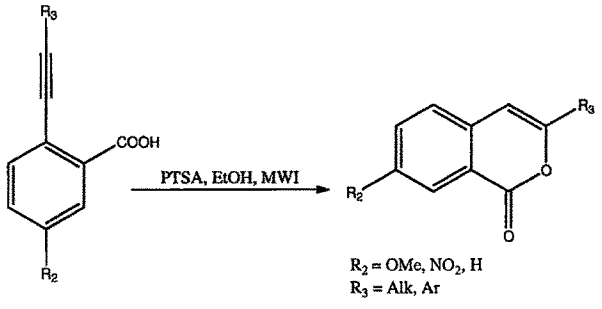
- A new class of C2-symmetric bis-isochromene derivatives with 3,3'-linkage has been synthesized from bis-propargyl sulfones. The method involves treatment of the sulfones with triethylamine to form the isochromene derivatives presumably via the intramolecular Michael addition to the intermediate bis-allenic sulfones.[117]



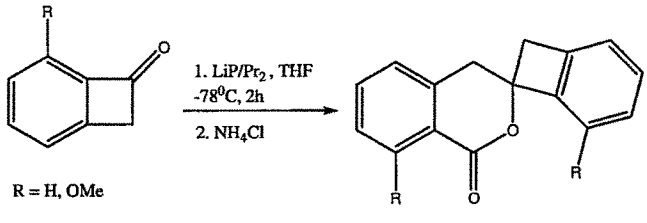
- Isocoumarins can be approached by building the homocyclic portion in the last carbon-carbon bond forming step. In biomimetic type synthesis of isocoumarins, the homocyclic portion is obtained by aldol or Claisen condensation of polyketide components.[118]



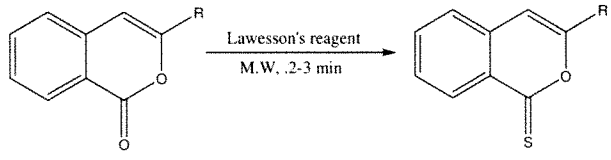
- An intra molecular cyclization reaction, promoted by PTSA, of *ortho*-substituted diarylalkynes, with substrates bearing an ethoxycarbonyl or a carboxylic acid function (CO_2Et, COOH) at the *ortho* position, yields 3-substituted isocoumarins in good to excellent yields.[119]



- When benzocyclobutenone is treated with LDP in presence of ammonium chloride under the usual reaction conditions, the spiro anellated isochromanone is obtained in high yield.[120]



- A rapid microwave-accelerated thionation of some 3-substitued isocoumarins to corresponding 1-thioisocoumarins was achieved employing Lawesson's reagent under solvent less conditions.[121]



R = Aryl, Alkyl

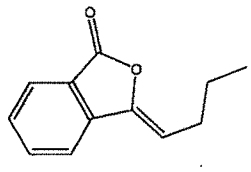
Isocoumarins encompass many interesting substances from the natural kingdom as well as useful synthetic intermediates in the synthesis of other classes of compounds. Owing to their borderline position between aromatic and aliphatic compounds, their chemistry is particularly rich and fascinating. Similar to isocoumarins is another class of compounds, Phthalides, which are also naturally occurring, having many biological applications.

The Phthalide group contains several closely related compounds such as butylphthalide, sedanolide, ligustilide, and sedanenolide. They are a group of secondary metabolites or phytochemical compounds classified under lactones. These compounds are found in celery, Dong Quai and lovage among other plants.

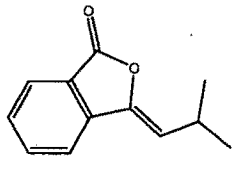
Phthalides are known to provide health benefits by stimulating and/or inhibiting various enzymes in the body. Studies have shown that these compounds can help lower blood pressure, provide an anti-inflammatory function, improve circulation, rid the body of toxins such as uric acid crystals, inhibit malignancy and offer a calming effect.

Many of the major phthalides have been isolated from plants mainly medicinal herbs, celery stalks, celery seeds and essential oils of their plant of origin. There are numerous reports on the bioactivities of phthalides but investigations concerning the mode of action are few.

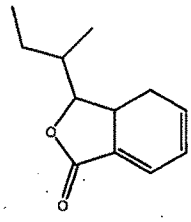
Following are the some examples showing naturally occurring phthalides: [122-140]



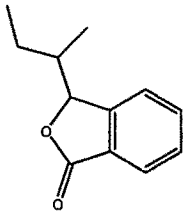
cis-Butylidene phthalide



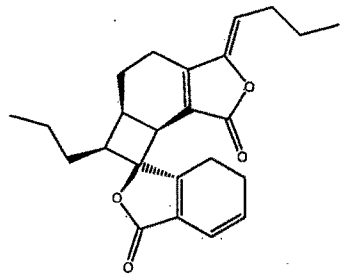
cis-3-Isobutylidene phthalide



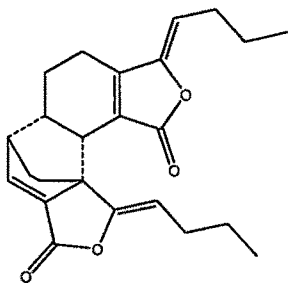
3- Butyl dihydro phthalide



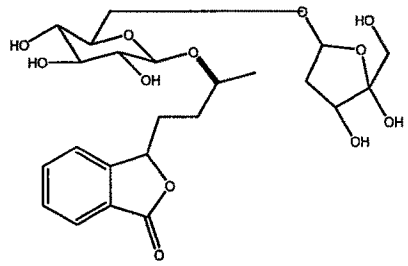
3- Butyl phthalide



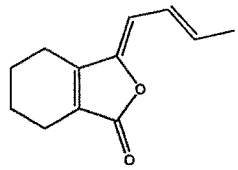
(Z)-6,8',7,3'-Diligustilide



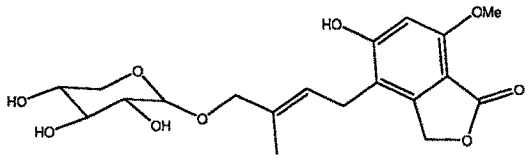
Z-Levistolide A

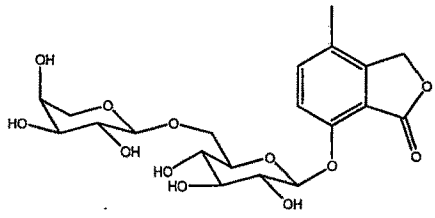


Celephthalide B

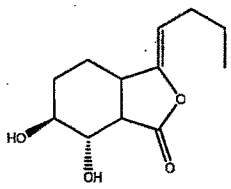


(3Z),(2'*E*)-3-but-2'-enylidene-4,5 6,7-tetrahydrophthalide

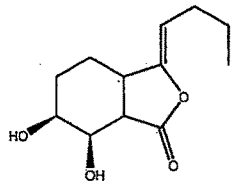




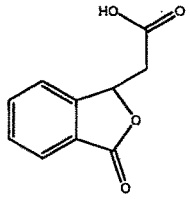
Arenophthalide B



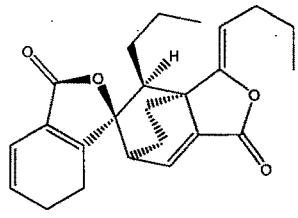
Senkyunolide I



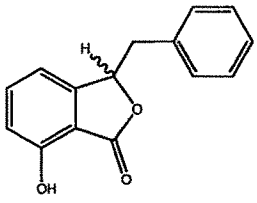
Senkyunolide H



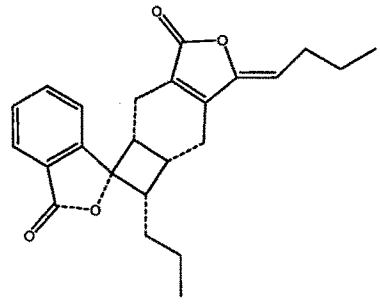
3-Carboxyrthyl-phthalide



(3Z)-(3aR,6S,3'R,8'S)-3a.8',6.3'-diligustilide



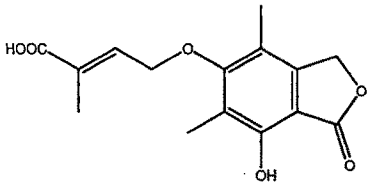
Typhaphthalide



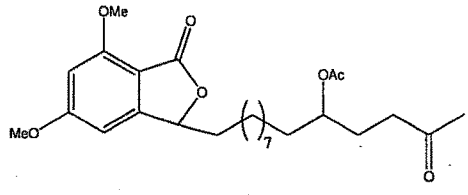
Gelispirolide

Following are the few recent examples of biologically active phthalides:

Antimicrobial Phthalides[141-142]

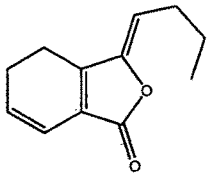


(E)-4-[(1,3-Dihydro-7-hydroxy-4,6-dimethyl] -2-methyl-2-butenoic acid phthalide

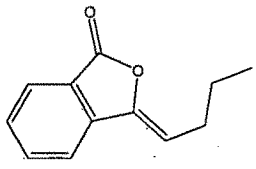


CJ-13,102

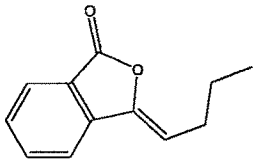
Anticholinergic Phthalide[143]



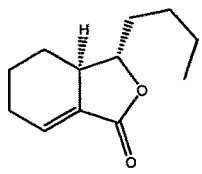
Vasodilator Phthalide[144-145]



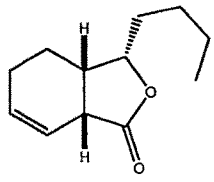
Insecticidal Phthalides[146-147]



Butylidienephthalide

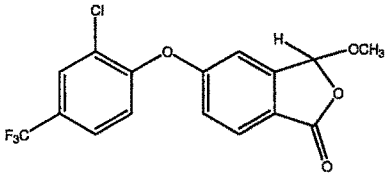


(3S) – Butylphthalide

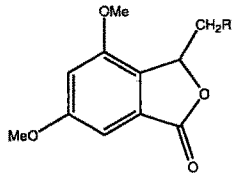


Cnidilide

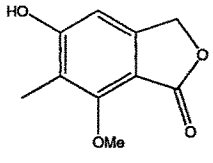
Peroxidizing Phthalide[148]

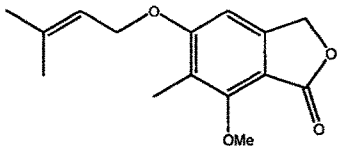


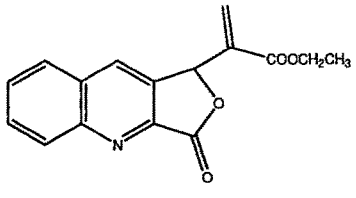
Helicobacterial Phthalide[149]



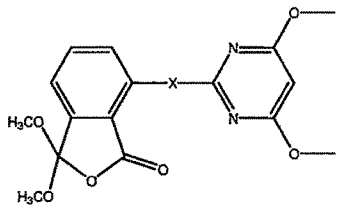
Anti tumour Phthalides[150-151]

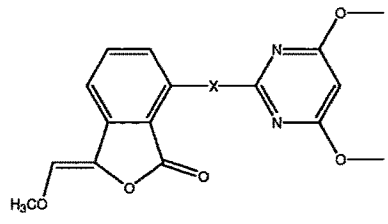






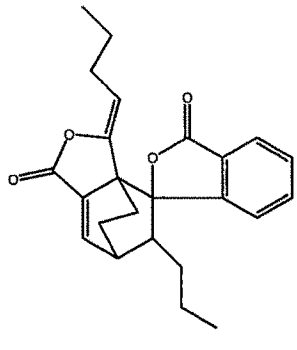
Herbicidal Phthalides[152]



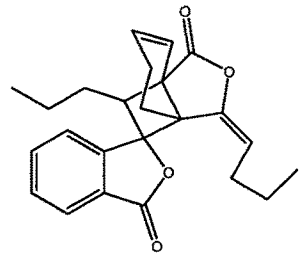


Pyrimidinyl Phthalides

Anti oxidant Phthalides[153]

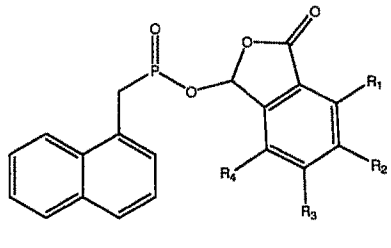


Anaspirolide



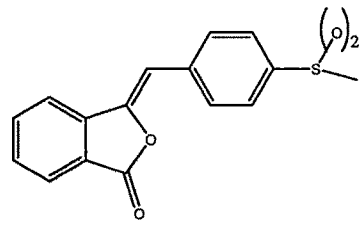
Sinaspirolide

Enzyme Inhibiting Phthalide[154]



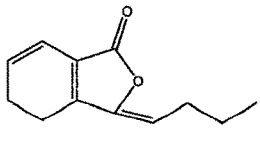
Phosphonate-3-phthalidyl esters

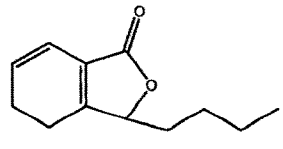
Anxiolytic Phthalide[155]



Benzalphthalides

Anti inflammatory Phthalides[156]



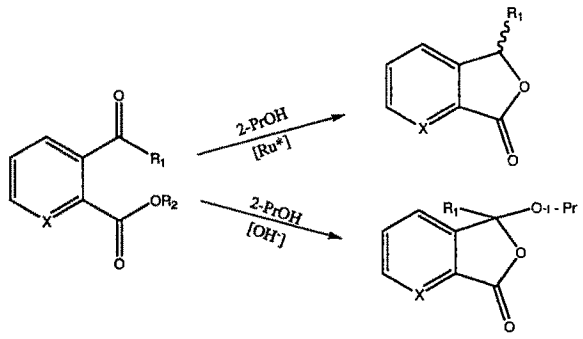


Dihydro Phthalides

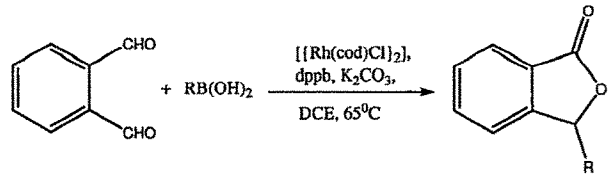
Phthalides (isobenzofuranone), a family of five-membered lactones in plants, are important building blocks in a large number of biologically active compounds. 3-Arylphthalides, for example, are useful intermediates for the synthesis of triand tetracyclic natural products such as anthracycline antibiotics. Phthalides are versatile starting materials for the synthesis of a variety of structures and the derivatives are also key intermediates for the synthesis of natural products. Approaches have been developed for the synthesis of such organic skeletons. Some 3-alkyl substituted phthalides exhibit pharmacological applications, and some others have been used as starting materials for the synthesis of carbo- and heterocycles. These facts led to an increased interest in the synthesis of these compounds.

Following are the recent methods deployed for the synthesis of different derivatives of phthalides:

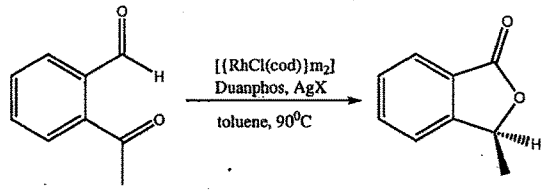
- Catalytic asymmetric reduction of 2-acylbenzoates using in situ combinations of $[RuCl_2$ (p-cymene)$]_2$ with either TsDPEN or β-amino alcohols as ligands, in the presence of a base promoter is a process of high interest to prepare chiral phthalides.[157]



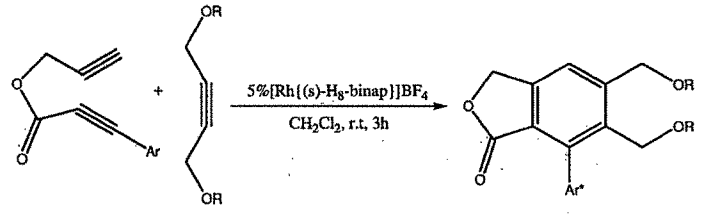
- Zhishi Ye et al reported a novel and facile strategy to obtain phthalide starting from commercially available phthalaldehyde and arylboronic acids based on the well-developed rhodium-catalyzed addition of arylboronic acids to aldehydes.[158]



- Enantioselective synthesis of phthalides using catalytic intramolecular ketone hydroacylation was demonstrated by Michael C. Willis.[159]

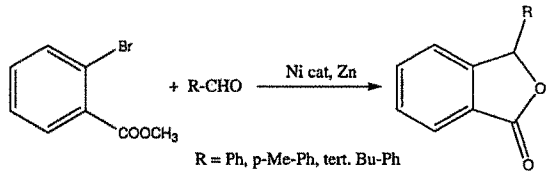


- Phthalides have been synthesized by a highly enantioselective reaction of the cationic [$Rh^{I}(H_8$-binap)] complex catalyzed cross alkyne cyclotrimerization of unsymmetrical α,ω-diynes and unsymmetrical or symmetrical monoynes.[160]



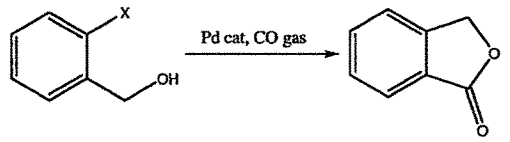
Ar = p-Me-Ph, Naphthyl
R = h, Ac

- Treatment of 2-iodobenzoate with benzaldehyde in the presence of a mixture of [CoI_2 (dppe)] (5 mol %) and zinc metal powder (2.75 mmol) in THF at 75^{0}C leads to the cocyclization of iodobenzoate with aldehyde and the formation of phthalide derivative.[161-162]

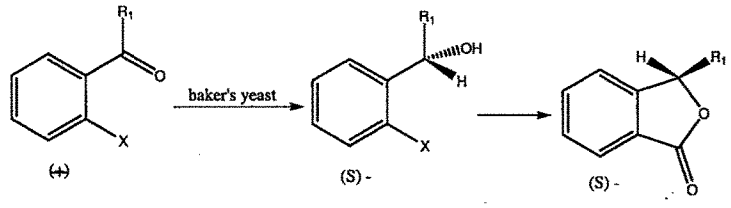


R = Ph, p-Me-Ph, tert. Bu-Ph

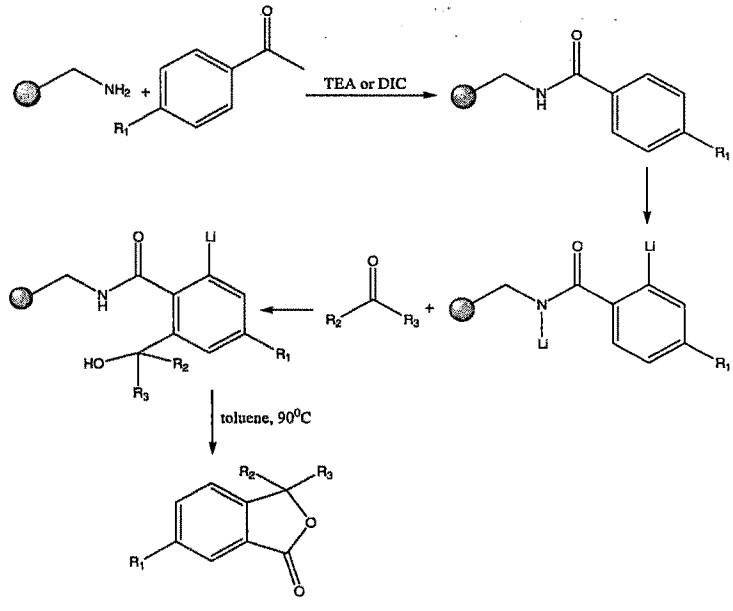
- Larock et al. and Kazuhiko Orito reported a two-step process for the synthesis of phthalide involving an ortho-thallation of o-iodobenzyl alcohols and a palladium- catalyzed cyclocarbonylation of the thallated intermediate.[163-164]



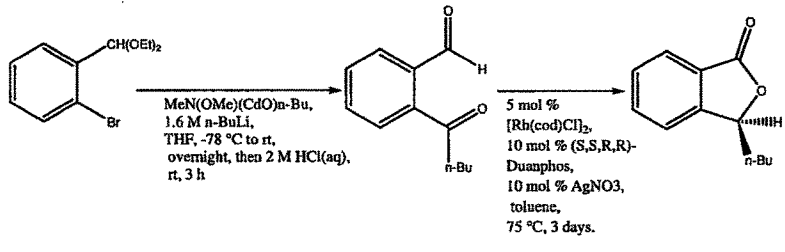
- The chemoenzymic synthesis of 3-substituted phthalides using baker's yeast, lipases and esterases was shown by Taeko Izumi et al.[165]



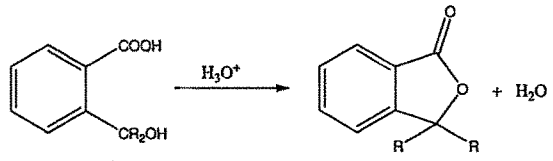
- Patrick Garibay et al have synthesized a phthalide library by using directed ortholithiation of resin-bound benzamides. Similar library was reported by Shimomura et al also. One of the scheme is shown below:[166-167]



- Total synthesis of Celery Extract (*S*)-(-)-3-*n*-Butylphthalide using Rh catalyst has also been reported recently.[168]

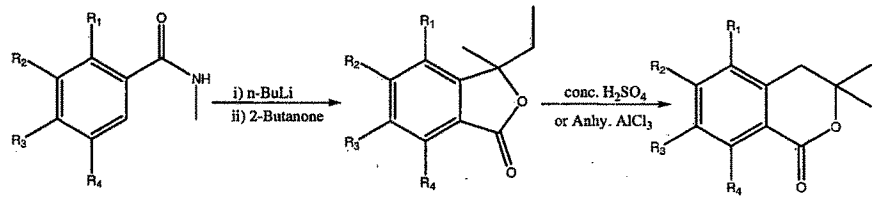


- Phthalides have also been synthesized by lactonization of hydroxyl acids in one step.[169]



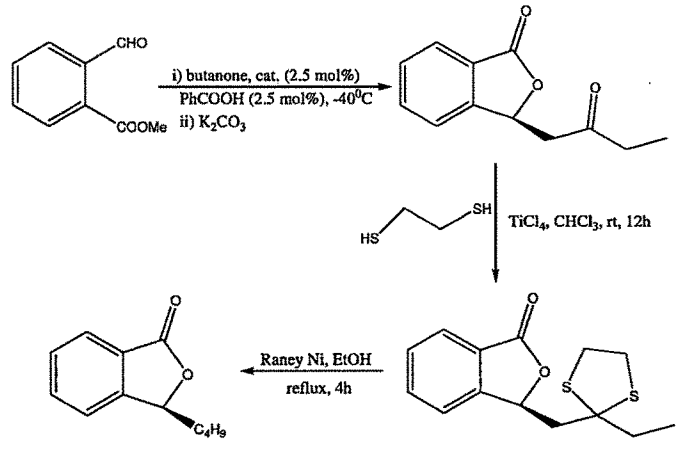
R = H, Me, Ph

- N-methyl benzamides have also led to different derivatives of phthalides which in turn have been used to synthesize dihydroisocoumarins.[170]

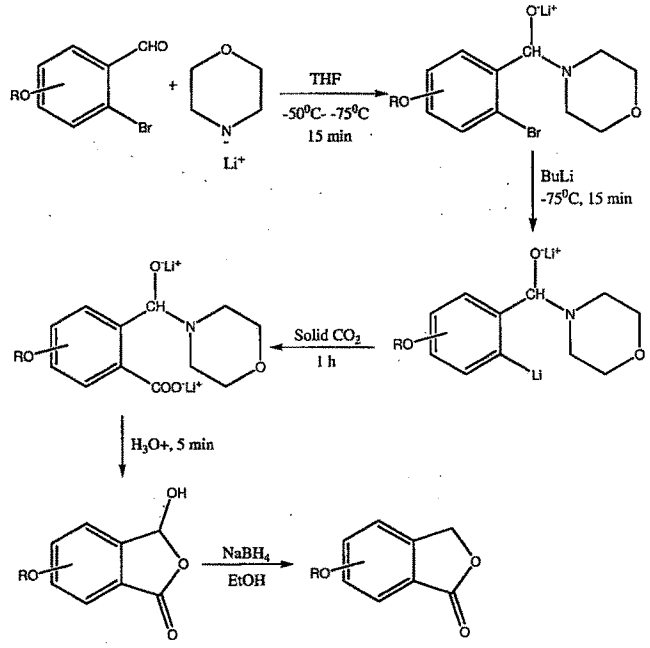


R_1 = H, OMe, OH
R_2 = H, Me
R_3 = H, Cl, Me
R_4 = H,Me

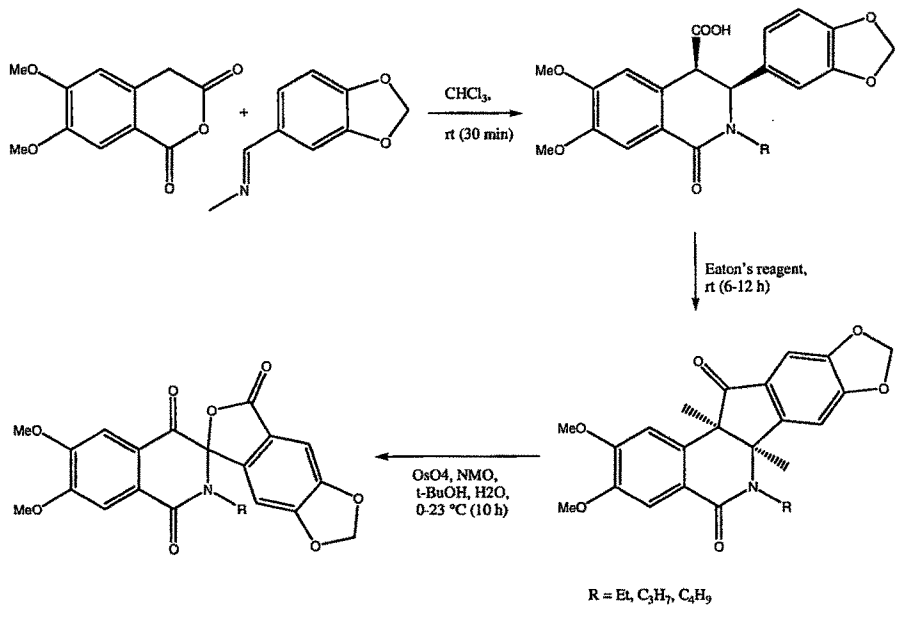
- New highly efficient organocatalytic enantioselective aldol-lactonization process for the facile preparation of chiral 3-substituted phthalides from simple achiral starting materials under mild reaction conditions was proposed by Zhang et al and they have successfully applied the powerful method for efficient synthesis of natural product (S) 3-butylphthalide in three steps with high yields and high enantioselectivity. [171]



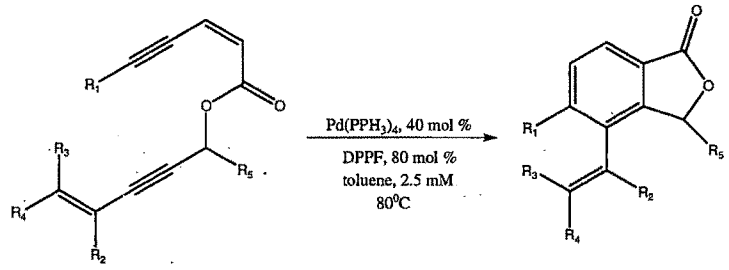
- A variety of phthalides have been synthesized starting from o-bromobenzaldehyde via ortho lithiated aminoalkoxides.[172]



- Isoquinoline-3-spiro-3'-phthalides were synthesized by oxidative transformation of indenoisoquinolines in the presence of osmium tetraoxide and 4-Methylmorpholine *N*-oxide.[173]

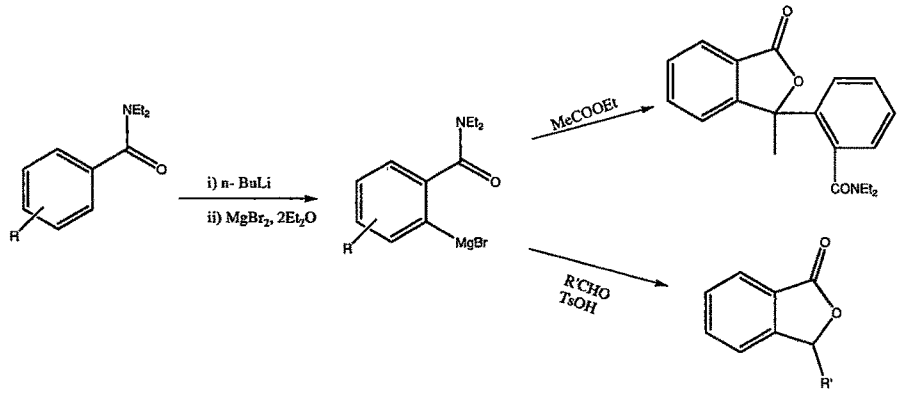


- A novel method for the synthesis of phthalides and 3,4-dihydroisocoumarins via the palladium catalyzed intramolecular benzannulation of bis-enyne and enyne-diyne systems was described by Taishi Kawasaki et al.[174]

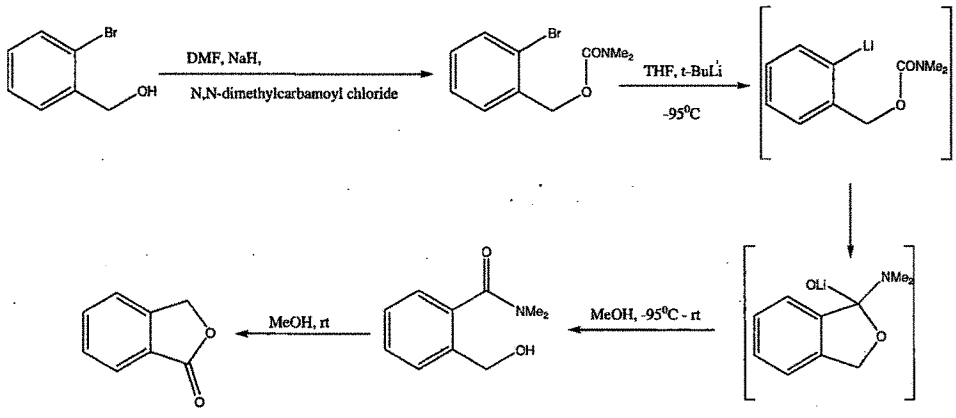


R_1 = H, Ph, C_3H_7, R_2 = H, Me, R_3 = H, CO_2Me
R_4 = H, C_6F_{13}, R_5 = H, Ph, C_4H_9

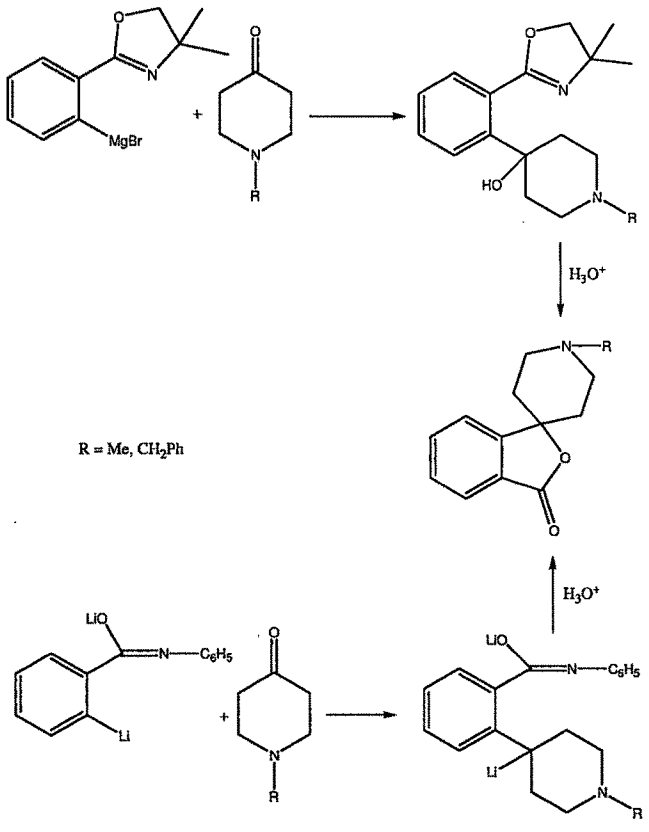
- Mukund P. Sibi et al have proved that the transmetalation of ortho-lithiated benzamides into the bromomagnesium counterpart allows introduction of allyl and hydroxyalkyl moieties, thereby extending the utility of the benzamide-directed metalation strategy. The resulting products may be economically converted into phthalides and isocoumarins.[175]



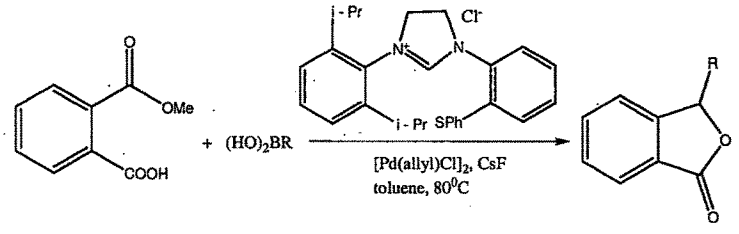
- A variety of phthalides can be obtained by lithium-halogen interchange followed by internal trapping from carbamates derived from o-bromobenzylic alcohols.[176]



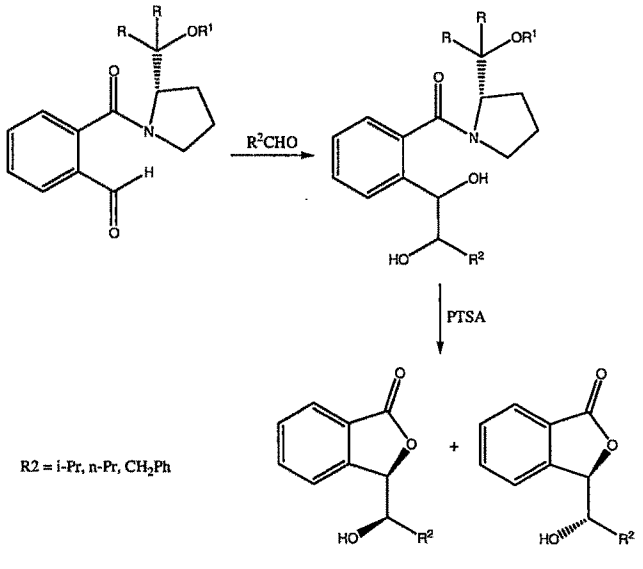
- Synthesis of spiro phthalides was established by Adrian Marxer et al from magnesium derivative of 2-(2-bromophenyl)-4,4-dimethyloxazoline with N-alkylpiperidones in 1975.[177]



- Efficient synthesis of 3-arylphthalides was achieved using the arylation of aldehydes with organoboronic acids catalyzed by the palladium/thioether imidazolinium chloride system.[178]

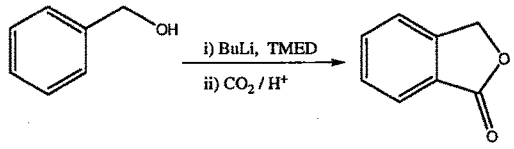


- Optically Active 3(1-Hydroxyalky1) phthalides have been synthesized by stereoselective pinacol cross-coupling in the presence of vanadium (II) species.[179-180]

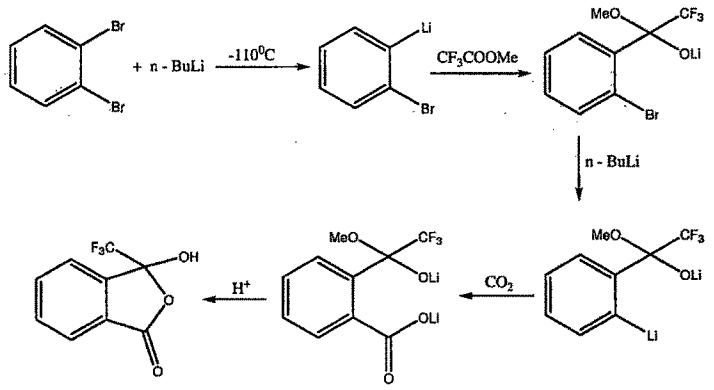


- The thallation and subsequent palladium catalyzed carbonylation of simple arenes, benzylic and o-phenethyl are known to afford benzoate esters, phthalides, 3, 4-dihydroisocoumarins respectively. The carbonylation reaction

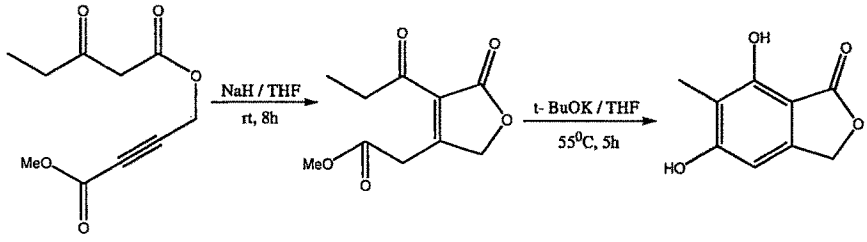
proceeds in excellent yield at room temperature and atmospheric pressure and is highly stereo- and regioselective.[181-182]



- Phthalides with substituents in the heterocyclic ring have been prepared starting from o-dibromobenzene via sequential metal-halogen exchange reactions and treatment with appropriate electrophiles. Low temperature is essential for the stability of the intermediates involved in this reaction.[183]

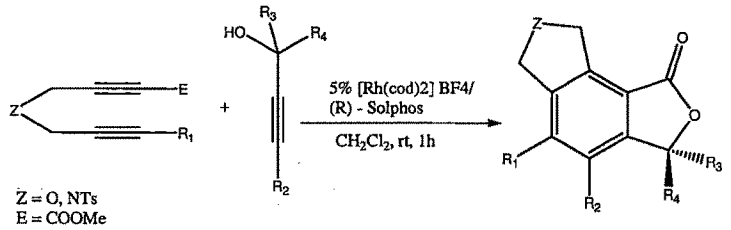


- Demethylnidulol isolated from natural sources, Aspergillus nidulans and Aspergillus duricaulis was synthesized via an intramolecular michael reaction by Rogelio Jime´nez et al.[184]



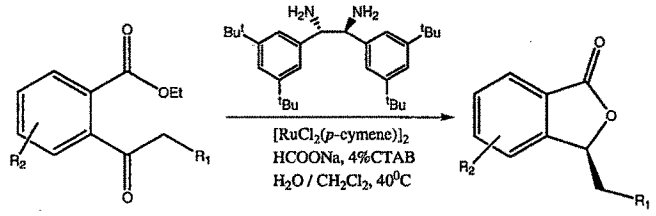
- Ken Tanaka et al developed a cationic rhodium (I)/Solphos complex-catalyzed asymmetric one-pot transesterification and [2 + 2 + 2] cycloaddition of 1, 6-

diyne esters with tertiary propargylic alcohols leading to enantioenriched tricyclic 3, 3-disubstituted phthalides.[185]



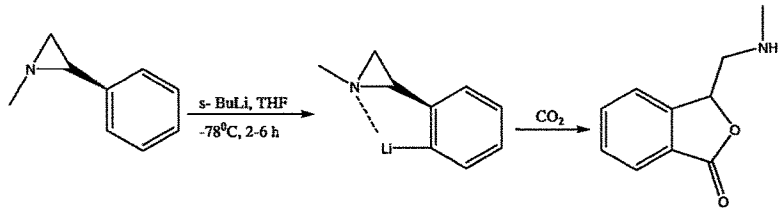
R_1 = Me, COOMe, R_2 = Ph, Me
R_3 = Me

- Catalytic enantioselective synthesis of chiral phthalides by efficient reductive cyclization of 2-Acylarylcarboxylates under aqueous transfer hydrogenation conditions is also well known.[186]



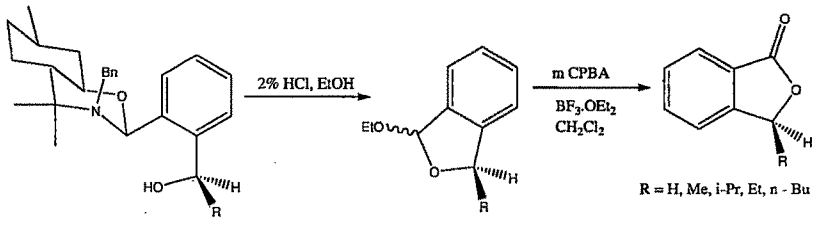
R_1 = Alkyl, Aryl

- Directed ortho lithiation of N-Alkylphenylaziridines also leads to phthalide. This methodology, which counts on the ability of the aziridino group to act as a directed metalation group (DMG), provides an easy access to functionalized arylaziridines as well as to phthalides.[187]

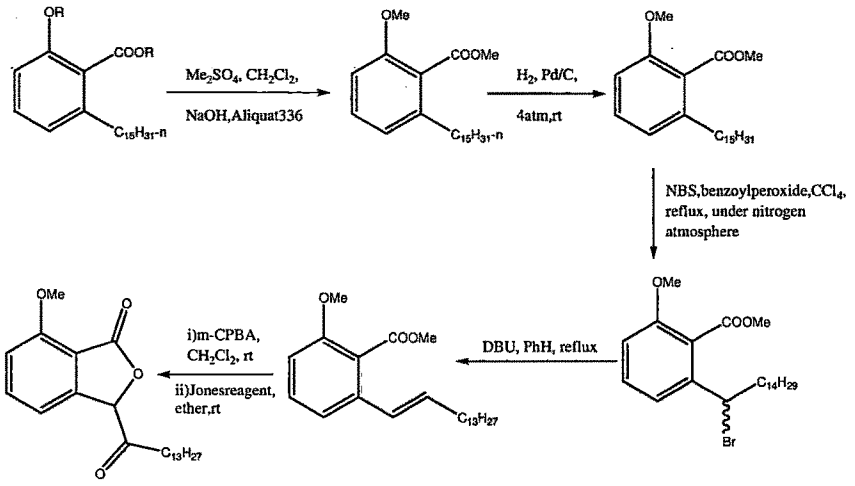


- A versatile diastereoselective synthesis of chiral 3-substituted phthalides in excellent enantiomeric excess (ee) by using a chiral perhydro-1, 3-

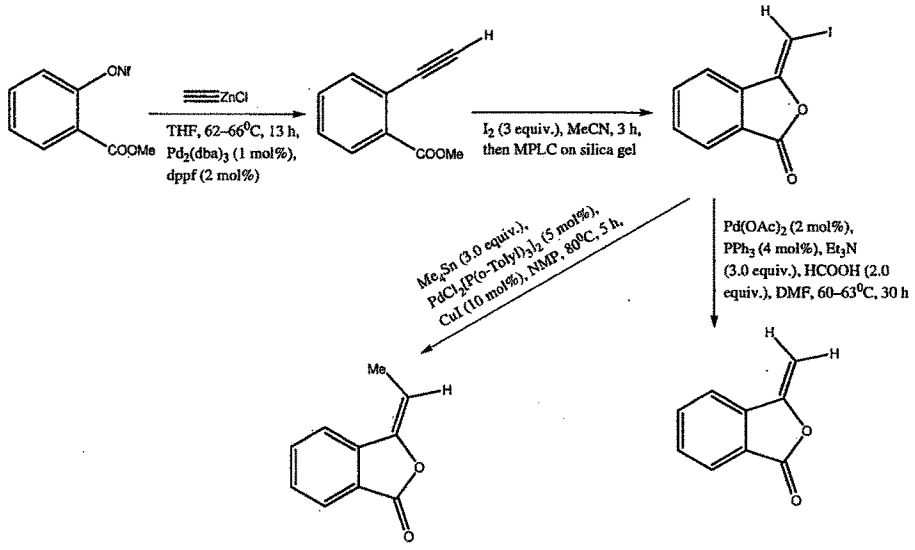
benzoxazine derived from (-)-8-benzylaminomenthol was reported by Rafael Pedrosa et al.[188]



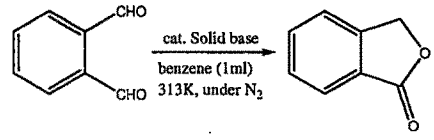
- New anti tumour phthalides were prepared from anacardic acids, the major natural cashew (*Anacardium occi- dentale*) nut-shell phenolic lipid.[189]



- 3-iodomethylidenephthalides, which are regioselectively prepared by iodolactonization of methyl 2-ethynylbenzoate, were employed as starting materials for the stereospecific synthesis of (Z)- and (E)-3-ylidenephthalides.[190]

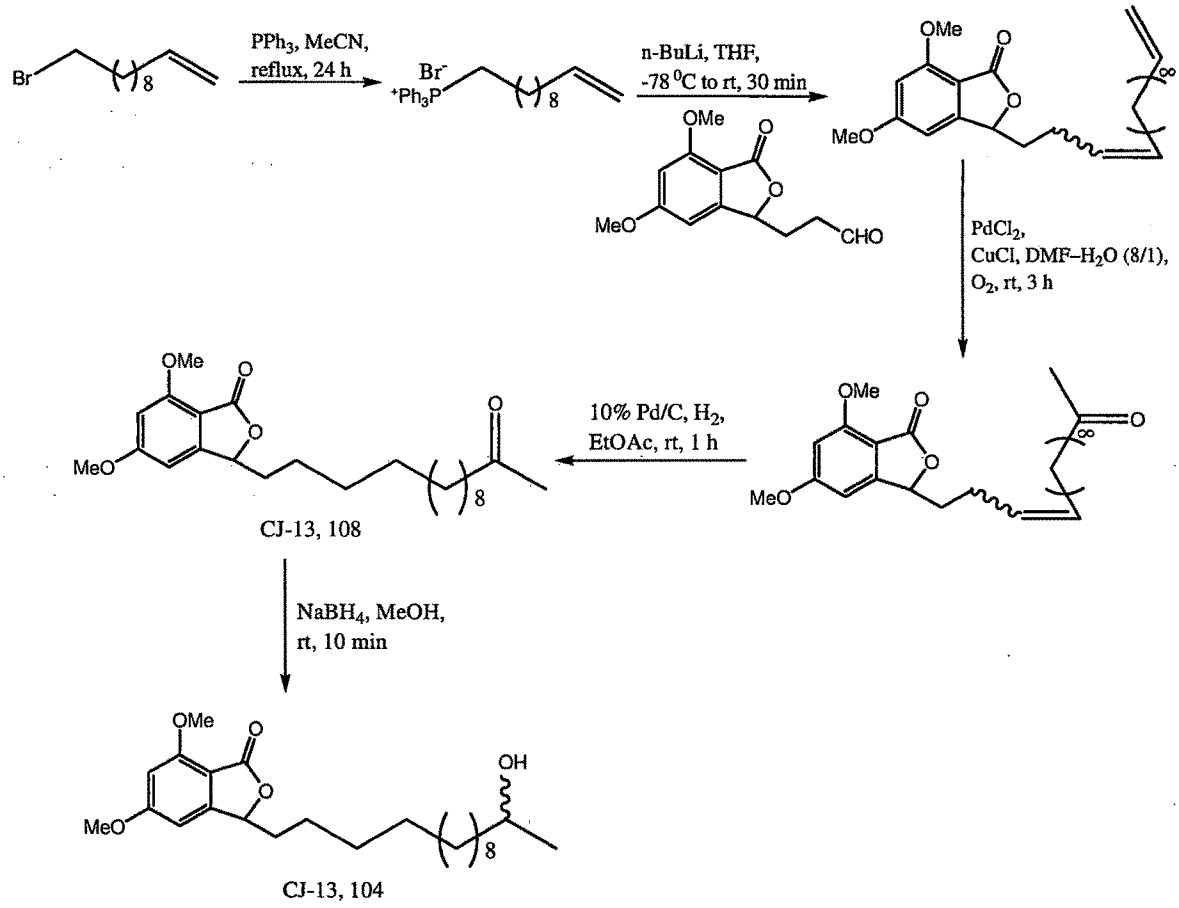


- An efficient, economical, and environmentally benign method for the synthesis of phthalide–skeleton using heterogeneous catalytic intramolecular Tishchenko reaction with solid bases was extensively described by Tsunetake Seki et al.[191]

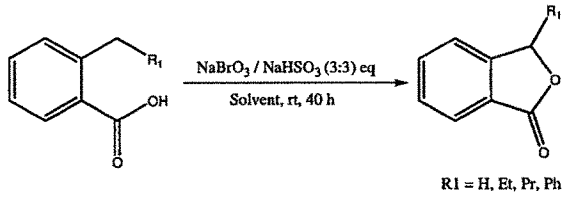


- Flexible racemic syntheses of the phthalide-containing antibiotics CJ-13,015, CJ-13,102, CJ-13,103, CJ-13,104 and CJ-13,108 that inhibit Helicobacter pylori have been carried out in a convergent fashion by Wittig coupling of a

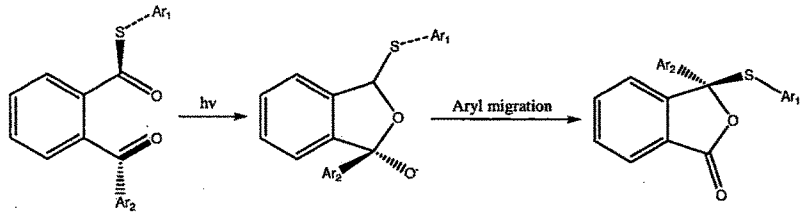
phthalide-containing aldehyde fragment with an appropriate phosphorous ylide.[192]



- γ-lactones have also been prepared by the conversion of *o*-alkyl aromatic carboxylic acids by using the $NaBrO_3$:$NaHSO_3$ reagent at room temperature under a two-phase system.[193]

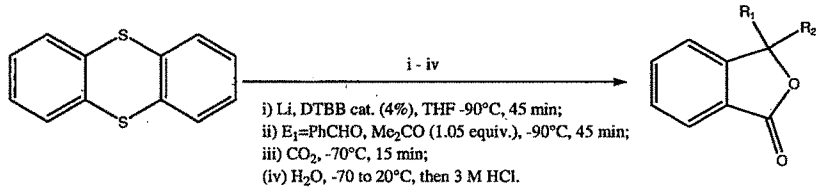


- Absolute asymmetric synthesis of phthalide via the solid-state photoreaction of *N,N*-disubstituted 2-Benzoylbenzamides involving a radical pair intermediate is also well known.[194-195]



Ar_1, Ar_2 = Ph, p-tolyl, m-tolyl

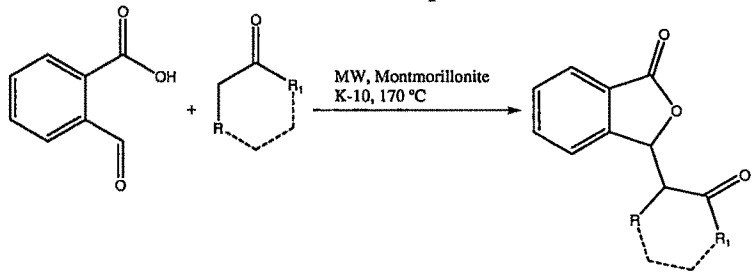
- 3- Substituted phthalides can also be synthesized from thiaanthrene following a four step procedure.[196-197]



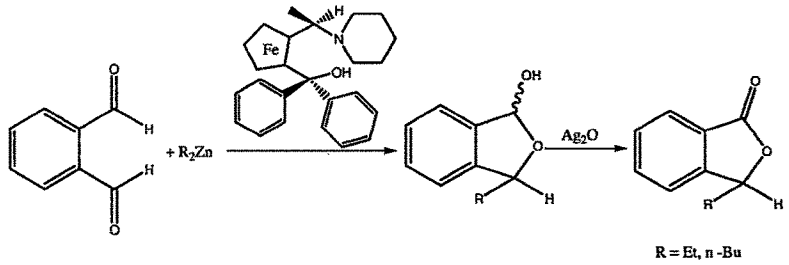
i) Li, DTBB cat. (4%), THF -90°C, 45 min;
ii) E_1=PhCHO, Me_2CO (1.05 equiv.), -90°C, 45 min;
iii) CO_2, -70°C, 15 min;
(iv) H_2O, -70 to 20°C, then 3 M HCl.

R_1 = H, Me
R_2 = Ph, Me

- The use of solid acid (Montmorillonite K-10) catalyzed microwave-assisted cyclization of phthalaldehydic acid with substituted acetophenones and cyclic ketones leads to different derivatives of phthalides.[198]

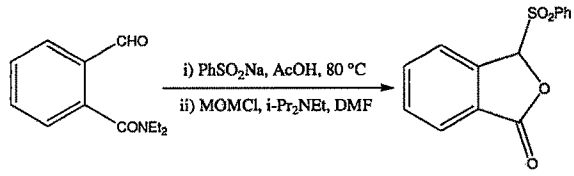


- Optically active 3-Ethyl and 3-n –Butylphthalides were also synthesized via catalytic enantioselective addition of dialkylzinc reagents to o-Phthalaldehyde.[199]

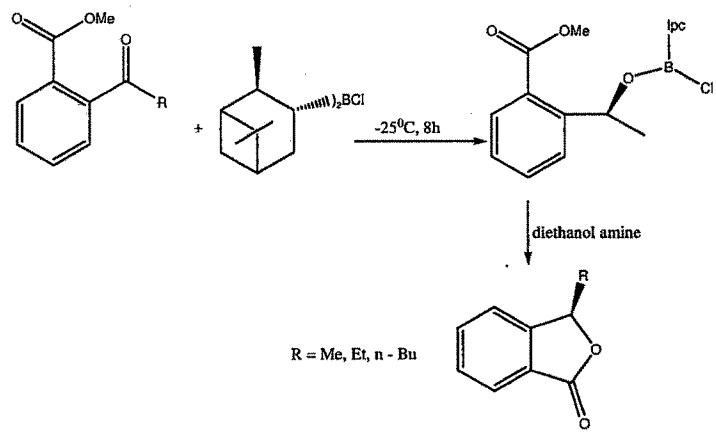


R = Et, n -Bu

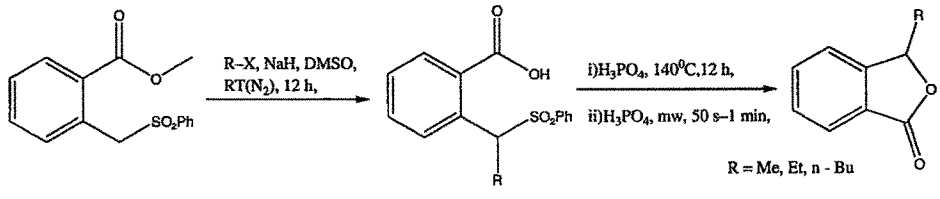
- Tatsuta et al showed that the furanone ring of an isobenzofuranone, can be directly built up from ortho-formylated benzamide by reacting with sodium benzenesulfinate at 80 °C in acetic acid medium. A similar principle has been used for phenolic phthalide sulfone.[200]



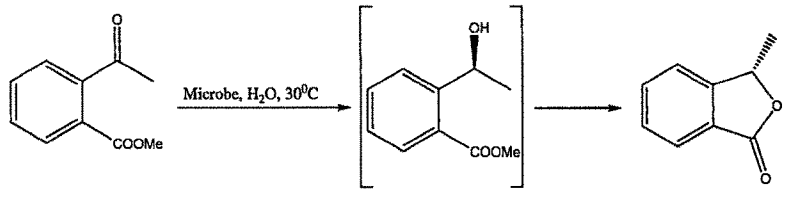
- Chiral 3- substituted phthalides in very high enantiomeric excess can also be synthesized via asymmetric reduction using (-) – B-chlorodiisopinocampheylborane in ethyl ether.[201]



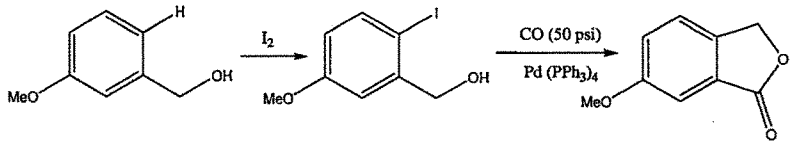
- Alkylated phthalide compounds from easily available sulfones as the starting material was studied by B. T. S. Thirumamagal et al.[202]



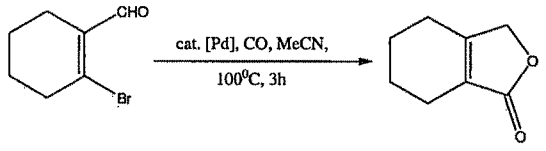
- Optically active (S) – 3-methyl phthalide was also synthesized by using asymmetric microbial reduction apart from conventional methods.[203]



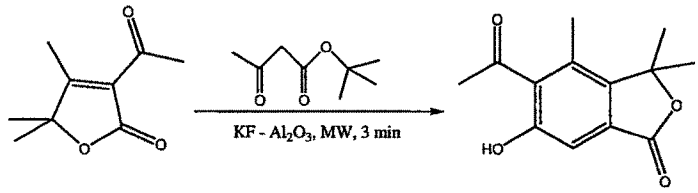
- A two step procedure for the synthesis of different phthalide derivatives have been developed based on Stille's palladium catalyzed tandem carbonylation lactonization reaction.[204]



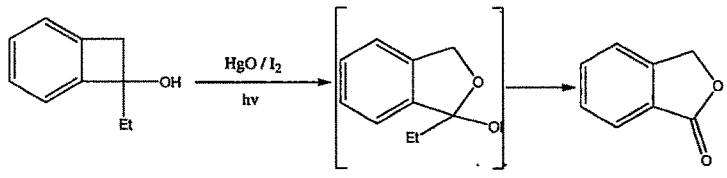
- An unusual palladium- catalyzed lactonization of β-bromovinyl aldehydes via intrinsic carbonylative cyclization leads to tetrahydro phthalide derivatives.[205]



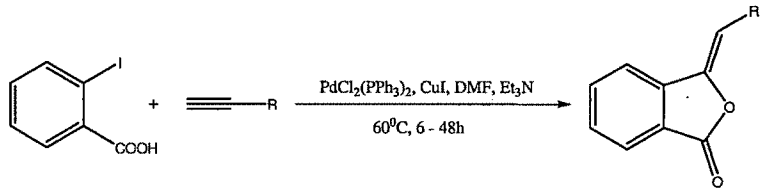
- The reaction of α-hydroxyketones with a double equivalent of t-butyl acetylacetonate in the presence of KF alumina under microwave irradiation afforded 6-acetyl-5-hydroxy-3,3-dialkyl-7-methyl phthalide instead of expected acetyl butenolides.[206]



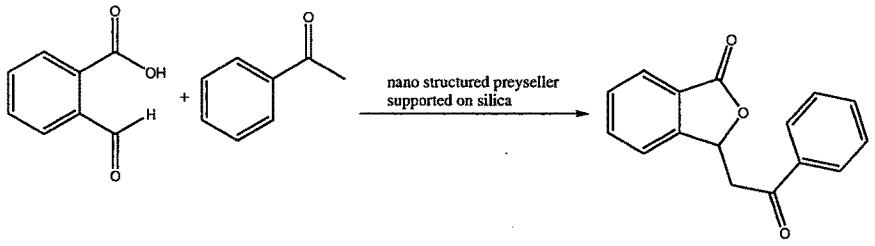
- Kobayashi et al have reported a general method for the synthesis of the phthalides from BCB derivatives. This methodology involves regioselective single or double β-scission of the alkoxy radicals generated by photolysis of the hypoiodite of 1-ethyl-benzocyclobuten-1-ol.[207]



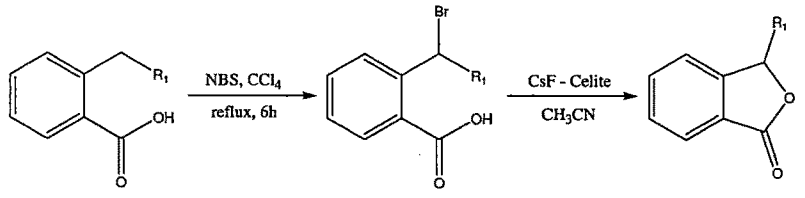
- Kundu and co-workers have shown that the reaction of *o*-iodobenzoic acid with the terminal alkynes in the presence of a catalytic amount of $PdCl_2(PPh_3)_2$, CuI, and Et_3N in DMF leads to phthalides in good yield. The process was found to be highly stereospecific since only the *Z* isomers were obtained.[208-210]



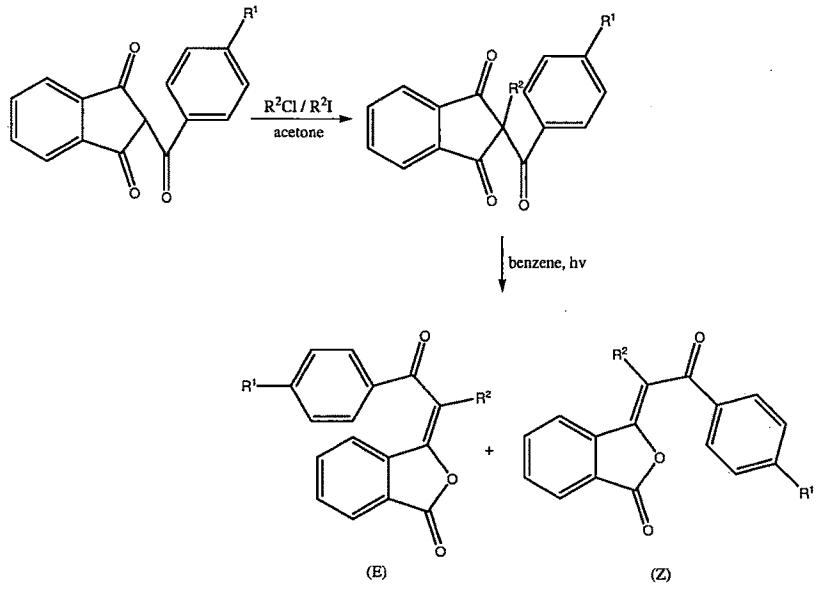
- A direct and efficient method for the preparation of substituted phthalides from the reaction of phthalaldehydic acid, substituted acetophenones, and cyclic ketones using silica-supported Preyssler nanoparticles as a novel and efficient catalytic system was reported by Majid M. Heravi.[211]



- 2-(1-bromoalkyl) aromatic carboxylic acids can be converted into its corresponding γ-lactones by using the CsF-Celite as a solid base in acetonitrile.[212]



- The photoisomerization of 2-aroyl-2-methyl/benzylindan-1,3-diones results in the formation of a mixture of isomeric (E/Z)-3-alkylidene-phthalides in 86–91% yields.[213]



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Chapter 2:

Synthesis of some new isocoumarins and phthalides

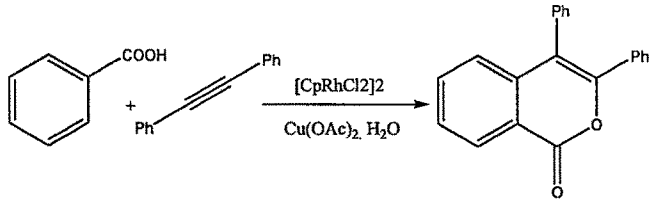
2.1 INTRODUCTION

o-Acyl / Aroyl benzoic / Phthalaldehydic acids are versatile building blocks serving a variety of applications such as pharmaceuticals, fine chemicals, specialty polymers etc. They are important starting materials in the synthesis of a wide variety of compounds like 1,3-thiazolidine-annulated systems[1], Dihydro isoindolin ones[2,3], ARQ 501 human blood metabolites[4], High Density Lipoprotein Cholesterol Enhancers[5], Alkaloid type compounds[6-7], Fused polycyclic compounds[8], Phthalazin-1(2*H*)-ones[9], benzo imidazo phthalazines[10-11], γ-Lactams as Part of a Multi-Ring System[12].

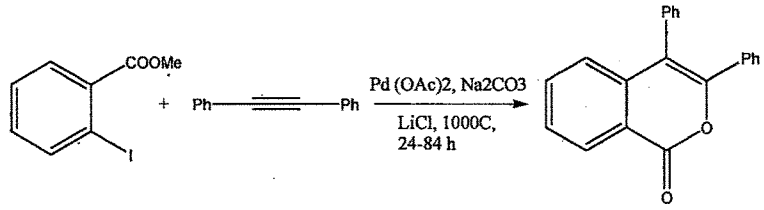
Isocoumarin and phthalide structures are important components in many natural products that exhibit a broad range of biological activities and are also useful intermediates in the synthesis of a variety of important compounds. In the last 10 years 3-substituted isocoumarins with no substituent at the 4-position and 3- aryl phthalides have been synthesized either by a variety of traditional approaches or by utilizing transition metal catalyzed reactions, as described in previous chapter. The synthesis of 3,4-disubstituted isocoumarins along with 3- aryl phthalides has received considerable attention recently, but the number is still very less.

- ❖ Few of the new methodologies developed for the synthesis of 3, 4 - disubstituted isocoumarins are:-

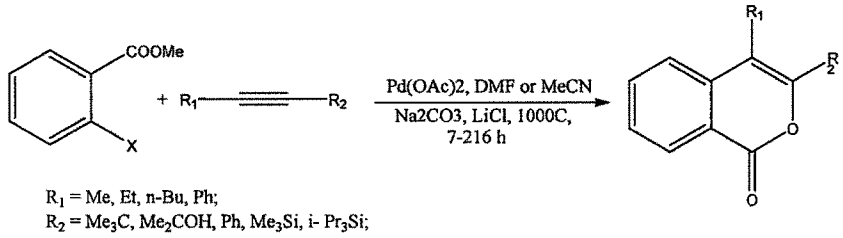
- ➢ Dehydrogenative Coupling of Benzoic Acid with Diphenylacetylene.[13]



- Heck et al. demonstrated that *o*-iodobenzoates reacted with internal acetylenes in the presence of palladium (II) acetate and a phosphine to give the corresponding isocoumarins in low to moderate yields.[14]

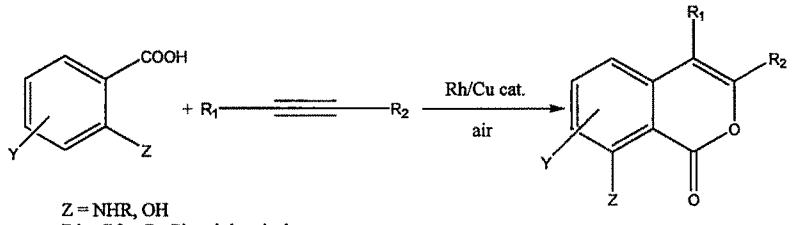


- 3,4-disubstituted isocoumarins were obtained in good yields by treating the halogen containing aromatic esters with internal alkynes in the presence of a palladium catalyst. Synthetically, this methodology provides an especially simple and convenient regioselective route to isocoumarins containing aryl, silyl, ester, *tert*-alkyl, and other hindered groups.[15-16]



R_1 = Me, Et, n-Bu, Ph;
R_2 = Me_3C, Me_2COH, Ph, Me_3Si, i- Pr_3Si;
X= I, Br

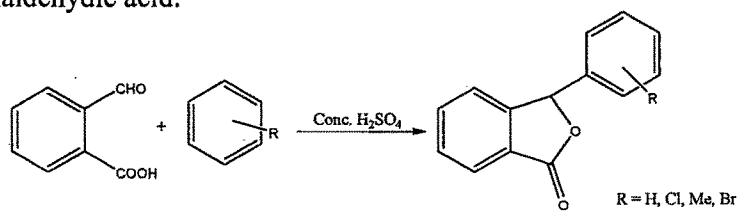
- 2- Substituted Benzoic acids can directly couple with alkynes under air in the presence of an Rh/Cu catalyst system to form isocoumarin derivatives.[17]



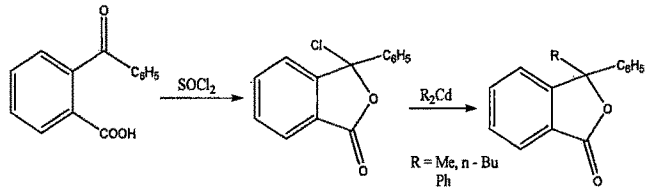
Z = NHR, OH
R1 = R2 = Pr, Ph, tolyl, anisole

❖ Recent examples of synthesis of 3-aryl phthalides:-

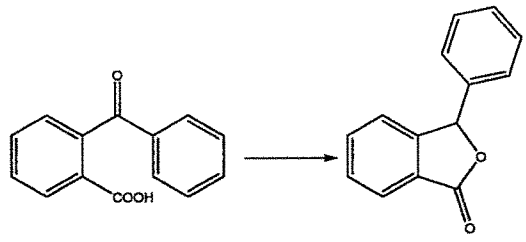
➢ Simple 3-aryl phthalides are known to be the condensation products of phthalaldehydic acid.[18]



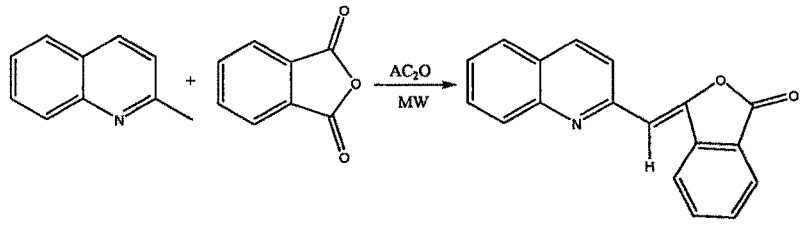
➢ 3-Substituted phenyl phthalides have also been prepared by using thionyl chloride and dialkyl or diphenyl cadmium.[19]



➢ Clemmenson reduction of 2- benzoyl benzoic acid had yielded 3- phenyl phthalide instead of expected 2-benzyl benzoic acid.[20]



➢ Synthesis of new 3-arylidene phthalide derivatives was achieved by condensation reaction of phthalic anhydride and quinoline derivatives under solvent-free condition and microwave irradiation in the presence of acetic anhydride as catalyst in excellent yield.[21]

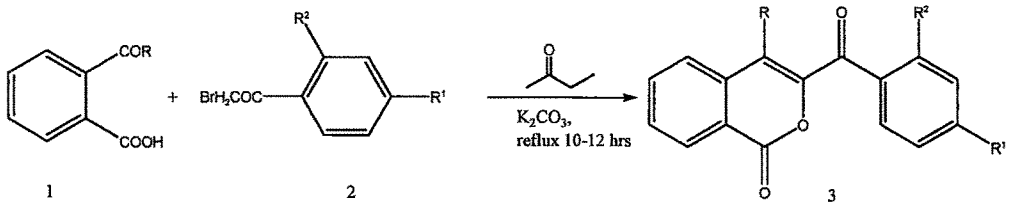


However, the methods described above are very expensive and somewhat limited in synthetic scope since they are highly regioselective. So, the development of a simple and efficient method to access 3,4 disubstituted isocoumarins and 3-aryl phthalides still remains a highly desirable goal in synthetic chemistry.

In the present work we report the synthesis of some new 3, 4 disubstituted isocoumarins by condensing different o-acyl benzoic acids with bromoacetophenone derivatives and chloroacetone, while 3- aryl phthalides by reaction of o- aroyl benzoic acids with bromoacetophenone derivatives in moderate yields.

The reaction sequences for different title compounds are outlined in Scheme I, II and III.

Scheme I

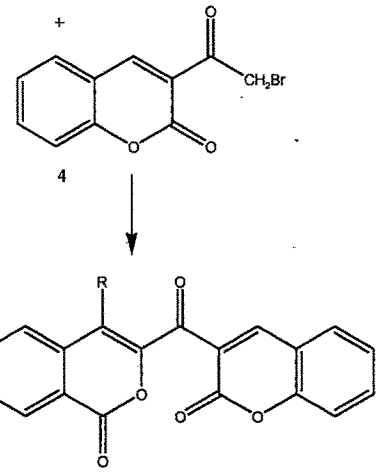


1 2 3

R = -CH_3 **(1a)**
-C_2H_5 **(1b)**
-C_3H_7 **(1c)**

R^1 = Br,OH, OCH_3,Ph
R^2 = H, OH

R = CH_3, R^1 = Br, R^2 = H **(3a)**
R = C_2H_5, R^1 = Br, R^2 = H **(3b)**
R = C_3H_7, R^1 = Br, R^2 = H **(3c)**
R = CH_3, R^1 = OH, R^2 = H **(3d)**
R = C_2H_5, R^1 =OH, R^2 = H **(3e)**
R = C_3H_7, R^1 = OH, R^2 = H **(3f)**
R = CH_3, R^1 = OH, R^2 =O H **(3g)**
R = C_2H_5, R^1 = OH, R^2 =O H **(3h)**
R = C_3H_7, R^1 = OH, R^2 = OH **(3i)**
R = CH_3, R^1 = OCH_3, R^2 = H **(3j)**
R = C_2H_5, R^1 = OCH_3, R^2 = H **(3k)**
R = C_3H_7, R^1 = OCH_3, R^2 = H **(3l)**
R = CH_3, R^1 = Ph R^2 = H **(3m)**
R = C_2H_5, R^1 = Ph R^2 = H **(3n)**
R = C_3H_7, R^1 = Ph, R^2 = H **(3o)**



5

R = CH_3 **(5a)**
R = C_2H_5 **(5b)**

2.2 RESULTS AND DISCUSSION

The procedure followed here for the synthesis of the title compounds was developed by Chatterjea et al[22].

o – acyl benzoic acids **1a-c** on condensation with different substituted bromoacetophenones **2** in presence of anhy. K_2CO_3 in ethyl methyl ketone as solvent gives 4-alkyl-3-aroyl isocoumarins **3a-o (Scheme I)**.

The frequencies obtained in IR spectrum are 1631, 1506 and 3185 cm^{-1} for γ lactone, -C=O and –OH group respectively (**Fig. 2.1**).

^1H NMR spectrum of **3c** shows signals at δ 1.0 (t, 3H, CH_3), 1.7 (m, 2H, CH_2), 2.8 (t, 2H, CH_2), 7.60-7.95 (m, 7H, aromatic protons), 8.40-8.45 (dd, 1H, C_8-H) (**Fig. 2.8**), **3f** at δ 1.1 (t, 3H, CH_3), 1.7 (m, 2H, CH_2), 2.7 (q, 2H, CH_2), 6.87 (s, 1H, OH), 7.50-7.90 (m, 7H, aromatic protons), 8.0 (d, 1H, C_8-H) (**Fig. 2.10**), **3g** at 2.5 (s, 3H, CH_3), 6.3-7.9 (m, 6H, aromatic protons), 8.2 (d, 1H, C_8-H), 12.4 (s, 1H), 12.6 (s, 1H, OH) (**Fig. 2. 2**), **3k** at δ 1.3 (t, 3H, CH_3), 2.8 (q, 2H, CH_2), 4.0 (s, 3H, OCH_3), 6.95-8.05 (m, 7H, aromatic protons), 8.41-8.43 (dd, 1H, C_8-H) (**Fig. 2. 4**), **3n** at δ 1.3 (t, 3H, CH_3), 2.8 (q, 2H, CH_2), 7.4-8.0 (m, 12H, aromatic protons), 8.43-8.45 (dd, 1H, C_8-H) (**Fig. 2.6**) and **3o** at δ 1.0 (t, 3H, CH_3), 1.7 (m, 2H, CH_2), 2.8 (t, 2H, CH_2), 7.40-7.95 (m, 12H, aromatic protons), 8.42-8.44 (d, 1H, C_8-H) (**Fig. 2.12**).

Mass spectrum of **3c** gives m/z: 370.9 (M^+), 300, 276, 262, 214, 187, 157, and 146 (**Fig. 2.9**), **3f** at m/z: 308 (M^+), 294, 280, 252, 236, 215, 186,172, 157, 146 and 121(**Fig. 2.11**), **3g** at m/z: 295 (M^+-1), 281, 236, 221, 185, 149, 121 and 110 (**Fig. 2.3**), **3k** at m/z: 308(M^+), 262, 187, 146, 135 and 108 (**Fig. 2.5**), **3n** at m/z: 355 (M^++1), 340, 277, 222, 202, 154 and 146 (**Fig. 2.7**) and **3o** at m/z: 369 (M^++1), 325, 297, 214, 154 and 146 (**Fig. 2.13**).

Series was extended when substituted bromoacetophenones **2** were replaced with 3-Bromoacetyl coumarin **4** and reacted with o-acyl benzoic acids **1a-b** following the same pathway i.e condensation in presence of K_2CO_3 and ethyl methyl ketone, to give 4-alkyl-3-coumarinoyl isocoumarins **5a-b**.

In ^1H NMR spectra, a singlet of methyl group at δ 2.6 confirms the CH_3 at 4th position of isocoumarin **5a**. Aromatic protons show signals between δ 6.8 – 8.4 and the proton at 8th position of isocoumarin ring shows a characteristic doublet at δ 8.4 (**Fig. 2.14**).

Mass spectra of **5a** gives m/z: at 330 (M^+ - 2), 317, 304, 257, 213, 173, 146 and 133 (**Fig. 2.15**).

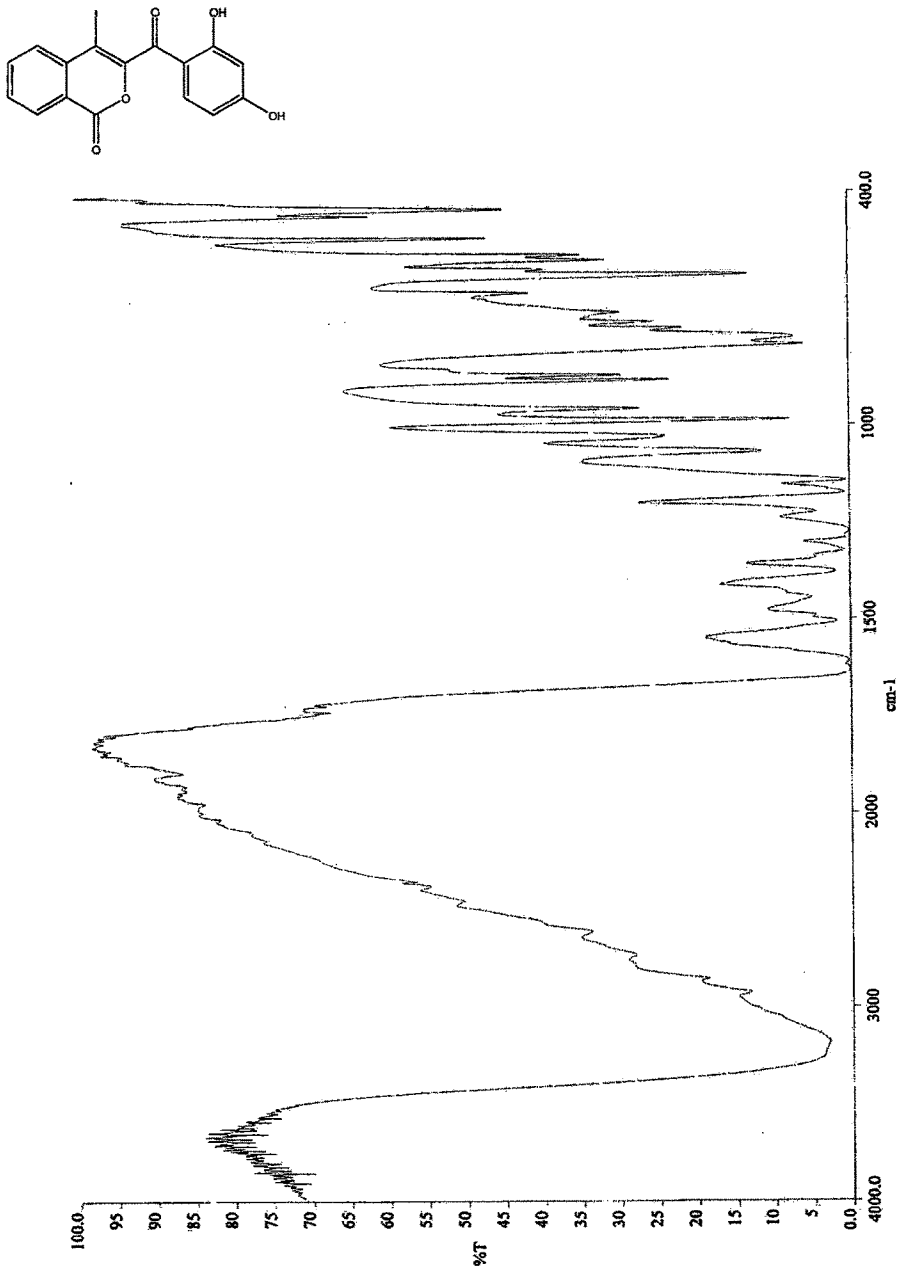


Fig. 2.1 – IR: **3-(2', 4'-Diydroxy benzoyl) - 4- methyl-isocoumarin 3g**

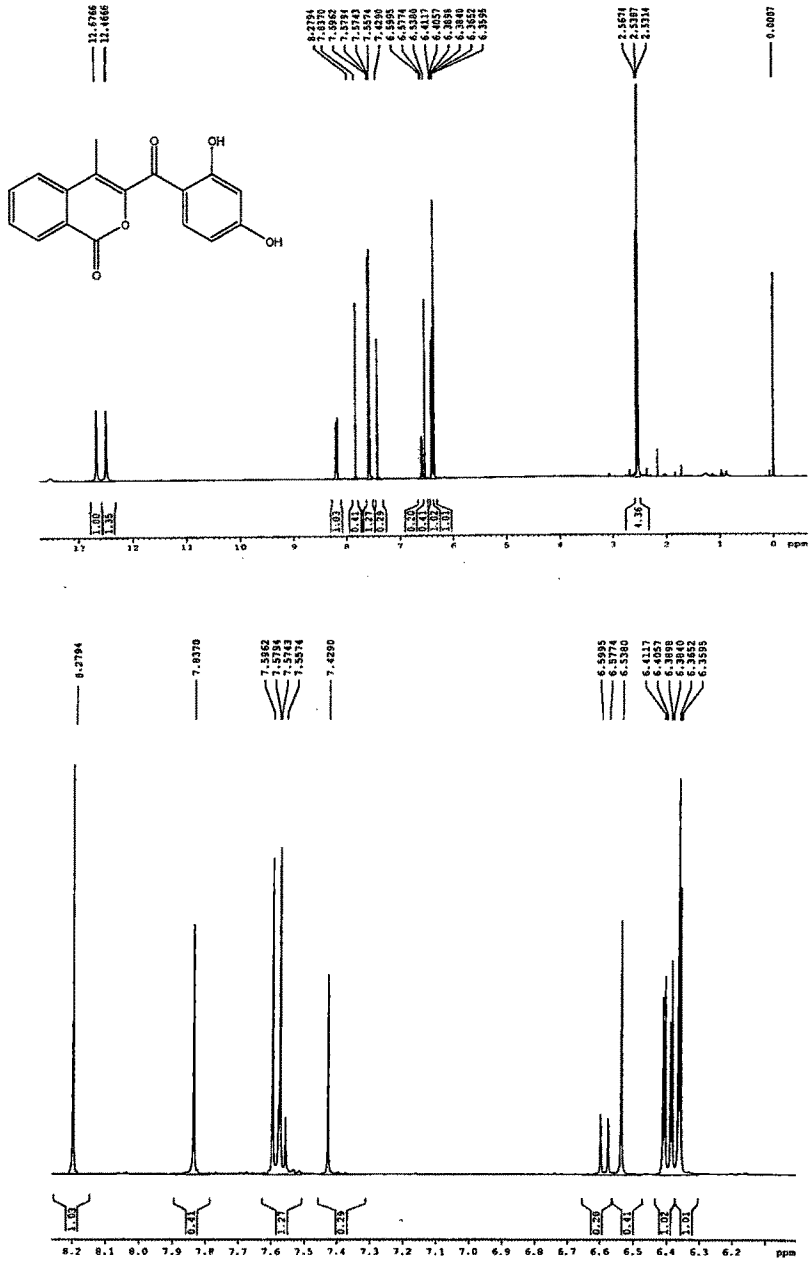


Fig. 2.2 - ^{1}H NMR: 3-(2', 4'-Diydroxy benzoyl) - 4- methyl-isocoumarin 3g

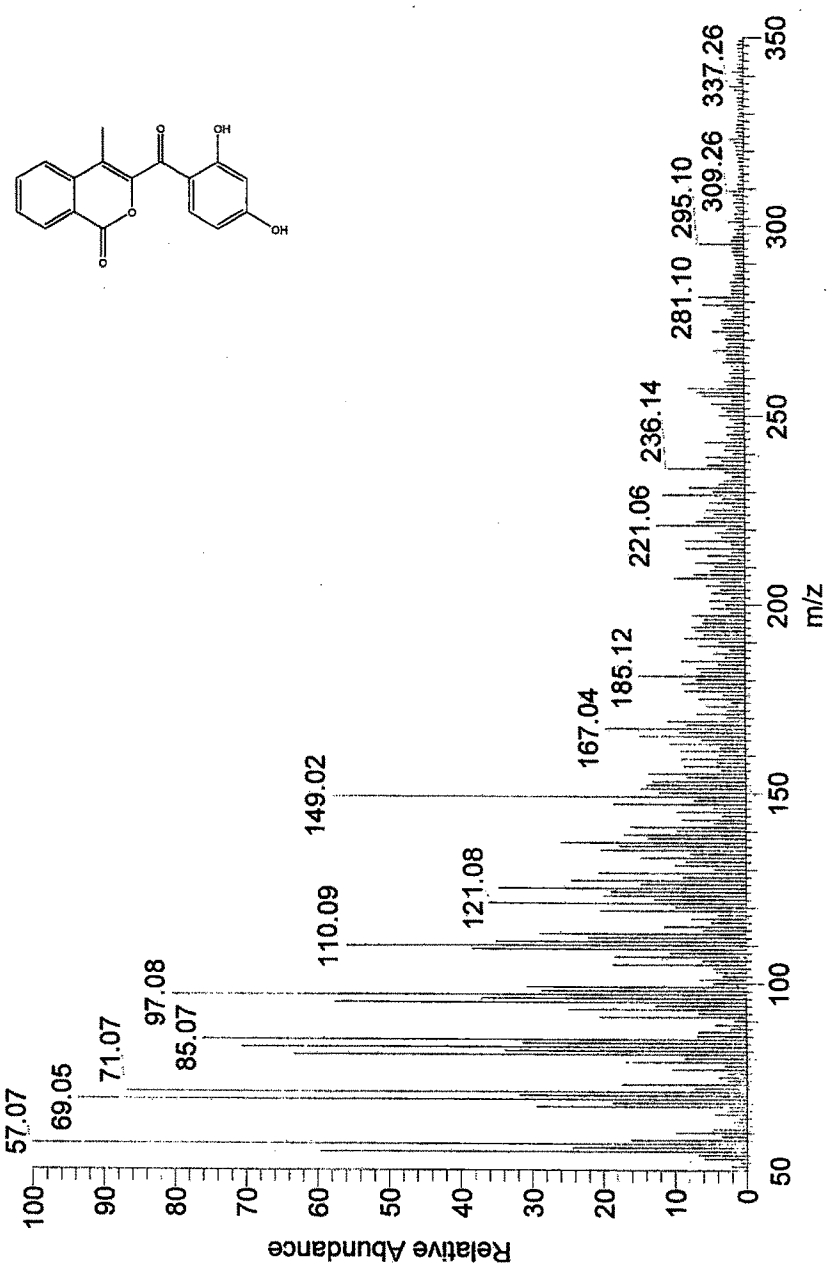
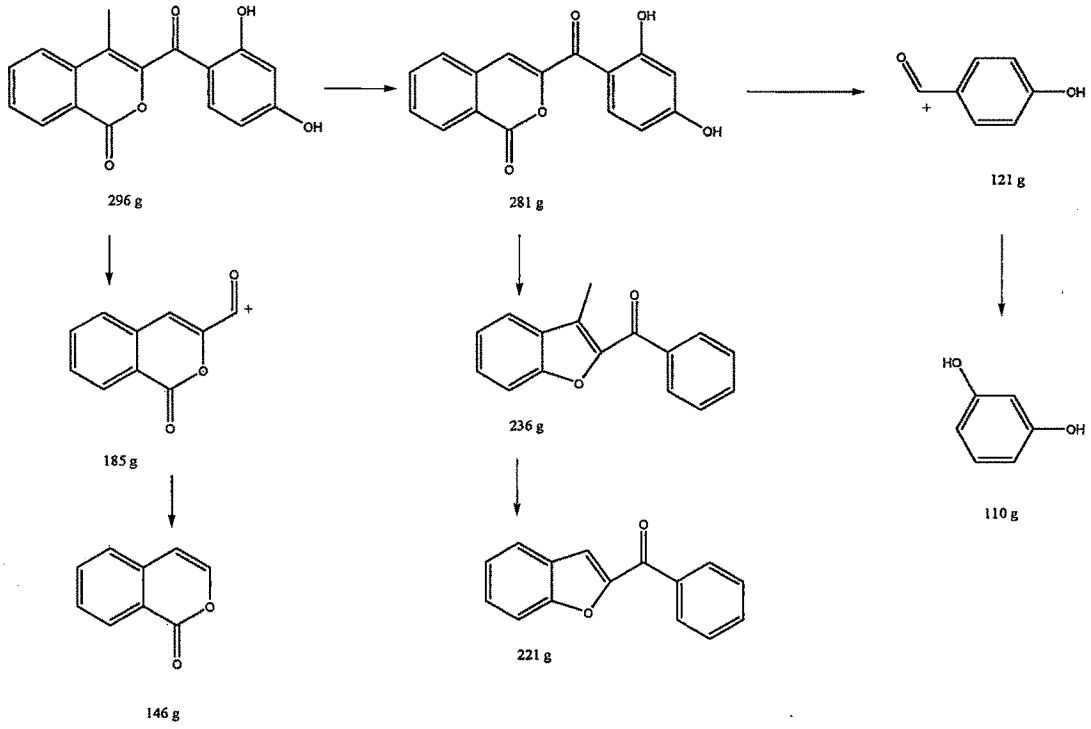


Fig. 2.3 - Mass spectrum: **3-(2', 4'-Diydroxy benzoyl) - 4- methyl-isocoumarin 3g**



Fragmentation Pattern: **3-(2', 4'-Diydroxy benzoyl) - 4- methyl-isocoumarin**

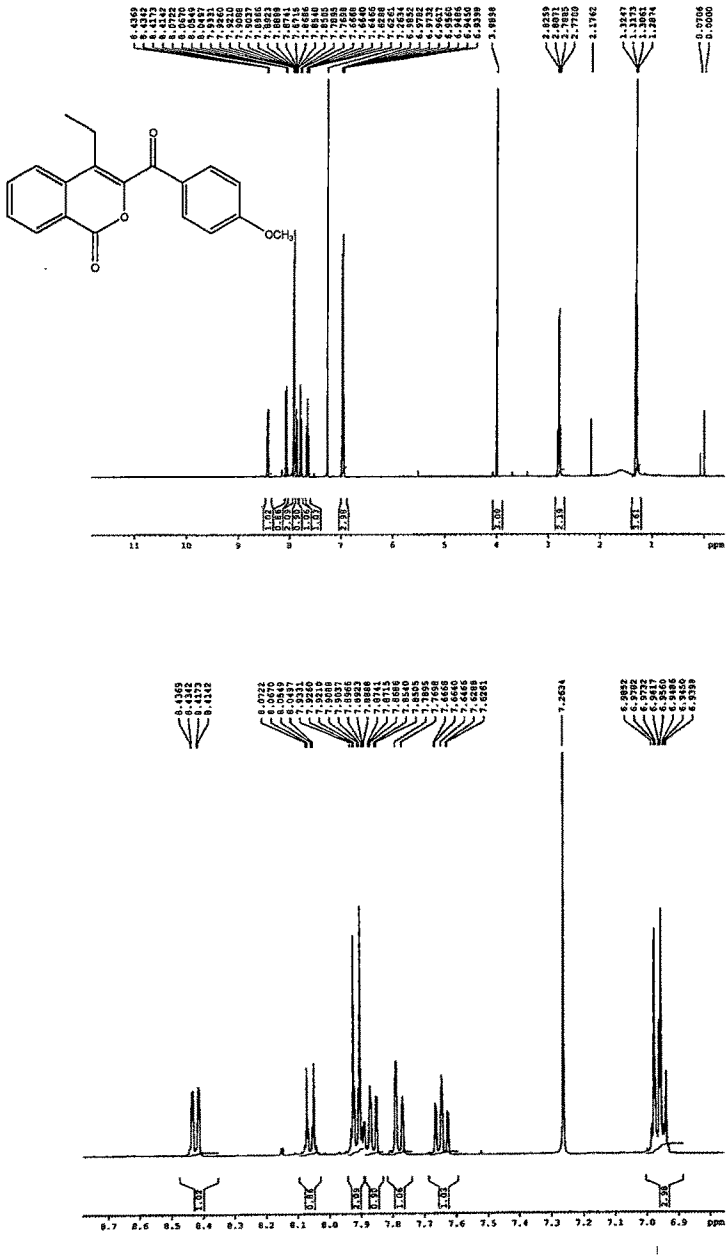


Fig. 2.4 - ^{1}H NMR: 3-(4'-Methoxy benzoyl) - 4- ethyl-isocoumarin 3k

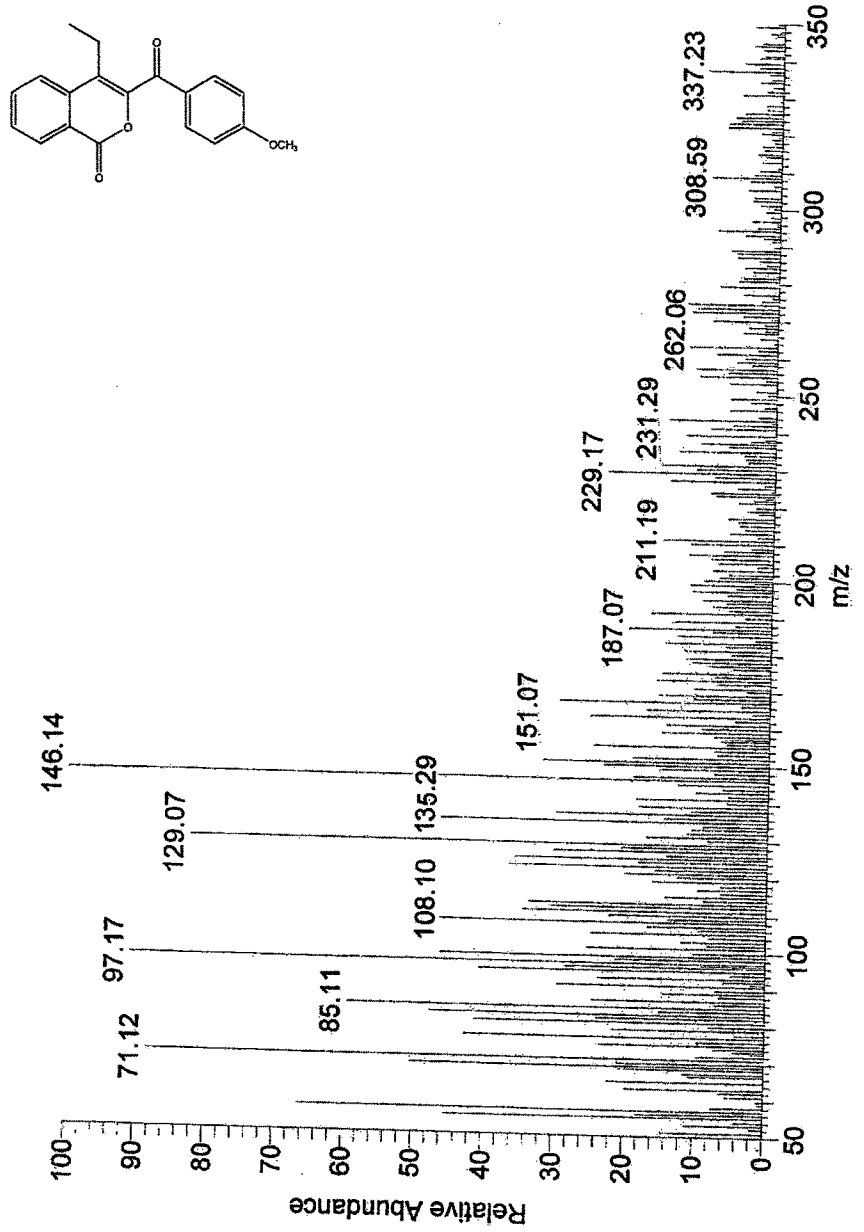
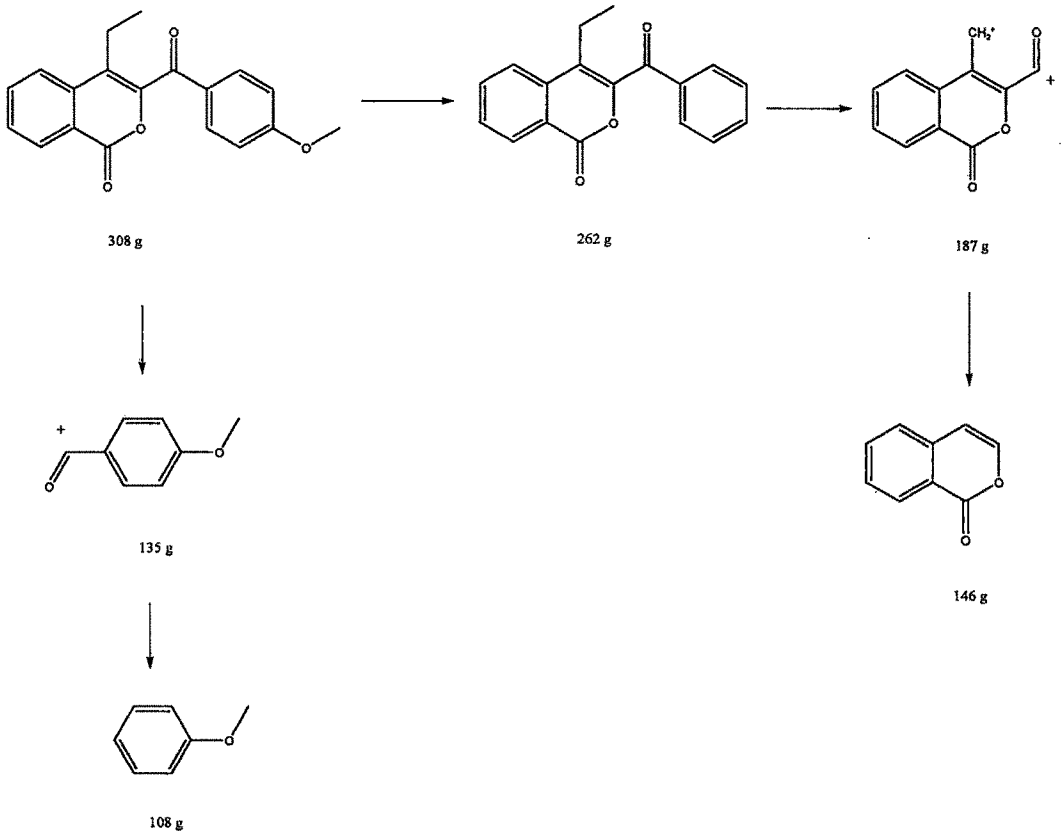
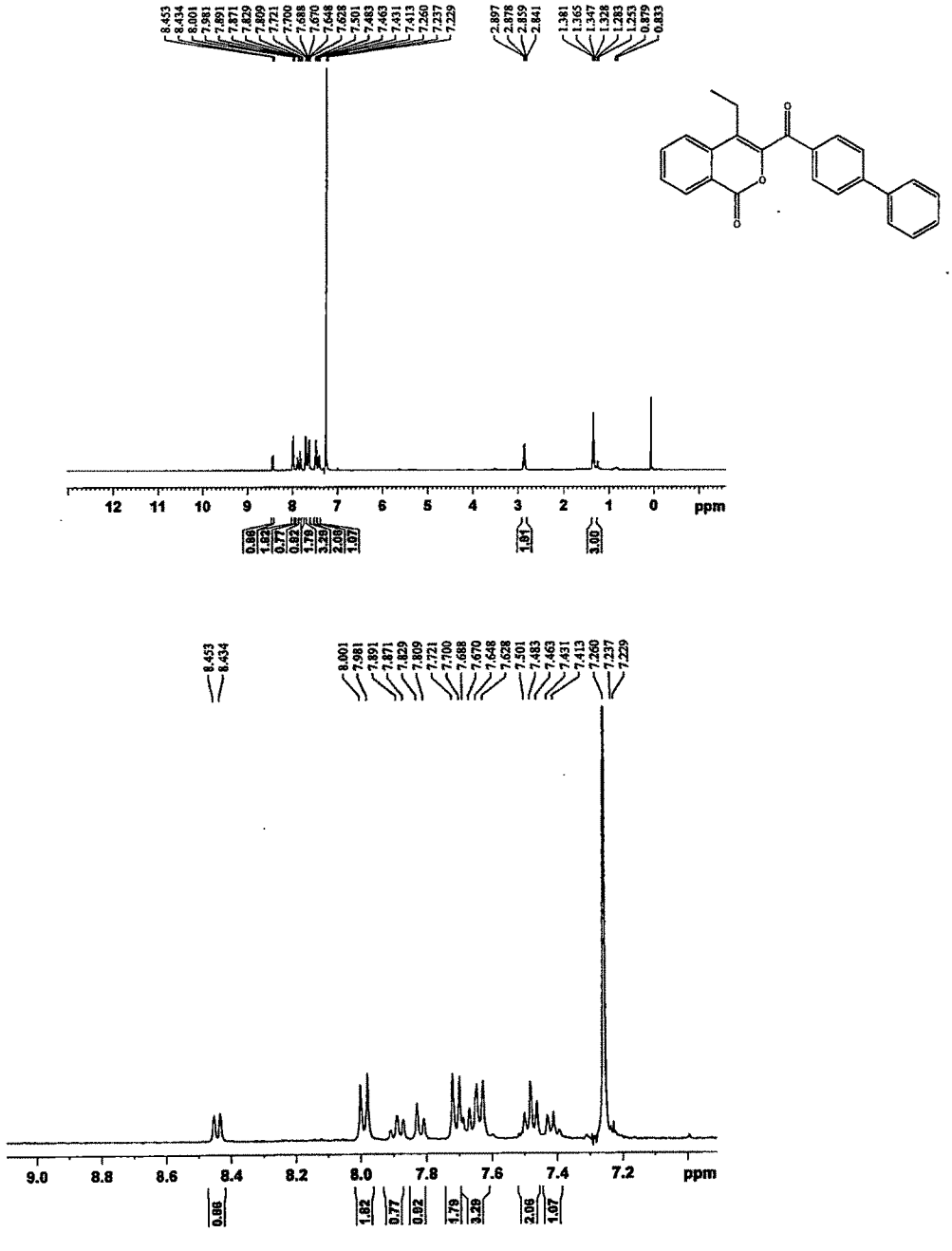


Fig. 2.5 - Mass spectrum: **3-(4'-Methoxy benzoyl) - 4- ethyl-isocoumarin 3k**



Fragmentation Pattern: **3-(4'-Methoxy benzoyl) - 4- ethyl-isocoumarin**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7.229
2.897
2.878
2.859
2.841
1.381
1.365
1.347
1.328
1.283
1.253
0.879
0.833
12 11 10 9 8 7 6 5 4 3 2 1 0 ppm
0.86 1.82 0.77 0.82 1.79 3.29 2.06 1.07
1.81
3.00
9.0 8.8 8.6 8.4 8.2 8.0 7.8 7.6 7.4 7.2 ppm
0.86 1.82 0.77 0.82 1.79 3.29 2.06 1.07

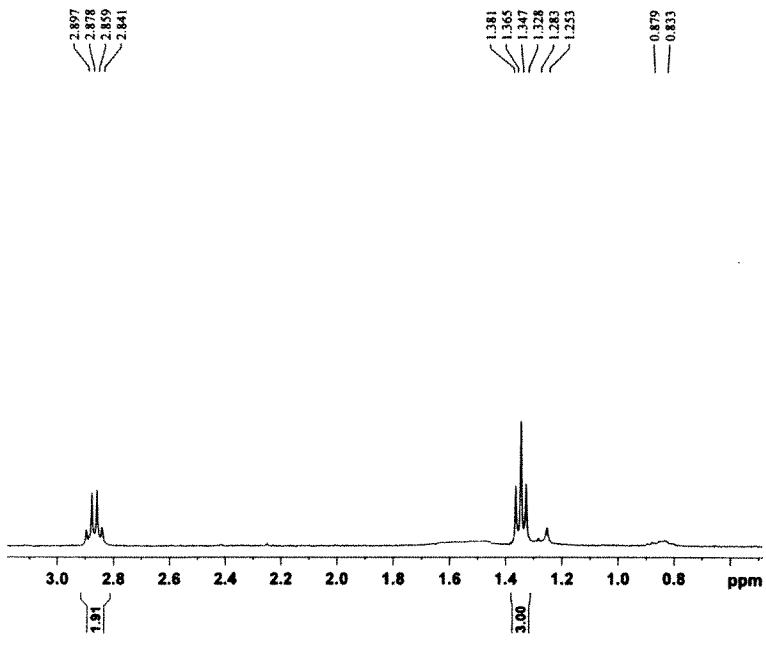


Fig. 2.6 - ^{1}H NMR: 4- Ethyl- 3-(4'- phenyl benzoyl) isocoumarin 3n

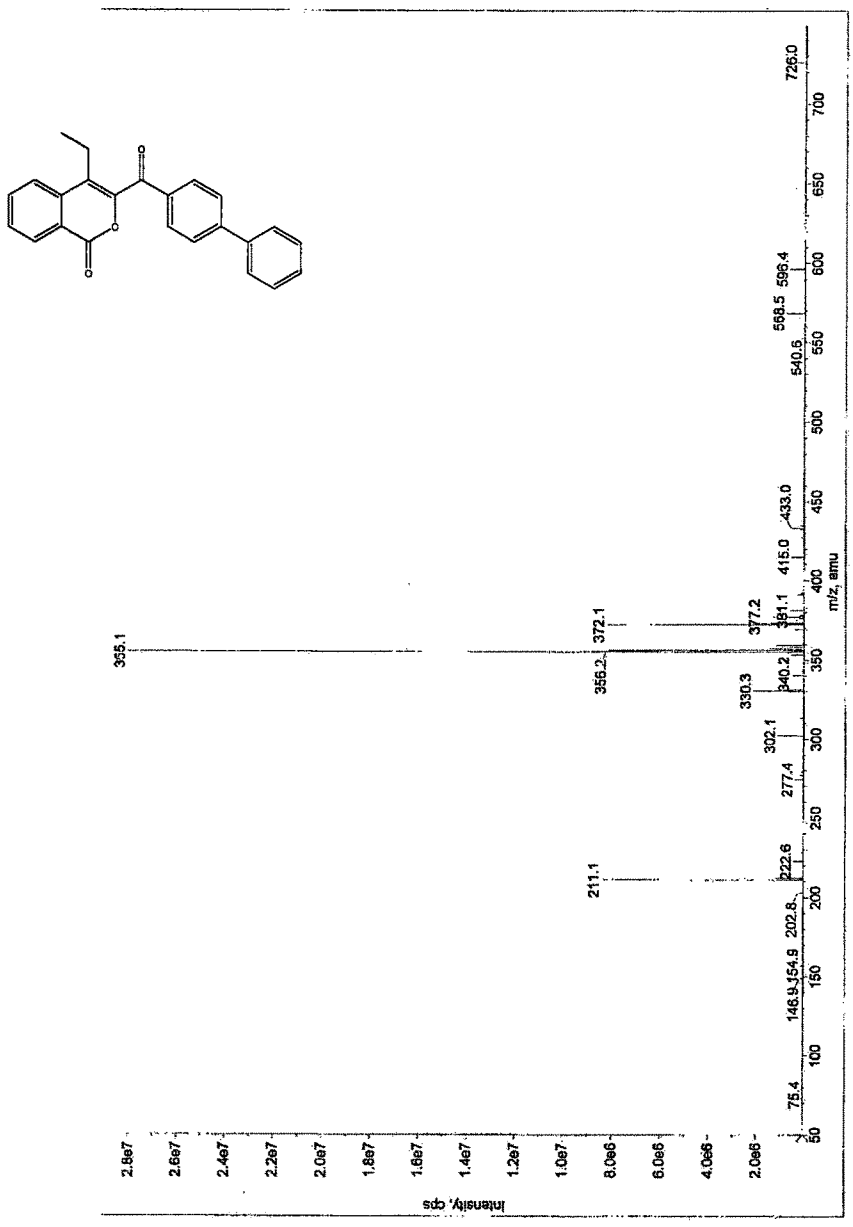
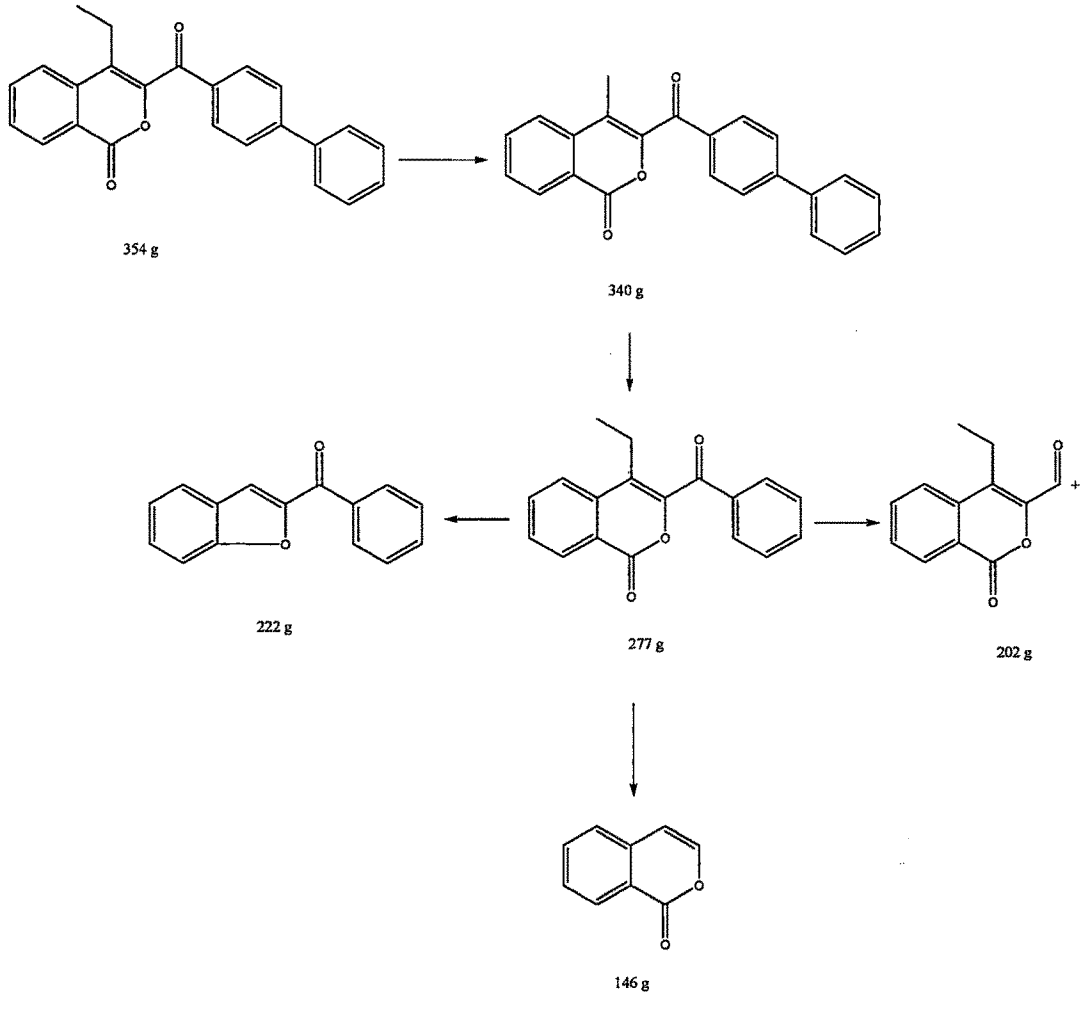


