Studies on Some Heterocyclic compounds

A THESIS SUBMITED

TO THE

SAURASHTRA UNIVERSITY

FOR THE DEGREE

OF

Doctor of Philosophy

IN

CHEMISTRY

BY

UNDER THE GUIDANCE

OF

Professor in Chemistry

Department of Chemistry

Saurashtra University

RAJKOT-360 005

(INDIA)

July-2005

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Statement under O.Ph.D.7 of Saurashtra University

The work included in the thesis is my own work under the supervision of **Prof. Anamik Shah** and leads to some contribution in the field in synthetic chemistry and is supported by sufficient number of recent references.

Date:
Place : Rajkot **Arun Kumar Mishra**

CERTIFICATE

This is to certify that the present work submitted for the Ph.D. degree of Saurashtra University by **Arun Kumar Mishra** has been the result of work carried out under my supervision and is a good contribution in the field of chemistry of Benzopyrans, pyranoquinolines, 1,4-dihydropyridines and related heterocycles with a special emphasis on possible biological activities.

Date :
Place:

Prof. Anamik Shah
Department of Chemistry,
Saurashtra University,
Rajkot- 360 005

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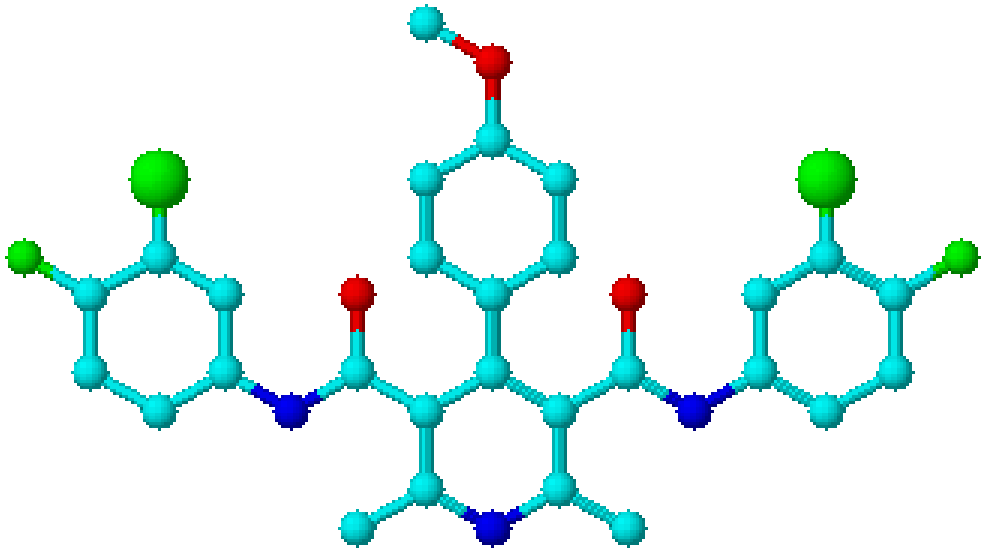
Preparation of 4-(4-methoxy phenyl)-bis(3-Cl,4-fluorophenyl)-2,6dimethyl-1,4dihydropyridine-3,5-dicarboxamides :



INTRODUCTION :

The chemistry of 1,4-dihydropyridine began with compounds having carboxylic alkyl ester groups at 3 and 5 position, when first 1, 4-dihydro pyridine was introduced by Hantzsch et al[1] in 1882. Later on, Arthur Phillips[2] of Welcome Research Laboratories, in his well-recognized work, used Hantzsch synthesis to get symmetrical compounds for the curare like activity who for the first time reported pharmacological activity of these compounds. Such compounds are devoid of undesirable side effects during clinical trials.

The discovery in the 1930's that a dihydropyridine was a "hydrogen-transfering coenzyme" and consequently of utmost importance in biological systems[3,4] has generated numerous studies of the biochemical properties of dihydropyridines. However, there have been relatively few studies of the pharmacological activites of such compounds. At the time this work was initiated, the only such reports described weak analgesic and curare-like properties[5]. Consequently, work had been explored to evaluate some of these compounds, in particular the "Hantzsch-type" dihydropyridines[6,7] in a number of standard test systems,. Subsequent to this work, antitumor[8]and coronary dilating activities have been reported[9] for certain dihydropyridines.

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1,4-dihydropyridine activators, since it does not shift phytohormone abscisic acid has prominent calcium channel activating properties in plant cells and this may extent to mammalian systems. In recent years, 1, 4-dihydropyridines, which are showing predominant pharmacological effects of these studies, appeared to correlate with physiological activities determined by in vivo studies[10]. Calcium Channel (Antagonists) or -blocking agents have made their way into clinical medicine for treatment of umpteen number of disorders. They are most commonly used for patients with cardiovascular diseases[11]. The predominant pharmacological effects of calcium channel antagonists are coronary, peripheral and cerebral vasodilation[12], negative ionotropic effect[13-15], and also as postitive ionotropic agents.

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Nifedipine (2,6-dimethyl-3,5-dicarbomethoxy-4-(2-nitro)phenyl-1,4-dihydropyridine is a first generation calcium channel blocker that interacts at a specific class of voltage-gated calcium channel-the-L-type channel to produce its cardiovascular effects, including the relief of hypertension and angina[16]. First introduced in 1975 nifedipine interacts at a discrete receptor site on the channel that is linked in complex allosteric relationships to other, and structurally quite distinct, calcium channel blocker binding sites (receptors) for the phenylalkylamine verapamil and the benzothiazepinone diltiazem The receptor for 1,4-dihydropyridines has been localized to segments IIIS5, IIIS6 and IVS6 of the major a1 submit of the channel and some nine critical amino acid residues have been identified as constituting the binding domain[17-18].

The receptor site for nifedipine on the L-type class of calcium channel is shared not only by the 1,4-dihydropyridine antagonists of Figure 1, but also by potent and structurally related 1,4-dihydropyridine activators, including Bay K 8644 that serve as vasoconstrictors, positive inotropic agents and secretagogues. The availability of extremely potent and structurally specific antagonist and activator ligands for this channel has raised the issue for the whether there exist endogenous regulatory species "endogenous ligands" for which the 1,4-dihydropyridines are merely surrogate species. However, such species have not, despite much efforts, been unambiguously indentified[19] .

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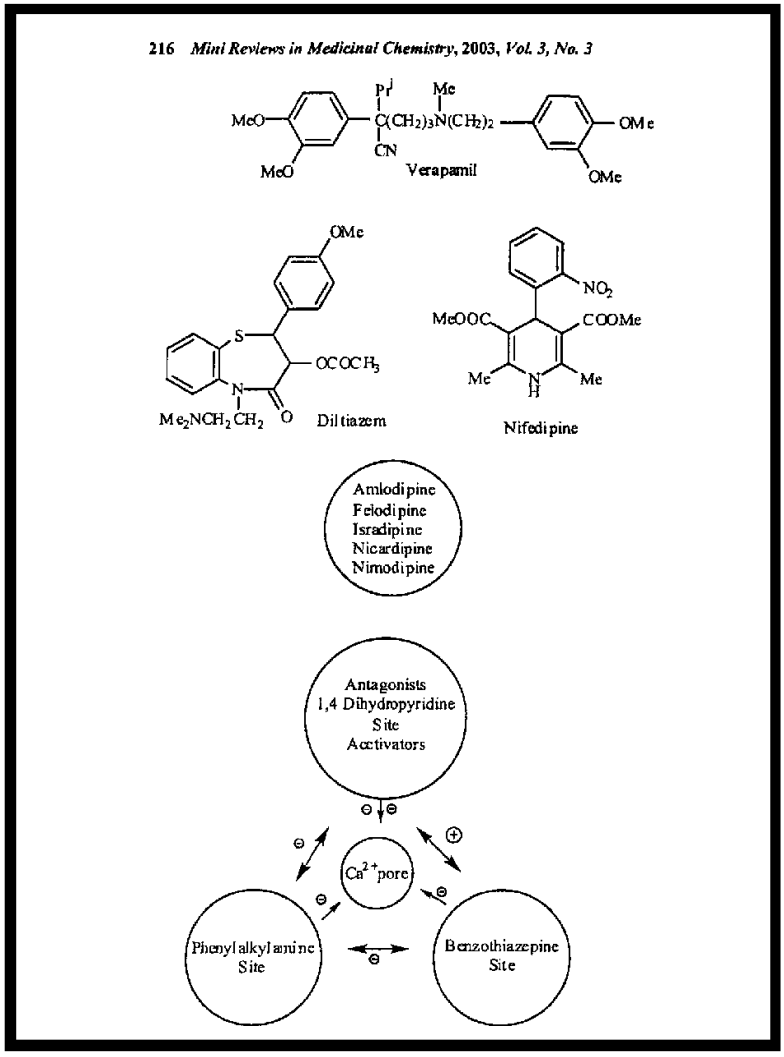

1,4-dihydropyridines, whether symmetrical or unsymmetrical are expected for their cardiovascular and other pharmacological property. Sufficient literature is available regarding structure-activity studies[20-24].

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24 Frigerio Marco, zaliani Andrea, Gandolfi CarmeloA, Germini, Mauro, Tofanetti,and Tangnella Sergio, *Fur. pat.272 ,693.Abstr.,* ***109****,190259f (1988).*

1,4-dihydropyridines may have tremendous applications with different modification in its structure. a number of good reviews on the structure, synthesis, stereochemistry and hydrogen transfer mechanism of the pyridine nucleoside are well reported in literature [25-30].

These compounds have been synthesized and developed as calcium antagonists which inhibit smooth and cardiac muscle contractions by blocking the influx of Ca^{+2} through calcium channels [31-39].

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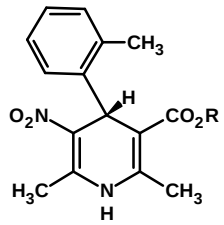
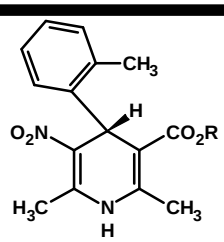
The design of tissue selective1,4-dihydropyridine(1,4-DHP) calcium channel agonists to treat congestive heart failure necessitates removal of their contraindicated smooth muscle vasoconstrictor effect while the target cardiac positive inotropic action is maintained. in this regard, racemic methyl 1,4-DHP (Bay K8644) produced a Ca^{+2} channel agonist effect on both smooth and cardic muscle since the agonist (-)-(*S*)-enantiomer (1) is about 10-fold more potent relative to the antagonist(+)-(*R*)-enantiomer .



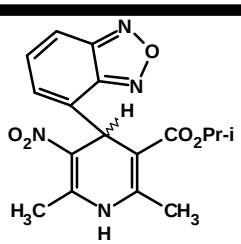
1, R=Me

2, R=$CH_2CH_2ONO_2$



3 ,R= Me

4, R=$CH_2CH_2ONO_2$



5 ,PN202-791

Figure structures for the (-)-(S)-1 and (+)-(R)-3 enantiomers of Bay K8644, the (-)-(S)-2 and (+) -(R)-4 enantiomers of 2-nitroxyethyl 1,4-dihydro-2,6dimethyl-3-nitro-4-(2-trifluoromethylphenyl)pyridine-5-carboxylate, and PN 202-791 (5).

Major investigation done by Tsuruo et.al.[41] on Ca^{2+} channel blockers (Verapamil and some 1, 4-dihydropyridines) activity suggested that 1, 4-DHP's possesses multidrug resistance (MDR) reversal activity[40]. This investigation further confirmed by Hollt V. and co-workers[42] in 1992. The development of multidrug resistance (MDR) of tumor cells is a major problem in malignant tumor chemotherapy, as tumor cells, by increasing drug efflux, acquire cross-resistance to many structurally and functionally unrelated anticancer agents, which therefore never achieve effective intracellular concentrations[43].

Multidrug resistance (MDR) of cancer cells has often been correlated with the over expression of P-glycoprotein (P-gp). An ABC transporter ATP binding cassette act as a cellular pump membrane transporter by extruding the anticancer agents and preventing their Antitumor effect.

The nitric oxide releasing action of the 1,4-dihydropyridines appear to be a class action, to be exerted at therapeutic concentrations and to involve the stimulation of calcium influx into endothelial cells though a anion-L-type process[43-a,b,c].

1,4-dihydropyridines may have at least two other effect on the nitric oxide mediated vasorelaxant system. Pranidipuine (0PC-13340, methyl-3-pheny 2(E)-propenyl-1,4-dihydro-2,6-dimethyl-4(3-nitrophenyl)-3,5-pyridine dicarboxylate) enhances nitric oxide-mediated vasorelaxation by a mechanism apparently distinct from NO release[43-d] additionally, a series of 1,4-dihydropyridines exist with "dual" pharmacological properties – blockade of L-type calcium channels and direct release of nitric oxide[43-e,f,g].

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43(b) Zhang X.and Hinke T.H., Amlodipine releases nitric oxide from canine coronary microvessel: and unexpected mechanism of a calcium channel-blocking agent, *Circulation.*, **97**, 576-580, (1998).

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Many researchers have synthesized 1,4-DHPs having aliphatic ester group[44], aromatic ester group[45] and heterocyclic ester[46] group at 3 and 5 position. After almost 80 years, later structure and hypotensive activity of Hantzsch type symmetrical 3,5-disubstituted 1,4-DHPs thoroughly investigated by Love and co-workers[47]. As a result, Nifedipine[36] came out as a promising compound which exerted its cardiovascular effects through a direct action on vascular smooth muscles. Several other drugs discovered and used such as Niludipine, Darodipine, Riodipine, Lacidipine, Flordipine in last two decades.

43(d) Mori T.,TakaseK., Toide K., Nakayama N. and Schwartz A., *Cardiovas., Drug Revs.*,**19**,1-8, (2001).

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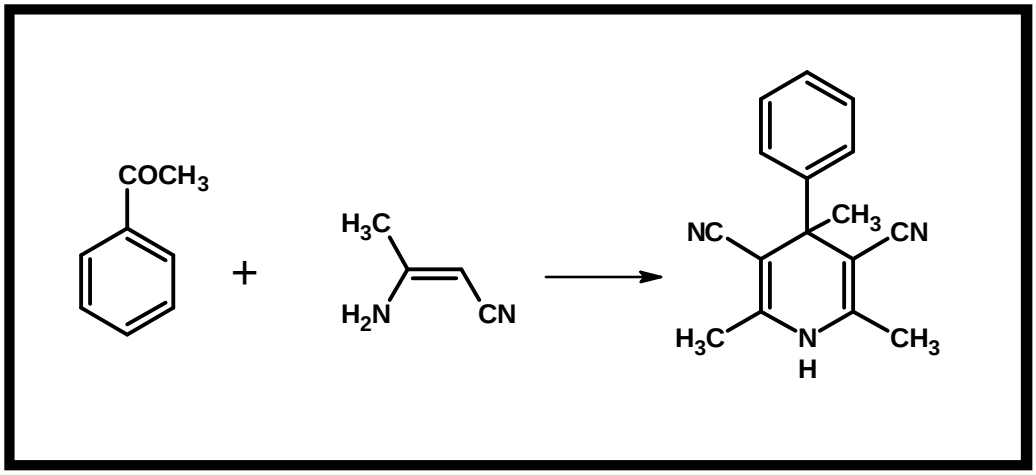
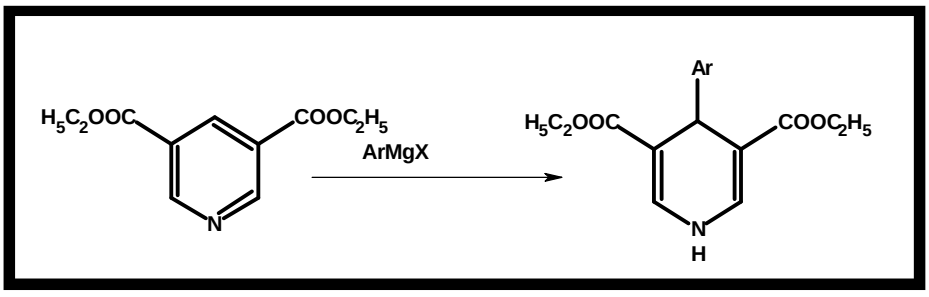
Similarly symmetrical & unsymmetrical 1,4-dihyropyridines[48] having cyano group at 3 and 5 position and para biphenyl group at 2 and 6 position are reported. When acetophenone reacted with 3-aminocrotonitrile gave sym. 3, 5-dicyano-2, 4, 6-trimethyl-4-phenyl-1, 4-dihydropyridine[49] (VI) and aldehyde condensation with 3-aminocrotonitrile in suitable acidic medium like acetic acid or hydrochloric acid gave sym. 3, 5-dicyano-2, 6-dimethyl-4-substituted phenyl-1, 4-dihydropyridine[50,51].



Reaction of Aryl Grignard reagents with pyridine-3,4-dicarboxylic ester gave the 4-aryl-2,6-disubstituted dihydropyridines(XXI). Methyl Grignard is reported to give mixtures of 2- and 4-substituted products[52].



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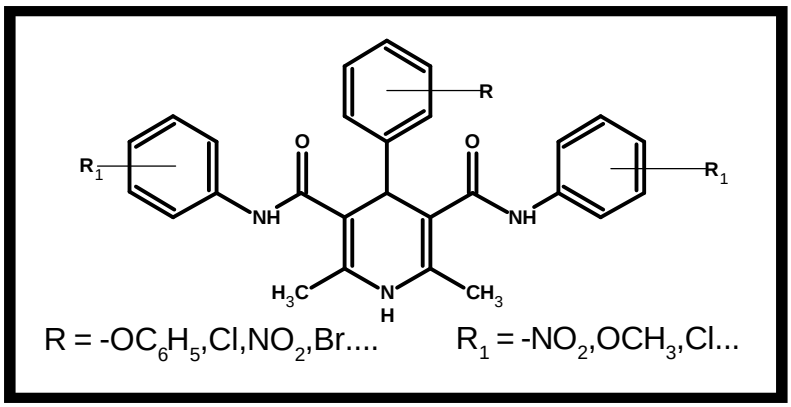
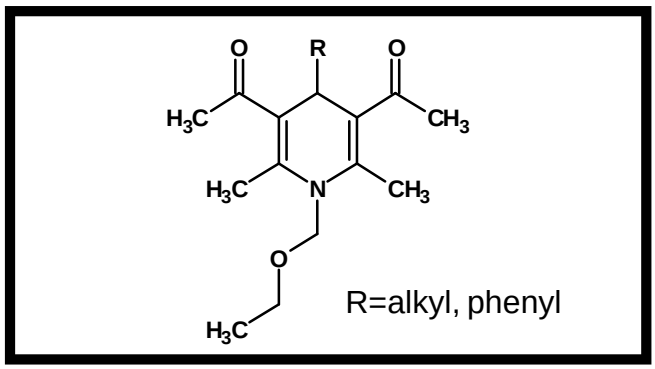
Shah and Naliapara[53] have synthesized few series of 3,5-disubstitute carbamoyl derivatives and studied 2D QSAR of 4-substituted phenyl 2,6-dimethyl-3,5-bis[(N-substituted phenyl)carbomoyl]-1,4-dihydropyridine as potent antituberculer agents. These compounds show >90% inhibition comparable to rifampicin and MIC value<12.5µg.



Ulrich et.al[53a]. has obtained 3, 5-dicarboxylic acid substituted 1, 4-DHP's.



The 1,4-dihydropyridine having acetyl or benzoyl groups on 3 and 5 position are still subject of intensive study due to recent development on MDR reversal activity in tumor cells which has a new dimension of application. This recent findings were contribution of this laboratory. Detail of its chemistry, pharmacological activity is given in synthetic aspects.

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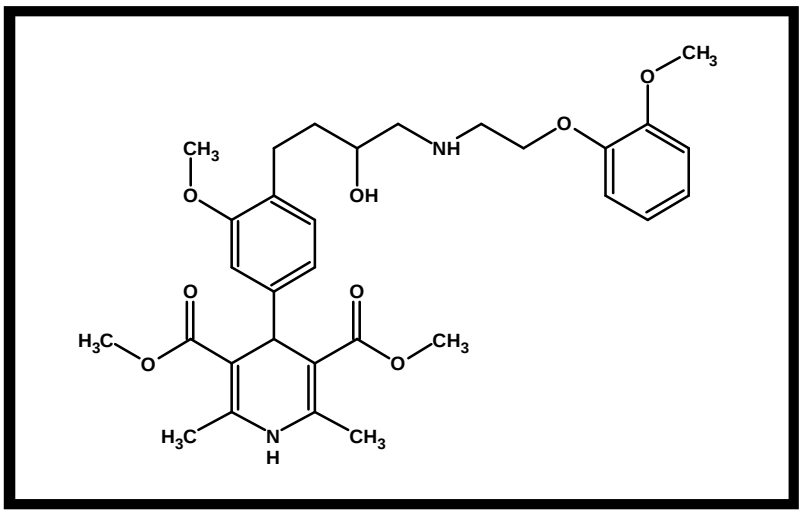
53(a) Ruediagerwolf Ulrich., Bernhard Kohl., Flockerzi Dieter., *WO* **8809,331** (1988).

The interest is growing towards pharmacological activities that are not connected with their calcium channel antagonist properties, like neurotropic (antiamnestic, anticonvulsant, neuroregulatory), antidiabetic, and membrane protectging as well as anticancer, antibacterial, and antiviral activities[54-57].

Labedipinedilol-A(X), a novel dihydropyridine-type calcium antagonist, has been shown to induce hypotension and vasorelaxation. Yeh JL et al.[58], investigated the effect of labedipinedilol-A on vascular function of rat aortic rings and cultured human umbilical vein endothelial cells (HUVECs).



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The pharmacology and clinical applications of these agents have been reviewed on numerous occasions. Nifedipine has now been joined in the clinical market place by several second and third-generation 1,4-dihydropyridines including – amlodipine, felodipine, isradipine, nicardipine, nimodipine, nitrendipine, lacidipine and lercanidipine. These agents differ in detail in their overall pharmacological and pharmacokinetic characteristics, although they do share a fundamental similarity of action.

Calcium channel blockers (CCBs) of the 1,4-dihydropyridine derivatives (DHPs), exemplified by nifedipine (Adalat) and nilvadipine (Nivadil), are well-know as clinically important drugs since they first appeared in the market in 1975. Till date, these compounds have become almost indispensable for the clinical treatment of cardiovascular diseases such as angina pectoris, hypertension, and cardiac arrhythmias[59,60] and anticancer[61].

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(c) Marchalin, S.; Chudik, M.: Mastihuba, V.; Decroix, B.*Heterocycles* **48**,1998 and references therein.

61. Chudik, M.; Marchalin, S,; Daich, A,; Decroix, B. *Res Adv. Synth. Org. Chem.* ***1***, 1,2000.

Interestingly, it has been now recognized that the absolute configuration at the C_4 position of the 1,4-DHP nucleus is indispensable for activity modulation. Indeed, enantiomers of an unsymmetrial, 1,4-DHPs usually differ in the their biological properties, and sometimes they could have exactly the opposite action profile (calcium antagonist vs calcium agonist for example)[62]. Consequently, the synthesis of enantiometrically pure 4-aryl 1,4-DHPs, and their biological evaluation continue to present significant challenge for the scientific community.

Rudong Shah & Edward E. Knaus[63] studied synthesis, calcium channel agonist-antagonist modulation activities and nitric oxide release of nitrooxyalkyl 1,4-dihydro-2,6-dimethyl-3-nitro-4-(2,1,3-benzoxadiazol-4-yl)pyridine-5-carboxylate racemates,enantiomers and diastereomers.

New 1,4-dihydropyridines endowed with NO-Donor and calcium channel agonist properties reported by Sonja Visentin et. al [64].

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62. Sibikev, A.; Franssen, M. C. R.; Vigante, B.; Cekvaicus, B.; Makarova, N.; Daburs G.; De Groot, A. *Tetrahedron: Asymmetry* , **12**, 3251 (2001).

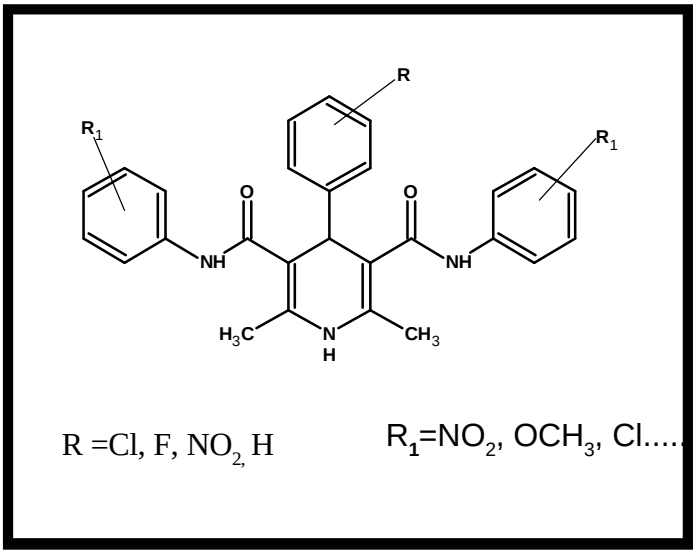
63. Rudong Shah, Carlos Velazquez, and Edward E. Knaus, *J .Med . chem ,**47**, 254-261 (2004).*

64. Albert Gasco, Sonja Visertin, Barbara Ronaldo,et. al., *J,. Med. Chem.,**47**, 2688-2693 (2004).*

65. Desai, B., Sureja, D., Naliapara,Y., Shah, A. And saxena, A. K. ; *Bioorg. Med. chem., **9** ,1993-1998 (2001).*

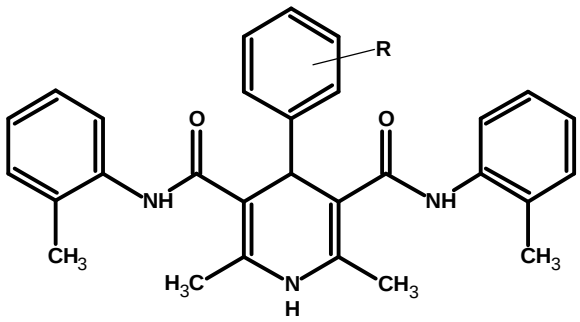
66. Swamy, S. K. Reddy, T. M., Reddy , V. M.; *Indian J. Pharm. Sci., **60**(2) , 102-06(1998).*

Later on Shah et al[65] synthesized some new series of 4-substituted phenyl-2,6-dimethyl-3,5-bis-(N-substitutedphenyl)-carbomyl-1,4-dihydropyridine and studied their 3D-QSAR as well as antituberculer activity aganist M.tuberculosis H_{37} Rv. Among them, some derivatives showed >90% inhibition comparable to Rifampicin.



R =Cl, F, NO_2, H R_1=NO_2, OCH_3, Cl.....

Almost parallel to Shah & co-workers, Reddy and coworkers[66] synthesized 4-aryl / heteroaryl,2,6 -dimethyl-3,5-bis-N-(2-methyl phenyl)carbamoyl-1,4-dihydropyridines and reported pharmacological screening of the new 1,4-dihyropyridines such as CNS depressant (anticonvulsant and analgesic) and cardiovascular activities by standard methods.



R=OH, NO_2, di-OCH_3, $N(CH_3)_2$, etc...

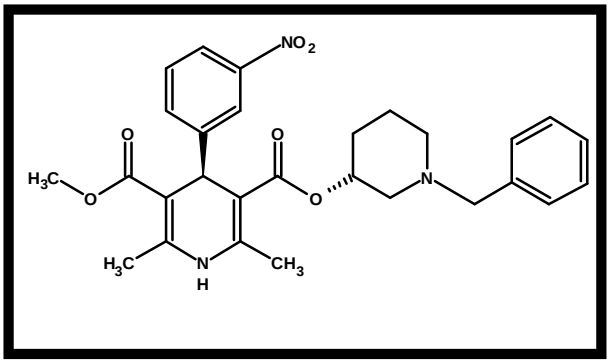
New Generation of 1,4-dihydropyridine Introduced:

Generic Name : Benidipine hydrochloride[67]

Highest Phase : Launched-1991

Therapeutic Group : Treatment of Hypertension

Chemical Structure :

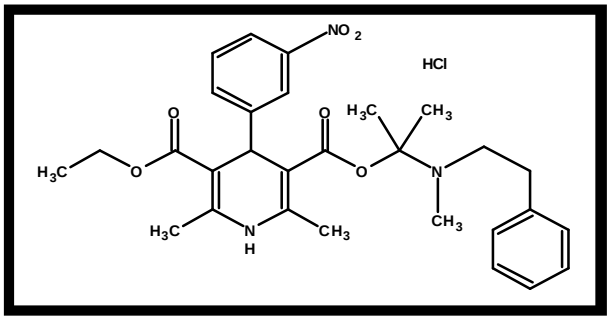


Generic Name : Nicardipine hydrochloride[68]

Highest Phase : Launched-1981

Therapeutic Group : Treatment of Hypertension

Chemical Structure :

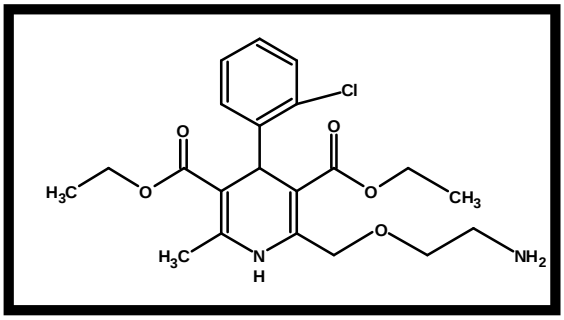


Generic Name : Amlodipine[69]

Highest Phase : Launched-1990

Therapeutic Group : Treatment of Hypertension and Angina Pectoris

Chemical Structure :

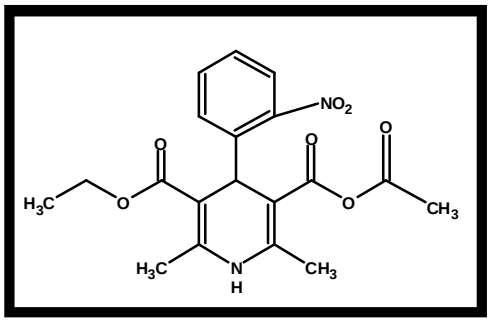


Generic Name : Aranidipine[70]

Highest Phase : Launched-1996

Therapeutic Group : Treatment of Hypertension

Chemical Structure :

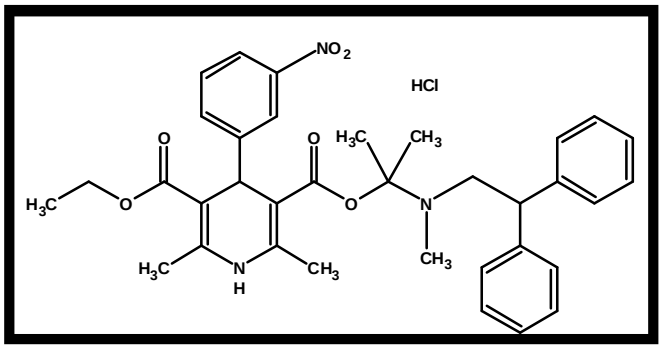


Generic Name : Lercanidipine hydrochloride[71]

Highest Phase : Launched-1997

Therapeutic Group : Treatment of Hypertension and Stroke

Chemical Structure :

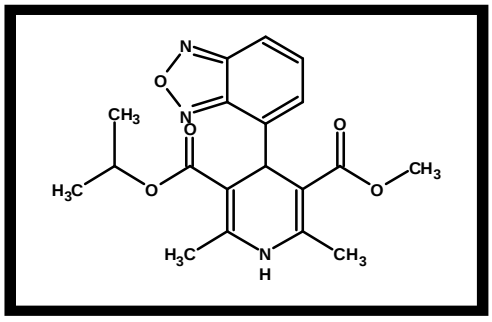


Generic Name : Isradipine[72]

Highest Phase : Launched-1989

Therapeutic Group : Treatment of Hypertension

Chemical Structure :

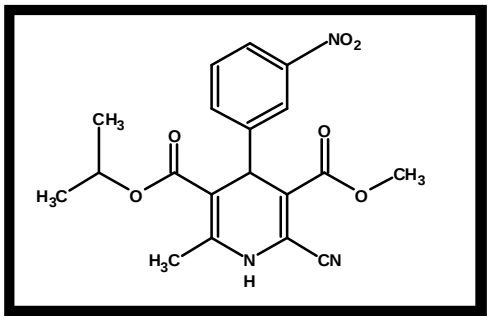


Generic Name : Nilvadipine[73]

Highest Phase : Launched-1989

Therapeutic Group : Treatment of Hypertension

Chemical Structure :

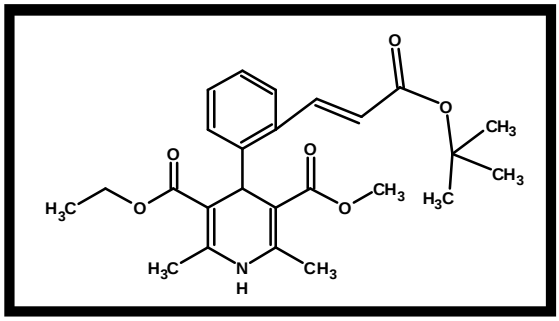


Generic Name : Lacidipine[74]

Highest Phase : Launched-1991

Therapeutic Group : Treatment of Hypertension

Chemical Structure :

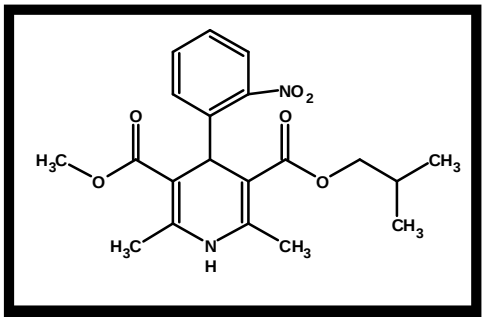


Generic Name : Nisoldipine[75]

Highest Phase : Launched-1990

Therapeutic Group : Treatment of Hypertension

Chemical Structure

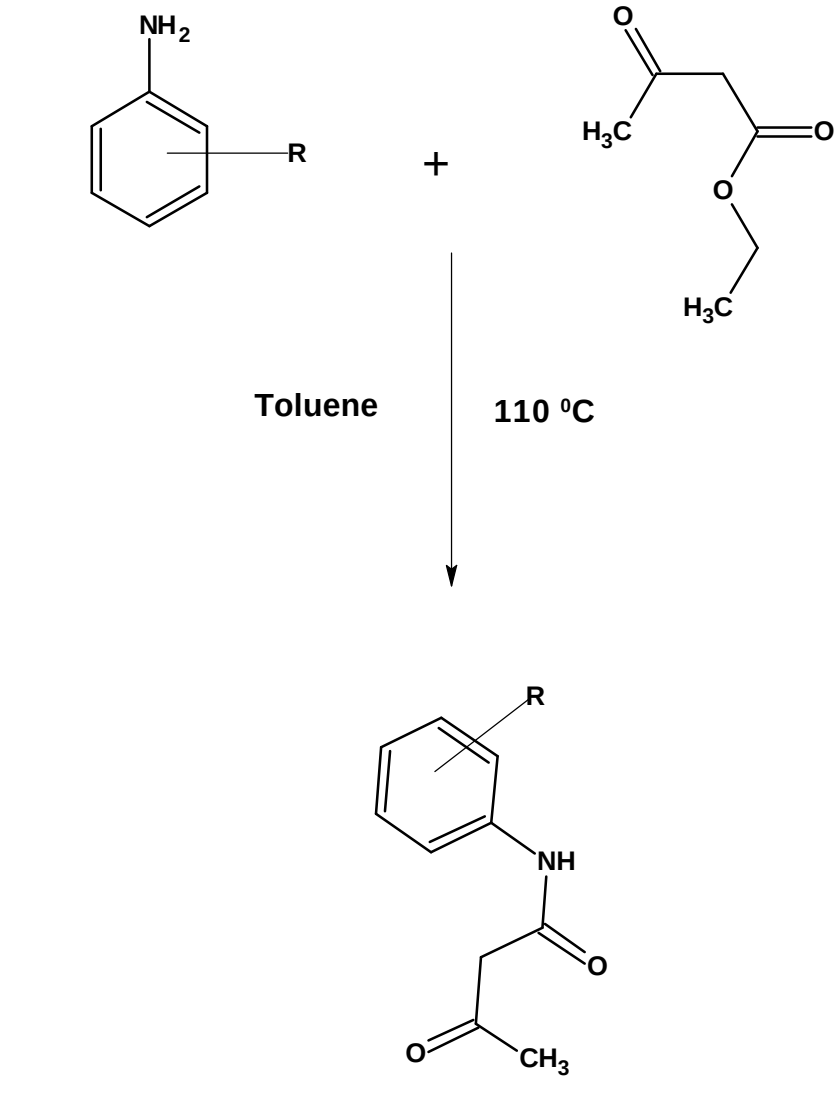


Looking to the synthetic work done so far in literature and in continution of our earlier work, few 1,4-dihydropyridines were synthesized using modified Hantzsch synthesis. Thus, the starting acetoacetanilide derivative was prepared &activated in appropriate solvent & furthur treated with appropriate aldehyde and ammonia at reflux temperature for several hour to obtain the desired compounds in this chapter. **(Reaction Scheme 1)**

67. Mehler P.S., Coll J.R., Estacio R., Esler A., Schrier R.W., and Hiatt R., *Circulation*, **107(5):** 753(2003).
68. Nomura M., Nakaya Y., and Uemora E. et al.,*Arzneim-Forsch Drug Res* , **53(5)**: 314(2003).
69. Martell N., *Am J Hypertens* , **16** (5, Part 2) Abst P-235(2003).
70. Fernandes C.M., Veiga, F.J.B., *BMC - Biomed Chromatogr*, **17(1)**: 33(2003)
71. Belal F., *J Pharm Biomed Anal* , **31(5)**: 989(2003).
72. *Drug Data Rep* , **14(4)**: 316(1992).
73. Pepine C.J., Cooper-DeHoff R.M.,and Weiss R.J. et al., *Am J Cardiol* , **91(3)**: 274(2003).
74. *Drug News Perspect* , **9(5):** 288 (1996).
75. *Drug Data Rep* , **13(11)**: 952 (1991).

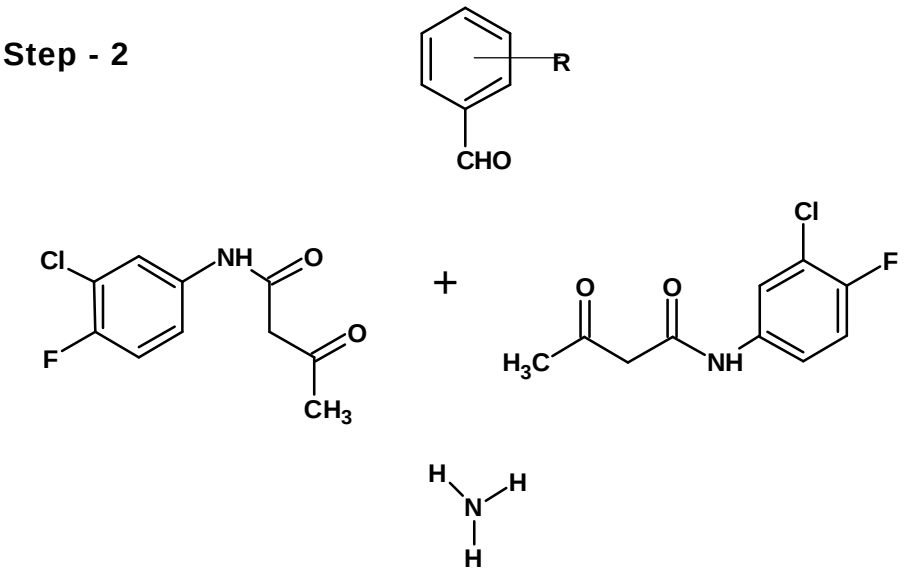
REACTION SCHEME : 1

Step - 1

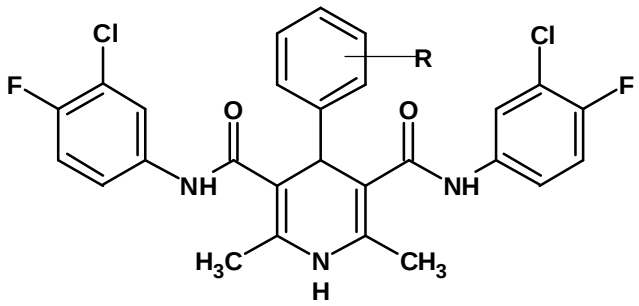


REACTION SCHEME : 1

Step - 2



Methanol | **Hantzsch synthesis**



Where R = Different substitute

Experimental :

Experimental protocols :

Except substituted acetoacetanilide, all the chemicals were obtained from industrial sources. The TLC was carried out on silica Gel-G as stationary phase purchased from Merck India Ltd. Ethyl acetate: Hexane (4:6) was used as a mobile phase. The other solvent system like acetone: benzene, methanol: chloroform was also employed but the best results were obtained in mixture of ethyl acetate and hexane(4:6) .

Preparation of Acetoacetanilide :

3-Chloro-4-Fluoroaniliine (0.1), ethylacetoacetate (0.1 mole) and caustic lye (0.2gm NaOH+0.5ml water) were heated at 110 oC in 50 ml toluene as solvent for 10-12 hrs. After completion of reaction, most of toluene was distilled out. The reaction mass was cooled at room temperature and then taken in an ice bath which formed light yellow crystals of crude acetoacetanilide. It was then extracted with diethyl ether/ petroleum ether and filtered. The ethereal solution was evaporated & afforded to ger pure acetoaceanilide. Yield 55-65 %.

Elemental Analysis

Calculated = C (52.30%) H(3.95%) N(6.10%)

Experimental = C (52.74%) H(3.51%) N(6.39%)

Molecular formula = C_{10} H_9ClFNO_2

Formula Weight = **229.63**

M.P. = **73-76^{o}C**.

TLC System = (Ethyl acetate: Hexane : 4: 6)

Yield = **55-65%**

Preparation of 4-(3-bromophenyl)-N3,N5-bis(3-Cl,4-fluorophenyl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxamide:

Acetoacetanilide(0.02mol) and 3-chlorobenzaldehyde(0.01mol) were dissolved in 25ml methanol and heated on water bath till the solid disappeared in the reaction mass. Concentrated ammonia(3ml) was added to the reaction and it was further refluxed on a water bath for a period of 10-12hrs. The completion was monitored by TLC(Merck 60 F_{254}). After completion of reaction it was allowed to cool to room temperature and solid mass appeared in the flask. The product was filtered and washed with ether. It was recrystallized from ethanol+acetone.

Elemental Analysis

Calculated = C (57.62%) H(3.58%) N(7.47%)

Experimental = C (57.74%) H(3.51%) N(7.39%)

Molecular formula = $C_{27}H_{20}Cl_3F_2N_3O_2$

Formula Weight = **562.82**

M.P. = **220-24°C**.

TLC System = (Ethyl acetate: Hexane : 5: 5)

Yield = **45%**

Similarly other compounds were prepared by taking different substituted aromatic aldehydes & acetoacetanilide.

Table 1.1 Physical data of N^3,N^5-bis(3-chloro-4-fluorophenyl)-4-(R-phenyl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxamides :

*** Values in parenthesis denotes the calculated % of composition .**

Table 1.2 Physical data of N^3,N^5-bis(3-chloro-4-fluorophenyl)-4-(R-phenyl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxamides :

[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]		
								[illegible]		[illegible]
[illegible]	AV [illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible] ([illegible])	[illegible] ([illegible])	[illegible] ([illegible])
[illegible]	AV [illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible] ([illegible])	[illegible] ([illegible])	[illegible] ([illegible])
[illegible]	AV [illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible] ([illegible])	[illegible] ([illegible])	[illegible] ([illegible])
[illegible]	AV [illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible] ([illegible])	[illegible] ([illegible])	[illegible] ([illegible])
[illegible]	AV [illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible] ([illegible])	[illegible] ([illegible])	[illegible] ([illegible])
[illegible]	AV [illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible] ([illegible])	[illegible] ([illegible])	[illegible] ([illegible])

*** Values in parenthesis denotes the calculated % of composition .**

Table 1.3 Physical data of N^3,N^5-bis(3-chloro-4-fluorophenyl)-4-(R-phenyl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxamides :

[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]		
								[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]		[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]

*** Values in parenthesis denotes the calculated % of composition .**

SPECTRAL STUDY :

The constitutions of newly synthesized compounds were supported by IR, ^{1}H NMR, Mass and ^{13}C NMR spectral study. The details are as under.

IR Spectral Study :

Instrument : SHIMADZU FT IR-8400 Spectrophotometer

Sample technique : KBr pellet

Frequency range : 400-4000 cm^{-1}

In dihydropyridines, theoretically carbamoyl carbonyl group is present at C_3 and C_5 observed between frequency 1900-1700 cm^{-1}, while amide linkage is confirmed due to -NH streching appeared at 3400-3200cm^{-1} and -CONH stretching appeared between 3400-3200 cm^{-1}. Aromatic skeleton frequencies are observed in the range 1600-1400 cm^{-1}. The halogen linkage(C-Cl &C-F) is observed at 800-700 &1200-1100 cm^{-1}.

In case of 4-(2-Chlorophenyl)-N^3,N^5-bis(3-Cloro ,4-Fluorophenyl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxamide (**AM-13**), a sharp band of -NH was observed at 3224 cm^{-1}. The sharp carbonyl band (>C=O) was seen at 1676cm^{-1} which is slightly lower due to presence of -NH linkage compare to 1700 cm^{-1}. The amide stretching was observed at 3292 cm^{-1}.The aromatic moiety and ring skeleton (like C-C double bond stretching) were observed at 1600.8, 1494.7, and 1442 cm^{-1}. The halogen(C-Cl&C-F) group was confirmed due to band observed at frequency 752 & 1122.5 cm^{-1}.

Similarly other compounds were characterized. The tabular form of IR frequency is given on page 34 & 35.

^{1}H NMR Spectral Study :-

Instrument **:BRUKER AC 300 MHz FT-NMR**

Internal reference **:TMS**

Solven **:$CDCl_3$ or DMSO d_6**

During ^{1}H NMR study of 1,4-DHP's synthesized in this chapter, a singlet of methyl proton(-CH_3) is observed in the range 2-3 δppm. The most important identity of 1,4-DHP is achiral proton at C_4 position, is observed as singlet in the region 5-6 δppm. In the aromatic region, two doublet of 4-Fluoro phenyl ring with *J* value 6-9Hz. should be observed but due to presence of fluorine at C_{23} and C_{31} position it is observed as triplet and quartet in the aromatic region. The aromatic protons of the phenyl ring joined at C_4 position shows the signal in the region of 7-9 δppm. Due to presence of methyl protons (-CH_3), the -NH of pyridine ring is very much in downfield and is observed in the region 1-10 δppm. Due to presence of carbonyl group (-CO), the -CONH protons are in the downfield and singal of this proton is observed around 9 δppm.

In ^{1}H NMR spectra of 4-(3-methoxyphenyl)-N^3,N^5-bis(3-chloro-4-fluorophenyl)-2,6-Dimethyl-1,4-dihydropyridine-3,5-dicarboxamide **(AM-34),** singlet at 2.09 δppm, confirms the presence of two identical protons of methyl -CH_3(C_{18},C_7) group. Achiral proton(C_4) is in downfield due to presence of electron withdrawing group(-OCH_3) at C_4 phenyl ring which is confirmed by presence of singlet at 5.07 δ ppm. ton is showing singlet in downfield at 9.22 δppm.

In the aromatic region, a triplet at 6.92 δppm(J= 8.6 Hz) and multiplet at 7.50 δppm(J=3.79Hz) confirms amide linkage of -CH (C_{22},C_{24},C_{31},C_{33}) and -CH(C_{21},C_{25},C_{34},C_{30}) of the phenyl ring with moiety. Meta coupling of Nitrophenyl at C_4 (-CH_{13}) is obtained at 7.67 δppm(J = 7.5 Hz) as doublet of doublet (d,d) due to coupling with -CH(C_{13}) and -CH(C_{14}), while one triplet of -CH(-C_{14})is seen at 7.39 δppm and singlet of -CH(-C_{11})at 8.79 δppm. The -NH (N_{19},N_{28})proton is showing singlet in downfield at 9.22 δppm .

Similarly other derivatives of the series are confirmed. The NMR data mentioned on page 36-39.

Mass Spectral Study :-

Instrument : VG 70-S (70eV) Spectrograph for El

Instrument : JEOL SX 102/DA-6000 Spectrograph for FAB

The molecular ion peak is in concormitant with the molecular weight. The newly synthesized compounds were subjected to FAB Mass study. The Fast bombardment study revealed the Molecular ion peak, base peaks and other relevant fragmentation pattern to confirm the structure of the molecules.

In the Fab Mass of 4-(3-Phenoxyphenyl)-bis(3-Chloro-4-Fluorophenyl)-2,6-dimethyl-1,4-Dihydropyridine-3,5 dicaboxamide **(AM-25),** base peak is observed at 620.0 m/z, while molecular ion peak is also at 620.00 m/z.

In the Fab Mass of 4-(2-chlorophenyl)-bis(3-Chloro-4-Fluorophenyl)-2,6-dimethyl-1,4-dihydropyridine-3,5 dicaboxamide **(AM-13),** base peak is observed at 417.0 m/z, while molecular ion peak is at 563.00 m/z as (M+1) peak.

In the FAB Mass of 4-(3-bromophenyl)-bis(3-chloro-4-fluorophenyl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicaboxamide **(AM-15)** base peak is at m/z 154.00 and the molecular ion peak is observed at m/z 608.00 (M+1) peak..

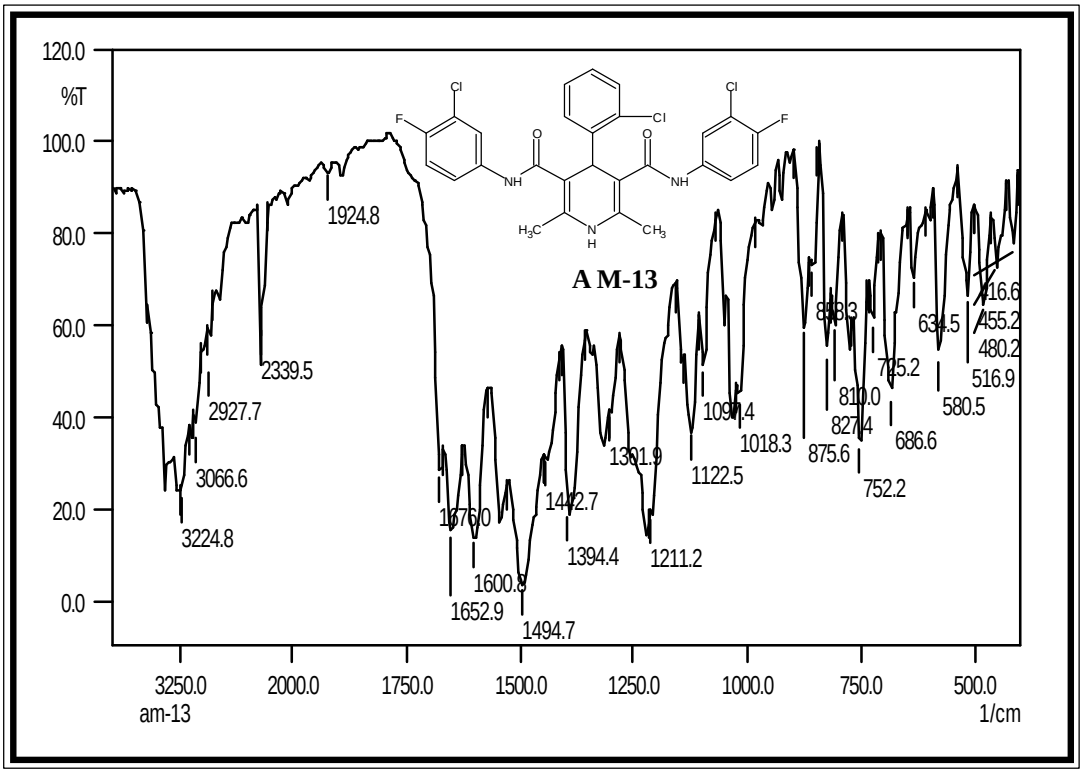
In the Fab Mass of 4-(4-methoxyphenyl)-bis(3-chloro-4-fluorophenyl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicaboxamide **(AM-26***)* base peak is at m/z 449.0, while the molecular ion peak is observed at m/z 558.0 (M+).

The mass fragmentation pattern of newly synthesized molecules are in total agreement with the suggested structures.

^{13}C NMR :

The compounds **AM-13, AM-15, AM-25 and AM-26** were subjected for ^{13}C NMR spctroscopy and the results of these data shows the predicted sturctures is in agreement with the carbon skeletons of respective compounds.

IR Spectrum of 4-(2-chlorophenyl)-N³,N⁵-bis (3-Chloro-4-fluorphenyl)-2, 6-dimethyl -1, 4-dihydro-3, 5-pyridinedicarboxamide (AM-13)



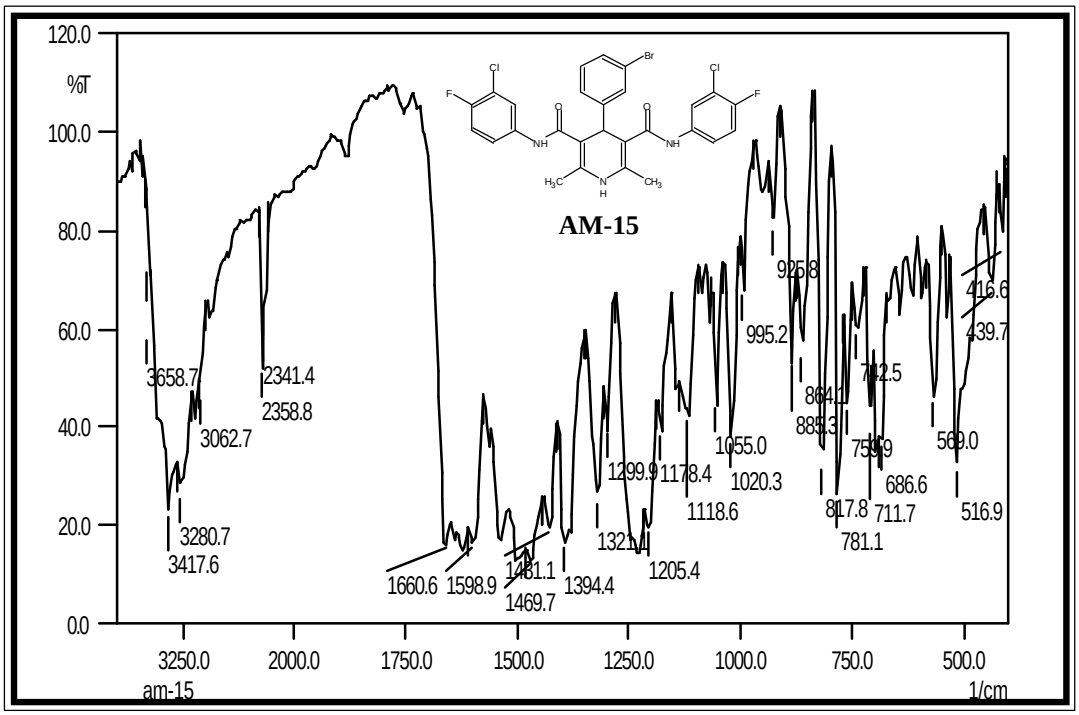
Instrument : SHIMADZU FT IR-8400

Sample technique : KBr Pellet

Frequency range : 4000-400 cm^{-1}

Type	Vibration mode	Frequency cm^{-1}
Amine	-NH str.	3224.8
Amide	>C=O str.	1676.0
	>C-N str.	1600.8
Methyl	-CH str. (Asym.)	2927.7
	-CH str. (sym.)	3066.8
Aromatic	>C=C< ring str. vibn.	1494.7 1652.8
	o.o.p.bending vib. (1,3,4-tri sub.)	752.2 810.0
Halogen	C-Cl & C-F str.	688 &1122.5

IR Spectrum of 4-(3-bromophenyl)-N³,N⁵-bis (3-Chloro-4-fluorphenyl)-2, 6-dimethyl -1, 4-dihydro-3, 5-pyridinedicarboxamide (AM-15)



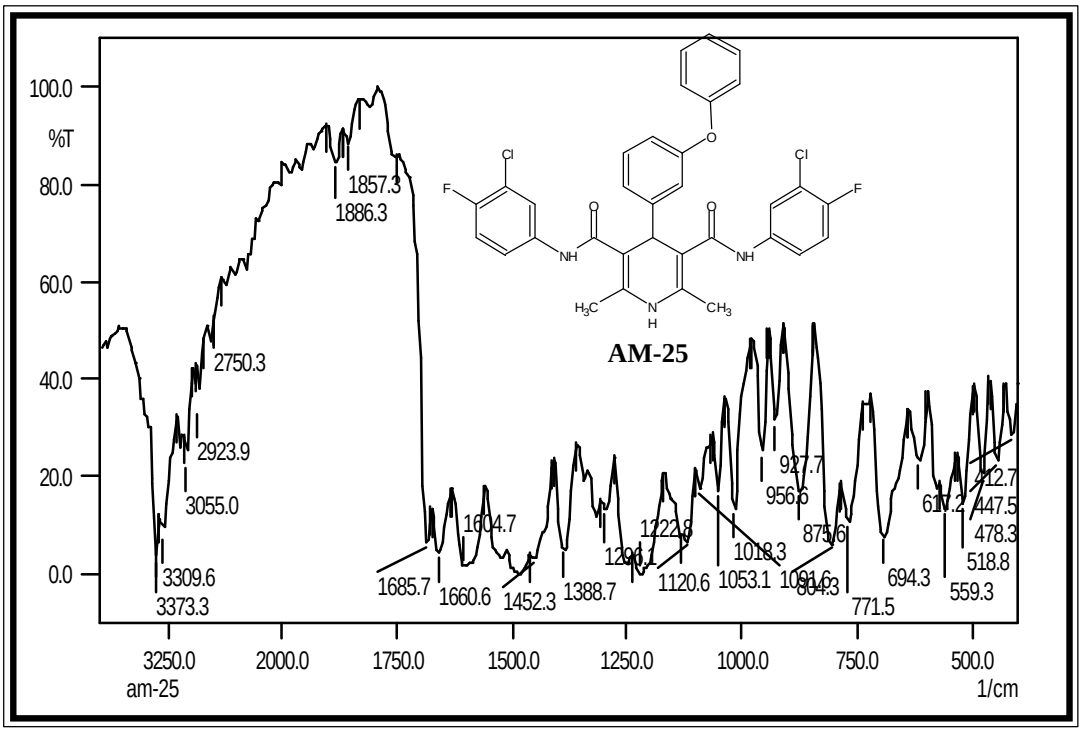
Instrument : SHIMADZU FT IR-8400

Sample technique : KBr Pellet

Frequency range : 4000-400 cm^{-1}

Type	Vibration mode	Frequency cm^{-1}
Amine	-NH str.	3280.7
Amide	>C=O str.	1660.6
	>C-N str.	1598.9
Methyl	-CH str. (Asym.)	~2900.0
	-CH str. (sym.)	3062.7
Aromatic	>C=C< ring str. vibn.	1461.1 1469.7
	o.o.p.bending vib. (1,3,4-tri sub.)	781.1 817.5
Halogen	C-Cl & C-F str.	~711 & 1118.6

IR Spectrum of 4-(3-phenoxyphenyl)-N³,N⁵-bis (3-Chloro-4-fluorphenyl)-2, 6-dimethyl -1, 4-dihydro-3, 5-pyridinedicarboxamide (AM-25)



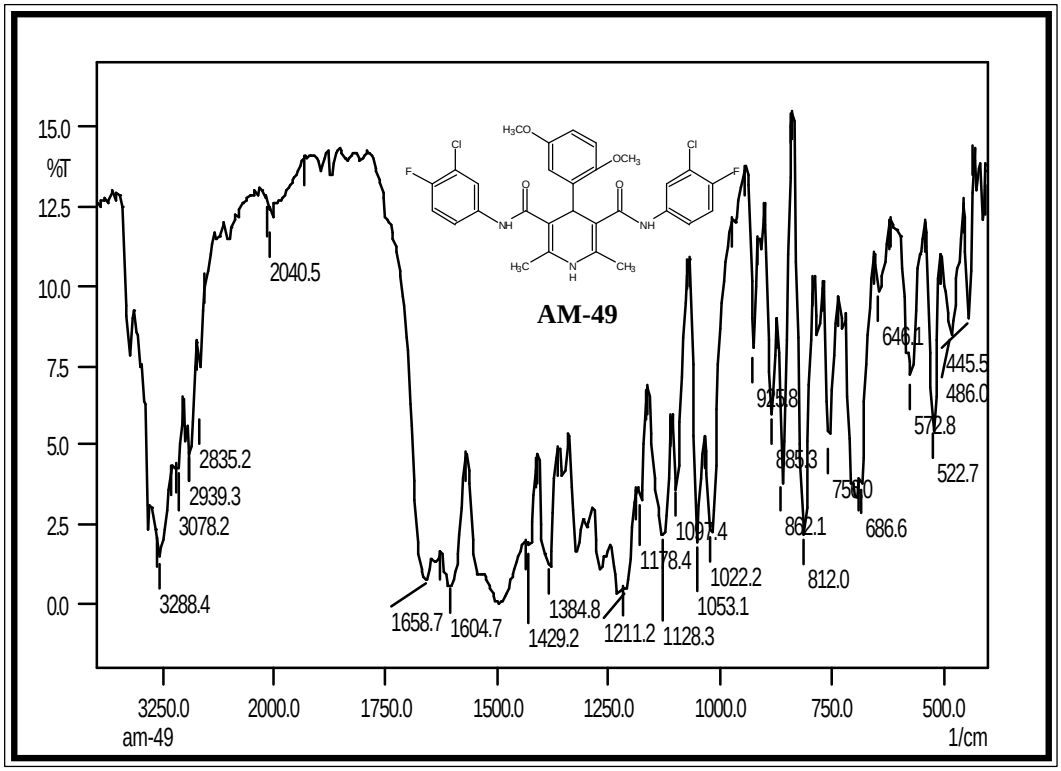
Instrument : SHIMADZU FT IR-8400

Sample technique : KBr Pellet

Frequency range : 4000-400 cm^{-1}

Type	Vibration mode	Frequency cm^{-1}
Amine	-NH str.	3309.6
Amide	>C=O str.	1685.7
	>C-N str.	1604.7
Methyl	-CH str. (Asym.)	2923.3
	-CH str. (sym.)	3055.0
Aromatic	>C=C< ring str. vibn.	1452.3 1660
	o.o.p.bending vib. (1,3,4-tri sub.)	771.5 875.6
Halogen	C-Cl & C-F str.	694 & 1120.6

IR Spectrum of 4-(2,5dimethoxyphenyl)-N³,N⁵-bis (3-Chloro-4-fluorphenyl)-2, 6-dimethyl -1, 4-dihydro-3,5-pyridinedicarboxamide (AM-49)



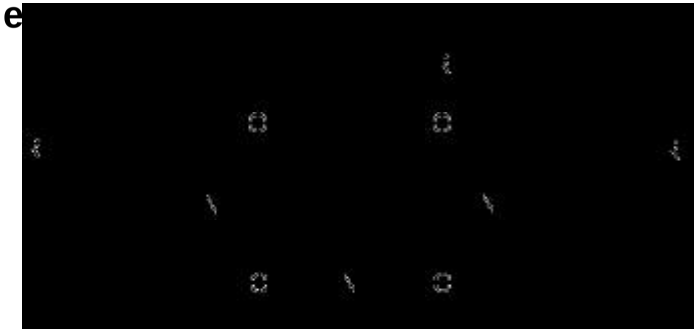
Instrument : SHIMADZU FT IR-8400

Sample technique : KBr Pellet

Frequency range : 4000-400 cm^{-1}

Type	Vibration mode	Frequency cm^{-1}
Amine	-NH str.	3288.4
Amide	>C=O str.	1668.7
	>C-N str.	1604.7
Methyl	-CH str. (Asym.)	2939.3
	-CH str. (sym.)	3078.2
Aromatic	>C=C< ring str. vibn.	1429.2
	o.o.p.bending vibn. (1,3,4-tri sub.)	756.0 812.0
Halogen	C-Cl & C-F str.	~686.0 &1128.3

Table 1.4 IR Spectral study of N^3,N^5-bis(3-chloro-4-fluorophenyl)-4-(R-phenyl)-2,6-di methyl-1,4-dihydropyridine-3,5-dicarboxamide



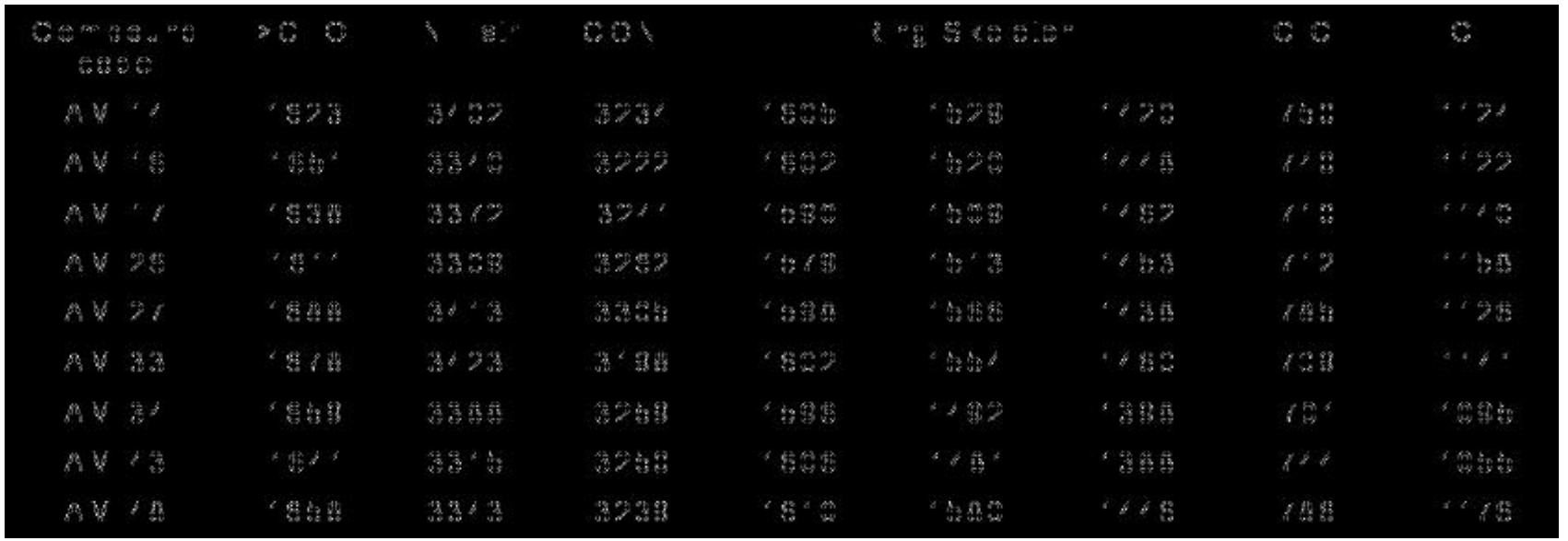
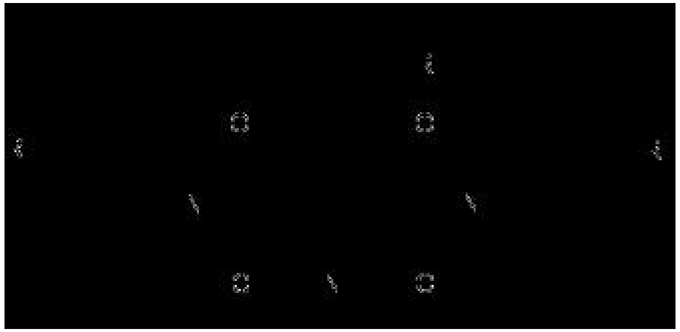
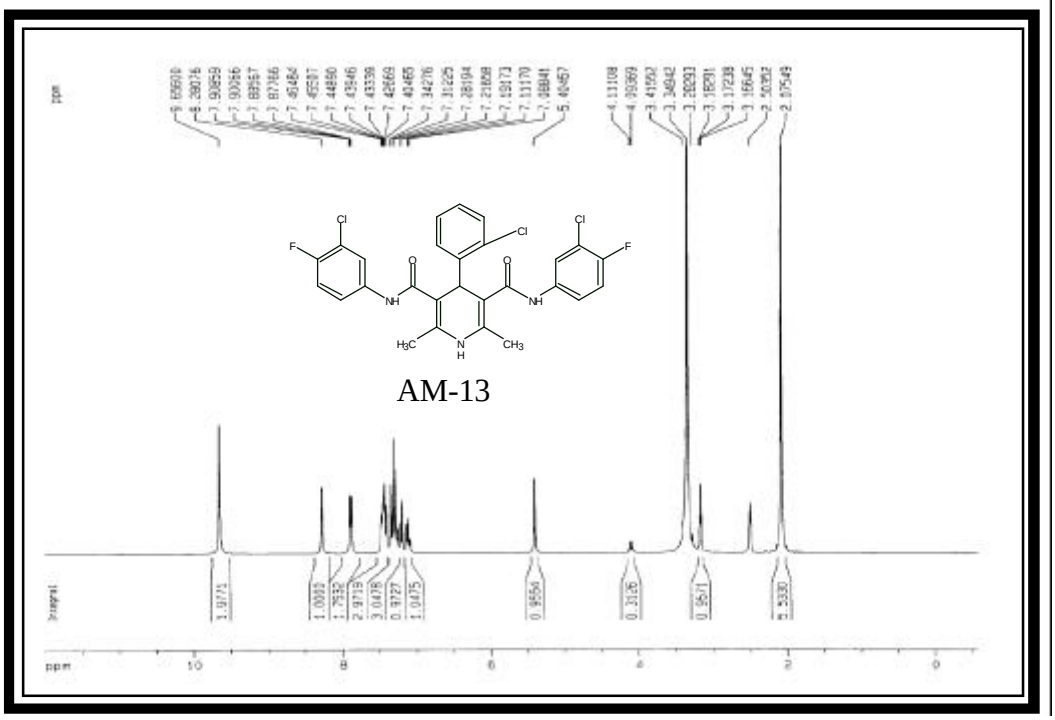


Table 1.5 IR Spectral study of N^3,N^5-bis(3-chloro-4-fluorophenyl)-4-(R-phenyl)-2,6-di methyl-1,4-dihydropyridine-3,5-dicarboxamides :



Compound code	>C=O	N-H str	CONH	Ring Skeleton			[illegible]	[illegible]
AV [illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
AV [illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
AV [illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
AV [illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
AV [illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
AV [illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
AV [illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
AV [illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
AV [illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]

^{1}H NMR Spectrum of 4-(2-chlorophenyl)-N,N-bis (3-chloro-4-fluoro phenyl)-2, 6-dimethyl -1, 4-dihydropyridine-3, 5-dicarboxamide (AM-13).



