Studies in synthesis and applications of O and N containing heterocycles

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BY

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CERTIFICATE

This is to certify that the work presented in the thesis entitled **"Studies in synthesis and applications of O and N containing heterocycles"** submitted to the Department of Chemistry, Faculty of Sciene, The Maharaja Sayajirao University of Baroda, for the award of **Ph.D.** degree by **Mr. Jigar Nareshkumar Soni**, is original research work carried out by him under my guidance and supervision.

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Dedicated to Dream of

My Father

Shri Nareshkumar Praddhumanlal Soni

DECLARATION

I state the work presented in this thesis entitled "**Studies in synthesis and applications of O and N containing heterocycles**", comprises of independent investigations carried out by me under the guidance of Dr. Shubhangi S. Soman and are true to the best of my knowledge. Whenever references have been made to the work of others, it has been clearly indicated with the source of information under the reference section. The work presented in this thesis has not been submitted elsewhere for the award of any other degree.

Signature of candidate

Jigar Nareshkumar Soni

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Jigar Nareshkumar Soni

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List of Abbreviations and Symbols

$(AcO)_2Hg$	Mercury acetate
µg	Micro gram
µl	Micro liter
µM	Micro molar
µv	Micro volt
AC	Alternate current
Ac_2O	Acetic anhydride
AcCl	Acetyl chloride
AcOH	Acetic acid
B. subtilis	Bacillus subtilis
BOP	Benzotriazol-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate
Br_2	Bromine
Bu_2SnCl_2	Dibutyl tin chloride
C. albicans	Candida albicans
$CDCl_3$	Deutarated chloroform
CH_3COONa	Sodium acetate
$ClSO_3H$	Chlorosulfonic acid
CNS	Central nervous system
COX	Cyclooxygenase
CS_2	Carbon disulfide
Cs_2CO_3	Cesium carbonate
DCC	N, N-Dicyclohexylcarbodiimide

DDQ	2,3-Dichloro-5,6-dicyano-1,4-benzoquinone
DIPEA	N, N-Diisopropylethyleneamine
DMAD	Dimethyl acetylenedicarboxylate
DMF	N, N-dimethyl formamide
DMSO	Dimethylsulfoxide
DMSO-d_6	Deutarated Dimethylsulfoxide
E. coli	Escherichia coli
EDC.HCl	1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride
ESI/MS	Electron spray ionization mass spectroscopy
EtOH	Ethyl alcohol, Ethanol
g	Gram
GABA	γ-amino butyric acid
h	Hour
H_2SO_4	Sulphuric acid
HATU	O-(7-Azabenzotriazol1-yl)-N,N,N',N'-tetramethyluroniumhexafluorophosphate
HCl	Hydrochloric acid
HCOH	Formic acid
HOBt	1-Hydroxybenzotriazole
HPLC	High performance liquid chromatography
I_2	Iodine
IC_{50}	50% Inhibitory concentration
IP	Intraperitoneal
FTIR	Fourier transform infrared spectroscopy

K_2CO_3	Potassium carbonate
KBr	Potassium bromide
KIO_3	Potassium iodate
KOH	Potassium hydroxide
LiCl	Lithium chloride
LiOH	Lithium hydroxide
Lit.	Literature
MAO	Monoamine oxidase
MeOH	Methyl alcohol, Methanol
MES	Maximal electroshock seizure
mg	Milligram
MHz	Mega hertz
MIC	Minimum inhibitory concentration
min	Minute
ml	Milliliter
mmol	Millimole
mp	Melting point
MS	Mass spectroscopy
Na	Sodium metal
$NaHCO_3$	Sodium bicarbonate
NaN_3	Sodium azide
NaOH	Sodium hydroxide
NBS	N-Bromosuccinimide

NCS	Isothiocyanate
$NH_2NH_2.H_2O$	Hydrazine hydrate
NH_3	Ammonia
NIH	National institute of health
NMR	Nuclear magnetic resonance
^oC	Degree celsius
P. aeruginosa	Psuedomonas aeruginosa
P_2O_5	Phosphorus pentoxide
Pd-C	Palladium on charcoal (carbon)
$PdCl_2$	Palladium chloride
$POCl_3$	Phosphorus oxychloride
PPAR	Peroxisome proliferator activated receptor
ppm	Parts per million
PyBOP	Benzotriazol-1-yloxy)tripyrrolidinophosphoniumhexafluorophosphate
RT	Room temperature
S. aureus	Staphylococcus aureus
TEA	Triethylamine
TFA	Trifluoroacetic acid
THF	Tetrahydrofuran
TLC	Thin layer chromatography
UV	Ultraviolet-visible
$ZnCl_2$	Zinc chloride

Summary

Chapter 1: Introduction

Cyclic organic compounds containing all carbon atoms in ring formation is referred as a *carbocyclic* compound. If at least one atom other than carbon forms a part of the ring system then it is designated as a *heterocyclic* compound. Pyrones are heterocyclic compounds containing oxygen as hetero atom other than carbon; its derivatives may contain sulphur, phosphorous, nitrogen etc.

Pyran-2-one or coumarin and pyran-4-one or chromone and its derivatives are widely distributed in nature and show various pharmacological activities. Various heterocycles have been synthesized from coumarin derivatives. Many Scientists are keen to work in this field due to better selectivity and pharmacological activity of these compounds in medicinal chemistry.

Coumarin derivatives are used for fragrances in cosmetics, pharmaceuticals and foods. Coumarin derivatives show various activities like anti bacterial, anti fungal, antiviral, insecticidal, CNS activity, analgesic, anti inflammatory, anti HIV-1 integrase inhibitory activity, antioxidant and anticoagulant. They also show anticancer activity against lungs cancer, gastric cancer, pancreatic cancer, skin cancer, intraocular melanoma, uterine cancer, breast cancer, cancer of the endocrine system, and also inhibit the growth of ovarian and nasopharyngeal tumor cells.

Various derivatives synthesized from coumarin show wide range of pharmacological activities like anticonvulsant activity, anti histaminic activity, antiepileptic, hypotensive, hypertension, non steroidal human progesterone receptor agonists, regulation of human cervical carcinoma cell proliferation, dermatological disorders like psoriasis, vitiligo, mycosis and atropic eczema and anti spasmodic, antiallergic agent particularly for bronchial asthma, uricosuric, anti diuretic hyperuricemia and edema.

The study of coumarin, its various derivatives and various heterocycles is of great interest for synthesis and biological activity.

Objectives of the Work:

- Synthesis of coumarin derivatives
- Synthesis of various heterocycles from various coumarin derivatives
- Biological evaluation of various compounds and find out possible QSAR.

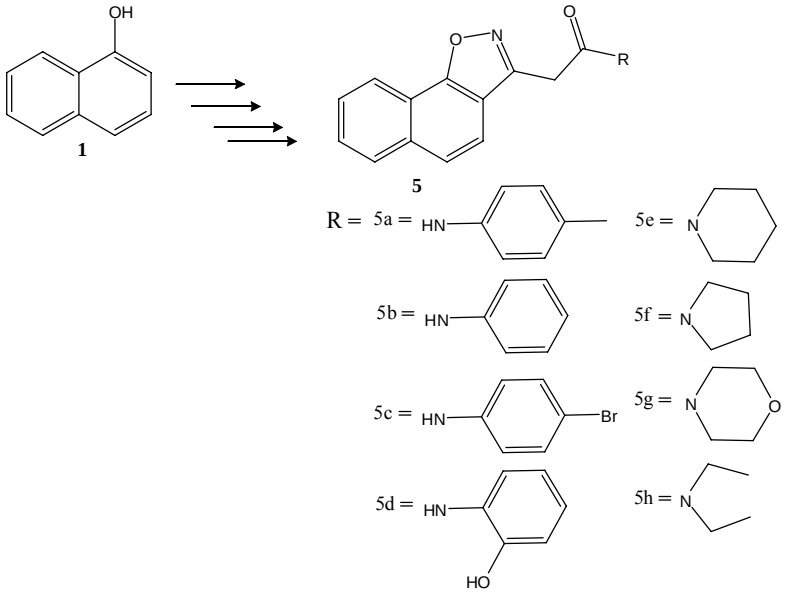
The structures of all the synthesized compounds have been established using various analytical methods like IR, ^{1}H NMR, ^{13}C NMR, Mass, Elemental analyses, HPLC and Single Crystal X-Ray Diffraction.

Chapter 2: Synthesis and biological evaluation of isoxazole derivatives

Derivatives of benzisoxazole are known to possess wide spectrum of biological activities such as anti microbial and anti herbicidal activities. 1, 2-benzisoxazole derivatives and various analogues show various activities like antidepressants, anti-inflammatory, antimalarial, antipsychotics, antiviral, general anesthetics, anticonvulsant, dopamine blocking properties and cytoprotective agents.

1-Naphthol on reaction with acetic acid in presence of zinc chloride gave 1-(1-hydroxynaphhthalen-2-yl)ethanone which on Hoesch reaction with diethyl carbonate in presence of pulverized sodium gave 4-hydroxy-2H-benzo[h]chromen-2-one. This on Posner reaction with hydroxylamine hydrochloride in presence of sodium bicarbonate afforded 2-(naphtho[2,1-d]isoxazole-3-yl)acetic acid which was then converted into different amides (Scheme 1) by reaction with different amines using dicyclohexylcarbodiimide (DCC) and N, N-dimethyl amino pyridine (DMAP) as catalyst.

Scheme 1:



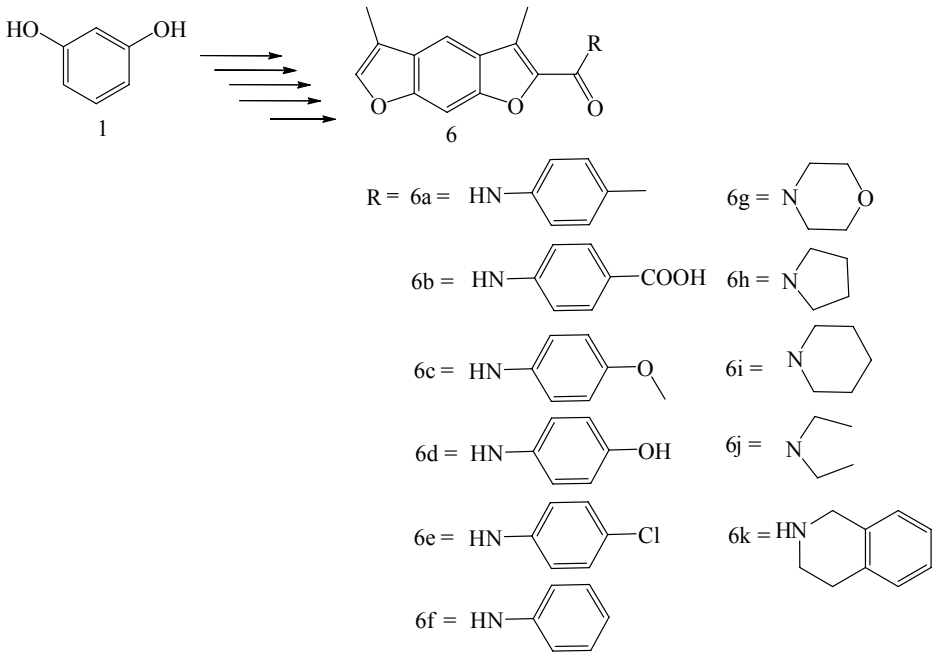
Biological Evaluation: All Synthesized compounds were screened against Melanoma Cancer Cell lines at Hershey Medical Centre, USA and also screened for anticonvulsant activity at Dharmsinh Desai University, Nadidad.

Chapter 3: Synthesis and biological evaluation of amide derivatives of difuran-2-carboxylic acid

Various esters, amide, ether and thioether derivatives of furan show antifungal, antimicrobial, cysteine protease inhibitor, ischemic cell death, orexin receptor antagonist, anti angiogenesis, anti osteoarthritis activity and also show CNS depressant effect.

7-hydroxy-4-methyl-coumarin was condensed with chloroacetone in presence of anhydrous K_2CO_3 in dry acetone gave 4-methyl-7-(2-oxopropoxy)-coumarin which was brominated using N-bromosuccinimide to give 3-bromo-4-methyl-7-(2-oxopropoxy)-coumarin. This bromo derivative was further refluxed with 10% alkaline ethanol gave cyclized 3, 5-dimethylbenzo[1,2-b:5,4-b']difuran-2-carboxylic acid **5** as major product, which was converted into corresponding amides (Scheme 2).

Scheme 2:



Biological Evaluation: All Synthesized compounds were screened against 2 gm +ve bacteria, 2 gm –ve bacteria and one fungi at Micro Care Lab, Surat and also screened for Orexin receptor binding study at University of Helsinki, Finland.

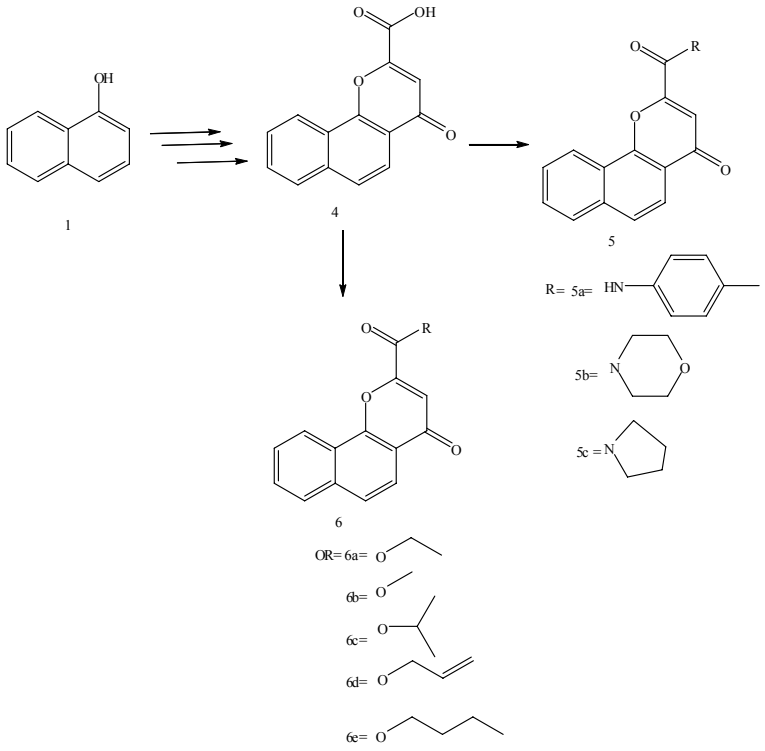
Chapter 4a: Synthesis of amide and ester derivatives of Naphthopyrone 2-carboxylic acid

Chromone derivatives are useful as antiallergic agent particularly for bronchial asthma they inhibit the release of mediators like histamine, several kinins etc. of immediate hypersensitivity reactions. Chromone derivatives have been reported as MAO-B inhibitors, melanin concentrating hormone receptor 1 antagonists and adenosine receptor ligand. Photodimerization and microwave assisted synthesis of Chromone-2-carboxylic acid derivatives have been reported. In this part we have synthesized various amide and ester derivatives of naphthopyrone-2-carboxylic acid.

Condensation of 1-naphthol **1** with dimethyl acetylenedicarboxylate (DMAD) in presence of anhydrous K_2CO_3 and dry acetone gave *E* and *Z* mixture of dimethyl 2-(naphthalen-1-yloxy)maleate. Since *E* and *Z* mixture was not possible to separate into *E*

and *Z* isomers, it on mild hydrolysis in aqueous KOH at room temperature gave 2-(Naphthalen-1-yloxy)maleic acid as corresponding mixture of *E* and *Z* isomers, which were also not separated. The diacid (*E*/*Z* mixture) on cyclization with concentrated sulphuric acid gave 4-Oxo-4H-benzo[h]chromene-2-carboxylic acid. This acid was converted into corresponding amide using two different methods. The chromen-2-acid was converted into various esters using various alcohols and dry HCl gas (Scheme 3).

Scheme 3:



Chapter 4b: Unusual deacetylation of 1-acetyl 2-naphthol in facile manner

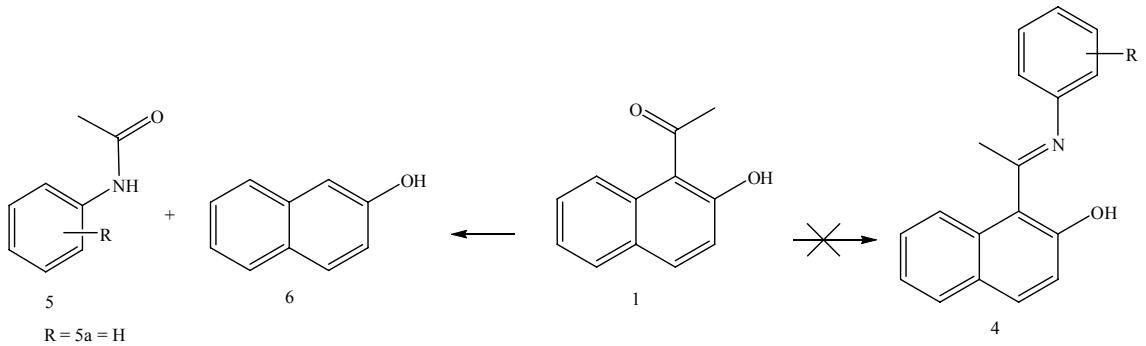
o- Hydroxy ketone is one of most the common starting material used for various reactions. Formation of chalcones or Schiff bases from *o*-hydroxy ketones is one of the most frequently used reactions in organic synthesis because various heterocyclic rings can be formed further from it. Chalcones, chromenes, imidazoles and other heterocyclic rings show various biological activities. Synthesis of chalcones[\] from various substituted *o*-hydroxy acetophenone derivatives[\] and formation of flavones from it was reported earlier from our laboratory. We were keen to form first Schiff bases and then heterocyclic ring

from 1-acetyl 2-naphthol. Instead of that during formation of Schiff base reaction, we have observed novel deacetylation of 1-acetyl 2-naphthol.

Reaction of acetic acid and acetic anhydride with 2-naphthol in presence of zinc chloride gave1-acetyl 2-naphthol which when refluxed in absolute ethanol with primary aromatic amines and 2-3 drops of acetic acid, we observed formation of two products on TLC, different from starting material. The two products were separated by column chromatography. One product was found to be different anilide derivatives and another product was found to be same in all reactions i.e. 2-naphthol (Scheme 4). Hence we observed deacetylation of 1-acetyl 2-naphthol which we can call it as either retero Friedel Crafts reaction or retero Diels Alder reaction.

Same reaction was repeated with secondary amines. In case of cyclic secondary amines, retero Friedel Crafts reaction occur and then acetyl group is removed as acetic acid from N-acetyl pyrrolidine or N-acetyl morpholine and 2-naphthol was obtained. No reaction was observed with p-nitro aniline, benzotriazole, indole and 3-acetyl naphthopyrone, only starting materials were recovered.

Scheme 4:



5

R = 5a = H
5b = 4-COOH
5c = 4-CH_3
5d = 2-OH
5e = 4-OH
5f = 4-OCH_3

6

1

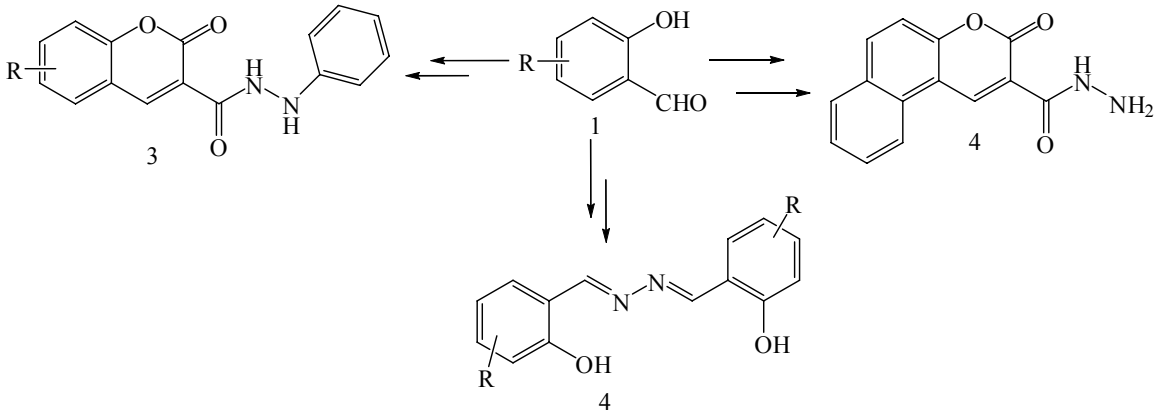
4

Chapter 5a: Novel Retro Knoevenagel reaction of Substituted Coumarin-3-carboxylate

Various derivatives of coumarin 3-caboxylate with various substitutions show various activities like anti-helicobacter pylori, human monoamine oxidase inhibition, α-Chymotrypsin inhibitory activity and inhibitory activity of gGAPDH enzyme.

Knoevenagel reaction of various salicylaldehyde derivatives in pyridine and diethyl malonate with catalytic amount of piperidine gave different coumarin-3-carboxylate derivatives, which on reaction with phenyl hydrazine in absolute ethanol gave different phenyl hydrazide derivatives. But to our surprise reaction of various coumarin-3-carboxylate derivatives with hydrazine hydrate gave various hydroxy schiff base derivatives (Scheme 5) which was proved by Single Crystal X-Ray Diffraction.

Scheme 5:



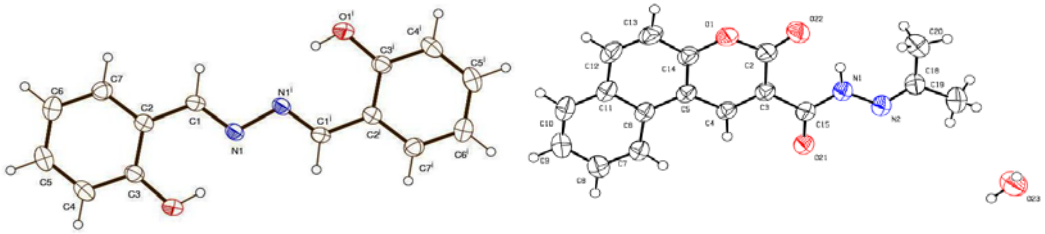


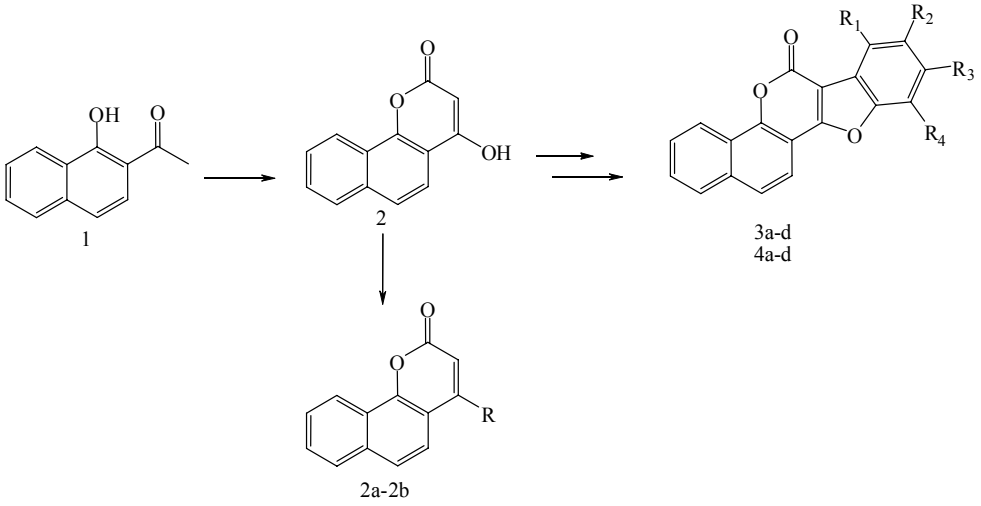
Figure 1: ORPET diagram of 2, 2'-(1E, 1'E)-hydrazine-1, 2-diylidenebis(methan-1-yl-1-ylidene)diphenol and 3-oxo-N'-(propan-2-ylidene)-3H-benzo[f]chromene-2-carbohydrazide

Chapter 5b: Synthesis and anticancer activity of 4-hydroxy Naphtho coumarin derivatives and naphtho coumestans

Coumestan ring system is present in number of natural products. It is found in coumestrol, psoralidine, wedelolactone, norwedelolactone and pterocarsin. Various coumestan derivatives show variety of pharmacological activities such as antihepatotoxic, anti-hypertensive, antitumor, antiphospholipase A_2 and antidote activities against snake venom, treatment for liver diseases and skin disorders, viral infections, antihemostatic activity, estrogenic activity and also binding to central benzodiazepine receptor.

Hoesch reaction of 2-acetyl 1-naphthol with diethyl carbonate in presence of pulverized sodium gave 4-hydroxy-2H-benzo[h]chromen-2-one. This on oxidative cyclization with catachole using sodium acetate and potassium iodate gave corresponding coumestan derivatives 8,9-dihydroxy-*6H*-benzo[*h*]benzofuro[3,2-c]chromen-6-one. These coumestan derivatives were condensed with dimethyl sulphate and different mono and di halides in presence of base like anhydrous K_2CO_3 and dry acetone gave corresponding condensed or cyclized coumestan (Scheme 6) compounds. 4-hydroxy-2H-benzo[h]chromen-2-one derivative was also condensed[7] with dimethyl sulphate and allyl bromide gave corresponding methoxy and allyloxy derivatives.

Scheme 6:



Comp. No.	R_1	R_2	R_3	R_4	R
2a	-	-	-	-	O-CH_3
2b	-	-	-	-	O-CH_2-CH=CH_2
3a	H	O-CH_3	O-CH_3	H	H
3b	H	O-CH_2-CH=CH_2	O-CH_2-CH=CH_2	H	H
3c	H	O-CH_2-CH_2-O		H	H
3d	H	O-CH_2-O		H	H
4a	H	O-CH_3	O-CH_3	O-CH_3	H
4b	H	O-CH_2-CH=CH_2	O-CH_2-CH=CH_2	O-CH_2-CH=CH_2	H
4c	H	O-CH_2-CH_2-O		OH	H
4d	H	O-CH_2-O		OH	H

Biological Evaluation: 2 Intermediate and 7 final synthesized compounds were screened against two melanoma Cancer cell lines UACC-903 and A375M, one breast cancer cell line MCF-7 and fibroblast (FF2441-Precursors of normal cells) at Hershey Medical Centre, USA.

Chapter 1

Introduction

1. Introduction

1.1 General Introduction

Heterocyclic compounds are widely distributed in nature and are essential for life. Both naturally and synthetically prepared heterocyclic compounds are pharmacologically active and are in clinical use.

The coumarin was first isolated by Vogel in Munich in 1820 from tonka beans. The name coumarin originates from a Caribbean word "coumarou" for the tonka tree. Coumarin is generally known as Benzo-2-pyrone or Benzo-**α**-pyrone or Chromen-2-one. Coumarin occurs in nature either in a free state or in combined state, as glycosides. They are abundant in grasses, legumes and citrus fruits. Coumarin derivatives have been reported neither in algae nor in mosses, however, there are few reports of coumarin derivatives present in bacteria and fungi. The principal sources have been classified into major families from which most of the coumarins are isolated.

Umbelliferae (e.g. Parsley, Parsnip, Celery, Ammi majus, Angelica archangelic)

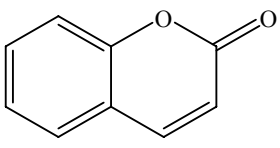
Rutaceae (e.g. Bergamot fruit, Lime grass plant, Cloves, Common rue)

Leguminosae (e.g. Psoralea Corylifolia, Xanthoxylum Flavum) and

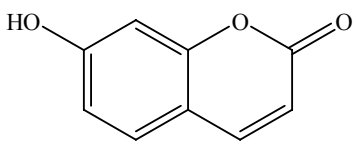
Moraceae (e.g. Ficus Carica)

Some important naturally occurring coumarin derivatives are shown below.

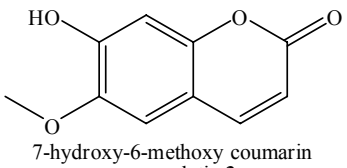
Coumarin **1** found in tonka beans with white clover. Umbelliferone **2** is 7-hydroxy coumarin. Scopoletin **3** is 7-hydroxy-6-methoxy coumarin, found in bark of the wild cherry.



chromen-2-one
or coumarin **1**

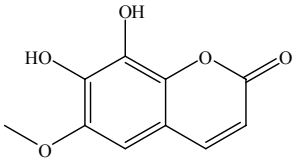


7-hydroxy coumarin
or umbelliferone **2**

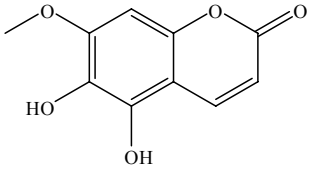


7-hydroxy-6-methoxy coumarin
or scopoletin **3**

Fraxetin **4** is 7, 8- dihydroxy-6-methoxy coumarin and Isofraxetin **5** is 5, 6-dihydroxy-7-methoxy coumarin.

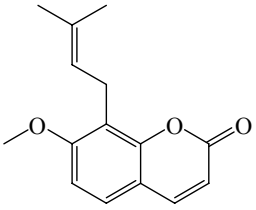


7,8-dihydroxy-6-methoxy coumarin
or fraxetin **4**

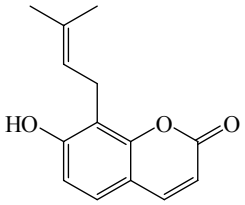


5,6-dihydroxy-7-methoxy coumarin
or isofraxetin **5**

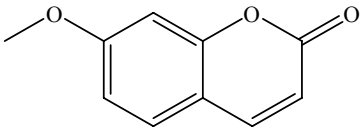
Some naturally occurring alkylated hydroxyl coumarins are Osthol and Osthanol. Osthol **6** is 7-methoxy-8-(3-methylbut-2-enyl) coumarin and Osthenol **7** is 7-hydroxy-8-(3-methylbut-2-enyl) coumarin. Herniarin **8** is 7- methoxy coumarin. Scoparone **9** (6, 7-dimethoxycoumarin) has been obtained from the Chinese herb Artemisia scoparia.



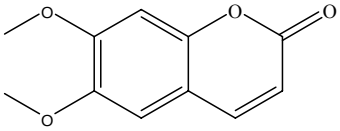
7-methoxy-8-(3-methylbut-2-enyl)coumarin
or osthol **6**



7-hydroxy-8-(3-methylbut-2-enyl)coumarin
or osthenol **7**

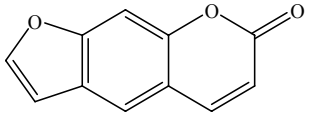


7-methoxy coumarin
or herniarin **8**

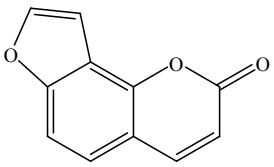


6,7-Dimethoxy coumarin
or scoparone **9**

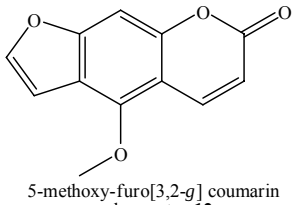
Some interesting naturally occurring coumarin derivatives like furocoumarins are; Psoralene **10**, Angelicin **11**, Bergapten **12**, Xanthotoxin **13**, Pimpinellin **14**, Isopimpinellin **15**, Aseculetin **16**, Daphnetin **17** and Ayapin **18** are few members of this group.



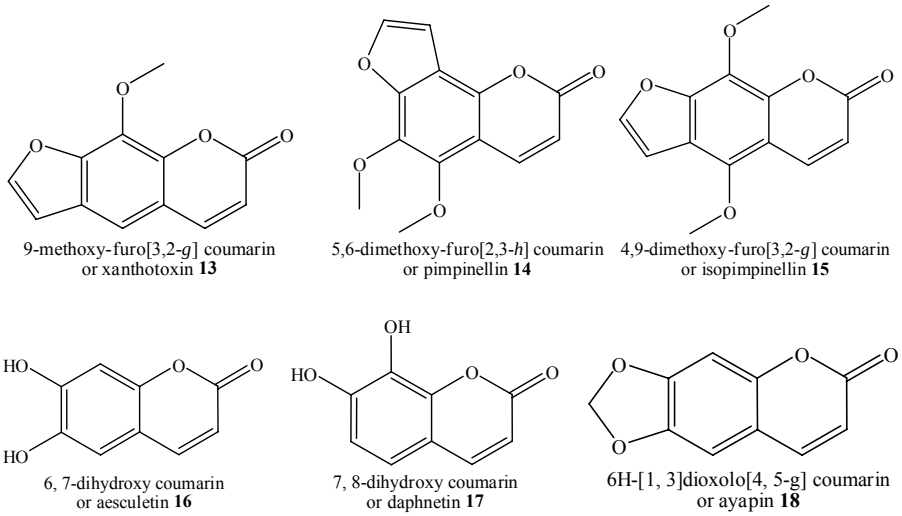
furo[3,2-*g*] coumarin or furocoumarin
or psoralene **10**



furo[2,3-*h*] coumarin
or angelicin **11**



5-methoxy-furo[3,2-*g*] coumarin
or bergapten **12**



9-methoxy-furo[3,2-*g*] coumarin or xanthotoxin **13**

5,6-dimethoxy-furo[2,3-*h*] coumarin or pimpinellin **14**

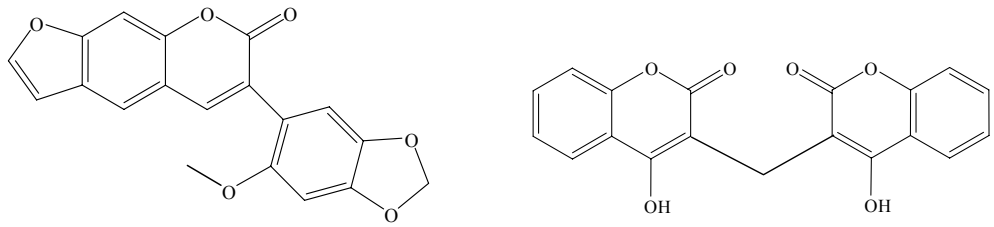
4,9-dimethoxy-furo[3,2-*g*] coumarin or isopimpinellin **15**

6, 7-dihydroxy coumarin or aesculetin **16**

7, 8-dihydroxy coumarin or daphnetin **17**

6H-[1, 3]dioxolo[4, 5-g] coumarin or ayapin **18**

Pachyrrhizin **19** is a naturally occurring 3-Phenylcoumarin derivative. Dicoumarol **20** is 3-alkyl-4-hydroxy coumarin derivative used as anticoagulant drug.



6-(6-methoxybenzo[*d*][1,3]dioxol-5-yl)-7*H*-furo[3,2-*g*] coumarin or pachyrrhizin **19**

dicoumarol **20**

1.2 Physiological Properties

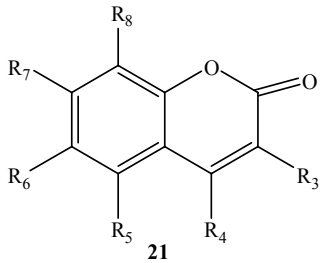
Coumarin is crystalline in nature and it has odor like vanilla hence it was used as food additive and perfumes. Coumarin itself was originally used as a flavoring substance until the direct use of coumarin in food was prohibited in the USA in 1954 due to reports of hepatotoxic effects in rats and dogs.

Coumarin has fungicidal as well as pesticidal properties. While several natural products with a coumarin moiety have been reported to have multiple biological activities. Due to concerns about coumarin as a potential liver and kidney toxin, its use as a food additive[1] was heavily restricted, although it is perfectly safe to eat food which contains natural coumarin.

When ingested, coumarin acts as a blood thinner and it also appears to be effective in treating some tumors.

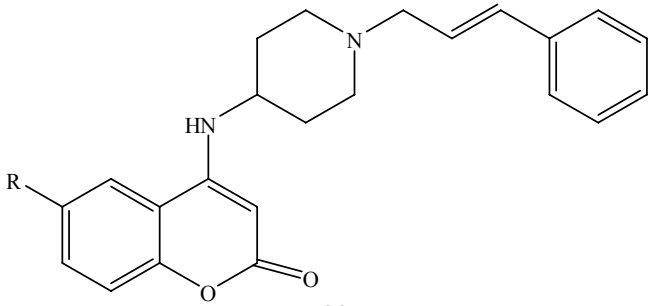
Coumarin based selective estrogen receptor modulators (SERMs) and coumarin-estrogen conjugates had been described as potential anti breast cancer agents.[2]

Kostova I. et al had synthesized synthetic and natural coumarins as cytotoxic agent **21**.[3]



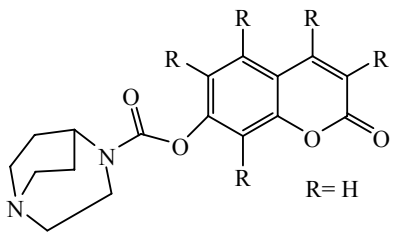
21

Kym P. R. et al had synthesized Aminopiperidinecoumarin **22** as Melanin concentrating hormone receptor 1 antagonists.[4]



22

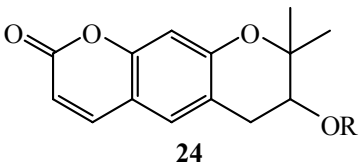
1, 4-diaza-bicyclo-(3, 3, 1) nonane coumarin derivatives **23** were found to be cholinergic ligand at the nicotinic acetylcholine receptors and modulators of monoamine receptors and transporters. Due to their pharmacological profile they may be useful for the treatment of disease of nervous system, disorders related to smooth muscle contraction, endocrine diseases, disorders related to neurodegeneration, inflammation and pain.[5]



23

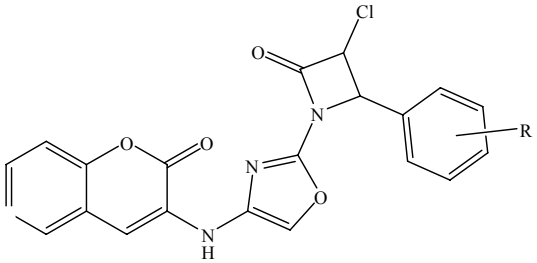
(+)-Decursinol esters[6] **24** were synthesized for therapeutic use as anti inflammatory and anti cancer agents which have been claimed for its use in the treatment of the lungs

cancer, pancreatic cancer, skin cancer, intraocular melanoma, uterine cancer, breast cancer, cancer of the endocrine system i.e., cancer of thyroid etc.



24

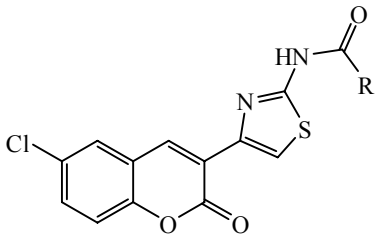
Compound **25** was found to be most potent antibacterial compound against *E. coli* and *K. pneumoniae* and compound **26** was found to be most potent antifungal agent against *C. albicans*.[7]



R= p-OCH_3 (**25**)

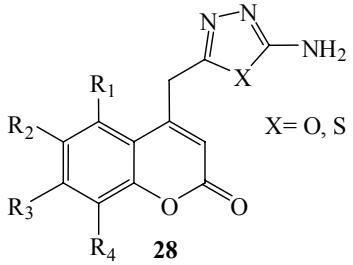
= o-OH (**26**)

Jayshree B. S. et al[8] had synthesized various coumarin thiazole derivatives **27** which were reported to have analgesic and anti inflammatory activity.

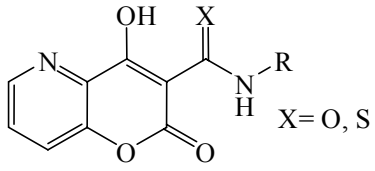


27

Some coumarin derivatives were found to have important pharmacological activities which had nitrogen as other heterocyclic element. Mashelkar U. C. et al[9] had reported some novel 4-substituted coumarin **28** as potential antibacterial agent. Lee S. U. and coworkers[10] had synthesized 4-Hydroxy-5-azacoumarin derivatives **29** which were reported as anti HIV-1 integrase inhibitors.



28



29

Coumarin can reduce tissue edema and inflammation. Its 7-hydroxy derivative inhibited prostaglandin biosynthesis.[11] They showed different physiological activities including anticancer, antioxidant, anti fungal, anti-HIV, anticoagulant.[3, 12]

7-Hydroxy-4-methylcoumarin conjugated with paclitaxel through an ester linkage, selectively inhibited growth of ovarian and nasopharyngeal tumor cells.[13]

7-Hydroxy-4-methyl coumarin nucleus with different secondary amines through two carbon spacing gave affinities for both dopamine and 5-HT receptors. It shows dopaminergic antagonistic activity.[14]

Free 6-OH in the coumarin nucleus has been found to be important for antifungal activity; while the free hydroxyl group at 7th position is important for antibacterial activity. 6-methylcoumarin is mainly used as a flavor enhancer and 7-hydroxycoumarin in sunscreens.

It has been shown that 4-hydroxycoumarin and 7-hydroxycoumarin inhibited cell proliferation in a gastric carcinoma cell lines.[15]

3-Substituted-4-hydroxy coumarin derivatives[16] and tricoumarol[17] possess HIV inhibitory potency.

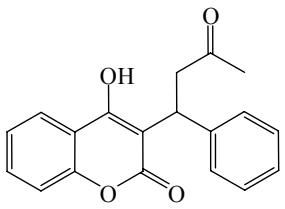
Naphthapyrones showed various biological activities like non steroidal human progesterone receptor agonists[18] and antihypertensive activity.[19]

Psoralens have been recently found to have application in the regulation of human cervical carcinoma cell proliferation in conjunction with anti-sense technology.[20]

Furocoumarins such as psoralens are well known photosentisizing drug used in PUV-A therapy (Psoralen Ultra Violet- A therapy) for the treatment of dermatological disorders like psoriasis, vitiligo, mycosis and atropic eczema[21] as well as fungal, viral and bacterial infections.[22] Psoralen derivatives are also used in treatment of cutaneous T cell lymphoma,[23] human immunodeficiency diseases[24] and prevention of rejection of organ transplant.[25]

The coumarins, Imperatorin and Osthole are known to exert anticonvulsant activity. Thus structure activity relationship of GABA receptor and various analogues of coumarins and furanocoumarins have been studied[26] by Singhuber J. and coworkers.

Warfarin **30** is a popular anticoagulant drug in market which can be ingested or injected, depending on the needs of the patient.

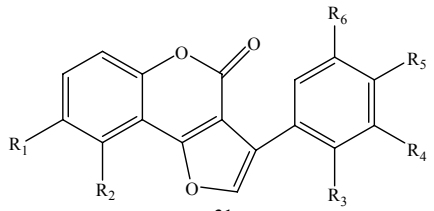


4-hydroxy-3-(3-oxo-1-phenylbutyl) coumarin
or warfarin **30**

Dicoumarol **20** or 3, 3'-methylene bis (4-hydroxy coumarin) behaves as anticoagulant agent. The inhibition of blood clotting by coumarin is predominantly linked to hydroxylation at 4th position. Dicoumarol is an effective rodenticide and also shows antimicrobial activity against a variety of bacteria.[27, 28]

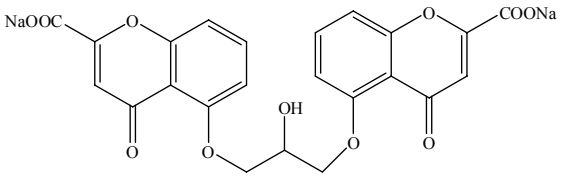
Coumarin-3-(N-aryl)sulfonamides derivatives were reported to show anticancer activity.[29]

Brahmabatt D. I. et al[30] reported synthesis of various 3-aryl furo [3,2-c] coumarins **31**.



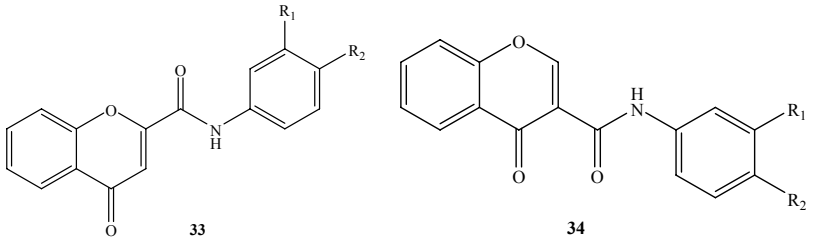
31

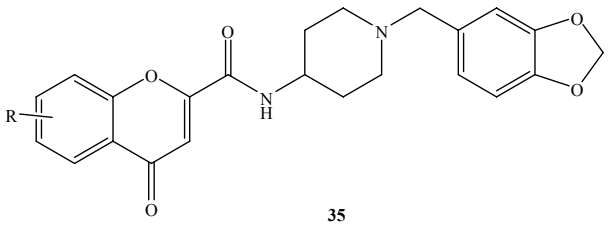
Disodium cromoglycate DSCG **32** is a clinically useful antiallergic agent particularly for bronchial asthma. It was reported to inhibit the release of mediators like histamine, several kinins etc. of immediate hypersensitivity reactions. Efforts have been made to find more potent and orally active compounds possessing pharmacological properties similar to DSCG.



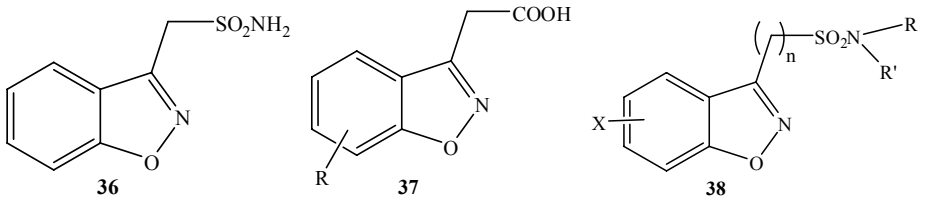
32

Various amide derivatives of benzopyrone carboxylic acid **33**, **34** and **35** have been reported as MAO-B inhibitors,[31] adenosine receptor,[32] and melanin concentrating hormone receptor 1 antagonists[33] respectively.





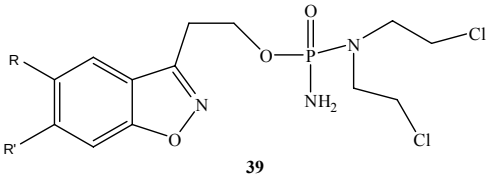
Zonisamide,[34] a 1, 2-benzisoxazole derivative **36** is an important antiepileptic agent available in the market. It has close resemblance to indole and 1, 2-benzisoxazole nucleus and can be substituted for indole nucleus for auxin like activity. Compound **37** has been synthesized by the reaction of 4-hydroxy coumarin with hydroxylamine.[35] Several 3-substituted 1, 2-benzisoxazole derivatives like 3-(sulfamoylmethyl) 1, 2-benzisoxazole **38** have been reported to show anticonvulsant activity.[36]



36 **37** **38**

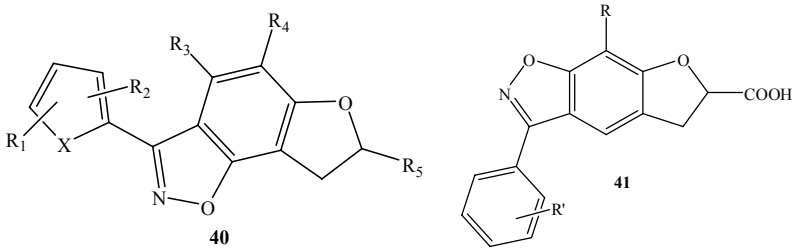
R, R'=different groups

Several 1, 2-benzisoxazole phosphorodiamidates have been designed as prodrug of phosphoramide requiring bioreductive activation.[37] Here enzymatic reduction of 1, 2-benzisoxazole moiety **39** is expected to result in formation of imine like intermediate due to cleavage of N-O bond.



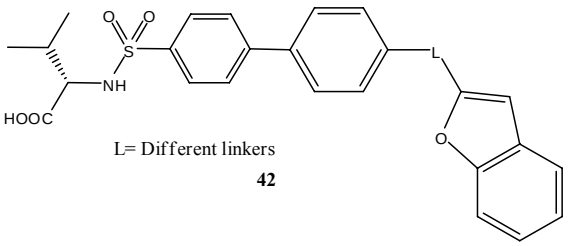
39

Some furobenzisoxazole derivatives **40** (R_1, R_2, R_3, R_4, R_5= different groups) and **41** (R, R'= different groups) were reported to posses hypotensive, uricosuric and diuretic activities and hence were useful as therapeutics for treatment of hyperuricemia, edema and hypertension.[38, 39]



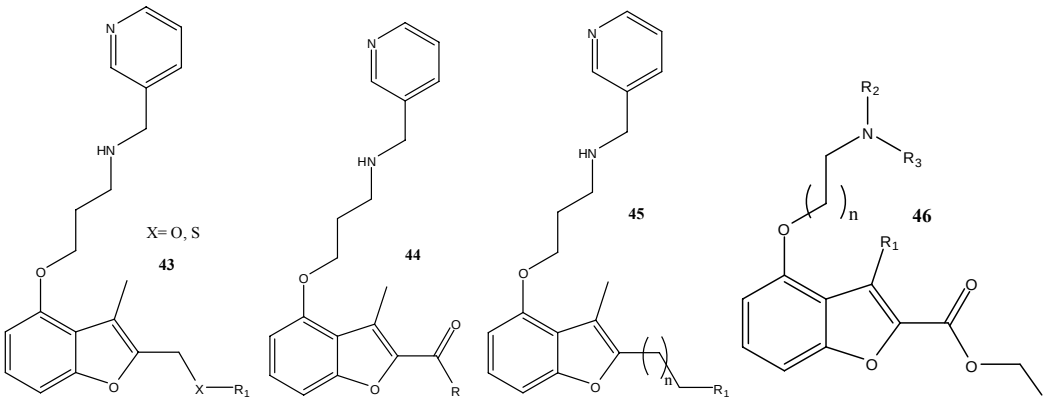
40 **41**

Various sulphonamide derivatives of benzofuran **42** were reported as potent, selective, and orally bioavailable matrix metalloproteinase-13 inhibitors for the treatment of osteoarthritis.[40]

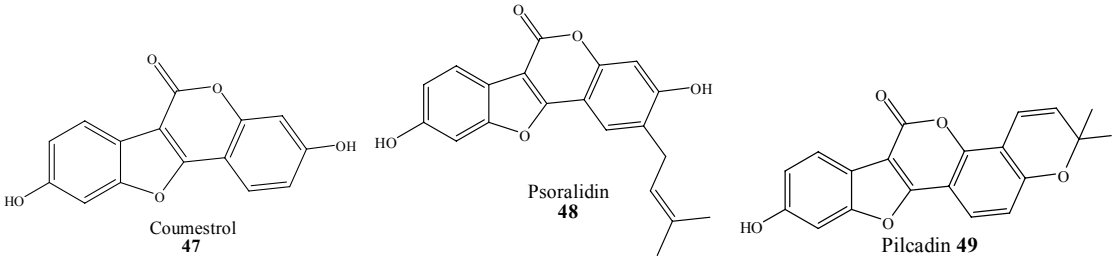


42

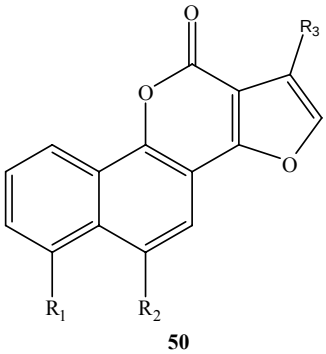
Various 3-methyl 4-hydroxy benzofuran derivatives **43**, **44**, **45** and **46** have been reported as antifungal agents targeting fungal N-myristoyltransferase.[41]



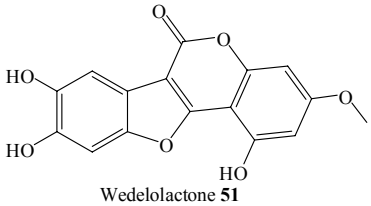
Number of natural products e.g. coumestrol **47**, psoralidine **48**, pilcadin **49** etc. belongs to coumestan class. Coumestan and its various derivatives have been reported to possess diverse pharmacological properties such as antihemorrhagic, antiproteolytic, antiphospholipase and snake antivenom.



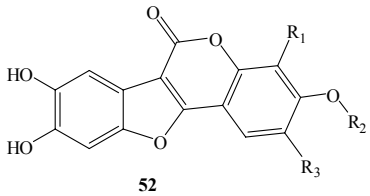
Xihong W. and coworkers[42] had synthesized novel neo-tanshinlactone analogues **50** as potent anti breast cancer agents.



Various wedelolactone analogues **51**, **52**, **53**, **54** and **55** were reported as Na^+ and K^+ ATPase inhibitors, binding to central benzodiazepine receptor.[43]



Wedelolactone **51**



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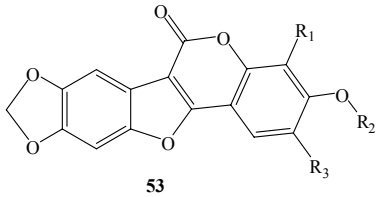

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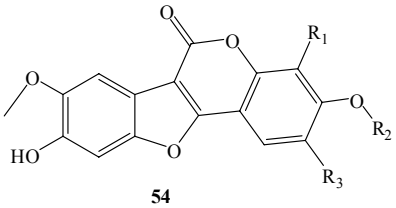
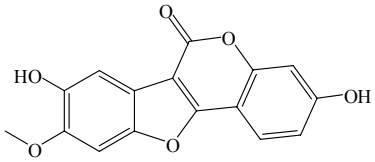


54



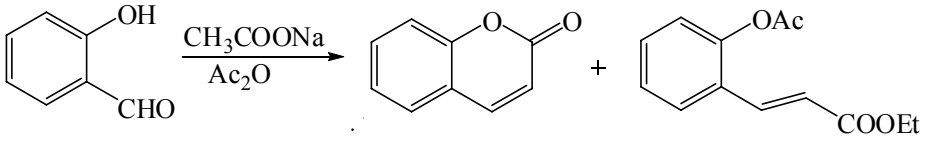
55

1.3 General methods for synthesis of Coumarin and its derivatives

Interest in naturally occurring coumarin and their derivatives have been revived for the past 30 years mainly due to the wide range of physiological properties that have been achieved by many workers. The key step of synthesis is formation of pyrone ring in which the phenol containing requisite substituent of the natural coumarin is prepared first and then modified by steps such as nuclear oxygenation, O- or C- alkylation, bromination, substitution by bases and finally building up of additional rings. Some of the approaches of recent past for the synthesis are depicted here.

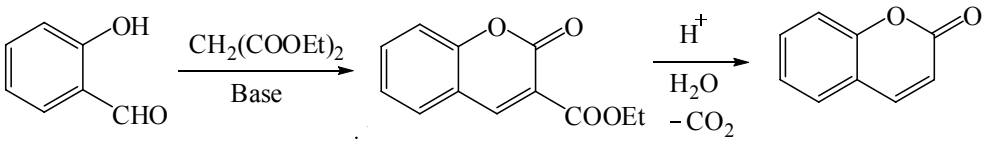
1.3.1 Perkin Condensation

Reaction[44] of salicylaldehyde with acetic anhydride in the presence of sodium acetate to give coumarin and acetyl cinnamic acid.



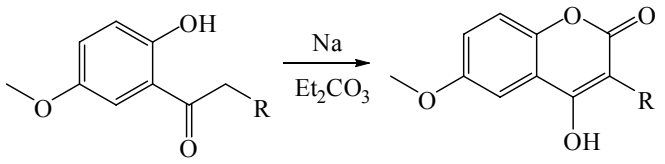
1.3.2 Knoevenagel Reaction

Salicylaldehyde in the presence of reactive methylene compound[45] like diethyl malonate in presence of pyridine and piperidine gives coumarin.



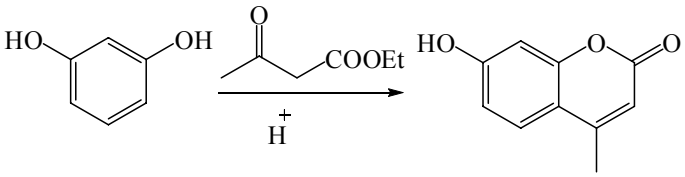
1.3.3 Hauben-Hosch Condensation

4-Hydroxy coumarin is prepared in good yield by condensation[46] of ortho-hydroxy phenyl ketone with diethyl carbonate and pulverized sodium.



1.3.4 Pechmann Condensation

Coumarin derivatives are prepared from β–ketoester[47] by condensation with phenol in the presence of H_2SO_4.



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Chapter 2

Synthesis and biological evaluation of isoxazole amide derivatives

2. Synthesis and biological evaluation of isoxazole amide derivatives

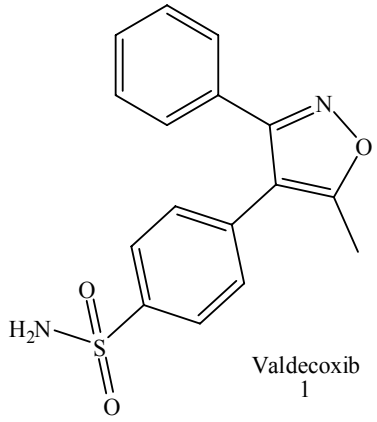
2.1 Introduction

Isoxazoles are class of heterocyclic compounds having remarkable pharmaceutical applications. They are very versatile building blocks in organic synthesis. Isoxazoles are useful intermediates, since the stability of the ring system allows manipulation of substituents on both the rings to give functionally complex derivatives. Isoxazoles can also be used as latent synthons in the synthesis of natural products.[1]

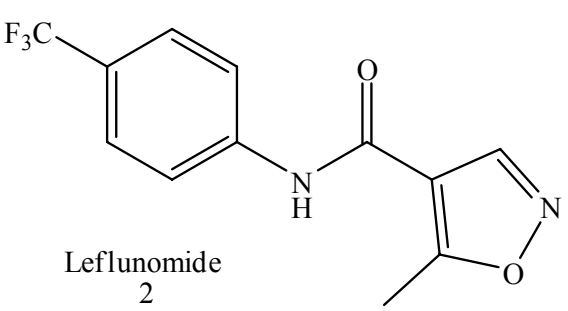
Derivatives of benzisoxazole are known to possess biological activities such as antimicrobial[2] and herbicidal activities[3]. The most extensively studied isoxazole containing anticonvulsant drug is zonisamide.[4] It is also used as antiepileptic agent in which substitution on benzene ring leads to increase in reactivity and also increase in neurotoxicity[5]. {1-[3-(6-fluoro-l,2-benzisoxazole-3-yl)propyl]-4-(2-oxo-l-benzimidazolinyl)}piperidine, haloperidol and thioridazine exhibit dopamine blocking properties.[6] Naphtho[2,3-d]isoxazole-4, 9-dione-3-carboxylates are potent cytoprotective agents.[7] Some isoxazole derivatives are used as prodrug for the treatment of cancer.[8] Furobenzisoxazole derivatives are reported to possess uricosuric and diuretic properties for treatment of hyperuricemia, edema and hypertension.[9,10] Trifluoromethyl benzisoxazole derivatives show affinity and potency for PPARα as well as better affinity for PPARγ.[11] Alkenyldiarylmethane derivatives of benzoxazolone and benzisoxazole show anti-HIV acvity.[12]

Anti inflammatory drugs, Valdecoxib **1** (Bextra) a COX-2 inhibitor and Leflunomide **2**[13, 14] are isoxazole derivatives. The other isoxazole derivatives like Sulfamethoxazole **3**, Sulfafurazole **4** and Oxacilline **5** are in commercial use for many years.[15] The antibiotic Cycloserine **6** is the best known example of isoxazole drug reported to possess antitubercular, antibacterial activities and is found to be of

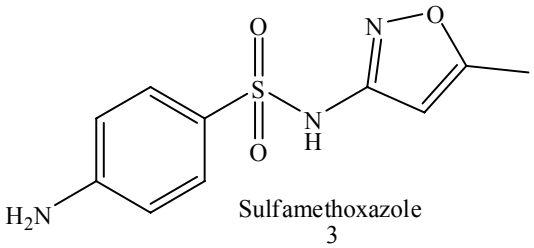
considerable use in treatment of leprosy.[16, 17] Acivicin **7** is an antitumor, anti leishmania drug, Isoxaflutole **8** is a herbicidal, possess isoxazole moiety.[18]



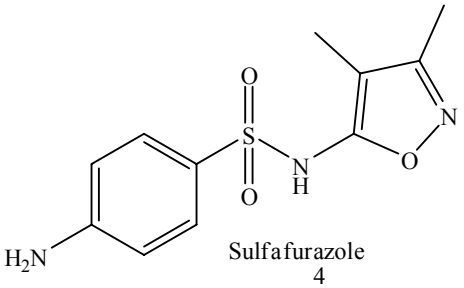
Valdecoxib
1



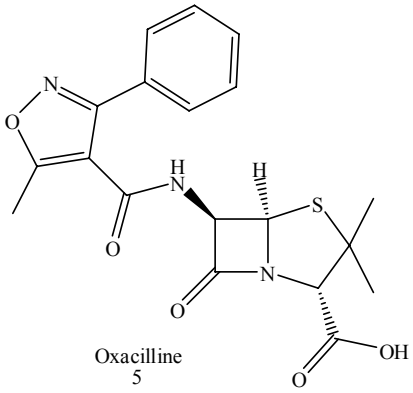
Leflunomide
2



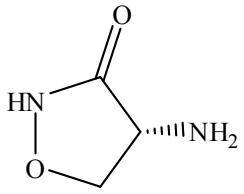
Sulfamethoxazole
3



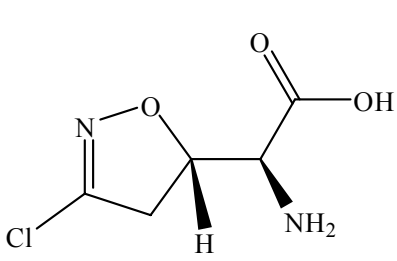
Sulfafurazole
4



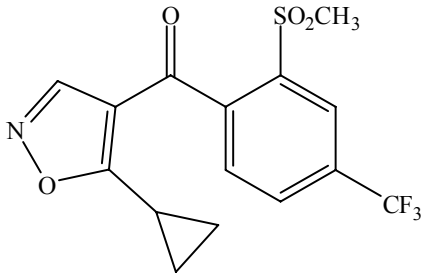
Oxacilline
5



Cycloserine
6



Acivicin
7



Isoxaflutole
8

Literature search reveals that all reported isoxazole derivatives have linear ring fusion with benzene or naphthalene but not a single report documented isoxazole

derivatives with angular ring fusion. Substitution on naphthalene ring may increase neurotoxicity.[6] Based on standard drugs Phenytoin, Zonisamide and Leflunomide, we have designed and synthesized new naphthoisoxazole derivatives keeping the active pharmacophore same as shown in Figure 1.

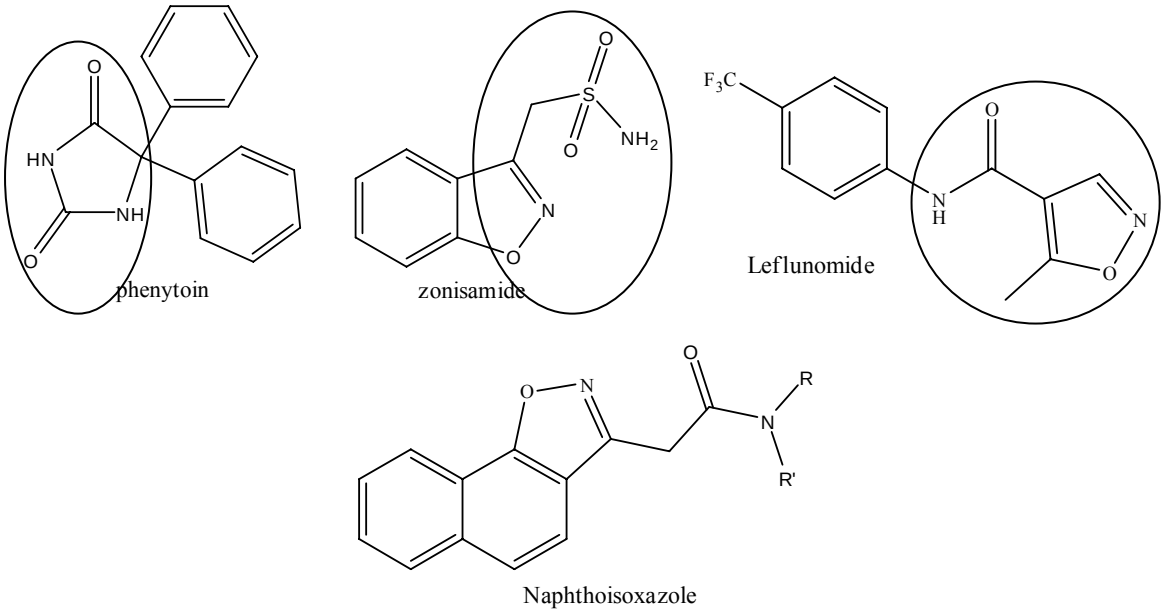
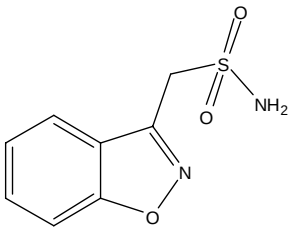


Figure 1: Design of Naphthoisoxazole derivatives

Zonisamide



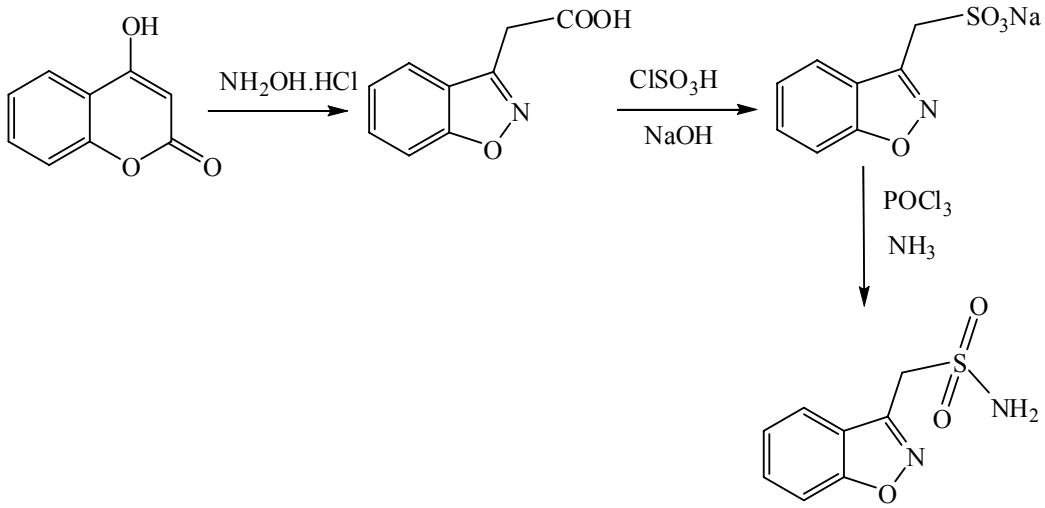
IUPAC Name: benzo[d]isoxazole-3-ylmethanesulfonamide

Researcher Company: Dainippon Pharmaceutical Co., Ltd.

The discovery of zonisamide (ZNS) did not result from a search for new antiepileptic drug (AED). A continuous random screening for anticonvulsant activity had been in progress for many years at the research laboratories of Dainippon Pharmaceutical Co., Ltd. Zonisamide was discovered by serendipity in 1974 during routine testing of 1,2-benzisoxazole derivatives which were synthesized for the management of psychiatric diseases. Among the derivatives tested, some compounds including Zonisamide, were found to have a potent anticonvulsant activity in experimental animals.

Marketed in: Japan, September 1989 and 1st introduced in Korean market in 1992

Scheme 1: Synthesis of Zonisamide



Zonisamide

Physical Properties

Melting Point: 164-168°C

Appearance: white to pale yellow, non hygroscopic crystalline powder

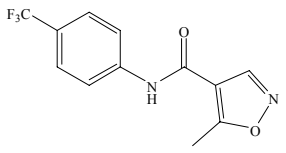
Solubility: Freely soluble in acetone, sparingly soluble in methanol, slightly soluble in ethanol, very slightly soluble in ether and chloroform, pH dependent solubility.