Fig. 2.7 - Mass spectrum: **4- Ethyl- 3-(4'- phenyl benzoyl) isocoumarin 3n**



Fragmentation Pattern: **4- Ethyl- 3-(4'- phenyl benzoyl) isocoumarin**

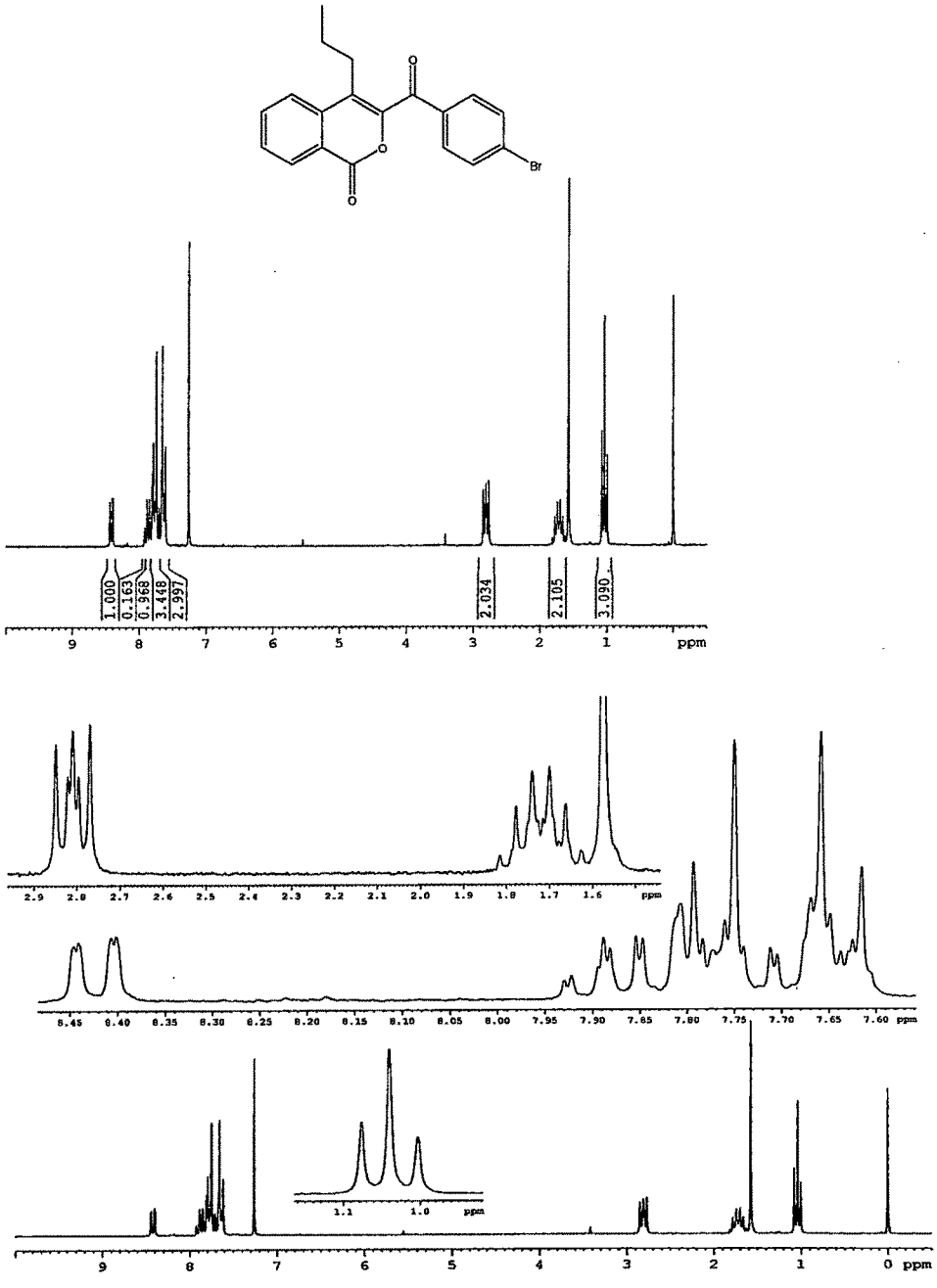


Fig. 2.8 - ^{1}H NMR: 3-(4'-Bromo benzoyl) - 4-propyl-isocoumarin 3c

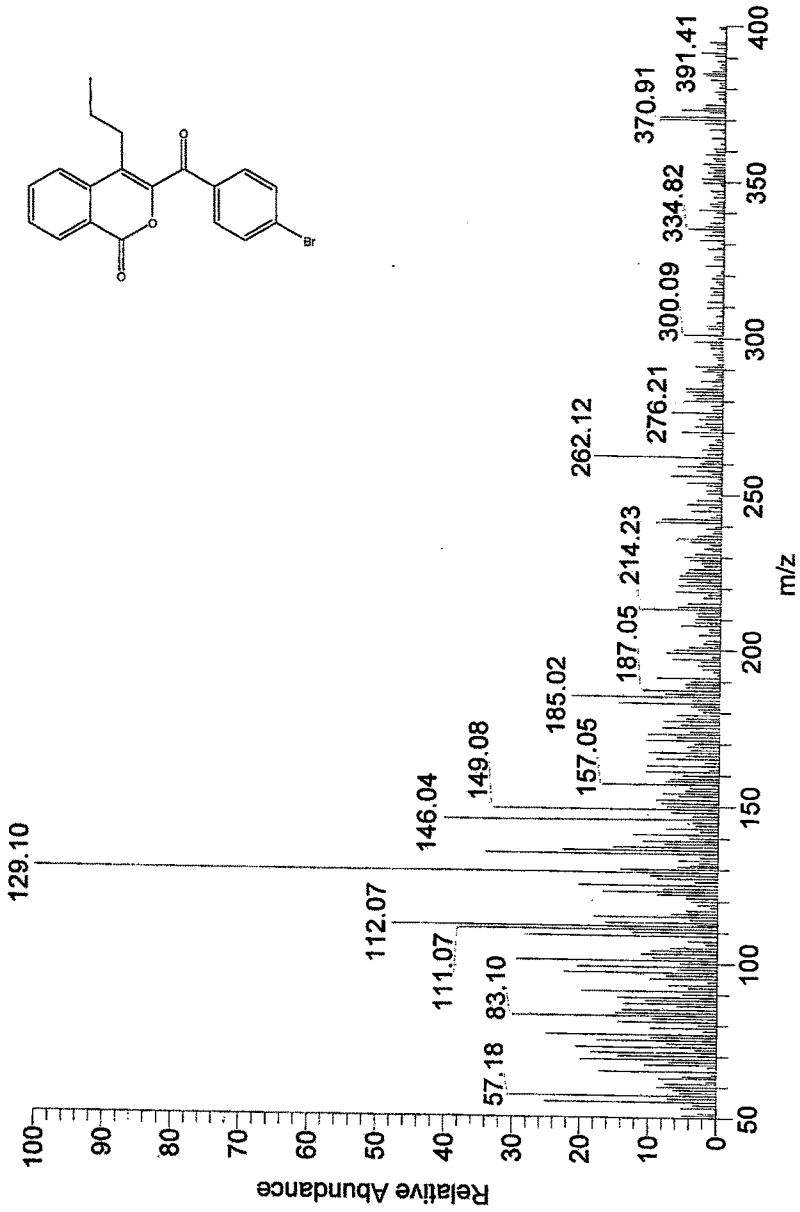
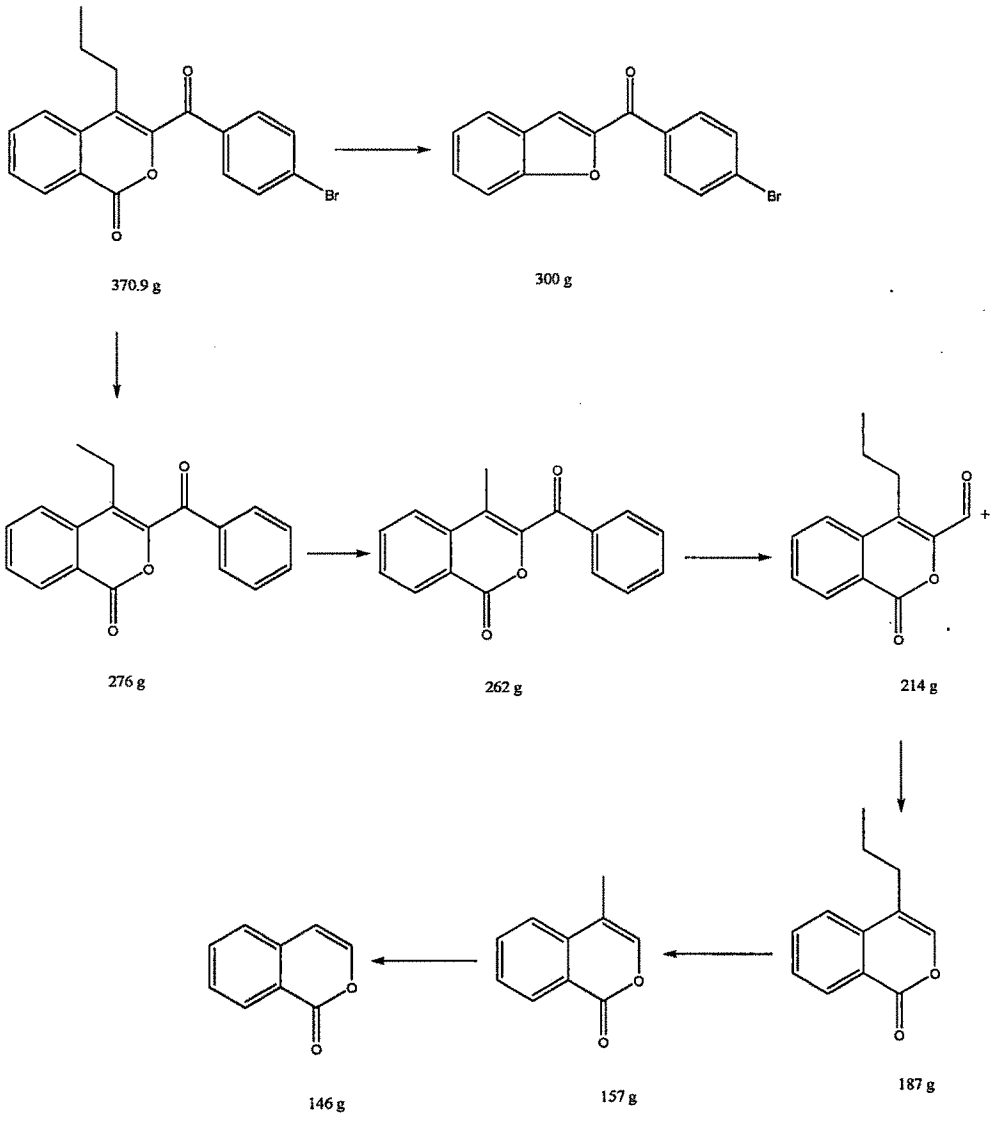
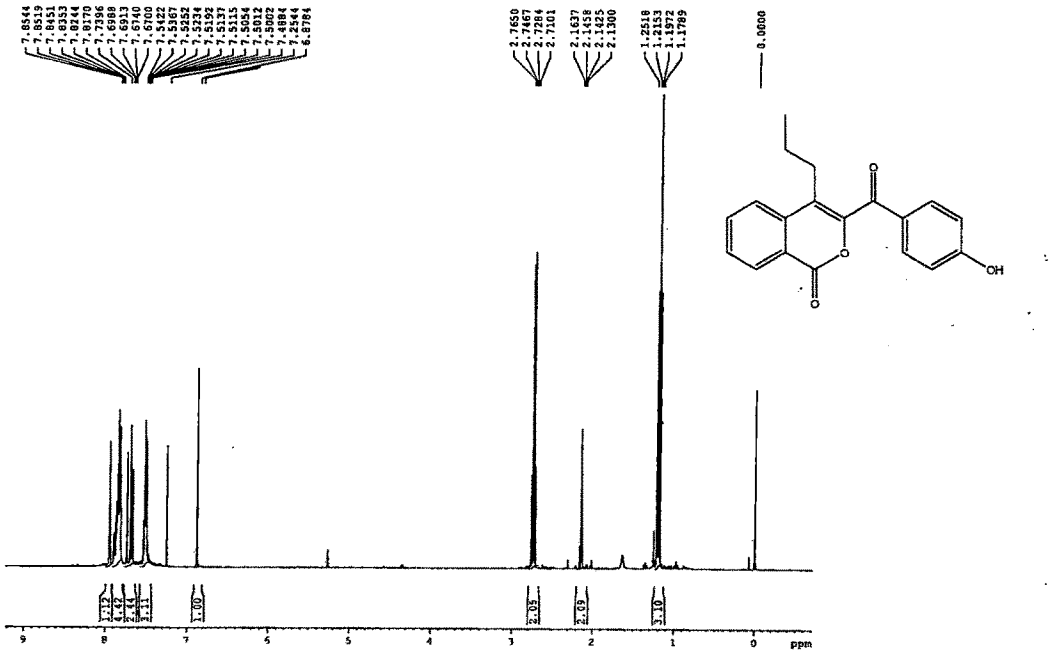


Fig. 2.9 - Mass spectrum : **3-(4'-Bromo benzoyl) - 4-propyl-isocoumarin 3c**



Fragmentation Pattern: **3-(4'-Bromo benzoyl) - 4-propyl-isocoumarin**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7.5192
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7.5054
7.5012
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6.8784
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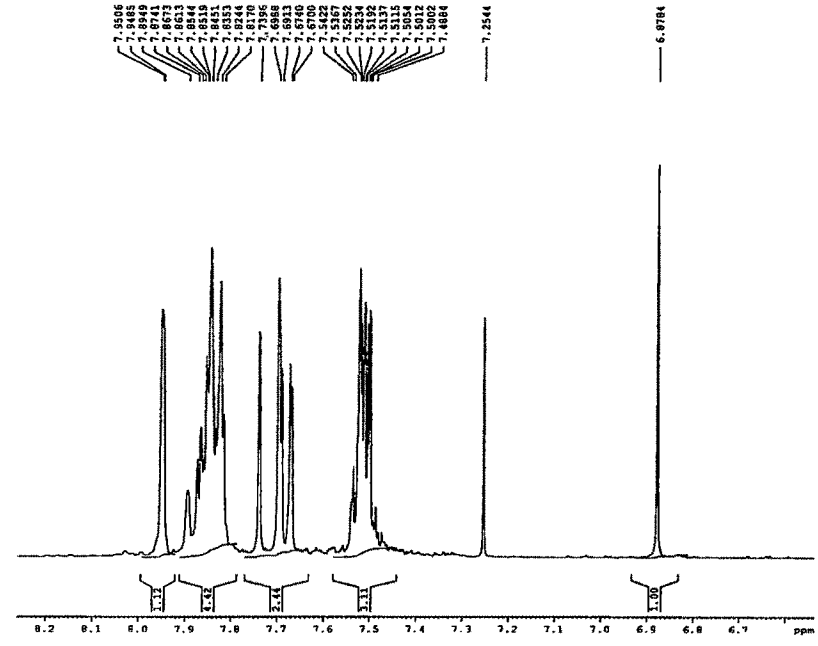
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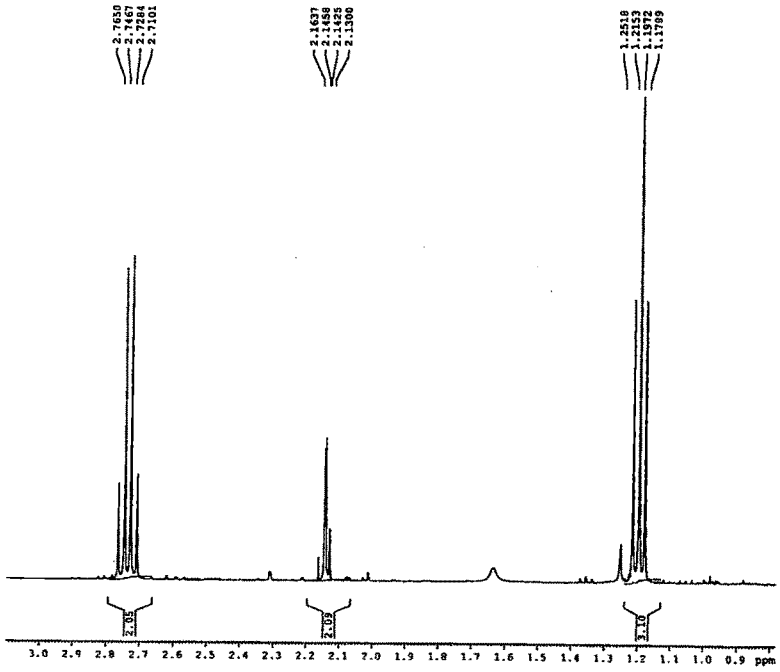


Fig. 2.10 - ^{1}H NMR: 3-(4'-Hydroxy benzoyl) - 4- propyl-isocoumarin 3f

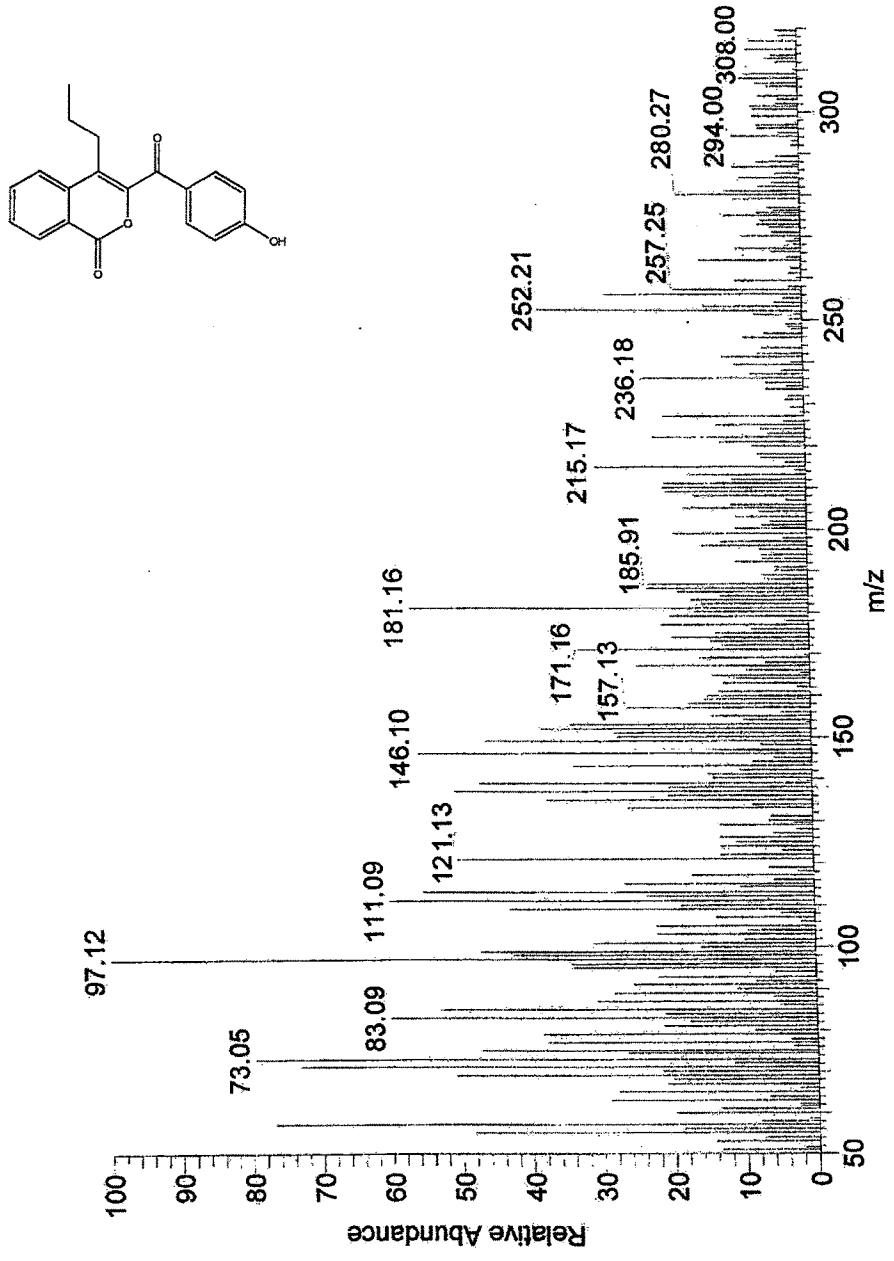
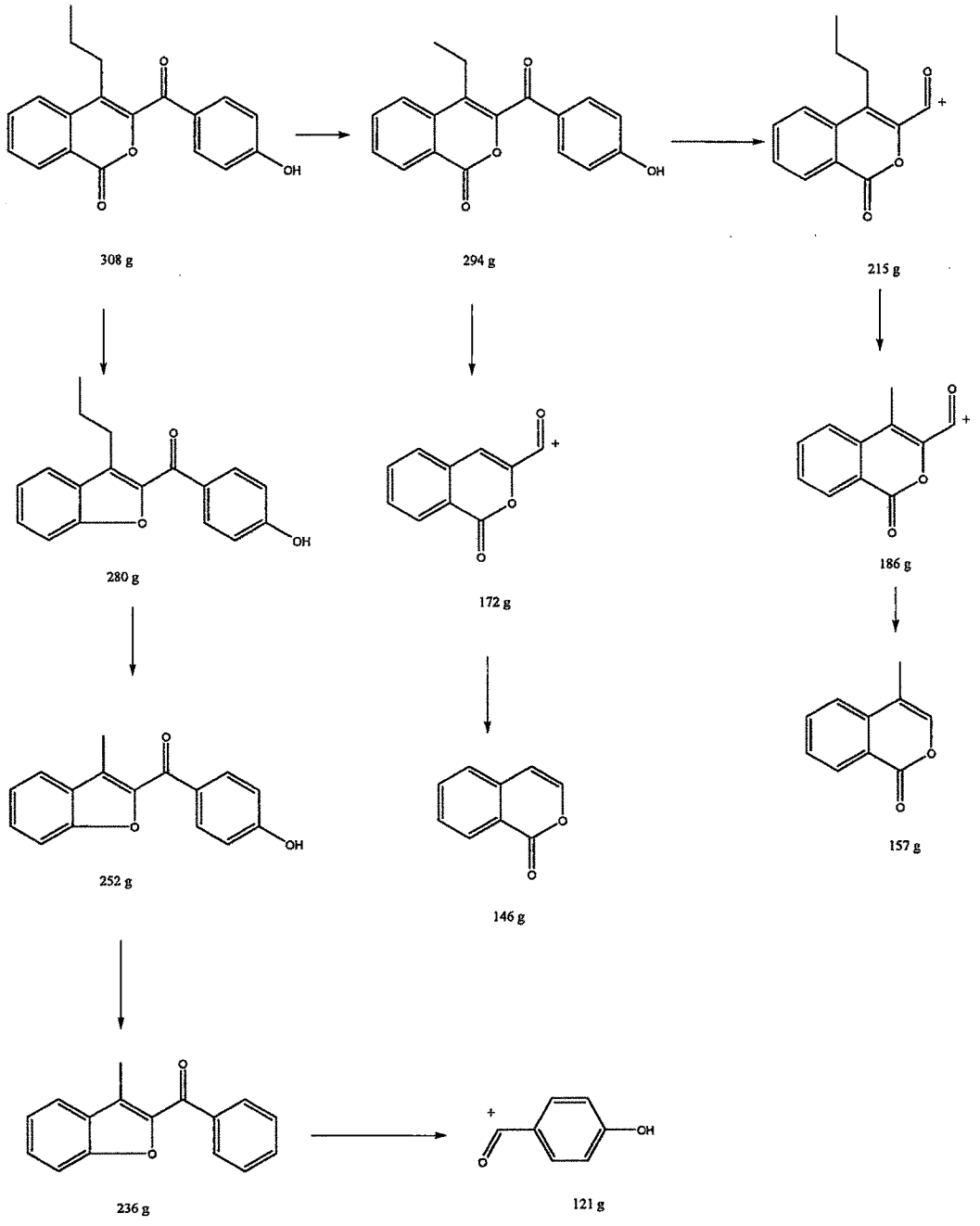


Fig. 2.11- Mass spectrum: **3-(4'-Hydroxy benzoyl) - 4- propyl-isocoumarin 3f**



Fragmentation Pattern: **3-(4'-Hydroxy benzoyl) - 4- propyl-isocoumarin**

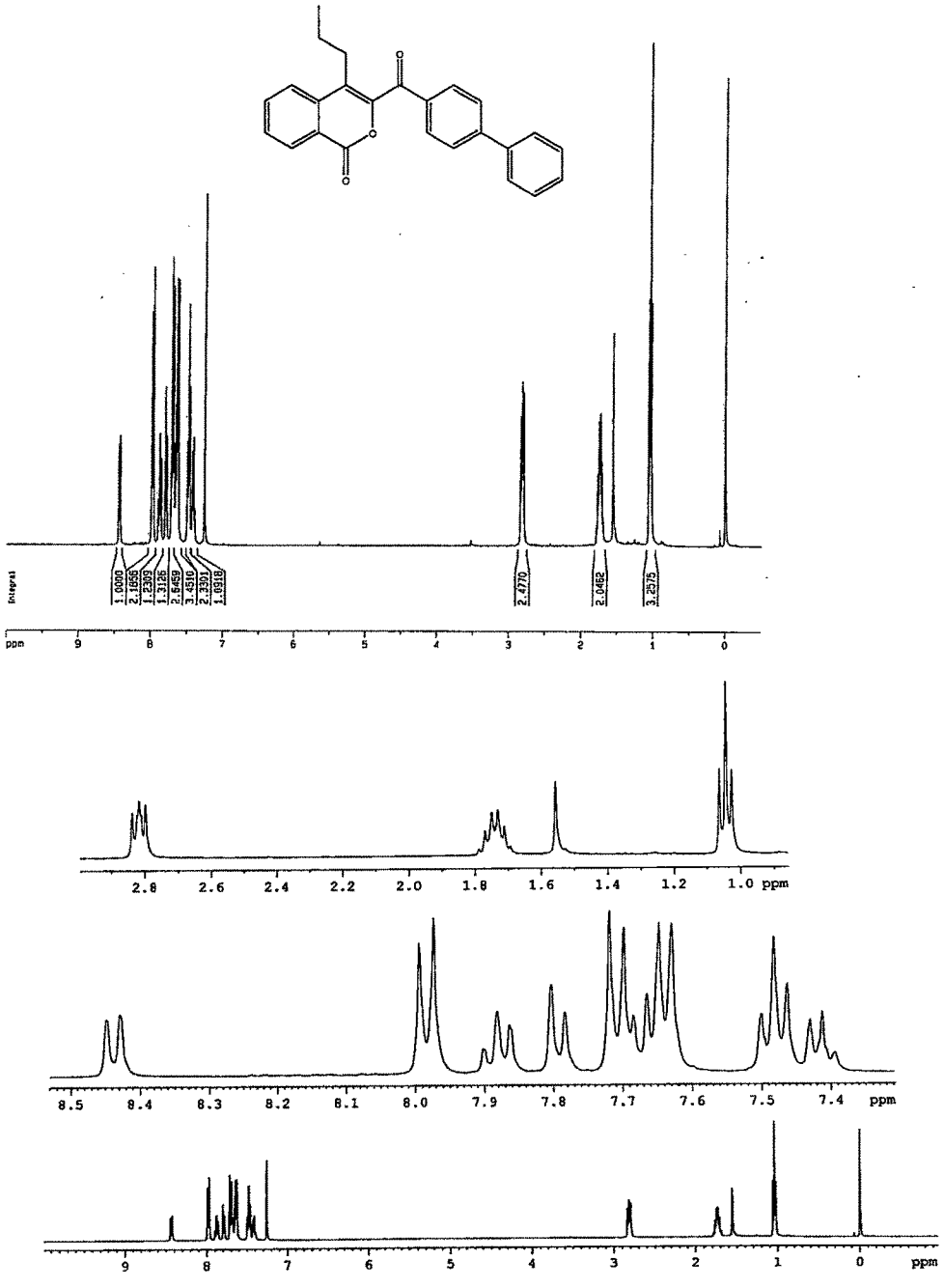


Fig. 2.12 - ^{1}H NMR: 3-(4'- Phenyl benzoyl)-4-propyl isocoumarin 3o

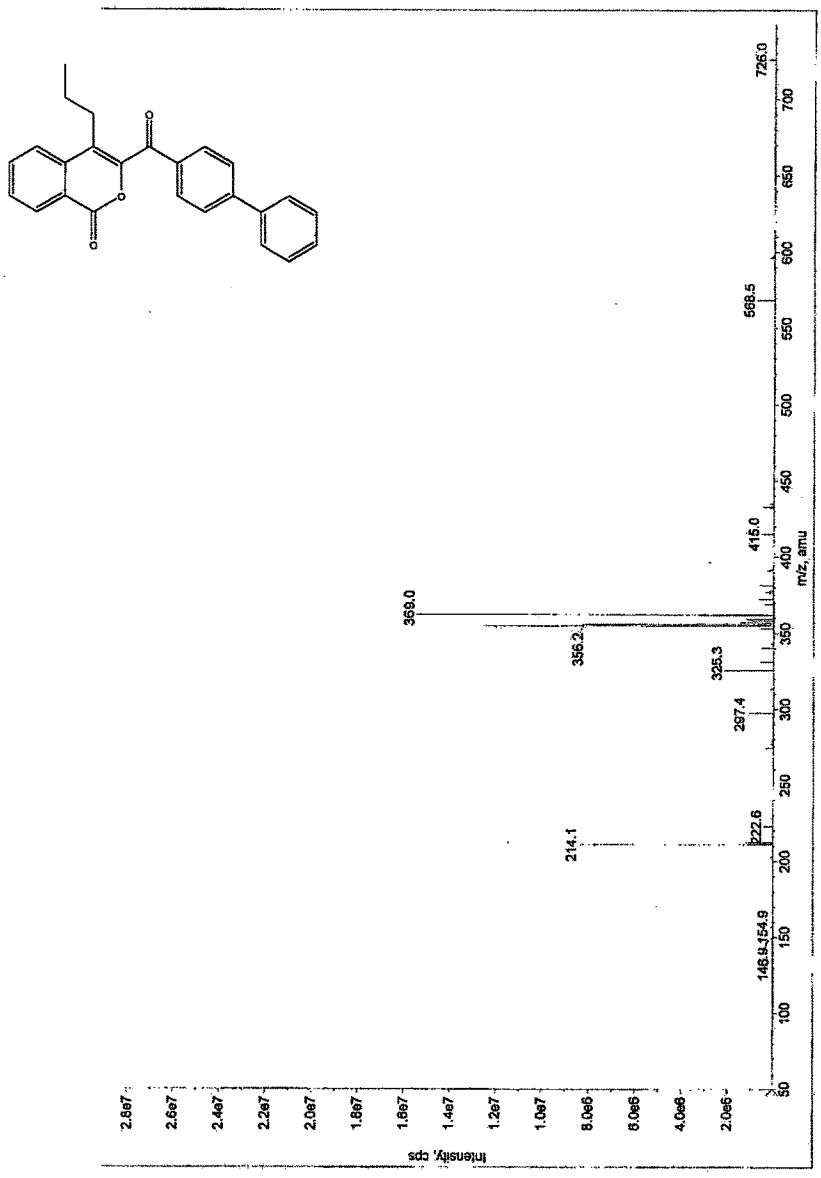
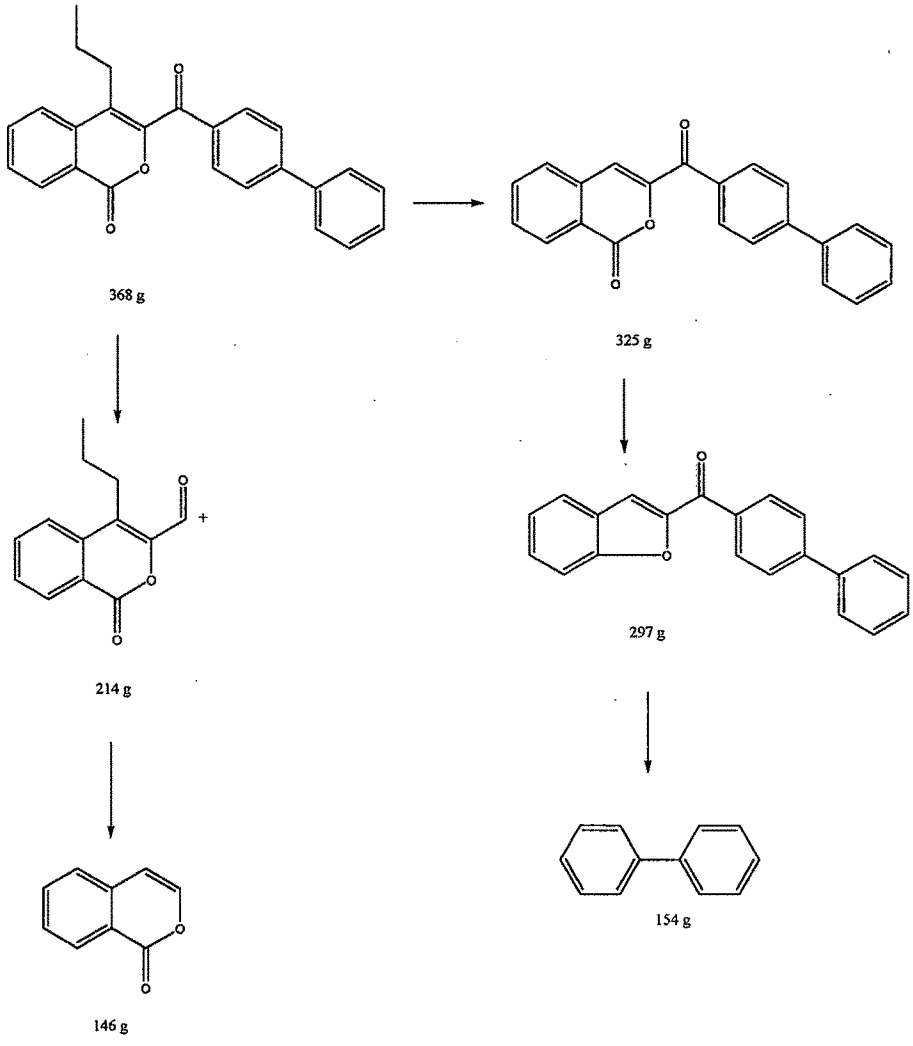


Fig. 2.13 - Mass spectrum: **3-(4'- Phenyl benzoyl)-4-propyl isocoumarin 3o**



Fragmentation Pattern: **3-(4'- Phenyl benzoyl)-4-propyl isocoumarin**

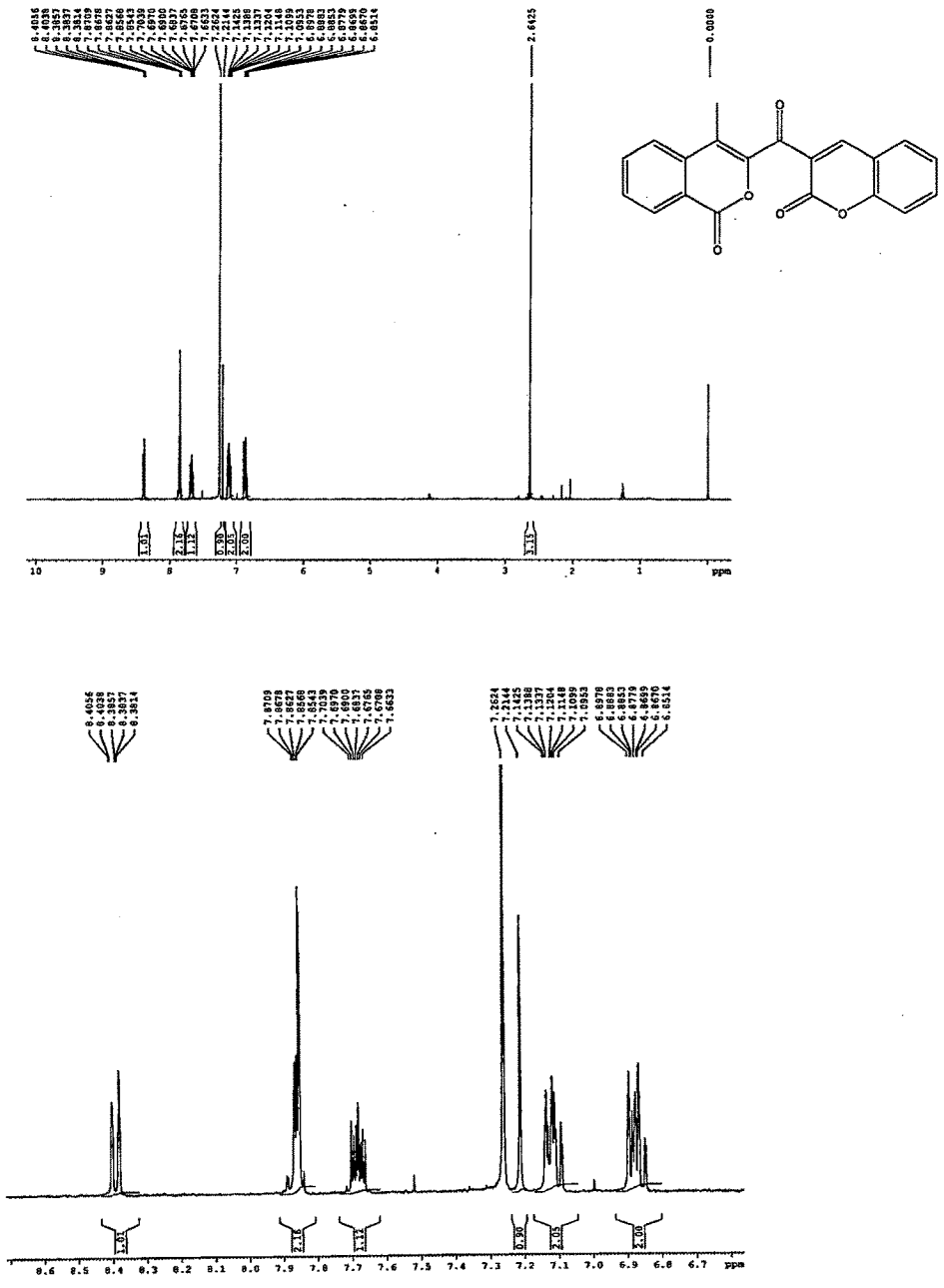


Fig. 2.14 - ^{1}H NMR: **3-Coumarinoyl-4-methyl isocoumarin 5a**

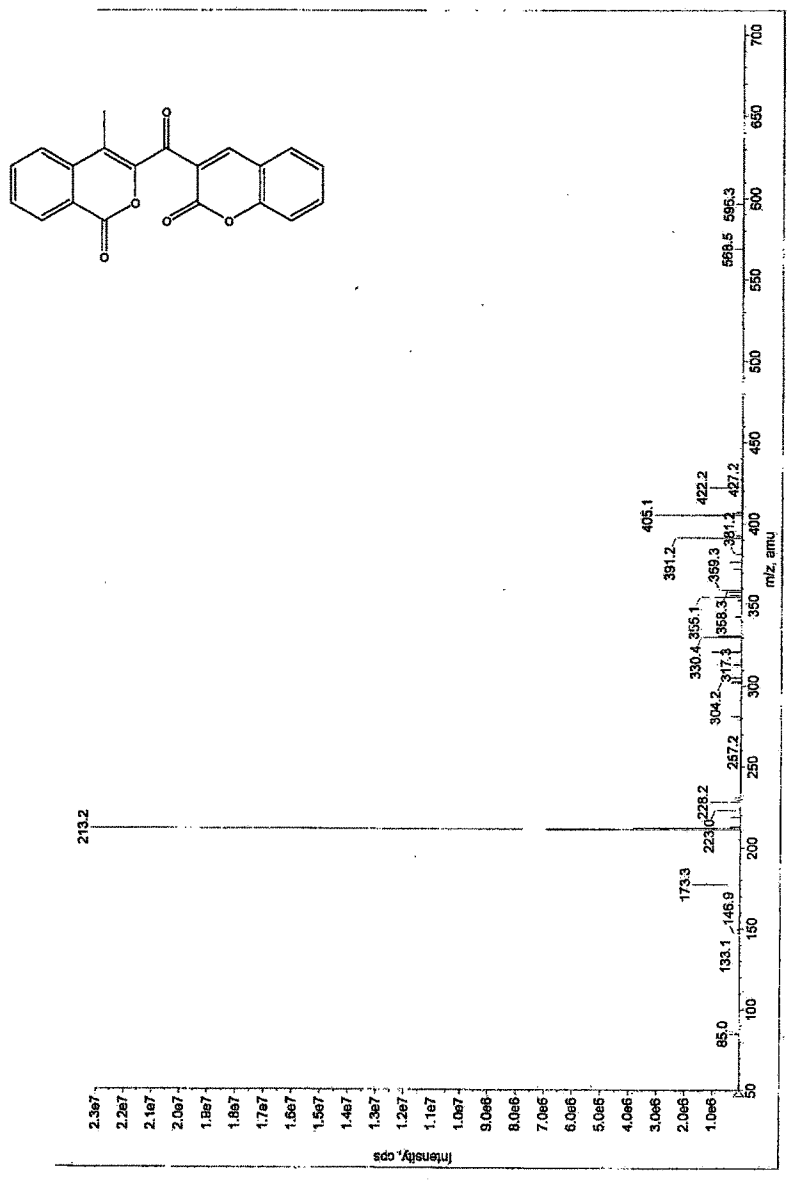
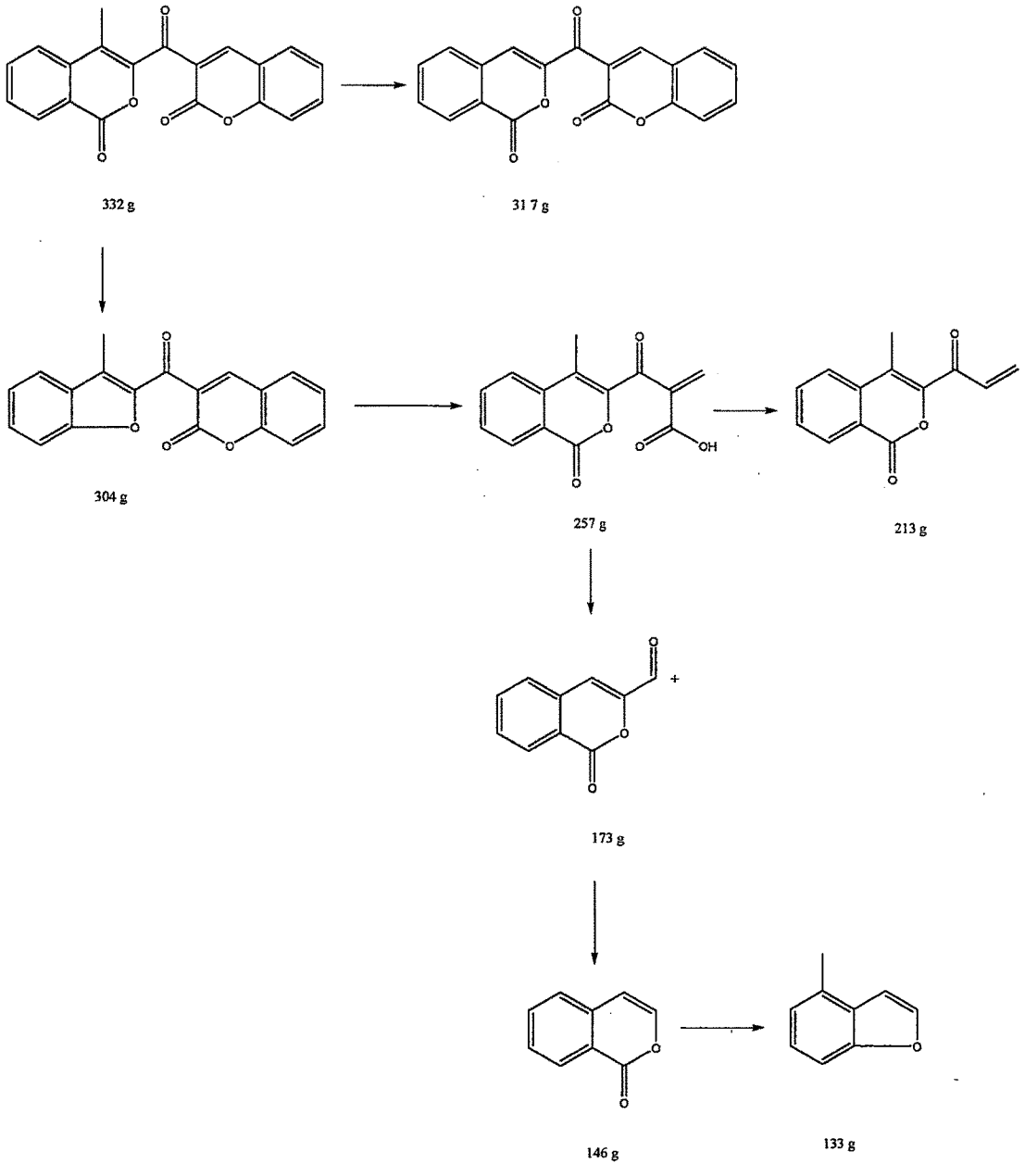
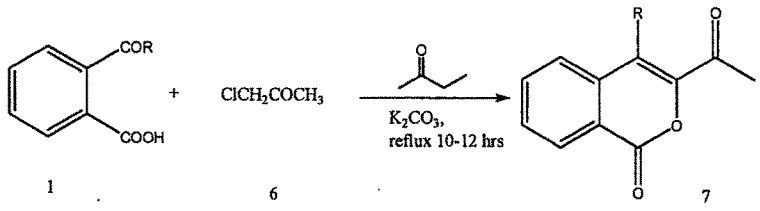


Fig. 2.15 - Mass spectrum: **3-Coumarinoyl-4-methyl isocoumarin 5a**



Fragmentation Pattern: **3-Coumarinoyl-4-methyl isocoumarin**

Scheme II



1 6 7

R = $-CH_3$ **(1a)**
$-C_2H_5$ **(1b)**
$-C_3H_7$ **(1c)**

R = $-CH_3$ **(7a)**
$-C_2H_5$ **(7b)**
$-C_3H_7$ **(7c)**

In **Scheme II**, here chloroacetone **6** was used instead of bromoacetophenone derivatives and refluxed with o – acyl benzoic acids **1a-c** in the presence of base leading to 4-alkyl-3-acetyl isocoumarin **7** in place of 4-alkyl-3-aroyl isocoumarins. The intention to synthesize isocoumarins **7** was to see the effect of acetyl and aroyl group on different biological studies.

IR spectrum shows signals at 1748 cm^{-1} and 1689 cm^{-1} for γ lactone and acetyl group respectively (**Fig. 2.16**).

The signals obtained in the ^1H NMR spectrum of **7a** are δ 2.60 (s, 3H, CH_3), 2.62 (s, 3H, $COCH_3$), 7.6-7.9 (m, 3H, aromatic protons), 8.39-8.41 (dd, 1H, C_8-H) (**Fig. 2.17**).

m/z peak for **7a** is obtained at 203(M^+ + 1), 187, 174, 159, 146 and 118 (**Fig. 2.18**).

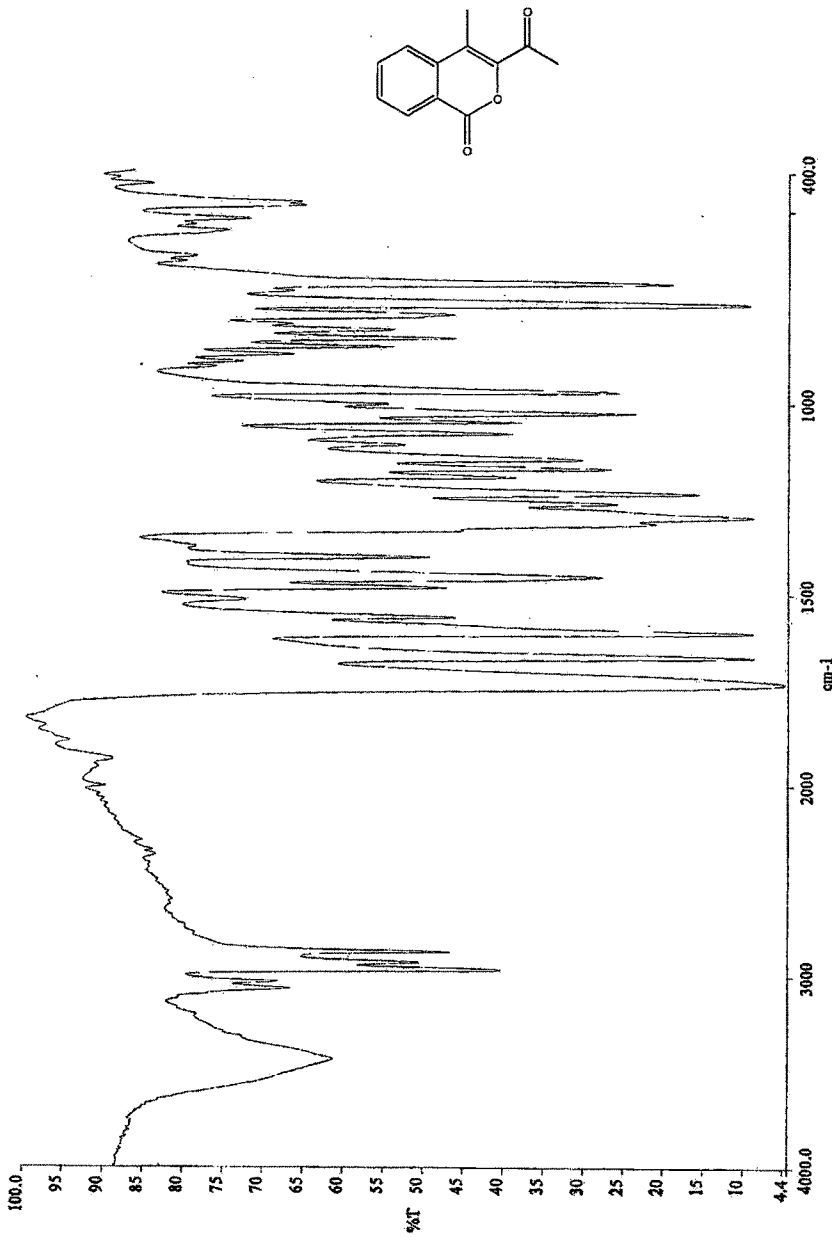
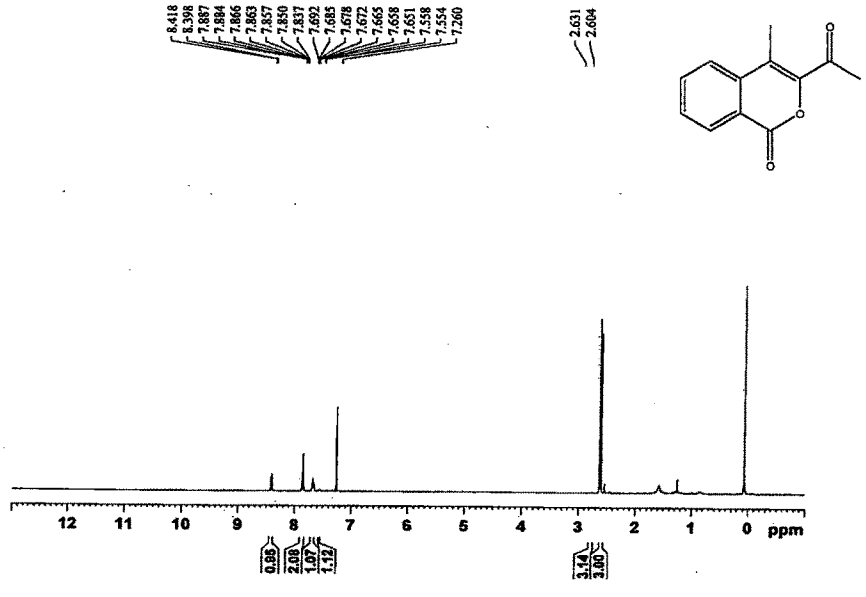


Fig. 2.16 – IR: **3-Acetyl-4-methyl isocoumarin 7a**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7.884
7.866
7.863
7.857
7.850
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7.665
7.658
7.651
7.558
7.554
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1.12
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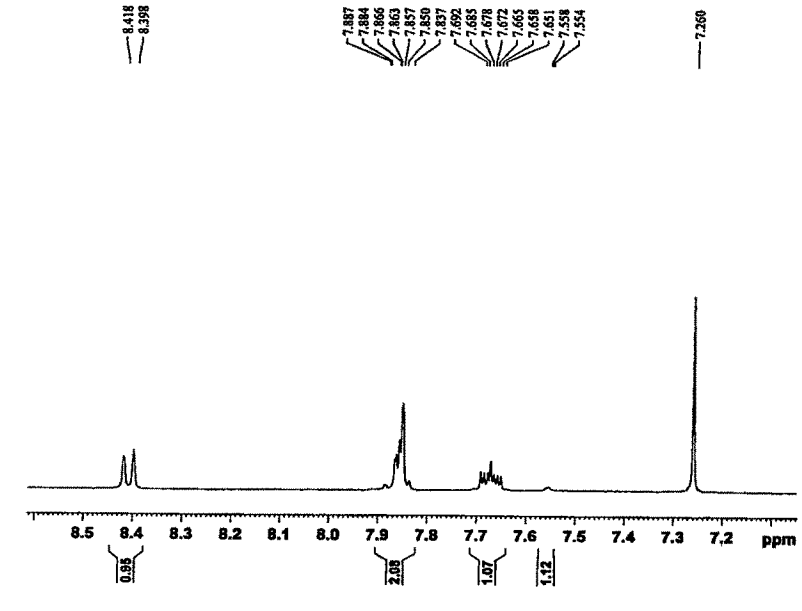
3.00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7.665
7.658
7.651
7.558
7.554
7.260
8.5 8.4 8.3 8.2 8.1 8.0 7.9 7.8 7.7 7.6 7.5 7.4 7.3 7.2 ppm
0.95
2.08
1.07
1.12

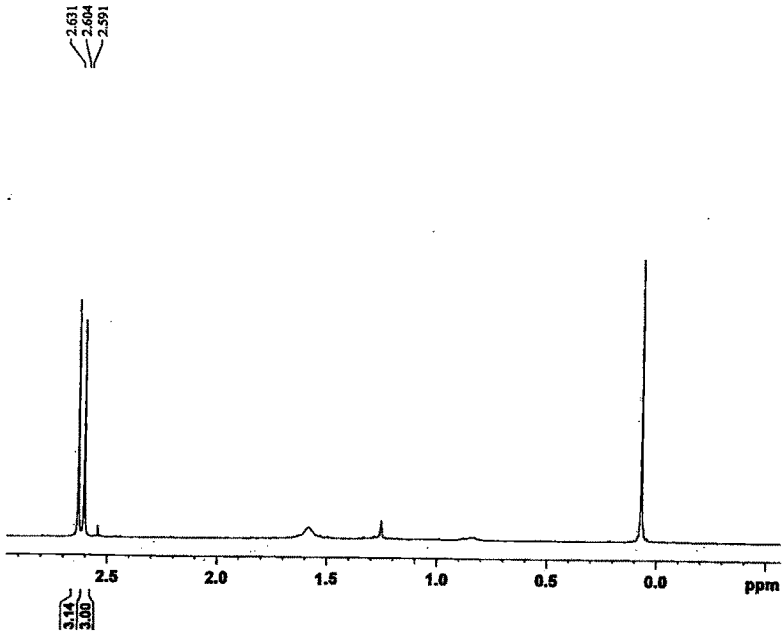


Fig. 2.17 – ^{1}H NMR: **3-Acetyl-4-methyl isocoumarin 7a**

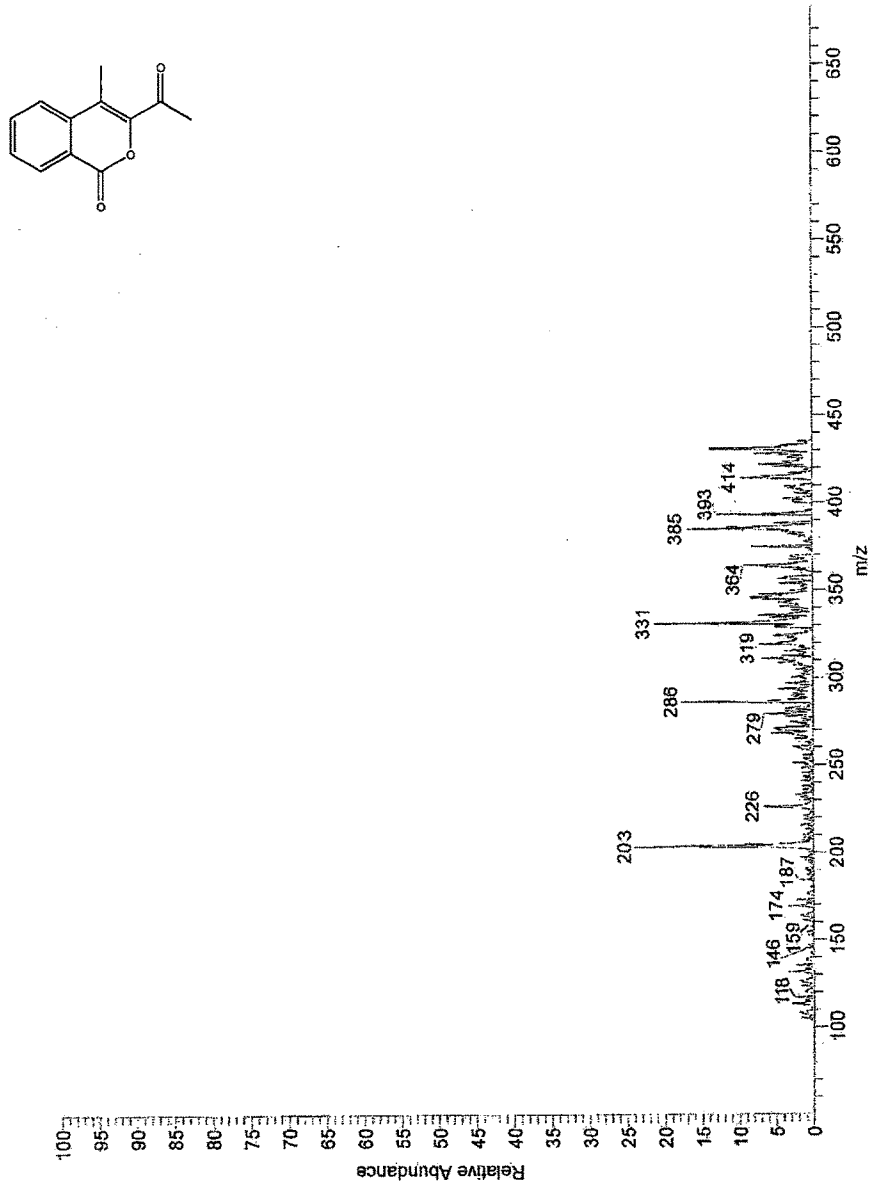
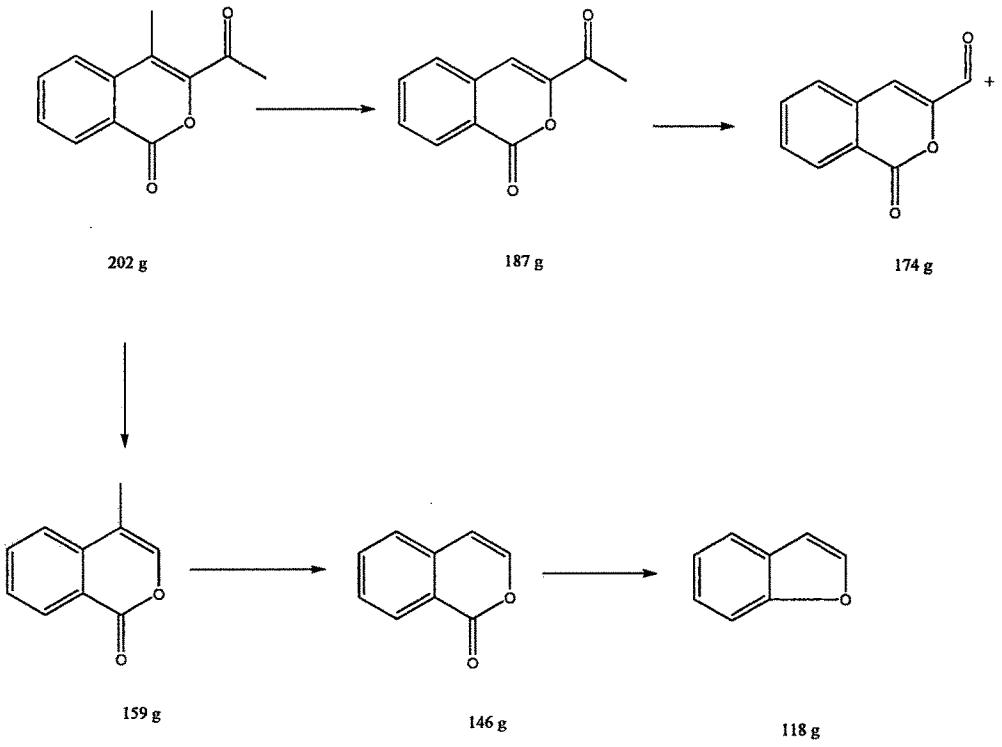
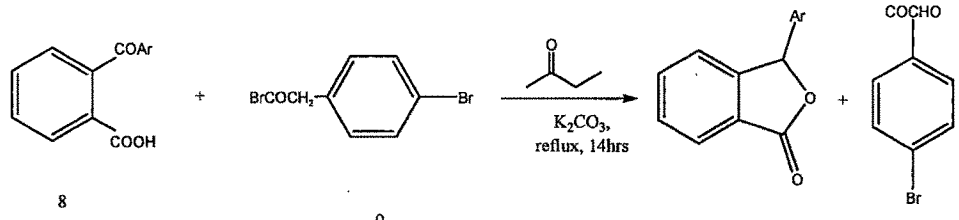


Fig. 2.18 - Mass spectrum: **3-Acetyl-4-methyl isocoumarin 7a**



Fragmentation Pattern: **3-Acetyl-4-methyl isocoumarin**

Scheme III



8 9 10 11

Ar = Biphenyl **10a)**
p-Tolyl **(10b)**
o- Xylol **(10c)**
Phenol **(10d)**
Methoxy benzene **(10e)**
Bromo benzene **(10f)**
Acetanilide **(10g)**
Napthalene **(10h)**
Anthracene **(10i)**
Dibenzofuran **(10j)**

In **Scheme III**, here o- aroyl benzoic acids **8** were used instead of o-acyl benzoic acids. o-aroyl benzoic acids on condensation with p- bromo phenacyl bromide **9** in presence of base in ethyl methyl ketone and following the same pathway resulted in the formation of a mixture of two products instead of single compound known by TLC. Here ring contraction takes place and instead of isocoumarins, phthalides **10** and phenyl glyoxal **11** was formed which was separated very carefully using column chromatography.

IR signals for different phthalides synthesized are at frequencies 1751, 1671, 1585, 3445 cm^{-1} for five memebered lactone, C=O, -NH respectively (**Fig. 2.21**).

^1H NMR of compounds **10f, 10g** and **10h** shows signals at δ 6.60 (s, 1H, C_3-H), 7.35-7.70 (m, 7H, aromatic protons), 8.11 (dd, 1H, C_7-H) (**Fig. 2.19**); δ 3.40 (s, 3H, $COCH_3$), 6.60 (s, 1H, C_3-H), 7.60-8.20 (m, 7H, aromatic protons), 8.40-8.45 (dd, 1H, C_7-H), 13.90 (s,

1H, NH) (**Fig. 2.22**); δ 6.50 (s, 1H, C_3-H), 7.40-7.70 (m, 10H, aromatic protons), 8.21 (dd, 1H, C_7-H) (**Fig. 2.24**) respectively.

Phenyl glyoxal (**11**) obtained gave all chemical tests satisfactorily. Formation of phenyl glyoxal was finally confirmed by its synthesis following the known procedure.[23]

Mass spectra of **10f, 10g** and **10h** shows m/z peaks at m/z : 209 (M^+ - Br), 181, 156, 134, 106 and 77 (**Fig. 2.20**), m/z : 268 (M^+ + 1), 224, 196 and 182 (**Fig. 2.23**) and m/z : 260 (M^+), 232, 132 and 128 (**Fig. 2.25**) respectively.

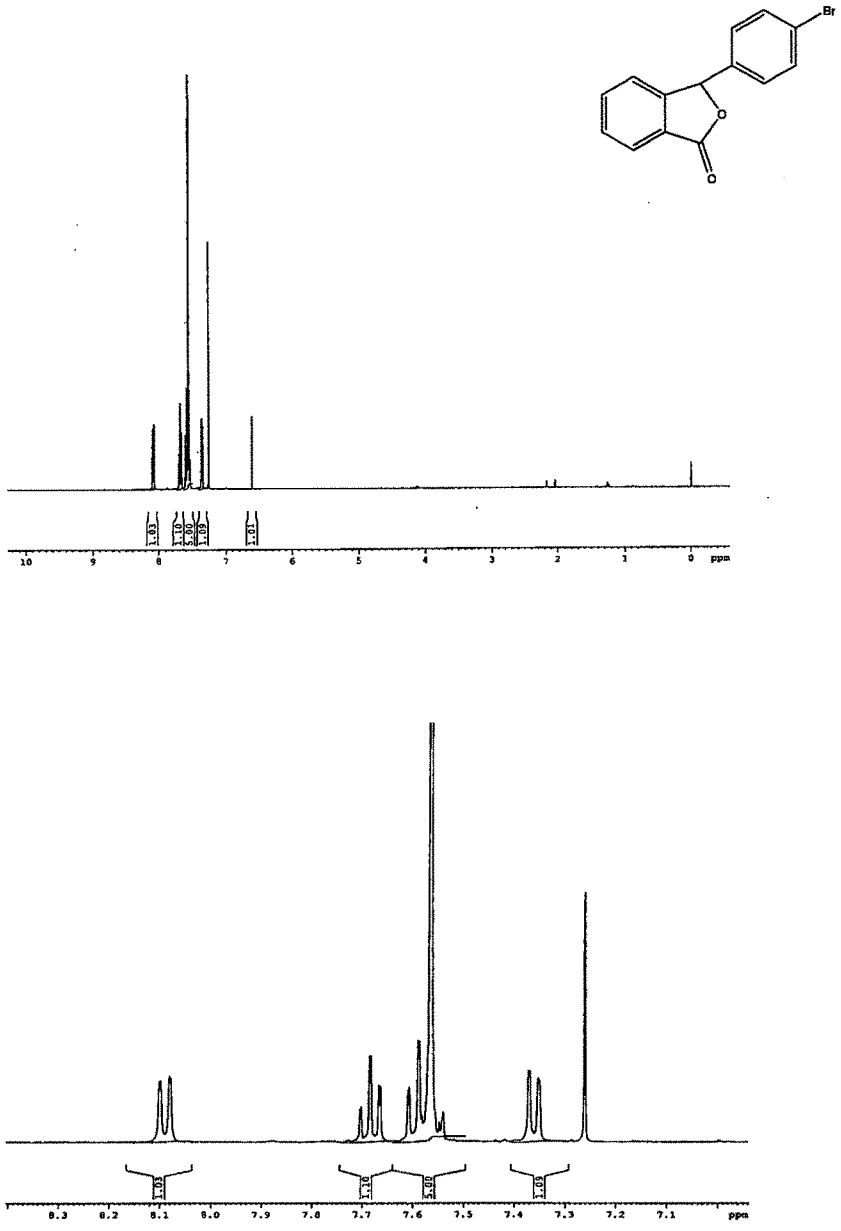


Fig. 2.19 - ^{1}H NMR: 3- (4'-Bromo phenyl) phthalide 10f

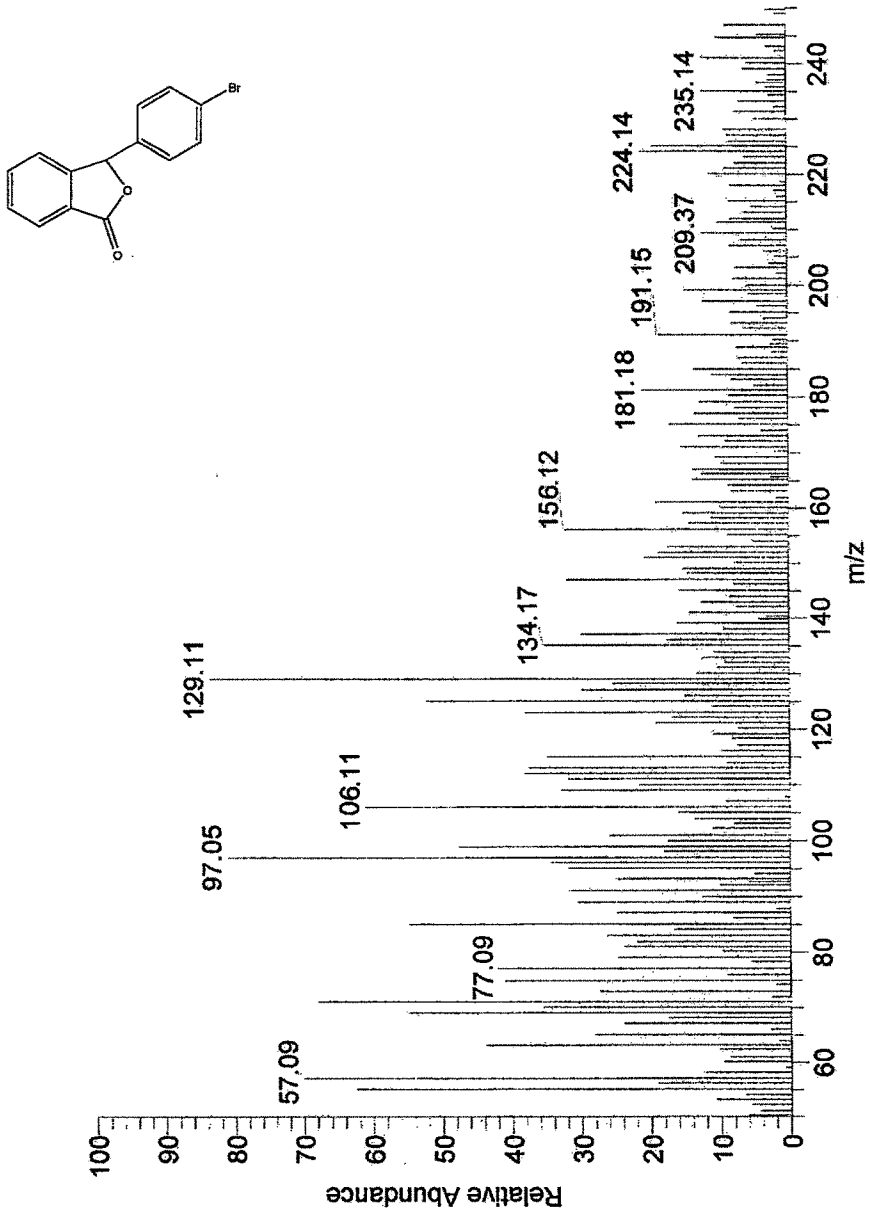
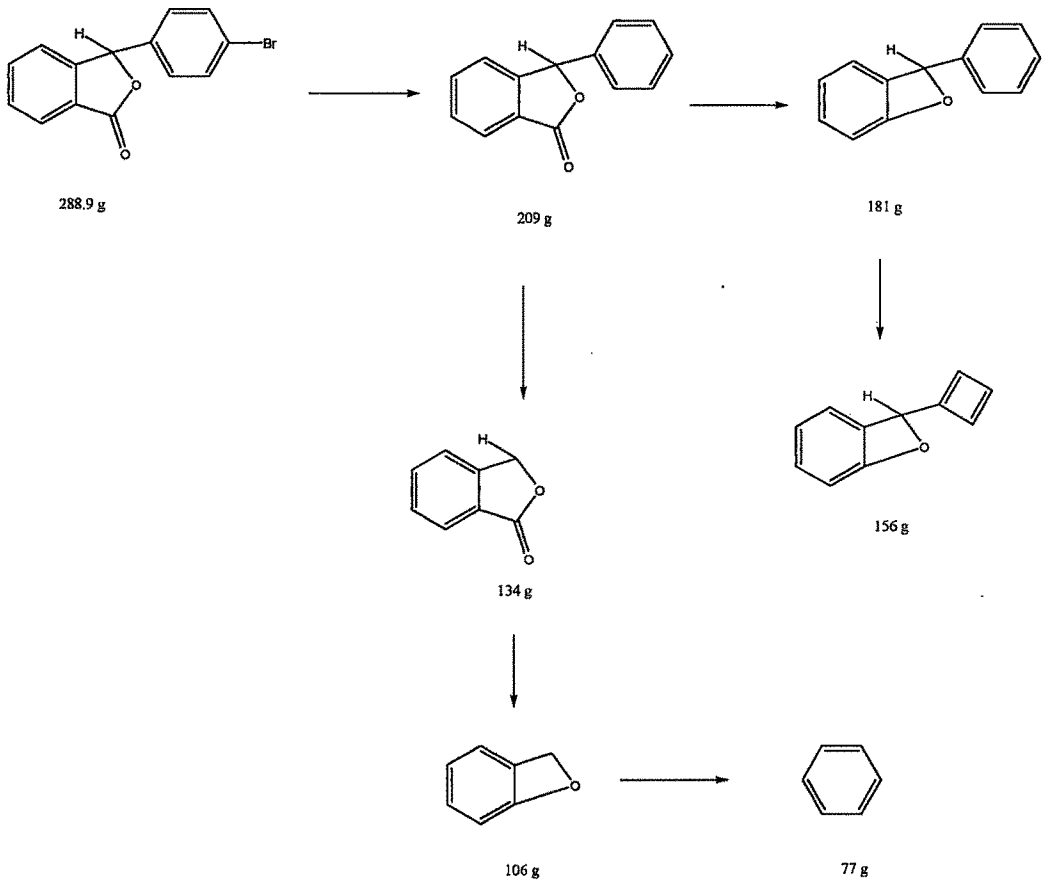


Fig. 2.20 - Mass spectrum: **3- (4'-Bromo phenyl) phthalide 10f**



Fragmentation Pattern: **3- (4'-Bromo phenyl) phthalide**

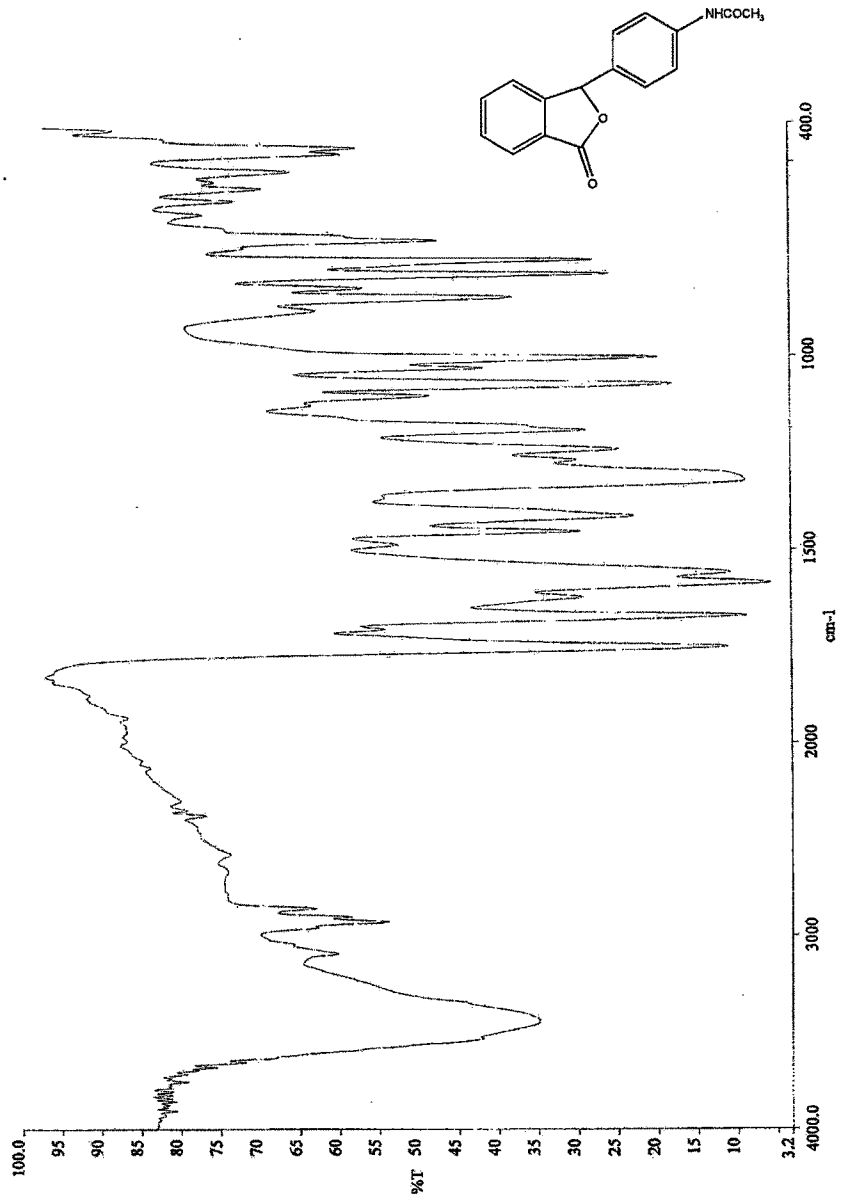


Fig. 2.21 – IR: **3- Acetanilidyl phthalide 10g**

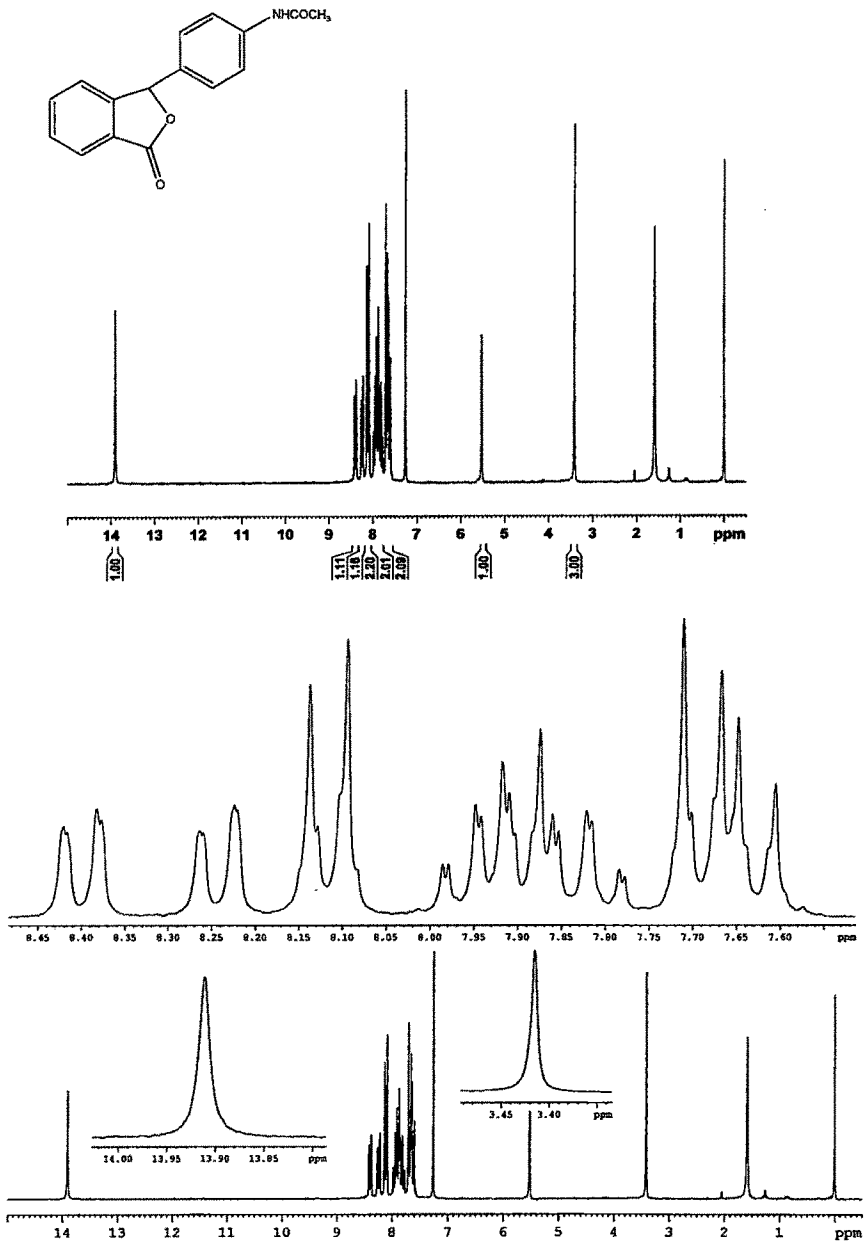


Fig. 2.22 - ^{1}H NMR: 3- Acetanilidyl phthalide 10g

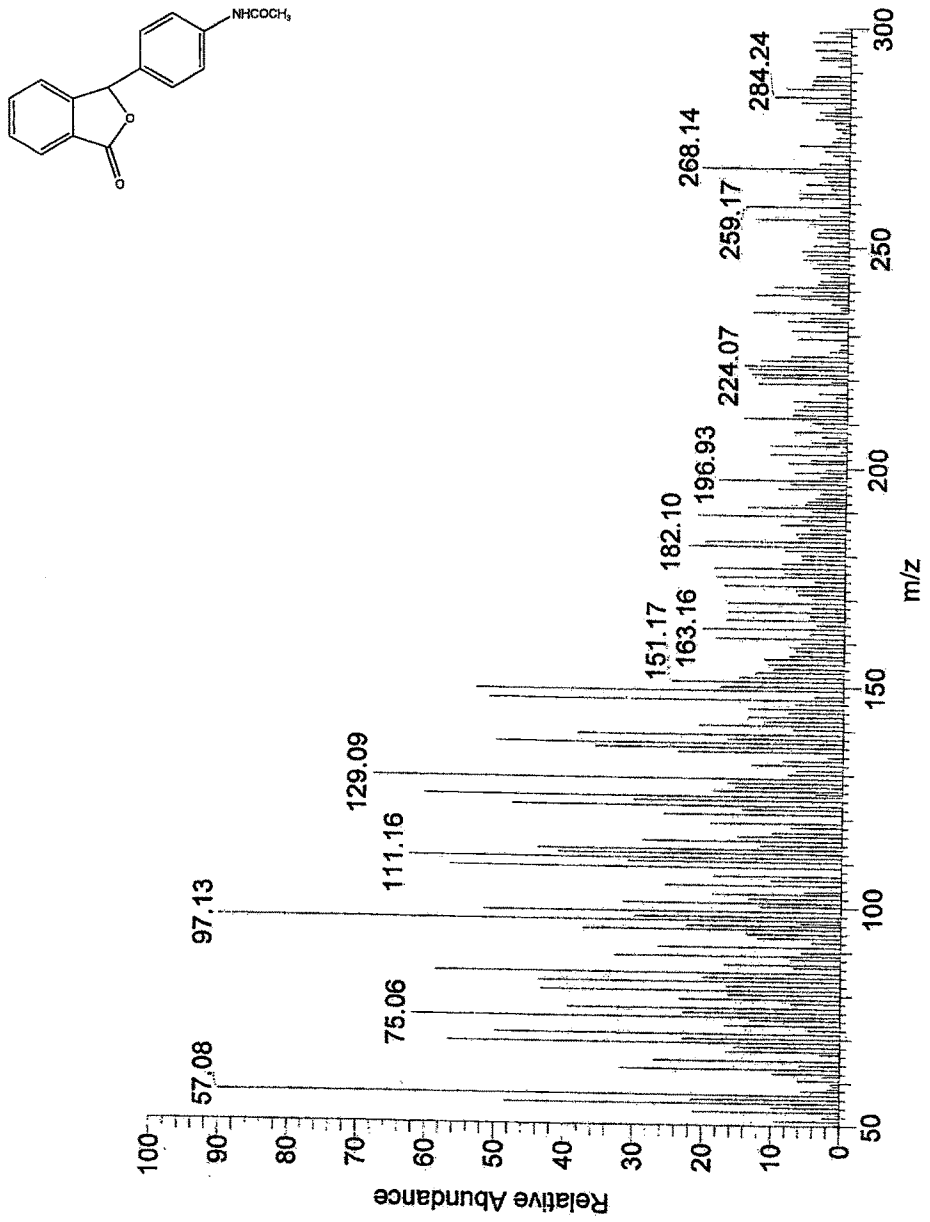
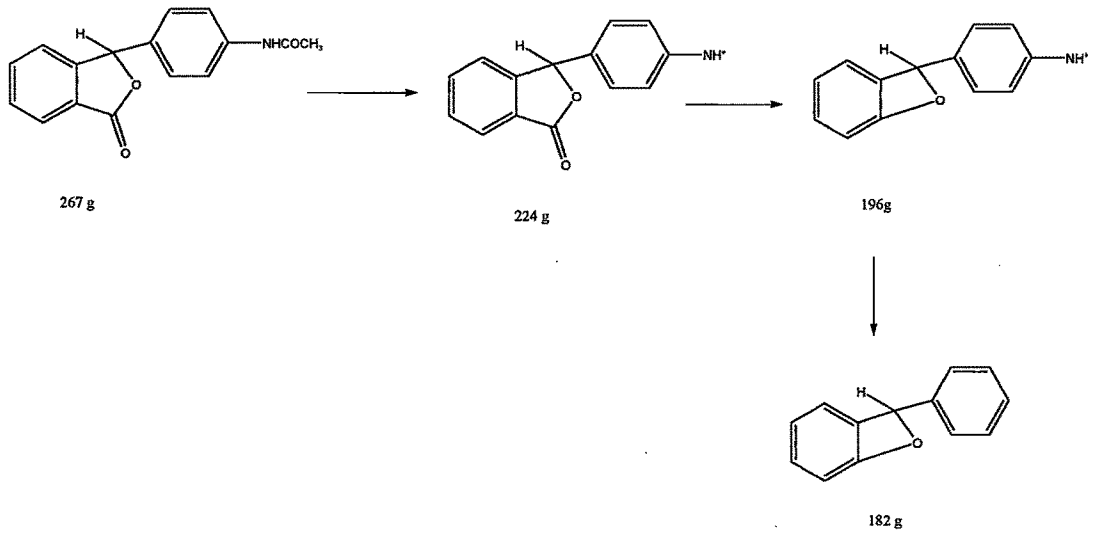


Fig. 2.23 - Mass spectrum: **3- Acetanilidyl phthalide 10g**



Fragmentation Pattern: **3- Acetanilidyl phthalide**

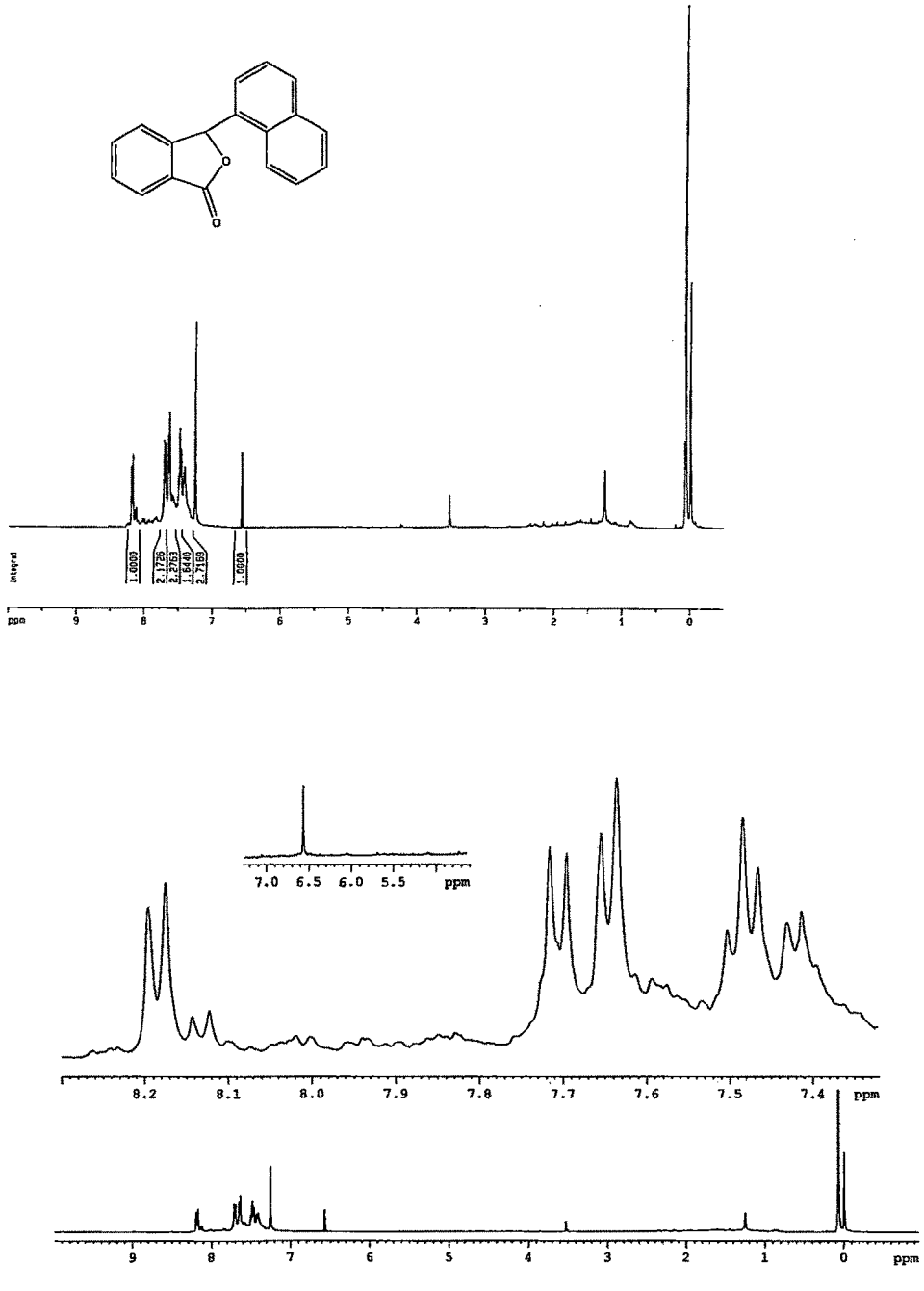


Fig. 2.24 - ^{1}H NMR: 3- Napthyl phthalide 10h

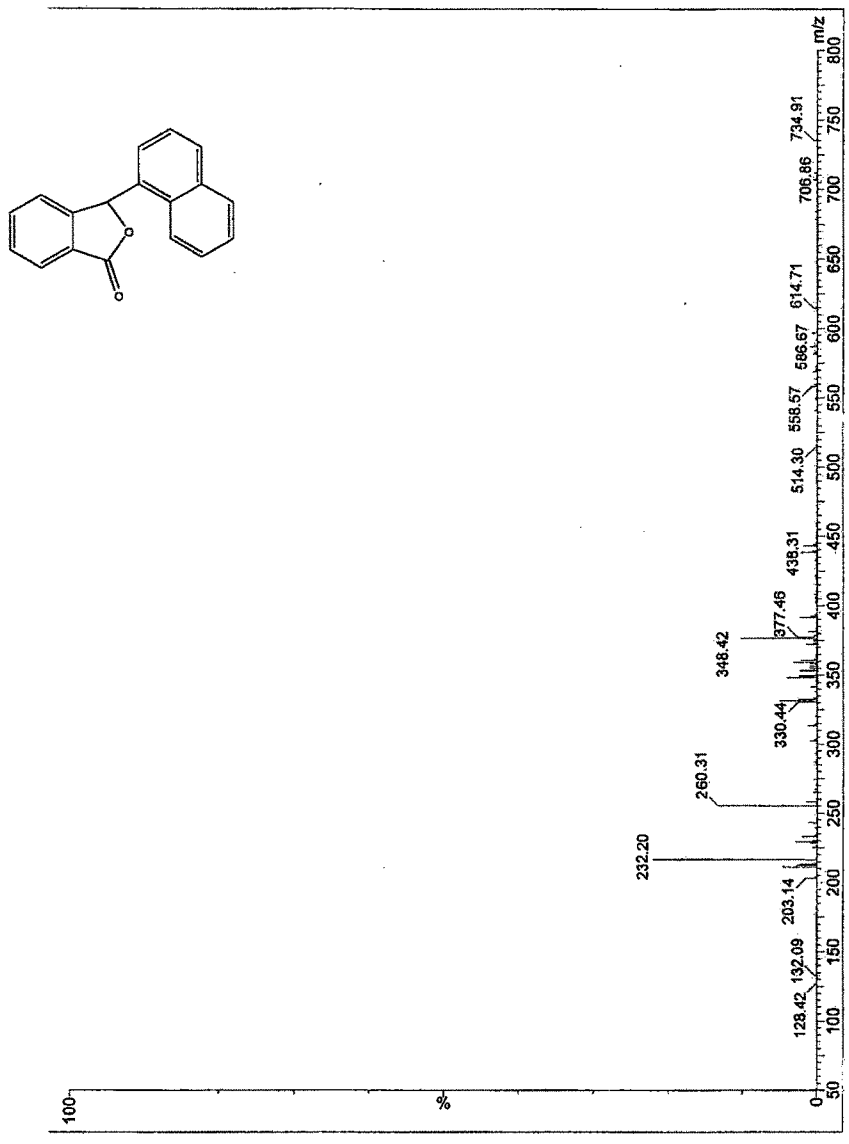
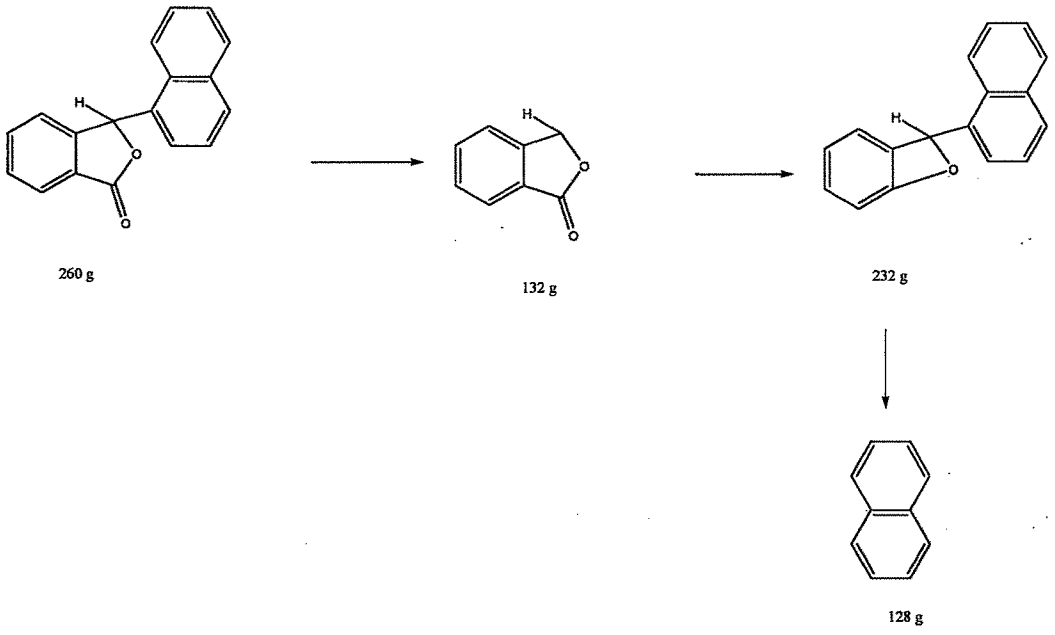


Fig. 2.25 - Mass spectrum: **3- Napthyl phthalide 10h**



Fragmentation Pattern: **3- Napthyl phthalide**

2.3 EXPERIMENTAL

The reagents and the solvents used in this study were of analytical grade and were used without further purification. Melting points were determined in open capillaries and are uncorrected. The purity of the compounds was checked by TLC on silica gel GF254 plates using UV/Iodine as visualizing agent and Merk's silica gel (60-120 mesh) was used for column chromatographic purification. Infrared spectra were recorded on FTIR Perkin Elmer spectrophotometer using potassium bromide optics. ^{1}H NMR spectra were recorded on a Bruker spectrometer (400 MHz) using TMS as internal standard and chemical shifts are given in ppm. Mass spectrums were obtained using Thermo Scientific Corporation, DSQ II Mass Spectrometer. Elemental analyses were carried out on Perkin-Elmer C, H, N, S analyzer (Model-2400). O-acyl benzoic acids and bromo acetophenone derivatives were prepared by literature method[23-27].

General procedure for 3a-3o

A mixture of o-acetyl benzoic acid **1a** (3 g, 0.018 moles), p-bromo phenacyl bromide **2** (5.08 g, 0.018 moles) anhy.K_2CO_3 (5.30 g, 0.038 moles) in ethyl methyl ketone was refluxed for 10- 12 hrs, solvent was removed completely; water added and reaction mixture was extracted with ethyl acetate. Solvent layer was first washed with sat. sodium bicarbonate and then with water and finally dried over anhy. Na_2SO_4. After removal of solvent the crude product was purified by column chromatography using petroleum ether (60-80^{0}C) -ethyl acetate mixture to give white crystals of **3a**

3-(4'-Bromo benzoyl) - 4-methyl-isocoumarin 3a

This compound was obtained as white crystals, mp 172^{0}C; 57.8%; Anal. Calcd $C_{17}H_{11}O_3Br$ (342.9 g): C, 59.49; H, 3.20; Found: C, 59.07; H, 3.30; ^{1}H NMR δ 1.50 (s, 3H, CH_3), 6.80- 8.00 (m, 7H, aromatic protons), 8.35 (d, 1H, C_8-H); ms: m/z: 343.9 (M^++1), 263, 220, 183.9, 155.9, 105 and 77.

3-(4'-Bromo benzoyl) - 4-ethyl-isocoumarin 3b

This compound was obtained as white crystals, mp 115^{0}C; 49.0%; Anal. Calcd $C_{18}H_{13}O_3Br$ (356.9g): C, 60.52; H, 3.64; Found: C, 60.50; H, 3.72; ^{1}H NMR δ 1.1 (t, 3H, CH_3), 1.7(q, 2H, CH_2), 7.60- 8.00 (m, 7H, aromatic protons), 8.40 (dd, 1H, C_8-H); ms: m/z: 357.9 (M^++1), 341.9, 277, 262, 234, 185, 182.9, 173, 154.9, 145, 117 and76.

3-(4'-Bromo benzoyl) - 4-propyl-isocoumarin 3c

This compound was obtained as white crystals, mp 131^{0}C; 52.0%; Anal. Calcd $C_{19}H_{15}O_3Br$ (370.9g): C, 61.47; H, 4.04; Found: C, 61.50; H, 4.21; ^{1}H NMR δ 1.0 (t, 3H, CH_3), 1.7 (m, 2H, CH_2), 2.8 (t, 2H, CH_2), 7.60-7.95 (m, 7H, aromatic protons), 8.40-8.45 (dd, 1H, C_8-H); ms: m/z: 370.9 (M^+), 300, 276, 262, 214, 187, 157, and 146.

3-(4'-Hydroxy benzoyl) - 4-methyl-isocoumarin 3d

This compound was obtained as yellow crystals, mp 217^{0}C; 69.30%; Anal. Calcd $C_{17}H_{12}O_4$ (280.0 g): C, 72.85; H, 4.04; Found: C, 72.82; H, 4.30; ^{1}H NMR δ 1.9 (s, 3H, CH_3), 7.30-7.90 (m, 7H, aromatic protons), 8.20 (dd, 1H, C_8-H), 12.5 (s, 1H, OH); ms: m/z: 280 (M^+), 265, 263, 187, 159, 146 and 121.

3-(4'-Hydroxy benzoyl) - 4-ethyl-isocoumarin 3e

This compound was obtained as pale yellow crystals, mp 177^{0}C; 70.00%; Anal. Calcd $C_{18}H_{14}O_4$ (294.0 g): C, 73.46; H, 4.76; Found: C, 73.28; H, 4.36; ^{1}H NMR δ 1.0 (t, 3H, CH_3), 1.8 (q, 2H, CH_2), 6.90-7.60 (m, 7H, aromatic protons), 8.30 (dd, 1H, C_8-H), 10.7 (s, 1H, OH); ms: m/z: 294 (M^+), 279, 238, 186, 173, 146 and121.

3-(4'-Hydroxy benzoyl) - 4- propyl-isocoumarin 3f

This compound was obtained as yellow crystals, mp 92^{0}C; 56.75%; Anal. Calcd $C_{19}H_{16}O_4$ (308.0 g): C, 74.02; H, 5.46; Found: C, 74.00; H, 4.21; ^{1}H NMR δ 1.1 (t, 3H, CH_3), 1.7 (m, 2H, CH_2), 2.7 (q, 2H, CH_2), 6.87 (s, 1H, OH), 7.50-7.90 (m, 7H, aromatic protons), 8.0 (d, 1H, C_8-H); ms: m/z 308 (M^+), 294, 280, 252, 236, 215, 186,172, 157, 146 and 121.

3-(2', 4'-Diydroxy benzoyl) - 4- methyl-isocoumarin 3g

This compound was obtained as white crystals, mp 110^{0}C; 76.05%; Anal. Calcd $C_{17}H_{12}O_5$ (296.0 g): C, 69.83; H, 4.05; Found: C, 69.85; H, 4.21; ^{1}H NMR δ 2 .5 (s, 3H, CH_3), 6.3-7.9 (m, 6H, aromatic protons), 8.2 (d, 1H, C_8-H), 12.4 (s, 1H, OH), 12.6 (s, 1H, OH); ms: m/z m/z: 295 (M^+-1), 281, 236, 221, 185, 149, 121 and 110.

3-(2', 4'-Diydroxy benzoyl) - 4- ethyl-isocoumarin 3h

This compound was obtained as pale white crystals, mp 113^{0}C; 76.39%; Anal. Calcd $C_{18}H_{14}O_5$ (310.0 g): C, 69.67; H, 4.51; Found: C, 69.65; H, 4.54; ^{1}H NMR δ 1 .1 (t, 3H, CH_3), 1.8(q, 2H, CH_2), 6.4-7.7 (m, 6H, aromatic protons), 8.1 (d, 1H, C_8-H), 11.6 (s, 1H, OH), 11.9 (s, 1H, OH); ms: m/z: 311 (M^++1), 295, 292, 281, 185, 157, 137 and 121.

3-(2', 4'-Diydroxy benzoyl) - 4- propyl-isocoumarin 3i

This compound was obtained as yellowish white crystals, mp 132^{0}C; 34.27%; Anal. Calcd $C_{19}H_{16}O_5$ (324.0 g): C, 70.37; H, 4.93; Found: C, 70.32; H, 4.90; ^{1}H NMR δ 1 .0 (t, 3H, CH_3), 1.9(m, 2H, CH_2), 2.6 (t, 2H, CH_2), 6.5-7.9 (m, 6H, aromatic protons), 8.45 (d, 1H, C_8-H), 9.0 (s, 1H, OH), 9.1 (s, 1H, OH); ms: m/z :325 (M^++1), 306, 296, 281, 275, 185, 157, 146, 121 and 110.

3-(4'-Methoxy benzoyl) - 4- methyl-isocoumarin 3j

This compound was obtained as white crystals, mp 128^{0}C; 45.83%; Anal. Calcd $C_{18}H_{14}O_4$ (294.0 g): C, 73.46; H, 4.76; Found: C, 73.50; H, 4.59; ^{1}H NMR δ 2 .0 (s, 3H, CH_3), 3.6 (s, 3H, OCH_3), 7.0-7.9 (m, 6H, aromatic protons), 8.55 (d, 1H, C_8-H); ms: m/z: 294 (M^+), 263, 252, 221, 173, 144, 135 and 104.

3-(4'-Methoxy benzoyl) - 4- ethyl-isocoumarin 3k

This compound was obtained as white crystals, mp 140^{0}C; 45.00%; Anal. Calcd $C_{19}H_{16}O_4$ (308.0 g): C, 74.02; H, 5.19; Found: C, 74.13; H, 4.99; ^{1}H NMR δ 1.3 (t, 3H,

CH_3), 2.8 (q, 2H, CH_2), 4.0 (s, 3H, OCH_3), 6.95-8.05 (m, 7H, aromatic protons), 8.41-8.43 (dd, 1H, C_8-H); ms: m/z 308 (M^+), 262, 187, 146, 135 and 108.

3-(4'-Methoxy benzoyl) - 4- propyl-isocoumarin 3l

This compound was obtained as pale white crystals, mp 110^{0}C; 37.92%; Anal. Calcd $C_{20}H_{18}O_4$ (322.0 g): C, 74.53; H, 5.59; Found: C, 74.61; H, 5.90; ^{1}H NMR δ 0.9 (t, 3H, CH_3), 1.7 (m, 2H, CH_2), 2.7 (q,2H, CH_2), 3.9 (s, 3H, OCH_3), 6.95-7.80 (m, 7H, aromatic protons), 8.43-8.44 (d, 1H, C_8-H); ms: m/z 322 (M^+), 291, 279, 173, 146 and 135.

4- Methyl- 3-(4'- phenyl benzoyl) isocoumarin 3m

This compound was obtained as whitish yellow crystals, mp 145^{0}C; 62.73%; Anal. Calcd $C_{23}H_{16}O_3$ (340.0 g): C, 81.11; H, 4.70; Found: C, 81.59; H, 4.83; ^{1}H NMR δ 2.2 (s, 3H, CH_3), 7.20-7.90 (m, 12H, aromatic protons), 8.43-8.45 (dd, 1H, C_8-H); ms: m/z: 342 (M^++2), 325, 312, 187, 159, 154 and 146.

4- Ethyl- 3-(4'- phenyl benzoyl) isocoumarin 3n

This compound was obtained as white crystals, mp 153^{0}C; 66.00%; Anal. Calcd $C_{24}H_{18}O_3$ (354.0 g): C, 81.35; H, 5.08; Found: C, 81.00; H, 5.39; ^{1}H NMR δ 1.3 (t, 3H, CH_3), 2.8 (q, 2H, CH_2), 7.4-8.0 (m, 12H, aromatic protons), 8.43-8.45 (dd, 1H, C_8-H); ms: m/z 355 (M^++1), 340, 277, 222, 202, 154 and 146.

