SYNTHESIS, CHARACTERIZATION AND PHOTOPHYSICAL AND BIOLOGICAL STUDIES ON SOME HETEROCYCLES

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requirements for the award of the degree of

DOCTOR OF PHILOSOPHY IN CHEMISTRY

By

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CERTIFICATE

This is to certify that the work embodied in this thesis entitled **"Synthesis, Characterization and Photophysical and Biological Studies on Some Heterocycles** " has been carried out by **Mr. Ranjith. C**., under my guidance, and the same has not been submitted elsewhere for a degree or diploma.

Calicut University,
05.04.2008.

Dr. K. K. Vijayan

DECLARATION

This is to certify that the thesis entitled "**Synthesis, Characterization and Photophysical and Biological Studies on Some Heterocycles** " is an authentic record of the research work carried out by me, under the guidance of **Dr. K. K. Vijayan**, Professor, Department of Chemistry, University of Calicut and the same has not been submitted elsewhere for any degree or diploma. In keeping with the general practice of reporting scientific observation, due acknowledgement has been made wherever the work described on the findings of other investigators.

It is also certified that I have fulfilled the course requirements and passed the qualifying examination for the Ph.D. degree of this university.

Calicut University, **Ranjith. C.**

05.04.2008

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Multicomponent Reactions

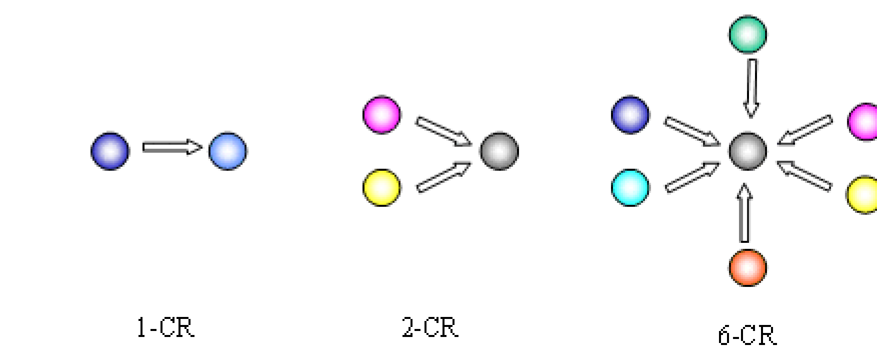
One of the major challenges in today's synthetic chemistry is the establishment of ideal step-economic processes (multi bond formation in one operation) with regio, chemo and stereo selectivity. Along with regio, stereo and chemo selectivity, an ideal chemical process should satisfy the criteria that it should be a process based on readily available starting material, operationally simple, easily automatable, resource effective, atom economical and ecologically benign. The complexity of organic target molecules is constantly increasing and novel strategies allowing the efficient formation of new carbon–carbon bonds between functionalised moieties are needed. The recent fashion in this scenario is the development of multicomponent reaction (MCR) processes. By definition, multicomponent reactions (MCR) are reaction processes in which three or more reactants are combined in a single chemical step to produce products that incorporate substantial portions of all the components reacted.

Multicomponent reactions are particularly effective at building functionalised drug-like structures from different families of compounds in a single step. Inventing and developing new MCR processes are important pursuits in academic, industrial and pharmaceutical chemistry. The last few years have seen a revolution in the development of new MCR reactions focused on the synthesis of enantiomerically pure or enriched compounds based on the use of new reagents and catalysts.

In an MCR, a product is assembled according to a cascade of elementary chemical reactions. Thus, there is a network of reaction equilibria, which all finally flow into an irreversible step yielding the product. The challenge is to conduct an MCR in such a way that the network of pre-equilibrated reactions channel into the main product and do not yield side products. The result is clearly dependent on the reaction conditions: solvent,

temperature, catalyst, concentration, the kind of starting materials and functional groups. Such considerations are of particular importance in connection with the design and discovery of novel MCRs.

Figure 1. Schematic presentation of a divergent one component reaction, a two component reaction and a highly convergent six component reaction.



In the light of chemical productivity and generation of molecular diversity an “ideal” MCR should, not only comprise more than two starting materials but also these starting materials would be different and all or most of the atoms of those starting materials would be incorporated into the final product.

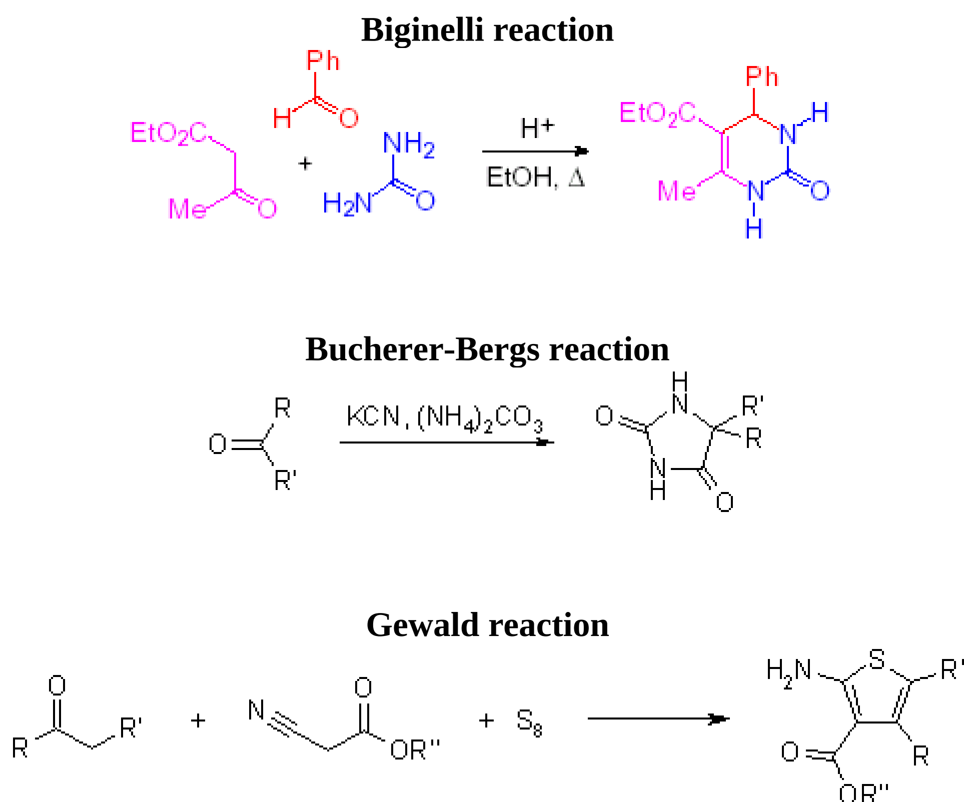
Most of the known older multicomponent reactions have been found by serendipity rather than by rational planning. It is interesting to note that many of them are constructed from only a few “prototypic” reactions that include amines and carbonyl functions of different reactivities like aldehydes, ketones, carboxylic acids, esters and amides – the main repertoire of chemists of those days. With the emerging automation of synthesis, purification and analysis in the area of combinatorial chemistry, we are entering into a new phase of our synthetic capabilities. New techniques like coupling liquid chromatography, mass spectroscopy and nuclear magnetic resonance (LC-MS-NMR) allow performing the fast and exhaustive investigation of rather

crude reaction products that may lead to new insights into "old" reaction mechanisms.

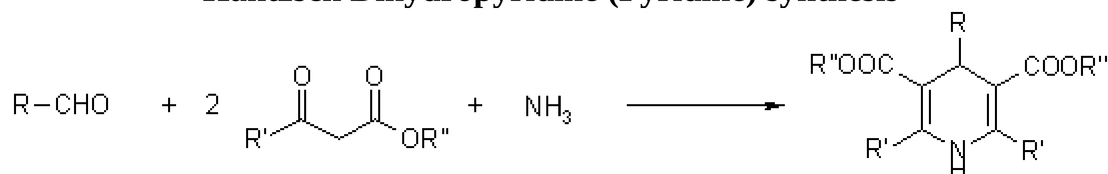
The parallel synthesis of large libraries of pure single compounds has been established in many pharmaceutical and agrochemical companies over the last years. Many otherwise "known" reactions have been investigated and used with a so far unseen wide range of starting materials, providing both insights into the breadth of these reactions as well as tales of the unexpected.

Carbonyl compounds played a crucial role in the early discovery of multicomponent reactions, as displayed by a number of following named reactions. The various multicomponent reactions involved by the carbonyl compounds are shown in Scheme 1.

Scheme 1. Multicomponent reactions of carbonyl compounds.



Hantzsch Dihydropyridine (Pyridine) synthesis



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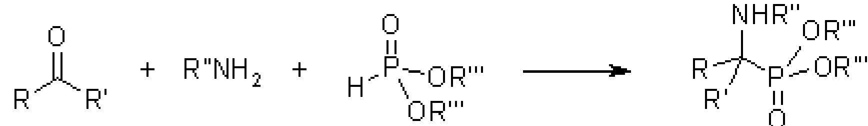
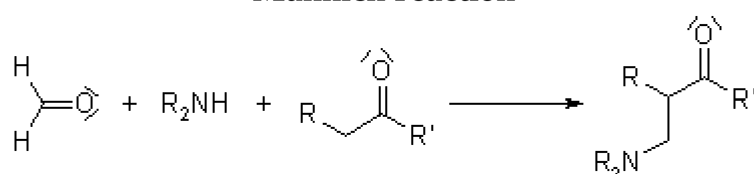
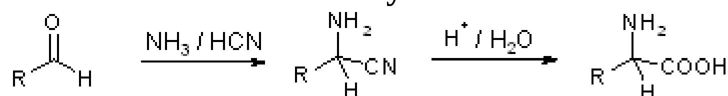


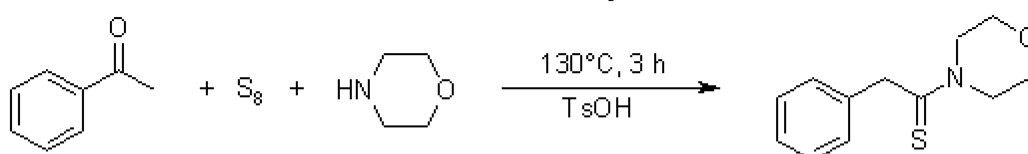
Mannich reaction



Strecker synthesis



Kindler thioamide synthesis



Multicomponent and sequential one-pot processes address very fundamental principles of synthetic efficiency and reaction design and they are steadily gaining a considerable and increasing academic, economic and ecological interest. Additionally, the aspect of a modular chemistry of one-pot reactions can be readily expanded into combinatorial and solid phase syntheses, promising manifold opportunities for developing novel lead

structures of pharmaceuticals, catalysts and even novel molecule based materials. Pioneering work by several research groups in this area has already established the versatility and uniqueness of one-pot multicomponent coupling protocols as a powerful methodology for the synthesis of diverse structural scaffolds required in the search of novel therapeutic molecules.

Microwave Assisted Organic Reactions

Microwave heating offers remarkable decrease in the time necessary to carryout reactions. It also appears that microwaves have a specific *microwave effect* that lowers the activation energy of reaction. During microwave-irradiated reactions many functional groups do not need protection. Very high yields and clean reaction have been obtained using only small amount of energy. The non-inert atmospheric conditions and simple experimental procedure are additional convenience. Microwave heating allows preparation of number of compounds at the same time in the microwave cavity. Therefore it is very useful in parallel and combinational synthesis. Microwave enhancement can take several forms. Reaction rate can be accelerated activated or suppressed. Fundamentally, microwaves heat things differently than conventional means.

Mechanism of Microwave heating

Microwaves are a form of electromagnetic energy lies in the region of the electromagnetic spectrum between infrared waves and radio waves. Specifically they are defined as those waves with wavelengths in between 0.01 and 1 metre, corresponding to frequency of 30 and 0.3 GHz. In order to avoid interference with RADAR equipment and telecommunications, the wavelengths at which industrial and domestic microwave apparatus may operate is regulated at both national and international levels. In majority of countries 2.40(± 0.050) GHz is the major operating frequency.

As with all electromagnetic radiation, microwave radiation can be divided in to an electric field component and a magnetic field component. The former component is responsible for the dielectric heating, which is effected via two major mechanisms, dipolar polarisation and conduction.

Dipolar Polarisation : A substance should possess a dipole moment in order to heat when irradiated with microwaves. A dipole is sensitive to external electric field and will attempt to align itself with the field by rotation. The applied filed provides the energy for this rotation. For a molecule in polar liquid such as water (methanol, ethanol, THF etc.), there are intermolecular forces, which give any motion of the molecule some inertia. The ability of molecules in a liquid to align with the applied electric field will vary with different frequencies and with the viscosity. Under a very high frequency electric field, the polar molecule will attempt to follow the field, but intermolecular inertia stops any significant motion before the field has reversed, and no net motion results. If the frequency of field oscillation is very low, then the molecule will be polarised uniformly and no random motion results. In the microwave radiation region, the frequency is however, not high enough for the rotation to precisely follow the field. Therefore as the dipoles re-orient to align itself with the electric field, the field is already changing and generates a phase difference between the orientation of the field and that of the dipole. This phase difference causes energy to be losing from the dipole by molecular friction and collisions, giving rise to dielectric heating.

Conduction mechanism: A solution containing ions, or even a single isolated ion with a hydrogen bonded cluster, in the sample the ions will move through the solution under the influence of an electric field, resulting in expenditure of energy due to an increased collision rate, converting the kinetic

energy to heat. The conductivity mechanism is a much stronger interaction than the dipolar mechanism with regard to the heat-generating capacity.

Solvent free organic synthesis

The chemists, for developing environment-friendly synthetic procedures has made them turn their attention to minimize or circumvent the use of solvents that are the major cause of pollution. It is believed that solvent free organic synthesis and transformation are industrially useful and largely green. This has led, in recent times, to vigorous research activity and reinvestigation of known reactions to achieve organic syntheses under solvent free conditions. The combination of solid support and microwave heating will be of importance in the search for green laboratory scale synthesis. The microwave strategy provides broad scope in the future development of clean and sustainable organic chemistry. Microwave synthesis represents one of the important dimensions of modern chemistry. The use of microwave processing is now the hot topic for combinatorial and parallel strategies.

The solvent free conditions under microwave irradiation, that is Microwave induced Organic Reaction Enhancement (MORE) chemistry offers several advantages. The cleanliness of microwave chemistry does away with the need for a solvent i.e. "The best solvent is no solvent". The solvents are often expensive, flammable, toxic, difficult to remove in the case of aprotic dipolar solvents with higher boiling points and are environmental polluting agents with disposal is often expensive. Waste solvents are a major problem for the chemical industry. More over liquid–liquid extraction is avoided for the isolation of reaction product.

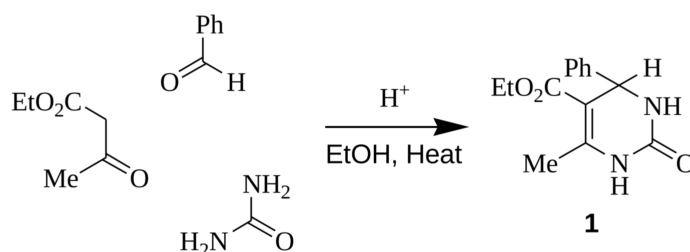
Solvent free reactions bring down handling cost due to simplification of experimental procedures (only simple glassware needed), work up technique and saving of labour. Microwave heating allows substantially

improved productivity of many chemical processes with reduced formation of byproducts caused buy overheating, also reduction in thermal degradation. Solvent less microwave synthesis is a springboard to clean, economical and safe industrial processing.

1.1.Introduction

Dihydropyrimidinones (DHPMs), commonly known as Biginelli compounds, have attained unprecedented attention due to its greater biological, pharmaceutical and therapeutic properties. In 1893, Pietro Biginelli reported the first synthesis of 3,4-dihydroprimidin-2(1*H*)ones (DHPM) by a very simple one-pot condensation reaction of an aromatic aldehyde, urea and ethyl acetoacetate in ethanolic solution[1] (Scheme 1.1). This efficient approach to partly reduced pyrimidines, termed the Biginelli reaction or condensation, was largely ignored in the following years, and therefore, also the synthetic potential of these multi-functionalized dihydropyrimidines remained unexplored. In recent years, however, interest in these compounds has increased rapidly, and the scope of the original cyclocondensation reaction has been widely extended by variation of all three components.

Scheme 1.1. Classical Biginelli condensation reaction.



The simple MCR for dihydropyrimidinone synthesis reported by Biginelli is being extensively exploited nowadays to have the maximum benefit to the scientific field especially in biological field. The synthesis and utilization of these multi-disciplined moieties is in full swing for the last few decades, which is clearly evident from the increasing number of publications and patents. The properties of these compounds can be changed considerably by varying the reactants of the reaction, which is of profound interest to contemporary scientific community.

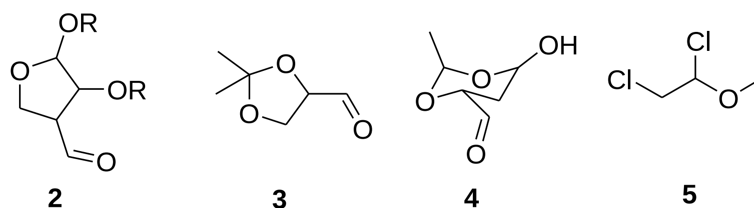
1.2. Review of Literature

Although the most straightforward protocol to synthesize DHPMs is the one-pot acid-catalyzed Biginelli condensation shown above (Scheme 1), this protocol– using ethanol and catalytic amounts of HCl– often provides only low to moderate yields of the desired target molecules, in particular, when substituted aromatic aldehydes or thioureas are employed.[2,3] This has led to the recent disclosure of several improved reaction protocols for the synthesis of DHPMs, either by modification of the classical one-pot Biginelli approach itself,[13] or by the development of novel, but more complex multistep strategies.[4]

1.2.1. Structural Variations of Reactants

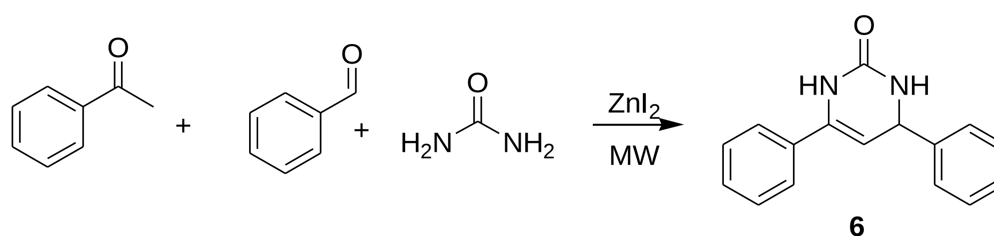
The original cyclocondensation reaction has been extended widely to include variations in all three components. Of these, the aldehyde component has been varied to the largest extent and now includes not only many aromatic,[5,6,7-9] but also aliphatic[7-13] and heterocyclic aldehydes.[14] Of particular interest are reactions where the aldehyde component is derived from a carbohydrate[15] (Figure 1.1). Another unusual substitute for an aldehyde in the standard Biginelli reaction is α, β-dichloroethyl ethyl ether[16] (Figure 1.1). The 4-unsubstituted derivative is prepared by reaction of methyleneurea with ethyl acetoacetate.[1,5,17] In some cases aldehyde diacetates have been used instead of the unprotected aldehydes.[11,14]

Figure 1.1. Substitute for aldehydes in Biginelli reaction



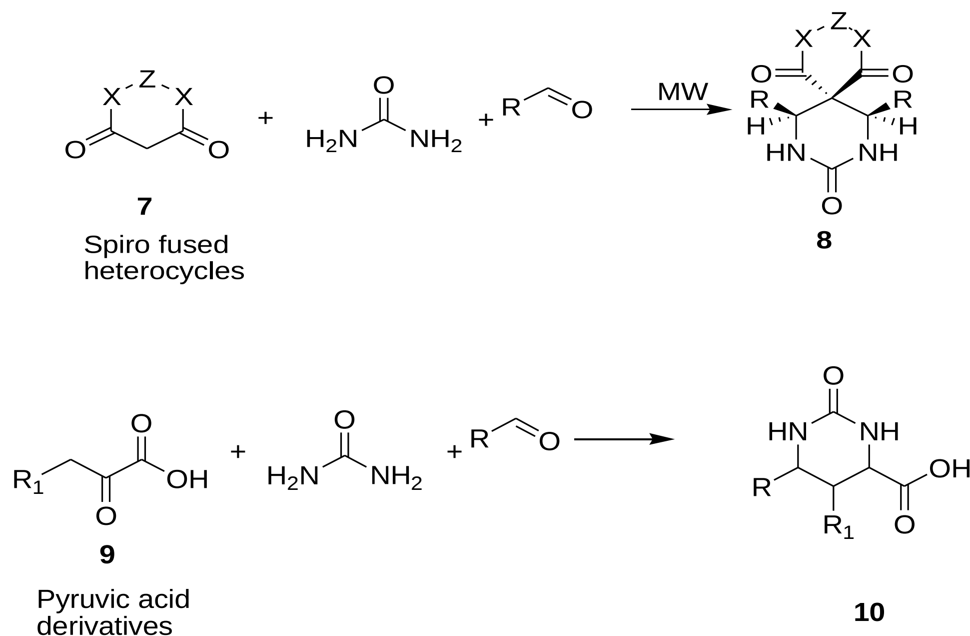
Apart from common alkyl acetoacetates which are employed frequently as the β-ketoester component, other acetoacetic acid esters such as benzyl acetoacetate,[10,18] methyl acetoacetate,[18] β-chloroethyl acetoacetate,[19] 2-furanylmethyl acetoacetate,[20] and ethylthioacetoacetate[20] and benzoylacetic acid esters[10] have been used successfully in the Biginelli reaction. Similarly, ethyl 4-bromoacetoacetate,[14] and ethyl trifluoromethylacetoacetate[7] afford the corresponding 6-functionalized dihydropyrimidinones. Liang *et al.*[21] used simple ketones instead of diketones to form DHPMs under a solvent free microwave assisted condition (Scheme 1.2).

Scheme 1.2. Simple Ketones for Dihydropyrimidinones



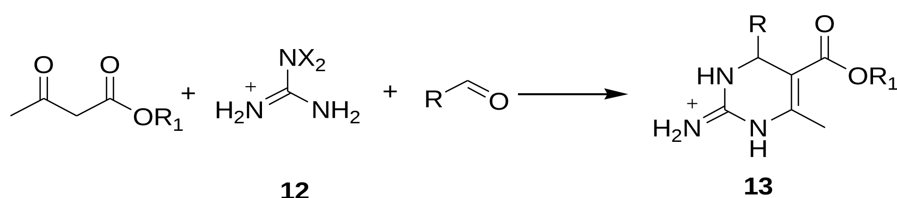
The diketones are substituted by spiro-fused heterocycles[22] and α-substituted ketoacids[23] for the successful formation of biginelli compounds as given in Scheme 1.3. Primary, secondary, and tertiary acetoacetamides have been used in place of esters to produce pyrimidine-5-carboxamides.[7,24-26]

Scheme 1.3. Spiro Fused Heterocycles and α- Ketoacids as Substitutes for Diketones in Biginelli reaction



Substituted ureas and thioureas can replace the urea component. It should be emphasized that monosubstituted ureas or thioureas form exclusively N-1 substituted dihydropyrimidines.[25-27] The N-3 alkylated products cannot be obtained by the standard Biginelli reaction or by alkylation of unsubstituted derivatives. N,N'-disubstituted ureas do not react at all under these conditions. Nilsson and Overman[28] effectively utilized the guanidine derivatives instead of urea for the formation of biologically active dihydropyrimidinones (Scheme 1.4).

Scheme 1.4. Guanidine Derivatives as Substitute for Urea in Biginelli Reaction



1.2.2. Variation of the Reagents and Methods

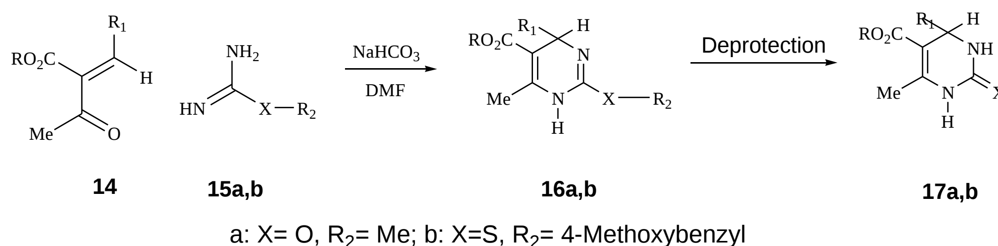
The classical Biginelli condensation was catalyzed by mineral acids. Later the mineral acids have been replaced successfully by various Lewis acids and metallic salts[30], polymer supported reagents[31], ionic liquids[32], non-metallic reagents[33] and solid catalysts such as clay[34], dowex[35], silica[36] etc. These reagents have been used under conventional as well as modern experimental conditions. The modern methodologies used for the Biginelli reaction involves the microwave assisted organic synthesis[30-36], ionic liquid phase organic synthesis[37] and sonochemical techniques[38]. Also combinatorial approaches[39] towards DHPMs have been advanced, under solid phase,[39a,b] or fluorous phase[39c,d] reaction conditions.

1.2.3. Alternative Synthetic Strategies

1.2.3.1. The Atwal Modification

Although the classical Biginelli reaction has been used widely in the past decades it is not always reliable and often gives only moderate yields, in particular when aliphatic or ortho-substituted aromatic aldehydes are employed. A more reliable approach to Biginelli compounds was reported in 1987 by K. Atwal and co-workers (Scheme 1.5).[40-42] In the first step an unsaturated ketoester (**14)** is condensed with a suitable protected urea (**15a**) or thiourea derivative (**15b**) in the presence of sodium bicarbonate. The reaction presumably proceeds through a Michael addition product and affords dihydropyrimidines (**16a,b)**. Deprotection with HCl (for 16a) or trifluoroacetic acid/ ethanethiol (for 16b) leads to the desired Biginelli compounds (**17a,b)** in high overall yield. Although this method requires prior synthesis of the unsaturated ketosters (14), its reliability and broad applicability makes it an attractive alternative to the standard Biginelli condensation.

Scheme 1.5. Atwal Modification for Biginelli Compounds.

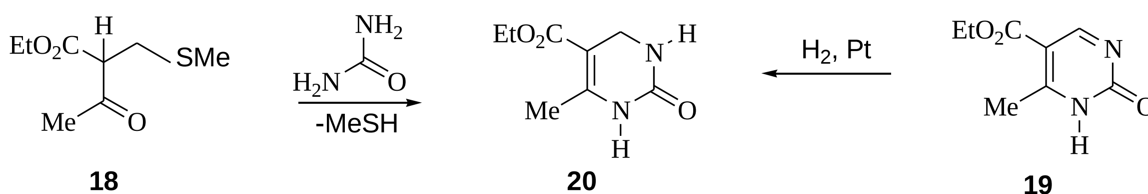


Similar results are obtained when **14** is condensed with guanidine or N,N-dimethylguanidine to give 2-amino-substituted pyrimidines, **16** (XR^2=NH_2, NMe_2).[43,44]

1.2.3.2. Other Procedures

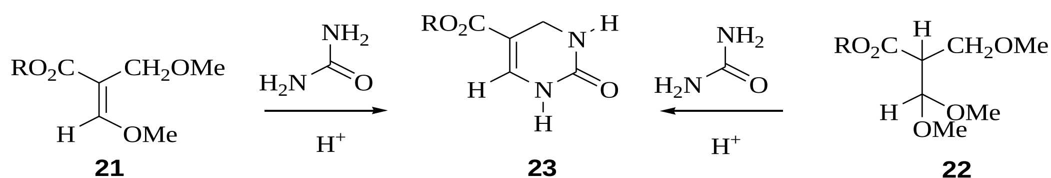
Apart from the procedure described in Scheme 1.1, there are a few other methods that lead to Biginelli compounds. Most of them, however, are limited in their scope and are hardly ever used for synthetic purposes. Thus, substituted acetoacetate can react with urea with elimination of MeSH to furnish dihydropyrimidinones (Scheme 1.6).[45] The same compound is obtained upon hydrogenation of pyrimidine with H_2/Pt.[46]

Scheme 1.6



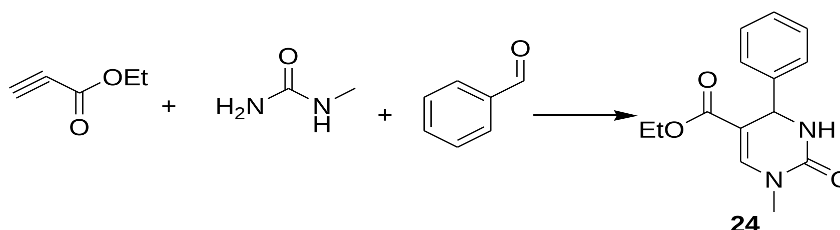
A route leading to dihydropyrimidinones, having a hydrogen atom in position 6 is shown in Scheme 1.7.[47]

Scheme 1.7



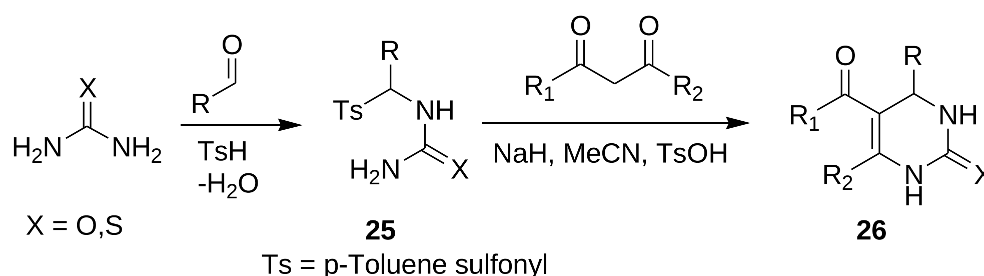
Another route leading to Biginelli compounds with a hydrogen atom in position 6 is the condensation of ethyl propiolate (H-C≡C-CO_2Et) with N-methyl urea and benzaldehyde (Scheme 1.8).[48]

Scheme 1.8



Yet another novel approach to DHPMs has been described by Shutalev *et al.*[4a] and is outlined below (Scheme 1.9). This synthesis is based on the condensation of readily available α-tosyl-substituted (thio) ureas with the (*in situ* prepared) enolates of aceto-acetates or 1,3-dicarbonyl compounds to give hexahydro pyrimidines which is then converted directly into DHPMs. This method works particularly well for aliphatic aldehydes and thioureas and produces high overall yields of the desired target compounds.

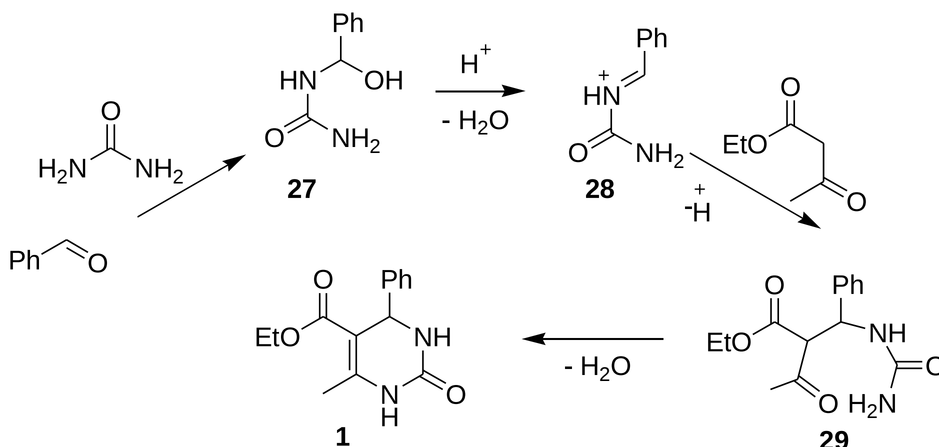
Scheme 1.9. Shutalev's Method for Dihydropyrimidinones



1.2.4. Mechanistic Aspects

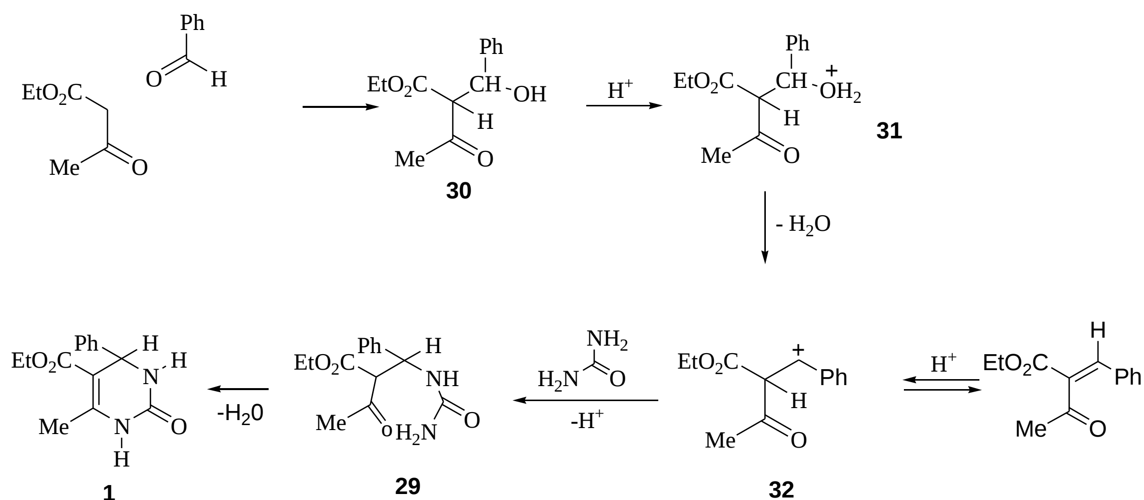
Several research groups have investigated the mechanism of the Biginelli reaction. Its dependence upon acid catalysis has been experimentally established[3-5] and a mechanism proposed by Folkers and co-workers[50] in 1933 was accepted. In the proposed mechanism, the first step is believed to be the condensation between the aldehydes and urea with some similarities to the Mannich condensation. The iminium intermediate generated act as an electrophile for the nucleophilic addition of the ketoester enol, and the ketone carbonyl of the resulting adduct undergoes condensation with the urea-NH_2 to give the cyclised product. The schematic representation of the mechanism is given in Scheme 1.10.

Scheme 1.10. Mechanism Proposed by Folkers for Acid Catalysed Biginelli Reaction.



The reaction mechanism was further reinvestigated by Sweet and Fissekis.[50] These authors suggested that an aldol condensation is the first and limiting step of the reaction, eventually leading to carbenium ion which was proposed as the key intermediate in the reaction. Interception of cation by urea affords an intermediate ready for cyclization to dihydropyrimidine as depicted in Scheme 1.11.

Scheme 1.11. Mechanism Proposed by Sweet And Fissekis.



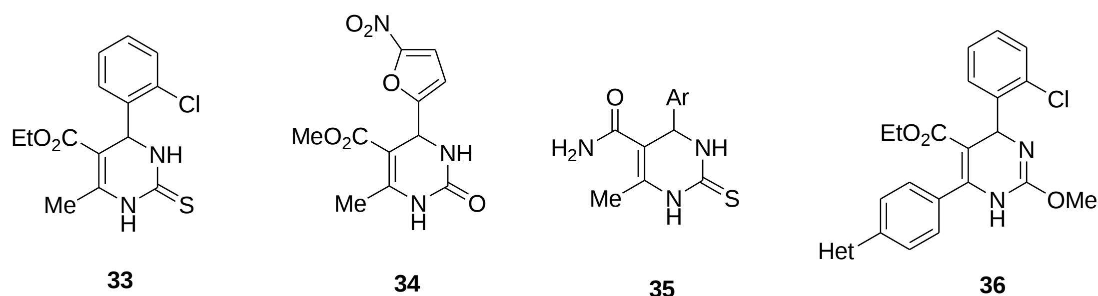
A reexamination of the mechanism by Kappe *et al.*[51] later showed that the original mechanistic proposal put forward by Folkers and Johnson in 1933, involving an aldehyde- urea condensation product as key intermediate in the Biginelli condensation is essentially correct. On the basis of the experimental evidences Hu *et al.*[3a] also established the same mechanism.

1.2.5. Biological Activity

Biginelli compounds show a diverse range of biological activities. As early as 1930 simple derivatives such as **33** were patented as agents for the protection of wool against moths.[52] Later, interest focused on the antiviral activity of Biginelli compounds,[53] eventually leading to the development of nitractin (**34**), which has excellent activity against the viruses of the trachoma group.[14,54] The Biginelli compounds also exhibits modest antibacterial activity. [55] Dihydropyrimidinone **1** and some of its analogs were screened as antitumor agents and found to be active against Walker carcinosarcoma in rats and mice.[55-57] Pyrimidine 5-carboxamides of type **35** are reported to possess anticarcinogenic[58] activity. Antinflammatory,[9,59] antioxidant,[59b] analgesic,[9] and blood platelet aggregation inhibitory activity [8] was found in a number of derivatives. 1,4 Dihydropyrimidine **36** is useful as platelet- activating factor

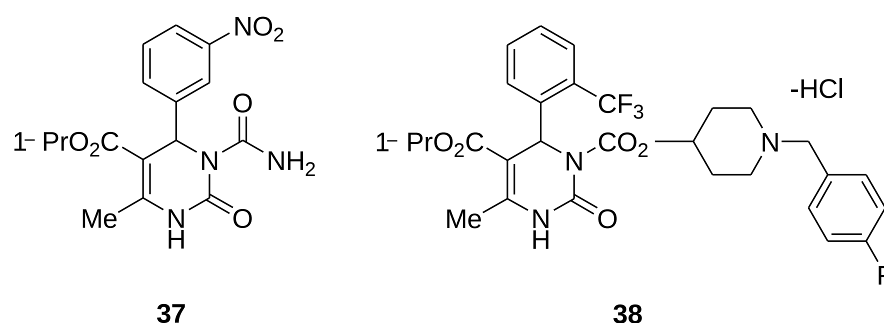
antagonist.[60] Other Biginelli compounds were shown to inhibit the uptake of adenosine by thrombocytes. [61]

Figure 1.2.

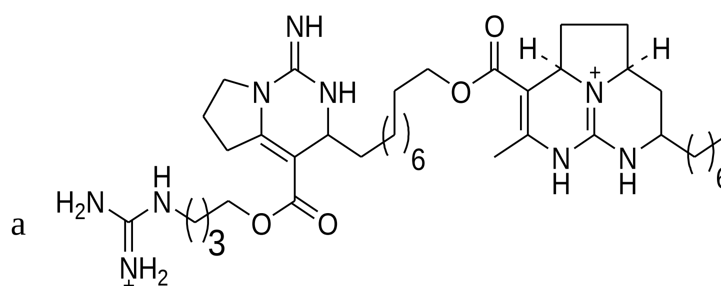


Dihydropyrimidinones have found widespread use in cardiovascular medicine[7,9,19] and have served as important tools for the study of calcium channel structure and function.[62] Structural modification of the substituent at N-3 of DHPMs led to the development of orally effective long-lasting antihypertensive agents (**37**).[65,66] Another derivative, **38** containing a basic amino group in the N-3 substituent was identified as a ‘lead compound’ for drug discovery.[67]

Figure 1.3.



Rovnyak reported a



general structure-activity relationship of dihydropyrimidinone calcium channel blockers.[67] Apart from their use as antihypertensive agents dihydropyrimidinone calcium channel blockers are also of interest as agents for treating anxiety,[68] and optic nerve dysfunction.[69]Several marine natural products with interesting biological activities containing the dihydropyrimidine-5-carboxylate core have recently been isolated.[70] Most notable among these are the batzelladine alkaloids (**39**) which inhibit the binding of HIV envelope protein gp-120 to human CD4 cells and, therefore, are potential new leads for AIDS therapy.[71]

1.3. Results and Discussion

Since the first report on the synthesis of dihydropyrimidinones by Biginelli in 1893, many improved procedures, have been developed for their formation under conventional, microwave assisted and ultrasonic pathways. Most of these methods reported describe the use of Lewis acids and salts, which mainly contain heavy metals. Other promoters include chloroacetic acid, ammonium chloride, tungstophosphoric acid, propane phosphonic acid anhydride, montmorillonite KSF clay, ZrO_2-pillard clay etc.

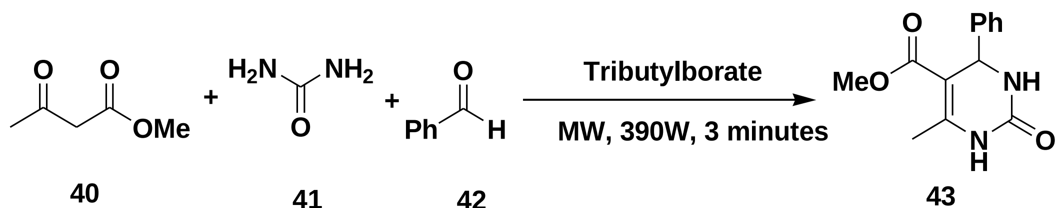
Although many revised protocols are available for the formation of dihydropyrimidinones, most of them suffer from low yields, long reaction time and cumbersome work up. In the present study we have devised tributylborate catalyzed two new synthetic strategies under microwave-assisted and reflux condition. The electron deficiency of boron and its ability to co-ordinate with the lone pair containing atoms or group make this reagent more efficient. Only very few boron-based reagents have been reported that catalyse the formation of DHPMs and they include BF_3-Etherate/CuCl/ HOAc, boric acid in glacial acetic acid, phenyl boronic acid, 1-n-butyl-3-methylimidazolium tetrafluoroborate and HBF_4.

It is noteworthy that tributylborate is a mild, efficient, cost-effective and non-metallic catalyst used for the first time to generate DHPMs library under conventional as well as microwave assisted conditions. To the best of our knowledge, it has not been much utilized as a catalyst in organic multicomponent reactions. In very few reactions it is used as a mild water scavenger[72].

In the present investigation my interest was to synthesis the pharmacologically active dihydropyrimidinone moieties without the possibility of any metallic contamination, which may alter its biological activity. Reports show that mono or di-substituted amide bearing DHPMs have the potential therapeutic properties such as anticancer, antioxidant, antimicrobial, analgesic, antihypertensive activities etc. With this basic knowledge I have synthesized some DHPMs with a secondary amide unit and screened for their bioactivity. The results of the biological studies have been discussed in Part 5.

The current studies were started by exposing a mixture of methyl acetoacetate (40), urea (41), benzaldehyde (42) and tributylborate to microwave in the absence of a solvent for 3 minutes at a power 390 W. It gave the target dihydropyrimidinone **43** in efficient yield of 95% (Scheme 1.12). A number of 1,3-diketones, urea/thiourea and aldehydes were found to be the suitable substrates for DHPMs synthesis under these conditions.

Scheme 1.12. Reaction of Methylacetoacetate, Urea And Benzaldehyde in Presence of Tributylborate.



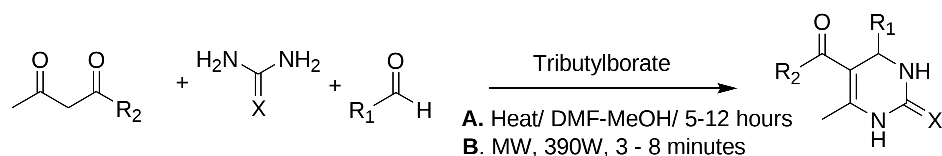
In the first part of the study, I have synthesized only known compounds to ascertain its applicability under different set of substrates and their melting points and other spectroscopic data are compared with that reported in the literature (Table 1.1). The microwave method gave good yields under micro-scale synthesis. However, in bulk synthesis the yield of the target compound was not very efficient. Hence, for large scale preparations, the reflux method has also been employed.

In the reflux method, the reaction was carried out in a dimethylformamide (DMF)- methanol solvent mixture at room temperature as well as under reflux condition. The summary of the optimization experiment carried out on the synthesis of compound **43** under conventional conditions has been shown in Table 1.2. From the table it could be understood that the room temperature experiments are not giving any satisfactory results. At the same time under reflux condition considerable yield was obtained. Also 1:1 DMF-methanol mixture was found to be a good solvent system for the reaction. All the compounds synthesized under solvent-free microwave assisted conditions have also been prepared under reflux condition and the yields are compared (Table 1.2).

From a comparative analysis of the microwave method and the reflux protocol for the synthesis of DHPMs, it is clear that the microwave method has merit over the other one in reaction time and yield. Also, the work up

under microwave method is very simple, easy and less time consuming than that of the reflux protocol. As the microwave strategy did not use any volatile organic solvents for synthesis it is an eco-friendly method. Except a few, most of the compounds are obtained in 95% purity. The formation of byproducts was almost negligible and the reaction was complete within 4-12 minutes. Although the microwave-assisted methods have many merits compared to the tributylborate catalyzed reflux technique, the latter is advantageous over the former when a large-scale synthesis is concerned. Though it is a time consuming process, the reflux method provides reasonable yields of DHPMs.

Table 1.1. Formation of DHPMs catalyzed by tributylborate.



Entries	R_1	R_2	X	Time		Yield (%)		Melting point (°C)	
				A (hours)	B (min)	A	B	Exptl.	Reported [Ref]
1	C_6H_5	Me	O	5	3	80	91	235-236	233-236 [30b]
2	4-OMe- C_6H_4	Me	O	7	4	72	87	174-176	178-180 [30e]
3	2-Cl-C_6H_4	Me	O	8	8	63	82	259-260	257-258 [60b]
4	C_6H_5	OEt	O	6	4	78	93	202-203	202-204 [29c]
5	4-OMe- C_6H_4	OEt	O	9	7	70	86	204-205	201-203 [3a]
6	2-OH- C_6H_4	OEt	O	10	6	68	69	198-200	201-203 [30d]
7	4-OH-3-OMe- C_6H_3	OEt	O	8	5	65	79	235-236	232-233 [30c]
8	Furyl	OEt	O	7	3	60	75	206-207	205-206 [38]
9	4-OH- C_6H_4	OEt	O	9	4	59	76	228-231	225-229[29c]
10	4-NMe_2- C_6H_4	OEt	O	5	2	58	83	258-260	256-258 [38]
11	3-NO_2- C_6H_4	OEt	O	11	8	61	72	227-229	229-231 [33a]
12	2-Naphthyl	OEt	O	12	3	60	81	247-249	248-250 [33d]
13	C_6H_5	OMe	O	5	4	85	95	206-207	207-210 [30b]
14	4-OMe- C_6H_4	OMe	O	6	4	70	83	188-191	191-193 [30b]
15	2-Cl-C_6H_4	OMe	O	8	6	55	71	247-249	252-253 [30b]
16	3-NO_2- C_6H_4	OMe	O	8	7	59	77	278-280	279-280 [30b]
17	C_6H_5	OEt	S	6	4	78	95	205-207	206-207 [30b]
18	4-OMe- C_6H_4	OEt	S	9	6	61	74	141-143	138-140 [30b]
19	C_6H_5	Me	S	6	7	58	87	199-201	220-222 [30e]
20	C_6H_5	C_6H_5	O	10	4	61	89	204-206	203-204 [30e]
21	4-OMe- C_6H_4	C_6H_5	O	7	5	55	82	222-224	218-220 [30e]

A – Reflux method; B – Microwave assisted method

Table 1.2. Optimization experiments under solution phase conditions.

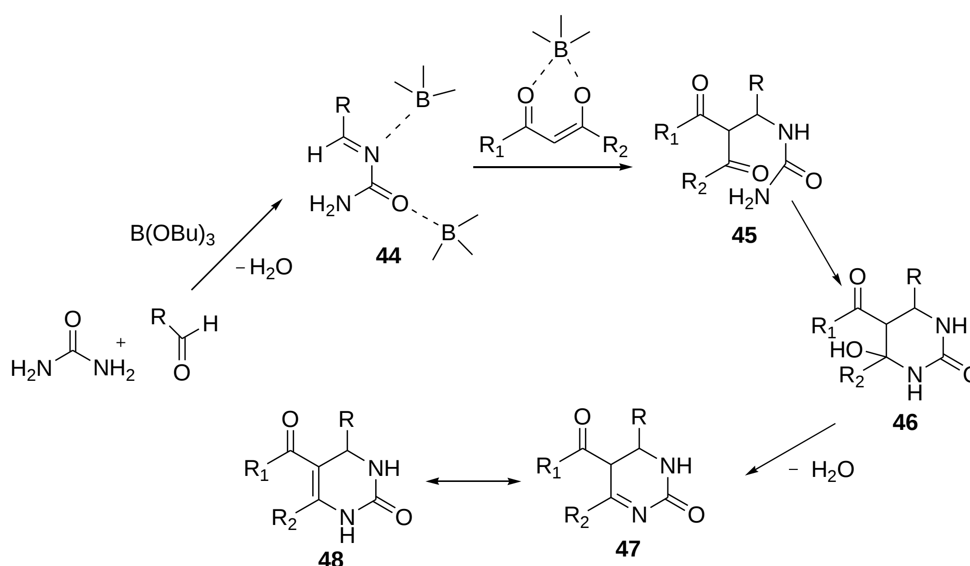
Sl.No	Solvent	Room temperature stirring		Reaction under reflux condition	
		Time (hours)	Yield (%)	A Time (hours)	Yield (%)
1	Methanol	18	38	10	78
2	Ethanol	20	35	12	73
3	Ethyl acetate	18	32	10	68
4	Acetonitrile	24	42	13	65
5	Toluene	26	12	14	52
6	DMF	16	41	9	79
7	DMF + Methanol (1: 1)	15	48	8	82

The purification procedure for the dihydropyrimidinones are simple as the impurities in the reaction product can be easily removed by repeated washing with cold solvents such as alcohols, ethyl acetate, acetonitrile, toluene, chloroform etc. since the dihydropyrimidinones are insoluble in above mentioned cold solvents, at the same time impurities are perfectly soluble. The final purification could be done by crystallization from hot alcohol or alcohol-DMF mixture. Hence it is clear that the protocol using tributylborate reagent, as a promoter for the Biginelli multicomponent reaction is very adaptable and could be used with different substrates. The aromatic aldehydes with both electron withdrawing and electron donating functionalities behave efficiently and even the acid sensitive aldehydes such as furfuraldehyde and thiophene-2-carbaldehyde also form DHPMs with considerable yields.

A plausible mechanism proposed for this tributylborate mediated Biginelli reaction is depicted in scheme 1.13. The water scavenging nature of the reagent may enhance the rate of condensation of aldehydes with the urea to form an acyliminium derivative **44**. Also the boron atom of the reagent co-

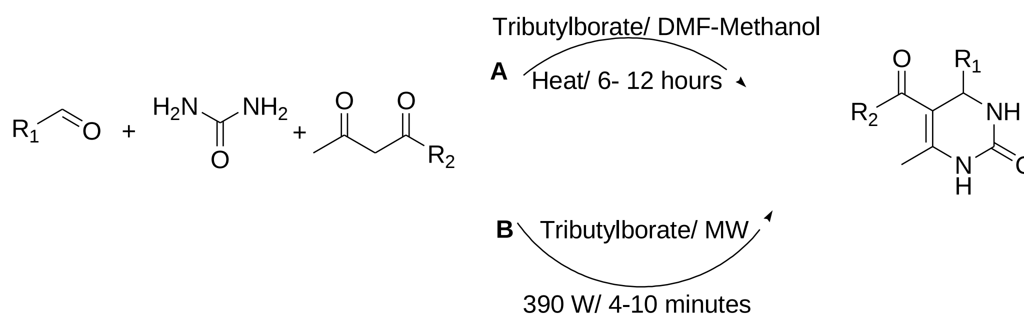
ordinates with the diketone, which will convert it into a nucleophilic keto-enolic structure and thus promote its nucleophilic addition to the acyliminium derivative to form the intermediate **45.** The final condensation-cyclisation of **45** to **47** is also speeded up by tributylborate. The excess reagent, if any, can be easily removed by the aqueous workup.

Scheme 1.13. Feasible Mechanism for Dihydropyrimidinone Formation in Presence of Tributylborate [B(OBu) $_3$].



In the second part of our study, new dihydropyrimidinones were synthesized using tributylborate both under reflux and microwave assisted method. The diketones mainly used are acetoacatanilide, benzoyl acetone and methyl acetoacetate. To the best of my knowledge, acetoacetanilide was rarely used for the DHPMs synthesis. The aldehydes utilized for the library synthesis of pyrimidinones were hydroxyl, chloro, methoxy, nitro and amino derivatives of benzaldehyde. Apart from these, heterocyclic aldehydes such as furfuraldehyde, thiophene-2-carbaldehyde and polynuclear aldehydes like naphthaldehyde have been successfully exploited. The details of the compounds are shown in Table 1.3.

Table 1.3. Synthesis of new dihydropyrimidinones



Entries	Compound	R_1	R_2	X	Time		Yield (%)		Melting point (°C)
					A (hours)	B (min)	A	B	
1	U21	C_6H_5	-NH C_6H_5	O	5	3	75	93	228-229
2	U22	4-OMe- C_6H_4	-NH C_6H_5	O	9	4	70	78	222-224
3	U24	4-OH-3-OMe- C_6H_3	-NH C_6H_5	O	8	5	68	85	221-223
4	U25	2-Cl-C_6H_4	-NH C_6H_5	O	10	8	58	73	168-170
5	U26	Furanyl	-NH C_6H_5	O	6	5	63	79	303-306
6	U27	4-OH- C_6H_4	-NH C_6H_5	O	8	3	65	82	241-243
7	U210	3-NO_2- C_6H_4	-NH C_6H_5	O	11	9	60	75	238-240
8	U211	2-Naphthyl	-NH C_6H_5	O	12	10	69	81	235-238
9	U217	Thiophenyl	-NH C_6H_5	O	7	4	72	85	220-222
10	T21	C_6H_5	-NH C_6H_5	S	6	3	78	90	202-203
11	U44	4-OH-3-OMe- C_6H_3	OCH_3	O	9	4	82	91	208-210
12	U47	4-OH- C_6H_4	OCH_3	O	8	4	73	87	235-238
13	U48	4-NMe_2- C_6H_4	OCH_3	O	6	3	60	73	230-232
14	U411	2-Naphthyl	OCH_3	O	11	7	63	81	260-263
15	U417	Thiophenyl	OCH_3	O	9	5	59	83	228-230
16	U56	Furanyl	C_6H_5	O	5	4	65	71	278-280
17	U57	4-OH- C_6H_4	C_6H_5	O	7	6	70	83	205-207
18	U511	2-Naphthyl	C_6H_5	O	10	9	72	81	206-207
19	U517	Thiophenyl	C_6H_5	O	8	6	54	75	259-261

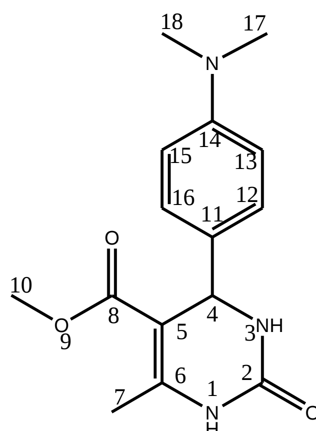
A- Reflux method; B- microwave assisted method

All the newly synthesized compounds have been characterized by IR, NMR and Mass spectrometric techniques. Characterization of one representative compound is discussed below.

1.3.1 Charaterisation

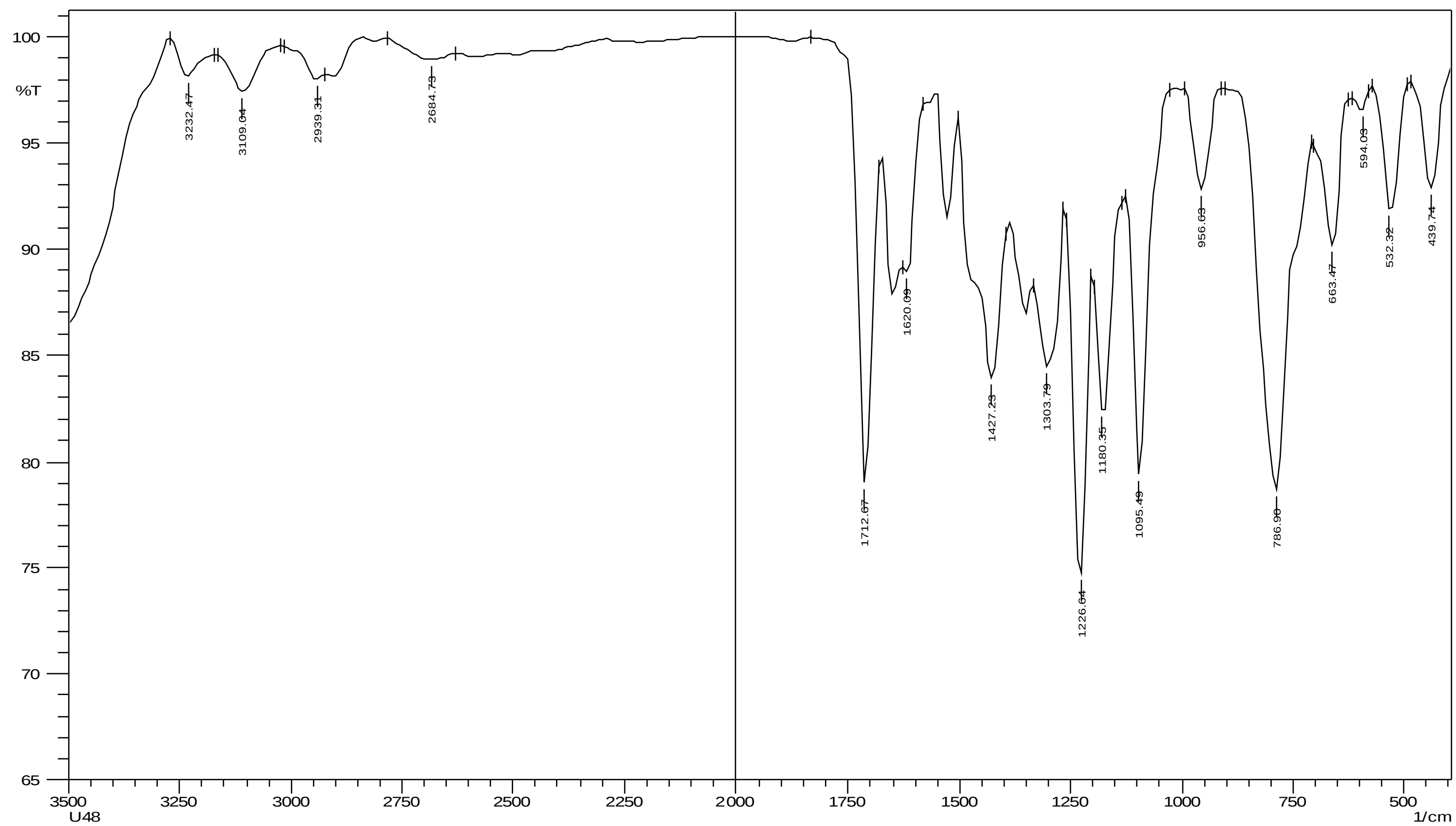
The dihydropyrimidinone synthesized were characterized by the conventional spectroscopic techniques. For the spectroscopic discussion let the compound U48 may be selected as a representative molecule. The tentative structure of the compound is shown in Figure 1.4. For convenience let the molecule may be numbered as shown in Figure 1.4.

Figure 1.4. 5-Methoxy carbonyl-4-(N, N-dimethylamino phenyl)-6-methyl-3, 4-dihydro – 1(H)-pyrimidin-2-one



In the infrared spectrum of the compound U48, the major absorption were seen at 3234.47, 3109.04, 1712.67, 1645, 1620, 1303.79 and 1180 cm^{-1}. The peaks at 3234.47 and 3109.04 cm^{-1} are due to the absorption of the two amide NH groups. The absorption of the ester carbonyl group (C_8) occurs at 1712 cm^{-1} and that of the ring carbonyl group (C_2) at 1645 cm^{-1}. The vibration due to the C=C (between C_5 and C_6), C-N and C-O of esters appears at 1620, 1379 and 1180 cm^{-1} respectively. The IR spectrum of the compound is shown in Figure 1.5.

Figure 1.5. IR spectrum of the compound U48



In the ^{1}H NMR spectrum there are seven distinct proton resonances. The down field resonances at δ 9.08 and δ 7.582 are ascribed to the two NH protons at position 1 and 3 of the pyrimidone ring. The NH proton at position 1 is more deshielded than the NH at position 3, as the former is flanked by an electron withdrawing carbonyl group and an electronegative sp^2 carbon, while the latter is near to a carbonyl group and an sp^3 carbon, which is less electronegative than C_6. The aromatic protons resonate at 7.0 and 6.6 ppm. The doublet at δ 7.0 is attributed to the protons on C_{12} and C_{16} of the aromatic ring. These protons are coupled to the protons at C_{13} and C_{15} with a coupling constant J = 7 Hz. Similarly the proton at C_{13} and C_{15} appears as a doublet at 6.6 ppm with a coupling constant J = 7.2 Hz. The similar J values for these protons confirm the mutual ^{3}J coupling with a coupling constant ~ 7 Hz. The proton on the tertiary carbon C_4 resonates at δ 5.068. In the present compound under consideration the peak of the proton on C_4 appears as a singlet. But in the spectra of most of the other dihydropyrimidinones (Appendix I) it appears as a doublet with a coupling constant J = 2-3 Hz. This splitting is due to the amide NH near to C_4. A singlet peak at 3.54 ppm is attributed to proton on C_{10} ($-OCH_3$). Similarly a singlet peak at δ2.864 is ascribed to the resonance of protons of the methyl groups at C_{17} and C_{18}. The methyl protons of C_7 resonate at 2.251 ppm. The proton NMR spectra of the compound U48 is shown in Figure 1.6.

Figure 1.6. a. H NMR spectrum of the compound U48

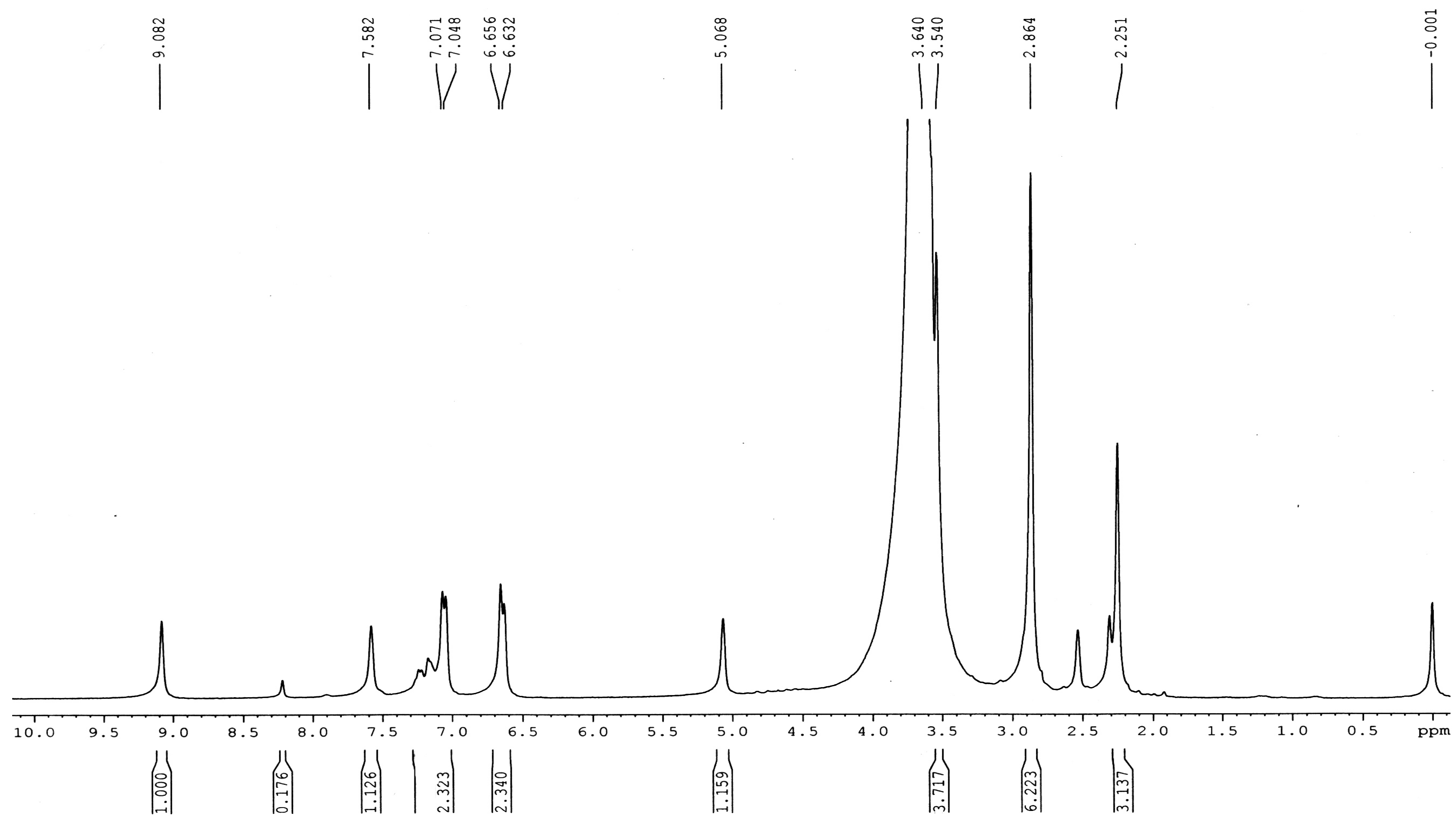
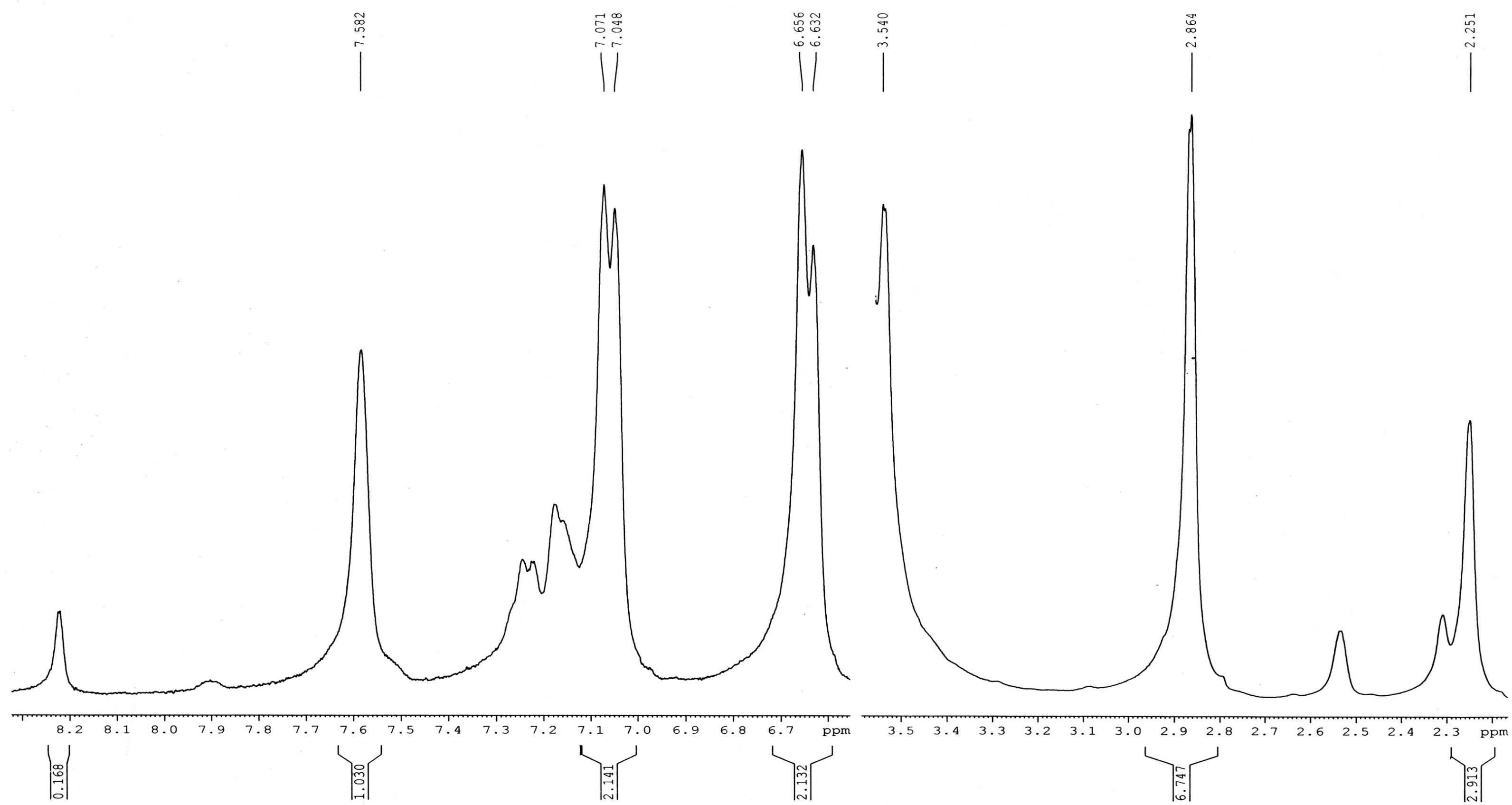


Figure 1.6.b. H NMR spectrum (Expanded) of the compound U48



The ^1H-^1H COSY (Homonulear Correlation Spectroscopy) spectrum of the compound U48 is shown in Figure 1.7. From the figure, it is clear that the proton correlation is between H_{12} and H_{13} or H_{15} and H_{16}, in which H_{12} & H_{16} and H_{13} & H_{15} are equivalent protons. Another correlation is observed between H_3 and H_4.

Figure 1.7.a. H-H COSY spectrum of the compound U48

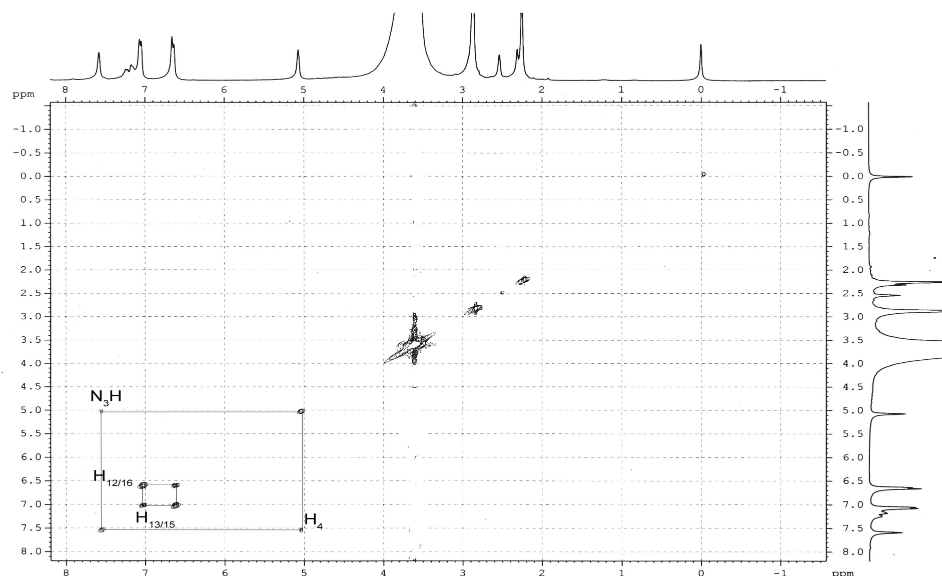
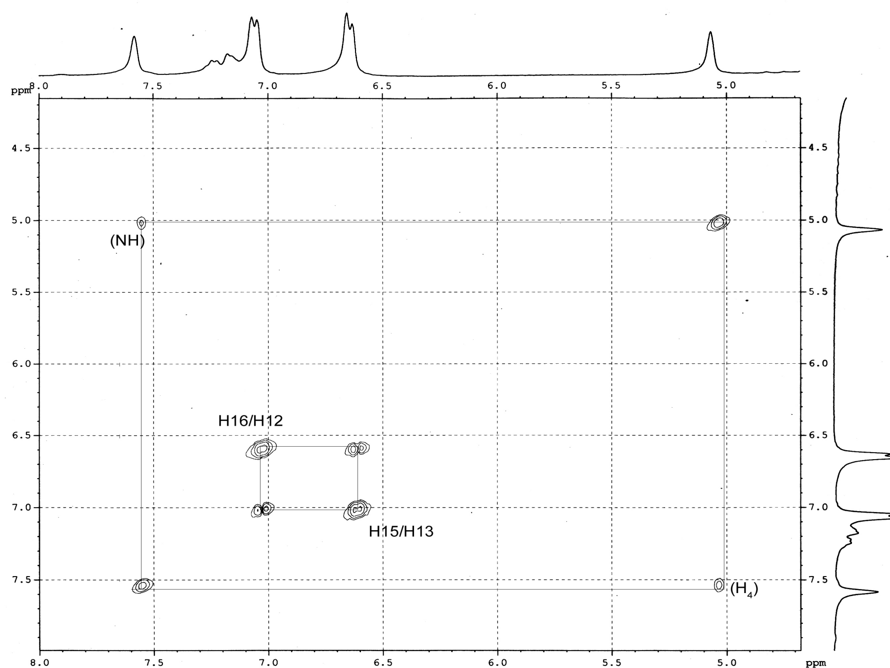
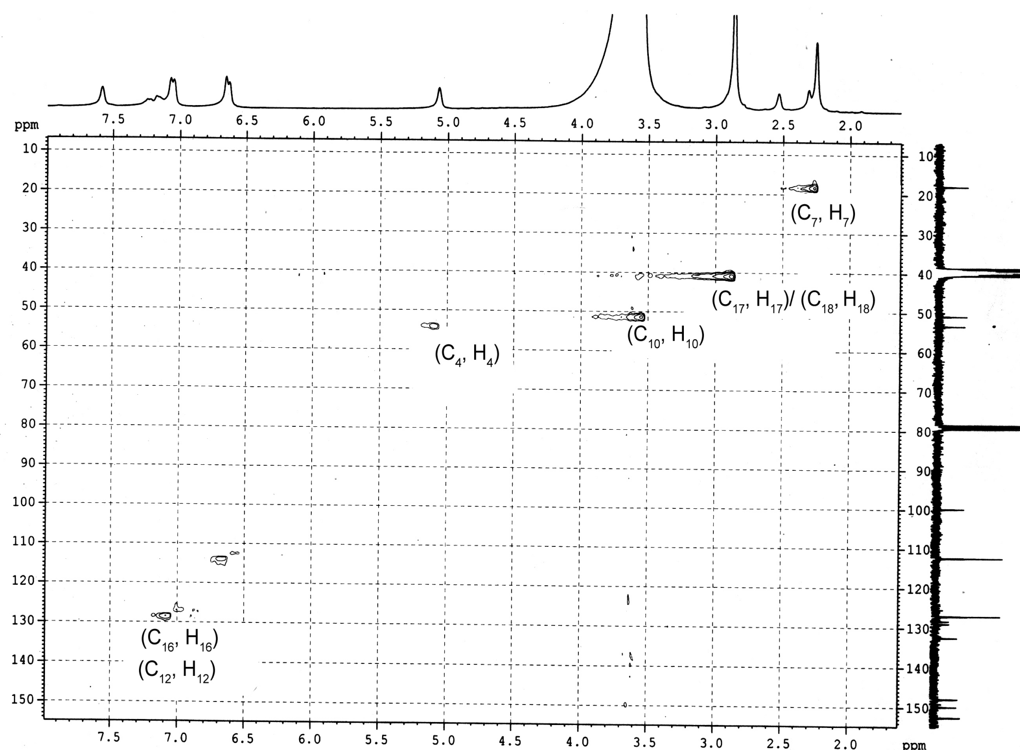


Figure 1.7.b. H-H COSY spectrum (expanded) of the compound U48



The ^{13}C-^{1}H COSY spectrum (Heteronuclear Multiple Quantum Coherence, HMQC) shows the direct correlation of different protons with carbons to which they are attached. The correlation obtained from the C-H COSY spectra are (C_{12}, H_{12}) / (C_{16}, H_{16}), (C_4, H_4), (C_{10}, H_{10}), (C_{18}, H_{18})/(C_{17}, H_{17}) and (C_7, H_7). The C-H COSY spectrum is shown in Figure 1.8.

Figure 1.8. C-H COSY spectra of the compound U48

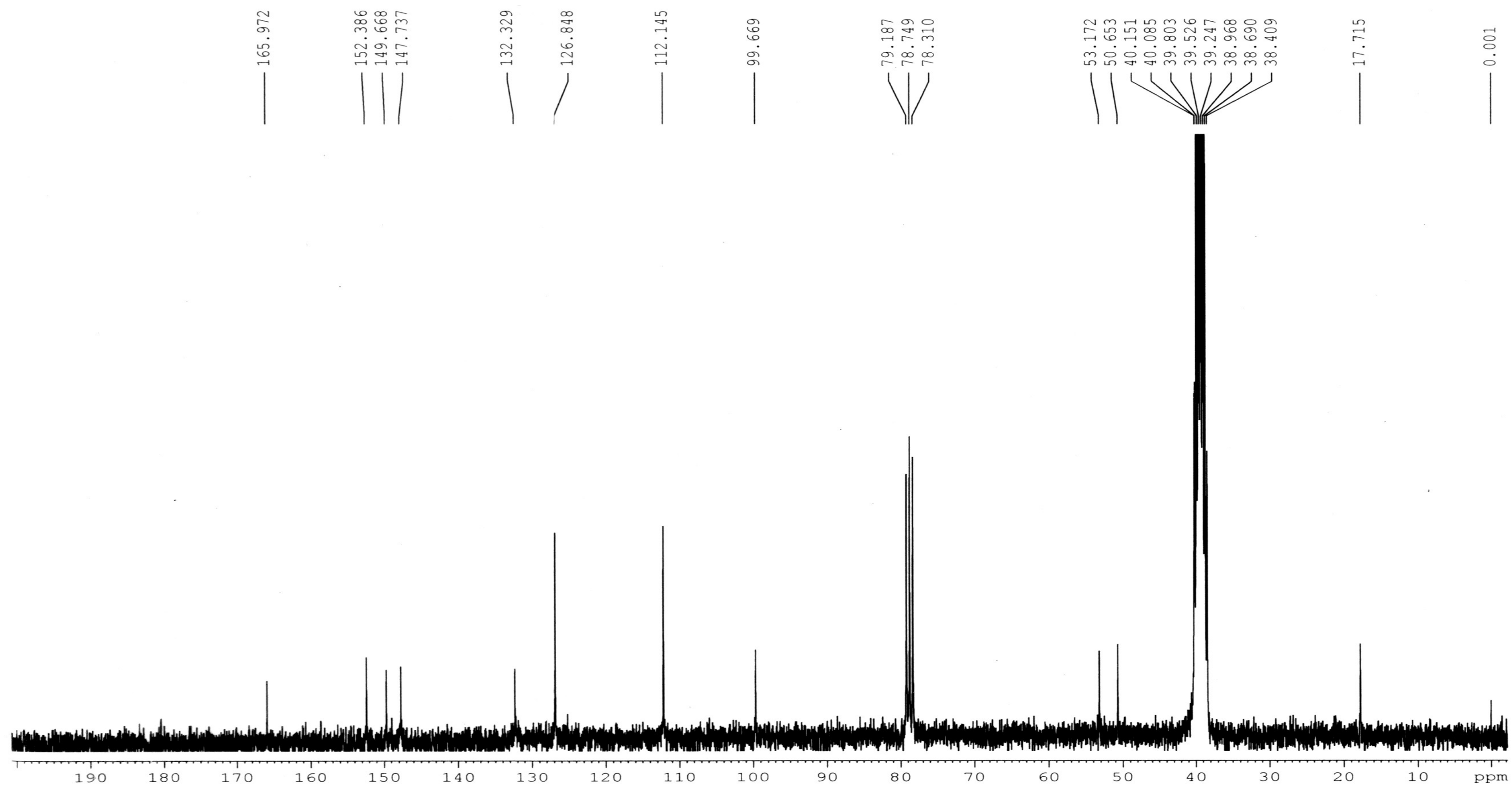


The ^{13}C NMR spectrum is shown in Figure 1.9.From the ^{13}C NMR and C-H COSY spectra the chemical shift values are ascribed to the carbon as shown in the Table 1.4.

Table 1.4

Carbons	C_7	C_{17} & C_{18}	C_4	C_{10}	C_5	C_{13} & C_{15}	C_{12} & C_{16}	C_{11}	C_2	C_6	C_8	C_{14}
Chemical shift (δ ppm)	17.715	40.145	53.172	50.653	99.669	112.145	126.848	132.329	147.737	149.668	165.972	152.386

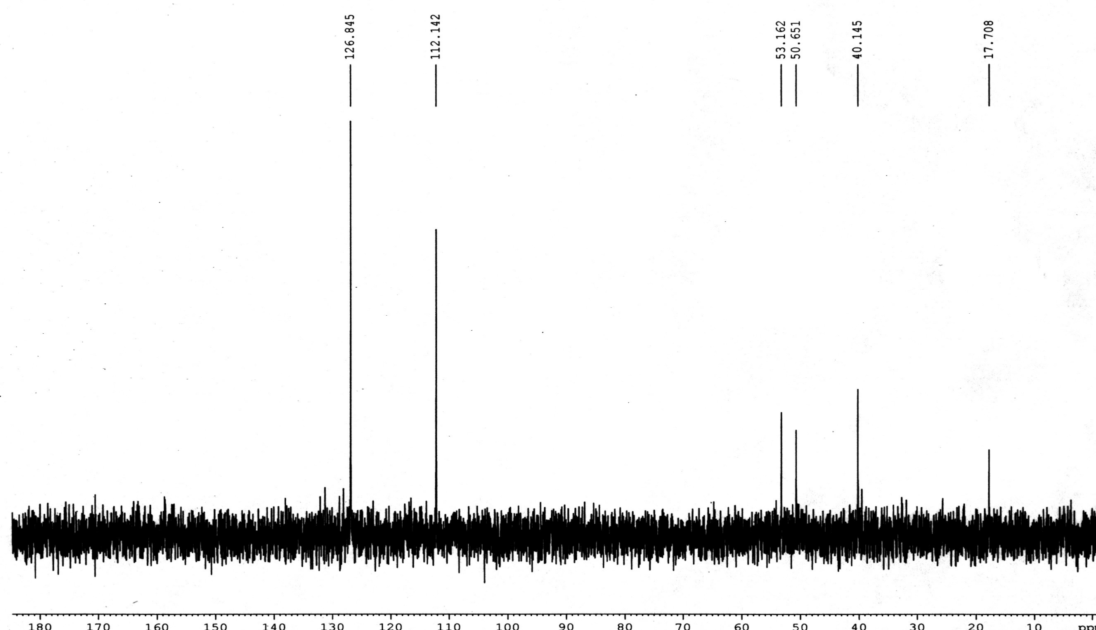
Figure 1.9. ^{13}C NMR spectrum of compound U48



The resonance of the N-methyl carbon is not seen in Figure 1.9, as it is merged with the solvent (DMSO) peak. But it is clearly visible in the DEPT spectrum (Figure 1.10).

The DEPT 135 spectrum of the compound is shown in Figure 1.10. The upward peaks at 17.708 ppm, 40.145 ppm, 50.651 ppm and 53.162 ppm represent the methyl carbons of C_7, C_{17} & C_{18}, C_4 and C_{10} respectively. The aromatic CH group at (C_{12} & C_{16}) and (C_{13} & C_{15}) are represented by the upward peaks at δ112.142 and δ 126.845 ppm.

Figure 1.10. The DEPT 135 spectrum of the compound U48



The Heteronuclear Multiple Bond Correlation (HMBC) spectrum of the compound shows the correlation of the protons with carbon separated by more than one bond. The different multiple bond correlations are (H_{16}, C_{15}), (H_{16}, C_{12}), (H_{16}, C_{14}), (H_{15}, C_{11}), (H_{15}, C_{13}), (H_{17}/ H_{18}, C_{14}), (H_7, C_6), and (H_7, C_5). The HMBC spectrum is shown in Figure 1.11.