Stability: Stable in aqueous acidic, neutral or alkaline solution, highly stable compound under severe conditions of heat and light exposure, highly reactive towards hydroxyl radicals.

Uses: Potent anticonvulsant drug

Doses: Available in tablets (100 mg) and powder form (200 mg/g).

Leflunomide

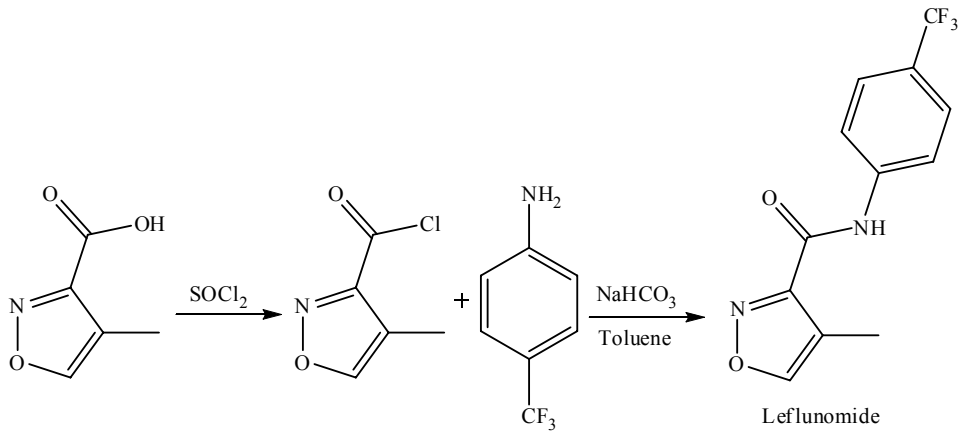


IUPAC Name: benzo[d]isoxazole-3-ylmethan N-(trifluoromethylphenyl)-5-methylisoxazol-4-carboxamide)

Researcher Company: Sanofi Aventis

Marketed in: USA, Europe and Canada in 1998

Scheme 2: Synthesis of Luflunomide



Physical Properties

Melting Point: 165-167°C

Appearance: white, non hygroscopic crystalline powder

Solubility: Insoluble in water and aqueous buffer systems but freely soluble in methanol, ethanol, isopropanol, ethyl acetate, propylene carbonate, acetone and acetonitrile.

Stability: Stable in aqueous acidic, neutral or alkaline solution, protect from light exposure.

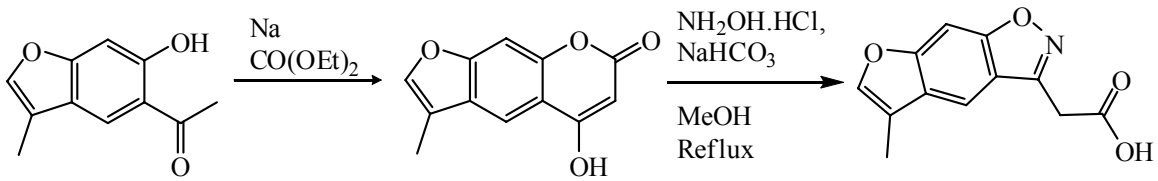
Uses: Potent anti inflammatory drug

Doses: Available in tablets (100 mg for 3 days).

Recent Work

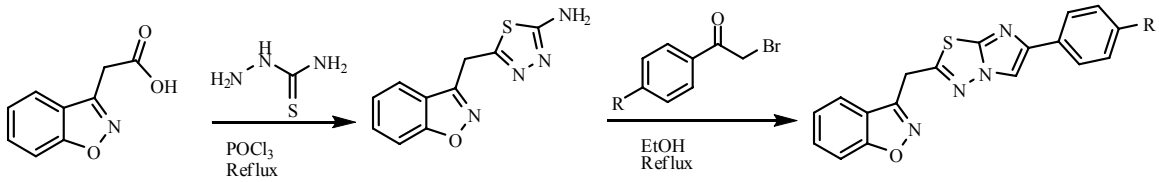
Patel J. M. and Soman S. S.[19] have synthesized isoxazole derivatives as shown in Scheme 3.

Scheme 3:



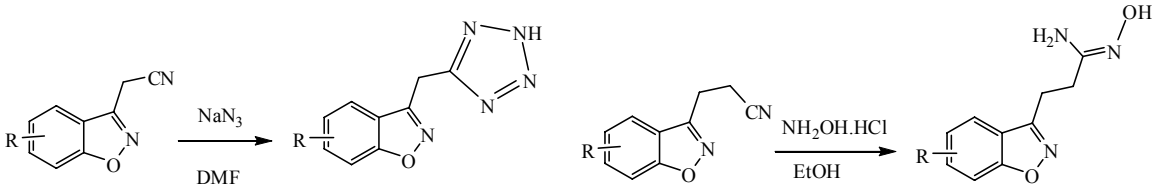
Laxmi R. S. and coworkers[2] have synthesized various methylene bridged benzisoxazolyl imidazo[2,1-b][1,3,4]thiadiazole derivatives as shown in Scheme 4.

Scheme 4:



Uno H. et al[20a] have synthesized various isoxazole derivatives as shown in Scheme 5.

Scheme 5:

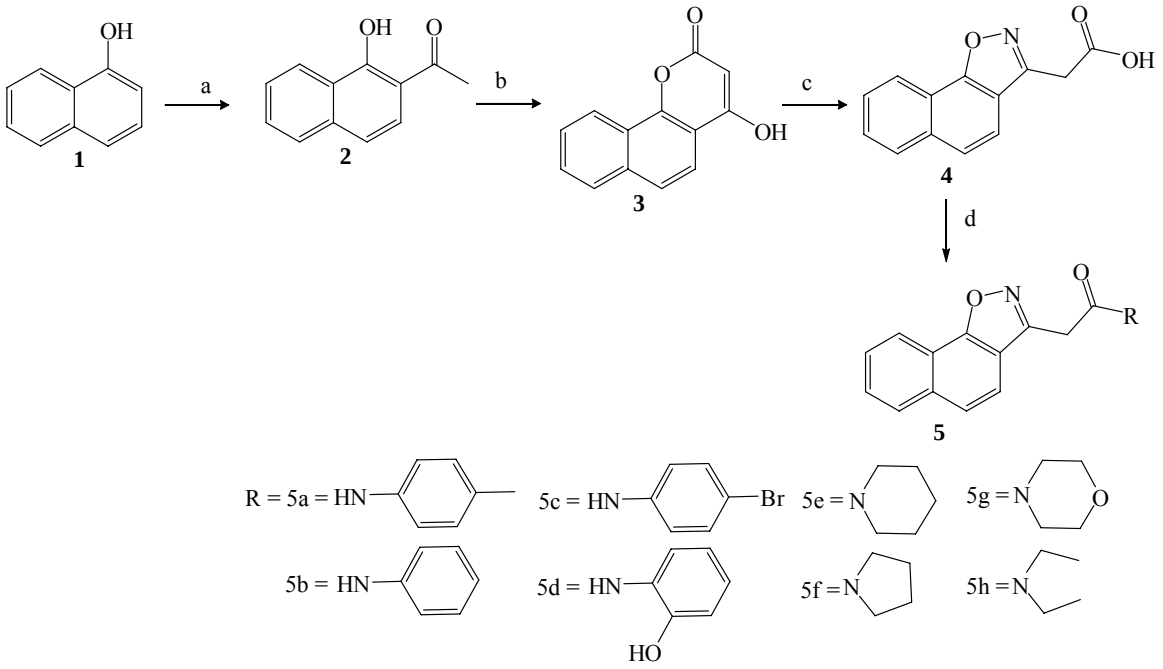


2.2 Result and discussion

2.2.1 Chemistry

1-Naphthol **1** on reaction with acetic acid and zinc chloride gave 1-(1-hydroxynaphhthalen-2-yl)ethanone[21] **2** which on Hoesch reaction[22] with diethyl carbonate in presence of pulverized sodium gave 4-hydroxy-2H-benzo[h]chromen-2-one **3** as shown in Scheme 6. **3** on Posner reaction[20, 23] with hydroxylamine hydrochloride in presence of sodium bicarbonate gave 2-(naphtho[2,1-d]isoxazole-3-yl)acetic acid **4** which was then converted into different naphthoisoxazole amide derivatives **5** by reaction with different amines using dicyclohexylcarbodiimide (DCC) as coupling agent and N, N-dimethyl amino pyridine[24] (DMAP) as catalyst as shown in scheme 6. All compounds were characterized by IR, ^{1}H NMR, ^{13}C NMR, elemental analyses and Mass while purity of compounds was checked by reverse phase HPLC.

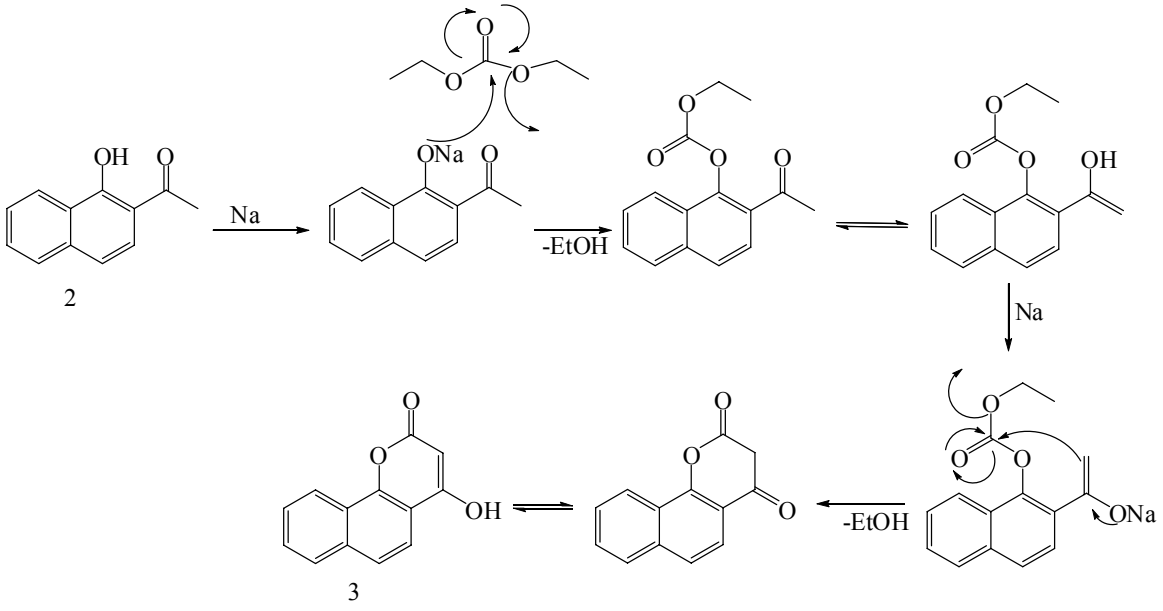
Scheme 6:



Reagents and conditions: (a) $ZnCl_2$, Glacial acetic acid, reflux, 8 h; (b) pulverized sodium, diethyl carbonate, 30 min; (c) $NH_2OH.HCl$, $NaHCO_3$, methanol, reflux, 15 h; (d) DCC, DMAP, amine, ethyl acetate, RT, 12 h

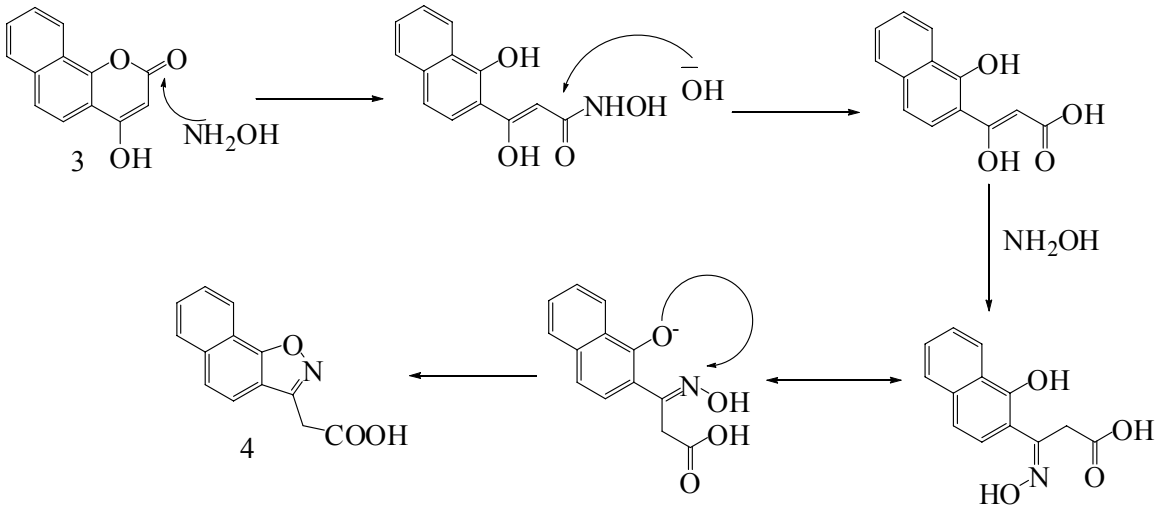
Formation of 4-hydroxy-2H-benzo[h]chromen-2-one **3** can be explained by following mechanism as shown in Scheme 7.

Scheme 7:



Formation of 2-(naphtho[2,1-d]isoxazole-3-yl)acetic acid **4** (Posner reaction) can be explained by following mechanism as shown in Scheme 8.

Scheme 8: Plausible mechanism for Posner reaction



The formation of compound **2** was confirmed by its melting point and its IR spectrum (Figure 2) which showed bands at 1625 cm^{-1} and 3421 cm^{-1} for ketone and hydroxyl groups respectively. The IR spectrum of compound **3** (Figure 3) exhibited band at 3423 cm^{-1} for hydroxyl group and 1604 cm^{-1} for carbonyl group. The ^{1}H NMR of compound **3** in DMSO-d_6 (Figure 4) showed singlet at δ 5.83 for C-3 proton, broad peak at δ 11.94 for –OH proton and all aromatic protons were observed between δ 7.60-8.53 confirmed the formation of compound **3**. The ^{13}C NMR spectrum of compound **3** in DMSO-d_6 (Figure 5) showed presence of 13 peaks which is in accordance with structure of compound **3**.

The IR spectrum of compound **4** (Figure 6) exhibited band at 3435 cm^{-1} for hydroxyl group, 1732 cm^{-1} for carbonyl group and 1641 cm^{-1} for C=N stretching frequency. In ^{1}H NMR of compound **4** in DMSO-d_6 (Figure 7) singlet observed at δ 4.17 for methylene protons (CH_2), broad peak at δ 12.92 for carboxylic acid proton. The disappearance of peak at δ 5.83 in ^{1}H NMR of compound **4** (Figure 7) confirmed the

absence of lactone ring. All aromatic protons were appeared between δ 7.75 to 8.38. The ^{13}C NMR spectrum of compound **4** in DMSO-d_6 (Figure 8) showed presence of 13 peaks which is in accordance with structure of compound **4** and *m/z* value at 227.8 for M^+ and 228.7 for $[M+1]^+$ in ESI/MS (Figure 9) confirmed formation of compound **4**.

The IR spectrum of compound **5a** (Figure 10) exhibited band at 3291 cm^{-1} for NH group and 1656 cm^{-1} for carbonyl group. The ^1H NMR of compound **5a** in $CDCl_3$ (Figure 11) showed peak at δ 2.29 for methyl protons, peak at δ 4.19 for methylene protons, peak between δ 7.09 to 8.44 for all aromatic protons and NH proton. The ^{13}C NMR spectrum of compound **5a** in $CDCl_3$ (Figure 12) showed presence of 16 peaks which is in accordance with structure of compound **5a** which further confirmed the structure. In mass spectrum of compound **5a** the *m/z* value at 316.6 for M^+, 317.7 for $[M+1]^+$ and 314.9 for $[M-1]^+$ in ESI/MS (Figure 13) confirmed formation of compound **5a**. HPLC Purity of compound **5a** (Figure 14) was found to be 100%.

Figure 15 to 19 shows IR, ^1H NMR, ^{13}C NMR, ESI/MS and HPLC purity of compound **5b**.

The IR spectrum of compound **5c** (Figure 20) exhibited band at 3345 cm^{-1} for NH group and 1684 cm^{-1} for carbonyl group. In ^1H NMR spectrum of compound **5c** in DMSO-d_6 (Figure 21) showed peak at δ 4.19 for methylene protons. All aromatic protons were observed between δ 7.37 to 8.41 and peak at δ 10.34 indicated NH proton thus confirmed the structure of compound **5c**. The ^{13}C NMR spectrum of compound **5c** in DMSO-d_6 (Figure 22) showed presence of 17 peaks which is in accordance with structure of compound **5c**. The DEPT-135 spectrum of compound **5c** in DMSO-d_6 (Figure 23) further confirmed the structure in which one secondary carbon -CH_2 observed below the base line. Mass spectrum of compound **5c** showed *m/z* value at 380.8 for M^+ and 380.1

for $[M-1]^+$ in ESI/MS (Figure 24) further confirmed formation of compound **5c**. HPLC Purity of compound **5c** (Figure 25) was found to be 99.73%.

The IR spectrum of compound **5h** (Figure 26) exhibited band at 1637 cm^{-1} for carbonyl group. In ^1H NMR spectrum of compound **5h** in DMSO-d_6 (Figure 27) multiplet at δ 1.11 to 1.25 indicated methyl protons, multiplet at δ 1.63 to 1.81 indicated methylene protons of ethyl group and singlet at δ 3.88 indicated methylene protons. All aromatic protons observed between δ 7.60 to 8.29 confirmed formation of compound **5h**. In ^{13}C NMR spectrum of compound **5h** in DMSO-d_6 (Figure 28) 19 different peaks for carbons confirmed the structure of compound **5h**. HPLC Purity of compound **5h** (Figure 29) was found to be 98.24%.

The IR, ^1H NMR, ^{13}C NMR, ESI/MS, HPLC data and elemental analyses for all synthesized isoxazole amide derivatives are given in experimental section. The other spectra are not attached here due to page constrain.

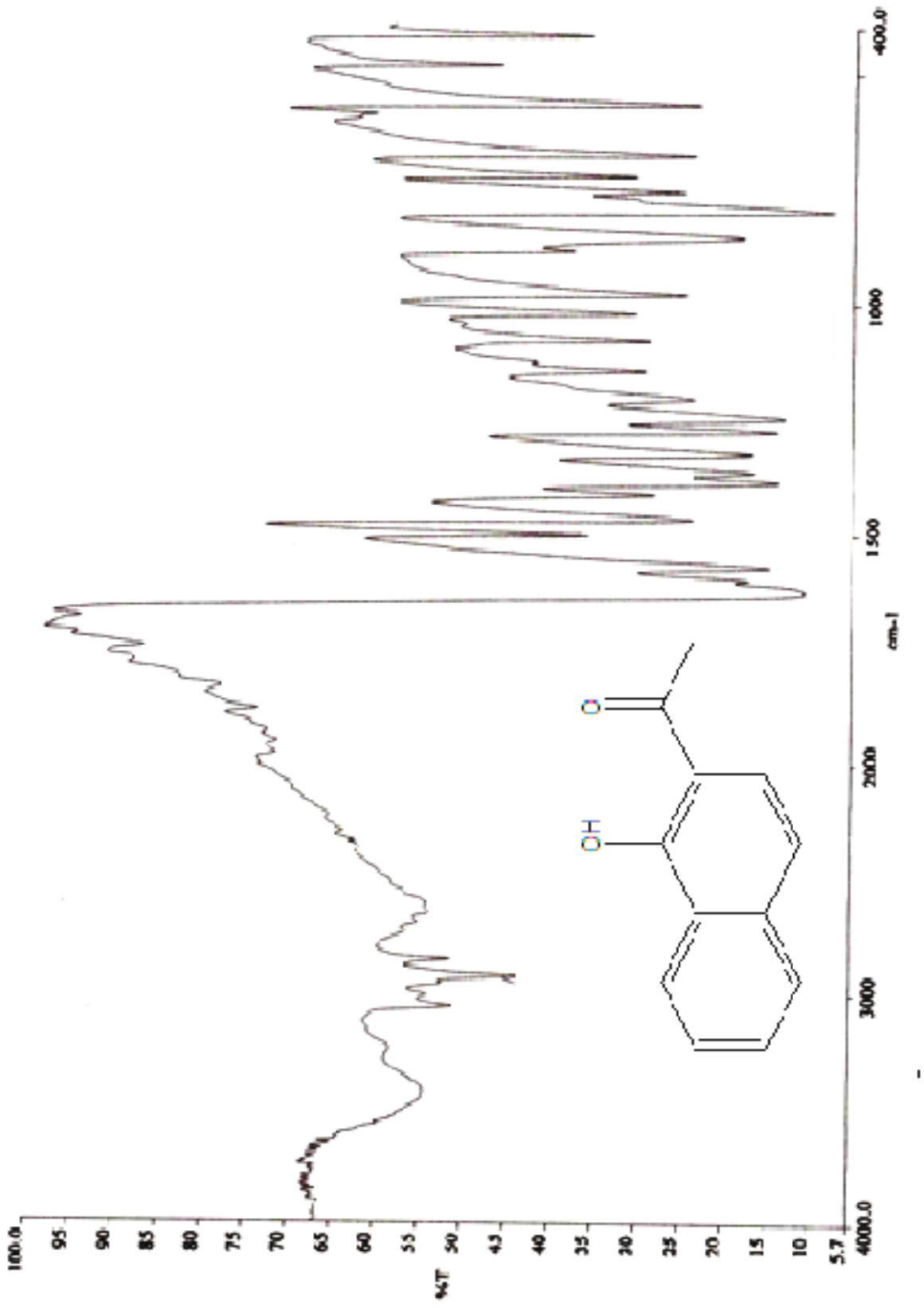


Figure 2: IR of 1-(1-hydroxynaphhthalen-2-yl)ethanone **2**

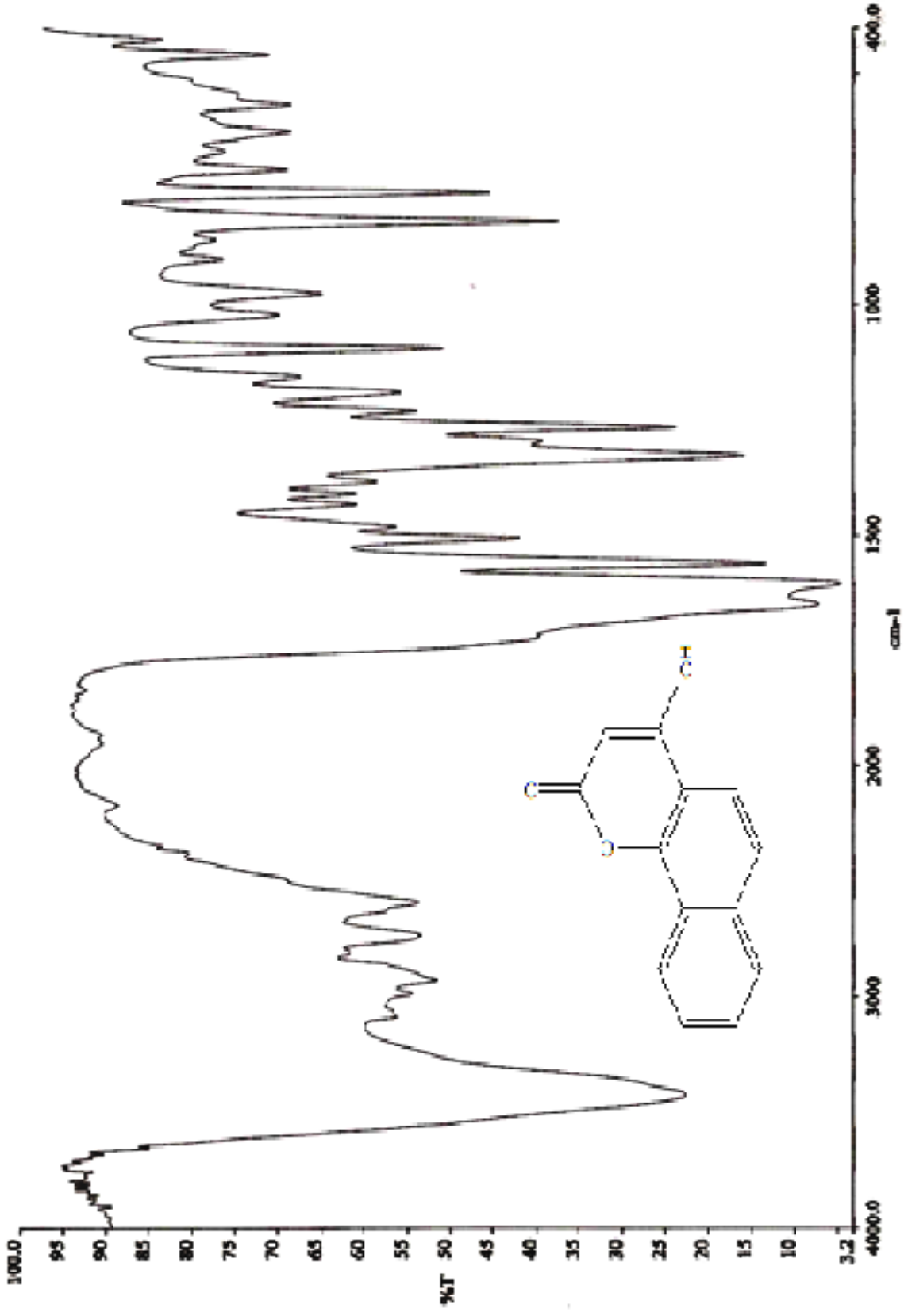


Figure 3: IR of 4-hydroxy-2H-benzo[h]chromen-2-one **3**

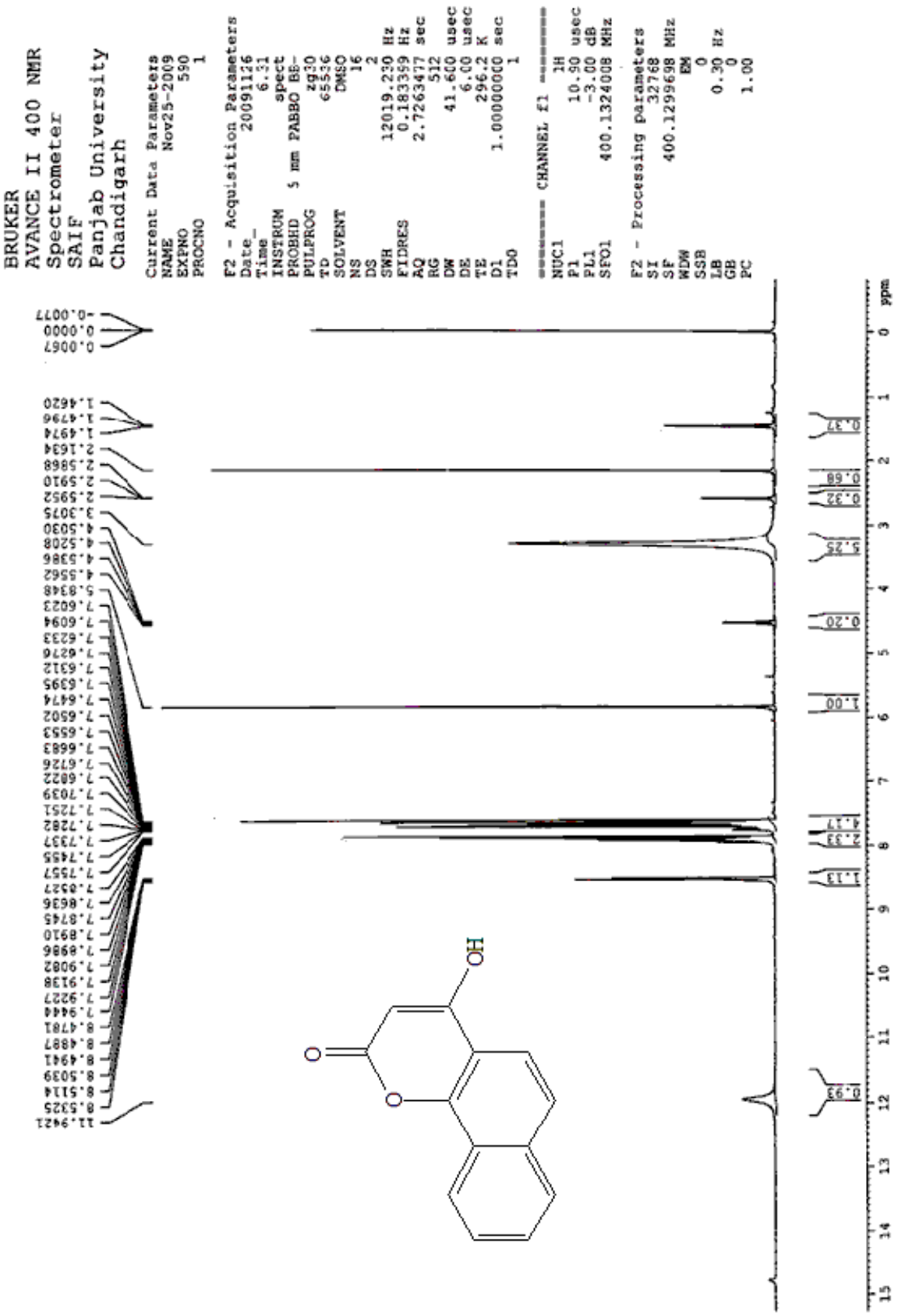


Figure 4: ^{1}H NMR of 4-hydroxy-2H-benzo[h]chromen-2-one **3**

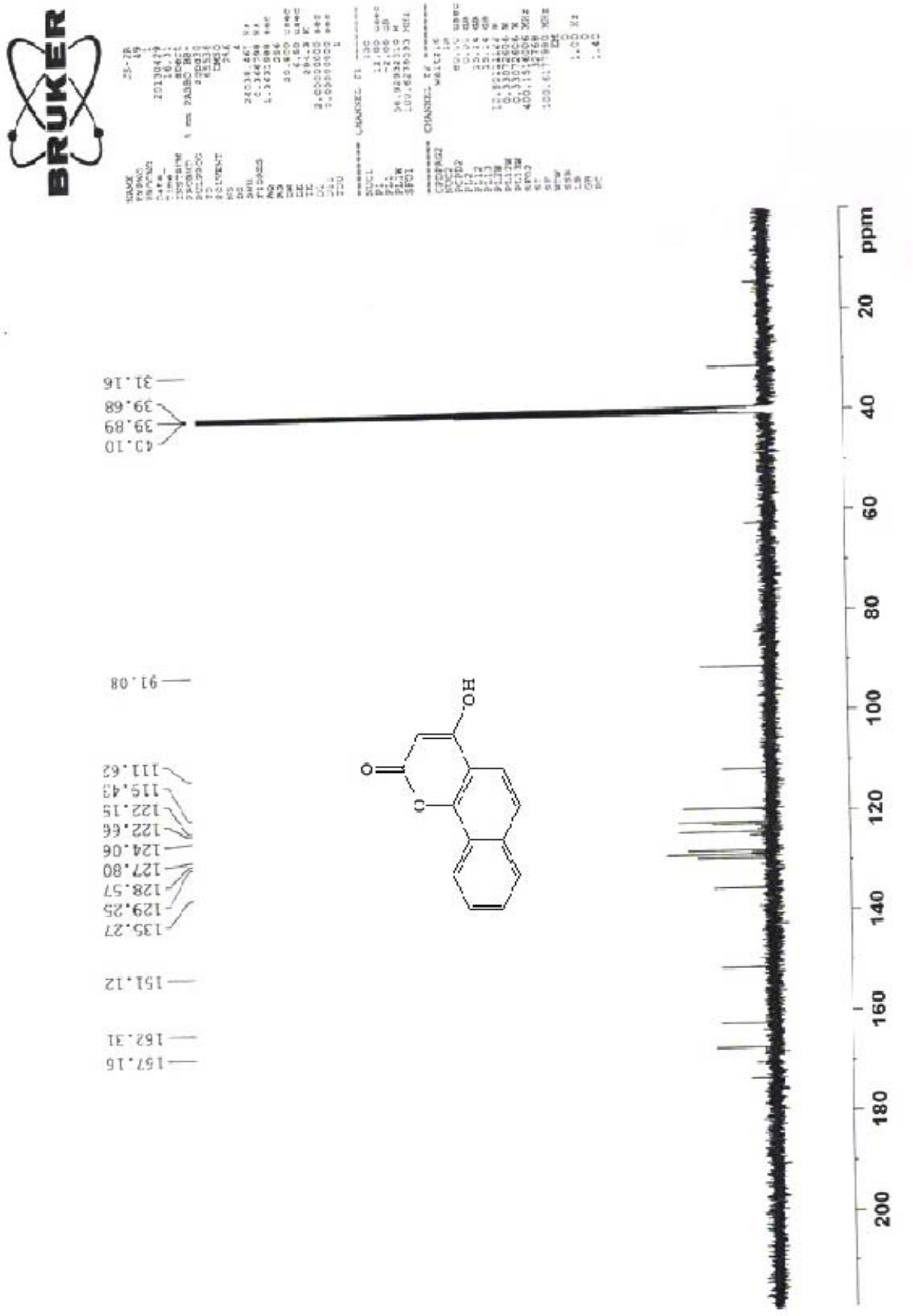


Figure 5: ^{13}C NMR of 4-hydroxy-2H-benzo[h]chromen-2-one **3**

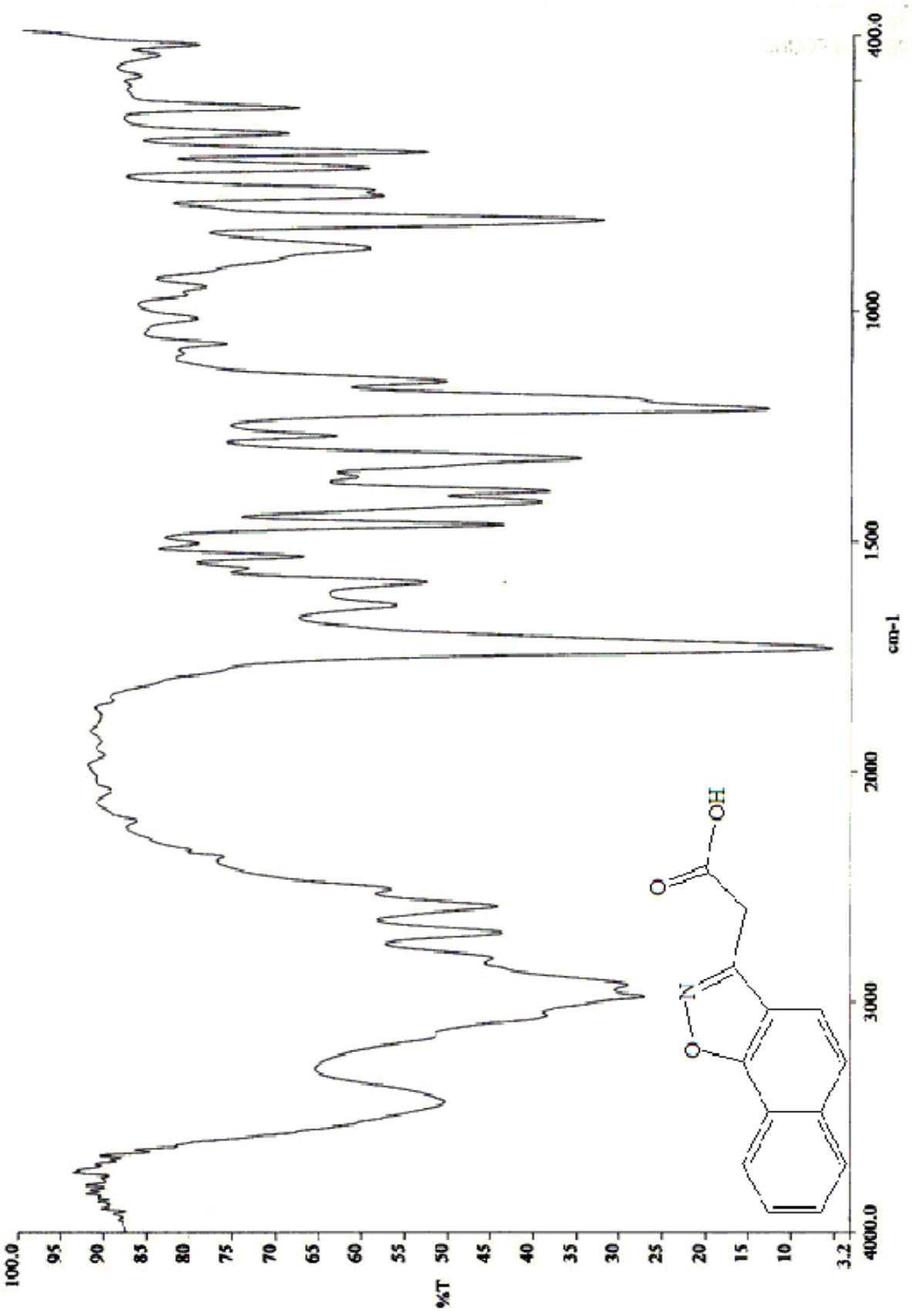


Figure 6: IR of 2-(naphtho[2,1-d]isoxazole-3-yl)acetic acid **4**

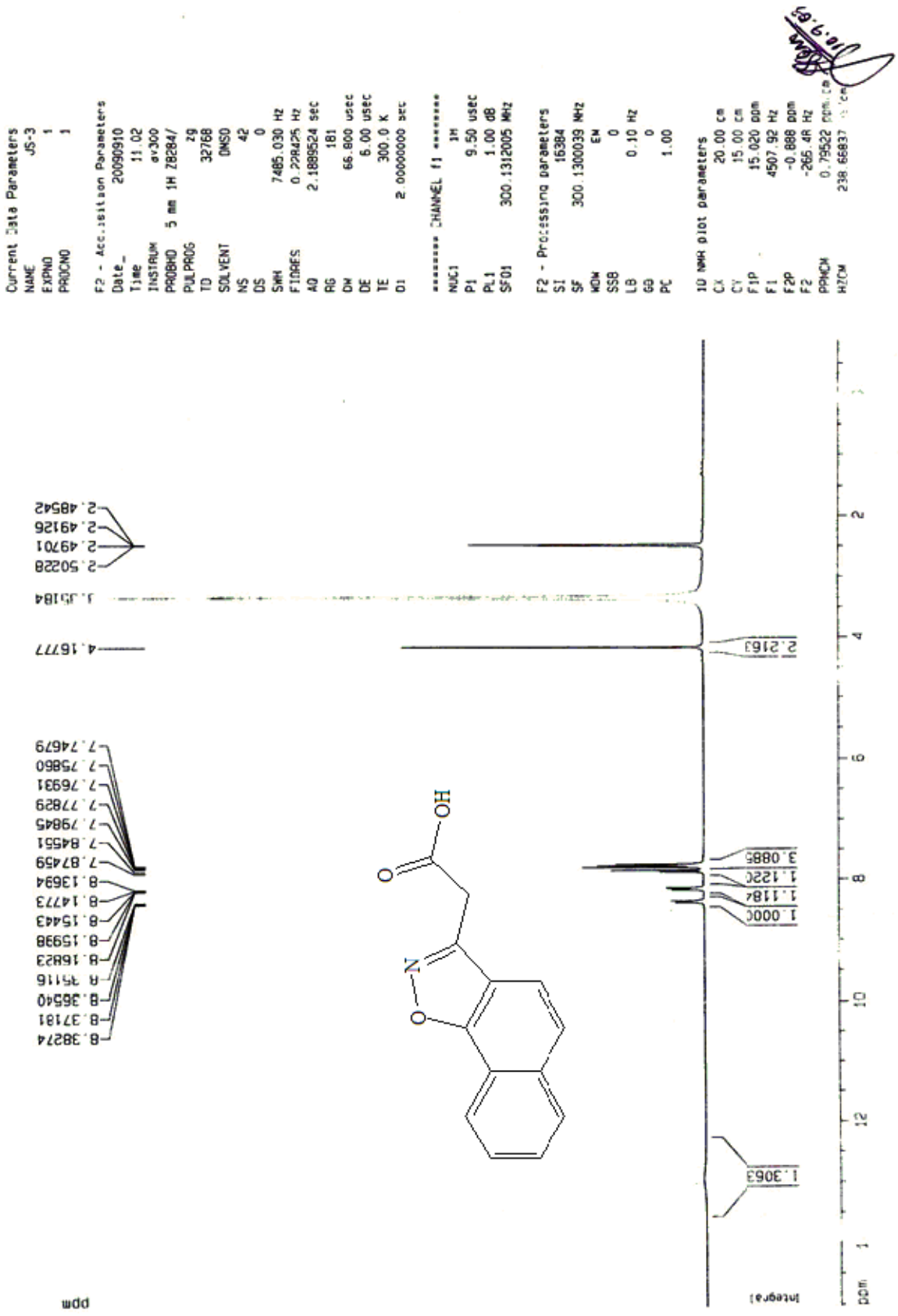


Figure 7: ^{1}H NMR of 2-(naphtho[2,1-d]isoxazole-3-yl)acetic acid **4**

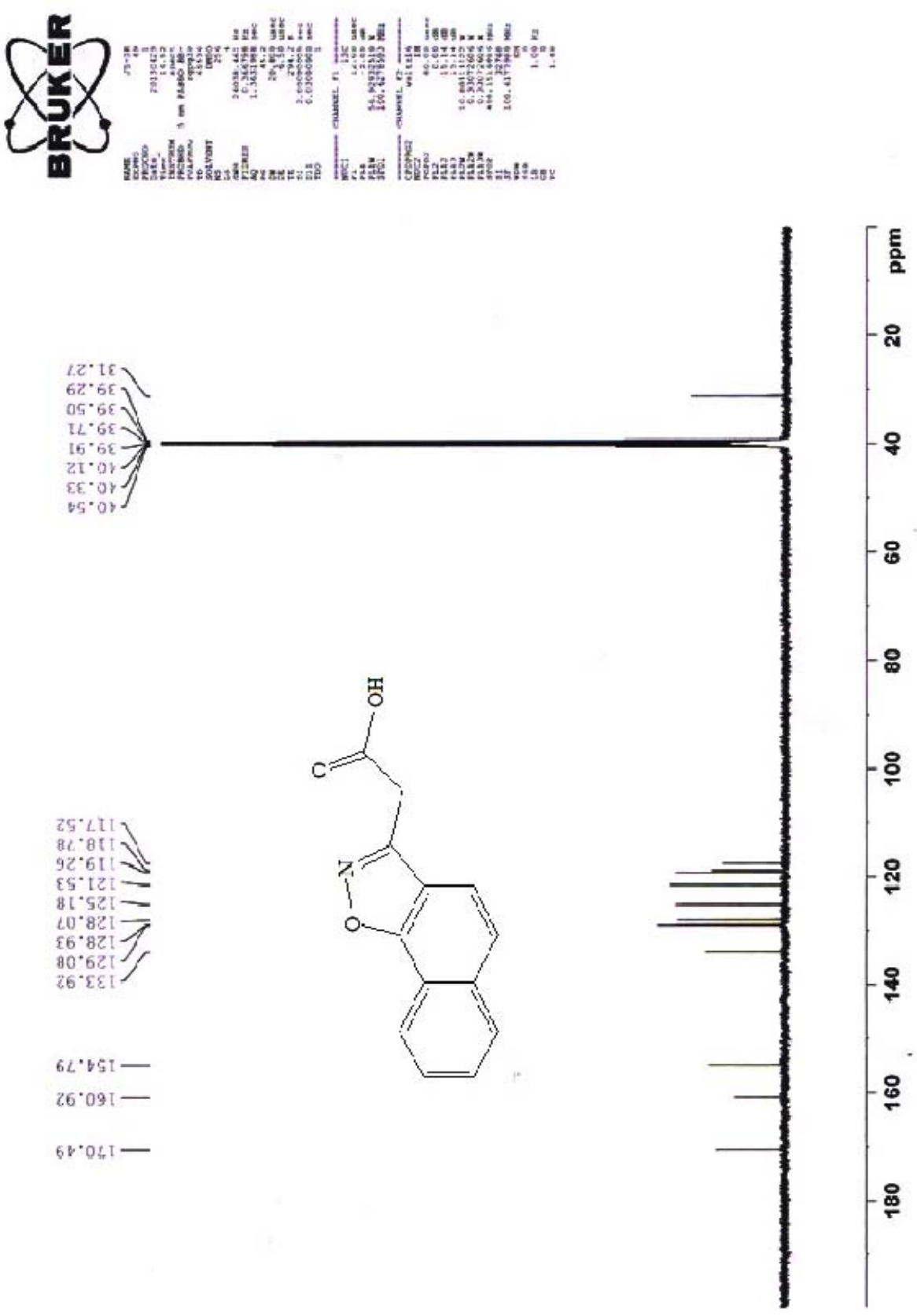


Figure 8: ^{13}C NMR of 2-(naphtho[2,1-d]isoxazole-3-yl)acetic acid **4**

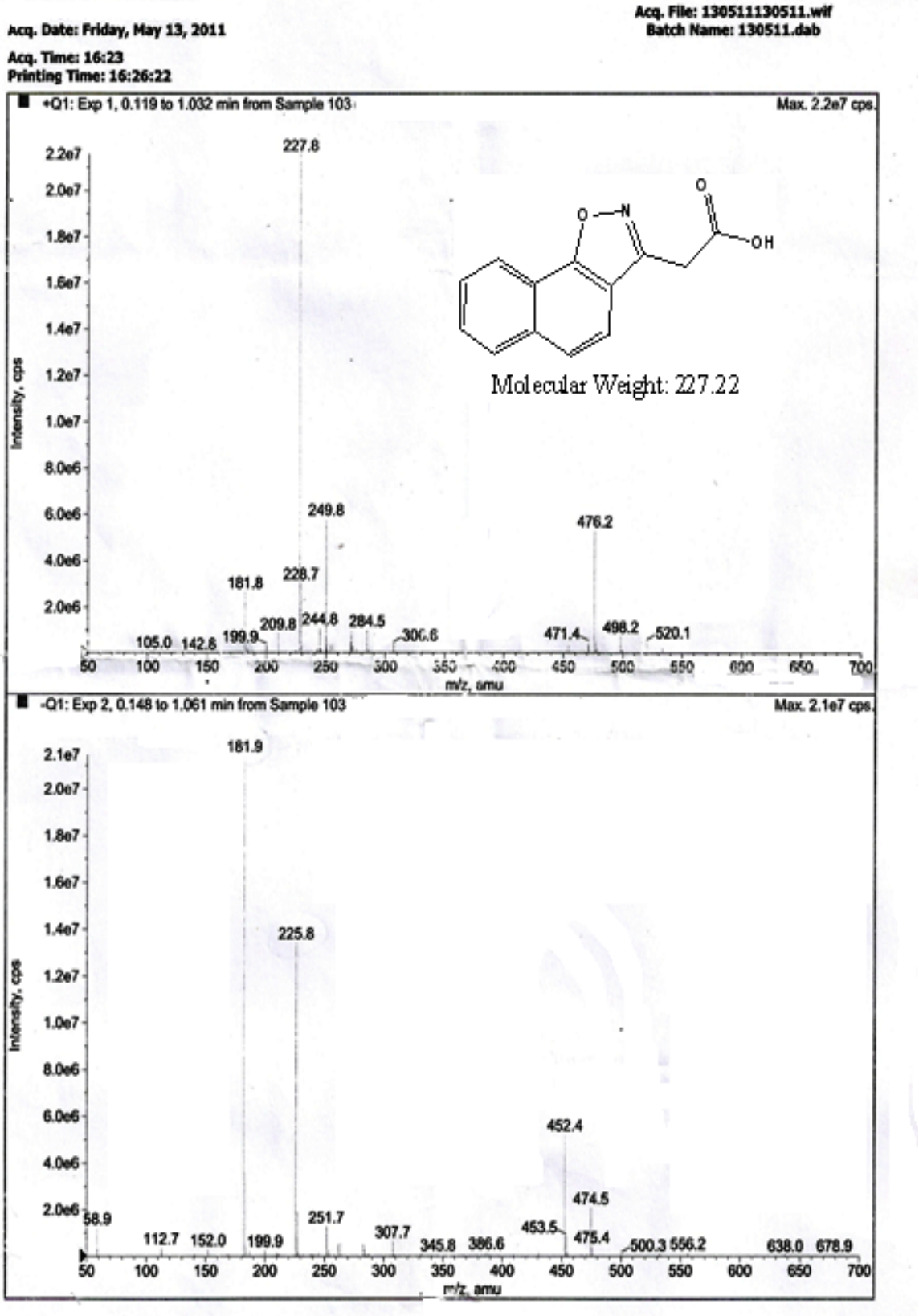


Figure 9: ESI/MS of 2-(naphtho[2,1-d]isoxazole-3-yl)acetic acid **4**

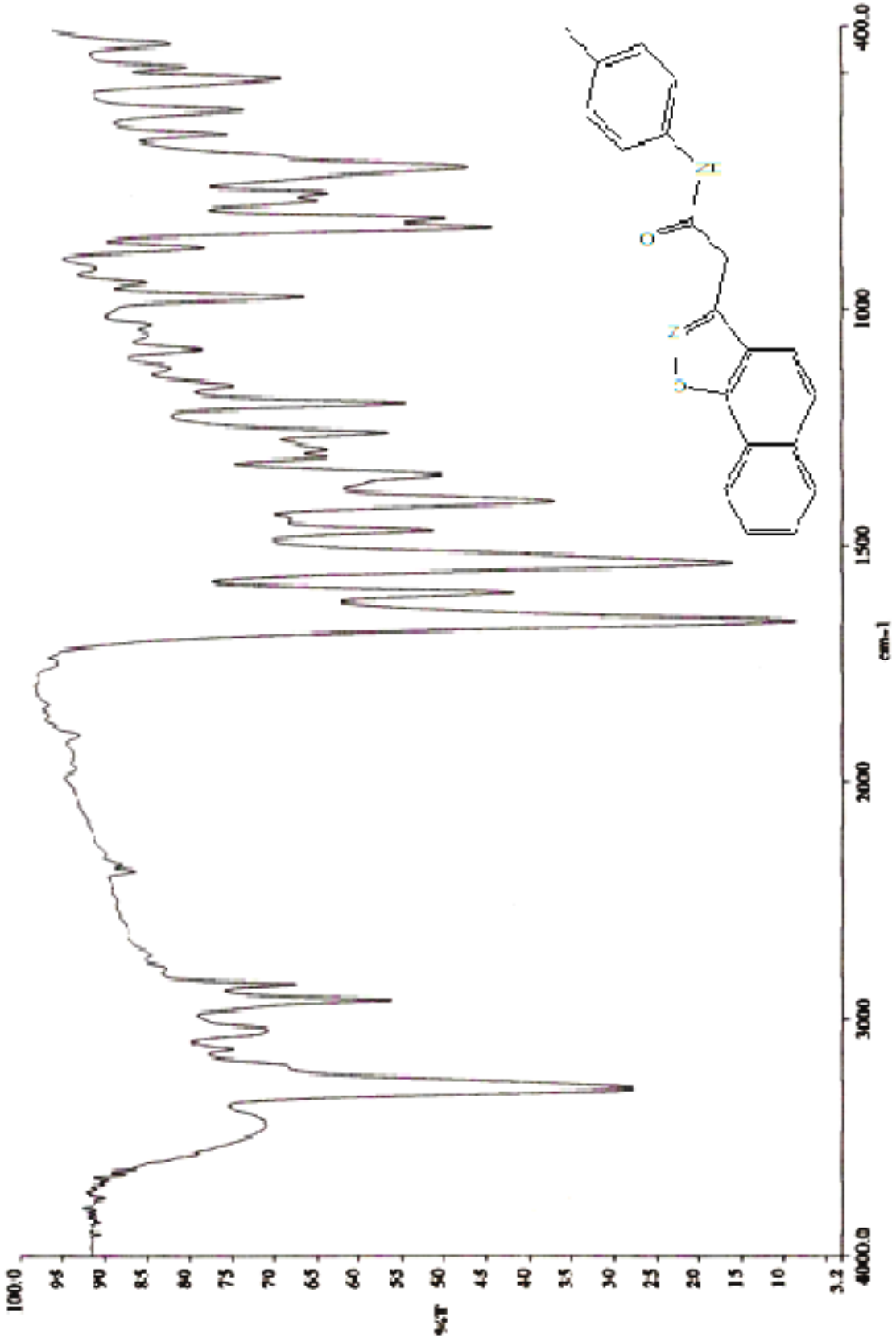


Figure 10: IR of 2-(naphtho[2,1-d]isoxazole-3-yl)-N-p-tolylacetamide **5a**

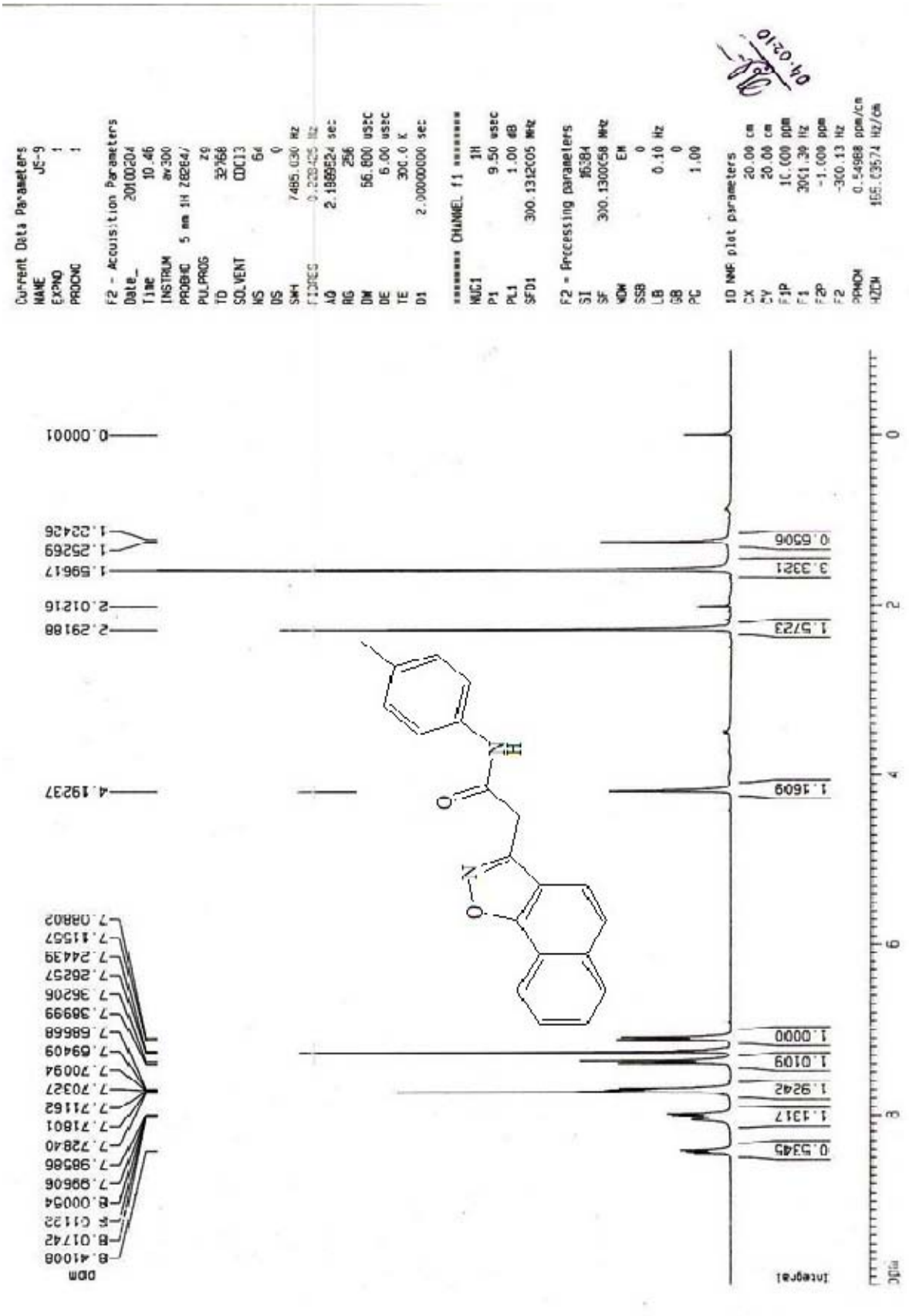


Figure 11: ^{1}H NMR of 2-(naphtho[2,1-d]isoxazole-3-yl)-N-p-tolylacetamide **5a**

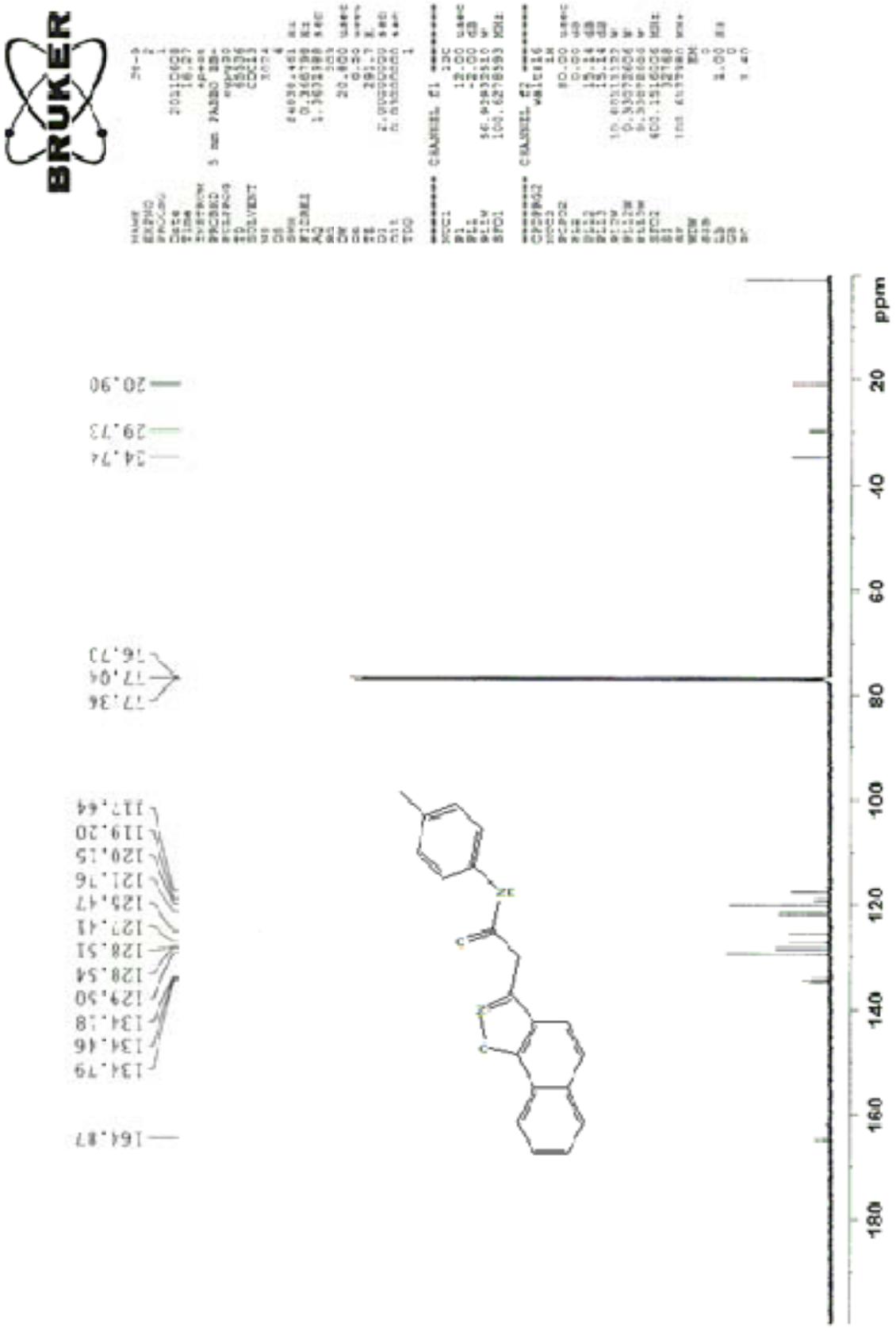


Figure 12: ^{13}C NMR of 2-(naphtho[2,1-d]isoxazole-3-yl)-N-p-tolylacetamide **5a**

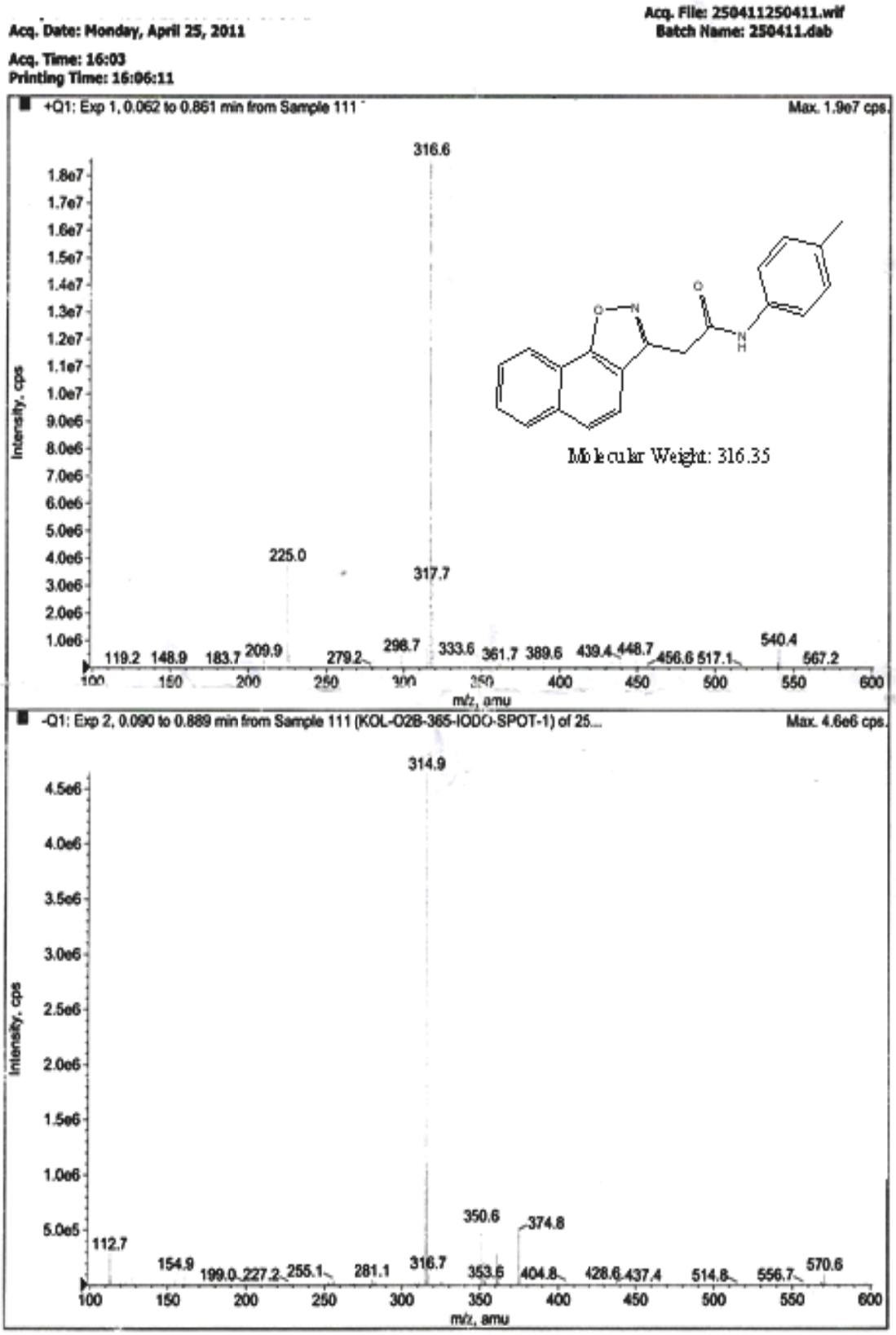


Figure 13: ESI/MS of 2-(naphtho[2,1-d]isoxazole-3-yl)-N-p-tolylacetamide **5a**

NPC DIVISION IIIM JAMMU

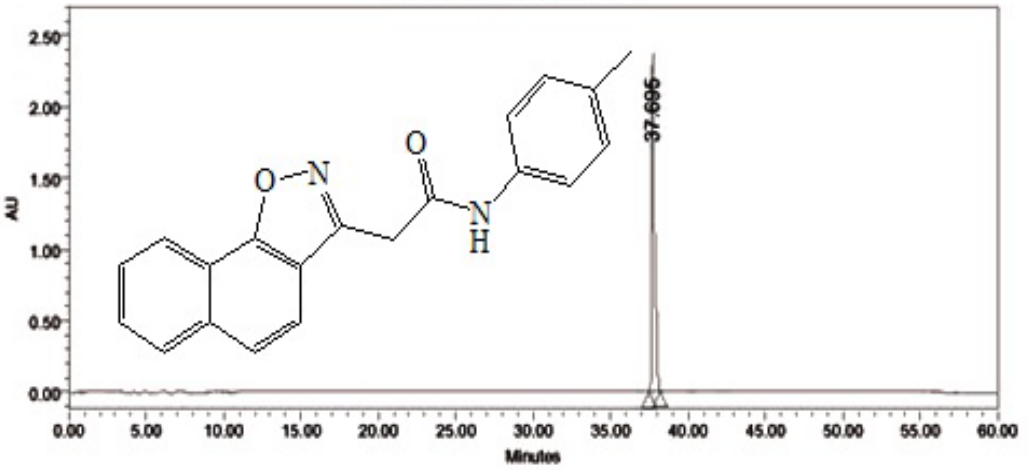
Reported by User: System

Project Name: NPC8

SAMPLE INFORMATION

Sample Name:	JS-09	Acquired By:	System
		Date Acquired:	11/17/2011 3:38:12 PM
Sample Type:	Unknown	Acq. Method Set:	Tinaspora1
Vial:	1	Date Processed:	11/19/2011 11:01:03 AM
Injection #:	1	Processing Method:	Sample
Injection Volume:	10.00 ul	Channel Name:	W2996 254.0nm-1.2
Run Time:	60.0 Minutes	Proc. Chnl. Descr.:	W2996 PDA 254.0 nm at 1.2
Sample Set Name:	Tinospora	Flow rate: 0.7 ml/min	
column_name	RP-18e,5um	Sample conc:0.5 mg/mL MeOH	
		Mobile phase: ACN:Water(gradient)	