3-(4'- Phenyl benzoyl)-4-propyl isocoumarin 3o

This compound was obtained as white crystals, mp 110^{0}C; 72.14%; Anal. Calcd $C_{25}H_{20}O_3$ (368.0 g): C, 81.52; H, 5.43; Found: C, 81.49; H, 5.40; ^{1}H NMR δ 1.0 (t, 3H, CH_3), 1.7 (m, 2H, CH_2), 2.8 (t, 2H, CH_2), 7.40-7.95 (m, 12H, aromatic protons), 8.42-8.44 (d, 1H, C_8-H); ms: m/z 369 (M^++1), 325, 297, 214, 154 and 146.

General procedure for 5a-5b

A mixture of o-acetyl benzoic acid **1a** (1 g, 0.006 moles), bromacetyl coumarin **4** (1.627 g, 0.006 moles) anhy.K_2CO_3 (1.76 g, 0.012 moles) in ethyl methyl ketone was refluxed for 10- 12 hrs, solvent removed after reaction was over; water added and extracted with ethyl acetate. Solvent layer was first washed with sodium bicarbonate and then with water and it was dried over anhy. Na_2SO_4. After removal of solvent the crude product was purified by column chromatography using petroleum ether (60-80^{0}C) -ethyl acetate mixture to give white crystals of **5a**

3-Coumarinoyl-4-methyl isocoumarin 5a

This compound was obtained as yellow crystals, mp 185^{0}C; 38.22%; Anal. Calcd $C_{20}H_{12}O_5$ (332.0 g): C, 61.01; H, 5.30 Found: C, 61.01; H, 5.08; ^{1}H NMR δ 2.6 (s, 3H, -CH_3), 6.9-7.9 (m, 8H, aromatic protons), 8.4 (d, 1H, C_8-H); ms: m/z 330 (M^+-2), 317, 304, 257, 213, 173, 146 and 133.

3-Coumarinoyl-4-ethyl isocoumarin 5b

This compound was obtained as pale white crystals, mp 200^{0}C; 40.02%; Anal. Calcd $C_{21}H_{14}O_5$ (346.0 g): C, 62.40; H, 5.60 Found: C, 62.00; H, 5.36; ^{1}H NMR δ 1.0 (t, 3H, -CH_3), 2.0 (q, 2H, CH_2), 7.4-7.7 (m, 8H, aromatic protons), 8.1-8.2 (d, 1H, C_8-H); ms: m/z: 346 (M^+), 331, 318, 317, 173, 146 and 118.

General procedure for 7a-7c

A mixture of o-acetyl benzoic acid **1a** (1g, 0.006 moles), chloroacetone **6** (0.834g, 0.006 moles), anhy.K_2CO_3 (1.72g, 0.0128 moles) and catalytic amount of potassium iodide in ethyl methyl ketone was refluxed for 10- 12 hrs, usual work up yielded dark yellow crystals of **7a**

3-Acetyl-4-methyl isocoumarin 7a

This compound was obtained as white crystals, mp 110^{0}C; 40.00%; Anal. Calcd $C_{12}H_{10}O_3$ (202.0 g): C, 71.28; H, 4.95; Found: C, 71.40; H, 4.80; ^{1}H NMR δ 2.60 (s, 3H, CH_3), 2.62 (s, 3H, $COCH_3$), 7.6-7.9 (m, 3H, aromatic protons), 8.39-8.41 (dd, 1H, C_8-H); ms: m/z 203 (M^+ + 1), 187, 174, 159, 146 and 118.

3-Acetyl-4-ethyl isocoumarin 7b

This compound was obtained as white crystals, bp □200^{0}C; 37.52%; Anal. Calcd $C_{13}H_{12}O_3$ (216.0 g): C, 72.22; H, 5.55; Found: C, 72.00; H, 5.57; ^{1}H NMR δ 1.1 (t, 3H, CH_3), 1.9 (q, 2H, CH_2), 2.65 (s, 3H, $COCH_3$), 7.3-7.6 (m, 3H, aromatic protons), 8.2 (d, 1H, C_8-H); ms: m/z 215 (M^+ - 1), 201, 188, 173, 159, 146 and 43.

3-Acetyl-4-proyl isocoumarin 7c

This compound was obtained as semi solid, 22.20%.

General procedure for 10a-10j

A mixture of o-biphenyl benzoic acid **8** (1g, 0.0033 moles) & p-bromo phenacyl bromide **9** (0.919 g 0.0033 moles), anhy. K_2CO_3 (0.959g, 0.0069 moles) in ethyl methyl ketone was refluxed for 12- 15 hrs, solvent was then removed, water added and it was extracted with ethyl acetate. Solvent layer was first washed with sat. sod. bicarbonate and then with water and finally dried over anhy. Na_2SO_4 resulting in a mixture containing two compounds **10a** & **11** (TLC). The two compounds were separated by column chromatography using petroleum ether (60-80^{0}C) -ethyl acetate to give white crystals of **10a**

3-Biphenyl phthalide 10a

This compound was obtained as white crystals, mp 180^{0}C; 72.00%; Anal. Calcd $C_{20}H_{14}O_2$ (286.0 g): C, 83.91; H, 4.89; Found: C, 83.86; H, 4.90; ^{1}H NMR δ 6.4 (s, 1H,

C_3-H), 7.2-7.8 (m, 12H, aromatic protons), 8.0 (d, 1H, C_7-H); ms: m/z: 287 (M^+ + 1), 285, 258, 209, 132 and 77.

3-(4'-Tolyl) phthalide 10b

This compound was obtained as white crystals, mp 130^{0}C; 66.70%; Anal. Calcd $C_{15}H_{12}O_2$ (224.0 g): C, 80.35; H, 5.35; Found: C, 80.40; H, 5.30; ^{1}H NMR δ 6.5 (s, 1H, C_3-H), 2.4 (s, 3H, CH_3), 6.9-7.5 (m, 7H, aromatic protons), 7.9 (d, 1H, C_7-H); ms: m/z: 224 (M^+), 209, 196, 147 and 76.

3-(3', 4'-Dimethyl) phthalide 10c

This compound was obtained as brown crystals, mp 127^{0}C; 51.00%; Anal. Calcd $C_{16}H_{14}O_2$ (238.0 g): C, 80.67; H, 5.88; Found: C, 80.10; H, 5.80; ^{1}H NMR δ 6.3 (s, 1H, C_3-H), 2.35-2.40 (s, 6H, CH_3), 6.8-7.5 (m, 6H, aromatic protons), 7.95 (d, 1H, C_7-H); ms: m/z: 240 (M^+ + 2), 237, 223, 210, 208, 92 and 77.

3- Phenyl phthalide 10d

This compound was obtained as yellow powder, mp 110^{0}C; 65.83%; Anal. Calcd $C_{14}H_{10}O_2$ (210 g): C, 80.00; H, 4.76; Found: C, 80.13; H, 4.88; ^{1}H NMR δ 6.4 (s, 1H, C_3-H), 7.2-7.6 (m, 8H, aromatic protons), 7.9 (d, 1H, C_7-H); ms: m/z: 211 (M^+ + 1), 133 and 77.

3- (4'-Methoxy phenyl) phthalide 10e

This compound was obtained as white powder, mp 119^{0}C; 53.28%; Anal. Calcd $C_{15}H_{12}O_3$ (240 g): C, 75.00; H, 5.00; Found: C, 74.34; H, 5.09; ^{1}H NMR δ 6.35 (s, 1H, C_3-H), 3.9(s, 3H, OCH_3), 6.8-7.5 (m, 7H, aromatic protons), 7.85 (d, 1H, C_7-H); ms: m/z: 240 (M^+), 239, 212, 135, 105 and 76.

3- (4'-Bromo phenyl) phthalide 10f

This compound was obtained as white crystals, mp 115^{0}C; 56.00%; Anal. Calcd $C_{14}H_9O_2Br$ (288.9 g): C, 58.15; H, 3.11; Found: C, 58.29; H, 3.46; ^{1}H NMR δ 6.60 (s,

1H, C_3-H), 7.35-7.70 (m, 7H, aromatic protons), 8.11 (dd, 1H, C_7-H); ms: m/z 209 (M^+ - Br), 181, 156, 134, 106 and 77.

3- Acetanilidyl phthalide 10g

This compound was obtained as yellow powder, mp 160^{0}C; 46.50%; Anal. Calcd $C_{16}H_{13}O_3N$ (267.0 g): C, 71.91; H, 4.86; N, 5.24; Found: C, 71.64; H, 5.24; N, 5.61; ^{1}H NMR δ 3.40 (s, 3H, $COCH_3$), 6.60 (s, 1H, C_3-H), 7.60-8.20 (m, 7H, aromatic protons), 8.40-8.45 (dd, 1H, C_7-H), 13.90 (s, 1H, NH); ms: m/z 268 (M^+ + 1), 224, 196 and 182.

3- Napthyl phthalide 10h

This compound was obtained as pale yellow powder, mp 140^{0}C; 40.19%; Anal. Calcd $C_{18}H_{12}O_2$ (260.0 g): C, 83.07; H, 4.61; Found: C, 83.19; H, 4.57; ^{1}H NMR δ 6.56 (s, 1H, C_3-H), 7.4-7.7 (m, 10H, aromatic protons), 8.20 (d, 1H, C_7-H); ms: m/z 260 (M^+), 232, 132 and 128.

3- Anthracenyl phthalide 10i

This compound was obtained as white powder, mp 72^{0}C; 37.58%; Anal. Calcd $C_{22}H_{14}O_2$ (310.0 g): C, 85.16; H, 4.51; Found: C, 84.83; H, 4.73; ^{1}H NMR δ 6.9 (s, 1H, C_3-H), 7.4-7.7 (m, 12H, aromatic protons), 8.20 (d, 1H, C_7-H); ms: m/z: 312 (M^+ + 2), 282, 178 and 132.

3- Dibenzofuryl phthalide 10j

This compound was obtained as white crystals, mp 108^{0}C; 68.00%; Anal. Calcd $C_{20}H_{12}O_3$ (300.0 g): C, 80.00; H, 4.00; Found: C, 80.36; H, 4.41; ^{1}H NMR δ 6.5 (s, 1H, C_3-H), 7.3-8.0 (m, 10H, aromatic protons), 8.1 (d, 1H, C_7-H); ms: m/z: 301 (M^+ + 1), 272, 168 and 133.

2.4 CONCLUSION

- 4-Alkyl-3-aroyl isocoumarins, 3-Acetyl-4-alkyl isocoumarins were synthesized in moderate to good yields from simple precussors like o-acyl acids by utilizing simple, efficient and economic reaction pathway.

- Following the similar pathway, differnt o-aroyl benzoic acids were employed to synthesize 3-Arly phthalides, again in a expeditious and streamlined manner.

- The title compounds synthesized in this chapter were used as building blocks for the synthesis of various heterocyclic compounds in subsequent chapters.

- The new synthesized compounds were screened for biological activities which is discussed in last chapter.

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Chapter 3:

Section A:

Synthesis of 3, 4-disubstituted nitro, aminyl benzoyl and amino carbonyl isocoumarins

3. A.1 INTRODUCTION

Isocoumarins represent an important class of naturally occurring lactones, isolated from a diverse range of natural organisms, including fungi, plants, and insects, that display a wide range of biological activities, as described in detail already.

High throughput screening of the selected chemical libraries having a heterocyclic or carbocyclic ring at their core is one of the most expeditious ways to search for useful medicinal activity. The heteroatoms improve binding and the rigid cyclic frame work imparts rigidity, enhancing the selectivity and further improving the binding, Nitrogen atom being one of them.

There are several examples reported in literature where the presence of nitrogen atom in compounds, in various forms, has shown tremendous therapeutic applications. In continuation of our efforts to adapt heterocyclization chemistry to a high-throughput format, we chose to introduce nitrogen atom in isocoumarin moiety in the form of nitro group and amino group, to see their effect on the remedial features of isocoumarin. These two assemblies were specifically chosen because of the following reasons:

- Organic nitrates are commonly used as NO-donors. The development of new NO-donors is an important field of research, aimed at improving the efficiency of NO-release and reducing cytotoxicity[1].

- Biological effects of NO can be grouped into three main categories: regulatory, protective and deleterious[2].

- Nitric oxide is an uncharged radical molecule and is about 70 times more soluble in hydrophobic solvents than in water[3]. This feature allows NO to easily diffuse among cellular compartments, transmit information as well as damage different targets. In biological systems, it can react with various molecules, e.g., molecular oxygen, superoxide anion (O_2^-), or transition metals, yielding reactive nitrogen oxide species (RNOS) and metal-nitrosyl adducts[4].

- NO has been shown to participate in a large number of pathological conditions such as arthritis, arteriosclerosis, cancer, diabetes, some neurodegenerative diseases and stroke, to name a few[5-8].

- The half-life of NO in vivo is a few milliseconds and it has been reported to diffuse at a rate of 50 μm s^{-1} (single direction) in biological systems[9]. This feature has suggested that NO could exert its effects within a few microns from its site of generation, as compared to other groups.

- Direct interaction of NO with the ferric heme group of cytochrome P450 or cytochrome oxidase as well as other metal-containing proteins has also been reported[10-11].

- Molecules containing nitro group are known to generate the nitro – anion free radical, RNO2*$^-$. The ability of these molecules to produce free radical species capable of inducing a cascade of reduced materials, which are toxic towards the parasite, has been the proposed mechanism of action of many nitro compounds[12].

- Tertiary amino group are not only liable to form hydrogen bonds, but also accept protons or form quaternary salts which result in the increase of water solubility[13], or coordinate with metal ions[14], which probably lead to enhance affinity, selectivity and potency in biological properties[15].

- Terminal primary amine side arms in organic molecules are critical for DNA binding affinity and drug potency[16-17].

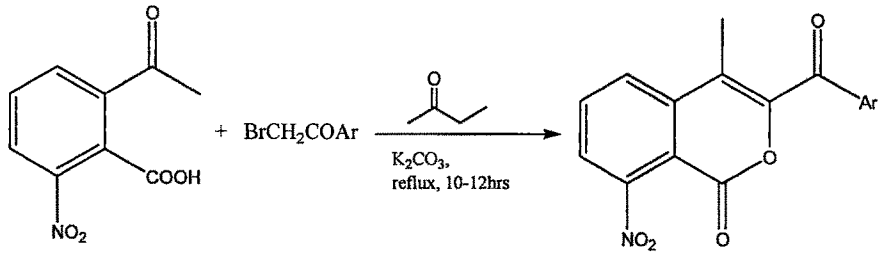
- The anti proliferative activities of many compounds are known to be influenced in a different way by the length and the number of alkyl chains of the amines, the coordinated number and the free amino groups presence, the planarity of the ligand and their electronic charge distribution there in[18].

- The lone pair of electrons on nitrogen imparts it the unique feature to act as a proton acceptor, which makes it one of the largest acid scavengers used in the synthesis of pharmaceuticals[19].

- Bacteriostatic activity in tertiary amines and quaternary ammonium salts, p-toludine moiety has been reported long back[20].

Hence, attempts were made to synthesize new oxygen-nitrogen containing heterocycles using different and simple pathways.

Scheme I



1 2 3

Ar = 4- Hydroxy phenyl (**3a**)
4- Bromo phenyl (**3b**)
2, 4- Dihydroxy phenyl (**3c**)
4- Methoxy phenyl (**3d**)
Biphenyl (**3e**)
Dibenzofuran (**3f**)

3. A.2 RESULTS AND DISCUSSION

From literature survey it is found that electron density plays an important role in biological activity. Here purposely we selected the nitro group to see the effect of electron withdrawing group on biological activity and therefore synthesized nitro substituted isocoumarins **3a-f**. These compounds were obtained by refluxing nitro substituted acyl benzoic acid[21] **1** (**Scheme I**) instead of o-acyl benzoic acid with substituted bromoacetophenone in presence of base following the same reaction path as given in chapter 2.

For preparation of **1** nitro phthalic anhydride was used instead of phthalic anhydride which was refluxed with malonic acid in pyridine, and after following the same route it resulted in isocoumarins **3a-f**.

IR shows absorption at 1344 cm^{-1} for nitro group, 1768 cm^{-1} for γ lactone and 1537 cm^{-1} for –C=O in almost all synthesized nitro isocoumarins (**Fig. 3.A.3**).

^{1}H NMR spectrum of **3a** shows signals at δ 2.3 (s, 3H, CH_3), 5.3 (s, 1H, OH), 6.8-7.8 (m, 6H, aromatic protons), 8.5 (d, 1H, C_7-H) (**Fig. 3.A.1**), **3b** at δ 1.7 (s, 3H, CH_3), 6.8-8.2 (m, 6H, aromatic protons) (**Fig. 3.A.4**), 8.5 (d, 1H, C_7-H) and **3d** at 2.1 (s, 3H, CH_3), 3.8 (s, 3H, OCH_3), 6.1-8.2 (m, 6H, aromatic protons), 8.5 (d, 1H, C_7-H) (**Fig. 3.A.6**).

Mass spectrum gives m/z peaks for **3a** at, m/z: 325 (M^+), 279, 266, 232, 191, 146 and 118 (**Fig. 3.A.2**), **3b** at m/z: 387.9 (M^+), 308, 232, 185, 184 and 146 (**Fig. 3.A.5**) and **3d** at m/z: 339 (M^+), 280, 192, 173, 146 and 118 (**Fig. 3.A.7**).

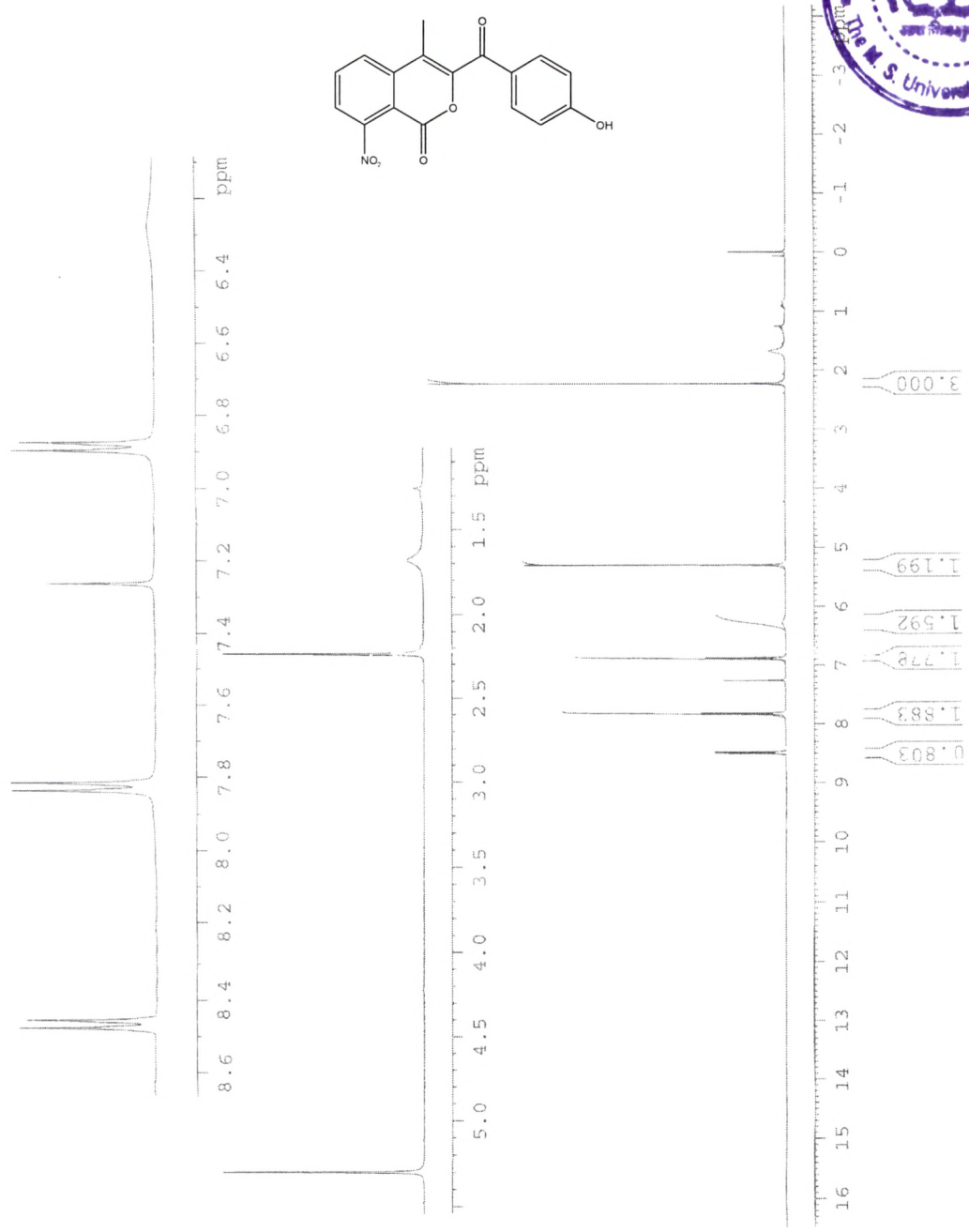


Fig. 3.A.2 - ^{1}H NMR: **3-(4'-Hydroxy benzoyl)-4-methyl-8-nitro isocoumarin 3a**

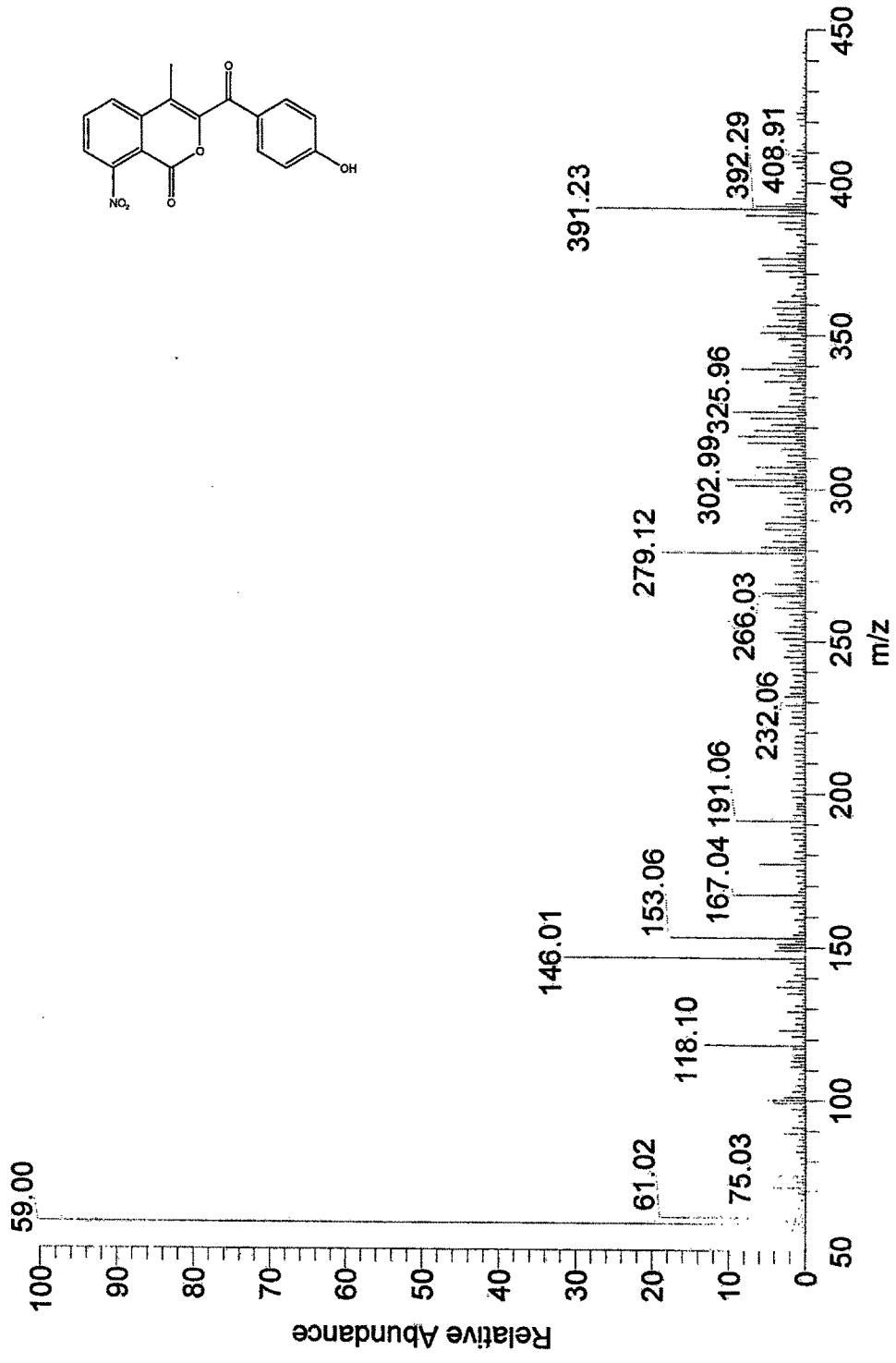
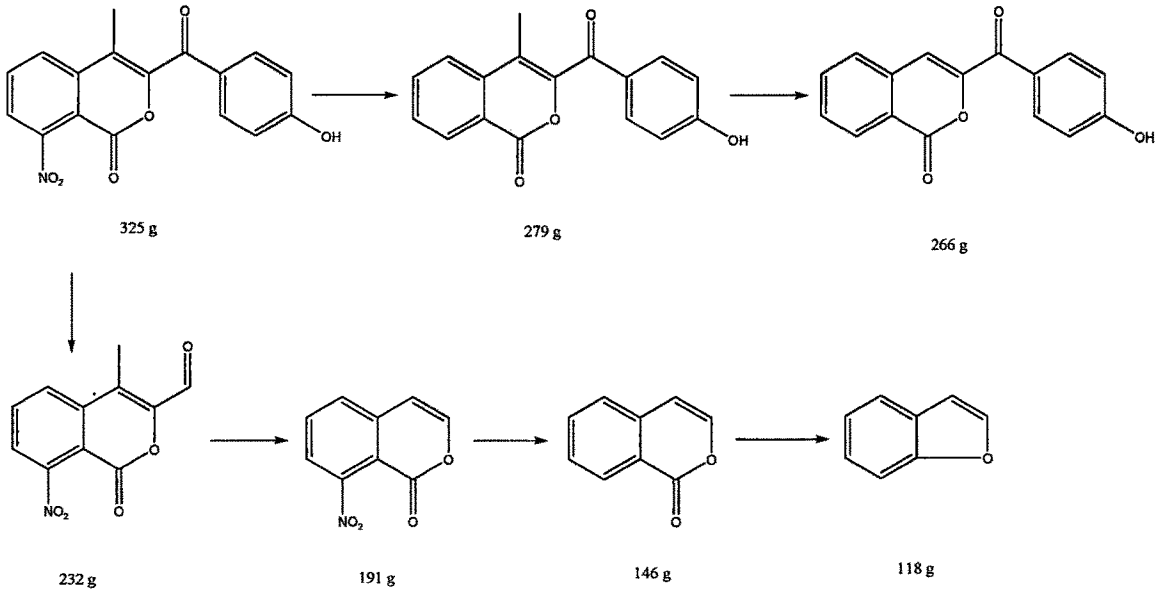


Fig. 3.A.2 - Mass spectrum: **3-(4'-Hydroxy benzoyl)-4-methyl-8-nitro isocoumarin 3a**



Fragmentation Pattern: **3-(4'-Hydroxy benzoyl)-4-methyl-8-nitro isocoumarin**

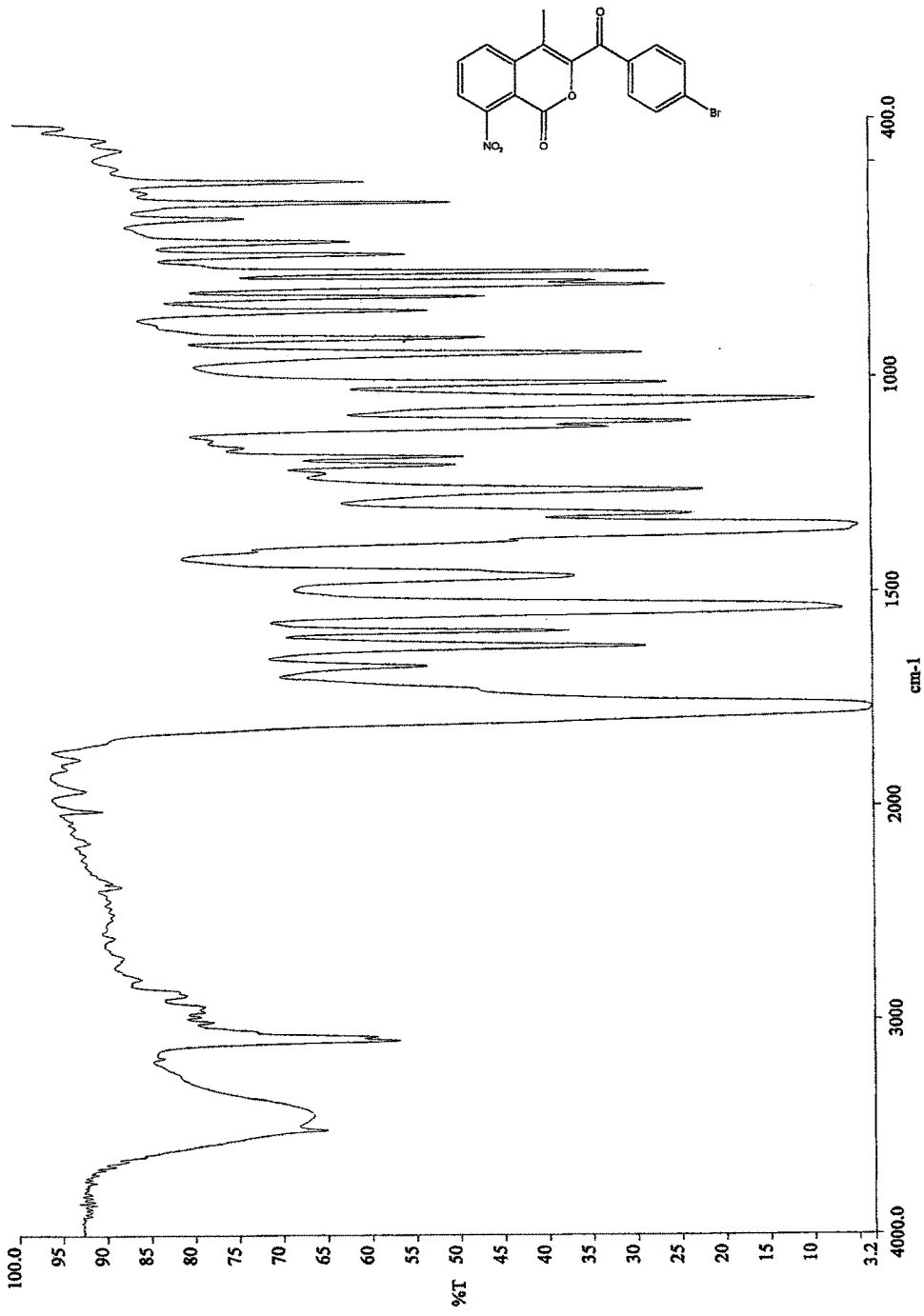


Fig. 3.A.3 – IR: **3-(4'-Bromo benzoyl)-4-methyl-8-nitro isocoumarin 3b**

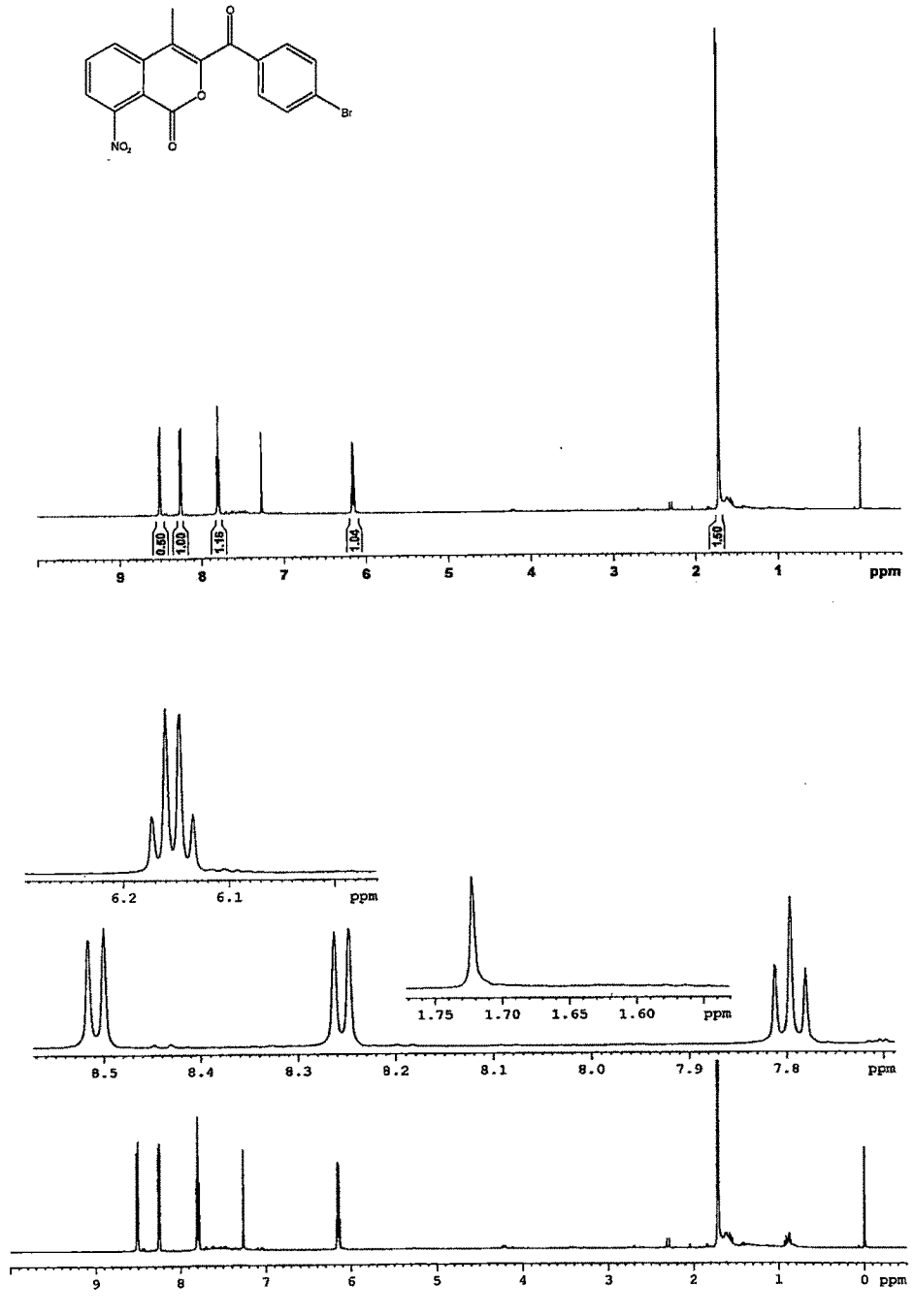


Fig. 3.A.4 - ^{1}H NMR: 3-(4'-Bromo benzoyl)-4-methyl-8-nitro isocoumarin 3b

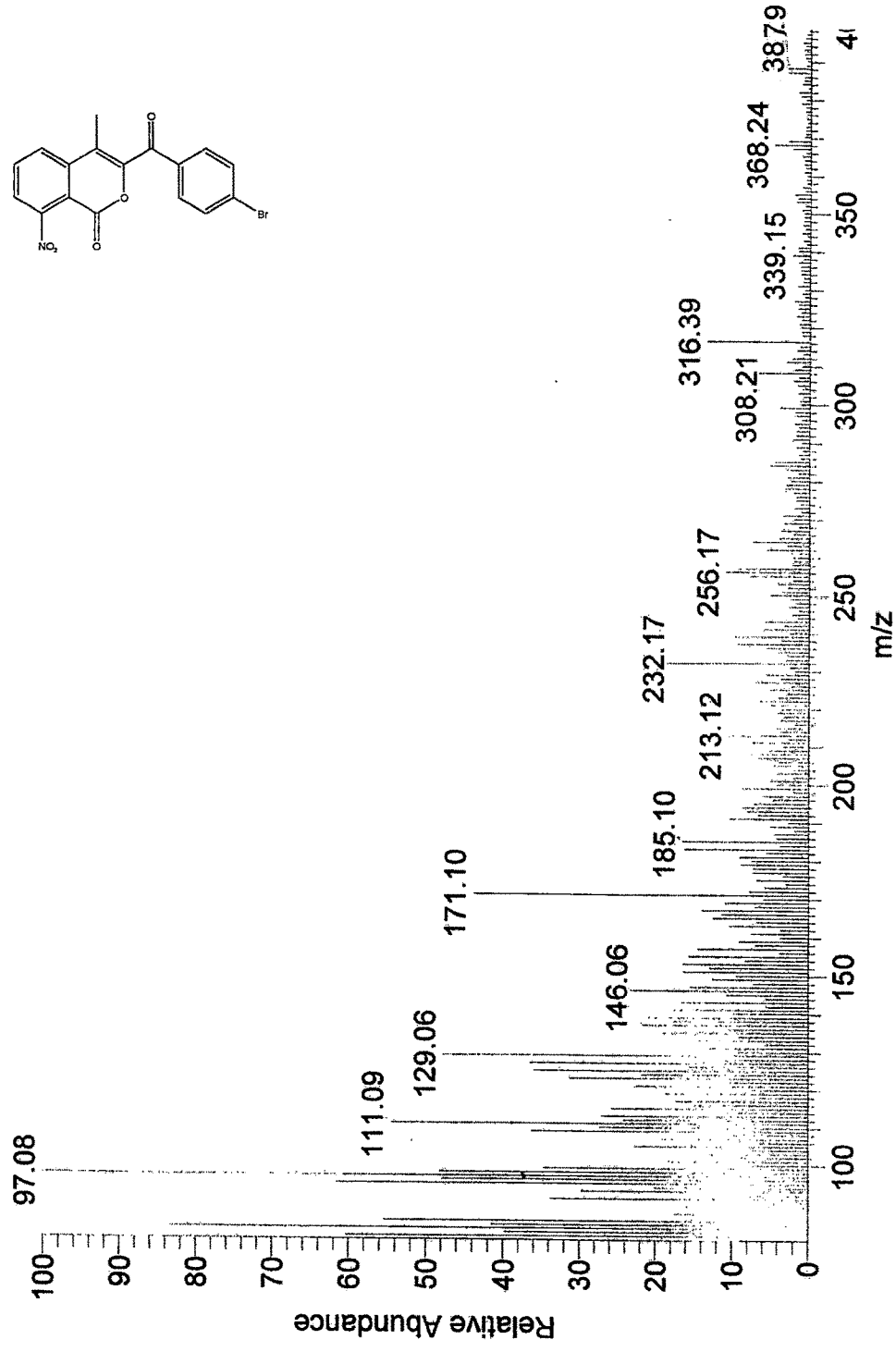
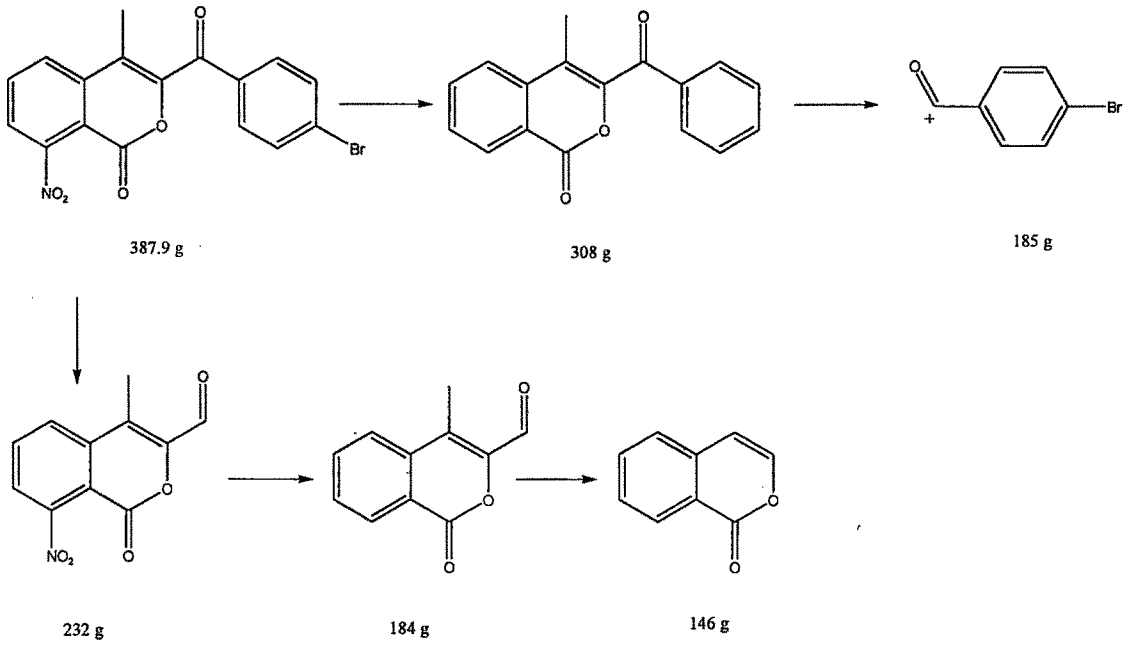


Fig. 3.A.5 - Mass spectrum: **3-(4'-Bromo benzoyl)-4-methyl-8-nitro isocoumarin 3b**



Fragmentation Pattern: **3-(4'-Bromo benzoyl)-4-methyl-8-nitro isocoumarin**

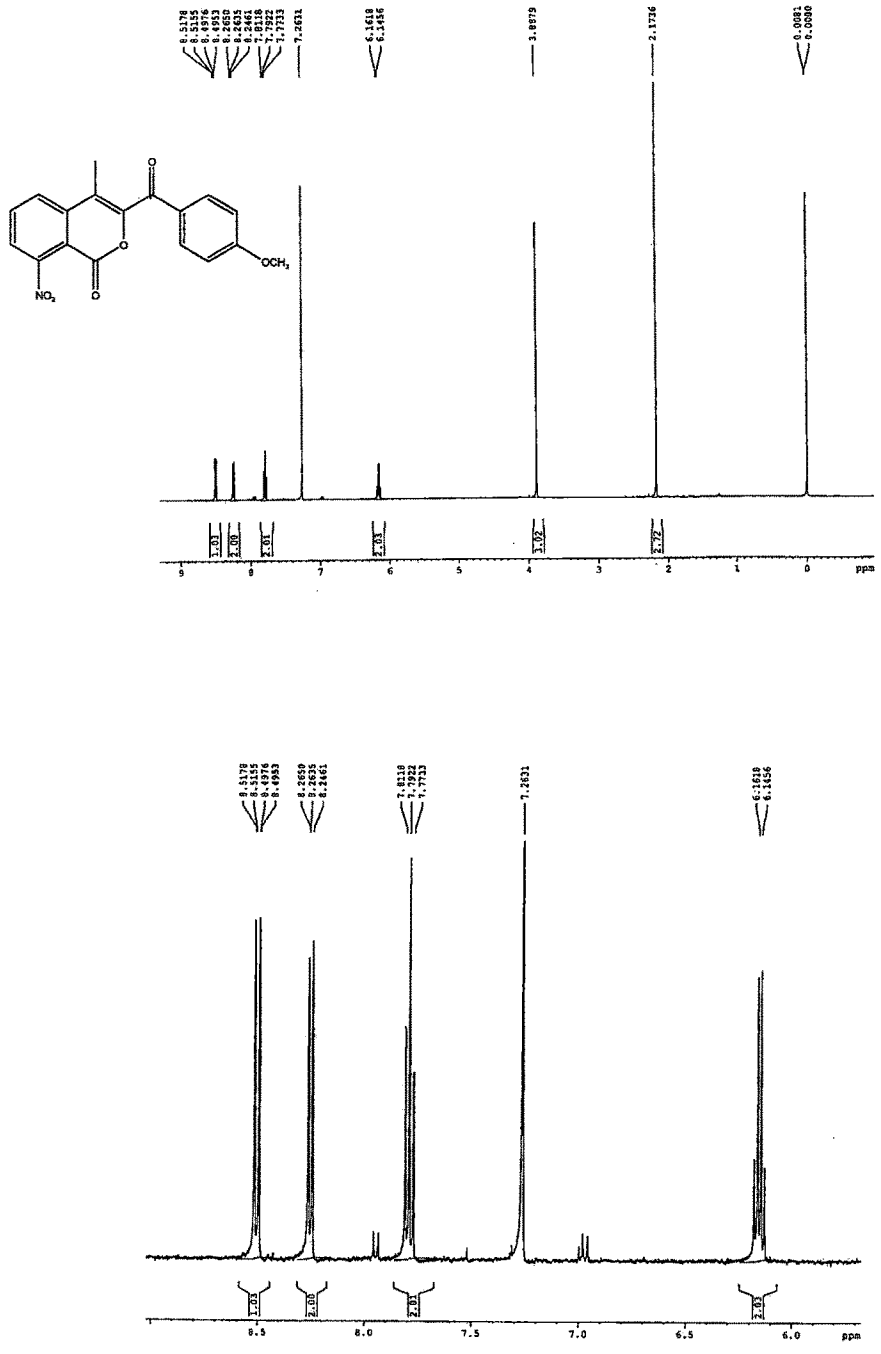


Fig. 3.A.6 - ^{1}H NMR: 4-Methyl-3-(4'-methoxy benzoyl)-8-nitro isocoumarin 3d

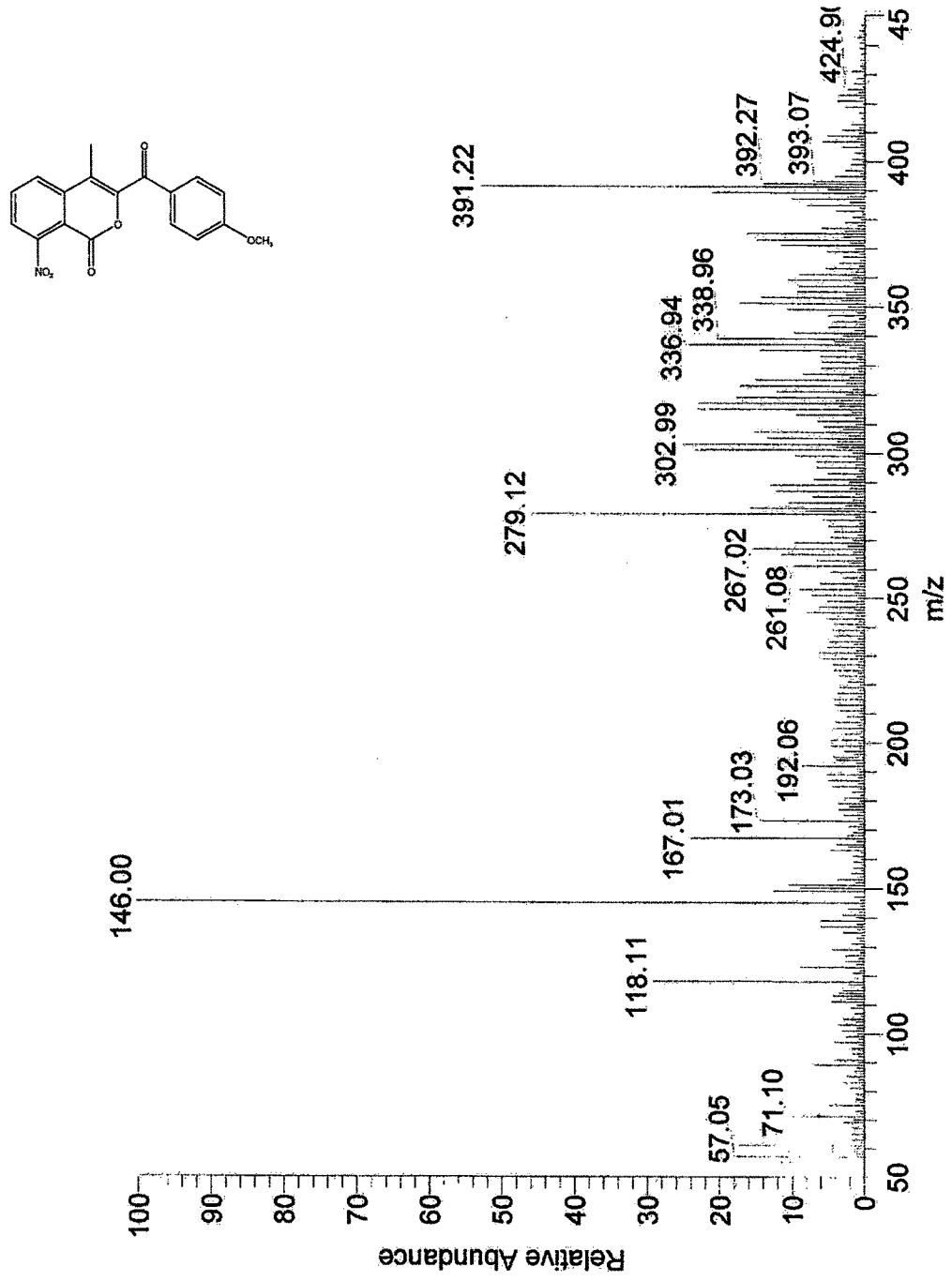
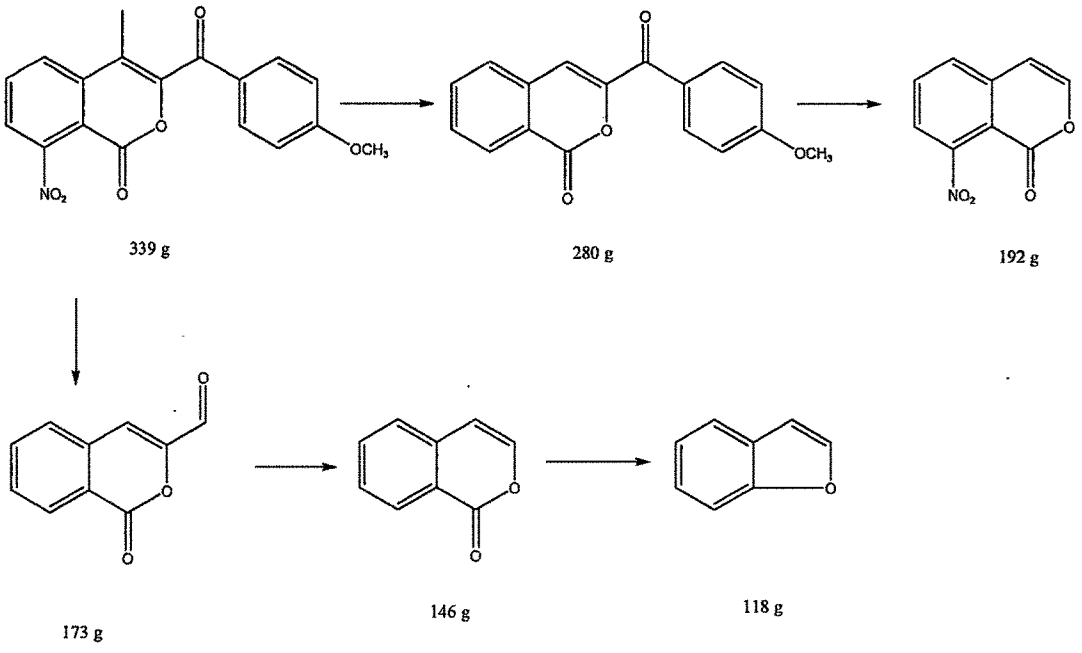
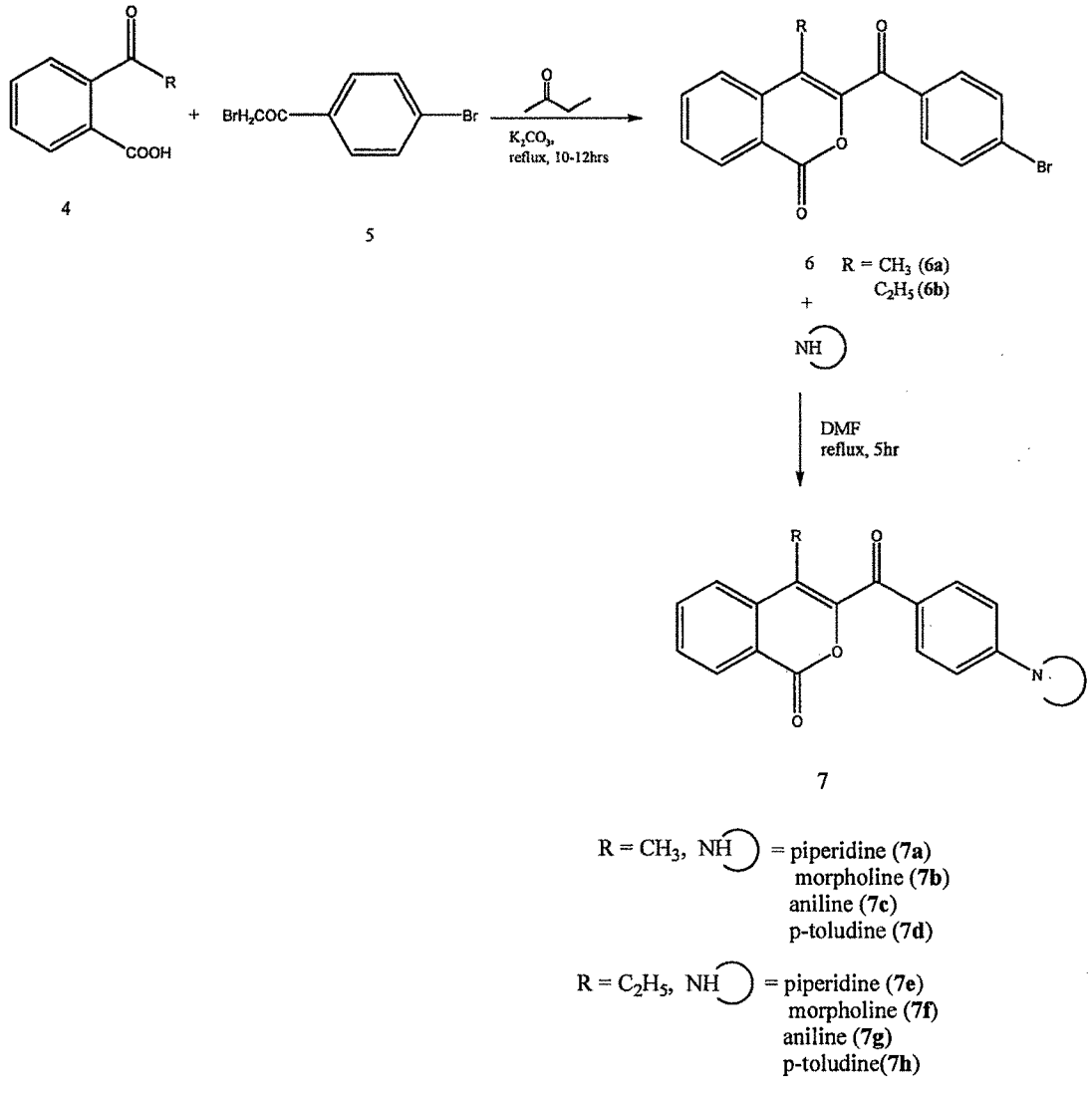


Fig. 3.A.7 - Mass spectrum: **4-Methyl-3-(4'-methoxy benzoyl)-8-nitro isocoumarin 3d**



Fragmentation Pattern: **4-Methyl-3-(4'-methoxy benzoyl)-8-nitro isocoumarin**

Scheme II



In (**Scheme II**), the aroyl moiety was chemically modified by the introduction of Br group at the para position of aroyl group **6** by using p-bromo phenacyl bromide **5.** Reaction of **4** with **5** in presence of base resulted in compounds **6a, b. 6a, b** was condensed with various primary amines like aniline, p- toluidine and secondary amines like piperidine, morpholine, in DMF, resulting in aminyl benzoyl isocoumarins **7a-h.**

All the compounds show absorption at 1238 cm^{-1} for C-N, 1295 cm^{-1} for C-O, 1669cm^{-1} for aroyl carbonyl and 1738 cm^{-1} for lactonic carbonyl (**Fig. 3.A.8**).

^{1}H NMR of compounds **7b** and **7e** shows signals at δ 1.5 (s, 3H, CH_3), 3.3(t, 4H,CH_2-N-CH_2), 3.9(t, 4H,CH_2-O-CH_2), 6.8-7.9 (m, 7H, aromatic protons), 8.4 (d, 1H, C_8-H) (**Fig. 3.A.9**) and δ 1.2 (t, 3H, CH_3), 1.6 (s, 6H, CH_2-CH_2-CH_2), 2.8 (q, 2H, CH_2), 3.4 (s, 4H, CH_2-N-CH_2) , 7.6-7.9 (m, 7H, aromatic protons), 8.42 (d, 1H, C_8-H) (**Fig. 3.A.11**) respectively. In case of 7e, the signals of piperidine ring are obtained as two singlets at δ 1.6 and 3.4 instead of usual triplet or multiplet suggesting merging of signals, though the spectrum was run at 500 MHz.

Mass spectrum of **7b** gives m/z: at 345 (M^+ - 2), 302, 279, 221, 190, 187, 146, 135 and 77 (**Fig. 3.A.10**) and **7e** at m/z: 361 (M^+), 277, 185, 146, 118 and 85 (**Fig. 3.A.12**).

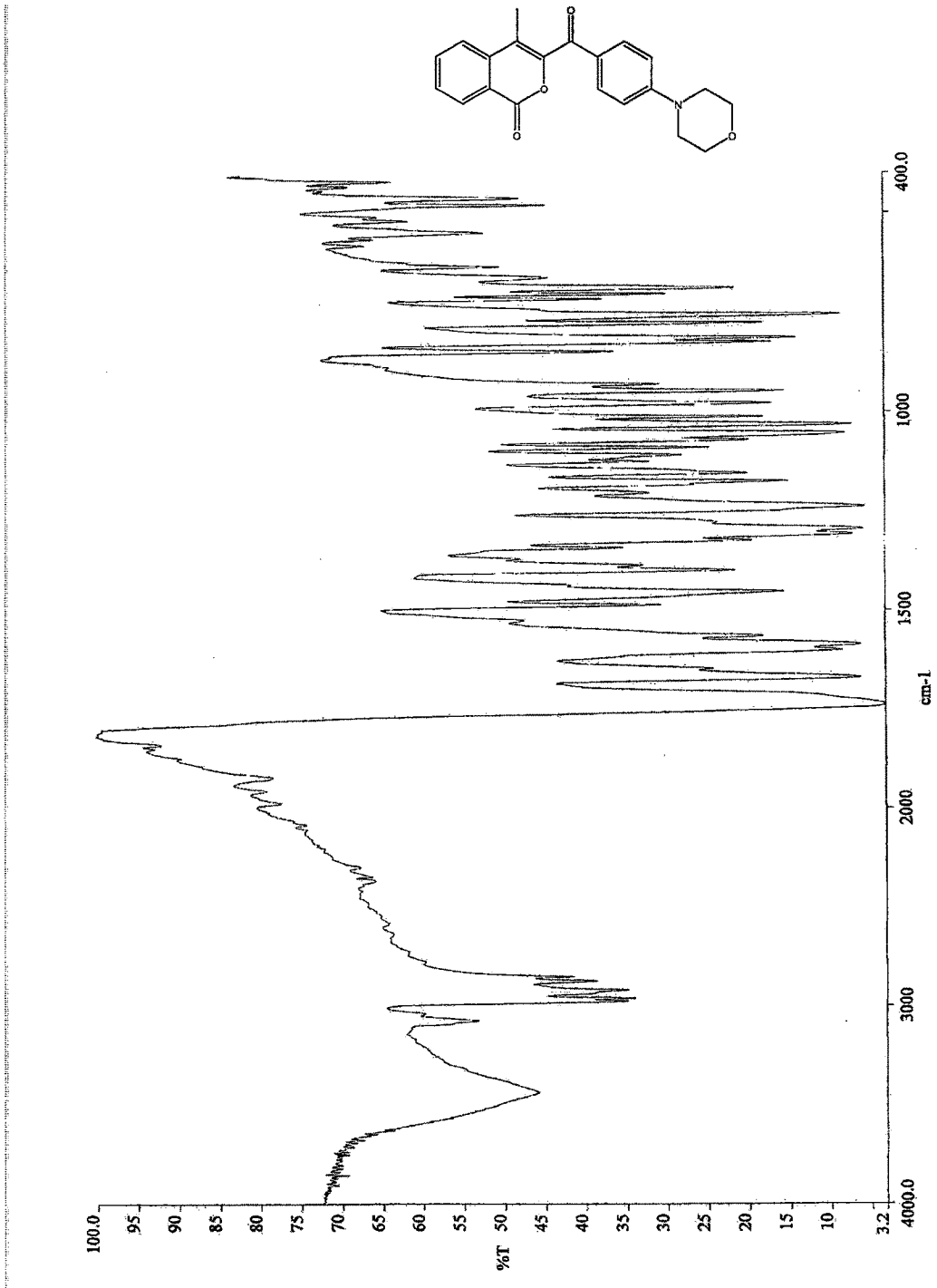
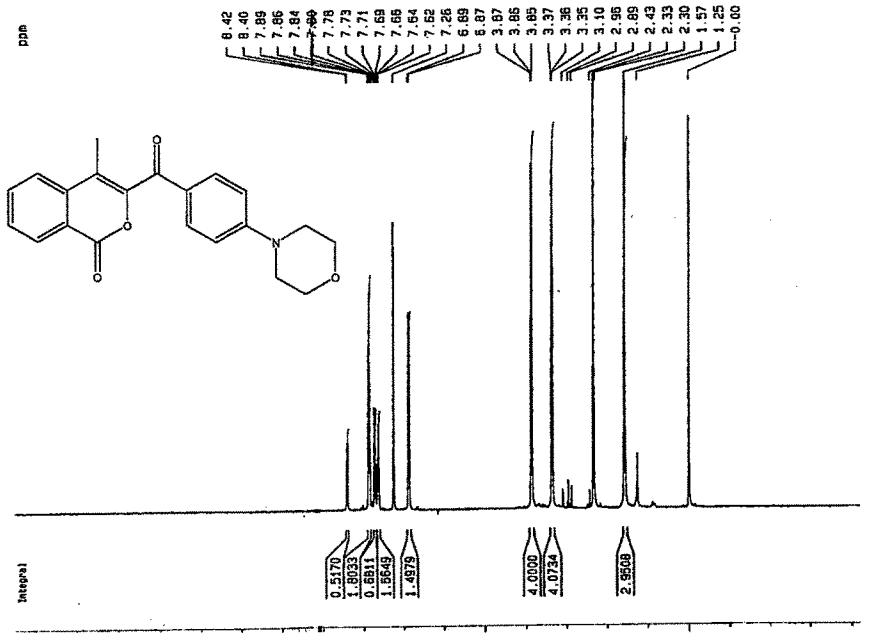


Fig. 3.A.8 – IR: **4-Methyl -3-(4'- morpholin-1-yl-benzoyl) isocoumarin 7b**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7.69
7.66
7.64
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6.89
6.87
3.87
3.86
3.85
3.37
3.36
3.35
3.10
2.95
2.89
2.43
2.33
2.30
1.57
1.25
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1.4979
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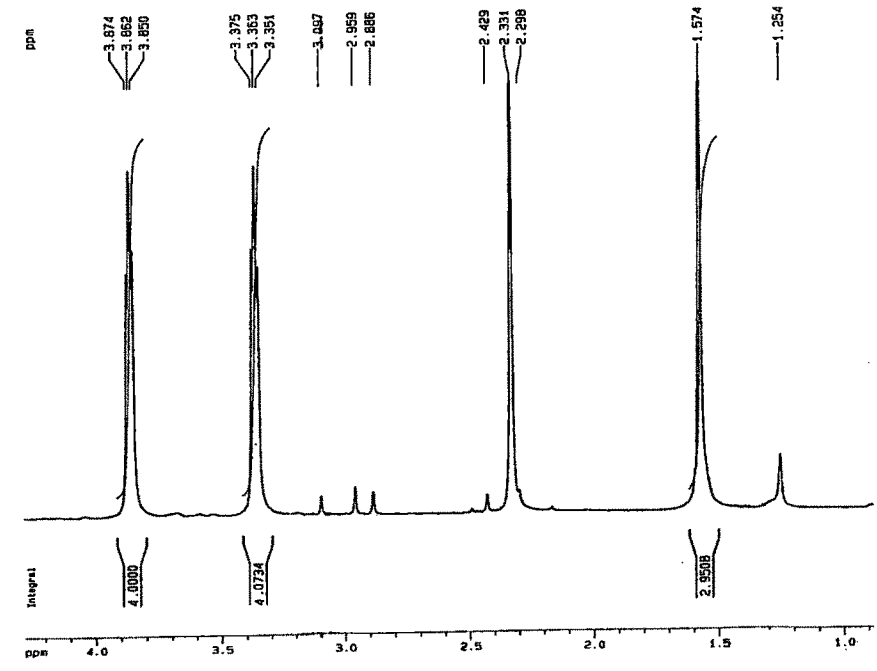
4.0734
2.9508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2.298
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1.254
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2.5
2.0
1.5
1.0

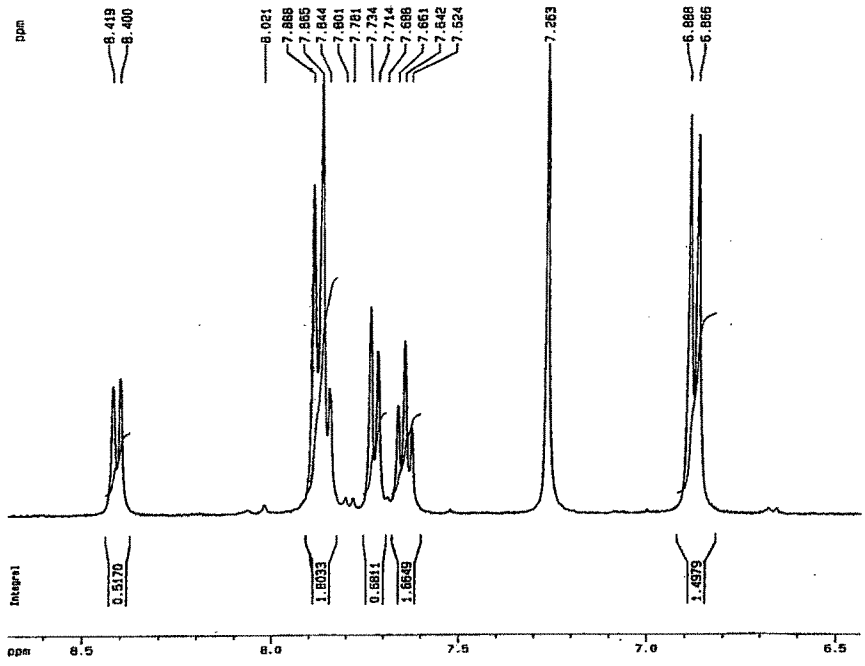


Fig. 3.A.9 - ^{1}H NMR: **4-Methyl -3-(4'- morpholin-1-yl-benzoyl) isocoumarin 7b**

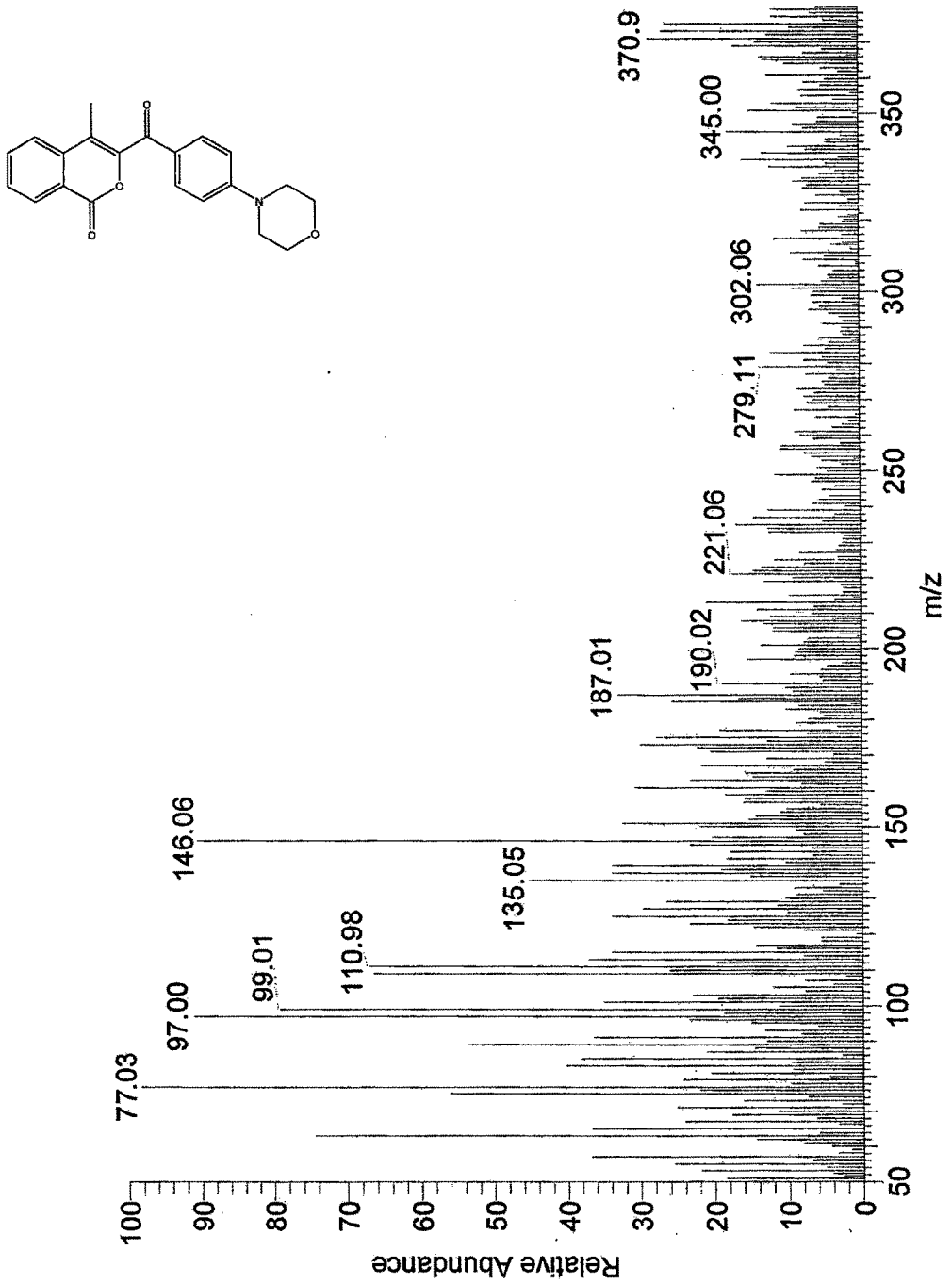
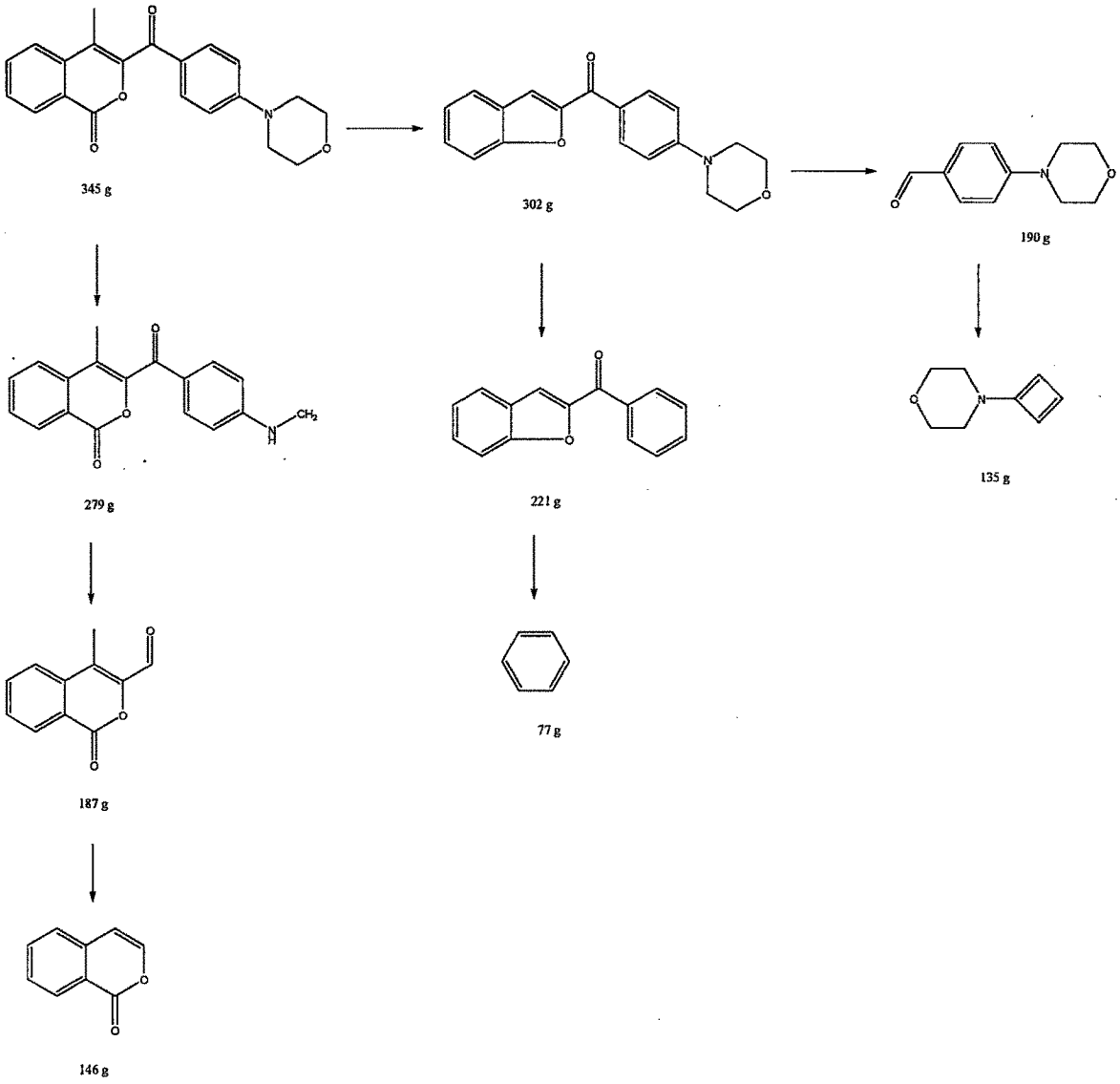


Fig. 3.A.10 – Mass spectrum: **4-Methyl -3-(4'- morpholin-1-yl-benzoyl) isocoumarin 7b**



Fragmentation Pattern: **4-Methyl -3-(4'- morpholin-1-yl-benzoyl) isocoumarin**

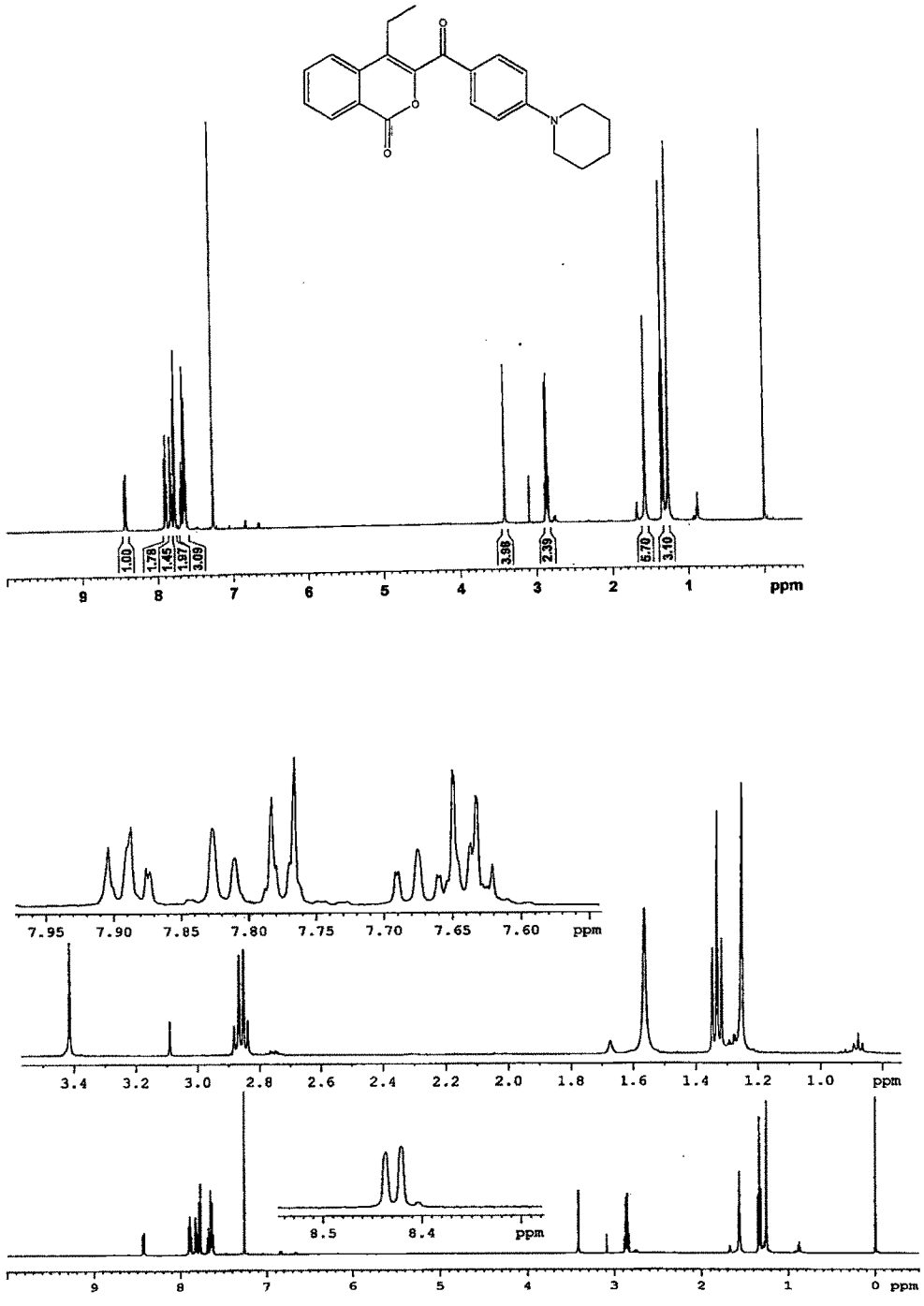


Fig. 3.A.11 - ^{1}H NMR: 4-Ethyl -3-(4'-piperidin-1-yl-benzoyl) isocoumarin 7e

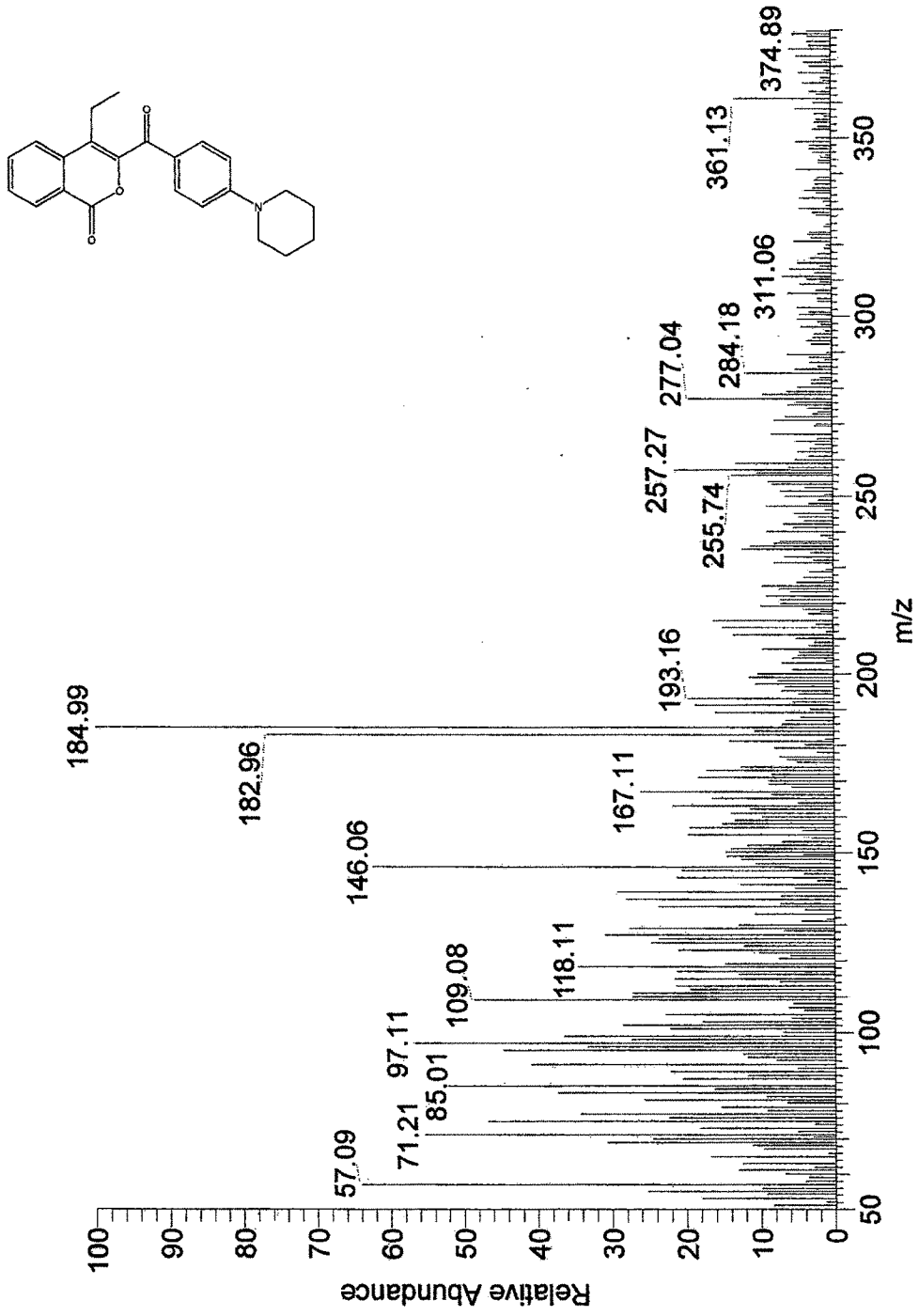
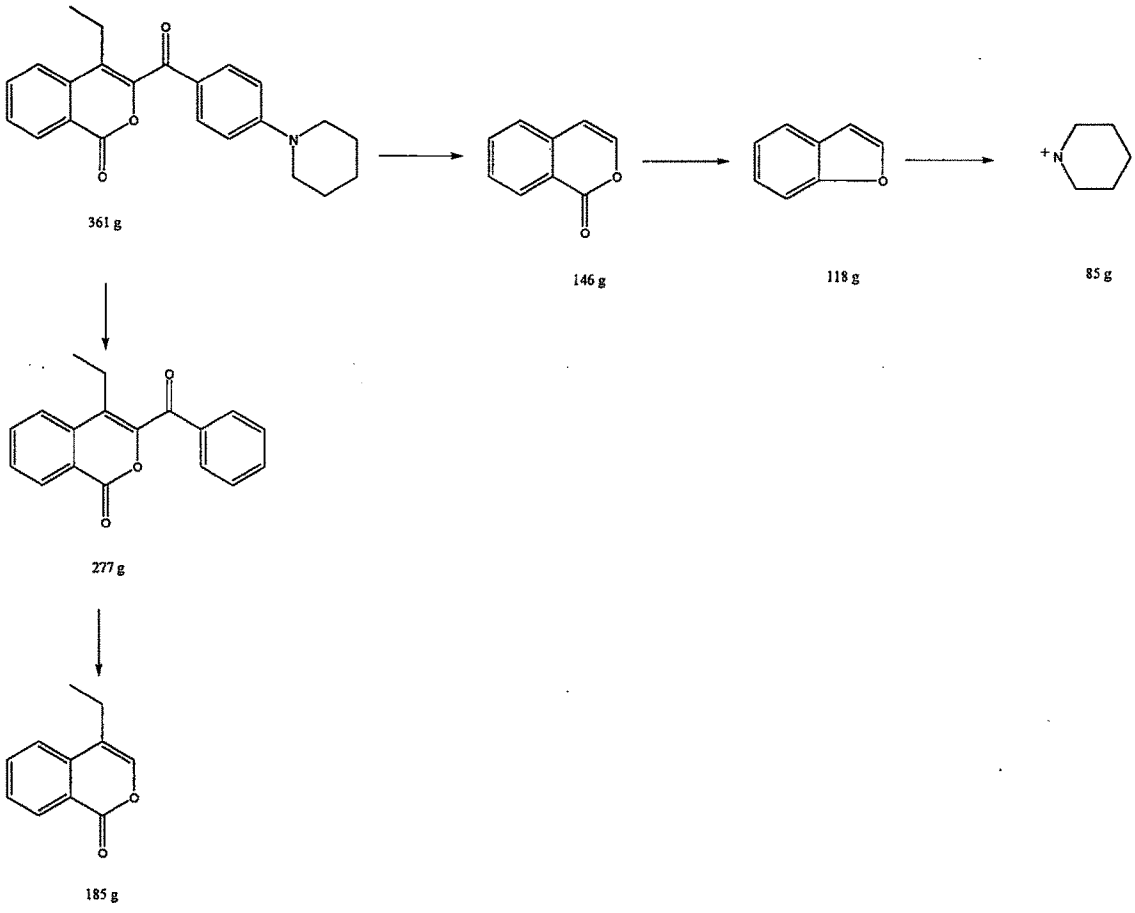
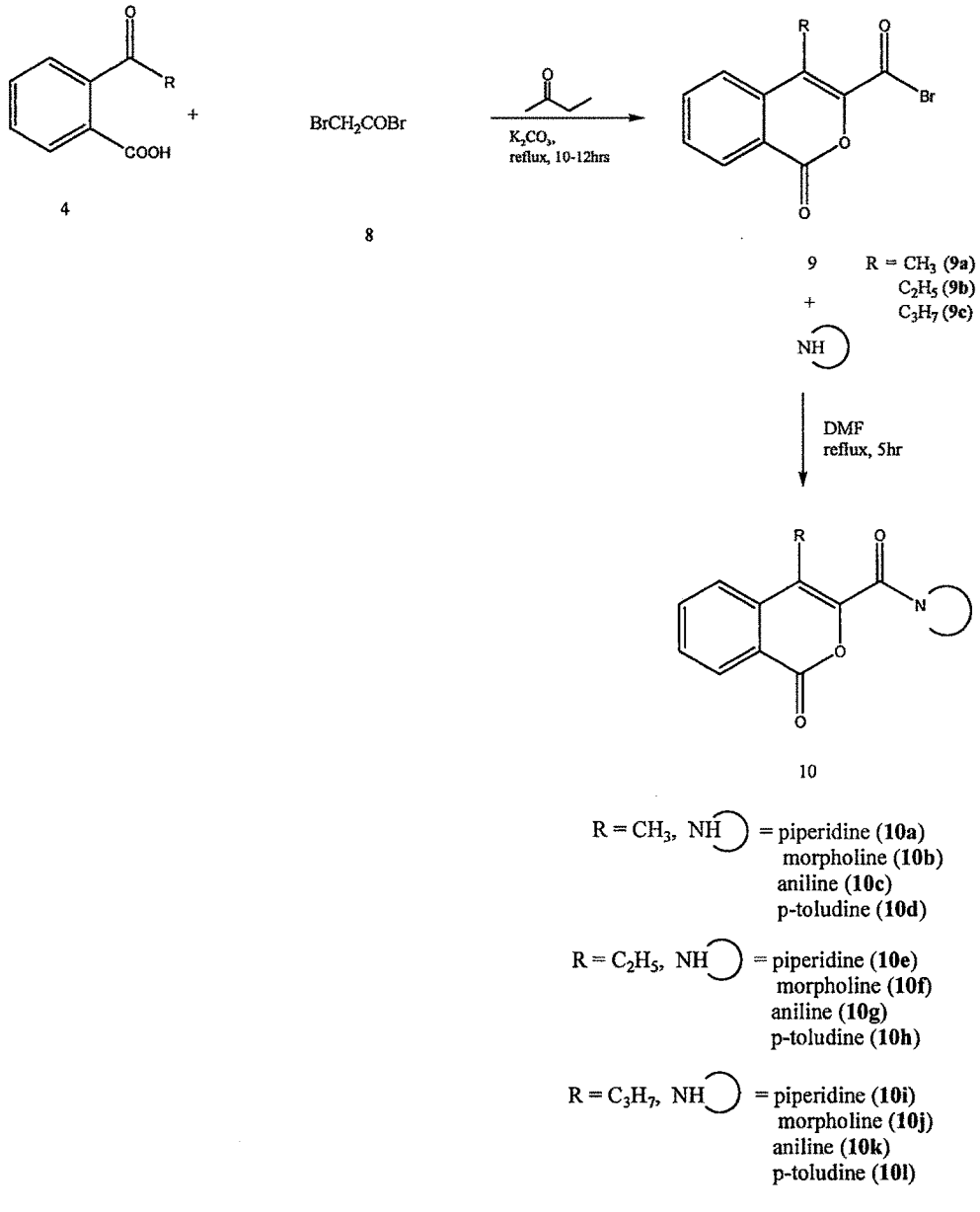


Fig. 3.A.12 – Mass spectrum: **4-Ethyl -3-(4'-piperidin-1-yl-benzoyl) isocoumarin 7e**



Fragmentation Pattern: **4-Ethyl -3-(4'-piperidin-1-yl-benzoyl) isocoumarin**

Scheme III



(**Scheme III**) was prepared to continue the extension of simple isocoumarins to its new derivatives containing amide functional groups by condensing it with various primary and secondary amines. First 4-alkyl-3-bromocarbonyl isocoumarins **9** were synthesized by usual method, i.e by condensing o-acyl acids **4** with bromoacetyl bromide **8** instead of p- bromo phenacyl bromide, in presence of K_2CO_3 and ethyl

methyl ketone as solvent. 4-alkyl-3-bromocarbonyl isocoumarins **9** were then reacted with various primary & secondary amines in DMF resulting in amino carbonyl isocoumarins **10a-l**. Primary and secondary amines used here were same as discussed in previous scheme.

IR signals for different amino carbonyl isocoumarins synthesized are at frequencies 1276, 1303, 1689 and 1569 cm^{-1} for C-N, C-O, lactonic carbonyl and –C=O groups respectively (**Fig. 3.A.13**).

^1H NMR spectrum of **10b** shows signals at δ 2.1 (s, 3H, CH_3), 3.2 (t, 4H, CH_2-N-CH_2), 3.6 (t, 4H, CH_2-O-CH_2), 7.3-7.6 (m, 3H, aromatic protons), 7.8-7.9 (dd, 1H, C_8-H) (**Fig. 3.A.14**), **10f** at δ 1.2 (t, 3H, CH_3), 2.9 (q, 2H, CH_2), 3.2 (t, 4H, CH_2-N-CH_2), 3.6 (t, 4H, CH_2-O-CH_2), 7.3-7.6 (m, 3H, aromatic protons), 7.8-7.9 (dd, 1H, C_8-H) (**Fig. 3.A.16**) and **10l** at δ 0.7 (t, 3H, CH_3),1.0 (m, 2H, CH_2), 2.0(m, 2H, CH_2), 2.4 (s, 3H, C_4· - CH_3), 3.4 (s, 1H, NH), 7.1-7.7 (m, 7H, aromatic protons), 7.8-7.9 (dd, 1H, C_8-H) (Fig. **3.A.18**).

Mass spectrum of **10b** gives m/z peak at 273 (M^+), 258, 245, 187, 159 and 146 (**Fig. 3.A.15**), **10f** at m/z: 287 (M^+), 272, 258, 201, 187 and 146 (**Fig. 3.A.17**) and **10l** at m/z: 322 ($M^+ + 1$), 306, 293, 278, 264 and 173 (**Fig. 3.A.19**).

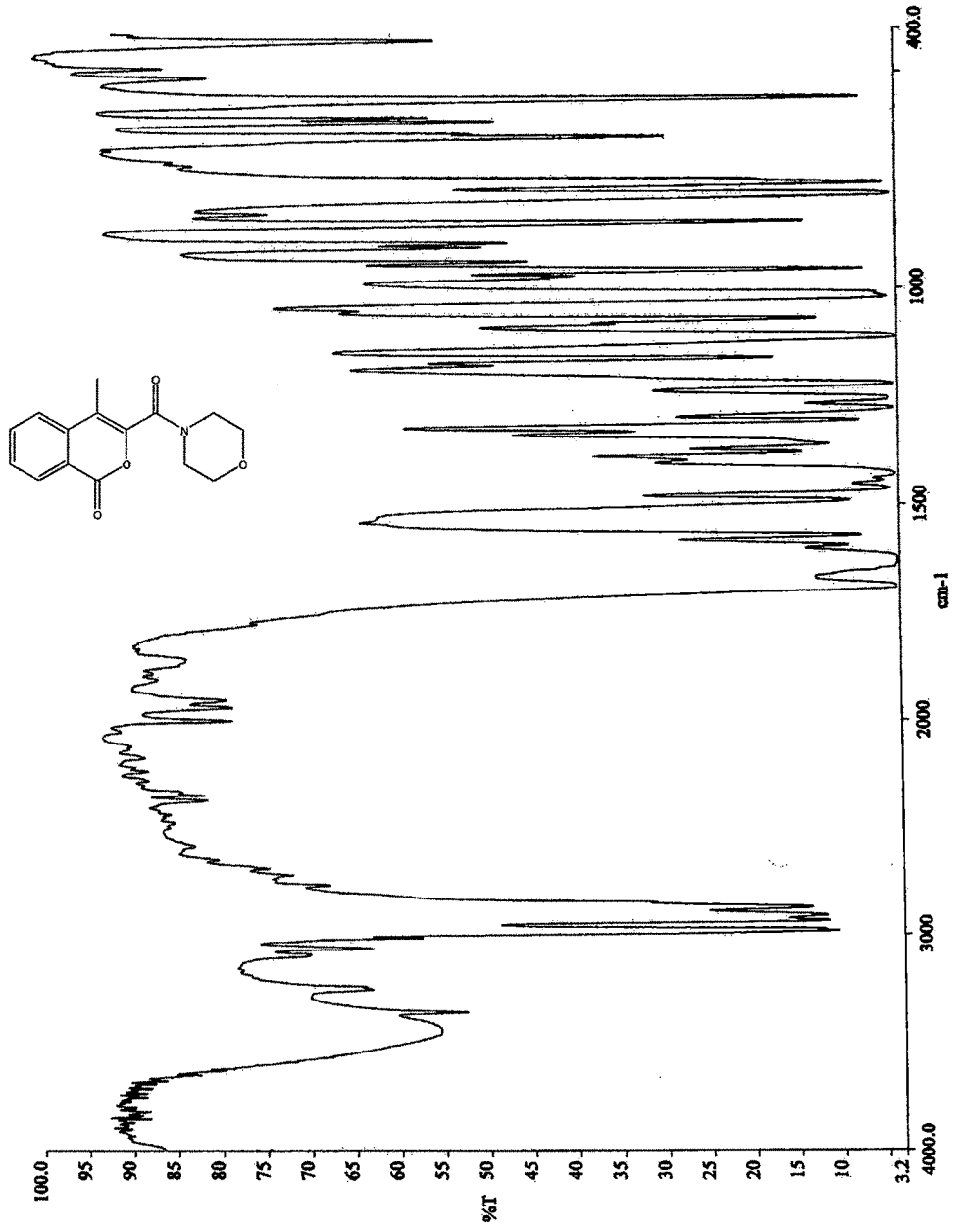


Fig. 3.A.13- IR: **4-Methyl-3-morphonlinyl-carbonyl isocoumarin 10b**

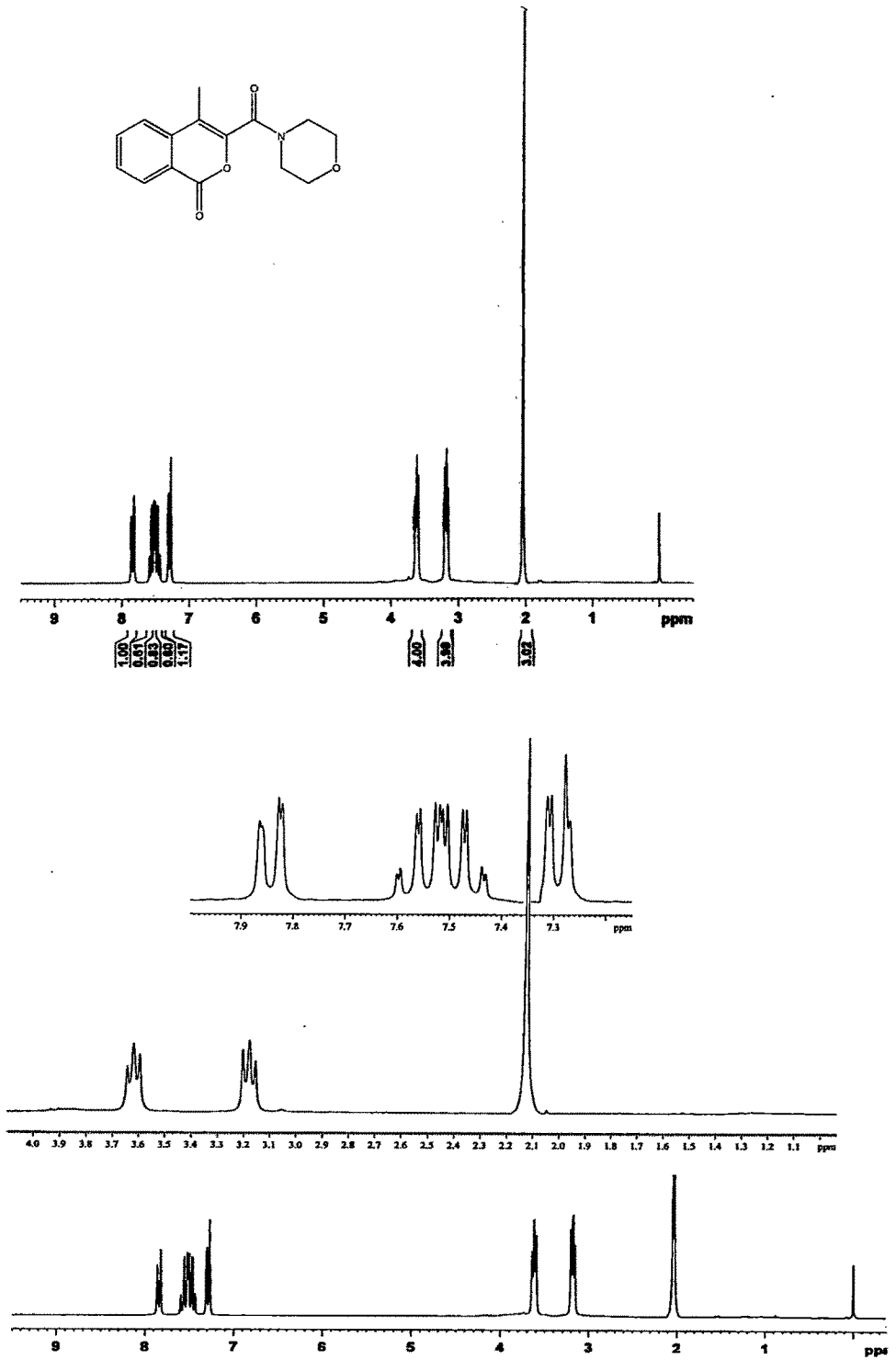


Fig. 3.A.14 - ^{1}H NMR : 4-Methyl-3-morphonlinyl-carbonyl isocoumarin 10b

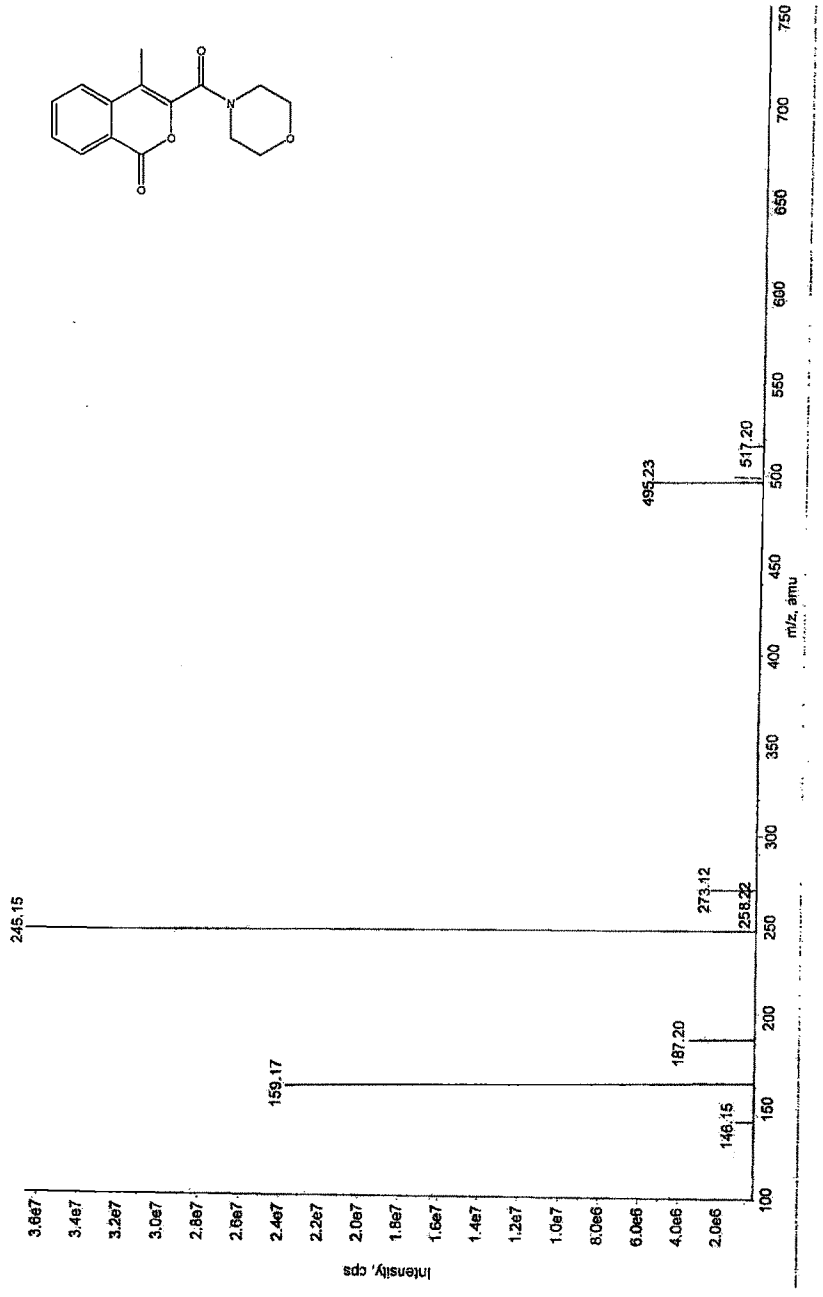
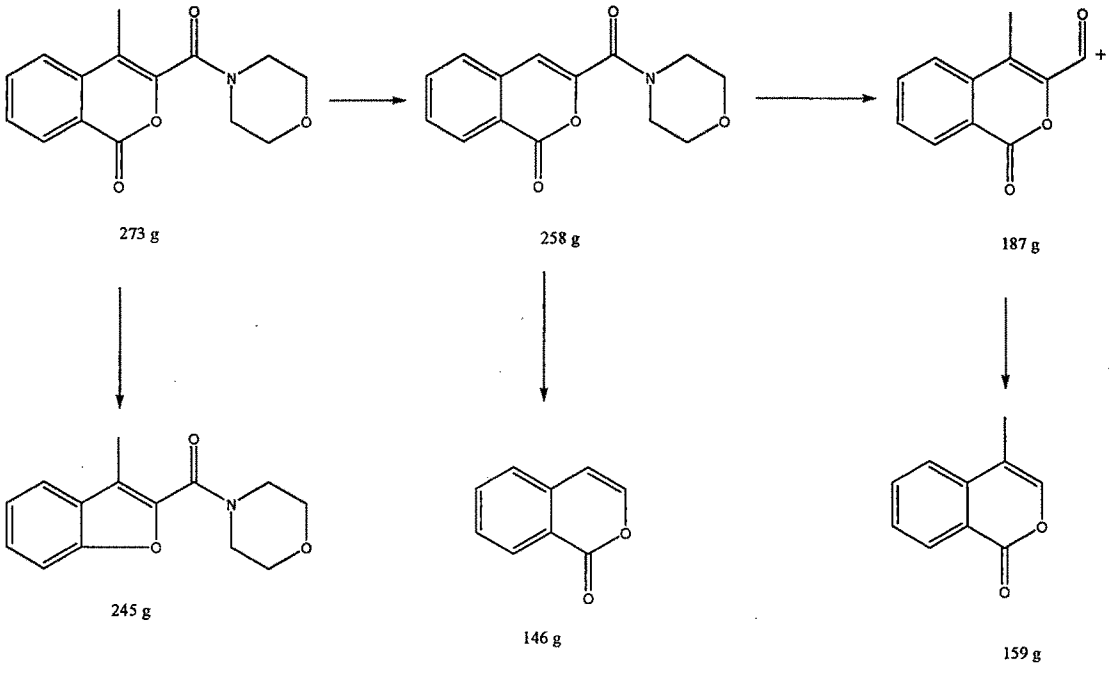