Figure 1.11.a. HMBC spectrum of the compound U48

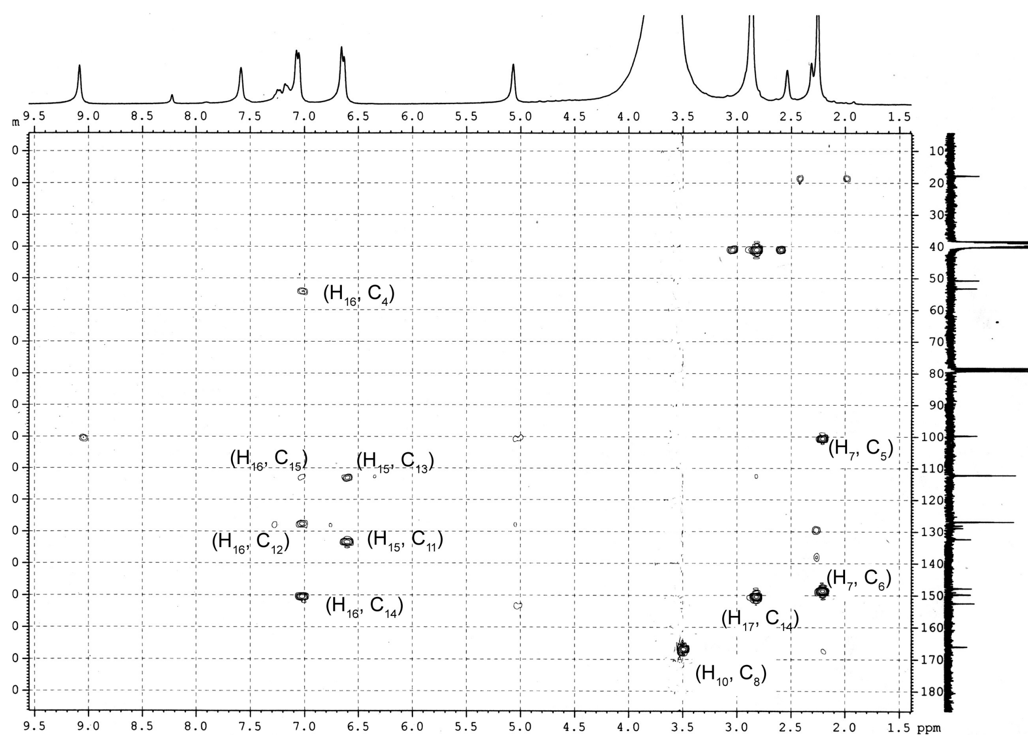
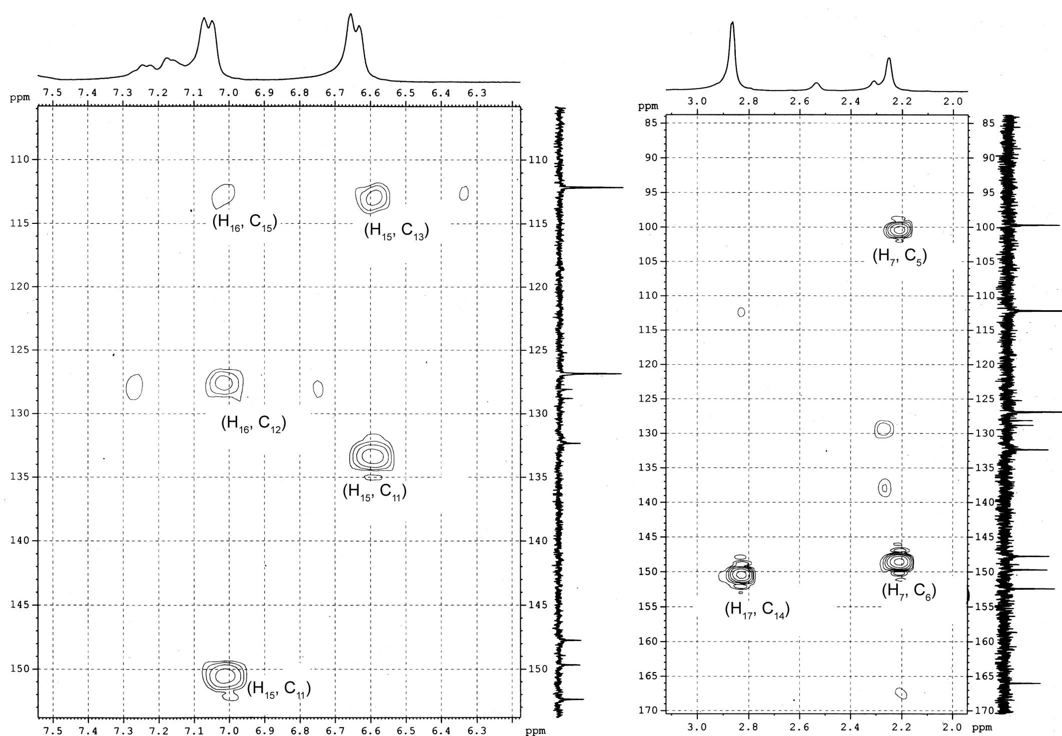
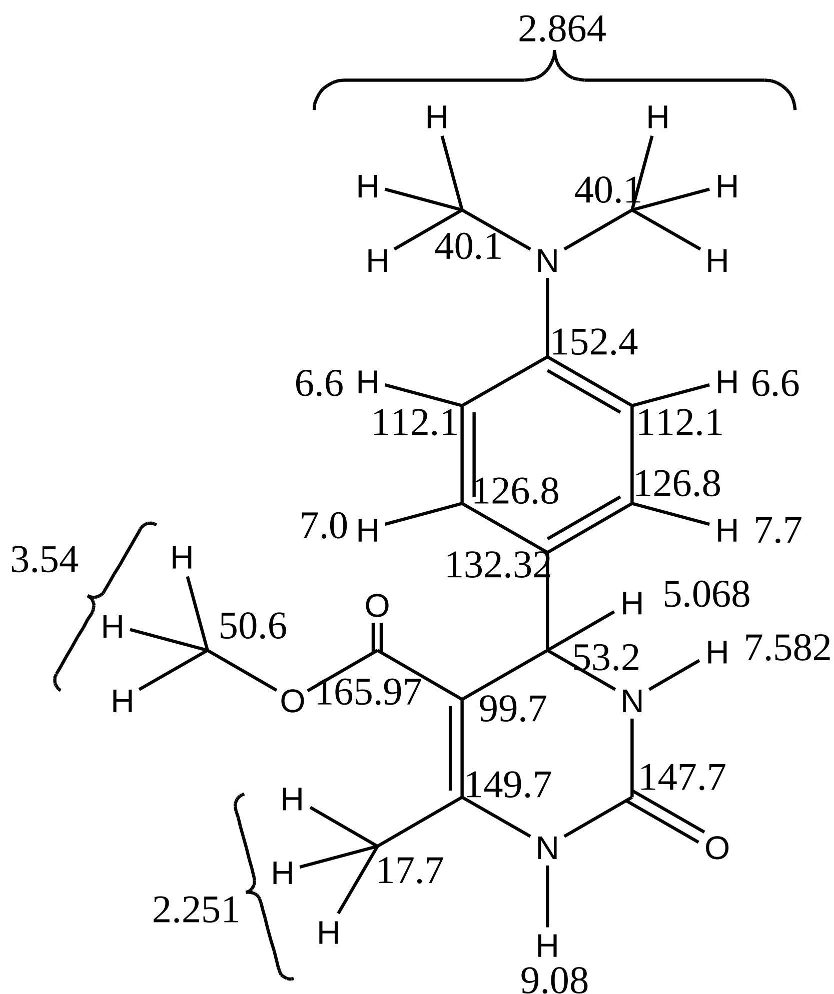


Figure 1.11.b. HMBC (expanded) spectrum of the compound U48



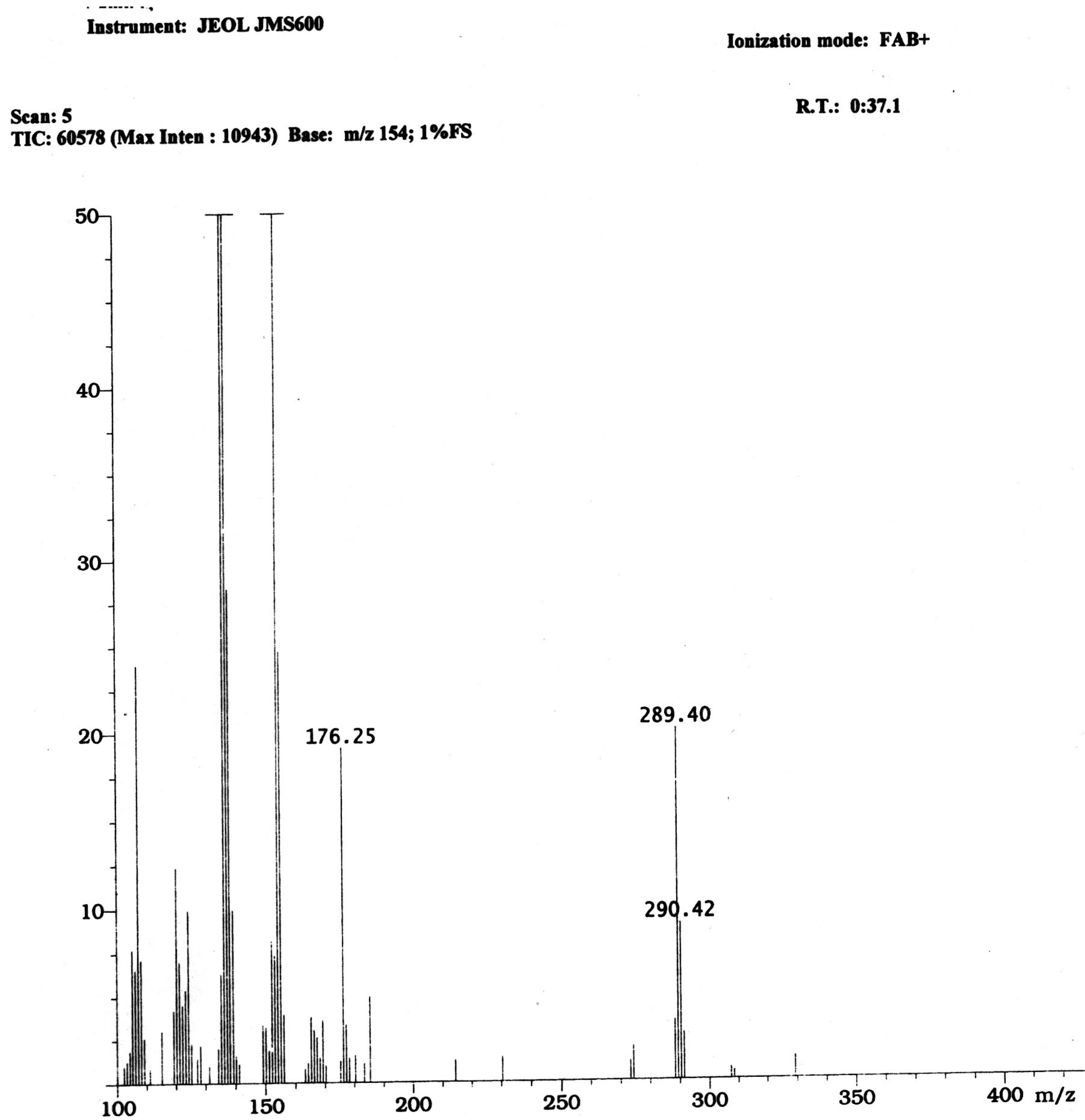
On the basis of the one and two-dimensional NMR experiments the chemical shift values ascribed to the protons are shown in Figure 1.12.

Figure 1.12. The NMR chemical shift values of the compound U48.

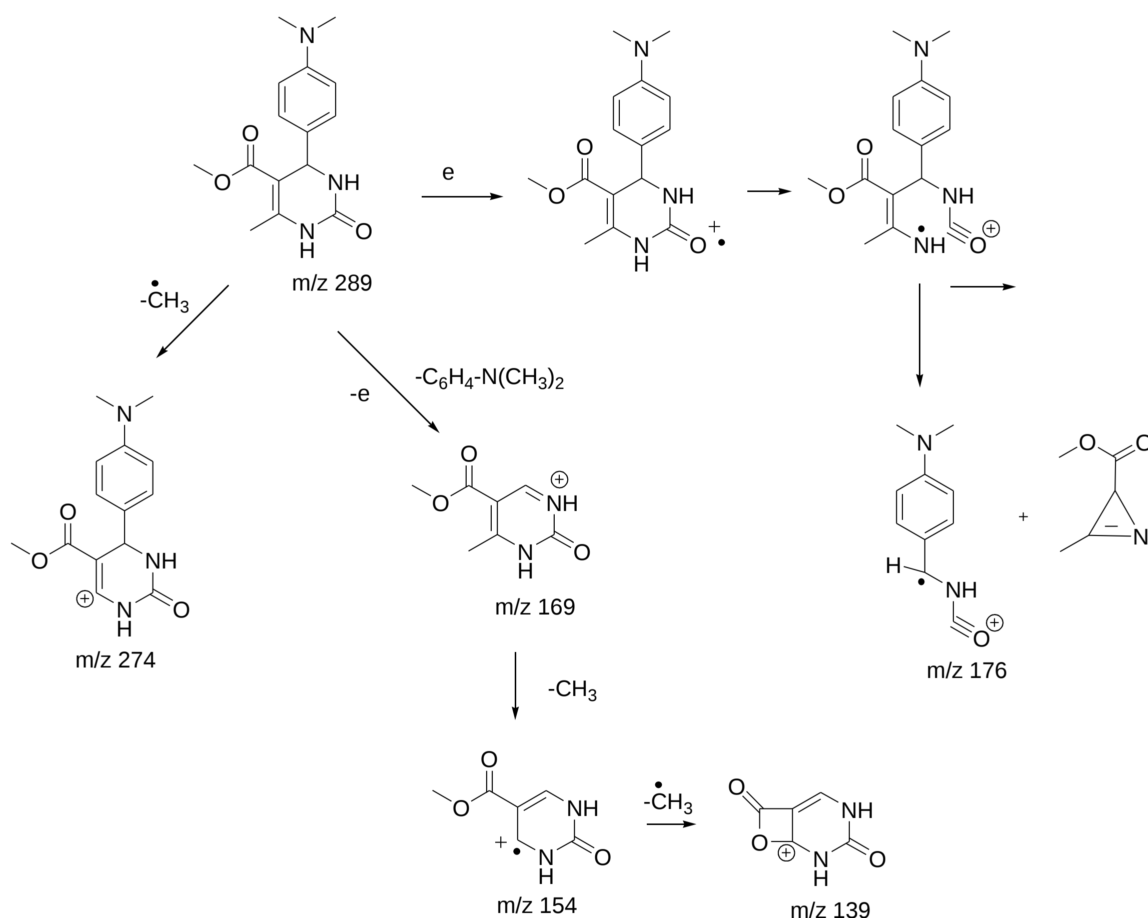


The structure is further confirmed by the mass spectrum. The molecular ion peak is obtained at m/z 289.40 and the base peak at m/z 154. The possible fragmentation pattern for the molecule is shown in scheme 1.14. The FAB mass spectrum of the compound U48 is shown in Figure 1.13.

Figure 1.13. FAB mass spectrum of the compound U48



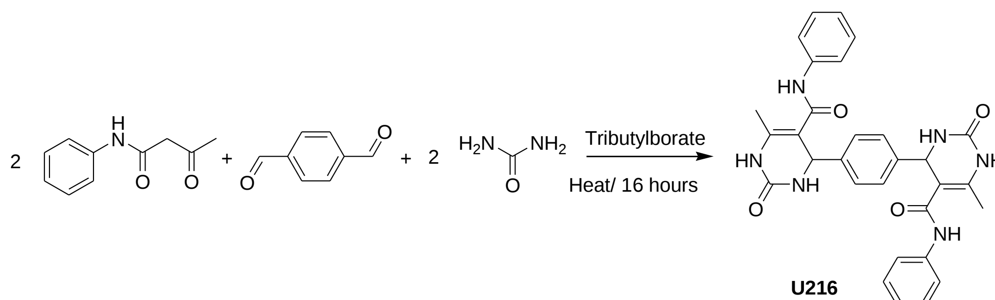
Scheme 1.14. Possible Mass Spectral Fragmentation for the Compound U48.



The spectroscopic details of all other compounds are given in experimental section 1.4 and the spectra are shown in the Appendix I.

When terephthalaldehyde was made to react with acetoacetanilide and urea (1:2:2) in refluxing DMF-methanol mixture a double Biginelli condensation occurred to form a symmetric bi-dihydropyrimidinone as shown in Scheme 1.15. NMR and mass spectra confirmed the structure of the compound.

Scheme 1.15



The spectra of this compound are shown in the Appendix I and the spectral data are given below.

Melting point - 327-329°C; IR (ν_{max}, KBr, cm^{-1}) 3394 (NH), 3271.05 (NH) 3116.75 (NH), 1666 (C=O), 1630 (ring C=O), 1527 (N-H bend), 1328 (C-N), ^{1}HNMR (δppm, 300 MHz, DMSO-d_6) 9.329 (s, 2H, two NH), 8.420 (s, 2H, Ar), 7.2-7.1 (m, 4H, Ar), 7.0 (m, 4H, Ar) 5.50 (S, 2H, 3°C-H), 2.121 (s, 6H, CH_3). ^{13}CNMR (δppm, 75 MHz, DMSO-d_6), 164.643, 152.327, 137.826, 137.452, 127.451, 125.765, 122.346, 118.906, 104.798, 55.256, 17.313; MS (FAB) m/z - 537.40 (M+1, 10), 444.4 (100), 429 (55) 323.25 (35), 217.16 (40).

1.4. Experimental

1.4.1. Materials and Methods

All the known compounds reported herein are characterized by comparing their melting points, NMR and mass spectroscopic data with those reported in the literature. The new compounds synthesized are characterized by IR, NMR (one dimensional and two dimensional) and FAB mass spectra. All the chemicals used are of synthetic grade and are purified before use. The melting points are determined using a GUNF melting point apparatus and are uncorrected. The IR spectra are recorded on a SHIMADZU FTIR 8400S and

JASCO FT/IR-4100 Spectrophotometer in KBr medium, NMR spectra are recorded on a BRUKER AVANCE DPX 300 MHz Spectrometer in DMSO-d_6 using TMS as the internal standard and high-resolution mass spectra recorded on a JEOL JMS600 instrument.

1.4.2. General Synthetic Procedure

1.4.2.1. *Synthesis of 5- methoxycarbonyl–6– methyl–4–phenyl-3, 4-dihydropyrimidin-2 (1H)-one (43) under solvent free microwave assisted condition.*

Methyl acetoacetate (0.69 g, 6 mmol), urea (0.48 g, 8 mmol), benzaldehyde (0.53 g, 5 mmol) and tributylborate (0.48 g, 2 mmol) are taken in a loosely stoppered borosil vessel and irradiated with microwave at a power level 390 W for 3 minutes, intermittently (6 x 30 seconds). The reaction mixture is transferred into crushed ice and stirred vigorously for 30 minutes. The crude solid separated is vacuum filtered, washed repeatedly with ice-cold ethyl acetate-petroleum ether mixture (1:1) and finally with distilled water. The dried product is further purified by crystallization from hot ethanol to obtain the pure product. The yield is 95%. Melting point- 206 -207°C.

For the synthesis of all other compounds the above procedure was followed with respective substrates, reagents and time as shown in Table 1.1 and 1.3.

1.4.2.2. *Synthesis of 5- methoxycarbonyl–6– methyl–4–phenyl-3, 4-dihydropyrimidin-2(1H)-one(43) using tributyl borate as catalyst under the reflux method.*

Methyl acetoacetate (0.69 g, 6 mmol), urea (0.48 g, 8 mmol), benzaldehyde (0.53 g, 5 mmol) and tributylborate (0.48 g, 2 mmol) are mixed in 5 ml dry solvent and stirred at room temperature/ refluxed for specified time as is indicated in Table 1.1. The reaction mixture is then poured into crushed ice and stirred vigorously for 1 hour keeping the temperature below

5°C. The solid separated out is vacuum filtered, washed repeatedly with ice-cold ethyl acetate and finally with distilled water. The crude product obtained is dried under vacuum and re-crystallised from ethanol for pure crystals. The yield is 85%.

In order to synthesis other compounds given Table 1.1 and 1.3 this procedure was adopted with appropriate substrates and reagents mentioned therein.

1.4.3. Spectroscopic Details

The spectral details of newly synthesized compounds are shown below

Compound	**Spectroscopic Data of the New Dihydropyrimidinones**
T21	IR (ν_{max}, KBr, cm^{-1}) 3278.76 (NH), 3186.18 (NH), 1674 (Carbonyl group) 1627 (C=S), 1334.65 (C-N), 1203 (C-O) HNMR (δ ppm, 300 MHz, DMSO-D_6) 9.415 (s, 1H, NH), 9.014 (s, 1H, NH), 8.8 (s, 1H, NH), 7.5 (m, 2H, Ar) 7.3 - 7.2 (m, 8H, Ar), 7.034 (m, 1H, Ar), 5.5 (d, J=2.4 Hz, 1H, 3°C-H), 2.186 (s, 3H, CH_3). ^{13}CNMR (δ ppm, 75 MHz, DMSO-D_6) - 173.915, 164.6, 141.9, 137.8, 135.2; 128.24, 128.03, 127.474, 126.10, 123.22, 119.539, 106.991, 55.517, 16.420, MS (FAB) m/z - 324.24 (M+1, 100), 217.16 (58), 172.25 (25), 149.16 (58).
U21	IR (ν_{max}, KBr, cm^{-1}) 3278 (NH), 3109 (NH), 1681(C=O), 1635(C-N) ^{1}HNMR (δ ppm, 300 MHz, DMSo-d_6) 8.584 (s, 1H, NH) 7.2 (d, J=8.4 Hz, 2H, Ar), 7.1 (d, J=7 Hz, 2H, Ar) 7.09-6.9 (m, 5H, Ar), 6.7 (s, 1H, Ar) 5.27 (s, 1H, 3°C-H) 1.925 (s, 3H, CH_3); ^{13}CNMR (δ ppm, 75 MH_2, DMSO-d_6). 165.0, 153.09, 142.8, 137.9, 128.1, 127.9, 127.2, 125.91, 123.030, 119.462, 105.280, 55.406, 16.822; MS (FAB) m/z-308.41 (M+1; 100), 265.35 (10), 215.24 (40), 172.28 (25).
U22	IR (ν_{max}, KBr, cm^{-1}) 3278.76 (NH), 3116.75(NH), 1700 and

1674 (C=O), 1630 (C=C), 1512 (NH-bend), 1326 (C-N) 1242.07 (C-O); HNMR (δppm, 300 MHz, DMSO-D_6), 8.594 (s, 1H, NH), 8.438 (s, 1H, NH), 7.4 (d, J=7.8 Hz, 2H, Ar), 7.3 (d, J=8.7 Hz, 2H, Ar), 7.2 (t, J= 8 Hz, 2H, Ar), 7.0 (t, J= 7Hz, 1H, Ar), 6.8 (d, J= 8.7 Hz, 2H, Ar), 3.7 (s, 3H, OCH_3), 2.181 (s, 3H, CH_3); ^{13}CNMR (δppm, 75 MHz, DMSO-d_6) 165.014, 158.616, 152.926, 138.311, 137.948, 135.022, 128.05, 127, 3; 123.1, 119.4, 113.5, 105.2, 55.03, 54.656, 16.924; MS (FAB) m/z - 338.30 (M+1, 100), 245.25 (43), 230.24 (85) 217.20 (45).

U24 IR ($\nu_{max,}$ KBr, cm^{-1}) 3286.48 (OH) 3209.33 (NH), 3093.61 (NH) 1681 (C=O), 1645 (C=O), 1272 (C-N), 1126 (C-O), HNMR (δppm, 300 MHz, DMSO-d_6) 9.312 (s, 1H, NH), 8.890 (s, 1H, OH), 8.556 (s, 1H, NH), 7.866 (s, 1H, NH) 7.5 (d, J=7.8 Hz, 2H, Ar), 7.2 (s, 2H, Ar), 7.0 (m, 1H, Ar) 6.9 (s, 1H, Ar), 6.7 (m, 2H, Ar); 5.4 (s, 1H, CH), 2.1 (s, 3H, CH_3); ^{13}CNMR (δppm, 75 MHz, DMSO-d_6) 164.57, 152.04, 146.3, 144.6, 137.54, 136.63, 133.67, 127.208, 122.136, 118660, 117.568, 114.054, 109.14, 104.718, 54.396, 54.173, 15.877; MS (FAB) m/z - 354.28 (M+1, 45), 230.21 (24), 217.20 (100).

U25 IR (ν_{max}, KBr, cm^{-1}) 3247.9, (NH), 1681 (C=O), 1527 (N-H bend) 1326 (C-N), 1249 (C-O), 756 (C-Cl), HNMR (δppm, 300 MHz, DMSO-D_6) 8.9 (s, 1H, NH) 8.6 (s, 1H, NH), 6.5 (s, 1H, NH), 7.5 (m, 1H, Ar) 7.49 (d, J=7.5 Hz, 2H, Ar), 7.3 (d, J=7.5 Hz, 1H, Ar), 7.3-7.2 (m, 4H, Ar), 7.014 (m, 1H, Ar) ^{13}C NMR (δ ppm, 75 MHz, DMSO-d_6) 164.51, 152.51, 139.84, 138.83, 137.91, 131.62, 129.08, 128.74, 128.47, 127.92, 127.0, 123.0, 119.4, 103.3, 52.337, 16.822; MS (FAB) m/z 342.44 (m+1, 100), 299.35 (15), 249.32 (35), 206.26 (25).

U 26 IR (ν_{max}, KBr, cm^{-1}) 3286.48 (NH), 3116 (NH) 1700 (C=O), 1674 (C=O), 1627 (C=C), 1326 (C-N) 1242 (C-O); ^{1}HNMR (δ ppm, 300 MHz, DMSO-d_6) 9.111 (s, 1H, NH) 8.536 (s, 1H, NH), 7.5 (d, J=7.8 Hz, 2H, Ar) 1.098 (S, 1H, NH), 7.2 (m, 2H, Ar), 7.09-7.0 (m, 2H, Ar), 6.3 (d, J=1.5 Hz, 2H, Ar), 5.5 (d,

J=2.7 Hz, 1H, 3ºCH), 2.191 (s, 3H, CH_3); ^{13}CNMR (δppm, 75 MHz, DMSO-d_6) 164.553, 154.345, 152.896, 141.419, 140.628, 138.014, 127.778, 122.762, 119.347, 109.618, 105.418, 102.163, 48.607, 16.738; MS (FAB) m/z - 298.27 (M+1, 100), 230.24 (75), 205.25 (30).

U27 IR (ν_{max}, cm^{-1}, KBr) 3425.34, (NH), 3247 (NH), 3116.75 (NH), 1700 (C=O), 1674 (C=O), 1519 (N-H bend), 1326 (C-N) 1249 (C-O); HNMR (δ ppm, 300 MHz, DMSO-d_6) 9.241 (s, 1H, NH), 8.827 (s, 1H, NH), 8.462 (s, 1H, NH), 7.467 (d, J=7.8 Hz, 2H, Ar), 7.2-7.1 (m, 4H, Ar) 7.0 - 6.9 (m, 2H, Ar), 6.7 (d, 1H, Ar); ^{13}CNMR (δ ppm, 75 MHz, DMSO-d_6) 164.936, 156.227, 152.677, 137.853, 137.707, 133.463, 127.787, 127.109, 122.784, 119.238, 114.956, 105.202, 54.785, 16.582; MS (FAB) m/z- 324.33 (M+1), 217.20 (100), 197.18 (20), 149.19 (15).

U210 IR (ν_{max}, KBr, cm^{-1}) 3278.76, 3070 (NH), 1650 (C=O) 1596 (C=C), 1535.23 (N=O), 1342 (C-N), HNMR (δppm, 300 MHz, DMSO-d_6), 10.142 (s; 1H, NH) 8.5 (S, 1H, NH), 8.05 (d, J=8.4 Hz, 1H, Ar), 7.9 (d, J=7.8 Hz, 1H, Ar) 7.5 (m, 2H, Ar), 7.3 (d, J=8.1 Hz, 2H, Ar), 7.2 (m, 2H, Ar) 7.0 (m, 1H, Ar), 4.83 (s, 1H, 3ºC-H), 2.6 (s, 3H, CH_3) ^{13}C NMR (δ ppm, 75 MHz, DMSO-d_6) 165.137, 153.932, 146.69, 137.29, 134.54, 129.83, 128.12, 123.85, 123.47, 123.47, 122.66, 119.63, 21.948; MS (FAB) m/z- 354.19 (M+1, 25) 276.21 (100), 217 (45).

U211 IR ($\nu_{max,}$ KBr, cm^{-1}) - 3402.2 (NH), 3294 (NH), 3232.47 (NH), 1700 (C=O), 1596 (C=C), 1311 (C-N), ^{1}HNMR (δppm, 300 MHz, DMSO-d_6) 7.8-7.7 (m, 2H, Ar), 7.5-7.4 (m, 7H, Ar), 7.2 (m, 2H, Ar) 7.0 (t, J=7.2 Hz, 1H, Ar), 5.6 (d, J= 2.1Hz, 1H, 3ºC -H) 2.1 (s, 3H, CH_3); ^{13}C NMR (δppm, 75MHz, DMSO-d_6) 164.563, 152.261, 139.942. 137.553, 137.025, 131.767, 131.411, 127.253, 126.691, 126.230, 124.960, 124.709, 123.783, 123.604, 122.233, 118.821, 104.632, 54.676, 16.045; MS (FAB) m/z- 358.22 (M+1, 100),

265.22 (35), 222.19 (30)

U217 IR (ν_{max}, cm^{-1}, KBr). 3471.63 (NH), 3394.48 (NH), 3263.33 (NH), 1666 (C=O), 1527 (N-H bend) 1319.22 (C-N); ^{1}HNMR (δ ppm, 300 MHZ, DMSO-d_6). 8.62 (s, 1H, NH), 8.414 (s, 1H, NH), 7.5, 7.4 (m, 2H, Ar) 7.2 (m, 3H, Ar), 7.0 (m, 1H, Ar), 7.016 (s, 1H, NH), 6.9 (m, 2H, Ar), 5.7 (d, J. 2.7 Hz, 1H, 3°C-H), 2.212 (s, 3H, CH_3) ^{13}CNMR (δ ppm, 75 MHz, DMSO-d_6) 164.727, 152.778, 147.293, 139.501, 138.059, 128.211, 126.487, 124.816, 123.972, 123.302, 119.700, 105.19, 5.98, 17.189; MS (FAB) m/z- 314 (M+1), 230.24, 217.2, 181.17.

U44 IR (ν_{max}, KBR, cm^{-1}) 3386.7 (OH), 3255.6 (NH), 3132.18 (NH), 1740 (C=O), 1674.10 (C=O), 1350 (C-N), 1234.36 (C-O); ^{1}HNMR (δ ppm, 300 MHz, DMSO-d_6) 9.111 (s, 1H, NH), 9.019 (s, 1H, NH), 7.636 (S, 1H, OH), 6.813 (S, 1H, Ar), 6.689 (d, J=8.1 Hz, 1H, Ar) 6.6 (d, J=8.7 Hz, 1H, Ar); ^{13}CNHMR (δppm, 75 MHz, DMSO-d_6) 167.597, 165.942, 152.318, 148.057, 147.234, 145.669, 135.595, 118.098, 115.109, 110.630, 99.328, 55.327, 53.390, 17.712.

U47 IR (ν_{max}, KBr, cm^{-1}) 3579.64 (OH), 3240 (NH), 3116 (NH) 1681 (C=O), 1320 (C-N), 1234 (C-O); ^{1}HNMR (δppm, 300 MHz, DMSO-d_6) 9.164 (s, 1H, NH), 8.873 (s, 1H, NH), 7.190 (s, 1H, OH), 7.1 (d, J=8.4 Hz, 2H, Ar), 6.7 (d, J = 8.4 Hz, 2H, Ar), 5.2 (d, J=3 Hz, 1H, 3°C-H) 3.6 (s, 3H, OCH_3 - it is found merged with the solvent peak), 2.3 (s, 3H, CH_3); ^{13}CNMR (δppm, 75 MHz, DMSO-d_6) 167.208, 165.355, 155.59, 152.04, 146.553, 134.357, 126.588, 114.219, 99.350, 53.043, 17.567; MS (FAB) m/z- 263.40 (M+1), 154.86 (100).

U411 IR (ν_{max}, KBr, cm^{-1}) 3317.34 (NH), 3201.6 (NH), 1670 (C=O) 1342.36 (C-N), 1249.79 (C-O); ^{1}HNMR (δppm, 300 MHz, DMSO-d_6) 9.262 (s, 1H, NH), 7.683 (s, 1H, NH), 7.9-7.8 (m, 5H, Ar) 7.5-7.4 (m, 7H, Ar), 5.364 (s, 1H, 3°C-H) 3.5 (s, 3H, OCH_3), 2.315 (s,

3H, CH_3); ^{13}CNMR (δppm, 75 MHz, DMSO-d_6) 165.882, 152.167, 148.737, 141.780, 132.646, 128.299, 127.779, 126.216, 125.874, 124.356, 98.884, 50.777, 17.831; MS (FAB) m/z - 297.37 (M+1), 217.24 (100), 181.24, 149.16.

U417 IR (ν_{max}, KBr, cm^{-1}) 3332.76 (NH), 3247.9 (NH), 1689.53 (C=O), 1643 (C=C), 1311.5 (C-N), 1226.6 (C-O); ^{1}HNMR (δppm, 300 MHz, DMSO-d_6) 9.167 (s, 1H, NH), 1.016 (s, 1H, NH), 7.1 (d, J= 4.5 Hz 1H, Ar), 7.0 (d, J= 4.8 Hz, 1H, Ar) 6.8 (t, J= 4.2 Hz, 1H, Ar), 5.5 (d, J=3.3 Hz, 1H, 3ºC-H), 3.6 (s, 3H, OCH_3), 2.3 (s, 3H, CH_3) ^{13}CNMR (δppm, 75 MHz, DMSO-d_6) 166.229, 164.523, 151.583, 147.370, 145.081, 125.219, 122.855, 122.238, 121.088, 100.222, 49.412, 17.078; MS (FAB) m/z - 253.20 (M+1, 100), 224.18, 217.16.

U56 IR (ν_{max}, KBr, cm^{-1}) 3325.05 (NH), 3271.05 (NH) 1689.5 (C=O), 1612 (C=C), 1334 (C-N), 1242 (C-O), ^{1}HNMR (δppm, 300 MHZ, DMSO-d_6) 8.876 (s, 1H, NH), 7.5-7.3 (m, 6H, Ar) 7.040 (s, 1H, NH) 6.2 (d, J=1.5 Hz, 1H, -H), 6.1 (d, J=2.7, 1H, furfuryl-H), 5.543 (s, 1H, 3ºC-H), 1.787 (S, 3H, CH_3) ^{13}CNMR (δppm, 75 MHZ, DMSO-d_6) 194.043, 154.635, 152.659, 145.458, 141.2, 140.2, 130.8, 127.7, 127.3, 109.6, 106.9, 105.17, 49.18, 18.19; MS (FAB) m/z 283.15 (M+1), 217.13 (100), 181.14, 149.1.

U57 IR (ν_{max}, cm^{-1}, KBr), 3350 (OH) 3224.76 (NH), 3109 (NH), 1681 (C=O), 1612 (C=C) 1334 (C-N); ^{1}HNMR (δppm, 300 MHz, DMSO-d_6) 8.957 (s, 1H, NH), 8.831 (s, 1H, NH), 7.4 (d, J= 6.6 Hz, 2H, Ar), 7.3 (m, 2H, Ar), 6.7(d, J= 8.4 Hz, 2H, Ar), 5.4 (s, 1H, 3ºC-H), 1.728 (s, 3H, CH_3), ^{13}CNMR (δ ppm, 75 MHz, DMSO-d_6) 194.625, 155.955, 152.429,142.32, 140, 159, 133.938, 130.769, 127.621, 126.885, 114.625, 109.991, 55.135, 17.872; MS (FAB) m/z 309.60 (M+1), 253.6, 169.44 (100).

U511 IR (ν_{max}, KBr, cm^{-1}) 3294 (NH), 3201(NH), 1704.96(C=O), 1596

	(C=O), 1360 (C-N) ^{1}HNMR (δppm, 300 MHZ, DMSO-d_6), 9.079 (NH), the other NH peak has been merged with the aromatic protons, 7.8 - 7.3 (m, 12H, Ar) 5.6 (d, 1H, 3ºC-H), 1.748 (s, 3H, CH_3), ^{13}CNMR (δppm, 75 MHZ, DMSO-d_6) 193.86, 15.181, 143.53, 140.24, 139.92, 131.77, 131.44, 130.32, 127.26, 126.71, 126.31, 124.98, 124.69, 123.71, 108.8, 55.13; 17.652.
U517	IR (ν_{max} KBr, cm^{-1}) - 3294.1 (NH), 3186.18 (NH), 1700 (C=O), 1581.5 (C=C), 1473 (N-H bend), 1357 (C-N), ^{1}HNMR (δppm, 300 MHz, DMSO-d_6) 9.077 (s, 1H, NH), 8.9 (s, 1H, NH), 7.6-7.3, (m, 5H, Ar), 7.2 (d, J=6 Hz, 1H, Thiophenyl-H), 7.0 (d, J=3.1 Hz, 1H, Thiophenyl-H), 6.7 (t, 1H, Thiophenyl-H) 5.7 (d, J=3.3 Hz, 1H, 3ºC-H), 1.9 (s, 3H, CH_3) ^{13}CNMR (δppm, 75 MHZ, DMSO-d_6) 195.585, 151.750, 147.194, 142.970, 140.022, 139.941, 130.016, 127.342, 126.850, 126.764, 125.466, 109.341, 50.066, 17.853; MS (FAB) m/z- 299.26 (M+1), 221.22, 157.12 (100).

1.5. Conclusions

In the present work I have adopted a new synthetic strategy for the dihydropyrimidinones under a solvent free microwave assisted green approach. Compared to the solution phase mode, the microwave-assisted method is found to be more advantageous. It is simple, easy, fast and high yielding. The workup procedure is simple and in most cases the products are obtained in high purity just by washing with cold solvents. The strategy is very versatile as it provides for any variation in the components of the reaction.

The method so far developed for the synthesis of Biginelli compounds extensively made use of metal containing reagents that may contaminate the highly pharmacologically active pyrimidinone moiety. Hence our aim was to

use a mild and efficient non-metallic reagent for the DHPM synthesis. Thus, a new boron-based reagent has been introduced for the expeditious synthesis of dihydropyrimidinones under the microwave-assisted protocol. The reagent can be successfully used under solution conditions also. Herein we have synthesized the DHPMs both under microwave assisted and solution phase experimental conditions and the yields were compared. As far as yield is concerned the two methodologies are almost equally efficient with slight predominance for the microwave method. But when rapidity and simplicity are considered the microwave method is the better of the two. The reactions, which require 5-12 hours under solution method, could be completed within 4-10 minutes by the microwave-assisted protocol. The main disadvantage with microwave method is the scaling up process. The method as such cannot be used for the large-scale synthesis with in the limitation of microwave synthesis.

The present protocol can adjust with the change in any components of the reaction. Five diketones, nine aromatic and two heterocyclic aldehydes, urea and thiourea have been used to generate a variety of DHPMs under the current experimental conditions. Twenty new compounds have been added to the Biginelli library using the new protocol. All the aromatic aldehydes with electron withdrawing and electron donating functional groups on the ring reacted effectively to give the desired product. Even acid sensitive heterocyclic aldehydes such as furan-2-carbaldhyde and thiophene-2-carbaldehyde formed DHPMs by this novel method.

From the pharmaceutical point of view, as the method does not involve the use of any heavy metal species, the products may be obtained free of any heavy metal contamination. Also when the reaction is followed by microwave-assisted route for synthesis, the product could be obtained by a simple, easy, fast, economic and clean strategy. The process is solvent-free and the simple workup procedure affords pure product .The quantity and numbers of byproducts formed are negligible and could be removed by

solvent washing. Hence this methodology may be claimed to be an eco-friendly ‘green protocol’ for Biginelli reaction.

1.6. References

1. Biginelli, P. *Gazz. Chim. Ital*.1893, *23,* 360.

2. (a) Review: Kappe, C. O. *Tetrahedron* **1993**, *49*, 6937.(b) Kappe, C. O. *Acc. Chem. Res.* **2000**, *33*, 879.

3. (a) Hu, E. H.; Sidler, D. R.; Dolling, U. H. *J. Org. Chem.* **1998**, *63*, 3454. (b) Ma, Y.; Qian, C.; Wang, L.; Yang, M. *J. Org. Chem.* **2000**, *65*, 3864. (c) Lu, J.; Ma, H. *Synlett* **2000**, 63. (d) Dandia, A.; Saha, M.; Taneja, H. *J. Fluorine Chem.* **1998**, *90*, 17. (e) Gupta, R.; Gupta, A. K.; Paul, S.; Kachroo, P. L. *Ind. J. Chem.* **1995**, *34B*, 151.

4. (a) Shutalev, A. D.; Kishko, E. A.; Sivova, N.; Kuznetsov, A. Y. *Molecules* **1998**, *3*, 100. (b) Atwal, K S.; O' Reilley, B. C.; Gougoutas, J. Z.; Malley, M. F. *Heterocycles* **1987**, *26*, 1189. (c) O'Reilly, B. C.; Atwal, K. S. *Heterocycles* **1987**, *26*, 1185.

5. Folkers, K.; Harwood, H. J.; Johnson, T. B. *J. Am. Chem. Soc.* **1932**, *54*, 3751.

6. Ehsan, A.; Karimullah, *Pak. J. Sci. Ind. Res.* **1967**, *10*, 83.

7. (a)Rutter, H. A.; Gustafson, L. O. *J. Franklin Inst.* **1954**, *258*, 413.(b) McKinstry, D. W.; Reading, E. H. *J. Franklin Inst.* **1944**, *237*, 203.(c) Duburs, G. Ya.; Khanina, E. L. *Khim, Geterotsikli. Soedin.* **1976**, 220. (d) Kastron, V. V.; Vitolin, R. A.; Khanina, E. L.; Duburs, G. Ya.; Kimenis, A. A *Khim- Farm. Zh.* **1987**, *21*, 948. (e) Jani, M. K.; Undavia, N. K.; Trivedi, P.B. J. *Ind. Chem. Soc.* **1990**, *67*, 847.

8. (a)George, T.; Tahilramani, R.; Metha, D. V. *Synthesis* **1975**, 405. (b) Konyukov, V. N.; Sakovich, G. S.; Krupnova, L. V.; Pushkareva, Z. V. *Zh. Org. Khim*. **1965**, *1*, 1487. (c) Kryukov, L. N.; Lebedeva, N. Yu.;

Kostrova S. M. *Zh. Obshch. Khim.* **1990**, *60*, 1066. (d) Ertan, M.; Balkan, A.; Sarac, S.; Uma, S.; Ruebseman, K.; Renaud, J. F. *Arzneim-Forsch.* **1991**, *41*, 725.

9. (a) Ertan, M.; Balkan, A.; Sarac, S.; Uma, S.; Renaud, J. F. Rolland, Y. *Arch. Pharm.* **1991**, *324*, 135. (b) Jain, S. M.; Khajuria, R. K.; Dhar, K. L.; Singh, S.; Singh, G. B. *Ind. J. Chem.* **1991**, *30B*, 805. (c) Sadanandam, Y. S.; Shetty, M. M.; Diwan, P. V. *Eur. J. Med. Chem.* **1992**, *27*, 87.

10. Zigeuner, G.; Knopp, C.; Blaschke, H. *Monatsh. Chem*. **1976**, *107*, 587.

11. Zigeuner, G.; Hamberger, H.; Blaschke, H.; Sterk, H. *Monatsh. Chem.* **1966**, *27*, 1408.

12. Kato, T.; Chiba, T.; Sasaki, M. *Yakugaku Zasshi* **1981**, *101*, 182.

13. Buzueva, A. M. *Khim. Geterotsikl. Soedin*. **1969**, 345.

14. (a)Hull, R.; Swain, G. (ICI Ltd.) Brit. Patent **1965**, 984, 365 [*Chem. Abstr*. **1965**, *62*, 13159f]. (b) Hull, R. (ICI Ltd.) Brit. Patent **1965**, 984, 365 [*Chem. Abstr.* **1965**, *62*, 13159f). (c) Chiba, T.; Sato, H.; Kato, T. *Heterocycles* **1984**, *22*, 493. (d) Hirao, I.; Kato, Y.; Hujimoto, T *Nippon Kagaku Zasshi* **1964**, *85*, 52.

15. (a) Lopez Sastre, J. A.; Molina Molina, J. *An. Quim.* **1978**, *74*, 353. (b) Lopez Aparicio, F. J.; Lopez Herrera, F. J. *Carbohydr. Res.* **1979**, *69*, 243. (c) Lopez Aparicio, F. J.; Lopez Sastre, A.; Molina Molina, J.; Lopez Herrera, F. J. *An. Quim. Ser. C* **1981**, *77*, 147. (d) Lopez Aparicio, F. J.; Lopez Sastre, J. A.; Molina Molina. J.; Romero-Avila Garcia, M. C. *An. Quim., Ser. C* **1981**, *77*, 348. (e) Lopez Aparicio, F. J.; Lopez Sastre, J. A.; Molina Molina. J. *Carbohydr. Res.* **1981**, *95*,

113. (e) Molina Molina, J.; Abad Lorenzo, J. P.; Lopez Sastre, J. A. *An. Quim., Ser. C* **1982**, *78*, 250. (f) Valpuestra Fernandez, M.; Lopez Herrera, F. J.; Lupion Cobios, T. *Heterocycles* **1986**, *24*, 679. (g) Valpuestra Fernandez, M.; Lopez Herrera, F. J.; Lupion Cobios, T. *Heterocycles* **1988**, *27*, 2133.

16. Ashby, J.; Griffiths, D. *J. Chem. Soc., Perkin Trans. I* **1975**, 657.

17. Khromov- Borisov, M. V.; Savchenko, A. M. *Zh. Obshch. Khim.* **1952**, *22*, 1680.

18. Kappe, C. O.; Uray, G; Roschger, P.; Lindner, W.; Kratky, C.; Keller, W *Tetrahedron* **1992**, *48*, 5473.

19. (a) Sujatha, K.; Shanmugam, P.; Perumal, T.; Muralidharan, D.; Rajendran, M. *Bioorg. Med. Chem. Lett.* **2006,** *16*, 4893. (b) Khanina, E. L.; Siliniece, G.; Ozols, J.; Duburs, G.; Kimenis, A. *Khim-Farm*. Zh, **1978**, *12*, 72.

20. Kadis, V.; Stradins, J.; Khanina, E. L.; Duburs, G.; Muceniece, D. *Khim. Geterotsikl. Soedin.* **1985**, 117.

21. Liang, B.; Wang, X.; Wang, J. X.; Du, Z. *Tetrahedron* **2007**, *63*, 1981.

22. Shaabani, A.; Bazgir, A. *Tetrahedron Lett.* **2004,** *45*, 2575.

23. Abelman, M. M.; Smith, S. C.; James, D. R. *Tetrahedron Lett.* **2003,** *44*, 4559.

24. Duburs, G.; Khanina, E. L.; Vitolins, R. USSR Patent **1974**, 422, 735 [*Chem. Abstr*. **1974**, *81*, 91549x].

25. Khanina, E. L.; Andaburskaya, M. B.; Duburs, G., Zolotoyabko, R. M. *Latv. PSR Zinat, Akad. Vestis, Kim. Ser.* **1978**, 197 [*Chem. Abstr.* **1978**, *89*, 43319r].

26. (a) Khanina, E. L.; Duburs, G. *Khim. Geterotsikl, Soedin.* **1982**, 535. (b) Khanina, E. L.; Mucenice, D.; Kadysh, V. P.; Duburs, G. *Khim. Geterotsikl, Soedin.* **1986**, 1223. (c) Khanina, E. L.; Zolotoyabko, R. M.; Muceniece, D.; Duburs, G. *Khim. Geterotsikl, Soedin.* **1989**, 1076.

27. (a) Folkers, K.; Johnson, T.B. *J. Am. Chem. Soc.* **1933**, *55*, 2886. (b) Kappe, C. O.; Roschger, P. *J. Heterocyl. Chem.* **1989**, *26*, 55.

28. Nilson, B. L.; Overman, E. L. *J. Org. Chem.* **2006**, *71*, 7706.

29. (a) G. Maiti, P. Kundu, C. Guin, *Tetrahedron Lett.* **2003**, *44*, 2757. (b) B. Liang, X. Wang, J. X. Wang, Z. Du, *Tetrahedron*. **2007,** *63*, 1981. (c) Mirza-Aghayan, M.; Bolourtchian, M.; Hosseini, M.; *Synth Commun*. **2004**, *34*, 3335. (d) Manjula, A.; Rao, B. V.; Neelakantan, P.; *Synth. Commun*. **2004**, *34*, 2665.

30. (a) Han, X.; Xu, F.; Luo, Y.; Shen, Q. *Eur. J. Org. Chem.* **2005**, *70*, 1500. (b) Shaabani, A.; Bazgir, A.; Teimouri, F, *Tetrahedron Lett.* **2003,** *44*, 857. (c) Kamal, A.; Krishnaji, T.; Azhar, M. A. *Catal.Commun.* **2007**, *8*, 1929. (d) Lu, J.; Bai, Y.; Wang, Z.; Yang, B.; Ma, H. *Tetrahedron Lett*. **2000**, *41*, 9075. (e) Kumar, D.; Sundaree, M. S.; Mishra, B. G.; *Chem. Lett.* **2006**, *35*, 1074. (f) Baruah, P. P.; Gadhwal, S.; Prajapati, D.; Sandhu, J. S.; *Chem. Lett.* **2002**, *31*, 1038.

31. Jain, S. L.; Singhal, S.; Sain, B. *Green Chemistry*, **2007**, *9*, 740.

32. (a) Peng, J.; Deng, Y. *Tetrahedron Lett.* **2001**, *42*, 5917. (b) Dong, F.; Jun, L.; Xinli, Z.; Zhiwen, Y.; Zuliang, L. *J. Mol. Cat. A* **2007,** *274*, 208.

33. (a) Tu, Fang, F.; Miao, C.; Jiang, H.; Feng, Y.; Shi, D.; Wang, X. *Tetrahedron Lett.* **2003**, *44*, 6153. (b) Debache, A.; Boumoud, B.; Amimour, M.; Belfaitah, A.; Rhouati, S.; Carboni, B.; *Tetrahedron Lett.* **2006**, *47*, 5697. (c) Chen, W. Y.; Qin, S. D.; Jin, J. R.; *Catal. Commun.* **2007,** *8*, 123. (d) Yu, Y.; Liu, D.; Liu, C.; Luo, G. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 3508.

34. (a) Bigi, F.; Carloni, S.; Frullanti, B.; Maggi, R.; Sartori, G.; *Tetrahedron Lett.* **1999**, *40*, 3465. (b) Singh, V.; Sapehiyia, V.; Srivastava, V.; Kaur, S. *Catal. Commun.* **2006**, *7*, 571.

35. Singh, K.; Arora, D.; Singh, S. *Tetrahedron Lett.* **2003**, *44*, 857.

36. Rafiee, E.; Shahbazi, F.; *J. Mol. Catal. A* **2006**, *250*, 57.

37. Ming, L.; Wei-Si, G.; Li-Rong, W.; Ya-Feng, L.; Zheng, Y. H.; *J. Mol. Catal. A* **2006**, *258*, 133.

38. Yadav, J. S.; Reddy, B. V. S.; Reddy, K. B.; Raj, K. S.; Prasad, A. R.; *J. Chem. Soc. Perkins Trans. 1* **2001**, 1939.

39. (a) Wipf, P.; Cunningham, A. *Tetrahedron Lett.* **1995**, *36*, 7819. (b) Kappe, C. O. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 49. (c) Studer, A.; Hadida, S.; Ferrito, R.; Kim, S. Y.; Jeger, P.; Wipf, P.; Curran, D. P. *Science* **1997**, *275*, 823. (d) Studer, A.; Jeger, P.; Wipf, P.; Curran, D. P. *J. Org. Chem.* **1997**, *62*, 2917 (e) Lewandowski, K.; Murer, P.; Svec, F.; Fréchet, J. M. J. *Chem. Commun.* **1998**, 2237.

40. O’Reilly, B. C.; Atwal, K. S *Heterocycles* **1987**, *26*, 1185.

41. Atwal, K. S.; O’Reilly, B. C.; Gougoutas, J. Z.; Malley, M. F. *Heterocycles* **1987**, *26*, 1189.

42. Atwal, K. S., Rovnyak, G. C., O'Reilly, B. C.; Schwartz, J. *J. Org. Chem.* **1989**, *54*, 5898.

43. Atwal, K. S., Rovnyak, G. C., Schwatz, J.; Moreland, S.; Hedberg, A; Gougoutas, J. Z.; Malley, M. F.; Floyd, D. M. *J. Med. Chem*. **1990**, *33*, 1510.

44. Cho, H.; Shima, K.; Hayashimatsu, M.; Ohnaka, Y; Mizuno, A.; Takeuchi, Y. *J. Org. Chem*. **1985**, *50*, 4227.

45. Bohme, H.; Mundlos, E. *Chem. Ber.* **1953**, *86*, 1414.

46. Bergmann, W.; Johnson, T. B. *Ber*, *Dtsch. Chem. Ges*. **1933**, *66*, 1492.

47. Fissekis, J. D.; Sweet, F.*J. Org. Chem*. **1973**, *38*, 1963.

48. Takamizawa, A.; Hirai, K. *Chem. Pharm. Bull.* **1964**, *12*, 804.

49. Folkers, K; Johnson, T. B. *J. Am. Chem. Soc.* **1933**, *55*, 3784.

50. Sweet, F.; Fissekis, J. D. *J. Am, Chem. Soc*. **1973**, *95*, 8741.

51. Kappe, C. O. *J. Org. Chem.* **1997**, *62*, 7201.

52. Hentrich, W.; Schepss, w (I.G Farbenind.) D.R.P. 1930, 547,057[Fortschr. Teerfarbenfabr. Verw Industriezweige (Friedliander, E.; Ed.) 1932, 25, 2590-2591].

53. McKinstry, D. W., Reading, E. H. J. *Franklin Inst.* **1944**, *237*, 422.

54. (a) Hurst, E. W.; Ann. N. Y.; *Acad. Sci.* **1962**, *98*, 275. (b) Hurst, E. W.; Hull, R. *J. Med. Pharm. Chem.* **1961**, *3*, 215.

55. Ashok, M.; Holla, B. S.; Kumari, N. S. *Eur. J . Med. Chem.* **2007**. *42*, 380.

56. *Chem Abstr*. 1968, 68, 103775q.

57. *Chem Abstr*. **1971**, *75* 47266e.

58. Kumsars, K.; Velena, A.; Duburs, G.; Uldrikis. J.; Zidermane, A. *Biokhimiya* **1971**, *36*, 1201.

59. (a)*Chem. Abstr.* **1991**, *114*, 247302 z]. (b) Stefani, H. A.; Oliveria, C. B.; Almeida, R. B.; Pereira, C. M. P.; Braga, R. C.; Cella, R.; Borges, V. C.; Savegnago, L.; Nogueira, C. W. *Euro. J. Med Chem.* **2006**, *41*, 513.

60. Kato, T. Japn. Kokai Tokkyo Koho JP **1984**, 59, 190, 974 [*Chem. Abstr.* **1985**, *102*, 132067].

61. Cooper, K. (Pfizer Ltd.) PCT Int. Appl. WO **1990**, 11,281 [*Chem. Abstr.* **1991**, *114*, 14347f].

62. Siliniece, G; Kimenis. A. *Farmakol. Neirotrophykh Sredstv.* **1978**, 135 [*Chem Abstr.* **1980**, *92*, 693686].

63. Vitolina, R.; Kimenis, A. *Khim* **-** *Farm Zh* **1989**, *23*, 285.

64. . Kurono, M; Hayashi, M; Miura, K.; Isogawa, Y. I.; Sawai, K. (Sanwa Kagaku Kenkyusho co.) Japn. Kokai Tokkyo Koho JP **1987**, 62 267, 272 [*Chem. Abstr.* **1988**, *109*, 378321].

65. Atwal, K. S.; Rovnyak, G. C., Kimball, S. D.; Floyd, D. M.; Moreland, S.; Swanson, B. N., Gougoutas, J. Z.; Schwartz, J.; Smillie, K. M. Malley, M. F. *J. Med. Chem.* **1990**, *33*, 2629.

66. Atwal, K. S.; Swanson, B. N., Unger, S. E.; Floyd, D. M.; Moreland, S.; Hedberg, A.; O'Reilly, B. C. *J. Med. Chem.* **1991**, *34*, 806.

67. Rovnyak, G. C.; Atwal, K. S.; Hedberg, A.; Kimball, S. D.; Moreland, S.; Gougoutas, J. Z.; O' Reilly, B. C.; Schwartz, J.; Malley, M. F. *J. Med. Chem. Soc.* **1934**, *56*, 1374.

68. Cho, H.; Ueda; Shima, K.; Mizino, A..; Hayashmatsu, M.; Ohnaka, Y.; Takeuchi, Y. I.; Hamaguchi, M.; *Aisaka. Med. Chem.* **1989**, 32, 2399.

69. Horovitz, Z. P. (Squibb, E.R. an Sons) Eur. Pat. Appl. Ep **1990**, 400, 665[*Chem. Abstr.* **1991**, *115*, 64793].

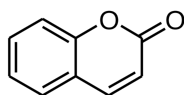
70. Review: Heys, L.; Moore, C. G.; Murphy, P. J. *Chem. Soc. Rev.* **2000**, *29*, 57.

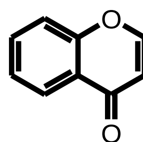
71. Patil, A. D.; Kumar, N. V.; Kokke, W. C.; Bean, M. F.; Freyer, A. J.; De Brosse, C.; Mai, S.; Truneh, A.; Faulkner, D. J.; Carte, B.; Breen, A. L.; Hertzberg, R. P.; Johnson, R. K.; Westley, J. W.; Potts, B. C. M. *J. Org. Chem.* **1995**, *60*, 1182.

72. Pabon, H. J. J. *Rec. Trav. Chim*. **1964**, *83*, 379.

2. 1. Introduction

Coumarins are a class of heterocyclic compounds containing oxygen as a member of the heterocyclic ring. The fusion of a pyrone ring with a benzene nucleus gives rise to a class of heterocyclic compounds known as benzopyrones, of which two distinct types are recognized: **(1)** benzo-α-pyrones, commonly called coumarins, and **(2)** benzo-γ-pyrones, called chromones, the latter differing from the former only in the position of the carbonyl group in the heterocyclic ring.





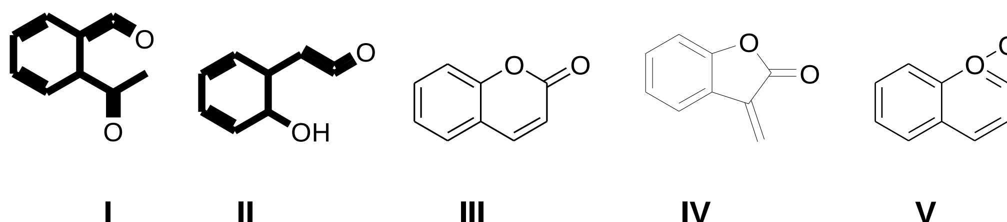
1 2

Benzo-α-pyrone Benzo-γ-pyrone

Representatives of these groups of compounds are found to occur in the vegetable kingdom, either in the free or in the combined state. Coumarin, the parent substance of the benzo-α-pyrone group, was first isolated from tonka beans in 1820. Several coumarin derivatives have been found to be widely distributed in the plant kingdom. Particularly the plants belonging to the families; Orchidaceae, Leguminoceae, Rutaceae, Umbelliferae, and Labiatae are rich sources of naturally occurring coumarins.[1]

Coumarin was initially considered to be a benzoic acid derivative, but its synthesis by W. H. Perkin, Sr.,[2] from salicylaldehyde by means of his classical reaction established its relation to o-hydroxycinnamic acid, which loses a molecule of water in forming the lactone ring. However, different constitutional formulae have been suggested from time to time. Of the various formulae proposed by Perkin -1868 (I), Basecke-1870 (II), Strecker -1867,

Fittig-1868, and Tiemann-1877 (III), Salkowski-1877 (IV), and Morgan and Micklethwait-1906 and Clayton-1906 (V), formula III has been found to be in complete accord with the known reactions of the coumarin derivatives and has been universally accepted as correct, vide Hugo Schiff.[3]



Thus coumarins and their derivatives are, from the point of view of their chemical constitution, a group of lactones derived from o-hydroxycinnamic acids. Alternately stated, a coumarin ring system is formed by the fusion of a benzene and an α-pyrone ring.

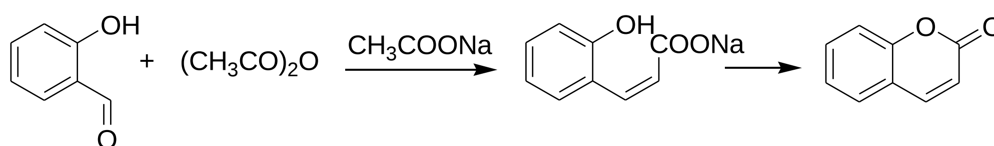
2.2. Review of Literature

2.2.1. Methods for the synthesis of coumarin derivatives

Many methods have been devised so far for the synthesis of coumarin derivatives, under conventional, microwave and sound assisted strategies. Some of them are described here.

2.2.1.1. Perkin reaction: This classical method has entered into every textbook of organic chemistry. As stated above, Perkin[2] first synthesized coumarin from salicylaldehyde by heating it with acetic anhydride and anhydrous sodium acetate (Scheme 2.1).

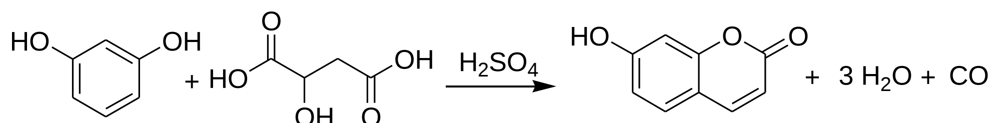
Scheme 2.1



This reaction occurs with the formation of an intermediate o-hydroxycinnamic acid derivative which passes spontaneously into the lactone when liberated from its sodium salt. This method was successfully used by Tiemann and Herzfield,[4] Taage,[5] Spath,[1] Yanagisawa and Kondo,[6] Dyson,[7] Dey and Sankaranarayanan[8] and later on, by numerous workers in the field[9]. Later K.K. Vijayan[10] introduced a procedural modification for the effective synthesis of phenyl coumarins under conventional condition in presence of triethylamine.

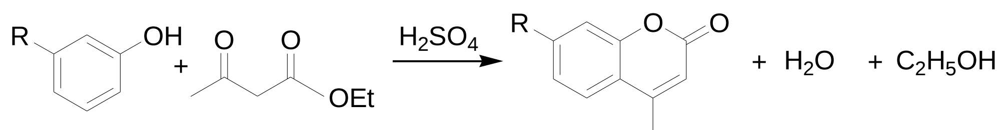
2.2.1.2. Pechmann reaction: Pechmann[11] found that a coumarin derivative is formed when a mixture of a phenol and malic acid is heated in the presence of concentrated sulfuric acid (Scheme 2.2).

Scheme 2.2



Later Pechmann and Duisberg[12] found that phenols can condense with β-ketonic esters in the presence of sulfuric acid, giving coumarin derivatives (Scheme 2.3).

Scheme 2.3

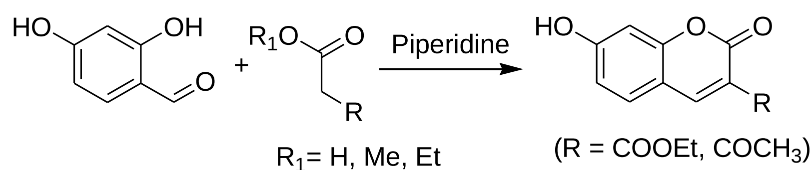


It is the simple and commonly used method for synthesizing coumarins from activated phenols and β-keto esters or an unsaturated carboxylic acid.[13,14] Conventionally, the Pechmann reaction is also carried out in presence of phosphorous pentoxide,[15] trifluoroacetic acid[16] and aluminum chloride.[17] Homogeneous metal chlorides such as $ZnCl_2$, $TiCl_4$, $InCl_3$, GaI_3,[18-21]

triflates[22], sulfonic acid [23] and ionic liquids [24-26] are reported to produce 7-hydroxy coumarin derivatives in high yield at ambient temperature. Due to non-reusability of these homogeneous catalysts different solid acid catalysts such as amberlyst ion-exchange resins,[27] zeolites,[28,29] montmorillonite K-10,[30] polyaniline sulfate salt,[31-32] heteropoly acids,[33] nafion resin/silica nanocomposites[34] and nano-crystalline sulfated-zirconia[35] have been studied for the synthesis of coumarin derivatives. Microwave irradiation has been found more useful for synthesis of these coumarin derivatives in order to minimize the reaction time.[36,37] Recently, ultrasound assisted Pechmann synthesis has also been reported producing good yields of 7-hydroxy -4-methyl coumarin, however, with homogenous $BiCl_3$ catalyst. [38]

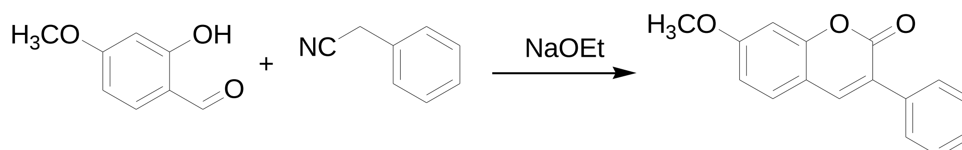
2.2.1.3. Knoevenagel reaction: Knoevenagel[39] developed a method for the synthesis of coumarin derivatives from o-hydroxyaldehydes by condensation with ethyl malonate, ethyl acetoacetate, ethyl cyanoacetate etc. in the presence of piperidine, pyridine, and other organic bases (Scheme 2.4).

Scheme 2.4



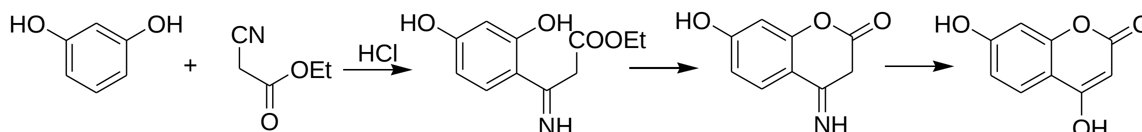
Various workers have successfully used this reaction by suitably changing the reactants, reagents and experimental conditions to improve the yield and reduce the complexity of the reactions [40-47] (Scheme 2.5).

Scheme 2.5



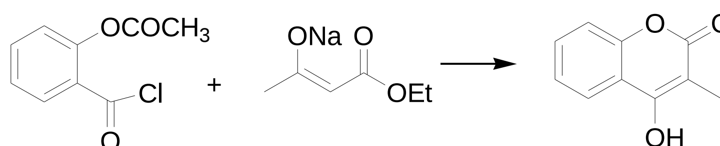
2.2.1.4. Sonn[48] found that resorcinol condenses with cyanoacetic ester under the conditions of the Hoesch reaction,[49] the ketimine hydrochloride obtained on hydrolysis gives ultimately 4,7-dihydroxycoumarin (Scheme 2.6)

Scheme 2.6



Still another method by which 4-hydroxycoumarins are obtained is due to Anschutz,[50,51] who condensed the sodium derivative of acetoacetic ester with 2-acetoxybenzoyl chloride in ethereal solution and obtained 4-hydroxycoumarin derivatives (Scheme 2.7). He extended his work by using the sodium derivatives of malonic ester and cyanoacetic ester with various substituted acid chlorides. Heilbron and Hill[52] have obtained 3-methyl, 3-benzoyl, and 3-benzyl coumarins by this method.

Scheme 2.7

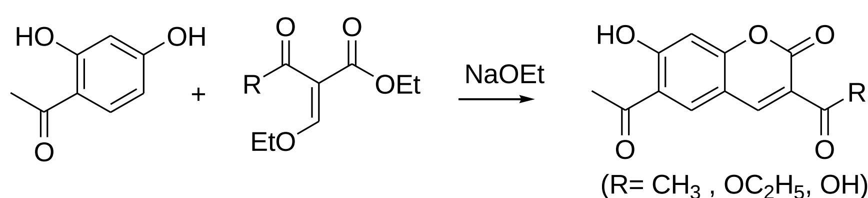


Pauly and Lockemann[53] synthesized 4-hydroxycoumarin from methyl acetylsalicylate by adding metallic sodium to the molten ester. Several

3-substituted 4-hydroxycoumarins have also been prepared by Stahmann *et al.*[54] from acylated derivatives of methyl salicylate.

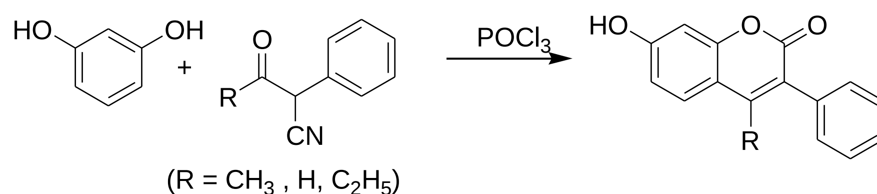
2.2.1.5. Weiss and Merksammer[55] found that resacetophenone on condensation with ethyl ethoxymethyleneacetoacetate by heating with alcoholic sodium ethoxide gave 7-hydroxy-3, 6-diacetylcoumarin (Scheme 2.8). Weiss and Kratz[56] extended the method and found that ethyl ethoxymethylenemalonate similarly condensed to give coumarin-3-carboxylates from resorcinol derivatives, the carbethoxyl group having hydrolyzed to the carboxyl group.

Scheme 2.8



2.2.1.6. Baker *et al.*[57] found that α-formylphenylacetonitrile and its derivatives condense with resorcinol and other phenols, in the presence of phosphorus oxychloride or dry hydrogen chloride as condensing agent, leading to the production of 3-phenylcoumarins in poor yields and not the isomeric 3-phenyl chromones (isoflavones) (Scheme 2.9).

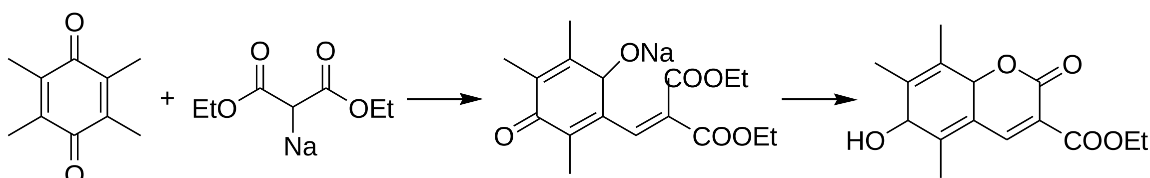
Scheme 2.9



2.2.1.7. Smith and Dobrovolny[58] have put forward one more method of general applicability but of limited interest, in which they showed that

3-carbethoxy-5,7,8-trimethyl-6-hydroxycoumarin was produced when duroquinone reacted with ethyl sodiomalonate in benzene solution (Scheme 2.10).

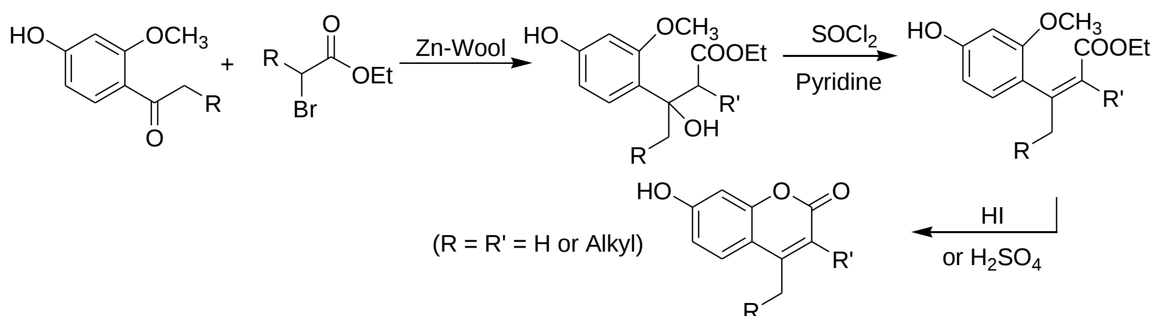
Scheme 2.10



Smith and coworkers[59-62] have exhaustively investigated this reaction with various brominated methylquinones and found that they may react with a metallic enolate to produce either a coumarin by reaction with a methyl group or a quinone malonic ester by direct replacement of a bromine atom.

2.2.1.8. **Reformatsky reaction:** Chakravarti and Majumdar[63] have developed a method by which 3,4-dialkyl-substituted coumarins not available by the usual methods may be synthesized, in which o-hydroxyaryl alkyl ketones, under the conditions of the Reformatsky reaction, are ultimately converted into coumarin derivatives[64] (Scheme 2.11)

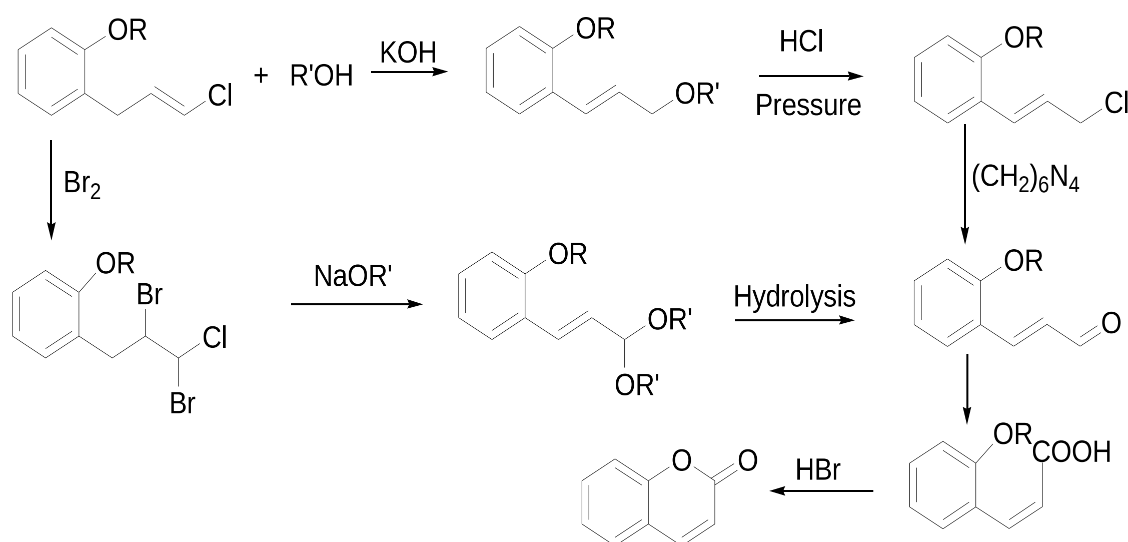
Scheme 2.11



2.2.1.9. Friedel-Crafts reaction: Bert[65] has developed a general method for synthesizing coumarins, which consists in condensing phenolic ethers with $CH_2C1CH=CHC1$ either by the Friedel-Crafts reaction or in the presence of

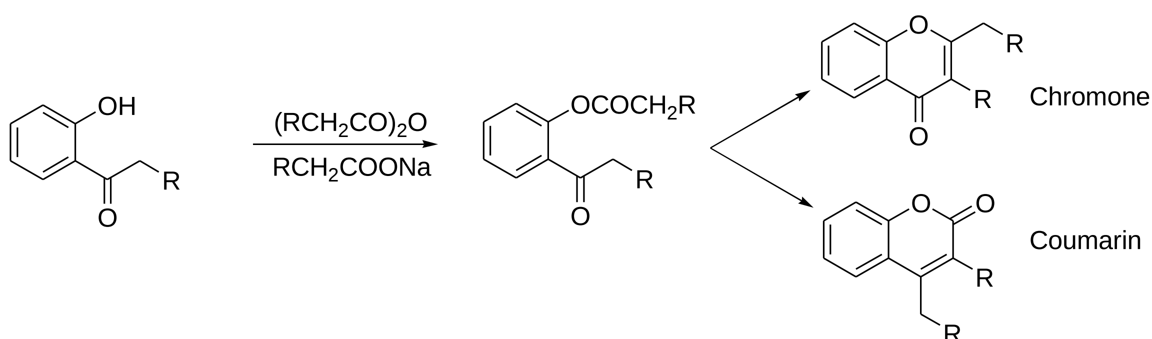
zinc dust to obtain $ROC_6H_4CH_2CH=CHCl$, which can also be synthesized by condensing $CH_2ClCH=CHCl$ with o-bromophenolic ether through the Grignard reaction. This is then converted into the corresponding coumarin in two ways, as shown below (Scheme 2.12).

Scheme 2.12



2.2.1.10. Kostanecki acylation of o-hydroxyketones: This is a method of coumarin formation with an element of uncertainty in it. Kostanecki and Rozycki[66] showed that the products obtained by Nagai[67] and Tahara[68] by heating resacetophenone and its monomethyl ether with acetic anhydride and sodium acetate were chromone derivatives (Scheme 2.13).

Scheme 2.13



Allan and Robinson[69] further developed this method for the synthesis of a large number of chromones and chromonols occurring in nature. It has been found, however, that this method is not exclusively applicable for chromone formation, inasmuch as chromones or coumarins or a mixture of both may result from the above reaction, since there are two ways, in which the intermediate acyl derivative may lose water, giving a chromone or a coumarin.

2.2.1.11. In addition to the above-mentioned methods, other reactions used for the coumarin synthesis are Claisen rearrangement,[70] Wittig[71] and catalytic cyclization reactions.[72] An approach via ring-closing metathesis (RCM) to synthesize versatile coumarin scaffolds have been described recently.[73-75]

2.2.2. Uses of coumarin derivatives and their biological activities

Coumarins are one of the most important classes of fluorescent molecules, and they are found to possess applications in industrial and biological fields.[76] The odoriferous and fluorescent nature of coumarin derivatives led to its wide spread use in industries as perfumery chemicals, food additives,[77] optical brightening agents and dispersed fluorescent and laser dyes.[78] For example, 7-hydroxy-4-methyl coumarin (β-methyl-umbelliferone) is used as fluorescent brightener, efficient laser dye, standard

for fluorimetric determination of enzymatic activity and as a starting material for the preparation of insecticide and furanocoumarins.[79-81] Similarly, 7-amino- 4-methylcoumarin is mainly used as laser dye and intermediate for the synthesis of bioactive compounds.[82]

2.2.3. Biological activities

Coumarins are worthful species and known to exhibit remarkable pharmacological and biological activities.[83] These include the anti-coagulant, dicoumarol[84], the antibiotics, novobiocin and chlorobiocin,[85] and the calanolides A and B, which have been shown to inhibit HIV-1 replication *in vitro*.[86] Coumarin derivatives also found applications as antimicrobial agents[87] and intrinsic probe for the labeling of peptides.[88] Juan Manuel Ferrer *et al.* have studied the binding of several compounds bearing the coumarin moieties to serum albumin by equilibrium dialysis.[89] A new fluorescent GTP analogue that has a coumarin fluorophores attached to the γ-phosphate (Cm-GTP) has been synthesized and tested in transcription assays using T7 RNA polymerase as a model system.[90]

2.3. Results and Discussions

2.3.1. Syntheses of Substituted Coumarin Derivatives

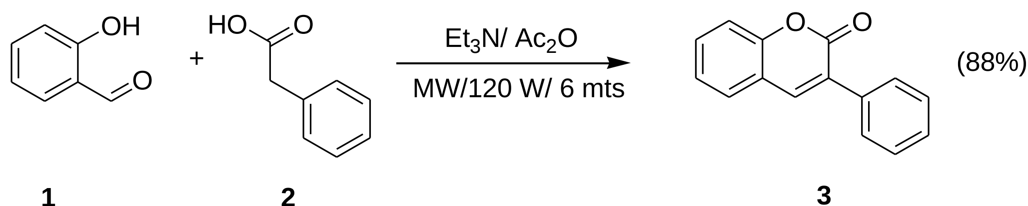
Many methods have been developed for the synthesis of these highly active moieties both under conventional and modern methodologies, which include Claisen rearrangement, Perkins reaction, Von Pechmann reaction, Knoevenagel condensation, Wittig reaction etc. and the modified version of these methods. The main drawbacks of these methods are low yields, longer reaction time, high temperature and formation of large number of byproducts. The modern protocol mainly comprises of microwave assisted and phase transfer catalyzed pathways to effect the above-mentioned reactions to form the coumarin derivatives with considerable yield.

In the present scenario a greener method for the synthesis of organic compounds are necessary, which eliminate or minimize the utilization of nonparticipating chemicals and reduce the amount of energy, efforts and resources. To a certain extent, the microwave assisted solvent free synthetic method is a tool to attain this goal. As part of an effort for the synthesis of oxygen heterocycles, I have explored a new microwave assisted approach for the coumarin derivatives.

Triethylamine in combination with other reagents like sodium acetate[9], $PhPOCl_2$[91], 2-chloro-1-methyl pyridinium iodide [92] etc. has proved to be good catalyst for coumarin formation. Vijayan[10] reported a conventional method for the formation of coumarins using triethylamine and acetic anhydride. A remarkable procedural modification from the conventional route, utilizing the flexibility of microwave assisted reaction and the same reagents as catalyst with some interesting results has been successfully explored here. Compared to the early reported conventional protocol, the newly modified microwave assisted method is fast, easy, clean with least number of byproducts and with good yield.

In the present study, a solvent free one-pot strategy for the synthesis of coumarin derivatives under microwave assisted conditions has been carried out. When salicylaldehyde (1) was mixed with phenyl acetic acid (2) in the presence of triethylamine and acetic anhydride and irradiated under microwave for 6 minutes (Scheme 2.14) using a domestic microwave oven under solvent free condition, an efficient reaction occurred and on subsequent work up gave one product that has been identified as 3-phenylcoumarin.

Scheme 2.14. Synthesis of 3-phenylcoumarin.

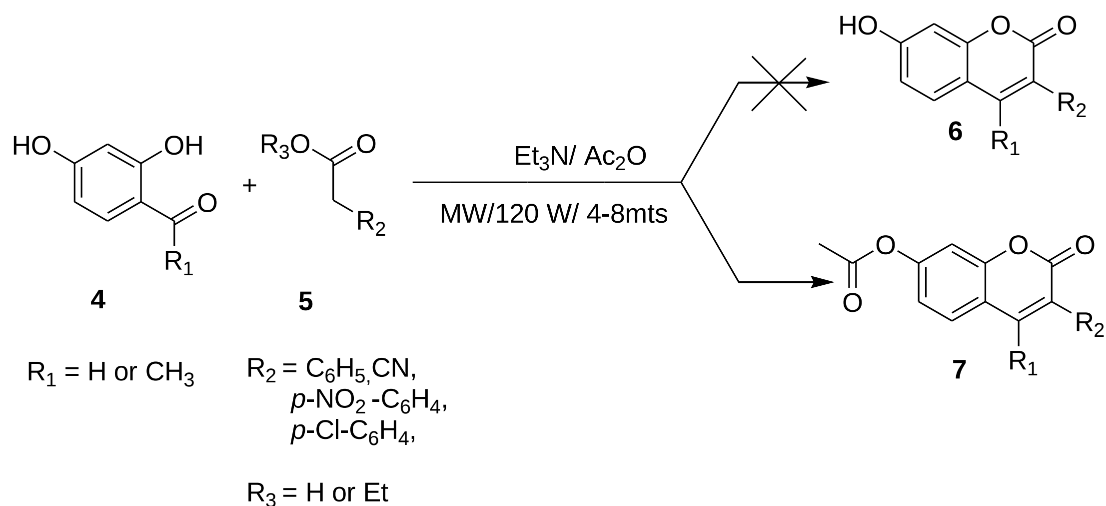


The reaction is further extended to other substituted acetic acid and their derivatives and 2-hydroxy aromatic aldehydes/ ketones for the synthesis of substituted coumarins. 3,4- and 7-substituted coumarins have been synthesized using this fast one-pot protocol. Along with the new coumarin derivatives many known coumarin compounds were synthesized and characterized by comparing their melting points IR, NMR and Mass spectral data with those reported in the literatures.

In the absence of acetic anhydride the reaction failed to yield the desired products. Also when the amount of acetic anhydride is reduced to half, the reaction did not proceed to completion. Thus, acetic anhydride is supposed to act as a dehydrating agent, enhancing the rate of condensation of carbonyl compound with active methylene species.

When 2,4-dihydroxy aromatic aldehydes/ ketones (**4**) were made to react with substituted acetic acid or its derivatives (**5**), instead of 7-hydroxy coumarins, 7-acetoxy coumarin derivatives (**7**) were obtained (Scheme 2.15). Thus it is a simple and easy method for the formation of acetoxycoumarins directly from hydroxy aldehydes/ketones in a single step.

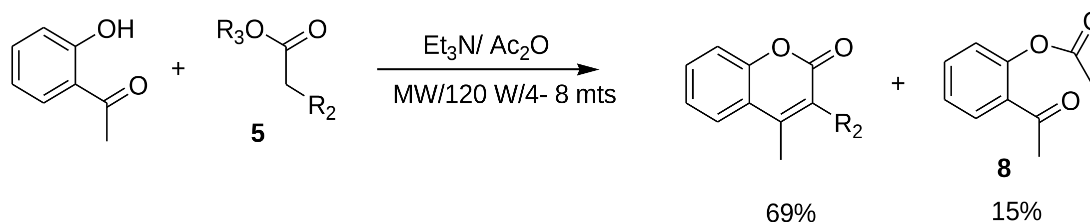
Scheme 2.15. Synthesis of 7-acetoxycoumarin Derivatives.



When ethyl acetate derivatives were used in place of the acetic acid derivatives it was observed that instead of a single product several byproducts were formed with decreased yield. Also, the reaction was incomplete and the starting materials were recovered.

When 2-hydroxyacetophenone was reacted with **5**, it was found on TLC monitoring that two products were formed. They were separated and identified as 2-acetoxyacetophenone (**8**) and the corresponding coumarin (Scheme 2.16). The acetylation of the hydroxyl group is observed only in the case of 2-hydroxyacetophenone. With other carbonyl compounds such as 2,4-dihydroxyacetophenone, 2-hydroxybenzaldehyde, 2,4-dihydroxybenzaldehyde etc. no acetylated product was formed. Also 3-acetyl-4-methylcoumarin derivative could not be synthesised when ethyl acetoacetate was made to react with 2-hydroxyacetophenone and only the compound **8** could be separated out from the reaction mixture.

Scheme 2.16. Formation of 2-acetoxyacetophenone in the Reaction.

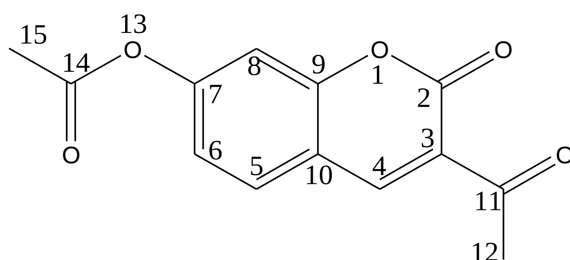


Ethyl acetate or acetic acid did not answer this reaction for the formation of simple coumarins using the present synthetic protocol. However, 3,4 and 7- trisubstituted coumarins (**7**) were synthesized in a single step with great easiness and rapidity.

2.3.2. Characterization

All the compounds synthesized were characterized using IR, NMR and Mass spectra. For convenience, the compound C45 has been selected for the spectroscopic discussion. On the basis of the spectroscopic data the structure proposed for the compound C45 is given in (Figure 2.1). Spectral details obtained for this compound are described below.

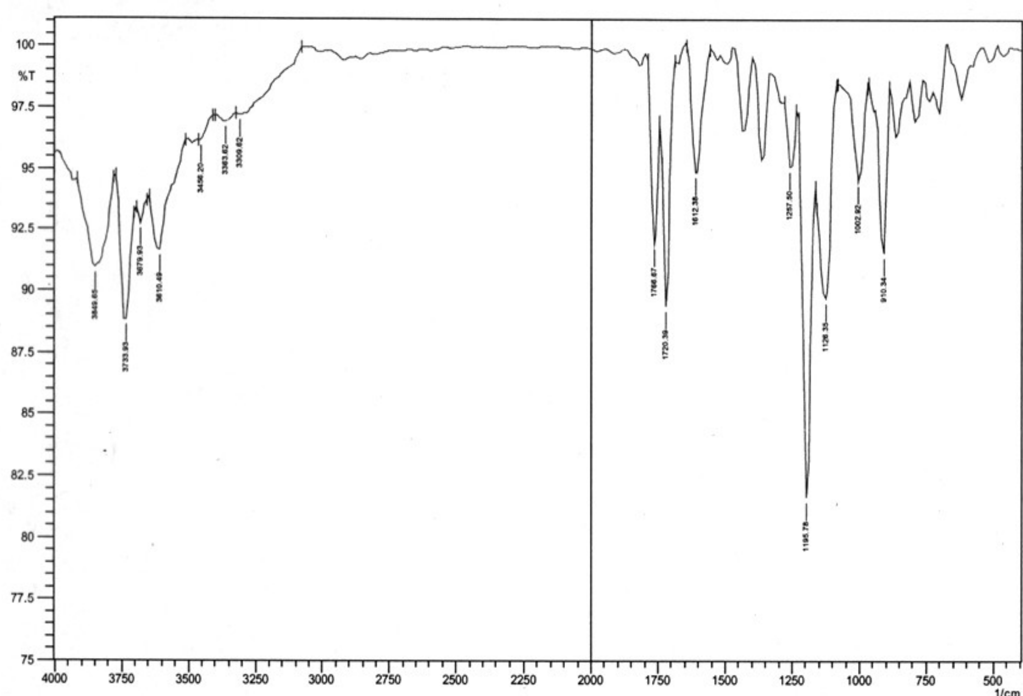
Figure 2.1. Structure of the Compound C45.



The UV spectrum of the compound shows two absorption maxima at 301 and 339 nm which are characteristics of coumarins, indicating the $\pi \longrightarrow \pi^*$ and $n \longrightarrow \pi^*$ transitions respectively. In the IR spectrum of C45, the major absorptions were found at 1766, 1720, 1612 and 1195 cm^{-1}. The peak at 1766 cm^{-1} is due to the absorption by ester carbonyl group. Phenyl esters are known to absorb at a higher frequency than a normal ester carbonyl.

No separate absorption could be observed for the ring carbonyl group (at C_2). It is possible that the absorption of carbonyl group of the coumarin ring and the acetyl group (C_{11}) might have merged to form a single peak at 1720 cm^{-1}. The peaks at 1612 cm^{-1} and 1195 cm^{-1} are due to the absorption by C = C between C_3 and C_4 and the vibration due to the C-O bond of the ester. The IR spectrum is shown in Figure 2.2.

Figure 2.2. **IR spectrum of the compound C45.**



In the NMR spectrum the downfield proton resonance at δ 8.505 is due to the vinyl proton at C_4, which is a singlet. Other peaks occur at δ 7.6, δ7.18 and δ7.1. These values are ascribed to the aromatic protons. The peak at δ7.6 is attributed to the proton at C_5. This peak is split into a doublet by the coupling with the proton at C_6 with a coupling constant, J = 8.7 Hz. The proton resonance with a doublet at δ 7.18 is ascribed to the proton at C_8 with J = 2.1 Hz. A doublet of doublet at δ 7.1 is observed for the proton at C_6.