Auto-Scaled Chromatogram



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Report Method: Parveen

Printed 11:11:39 AM 11/19/2011

Page: 1 of 1

Figure 14: HPLC of 2-(naphtho[2,1-d]isoxazole-3-yl)-N-p-tolylacetamide **5a**

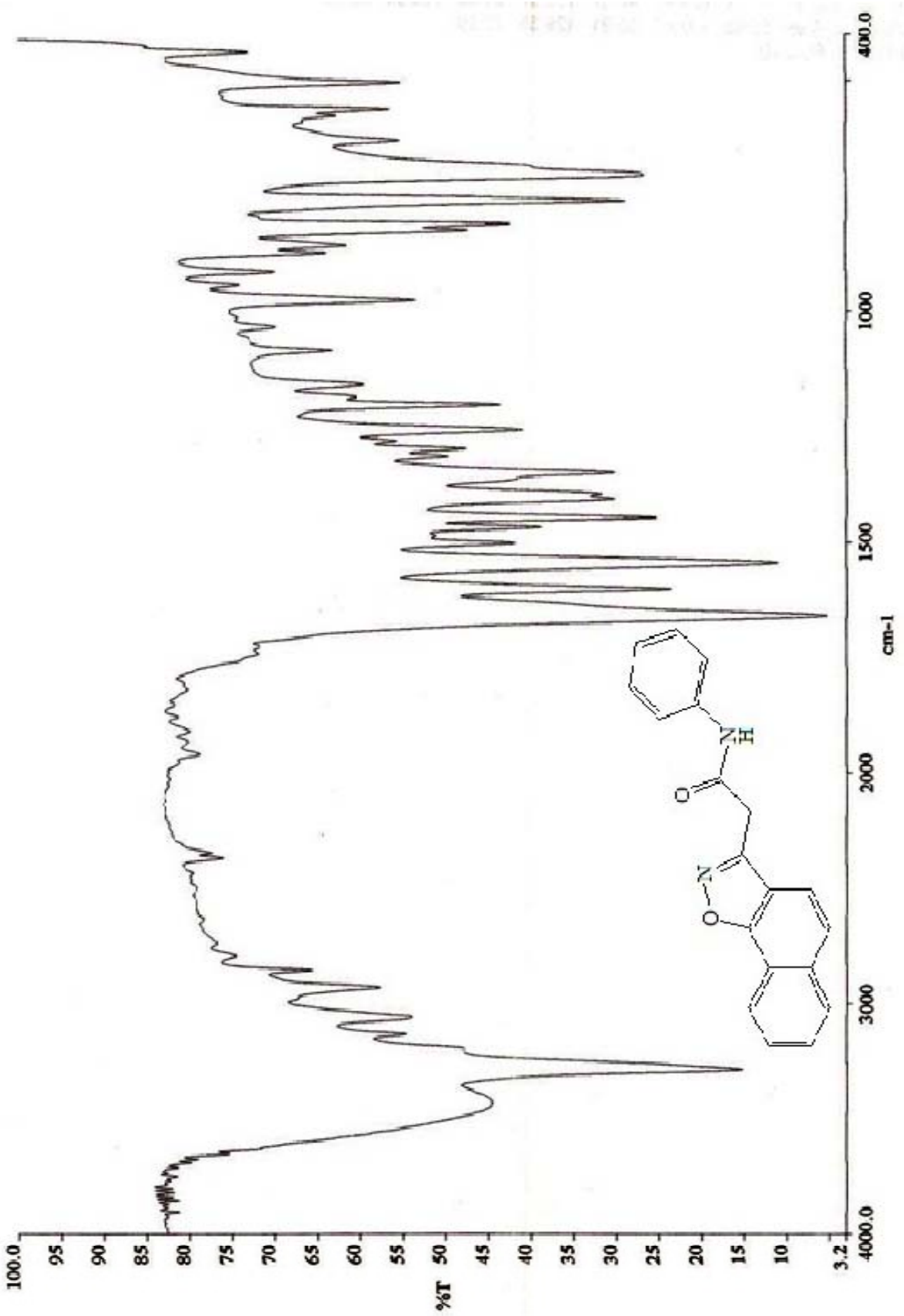

Figure 15: IR of 2-(naphtho[2,1-d]isoxazole-3-yl)-N-phenylacetamide **5b**

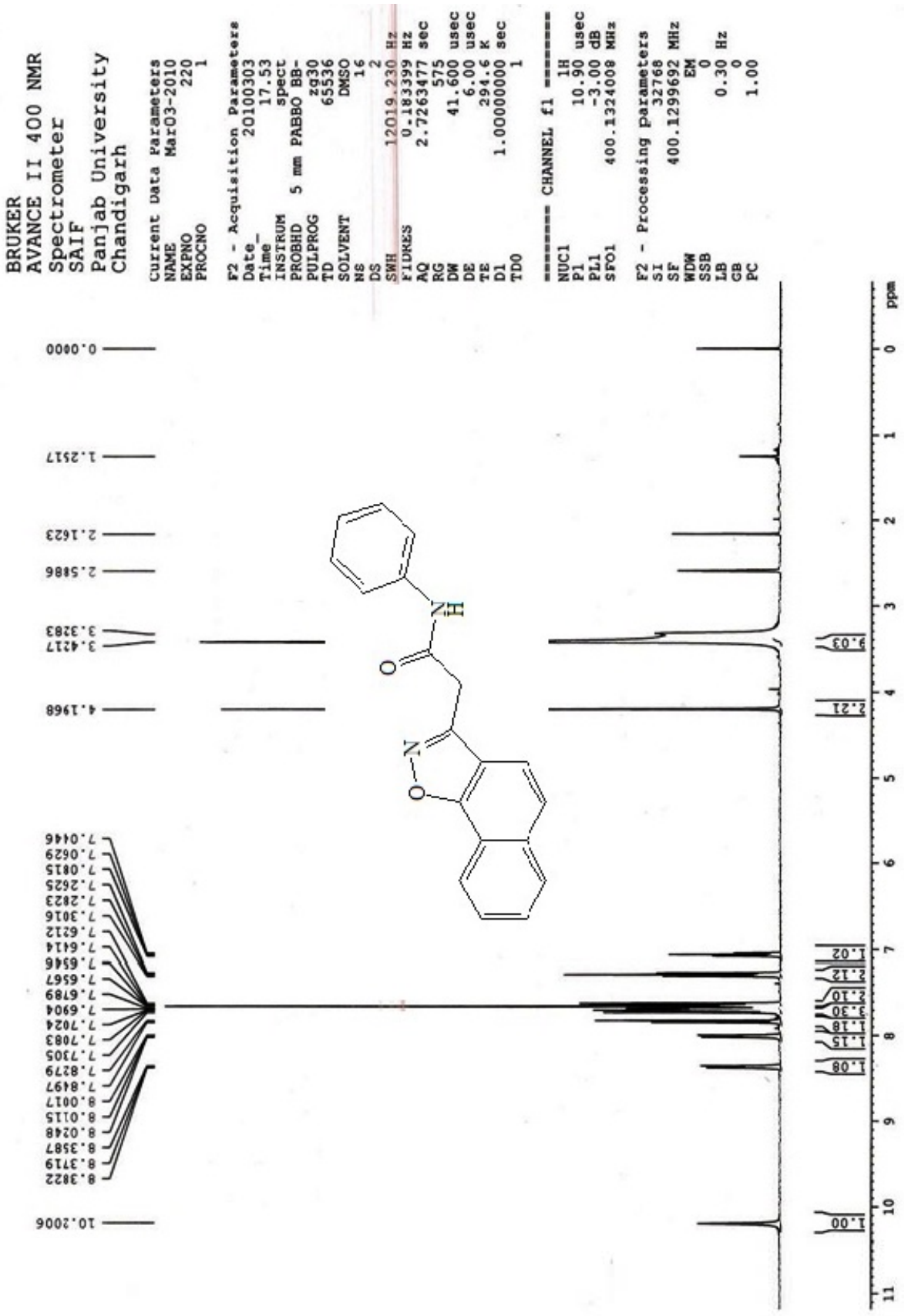

Figure 16: ^{1}H NMR of 2-(naphtho[2,1-d]isoxazole-3-yl)-N-phenylacetamide **5b**

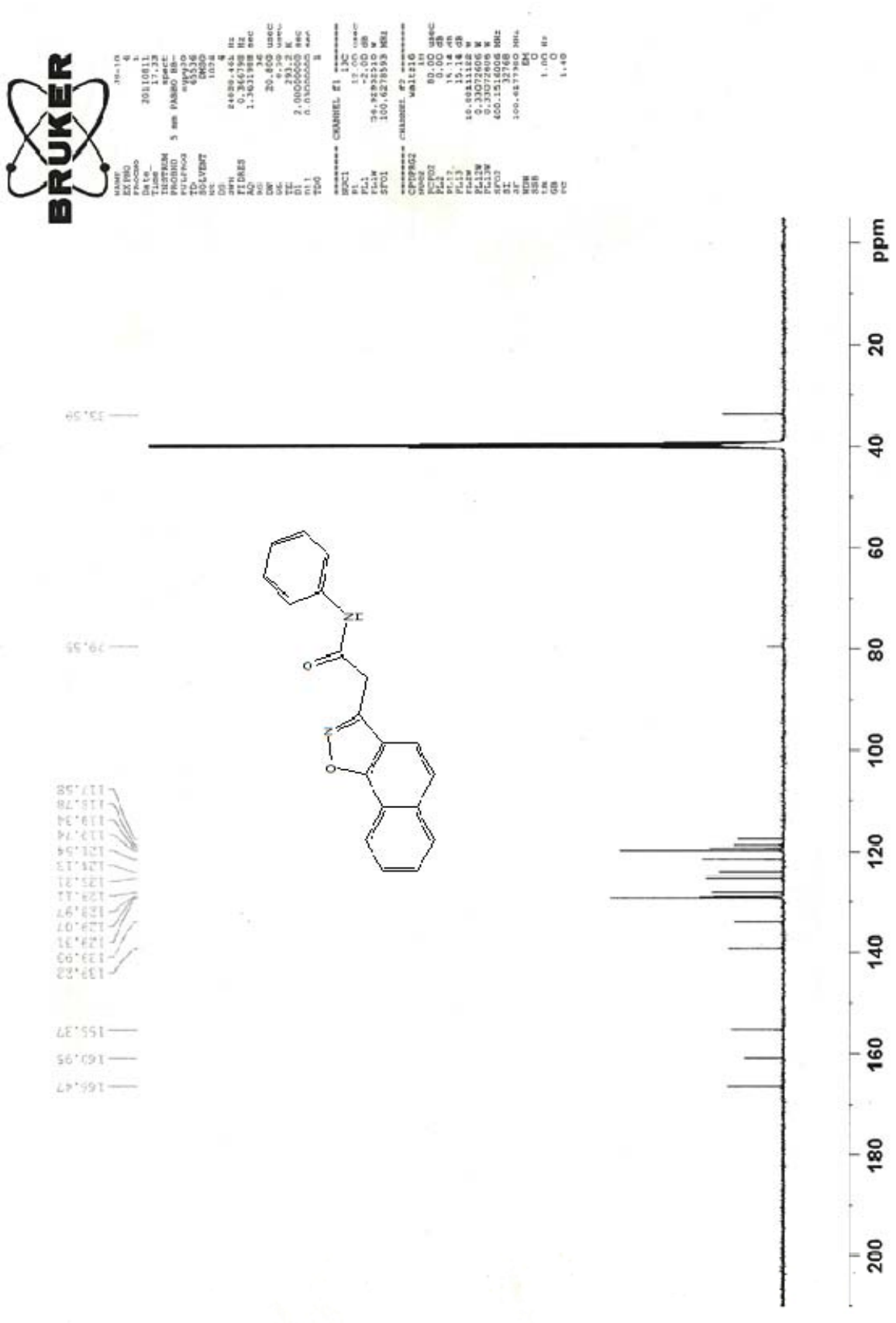

Figure 17: ^{13}C NMR of 2-(naphtho[2,1-d]isoxazole-3-yl)-N-phenylacetamide **5b**

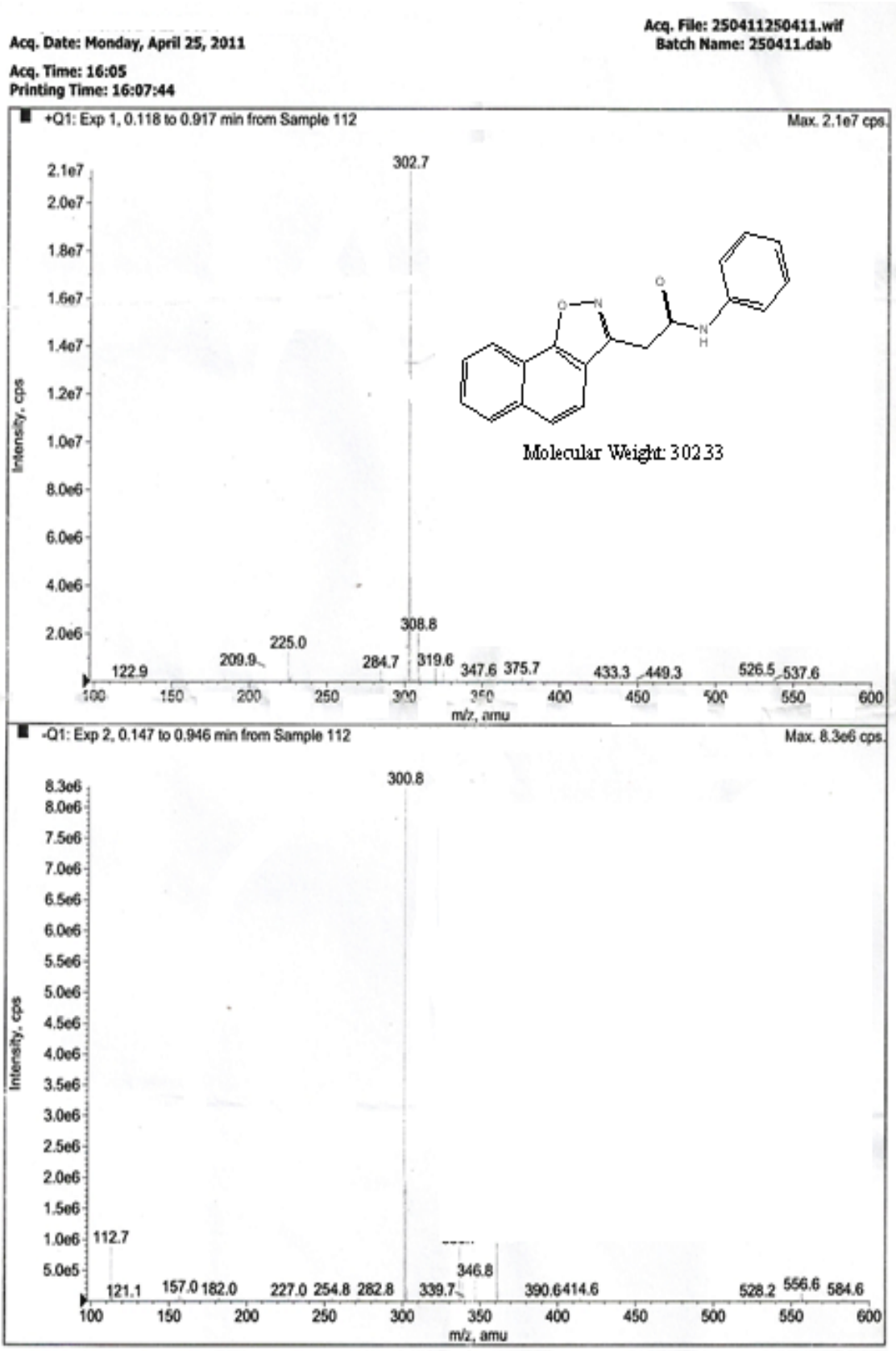

Figure 18: ESI/MS of 2-(naphtho[2,1-d]isoxazole-3-yl)-N-phenylacetamide **5b**

NPC DIVISION IIIM JAMMU

Reported by User: System

Project Name: NPC8

SAMPLE INFORMATION

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Sample Type:	Unknown	Date Acquired:	11/17/2011 4:44:20 PM
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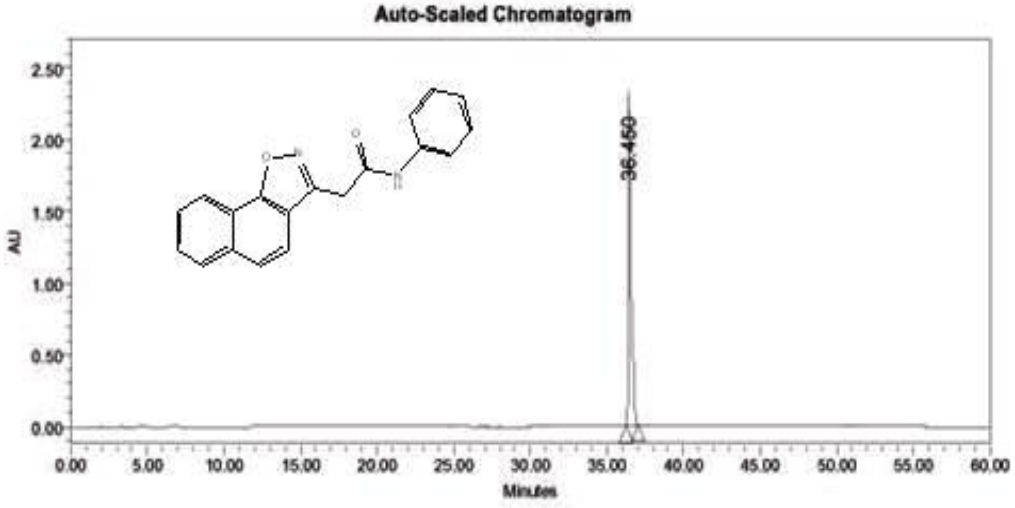
Injection #:	1	Date Processed:	11/19/2011 11:01:41 AM
Injection Volume:	10.00 ul	Processing Method:	Sample
Run Time:	60.0 Minutes	Channel Name:	W2996 254.0nm-1.2
Sample Set Name:	Tinospora	Proc. Chnl. Descr.:	W2996 PDA 254.0 nm at 1.2
column_name	RP-18e,5um	Flow rate: 0.7 ml/min	
		Sample conc:0.6 mg/mL MeOH	
		Mobile phase: ACN:Water(gradient)	



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Report Method: Parveen Printed 11:13:20 AM 11/19/2011 Page: 1 of 1

Figure 19: HPLC of 2-(naphtho[2,1-d]isoxazole-3-yl)-N-phenylacetamide **5b**

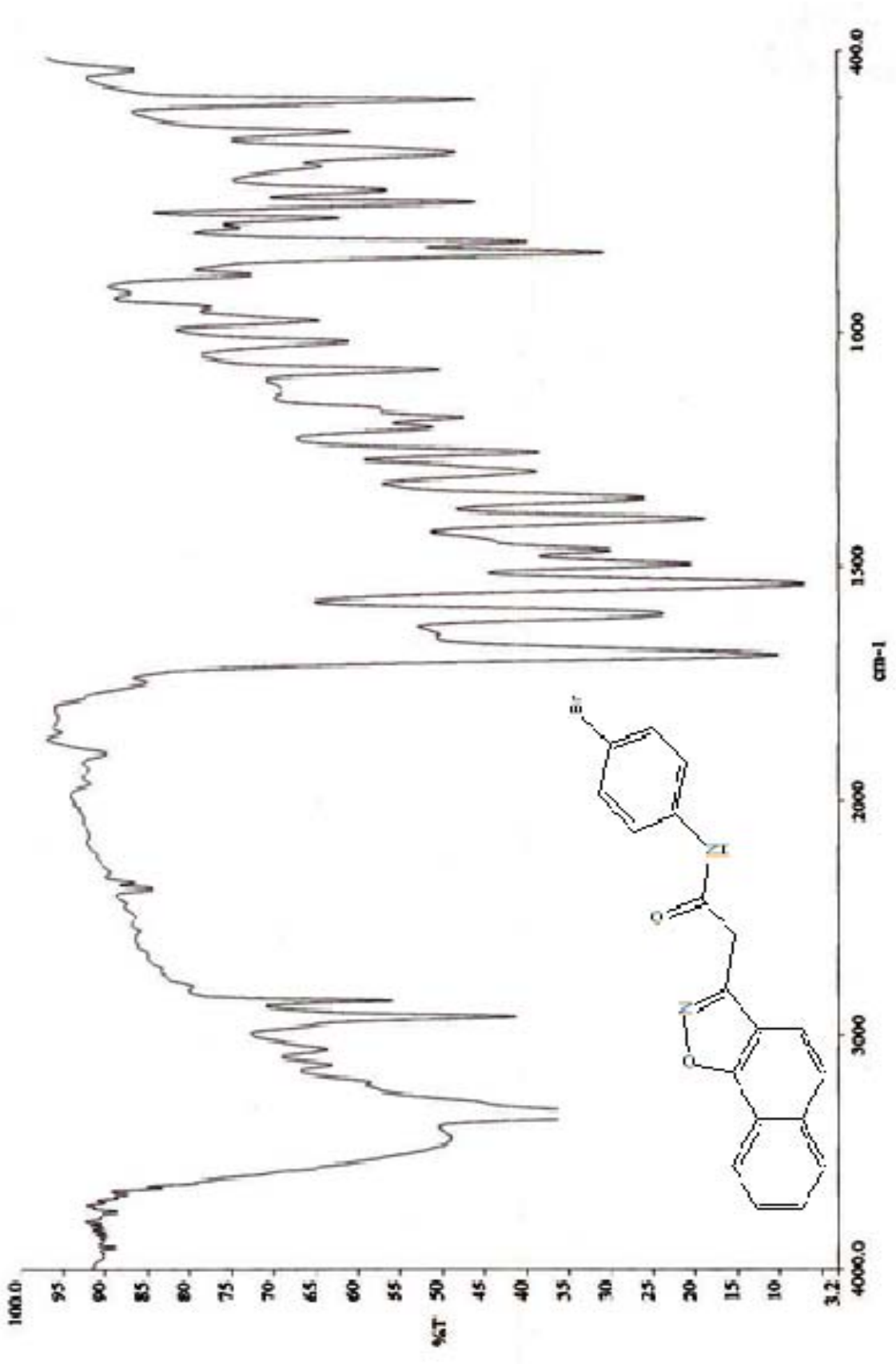


Figure 20: IR of N-(4-bromophenyl)-2-(naphtho[2,1-d]isoxazole-3-yl)acetamide **5c**

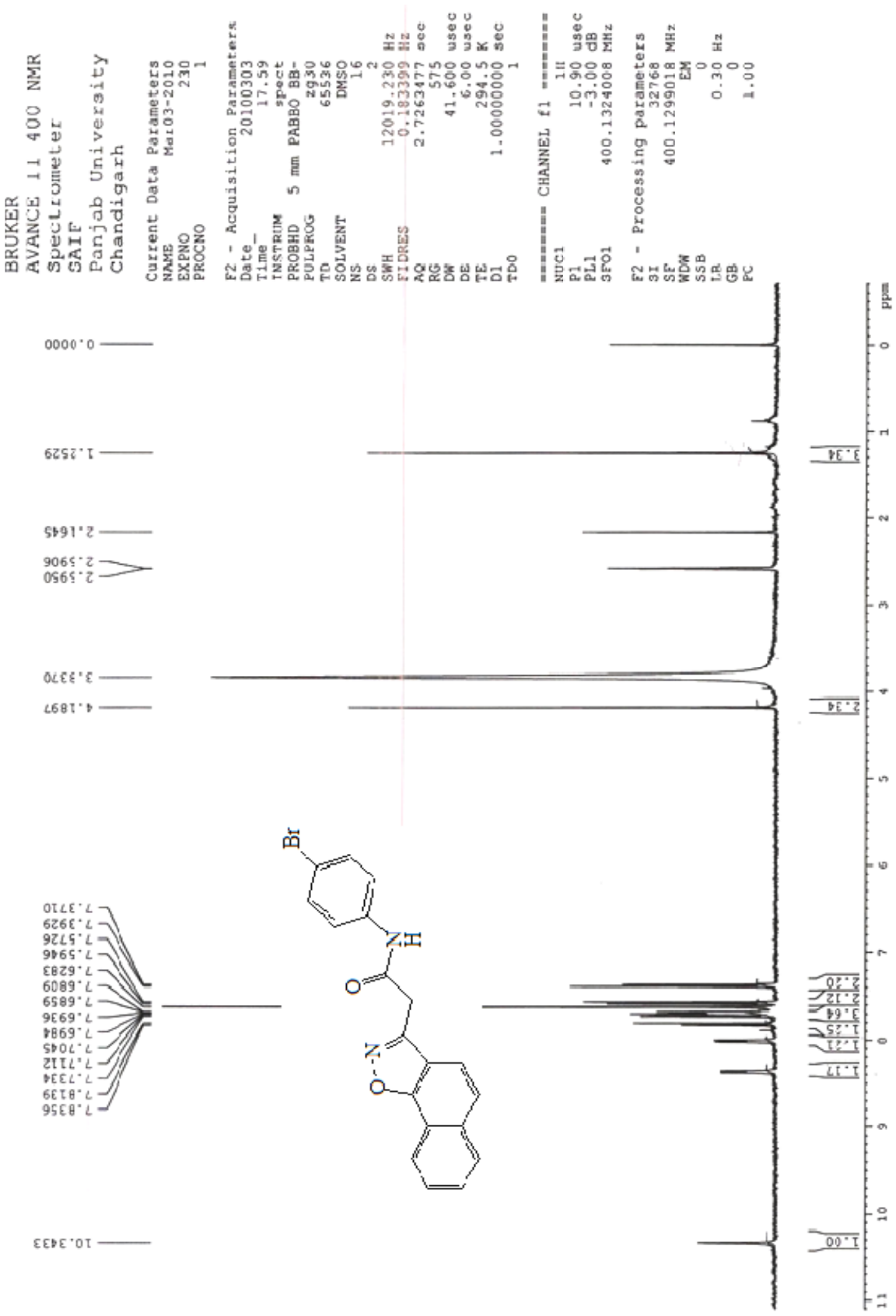


Figure 21: ^{1}H NMR of N-(4-bromophenyl)-2-(naphtho[2,1-d]isoxazole-3-yl)acetamide **5c**

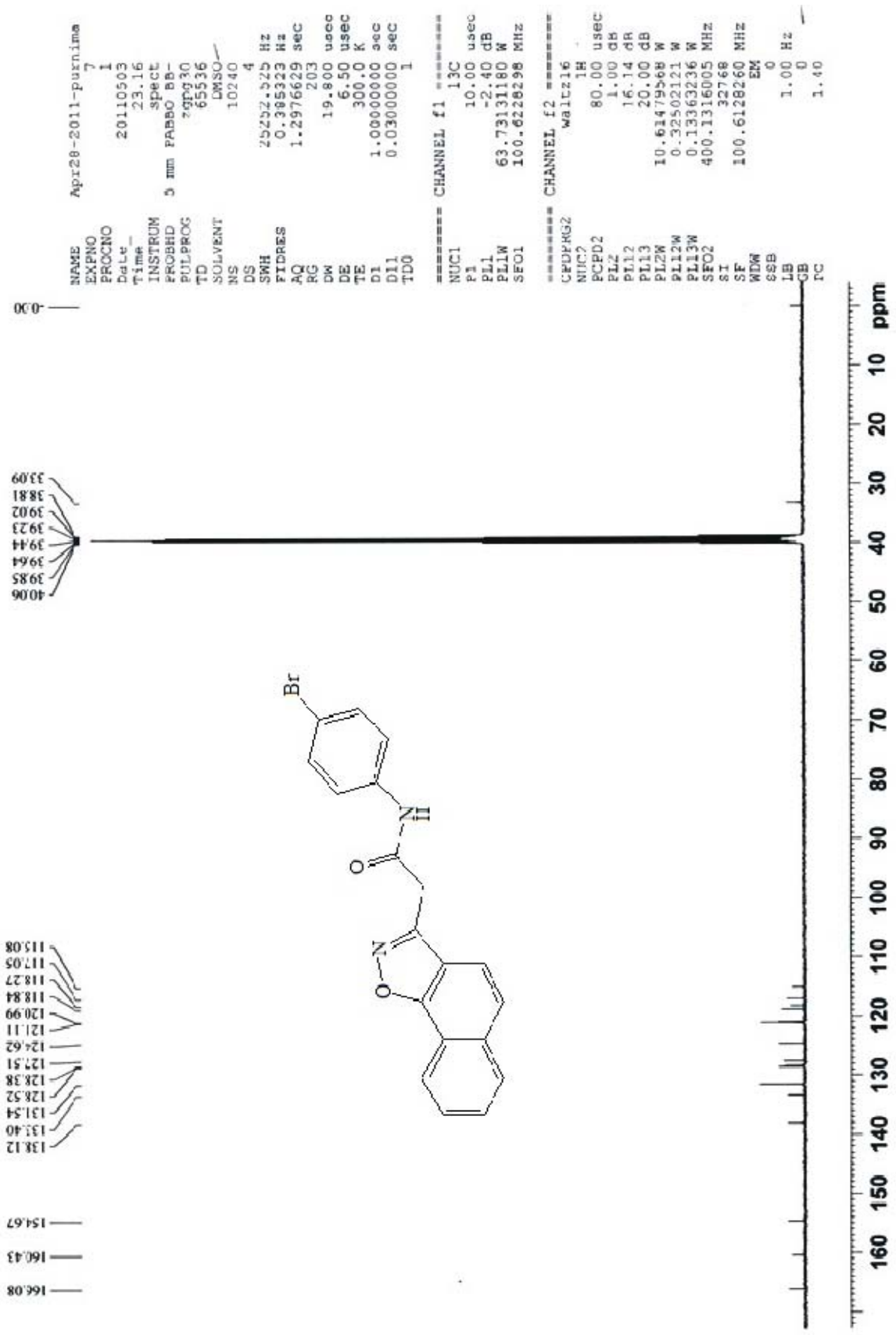


Figure 22: ^{13}C NMR of N-(4-bromophenyl)-2-(naphtho[2,1-d]isoxazole-3-yl)acetamide **5c**

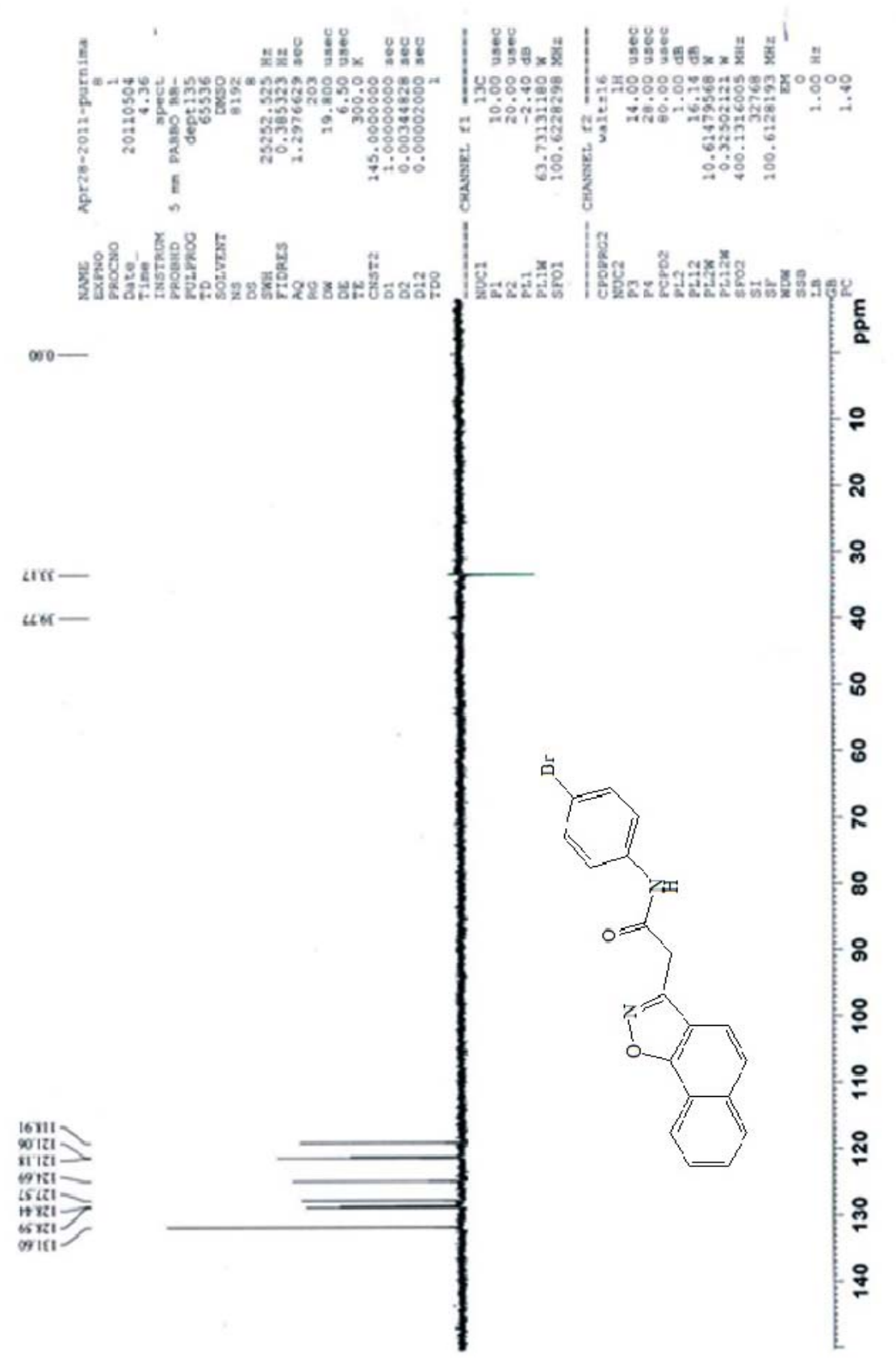


Figure 23: DEPT-135 of N-(4-brom ophenyl)-2-(naphtho[2,1-d]isoxazole-3-yl)acetamide **5c**

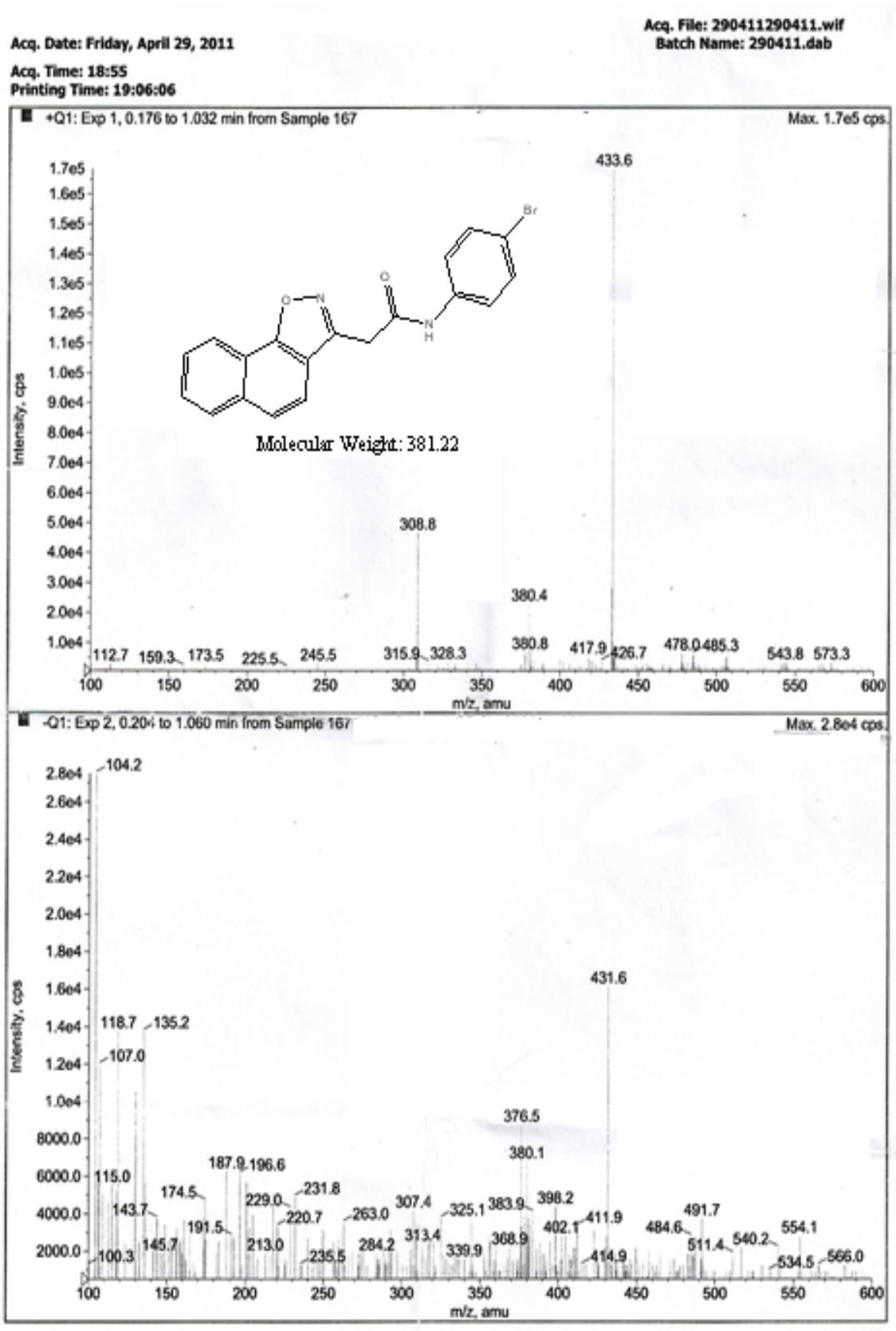
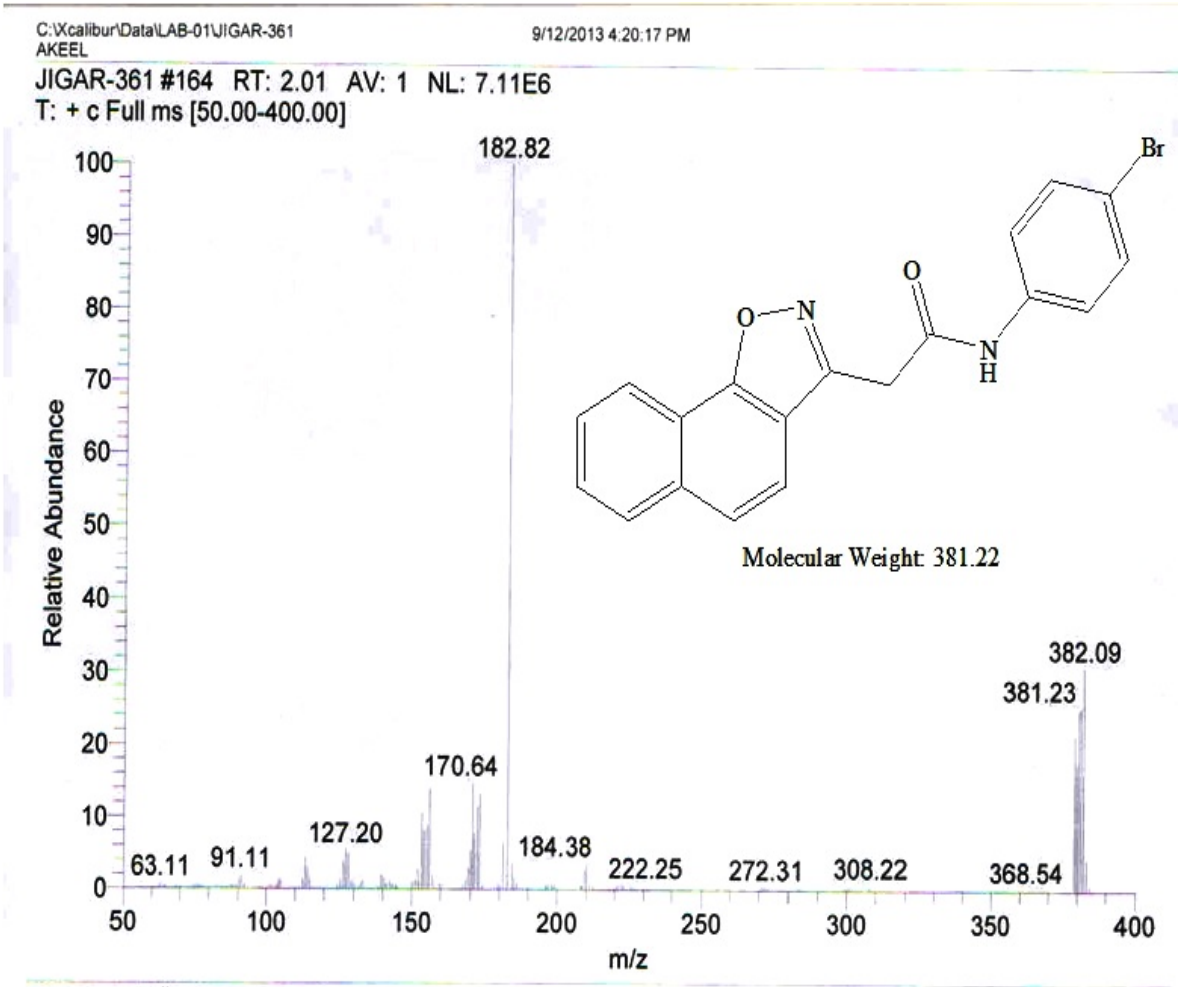


Figure 24: ESI/MS of N-(4-bromophenyl)-2-(naphtho[2,1-d]isoxazole-3-yl)acetamide **5c**



JIGAR-361#164 RT: 2.01
T: + c Full ms [50.00-400.00]

m/z	Intensity	Relative
113.53	296691.0	4.18
127.20	381005.0	5.36
128.08	334511.0	4.71
153.51	761652.0	10.72
154.26	586910.0	8.26
155.22	625180.0	8.80
156.03	991430.0	13.95
170.00	382283.0	5.38
170.64	1040572.0	14.65
171.25	543971.0	7.66
172.26	818186.0	11.52
173.02	948430.0	13.35
181.31	473835.0	6.67
182.82	7105180.0	100.00
378.98	1524691.0	21.46
379.69	1258023.0	17.71
380.35	1776918.0	25.01
381.23	1805378.0	25.41
382.09	2197798.0	30.93
383.23	293471.0	4.13

Figure 24 a: GC/MS of N-(4-bromophenyl)-2-(naphtho[2,1-d]isoxazole-3-yl)acetamide **5c**

NPC DIVISION IIIM JAMMU

Reported by User: System

Project Name: NPC8

SAMPLE INFORMATION

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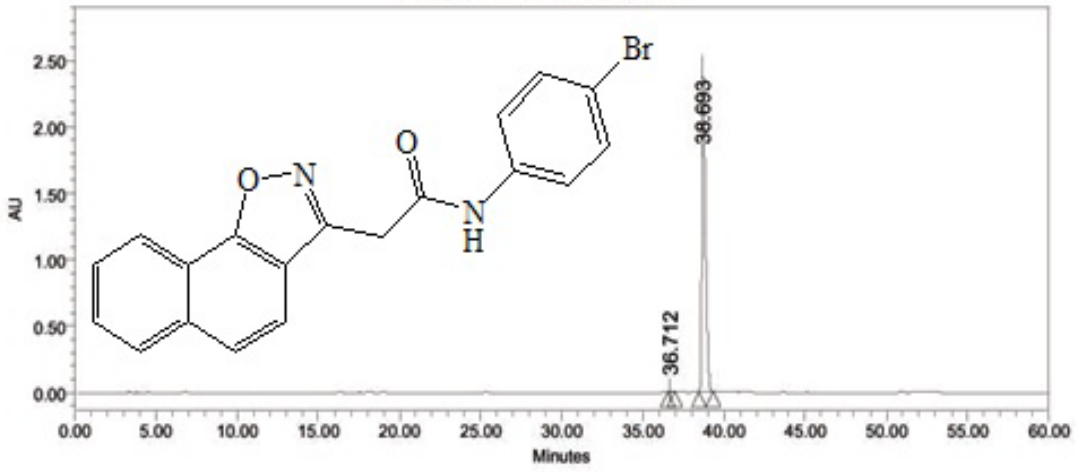
Injection #:	1	Processing Method:	Sample
Injection Volume:	10.00 ul	Channel Name:	W2996 254.0nm-1.2
Run Time:	60.0 Minutes	Proc. Chnl. Descr.:	W2996 PDA 254.0 nm at 1.2
Sample Set Name:	Tinospora	Flow rate: 0.7 ml/min	
column_name	RP-18e,5um	Sample conc:0.5 mg/mL MeOH	
		Mobile phase: ACN:Water(gradient)	

Auto-Scaled Chromatogram



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1	36.712	101780	0.27	9197
2	38.693	38012346	99.73	2453417

Report Method: Parveen

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Figure 25: HPLC of N-(4-bromophenyl)-2-(naphtho[2,1-d]isoxazole-3-yl)acetamide **5c**

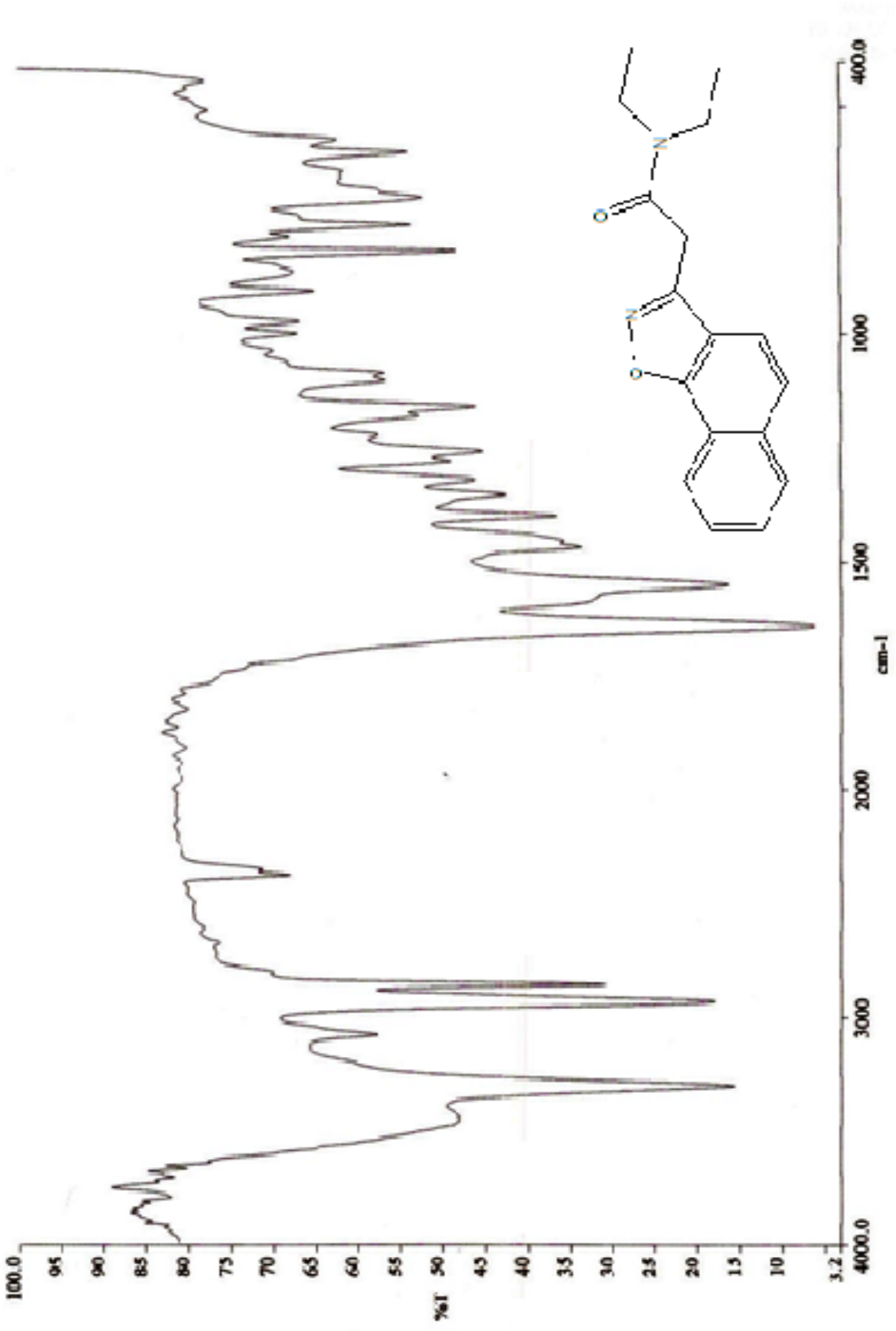


Figure 26: IR of N, N-diethyl-2-(naphtho[2,1-d]isoxazole-3-yl)acetamide **5h**

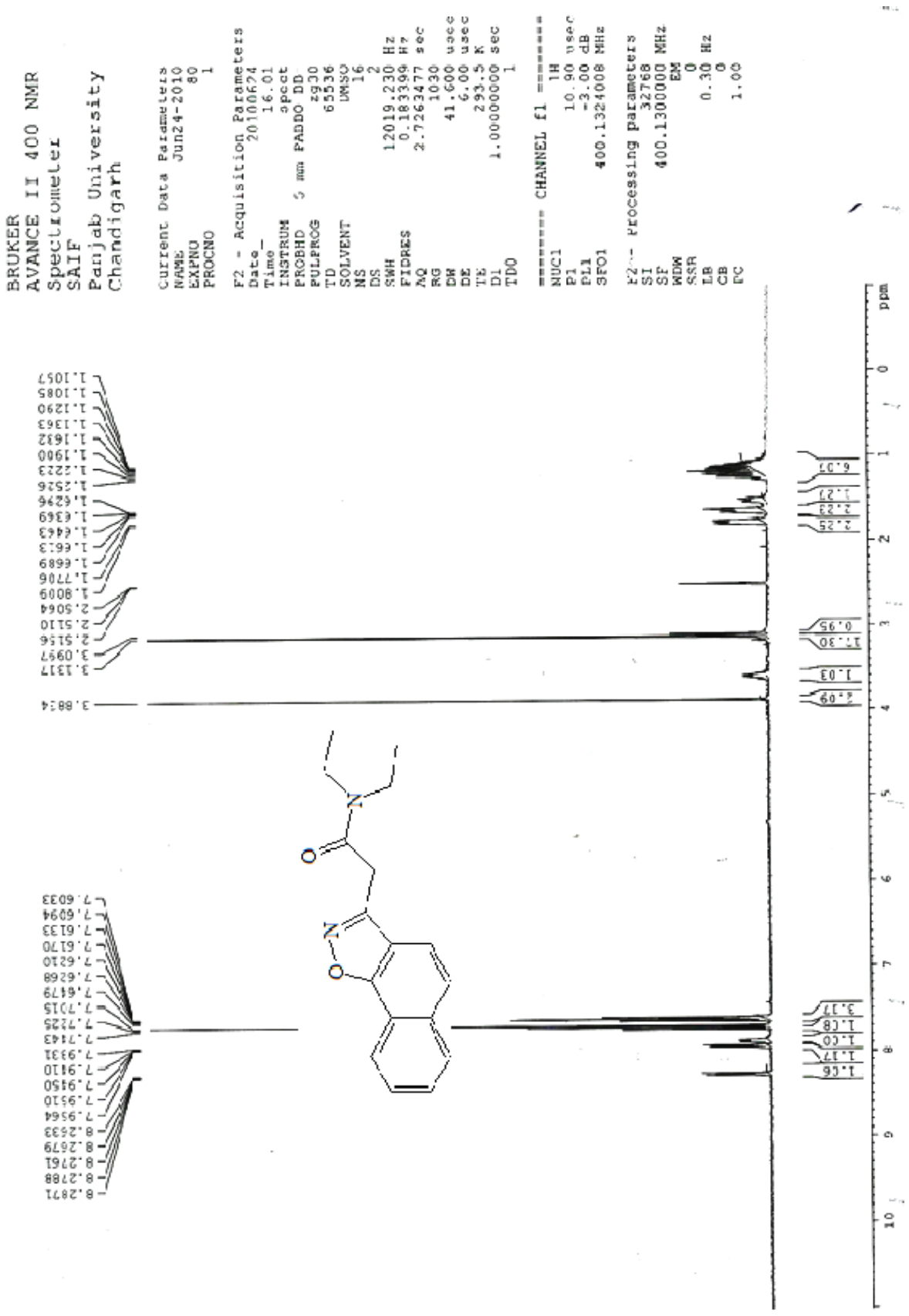


Figure 27: ^{1}H NMR of N, N-diethyl-2-(naphtho[2,1-d]isoxazole-3-yl)acetamide **5h**

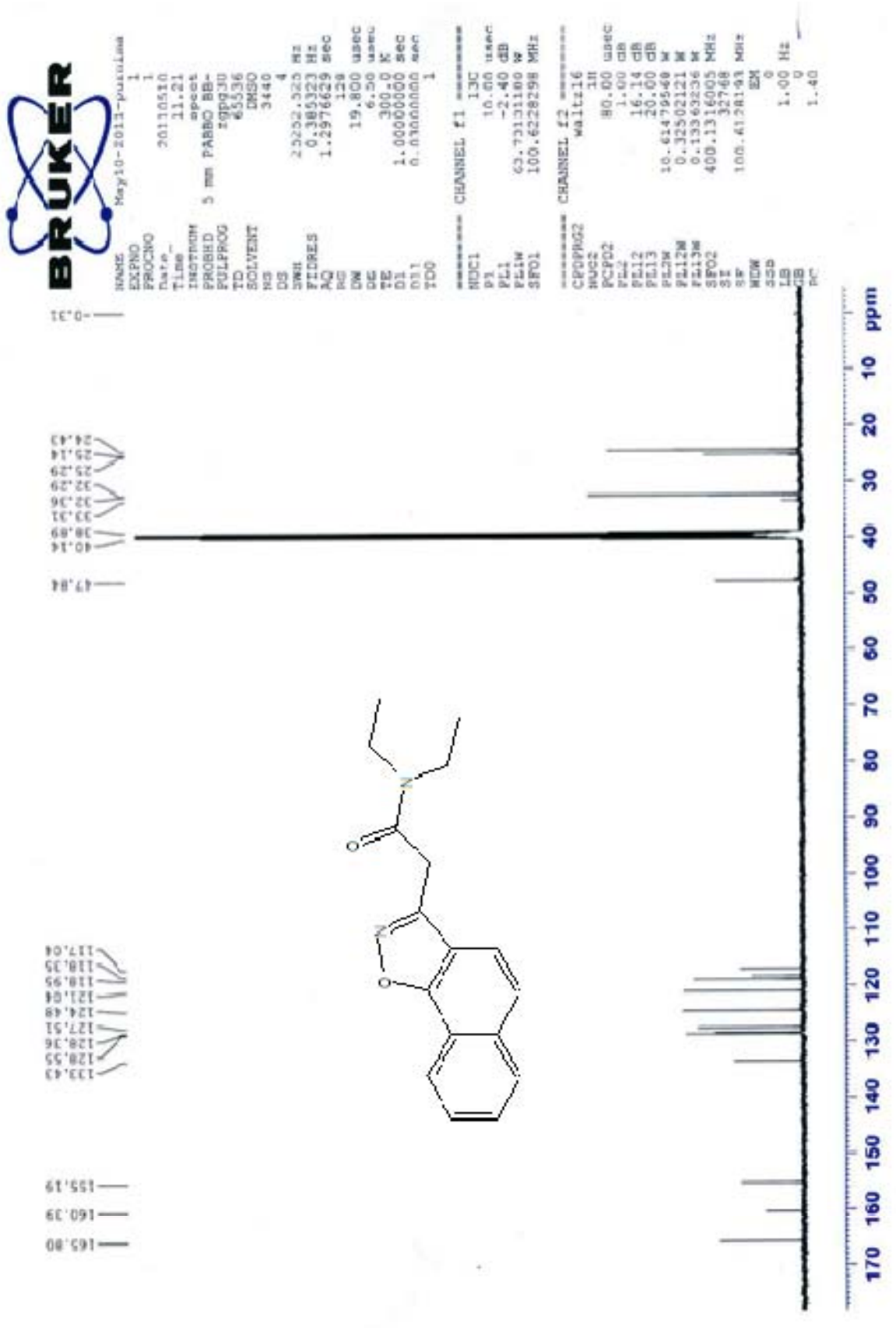


Figure 28: ^{13}C NMR of N, N-diethyl-2-(naphtho[2,1-d]isoxazole-3-yl)acetamide **5h**

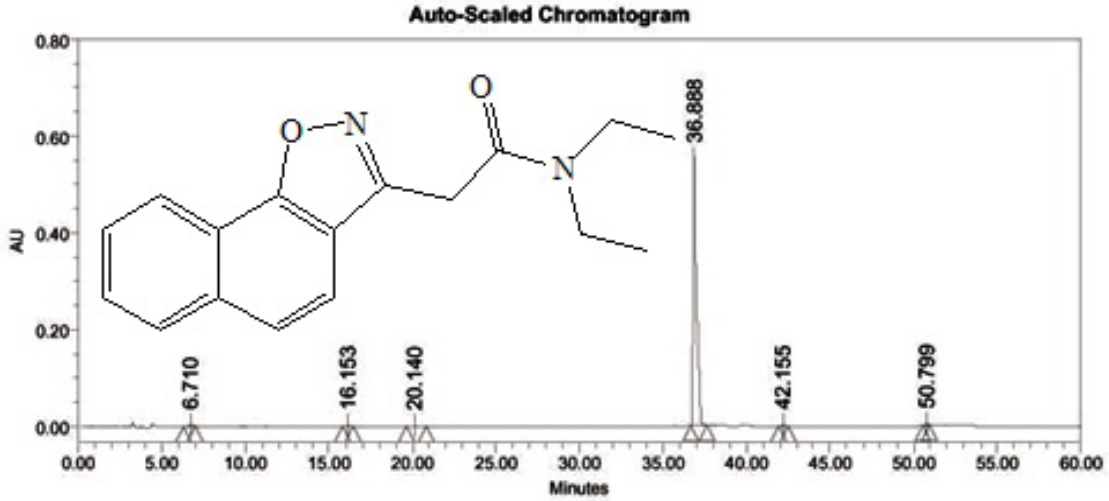
NPC DIVISION IIIM JAMMU

Reported by User: System

Project Name: NPC8

SAMPLE INFORMATION

Sample Name:	JS-16	Acquired By:	System
Sample Type:	Unknown	Date Acquired:	11/18/2011 5:03:14 PM
Vial:	8	Acq. Method Set:	Tinaspora1
Injection #:	1	Date Processed:	11/19/2011 11:06:42 AM
Injection Volume:	10.00 ul	Processing Method:	Sample
Run Time:	60.0 Minutes	Channel Name:	W2996 254.0nm-1.2
Sample Set Name:	Tinospora	Proc. Chnl. Descr.:	W2996 PDA 254.0 nm at 1.2
column_name	RP-18e,5um	Flow rate: 0.7 ml/min	
		Sample conc:0.5 mg/mL MeOH	
		Mobile phase: ACN:Water(gradient)	



	RT	Area (µV*sec)	% Area	Height (µV)
1	6.710	47251	0.59	2931
2	16.153	12058	0.15	1162
3	20.140	23384	0.29	595
4	36.888	7836701	98.24	556141
5	42.155	26324	0.33	2000
6	50.799	31180	0.39	3582

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Figure 29: HPLC of N, N-diethyl-2-(naphtho[2,1-d]isoxazole-3-yl)acetamide **5h**

2.2.2 Biological Evaluation

2.2.2.1 Anticonvulsant activity

The anticonvulsant activity study of all synthesized isoxazole amide derivatives have been carried out by the MES method[25] in Wistar rats and is summarized in Table 1. The experiments were carried out on male Wistar rats (150–180 g). Animals were housed in plastic cages at a constant temperature of 20-28°C with natural light-dark cycles. The animals had free access to standard pellet diet and water, and were used after a minimum of 3 days of acclimatization to the housing conditions. Control and experimental groups consisted of 6 animals each. All the procedures used followed the NIH Animal Care and Use Committee guidelines and were approved by the Ethics Committee at the Dharmsinh University, Nadiad, India. The examined compounds were administered as solution in

Table 1: Anticonvulsant activity of isoxazole derivatives by MES method in Wistar rats.

Phases in Sec. / Comp. No.	Flexion	Extension	Clonus	No. of Jerks	Stupor Phase
5a	1.86 ± 0.08	10.41 ± 0.33	10.3 ± 0.32	15 ± 0.57	7.33 ± 0.39
5b	9.16 ± 0.34	1.91 ± 0.06	30.25 ± 0.6	91.33 ± 0.36	17.53 ± 0.43
5c	0.51 ± 0.07	10.58 ± 0.14	8.93 ± 0.2	7 ± 0.36	16.51 ± 0.47
5d	1.02 ± 0.07	10.05 ± 0.15	15.28 ± 0.24	25.33 ± 0.76	15.11 ± 0.26
5e	2.0 ± 0.05	11.03 ± 0.14	33.3 ± 0.21	65.5 ± 1.61	31.58 ± 0.26
5f	6.91 ± 0.09	0.33 ± 0.09	34.63 ± 0.42	84.5 ± 1.14	13.11 ± 0.32
5g	3.0 ± 0.06	3.18 ± 0.12	21.98 ± 0.26	42.66 ± 0.88	61.35 ± 2.73
5h	0.41 ± 0.07	2.96 ± 0.08	3.9 ± 0.2	5.83 ± 0.94	4.63 ± 0.32
DMSO	2.98 ± 0.09	13.45 ± 0.18	22.98 ± 0.55	18 ± 0.57	28.46 ± 1.18
Phenytoin	0.4 ± 0.15	0.38 ± 0.17	3.26 ± 0.18	1.83 ± 0.3	2.76±0.27

0.1 ml DMSO via IP route at a constant dose of 5, 10 and 15 $mgkg^{-1}$ of body weight. The anticonvulsant activity observed for synthesized compounds is given in Table 1.

Different phases of activity were observed. In case of compound **5c** and **5h** i.e. amines used were p-bromo aniline (**5c**) and N, N-diethyl amine (**5h**), the flexion phase was found to be very comparable to the standard drug phenytoin. For compound **5f** i.e. when amine used was pyrrolidine (**5f**), the extension phase was found to be very low. In case of compound **5h** i.e. when amine used was N, N-diethyl amine (**5h**), the clonus phase was found to be comparable with standard drug.

2.2.2.2 Anticancer Activity

Since isoxazole amide derivatives can also show anticancer activity, we have tried to determine IC_{50} values against melanoma cancer cell lines. The IC_{50} values of all synthesized compounds were determined by *in vitro* screening on a series of various melanoma cell lines i.e. Melan-A, WM3211, WM278, UACC903 and 1205Lu using a MTS assay[26] to establish the efficacy of the compound for alter cell survival. The values are given in Table 2. All compounds exhibited low toxicity with IC_{50} values > 20 μM. In the series **5a-h**, when the amines used were p-toludine **5a** and p-bromo aniline **5c**, showed IC_{50} values 24.31 for WM3211 cell line and 25.51 for WM278 cell line. Since IC_{50} values are greater than 20 μM, further detail study of anticancer activity was not carried out.

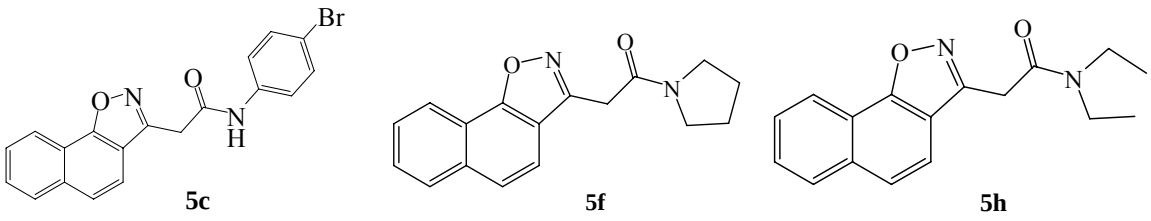
Table 2: IC_{50} value of isoxazole derivatives in µM concentration against melanoma cancer cell lines

Cell lines	Melan-A$	WM3211*	WM278*	UACC903^	1205Lu^
Compound Name			**IC_{50} in µM**		
5a	>50	24.31	>50	>50	>50
5b	>50	34.89	>50	>50	>50
5c	>50	>50	25.51	>50	>50
5d	>50	>50	>50	>50	>50
5e	>50	95.05	>50	>50	>50
5f	>50	91.05	>50	>50	>50
5g	>50	>50	>50	>50	>50
5h	>50	80.40	44.87	>50	>50

$ are mouse melanocyte (normal cells)

* are early stage melanoma cell-lines

^ are metastatic melanoma cell-lines



5c **5f** **5h**

Figure 30: Structure Activity Relationship of naphthoisoxazole amide derivatives

2.3 Conclusion

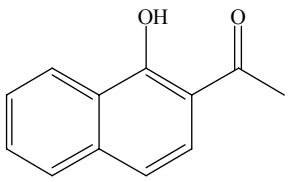
We have concluded that angular isoxazole amide derivatives could be a good scaffold for CNS activities. Since all compounds have IC_{50} values more than 20 µM in cytotoxicity study we cannot use them as anticancer agent but we have studied anticonvulsant activity of all compounds. Compound **5h** showed excellent anticonvulsant

activity as compare with the standard drug Phenytoin in all phases. Compound **5c** has low flexion phase and **5f** has low extension phase as compare to the standard drug which indicated that such scaffolds can be useful in the treatment of various CNS diseases.

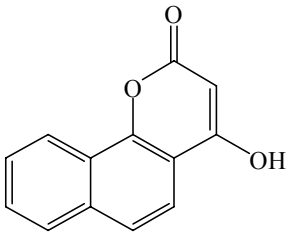
2.4 Experimental

2.4.1 Chemistry

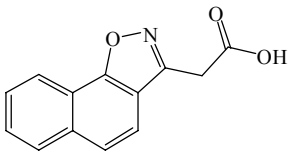
Reagent grade chemicals and solvents were purchased from commercial supplier and used without purification. TLC was performed on silica gel F254 plates (Merck). Melting points are uncorrected and were measured in open capillary tubes, using a Rolex melting point apparatus. IR spectra were recorded as KBr pellets on Perkin Elmer RX 1 spectrometer. ^{1}H NMR and ^{13}C NMR spectral data were recorded on Bruker Advance 300 spectrometer (300 MHz) and Advance 400 spectrometer (400 MHz) with $CDCl_3$ or DMSO-d_6 as solvent and TMS as internal standard. J values are in Hz. Mass spectra were determined by ESI/MS, using a Shimadzu LCMS 2020 apparatus. Purity of compounds was checked by reverse phase HPLC using W2996 PDA detector at 254 nm wavelength using Acetonitrile: Water as solvent. CHN elemental analyses were recorded on Thermosinnigan Flash 11-12 series EA.



1-(1-hydroxynaphhthalen-2-yl)ethanone 2: Zinc chloride (0.29351 mol) was dissolved in Acetic acid (1.049 mol) and 1-Naphthol **1** (0.06936 mol) was added. Reaction mixture was refluxed for 16 hours. Reaction mixture was poured in 500 gm ice HCl to obtain solid. The product was purified by column chromatography using petroleum ether (60-80°C) as eluent to give 1-(1-hydroxynaphthalen-2-yl)ethanone **2** as greenish yellow solid. Yield: 63.5%; mp 98°C (Lit. 98-100°C[21]); IR (KBr) (Figure 2): 3429, 3058, 2924, 2853, 1625 cm^{-1}; Molecular formula $C_{12}H_{10}O_2$.



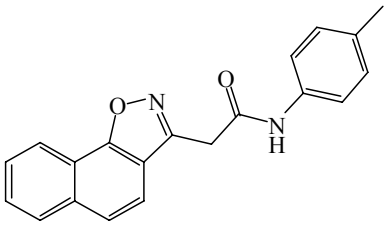
4-hydroxy-2H-benzo[h]chromen-2-one 3: A solution of **2** (0.00107 mol) in diethyl carbonate (30 ml) was slowly added to pulverized sodium (0.01739 mol) under anhydrous conditions. Highly exothermic reaction was observed. It was then allowed to cool to room temperature. Ethanol (50 ml) was added to decompose the unreacted sodium. The reaction mass was then poured into water (250 ml) and the aqueous layer washed twice with petroleum ether (50 ml). Concentrated hydrochloric acid was slowly added to the aqueous layer until pH 2 and the solid obtained was collected by filtration. The crude product was crystallized from ethanol to give 4-hydroxy-2H-benzo[h]chromen-2-one **3** as light yellow solid. Yield: 96%; mp 283-285°C (Lit. 284°C[22]); IR (KBr) (Figure 3): 3423, 2926, 1604, 1561 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) (Figure 4): δ 5.83 (1H, s, C-3 proton), 7.60-7.76 (3H, m, ArH), 7.86-7.94 (2H, m, ArH), 8.48-8.53 (1H, m, ArH), 11.94 (1H, s, OH); ^{13}C NMR (400 MHz, DMSO-d_6) (Figure 5): δ 91.1, 111.6, 119.3, 122.1, 122.7, 124.1, 127.8, 128.6, 129.2, 135.3, 151.1, 162.3, 167.2; Molecular formula $C_{13}H_8O_3$.