Fig. 3.A.15 – Mass spectrum: **4-Methyl-3-morphonlinyl-carbonyl isocoumarin**



Fragmentation Pattern: **4-Methyl-3-morphonlinyl-carbonyl isocoumarin**

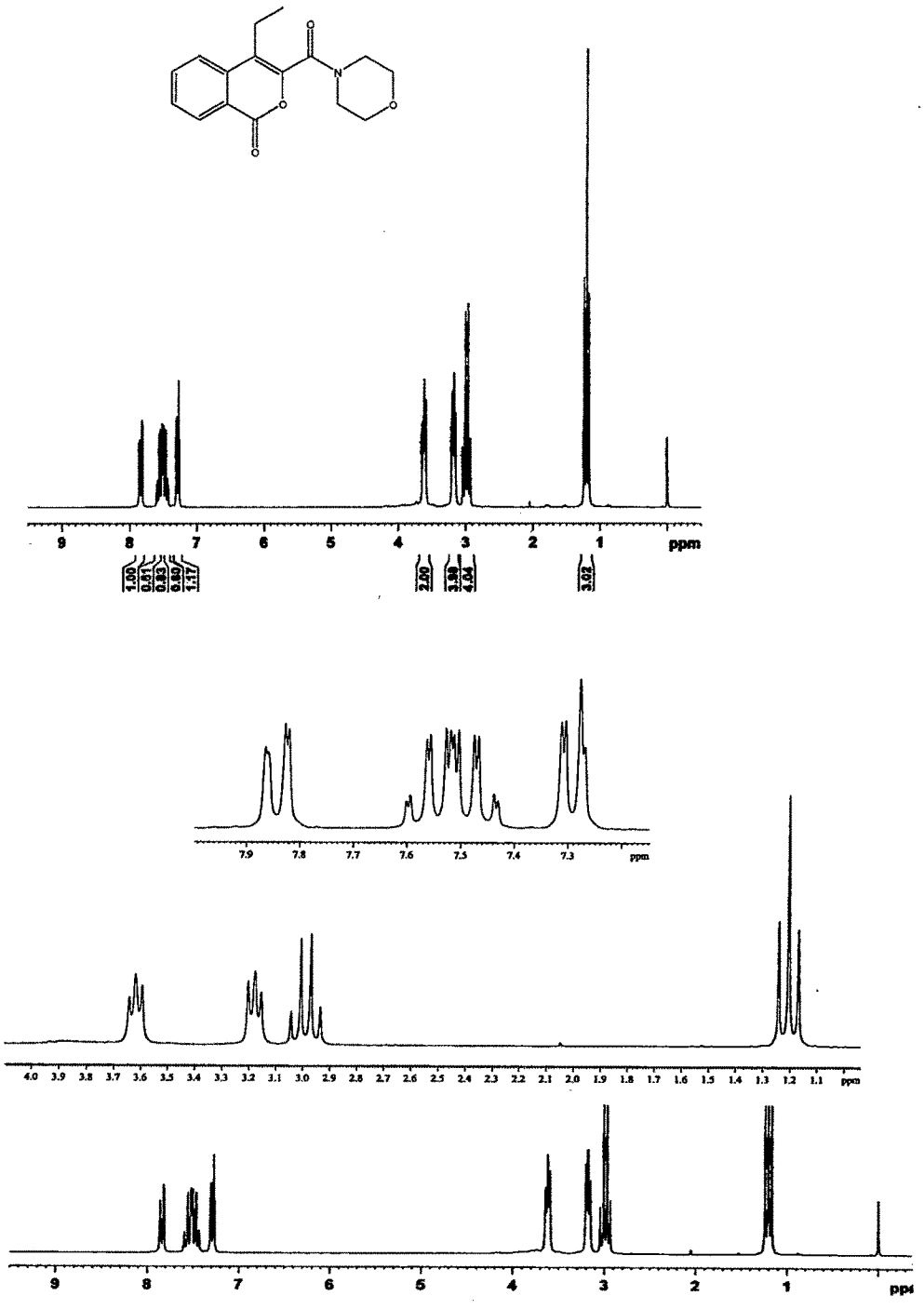


Fig. 3.A.16 - ^{1}H NMR: 4-Ethyl-3-morphonlinyl-carbonyl isocoumarin 10f

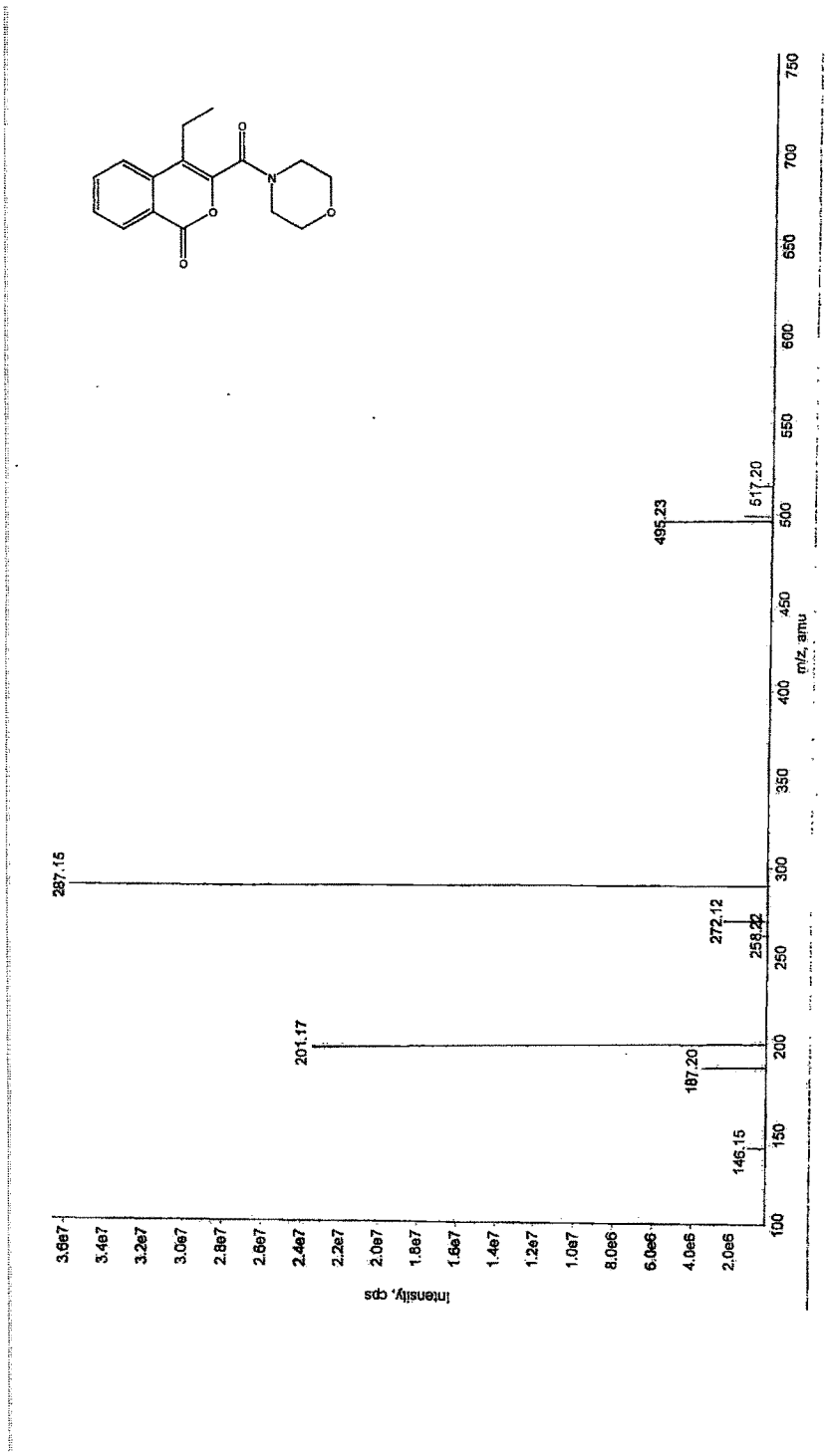
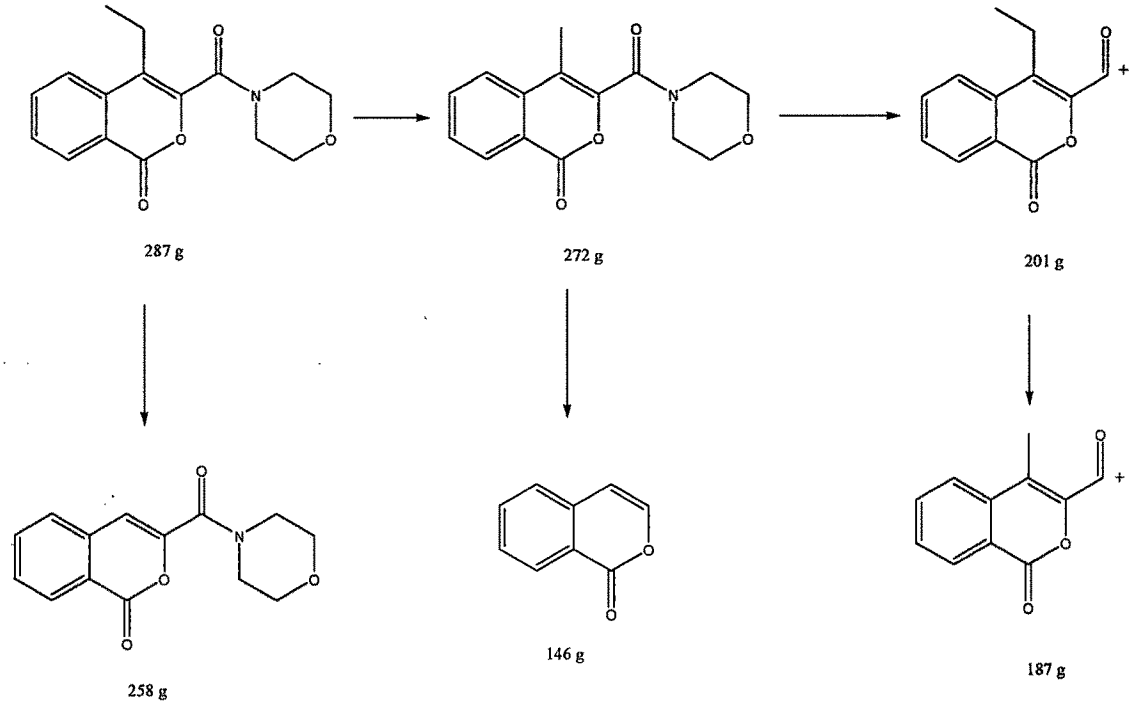


Fig. 3.A.17 – Mass spectrum: **4-Ethyl-3-morphonlinyl-carbonyl isocoumarin 10f**



Fragmentation Pattern: **4-Ethyl-3-morphonlinyl-carbonyl isocoumarin**

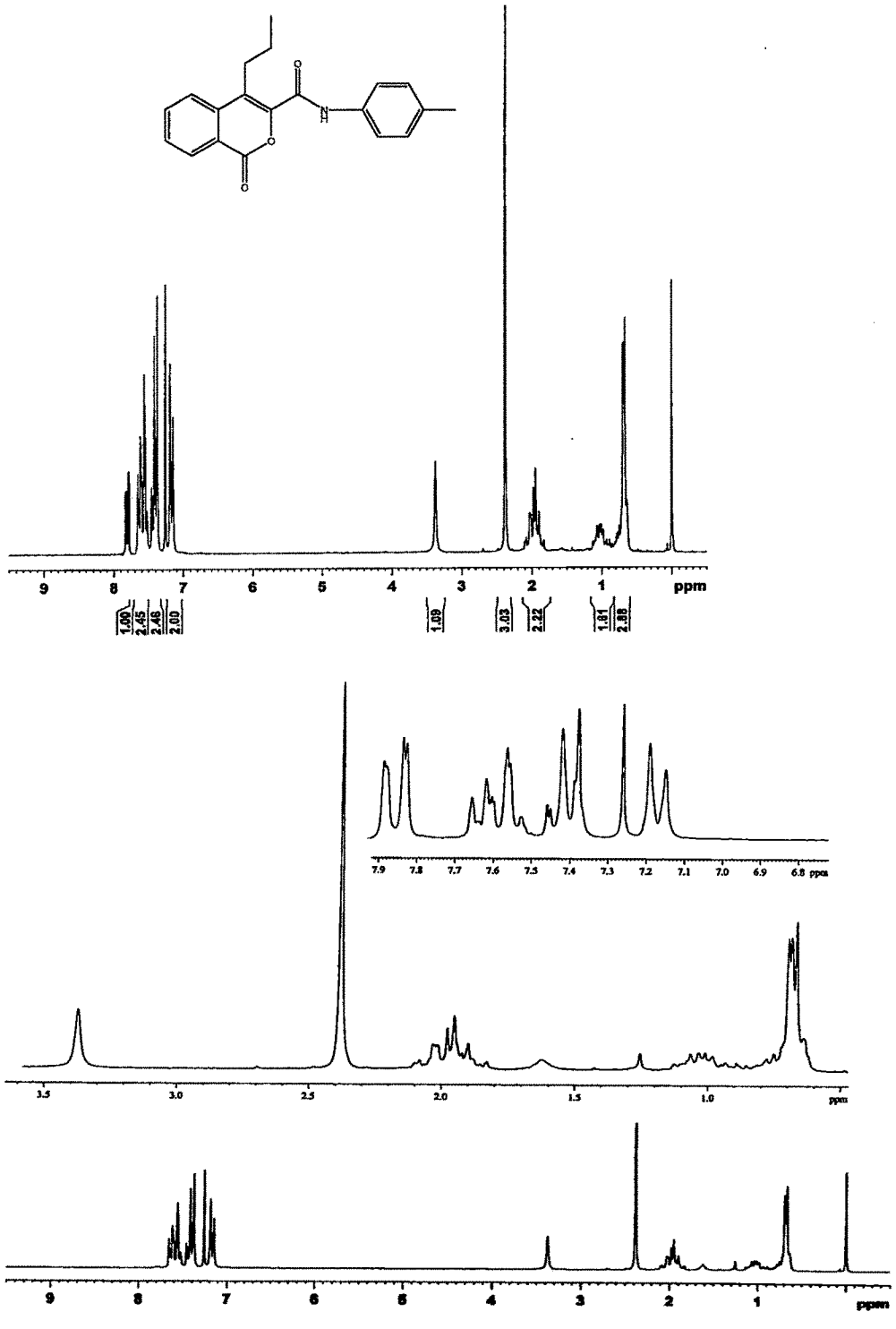


Fig. 3.A.18 - ^{1}H NMR: 4-Propyl-3-4'-tolylamino -carbonyl isocoumarin 10l

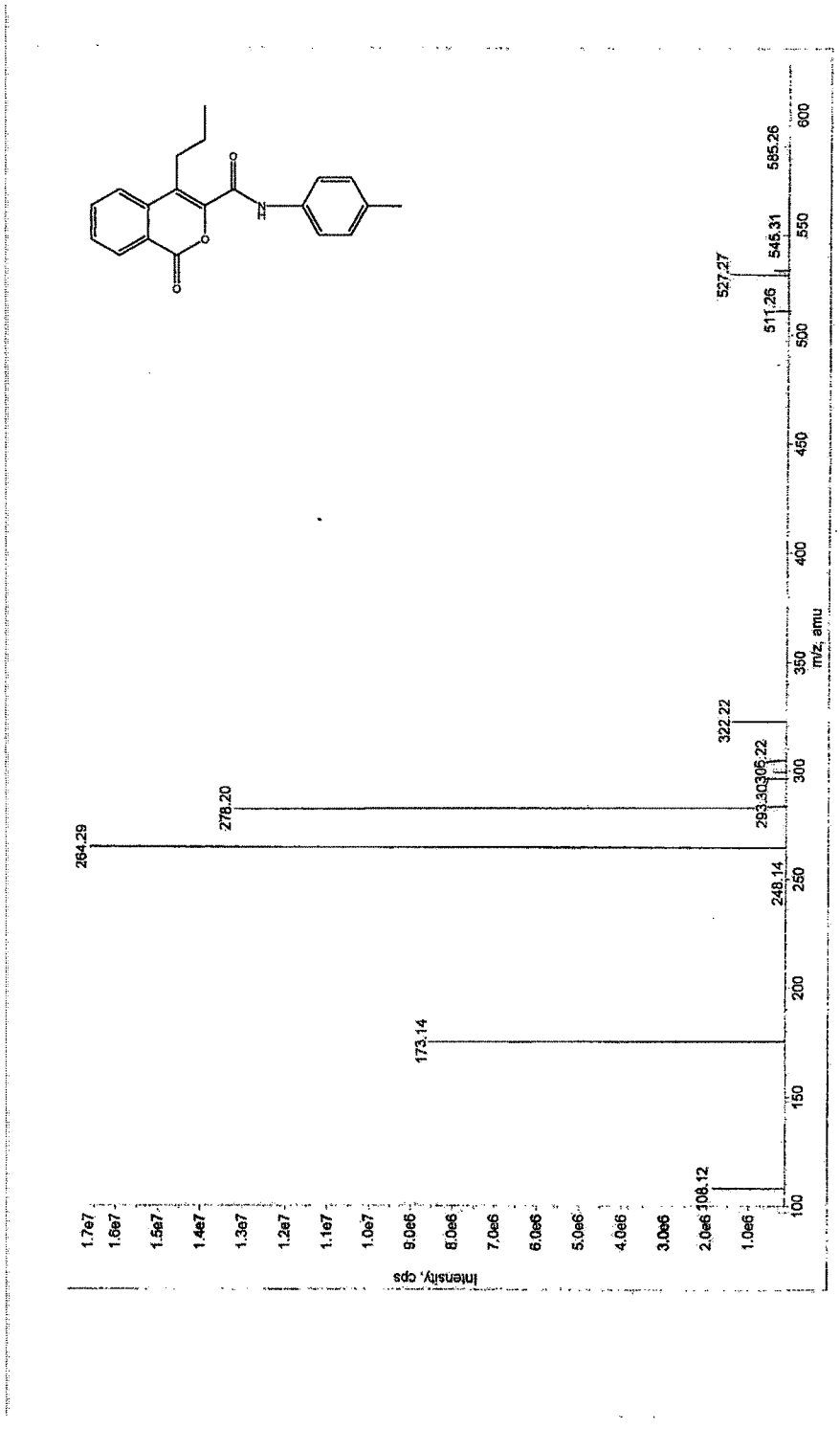
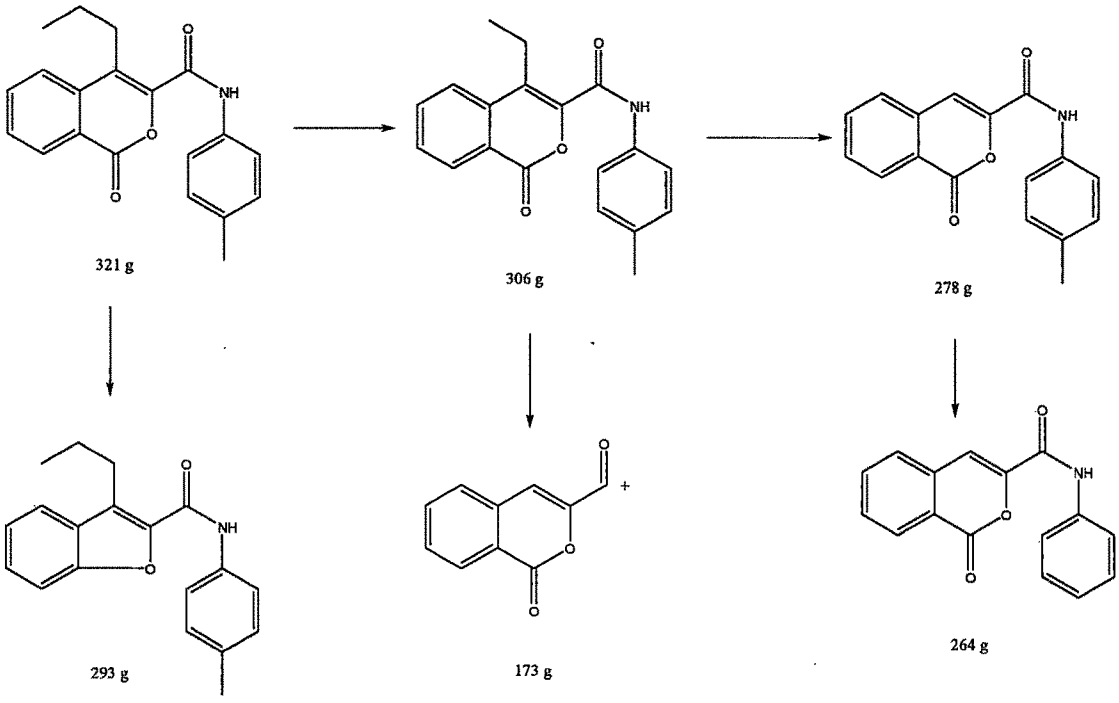


Fig. 3.A.19 – Mass spectrum: **4-Propyl-3-4'-tolylamino -carbonyl isocoumarin 10l**



Fragmentation Pattern: **4-Propyl-3-4'-tolylamino -carbonyl isocoumarin**

3. A.3 EXPERIMENTAL

The reagents and the solvents used in this study were of analytical grade and were used without further purification. Melting points were determined in open capillaries and are uncorrected. The purity of the compounds was checked by TLC on silica gel GF254 plates using UV/Iodine as visualizing agent and Merck's silica gel (60-120 mesh) was used for column chromatographic purification. Infrared spectra were recorded on FTIR Perkin Elmer spectrophotometer using potassium bromide optics. ^{1}H NMR spectra were recorded on a Bruker spectrometer (400 MHz) using TMS as internal standard and chemical shifts are given in ppm. Mass spectrums were obtained using Thermo Scientific Corporation, DSQ II Mass Spectrometer. Elemental analyses were carried out on Perkin-Elmer C, H, N, S analyzer (Model-2400). O-acyl benzoic acids and bromo acetophenone derivatives were prepared by literature method[21-26].

General procedure for 3a-3f

6-Nitro acetyl benzoic acid (1 g, 0.0047 mole) **1**, p-hydroxy bromo acetophenone (1.02 g, 0.0047 mole) **2**, K_2CO_3 (1.38 g, 0.01 mole) in ethyl methyl ketone was taken in a round bottom flask and refluxed for 10-12 hrs. Solvent was then removed, water added and extracted with ethyl acetate. Solvent layer was first washed with sod. bicarbonate and then with water and dried over anhy. Na_2SO_4. After removal of solvent the crude product was purified by column chromatography using petroleum ether (60-80^{0}C)-ethyl acetate to give yellow crystals of **3a**.

3-(4'-Hydroxy benzoyl)-4-methyl-8-nitro isocoumarin 3a

This compound was obtained as yellow crystals, mp: 124^{0}C; 62.34% yield; Anal. Calcd $C_{17}H_{11}NO_6$ (325.0 g): C, 62.76; H, 3.38; N, 4.30; Found: C, 62.76; H, 3.36; N, 4.37; ^{1}H NMR δ 2.3 (s, 3H, CH_3), 5.3 (s, 1H, OH), 6.8-7.8 (m, 6H, aromatic protons), 8.5 (d, 1H, C_7-H); ms : m/z: 325 (M^+), 279, 266, 232, 191, 146 and 118.

3-(4'-Bromo benzoyl)-4-methyl-8-nitro isocoumarin 3b

This compound was obtained as orange crystals, mp: 157^{0}C; 61.19% yield; Anal. Calcd $C_{17}H_{10}NO_5Br$ (387.9 g): C, 52.59; H, 2.57; N, 3.60; Found: C, 52.12; H, 2.45; N, 4.08; ^{1}H NMR δ 1.7 (s, 3H, CH_3), 6.8-8.2 (m, 6H, aromatic protons), 8.5 (d, 1H, C_7-H); ms: m/z: 387.9 (M^+), 308, 232, 185, 184 and 146.

3-(2', 4'-Dihydroxy benzoyl) - 4-methyl-8-nitro isocoumarin 3c

This compound was obtained as white crystals, mp: 105-107^{0}C; 60.98% yield; Anal. Calcd $C_{17}H_{11}NO_7$ (341.0 g): C, 59.82; H, 3.22; N, 4.10; Found: C, 60.05; H, 4.00; N, 4.22; ^{1}H NMR δ 2.0 (s, 3H, CH_3), 6.8-8.0 (m, 5H, aromatic protons), 8.5 (d, 1H, C_7-H), 9.4 (s, 1H, OH), 9.6 (s, 1H, OH); ms: m/z 341 (M^+), 295, 233, 191, 145, 137 and 110.

4-Methyl-3-(4'-methoxy benzoyl)-8-nitro isocoumarin 3d

This compound was obtained as yellow crystals, mp: 156^{0}C; 60.25% yield; Anal. Calcd $C_{18}H_{13}NO_6$ (339.0 g): C, 63.71; H, 3.83; N, 4.12; Found: C, 63.41; H, 3.82; N, 4.33; ^{1}H NMR δ 2.1 (s, 3H, CH_3), 3.8 (s, 3H, OCH_3), 6.1-8.2 (m, 6H, aromatic protons), 8.5 (d, 1H, C_7-H); ms: m/z: 339 (M^+), 279, 192, 173, 146 and 118.

4-Methyl-3-(4'-phenyl benzoyl)-8-nitro isocoumarin 3e

This compound was obtained as pale yellow crystals, mp: 76^{0}C; 70.37% yield; Anal. Calcd $C_{23}H_{15}NO_5$ (385.0 g): C, 71.68; H, 3.89; N, 3.63; Found: C, 71.24; H, 3.82; N, 3.92; ^{1}H NMR δ 1.9 (s, 3H, CH_3), 7.3-8.0 (m, 11H, aromatic protons), 8.45 (d, 1H, C_7-H); ms: m/z 385 (M^+), 370, 357, 339, 324, 308, 231, 187, 173, 146 and 118.

3-Dibenzofuroyl -4-methyl- 8-nitro isocoumarin 3f

This compound was obtained as pale yellow crystals, mp: 100^{0}C; 72.04% yield; Anal. Calcd $C_{23}H_{13}NO_6$ (399.0 g): C, 69.17; H, 3.25; N, 3.50; Found: C, 68.94; H, 3.06; N, 3.47; ^{1}H NMR δ 2.2 (s, 3H, CH_3), 7.1-8.0 (m, 9H, aromatic protons), 8.4 (d, 1H, C_7-H); ms: m/z 399 (M^+), 385, 370, 340, 339, 323, 279, 213, 187, 173, 146 and 106.

General procedure for 7a-7h

3-(4'-Bromo-benzoyl) - 4-methyl-isocoumarin (0.1 g, 0.00029 mole) (**6a**), piperidine (0.06 ml, 0.00059 mole) and DMF were taken in a round bottom flask and refluxed for 8 hrs. After the reaction was over the reaction mixture was poured into ice and the solid obtained was filtered. The crude product was purified by column chromatography using petroleum ether (60-80^{0}C)-ethyl acetate to give yellow crystals of **7a**.

4-Methyl -3-(4'-piperidin-1-yl-benzoyl) isocoumarin 7a

This compound was obtained as yellow crystals, mp: 155^{0}C; 86.23% yield; Anal. Calcd $C_{22}H_{21}NO_3$ (347.0 g): C, 76.08; H, 6.05; N, 4.03; Found: C, 76.00; H, 6.11; N, 3.87; ^{1}H NMR δ 1.7 (s, 3H, CH_3), 2.8 (m, 6H, CH_2-CH_2-CH_2), 3.3 (q, 4H, CH_2-N-CH_2) 6.8-7.7 (m, 7H, aromatic protons), 8.4 (d, 1H, C_8-H); ms: m/z: 347 (M^+), 263, 160, 146, 118 and 85.

4-Methyl -3-(4'- morpholin-1-yl-benzoyl) isocoumarin 7b

This compound was obtained as yellow crystals, mp: 245^{0}C; 68.00% yield; Anal. Calcd $C_{21}H_{19}NO_4$ (349.0 g): C, 72.20; H, 5.44; N, 4.01 Found: C, 72.26; H, 5.52; N, 4.31; ^{1}H NMR δ 1.5 (s, 3H, CH_3), 3.3(t, 4H,CH_2-N-CH_2), 3.9(t, 4H,CH_2-O-CH_2), 6.8-7.9 (m, 7H, aromatic protons), 8.4 (d, 1H, C_8-H); ms: m/z: 345 (M^+ - 2), 302, 279, 221, 190, 187, 146, 135 and 77.

4-Methyl -3-(4'-phenylamino -benzoyl) isocoumarin 7c

This compound was obtained as yellow crystals, mp: 155^{0}C; 68.43% yield; Anal. Calcd $C_{23}H_{17}NO_3$ (355.0 g): C, 77.74; H, 4.78; N, 3.94 ; Found: C, 78.01; H, 5.02; N, 4.11; ^{1}H NMR δ 2.4 (s, 3H, CH_3), 3.4 (s, 1H, NH), 7.1-7.7 (m, 12H, aromatic protons), 8.2 (d, 1H, C_8-H); ms: m/z: 355 (M^+), 341, 286, 263, 236, 221, 197, 187, 168, 146, 132 and 118.

4-Methyl -3-(4'- tolylamino -benzoyl) isocoumarin 7d

This compound was obtained as yellow crystals, mp: 145^{0}C; 65.13% yield; Anal. Calcd $C_{24}H_{19}NO_3$ (369.0 g): C, 78.04; H, 5.14; N, 3.79; Found: C, 78.01; H, 5.52; N, 4.11; ^{1}H NMR δ 1.9 (s, 3H, CH_3), 2.4 (s, 3H, CH_3), 3.4 (s, 1H, NH), 6.4-7.7 (m, 11H,

aromatic protons), 8.0 (d, 1H, C_8-H); ms: m/z: 368 (M^+ - 1), 354, 264, 187, 159, 146 and 118.

4-Ethyl -3-(4'-piperidin-1-yl-benzoyl) isocoumarin 7e

This compound was obtained as pale yellow crystals, mp: 130^{0}C; 74.68% yield; Anal. Calcd $C_{23}H_{23}NO_3$ (361.0 g): C, 76.45; H, 6.37; N, 3.87; Found: C, 76.46; H, 6.77; N, 3.92; ^{1}H NMR δ 1.2 (t, 3H, CH_3), 1.6 (s, 6H, CH_2-CH_2-CH_2), 2.8 (q, 2H, CH_2), 3.4 (s, 4H, CH_2-N-CH_2) , 7.6-7.9 (m, 7H, aromatic protons), 8.42 (d, 1H, C_8-H); ms: m/z: 361 (M^+), 277, 185, 146, 118 and 85.

4-Ethyl -3-(4'- morpholin-1-yl-benzoyl) isocoumarin 7f

This compound was obtained as yellow crystals, mp: 220^{0}C; 66.00% yield; Anal. Calcd $C_{22}H_{21}NO_4$ (363.0 g) : C, 72.72; H, 5.78; N, 3.85; Found: C, 72.76; H, 5.79; N, 4.00; ^{1}H NMR δ 1.0 (t, 3H, CH_3), 2.1 (q, 2H, CH_2), 3.2(t, 4H,CH_2-N-CH_2), 3.6(t, 4H,CH_2-O-CH_2), 6.8-7.9 (m, 7H, aromatic protons), 8.4 (d, 1H, C_8-H); ms: m/z: 363 (M^+), 334, 306, 221, 200, 190, 146 and 86.

4-Ethyl -3-(4'-phenylamino -benzoyl) isocoumarin 7g

This compound was obtained as yellow crystals, mp: 145^{0}C; 61.85% yield; Anal. Calcd $C_{24}H_{19}NO_3$ (369.0 g) : C, 78.04; H, 5.14; N, 3.79; Found: C, 78.23; H, 5.34; N, 4.29; ^{1}H NMR δ 1.1 (t, 3H, CH_3), 1.9 (q, 2H, CH_2), 4.5 (s, 1H, NH), 7.4-7.9 (m, 12H, aromatic protons), 8.45 (d, 1H, C_8-H); ms: m/z: 368 (M^+ - 1), 340, 313, 264, 236, 201, 186, 172 and 117.

4-Ethyl -3-(4'- tolylamino -benzoyl) isocoumarin 7h

This compound was obtained as yellow crystals, mp: 138^{0}C; 63.79% yield; Anal. Calcd $C_{25}H_{21}NO_3$ (383.0 g) : C, 78.32; H, 5.48; N, 3.65; Found: C, 78.16; H, 5.62; N, 3.88; ^{1}H NMR δ 1.0 (t, 3H, CH_3), 2.1 (q, 2H, CH_2), 2.4 (s, 3H, CH_3), 3.4 (s, 1H, NH), 6.9-7.7 (m, 11H, aromatic protons), 8.4 (d, 1H, C_8-H); ms: m/z: 383 (M^+), 355, 278, 277, 263, 186, 158 and 118.

General procedure for 9a-9c

O-acetyl benzoic acid (2 g, .012 mole) **4a,** Bromoacetyl bromide (1.06 ml, .012 mole) **8,** K_2CO_3 (3.53 g, 0.025 mole) and ethyl methyl ketone were taken in a round bottom flask and refluxed for 10-12 hrs, solvent removed after reaction was over; water added and extracted with ethyl acetate. Solvent layer was first washed with sodium bicarbonate and then with water and it was dried over anhy. Na_2SO_4. After removal of solvent the crude product was purified by column chromatography using petroleum ether (60-80^{0}C) -ethyl acetate mixture to give white crystals of **9a**

3-Bromo carbonyl-4-methyl isocoumarin 9a

This compound was obtained as white crystals, mp: 94^{0}C; 60.93% yield; Anal. Calcd $C_{11}H_7O_3Br$ (266.9 g): C, 49.45; H, 2.62; Found: C, 49.00; H, 2.60.

3-Bromo carbonyl-4-methyl isocoumarin 9b

This compound was obtained as white crystals, mp: 52^{0}C; 56.86 % yield; Anal. Calcd $C_{12}H_9O_3Br$ (280.9 g): C, 51.26; H, 3.20; Found: C, 51.30; H, 3.41.

3-Bromo carbonyl-4-propyl isocoumarin 9c

This compound was obtained as yellow liquid, bp: > 200^{0}C; 47.95 % yield; Anal. Calcd $C_{13}H_{11}O_3Br$ (294.9 g): C, 52.89; H, 3.73; Found: C, 52.54; H, 3.70.

General procedure for 10a-10l

3-Bromo carbonyl -4-methyl isocoumarin (2.0g, 0.0074 mole) **9a,** piperidine (1.55 ml, 0.015 mole) and DMF was refluxed on sand bath for 3-5 h. The reaction mixture was cooled and poured on crushed ice. The product was filtered and purified by column chromatography using petroleum ether (60-80^{0}C) -ethyl acetate mixture to give white crystals of **10a**

4-Methyl-3-piperdinyl carbonyl isocoumarin 10a

This compound was obtained as pale yellow crystals, mp: 79^{0}C; 65.32% yield; Anal. Calcd $C_{16}H_{17}NO_3$ (271.0 g): C, 70.84; H, 6.27; N, 5.16; Found: C, 70.52; H, 6.38; N, 4.94; ^{1}H NMR δ 1.9 (s, 3H, CH_3), 1.5 (m, 6H, CH_2-CH_2-CH_2), 3.4 (t, 4H, CH_2-N-

CH_2) 7.2-7.6 (m, 3H, aromatic protons), 7.9 (d, 1H, C_8-H); ms: m/z: 271 (M^+), 256, 186, 160, 146 and 118.

4-Methyl-3-morphonlinyl-carbonyl isocoumarin 10b

This compound was obtained as white crystals, mp: 61^{0}C; 65.32% yield; Anal. Calcd $C_{15}H_{15}NO_4$ (273.0 g): C, 65.93; H, 5.49; N, 5.12; Found: C, 65.46; H, 5.53; N, 4.97; ^{1}H NMR δ 2.1 (s, 3H, CH_3), 3.2 (t, 4H, CH_2-N-CH_2), 3.6 (t, 4H, CH_2-O-CH_2), 7.3-7.6 (m, 3H, aromatic protons), 7.8-7.9 (dd, 1H, C_8-H); ms: m/z: 273 (M^+), 258, 245, 187, 159 and 146.

4-Methyl-3-N-phenyl amino-carbonyl isocoumarin 10c

This compound was obtained as semi solid, mp: -^{0}C; 35.61% yield; ^{1}H NMR δ 1.8 (s, 3H, CH_3), 7.3-7.6 (m, 8H, aromatic protons), 7.9 (d, 1H, C_8-H), 9.5 (s, 1H, NH); ms: m/z: 279 (M^+), 264, 188, 187, 159 and 146.

4-Methyl-3-4'-tolylamino -carbonyl isocoumarin 10d

This compound was obtained as yellow powder, mp: 78^{0}C; 35.00 % yield; Anal. Calcd $C_{18}H_{15}NO_3$ (293.0 g): C, 73.72; H, 5.11; N, 4.77; Found: C, 73.86; H, 5.50; N, 4.48; ^{1}H NMR δ 1.9 (s, 3H, CH_3), 2.4(s, 3H, CH_3), 7.1-7.6 (m, 7H, aromatic protons), 8.0 (d, 1H, C_8-H), 13.1 (s, 1H, NH); ms: m/z: 293 (M^+), 265, 263, 203, 159, 146, 120 and 77.

4-Ethyl-3-piperdinyl carbonyl isocoumarin 10e

This compound was obtained as yellow liquid, bp: 130^{0}C; 52.27% yield; Anal. Calcd $C_{17}H_{19}NO_3$ (285.0 g): C, 71.57; H, 6.66; N, 4.91; Found: C, 71.12; H, 6.38; N, 4.94; ^{1}H NMR δ 1.3 (t, 3H, CH_3),2.2 (q, 2H, CH_2), 1.6 (m, 6H, CH_2-CH_2-CH_2), 3.4 (t, 4H, CH_2-N-CH_2) 7.3-7.5 (m, 3H, aromatic protons), 8.0 (d, 1H, C_8-H); ms: m/z: 28 (M^+ - 1), 257, 256, 201, 173 and 146.

4-Ethyl-3-morphonlinyl-carbonyl isocoumarin 10f

This compound was obtained as white crystals, mp: 115^{0}C; 75.97% yield; Anal. Calcd $C_{16}H_{17}NO_4$ (287.0 g): C, 66.89; H, 5.92; N, 4.87; Found: C, 67.09; H, 5.84; N, 4.91; ^{1}H NMR δ 1.2 (t, 3H, CH_3), 2.9 (q, 2H, CH_2), 3.2 (t, 4H, CH_2-N-CH_2), 3.6 (t, 4H,

CH_2-O-CH_2), 7.3-7.6 (m, 3H, aromatic protons), 7.8-7.9 (dd, 1H, C_8-H); ms: m/z: 287 (M^+), 272, 258, 201, 187 and 146.

4-Ethyl-3-N-phenyl amino-carbonyl isocoumarin 10g

This compound was obtained as brown powder, mp: 140^{0}C; 55.61% yield; Anal. Calcd $C_{18}H_{15}NO_3$ (293.0 g): C, 73.72; H, 5.11; N, 4.77; Found: C, 73.54; H, 5.42; N, 4.97; ^{1}H NMR δ 1.1 (t, 3H, CH_3), 1.9 (q, 2H, CH_2), 7.3-7.7 (m, 8H, aromatic protons), 8.0 (d, 1H, C_8-H), 11.0 (s, 1H, NH); ms: m/z: 293 (M^+), 251, 216, 161, 118 and 77.

4-Ethyl-3-4'-tolylamino -carbonyl isocoumarin 10h

This compound was obtained as white powder, mp: 145^{0}C; 37.82 % yield; Anal. Calcd $C_{19}H_{17}NO_3$ (307.0 g): C, 74.26; H, 5.53; N, 4.56; Found: C, 74.02; H, 5.42; N, 4.92; ^{1}H NMR δ 1.0 (t, 3H, CH_3), 2.1 (q, 2H, CH_2), 2.4(s, 3H, CH_3), 7.1-7.5 (m, 7H, aromatic protons), 8.0 (d, 1H, C_8-H), 9.8 (s, 1H, NH); ms: m/z: 307 (M^+), 292, 279, 277, 173, 146 and 134.

4-Propyl-3-piperdinyl carbonyl isocoumarin 10i

This compound was obtained as yellow liquid, bp: >220^{0}C; 44.63.27% yield; Anal. Calcd $C_{18}H_{21}NO_3$ (299.0 g): C, 72.24; H, 7.02; N, 4.68; Found: C, 72.12; H, 6.98; N, 4.90; ^{1}H NMR δ 1.0 (t, 3H, CH_3), 1.5 (m, 6H, CH_2-CH_2-CH_2), 1.7 (m, 2H, CH_2), 2.1 (t, 2H, CH_2), 3.6 (t, 4H, CH_2-N-CH_2) 7.3-7.5 (m, 3H, aromatic protons), 8.1 (d, 1H, C_8-H); ms: m/z: 298 (M^+ - 1), 265, 256, 208, 146 and 86.

4-Propyl-3-morphonlinyl-carbonyl isocoumarin 10j

This compound was obtained as white crystals, mp: 75-80^{0}C; 38.31% yield; Anal. Calcd $C_{17}H_{19}NO_4$ (301.0 g): C, 67.77; H, 6.31; N, 4.65; Found: C, 67.54; H, 6.38; N, 4.58; ^{1}H NMR δ 1.0 (t, 3H, CH_3),1.8 (m, 2H, CH_2), 2.4 (q, 2H, CH_2), 3.4 (t, 4H, CH_2-N-CH_2), 3.7 (t, 4H, CH_2-O-CH_2), 7.4-7.6 (m, 3H, aromatic protons), 7.9 (d, 1H, C_8-H); ms: m/z: 301 (M^+), 258, 215, 173, 146, 86 and 77.

4-Propyl-3-N-phenyl amino-carbonyl isocoumarin 10k

This compound was obtained as yellow powder, mp: 135^{0}C; 52.59% yield; Anal. Calcd $C_{19}H_{17}NO_3$ (307.0 g): C, 74.26; H, 5.53; N, 4.56; Found: C, 74.09; H, 5.46; N,

4.32; ^{1}H NMR δ 1.0 (t, 3H, CH_3), 1.7 (m, 2H, CH_2), 2.0 (q, 2H, CH_2), 7.2-7.7 (m, 8H, aromatic protons), 8.0 (d, 1H, C_8-H), 9.0 (s, 1H, NH); ms: m/z: 307 (M^+), 264, 236, 73, 145 and 77.

4-Propyl-3-4'-tolylamino -carbonyl isocoumarin 10l

This compound was obtained as yellow crystals, mp: 190^{0}C; 69.24 % yield; Anal. Calcd $C_{20}H_{19}NO_3$ (321.0 g): C, 74.76; H, 5.91; N, 4.36; Found: C, 75.75; H, 5.78; N, 4.95; ^{1}H NMR δ 0.7 (t, 3H, CH_3),1.0 (m, 2H, CH_2), 2.0(m, 2H, CH_2), 2.4 (s, 3H, $C_{4'}$ - CH_3), 3.4 (s, 1H, NH), 7.1-7.7 (m, 7H, aromatic protons), 7.8-7.9 (dd, 1H, C_8-H); ms: m/z: 322 (M^+ + 1), 306, 293, 278, 264 and 173.

3. A.4 CONCLUSION

- The different series were synthesized to introduce the nitrogen atom in the isocoumarin moiety by following effortless procedure, keeping in view the importance of nitrogen atom in biological studies.

- The nitrogen atom was introduced in the form of NO_2 group and in the form of primary and secondary amines, which resulted in 4-Alkyl-3-aroyl-8-nitro isocoumarins, 4-Alkyl-3-aminobenzoyl isocoumarins and 4-Alkyl-3-aminocarbonyl isocoumarins respectively.

- All the title compounds synthesized were screened for biological activities which are given in last chapter.

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Chapter 3:

Section B:

Introduction of six membered lactone ring in isocoumarin moiety

3. B.1 INTRODUCTION

In recent years there has been a rapid increase in resistance to antibiotics by pathogenic bacteria[1]. Infectious diseases are the leading cause of death worldwide. Conventional antibiotics are designed to possess static or bactericidal properties[2]. Mutations in bacteria are responsible for drug resistance which can take a variety of forms. As a result, these resistant pathogens are gradually rendering ineffective the traditional treatments.

The lactones comprise an important class of compounds that participates in many biological pathways, like bacterial cell communications[3], thus affecting the system in either way.

There have been many known examples where the presence of lactone ring has affected the action of the drug against various disease models. Few of the examples are described below:

- A series of C19-steroids bearing a spiro-δ-lactone, tested for their ability to inhibit the reductive activity of 17β-HSD5 transfected in HEK-293 cells, because they are known inhibitors of 17β-HSD2[4].

- Lactones are usually added as Flavour ingredients to foods at levels less than 20 ppm, with a range of 0.05 to 80 ppm[5].

- Camptothecin is an anti cancer drug, in which earlier reports have suggested that the E-ring lactone is not essential for its activity. However, this ring in the lactone form with specific C-20 configuration is required for better activity. Conformation at C-20 is crucial for better activity as the 20(S) isomer is 10- to 100-fold more active than 20(R) [6].

- Discodermolide (DDM) is an antimitotic polyketide, isolated from a deep-sea sponge, which displays potent cytotoxic activity against a number of human

tumor cell lines and suppresses normal microtubule (MT) dynamics and disrupts the formation of mitotic spindles, thus leading to apoptosis[7].

- The one-dimensional ^1H and NOESY spectra showed that the δ -lactone ring of DDM opens up slowly by hydrolysis in the buffer. Comparison of the intensities of cross- and diagonal peaks reveals that the NOE rates of hydrolyzed DDM are only 15% of the NOE rates of intact DDM, thus indicating that binding of hydrolyzed DDM to tubulin is much weaker. This finding is in good agreement with activity data showing that the potency of DDM decreases approximately by a factor of six upon opening of the lactone[8].

Coumarins, also contain a six membered lactone ring, and represent one of the most active classes of compounds possessing a wide spectrum of biological activity[9].

- Coumarins comprise a group of natural compounds found in a variety of plant sources. The distribution of biologically active coumarins in a wide range of plants seems to correlate with their ability to act as phytoalexins, *i.e.* they are formed as a response to traumatic injury, during the wilting process, by plant diseases or through drying, they accumulate on the surface of the leaves, fruits and seeds, and they inhibit the growth and sporulation of fungal plant pathogens and act as repellents against beetles and other terrestrial invertebrates[10].

- Coumarins have important effects in plant biochemistry and physiology, acting as antioxidants, enzyme inhibitors and precursors of toxic substances[11].

- The coumarins are extremely variable in structure and due to the various types of substitutions in the basic structural form their biological activity is influenced[12].

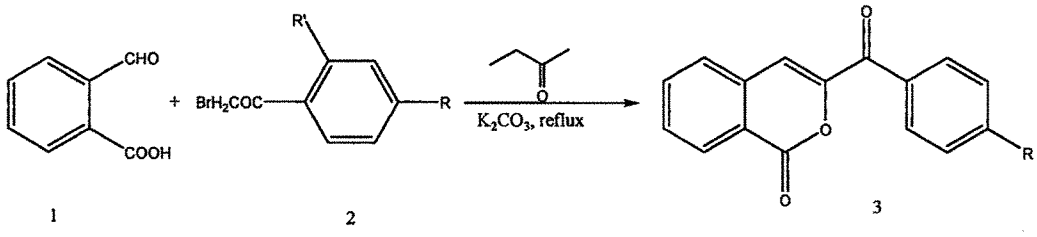
- The presence of phenolic, hydroxy and carboxylic acid groups on the coumarin nucleus has been considered necessary for antimicrobial activity[13].

- Coumarins are known to possess cardio protective properties: many of them are selective coronary vasodilators, an effect that may be related to a Ca^{2+}antagonistic activity[14].

From the above line of reasoning we directed this chapter towards synthesis of various isocoumarin derivatives containing one more six membered lactone ring, of biological interest, using isocoumarins itself as a key starting material.

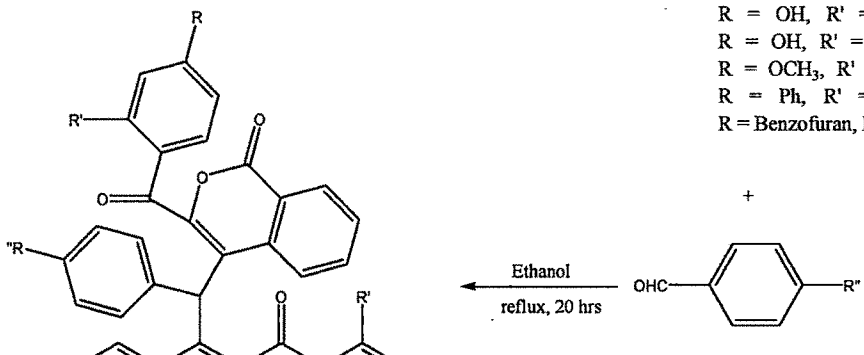
The reaction sequences for title compounds are outlined in Scheme I and II.

Scheme I



1 2 3

R = Br , R' = H **(3a)**
R = OH, R' = H **(3b)**
R = OH, R' = OH **(3c)**
R = OCH_3, R' = H **(3d)**
R = Ph, R' = H **(3e)**
R = Benzofuran, R' = H **(3f)**



4

5

R = OH, R' = H, R" = Cl **(5a)**
R = OH, R' = H, R" = H **(5b)**
R = OH, R' = H, R" = NO_2 **(5c)**
R = OH, R' = H, R" = OCH_3 **(5d)**
R = Br, R' = H, R" = NO_2 **(5e)**
R = Br, R' = H, R" = OH **(5f)**
R = Br, R' = H, R" = H **(5g)**
R = OCH_3, R' = H, R" = NO_2 **(5h)**
R = OCH_3, R' = H, R" = OH **(5i)**
R = OCH_3, R' = H, R" = OCH_3 **(5j)**
R = OH, R' = OH, R" = NO_2 **(5k)**
R = OH, R' = OH, R" = OH **(5l)**
R = OH, R' = OH, R" = OCH_3 **(5m)**

3. B.2 RESULTS AND DISCUSSION

(**Schemel**) deals with the synthesis of Bis isocoumarins. Till now we have synthesized 3,4 – disubstituted isocoumarins, but now our aim was to synthesize only 3- substituted isocoumarins so that the 4th position is vacant and on condensing with different aromatic aldehydes we can get the desired bis isocoumarins. Reaction route followed was same but starting materials used were different for the synthesis of isocoumarins.

To get 3-aroyl isocoumarins **3**, 2-carboxy benzaldehyde **1** was refluxed with different substituted bromo acetophenones **2** in ethyl methyl ketone in presence of anhydrous K_2CO_3 for 10-12 hrs. Characterization of the synthesized compounds was done by IR, NMR and Mass spectral studies.

3a shows absorption at 1720cm^{-1} for lactonic carbonyl, 1655 cm^{-1} for –C=O and 620 cm^{-1} for C-Br respectively (**Fig. 3.B.1**).

^{1}H NMR spectrum of **3a** and **3b** shows signals at δ 7.4 (s, 1H, C_4-H), 7.6-7.9 (m, 7H, aromatic protons), 8.4 (dd, 1H, C_8-H) (**Fig. 3.B.2**) and δ 5.5 (s, 1H, OH), 7.4 (s, 1H, C_4-H), 7.6-7.9 (m, 7H, aromatic protons), 8.4 (dd, 1H, C_8-H) (**Fig. 3.B.4**).

Mass spectrum of **3a** shows M^+ peak at m/z 328.97 (M^+), 249, 174 and 146 (**Fig. 3.B.3**).
Mass spectrum of **3b** shows M^+ peak at m/z 266.04 (M^+), 145 and 121 (**Fig. 3.B.5**).

As there is no substituent at the 4th position in 3- aroyl isocoumarins **3**, the **H** present can be replaced with other groups. Therefore, 3-aroyl isocoumarins **3** when were condensed with different aryl aldehydes **4** in the ratio 2:1 in ethanol, we get bis isocoumarins **5**.

The bis isocoumarins shows IR signals at 3130 cm^{-1}, 1573 cm^{-1}, 1452 cm^{-1} for –OH, lactonic carbonyl and –C=O respectively (**Fig. 3.B.6**).

^{1}H NMR spectrum of **5a** shows signals at δ 5.7 (s, 1H, CH), 6.9-8.1(m, 18H, aromatic protons), 8.4 (d, 2H, C_8-H) 10.0 (s, 2H, OH) (**Fig. 3.B.7**), **5b** at δ 3.6 (s, 2H, OH), 5.8 (s, 1H, CH), 6.9-8.0 (m, 19H, aromatic protons), 8.2 (d, 2H, C_8-H) (**Fig. 3.B.9**) and **5c** δ 6.7 (s, 1H, CH), 7.5-8.2(m, 18H, aromatic protons), 8.3 (d, 2H, C_8-H) 9.3 (s, 2H, OH) (**Fig.3.B.11**).

Mass spectrum of **5a** gives m/z peak at 480, 410, 390, 375, 298, 266, 145, 121, 118 and 77 (**Fig. 3.B.8**), **5b** at m/z: 620 (M^+), 603, 577, 551, 509, 423, 368, 264 and 121 (**Fig.3.B.10**) and **5c** at m/z: 663 (M^+ - 2), 619, 525, 479, 405, 266, 145 and 121 (**Fig. 3.B.12**).

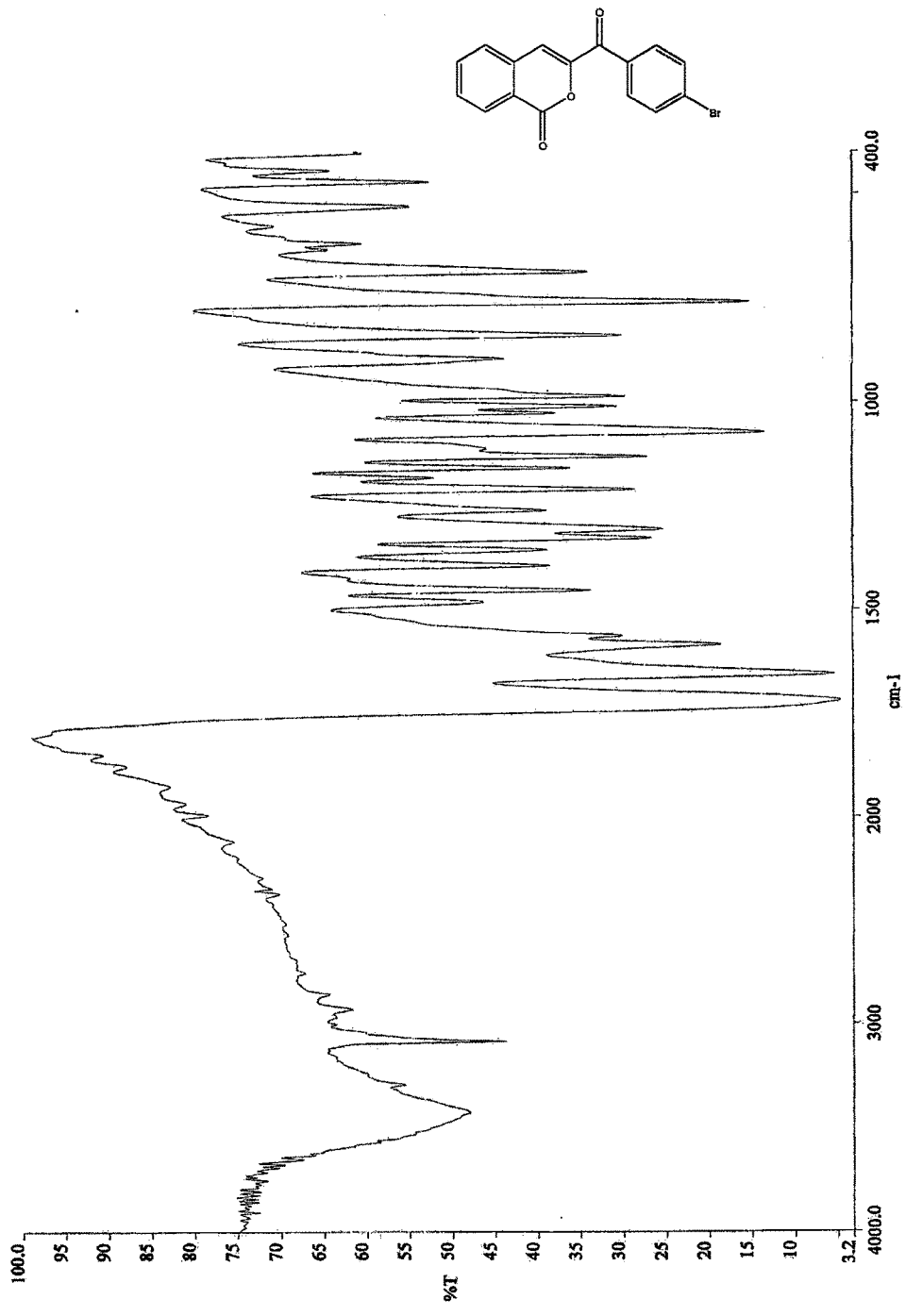


Fig. 3.B.1 – IR: **3-(4'-Bromo benzoyl) isocoumarin 3a**

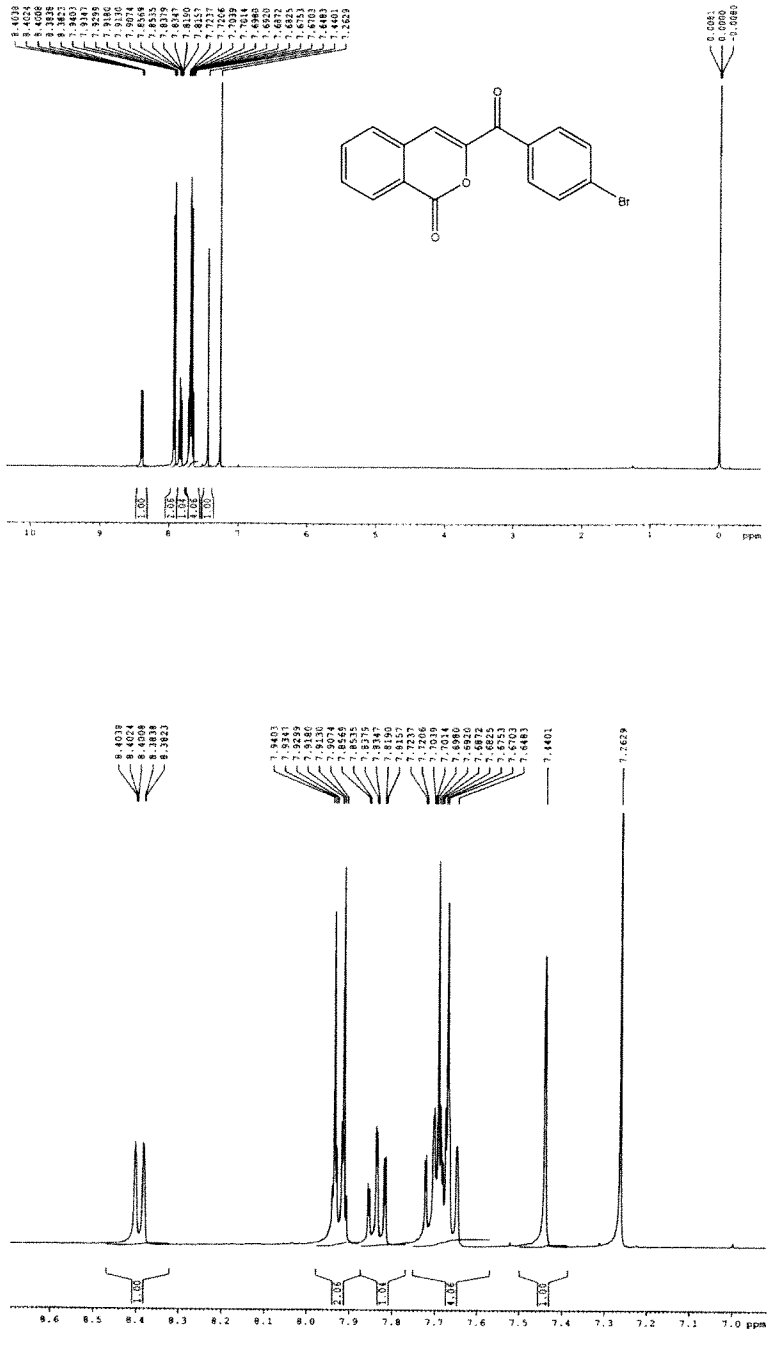


Fig. 3.B.2 – ^{1}H NMR: **3-(4'-Bromo benzoyl) isocoumarin 3a**

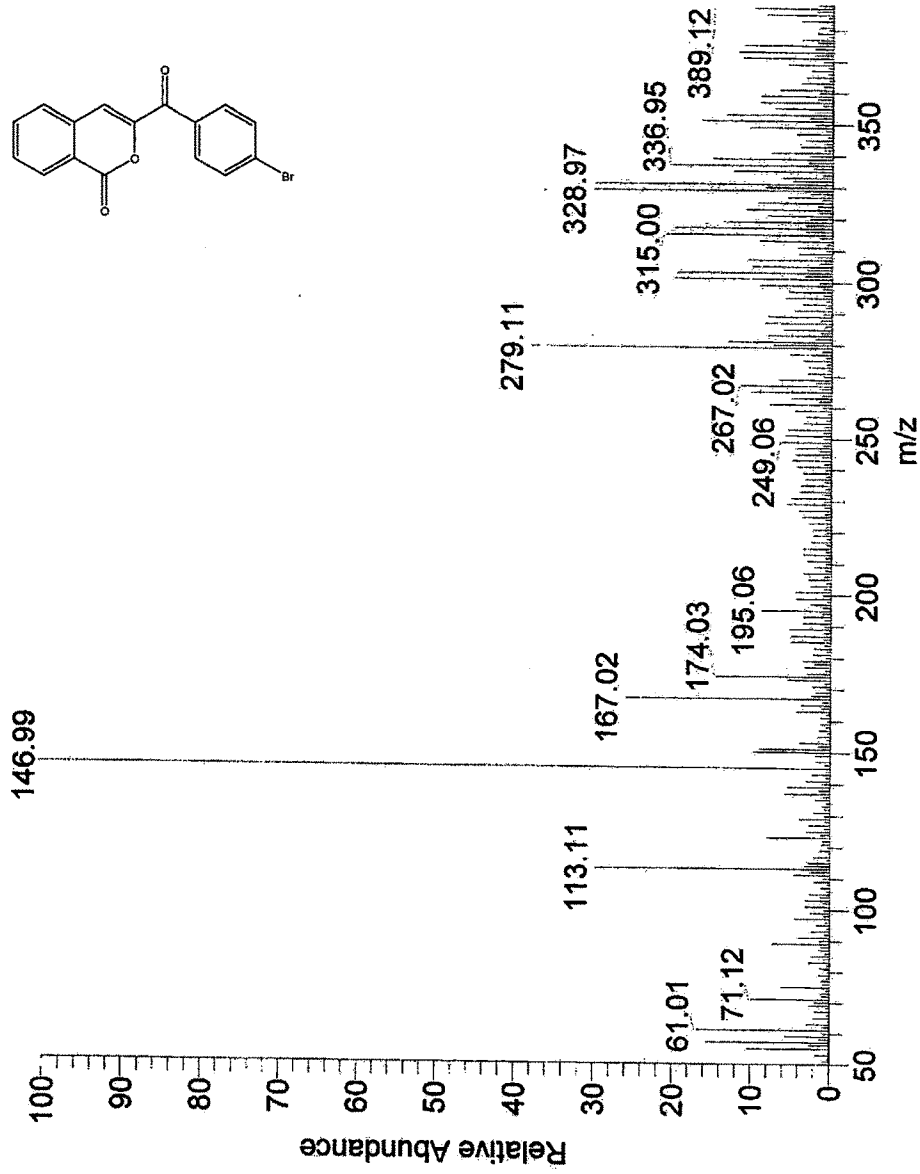
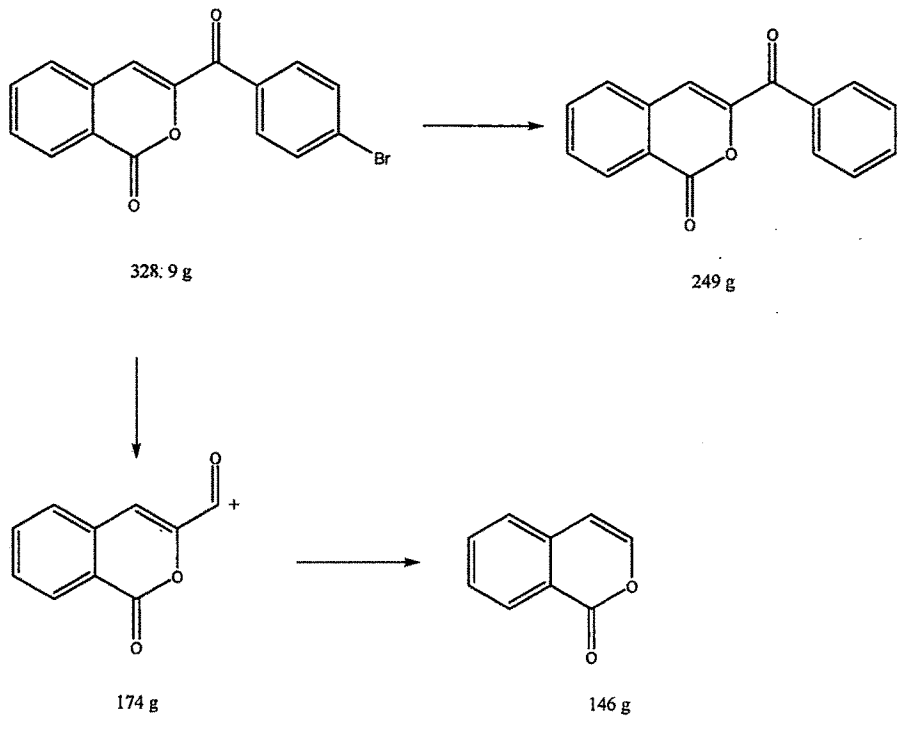


Fig. 3.B.3 - Mass spectrum: **3-(4'-Bromo benzoyl) isocoumarin 3a**



Fragmentation Pattern: **3-(4'-Bromo benzoyl) isocoumarin**

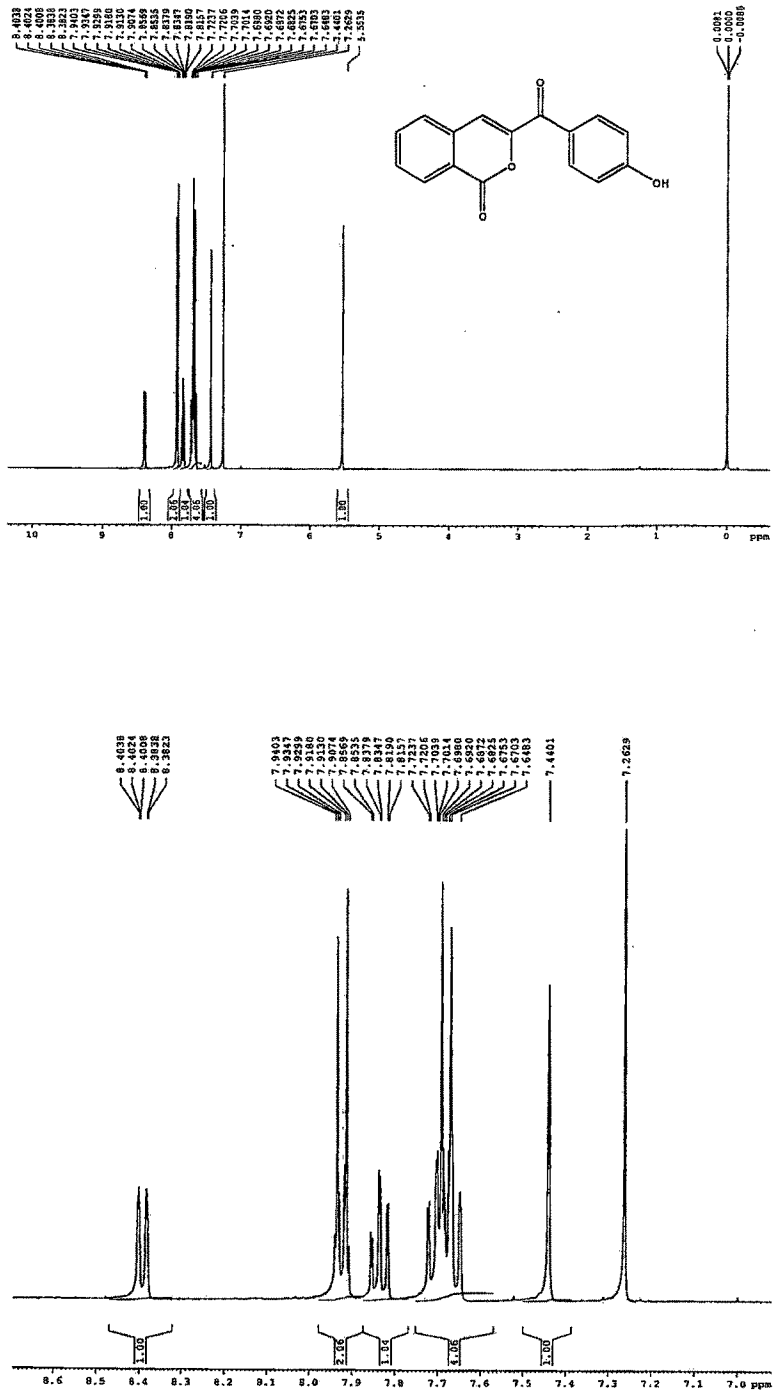


Fig. 3.B.4 – ^{1}H NMR: 3-(4'-Hydroxy benzoyl) isocoumarin 3b

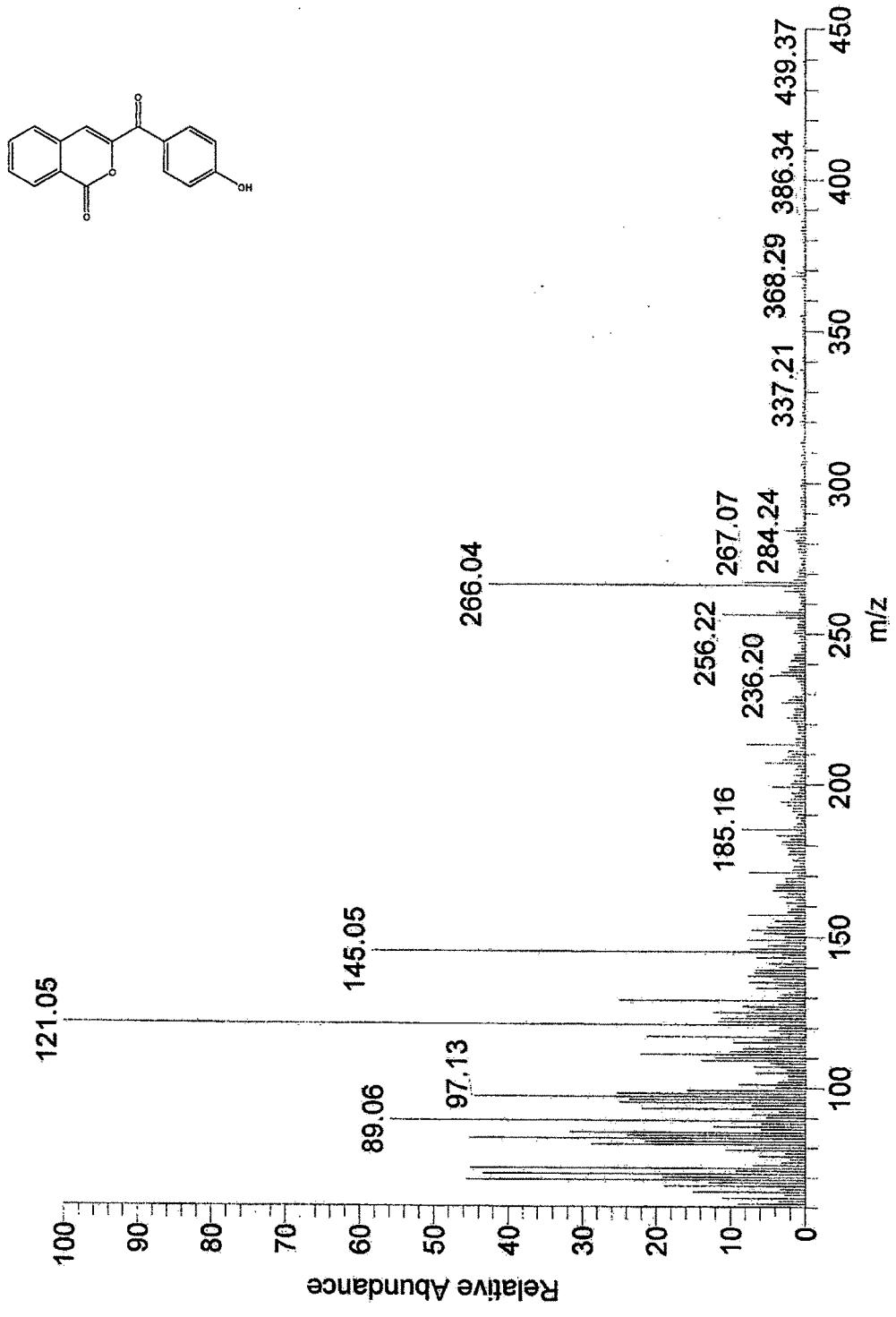
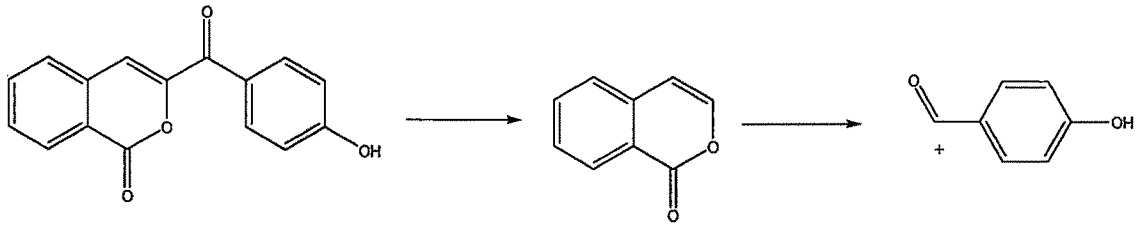


Fig. 3.B.5 – Mass spectrum: **3-(4'-Hydroxy benzoyl) isocoumarin 3b**



266 g 145 g 121 g

Fragmentation Pattern: **3-(4'-Hydroxy benzoyl) isocoumarin**

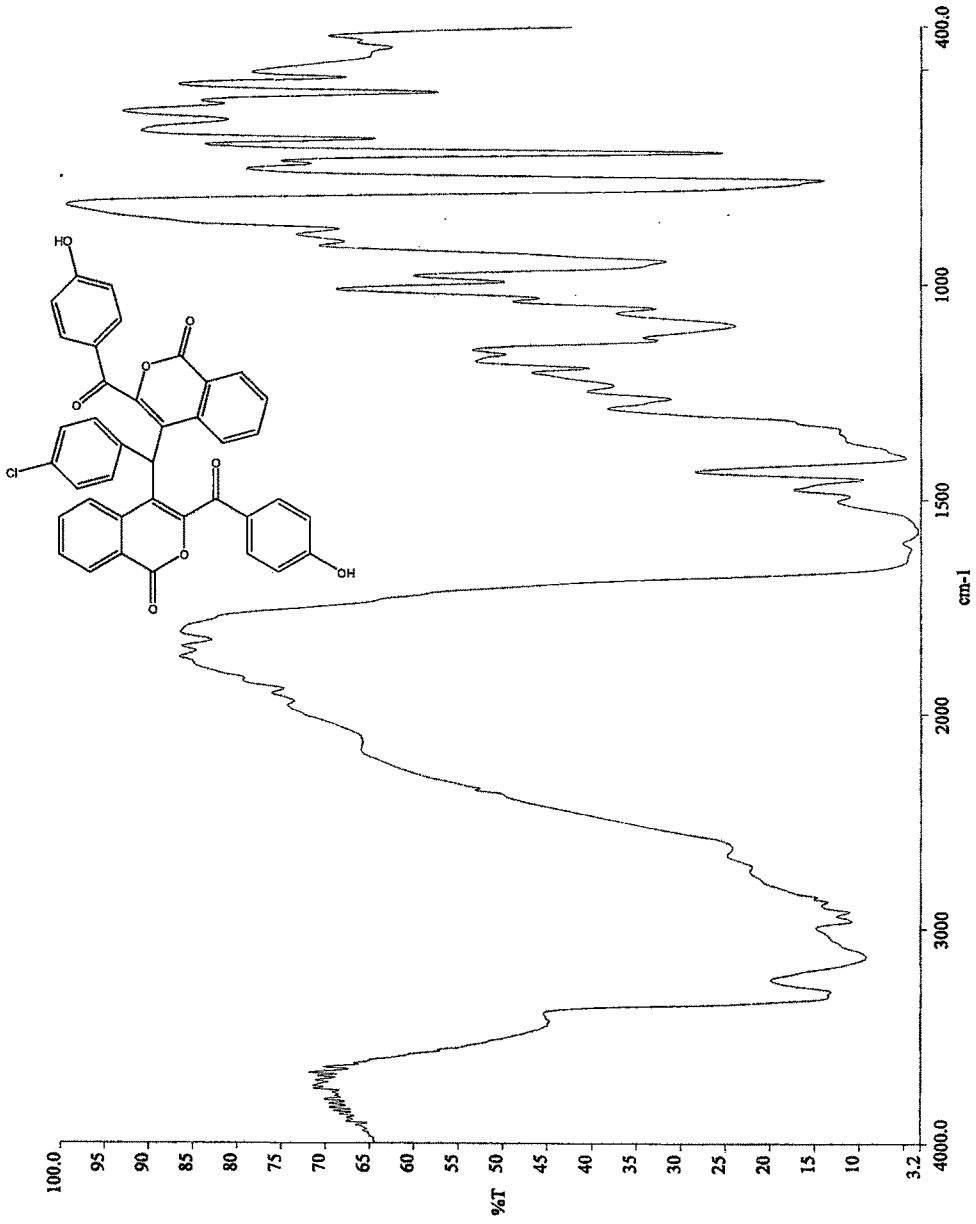


Fig. 3.B.6 – IR: **4 - (4''- Chlorobenzylidene)-bis-3-(4'-hydroxy benzoyl) isocoumarin 5a**

Fig. 3.B.7 – ^{1}H NMR: **4 - (4''- Chlorobenzylidene)-bis-3-(4'-hydroxy benzoyl) isocoumarin 5a**

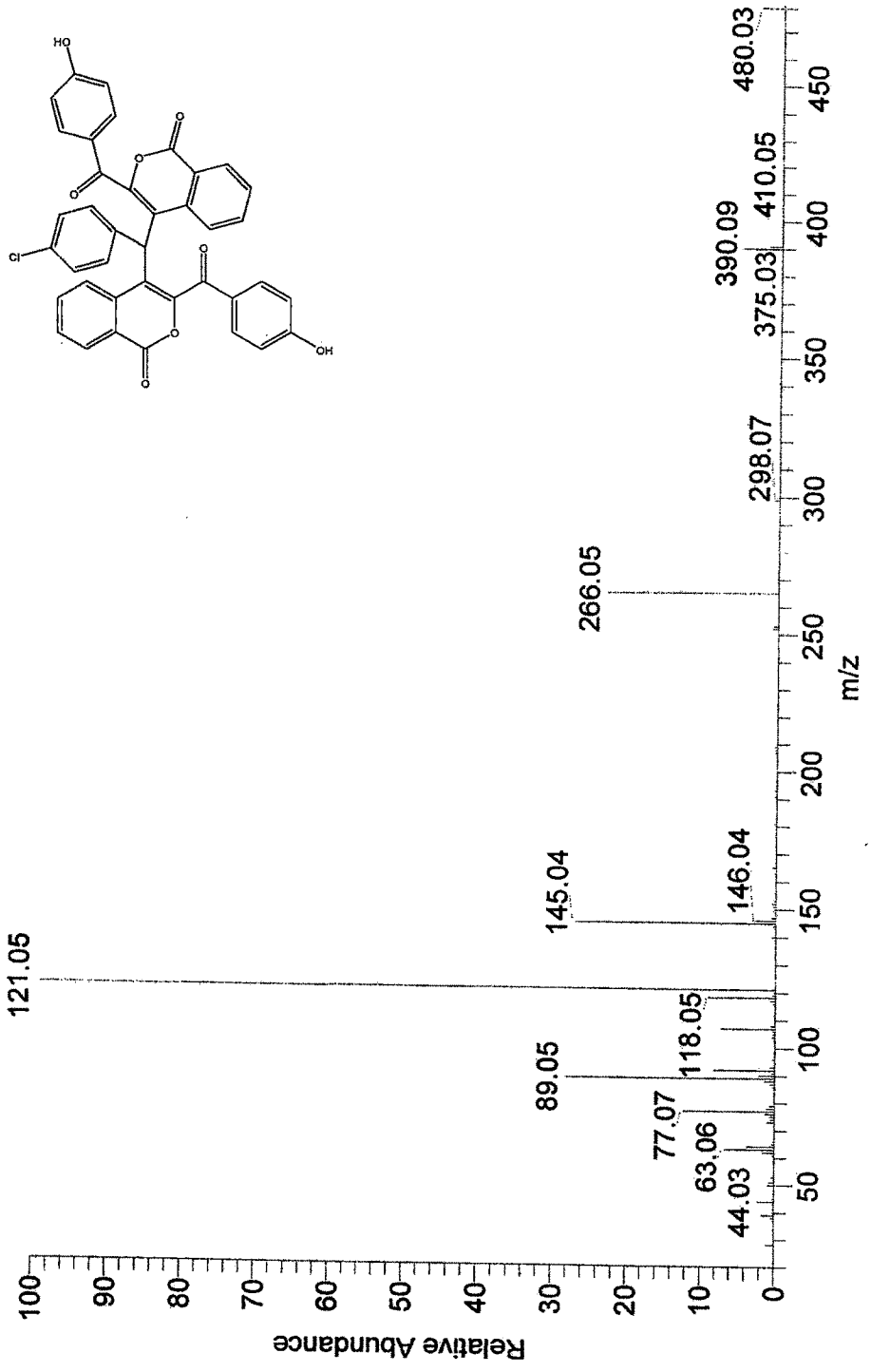
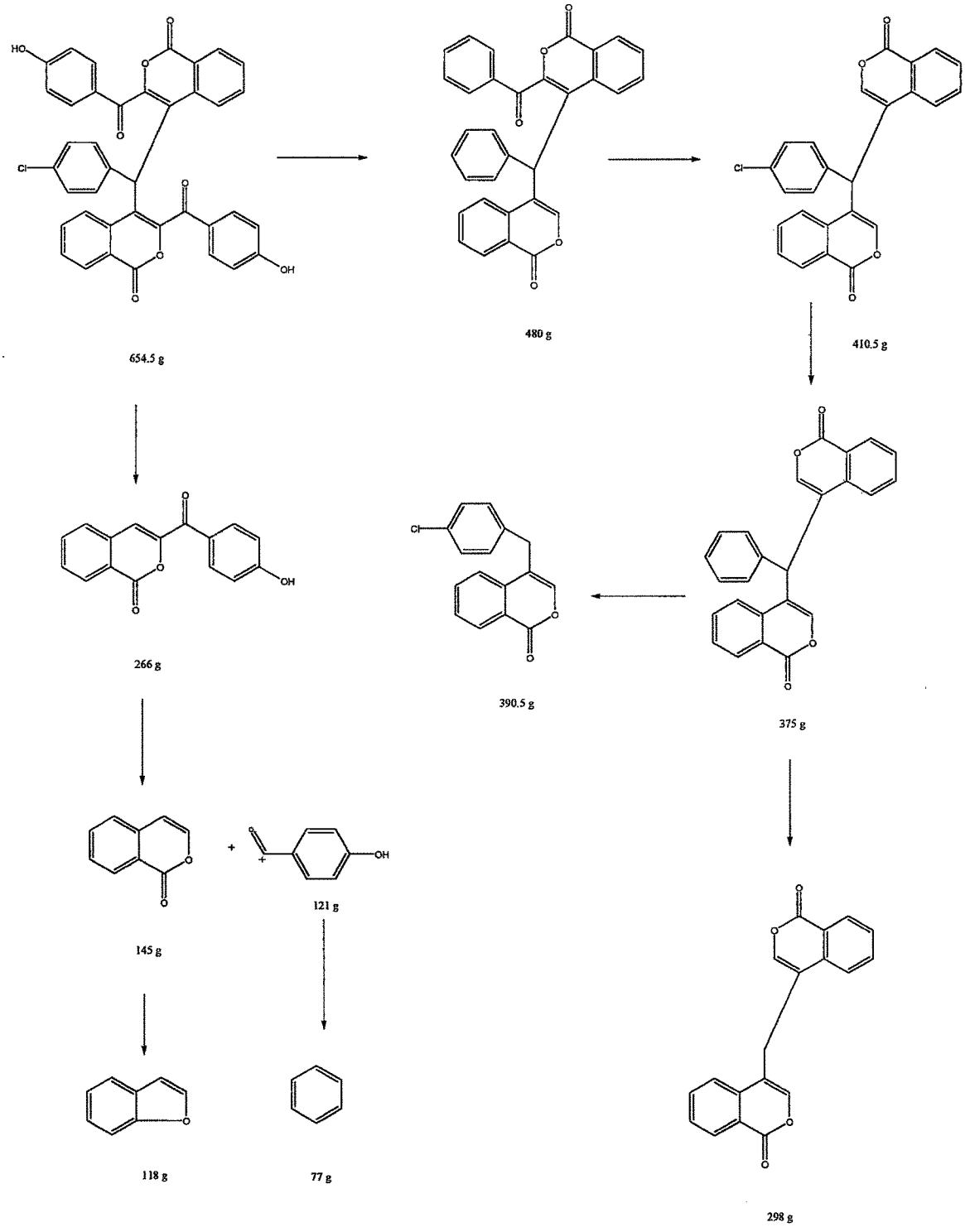


Fig. 3.B.8 – Mass spectrum: **4 - (4''- Chlorobenzylidene)-bis-3-(4'-hydroxy benzoyl) isocoumarin 5a**



Fragmentation Pattern: **4 - (4''- Chlorobenzylidene)-bis-3-(4'-hydroxy benzoyl) isocoumarin**

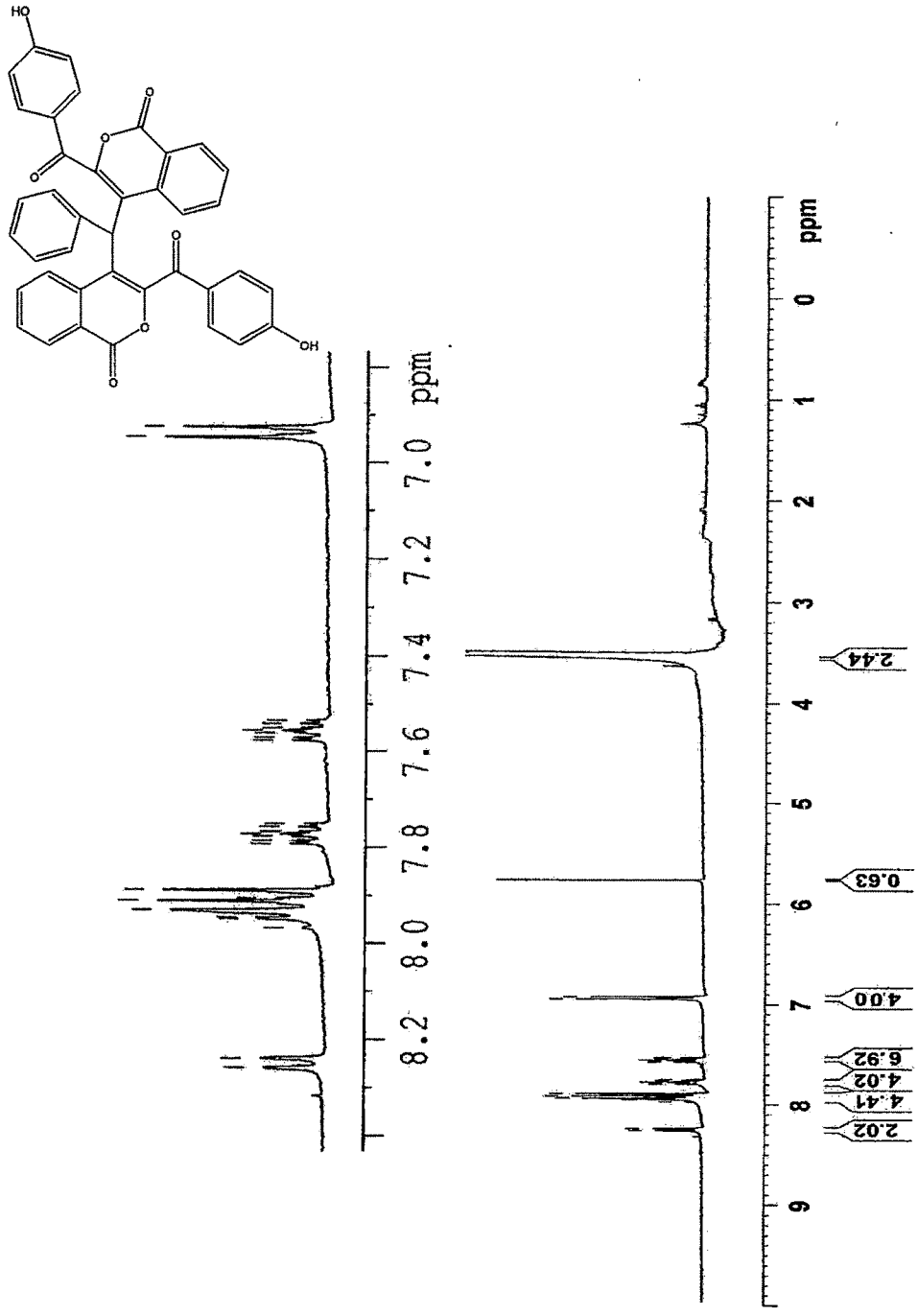


Fig. 3.B.9 – ^{1}H NMR: **4 - (Benzylidene)-bis-3-(4'-hydroxy benzoyl) isocoumarin 5b**

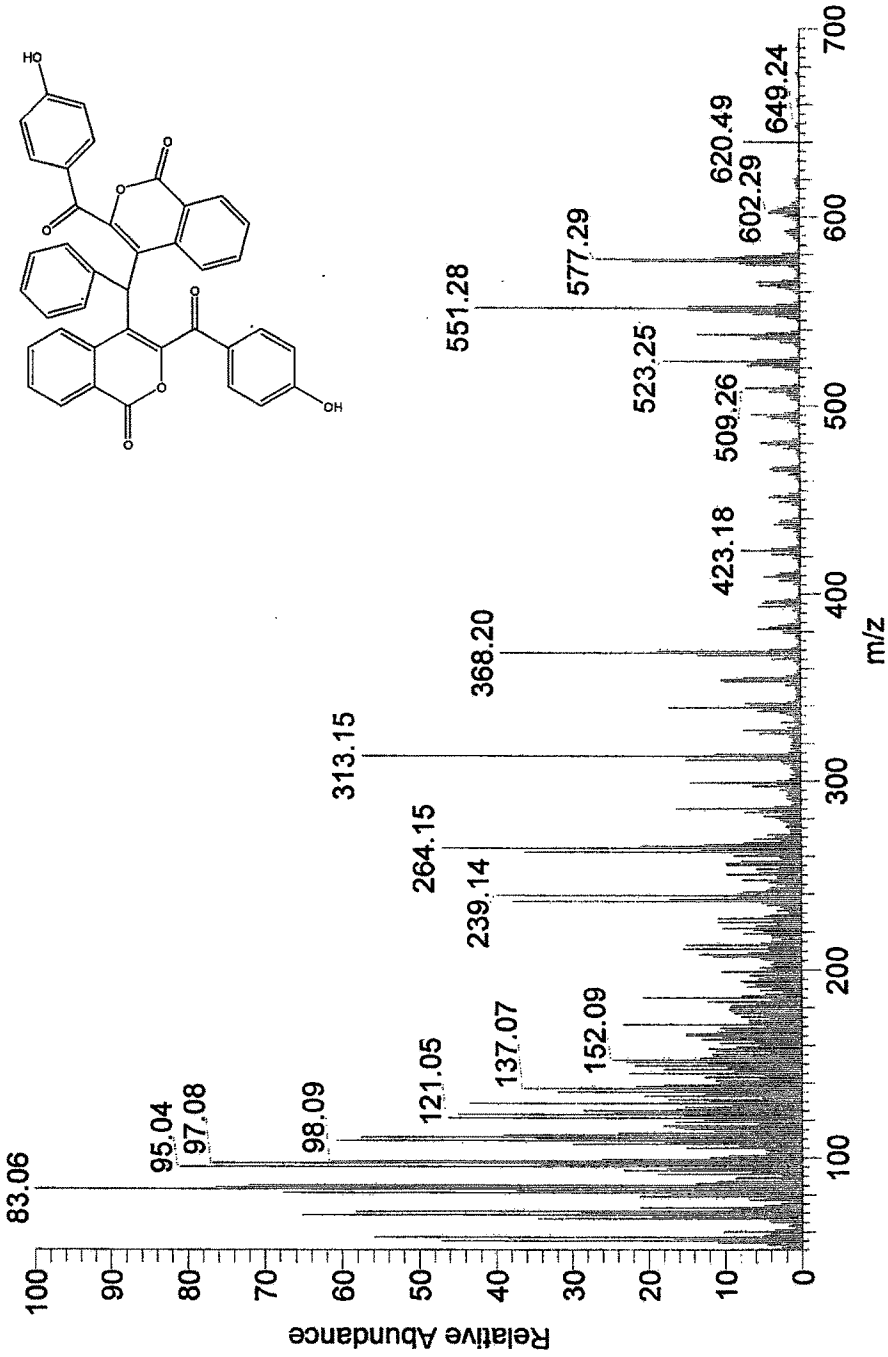
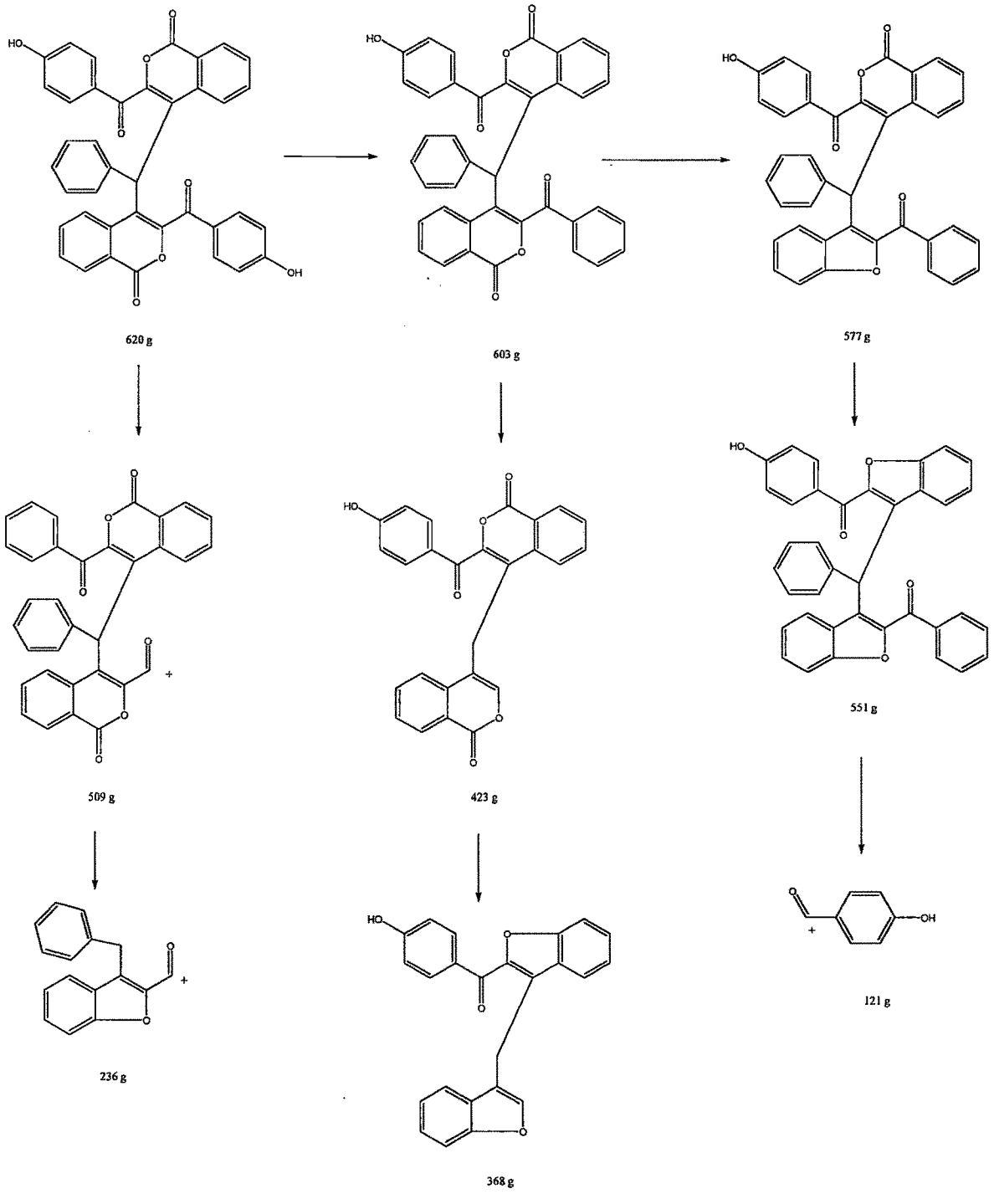


Fig. 3.B.10 – Mass spectrum: **4 - (Benzylidene)-bis-3-(4'-hydroxy benzoyl) isocoumarin 5b**



Fragmentation Pattern: **4 - (Benzylidene)-bis-3-(4'-hydroxy benzoyl) isocoumarin**

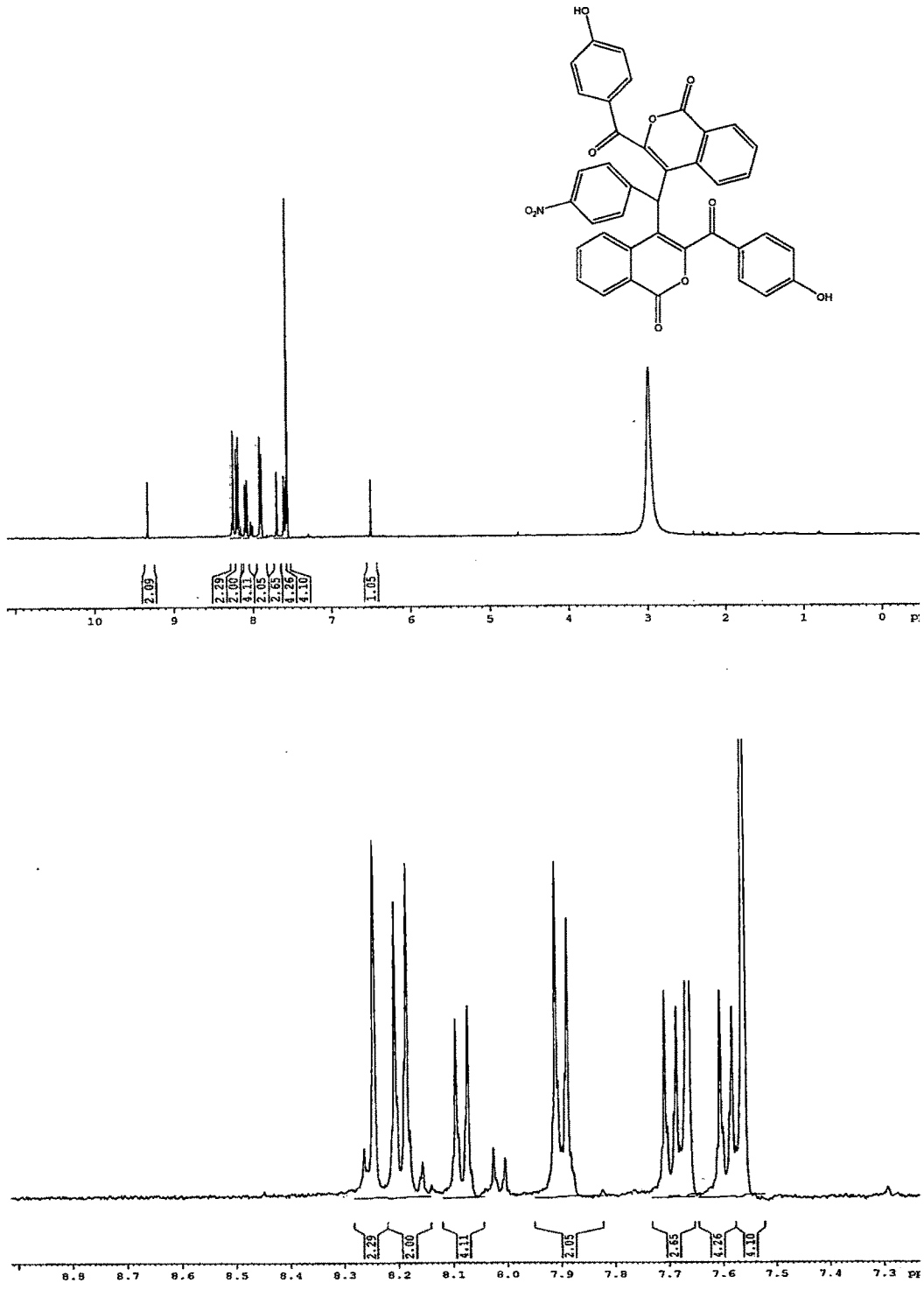


Fig. 3.B.11– ^{1}H NMR: **4 - (4"- Nitrobenzylidene)-bis-3-(4'-hydroxy benzoyl) isocoumarin 5c**

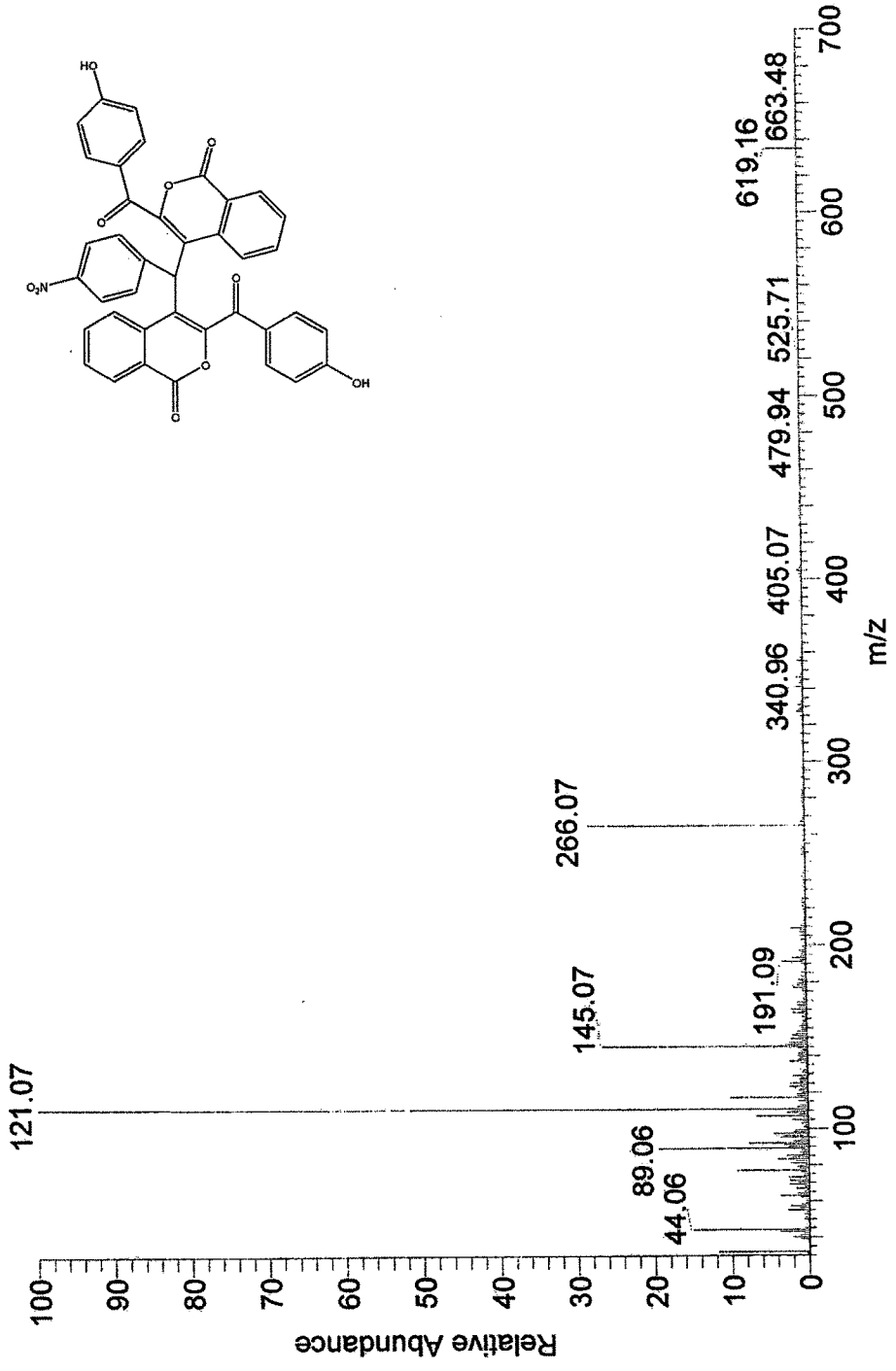
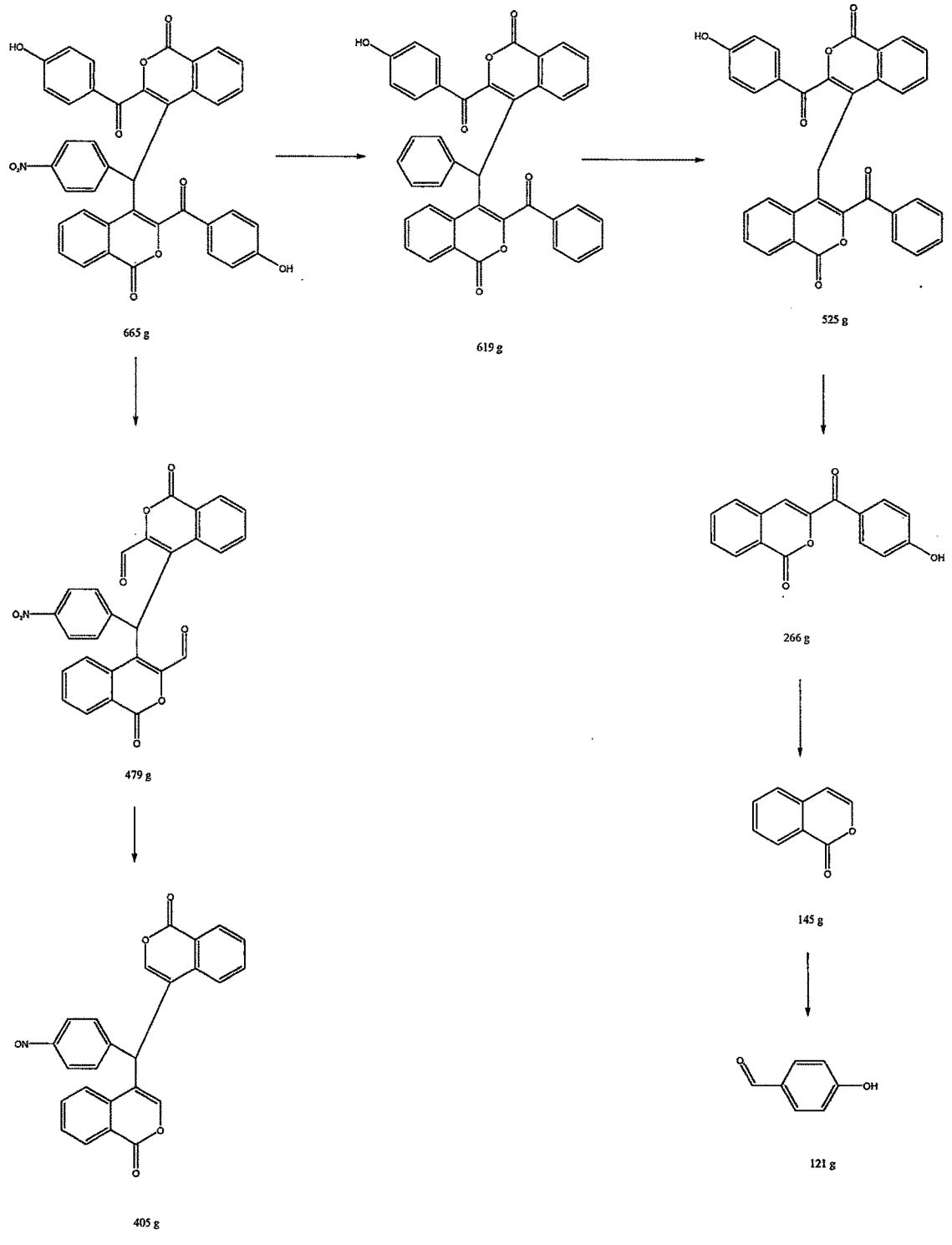
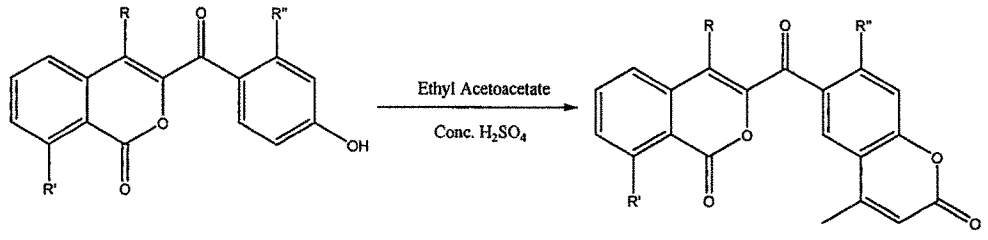


Fig. 3.B.12 – Mass spectrum: **4 - (4"- Nitrobenzylidene)-bis-3-(4'-hydroxy benzoyl) isocoumarin 5c**



Fragmentation Pattern: **4 - (4"- Nitrobenzylidene)-bis-3-(4'-hydroxy benzoyl) isocoumarin**

Scheme II

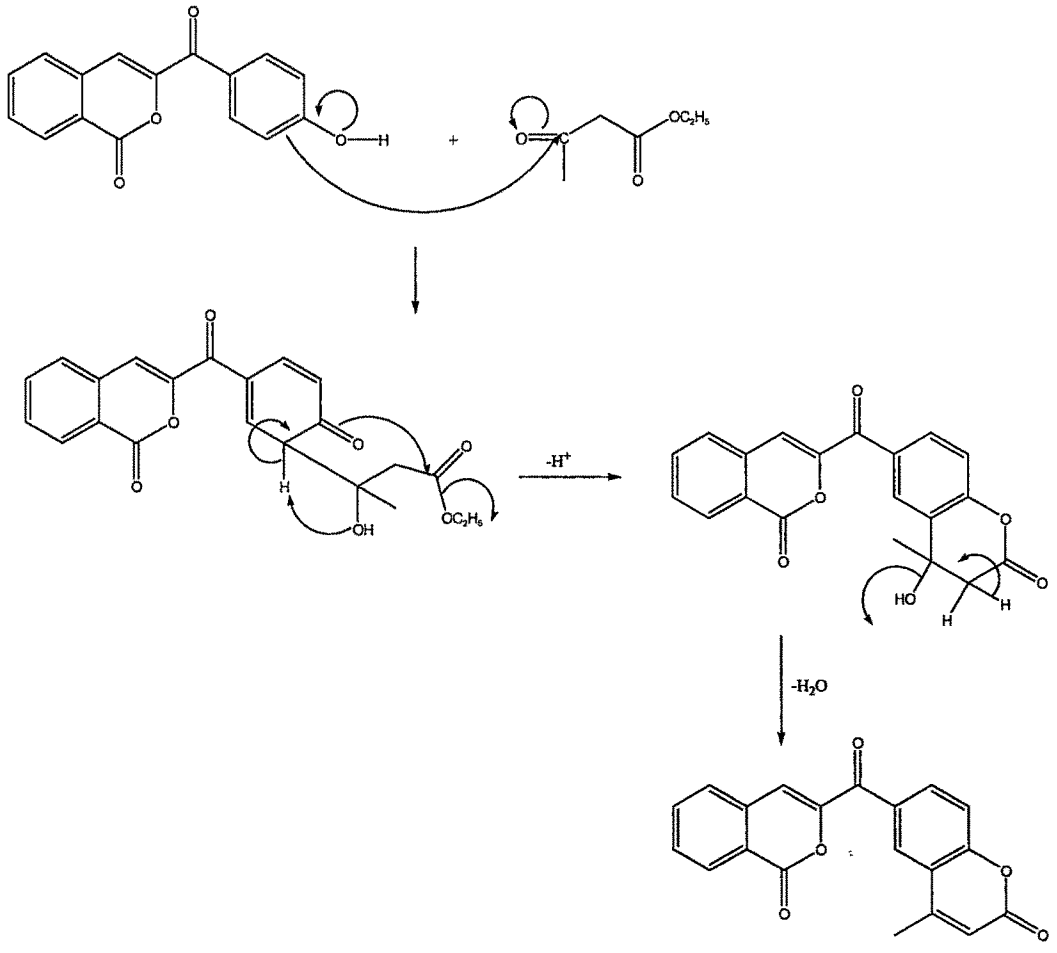


6

7

R= CH_3, R' = H, R" = H (**6a & 7a**)
R= CH_3, R' = H, R" = OH (**6b & 7b**)
R= CH_3, R' = NO_2, R" = H (**6c & 7c**)
R= CH_3, R' = NO_2, R" = OH (**6d & 7d**)
R= C_2H_5, R' = H, R" = H (**6e & 7e**)
R= H, R' = H, R" = H (**6f & 7f**)
R= H, R' = H, R" = OH (**6g & 7g**)

Mechanism: **3-(4'-methyl coumarin-6'-carbonyl) isocoumarin**



Earlier we have synthesized bis isocoumarins and now in this scheme, coumarin moiety was introduced in the isocoumarins. As both the moieties are well known for their biological importance, therefore they were chosen, so that their effect on different biological studies can be seen in presence of each other.