Figure 2.3a. Proton NMR spectrum of the compound C45.

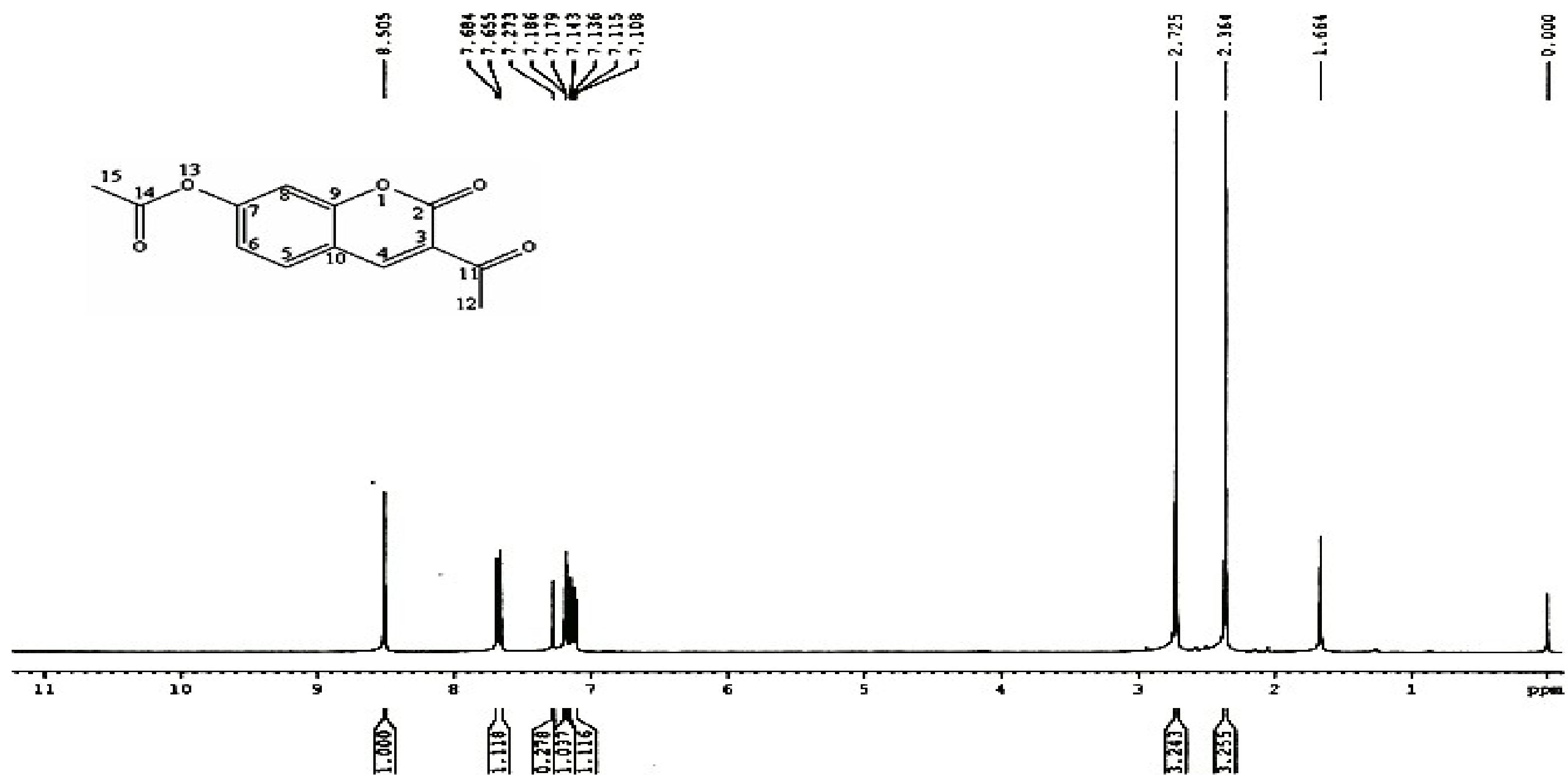
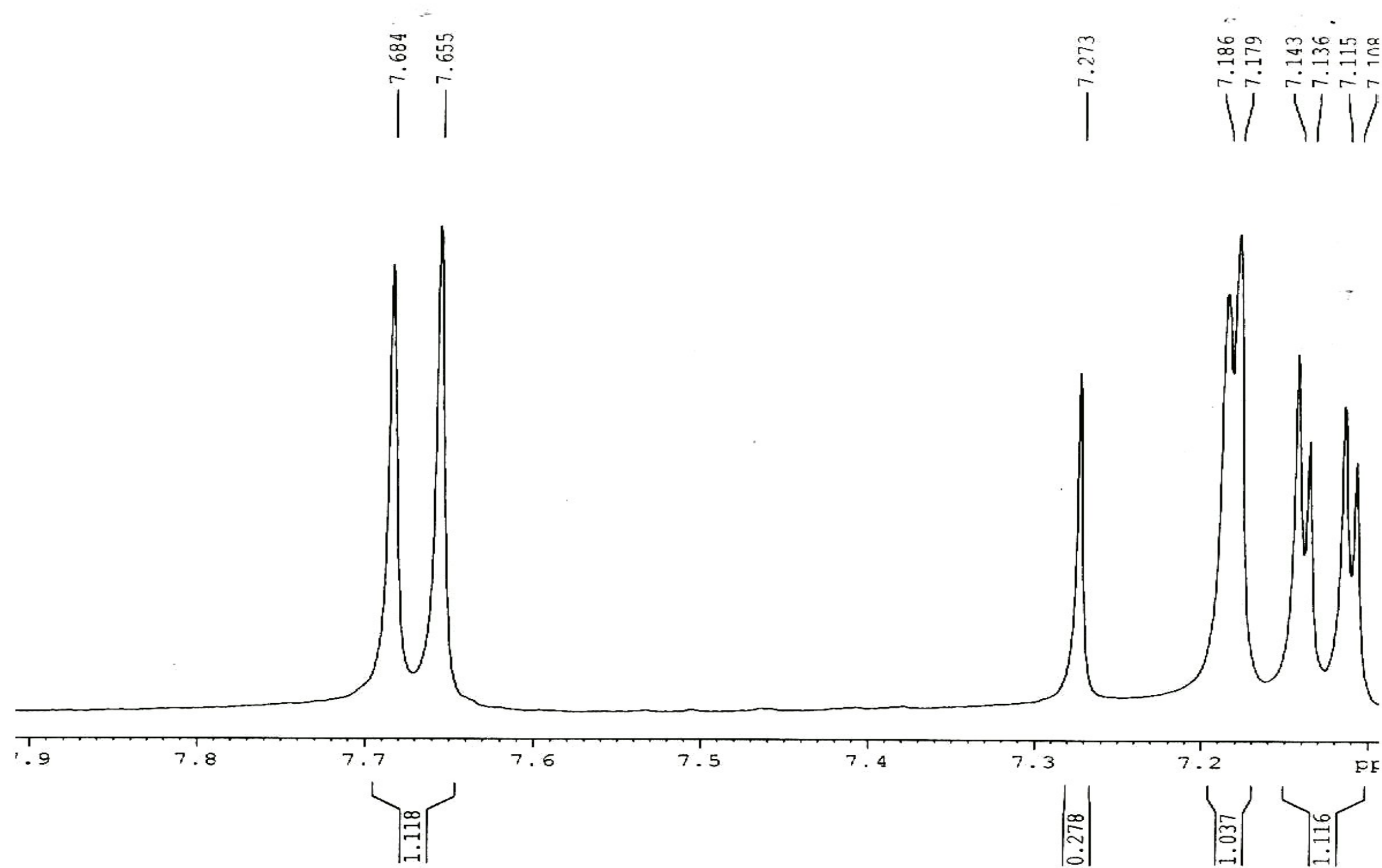


Figure 2.3b. Proton NMR spectrum (expanded) of the compound C45.



The peak is first split into a doublet by the proton at C_5 with a coupling constant, J = 8.4 Hz. This coupling is evident from the similar J values (8.7 and 8.4 Hz). The proton at C_8 further splits each peak of the doublet into a doublet of doublet with coupling constant 2.1 Hz. The low J-value suggests meta coupling. A singlet peak at 2.725 ppm corresponds to the resonance of proton at C_{12}. Similarly a singlet at δ 2.364 is attributed to the proton at C_{15}.The proton NMR spectrum is shown in Figure 2.3.

The chemical shift values attributed to protons are confirmed by two-dimensional proton correlation spectra. The correlation between the protons on C_5 and C_6 is clear from the H-H COSY spectra given in Figure 2.4.

Figure 2.4a. ^1H-^1H COSY spectrum of the compound C45.

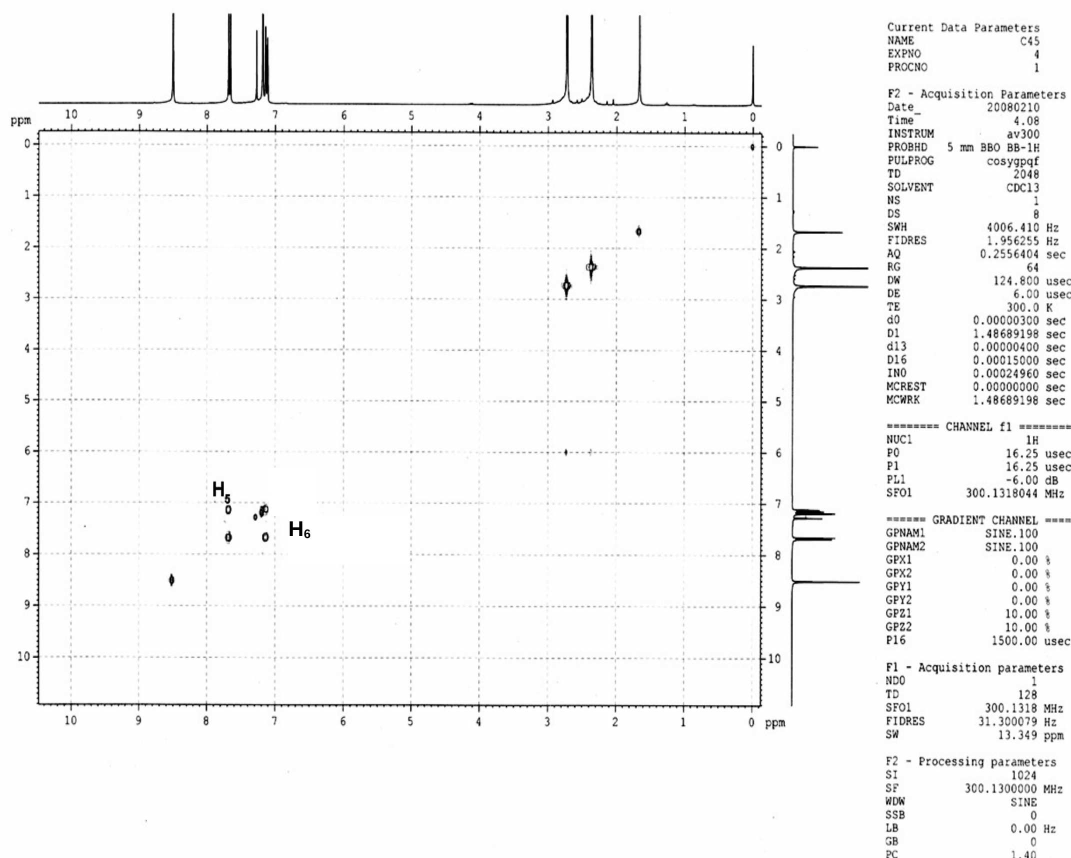
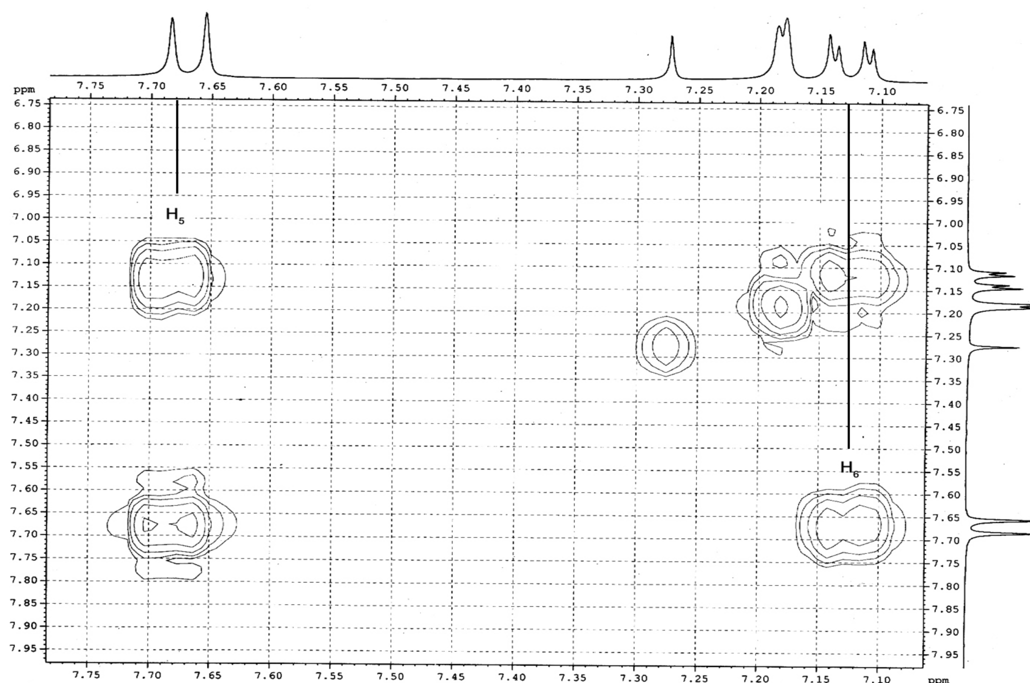


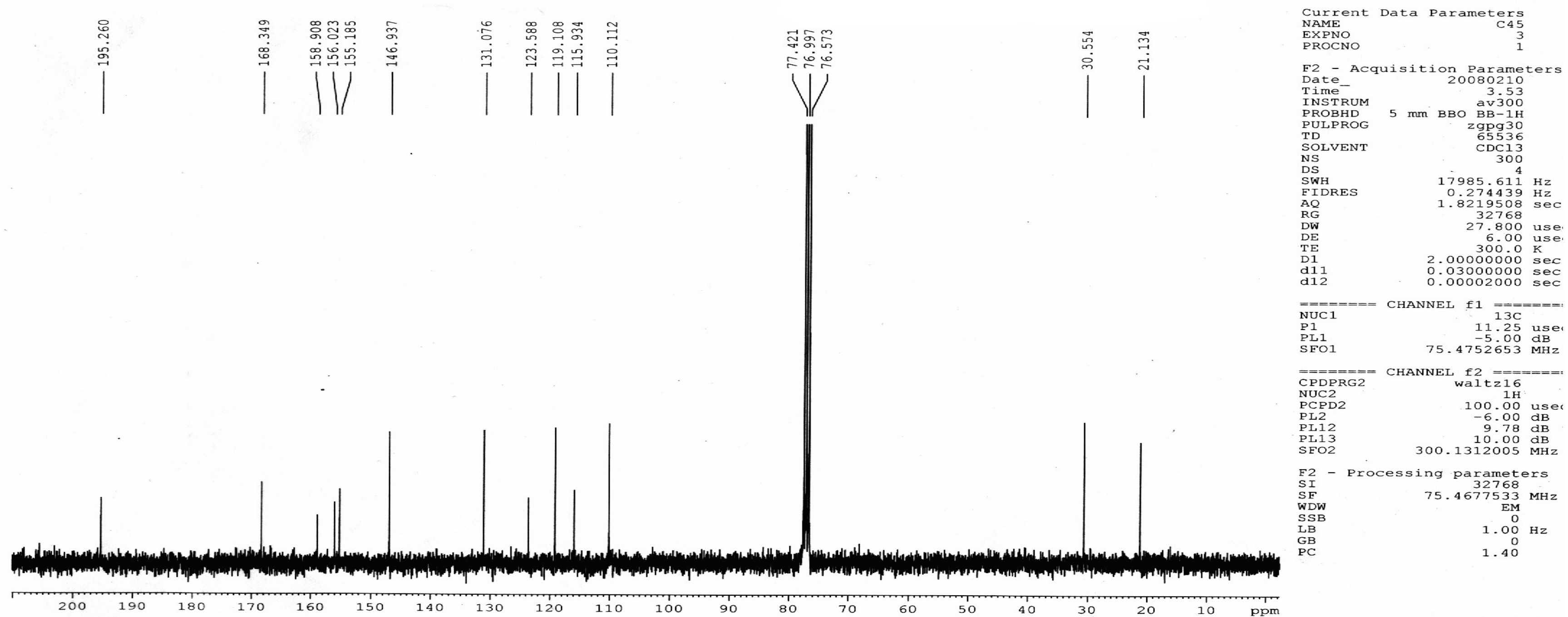
Figure 2.4b. H-H COSY spectrum (Expanded) of the compound C45.



On the basis of the ^{13}CNMR and the two dimensional NMR such as HMBC (Heteronuclear Multiple Bond Coherence) and C-H COSY spectra (Heteronuclear Multiple Quantum Coherence, HMQC) the carbons in the compound C45 have been ascribed the chemical shift values as follows.

The ring carbonyl group (coumarin carbonyl) resonates at 158.908 ppm and the carbonyl group of the ester (C_{14}) has a chemical shift δ 168.349. The resonance of the carbonyl group of the ketone, C_{11} occurs at δ 195.260. The C_4 carbon has got a resonance at 146.937 ppm and for C_3 at 123.588 ppm. The carbons C_7 and C_9 have chemical shift values δ 155.18 and δ 156.023 respectively. This downfield resonance is due to the deshielding of these carbons in the presence of oxygen atoms bonded to it. Other aromatic carbons C_5, C_6, C_8 and C_{10} resonate at δ 131.076, δ 119.108, δ 110.112 and δ 115.934 respectively. The ^{13}CNMR spectrum is shown in Figure 2.5.

Figure 2.5. ^{13}C NMR Spectrum of the compound C45.



The direct correlation of the protons with carbon is evident from the ^{13}C-^{1}H COSY spectrum shown in Figure 2.6. Also the correlations of the protons with the carbons that are separated by more than one bond have been obtained from the HMBC spectrum as shown in Figure 2.7. The different correlations of H_4 observed are (H_4, C_{11}), (H_4, C_2), (H_4, C_5) and (H_4, C_9). Similarly H_6 shows coherence with C_8 and C_{10}. The multiple bond correlation for H_{15} is (H_{15}, C_{14}) and that of H_{12} is (H_{12}, C_{11}).

Figure 2.6a. C-H COSY spectrum of the compound C45.

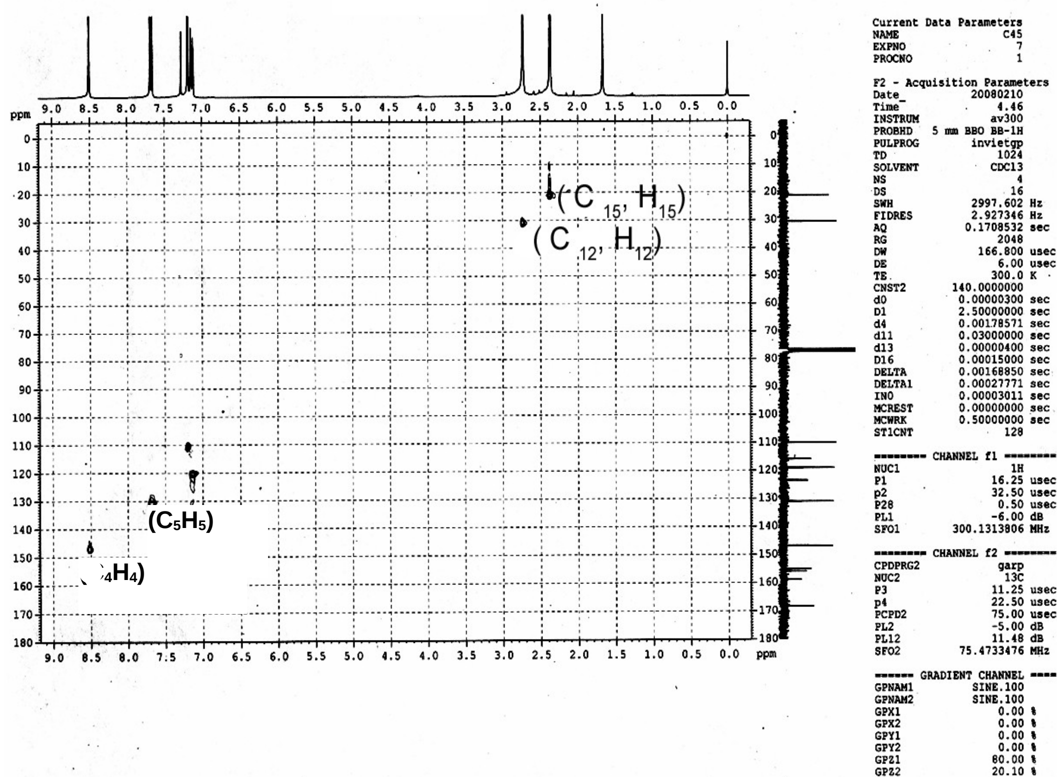


Figure 2.6b. C-H COSY spectrum (expanded) of the compound C45.

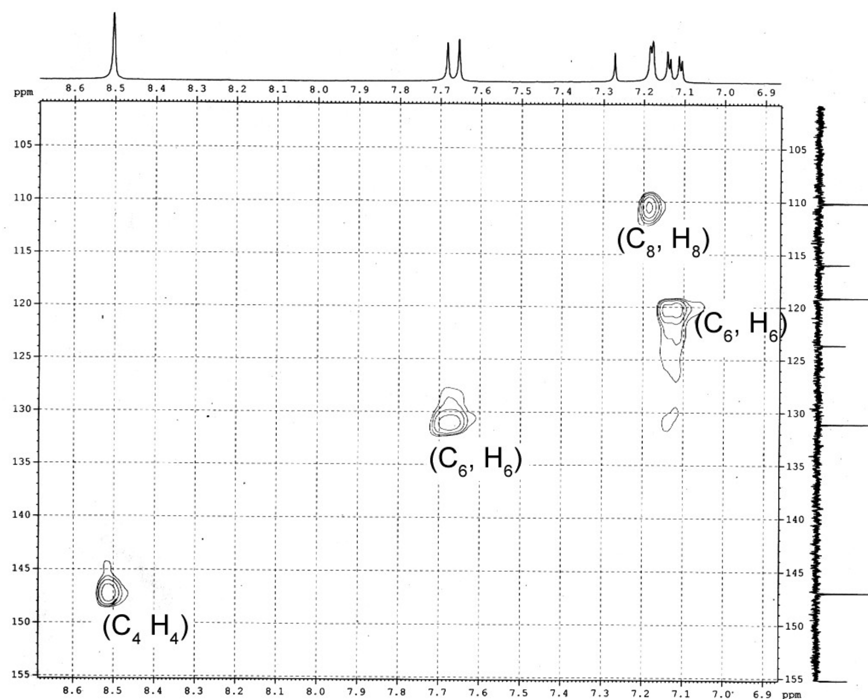


Figure 2.7a. HMBC spectrum of the compound C45.

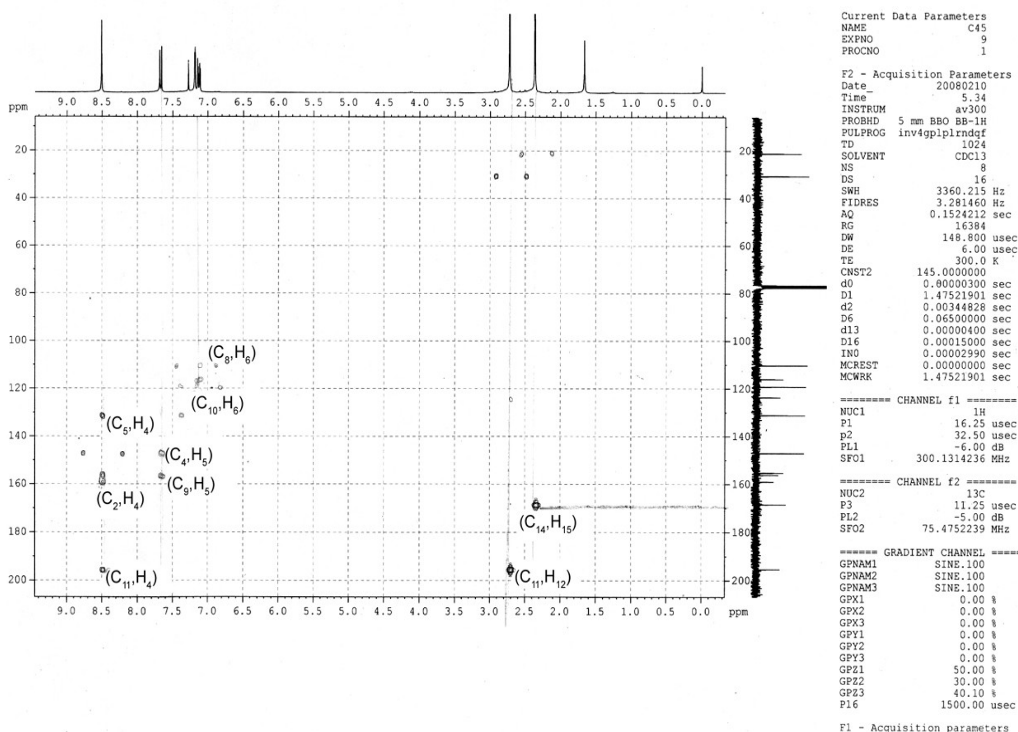
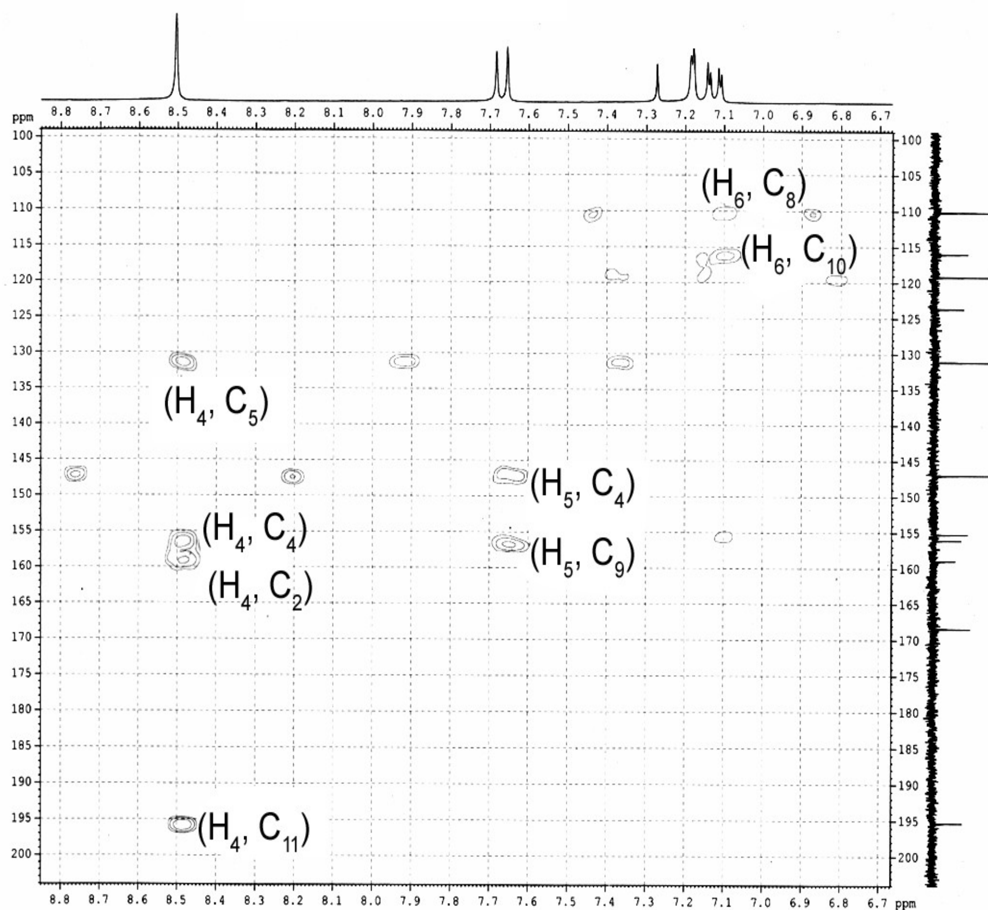
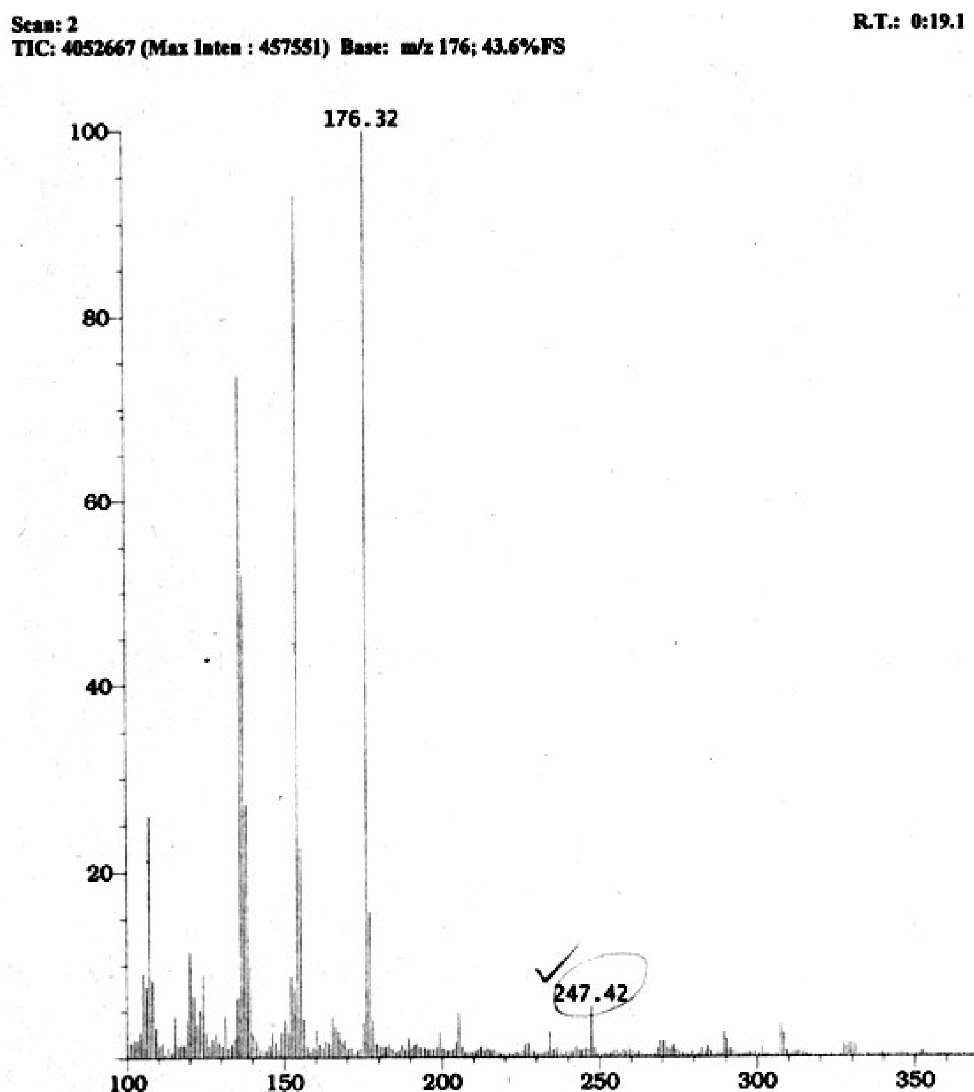


Figure 2.7b. HMBC spectrum of the compound C45.



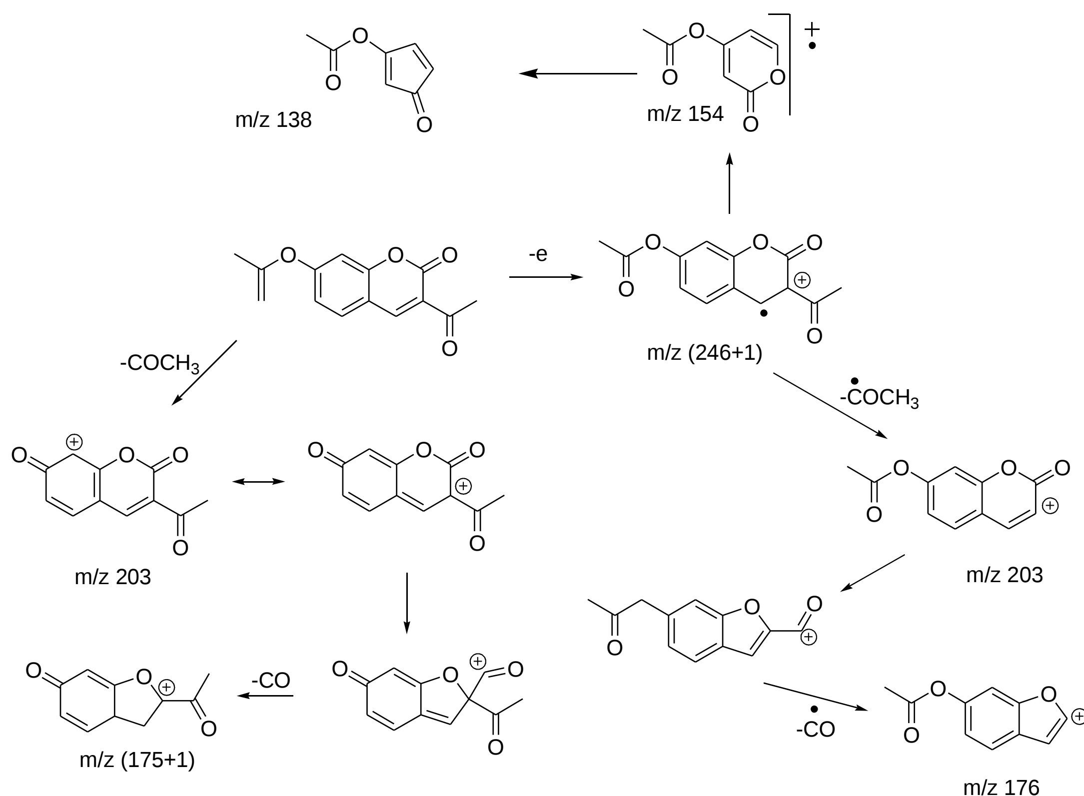
The structure of the compound is further confirmed by mass spectrum. The FAB spectrum obtained for the compound C45 is given in Figure 2.8.

Figure 2.8. FAB Mass spectrum for the compound C45.



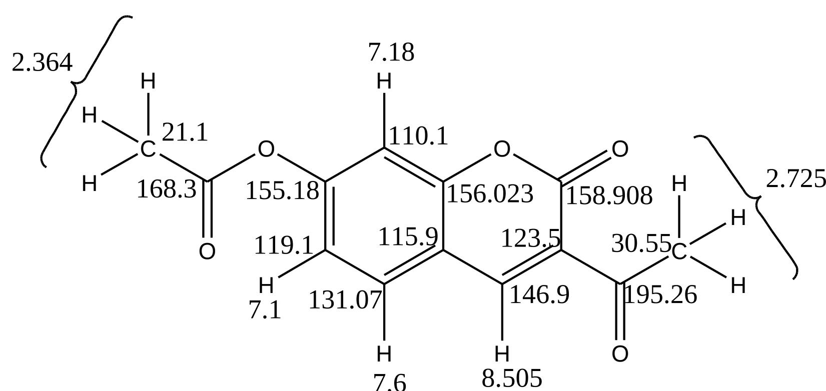
The molecular ion peak is obtained at m/z = 247.42 (M+1) and the base peak at m/z = 176.32. Other major fragments obtained are m/z = 203, 154 and 138. The possible mass spectral fragmentation pattern can be formulated as shown in Scheme 2.17.

Scheme 2.17. Possible Mass Spectral (FAB) Fragmentation Pattern for the Compound C45.



On the basis of the various spectroscopic techniques the final structure arrived at for the compound C45 with the ^{1}HNMR and ^{13}CNMR values is shown in Figure 2.9.

Figure 2.9. Structure of the compound C45 with the NMR chemical shift values.



Spectral data for all other compounds have been tabulated in Table 2.3 and the spectra are enclosed in the Appendix.II.

2.4. Experimental

2.4.1. Materials and Methods

All the chemicals used are of synthetic grade and are purified before use. The melting points are determined using a GUNF melting point apparatus and are uncorrected. The UV spectra are recorded on SYSTRONIC Double beam UV-VIS Spectrophotometer, IR on a JASCO FT/IR- 4100 and SHIMADZU 8400S Spectrophotometer in KBr medium, NMR spectra recorded on a BRUKER AVANCE DPX 300 MHz Spectrometer using TMS as internal standard and mass spectra recorded on a SHIMADZU GC-MS-QP 2010 mass spectrometer and JEOLJMS600 FAB mass spectrometer. Microwave irradiation is done using an ELECTOLUX 700 W domestic microwave oven.

2.4.2. General synthetic procedure

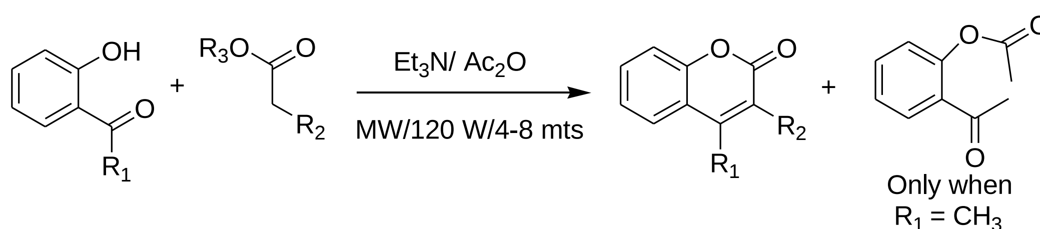
2.4.2.1. *Synthesis of 7-acetoxy-3-acetyl-4-methyl coumarin (C45)*

2,4-dihydroxybenzaldehyde (1.38 g, 10 mmol), ethylacetoacetate (1.3 ml, 10 mmol), triethylamine (0.7 ml, 5 mmol) and acetic anhydride (5 ml) are taken in a 50 ml stoppered flask, mixed well and irradiated under 120 W microwave for 5 minutes intermittently. The reaction mixture is poured into ice-cold water, stirred for half an hour at low temperature (0-10˚C), separated solid is washed repeatedly with dilute $NaHCO_3$ solution and distilled water, dried and re-crystallized from ethanol. The crystals obtained have more than 90% purity. The final purification is done with chromatography using a 100-200 mesh silica gel column with light petroleum -ethyl acetate as the eluent

by gradient elution technique. Melting point; 159-161°C. The yield is found to be 69 %.

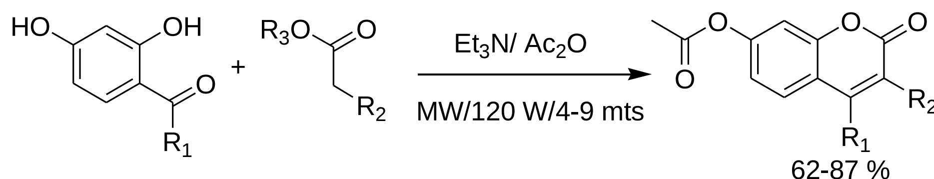
All other coumarin derivatives have been synthesized using the same procedure with different reaction time. The structural details with experimental condition and melting points of the various coumarin derivatives have been tabulated in Table 2.1and Table 2.2. The spectroscopic details of all the new compounds have been shown in Table 2.3. The spectra of the known compounds used for comparison and spectra of the new compounds have been included in Appedix.II.

Table 2.1. Experimental Details of The Microwave-Assisted Synthesis of 3 and/or 4-substituted coumarin derivatives.



Sl.No.	Comp-ound	R_1	R_2	R_3	Time of Irradiation (Minutes)	Yield (%)	Melting point	Reported Melting point
1	**C11**	H	C_6H_5	H	6	89	138-140	137-139
2	**C12**	H	CN	Et	4	75	180-182	182-184
3	**C13**	H	4-Cl- C_6H_4	H	7	90	193-194	--
4	**C15**	H	COMe	Et	6	78	118-120	120-122
5	**C18**	H	4-NO_2- C_6H_4	H	5	88	259-261	262-264
6	**C21**	Me	C_6H_5	H	8	69	149-150	153-155
7	**C23**	Me	4-Cl- C_6H_4	H	4	73	156-158	--
8	**C22**	Me	CN	Et	6	61	164-168	--
9	**C27**	Me	2-OMe- C_6H_4	H	7	79	121-122	120-122

Table 2.2. Experimental details of the synthesis of 7-acetoxy-3 and/or 4-substituted coumarin derivatives.



Sl. No.	Comp-ound	R_1	R_2	R_3	Time of Irradiation (Minutes)	Yield (%)	Melting Point
1	**C31**	Me	C_6H_5	H	8	62	169-171
2	**C41**	H	C_6H_5	H	5	87	172-175
3	**C42**	H	CN	Et	4	73	135-136
4	**C45**	H	COMe	Et	5	69	159-161
5	**C48**	H	4-NO_2- C_6H_4	H	9	85	222-224

2.4.3 Spectroscopic Data

All the known compounds have been characterized by comparing the melting points, IR, NMR and Mass spectrometric data with that of the available literature. Newly synthesized compounds are characterized using the UV, IR, NMR and Mass spectrometric data given in Table 2.3. The spectra are enclosed in Appendix II.

Table 2.3. Spectroscopic data of the newly synthesized coumarin derivatives.

Compound	Spectral data
C13	UV (λ_{max}(nm), methanol) 323, 293, 214; IR ($\upsilon_{max,}$ KBr, cm^{-1}) 3055 (=C-H), 1711(C=O), 1608(C=C), 749 (C-Cl); ^{1}HNMR (δppm, 300MHz, $CDCl_3$) 7.8042(s,1H, =CH), 7.6(d, J=8.7 Hz, 2H, Ar), 7.5(d, J=7.5 Hz, 2H, Ar), 7.4(d, J=8.5 Hz, 2H, Ar), 7.3-7.2 (m, 2H, Ar); ^{13}CNMR (δ ppm, 75MHz, $CDCl_3$)

160.282, 153.396, 139.884, 134.797, 132.944, 131.595, 129.730, 128.581, 127.883, 126.996, 124.540, 119.352, 116.405; MS (EI) *m/z* 256(M^+, 100), 228(70), 165(55).

C22 UV [λ_{max} (nm), methanol] 323, 293, 214; IR ($\upsilon_{max,}$ KBr, cm^{-1})- 2229(CN), 1724(C=O), 1602.85 (C=C); ^{1}HNMR (δppm, 300MHz, $CDCl_3$) 7.7 (m,2H, Ar), 7.4(m,2H,Ar), 2.7 (s,3H, CH_3); ^{13}CNMR (δppm, 300MHz,$CDCl_3$)- 162.3, 158.6 154.2, 135, 125.9, 125.4, 119, 118.1, 113.1, 102, 18.2; MS(EI) *m/z* 185(M^+,100), 156(47), 140(35),102(45).

C23 UV [λ_{max} (nm), methanol] 311, 252, 209; IR ($\upsilon_{max,}$ KBr, cm^{-1}) 1698 (C=O), 1597(C=C), 775 (C-Cl); ^{1}HNMR(δppm, 300MHz,CDCl3) 7.6 (dd, J=8.1, 1H, Ar), 7.5(m, 1H, Ar), 7.4-7.2(m, 6H, Ar), 2.3339(s, 3H, CH_3); ^{13}CNMR (δppm, 75MHz, $CDCl_3$) 160.786, 152.624, 148.071, 134.272, 132.777, 131.606, 131.506, 128.711, 126.130, 125.151, 124.397, 120.334, 116.915,16.2; MS (EI) *m/z* 270(M^+, 100), 241(50), 178(40).

C31 UV [λ_{max} (nm), methanol] 369, 311, 207; IR ($\upsilon_{max,}$ KBr, cm^{-1}) 1761 (ester C=O), 1714(coumarin C=O), 1608(C=C); ^{1}HNMR (δppm, 300MHz, DMSO-d_6) 7.6(d, J=8.7, 1H, Ar), 7.4-7.3 (m, 3H, Ar), 7.2(d, J=10, 2H, Ar), 7.1(m, 2H, Ar), 2.3541 (s, 3H, CH_3CO), 2.3138 (s, 3H, CH_3); ^{13}CNMR (δppm, 75MHz, DMSO-d_6) 169.3, 161.2, 153.6, 153.0, 147.6, 134.6, 130.4, 128.9, 128.7, 127.2, 126.4, 118.6, 110.6, 21.6, 17.1; MS(EI) *m/z* 294 (M^+,25), 252 (100), 224 (75).

C41 UV [λ_{max} (nm), DMSO] 328,300; IR ($\upsilon_{max,}$ KBr, cm^{-1}) 1751.24 (ester C=O), 1704.96 (coumarin C=O), 1612.38 (C=C); ^{1}HNMR (δ ppm, 300MHz, DMSO-d_6) 7.8 (s, 1H, =CH), 7.7-7.6 (dd, 2H, Ar), 7.5(d, 1H, Ar), 7.4(m, 3H, Ar), 7.1(d, , 1H, Ar), 7.09-7.06 (dd, , 1H, Ar), 2.357 (s, 3H, CH_3CO), 2.3138 (s, 3H, CH_3); ^{13}CNMR (δppm, 75MHz, DMSO-d_6) 168.794, 160.255, 154.006, 152.779, 139.250, 134.480, 128.931, 128.579, 128.495, 127.758, 118.516,117.494, 110.001, 21.143, 17.1; MS (FAB) m/z 281.37 (M+1, 20), 238.33 (15), 176.28 (25), 154 (100), 138 (85).

C42 UV [λ_{max} (nm), DMSO] 358, 285; IR ($\upsilon_{max,}$ KBr, cm^{-1}) 2248.59 (C≡N), 1734.66 (C=O), 1613.16 (C=C), 1225.54 (C-O); ^{1}HNMR (δ ppm, 300MHz, DMSO-d_6) 7.72 (s, 1H, CH), 7.6-7.5 (m, 2H, Ar), 7.3 (d, J=3.4Hz, 1H, Ar), 2.426 (s, 3H, $COCH_3$); ^{13}CNMR (δppm, 75MHz, DMSO-d_6) 172.7, 158.3, 152.4, 149.9, 129.8, 122.4, 119.6, 118.7, 114.7, 113.6, 99.2, 21.4; MS (FAB) m/z – 213.73 (M+1, 100), 203, 186, 160.

C48 UV [λ_{max} (nm), DMSO] – 339, 288; IR ($\upsilon_{max,}$ KBr, cm^{-1}) 3093.61 (=C-H)

1731.81 (ester C=O), 1681.81, (coumarin C=O), 1512 (NO_2), 1350.08 (C-N); ^{1}HNMR not obtained due to least solubility. Sold state ^{1}HNMR has been recorded but not resolved; ^{13}CNMR (Solid state) 173.07, 158.24, 154, 145.93, 142, 139.55, 130.46, 128, 121, 119.38, 115.39, 110.29, 21, MS (FAB) m/z – 313.66 (M+), 154.28, 136.22, 107.23 (100); Elemental analysis:- C = 62.82 (Cal. 62.77); H = 3.60 (Cal. 3.41); N = 4.73 (Cal. 4.31).

2.5. Conclusions

In the present work a new microwave assisted synthetic strategy for the synthesis of substituted coumarin derivatives have been devised. This method is devoid of any solvent. Also it is fast and the work up procedure is very simple and easy. In most cases the products are obtained in 90% purity by two or three step crystallization. Column purification is needed only for few compounds. By this method the acetoxy coumarins can be obtained directly from hydroxyl carbonyl compounds. Hence compared to any of the conventional method it possesses the advantage of high yield, low reaction time, easy workup procedure and completeness of the reaction.

In conclusion the new method that is devoloped by me is an environmentally benign method as it is destitute of toxic volatile organic solvents to a greater extent. This simple protocol can be employed for the expeditious synthesis of 3-phenyl, 3-cyano, 3-chloro, 3-amino, 3-acetyl, 4-methyl, 7-acetoxy coumarins etc. which are very versatile platforms for many synthetic conversions to form high potential pharmacophores. These new compounds are studied in detail for its photophysical and biological activities, which are described in the succeeding parts.

2. 6. References

1. Spath, E. *Ber*. **1937**,*70a*, 53.

2. Perkin,W. H. *J . Chem. Soc.* **1868**, *53* ; **1877**, **388**.

3. Schiff, H . *Ber*. **1872**, *6*, 665.

4. Tiemann, F.; Herzfield, H. ; *Ber.* **1877**, *10*, 283.

5. Taege, C . *Ber.* **1877,** *20*, 2109.

6. Yanagisawa, H.; Kondo, H . *J. Pharm. Soc. Japan* **1921**, *472*, 498.

7. Dyson, G . *J. Chem. Soc.* **1887**, *63*.

8. Dey, B. B.; Sankaranarayanan, Y. *J. Indian Chem. Soc.* **1931**, *8*, 819.

9. (a) Perkin, W. H.; Henry Sr., W. *J. Chem. Soc.* **1875,** *28*, 10. (b) Perkin, W.H. *J. Chem. Soc.* **1887**, *31*, 388. (C) Johnson, J. R. *Org. React.* **1942**, *1*, 210.

10. Vijayan, K.K. *Ind. J. Het. Chem*. **1998**, *8*, 01.

11. Pechmann, H. *Ber*. **1884**, *17*, 929.

12. Pechmann, H.; Duisberg, C.; *Ber.* **1883**, *16*, 2119.

13 (a) von Pechmann, H. *Chem. Ber.* **1884**, 17, 929. (b) Horning, E. C. *Organic Synthesis*, vol. 3, p.281, Wiley, New York, **1955.** (c) John, E. V. O.; Israelstam, S. S. *J. Org. Chem.* **1961**, 26. (d) Bose, A.K.; Pednekar, S.; Ganguly, S. N.; Chakraborty, G.; Manhas, M. S. *Tetrahedron Lett*. **2004**, *45*, 8351. (e) Singh, P. R.; Singh, D. U.; Samant, S. D.; *Synlett.* **2004**, *11*, 1909.

14 Singh, V.; Singh, J.; Kaur, K. P.; Kad, G. L. *J. Chem. Res. (S)* **1997**, 58.

15 Canter, F. W.; Curd, F. H.; Robertson, A. *J. Chem. Soc.* **1931,** 1255.

16 Woods, L. L.; Sapp, J. *J. Org. Chem.* **1962**, 27, 3703.

17 Gupta, D. A. K.; Chatterje, R. M.; Das, K.R.; Green, B. *J. Chem. Soc.* **1969**, 29.

18 Smitha, G.; Reddy, C. S. *Synth. Commun.* **2004**, *34*, 3997.

19 Valizadeha, H.; Shockravi, A. *Tetrahedron Lett.* **2005**, *46*, 3501.

20 Bose, D. S.; Rudradas, A.P.; Babu, M. H. *Tetrahedron Lett.* **2002**, *43*, 9195.

21 Sun, P.; Hu, Z. *Synth. Commun.* **2005**, *35*, 1875.

22 Wang, L.; Xia, J.; Tian, H.; Qian, C.; Ma, Y. *Ind. J. Chem. B.* **2003**, *42* 2097.

23 Sugino, T.; Tanaka, K. *Chem. Lett.* **2001,** 110.

24 Harjani, J. R.; Nara, S. J.; Salunkhe, M. M. *Tetrahedron Lett.* **2002**, *43*, 1127.

25 Singh, V.; Kaur, S.; Sapehiyia, V.; Singh, J.; Kad, G. L. *Catal. Commun*. **2005**, *6*, 57.

26 Gu, Y.; Zhang, J.; Duan, Z.; Deng, Y. *Adv. Synth. Catal.* **2005**, *347*, 512.

27 Sabou, R.; Hoelderich, W. F.; Ramprasad, D.; Weinand, R. *J. Catal.* **2005**, *232*, 34.

28 Gunnewegh, E. A.; Hoefnagel, A. J.; van Bekkum, H. *J. Mol. Catal. A: Chem.* **1995**, *100*, 87.

29 Hoefnagel, A. J.; Gunnewegh, E. A.; Downing, R. S.; van Bekkum, H.

J. Chem. Soc. Chem. Commun. **1995**, 225.

30 Li, T. S.; Zhang, Z. H.; Yang, F.; Fu, C. G. *J. Chem. Res. (S).* **1998**, 38.

31 Palaniappan, S.; Shekhar, R. C. *J. Mol. Catal. A: Chem.* **2004**, *209*, 117.

32 Palaniappan, S.; Saravanan, C.; Rao,V. J. *Polym. Adv. Technol.* **2005**,*16*, 42.

33 Romanelli, G.P.; Bennardi, D.; Ruiz, D. M.; Baronetti, G.; Thomas, H.J.; Autino, J.C. *Tetrahedron Lett.* **2004**, *45*, 8935.

34 Laufer, M.C.; Hausmann, H.; Holherich, W. F. *J. Catal.* **2003**, *218*, 315.

35 Tyagi, B.; Mishra, M. K.; Jasra, R V. *J. Mole. Cata. A: Chemical.* **2007**, *276*, 47.

36 Frere, S.; Thiery, V.; Besson, T. *Tetrahedron Lett.* **2001**, *42*, 2791.

37 de la Hoz, A.; Moreno, A.; Vazquez, E. *Synlett* **1999**, 608.

38 Patil, S. B.; Bhat, R. P.; Raje, V. P.; Samant, S. D. *Synth. Commun.* **2006,** *36*, 525.

39 Knoevenagel, E. *Ber.* **1898**, *31*, 2585.; **1904**, *37*,4461.

40 Borsche,W.; Streitberger, F.; *Ber.* **1904**, *37*, 3165.

41 Ghosal, S. C. *J. Indian Chem. Soc.* **1926**, *3*,105.

42 Khan, A. A.; Kurien, P. N.; Pandya, K. C. *Proc. Indian Acad. Sci.1A,* **1935**, 440.

43 Kiewiet,T.; Stephens, H . *J. Chem. Soc*. **1931**, 639.

44 Kurien, N. P.; Andyka., C.; Surangev, R. *J. Indian Chem. Soc*.**1934**, *11*, 823.

45 Pandya, K. C.; Sodhi, T. S. *J. Univ. Bombay* **1939**, *8*,173.

46 Shah, H. A.; Shah, R. C. *J. Chem. Soc.* **1939**, *132*, 949; **1940**, 245.

47 (a) Knoevenagel, E. *Chem. Ber.* **1896**, *29*, 172. (b) Knoevenagel, E. *Ber. Dtsch. Chem. Ges.* **1904**, *37*, 446. (c) Bogdal, D. *J. Chem. Research (S)*, **1998**, 468. (d) Shockravi, A.; Sharghi, H.; Valizadeh, H.; Heravi, M. M. *Relat. Elem.* **2002**, *177*, 2555. (e) Song, A.; Wang, X.; Lam, K. S. *Tetrahedron Lett.* **2003**, *44*, 1755.

48 Sonn, A. *Ber.* **1917**, *60*, 1292.

49 Hoesch, K. *Ber.* **1915**, *48*, 1122.

50 Anschutz, R. *Ann.* **1909**, *367*, 169.

51 Anschutrz, R. *Ann*. **1910**, *368*, 23.

52 Heilbron, I. M.; Hill, D. W. *J. Chem. Soc.* **1927**, 1705.

53 Pauli, H.; Lockemann, K. *Ber*. **1915**, *48*, 28.

54 Stahmann, M. A.; Wolff, I.; Link, K. P. *J. Am. Chem. Soc.* **1943**, *65*, 2285.

55 Weiss,R .; Merksammer, E . *Monatsh.* **1928,** *60*, 115.

56 Weiss, R.; Kratz, A *Monatsh.* **1929**, *61*, 386.

57 Badhwar, I, C.; Baker, W.; Menon, B . K.; Venkatraman, K. *J. Chem. Soc.* **1931**, 1541.

58 Smith, L. I.; Dobrovolny, F. J. *J. Am. Chem. Soc.* **1926**, *48*, 1693.

59 Smith, L. I.; Austin, F . L. *J. Am. Chem. Soc.* **1942**, 64, 528.

60 Smith, L. I.; Byers, D. J. *J. Am.Chem. Soc.* **1941**, *63*, 612.

61 Smith, L. I.; Denyes, R .O . *J . Am. Chem. Soc.* **1936**, *58*, 304.

62 Smith, L. I.; Nichols, J. *J. Am. Chem. Soc.* **1943**, *65*, 1739.

63 Chakravarti, D.; Majumdar, B. *J. Indian Chem. Soc.* **1938**, *15*, 136; **1939**, *16*, 389.

64 Shriner, R. L. *Org. React.* **1942**, *1*, 1.

65 Bert, L. *Compt. Rend.* **1942**, *214*, 230.

66 Kostanecki, S.; Rozycki, A. *Ber.* **1901**, *34*, 102.

67 Nagai, N . *Ber.* **1892**, *26*, 1254.

68 Tahara, Y. *Ber.* **1892**, *26*,1292.

69 Allan, J.; Robinson, R . *J. Chem. Soc.* **1924**, 2192.

70 Cairns, N.; Harwood, L. M.; Astles, D. P. *J. Chem. Soc. Perkin Trans. 1* **1994,** 3101.

71 (a) Maercker, A. *Org. Synth.* **1934**, *14*, 270. (b) Wittig, G.; Hagg, W. *Chem. Ber.* **1955**, *88*, 1654. (c) Yavari, I.; Hekmat-shoar, R.; Zonouzi, A. *Tetrahedron Lett.* **1998**, *39*, 2391. (d) Shockravi, A.; Valizadeh, H.; Heravi, M. M.; Ghadim, H. A. *J. Chem. Res. (S).* **2003**, 718.

72 Saidi, M. R.; Bigdeli, K. *J. Chem. Res. (S)*. **1998**, 800.

73 Van, T. N.; Debenedetti, S.; De Kimpe, N. *Tetrahedron Lett.* **2003**, *44*, 4199.

74 Chatterjee, A. K.; Toste, F. D.; Goldberg, S. D.; Grubbs, R. H. *Pure Appl. Chem.* **2003**, *75*, 421.

75 Polito, L.; Cravini, M.; Poletti, L.; Lay, L. *Synth. Commun.* **2006**, *36*, 2203.

76 a) Christie, R.M. *Review of Progress in Coloration.* **1993,** *23*, 1.

b) O'-Kennedy, R. *Coumarins;* John Wiley: New York, 1997.

77 Yoneda, E.; Sugioka, T.; Hirao, K.; Zhang, S. W.; Takahashi, S. *J. Chem. Soc. Perkins Trans. I.* **1998**, 477.

78 (a) Zahradnik, M. *The Production and Application of Fluorescent Brightening Agents,* Wiley & Sons, **1992**. (b) Rao, C. P.; Krupadanam, G. L. D.; Srimannarayanna, G.; *Ind. J. Chem. Sec. B.* **1991**, *30*, 666. (c) Majumdar, K. C.; Chatterjee, P. *J. Chem. Res. (S).* **1996**, 462.

79 Kuznetsova, N. A.; Kaliya, O. L. *Russ. Chem. Rev.* **1992**, *61(7)*, 683.

80 Musgrove, E.; Rugg, C.; Hedley, D. *Cytometry* **1986**, *7*, 347.

81 Murray, R. D. H.; Mendez, J.; Brown, S. A. *The Natural Coumarins: Occurrence, Chemistry and Biochemistry;* Wiley: New York, **1982**.

82 Nowakowska, M.; Smoluch, M.; Sendor, D. *J. Inclusion Phenom. Macrocyclic Chem.* **2001**, *40*, 213.

83 Some current references: (a) Curini, M.; Cravotto, G.; Epifano, F.; Giannone, G. *Curr. Med. Chem.* **2006**, *13*, 199. (b) Borges, F.; Roleira, F.; Milhazes, N.; Santana, L.; Uriarte, E. *Curr. Med. Chem.* **2005**, *12*, 887. (c) Fylaktakidou, K. C.; Hadjipavlou-Litina, D. J.; Litinas, K. E.; Nicolaides, D. N. *Curr. Pharm. Design.* **2004**, *10*, 3813. (d) Xie, L.; Takeuchi, Y.; Cosentino, L. M.; McPhail, A. T.; Lee, K. -H. *J.Med.*

Chem. **2001**, *44*, 664. (e) Yang, Z. Y.; Xia, Y.; Xia, P.; Brossi, A.; Cosentino, L. M.; Lee, K.-H. *Bioorg. Med. Chem. Lett*. **2000**, *10*, 1003.

84 (a) Hermodson, M. A.; Barker, W. M.; Link, K. P. *J. Med. Chem.* **1971**, *14*, 167. (b) Conlin, G. M.; Gear, J. R. *J. Nat. Prod.* **1993**, *56(8)*, 1402. (c) *Chem. Abstr.* **1981**, *95*, 1914e.

85 (a) Periers, A. M.; Laurin, P.; Benedetti, Y.; Lachaud, S.; Ferroud, D.; Iltis, A.; Haesslein, J. L.; Klich, M.; L'Hermite, G.; Musicki, B. *Tetrahedron Lett.* **2000**, *41*, 867. (b) Peixoto, C.; Laurin, P.; Klich, M.; Dupuis-Hamelin, C.; Mauvais, P.; Lassaigne, P.; Bonnefoy, A.; Musicki, B. *Tetrahedron Lett.* **2000**, *41*, 1741.

86 Fuller, R. W.; Bokesch, H. R.; Gustafson, K. R.; McKee, T. C.; Cardellina, J. H.; McMahon, J. B.; Cragg, G. M.; Soejarto, D.D.; Boyd, M. R. *Bioinorg. Med. Chem. Lett.* **1994**, *4 (16)*, 1961.

87 Majumdar, K. C.; Guptha, A. K. *Ind. J. Chem. Sec. B.* **1990**, *29*, 752.

88 Bennett, F. A.; Barlow, D. J.; Dodoo, A. N. O.; Hider, R C.; Lansley, A. B.; Lawrence, M. J.; Marriott, C.; Bensal, S. S. *Tetrahedron Lett.* **1997**. *38(42)*, 7449.

89 Ferrer, J. M.; Leiton, M. J.; Zaton, A. M. L. *J. Protein Chem.* **1998**, *17(2)*, 115.

90 Sastry, S. *Biophys. Chem.* **2001**, *91*, 191.

91 Gallastegui, D. J.; Lago, J. M.; Palomo, C. *J. Chem. Res.(s)*, **1984**, 170.

92 Mashraqui, S. H.; Vashi, D.; Mistry, H. D. *Synth. Commun.*, **2004**, *34*, 3129.

3.1 Introduction

3.1.1. Introduction to Photochemistry

During the last 25 years there has been a remarkable growth in the use of fluorescence in biological sciences- biochemistry and biophysics. Fluorescence also finds application in environmental monitoring, clinical chemistry, DNA sequencing and genetic analysis by fluorescence *in situ* hybridization (FISH). In molecular biology, fluorescence is used for cell identification and sorting in flow cytometry, and in cellular imaging to reveal the localization and movement of intracellular substances by means of fluorescence microscopy. Because of the high sensitivity of fluorescence detection, expense and difficulties of handling radioactive substances, there is continuing development of medical tests based on the phenomenon of fluorescence. These tests include the widely used enzyme linked immunoassays (ELISA) and fluorescence polarization immunoassays.

As a result of light absorption, molecules attain higher singlet energy electronic levels, and in most cases this is accompanied by adjustment to a new equilibrium between the molecule and its environment. Deactivation of the excited molecule can take place either by the emission of fluorescence quanta (or, after inter-system crossing to triplet levels, phosphorescence) or by non-radiative energy dissipation to the solvent. Transitions between energy states occur in about 10^{-15} s, a time too short for significant displacement of nuclei. This is the Franck-Condon principle.

The luminescent property of a molecule is mainly determined by the measurement of absorption wavelength (λ_A), emission wavelength (λ_F), quantum yield (ϕ_F), fluorescent decay lifetime (τ_F), radiative (k_R) and non-radiative decay (k_{NR}) constants. The fluorescence life time and quantum yields are the most

important characteristics of a fluorophore. The quantum yield is the number of emitted photons relative to the number of absorbed photons.[1] The quantum yield is found by recording the total number of photons emitted by the fluorescing molecules throughout its fluorescence emission and comparing it with the number of photons absorbed.[2] Thus if I_f is the fluorescence intensity and I_a is the intensity of the absorbed light, the quantum yield is then defined as

$$\phi_F = I_f/I_a \quad \text{--------} \ (3.1)$$

The maximum theoretical value of quantum efficiency ϕ is unity, since each quantum absorbed excites only one molecule, which can emit only one quantum of light on emission while decaying to the ground state. Since the quantum of light re-emitted is generally of longer wavelength than the one absorbed, the amount of energy re-emitted even at $\phi = 1$ is less than the amount absorbed, and the excess energy in transformed into heat. The quantum yield for most molecules decreases with increasing temperature.

The lifetime of the excited state is also an important photophysical parameter of any fluorophores as it determines the time available for the fluorophore to interact with or diffuse in its environment, and hence the information available from its emission. The lifetime of the fluorophore in the absence of non-radiative process is called the intrinsic or true life time, τ_F^0 .The intrinsic radiative life time τ_F^0 of a state is the reciprocal of the rate constant for the disappearance of this state if emission were the only path of energy dissipation.

$$\text{i.e., } k_R = 1/\tau_F^0 \quad \text{---------} \ (3.2)$$

where k_R is the radiative decay constant. However for most molecules measured fluorescence lifetime τ_F, that is the mean lifetime is always less than τ_F^0. The relationship between the two life times is given by

$$\tau_F^0 = \tau_F / \phi_f \quad \text{----------} \quad (3.3)$$

Where ϕ_f is the fluorescence quantum yield

Substituting in (3.2)

$$k_R = \phi_f / \tau_F \quad \text{----------} \ (3.4)$$

k_{NR}, the non radiative decay constant, can be related to the k_R and τ_F by the equation;

$$k_{NR} = k_{IC} + k_{ISC} + k_Q = (1- \phi_f) / \tau_F = \tau_F^{-1} - k_R \quad \text{----------} \ (3.5)$$

Where k_{IC}, k_{ISC} and k_Q the decay constants due to internal conversion, intersystem crossing and quenching due to other mechanisms respectively.

The lifetime of the lowest singlet excited state, τ_F is of importance in many applications. For strongly forbidden transitions, the lifetime is longer (10^{-3} s or more). Observed lifetime as noted before are generally less than the calculated values because of the other competing processes. Evidently, for molecules which do not fluoresce, the deactivation take place by other processes in a period less than 10^{-6} to 10^{-9} s.

3.1.2. Time-Correlated Single-Photon Counting (TCSPC)

At present almost all time-domain measurements are performed using TCSPC. Several comprehensive monographs dealing with TCSPC have appeared.[3-6] The insightful monograph of Ware[6] clearly describes the concept of

TCSPC, and anticipated many of its present applications. These instruments use high-repetition-rate picosecond or femtosecond laser light sources and high-speed MCP PMTs.

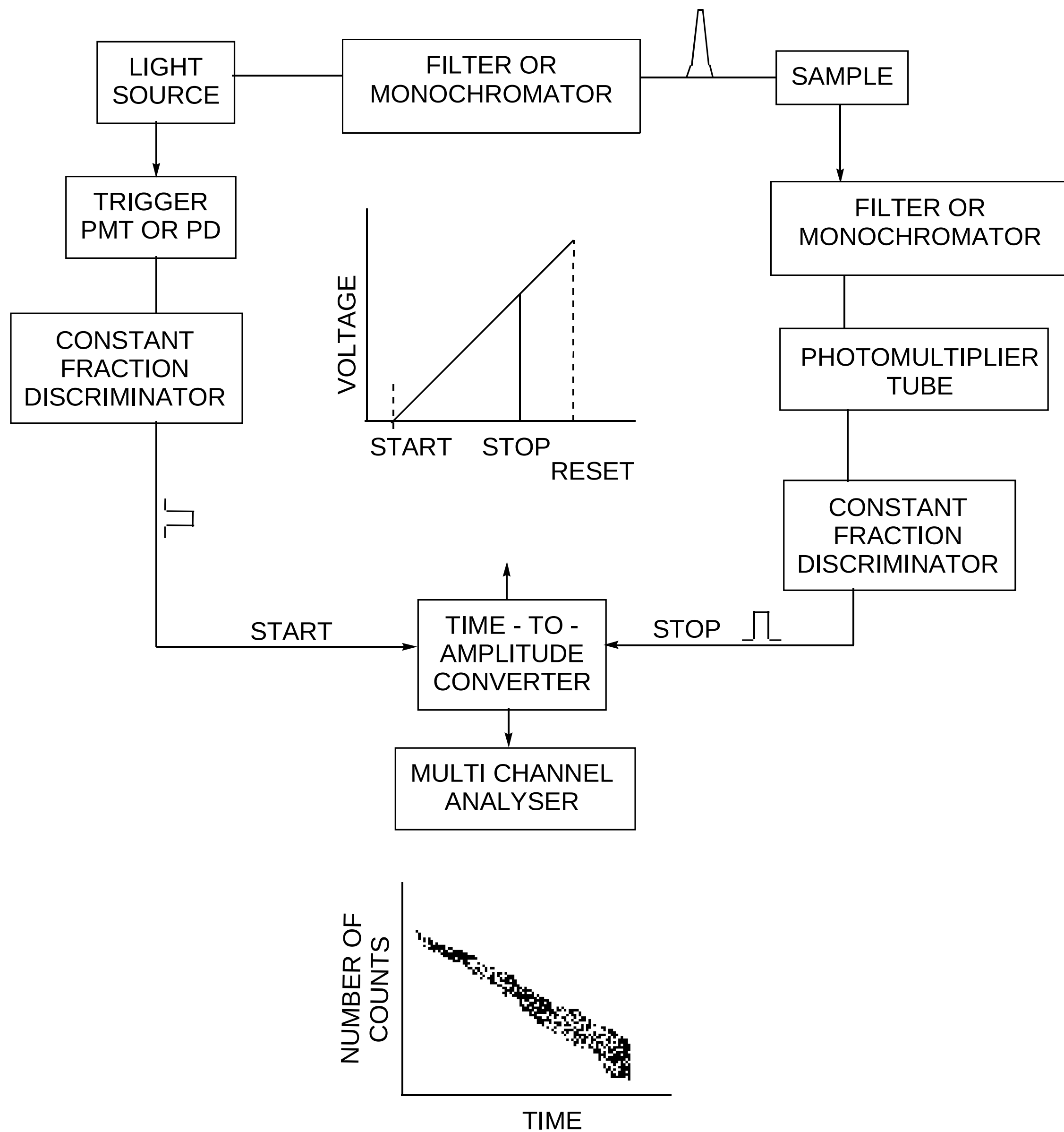
3.1.3. Principles of TCSPC

TCSPC is a digital technique for counting photons, which are time-correlated in relation to the excitation pulse (Figure 3.1). The heart of the method is a time-to-amplitude converter (TAC), which can be considered to be analogous to a fast stopwatch. The sample is repetitively excited using a pulse light source, often from a laser or flash lamp. Each pulse is optically monitored, by a high-speed photodiode or photo multiplier, to produce a start signal, which is used to trigger the voltage ramp of the TAC. The voltage ramp is stopped when the first fluorescence photon from the sample is detected. The TAC provides an output pulse whose voltage is proportional to the time between the start and stop signals. A multichannel analyzer (MCA) converts this voltage to a time channel using an analog-to-digital converter (ADC). Summing over many pulses, the MCA builds up a probability histogram of counts versus time channels. The experiment is continued until one has collected more than 10,000 counts in the peak channel. There can be no more than one photon detected per 100 laser pulses. Under these conditions, the histogram of photon arrival times represents the intensity decay of the sample.

Another important feature of TCSPC is the use of the rising edge of the photoelectron pulse for timing. This allows phototubes with nanosecond pulse widths to provide sub nanosecond resolution. This is possible because the rising edges of the single photon pulses are usually steeper than one would expect from the time response of the PMT. Also, the use of a constant fraction discriminator

provides improved time resolution by removing the variability due to the amplitude of each pulse.

Figure 3.1. Schematic block diagram for TCSPC.



3. 2. Review of Literature

Coumarin derivatives are the subject of photophysical studies during the last few decades as they are highly fluorescing moieties. The nature and position of the substituents on coumarin ring has profound importance in deciding the photophysical behaviour of the substituted coumarin compounds.

Coumarins substituted at 7-position with an electron donating group are known to exhibit strong fluorescence.[7] Since 7-aminocoumarins are highly fluorescent, they have been used as optical brighteners and fluorescent probes. Substituted 7-aminocoumarins also form an important class of laser dyes for the blue-green region. The photophysical properties of these compounds depend on the nature and position of a substituent group in the parent molecule and also change due to a change in the surrounding media. Coumarins are used as non-linear optical chromophores and as excellent probe to study solvation dynamics in the homogeneous solutions as well as organized media.[8-13] In the recent past, numerous coumarin heterodimers have been synthesized and explored the possibility of their applications as laser dyes[14] as organic scintillators[15] and as triplet sensitizers.[16,17] In a series of earlier works the effect of solvents, substituents and temperature on the various photophysical properties of coumarin compounds have been reported.[13,18-21] It is found that the nature of solvents and substituents brings about a change in the values of fluorescence wavelength maxima, quantum yield, lifetime, polarization and excited state dipole moment of the coumarins. A systematic study of fluorescence quenching of 4- and 7- substituted coumarins by halide ions in aqueous media have also been studied in detail.[22] A series of 2H-pyrano[3,2-c]chromen-5-one derivatives were synthesized, characterized and their photochromic and redox properties were investigated by the UV–Vis absorption spectroscopy recently.[23]

The influence of the alkoxy substituent at position 7 and alkyl group at 4 of the coumarins have been investigated by Diehl *et al.*[24] and the spectroscopic properties of 7-dialkylamino and 3-styryl substituted coumarins have been studied by Raju et al.[25] The solvent effect on the absorption and fluorescent spectra of some 6-alkylamino-7-alkyl coumarin derivatives have been reported recently.[26] The coumarins with bulky groups such as phenyl, phenylthio, benzylthio etc. substituted at position 3 were spectroscopically analyzed in solvents of different viscosity and polymer matrices.[12a] The absorption-emission properties, fluorescent decay, quantum yield and other photophysical parameters and their dependance on concentration[27] , polarity and viscosity of the solvents and effect of various substituents on some biscoumarins [28], cyclopenta coumarins[18] amino coumarins [29], etc. have been reported in the preceding years.

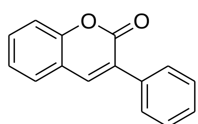
3.3. Results and discussions

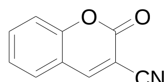
In the present work an array of coumarin derivatives have been subjected to photophysical analysis. All these molecules possess the fluorescent property. Hence our interest was to exploit this property of coumarins to find application in industries and biological field. Nowadays coumarins are used in dye-laser techniques, cell imaging, transcription assays, intrinsic probe for labeling peptides etc. To know the applicability based on fluorescence of any molecule, one should know the photophysical parameters and the factors governing it.

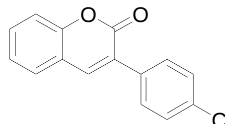
The aim of the present study is to determine the basic spectroscopic and photophysical data for the compounds given in Figure 3.2. The photophysical constants of the molecule $\phi_{F,}$ $\tau_{F,}$ k_R and k_{NR}, and also the absorption spectrum (as expressed by the molar absorption coefficient, ε_A) and the fluorescence spectral distribution normalized to the quantum yield, form a basic set of data characterizing a luminescent molecule. The determination of these parameters of

the newly synthesized coumarin derivatives and its variation in the presence and absence of some functional group is the aim of the present investigation. A comparison of these data of the new compounds with that of the known compounds (whose photo-physical studies are not extensively carried out) reveals some interesting results.

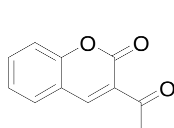
Figure 3.2. Substituted coumarin derivatives under study.

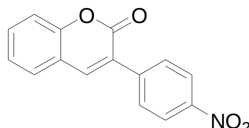


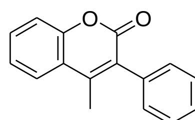


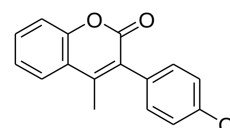


C 11 **C 12** **C 13**

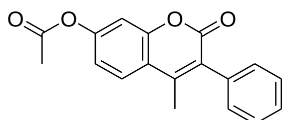


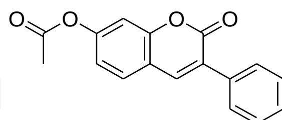


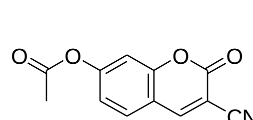


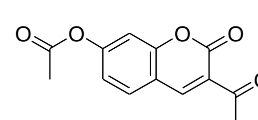


C 15 **C 18** **C 21** **C 23**









C 31 **C 41** **C 42** **C 45**

In the present study, the optical properties of newly synthesized coumarin derivatives have been investigated in detail by measuring the UV/Vis absorption, steady state and time resolved fluorescence. From these studies we have calculated the photophysical parameters like extinction coefficient (ε_A), quantum yield (ϕ_F), lifetime values (τ_F) radiative (k_R) and non-radiative decay constants (k_{NR}). An analysis on the correlation between the optical properties and structural characteristics of newly synthesized coumarin derivatives explores some interesting aspects.

3.3.1 Absorption and Emission Properties of Coumarin Derivatives

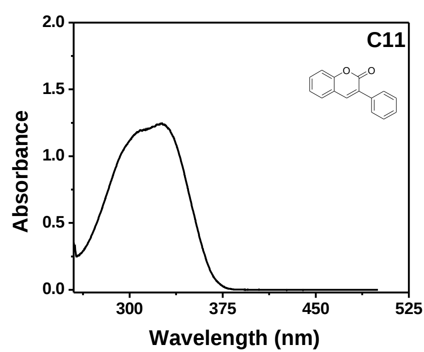
All the measurements have been made in the polar solvent, dimethyl sulfoxide, as it is the only solvent in which all the compounds are soluble. Absorption spectra are less sensitive to solvent polarity than emission spectra. Absorption of light occurs in about 10^{-15} s, a time that is too short for motion of the fluorophore or solvent. Absorption spectra are not affected by the decrease in the excited-state energy, which occurs after absorption has occurred. The absorption maxima (λ_A) and the molar extinction coefficient (ε_A) and absorption spectra are shown in Table 3.1 and Figure 3.3 respectively.

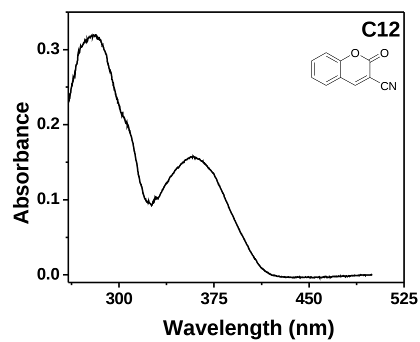
The effects of solvent and environment on fluorescence spectra are complex. Spectral shifts result from the general effect of solvent polarity whereby the energy of the excited state decreases with increasing solvent polarity. However, spectral shifts also occur due to specific fluorophore-solvent interactions and due to charge separation in the excited state. Emission from fluorophores generally occurs at wavelengths, which are longer than those at which absorption occurs. This loss of energy is due to a variety of dynamic processes, which occur following light absorption.

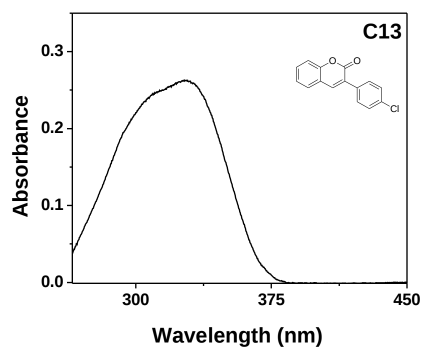
Table 3.1. Photophysical parameters of coumarin derivatives.

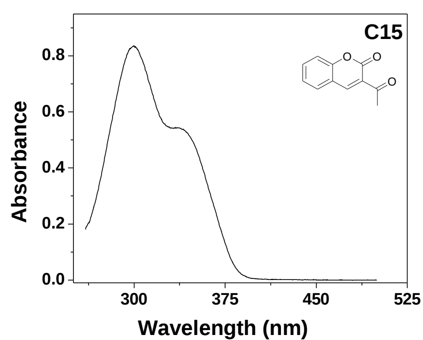
Entries	λ_A (nm)	ε ($M^{-1}cm^{-1}$)	λ_F (nm)	Stokes' Shift, μ_s (cm^{-1})	ϕ_F	τ_{av} (ns)	K_R (10^7 s^{-1})	K_{NR} (10^7 s^{-1})
C11	304	11601	404	6922	0.06	0.95	6.315	98.94
	326	12431	421			1.36	4.411	95.59
C12	358	1573	435	4944	0.006	3.74	0.160	99.83
C13	326	14938	405	5984	0.153	0.503	30.417	168.39
	304	13300	423			0.413	37.046	205.08
C15	300	9800	435	6862	0.003	3.66	0.082	27.24
	345	6200						
C18	343	19657	435	6166	0.002	2.475	0.081	40.32
C21	285	11600	406	7115	0.003	3.09	0.104	32.25
	317	9980						
C23	286	14600	403	6932	0.007	0.975	0.718	101.85
	319	12500						
C31	288	14136	431	8344	0.006	2.56	0.234	38.83
	317	11900						
C41	328	13400	428	7124	0.25	2.22	11.261	33.78
C42	298	7700	408	9048	0.024	0.8	3.00	122.00
	329	6800						
C45	301	8600	466	11764	0.04	3.25	1.231	29.53
	339	8400						

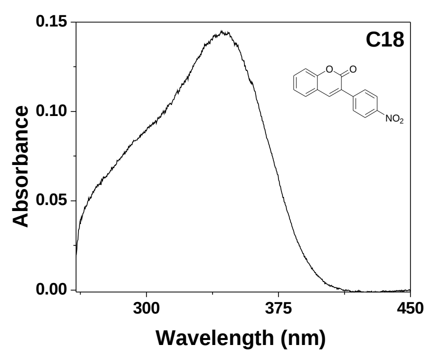
Figure 3.3a. Absorption spectra of coumarin derivatives.











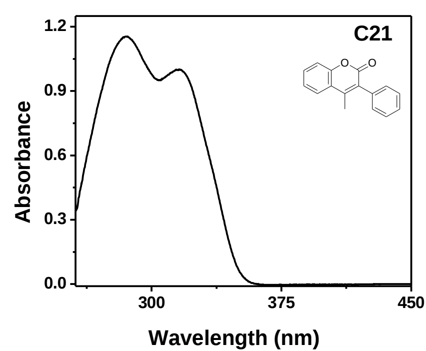
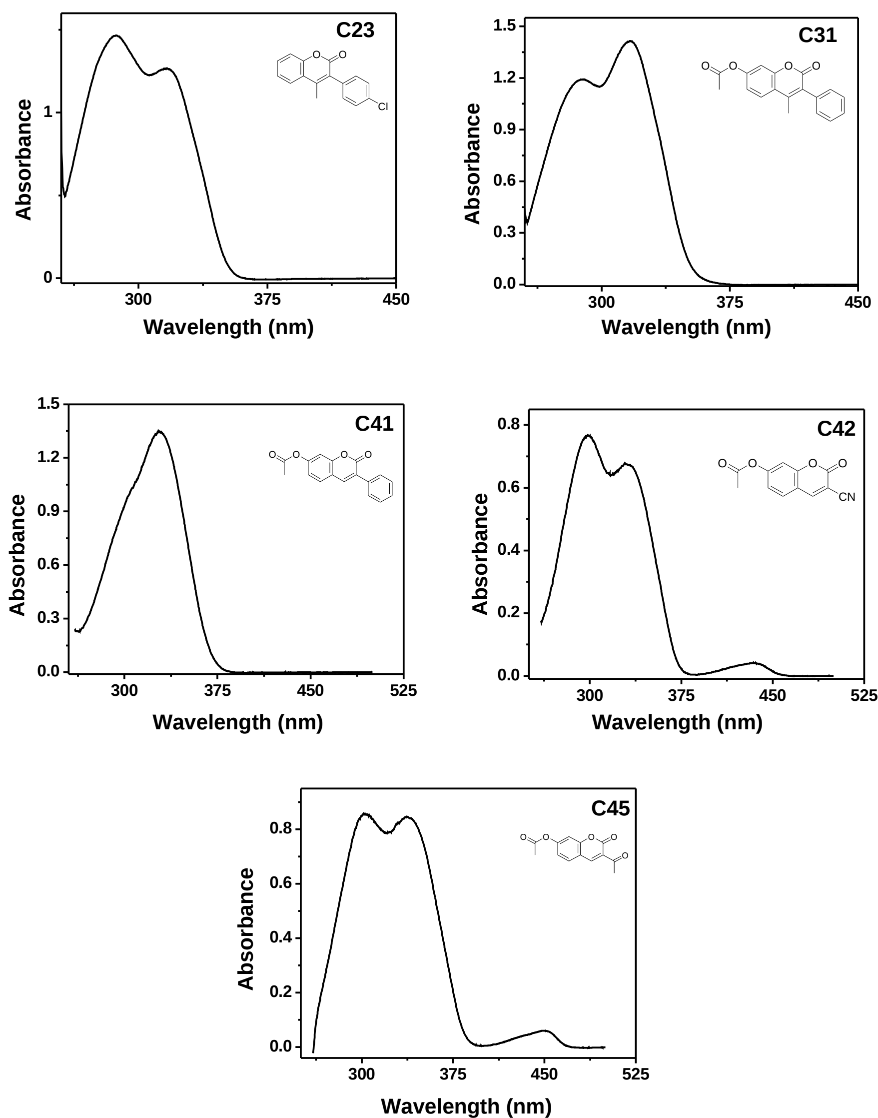


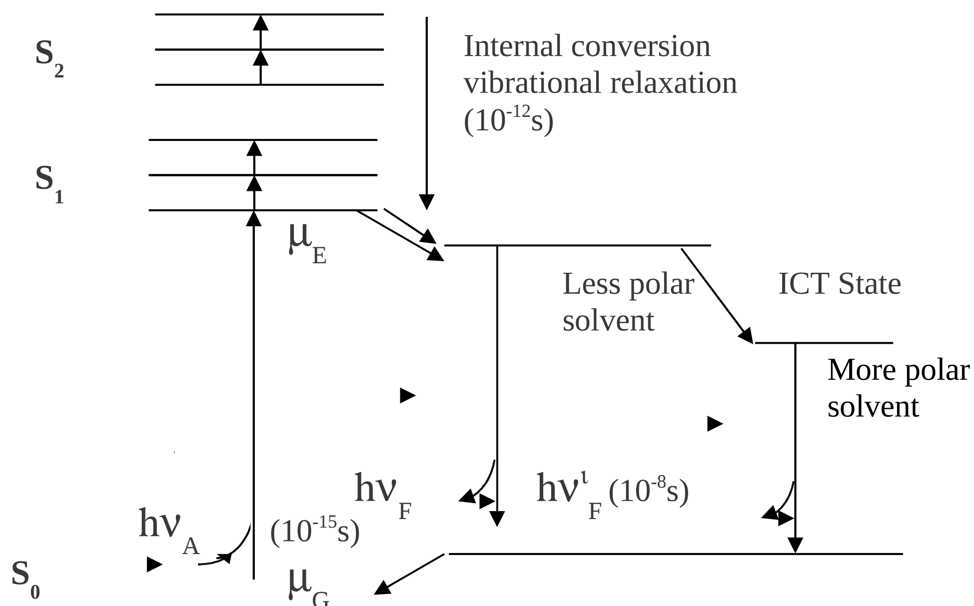
Figure 3.3b. Absorption spectra of the coumarin derivatives.