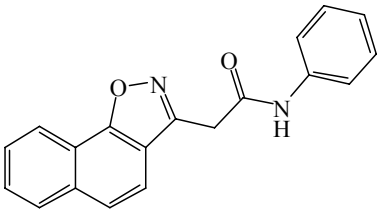
2-(naphtho[2,1-d]isoxazole-3-yl)acetic acid 4: To a solution of compound **3** (0.0236 mol) in methanol (50 ml) hydroxylamine hydrochloride (0.0825 mol) and sodium bicarbonate (0.0825 mol) were added and the reaction mixture was refluxed for 15 h. Excess methanol was distilled off and the reaction mass was dissolved in 10% sodium bicarbonate solution (200 ml) and filtered. Filtrate was acidified with concentrated hydrochloric acid till pH 2; the off white solid thus obtained was filtered off and crystallized in 5% hot ethyl acetate in petroleum ether to give 2-(naphtho[2,1-d]isoxazole-3-yl)acetic acid **4** as off white solid. Yield: 43%; mp 189-192°C; IR (KBr) (Figure 6): 3435, 2978, 2703, 1732, 1641, 1592 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6) (Figure 7): δ 4.17 (2H, s, CH_2), 7.75-7.80 (3H, m, ArH), 7.85-7.87

(1H, d, *J*= 8.72 Hz, ArH), 8.14-8.17 (1H, m, ArH), 8.35-8.38 (1H, dd, *J*= 3.28, 4.27 Hz, ArH), 12.92 (1H, s, COOH); ^{13}C NMR (400 MHz, DMSO-d_6) (Figure 8): δ 31.3, 117.5, 118.8, 119.3, 121.5, 125.2, 128.1, 128.9, 129.1, 133.9, 154.8, 160.9, 170.5; MS (ESI, *m/z*) (Figure 9): 227.8 $[M+1]^+$ Calculated for $C_{13}H_9NO_3$.

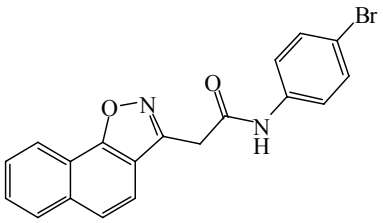
General Procedure for 5a-5h: To a solution of **4** (0.002 mol) in ethyl acetate (50 ml), DMAP (0.050 gm, 10%), primary amine/ secondary amine (0.0022 mol) and DCC (0.0026 mol) were added and the reaction mixture was allowed to stir at room temperature for 12 hours. Solid was filtered off and solvent evaporated under reduced pressure to give solid which was treated with 20 ml saturated sodium bicarbonate solution to remove unreacted acid then 20 ml conc. HCl (10%) to remove excess primary amine (20 ml brine solution in case of secondary amine only) and then 20 ml water. Product was recrystallized from ethanol.



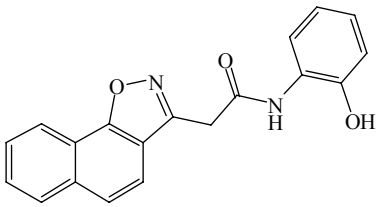
2-(naphtho[2,1-d]isoxazole-3-yl)-N-p-tolylacetamide 5a: This compound was obtained as off white solid. Yield: 57%; mp: 257-259°C; IR (KBr) (Figure 10): 3291, 3045, 2918, 2850, 1656, 1594 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) (Figure 11): δ 2.29 (3H, s, CH_3), 4.19 (2H, s, CH_2), 7.09-7.12 (2H, d, *J*= 8.26 Hz, ArH), 7.36-7.39 (2H, d, *J*= 8.38 Hz, ArH), 7.67-7.76 (4H, m, ArH), 7.98-8.05 (2H, m, ArH and NH), 8.41-8.44 (1H, dd, *J*= 2.0, 4.28 Hz, ArH); ^{13}C NMR (400 MHz, DMSO-d_6) (Figure 12): δ 20.9, 29.7, 34.7, 117.6, 119.2, 120.1, 121.8, 125.5, 127.4, 128.5, 128.5, 129.5, 134.2, 134.5, 134.8, 164.9; MS (ESI, *m/z*) (Figure 13) 316.6 $[M]^+$ and 317.7 $[M+1]^+$ Calculated for $C_{20}H_{16}N_2O_2$; HPLC Purity 100% (Figure 14); Ele. Anal. Calcd. for $C_{20}H_{16}N_2O_2$; Requires (Found) %: C, 75.32 (75.53); H, 5.26 (5.10); N, 8.40 (8.66).



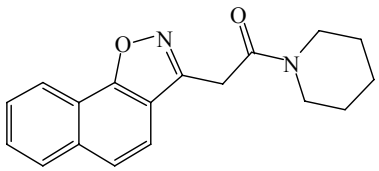
2-(naphtho[2,1-d]isoxazole-3-yl)-N-phenylacetamide 5b: This compound was obtained as off white solid. Yield: 77%; mp: 250-252°C; IR (KBr) (Figure 15): 3289, 3056, 2926, 2851, 1657, 1598, 1542 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) (Figure 16): δ 4.19 (2H, s, CH_2), 7.04-7.08 (1H, m, ArH, 7.44 Hz), 7.26-7.30 (2H, m, ArH, 7.72 Hz), 7.62-7.73 (5H, m, ArH), 7.83-7.85 (1H, d, *J*= 8.72 Hz, ArH), 8.00-8.02 (1H, dd, *J*= 3.92, 5.32 Hz, ArH), 8.36-8.38 (1H, dd, *J*= 4.12, 5.28 Hz, ArH), 10.20 (1H, s, NH); ^{13}C NMR (400 MHz, DMSO-d_6) (Figure 17): δ 33.1, 115.1, 117.0, 118.3, 118.8, 121.0, 121.1, 124.6, 127.5, 128.4, 128.5, 131.5, 133.4, 138.1, 154.7, 160.4, 166.1; MS (ESI, *m/z*) (Figure 18) 302.7 $[M]^+$ and 300.8 $[M-2]^+$ Calculated for $C_{19}H_{14}N_2O_2$; HPLC Purity (Figure 19) 100%; Ele. Anal. Calcd. for $C_{19}H_{14}N_2O_2$; Requires (Found) %: C, 75.69 (75.48); H, 4.73 (4.67); N, 9.53 (9.27).



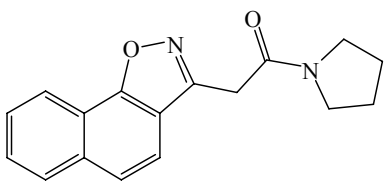
N-(4-bromophenyl)-2-(naphtho[2,1-d]isoxazole-3-yl)acetamide 5c: This compound was obtained as light yellow solid. Yield: 22%; mp: 267-269ºC; IR (KBr) (Figure 20): 3345, 3056, 2919, 2850, 1684, 1596 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) (Figure 21): δ 4.19 (2H, s, CH_2), 7.37-7.39 (2H, d, *J*= 8.76 Hz, ArH), 7.57-7.59 (2H, d, *J*= 8.8 Hz, ArH), 7.68-7.73 (3H, m, ArH), 7.81-7.83 (1H, d, *J*= 8.68 Hz, ArH), 7.98-8.04 (1H, m, ArH), 8.34-8.41 (1H, m, ArH), 10.34 (1H, s, NH); ^{13}C NMR (400 MHz, DMSO-d_6) (Figure 22): δ 33.1, 115.1, 117.0, 118.3, 118.8, 121.0, 121.1, 124.6, 127.5, 128.4, 128.5, 131.5, 133.4, 138.1, 154.7, 160.4, 166.1; MS (ESI, *m/z*) (Figure 24) 380.8 $[M]^+$ and 380.1 $[M-1]^+$ Calculated for $C_{19}H_{13}BrN_2O_2$; HPLC Purity 99.73% (Figure 25); Ele. Anal. Calcd. for $C_{19}H_{13}BrN_2O_2$; Requires (Found) %: C, 59.59 (59.86); H, 3.53 (3.44); N, 7.63 (7.35).



N-(2-hydroxyphenyl)-2-(naphtho[2,1-d]isoxazole-3-yl)acetamide 5d: This compound was obtained as light brown solid. Yield: 47%; mp: 208-210°C; IR (KBr): 3323, 2928, 2851, 1763, 1626, 1542 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ 4.36 (2H, s, CH_2), 6.76-6.78 (1H, t, *J*= 1.2, 6.8 Hz, ArH), 6.89-6.98 (2H, m, ArH), 7.75-7.89 (3H, m, ArH), 8.14-8.17 (1H, m, ArH), 8.37-8.40 (1H, dd, *J*= 2.0, 4.0 Hz, ArH), 9.77 (1H, s, NH), 9.89 (1H, s, OH); ^{13}C NMR 400 MHz (DMSO-d_6): δ 48.0, 115.8, 117.6, 118.8, 119.3, 119.5, 121.6, 122.8, 125.1, 125.3, 126.4, 128.1, 128.9, 129.1, 133.9, 148.4, 155.6, 160.9, 166.7; HPLC Purity 96.74%; Molecular formula $C_{19}H_{14}N_2O_3$.

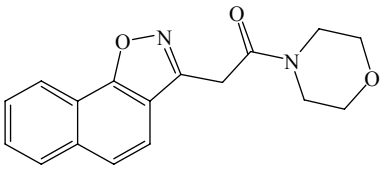


2-(naphtho[2,1-d]isoxazole-3-yl)-1-(piperidine-1-yl)ethanone 5e: This compound was obtained as white solid. Yield: 64%; mp: 213-215°C; IR (KBr): 3305, 3052, 2925, 2851, 1689, 1658, 1589 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ 1.25-1.38 (2H, m, CH_2), 1.62-1.67 (2H, m, CH_2), 1.75-1.79 (2H, m, CH_2), 1.89-1.92 (2H, d, CH_2), 1.97-2.00 (2H, d, CH_2), 4.20 (2H, s, CH_2), 7.67-7.74 (4H, m, ArH), 7.87-7.89 (1H, d, *J*= 8.0 Hz, ArH), 7.99-8.01 (1H, dd, *J*= 2.12, 4.08 Hz, ArH), 8.36-8.38 (1H, dd, *J*= 3.64, 5.8 Hz, ArH); ^{13}C NMR 400 MHz (DMSO-d_6): δ 24.8, 25.5, 25.8, 31.8, 32.1, 50.2, 117.6, 118.8, 119.3, 121.5, 125.0, 128.1, 128.9, 129.1, 133.9, 155.2, 160.8, 165.3; HPLC Purity 97.7%; Molecular formula $C_{18}H_{18}N_2O_2$.

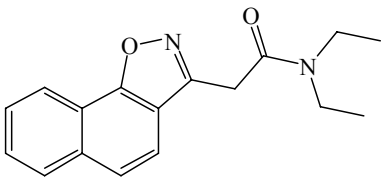


2-(naphtho[2,1-d]isoxazole-3-yl)-1-(pyrrolidin-1-yl)ethanone 5f : This compound was obtained as white solid. Yield: 95%; mp: 198-200°C; IR (KBr): 3306, 3053, 2927, 2852, 2361, 1659, 1589 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ 1.60-1.65 (2H, m, CH_2), 1.77-1.81 (2H, m, CH_2), 1.87-1.90 (2H, m, CH_2), 1.96-1.98 (2H, m, CH_2), 4.18 (2H, s, CH_2), 7.68-7.72 (3H, m, ArH), 8.02- 8.04 (1H, dd, *J*= 1.88, 2.52 Hz, ArH), 8.29-

8.31 (1H, d, *J*= 7.76 Hz, ArH), 8.34-8.36 (1H, m, ArH); ^{13}C NMR 400 MHz (DMSO-d_6): δ 25.5, 25.8, 31.8, 32.1, 50.2, 117.6, 118.8, 119.3, 121.5, 125.0, 128.1, 128.9, 129.1, 133.9, 155.2, 160.8, 165.3; HPLC Purity 97.6%; Molecular formula $C_{17}H_{16}N_2O_2$.



1-morpholino-2-(naphtho[2,1-d]isoxazole-3-yl)ethanone 5g: This compound was obtained as light brown solid. Yield: 51%; mp: >260°C; IR (KBr): 3299, 3071, 2927, 2853, 1636, 1544 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.54-1.56 (2H, m, CH_2), 1.65-1.68 (2H, d, CH_2), 1.82 (2H, m, CH_2), 3.94 (2H, s, CH_2), 7.65 (3H, m, ArH), 7.75-7.77 (1H, d, *J*= 9.72 Hz, ArH), 7.95 (1H, m, ArH), 8.33 (1H, m, ArH); ^{13}C NMR 400 MHz (DMSO-d_6): δ 24.9, 25.6, 32.7, 32.8, 48.3, 117.5, 118.8, 119.4, 121.5, 125.0, 128.0, 128.9, 129.1, 133.9, 155.7, 160.8, 166.4; HPLC Purity 96.92%; Molecular formula $C_{17}H_{16}N_2O_3$.



N,N-diethyl-2-(naphtho[2,1-d]isoxazole-3-yl) acetamide 5h: This compound was obtained as light brown solid. Yield: 58%; mp: 236-238°C; IR (KBr) (Figure 26): 3299, 3071, 2927, 2853, 2362, 1637, 1545 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) (Figure 27): δ 1.11-1.25 (6H, m, CH_3), 1.63-1.67 (2H, m, CH_2), 1.77-1.81 (2H, d, CH_2), 3.88 (2H, s, CH_2), 7.60-7.65 (3H, m ArH), 7.93-7.96 (2H, m, ArH), 8.26, 8.29 (1H, m, ArH); ^{13}C NMR (400 MHz, DMSO-d_6) (Figure 28): δ 24.4, 25.3, 32.4, 33.3, 47.8, 117.0, 118.3, 118.9, 121.0, 121.5, 127.5, 128.4, 128.5, 133.4, 155.2, 160.4, 165.8; HPLC Purity 98.24% (Figure 29); Molecular formula $C_{17}H_{18}N_2O_2$.

2.4.2 Biological Evaluation

2.3.2.1 Procedure of MES test for anticonvulsant activity

The examined compounds were administered as solution in 0.1 ml DMSO at a constant dose of 5, 10 and15 mg/kg of body weight via IP route. After 30 minutes 150 µv

AC current for 0.2 second was given for convulsion. Phenytoin was used as standard drug. Different phases were observed as given in Table 1.

2.4.2.2 Procedure to assess the effect of the isoxazole derivatives on melanoma cell survival using the MTS assay

96 well plates were plated with 100 µl Media (DMEM + 10% Fetal bovine serum and L-Glutamine) containing 5000 cells/well. Stock of 20 mM solution was prepared for compounds to get a series of concentration ranging from 50 µM to 0.625 µM. 100µl of these compounds were added to the 96 well plates. These 96 well plates were incubated at 37°C in humidified incubator under 5% CO_2 atmosphere for 24, 48 and 72 hours.

In vitro inhibitory efficacy of cancer cell lines representing different cancer types following treatment with compounds was measured using the 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2*H*-tetrazolium (MTS) assay (Promega, Madison, WI). In brief, 5×10^3 cells per well in 100 μl of DMEM containing 10% FBS were grown in a 96-well plate for 24 h and treated with either control DMSO vehicle or increasing concentrations (0.625-50 μM) of these compounds for 24, 48 and 72 h. The proportion of viable cells compared to control DMSO treated cells were determined using MTS assay and IC_{50} values calculated using GraphPad Prism, version 4.01 (GraphPad software, San Diego, CA). The IC_{50} value for each compound was determined by at least three independent experiments and represented with a standard error. IC_{50} in µM concentration of all compounds is given in Table 2.

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Chapter 3

Synthesis and biological evaluation of amide derivatives of Benzodifuran-2-carboxylic acid

3. Synthesis and biological evaluation of amide derivatives of Benzodifuran-2-carboxylic acid

3.1 Introduction

The incidences of bacterial and fungal infections have increased significantly in the past 25 years. The evolution of antibacterial resistance in bacterial strains against the currently available antibacterial agents is an increasing concern in recent years. For instance Gram positive bacterial pathogens such as *Staphylococus aureus* (*S. aureus*) is resistant to Methicillin, *Streptococus pneumoniae* and *Enterococci* are resistant to Penicillin and Vancomycin respectively[1] while Gram negative bacteria are resistant to β lactams, quinolones and macrolides.[2] Since *Candida albicans* (*C. albicans*) and *Aspergillus fumigatus* (*A. fumigatus*) are the main causative fungi of the systemic mycosis, antifungal drugs for treating patients of deep mycosis should have a broad antifungal spectrum including at least these microorganisms. Currently only four classes of antifungal drugs are available which include polyene macrolides (amphotericin B), azoles (fluconazole, miconazole, itraconazole and voriconazole), flucytosine and candins (caspofungin acetate and micafungin) for treatment of systemic mycosis. Unfortunately none of them is ideal in terms of efficacy, antifungal spectrum or safety. Although amphotericin B is efficacious against both *candidiasis* and *aspergillosis*, it shows severe renal toxicity. The antifungal spectra of fluconazole and flucytosine are narrow (mainly against *C. albicans*) and they are prone to develop drug resistance.

In order to overcome the threat of wide spread multi drug resistance in Gram positive and Gram negative bacterial strains as well as fungi, there is ongoing demand for new antimicrobial agents.

Furan and its derivatives show wide range of activity. Benzofurans having various amide, ester, ether and thioether derivatives with varying functional groups show

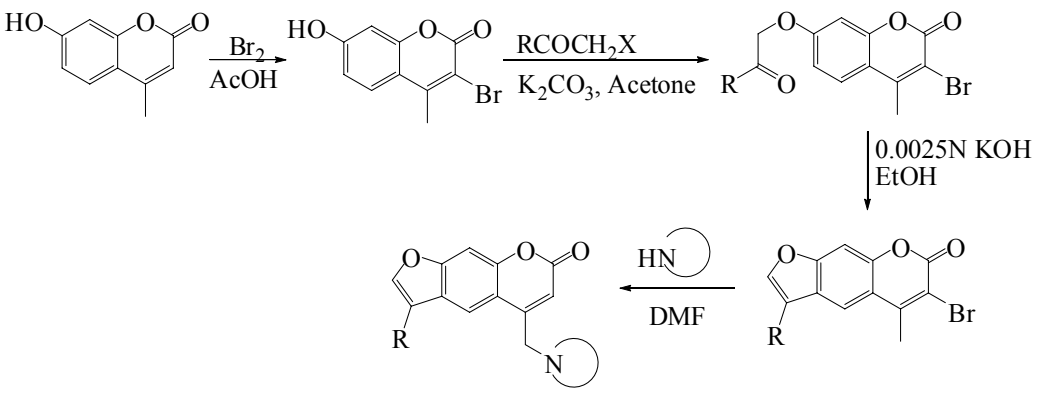
antifungal activity.[3-6] 3-substituted benzofuran-2-amide derivatives are reported as cysteine protease inhibitors.[7] Benzofuran and benzothiophene derivatives substituted with amide effectively inhibit ischemic cell death and can be useful in many other diseases.[8] Various 2-substituted amide derivatives of benzofuran are reported as potent orexin receptor antagonist.[9] Various 2-substituted benzofuran amide derivatives are reported to show good antimicrobial activity.[10] Ethyl ester derivatives of 4-hydroxy-3-methyl-6-phenylbenzofuran-2-carboxylic acid have been reported as antitumor agents.[11] Various 2-substituted vinyl ester derivatives of benzofuran and benzodifuran have been reported as angiogenesis inhibitors.[12] Benzofuran-2-biphenyl sulfonamide derivatives are useful in treatment of osteoarthritis.[13] Substituted benzofuran-1, 3-diazepin derivatives are reported to show CNS depressant effect.[14]

Literature survey reveals that various benzofuran amide derivatives show antimicrobial as well as CNS activities, while very little work is reported on benzodifuran derivatives. In continuation of our work on search for antimicrobial agents[15, 16] we report herein synthesis and antimicrobial evaluation of series of amide derivatives of benzodifuran carboxylic acid.

Recent Work

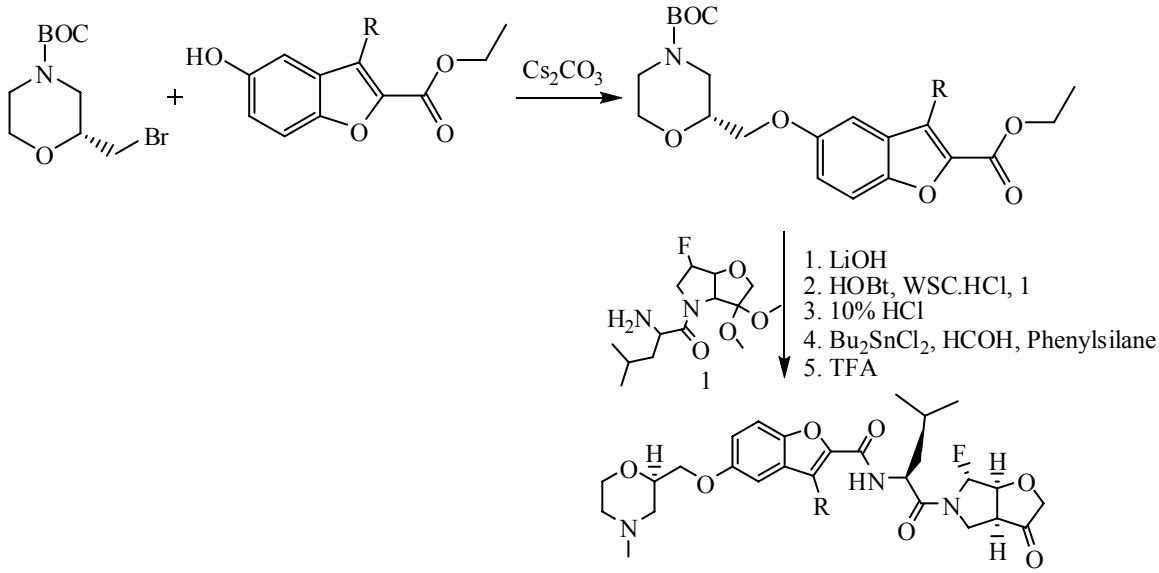
Patel J. M. and Soman S. S.[17] have synthesized furocoumarin derivatives as shown in Scheme 1.

Scheme 1:



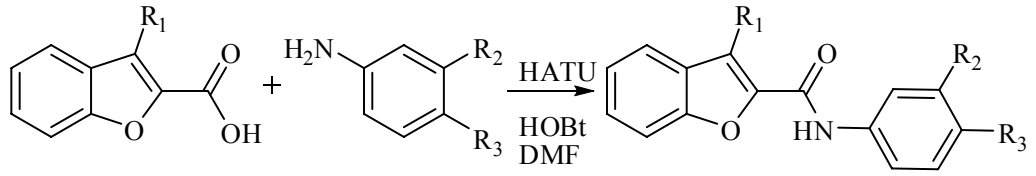
Kangasmesta J. and Liley M.[7] have synthesized various benzofuran derivatives as shown in scheme 2.

Scheme 2:



Bentley J. M. et al[9] have synthesized various benzofuran amide derivatives as shown in scheme 3.

Scheme 3:



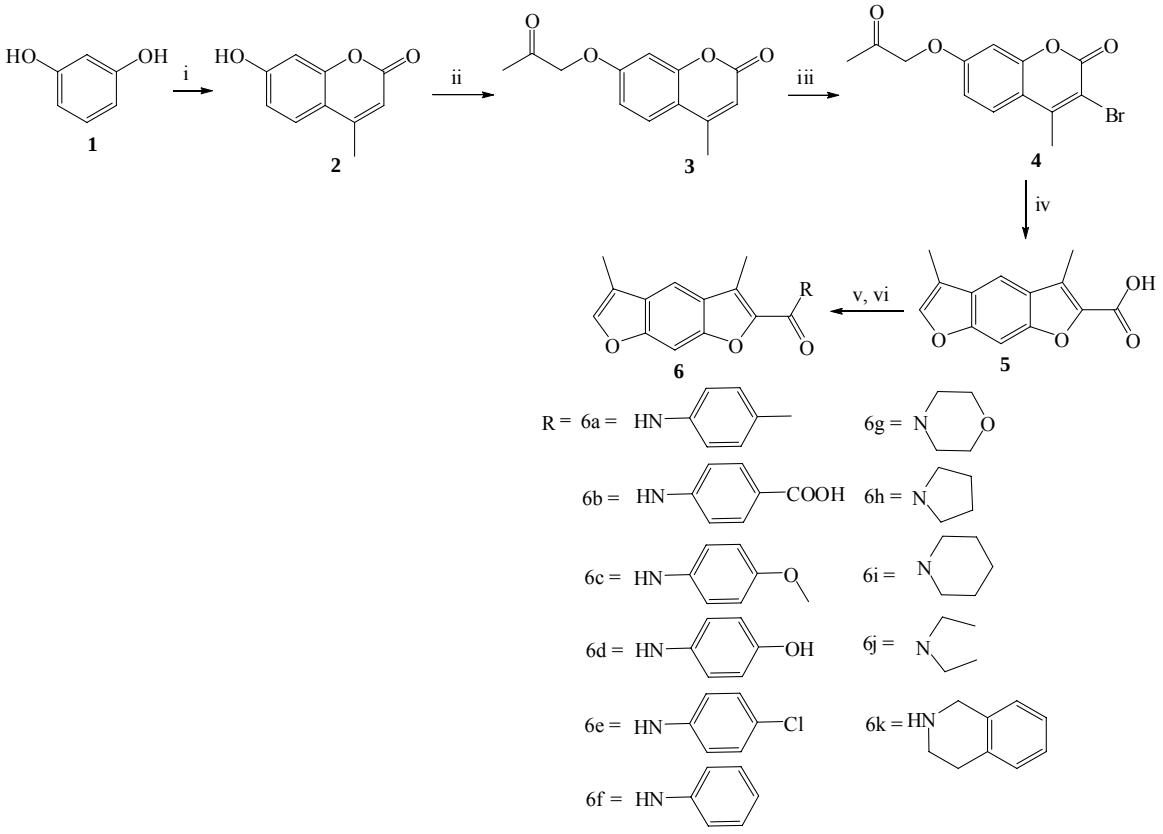
3.2 Result and discussion

3.2.1 Chemistry

Pechmann reaction of resorcinol with ethyl acetoacetate in presence of sulphuric acid gave 7-hydroxy-4-methyl coumarin[18] **2** which was condensed[17] with chloroacetone in presence of anhydrous K_2CO_3 and dry acetone gave 4-methyl-7-(2-oxopropoxy)-coumarin **3**. Bromination[19] of **3** using N-bromosuccinimide in chloroform gave unexpected product 3-bromo-4-methyl-7-(2-oxopropoxy)coumarin **4** (Scheme 4). Cyclization of **4** in 10% alkaline ethanol[17] gave 3, 5-dimethylbenzo[1,2-b:5,4-b']difuran-

2-carboxylic acid **5** as major product. Acid **5** was converted into acid chloride using oxalyl chloride and DMF in dry dichloromethane which on substitution reaction with different amines in dry dichloromethane in presence of base, triethylamine gave corresponding amides as shown in Scheme 4.

Scheme 4:



Reagents and conditions: (i) Ethyl acetoacetate, H_2SO_4, 12 h; (ii) Chloroacetone, K_2CO_3, Dry acetone, 6h; (iii) NBS, Chloroform, 6h; (iv) 10% KOH in ethanol, 3h; (v) Dichloromethane, DMF, Oxalyl chloride, 4h; (vi) Dichloromethane, triethylamine, Various amines, 12h

The formation of compound **2** was confirmed by its melting point. The IR spectrum of compound **3** (Figure 1) exhibited bands at 1736 and 1710 cm^{-1} indicated presence of lactone and ketone carbonyl group respectively. In ^1H NMR of compound **3** in $CDCl_3$ (Figure 2) singlet at δ 2.32 and singlet at δ 2.42 for three protons each confirmed the presence of two methyl groups at C-4 position and $COCH_3$ group respectively. Singlet at δ 4.66 for two protons indicated presence of methylene group of

OCH_2CO and singlet at δ 6.17 for one proton indicated proton at C-3 position. All aromatic protons observed between δ 6.76 to 7.55 confirmed the formation of compound **3**. The ^{13}C NMR spectrum of compound **3** in $CDCl_3$ (Figure 3) showed peak at δ 203 indicated lactone carbon. The presence of 13 peaks are in accordance with structure of compound **3**. Figure 4 indicates mass spectrum of compound **3** which also confirmed the structure of compound **3** which showed *m/z* value at 177.0 $[M-55]^+$ in ESI/MS indicated $M-OCH_2CH_3$ fragment.

After bromination, the IR spectrum of compound **4** (Figure 5) exhibited bands at 1710 and 1681 cm^{-1} indicated presence of lactone and ketone carbonyl group respectively. In ^1H NMR of compound **4** in $CDCl_3$ (Figure 6) two doublets at δ 2.33 and 2.62 for three protons each confirmed the presence of two methyl groups. Singlet at δ 4.67 for two protons indicated presence of methylene group of OCH_2CO. The disappearance of peak at δ 6.17 indicated the presence of Br at C-3. All aromatic protons observed between δ 6.79 to 7.62 confirmed the formation of compound **4**. The ^{13}C NMR spectrum of compound **4** in DMSO-d_6 (Figure 7) showed presence of 13 peaks which is in accordance with structure of compound **4** which was further confirmed by its mass spectrum (Figure 8) which showed M^+ and $[M+2]^+$ peaks at *m/z* value at 311 and 312.9 of equal intensity in ESI/MS.

The IR spectrum of compound **5** (Figure 9) exhibited bands at 3428 and 1680 cm^{-1} indicated presence of carboxylic acid group which was further confirmed by solubility of compound **5** in saturated $NaHCO_3$ solution and reprecipitation of it by HCl. In ^1H NMR spectrum of compound **5** in DMSO-d_6 (Figure 10), the singlet observed at δ 2.27 for three protons and singlet at δ 2.60 for three protons each indicated presence of two methyl groups at C-5 and C-3 position respectively. The singlet at δ 7.82 for one proton indicated proton at C-6 and two singlets at δ 7.84 and 7.92 for one proton each confirmed the linear

ring fusion. In ^{13}C NMR spectrum of compound **5** in DMSO-d_6 (Figure 11) two methyl carbons observed at δ 8.1 and 9.7, the carboxylic acid carbon observed at δ 161.5 and total presence of 13 peaks is in accordance with structure of compound **5**. The mass spectrum of compound **5** (Figure 12) showed *m/z* value at 231.0 for $[M+1]^+$ peak in ESI/MS confirmed the formation of compound **5**.

Various amide derivatives of difuran carboxylic acid **5** were prepared by converting acid **5** in to corresponding acid chloride by using oxalyl chloride and then substituting it with various amines in presence of triethylamine. The amides **6** now insoluble in $NaHCO_3$ indicated the formation of **6** which was further confirmed by IR, ^1H NMR, ^{13}C NMR and mass.

The IR spectrum of compound **6a** (Figure 13) exhibited band at 3421 cm^{-1} indicated presence of NH group and band at 1670 cm^{-1} indicated presence of carbonyl group of amide. The ^1H NMR of compound **6a** in $CDCl_3$ (Figure 14) showed peak at δ 2.34, 2.37 and 2.77 for three protons each confirmed the presence of three methyl protons. All aromatic protons observed between δ 7.22 to 7.68 and peak at δ 8.34 for one NH proton confirmed formation of compound **6a**. The ^{13}C NMR spectrum of compound **6a** in $CDCl_3$ (Figure 15) now showed three peaks at 8.0, 9.2 and 20.9 indicated presence of three methyl carbons and presence of 15 peaks indicated remaining aromatic carbons and carbonyl carbon thus confirmed the structure of compound **6a**.The mass spectrum of compound **6a** (Figure 16) showed *m/z* value at 320.0 for $[M+1]^+$ peak in ESI/MS confirmed formation of compound **6a**.

The IR spectrum of compound **6c** (Figure 17) exhibited band at 3419 cm^{-1} indicated presence of NH group and band at 1671 cm^{-1} indicated presence of carbonyl group of amide. The ^1H NMR of compound **6c** in $CDCl_3$ (Figure 18) showed two singlets at δ 2.33 and 2.76 for three protons each confirmed presence of two methyl groups,

singlet at δ 3.84 for three protons confirmed the presence of methoxy group. All aromatic protons observed between δ 6.93 to 7.67 and peak δ 8.30 for one NH proton confirmed the formation of compound **6c**. The ^{13}C NMR spectrum of compound **6c** in $CDCl_3$ (Figure 19) showed presence of 18 peaks for different carbons confirmed the structure of **6c**. The mass spectrum of compound **6c** (Figure 20) showed *m/z* value at 336.0 for $[M+1]^+$ peak in ESI/MS further confirmed formation of compound **6c**.

The IR spectrum of compound **6g** (Figure 21) exhibited band at 1623 cm^{-1} confirmed presence of carbonyl group of amide. The ^1H NMR of compound **6g** in $CDCl_3$ (Figure 22) showed two singlets at δ 2.32 and 2.56 for three protons each confirmed presence of two methyl groups. Peak at δ 3.83 for eight protons confirmed presence of methylene protons of morpholine. All aromatic protons observed between δ 7.46 to 7.63 confirmed the formation of compound **6g**. The ^{13}C NMR spectrum of compound **6g** in $CDCl_3$ (Figure 23) also supported the structure of compound **6g**. The mass spectrum of compound **6g** (Figure 24) showed *m/z* value at 300.0 for $[M+1]^+$ peak in ESI/MS confirmed formation of compound **6g**.

The IR spectrum of compound **6h** (Figure 25) exhibited band at 1617 cm^{-1} confirmed presence of carbonyl group of amide. The ^1H NMR of compound **6h** in $CDCl_3$ (Figure 26) showed singlet at δ 1.99 for four protons indicated two methylene groups of pyrrolidine, two peaks at δ 2.32 and 2.66 for three protons each confirmed presence of two methyl groups. Triplet at δ 3.84 to 3.87 for four protons indicated two methylene groups of pyrrolidine and all aromatic protons observed between δ 7.45 to 7.64 for three protons confirmed the formation of compound **6h**. The ^{13}C NMR spectrum of compound **6h** in $CDCl_3$ (Figure 27) showed presence of 15 peaks which is in accordance with structure thus support the structure of compound **6h**. The mass spectrum of compound **6h**

(Figure 28) showed *m/z* value at 284.0 for $[M+1]^+$ peak in ESI/MS confirmed formation of compound **6h**.

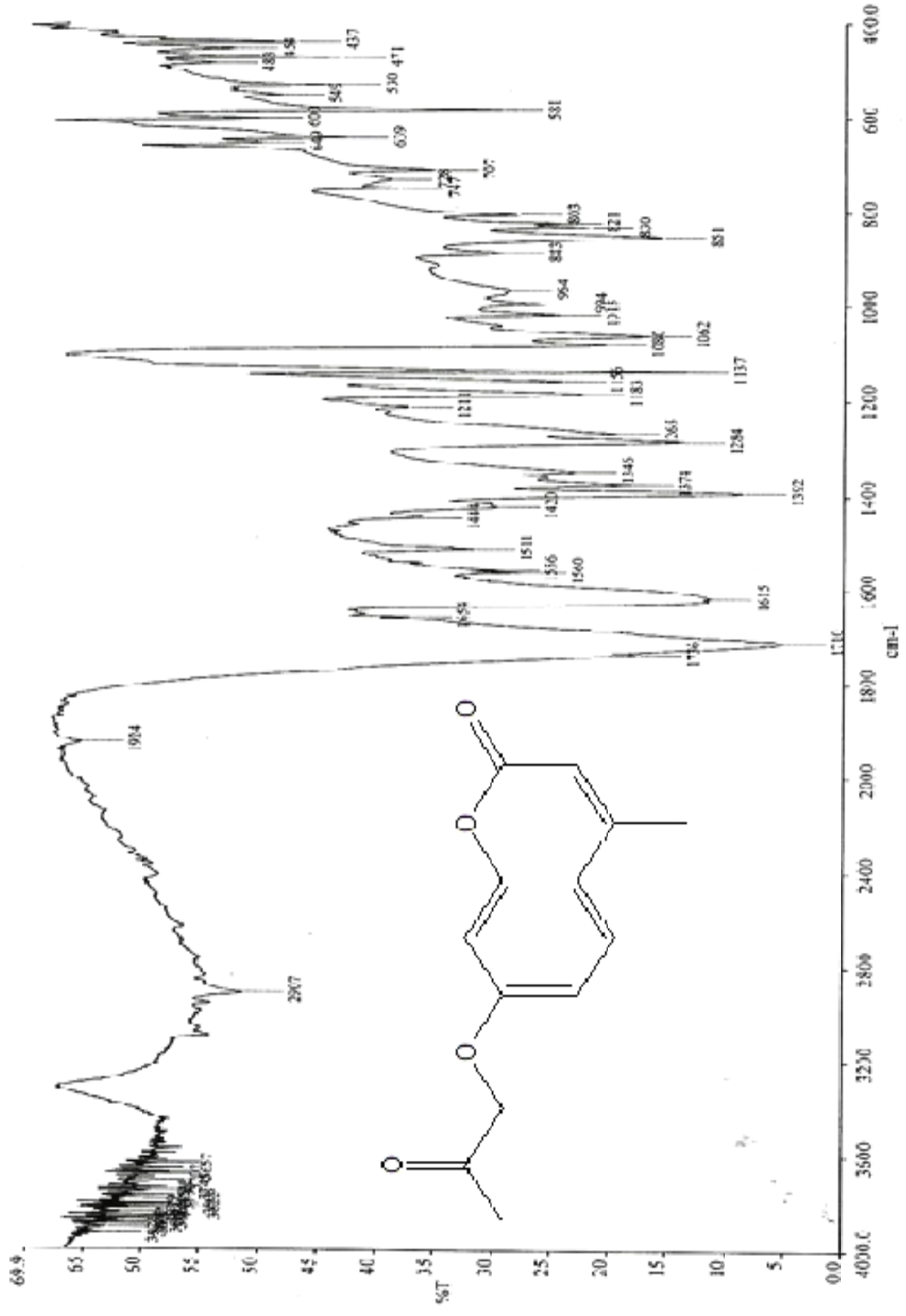


Figure 1: IR of 4-methyl-7-(2-oxopropoxy)-2H-chromen-2-one **3**

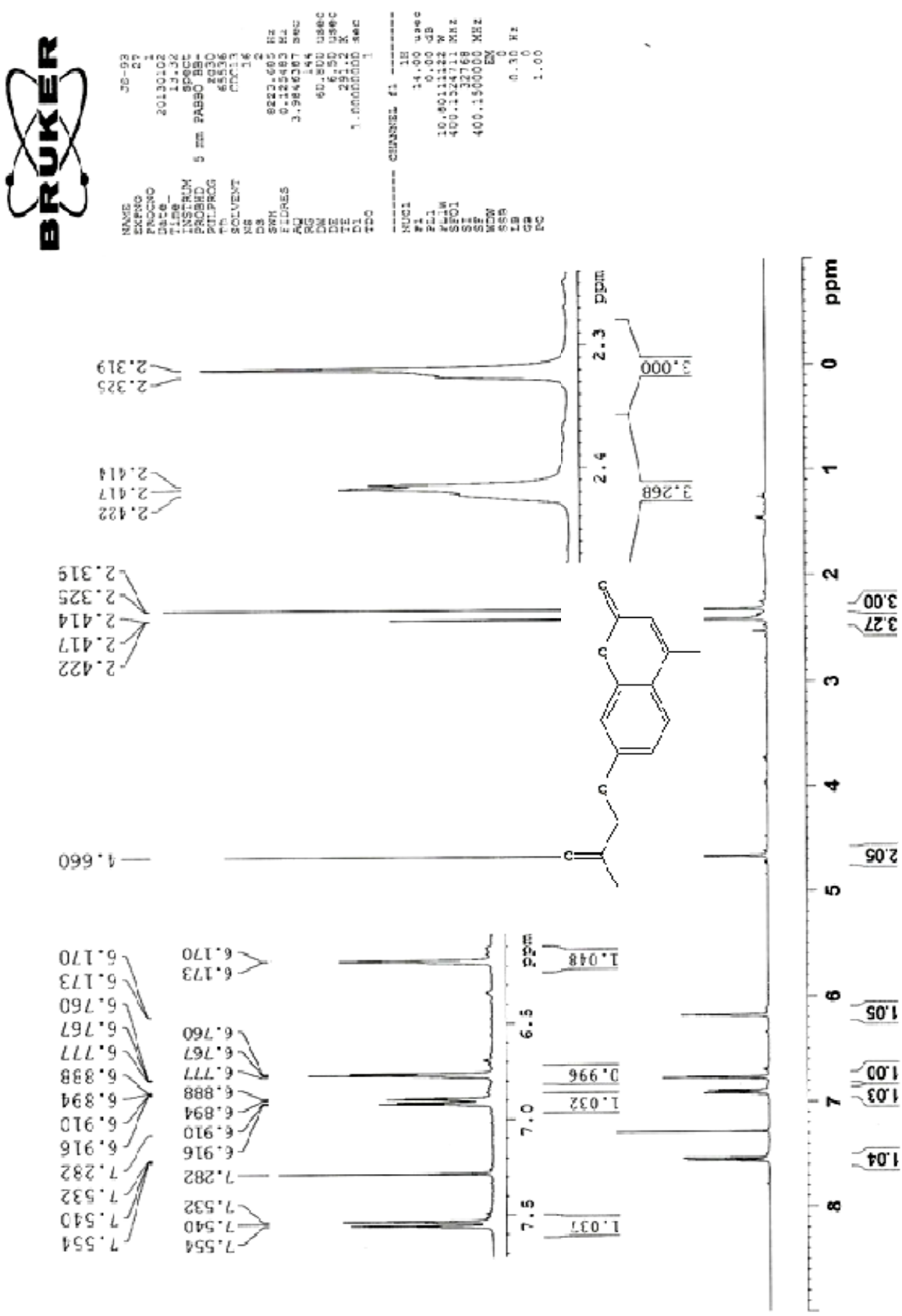


Figure 2: ^{1}H NMR of 4-methyl-7-(2-oxopropoxy)-2H-chromen-2-one **3**

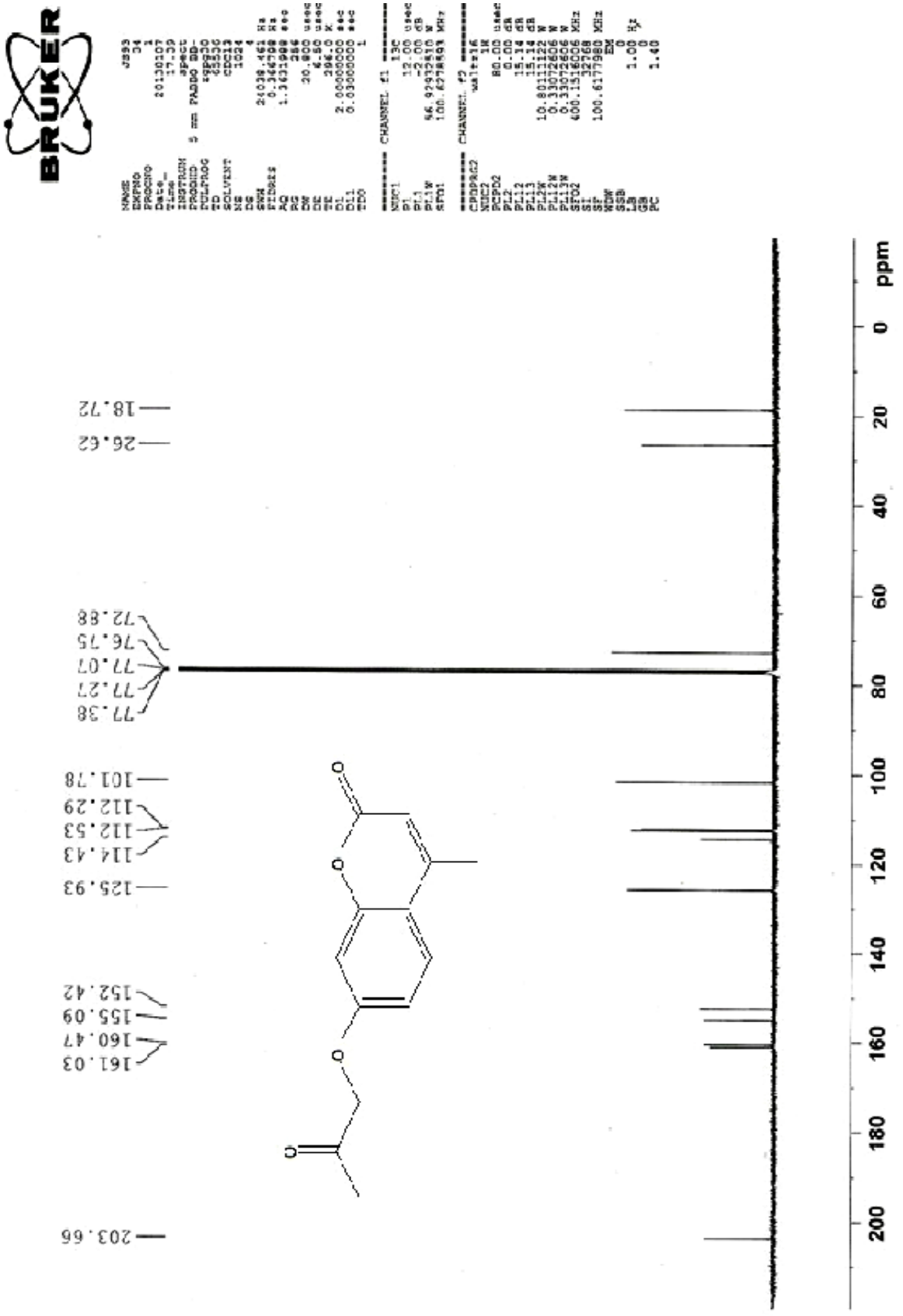


Figure 3: ^{13}C NMR of 4-methyl-7-(2-oxopropoxy)-2H-chromen-2-one **3**

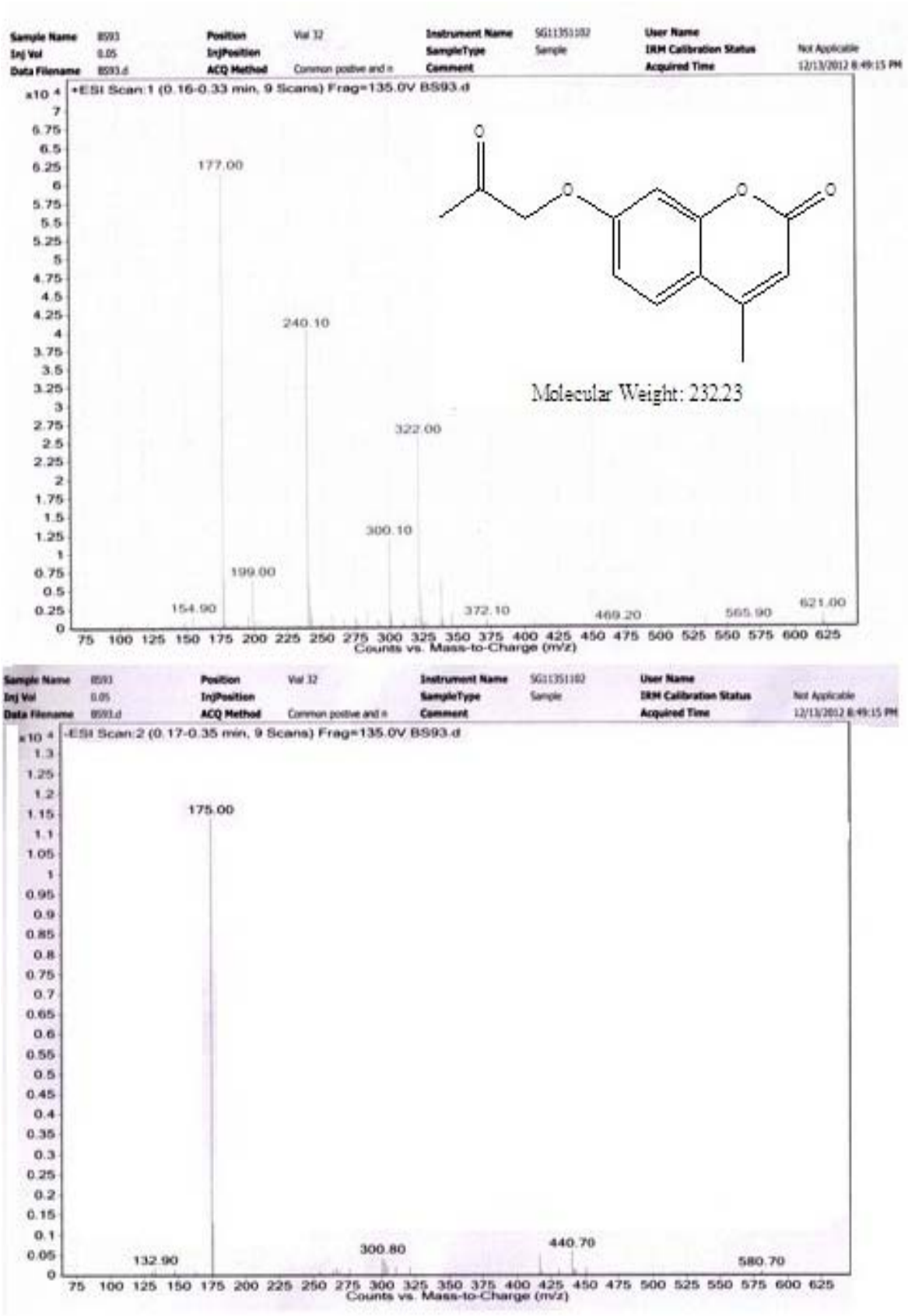


Figure 4: ESI/MS of 4-methyl-7-(2-oxopropoxy)-2H-chromen-2-one **3**

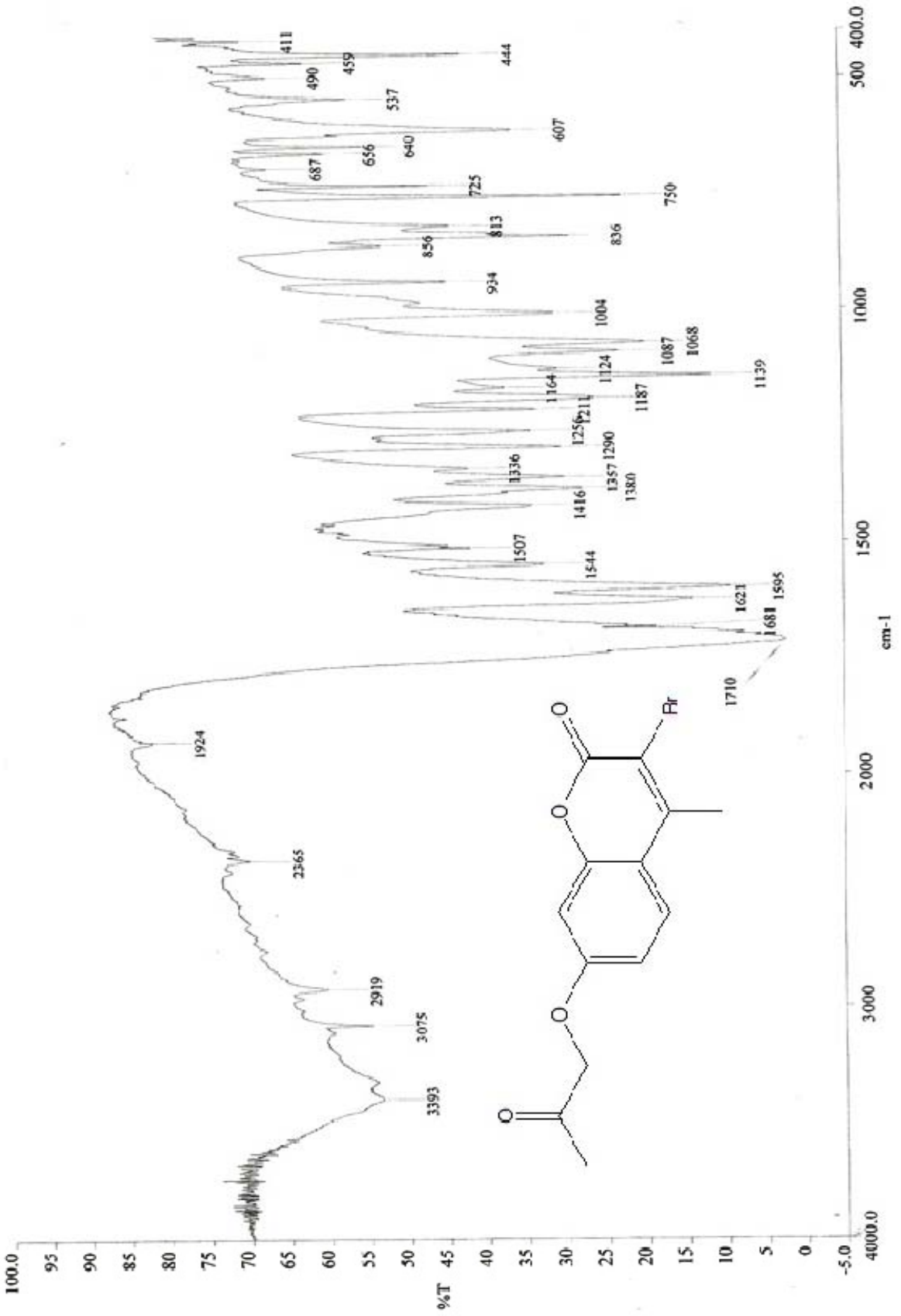


Figure 5: IR of 3-bromo-4-methyl-7-(2-oxopropoxy)-2H-chromen-2-one **4**

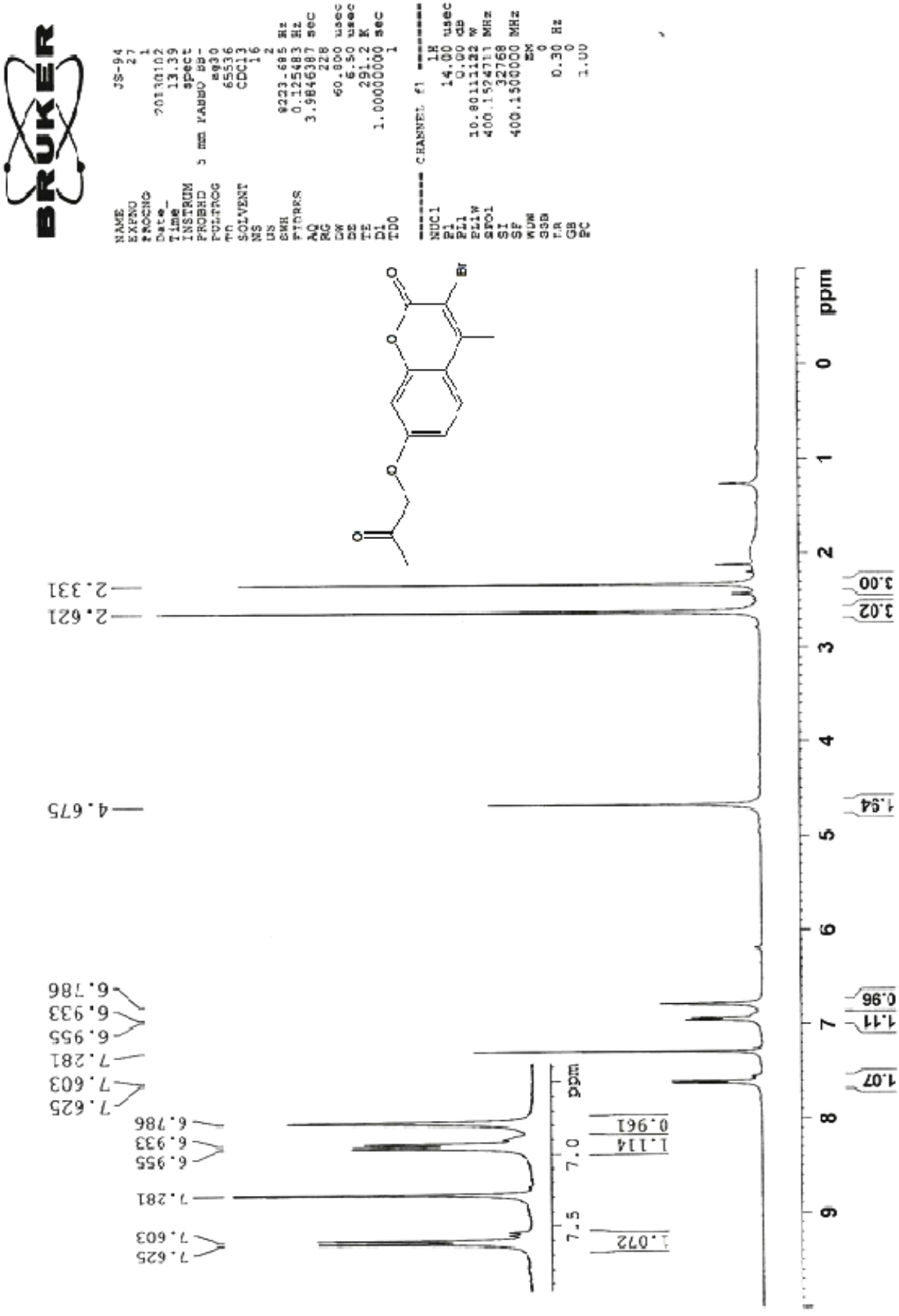


Figure 6: [1]H NMR of 3-bromo-4-methyl-7-(2-oxopropoxy)-2H-chromen-2-one **4**

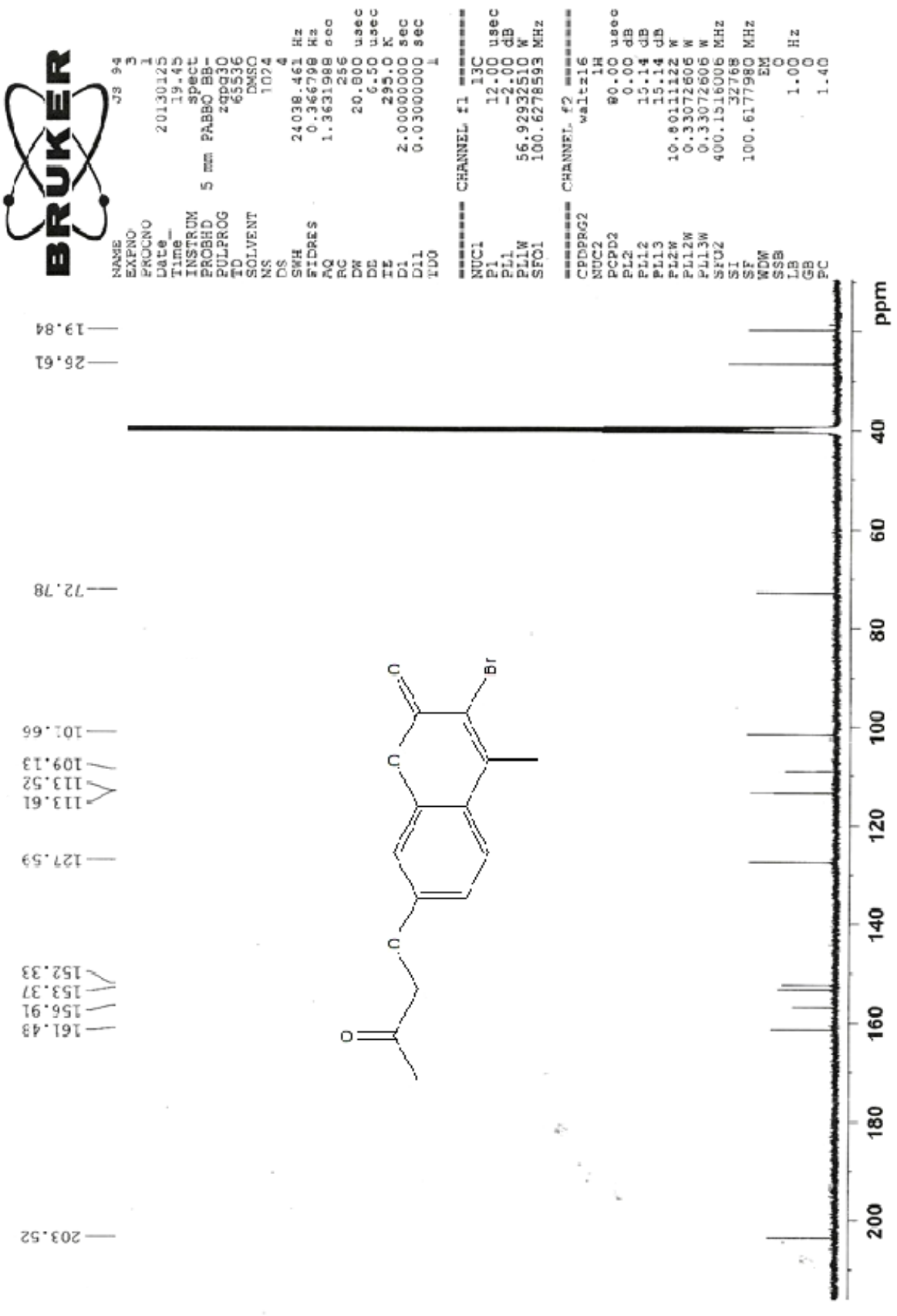


Figure 7: ^{13}C NMR of 3-bromo-4-methyl-7-(2-oxopropoxy)-2H-chromen-2-one **4**

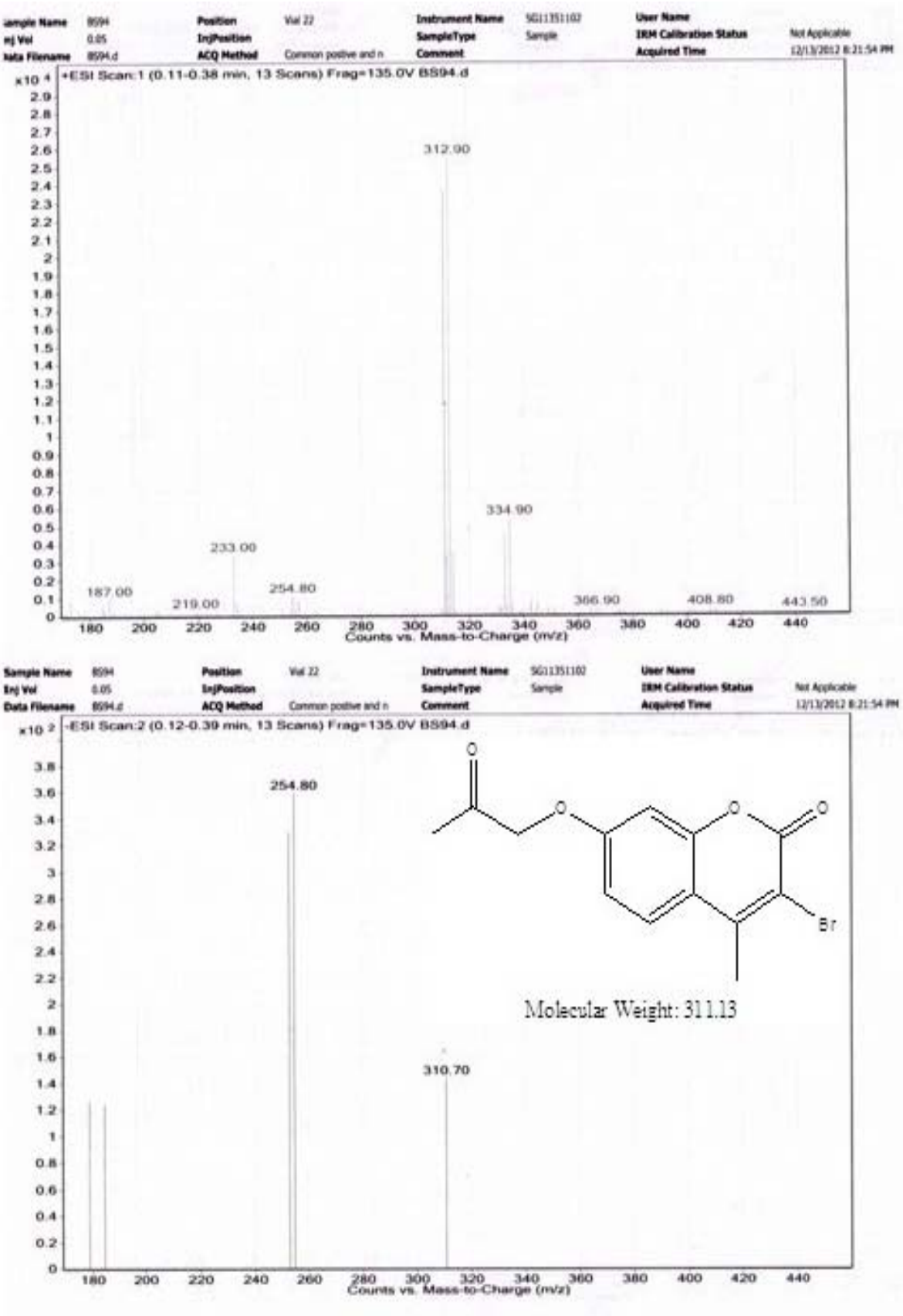


Figure 8: ESI/MS of 3-bromo-4-methyl-7-(2-oxopropoxy)-2H-chromen-2-one **4**

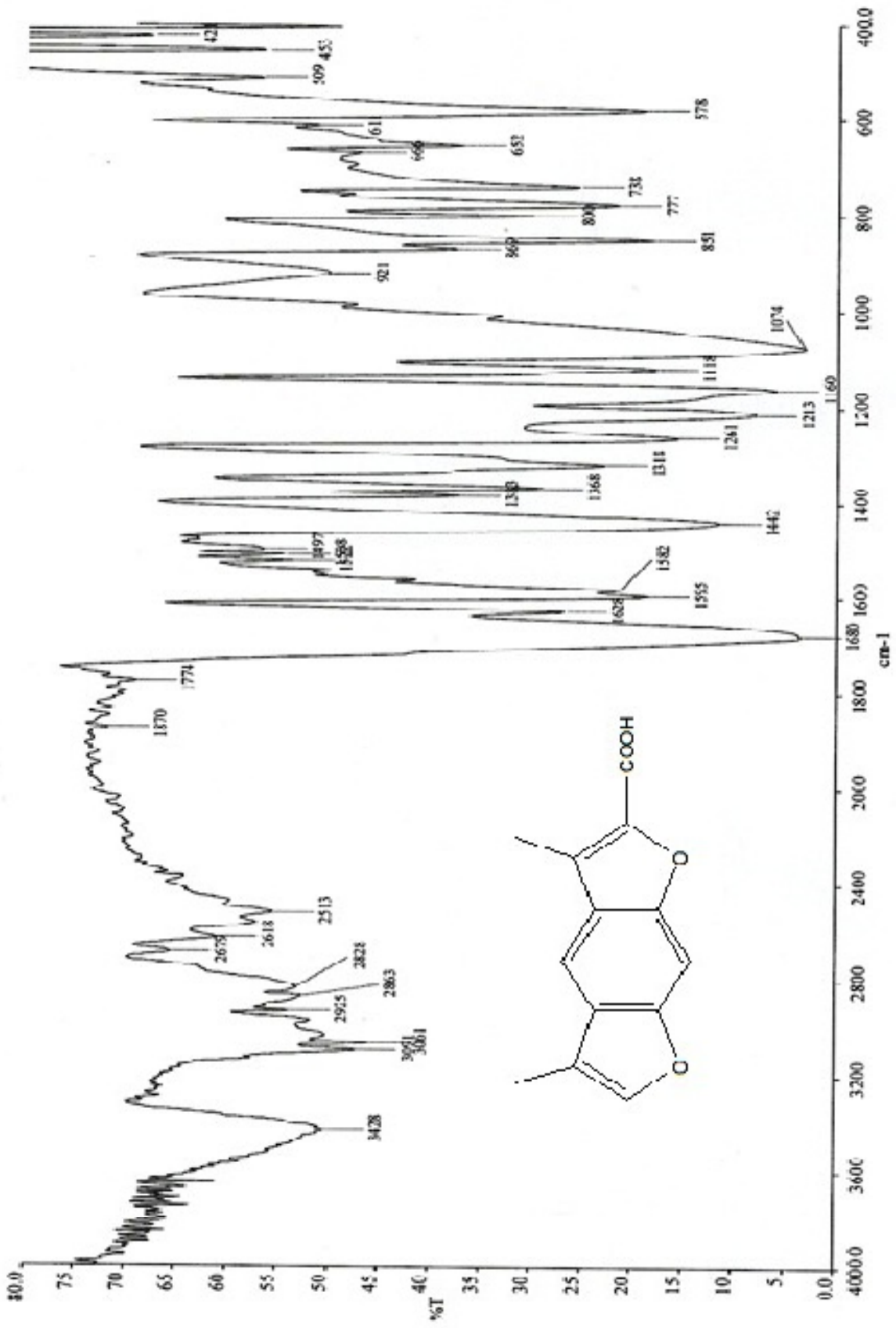


Figure 9: IR of 3, 5-dimethylbenzo[1,2-b:5,4-b']difuran-2-carboxylic acid **5**

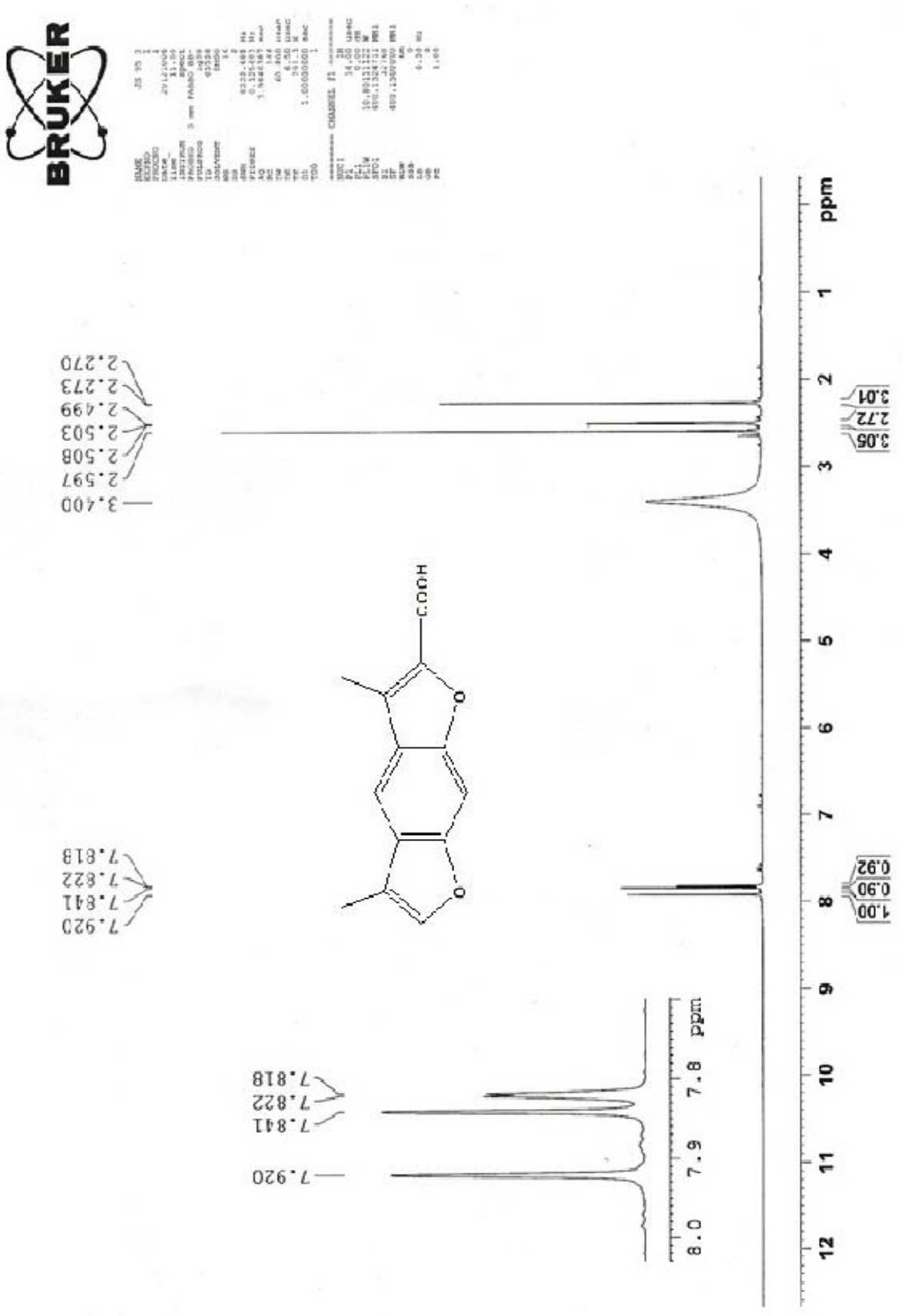


Figure 10: ^{1}H NMR of 3, 5-dimethylbenzo[1,2-b:5,4-b']difuran-2-carboxylic acid **5**