The synthesis of (coumarin -3-carbonyl) - isocoumarins **7** was achieved with an efficient synthetic route outlined in (**Scheme II**). The isocoumarins **6a-g** were prepared by condensing different o-acyl benzoic acids with p-hydroxy bromoacetophenone in presence of anhy. K_2CO_3 using ethyl methyl ketone as solvent, as described in earlier chapetrs. These isocoumarins were then subjected to cyclization by reacting them with ethyl acetoacetate in presence of conc. sulphuric acid (Pal Knorr synthesis), used as a catalyst and solvent both, to get the target compounds **7a-g** in good yield.

IR spectrum of **7a** shows frequencies at 1706.24 cm^{-1} for γ lactone and at 1604.26 cm^{-1} for –C=O (**Fig. 3.B.13**).

The signals obtained in the ^1H NMR spectrum of **7a** are δ 2.3 (s, 3H, C_4' –H), 2.9 (s, 3H, C_4-H), 6.8-8.1 (m, 7H, aromatic protons), 8.2 (d, 1H, C_8-H) (**Fig. 3.B.14**) and **7b** are δ 2.3 (s, 3H, C_4' –H), 2.9 (s, 3H, C_4-H), 6.8-8.1 (m, 7H, aromatic protons), 8.2 (d, 1H, C_8-H), 9.0 (s, 1H, OH) (**Fig. 3.B.16**).

Mass spectra of **7a** and **7b** shows m/z peaks at 346 (M^+), 187, 159 and 145 (**Fig. 3.B.15**), 362 (M^+), 287, 259, 203, 187 and 146 (**Fig. 3.B.17**) respectively.

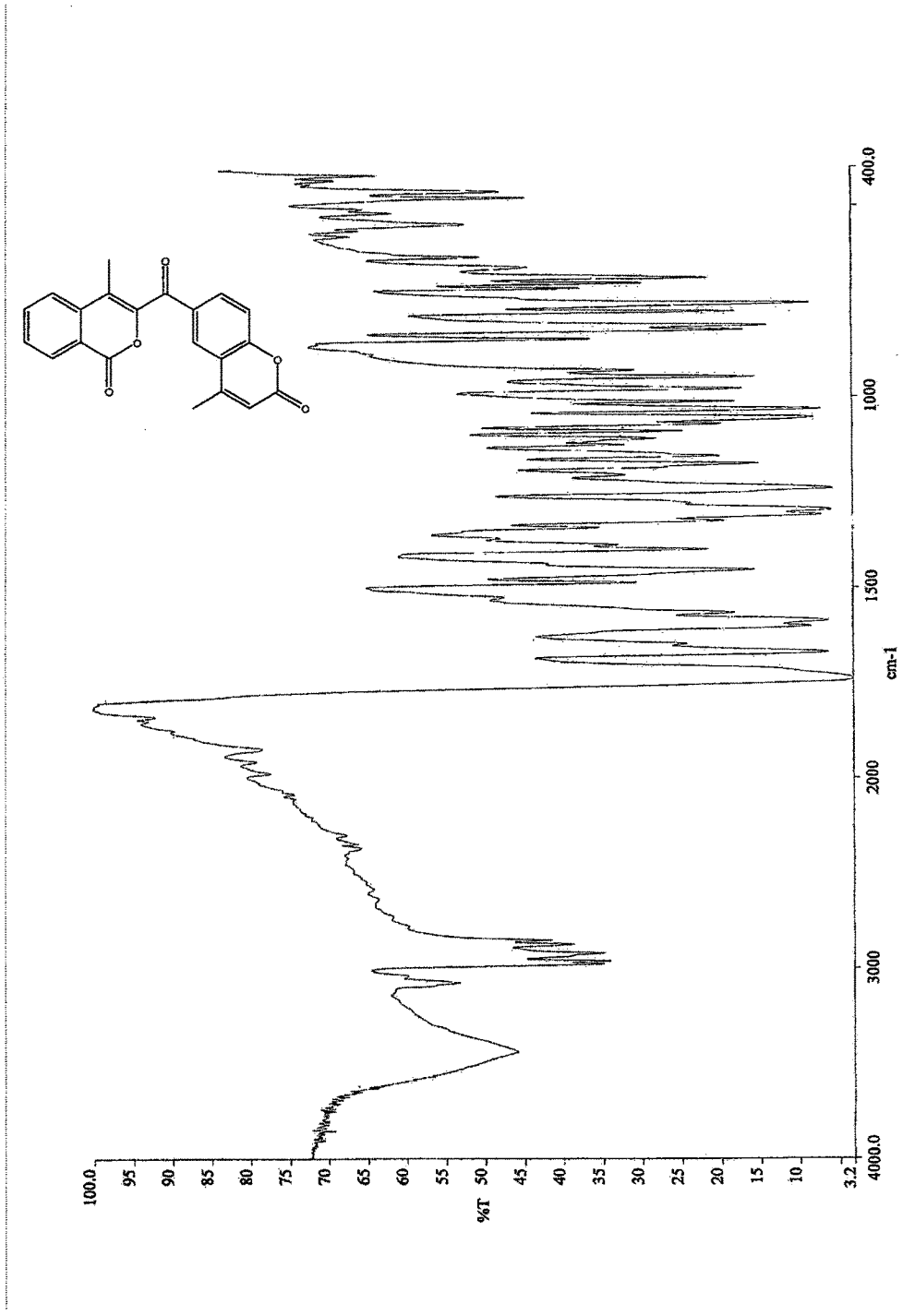
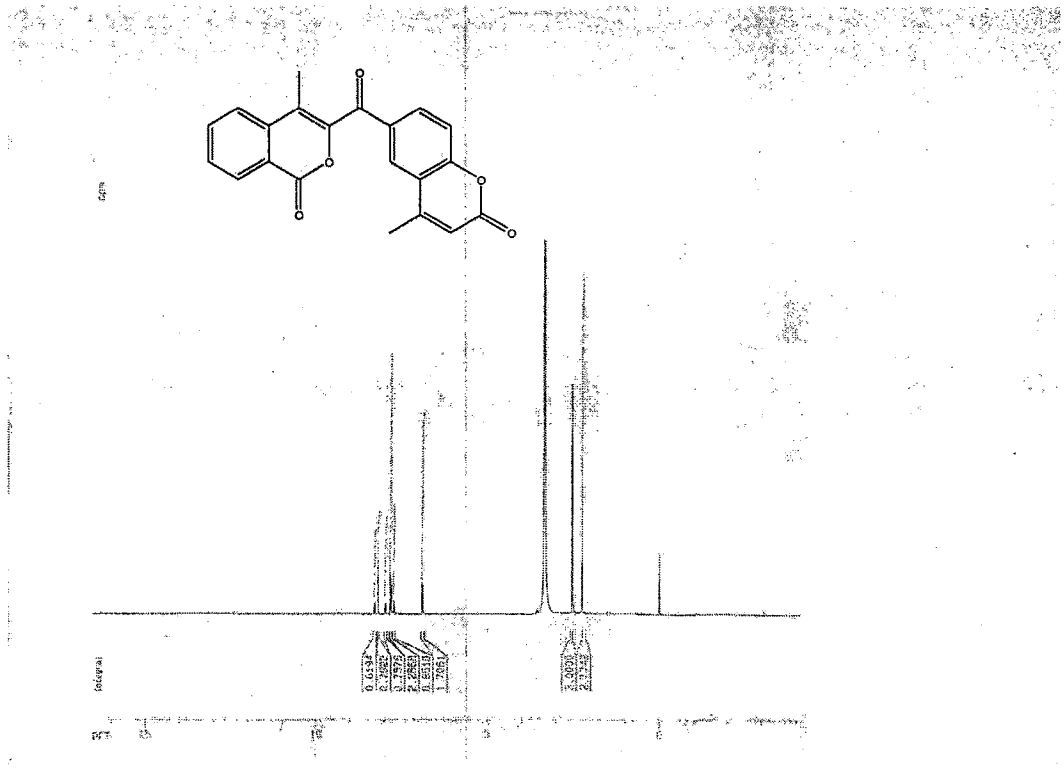
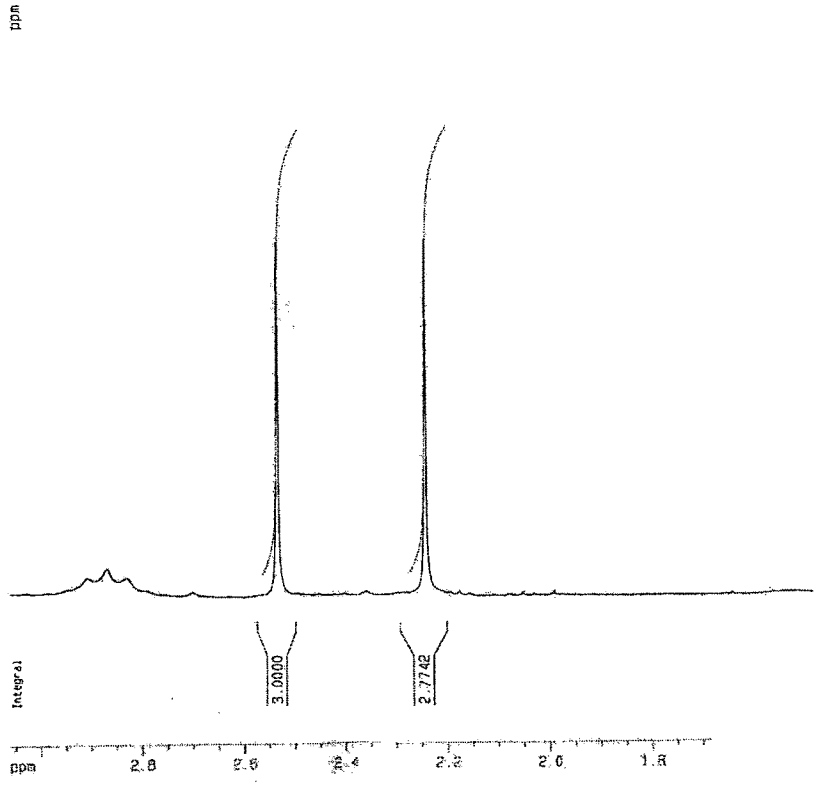


Fig. 3.B.13 – IR: **4-Methyl-3-(4'-methyl coumarin-6'-carbonyl) isocoumarin 7a**

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Integral
ppm
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2.6
2.4
2.2
2.0
1.8

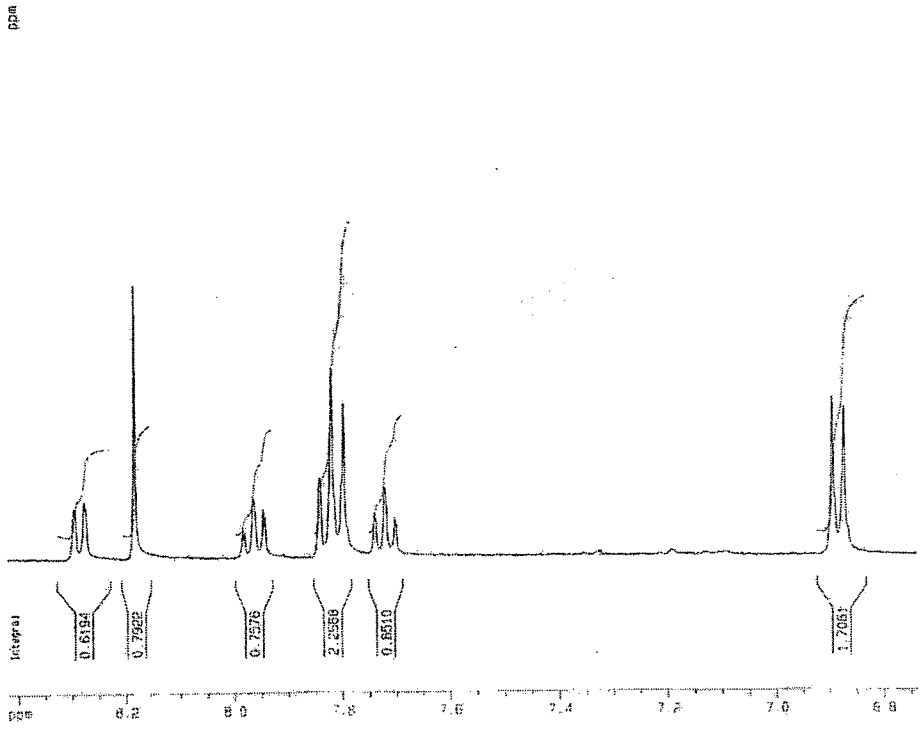


Fig. 3.B.14 – ^{1}H NMR : 4-Methyl-3-(4'-methyl coumarin-6'-carbonyl) isocoumarin 7a

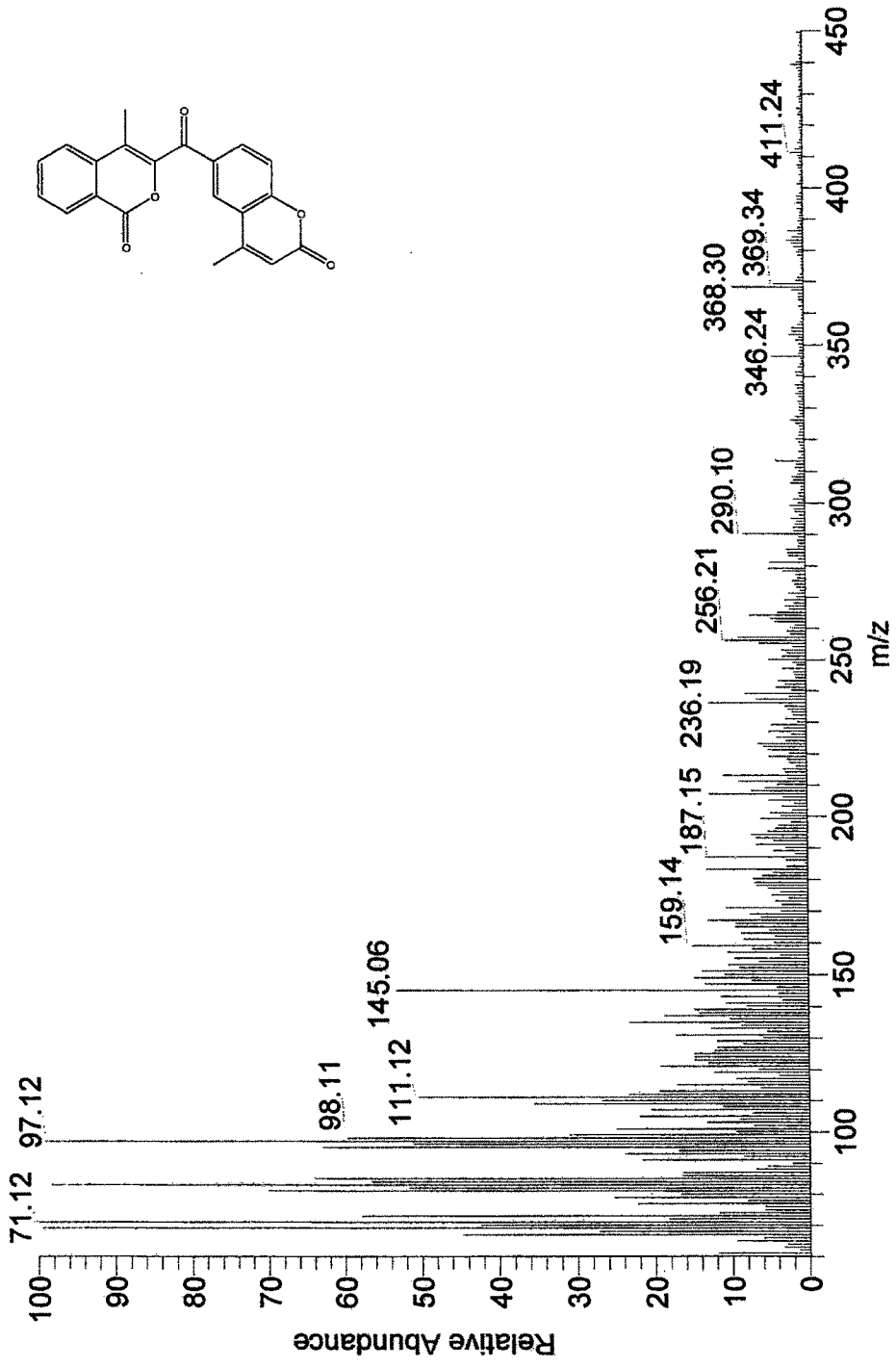
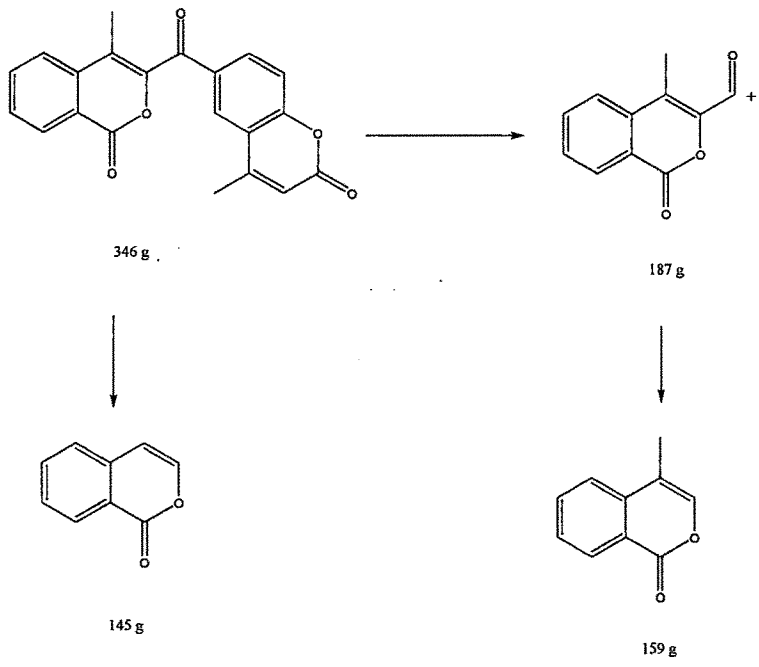
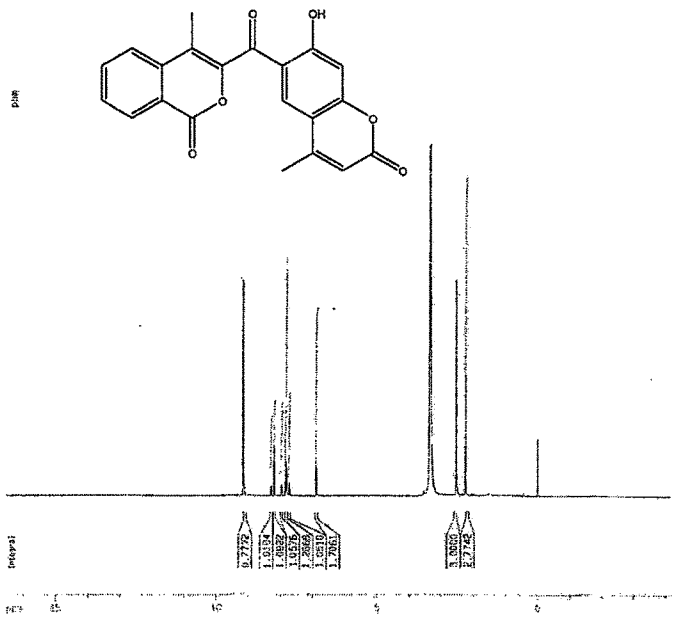


Fig. 3.B.15 – Mass spectrum: **4-Methyl-3-(4'-methyl coumarin-6'-carbonyl) isocoumarin 7a**



Fragmentation Pattern: **4-Methyl-3-(4'-methyl coumarin-6'-carbonyl) isocoumarin**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O
O
O
O
ppm
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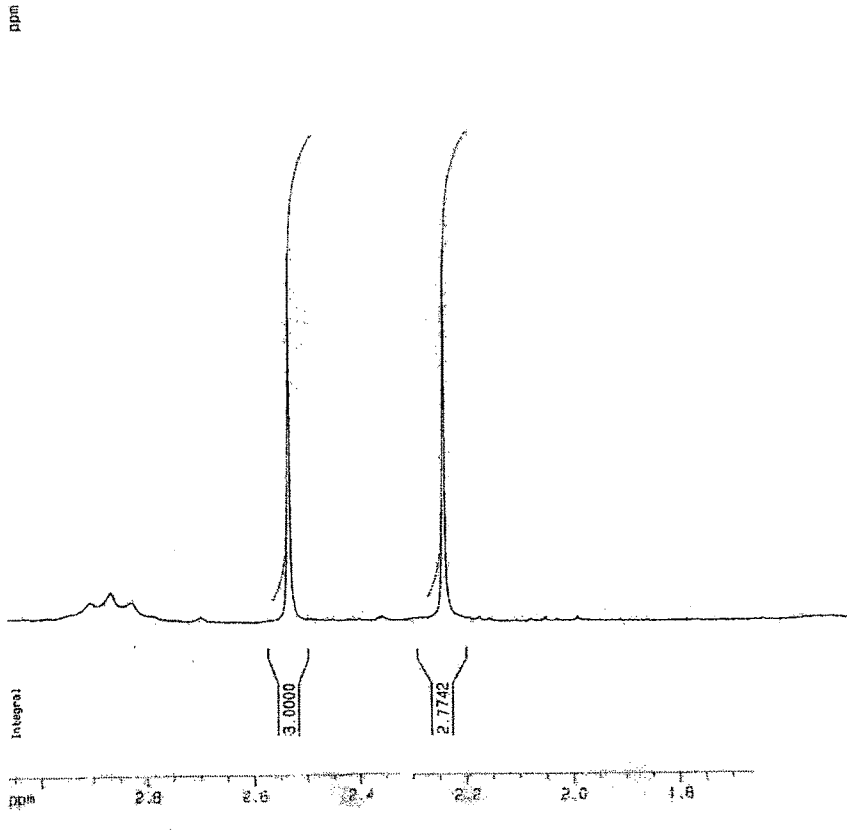
1.0510
1.7065
3.0000
2.7742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ppm
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2.6
2.4
2.2
2.0
1.8

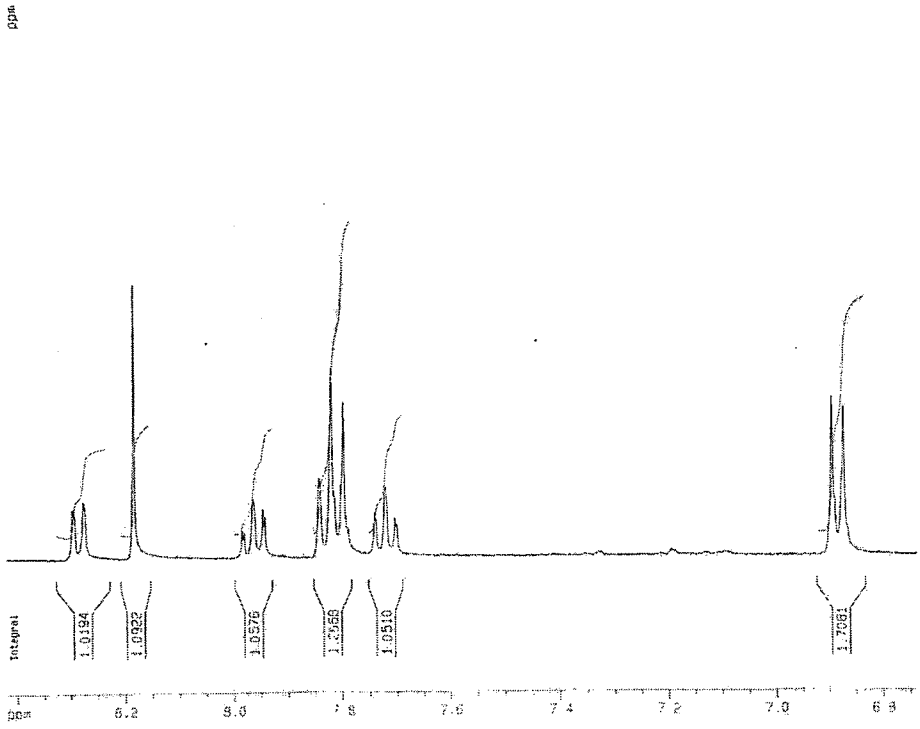


Fig. 3.B.16 – ^{1}H NMR : **4-Methyl-3-(4'-methyl-7'-hydroxy coumarin-6'-carbonyl) isocoumarin 7b**

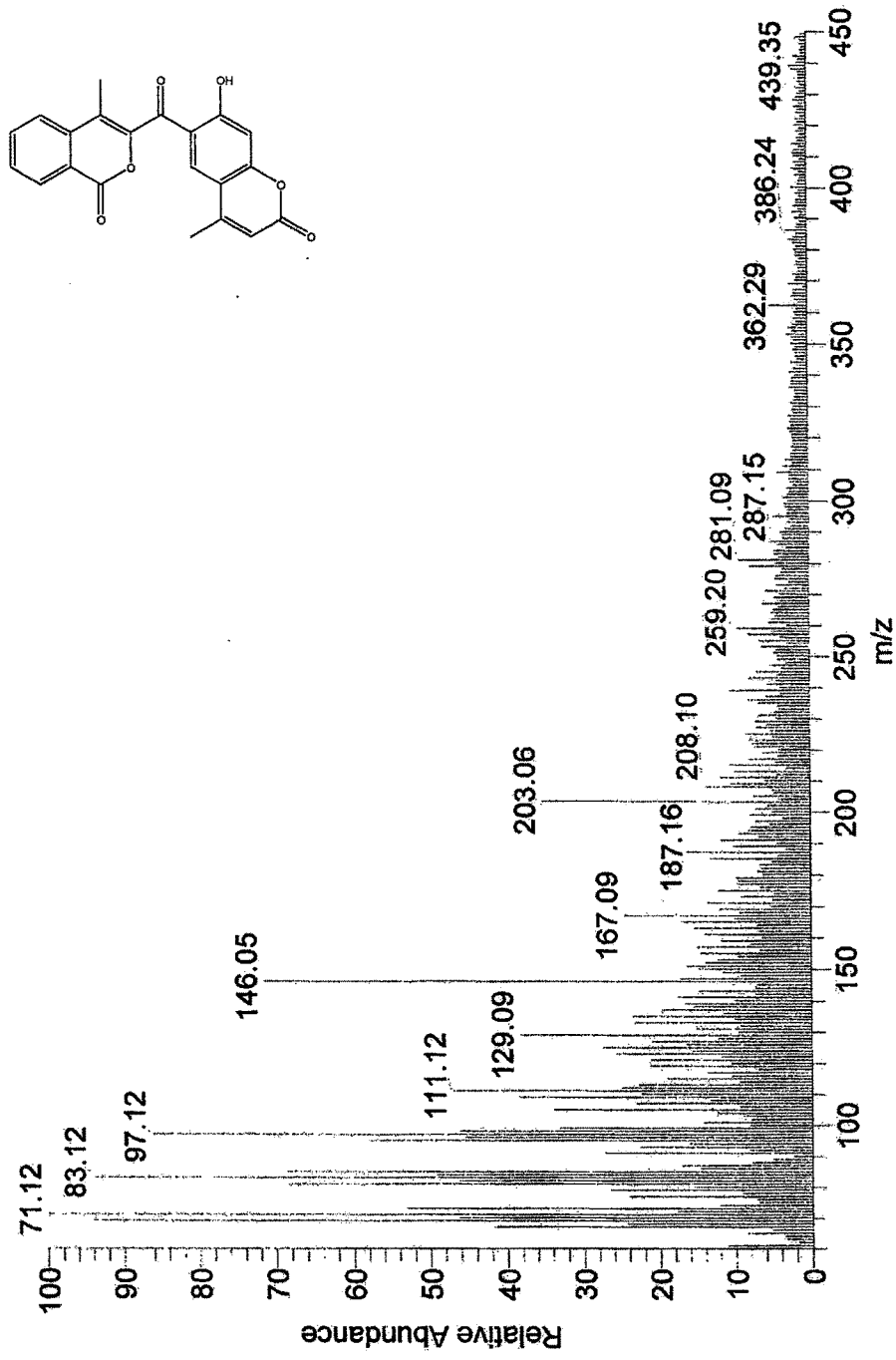
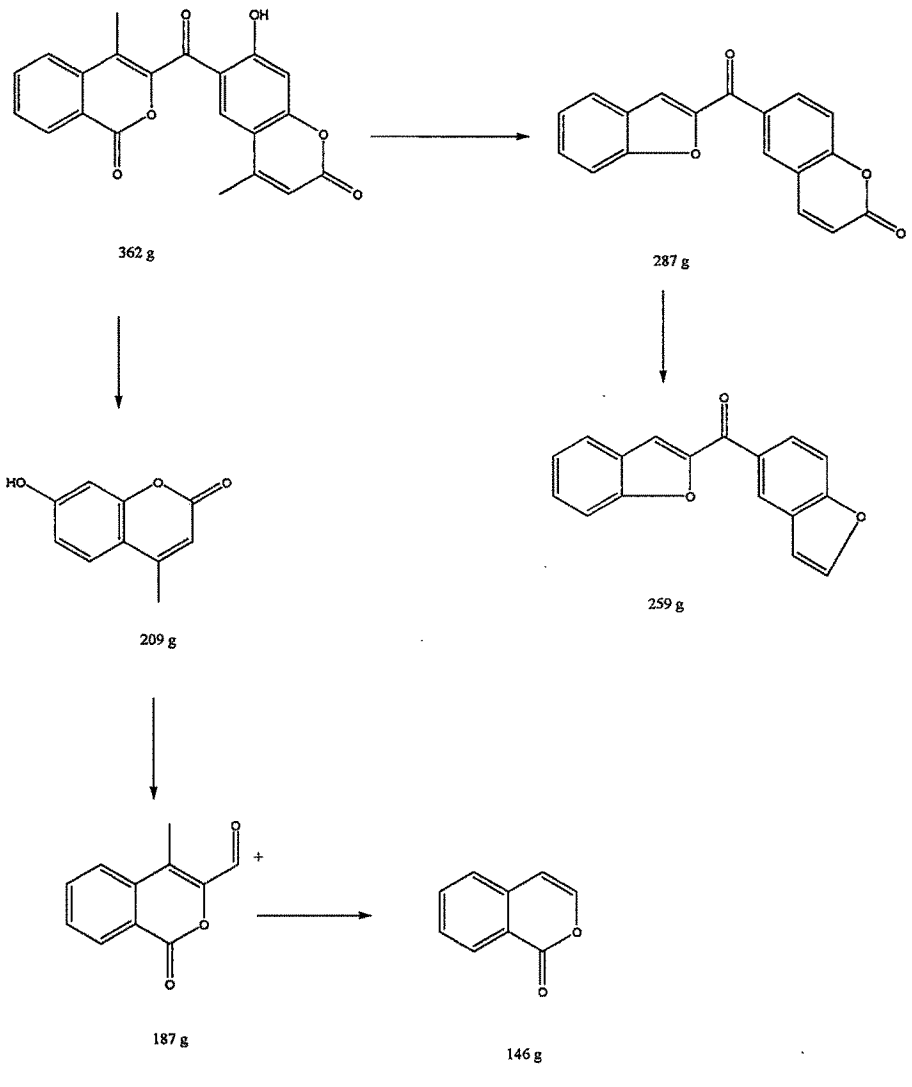


Fig. 3.B.17 – Mass spectrum : **4-Methyl-3-(4'-methyl-7'-hydroxy coumarin-6'-carbonyl) isocoumarin 7b**



Fragmentation Pattern: **4-Methyl-3-(4'-methyl-7'-hydroxy coumarin-6'-carbonyl) isocoumarin**

3. B.3 EXPERIMENTAL

The reagents and the solvents used in this study were of analytical grade and were used without further purification. Melting points were determined in open capillaries and are uncorrected. The purity of the compounds was checked by TLC on silica gel GF254 plates using UV/Iodine as visualizing agent and Merk's silica gel (60-120 mesh) was used for column chromatographic purification. Infrared spectra were recorded on FTIR Perkin Elmer spectrophotometer using potassium bromide optics. ^{1}H NMR spectra were recorded on a Bruker spectrometer (400 MHz) using TMS as internal standard and chemical shifts are given in ppm. Mass spectrums were obtained using Thermo Scientific Corporation, DSQ II Mass Spectrometer. Elemental analyses were carried out on Perkin-Elmer C, H, N, S analyzer (Model-2400). Bromo acetophenone derivatives were prepared by literature method[15].

General procedure for 3a-3f

2-carboxy benzaldehyde (1 g, 0.006 mole) **1**, 4-bromo bromoacetophenone (1.85 g, 0.006 mole) **2**, K_2CO_3 (2.00 g, 0.012 mole) and ethyl methyl ketone were taken in a round bottom flask and refluxed for 10-12 hrs. Solvent was then removed, water added and extracted with ethyl acetate. Solvent layer was first washed with sod. bicarbonate and then with water and dried over anhy. Na_2SO_4. After removal of solvent the crude product was purified by column chromatography using petroleum ether (60-80^{0}C)-ethyl acetate to yield white crystals of **3a**

3-(4'-Bromo benzoyl) isocoumarin 3a

This compound was obtained as white crystals, mp: 171^{0}C; 76.05% yield; Anal. Calcd $C_{16}H_9O_3Br$ (328.9 g): C, 58.37; H, 2.73; Found: C, 58.42; H, 3.06; ^{1}H NMR δ 7.4 (s, 1H, C_4-H), 7.6-7.9 (m, 7H, aromatic protons), 8.4 (dd, 1H, C_8-H); ms: m/z: 328.97 (M^+), 249, 174 and 146.

3-(4'-Hydroxy benzoyl) isocoumarin 3b

This compound was obtained as white crystals, mp: 180^0C; 64.21% yield; Anal. Calcd $C_{16}H_{10}O_4$ (266.0 g): C, 72.18; H, 3.75; Found: C, 72.00; H, 3.18; ^{1}H NMR δ 5.5 (s, 1H, OH), 7.6 (s, 1H, C_4-H), 7.0-7.8 (m, 7H, aromatic protons), 8.3 (d, 1H, C_8-H); ms: m/z: 266.04 (M^+), 185, 145 and 121.

3-(2', 4'-Hydroxy benzoyl) isocoumarin 3c

This compound was obtained as pinkish white crystals, mp: 116^0C; 44.69% yield; Anal. Calcd $C_{16}H_{10}O_5$ (282.0 g): C, 68.08; H, 3.54; Found: C, 68.37; H, 4.08; ^{1}H NMR δ 5.5 (s, 1H, OH), 5.8 (s, 1H, OH), 7.5 (s, 1H, C_4-H), 6.7-7.7 (m, 6H, aromatic protons), 8.2 (d, 1H, C_8-H); ms: m/z: 282 (M^+), 264, 238, 173, 146, 137 and 57.

3-(4'-Methoxy benzoyl) isocoumarin 3d

This compound was obtained as yellow crystals, mp: 134^0C; 62.84% yield; Anal. Calcd $C_{17}H_{12}O_4$ (280.0 g): C, 72.85; H, 4.28; Found: C, 73.24; H, 3.94; ^{1}H NMR δ 3.9(s, 3H, OCH_3), 7.4 (s, 1H, C_4-H), 7.6-7.9 (m, 7H, aromatic protons), 8.4 (dd, 1H, C_8-H); ms: m/z: 280 (M^+), 252, 249, 145, 135 and 118.

3-(4'-Phenyl benzoyl) isocoumarin 3e

This compound was obtained as white crystals, mp: 64^0C; 65.15% yield; Anal. Calcd $C_{22}H_{14}O_3$ (326.0 g): C, 80.98; H, 4.29; Found: C, 81.29; H, 4.31; ^{1}H NMR δ 7.4 (s, 1H, C_4-H), 7.3-7.9 (m, 12H, aromatic protons), 8.4 (dd, 1H, C_8-H); ms: m/z: 325 (M^+ -1), 300, 272, 221, 195, 174 and 146.

3-Dibenzofuroyl isocoumarin 3f

This compound was obtained as white crystals, mp: 110^0C; 65.63% yield; Anal. Calcd $C_{22}H_{12}O_4$ (340.0 g): C, 77.64; H, 3.52; Found: C, 77.51; H, 4.01; ^{1}H NMR δ 7.7 (s, 1H, C_4-H), 7.2-8.0 (m, 10H, aromatic protons), 8.4 (d, 1H, C_8-H); ms: m/z: 340 (M^+), 312, 174, 168 and 146.

General procedure for 5a-5m

A mixture of 3- aroyl isocoumarin **3b** (1g, 0.0037 mole) was dissolved in ethanol and heated on water bath to get a clear solution. p- Chlorobenzaldehyde **4** (0.264g, 0.0018 mole) was added to this hot solution and refluxed for 18-20 hrs. After the reaction was over, the solvent was distilled off and product recrystallised from ethanol to yield pale white crystals of **5a**

4 - (4''- Chlorobenzylidene)-bis-3-(4'-hydroxy benzoyl) isocoumarin 5a

This compound was obtained as pale white crystals, mp: 200^{0}C; 74.98% yield; Anal. Calcd $C_{39}H_{23}O_8Cl$ (654.5 g): C, 71.61; H, 3.51; Found: C, 71.95; H, 3.14; ^{1}H NMR δ 5.7 (s, 1H, CH), 6.9-8.1(m, 18H, aromatic protons), 8.4 (d, 2H, C_8-H) 10.0 (s, 2H, OH); ms: m/z: 480, 410, 390, 375, 298, 266, 145, 121, 118 and 77.

4 - (Benzylidene)-bis-3-(4'-hydroxy benzoyl) isocoumarin 5b

This compound was obtained as white crystals, mp: 197^{0}C; 70.00% yield; Anal. Calcd $C_{39}H_{24}O_8$ (620.0 g): C, 75.48; H, 3.87; Found: C, 75.03; H, 4.17; ^{1}H NMR δ 3.6 (s, 2H, OH), 5.8 (s, 1H, CH), 6.9-8.0 (m, 19H, aromatic protons), 8.2 (d, 2H, C_8-H); ms: m/z: 620 (M^+), 603, 577, 551, 509, 423, 368, 264 and 121.

4 - (4"- Nitrobenzylidene)-bis-3-(4'-hydroxy benzoyl) isocoumarin 5c

This compound was obtained as white crystals, mp: 198^{0}C; 68.27% yield; Anal. Calcd $C_{39}H_{23}O_{10}N$ (665.0 g): C, 70.37; H, 3.45; N, 2.10; Found: C, 70.71; H, 3.92; N, 2.53; ^{1}H NMR δ 6.7 (s, 1H, CH), 7.5-8.2(m, 18H, aromatic protons), 8.3 (d, 2H, C_8-H) 9.3 (s, 2H, OH); ms: m/z: 663 (M^+ - 2), 619, 525, 479, 405, 266, 145 and 121.

4 - (4"- Methoxybenzylidene)-bis-3-(4'-hydroxy benzoyl) isocoumarin 5d

This compound was obtained as yellow crystals, mp: 200^{0}C; 72.03% yield; Anal. Calcd $C_{40}H_{26}O_9$ (650.0 g): C, 73.84; H, 4.00; Found: C, 74.21; H, 4.38; ^{1}H NMR δ 3.8 (s, 3H, OCH_3), 7.4 (s, 1H, CH), 7.3-8.0 (m, 18H, aromatic protons), 8.3 (d, 2H, C_8-H) 8.7 (s, 2H, OH); ms: m/z: 649 (M^+ -1), 616, 588, 560, 529, 406, 383, 329, 280, 145 and 135.

4 - (4"- Nitrobenzylidene)-bis-3-(4'-bromo benzoyl) isocoumarin 5e

This compound was obtained as yellow crystals, mp: 144^{0}C; 68.27% yield; Anal. Calcd $C_{39}H_{21}O_8NBr_2$ (776.8 g): C, 59.18; H, 2.65; N, 1.77; Found: C, 58.97; H, 2.74; N, 2.05; ^{1}H NMR δ 7.5 (s, 1H, CH), 7.3-8.1 (m, 18H, aromatic protons), 8.44 (d, 2H, C_8-H); ms: m/z: 776.8 (M^+), 617, 571, 540, 447.9, 435, 407, 328.9, 187, 183.9, 146, and 77.

4 - (4"- Hydroxybenzylidene)-bis-3-(4'-bromo benzoyl) isocoumarin 5f

This compound was obtained as yellow crystals, mp: 150^{0}C; 69.00% yield; Anal. Calcd $C_{39}H_{22}O_7Br_2$ (761.8 g): C, 61.43; H, 2.88; Found: C, 61.72; H, 3.12; ^{1}H NMR δ 6.0 (s, 1H, OH), 7.5 (s, 1H, CH), 7.3-8.1 (m, 18H, aromatic protons), 8.3 (d, 2H, C_8-H); ms: m/z: 761.8 (M^+), 602, 585, 508, 432.9, 392, 183.9, 146 and 118.

4 - (Benzylidene)-bis-3-(4'-bromo benzoyl) isocoumarin 5g

This compound was obtained as white crystals, mp: 176^{0}C; 62.76% yield; Anal. Calcd $C_{39}H_{22}O_6Br_2$ (745.8 g): C, 62.75; H, 2.94; Found: C, 63.18; H, 3.36; ^{1}H NMR δ 7.4 (s, 1H, CH), 7.4-8.1 (m, 18H, aromatic protons), 8.45 (d, 2H, C_8-H); ms: m/z: 745.8 (M^+), 665.9, 509, 481, 376, 483.9, 416.9, 328.9, 183.9 and 146.

4 - (4"- Nitrobenzylidene)-bis-3-(4'-methoxy benzoyl) isocoumarin 5h

This compound was obtained as yellow crystals, mp: 128^{0}C; 54.39% yield; Anal. Calcd $C_{41}H_{27}O_{10}N$ (693.0 g): C, 70.99; H, 3.89; N, 2.02; Found: C, 71.23; H, 4.07; N, 1.94; ^{1}H NMR δ 3.7 (s, 6H, OCH_3), 7.55 (s, 1H, CH), 7.0-7.9 (m, 18H, aromatic protons), 8.2 (d, 2H, C_8-H); ms: m/z: 693 (M^+), 662, 525, 479, 405, 280, 145 and 135.

4 - (4"- Hydroxybenzylidene)-bis-3-(4'-methoxy benzoyl) isocoumarin 5i

This compound was obtained as yellow crystals, mp: 90^{0}C; 61.75% yield; Anal. Calcd $C_{41}H_{28}O_9$ (664.0 g): C, 74.09; H, 4.21; Found: C, 73.86; H, 4.65; ^{1}H NMR δ 3.9 (s, 6H, OCH_3), 7.3 (s, 1H, CH), 7.0-7.7 (m, 18H, aromatic protons), 8.3 (dd, 2H, C_8-H) 12.7 (s, 1H, OH); ms: m/z: 662 (M^+ - 2), 647, 585, 508, 497, 384, 392, 145 and 135.

4 - (4"- Methoxybenzylidene)-bis-3-(4'-methoxy benzoyl) isocoumarin 5j

This compound was obtained as yellow crystals, mp: 134^{0}C; 60.00% yield; Anal. Calcd $C_{42}H_{30}O_9$ (678.0 g): C, 74.33; H, 4.42; Found: C, 74.47; H, 4.68; ^{1}H NMR δ 4.1 (s, 9H, OCH_3), 7.45 (s, 1H, CH), 7.3-7.8 (m, 18H, aromatic protons), 8.4 (d, 2H, C_8-H); ms: m/z: 678 (M^+), 398, 280, 146, 135 and 118.

4 - (4"- Nitrobenzylidene)-bis-3-(2', 4'-dihyroxy benzoyl) isocoumarin 5k

This compound was obtained as yellow crystals, mp: 75^{0}C; 34.48% yield; Anal. Calcd $C_{39}H_{23}O_{12}N$ (697.0 g): C, 67.14; H, 3.29; N, 2.00; Found: C, 66.29; H, 3.57; N, 1.84; ^{1}H NMR δ 7.4 (s, 1H, CH), 6.8-8.1 (m, 16H, aromatic protons), 8.4 (d, 2H, C_8-H), 11.6 (s, 2H, OH), 11.8 (s, 2H, OH) ; ms: m/z: 698 (M^++1), 680, 617, 558, 540, 453, 415, 264, 173, 146 and 137.

4 - (4"- Hydroxybenzylidene)-bis-3-(2', 4'-dihydroxy benzoyl) isocoumarin 5l

This compound was obtained as white crystals, mp: 126^{0}C; 38.72% yield; Anal. Calcd $C_{39}H_{25}O_{11}$ (669.0 g): C, 69.95; H, 3.73; Found: C, 70.21; H, 3.70; ^{1}H NMR δ 7.4 (s, 1H, CH), 6.8-7.7 (m, 17H, aromatic protons), 8.3 (d, 2H, C_8-H), 9.8 (s, 1H, OH), 12.5 (s, 2H, OH), 12.7 (s, 2H, OH) ; ms: m/z: 668 (M^+ - 1), 618, 541, 436, 387, 331, 282, 173, 146 and 77.

4 - (4"- Methoxybenzylidene)-bis-3-(2', 4'-dihydroxy benzoyl) isocoumarin 5m

This compound was obtained as yellow crystals, mp: 144^{0}C; 35.21% yield; Anal. Calcd $C_{40}H_{26}O_{11}$ (683.0 g): C, 70.27; H, 3.95; Found: C, 69.84; H, 3.78; ^{1}H NMR δ 3.8 (s, 3H, OCH_3), 5.7 (s, 4H, OH), 7.4 (s, 1H, CH), 6.6-7.5 (m, 16H, aromatic protons), 8.4 (d, 2H, C_8-H); ms: m/z: 683 (M^+), 666, 618, 541, 495, 439, 401 and 146.

General procedure for 7a-7g

A mixture of isocoumarin **6** (100mg, 0.00033mole), ethyl acetoacetate (0.043ml, 0.00033mole) was added slowly to conc. sulphuric acid (2ml) cooled in ice within 15 mins. The reaction mixture was left overnight. After the reaction was complete, the reaction mass was poured into ice and solid product obtained was filtered and purified

by column chromatography using petroleum ether (60-80^{0}C)-ethyl acetate to yield white crystals of **7a**

4-Methyl-3-(4'-methyl coumarin-6'-carbonyl) isocoumarin 7a

This compound was obtained as white crystals, mp: 192^{0}C; 49.37% yield; Anal. Calcd $C_{21}H_{14}O_5$ (346.0 g): C, 72.83; H, 4.04; Found: C, 73.19; H, 4.26; ^{1}H NMR δ 2.3 (s, 3H, C_4' –H), 2.9 (s, 3H, C_4-H), 6.8-8.1 (m, 7H, aromatic protons), 8.2 (d, 1H, C_8-H); ms: m/z: 346 (M^+), 187, 159 and 145.

4-Methyl-3-(4'-methyl-7'-hydroxy coumarin-6'-carbonyl) isocoumarin 7b

This compound was obtained as white crystals, mp: 158^{0}C; 38.09% yield; Anal. Calcd $C_{21}H_{14}O_6$ (362.0 g): C, 69.61; H, 3.86; Found: C, 69.48; H, 4.13; ^{1}H NMR δ 2.3 (s, 3H, C_4' –H), 2.9 (s, 3H, C_4-H), 6.4 (s, 1H, OH), 6.8-8.1 (m, 6H, aromatic protons), 8.2 (d, 1H, C_8-H); ms: m/z: 362 (M^+), 287, 259, 203, 187 and 146.

4-Methyl-3-(4'-methyl coumarin-6'-carbonyl)-8-nitro isocoumarin 7c

This compound was obtained as yellow crystals, mp: 128^{0}C; 40.28% yield; Anal. Calcd $C_{21}H_{13}O_7N$ (391.0 g): C, 64.45; H, 3.32; N, 3.58; Found: C, 64.19; H, 3.68; N, 3.82; ^{1}H NMR δ 2.0 (s, 3H, C_4' –H), 2.5 (s, 3H, C_4-H), 6.5-8.1 (m, 6H, aromatic protons), 8.4 (d, 1H, C_7-H); ms: m/z: 391(M^+), 207 and 160.

4-Methyl-3-(4'-methyl-7'-hydroxy coumarin-6'-carbonyl)-8-nitro isocoumarin 7d

This compound was obtained as yellow crystals, mp: 134^{0}C; 40.00% yield; Anal. Calcd $C_{21}H_{13}O_8N$ (407.0 g): C, 61.91; H, 3.19; N, 3.43; Found: C, 62.35; H, 3.30; N, 3.72; ^{1}H NMR δ 1.9 (s, 3H, C_4' –H), 2.3 (s, 3H, C_4-H),5.3 (s, 1H, OH), 6.5-8.2 (m, 5H, aromatic protons), 8.4 (d, 1H, C_7-H); ms: m/z: 407 (M^+), 377, 361, 331, 209, 187, 159, 145 and 77.

4-Ethyl-3-(4'-methyl coumarin-6'-carbonyl) isocoumarin 7e

This compound was obtained as white crystals, mp: 180^{0}C; 57.83% yield; Anal. Calcd $C_{22}H_{16}O_5$ (360.0 g): C, 73.33; H, 4.44; Found: C, 73.30; H, 3.71; ^{1}H NMR δ 1.3 (t,

3H, CH_3), 2.3 (s, 3H, C_4' –H), 2.9 (q, 2H, CH_2), 6.8-8.1 (m, 7H, aromatic protons), 8.2 (d, 1H, C_8-H); ms: m/z: 361 (M^+ + 1), 354, 331, 316, 201, 174 and 118.

3-(4'-Methyl coumarin-6'-carbonyl) isocoumarin 7f

This compound was obtained as white crystals, mp: 190^{0}C; 50.71% yield; Anal. Calcd $C_{20}H_{12}O_5$ (332.0 g): C, 72.28; H, 3.61; Found: C, 72.40; H, 3.89; ^{1}H NMR δ 2.3 (s, 3H, C_4' –H), 7.4 (s, 1H, C_4-H), 6.5-8.1 (m, 7H, aromatic protons), 8.4 (dd, 1H, C_8-H); ms: m/z: 330 (M^+ - 2), 187, 146 and 77.

3-(4'-Methyl-7'-hydroxy coumarin-6'-carbonyl) isocoumarin 7g

This compound was obtained as white crystals, mp: 230^{0}C; 47.83% yield; Anal. Calcd $C_{20}H_{12}O_6$ (348.0 g): C, 70.58; H, 3.52. Found: C, 70.72; H, 3.88; ^{1}H NMR δ 2.1 (s, 3H, C_4' –H), 5.1(s, 1H, OH), 7.4 (s, 1H, C_4-H), 6.5-7.9 (m, 6H, aromatic protons), 8.4 (d, 1H, C_8-H); ms: m/z: 348 (M^+), 333, 173 and 146.

3. B.4 CONCLUSION

- The chapter deals with the synthesis of some new isocoumarin derivatives having more than one heterocyclic ring introduced into it.

- The more than one lactone ring was introduced to see the biological effects due to the presence of both rings.

- The synthesis was divided in two schemes; in first scheme, the final compound contains two isocoumarin moieties which were obtained by condensation of 3-aroyl isocoumarins with different aromatic aldehydes. In second scheme, coumarin ring was introduced in the isocoumarin moiety, by Pechmann condensation using ethyl acetoacetate.

- All the final compounds obtained were screened for biological applications which are given in last chapter.

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Chapter 4:

Section A:

Synthesis of 3, 4-disubstituted triazole and tetrazole isoquinolines

4. A.1 INTRODUCTION

The starting point of this work stems from research on the preparation of heterocyclic compounds with a high-nitrogen content in addition to isocoumarins, for their potential applications in a variety of fields including energetic materials featuring triazole and tetrazole rings.

Among the various heterocycles reported to date, tetrazole and its derivatives have received much attention in recent years due to their widespread applications in biology.

- The tetrazole group is similar to the carboxylic function in terms of size and acidity but is apparently more stable metabolically; its use as a carboxylic acid mimic in analogues of biologically active compounds has therefore attracted increasing interest[1]. This ability of tetrazole compounds to mimic the carboxylic functionality has motivated the incorporation of tetrazole derivatives into biologically active molecules[2].

- Some researchers have proved that tetrazole groups in drug molecules could improve the interaction of drugs with receptors in cell membrane[3-4].

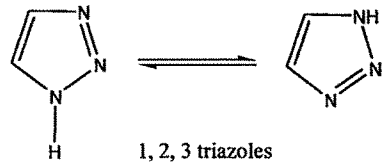
- Tetrazoles are important precursors in medicinal chemistry owing to their increased resistance towards metabolic degradation pathways and have found increasing applications as catalysts in asymmetric synthesis[5-6].

- Metal derivatives of nitrogen-rich compounds, and in particular tetrazoles, have great potential for energetic applications. Various metal complexes of tetrazole itself and its amino, nitro and nitrimino-derivatives as well as of bistetrazole, azotetrazole, bistetrazolylhydrazine, bistetrazolylamine have been intensively studied as low-smoke producing pyrotechnic compositions, gas generators, propellants or primer explosives[7].

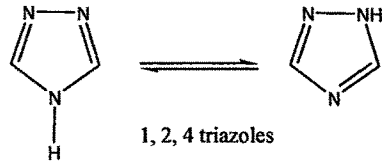
- These ring systems are present in drugs patented for the treatment of central nervous system disorders, HIV, sexual dysfunction, asthma, obesity and diabetes as well as for injuries caused by exposure to chemical warfare agents[8].

- Tetrazoles are also useful reagents in heterocyclic synthesis and are widely used in ring cleavage/ring closure reactions with electrophilic reagents to form new C–N and N–N bonds. These reactions usually proceed *via* a dipolar nitrilimine intermediate; the resulting dipole frequently being designed to react further in a tandem electrocyclic cyclization process to generate a new heterocyclic ring[9].

Triazoles are five membered heterocyclic compounds having three nitrogen atoms. They are of two types:



1, 2, 3 triazoles



1, 2, 4 triazoles

Various 1, 2, 4-triazols are found to be linked with diverse pharmacological activities. Apart from this activity, these are also used for metal organic frameworks and have attracted scientists working in the field of coordination chemistry as given below:

- 1, 2, 4 triazole has been incorporated into a wide variety of therapeutically interesting drug candidates including H_1/H_2 histamine receptor blockers,

cholinesterase active agents, CNS stimulants, anti anxiety agents and sedative[10].

- It has been reported that the major effect of triazoles on fungi is inhibition of sterol 14-α- demethylase, a microsomal cytochrome P-450 dependent enzyme system[11].

- Several potent drugs possessing triazole nucleus have been applied in medicine, like, Alprazolam (anxiolytic agent, tranquilizer), Anastrozole, Letrozole, Vorozole (antineoplastics, nonsteroidal competitive aromatase inhibitors), Fluconazole, Itraconazole, Terconazole (antifungal agents) etc[12].

- Apart from their pharmacological significance, 1, 2, 4-triazole derivatives exhibit interesting chemical properties. The ability of triazoles to form a bridge between metal ions makes such ligands very important for magneto chemical applications. Some complexes containing substituted 1, 2, 4-triazole ligands have potential uses as optical sensors or molecular-based memory devices[13].

- An optimised synthesis of 3, 4, 5-trisubstituted 1, 2, 4-triazoles, that can be used as linkers for metal organic frameworks (MOFs) has also been described[14].

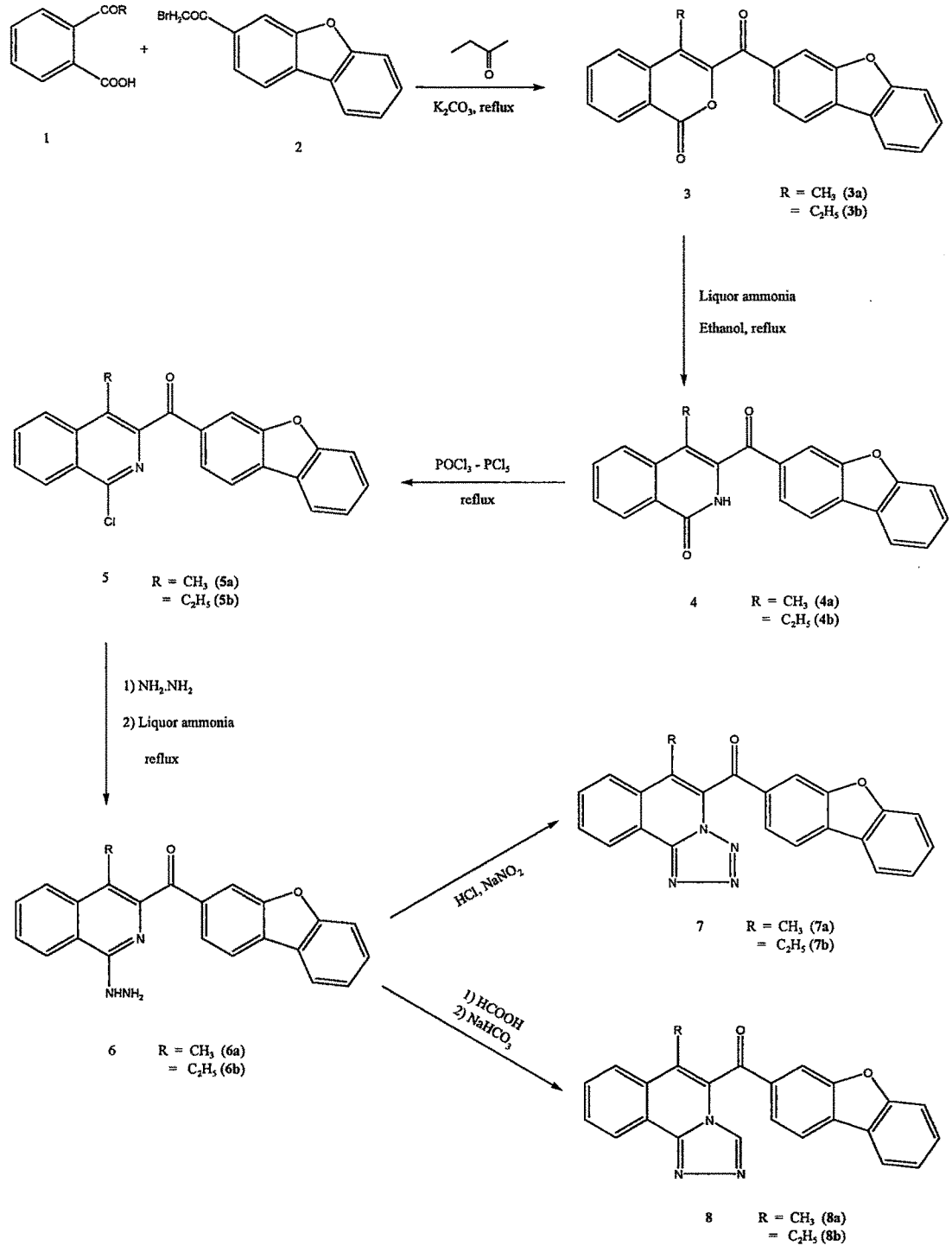
- 1,2,4-Triazoles have attracted great and growing interest in the coordination chemistry because of the fact that they can synthesize transition metal coordination polymers with the two bridging close adjacent nitrogen atoms (N1 and N2) or 4-positioned one (N4) , and can effectively transmit magnetic interaction between paramagnetic centers[15].

- 1,2,4-triazole unit serve as a good electron-transporting and hole-blocking material in organic light-emitting diodes (OLEDs), because of the electron

accepting capabilities of the unit associated with an electron withdrawing imine –C=N- group in the unit[16].

Keeping these observations in mind and in continuation of our work on the synthesis of heterocyclic compounds containing nitrogen with expected biological activity we report herein the synthesis of the 3, 4- disubstituted triazole and tetrazole isoquinolines by converting isocoumarins first into isoquinolone derivative and then introducing triazole and tetrazole nucleus into it.

Scheme I



1 2

3 R = CH_3 (3a) = C_2H_5 (3b)

4 R = CH_3 (4a) = C_2H_5 (4b)

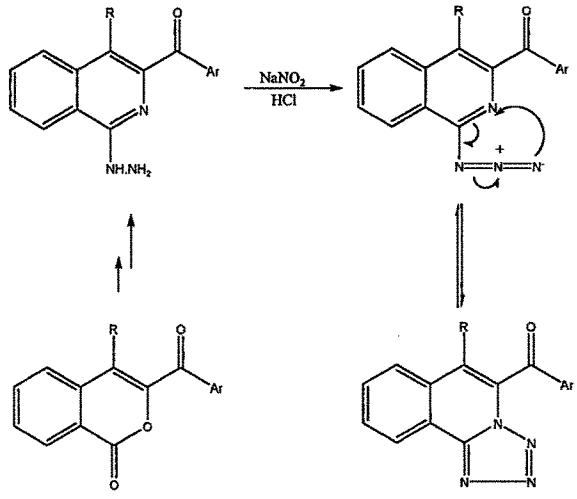
5 R = CH_3 (5a) = C_2H_5 (5b)

6 R = CH_3 (6a) = C_2H_5 (6b)

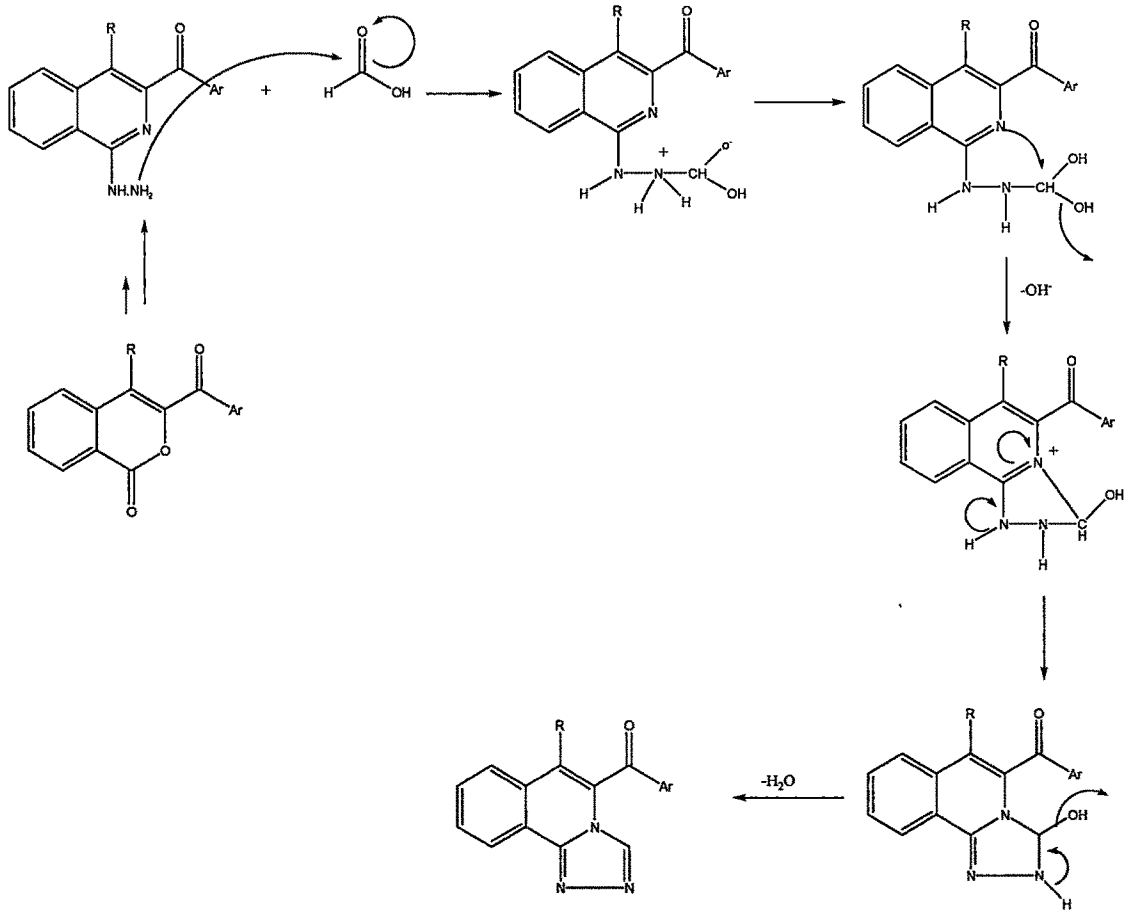
7 R = CH_3 (7a) = C_2H_5 (7b)

8 R = CH_3 (8a) = C_2H_5 (8b)

Mechanism: **Tetrazole isoqunolines** (Tetrazole – Azide Tautomerism)



Mechanism: **Triazole isoqunolines**



4. A.2 RESULTS AND DISCUSSION

During our studies on isocoumarins we have found that isocoumarins can be easily converted to 1-hydrazinoisoquinoline. This promoted us to carry out synthesis of some triazolo- and tetrazolo – isoquinolines by making use of isocoumarins as starting materials and also to introduce biologically important heterocyclic moiety in the isocoumarin ring . The isocoumarin **3** was prepared from o-acyl benzoic acid **1** and 2-bromo acetyl dibenzofuran **2** using the same method as described in previous chapters (**2 – 3B**). The isocoumarins **3a-b** were converted to isoquinolones on reaction with liquor ammonia in ethanol. The resultant isoquinolone **4** were then converted to 1-chloro isoquinolines **5** by treatment with $POCl_3$ - PCl_5. The later on treatment with hydrazine afforded the required 1- hydrazino isoquinolines **6** which were finally converted to the corresponding tetrazolo isoquinoline **7** and triazolo isoquinoline **8** by treatment with $NaNO_2$/HCl and HCOOH respectively (**Scheme I**).

The isocoumarin **3a** shows IR absorption frequencies at 1735, 1600 and 1266 cm^{-1} for γ lactone, -C=O and C-O respectively (**Fig. 4.A.1**).

^1H NMR spectrum of **3a** shows signals at δ 2.1 (s, 3H, CH_3), 7.3-8.0 (m, 10H, aromatic protons), 8.2 (d, 1H, C_8-H) (**Fig. 4.A.2**) and **3b** at δ 1.2 (t, 3H, CH_3), 2.7 (q, 2H, CH_2), 6.8-7.9 (m, 10H, aromatic protons), 8.16 (s, 1H, C_8-H) (**Fig.4.A.4**).

Mass spectrum of **3a** shows m/z at 355 (M^+ + 1), 340, 311, 264, 174, 160 and 146 (**Fig. 4.A.3**), **3b** at 368 (M^+), 312, 278, 250, 186 and 146 (**Fig. 4.A.5**).

The melting point of all the three intermediates i.e isoquinolones **4**, 1-chloro isoquinolines **5** and 1- hydrazino isoquinolines **6** were taken and were characterized by elemental analysis.

The IR spectras of tetrazolo **7a** and triazolo isoquinolines **8a** shows absorption at 1692, 1645, 1567, 1497 and 1675 (**Fig. 4.A.6**), 1620, 1559, 1494 cm^{-1} for –C=O, C=N, C=C, C-N respectively (**Fig. 4.A.7**).

The signals obtained in ^1H NMR spectra's of **7a** are δ 2.2 (s, 3H, CH_3), 7.25-7.99 (m, 10H, aromatic protons), 8.1 (d, 1H, C_1-H) (**Fig. 4.A.8**), and **8a** are δ 2.1 (s, 3H, CH_3), 7.25-7.99 (m, 10H, aromatic protons), 8.00 (d, 1H, C_1-H), 8.07 (d, 1H, N-CH=N) (**Fig. 4.A.10**) respectively.

In **7a** and **8a** the absence of lactone frequency at around 1700 cm^{-1} in IR spectrum and the absence of a doublet at δ 8.2 or 8.4 in the NMR spectrum, a characteristic signal of C_8 in isocoumarin moiety which shifted to δ 7.99 shows that the tetrazole / triazole ring formation has taken place.

Mass spectra's of **7a** and **8a** gives m/z at 378 (M^+), 363, 316, 289, 212, 184, 169 and 168 (**Fig. 4.A.9**) and 368 ($M^+ + 3$), 327, 287, 210, 183 and 169 (**Fig. 4.A.11**) which also supports the formation of triazole / tetrazole ring in isoquinolone nucleus.

Further m/z value at 146 which is molecular weight of isocoumarin, is not seen in any of the mass spectra's of triazolo and tetrazolo isoquinolines, confirms the absence of isocoumarin nucleus from the synthesized compounds.

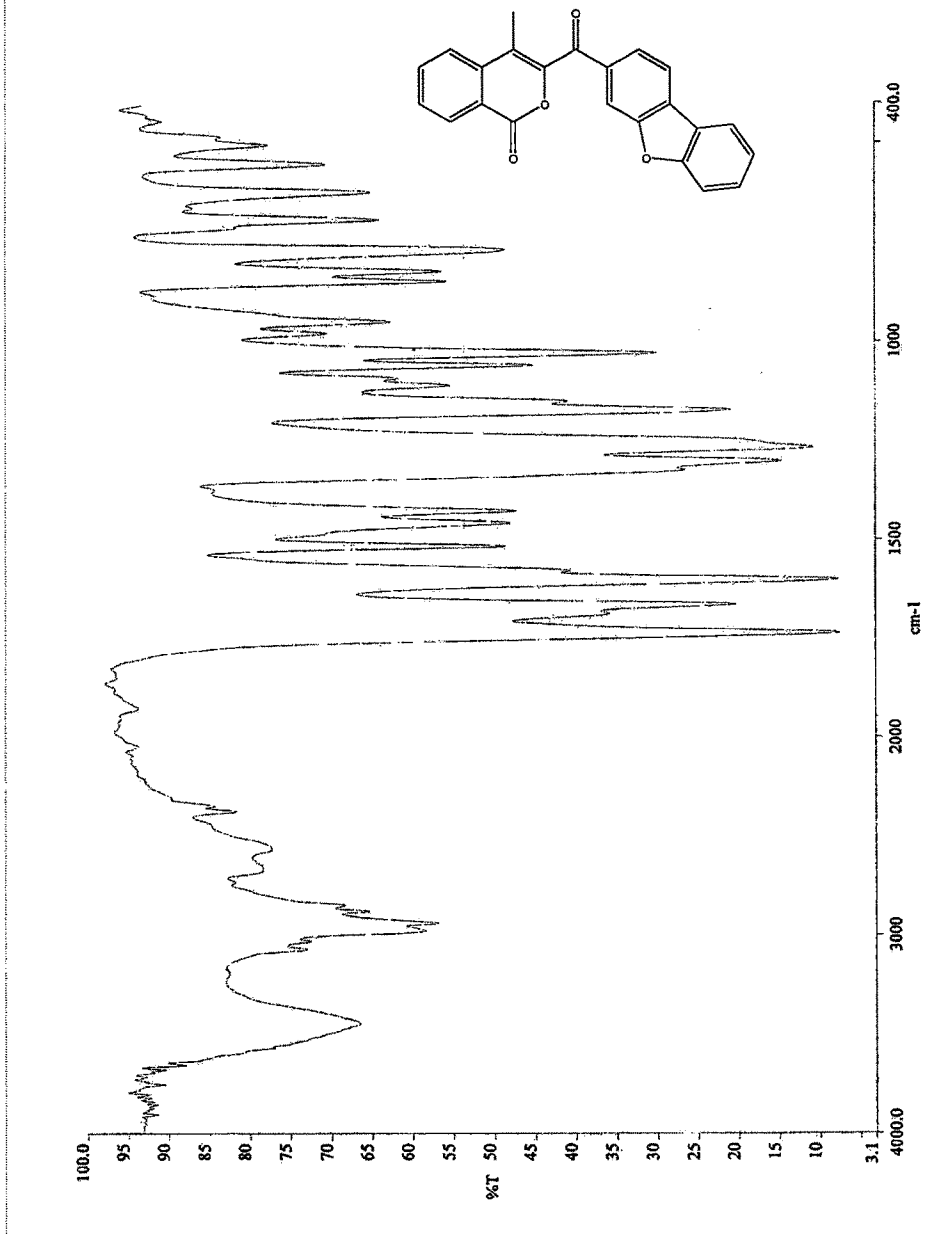


Fig. 4.A.1 – IR: **3- Dibenzofuryl-4-methyl isocoumarin 3a**

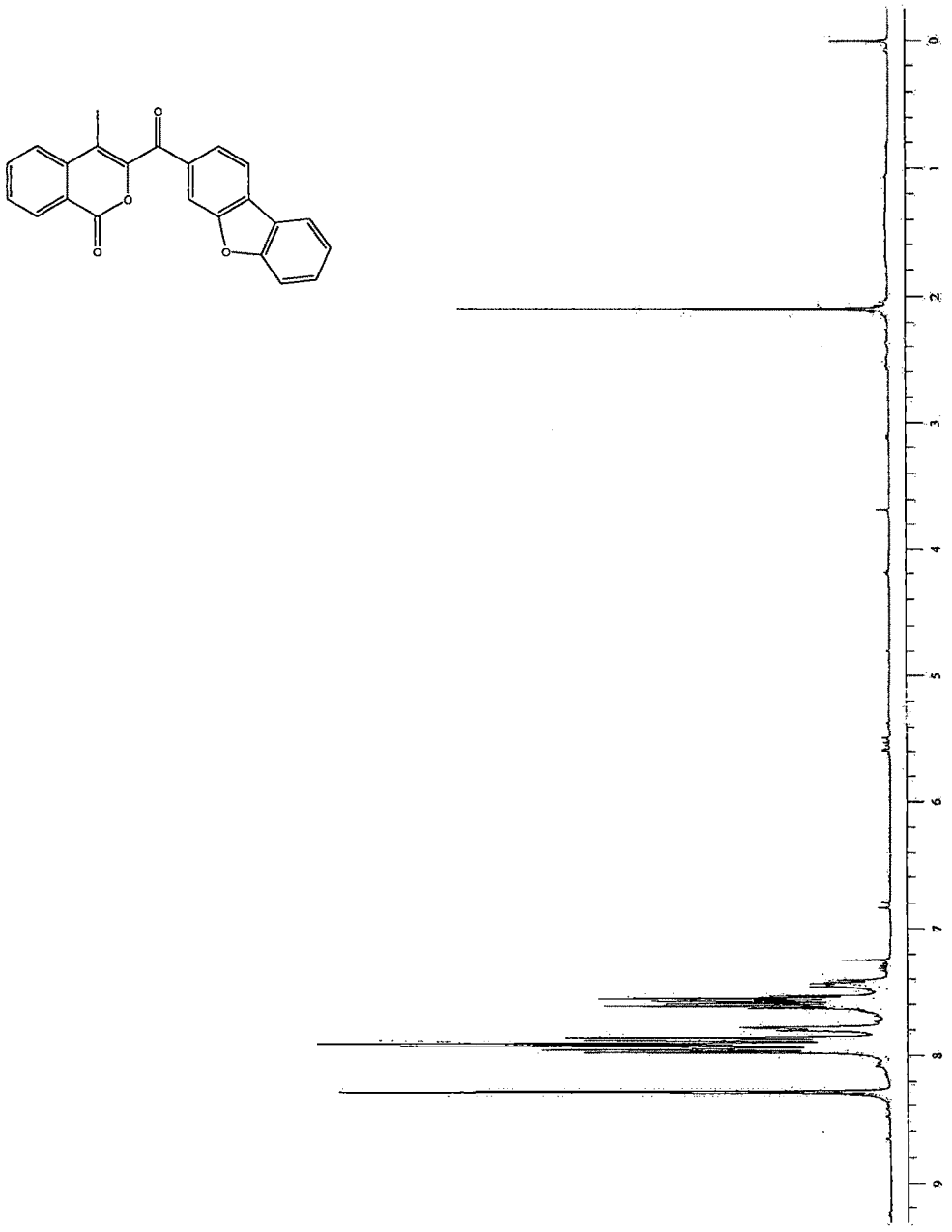


Fig. 4.A.2 – ^{1}H NMR: **3- Dibenzofuryl-4-methyl isocoumarin 3a**

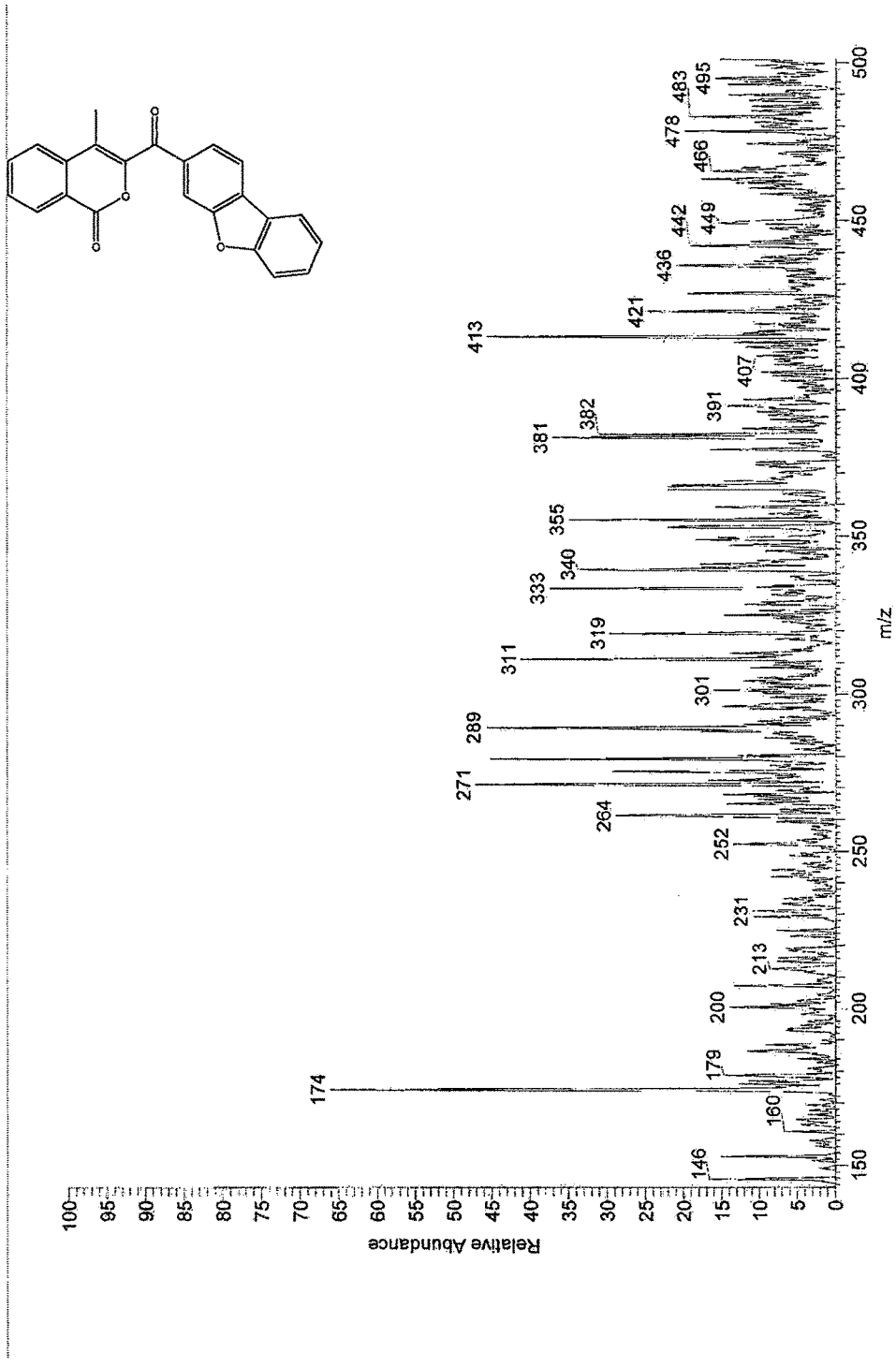
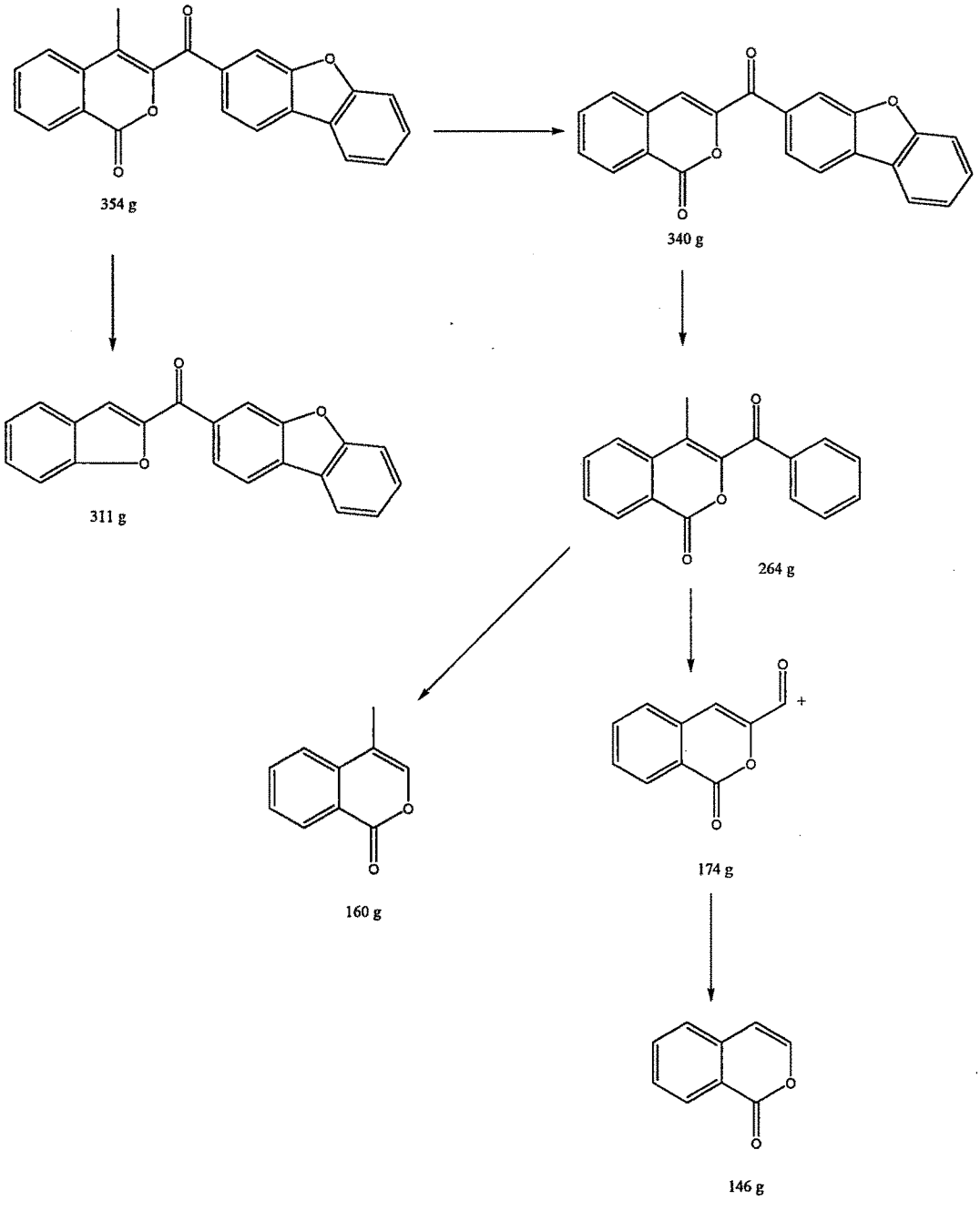
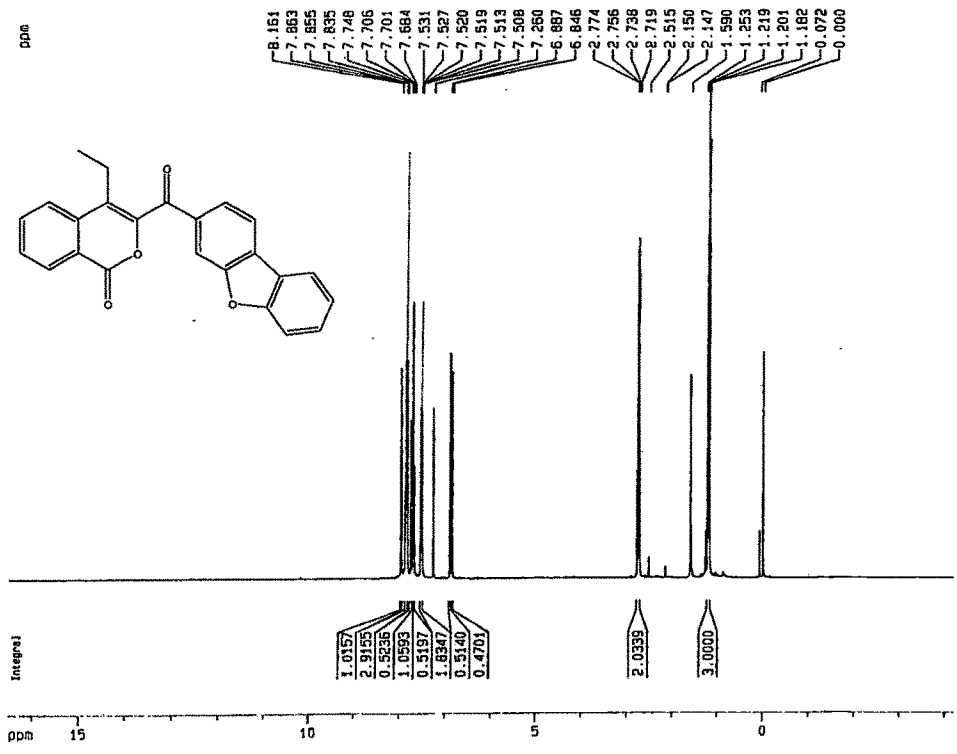


Fig. 4.A.3 - Mass spectrum: **3- Dibenzofuryl-4-methyl isocoumarin 3a**



Fragmentation Pattern: **3- Dibenzofuryl-4-methyl isocoumarin**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7.508
7.260
6.887
6.846
2.774
2.756
2.738
2.719
2.515
2.150
2.147
1.590
1.253
1.219
1.201
1.182
0.072
0.000
Integral
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0.5197
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0.5140
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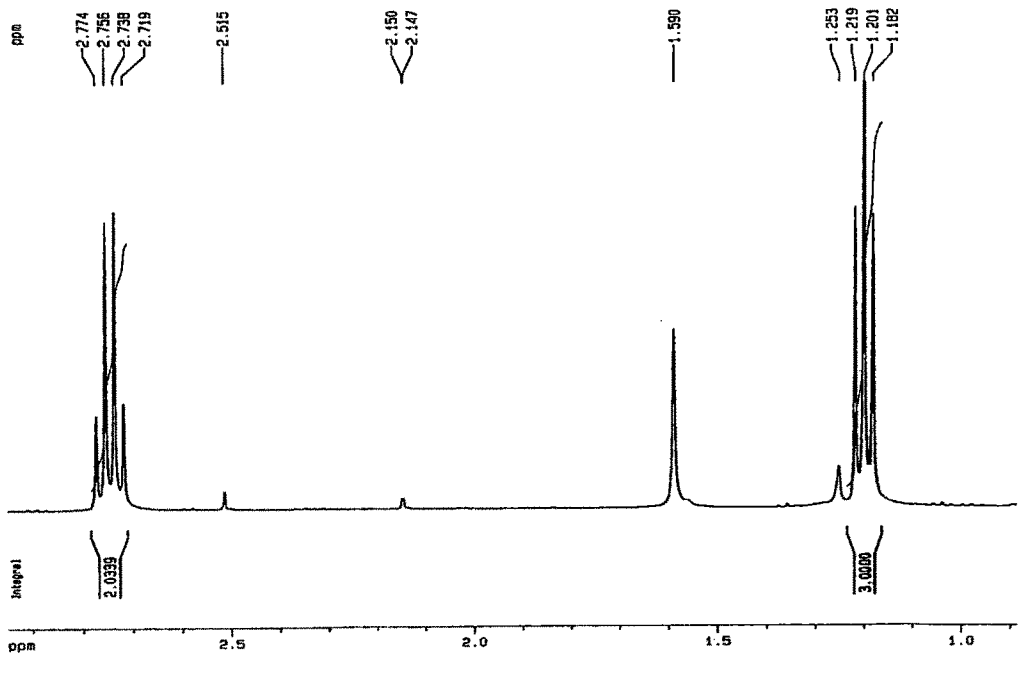
3.0000
ppm
15
10
5
0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2.0
1.5
1.0

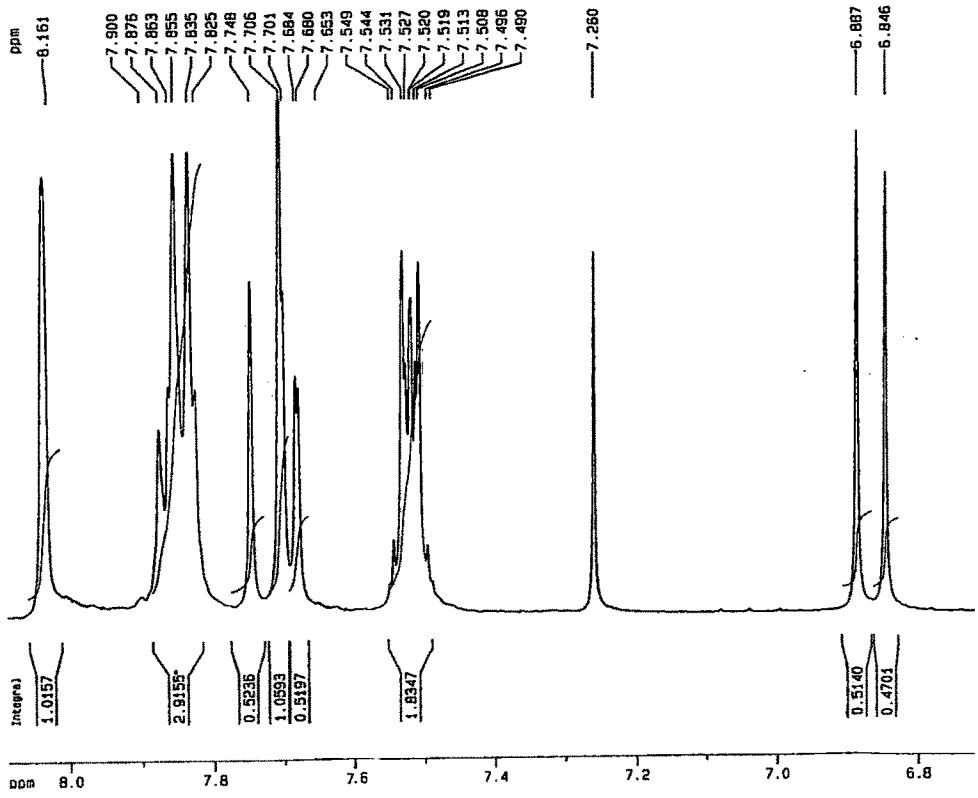


Fig. 4.A.4 – ^{1}H NMR: 3- Dibenzofuryl-4-ethyl isocoumarin 3b

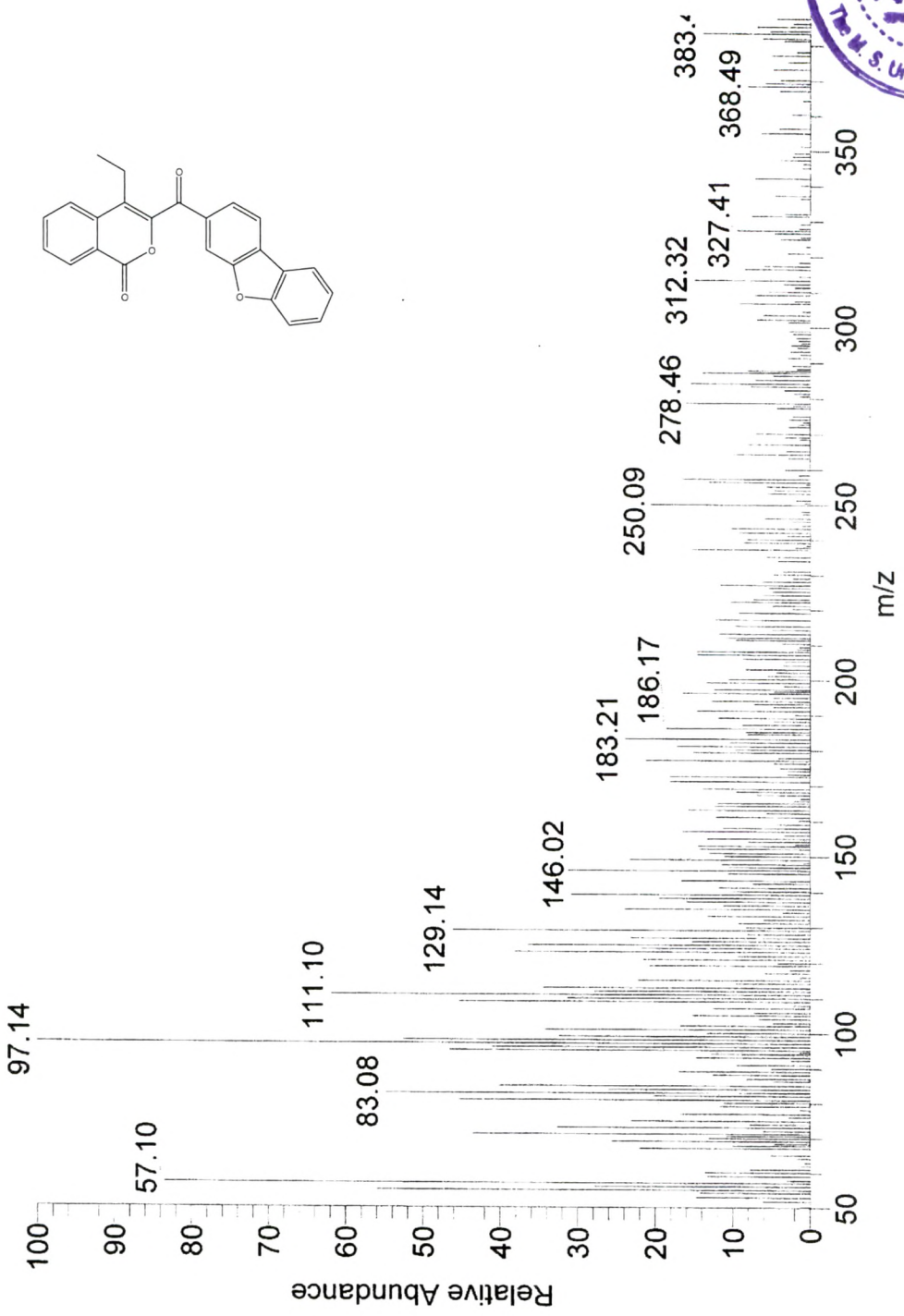
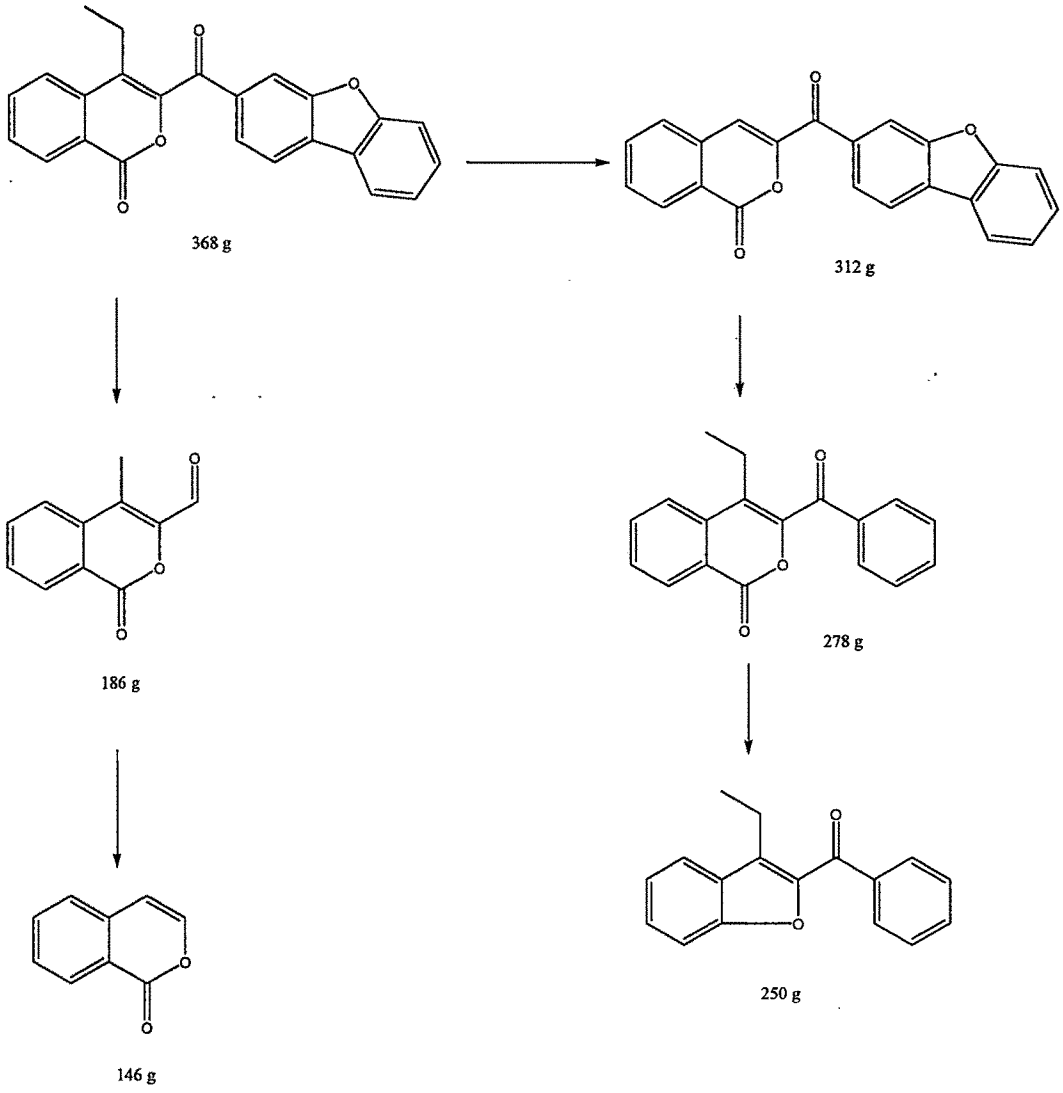


Fig. 4.A.5 - Mass spectrum : **3- Dibenzofuryl-4-ethyl isocoumarin 3b**



Fragmentation Pattern: **3- Dibenzofuryl-4-ethyl isocoumarin**

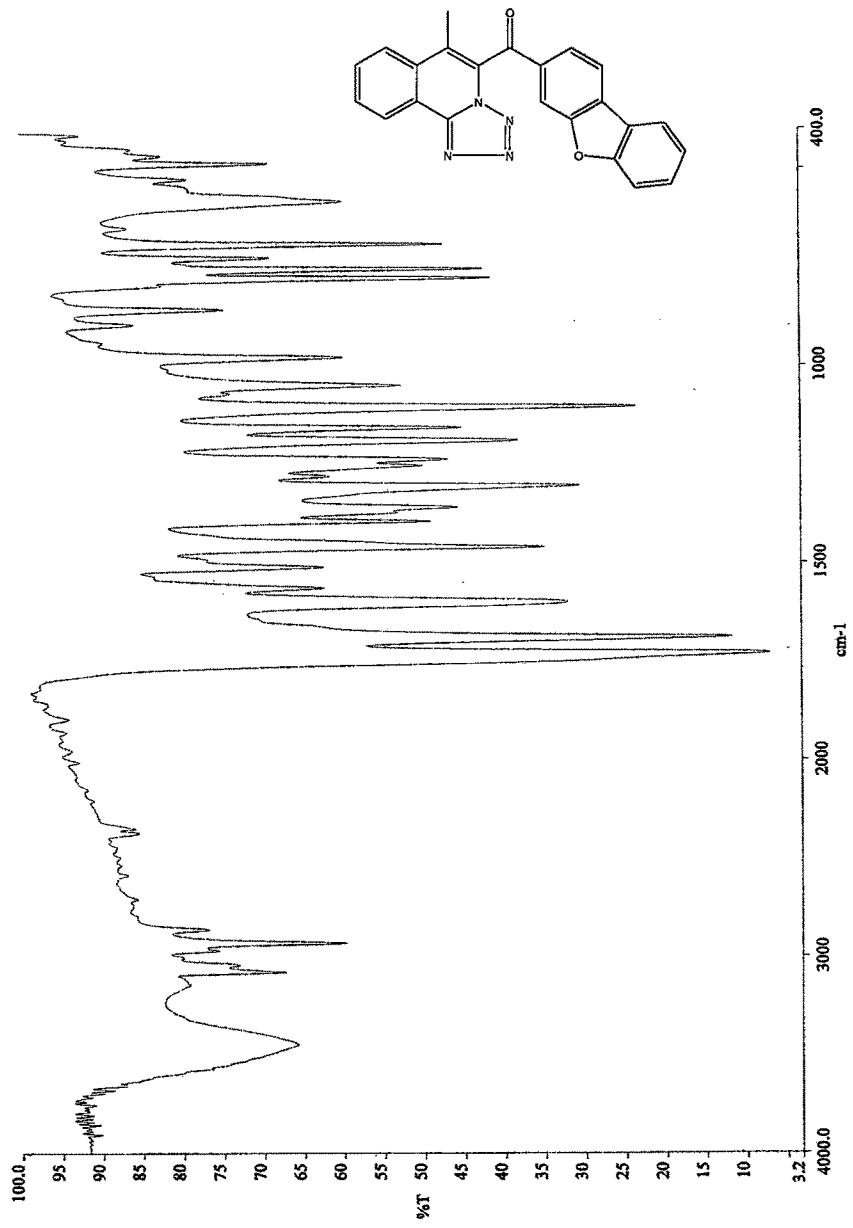


Fig. 4.A.6 – IR: **3 - (Dibenzofuran– 3-carbonyl) – 4 - methyl – Tetrazolo – (5, 1 – a) isoquinoline 7a**

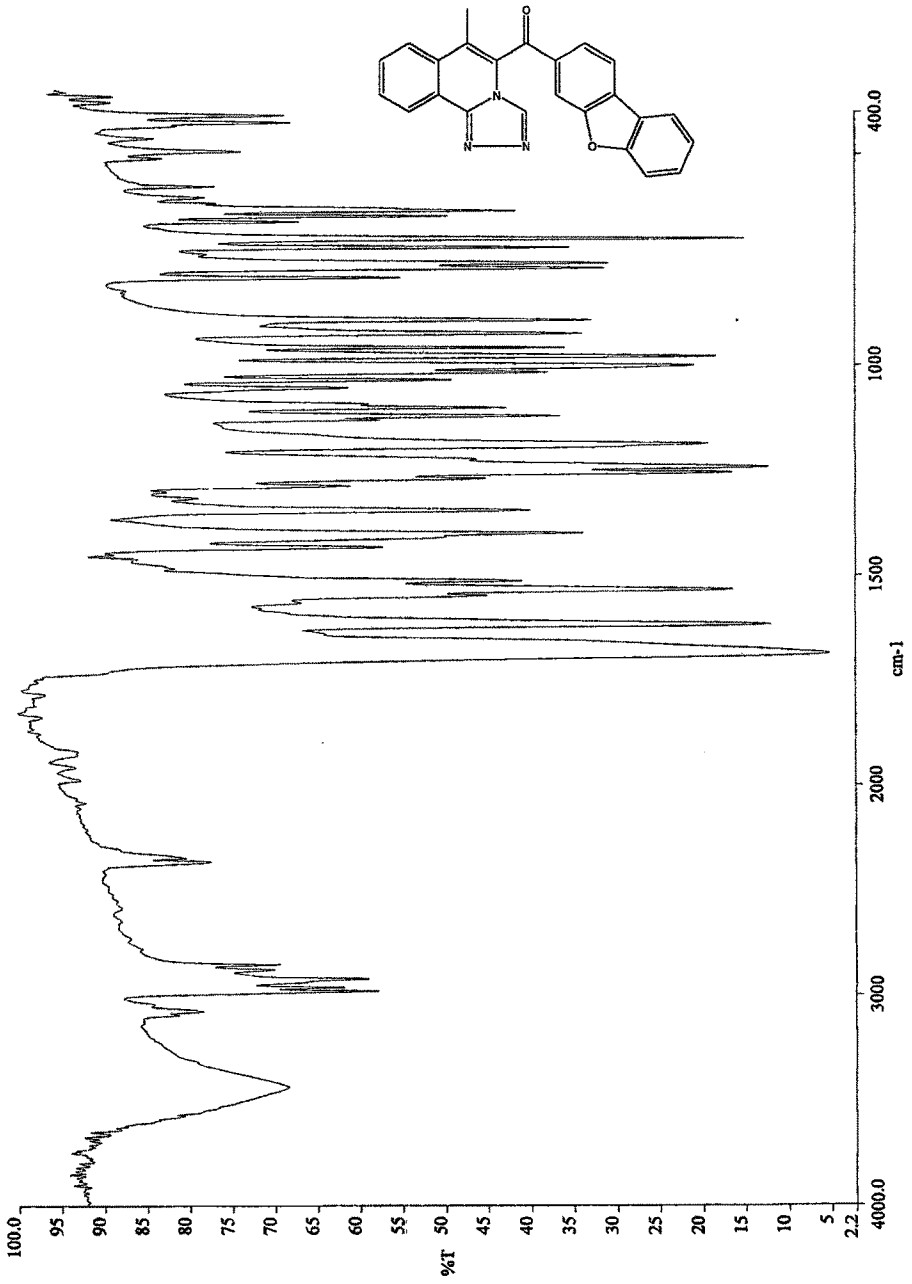


Fig. 4.A.7 – IR: **3 - (Dibenzofuran– 3-carbonyl) – 4 - methyl – 1,2,4 -Triazolo – (3, 4 – a) isoquinoline 8a**