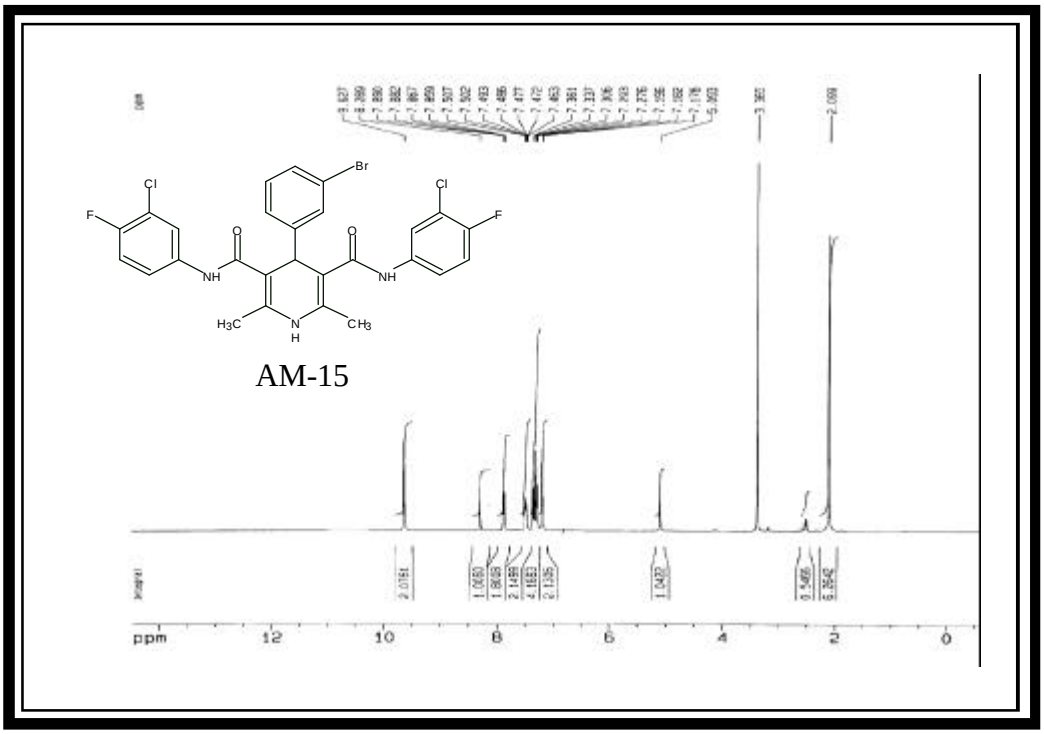
Instrument : BRUKER AC 300 MHz FT-NMR

Standard : TMS

Solvent : DMSO d_6

Chemical Shift d ppm	No. of Proton	Muliplicity	Interence	J. Value
2.07	6H	s	$-CH_3$	-
5.40	1H	s	H_1	-
7.08-7.13	21H	m	H_{27}	-
7.19-7.21	1H	d	H_{13}	$J_{13,14}=H_2$
7.25-7.34	3H	q	$C_{21,21,19}$	-
7.42-7.47	3H	m	C_{22} C_{31} C_{17}	-
7.87-7.90	2H	d(d)	C_{14} C_{28}	$J_{14,13}=9H$
8.28	1H	s	NH	-

^{1}H NMR Spectra of 4-(3-bromophenyl)-N^{3},N^{5}-bis (3-chloro-4-fluoro phenyl)-2, 6-dimethyl -1, 4-dihydropyridine-3,5-dicarboxamide (AM-15)



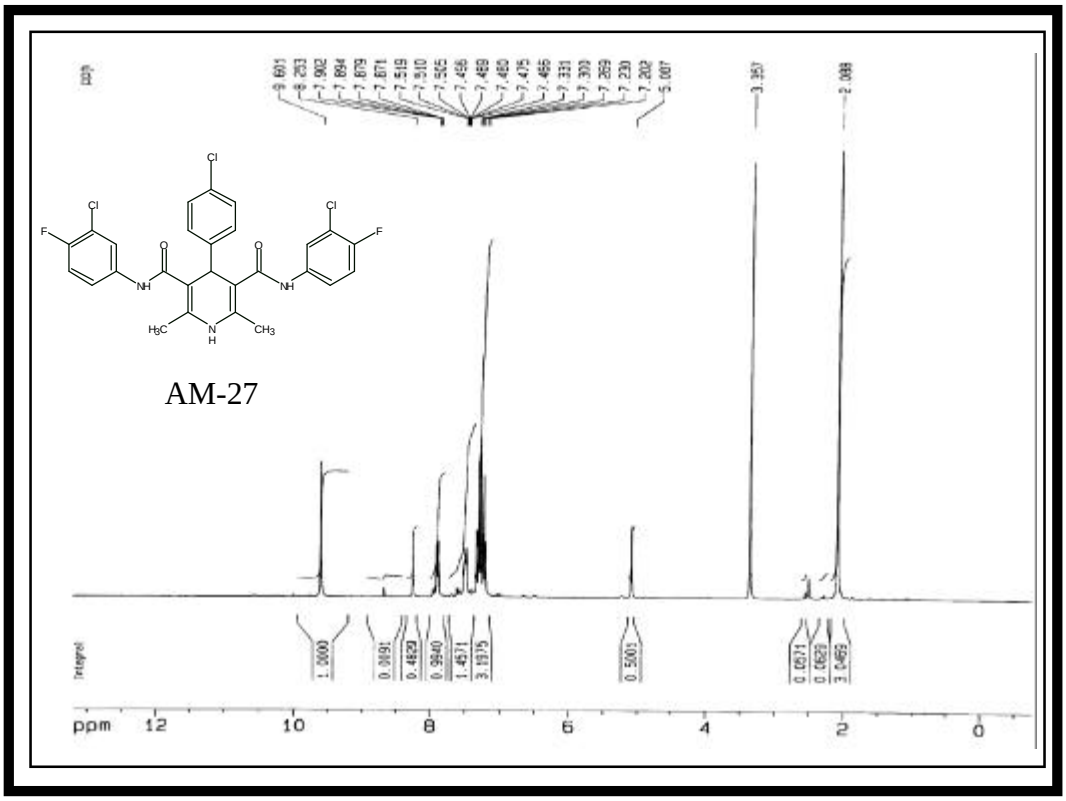
Instrument :BRUKER AC 300 MHz FT-NMR

Standard : TMS

Solvent : DMSO d_6

Chemical Shift d ppm	No. of Proton	Muliplicity	Interence	J. Value
2.099	3HX2	s	C-CH_3	---
5.093	1H	s	Ar-H_1	---
7.19	2H	d (b)	Ar-$H_{8,9}$	---
7.27-7.36	5H	m	Ar-$H_{7,11,9}$ Ar-$H_{23,16}$	J,23,24=9H_2 J,16,17=9H_2
7.46-7.51	2H	m	Ar-$H_{27,20}$	---
7.87	2H	d (b)	Ar-H_{24} A-rH_{17}	J,24,23=H_2 J,17,16=9H_2
8.27	-NH	s (b)	-NH	--

^{1}H NMR Spectra of 4-(4-chlorophenyl)-N^{3},N^{5}-bis (3-chloro-4-fluoro phenyl)-2, 6-dimethyl -1, 4-dihydropyridine-3, 5-dicarboxamide (AM-27)



Instrument : BRUKER AC 300 MHz FT-NMR

Standard : TMS

Solvent : DMSO d_6

Chemical Shift d ppm	No. of Proton	Muliplicity	Interence	J. Value
2.08	3HX 2	Singlet	-C-CH3	-
5.08	1H	Singlet	Ar H1	-
7.20-7.33	6H	pentlet	Ar $H_{7,8\,10}$ Ar $H_{16,23}$	J10,11=J11-,10= 9Hz
7.46-7.51	2H	multiplate	Ar H_{27} Ar H_{20}	-
7.86-7.89	2H	double doublet	Ar H_{24} Ar H_{17}	Ar H 24,23= 8Hz Ar h 17,6= 8.7 HZ
8.24	-NH	Singlet	-NH-	

^{1}H NMR Spectra of 4-(3-methoxyphenyl)-N^{3},N^{5}-bis (3-chloro-4-fluoro phenyl)-2, 6-dimethyl -1, 4-dihydropyridine-3, 5-dicarboxamide (AM-34)

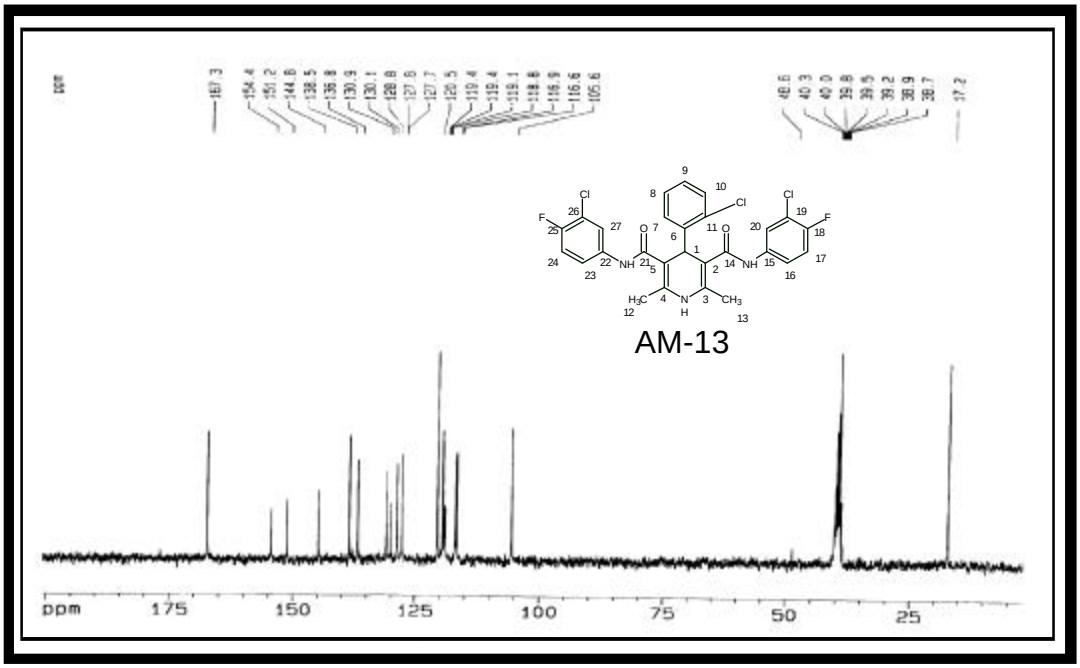
Instrument : BRUKER AC 300 MHz FT-NMR

Standard : TMS

Solvent : DMSO d_6

Chemical Shift d ppm	No. of Proton	Muliplicity	Interence	J. Value
2.09	6H	Singlet	-C-CH3	-
3.62	3H	Singlet	-OCH3	-
5.07	1H	Singlet	-Ar H1	-
6.66-6.69	1H	Double Doublet	Ar H10	J10,9=9Hz J10,8=2-4Hz
6.75	1H	Singlet	Ar H2	-
6.80	1H	Doublet	Ar H8	J8,9=7-8Hz
7.138	1H	Triplet	Ar H9	J9,10=9Hz J9,8=7-8Hz
7.30	2H	Triplet	Ar H16 Ar H27	J16,17=9Hz J27,26=9Hz
7.49	2H	Multiplate	Ar H20 Ar H23	-
7.905	2H	Double Doublet	Ar H17 Ar H26	J17,16-9Hz J26,27=9Hz
8.18	-	Singlet	-NH-	-

^{13}C Spectra of 4-(2-chlorophenyl)-N^3,N^5-bis(3-chloro-4-fluoro phenyl)-2,6 dimethyl -1,4-dihydropyridine-3,5-dicarboxamide (AM-13)

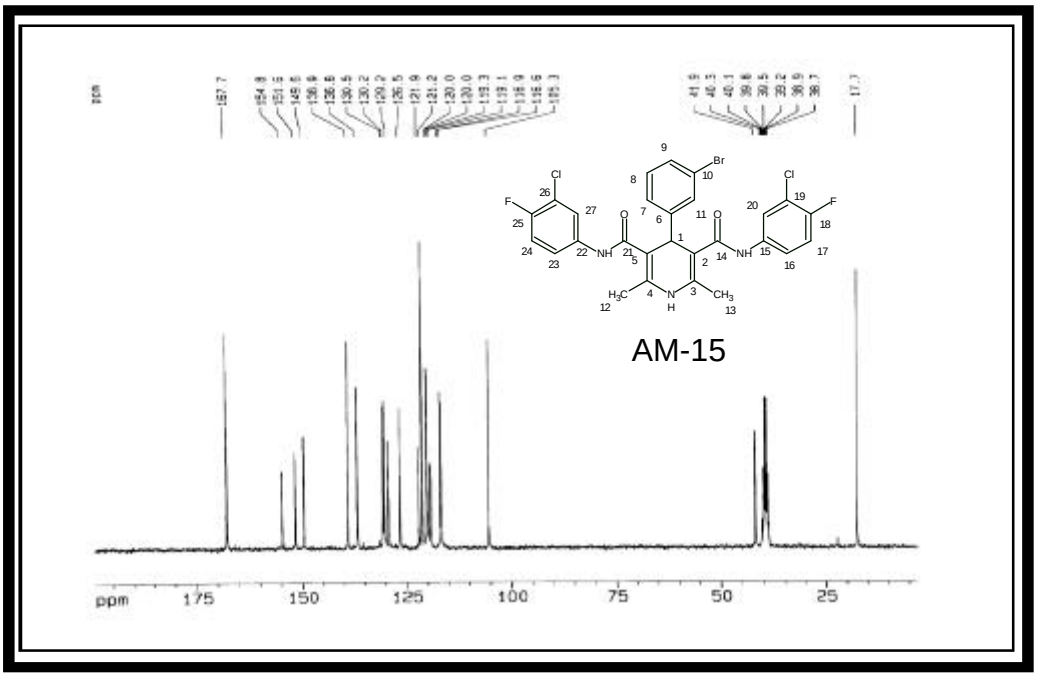


Instrument : BRUKER AC 300 MHz FT-NMR

Solvent : DMSO d_6

Chemical Shift d ppm	No.of carbons	Interference
39.8	2C	-CH$_3$(12,13)
105.6	1C	-CH(1)
119.6	1C	-CH(6)
118.4	4C	-CH(7,8,9,10)
136.8	2C	-CH(18,25)
138.5	2C	-CH(19,26)
144.8	1C	-CH(11)
130.1	2C	-CH(3,4)
130.9	2C	-CH(2,5)
128.8	2C	-CH(15,22)
127.7	2C	-CH(20,27)
119.4	2C	-CH(16,23)
151.2	1C	-CH(14)
154.4	1C	-CH(21)

^{13}C Spectra of 4-(3-bromophenyl)-N^3,N^5-bis(3-chloro-4-fluoro phenyl)-2,6-dimethyl -1,4-dihydropyridine-3,5-dicarboxamide (AM-15)

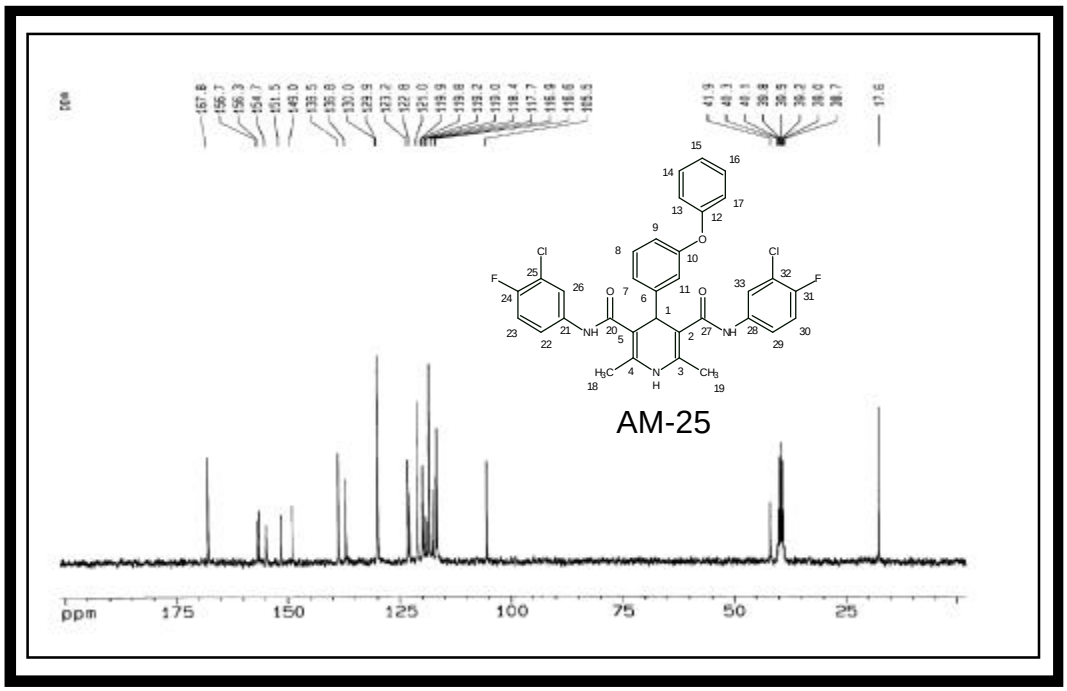


Instrument : BRUKER AC 300 MHz FT-NMR

Solvent : DMSO d_6

Chemical Shift d ppm	No.of carbons	Interference
38.8	2C	-CH$_3$(12,13)
105.3	1C	-CH(1)
116.6	1C	-CH(1)
136.8	2C	-CH(3,4)
138.9	2C	-CH(2,5)
129.2	2C	-CH(22,15)
130.2	2C	-CH(16,23)
121.1	2C	-CH(20,27)
121.9	2C	-CH(19,16)
121.2	4C	-CH(7,8,9,11)
149.8	2C	-CH(18,25)
151.6	2C	-CH(17,24)
154.8	1C	-CH(10)
167.7	2C	-CH(14,21)

^{13}C Spectra of 4-(3-phenoxyphenyl)-N^3, N^5-bis(3-chloro-4-fluoro phenyl)-2,6-dimethyl -1,4-dihydropyridine-3,5-dicarboxamide (AM-25)

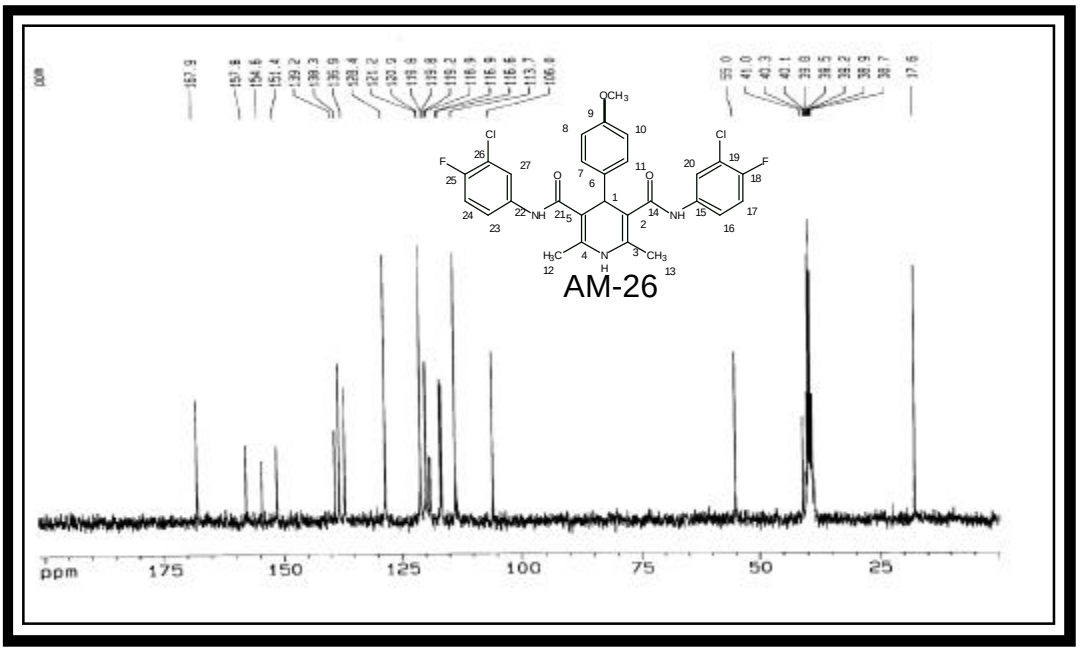


Instrument : BRUKER AC 300 MHz FT-NMR

Solvent : DMSO d_6

Chemical Shift d ppm	No.of carbons	Interference
39.2	2C	-CH$_3$(18,19)
105.5	1C	-CH(6)
119.2	1C	-CH(1)
116.6	2C	-CH(22,29)
117.8	2C	-CH(26,23)
118.4	2C	-CH(25,32)
119.8	4C	-CH(7,8,9,11)
121.0	2C	-CH(13,17)
122.8	2C	-CH(14,16)
126.9	1C	-CH(15)
129.9	1C	-CH(21)
130.0	1C	-CH(28)
136.8	1C	-CH(23,30)
138.5	1C	-CH(24,31)
156.3	1C	-CH(10)
156.8	1C	-CH(20)
156.9	1C	-CH(27)
167.8	1C	-CH(12)

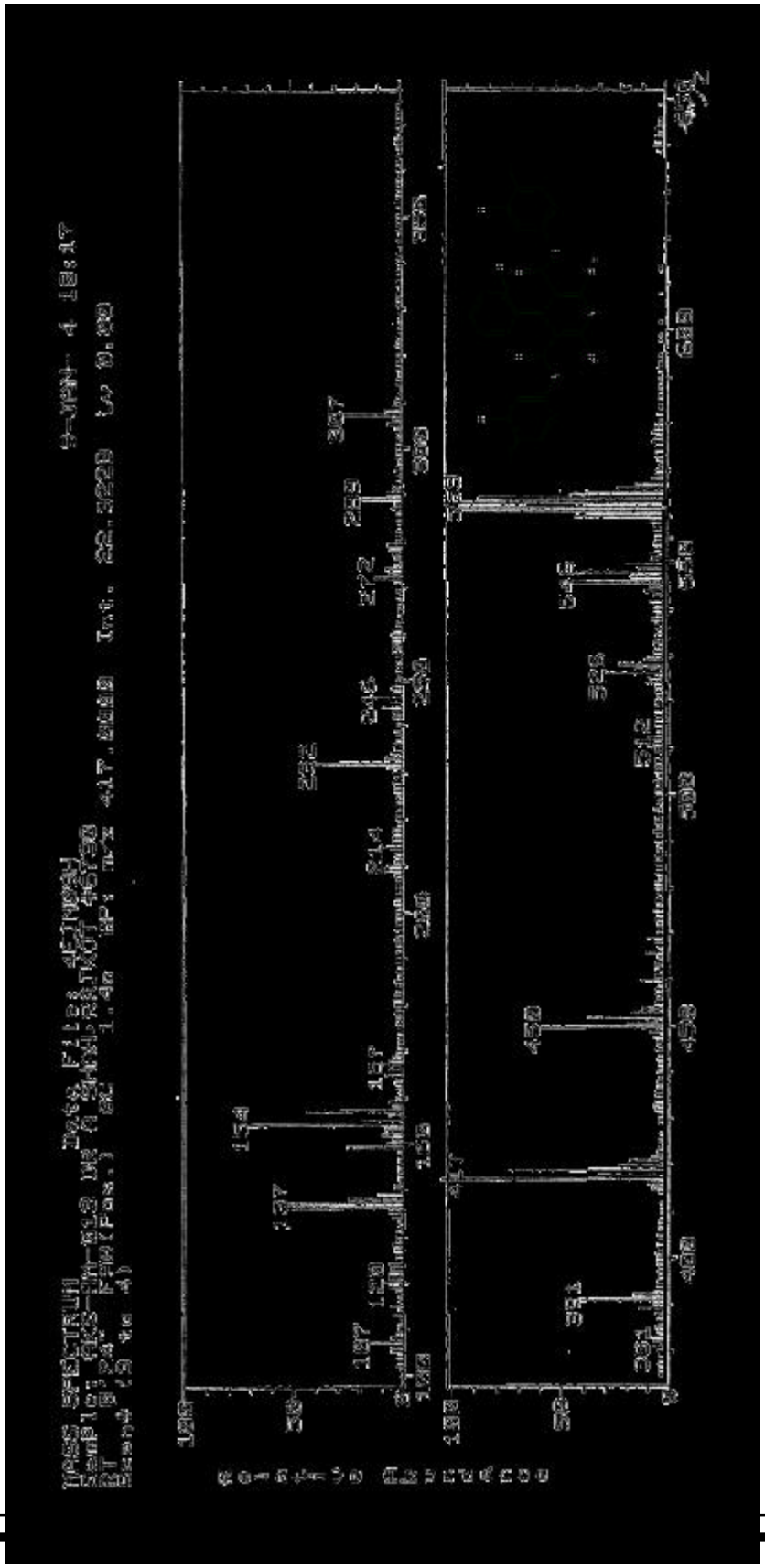
^{13}C Spectra of 4-(4-methoxyphenyl)-N^3,N^5-bis(3-chloro-4-fluoro phenyl)-2,6-dimethyl -1,4-dihydropyridine-3,5-dicarboxamide (AM-26)



Instrument : BRUKER AC 300 MHz FT-NMR

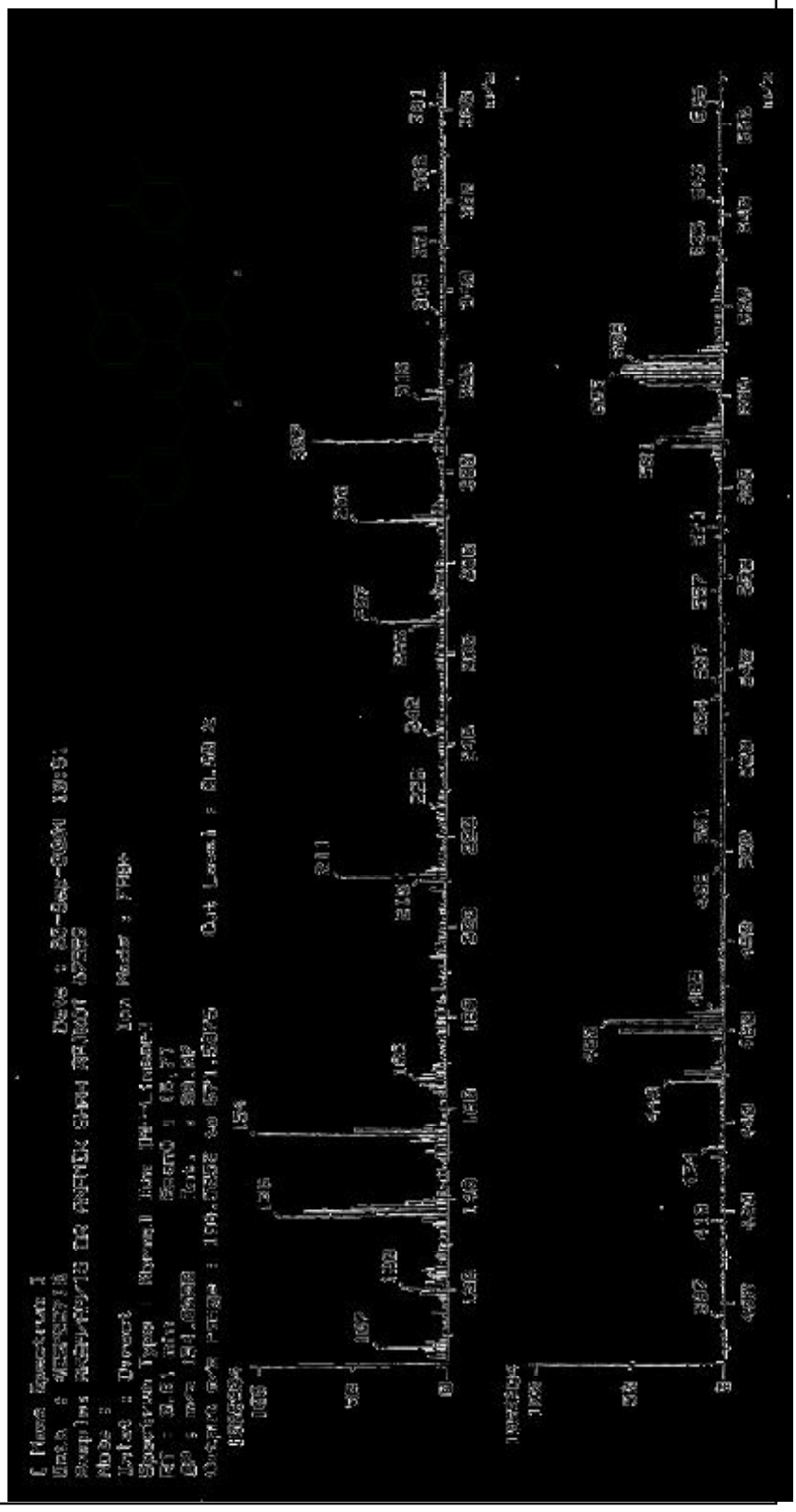
Solvent : DMSO d_6

Chemical Shift d ppm	No.of carbons	Interference
39.5	2C	-CH$_3$(12,13)
55.5	1C	-CH(28)
106.2	1C	-CH(6)
116.6	1C	-CH(1)
118.8	1C	-CH(26,19)
119.2	2C	-CH(20,27)
119.6	2C	-CH(16,23)
120.8	2C	-CH(7,11)
119.8	1C	-CH(8,10)
138.3	2C	-CH(3,4)
136.9	2C	-CH(2,5)
151.4	2C	-CH(22,15)
154.6	2C	-CH(18,25)
157.8	2C	-CH(17,24)
167.8	2C	-CH(14,21)

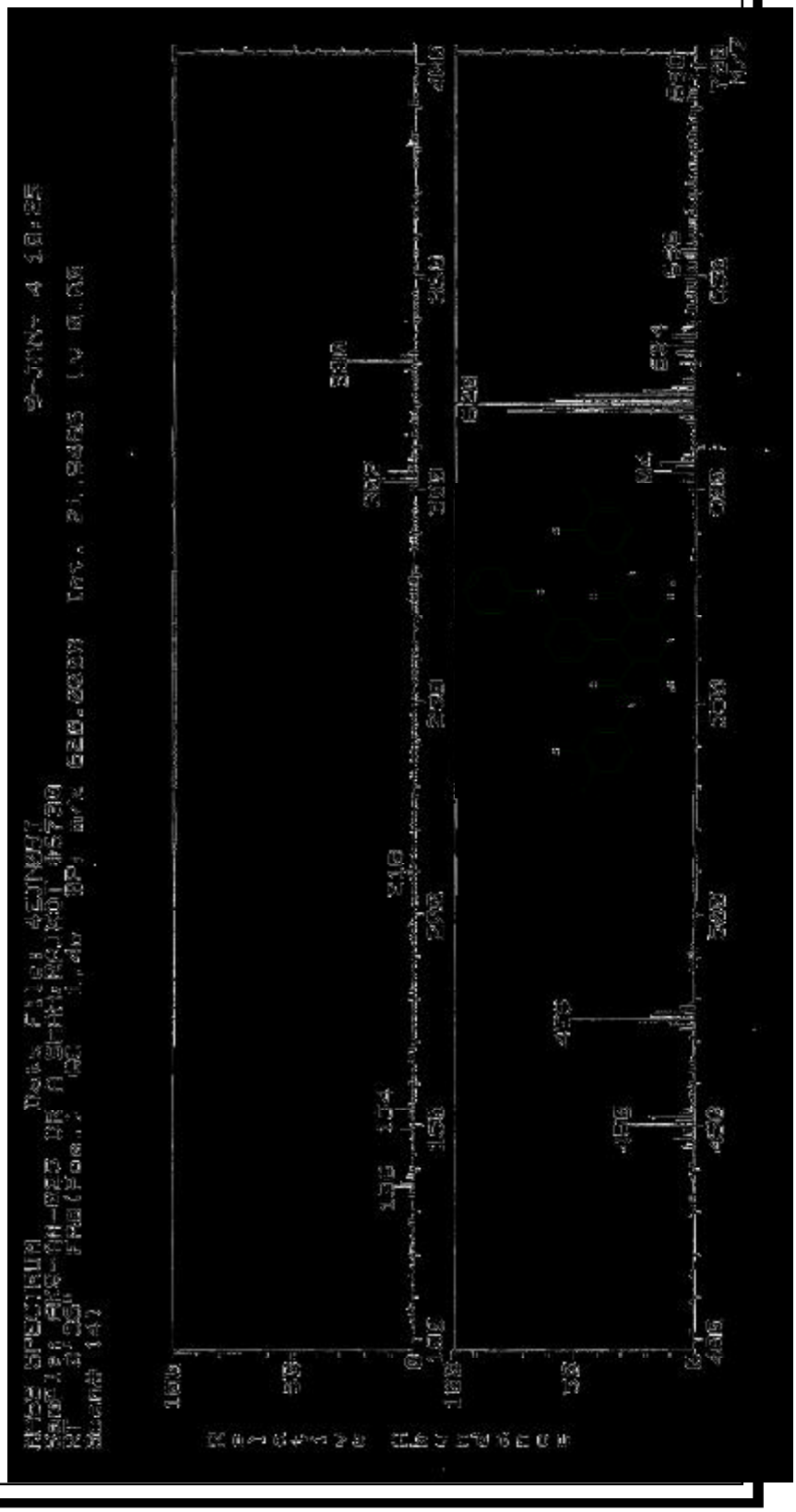


Fab Mass Spectra of 4-(2-chlorophenyl)-N^3,N^5-bis (3-Chloro-4-fluorophenyl)-2, 6-dimethyl -1, 4-dihydropyridine-3, 5-dicarboxamide (AM-13)

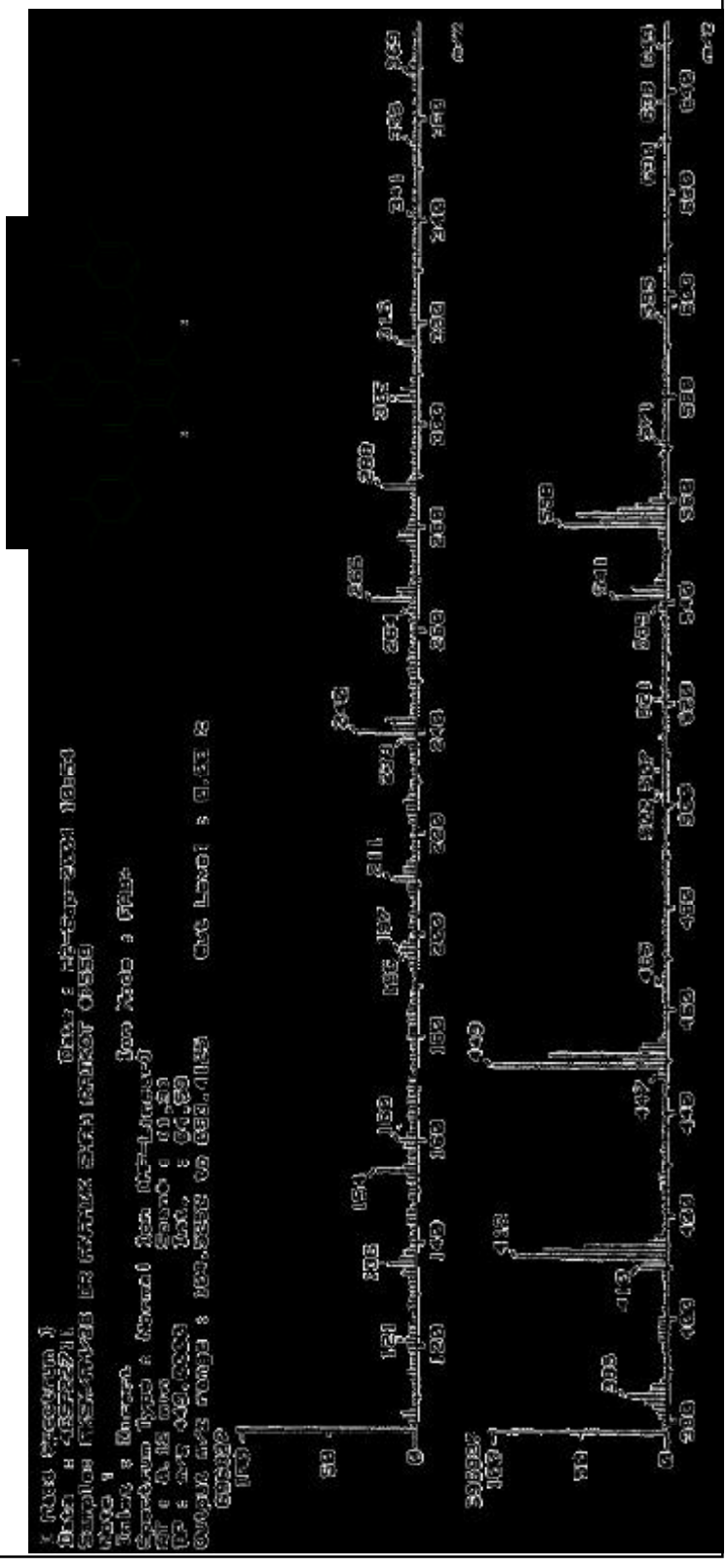
Fab Mass Spectra of 4-(3-bromophenyl)-N^3,N^5-bis (3-Chloro-4-fluorphenyl)-2, 6-dimethyl -1, 4-dihydropyridine-3, 5-dicarboxamide (AM-15)



Fab Mass Spectra of 4-(3-phenoxyphenyl)-N^3,N^5-bis (3-chloro-4-fluorphenyl)-2, 6-dimethyl -1, 4-dihydropyridine-3, 5-dicarboxamide (AM-25)

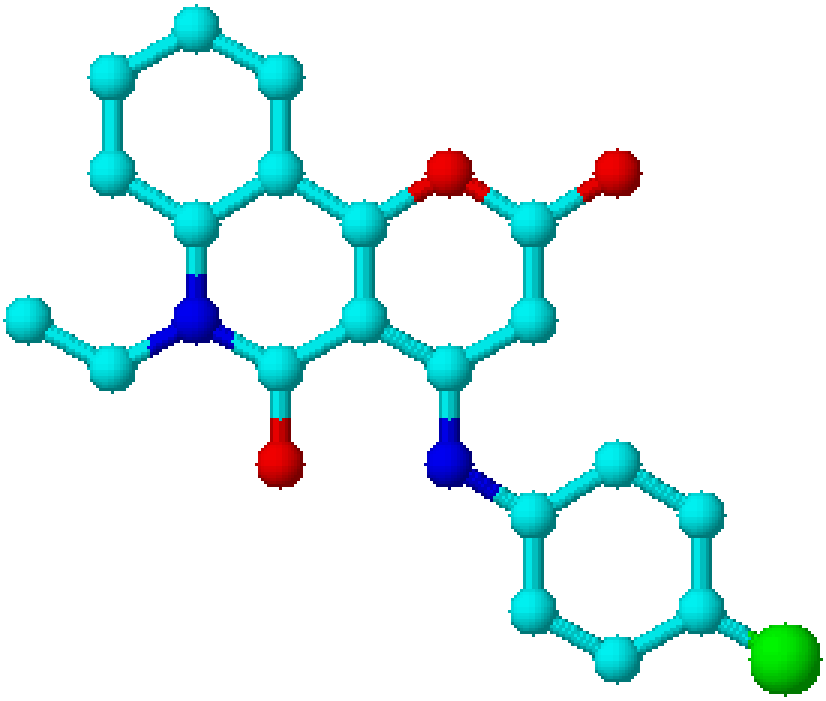


Fab Mass Spectra of 4-(3-chlorophenyl)-N^3,N^5-bis (3-Chloro-4-fluorophenyl)-2, 6-dimethyl -1 4 - dihydropyridine-3, 5-dicarboxamide (AM-26)



Chapter-2

Preparation of 4-[(4-chlorophenyl)amino]-6-ethyl-2H-pyrano[3,2-c]quinoline-2,5(6H)-diones :



INTRODUCTION :

Pyrano fused heterocycles are commercially important as antibacterials[1-4], antihistamines[5], antimicrobials[6], enzyme substrates[7] and alkaloids[8]. Several patents describe the synthesis and technical importance of pyrano fused derivatives in high technology applications such as liquid crystal display devices[9], ink-jets[10], photochromic materials[11,] electroluminescent materials[12], and fluorescent whitening agents[13].

Yoshiuki Kawase et al[14], has studied some benzofuro[2,3-b]quinolines and benzofuro[3,2-c]quinolines activities as mutagens, carcinogens and antitumour polycyclic heteroaromatic compounds.

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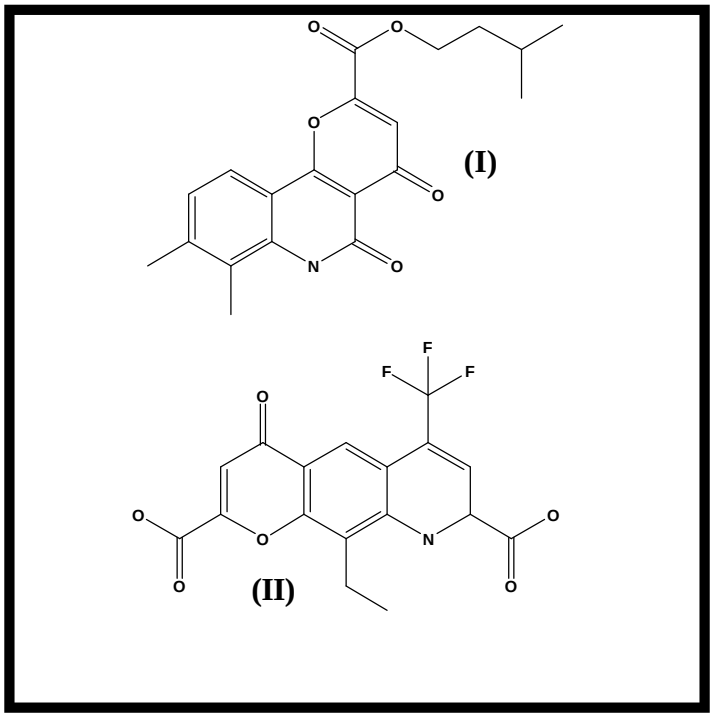
Pyrano quinolones are structurally related to antihistaminic and anti-allergic drug Romet (I)[12-15] and 4-oxo-10-pyrano[3,2-g]quinoline-2,8-dicarboxylic acid[16-17] (II).



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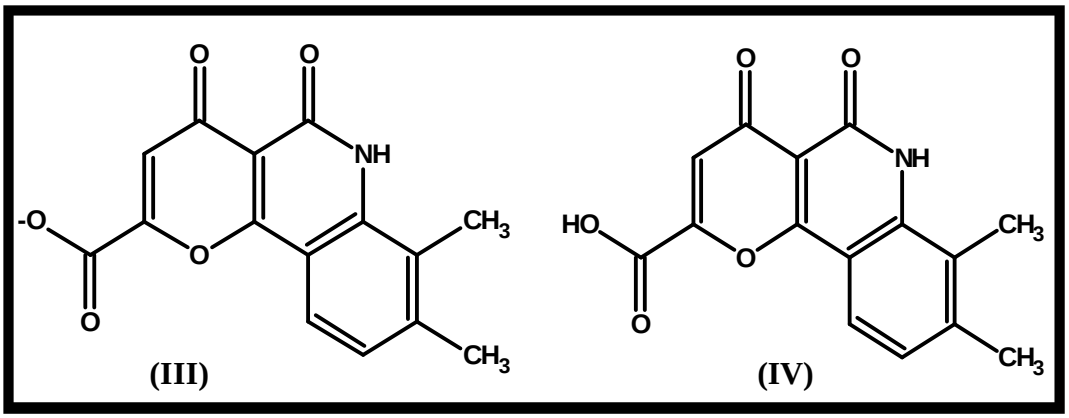
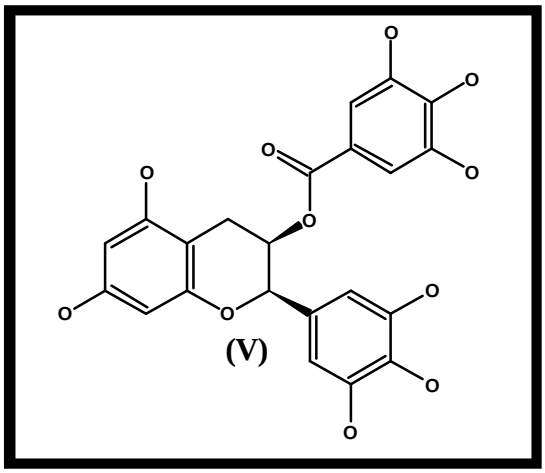
18. Yamada N, Kadowaki S, Takahashi K, and Umezu K.,*Biochem Pharmacol*. 1992 Sep 25;**44(6)**:1211-3

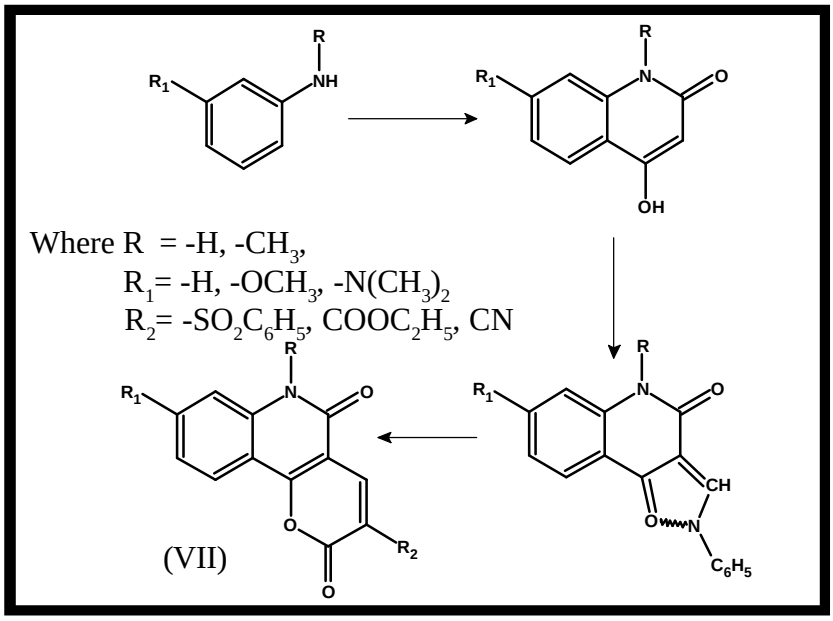


Structurally different pyrano quinolines are reported in literature. For e.g. Inositol-1,4,5-trisphosphate-3-kinases (IP_3K) A, B and C as well as inositol polyphosphate multikinase (IPMK) catalyze the first step in the formation of the higher phosphorylated inositols $InsP_5$ and $InsP_6$ by metabolizing $Ins(1,4,5)P_3$ to $Ins(1,3,4,5)P_4$. In order to clarify the special role of these $InsP_3$ phosphorylating enzymes and of subsequent anabolic inositol phosphate reactions, a search was conducted by Hillemeier K et al[19], for potent enzyme inhibitors starting with a fully active IP3K-A catalytic domain. In this search epigallocatechin-3-gallate (EGCG, 120 nM)(V), was identified as potent inhibitors with IC_{50} < 200 nM.

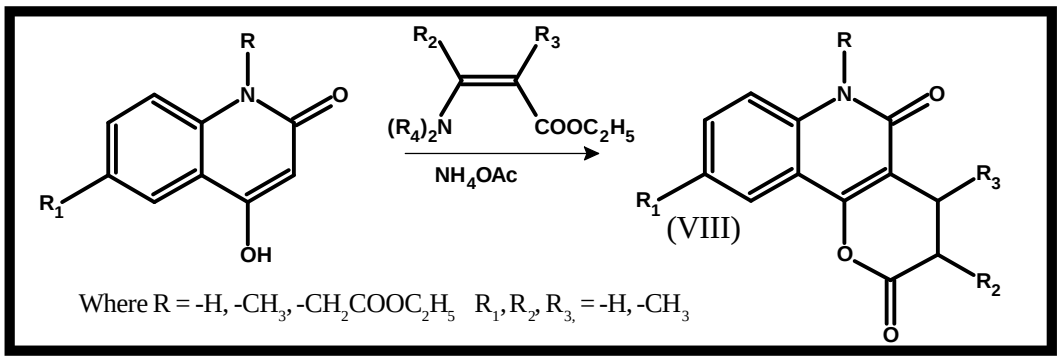


19. Mayr GW, Windhorst S, Hillemeier K., *J Biol Chem.* ***2005 Jan 19.***
20. Knierzinger A. and Wolfbeis O. S., *J. Het. Chem.,* **17**, 225 (1980).
21. Kappe T., and Mayer C., *Synthesis*, 524 (1981).

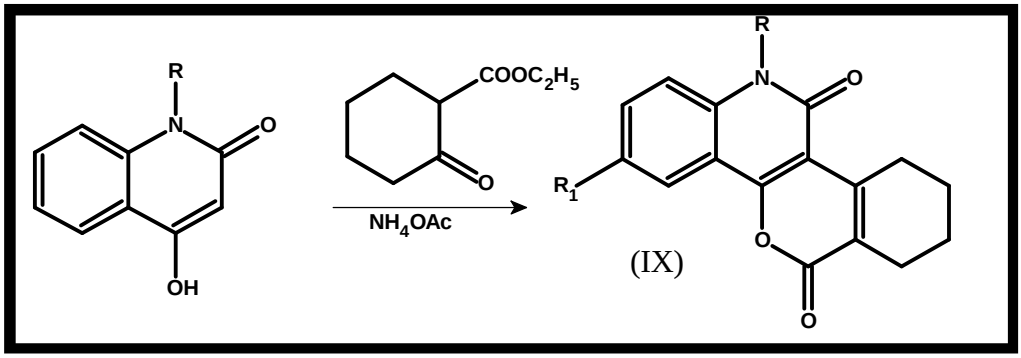
Knierzinger and Wolfbeis[20] reported 2H, 5H-pyrano[3,2-c]quinoline diones(VII).



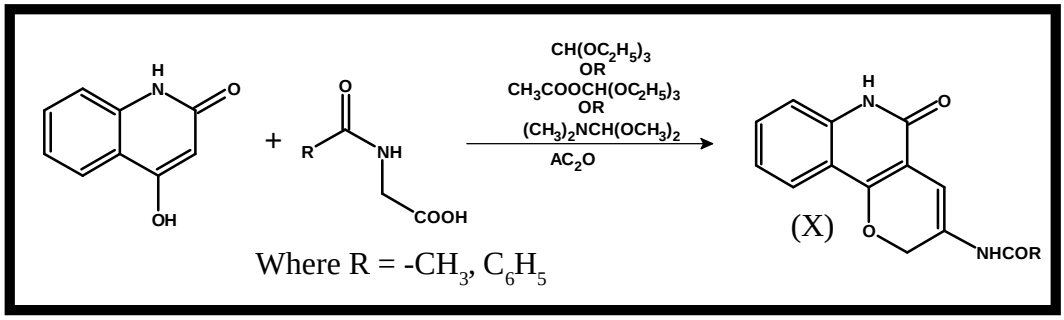
Kappe and Mayer[21] studied formation of heterocycles at C_3-C_4 position by various β-keto esters. They prepared many pyranoquinolines(VIII) as under.



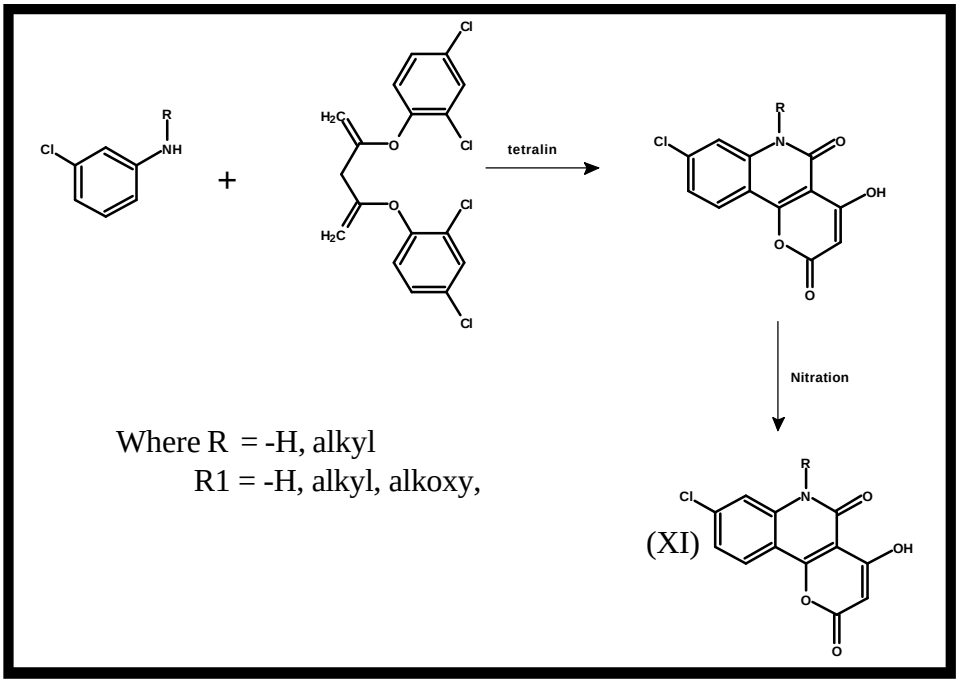
They have also reported modified Pechmann condensation[5] (IX) with respect to different condensates in presence of ammonium acetate.



Vladimir and coworkers[22] have synthesized some fused derivative from pyrano-2-ones as well as 4-hydroxy-2-quinolones (X). It possesses an unsymmetrically substituted diactivated methylene group and might exist in two or more tautomeric forms.



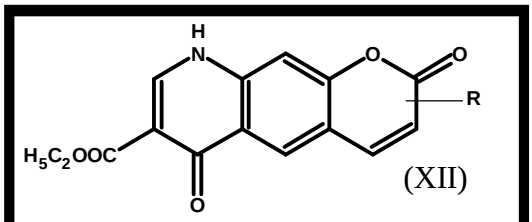
Karemmerer and coworkers[23] prepared substituted N-alkyl-3-nitro -pyrano[3,2-c]quinoline-2-ones (XI) that were tested as antiallergic agents. Thus, N-ethyl-m-chloroaniline was reacted with 2,4-dichlorodiphenyl malonate in tetralin and the product was nitrated to obtain above compounds.



Trkovnik et al.[24] prepared fused pyrano-4-quinolones derivatives (XII). These derivatives were tested for antibacterial against various microorganism like

22. Kepe Vladmir, Kocevar Marijan, and Polanc Slovenko, *Heterocycles*, **41(6),** (1995).

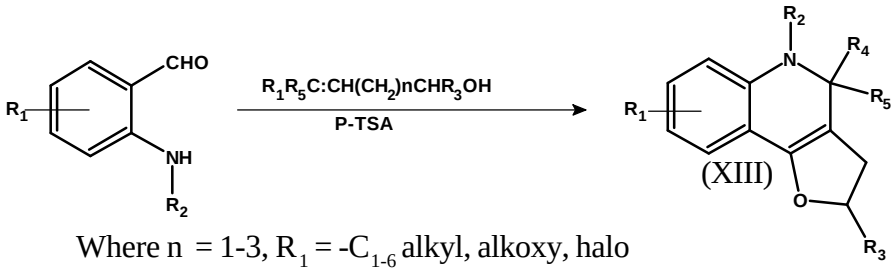
S. aureus, *E. faecalis*, *P. aenrgiizosa*, *Streptococcus pyogens*, *Streptococcus faecalis*, *Staphylococcus aureus*, *Staphylococcus epiderms* etc.



(XII)

Where R = -H, OH, $-CH_3$

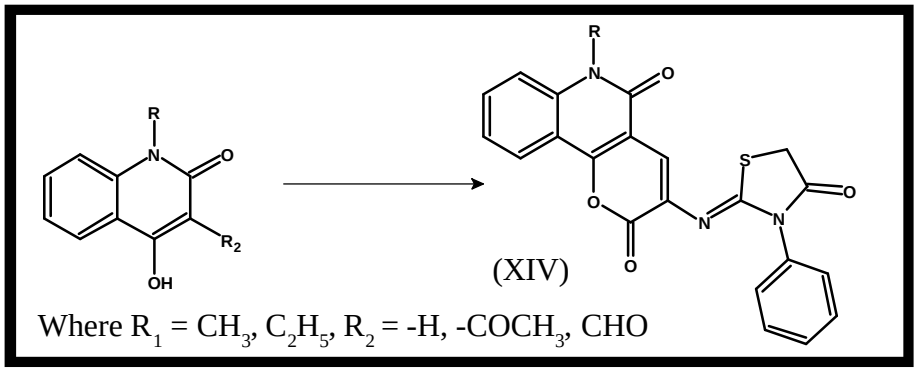
Masotoshi et al.[25] have reported pyranoquinolines and furoquinolines derivatives(XIII) for antibacterial agents against methicillin resistant *Staphylococcus aureus*. These derivatives were prepared by treatment of o-amino benzaldehydes with $R_4R_5C:(CH_2)_nCHR_3OH$ in the presence of acid catalysts and alkyl orthoformates.



(XIII)

Where n = 1-3, R_1 = $-C_{1-6}$ alkyl, alkoxy, halo
R_2= $-R_6SO_2$, R_6 = $-C_{1-6}$alkyl, C_{1-10}aryl
R_3= $-C_{1-6}$ alkyl, R_4, R_5 = $-C_{1-6}$alkyl, C_{6-10} aryl, heterocyclyl

El-Taweel and Ibrahim[26] synthesized several polysubstituted pyrano[3,2-c]quinolones from readily available 4-hydroxy-2-quinolinones. These compounds were evaluted for bactericidal activity against Gram negative and Gram positive bacteria.