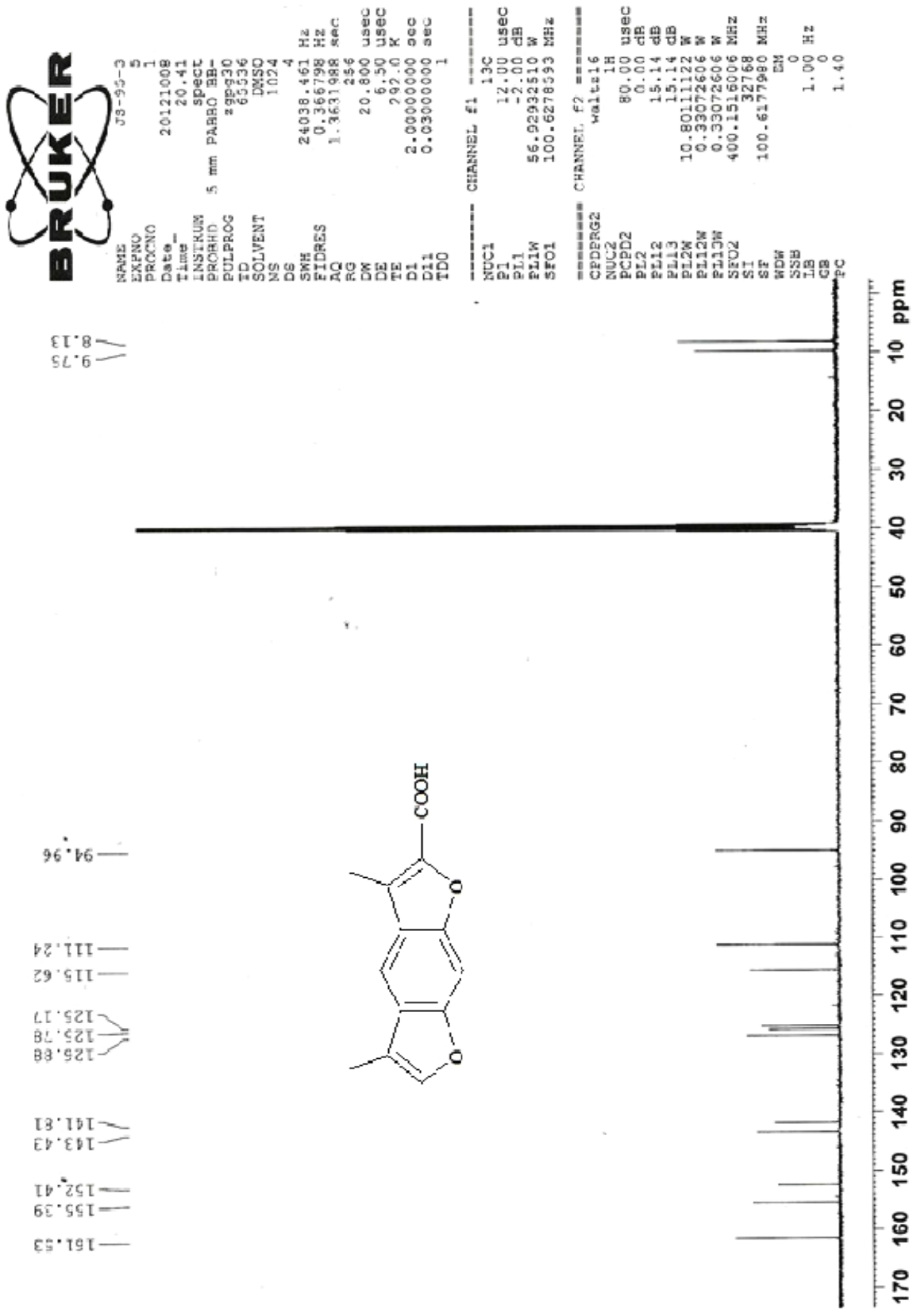


Figure 11: ^{13}C NMR of 3, 5-dimethylbenzo[1,2-b:5,4-b']difuran-2-carboxylic acid **5**

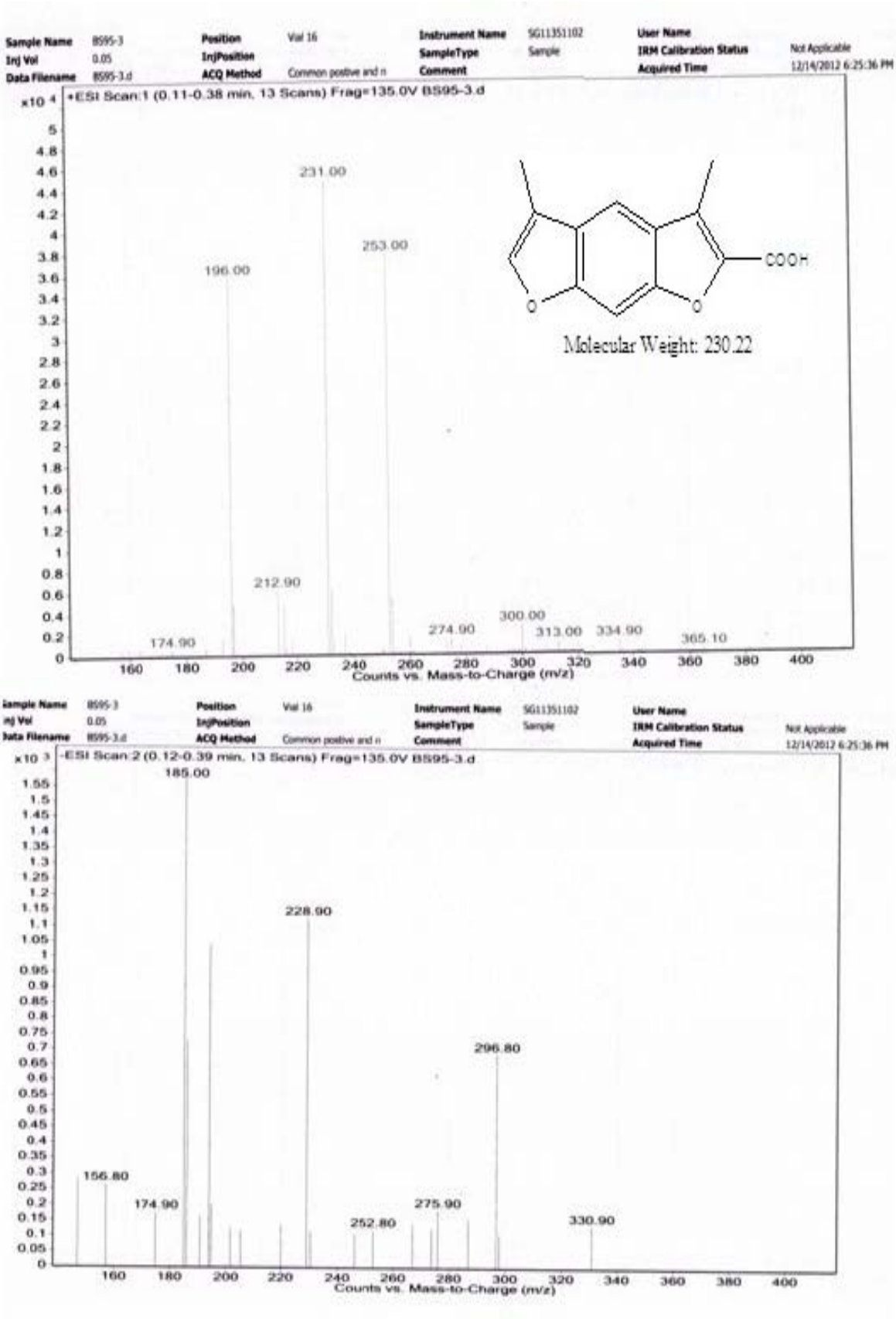


Figure 12: ESI/MS of 3, 5-dimethylbenzo[1,2-b:5,4-b']difuran-2-carboxylic acid **5**

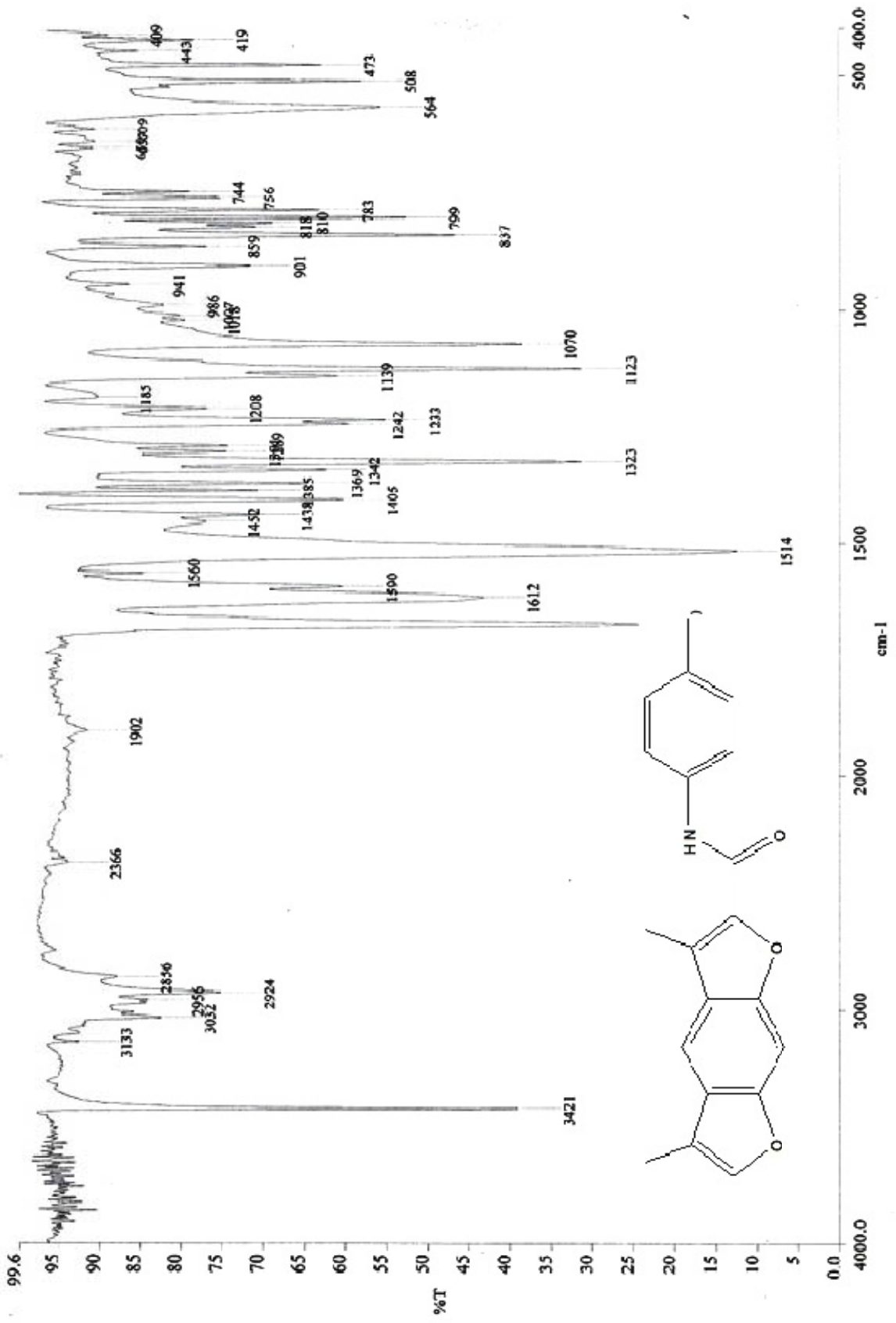


Figure 13: IR of 3, 5-dimethyl-N-(p-tolyl)benzo[1,2-b:5,4-b']difuran-2-carboxamide **6a**

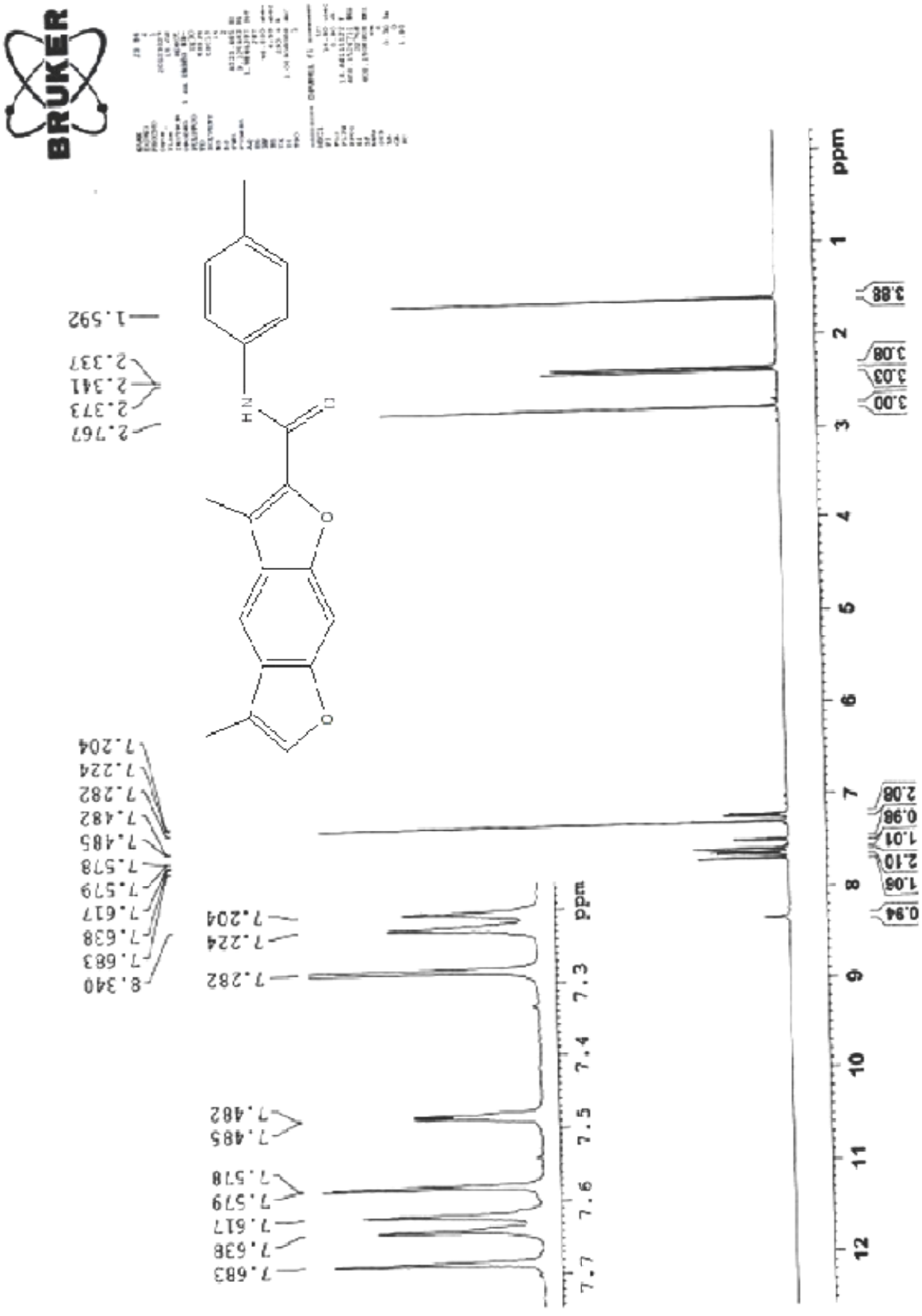


Figure 14: ^{1}H NMR of 3, 5-dimethyl-N-(p-tolyl)benzo[1,2-b:5,4-b']difuran-2-carboxamide **6a**

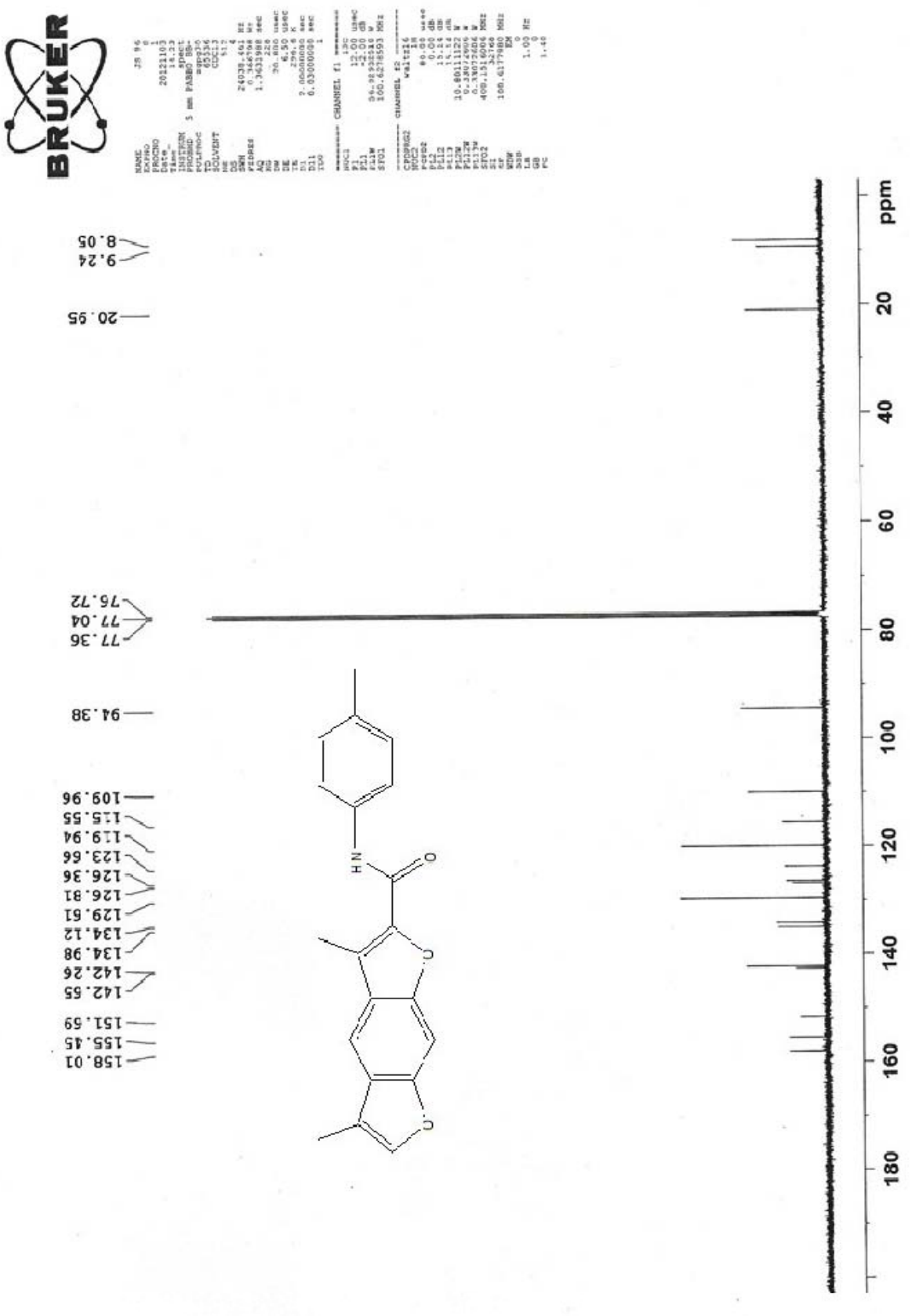


Figure 15: ^{13}C NMR of 3, 5-dimethyl-N-(p-tolyl)benzo[1,2-b:5,4-b']difuran-2-carboxamide **6a**

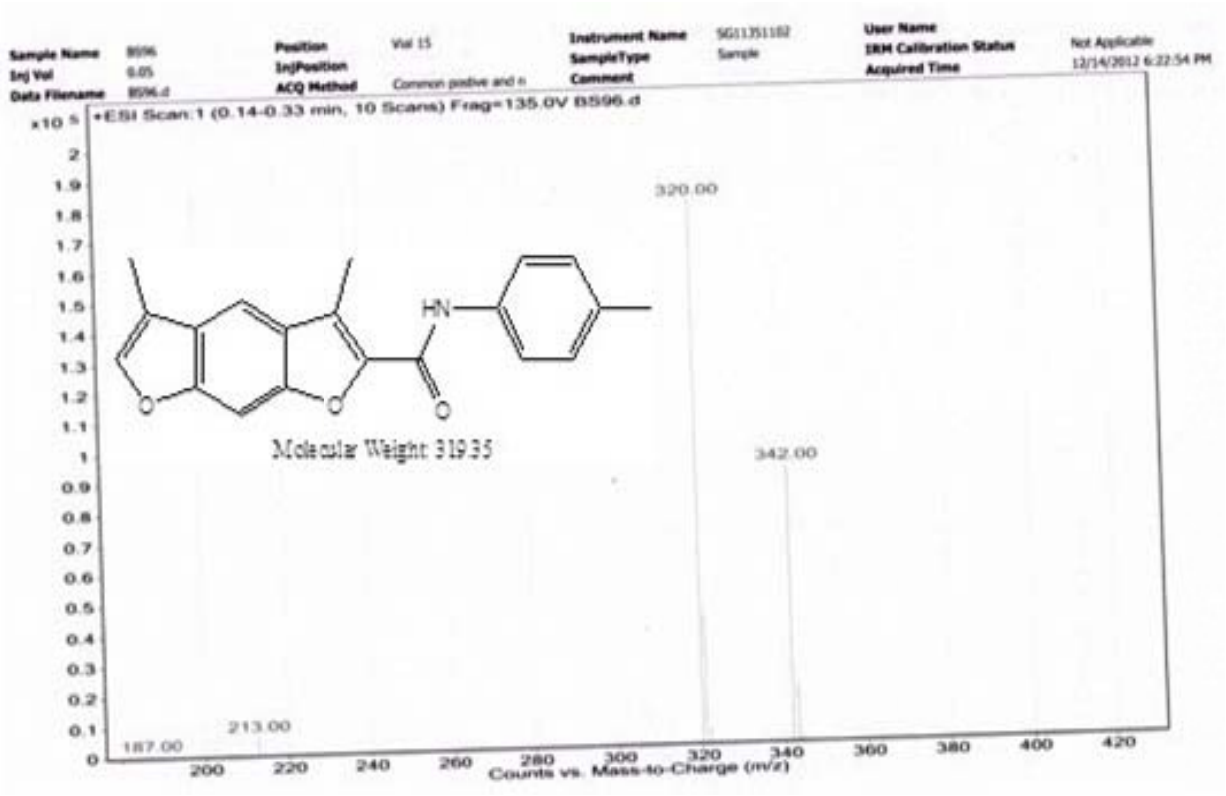


Figure 16: ESI/MS of 3, 5-dimethyl-N-(p-tolyl)benzo[1,2-b:5,4-b']difuran-2-carboxamide **6a**

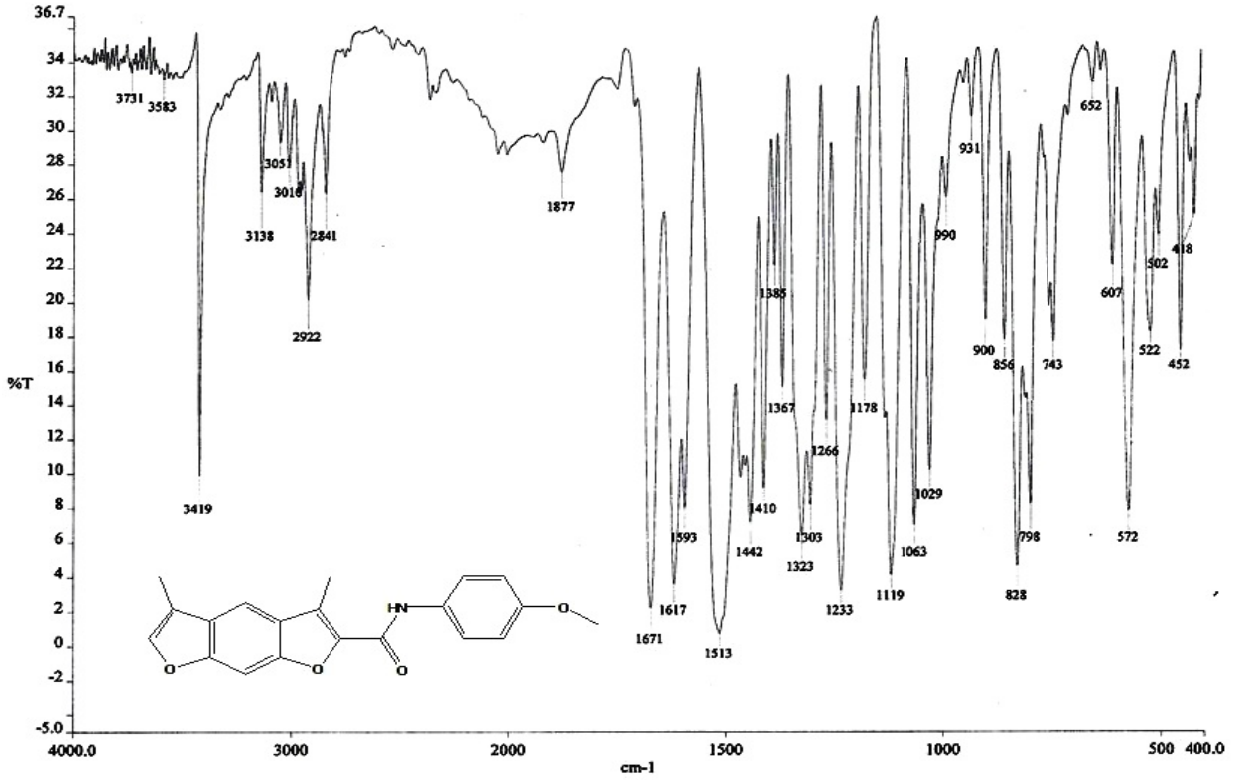


Figure 17: IR of N-(4-methoxyphenyl)-3,5-dimethylbenzo[1,2-b:5,4-b']difuran-2-carboxamide **6c**

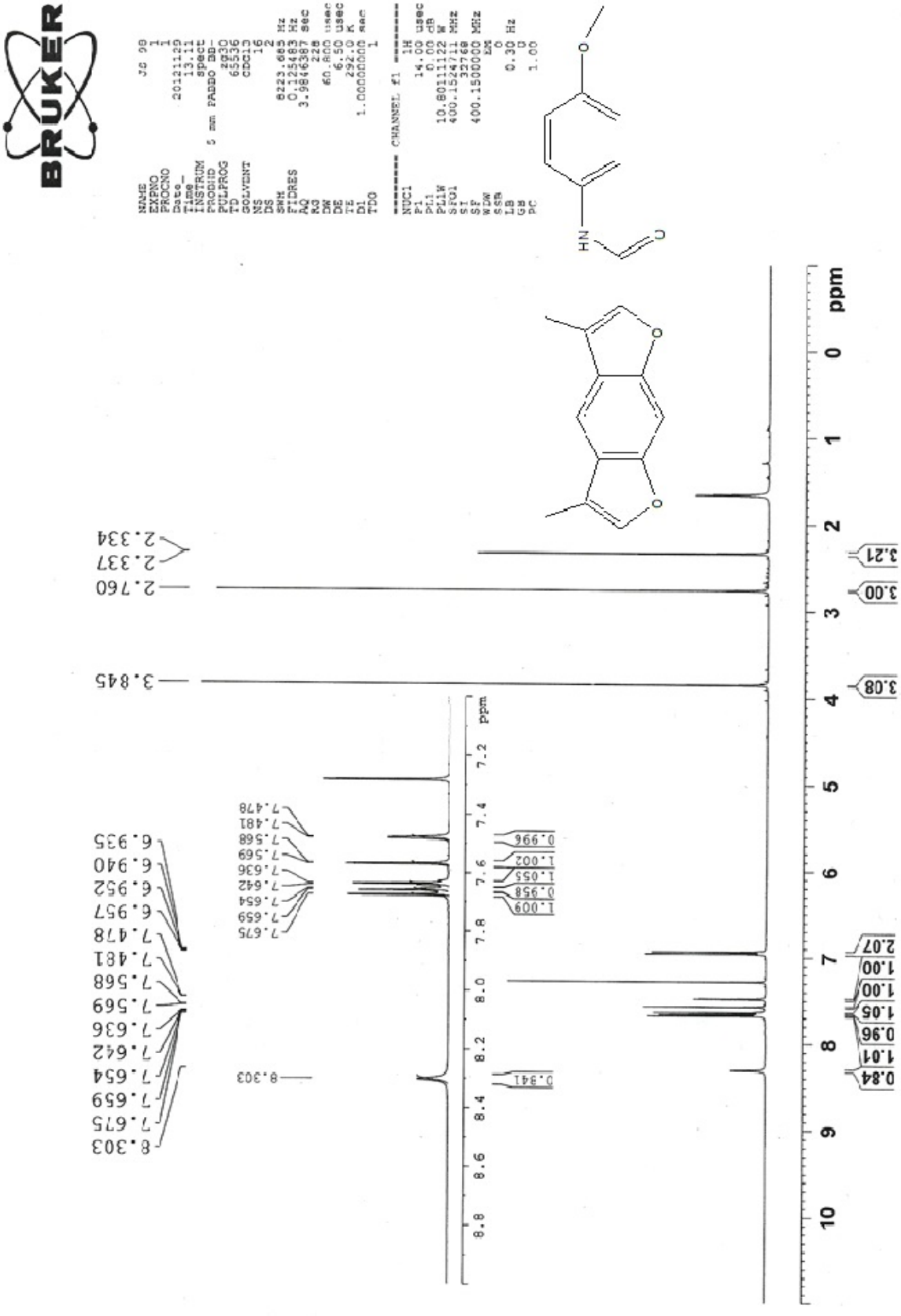


Figure 18: ^{1}H NMR of N-(4-methoxyphenyl)-3,5-dimethylbenzo[1,2-b:5,4-b']difuran-2-carboxamide **6c**

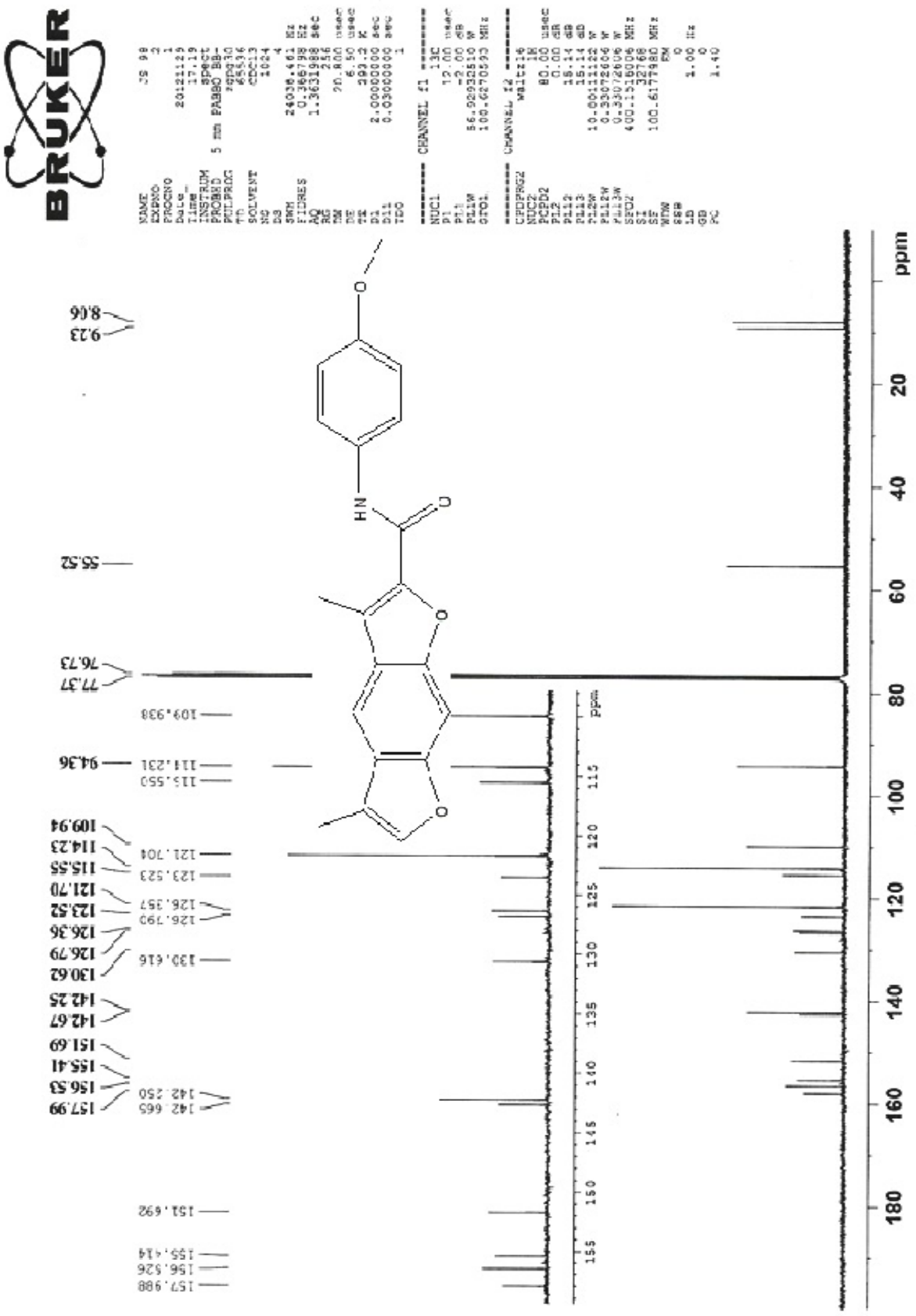


Figure 19: ^{13}C NMR of N-(4-methoxyphenyl)-3,5-dimethylbenzo[1,2-b:5,4-b']difuran-2-carboxamide **6c**

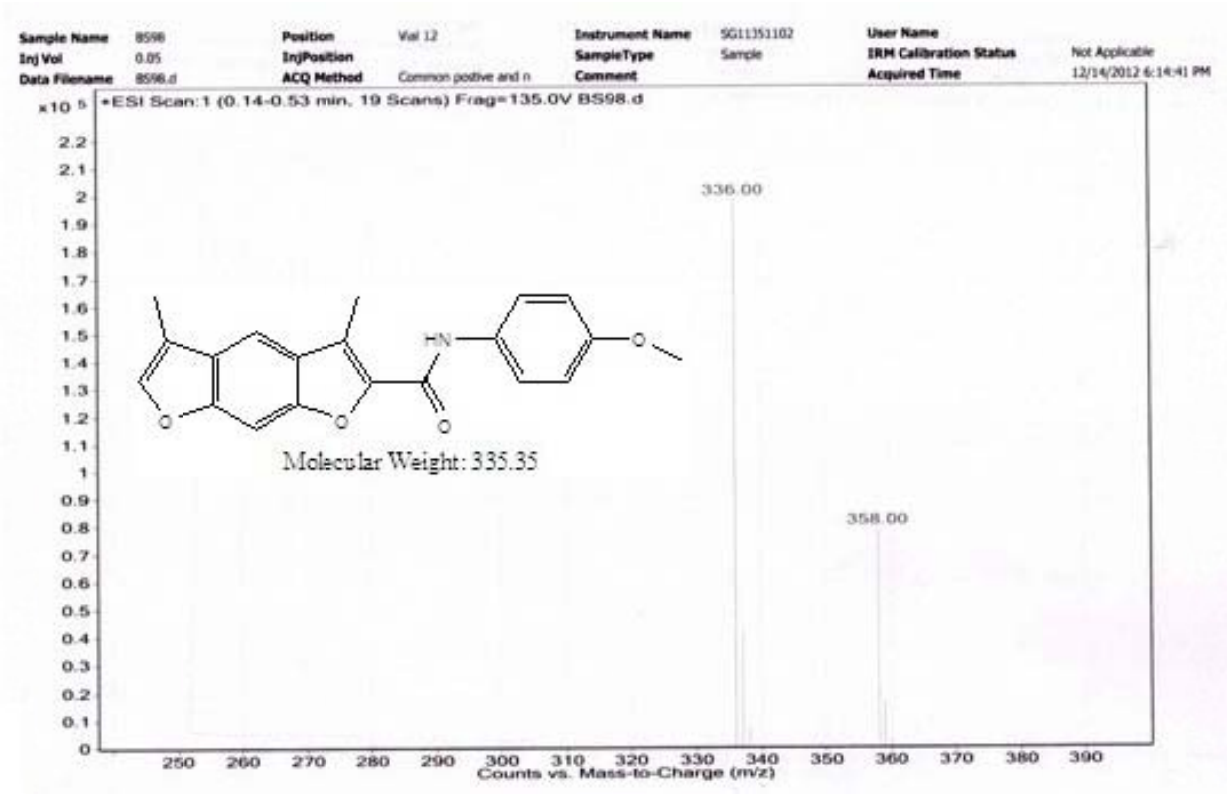


Figure 20: ESI/MS of N-(4-methoxyphenyl)-3,5-dimethylbenzo[1,2-b:5,4-b']difuran-2-carboxamide **6c**

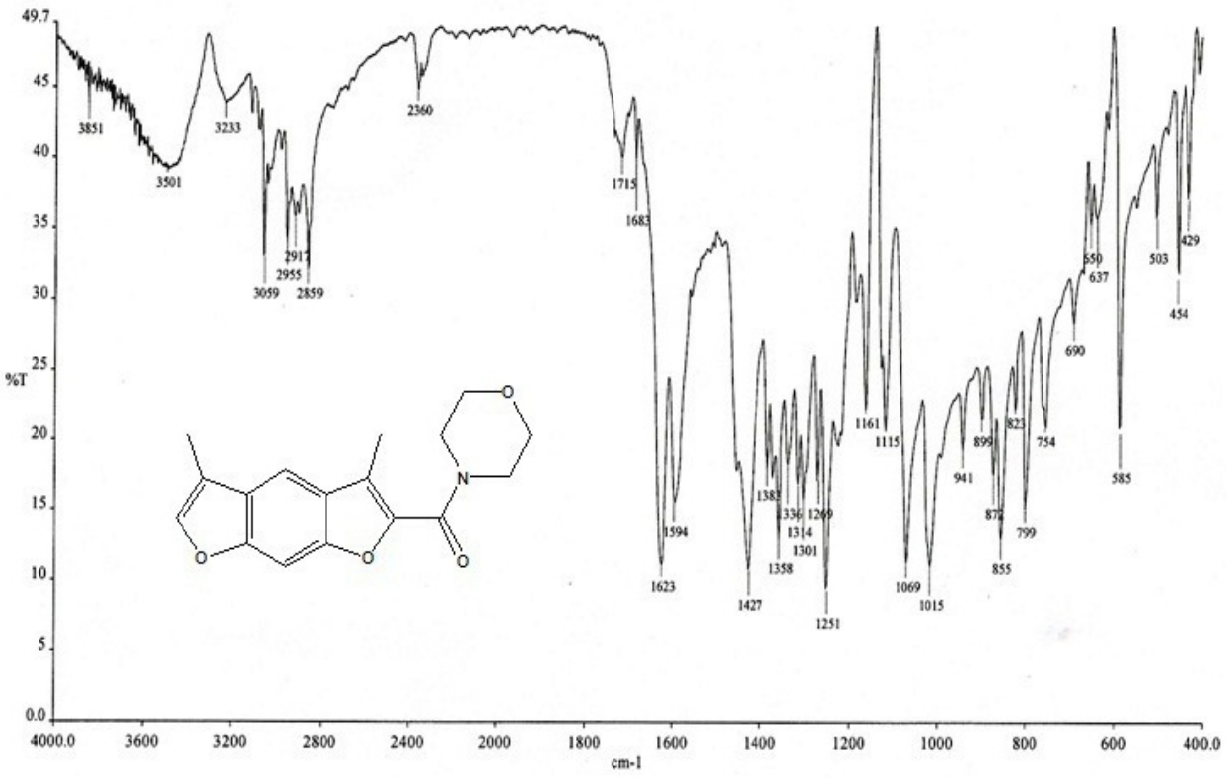


Figure 21: IR of (3, 5-dimethylbenzo[1,2-b:5,4-b']difuran-2-yl)(morpholino) methanone **6g**

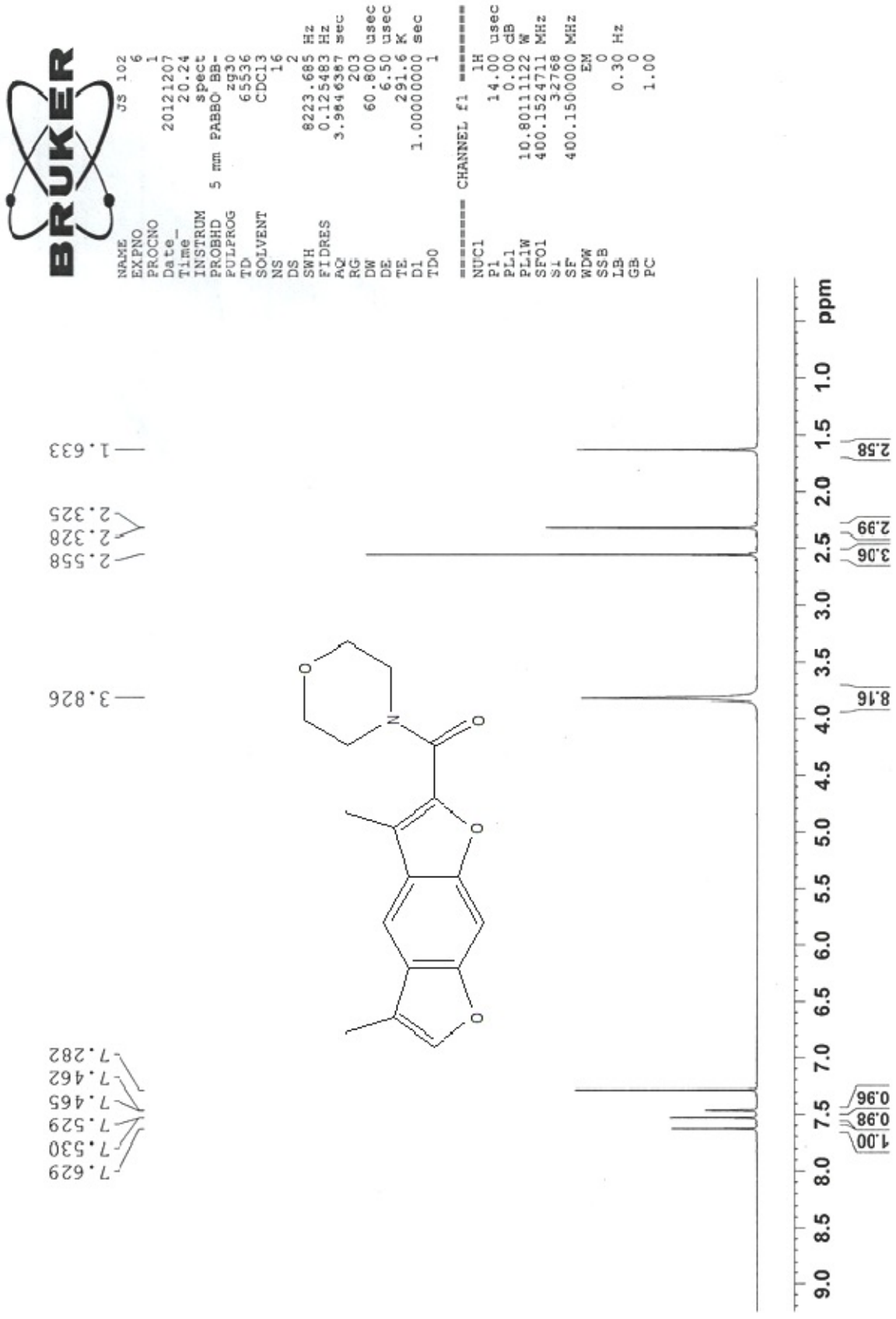


Figure 22: ^{1}H NMR of (3, 5-dimethylbenzo[1,2-b:5,4-b']difuran-2-yl)(morpholino) methanone **6g**

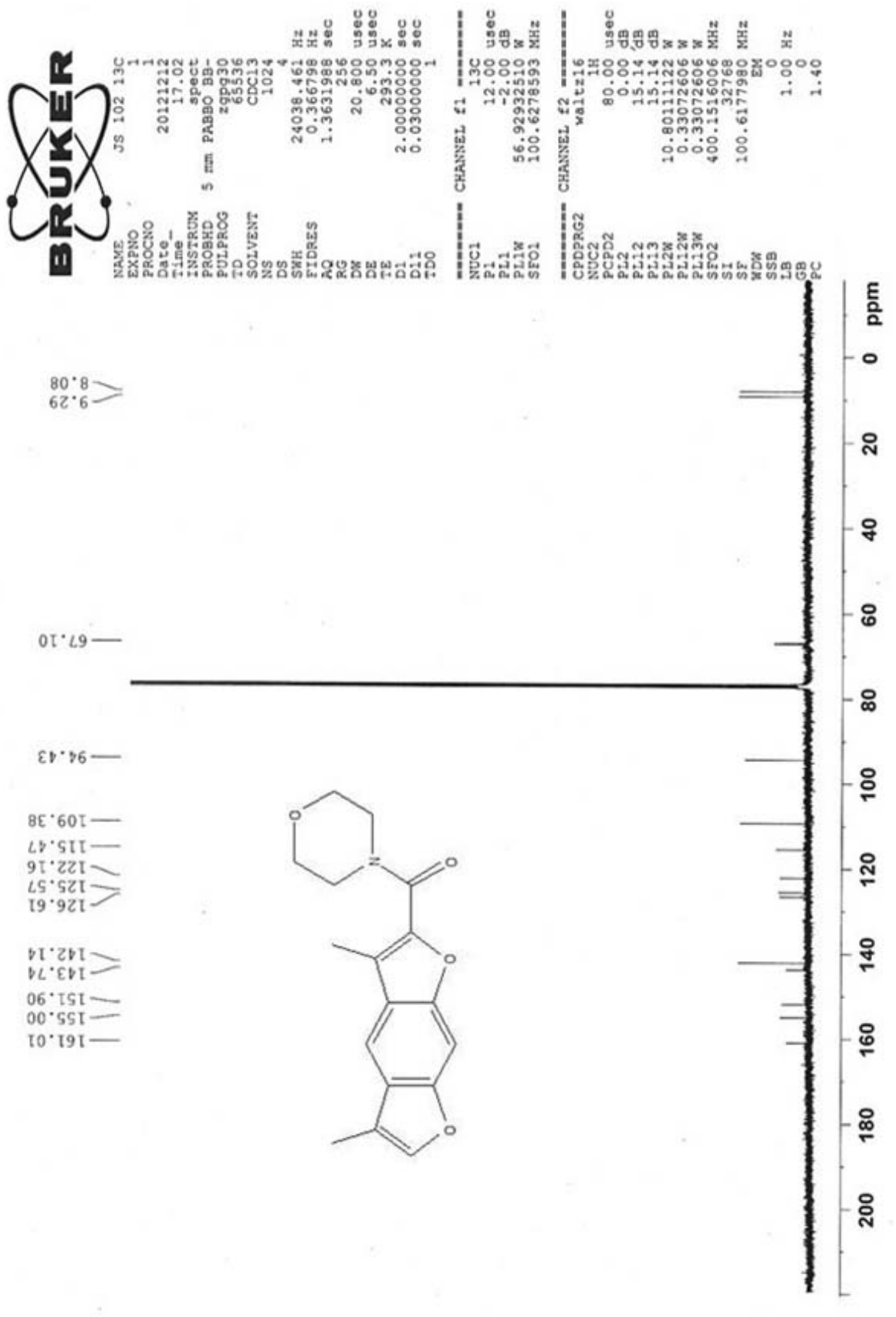


Figure 23: ^{13}C NMR of (3, 5-dimethylbenzo[1,2-b:5,4-b']difuran-2-yl)(morpholino) methanone **6g**

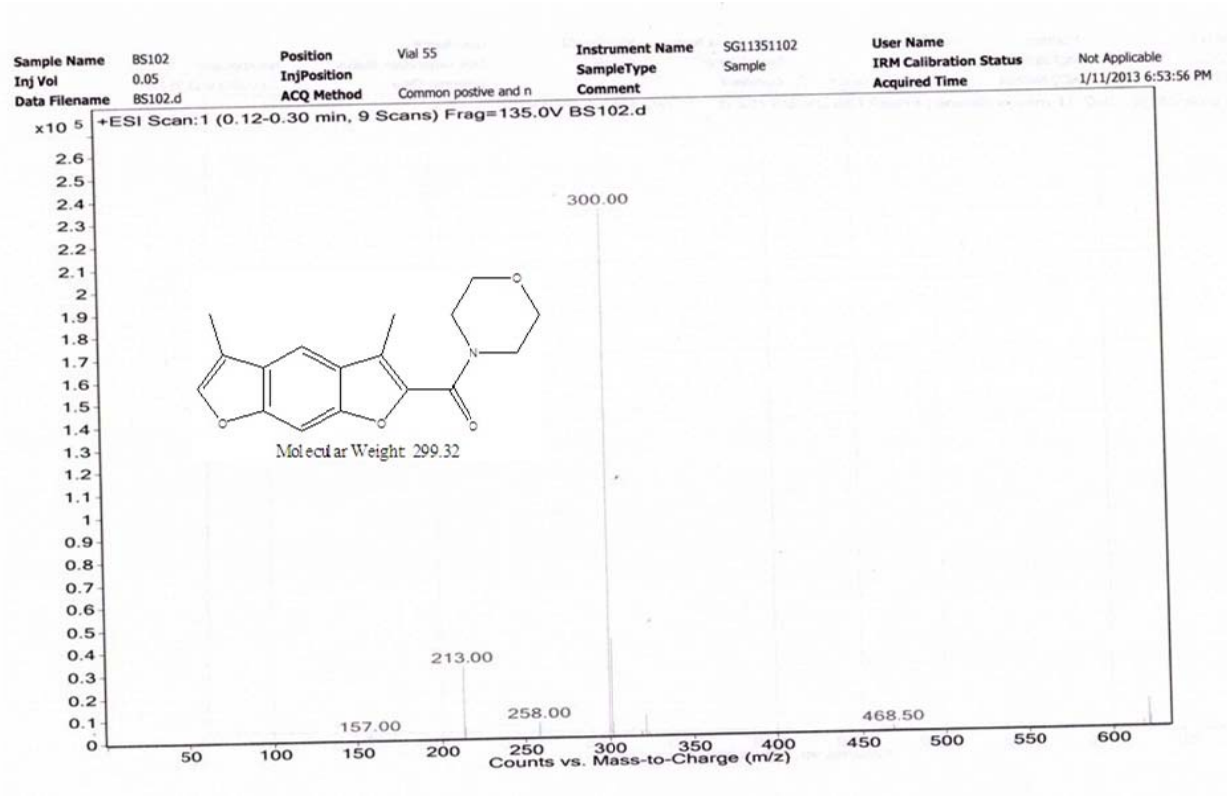


Figure 24: ESI/MS of (3, 5-dimethylbenzo[1,2-b:5,4-b']difuran-2-yl)(morpholino) methanone **6g**

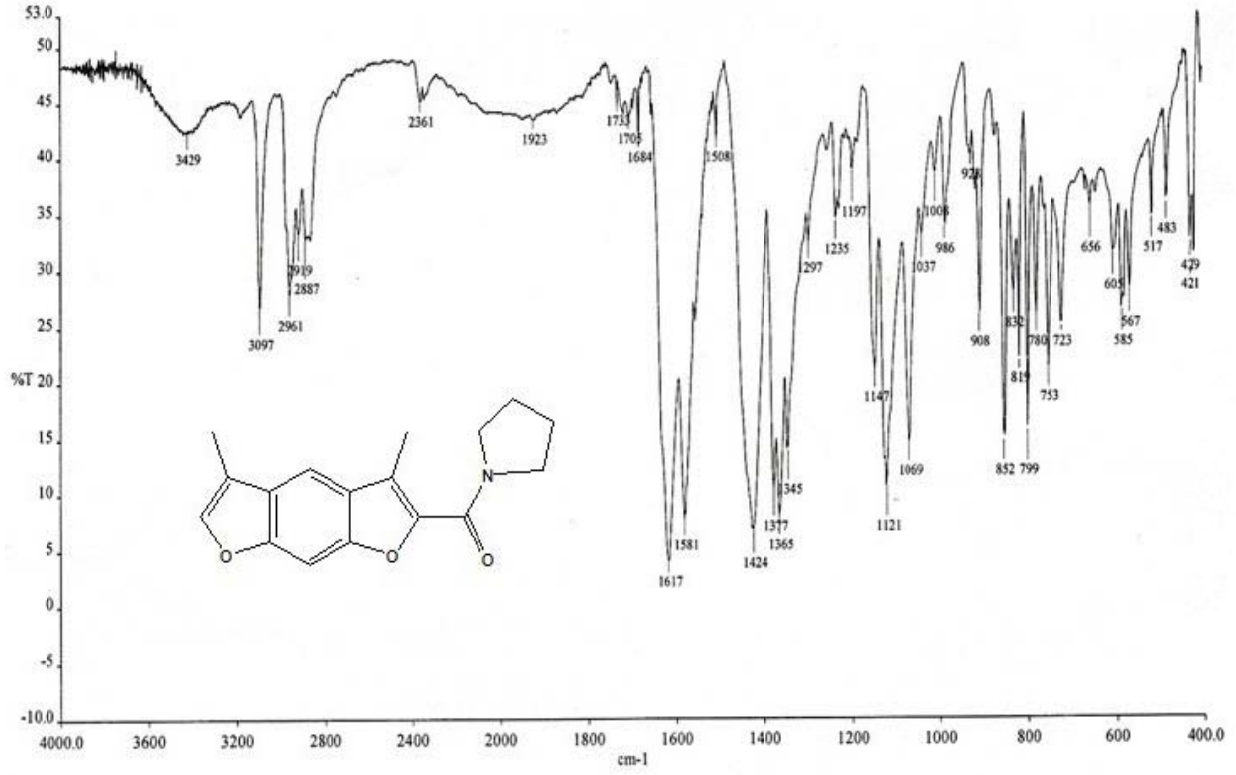


Figure 25: IR of (3, 5-dimethylbenzo[1,2-b:5,4-b']difuran-2-yl) (pyrrolidin-1-yl)methanone **6h**

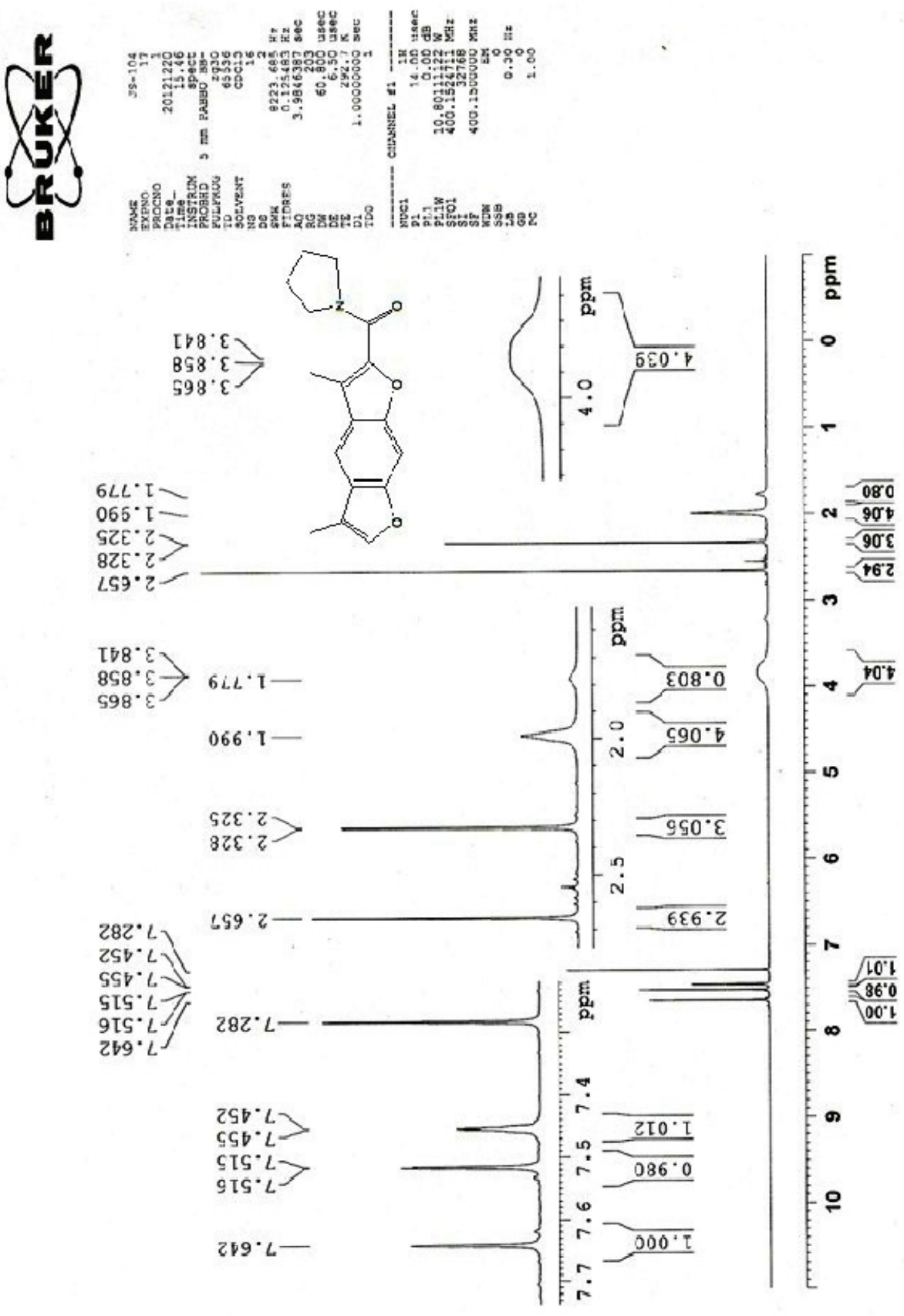


Figure 26: ^{1}H NMR of (3, 5-dimethylbenzo[1,2-b:5,4-b']difuran-2-yl) (pyrrolidin-1-yl)methanone **6h**

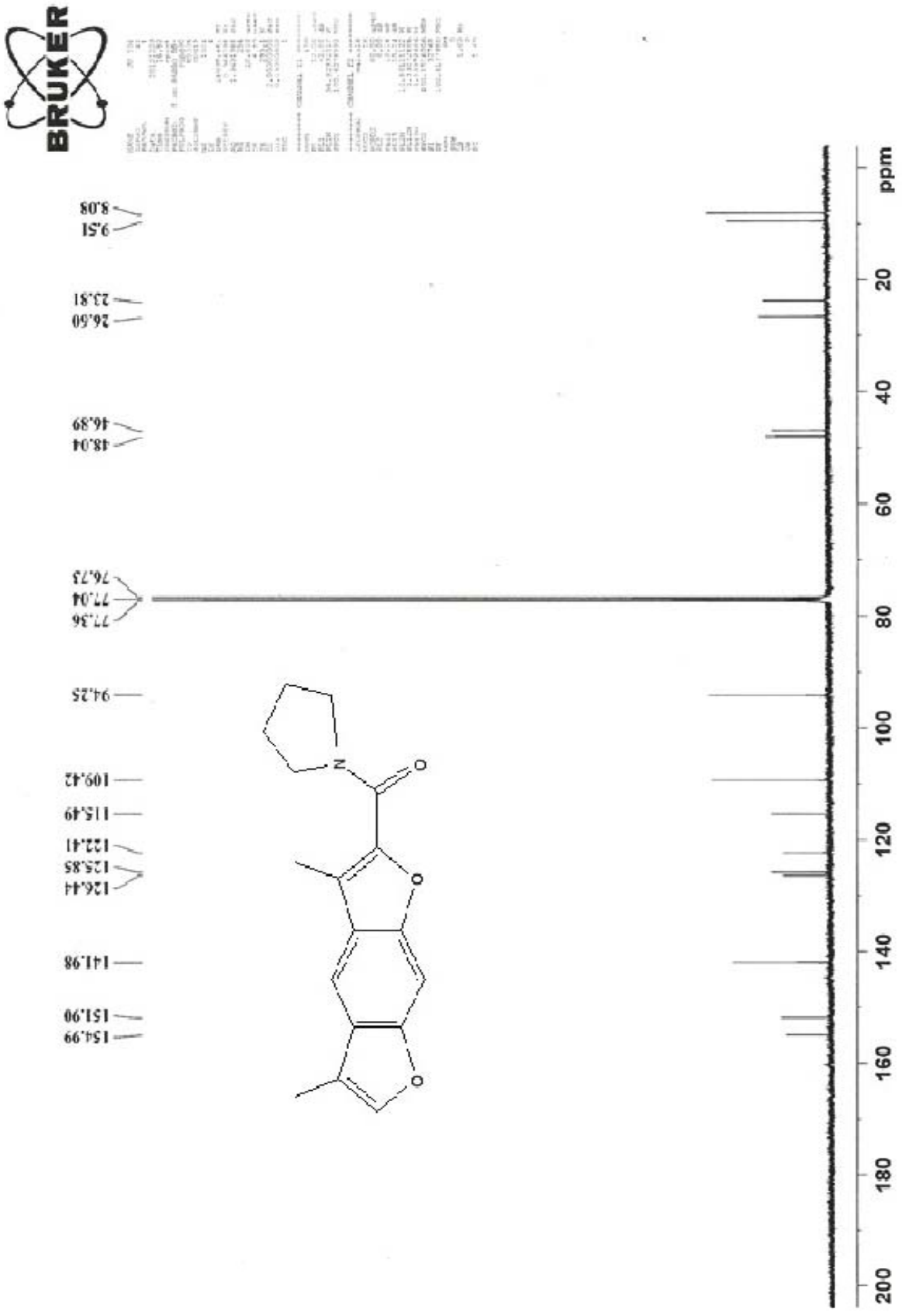


Figure 27: ^{13}C NMR of (3, 5-dimethylbenzo[1,2-b:5,4-b']difuran-2-yl) (pyrrolidin-1-yl)methanone **6h**

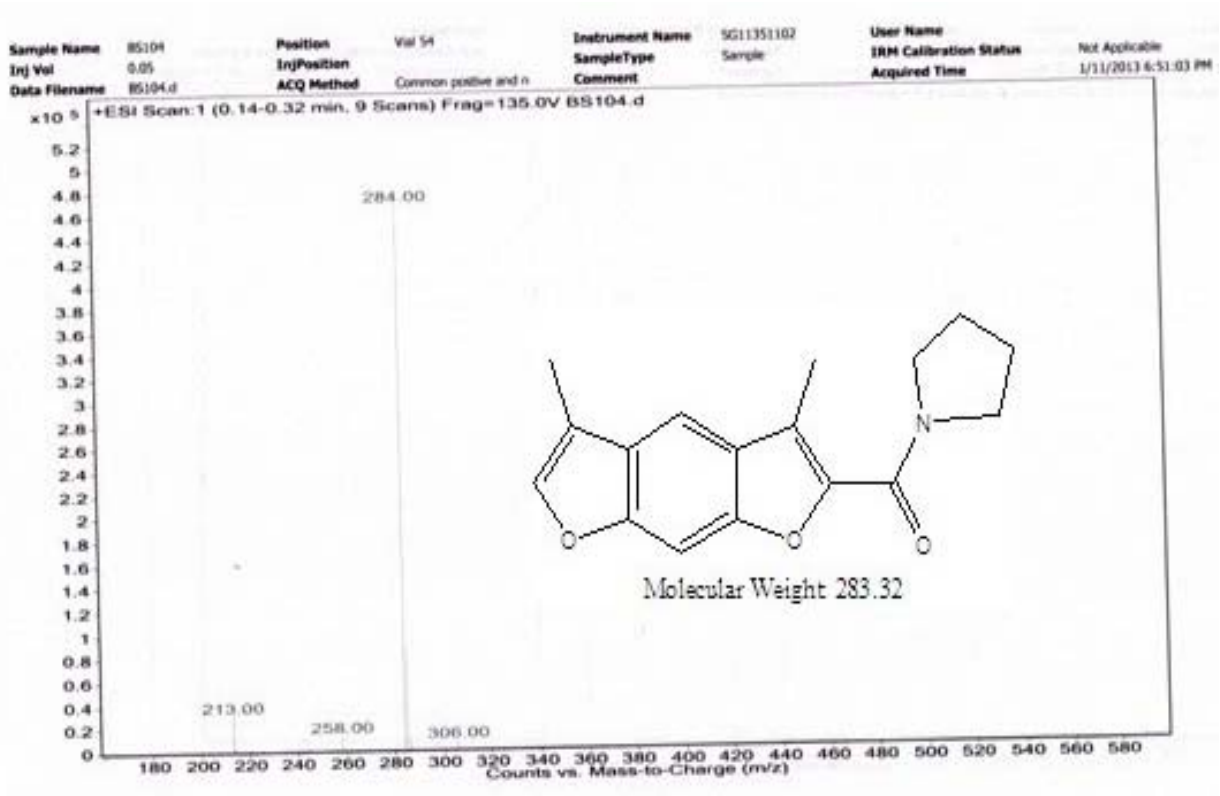


Figure 28: ESI/MS of (3, 5-dimethylbenzo[1,2-b:5,4-b']difuran-2-yl) (pyrrolidin-1-yl)methanone **6h**

3.2.2 Biological Evaluation

3.2.2.1 Antimicrobial activity

All the synthesized compounds were screened by Broth dilution method[20] for their antibacterial activity against two Gram positive bacteria *S. aureus* and *B. subtilis*, two Gram negative bacteria *E. coli* and *P. aeruginosa* and one fungus *C. albicans*. Concentrations of compounds were ranging from 40 µg to 600 µg. The lowest concentration of compounds that prevented visible growth is given in Table 1. It was determined that the solvent has no antibacterial or antifungal activities against any of the test organisms. Ciprofloxacin and Flucanazole were used as standard drugs, also tested under the similar conditions for comparison. The results of minimum inhibitory concentration (MIC) of the synthesized compounds against highly inhibited organisms are reported in Table 1. Compounds **6c**, **6h** and **6j** showed moderate effects against Gram positive bacteria *S. aureus* while compounds **6a**, **6b**, **6d**, **6e**, **6g** and **6k** showed moderate

activity against Gram positive bacteria *B. subtilis.* Only compound **6e** showed moderate activity against Gram negative bacteria *E. coli* while all these compounds found less active against *P. aeruginosa* (MIC 600 μg/ml).