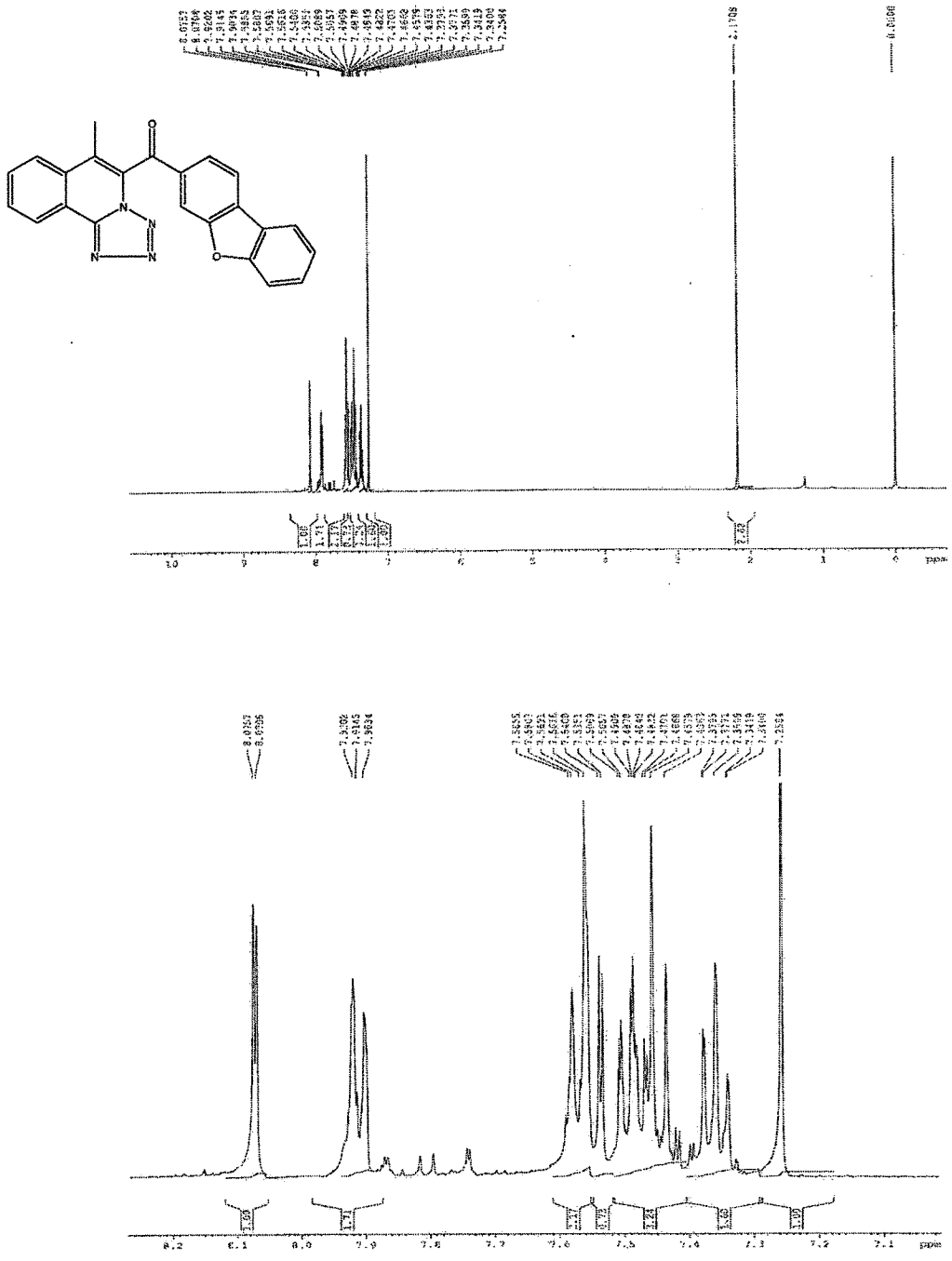


Fig. 4.A.8 – ^{1}H NMR: 3 - (Dibenzofuran– 3-carbonyl) – 4 - methyl – Tetrazolo – (5, 1 – a) isoquinoline 7a

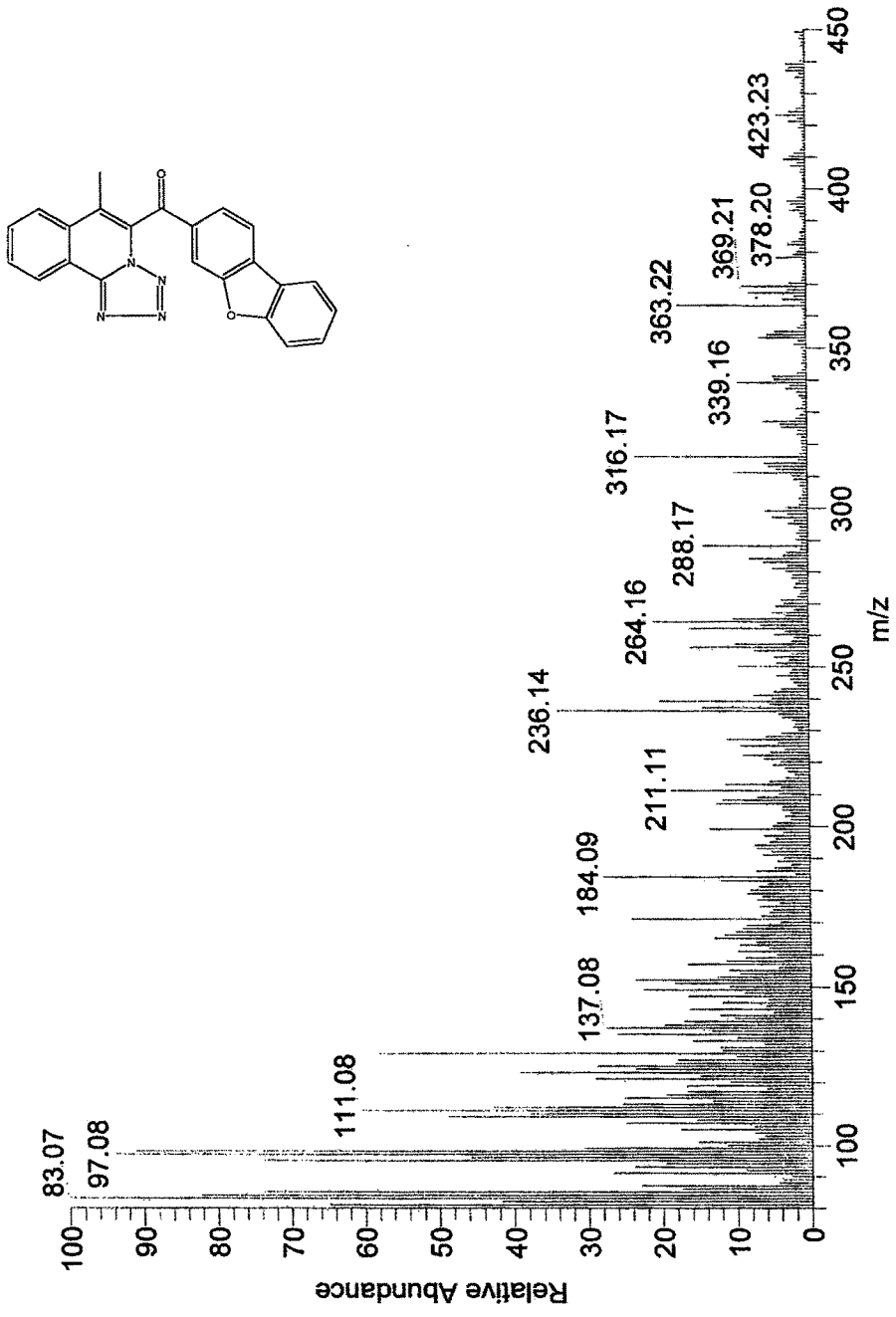
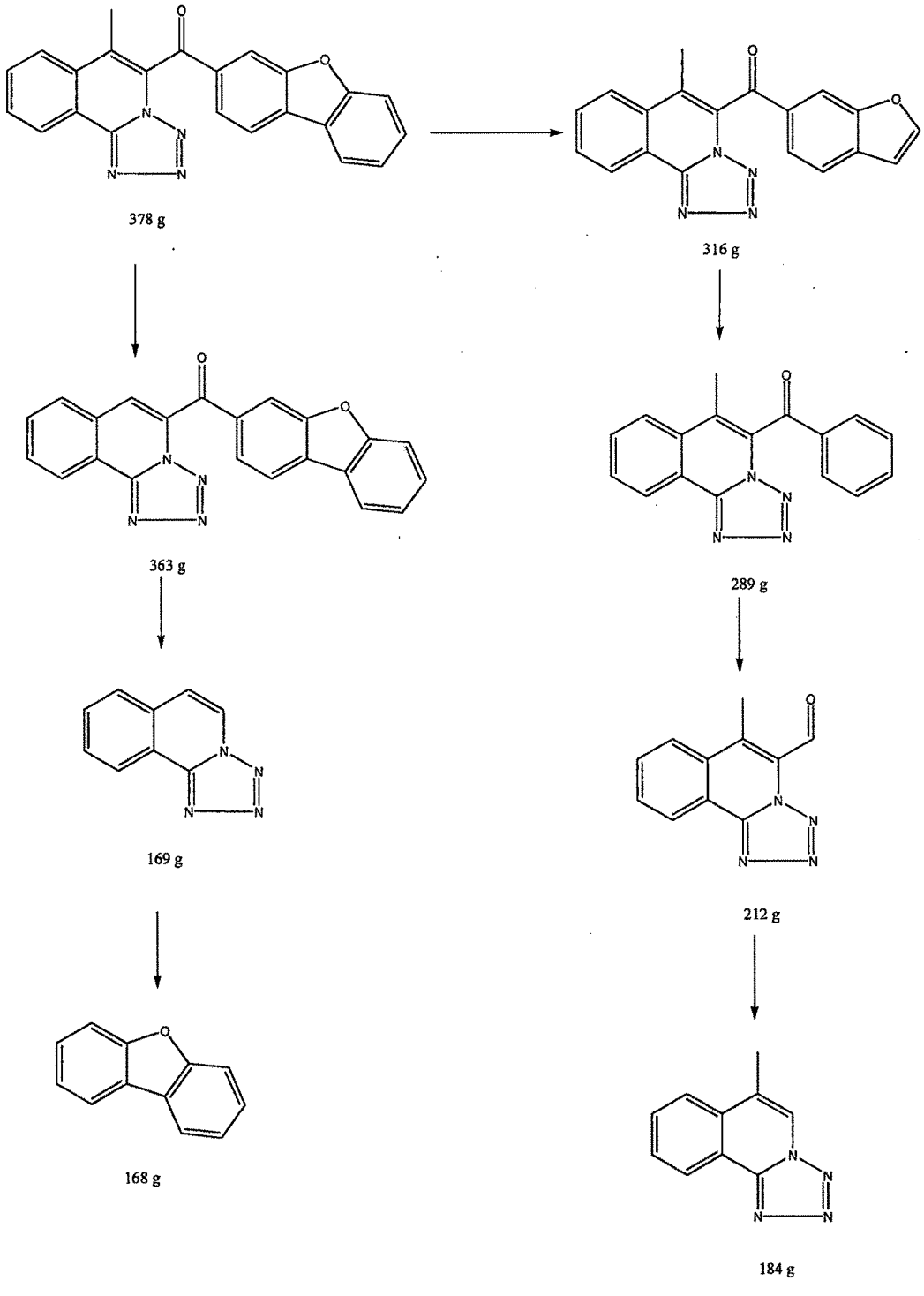


Fig. 4.A.9 - Mass spectrum: **3 - (Dibenzofuran– 3-carbonyl) – 4 - methyl – Tetrazolo – (5, 1 – a) isoquinoline 7[a]**



Fragmentation Pattern: **3 - (Dibenzofuran– 3-carbonyl) – 4 - methyl – Tetrazolo – (5, 1 – a) isoquinoline**

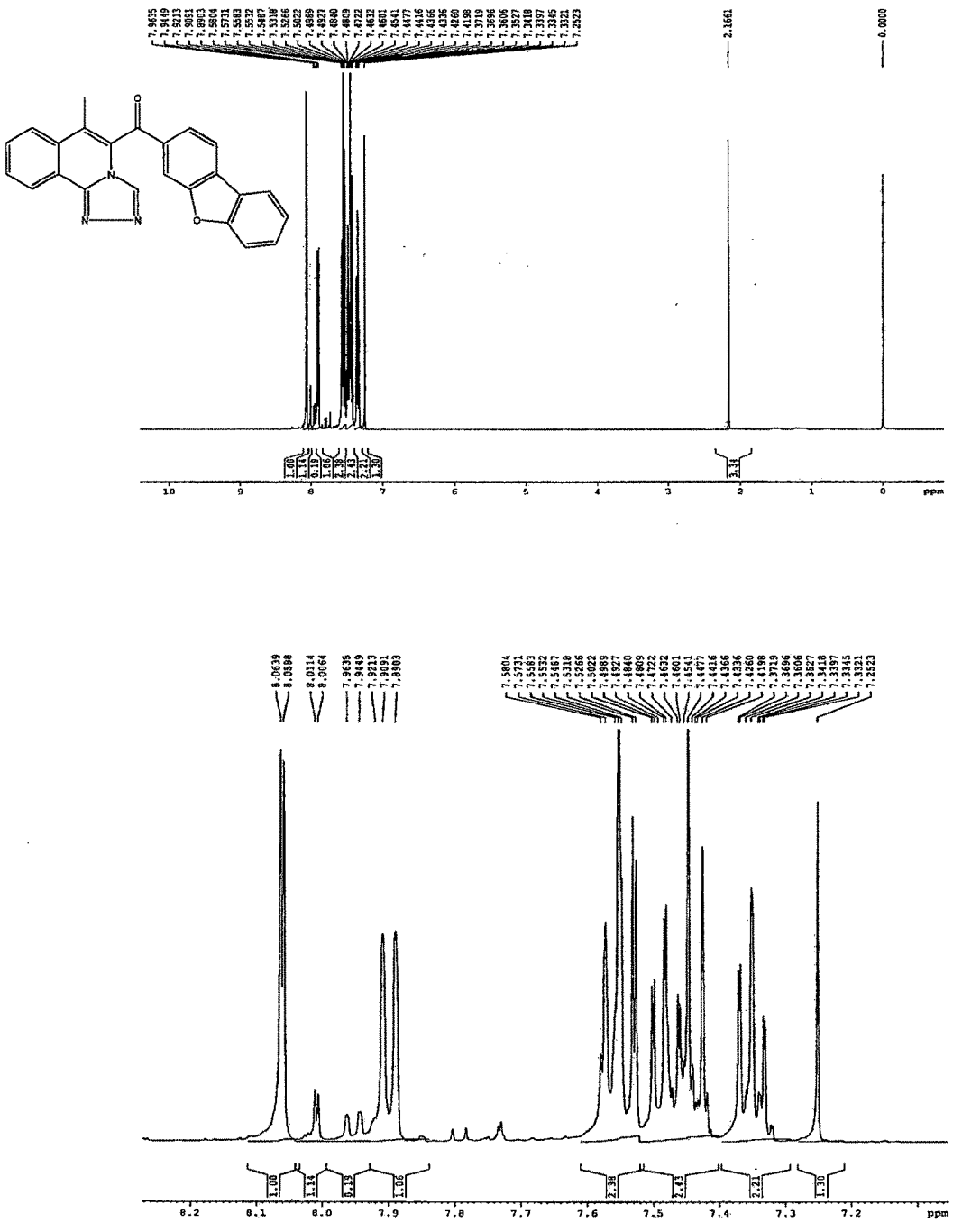


Fig. 4.A.10 – ^{1}H NMR: **3 - (Dibenzofuran– 3-carbonyl) – 4 - methyl – 1,2,4 - Triazolo – (3, 4 – a) isoquinoline 8a**

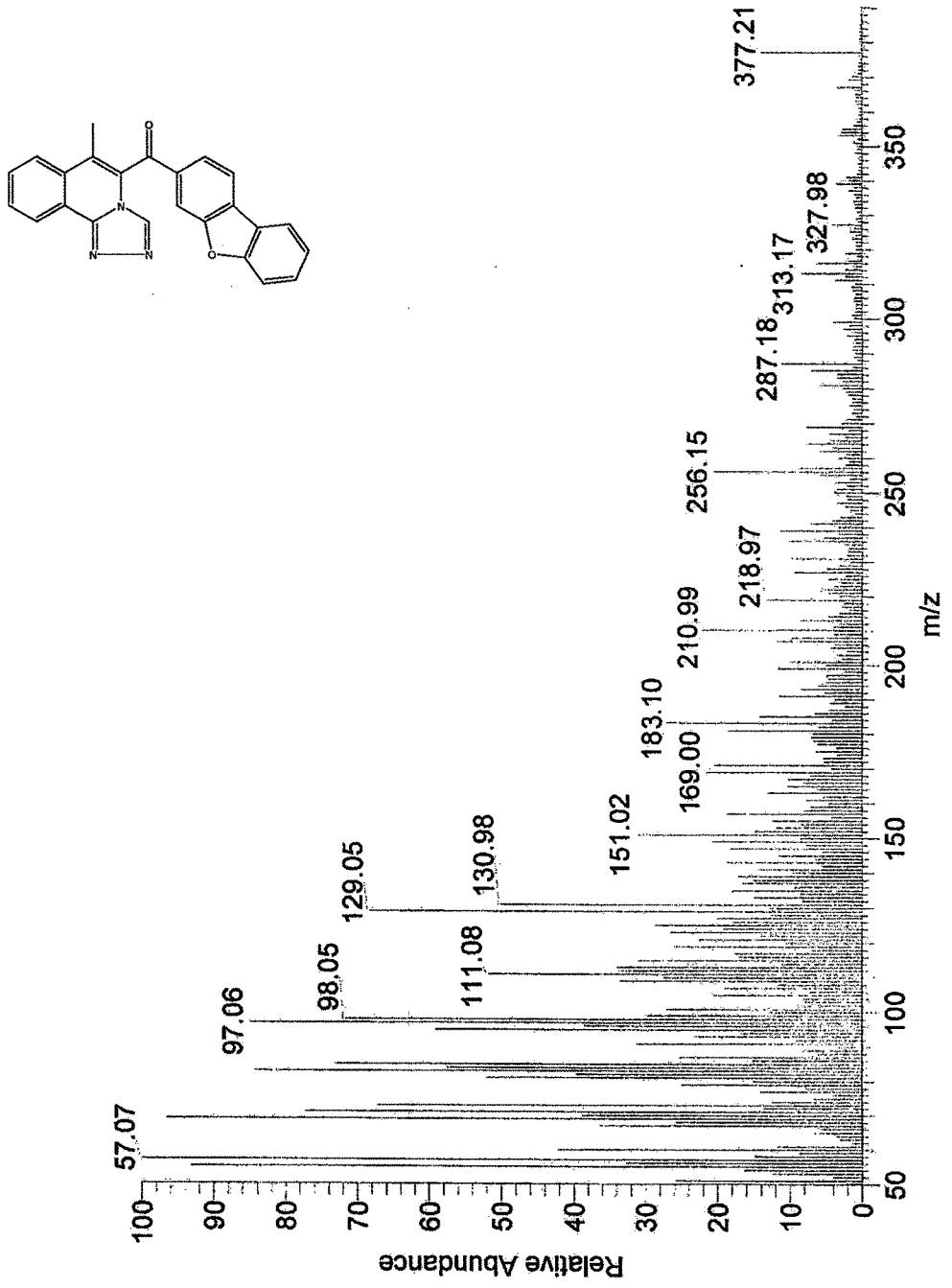
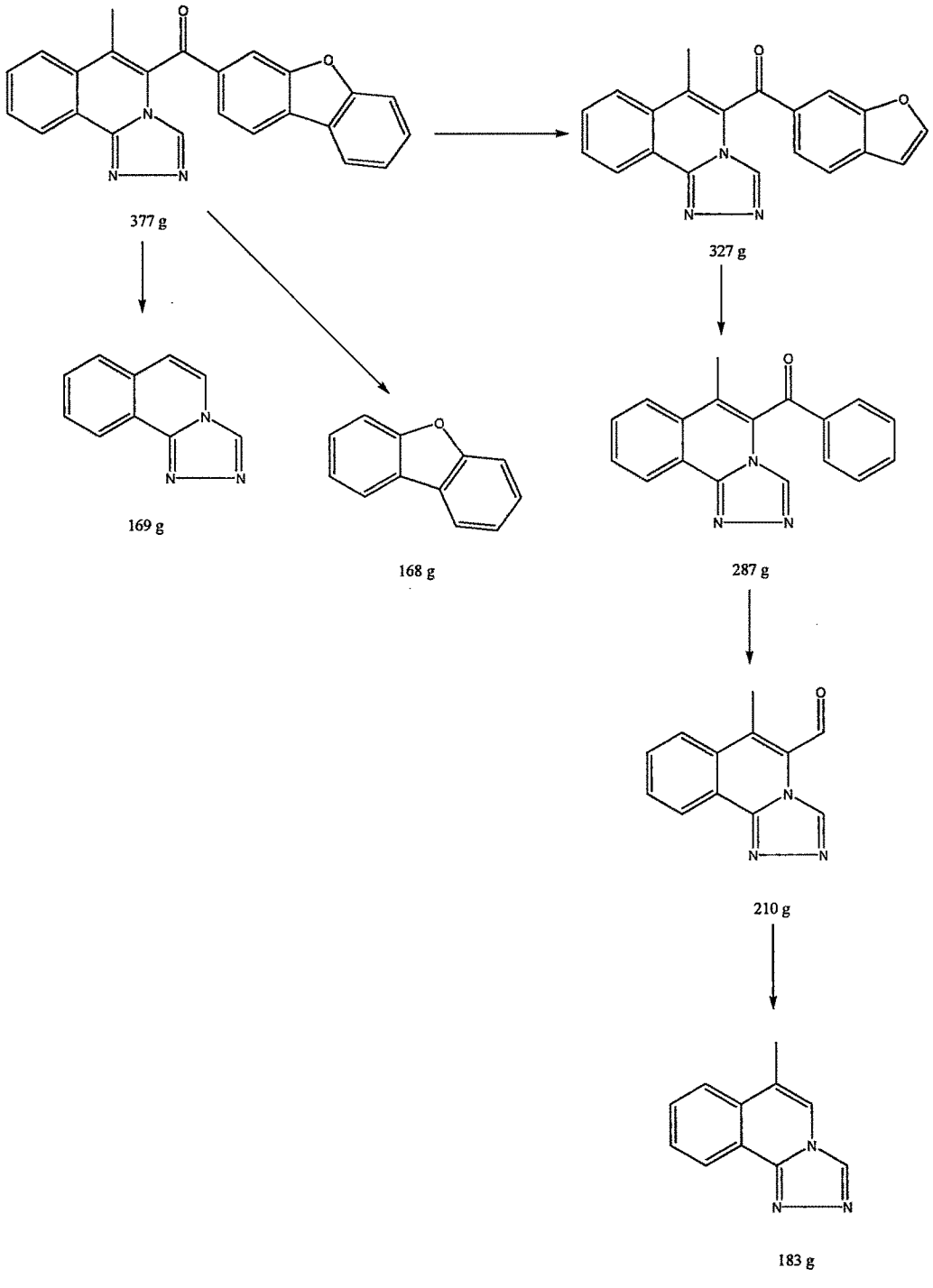


Fig. 4.A.11 – Mass spectrum: **3 - (Dibenzofuran– 3-carbonyl) – 4 - methyl – 1,2,4 - Triazolo – (3, 4 – a) isoquinoline 8a**



Fragmentation Pattern: **3 - (Dibenzofuran– 3-carbonyl) – 4 - methyl – 1,2,4 - Triazolo – (3, 4 – a) isoquinoline**

4. A.3 EXPERIMENTAL

The reagents and the solvents used in this study were of analytical grade and were used without further purification. Melting points were determined in open capillaries and are uncorrected. The purity of the compounds was checked by TLC on silica gel GF254 plates using UV/Iodine as visualizing agent and Merk's silica gel (60-120 mesh) was used for column chromatographic purification. Infrared spectra were recorded on FTIR Perkin Elmer spectrophotometer using potassium bromide optics. ^{1}H NMR spectra were recorded on a Bruker spectrometer (400 MHz) using TMS as internal standard and chemical shifts are given in ppm. Mass spectrums were obtained using Thermo Scientific Corporation, DSQ II Mass Spectrometer. Elemental analyses were carried out on Perkin-Elmer C, H, N, S analyzer (Model-2400).

General procedure for 3a-3b

o-propionyl benzoic acid **1b** (1.91g, 0.01mole), 2-bromo acetyl dibenzofuran **2** (3.1g. 0.01mole) and anhy. K_2CO_3, (3.1g, 0.022mole) was refluxed for 10-12 hrs in ethyl methyl ketone. Solvent was then removed, water was added and it was extracted with ethyl acetate. Solvent layer was first washed with sod. bicarbonate and then with water and finally dried over anhy. Na_2SO_4. After removal of solvent the crude product was purified by column chromatography using petroleum ether (60-80^{0}C)-ethyl acetate to yield white crystals of **3a**

3- Dibenzofuryl-4-methyl isocoumarin 3a

This compound was obtained as white crystals, mp: 55^{0}C; 80.26% yield; Anal. Calcd $C_{23}H_{14}O_4$ (354.0g): C, 77.96; H, 3.95; Found: C, 77.58; H, 4.10; ^{1}H NMR δ 2.1 (s, 3H, CH_3), 7.3-8.0 (m, 10H, aromatic protons), 8.2 (d, 1H, C_8-H); ms: m/z: 355 (M^+ + 1), 340, 311, 264, 174, 160 and 146.

3- Dibenzofuryl-4-ethyl isocoumarin 3b

This compound was obtained as white crystals, mp: 86^{0}C; 80.00% yield; Anal. Calcd $C_{24}H_{16}O_4$ (368.0g): C, 78.26; H, 4.34. Found: C, 78.43; H, 4.71; ^{1}H NMR δ 1.2 (t, 3H, CH_3), 2.7 (q, 2H, CH_2), 6.8-7.9 (m, 10H, aromatic protons), 8.16 (s, 1H, C_8-H); ms: m/z: 368 (M^+), 312, 278, 250, 186 and 146.

General procedure for 4a-4b

A solution of isocoumarin **3** (0.1g, 0.00027mole) in ethanol (3 ml) was refluxed with liquor ammonia (4ml) for 2.5 hrs. The reaction mixture was left overnight at room temperature. The resultant solid was filtered, washed with water and recrystallised from ethanol to give white crystals of **4a**

3-(Dibenzofuran– 3-carbonyl)-4-methyl-2*H*-isoquinoline-1-one 4a

This compound was obtained as white crystals, mp: 80^{0}C; 90.15% yield; Anal. Calcd $C_{23}H_{15}O_3N$ (353.0 g): C, 78.18; H, 4.24; N, 3.96; Found: C, 78.02; H, 4.58; N, 4.03.

3-(Dibenzofuran– 3-carbonyl)-4-ethyl-2*H*-isoquinoline-1-one 4b

This compound was obtained as white crystals, mp: 75^{0}C; 91.00% yield; Anal. Calcd $C_{24}H_{17}O_3N$ (367.0 g): C, 78.47; H, 4.63; N, 3.81; Found: C, 78.50; H, 4.81; N, 3.99.

General procedure for 5a-5b

Isoqinolone **4** (64mg, 0.00017mole), PCl_5 (42mg) and $POCl_3$ (0.2 ml) were mixed and the mixture refluxed for 4hrs at 130 ^{0}C. Excess of $POCL_3$ was removed by distillation under reduced pressure. The residue was decomposed with ice water containing excess of sodium bicarbonate. The product was filtered, washed with water and recrystallised from ether-hexane to give white crystals of **5a**

3-(Dibenzofuran– 3-carbonyl)-1-chloro-4-methyl-2*H*-isoquinoline 5a

This compound was obtained as white crystals, mp: 92^{0}C; 87.36% yield; Anal. Calcd $C_{23}H_{14}O_2NCl$ (371.5 g): C, 74.29; H, 3.76; N, 3.76; Found: C, 74.49; H, 3.54; N, 3.72.

3-(Dibenzofuran– 3-carbonyl)-1-chloro-4-ethyl-2*H*-isoquinoline 5b

This compound was obtained as white crystals, mp: 75^{0}C; 87.33% yield; Anal. Calcd $C_{24}H_{16}O_2NCl$ (385.5 g): C, 74.70; H, 4.15; N, 3.63; Found: C, 74.46; H, 4.39; N, 3.91.

General procedure for 6a-6b

A mixture of **5** (47mg, 0.00012mole), 80% aq. Hydrazine hydrate (0.5ml) and ethanol (1ml) was refluxed at 100^{0}c for 1 hr. The alcohol was distilled off and the residue treated with excess of ammonia, filtered, washed with water and recrystallised from ethanol to give white crystals of **6a**

3-(Dibenzofuran– 3-carbonyl)-1-hydrazino -4-methyl-2*H*-isoquinoline 6a

This compound was obtained as white crystals, mp: 90^{0}C; 74.00% yield; Anal. Calcd $C_{23}H_{17}O_2N_3$ (367.0 g): C, 75.20; H, 4.63; N, 11.44. Found: C, 75.52; H, 4.60; N, 11.61.

3-(Dibenzofuran– 3-carbonyl)-1-hydrazino -4-ethyl-2*H*-isoquinoline 6b

This compound was obtained as white crystals, mp: 95^{0}C; 74.23% yield; Anal. Calcd $C_{24}H_{19}O_2N_3$ (381.0 g): C, 75.59; H, 4.98; N, 11.02. Found: C, 75.70; H, 5.21; N, 11.27.

General procedure for 7a-7b

To a solution of **6** (50mg) (0.00013mole) in 50% aq. HCl (1ml) cooled to 0^{0}C, an aq. solution of sodium nitrite (29mg) in 1ml water was added drop wise maintaining the temperature below 5^{0}C. After the addition was over, the reaction mixture was heated

for 1 hr, cooled, the solid product formed was filtered, washed with water, dried and recrystallised from ethanol to give white crystals of **7a**

3 - (Dibenzofuran– 3-carbonyl) – 4 - methyl – Tetrazolo – (5, 1 – a) isoquinoline 7a

This compound was obtained as white crystals, mp: 84^{0}C; 60.00% yield; Anal. Calcd $C_{23}H_{14}O_2N_4$ (378.0 g): C, 73.00; H, 3.70; N, 14.81; Found: C, 73.30; H, 3.49; N, 15.01; ^{1}H NMR δ 2.2 (s, 3H, CH_3), 7.25-7.99 (m, 10H, aromatic protons), 8.1 (d, 1H, C_1-H); ms: m/z: 378 (M^+), 363, 316, 289, 212, 184, 169 and 168.

3 - (Dibenzofuran– 3-carbonyl) – 4 - ethyl – Tetrazolo – (5, 1 – a) isoquinoline 7b

This compound was obtained as white crystals, mp: 75^{0}C; 60.63% yield; Anal. Calcd $C_{24}H_{16}O_2N_4$ (392.0 g): C, 73.46; H, 4.08; N, 14.28; Found: C, 73.72; H, 4.29; N, 14.34; ^{1}H NMR δ 1.5 (t, 3H, CH_3), 2.3 (q, 2H, CH_2), 7.24-8.0 (m, 10H, aromatic protons), 8.05 (d, 1H, C_1-H); ms: m/z: 393 (M^+ + 1), 363, 330, 302, 224, 210, 198 and 183.

General procedure for 8a-8b

A mixture of **6** (50mg, 0.00013mole) and formic acid (0.1 ml) was heated at 130-135^{0}C for 3 hr, and poured into ice cold water. The formic acid was neutralized with sodium bicarbonate. The solid product was filtered, washed with water and recrystallized from ethanol to give white crystals of **8a**

3 - (Dibenzofuran– 3-carbonyl) – 4 - methyl – 1,2,4 -Triazolo – (3, 4 – a) isoquinoline 8a

This compound was obtained as white crystals, mp: 78^{0}C; 55.86% yield; Anal. Calcd $C_{24}H_{15}O_2N_3$ (377.0 g): C, 76.39; H, 3.97; N, 11.14; Found: C, 76.01; H, 4.25; N, 11.38; ^{1}H NMR δ 2.1 (s, 3H, CH_3), 7.25-7.99 (m, 10H, aromatic protons), 8.00 (d, 1H, C_1-H), 8.07 (d, 1H, N-CH=N); ms: m/z: 377 (M^+), 327, 287, 210, 183 and 169.

3 - (Dibenzofuran– 3-carbonyl) – 4 - ethyl – 1,2,4 -Triazolo – (3, 4 – a) isoquinoline 8b

This compound was obtained as white crystals, mp: 92^0C; 55.00% yield; Anal. Calcd $C_{25}H_{17}O_2N_3$ (391.0 g): C, 76.72; H, 4.34; N, 10.74; Found: C, 76.58; H, 4.50; N, 10.92; ^{1}H NMR δ 1.3 (t, 3H, CH_3), 2.7 (q, 2H, CH_2), 7.25-8.0 (m, 10H, aromatic protons), 8.06 (d, 2H, C_1-H and N-CH=N); ms: m/z: 391(M^+), 341, 301, 223, 210, 196 and 169.

4. A.4 CONCLUSION

- In the course of our programme focused on the preparation of high-nitrogen content heterocyclic compounds, we prepared tetrazolo and triazolo isoquinolines from 4-alkyl-3-dibenzofuroyl isocoumarins.

- The tetrazolo and triazolo isoquinolines were obtained by first converting 4-alkyl-3-dibenzofuroyl isocoumarins to isoquinolones, then to 1-chloro isoquinolines followed by 1-hydrazino isoquinoline which led to the target compounds.

- Apart from 2-Bromoacetyl dibenzofuran, isocoumarins containing other bromo substituents were also used to synthesize tetrazolo and triazolo isoquinolines, but because of low yield could not be characterized and therefore were not included in this chapter and 2-Bromoacetyl dibenzofuran was found to be most suitable reagent for the synthesis of triazole / tetrazole isoquinolines.

- The title compounds were screened against various disease models for therapeutic activities which is given in later chapter.

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Chapter 4:

Section B:

Introduction of nitrogen heterocyclic moiety in isocoumarin-3-carboxylic acid

4. B.1 INTRODUCTION

Chemical modification of bioactive components of naturally occurring metabolites is one of the most common approaches in drug discovery for new drugs and improved therapeutic properties. Therefore, in continuation of our research programme aimed to obtain new beneficial agents, some new isocoumarin derivatives were synthesized containing different nitrogen heterocyclic moieties. The nitrogen heterocyclic moieties which were chosen to be introduced in isocoumarins were pyrazole, Schiff base functionality, mercapto triazole and mercapto imidazole. These moieties were selected due to the following reasons:

- Pyrazole derivatives are well established in the literatures as important biologically effective heterocyclic compounds. These derivatives are the subject of many research studies due to their widespread potential pharmacological activities such as anti inflammatory[1], antipyretic[2], antimicrobial[3], anti cancer[4], antiviral[5], antioxidant[6], and anticonvulsant[7] activities.

- Some nickel pyrazolyl borate complexes has been investigated as anion selective sensors with analytical application in biological and environmental samples[8].

- Apart from biological activity disazo dyes having pyrazole moiety are also well known[9].

- Pyrazole-tethered phosphine ligands are useful catalysts for Stille, Kumada cross coupling reactions which has wide application in natural product synthesis, carbohydrate chemistry and biological research where as Hiyama cross-coupling reactions are important and selective method for producing carbon – carbon bonds[10].

Schiff bases, named for Hugo Schiff, are formed when any primary amine reacts with an aldehyde or a ketone under specific conditions. Structurally, a Schiff base (also

known as imine or azomethine) is a nitrogen analogue of an aldehyde or ketone in which the carbonyl group (C= O) has been replaced by an imine or azomethine group.

They are widely used for industrial purposes and also exhibit a broad range of biological activities as shown by examples given below:

- Imine or azomethine groups are present in various natural, natural-derived and non-natural compounds. The imine group present in such compounds has been shown to be critical to their biological activities[11].

- Schiff bases have been shown to exhibit a broad range of biological activities, including antifungal, antibacterial, anti malarial, anti proliferative, anti inflammatory, antiviral, and antipyretic properties[12].

- Chiral metal complexes of binapthyl schiff base ligands are active catalysts for stereoselective organic transformations including hydroxylation of styrene, aldol reactions, alkene epoxidation, trimethylsilylcyanation of aldehydes, desymmetrization of meso-N-sulfonylaziridine, Baeyer-Villiger oxidation of aryl cyclobutanone, Diels-Alder reactions of 1,2-dihydropyridine, and ring-opening polymerization of lactide[13].

- Schiff base-transition metal complexes have also applications in clinical and analytical fields[14].

The Chemistry of N-bridged heterocycles derived from 1, 2, 4- triazole has received considerable attention in recent years due to their usefulness in different areas of biological activities and as industrial intermediates.

- An interesting heterocyclic compound of this group is 3-mercapto-1, 2, 4-triazole due to the virtue of its vicinal nucleophile mercapto group constitutes. This structure is a ready-made building block for construction of various others organic heterocycles[15].

- Various 3-substituted-4-amino-5-mercapto-1,2,4-triazoles have been reported to possess analgesic, antibacterial, antifungal, anti inflammatory, antitubercular, antiviral, herbicidal and sedative properties[16-17].

- In recent years, N- and S-containing triazole derivatives have attracted more attention for their excellent corrosion inhibition performance. In contrast to most commercial acid corrosion inhibitors which are highly toxic, many N- and S-containing triazole derivatives are environmental friendly corrosion inhibitors[18].

Imidazole and its derivatives are of great significance due to their important roles in biological systems, particularly in enzymes, as proton donors and/or acceptors, coordination system ligands and the base of charge–transfer processes.

- The imidazole nucleus appears in a number of naturally occurring products like the amino acids, histidine and purines which comprise many of the most important bases in nucleic acids[19].

- Imidazoles and its derivatives have been shown to exhibit interesting biological activities such as antimicrobial, anticryptococcal, inhibition of nitric oxide synthase, antibiotic, antifungal, and antiulcerative activities and cytotoxic activities[20].

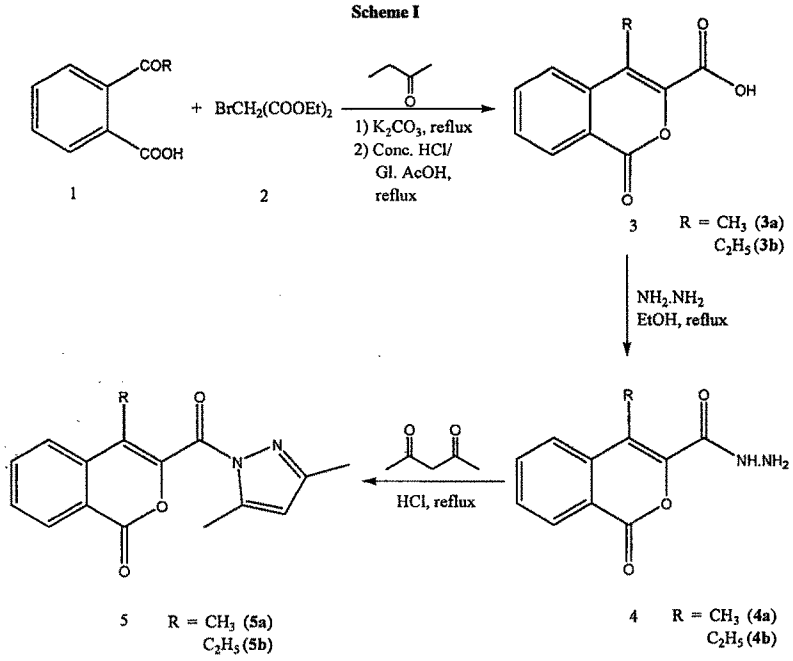
- Mercapto imidazole derivative inhibits the corrosion of copper in 1M HCl by adsorbing on its surface and blocking its active sites[21].

- More recently, imidazole and its derivatives became of interest due to their ability to bind various transition metals. In such complexes, the imidazole with its two nitrogen atoms serves as a coordination part of the molecule whereas the chiral auxiliaries at positions 1, 2, 4 or 5 provide an overall asymmetrical environment. This way, designed complexes were able to perform as promising candidates for application in a wide range of asymmetric reactions involving e.g. the Henry reaction, conjugate addition, addition of dialkylzinc

to aldehydes, allylation, epoxidation and cyclopropanation, oxidation or transfer hydrogenation[22].

Prompted from the observations described above and the chemotherapeutic value of the nitrogenous chemical scaffolds, synthesis of some novel isocoumarin derivatives containing different nitrogen heterocycles were examined in this study.

Scheme I

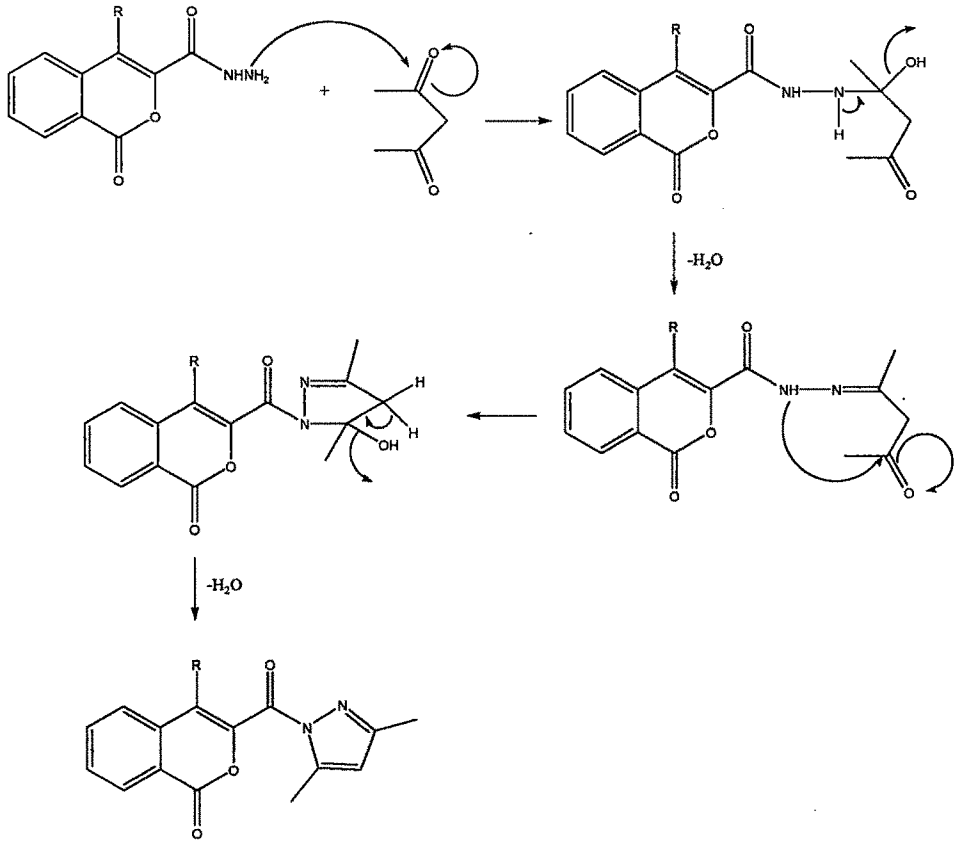


1 2 3 R = CH_3 (3a), C_2H_5 (3b)

4 R = CH_3 (4a), C_2H_5 (4b)

5 R = CH_3 (5a), C_2H_5 (5b)

Mechanism: **Dimethyl pyrazole -1-carbonyl isocoumarin derivative**



4. B.2 RESULTS AND DISCUSSION

In this chapter 4-alkyl-isocoumarin-3-carboxylic acids were synthesized instead of 4-alkyl-3-aroyl isocoumarins which were discussed in previous chapters. 4-alkyl-isocoumarin-3-carboxylic acids were used as starting material to introduce different heterocyclic moieties (pyrazole, mercapto triazole, mercapto imidazole) in it.

o-acyl benzoic acids **1** on condensation with bromodiethyl malonate **2** in presence of anhy. K_2CO_3 gives 4-alkyl-isocoumarin-3-carboxylic acids. First a diethyl malonate intermediate of isocoumarin was obtained, which on hydrolysis with glacial acetic acid and conc. hydrochloric acid leads to the desired acids **3a-b**[23] (**Scheme I**).

Isocoumarin-3-carboxylic acid on treatment with hydrazine hydrate in ethyl alcohol gets converted to isocoumarin-3-carboxylic acid hydrazide **4a-b**.

The frequencies obtained in IR spectra are 1694, 1652, 3269, 1588 for γ lactone, C=O and N-H (amide) and N-H (amine) respectively. (**Fig. 4.B.1**)

The signals obtained in the ^{1}H NMR spectrum of **4a** are δ 2.2 (s, 3H, CH_3), 6.9 (d, 2H, NH_2), 7.9 (t, 1H, NH), 7.6-7.8 (m, 3H, aromatic protons), 8.4 (d, 1H, C_8-H) (**Fig. 4.B.2**) and m/z at 218 (M^+), 202, 187, 173, 159, 146, 77 and 59 (**Fig. 4.B.3**).

Isocoumarin-3-carboxylic acid hydrazide on condensation with acetylacetone in hydrochloric acid leads to cyclization and Dimethyl pyrazole -1-carbonyl isocoumarin **5a-b** are obtained following Pal knorr pathway.

IR frequencies obtained in the spectra are 1709, 1644, 1586, 1489 and 1244 cm^{-1} for γ lactone, C=O, C=C, C=N and C-N respectively. (**Fig. 4.B.4**)

5b shows signals in ^{1}H NMR at δ 1.3 (t, 3H, CH_3), 2.7 (q, 2H, CH_2), 2.9 (s, 6H, CH_3), 6.2 (s, 1H, =CH), 7.5-7.8 (m, 3H, aromatic protons), 8.3 (d, 1H, C_8-H) (**Fig. 4.B.5**) and in mass spectra m/z at 295 (M^+ - 1), 267, 254, 209 and 146 (**Fig. 4.B.6**).

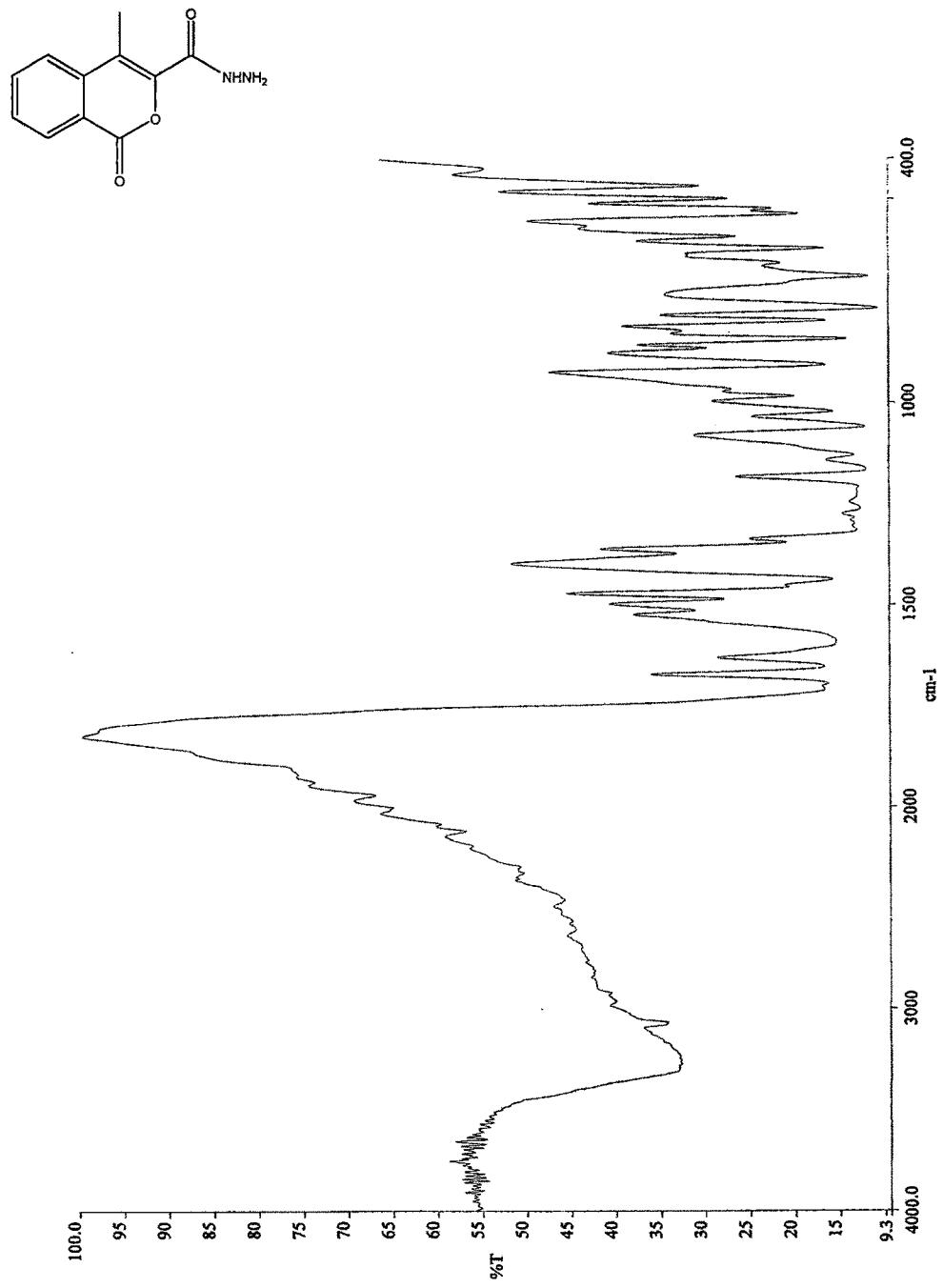


Fig. 4.B.1 – IR: **4-Methyl-isocoumarin-3-carboxylic acid hydrazide 4b**

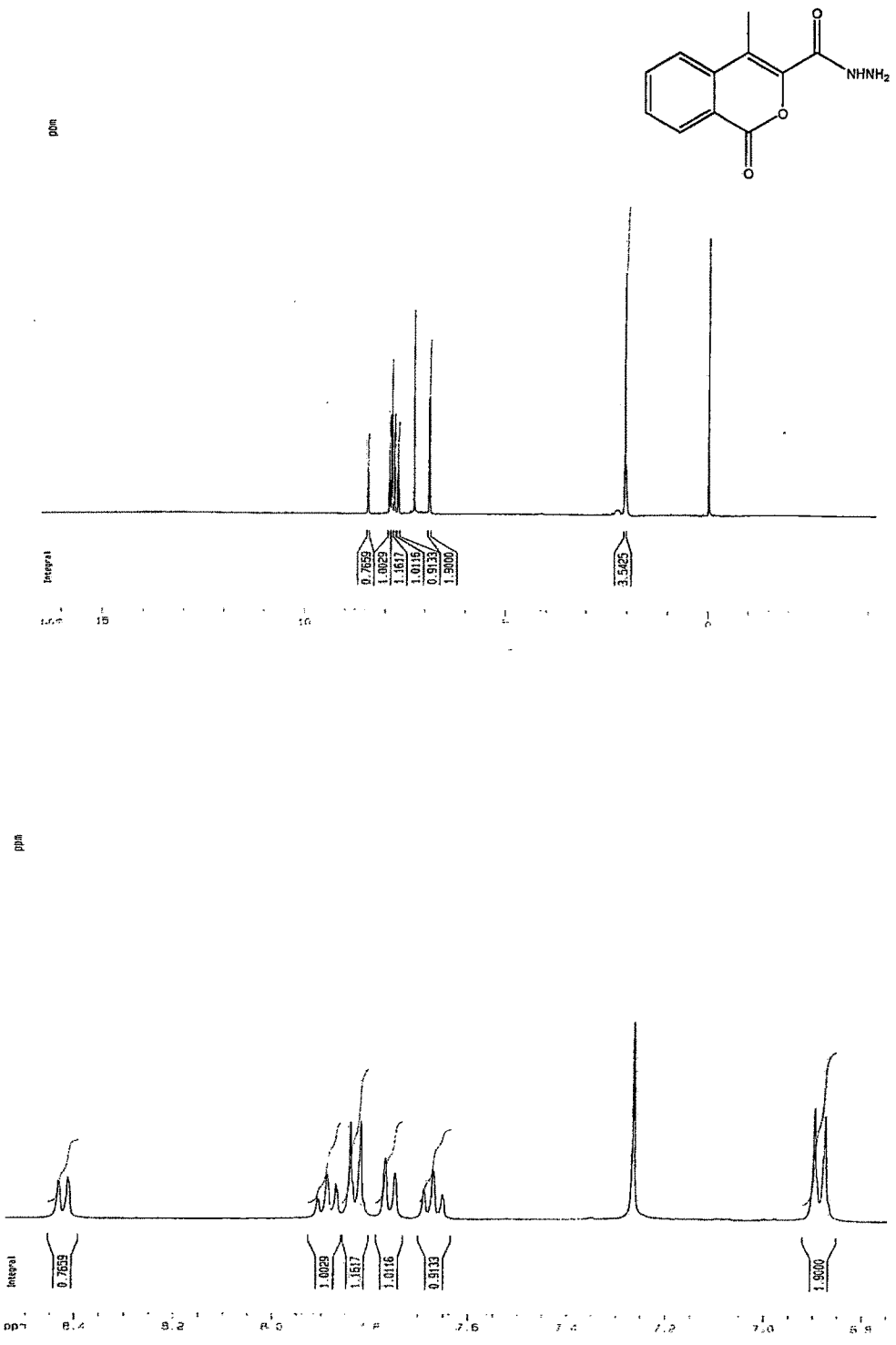


Fig. 4.B.2 – ^{1}H NMR : 4-Methyl-isocoumarin-3-carboxylic acid hydrazide 4b

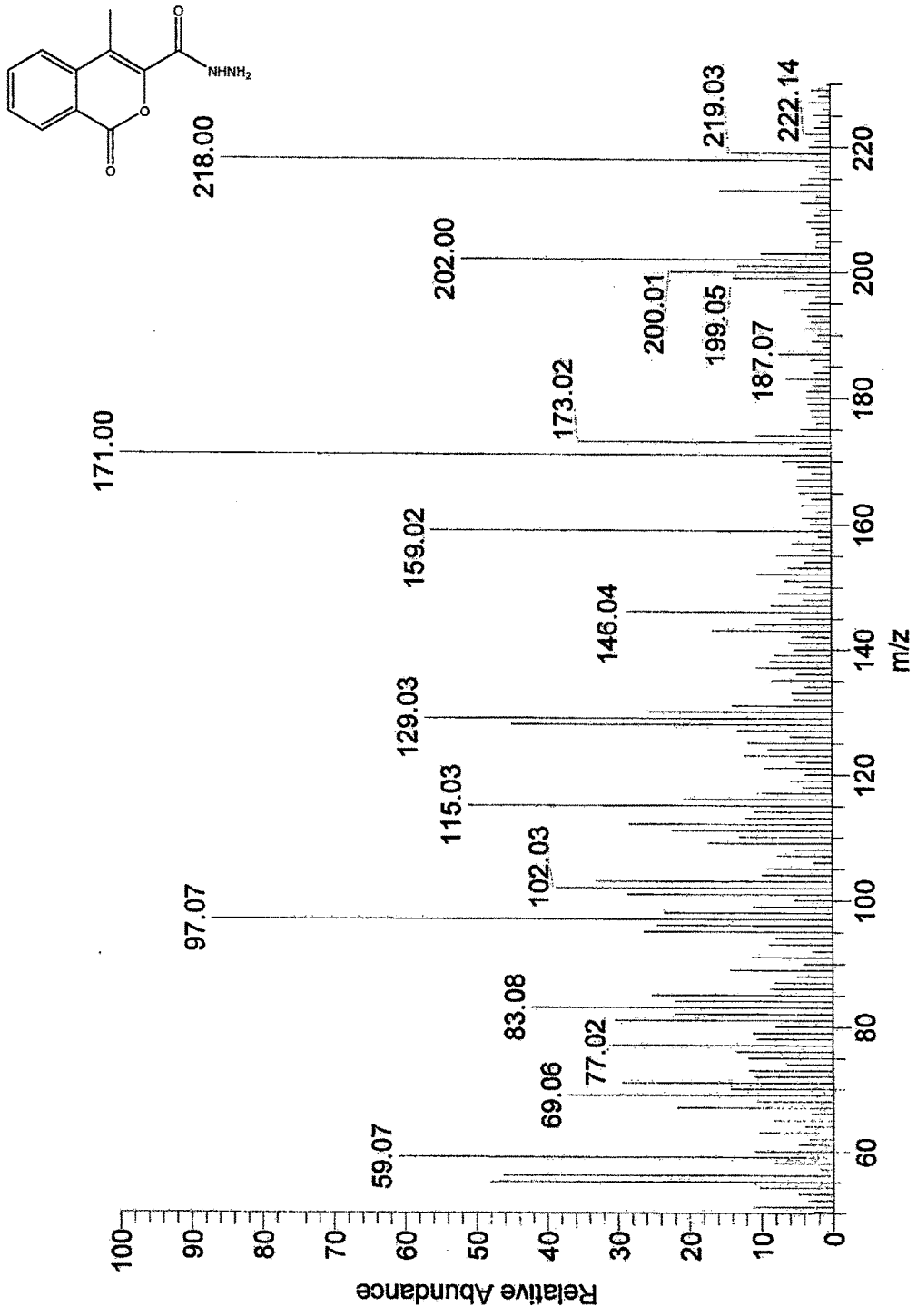
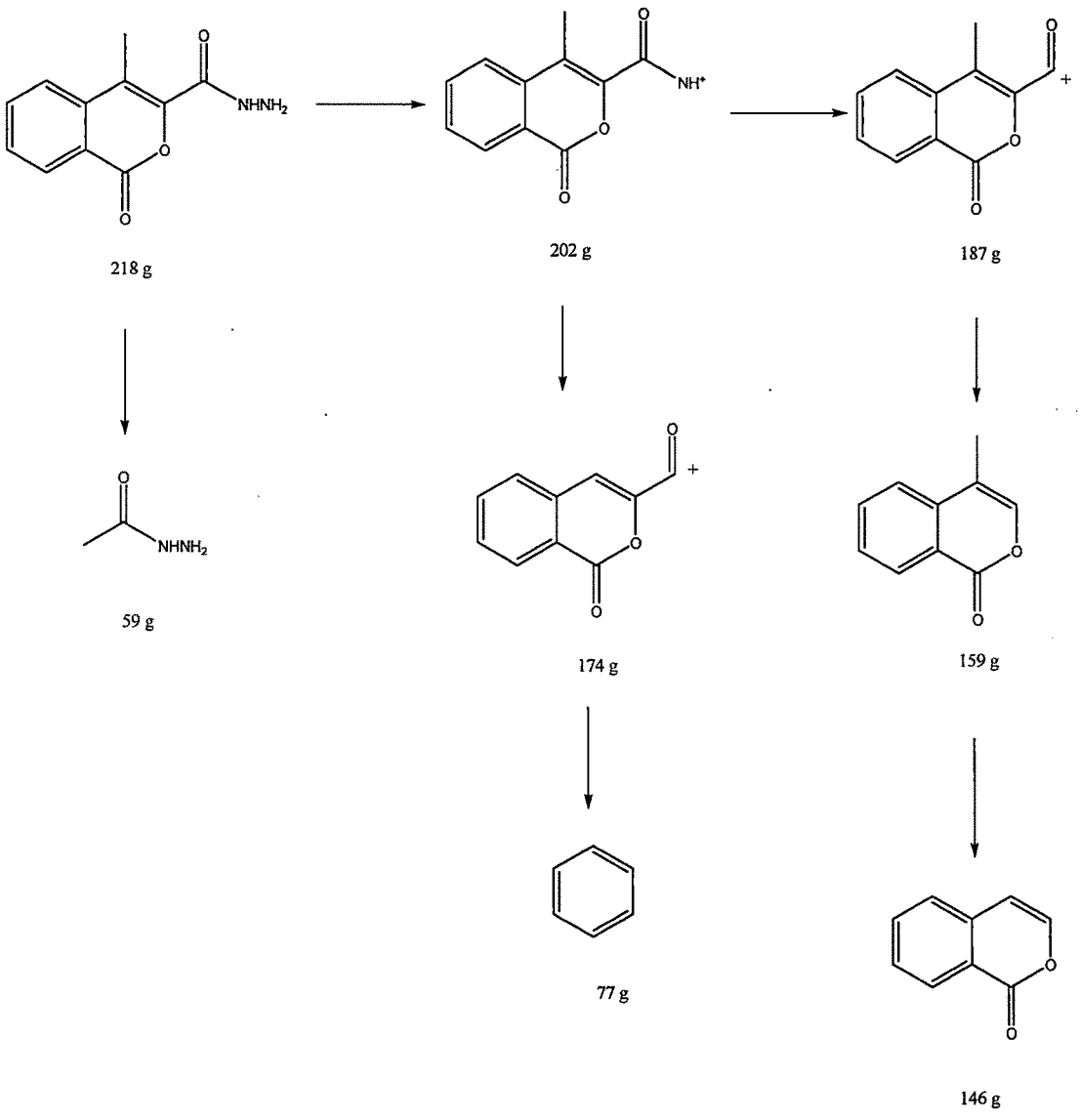


Fig. 4.B.3 – Mass spectrum: **4-Methyl-isocoumarin-3-carboxylic acid hydrazide 4b**



Fragmentation Pattern: **4-Methyl-isocoumarin-3-carboxylic acid hydrazide**

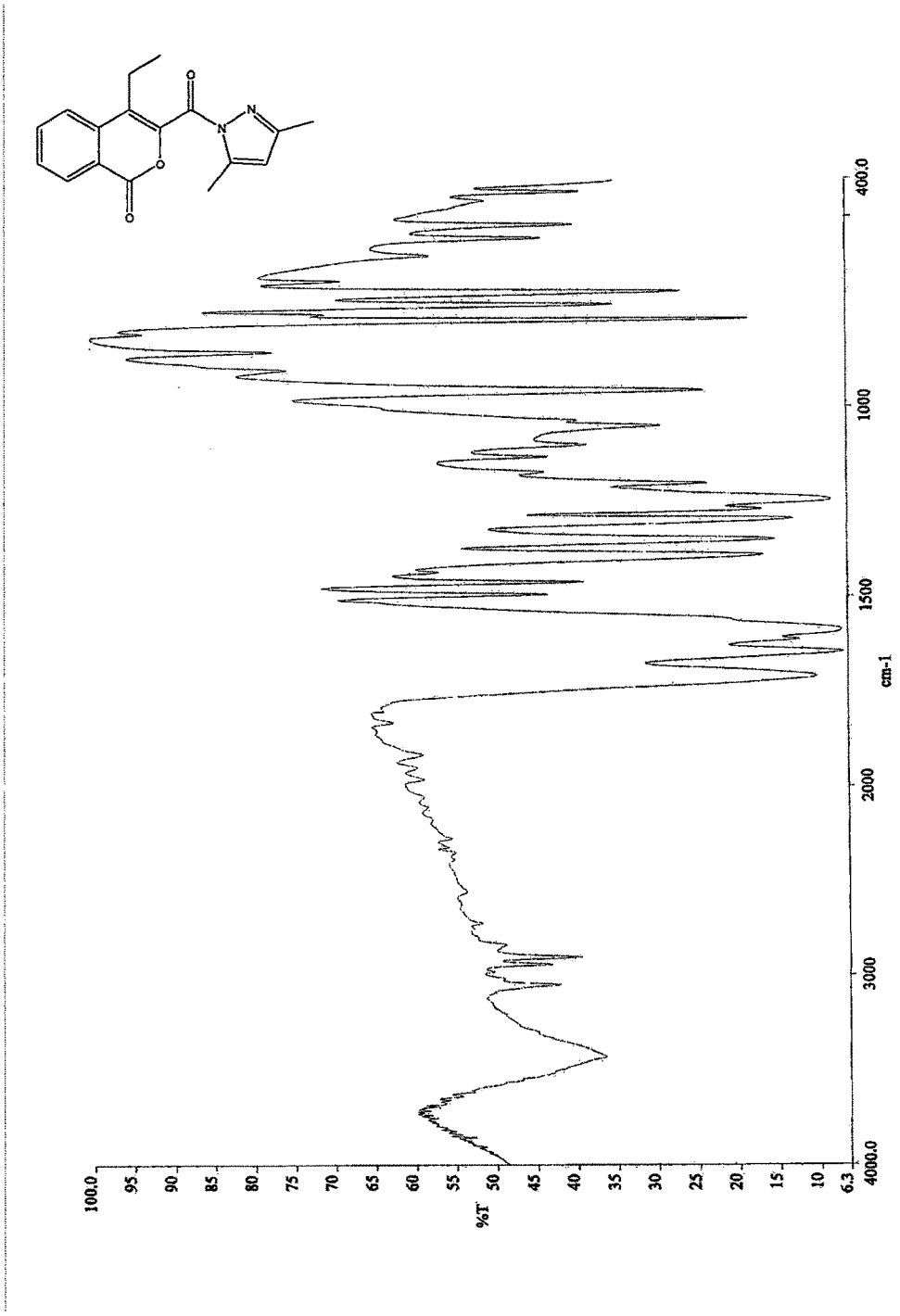
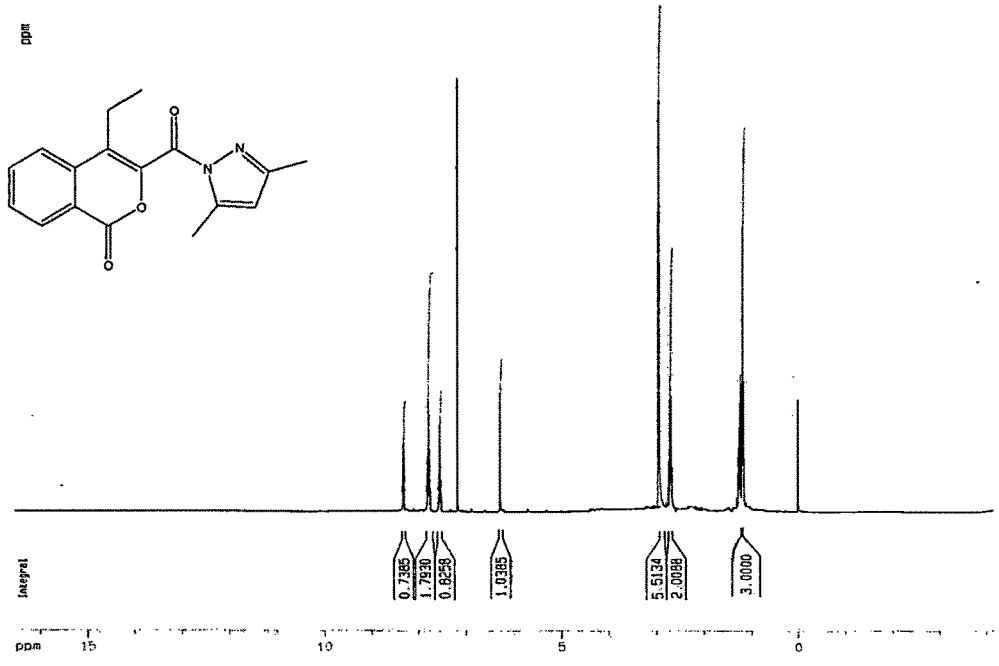
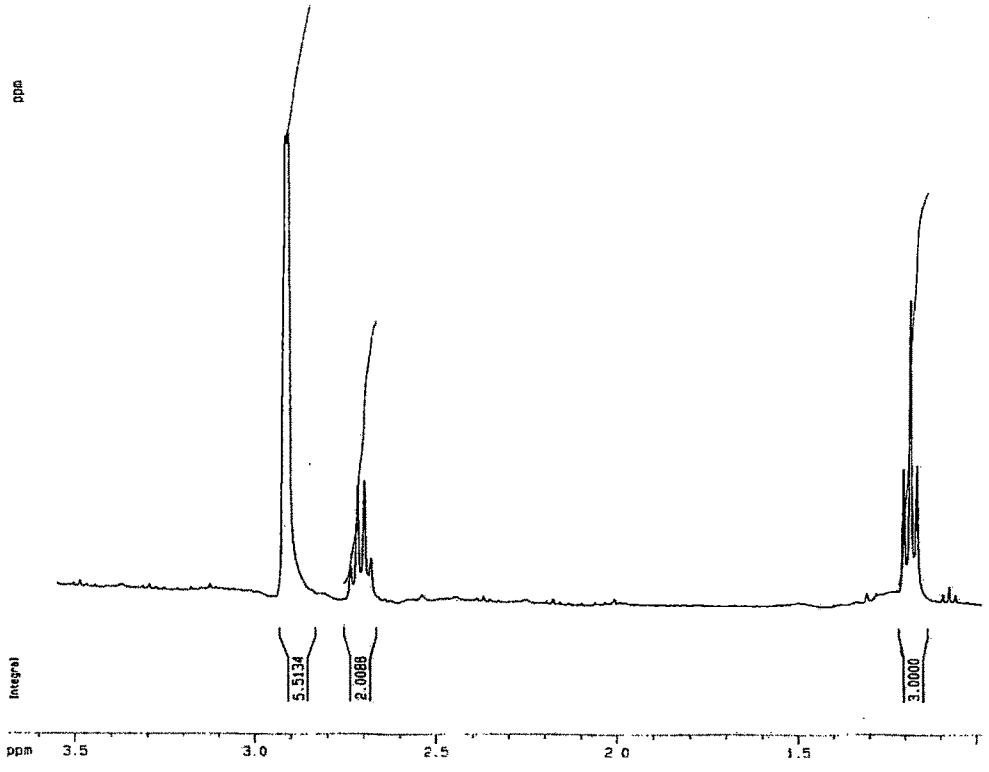


Fig. 4.B.4 – IR: **3-(3', 5'-Dimethyl pyrazole-1-carbonyl)-4-ethyl isocoumarin 5b**


ppm
Integral
0.7385
1.7930
0.8258
1.0385
5.5134
2.0088
3.0000
ppm 15 10 5 0


ppm
Integral
5.5134
2.0088
3.0000
ppm 3.5 3.0 2.5 2.0 1.5

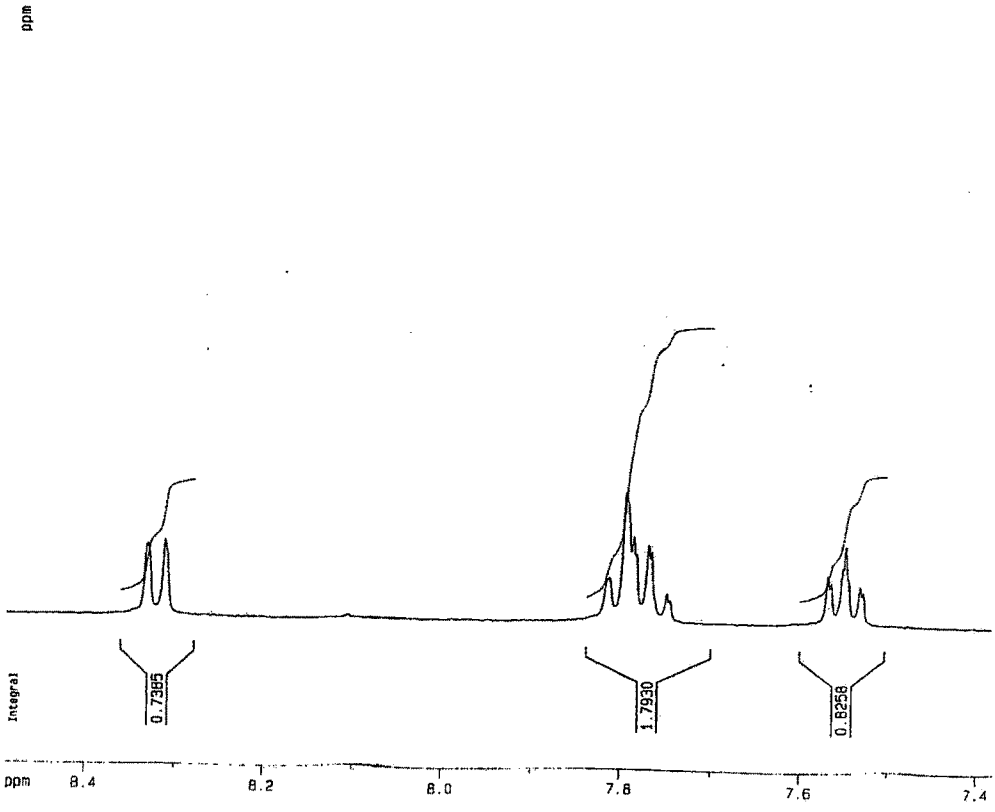


Fig. 4.B.5 – ^{1}H NMR: 3-(3', 5'-Dimethyl pyrazole-1-carbonyl)-4-ethyl isocoumarin 5b

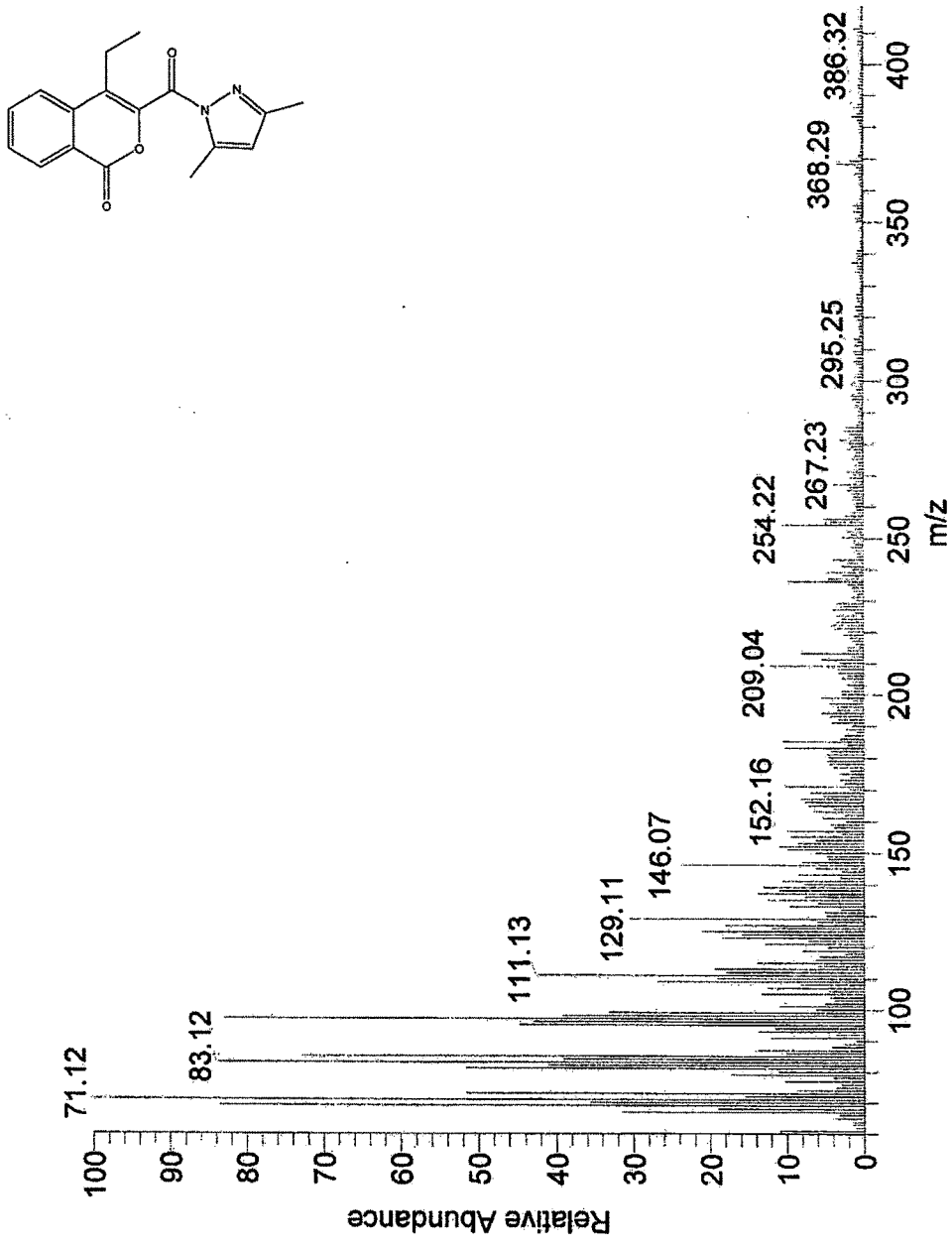
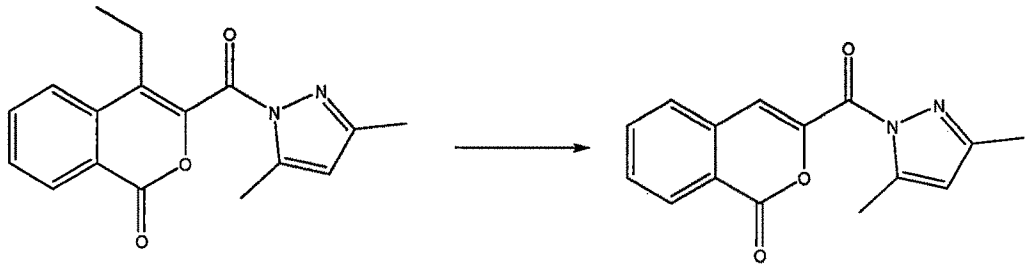
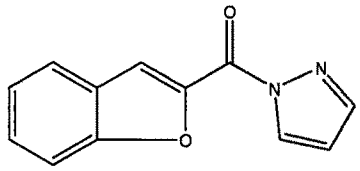


Fig. 4.B.6 - Mass spectrum: **3-(3', 5'-Dimethyl pyrazole-1-carbonyl)-4-ethyl isocoumarin 5b**

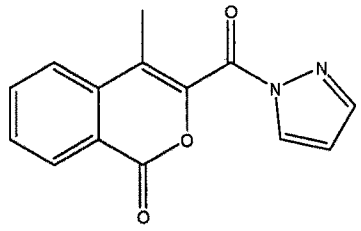


296 g

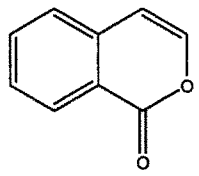
267 g



209 g



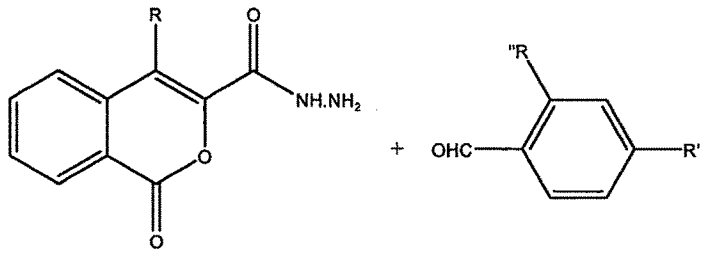
254 g



146 g

Fragmentation Pattern: **3-(3', 5'-Dimethyl pyrazole-1-carbonyl)-4-ethyl isocoumarin**

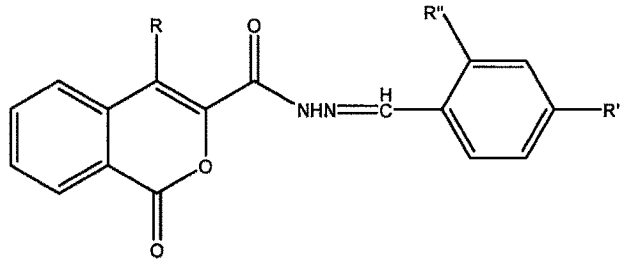
Scheme II



4 R = CH_3 (**4a**)
C_2H_5 (**4b**)

6

EtOH
reflux



7

R = CH_3, R' = NO_2, R" = H (**7a**)
R = CH_3, R' = OH, R" = H (**7b**)
R = CH_3, R' = OCH_3, R" = H (**7c**)
R = CH_3, R' = H, R" = H (**7d**)
R = CH_3, R' = H, R" = OH (**7e**)
R = CH_3 & cinnamaldehyde (**7f**)
R = C_2H_5, R' = NO_2, R" = H (**7g**)
R = C_2H_5, R' = OH, R" = H (**7h**)
R = C_2H_5, R' = OCH_3, R" = H (**7i**)
R = C_2H_5, R' = H, R" = H (**7j**)
R = C_2H_5, R' = H, R" = OH (**7k**)
R = C_2H_5 & cinnamaldehyde (**7l**)

(**Scheme II**) shows the synthesis of Schiff base derivatives of isocoumarin. The Schiff bases were prepared keeping in view the importance of them in different biological systems.

Isocoumarin-3-carboxylic acid hydrazide **4** when refluxed with different substituted benzaldehyde **6** in ethanol in presence of conc. sulphuric acid leads to formation of Schiff bases **7a-l**.

The frequencies obtained in IR spectra are 1703, 1623, 1597, 1296 cm^{-1} for γ lactone, C=O, C=N and C-N respectively (**Fig. 4.B.7**).

^1H NMR spectra of **7c** and **7h** shows signals at δ 2.0 (s, 3H, CH_3), 4.0 (s, 3H, OCH_3), 7.3 (s, 1H, -C=H), 7.1-8.3 (m, 7H, aromatic protons), 8.4 (dd, 1H, C_8-H), 9.3 (s, 1H, NH) (**Fig. 4.B.8**) and δ 1.1 (t, 3H, CH_3), 2.5(q, 2H, CH_2), 4.9 (s, 1H, OH), 7.3 (s, 1H, =CH), 7.1-8.3 (m, 7H, aromatic protons), 8.45 (d, 1H, C_8-H), 10.1(s, 1H, NH) (**Fig. 4.B.10**).

Mass spectra of **7c** shows m/z at 336 (M^+), 264, 215, 187, 135, 71 and 57 (**Fig. 4.B.9**) and **7h** at m/z 336 (M^+), 308, 278, 213, 165 and 122 (**Fig. 4.B.11**).

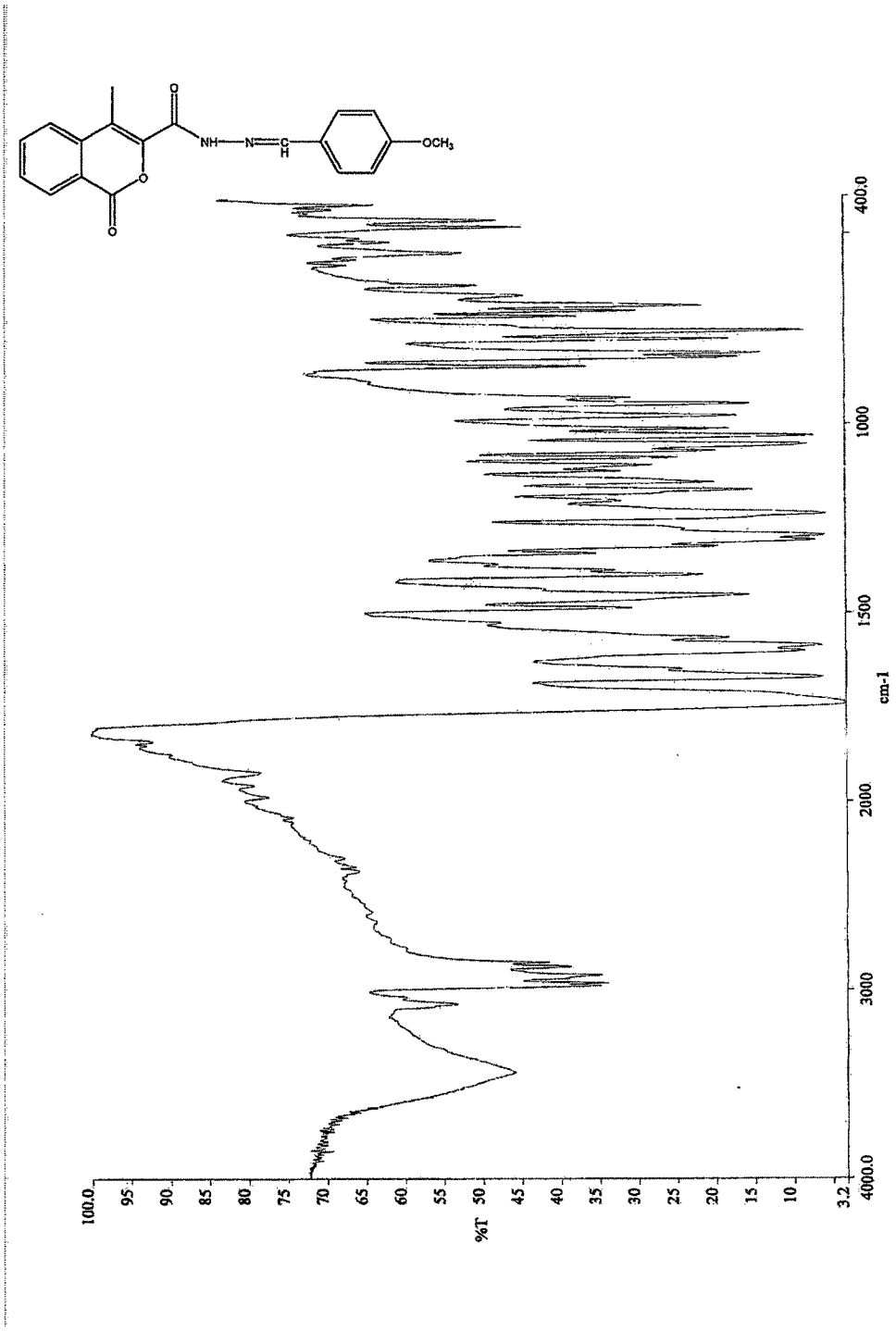


Fig. 4.B.7 – IR: **4-Methyl-isocoumarin-3-carboxylic acid (4'-methoxy benzylidene)-hydrazide 7c**

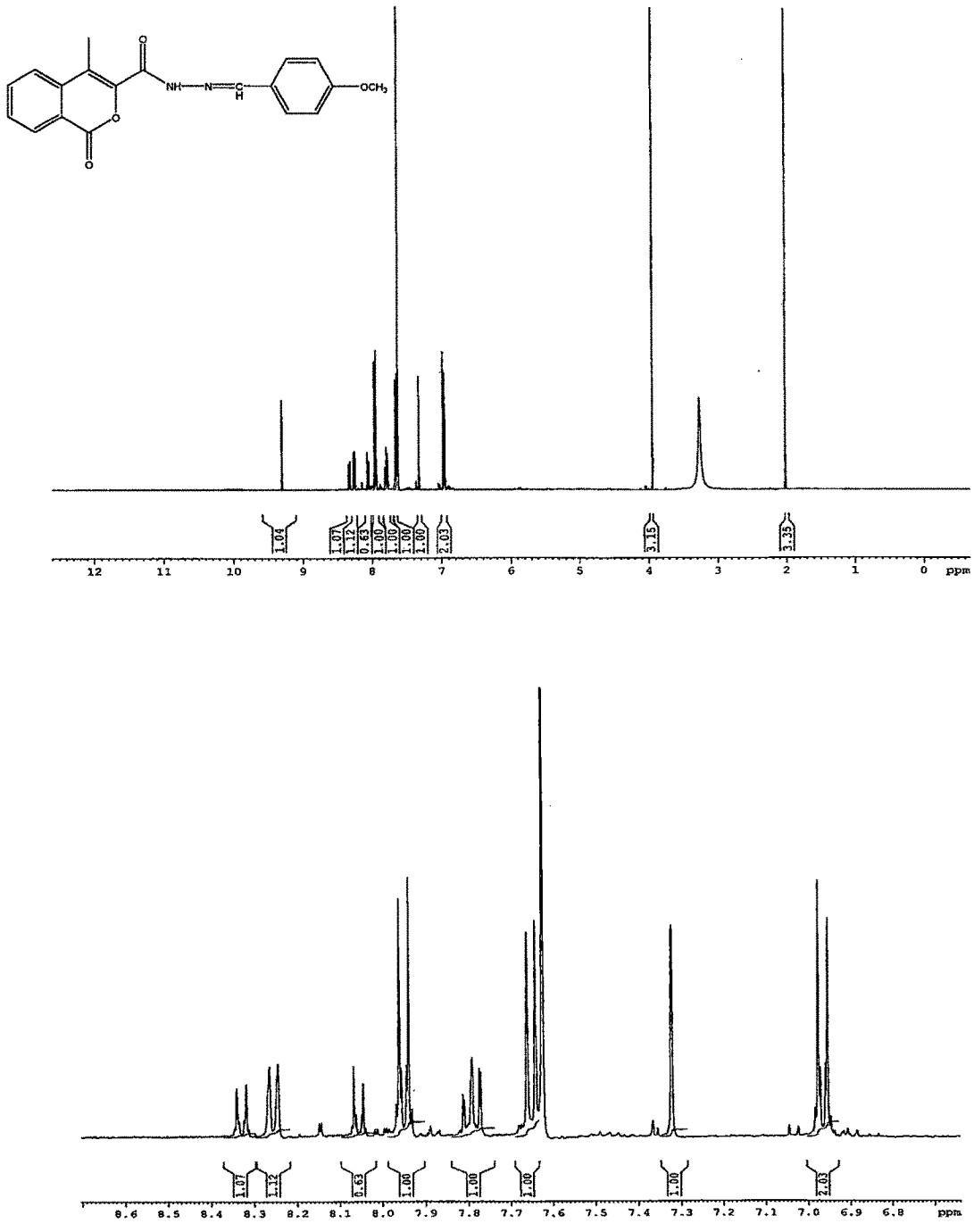


Fig. 4.B.8 – ^{1}H NMR: **4-Methyl-isocoumarin-3-carboxylic acid (4'-methoxy benzylidene)-hydrazide 7c**

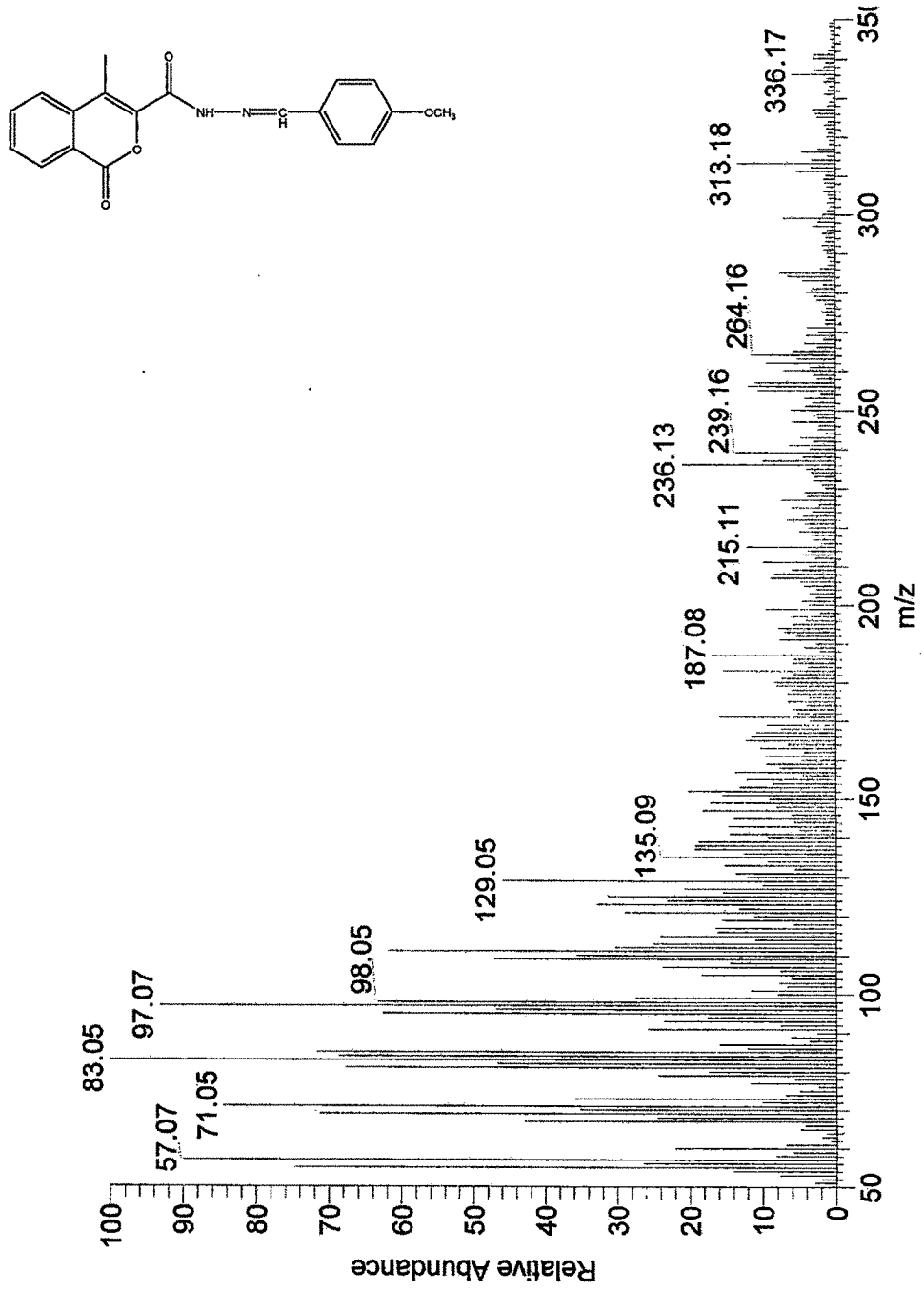
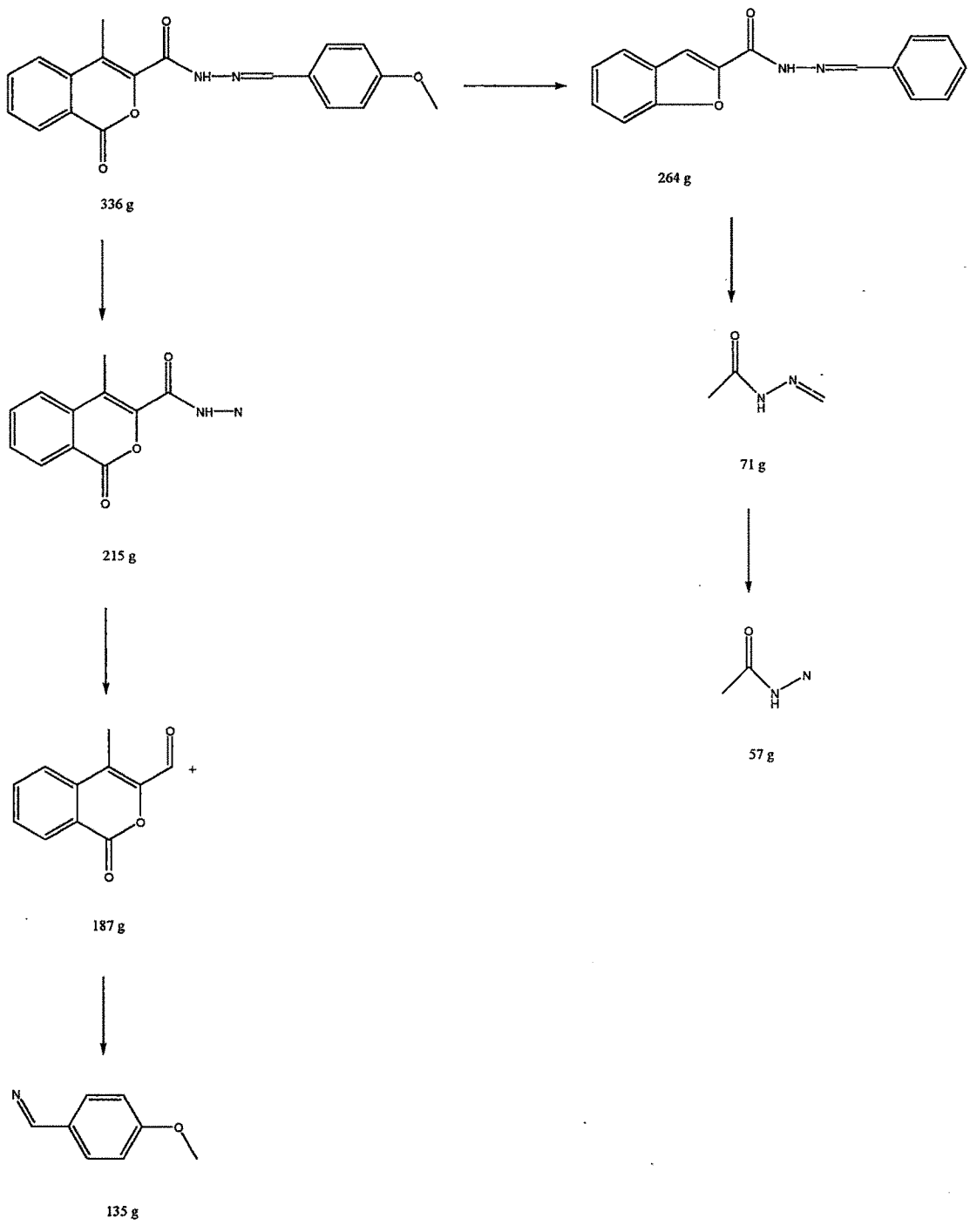


Fig. 4.B.9 – Mass spectrum: **4-Methyl-isocoumarin-3-carboxylic acid (4'-methoxy benzylidene)-hydrazide 7c**



Fragmentation Pattern: **4-Methyl-isocoumarin-3-carboxylic acid (4'-methoxy benzylidene)-hydrazide**

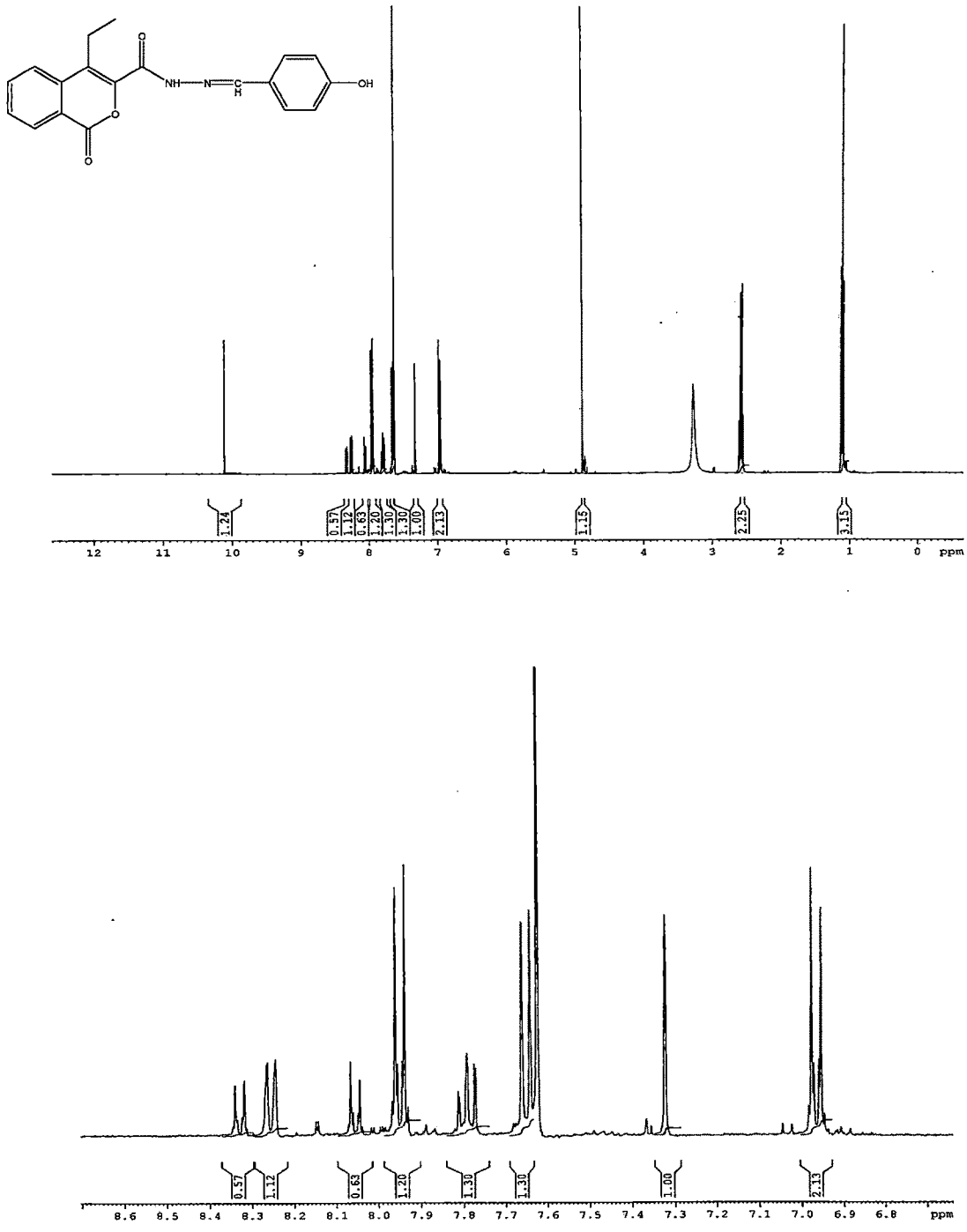


Fig. 4.B.10 – ^{1}H NMR: 4-Ethyl-isocoumarin-3-carboxylic acid (4'-hydroxy benzylidene)-hydrazide 7h

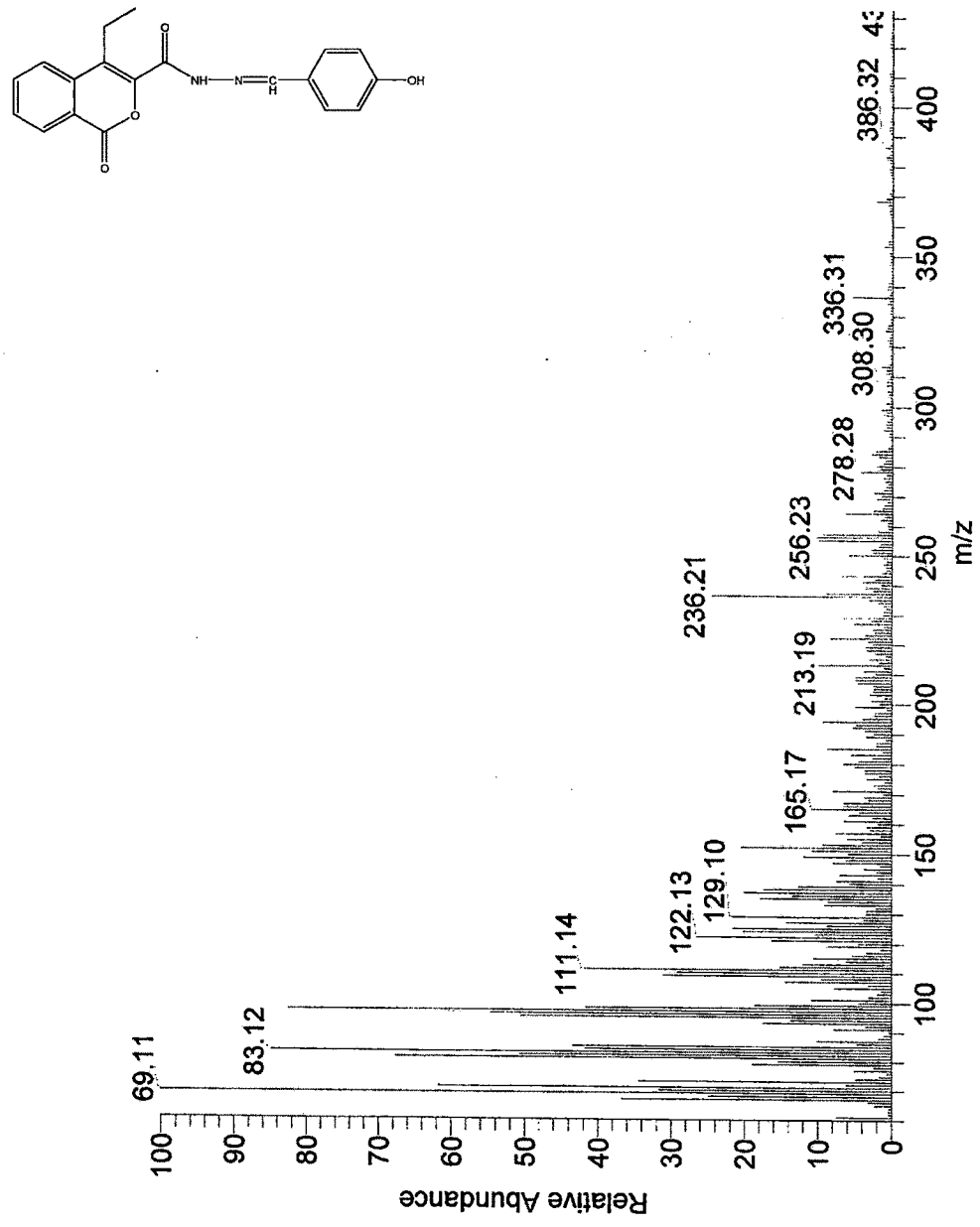
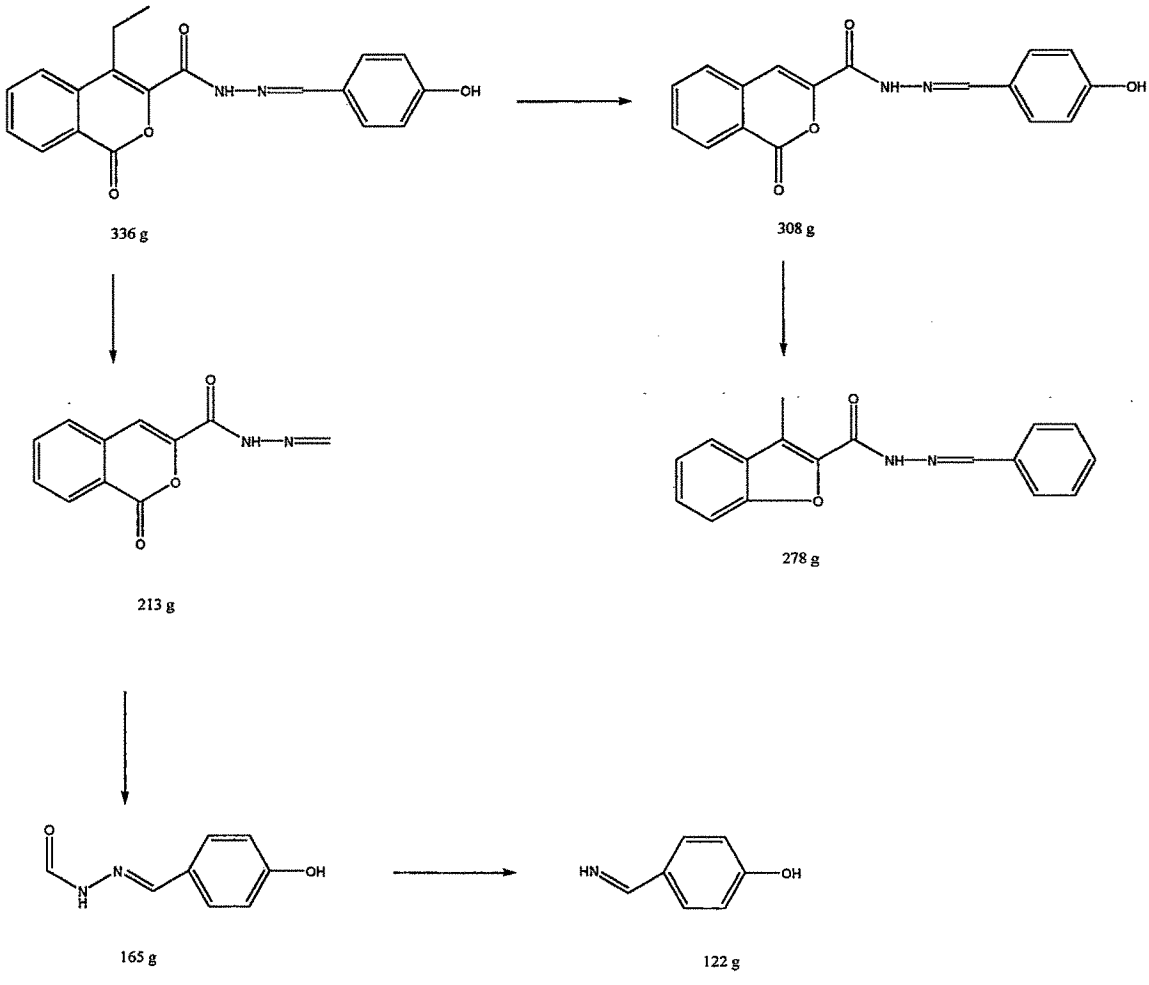
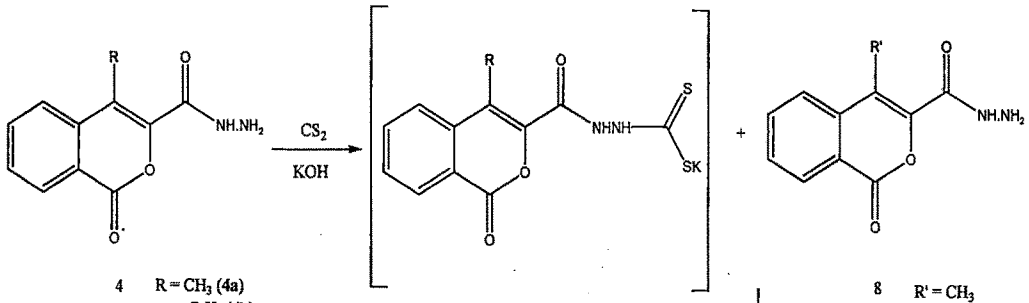


Fig. 4.B.11 – Mass spectrum: **4-Ethyl-isocoumarin-3-carboxylic acid (4'-hydroxy benzylidene)-hydrazide 7h**



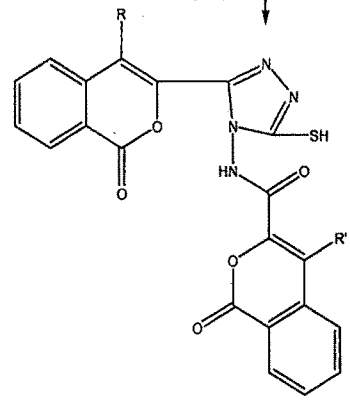
Fragmentation Pattern: **4-Ethyl-isocoumarin-3-carboxylic acid (4'-hydroxy benzylidene)-hydrazide**

Scheme III



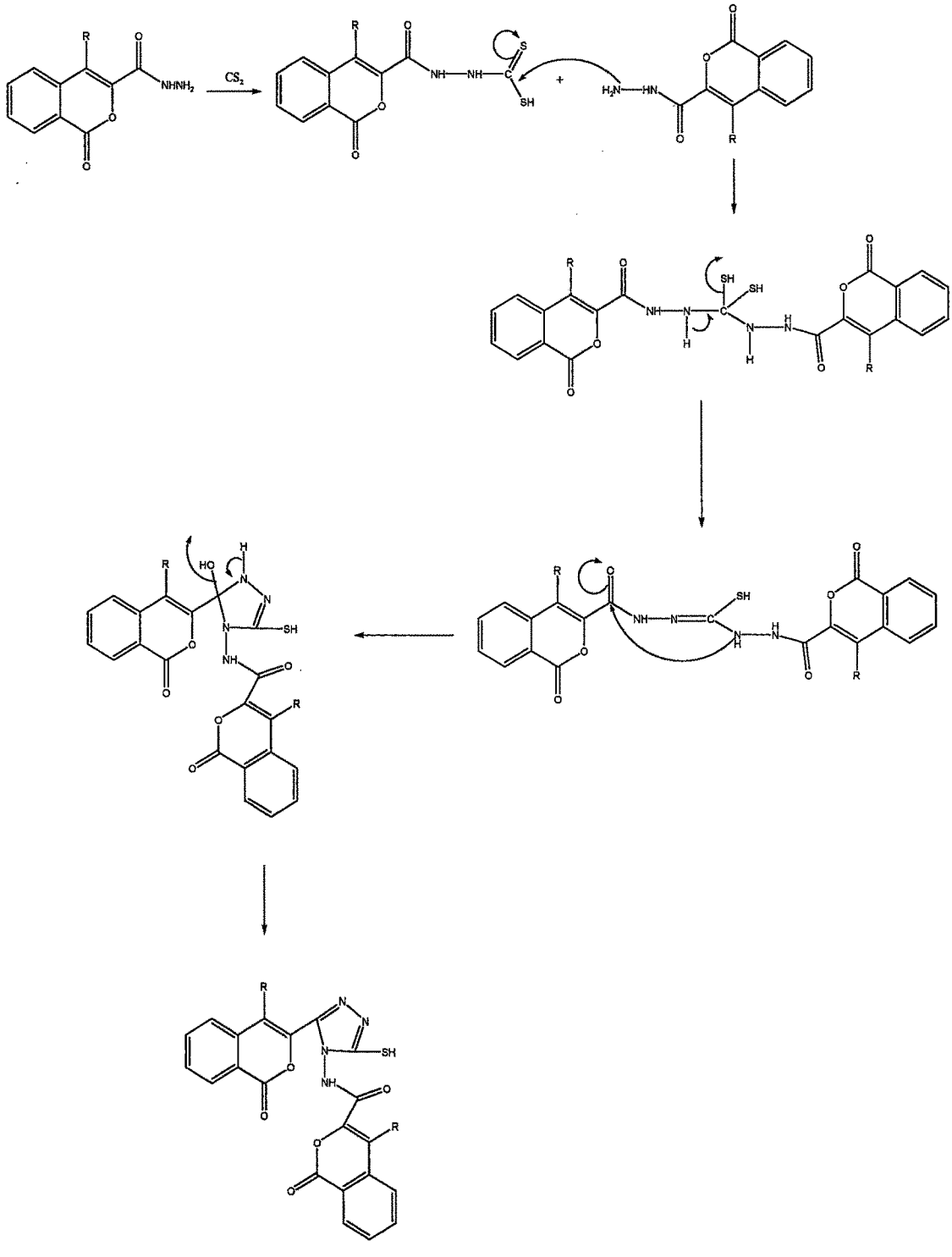
4 R = CH_3 (4a)
C_2H_5 (4b)

8 R' = CH_3
C_2H_5



9 R = R' = CH_3 (9a)
R = R' = C_2H_5 (9b)
R = CH_3, R' = C_2H_5 (9c)
R = C_2H_5, R' = CH_3 (9d)

Mechanism: **4-Alkyl-isocoumarin-3-carboxylic acid [3'-mercapto-5-(4''-alkyl-isocoumarin-3''-yl) - (1', 2', 4') triazole-4'-yl] amide**



After the introduction of pyrazole and Schiff base functionality in isocoumarin ring, the next moiety to be introduced in isocoumarin via isocoumarin-3-carboxylic acid was mercapto triazole. Mercapto triazole moiety was chosen because of the presence of both sulphur and nitrogen atom in it, so that the effect of both the hetero atoms together can be seen in different biological activities, as until now the effect of sulphur atom was not studied in any of the series and hence can be compared with other synthesized compounds in SAR studies.