The fluorophore is typically excited to the first singlet state (S_1), usually to an excited vibrational level within S_1. The excess vibrational energy is rapidly lost to the solvent. If the fluorophore is excited to the second singlet state (S_2), it

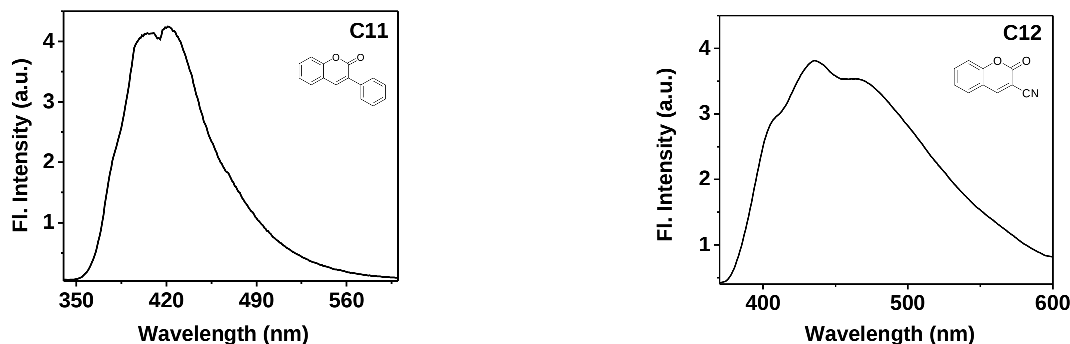
rapidly decays to the S_1 state in 10^{-2} s due to internal conversion. Solvent effects shift the emission to still lower energy owing to stabilization of the excited state by the polar solvent molecules (Figure 3.4). Typically, the fluorophore has a larger dipole moment in the excited state (μ_E) than in the ground state (μ_G). Following excitation, the solvent dipoles can reorient or relax around μ_E, which lowers the energy of the excited state. As the solvent polarity is increased, this effect becomes larger, resulting in emission at lower energies or longer wavelengths.

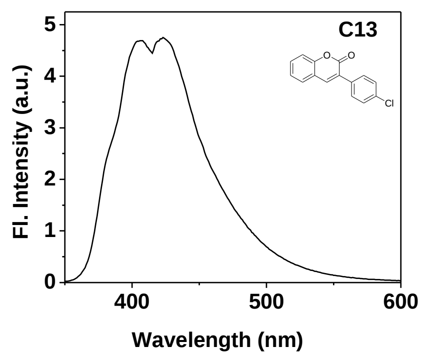
Figure 3.4. Jablonski diagram for fluorescence with solvent relaxation.



The coumarins are itself polar in nature and hence display large sensitivity to the solvent polarity. Solvent polarity and the local environment have profound effects on the emission spectra of polar fluorophores. Literatures show that the substituted coumarin molecules exhibit solvatochromic effect in different solvent with varying polarity due to the intramolecular charge transfer (ICT). In the current investigation the solvatochromic effect of all the coumarin derivatives in different solvents could not be studied as the highly polar dimethyl sulfoxide (DMSO) is the only solvent in which all the compounds are soluble. The emission maxima ((λ_F) and emission spectra are shown in Table 3.1 and Figure 3.5 respectively.

Figure 3.5a. Emission spectra of coumarin derivatives.





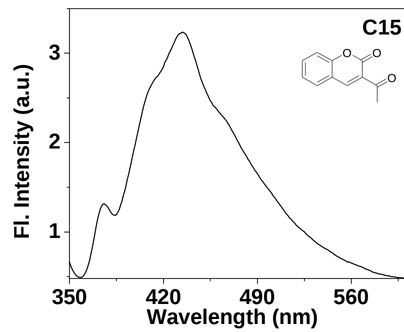
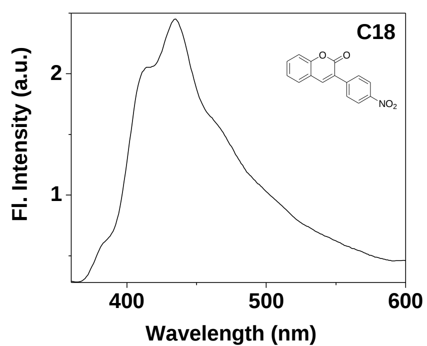
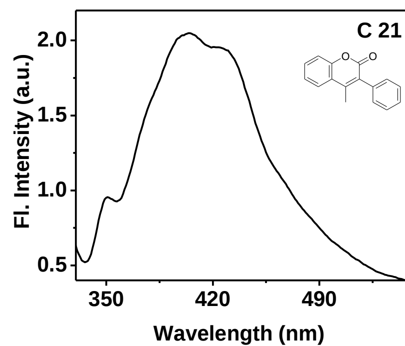
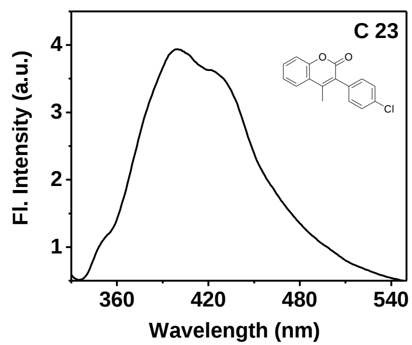
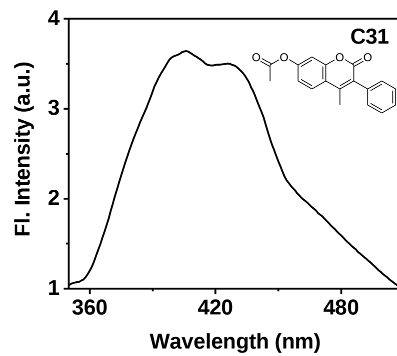


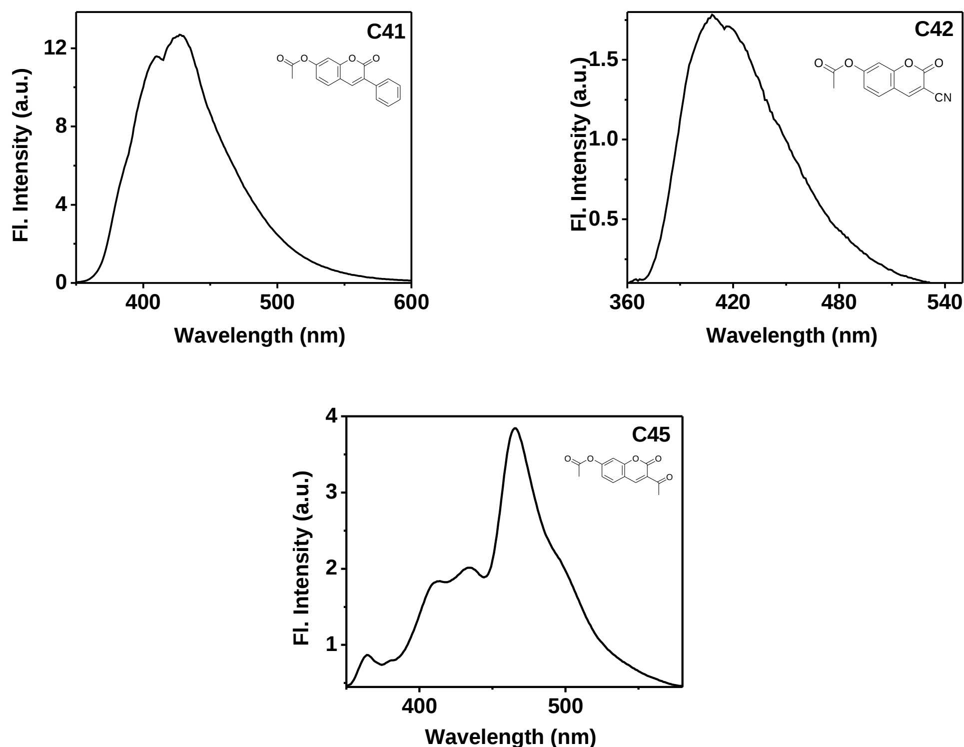
Figure 3.5b. Emission spectra of coumarin derivatives.











An interesting consequence of emission to higher vibrational ground states is that the emission spectrum is usually a mirror image of the absorption spectrum of the S_0 to S_1 transition. This similarity occurs because electronic excitation does not greatly alter the nuclear geometry. Hence the spacing of the vibrational energy levels of the excited state is similar to that of the ground state. As a result, the vibrational structures seen in the absorption and emission spectra are similar. Observations of the combined absorption-emission spectra of the coumarin derivatives (**Figure 3. 6**) reveal that except few most of the molecules hold the mirror image rule. Although the mirror image rule is obeyed, an exact symmetry in spectra is not seen. It may be due to a faintly distorted geometry in the excited state. The generally symmetric nature of these spectra is a result of the same transition is being involved in both the absorption and emission and the similarities of the vibrational energy levels of S_0 and S_1.

Figure 3.6a. Combined absorption-emission spectra of coumarin derivatives.

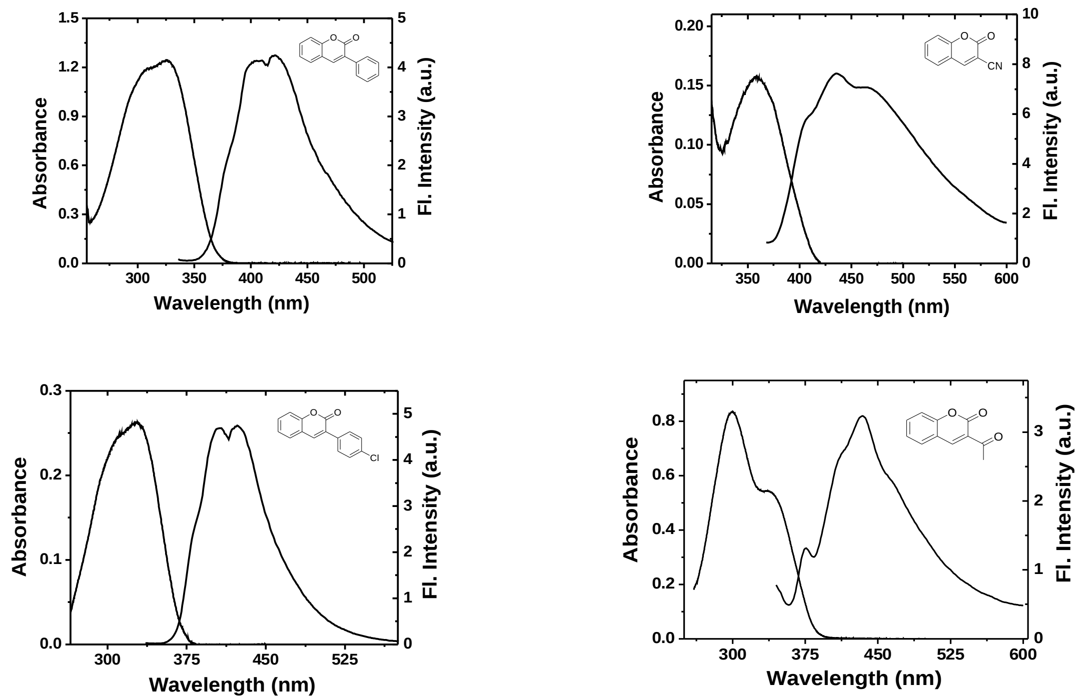
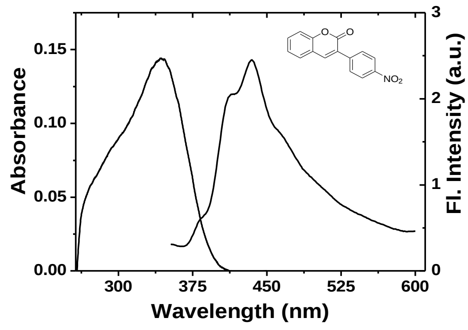
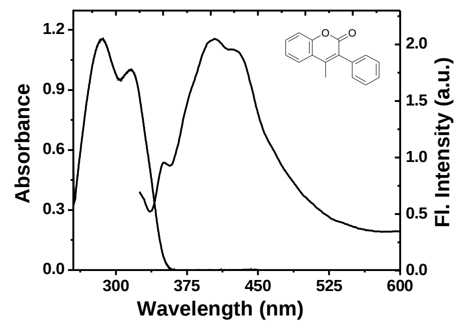
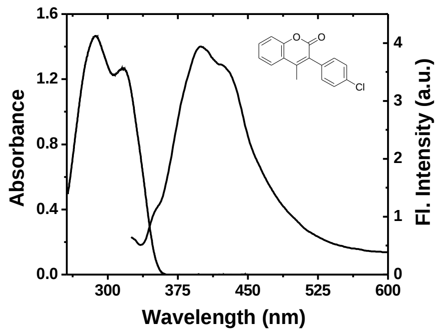
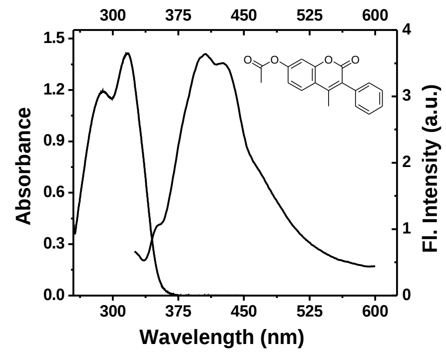


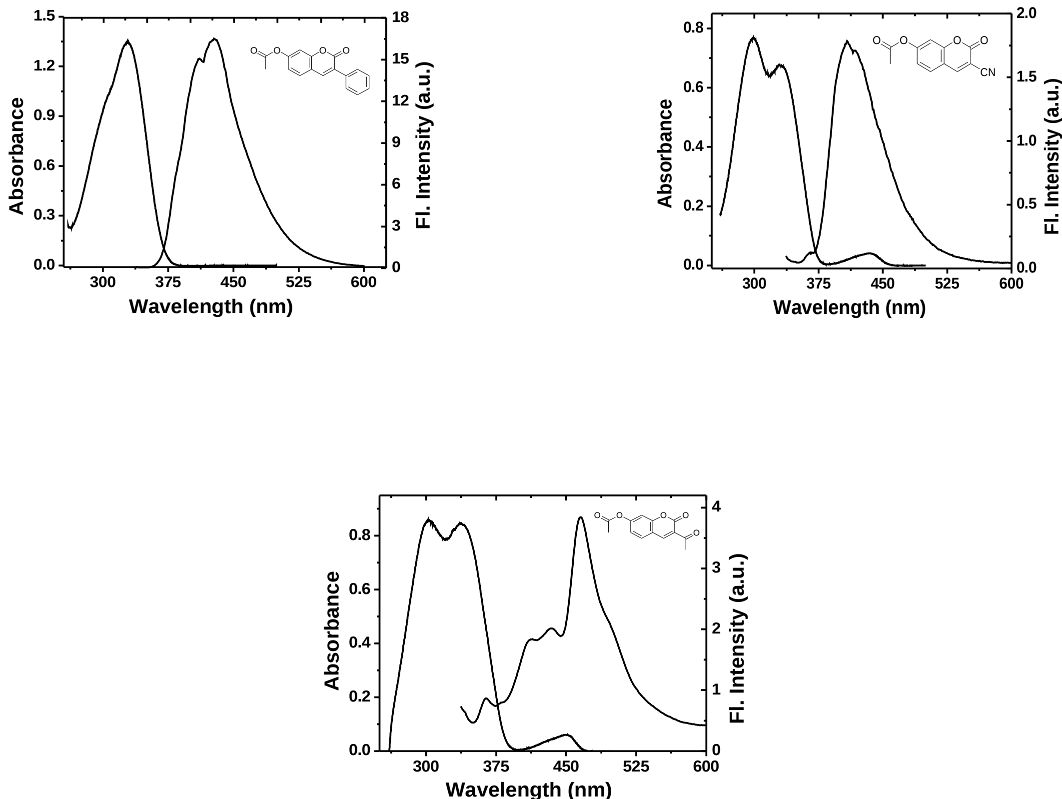
Figure 3.6b. Combined absorption-emission spectra of coumarin derivatives.


Absorbance
Fl. Intensity (a.u.)
Wavelength (nm)
NO2


Absorbance
Fl. Intensity (a.u.)
Wavelength (nm)


Absorbance
Fl. Intensity (a.u.)
Wavelength (nm)
Cl


Absorbance
Fl. Intensity (a.u.)
Wavelength (nm)



The deviation from the mirror image rule in the case of few molecules (C12, C45 etc.) indicates a different geometric arrangement of the nuclei in the excited state as compared to the ground state.

3.3.2 Stokes' Shift

Energy losses between excitation and emission are observed generally for fluorescent molecules in solution. One cause of Stokes' shift is the rapid decay to the lowest vibrational level of $S_{1.}$ Furthermore, fluorophores generally decay to higher vibrational levels of S_0, resulting in further loss of excitation energy by thermalisation of the excess vibrational energy. In addition to these effects, fluorophores can display further Stokes' shifts due to solvent effects, excited-state reactions, complex formation, and/or energy transfer. Some of the dynamic

processes in solution involve fluorophore-solvent interactions and rotational diffusion. As was observed by Stokes, most fluorophores display emission at lower energies than their absorption. Rotational motions of small solvent molecules in fluid solution are rapid, typically occurring on a timescale of 40 ps or less. The relatively long timescale of fluorescence allows ample time for the solvent molecules to reorient around the excited-state dipole, which lowers its energy and shifts the emission to longer wavelengths. This process called solvent relaxation occurs in 10^{-10}s in fluid solution. It is these differences between absorption and emission that result in the high sensitivity of emission spectra to solvent polarity, and the smaller spectral changes seen in absorption spectra. Solvent relaxation can result in substantial Stokes' shifts. Thus one of the reasons for high Stokes' shift values observed for the coumarin derivatives are ascribed to the polarity of the solvent, DMSO. The Stokes' shift values calculated for all the coumarin derivatives are represented in Table 3.2. The Stokes' shift values are expressed in wavenumber and are calculated from the experimental parameters by taking the difference between absorption and emission maxima expressed in wave number using the equation,

$$\text{Stokes' Shift } \eta_S = (\bar{\upsilon}_A - \bar{\upsilon}_F) \times 10^{7} \text{ cm}^{-1},$$

$$\text{Where, } \bar{\upsilon}_A = 1/\lambda_A \text{ (nm) and}$$

$$\bar{\upsilon}_F = 1/\lambda_F \text{ (nm)}$$

Table 3.2. Stokes' shift values of coumarin derivatives.

Compounds	Stoke's Shift; Wave number, cm^{-1}
C11	6922
C12	4944
C13	5984
C15	6862
C18	6166

C21	7115
C23	6932
C31	8344
C41	7124
C42	9048
C45	11764

3.3.3. Quantum Yield

Quantum yield value of a molecule is a direct measure of its luminescent property. If the absolute fluorescence efficiency of one substance (reference ϕ_r) is known, then that of the other (sample ϕ_s) can simply be calculated. Quinine sulfate in dilute H_2SO_4 (0.1 M) has been used as a standard substance for quantum yield measurements. The quantum yield value of the solution of quinine sulfate in 0.1 M H_2SO_4 is determined as 0.546 and hence the ϕ values of the coumarin derivatives are calculated knowing the other parameters. The values of ϕ_s are determined by the measurements of the area under the fluorescence curve (which have been suitably corrected for instrumental factors) using the equation;

$$\phi_s = \phi_r [A_r F_s / A_s F_r] [\eta_s^2 / \eta_r^2]$$

Where ϕ_r is the quantum yield of the reference (ϕ_r = 0.546). A_r and A_s are the absorbance of the 'reference standard' and 'sample' respectively at the excitation wavelength, F_r and F_s are the relative integrated fluorescent intensities (peak area) of the reference and samples respectively and η_r and η_s are respectively the refractive indices of the solvents in which the reference standard and samples are prepared. The measured values of the parameters and the calculated quantum yield value for all the coumarin derivatives have been tabulated in Table 3.3.

Table 3.3. Quantum yield values of coumarin derivatives.

ϕ_r = 0.546; η_s = 1.4785; η_r = 1.33

Compound	A_r	A_s	F_r	F_s	ϕ_S
C11	0.108	0.104	4.63461×10^8	3.67907×10^7	0.06
C12	0.109	0.116	5.09399×10^8	7.58255×10^6	0.006
C13	0.108	0.123	3.01383×10^8	7.78622×10^7	0.153
C15	0.111	0.116	3.97736×10^8	1.92447×10^6	0.003
C18	0.108	0.118	4.23774×10^8	1.62761×10^6	0.002
C21	0.110	0.112	2.26758×10^8	1.08984×10^6	0.003
C23	0.110	0.126	2.26758×10^8	2.84662×10^6	0.007
C31	0.110	0.139	2.26758×10^8	2.78665×10^6	0.006
C41	0.099	0.125	2.96364×10^8	1.36624×10^8	0.25
C42	0.099	0.114	2.96364×10^8	1.19625×10^7	0.02
C45	0.111	0.118	3.97736×10^8	2.42806×10^6	0.04

A general trend of decrease in quantum yield value is observed for all these compounds in DMSO. This is supposed to be due to the solvent relaxation resulting from the excited state internal charge transfer (ICT) of fluorophores. The high Stokes' shift values of these fluorophores also point towards an ICT emission in highly polar solvents. In addition to specific solvent-fluorophore interactions and an internal charge-transfer (ICT) state formation, the fluorophores can form a twisted internal charge-transfer (TICT) state.[4]

A keen examination of the quantum yield values also reveals some interesting results regarding the influence of substituents on the benzopyrone ring as well as the aromatic ring. The presence of electron withdrawing groups (CN, -$COCH_3$, *p*-NO_2-C_6H_4) at position 3 of the benzopyrone ring, found to decrease the fluorescent quantum efficiency and electron-donating functionalities (phenyl, *p*-Cl-phenyl) cause an increase in ϕ value of the molecules. Another

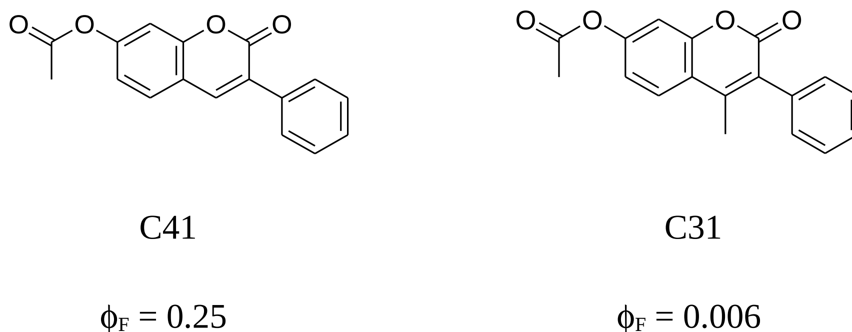
observation that could be made from the study is that an increase in the fluorescent property of the molecule with the presence of an electron donating acetoxy group at position 7 of the coumarin ring. These results are in conjunction with the reported literatures.[13, 24 -25]

3.3.4. Abnormal Photophysical Observation in Presence of Methyl Group

On a perusal of the photophysical values (Table 3.1) of the coumarin derivatives under investigation, the noteworthy observations that can be made is that, when an alkyl group (methyl) is present at position 4 of the benzopyrone ring, the fluorescent property of the compound has diminished to a great extent, which is not in agreement with the early reports. This can be discussed in detail in view of the photophysical properties of the representative compounds C41 and C31, as given below (Figure 3.7).

Consider the compounds C41 and C31. The only difference between these two is the presence of one $-CH_3$ group at position 4. But the quantum yield value of C31 is found to be decreased 40 times to that of the compound C41. The methyl group, which is an electron donating group on the benzopyrone ring of the

Figure 3.7

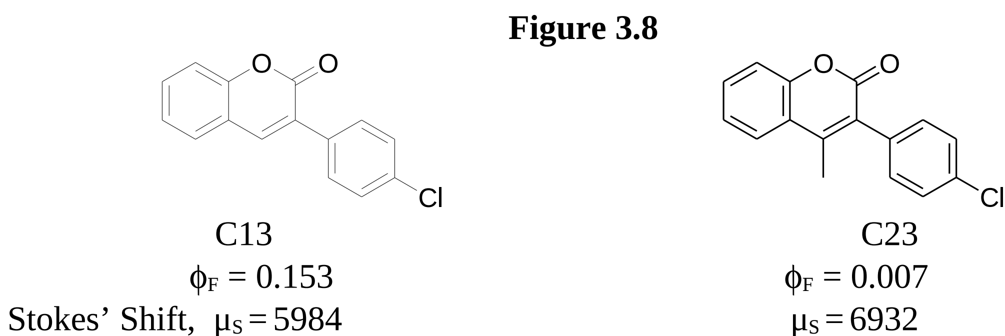


Stokes Shift (cm^{-1}), $\mu_S = 7124$ $\mu_S = 8344$

compound C31 can lead to an enhanced donor- acceptor capability on it compared to C 41 and thus an improved charge transfer (by the formation of a better donor-accepter system). In this better donor-acceptor system C31, the excited state dipole moment of the molecule would be high compared to C41. This may also result to a higher stabilization of the excited state of C31 by solvent relaxation through charge transfer. From the Table 3.2 it is clear that compound C 31 is more red-shifted and has got high Stokes shift value. It is another evidence for the possible ICT due to the donor-acceptor pair formation in the presence of a methyl group. The k_{NR} value for most of the compounds in Table 3.1 are found to have a considerable value, which may be ascribed due to the vibrational relaxation during the charge transfer.

Similarly a marginal decrease in the fluorescent property with the presence of a methyl group could be observed in the case of compounds C13 and C23 also.

Figure 3.8



C13 C23

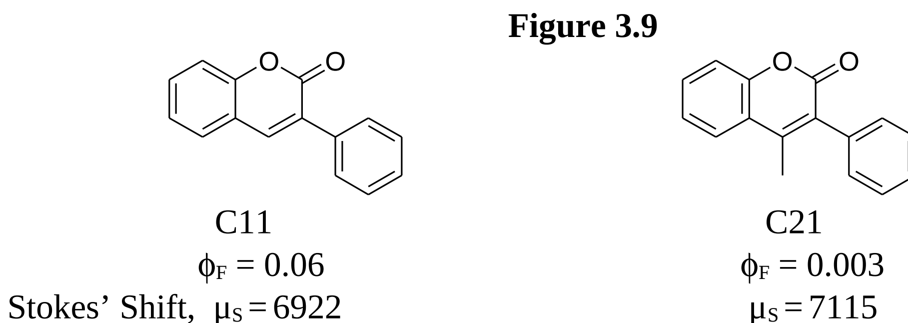
$\phi_F = 0.153$ $\phi_F = 0.007$

Stokes' Shift, $\mu_S = 5984$ $\mu_S = 6932$

Compounds C11 and C21 are considered as less emissive species in DMSO on the basis of the ϕ_F values obtained for them. Even in these low values a well-defined decrease in fluorescence in the presence of methyl group is apparent.

The ϕ_F value of the C21, which is having a methyl group at position 4, is nearly 20 times less than that of the compound C11 that lacks it.

Figure 3.9



3.3.5. Fluorescent Lifetime

The fluorescent lifetime (decay time) of the excited state is defined by the average time the molecule spends in the excited state prior to return to the ground state. The fluorescent decay of all the substituted coumarin derivatives have been measured and plotted in Figure 3.10. From this figure, it is obvious that all the compounds follow a characteristic biexponential fluorescent decay, which reveal the existence of two different emissive states for the molecule, which could be the locally excited state (LE, Franck-Condon state) and charge transfer state (CT). The χ^2 value, which is known as the fitting parameter, determine fine fit for the biexponential decay and is in between 1.0 to 1.3. The average lifetime values are calculated using the equation;[1]

$$\tau_{av} = (\alpha_1\tau_1^2 + \alpha_2\tau_2^2) / (\alpha_1\tau_1 + \alpha_2\tau_2)$$

Where τ_1 and τ_2 are the lifetime values of the two emissive states and α_1 and α_2 are called the pre-exponential factors, which give the abundance of each emissive states. The average lifetime values calculated for each compound are shown in Table 3.4.

Figure 3.10a. Fluorescent lifetime decay plots of coumarin derivatives.

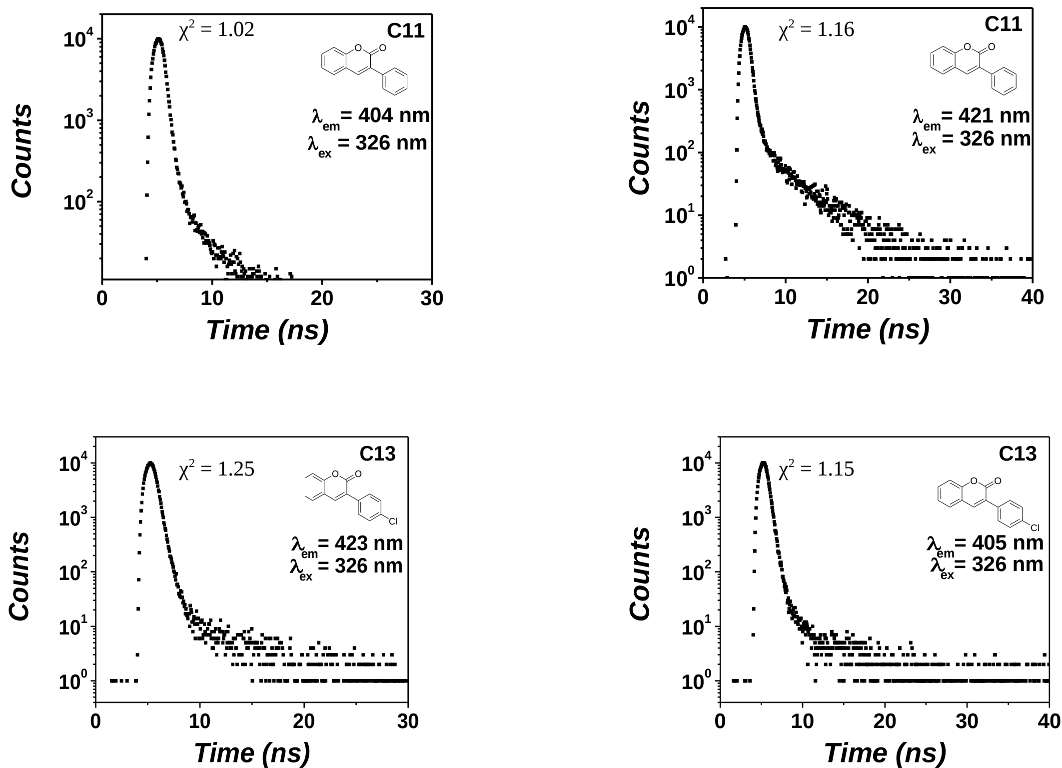
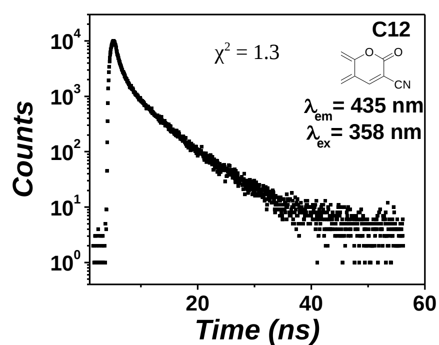
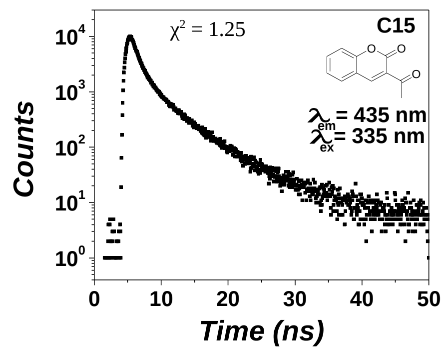
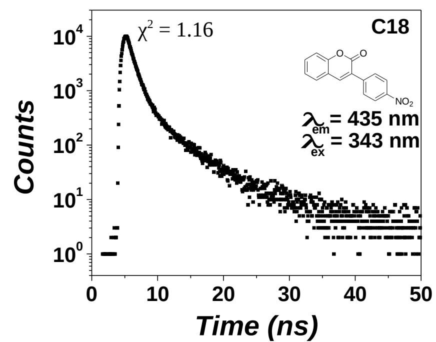


Figure 3.10b. Fluorescent lifetime decay plots of coumarin derivatives.

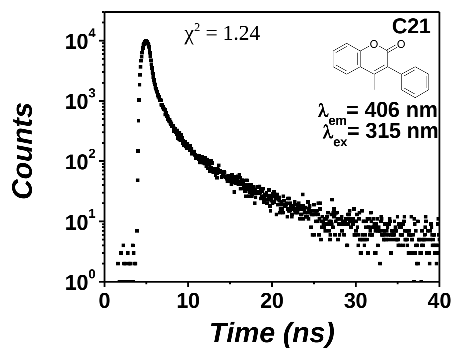




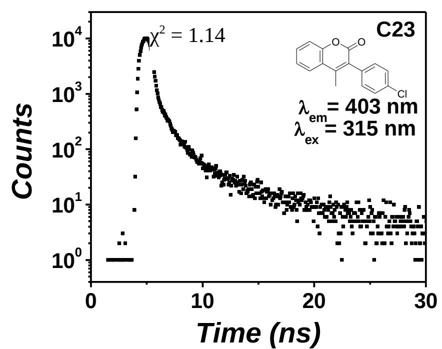
χ² = 1.25
C15
λem = 435 nm
λex = 335 nm
Counts
Time (ns)



χ² = 1.16
C18
λem = 435 nm
λex = 343 nm
Counts
Time (ns)

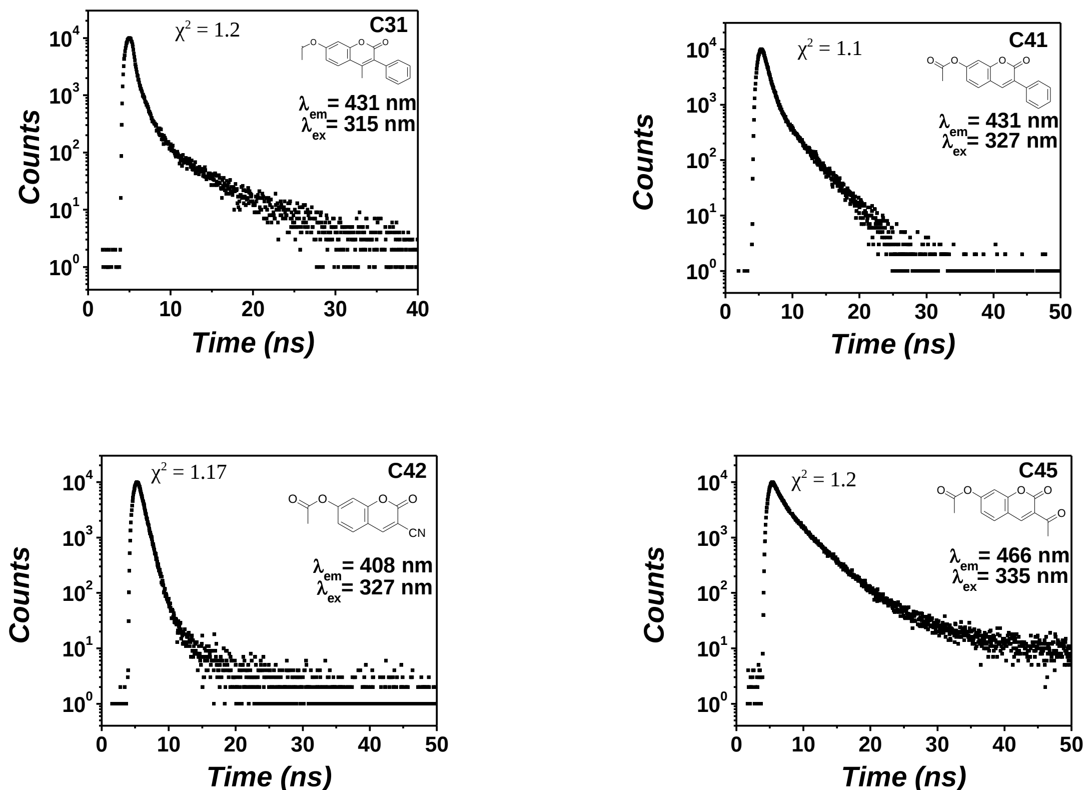


χ² = 1.24
C21
λem = 406 nm
λex = 315 nm
Counts
Time (ns)



χ² = 1.14
C23
λem = 403 nm
λex = 315 nm
Counts
Time (ns)

Figure 3.10c. Fluorescent lifetime decay plots of coumarin derivatives.



3.3.6. Radiative and Nonradiative Decay Constants

The fluorescence emission is a random process and emission occurs by a unimolecular process. The radiative lifetime of the excited state may be defined in terms of a first order decay process. The radiative decay constant (k_R) and non-radiative decay constant (k_{NR}) can be calculated by knowing the quantum yield (ϕ_F) and lifetime values (τ), which are related by the equation,

$$k_R = \phi_F / \tau \qquad \text{and}$$

$$k_{NR} = \tau^{-1} - k_R$$

The calculated values of the k_R and k_{NR} are shown in Table 3.1. The high k_{NR} values for few molecules (C13, C14, C42) may be due to the torsional vibrations of the substituents on the coumarin ring and vibrational relaxation during the charge transfer (CT).

Table 3.4. Average lifetime values for coumarin derivatives.

Compound	λ_{ex}(nm)	λ_{em}(nm)	τ_1(ns)	α_1 (%)	τ_2(ns)	α_2 (%)	τ_{av} (ns)	χ^2
C11	326	404	0.216	97	2.79	3	0.95	1.02
		421	0.21	95	2.93	5	1.36	1.16
C12	358	435	0.75	48	4.23	52	3.74	1.3
C13	326	405	0.30	99	2.72	1	0.503	1.15
		423	0.30	99	2.05	1	0.413	1.25
C15	335	435	0.99	57	4.45	43	3.66	1.25
C18	343	435	0.91	83	4.15	17	2.475	1.16
C21	315	406	0.83	66	4.0	34	3.09	1.24
C23	315	403	0.025	97	1.49	3	0.975	1.14
C31	315	431	0.77	76	3.73	24	2.56	1.2
C41	327	428	0.78	76	3.29	24	2.22	1.1
C42	327	408	0.58	66	1.04	34	0.80	1.17
C45	335	466	1.01	36	3.6	64	3.25	1.25

3.4. Experimental

3.4.1. Electronic Spectral Measurements

Electronic absorption spectra were recorded on a Shimadzu UV-3101 PC UV-VIS-NIR Scanning Spectrophotometer and the emission spectra were recorded on a SPEX-Fluorolog F112x Spectrofluorimeter. The absorption measurements were carried out using 1 mm cuvette and fluorescence measurements were carried out using 1 × 1 cm cuvette. The fluorescence quantum

yields of all the coumarin derivatives in DMSO were estimated by comparison with Quinine sulfate in 0.1 M dilute sulfuric acid (Φ_F = 0.546) as the standard reference.

3.4.2. Fluorescence Lifetime Measurements

Fluorescence lifetimes were measured using IBH (FluoroCube) Time-Correlated Picosecond Single Photon Counting (TCSPC) system. Solutions were excited with a pulsed diode laser (<100 ps pulse duration) at a wavelength of 375 nm (NanoLED-11) with a repetition rate of 1 MHz. The detection system consisted of a micro channel plate photomultiplier (5000U-09B, Hamamatsu) with a 38.6 ps response time coupled to a monochromator (5000M) and TCSPC electronics (Data station Hub including Hub-NL, NanoLED controller and preinstalled fluorescence Measurement and Analysis Studio (FMAS) Software). The fluorescence lifetime values were obtained using DAS6 decay analysis software.

3.5. Conclusions

In the present work newly synthesized coumarin derivatives have been subjected to the photophysical evaluation by studying the luminescence parameters. These data are compared with that of the values of the existing known molecules, the photophysical properties of which are not much investigated. Preliminary studies on the effect of substituents on the fluorescent properties of these coumarin derivatives have been carried out. Although the influence of the electron donating groups such as amino, substituted amino, hydroxy, alkoxy groups etc. at position 7 of the coumarin ring system have been extensively studied, the luminescent properties of the coumarin moieties with an acetoxy substituent have not been explored.

Most of the results obtained here are in close agreement with the early reports. However the interesting results regarding the substituent effect that emerged out from the present study are;

a) Presence of an electron donating substituent at position 3 of the coumarin ring increase the fluorescent properties of the molecules;

b) An electron withdrawing group at position 3 of the coumarin moiety found to decrease the fluorescence;

c) An electron donating acetoxy group at position 7 enhances the luminescent property to high extent and

d) Extraordinary declines in the fluorescence of coumarin derivatives are observed when a methyl group is present at position 4.

The first three results are in full concurrence with the literature, but last result is contradictory to the report of an enhanced quantum efficiency of the molecule with the presence of alkyl group at position 4. This discrepancy in fluorescence with the presence of a methyl group require an extensive experimental investigation with the support of a theoretical evaluation, which are beyond the scope of the present analysis. Thus it can be concluded that, depending upon the need for its application in industries, the photophysical properties can be varied suitably by incorporating electron donating or withdrawing substituents at different positions of coumarin scaffold. The compounds C13 and C41 have high Stokes' shift value and reasonable quantum yield, which implies its possibility to use these compounds in cell imaging and dye-laser techniques.

3.6. References

1. Lackowicz, J. R. *Principles of Fluorescence Spectroscopy*, 2nd ed.; Kluwer Academic (Plenum Publishers): New York, 1999.

2. Sathyanarayana, D. N., *Electronic Absorption Spectroscopy and Related Techniques*; Universities Press (India) Limited: Hyderabad, 2001.

3. Demas, J. N. *Excited State Life Time Measurements*; Academic Press: New York, 1983.

4. O'Connor, D. V.; Phillips, D. *Time-Correlated Single Photon Counting*; Academic Press: New York, 1984.

5. Birch, D, J. S.; Imhof, R. E., *Time-domain fluorescence Spectroscopy using time-correlated single photon counting, in Topics in fluorescence spectroscopy, Volume 1, Techniques*; Lakowicz, J. R. Ed.; Plenum Press: New York, 1991; pp 1-95.

6. Ware, W. R. *Transient luminescence measurements,in creation and detection of the excited state*; Vol. 1A, Lamola Ed.; Marcel Dekker: New York, 1971; pp 213-302.

7. Drexhage, K. H. *Dye-Lasers*, *Topics in Applied Physics*, 2nd ed; in: F. P. Schafer Ed.; Springer-Verlag: New York.

8. Moylan, C. R. *J. Phys. Chem*. **1994**, *98*, 13513.

9. Sarkar, N.; Datta, A.; Das, S.; Bhattacharya, K. *J. Phys. Chem.* **1996**, *100*,15483.

10. Jones II, G.; Jimenez, J. A. C. *J. Photochem. Photobiol. B* ***2001***, *65*, 5.

11. Raju, B. B.; Costa, S. M. B. *J. Phys. Chem. B* **1999**, *103*, 4309.

12. (a) Kaholek, M.; Hrdovie, P. *J. Photochem. Photobiol. A* **1997**,*108*, 283; (b) Moriya, T. *Bull. Chem. Soc. Jpn*. **1986**, *59*, 961; (c) Fletcher, A. N.; Bliss, D. *Appl Phys.* **1986**, *16*, 289; (d) Bush, T. E.; Scott, G. W. *J. Phys. Chem.* **1981**, *85*, 144; (e) Wolfbeis, O. S.; Furlinger, E.; Kroneis, H.; Marsoner, H. *Z. Anal. Chem.* **1983**, *314*, 577.

13. Kumar, S.; Giri, R.; Mishra, S. C.; Machwe, M. K. *Ind. J. Pure Appl. Phys.* **1998,** *36*,622.

14. Padhye, M. R.; Varadarajan, T. S.; Deshpande, A. *Spectrosc. Lett.* **1982**, *15*, 597.

15. Bourson, J.; Mugnier, J.; Valeur, B. *Chem. Phys. Lett.* **1982**, *92*, 430.

16. Sprecht, P.; Martic, P. A.; Farid, S. *Tetrahedron* **1982**, *38*, 1203.

17. Urano, T.; Nagasaka, H.; Tsuchiyama, M.; Shimizu, S.; Kawazoe, K.; Shimizu, M.; Yamaoka, T. *Bull. Chem. Soc. Jpn.* **1995**, *68*, 1661.

18. Kumar, S.; Giri, R.; Mishra, S. C. ; Machwe, M.K. *Spectrochim. Acta A* **1995**, *51*, 1459.

19. Kumar, S.; Giri, R.; Mishra, S. C.; Machwe, M.K. *Ind. J. Pure Appl. Phys.* **1995**, *33*, 615.

20. Kumar, S.; Giri, R.; Mishra, S.C.; Machwe, M.K. *Ind. J. Pure Appl. Phys.* **1994**, *32*, 730.

21. Giri, R. *Spectrochim. Acta A* **1992**, *48*, 843.

22. Giri, R. *Spectrochimica Acta Part A* **2004**, *60*, 757.

23. Huang, C.N.; Kuo, P.Y.; Lin,C.H.; Yang, D.Y. *Tetrahedron* **2007**, *63*,10025.

24. Heldt, J. R.; Heldt, J.; Stofi, M.; Diehl, H. A. *Spectrochimica Acta Part A* **1995,** *51*, 1549.

25. Raju, B. B.; Varadarajan, T.S. *J.Photochem.Photobio. A* **1995**, *85*, 263.

26. Mannekutla, J. R.; Mulimani, B.G.; Inamdar, S. R. *Spectrochim. Acta Part A: Mol. Biomol. Spectrosc* **2007**, doi:10.1016/j.saa.2007.04.016.

27. Taneja, L.; Sharma A. K.; Singh, R. D. *Journal of Luminescence* **1995**, *63*, 203.

28. Ammara, H.; Forguesb, S. F.; Gharbia, R. E. *Dyes and Pigments* **2003**, *57*, 259.

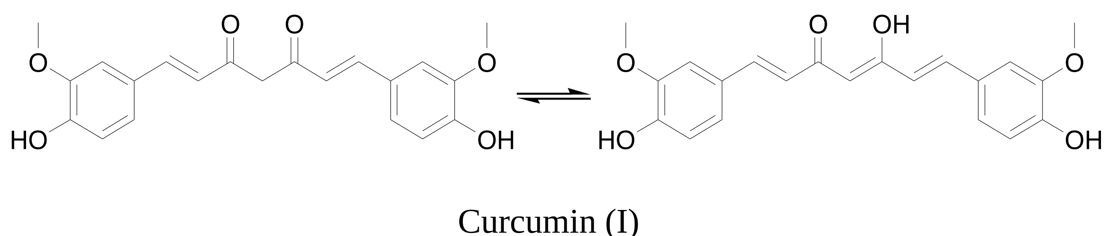
29. Kitamura, N.; Fukagawa, T.; Kohtani , S.; Kitoh, S.; Kunimoto, K. K.; Nakagaki, R . . *J.Photochem.Photobio. A* **2007**, *188*, 378.

4.1. Introduction

4.1.1. Chemistry of Curcumin

Curcumin (diferuloylmethane), a polyphenol, is an active principle of the perennial herb *Curcuma longa* (commonly known as turmeric). The yellow-pigmented fraction of turmeric contains curcuminoids, which are curcumin I, 1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione-(1E,6E) and demethoxy curcumin (curcumin II), bisdemethoxy curcumin (curcumin III), and the recently identified cyclocurcumin.[1] The major components of commercial curcumin are curcumin I (77%), curcumin II (17%), and curcumin III (3%) (Figure 4.1). The curcuminoid complex is also referred to as Indian saffron, yellow ginger, yellow root, kacha haldi, ukon, or natural yellow. Though principally cultivated in India, South east Asia, China, and other Asian and tropical countries and regions, turmeric is also common in other parts of the world and is recognized by different names in different languages worldwide. Turmeric has been described in Ayurveda for the treatment of many diseases. The Ayurvedic medicines derived from plants have played a pivotal role in the health care of many cultures, both ancient and modern.[2-7]

Figure 4.1. **Structure of Curcumin (I) , (II) and (III).**



Curcumin (I)

Curcumin (II) Curcumin (III)

Curcumin was first isolated in 1815, obtained in crystalline form in 1870,[8,9] and ultimately identified as 1,6-heptadiene-3,5-dione-1,7-bis(4-hydroxy-3-methoxyphenyl)-(1E,6E) or diferuloylmethane. In 1910, the feruloylmethane skeleton of curcumin was confirmed and synthesized by Lampe.[10] Curcumin is an yellow-orange powder that is insoluble in water and ether but soluble in ethanol, dimethylsulfoxide (DMSO) and acetone. Curcumin has a melting point of 183 ºC.

4.1.2. Uses and biological activities of curcumin

Traditionally, turmeric has been put to use as a foodstuff, cosmetic, and medicine. As a spice, it is used to provide curry with its distinctive yellow colour and flavour. It is used as a coloring agent in cheese, butter, and other foods.[11,12] In folk medicine, turmeric and natural curcuminoids have been applied as therapeutic preparations over the centuries in different parts of the world. In Ayurvedic medicine, curcumin is well-documented for the treatment of various respiratory conditions (e.g., asthma, bronchial hyperactivity and allergy) as well as for liver disorders, anorexia, rheumatism, diabetic wounds, runny nose, cough and sinusitis.[13] In traditional Chinese medicine, it is used to treat diseases associated with abdominal pain.[14] In ancient Hindu medicine, it was used to treat sprains and swelling.[13] Throughout the Orient, it has traditionally been used to good therapeutic effect, particularly as an anti-inflammatory,[15] and many of its therapeutic effects have been confirmed by modern scientific research. Such effects include antioxidant,[16] anti-inflammatory,[12,17,18] anticarcinogenic and

antimicrobial,[19-21] hepatoprotective,[21] thrombosuppressive,[22] cardiovascular (i.e., as protection against myocardial infarction),[18,23,24] hypoglycemic[25-27] and antiarthritic (i.e., as protection against rheumatoid arthritis).[28] The most compelling and key rationale for the continuing traditional therapeutic use of curcumin is its extremely good safety profile. To date, no studies in either animals[29,30] or humans[31] have discovered any toxicity associated with the use of curcumin, and it is clear that curcumin is not toxic even at very high doses.

4.2. Review of Literature

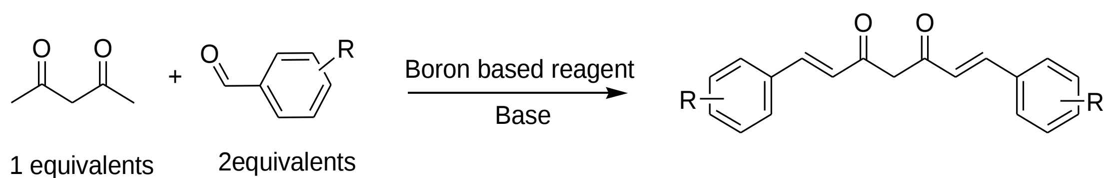
4.2.1. Synthesis of Curcuminoids

Curcumin has attracted a lot of attention due to its promising biological properties to treat cancer[32,] Alzheimer's disease[33], HIV,[34,35] chronic inflammations,[33] oxidative stress,[36] and cystic fibrosis.[37] Curcumin underwent clinical trial for cancer owing to its prominent activity as antitumour and chemopreventive agent.[38] However this trial ceased due to poor bioavailability of the molecule.[39,40] Clinical trials are ongoing to test the efficacy of curcumin against Alzheimer's disease,[41] and cystic fibrosis. [42] Intense research is also being undertaken to modify the structure of curcumin so as to increase the bioavailability and potency while maintaining the relative non-toxic nature of this natural product.[35,43-48]

Although curcumin is a simple symmetrical β-diketone, its synthesis is not possible by a straightforward di-aldol condensation on 2,4-pentanedione.[49] The C-3 of 2,4-pentanedione bears more acidic protons than those on C-1/C-5 and therefore aldol condensations on terminal methyl groups (C-1 and C-5) must be carried out successively via the dienolate. This is hard to obtain and reaction at C-3 often leads to side products. Use of boron-based protection of the 1,3-diketone circumvents the Knoevenagel condensation at C-3 and facilitates aldol condensations at C-1 and C-5 of 2,4-pentanedione.[49] A boron-

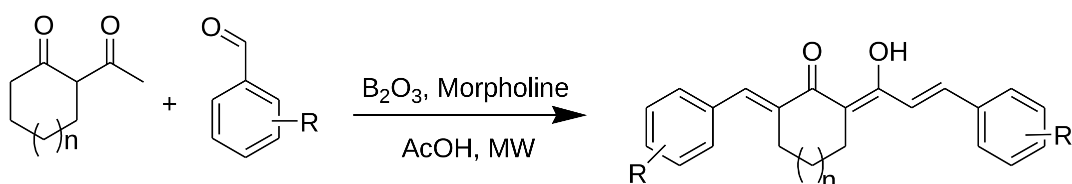
based reagent such as boron oxide, boric acid and tributoxyboron complex as Lewis acids with the β-diketone systems and consequently reduces the nucleophilicity of the C-3 position and the reaction occurs at the terminal active methylenes resulting in diarylheptanoids (Scheme 4.1).[48-50]

Scheme 4.1. General reaction for the preparation of diarylheptanoides like curcumin.



Recently, Nicholas *et al.*[51] reported a new microwave assisted method for the curcuminoids from cyclic diketones and aldehydes using a primary amine morpholine in glacial acetic acid (Scheme 4.2). Although this method gave curcuminoids from cyclic diketones it cannot be satisfactorily used for the synthesis of curcumin and their derivatives from acetylacetone and aldehydes.

Scheme 4.2.



4.3. Results and Discussion

4.3.1. Synthesis of Curcumin

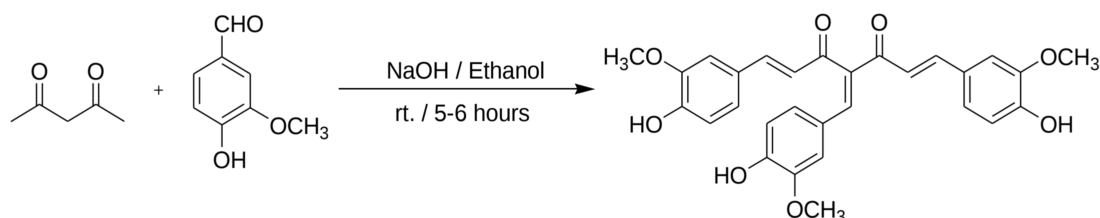
In the present work, two novel microwave assisted solvent-free protocol for the synthesis of curcumin are described. The first method is a microwave version of the early strategy developed by Pavolini *et al*,[49a] that is

suitably modified to reduce the reaction time and suppress the formation of byproducts. The second one is a new microwave assisted fast and simple method for the synthesis of curcumin.

A method that was reported for the synthesis of curcumin from vanillin and acetylacetone, used tributylborate, n-butylamine, and boric oxide as the catalyst and condensing agents. The reported method suffered from long reaction time and formation of Schiff's bases between carbonyl compounds and n-butylamine. The Schiff's bases formed were highly coloured and imposed difficulties in chromatographic separation. The yield was also found to be low. Thus a fast, simple, and cost effective method for the synthesis of curcumin is necessary.

The present attempt started by reacting acetylacetone with 4-hydroxy-3-methoxybenzaldehyde (vanillin) in presence of bases such as NaOH, KOH, pyrrolidine, piperidine or sodium acetate. Aldehydes and diketone (2:1) were dissolved in ethanol, the condensing agent was added drop wise with stirring for 30 minutes and the stirring was continued for 5- 6 hours. The reaction mixture was kept overnight, precipitated from ice-cold water and the dried product was crystallized from hot benzene. The products obtained were characterized by IR and NMR spectroscopy. It was identified as a tricondensation product as given in Scheme 4.3.

Scheme 4.3

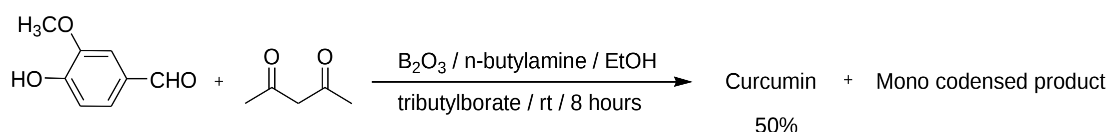


The same reaction was carried out under reflux condition. Then also the result obtained was a tricondensed product. Other bases such as,

piperidine, pyridine and sodium acetate were also tried as condensing agent. No change could be observed in the products. Hence it was concluded that only tricondensation product would be obtained by the condensation of acetylacetone with vanillin using common strong and mild bases. No curcumin were formed under this condensation protocol. Thus in order to have selective condensation at C_1 and C_5 of the pentane-2,4-dione the active methylene group at C_3 is to be protected.

The method reported by Pavolini made use of boron based reagent, to protect the active methylene group of pentanedione (Scheme 4.4). This method described the synthesis of curcumin from acetylacetone and vanillin

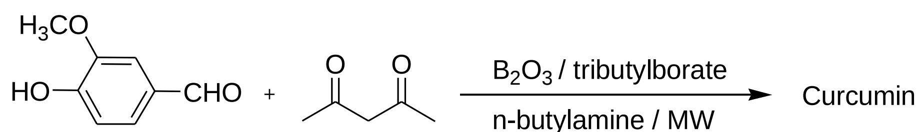
Scheme 4.4



in the presence of B_2O_3, tributylborate and n-butylamine. Along with curcumin the mono condensed product and Schiff's bases were also formed. The formations of byproducts are on the higher side. So a procedural modification of this method under a solventless microwave condition has been tried.

In the current work, acetylacetone was made to react with boric oxide (B_2O_3) by irradiating under microwave and to the reaction mixture, aldehyde, tributylborate and n-butylamine was added and further irradiated for few minutes (Scheme 4.5).

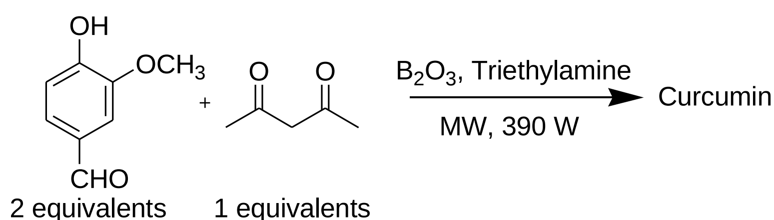
Scheme 4.5



On TLC monitoring it was found that curcumin along with the coloured Schiff's base impurities were formed. No mono condensed product could be identified by chromatography. Thus the reaction, which took 5 hours under conventional method, could be carried out within 10 minutes under microwave-assisted method with least number of byproducts. However yield of this solvent free method was comparable to that of the solution phase method. Here also the primay amine (n-butylamine) used in the reaction formed Schiff's base with the aldehydes as well as ketones. This caused the low yield in this protocol. These difficulties demanded a revised route for the synthesis of curcumin.

In the new method, a stepwise synthesis of curcumin under a solvent free microwave assisted method have been devised that can overcome the above difficulties to a certain extent. The acetylacetone was reacted with B_2O_3 under microwave irradiation for 3 minutes. To this reaction mixture, triethylamine and vanillin were added and irradiated for further 5 minutes (Scheme 4.6).

Scheme 4.6.



TLC and co-chromatography confirmed the formation of curcumin. The reaction was found to be incomplete. The completion of the reaction could not be attained by increasing the time of irradiation or power of microwave.

The merits of the present work are;

a) No mono condensation product was obtained.

b) No Schiff's base formation.

c) Column purification was easy.

d) The reaction gave a considerable yield of 30-40%.

e) The reaction was fast as it was completed within 8 minutes.

Although the yield was slightly low compared to the conventional methods, considering its simplicity, rapidity and easiness, the method is advantageous over the others so far reported.

4.3.2. Attempted work on the synthetic conversion of curcumin

In the second part of the study an attempt was made to carry out some synthetic conversions on curcumin. My aim was to know how its biological properties are changed with the synthetic conversions. So far it was reported that the substituent variation on the aromatic ring marginally alter the biological properties of the curcumin moiety. Here the attempt was to incorporate the diketone moiety of the curcumin into reaction and study how the properties of the products vary from the curcumin.

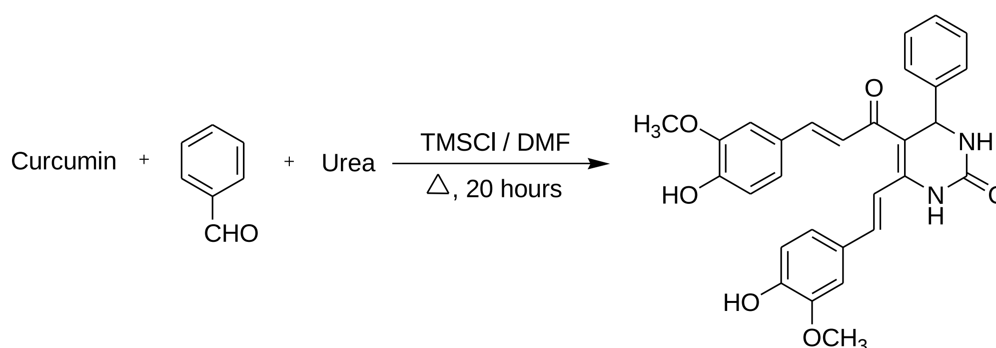
The various reactions tried on curcumin are shown in Scheme 4.7. TLC analyses clearly indicate its conversion and chromatographic separation afforded pure products. Unfortunately, the characterization by spectroscopy

could not be carried out due to the failure in getting NMR spectra of these compounds in DMSO/$CDCl_3$. The Solid State NMR results are awaited.

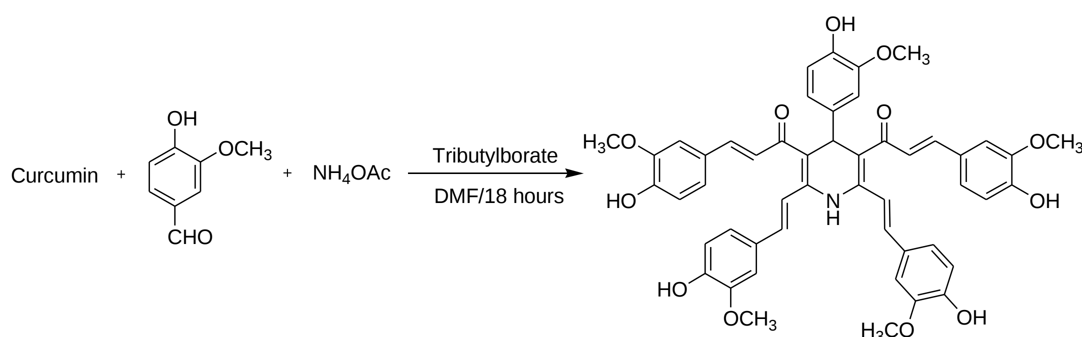
The various reactions carried out on the curcumin and the envisaged products can be formulated as shown in Scheme 4.7. But no complete spectroscopic evidences are available to ascertain the structural characteristics of the formed product.

Scheme 4.7. Attempted Synthetic Reactions on Curcumin

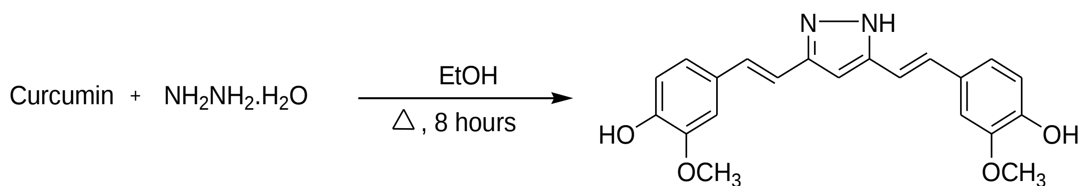
Reaction 1: Biginelli reaction



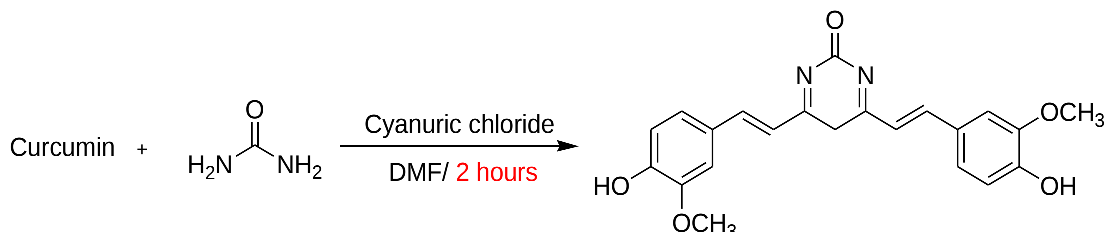
Reaction 2: Hantsch reaction



Reaction 3



Reaction 4



4.4. Experimental

4.4.1 ***Synthesis of β-bis (3-methoxy-4-hydroxyphenyl)-α'-(3-methoxy-4-hydroxybenzal)-hept-1, 6-dien-3, 5-dione (Tricondensation Product):***

4-Hydroxy-3-methoxybanzaldehyde (1.5 g, 10 mmol) and acetylacetone (9.6 g, 10 mmol) were dissolved in ethanol (20 mL) and stirred at room temperature. Sodium hydroxide (20% solution, 10 mL) was added drop wise within 30 minutes and stirred well for 3 hours at room temperature. The reaction was monitored using TLC. The reaction mixture was kept overnight (12 hours), poured into crushed ice and neutralized with 0.1 M HCl. The precipitate formed was filtered, washed several times with distilled water, dried and re-crystallized from hot benzene; melting point 128-130°C.

The spectroscopic data are shown below.

UV (λ_{max}(nm), methanol) 416; IR (υ_{max}, KBr , cm^{-1}) 1646 (C=O), 1613 (C=C stretch), 1580 (aromatic C=C), 1268 (C-O); ^{1}H NMR (δ ppm, 300 MHz, $CDCl_3$) 7.1 (d, J= 2.4 Hz, 1H), 8.1 (d, J= 2.5 Hz, 1H), 8.0 (s, 1H), 7.58 (m, 3 H, Ar), 6.5 (m, 3H, Ar), 6.4 (m, 3 H, Ar), 7.0 (m, 3 H, Ar), 3.8 (s, 9 H,

OCH_3,); ^{13}C NMR (δ ppm, 75 MHz, DMSO) 190.0, 160.1, 162.8, 137.9, 130.3, 124.2 117.3, 105.5, , 98.5, 55; Mass (FAB) m/z 503.217(M+1)

4.4.2 Synthesis of Curcumin under microwave-assisted condition

4.4.2.1 *A one-pot solvent free microwave strategy for1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione(Curcumin I) - Procedural modification of the conventional protocol.*

Acetyl acetone (1 mL, 10 mmol) and boric oxide (0.5 g, 7 mmol) were taken in a small beaker, mixed well and irradiated under microwave at a power 390 W for 3 minutes intermittently (6 x 30 sec). To the thick paste obtained added vanillin (1.5 g, 10 mmol) and tributylborate (1 mL, 3 mmol). Mixed thoroughly and irradiated under microwave for 2 minutes at 390 W. Add 5 drops of n-butylamine and irradiated for further 3 minutes in an intermittent fashion. The reaction mixture is cooled, added 2 mL of 4 N HCl and subjected to microwave irradiation for 2 minutes at 120 W. The product obtained was extracted with ethyl acetate and final purification was done by chromatography on a column packed with silica gel (100-200 mesh) using chloroform- methanol mixture (25:1) as the eluent.

The formation of curcumin was identified by comparing the R_f values of the synthesized curcumin with that of the authentic sample. Also a co-chromatography confirmed the same. The melting point of the synthesized curcumin (179-181ºC) was comparable with that of reported melting point (183-185ºC).

4.4.2.1. *A novel microwave-assisted solvent free protocol for 1,7-bis (4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione (curcumin).*

Acetyl acetone (1 mL, 10 mmol) and boric oxide (0.5 g, 7 mmol) were taken in a loosely stoppered borosil vessel, mixed well and irradiated under

microwave at a power 390 W for 2 minutes intermittently (6 x 30 sec). Added vanillin (1.5 g, 10 mmol) and triethylamine (0.5 ml) and irradiated for further 5 minutes with occasional cooling. The reaction mixture was poured into ice-cold water. The product obtained was washed with dilute HCl solution, extracted with ethyl acetate and the solvent was evaporated off to get the desired curcumin. Further purification was done by chromatography. The yield was found to be 30-40%. Melting point; 179-182°C.

The formation of the product was identified by TLC and co-chromatography and confirmed by the comparison of IR and NMR data with that of the literature. The spectra used for comparison have been enclosed in Appendix III.

4.4.3. Attempted synthetic conversions on curcumin

4.4.3.1. Reaction 1 – Biginelli reaction

Curcumin (1.1 g, 3 mmol), urea (0.3 g, 5 mmol) and benzaldehyde (0.3 mL, 3 mmol) are dissolved in DMF and added 0.5 ml of trimethylsilyl chloride (TMSCl) into this reaction mixture. Refluxed this mixture for 20 hours at 110^{0}C and monitored the reaction by TLC. The reaction mixture was cooled and transferred to ice cold water. The precipitated product is filtered, washed repeatedly with sodium bicarbonate solution and dried at 60^{0}C.

The TLC analysis showed the presence of 4 products. The crude products further subjected to chromatography on a silica gel (100-200 mesh) packed column using chloroform-methanol solvent system under gradient elution condition. One product was obtained in major quantity and all others are in negligibly small amount. The major product obtained was recrystallised from hot ethanol and the yield was 500 mg. NMR spectra could not be recorded in solution. The available mass and IR spectral values are given below.

Melting Point	-	238-242^{0}C
UV (λ_{max} (nm), methanol)	-	353, 326
IR (ν_{max}, kBr, cm^{-1})	-	3278, 3224, 3070, 2923.56,1697.24, 1596.95, 1512.09, 1265.22 cm^{-1}
Mass (FAB)	-	428.62, 390.88, 358.93, 322.71, 256.94, 216.92 (100), 180.92, 148.99
NMR	-	Not available

4.4.3.2. Reaction 2 - Hantsch Reaction

Curcumin (2.2 g, 6 mmol), vanillin (0.5 g, 3 mmol) ammonium acetate (0.23 g, 3 mmol) and tributylborate (0.5 ml) are taken in an RB flask and refluxed in DMF at 110^{0}C for 18 hours. The mixture obtained are poured into ice-cold water and stirred for 1 hour at 10^{0}C. The reaction mixture was kept overnight and the precipitated product was filtered and washed repeatedly with distilled water and 1% HCl. The solid is then dried under IR lamp. Purification was done using column chromatography over silica gel (100-200 mesh) and the solvent system chloroform-methanol (gradient elution). The yield of the major product was found to be 1.32g.

Melting point	-	160-163^{0}C
UV (λ_{nm}, Methanol)	-	331, 285, 210
IR (ν_{max}, kBr, cm^{-1})	-	3317,1666,1596.95,1272.93
Mass (FAB)	-	429.16, 409.37,181.17.
NMR	-	Not obtained

4.4.3.3. Reaction 3

Curcumin (1.1 g, 3 m mol) and hydrazine hydrate (0.2 ml, 5 mmol) are dissolved in ethanol and refluxed for 8 hours. The reaction mixture was cooled, transferred into ice-cold water, stirred for 30 minutes and kept for 5 hours at 0^0C. The solid product precipitated was filtered and dried under IR lamp. Yield of the crude product was 450 mg. The crude product was further purified by column chromatography over silica gel using chloroform-methanol system as eluent. Melting point of the major product was 185-189^0C.

Melting point	-	185-189^0C
UV (λ_{max}(nm), methanol)	-	320, 285 nm
IR (ν_{max}, kBr, cm^{-1})	-	3304 (NH), 1604.48, 1514.8, 1462.74 (2^0NH), 1275 (C-N)
^{1}HNMR (δ ppm, 300MHz, DMSO)	-	8.94, (S, 2H, OH), 7.93 (S, 1H, NH), 7.04-6.79 (m, Ar), 6.7 - 6.5 (Vinyl proton), 6.1 (S, 1H, CH), 3.89 (OCH_3) Although peak position of the protons were satisfied, the integrations were not agreeable in ^{1}HNMR.
^{13}CNMR	-	Not obtained
Mass (FAB)	-	365.24 (M+1), 323, 274, 257.27, 217, 197.15, 181.17.

4.4.3.4. Reaction 4

Curcumin (1.1 g, 3 mmol), urea (0.3 g, 5 mmol) and cyanuric chloride (catalytic amount) are dissolved in DMF and refluxed for 2 hours at 100^0C. The reaction mixture is kept overnight and poured into ice cold water. Stirred for 1 hours at 5^0C and the precipitated product was filtered and washed repeatedly with saturated solution of potassium hydrogen sulfate. The solid

was recrystallised from hot ethanol and subjected to column chromatographic purification. The yield of the major product was 370 mg.

Melting point - 167 - 171^{0}C

UV (λ_{max}(nm), methanol) - 310, 275nm

IR (ν_{max}, kBr, cm^{-1}) - 3371.4, 2229.6, 1512.09,1272.93

Mass (FAB) - 392.05 (M+1), 217, 1971.54, 149.41.

4.5. Conclusions

In the current study, we have introduced two protocols for the synthesis of curcumin, under solvent free microwave assisted conditions. Of the two methods one is a procedural modification of the early reported method, while the other is a newly introduced one. The methods described so far under solution phase mode suffer from the low yield, formation of large number of byproducts and cumbersome work-up procedures. The new methods introduced here are solvent free and number of byproducts formed is minimum. Also the purification work up is easy. The yield is comparable to other methods reported earlier. The novel strategy has the added benefit of rapidity and cleanliness.

4.6. References

1 Kiuchi, F.; Goto ,Y.; Sugimoto, N.; Akao, N.; Kondo, K.; Tsuda, Y. *Chem Pharm Bull. (Tokyo)* **1993,** *41*,1640.

2 Newman, D.J.; Cragg, G.M.; Snader, K.M. *J Nat Prod.* **2003**,*66*,1022.

3 Butler, M.S. *J Nat Prod*, **2004**, *67*, 2141.

4 Balunas, M.J.; Kinghorn, A.D. *Life Sci.* **2005**, 78, 431.

5 Gurib-Fakim, A.. *Mol Aspects Med*. **2006**,*27*,1.

6 Newman, D.J.; Cragg ,G.M. *J Nat Prod*. **2007**, *70*, 461.

7 Mukherjee, P.K.; Wahile, A. *J Ethnopharmacol.* **2006**,*103*,25.

8 Aggarwal, B.B.; Surh, Y.J.; Shishodia, S. *Adv Exp Med Biol.* **2007**,595. [9]

9 Daybe, F.V. *Ber*.**1870**.*3*, 609. [10]

10 Lampe V. Milobedzka. *J Ver Dtsch Chem Ges*. **1913**, 46, 2235. [11]

11 Govindarajan, V. S. *Crit Rev Food Sci Nutr*. **1980**,*12*,199.

12 Ammon, H. P.; Wahl, M.A. *Planta Med.* **1991**,*57*,1.

13 Araujo, C. C.; Leon, L.L. *Mem Inst Oswaldo Cruz.* **2001**,*96*,723.

14 Aggarwal, B. B.; Takada, Y.; Oommen, O.V. *Expert Opin Investig Drugs* **2004**,*13*,1327.

15 Aggarwal, B. B.; Kumar, A.; Bharti ,A. C. *Anticancer Res.* **2003**, *23*, 363

16 Sreejayan.; Rao, M. N. *J Pharm Pharmacol*. **1997**,*49*,105.

17 Brouet, I.; Ohshima, H. *Biochem Biophys Res Commun.* **1995**, *206*,533.

18 Dikshit, M.; Rastogi, L.; Shukla, R.; Srimal, R. C. *Indian J Med Res.* **1995**, *101*, 31.

19 Rao ,C. V.; Rivenson, A.; Simi, B.; Reddy, B. S. *Cancer Res.***1995**, *55*, 259.

20 Limtrakul, P.; Lipigorngoson, S.; Namwong, O.; Apisariyakul, A.; Dunn, F. W. *Cancer Lett*. **1997**, 116, 197.

21 Kiso, Y.; Suzuki ,Y.; Watanabe, N.; Oshima, Y.; Hikino, H. *Planta Med.* **1983**, 49, 185.

22 Srivastava, R.; Dikshit, M.; Srimal, R. C.; Dhawan, B. N. *Thromb Res*. **1985**, *40*, 413.

23 Nirmala, C.; Puvanakrishnan , R. *Mol Cell Biochem*. **1996,** *159,* 85.

24 Venkatesan, N. *Br. J. Pharmacol*. **1998**,*124*,425.

25 Srinivasan, M. *Indian J. Med. Sci.* **1972**, 26, 269.

26 Babu ,P. S.; Srinivasan, K. *Mol Cell Biochem*. **1995**, *152*, 13.

27 Arun ,N.; Nalini, N. *Plant Foods Hum Nutr.* **2002**, 57, 41.

28 Deodhar, S. D.; Sethi, R.; Srimal, R. C. *Indian J Med Res*. **1980**,*71*, 632.

29 Shankar, T. N,; Shantha, N. V.; Ramesh, H. P.; Murthy, I. A.; Murthy, V. S. *Indian J Exp Biol*. **1980**,18, 73.

30 Qureshi, S.; Shah, A. H.; Ageel, A. M. *Planta Med.* **1992**, 58, 124.

31 Lao, C. D.; Ruffin, M. T.; Normolle, D.; Heath, D. D.; Murray, S. I.; Bailey, J. M. *Altern Med.* **2006,** *6*, 10.

32 Sharma, R. A.; Gescher, A. J.; Steward, W. P. *Eur. J. Cancer* **2005**, *41*, 1955.

33 Lim, G. P.; Chu, T.; Yang, F.; Beech, W.; Frautschy, S. A.; Cole, G. M. *J. Neurosci.* **2001**,*21*, 8370.

34 De Clercq, E. *Med. Res. Rev.* **2000**, *20*, 323.

35 Mazumder, A.; Neamati, N.; Sunder, S.; Schulz, J.; Pertz, H.; Eich, E.; Pommier, Y. *J. Med.Chem.* **1997**, *40*, 3057.

36 Kopani, M.; Celec, P.; Danisovic, L.; Michalka, P.; Biro, C. *Clin. Chim. Acta* **2006**, *364*, 61.

37 Zeitlin, P. *New Engl. J. Med.* **2004**, *351*, 606.

38 National Cancer Institute, Clinical Development Plan: Curcumin. *J. Cell. Biochem.* **1996**,*26S*, 72.

39 Shoba, G.; Joy, D.; Joseph, T.; Majeed, M.; Rajendran, R.; Srinivas, P. S. *Planta Med.* **1998**,*64*, 353.

40 Sharma, R. A.; McLelland, H. R.; Hill, K. A.; Ireson, C. R.; Euden, S. A.; Manson, M. M.; Pirmohamed, M.; Marnett, L. J.; Gescher, A. J.; Steward, W. P. *Clin. Cancer Res.* **2001**, *7*,1894.

41 National Institute of Aging, A phase II, double-blind, placebo-controlled study of the safety and tolerability of two doses of curcumin C3 complex versus placebo in patients with mild to moderate Alzheimer's disease,2006, *http://clinicaltrials.gov/show/NCT000997 0*.

42 Ramsey, B. A phase I safety and dose finding study of orally administered curcuminoids in adult subjects with cystic fibrosis who are homozygous for delta F508 cystic fibrosis transmembrane conductance regulator (.F508 CFTR) mutation, **2005**, *http://www.clinicaltrials.gov/ct/show/NCT00219882*.

43 Lee, S. L.; Huang, W. J.; Lin, W. W.; Lee, S. S.; Chen, C. H. *Bioorg. Med. Chem.* **2005**, *13*, 6175.

44 Ishida, J.; Ohtsu, H.; Tachibana, Y.; Nakanishi, Y.; Bastow, K. F. Nagai, M.; Wang, H. K.; Itokawa, H.; Lee, K. H. *Bioorg. Med. Chem.* **2002**, *10*, 3481.

45 Youssef, K. M.; El-Sherbeny, M. A.; El-Shafie, F. S.; Farag, H. A.; Al-Deeb, O.A.; Awadalla, S. A. A. *Arch. Pharm. Pharm. Med. Chem.* **2004**, *337*, 42.

46 Ohtsu, H.; Xiao, Z.; Ishida, J.; Nagai, M.; Wang, H.K.; Itokawa, H.; Su, C.Y.; Shih, C.; Chiang, T.; Chang, E.; Lee, Y.F.; Tsai, M.Y.; Chang, C.; Lee, K.H. *J. Med. Chem.* **2002**, *45*, 5037.

47 Jha, A.; Zhao, J.; Cameron, T. S.; DeClercq, E.; Manavathu, E. K.; Stables, J. P. *Lett. Drug Design Discov.* **2006**, *3*, 304.

48 Ohtsu, H.; Itokawa, H.; Xiao, Z.; Su, C.Y.; Shih, C.C.Y.; Chiang, T.; Chang, E.; Lee, Y.; Chiu, S.Y.; Chang, C.; Lee, K.H. *Bioorg. Med. Chem.* **2003**, *11*, 5083.

49 (a) Pavolini, J.; Gambarin, F.; Grintzo, A.M. *Ann. Chim(Rom)*, **1950**, *40*, 280; (b) Pabon, H.J.J. *Rec. Trav.Chim*. **1964**,*83*, 379; (c) Babu, K. V. D.; Rajasekharan, K. N. *Org. Prep. Proc. Int'l.* **1994**, *26*, 674.

50 Samula, K.; Kardasz, K. *Pol. J. Chem.* **1985**, *59*, 73.

51 Nichols, C.E.; Youssef, D.; Harris, R. B.; Jha, A. *ARKIVOC* **2006**, *13*, 64.

5.1.1. Introduction

5.1.1.1. Cancer

Cancer is a class of diseases or disorders characterized by uncontrolled or inappropriate division of cells and the ability of these cells to invade other tissues either by direct growth into adjacent tissues through invasion or by implantation into distant sites by metastasis. The name 'Cancer' coined by Hippocrates is derived from Greek word "Karkinos" meaning 'Creb' describing malignant growth. Cancer may affect people at all ages, risk, tends to increase with age, due to the fact that DNA damage become more apparent in ageing DNA.

Unregulated growth that characterizes cancer is caused by damage of DNA, resulting in mutation of genes, which codes for protein controlling cell division. These mutations can be caused by chemical agents or physical agents or by biological agents such as certain viruses. They are called carcinogens. The event of cascade that converts normal cells to cancer cells by carcinogen is known as carcinogenesis

5.1.1.2. Different Kinds of Cancer

Cancer can originate almost any where in the body. Cancer is classified on the basis of the part of the body in which it begins and by its appearance under microscope.

‘Carcinomas**’,** the most common types of cancer arise from the cells that cover external and internal body surfaces. Oral cavity, cervix, lung, breast and colon cancers are the most frequent of this type. ‘Sarcomas’ are cancers arising from cells found in the supporting tissues of the body such as bone, cartilage, fat, connective tissues and muscles. The cancers that arise in the lymph nodes and tissues of the body's immune systems are called

‘lymphomas’ and cancers of the immature blood cells that grow in the bone marrow and laid to accumulate in large numbers in the blood stream are called ‘leukemia’.

In normal tissues, the rate of new cell growth and old cell death are kept in balance known as tissue homeostasis. In cancer, this balance is disrupted and can result in uncontrolled cell growth or loss of a cell's ability to undergo 'apoptosis'. Apoptosis, or programmed cell death is the mechanism by which old or damaged cell normally self-destruct.

5.1.1.3. Apoptosis

Apoptosis is a morphologically distinct form of programmed cell death. It plays a major role in development and tissue homeostasis of multicellular organisms and in many diseases including cancer. Apoptosis can be initiated by a wide array of stimuli including multiple signalling pathways[1]. It is an active gene directed process that seems to have conserved throughout animal evaluation[2]. Apoptosis is required by normal tissues for remodeling, proper development and function.[3] Cells are eliminated in a variety of physiological settings by apoptosis, which appears as cellular suicide.

The principal biochemical effectors of apoptosis are cystein aspartyl serine proteases (caspases), which are synthesized as inactive zymogens that once activated systematically and in hierarchal process degrade DNA and vital house keeping proteins.[4]

5.1.1.4. Molecular biology of Cancer

Cancer is ultimately a disease of genes. The main genes involving in these processes are proto-oncogens and tumour suppressor genes. Normally proto-oncogenes are genes, which promote cell growth and mitosis, a process of cell division, and tumour suppressor genes discourage cell growth.

Mutation in proto-oncogenes can modify their expression and function, increasing the amount or activity of the product protein. When this happens they become oncogenes, and these cells have a higher chance to divide excessively and uncontrollably. A mutation can damage the tumour suppressor gene itself, or the signal pathway, which activates it, "switching it off". The invariable consequence of this is that DNA repair is hindered. DNA damage accumulates without repair, leading to cancer.[5,6]

5.1.1.5. Treatment of Cancer

Cancer can be treated by many ways. The choice of therapy depends upon the location and grade of the tumour and the stage of the disease as well as the general state of the patients. Surgery, radiation therapy, chemotherapy, immunotherapy, hormone therapy, cancer vaccines etc. are the common means of treatment of cancer.

5.1.1.6. Chemotherapy

It is the treatment of cancer with drugs that destroy cancer cells. These drugs go into the blood stream and are carried to cancer cells any where in the body.

5.1.1.6.1. Importance of chemotherapy

Chemotherapy is the only method of treatment for metastatic cancer. Chemotherapy could be used along with surgery and radiation therapy or used alone.

5.1.1.6.2. Types of chemotherapeutic drugs

There are mainly two types of chemotherapeutic drugs. They are ‘Cell cycle phase-specific’ and ‘Cell cycle phase-non-specific’. Cell-cycle Phase - Specific drugs affect cells in phases G_1, S, G_2 or M but not G_0. Since these drugs exert their cytotoxic effects during the cell cycle, they are most effective against actively growing tumours. They are given in minimal concentrations using continuous dosing methods. Antimetabolites, Vinca plant alkaloids, asparginase, dacarbazine etc. are some of the examples of these drugs.

Cell Cycle Phase-non-Specific Drugs are active in all phases of the cell cycle, so they may be effective in large tumours that have fewer active cells. They are given as a single injection. Some of the examples are nitrosoureas, procarbazine, steroid drugs etc.

5.1.1.6.3. Advantages of chemotherapy

The major advantages of chemotherapy are:

a) shrink a tumour before surgery or radiation.

b) help to destroy cancer cells that may remain after surgery or radiation.

c) make radiation and immunotherapy work better.

d) help to destroy cancer if it reoccurs or has spread to other parts of the body from the original tumour.

5.1.1.6.4 Side effects

When chemotherapy acts on normal cells, 1) in the stomach it can cause loss of appetite, nausea and vomiting, 2) mouth sores, 3) tingling or shooting pain in the fingers and toes due to nerve damage, 4) Temporary hair loss, 5) can affect the central nervous system causing tiredness and

depression. Some phytochemicals used as anticancer drugs includes *Catharanthus* alkaloid; vincristine alkaloid, pyrrolizidine alkaloid monocrotaline, taxol, harringtonine, homoharringtonine etc.

5.1.1.7 *Invitro* tests for Anticancer Studies

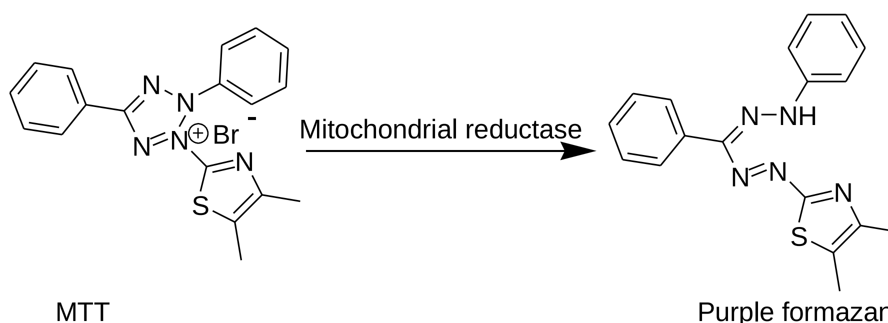
MTT assay and trypanblue exclusion assay are the proliferation tests, by which the rate of proliferation and rate of growth inhibition by the drug is measured. Acridine orange staining and geimsa staining are morphological tests that measures the morphological changes produced by the drug action like membrane blebbing, nuclear condensation etc. These morphological changes ultimately lead to the death of the cells through apoptosis. DNA laddering is usually carried out for continuing the apoptotic effect of the drug on cancer cells.