Table 1: MIC determination of antibacterial and antifungal agent (μg)

Sr. No.	MIC (μg)				
	S. aureus	*B. subtilis*	*E. coli*	*P. aeruginosa*	*C. albicans*
	(gm +ve bacteria)		(gm –ve bacteria)		Fungi
6a	200	200	≥ 600	≥ 600	≥ 600
6b	600	160	400	600	600
6c	160	400	≥ 600	≥ 600	≥ 600
6d	600	200	600	≥ 600	≥ 600
6e	400	200	200	≥ 600	≥ 600
6f	600	400	600	600	600
6g	400	200	600	≥ 600	≥ 600
6h	160	400	400	≥ 600	600
6i	400	200	600	≥ 600	≥ 600
6j	160	400	400	600	600
6k	400	160	≥ 600	≥ 600	600
Ciprofloxacin	5	2	15	7.5	-
Flucanazole	-	-	-	-	5

It was observed that methyl or methoxy group at p- position of amine showed moderate activity while pyrrolidine ring also showed moderate activity against Gram positive bacteria *S. aureus*, while all other amines except **6c**, **6f**, **6h** and **6k** when used showed moderate activity against Gram positive bacteria *B. subtilis*. All these amide

derivatives of benzodifuran carboxylic acid showed high MIC values (600 µg/ml) against Gram negative bacteria *E. coli* and *P. aeruginosa*, as well as fungus *C. albicans*.

3.2.2.2 Orexin receptor binding assay

All synthesized amide derivatives of benzodifuran carboxylic acid were screened against OX_1 and OX_2 receptors isolated from chinese hamster ovary (CHO) cells. For the functional orexin receptor assay, they were cultured on black, clear bottom 96 well plates (Costar XX, Corning, XX), coated with polyethylene imine. 1 µM solution in DMSO was used for evaluation of orexin receptor antagonist as well as agonist activity by Ca^{+2} binding assay.[21] Results are summarized as given below.

Figure 29: Response of compounds on Orexin receptor OX_1 and OX_2

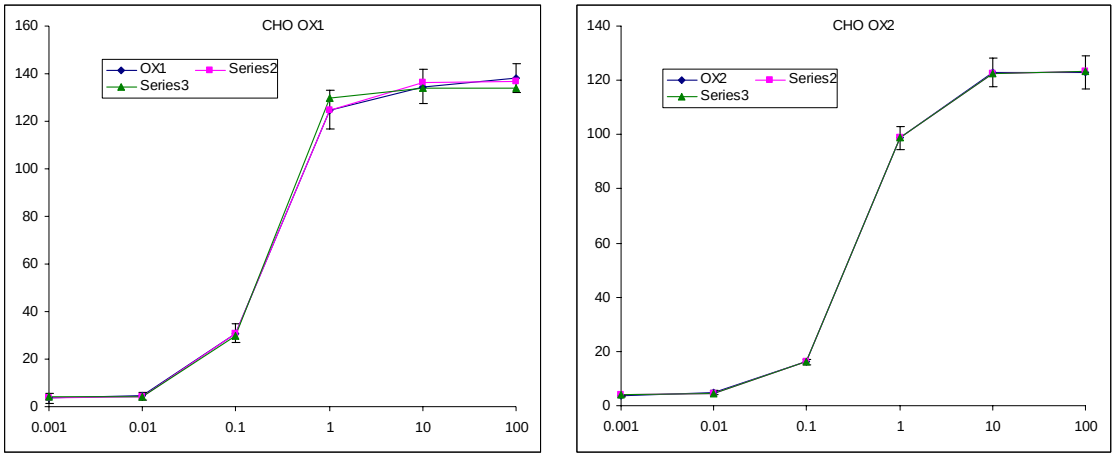
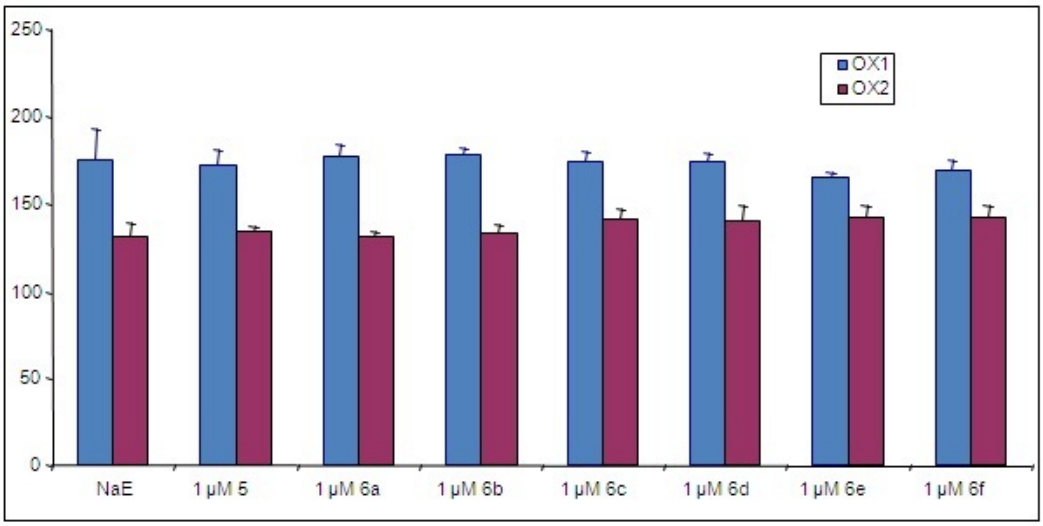
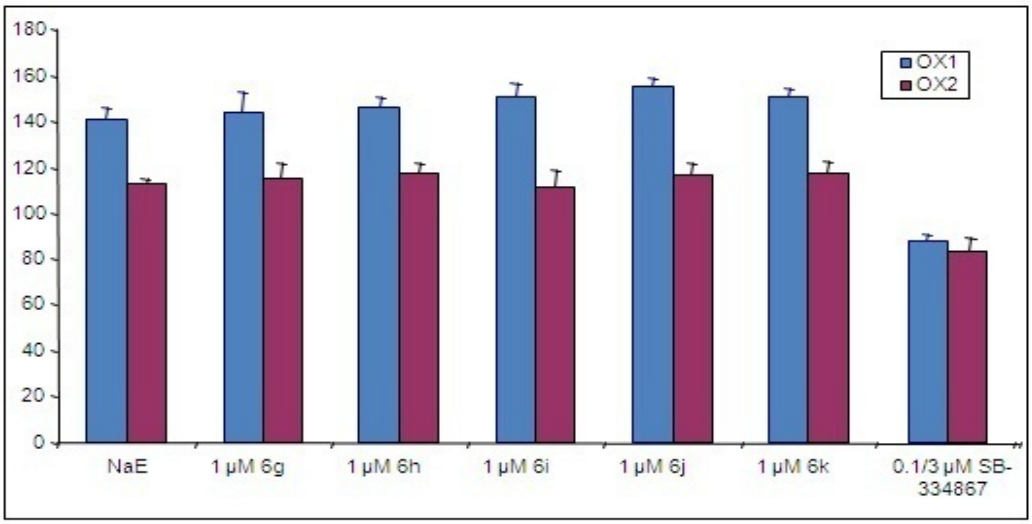


Figure 30: Ability of compounds for binding on Orexin receptor OX_1 and OX_2





Orexin-A potently activated both OX_1 and OX_2 receptors with an $EC_{50} \approx 0.2$-0.4 nM. In contrast, no response to any of the compounds was obtained at 0.8 and 2.4 µM concentrations and only one result is shown here. SB-334867 inhibited the OX_1 and OX_2 responses with the expected potency (Ki ≈ 10 and 1000 nM). In contrast, the compounds did not produce any inhibition of the orexin-A response at 0.8 and 2.4 µM concentrations. Taking into account the resolving power of the method, this indicated that the binding affinity of the compounds to both orexin receptors is less than 2 µM.

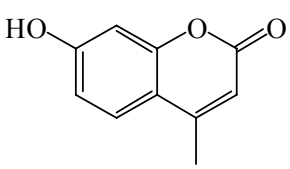
3.3 Conclusion

We have synthesized eleven benzodifuran amides and screened for their antimicrobial activity as well as their MIC against all test organisms. The presence of electron withdrawing group at para position of amine showed moderate activity against Gram positive bacteria *B. subtilis* (**6b**, **6d**, **6e** and **6g**) while presence of electron releasing group at para position of amine showed moderate activity against Gram positive bacteria *S. aureus*. All the synthesized amide derivatives of benzodifuran carboxylic acid showed higher MIC values (600 µg/ml) against Gram negative bacteria *P. aeruginosa*, and fungus *C. albicans*. All synthesized compounds show very less binding affinity towards orexin receptors in the range of 0.8 and 2.4 µM concentrations.

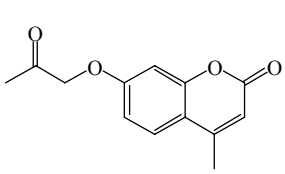
3.4 Experimental

3.4.1 Chemistry

Reagent grade chemicals and solvents were purchased from commercial supplier and used without purification. TLC was performed on silica gel F254 plates (Merck). Silica gel (100-200 mesh) was used for column chromatographic purification. Melting points are uncorrected and were measured in open capillary tubes, using a Rolex melting point apparatus. IR spectra were recorded as KBr pellets on Perkin Elmer RX 1 spectrometer. ^{1}H NMR and ^{13}C NMR spectral data were recorded on Advance Bruker 400 spectrometer (400 MHz) with $CDCl_3$ or DMSO-d_6 as solvent and TMS as internal standard. *J* values are in Hz. Elemental analyses were recorded on Thermosinnigan Flash 11-12 series EA. Mass spectra were determined by ESI/MS, using a Shimadzu LCMS 2020 apparatus.

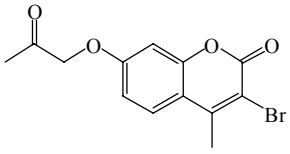


7-hydroxy-4-methyl-2H-chromen-2-one 2: Resorcinol **1** (10 g, 0.0908 mol) was dissolved in ethyl acetoacetate (12 ml, 0.095 mol) with constant shaking in reaction flask. Concentrated sulphuric acid (20 ml) was added in 3 to 4 portions and reaction mixture was kept overnight at room temperature. Reaction mass was poured into crushed ice to obtain pale yellow colored solid which was filtered, dried and recrystallized using ethanol gave yellow crystalline product. Yield: 74%; mp: 190-193ºC (Lit. 185 ºC[18]).

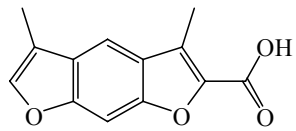


4-methyl-7-(2-oxopropoxy)-2H-chromen-2-one 3: To the solution of **2** (5 g, 0.0289 mol) in dry acetone (50 ml) and anhydrous potassium carbonate (13.98 g, 0.1011 mol), chloroacetone (2.54 ml, 0.0318 mol) was added very slowly. Reaction mixture was refluxed for 6 hours in water bath. Reaction mass was poured in crushed ice to obtain brown solid product, which was filtered, dried and crystallized using ethanol to afford

light brown solid. Yield: 49%; mp: 155-158ºC (Lit. 157 ºC[17]); IR (KBr) (Figure 1): 2907, 1736, 1710 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) (Figure 2): δ 2.32 (3H, s, CH_3), 2.41 (3H, s, CH_3), 4.66 (2H, s, CH_2), 6.17 (1H, s, ArH), 6.76-6.78 (1H, t, *J*= 2.8, 4 Hz, ArH), 6.89-6.92 (1H, dd, *J*= 2.4, 8.8 Hz, ArH), 7.53-7.55 (1H, t, *J*= 3.2, 5.6 Hz, ArH); ^{13}C NMR (400 MHz, $CDCl_3$) (Figure 3): δ 18.7, 26.6, 72.9, 101.8, 112.3, 112.5, 114.4, 125.9, 152.4, 155.1, 160.5, 161.0, 203.7; MS (ESI, *m/z*) (Figure 4) 177.0 $[M-55]^+$ and 175 $[M-57]^+$ calculated for $C_{13}H_{12}O_4$.



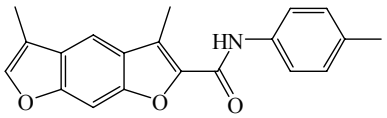
3-bromo-4-methyl-7-(2-oxopropoxy)-2H-chromen-2-one 4: 3 (5 g, 0.0216 mol) dissolved in chloroform (50 ml) and N-bromosuccinimide (4.24 g, 0.0238 mol) added in the reaction flask. Reaction mixture was refluxed for 6 hours in water bath. Excess solvent distilled under reduced pressure to obtain solid which was washed with hot water thrice, filtered, dried and crystallized using glacial acetic acid to obtain pale yellow solid. Yield: 63%; mp: 189-193ºC; IR (KBr) (Figure 5): 3075, 2919, 1710, 1681 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) (Figure 6): δ 2.33 (3H, s, CH_3), 2.62 (3H, s, CH_3), 4.67 (2H, s, CH_2), 6.78 (1H, s, ArH), 6.93-6.95 (1H, d, *J*= 8.8 Hz, ArH), 7.60-7.62 (1H, d, *J*= 8.8 Hz, ArH); ^{13}C NMR (400 MHz, DMSO-d_6) (Figure 7): δ 19.8, 26.6, 72.8, 101.7, 109.1, 113.5, 113.6, 127.6, 152.3, 153.4, 156.9, 161.5, 203.5; MS (ESI, *m/z*) (Figure 8) 311 $[M]^+$, 312.9 $[M+2]^+$ and 310.7 $[M-1]^+$ calculated for $C_{13}H_{11}BrO_4$.



3, 5-dimethylbenzo[1,2-b:5,4-b']difuran-2-carboxylic acid 5: 4 (5 g, 0.0161 mol) was dissolved in 50 ml 10% ethanolic potassium hydroxide solution and refluxed for 3 hours in water bath. Excess ethanol was distilled under reduced pressure and reaction mixture poured into crushed ice, concentrated hydrochloric acid was slowly added until pH 2. The solid obtained was filtered, dissolved in saturated solution of sodium bicarbonate, filtered and reprecipitated

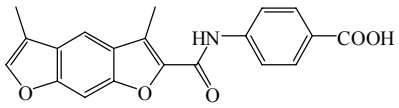
with concentrated HCl. Then it was filtered and dried. The crude product was purified using column chromatography by using petroleum ether: ethyl acetate (7:3) as eluent gave light brown solid. Yield: 42%; mp: 260-264ºC; IR (KBr) (Figure 9): 3428 (b), 3091, 3061, 2925, 2863, 2828, 1680 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) (Figure 10): δ 2.27 (3H, s, CH_3), 2.59 (3H, s, CH_3), 7.82-7.82 (1H, d, *J*= 1.6 Hz, ArH), 7.84 (s, 1H, ArH), 7.92 (1H, s, ArH); ^{13}C NMR (400 MHz, DMSO-d_6) (Figure 11): δ 8.1, 9.7, 94.9, 111.2, 115.6, 125.2, 125.8, 126.9, 141.8, 155.4, 161.5; MS (ESI, *m/z*) (Figure 12) 231.0 $[M+1]^+$ and 228.9 $[M-1]^+$ calculated for $C_{13}H_{10}O_4$.

General Procedure for 6a-6k: 5 (0.5 g, 0.0022 mol) dissolved in dichloromethane (25 ml) and 3-4 drops of DMF slowly added in reaction flask. Oxalyl chloride (0.75 ml, 0.0088 mol) was added slowly in the reaction mixture and stirred at room temperature for 3 h. Solvent distilled under reduced pressure and dried under high vacuum to remove oxalyl chloride. Reaction mass was dissolved in dichloromethane (30 ml), and amine (1.1 eq.) along with catalytic amount of triethylamine (0.64 ml, 0.0046 mol) were added and allowed to stir at room temperature for overnight. Reaction mixture was washed with saturated sodium bicarbonate (20 ml) and then with 10% concentrated hydrochloride solution (20 ml). Solvent was passed through Na_2SO_4 and removed under reduced pressure to give solid. Crude solid product was purified using column chromatography by using petroleum ether: ethyl acetate (9:1) as eluent.

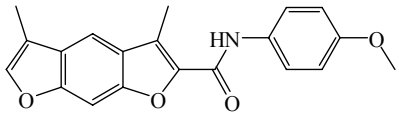


3, 5-dimethyl-N-(p-tolyl)benzo[1,2-b:5,4-b']difuran-2-carboxamide 6a: This compound obtained as white solid. Yield: 35%; mp: 192-194ºC; IR (KBr) (Figure 13): 3421, 3133, 3032, 2956, 2924, 2856, 1670 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) (Figure 14): δ 2.34 (3H, s, CH_3), 2.37 (3H, s, CH_3), 2.77 (3H, s, CH_3), 7.22-7.24 (2H, d, *J*= 8 Hz, ArH), 7.48 (1H, s, ArH), 7.58 (1H, s, ArH), 7.62-7.64 (2H, d, *J*= 8.4 Hz, ArH), 7.68 (1H, s, ArH), 8.34 (1H, s, NH); ^{13}C NMR

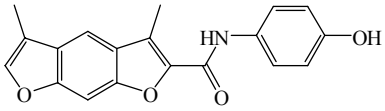
(400 MHz, $CDCl_3$) (Figure 15): δ 8.0, 9.2, 20.9, 94.4, 110.0, 115.5, 119.9, 123.7, 126.4, 126.8, 129.6, 134.1, 135.0, 142.3, 142.6, 151.7, 155.4, 158.0; MS (ESI, *m/z*) (Figure 16) 320.0 $[M+1]^+$ calculated for $C_{20}H_{17}NO_3$; Ele. Anal. Calcd. for $C_{20}H_{17}NO_3$; Requires (Found) %: C, 75.22 (75.43); H, 5.37 (5.28); N, 4.39 (4.27).



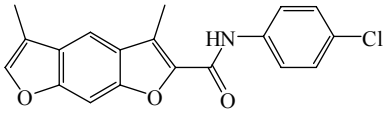
4-(3,5-dimethylbenzo[1,2-b:5,4-b']difuran-2-carboxamido)benzoic acid 6b: This compound obtained as yellow solid. Yield: 29%; mp: > 300ºC; IR (KBr): 3676 (b), 2922, 2853, 1694, 1684 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ 2.28 (3H, s, CH_3), 2.66 (3H, s, CH_3), 7.83-7.84 (2H, m, ArH), 7.92-7.99 (5H, m, ArH), 10.69 (1H, s, COOH); ^{13}C NMR (400 MHz, DMSO-d_6): δ 8.1, 9.6, 95.0, 111.1, 115.7, 120.2, 123.9, 126.1, 126.2, 127.0, 130.6, 143.0, 143.1, 143.5, 151.7, 155.2, 158.6, 167.4; MS (ESI, *m/z*) 252.9 $[M+1-98]^+$ calculated for $C_{20}H_{15}NO_5$; Ele. Anal. Calcd. for $C_{20}H_{15}NO_5$; Requires (Found) %: C, 68.76 (68.43); H, 4.33 (4.33); N, 4.01 (3.69).



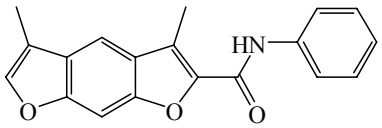
N-(4-methoxyphenyl)-3,5-dimethylbenzo[1,2-b:5,4-b']difuran-2-carboxamide 6c: This compound obtained as light brown solid. Yield: 43%; mp: 178-180ºC; IR (KBr) (Figure 17): 3419 (s), 3138, 3051, 3010, 2922, 2841, 1671 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) (Figure 18): δ 2.33 (3H, s, CH_3), 2.76 (3H, s, CH_3), 3.84 (3H, s, -OCH_3), 6.93-6.96 (2H, dd, *J*= 2, 6.8 Hz ArH), 7.48 (1H, s, ArH), 7.57 (1H, s, ArH), 7.64 (1H, s, ArH), 7.65-7.66 (1H, d, *J*= 2 Hz, ArH), 7.67 (1H, s, ArH), 8.30 (1H, s, NH); ^{13}C NMR (400 MHz, $CDCl_3$) (Figure 19): δ 8.1, 9.2, 55.5, 94.4, 109.9, 114.2, 115.5, 121.7, 123.5, 126.4, 126.8, 130.6, 142.2, 142.7, 151.7, 155.4, 156.5, 158.0; MS (ESI, *m/z*) (Figure 20) 336.0 $[M+1]^+$ calculated for $C_{20}H_{17}NO_4$; Ele. Anal. Calcd. for $C_{20}H_{17}NO_4$; Requires (Found) %: C, 71.63 (71.40); H, 5.11 (5.04); N, 4.18 (4.04).



N-(4-hydroxyphenyl)-3,5-dimethylbenzo[1,2-b:5,4-b']difuran-2-carboxamide 6d: This compound obtained as light yellow solid. Yield: 39%; mp: 244-246ºC; IR (KBr): 3418, 3372, 2924, 1644 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ 2.28 (3H, s, CH_3), 2.63 (3H, s, CH_3), 6.73-6.75 (2H, dd, *J*= 2.4, 6.8 Hz, ArH), 7.57-7.59 (2H, dd, *J*= 2.0, 6.8 Hz, ArH), 7.81-7.83 (2H, m, ArH), 7.91 (1H, s, ArH), 9.38 (1H, s, NH), 10.19 (1H, s, OH); ^{13}C NMR (400 MHz, DMSO-d_6): δ 8.2, 9.5, 94.9, 110.8, 115.4, 115.7, 122.3, 122.9, 126.4, 126.8, 130.3, 143.4, 143.7, 151.6, 154.3, 155.0, 158.0; MS (ESI, *m/z*) 322.0 $[M+1]^+$ and 319.8 $[M-1]^+$ calculated for $C_{19}H_{15}NO_4$; Ele. Anal. Calcd. for $C_{19}H_{15}NO_4$; Requires (Found) %: C, 71.02 (71.19); H, 4.71 (4.69); N, 4.36 (4.00).

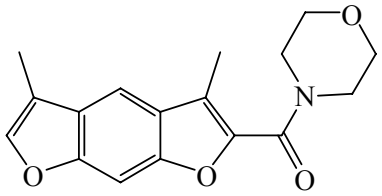


N-(4-chlorophenyl)-3,5-dimethylbenzo[1,2-b:5,4-b']difuran-2-carboxamide 6e: This compound obtained as light yellow solid. Yield: 51%; mp: 190-192ºC; IR (KBr): 3389, 2925, 2853, 1684 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 2.34 (3H, s, CH_3), 2.76 (3H, s, CH_3), 7.36-7.38 (2H, m, ArH), 7.49-7.49 (1H, m, ArH), 7.58-7.58 (1H, m, ArH), 7.69-7.72 (3H, m, ArH), 8.38 (1H, s, NH); ^{13}C NMR (400 MHz, $CDCl_3$): δ 8.0, 9.3, 94.4, 110.1, 115.6, 121.1, 124.3, 126.2, 127.0, 129.1, 129.4, 136.1, 142.3, 142.4, 151.7, 155.6, 158.0; MS (ESI, *m/z*) 340.0 $[M+1]^+$ and 337.8 $[M-2]^+$ calculated for $C_{19}H_{14}ClNO_3$; Ele. Anal. Calcd. for $C_{19}H_{14}ClNO_3$; Requires (Found) %: C, 67.16 (66.89); H, 4.15 (4.33); N, 4.12 (3.98).

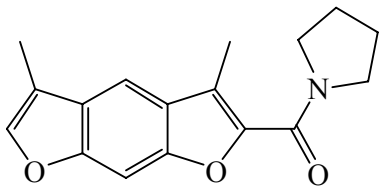


3,5-dimethyl-N-phenylbenzo[1,2-b:5,4-b']difuran-2-carboxamide 6f: This compound obtained as white solid. Yield: 42%; mp: 182-184ºC; IR (KBr): 3421, 3119, 3050, 2923, 2852, 1669 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 2.34 (3H, s, CH_3), 2.77 (3H, s, CH_3), 7.17-7.20 (1H, m, ArH), 7.40-7.43 (2H, m, ArH), 7.48 (1H, s, ArH), 7.59 (1H, s, ArH), 7.69 (1H, s, ArH), 7.74-7.76 (2H, m, ArH), 8.39 (1H, s, NH); ^{13}C NMR (400 MHz, $CDCl_3$): δ 8.1, 9.3, 94.4,

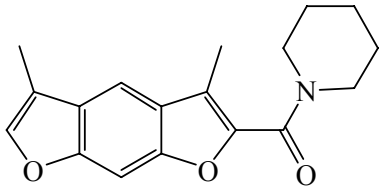
110.0, 115.6, 119.9, 124.0, 124.5, 126.3, 126.9, 129.1, 137.5, 142.3, 142.5, 151.7, 155.5, 158.1; MS (ESI, *m/z*) 306.0 $[M+1]^+$ calculated for $C_{19}H_{15}NO_3$; Ele. Anal. Calcd. for $C_{19}H_{15}NO_3$; Requires (Found) %: C, 74.74 (74.84); H, 4.95 (5.15); N, 4.59 (4.65).



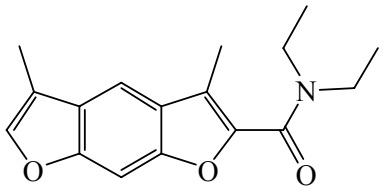
(3,5-dimethylbenzo[1,2-b:5,4-b']difuran-2-yl)(morpholino)methanone 6g: This compound obtained as white solid. Yield: 40%; mp: 136-140ºC; IR (KBr) (Figure 21): 3059, 2955, 2917, 2859, 1623 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) (Figure 22): δ 2.32 (3H, s, CH_3), 2.56 (3H, s, CH_3), 3.83 (8H, s, CH_2), 7.46-7.46 (1H, d, *J*= 1.2 Hz, ArH), 7.53-7.53 (1H, d, *J*= 0.4 Hz, ArH), 7.63 (1H, s, ArH); ^{13}C NMR (400 MHz, $CDCl_3$) (Figure 23): δ 8.1, 9.3, 67.1, 94.4, 109.4, 115.5, 122.2, 125.6, 126.6, 142.1, 143.7, 151.9, 155.0, 161.0; MS (ESI, *m/z*) (Figure 24) 300.0 $[M+1]^+$ calculated for $C_{17}H_{17}NO_4$; Ele. Anal. Calcd. for $C_{17}H_{17}NO_4$; Requires (Found) %: C, 68.21 (68.54); H, 5.72 (5.67); N, 4.68 (4.85).



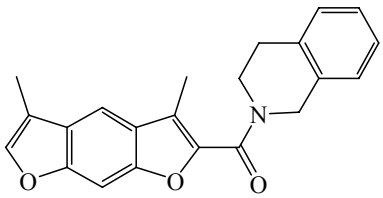
(3,5-dimethylbenzo[1,2-b:5,4-b']difuran-2-yl) (pyrrolidin-1-yl)methanone 6h: This compound obtained as light yellow solid. Yield: 38%; mp: 108-110ºC; IR (KBr) (Figure 25): 3097, 2961, 2919, 2887, 1617 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) (Figure 26): δ 1.99 (4H, s, CH_2), 2.32 (3H, s, CH_3), 2.66 (3H, s, CH_3), 3.84-3.87 (4H, m, CH_2), 7.45 (1H, s, ArH), 7.52 (1H, s, ArH), 7.64 (1H, s, ArH); ^{13}C NMR (400 MHz, $CDCl_3$) (Figure 27): δ 8.1, 9.5, 23.8, 26.6, 46.9, 48.0, 94.2, 109.4, 115.5, 122.4, 125.8, 126.4, 142.0, 151.9, 155.0; MS (ESI, *m/z*) (Figure 28) 284.0 $[M+1]^+$ calculated for $C_{17}H_{17}NO_3$; Ele. Anal. Calcd. for $C_{17}H_{17}NO_3$; Requires (Found) %: C, 72.07 (71.89); H, 6.05 (6.03); N, 4.94 (5.14).



(3,5-dimethylbenzo[1,2-b:5,4-b']difuran-2-yl)(piperidin-1-yl)methanone 6i: This compound obtained as light yellow solid. Yield: 33%; mp: 110-112ºC; IR (KBr): 3106, 2942, 2857, 1635 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 1.7-1.72 (6H, CH_2), 2.32 (3H, s, CH_3), 2.51 (3H, s, CH_3), 3.69 (4H, m, CH_2), 7.45 (1H, s, ArH), 7.53 (1H, s, ArH), 7.61 (1H, s, ArH); ^{13}C NMR (400 MHz, $CDCl_3$): δ 8.1, 9.1, 24.7, 94.4, 109.1, 115.4, 120.0, 125.7, 126.4, 142.0, 144.7, 152.0, 154.7, 161.0; MS (ESI, *m/z*) 298.0 $[M+1]^+$ calculated for $C_{18}H_{19}NO_3$; Ele. Anal. Calcd. for $C_{18}H_{19}NO_3$; Requires (Found) %: C, 72.71 (72.44); H, 6.44 (6.29); N, 4.71 (5.02).



N,N-diethyl-3,5-dimethylbenzo[1,2-b:5,4-b']difuran-2-carboxamide 6j: This compound obtained as brown viscous liquid. Yield: 51%; IR (Neat): 3108, 3062, 2973, 2932, 1627 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 1.27-1.34 (6H, t, CH_3), 2.33 (3H, s, CH_3), 2.54 (3H, s, CH_3), 3.54-3.59 (4H, q, CH_2), 7.45-7.46 (1H, d, *J*= 1.2 Hz, ArH), 7.52 (1H, s, ArH), 7.61 (1H, s, ArH); ^{13}C NMR (400 MHz, $CDCl_3$): δ 8.1, 9.2, 94.3, 109.1, 115.5, 120.6, 125.8, 126.4, 141.9, 145.0, 151.8, 154.8, 161.6; MS (ESI, *m/z*) 286.0 $[M+1]^+$ calculated for $C_{17}H_{19}NO_3$.



(3,4-dihydroisoquinolin-2(1H)-yl)(3,5-dimethylbenzo[1,2-b:5,4-b']difuran-2-yl)methanone 6k: This compound obtained as light yellow solid. Yield: 31%; mp: 96-98ºC; IR (KBr): 3063, 3023, 2921, 2854, 1628 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 2.33 (3H, s, CH_3), 2.55 (3H, s, CH_3), 3.06 (2H, S, CH_2), 3.98-3.99 (2H, d, CH_2), 4.93 (2H, s, CH_2), 7.22-7.23 (3H, m, ArH), 7.47 (1H, s, ArH), 7.56 (1H, s, ArH), 7.64 (1H, s, ArH); ^{13}C NMR (400 MHz, $CDCl_3$): δ 8.1, 9.3, 94.5, 109.3, 115.5, 121.7, 125.7, 126.5, 126.7, 142.1, 144.3, 152.0, 154.9; MS (ESI, *m/z*) 346.0 $[M+1]^+$ calculated for $C_{22}H_{19}NO_3$;

Ele. Anal. Calcd. for $C_{22}H_{19}NO_3$; Requires (Found) %: C, 76.50 (76.37); H, 5.54 (5.61); N, 4.06 (4.20).

3.4.2 Biological Evaluation

3.4.2.1 Antimicrobial activity

All the synthesized compounds were screened by Broth dilution method[20] for their antibacterial activity against two Gram positive bacteria *S. Aureus* and *B. Subtilis*, two Gram negative bacteria *E. Coli* ATCC 25922 and *P. Aeruginosa* ATCC 27853. They were also evaluated for their *in vitro* antifungal activity against *C. Albicans*. Concentration of compounds was ranging from 40 µg to 600 µg. The lowest concentrations of the compounds that prevented visible growth are given in Table-1. It was determined that the solvent had no antibacterial and antifungal activities against any of the test microorganisms. Ciprofloxacin and Flucanazole were used as standard drugs also tested under the similar conditions for comparison.

3.4.2.2 Orexin receptor binding assay

The binding of the compounds on human orexin receptors was tested in a functional assay. The cells, on 96 well plates, were loaded with the fluorescent Ca^{2+} indicator fluo-4 AM (1 mM) in NaE, pluronic acid and 2 mM probenecid for 1 h at 37℃. They were washed once and 80-100 µl NaE was dispensed in each well. The measurements were performed with the fluorescent plate reader FlexStation 3 (Molecular Devices). Both the agonistic and antagonistic properties of the compounds were tested, the former by measuring the ability of the compounds to induce a Ca^{2+} elevations and the latter by measuring the ability of the compounds to inhibit the orexin A response 30 minutes later. The results were visualized and analyzed in Microsoft Excel as shown in Figure 29 and 30.

3.5 References

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Chapter 4a

Synthesis of amide and ester derivatives of Naphthopyrone-2-carboxylic acid

4a. Synthesis of amide and ester derivatives of Naphthopyrone-2-carboxylic acid

4a.1 Introduction

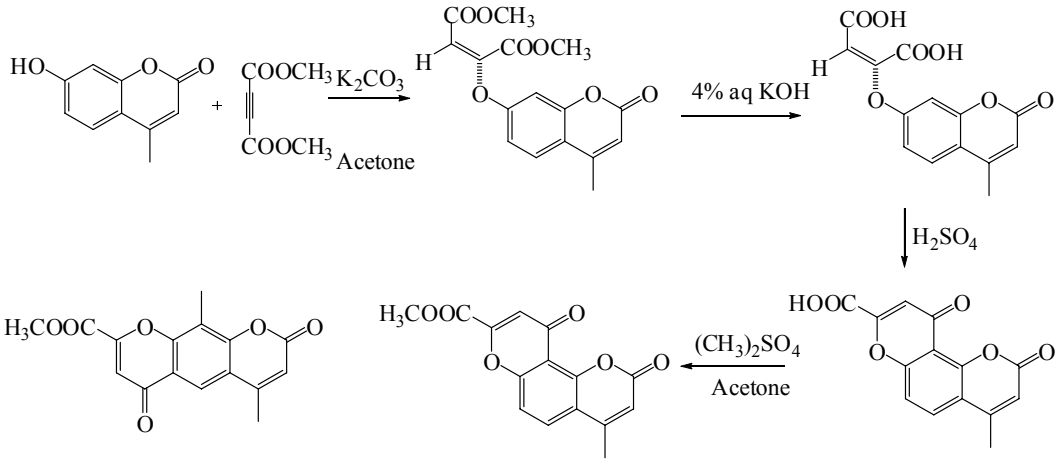
Chromones (benzopyran-4-ones) are one of the most abundant groups of naturally occurring heterocyclic compounds.[1] Chromones have remarkable biological properties like anti tuberculosis.[2, 3] Chromone scaffold has been recognized as a pharmacophore of number of bioactive molecules. Disodium cromoglycate, sodium salt of cromoglycic acid, is a clinically useful antiallergic agent, particularly for bronchial asthma and reported to inhibit the release of mediators like histamine, several kinins etc. of immediate hypersensitivity reactions[4] contains chromone ring. Heterocyclic analogues of Ritonavir, HIV-1 protease inhibitor are amide derivatives of chromone-2-carboxylic acid.[5] Chromone-3-carboxamide derivatives have been reported as MAO-B inhibitors[6, 7] while chromone-2-carboxylic acid derivatives are reported as melanin concentrating hormone receptor 1 antagonists[8] or adenosine receptor ligand.[9] Photodimerization[10] reactions of chromone-2-carboxylic esters and microwave assisted synthesis of various amide derivatives of chromone-2-carboxylic acids[11] have been reported. Vercauteren et al[12] have reported the use of activated alkynes for condensing it with tryptamine in Pictel-Spengler synthesis of tetrahydro-β-carbolines which involves acid catalyzed ring closure.

Synthesis of chromone-2-carboxylic acid was reported from our laboratory earlier.[13] Present investigation reports on synthesis of new class of various amide and ester derivatives of naphthopyrone-2-carboxylic acid. These naphthopyrone amides and esters are expected to show MAO inhibitor activity and anti allergic activity.

Recent Work

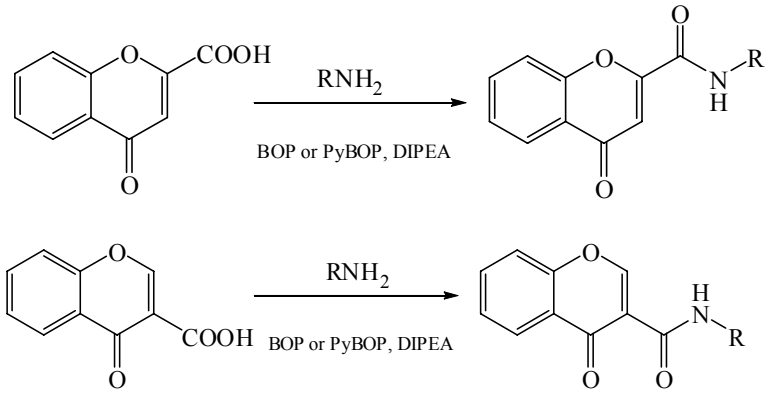
Soman S. S.[13] has synthesized various coumarinochromone acid and its ester derivatives as shown in scheme 1.

Scheme 1:

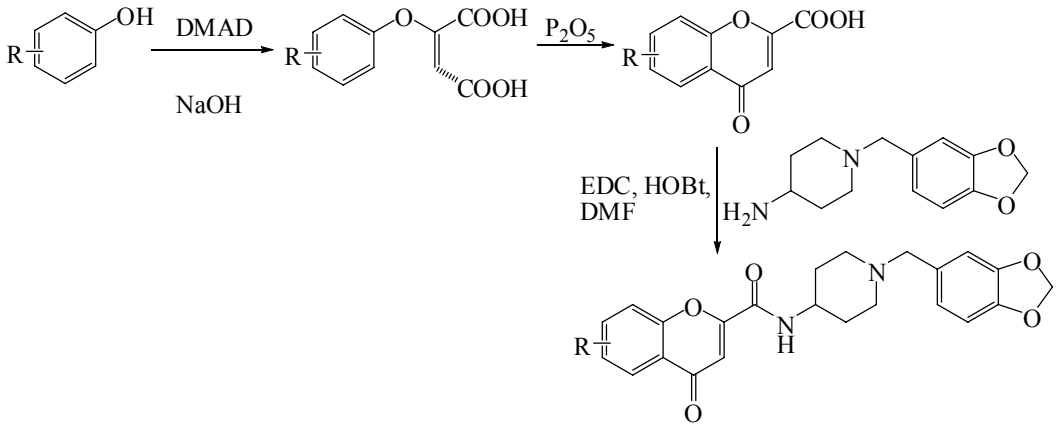


Gaspar A. and coworkers[6, 7] have synthesized various amide derivatives of benzopyrone as shown in scheme 2.

Scheme 2:



Lynch J. K. et al[8] have synthesized various substituted amide derivatives of benzopyrone as shown in **Scheme 3**.



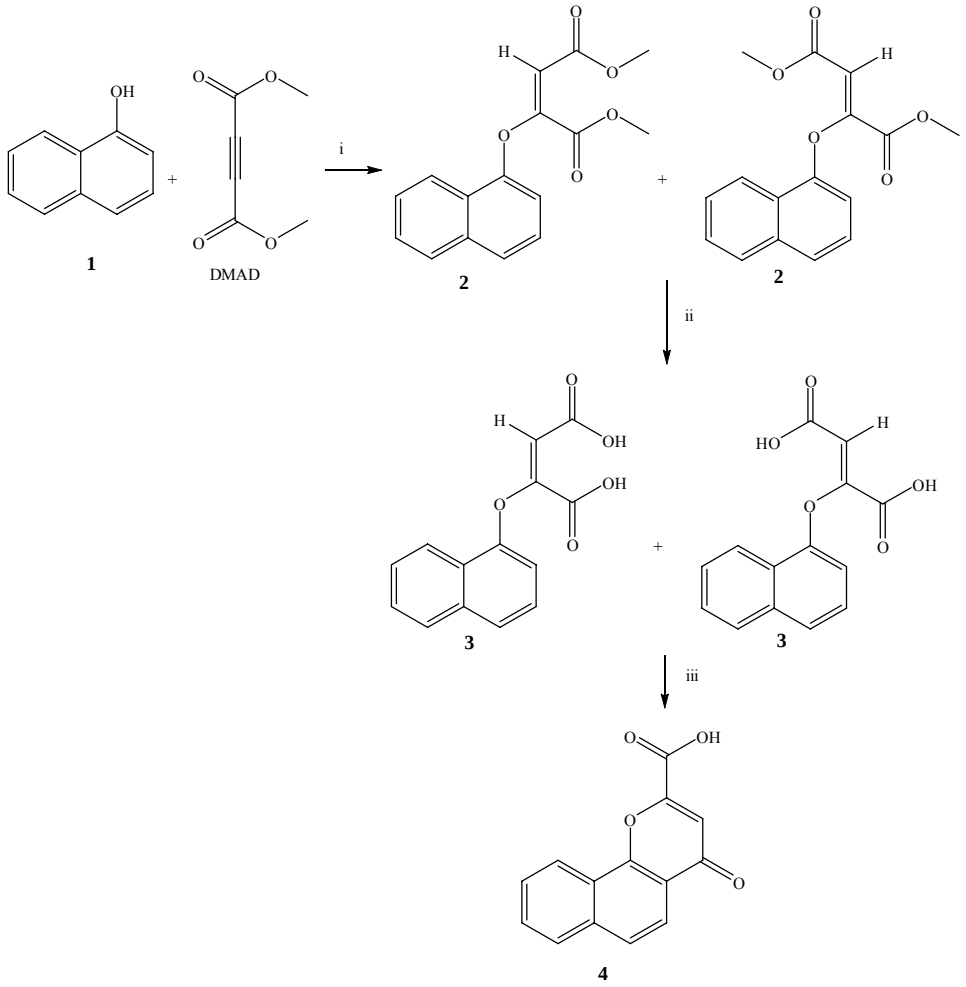
In view of these findings herein we have synthesized various amide and ester derivatives of naphthopyrone-2-carboxylic acid.

4a.2 Result and discussion

4a.2.1 Chemistry

Condensation[13] of 1-naphthol **1** (Scheme 4) with dimethyl acetylenedicarboxylate (DMAD) in presence of anhydrous K_2CO_3 and dry acetone gave *Z* or cis and *E* or trans mixture of dimethyl 2-(naphthalen-1-yloxy)maleate **2**. It was not possible to separate the *Z* and *E* mixture either by column chromatography or by TLC method as shown in Scheme 4.

Scheme 4:



Reagents and conditions: (i) K_2CO_3, dry acetone, reflux, 8 h; (ii) 1% aqueous KOH, RT, 12 h; (iii) H_2SO_4, 60-70ºC, 2 h

Earlier S. S. Soman[13] and J. K. Lynch[8] also could not separate the *Z* and *E* isomer of adducts obtained by condensation of DMAD with coumarin and phenol respectively. The IR spectrum of compound **2**/ **2'** exhibited band at 1735 cm^{-1} for ester group. In ^1H NMR spectrum of compound **2**/ **2'** in $CDCl_3$ (Figure 1), four singlets at δ 3.65, 3.68, 3.70 and 4.03 for three protons each indicated presence of four methoxy groups clearly indicated it is *Z* and *E* mixture. Singlet at δ 5.01 for one proton indicated *Z* or cis vinylic proton while singlet at δ 6.72 indicated *E* or trans vinylic proton. All aromatic protons appeared between δ 6.75 to 8.36 confirmed the formation of compound **2** as cis-trans mixture. The ratio of *E* and *Z* isomer of compound **2** was found to be 57:43 from ^1H NMR.

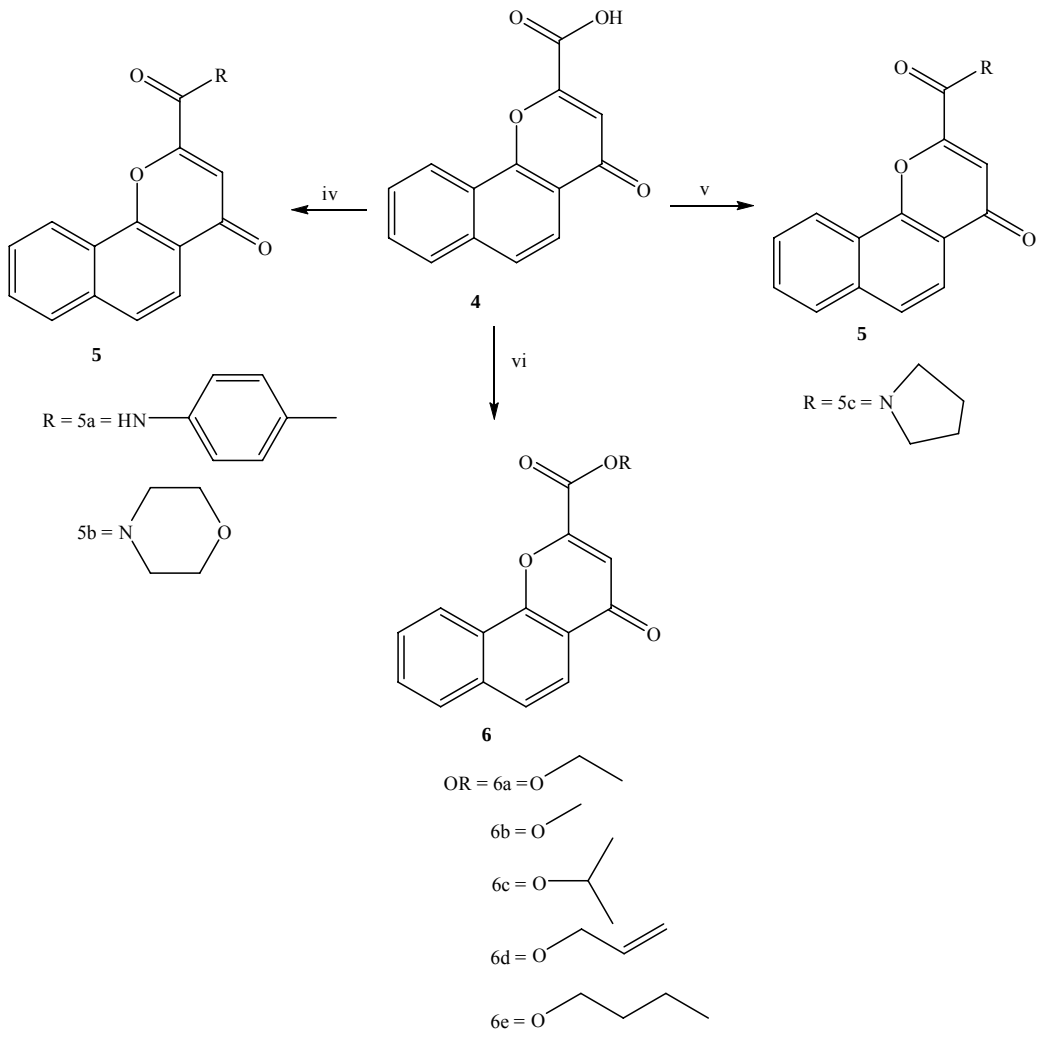
Mild hydrolysis of **2**/ **2'** in aqueous KOH (1%) at room temperature gave mixture of *Z* and *E* or cis and trans 2-(Naphthalen-1-yloxy)maleic acid **3**/ **3'**, which also could not be separated into *Z* and *E* isomer. The IR spectrum of compound **3**/ **3'** (Figure 2) showed bands at 1713 cm^{-1} and broad band at 2500-3053 cm^{-1} for carbonyl and hydroxy group of carboxylic acid respectively. Now absence of singlet for methoxy group of ester in ^1H NMR spectrum of compound **3**/ **3'** in DMSO-d_6 (Figure 3) confirmed the hydrolysis of esters. The presence of singlet for one proton each at δ 4.95 and 6.66 indicated cis and trans vinylic protons confirming it as *Z* and *E* mixture. All aromatic protons observed between δ 6.76 to 8.19 confirmed the formation of compound **3**.

Compound **3**/ **3'**on cyclization with concentrated sulphuric acid gave 4-oxo-4H-benzo[h]chromene-2-carboxylic acid **4**. The IR spectrum of compound **4** (Figure 4) showed bands at 1725 cm^{-1} and broad band from 2500-3500 cm^{-1} indicated presence of carboxylic acid group. The other sharp band at 1641 cm^{-1} indicated presence of carbonyl group of chromone. In ^1H NMR spectrum of compound **4** in DMSO-d_6 (Figure 5), the disappearance of two singlets at δ 4.95 and 6.66 which were for cis and trans protons in ^1H NMR of compound **3** (Figure 3) and appearance of one singlet at δ 7.07 indicated

aromatic proton at C-3, thus confirmed the cyclization. The multiplet from δ 7.81 to 8.53 for six protons indicated remaining aromatic protons.

This acid **4** is converted into corresponding amide **5** using two different methods.[14] Formation of amide **5a-b** using 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC.HCl), 4, 4'-dimethylaminopyridine (DMAP) and amine gave very low yield of amide hence we have used oxalylchloride to prepare acid chloride of **4** and its reaction with pyrrolidine gave **5c** as shown in Scheme 5.

Scheme 5:



Reagents and conditions: (iv) EDC.HCl, DMAP, Triethylamine, Amine, dry THF, RT, 8h; (v) Oxalyl chloride, DMF, Dichloromethane, 2h; (vi) Amine, Triethylamine, Dichloromethane, 8h; (vii) Alcohol, Dry HCl gas, 2h

The compound **5a** was prepared by reaction of acid with para toludine using EDC.HCl and DMAP gave very poor yield. The IR spectrum of compound **5a** (Figure 6) exhibited band at 1694 and 1651 cm^{-1} for carbonyl group of chromone and carbonyl group of amide respectively. The ^{1}H NMR spectrum of compound **5a** in $CDCl_3$ (Figure 7) showed singlet at δ 2.41 for three protons confirmed presence of methyl group, all aromatic protons appeared between δ 7.27 to 8.48 and singlet at δ 8.57 for NH proton confirmed formation of compound **5a**. The ^{13}C NMR spectrum of compound **5a** in $CDCl_3$ (Figure 8) showed presence of 19 peaks which is in accordance with the structure of compound **5a**. The mass spectrum of compound **5a** (Figure 9) showed *m/z* value at 330.0 for $[M+1]^+$ in ESI/MS confirmed the formation of compound **5a**.

The compound **5c** was prepared by carrying out reaction of acid with oxalyl chloride and converting in to acid chloride then its reaction with pyrrolidine. The IR spectrum of compound **5c** (Figure 10) exhibited band at 1629 cm^{-1} for carbonyl group. In ^{1}H NMR spectrum of compound **5c** in $CDCl_3$ (Figure 11) showed peaks at δ 2.05 to 3.88 for four protons each indicated methylene protons of pyrrolidine ring and all aromatic protons observed between δ 6.95 to 8.52 confirmed the formation of compound **5c**.

We have synthesized various esters[15] **6a-e** of naphthopyrone-2-carboxylic acid by using various alcohols and passing dry HCl gas as shown in Scheme 5. The IR spectrum of compound **6c** (Figure 12) exhibited band at 1732 and 1683 cm^{-1} for carbonyl group of ester and chromone respectively. In ^{1}H NMR spectrum of compound **6c** in $CDCl_3$ (Figure 13) showed two singlets at δ 1.48 and 1.49 for three protons each confirmed presence of two methyl groups of isopropoxy group, multiplet at δ 5.32 to 5.40 for one proton confirmed presence of CH proton of isopropoxy group and all aromatic protons observed between δ 7.73 to 8.67 confirmed formation of compound **6c**. The ^{13}C NMR spectrum of compound **6c** in $CDCl_3$ (Figure 14) showed presence of 17 peaks indicated 17 different

carbons present in compound **6c** thus confirmed the structure of compound **6c**. The mass spectrum of compound **6c** (Figure 15) showed *m/z* value at 282.9 for $[M+1]^+$ in ESI/MS confirmed formation of compound **6c**.

The IR spectrum of compound **6d** (Figure 16) exhibited band at 1732 and 1683 cm^{-1} for carbonyl group of ester and chromone respectively. In ^1H NMR spectrum of compound **6d** in $CDCl_3$ (Figure 17) showed triplet at δ 4.96 to 4.97 for two protons confirmed presence of $–OCH_2$ group of allyloxy group. The doublet of doublet at δ 5.41 to 5.44 for one proton indicated presence of terminal proton of $=CH_2$ of allyloxy group, another doublet of doublet at δ 5.51 to 5.56 for one proton confirmed presence of another terminal proton of $=CH_2$ of allyloxy group. The multiplet at δ 6.05 to 6.13 for one proton confirmed presence of –CH= proton of allyloxy group. Singlet at δ 7.30 for one proton indicated aromatic C-3 proton and all aromatic protons observed between δ 7.72 to 8.63 confirmed formation of compound **6d**. The ^{13}C NMR spectrum of compound **6d** in $CDCl_3$ (Figure 18) showed presence of 17 peaks which is in accordance with structure of **6d**. The mass spectrum of compound **6d** (Figure 19) showed *m/z* value at 281.0 for $[M+1]^+$ in ESI/MS confirmed the formation of compound **6d**.