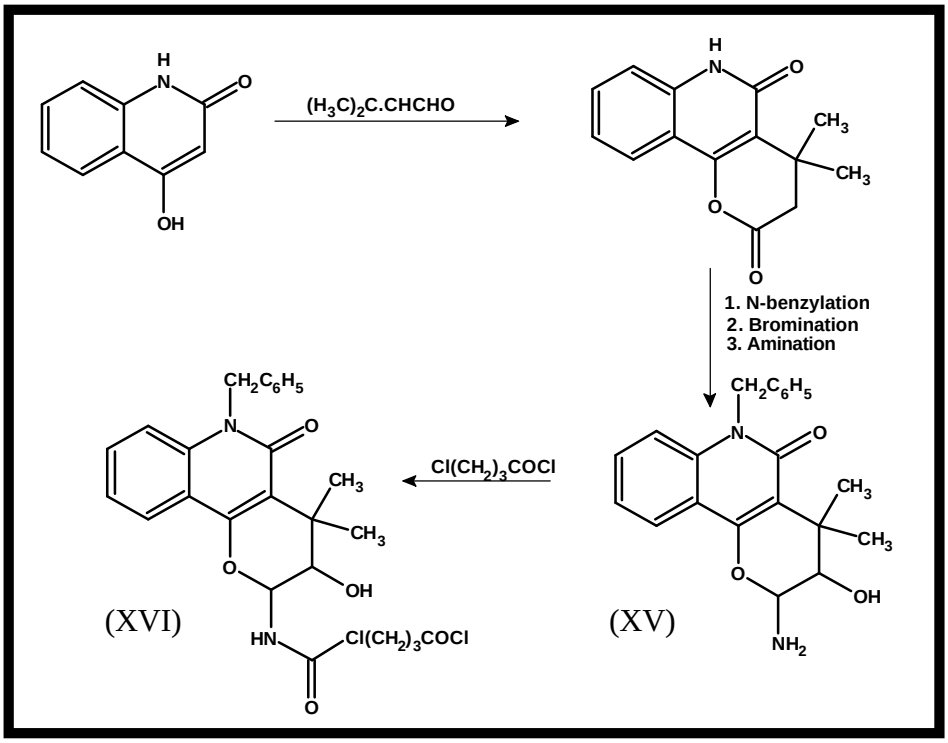


(XIV)

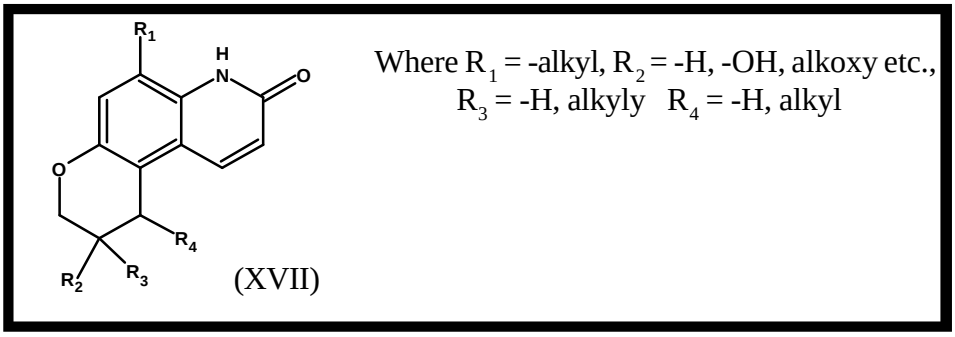
Where R_1 = CH_3, C_2H_5, R_2 = -H, $-COCH_3$, CHO

23. Karemmerer F. J., Ulrich G., Alpermann H. G., *Ger. Offen 2*, **836**, 470 (1980).

Atwal Karnail[27] in 1991 have prepared pyrano[3,2-c]quinolin-5-ones as calcium channel blockers. The 4-hydroxy-2-quinolone was cyclocondensed with 3-methyl-2-butenal and the product N-benzylated to give after bromination pyranoquinolinone, which was aminated to give (XV) (R_2= -OH, R_7= -NH_2). It was condensed with 4-chlorobutanoyl chloride to give (XVI).



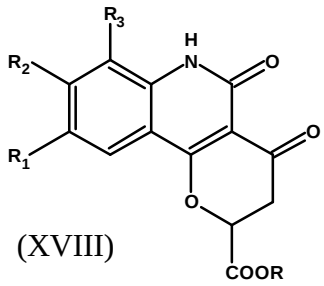
Kyotani and coworkers[28] have reported pyranoquinolines (XVII) as cardiovascular agents. The general structure of the compound is as under,



24. Trkovnik Mladen, Iveric Z., Kelneric Z., **EP 820**, 998 (1998).

25. Masotoshi A., **JP 2002 322**,181 (2002).

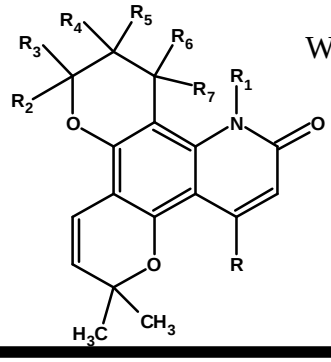
Morinaka et.al[29] had synthesized several ester derivatives of quinolopyran-4-one, 2-carboxylic acid (XVIII). These novel compouds are especially useful as antiallergic and for treatment of asthma.



(XVIII)

Where R = alkyl, sub. alkyl
R1, R2, R3, = H, alkyl, sub. alkyl, alkoxy, aryl, halo etc.

Gurjar and coworkers[30] have reported calanolide A analogs(XIX) as an anti-HIV agents, these analogs have pyranoquinolone skeleton.



(XIX)

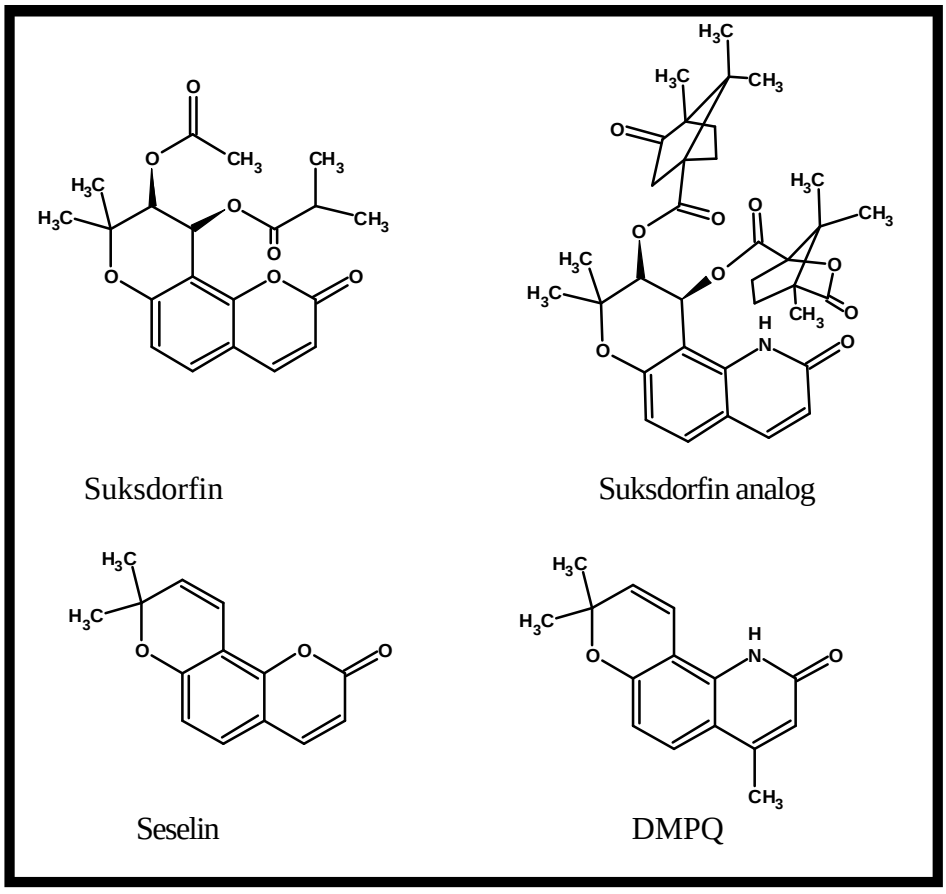
Where R = CH_3, R_1 = H or Sub. aryl
R_2- R_5 = H or CH_3
R_6, R_7 = -H, OH, aryloxy

Kuo-Hsiung Lee and coworkers[31] have synthesized 3',4'-di-O-(-)-camphanoyl-(+)-cis-Khellactone(DCK) lactam analogs. These analogs were prepared by doing bioisosteric replacement in Suksdorfin derivative. Suksdorfin isolated from the fruit of Lomatium suksdorfil. It is a Khellactone which possesses anti-HIV activity. Modification of khellactone yielded 3',4'-di-O-(-)-camphanoyl-(+)-cis-Khellactone. This compound has very potent anti-HIV activity in acutely infected H9 lymphocytes with an EC_{50} value of 0.00024 mM and a therapeutic index of 119, 333 compound was about

26. **El-Taweel F.M.A., Ibrahim D.A.,** *Bollettino Chimico. Farmaceutico*, **140(5)**, 287-296 (2001).
27. Karnail Atwal, **US 5**,070,088 (1991).

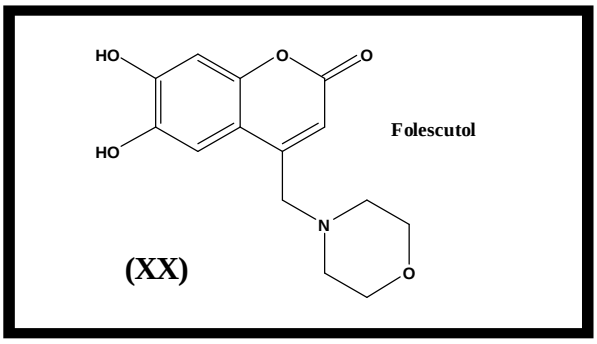
225 fold more active than AZT as comparing their EC_{50} values in assay.

Kuo-Hsiung and coworkers[32] reported Seselin analogs which are pyrano[6,5-h]quinoline-2-ones derivatives. Seselin an angular pyranocoumarin displays various biological activities including antifungal and anti-HIV activities. In particular, Seselin exhibited moderate cytotoxicity in a mechanism based anticancer bioassay employing DNA repair deficient and repair proficient yeasts. The bioisosteres of Seselin were prepared by replacing oxygen atom of the pyranocoumarin nucleus by nitrogen atom. These compounds were evaluated for their in vitro cytotoxicity against a panel of human tumor cell lines. The most active compound showed significant cytotoxic activity with GI_{50} values in the micromolar range.



28. Kyotani Y., Tsutomu T., Juji K., **JP 05**, 310,744 (1993).

Folescutol(XX), which posses a morpholine ring attached to coumarin via -CH_2- link proved to be a capillary protectant drug.[33]



(XX)

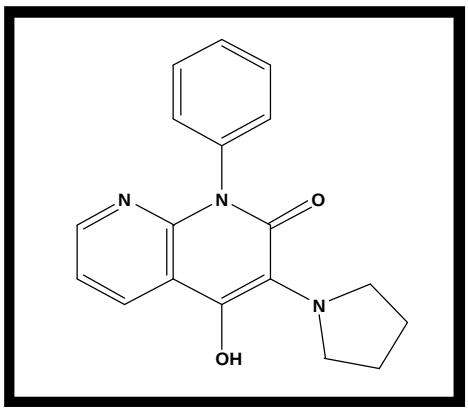
NEW DRUG MOLECULES UNDER CLINICAL STUDY:

In recent years, many new drug molecules which are under study from phase-I to Phase-IV clinical trials for different pharmacological action have shown that the basic characteristic of aryl amine behave as hidden amine and has attracted many medicinal chemists to incorporate this feature in drug design.

Few such examples are as under:

Drug Name : Pirodomas[35]

Chemical Structure :



Chemical Name : 4-Hydroxy-1-phenyl-3-(1-pyrrolidinyl)-1,8-naphthyridin-2(1H)-one

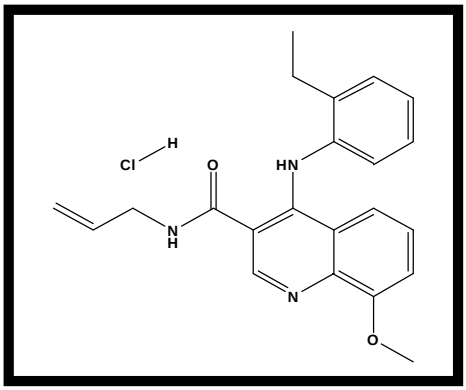
Phase : Phase II

Activity : Antiallergy/Antiasthamatic Drug

29. Morinaka Y., Takahashi K., **US 4,** 298, 610 (1981).

Drug Name : 138586-41-1(CAS No.)[36]

Chemical Structure :



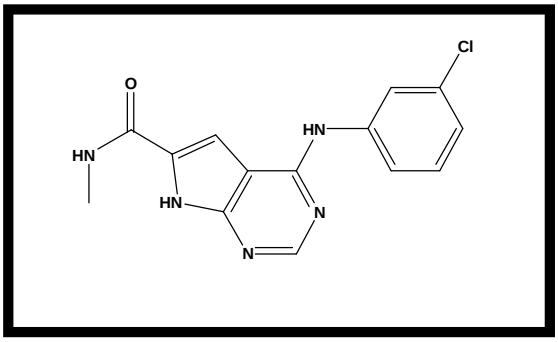
Chemical Name : N-Allyl-4-(2-ethylphenylamino)-8-thoxyquinoline-3-carboxamide hydrochloride

Phase : Preclinical

Activity : Antiulcer Drug

Drug Name : CGP-74321(Company Code, Novartis)[39]

Chemical Structure :



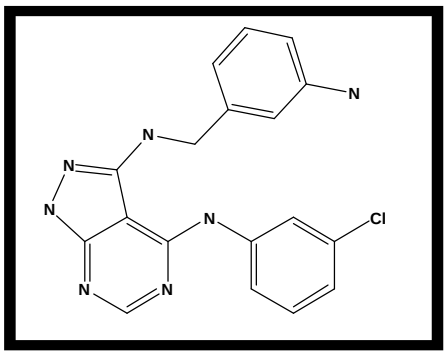
Chemical Name : 4-(3-Chlorophenylamino)-N-methylyrrolo yrrolo [2,3- d]pyrimidine-6-carboxamide

Phase : Biological Testing

Activity : Oncolytic Drug

Drug Name : CGP-76627(Company Code, Novartis)[40]

Chemical Structure



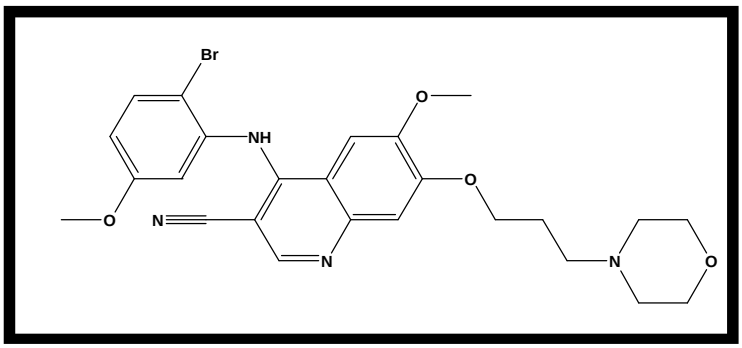
Chemical Name : N^3-(3-Aminobenzyl)-N^4-(3-chlorophenyl)-1H-pyrazolo[3,4-d]pyrimidine-3,4- diamine

Phase : Biological Testing

Activity : Oncolytic Drug

Drug Name : Not Reported[41,42]

Chemical Structure :



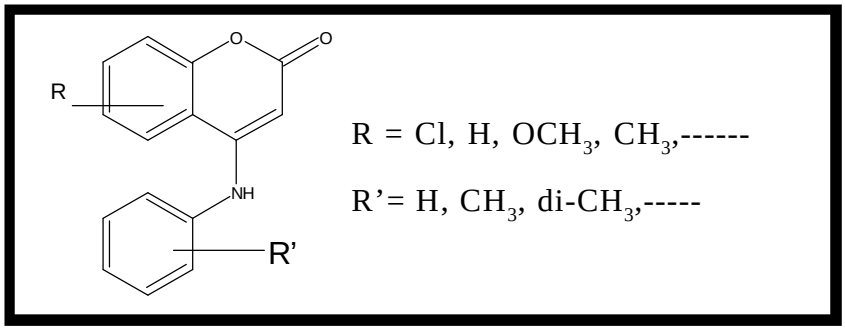
Chemical Name : 4-(2-Bromo-5-methoxyphenylamino)-6-methoxy-7-[3-(4-morpholinyl)propoxy] quinoline-3-carbonitrile

Phase : Biological Testing

Activity : Oncolytic Drug

SYNTHETIC ASPECTS:

Shah and Vora[43] synthesized many 4-aryl amino coumarins and studied their anti-HIV activity. All the target compounds were tested against the replication of HIV-1 (III-B)[44] and HIV-2 (ROD)[45] at subtoxic concentration in acutely infected MT-4 cell lines.[46,47]



The tested compounds were found to be very less active. The poor activity may be attributed to the poor solubility of the compounds. From the activity data of the above compounds, it may concluded that the substitutions present on the coumarin skeleton did not play any important role in anti-HIV activity. These results help us to desing new set of synthetic molecules possessing improved solublity and with basic structue modification required for anti-HIV acitivity.

The compounds were studeid for the antituberculosis activity at TAACF. Many compounds were synthesized for obtaining better structure-activity reationship.

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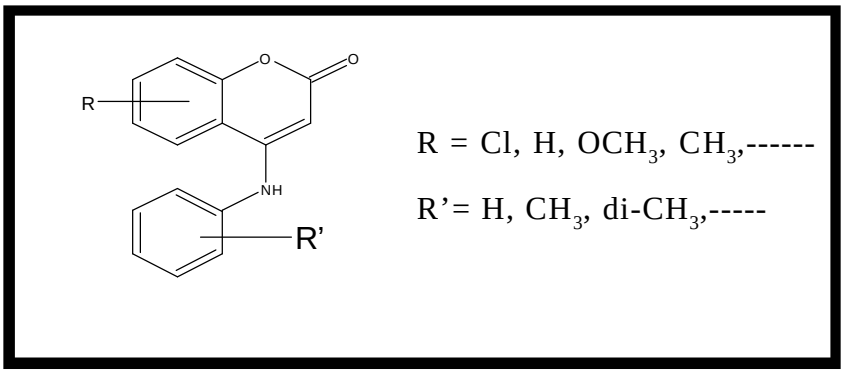
30. Gurjar M. K., Sharma G. V. M., Illangovan A., Narayanan V., **US 6**,191,279 (2001).

31. Yang Zheng-Yu, Xia Yi, Xia Peng, Yoko Tachibana, Kenneth F. Bastow and Lee. Kuo-Hsiung, *Bioorg. Med.Chem.Lett.*, **9**, 713-716 (1999).

32. Yang Zheng-Yu, Xia Yi, Xia Peng, Brossi Arnold, Cosentino L. M., and Lee KuoHsiung, *Bioorg. Med.Chem. Lett.*, **10,** 1003-1005 (2000).

WORK DONE FROM THIS LABORATORY:



No.	R	R'	Reference
1	Different 4-chloro-coumarins	Different aromatic amines	48
2	5,6-benzo-4-chloro coumarins	Different aromatic amines	49
3	Different 4-hydroxycoumarins	Different aromatic amines	50
4	Different 4-hydroxycoumarins	3-choloro-4fluoroanilin-e,4-methoxyaniline, 3-choloraniline	51
5	Different 4-hydroxycoumarins	4-fluoroaniline, cyclohexylamine, 4-morpholine	52

33. Tarayre J.P., et al., *Anna Pharm, France*, **33**, 467 (1975).

34. Rampa A., and Carrara M.; *Drug Fut*, **012** (01), 22 (1987).

35. Blythin D.J., and Gala D.; *Drugs Fut*, **16** (12): 1099 (1991).

36. Uchida M., Morita S., Otsubo K.,Shimizu T., and Yamasaki K, **US 5215999**, June 1, 1993 *Drug Data Rep* 1995, **17 (10)**: 919.

38. Fischer R.W., and Misun M.; *Org Process Res Dev* , **5(6):** 581 (2001).

39. *Drug Data Rep* 1998, **20** (5): 444.

The present chapter aims at the bio active properties of pyranoquinolines, if appended with the arylamine residue at C_4 position of the pyranoquinoline skeleton, will give novel compounds which were not studied earlier for their pharmacological profile.

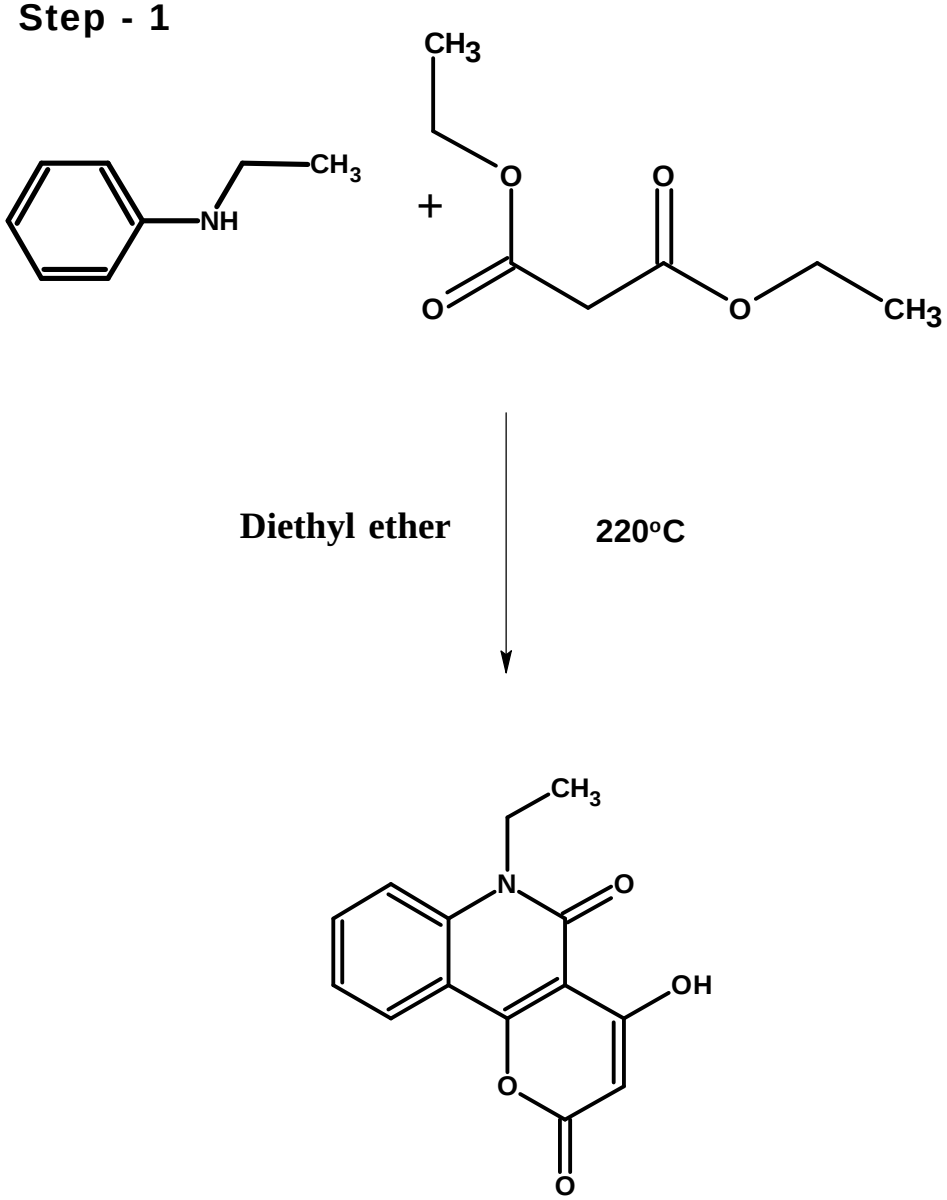
Thus, the starting material was prepared form N-ethyl aniline to obtain 6-ethyl-4-hydroxy-2*H*-pyrano[3,2-*c*]quinolin-2,5(6*H*)-dione which on furthur treatment with various amines at high temperature afforded a series of congeners mentioned in subsequent pages.

(Reaction Scheme - 2)

40. *Drug Data Rep* , **20**(5), 444 (1998)
41. *Drug Data Rep* , **23**(7), 710 (2001)
42. Wang Y.D., Boschelli D.H., and Ye F. ; *221st ACS Natl Meet* (April 1-5, San Diego) 2001, Abst MEDI 144.
43. *Vipul Vora, Ph.D Thesis, Saurashtra University, Rajkot (2000).*
44. Popovic M.M. G., Read S.E., and Gallo R.C., Detection, Isolation and continous production of cytopathic retroviruses(HTLV-III) from patients with AIDS and pre-AIDS. *Science*, **224**, 497-500 (1984).
45. Clavel F., Guyader M., Guetard D., Salle M., Montagnier L., and Alizon H., Molecular cloning and polymorphosm of the human immunodeficiency virus type2. *Nature*, **324**, 691-695 (1986).
46. Pauwels R., Clercq E. De., Desmyter J., Balzarini J., Goubau P., Herdewijn P., Vanderhaeghe H., and Vandeputte M., Sensitive and rapid assay on MT-4 cells for the detection of antiviral compounds against the AIDS virus., *J. Virol. Methods*, **16**, 171-185 (1987).
47. Schols D., Bada M., Pauwels R., Desmyter J., and Clercq E De., Specific inter action of aurintri-caboxylic acid with the human Immunodeficiency/CD4 cell recepter., *Proc. Natl. Acad. Sci*. USA, **86**, 3322-3326 (1989).
48. Bhatt N.S., *Ph. D Thesis*, Saurashtra University, Rajkot (1983).
49. Thaker D., *Ph. D Thesis*, Saurashtra University, Rajkot (1996).
50. Vipul Vora, *Ph.D Thesis*, Saurashtra University, Rajkot (2000).
51. Desai B., *Ph. D Thesis*, Saurashtra University, Rajkot (2000).
52. Loriya R., *Ph. D Thesis*, Saurashtra University, Rajkot (2002).

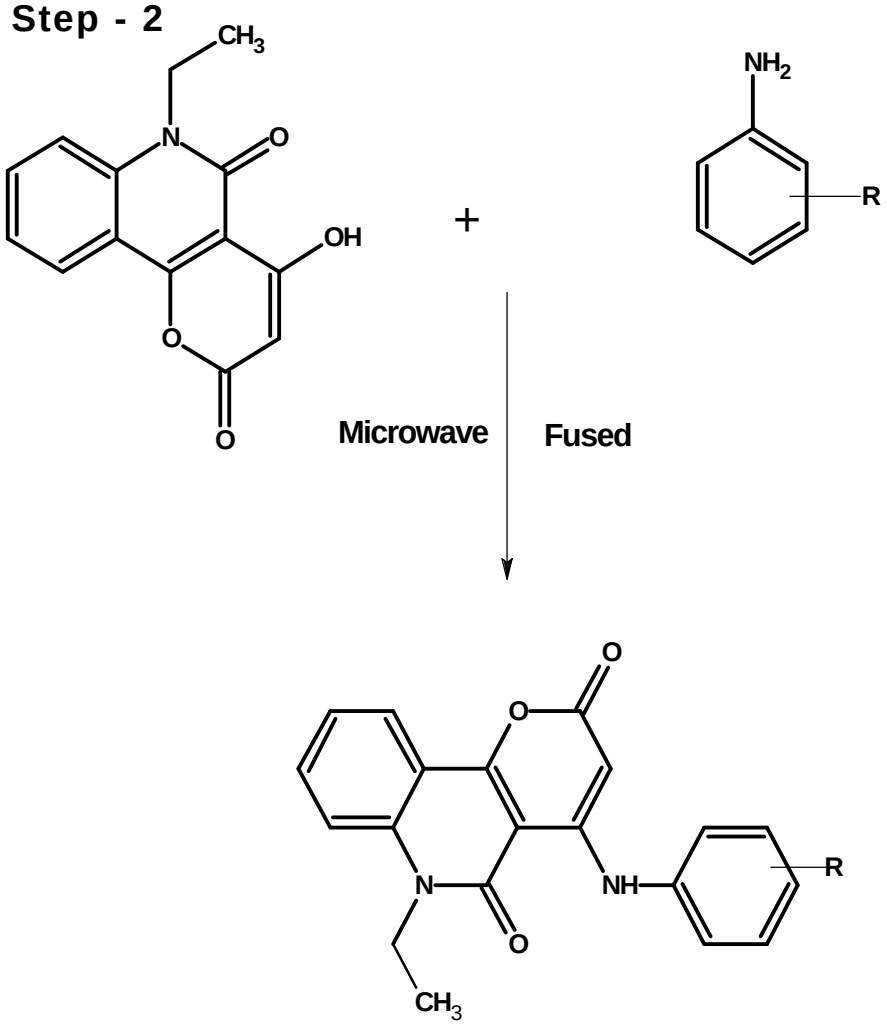
REACTION SCHEME : 2

Step - 1



REACTION SCHEME : 2

Step - 2



Where R = H, CH_3, F, Cl, etc.....

EXPERIMENTAL :

Peparation of 6-ethyl-4-hydroxy-2*H*-pyrano[3,2-*c*]quinoline-2,5(6*H*)-dione:

A mixture of N-ethylaniline(12.1gm 0.1mole) and diethyl malonate(32.0gm 0.2 mole) was taken in the three necked round bottom flask and to this mixture, diphenyl ether (40ml) was added. The reaction mixture was heated to 220^{o}C for 12hrs. During the reaction liberated ethanol was collected by distillation. After the completion of the reaction the product was isolated by addition of 1,4 Dioxane and it was washed with diethyl ether.The product was recrystalized in ethanol.

Elemental Analysis

Calculated = C (65.37%) H(4.31%) N(5.44)

Experimental = C (65.41%) H(4.27%) N(5.41)

Molecular formula = $C_{14}H_{11}NO_4$

Formula Weight = **257.24**

M.P. = **255-258^{o}C**.

TLC System = (Ethyl acetate: Hexane : 4: 6)

Yield = **52-65%**

Peparation of 4-[(4-chlorophenyl)amino]-6-ethyl-2*H*-pyrano[3,2-*c*]quinoline-2,5(6*H*)-dione.

6-ethyl-4-hydroxy-2H-pyrano[3,2-c]quinoline-2,5(6H)-dione (0.01mole) was taken in the flask and 4-chloroaniline (0.01mole) is addded and put it in the microwave oven for 4-6 minutes. The resultant mass was treated with methanol and product was filterd . The product was dried and recrystallized from DMF in hot air oven. The yield recorded between 35-40%. Light-yellow amorphous product was obtained after crystallization.

Elemental Analysis

Calculated = C (65.49%) H(4.12%) N(7.64)

Experimental = C (65.53%) H(4.8%) N(7.59)

Molecular formula = $C_{20}H_{15}Cl\ N_2\ O_3$

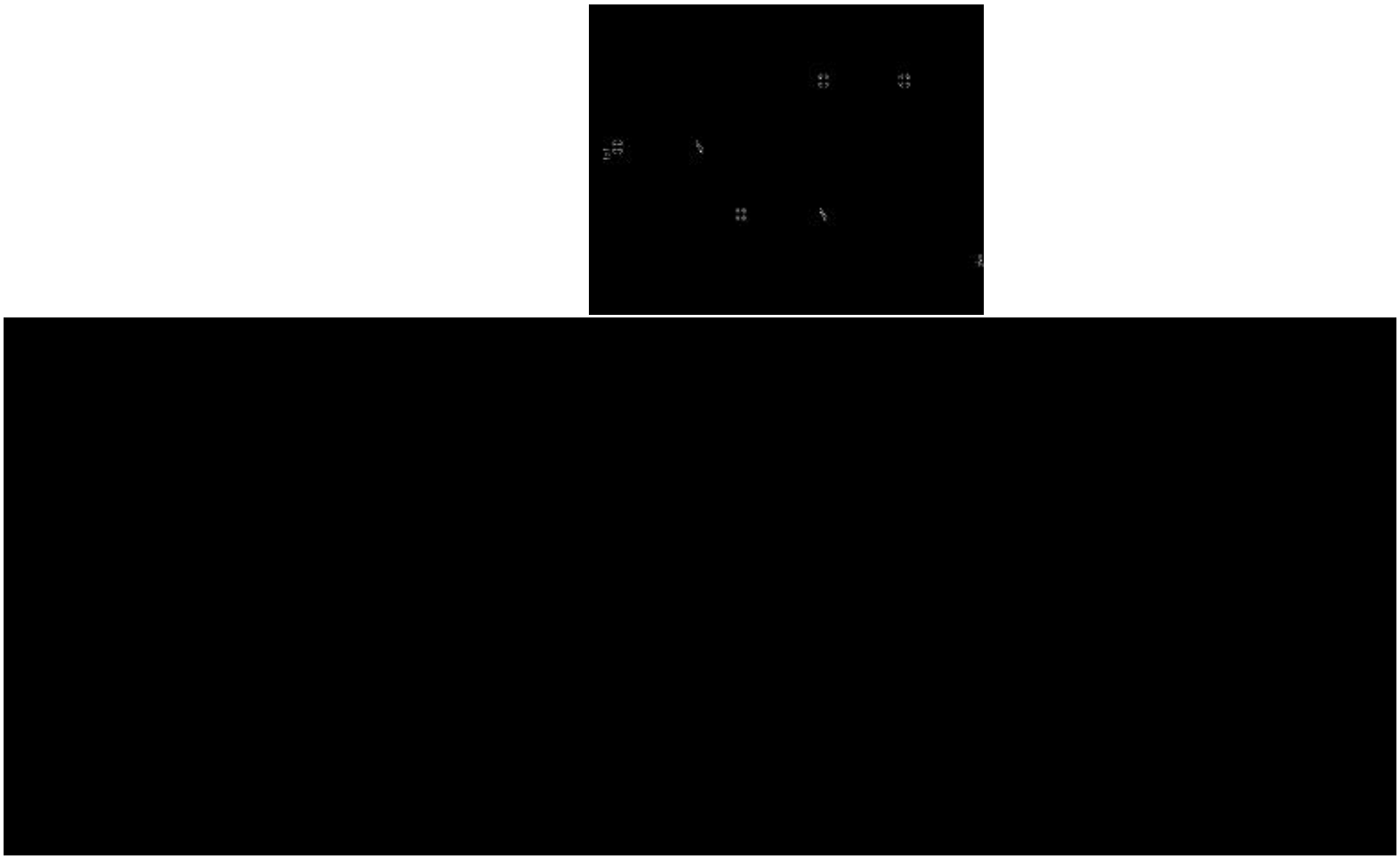
Formula Weight = **366.79**

M.P. = **167-170°C**.

TLC System = (Ethyl acetate: Hexane : 4: 6)

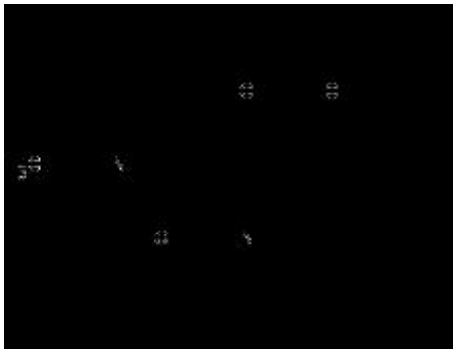
Yield = **18-30%**

Table 2.1 Physical data of 4-anilino-6-ethyl-2*H*-pyrano[3,2-*c*]quinoline-2,5(6*H*)-diones



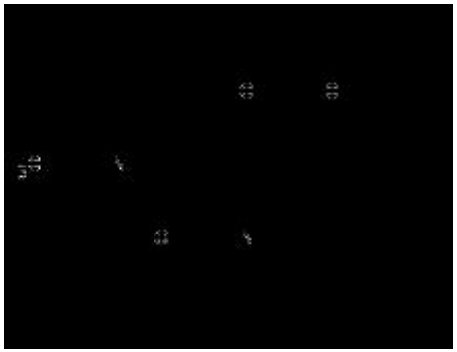
*** Values in parenthesis denotes the calculated % of composition . (d=decompose)**

Table 2.2 Physical data of 4-anilino-6-ethyl-2*H*-pyrano[3,2-*c*]quinoline-2,5(6*H*)-diones (cont.)



*** Values in parenthesis denotes the calculated % of composition .**

Table 2.3 Physcial data of 4-anilino-6-ethyl-2*H*-pyrano[3,2-*c*]quinoline-2,5(6*H*)-diones (cont.)



*** Values in parenthesis denotes the calculated % of composition .**

SPECTRAL STUDY :

The constitutions of newly synthesized compounds were supported by IR, NMR, and LC-MS study. The details are as under.

IR Spectral Study :

Instrument : SHIMADZU FT-IR-8400 Spectrophotometer

Sample technique : KBr pellet

Frequency range : 400-4000 cm^{-1}

It is observed that in the IR spectrum of 4-[(2-Chlorophenyl)Amino]-6-ethyl-2H-pyrano[3,2-c]Quinoline-2,5(6H)-Dione **(ASM-36),** -NH band is observed at 3440.8 cm^{-1}. It was found that both carbonyl (-C-C=O, and -N-C=O-) group seperates at low resolution frequency (at **0.84**) 1738 and 1676 cm^{-1} repectively. Initially, when IR was taken at high resolution (**4.00**), and both carbonyl merged only one carbonyl (-C-C=O-) was seen at 1742 cm^{-1}. The ether (C-O-C) of lactone appeared at 1275 cm^{-1}.

The aromatic moiety and ring skeleton (like C-C multiple bond stretching, C-H i.p. def. and (C-H o.o.p. def.) were observed at 1575,1490, 1442 cm^{-1}. The halogen(C-Cl) was confirmed due a band appeared at 819.7 cm^{-1}. Similar observations are made in all IR spectral data of the compounds of this series. The range of frequencies of othr compounds are reported in Tabular form on page 76 & 77.

^{1}H NMR Spectral Study :-

Instrument : BRUKER AC 300 MHz FT-NMR Spectrometer

Internal reference : TMS

Solvent : $CDCl_3$+DMSO d_6

In ^{1}H NMR study of 6-ethyl--4-[(2,3-dimethylphenyl)amino]-2H-pyrano-[3,2-c]-quinoline-2,5(6H)-dione (**ASM-44**) confirms structure as singlet of two identical methyl(-CH_3)protons is observed at δ 1.59 ppm. Another singlet is observed at δ 5.68 ppm confirms the presece of proton of C_3 position. In the aromatic region, two triplets were observed at δ 7.36 ppm(J=8.7 Hz) and δ 7.82 ppm (J=8.1 Hz), while two doublets are observed at δ 7.55ppm and δ 7.82 ppm.

In ^{1}H NMR Spectra of 4-[(2-Chlorophenyl)amino]-6-Ethyl-2*H*-pyrano[3,2-*c*]Quinoline-2,5(6*H*)-Dione (**ASM-36**), a singlet at δ 5.68 ppm confirms presence of C_3 proton of coumarin ring. A singlet of -NH proton is observed at d 7.26 ppm in the downfield due to substitution of chlorine at C_{25} position. A triplet and quartet pattern for the ethyl group at (C_{24} & C_{25}) is observed at d 1.43 and 4.45 ppm. The value for the δ obtained for ethyl group is higher, can be explained by the attatchment of ethyl group at N atom. Moreover the aromatic ring skeleton are observed as multiplet at δ 7.43-7.50 ppm ($CH_{12,13,21,20}$) with coupling constant (J=8.7 Hz). A triplet is observed for the protons of $C_{11,14}$ at 7.799 δppm with (J=8.7 Hz.).Similar observations are seen in ^{1}H NMR spectra of other derivatives prepared.

The compounds, 4-[(2-Chlorophenyl)Amino]-6-ethyl-2H-Pyrano[3,2-c]Quinolin-2,5 (6H)-Dione **(ASM-36)** and 4-[(2,3-dimethyl phenyl)Amino]-6-ethyl-2*H*-Pyrano[3,2-*c*]Quinoline-2,5(6*H*)-12345Dione **(ASM-44)** are also confimed by ^{13}C NMR and Mass spectra.

Mass Spectral Study :-

Instrument : VG 70-S (70eV) Spectrograph for El

Instrument : JEOL SX 102/DA-6000 Spectrograph for FAB

The molecular ion peak is concormitant with the molecular weight. The newly synthesized compounds were subjected to FAB Mass study. The Fast bombardment study revealed the Molecular ion peak, base peaks and other relevant fragmentation pattern to confirm the structure of the molecules.

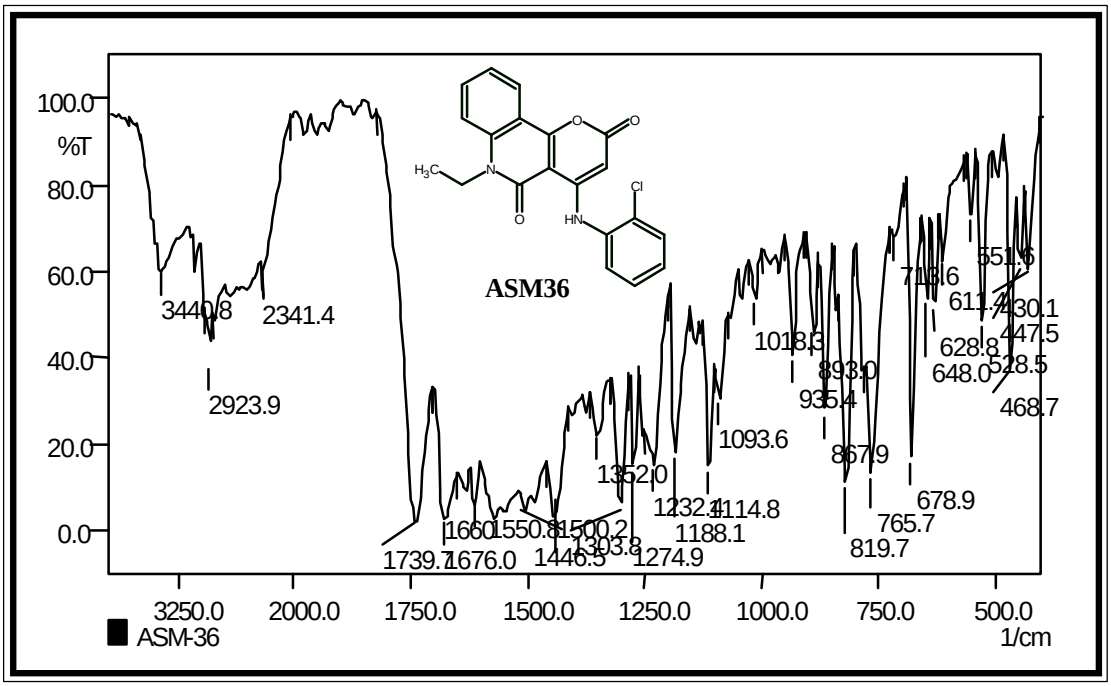
In the Fab Mass of 6-Ethyl-4-[(2,3-dimethylphenyl)amino]-2H-pyrano[3,2-c]quinoline-2,5(6H)-dione **(ASM-44),** base peak is observed at 361.0 m/z, while molecular ion peak is also at 361.00 m/z as (M+1) peak, while in (ASM-40) structure is confirmed by base peak at 258.00 m/z and 347.00 m/z (M+1) peak.

The details of other FAB-Mass spectra are recorded.

^{13}C NMR :

The compound ASM-36 was subjected for ^{13}C NMR spctroscopy and the results of these data shows the predicted sturcture is in agreement with the carbon skeleton of respective compound.

IR Spectrum of 6-ethyl-4-[(2-chlorophenyl)amino]-2*H*-pyrano[3,2-*c*]quinoline-2,5(6*H*)-dione(ASM-36)



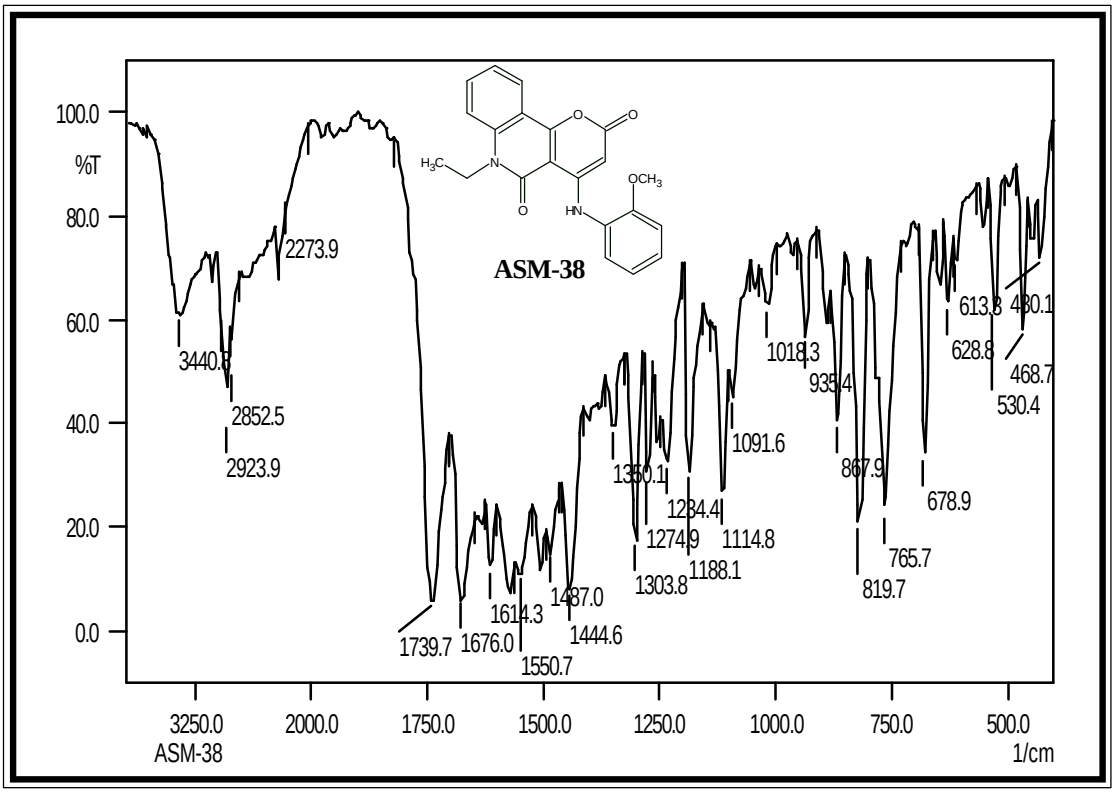
Instrument : SHIMADZU FT IR-8400

Sample technique : KBr Pellet

Frequency range : 4000-400 cm^{-1}

Type	Vibration mode	Frequency cm^{-1}
Carbonyl	-C-C=O & -N-C=O	1739.7 & 1676
Amine	-N-H Str.	3440.8
Aromatic	ring skeleton vib.	1660,1550, 1500,1446
	o.o.p.bending vib. (1,2,3-tri sub.)	765.7 819.7
Halogen	C-Cl str.	1114.8
Alkyl	$-CH_3$	1352.0

IR Spectrum of 6-ethyl-4-[(2-methoxyphenyl)amino]-2*H*-pyrano[3,2-*c*]quinoline-2,5(6*H*)-dione(ASM-38)



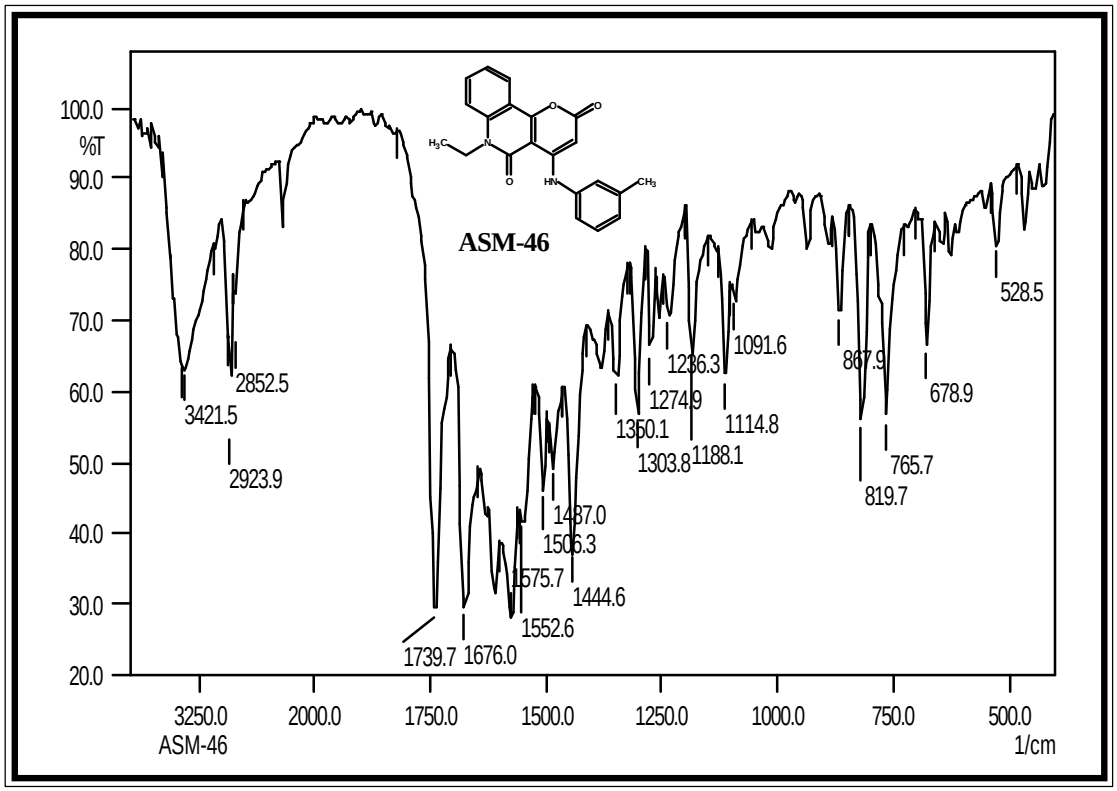
Instrument : SHIMADZU FT IR-8400

Sample technique : KBr Pellet

Frequency range : 4000-400 cm^{-1}

Type	Vibration mode	Frequency cm^{-1}
Carbonyl	-C-C=O & -N-C=O	1739.7 & 1676
Amine	-N-H Str.	3440
Aromatic	ring skeleton vib.	1550 1488 1444
	o.o.p.bending vib. (1,2,3-tri sub.)	765 819
Alkyl	$-CH_3$ str.	1350
Ether	C-O-C	1274

IR Spectrum of 6-ethyl-4-[(3-methylphenyl)amino]-2*H*-pyrano[3,2-*c*]quinoline-2,5(6*H*)-dione(ASM-46)



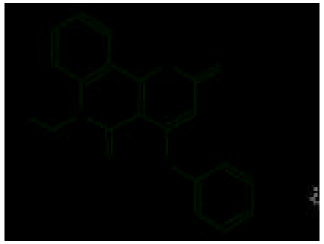
Instrument : SHIMADZU FT IR-8400

Sample technique : KBr Pellet

Frequency range : 4000-400 cm^{-1}

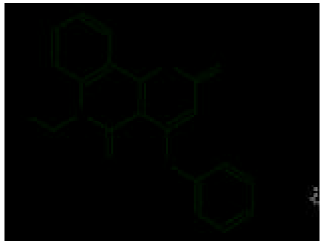
Type	Vibration mode	Frequency cm^{-1}
Carbonyl	-C-C=O &-N-C=O	1739.7 & 1676
Amine	-N-H Str.	3421.5
Aromatic	ring skeleton vib.	1575 1552 1487 1444
	o.o.p.bending vib. (1,2,3-tri sub.)	819 867
Alkyl	$-CH_3$ str.	1350

Table 2.4 IR frequency data of newly syntehsized 6-ethyl-4-[(substituted phenyl) amino]-2*H*-pyrano[3,2-*c*]quinoline-2,5(6*H*)-diones (ASM series).



[illegible]	[illegible]	[illegible]	[illegible]	[illegible]			[illegible]	[illegible]
ASM 30	1738	1678	3118	1553	1180	1117		1233
ASM 31	1738	1668	3112	1550	1111	1111		1232
ASM 32	1770	1681	3110	1578	1188	1119	768	1231
ASM 33	1738	1678	3162	1552	1181	1117	756 1111	1228 1231
ASM 34	1736	1678	3138	1561	1182	1116	1181	1231
ASM 35	1733	1678	3111	1552	1182	1112	1111	1238

Table 2.5 IR frequency data of newly syntehsized 6-ethyl-4-[(substituted phenyl) amino]-2*H*-pyrano[3,2-*c*]quinoline-2,5(6*H*)-diones (ASM series).



[illegible]	[illegible]	[illegible]	[illegible]	[illegible]			[illegible]	[illegible]
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[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]		[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]		[illegible]
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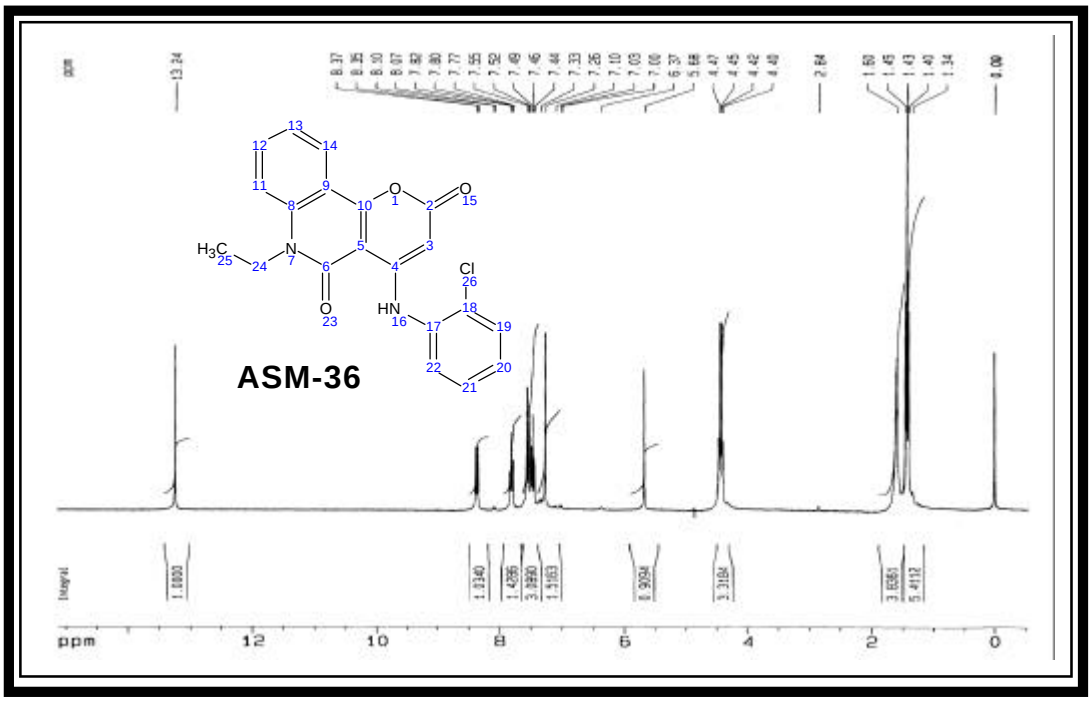
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]		[illegible]

^{1}H NMR Spectra of 6-ethyl-4-[(2-chlorophenyl)amino]-2*H*-pyrano[3,2-*c*]quinoline-2,5(6*H*)-dione(ASM-36)



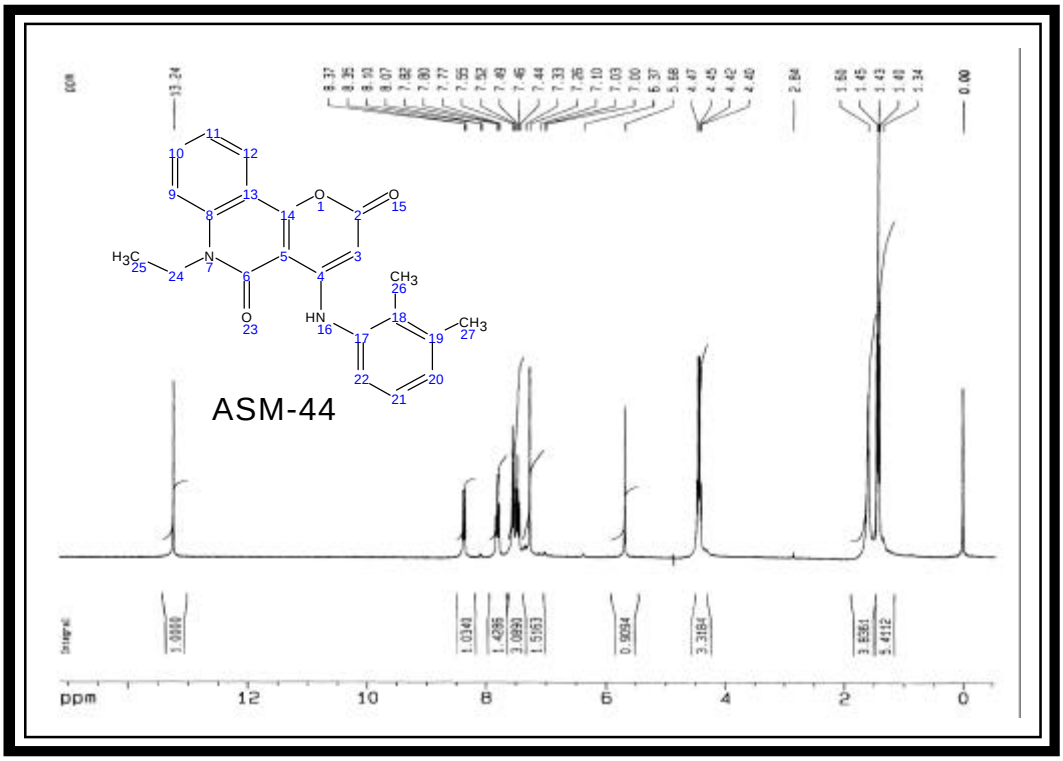
Instrument : BRUKER AC 300 MHz FT-NMR

Standard : TMS

Solvent : $CDCl_3$+ DMSO d_6

Chemical Shift d ppm	No. of Proton	Muliplicity	Inference	J. Value
1.43	3H	Triplet	CH(25)	-
4.45	2H	Quartet	CH(24)	-
5.68	1H	Singlet	CH(3)	-
7.26	1H	Singlet	NH(16)	-
7.43-7.50	4H	Multiplet	CH(12,13) CH(21,20)	8.7
7.799	2H	Triplet	CH(11,14)	8.7
8.35-8.37	2H	Doublet	CH(19,12)	8.1

^{1}H NMR Spectra of 6-ethyl-4-[(2,3-dimethylphenyl)amino]-2*H*-pyrano[3,2-*c*]quinoline-2,5(6*H*)-dione(ASM-44)



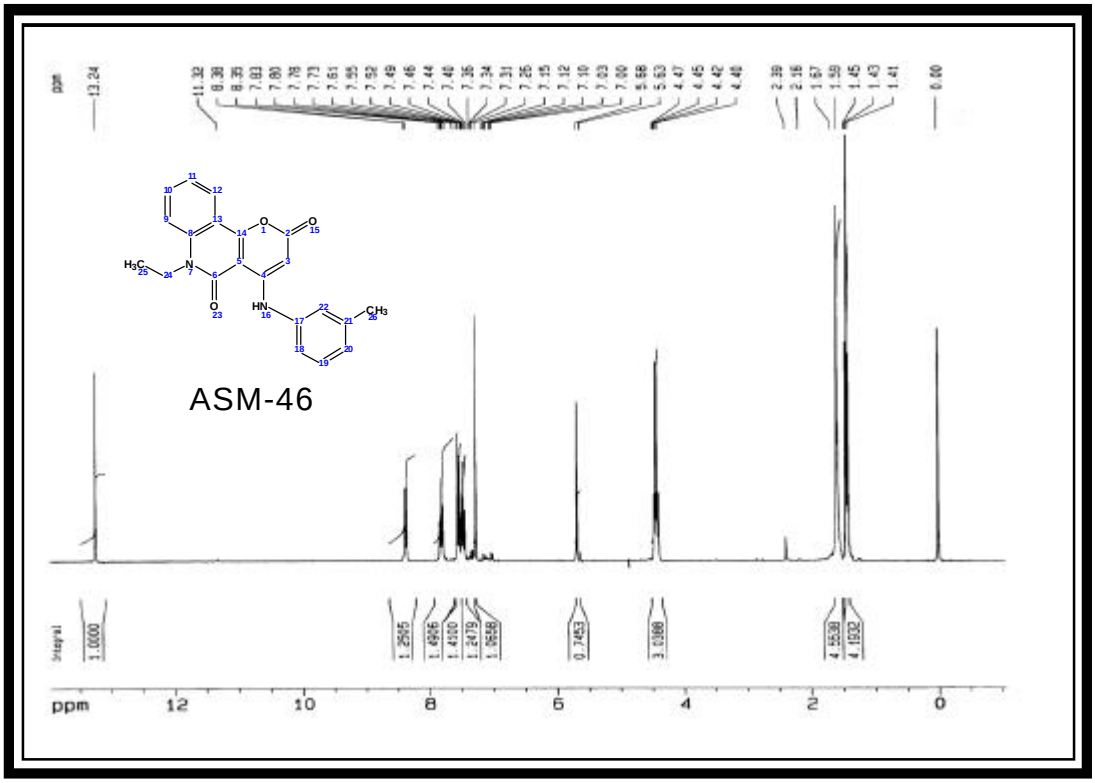
Instrument : BRUKER AC 300 MHz FT-NMR

Standard : TMS

Solvent : $CDCl_3$+ DMSO d_6

Chemical Shift d ppm	No. of Proton	Muliplicity	Interence	J. Value
1.43	3H	Triplet	CH(25)	-
1.59	6H	Singlet	CH(26,27)	-
4.47	2H	Quartet	CH(24)	-
5.68	1H	Singlet	CH(3)	-
7.26	1H	Singlet	NH(16)	-
7.36	1H	Triplet	CH(21)	8.7
7.55	1H	Doublet	CH(10,11)	
7.77-7.82	2H	Triplet	CH(20,22)	
8.37	2H	Doublet	CH(9,12)	8.1

^{1}H NMR Spectra of 6-ethyl-4-[(3-methylphenyl)amino]-2*H*-pyrano[3,2-*c*]quinoline-2,5(6*H*)-dione(ASM-46)