MTT assay is a laboratory test and a standard colourimetric assay, which measures changes in colour for measuring cellular proliferation or cell growth. It is used to determine the cytotoxicity of potential medicinal agents and other toxic materials.

5.1.1.7.1. MTT Assay-Principle

MTT (3-(4-5-dimethyl thiazole-2-yl)-2,5-diphenyltetrazolium bromide), a tetrazole is reduced to purple formazan in the mitochondria of living cells (Figure 5.1.1). A solubilization solution (usually DMSO) is added to dissolve the insoluble purple formazan product into a coloured solution. The absorbance of the coloured solution can be quantified by measuring at a particular wavelength (500-600 nm, specifically at 570 nm) by a spectrophotometer.

Figure 5.1.1. Recuction of MTT to Formazan



The reduction of MTT to formazan takes place only when mitochondrial reductase enzymes are active, and therefore conversion is indirectly related to the number of viable (living) cells. When the amount of purple formazan produced by cells treated with an agent is compared with the amount of formazan produced by untreated control cells, the effectiveness of the agent causing death of cells can be deduced, through the production of a close-response curve.

The percentage of growth inhibition can be calculated using the formula,

% Growth inhibition = [100-(A_{CD}-A_D) x 100] / A_{CU}

A_{CD} - absorbance of drug treated cells

A_D - absorbance of drug in the medium

A_{CU} - absorbance of untreated cells

5.1.2. Review of Literature

Anticancer Studies on Coumarins and Dihydropyrimidinones.

Coumarins and related compounds are the subject of cancer studies even from the early times. Many compounds bearing the coumarin unit, which are obtained from the plant kingdom as well as synthesized in the laboratory are

reported to have various biological properties including anticancer activities. Seokjoon *et al.* [9] have synthesized a series of 7-diethylaminocoumarin compounds and the cytotoxicities were tested against human umbilical vein endothelial cell (HUVEC) and cancer cell lines such as U87 MG,B16 BL6, HeLa,DLD-1, SiHa and NIH 3T3[7]. Chemoprevention of Aflatoxin B1 hepatocarcinogenesis by coumarin has been investigated by Kelly *et al.*[8] Coumarin, umbelliferone (7-hydroxycoumarin), scopoletin (7-hydroxy-6-methoxycoumarin), and limettin (5,7-dimethoxy coumarin), four naturally occurring plant constituents, were studied for their effects on 7,12-dimethylbenz(α)anthracene induced neoplasia of the rat mammary gland.[9] In addition, 6,7-dimethylcoumarin-based novel angiogenesis inhibitors[10] and some tumour inhibitors have been reported in the preceding years.[11]

Dihydropyrimidinones (DHPMs) are heterocycles with high pharmacological and therapeutic potential. Some of the compounds with pyrimidone unit are reported to exhibit antitumour and anticancer activities. Dihydropyrimidine 1 and some of its analogs were screened as antitumour agents and found to be active against Walker carcinosarcoma in rats and mice.[12-14] Pyrimidine-5-carboxamides are reported to possess anticarcinogenic activity.[15]Although many compounds that are isolated from the plants and animals bearing the pyrimidone moieties are reported to have anticancer activities, not much work has been carried out on the simple synthetic dihydropyrimidones for its cellular viability assessment. Recently Russowsky *et al.*[16] reported the differential antiproliferative activity of monatrol, oxomonastrol and some oxygenated analogues of it against cancer cell lines.

5.1.3. Results and Discussion

The present study was carried out to determine the anticancer activity of some oxygen and nitrogen heterocycles synthesized by me. The method adopted for this was the cellular viability assessment through MTT assay. The compounds under consideration were tested in two different cancer cell lines-leukemia cell lines K562, obtained from the National Centre for Cell Sciences (NCCS), Pune and Ehrlich Ascites Carcinoma (EAC) cells that are developed in the peritoneal cavity of BALB/c mice.

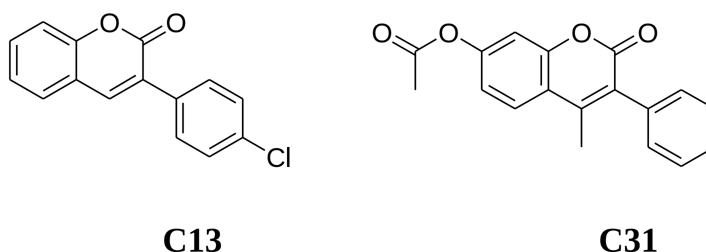
Nine compounds were screened for its cytotoxicity on two cancerous cell lines. Two coumarin derivatives (C13 and C31) and seven dihydropyrimidinones (DHPMs) were selected randomly for analysis and the results obtained are summarized in Table 5.1.1.

Table 5.1.1. Cytotoxicity assay of coumarins and dihydropyrimidinones on cancer cell lines

Compound	Cytotoxicity assay on			
	K562 cells		EAC cells	
	Maximum % Inhibition	Concentration (μg/mL)	Maximum % Inhibition	Concentration (μg/mL)
C13	**7.4**	**10**	**37.4**	**1**
C31	3.5	1-10	14	1000
U21	**4.8**	**10**	**27.4**	**1**
U24	10.8	1	13.1	1000
U216	4.0	1000	10	100
U217	4.5	1-10	10	200
T21	**22.5**	**1**	**26**	**800**
U416	7.4	10	12.3	800
U517	5.6	1	17	200

A preliminary analysis of the cytotoxicity of these compounds are carried out with varying concentration ranging from 0.01-1000 μg/mL against K562 and 1-1000 μg/mL against EAC cell lines.

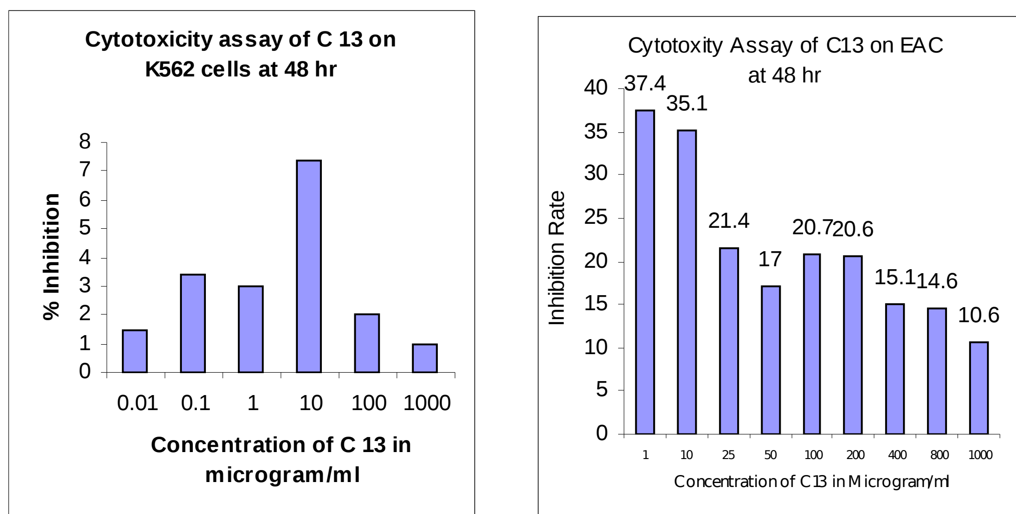
Figure 2.1.2



C13 **C31**

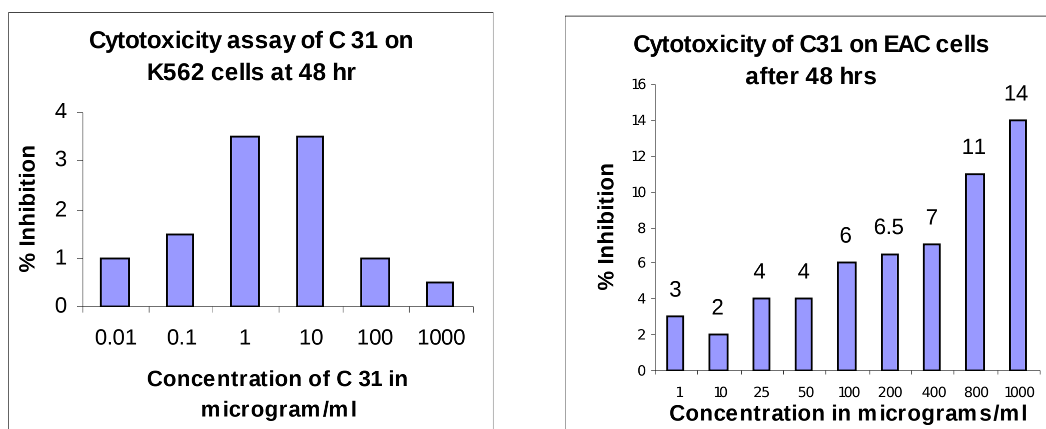
Among the coumarins C13 and C31 studied, (Figure 5.1.2) the former showed a growth inhibition of 7.4% against K562 cells at a concentration of 10 μg/ml, whereas it has a prominent growth inhibition rate of 37.4% against EAC cells at a concentration 1 μg/mL. On increasing the concentration the growth inhibitory activity on EAC is found to be decreased. Thus at low concentrations C13 has promising activity on EAC at the same time the inhibition on K562 cells found to increase up to a concentration of 10 μg/mL with a maximum inhibition of 7.4% and on further increasing the concentration decreases the percentage inhibition (Figure 5.1.3).

Figure 5.1.3. Cytotoxicity assay of C13



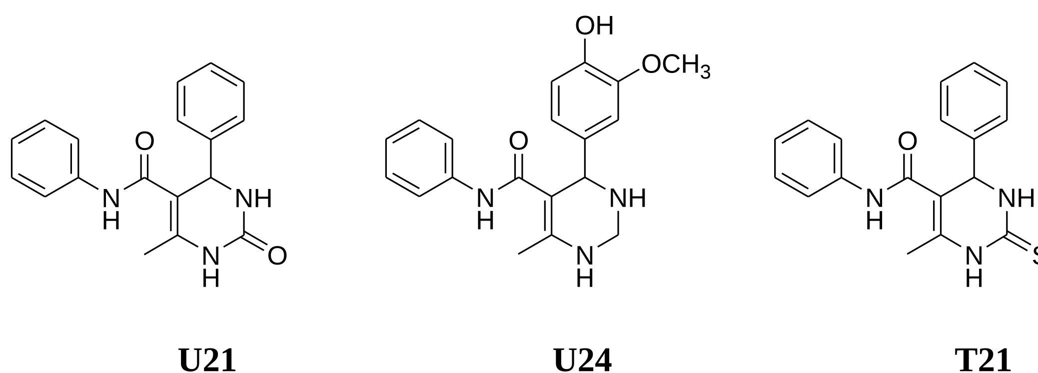
The compound C31 exhibits a maximum inhibition of 3.5% at concentration 1-10 µg/mL on K562 cells and a 14% inhibition on EAC cells at higher concentration of 1000 µg/mL (Figure 5.1.4). Accordingly it can be summed up that among the coumarins tested only C13 has a promising inhibitory activity and that is only against EAC cells, but not against K562 cells.

Figure 5.1.4. Cytotoxicity assay of C31



From a perusal of the available literature, except monastrol and its derivatives, the simple dihydropyrimidinones (DHPMs) are not been screened for its cytotoxicity. Thus here an attempt is made to have a preliminary assessment of the DHPMs for the cellular viability using only two cancer cell lines by MTT assay method. The compounds with the dihydropyrimidinone unit selected for analysis are U21, U24, U216, U217, U416, U517 and T21 that have different substitutions at 4 and 5 positions of dihydropyrimidinone ring.

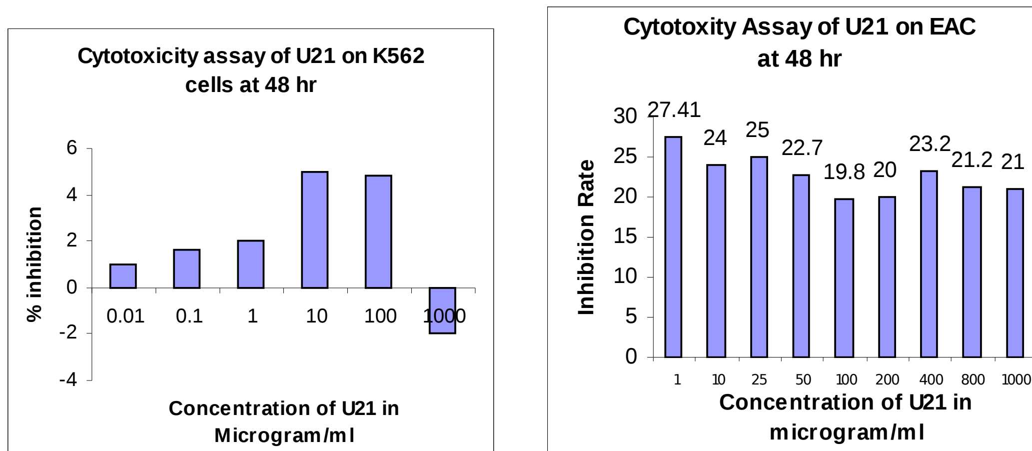
Figure 5.1.5



Consider the compound U21 (Figure 5.1.5). The maximum growth inhibition rate against K562 cell lines is found to be 4.8% at a concentration 10-100 μg/mL. At the same time it has an inhibition of 27.4% against EAC cells at a concentration 1 μg/mL, and on increasing the concentration inhibition rate is decreased.

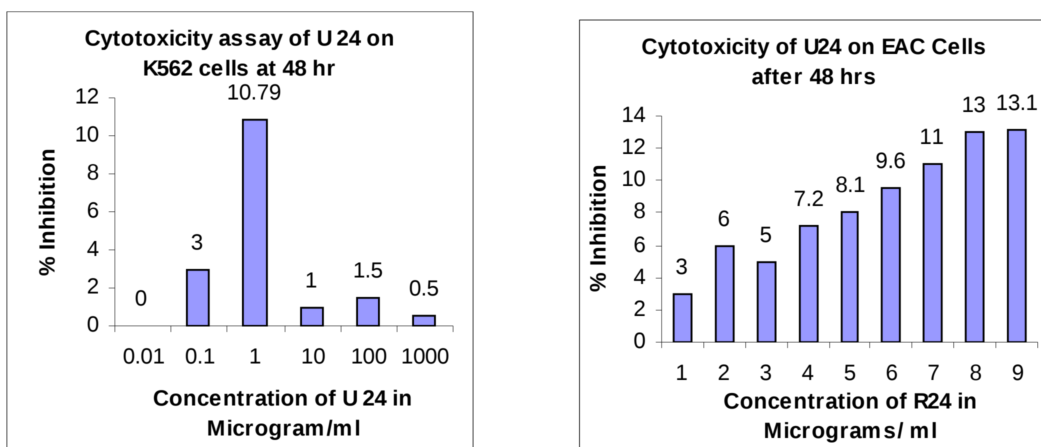
Yet another interesting feature on screening this compound is the exhibition of proliferation against K562 cells. The proliferation started at concentration 1000 μg/ ml with a value 2% (Figure 5.1.6).

Figure 5.1.6. Cytotoxicity assay of U21



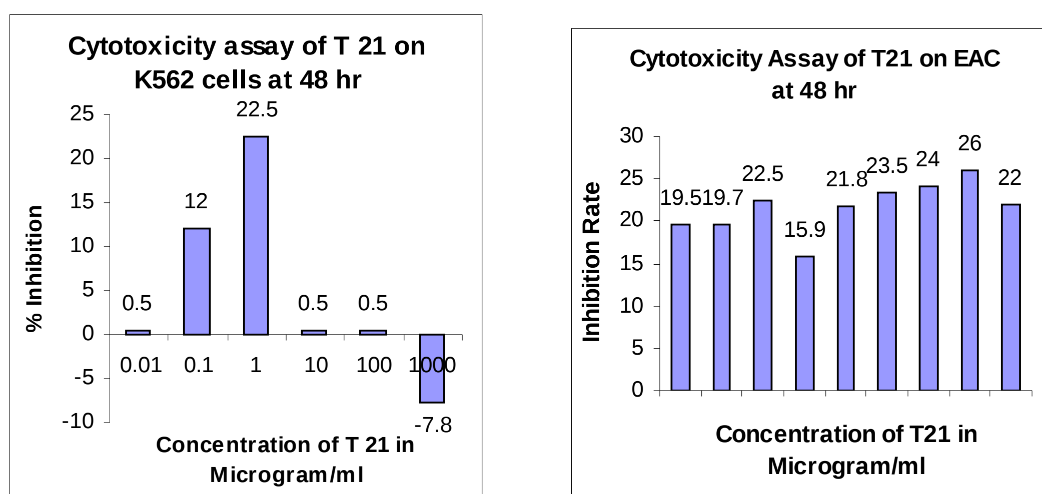
Compound U24 (Figure 5.1.5) displays a maximum inhibition of 13.1% against EAC cells at concentration 1000 µg/mL and 10.8% against K562 cells at concentration 1 µg/mL. When concentration was increased from 1 µg/mL the growth inhibition rate was decreased on K562 cells (Figure 5.1.6). Same results were obtained when concentration was decreased from 1 µg/mL.

Figure 5.1.7. Cytotoxicity assay of U24



Another compound selected was a pyrimidinone with a thione unit, T21 (Figure 5.1.4). It exhibited a maximum growth inhibition of 26% around the concentration 800 μg/ mL against EAC cells and 22.5% against K562 cells at a concentration 1μg/ mL. It found to display a proliferation of 7.8% at a concentration 1000 μg/ ml.

Figure 5.1.8. Cytotoxicity assay of T21



U216 (Figure 5.1.9) is a molecule with two dihydropyrimidone moieties. It has a maximum inhibitory rate of 10% against EAC cells at concentration 200 μg/mL, but a very low inhibition maximum of 4% at high concentration 1000 μg/mL on K562 cells (Figure 5.1.10).

Figure 5.1.9

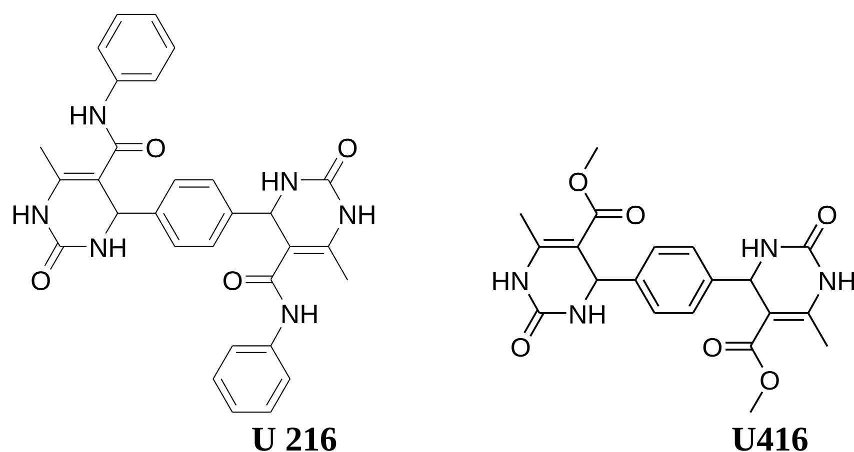
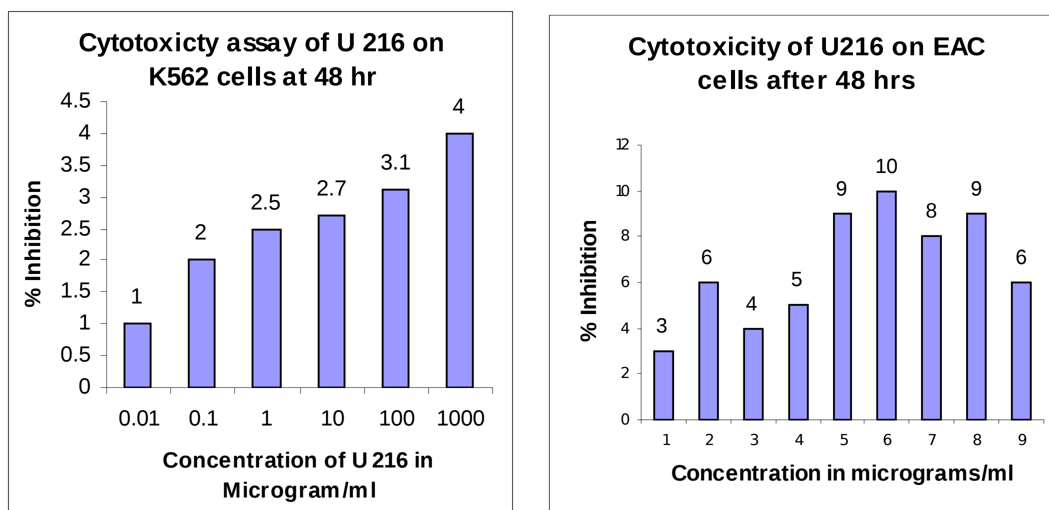


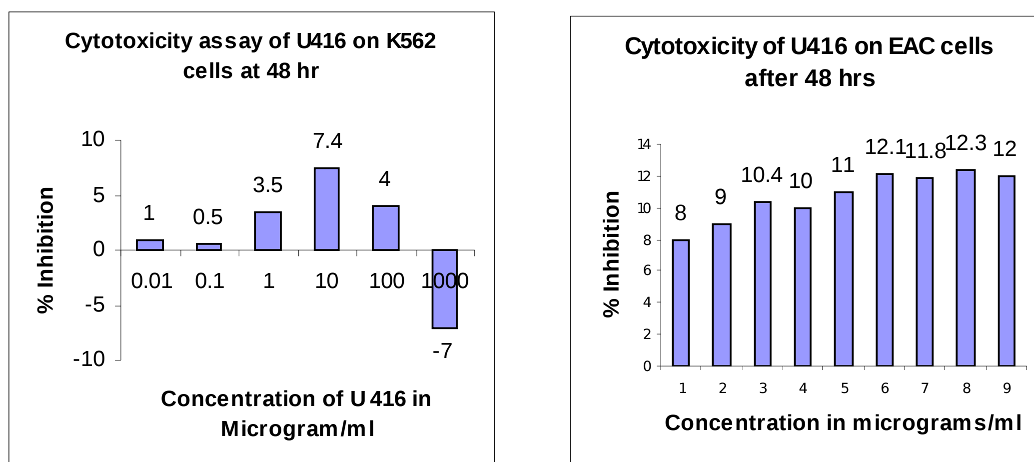
Figure 5.1.10. Cytotoxicity assay of U216



U416 (**Figure 9**) is another bidihydropyrimidinones with two CH_3OCO- group at positions 5 and 5'. On cytotoxicity assay it showed a maximum inhibition of 7.4% at 10 μg/mL concentration against K562 cells and 12.3% on EAC cells at a concentration of 800 μg/mL. Also it displayed a

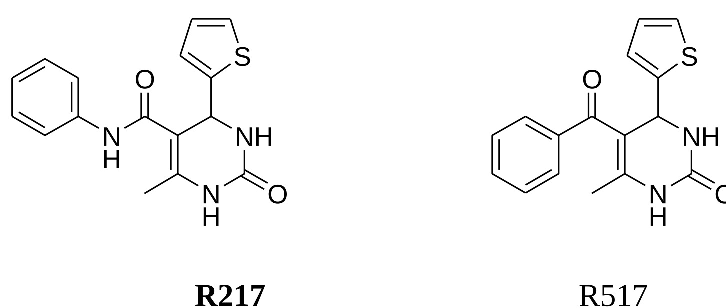
proliferation 7% against K562 cells at higher concentration, 1000 μg/mL (Figure 5.1.11).

Figure 5.1.11. Cytotoxicity assay of U416



U217 and U517 are sulfur-containing compounds with thienyl moiety on dihydropyrimidinone ring under consideration as shown in Figure 5.1.12.

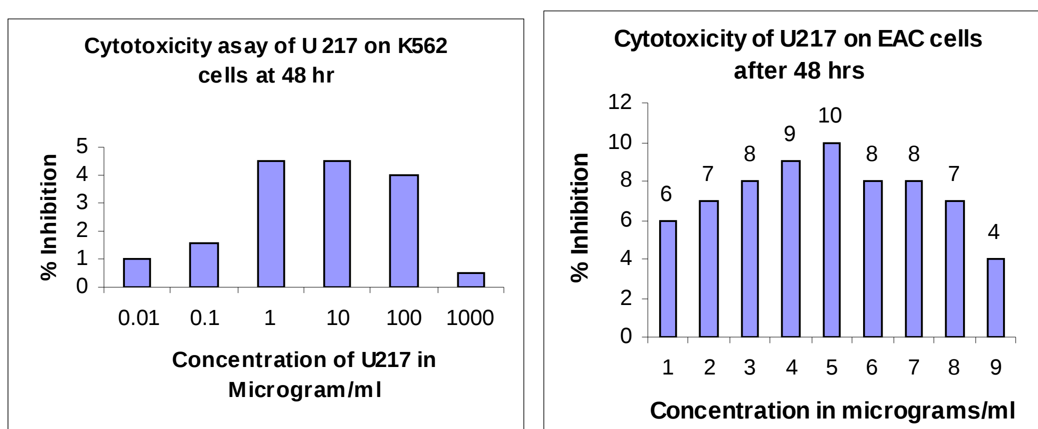
Figure 5.1.12



R217 R517

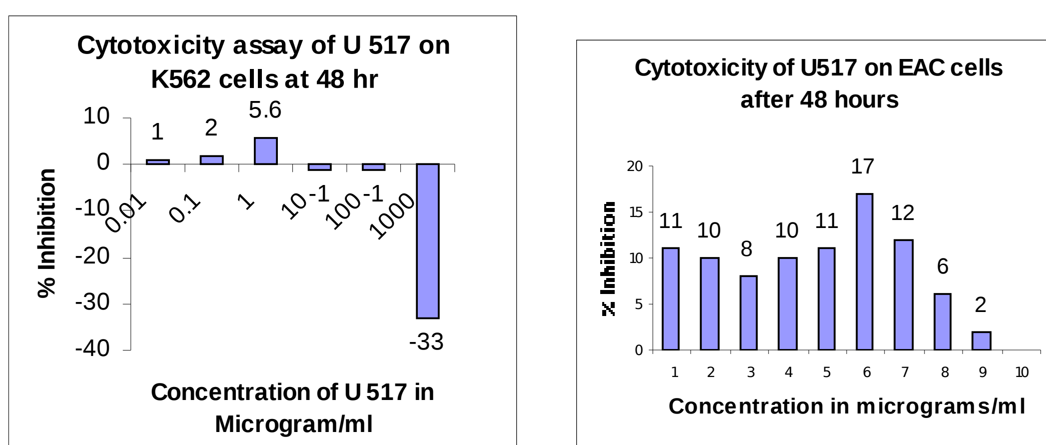
The cytotoxicity screening of compound U217 showed that it has a maximum inhibition rate of 10% at a concentration 200 μg/mL against EAC cells and 4.5% against K562 cells at 1 μg/mL concentration (Figure 5.1.13).

5.1.13. Cytotoxicity assay of U217



The compound, U517 have a C_6H_5CO group at position 5 of the DHPM ring. When cytotoxicity assay of this compound was carried out on K562 cells some interesting results were obtained. It showed a maximum inhibition of only 5.6% at concentration 1 µg/mL but when concentration increased to1000 µg/mL a proliferation of 33% was observed. Against EAC cells it has no proliferation, but has a maximum inhibition of 17% at concentration 200 µg/mL (Figure 5.1.14). The negative value of growth inhibition against K562 cells may be confirmed as proliferation only after the cell viability assessment on noncancerous lymphocytic cells.

Figure 5.1.14. Cytotoxicity assay of U517



5.1.4 Experimental

All the compounds under consideration for screening have been synthesized using the procedures described in Part I and Part II. All the compounds were accurately weighed and prepared solutions in DMSO with concentration ranging from 0.01- 1000 μg/mL. Two cancer cell lines, K562 the leukemia cell lines obtained from the National Centre for Cell Sciences (NCCS), Pune and Ehrlich Ascites Carcinoma (EAC) cells that are developed in the peritoneal cavity of BALB/c mice, were used for the cytotoxicity assay. Microtitre plates (Invitrogen, India) with 96 well were used for seeding the cells. The incubation at different period is carried out using a Heraeus CO_2 incubator and final absorbance reading was taken using an ELISA reader (Multiscan India).

5.1.4.1 MTT cellular viability assessment protocol:

Compound at different concentrations were added into a microtitre plate (5000 cells/well) seeded with 100 μL of cells. Incubated for different time intervals at 37°C in 5% CO_2. Outer wells were covered with OPBS buffer, in order to prevent the evaporation of the samples. Medium along with cells (100 μL) and medium along with compound (100 μL) together have a

volume 200 μL. Plates were incubated in CO_2 incubator for 48 hours and later centrifuged in a plate centrifuger at 2000 rpm for 10 minutes. The supernatant solution is discarded and the pellet obtained is washed with 100μL PBS buffer. 100 μL MTT dye along with RPMI medium and 100 μL MTT lysis buffer were added and incubated for 4 hours, in a CO_2 incubator. The absorbances were measured using ELISA reader at 570 nm. All the results obtained are summarized in Table 5.1.1.

5.1.5 Conclusions

In the present study a pleliminary screening on the anticancer activity of some coumarins and dihydropyrimidinones have been carried out. Some interesting results were obtained from the cellular viability assessment of these molecule on cancer cell lines K562 and EAC cells, through MTT assay. Among the tested compounds C13, U21 and T21 are more active compounds. These three compounds showed growth inhibition against both the cell lines with different extent. However growth of EAC cells were more inhibited than the K562 cells. The compound T21 is having high potential as anticancer agent as it has inhibition against both the cell lines tested. Activities of other compounds vary with cell lines on which the assessment has been carried out. Compounds C31, U24, U216, U217, U416 and U517 has more inhibition on the EAC cells. At the same time on K562 it is not having much growth inhibiton. The cytotoxicity assessment of the above mentioned molecules and the remaining molecules on more cancer cell lines are in progress in collaboration with the Regional Cancer Centre (RCC), Trivandrum.

5.1.6. References

1. Mishra, N.C.; Kumar, S. *Ind. J. Exp. Biology*, **2005**,*43*, 25.

2. Steller, H. *Science* **1995**, *267*, 1445.

3. Kerr, J.F.R.; Wylie, A. H.; Currie, A. R. *Br. J. Cancer* **1972,** *26*, 239.

4. Hill, R.P. *Clinical oneology*: "The biology of Cancer: A multidisciplinary approach for physician and students **2001,** 6, 8, 32-45.

5. Banchereau, J.; Steinman, R. M. *Nature* **1998**, *392*, 245, 1168.

6. Ian, F. T.; Richard, P. H.; Robert, G. B.; Lea, H. **2005,** Introduction to caner biology. The basic science of oncology, 4; 1-2.

7. William, B.C., Gregory, J.T. 2002, "Cancer genes, The molecular Basis of Human Cancer, 14: 45-47" (2002).

8. Raphael, E.P., James, H.P., James, G.G., David, K., Jin, P.K., Brian, O.S "Molecular Biology of Cancer. *Manual of Clinical Oncology*" 7: 48-51, (1999).

9. Lee, S.; Sivakumar, K.; Shin, W. S.; Xiea, F.; Wanga, Q. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 4596.

10. Kelly, V. P.; Ellis, E. M.; Manson, M. M.; Chanas, S. A.; Moffat, G. J.; McLeod, R.; Judah, D. J.; Neal, G. E.; Hayes, J. D. *Cancer Res.* **2000**, *60*, 957.

11. Wattenberg, L. W.; Lam, L. K. T.; Fladmoe, A. V. *Cancer Res*. **1979**, *39*, 1651.

12. Nam, N. H.; Kim, Y.; You, Y. J.; Hong, D. H.; Kim, H. M.; Ahn, B. Z. *Bioorg. Med. Chem. Lett*. **2002**, *12*, 2345.

13. Cai, Y.; Kleiner, H.; Johnston, D.; Dubowski, A.; Bostic, S.; Ivie, W.; DiGiovanni, J. *Carcinogenesis* **1997**, *18*, 1521.

14. Bokaeva, s.S. r. Kaz. Nauch. 0 Issled. Inst. - Onkol. Radiol. 1967, 3, 305-309 [*Chem Abstr*. 1968, 68, 103775q].

15. Matsuda, T; Hirao, I. Nippon Kagaku Zasshi 1965, 86, 1195-1197.

16. Kumsars, K.; Velena, A.; Duburs, G.; Uldrikis, J.; Zidermane, A. *Biokhimiya* **1971**, *36*, 1201.

17. Kato, T. Japn. Kokai Tokkyo Koho JP **1984**, 59, 190, 974 [*Chem. Abstr*. **1985**, *102,* 132067].

18. Russowsky, D.; Canto, R. F. S.; Sanches, S. A. A.; D’Oca, M. G. M.; Fa´tima, A.; Pilli, R. A.; Kohn, L.K.; Antoˆnio, M. A.; Carvalho, J. E. *Bioorg. Chem*. **2006**, *34*, 173.

19. Jim, C.; Donald, B.; Roy, A. J.; Spence, O. "Principles of treatment, Oxford Hand Book of oncology, 83, pp.189 (2002). B. Pat, P., Karol, S. "Therapy, Treatment of Cancer" 3rd edition, 11-13.

5. 2.1 Introduction

5.2.1.1. Antioxidants

Antioxidants are substance when present in trace amounts inhibits oxidation of the bulk. The main characteristic of an antioxidant is its ability to trap free radicals. There are two broad classes of antioxidants, which are ‘preventive’ and ‘chain-breaking’. Preventive antioxidants reduce the rate of chain initiation and chain-breaking antioxidants reduce the rate of chain propagation in a free radical reaction. Highly reactive free radicals and oxygen species are present in biological systems from a wide variety of sources. These free radicals may oxidize nucleic acids, proteins, lipids or DNA and can initiate degenerative diseases. Antioxidant compounds like phenolic acids, polyphenols and flavonoids scavenge free radicals such as peroxide, hydroperoxide or lipid peroxyl and thus inhibit the oxidative mechanisms that lead to degenerative diseases. These compounds in food play an important role as a health-protecting factor. Scientific evidence suggests that antioxidants reduce risk for chronic diseases including cancer and heart disease.

Primary sources of naturally occurring antioxidants are whole grains, fruits and vegetables. Plant originated food antioxidants like vitamin C, vitamin E, carotenes, phenolic acids, phytate and phytoestrogens have been recognized as having the potential to reduce disease risk. Most of the antioxidant compounds in a typical diet are derived from plant sources and belong to various classes of compounds with a wide variety of physical and chemical properties. Some compounds, such as gallates, have strong antioxidant activity, while others, such as the mono-phenols are weak antioxidants. The free radical scavenging activity of antioxidants in foods have been substantially investigated and reported in the literature by Miller

and Rigelhof *et.al*.[1] The commonly available synthetic antioxidants are t-butyl hydroxy toluene (BHT) and t-butyl hydroxy anisole (BHA).

5.2.1.2. Method Considerations

Various antioxidant activity measurements have been used to monitor and compare the antioxidant activity. In recent years, oxygen radical absorbance capacity assays and enhanced chemiluminescence assays have been used to evaluate antioxidant activity of foods, serum and other biological fluids. These methods need special equipment and technical skills for the analysis. These types of methods published in the literature for the determinations of antioxidant activity involve electron spin resonance (ESR) and chemiluminescence methods. These analytical methods measure the radical scavenging activity of antioxidants against free radicals like 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical, superoxide anion radical (O_2), hydroxyl radical (OH), or peroxyl radical (ROO).

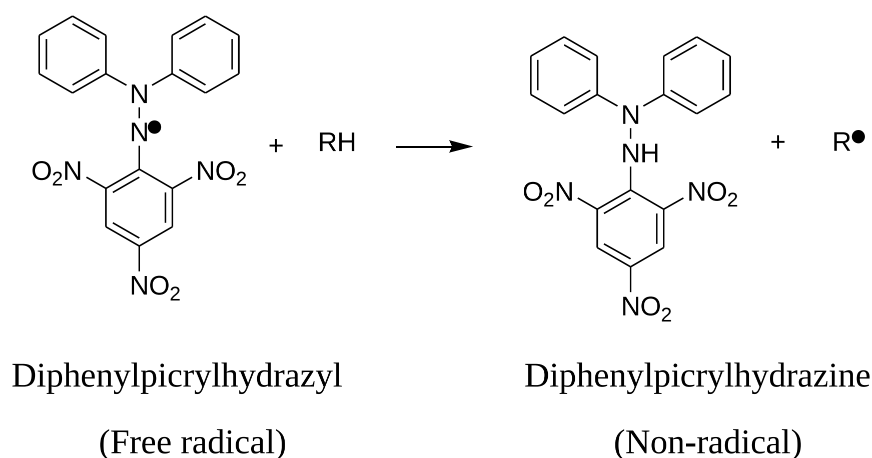
Antioxidant activity methods using free radicals are fast, easy and simple. The ABTS [2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid)] radical cation[1] has been used to screen the relative radical-scavenging abilities of flavonoids and phenolics through their properties as electron- or H-donating agents. Prior *et al*.[2] have used the Oxygen Radical Absorbance Capacity (ORAC) procedure to determine antioxidant capacities of fruits and vegetables. In the ORAC method, a sample is added to the peroxyl radical generator, 2,2'-azobis(2-amidinopropane)dihydrochloride (AAPH) and inhibition of the free radical action is measured[3] using the fluorescent compound, B-phycoerythrin or R-phycoerythrin.

5.2.1.3. The DPPH Method - Principle

A rapid, simple and inexpensive method to measure antioxidant capacity involves the use of the free radical, 1,1-diphenyl-2-picrylhydrazyl

(DPPH). DPPH is widely used to test the ability of the compounds to act as free radical scavengers or hydrogen donors, and to evaluate antioxidant activity of foods and to quantify antioxidants in complex biological systems. The DPPH method can be used for solid or liquid samples and is not specific to any particular antioxidant component, but applies to the overall antioxidant capacity of the sample. The structure of DPPH and its reduction by an antioxidant are shown in Figure 5.2.1.

Figure 5.2.1



Diphenylpicrylhydrazyl (Free radical) | Diphenylpicrylhydrazine (Non-radical)

The odd electron in the DPPH free radical gives a strong absorption maximum at 517 nm and is purple in color. The color turns from purple to yellow as the molar absorptivity of the DPPH radical at 517 nm reduces from 9660 to 1640 when the odd electron of DPPH radical becomes paired with hydrogen from a free radical scavenging antioxidant to form the reduced DPPH-H. The resulting decolorization is stoichiometric with respect to number of electrons captured.

5.2.1.4. The Parameter EC_{50} or IC_{50}

One of the parameters that has been used to express the antioxidant activity of the compounds using the results from DPPH method is the 'efficient concentration', EC_{50} or 'inhibition coefficient', IC_{50} value. This is defined as the concentration of substrate that causes 50% loss of the DPPH activity (colour). Antioxidants react at different rates i.e. differing kinetics, and the reaction will often not go to completion in a reasonable assay time. Therefore, sample size that can lower the initial absorbance of DPPH solution by 50% has been selected as the endpoint for measuring the antioxidant activity. This parameter was apparently introduced by Brand-Williams and his colleagues[4] and has been used subsequently by several groups of workers for presenting their results.[5]

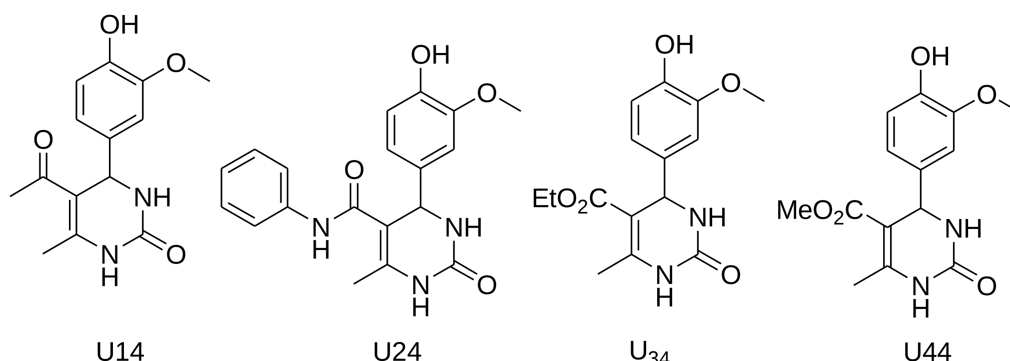
5.2.2. Review of Literature

Dihydropyrimidinones and coumarin derivatives have attracted interest in medicinal chemistry, exhibiting pharmacological and therapeutic properties. Although, during the previous years extensive studies on biological and pharmacological activity of these scaffolds have been reported, only very few reports have been appeared on the antioxidant activity. In one of the recent reports Stefani *et al.*[6] have reported the antioxidant activity of some DHPMs by lipid peroxidation, reactive oxygen species (ROS) measurement and thiol-peroxidase activity. No other relevant report on the antioxidant activity of coumarins and DHPMs could be found. The radical scavenging activity of these compounds on DPPH has never been appeared in the literature. In the present study the determination of the radical scavenging activity of the coumarins and dihydropyrimidinones on DPPH has been attempted.

5.2.3. Results and Discussion

In the present analysis a series of coumarin derivatives and dihydropyrimidinones described in Part 1 and 2 respectively are subjected to antioxidant assay by DPPH method. None of the coumarin derivatives found to have the antioxidant activity. Among the library of 40 dihydropyrimidinones, only four compounds showed the antioxidant activity. These compounds are U14, U24, U34 and U44. These compounds have similar structure with a variation of the substitution at position 5 of the dihydropyrimidinone ring as shown below in Figure 5.2.2.

Figure 5.2.2



The results obtained for these active compounds have been shown in Figures 5.2.3 to 5.2.6 and Table 1-4. Using the same method the antioxidant activity of two standards, a natural antioxidant, gallic acid and a synthetic antioxidant BHT have been assessed and the values obtained have been expressed in Figures 5.2.7- 5.2.8 and Table 6. A comparison of the activity of the investigated compounds with that of the standards have been made and tabulated in Table 7.

In this method a fixed volume of the DPPH solution whose absorbance is less than 1 is mixed with different volumes of the sample solution and the decrease in the absorbance is noted at 517 nm. From the absorbance value the 'Percentage difference' and 'Percentage Inhibition' in each case can be calculated using the equations;

% Difference = [Sample absorbance/ DPPH absorbance (blank)] x100

% Inhibition = 100 – (% Difference)

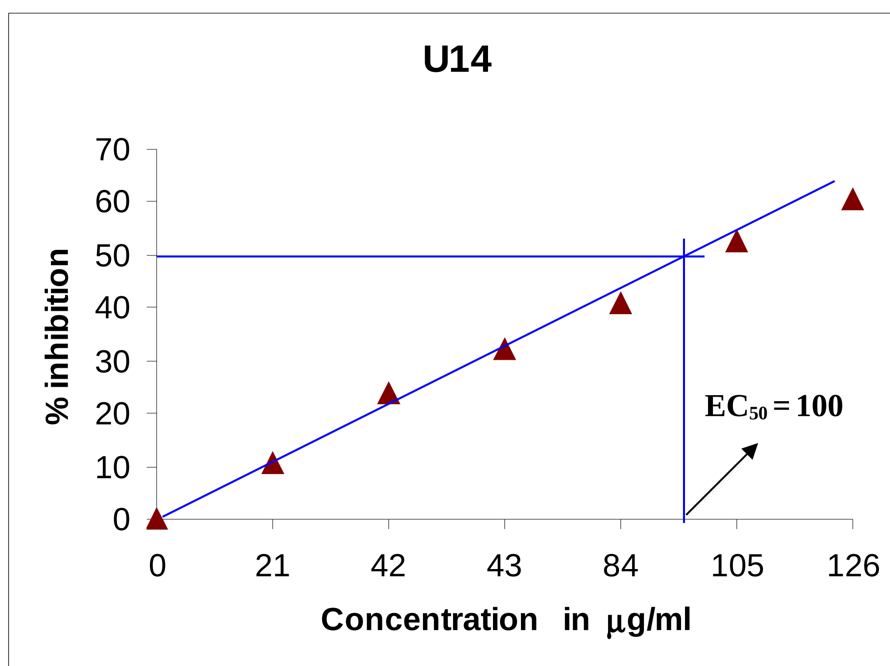
The EC_{50} values are determined from the calibration curve in which the % inhibition is plotted against concentration.

5.2.3.1. Antioxidant Assay of Compound U14

Table 5.2.1

Sample Volume (µL)	Absorbance	% Difference	% Inhibition	Concentration (µg/ml)	EC_{50} (µg/ml)
0	0.8721	--	--	--	
100	0.7801	89.5	10.5	21	
200	0.6627	76.0	24.0	42	
300	0.5937	68.0	32.0	63	100
400	0.5149	59.0	41.0	84	
500	0.4136	47.4	52.6	105	
600	0.3429	39.3	60.7	126	

Figure 5.2.3

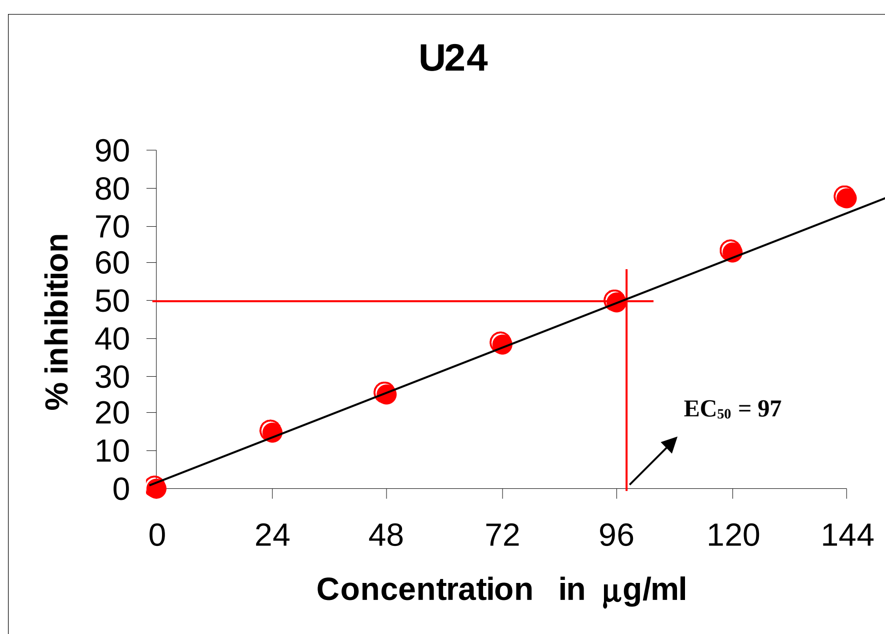


5.2.3.2. Antioxidant Assay of Compound U24

Table 5.2.2

Sample Volume (µL)	Absorbance	% Difference	% Inhibition	Concentration (µg/ml)	EC_{50} (µg/ml)
0	0.9164	--	--	--	
100	0.7811	85.2	14.8	24	
200	0.6883	75.1	24.9	48	
300	0.5648	61.6	38.4	72	97
400	0.4635	50.6	49.4	96	
500	0.3380	36.9	63.1	120	
600	0.2094	22.9	77.1	144	

Figure 5.2.4

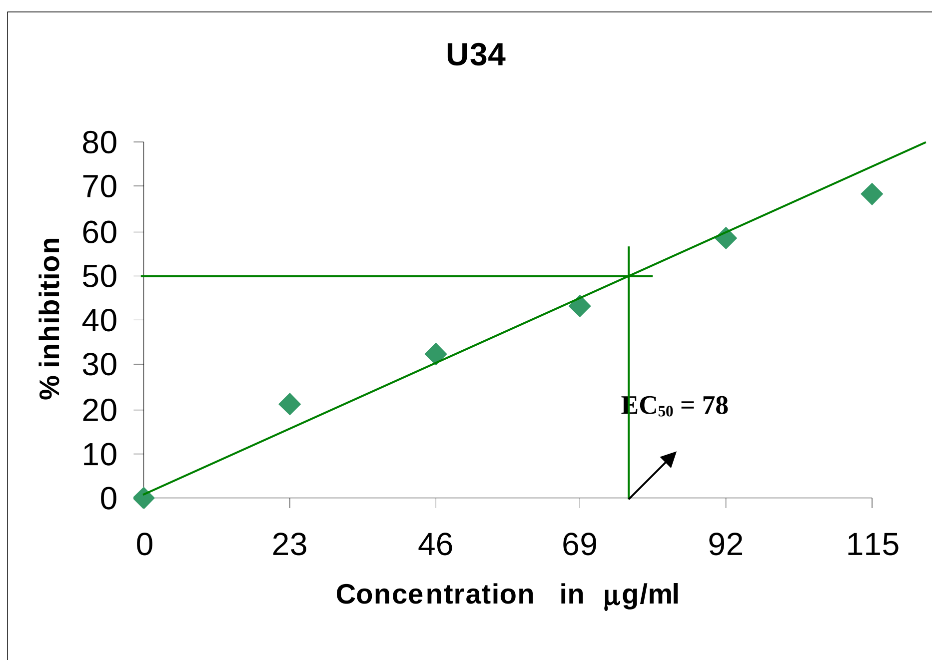


5.2.3.3. Antioxidant Assay of Compound U34

Table 5.2.3

Sample Volume (Microlitres)	Absorbance	% Difference	% Inhibition	Concentration (μg/ml)	EC_{50} (μg/ml)
0	0.9346				
100	0.7386	79.0	21.0	23	
200	0.6319	67.6	32.4	46	78
300	0.5333	57.0	43.0	69	
400	0.3901	41.7	58.3	92	
500	0.2968	31.8	68.2	115	

Figure 5.2.5

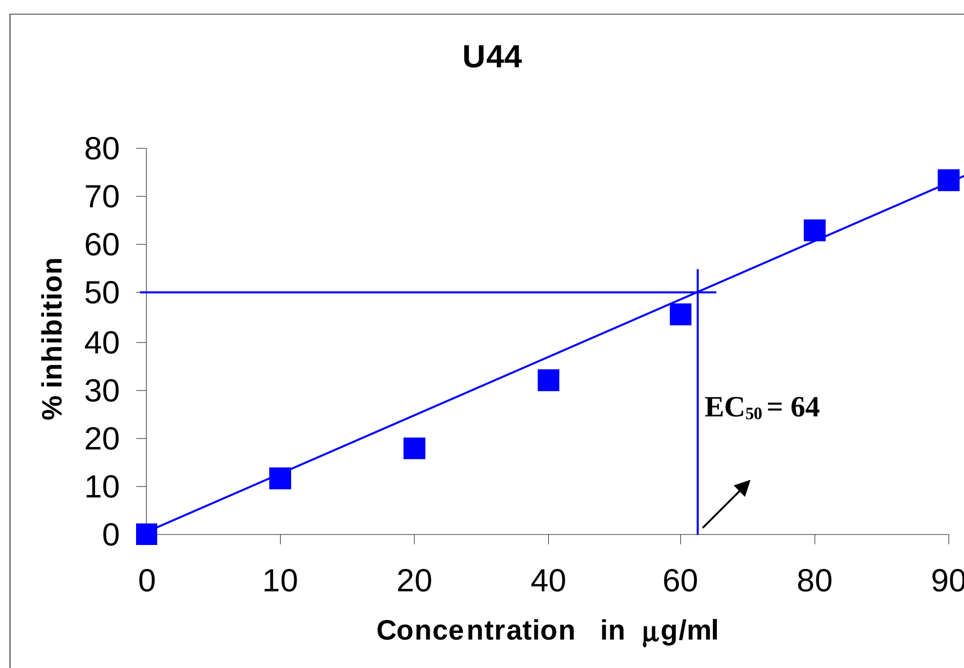


5.2.3.4. Antioxidant Assay of Compound U44

Table 5.2.4

Sample Volume (Microlitres)	Absorbance	% Difference	% Inhibition	Concentration (µg/ml)	EC_{50} (µg/ml)
0	0.8845				
50	0.7820	88.4	11.6	10	
100	0.7276	82.3	17.7	20	
200	0.6032	68.2	31.8	40	64
300	0.4829	54.6	45.4	60	
400	0.3262	36.9	63.1	80	
450	0.2373	26.8	73.2	90	

Figure 5.2.6

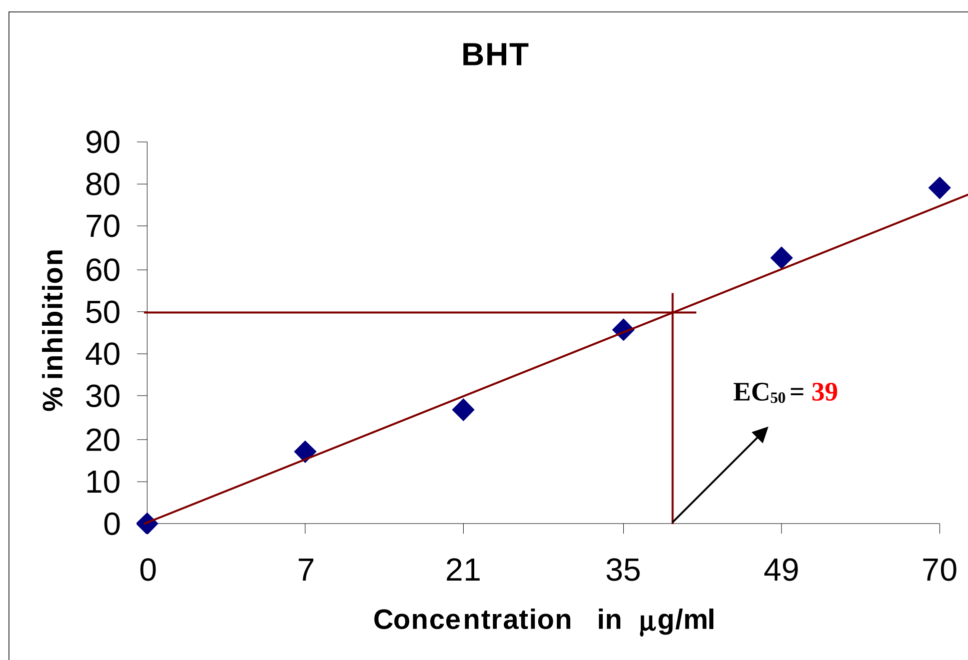


5.2.3.5. Antioxidant Assay of Standard 2 (BHT)

Table 5.2.5

Sample Volume (Microlitres)	Absorbance	% Difference	% Inhibition	Concentration (µg/ml)	EC_{50} (µg/ml)
0	0.7741	--	--	--	
10	0.6424	83	17	7	
30	0.5728	74	26	21	39
50	0.4216	54.5	45.5	35	
70	0.2869	37.1	62.9	49	
100	0.1606	2.07	79.3	70	

Figure 5.2.7



5.2.3.6. Antioxidant Assay of Standard 1(Gallic acid)

Table 5.2.6

Sample Volume (Microlitres)	Absorbance	% Difference	% Inhibition	Concentration (µg/ml)	EC_{50} (µg/ml)
0	0.7741				
50	0.6922	89.4	10.6	2.35	
100	0.6116	79	21	4.7	
150	0.5434	70.2	30	7.05	11.6
200	0.4753	61.4	38.6	9.4	
250	0.3714	48	52	11.75	
300	0.3273	42.3	57.7	14.1	
400	0.2274	29.3	70.7	18.8	

Figure 5.2.8

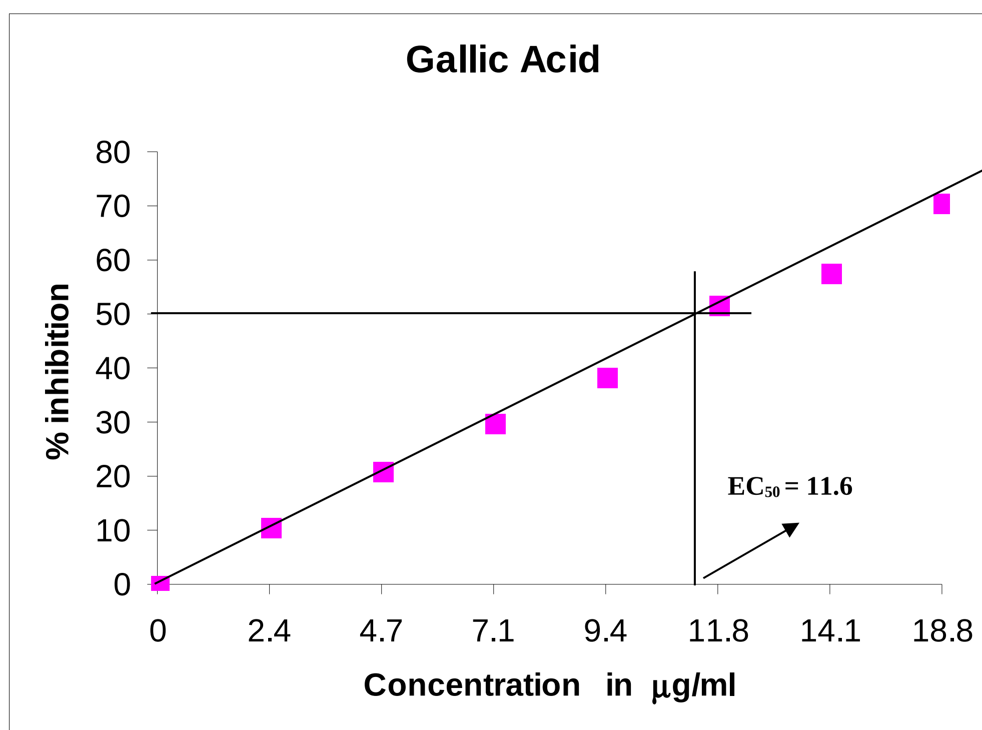
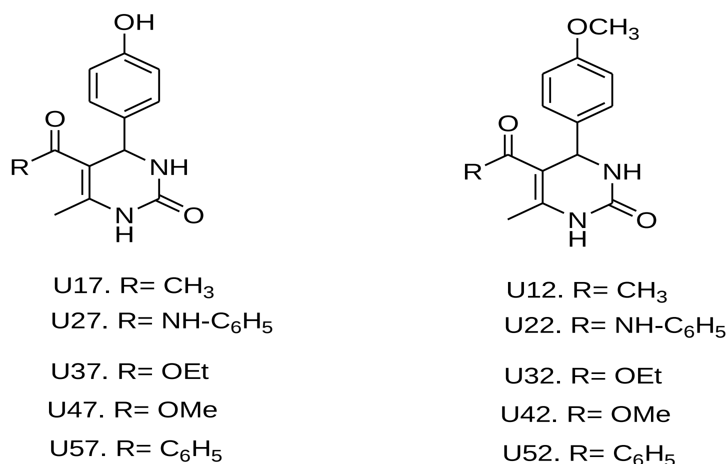


Table 5.2.7 Comparison of antioxidant activity of DHPMs with the Standard antioxidants, gallic acid and BHT

Compound	**EC_{50} (µg/mL)**	**% Activity compared to Gallic acid EC_{50} = 11.6 µg/mL**	**% Activity compared to BHT EC_{50} = 39 µg/mL**
U14	100	11.6	39
U24	97	12	40
U34	78	15	50
U44	64	18	61

From the above results it is evident that the dihydropyrimidinones with a methoxy substituted phenolic moiety is exhibiting better free radical scavenging activity. The pyrimidone ring is not contributing to the radical inhibition, which is apparent from the analysis carried out on other DHPMs. Also the phenolic unit alone cannot impart antioxidant activity to the molecule. It is clear from antioxidant analysis carried out on the molecules U17, U27, U37, U47 and U57 (Figure 5.2.9). These molecules didn't show any radical inhibition on DPPH. Similarly the methoxy-substituted derivatives (U12, U22, U32, U42 and U52) are also inactive. Only when both the methoxy and hydroxyl groups are present on the aromatic ring substituted at position 4 of the dihydropyrimidinone ring the compounds have remarkable antioxidant activity. The presence of other substituents like chloro, nito, amino, etc. substituents did not have any influence on these activities.

Figure 5.2.9



5.2.4. Experimental

All the compounds have been synthesized using the procedure described in Part I and II and purified by chromatographic techniques and crystallization. The purity of all the reagents and solvents has been ensured before analysis. The absorbance was measured using a JASCO V-530 FT/UV-Visible Spectrophotometer. Dimethylsulfoxide (DMSO) and methanol were the solvents used for the preparation of solutions.

A solution of DPPH is prepared such that 1 ml of it in 10 ml methanol gives an absorbance of less than one. Mix 1 ml of DPPH solution with the sample solution with varying volumes (50-600 μL) and made up to 10 ml with methanol. Keep this solution in dark for 30 minutes and measure the absorbance at wavelength 517 nm. Measure the absorbance of the DPPH solution in the same manner without the sample, which give the blank absorption. By knowing the absorbance of the sample and the blank the percentage difference, percentage inhibition and hence EC_{50} can be calculated.

5.2.5. Conclusions

A library of coumarins and dihydropyrimidinones has been screened for its antioxidant property by studying the radical scavenging activity against

free radical, DPPH. None of the coumarin derivatives have got the radical inhibition against DPPH. Among the dihydropyrimidinones, four compounds were found to be potent antioxidants. The radical scavenging activities of these compounds were compared with that of the known antioxidants gallic acid and BHT. From this comparison it is found that the compound U44 is having an antioxidant activity of 61% and U34 possess an activity of 50% to that of the commonly available synthetic antioxidant BHT. Similarly U24 and U14 have 40% and 39% radical scavenging activity to that of BHT. On comparison with naturally occurring strong antioxidant, gallic acid, it was observed that all these compounds have a radical inhibition of 11-18% to that of gallic acid. Thus it is apparent from the present analysis that these compounds have extensive antioxidant potentials comparable to any synthetic antioxidants, which are being used widely.

The antioxidant screening of the dihydropyrimidinones unveil some interesting facts regarding structure and activity. These are,

a) The dihydropyrimidinone unit has a least contribution towards the radical inhibition.

b) The presence of methoxy substituted phenol unit has a profound role in determining antioxidant activity.

c) When hydroxyl or methoxy group alone are present on the aromatic ring at position 4 of the DHPM ring no activity was found.

d) Presence of other functional groups like chloro, amino and nitro groups cannot impart any antioxidant activity to the dihydropyrimidinones.

e) The substituents at position 5 of the pyrimidinone ring of the active DHPMs have only mild influence on the antioxidant activity.

f) Thus from the results obtained by the antioxidant evaluation it can be proposed that the high radical scavenging potential of the compound with a methoxy substituted phenolic moiety is due to the synergic effect of both the hydroxyl and methoxy group.

5. 2.6. References

1. Miller, H. E.; Rigelhof, F.; Marquart, L.; Prakash, A.; Kanter, M. *J. Am.Coll. Nutr*. **2000,** *19(3)***,** 312.

2. Hodges, D. M.; DeLong, J. M.; Forney, C. F.; Prange, R. K. *Planta* **1999**, *207*, 604.

3. Pellegrini, N.; Re, R.; Yang, M.; Rice Evans, C.; *Methods in Enzymology* **1999**, *299*, 379.

4. (a) Brand-Williams, W.; Cuvelier, M. E.; Berset, C. *Food Science and Technology* **1995**, *28*, 25. (b) Bondet, V.; Brand-Williams, W.; Berset, C. *Food Science and Technology* **1997,** *30*, 609.

5. (a) Kim, J. -K.; Noh, J. H.; Lee, S.; Choi, J. S.; Suh, H.; Chung, H. Y.; Song, Y.-O.; Choi, W. C. *Bull. Korean Chem. Soc*. **2002**, *23(5)*, 661.

   (b) Lebeau, J.; Furman, C.; Bernier, J.-L.; Duriez, P.; Teissier, E.; Cotelle, N. *Free Radic. Biol. Med.* **2000,** *29(9)*, 900. (c) Lu, Y.; Foo, L.Y. *Food Chemistry* **2000,** *68*, 81.

6. Stefani, H.A.; Oliveria, C. B.; Almeida, R. B.; Pereira, C. M. P.; Braga, R. C.; Cella, R.; Borges, V. C.; Savegnago, L.; Nogueira, C.W. *Euro. J. Med. Chem.* **2006**, *41*, 513.

5.3.1. Introduction

5.3.1.1. Introduction to Anti-microbial Studies

Microorganisms are universally associated with the lives of humans, other animals and plants. Some of them are beneficial and others are detrimental. They play an important role in the food and pharmaceutical industry. They are involved in the making of yogurt, cheese, wine, buttermilk and in the production of antibiotics. Microorganisms are essential for the digestive process in ruminant animals such as cattle and sheep. Legumes, which live in close association with special bacteria that form nodules on their roots. In these root nodules, atmospheric nitrogen is converted to fixed nitrogen compounds that the plants can use for growth. Besides their role as a beneficiary, microorganisms can cause disease, spoil food and deteriorate materials like iron pipes, glass lenses and wood pilings.

Each kind of microorganism has specific growth requirements. Many of them can be grown in the laboratory culture medium containing necessary nutrients for their growth and multiplication. Some of them require a supply of inorganic salts, particularly the anions, phosphate and sulphate, and the cations sodium, potassium, iron, etc. whereas others can grow in a medium containing organic compounds (amino acids, vitamins or enzymes) in minute quantities. Some others require complex natural substance (peptone, blood, serum, etc.) and microorganisms like rickettsias cannot be grown in an artificial laboratory medium. On solid culture media, microbial cells can grow and form visible masses called colonies.

5.3.1.2. Modes of action of anti-microbial agents

Microorganisms can be inhibited or killed by various physical and chemical agents. The agents that kill or destroy the organisms are known as 'cidal' whereas the one that merely prevents the growth of the microorganism

is called 'static'. If a static agent is removed from a culture, the organism will resume growth, but the effects of cidal agents are irreversible.

Several types of chemical agents damage the cell wall by blocking its synthesis. Some of them will disrupt the cell membrane, so that the cell loses its selective permeability and can neither prevent the loss of vital molecules nor bar the entry of damaging chemicals. Some others will inhibit the enzyme action and will damage the microbial life. Chemicals such as strong solvents (alcohols, acids and phenolics) coagulate bacterial proteins; some agents disrupt or denature proteins. Such losses in normal protein function can arrest bacterial metabolism, thereby inhibit the growth or kill them.

5.2. Results and Discussion

Among the tested compounds only few coumarins and dihydropyrimidinones have potential inhibition against bacteria. Only a preliminary screening was carried out and the results are summarized in Table 1.

Compound	Organisms (bacteria)			
	E. Coli	***Klebsiella sps.***	***Serratia sps.***	***S. aureus***
U14	-	-	-	-
U15	-	-	-	-
U16	-	-	-	-
U17	-	-	-	-
U21	-	-	-	-
U22	-	-	-	-
U24	-	-	-	-
U25	-	-	-	+
U26	-	-	-	-
U27	-	-	-	+
U210	-	-	-	-
U216	-	-	-	-
U217	-	-	-	-
T21	+	-	-	+
T11	-	-	-	-
U51	-	-	-	+
U52	+	-	-	+
U55	-	-	-	-
U56	-	-	-	-
U57	-	-	-	-
U510	-	-	-	-
U511	-	-	-	-
U517	-	-	-	-
C12	-	+	-	-
C21	-	+	-	-
C31	-	+	-	-

5.3. Experimental Protocol

5.3.3.1. Methods and materials

The solutions of the compounds in DMSO were prepared such that the concentration was around 5μg /mL. All the glass wares, gel puncher etc. were sterilised before use. The nutrient agar media was used to maintain the pure bacterial culture and their lawn cultures for the detection of antibacterial activity. Peptone water was used for making suspension cultures of test bacteria.

5.3.3.2. Preparation of inoculums

Each culture to be tested was streaked on to nutrient agar medium and incubated at 35^0C overnight. Using a sterile inoculation loop, a small portion of the colonies was inoculated into a conical flask containing sterile peptone water.

5.3.3.3. Screening of antibacterial activity

The agar well diffusion method by Perezetal was followed to conduct the antibacterial activity. The test organisms used were *Escherichia coli*, *Klebsiella*, *Serratia* and *Staphylococcus aureus*. The nutrient agar was prepared and the test organisms were inoculated so as to get a lawn growth. The wells were cut on the agar by using gel puncher. 25 μL of the test compound in DMSO was added to the appropriately labeled wells. Following incubation at 37^0C for overnight the plates were analysed for zones of growth inhibition.

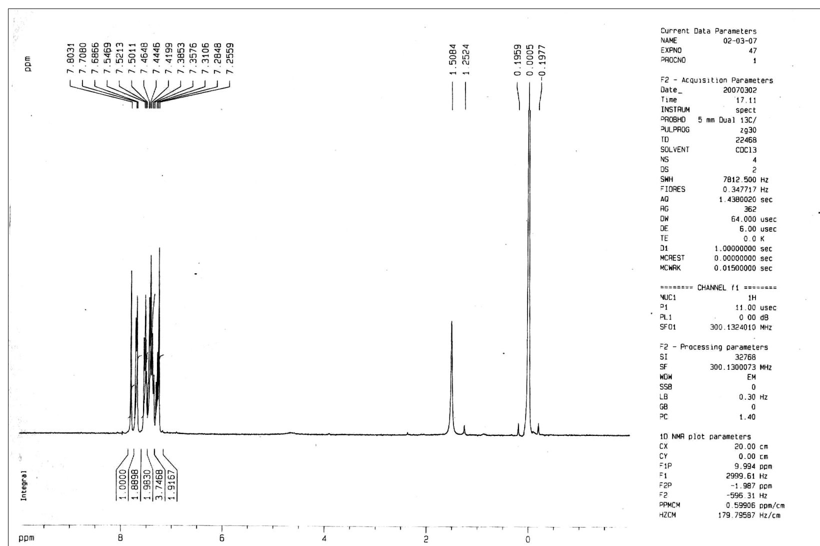
5.4. Conclusions

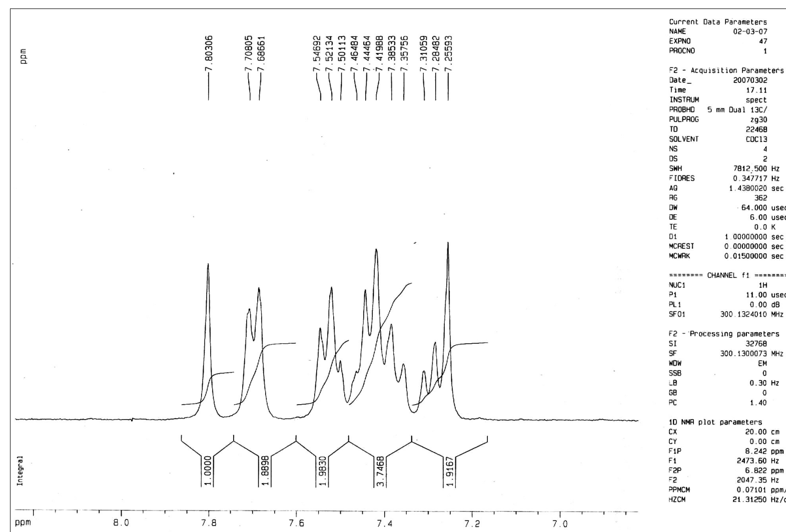
Coumarins and dihydropyrimidinones were screened for its antibacterial activities. Among the compounds tested U25, U27, T21, U51 and U52 are the DHPMs having inhibitory activity against *S.aureus*. T21 and U52 shows growth inhibitory activity against E.coli also. None of the DHPMs are active against *Klebsiella* and *Serratia.*

Among the coumarins C12, C23 and C31 are active against the gram negative bacteria *Klebsiella*. At the same time these compounds did not show any growth inhibition against other organisms considered.

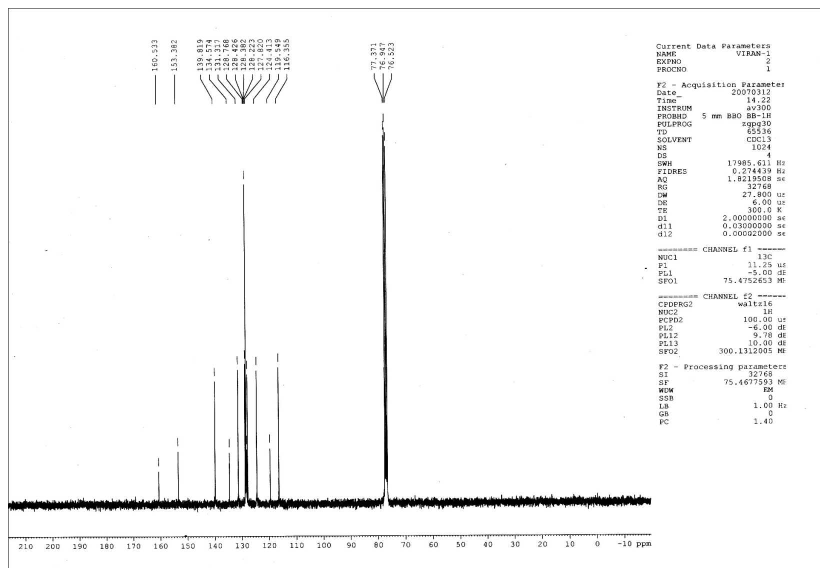
Spectra of known coumarins

Spectra of C11



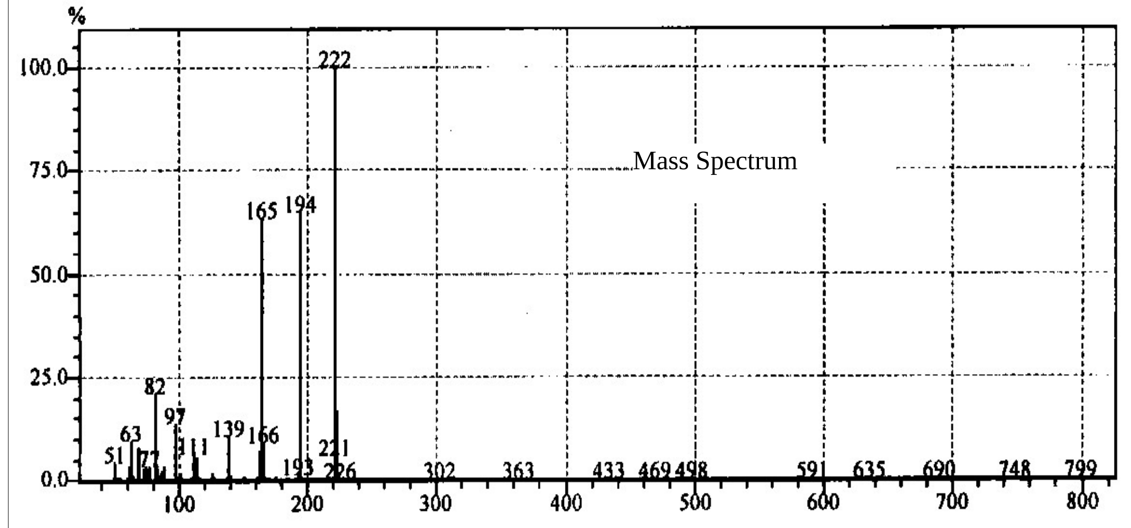


^{1}H NMR Spectrum (Expanded)



^{13}C NMR Spectrum

^{13}C NMR Spectrum (Expanded)



%
100.0
75.0
50.0
25.0
0.0
222
Mass Spectrum
165
194
82
97
63
139
166
51
77
111
221
193
226
302
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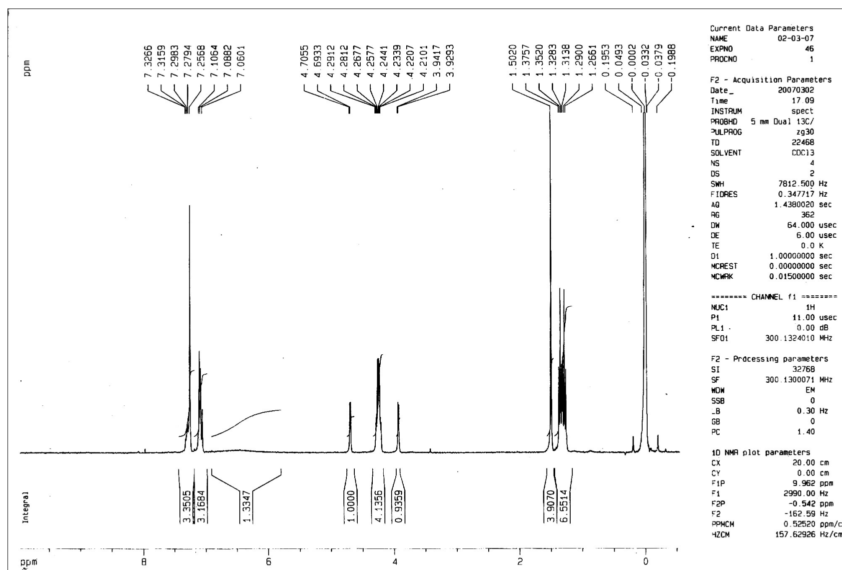
600
700
800

Spectra of C12



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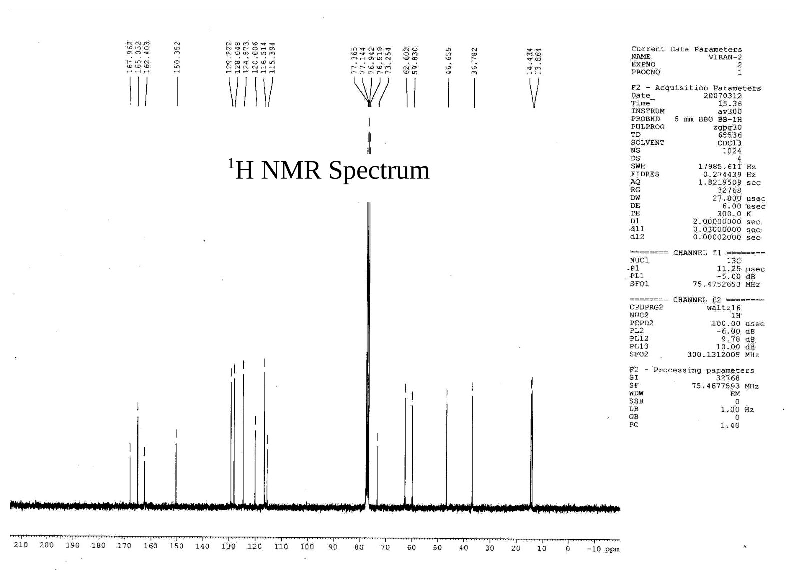

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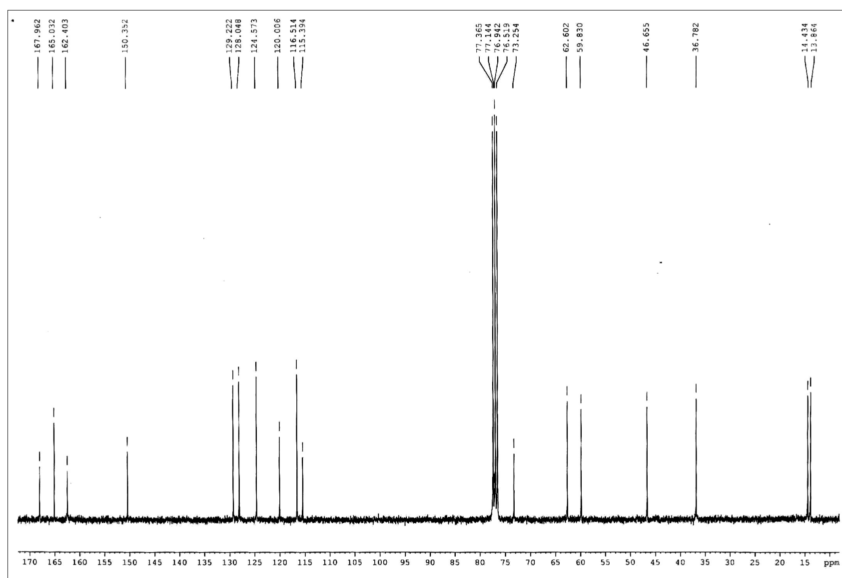

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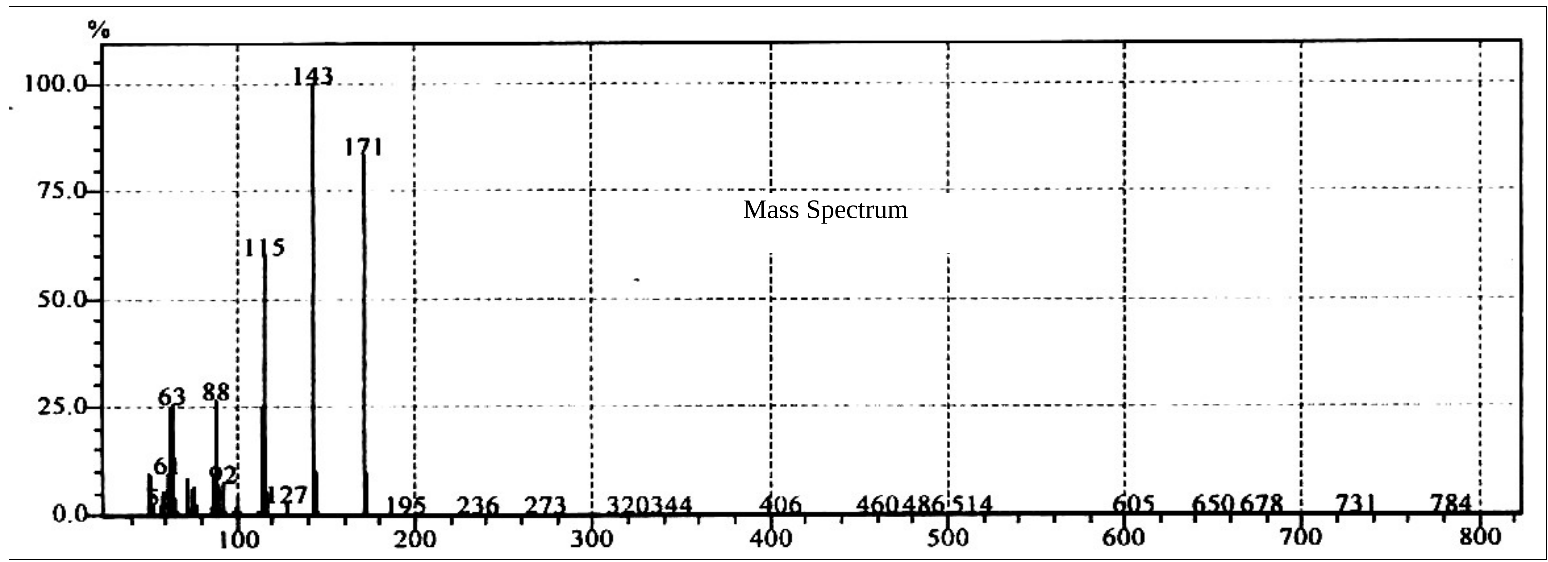
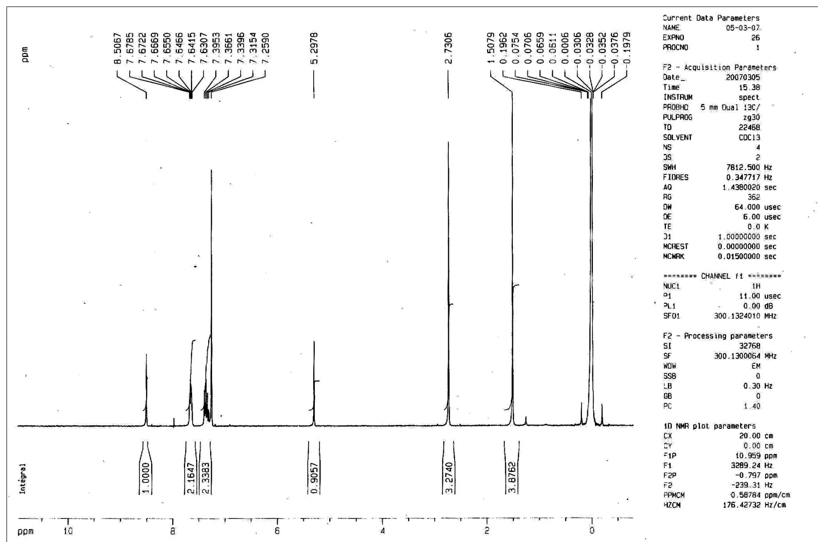
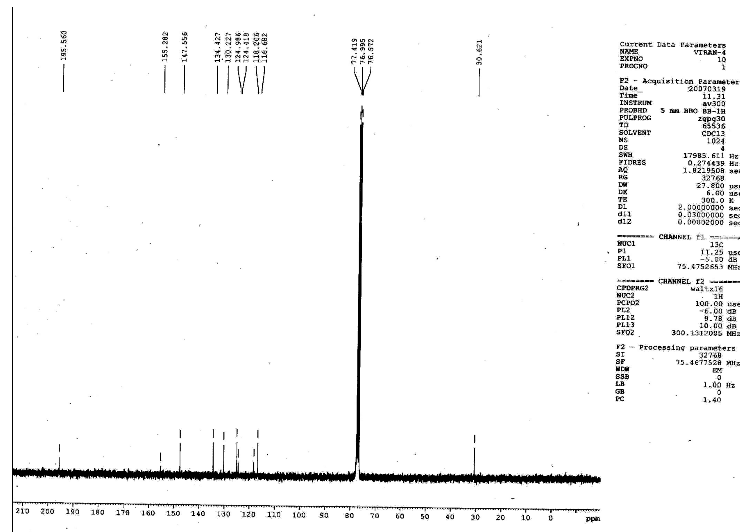