To introduce mercapto triazole in isocoumarins, Isocoumarin-3-carboxylic acid hydrazide **4** was stirred with carbon disulphide and potassium hydroxide to give an intermediate which on further reaction, in situ, with second mole of isocoumarin-3-carboxylic acid hydrazide **8** leads to isocoumarin-3-carboxylic acid-mercapto triazole amide derivatives **9a-d** (**Scheme III**).

IR spectra show absorption frequencies for γ lactone at 1710, C=O at 1682, C=N at 1586, CONH at 3315, C=S at 1347 and SH at 2469 cm^{-1} (**Fig. 4.B.12**).

9a and **9c** shows signals in ^1H NMR at δ 2.1 (s, 6H, CH_3), 2.5 (s, 1H, SH), 7.7-8.1 (m, 6H, aromatic protons), 8.35 (d, 2H, C_8-H), 10.1 (s, 1H, NH) (**Fig. 4.B.13**) and δ 1.2 (t, 3H, CH_3), 2.5 (s, 3H, CH_3), 2.7-2.8 (q, 4H, CH_2), 5.4 (s, 1H, SH), 7.6-7.9 (m, 6H, aromatic protons), 8.4 (d, 2H, C_8-H), 9.5 (s, 1H, NH) (**Fig. 4.B.15**).

The m/z obtained in mass spectra of **9a** is 460 (M^+), 428, 383,369, 368, 344, 284, 256, 224, 186, 109 and 57 (**Fig. 4.B.14**) and **9c** at m/z: 472 (M^+ - 2), 430, 386, 341, 274, 256, 160, 109 and 78 (**Fig. 4.B. 16**).

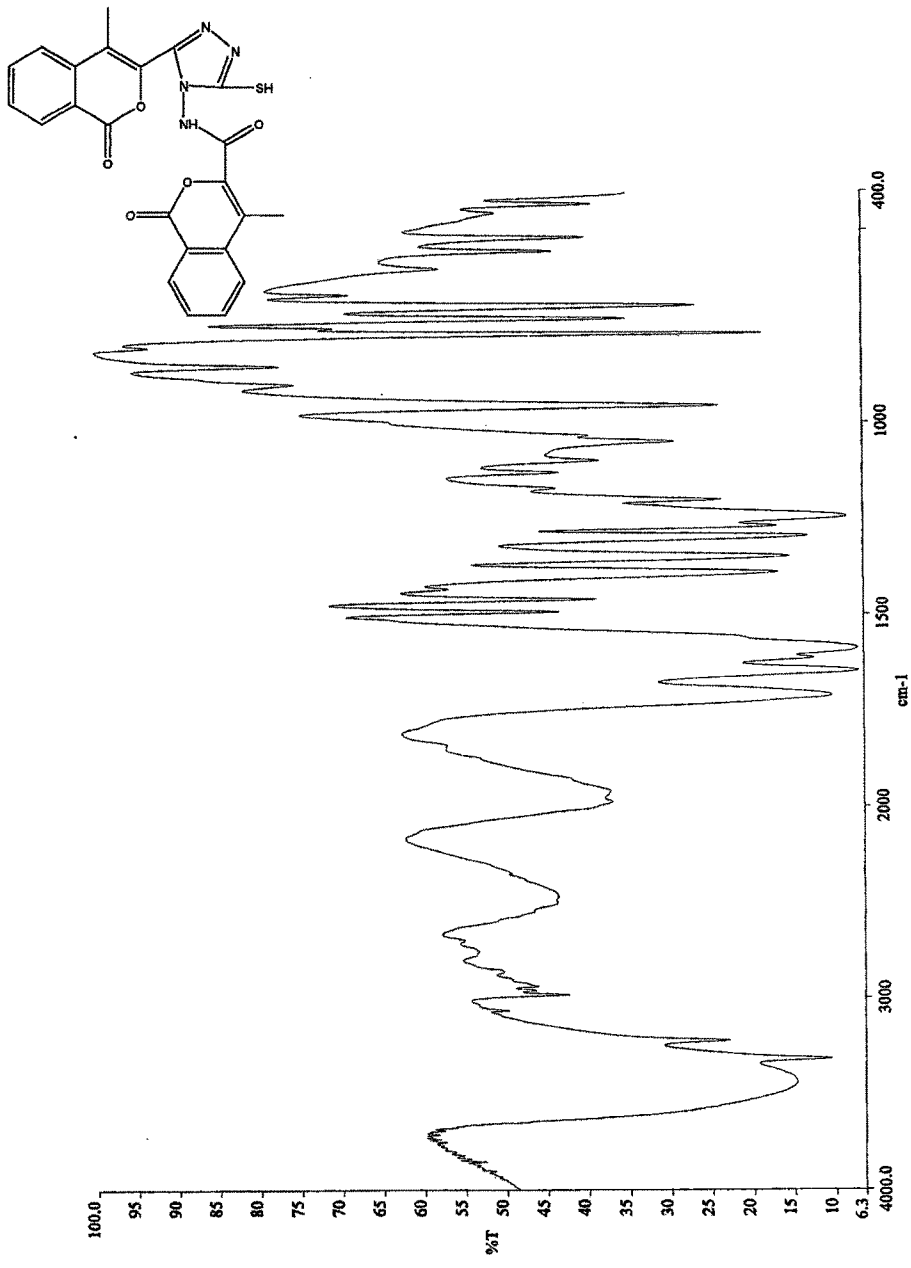
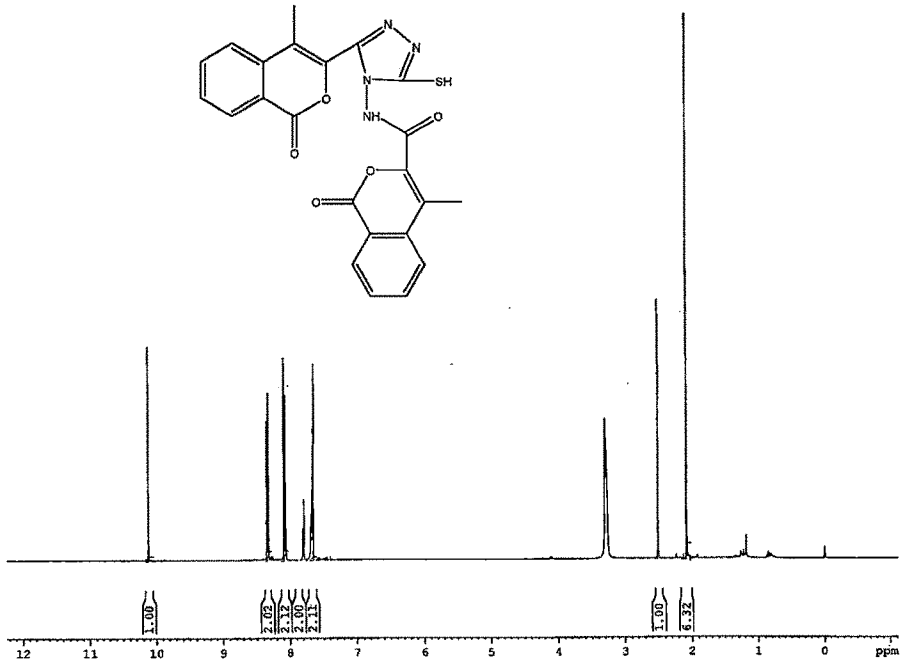


Fig. 4.B.12 – IR: **4-Methyl-isocoumarin-3-carboxylic acid [3'-mercapto-5-(4''-methyl-isocoumarin-3''-yl) - (1', 2', 4') triazole-4'-yl] amide 9a**



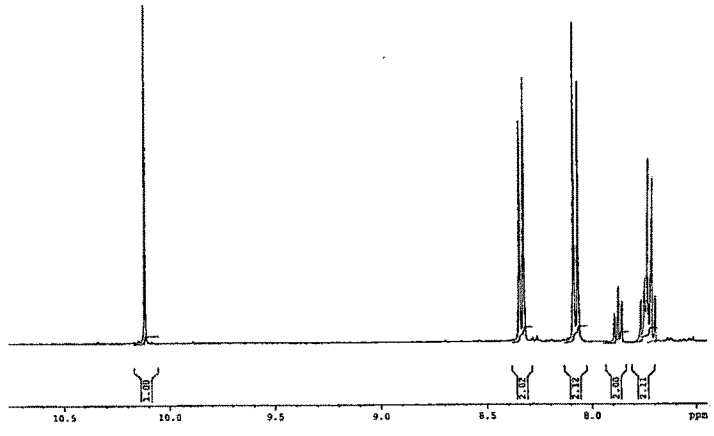


Fig. 4.B.13 – ^{1}H NMR: **4-Methyl-isocoumarin-3-carboxylic acid [3'-mercapto-5-(4''-methyl-isocoumarin-3''-yl) - (1', 2', 4') triazole-4'-yl] amide 9a**

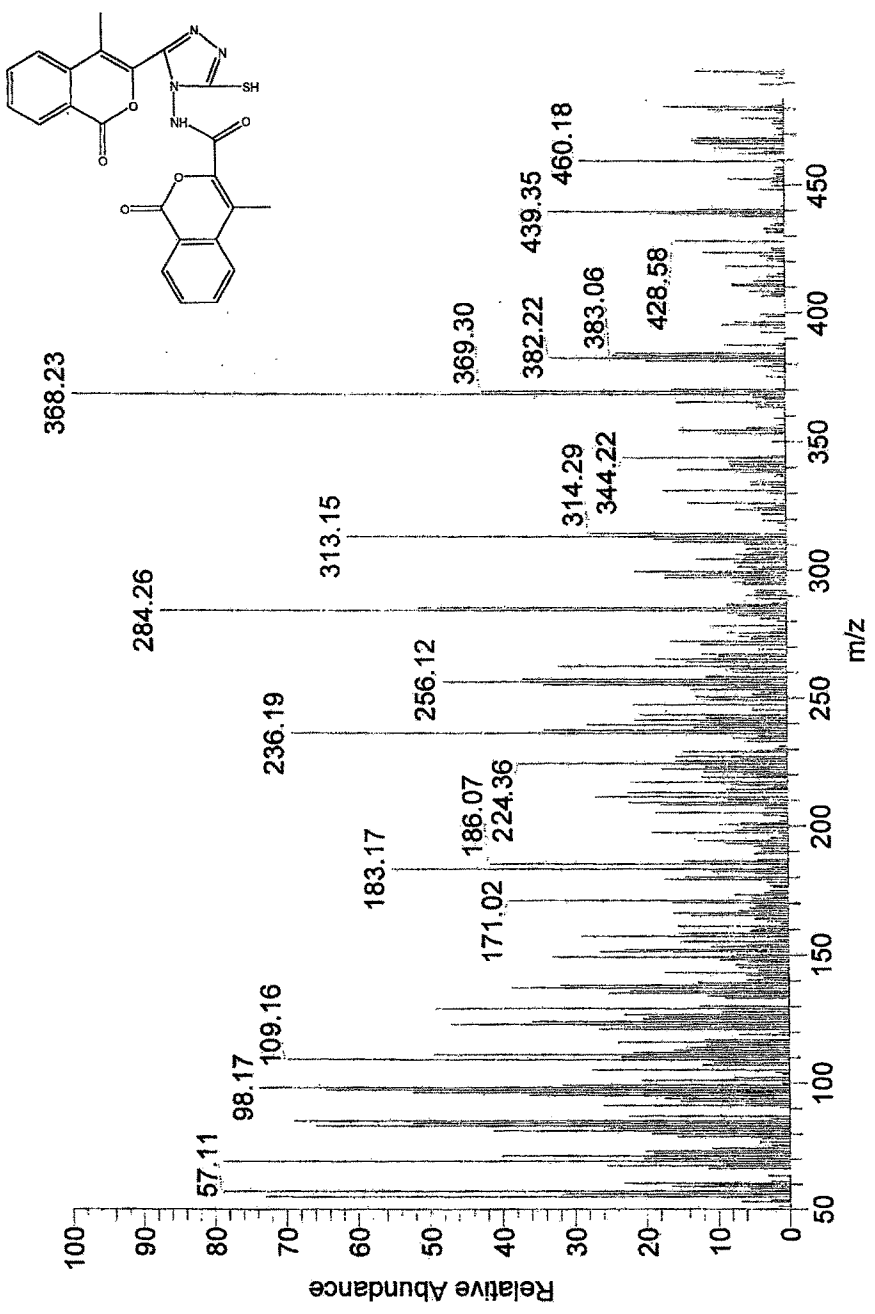
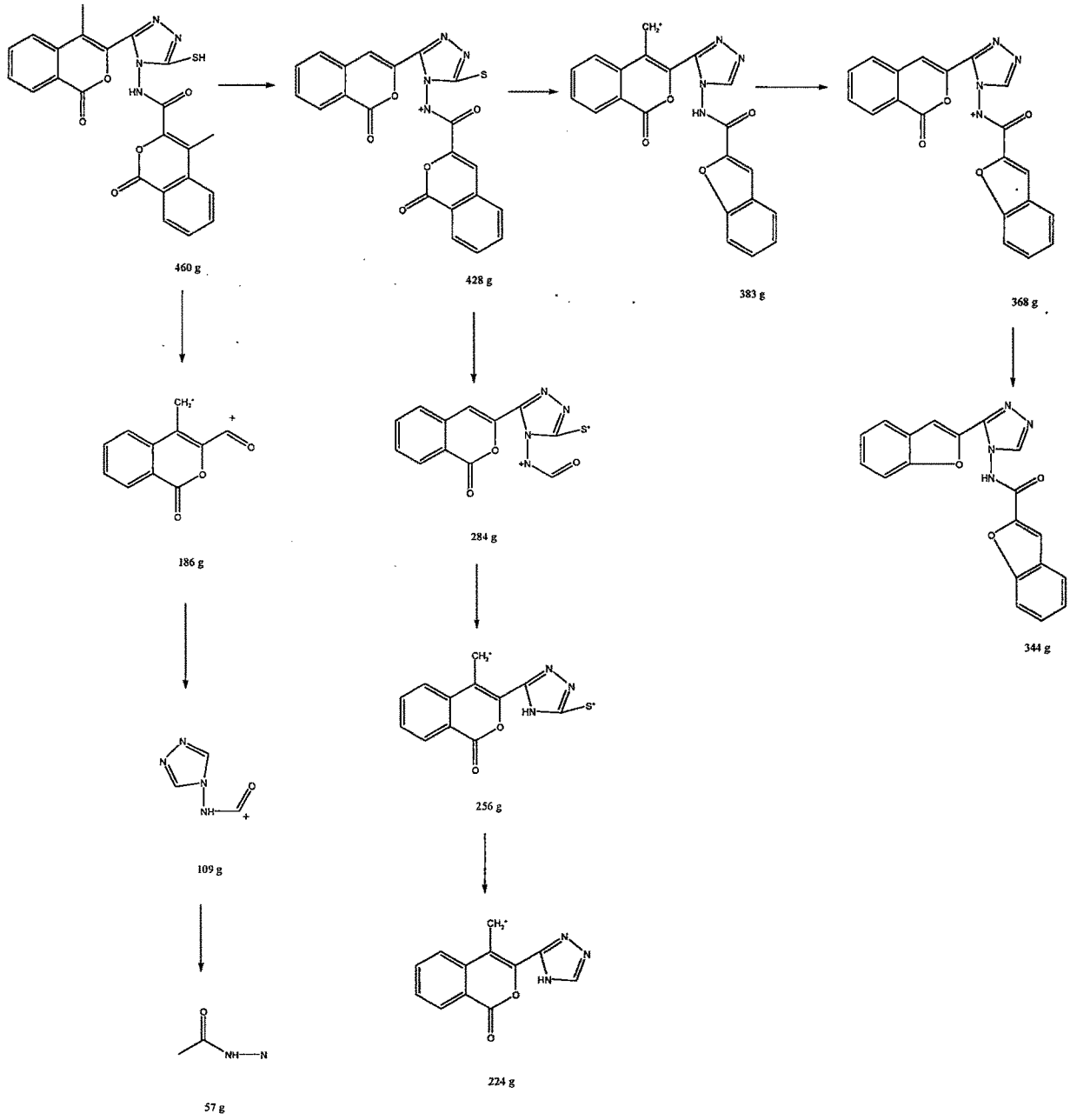


Fig.4.B.14 – Mass spectrum: **4-Methyl-isocoumarin-3-carboxylic acid [3'-mercapto-5-(4''-methyl-isocoumarin-3''-yl) - (1', 2', 4') triazole-4'-yl] amide 9a**



Fragmentation Pattern: **4-Methyl-isocoumarin-3-carboxylic acid [3'-mercapto-5-(4''-methyl-isocoumarin-3''-yl) - (1', 2', 4') triazole-4'-yl] amide**

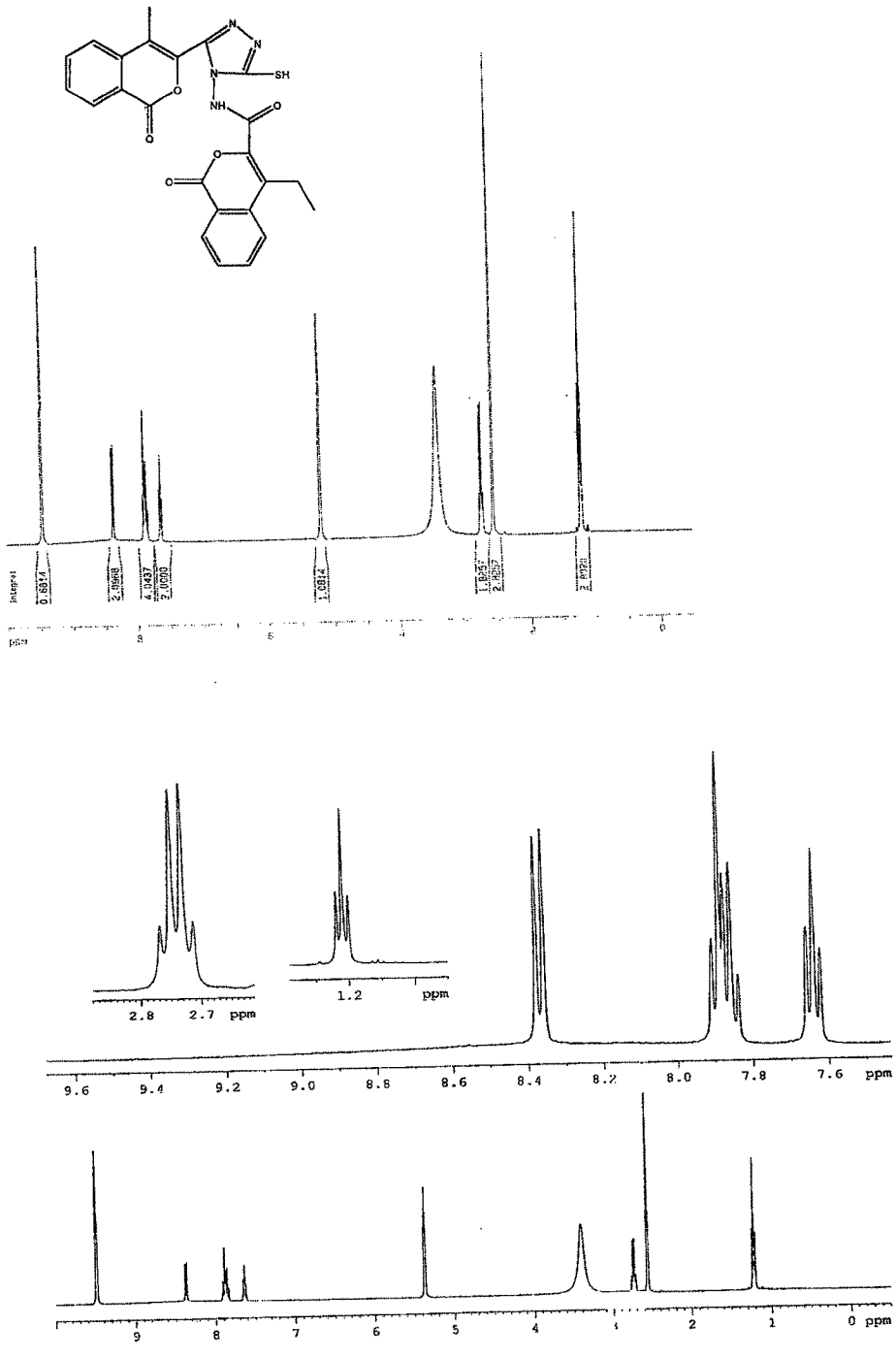


Fig.4.B.15 - ^{1}H NMR: **4-Methyl-isocoumarin-3-carboxylic acid [3'-mercapto-5-(4''-ethyl-isocoumarin-3''-yl) - (1', 2', 4') triazole-4'-yl] amide 9c**

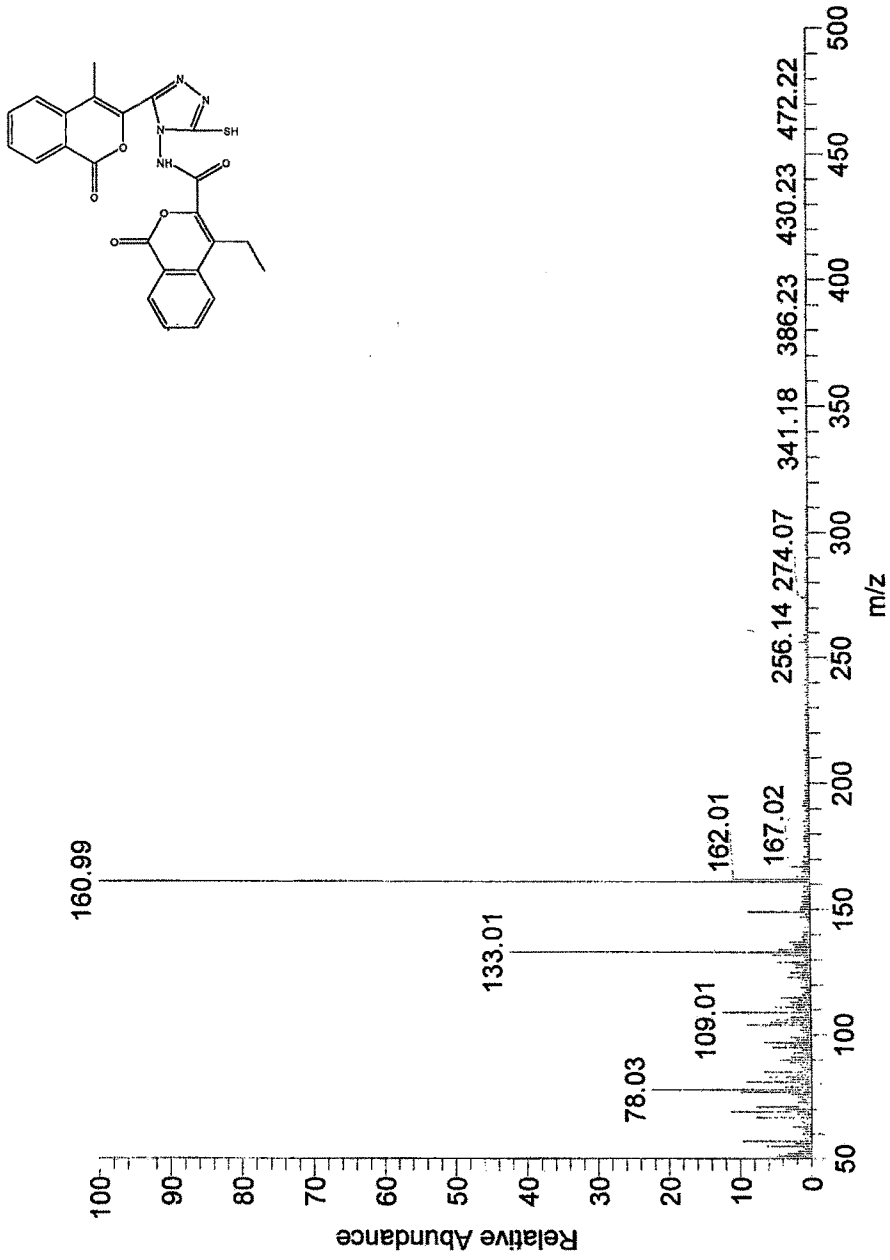
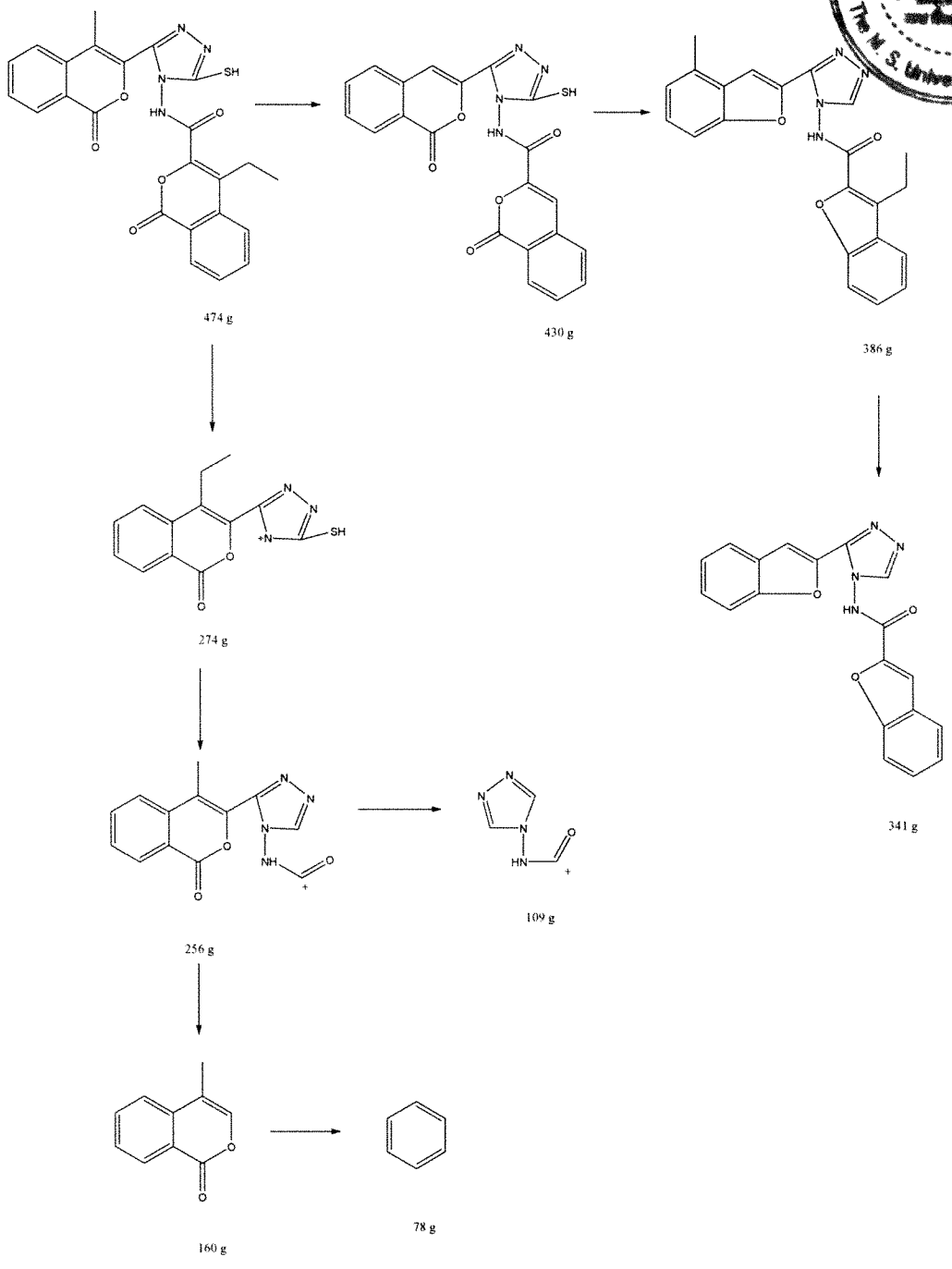
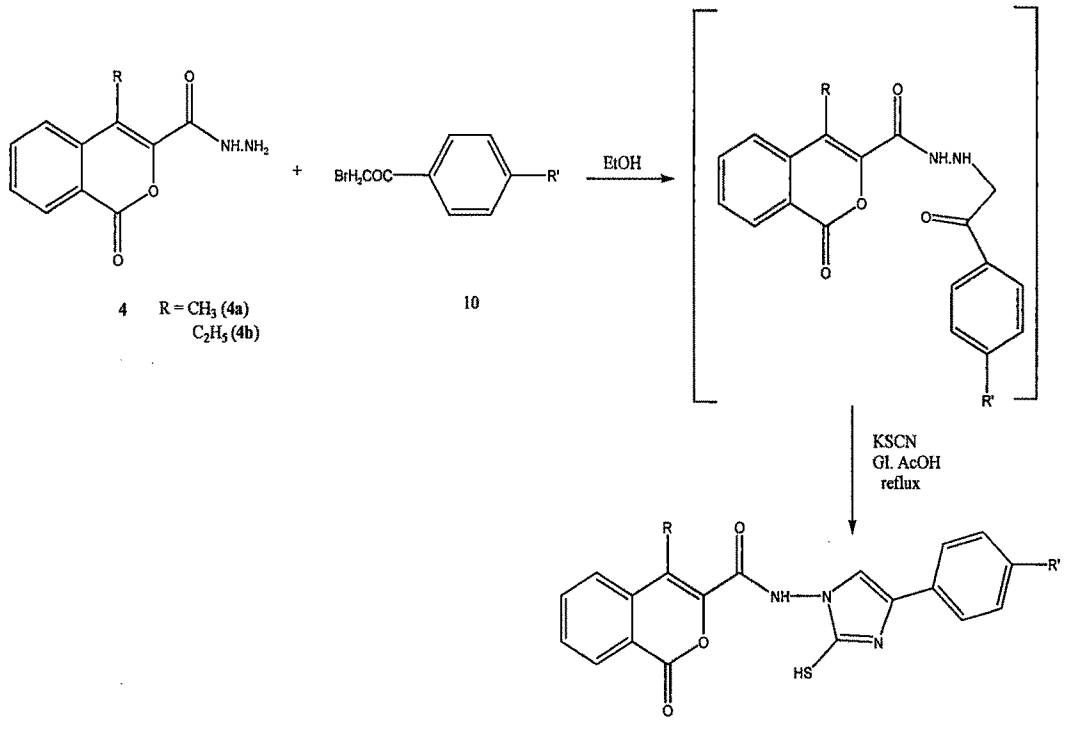


Fig. 4.B.16 – Mass spectrum: **4-Methyl-isocoumarin-3-carboxylic acid [3'-mercapto-5-(4''-ethyl-isocoumarin-3''-yl) - (1', 2', 4') triazole-4'-yl] amide 9c**



Fragmentation Pattern: **4-Methyl-isocoumarin-3-carboxylic acid [3'-mercapto-5-(4''-ethyl-isocoumarin-3''-yl) - (1', 2', 4') triazole-4'-yl] amide**

Scheme IV



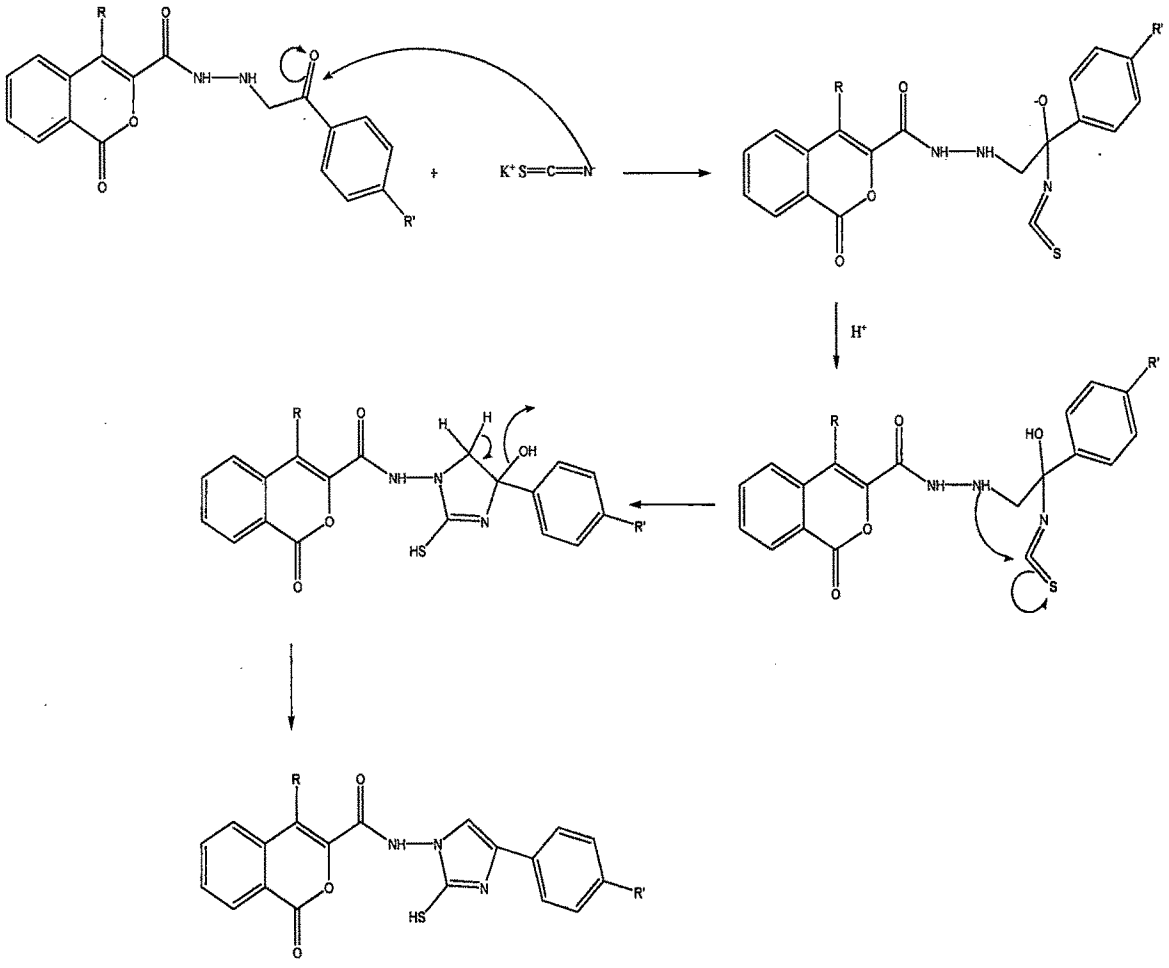
4 R = CH_3 (4a)
C_2H_5 (4b)

10

11

R = CH_3, R' = OH (**11a**)
R = CH_3, R' = Br (**11b**)
R = CH_3, R' = OCH_3 (**11c**)
R = CH_3, R' = Benzofuran (**11d**)
R = C_2H_5 , R' = OH (**11e**)
R = C_2H_5, R' = B r (1 1f)
R = C_2H_5, R' = OCH_3 (**11g**)
R = C_2H_5, R' = Benzofuran (**11h**)

Mechanism: **4-Alkyl-isocoumarin-3-carboxylic acid [4'- (4"-substituted phenyl)-2'mercapto-imidazol-1-yl] amide**



Mercapto imidazole was the next nitrogen heterocyclic moiety to be inserted in the isocoumarins. This moiety again was chosen because of the biological importance of nitrogen and sulphur atoms present in it.

To introduce mercapto imidazole in isocoumarins, isocoumarin – 3- carboxylic acid hydrazide **4a-b** was reacted with different substituted bromoacetophenones **10** to give intermediate. The intermediate was not able to separate due to its instability outside the solvent. Therefore, intermediate was refluxed in situ, with potassium thiocyanate in presence of glacial acetic acid to yield isocoumarin – 3 – carboxylic acid – mercapto imidazole amide derivatives **11**.

IR spectra show absorption frequencies for γ lactone at 1705, C=O at 1688, C=N at 1590, CONH at 3347, C=S at 1344 and SH at 2523 cm^{-1} (**Fig. 4.B.17**).

11b and **11h** shows signals in ^1H NMR at δ 2.1 (s, 3H, CH_3), 4.0 (s, 1H, SH), 7.45 (s, 1H, C=CH), 7.65-7.95 (m, 7H, aromatic protons), 8.4 (d, 1H, C_8-H), 10.0 (s, 1H, NH) (**Fig. 4.B.18**) and δ 1.2 (t, 3H, CH_3), 2.65 (q, 2H, CH_2), 4.5 (s, 1H, SH), 7.1 (s, 1H, C=CH), 6.8-7.9 (m, 10H, aromatic protons), 8.1 (s, 1H, C_8-H), 11.0 (s, 1H, NH) (**Fig. 4.B.20**).

The m/z obtained in mass spectra of **11b** is 457.9 (M^+ + 2), 440.9, 412.9, 364, 331, 284, 238, 191, 187, 113, 98 and 57 (**Fig. 4.B.19**) and **11h** at m/z: 483 (M^+ + 2), 430, 386, 341, 274, 256, 160, 109 and 78 (**Fig. 4.B. 21**).

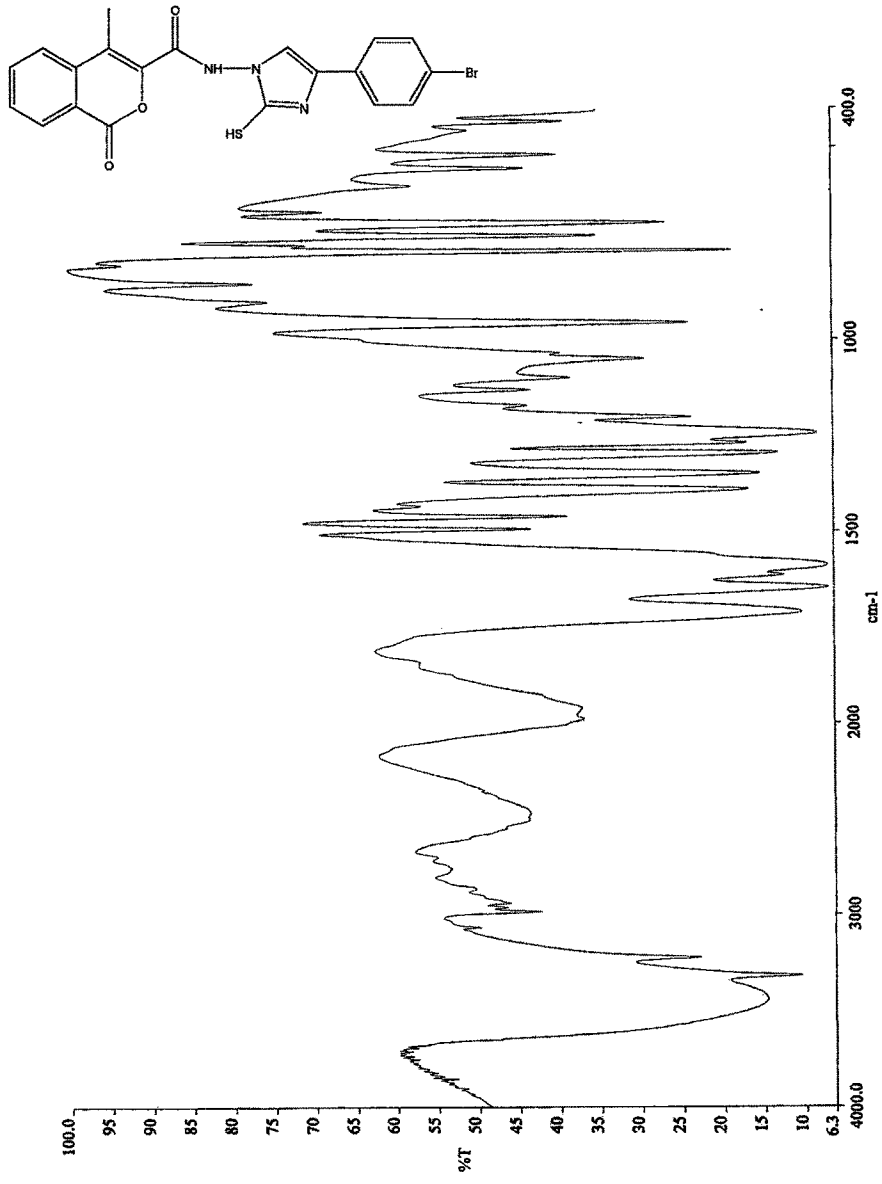


Fig. 4.B.17 – IR: **4-Methyl-isocoumarin-3-carboxylic acid [4'- (4"-bromo phenyl)-2'mercapto-imidazol-1-yl] amide 11b**

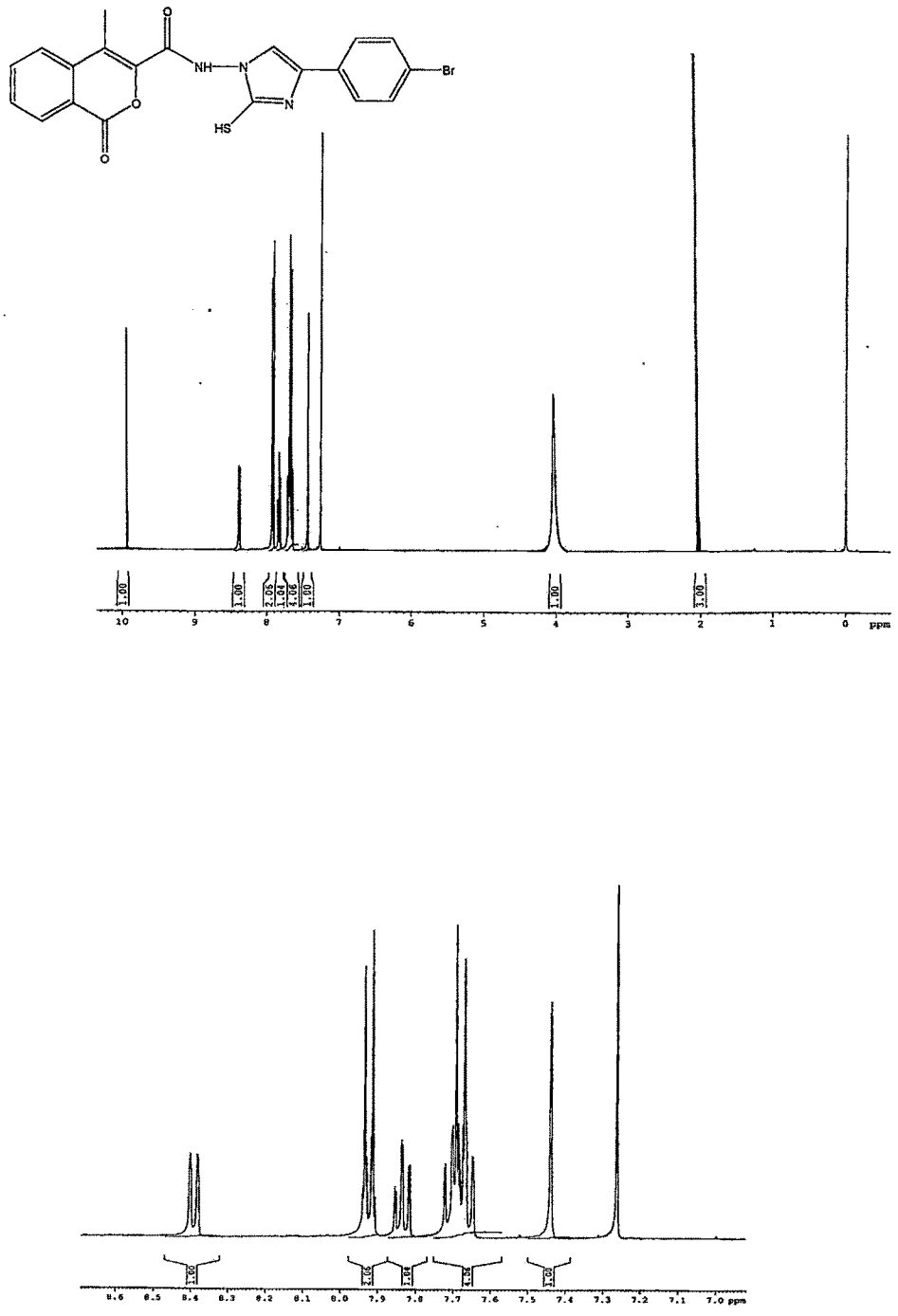


Fig. 4.B.18 – ^{1}H NMR: 4-Methyl-isocoumarin-3-carboxylic acid [4'- (4"-bromo phenyl)-2'mercapto-imidazol-1-yl] amide 11b

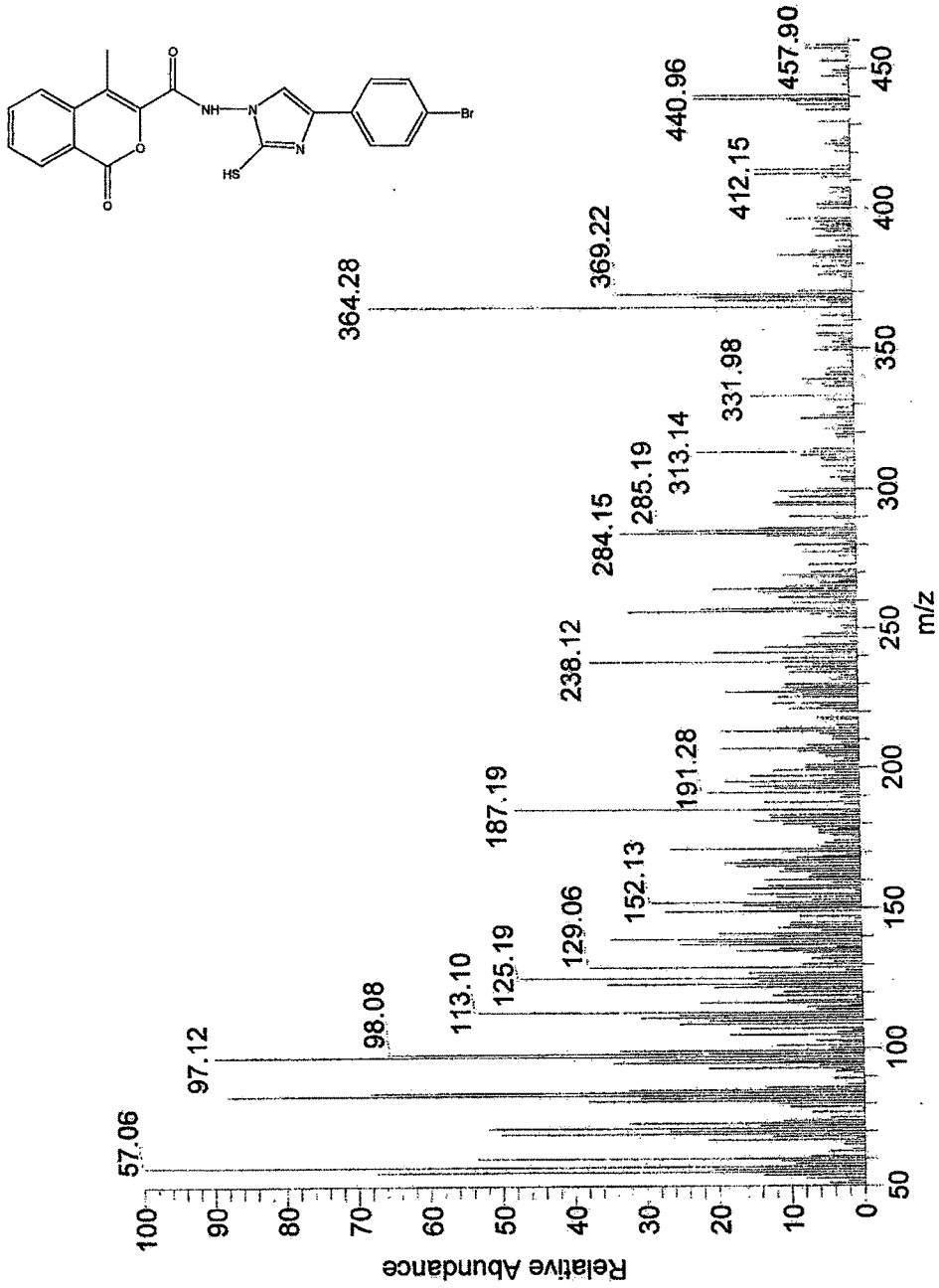
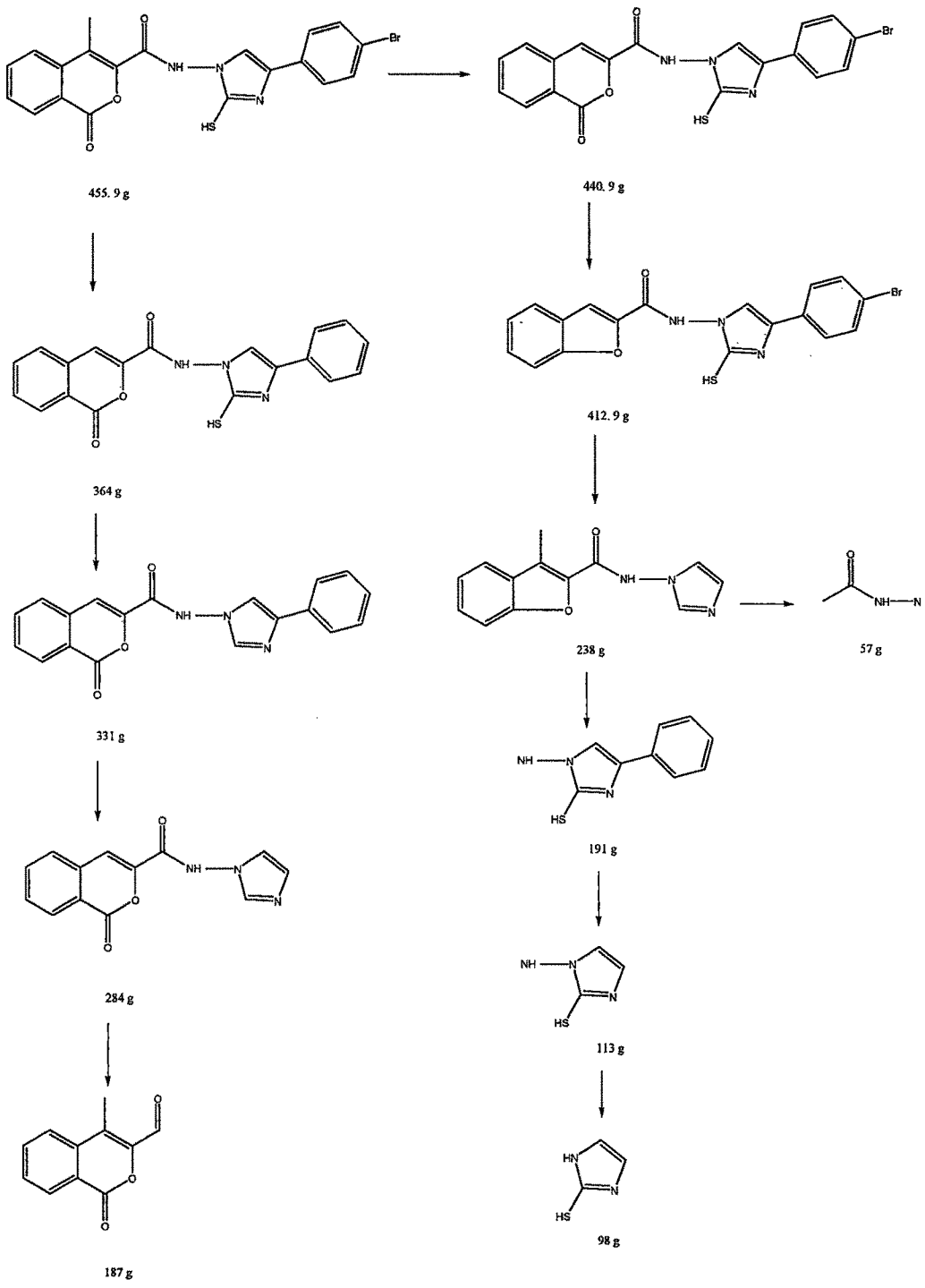
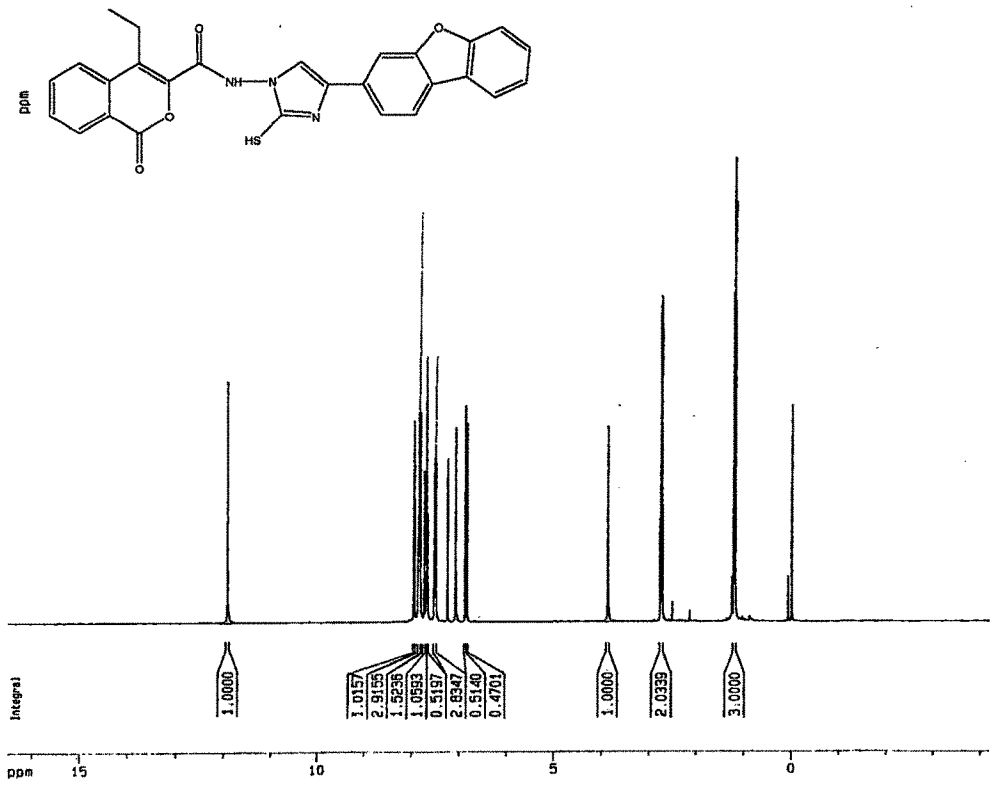


Fig. 4.B.19 – Mass spectrum: **4-Methyl-isocoumarin-3-carboxylic acid [4'- (4"-bromo phenyl)-2'mercapto-imidazol-1-yl] amide 11b**



Fragmentation Pattern: **4-Methyl-isocoumarin-3-carboxylic acid [4'- (4"-bromo phenyl)-2'mercapto-imidazol-1-yl] amide**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O
NH
N
N
HS
O
O
O
Integral
1.0000
1.0157
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15
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5
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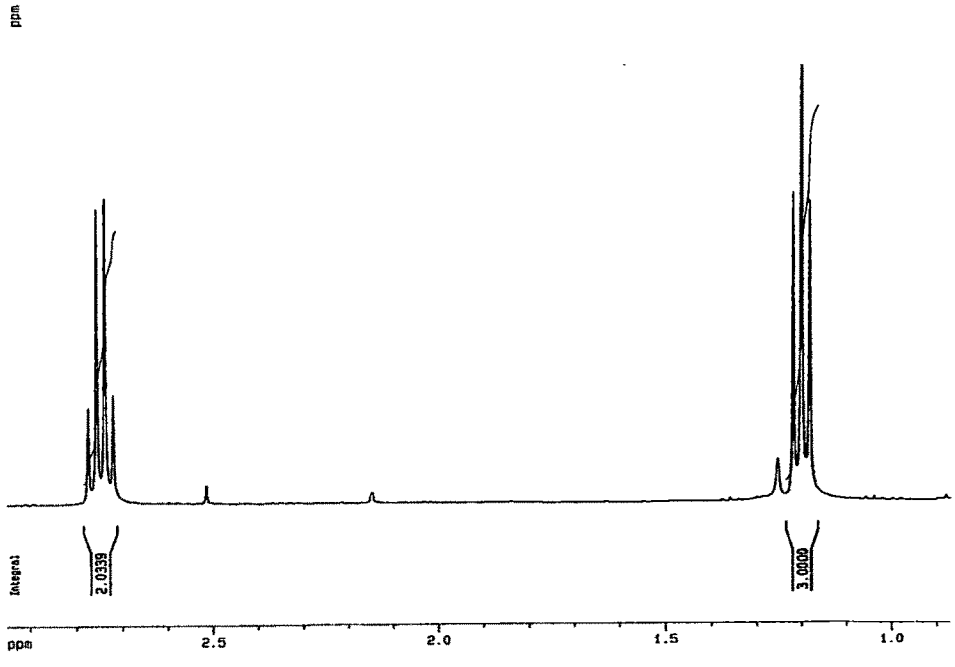
ppm
Integral
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3.0000
ppm
2.5
2.0
1.5
1.0

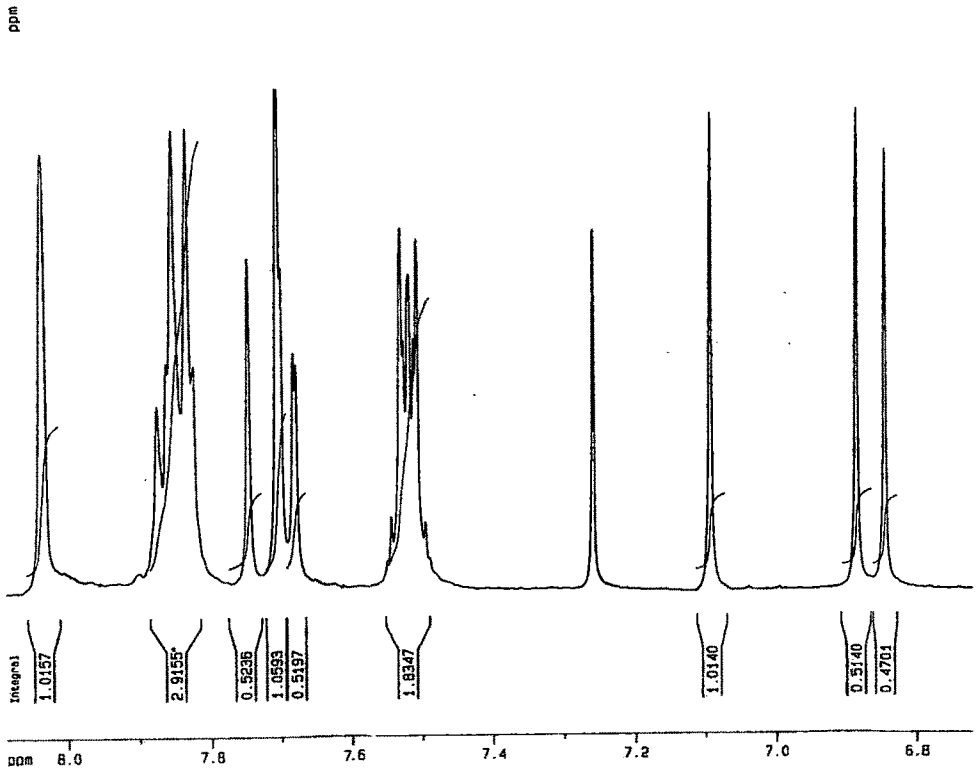


Fig. 4.B.20 – ^{1}H NMR: **4-Ethyl-isocoumarin-3-carboxylic acid [4'- (4"-dibenzofuran-4'-yl)-2'mercapto-imidazol-1-yl] amide 11h**

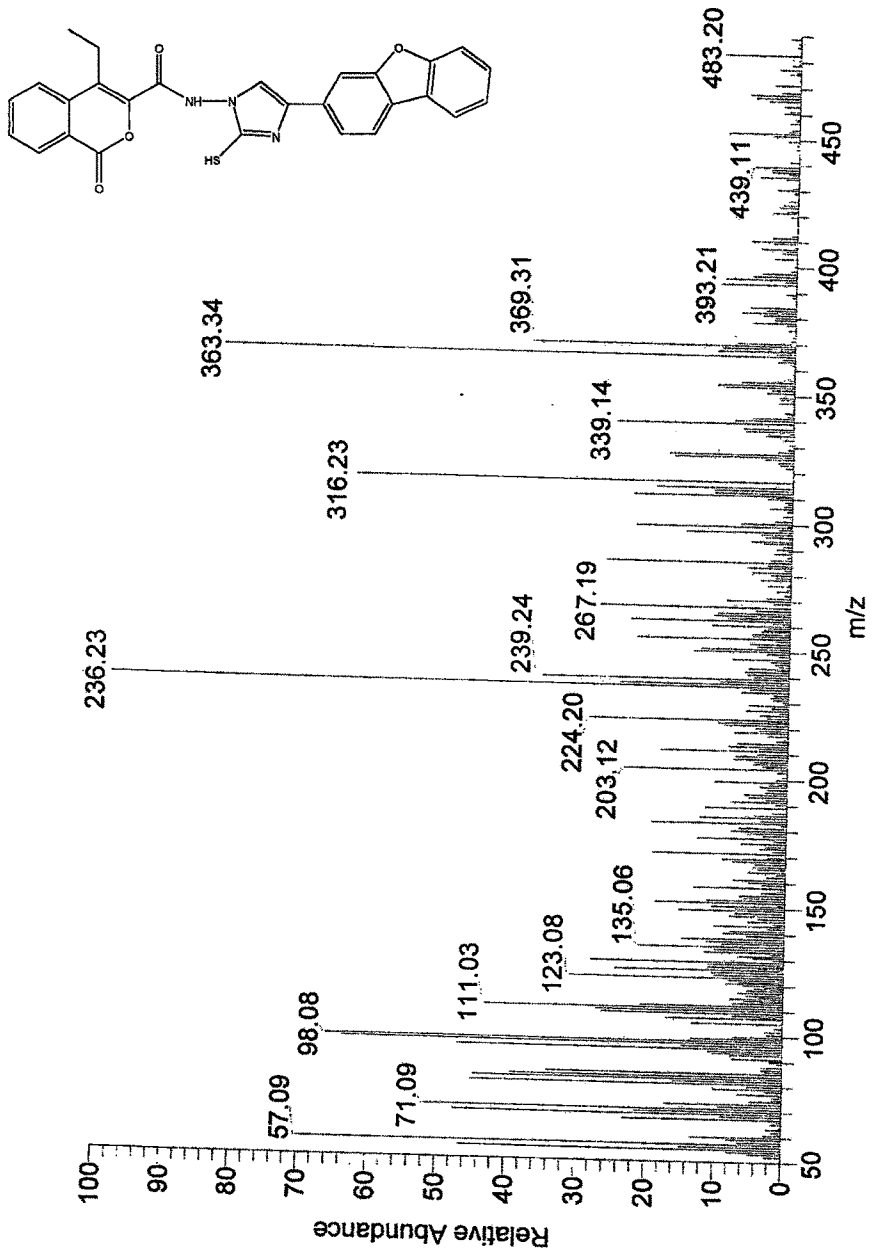
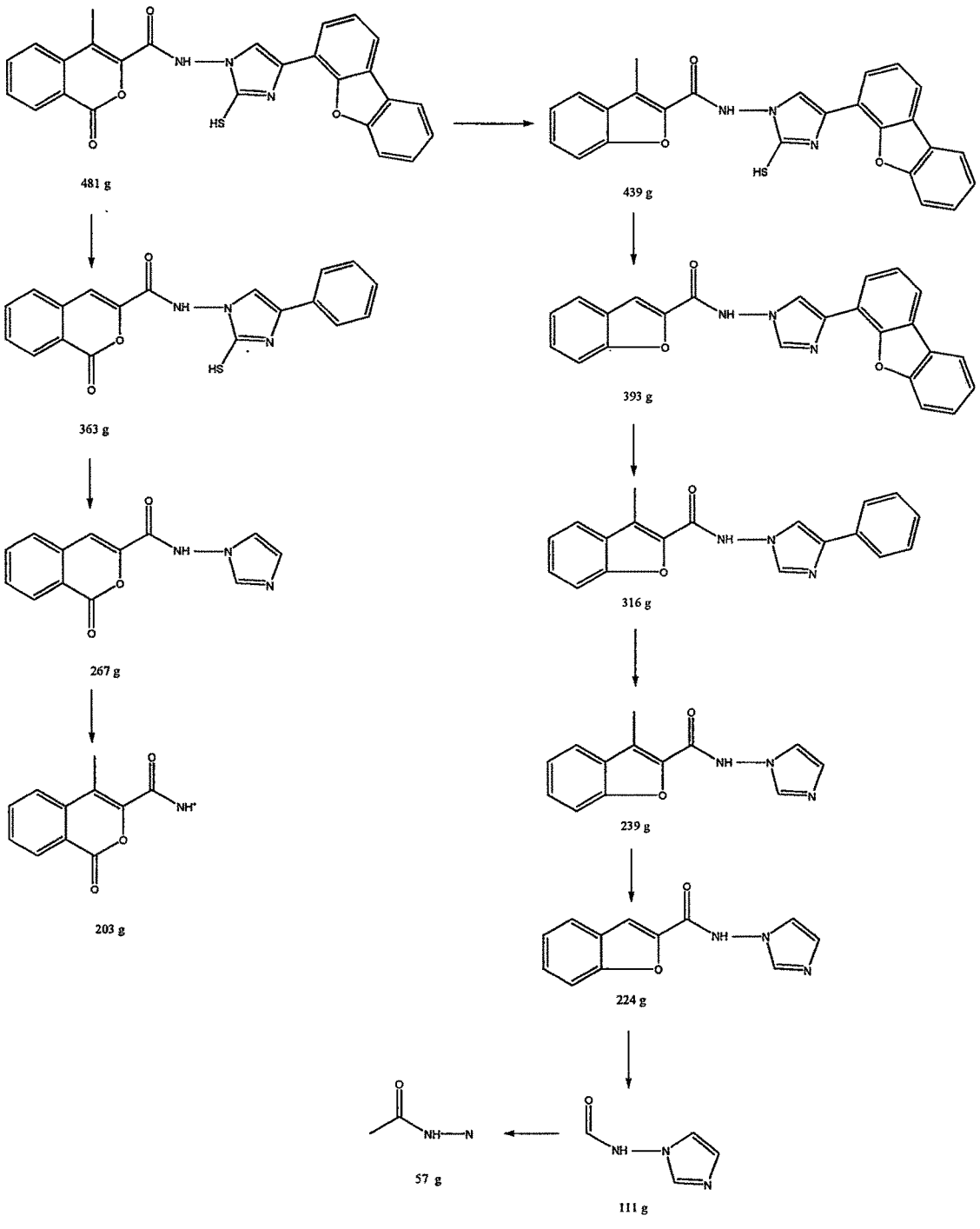


Fig. 4.B.21 – Mass spectrum: **4-Ethyl-isocoumarin-3-carboxylic acid [4'- (4"-dibenzofuran-4'-yl)-2'mercapto-imidazol-1-yl] amide 11h**



Fragmentation Pattern: **4-Ethyl-isocoumarin-3-carboxylic acid [4'- (4"-dibenzofuran-4'-yl)-2'mercapto-imidazol-1-yl] amide**

4. B.3 EXPERIMENTAL

The reagents and the solvents used in this study were of analytical grade and were used without further purification. Melting points were determined in open capillaries and are uncorrected. The purity of the compounds was checked by TLC on silica gel GF254 plates using UV/Iodine as visualizing agent and Merk's silica gel (60-120 mesh) was used for column chromatographic purification. Infrared spectra were recorded on FTIR Perkin Elmer spectrophotometer using potassium bromide optics. ^{1}H NMR spectra were recorded on a Bruker spectrometer (400 MHz) using TMS as internal standard and chemical shifts are given in ppm. Mass spectrums were obtained using Thermo Scientific Corporation, DSQ II Mass Spectrometer. Elemental analyses were carried out on Perkin-Elmer C, H, N, S analyzer (Model-2400). O-acyl benzoic acid, bromodiethyl malonate and substituted bromo acetophenone derivatives were prepared by literature method[24-28].

General procedure for 3a-3b

o-acetyl benzoic acid **1** (2.0 g, 0.012 mole), bromo diethyl malonate **2** (2.0 ml. 0.012 mole) and anhy. K_2CO_3, (3.53 g, 0.022 mole) was refluxed for 10-12 hrs in ethyl methyl ketone. Solvent was then removed, water added and extracted with ethyl acetate. Solvent layer was first washed with sod. bicarbonate and then with water and dried over anhy. Na_2SO_4. After removal of solvent the crude product was purified by column chromatography using petroleum ether (60-80^{0}C)-ethyl acetate to yield intermediate which then refluxed with conc. HCl (9.0 ml) and glacial acetic acid (12.0 ml) for 6 hrs. After that the reaction mixture was cooled and poured in crushed ice and left overnight. The solid product obtained was filtered and recrystallised with methanol to give brown crystals of **3a**

4-Methyl-isocoumarin-3-carboxylic acid[23] 3a

This compound was obtained as brown crystals, mp: 242^{0}C; 63.17% yield; Anal. Calcd $C_{11}H_8O_4$ (204.0 g): C, 64.70; H, 3.92; Found: C, 64.58; H, 4.26; ^{1}H NMR δ 2.3

(s, 3H, CH_3), 7.7-8.0 (m, 3H, aromatic protons), 8.39 (d, 1H, C_8-H) 11.0 (s, 1H, OH); ms: m/z: 204 (M^+), 203, 189, 146, 118, 77 and 57.

4-Ethyl-isocoumarin-3-carboxylic acid[23] 3b

This compound was obtained as brown crystals, mp: 182^{0}C; 56.30% yield; Anal. Calcd $C_{12}H_{10}O_4$ (218.0 g): C, 66.05; H, 4.58; Found: C, 66.38; H, 4.86; ^{1}H NMR δ 1.1 (t, 3H, CH_3), 1.7 (q, 2H, CH_2), 7.7-7.9 (m, 3H, aromatic protons), 8.4-8.45 (dd, 1H, C_8-H) 11.2 (s, 1H, OH); ms : m/z: 218 (M^+), 217, 190, 174 and 146.

General procedure for 4a-4b

To a solution of 80% aq. hydrazine hydrate (0.3ml), isocoumarin-3-carboxylic acid **3** (0.1 g, 0.00049 mole) was added portion wise and after addition was complete, it was refluxed for 15 mins. After that, absolute alcohol was added to the reaction mixture which was just enough to get a clear solution and reaction mixture was refluxed for 5 hrs. After completion of reaction, solvent was distilled off and solid product obtained was recrystallised from ethanol to yellow crystals of **4a**

4-Methyl isocoumarin-3- carboxylic acid hydrazide 4a

This compound was obtained as yellow crystals, mp: 242^{0}C; 65.00% yield; Anal. Calcd $C_{11}H_{10}N_2O_3$ (218.0 g): C, 60.55; H, 4.58; N, 12.84; Found: C, 60.14; H, 4.73; N, 12.96; ^{1}H NMR δ 2.2 (s, 3H, CH_3), 6.9 (d, 2H, NH_2), 7.9 (t, 1H, NH), 7.6-7.8 (m, 3H, aromatic protons), 8.4 (d, 1H, C_8-H); ms : m/z: 218 (M^+), 202, 187, 173, 159, 146, 77 and 59.

4-Ethyl isocoumarin -3-carboxylic acid hydrazide 4b

This compound was obtained as yellow crystals, mp: 182^{0}C; 48.71% yield; Anal. Calcd $C_{12}H_{12}N_2O_3$ (232.0 g): C, 62.06; H, 5.17; N, 12.06; Found: C, 62.31; H, 5.42; N, 12.46; ^{1}H NMR δ 1.1 (t, 3H, CH_3), 1.8 (q, 2H, CH_2), 7.0 (d, 2H, NH_2), 7.9 (t, 1H, NH), 7.6-7.8 (m, 3H, aromatic protons), 8.45 (dd, 1H, C_8-H); ms : m/z: 232 (M^+), 216, 201, 173, 172, 146 and 59.

General procedure for 5a-5b

A mixture of isocoumarin carboxylic acid hydrazide **4** (0.106 g, 0.00048 mole), acetylacetone (0.05ml, 0.00048 mole) and 2M HCl was refluxed in methanol for 15hr. The solvent then distilled off and the residue obtained was recrystallised from ethanol to give white crystals of **5a**

3-(3', 5'-Dimethyl pyrazole-1-carbonyl)-4-methyl isocoumarin 5a

This compound was obtained as white crystals, mp: 220^0C; 72.69% yield; Anal. Calcd $C_{16}H_{14}N_2O_3$ (282.0 g): C, 68.08; H, 4.96; N, 9.92; Found: C, 68.21; H, 4.53; N, 10.21; ^{1}H NMR δ 2.3 (s, 3H, CH_3), 2.9 (s, 6H, CH_3), 7.0 (s, 1H, =CH), 7.5-7.8 (m, 3H, aromatic protons), 8.3 (d, 1H, C_8-H); ms : m/z 282 (M^+), 267, 237, 209, 173 and 146.

3-(3', 5'-Dimethyl pyrazole-1-carbonyl)-4-ethyl isocoumarin 5b

This compound was obtained as white crystals, mp: 230^0C; 73.45% yield; Anal. Calcd $C_{17}H_{16}N_2O_3$ (296.0 g): C, 68.91; H, 5.40; N, 9.45; Found: C, 68.61; H, 5.73; N, 9.81; ^{1}H NMR δ 1.3 (t, 3H, CH_3), 2.7 (q, 2H, CH_2), 2.9 (s, 6H, CH_3), 6.2 (s, 1H, =CH), 7.5-7.8 (m, 3H, aromatic protons), 8.3 (d, 1H, C_8-H) ; ms : m/z 295 (M^+ -1), 267, 254, 209 and 146.

General procedure for 7a-7l

A mixture of isocoumarin-3-carboxylic acid hydrazide **4** (0.106 g, 0.00048 mole), p-nitro benzaldehyde **6** (0.0734 g, 0.00048 mole) and few drops of conc. H_2SO_4 was refluxed in ethanol for 4hr. After the reaction was over, reaction mass was poured into ice, product filtered and recrystallised from ethanol to give white crystals of **7a**

4-Methyl-isocoumarin-3-carboxylic acid (4'-nitro benzylidene)-hydrazide 7a

This compound was obtained as yellow crystals, mp: 140^0C; 76.28% yield; Anal. Calcd $C_{18}H_{13}N_3O_5$ (351.0 g): C, 61.53; H, 3.70; N, 11.96; Found: C, 61.82; H, 3.84; N, 12.08; ^{1}H NMR δ 2.1 (s, 3H, CH_3), 7.4(s, 1H, =CH), 7.5-8.3 (m, 7H, aromatic

protons), 8.4 (d, 1H, C_8-H), 9.0(s, 1H, NH); ms : m/z: 353 (M^+ +2), 229, 213, 171, 135, 71 and 57.

4-Methyl-isocoumarin-3-carboxylic acid (4'-hydroxy benzylidene)-hydrazide 7b

This compound was obtained as white crystals, mp: 242^{0}C; 74.53% yield; Anal. Calcd $C_{18}H_{14}N_2O_4$ (322.0 g): C, 67.08; H, 4.34; N, 8.69; Found: C, 67.15; H, 4.30; N, 8.81; ^{1}H NMR δ 2.0 (s, 3H, CH_3), 3.9 (s, 1H, OH), 7.3(s, 1H, =CH), 6.95-8.25 (m, 7H, aromatic protons), 8.35 (d, 1H, C_8-H), 10.1(s, 1H, NH); ms : m/z: 321 (M^+ -1), 307, 294, 290, 213, 122, 71 and 57.

4-Methyl-isocoumarin-3-carboxylic acid (4'-methoxy benzylidene)-hydrazide 7c

This compound was obtained as yellow crystals, mp: 160^{0}C; 83.47% yield; Anal. Calcd $C_{19}H_{16}N_2O_4$ (336.0 g): C, 67.85; H, 4.76; N, 8.33; Found: C, 67.53; H, 4.77; N, 8.39; ^{1}H NMR δ 2.0 (s, 3H, CH_3), 4.0 (s, 3H, OCH_3), 7.3 (s, 1H, -C=H), 7.1-8.3 (m, 7H, aromatic protons), 8.4 (dd, 1H, C_8-H), 9.3 (s, 1H, NH); ms : m/z: 336 (M^+), 264, 215, 187, 135, 71 and 57.

4-Methyl-isocoumarin-3-carboxylic acid benzylidene-hydrazide 7d

This compound was obtained as white crystals, mp: 230^{0}C; 83.00% yield; Anal. Calcd $C_{18}H_{14}N_2O_3$ (306.0 g): C, 70.58; H, 4.57; N, 9.15; Found: C, 70.20; H, 4.90; N, 9.11; ^{1}H NMR δ 1.8 (s, 3H, CH_3), 6.8 (s, 1H, -C=H), 7.3-7.7 (m, 8H, aromatic protons), 8.36-8.4 (d, 1H, C_8-H), 9.5 (s, 1H, NH) ; ms : m/z: 306 (M^+), 215, 202, 187, 172, 147 and 145.

4-Methyl-isocoumarin-3-carboxylic acid (2'-hydroxy benzylidene)-hydrazide 7e

This compound was obtained as white crystals, mp: 208^{0}C; 59.71% yield; Anal. Calcd $C_{18}H_{14}N_2O_4$ (322.0 g): C, 67.08; H, 4.34; N, 8.69; Found: C, 67.00; H, 4.46; N, 8.75; ^{1}H NMR δ 2.0 (s, 3H, CH_3), 5.4 (s, 1H, OH), 7.0-7.8 (m, 7H, aromatic protons), 8.2 (s, 1H, =CH), 8.3 (d, 1H, C_8-H), 8.8 (s, 1H, NH); ms : m/z: 322 (M^+), 216, 147, 121, 93 and 77.

4-Methyl-isocoumarin-3-carboxylic acid (3'-phenyl allylidene)-hydrazide 7f

This compound was obtained as yellow crystals, mp: 180^{0}C; 50.95% yield; Anal. Calcd $C_{20}H_{16}N_2O_3$ (332.0 g): C, 72.28; H, 4.81; N, 8.43; Found: C, 72.26; H, 5.03; N, 8.60; ^{1}H NMR δ 2.1 (s, 3H, CH_3), 5.8 (s, 1H, =C_2-H), 6.5(s, 1H, =C_3-H), 7.5(s, 1H, =C_1-H), 7.0-7.6 (m, 8H, aromatic protons), 8.2 (s, 1H, NH), 8.3 (d, 1H, C_8-H); ms : m/z: 332 (M^+), 317, 240, 228, 214, 130, 116 and 103.

4-Ethyl-isocoumarin-3-carboxylic acid (4'-nitro benzylidene)-hydrazide 7g

This compound was obtained as yellow crystals, mp: 230^{0}C; 70.93% yield; Anal. Calcd $C_{19}H_{15}N_3O_5$ (365.0 g): C, 62.46; H, 4.10; N, 11.50; Found: C, 62.51; H, 4.00; N, 11.72; ^{1}H NMR δ 1.4 (t, 3H, CH_3), 2.5 (q, 2H, CH_2), 7.3 (s, 1H, =CH), 7.5-8.3 (m, 7H, aromatic protons), 8.4 (d, 1H, C_8-H), 8.8 (s, 1H, NH); ms : m/z: 366 (M^+ +1), 319, 305, 242, 187, 122, 71 and 57.

4-Ethyl-isocoumarin-3-carboxylic acid (4'-hydroxy benzylidene)-hydrazide 7h

This compound was obtained as white crystals, mp: 110^{0}C; 74.00% yield; Anal. Calcd $C_{19}H_{16}N_2O_4$ (336.0 g): C, 67.85; H, 4.76; N, 8.33; Found: C, 67.60; H, 4.93; N, 8.65; ^{1}H NMR δ 1.1 (t, 3H, CH_3), 2.5(q, 2H, CH_2), 4.9 (s, 1H, OH), 7.3 (s, 1H, =CH), 7.1-8.3 (m, 7H, aromatic protons), 8.45 (d, 1H, C_8-H), 10.1(s, 1H, NH); ms : m/z: 336 (M^+), 308, 278, 213, 165 and 122.

4-Ethyl-isocoumarin-3-carboxylic acid (4'-methoxy benzylidene)-hydrazide 7i

This compound was obtained as yellow crystals, mp: 170^{0}C; 83.69% yield; Anal. Calcd $C_{20}H_{18}N_2O_4$ (350.0 g): C, 68.57; H, 5.14; N, 8.00; Found: C, 68.70; H, 5.44; N, 8.26; ^{1}H NMR δ 1.0 (t, 3H, CH_3), 2.0 (q, 2H, CH_2), 3.5 (s, 3H, OCH_3), 6.9 (s, 1H, -C=H), 7.1-7.6 (m, 7H, aromatic protons), 8.4 (d, 1H, C_8-H), 10.3 (s, 1H, NH); ms : m/z: 350 (M^+), 262, 202, 199, 187, 185, 160 and 146.

4-Ethyl-isocoumarin-3-carboxylic acid benzylidene-hydrazide 7j

This compound was obtained as white crystals, mp: 170^{0}C; 76.94% yield; Anal. Calcd $C_{19}H_{16}N_2O_3$ (320.0 g): C, 71.25; H, 5.00; N, 8.75; Found: C, 71.33; H, 5.34; N, 9.04; ^{1}H NMR δ 1.1 (t, 3H, CH_3), 2.0 (q, 2H, CH_2), 6.8 (s, 1H, -C=H), 7.3-7.7 (m, 8H,

aromatic protons), 8.36-8.4 (d, 1H, C_8-H), 9.0 (s, 1H, NH) ; ms : m/z: 320 (M^+), 305, 202, 173, 146, 77 and 57.

4-Ethyl-isocoumarin-3-carboxylic acid (2'-hydroxy benzylidene)-hydrazide 7k

This compound was obtained as white crystals, mp: 220^{0}C; 58.01% yield; Anal. Calcd $C_{19}H_{16}N_2O_4$ (336.0 g): C, 67.85; H, 4.76; N, 8.33; Found: C, 67.89; H, 4.72; N, 8.49; ^{1}H NMR δ 1.2 (t, 3H, CH_3), 2.1(q, 2H, CH_2), 5.6 (s, 1H, OH), 7.0-7.8 (m, 7H, aromatic protons), 8.0 (s, 1H, =CH), 8.3 (d, 1H, C_8-H), 9.5 (s, 1H, NH) ; ms : m/z : 336 (M^+), 305, 240, 200, 168, 148, 71, 69 and 57.

4-Ethyl-isocoumarin-3-carboxylic acid (3'-phenyl allylidene)-hydrazide 7l

This compound was obtained as yellow crystals, mp: 172^{0}C; 50.00% yield; Anal. Calcd $C_{21}H_{18}N_2O_3$ (346.0 g): C, 72.83; H, 5.20; N, 8.09; Found: C, 73.10; H, 5.41; N, 8.37; ^{1}H NMR δ 1.1 (t, 3H, CH_3), 1.9 (q, 2H, CH_2), 5.8 (s, 1H, =C_2-H), 6.5 (s, 1H, =C_3-H), 7.5 (s, 1H, =C_1-H), 7.0-7.6 (m, 8H, aromatic protons), 8.0 (s, 1H, NH), 8.4 (d, 1H, C_8-H) ; ms : m/z: 347 (M^+ +1), 259, 232, 217, 145, 130, 116, 115, 103, 77 and 76.

General procedure for 9a-9d

A mixture of isocoumarin carboxylic acid hydrazide **4** (0.106 g, 0.00048 mole), carbon disulfide (0.0438 ml, 0.00072 mole) and potssium hydroxide (0.136 g, 0.0024 mole) was stirred in ethanol for 15 hrs. After that the second mole of isocoumarin carboxylic acid hydrazide **8** (0.105 g, 0.00048 mole) was added and refluxed in ethanol for 5 hrs. After the reaction was over, reaction mass was poured into ice, product filtered and recrystallised from ethanol to give white crystals of **9a**

4-Methyl-isocoumarin-3-carboxylic acid [3'-mercapto-5-(4''-methyl-isocoumarin-3''-yl) - (1', 2', 4') triazole-4'-yl] amide 9a

This compound was obtained as white crystals, mp: 230^{0}C; 69.51% yield; Anal. Calcd $C_{23}H_{16}N_4SO_5$ (460.0 g): C, 60.00; H, 3.47; N, 12.17; Found: C, 60.29; H, 3.70; N, 12.42; ^{1}H NMR δ 2.1 (s, 6H, CH_3), 2.5 (s, 1H, SH), 7.7-8.1 (m, 6H, aromatic

protons), 8.35 (d, 2H, C_8-H), 10.1 (s, 1H, NH); ms: m/z: 460 (M^+), 428, 383,369, 368, 344, 284, 256, 224, 186, 109 and 57.

4-Ethyl-isocoumarin-3-carboxylic acid [3'-mercapto-5-(4''-ethyl-isocoumarin-3''-yl) - (1', 2', 4') triazole-4'-yl] amide 9b

This compound was obtained as white crystals, mp: 226^{0}C; 58.47% yield; Anal. Calcd $C_{25}H_{20}N_4SO_5$ (488.0 g): C, 61.47; H, 4.09; N, 11.47; Found: C, 61.75; H, 4.38; N, 11.72; ^{1}H NMR δ 1.2 (t, 6H, CH_3), 2.6 (s, 1H, SH), 2.7-2.8 (q, 4H, CH_2), 7.6-7.9 (m, 6H, aromatic protons), 8.4 (d, 2H, C_8-H), 10.0 (s, 1H, NH); ms : m/: 486 (M^+ - 2), 440, 383, 369, 368, 355, 315, 284, 257, 239, 216, 211, 172 and 57.

4-Methyl-isocoumarin-3-carboxylic acid [3'-mercapto-5-(4''-ethyl-isocoumarin-3''-yl) - (1', 2', 4') triazole-4'-yl] amide 9c

This compound was obtained as white crystals, mp: 234^{0}C; 75.02% yield; Anal. Calcd $C_{24}H_{18}N_4SO_5$ (474.0 g): C, 60.75; H, 3.79; N, 11.81; Found: C, 60.98; H, 3.66; N, 12.08; ^{1}H NMR δ 1.2 (t, 3H, CH_3), 2.5 (s, 3H, CH_3), 2.7-2.8 (q, 4H, CH_2), 5.4 (s, 1H, SH), 7.6-7.9 (m, 6H, aromatic protons), 8.4 (d, 2H, C_8-H), 9.5 (s, 1H, NH); ms : m/z: 472 (M^+ - 2), 430, 386, 341, 274, 256, 160, 109 and 78.

4-Ethyl-isocoumarin-3-carboxylic acid [3'-mercapto-5-(4''-methyl-isocoumarin-3''-yl) - (1', 2', 4') triazole-4'-yl] amide 9d

This compound was obtained as white crystals, mp: 240^{0}C; 68.41% yield; Anal. Calcd $C_{24}H_{18}N_4SO_5$ (474.0 g): C, 60.75; H, 3.79; N, 11.81; Found: C, 61.07; H, 4.11; N, 11.94; ^{1}H NMR δ 1.0 (t, 3H, CH_3), 2.0 (s, 3H, CH_3), 2.1 (q, 2H, CH_2), 2.9 (s, 1H, SH), 7.6-7.9 (m, 6H, aromatic protons), 8.4 (d, 2H, C_8-H), 10.1 (s, 1H, NH); ms : m/z: 472 (M^+ - 2), 430, 386, 341, 274, 256, 160, 109 and 77.

General procedure for 11a-11h

A mixture of isocoumarin carboxylic acid hydrazide **4** (0.106 g, 0.00048 mole), bromophenacyl bromide **10** (0.203 g, 0.00073 mole) was refluxed in ethanol for 5 hrs. After reaction was over, reaction mass was poured into ice and extracted with ethyl

acetate. The solvent was then removed to get the intermediate which was further reacted with KSCN (0.0711 g, 0.00073 mole) and refluxed in glacial acetic acid for 5 hrs. Reaction mass was poured into ie and product filtered, recrystallised from ethanol to give yellow crystals of **11a**

4-Methyl-isocoumarin-3-carboxylic acid [4'- (4"-hydroxy phenyl)-2'mercapto-imidazol-1-yl] amide 11a

This compound was obtained as yellow crystals, mp: 174^{0}C; 57.26% yield; Anal. Calcd $C_{20}H_{15}N_3SO_4$ (393.0 g): C, 61.06; H, 3.81; N, 10.68; Found: C, 61.24; H, 3.76; N, 10.83; ^{1}H NMR δ 2.2 (s, 3H, CH_3), 3.9 (s, 1H, SH), 6.7 (s, 1H, OH), 7.3 (s, 1H, C=CH), 7.7-8.0 (m, 7H, aromatic protons), 8.3 (d, 1H, C_8-H), 12.4 (s, 1H, NH) ; ms : m/z: 393 (M^+), 316, 299, 284, 256, 238, 187, 98, 97 and 57.

4-Methyl-isocoumarin-3-carboxylic acid [4'- (4"-bromo phenyl)-2'mercapto-imidazol-1-yl] amide 11b

This compound was obtained as yellow crystals, mp: 220^{0}C; 62.48% yield; Anal. Calcd $C_{20}H_{14}N_3SBrO_3$ (455.9 g): C, 52.64; H, 3.07; N, 9.21; Found: C, 52.48; H, 3.27; N, 9.38; ^{1}H NMR δ 2.1 (s, 3H, CH_3), 4.0 (s, 1H, SH), 7.45 (s, 1H, C=CH), 7.65-7.95 (m, 7H, aromatic protons), 8.4 (d, 1H, C_8-H), 10.0 (s, 1H, NH); ms : m/z: 457.9 ($M^+ + 2$), 440.9, 412.9, 364, 331, 284, 238, 191, 187, 113, 98 and 57.

4-Methyl-isocoumarin-3-carboxylic acid [4'- (4"-methoxy phenyl)-2'mercapto-imidazol-1-yl] amide 11c

This compound was obtained as yellow crystals, mp: 168^{0}C; 70.06% yield; Anal. Calcd $C_{21}H_{17}N_3SO_4$ (407.0 g): C, 61.91; H, 4.17; N, 10.31; Found: C, 62.27; H, 4.00; N, 10.56; ^{1}H NMR δ 2.1 (s, 3H, CH_3), 3.9 (s, 3H, OCH_3), 4.7 (s, 1H, SH), 7.4 (s, 1H, C=CH), 7.6-7.9 (m, 7H, aromatic protons), 8.45 (d, 1H, C_8-H), 13.5 (s, 1H, NH) ; ms : m/z: 409 ($M^+ + 2$), 366, 350, 285, 284, 255, 238, 187, 159, 112 and 98.

4-Methyl-isocoumarin-3-carboxylic acid [4'- (4"-dibenzofuran-4-yl)-2'mercapto-imidazol-1-yl] amide 11d

This compound was obtained as yellow crystals, mp: 114^{0}C; 50.39% yield; Anal. Calcd $C_{26}H_{17}N_3SO_4$ (467.0 g): C, 66.80; H, 3.64; N, 8.99; Found: C, 66.59; H, 3.35;

N, 9.27; ^{1}H NMR δ 2.1(s, 3H, CH_3), 4.8 (s, 1H, SH), 7.3 (s, 1H, C=CH), 6.8-7.9 (m, 10H, aromatic protons), 8.1 (d, 1H, C_8-H), 9.7 (s, 1H, NH) ; ms : m/z: 465 (M+ - 2), 452, 438, 393, 363, 267, 224, 111 and 98.

4-Ethyl-isocoumarin-3-carboxylic acid [4'- (4"-hydroxy phenyl)-2'mercapto-imidazol-1-yl] amide 11e

This compound was obtained as yellow crystals, mp: 206^{0}C; 53.79% yield; Anal. Calcd $C_{21}H_{17}N_3SO_4$ (407.0 g): C, 61.91; H, 4.17; N, 10.31; Found: C, 62.08; H, 4.39; N, 10.61; ^{1}H NMR δ 1.1 (t, 3H, CH_3), 2.7 (q, 2H, CH_2), 3.0 (s, 1H, SH), 5.5 (s, 1H, OH), 6.9 (s, 1H, C=CH), 7.6-8.9 (m, 7H, aromatic protons), 8.35 (d, 1H, C_8-H), 11.9 (s, 1H, NH) ; ms : m/z: 409 (M^+ + 2), 316, 284, 256, 215, 97 and 57.

4-Ethyl-isocoumarin-3-carboxylic acid [4'- (4"-bromo phenyl)-2'mercapto-imidazol-1-yl] amide 11f

This compound was obtained as yellow crystals, mp: 260^{0}C; 62.00% yield; Anal. Calcd $C_{21}H_{16}N_3SBrO_3$ (469.9 g): C, 53.62; H, 3.40; N, 8.93; Found: C, 53.81; H, 3.51; N, 8.77; ^{1}H NMR δ 1.2 (t, 3H, CH_3), 2.6 (q, 2H, CH_2), 4.4 (s, 1H, SH), 7.1 (s, 1H, C=CH), 7.65-7.95 (m, 7H, aromatic protons), 8.2 (d, 1H, C_8-H), 10.1 (s, 1H, NH); ms : m/z: 469.9 (M^+), 440.9, 390, 361, 284, 266, 113, 98 and 57.

4-Ethyl-isocoumarin-3-carboxylic acid [4'- (4"-methoxy phenyl)-2'mercapto-imidazol-1-yl] amide 11g

This compound was obtained as yellow crystals, mp: 220^{0}C; 70.00% yield; Anal. Calcd $C_{22}H_{19}N_3SO_4$ (421.0 g): C, 62.70; H, 4.51; N, 9.97; Found: C, 63.00; H, 4.88; N, 10.07; ^{1}H NMR δ 1.1 (t, 3H, CH_3), 2.7 (q, 2H, CH_2), 4.0 (s, 3H, OCH_3), 5.1 (s, 1H, SH), 7.1 (s, 1H, C=CH), 7.6-7.9 (m, 7H, aromatic protons), 8.3 (d, 1H, C_8-H), 10.5 (s, 1H, NH) ; ms : m/z: 421 (M^+), 393, 284, 255, 187, 98 and 57.

4-Ethyl-isocoumarin-3-carboxylic acid [4'- (4"-dibenzofuran-4'-yl)-2'mercapto-imidazol-1-yl] amide 11h

This compound was obtained as yellow crystals, mp: 166^{0}C; 47.28% yield; Anal. Calcd $C_{27}H_{19}N_3SO_4$ (481.0 g): C, 67.35; H, 3.95; N, 8.73; Found: C, 67.71; H, 4.07; N, 8.99; ^{1}H NMR δ 1.2 (t, 3H, CH_3), 2.65 (q, 2H, CH_2), 4.5 (s, 1H, SH), 7.1 (s, 1H,

C=CH), 6.8-7.9 (m, 10H, aromatic protons), 8.1 (s, 1H, C_8-H), 11.0 (s, 1H, NH) ; ms : m/z: 483 (M^+ + 2), 430, 386, 341, 274, 256, 160, 109 and 78.

4. B.4 CONCLUSION

- This chapter deals with the synthesis of new isocoumarin derivatives containing different nitrogen heterocyclic moieties starting with easily available compounds.

- The synthesis was divided into four different schemes leading to synthesis of dimethyl pyrazole-1-carbonyl isocoumarins, Schiff bases, mercapto triazole and mercapto imidazole derivatives of isocoumarins following different and effortless reaction pathways.

- All the schemes followed had isocoumarin-3-carboxylic acid hydrazide as starting material, which was an easy target to introduce other nitrogen heterocyclic moieties via cyclization.

- All the title compounds synthesized were tested for their biological potential which is given in last chapter.

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5.1 INTRODUCTION

While it is true that infectious diseases are the second leading cause of death worldwide and the third leading cause of death in developed countries, the main rationale for the development of novel antibacterial agents is the emergence and dissemination of resistant strains. In fact, it has been difficult to demonstrate that patients infected with resistant bacteria have poorer clinical outcomes than patients infected with susceptible bacteria and this is especially true when other co-morbidities are taken into account[1].

Despite the enormous improvements in public health that occurred following the development and commercialization of antibiotics in the 1940s, infectious diseases re-emerged as a significant cause of morbidity and mortality at the end of the twentieth century due to a variety of environmental, medical, and social factors[2]. Infectious diseases now constitute one of the leading causes of death worldwide, accounting for 13.3 million deaths in 1998[3].

When penicillin was originally introduced for therapy, almost all isolates of *Staphylococcus aureus* were susceptible and infections caused by these organisms were well controlled. After few decades of using antibiotics, almost 90% of S.aureus clinical isolates now produce a β-lactamase and are resistant to many β- lactams, particularly penicillin[4]. Soon after introduction of methicillin, the first methicillin resistant *S. Aureus* (MRSA) strains were isolated. As a result of extensive use of this antibiotic and other antimicrobial agents over the years, MRSA strains have accumulated resistance markers to almost all classes of antibiotics[5].

Pan-resistant strains are also starting to emerge, especially among multidrug resistant strains of Gram negative bacteria[6].

Organisms with apparently low pathogenic potential have evolved recently to produce strains with new potency and a different pathogenic profile, such as the food-borne pathogen *Escherichia coli* O157:H7, or have been shown to cause opportunistic

infections in severely immune-compromised individuals, including acquired immunodeficiency syndrome patients and those receiving immunosuppressive drugs during organ transplantation or chemotherapy.

The seriousness of the situation was highlighted by a recent study showing that bacteria collected from diverse soil samples were intrinsically resistant to multiple natural and synthetic antibiotics[7].

When faced with antifungal drugs, fungal pathogens have, in principle, the capacity to overcome their inhibitory action through specific resistance mechanisms. In a clinical context, whenever antifungal agents are used to combat fungal infections, the exposure of fungal pathogens to these agents is therefore expected to give rise to resistant isolates[8].

The evolution of drug resistance in fungal pathogens poses grave concern given the limited number of clinically useful antifungal drugs available and the prevalence of cross-resistance to drugs with a common target. The inherent challenges of antimicrobial drug discovery are exacerbated in the context of antifungals, given the close evolutionary relationships that fungi share with their human host making it difficult to identify fungal selective drug targets[9].

Fungi have evolved an elegant repertoire of mechanisms to survive the cellular stress exerted by antifungal drugs such as azoles, which inhibit ergosterol biosynthesis inducing cell membrane stress[10] and recent mass attacks on citizens also make it necessary to consider the implications of fungi as weapons[11].

Therefore, there is a crucial and urgent need to develop new classes of antibiotics or to revitalize existing antibiotics and to bring novel effective therapies to patients while being careful to minimize the emergence of resistance.

Pain is an unpleasant and subjective sensation resulting from a harmful sensorial stimulation that alerts the body about a current or potential damage to its tissues and

organs[12]. It is estimated that more than 75 million people refer to health services annually, presenting some form of recurrent or persistent pain[13]. In spite of painful sensation that can be solved most efficiently by removal of the underlying cause, the pain-causing stimulus cannot always be either easily defined or quickly removed.

Therefore, the health professionals are usually faced with the necessity to manage the symptomatology of the pain[14].

Chronic pain conditions entail a heterogeneous patient population in which the underlying disease process or precipitant injury can be functionally diverse[15].

Clinically, chronic pain from various etiologies, such as inflammation and neural destruction, is generally resistant to the treatments of simple analgesics or traditional agents[16].

Consequently, chronic pain, due to relative lack of response to current analgesics, represents an unmet medical need.

Oxidation is a basic part of the aerobic life and our metabolism. During oxidation, many free radicals are produced which have an unpaired, nascent electron. Atoms of oxygen or nitrogen having central unpaired electron, are called reactive oxygen or nitrogen species[17].

It is increasingly being realized that many of today's diseases are due to the "oxidative stress" that results from an imbalance between formation and neutralization of pro-oxidants. Oxidative stress is initiated by free radicals, which seek stability through electron pairing with biological macromolecules such as proteins, lipids and DNA in healthy human cells and cause protein and DNA damage along with lipid peroxidation. These changes contribute to cancer, atherosclerosis, cardiovascular diseases, ageing and inflammatory diseases[18-19].

The role of nitric oxide (NO) in numerous disease states has generated a considerable discussion over the past several years since the journal *Science* named it the molecule of the year in 1992. NO is an important bio regulatory molecule, which has a number of physiological effects including control of blood pressure, neural signal transduction, platelet function, antimicrobial and antitumor activity. Low concentrations of NO are sufficient, in most cases, to effect these beneficial functions. However, during infections and inflammations, formation of NO is elevated and may bring about some undesired deleterious effects[20].

An antioxidant is defined as 'any substance that when present at low concentrations compared to those of an oxidisable substrate significantly delays or prevents oxidation of that substrate[21].

Antioxidants, used to prevent or inhibit the natural phenomena of oxidation, also have a broad application in diverse industrial fields as they have a huge importance either as industrial additives or health agents[22].

Inflammation is a defensive response of body, which induces physiological adaptations to minimize tissue damage and to remove the pathogenic infections[23]. Inflammation is a local reaction of the vascular and supporting elements of a tissue to injury resulting in the formation of a protein-rich exudates; it is a protective response of the nonspecific immune system that serves to localize, neutralize, or to destroy an injurious agent in preparation for the process of healing[24].

Inflammation is divided into acute and chronic patterns. The cardinal signs of inflammation are rubor (redness), calor (heat), dolor (pain), tumor (swelling), and function laesa (loss of function).

A chronic inflammation is however an important contributory factor in morbidity and mortality. Such inflammatory disorders include rheumatoid arthritis, osteoarthritis, inflammatory bowl diseases, retinitis, multiple sclerosis, psoriasis and atherosclerosis[25].

Cause of inflammation includes physical agents, chemical agents, immunological reactions, and infection by pathogenic organism.

NO is produced from L-arginine by nitric oxide synthase (NOS). There are three isoforms of NOS. The constitutive NOS (cNOS) found in neuronal tissues (nNOS, type I) and vascular endothelium (eNOS, type III) release the small amounts of NO required for physiological functions[26]. NO activates the inducible cyclooxygenase (COX-2) resulting in the markedly increased release of proinflammatory PGs[27]. Large amounts of NO can be converted into peroxynitrite ($ONOO^-$) in the presence of superoxide anion (O_2^-). These neurotoxic RNS mediates neurodegenerative diseases[28].

Therefore, the inhibition of NO production by activated microglia and the scavenging of it might be beneficial for the treatment of neuronal inflammatory diseases.

Mosquitoes (one of the most important single group of insects) are not only the cause of nuisance by their bites but also transmit deadly diseases like malaria, filariasis, yellow fever, dengue, and Japanese encephalitis, which contribute significantly to poverty and social debility in tropical countries, causing millions of death every year[29].

Mosquito borne diseases with an economic impact create loss in commercial and labour outputs, particularly in countries with tropical and subtropical climates. In India, there may be up to 31 million microfilareamics, 23 million cases of symptomatic filariasis and about 473 million individuals potentially at risk of infection[30].

Despite significant advances in the techniques used for its control during recent decades, the mosquito continues to pose serious public health problems.

Repeated use of synthetic insecticides for mosquito control has fostered several environmental and human health concerns, including disruption of natural-biological

control systems, resurgences in mosquito populations, widespread development of resistance, and undesirable effects on non-target organisms[31].

These problems have highlighted the need of new strategies for mosquito control and the current emphasis for chemists is on the production of novel types of insecticide that prevent insect resistance and that are environmentally friendly with relatively long-term effects.

It is unusual for a natural product to be a plant growth promoter, and a search of the literature showed that this is a rare phenomenon. The usual natural product plant growth promoters are IAA, gibberellins, cytokinins, ethylene[32] and brassinosteroids[33].

Promoting plant growth in crops is of significant importance in global food production. Anytime crop production is increased, or sped up, economic savings may be realized. The shorter period of time that a crop is in the field also means reduced time of exposure to phytopathogens[34].

Improving plant vigour and maintaining optimal growth conditions can reduce host susceptibility to pathogen attack and the best way to maintain plant health is to manage its nutrient availability. By affecting the growth pattern, anatomy, morphology, and chemical composition in particular, nutritional availability to plants may contribute either to an increase or to a decrease of resistance and/or tolerance to pests and diseases[35].

So, the performance of few of the synthesized phthalides as growth promoters was checked, along with other biological screenings of the isocoumarin derivatives synthesized in earlier chapters.

5.2 RESULTS AND DISCUSSION

ANTIBACTERIAL ACTIVITY

The title compounds synthesized in earlier chapters were screened in vitro, against various disease models and their activities were compared. The compounds were first tested for antibacterial activity against bacterial strains *Staphylococcus Aureus* (gram positive) and *Escherichia Coli* (gram negative). Zone of inhibition was measured to determine the activity and ampicillin was used as the standard drug and DMF as control.

The experiments revealed that all compounds screened were found to be active against *S. Aureus* (gram positive) as compared to control. In 4-alkyl-3-aroyl isocoumarins, the presence of electron withdrawing group like –Br or electron releasing groups $–OCH_3$ and OH, does not lead to much difference in the activity towards the gram positive bacteria. Also, presence of two electron releasing groups does not help much in increasing the activity of the compounds. Same is the case when the number of phenyl rings is increased. However, activity is reduced when the length of alkyl chain at the fourth position of isocoumarin moiety increases from C_1 to C_3. Against gram negative bacteria, isocoumarins show better results when compared to control. The presence of electron releasing groups escalates the activity rather than the isocoumarins having electron withdrawing group.