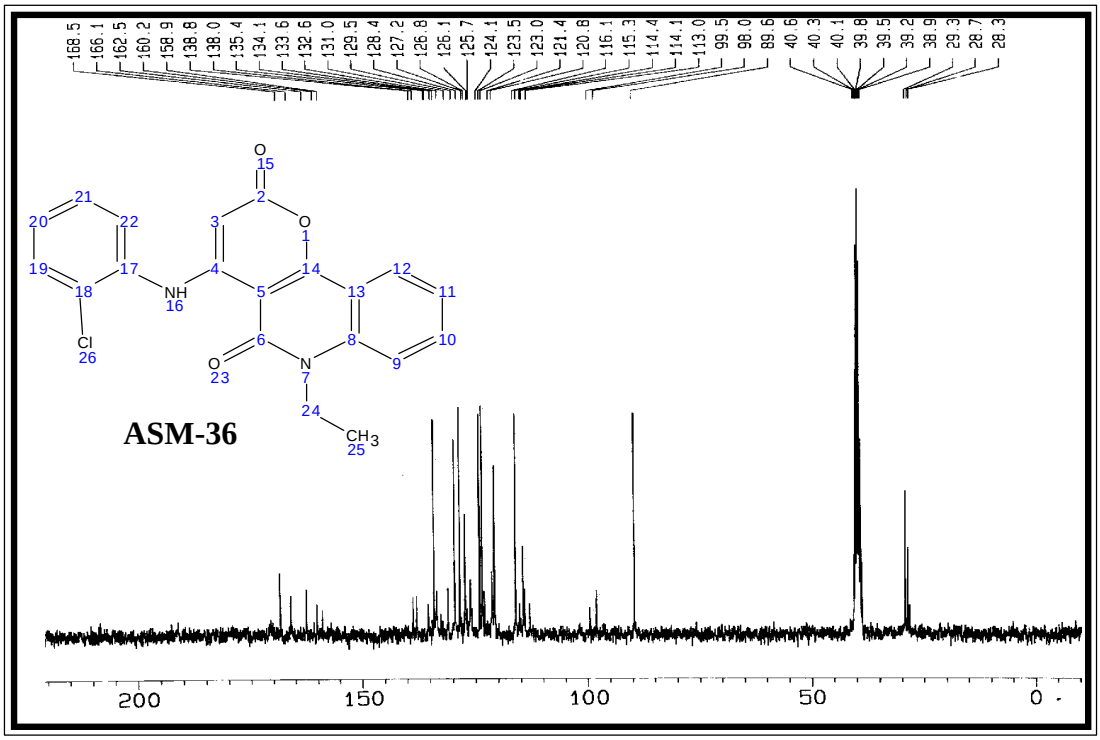
Instrument : BRUKER AC 300 MHz FT-NMR

Standard : TMS

Solvent : $CDCl_3$+ DMSO d_6

Chemical Shift d ppm	No. of Proton	Muliplicity	Interence	J. Value
1.41	3H	Triplet	CH(25)	-
1.51	3H	Singlet	CH(26)	-
4.45	2H	Quartet	CH(24)	-
5.68	1H	Singlet	CH(3)	-
7.26	1H	Singlet	NH(16)	-
7.40	2H	Triplet	CH(18,19)	-
7.51-7.61	2H	Doublet	CH(10,11)	8.7
7.72-7.82	2H	Triplet	CH(20,22)	8.7
8.35-8.37	2H	Doublet	CH(9,12)	8.1

^{13}C NMR Spectral study of 4-[(2-CHLOROPHENYL)AMINO]-6-ETHYL-2*H*-PYRANO[3,2-*C*]QUINOLINE-2,5(6*H*)-DIONE (ASM-36)

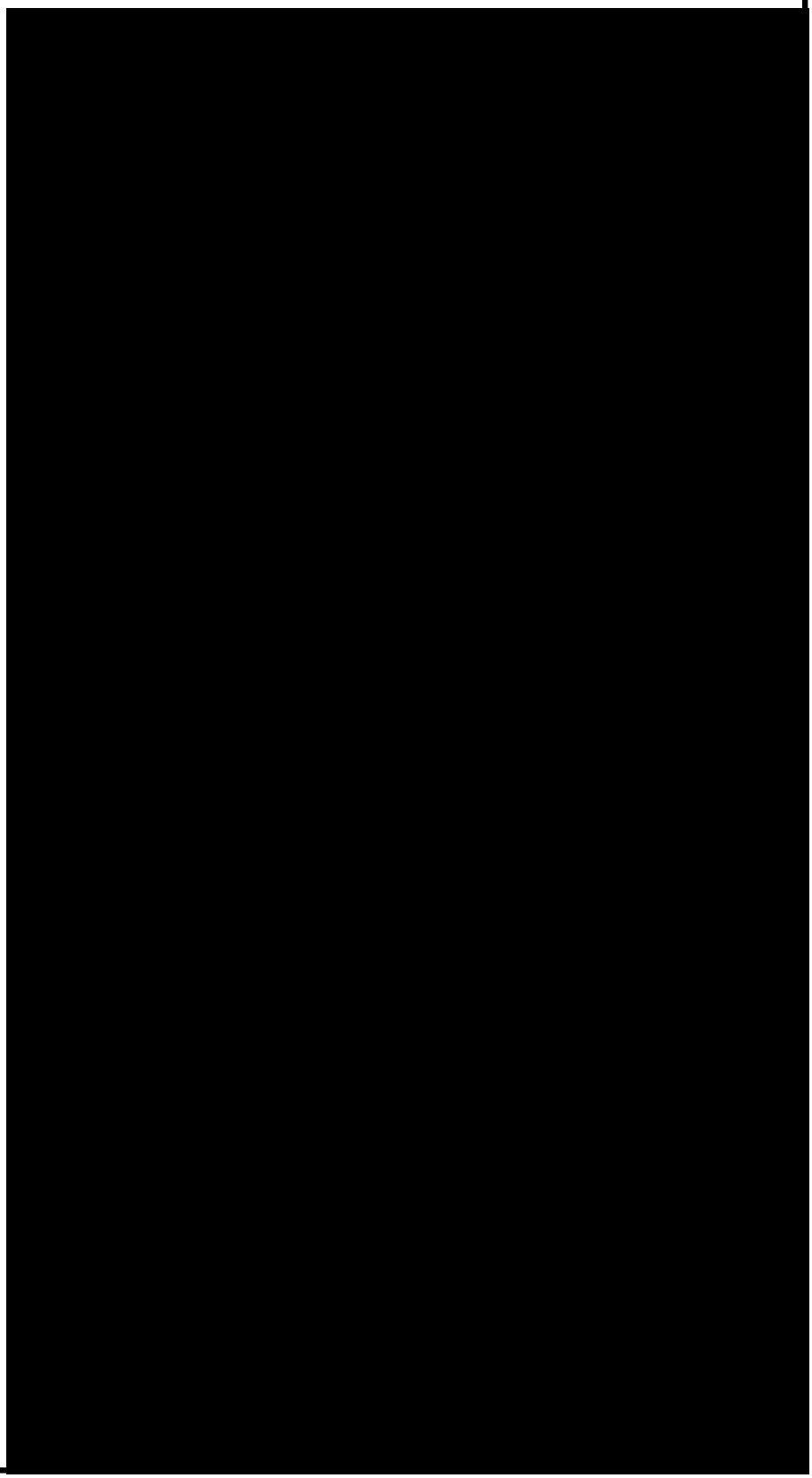


Instrument :BRUKER AC 300 MHz FT-NMR

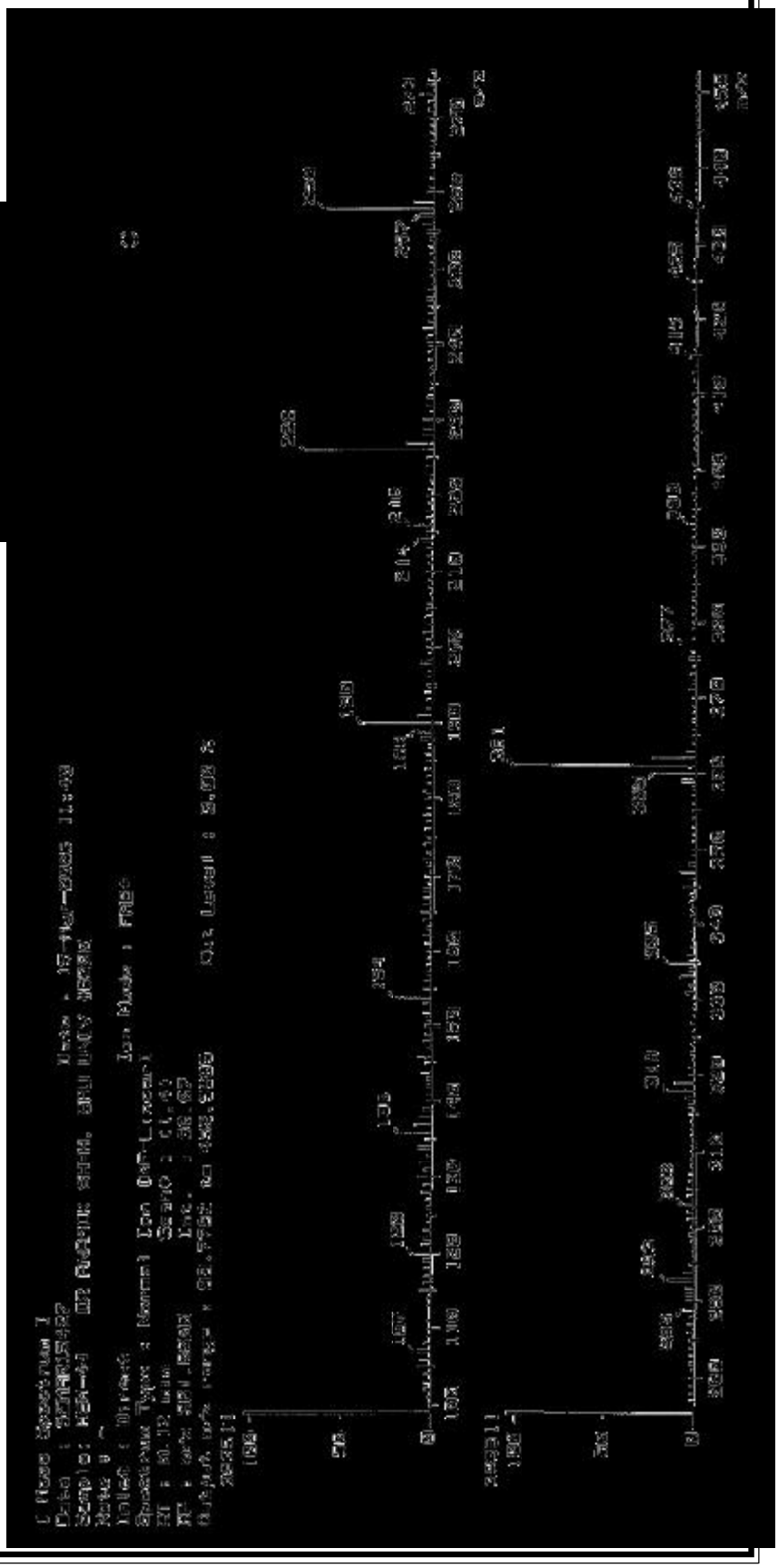
Solvent : DMSO- d_6

δ PPM	No. of carbons	Inference
28.7	1C	-CH_3(13)
89.6	1C	-CH(12)
98.0	1C	-C(9)
114.1	1C	-C(3)
115.3	2C	-CH(15,16)
116.1	1C	-C(2)
120.8	1C	-CH(6,)
123.0	1C	-CH(4)
121.4	1C	-CH(19)
129.5	2C	-CH(17,18)
134.6	1C	-C(5)
135.4	1C	-C(1)
138.8	1C	-C(14)
158.8	1C	-C=O(10)
162.5	1C	-C(7)
168.5	1C	-C(8)

Fab Mass Spectra of 6-ethyl-4-[(2-methylphenyl)amino]-2*H*-pyrano[3,2-*c*]quinoline-2,5(6*H*)-dione(ASM-40)

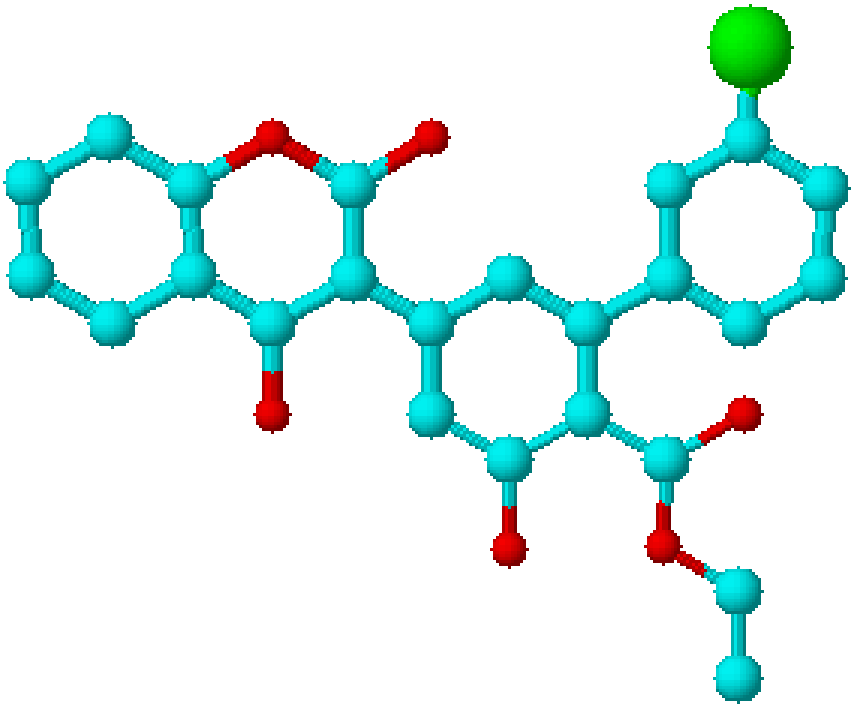


Fab Maas Spectra of 6-ethyl-4-[(2,3-dimethylphenyl)amino]-2*H*-pyrano[3,2-c]quinoline-2,5(6*H*)-dione (ASM-44)



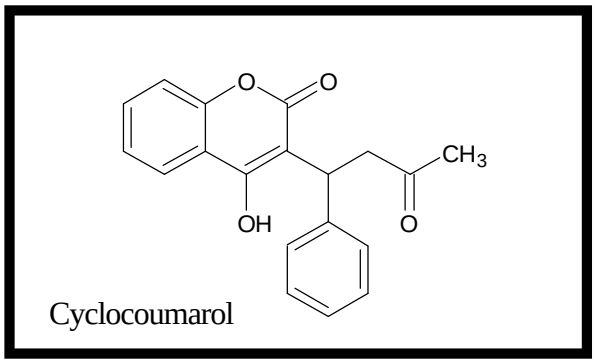
Chapter-3

Preparation of Ethyl 4-(3-bromo -2-oxo-2H-chromen-3-yl)-2-oxo-6-(2-methoxy phenyl) cyclohex-3-enecarboxylates :



Introduction

The coumarins are large group of naturally occurring oxygen heterocycles. Many natural coumarins have been reported for their wide range of biological activities[1-5]. As a result, several methods for their synthesis have been developed and anticoagulants like Tromexan[6], Warfarin[7], Cyclocoumarol[8], Marcoumar[9], came into market.



Cyclocoumarol

Coumarin and its annulated derivatives are reported to possess bactericidal and fungicidal properties[10,11] coronary dilatory[12], hypothermal[13], anticoagulant[14], antiviral[15-17], and antiinflamatory[18-21] activities.

---

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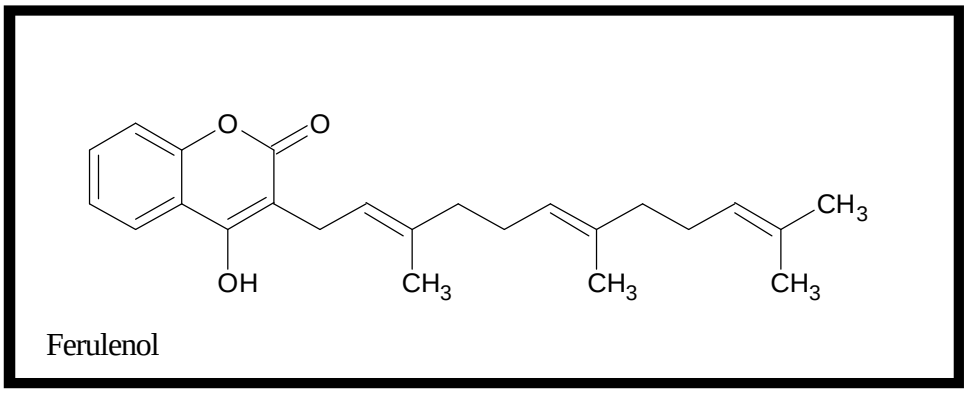
4-Hydroxy coumarins are only a minor constituent of natural coumarins. Some important derivatives have oxygen function at C_4 almost free. Though more often, it is methylated or sometimes involved in a pyran or furan ring formulation with an isoprene unit at C_3. The 3-position is free or occupied by an isoprene or multi isoprene units. However the physiological active compounds have various substituents. Ferulenol is one of such important natural product.



Ferulenol

10. i) Firanz, E., Klaubo, E. & Hammann, I., *Ger. Pat. 3012642 (1981); Chem. Abstr., **96**, 14276 (1982).*
ii) Reddy, Y. D. & Somayayulu, V V.; *J. Indian Chem. Soc., **58**, 599 (1981).*
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SYNTHESIS OF 4-HYDROXY COUMARINS :-

Several methods are reported for the synthesis of 4-hydorxy coumarins and their substituted derivatives namely : Anschitz[22], Kaneyuki[23] , Pauli Lockemann synthesis[24] ,Robertson synthesis[25], Sonn's synthesis[26] , Mentzer's synthesis[27], Garden's method[28], Ziegler and Junek[29], Resplandy's method[30], Jain, Rohtag and Sheshadri's method[31], and Bose and Shah's method[32].

Shah and co-workers[32] have prepared 4-hydroxy coumarin derivatives in good yield by condensation of different phenols with malonic acid. The method is useful as single step-preparation of many 4-hydroxy coumarin derivatives substituted on benzenoid part.

Synthesis of chalcones:-

A general method for synthesis of chalcone consists in condensing a ketone with an aromatic aldehyde in presence of appropriate condensing agent. Several condensing agents are employed. Out of these, dry hydrogen chloride and ethanolic aqueous potassium hydroxide are found to be most satisfactory. During the course of reaction a water molecule its eliminated.

---

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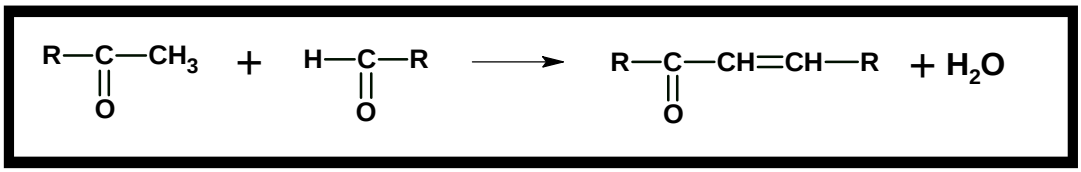
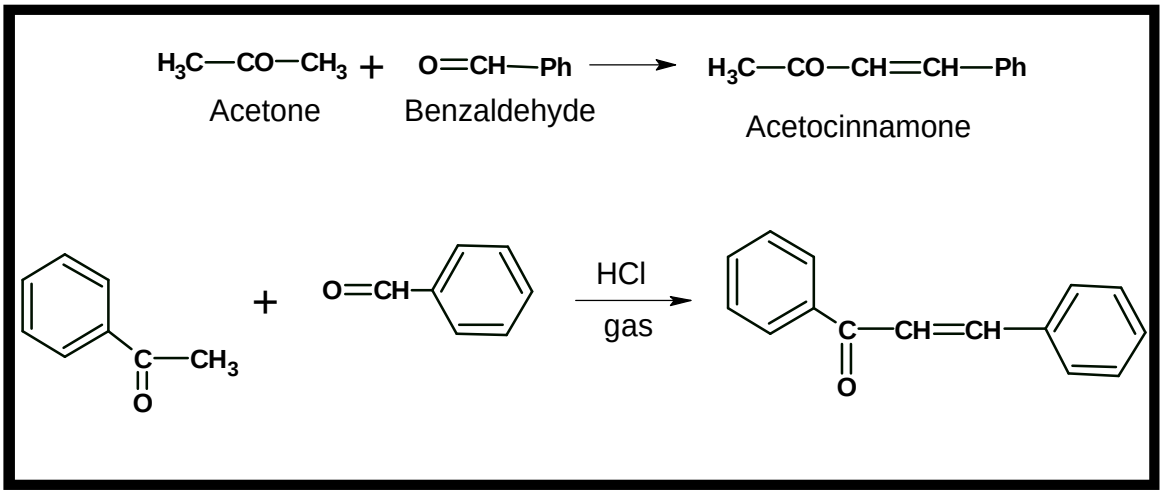


On condensing acetone with benzaldehyde in presence of zinc chloride and acetic anthydride, Claisen and Deparede[33] prepared benzylidene acetone identical with Engler and Leist' acetocinnamone[34].



Extending the above condensation to on aromatic ketone, benzylidine acetophenone were Prepared by using hydrogen chloride gas as a condensing agent. During the condensation of 4-nitro benzaldehyde with acetone, Bayer and Balker[35] isolated an intermediate Aldol type product.

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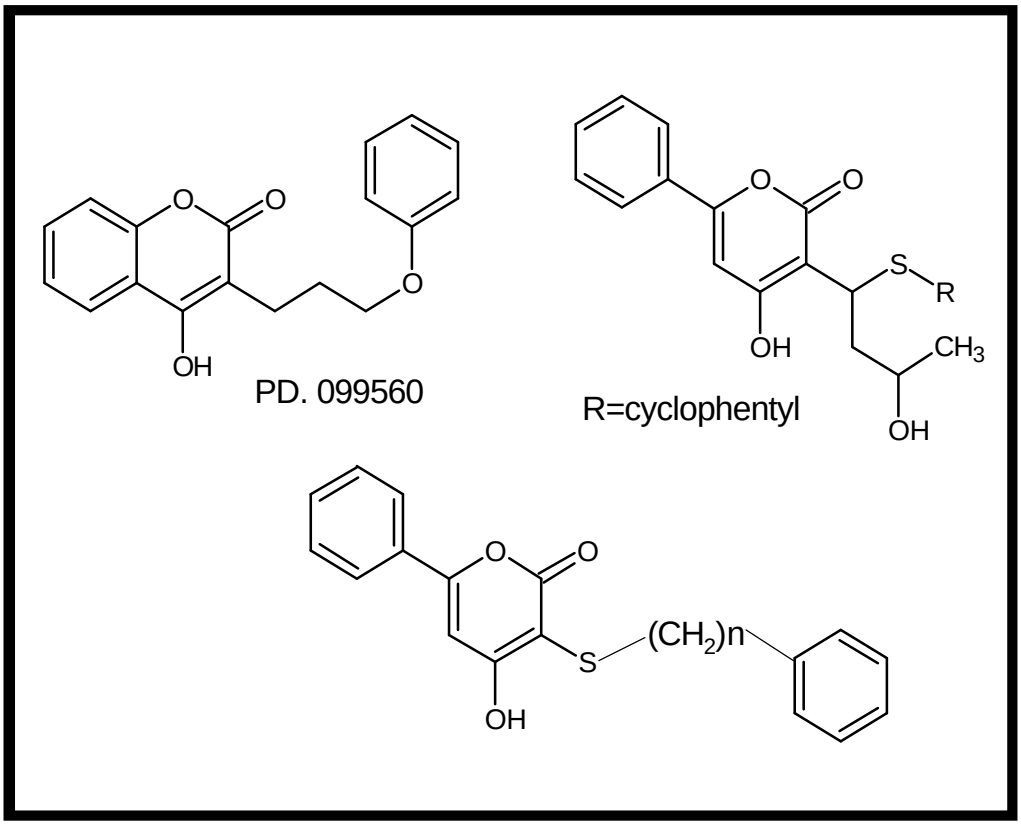
Recently screening work by Tummino and co-workers[36] identified several related structures with significantly better anti-HIV activity, including the 4-hydroxy coumarin PD 099560 and the 4-hydroxypyrone.This has given new impetus to this pyran skeleton.



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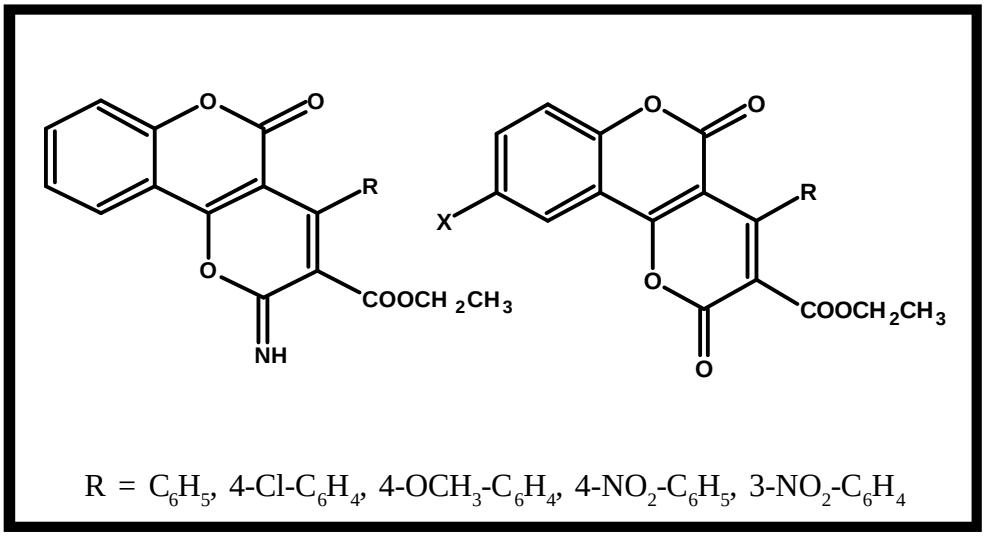
Kudo and coworkers[45] reported the formation of 2-imino-3-ethoxycarbonyl-4-phenyl-5-oxodihydro-pyrano[3,2 c] benzopyran[46], from condensation reaction of -4-hydroxy cournarin and ethylbenzylidene-cyanoacetat in the presence of water. Another structure was also reported by Darbarwar and coworkers[47].



R = C_6H_5, 4-Cl-C_6H_4, 4-OCH_3-C_6H_4, 4-NO_2-C_6H_5, 3-NO_2-C_6H_4

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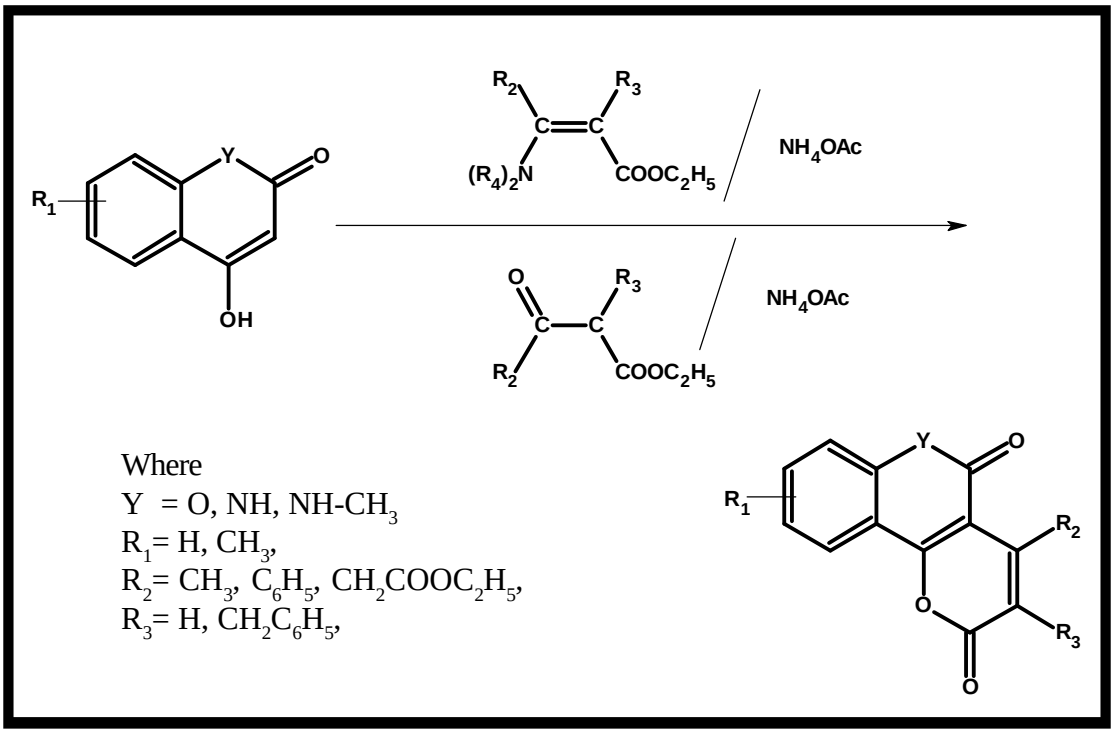
Kappe and coworkers[48] reported the reaction of 4-hydroxycoumarin and some 4-hydroxy-2-quinolones with β-enamino esters and with β-Keto esters in the presence of equivalents of ammonium acetate to prepare compounds .



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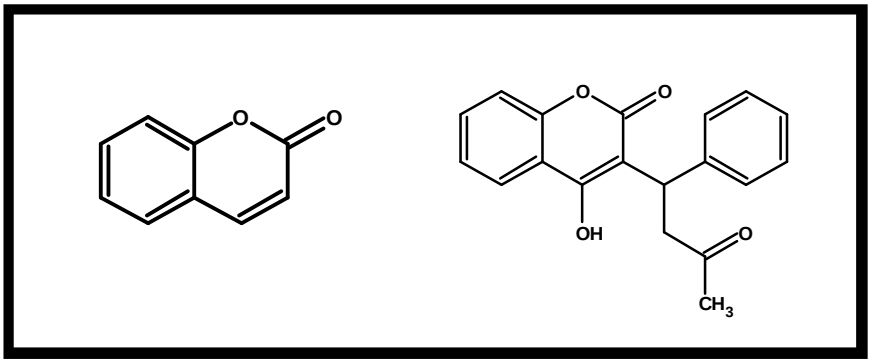
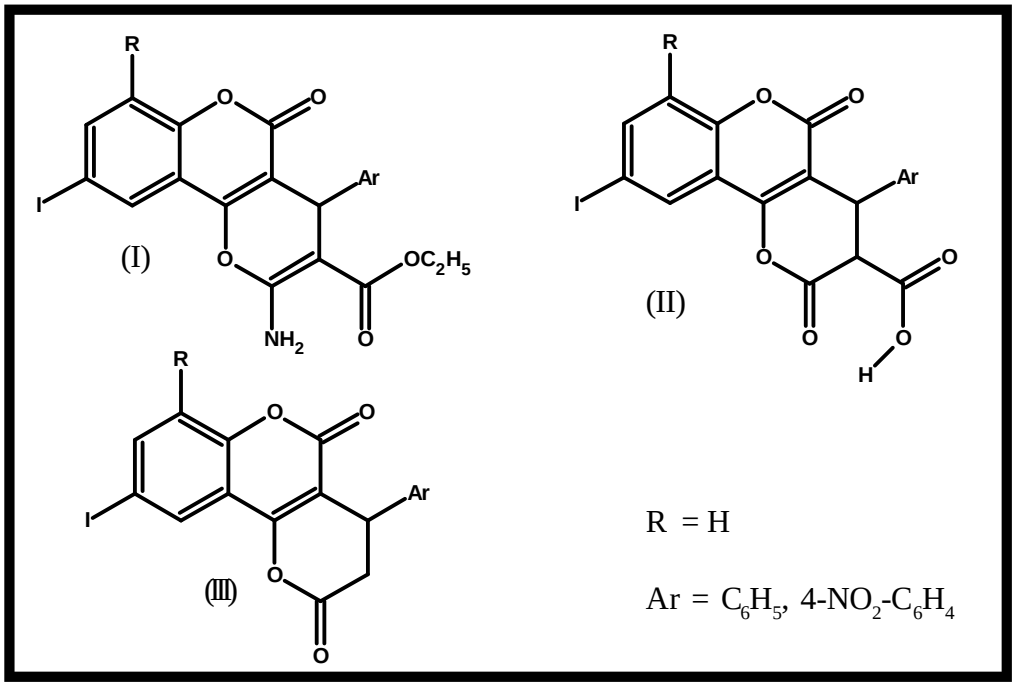
Coumarins are members among the most important classes of natural products. The first member of this class, unsaturated coumarin (a natural flavouring agent), was discovered in 1820. This scaffold is prolific in the plant kingdom but may also be found in fungi and bacteria, providing an enormous diversity in substitution patterns on the core scaffold.[49-54]



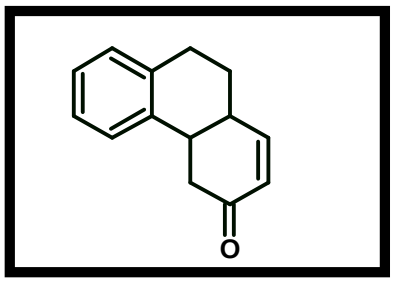
The therapeutic potential of these compounds are immense. Coumarin is the parent molecule of warfarin, which is used clinically as an anticoagulant and as a rodenticide. Coumarins have been reported to exhibit antibacterial and antifungal[41]activity and to act as diuretics and analgesics[42]. There have also been reports that structures containing the coumarin ring reduce tissue swelling due to various kinds of trauma or disease.

Mario and coworkers[57] reported that equimolar amount of 6-iodo-4-hydroxycoumarin and ethyl α-cyano-β-phenylacrylate in methyl cellulose when treated with piperidine, the formation of 2-amino-3-ethoxycarbonyl-4-phenyl-5-oxo-9-iodo-4*H*, 5*H*-pyrano[3,2-c]benzopyran is obtained. Further, Warfarin is refluxed in 80% CH_3COOH yeilded corresponding 2,4-dioxo-3-carboxy-4-aryl-2,3-dihydro-4*H*, 5*H*-pyrano[3,2-c]benzopyran.

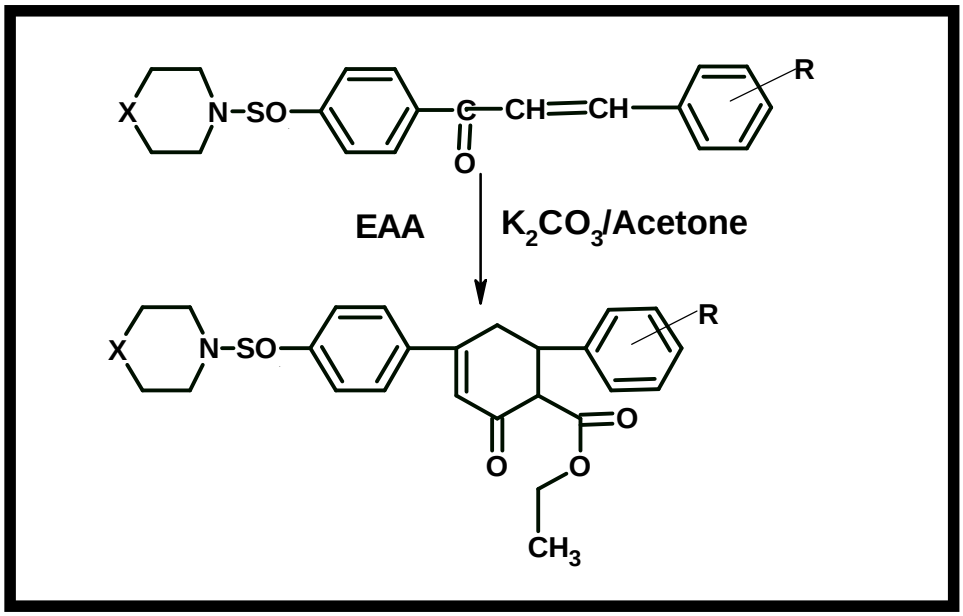
Warfarin is heated in vaccum at 180^{o}C with evolution of gas, gave 2,5-dioxo-4-aryl-2,3-dihydro 4*H*,5*H*-pyrano[3,2-c]benzopyrans.



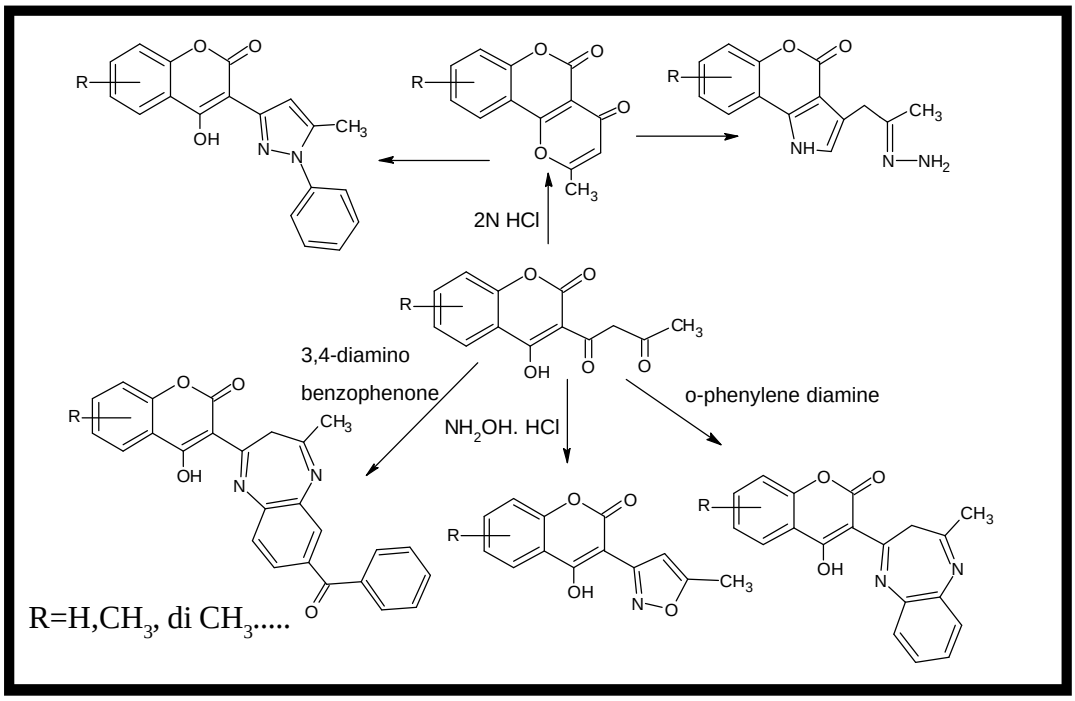
(1) A review of the earlier litrature by Gerald et al[58] describes representative synthetic procedure of cyclohexeonone derivative (I).



Eman H. A. et al.[59] have prepared cyclohexenone derivative (II) for chalcone.



Shah et. al.[79-81] have prepared various heterocycles derivatives from 3-acetoacetyl-4-hydroxy coumarin like pyrano chromendiones, benzopyrano pyrazoles, 1-4diazepine, isoxazoles, phenyl pyrazoles by known methods[38-40]. They also studied antimicrobial[41], antifungal[41], anti HIV[42,43] and antitubercular activity of these compounds. This suggests the importance of nitrogen containing heterocycles in most of the cases with 5 or 7 member systems. It was essential to study the xoygen containing heterocycles, if appended with 4-hydrosy skeleton, some pharmacological profile may alter.



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Usefulness of cyclohexenone clubbed with other heterocycle in various medicinal applications such as anthelmintic, hypoglycemic, nematocidal, antibacterial, antifungal, antiviral, analgesic etc are wellknown. Anti-arrhythmic activity[60] of some cyclohexenone derivatives have been reported. Cyclohexenone possess Cardiovascular, Osteoporosis, Menpausal Symptoms, estrogen dependent, cancers activities, which was reported by Jacobsen Poul et al[61].

These derivatives are reported for large spectrum of physiological properties viz. antibiotic[62-63,] bactericidal[64], herbicidal[65], antimicrobial[66], and anticonvulsant[67]. Alekseeva L.M. and co-workers[68], have reported this class as neurotropic activity.Toshiyuki et al.[69], have prepared some novel cyclohexenone and screened for allergy inhibitor, antithrombitic platelet aggregation inhibitors and fibrinogen antagonist activity.

Collins David J. et al.[70], have documented cyclohexenone derivatives which possess estrogenic activity As reported by V. K. Ahluwalia et al.[71] also as some new cyclohexenone are active as anti-HIV-1, gastric secretion inhibitors and pesticidal activity. Nagarajan and Shenoy[72] have prepared substituted cyclohexenone which has shown to possess marked antiinflammatory activity. Nagao et al[73] have reported antiarrhythmics activity. Inverse agonist for GABA activity[74] of some derivatives have been investigated.

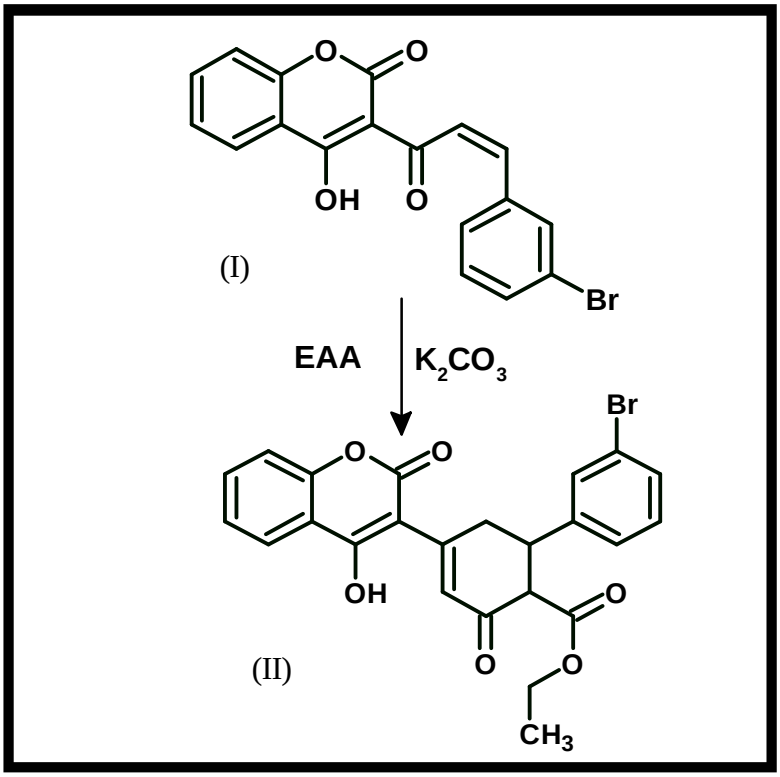
Recently, antimicrobial activity have been studied by Salama and Atshikh[75], cyclohexenone possess neutro peptide γ- receptor antagonist activity which was reported by Takehiro & co-workers[76].

Broughton Howard[77] have demonstrated these molocules as GABA α5 receptor ligands for enhancing cognition properties. Cyclohexenone possess inhibitory activity against the growth of lettuce seedling found by Kimura & co-workers[78].

(1) The current chapter deals with a 5 step synthesis of the coumarin com pounds possessing many oxygen atoms in the scaffold at C_3 position believed to be essential pharmacophore for various biological activiti es including chemotherapeutics (anti-tubercular,anti-viral & anti-cancer).

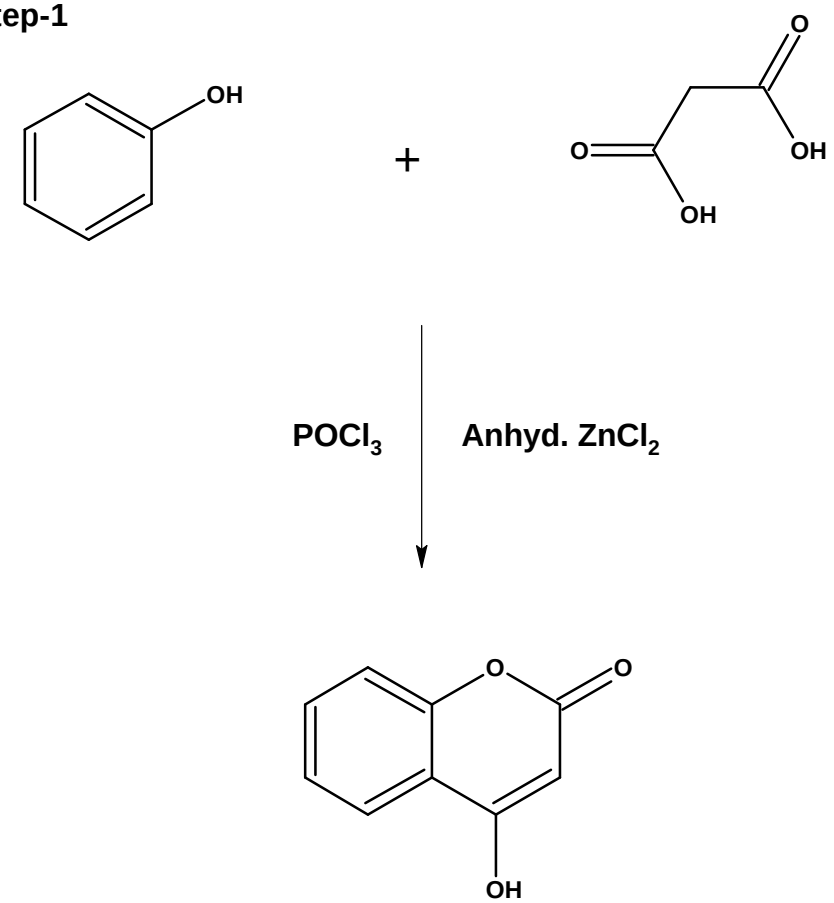
(2) Thus, 4-hydroxy coumarin prepared was acetylated to get 3-acetyl-4-hydroxy coumarin which was furthur converted into respective chalcone derivative which was furthur on cyclocondensation with ethylacetoacetate to give the final compounds.

(3) A series of Ethyl 6-(3-bromophenyl)-4-(4-hydroxy-2-oxo-2H- chromen-3-yl) -2-oxocyclohex-3- ene-1-carboxylates have been synthesized in this chapter.

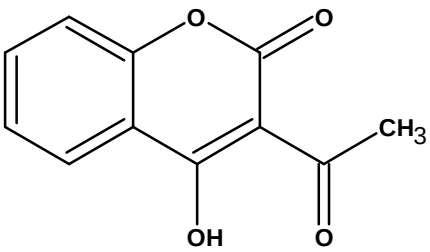


REACTION SCHEME : 3

Step-1

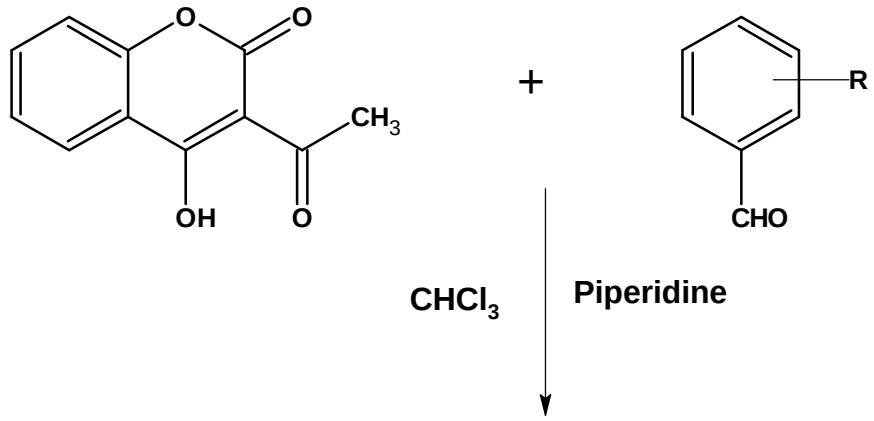


Step-2

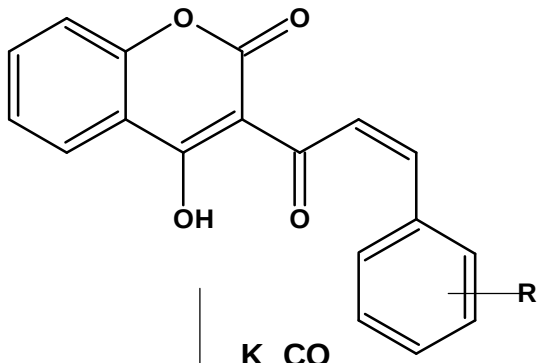


REACTION SCHEME : 3

Step-3



Step-4

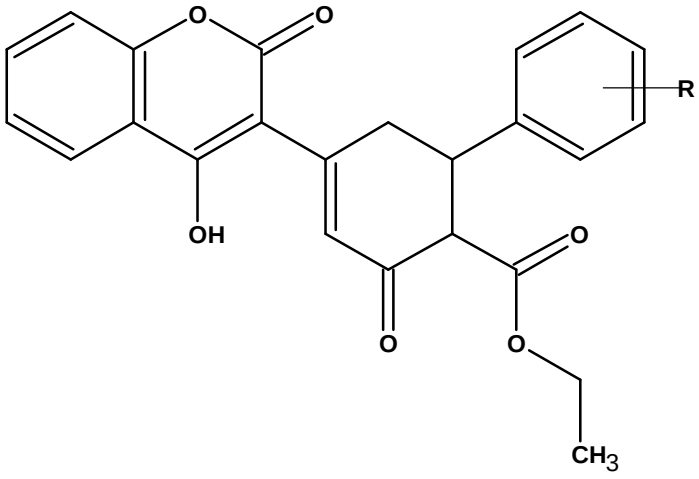


E A A

$K_2 CO_3$

+

Solvent



EXPERIMENTAL :

(1) Preparation of 4-hydroxy coumarin :

It was prepared according to method of Shah and coworkers[79-80]. Phenol (9.4 gm, 0.1 mole) and malonic acid (10.8 gm, 0.1 mole) was added to a mixture of phosphorous oxychloride (40 ml) and anhydrous zinc chloride (30 gm) which was preheated to 60-70 ^{0}C and the reaction mixture was heated on water bath at 70 ^{0}C for 10 hours. It was then cooled to room temperature and decomposed with ice and chilled water to afford solid, which was filtered and washed with water. It was then treated with 10% sodium bicarbonate solution and filtered. The filterate was slowely acidified with diluted hydrochloric acid. At the neutral point, the precipitates obtained were washed with water and dried. The product was recrystallized from ethanol with 72% yield obtained, m.p. 210-212 ^{0}C (Reported[80] m.p. 209-210).

(2) Preparation of 3-Acetyl-4-hydroxy coumarin :

4-hydroxy coumarin (5 gm,) was mixed with glacial acetic acid (25 ml). Phosphorous oxychloride (20 ml) was added slowely to the mixture and it was heated on water bath for 4 hours. Then the reaction mixture was cooled and poured into crushed ice, filtered, dried and recrystallized from ethanol. The product obtained in 75% Yield, m. p. 112-15^{o}C (Reported[81] M.P. 113-114 ^{o}C)

(3) Preparation of 3-[(2*Z*)-3-(4-bromophenyl)prop-2-enoyl]-4-hydroxy-2*H*-chromen-2-one : (General Method)

3-acetyl-4-hydroxy coumarin (0.05 mole) and unsaturated aromatic aldehyde (0.05 mole) were dissolved in 30 ml of chloroform. The catalytic amount of piperidine (0.02 ml) was added and the reaction mixture was refluxed for 6 hours on a water bath, then it was allowed to cool at room temperature. The chloroform was distilled out and the residue was washed with methanol. It was then dried and recrystallised from chloroform.

Elemental Analysis

Calculated = C (58.24%) H(2.99%)

Experimental = C (58.71%) H(2.91%)

Molecular Formula = $C_{18}H_{11}ClBrO_4$

Formula Weight = **371.18**

M.P. = **170-72°C**.

TLC System = (Ethyl acetate: Hexane : 4: 6)

Yield = **65-75%**

Synthesis of Ethyl 4-(3-bromo-2-oxo-2H- chromen-3-yl)-2-oxo-6-(2-methoxyphenyl) cyclohex-3-enecarboxylate. (General Method)

A mixture of chalcone [3-[(2Z)-3-(3-bromophenyl)prop-2-enoyl]-4-hydroxy-2H-chromen-2-one](3.71g,0.01 mol) in 50ml of dry acetone, anhydrous K_2CO_3(5.42g, 0.04mol)and ethyl acetoacetate(2.60g,0.02mol)was stirred at room temperature overnight and was filtered. The solvent from the filtrate on distillation gave a solid which was crystallized from ethanol .

Elemental Analysis

Calculated = C (59.64%) H(3.95%)

Experimental = C (52.71%) H(3.88%)

Molecular formula = $C_{24}H_{19}ClBrO_6$

Formula Weight = **483.30**

M.P. = **108-10°C**.

TLC System = (Ethyl acetate: Hexane : 4: 6)

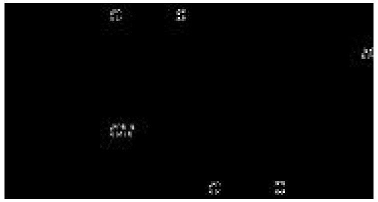
Yield = **58-65%**

Similarly other compounds were synthesized (Table 3.1,3.2,3.3).

Table 3.1 Physical data of Ethyl 4-(4-hydroxy-2-oxo-2*H*-chromen-3-yl)-2-oxo-6-substituted phenylcyclohex-3-ene-1-carboxylates

*** Values in parenthesis denotes the calculated % of composition .**

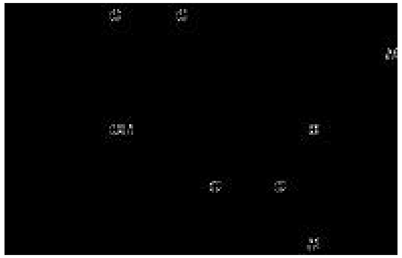
Table 3.2 Physcial data of Ethyl 4-(4-hydroxy-2-oxa-2H-chromen-3-yl)-2-oxa-6-substituted phenylcyclohex-3-enecarboxylates (cont.)



Sr No	Code	Substitution [illegible]	Substitution [illegible]	Molecular Weight	M.P. [illegible]	[illegible] Value	Elemental Analysis %	
							C	[illegible]
[illegible]	[illegible]	[illegible]		[illegible]	[illegible]	[illegible]	[illegible] ([illegible])	[illegible] ([illegible])
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible] ([illegible])	[illegible] ([illegible])
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible] ([illegible])	[illegible] ([illegible])
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible] ([illegible])	[illegible] ([illegible])
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible] ([illegible])	[illegible] ([illegible])
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible] ([illegible])	[illegible] ([illegible])
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible] ([illegible])	[illegible] ([illegible])

*** Values in parenthesis denotes the calculated % of composition .**

Table 3.3 Physical data of thyl 4-(4-hydroxy-2-oxa-2H-chromen-3-yl)-2-oxa-6-substituted phenylcyclohex-3-enecarboxylates (cont.)



Sr. No	Code	Substitution R	Substitution R	Molecular Weight	M.P. °C	Rf Value	Elemental Analysis % C	Elemental Analysis %
[illegible]	ASV-[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	ASV-[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	ASV-[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	ASV-[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	ASV-[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	ASV-[illegible]	[illegible]		[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	ASV-[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]

*** Values in parenthesis denotes the calculated % of composition .**

SPECTRAL STUDY :

The constitution of newly synthesized compounds were supported by IR, ^{1}H NMR, Mass and ^{13}C NMRspectral study. The details are as under.

IR Spectral Study :

Instrument : SHIMADZU FT IR-8400 Spectrophotometer

Sample technique : KBr pellet

Frequency range : 400-4000 cm^{-1}

As per spectral study of the newly synthesized coumarin compounds, the carbonyl (>C=O) for the ring and the ester stretching is observed at range of ~1680-1620 cm^{-1} and 1740-1710 cm^{-1} respectively for the carbonyl functionality.The two carbonyl (>C=O) for the chromone & coumarin ring does not get sparate due to merging in some cases. The confirmation of hydroxyl group is the O-H stretching in range of ~3400-3200 cm^{-1}.

In case of ethyl 6-(3-hydroxyphenyl)-4-(4-hydroxy-2-oxo-2*H*-chromen-3-yl)-2-oxocyclohex-3-ene-1-carboxylate **(ASM-12)**, a broad band of -OH (hydroxyl) is observed at 3413 cm^{-1} & 3207 cm^{-1} indicating the presence of two -OH group. The sharp carbonyl band (>C=O) is seen at 1710.7cm^{-1} which indicates the presence of ester(>COOR) group compare to 1750 cm^{-1}. The ring carbonyl (>CO) stretching is observed at 1620.7cm^{-1}.

The aromatic moiety and ring skeleton (like C-C multiple bond stretching, C-H i.p. def. and C-H o.o.p. def.) were observed at 1592, 1528 and 1402 cm^{-1} & 762 cm^{-1} .

Similarly, other compounds were studies for their characteristic study.

^{1}H NMR Spectral Study :-

Instrument :BRUKER AC 300 MHz FT-NMR

Internal reference :TMS

Solvent :$CDCl_3$ or DMSO d_6

^{1}H NMR analysis of Ethyl 4-(4-hydroxy-2-oxo-2H-chromen-3-yl)-2-oxo-6-phenyl cyclohex-3-enecarboxylate (ASM-25).

During ^{1}H NMR study of compounds synthesized in this Chapter, the methyl triplet of CH_{3a} is observed at δ 0.824 ppm along with a quartet for CH_{2b} at δ 3.91ppm respectively, confirming the presence for the ethyl group (CH_2-CH_3). The high value of CH_2 signal is due to attatchment of ethyl to the ester group. A doublet for the Hc & He is observed at δ 3.017 & 2.23 ppm indicating the presence for the single proton present at the cyclohexenone ring. This single proton Hd gives the signal at δ 3.6ppm as multiplet.The signal for the ethylene H atom of the cyclohexenone ring as Hg is observed as singlet at δ 1.896ppm. These signals confirms the proof for the cyclohexanone substituted ring. Refering to the protons of the substituted phenyl ring of the cyclohexenone ring, the multiplet obtained in the spectra shows coupling between the ortho-protons at the value δ 7.15-7.28 along with the multiplet for the coumarin phenyl ring protons Hm,l at δ 7.08ppm. The remaining proton Hj of this ring gives multiplet at δ 7.31ppm due to ortho- and para- coupling protons. The doublet format in the spectra is seen for the protons at the coumarin ring for the Hk & Hn at δ 7.85ppm.

The hydroxyl group present in the coumarin ring is confirmed by the IR spectral value along with the signal obtained as singlet in ^{1}H NMR spectra of **(ASM-25)** at δ 2.502ppm.

In the ^{1}H NMR spectra of **(ASM-6),** the substitution of methoxy group at the 2nd position of the phenyl ring exhibits the singlet obtained in the downfield region δ 3.334ppm. In the similar spectra, a pair of doublet and quartet for the ethyl group is also observed at δ 0.937-0.984ppm & δ 4.06ppm respectively. The remaining proton signals came out to be same as the above pattern of other compounds of the series.

The ^{1}H NMR spectra of compounds **(AM-6)** and **(AM-25)** are given on page no. **22 & 23.**

Mass Spectral Study :-

Instrument : VG 70-S (70eV) Spectrograph for EI

Instrument : JEOL SX 102/DA-6000 Spectrograph for FAB

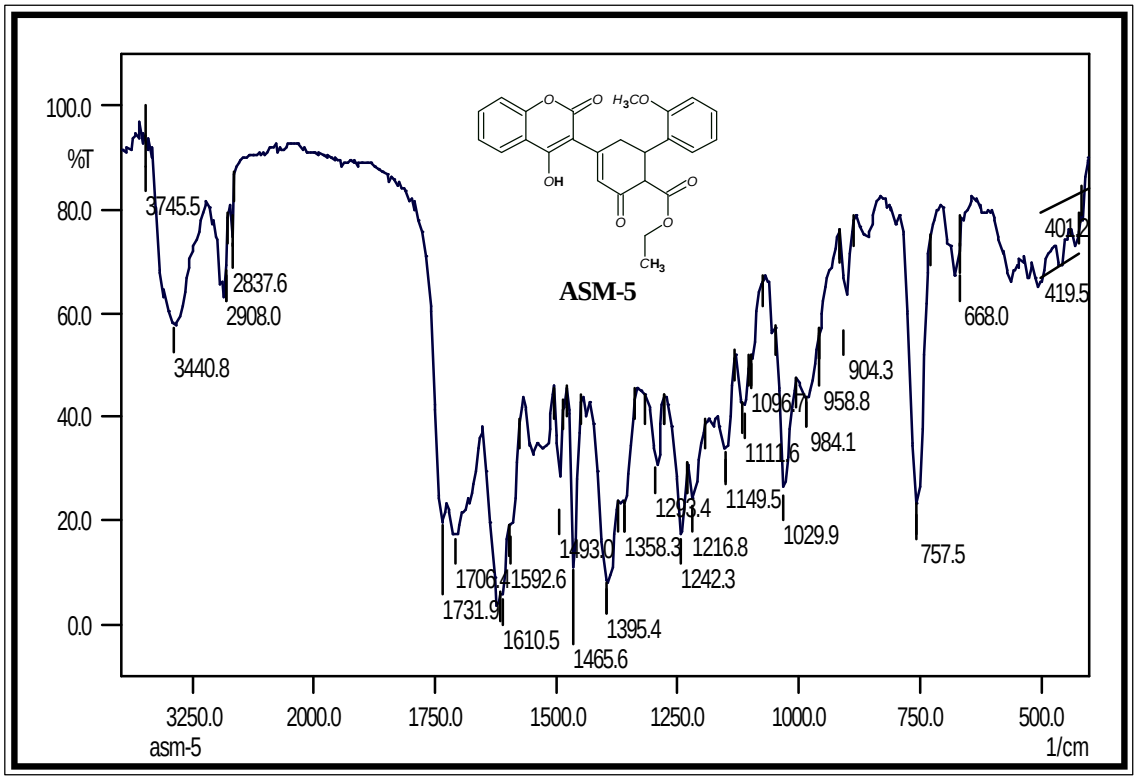
The newly synthesized compounds were subjected to FAB Mass study.The Fast bombardment study revealed the Molecular ion peak, base peaks and other relevant fragmentation pattern to confirm the structure of the molecules.The molecular ion peak is concormitant with the molecular weight of the compounds.

While in Fab Mass of Ethyl 4-(4-hydroxy-2-oxo-2H-chromen-3-yl)-2-oxo-6-(3-chloro phenyl) cyclohex-3-enecarboxylate **(ASM-12)** base peak is observed at 154.00 m/z, while molecular ion peak is also at 438.00 m/z.

In the Fab Mass of Ethyl 4-(4-hydroxy-2-oxo-2H-cromen-3-yl)-2-oxo-6-(3-methoxyphenyl)cyclohex-3-enecarboxylate **(ASM-6),** a base peak is observed at 361.0 m/z, while molecular ion peak is at 433.00 m/z (M+1) peak.

The mass spectra of representative compounds are given on page no. **115** & **117**.