Mass Spectrum

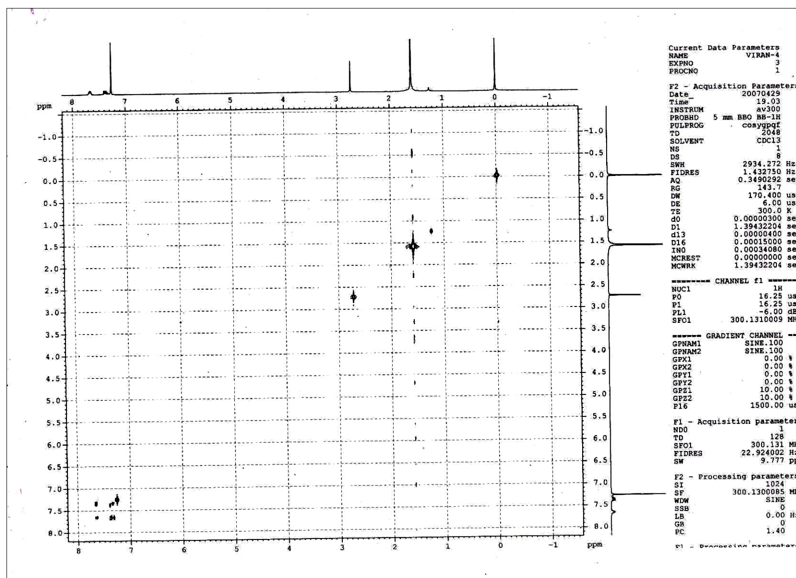
Spectra of C15

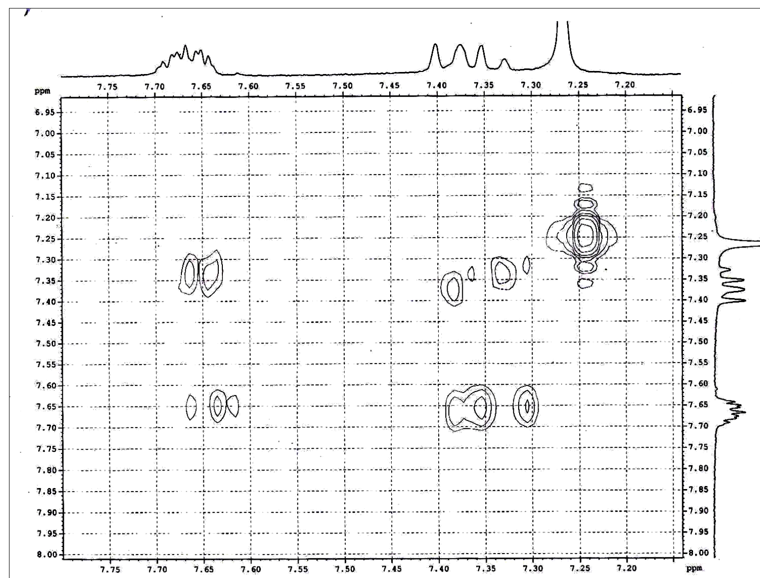




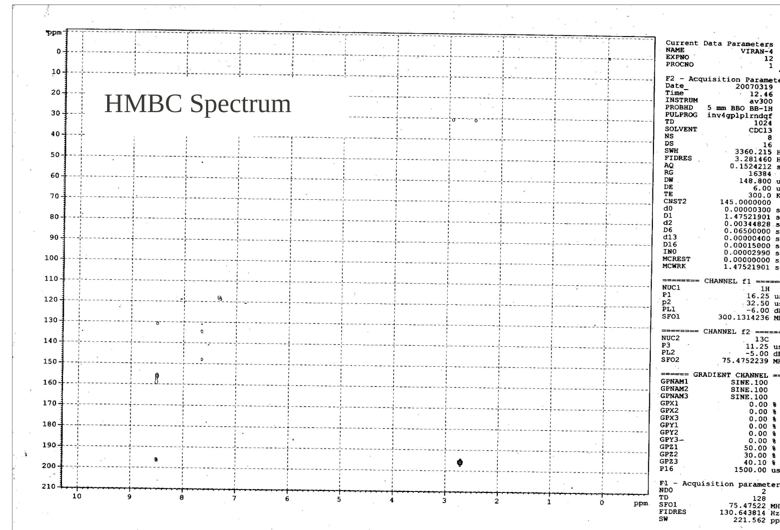
^{1}H NMR Spectrum

^{13}C NMR Spectrum





HH COSY Spectrum (Expanded)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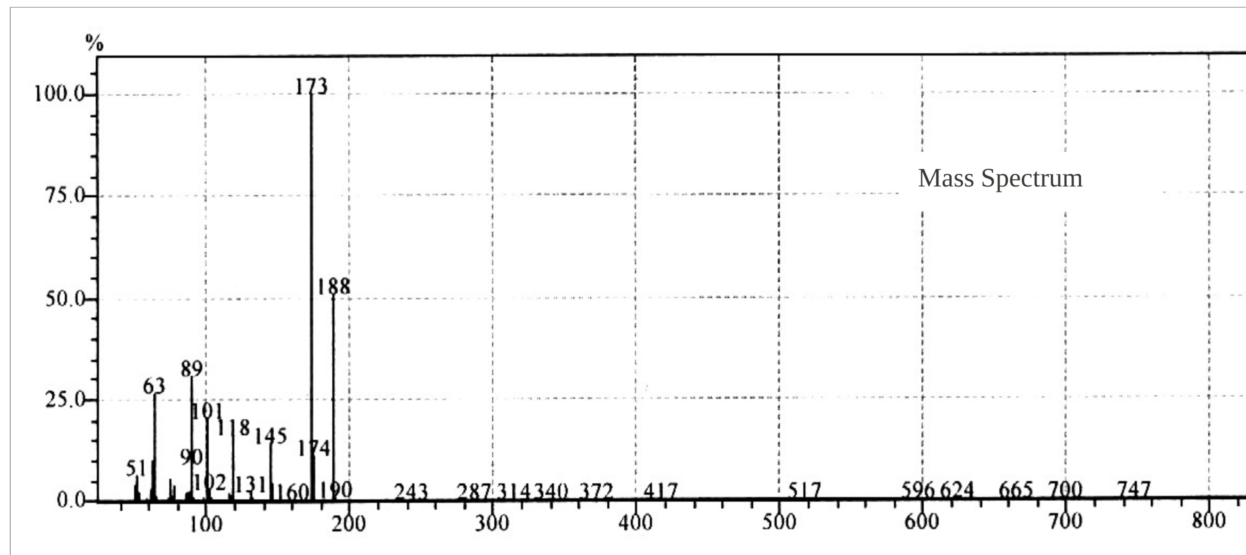
Mass Spectrum
%
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63
101
118
145
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160
190
243
287
314
340
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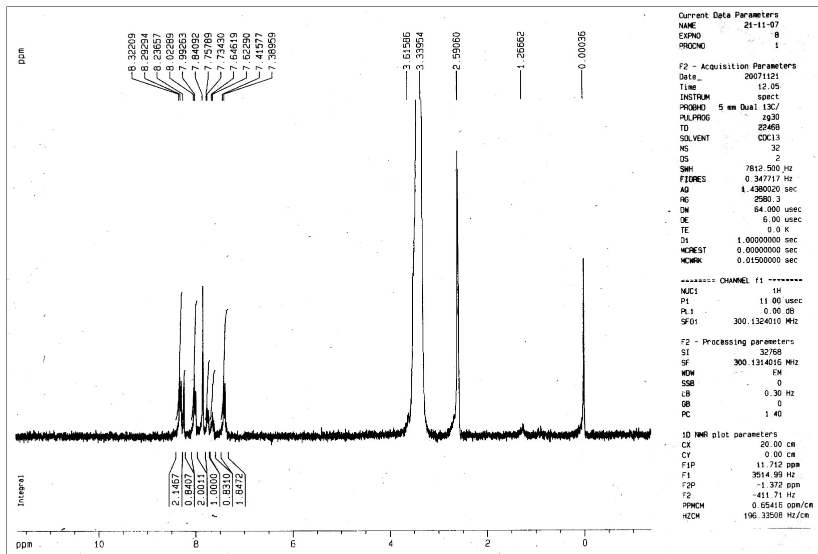
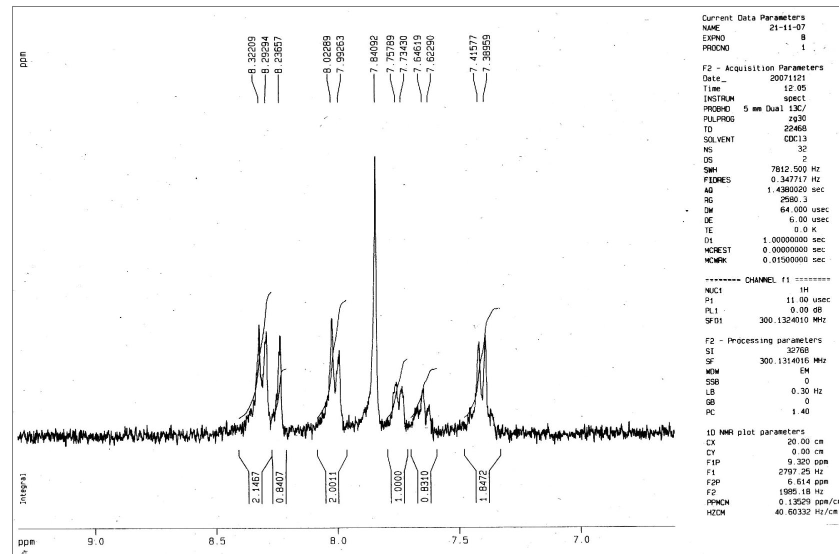
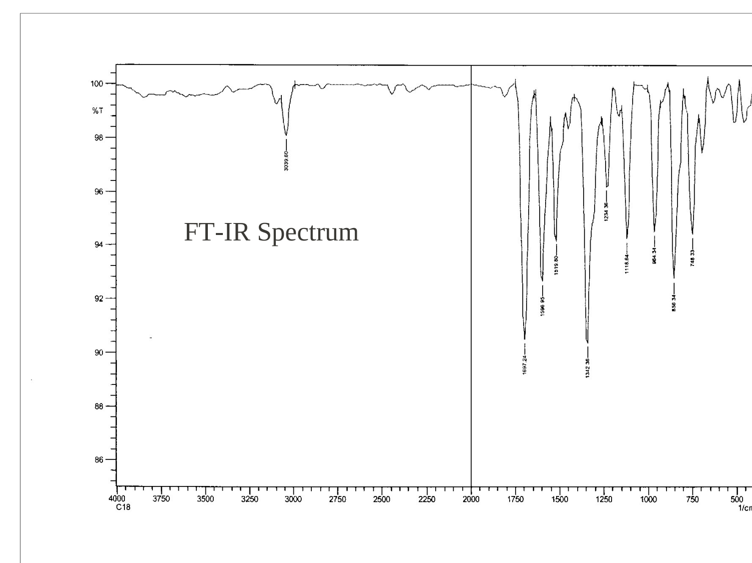
100
200
300
400
500
600
700
800

Spectra of C18





^{1}H NMR Spectrum (Expanded)

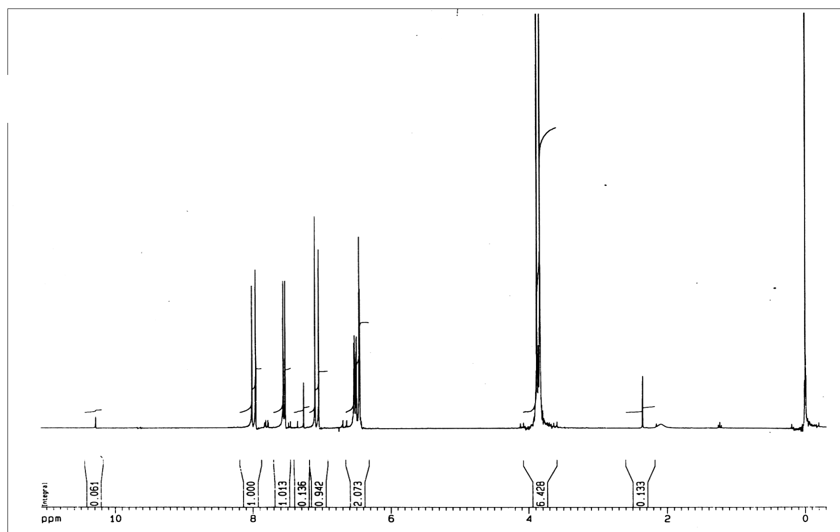


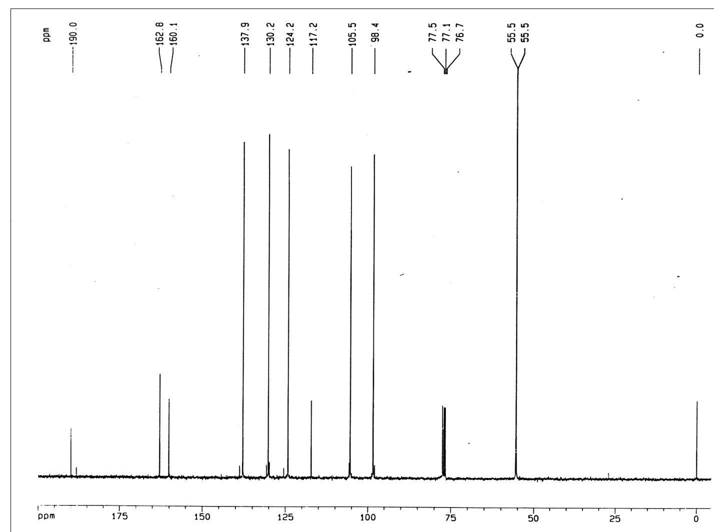
FT-IR Spectrum

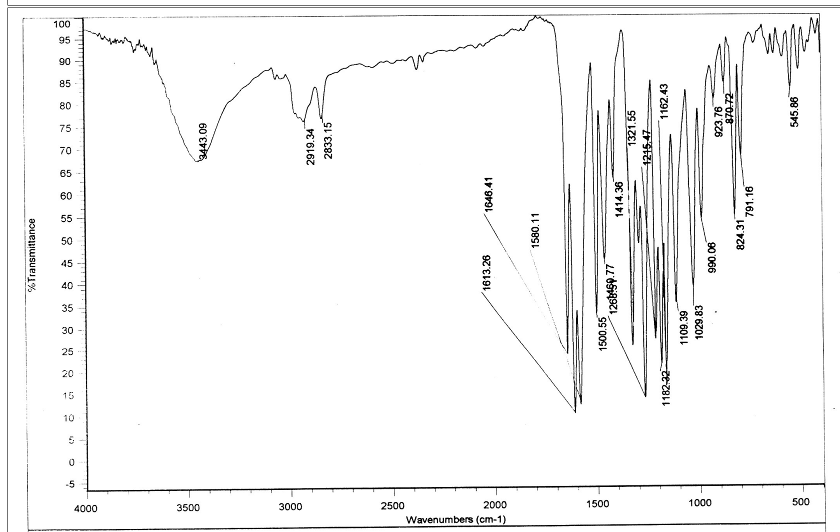
Spectra of β-bis(3-methoxy-4-hydroxyphenyl)-α’-(3-methoxy-4-hydroxybenzyl)-hept-1,6-dien-3,5-dione

^{1}H NMR Spectrum

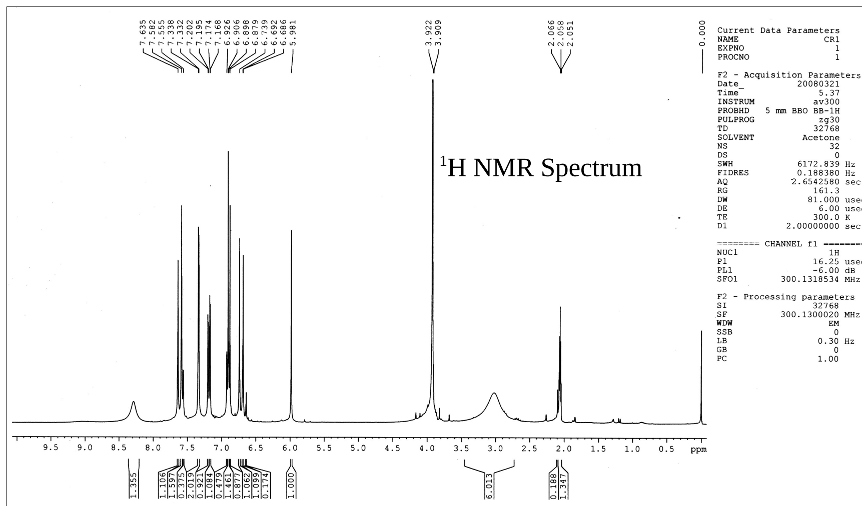
^{13}C NMR Spectrum

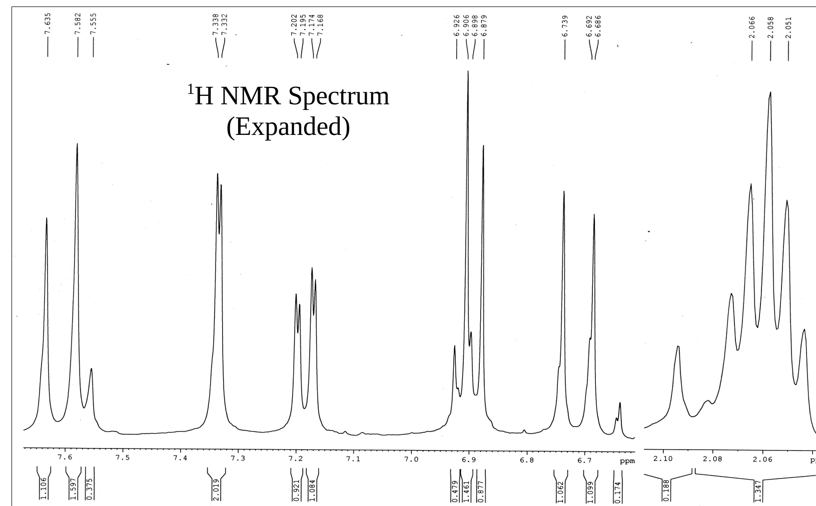


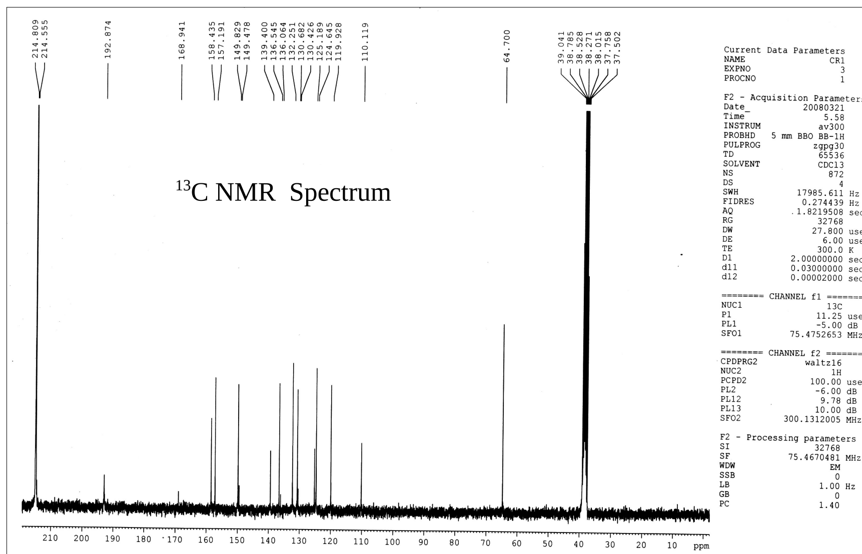


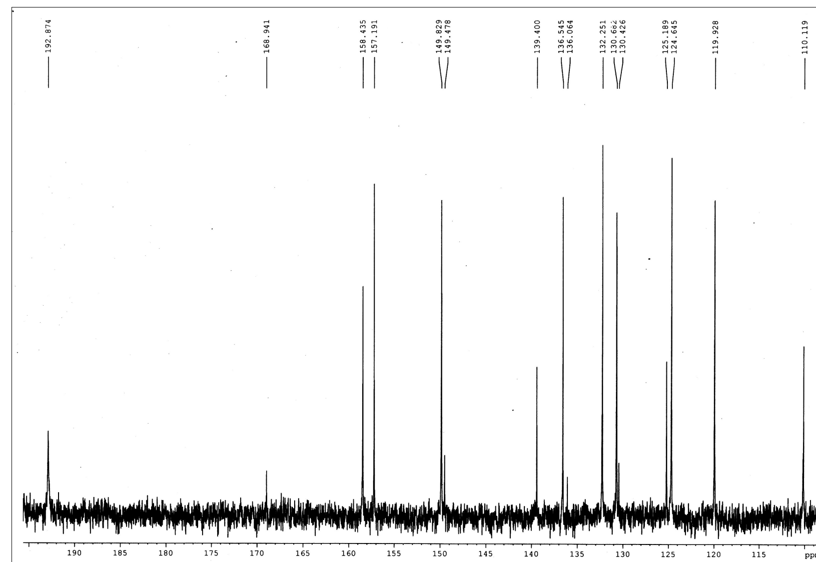


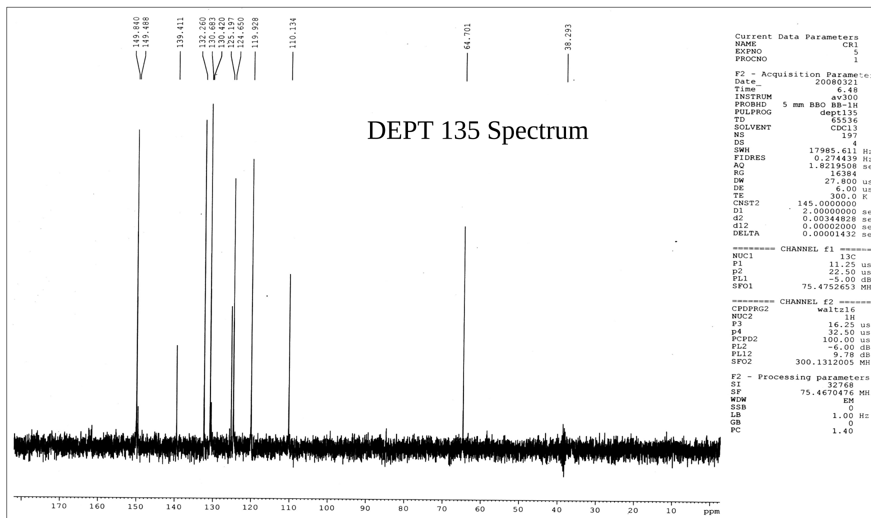
Spectra of Synthesized Curcumin I







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119.928
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38.293
Current Data Parameters
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PROCNO 1
F2 - Acquisition Paramete
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Time 6.48
INSTRUM av300
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PULPROG dept135
TD 65536
SOLVENT CDCl3
NS 197
DS 4
SWH 17985.611 Hz
FIDRES 0.274439 Hz
AQ 1.8219508 se
RG 16384
DW 27.800 us
DE 6.00 us
TE 300.0 K
CNST2 145.0000000
D1 2.00000000 se
d2 0.00344828 se
d12 0.00002000 se
DELTA 0.00001432 se
======== CHANNEL f1 ======
NUC1 13C
P1 11.25 us
p2 22.50 us
PL1 -5.00 dB
SFO1 75.4752653 MH
======== CHANNEL f2 ======
CPDPRG2 waltz16
NUC2 1H
P3 16.25 us
p4 32.50 us
PCPD2 100.00 us
PL2 -6.00 dB
PL12 9.78 dB
SFO2 300.1312005 MH
F2 - Processing parameters
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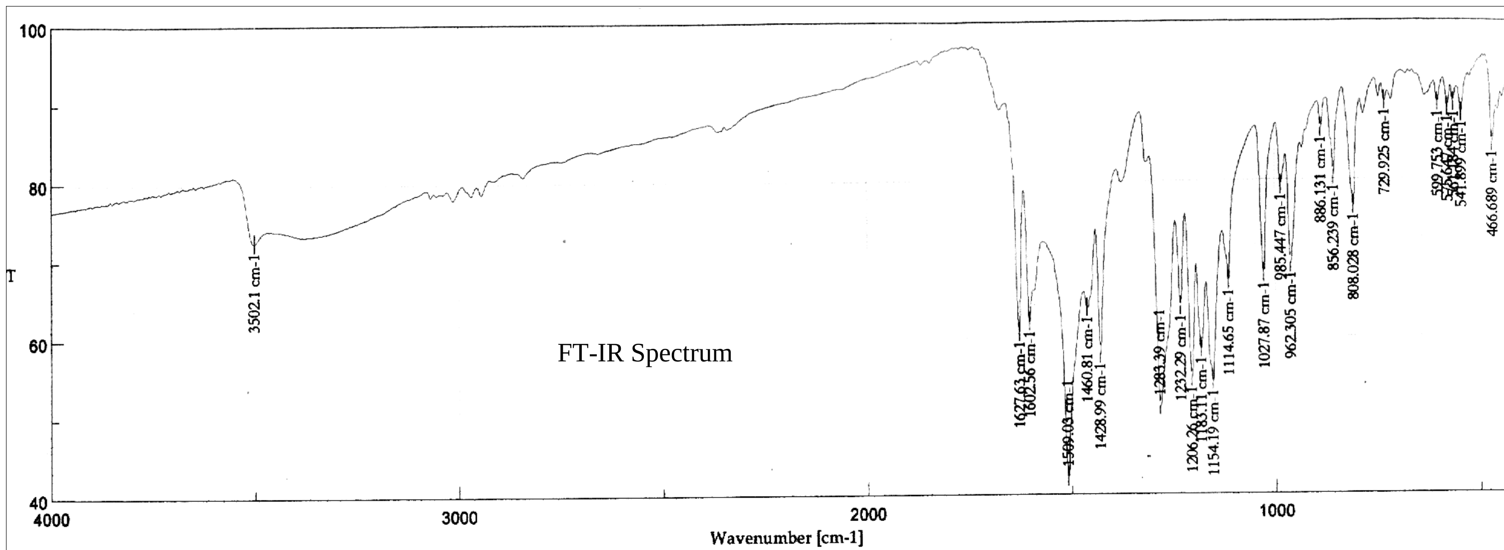
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40
170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 ppm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60
40
T
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1627.63 cm-1
1602.56 cm-1
1509.03 cm-1
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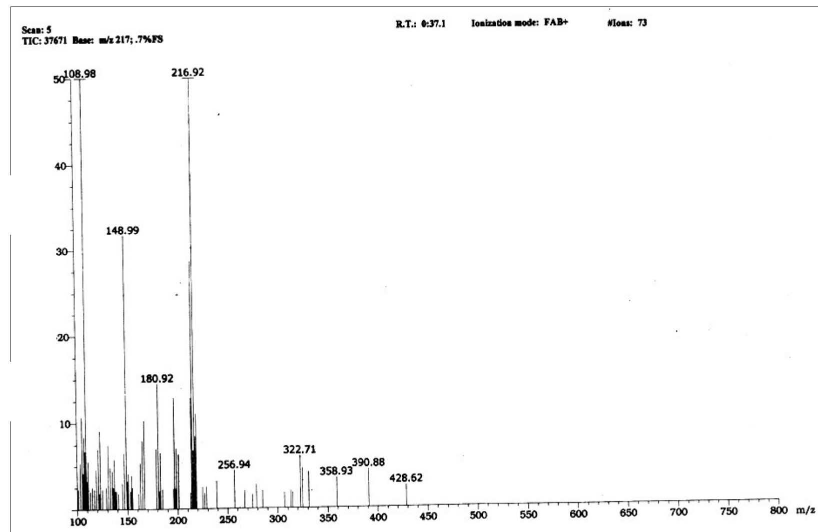
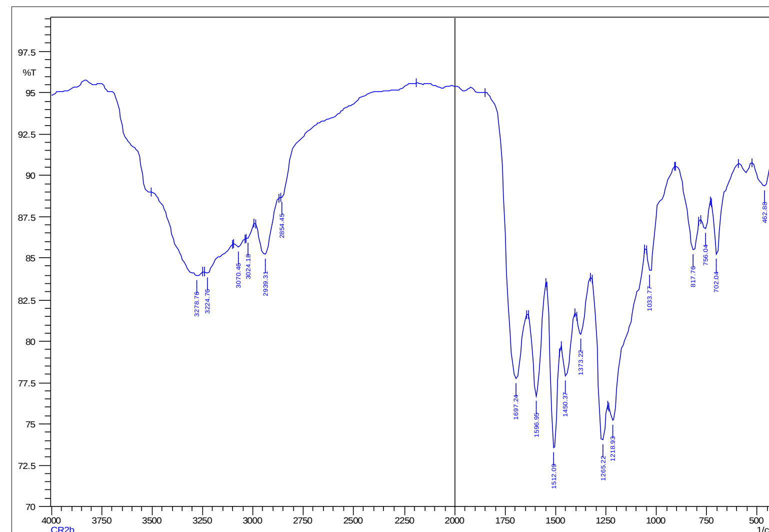
1428.99 cm-1
1283.39 cm-1
1232.29 cm-1
1206.26 cm-1
1183.11 cm-1
1154.19 cm-1
1114.65 cm-1
1027.87 cm-1
985.447 cm-1
962.305 cm-1
886.131 cm-1
856.239 cm-1
808.028 cm-1
729.925 cm-1
599.753 cm-1
541.899 cm-1
466.689 cm-1
4000
3000
2000
1000
Wavenumber [cm-1]

Spectra of the Product Under Section 4.4.3.1

Mass Spectrum

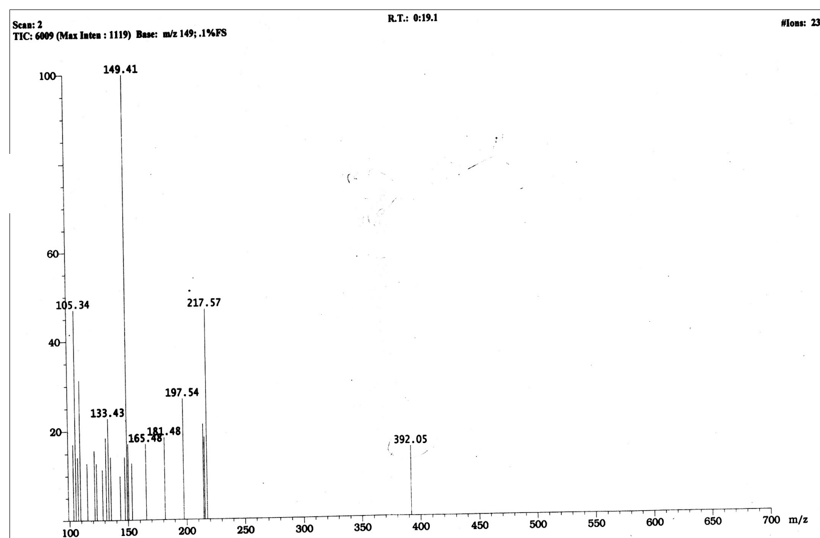
FT-IR Spectrum

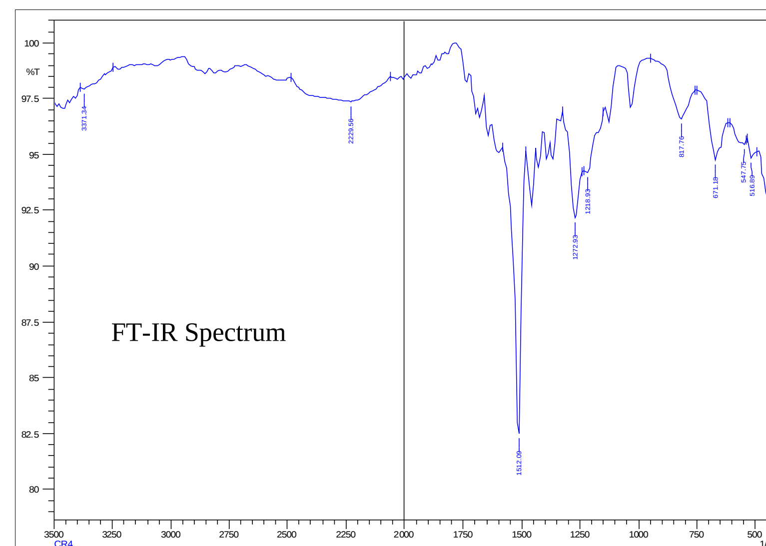




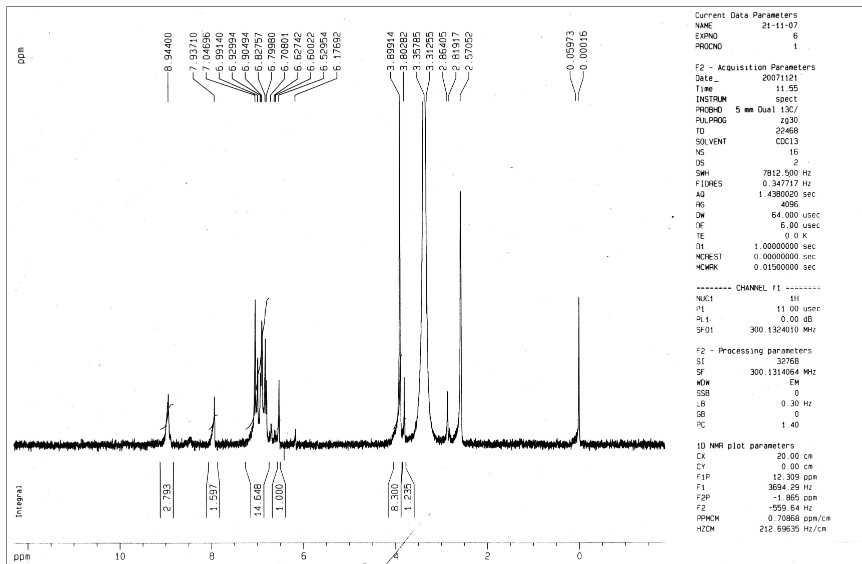
Spectra of Product of Reaction Under Section 4.4.3.4

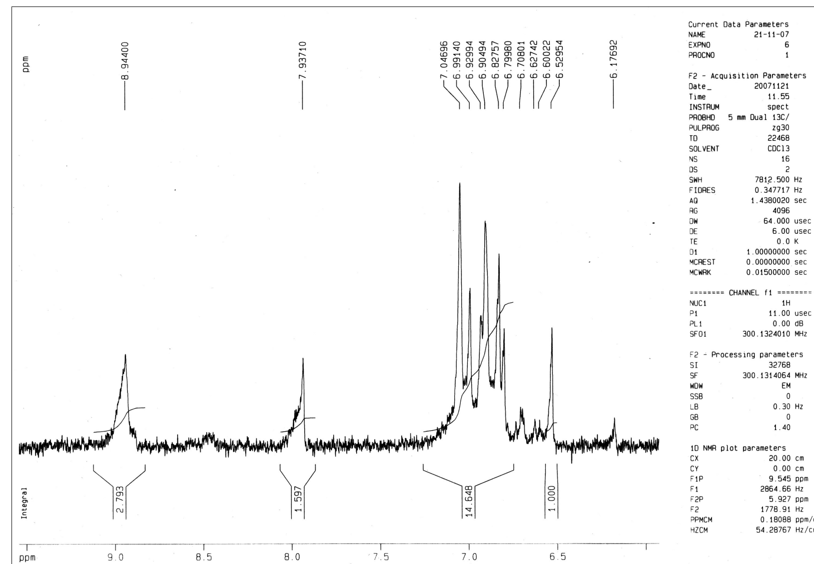
Mass Spectrum

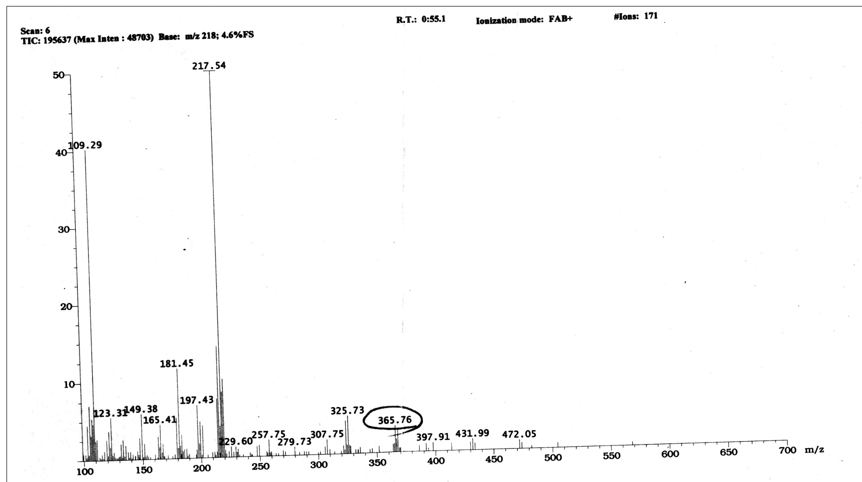


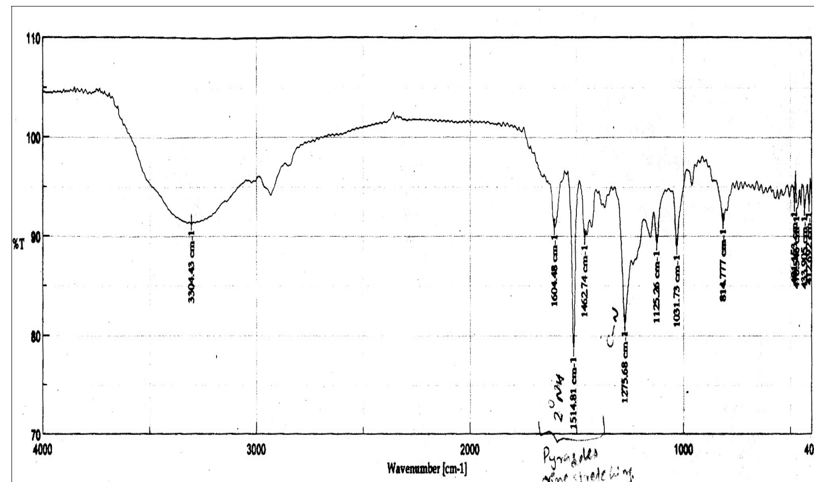


Spectra of the product of Reaction Under Section 4.4.3.3

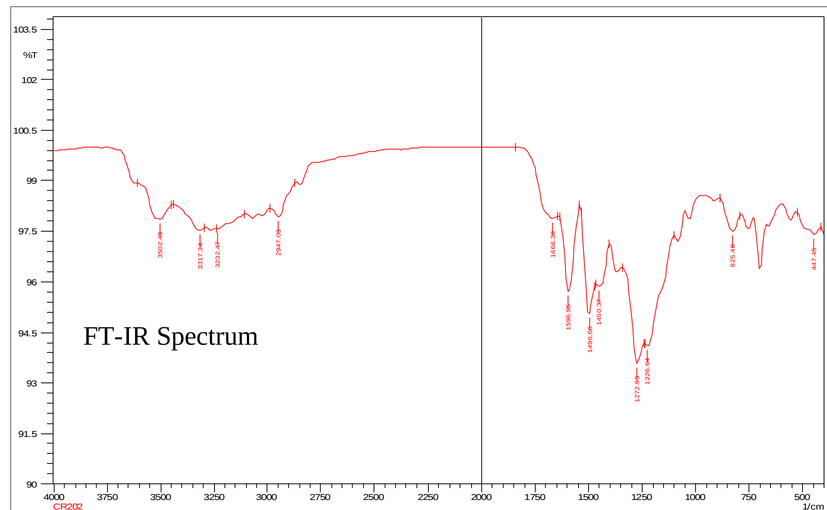


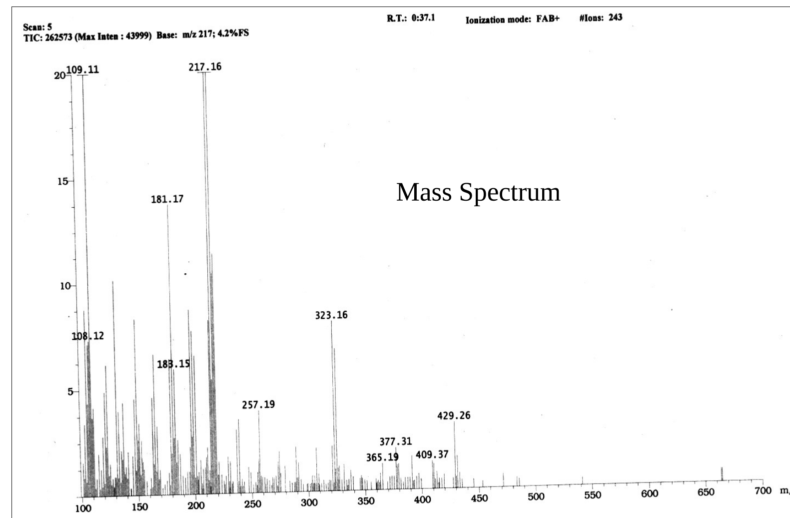




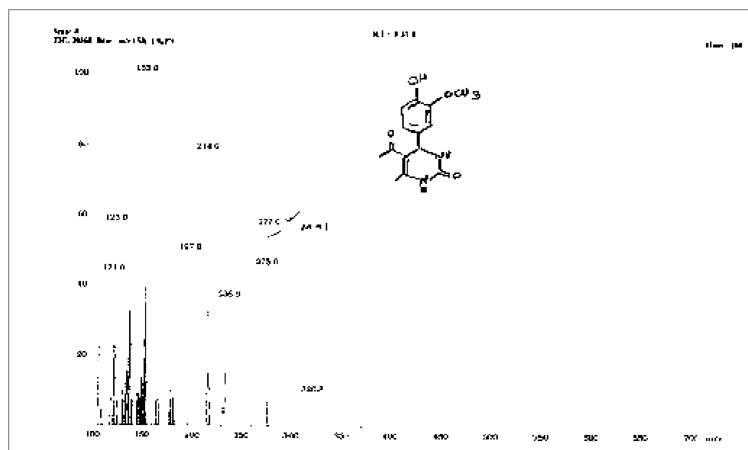


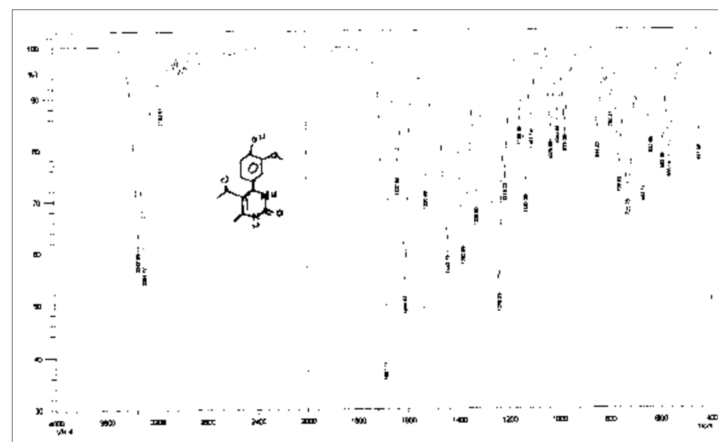
Spectra of the Product of Reaction Under Section 4.4.3.2

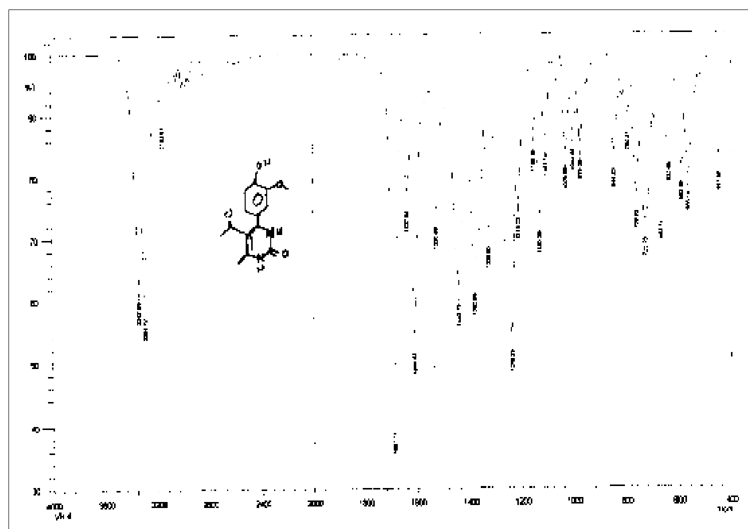


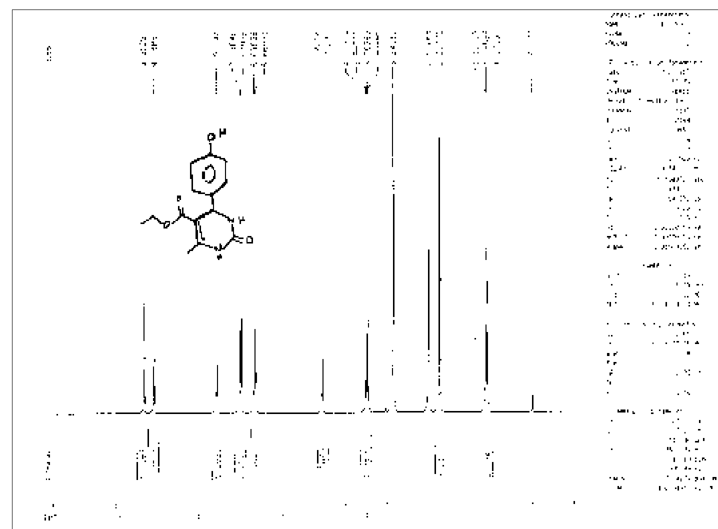


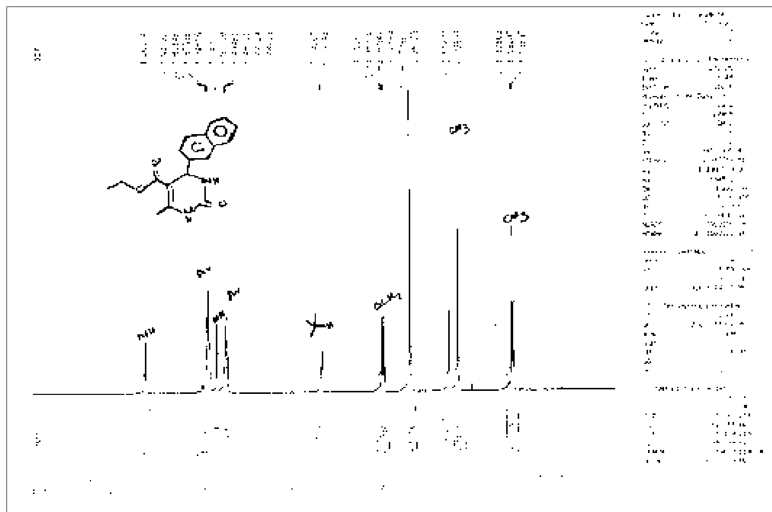
Spectra of Known Compounds Used for Comparison with Literature Data

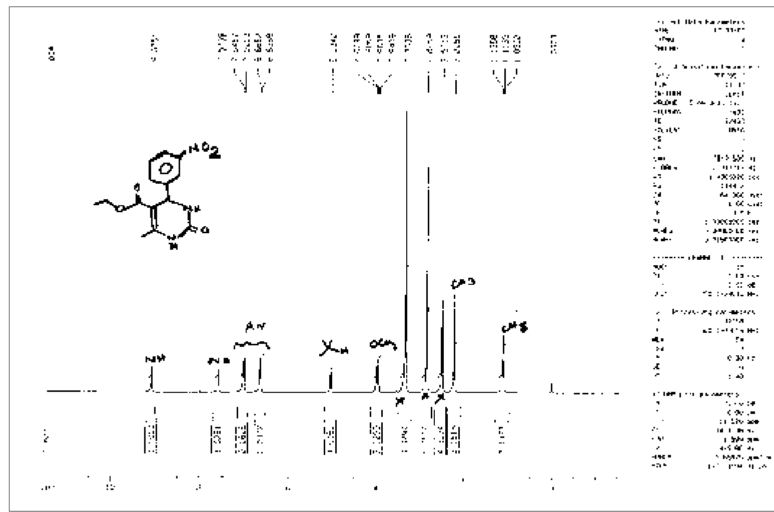


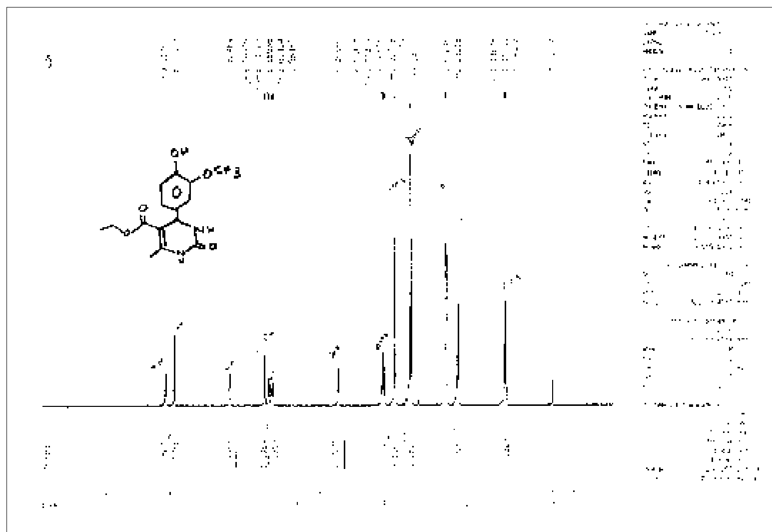


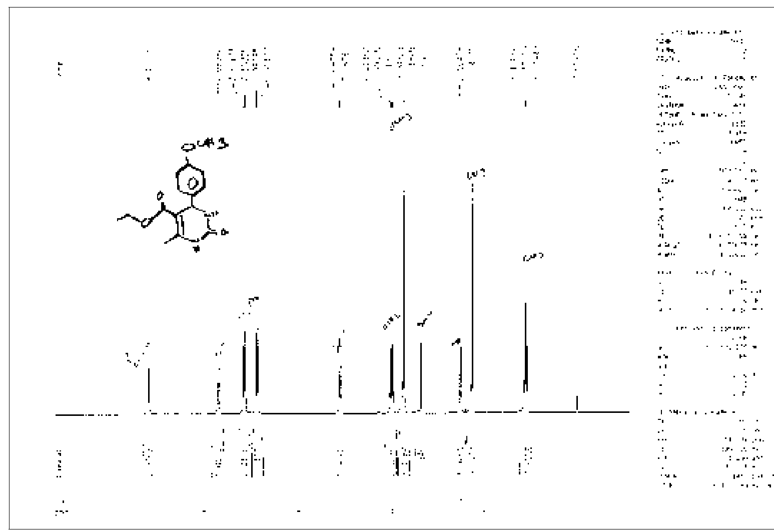


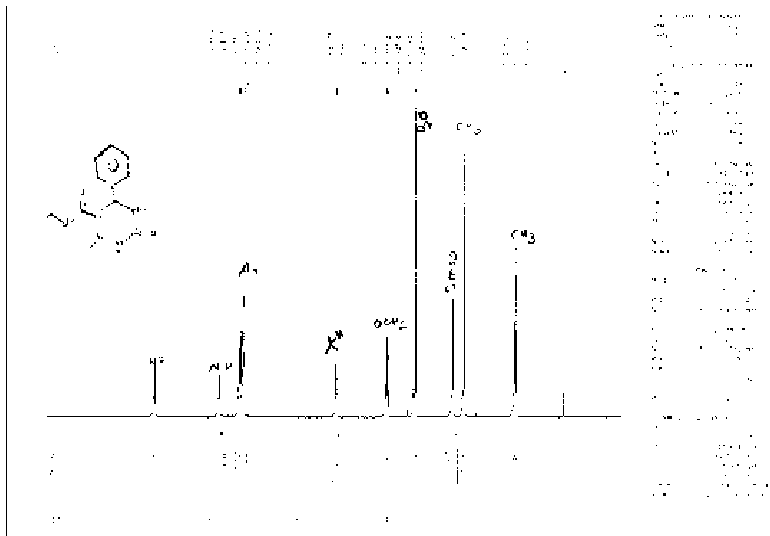


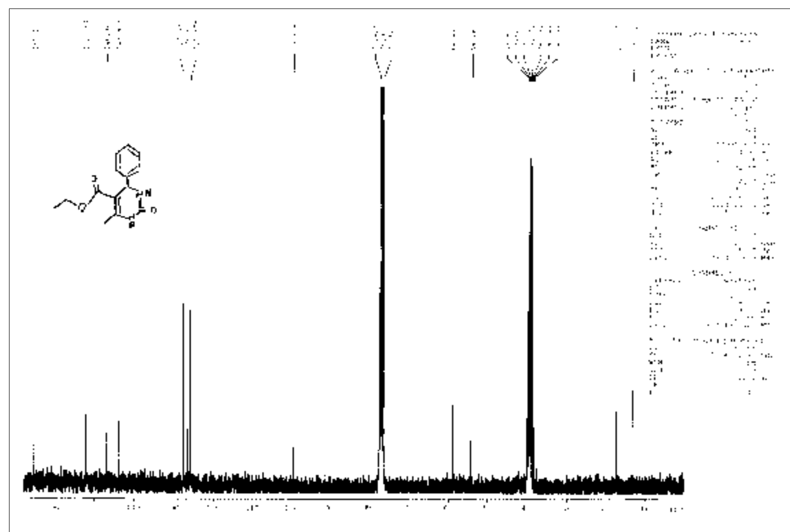


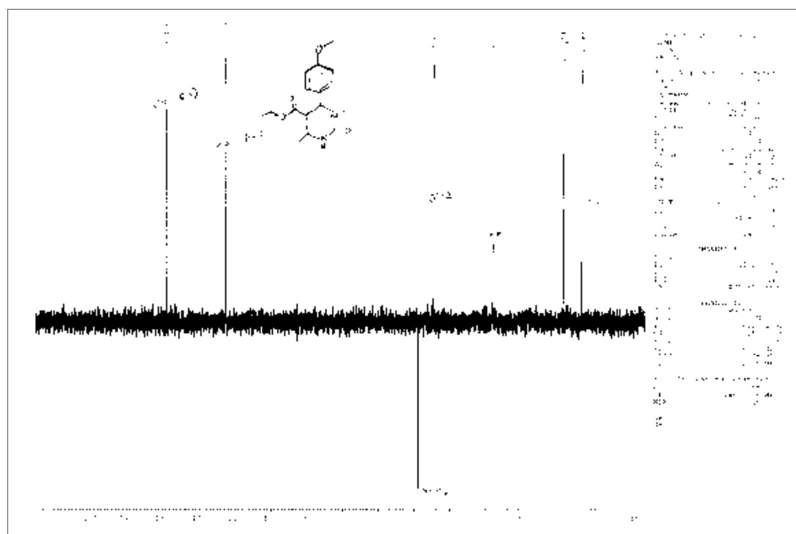


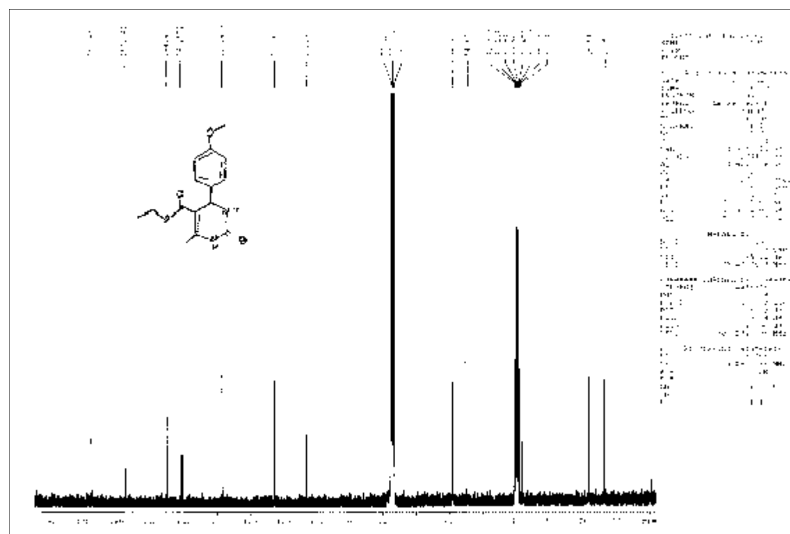


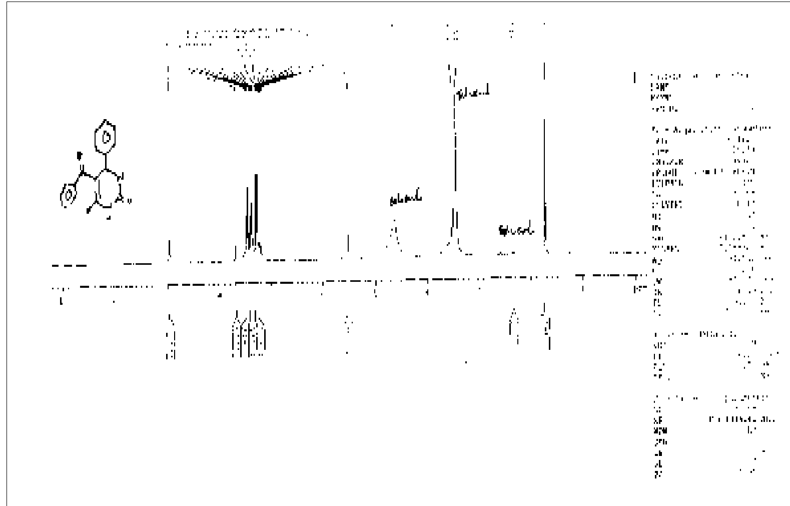


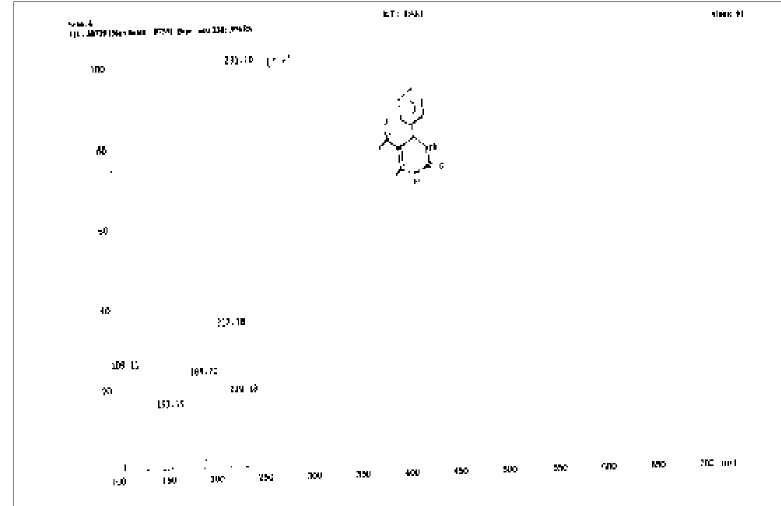


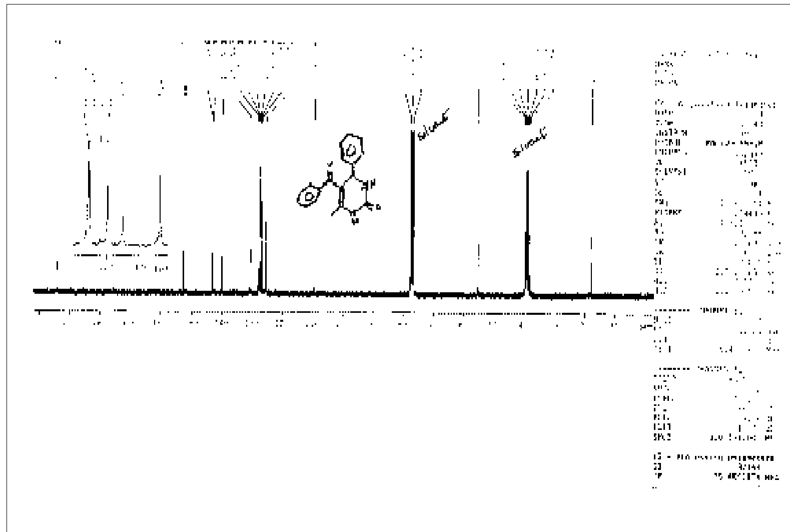


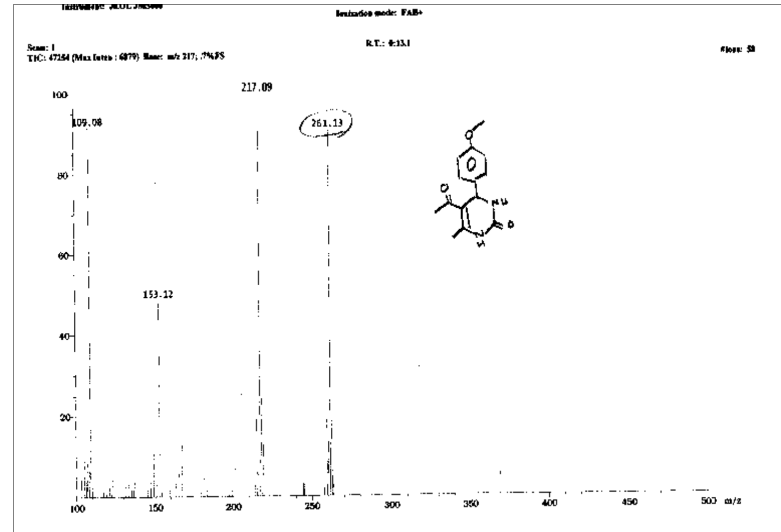


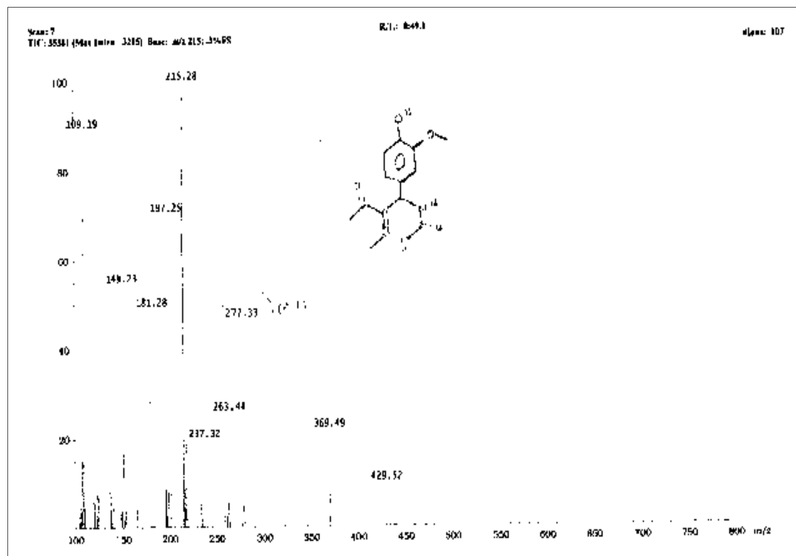




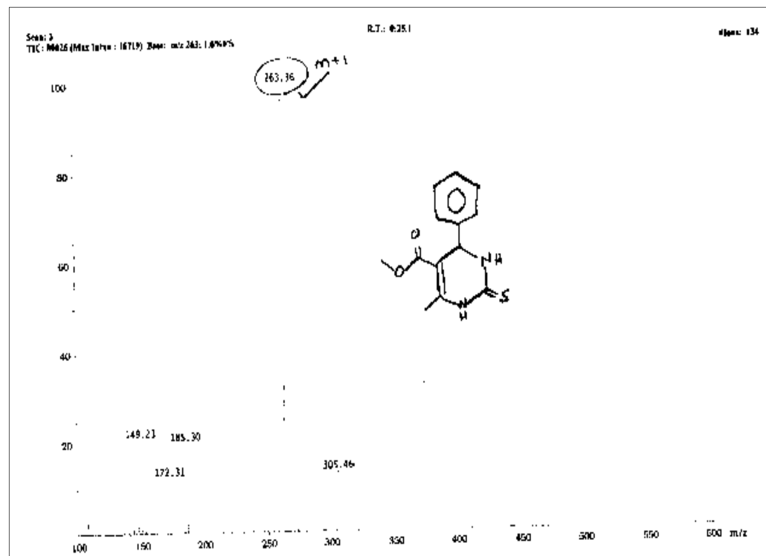





Ionization mode: FAB+
217.09
109.08
261.13
153.12



215.28
109.19
197.25
149.23
181.28
277.33
263.44
237.32
369.49
429.52
m/z



263.36
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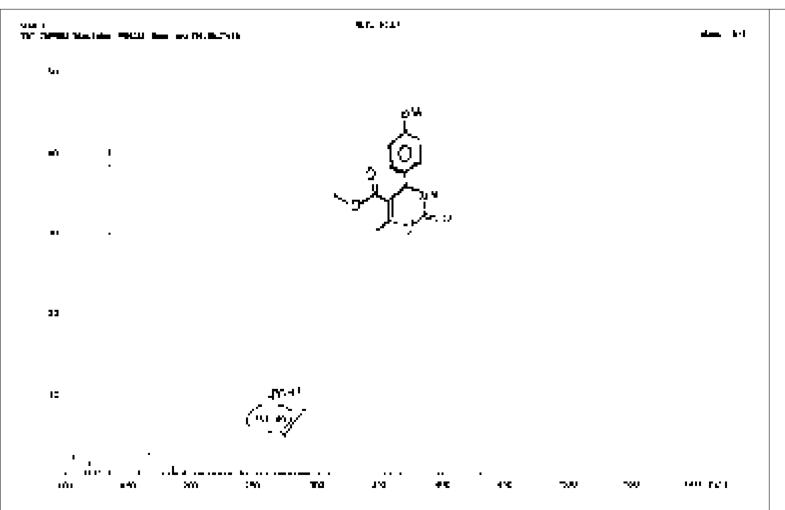
149.23
185.30
172.31
305.46
m/z



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R.T.: [illegible]

Scan: [illegible]

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[illegible] (M + 1)

100 150 200 250 300 350 400 450 500 550 600 m/z

Scan: [illegible]
TIC: [illegible] (Max Inten: [illegible]) Base: m/z [illegible]

R.T.: [illegible]

Scan: [illegible]

100 80 60 40 20

217.51

[illegible] (M + 1)

100 150 200 250 300 350 400 450 500 550 600 m/z

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R.T.: [illegible]

Scan: [illegible]

100 80 60 40 20

[illegible]

100 150 200 250 300 350 400 450 500 550 600 m/z

Scan: [illegible]
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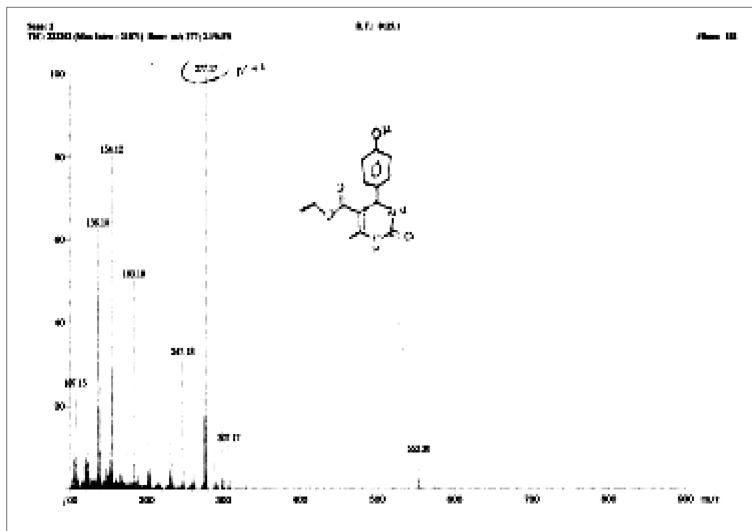
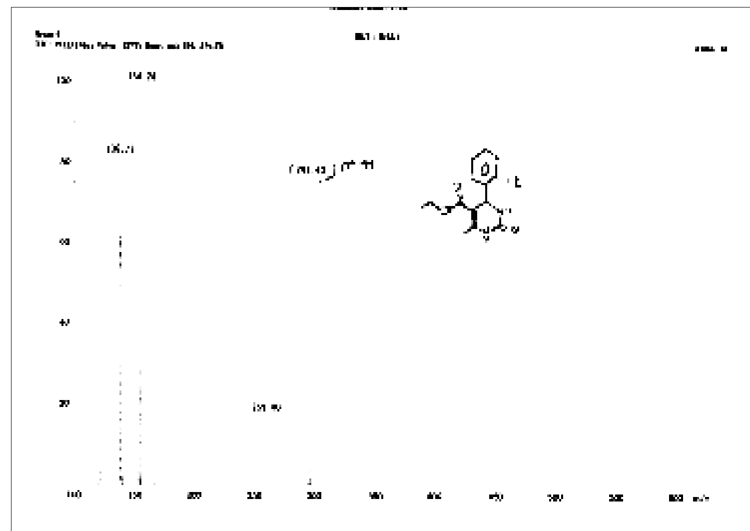
Scan: [illegible]

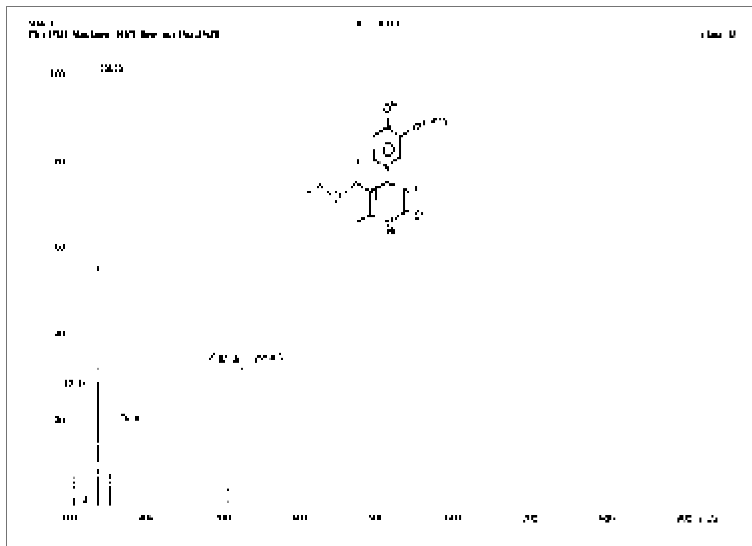
100 80 60 40 20

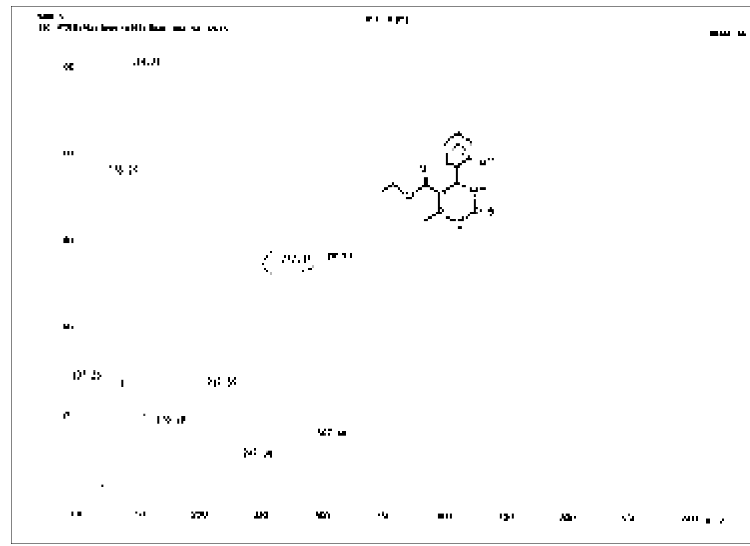
[illegible] (M + 1)

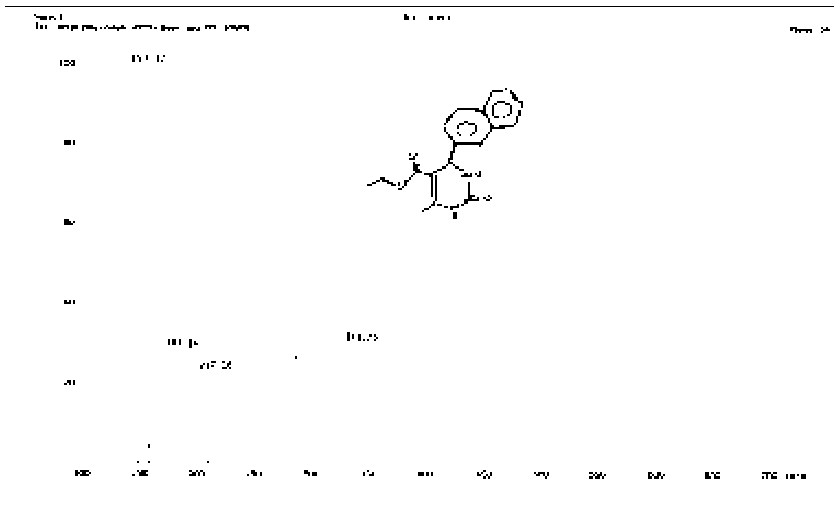
100 150 200 250 300 350 400 450 500 550 600 m/z

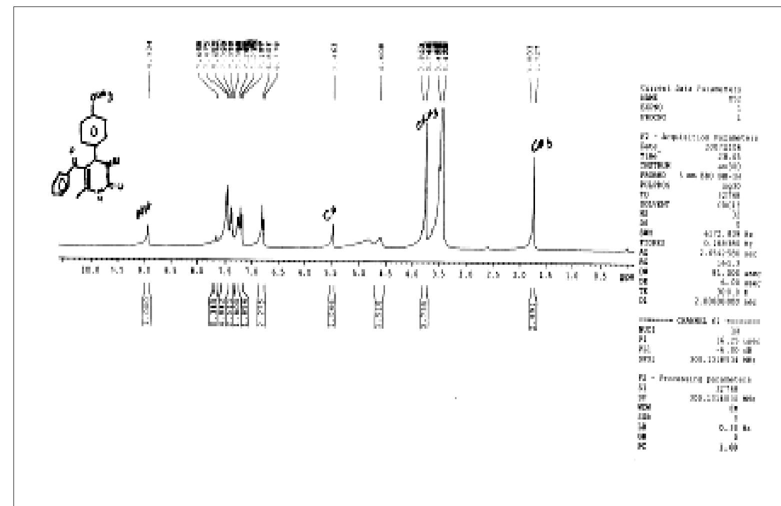


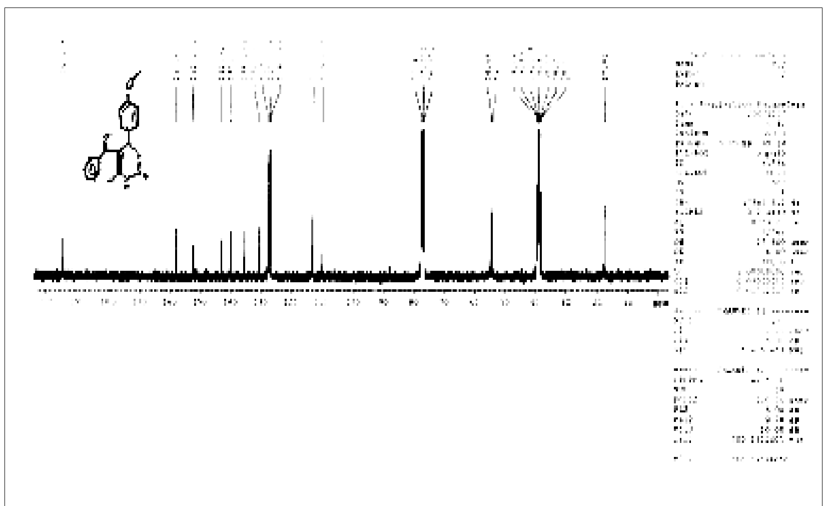


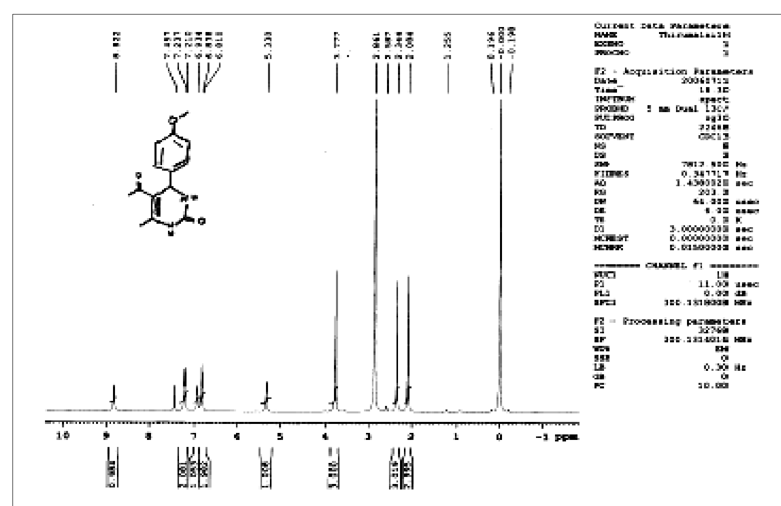


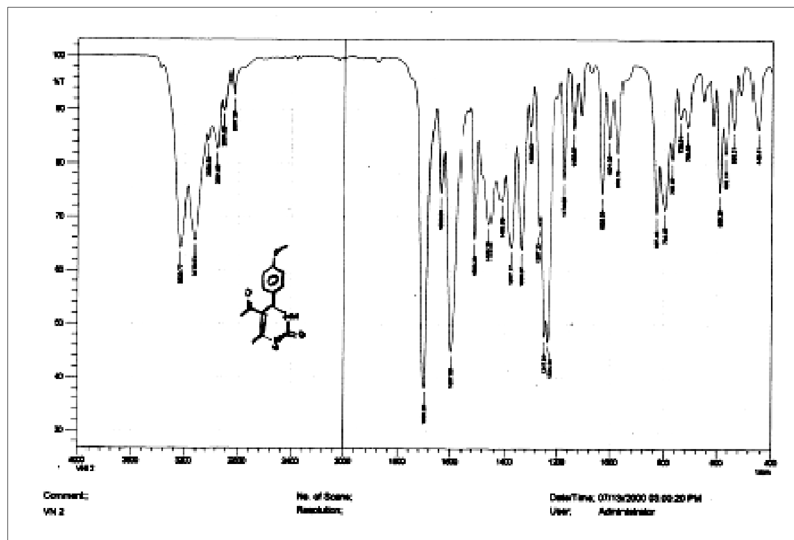








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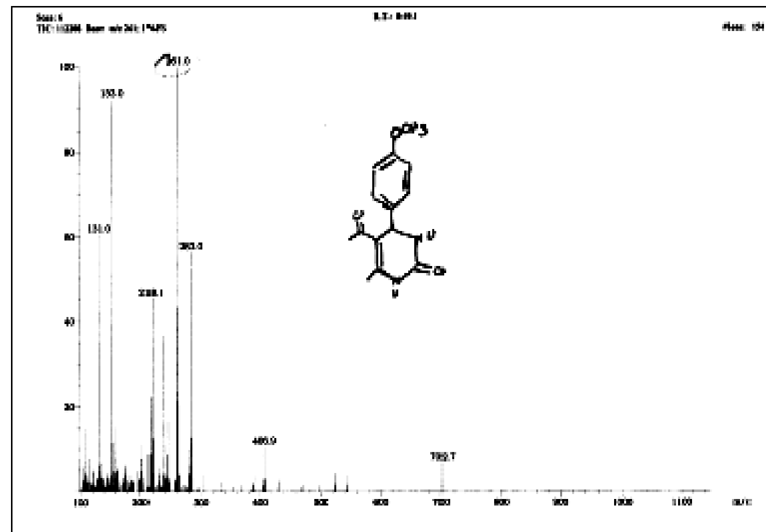
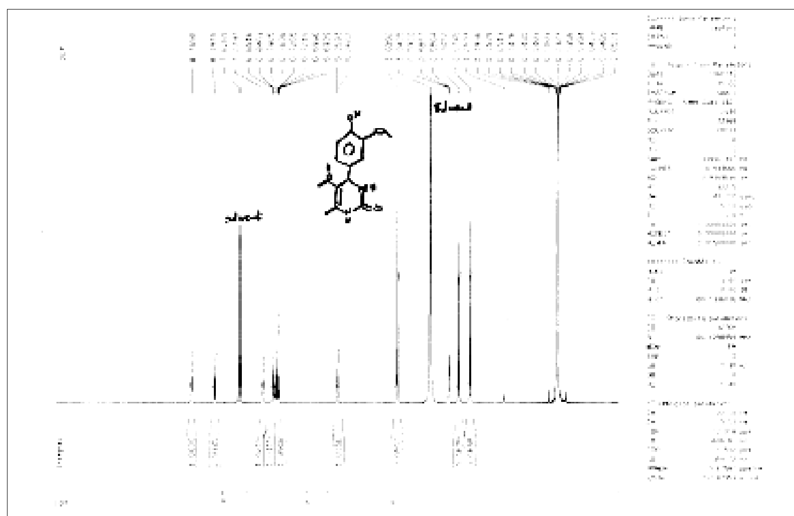
VN 2
No. of Scans;
Resolution;
User;
Administrator


485.9

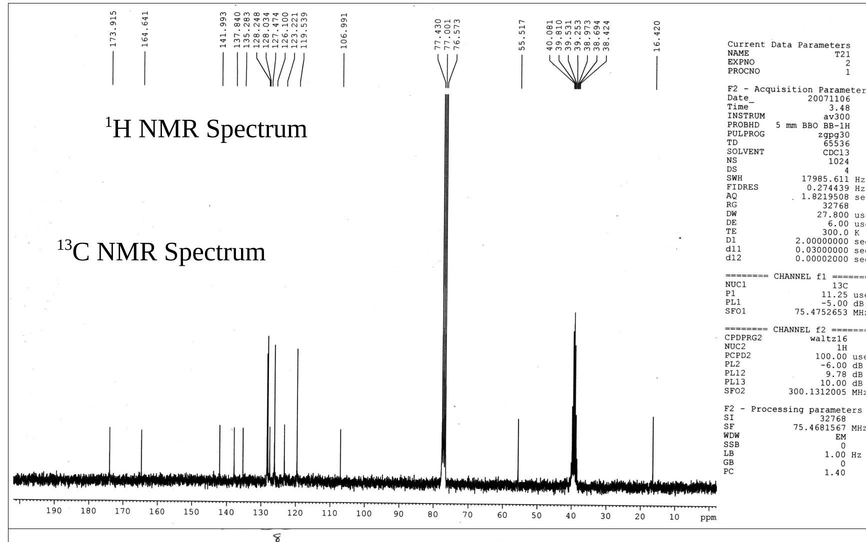


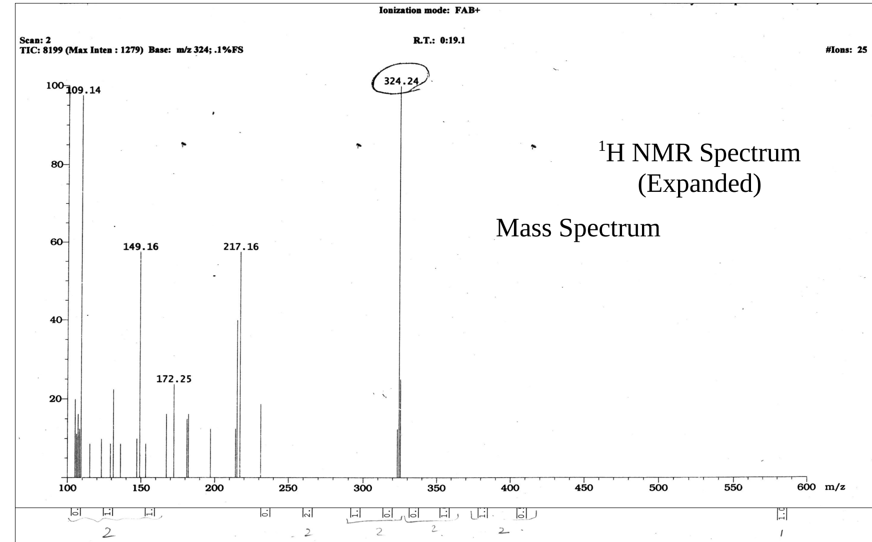
^{13}C NMR Spectrum

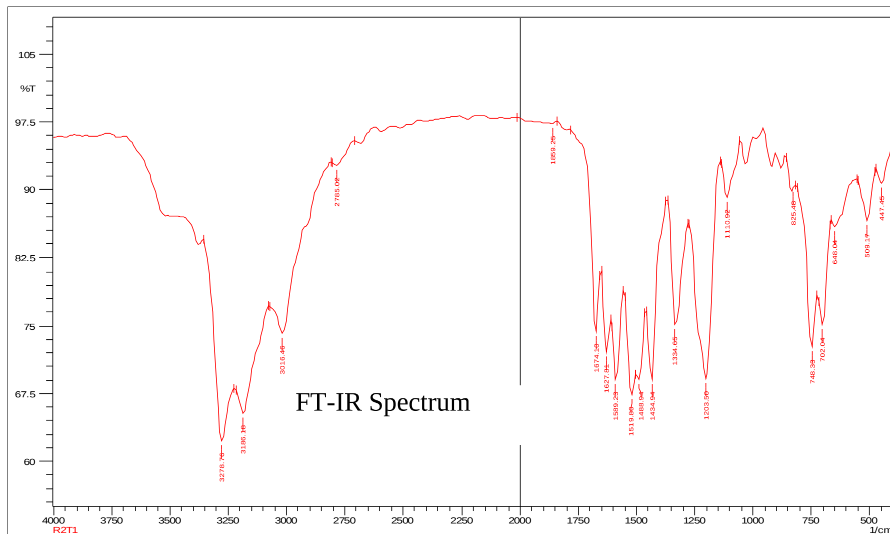
Mass Spectrum

Spectra of new dihydropyrimidinones

Spectra of T21







FT-IR Spectrum

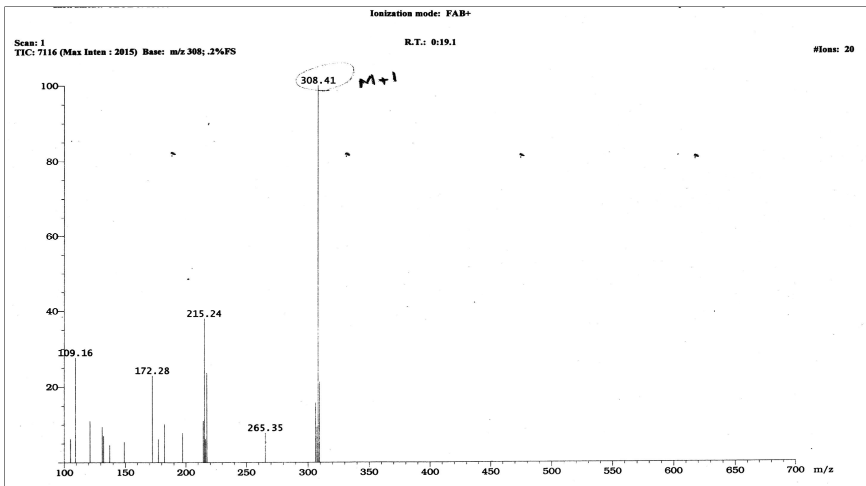
Spectra of U21

H NMR Spectrum

H NMR Spectrum (Expanded)