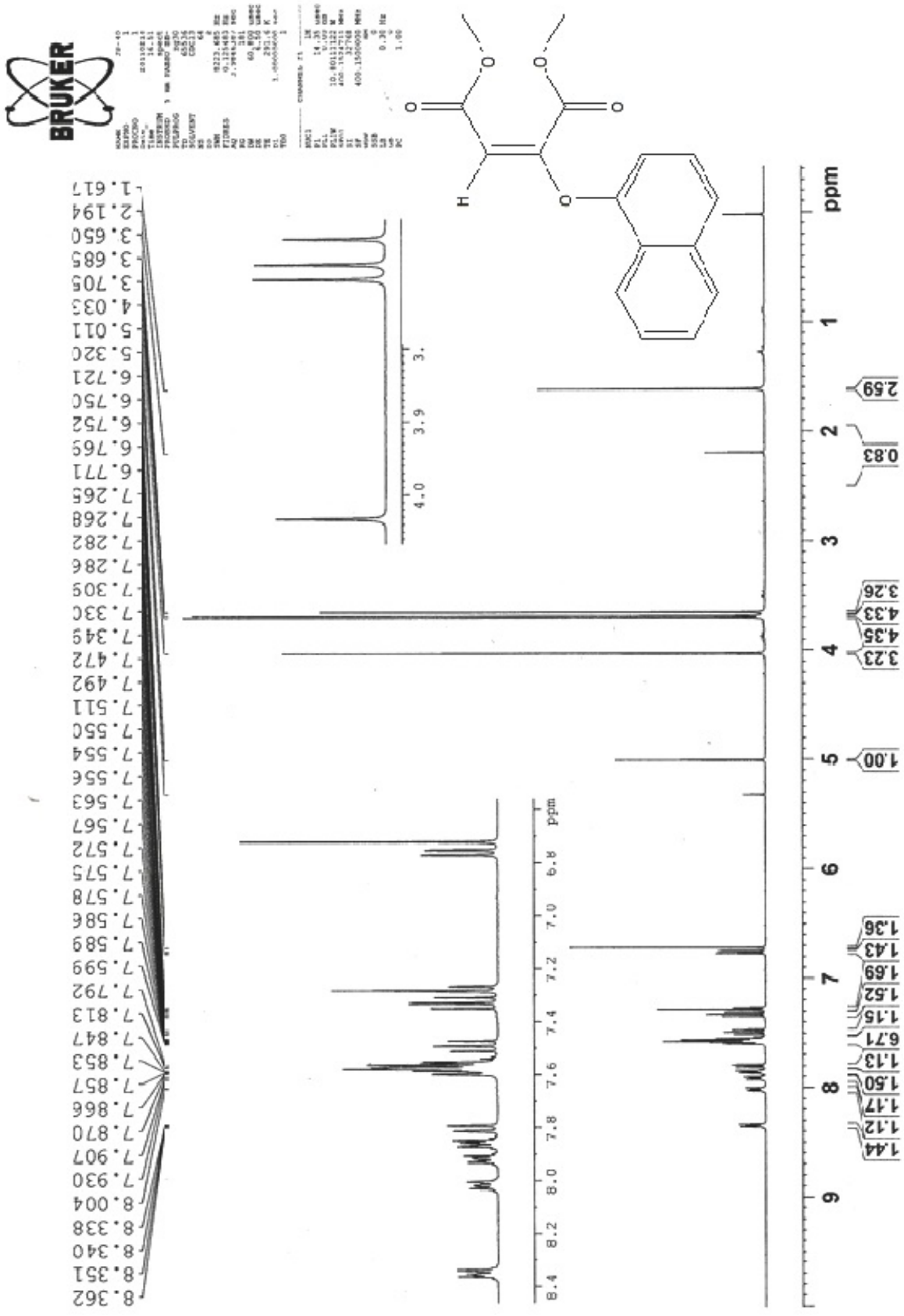


Figure 1: ^{1}H NMR of Dimethyl 2-(naphthalen-1-yloxy)maleate **2**

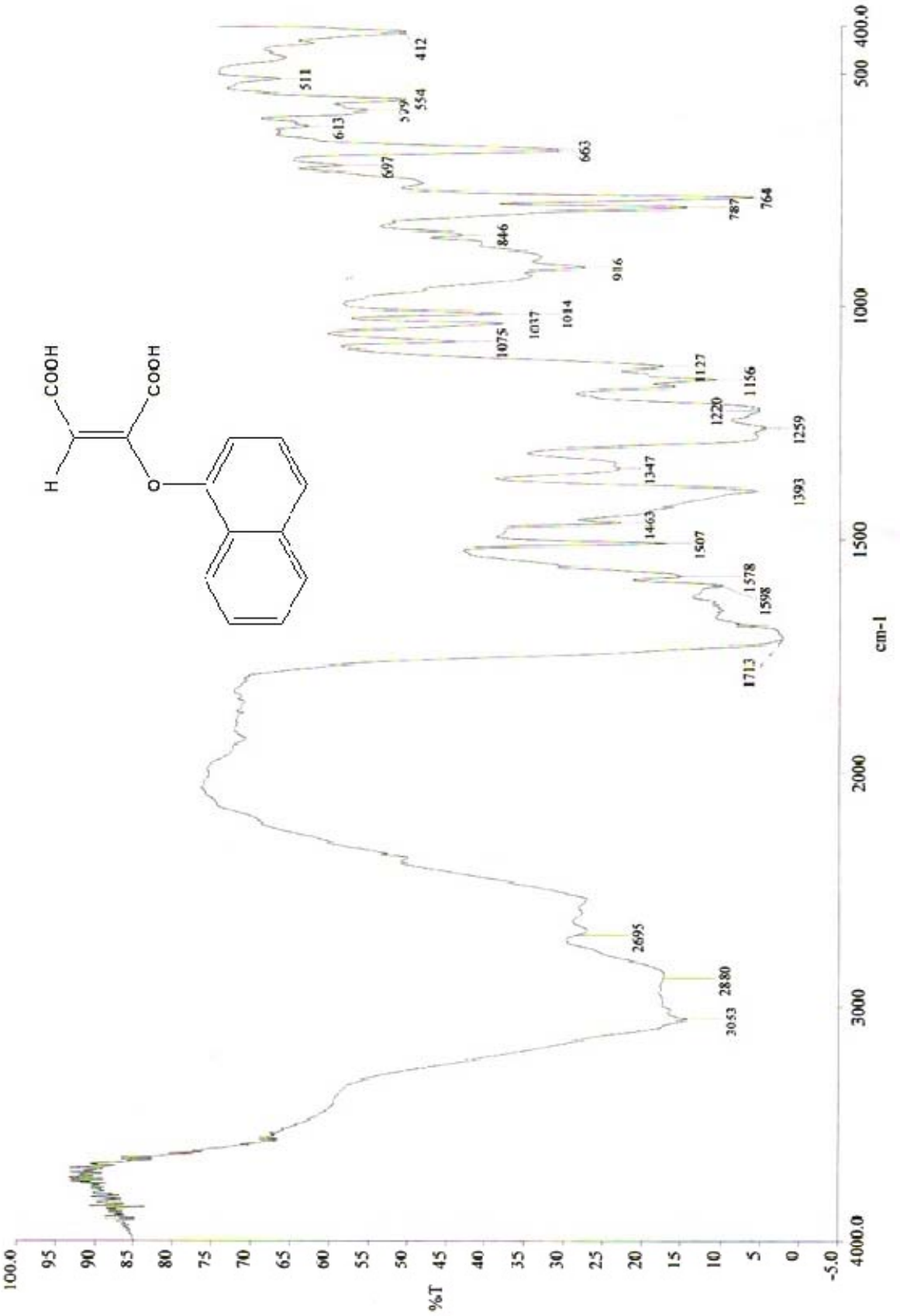


Figure 2: IR of 2-(Naphthalen-1-yloxy)maleic acid **3**

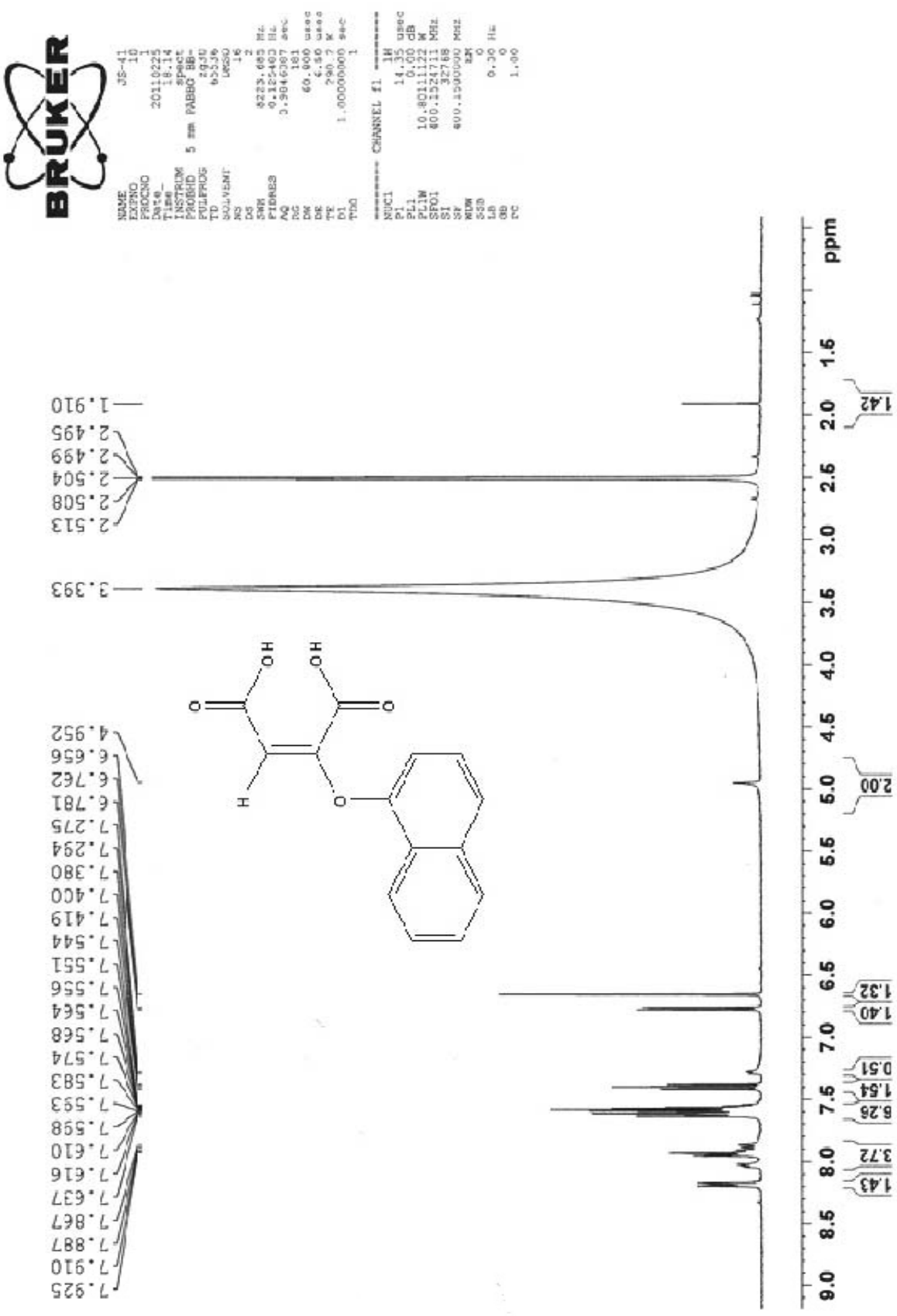


Figure 3: ^{1}H NMR of 2-(Naphthalen-1-yloxy)maleic acid **3**

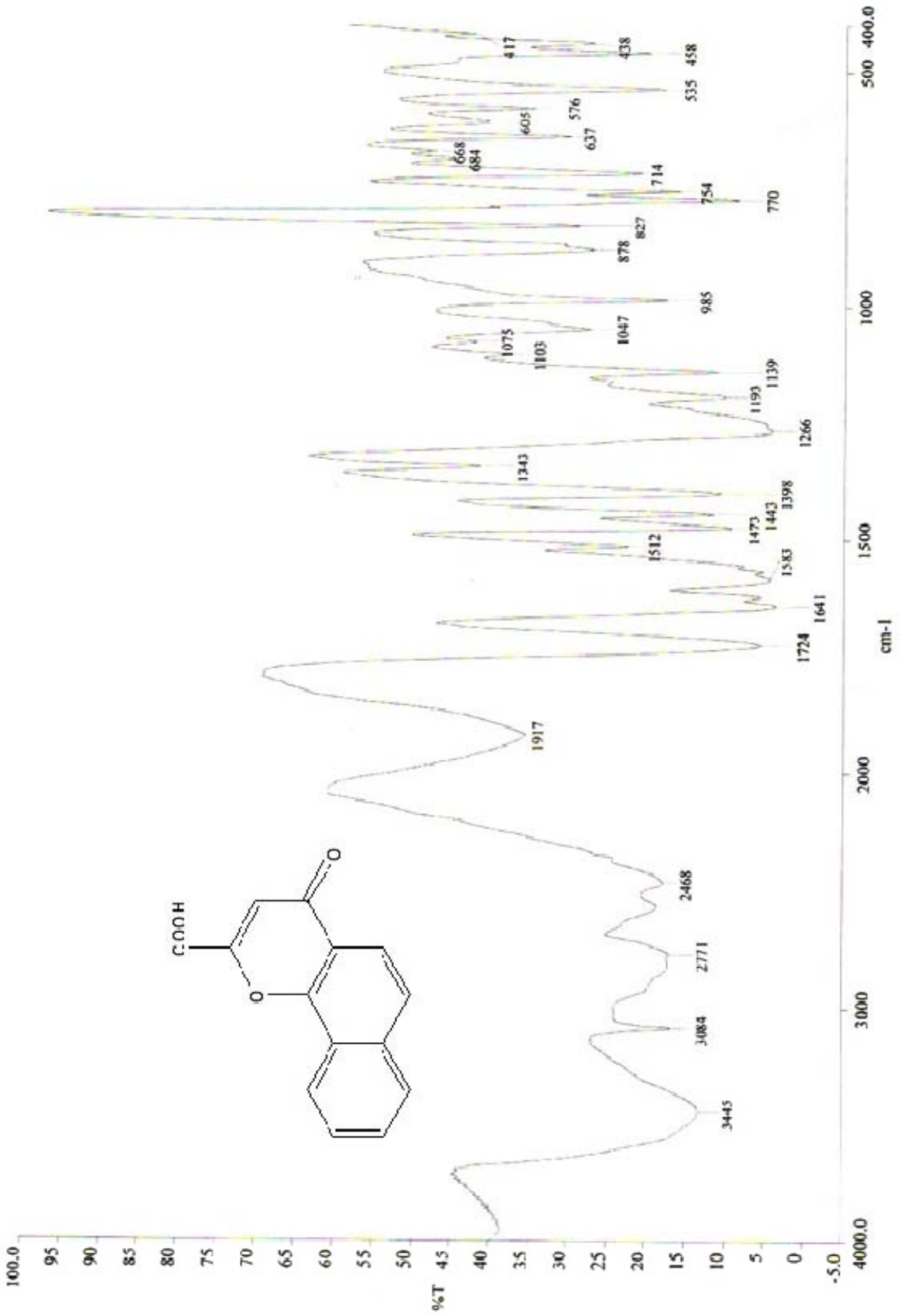


Figure 4: IR of 4-Oxo-4H-benzo[h]chromene-2-carboxylic acid **4**

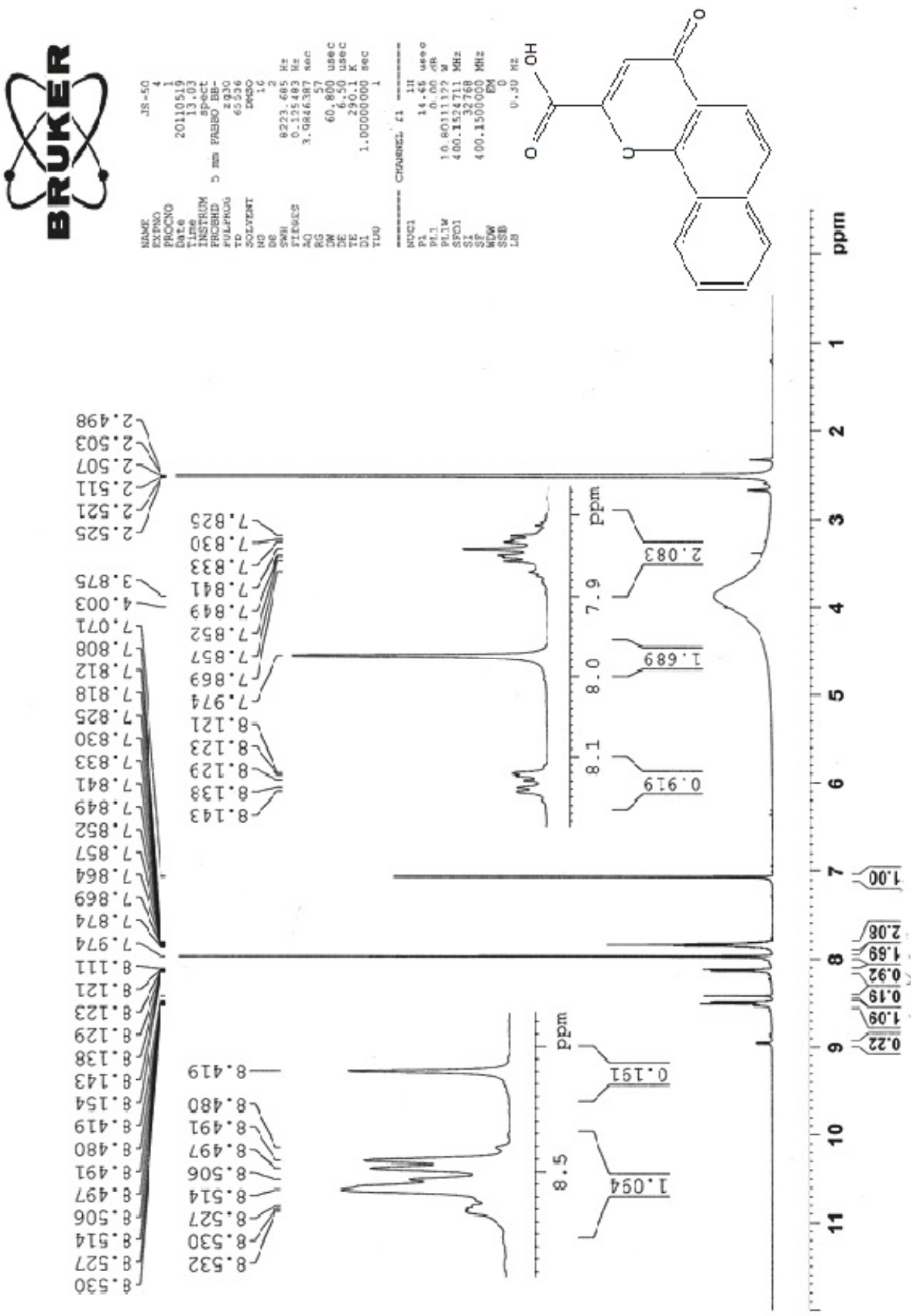


Figure 5: ^{1}H NMR of 4-Oxo-4H-benzo[h]chromene-2-carboxylic acid **4**

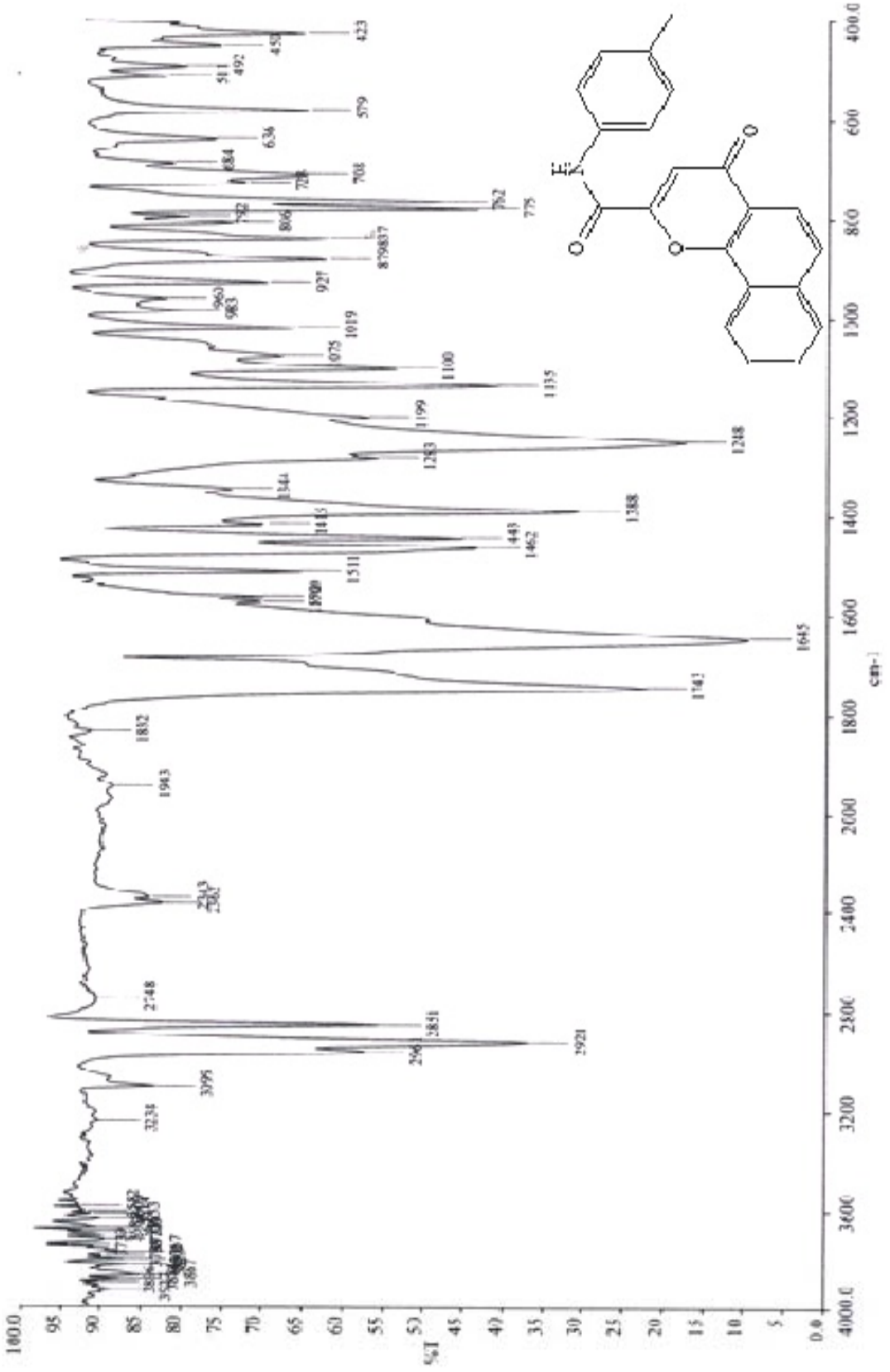


Figure 6: IR of 4-Oxo-N-p-tolyl-4H-benzo[h]chromene-2-carboxamide **5a**

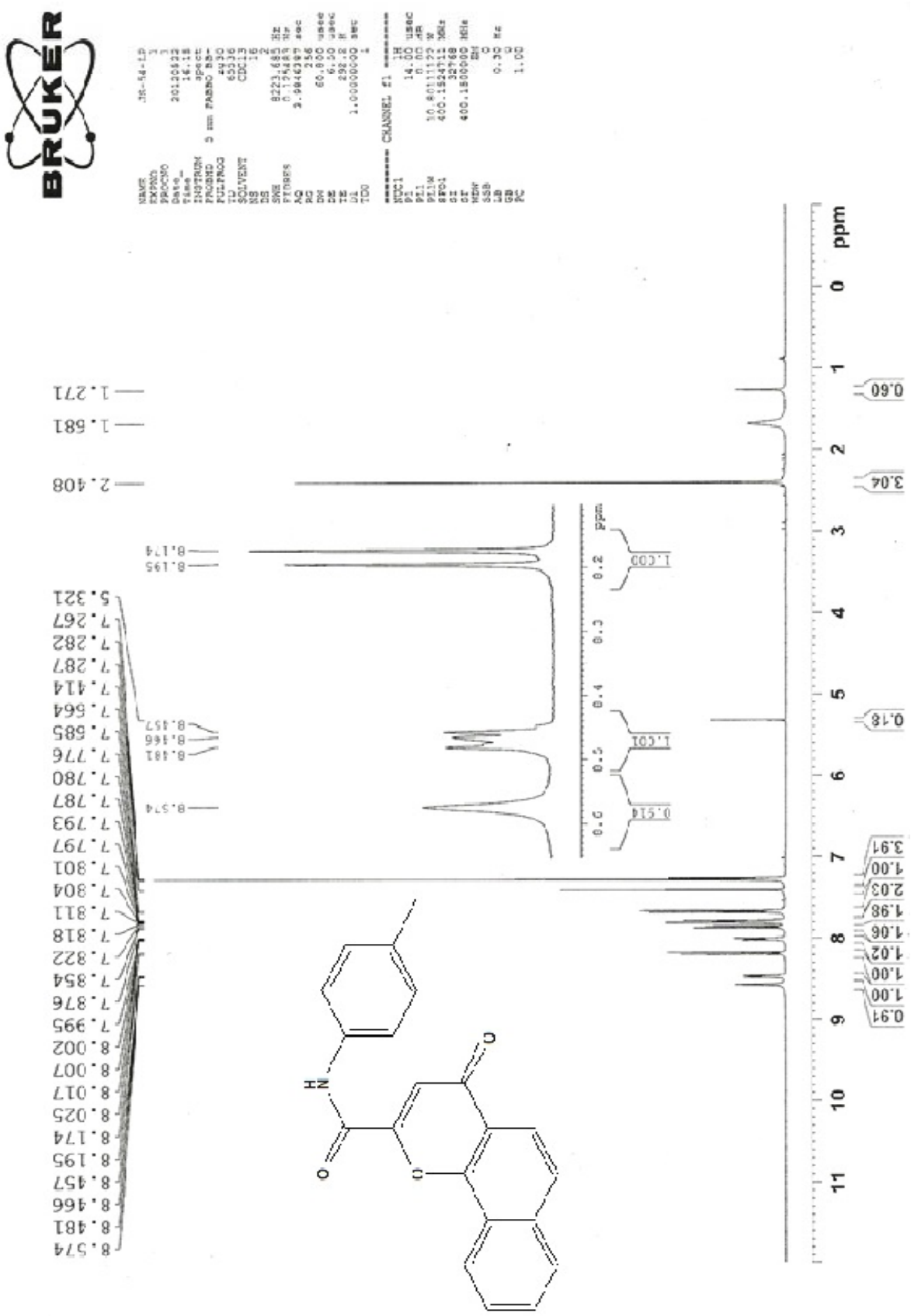


Figure 7: ^{1}H NMR of 4-Oxo-N-p-tolyl-4H-benzo[h]chromene-2-carboxamide **5a**

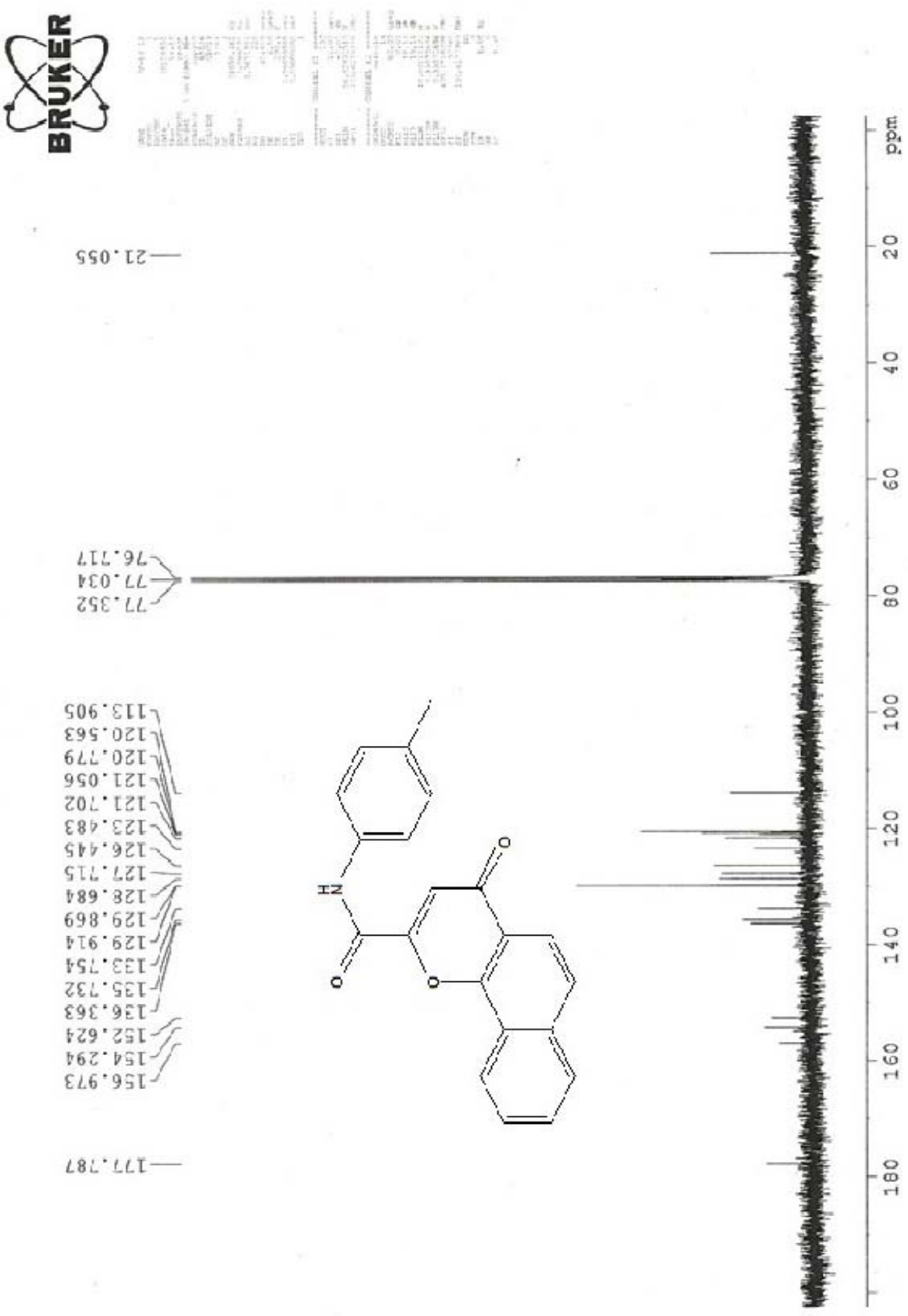


Figure 8: ^{13}C NMR of 4-Oxo-N-p-tolyl-4H-benzo[h]chromene-2-carboxamide **5a**

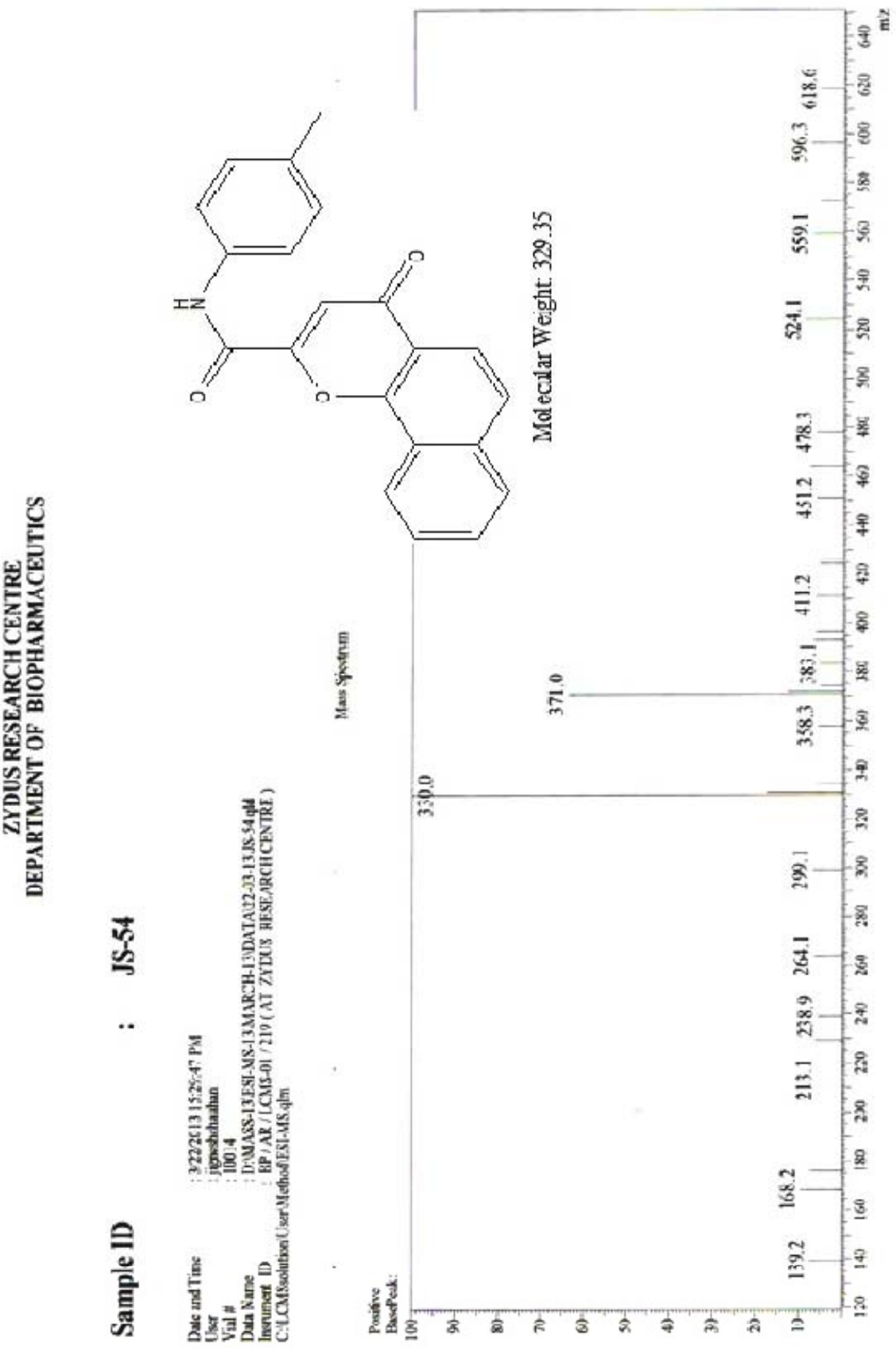


Figure 9: ESI/MS of 4-Oxo-N-p-tolyl-4H-benzo[h]chromene-2-carboxamide **5a**

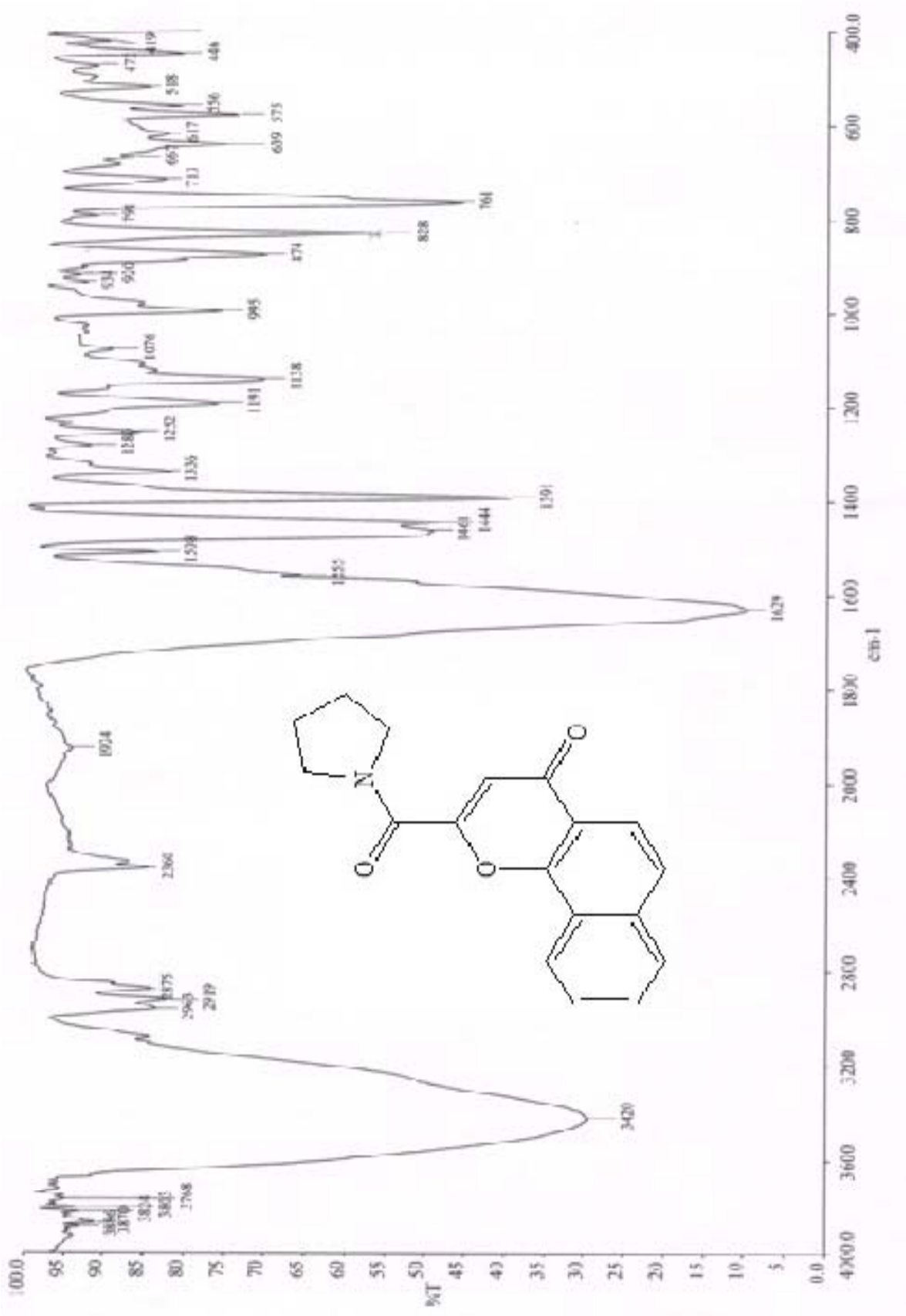


Figure 10: IR of 2-(Pyrrolidine-1-carbonyl)-4H-benzo[h]chromen-4-one **5c**

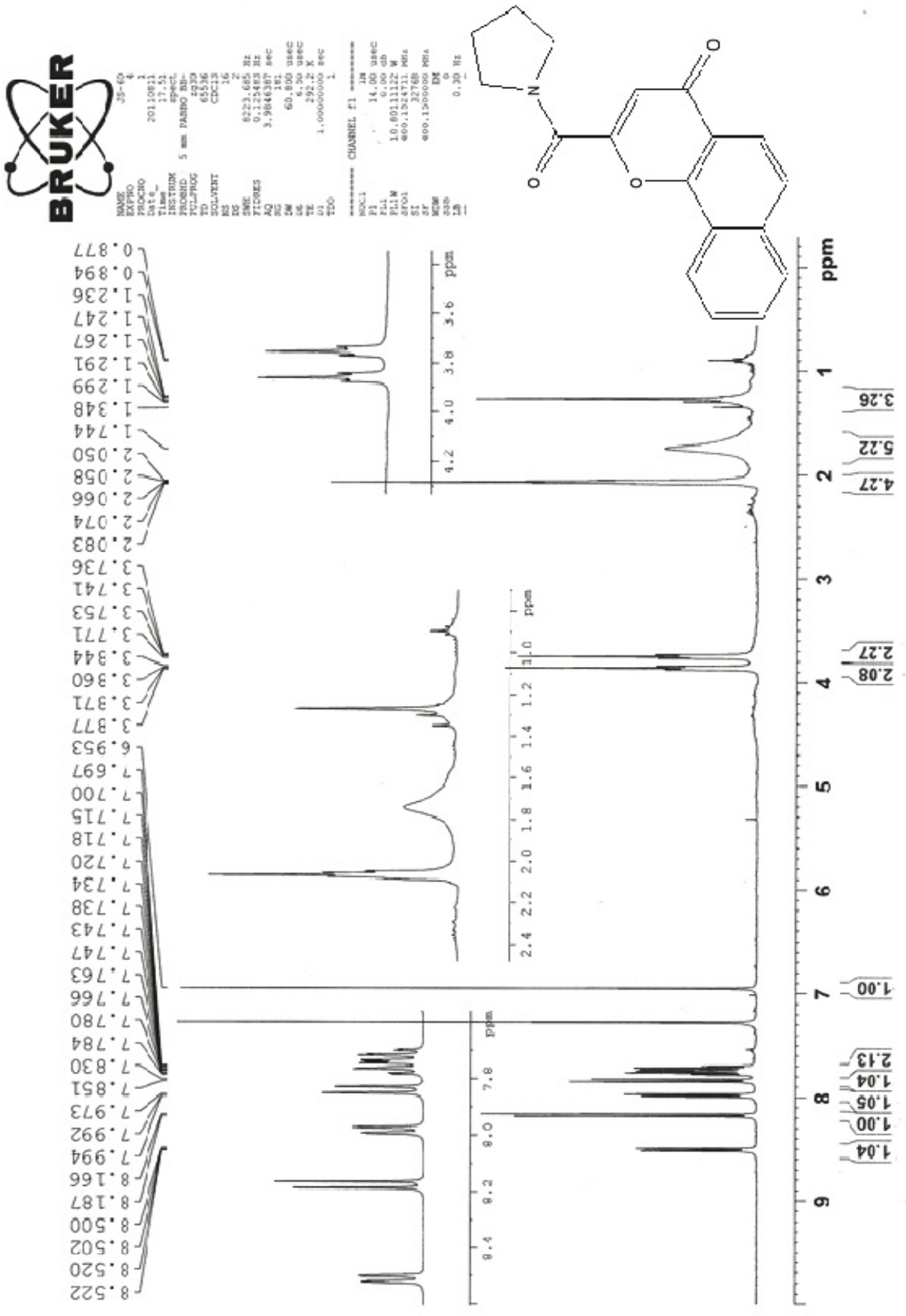


Figure 11: ^{1}H NMR of 2-(Pyrrolidine-1-carbonyl)-4H-benzo[h]chromen-4-one **5c**

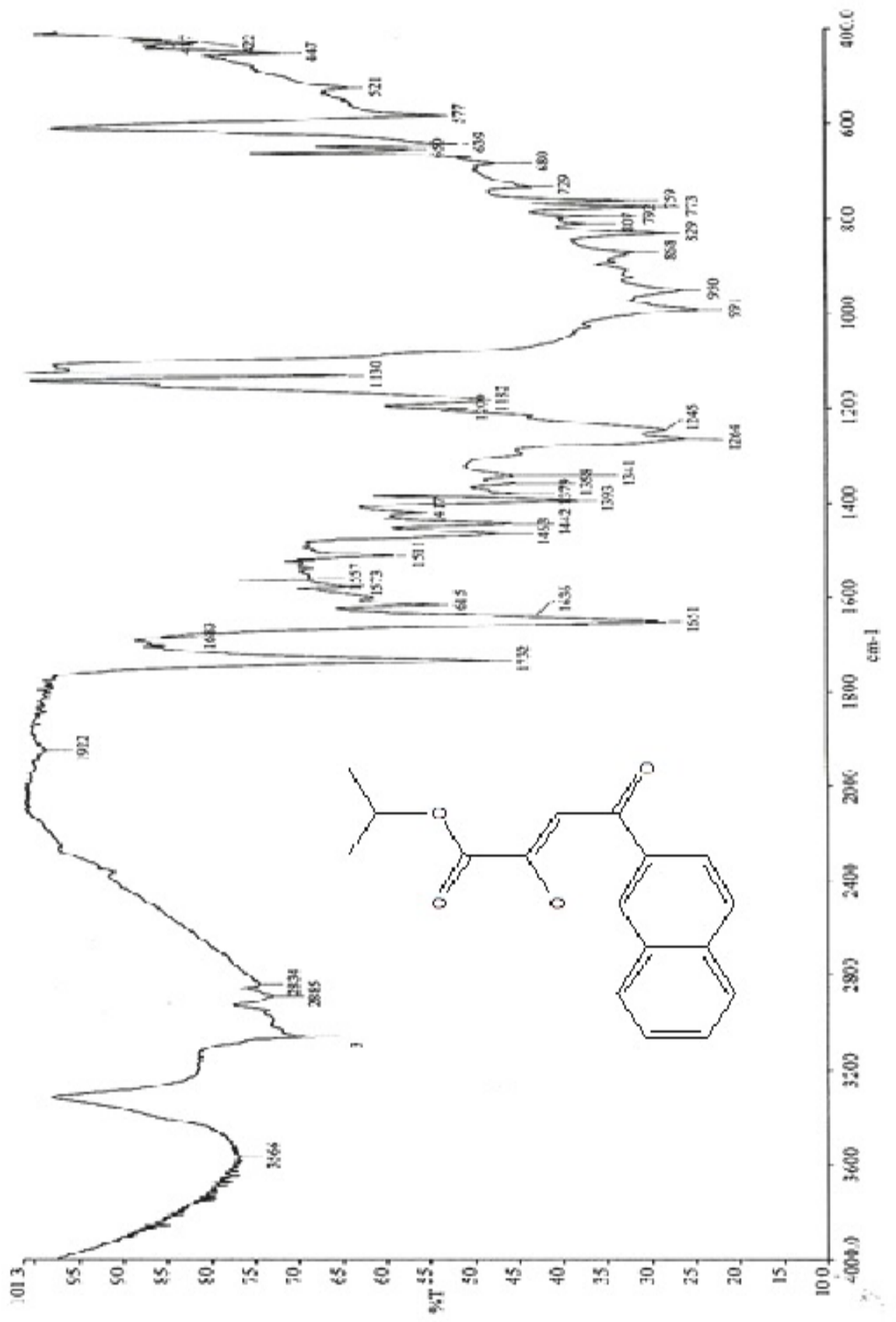


Figure 12: IR of Isopropyl 4-oxo-4H-benzo[h]chromene-2-carboxylate **6c**

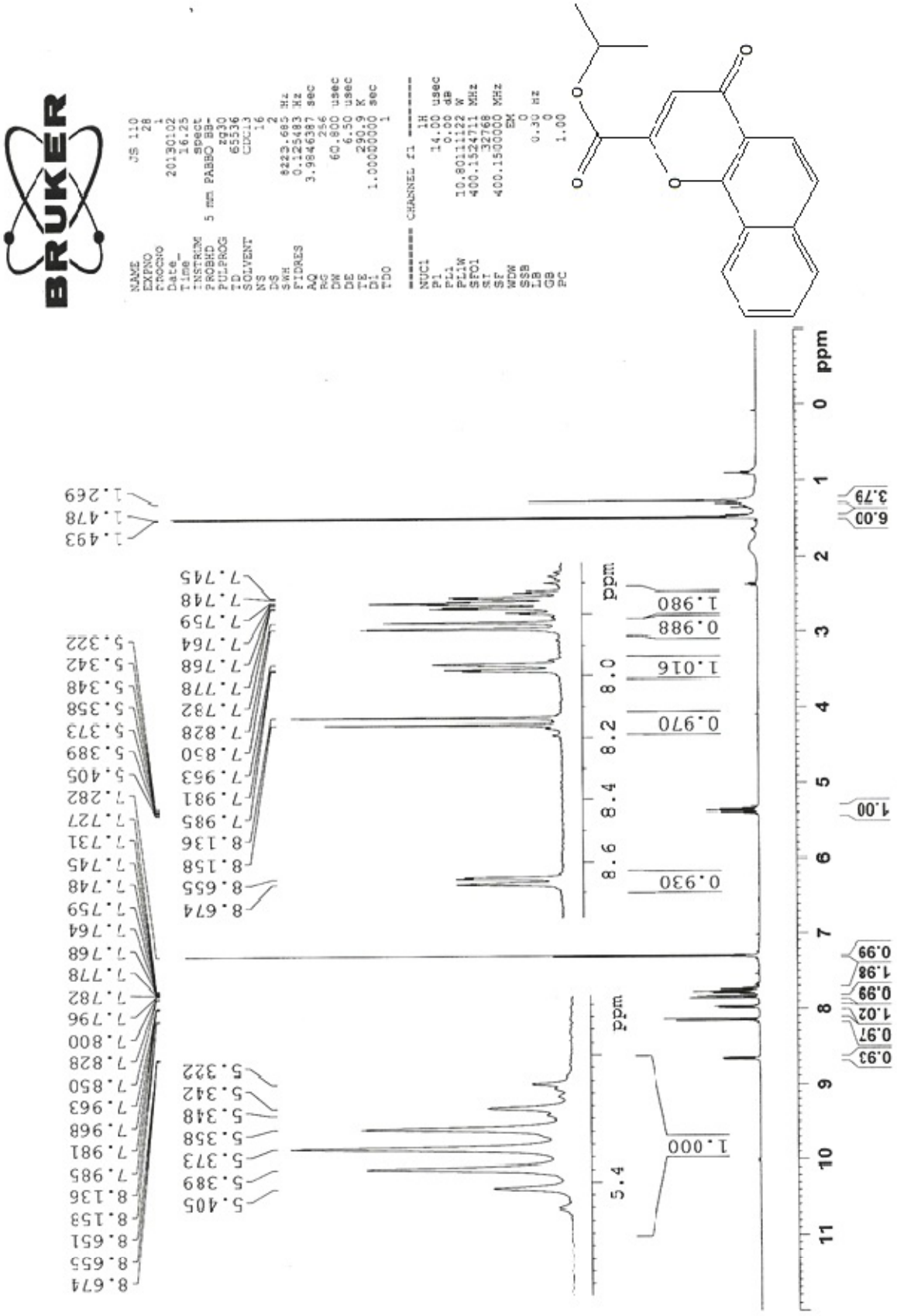


Figure 13: ^{1}H NMR of Isopropyl 4-oxo-4H-benzo[h]chromene-2-carboxylate **6c**

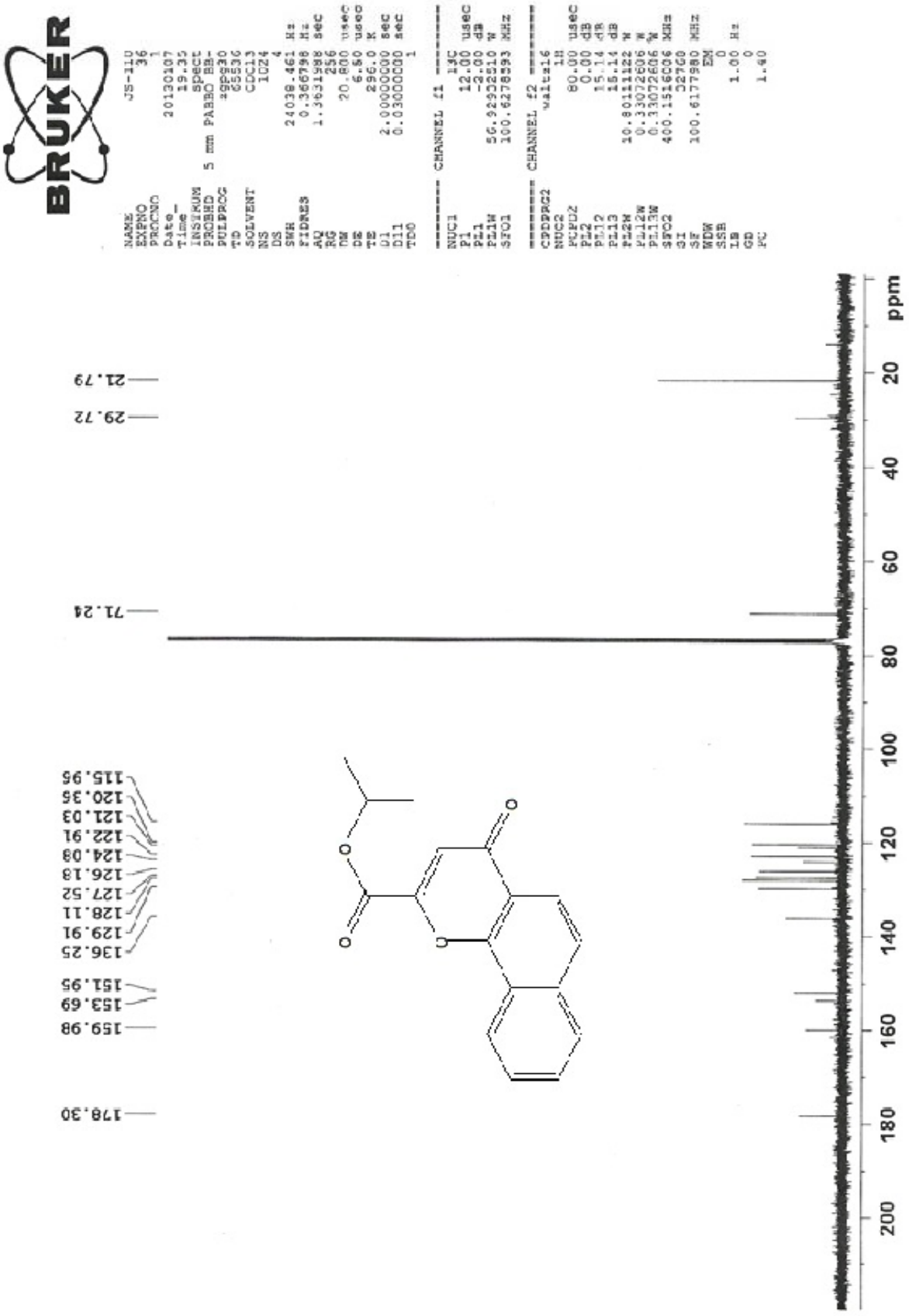


Figure 14: ^{13}C NMR of Isopropyl 4-oxo-4H-benzo[h]chromene-2-carboxylate **6c**

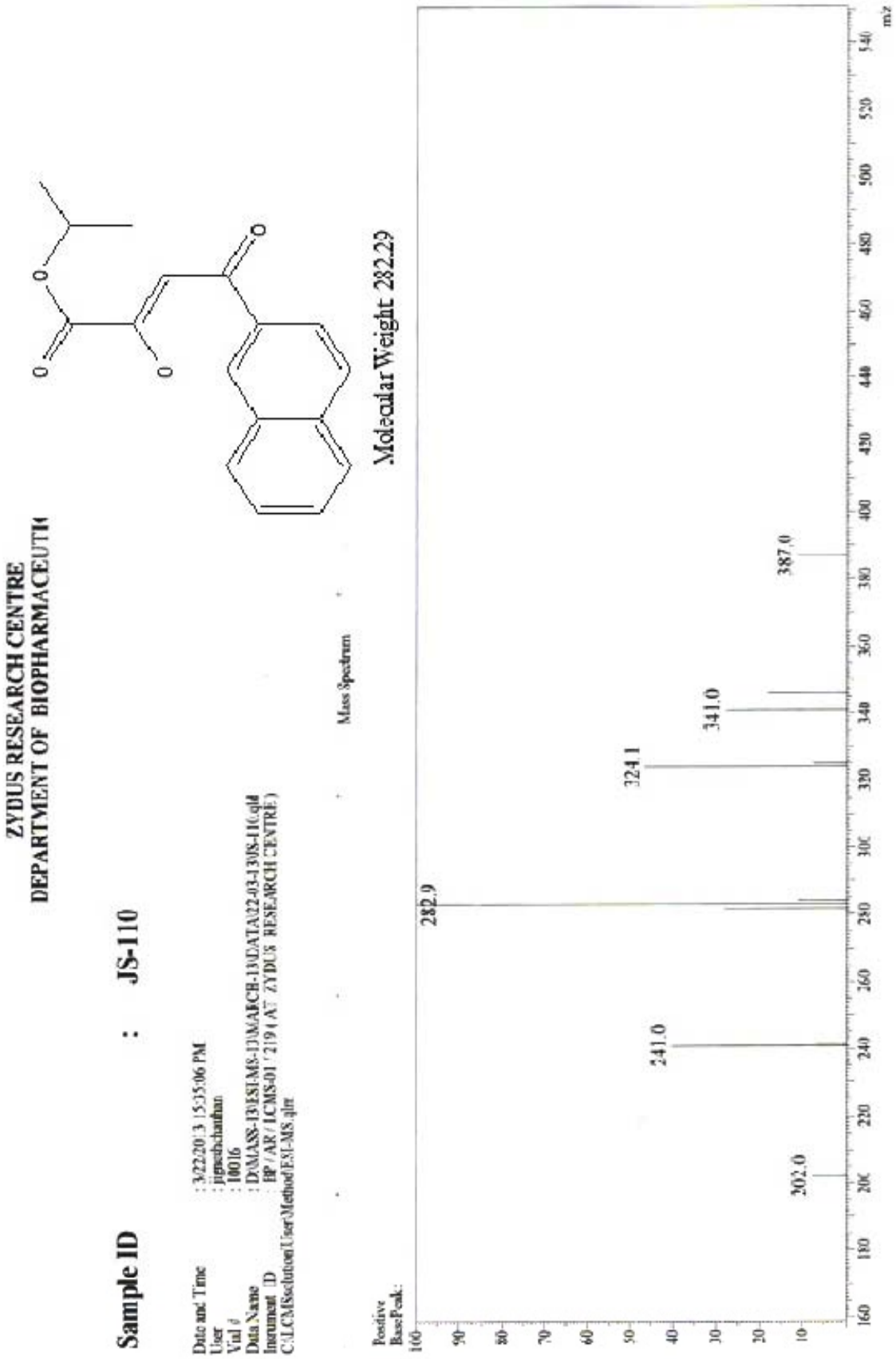


Figure 15: ESI/MS of Isopropyl 4-oxo-4H-benzo[h]chromene-2-carboxylate **6c**

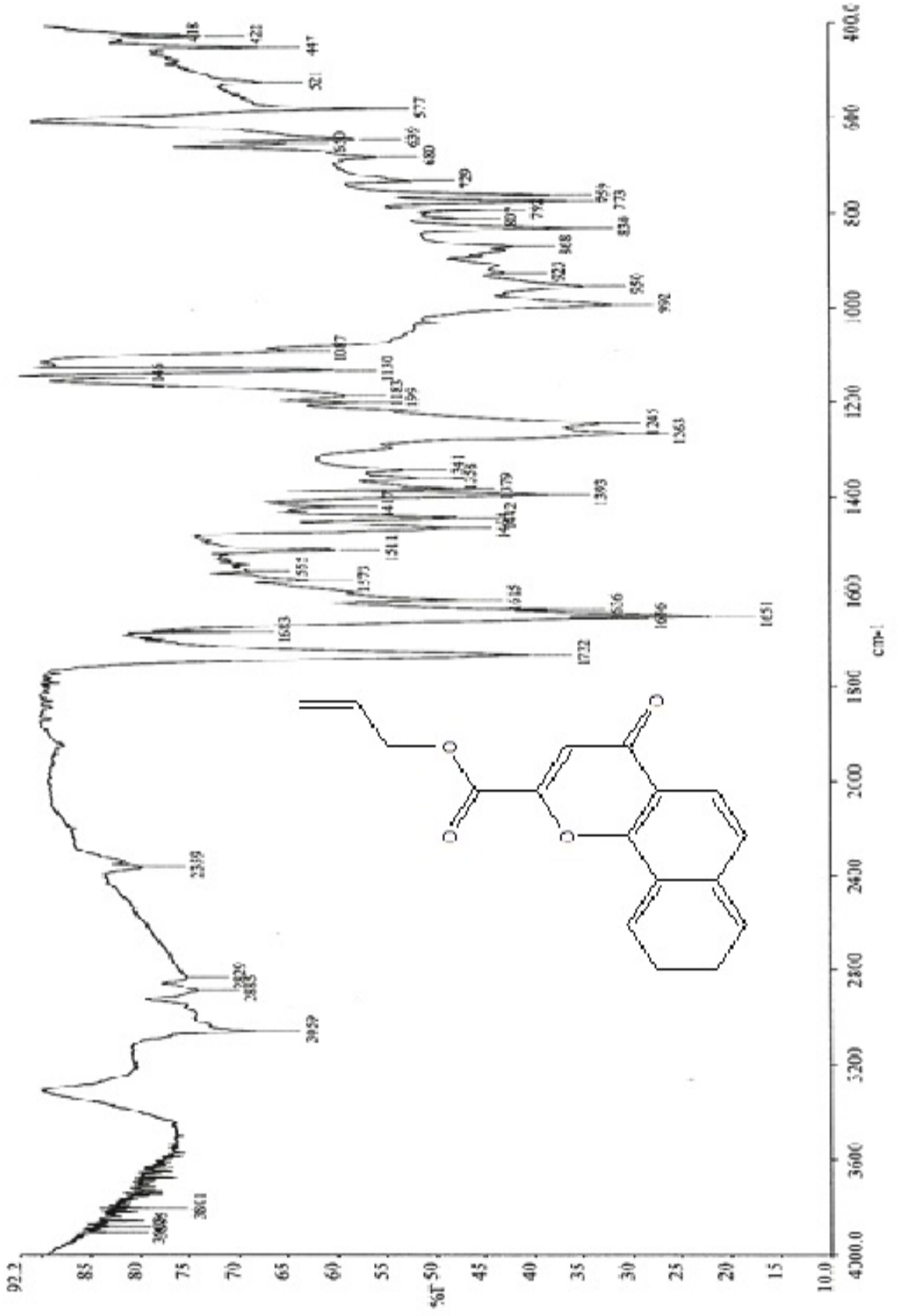


Figure 16: IR of Allyl 4-oxo-4H-benzo[h]chromene-2-carboxylate **6d**

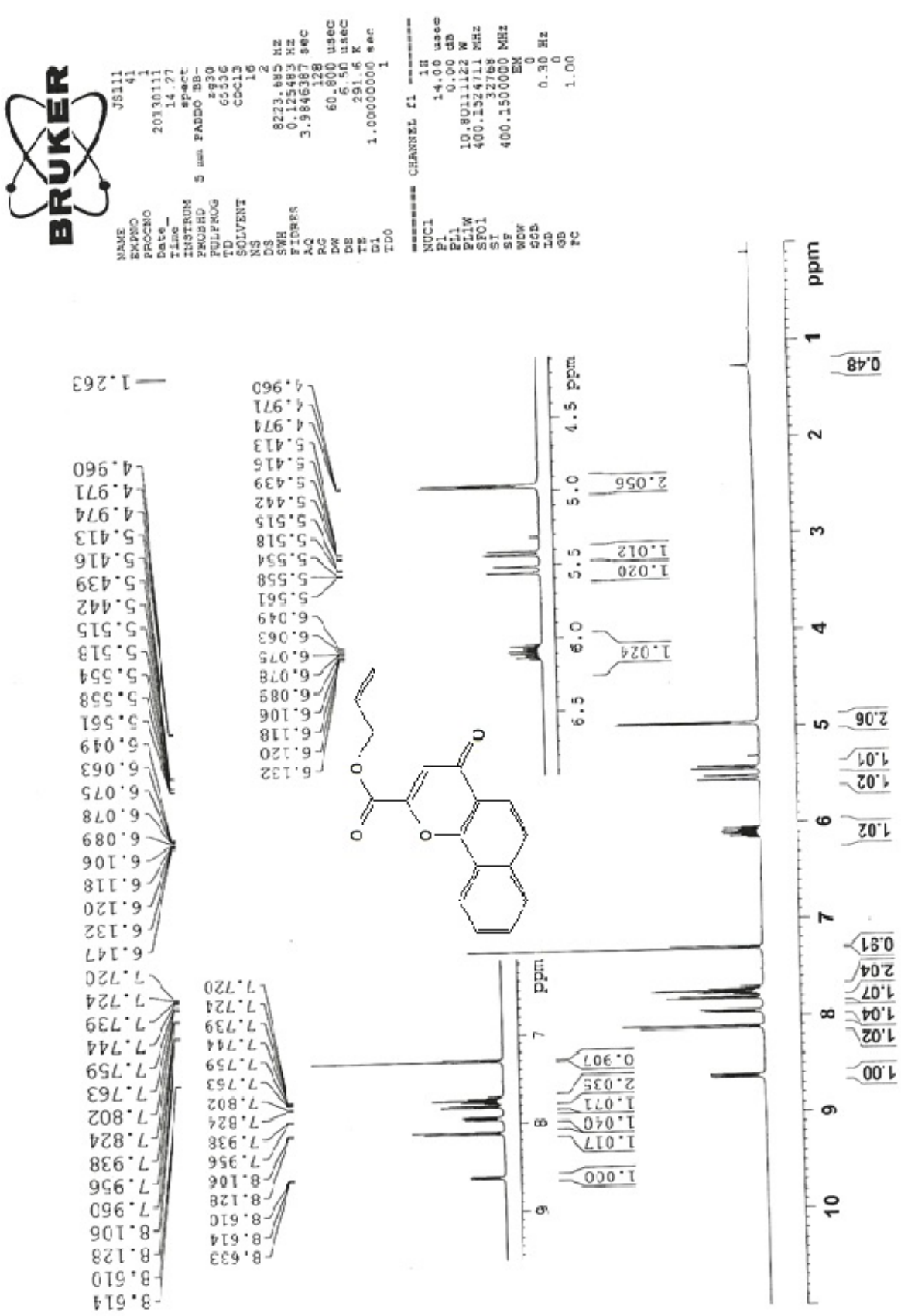


Figure 17: ^{1}H NMR of Allyl 4-oxo-4H-benzo[h]chromene-2-carboxylate **6d**

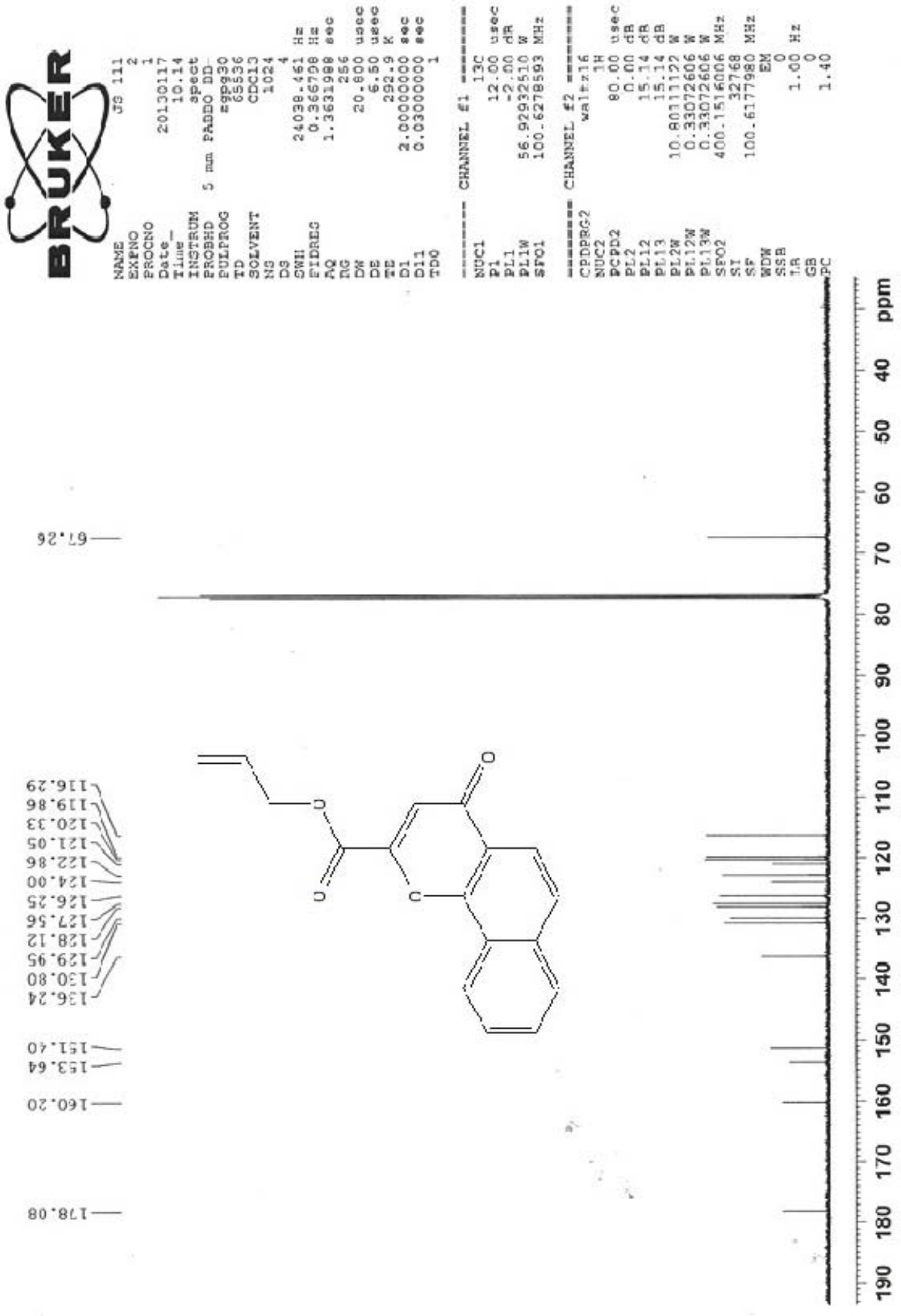


Figure 18: ^{13}C NMR of Allyl 4-oxo-4H-benzo[h]chromene-2-carboxylate **6d**

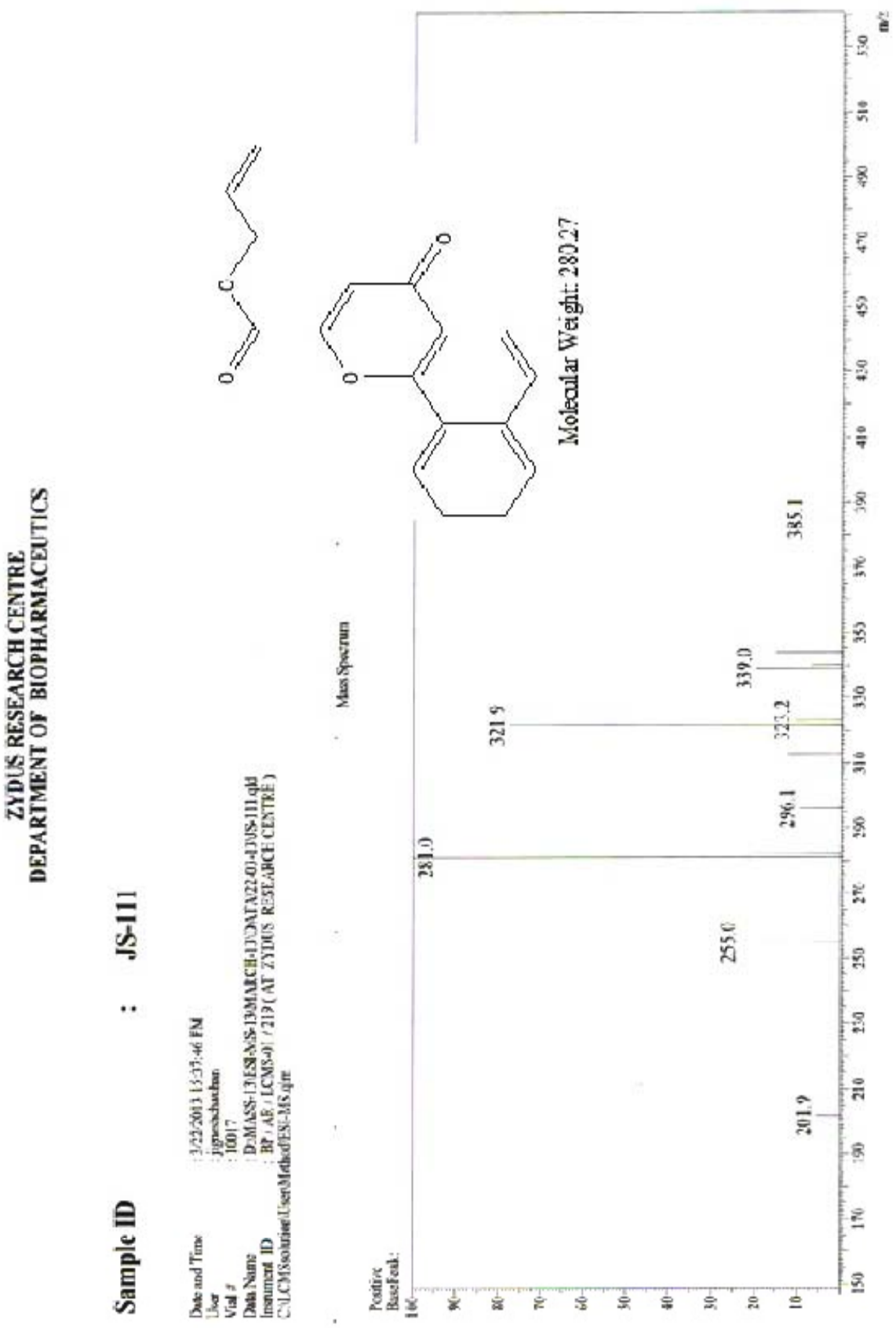


Figure 19: ESI/MS of Allyl 4-oxo-4H-benzo[h]chromene-2-carboxylate **6d**

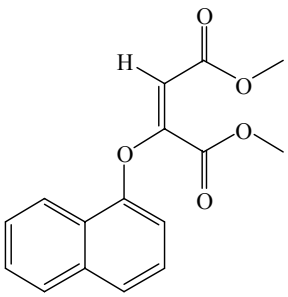
4a.3 Conclusion

We have synthesized naphthopyrone-2-carboxylic acid from corresponding naphthyloxy diacid. The diester **2** obtained by reaction of 1-naphthol with DMAD was found to be inseparable cis and trans mixture. From ^{1}H NMR the trans isomer was found to be major product than cis isomer. The corresponding diacid **3** obtained by hydrolysis of diester **2** with aqueous KOH was also found to be cis and trans mixture. From ^{1}H NMR of both compounds the trans isomer was found to be major product. Hence the yield of naphthopyrone-2-carboxylic acid obtained by cyclization in H_2SO_4 was found to be less. The cyclized naphthopyrone-2-carboxylic acid was converted into corresponding amides using EDC.HCl. Since the yields were very low, the naphthopyrone-2-carboxylic acid was converted into corresponding acid chloride and then treated with amine to get corresponding amide. Naphthopyrone-2-carboxylates were prepared by using various alcohols and passing dry HCl gas.

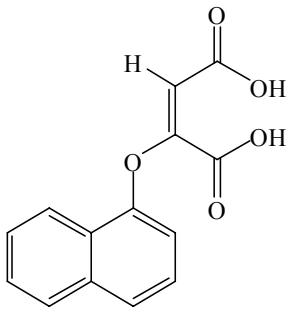
4a.4 Experimental

4a.4.1 Chemistry

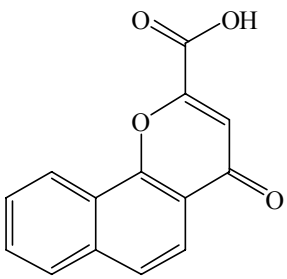
Reagent grade chemicals and solvents were purchased from commercial supplier and used without purification. TLC was performed on silica gel F254 plates (Merck). Acme's silica gel (60-120 mesh) was used for column chromatographic purification. Melting points are uncorrected and were measured in open capillary tubes, using a Rolex melting point apparatus. IR spectra were recorded as KBr pellets on Perkin Elmer RX 1 spectrometer. ^{1}H NMR and ^{13}C NMR spectral data were recorded on Bruker Advance 400 spectrometer (400 MHz) with $CDCl_3$ or DMSO-d_6 as solvent and TMS as internal standard. *J* values are in Hz. Elemental analyses were recorded on Thermosinnigan Flash 11-12 series EA. Mass spectra were determined by ESI/MS, using a Shimadzu LCMS 2020 apparatus.



Dimethyl 2-(naphthalen-1-yloxy)maleate 2/ **2'**: To the solution of 1-naphthol **1** (5 g, 3.47 mmol) in dry acetone (50 ml), anhydrous potassium carbonate (16.77 g, 12.13 mmol), a solution of dimethyl acetylenedicarboxylate (4.26 ml, 3.47 mmol) in dry acetone (100 ml) was added very slowly. Reaction mixture refluxed for 8 hours in water bath. Then poured in to crushed ice and extracted with ethyl acetate gave viscous liquid after removal of ethyl acetate. It was purified by column chromatography using 5% ethyl acetate in petroleum ether gave brown liquid. Yield: 72.5%; IR (Neat): 3053, 2880, 1735 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) (Figure 1): δ 3.65 (3H, s), 3.68 (3H, s), 3.70 (3H, s), 4.03 (3H, s), 5.01 (1H, s, cis vinylic proton), 6.72 (1H, s, trans vinylic proton), 6.75-6.77 (1H, dd, *J*= 8.0 Hz, ArH), 7.26-7.29 (1H, m, ArH), 7.31-7.35 (1H, t, *J*= 7.6, 8.4 Hz, ArH), 7.47-7.51 (1H, t, *J*= 7.6, 8.4 Hz, ArH), 7.55-7.60 (5H, m, ArH), 7.79-7.81 (1H, d, *J*= 8.4 Hz, ArH), 7.84-7.87 (1H, m, ArH), 7.91-7.93 (1H, m, ArH), 8.00-8.04 (1H, m, ArH), 8.34-8.36 (1H, m, ArH).

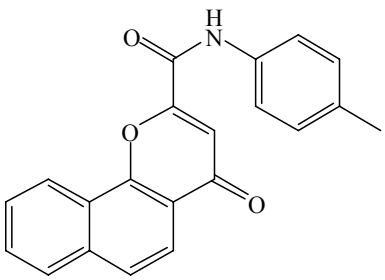


2-(Naphthalen-1-yloxy)maleic acid 3/ **3':** The adduct **2**/ **2'** (7.2 g, 2.51 mmol) was stirred with 1% aqueous solution of KOH (350 ml) for 3 hours and then left at room temperature for 12 h. Reaction mixture was extracted with dichloromethane (50 ml) thrice, the aqueous solution acidified with concentrated hydrochloric acid till pH 2; the solid obtained was filtered, crude product dissolved in saturated sodium bicarbonate, filtered and reprecipitated using concentrated hydrochloric acid to give yellow solid. Yield: 92.4%; mp: 165-170°C; IR (KBr) (Figure 2): 3053, 2880, 1713 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) (Figure 3): δ 4.95 (1H, s, cis vinylic proton), 6.66 (1H, s, trans vinylic proton), 6.76-.678 (1H, d, *J*= 7.6 Hz, ArH), 7.38-7.42 (2H, m, ArH), 7.54-7.64 (6H, m, ArH), 7.87-8.04 (4H, m, ArH), 8.17-8.19 (1H, m, ArH).



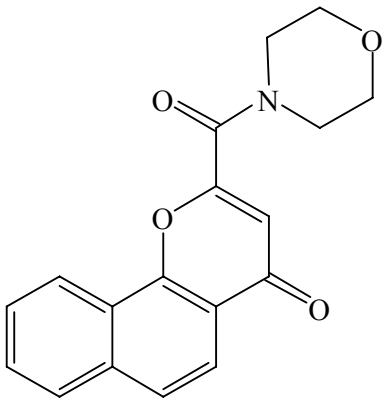
4-Oxo-4H-benzo[h]chromene-2-carboxylic acid 4: Mixture of **3**/ **3'** (1 g, 0.38 mmol) and concentrated sulphuric acid (10 ml) heated at 60-70ºC for 2 h. The reaction mixture poured into ice. The solid product filtered, washed with plenty of water and dried which gave yellow solid. Yield: 21.5%; mp: 148-150°C; IR (KBr) (Figure 4): 3445, 3084, 2984, 1724, 1641 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) (Figure 5): δ 7.07 (1H, s, ArH), 7.81-7.87 (2H, m ArH), 7.97 (2H, s, ArH), 8.12-8.15 (1H, m, ArH), 8.48-8.53 (1H, m, ArH).

General method for 5a-5b: In a solution of **4** (0.5 g, 0.21 mmol) in dry THF (50 ml) EDC.HCl (0.4 g, 0.21 mmol), DMAP (0.05 g, 10%), triethylamine (0.61 ml, 0.43 mmol) and p-toluidine/ morpholine (0.21 mmol) were added. Reaction mass stirred at room temperature for 8 h then poured into ice and extracted with ethyl acetate. After removal of solvent it gave brown liquid. Crude product was purified by column chromatography using 5% ethyl acetate in petroleum ether.