IR Spectrum of Ethyl 4-(4-hydroxy-2-oxo-2H-chromen-3-yl)-2-oxo-6-(2-methoxyphenyl) cyclohex-3-enecarboxylate (ASM-5)



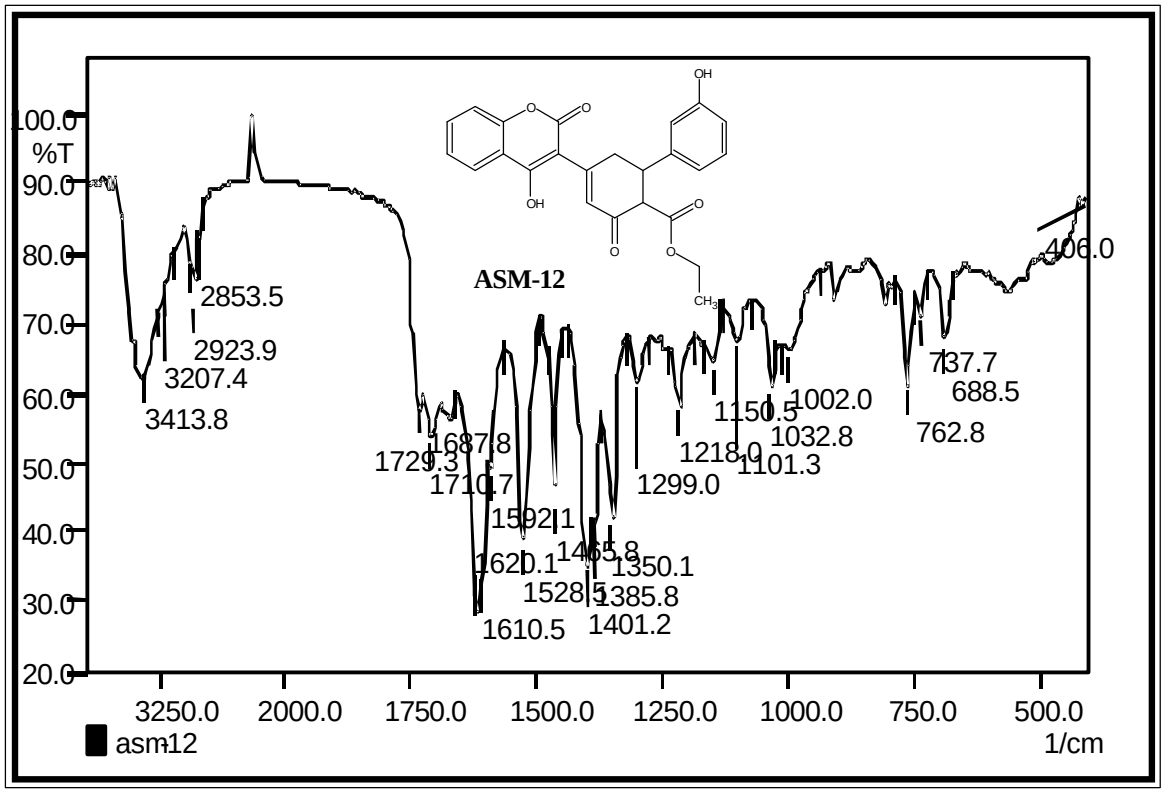
Sample technique : KBr Pellet

Instrument : SHIMADZU FT IR-8400

Frequency range : 4000-400 cm^{-1}

Type	Vibration mode	Frequency cm^{-1}
Carbonyl	>C=O str. (ester).	1731.9
	>C=O str. (ring).	1706.4
Hydroxy	-OH str.	3440.8
Methyl	Assymetric str.	2837.6
	Symmetric str.	2908.0
	Bending	1358.3
Aromatic	ring skeleton vib.	1465.5 1493.0 1592.6
	o.o.p.bending vib. (1,2,3-tri sub.)	757.5

IR Spectrum of Ethyl 4-(4-hydroxy-2-oxo-2H-chromen-3-yl)-2-oxo-6-(3-hydroxyphenyl) cyclohex-3-enecarboxylate (ASM-12)



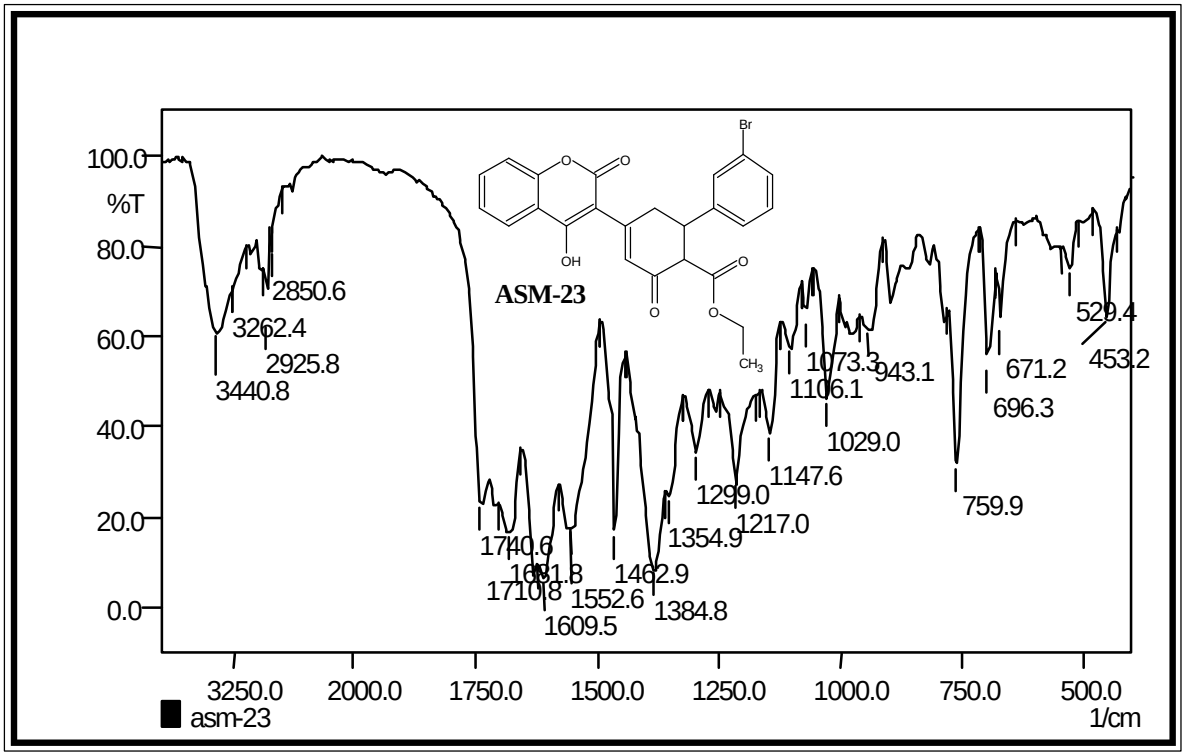
Sample technique : KBr Pellet

Instrument : SHIMADZU FT IR-8400

Frequency range : 4000-400 cm^{-1}

Type	Vibration mode	Frequency cm^{-1}
Carbonyl	>C=O str. (ester).	1729.3
	>C=O str. (ring).	1710.7
	>C=O str. (chromone)	1687.8
Hydroxy	-OH str.	3413 & 3207
Methyl	Assymetric str.	2853.5
	Symmetric str.	2923.5
	Bending	13858
Aromatic	ring skeleton vib.	1401.2 1528.5 1592.1
	o.o.p.bending vib. (1,2,3-tri sub.)	762.8
Ether C-O-C	bending	1218.0

IR Spectrum of Ethyl 4-(4-hydroxy-2-oxo-2H-chromen-3-yl)-2-oxo-6-(3-bromophenyl) cyclohex-3-enecarboxylate (ASM-23)



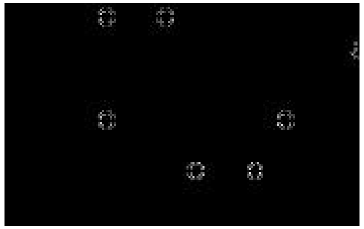
Sample technique : KBr Pellet

Instrument : SHIMADZU FT IR-8400

Frequency range : 4000-400 cm^{-1}

Type	Vibration mode	Frequency cm^{-1}
Carbonyl	>C=O str. (ester).	1740.6
	>C=O str. (ring). >C=O str. (chromone)	1681.8 1710.8
Hydroxy	-OH str.	3440.8
Methyl	Assymetric str.	2850.6
	Symmetric str.	2925.8
	Bending	1384.8
Aromatic	ring skeleton vib.	1462.9 1552.6 1609.5
	o.o.p.bending vib. (1,2,3-tri sub.)	759.9
Halogen	C-Br str.	529.4

Table 3.4 IR frequency of newly synthesized Ethyl 4-(4-hydroxy-2-oxa-2H-chromen-3-yl)-2-oxa-6-substituted phenyl cyclohex-3-enecarboxylates

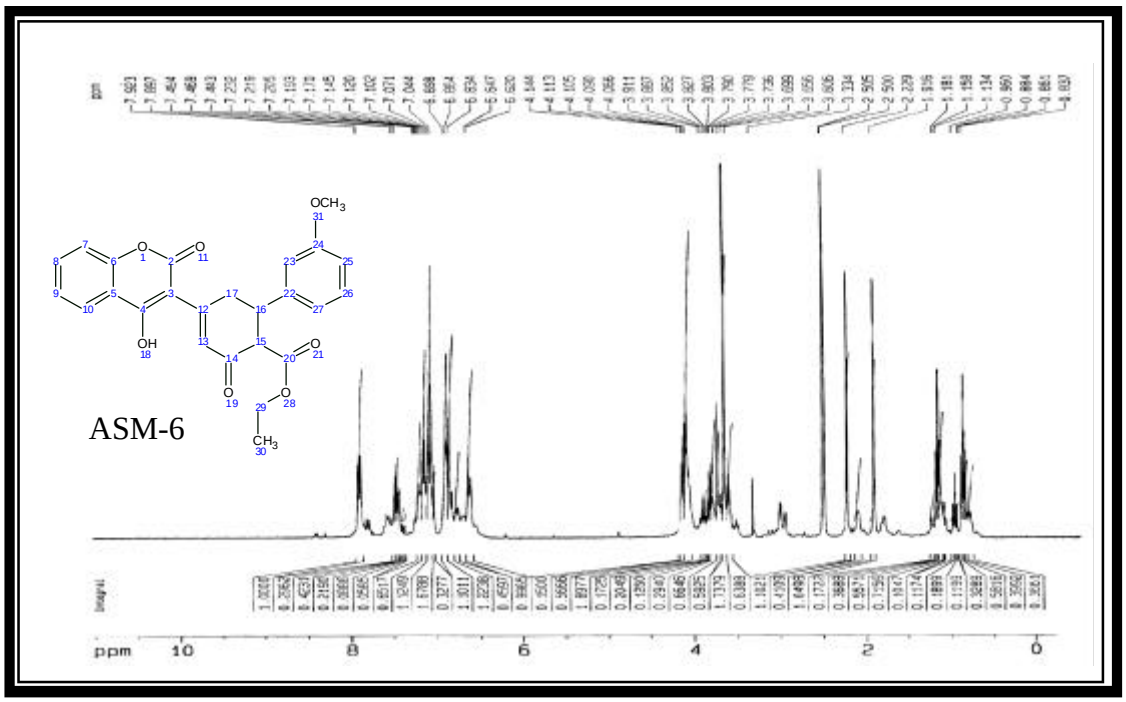


Compound code	>C=O str (lact) cm⁻¹	>C=O str (ring) cm⁻¹	O-H str cm⁻¹	Ring Skeleton cm⁻¹		
ASV 6	1731	1888	3112	1553	1765	1381
ASV 7	1702	1818	3781	1572	1518	1750
ASV 8	1857	1507	3383	1557	1783	1721
ASV 9	1710	1808	3388	1517	1761	1702
ASV 10	1887	1807	3263	1580	1768	1387
ASV 11	1873	1580	3711	1601	1778	1753
ASV 13	1856	1817	3387	1555	1578	1707
ASV 14	1887	1808	3388	1578	1783	1381
ASV 15	1877	1580	3387	1567	1723	1708

Table 3.5 IR frequency of newly synthesized Ethyl 4-(4-hydroxy-2-oxa-2H-chromen-3-yl)-2-oxa-6-substituted phenyl cyclohex-3-enecarboxylates (cont.)

[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
ASV 16	1681	1632	3102	1605	1528	1120
ASV 17	1681	1621	3310	1602	1528	1118
ASV 18	1681	1588	3317	1580	1508	1162
ASV 19	1711	1603	3308	1613	1513	1153
ASV 20	1686	1587	3114	1598	1568	1138
ASV 21	1686	1608	3123	1602	1514	1160
ASV 22	1710	1613	3308	1598	1182	1398
ASV 27	1682	1588	3315	1608	1181	1388
ASV 28	1723	1613	3123	1613	1583	1176

^{1}H NMR Spectra of Ethyl 4-(4-hydroxy-2-oxo-2H-chromen-3-yl)-2-oxo-6-(3-methoxyphenyl) cyclohex-3-enecarboxylate (ASM-6)



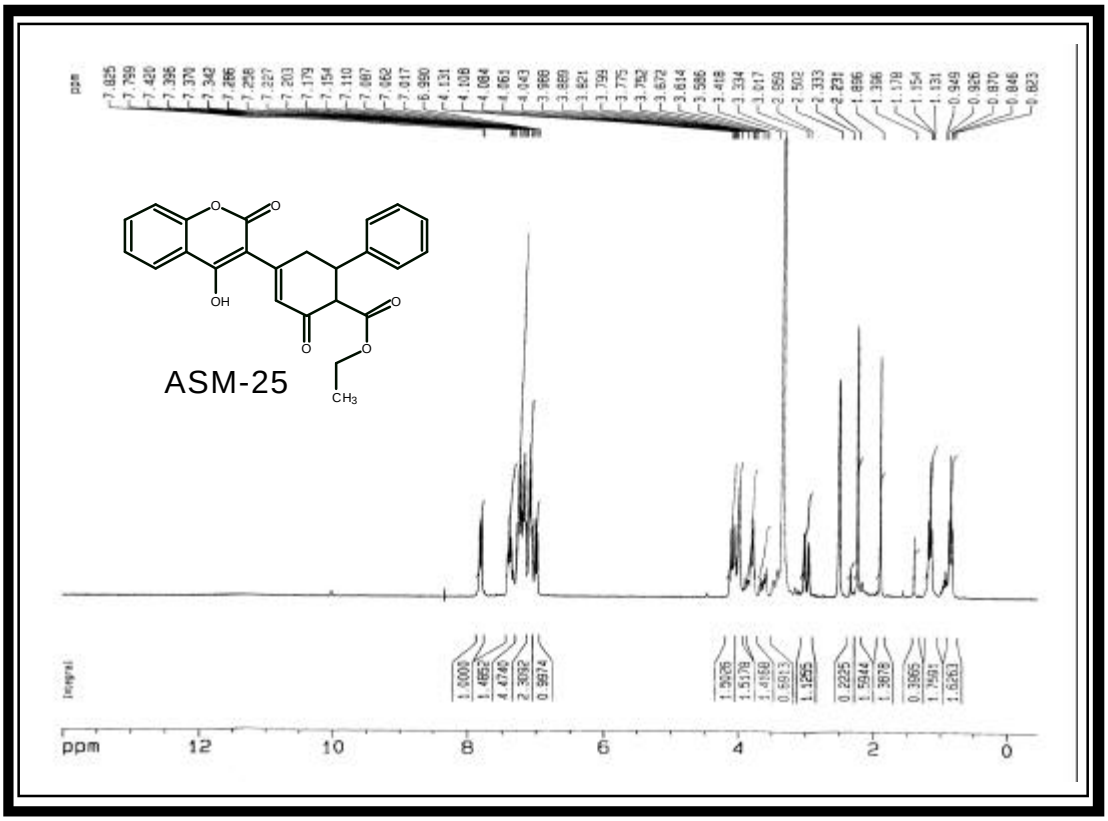
Instrument : BRUKER AC 300 MHz FT-NMR

Standard : TMS

Solvent : $CDCl_3$ + DMSO d_6

Chemical Shift d ppm	No. of Proton	Mulipliclity	Inference
0.845	3 H	T	H_a
1.97	1 H	S	H_f
2.196	2 H	D	H_e
3.91	2 H	Q	H_b
4.05	3 H	S	-O C H_3
4.15	1 H	D	H_c
4.26	1 H	M	H_d
6.74	1 H	Q	H_k
6.86	1 H	M	H_j
7.06	3 H	"	H_i

^{1}H NMR Spectra of Ethyl 4-(4-hydroxy-2-oxo-2*H*-chromen-3-yl)-2-oxo-6-phenylcyclohex-3-ene-1-carboxylate (ASM-25)



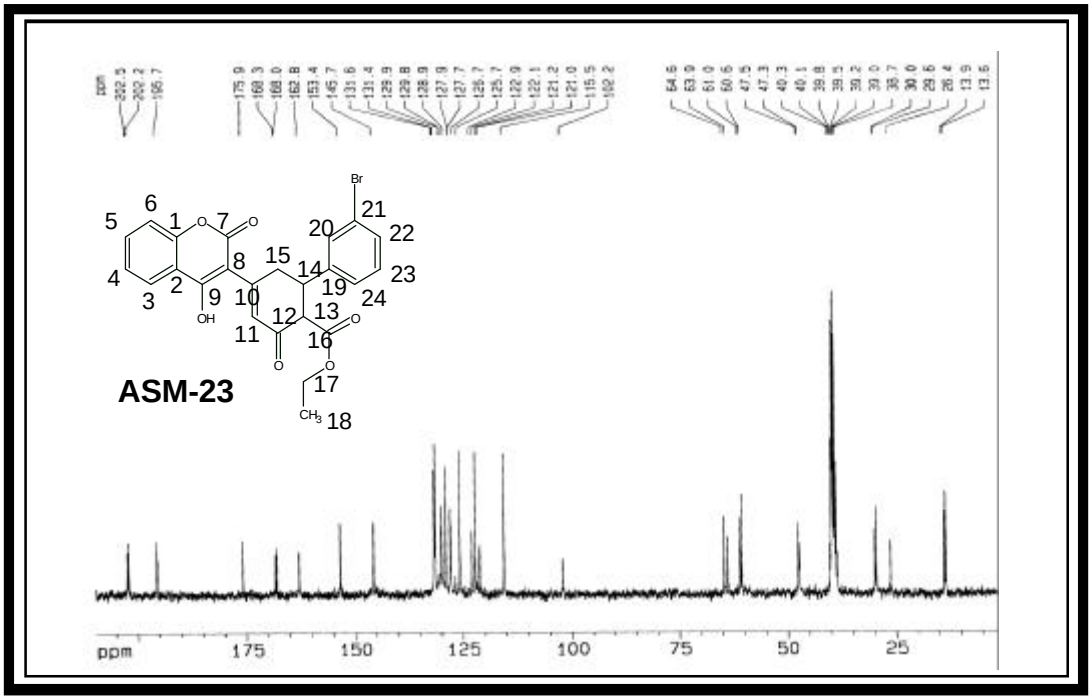
Instrument : BRUKER AC 300 MHz FT-NMR

Standard : TMS

Solvent : $CDCl_3$ + DMSO d_6

Chemical Shift d ppm	No. of Proton	Mulipliclity	Inference
0.824	3 H	T	C H_{3a}
1.896	1 H	S	H_g
2.23	2 H	D	H_e
3.017	1 H	D	H_c
3.6	1 H	M	H_d
3.77	2 H	Q	H_b
6.90	1 H	D	H_k
7.08	2 H	M	$H_{m,l}$
7.15-7.28	2 H 4 H	M	H h,h,i,i
7.31	1 H	M	H j

^{13}C Spectra of Ethyl 4-(4-hydroxy-2-oxo-2H-chromen-3-yl)-2-oxo-6-(3-bromophenyl) cyclohex-3-enecarboxylate (ASM-23)

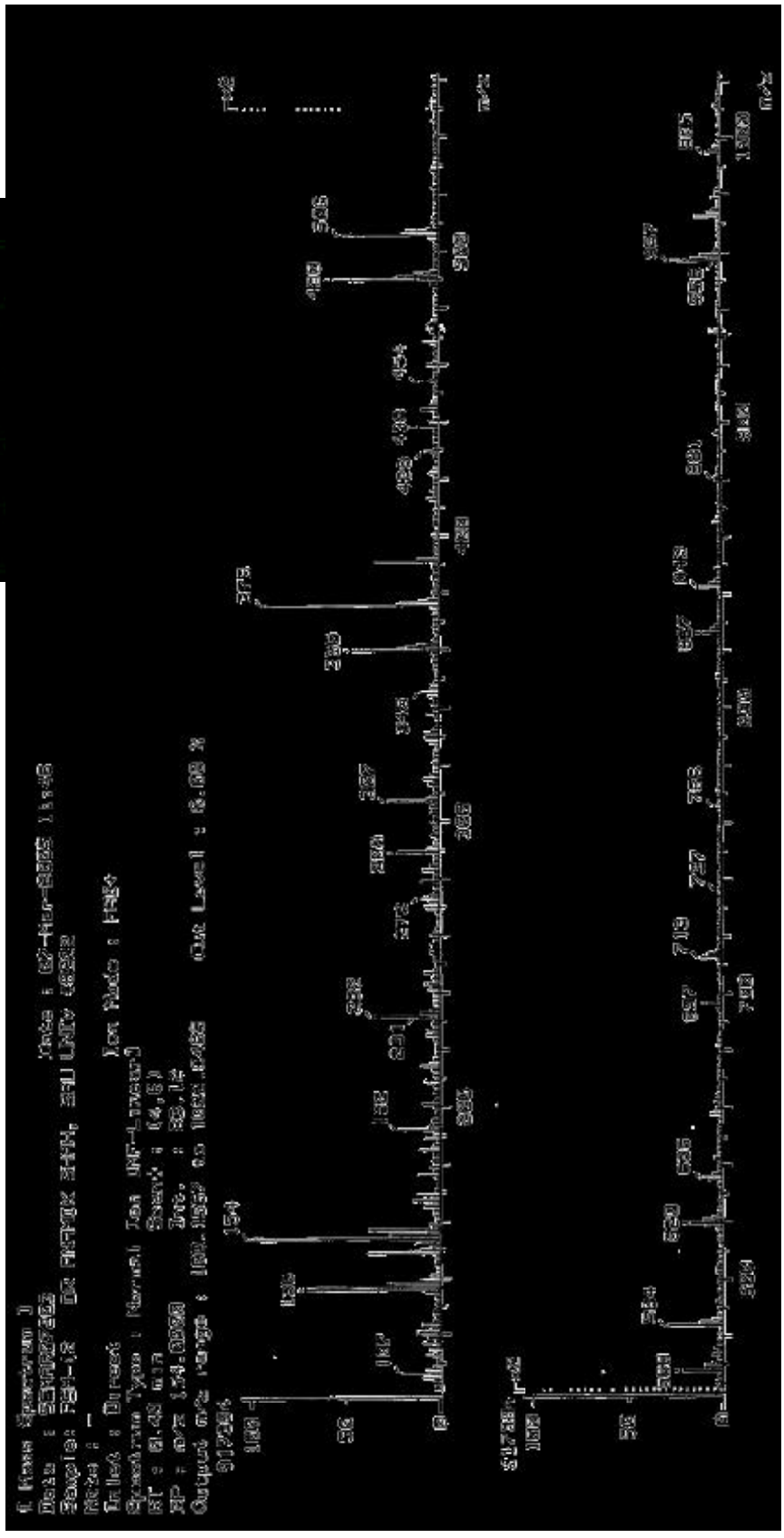


Instrument : BRUKER AV 300 MHz FT-NMR

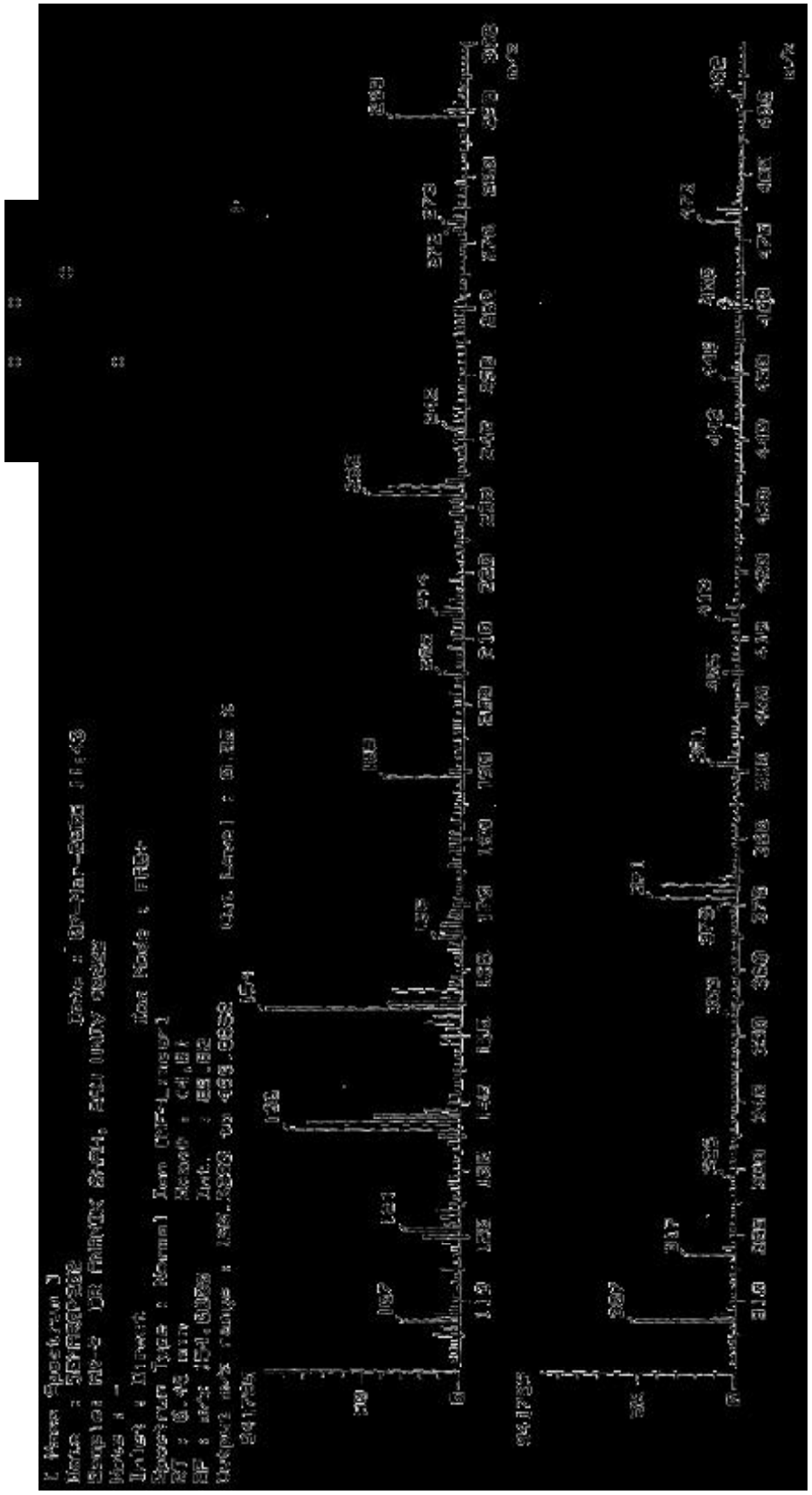
Solvent : $CDCl_3$ + DMSO d_6

Chemical Shift d ppm	No.of carbons	Inference
26.4	1C	-CH$_3$(18)
29.6	1C	-CH(14)
39.8	1C	-CH(17)
61.0	1C	-CH(15)
63.9	1C	-CH(2)
64.6	1C	-CH(3)
121.2	2C	-CH(4,5)
122.9	1C	-CH(20)
125.7	1C	-CH(21)
127.7	2C	-CH(21,22)
128.9	2C	-CH(22,23)
129.9	1C	-CH(24)
102.2	1C	-CH(19)
145.7	1C	-CH(1)
153.4	1C	-CH(13)
162.8	1C	-CH(9)
168.0	1C	-CH(7)
168.3	1C	-CH(12)
175.9	1C	-CH(16)

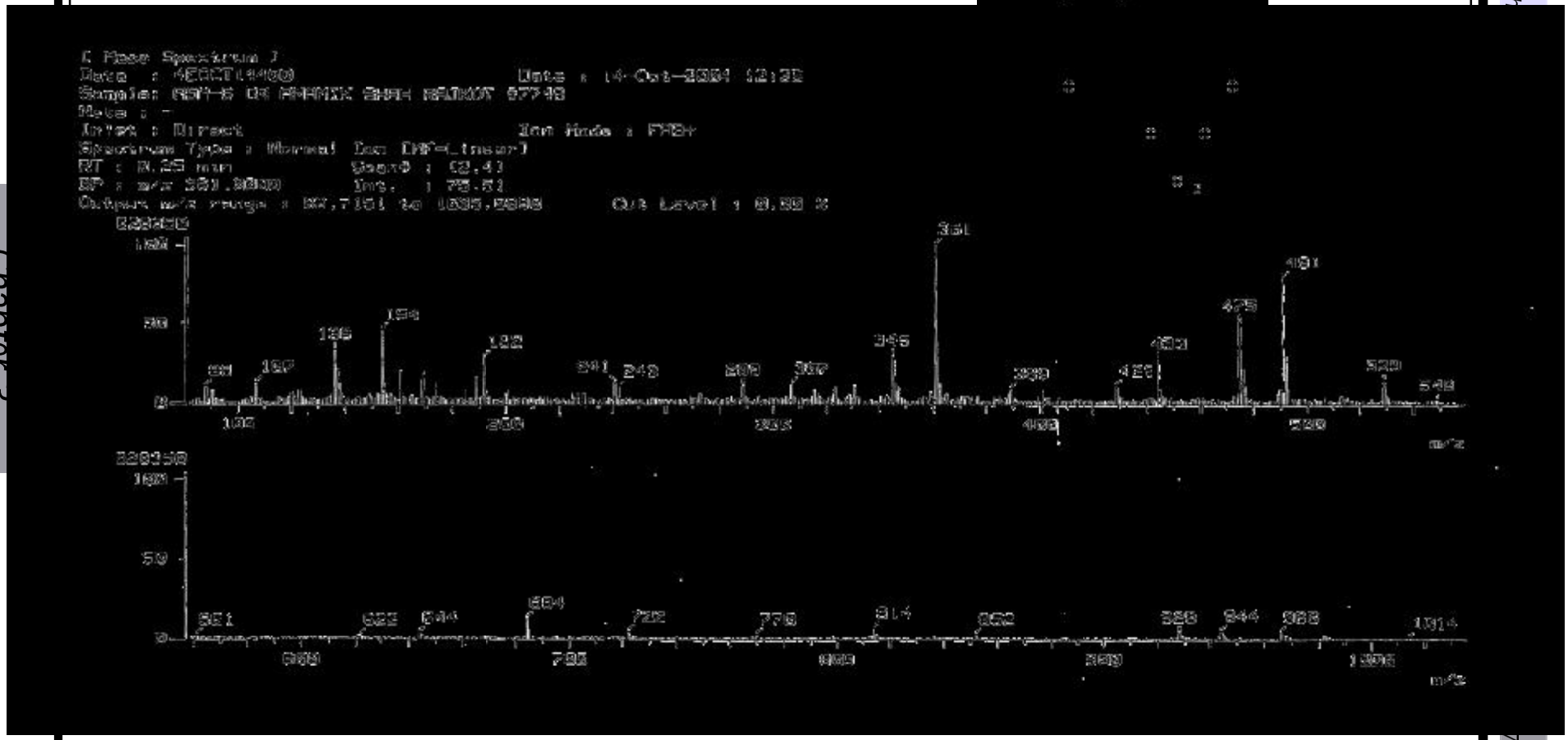
Fab Maas Spectra of Ethyl 4-(4-hydrxoy-2-oxo-2H-chromen-3-yl)-2-oxo-6-(4-chlorophenyl) cyclohex-3-enecarboxylate (ASM-12)



Fab Maas spectra of 3-[(2Z)-3-(3-bromophenyl)prop-2-enoyl]-4-hydroxy-2*H*-chromen-2-one (AR-2)

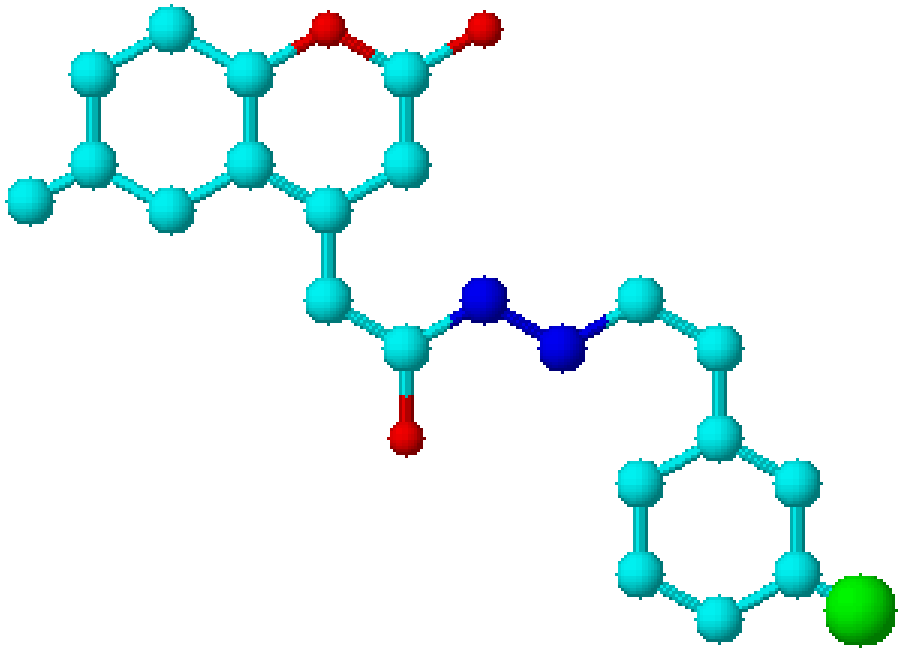


Fab Maas Spectra of Ethyl 4-(4-hydroxy-2-oxo-2H-chromen-3-yl)-2-oxo-6-(3-methoxyphenyl) cyclohex-3-enecarboxylate (ASM-6)



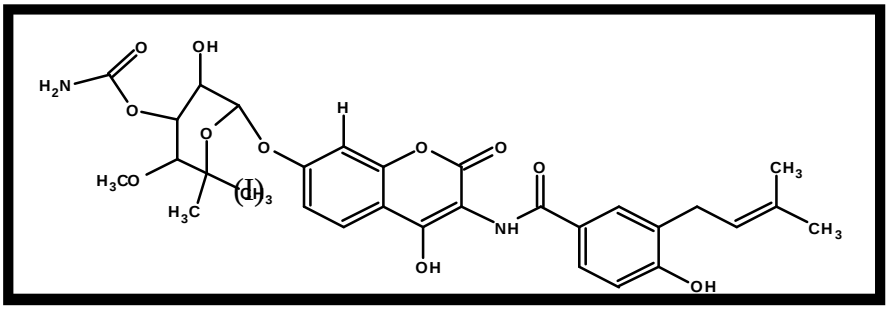
Chapter-4

Preparation of N'-[(1E)-(3-chlorophenyl) methylene]-6-methyl-2-oxo-2H-chromene-4-carbohydrazides :



Introduction

Coumarin-containing antibiotics such as novobiocin (I)amino coumarin core produced by*Streptomyces* species, have gained renewed interest since the discovery that they are potent inhibitors of bacterial DNA gyrase, which is essential for cell viability[1a,b]. In addition,these coumarin antibiotics are potent against methicilin resistant strains (mrs) of S*taphylococci* species, currently one of the major concerns in the treatment of bacterial infections.[2]

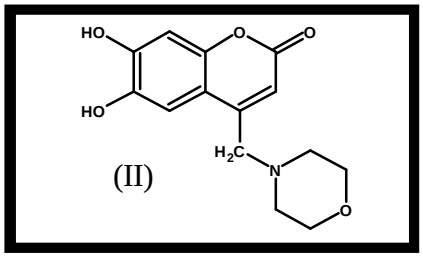


Effective reversal of breast cancer resistant protein(BCRP)-mediated drug resistance by novobiocin was studied by Shozawa et al.,[3] and Nakatomi et al.[4] This has renewed interest in coumarin based compounds.

Folescutol (2), a known capillary protectant drug[5], 6,7-dihydroxy-4-(4-morpholinyl methyl)-2H-1-benzopyran-2-one has been prepared by the reaction of 4-chloromethyl-6,7-dihydroxy coumarin with morpholine. Folescutol has -CH_2- linkage between coumarin and morpholine.

---

1. (a) Maxwell, A. *Trends Microbiol*. , ***5***, 102, (1997).
(b) Calia, H.;Hoerman, L.; Schultz, P.; Lebeau, L.; Mallouh, V.; Wigley, D. B.; Wang,J.C.; Mioskowski, C.; Oudet, P. *J. Mol. Biol*. , **236**, 618,1994).
2. Boehm H. J.; Boehringer M.; Bur D.; Gmeunder H.; Huber W.;Klaus W.; Kostrewa D.;Kuehne H.; Luebbers T.; Meunier-Keller N.; and Mueller F. *J. Med. Chem.* , **43**, 664,(2000).
3. Shiozawa et al., *Proc. Am. Assoc. Cancer Res*. **43**, 2460, (2002).
4. Nakatomi et al., *Jpn. J. Cancer Res.*, **92**, 1973, (2001).
5. Taraye J.P., *Am. Pharm. Franc.*, **33**, 467(1975); Merck Index 12th Ed., p.715,4252(1996); C.A., **60**, 4113, (1964).



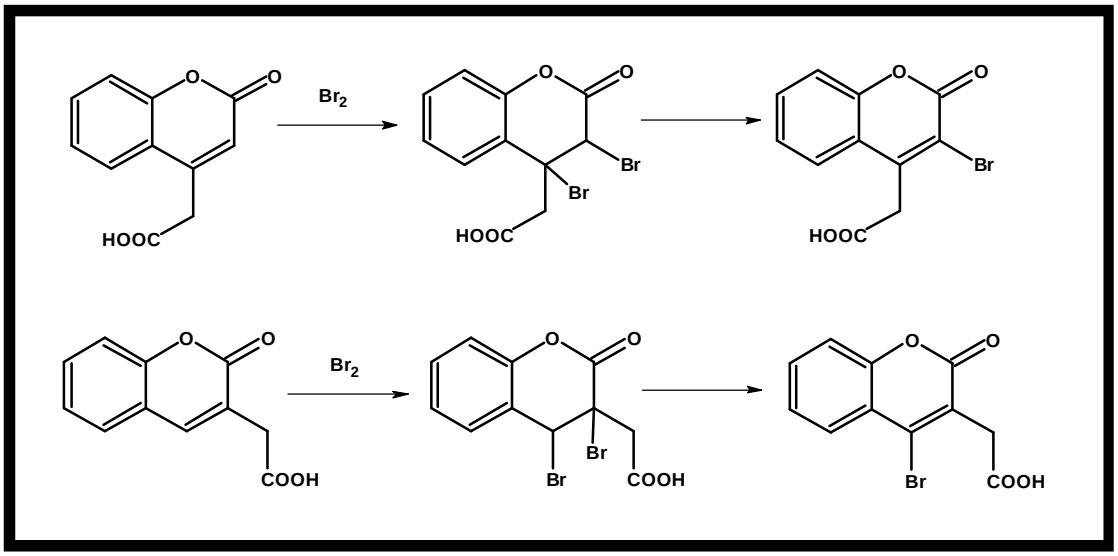
(II)

The chemical reaction of 4-methyl-2-oxo-2*H*-benzopyran-7-yloxy acetic acid hydrazide with some different reagents, such as anhydride compounds, aromatic aldehydes, carbon disulphide, and nitrous acid, yielded the corresponding phthalazine derivatives, hydrazone derivatives, 1,3,4-oxadiazol derivative and acid azide respectively. Treatment of these compounds with absolute alcohols, amines and ethyl amino acid esters gave the corresponding carbamate derivatives, substituted urea derivatives and substituted ethyl acetate respectively. The biological activities of some of the synthesized compounds were evaluated[6-13] in this reported work.

Coumarin-3 and 4-acetic acids comprises within their molecules a double bond between carbon atoms 3 and 4 in the pyrone ring and a reactive methylene group attatched to either of these atoms. A study of the action of chlofine and bromine on these acids was, therefore, expected to yield results of considerable interest by revealing the relative affinities for halogen of the methylene group and the ethenoid linkage.

6. M.E. Abdel-Fateh, Chemistry Department, Faculty of Science, Suez Canal University, Ismallia,Egypt (1998)
7. Gaplovsky, A.; Donovalova, J.; Lacova, M.; Mracnova, R.; El-Shaaer H. M., Journal of Photochemistry and Photobiology- A: Chemistry, **61**,136,(2000).
8. Eicher, T.; Hauptmann, S., The Chemistry of Heterocycles, Georg Thieme Verlag Stuttgard, New York ,(1995).
9. Edwards, A.M.; Howell, J.B.L., Clinical and Experimental Allergy: , 30, 756, (2000).
10. Nohara, A.; Ishiguto, T.; Sanno, Y., Tetrahedron,**13**, 1183, (1974).
11. Stankovicova,H.; Lacova, M.; Gaplovsky, A.; Chovancova J.; Pronayova, N., Tetrahedron **57**,3455, (2001).
12. Sabitha G., Aldrichimica Acta,**29**,15,(1996)
13. Galema, A.S., Chemical Society Reviews,**26**, 233, (1997).

If the usual rule of the halogen attacking primarily the doublebond in the pyrone ring were followed in this case without the interference of any possible influence exerted by the reactive methylene group, an unstable dihalide readily changing, in the case of the coumarin-3-acetic acids into the 4-halogen acids should result thus : -



It is to be noted, however, that if the induced polarities of carbon atoms 3 and 4 in the crotonoid system -CH=CH=C=O, contained in coumarins be taken into account, they would be expected to influence materiallyu these reactions and diminish greatly the probability of bromine being added to the double bond, particularly, in coumarin-3-acetic acids.

Premilinary experiments showed that there was very litttle action at laboratory temperature and exposure to direct sunlight did not effect any improvement.

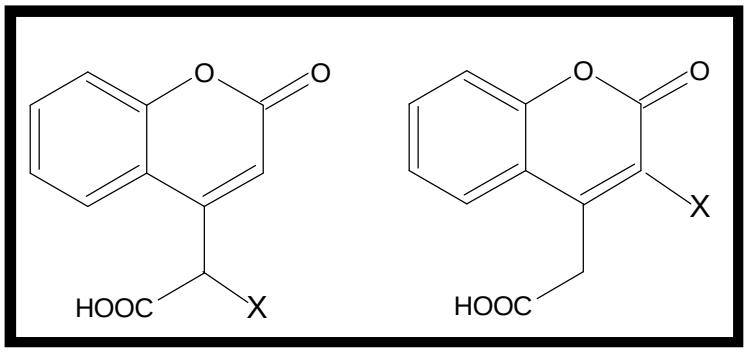
The best results were obtained by heating in glacial acetic acid at the temperature of boiling water bath though in the case of the coumarin-4-acetic acids, a considerable amount of decarboxylation ensued and the product, a mixture of the halogenated acid and the decarboxylated body, had to be separated finally by cold sodium carbonate. It was observed as a general rule that decarboxylation occured to a smaller extent with the chloro than with the bromo acids, the neutral compounds being the main product obtained in the later case under the conditions of experiment. B-Napthapyrone-4-acetic acid provided the only exceptional case in which neither chlorination nor bromination caused any appreciable decarboxylation, but the product in this case is differently constituted.

The halogenation of the coumarin-3-acetic acids was found to proceed much more slowly, a period for 5-6 hours heating at 100^{o} C being normally required for completing the reaction. There was, however, no decarboxylation in this case and the product dissolved completely in cold sodium carbonate. This is to be explained by the superior stability of hte 3-acetic acids which unlike the 4-acetic acids, do not decompose even at their melting points.

The halogenated acids, as is to be expected, proved to be stronger acids than the parent compounds, while the latter were easily liberated from a solution of their sodium salts by dilute acetic acids, at the halogenated bodies did not separate from the alkaline solutions until they were treated with mineral acids. This difference has been utilised in working out an excellent method of separating a mixture of the two acids. The halogencoumarin-4-acetic acids decomposed at their melting points quantitatively into CO_2 and halogen-4-methyl-coumarins which were found in every case to be identical with the product, insoluble in sodium carbonate, obtained during halogenation.

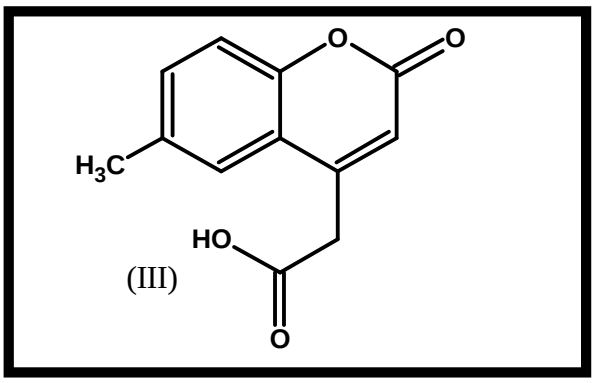
These were neutral compounds, very sparingly soluble in boiling alcohol and having an irritant action on the skin. Nascent hydrogen (zinc-copper couple) reduced them to the 4-methyl coumarins, the replacement of halogen by hydrogen occuring smoothly and quantitatively.

The position of the halogen in the products is deduced from the following considerations : That the halogen could not have entered the benzene ring is proved by (a) their behaviour with boiling aqueous alkali which completely removes the halogen, and (b) oxidation, halogen-free salicyclic acid and hydroxynapthoic acids or their homologues being formed. Their remains little doubt, therefore, that the halogen has either entered the pyrone ring or substituted one of the methylene hydrogen atoms, the following alternative structure s for the halogenated coumarin-4-acetic acids thus predicted.



SYNTHETIC ASPECT :

Coumarin 4-acetic acid were synthesized by many researchers using different methods, Limaye[14] synthesized coumarin-4-acetic acid from phenol and citric acid using concentrated sulfuric acid. Dixit and Gokhale[15,16] condensed, phenols with coumarin-4-acetic acids. Dixit and Padukone[17,18] prepared 6-methyl coumarin-4-acetic acid from citric acid and hydroquinone.6-methyl coumarin-4-acetic acid was prepared from resorcinol and acetone-dicarboxylic acid in presence of different Lewis catalysts like aluminium chloride and also either phosphorous oxychloride or phosphorous pentoxide or thionyl chloride under different reaction time and temperature conditions.



(III)

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Dey[19], Dixit[20], Fries[21], and Radhabai[22], have studied the reaction of phenol with acetone dicarboxylic acid and also using citric acid. Gokhale, Ghosh[23], Chakravati[24], and Banerjee[25] have studied the formation of coumarin-4-acetic acid from substituted phenol and acetone dicarboxylic acid using sulfuric acid as condensing agent. Burton and Muller[26-27] have found that condensation of resorcinol with diethyl acetone dicarboxylate afforded 6-methyl coumarin-4-acetic acid(I).

Schiff bases are important intermediates for the synthesis of some bioactive compounds such as β-lactams[28-31]. Furthermore, they are reported to show a variety of interesting biological actions, including antibacterial[32-37], antifungal[17,18,23], anti mouse hepatitis virus (MHV)[38], inhibition of herpes simplex virus type 1 (HSV-1) and adenovirus type 5 (Ad 5)[39], anticancer[40-43], anti-mosquito larvae[44] and herbicidal activities[45].

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Some Coumarin structures as lead molecules:

Looking to the importance of coumarin system [51-55]with 7-Hydroxy group, number of derivations have successfully entered into the clinical trial. It is very interesting to note that in all cases, the majority of such lead molecules bear a hydroxy group for a wider pharmacological spectrum like antiplatelet, antipsychotic, antidiabetic, antiparkinson, and ischemic disease.

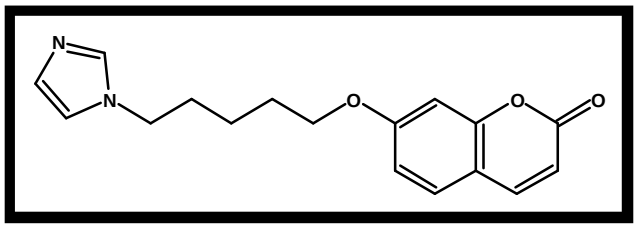
Few such lead molecules are summarised as bewlow :

Chemical Name : 7-[5-(1-Imidazolyl)pentyloxy]coumarin[46]

Therapeutic Group : Antiplatelet Therapy

Highest Phase : Biological Testing

Chemical Structure :

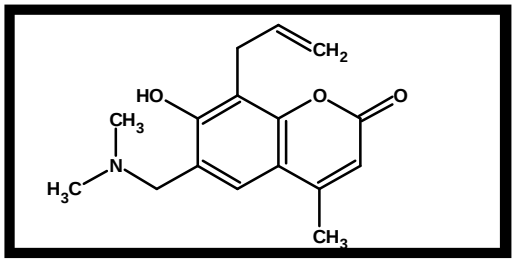


Chemical Name : 4-Methyl-6-(dimethylaminomethyl)-7-hydroxy-8-allyl-coumarin[47]

Therapeutic Group : Antiplatelet Therapy

Highest Phase : Biological Testing

Chemical Structure :

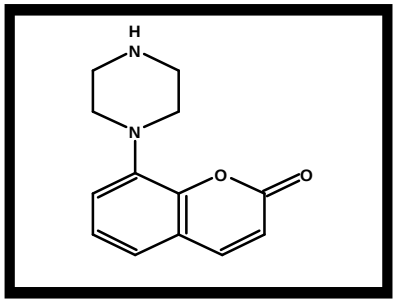


Chemical Name : 8-Piperazino-2H-1-benzopyran-2-one[48]

Therapeutic Group : Antipsychotic Drug

Highest Phase : Biological Testing

Chemical Structure :

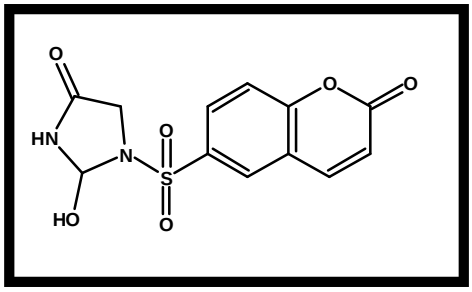


Chemical Name : 1-(2-Oxobenzo[b]pyran-6-ylsulfonyl)imidazolidine-2,4-dione

Therapeutic Group : Antidiabetic Agents

Highest Phase : Biological Testing

Chemical Structure :

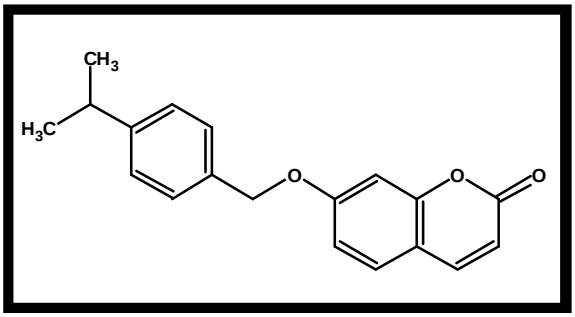


Chemical Name : 7-(4-Isopropylbenzyloxy)coumarin[49]

Therapeutic Group : Antiparkinson Drug

Highest Phase : Biological Testing

Chemical Structure :

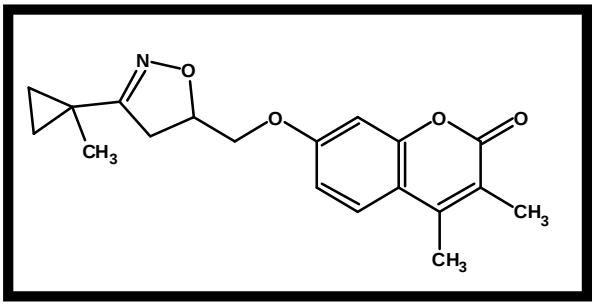


Chemical Name : 3,4-Dimethyl-7-[3-(1-methylcyclopropyl)isoxazol-5-ylmethoxy]coumarin[50]

Therapeutic Group : Antiparkinson Drug

Highest Phase : Biological Testing

Chemical Structure :



The hydrazides attatched to heterocycles like pyridine, pyrazines is believed to be wonderful chemotherapeutic agents. The isoniazide and pyrazinamide are classical examples of such drugs used as antitubercular agents. In the same vein, the coumarin scaffold when appended with -CH_2-C=O- linker gave very good antiviral properties.

On the basis of these findings, in the present work, it was planned to club coumarin moiety with a spacer -CH_2-C=O- with an aim to prepare compounds which mimick "hydrazide" like properties.

In the present chapter, coumarin-4-acetic acids were prepared by literature method & the acids were furthur converted into respective esters, which on treatment with hydrazine hydrate afforded to give 22 new compounds.

In the present work 6-methyl coumarin-4-acetic acid (III) was prepared by Pechmann condensation of resorcinol and citric acid using sulfuric acid as condensing agent.

The compounds were prepared by the hydrazide with appropriately reacting aldehydes to obtain *N*'-[(1)-ethylidene]-2-(6-methyl-2-oxo-2*H*-chromen-4-yl)acetohydrazide in good yields.

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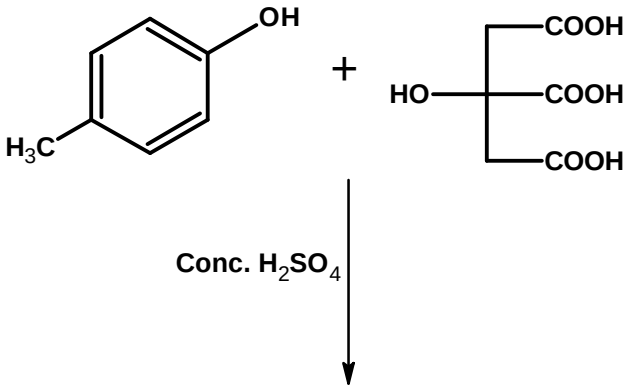
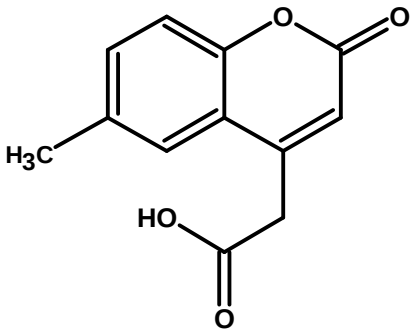
REACTION SCHEME - 4

Step-1



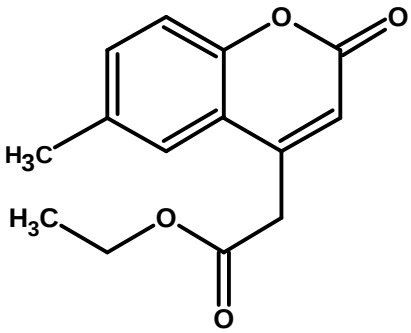


Step-2

Reflux

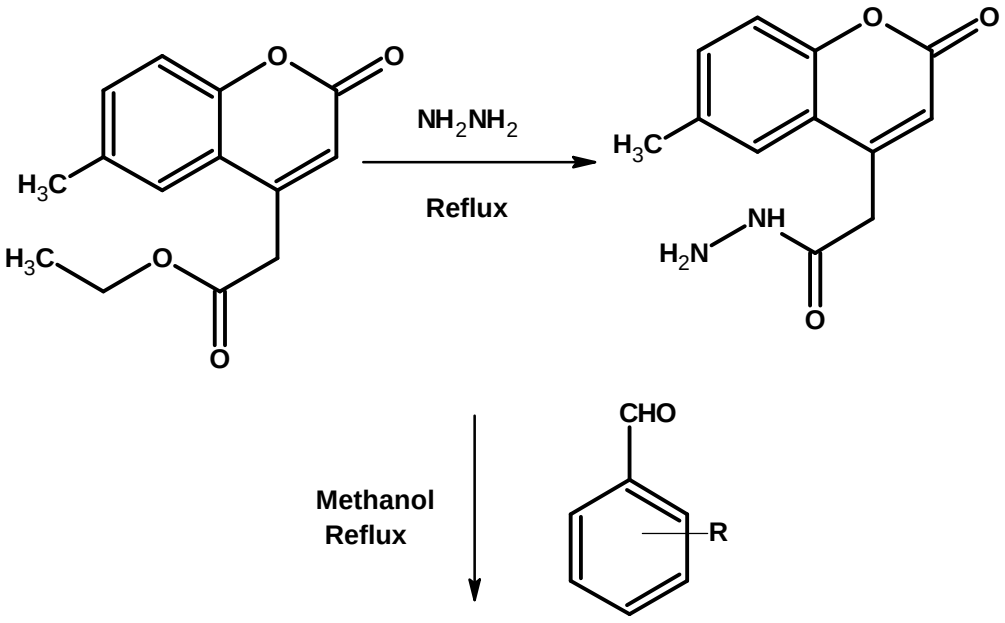
CH_3CH_2OH

Conc. H_2SO_4

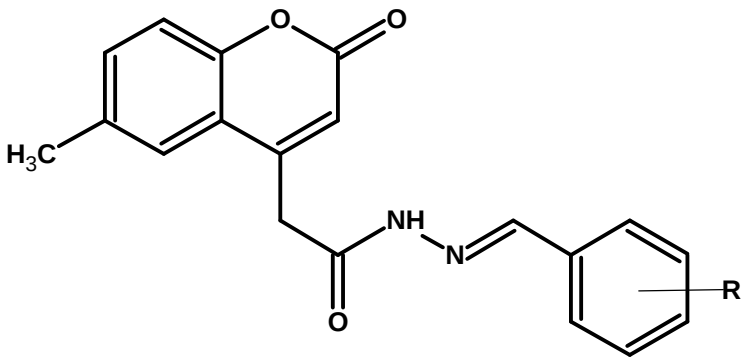


REACTION SCHEME - 4

Step-3



Step-4



EXPERIMENTS:

Preparation of (6-metheyl-2-oxo-2H-chromen-4-yl)acetic acid:

Citric acid (0.1M) and concentrated sulfuric acid (32 ml) were mixed in a RBF and shaken for half an hour. The mixture was then slowly warmed on a water bath up to 70^0C temperature. The mixture was left at this temperature for 10-15 minutes and as soon as gas slackened, the flask was removed from the bath allowed to stand for 15 minutes, till the liquid becomes clear, free from gas bubbles and then cooled to 10^oC in ice bath. p - cresol (0.1M) and concentrated sulfuric acid (14ml) were added gradually to the solution and the mixture was shaken, taking care that the temperature doesn't rise above 10^oC. The dark color solution was left for 48 hour at room temperature. It was then poured into ice-cold water, when a bulky solid separated. It was filtered and treated with sodium bicarbonate (10%) solution and then insoluble matter was removed by filtration. The filtrate, on acidification gave (6-methyl-2-oxo-2*H*-chromen-4-yl) acetic acid. The purity of the compound was checked by TLC(Acetone: Benzene: 5:5).

M.P. 185-187^{o}C(M.P. Reported = 181^{o}C[73]) {R_f Value : 0.54}

Preparation of ethyl (6-methyl-2-oxo-2H-chromen-4-yl) acetate:

(6-methyl-2-oxo-2*H*-chromen-4-yl) acetic acid (10 gm) was taken into 250 ml RBF and to this, 70 ml ethanol along with few drops of sulfuric acid was added and this reaction mixture was allowed to reflux on water bath for 4hr and the reaction was monitored with TLC (Ethyl Acetate: Hexane : 4:6). The reaction mixture was poured into ice-water to obtain the product which was filtered and washed with distilled water. M.P.136^{o}C

Preparation of 2-(6-methyl-2-oxo-2H-chromen-4-yl) acetohydrazide:

A mixture of ethyl (6-methyl-2-oxo-2H-chromen-4-yl) acetate(10gm) and hydrazine hydrate(98% 20ml) was allowed to reflux for 8hr in the heating metal. The completion of reaction was observed by TLC. The reactio mixture was poured in ice, filtered and crystallised from alcohol. M.P. >300^{o}C.

Preparation of *N*'-[(1)-ethylidene]-2-(6-methyl-2-oxo-2*H*-chromen-4-yl)acetohydrazide:

A mixture of 2-(6-methyl-2-oxo-2H-chromen-4-yl)acetohydrazide (0.01mol) and aldehyde (0.01 mol) was refulxed in absolute alcohol for 12hrs. The reaction mixture was cooled to room temperature and was poured to ice and filtered the product. The product was recrystallised from alcohol.

Elemental Analysis

Calculated	= C (71.24%) H(5.04%) N(8.74%)
Experimental	= C (71.20%) H(5.00%) N(8.70%)
Molecular formula	= $C_{19}H_{16}N_2O_3$
Formula Weight	= **320.34**
M.P.	= **209-211^{o}C**.
TLC System	= (Ethyl acetate: Hexane : 4: 6)
R_f Value	= **0.32**
Yield	= **58-65%**

Table 4.1 Physical data of *N* - benzylidene -6-methyl-2-oxa-2H-chromene -4- carbohydrazides.

*** Values in parenthesis denotes the calculated % of composition .**

Table 4.2 Physical data of *N* - benzylidene -6-methyl-2-oxa-2H-chromene -4- carbohydrazides (cont.)

*** Values in parenthesis denotes the calculated % of composition .**

Table 4.3 Physical data of *N* - benzylidene -6-methyl-2-oxa-2H-chromene -4- carbohydrazides (cont.)

*** Values in parenthesis denotes the calculated % of composition .**

SPECTRAL STUDY :

The constitutions of newly synthesized compounds were supported by IR, ^{1}H NMR, Mass and ^{13}C NMR spectral study. The details are as under.

IR Spectral Study :

Instrument : SHIMADZU FT IR-8400 Spectrophotometer

Sample technique : KBr pellet

Frequency range : 400-4000 cm^{-1}

As per the spectral study of the newly synthesized 2H-chromene-4-carbohydrazide ,the carbonyl (>C=O) for the ring and the ester stretching of side chain is observed at range of ~1720-1700 cm^{-1} and 1740-1710 cm^{-1} respectively. The confirmation of methyl group in the ring shows the CH_3 bending in range of ~1385-1360 cm^{-1} and 2854-2823 cm^{-1} **(MSB-1)**.

In case of N'-[(1E)-(Phenyl)methylene]-6-methyl-2-oxo-2H-chromene-4-carbohydrazide **(MSB-1),** a sharp band of -NH stretch is observed at 3423 cm^{-1}. The sharp carbonyl band (>C=O) is seen at 1718 cm^{-1} which indicates the presence of carbonyl (>C=O) group in the ring . The (>C=O) for the amide carbonyl bending is observed at 1654 cm^{-1}.

The aromatic moiety and ring skeleton (like C-C multiple bond stretching, C-H i.p. def. and C-H o.o.p. def.) were observed at 1604 ,1535 , and 1425 cm^{-1} & 759 cm^{-1} .

Similar important values for the various functional group representative peak of the aromatic moiety are seen in the other IR graphs.(Please see Table 4.4)

^{1}H NMR Spectral Study :-

Instrument :BRUKER AC 300 MHz FT-NMR

Internal reference :TMS

Solvent :$CDCl_3$ or DMSO+ d_6

In ^{1}H NMR Spectrum of *N*'-[(1*E*)-(phenyl)methylene]-6-methyl-2-oxo-2*H*-chromene-4-acetohydrazide **(MSB-1)**, presence of methylene protons-CH_2(C_{12}) is confirmed by observing a singlet in upfield at 3.52 δppm, another singlet at 2.26δppm, indicates the presence of C_3 proton of coumarin ring (C_{15}). The protons of the substituted phenyl ring gives different signal pattern. The signal at 7.46 δppm observed as multiplet for the $C_{20,24}$ protons exhibiting ortho-, meta-coupling. Along with this signal, the the signal for the proton of N_{16} is merged and obtained as multiplet which can be calculated by the number of protons. The signal for the protons of $C_{21,22,23}$ is observed as multiplet at 7.91 δppm. A singlet for the proton of C_{18} is observed in very down field at 9.55 δppm due to the presence of neighbouring N atom.

For the signals of coumarin ring it can be observed that a doublet for the C_{10} proton is seen at 7.258 δppm due to meta coupling with the C_8 proton. A quartet is observed at 6.70 δppm for the C_7 & C_8 protons due to ortho and meta coupling with each other with (J=8.1 Hz and J=2.0 Hz). The proton at the C_{18} is observed as singlet in the down field region due to presence of neighbouring N atom at 9.55 δppm.

In ^{1}H NMR of N'-[(1E)-(4-chlorophenyl)methylene]-6-methyl-2-oxo-2H-chromene-4-acetohydrazide **(MSB-11)**, the singlet for -CH_3 (C_{15}) group is observed at 2.24 δppm due to conjugation with ring double bond. The signal for the protons of C_{12} is observed at 3.45 δppm due to presence of >C=O group. For the signal of substituted phenyl ring, a multiplet is seen in the spectra at 6.9 dppm for the protons of $C_{20,21,23,24}$ due to internal ortho and meta coupling with each other. (J=8.4 Hz & J=2.1 Hz). For the proton signal of coumarin ring , a multiplet is observed at 7.29 δppm for the $C_{7,8,10}$ with internal coupling. The signal for the C_{18} proton is observed at 7.414 δppm. A singlet for the -NH proton N_{16} is observed at 6.24 δppm.

Mass Spectral Study :-

Instrument : VG 70-S (70eV) Spectrograph for El

Instrument : JEOL SX 102/DA-6000 Spectrograph for FAB

The molecular ion peak is in concommitant with the molecular weight of the compounds. The newly synthesized compounds were subjected to FAB Mass study. The Fast Atom Bombardment study revealed the molecular ion peak, base peak and ohter relevant fragmentation pattern ot confirm the structure of the molecules.