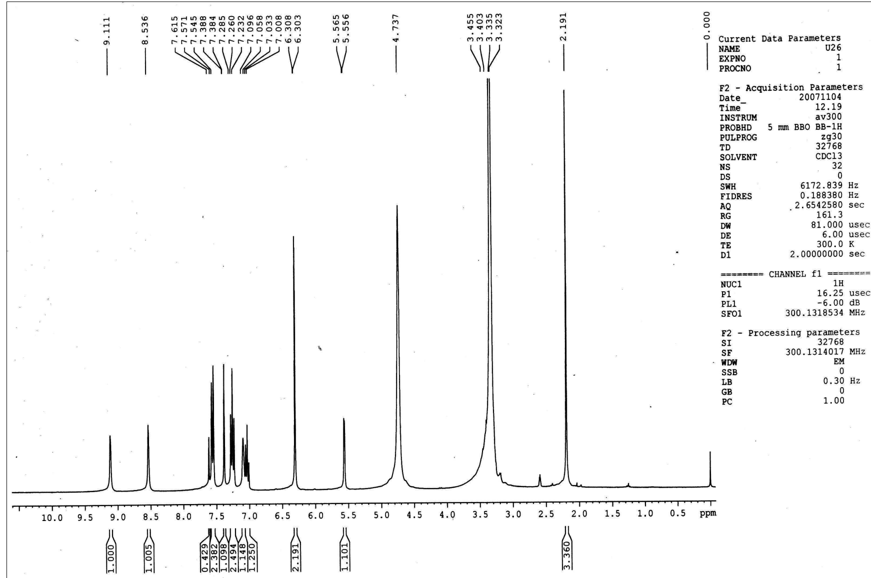
HMQC Spectrum

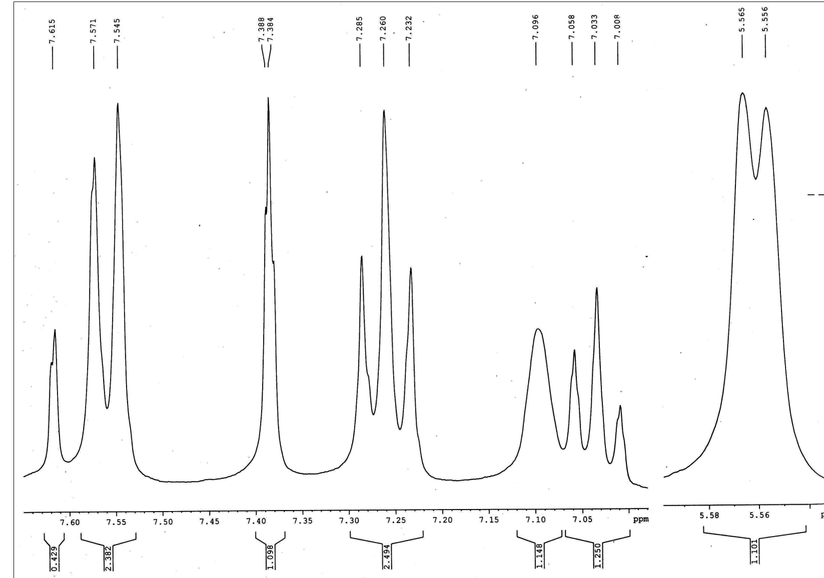
FT-IR Spectrum



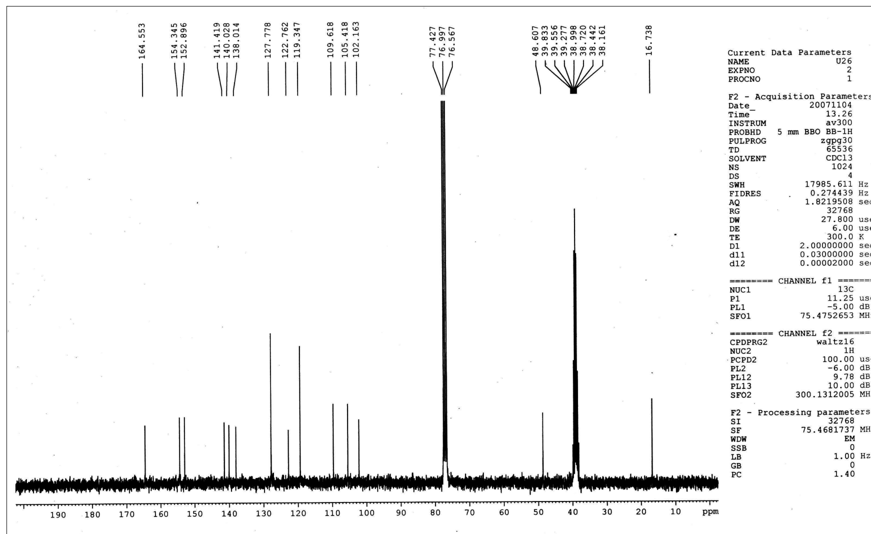
Mass Spectrum

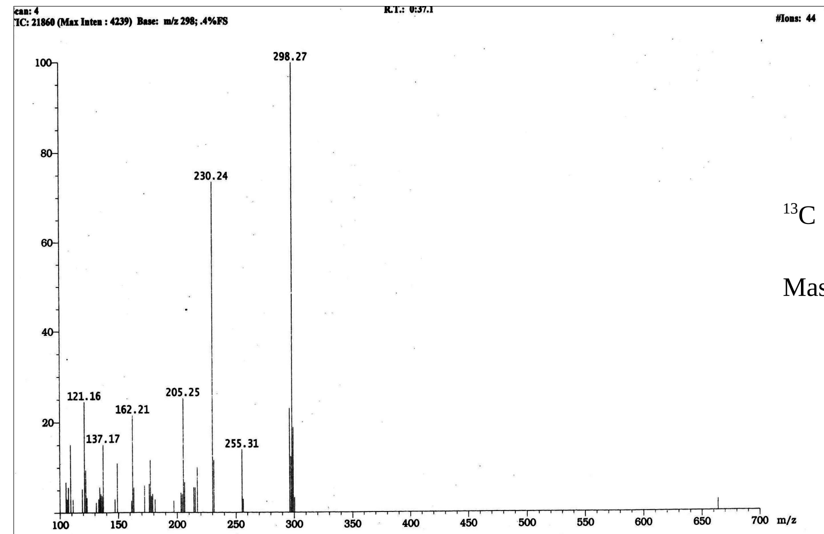
Spectra of U26





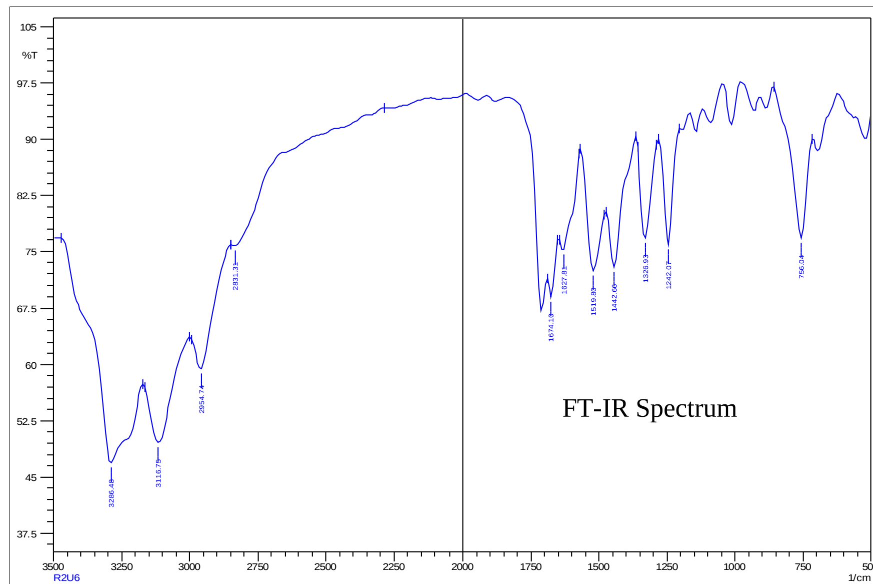
H NMR Spectrum
(Expanded)





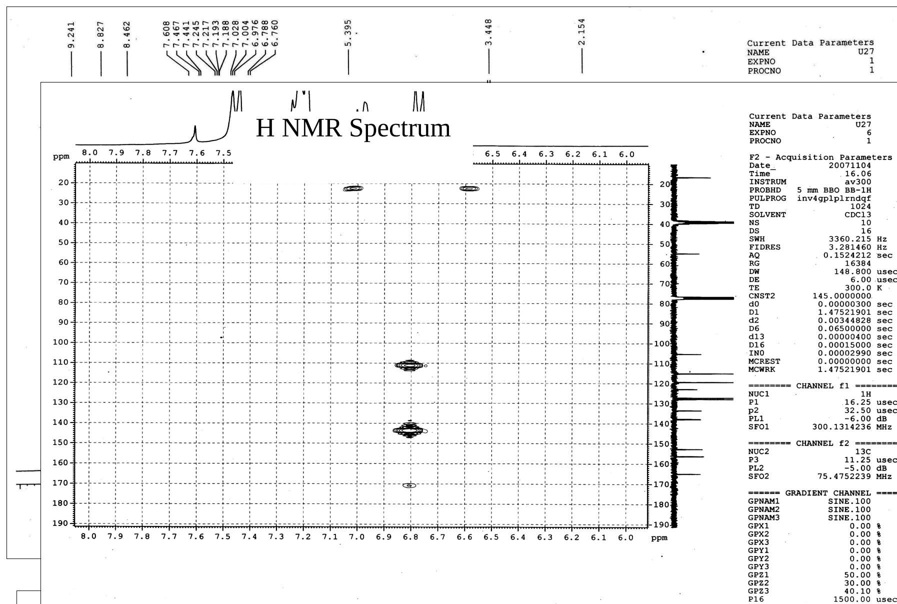
^{13}C NMR Spectrum

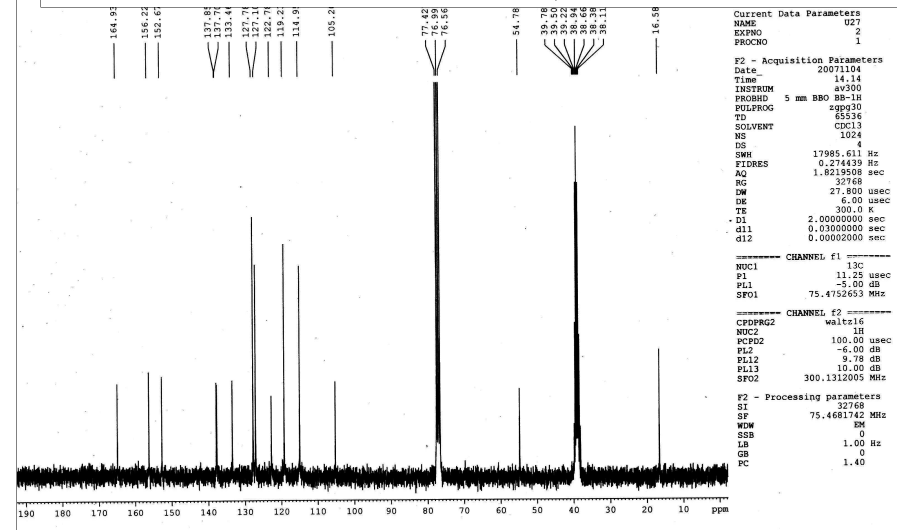
Mass Spectrum

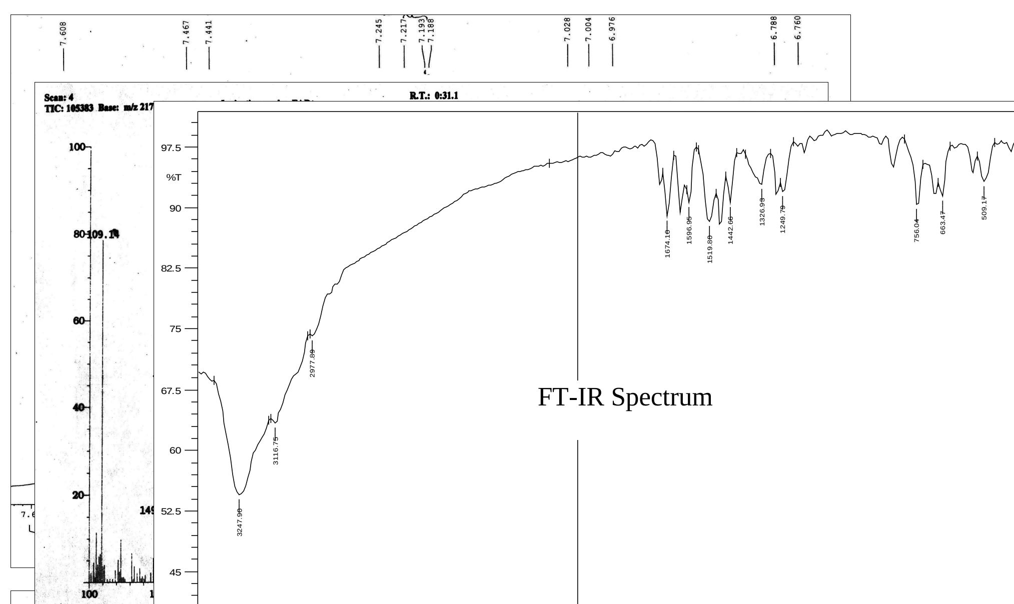

FT-IR Spectrum
%T
105
97.5
90
82.5
75
67.5
60
52.5
45
37.5
3500
3250
3000
2750
2500
2250
2000
1750
1500
1250
1000
750
1/cm
R2U6
3286.49
3116.75
2954.74
2831.31
1674.18
1627.81
1519.80
1442.66
1326.93
1242.07
756.04

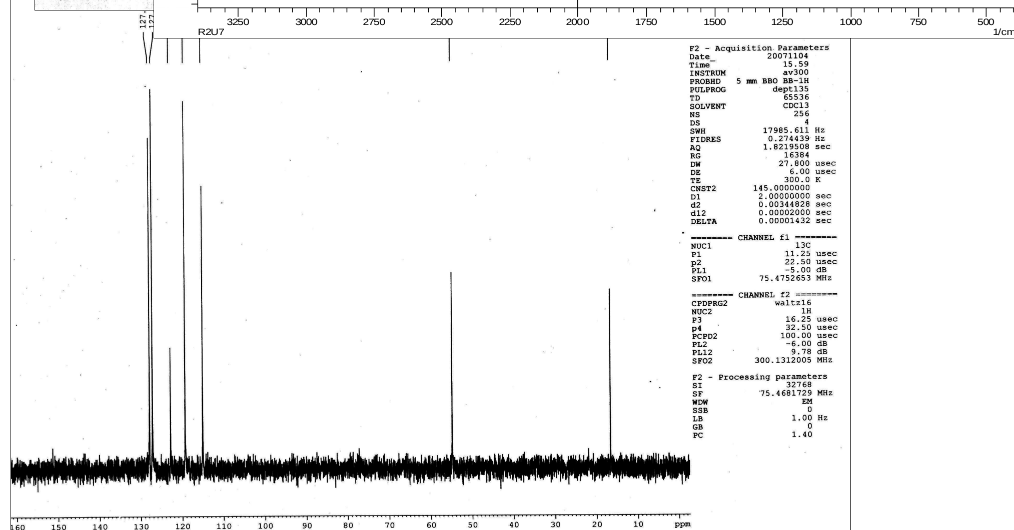
H NMR Spectrum ^{13}C Spectrum Mass Spectrum

Spectra of U27

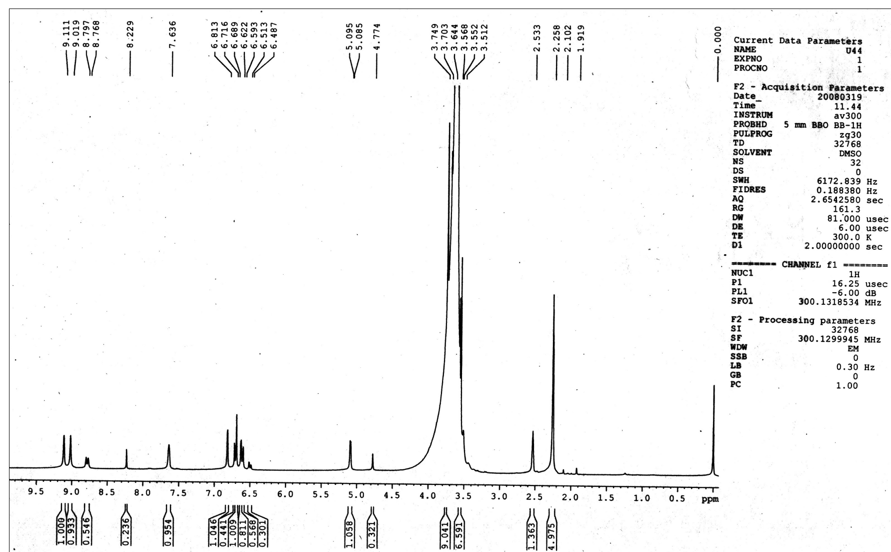


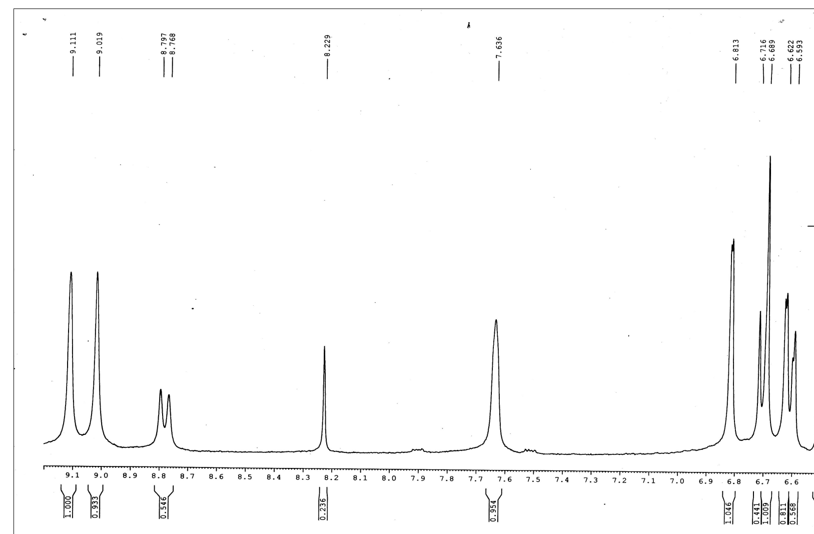




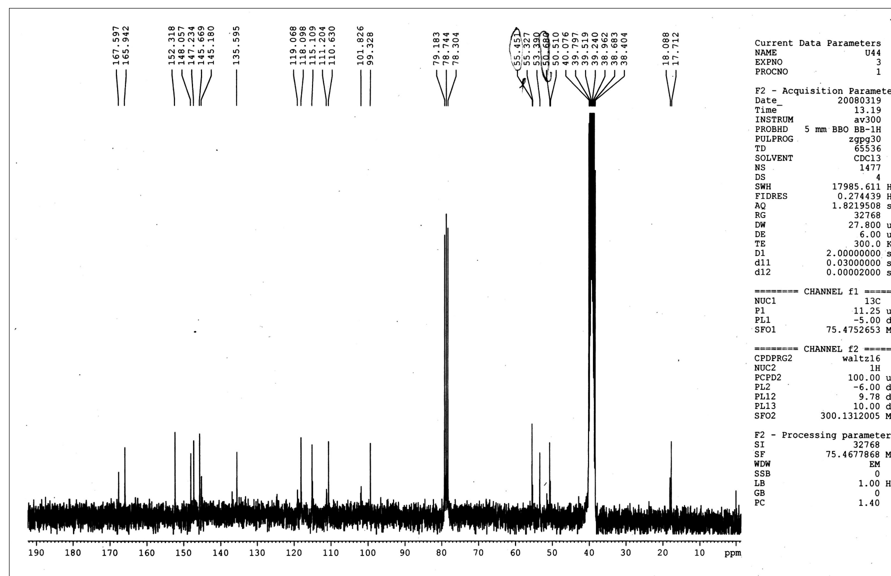


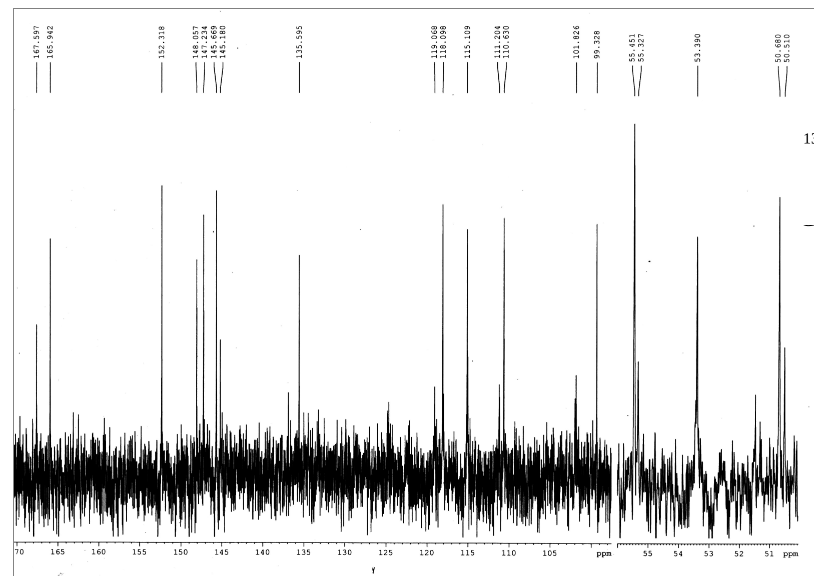
Spectra of U44



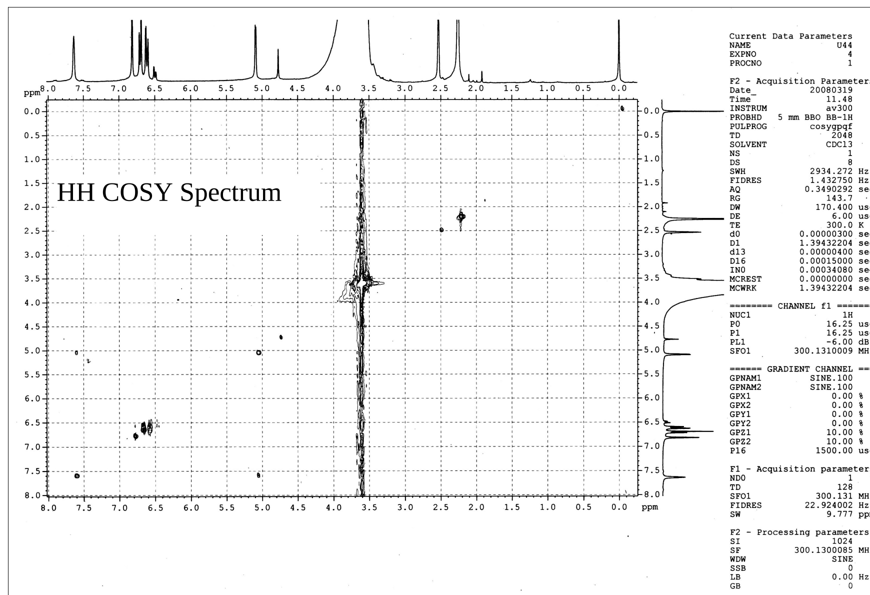


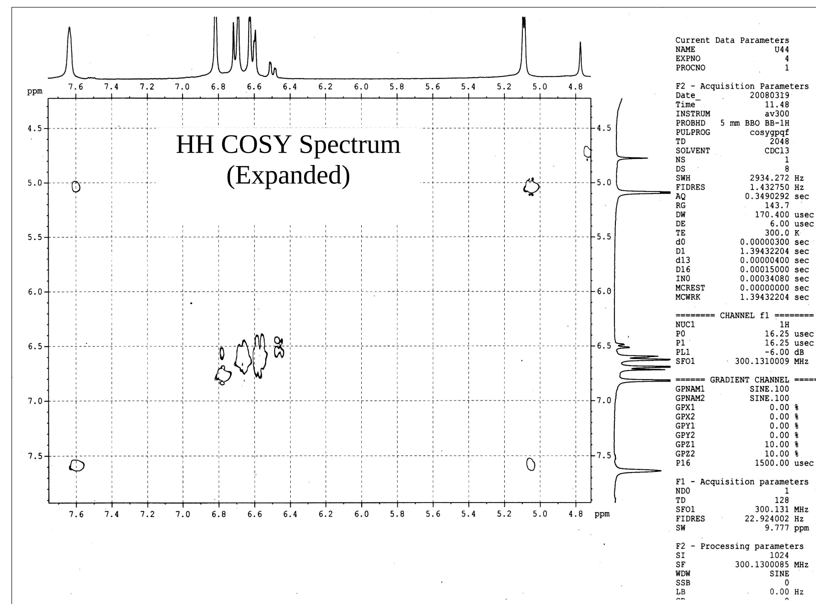
H NMR Spectrum (Expanded)

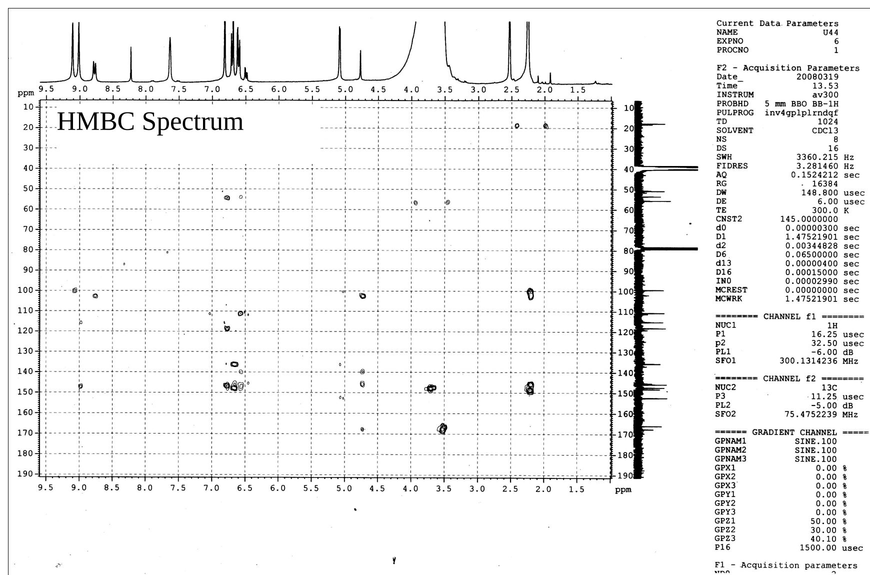


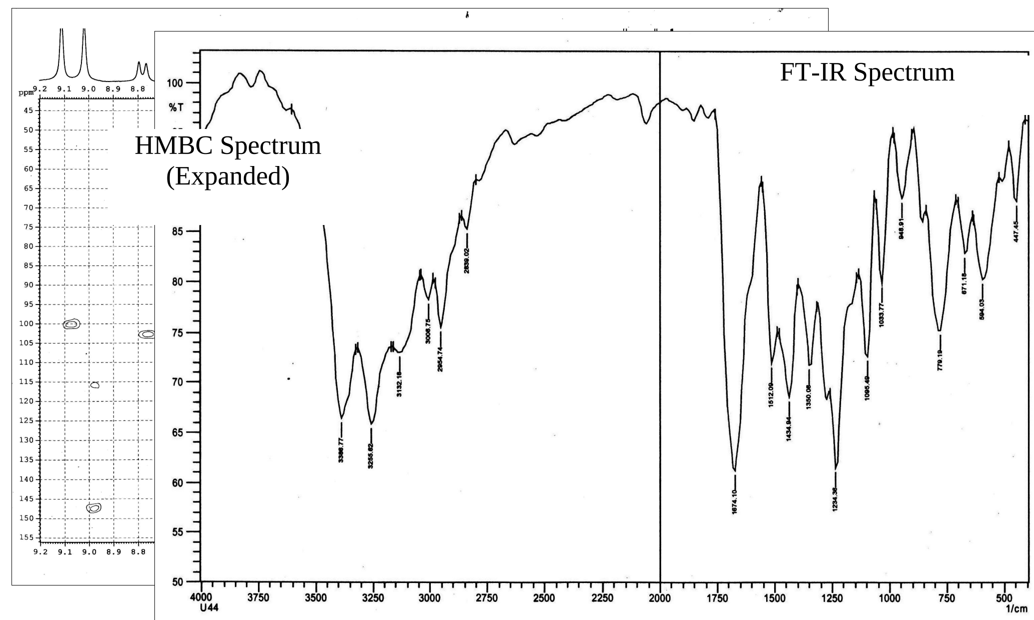


^{13}C NMR Spectrum (Expanded)

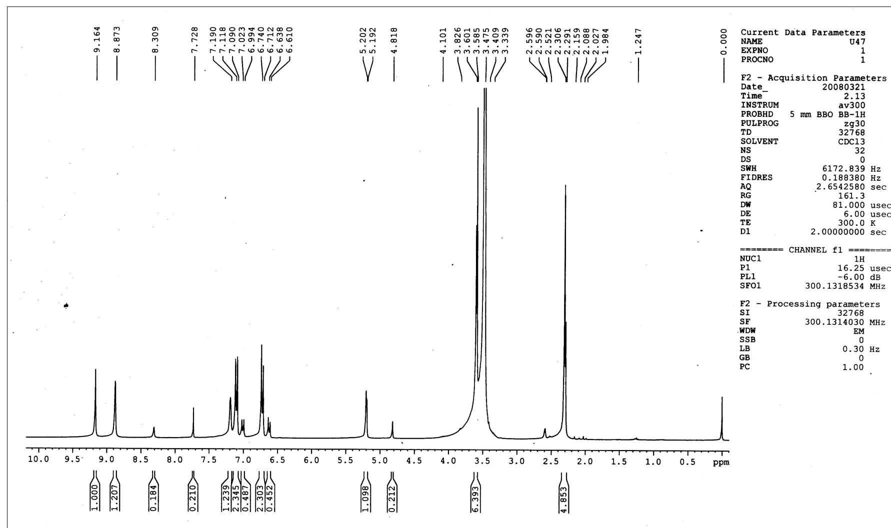

HH COSY Spectrum
Current Data Parameters
NAME U44
EXPNO 4
PROCNO 1
ppm

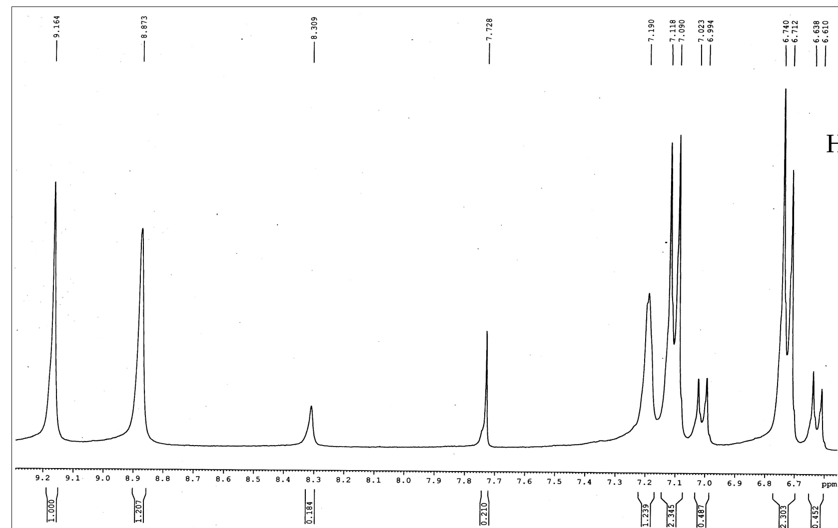

HH COSY Spectrum
(Expanded)
Current Data Parameters
NAME U44
EXPNO 4
PROCNO 1
ppm


HMBC Spectrum
Current Data Parameters
NAME U44
EXPNO 6
PROCNO 1
ppm

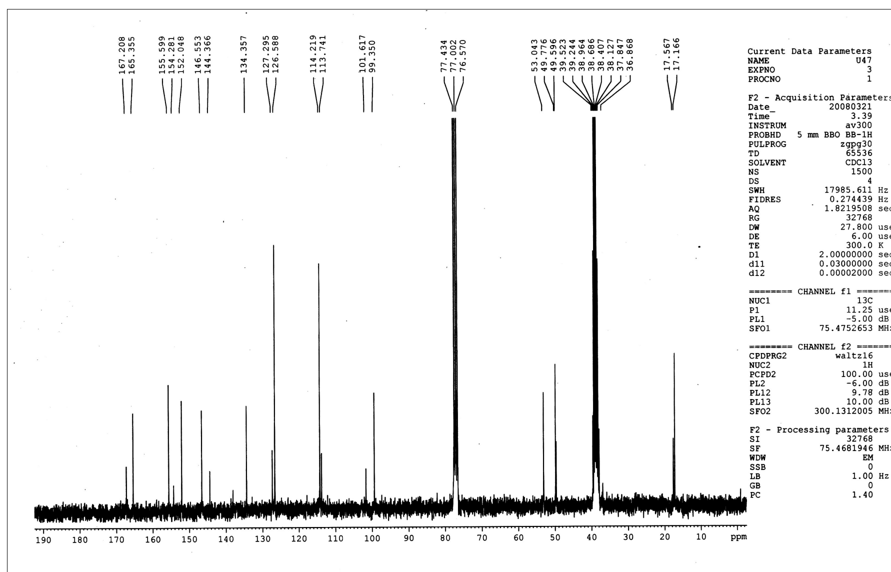

HMBC Spectrum
(Expanded)
FT-IR Spectrum
%T
U44
1/cm

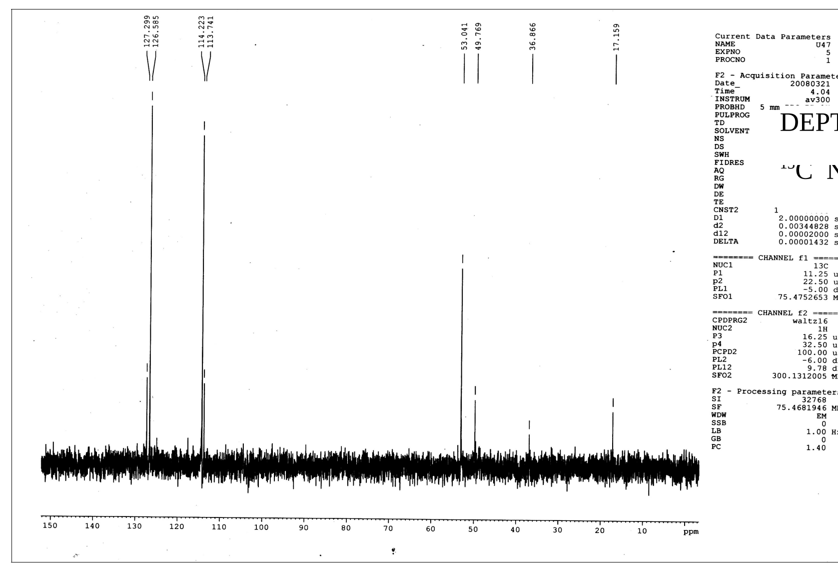
Spectra of U47





H NMR Spectrum
(Expanded)

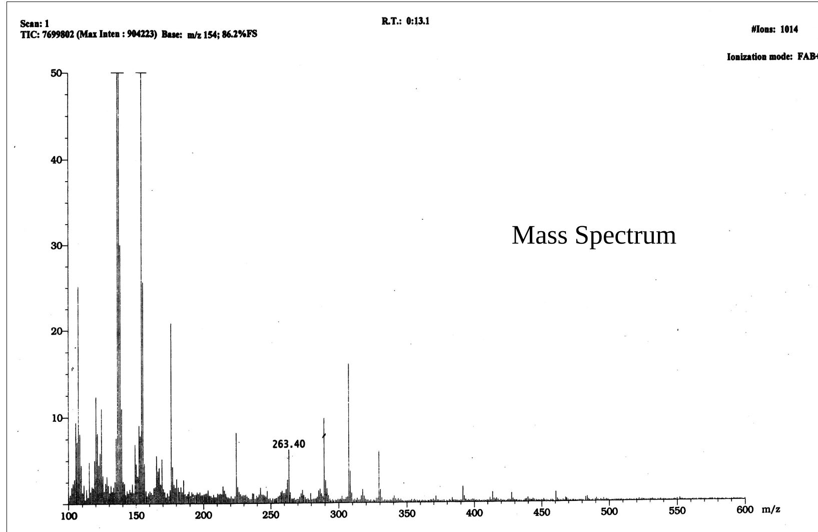


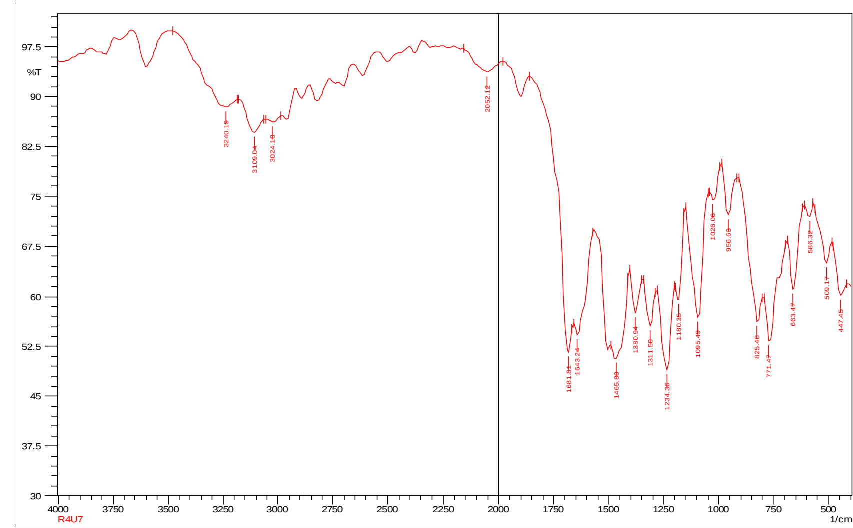


DEPT 135 Spectrum

^{13}C NMR Spectrum

FT-IR Spectrum

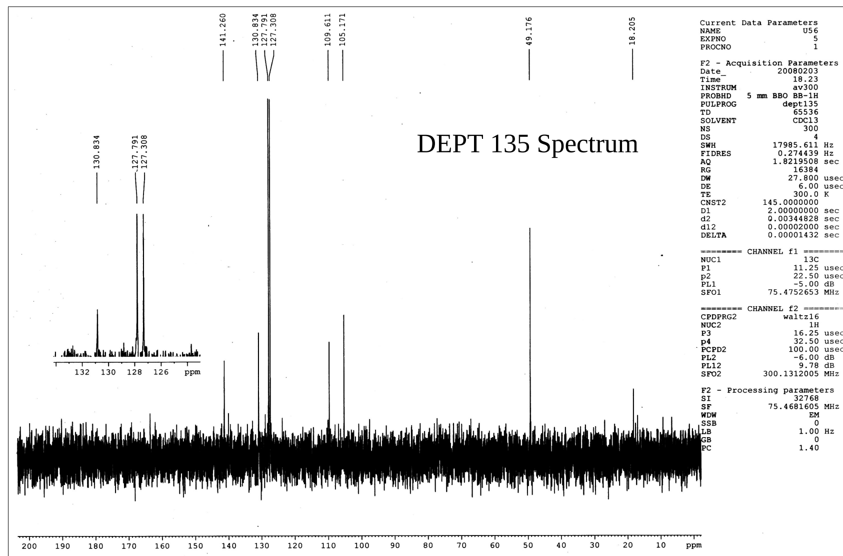


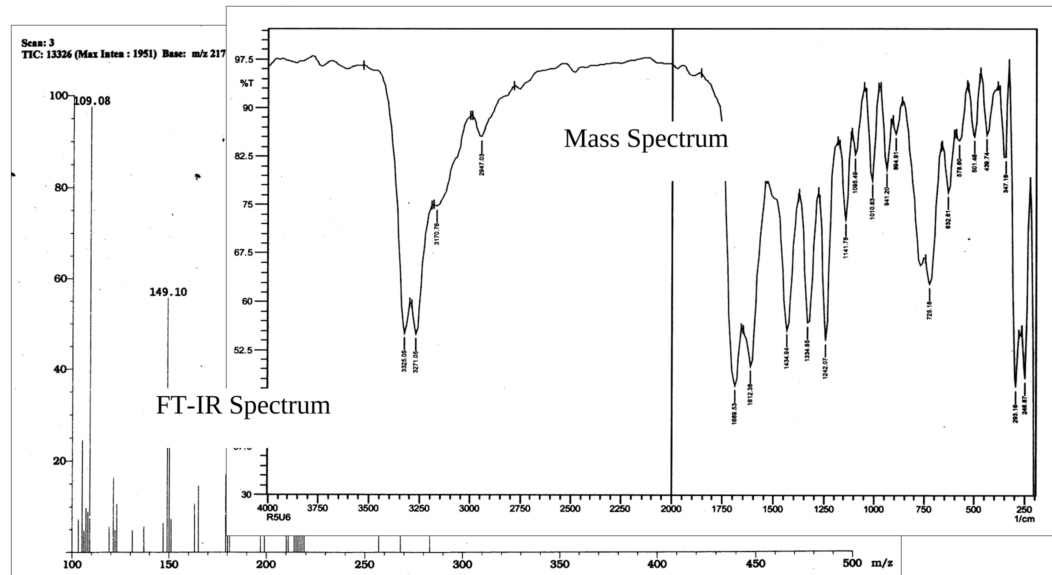


Spectra of U56

H NMR Spectrum
(Expanded)

^{13}C NMR Spectrum
(Expanded)


DEPT 135 Spectrum
Current Data Parameters

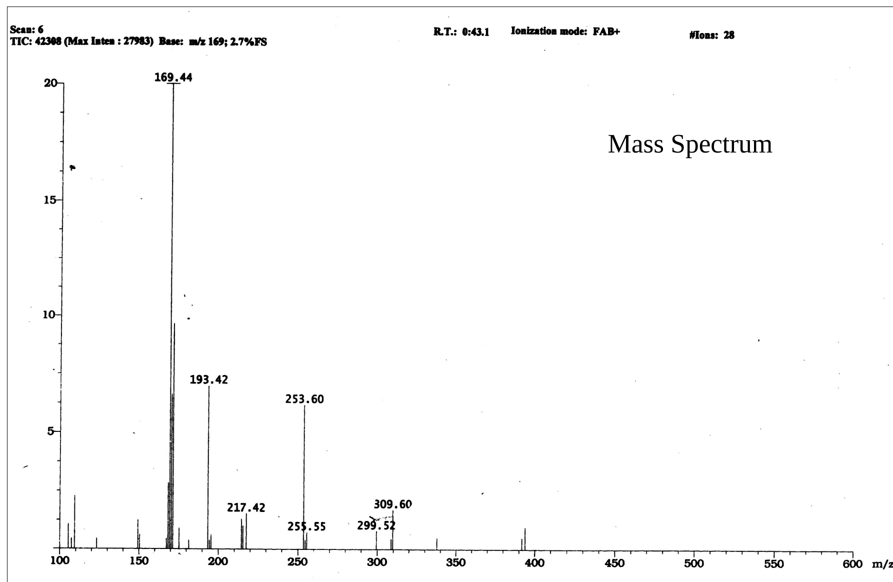

Mass Spectrum
FT-IR Spectrum
109.08
149.10
m/z
%T
1/cm

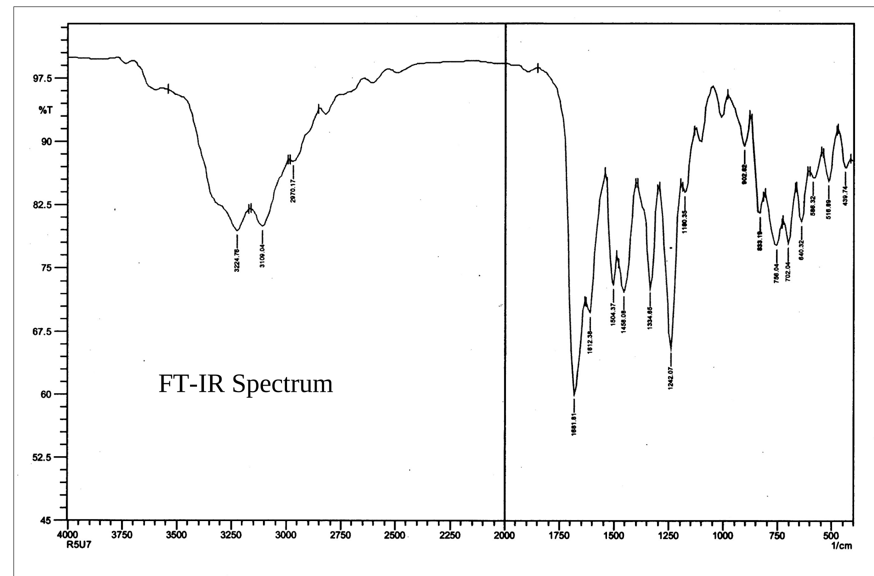
H NMR Spectrum

Spectra of U57

H NMR Spectrum (Expanded)

^{13}C NMR Spectrum


Scan: 6
TIC: 42308 (Max Inten : 27983) Base: m/z 169; 2.7%FS
R.T.: 0:43.1
Ionization mode: FAB+
#Ions: 28
Mass Spectrum
169.44
193.42
253.60
217.42
255.55
309.60
299.52
m/z


%T
FT-IR Spectrum
R5U7
1/cm

^{13}C NMR Spectrum
(Expanded)

Spectra of U210

^{1}H NMR Spectrum

^{1}H NMR Spectrum
(Expanded)

Mass Spectrum

FT-IR Spectrum

Spectra of U211

H NMR Spectrum (Expanded)

H NMR Spectrum

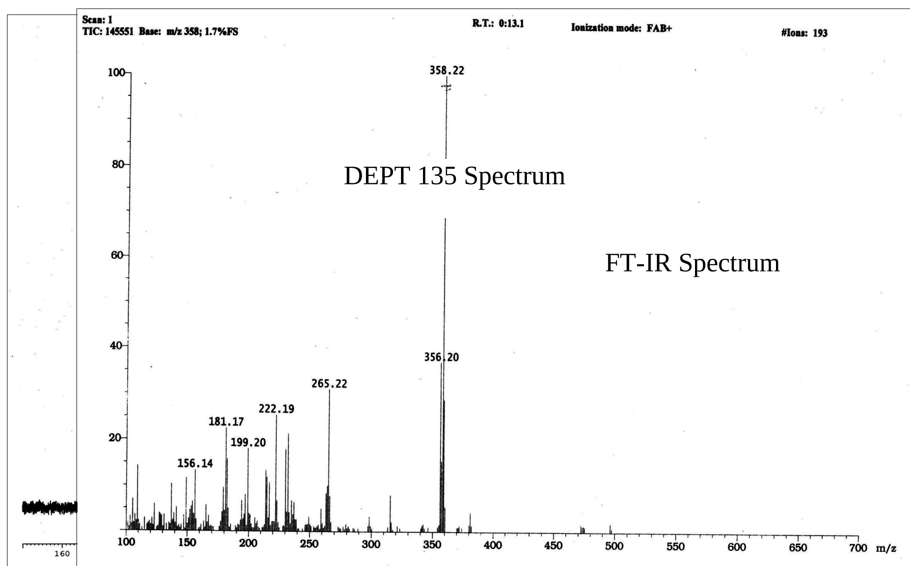
^{13}C NMR Spectrum

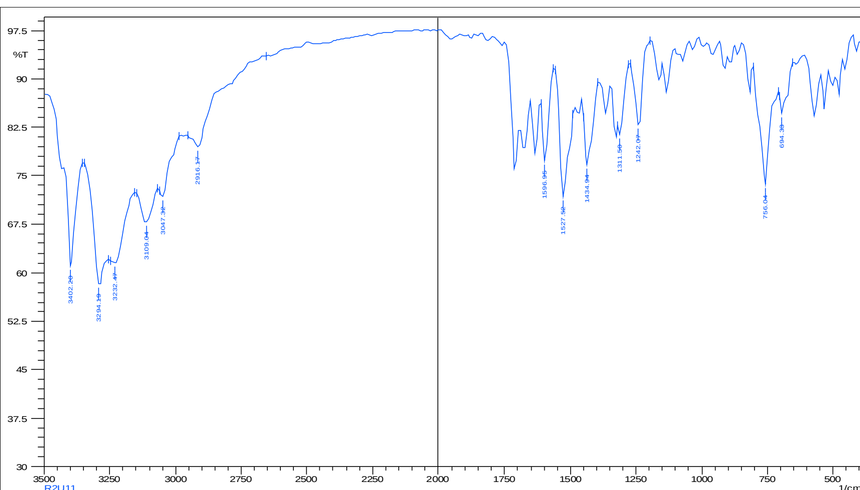
^{13}C NMR Spectrum (Expanded)

DEPT 135 Spectrum

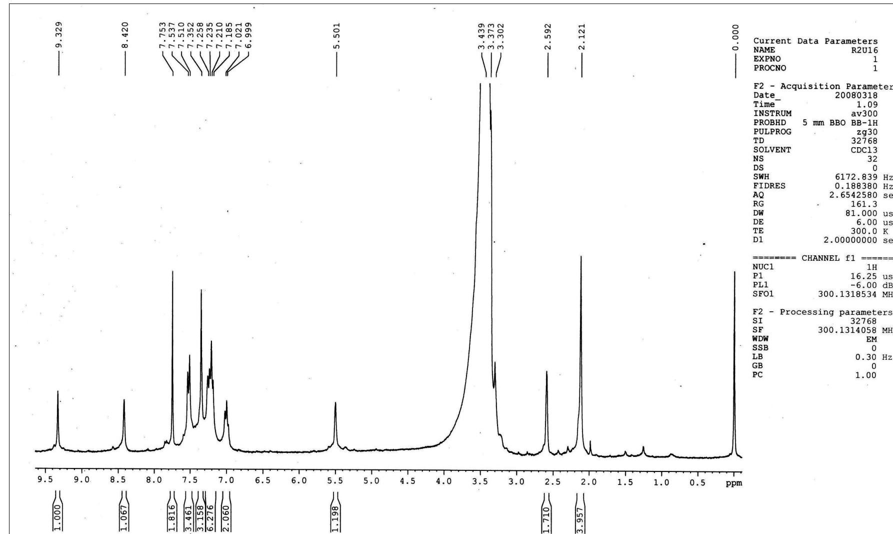
FT-IR Spectrum

Mass Spectrum

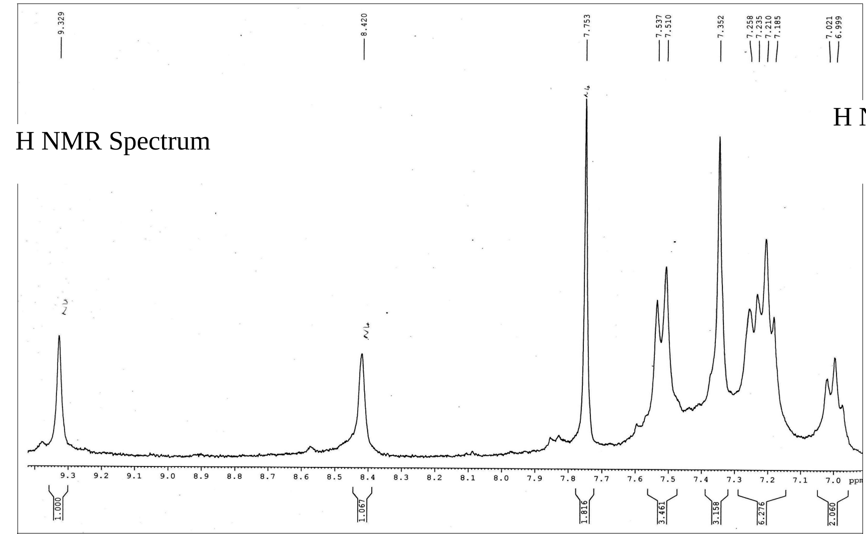




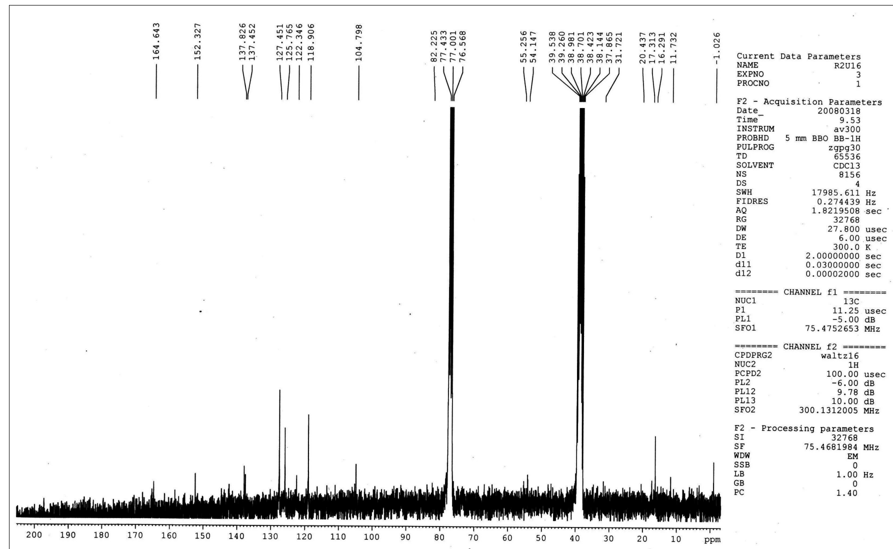
Spectra of U216



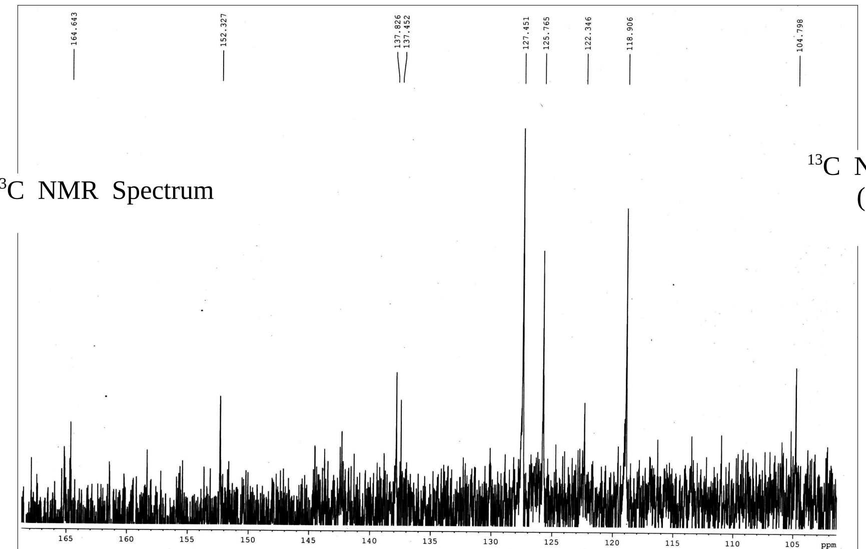
H NMR Spectrum



H NMR Spectrum (Expanded)

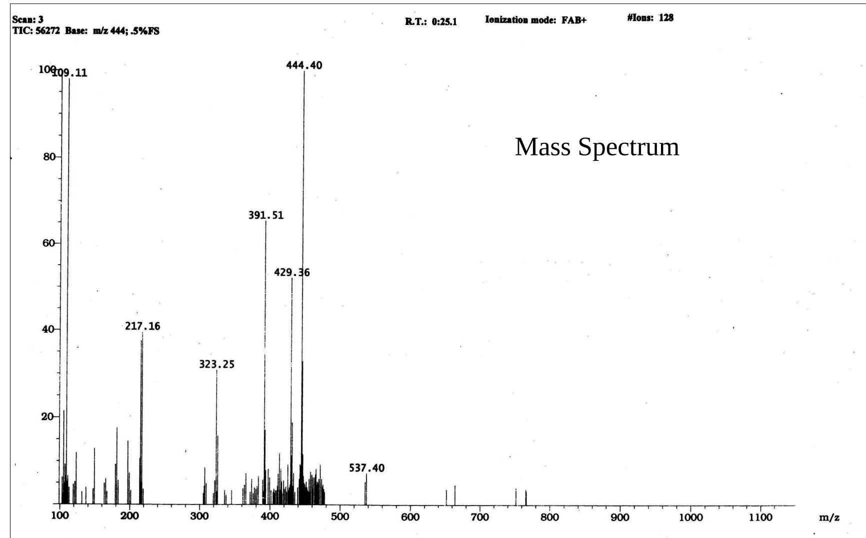


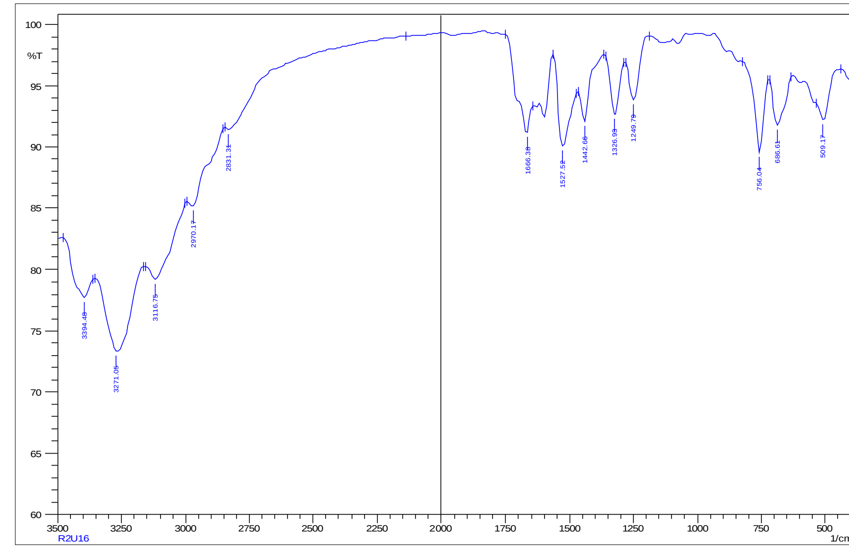
^{13}C NMR Spectrum



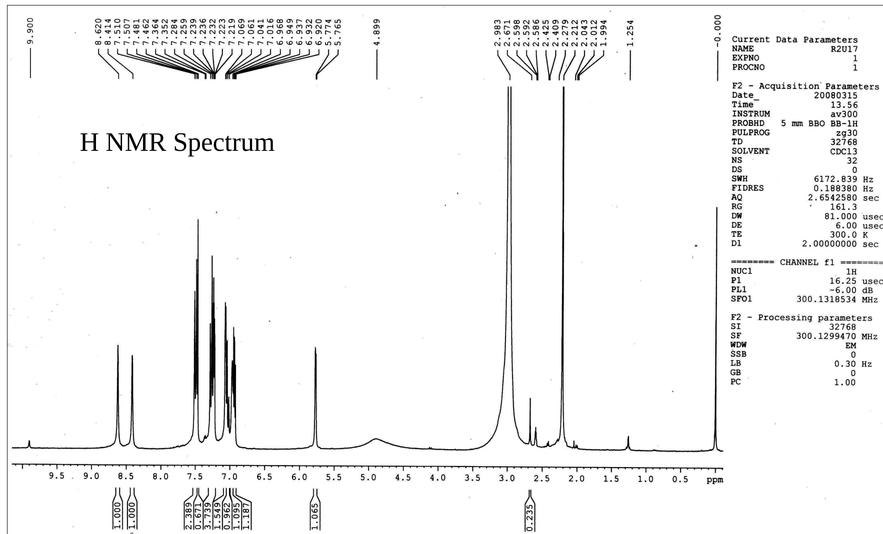
^{13}C NMR Spectrum (Expanded)

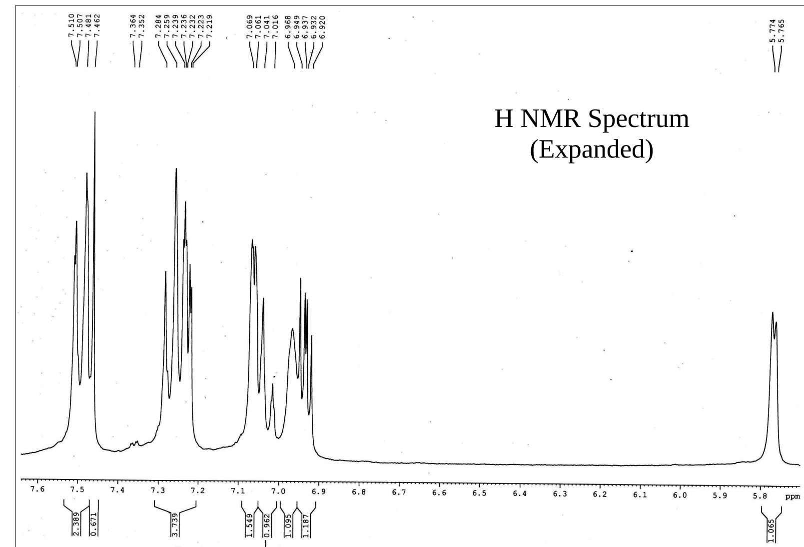
FT-IR Spectrum

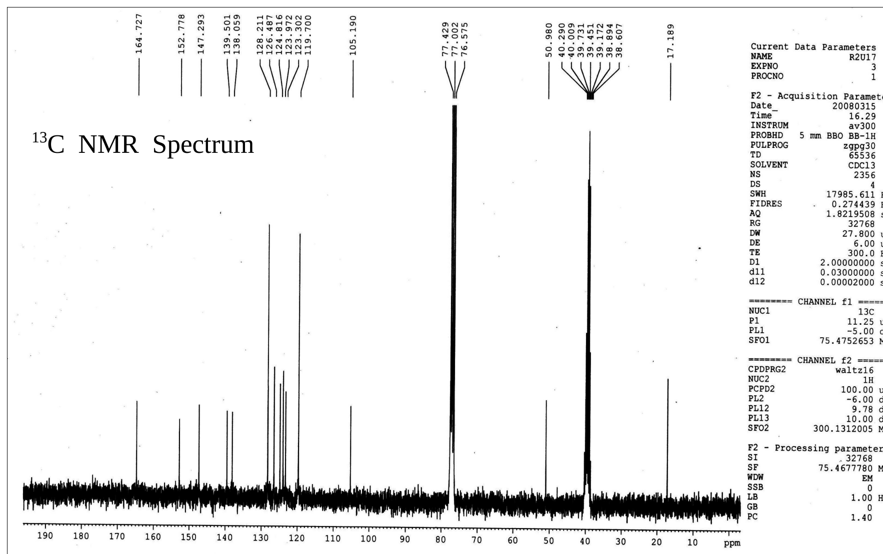


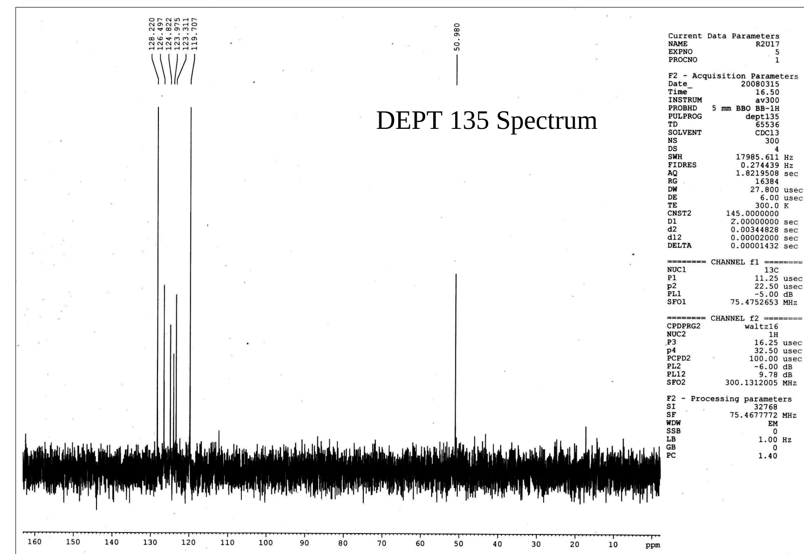


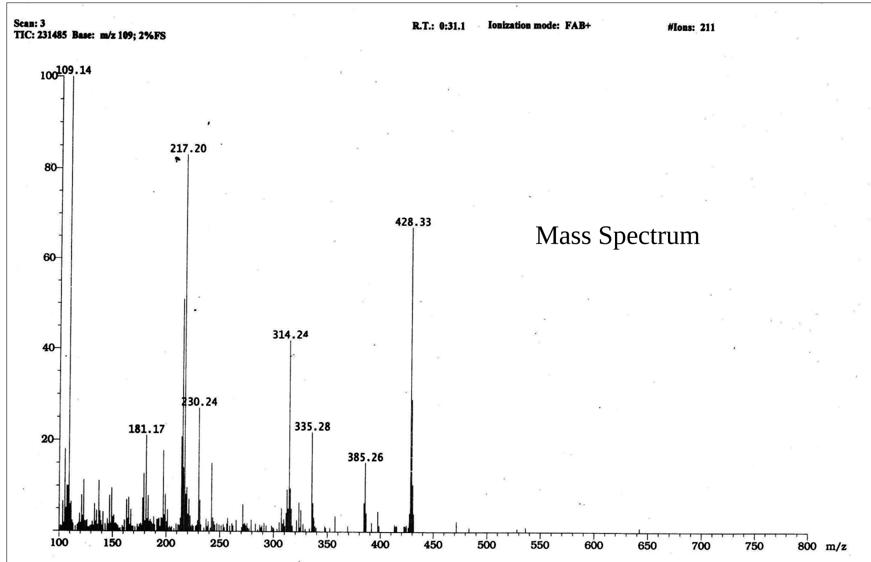
Spectra of U217

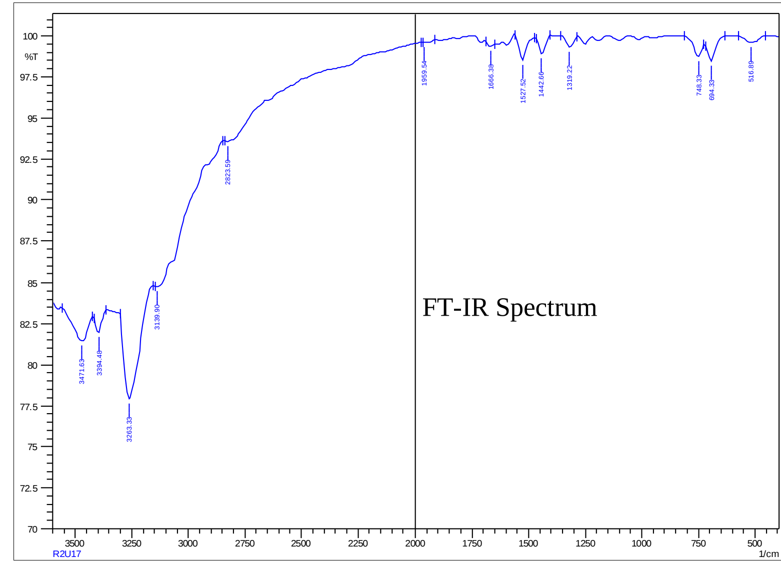




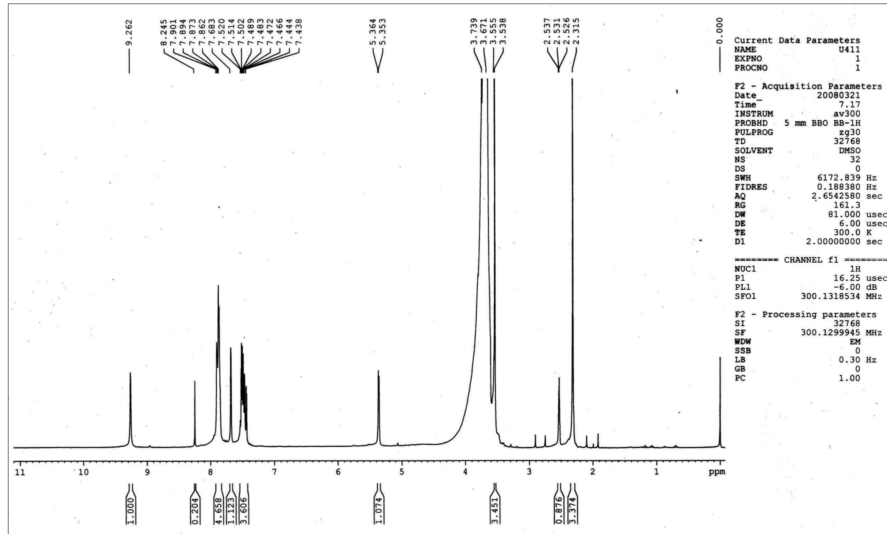


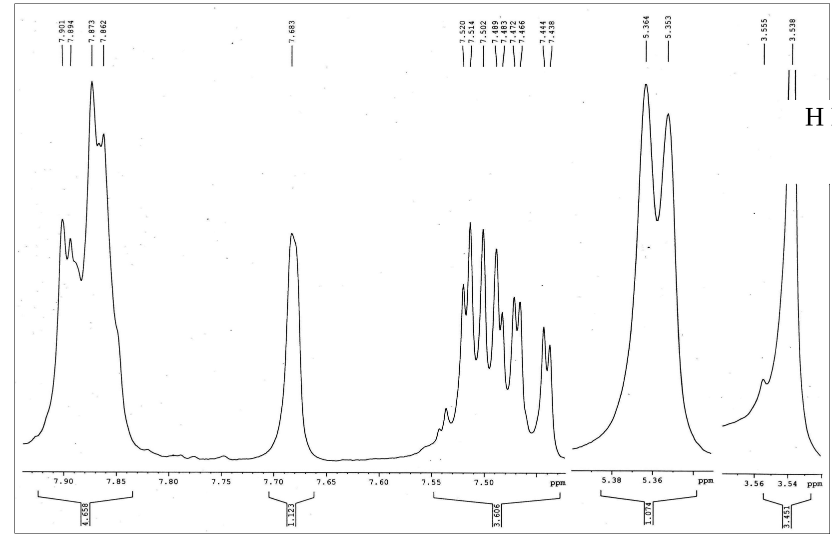



Scan: 3
TIC: 231485 Base: m/z 109; 2%FS
R.T.: 0:31.1
Ionization mode: FAB+
#Ions: 211
109.14
217.20
428.33
314.24
230.24
181.17
335.28
385.26
Mass Spectrum
m/z

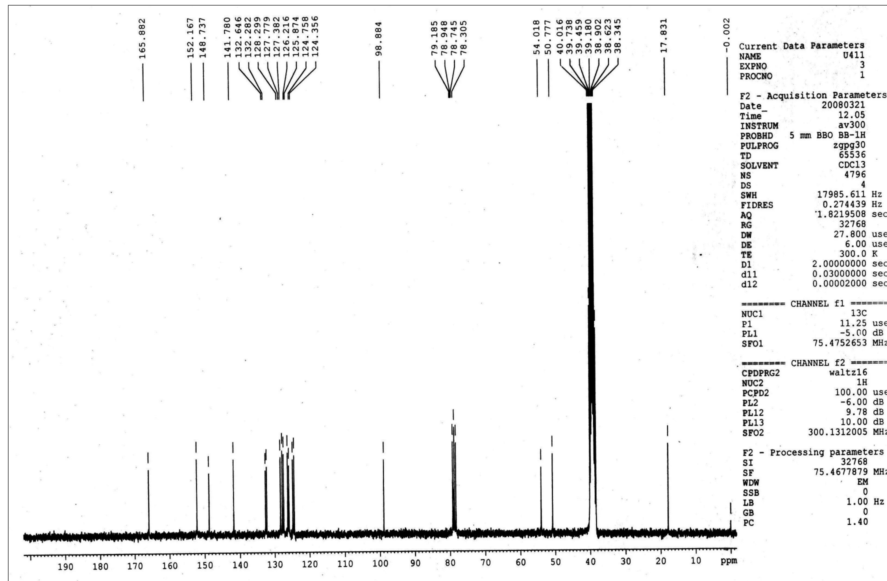

%T
FT-IR Spectrum
R2U17
1/cm

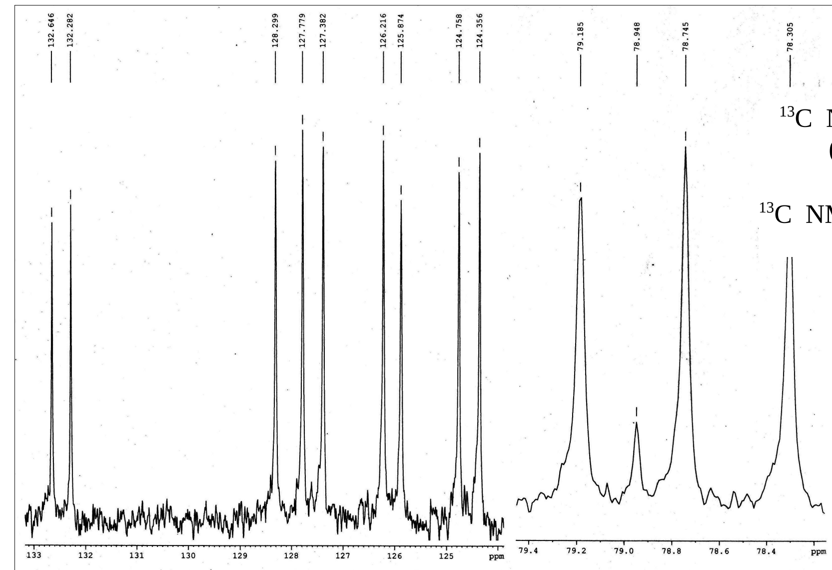
Spectra of U411





H NMR Spectrum
(Expanded)

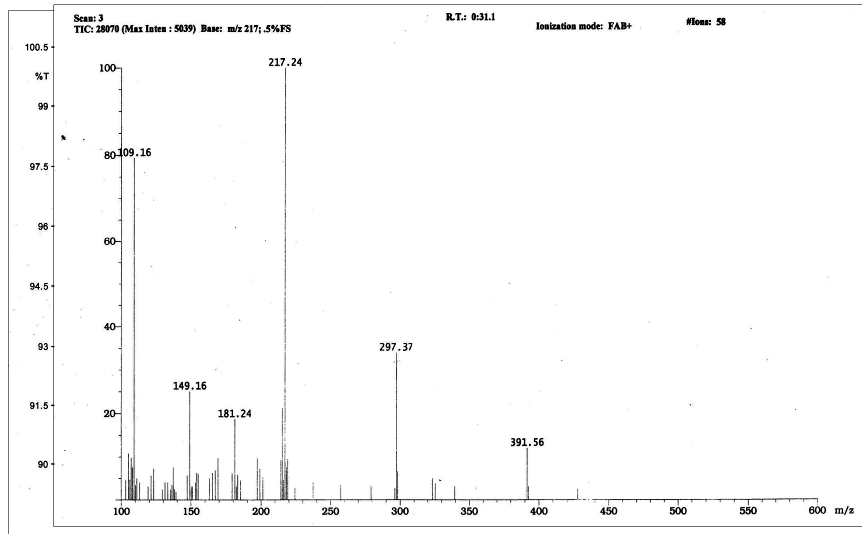




^{13}C NMR Spectrum
(Expanded)

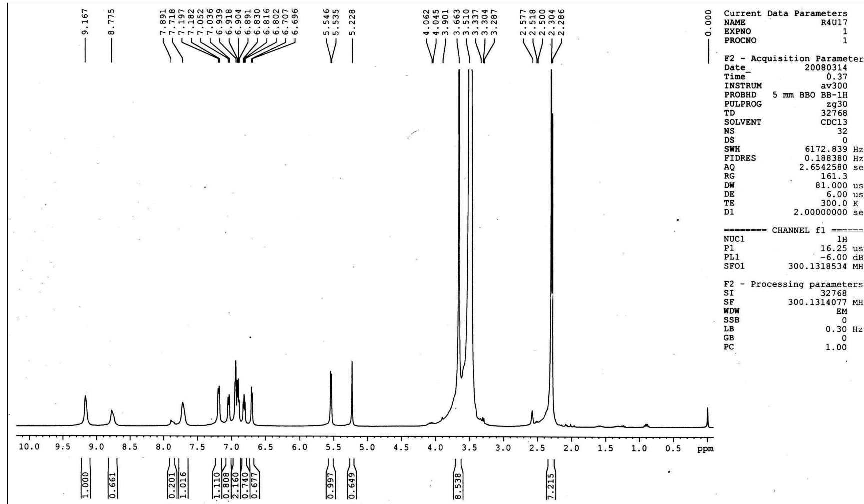
^{13}C NMR Spectrum

FT-IR Spectrum



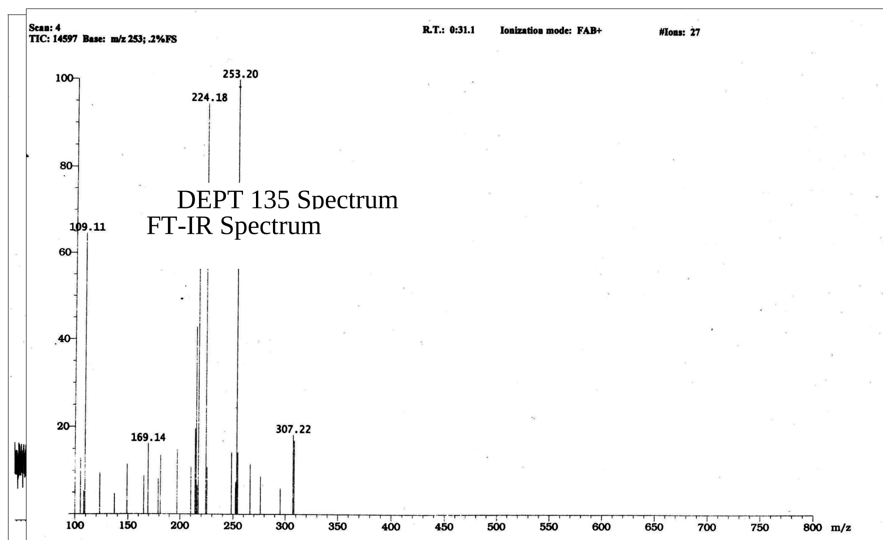
Mass Spectrum

Spectra of U417



^{1}H NMR Spectrum (Expanded)

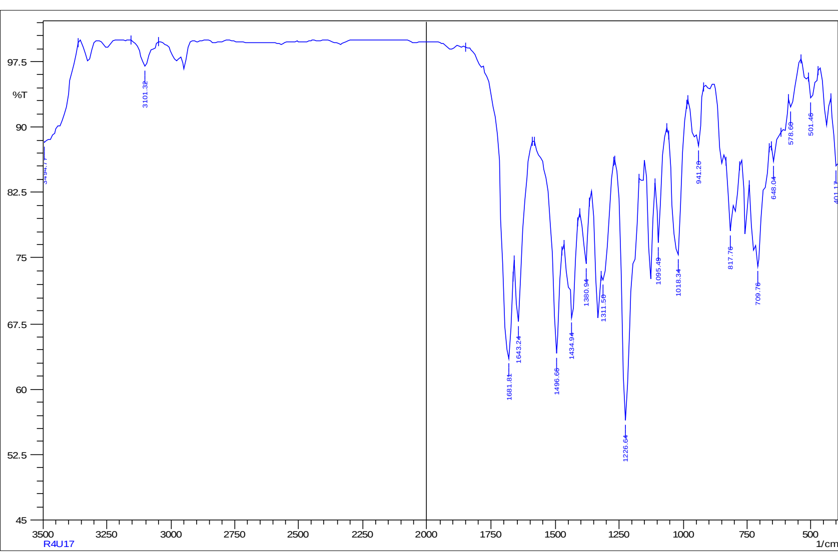
^{13}C NMR Spectrum (Expanded)



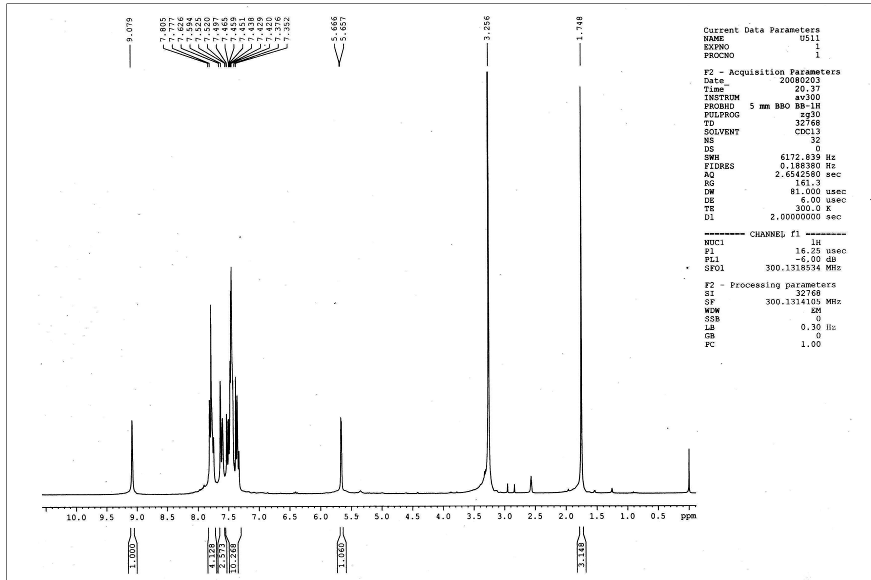
DEPT 135 Spectrum

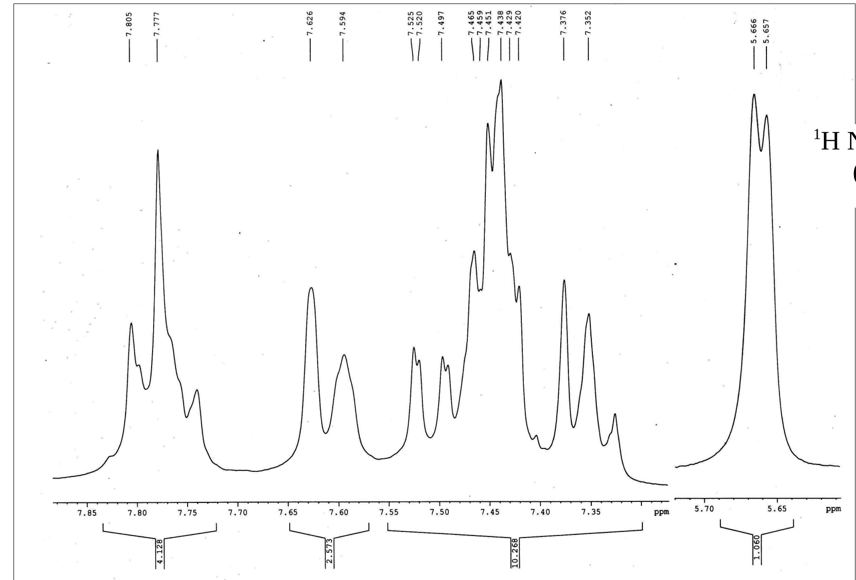
FT-IR Spectrum

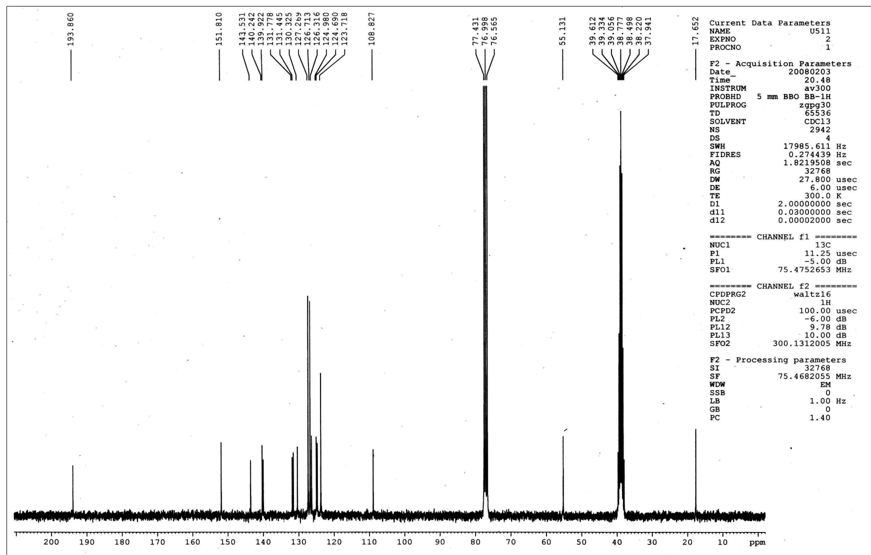
Mass Spectrum

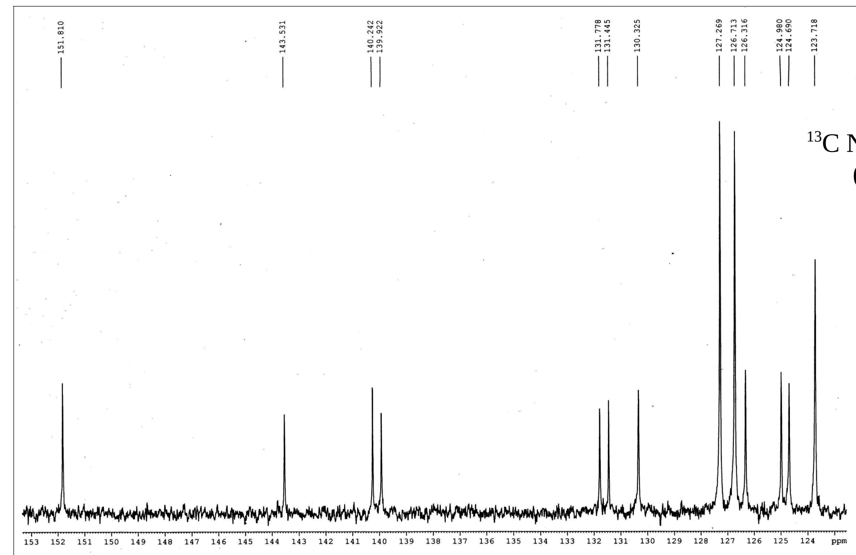


Spectra of U511









130.328
127.275
126.713
126.316
124.984
124.692
123.724
55.131

DEPT 135 Spectrum

180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 ppm

130 129 128 127 126 125 ppm

```
Current Data Parameters
NAME                U511
EXPNO                  5
PROCNO                 1

F2 - Acquisition Parameters
Date_           20080204
Time                0.09
INSTRUM            av300
PROBHD   5 mm BBO BB-1H
PULPROG          dept135
TD                 65536
SOLVENT            CDCl3
NS                   300
DS                     4
SWH            17985.611 Hz
FIDRES          0.274439 Hz
AQ             1.8219508 sec
RG                 16384
DW                27.800 usec
DE                  6.00 usec
TE                 300.0 K
CNST2        145.0000000
D1            2.00000000 sec
d2            0.00344828 sec
d12           0.00002000 sec
DELTA         0.00001432 sec

======== CHANNEL f1 ========
NUC1                 13C
P1                 11.25 usec
p2                 22.50 usec
PL1                -5.00 dB
SFO1          75.4752653 MHz

======== CHANNEL f2 ========
CPDPRG2         waltz16
NUC2                  1H
P3                 16.25 usec
p4                 32.50 usec
PCPD2             100.00 usec
PL2                -6.00 dB
PL12                9.78 dB
SFO2         300.1312005 MHz

F2 - Processing parameters
SI                 32768
SF            75.4682055 MHz
WDW                   EM
SSB                    0
LB                  1.00 Hz
GB                     0
PC                  1.40
```

FT-IR Spectrum

%T

100 95 90 85 80 75 70 65 60

3425.34
3294.19
3201.61
3085.89
1959.54
1905.54
1704.90
1596.95
1419.51
1350.09
1249.79
709.76

3500 3250 3000 2750 2500 2250 2000 1750 1500 1250 1000 750 500

R5U1

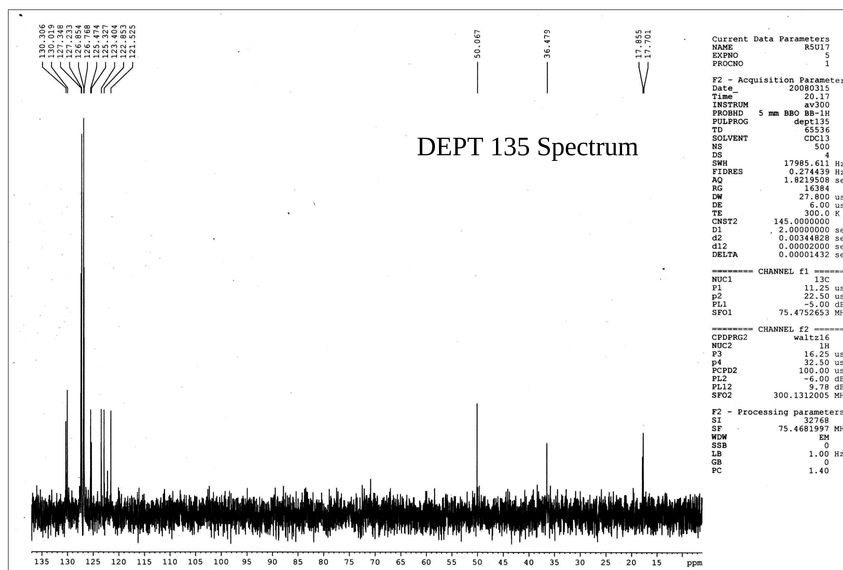
1/cm

Spectra of U517

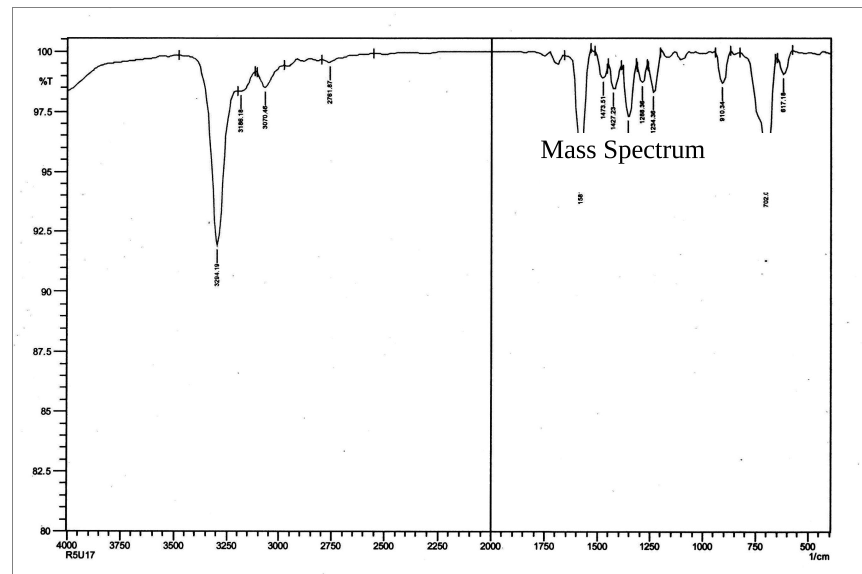
^{1}H NMR Spectrum (Expanded)