Replacement of aroyl group with acetyl group at 3rd position of the isocoumarin leads to decrease in inhibition towards both *S. Aureus* and *E. Coli*.

Phthalides on the other hand gives results similar to that of isocoumarins, with presence or absence of groups not making much differentiation in the activity.

Introduction of nitro group in the isocoumarins leads to drastic increase in the action against *S. Aureus*, especially in the presence of electron withdrawing group.

Aminyl benzoyl isocoumarins shows same zone of inhibition against *S. Aureus* and no activity against *E. Coli* showing that presence of different groups does not make much difference.

Bromocarbonyl isocoumarins is seen to inhibit both the bacteria effectively, but the activity slightly decreases in case of amino carbonyl isocoumarins and a set pattern of activity could not be established in them.

Absence of alkyl chain at 4^{th} position gives very good results against both the bacteria, particularly in presence of electron withdrawing group.

Condensation of two isocoumarin scaffolds in bisisocoumarins leads to brilliant results, against both the bacteria, irrespective of the groups present.

Presence of both coumarin and isocoumarin moieties also shows good activity, more in opposition to gram positive bacteria.

From literature survey it is known that tetrazole moiety is good antibacterial agent but here conversion of isocoumarins to tetrazole isoquinolines shows much reduced inhibition. On the other hand, the results are much admirable with triazole isoquinolines.

Isocoumarin – 3 - carboxylic acid hydrazide has enhanced zone of inhibition than corresponding acid.

Pyrazole derivative of isocoumarins seems to be more effective against *S. Aureus*.

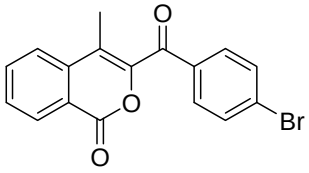
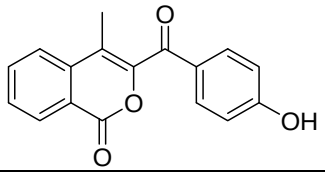
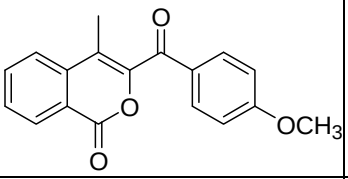
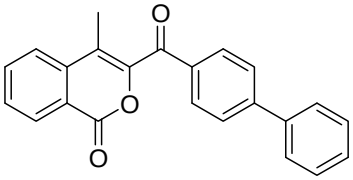
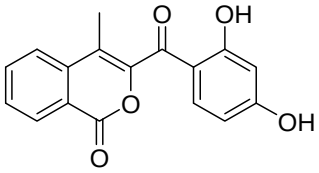
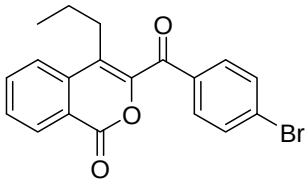
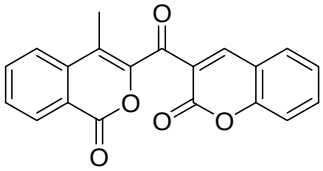
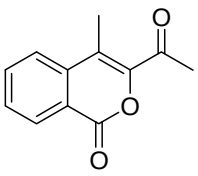
Schiff bases of isocoumarins shows exceptional results against *S. Aureus* and better activity with *E. Coli*, enhancing the importance of carbon-nitrogen double bond.

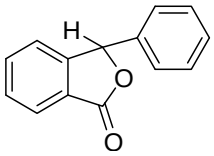
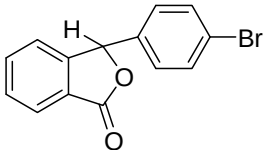
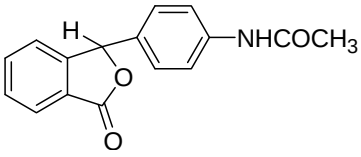
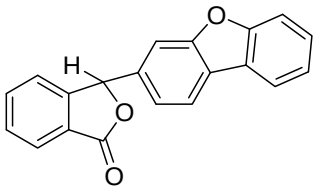
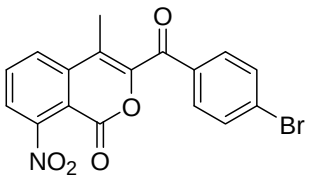
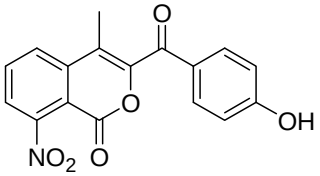
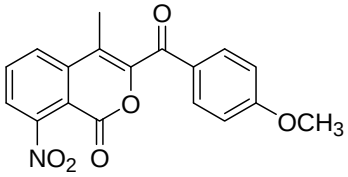
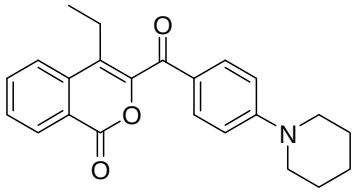
Existence of mercapto triazole ring along with two similar isocoumarin moieties gives improved action than that having two different moieties.

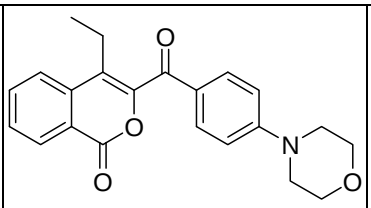
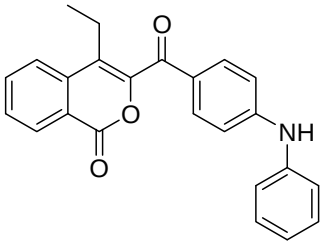
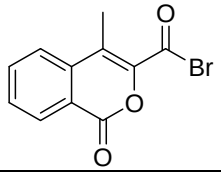
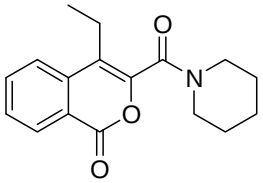
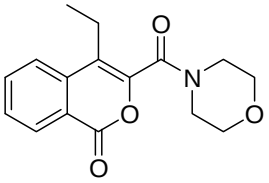
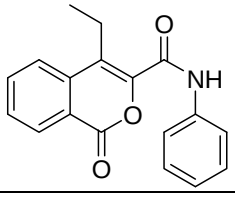
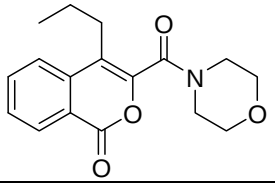
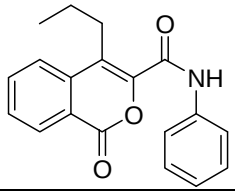
Mercapto imidazole derivatives show analogous results with both electron releasing and electron withdrawing groups against both the bacteria.

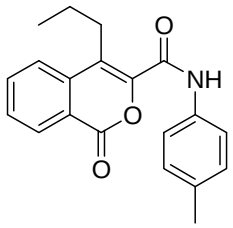
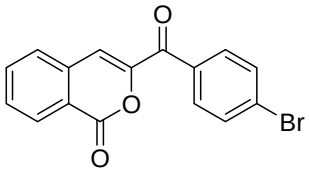
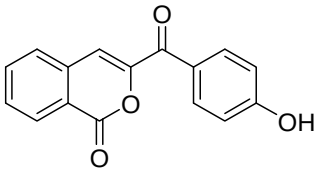
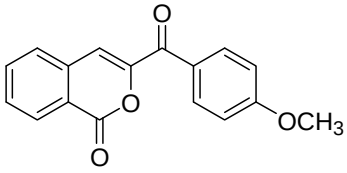
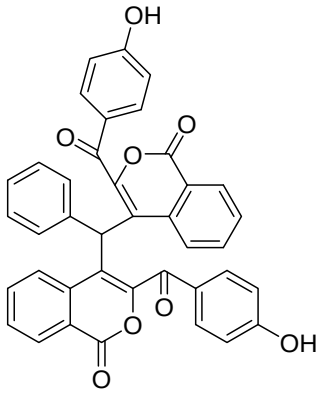
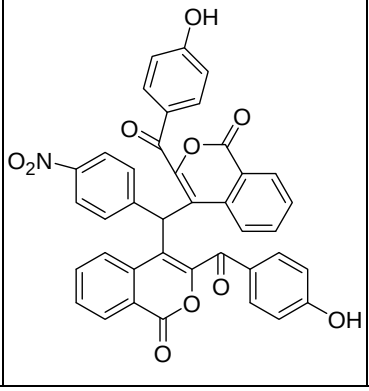
All the synthesized compounds lead to better activity when compared to standard drug ampicillin for gram negative bacteria. Against gram positive bacteria, some compounds gave comparable results while others gave better results when evaluated with the standard drug.

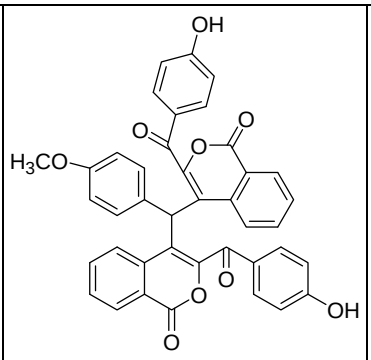
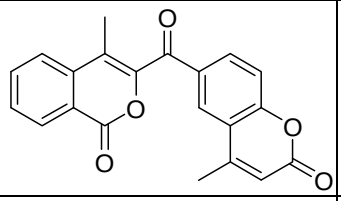
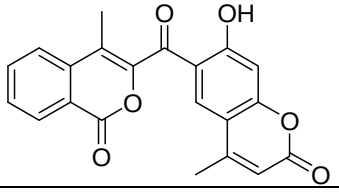
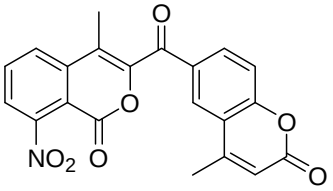
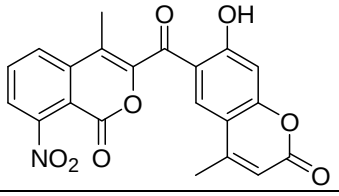
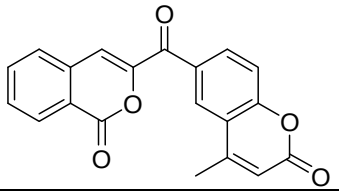
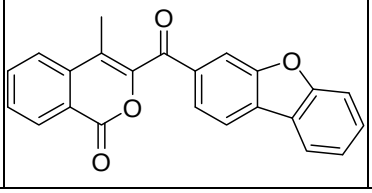
Zone of Inhibition (mm)

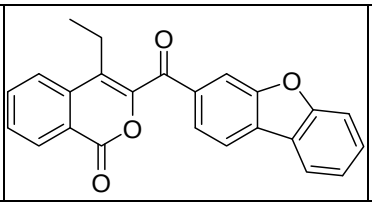
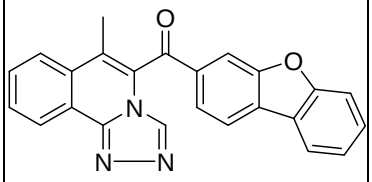
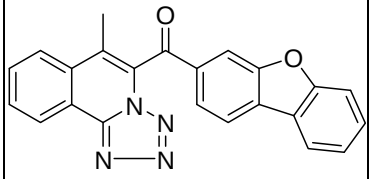
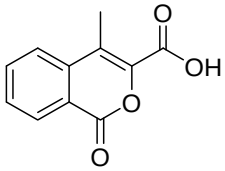
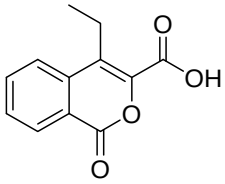
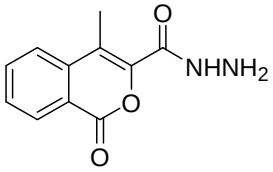
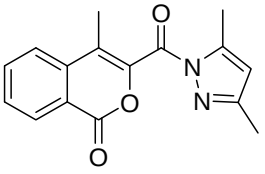
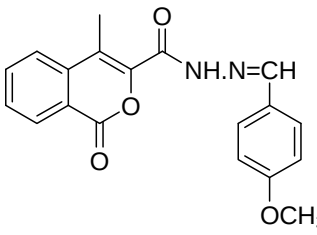
Sr. No.	**Compound**	***S. Aureus***	***E. Coli***
1.		11	12
2.		12	14
3.		13	14
4.		13	13
5.		12	14
6.		11	13
7.		14	14
8.		10	11

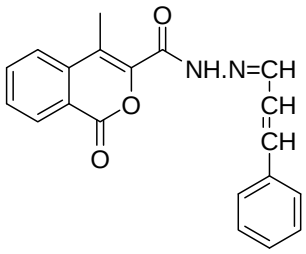
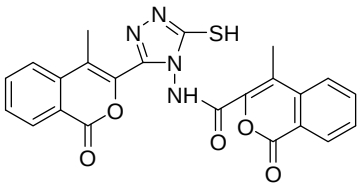
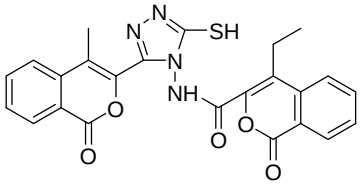
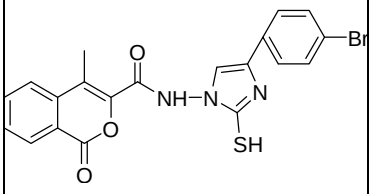
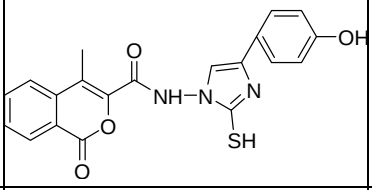
9.		13	13
10.		11	13
11.		10	13
12.		12	13
13.		17	11
14.		12	12
15.		15	11
16.		11	11

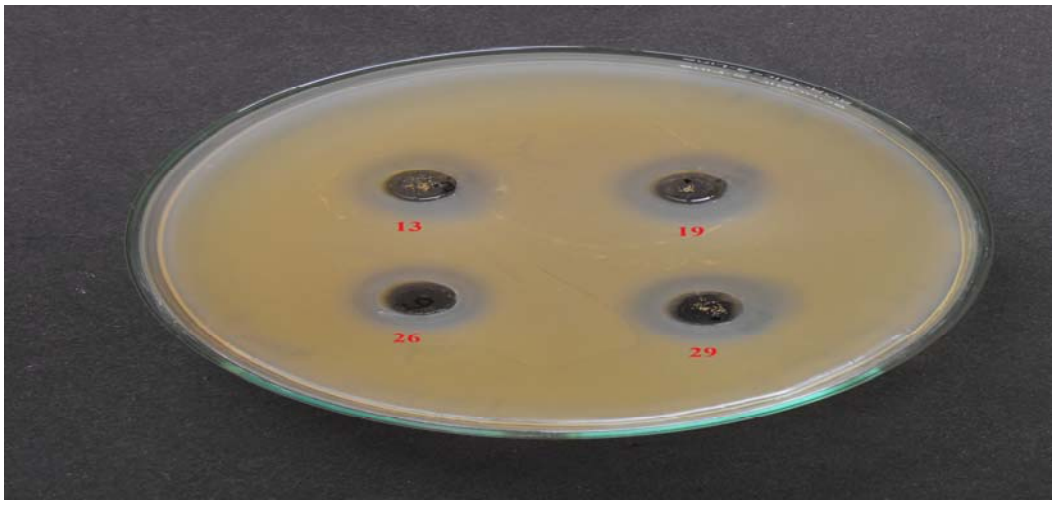
17.		11	11
18.		11	11
19.		16	14
20.		12	11
21.		0	15
22.		11	11
23.		11	15
24.		11	11

25.		0	17
26.		16	14
27.		15	11
28.		11	14
29.		15	14
30.		17	14

31.		15	15
32.		15	14
33.		15	14
34.		15	12
35.		16	12
36.		15	13
37.		12	12

38.		12	13
39.		15	15
40.		10	13
41.		10	12
42.		11	14
43.		14	14
44.		11	15
45.		15	14

46.		17	17
47.		15	15
48.		13	12
49.		12	12
50.		11	12
51.	CONTROL (DMF)	0	10
52.	AMPICILLIN	15	5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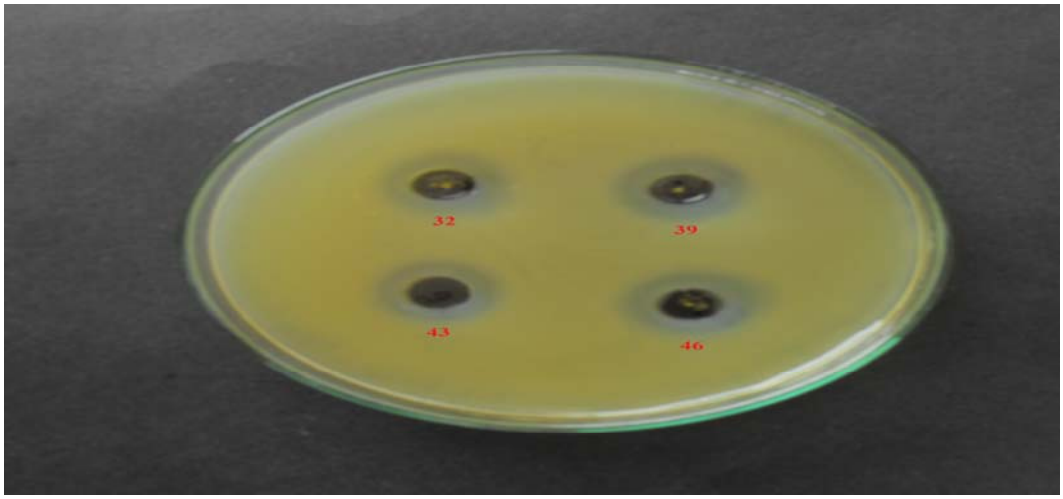
32
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46


7
1
8
CONTROL

Nitrogen as heteroatom does not show much difference in activity against both bacteria but in the form of functional group such as NO_2, it does affect the activity against both bacteria, which is shown in compounds **17**, **25**, **30**, **34**, and **35**.

Br group as such helps in activity (**6**, **10**, **19**, **26)** but in presence of NO_2 activity is significant (**13**).

Increase in lactone ring also shows increase in activity against both the bacteria (**7**, **29**, **31**, **32**, **36**).

Conjugation in the form of Schiff base between isocoumarins and phenyl ring were also found to enhance the activity (**45**, **46**).

Presence of SH group does help in showing the activity but not upto the mark as expected.

ANTIFUNGAL ACTIVITY

The synthesized compounds were screened for the antifungal activity against fungal strains *Thielaviopsis paradoxa, Phomopsis mangiferae, Fusarium pallidoroseum, Colletotrichum capsici*, belonging to the same family, using Potato Dextrose Agar Medium[36].

4-alkyl-3-aroyl isocoumarins were found to show increased activity against all the fungal strains, from moderate to excellent with groups changing from electron withdrawing to electron releasing. Presence of more than one electron releasing group and aromatic rings enhances the activity, while the increase in the length of alkyl chain reduces the activity slightly. Acetyl group in place of aroyl group at the third position of isocoumarin moiety does not give much different activity while Phthalides show good activity in presence of electron withdrawing group.

In contrast to antibacterial activity, introduction of nitrogen heteroatom in the form of nitro group or aminyl benzoyl reduces the activity drastically with inhibition being less than 15.00%. However, when aminyl benzoyl isocoumarin contain morpholine ring, the inhibition is 100.00%. Reason for this exceptional activity could not be ascertained. On the other hand, aminocarbonyl isocoumarins gave moderate to good results, excluding the one having morpholine ring. Increase in length of alkyl chain does increase the activity.

Absence of alkyl chain in 3- aroyl isocoumarins reduces the activity when compared with the corresponding 4-alkyl-3-aroyl isocoumarins.

In support to antibacterial activity, existence of two six membered lactone rings in the same moiety like in bis isocouamrins and coumarinoyl isocoumarins show excellent results irrespective of the groups present in them towards *Chaetomium* and *F. Pallidorosoeum*.

Dibenzofuran ring in attendance of lactone ring inhibits the fungi poorly.

Triazole and tetrazole isoquinolines shows moderate inhibition towards *Chaetomium* and *F. Pallidorosoeum*.

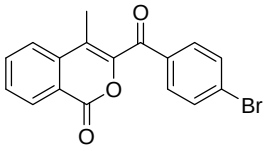
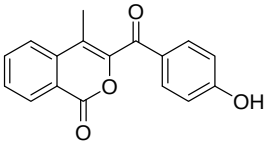
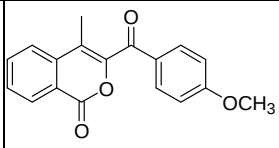
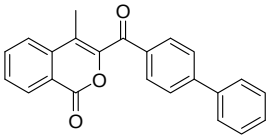
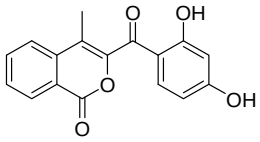
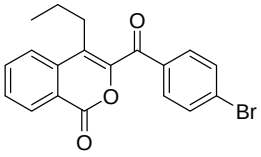
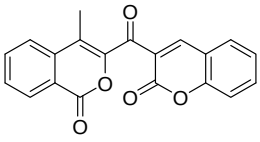
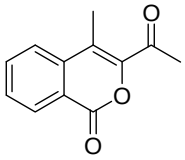
With increase in length of alkyl chain at 4^{th} position of 4-alkyl-isocoumarin-3-carboxylic acid, slight increase in activity is observed.

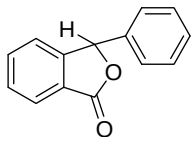
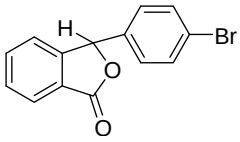
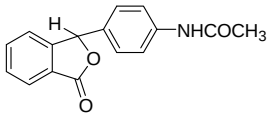
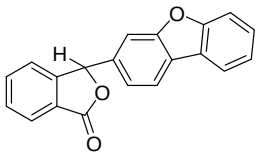
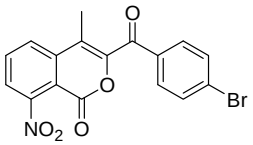
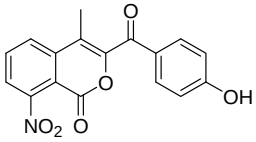
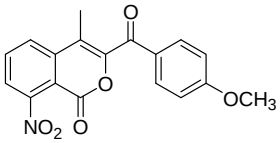
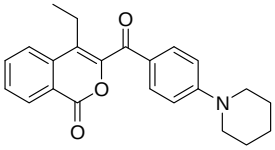
Acid hydrazide is found to show the same activity as the parent compound acid has.

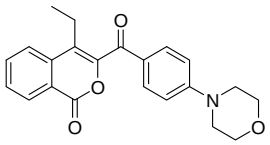
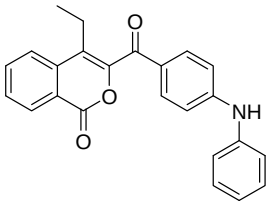
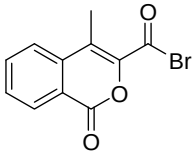
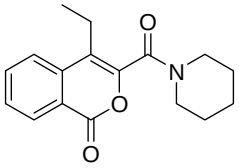
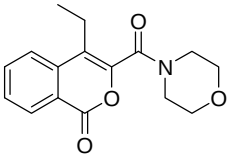
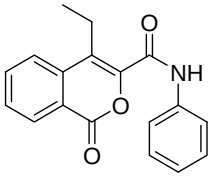
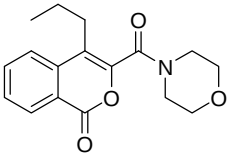
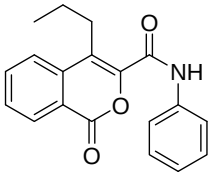
Schiff bases of isocoumarins furnish exceptionally good quality results in opposition *Chaetomium* and *F. Pallidorosoeum* strains.

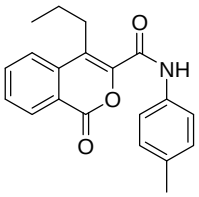
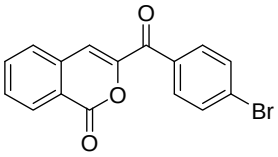
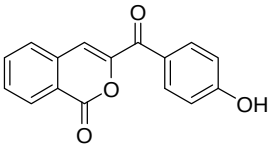
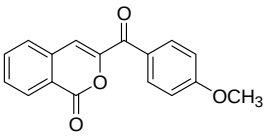
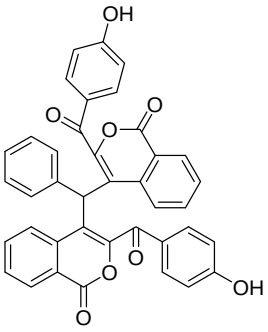
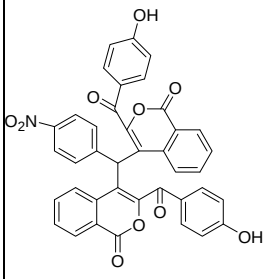
Insignificant activity is found when nitrogen heterocyclic moiety is introduced in the form of pyrazole, mercapto triazole and mercapto imidazole rings against *Chaetomium* and *F. Pallidorosoeum*.

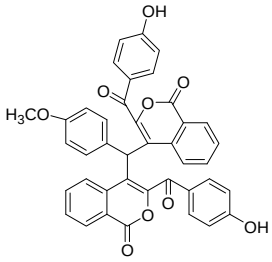
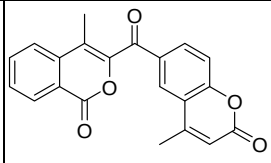
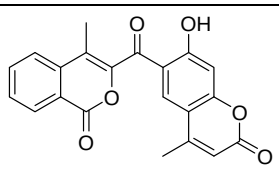
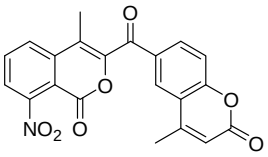
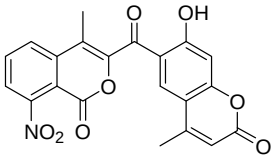
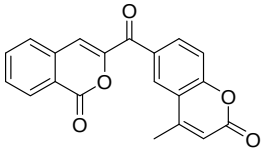
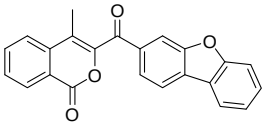
% Inhibition of Growth

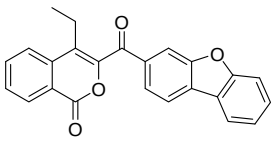
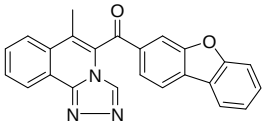
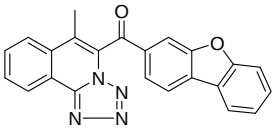
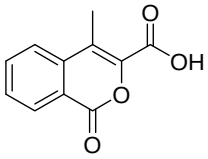
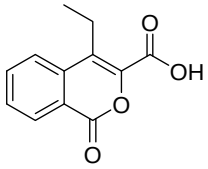
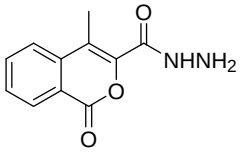
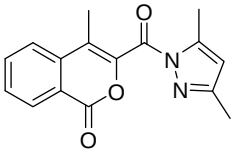
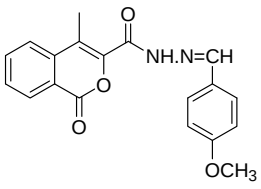
Sr. No.	Compound	*T. Paradoxa*	*P. Mangiferae*	*F. Pallidoroseum*	*Chaetonium*
1.		22.29	43.61	-	-
2.		-	-	70.00	-
3.		23.68	66.66	-	-
4.		38.63	59.15	-	-
5.		-	-	77.80	-
6.		21.21	20.58	-	-
7.		66.40	58.98	-	-
8.		29.34	44.10	-	-

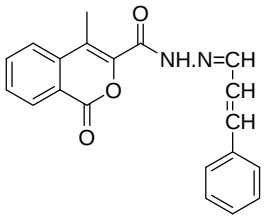
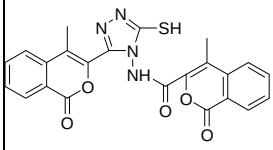
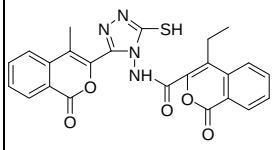
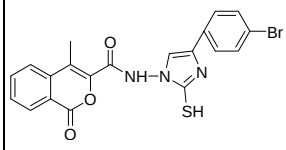
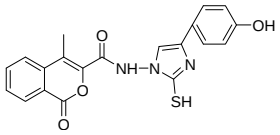
9.		55.23	44.68	-	-
10.		24.93	36.04	-	-
11.		40.21	42.65	-	-
12.		41.48	46.00	-	-
13.		-	-	0.00	0.00
14.		-	-	39.97	45.00
15.		-	-	0.00	0.00
16.		-	-	0.00	11.45

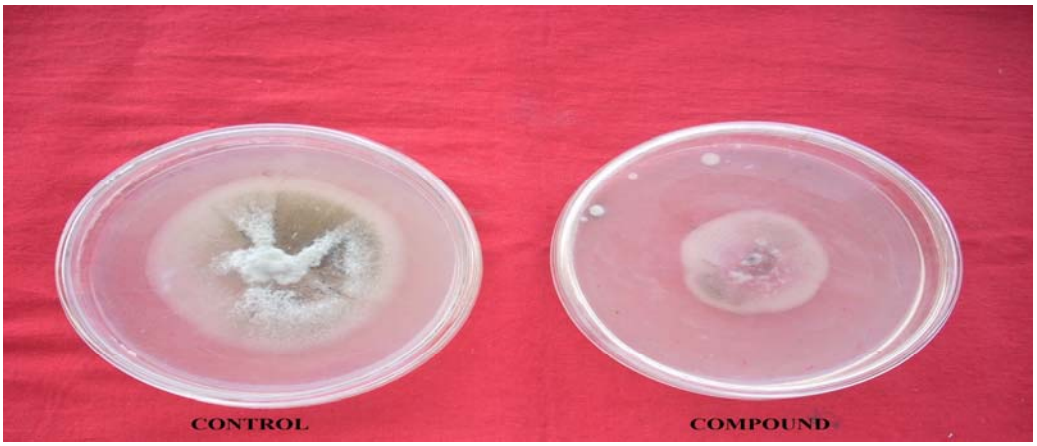
17.		-	-	0.00	100.00
18.		-	-	0.00	12.50
19.		80.33	81.74	-	-
20.		79.21	80.53	-	-
21.		0.00	35.55	-	-
22.		48.62	44.52	-	-
23.		11.90	38.00	-	-
24.		57.27	33.27	-	-

25.		22.58	46.07	-	-
26.		-	-	13.75	6.83
27.		-	-	29.60	17.76
28.		-	-	48.97	43.10
29.		-	-	64.87	70.60
30.		-	-	57.34	66.64

31.		-	-	64.08	70.00
32.		-	-	80.53	81.06
33.		-	-	80.39	79.55
34.		-	-	65.87	61.83
35.		-	-	77.69	68.37
36.		-	-	84.37	82.49
37.		-	-	36.81	27.99

38.		-	-	22.85	26.30
39.		-	-	53.00	51.89
40.		-	-	62.48	60.03
41.		-	-	61.12	60.75
42.		-	-	64.45	63.76
43.		-	-	58.73	55.30
44.		-	-	44.76	49.11
45.		-	-	75.07	77.90

46.		-	-	81.00	78.80
47.		-	-	31.96	38.00
48.		-	-	39.65	35.04
49.		-	-	22.87	30.01
50.		-	-	33.72	38.57


CONTROL
COMPOUND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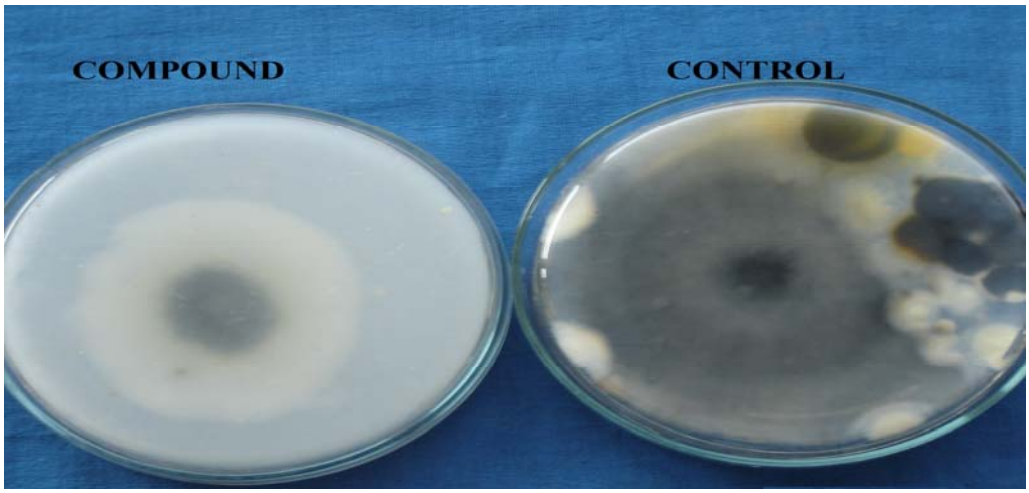
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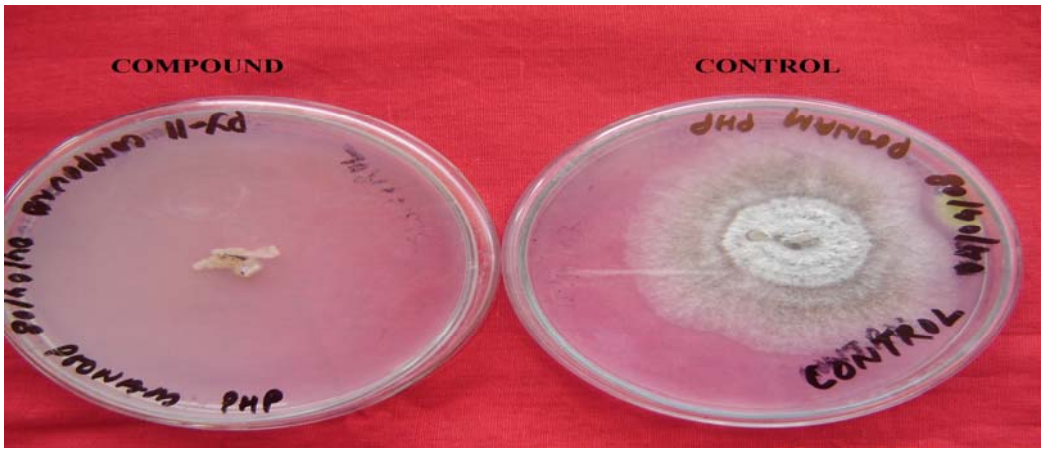
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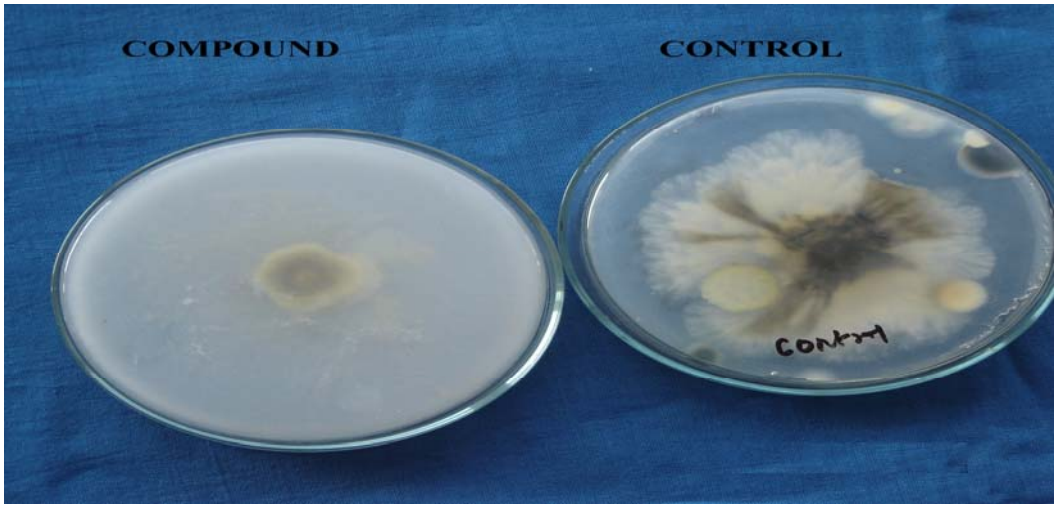

COMPOUND
CONTROL

Electron releasing group plays an important role in showing antifungal activity (**2**, **5**, **29**, **30**, **31**, **33**, **45**)

Morpholine ring shows excellent activity against *Chaetomium* i.e 100% when attached to aroyl ring (**17**) but activity is very less when morpholine ring is attached to carbonyl group directly (**21**, **23**). In the same way –Br group when directly attached to carbonyl group shows significant activity against *T. Paradoxa* and *P. Mangiferae* (**19**). This shows the importance of phenyl ring in showing the activity in this particular instance.

Schiff base directly attached to aroyl carbonyl group shows excellent activity against *Chaetomium* and *F. Pallidorosoeum.*

Conjugation, Lactone ring and electron releasing groups show much better antifungal activity.

ANALGESIC ACTIVITY

Analgesic activity of the compounds was determined by tail flick method[37] on mice of either sex. 2 % gum acacia was used as the control, Analgin was the standard drug and their reaction time being 3.00 and 9.00 seconds respectively.

4-alkyl-3-aroyl isocoumarins shows good reaction time as compared to the standard, the reaction time increases with the presence of electron releasing groups and number of phenyl rings. Increase in the length of alkyl chain also boosts the reaction time by few seconds.

With acetyl group at the 3rd position, the average response time dips a little.

The pattern for the phthalides is similar to 4-alkyl-3-aroyl isocoumarins, the reaction time is maximum without any substitutions, more with electron releasing group and minimum with electron withdrawing group.

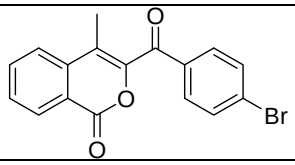
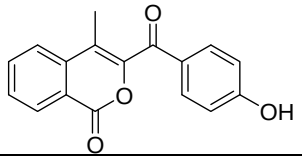
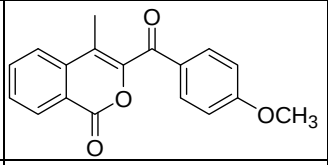
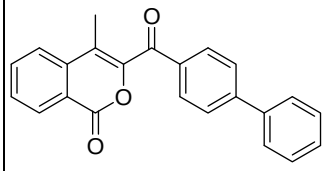
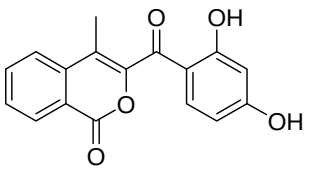
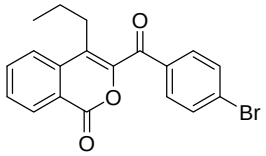
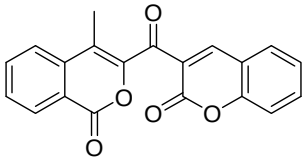
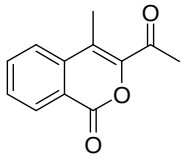
The response time decreases substantially with nitro and aminyl benzoyl derivatives, but escalates again on introduction of bromo carbonyl and amino carbonyl moieties.

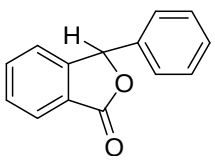
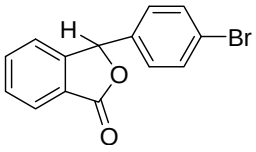
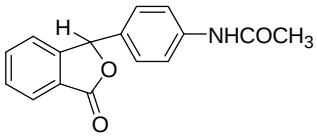
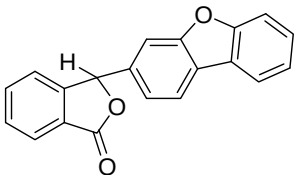
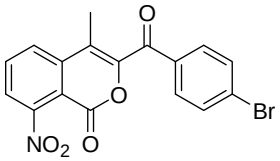
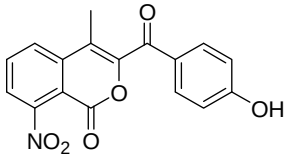
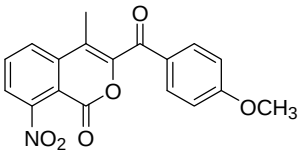
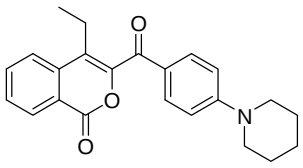
3-aroyl isocoumarins also fair badly in reaction time when compared to corresponding 4-alkyl-3-aroyl isocoumarins, ascertaining the importance of alkyl chain.

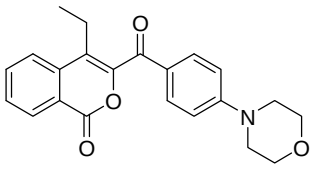
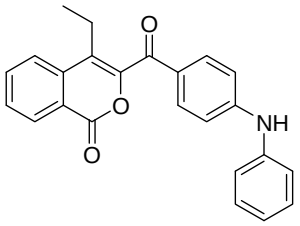
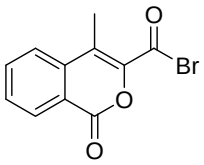
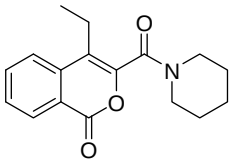
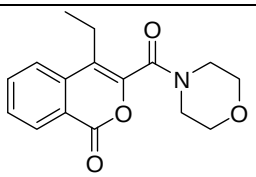
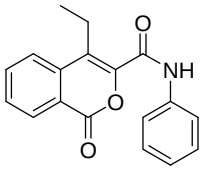
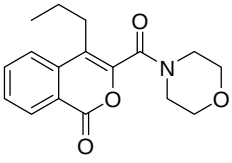
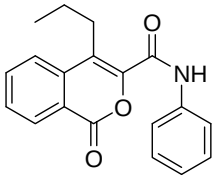
The response time is tremendous, almost equivalent to standard drug when two six membered lactone rings are present together, irrespective of the other groups present in them. This shows that the lactone ring in itself is gifted for biological applications.

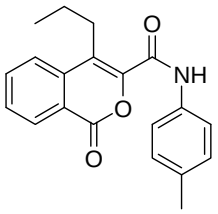
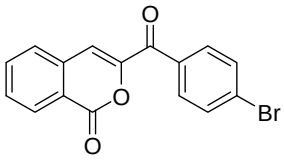
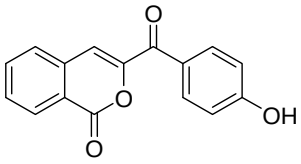
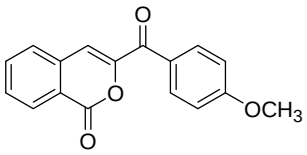
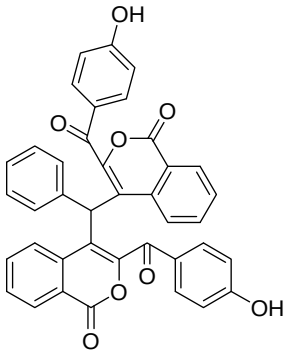
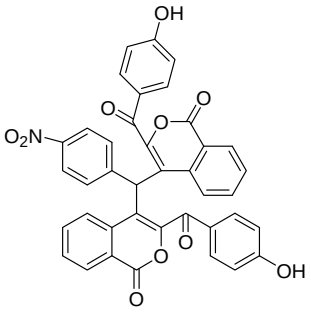
Triazole, Tetrazole isoquinolines, Isocoumarin-3-carboxylic acid hydrazide, Pyrazole and Schiff base derivatives also gives extremely good results, very close to the reaction time of standard, enhancing the importance of C=N bond in biological systems.

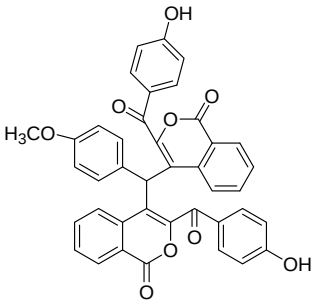
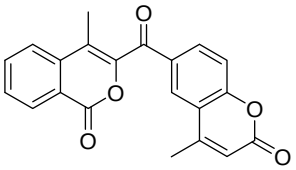
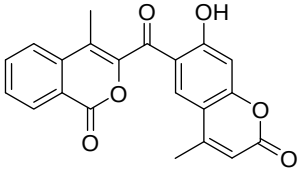
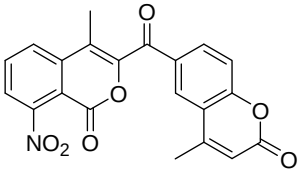
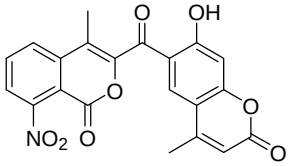
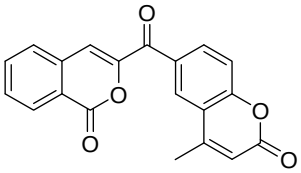
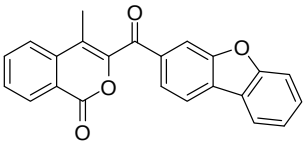
Introduction of mercapto triazole and mercapto imidazole rings in isocoumarins, however, decreases the activity considerably with response time being less than 5.0 seconds for majority of compounds, concluding that the presence of nitrogen and sulphur atoms together in isocoumarin moieties do not help in escalating the activity.

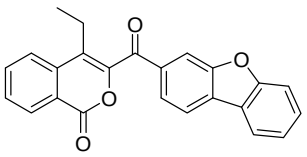
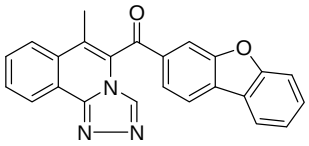
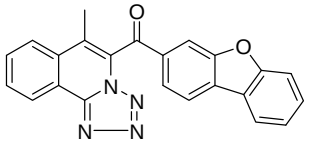
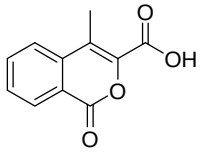
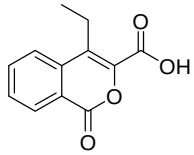
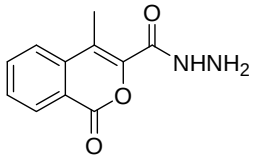
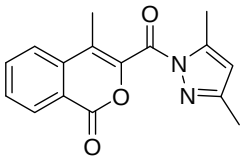
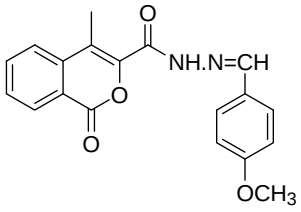
Sr. No.	Compound	Average (± SE) reaction time (sec.) Time after drug treatment (min.)			
		0	30	60	90
1.		3.69 (± 0.408)	5.34 (± 0.249)	6.51 (± 0.408)	5.56 (± 0.408)
2.		3.01 (± 0.00)	4.08 (± 0.408)	4.02 (± 0.408)	4.26 (± 0.408)
3.		3.00 (± 0.408)	4.50 (± 0.408)	5.50 (± 0.577)	6.25 (± 0.249)
4.		4.04 (± 0.408)	4.58 (± 5.770)	6.35 (± 0.500)	7.70 (± 0.249)
5.		4.04 (± 0.408)	4.58 (± 5.770)	6.35 (± 0.500)	7.70 (± 0.249)
6.		2.71 (± 0.245)	3.72 (± 0.245)	4.78 (± 0.381)	6.06 (± 0.577)
7.		2.72 (± 0.249)	5.33 (±0.577)	8.35 (± 0.456)	8.22 (± 0.456)
8.		2.31 (± 0.249)	4.02 (± 0.408)	4.59 (± 0.408)	5.03 (± 0.303)

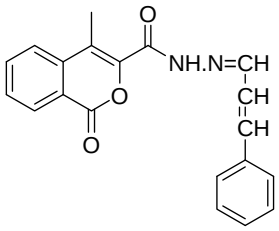
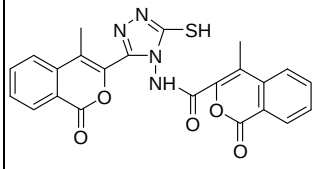
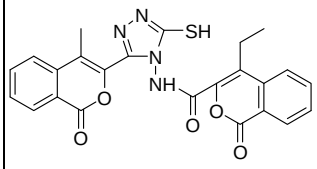
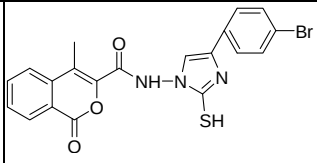
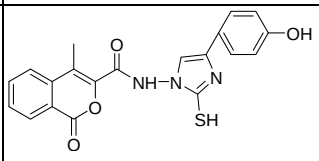
9.		3.75 (± 0.249)	5.25 (± 0.322)	8.25 (± 0.249)	8.50 (±0.249)
10.		3.51 (± 0.418)	4.00 (± 0.200)	5.59 (± 0.142)	6.34 (± 0.208)
11.		2.51 (± 0.408)	4.06 (± 0.408)	5.77 (± 0.749)	7.10 (± 0.408)
12.		2.31 (± 0.249)	4.52 (± 0.408)	6.52 (± 0.408)	6.26 (± 0.353)
13.		3.21 (± 0.000)	4.00 (± 0.408)	4.02 (± 0.408)	4.16 (± 0.408)
14.		3.21 (± 0.000)	4.00 (± 0.408)	4.02 (± 0.408)	4.16 (± 0.408)
15.		2.77 (± 0.200)	2.90 (± 0.423)	3.02 (± 0.408)	3.54 (± 0.211)
16.		3.00 (± 0.358)	3.20 (± 0.288)	3.55 (± 0.358)	3.79 (± 0.000)

17.		2.00 (± 0.158)	3.33 (± 0.200)	4.55 (± 0.338)	4.06 (± 0.100)
18.		1.09 (± 0.100)	3.05 (± 0.230)	3.69 (± 0.176)	3.99 (± 0.140)
19.		3.11 (± 0.008)	3.67 (± 0.308)	5.64 (±1.249)	8.00 (± 0.229)
20.		2.00 (± 0.308)	4.00 (± 0.500)	5.19 (± 0.353)	6.84 (± 0.500)
21.		3.09 (± 0.408)	4.40 (± 0.408)	7.77 (±6.249)	8.65 (± 0.249)
22.		4.00 (± 0.408)	4.75 (± 0.500)	6.25 (± 0.353)	7.25 (± 0.500)
23.		3.21 (± 0.208)	4.00 (± 0.578)	7.96 (±0.249)	8.69 (± 1.249)
24.		3.65 (± 0.400)	4.66 (± 0.540)	6.87 (± 0.353)	7.59 (± 0.300)

25.		3.00 (± 0.200)	4.48 (± 0.240)	5.95 (± 0.203)	7.06 (± 0.229)
26.		1.32 (± 0.100)	3.28 (± 0.230)	3.69 (± 0.176)	4.77 (± 0.140)
27.		2.46 (± 0.158)	3.00 (± 0.288)	3.04 (± 0.358)	3.88 (± 0.000)
28.		3.62 (± 0.158)	3.97 (± 0.205)	4.01 (± 0.088)	5.35 (± 0.400)
29.		4.00 (± 0.100)	4.53 (± 0.265)	5.98 (± 0.888)	7.44 (± 0.000)
30.		3.53 (± 0.110)	4.20 (± 0.200)	5.68 (± 0.138)	6.52 (± 0.030)

31.		3.41 (± 0.365)	4.73 (± 0.249)	5.05 (± 0.138)	6.93 (± 0.000)
32.		3.32 (± 0.249)	5.81 (±0.577)	7.08 (± 0.456)	8.49 (± 0.456)
33.		3.49 (± 0.408)	4.40 (± 0.408)	7.26 (±6.249)	8.71 (± 0.249)
34.		4.03 (± 0.458)	5.39 (± 0.540)	6.74 (± 0.353)	7.09 (± 0.50)
35.		2.79 (± 0.849)	4.61 (±0.577)	7.34 (± 0.416)	8.50 (± 0.456)
36.		3.00 (± 0.447)	5.93 (±0.784)	8.00 (± 0.765)	8.34 (± 0.366)
37.		2.98 (± 0.240)	3.70 (± 0.245)	4.17 (± 0.371)	6.00 (± 0.517)

38.		2.87 (± 0.200)	3.75 (± 0.265)	4.27 (± 0.374)	6.06 (± 0.537)
39.		2.59 (± 0.247)	5.83 (±0.584)	8.15 (± 0.498)	8.26 (± 0.456)
40.		4.14 (± 0.408)	4.38 (± 5.77)	6.55 (± 0.500)	7.78 (± 0.249)
41.		3.35 (± 0.353)	4.76 (± 0.353)	6.73 (± 6.540)	7.22 (± 0.50)
42.		3.55 (± 0.353)	4.73 (± 0.353)	5.70 (± 0.408)	3.71 (± 0.245)
43.		3.27 (± 0.277)	3.98 (± 0.153)	6.74 (± .540)	8.14 (± 0.560)
44.		3.03 (± 0.008)	5.49 (± 0.049)	7.02 (± 0.195)	8.63 (± 0.403)
45.		3.00 (± 0.408)	5.32 (± 0.408)	5.59 (± 0.549)	7.31 (± 0.408)

46.		3.12 (± 0.049)	4.93 (± 0.322)	8.00 (± 0.249)	8.50 (±0.249)
47.		2.52 (± 0.220)	4.33 (± 0.048)	4.09 (± 0.339)	4.91 (± 0.748)
48.		2.58 (± 0.000)	3.59 (± 0.408)	4.26 (± 0.408)	4.40 (± 0.408)
49.		2.91 (± 0.238)	4.85 (± 0.249)	5.33 (± 0.408)	5.87 (± 0.988)
50.		3.00 (± 0.353)	5.73 (± 0.353)	5.70 (± 0.408)	4.49 (± 0.245)
51.	CONTROL	3.01 (± 0.358)	3.20 (± 0.288)	3.10 (± 0.358)	3.02 (± 0.00)
52.	ANALGIN	3.09 (± 0.408)	5.25 (± 0.249)	7.75 (± 0.249)	9.00 (± 0.000)

Introduction of Nitrogen atom in the form of functional group such as NO_2 (**13-15**), or when attached to the aroyl group (**17-19**) does not show good activity.

Significant results are obtained when nitrogen introduced as heteroatom is directly attached to carbonyl group (**20, 21, 23**) and in the form of amide (**22**, **24**, **25**, **43**, **45**, **46**).

Presence of –Br attached to phenyl ring shows moderate activity (**1**,**6**) but when attached to the C=O in the form of bromocarbonyl group (**19**), the activity is significant.

Nitrogen and sulphur atom together in compounds does not show any significant activity similar to the antibacterial and antifungal activity (**47-50**).

In accordance to antimicrobial activity, presence of two lactone rings show excellent activity (**29-36**).

Phthalides (**9-11**), show comparable response but the result is excellent without any substitution in the phenyl ring (**9**).

ANTIOXIDANT ACTIVITY

The synthesized compounds were tested for nitrous oxide scavenging by using Griess reagent[38]. The antioxidant activity was determined by measuring the absorbance of the chromophore formed by nitrous oxide with the Griess reagent at λ 546nm.

Almost all the compounds checked for the activity shows good % nitrous oxide scavenging.

The results obtained for antioxidant activity are opposite to that of earlier studies, in case of 4-alkyl-3-aroyl isocoumarins. The maximum scavenging is shown by isocoumarin containing electron withdrawing group with small alkyl chain, and as the number of electron releasing groups, phenyl ring or carbon atoms in the alkyl chain increases, the activity decreases.

Phthalide shows almost 85% of NO scavenging.

In case of nitro derivatives, the tendency is repeated with maximum NO scavenging obtained by the presence of electron withdrawing group.

In opposition to the other biological activities earlier, aminyl benzoyl isocoumarins here shows very good results for NO scavenging, except when electron releasing groups are introduced in the compound.

Aminocarbonyl isocoumarins on the other hand fair badly and the % NO scavenging obtained is less.

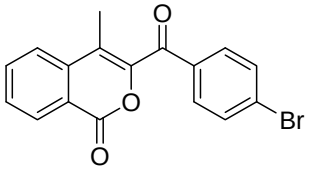
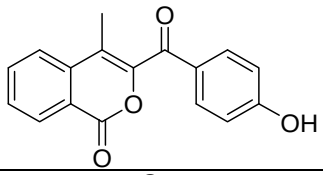
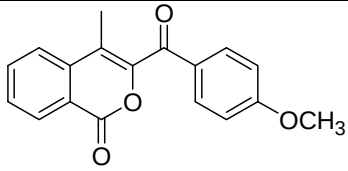
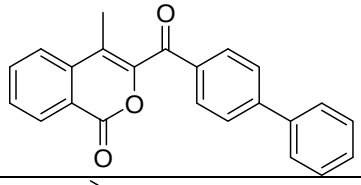
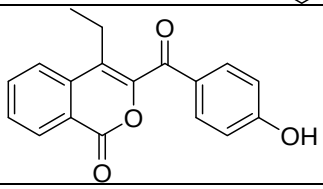
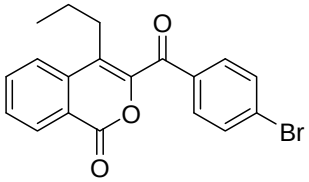
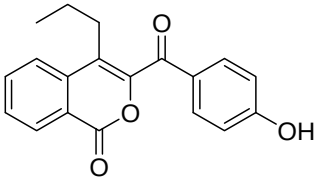
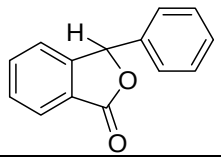
The trend gets reversed when the alkyl chain is removed from the isocoumarin moiety and the % NO scavenging is more with electron releasing groups.

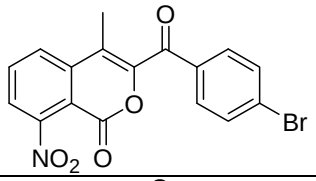
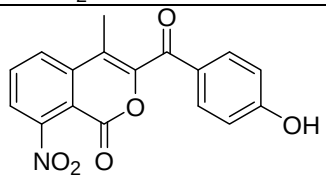
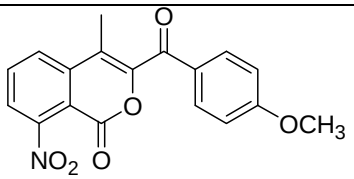
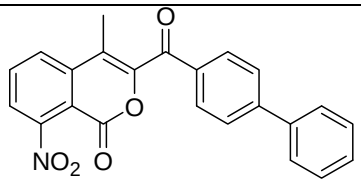
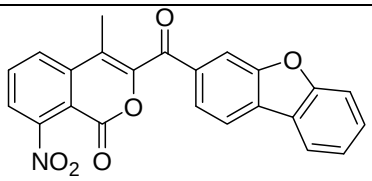
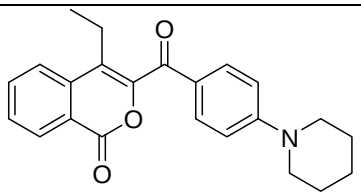
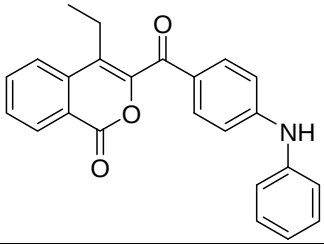
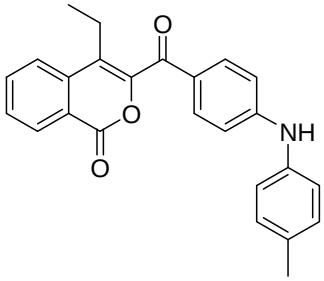
Presence of two isocoumarin rings or one isocoumarin and one coumarin ring together increases the % NO scavenging significantly with activity being around 100% in some cases.

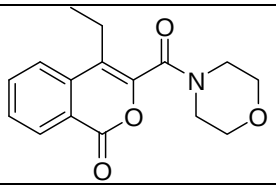
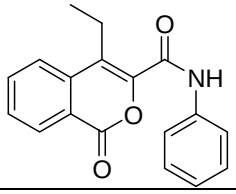
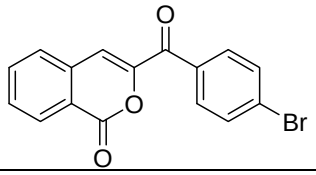
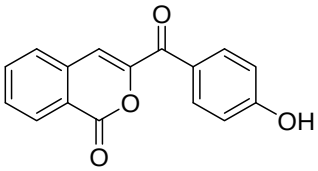
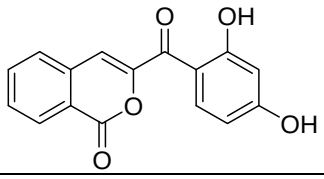
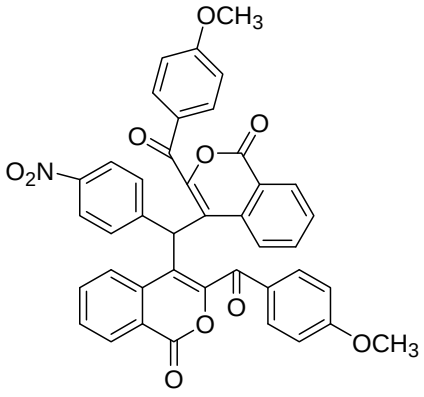
Triazole and Tetrazole nucleus in place of lactone ring shows poor results.

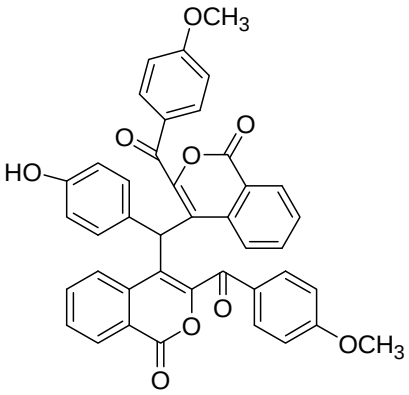
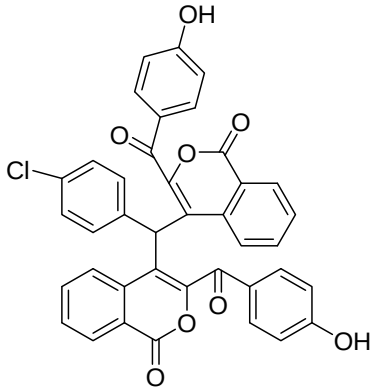
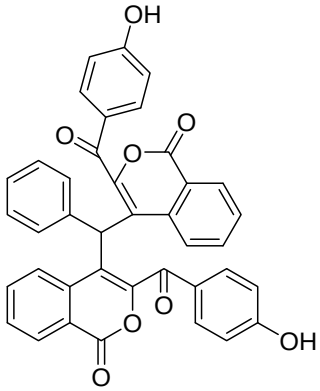
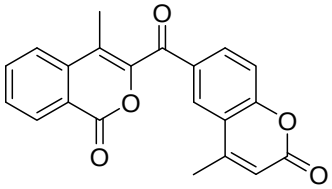
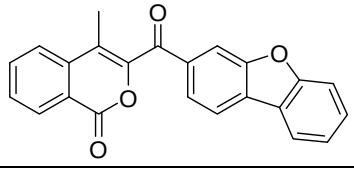
Exceptionally brilliant % NO scavenging is obtained with isocoumarin -3- carboxylic acid hydrazide, pyrazole and Schiff base derivatives, with more than 100% scavenging in most of the compounds.

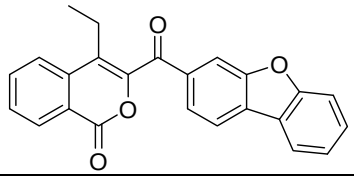
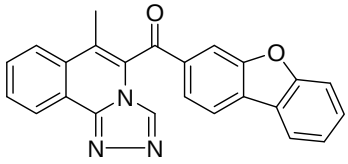
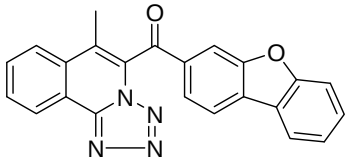
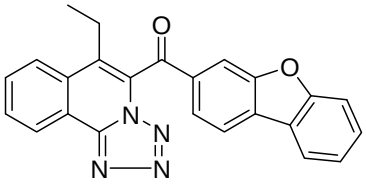
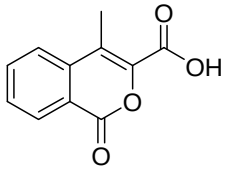
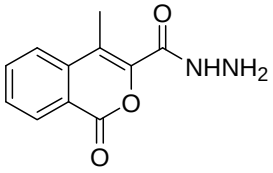
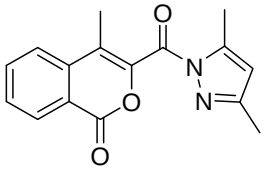
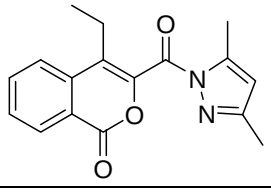
The activity decreases slightly with the introduction of sulphur atom in the isocoumarin derivatives, especially in the case of mercapto imidazole derivatives where the % NO scavenging is less than 50%.

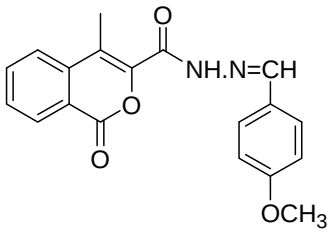
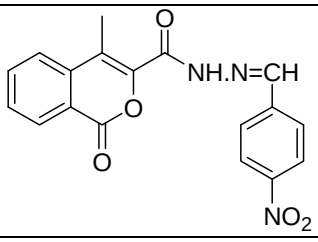
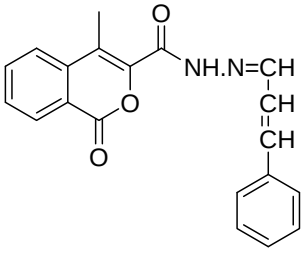
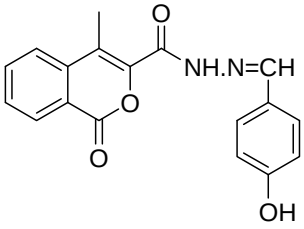
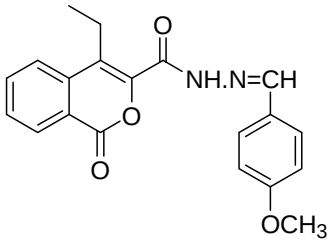
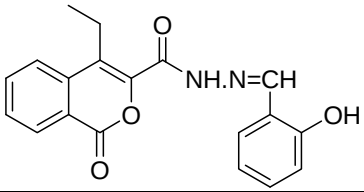
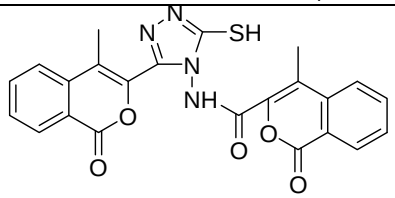
Sr. No.	Compound	Absorbance	% NO Scavenging
1.		-0.1110	109.55
2.		0.0474	95.91
3.		0.3821	67.10
4.		0.3378	70.91
5.		0.5412	53.40
6.		0.9071	21.90
7.		0.6605	47.86
8.		0.1806	84.45

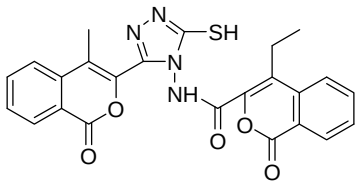
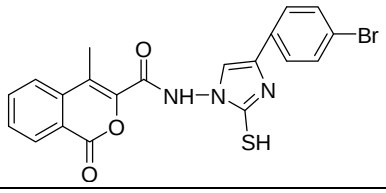
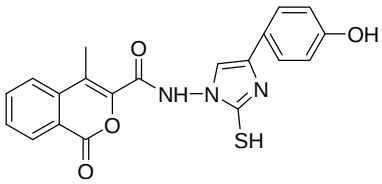
9.		0.4798	58.69
10.		0.7479	35.60
11.		0.8295	28.58
12.		0.5242	54.86
13.		0.7775	33.06
14.		0.2582	77.77
15.		0.3989	65.65
16.		0.8753	24.64

17.		0.9725	16.27
18.		0.3971	65.81
19.		1.0103	13.01
20.		0.5495	52.69
21.		0.6251	46.18
22.		0.6576	43.38

23.		0.3517	69.72
24.		0.1651	85.78
25.		-0.190	116.35
26.		0.0207	98.21
27.		0.3052	73.72

28.		0.6919	40.43
29.		0.3591	69.08
30.		1.0210	12.09
31.		0.7228	37.77
32.		0.2808	75.82
33.		-0.6240	153.72
34.		-0.7860	167.67
35.		-0.5820	150.10

36.		0.3614	68.80
37.		-0.0490	104.21
38.		-0.4370	137.62
39.		-0.7860	167.67
40.		0.7995	31.16
41.		0.3191	72.52
42.		0.4652	59.75

43.		0.3462	70.19
44.		0.7292	37.21
45.		0.8036	30.81
46.	CONTROL	1.1615	-

Similar to other activities nitrogen and sulphur atoms together does not show any significant activity (**43**, **44**, **45**) and excellent activity is seen in the form of amide (**33**, **37**, **39**).

Conjugation enhances the activity (**38**).

Pyrazoles attached directly to the aroyl carbonyl shows tremendous effect as antioxidant agents (**34**, **35**).

All bisisocoumarins (**23**, **24**, **25**) shows antioxidant activity.

With the substitution in the benzylidene ring i.e Cl, OH affects the activity but without any substitution in the benzylidene ring (**25**) the activity is significant.

Effect of alkyl chain length is seen with compounds (**1**, **2**, **26**).

ANTI-INFLAMMATORY ACTIVITY

Anti inflammatory activity of the title compounds was determined by measuring the concentration of nitrite in blood plasma, a NO metabolite by Griess reagent[39]. Acetone was used as the control. The concentration of nitrite in normal plasma was less than 20 μm. The level of nitrite increases approximately 9 times on treatment with Lipo Polysaccharide and the control alone decreases the level slightly.

The results obtained for anti inflammatory activity are similar to that of anti oxidant activity. 4- alkyl-3-aroyl isocoumarins reduces the level of nitrite drastically, very close to the level in normal plasma, only when it contains electron withdrawing group. Increase in the length of alkyl chain affects the activity unfavourably.

Presence of nitro group in isocoumarins decreases the level of nitrite many times when compared with LPS treated blood.

Aminyl benzoyl and aminocarbonyl derivatives of isocoumarins are also found to be very good anti inflammatory agents, the level being almost equivalent to normal plasma levels with the aminocarbonyl isocoumarins.

Removal of alkyl chain from the 4^{th} position of isocoumarin or the introduction of two six membered lactone rings shows admirable levels of nitrite concentration in plasma on comparison with the LPS treated blood and control.

Increases in the number of aromatic moieties in the compounds also help in dropping the nitrite level from the plasma.

Triazole and Tetrazole isoquinolines again shows very good action in controlling the concentration of nitrite and is capable of dipping it to the level of normal plasma.

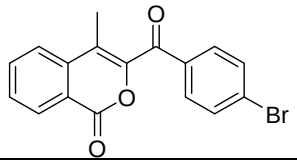
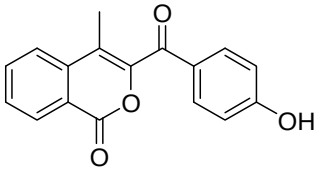
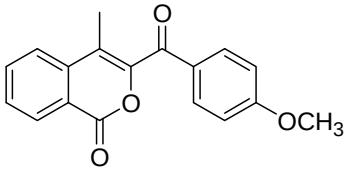
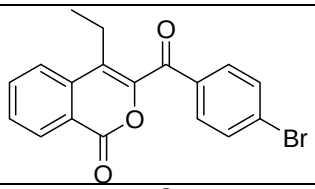
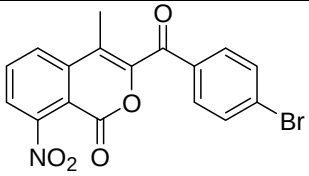
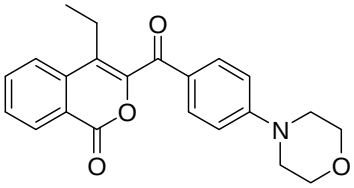
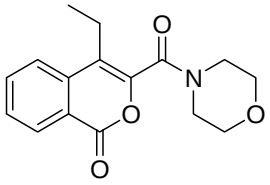
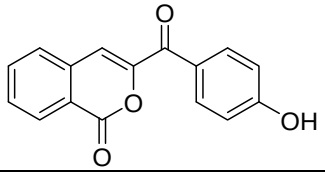
Iscoumarin-3-carboxylic acid hydrazide are slightly less active than the corresponding acid but were good enough when compared with control.

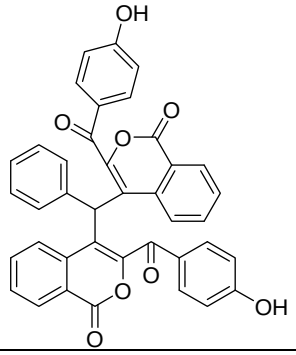
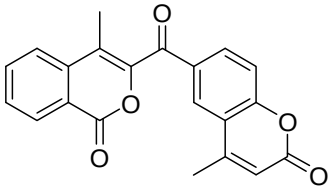
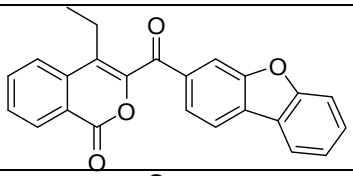
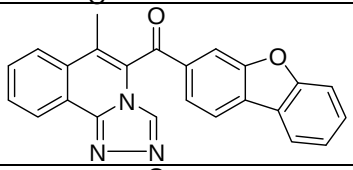
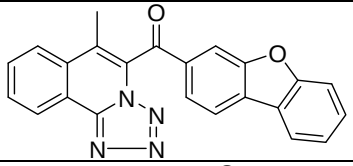
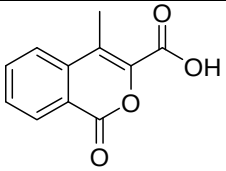
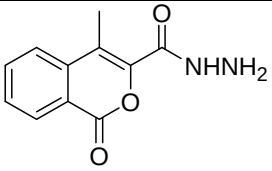
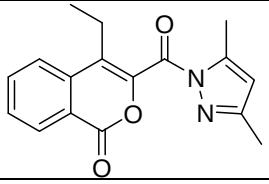
Pyrazole derivatives of isocoumarin are ineffective as anti inflammatory agents as the nitrite levels being equivalent to that of control.

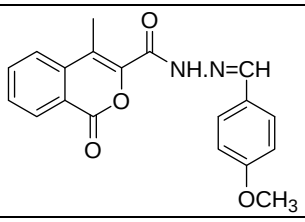
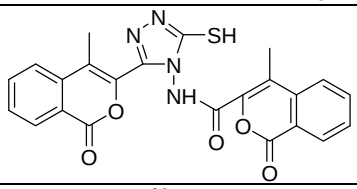
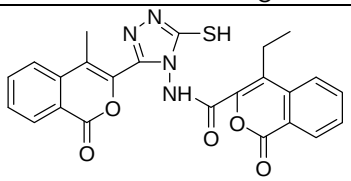
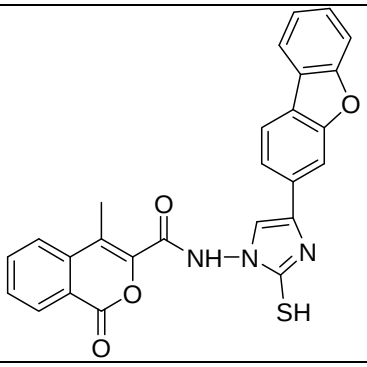
Schiff bases of isocoumarin are quite effective in controlling the concentration of nitrite after its release on LPS treatment.

Two isocoumarin moieties along with the mercapto triazole ring in the compound are seem to inhibit the nitrite concentration only when they are substituted with the same alkyl group. But with different alkyl groups in isocoumarins, it leads to more inflammation in blood and nitrite concentration shoots up more than that of LPS treated blood.

Same result is obtained with the mercapto imidazole derivative where the concentration of nitrite in plasma is found to be more than concentration of nitrite in control.

Sr. No.	Compound	Absorbance	Concentration of NO in blood (µm)
1.		0.0517	25.52
2.		0.3182	152.43
3.		0.2757	132.19
4.		0.0867	42.19
5.		0.1134	54.90
6.		0.1429	68.95
7.		0.0419	20.86
8.		0.1058	51.29

9.		0.1265	61.14
10.		0.1507	72.67
11.		0.1047	50.76
12.		0.0691	33.81
13.		0.0847	41.24
14.		0.187	89.95
15.		0.1902	91.48
16.		0.2545	122.10

17.		0.0925	44.95
18.		0.2014	96.81
19.		0.5545	264.95
20.		0.2843	136.29
21.	BLOOD	0.0347	17.43
22.	LPS TREATED BLOOD	0.3307	158.38
23.	CONTROL	0.2643	126.76

Nitrogen shows good activity in the form of heteroatom, when attached to carbonyl (**7**) than as NO_2 functional group (**5**).

Presence of electron withdrawing group like –Br shows good result (**1**,**4**).

In contrast to all other activities, here triazole and tetrazole isoquinolines show significant results which was not found earlier.

LARVICIDAL ACTIVITY

Larvicidal activity of the title compounds was investigated against mosquito larvae, culex pipens, and the lethal concentration (LC_{50}) values were calculated using Probit Analysis (Finney Method) [40].

Most of the synthesized compounds do not show any activity against the mosquito larvae with the LC_{50} values above 2000 ppm for the isocoumarins containing nitrogen atom in the form of nitro, aminyl benzoyl, amino carbonyl, isocoumarin-3-carboxylic acid hydrazide, schiff base and pyrazole groups.

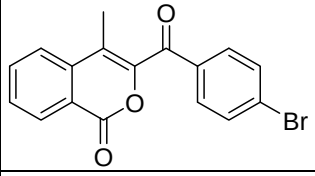
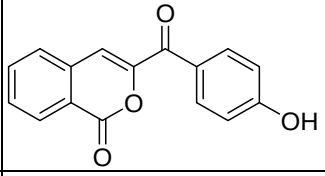
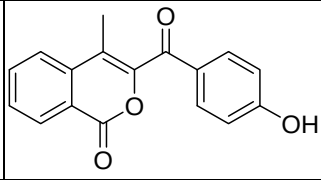
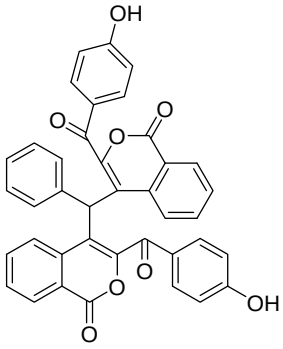
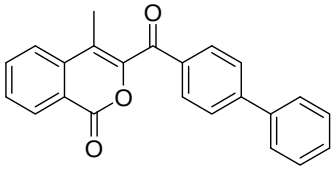
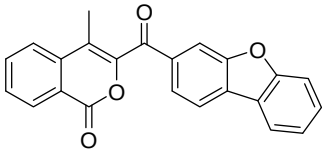
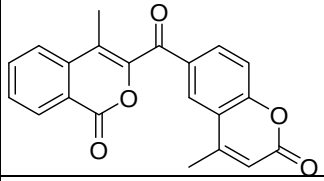
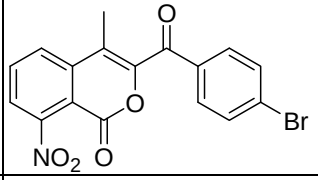
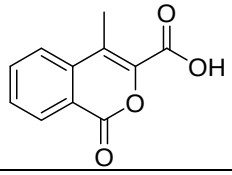
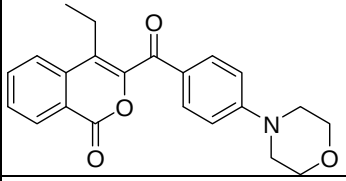
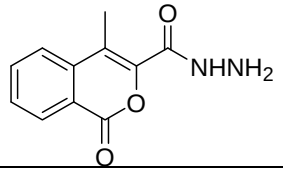
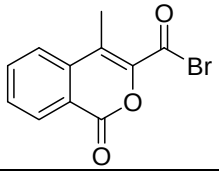
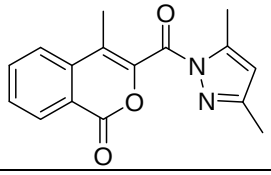
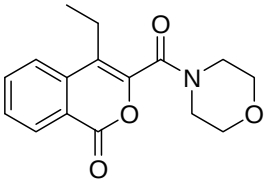
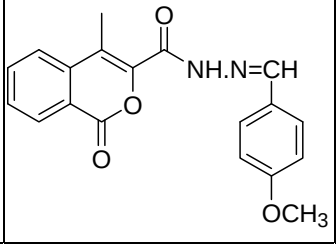
The 4-alkyl-3-aroyl isocoumarins were also found to be less lethal with LC_{50} values around 1000 ppm irrespective of the groups present in them.

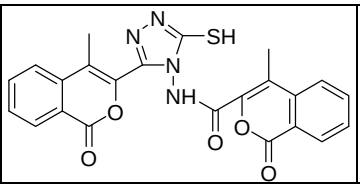
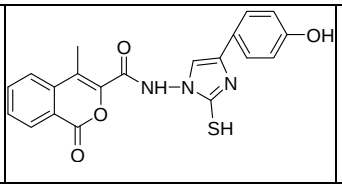
Introduction of mercapto triazole and mercapto imidazole rings in the isocoumarin moiety increases the activity against the larvae exceptionally and the LC_{50} value changes to 264 & 500 ppm respectively.

Removal of alkyl chain from the 4^{th} position of isocoumarins and presence of –COOH group also leads to very good action against the larvae.

When the numbers of phenyl rings are increased in the isocoumarin or existence of two lactone rings together, the activity increases many folds and the LC_{50} values are found to be less than 50 ppm.

Presence of dibenzofuran nucleus shows almost 100% activity with LC_{50} being 1.00 ppm.

Sr. No.	COMPOUND	LC_{50} value (ppm)	Sr. No.	COMPOUND	LC_{50} value (ppm)
1.		1000.00	9.		341.76
2.		1083.92	10.		23.88
3.		1.00			
4.		3.37	11.		22.40
5.		3269.54	12.		300.00
6.		2244.44	13.		2250.00
7.		1500.00	14.		1986.33
8.		2209.23	15.		2932.17

16.		264.51	17.		500.00

All the compounds investigated for the larvicidal activity, only four compounds are showing good results.

Compounds with –OH group enhances the activity (**9**, **12**). Increase in number of lactone ring promotes the larvicidal activity (**10**, **11**).

Excellent result is obtained by the presence of biphenyl ring (**3**).

GROWTH PROMOTING PROPERTY

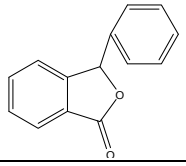
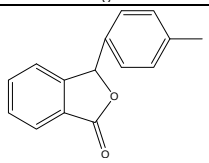
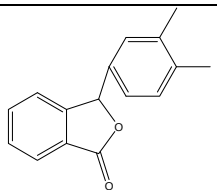
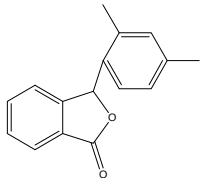
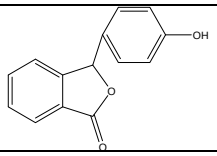
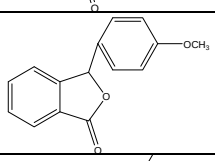
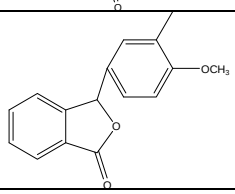
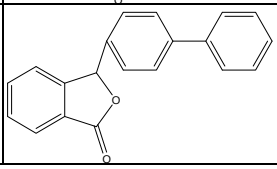
Growth promoting property of phthalides in Moong, Gram and Wheat seeds were determined and the % germination of seeds was noted after 24h, 48h respectively for Gram and Moong seeds and for Wheat seeds after 48h, 96 h. The % germination in control was found to be less than the test compounds.

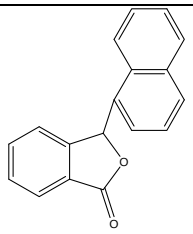
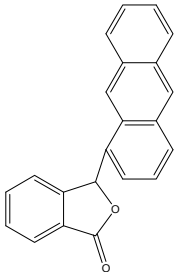
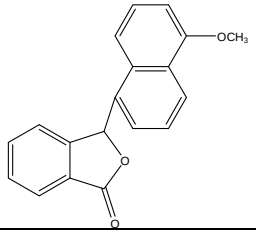
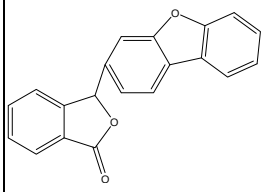
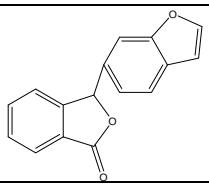
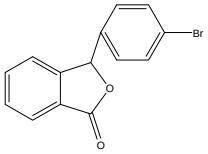
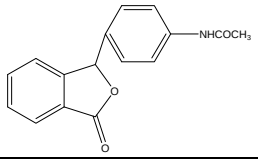
Fused phenyl rings with increasing number of phenyl group helps in germination in moong and gram seeds which is significant even after 24 hrs. After 48 hrs growth is seen in all compounds but it is insignificant for other groups such as dibenzofuran, benzofuran and biphenyl.

Electron releasing groups like –OH, OMe, $-CH_3$ helps in germination (moong and gram seeds). When two $-CH_3$ groups are substituted in phenyl ring than effect of ortho is better than meta substitution. In many cases after 48 hrs, 100% germination is obtained. The same result is observed with dibenzofuran as substitution at 3rd position of phthalide.

Germination in wheat seeds is very much insignificant even after 48 hrs. Better result was obtained with phenyl group substituted with two $-CH_3$ at ortho position or with -OMe group ortho to methyl group. In other phthalides substantial germination was seen after 96 hrs which was not comparable with gram and moong seeds.

% Germination in Seeds

Sr. No.	COMPOUND	Gram 24 hrs	Gram 48 hrs	Moong 24 hrs	Moong 48 hrs	Wheat 48 hrs	Wheat 96 hrs
1.		50	100	50	100	10	30
2.		40	80	30	60	10	40
3.		50	90	30	80	40	70
4.		40	80	40	60	00	00
5.		60	100	50	90	20	60
6.		30	80	50	100	10	50
7.		50	90	50	100	30	70
8.		40	60	45	100	20	90

9.		55	100	55	100	00	00
10.		60	100	60	100	00	00
11.		40	80	20	50	00	00
12.		60	100	40	100	10	20
13.		30	70	50	100	00	10
14.		20	40	40	80	00	00
15.		20	50	50	100	10	30
16.	CONTROL	20	60	40	80	00	00

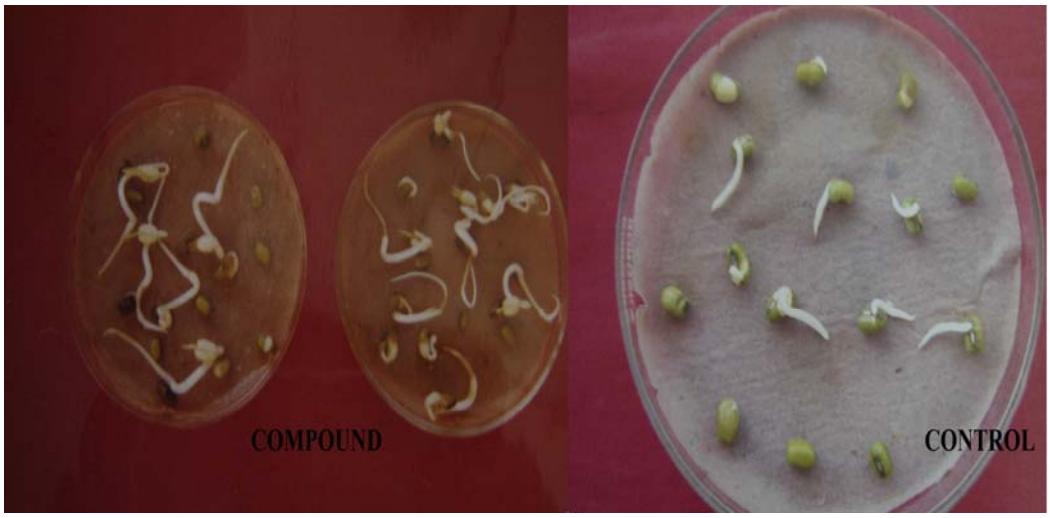
In all the phthalides the % germination in gram and moong seeds was good in 24 hrs compared to wheat and was excellent in 48 hrs.
Germination with wheat seeds was insignificant even in 48 hrs.

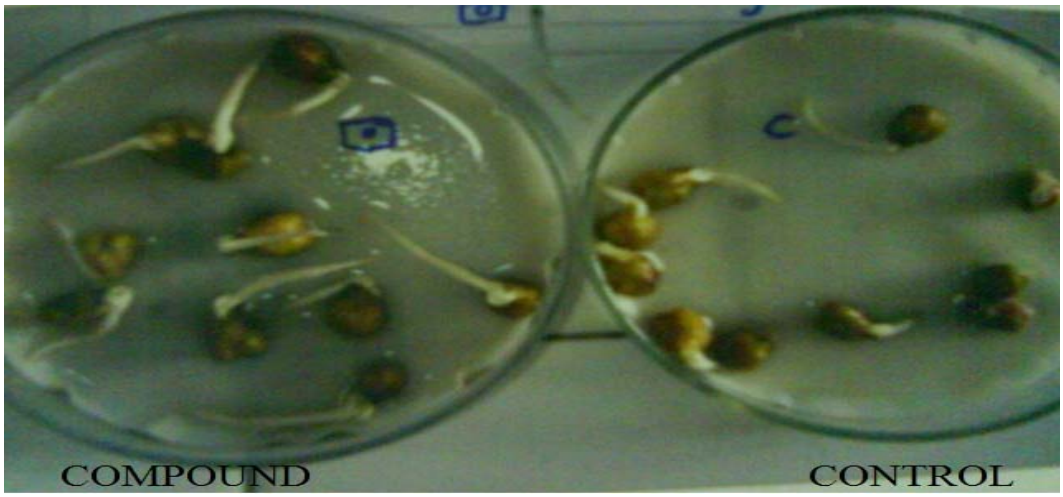
Fused phenyl rings help a lot in germination of both gram and moong seeds (**9, 10**) and 100% germination in 48 hrs is observed.

Isolated phenyl rings or only phenyl ring substituted but less than fused phenyl ring (**1**, **8**).

In moong seeds, phenyl ring substituted with $NHCOCH_3$ at 3^{rd} position of phthalide was found to be excellent after 48 hrs and significant growth was observed after 24 hrs when compared to other electron releasing groups.

In moong seeds, all the phthalides except few show 100% germination after 48 hrs (**1**, **6**, **7**, **8**, **9**, **10**, **12**, **13**, **15**).


COMPOUND
CONTROL


COMPOUND
CONTROL


E 3
COMPOUND
CONTROL

5.3 EXPERIMENTAL

All the biochemicals were procured from M/s Sigma Chemical Company USA. Other chemicals were of analytical grade of Qualigens or equivalent. Antimicrobial screening was performed in vitro against bacterial strains *Staphylococcus aureus* (gram positive), *Escherichia coli* (gram negative) and fungal strains *Thielaviopsis paradoxa, Phomopsis mangiferae, Fusarium pallidoroseum, Colletotrichum capsici.* Analgesic activity was done, in vivo, in mice of either sex weighing 20-25g. Fresh venous blood was collected from healthy volunteers for anti inflammatory activity. Larvae of *Culex pipens* mosquito was used in larvicidal activity.

General procedure for Antibacterial activity

Antibacterial activity of newly synthesized compounds was tested in vitro in bacterial strains, *Staphylococcus aureus* and *Escherichia coli* using serial agar dilution (cup plate method). [41]

The two microorganisms were cultured in dishes containing agar medium, four cups (8 mm) were put onto the dishes and each tested compound (0.1ml of 2mg/ml) was added into the cups under aseptic condition. Then the dishes were incubated at 37^0C for 24h. The zone of inhibition of the growth of the bacteria, which were produced by diffusion of the compounds from the cup into the surrounding medium, was measured to evaluate the antibacterial activity. Each experiment was repeated twice. DMF was used as a positive control for the experiments and the results were compared against standard drug ampicillin.

General procedure for Antifungal activity

Antifungal activity was performed in vitro against fungal strains *Thielaviopsis paradoxa, Phomopsis mangiferae, Fusarium pallidoroseum, Colletotrichum capsici* using Potato Dextrose Agar Medium (Poisoned Food Technique) [36].

The standard fungal culture *T. paradoxa, P. mangiferae, F. pallidoroseum & C. capsici* were grown on PDA slants at room temperature.

Mycelial growth inhibition of *T. paradoxa, P. mangiferae, F. pallidoroseum & C. capsici* was evaluated by the poisoned food technique, where the inhibition in growth of the fungal strain was observed on PDA. The stock solutions (1000ppm) were made from each of the test compounds. The required % concentrations of the compounds (mg/ml) were obtained by mixing the appropriate amount of the stock solution with 20 ml of molten PDA. The amended PDA was poured into petri dishes and allowed to set.

An inoculum of the fungus obtained from 7 days old axenic culture, grown as above, was placed at the centre of the amended agar medium. Each experiment was performed in triplicate. The diameter of the fungal colony was measured after 4 days and then 7 days at $26+1^0$C and the % inhibition was calculated using the following equation:

$$\% \text{ inhibition} = \frac{\text{Growth area in reference - growth area in sample}}{\text{Growth area in reference}} \times 100$$

General procedure for Analgesic Activity

Analgesic activity of the compounds was determined by Tail flick method [37]. Mice of either sex weighing between 20-25 g which shows positive response were selected and divided into different groups with four mice in each group. The first group served as control which received 2% gum acacia. Second group served as standard which received analgin at a dose of 50 mg/kg body weight orally. Rest groups received test compounds at a dose of 50 mg/kg body weight of mouse, orally.

The tail of the mouse was dipped (up to 5 cm) in a water bath at 55 ± 0.7^0C. The time taken to withdraw the tail clearly out of water was considered as the reaction time with the cut-off time being 60 seconds. The first reading was taken immediately after administration of the standard drug and test compounds and afterwards at the intervals of 30 minutes. The response time was recorded and the results are described in tabular form.

General procedure for Antioxidant Activity

Antioxidant activity was tested by estimating scavenging activity for nitrous oxide using Griess reagent[38] by test compounds.

2.0 ml of sodium nitroprusside (10 mM) in 0.5ml phosphate buffer pH 7.4 was incubated with 0.5 ml, 1000 ppm concentration of test compounds dissolved in a suitable solvent (DMSO) and tubes were incubated at 25^0C for 150 min. Control experiment was conducted with equal amount of solvent in an identical manner. After 150 mins, 1.0 ml of incubation solution was taken and diluted with 4.0 ml of Griess reagent (1.0 ml 1% sulfanilamide, 1.0 ml 5% o-phosphoric acid and 2.0 ml 0.1% N-naphthylethylenediamine dihydrochloride dissolved in distilled water). The absorbance of the chromophore formed during diazotization of nitrite with sulfanilamide and subsequent N-naphthylethylenediamine dihydrochloride was read at λ 546 nm after 30 mins. The experiment was repeated in triplicate. % NO scavenging was calculated using the following equation:

$$\% \text{ NO scavenging} = \frac{A_{control} - A_{test}}{A_{control}} \times 100$$

General procedure for Anti-inflammatory Activity

Anti inflammatory activity was measured as function of nitrite in human blood plasma, a NO metabolite, in control and test samples[39]. 0.5ml blood was treated with 100µl, 500 ppm, test samples dissolved in solvent and 100µl, 200 ppm, LPS solution for 24 hrs. 100µl blood plasma, separated after 24 hrs was then reacted with 100µl phosphate buffer (7.5 pH), 100µl 4N HCl and 100µl sulfanilic acid (0.04N HCl containing 2g/L sulphanilamide). After 30 mins, coloured dye was formed by adding 100µl N-naphthylethylenediamine dihydrochloride (1g/L NEDD). The formation of the azo dye was measured 30 mins later by spectrophotometry at 540 nm. The concentration of nitrite was calculated from a standard curve established with serial dilutions of sodium nitrite (0-200 µm). Acetone was used as the solvent.

General procedure for Larvicidal Activity

Batches of 20 larvae were exposed to 249 ml conductivity water and 1ml of acetone dissolved concentration of the test compounds in glass beakers of 500 ml capacity. It was made sure that the compounds were completely miscible in water.

Four replicate sets were tested with a final tally of 100 larvae for each concentration. The toxicity of each compound was determined, with at least sixteen concentrations ranging in between 10 and 1500 ppm to obtain a range of 10–98% mortality. Solutions containing 249 ml water and 1ml of acetone, without test sample served as controls. Food was provided once to the larvae during the test period. Mortality and survival were monitored after 24 h and 48 h of treatment. The moribund and dead larvae in replicates were combined and expressed as percentage mortality. The larvae were considered as dead or moribund, if they were not responsive to a gentle prodding with a fine needle. Experiments were conducted at 25 ± 2^0 C, 12 h light/dark regime. The final lethal concentration of the test compounds was calculated using Probit Analysis Method[40] with 95% confidence interval. Acetone was used as control and mortality rate of larvae was 0% in it.

General procedure for Growth Promoting Property

Growth promoting property of synthesized phthalides was checked against Moong, Wheat and Gram seeds. Seeds were placed in 90 mm diameter petridishes on Whatmann no.1 filter paper wetted with water. 20 seeds per petridish were used. 0.1 mg/ml solution of phthalides in suitable solvent (70% acetone-30% water) was used and control was only solvent without any compound. Seeds were first soaked in solution for 12 hrs and after that were transferred to petridish with few ml of solution just enough to wet the paper. Seeds were incubated at 24 ± 1^0C and readings were taken after 24 and 48 hrs for moong and gram seeds and for wheat seeds after 48 and 96 hrs during the assay. Radical emergence was used as the criterion for the germination.

5.4 CONCLUSION

- The synthesized compounds were investigated for different biological applications like antimicrobial, analgesic, antioxidant, anti inflammatory, larvicidal and for growth promoting properties.

- Most of the compounds were found to be good biological agents.

- The trend of activity was similar, for antimicrobial and analgesic activity i.e with the presence of electron releasing groups enhancing the activity.

- However, the trend gets reversed for antioxidant and anti inflammatory activity where electron withdrawing groups shows better action than electron withdrawing groups.

- The compounds investigated for larvicidal activity except few fair badly against the mosquito larvae with LC_{50} values, which is very high for showing activity.

- All the phthalides checked for growth promoting properties were seemed to help in increasing the % germination of seeds.

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Appendix - 1

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SYNTHESIS AND ANTIBACTERIAL ACTIVITY OF SOME NEW ISOCOUMARINS, PHTHALIDES AND PHENYL GLYOXALS

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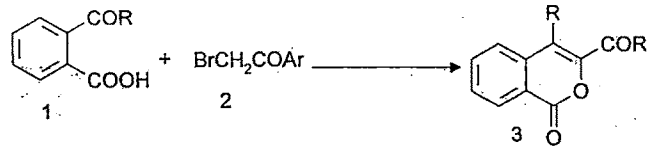
A new series of 3,4-disubstituted isocoumarins, 3-substituted phthalides and phenyl glyoxals has been synthesized conveniently in moderate yield. Structure of all these compounds have been confirmed by elemental analysis, melting point, IR and NMR spectral data. The bioassay result indicated that some compounds have high antibacterial activity and other compounds have bioactivity for improving plant growth.

Isocoumarin and phthalides have exhibited varied biological/physiological activities such as antibacterial[1-4] antifungal[5-6], blood anticoagulant[2], blood pressure lowering effect[2] and antiallergic effect[5].

In this paper we have reported synthesis and biological properties of some new isocoumarins by condensing (Scheme-1) *o*-acetyl benzoic acid (**1**) with bromo derivative of different substituted acetophenones



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1b R=C_2H_5

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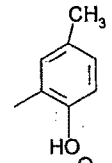

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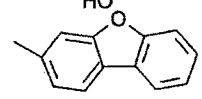

SCHEME-1

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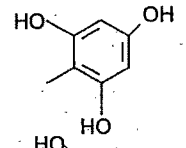

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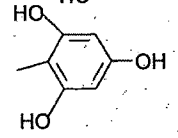
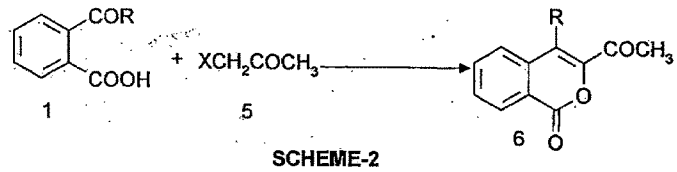




SCHEME-2

6a, R=CH_3
6b, R=C_2H_5 X=Cl or Br
6c, R=*n*-C_3H_7

Table-1
Characterization of new compounds prepared

Compd	Yield (%)	M.P. (°C)
3b	57	87
3c	62	140
3d	54	<200
6b	35	<200
6c	22	Semi solid
8b	66	130
8c	51	127
8d	60	135
8e	53	119
9c	51	94
9d	68	88

(2) in ethyl methyl ketone as solvent in presence of anhyd K_2CO_3 as base. Similarly isocoumarins (6a-c) (Scheme-2) were also formed when chloro/bromo acetone (5a, 5b) was taken in place of bromoacetophenone (2a-d) in presence of base. Isocoumarin formed with bromoacetone gave good yield but chloroacetone was found to be more suitable intermediate. Having taken o-aroyl benzoic acid (7) in place of o-acyl benzoic acid (1) (Scheme-3) and condensing it with different bromoacetophenones (2) resulted in the ring contraction and a mixture of phthalide and phenyl glyxal[10] was formed instead of isocoumarin. Every time condensation was between different aroyl acid with different bromoacetophenone derivatives, so that different phthalide and phenyl glyoxal were obtained, depending on the intermediates used. Some of the phthalides & phenyl glyoxals formed by different combination are reported earlier too[10,12]. Compounds synthesized were characterized by elemental analysis, IR and NMR spectral data after going through TLC.

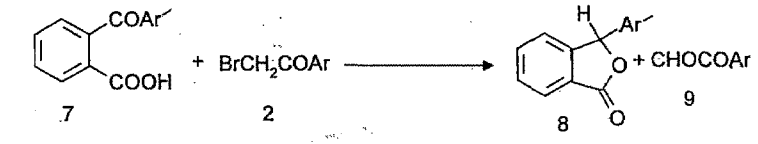
Phthalides were evaluated for their activity of plant growth promotion[2]. Some selected compounds were tested on wheat; moong seeds and average length of radicle were recorded. The concentration chosen were 0.001 to 100 ppm. It showed good result on moong growth.

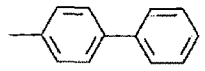
Antibacterial activity

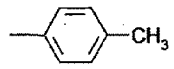
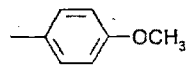
Isocoumarins, phthalides and phenyl glyoxals were evaluated for their antibacterial activity *in vitro* against *Escherichia coli* and *S. aureus* using standard cup plate method[11] and nutrient agar as culture medium. Compounds were found to be active, on comparing with control. Test solution was prepared by dissolving 1 mg (1000µg) in 1 ml of DMF and 0.1 ml (100µg) of this solution was used for testing. The zone of inhibition was measured in mm. 3c and 3d were found to be active against gram (+ve) bacteria, compound 3b showed slight activity against *S. aureus* whereas 3a was found to be active against *E.coli* and *S. aureus* both. The result was found to be excellent with 6b, 6c and 6d against gram+ve bacteria. In isocoumarin best result was obtained with gram (+ve) except 3b. Among phhalides and substituted phenyl glyoxals, all were found to be active against both gram+ve and gram-ve bacteria except 9a which was found to be active against gram +ve bacteria only. But excellent result was with phthalide having OCH_3 as terminal group (8e) against *S. aureus* and phthalide having biphenyl substituent group at 3rd position (8a) with *E.coli*. In general all compounds showed good activity specially towards gram (+ve) bacteria.

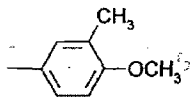
Growth promoting properties

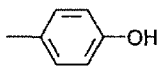
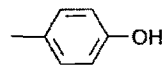
Phthalides are known for their growth promoting

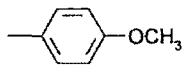


8a Ar' =  9a Ar = as in 3d

8b Ar' =  9b Ar = 

8c Ar' =  9c Ar = as in 3b

8d Ar' =  9d Ar = 

8e Ar' = 

SCHEME-3

properties[2]. In our earlier work compounds were tested on seeds pigeon pea and wheat. Encouraged from that result here phthalides were tested on moong seeds and a comparison with control showed good results. In comparison to wheat, with moong result was better. Compound 8b-e and 9a showed 50% germination after 24 hr and almost 100% after 48 hr except few. Germination with 8d was 90% after 48 hr. Radicle size was good with 8d, 9a and 8a but was excellent with 9a.

Experimental

Melting points were determined in open capillaries and are uncorrected. The purity of the compounds was checked by tlc on silica gel GF254. IR spectra were recorded on a FTIR Perkin Elmer spectrophotometer and ^{1}H NMR spectra were recorded on a Bruker spectrometer (400 MHz) using TMS as internal standard. All the compounds gave satisfactory elemental analysis. *o*-Acyl benzoic acid **1a**[12], b-c[13], *o*-aroyl benzoic acid[14] **7***a-e* and bromo compounds[14] **2** and **5** were prepared by literature method.

4-Methyl-3-(2'-hydroxy-tolyl) isocoumarin (3a)

A mixture of o-acetyl benzoic acid 1a (1g, 0.006

mol), 2-hydroxy-5-methylbromoacetophenone (1.39g, 0.006 mol) and anhyd K_2CO_3 (1.72g, 0.0128 mol) in ethyl methyl ketone was refluxed for 10-12 hr on a water bath, solvent was then removed, water added and extracted with ethyl acetate. Solvent layer was first washed with sod. bicarbonate and then with water and it was dried over anhyd. Na_2SO_4. After removal of solvent the crude product was purified by column chromatography using pet. ether-ethyl acetate. **3a** Yield 66.8%, m.p. 60°. IR : 3426 cm^{-1} (OH), 1758 (C=O), 1731 (lactone). ^1H NMR 1.5 (s, 3H, C_4-CH_3), 2.1 (s, 3H, C_5-CH_3), 5.0 (d, OH, C_2), 6.8-8.0 (m, 6H, C_5-H, C_6-H, C_7-H, C_8-H, C_3-H, C_6-H).

4-Methyl-3-acetyl isocoumarin 6a

A mixture of *o*-acetyl benzoic acid **1a** (1g, 0.006 mol), chloroacetone **5a** (0.0834g, 0.006 mol) and anhyd K_2CO_3 (1.72g, 0.0128 mol) in ethyl methyl ketone was refluxed for 10-12 hr, usual work up yielded **6a** which was purified by column chromatography.

6a: Yield 40%, m.p. 110°. ^1H NMR 2.6 (s, 3H, C_2-CH_3), 2.62 (s, 3H, C_3-CH_3), 7.6-8.4 (m, 4H, C_5-H, H, C_5-H, C_7-H, C_8-H).

3-Biphenyl phthalide[15] 8a, trihydroxy phenyl glyoxal 9a

A mixture of **7a** (1g, 0.0033 mol) & 2,4,6-trihydroxy bromoacetophenone **2e** (0.738, 0.0033 mol) and anhyd. K_2CO_3 (0.959g, 0.0069 mol) in ethyl methyl ketone was refluxed for 12-15 hr on a water bath, solvent was then removed, water was added and extracted with ethyl acetate. Solvent layer was first washed with sod. bicarbonate and then with water and it was dried over anhyd. Na_2SO_4 which resulted in a mixture containing two compounds **8a** & **9a** (TLC). The two compounds were separated by column chromatography using pet. ether-ethyl acetate.

Phenyl glyoxal (**9a**) obtained gave all chemical tests satisfactorily. Formation of phenyl glyoxal was finally confirmed by its synthesis following the known procedure[14].

8a: Yield 72%, m.p. 180°, ^1H NMR : 6.4 (s, 1H, C_3-H), 7.2-7.9 (m, 13H, C_4-H, C_5-H, C_6-H, C_7-H, C_2-H, C_3-H, C_5-H, C_6-H, C_2-H, C_3-H, C_4-H, C_5-H, C_6-H).

9a : Yield 48%, semi solid. IR : 1730, (C=O), 3428 (-OH). ^1H NMR : 9.55 (s, 1H, CHO), 4.7-4.9 (br, s, 3H, -OH), 6.1 (s, 2H, C_3-H, C_5-H).

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