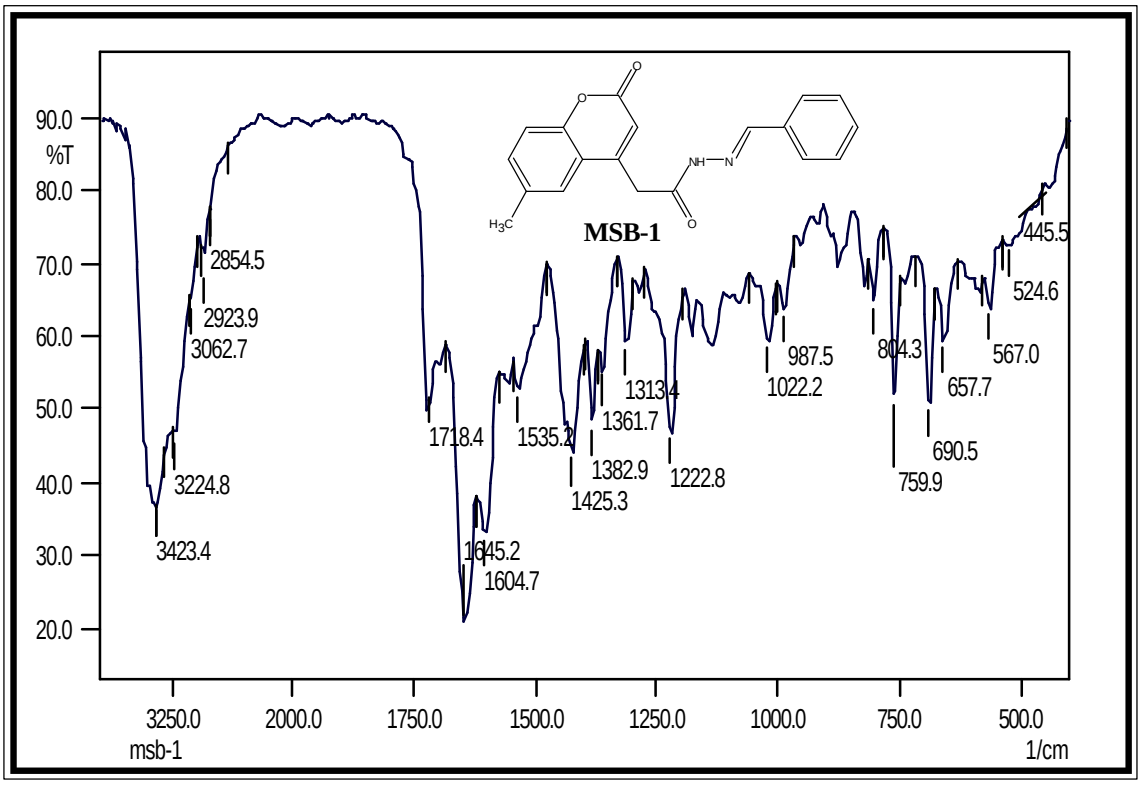
In the Fab Mass of *N*'-[(1*E*)-(3-methoxyphenyl)methylene]-2-(6-methyl-2-oxo-2*H*-chromen-4-yl)acetohydrazide **(MSB-8)** base peak and molecular ion peak both are observed at 351.00 m/z.

In the Fab Mass of *N*'-[(1*E*)-(4-chlorophenyl)methylene]-2-(6-methyl-2-oxo-2*H*-chromen-4-yl)acetohydrazide **(MSB-11)** base peak is observed at 157.00 m/z, while molecular ion peak(m+1) is at 355.00 m/z.

In the Fab Mass of *N*'-[(1*E*)-(3,4-dimethoxyphenyl)methylene]-2-(6-methyl-2-oxo-2*H*-chromen-4-yl)acetohydrazide **(MSB-7)** base peak is observed at 154.0 m/z, while molecular ion peak is at 379.00 m/z as (M+1) peak.

Similarly other compounds were studied for Mass spectral details.

IR Spectrum of *N*'-[(1*E*)-(phenyl)methylene]-6-methyl-2-oxo-2*H*-chromene-4-acetohydrazide(MSB-1)



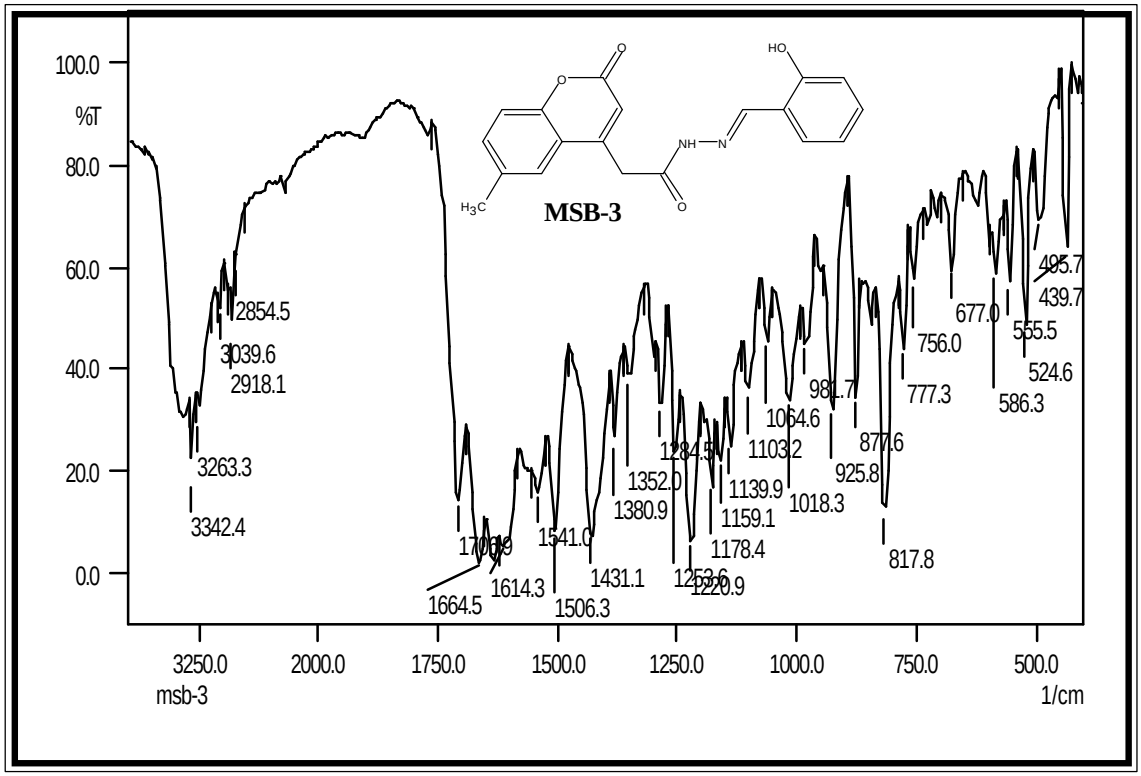
Instrument : SHIMADZU FT IR-8400

Sample technique : KBr Pellet

Frequency range : 4000-400 cm^{-1}

Type	Vibration mode	Frequency cm^{-1}
Carbonyl	>C=O Str (Ring)	1718.4
	>C=O Str (Amide)	1654.2
Amine	-NH Str.	3423.4
Aromatic	ring skeleton vib.	1604.7 1535.2 1425.3
	o.o.p.bending vib. (1,2,3-tri sub.)	804.3 759.9
Alkyl	-CH3 (Assymetric)	2854.5
	-CH3 (symetric)	2923.9
	$-CH_3$ (Bend.)	1361.7

IR Spectrum of *N*'-[(1*E*)-(2-hydroxyphenyl)methylene]-6-methyl-2-oxo-2*H*-chromene-4-acetohydrazide(MSB-3)



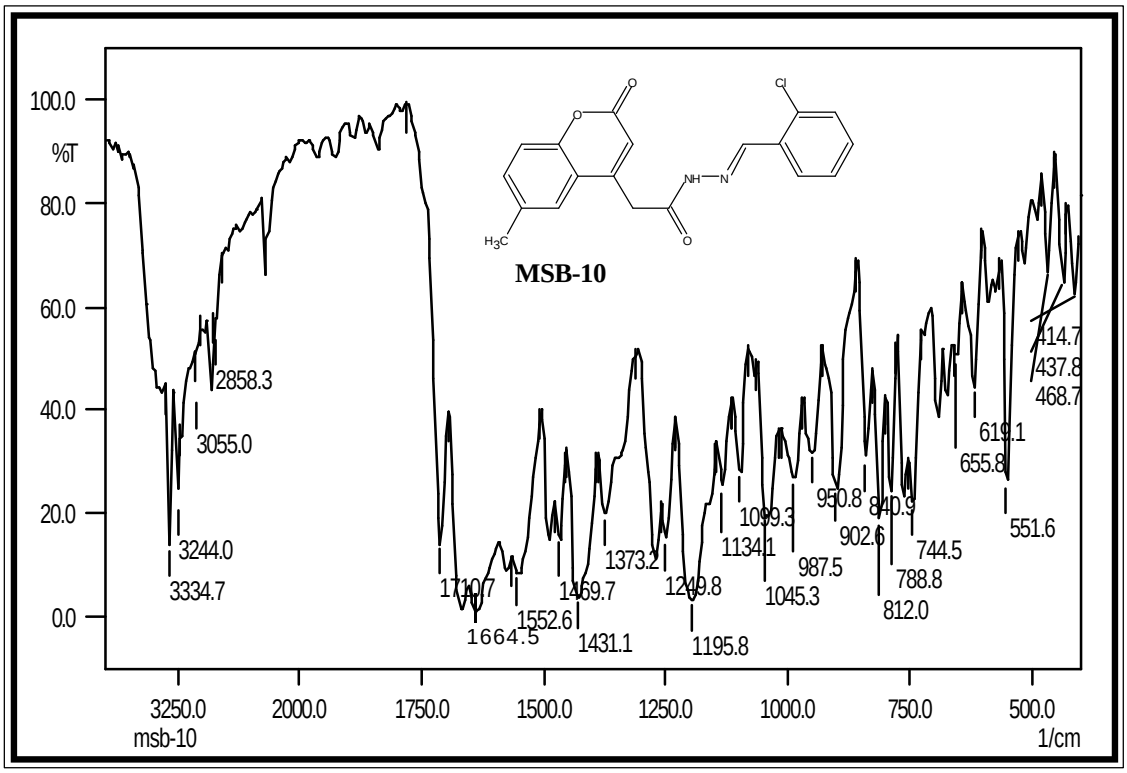
Instrument : SHIMADZU FT IR-8400

Sample technique : KBr Pellet

Frequency range : 4000-400 cm^{-1}

Type	Vibration mode	Frequency cm^{-1}
Carbonyl	>C=O Str (Ring)	1706.9
	>C=O Str (Amide)	1664.5
Alkyl	$-CH_3$ (Assymetric)	2854.5
	$-CH_3$ (symmetric)	3039.6
Amin	-NH Str.	3342.4
Aromatic	ring skeleton vib.	1541.0 1506.3 1431.1
	o.o.p.bending vib. (1,2,3-tri sub.)	777.3 756.0

IR Spectrum of *N*'-[(1*E*)-(2-chlorophenyl)methylene]-6-methyl-2-oxo-2*H*-chromene-4-carbohydrazide(MSB-10)



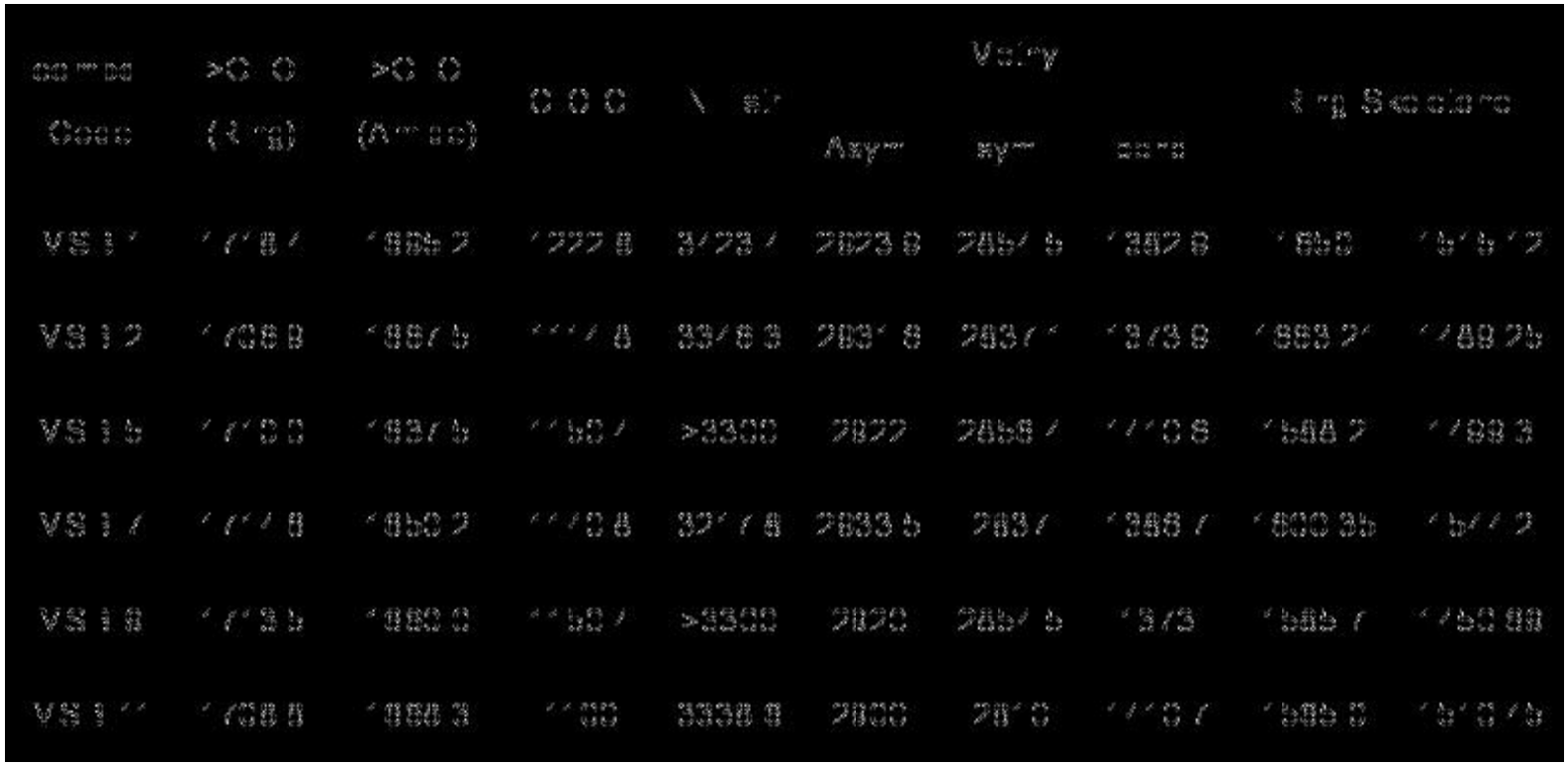
Instrument : SHIMADZU FT IR-8400

Sample technique : KBr Pellet

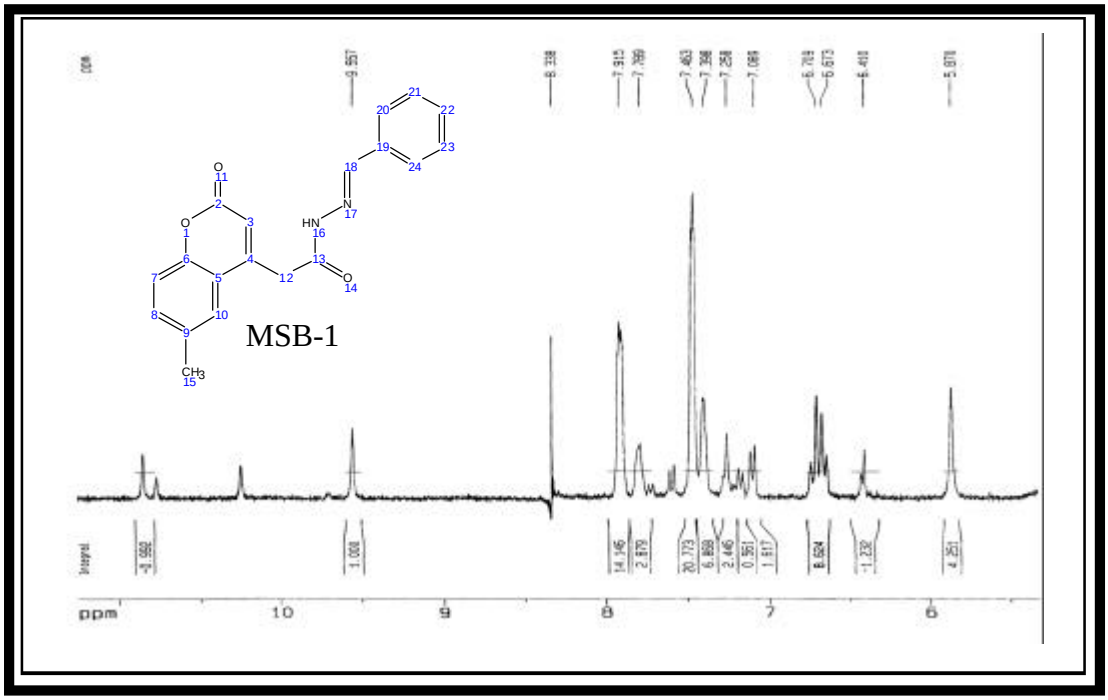
Frequency range : 4000-400 cm^{-1}

Type	Vibration mode	Frequency cm^{-1}
Carbonyl	>C=O Str (Ring)	1710.7
	>C=O Str (Amide)	1664.5
Amine	-NH Str.	3334.7
Aromatic	ring skeleton vib.	1552.6 1431.1 1431.1
	o.o.p.bending vib. (1,2,3-tri sub.)	788.8 744.5
Alkyl	$-CH_3$ (Assymetric)	2858.3
	$-CH_3$ (symmetric)	3055.0
	$-CH_3$ (Bend.)	1373.2

Table 4.4 IR Spectral data of *N*'-[(1*E*)-(substituted phenyl)methylene]-6-methyl-2-oxo-2*H*-chromene-4-carbohydrazides (MSB series



^{1}H NMR Spectra of *N*'-[(1*E*)-(phenyl)methylene]-6-methyl-2-oxo-2*H*-chromene-4-acetohydrazide (MSB-1)



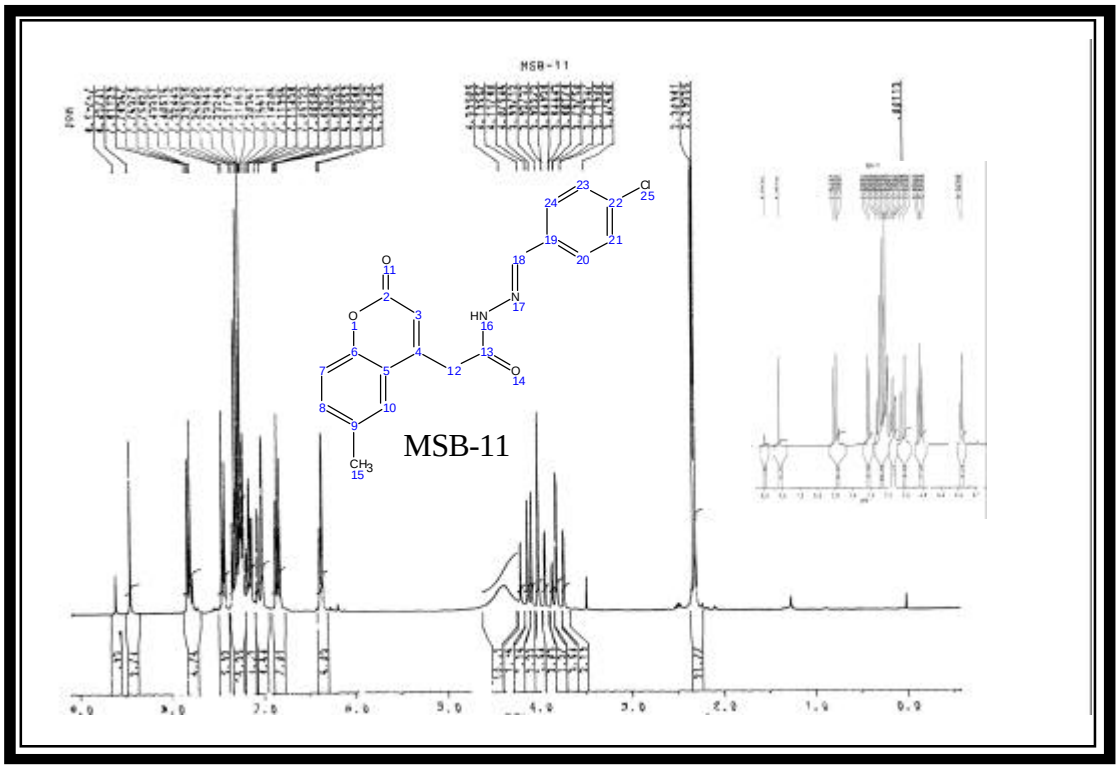
Instrument :BRUKER AC 300 MHz FT-NMR

Standard :TMS

Solvent :DMSO d_6

Chemical Shift d ppm	No. of Proton	Muliplicity	Inference	J. Value
2.266	3H	S	C(15)	
3.52	2H	S	C(12)	
5.87	1H	S	C(3)	
6.67-6.70	2H	Q	C(7,8)	J=8.1 Hz & J=2.0 Hz
7.258	1H	D	C(10)	
7.39-7.46	2H+1H	M	C(20,24 N(16))	
7.78-7.91	3H	M	C(21,22,23)	
9.55	1H	S	C(18)	

^{1}H NMR Spectra of *N*'-[(1*E*)-(4-chlorophenyl)methylene]-6-methyl-2-oxo-2*H*-chromene-4-acetohydrazide (MSB-11)



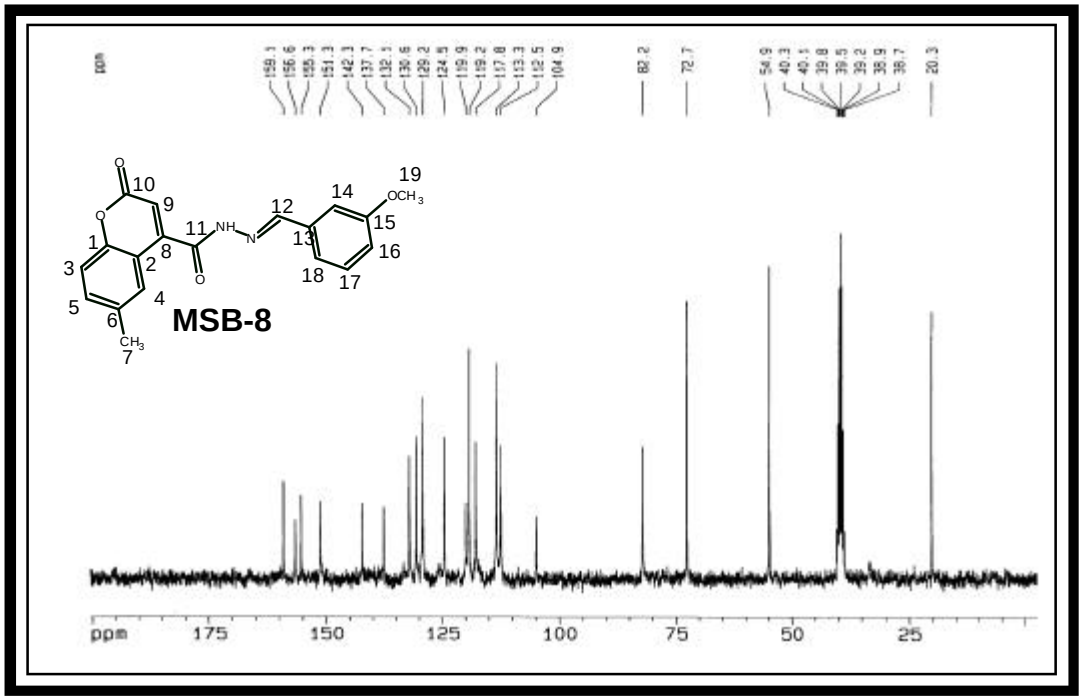
Instrument :BRUKER AC 300 MHz FT-NMR

Standard :TMS

Solvent :DMSO d_6

Chemical Shift d ppm	No. of Proton	Muliplicity	Inference	J. Value
2.24	3H	Single	C(15)	
3.45	2H	s	C(12)	
6.02	1H	s	C(3)	
6.24	1H	s	N(16)	
6.79-690	4H	m	C(20,21, 23,24)	(J=8.4 Hz & J=2.1 Hz)
7.06-7.29	3H	m	C(7,8,10)	
7.414	1H	s	C(18)	

^{13}C Spectra of *N*'-[(1*E*)-(3-methoxyphenyl)methylene]-6-methyl-2-oxo-2*H*-chromene-4-carbohydrazide(MSB-8)



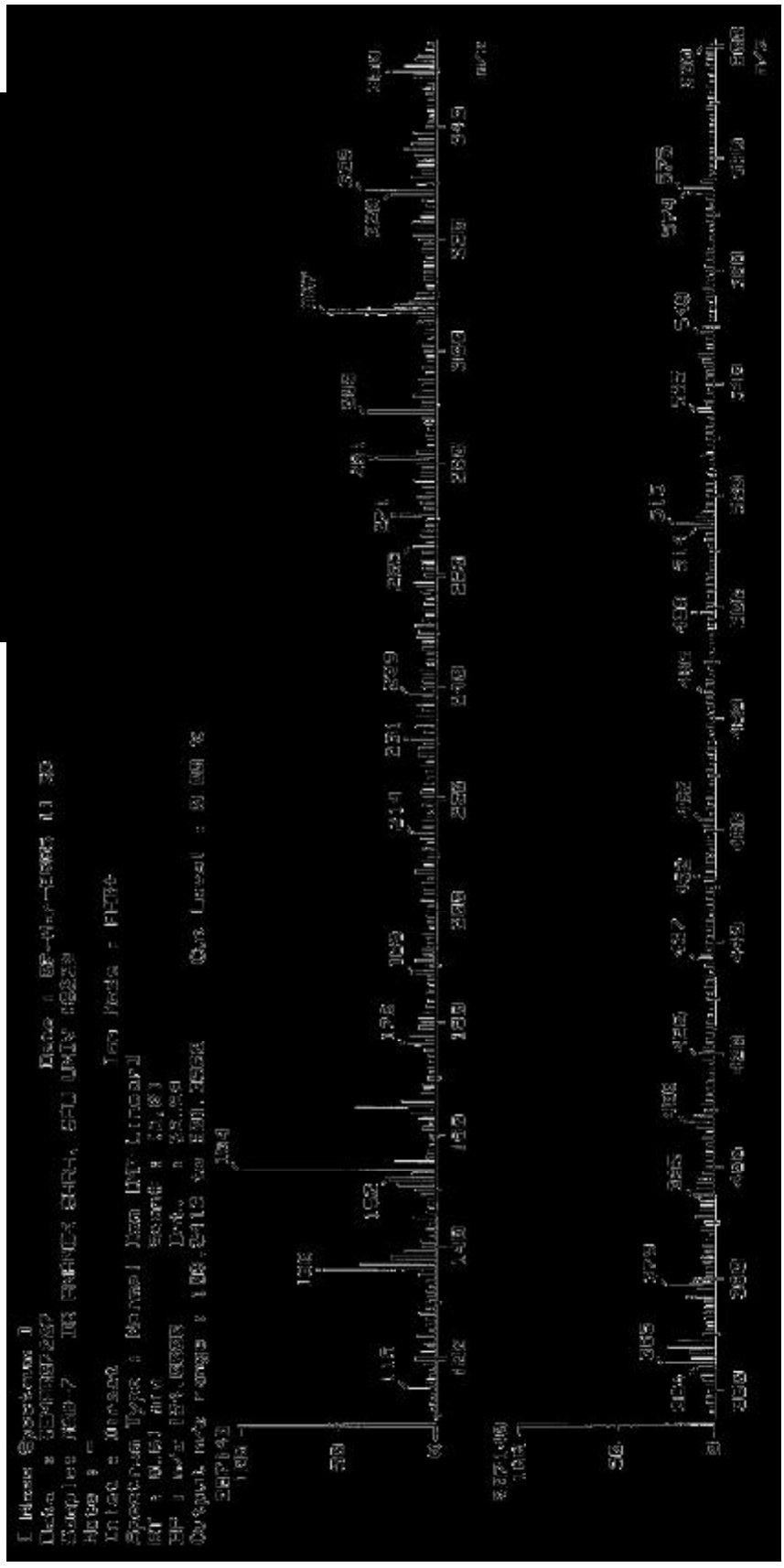
Instrument :BRUKER AC 300 MHz FT-NMR

Standard :TMS

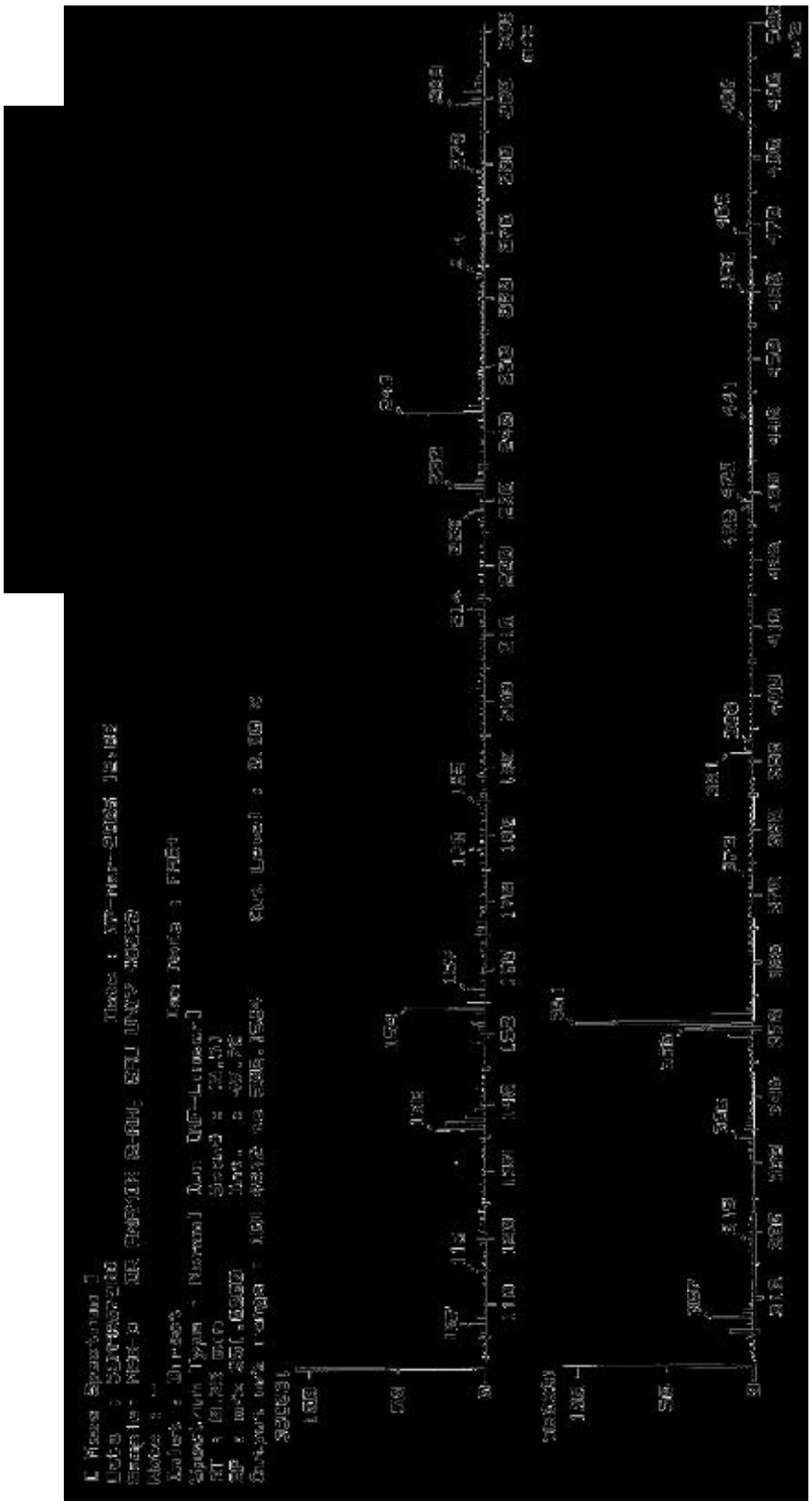
Solvent :DMSO d_6

Chemical Shift d ppm	No.of carbons	Inference
39.5	1C	-CH_3(7)
54.6	1C	-CH(19)
82.2	1C	-CH(8)
104.9	1C	-CH(12)
112.5	1C	-CH(3,4)
113.3	1C	-CH(5)
117.8	1C	-CH(1)
119.2	1C	-CH(2)
129.9	1C	-CH(6)
124.5	1C	-CH(13)
130.0	1C	-CH(14)
137.2	1C	-CH(18)
142.1	1C	-CH(16)
151.3	1C	-CH(17)
155.3	1C	-CH(15)
156.2	1C	-CH(9)
159.0	1C	-CH(10)
159.1	1C	-CH(11)

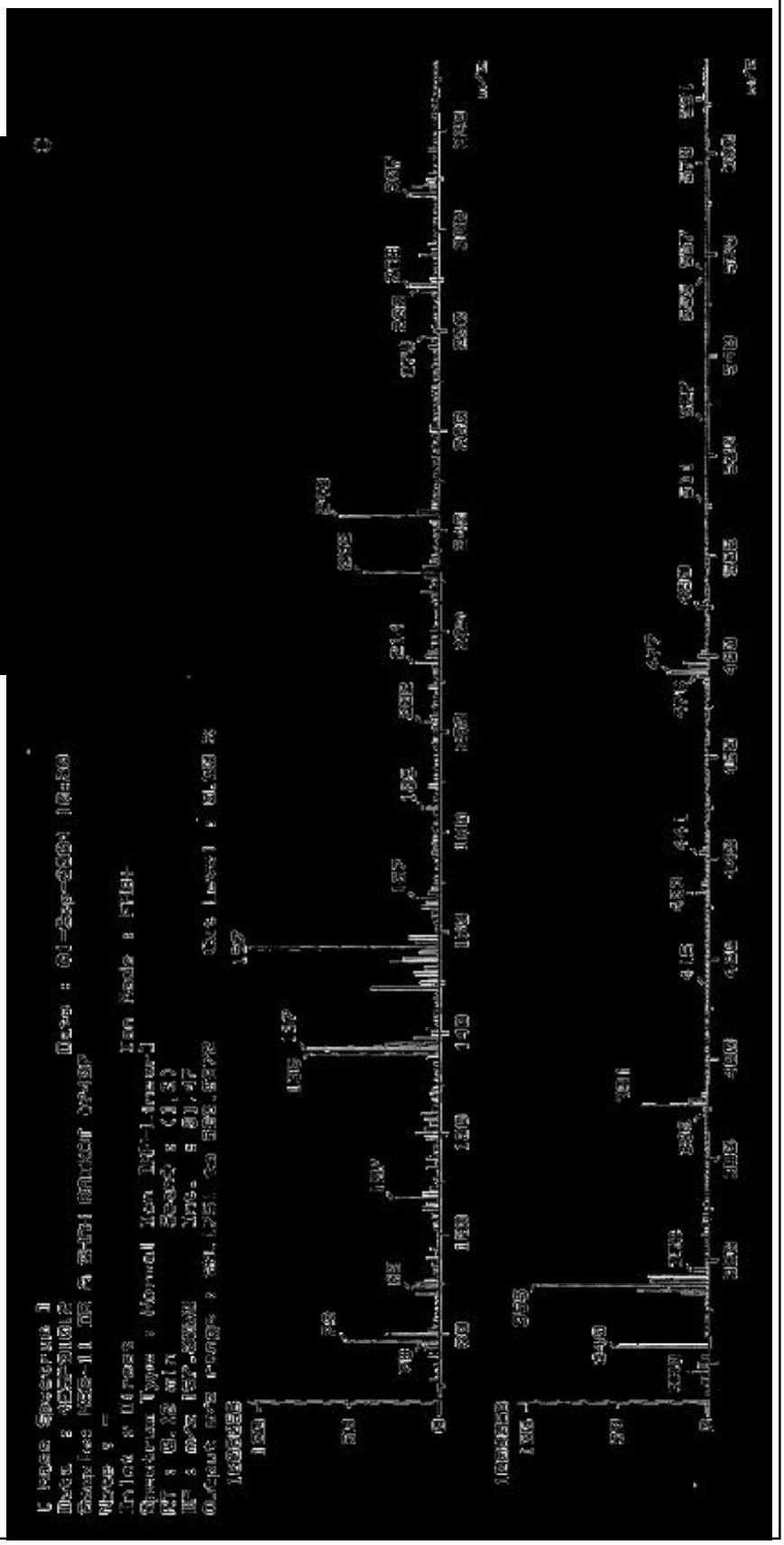
Fab Mass Spectra of *N'*-[(1*E*)-(3,4-dimethoxyphenyl)methylene]-6-methyl-2-oxo-2*H*-chromene-4-acetohydrazide (MSB-7)



Fab Mass Spectra of *N'*-[(1*E*)-(3-methoxyphenyl)methylene]-6-methyl-2-oxo-2*H*-chromene-4-aceto-hydrazide (MSB-8)

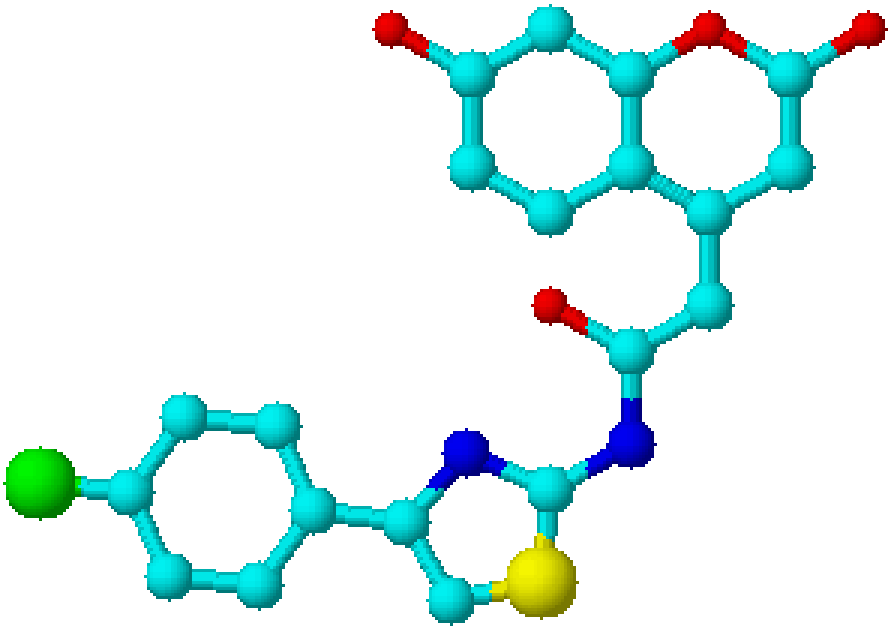


Fab Mass Spectra of *N'*-[(1*E*)-(4-chlorophenyl)methylene]-6-methyl-2-oxo-2*H*-chromene-4-acetohydrazide(MSB-11)



Chapter-5

Preparation N-[4-(4-chlorophenyl)-1,3-thiazol-2-yl]-2-(7-hydroxy-2-oxo-2H-chromen-4-yl)acetamides :



Introduction:

Coumarin nucleus is found in a variety of natural products, which exhibit various pharmacological effects. Derivatives of coumarin are also important drugs having varied properties. There are excellent monographs and review articles[1-5] describing the structure, synthetic reactions and properties of coumarins. Numerous reports have appeared in the literature describing anitmicrobial[6,7], antiradiation[8,9]and antiparasitic properties of the thiazole ring.

Pechmann reaction have been studied by many resesrchers, using various substituted phenols and different β -ketonic esters.

Biginelli[10] condensed quinole with ethyl oxalo acetate in presence of sulfuric acid and obtained 6-hydroxy-coumarin-4-acetic acid . Pechmann, Kraft[11] and Graeger[12] extended this reaction to other phenols.

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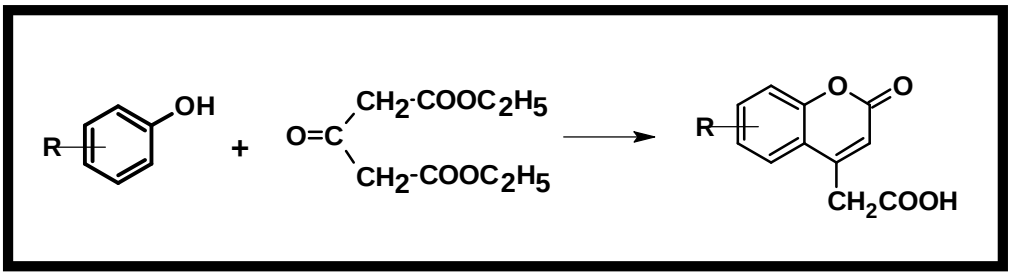
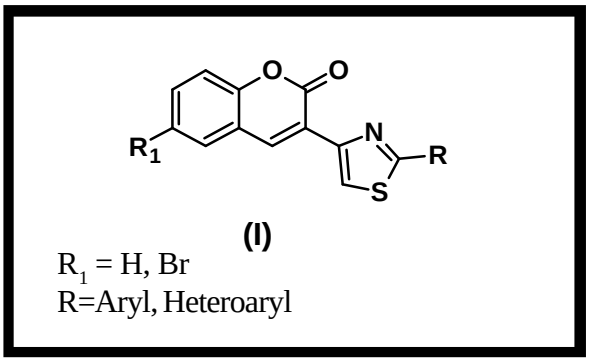
11. Pechmann H, Kraft E.; *Ber.*, 421,**34**,(1901).
12. Pechmann H, Graeger E.; *Ber*.,378, **34** (1901).



Thiazole and coumarin derivatives have been associted with diverse pharmacological activities such as antibiotics, antiinflammatory,antidiabetic, antimicrobial and fungicidal properties.Coumarin associated with thiazole at 3-position exhibited the antibacterial activity against both Gram positive and Gram negative bacteria. Kalluraya and co-workers[13] found that 2-aryl-4-(3-coumarin) thiazoles (I) showed antibacterial activity against *S. aureus, E.coli, B. subtilis*, *P. aurogenosa, Klebesiella* and *Shigella* in compare to nitrofurazone as a standard drug.



(I)

R_1 = H, Br
R=Aryl, Heteroaryl

Coumarin ring linked through $–CH_2$-NH group with benzothiazoles were also studied and gave very good antimicrobial activity. Shinde et al[14]have proved that the Mannich bases of 4-methyl-7-hydroxy coumarins with various aliphatic amines[15] and those derived from benzoxaoles have been found to possess antimicrobial and also CNS stimulating activity. Antifungal activity of 4-methyl-7-hydroxy/acetoxy coumarins and substituted amino benzothiazoles (II) have been reported[16-17].

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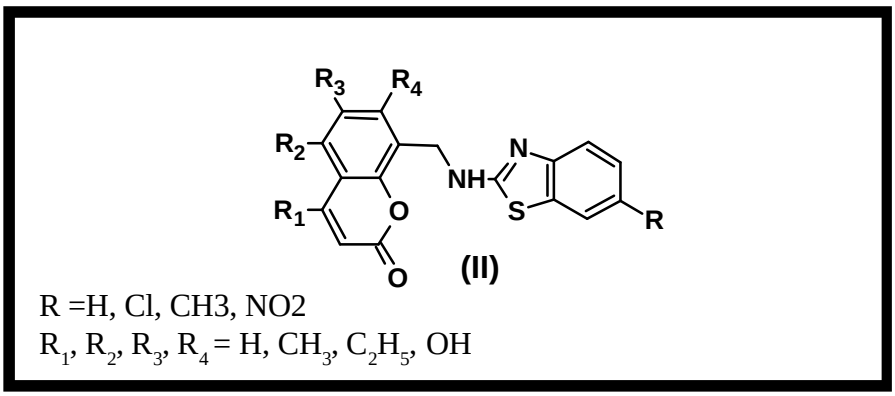
13. Kalluraya B, Chimbalkar R, Vishwanatha P and Kotain Mohan; Ind. *J. Het. Chem.,* **5**, 153-54 (1995).

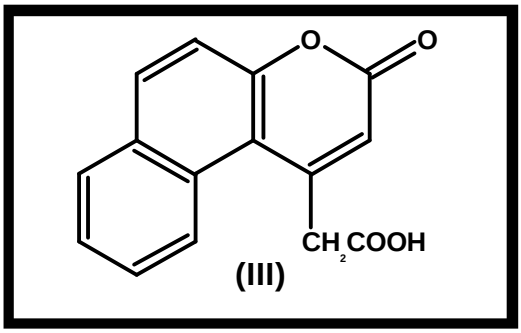


(II)

R =H, Cl, CH3, NO2

R_1, R_2, R_3, R_4 = H, CH_3, C_2H_5, OH

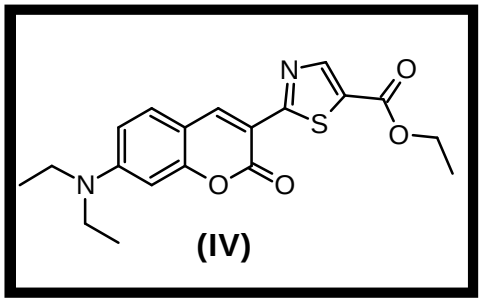
Werder have synthesised over one hundred derivatives of coumarin-3-carboxylic acid. These acids have not yet been found to occur in the vegetable kingdom. He has investigated their utility as medicines. They are sedative in small doses and hypnotic in large doses. Among the derivatives of these acids, the diethylamide has proved to be a good drug in general nervous disease and in varoius neurasthenic and hysterical ailments mannich has found that some hydroxy coumarins possenssing the power of absorbibg ultraviolet light are extensively used as medicinals in skin diseases.



(III)

Alrestatin had an IC_{50} of 10^{-5} - 10^{-6} m against bovine lens aldose reductase with glyceraldehyde or galactose as substrate. Not only did alrestatin at a concentration of 10^{-3} m prevent swelling and the formation of galactinol in lens tissue cultures exposed to 30 mm galactose, it was the first aldose reductase inhibitor to display in vivo activity after oral dosing. 5,6-benzo coumarin-4-acetic acid being the most potent representative had IC_{50} of 20 μm against boving lens aldose reductase.

Recently thiazole & coumarin bearing compound has other uses.In order to find a highly sensitive fluorophore, 3-azolyl-7-diethylaminocoumarin derivatives were synthesized. Both the absorption and fluorescence maxima of the coumarin-thiazole compounds showed red shifts with increase of the molar absorptivities and fluorescence intensities, in comparison with those of the corresponding coumarin-oxazole compounds. Among them, 3-(5-ethoxycarbonyl-1,3-thiazol-2-yl)-7-diethylamino-2H-chromen-2-one **(IV)** was one of the most promising candidate as a fluorophore accessible for analytical purposes in the fields of analytical and biological chemistry.



(IV)

The introduction of a phenyl group at the 3-position of the coumarin ring causes bathochromic shifts in absorption and fluorescence maxima with increases in the molar absorptivity and fluorescence intensity. Development of 7-diethylamino-3-[4-(bromomethyl)-phenyl]-2*H*-chromen-2-one(MPAC-Br) and 4-(7-diethylaminocoumarin-3-yl)benzoyl cyanide (DACB-CN), which are among the most sensitive and practically useful fluorescent derivatization reagents for carboxylic acids and for alcohols, respectively. In order to develop more highly sensitive fluorophores, the introduction of an azole in the place of the phenyl group at the 3-position of a coumarin ring was considered.

There are a few examples of studies on 3-(benzazol-2-yl) coumarin derivatives, but little is known about the systematic study of 3-azolyl-substituted coumarins.The synthesis and spectroscopic properties of 3-azolyl-7-diethylamino coumarins as screening in for fluorophores.

2-Aminothiazoles and their derivatives have long been used as precursors for the synthesis of biologically active molecules. Because of the wide spectrum of activity shown by the thiazole moiety, numerous thiazoles substituted with different groups at various positions have been prepared. In recent years, several new methods for the preparation of 2-aminothiazole derivatives and reactions have been reported, including waste-free techniques. 2-Amino-4-substituted-1,3-thiazoles, in the presence of various reagents, undergo different types of reactions to yield other heterocyclic compounds, e.g., thiazolo[3,2-a]pyrimidine-5-ones, thiazolo[3,2-a]-pyrimidine-7-ones, imidazo[2,1-b]thiazoles, thiazolo[3,2-a]benzimidazoles, etc. Consequently we were interested in surveying the synthetic utility of 2-amino-4-substituted-thiazoles.

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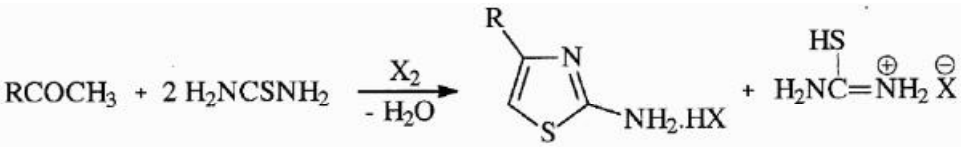
Synthesis of 2-amino-4-substituted-1,3-thiazoles from ketones

(1) Using halogen and thiourea

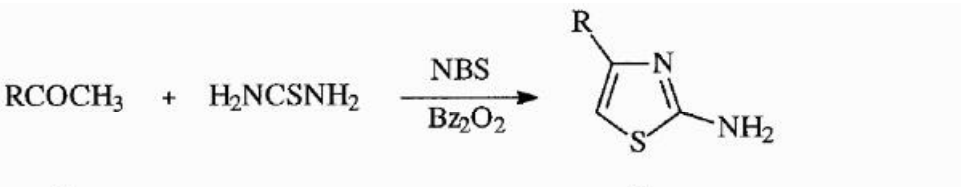
Methyl ketones of the type **1** react directly with one mole of halogen and two moles of thiourea to give 2-aminothiazoles (**2**) in excellent yield[19, 20] (R=phenyl)



1 **2**

(2) Using NBS and thiourea

Methyl ketones **1** react with thiourea in the presence of *N*-bromosuccinimide (NBS) using benzoyl peroxide as radical initiator to furnish 2-aminothiazoles (**3**)[21,22.]

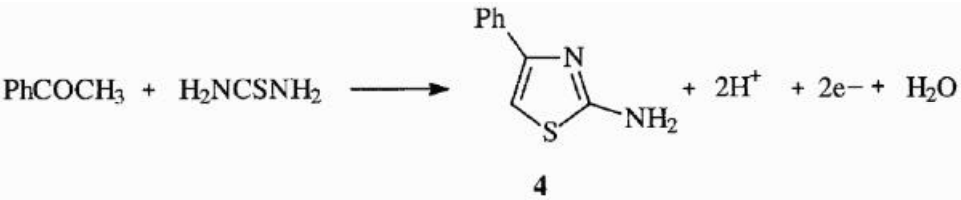


1 **3**

R = (un)substituted-3-coumarinyl, (un)substituted phenyl

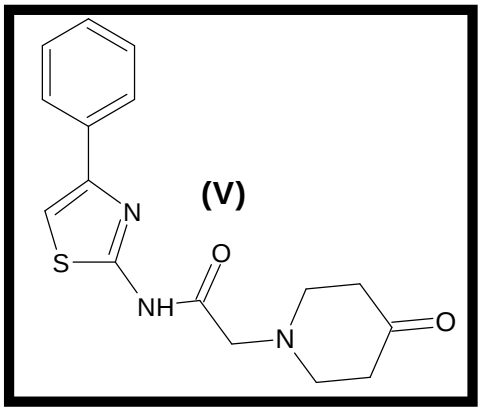
(3) Using oxidizing agents and thiourea

An oxidative process can accomplish the formation of 2-aminothiazoles from a ketone and thiourea. Thus, mixtures of thiourea and acetophenone have been treated with various oxidizing agents, namely sulfuryl chloride, chlorosulfonic acid, thionyl chloride, sulfur monochloride, sulfur trioxide, sulfuric acid, nitric acid and sulfur. In each case a considerable quantity of 2-amino-4-phenylthiazole (4) was obtained[23.]



4

N-Phenyl-N'-(2-thiazolyl) urea have displayed potential anti-parkinson, anthelmintic and trichimodial activities.[24,25] Bhargava[26] have synthesized 2,6-diaryl-3-ethoxy cabonyl-4-piperidino acetyl-2'-amino-4'-phenyl thiazole **(V)** as local anaesthetic.



2-substuituted amino thiazole derivatives were prepared as antipsychotics agent by Rao[27],N.Atsuo[28,29] and S.Masaru[30], et.al. have synthesized 2-amino 4,5-diphenyl derivatives and are useful as inhibitors of blood platelet aggregation.

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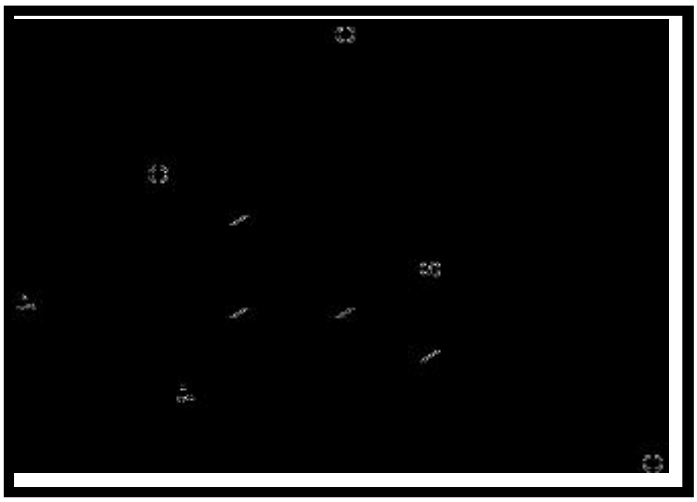
30. S.Masara, I. Hisataka,I. Kouichi, F.Noriyaki, S. Inko, T. Yasuaki, M. Hidenao, N. Kanji; *PCT Int. Appl.* **WO** 92,15,570; *Chem. Abstr.* **118**,191727g (1993).

A new series of such thiazole compounds were studied by Patnaik[31] bearing 2-substituted amino thiazoles moiety in the structure having fungicidal activity.



Few important thiazole bearing drug molecules are reported here which have desired pharmacological profile.

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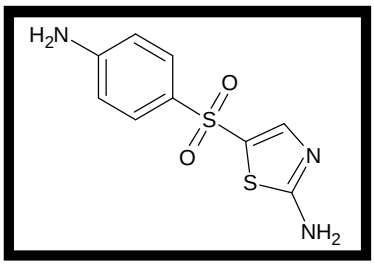
Some thiazole containing drugs :

Drug Name : Thiazol-sulfone[32]

Chemical Structure :

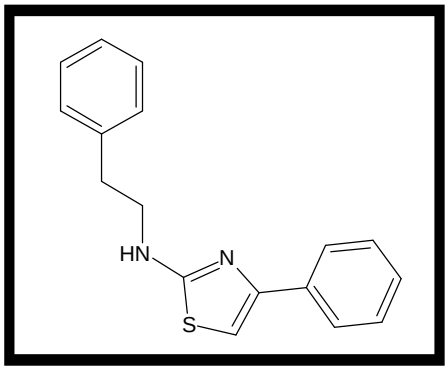


Chemical Name : 5-[(4-aminophenyl)sulfonyl]-1,3-thiazol-2-amine

Activity : Anti-bacterial Drug

Drug Name : Fanetizole[33]

Chemical Structure :

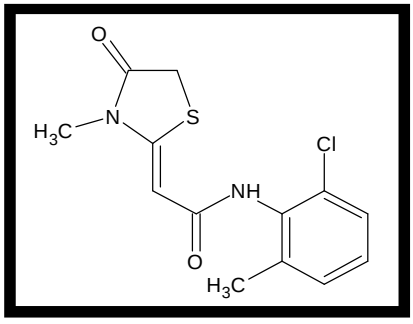


Chemical Name : 4-phenyl-*N*-(2-phenylethyl)-1,3-thiazol-2-amine

Activity : Immuno regulating

Drug Name : Ralitoline[34]

Chemical Structure :

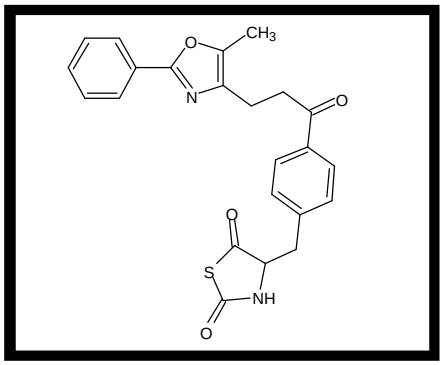


Chemical Name : (2*Z*)-*N*-(2-chloro-6-methylphenyl)-2-(3-methyl-4-oxo-1,3-thiazolidin-2-ylidene)acetamide

Activity : Anti-convulsant

Drug Name : Dargliatzone[35]

Chemical Structure :

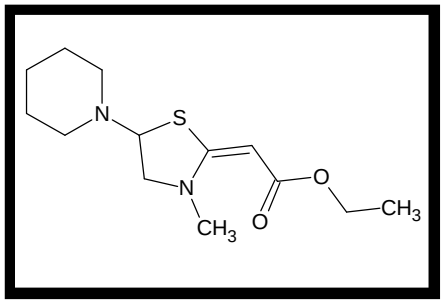


Chemical Name :4-{4-[3-(5-methyl-2-phenyl-1,3-oxazol-4-yl)propanoyl] benzyl}-1,3-thiazolidine-2,5-dione

Activity : Antidiabetic

Drug Name : Etozoline[36]

Chemical Structure :



Chemical Name : Ethyl (2*E*)-(3-methyl-5-piperidin-1-yl-1,3-thiazolidin-2-ylidene)acetate

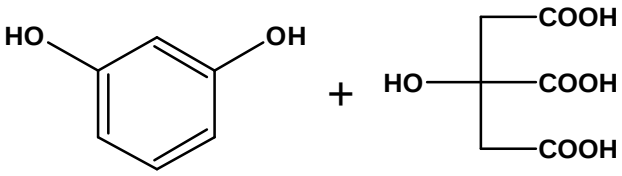
Activity : Diuretic

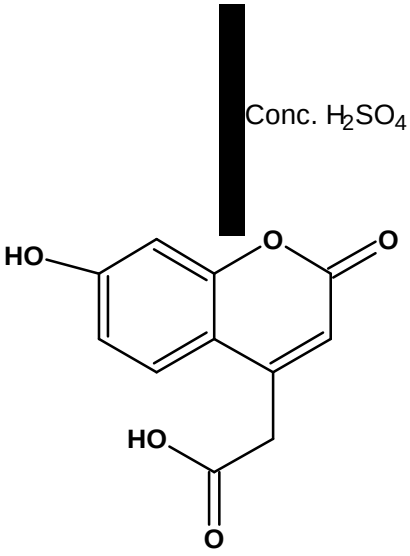
As mentioned earlier, some important findings were obtained in this laboratory for the antiviral properties of coumarin derivatives linked by -CH_2-C=O-NH spacer with thiazole units. The promising results has encouraged us for the furthur derivatization of these compounds.

Thus in the current chapter, 7-hydroxy-4-acetic acid is prepared by Pechmann condensation which was converted into ethyl ester & it was treated with substituted 2-amino thiazoles to obtain the title compounds.

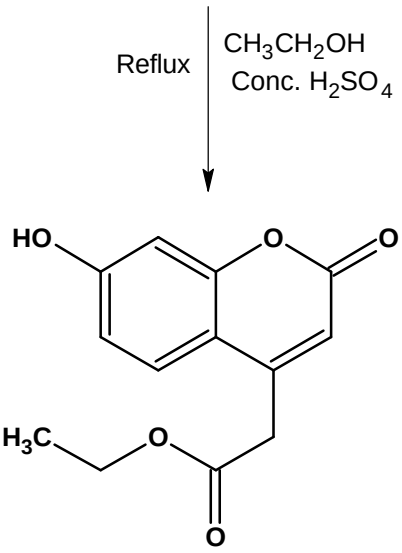
REACTION SCHEME : 5

Step - 1



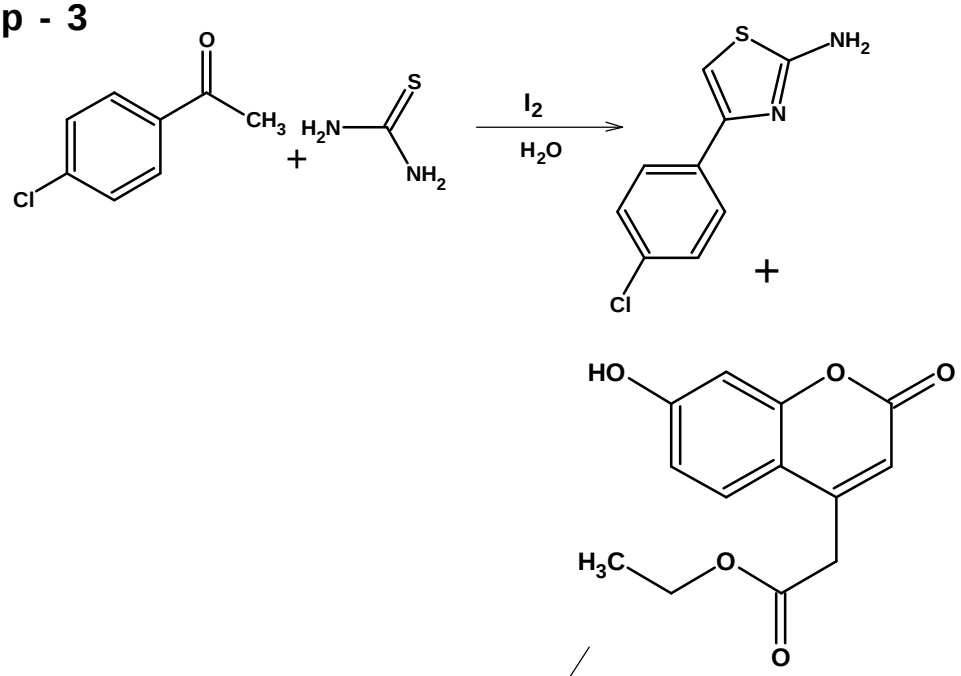


Step - 2



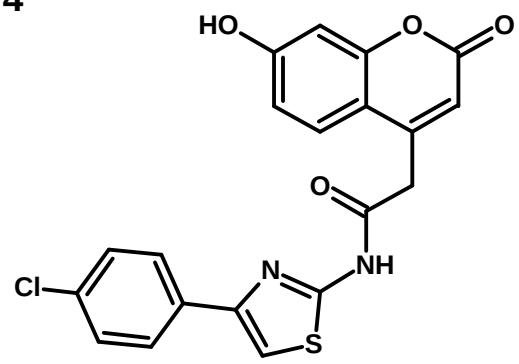
REACTION SCHEME : 5

Step - 3



$POCl_3$ Diphenyl Ether

Step - 4



EXPERIMENTS:

Preparation of (7-hydroxy-2-oxo-2H-chromen-4-yl)acetic acid:

Citric acid (0.1M) and concentrated sulfuric acid (32 ml) were mixed in a RBF and shaken for half an hour. The mixture was then slowly warmed on a water bath up to 70^0C temperature. The mixture was left at this temperature for 10-15 minutes and as soon as gas slackened, the flask was removed from the bath allowed to stand for 15 minutes, till the liquid becomes clear, free from gas bubbles and then cooled to 10^oc in ice bath. Resorcinol (0.1M) and concentrated sulfuric acid (14ml) were added gradually to the solution and the mixture was shaken, taking care that the temperature doesn't rise above 10^oC. The dark color solution was left for 48 hour at room temperature. It was then poured into ice-cold water, when a bulky solid separated. It was filtered and treated with sodium bicarbonate (10%) solution and then insoluble matter was removed by filtration. The filtrate, on acidification gave (7-hydroxy-2-oxo-2*H*-chromen-4-yl) acetic acid. The purity of the compound was checked by TLC(Acetone: Benzene: 5:5).
M.P. 205-207^{o}C(M.P. Reported = 209-210^{o}C[36])

Preparation of ethyl (7-hydroxy-2-oxo-2H-chromen-4-yl) acetate:

(7-hydroxy-2-oxo-2*H*-chromen-4-yl) acetic acid (10 gm) was taken into 250 ml RBF and to this, 70 ml ethanol along with few drops of sulfuric acid was added. The reaction mixture was allowed to reflux on water bath for 4hr and the reaction was monitored with TLC(Ethyl Acetate: Hexane : 4:6). The reaction mixture was poured into ice-water to obtain the product which was filtered, washed with distilled water and dried.
M.P.146^{o}C (Reported = 147-149 ^{o}C[36]).

Preparation of 4-(4-chlorophenyl)-1,3-thiazol-2-amine:

They were prepared by reported methods[50]. Following compound were repared.