^{13}C NMR Spectrum (Expanded)

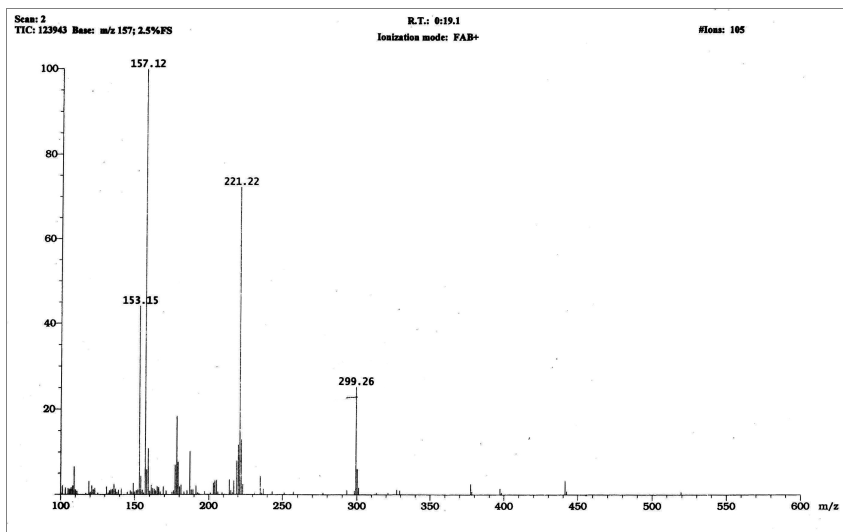
FT-IR Spectrum



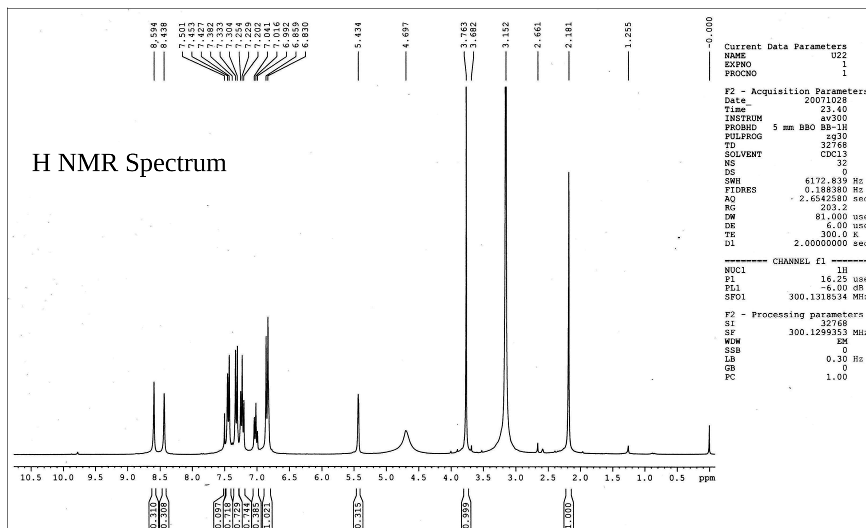
DEPT 135 Spectrum

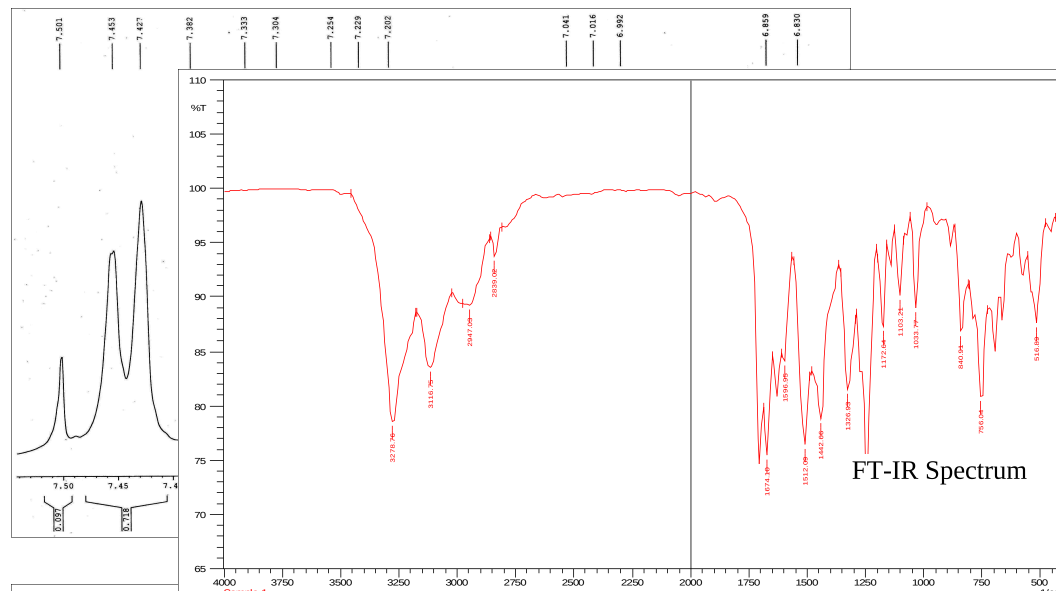


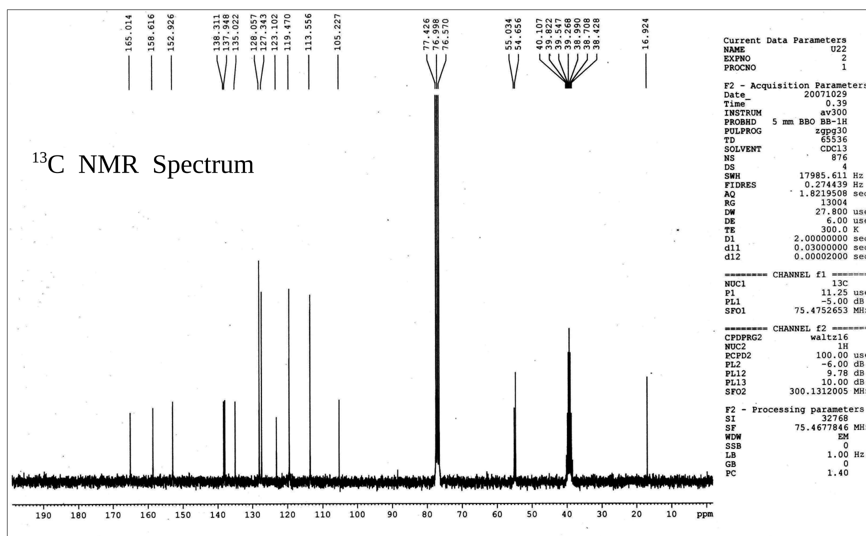
Mass Spectrum

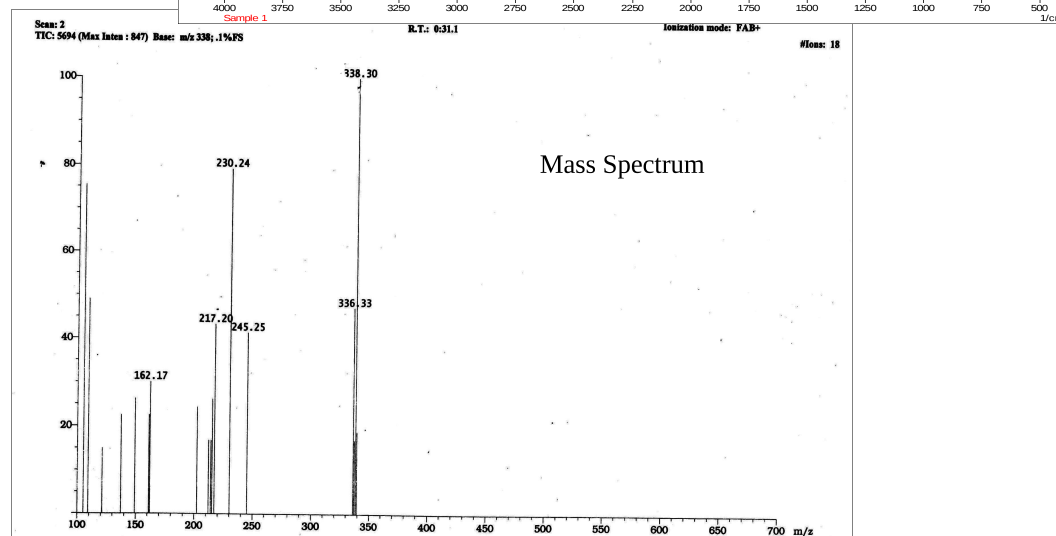


Spectra of U22

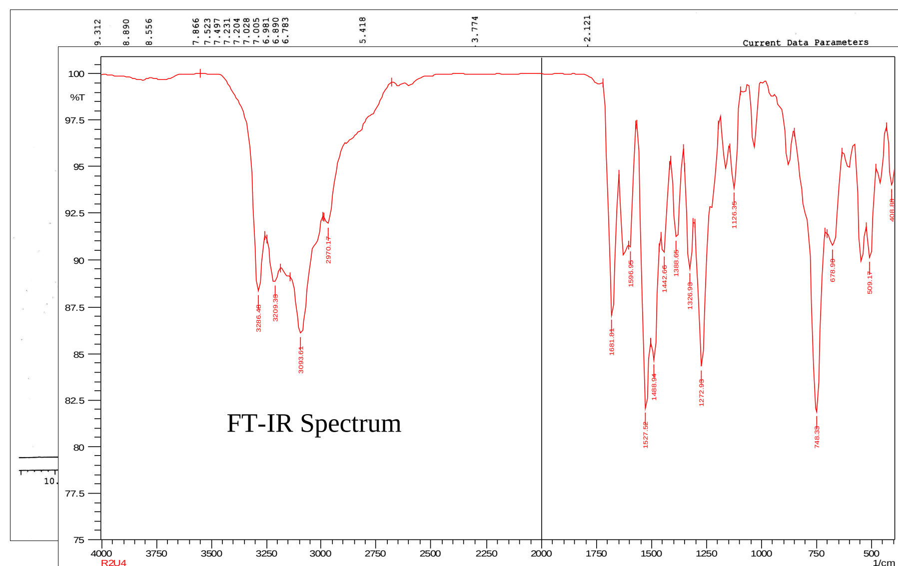


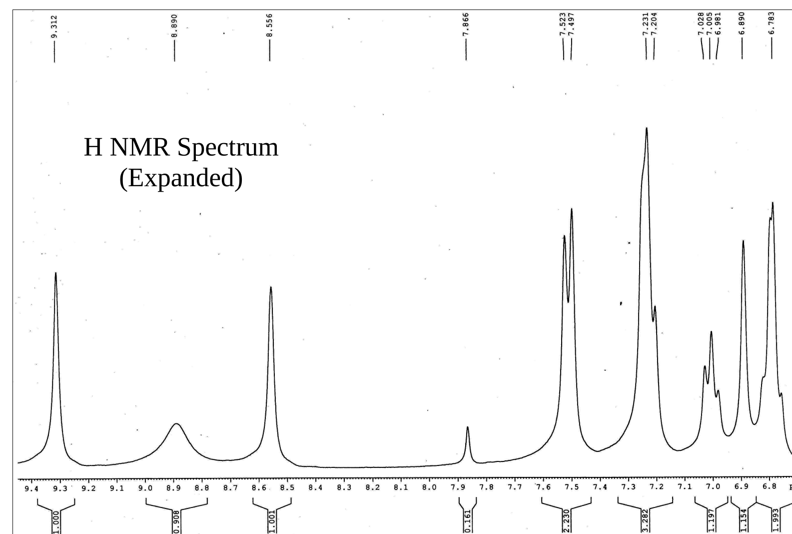


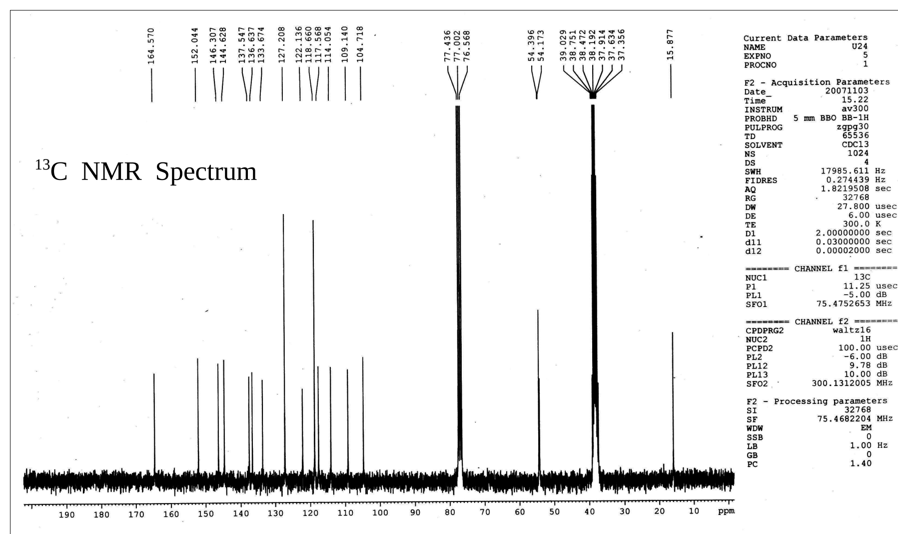


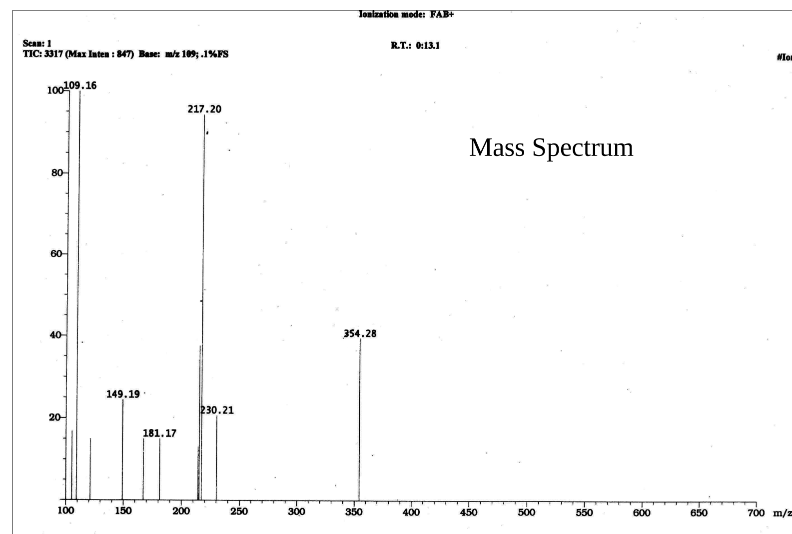


Spectra of U24









Spectra of U25

H NMR Spectrum

^{13}C NMR Spectrum

Mass Spectrum

FT-IR Spectrum

Current Data Parameters
NAME U25
EXPNO 1
PROCNO 1

F2 - Acquisition Parameters
Date_ 20071104
Time 11.00
INSTRUM av300
PROBHD 5 mm BBO BB-1H
PULPROG zg30
TD 32768
SOLVENT CDCl3
NS 32
DS 0
SWH 6172.839 Hz
FIDRES 0.188380 Hz
AQ 2.6542580 sec
RG 161.3
DW 81.000 usec
DE 6.00 usec
TE 300.0 K
D1 2.00000000 sec

NUC1
P1
PL1
SFO1

F2 - Processing parameters
SI 32768
SF 300.1299222 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00

Current Data Parameters
NAME U25
EXPNO 2
PROCNO 1

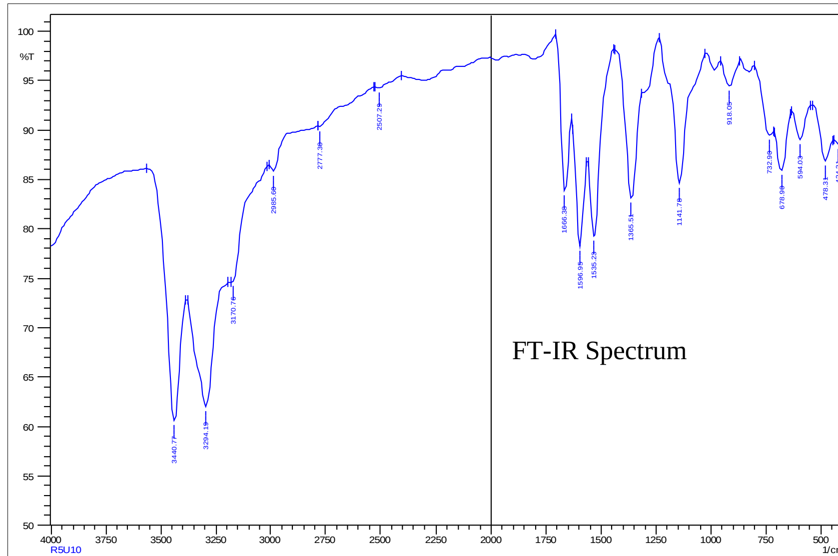
F2 - Acquisition Parameters
Date_ 20071104
Time 12.08
INSTRUM av300
PROBHD 5 mm BBO BB-1H
PULPROG zgpg30
TD 65536
SOLVENT CDCl3
NS 1024
DS 4
SWH 17985.611 Hz
FIDRES 0.274439 Hz
AQ 1.8219508 sec
RG 32768
DW 27.800 usec
DE 6.00 usec
TE 300.0 K
D1 2.00000000 sec
d11 0.03000000 sec
d12 0.00002000 sec

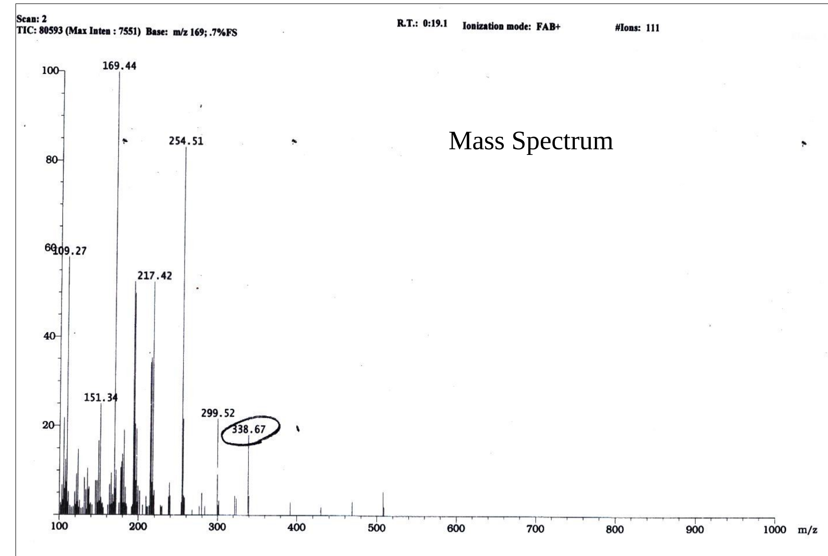
======== CHANNEL f1 ========
NUC1 13C
P1 11.25 usec
PL1 -5.00 dB
SFO1 75.4752653 MHz

======== CHANNEL f2 ========
CPDPRG2 waltz16
NUC2 1H
PCPD2 100.00 usec
PL2 -6.00 dB
PL12 9.78 dB
PL13 10.00 dB
SFO2 300.1312005 MHz

F2 - Processing parameters
SI 32768
SF 75.4677895 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40

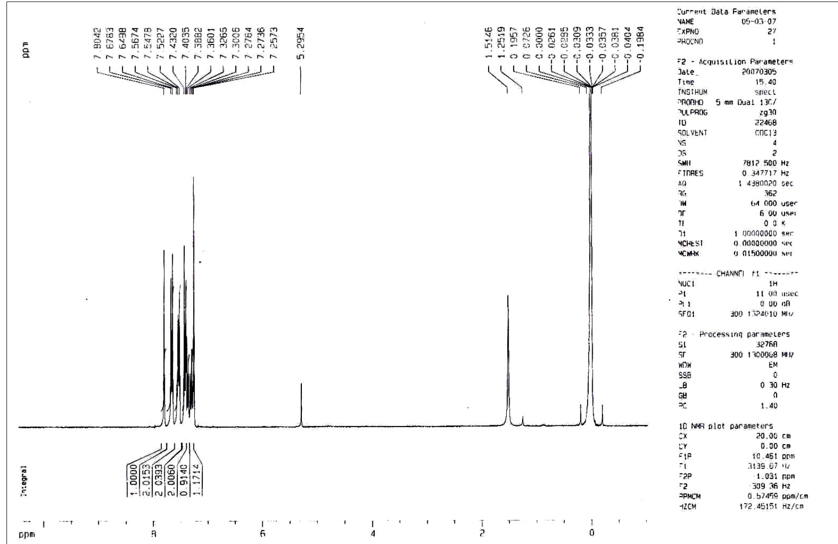
Spectra of U510

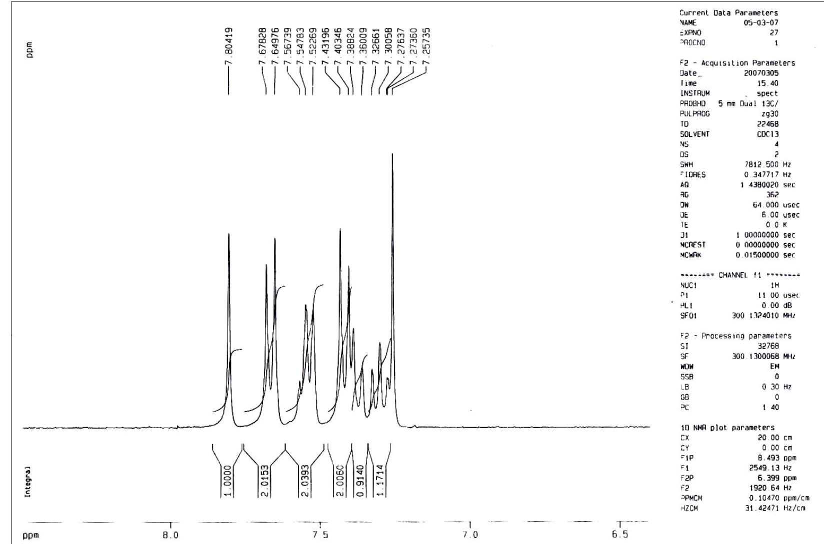




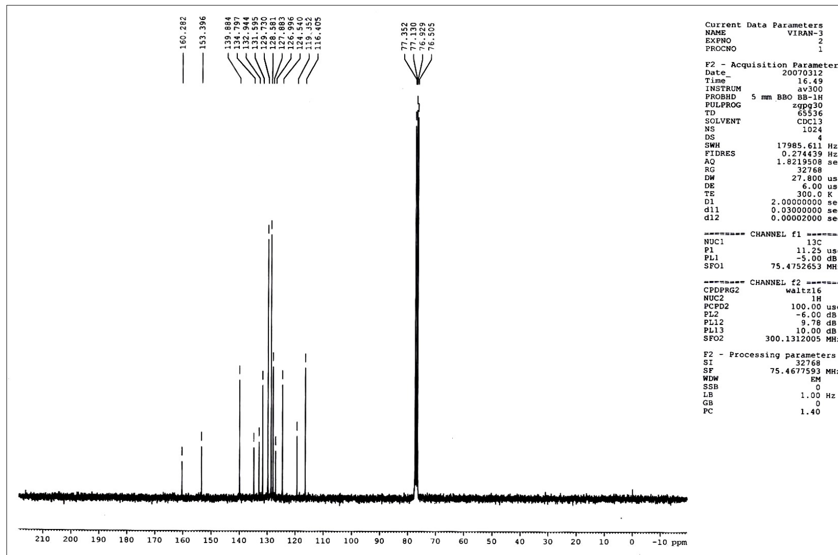
Spectra of New Coumarins

Spectra of C13



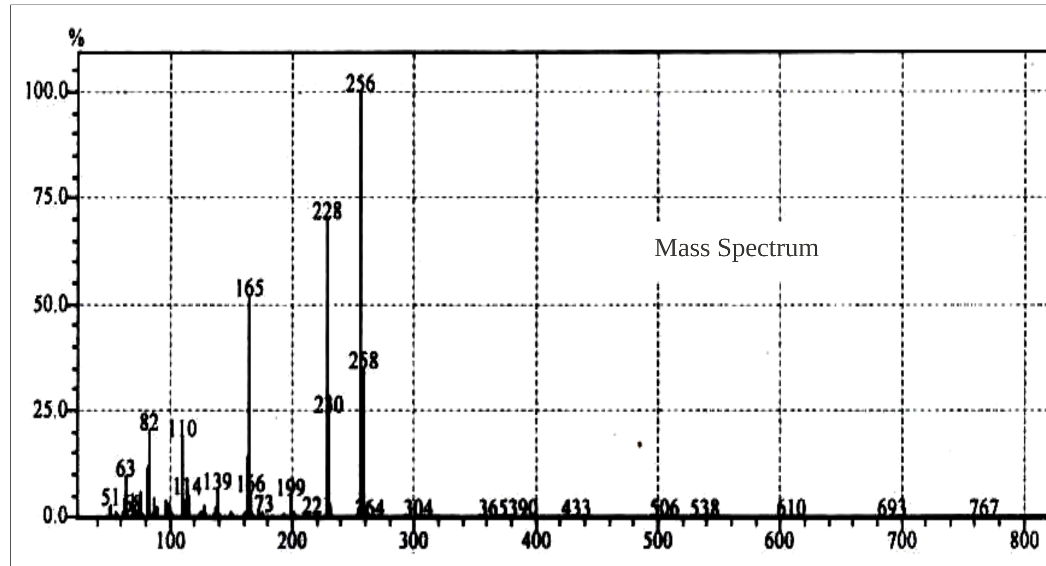


^{1}H NMR Spectrum
(Expanded)

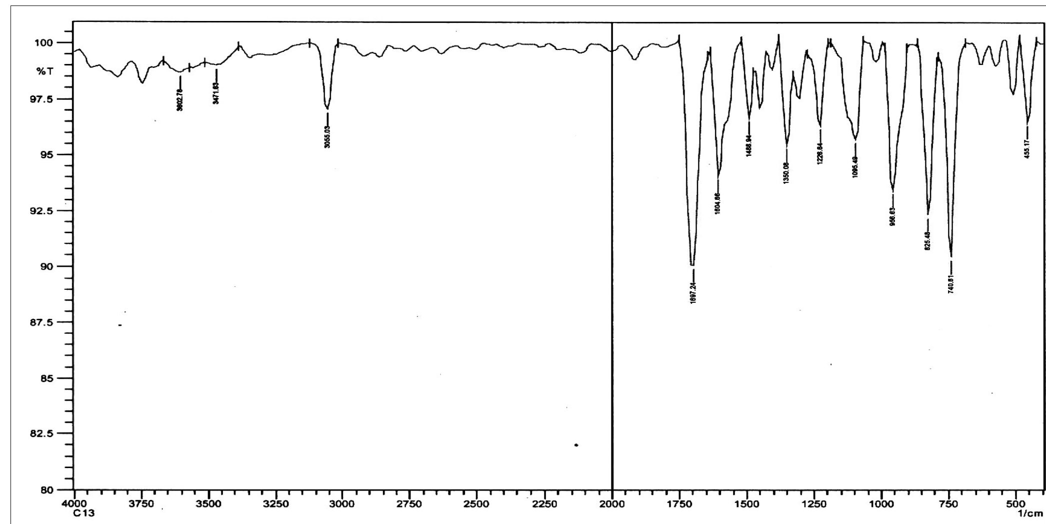


^{13}C NMR Spectrum

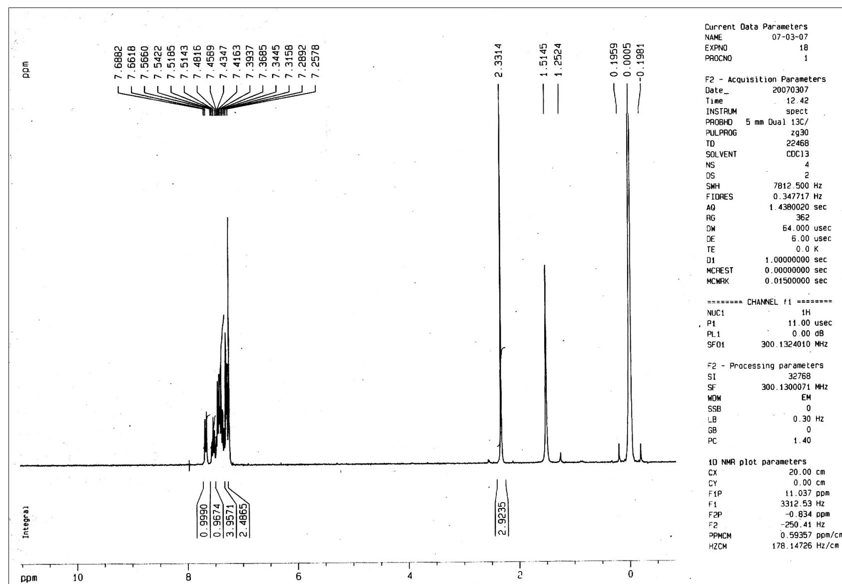
^{13}C NMR Spectrum
(Expanded)

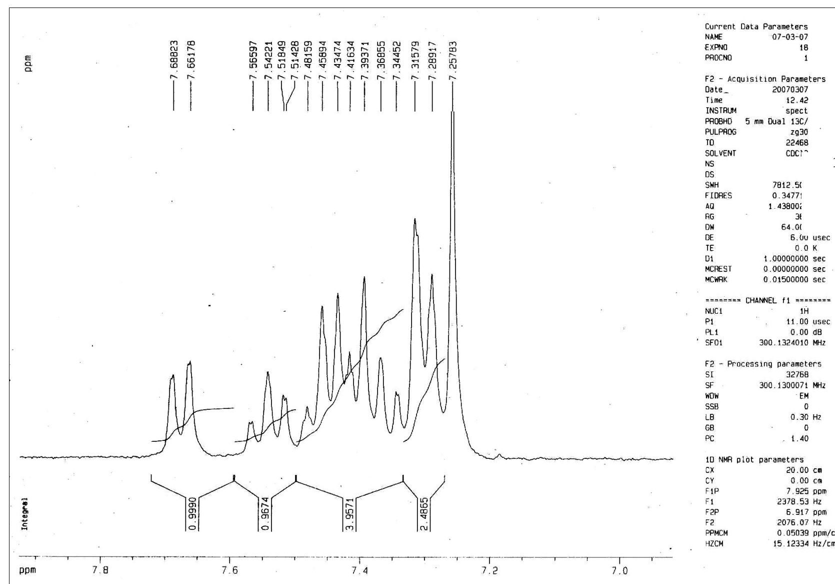


FT-IR Spectrum

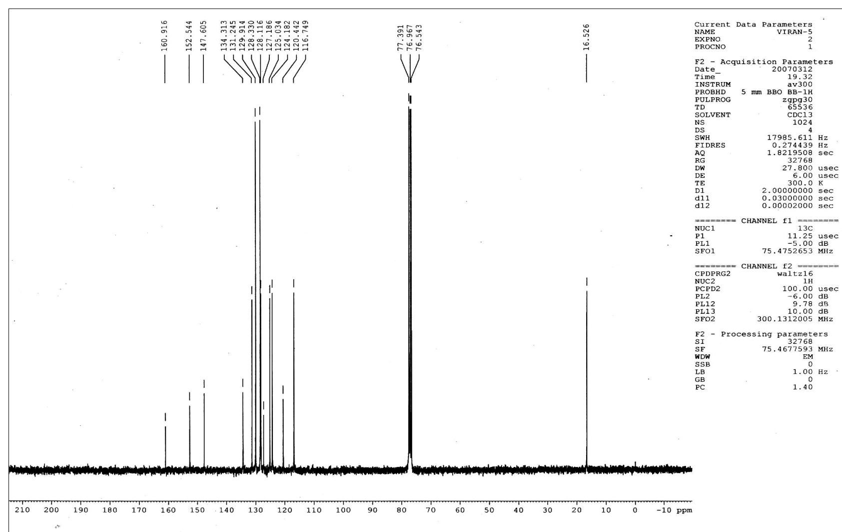


Spectra of C21

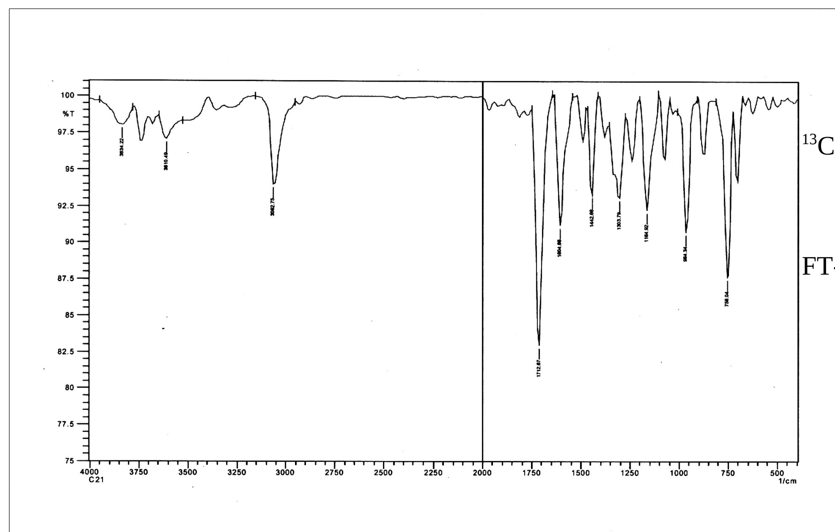




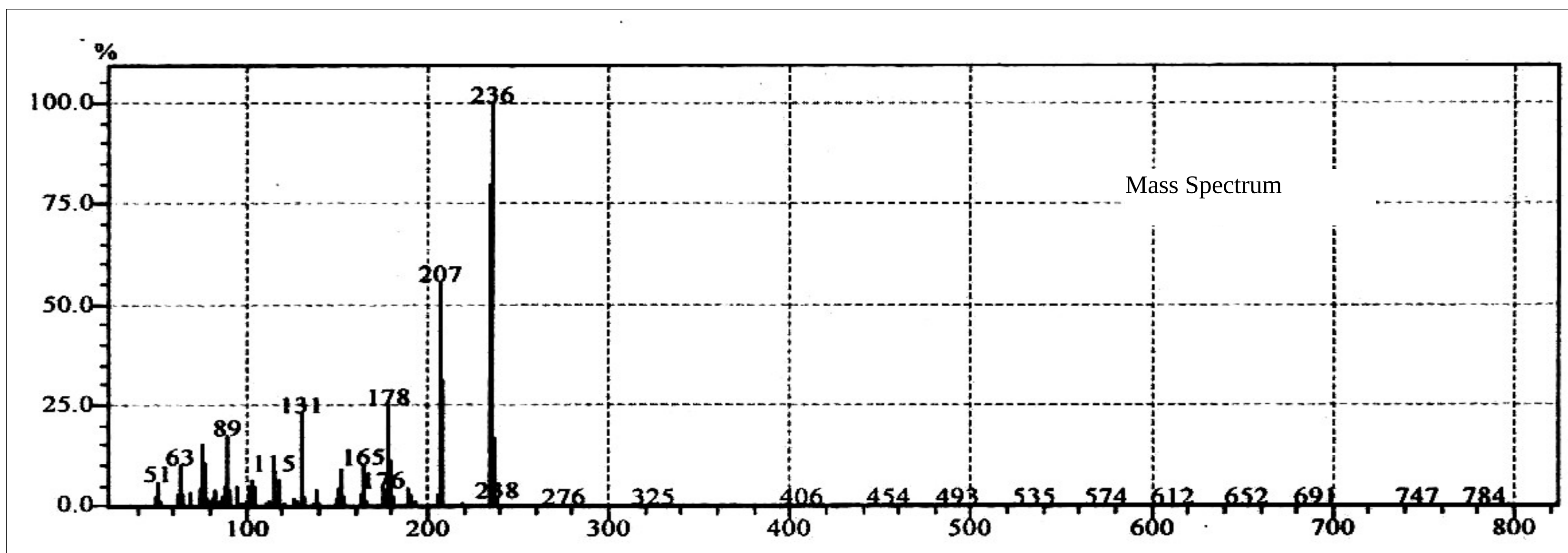
^{1}H NMR Spectrum (Expanded)



^{13}C NMR Spectrum

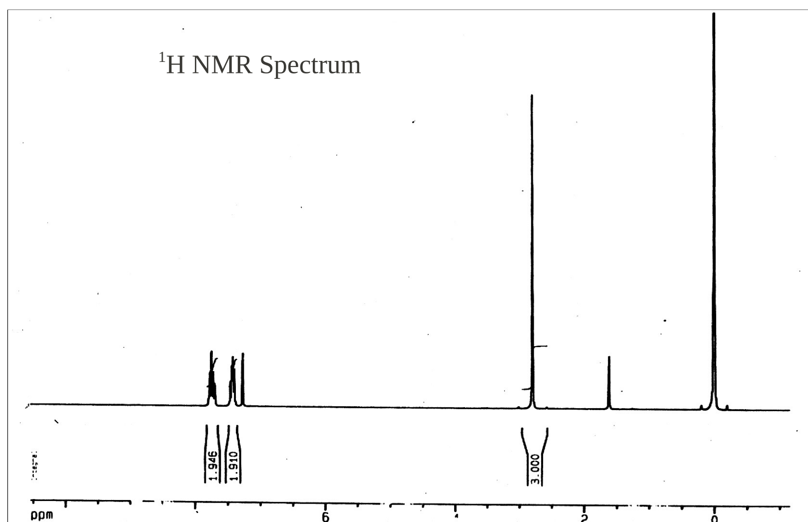


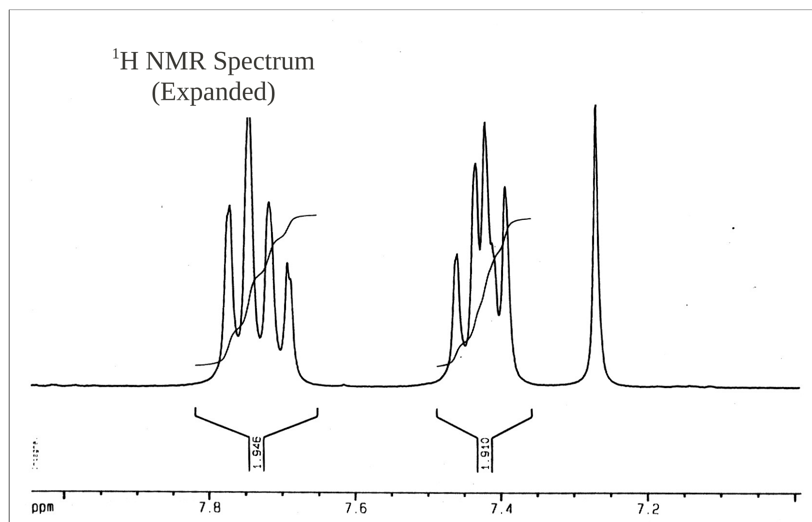
FT-IR Spectrum

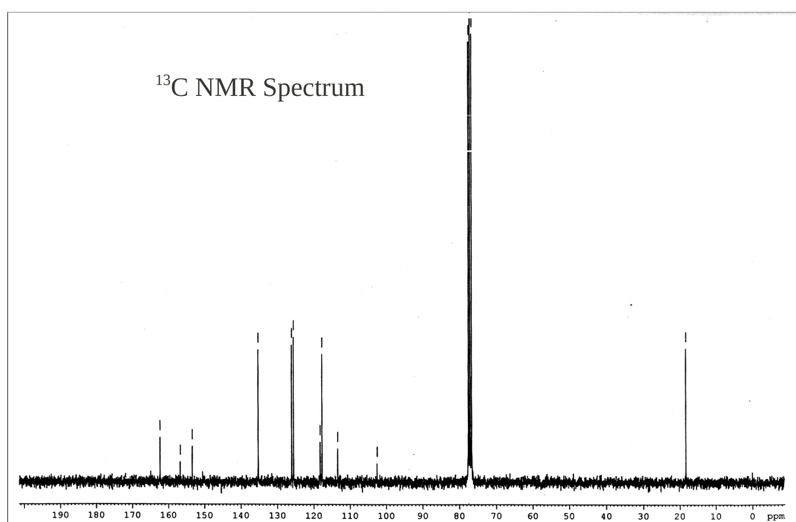

%
100.0
75.0
50.0
25.0
0.0
236
207
178
131
89
63
51
165
176
238
276
325
406
454
493
535
574
612
652
691
747
784
100
200
300
400
500
600
700
800
Mass Spectrum

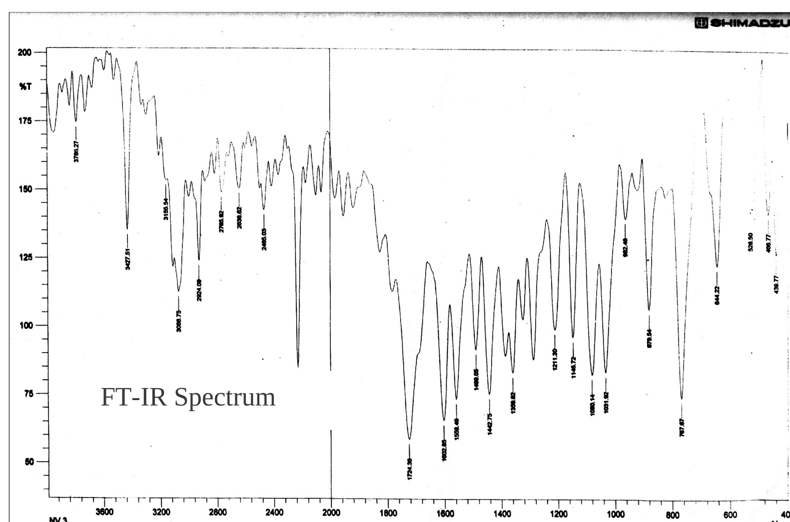
¹³C NMR Spectrum

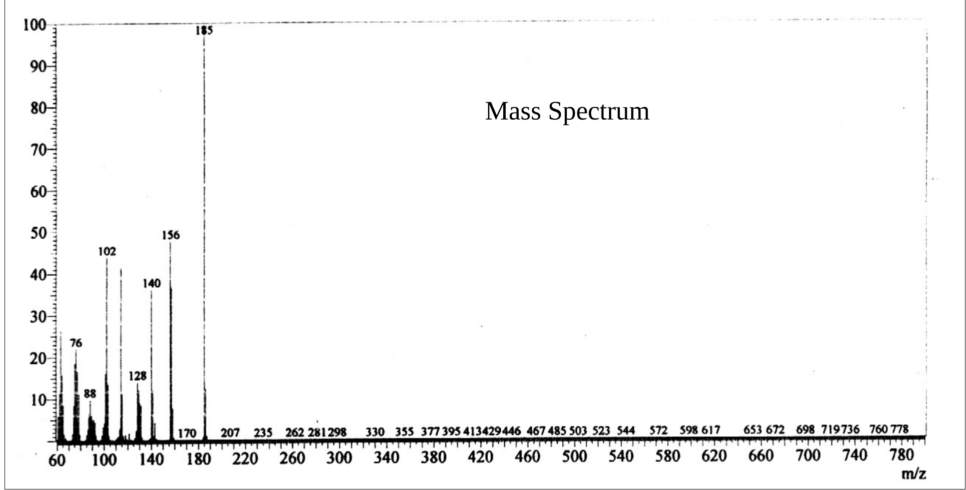
Spectra of C22



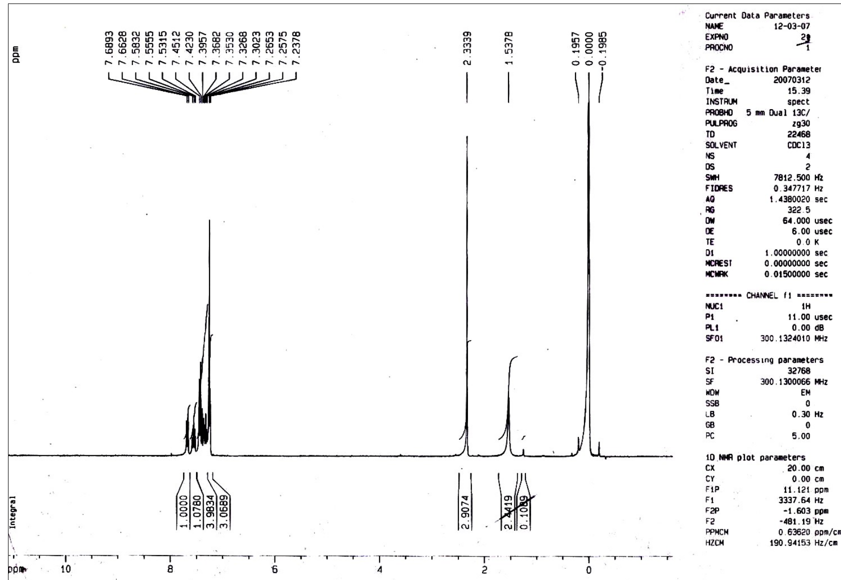


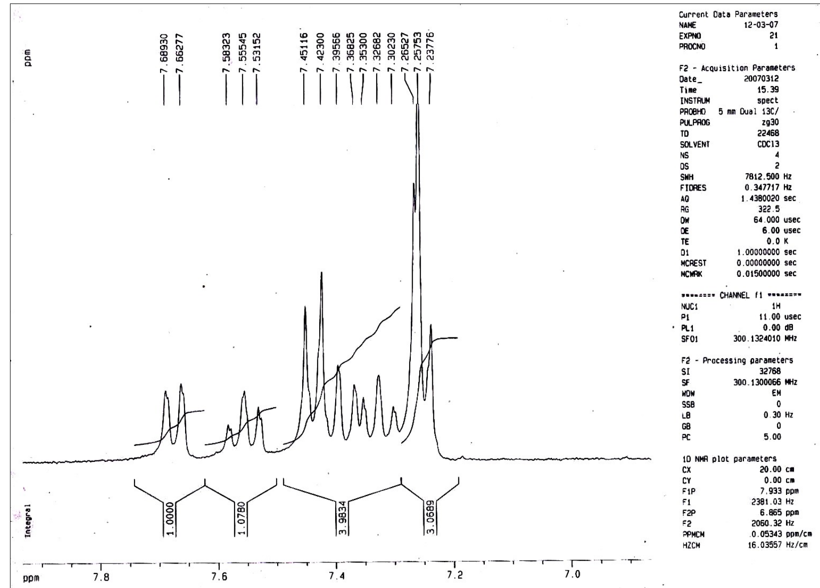





Mass Spectrum
m/z

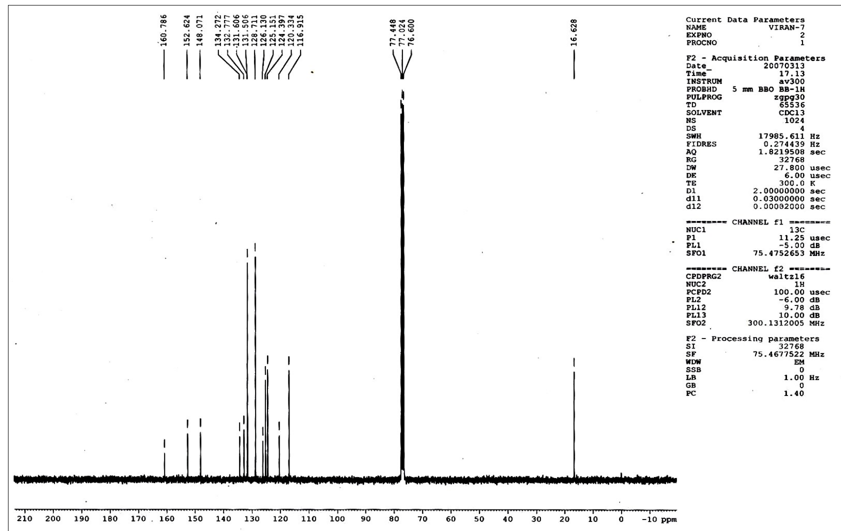
Spectra of C23

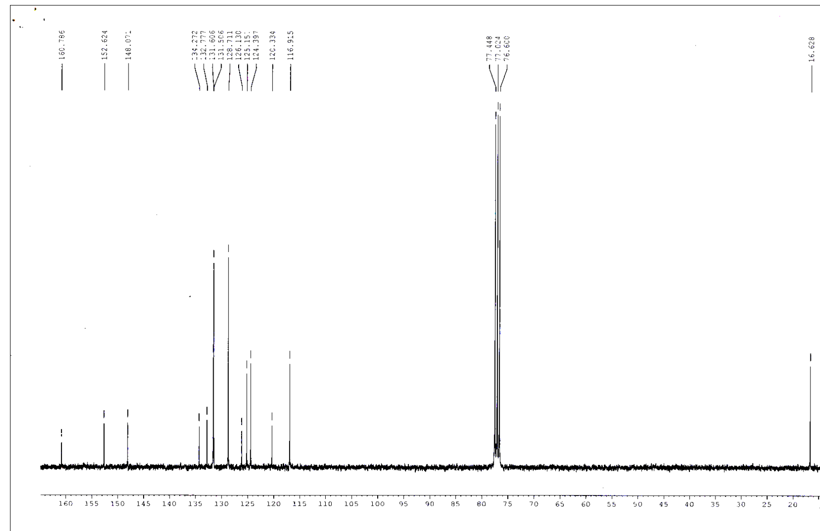




^{1}H NMR Spectrum

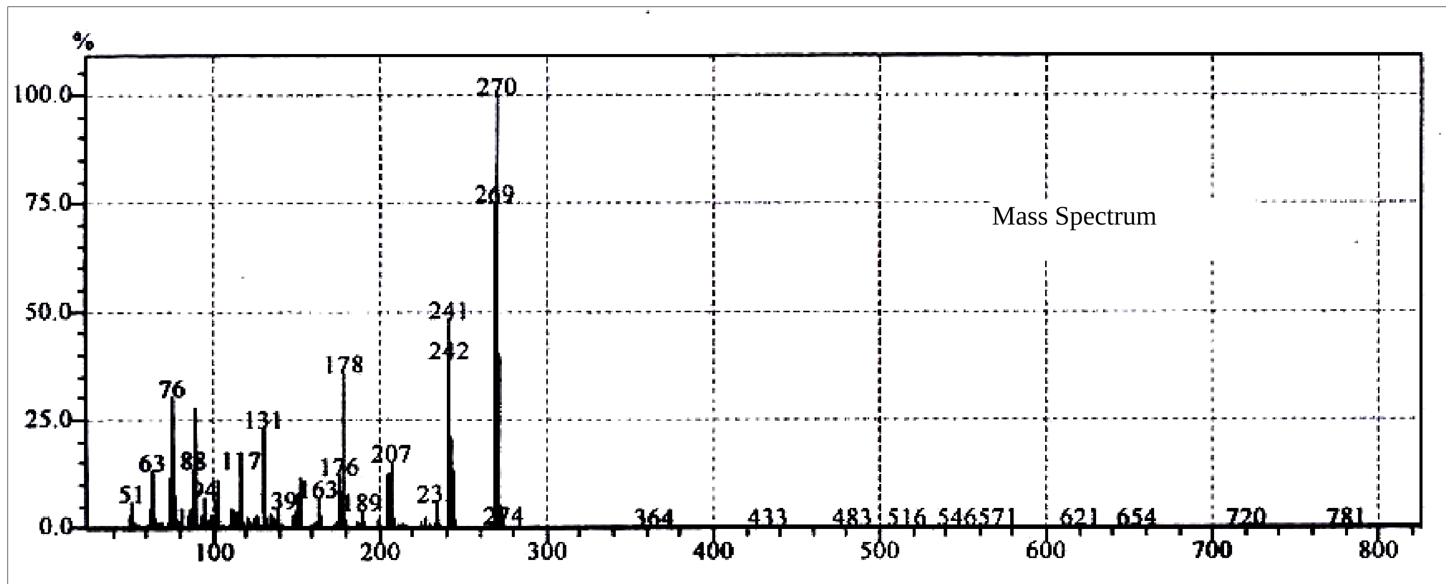
^{1}H NMR Spectrum (Expanded)





^{13}C NMR Spectrum

^{13}C NMR Spectrum (Expanded)


Mass Spectrum
%
100.0
75.0
50.0
25.0
0.0
270
269
241
242
178
76
131
63
88
117
176
207
51
163
189
274
364
433
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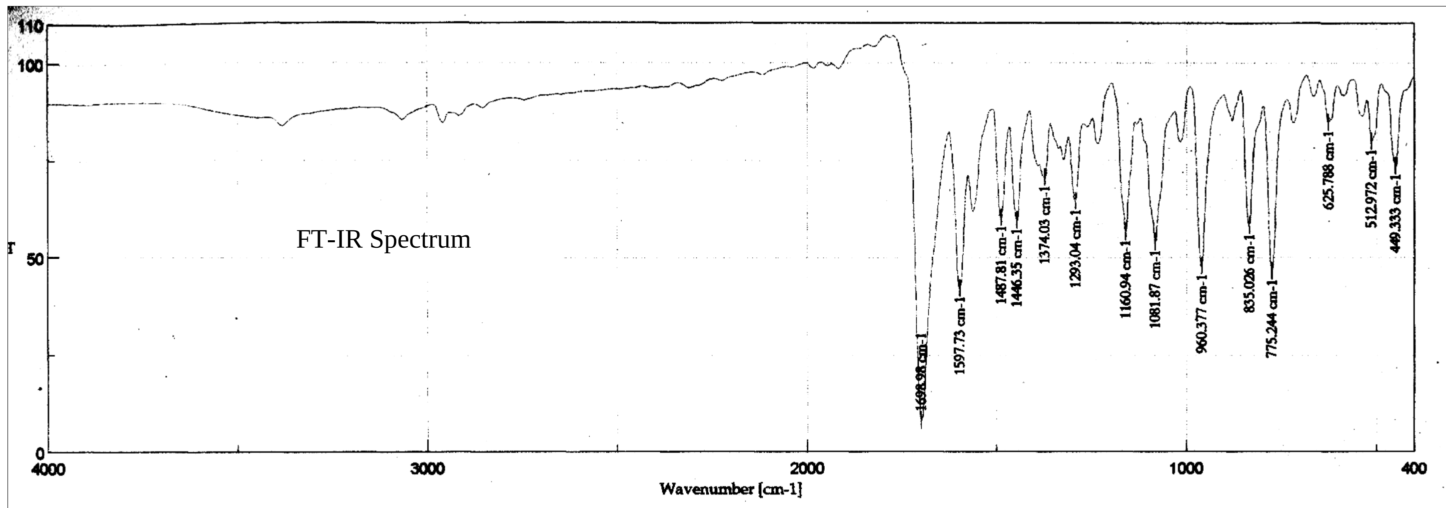
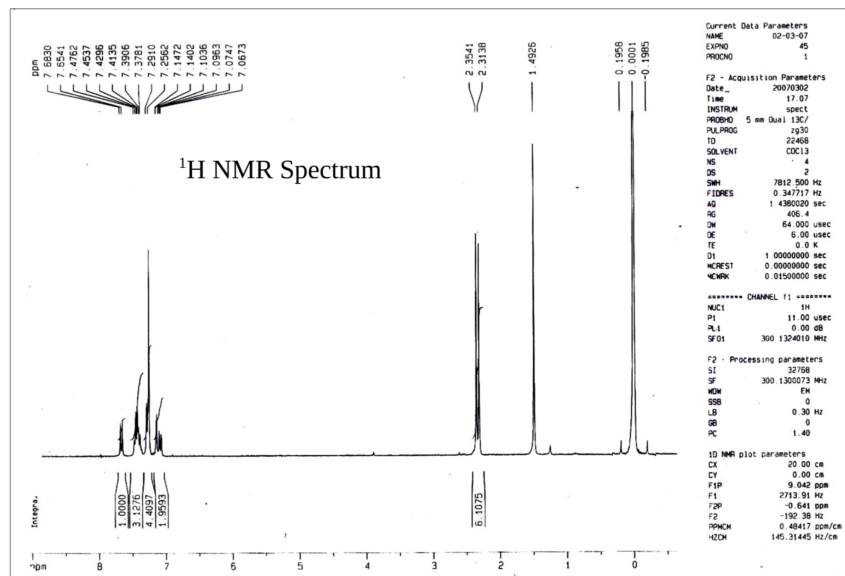
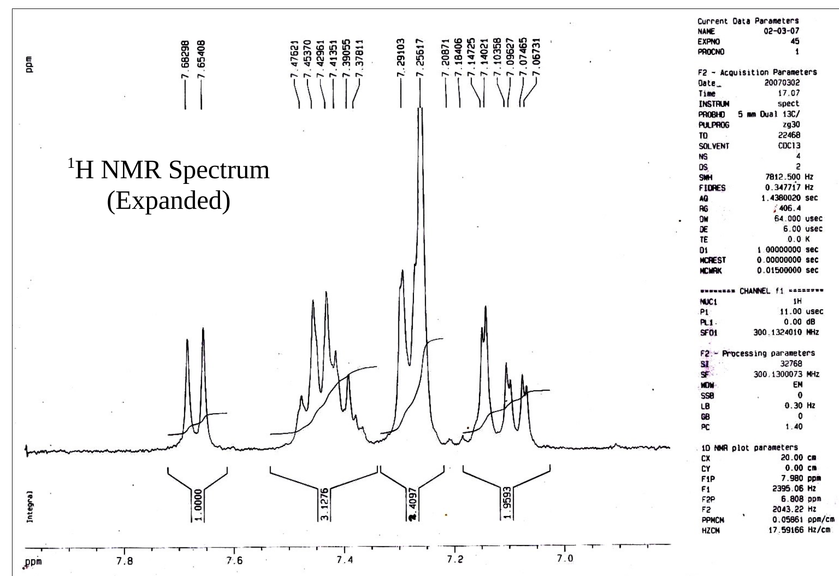
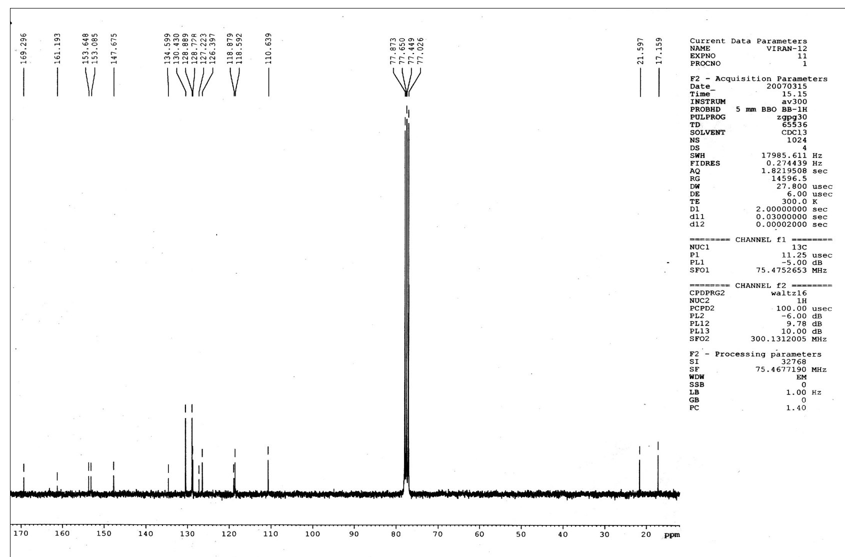
654
720
781
100
200
300
400
500
600
700
800

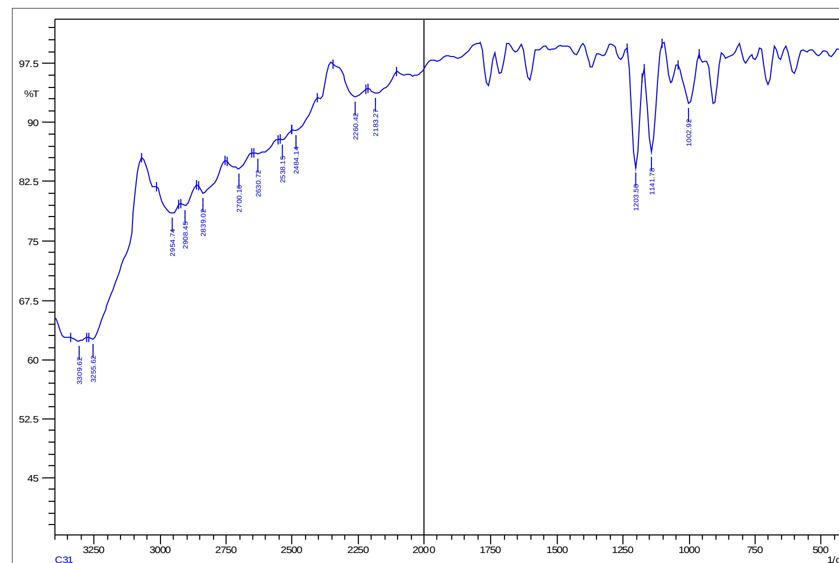

FT-IR Spectrum
110
100
50
0
4000
3000
2000
1000
400
Wavenumber [cm-1]
1597.73 cm-1
1487.81 cm-1
1446.35 cm-1
1374.03 cm-1
1293.04 cm-1
1160.94 cm-1
1081.87 cm-1
960.377 cm-1
835.026 cm-1
775.244 cm-1
625.788 cm-1
512.972 cm-1
449.333 cm-1

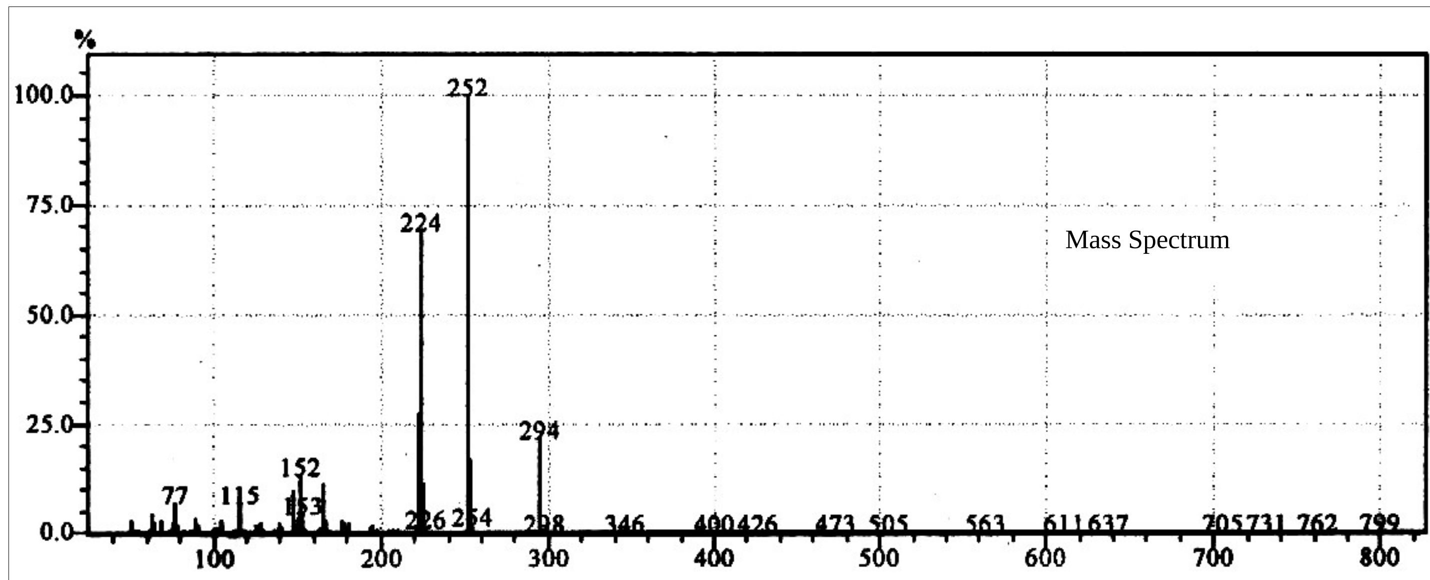
Spectra of C31









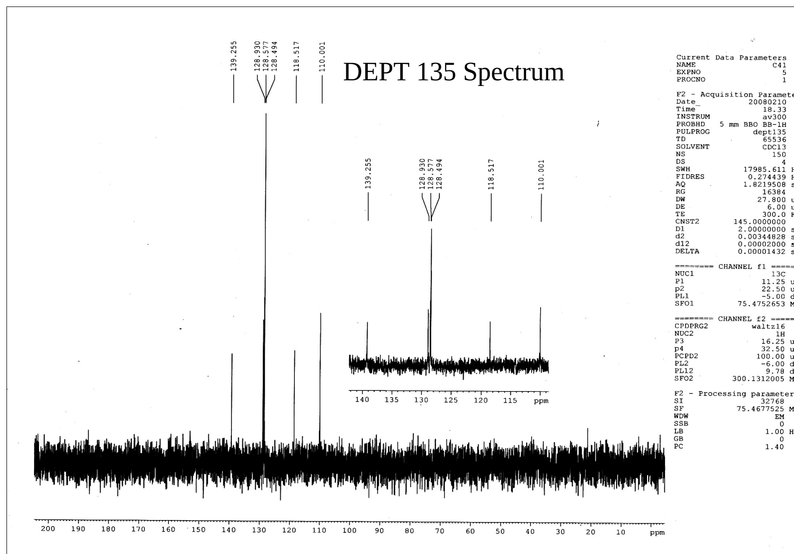


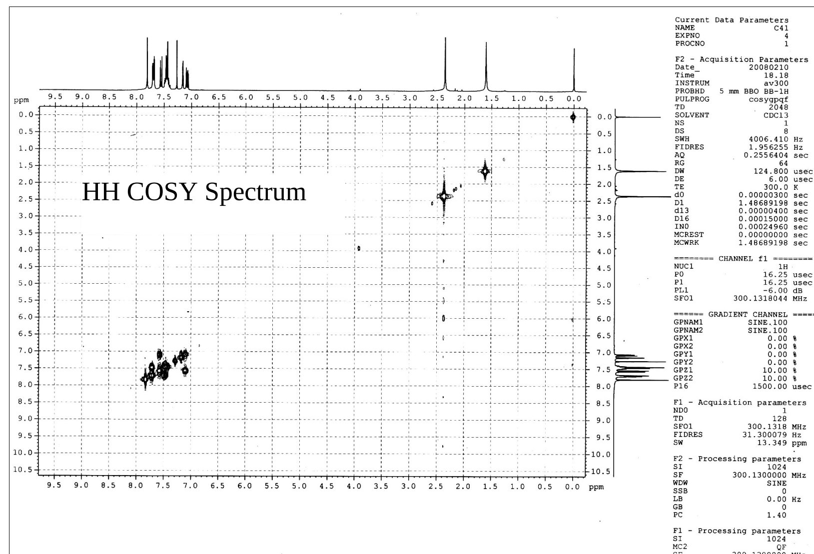
%
100.0
75.0
50.0
25.0
0.0
252
224
294
152
77
115
153
226
254
298
346
400
426
473
505
563
611
637
705
731
762
799
100
200
300
400
500
600
700
800
Mass Spectrum

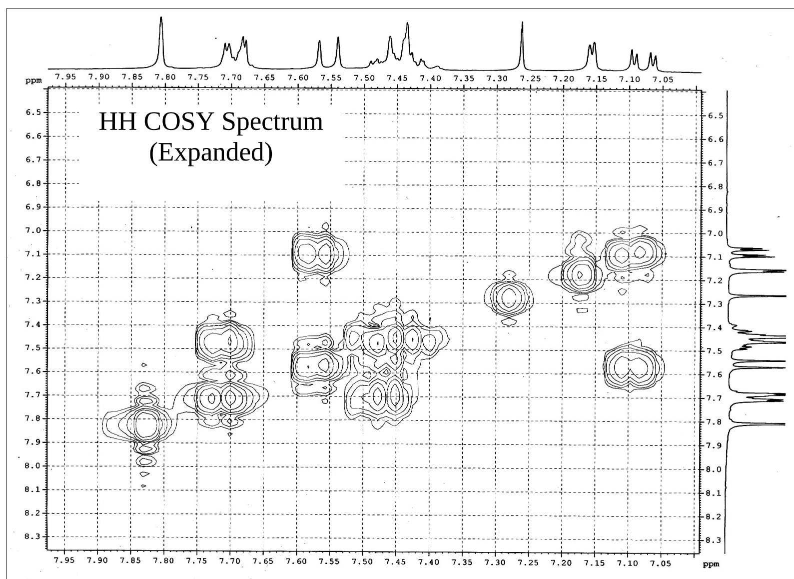
Spectra of C41

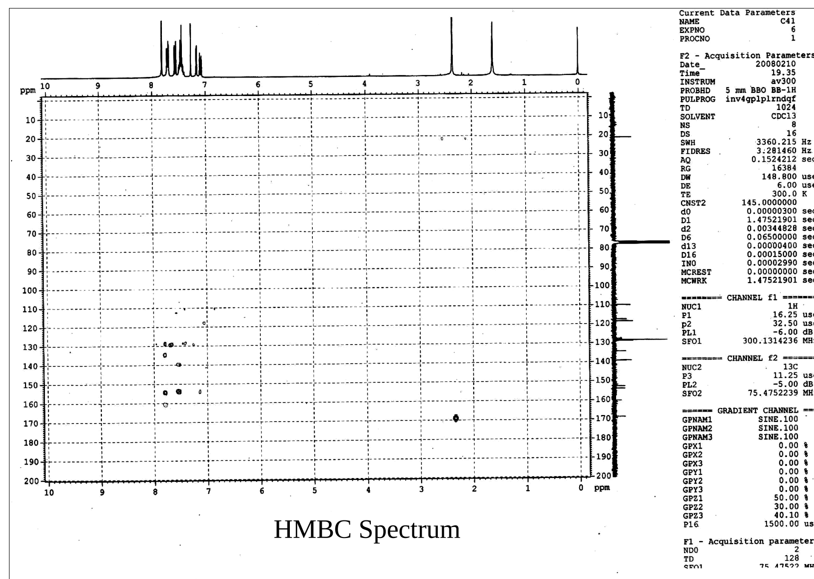
^{1}H NMR Spectrum (Expanded)

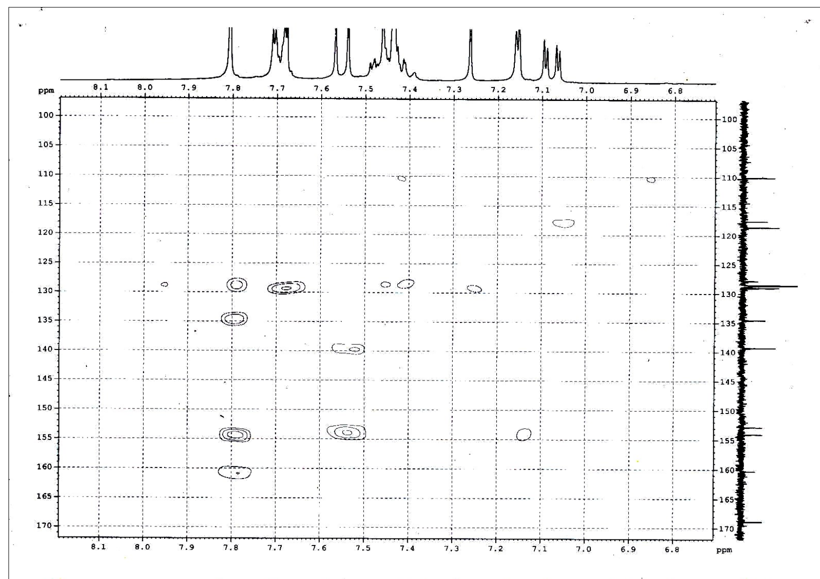
^{13}C NMR Spectrum (Expanded)

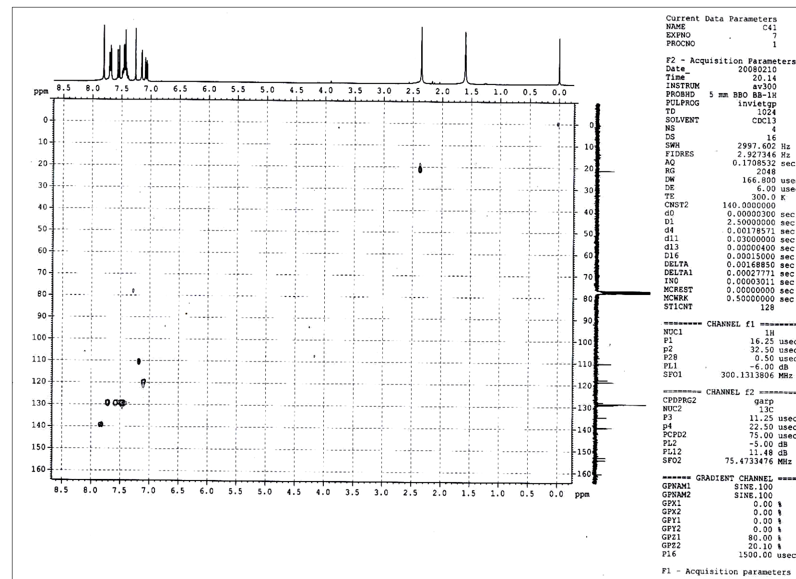

DEPT 135 Spectrum
139.255
128.930
128.577
128.494
118.517
110.001


HH COSY Spectrum

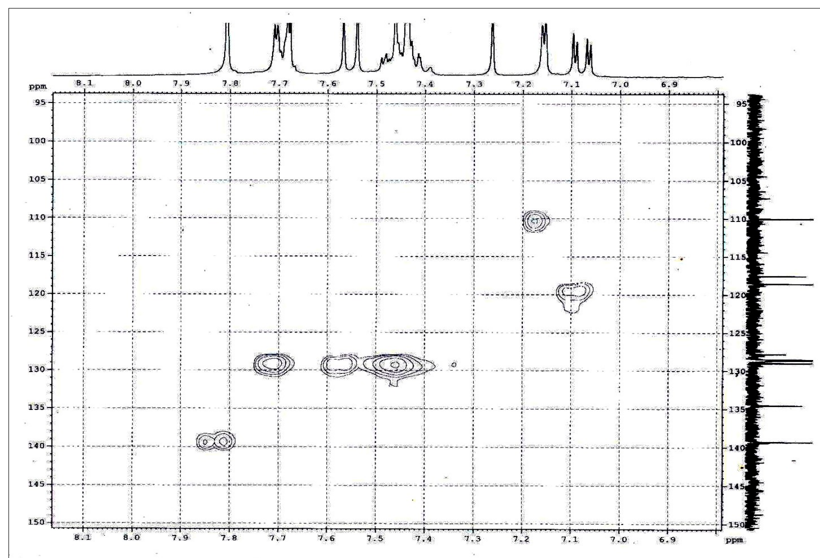

HH COSY Spectrum
(Expanded)


HMBC Spectrum

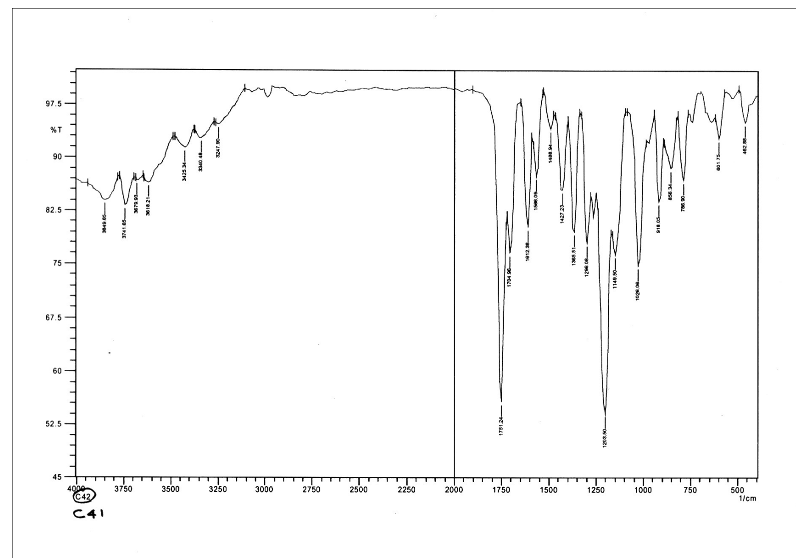




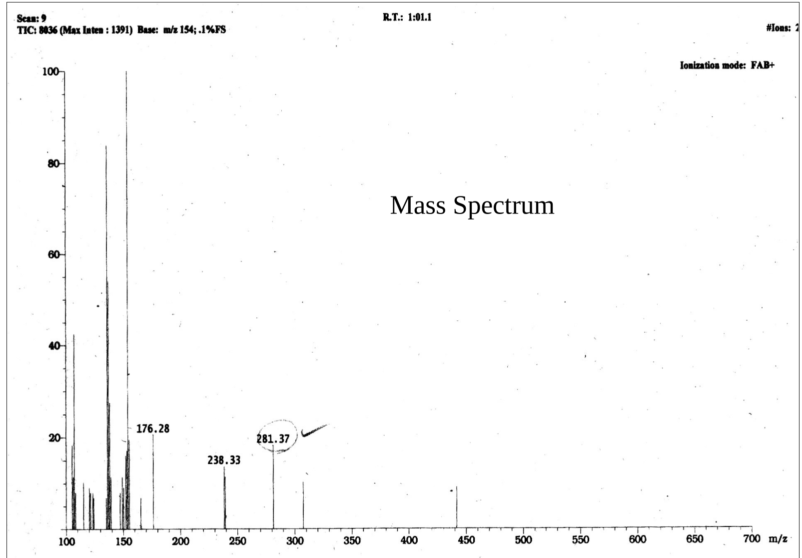
HMQC Spectrum



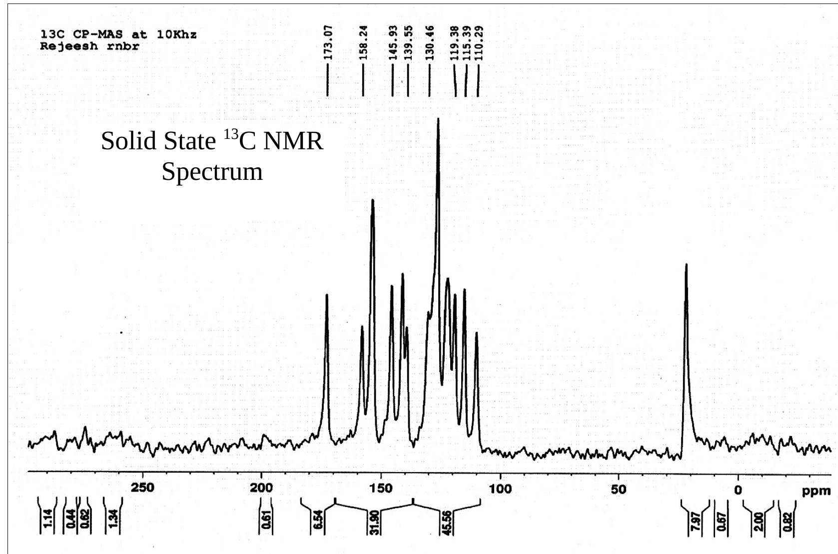
HMQC Spectrum (Expanded)

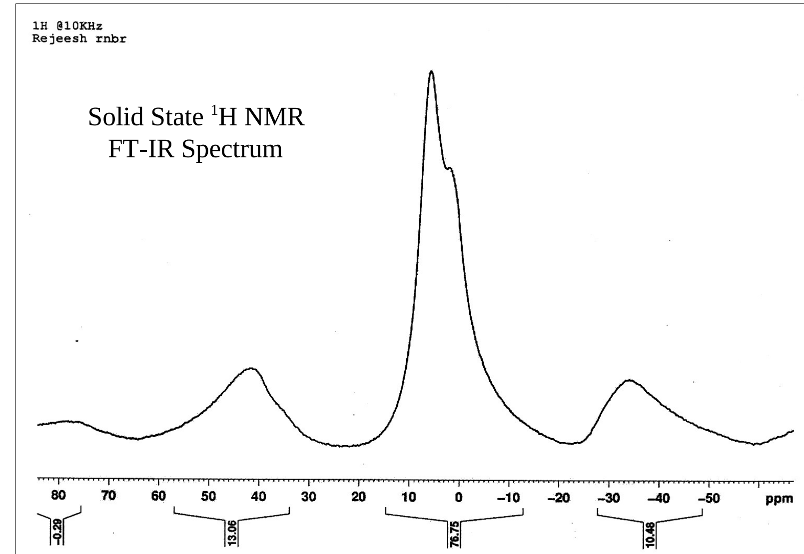


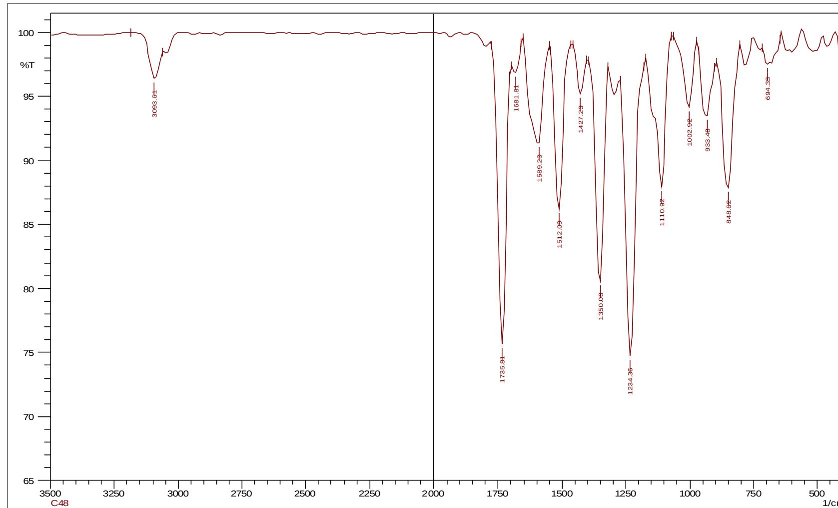
FT-IR Spectrum

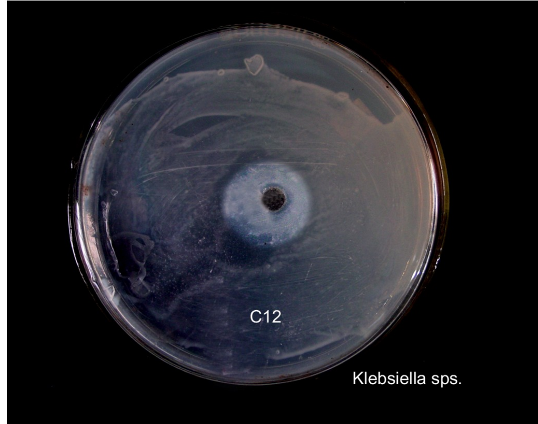

Scan: 9
TIC: 8036 (Max Inten : 1391) Base: m/z 154; .1%FS
R.T.: 1:01.1
#Ions: 2
Ionization mode: FAB+
Mass Spectrum
100
80
60
40
20
176.28
238.33
281.37
100
150
200
250
300
350
400
450
500
550
600
650
700 m/z

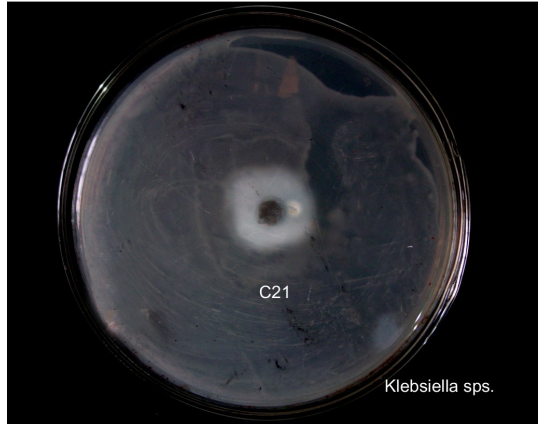
Spectra of C48

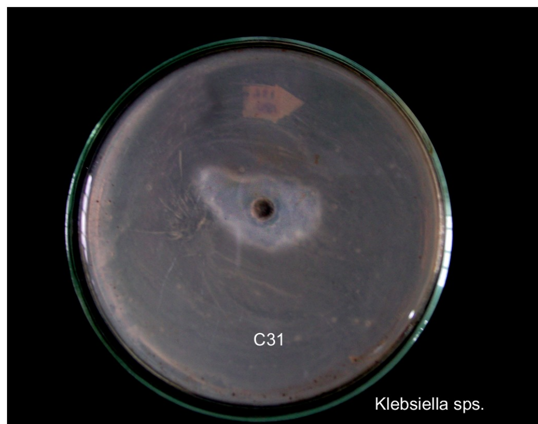


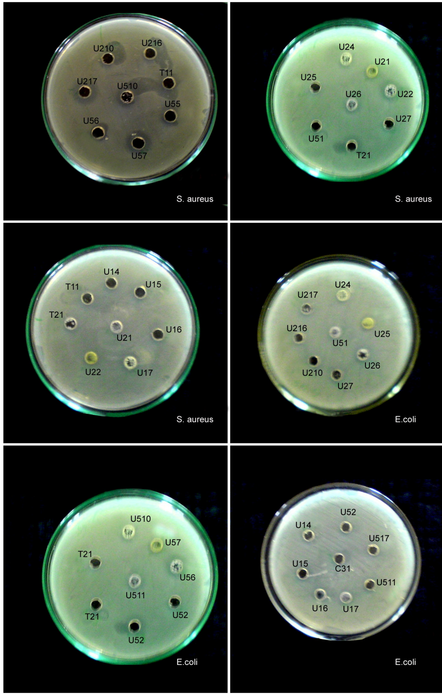


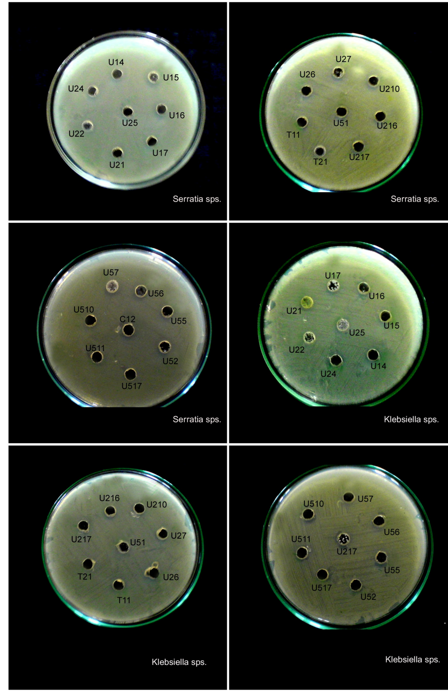



C12
Klebsiella sps.


C21
Klebsiella sps.


C31
Klebsiella sps.


U210
U216
U217
U510
T11
U55
U56
U57
S. aureus
U24
U21
U25
U26
U22
U27
U51
T21
S. aureus
U14
T11
U15
T21
U21
U16
U22
U17
S. aureus
U24
U217
U216
U51
U25
U26
U210
U27
E.coli
U510
U57
T21
U56
U511
T21
U52
U52
E.coli
U52
U14
U517
U15
C31
U511
U16
U17
E.coli


U14
U15
U24
U25
U16
U22
U21
U17
Serratia sps.
U27
U26
U210
U51
U216
T11
T21
U217
Serratia sps.
U57
U56
U510
C12
U55
U511
U52
U517
Serratia sps.
U17
U16
U21
U15
U25
U22
U24
U14
Klebsiella sps.
U216
U210
U217
U27
U51
T21
U26
T11
Klebsiella sps.
U510
U57
U56
U511
U217
U55
U517
U52
Klebsiella sps.