	Melting Point
2-Aminothiazole	88-91 ^{0}C (Repoted m.p.164-65^{0}C)
4-Chlorophenyl-2-aminothiazole	160-61^{0}C(Repoted m.p.164-65^{0}C)
4-Metheylphenyl-2-aminothiazole	123-25^{0}C
2-Hydroxyphenyl-2-aminothiazole	168-69^{0}C

Preparation of *N*-[4-(4-chlorophenyl)-1,3-thiazol-2-yl]-2-(7-hydroxy-2-oxo-2*H*-chromen-4-yl) acetamide :

A mixture of 7-hydroxy coumarin -4-ethyl ester(0.01mole) and 4-(chlorophenyl)-2-amino thiazole (0.01 mole) was taken in diphenyl ether (6-8 ml. along with 2-4 drops of $POCl_3$. The mass was refluxed for 10 hours with stirring and excess of diphenyl ether was distilled out the resultant mass was poured into solvent. It is then washed, filtered and then dried for the cyrstallization. The crystallization carried out in ethanol.

Elemental Analysis

Calculated = C (58.18%) H(3.17%) N(6.79%)

Experimental = C (58.02%) H(3.15%) N(6.75%)

Molecular formula = $C_{20}H_{13}ClN_2O_4S$

Formula Weight = **412.84**

M.P. = **153-155^{o}C**.

TLC System = (Ethyl acetate: Hexane : 4::6)

R_f Value = 0.41

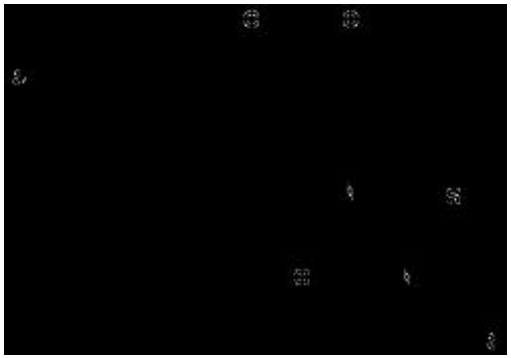
Yield = **33.2%**

Similarly all other compounds were prepared by employing different substituted 2-amino thiazoles.

Table 5.1 Physical data of 2-(2-oxo-2*H*-chromen-4-yl)-*N*-(4-substituted phenyl-1,3-thiazol--2-yl)acetamides

*** Values in parenthesis denotes the calculated % of composition .**

Table 5.2 Physical data of 2-(2-oxo-2*H*-chromen-4-yl)-*N*-(4-substituted phenyl-1,3-thiazol--2-yl)acetamides (cont.)



*** Values in parenthesis denotes the calculated % of composition .**

Table 5.3 Physical data of 2-(2-oxo-2*H*-chromen-4-yl)-*N*-(4-substituted phenyl-1,3-thiazol--2-yl)acetamides (cont.)

*** Values in parenthesis denotes the calculated % of composition .**

SPECTRAL STUDY :

The constitutions of newly synthesized compounds were supported by IR, ^{1}H NMR, Mass and ^{13}C NMRspectral study. The details are as under.

IR Spectral Study :

Instrument : SHIMADZU FT IR-8400 Spectrophotometer

Sample technique : KBr pellet

Frequency range : 400-4000 cm^{-1}

In coumarin, fused thiazole ring the values for the ring carbonyl and the amide carbonyl group are recognised in the IR peaks at around 1730 cm^{-1} and 1668.3 cm^{-1} respectively. The peak for the -NH stretch group is observed at around 3300 cm^{-1}.The aromatic ring stretching are observed at around 2990-2800 cm^{-1}. The ring C=C skeleton bending vibrations are observed at around 1600-1450 cm^{-1}.

In case of *N*-[4-(2-hydroxyphenyl)-1,3-thiazol-2-yl]-2-(6-methyl-2-oxo-2*H*-chromen-4-yl) acetamide **(ASM-55),** a broad band of -NH is observed at 3423.4cm^{-1}. The sharp carbonyl band (>C=O) for the ring is seen at 1735cm^{-1} which is slightly higher due to presence of O atom while the amide linkage carbonyl (>C=O) is observed at 1654.2 cm^{-1}. The hydroxy group present is identified by a broad band at 3353 cm^{-1}. The methyl group present is observed as bending at 1375.2 cm^{-1}.

The aromatic moiety and ring skeleton (like C-C multiple bond stretching, C-H stretch and bending) were observed at 1625, 1571, and 1494 cm^{-1}. The band for (C-S) inthe inplane bending region is observed at 1242-1163 cm^{-1}.

The characteristic bands and comparative chart of other synthesized compounds are rePorted in Table 5.4 on page 171.

^{1}H NMR Spectral Study :-

Instrument :**BRUKER AC 300 MHz FT-NMR**

Internal reference :**TMS**

Solvent :**$CDCl_3$ or DMSO d_6**

^{1}H NMR analysis of 2-(7-hydroxy-2-oxo-2*H*-chromen-4-yl)-*N*-1,3-thiazol-2-ylacetamide (ASM-50).

The spectra of compound **(ASM-50),** shows the presence of the thiazole ring as confirmed by signals obtained as doublet in the down field region at 7.225 δppm & 7.485 δppm for the protons at C_4 and C_5 with coupling constant (J=3.6 Hz).

The -CH_2 group linkage at the C_9 position gives signal as singlet at 4.020 δppm due to presence of (>C=O) group of amide linkage.The amide linkage -NH gives signal at 7.948 δppm as a broad singlet.

The signal for the -OH group at 7th postition of coumarin is obtained at 10.5 δppm as broad singlet.The proton at C_{15} is obtained as singlet at 6.25 δppm. The signal for the proton at C_{19} position is obtained as doublet due to meta-coupling with proton of C_{17} at 6.73 δppm with (J=2.1 Hz). Similarly, the proton at C_{17} gives signal at 6.79-6.82 δppm as double of doublet with (J=6.3 Hz & J=2.1 Hz). This is because this proton undergoes ortho-coupling with proton of C_{16} and meta-coupling C_{19} proton.

The signal for the C_{16} proton is obtained at 7.6 δppm with (J=8.1 Hz) bearing ortho-coupling with C_{17} proton.

Similarly, other protons were identified in other compounds and are recorded.

Mass Spectral Study :-

Instrument : VG 70-S (70eV) Spectrograph for El

Instrument : JEOL SX 102/DA-6000 Spectrograph for FAB

The newly synthesized compounds were subjected to FAB Mass study. The Fast bombardment study revealed the Molecular ion peak, base peaks and other relevant fragmentation pattern to confirm the structure of the molecules. The molecular ion peak is in concormitant with the molecular weight of the compounds.

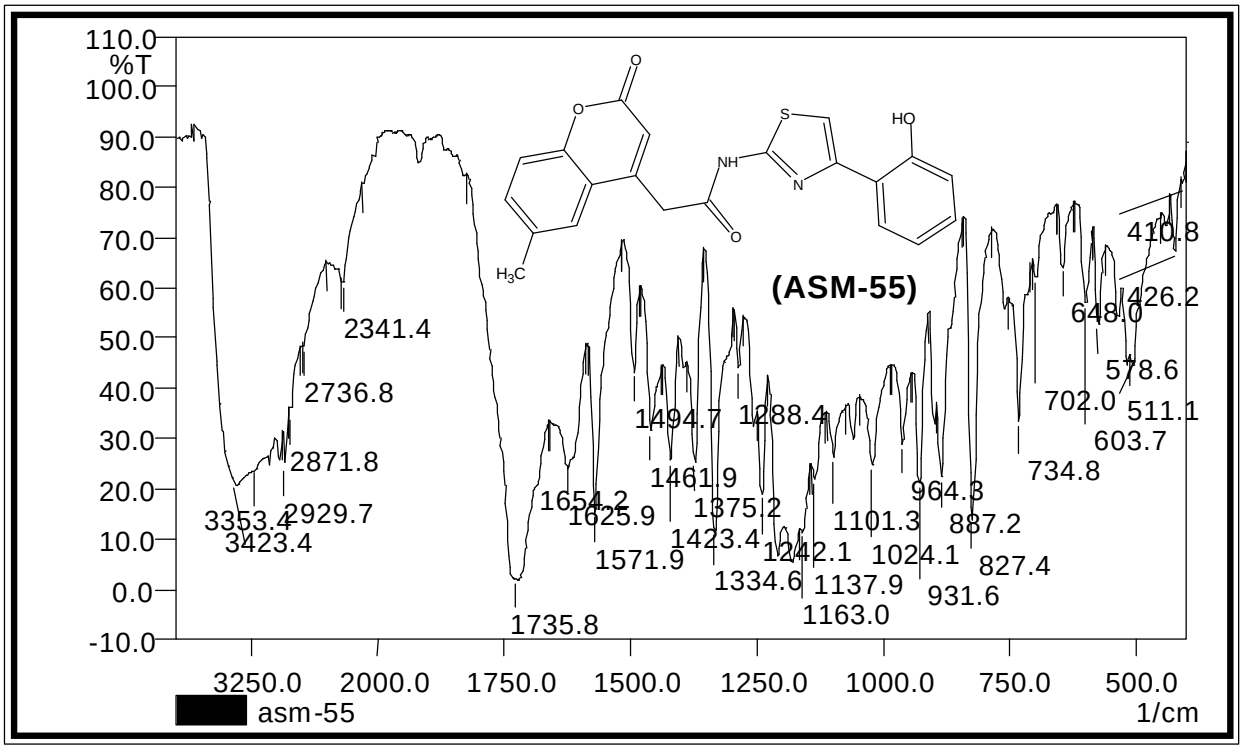
In case of Fab Mass of 2-(7-hydroxy-2-oxo-2H-chromen-4-yl)-N-1,3-thiazol-2-ylacetamide **(ASM-50),** base peak is observed at 154.0 m/z, while molecular ion peak is at 303.00 m/z (M+1) peak.

In case of Fab Mass of 2-(7-hydroxy-2-oxo-2H-chromen-4-yl)-N-[4-(2-hydroxyphenyl)1,3-thiazol-2-yl]acetamide **(ASM-52)** base peak is observed at 259.0 m/z, while molecular ion peak at 394.00 m/z.

In the Fab Mass of 2-(2-oxo-2H-benzo[h]chromen-4-yl)-N-1,3-thiazol-2-ylacetamide **(ASM-62)** base peak is observed at 259.0 m/z, while molecular ion peak is at 394.00 m/z.

The mass fragmentation pattern of newly synthesized molecules is in total agreement with the suggested structures.

IR Spectrum of *N*-[4-(2-hydroxyphenyl)-1,3-thiazol-2-yl]-2-(6-methyl-2-oxo-2*H*-chromen-4-yl)acetamide (ASM-55)



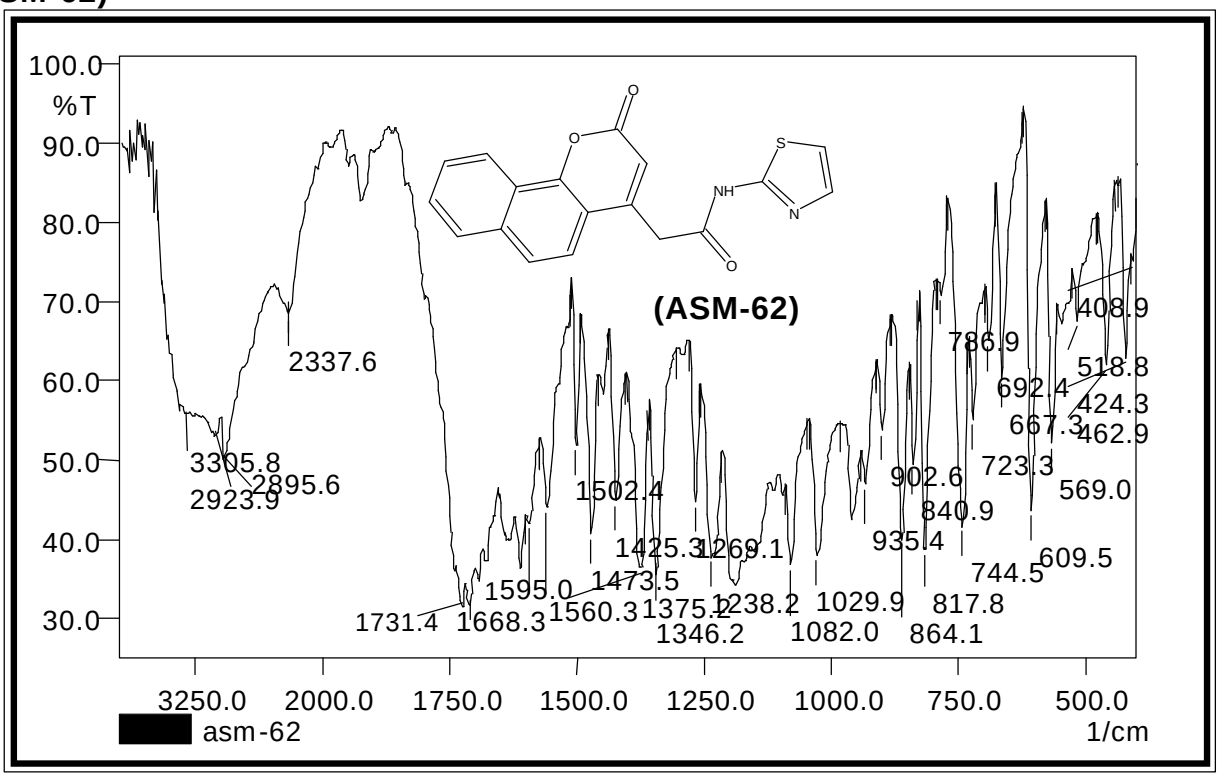
Instrument : SHIMADZU FT IR-8400

Sample technique : KBr Pellet

Frequency range : 4000-400 cm^{-1}

Type Of Functional Group	Mode Of Vibration	Frequency Cm^{-1}
Carbonyl	>C=O Str. (Ring)	1735.8
	>C=O Str. (Amide)	1654.2
Hydroxy	-OH Stre.	3354.4
Amine	-N-H Str.	3423.4
Methyl	Assymetric Stre.	2871.8
	Symmetric Stre.	2929.7
	$-CH_3$(Bending)	1375.2
Aromatic	Ring Skeleton Vib.	1571.9 1494.7 1461.9
	O.O.P. Bending Vib.	827.4

IR Spectrum of 2-(2-oxo-2*H*-benzo[*h*]chromen-4-yl)-*N*-1,3-thiazol-2-ylacetamide (ASM-62)



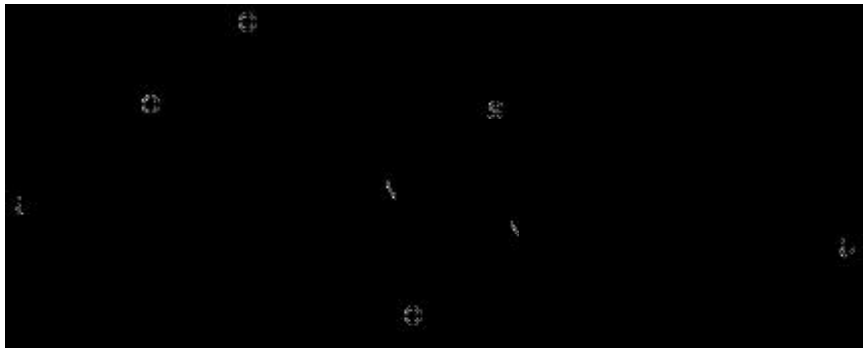
Instrument : SHIMADZU FT IR-8400

Sample technique : KBr Pellet

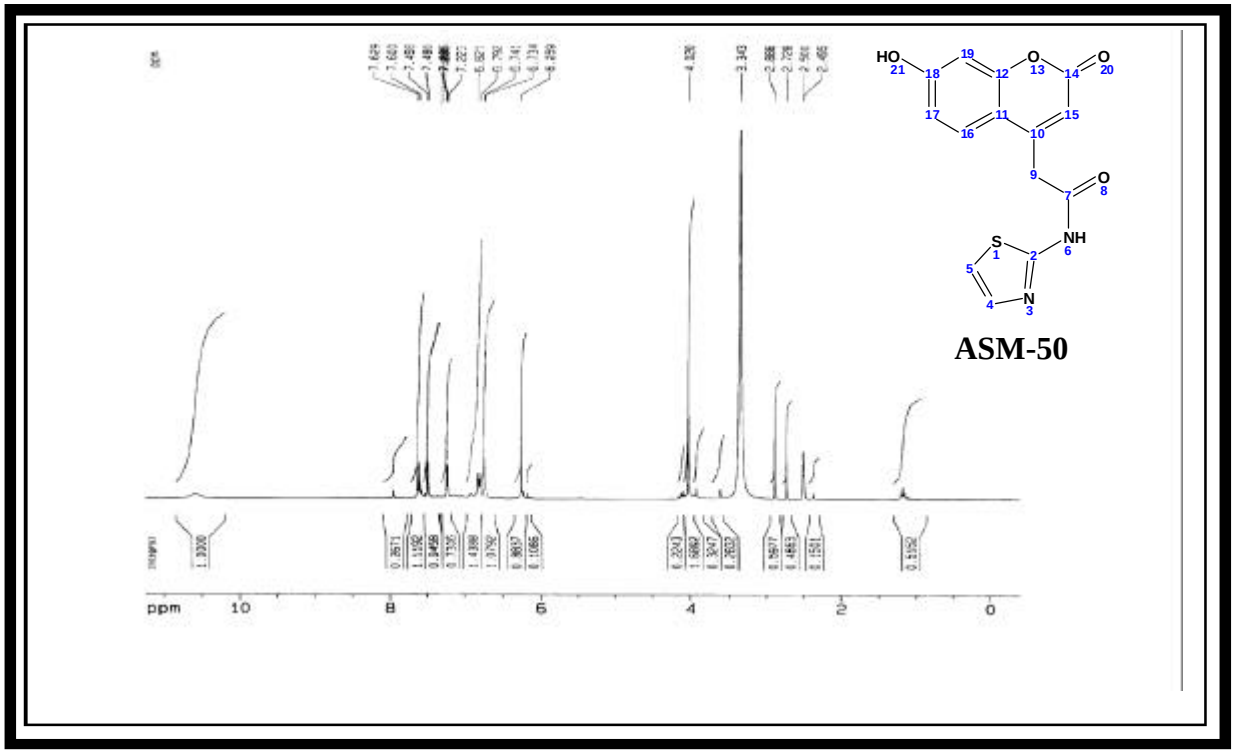
Frequency range : 4000-400 cm^{-1}

Type Of Functional Group	Mode Of Vibration	Frequency Cm^{-1}
Carbonyl	>C=O Str. (Ring)	1731.4
	>C=O Str. (Amide)	1668.3
Amine	-N-H Str.	3305.8
Methyl	Assymetric Stre.	2895.6
	Symmetric Stre.	2923.9
	$-CH_3$(Bending)	1375.2
Aromatic	Ring Skeleton Vib.	1595.0 1560.3 1473.5
	O.O.P. Bending Vib.	744.5

Table 5.4 IR Spectral data of 2-(2-oxo-2*H*-chromen-4-yl)-*N*-(4-substituted phenyl-1,3-thiazol-2-yl)acetamides.



^{1}H NMR Spectra of 2-(7-hydroxy-2-oxo-2H-chromen-4-yl)-N-(1,3-thiazol-2-yl) acetamide (ASM-50)

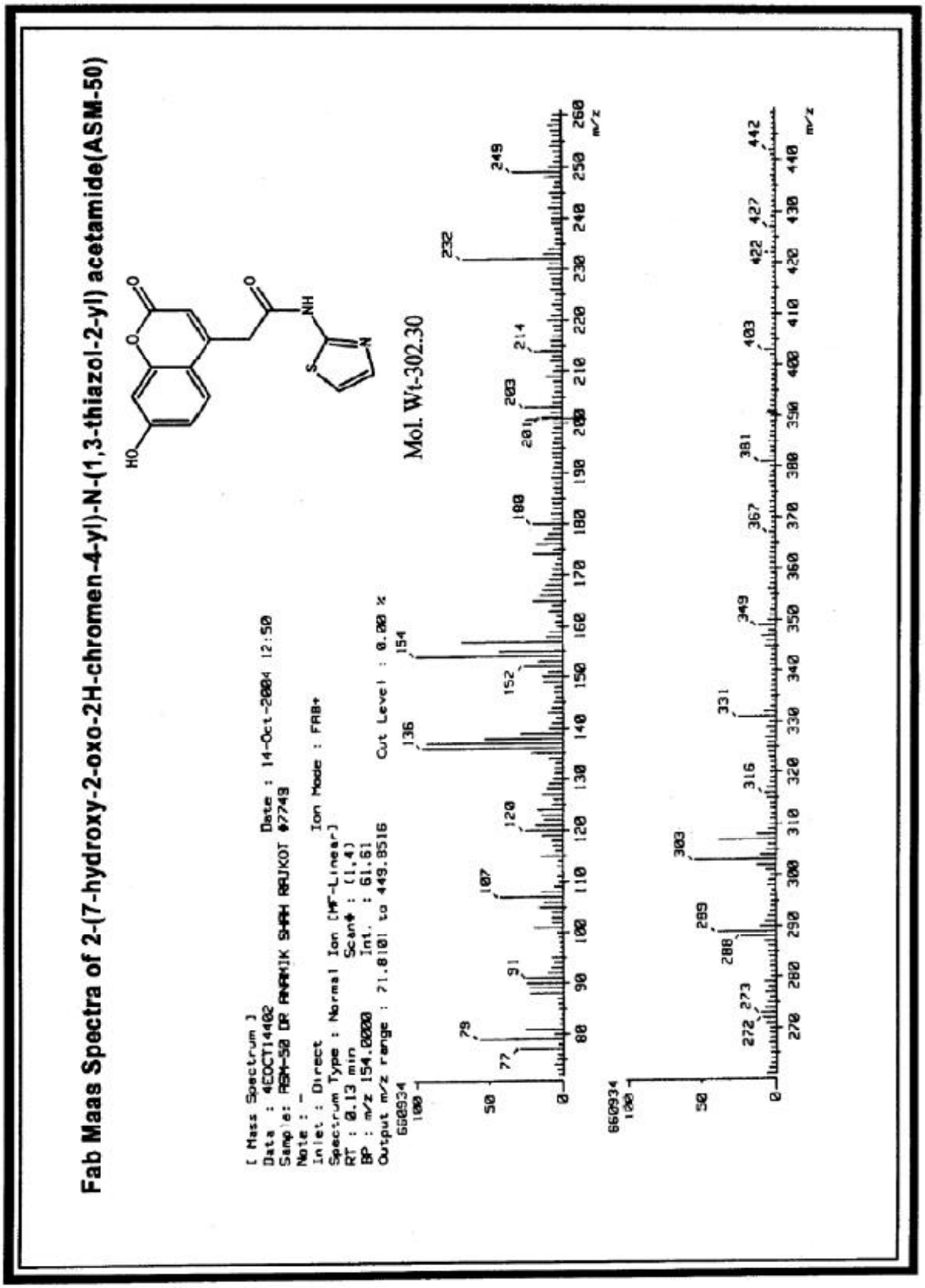


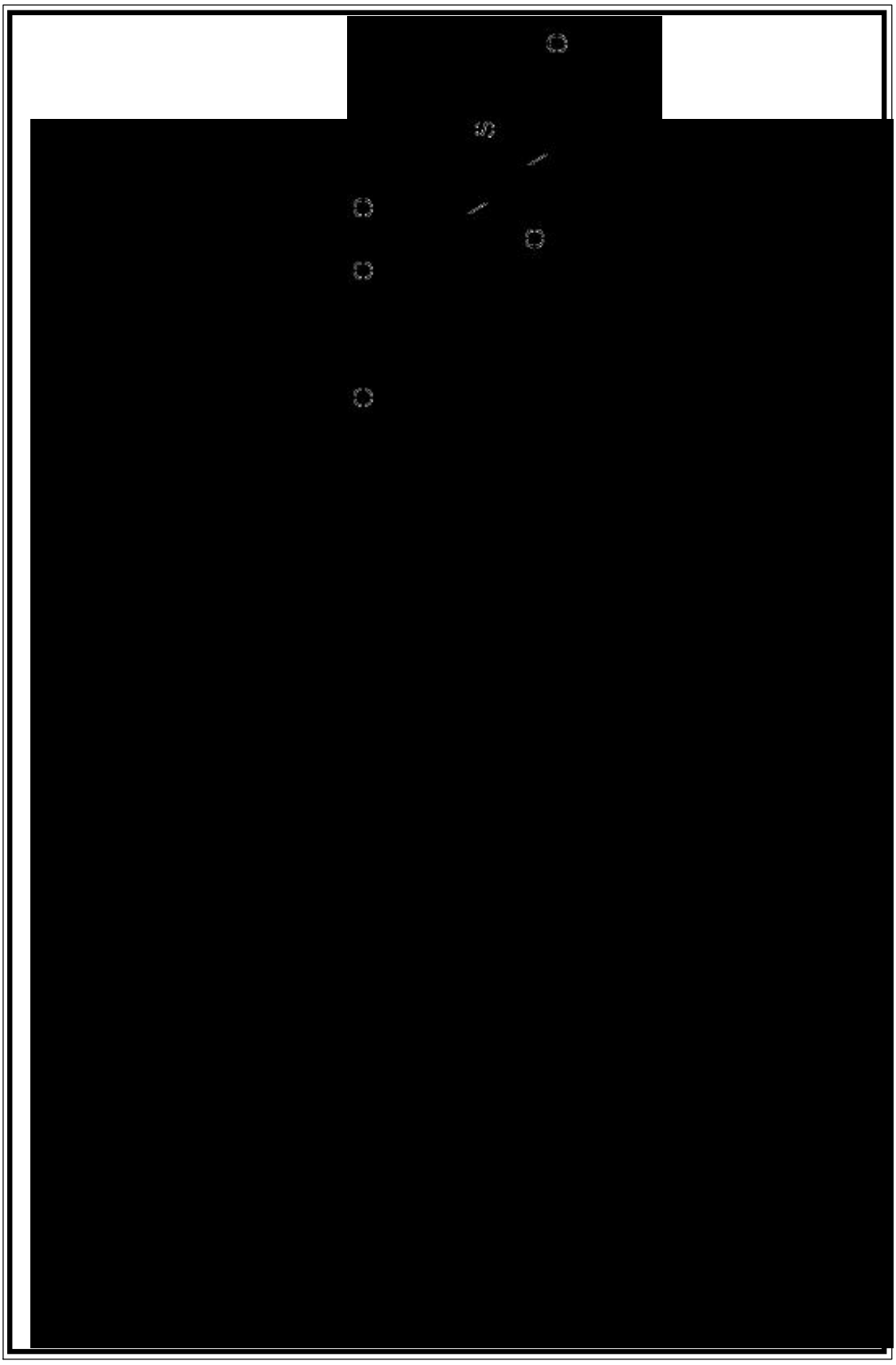
Instrument : BRUKER AC 300 MHz FT-NMR

Standard : TMS

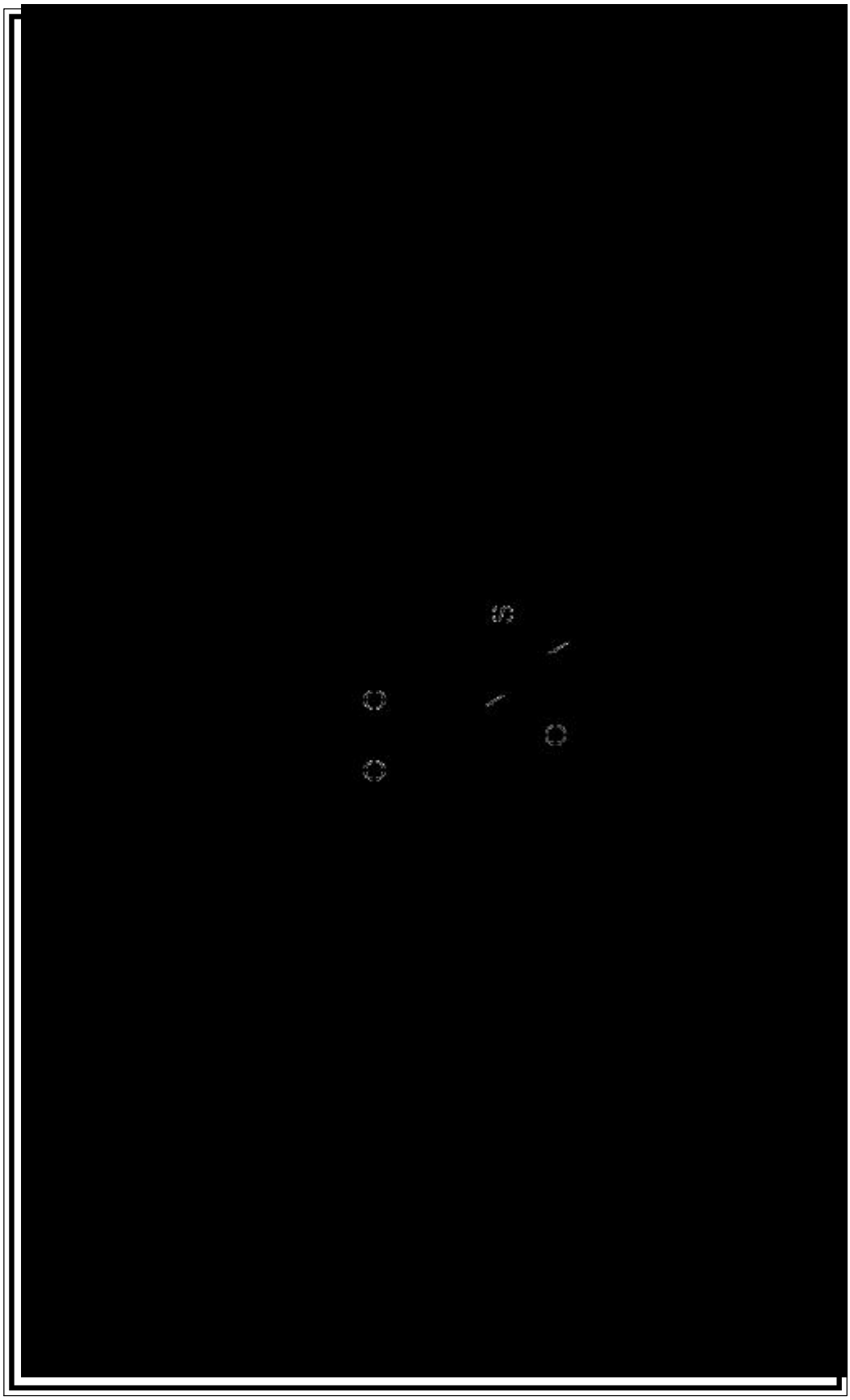
Solvent : DMSO d_6

Chemical Shift d ppm	No. of Proton	Muliplicity	J. Value
4.020	2H	Singlet	C_9
6.25	1H	Singlet	C_{15}
6.73	1H	Doublet	$C_{19}J_{19,17}=2.1H_2$
6.82-6.79	1H	Double Doublet	$C_{17}J_{17,16}=6.3H_2$ $J_{17,19}=2.4H_2$
7.225	1H	Doublet	$C_4J_{4,5}=3.6H_2$
7.485	1H	Doublet	$C_5J_{5,46}=3.6H_2$
7.6	1H	Doublet	$C_{16}J_{16,17}=8.1H_2$
7.948	1H	Broad Singlet	C_6(-NH-)
10.5	1H	Broad Singlet	-OH


Fab Maas Spectra of 2-(7-hydroxy-2-oxo-2H-chromen-4-yl)-N-(1,3-thiazol-2-yl) acetamide(ASM-50)
Mol. Wt-302.30
[Mass Spectrum]
Data : 4EOCT14402
Sample: RSM-50 DR ANNIK SHH RAJKOT #7743
Note : -
Inlet : Direct
Spectrum Type : Normal Ion (MT-Linear)
RT : 8.13 min
BP : m/z 154.0000
Output m/z range : 71.8101 to 449.8516
Date : 14-Oct-2004 12:50
Ion Mode : FAB+
Scan# : (1,4)
Int. : 61.61
Cut Level : 0.00 %
660934
m/z

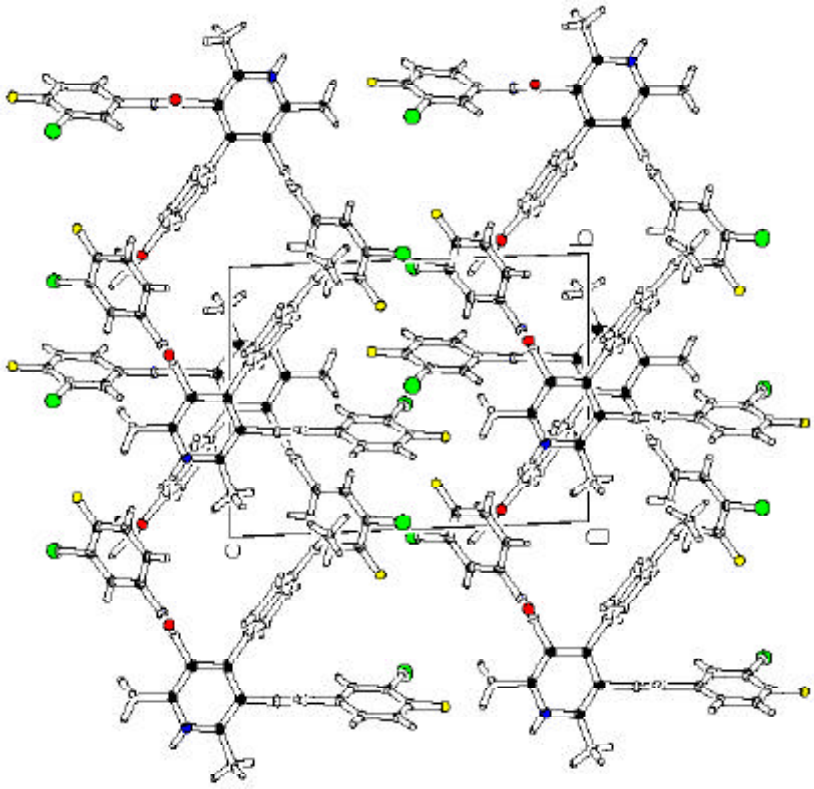


Fab Mass Spectra of 2-(7-hydroxy-2-oxo-2*H*-chromen-4-yl)-*N*-[4-(2-hydroxyphenyl)-1,3-thiazol-2-yl]acetamide (ASM-52).



Fab Mass Spectra of 2-(2-oxo-2*H*-benzo[*h*]chromen-4-yl)-*N*-1,3-thiazol-2-ylacetamide (ASM-62).

“ Synthesis and X-ray Crystallographic study of some important molecules”



Introduction of X-ray Crystalography & Polymorphism:

Definition:

Present chapter deals with X-ray crystallography of molecules which have been synthesized. This includes defining molecular structure, identifying all the atoms present along with their space patterns, and establishing correlation with physical and chemical properties of the substances,arrangement of ions, atoms,and molecules in a crystal. The symmetry and geometry exhibited by their arrangement are part of the structural properties so it is very important to have detailed study of important molecules.

The first step for the crystallographer is often the hunt for a decent single crystal, and in the macromolecular field, even this is being automated. **Julie Wilson** (New York) on the *Automatic Evaluation of High-Throughput Crystallisation Trials* gave a mathematical approach to finding crystals. The wells of crystallisation plates were scanned for “interesting objects” which were evaluated according to the shape of the object’s boundary. The parameters were chosen such that single well-formed crystals gave the best score, and with each well indexed, the best samples could be traced more quickly and save the crystallographer’s time, eyesight and sanity.

The study of crystal structures through X-ray diffraction techniques.

When an X-ray beam bombards a crystalline lattice in a given orientation, the beam is scattered in a definite manner characterized by the atomic structure of the lattice. This phenomenon, known as X-ray diffraction, occurs when the wavelength of X-rays and the interatomic distances in the lattice have the same order of magnitude.

In 1912, the German scientist Max von Laue predicted that crystals exhibit diffraction qualities. Concurrently, W. Friedrich and P. Knipping created the first photographic diffraction patterns. A year later Lawrence Bragg successfully analyzed the crystalline structures of potassium chloride and sodium chloride using X-ray crystallography, and developed a rudimentary treatment for X-ray/crystal interaction (Bragg's Law). Bragg's research provided a method to determine a number of simple crystal structures for the next 50 years. In the 1960s, the capabilities of X-ray crystallography were greatly improved by the incorporation of computer technology. Modern X-ray crystallography provides the most powerful and accurate method for determining single-crystal structures. Structures containing 100–200 atoms now can be analyzed on the order of 1–2 days, whereas before the 1960s a 20-atom structure required 1–2 years for analysis. Through X-ray crystallography the chemical structure of thousands of organic, inorganic, organometallic, and biological compounds are determined every year.

Theory

In many cases, an image of a microscopic object is generated by focusing the rays of the visible spectrum using a lens as in light microscopy. However, because the wavelength of visible light is long compared to atomic bond lengths and atoms themselves, it is necessary to use radiation with shorter wavelengths, such as X-rays. Employing shorter wavelengths implies abandoning microscopy and true imaging, however, because there exists no material from which a lens capable of focusing this type of radiation can be created. (That said, scientists have had some success focusing X-rays with microscopic Fresnel zone plates made from gold).

Generally, in diffraction-based imaging, the only wavelengths used are those that are too short to be focused. This difficulty is the reason that crystals must be used.

Because of their highly ordered and repetitive structure, crystals are an ideal material for analyzing the structure of solids. To use X-ray diffraction as an example, a single X-ray photon diffracting of one electron cloud will not generate a strong enough signal for the equipment to detect. However, many X-rays diffracting of many electron clouds in approximately the same relative position and orientation throughout the crystal will result in constructive interference and hence a detectable signal.

Technique

Some materials studied using crystallography, DNA for example, do not occur naturally as crystals. Typically, such molecules are placed in solution and allowed to crystallize over days, weeks, or months through vapor diffusion. A drop of solution containing the molecule, buffer, and precipitants is sealed in a container with a reservoir containing a hygroscopic solution. Water in the drop diffuses to the reservoir, slowly increasing the concentration and allowing a crystal to form. If the concentration were to rise more quickly, the molecule would simiply precipitate out of solution, resulting in disorderly granules rather than an orderly and hence usable crystal.

Once a crystal is obtained, data can be collected using a beam of radiation. Although many Universities that are engage in crystallographic research have their own X-ray producing equipment. Synchrotrons are often used as X-ray sources,because of the purer and more complete patterns such sources can generate.

Synchrotron sources also have a much higher intensity of x-ray beams, so data collection takes a fraction of the time normally necessary at weaker sources.

The first protein crystal structure was of sperm whale myoglobin, as determined by Max Perutz and Sir John Cowdery Kendrew in 1958, which led to a Nobel Prize in Chemistry. The X-ray diffraction analysis of myoglobin was originally motivated by the observation of myoglobin crystals in dried pools of blood on the decks of whaling ships. X-ray crystallography played a major role in elucidating the double-helix structure of DNA.Today X-ray crystallography is often used to determine how drugs, such as anti-cancer medications, can be improved to better influence their protein targets.

Biology :

X-ray crystallography is the primary method for determining the molecular conformations of biological macromolecules, particularly protein and nucleic acids such as DNA and RNA. In fact, the double-helical structure of DNA was deduced from crystallographic data. The first crystal structure of a macromolecule was solved in 1958 {Kendrew, J.C. et al. (1958). A three-dimensional model of the myoglobin molecule obtained by X-ray analysis. Nature 181, 662-666.)}.

The molecule must be crystallized because one photon diffracted by one electron cannot be reliably detected. However, because of the regular crystalline structure, the photons are diffracted by corresponding electrons in many symmetrically arranged molecules. Because waves of the same frequency whose peaks match reinforce each other, the signal becomes detectable.

To determine a structure, one must first grow crystals of the molecule of interest using some method of crystallization. This can be a painstaking procedure for macromolecules such as protein and DNA complexes. The crystals are harvested and often frozen with liquid nitrogen. Freezing crystals both reduces radiation damage incurred during data collection and decreases thermal motion within the crystal. Crystals are placed on a diffractometer, a machine that emits a beam of x-rays. The x-rays diffract off the electrons in the crystal, and the pattern of diffraction is recorded on film and scanned into a computer. These diffraction images are combined and eventually used to construct a map of the electron density of the molecule that was crystallized, atoms are then fit to the electron density map and various parameters such as position are refined to best fit the observed diffraction data.

Before the development of X-ray diffraction crystallography (see below), the study of crystals was based on the geometry of the crystals. This involves measuring the angles of crystal faces relative to theoretical reference axes (crystallographic axes), and establishing the symmetry of the crystal in question. The former is carried out using a goniometer.

The position in 3D space of each crystal face is plotted on a stereoraphic net, e.g. Wolff net or Lambert net. In fact, the pole to each face is plotted on the net. Each point is labelled with its Miller Index. The final plot allows the symmetry of the crystal to be established.

Crystallographic methods now rely on the analysis of the diffraction patterns that emerge from a sample that is targeted by a beam of some type. The beam is not always electromagnetic radiation, even though X-rays are the most common choice.

For some purposes electrons or neutrons are used, which is possible due to the wave properties of particles that are described by quantum mechanics. Crystallographers often explicitly state the type of illumination used when referring to a method, as with the terms.

X-ray diffraction, neutron diffraction and **electron diffraction.** X-rays are useful for visualizing the electron clouds around atoms, whereas neutron diffraction methods will reveal the atomic nuclei. Thus far, electron diffraction has not been widely used. *Crystallography* by itself typically implies X-rays.

It is important to note that even after obtaining crystals suitable for diffraction analysis, current X-ray sources and detectors limit the measurement of only the diffracted photon intensities and not their respective phases, the latter encoding the majority of the information about the actual shape of electron density. A combination of experimental and computational methods are typically used to solve the phase problem, in order to estimate phases and obtain an initial map of the electron density.

After phases are estimated, a model made up of atoms is built and refined against the observed data. Once a model of a molecule's structure has been determined, it is often deposited in a crystallographic database .

Polymorphism : "Ability of a molecule to crystallise in to more than one different crystal structure".

Drugs which form crystalline solids often exist in more than one crystal form, each of which many have distinct properties in terms of solubility, melting point etc. Invariably, one of the crystal forms may be more stable or easier to handle than another although the conditions under which the various crystal forms appears may be so close as to be very difficult to control on the large scale. This effect can create differences in the bioavailability of the drug which leads to inconsistencies in efficacy. In some cases, one crystal form can be transformed into another during storage and this called as polymorphism.

Polymorphs of a drug differ in properties that affect its shelf life or ease of manufacture. A newly discovered polymorph may turn out to be a more effective and convenient than the original product.

The Food and Drug Administration requires all companies to register the precise polymorph of any drug that they produce. Pharmaceutical manufacturers also have to demonstrate that each polymorph is stable and can be reproduced reliably. Otherwise, it would be hard to set a drug's effective dosage.

It also comes down to fundamental chemistry. Polymorphism has elicited enough excitement and fear in the drug business that a growing number of reseachers in academia and in private companies are taking a closer look at how crystals grow, and what these scientists discover could shape an **entire industry**[1,2]

1. Threfall, T. L. *Analyst*, **120**, 2435-2460, 1995,
2. Brittain, H. G. *J. Pharm. Sci.* , **86**, 405-412.1997,

Drug companies are becoming increasingly aware that different polymorphs can translate into more or less profit. Because each polymorph is legally defined as a unique, patentable composition of matter, a company that develops a new drug will patent all the polymorphs that it has discovered and produced[3-5].

Polymorphs and patents from a chemist's point of view :-

This talk will present some of the chemical issues that were raised in a number of recent patent litigations involving crystal forms (polymorphs and solvates).

(1) Disappearing and reappearing polymorphs .

(2) Seeding: intentional and non-intentional .

(3) Polymorph identity and polymorph purity .

(4) Polymorphic stability and polymorphic conversion .

A generic perspective on crystal polymorphs :-

The importance of the physical form and polymorphism of active pharmaceutical ingredients has grown dramatically over the last few years. Obviously the physical form is vital for obtaining the desired therapeutically effective product.However, the generic pharmaceutical industry is increasingly researching into novel polymorphic forms of actives to allow early entry into the market place and also to maintain their market position.

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3. N.Hall, Predicting Polymorphism. Pharmaceutical formulation & quality, February/ March-2000.

4. Bernstein, J. *Polymorphism in Molecular Crystals*; IUCR Monographs on Crystallography **14**; Oxford Science Publications: OUP, Oxford, U.K., 2002.

5. Byrne, S. R.; Pfeiffer, R. R.; Stowell, J. G. *Solid State Chemistry of Drugs*, **2nd** ed.; SSCI Inc.: West Lafayette, IN, pp 489-498.1999.

The focus is on some important aspects relating to the development of a generic pharmaceutical.

(1) The commercial benefits for polymorphism discovery and patents for a generic company

(2) Techniques used for the discovery and characterisation of polymorphs

(3) Recent litigation case studies to illustrate the importance of polymorph protection and patents.

Computational prediction of crystal structures and polymorphism :-

Computational methods of predicting organic crystal structures have the potential to confirm that all polymorphs have been identified, and aid characterisation of polymorphs from low quality powder diffraction data. The development of such methods presents some fundamental challenges to our understanding of polymorphism, and this session will explore the progress that has been made and how computation can currently aid polymorphism studies.

(1) Exploring the practicalities of computer modelling of polymorphism .

(2) Example case studies on generic pharmaceuticals .

(3) Progress, problems to date and future prospects .

Crystallography as a strong tool for comprehensive solid state studies:-

Using single crystals or micro-crystalline powders, crystallography represents the ultimate tool for the study of subtle phenomena occurring at the atomic level. This technique combined with molecular modelling is able to elucidate the main issues of the solid state such as polymorphism, phase transitions, solid solutions or chirality. This session will illustrate the contribution of crystallography to the fine characterisation of pharmaceutical compounds by way of:

(1) The description of state of the art x-ray sources and detectors that allow the investigation of the tiny crystalline samples.

(2) The application of computational methods to the simulation of crystal packing and the prediction of polymorph stability.

(3) Rehabilitating the role of the human eye in the detection and isolation of concomitant polymorphs or furtive species.

There are various methods of growing single crystals:

For X-ray crystallography, it is necessary to grow crystals with edges around 0.1-0.3 mm. Crystals are formed as the conditions in a supersaturated solution slowly change. There are three degrees of saturation in solution, and crystallographers take advantage of these when growing crystals:

Unsaturated - where no crystals will form or grow.

Low supersaturated - where crystals will grow but no new ones will form.

High supersaturated - where crystals will both form and grow.

One theory of crystal growth is to start by getting a few crystals to grow in the highly supersaturated solution. Then the crystals are exposed to a less saturated solution so they can grow. This is done either by moving the crystals or changing the saturation of the solution.

For small molecules, growing large enough crystals is relatively simple then other molecules. By taking a supersaturated solution and gradually changing the conditions, crystals will begin to grow. If left undisturbed for a few days ideally a few large crystals will grow.

Some known techniques for growing single crystals:

Batch crystallization : A saturated solution left in a sealed container to let the crystals grow.

Microbatch crystallization: A drop of solution is put in inert oil and left to grow. Here there probably is some diffusion of proteins into the oil, lowering the saturation over time.

Macroseeding: A crystal is grown in a highly saturated solution and placed in a less saturated one where only growth of the crystal will occur.

Microseeding: A few crystals are grown, then crushed, and put into a final solution that combines them into a few nice crystals. This involves quite a bit of experimentation with solution's concentrations to get the desired number of crystals.

X-ray Crystallography of some important molecules :-

Looking to the importance of our earlier molecules which were very very important research molecule in various pharmacological study. We planned to get the molecular structure and some information about the skeleton of the molecules. The work of X-ray crystalography of molecules synthesized by our research group was undertaken. This includes defining molecular structure, identifying all the atoms present along with their space patterns, and establishing correlation with physical and chemical properties of the substances, arrangement of ions, atoms, and molecules in a crystal. The symmetry and geometry exhibited by their arrangement are part of the structural properties.

We studied the different molecules especially the 1,4-dihydropyridines class. Moreover we have proved the crystal structure of 1,4-DHPs. The crystal structure of the same class was reported by our reseach group.

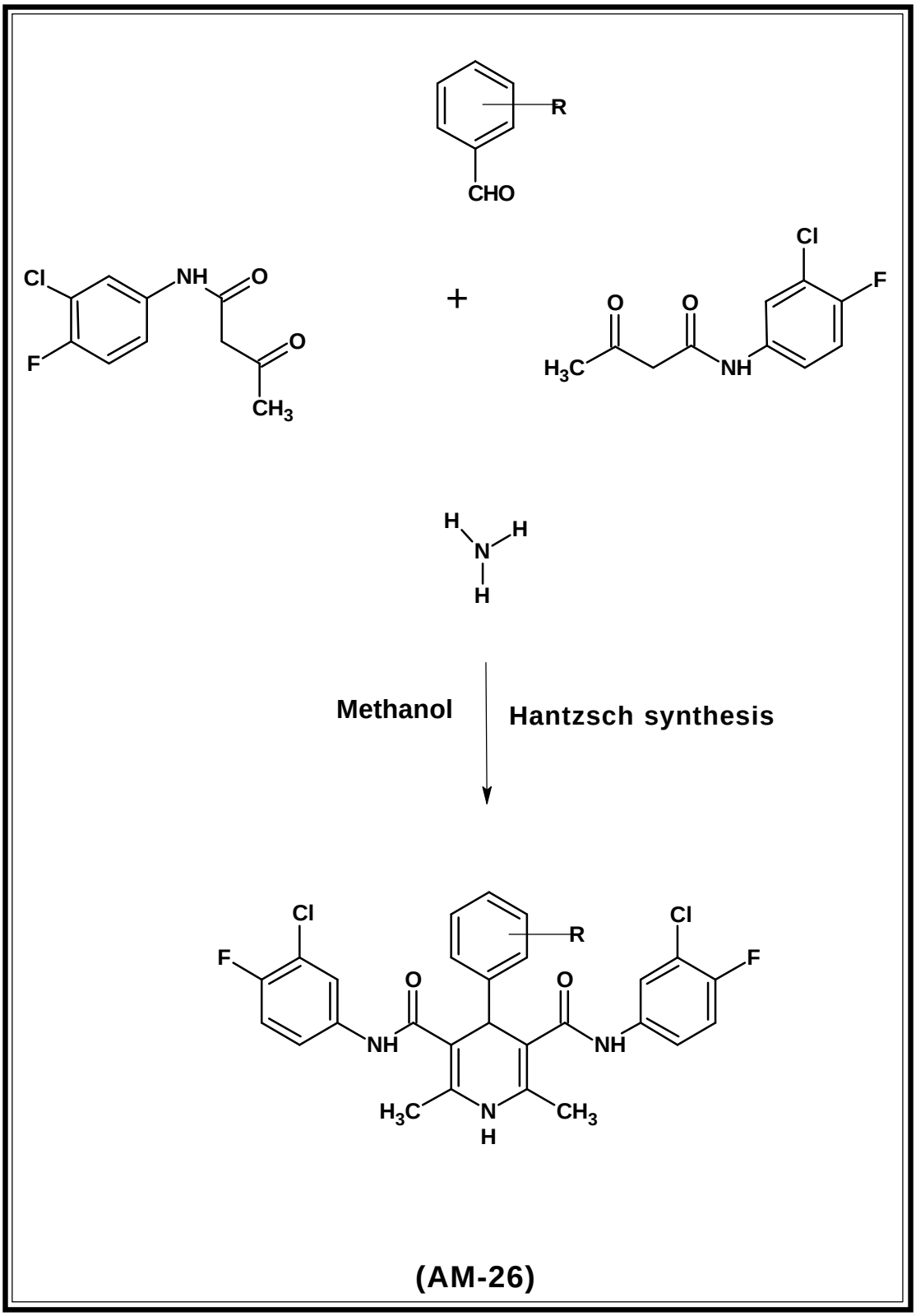
We undertake the work for following three molecules.

(1) 4-(4-methoxyphenyl)-N^3,N^5-bis (3-Chloro-4-fluorphenyl)-2,6-dimethyl -1, 4-dihydro-3,5-pyridinedicarboxamide (**AM-26**).

(2) 4-(N,N-dimethylphenyl)-N^3,N^5-bis (4-fluorphenyl)-2, 6-dimethyl -1, 4-dihydro-3,5-pyridinedicarboxamide (**AM-60**).

(3) 4-(3-Chlorophenyl)-N^3,N^5-bis (4-fluorphenyl)-2, 6-dimethyl -1, 4-dihydro-3,5-pyridinedicarboxamide (**AM-46**).

Reaction scheme for (AM-26):-



(AM-26)

Experimental protocols :

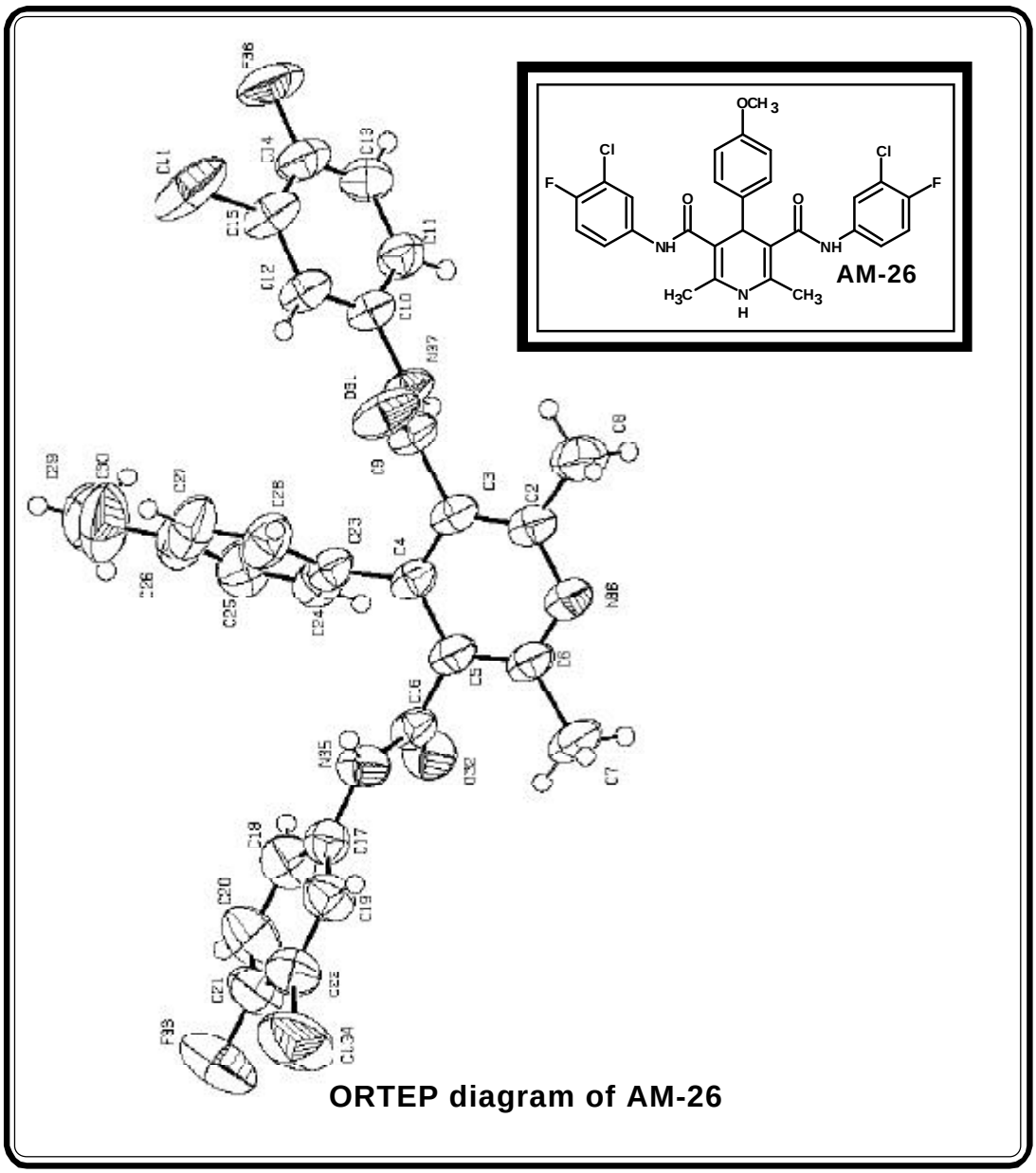
The original Hantzsch pyridine synthesis, which consists of condensation of acetoacetic ester with aldehyde and ammonia is now extended and substituted acetoaetanilide was used instead of acetoacetic ester. All the chemicals obtained from industrial sources. The crystallization of all carried out on silica Gel-G as stationary phase and purchased from MERCK India Ltd. Ethyl acetate: Hexane (4:6) was used as a Mobil phase. The other solvent system lime acetone: benzene, methanol: chloroform was also employed but the best results were obtained for mixture of ethyl acetate and hexane(4:6) .

Preparation of 4-(4-methoxyphenyl)-N^3,N^5-bis(3-chloro,4-fluorophenyl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxamide:

Acetoacetanilide(0.02 mol), 4-methoxybenzaldehyde(0.01mol) were dissolved in 25ml methanol and heated on water bath till the solid disappeared in the reaction mass. Concentrated ammonia(3ml) was added to the reaction and it was further refluxed on a water bath for a period of 10-12hrs. The completion was monitored by TLC(Merck 60 F_{254}). After completion of reaction it was allow to come to room temperature and solid mass will appear in the flask. The product was filtered and washed with ether. It was recrystallized from ethanol+acetone.

Method for obtaining single crystal:

4-(4-methoxyphenyl)-N^3,N^5-bis(3-Cl,4-fluorophenyl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxamide (2.5 gm) was taken in 30 ml solvent mixture [Ethanol + DMF (7.5:2.5)]. 1 gm Charcoal was added and heated on a heating device for 6 minutes. The solution was filtered while hot through whatmann 41 filter paper. The solution was kept in a stopper conical flask slightly opened. The Crystals was grown up due to thin film evaporation.



ORTEP diagram of AM-26

Spectral Data for AM-26 :

IR Spectral study:

The infrared spectrum was recorded on Shimadzu FT-IR-8400 on KBr pellet. The frequency range is 400-4000cm^{-1}

1611(>C=O), 3262 (N-H str.), 3309 (CONH atom. str,), 1453 (C-H ben., alk.), 1158, 1081 (C-O-C str. asy. sym.)

NMR spectral Data:

The ^{1}H NMR spectrum was analyzed on Bruker AC (300 MHz) FT-NMR and deuterated chloroform ($CDCl_3$) was used as solvent, TMS being internal standard. [2.073 (s, 6H, 2xCH3), 3.654 (s, 3H,-OCH_3), 6.785 (d, 2H, Ar-H), 7.136 (d, 2H, Ar-H),7.333(t, 2H,Ar-H), 7.510 (m, 4H,C_6H_4), 7.907(q, 2H Ar-H),8.150 (s, 1H,dhp-NH),9.508 (s, 2H,Ar-NH)].

MASS Spectral Data:

The FAB MASS was recorded on JEOL SX 102/DA-6000.

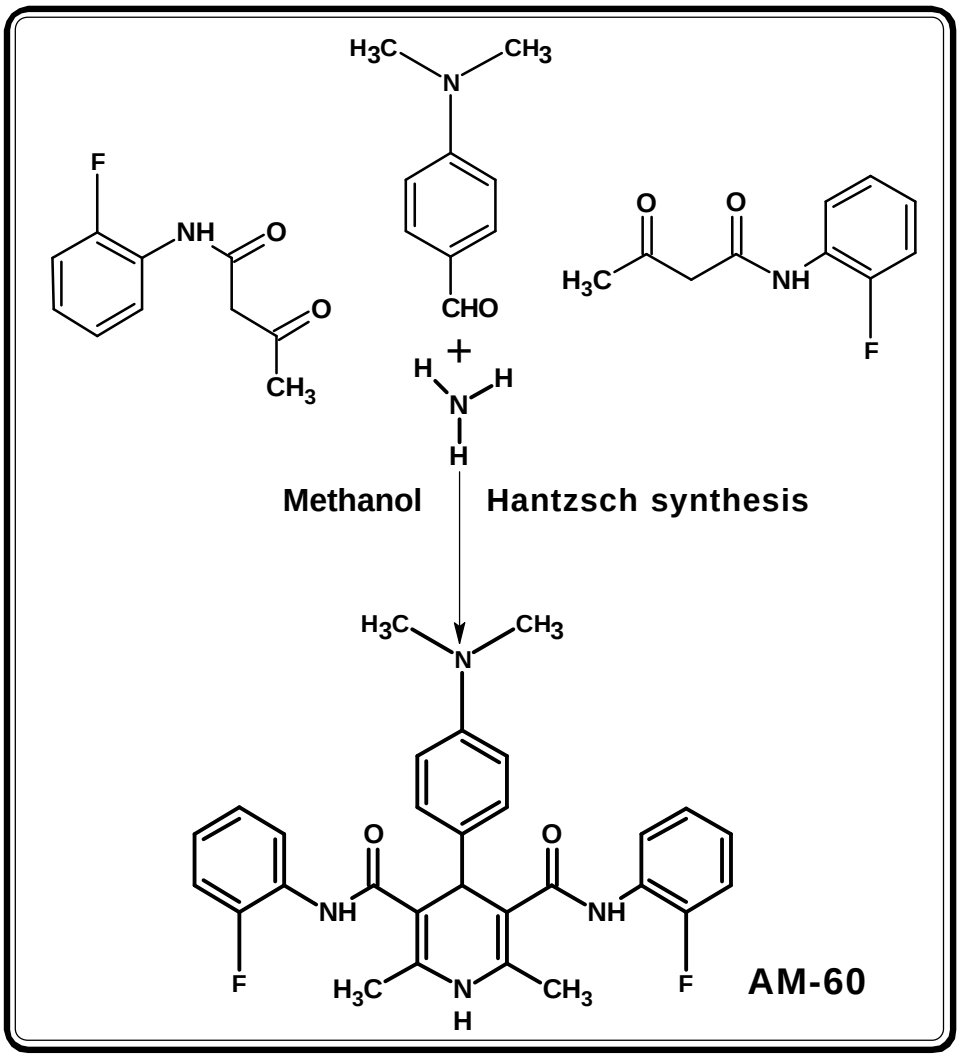
Mwt.558.40 [m/e (%)] : M^+ 559 (70), 449 (Base peak), 412 (86),280 (29),266 (21), 240 (20),154 (12), 136 (10).

Elemental Analysis:

[Calculated % C (60.27%)H (4.19%) N (7.58%) Cl (12.73%) F(6.85%)]

[Obtained % C (60.23%)H (4.15%) N (7.53%) Cl (12.70) F(6.80%)]

Reaction scheme for (AM-60):-

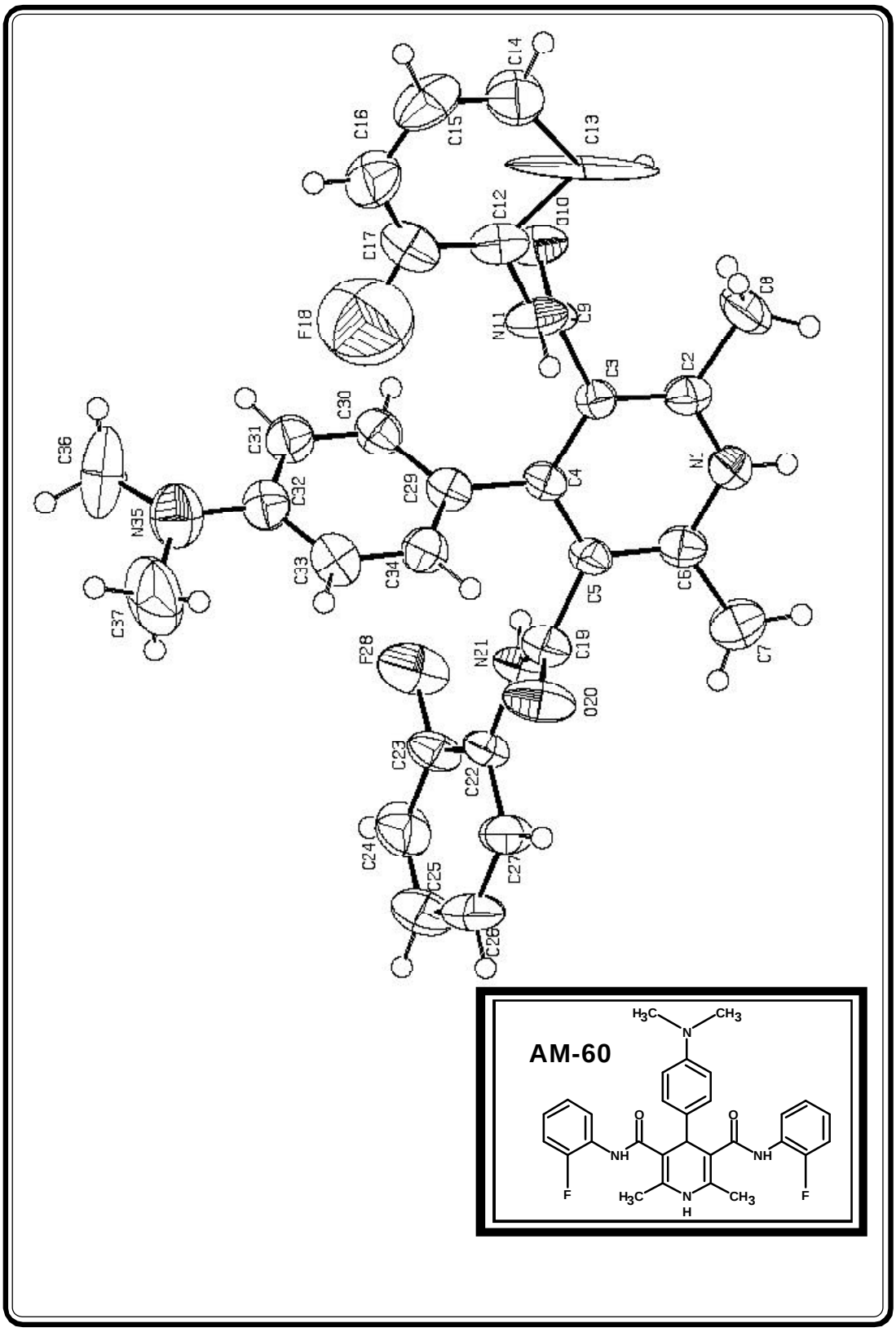


Experimental:

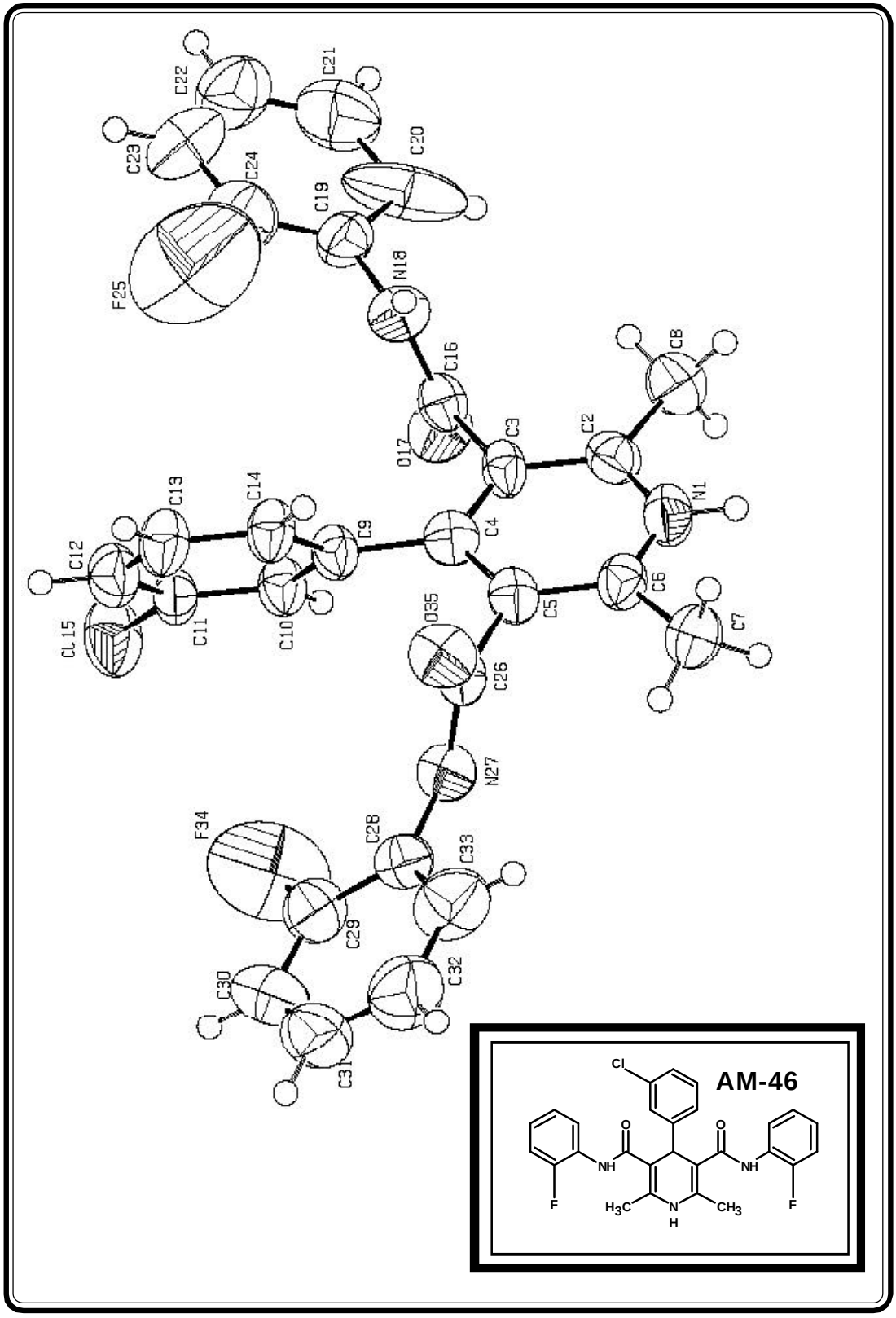
Preparation of 4-(N,N-dimethylphenyl)-N^3,N^5-bis(2-fluorophenyl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxamide (Scheme-2):

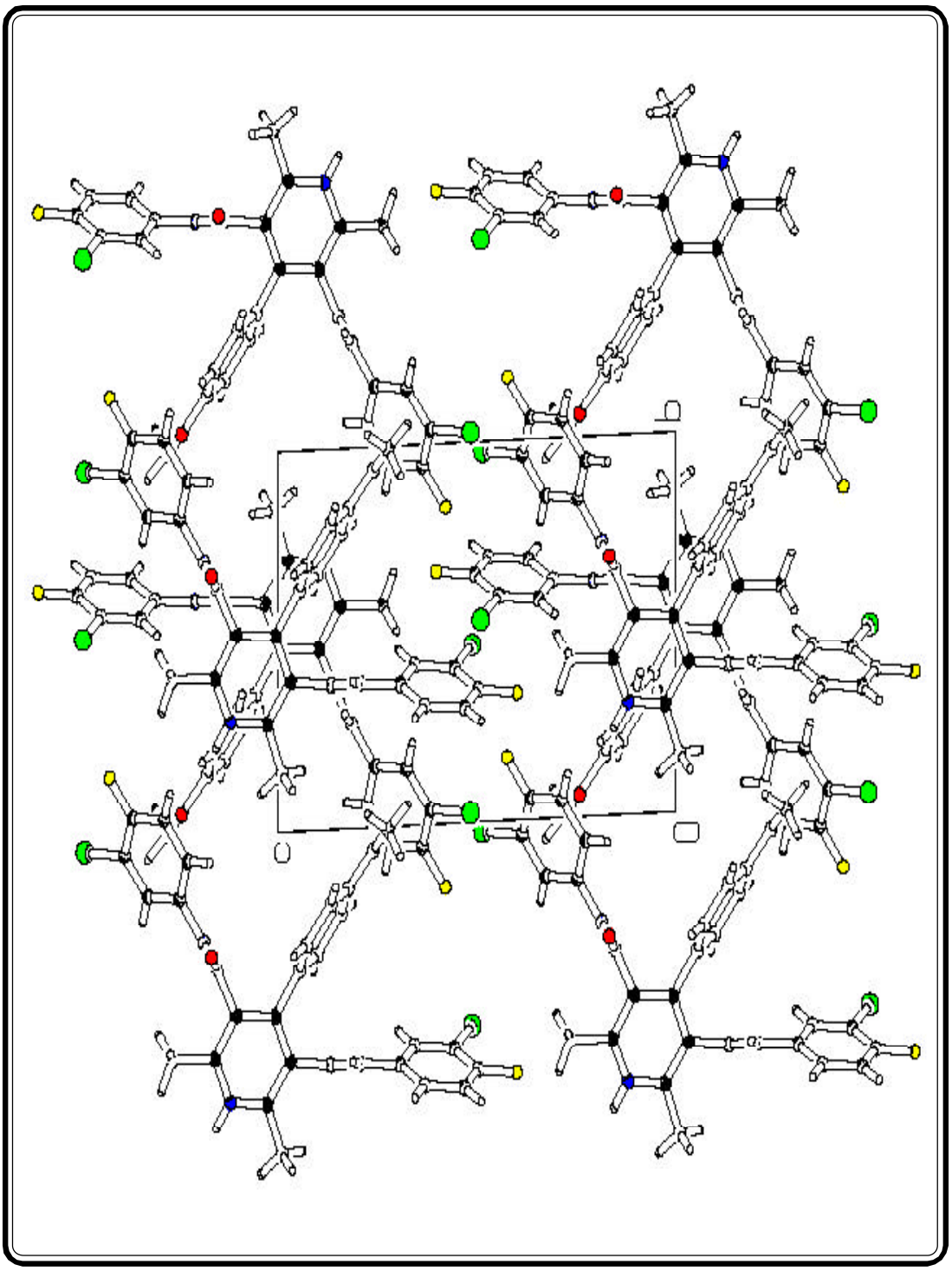
Sub.acetoacetanilide(0.02mol),N,N-dimethylaminobenzaldehyde (0.01mol) were dissolved in 25ml methanol and heated on water bath till the solid disappeared in the reaction mass. Concentrated ammonia(3ml) was added to the reaction and it was further refluxed on a water bath for a period of 10-12hrs. and solid mass will appear in the flask. and washed with ether. It was recrystallized from ethanol+acetone.

ORTEP diagram for AM-60

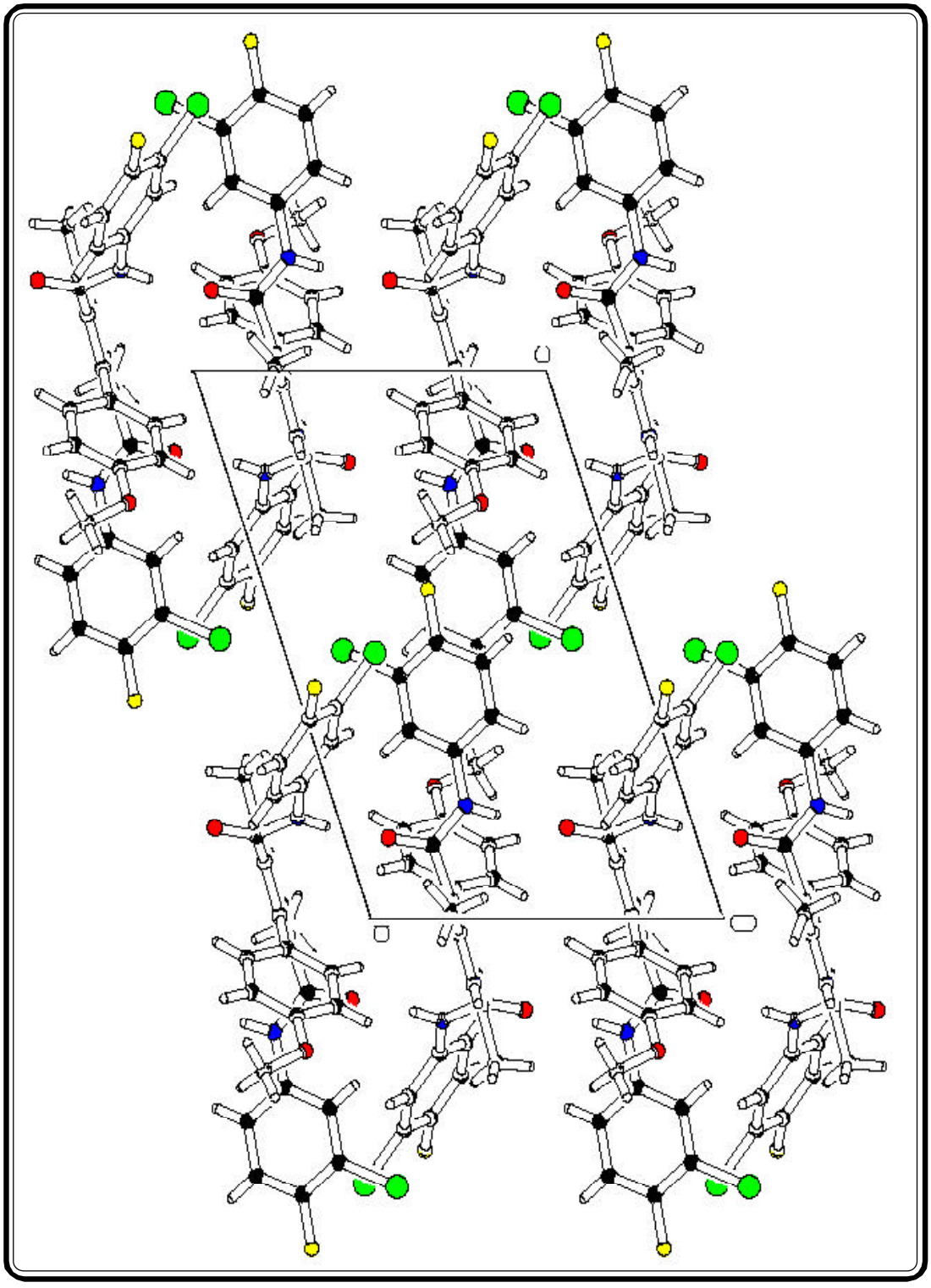


ORTEP diagram for AM-46

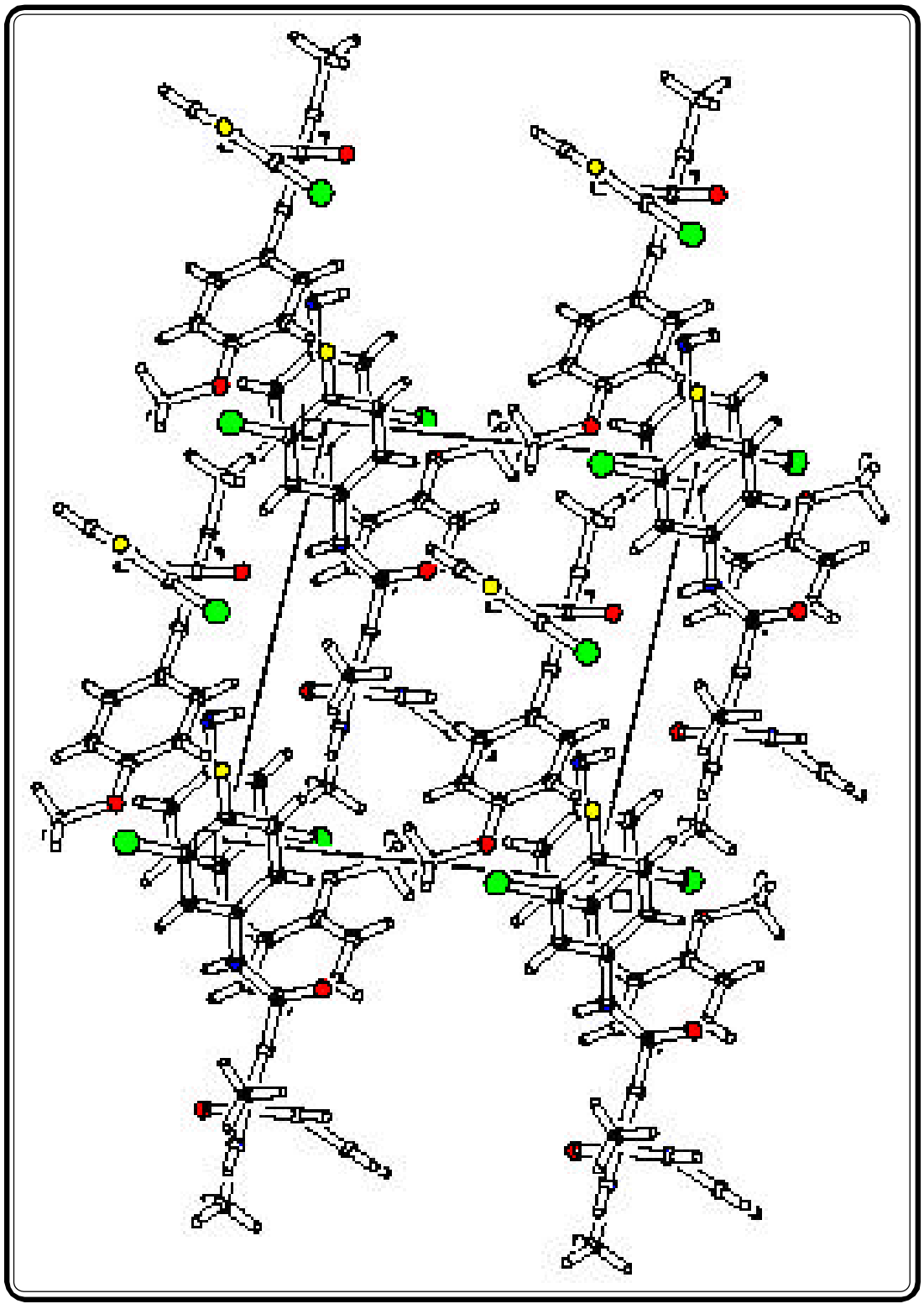




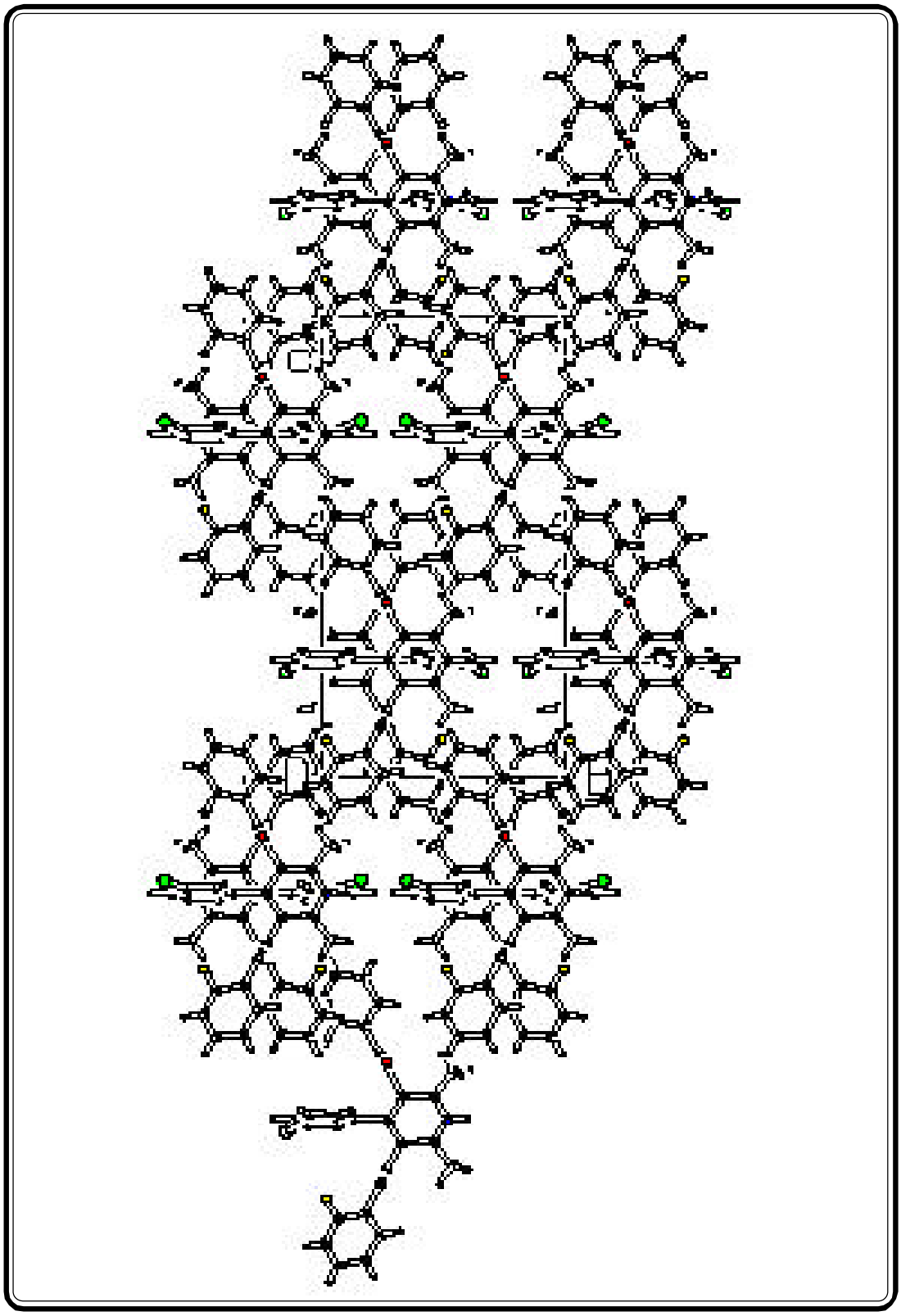
Packing of Molecule (AM-26) down a



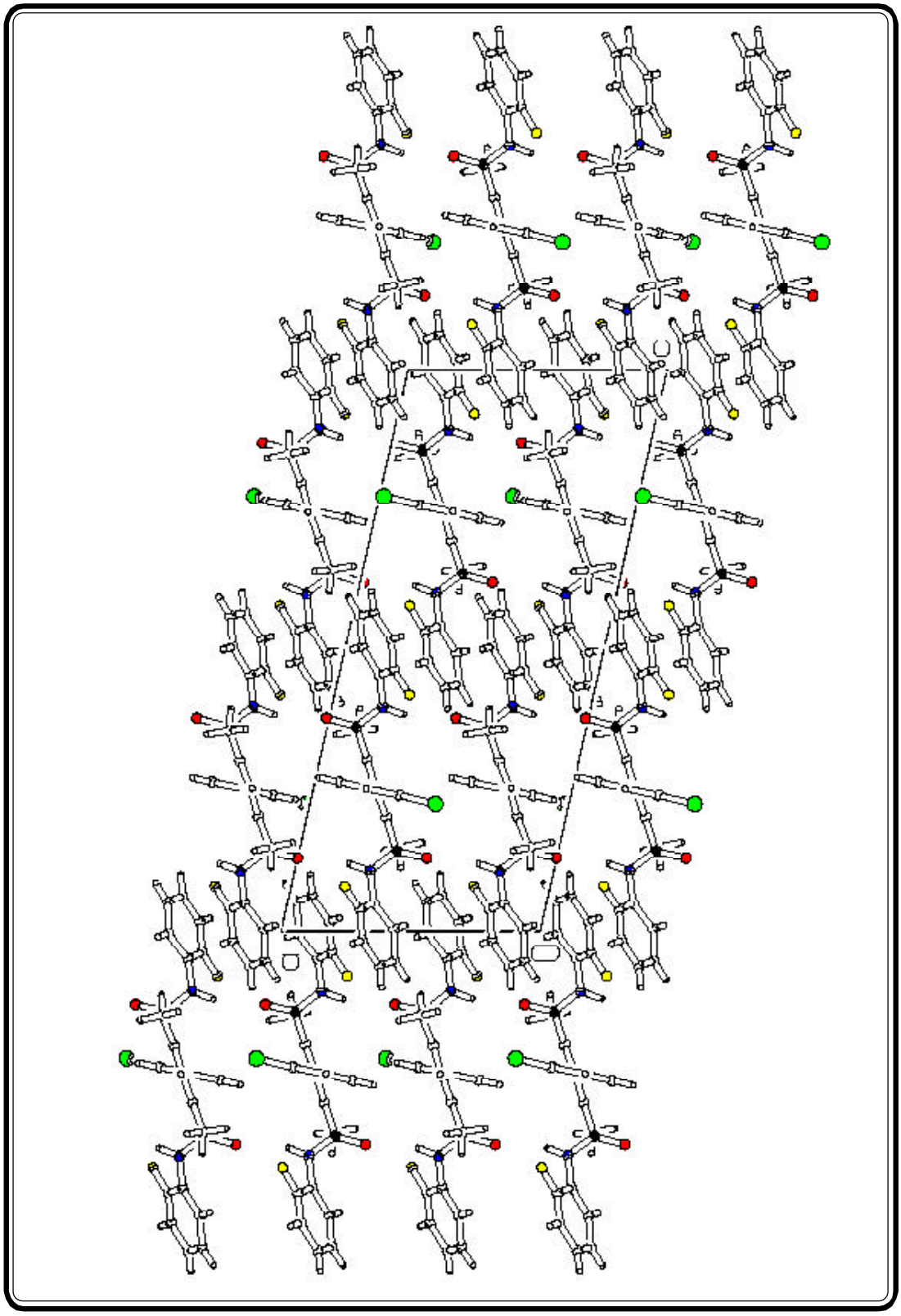
Packing of Molecule (AM-26) down b



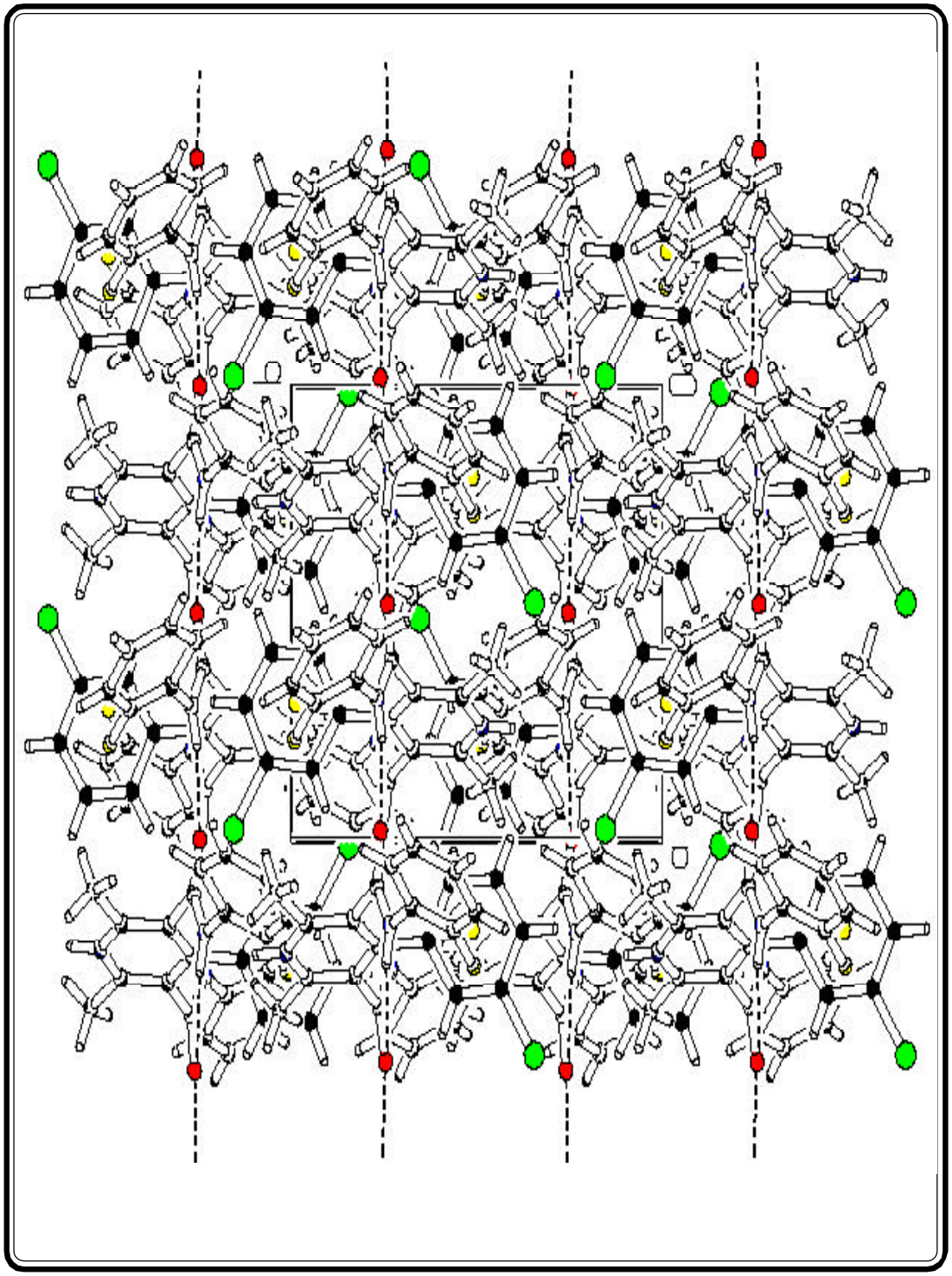
Packing of Molecule (AM-26) down c



Packing of Molecule (AM-46) down a



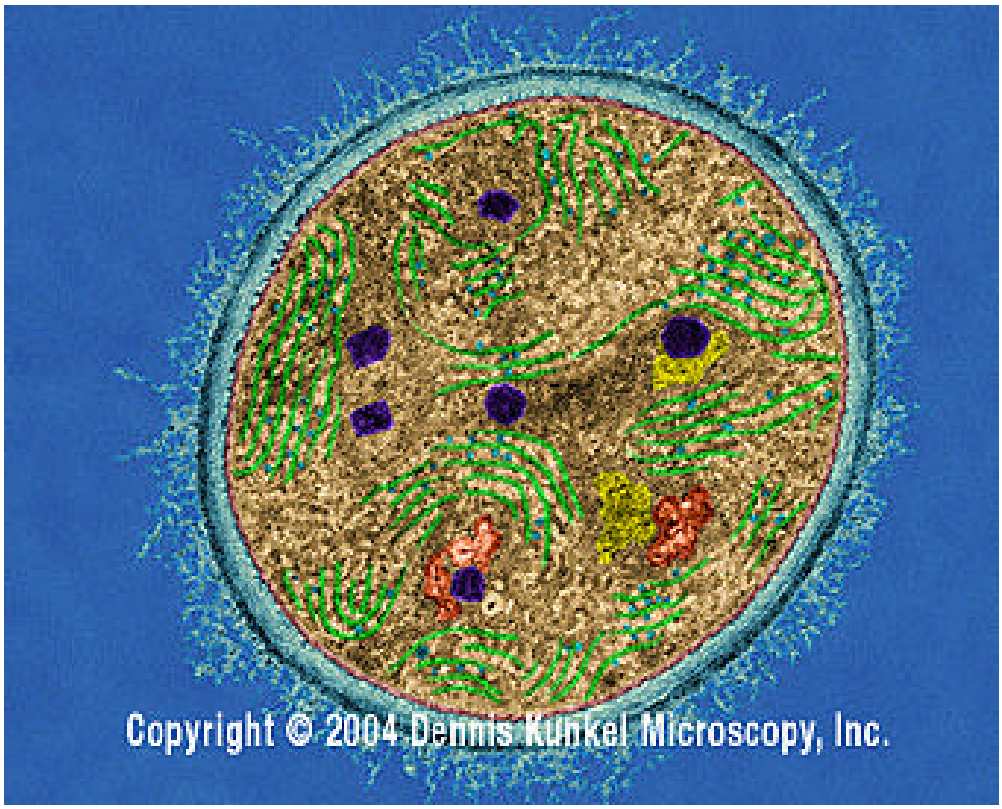
Packing of Molecule (AM-46) down b



Packing of Molecule (AM-46) down c

Chapter-7

Biological Profile of Newly Synthesized Compounds



Biological Activity Study:

The present work deals with the antimicrobial screening of the compounds (AM,ASM, MS & AKSM sereris) synthesized in earlier Chapters.

The minimum inhibition concentration (MIC) values were determined in the present study.

Protocol-1

Determination of MIC by Agar-dilution method was carried out as per the NCCLS guidelines M7-A5

Preparation of plates containing the antimicrobial agent:

To Mueller-Hinton agar (19 ml) medium (Hi Media), cooled to approximately 50^0C, 1 ml of antimicrobial agent, dissolved in DMSO is added. The antimicrobial agent is mixed thoroughly into the medium and poured into Borosil glass petriplates of 9 cm diameter and allowed to solidify. The 7 test cultures selected for the determination of MIC are spot-inoculated (2 ml/spot) on a single plate prepared.

Preparation of solutions of antimicrobial agents to be incorporated into the agar-based medium

10mg of the antimicrobial agent is dissolved in 5ml of DMSO to prepare the main stock of the compound to be tested. 1ml of this main stock is added to 19 ml of Mueller Hinton agar medium to make the final concentration of 100 µg/ml in the agar medium. The main stock solution is further diluted in DMSO by two-fold dilution procedure to obtain the desired concentration in the agar medium.

Preparation of inoculum of the test cultures:

For all cultures other than M.smegmatis strains

One loopful of culture from slant is inoculated into 5mL Muellar Hinton broth (HiMedia) in a test tube. The tube is incubated at 37 ^{0}C till the absorbance at 625nm equals that of 0.5 Mc. Farland standard (Section 5). The absorbance readings are taken against a sterile Mueller Hinton broth blank. The cultures are then transferred to 2-8^{o}C temperature and maintained this temperature till the time of inoculating the plates. Appropriate dilutions of the cultures (either 10 or 100 fold, depending on the culture) are made based on the viable count of the cultures, previously done to establish the relationship between absorbance at 625mn and viable count, before inoculating the plates containing the antimicrobial test agents. 2ml of this diluted culture is used to spot-inoculate the plates with antimicrobial test agents.

For *M.smegmatis* strains

One loopful of the culture from slant is used to inoculate 25 ml Mycoseed brotha with 2-3 glass beads. The broth is incubated at 35 ^{0}C for 5 days on 250rpm shaker. 2 ml of undiluted, 1:10 and 1:100 fold diluted culture is spot-inoculated on a Mueller Hinton agar control plate (section 6) and the spots are observed for confluent growth. The maximum dilution of the culture that gives a confluent growth of the spot on the Mueller Hinton agar control plates is selected for spot inoculation of the plates containing the antimicrobial test agents.

Preparation of 0.5 Mc. Farland standard

The 0.5 Mc. Farland standard solution as a reference for turbidity measurement is prepared as per the NCCLS guidelines M7-A5.

Briefly, 0.5mL of 1.175% w/v $BaCl_2$ solution is added to 99.5 ml of 1% v/v H_2SO_4 solution with constant stirring. The absorbance of the solution is measured at 625nm against a D.M.water blank by a uv-visible spectrophotometer. The absorbance lies in the range of 0.08 to 0.1. The Mc. Farland standard solution is stored in dark at room temperature and vortexed vigorously prior to use

Controls:

Two control plates (i.e.plates without any antimicrobial agent incorporated into the agar medium) with 1ml DMSO in 19mL Mueller Hinton agar medium are kept with each MIC experiment to check the growth of the cultures inoculated on the plates and the effect of the solvent (DMSO) on the cultures. 2 ml of all the test cultures are spot-inoculated onto these two plates.

Incubation time and temperature:

The plates are incubated at 35^{o}C for 48 hours for *M.smegmatis* and 24 hours for all other cultures before taking the results of MIC.

Evaluation of the results:

The antimicrobial test compounds that show the growth of all the cultures at a concentration of 100 µg/ml are not tested further for the determination of the MIC. The compounds that show no growth of any of the culture at 100 µg/ml concentration are further tested with lower doses of the test compounds for the determination of the MIC values.

If the test culture that show 1-5 colonies per spot instead of the confluent growth as in the control plate, it is considered to be inhibited by the test compound.

Table 7.1 :- Antimicrobial Activity data (as per Protocol 1)

Sr. No	Code	Microorganism (MIC, *mg/ml*)					
		S.coccus pyogens A77	*S. aureus SG511*	*S. aureus E710*	*E.coccus faecalis*	*M.smegm-atis (MY-1)*	*M.smegmat-is (MY-2)*
1	AM-13	2.5	5.0	5.0	10.0	5.0	5.0
2	AM-14	2.5	5.0	5.0	10.0	5.0	5.0
3	AM-15	1.25	5.0	5.0	-	5.0	5.0
4	AM-16	100	-	-	-	-	50
5	AM-17	100	-	-	-	-	100
6	AM-25	2.5	-	-	-	12.5	10.0
7	AM-26	5.0	-	-	-	12.5	12.5
8	AM-27	1.25	2.5	2.5	5.0	5.0	5.0
9	AM-33	25	-	50	-	100	50.0
10	AM-34	2.5	-	-	-	12.5	10.0
11	AM-43	12.5	-	-	-	100	12.5
12	AM-48	-	-	-	-	-	-

Table 7.1 :- Antimicrobial Activity data (as per Protocol 1) (continue)

Sr. No	Code	Microrganism (MIC in µg/ml)					
		S.coccus pyogens A77	*S. aureus SG511*	*S. aureus E710*	*E.coccus faecalis*	*M.smeg-matis (MY-1)*	*M.sme-gmatis (MY-2)*
13	AM-49	2.5	-	-	-	-	50
14	AM-61	-	-	-	-	-	-
15	AM-68	-	-	-	-	100	100
16	AM-69	-	-	-	-	50	-
17	AM-70	-	-	-	-	-	-
18	AM-71	-	-	-	-	100	100
19	AM-72	-	-	-	-	-	-
20	AM-73	-	-	-	-	-	-
21	AM-74	-	-	-	-	-	-
22	AM-75	-	-	-	-	-	-
23	AM-76	-	-	-	-	-	-

Table 7.1 :- Antimicrobial Activity data as per (Protocol 1)

(Continue)

Sr No	Code	Microorganism (MIC in mg/ml)					
		S.coccus pyogens A77	*S. aureus SG511*	*S. aureus E710*	*E.coccus faecalis*	*M.smeg-matis (MY-1)*	*M.smeg-matis (MY-2)*
24	AKSM-7	25.0	-	-	-	100	
25	AKSM-12	5.0	-	-	-	12.5	12.5
26	AKSM-19	5.0	-	-	-	100	100
27	AKSM-20	2.5	-	-	-	50	-
28	AKSM-28	12.5	-	-	-	-	-
29	AKSM-32	2.5	-	-	-	-	-
30	AKSM-33	-	-	-	-	-	-
31	AM-35	5.0	-	-	-	-	12.5
32	AM-37	-	-	-	-	-	-
33	AM-38	100	-	-	-	-	-
34	AM-39	-	-	-	-	100	100
35	AM-40	100	-	-	-	100	-
36	AM-41	-	-	-	-	-	-
37	AM-46	100	-	-	-	-	-
38	AM-47	100	-	-	-	-	-
39	AM-55	-	-	-	-	100	-

Protocol-2

All of these compounds were screened for their antibacterial activity using strains like *E. Coli, S. aureus.*

For these screening:

• All compounds were dissolved in 10% DMSO in methanol.

• Fluconazole (0.5 mg/ml) was used as control.

• (h) denotes hazy zone.

• Solvent control was taken into consideration 10% DMSO in methanol.

It was carried out by using cup-plate method[2-4] which has been described as under:

The purified products were screened for their antibacterial activity. The nutrient agar broth prepared by the usual method was inoculated espetiallly with 0.5 ml for 24 hrs. Old subcultures of *E. coli/ S. aureus/ S. aureus 209p* were taken in separate conical flask at 40°-50°C and mixed well by gentle shaking. About 25ml of the content of the flask were poured and evenly spread in petridish (13 cm in diameter) and 10mm bore in agar medium and filled with 0.05ml solution of sample in 10% DMSO in methanol.The plates were incubated at 37°C for 24 hrs and the control wasalso maintained with 10% DMSO in methanol in similar manner.

The zones of inhibition of the bacterial growth were measured in mm diameter.

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2. F. Simoncini et al.; Farmaco, 23, 559 (1968); C. A. 69, 109851 (1968).

3. Bhatt, S., Deo, K., Kundu, P., Chavda, M. & Shah, A.; Indian J. Chem., Sect 42B, 1502 (2003).

4. Chavda, M., Shah, A., Bhatt, S., Deo, K. & Kundu, P.; Arzneim-Forsch, Drug, Res., 53, 196 (2003).

Table 7.2 :- Antimicrobial Activity data (as per Protocol 2)

* Diameter zones of growth inhibition in mm.

Compound 1mg/100ml	**Gram Positive *S. aureus***	**Gram Negative *E. coli***
AKSM-3	15	-
AKSM-8	14	-
AKSM-11	29	-
AKSM-12	25	-
AKSM-13	16	-
AKSM-14	-	-
AKSM-16	26	-
AKSM-18	11	-
AKSM-22	-	-

Protocol - 3

It was carried out using agar diffusion method. The purified products totaling nine compounds from ASM and MSB series (Chapter-2,3 & Chapter-4) were screened for their detailed antibacterial activity.

Preparation of Muller Hinton agar plates with antibacterial test agents:

10 mg of compound was dissolved in 2 ml of DMSO to prepare stock solution of the compound to be tested. 1 ml of this stock was added to 19 ml of molten Muller Hinton agar medium and poured in a sterile empty plate, allowed it to solidify. It gave the final concentration of the compound 1000 µg/ml in the agar medium. The stock solution was further diluted by two fold dilution procedure to obtain the desired concentration of the compound in the agar medium plate. i.e. 1000 µg/ml, 500 µg/ml, 250 µg/ml, 125 µg/ml , 75 µg/ml , 37 µg/ml and 18 µg/ml.

Incubation of Microbial Cultures:

5 ml of Muller Hinton broth media were innoculated with the needed microbial cultures and kept for 2 to 6 hrs for incubation at 37^{O}C temperature. After getting proper growth the culture solution were read against sterile Muller Hinton broth blank, using 0.5 Mc farlan standard at 620nm wavelength in spectophotometer.

Appropriate dilution of microbial culture were made to match 0.5 Mc farland standard. The final dilution of microbial cultures contains 10^4 cells/ml. 2 µl of this diluted microbial culture were used to spot innoculate the plates.

The plates with antimicrobial test agents:

a) Controls:

Solvent control was kept adding 1 ml of DMSO only to check the inhibitory effect on microbes. Organizm control was kept without adding compound.

b) Evaluation:

The plates were kept at 37^{O}C temperature for 24 hrs to get visible growth of microorganisms. Plates were check for growth. Microbes grown in big colony if not inhibited, grown in small size colonies or scanty growth if inhibited, no growth was found in case when they were killed by cideal effect of synthetic compound.

Various organisms used in study of antibacterial activity are:

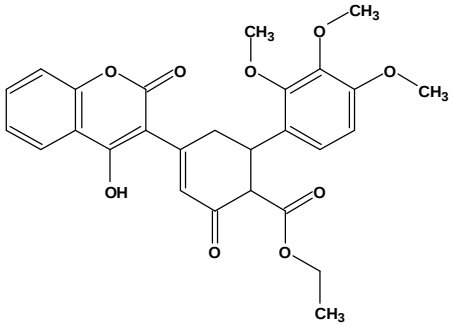
Gram positive:

1. *S.aureus*
2. *B.subtilis*

Gram negative:

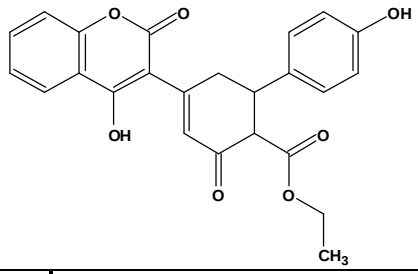
1. *E.coli*
2. *P.aeruginosa*
3. *K.pneunonae*
4. *Enterobacter*

Table 7.3 :- Antibacterial Activity data (as per Protocol 3)



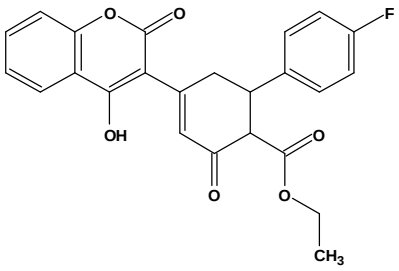
ASM-8

Sr. No.	Strain	Minimum Inhibitory Concentration (MIC, µg/ml)					
1	*Escherichia Coli*	500	-	125	>75	-	-
2	*Enterobacter*	-	-	-	-	-	-
3	*Pseudomonas Aeruginosa*	+/-	-	-	-	-	-
4	*Staphylococcus Aureus*	500	250	125	75	37	>18
5	*Kleibsiell Pneumonae*	-	-	-	-	-	-
6	*Bacillus Subtilis*	500	250	125	75	37	>18



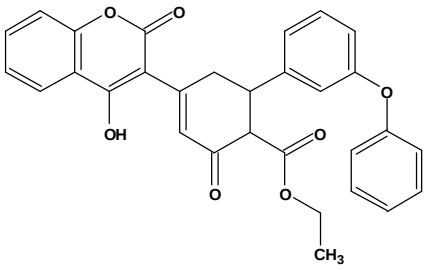
ASM-15

Sr. No.	Strain	Minimum Inhibitory Concentration (MIC, µg/ml)					
1	*Escherichia Coli*	-	-	-	-	-	-
2	*Enterobacter*	-	-	-	-	-	-
3	*Pseudomonas Aeruginosa*	500	+/-	+/-	>75	-	-
4	*Staphylococcus Aureus*	500	250	125	>75	-	-
5	*Kleibsiell Pneumonae*	500	250	-	-	-	-
6	*Bacillus Subtilis*	ND					



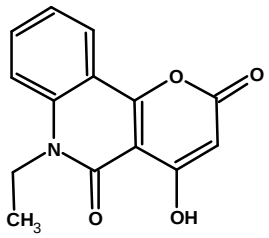
ASM-20

Sr. No.	Strain	Minimum Inhibitory Concentration (MIC, mg/ml)					
1	*Escherichia Coli*	-	-	-	-	-	-
2	*Enterobacter*	500	250	-	-	-	-
3	*Pseudomonas Aeruginosa*	500	250	125	>75	-	-
4	*Staphylococcus Aureus*	500	250	125	>75	-	-
5	*Kleibsiella Pheumonae*	500	250	125	>75	-	-
6	*Bacillus Subtilis*	500	250	125	>75	-	-



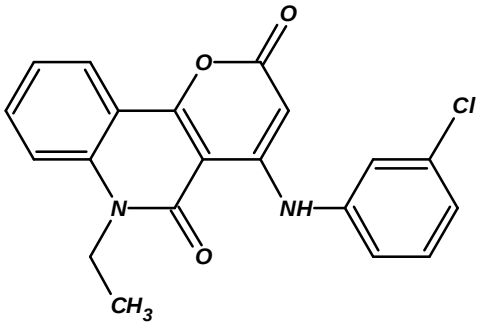
ASM-26

Sr. No.	Strain	Minimum Inhibitory Concentration (MIC, mg/ml)					
1	*Escherichia Coli*	-	-	-	-	-	-
2	*Enterobacter*	-	-	-	-	-	-
3	*Pseudomonas Aeruginosa*	++/-	++/-	125	75	-	-
4	*Staphylococcus Aureus*	500	250	125	75	-	-
5	*Kleibsiell Pneumonae*	-	-	-	-	-	-
6	*Bacillus Subtilis*	ND	ND	ND	ND	ND	ND



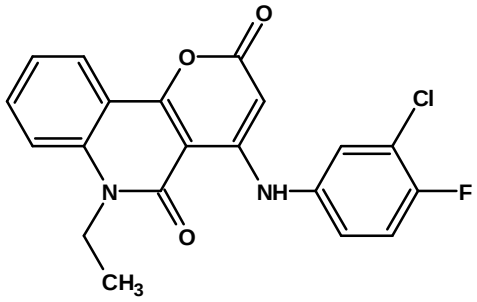
ASM-30

Sr. No.	Strain	Minimum Inhibitory Concentration (MIC, mg/ml)					
1	*Escherichia Coli*	-	-	-	-	-	-
2	*Enterobacter*	500	250	>125	-	-	-
3	*Pseudomonas Aeruginosa*	500	250	>125	-	-	-
4	*Staphylococcus Aureus*	500	250	125	>75	-	-
5	*Kleibsiell Pneumonae*	500	250	-	>75	-	-
6	*Bacillus Subtilis*	500	250	125	>75	-	-



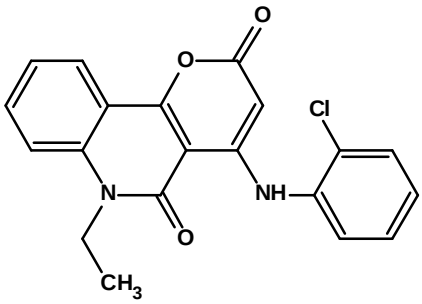
ASM-32

Sr. No.	Strain	Minimum Inhibitory Concentration (MIC, mg/ml)					
1	*Escherichia Coli*	-	-	-	-	-	-
2	*Enterobacter*	500	-	125	-	-	-
3	*Pseudomonas Aeruginosa*	500	250	-	-	-	-
4	*Staphylococcus Aureus*	500	250	125	-	-	-
5	*Kleibsiella Pneumonae*	500	250	125	-	-	-
6	*Bacilluc Subtilis*	500	250	125	75	-	-



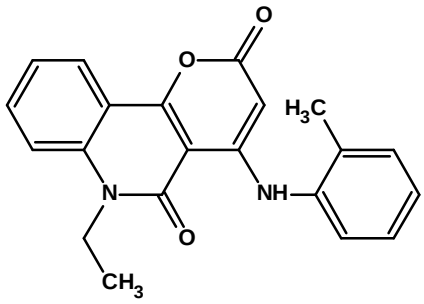
ASM-33

Sr. No.	Strain	Minimum Inhibitory Concentration (MIC, µg/ml)					
1	*Escherichia Coli*	500	+/-	+/-	-	-	-
2	*Enterobacter*	500	250	125	-	-	-
3	*Pseudomonas Aeruginosa*	500	+/-	-	-	-	-
4	*Staphylococcus Aureus*	500	250	125	75	<37	-
5	*Kleibsiella Pheumonae*	500	250	125	75	<37	-
6	*Bacillus Subtilis*	500	250	+/-	75	<37	-



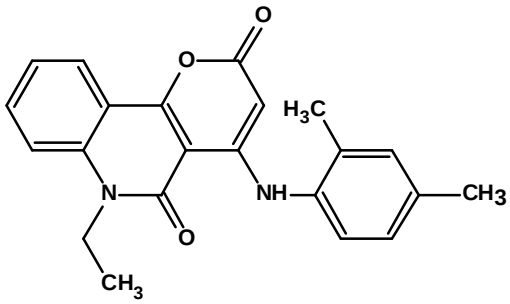
ASM-36

Sr. No.	Strain	Minimum Inhibitory Concentration (MIC, µg/ml)					
1	*Escherichia Coli*	-	-	-	-	-	-
2	*Enterobacter*	-	-	-	-	-	-
3	*Pseudomonas Aeruginosa*	-	-	-	-	-	-
4	*Staphylococcus Aureus*	500	250	125	<75	-	-
5	*Kleibsiella Pheumonae*	-	-	-	-	-	-
6	*Bacillus Subtilis*	500	250	125	<75	<37	-



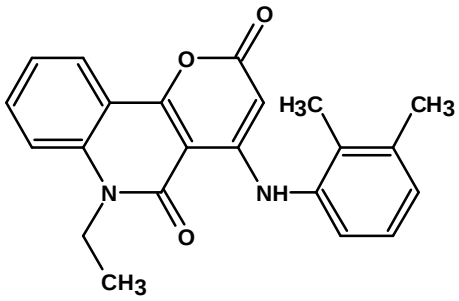
ASM-40

Sr. No.	Strain	Minimum Inhibitory Concentration (MIC, mg/ml)					
1	*Escherichia Coli*	500	-	-	-	-	-
2	*Enterobacter*	-	-	-	-	-	-
3	*Pseudomonas Aeruginosa*	-	-	<125	-	-	-
4	*Staphylococcus Aureus*	500	250	125	75	37	<18
5	*Kleibsiella Pheumonae*	500	250	125	75	+/-	-
6	*Bacillus Subtilis*	500	250	125	75	37	<18



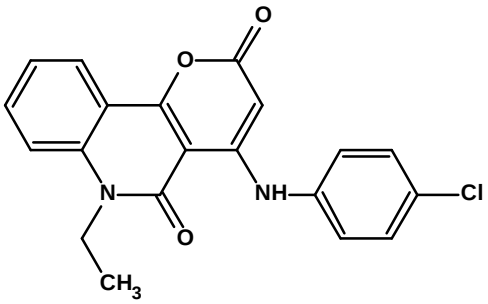
ASM-43

Sr. No.	Strain	Minimum Inhibitory Concentration (MIC, mg/ml)					
1	*Escherichia Coli*	-	-	-	-	-	-
2	*Enterobacter*	500	250	125	-	-	-
3	*Pseudomonas Aeruginosa*	500	250	-	-	-	-
4	*Staphylococcus Aureus*	500	250	125	<75	-	-
5	*Kleibsiell Pneumonae*	500	250	125	<75	-	-
6	*Bacillus Subtilis*	500	250	125	<75	-	-



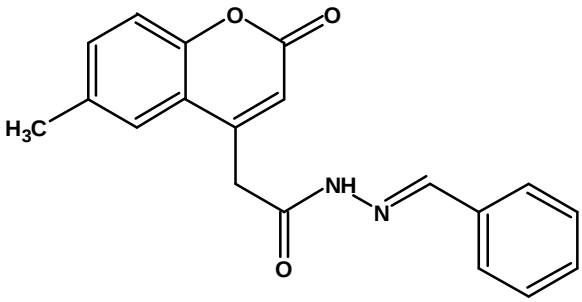
ASM-44

Sr. No.	Strain	Minimum Inhibitory Concentration (MIC, mg/ml)						
1	*Escherichia Coli*	1000	500	+/-	+/-	>75	+/-	-
2	*Enterobacter*	-	-	-	-	-	-	-
3	*Pseudomonas Aeruginosa*	1000	-	-	-	-	-	-
4	*Staphylococc-us Aureus*	1000	500	250	125	75	37	<18
5	*Kleibsiella Pheumonae*	1000	500	250	125	75	37	+/-
6	*Bacillus Subtilis*	1000	500	250	125	75	37	<18



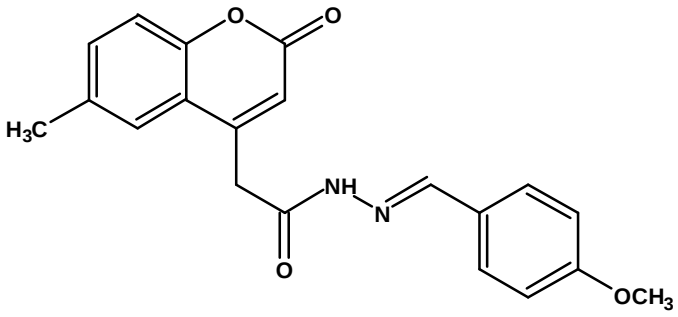
ASM-47

Sr. No.	Strain	Minimum Inhibitory Concentration (MIC, mg/ml)					
1	*Escherichia Coli*	500	250	125	75	37	<18
2	*Enterobacter*	500	250	-	-	<37	-
3	*Pseudomonas Aeruginosa*	-	250	-	-	-	-
4	*Staphylococcus Aureus*	500	250	125	75	37	<18
5	*Kleibsiella Pheumonae*	500	250	125	-	37	<18
6	*Bacillus Subtilis*	500	250	125	75	37	<18



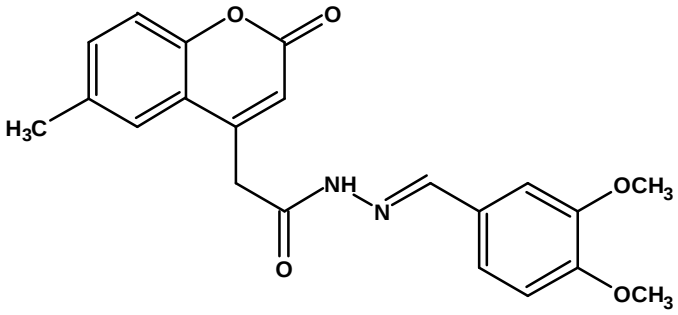
MSB-1

Sr. No.	Strain	Minimum Inhibitory Concentration (MIC, mg/ml)					
1	*Escherichia Coli*	500	250	125	75	37	<18
2	*Enterobacter*	500	-	125	75	37	<18
3	*Pseudomonas Aeruginosa*	-	-	-	-	-	-
4	*Staphylococcus Aureus*	500	250	125	75	37	<18
5	*Kleibsiella Pheumonae*	-	250	125	-	-	<18
6	*Bacillus Subtilis*	500	250	125	75	37	<18



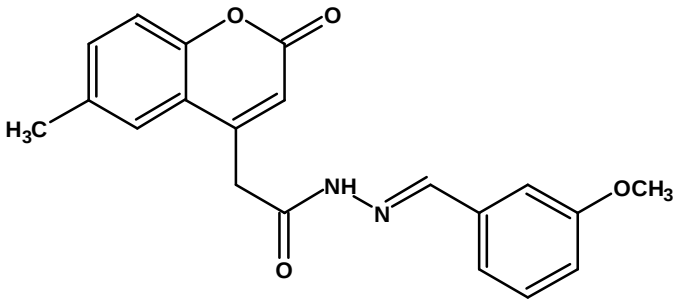
MSB-2

Sr. No.	Strain	Minimum Inhibitory Concentration (MIC, mg/ml)					
1	*Escherichia Coli*	-	-	-	-	-	-
2	*Enterobacter*	-	-	-	-	-	-
3	*Pseudomonas Aeruginosa*	500	250	-	-	-	-
4	*Staphylococcus Aureus*	500	250	<125	-	-	-
5	*Kleibsiell Pneumonae*	-	-	-	-	-	-
6	*Bacillus Subtilis*	ND					



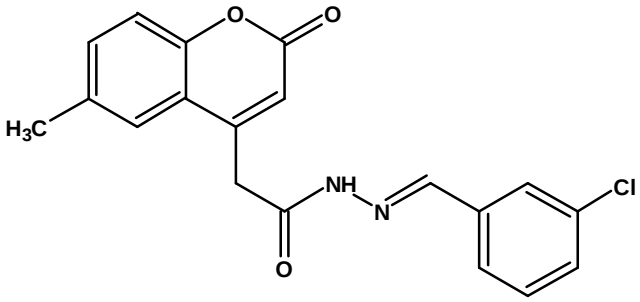
MSB-7

Sr. No.	Strain	Minimum Inhibitory Concentration (MIC, mg/ml)					
1	*Escherichia Coli*	-	-	-	-	-	-
2	*Enterobacter*	-	-	-	-	-	-
3	*Pseudomonas Aeruginosa*	500	<250	-	-	-	-
4	*Staphylococcus Aureus*	-	-	-	-	-	-
5	*Kleibsiella Pneumonae*	-	-	-	-	-	-
6	*Bacillus Subtilis*	ND	ND	ND	ND	ND	ND



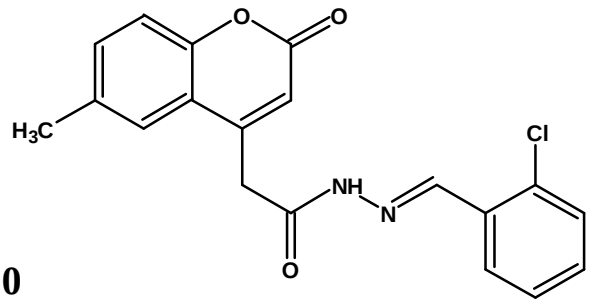
MSB-8

Sr. No.	Strain	Minimum Inhibitory Concentration (MIC, mg/ml)					
1	*Escherichia Coli*	500	250	125	>75	-	-
2	*Enterobacter*	-	-	-	-	-	-
3	*Pseudomonas Aeruginosa*	<500	-	-	-	-	-
4	*Staphylococcus Aureus*	-	-	-	-	-	-
5	*Kleibsiella Pheumonae*	500	-	-	-	-	-
6	*Bacillus Subtilis*	500	-	-	-	-	-



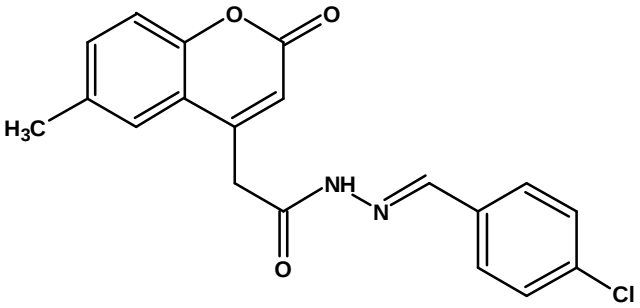
MSB-9

Sr. No.	Strain	Minimum Inhibitory Concentration (MIC, mg/ml)					
1	*Escherichia Coli*	-	-	-	-	-	-
2	*Enterobacter*	-	-	-	-	-	-
3	*Pseudomonas Aeruginosa*	500	250	125	>75	-	-
4	*Staphylococcus Aureus*	500	250	125	>75	-	-
5	*Kleibsiella Pheumonae*	500	250	125	>75	-	-
6	*Bacillus Subtilis*	500	250	125	>75	-	-



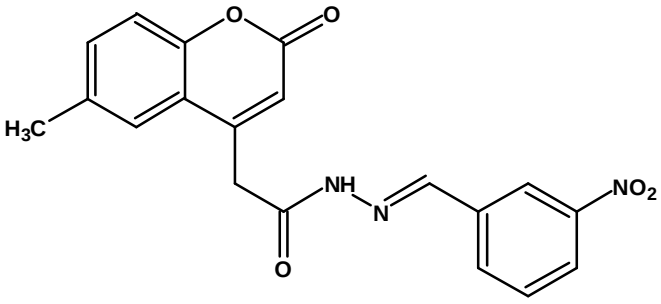
MSB-10

Sr. No.	Strain	Minimum Inhibitory Concentration (MIC, mg/ml)					
1	*Escherichia Coli*	-	-	-	-	-	-
2	*Enterobacter*	-	-	-	-	-	-
3	*Pseudomonas Aeruginosa*	500	250	-	-	-	-
4	*Staphylococcus Aureus*	500	250	125	<75	-	-
5	*Kleibsiell Pneumonae*	-	-	-	-	-	-
6	*Bacillus Subtilis*	ND					



MSB-11

Sr. No.	Strain	Minimum Inhibitory Concentration (MIC, µg/ml)					
1	*Escherichia Coli*	-	-	-	-	-	-
2	*Enterobacter*	500	250	-	-	-	-
3	*Pseudomonas Aeruginosa*	500	-	-	-	-	-
4	*Staphylococcus Aureus*	500	250	125	75	37	<18
5	*Kleibsiella Pheumonae*	500	250	125	75	37	<18
6	*Bacillus Subtilis*	500	250	125	75	37	<18



MSB-12

Sr. No.	Strain	Minimum Inhibitory Concentration (MIC, µg/ml)					
1	*Escherichia Coli*	-	250	125	>75	-	-
2	*Enterobacter*	500	-	-	-	-	-
3	*Pseudomonas Aeruginosa*	-	-	<125	-	-	-
4	*Staphylococcus Aureus*	-	-	-	-	-	-
5	*Kleibsiella Pheumonae*	500	250	-	-	-	-
6	*Bacillus Subtilis*	500	250	-	-	-	-

Conclusion:

Three types of antimicrobial studies were carried out.

(1) In the FIRST study, Protocol-1 was followed and the minimum inhibition concentration were determined of 40 compounds against *Streptococcus pyogens A77, S.aureus SG511, S.aureus E710, Enterococcus Faecalis,M. Smegmatis MY-1 and M. Smegmatis MY-2.*

Many compounds have shown very proming MIC at 1.25 µg/ml, 2.5 µg/ml, 5 µg/ml and 10 µg/ml against some of the species discussed above.

(2) In the SECOND study, the biological activity was studied against Gram positive and Gram negative strains like *S. aureus and E. coli.* The compounds were screened for preliminary data collection. Zone of inhibition are reported as **Table 7.2.**

(3) The THIRD study is based on the antibacterial study of individual compounds at various concentration.

(4) The compound AM-13 shows MIC at 50 µg (Protocol-3), against three different strains which is found most active in the present series. Many of the compounds has shown MIC value upto 100 µg/ml against two or three strains. While some compounds like (AM-14, AM-15, AM-25, AM-26, AM-27, AM-34 and AKSM-12,20 &32) do not show any activity aganist any strain.