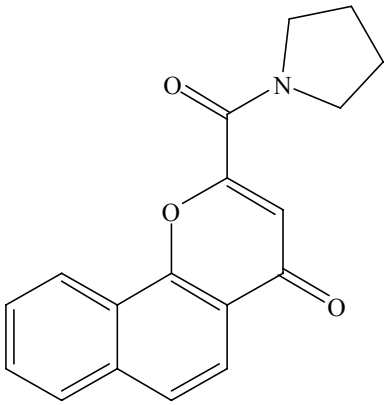


4-Oxo-N-p-tolyl-4H-benzo[h]chromene-2-carboxamide 5a: This compound obtained as brown solid. Yield: 14%; mp: 205-210°C; IR (KBr) (Figure 6): 3348, 3063, 2919, 2855, 1694, 1651 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) (Figure 7): δ 2.41 (3H, s, CH_3), 7.27-7.29 (2H, d, *J*= 8 Hz, ArH), 7.41 (1H, s, ArH), 7.66-7.68 (2H, d, *J*= 8.4 Hz, ArH), 7.78-7.82 (2H, m, ArH), 7.85-7.88 (1H, d, *J*= 8.8 Hz, ArH), 7.99-8.02 (1H, m, ArH), 8.17-8.20 (1H, d, *J*= 8.4 Hz, ArH), 8.46-8.48 (1H, dd, *J*= 3.6, 6.0 Hz, ArH), 8.57 (1H, s, NH); ^{13}C NMR (400 MHz, $CDCl_3$) (Figure 8): δ 21.0, 113.9, 120.6, 120.8, 121.1, 121.7, 123.5, 126.4, 127.7, 128.7, 129.8, 129.9, 133.7, 135.7, 136.4, 152.6, 154.3, 157.0, 177.8; MS (ESI, *m/z*) (Figure 9) 330.0 $[M+1]^+$ calculated for

$C_{21}H_{15}NO_3$; Ele. Anal. Calcd. for $C_{21}H_{15}NO_3$; Requires (Found) %: C, 76.58 (76.45); H, 4.59 (4.69); N, 4.25 (4.41).



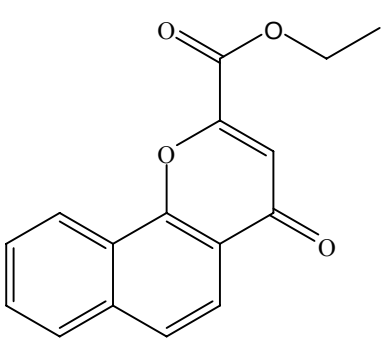
2-(Morpholine-4-carbonyl)-4H-benzo[h]chromen-4-one 5b: This compound obtained as brown solid. Yield: 12%; mp: 96-100°C; IR (KBr): 3099, 2961, 2921, 2851, 1743, 1645 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 2.03-2.06 (4H, m, -N- CH_2), 3.67-3.70 (2H, t, -O- CH_2), 4.51-4.54 (2H, t, -O- CH_2), 7.30 (1H, s, ArH), 7,73-7.80 (2H, m, ArH), 7.84-7.86 (1H, d, *J*= 8.8 Hz, ArH), 7.97-7.99 (1H, dd, *J*= 1.6, 6.8 Hz, ArH), 8.14-8.16 (1H, d, *J*= 8.8 Hz, ArH), 8.64-8.66 (1H, dd, *J*= 1.6, 7.6 Hz); Ele. Anal. Calcd. for $C_{18}H_{15}NO_4$; Requires (Found) %: C, 69.89 (70.15); H, 4.89 (4.63); N, 4.53 (4.71).



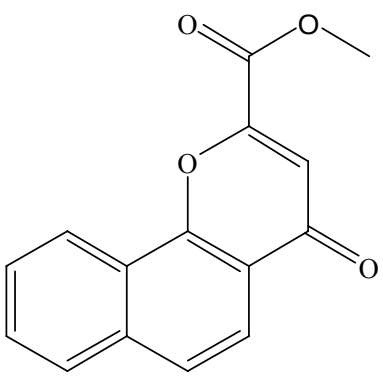
2-(Pyrrolidine-1-carbonyl)-4H-benzo[h]chromen-4-one 5c: 4 (0.5 g, 0.21 mmol) dissolved in dichloromethane (50 ml) and dimethylformamide (2-3 drops), oxalyl chloride (0.79 ml, 0.83 mmol) added slowly to the reaction flask and stirred at room temperature for 2 h. Solvent removed under reduced pressure. The acid chloride obtained dissolved in dichloromethane (50 ml), triethylamine (0.56 ml, 0.40 mmol) and pyrrolidine (0.17 ml, 0.21 mmol) were added and stirred at room temperature for 8 h. Reaction mixture poured into water and extracted with dichloromethane (50 ml) thrice. Solvent removed under vacuum and compound purified by column chromatography using 5% ethyl acetate in petroleum ether gave yellow solid. Yield: 23%; mp: 105-110°C; IR (KBr) (Figure 10): 2963, 2919, 2875, 1629 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) (Figure 11): δ 2.05-2.08 (4H, m, CH_2), 3.74-3.77 (2H, t, CH_2), 3.84-3.88 (2H, t, CH_2), 6.95 (1H, s, ArH), 7.69-7.78 (2H, m, ArH), 7.83-7.85 (1H, d, *J*=

8.4 Hz, ArH), 7.97-7.99 (1H, dd, *J*= 8.0 Hz, ArH), 8.17-8.19 (1H, d, *J*= 8.4 Hz, ArH), 8.50-8.52 (1H, dd, *J*= 8.0 Hz, ArH); Ele. Anal. Calcd. for $C_{18}H_{15}NO_3$; Requires (Found) %: C, 73.71 (73.45); H, 5.15 (5.33); N, 4.78 (4.91).

General Procedure for 6a-6e: Compound **4** (0.1 g, 0.04 mmol) dissolved in different alcohols and dry HCl gas purged for 2 h. Different alcohols removed under vacuum, poured into ice then extracted with dichloromethane, after removal of solvent it gave brown liquid. Crude compound was purified by column chromatography using 5% ethyl acetate in petroleum ether to obtain corresponding ester derivatives.

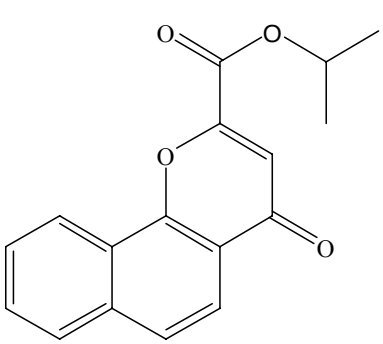


Ethyl 4-oxo-4H-benzo[h]chromene-2-carboxylate 6a: This compound obtained as yellow solid. Yield: 27%; mp: 126-128°C; IR (KBr): 2994, 2904, 1739, 1651 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 1.49-1.53 (3H, t, CH_3), 4.53-4.55 (2H, q, CH_2), 7.29 (1H, s, ArH), 7.73-7.77 (2H, m, ArH), 7.81-7.83 (1H, d, *J*= 8.8 Hz, ArH), 7.94-7.97 (1H, dd, *J*= 1.6, 7.2 Hz, ArH), 8.12-8.14 (1H, d, *J*= 8.4 Hz, ArH), 8.63-8.65 (1H, dd, *J*= 1.6, 7.6 Hz, ArH); ^{13}C NMR (400 MHz, $CDCl_3$): δ 14.2, 63.0, 116.1, 120.3, 121.0, 122.9, 124.0, 126.2, 127.5, 128.1, 129.9, 136.2, 151.6, 153.6, 160.5, 178.2; MS (ESI, *m/z*) 269.0 $[M+1]^+$ calculated for $C_{16}H_{12}O_4$; Ele. Anal. Calcd. for $C_{16}H_{12}O_4$; Requires (Found) %: C, 71.64 (71.35); H, 4.51 (4.72).

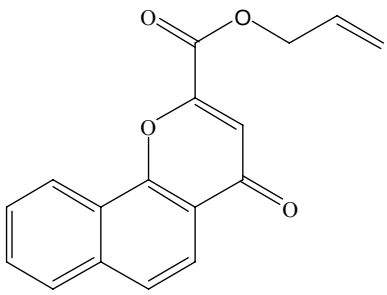


Methyl 4-oxo-4H-benzo[h]chromene-2-carboxylate 6b: This compound obtained as yellow solid. Yield: 24%; mp: 148-150°C; IR (KBr): 3072, 2956, 2922, 2853, 1746, 1655 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 4.01 (3H, s, $-OCH_3$), 7.29 (1H, d, *J*= 1.2 Hz, ArH), 7.72-7.79 (2H, m, ArH), 7.82-7.84 (1H, d, *J*= 8.8 Hz, ArH), 7.95-7.98 (1H, dd, *J*= 1.6, 7.2Hz, ArH), 8.13-8.15 (1H, d, *J*= 8.8 Hz, ArH), 8.64-8.65 (1H, s, ArH);

^{13}C NMR (400 MHz, $CDCl_3$): δ 53.6, 116.2, 120.3, 121.0, 122.8, 123.9, 126.2, 127.5, 128.1, 129.9, 136.2, 151.3, 153.6, 161.0, 178.0; Ele. Anal. Calcd. for $C_{15}H_{10}O_4$; Requires (Found) %: C, 70.86 (70.55); H, 3.96 (4.29).

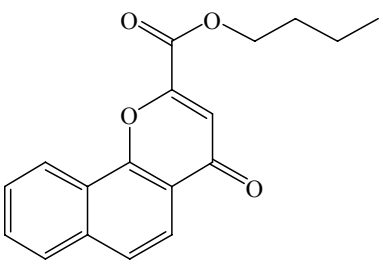


Isopropyl 4-oxo-4H-benzo[h]chromene-2-carboxylate 6c: This compound obtained as yellow solid. Yield: 23%; mp: 122-124°C; IR (KBr) (Figure 12): 3059, 2885, 2834, 1732, 1683 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) (Figure 13): δ 1.48 (3H, s, CH_3), 1.49 (3H, s, CH_3), 5.32-5.40 (1H, m, CH), 7.73-7.73 (1H, d, *J*= 1.6 Hz, ArH), 7.74-7.78 (2H, m, *J*= 1.6, 8.8 Hz, ArH), 7.83-7.85 (1H, d, *J*= 8.8 Hz, ArH), 7.96-7.99 (1H, dd, *J*= 1.6, 8.8 Hz, ArH), 8.14-8.16 (1H, d, *J*= 8.8 Hz, ArH), 8.65-8.67 (1H, d, *J*= 7.6 Hz, ArH); ^{13}C NMR (400 MHz, $CDCl_3$) (Figure 14): δ 21.8, 29.7, 71.2, 116.0, 120.4, 121.0, 122.9, 124.1, 126.2, 127.5, 128.1, 129.9, 136.2, 151.9, 153.7, 160.0, 178.3; MS (ESI, *m/z*) (Figure 15) 282.9 $[M+1]^+$ calculated for $C_{17}H_{14}O_4$; Ele. Ana. for $C_{17}H_{14}O_4$; Requires (Found) %: Cal. C, 72.33 (72.76); H, 5.00 (4.51).



Allyl 4-oxo-4H-benzo[h]chromene-2-carboxylate 6d: This compound obtained as yellow solid. Yield: 34%; mp: 96-98°C; IR (KBr) (Figure 16): 3059, 2885, 2829, 1732, 1683 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) (Figure 17): δ 4.96-4.97 (2H, t, -OCH_2), 5.41-5.44 (1H, dd, vinylic terminal proton), 5.51-5.56 (1H, m, vinylic terminal proton), 6.02-6.13 (1H, m, vinylic proton), 7.30 (1H, s, ArH), 7.72-7.76 (2H, m, ArH), 7.80-7.82 (1H, d, *J*= 8.8 Hz, ArH), 7.94-7.96 (1H, d, *J*= 7.6 Hz, ArH), 8.11-8.13 (1H, d, *J*= 8.8 Hz, ArH), 8.61-8.63 (1H, dd, *J*= 1.6, 7.6 Hz, ArH); ^{13}C NMR (400 MHz, $CDCl_3$) (Figure 18): δ 67.3, 116.3, 119.9, 120.3, 121.0, 122.9, 124.0, 126.2, 127.6, 128.1, 129.9, 130.8, 136.2, 151.4, 153.6, 160.2, 178.1; MS (ESI, *m/z*)

(Figure 19) 281.0 $[M+1]^+$ calculated for $C_{17}H_{12}O_4$; Ele. Anal. Calcd. for $C_{17}H_{12}O_4$; Requires (Found) %: C, 72.85 (72.63); H, 4.32 (4.50).



Butyl 4-oxo-4H-benzo[h]chromene-2-carboxylate 6e: This compound obtained as yellow solid. Yield: 32%; mp: 84-86°C; IR (KBr): 3099, 3056, 2959, 2872, 1744, 1651 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 1.02-1.06 (3H, t, CH_3), 1.52-1.57 (2H, q, CH_2), 1.83-1.86 (2H, m, CH_2), 4.46-4.49 (2H, t, -OCH_2), 7.26 (1H, s, ArH), 7.71-7.75 (2H, m, ArH), 7.77-7.81 (1H, d, *J*= 8.8 Hz, ArH), 7.92-7.95 (1H, dd, *J*= 1.2, 8.8 Hz, ArH), 8.09-8.11 (1H, d, *J*= 8.8 Hz, ArH), 8.59-8.62 (1H, dd, *J*= 1.2, 8.8 Hz, ArH); ^{13}C NMR (400 MHz, $CDCl_3$): δ 13.7, 19.2, 30.5, 66.7, 116.1, 120.3, 121.0, 122.9, 124.0, 126.2, 127.5, 128.1, 129.9, 136.2, 151.6, 153.6, 160.5, 178.2; MS (ESI, *m/z*) 296.9 $[M+1]^+$ calculated for $C_{18}H_{16}O_4$; Ele. Anal. Calcd. for $C_{18}H_{16}O_4$; Requires (Found) %: C, 72.96 (72.67); H, 5.44 (5.61).

4a.5 References

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Chapter 4b

Unusual deacetylation of 1-acetyl 2-naphthol in facile manner

4b. Unusual deacetylation of 1-acetyl 2-naphthol in facile manner

4b.1 Introduction

Acetylation is very common and useful reaction in organic synthesis. Protection of amine and hydroxyl group is very common and usually carried out by acetylation reaction. Acetylation using acetic anhydride or acetyl chloride is the preferred one while literature also reports acetylation reactions using catalyst.[1, 2]

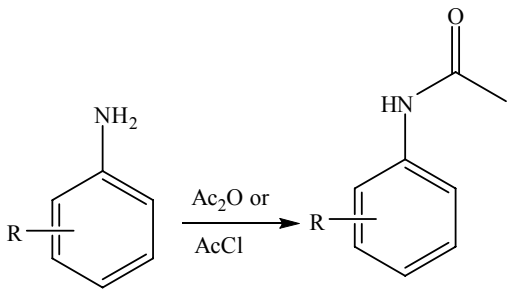
Formation of chalcones[3, 4] or Schiff bases[5-8] from o-hydroxy ketones is one of the most frequently used reaction in organic synthesis because various heterocyclic rings can be formed further from it. Chalcones, chromenes, imidazoles and other heterocyclic rings show various biological activities.[9-12]

We have already reported formation of chalcones[13] from various substituted o-hydroxy acetophenone derivatives[14] and further formation of flavones[13] from it. Hence we were keen to form Schiff bases **4** from 1-acetyl 2-naphthol **2** (Scheme 4) and then further formation of heterocyclic ring. Here we report novel retro Friedel Crafts reaction of 1-acetyl 2-naphthol **2** when we have tried to prepare Schiff bases **4** from 1-acetyl 2-naphthol **2** (Scheme 4).

General methods and recent modifications for acetylation

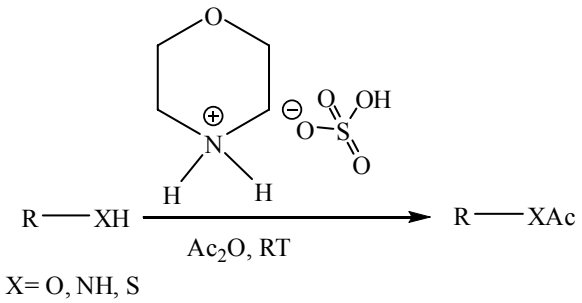
Acetylation using acetic anhydride in presence of solvent or neat is very common in organic synthesis while reaction of amine in presence of base and acetyl chloride is also used in many reactions when compounds are acid sensitive as shown in scheme 1.

Scheme 1:



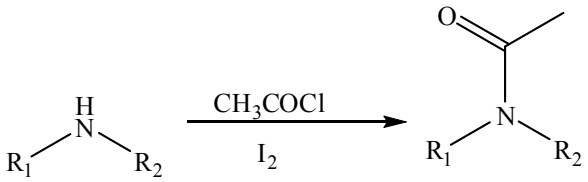
Balaskar R. S. and co workers[15] have synthesized various amide derivatives in short time and higher yields using morpholinium bisulfate as catalyst as shown in scheme 2.

Scheme 2:



Phukan K. and co workers[16] have reported iodine promoted very quick acylation using acetyl chloride as shown in scheme 3.

Scheme 3:

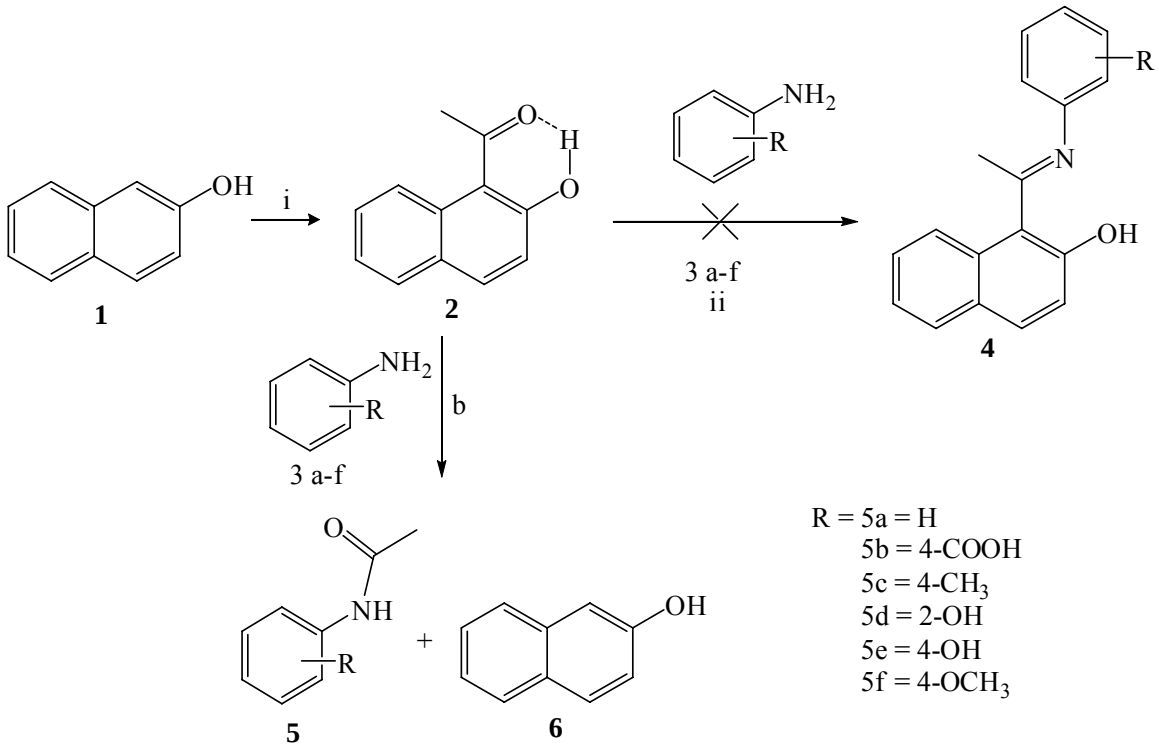


4b.2 Result and discussion

4b.2.1 Chemistry

Reaction of acetic anhydride with 2-naphthol **1** in acetic acid and acetic anhydride in presence of zinc chloride gave 1-acetyl 2-naphthol[17] **2** (Scheme 4). When **2** was refluxed in absolute ethanol with primary aromatic amines (3a-f) and 2-3 drops of acetic acid, we observed formation of two products on TLC, different from starting material. The expected product was to be **4** (Schiff base) but when the products were separated by column chromatography, they were found to be N-acetylated anilines **5a-f** and 2-naphthol **6** as shown in Scheme 4.

Scheme 4:



Reagents and conditions: (i) $ZnCl_2$, acetic acid, acetic anhydride, reflux, 12 h; (ii) ethanol, acetic acid, reflux, 8 h

The IR spectrum of compound **2** (Figure 1) exhibited band at 3431 cm^{-1} for hydroxyl group and 1755 cm^{-1} for carbonyl group (intramolecular hydrogen bonded). In ^1H NMR of compound **2** in $CDCl_3$ (Figure 2), singlet observed at δ 3.03 for three protons indicated presence of -$COCH_3$ group. All aromatic protons were appeared between δ 7.41 to 8.44 confirmed formation of compound **2**.

The IR spectrum of compound **5b** (Figure 3) exhibited bands at 3306 cm^{-1} for hydroxy group and 1681 cm^{-1} for carbonyl group of anilide. The ^1H NMR of compound **5b** in DMSO-d_6 (Figure 4) showed singlet at δ 2.13 for three protons indicated presence of -$COCH_3$ group and two aromatic protons were observed at δ 7.08 while the other two aromatic protons were observed at δ 7.51. Singlet at δ 10.30 for one proton indicated presence of NH proton and broad peak at δ 12.74 for one proton indicated presence of carboxylic acid group thus confirmed the formation of compound **5b**. The ^{13}C NMR

spectrum of compound **5b** in DMSO-d_6 (Figure 5) showed presence of 7 peaks which is consistent with structure of compound **5b**. More over the formation of N-acetylated product was further confirmed by melting point and mix melting point of compound **5b** with the sample **5b** prepared by routine method (reaction of acetic anhydride with para amino benzoic acid) which were found to be same.

The IR spectrum of compound **5e** (Figure 6) exhibited broad band from 3161 to 3326 cm^{-1} for hydroxy group and band at 1657 cm^{-1} for carbonyl group of anilide. The ^1H NMR of compound **5e** in DMSO-d_6 (Figure 7) showed singlet at δ 1.98 for three protons indicated presence of -$COCH_3$ group. Two doublets at δ 6.6 and δ 7.33 for two protons each indicated ortho coupled aromatic protons. Singlet at δ 9.16 and 9.66 for one proton each indicated presence of NH proton and OH proton respectively thus confirmed the formation of compound **5e**. The ^{13}C NMR spectrum of compound **5e** in DMSO-d_6 (Figure 8) showed presence of 6 peaks which is in accordance with the structure of compound **5e**.

The IR spectrum of compound **5f** (Figure 9) exhibited bands at 3191 cm^{-1} for NH group and 1650 cm^{-1} for carbonyl group of anilide. The ^1H NMR of compound **5f** (Figure 10) showed singlet at δ 2.17 for three protons indicated presence of -$COCH_3$ group, singlet at δ 3.79 for three protons indicated presence of methoxy group. Two doublets at δ 6.86 and δ 7.42 for two protons each with *J* value 8.0 Hz indicated ortho coupled aromatic protons. Singlet at δ 7.53 for one proton indicated presence of NH proton thus confirmed the formation of compound **5f**. The ^{13}C NMR spectrum of compound **5f** in DMSO-d_6 (Figure 11) showed presence of 7 peaks which is in accordance with the structure of compound **5f**.

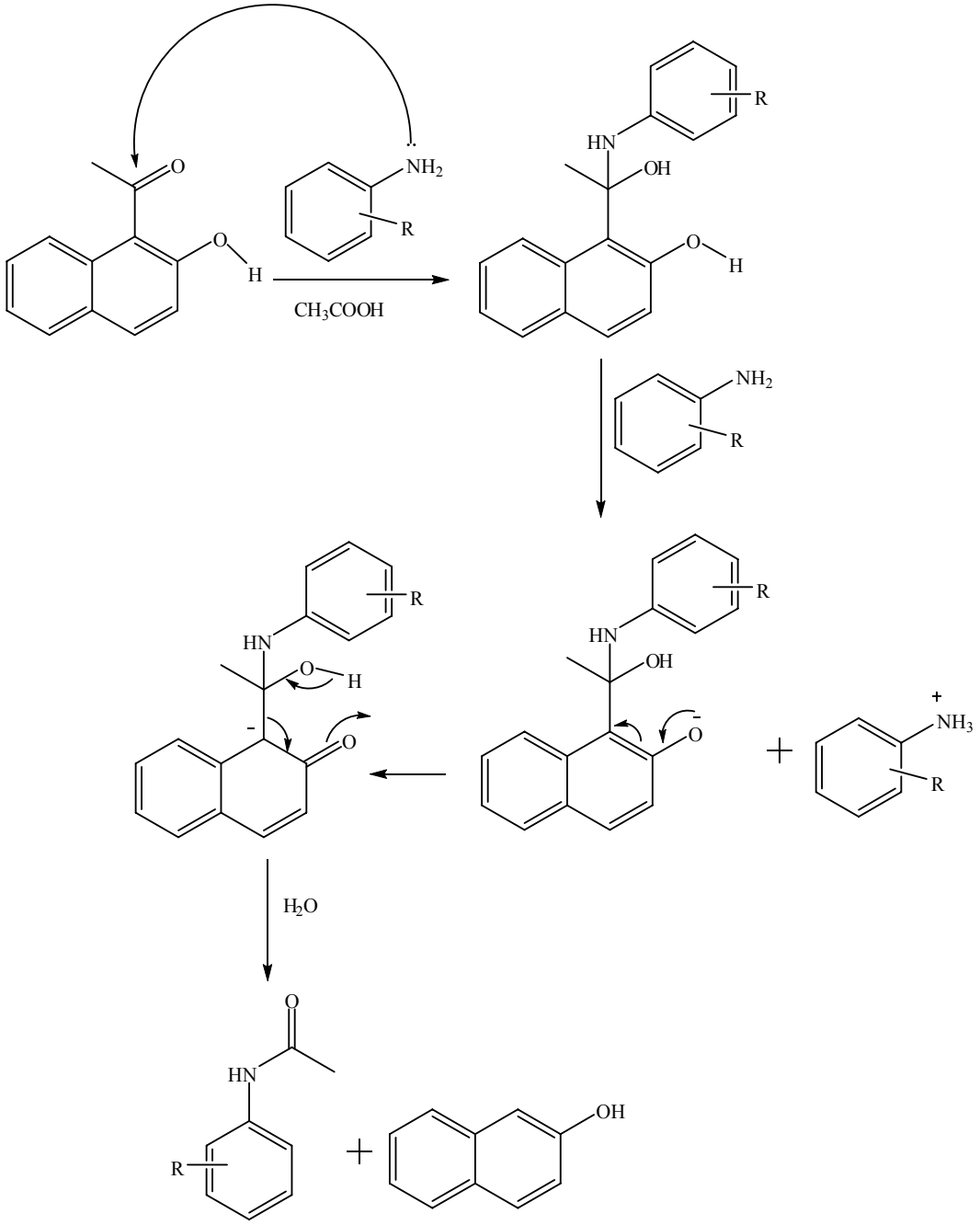
Compound **6** obtained by column chromatography was found to be 2-naphthol which was confirmed by its IR, ^1H NMR spectrum, melting point and mix melting point with standard sample. The IR spectrum of compound **6** (Figure 12) exhibited broad band

at 3254 cm^{-1} for OH group. The ^{1}H NMR of compound **6** (Figure 13) showed singlet at δ 5.04 for one proton indicated presence of OH group. All aromatic protons were observed between δ 7.08 to 7.78 confirmed the compound **6** to be 2-naphthol.

We carried out reaction with different primary aromatic amines with electron releasing and electron withdrawing groups and in all cases with electron releasing groups on aniline, the formation of N-acetylated product was observed. When electron withdrawing nitro group was present the reaction doesn't take place, while with carboxylic acid group on aniline the reaction was observed with less yield. When methoxy group was there on aniline the yield was very poor.

Since with primary aromatic amines N-acetylated aniline product formation was observed, we have extended the reaction with secondary and tertiary amines. When the reaction was carried out with cyclic saturated secondary amines e.g. pyrrolidine and morpholine, 2-naphthol was obtained as major product and small amount of secondary amine was obtained back while in case of aromatic secondary amines e.g. indole and benzotriazole, the reaction doesn't take place and starting material **2** was recovered. The formation of 2-naphthol **6** was more than 50% in all cases as expected from molar ratios. There were several other and easy methods available for formation of N-acetylated products of aromatic amines.[15, 16, 18, 19] Though our purpose was not to form N-acetylated amines, this is found to be new method for formation of N-acetylated amines. Formation of various anilide derivatives **5a-f** from 1-acetyl 2-naphthol **2** can be explained by following mechanism as shown in Scheme 5.

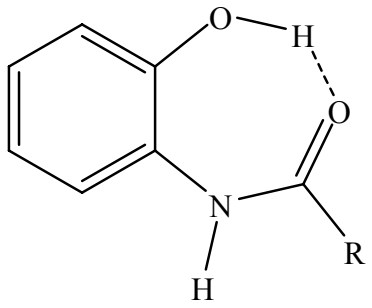
Scheme 5: Plausible mechanism for novel retro Friedel Crafts acylation



The proposed mechanism (Scheme 5) for formation of N-acetylated product can be explained by the fact that aromatic amines react with acetyl group and then instead of dehydration, since hydroxyl group is there at ortho position of acetyl group, it participates in the reaction and due to which retro Friedel Crafts reaction has occurred. One can call it as reverse Claisen rearrangement. In case of cyclic secondary amines, retro Friedel Crafts

reaction has occured and then acetyl group is removed as acetic acid from N-acetyl pyrrolidine or N-acetyl morpholine.

The involvement of hydroxyl group at ortho position of acetyl group in this reaction is supported by the fact that when reaction was carried out with 3-acetyl naphthopyrone where no hydroxy group was there at ortho position of acetyl group, under the same conditions, the starting material remained as such i.e. retro Friedel Crafts reaction has not been observed, also Schiff base formation was not observed. In case of ortho hydroxy aniline the product obtained was highly stabilized by hydrogen bonding[20] hence the reaction yield is good (Structure 7).



7

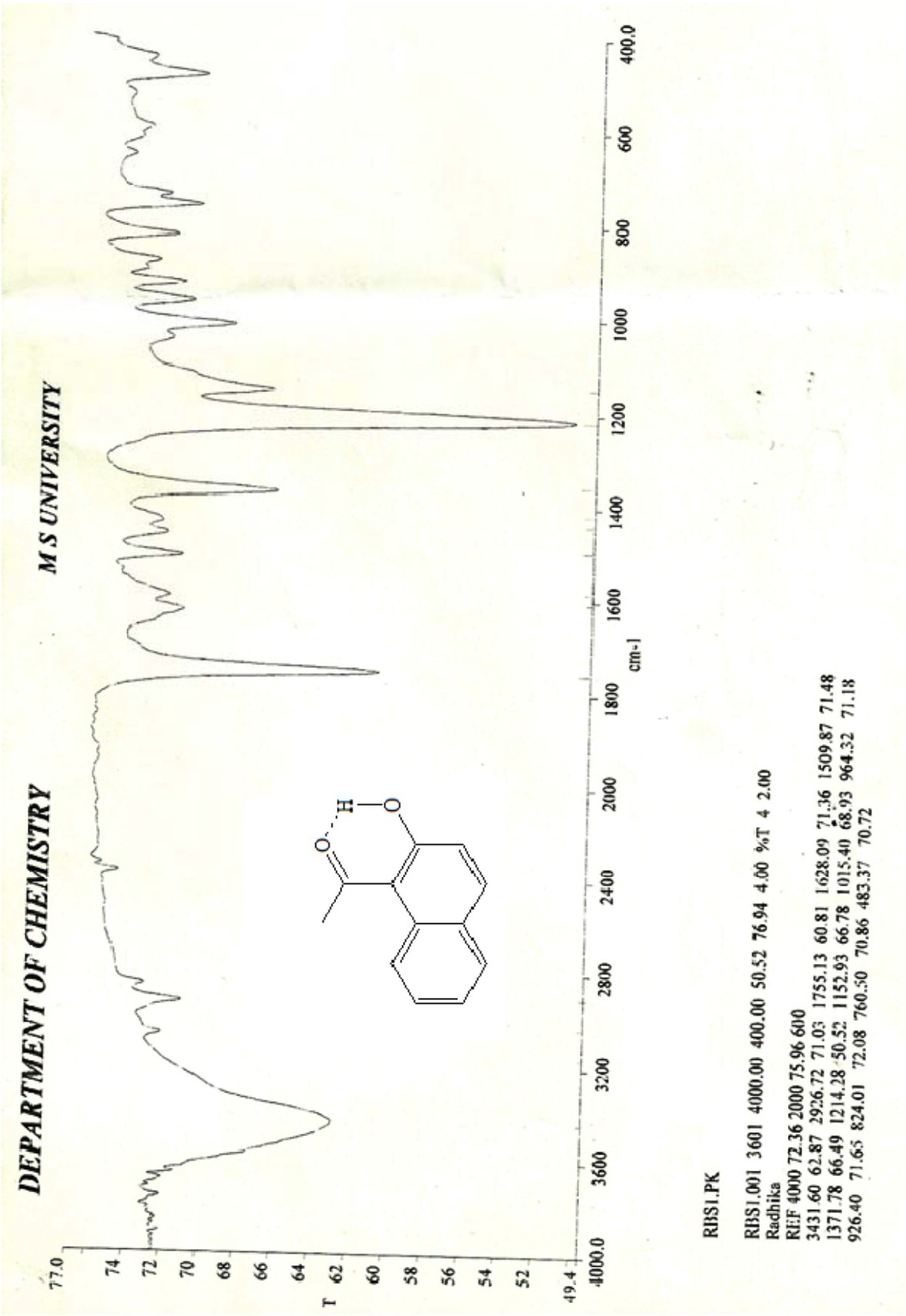


Figure 1: IR of 1-(2-hydroxynaphthalen-1-yl)ethanone **2**

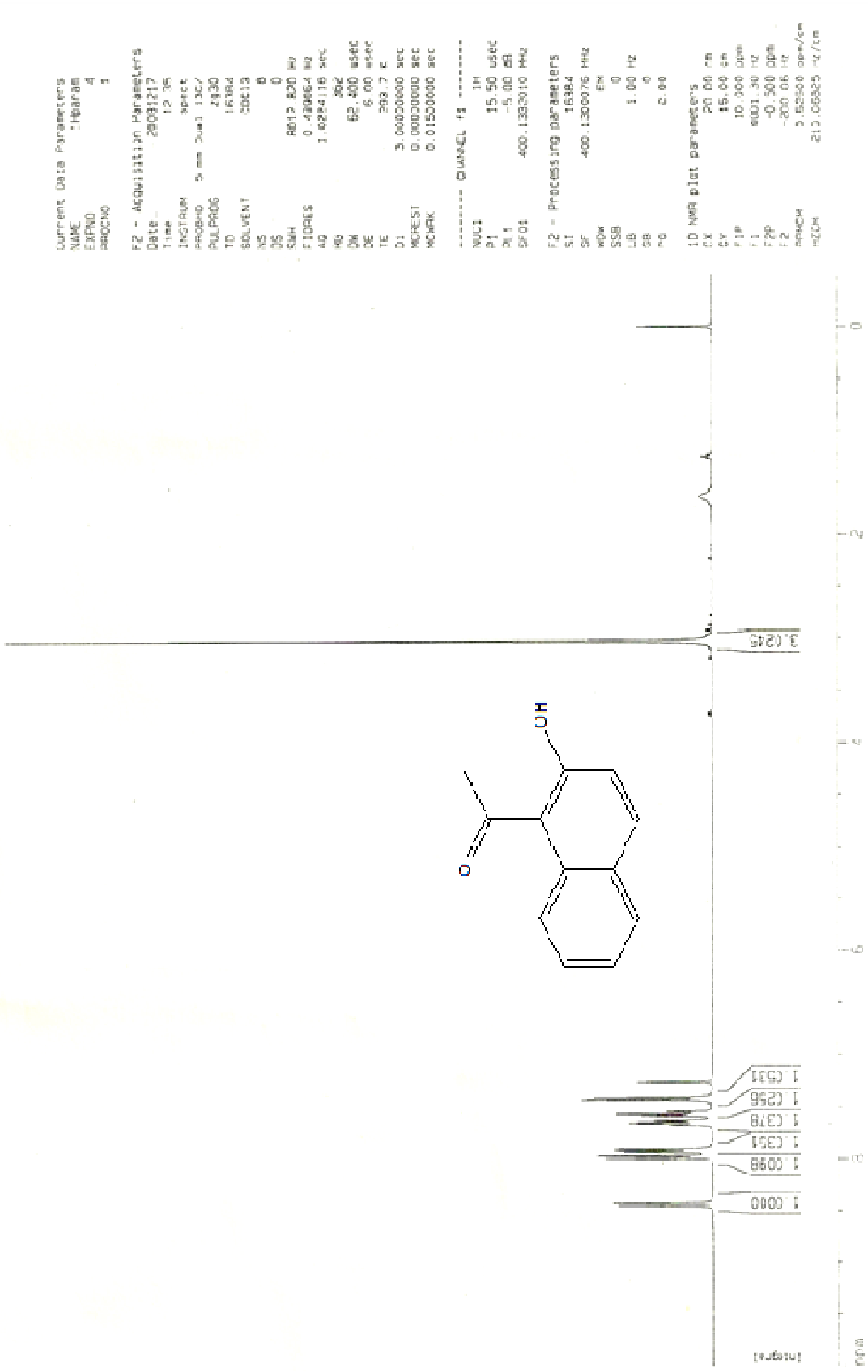


Figure 2: ^{1}H NMR of 1-(2-hydroxynaphthalen-1-yl)ethanone **2**

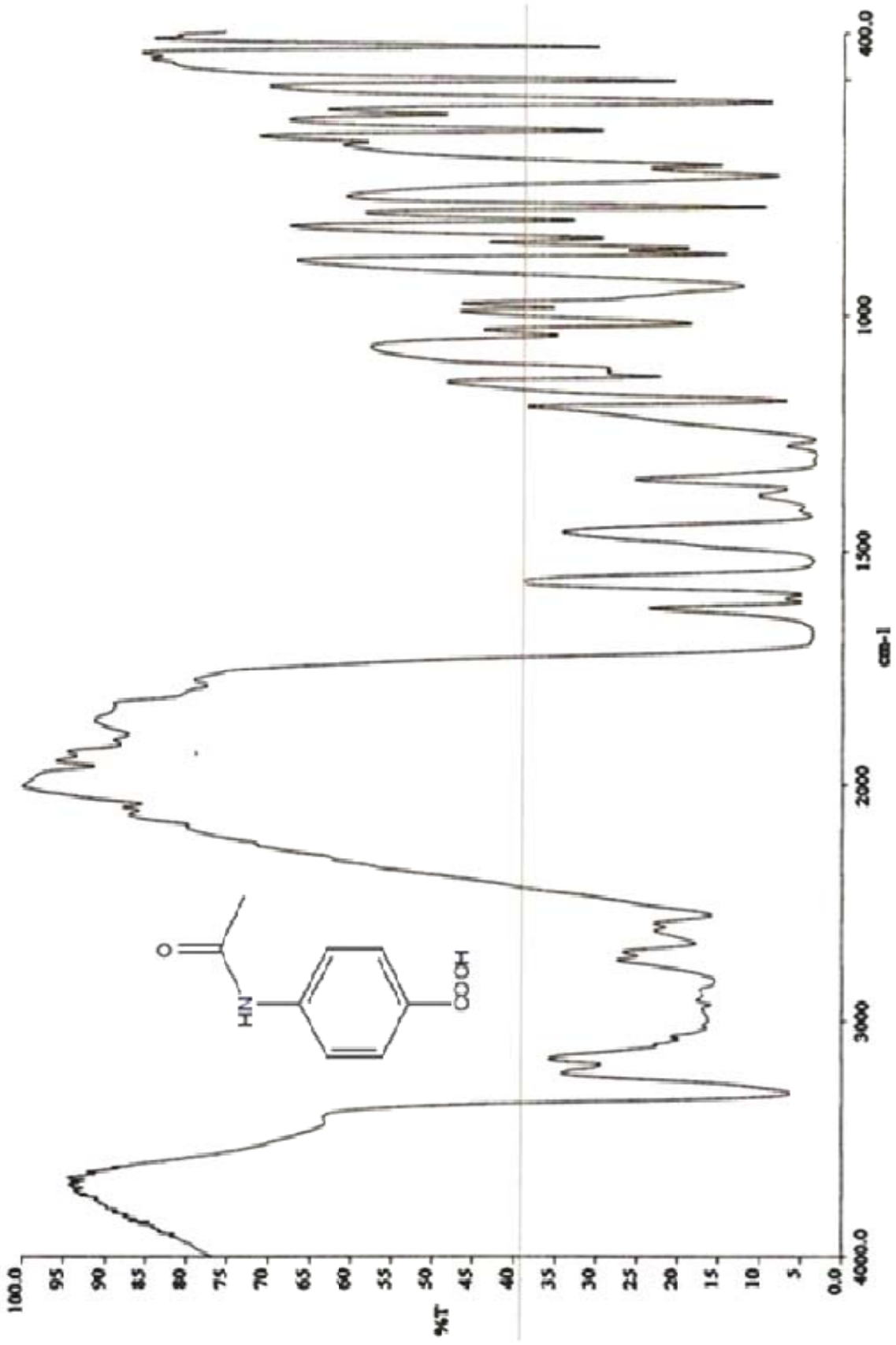


Figure 3: IR of 4-acetamidobenzoic acid **5b**

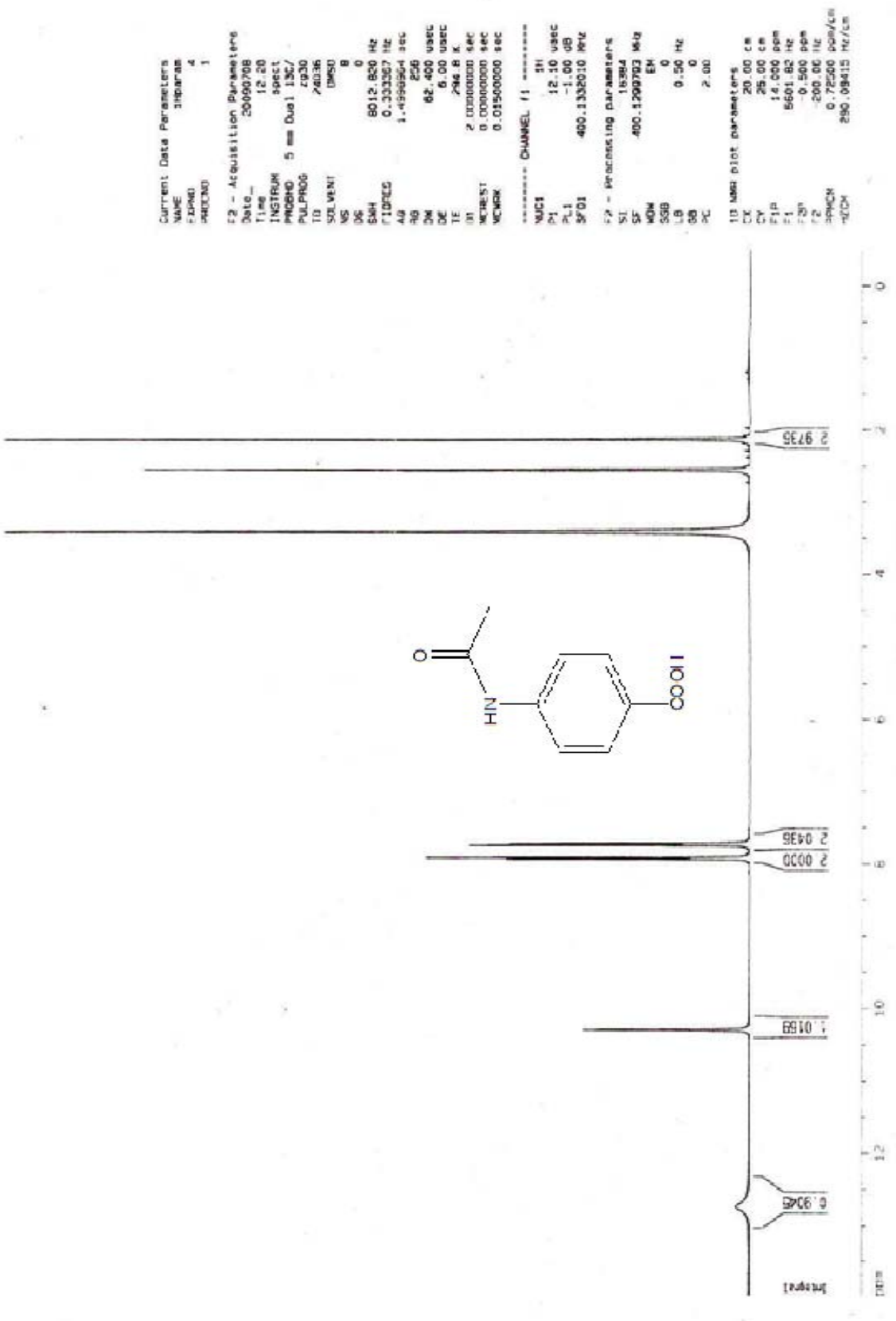


Figure 4: ^{1}H NMR of 4-acetamidobenzoic acid **5b**

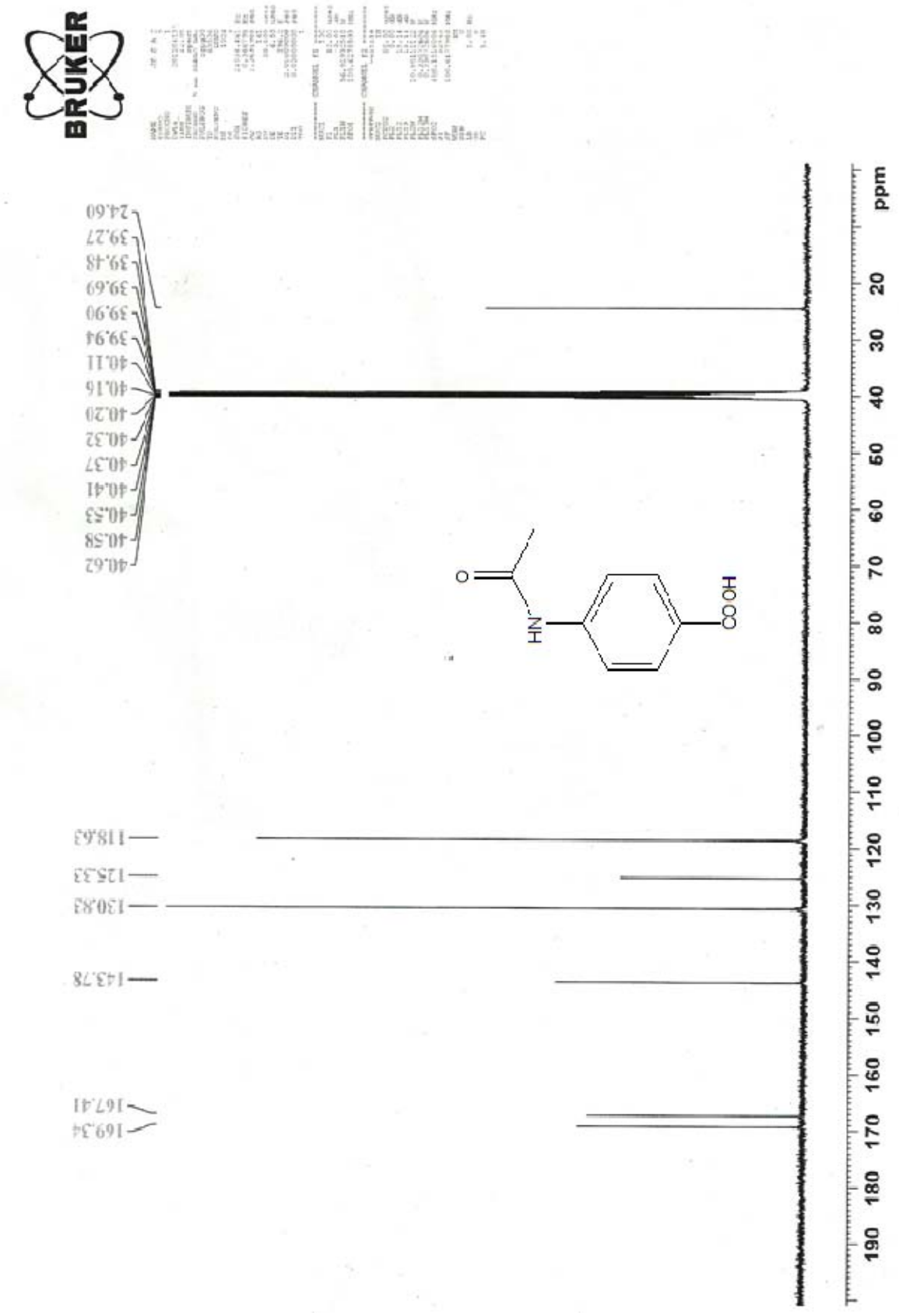


Figure 5: ^{13}C NMR of 4-acetamidobenzoic acid **5b**

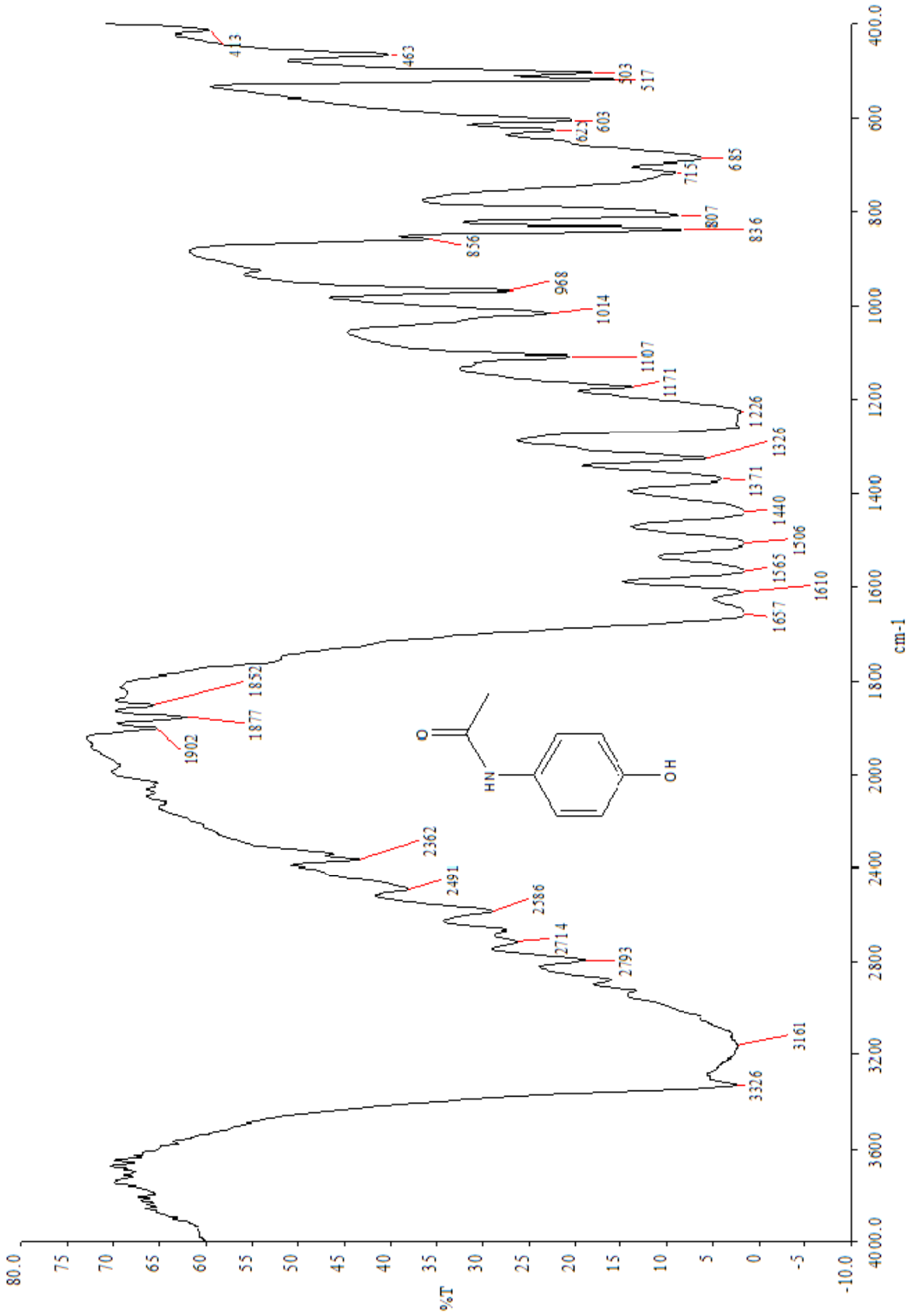


Figure 6: IR of N-(4-hydroxyphenyl)acetamide **5e**

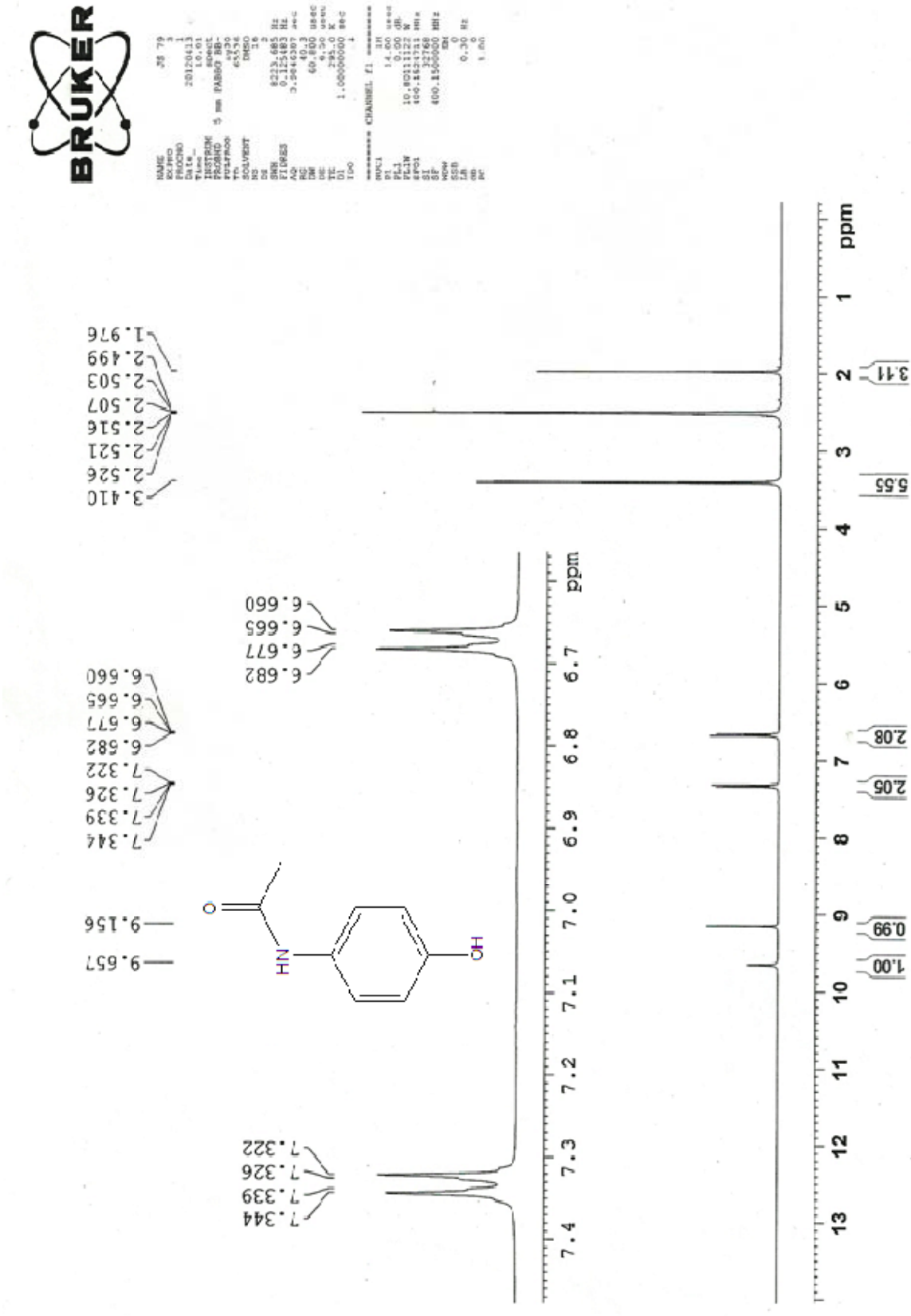


Figure 7: ^{1}H NMR of N-(4-hydroxyphenyl)acetamide **5e**

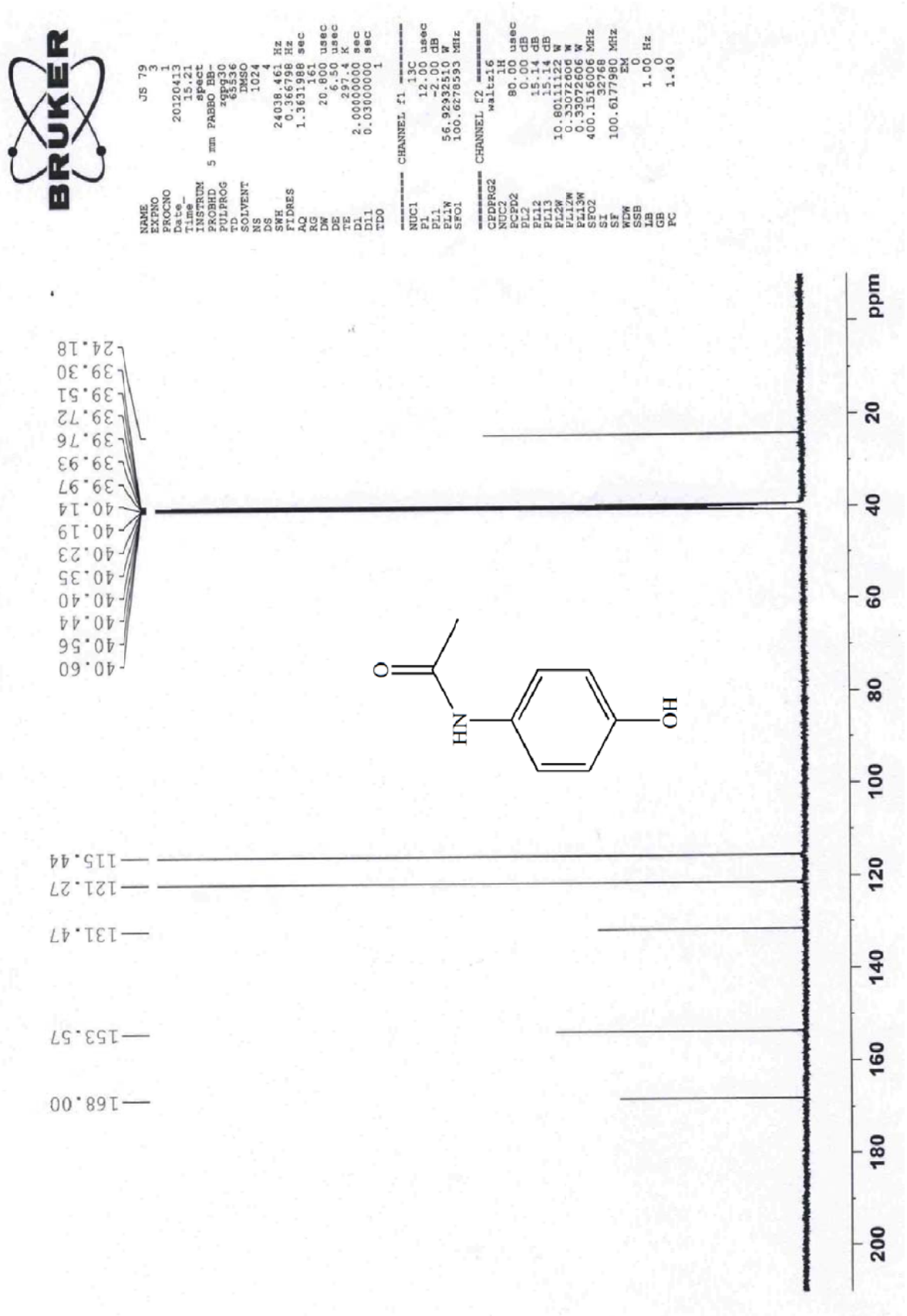


Figure 8: ^{13}C NMR of N-(4-hydroxyphenyl)acetamide **5e**

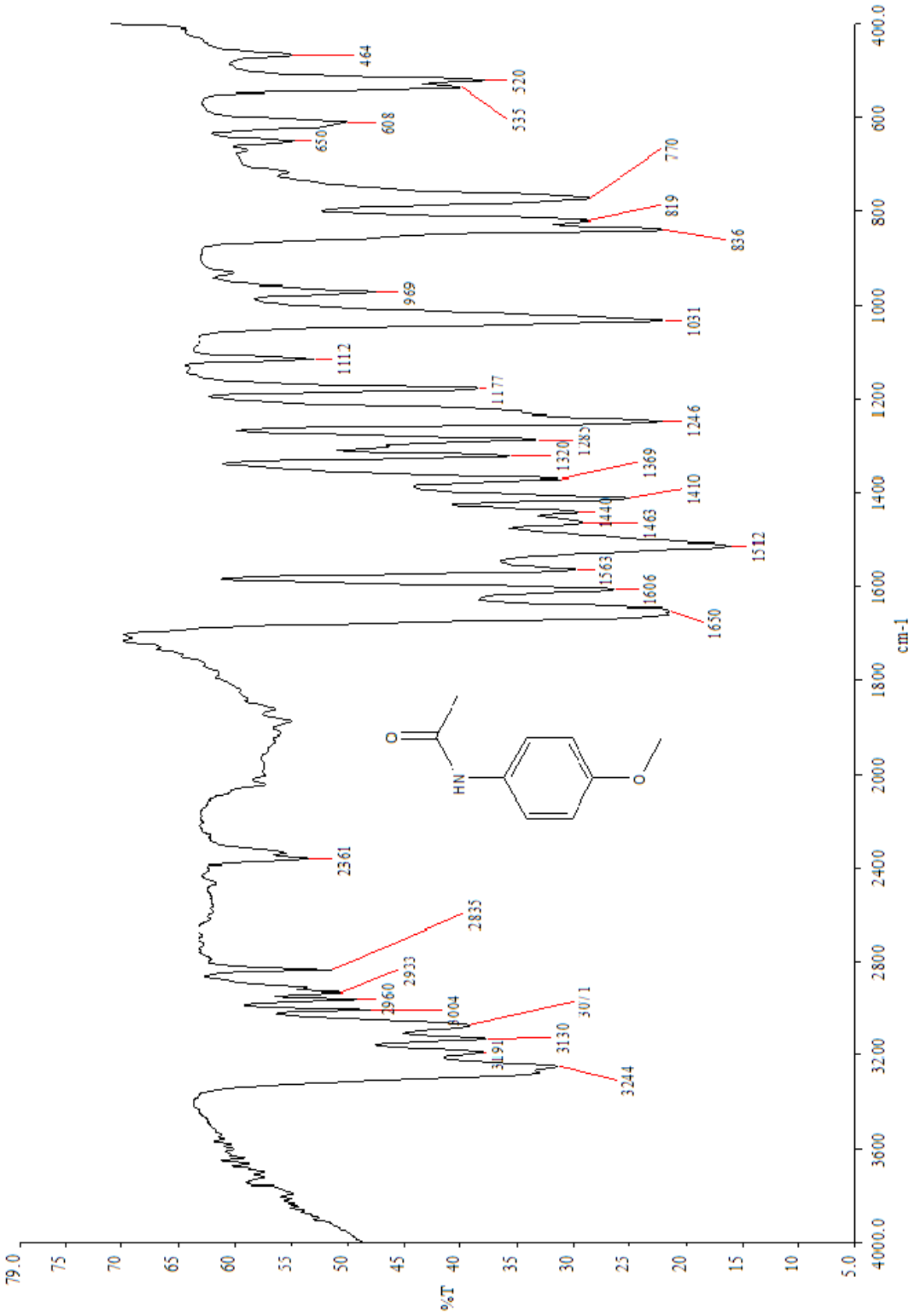


Figure 9: IR of N-(4-methoxyphenyl)acetamide **5f**

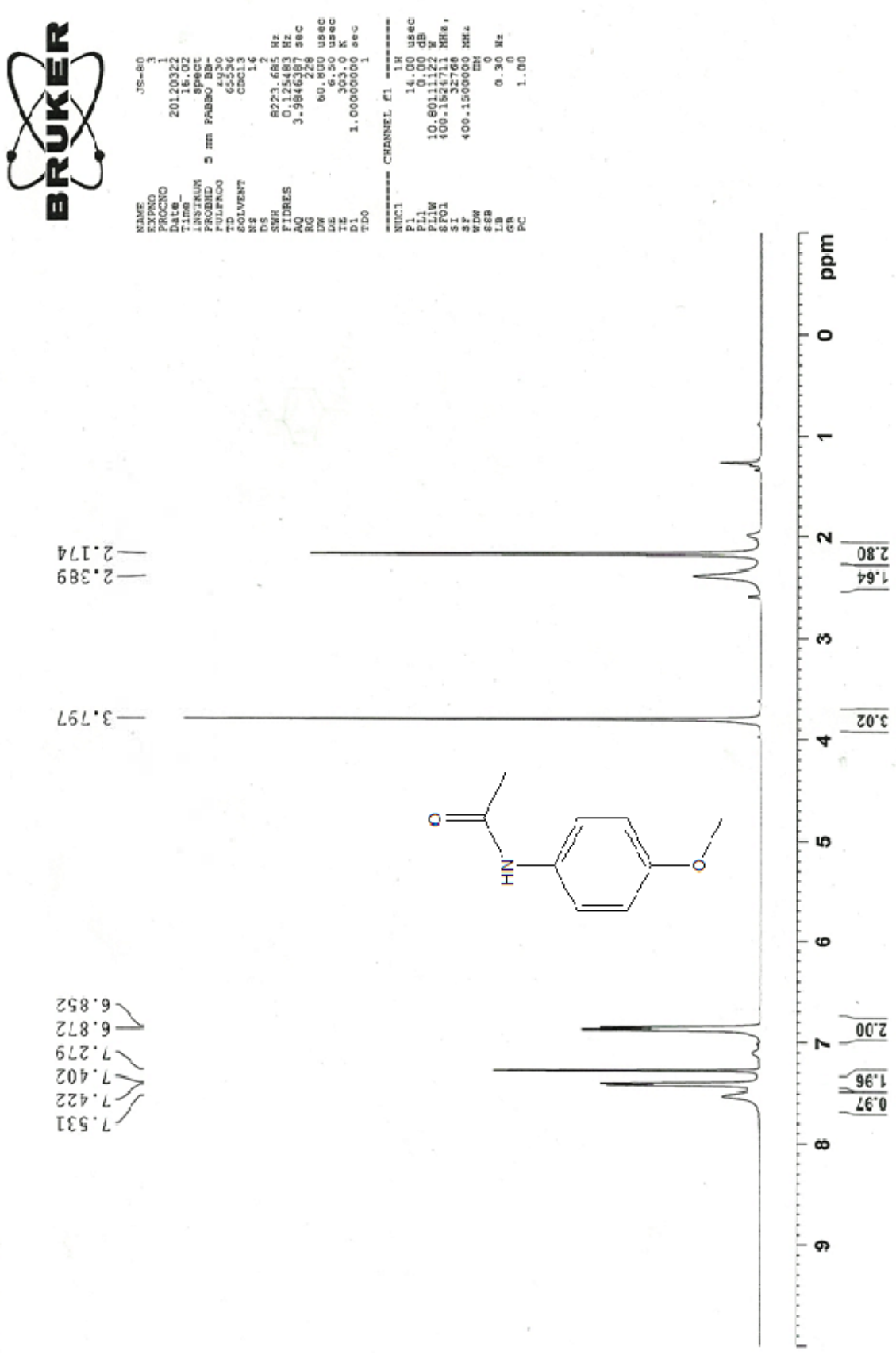


Figure 10: ^{1}H NMR of N-(4-methoxyphenyl)acetamide **5f**

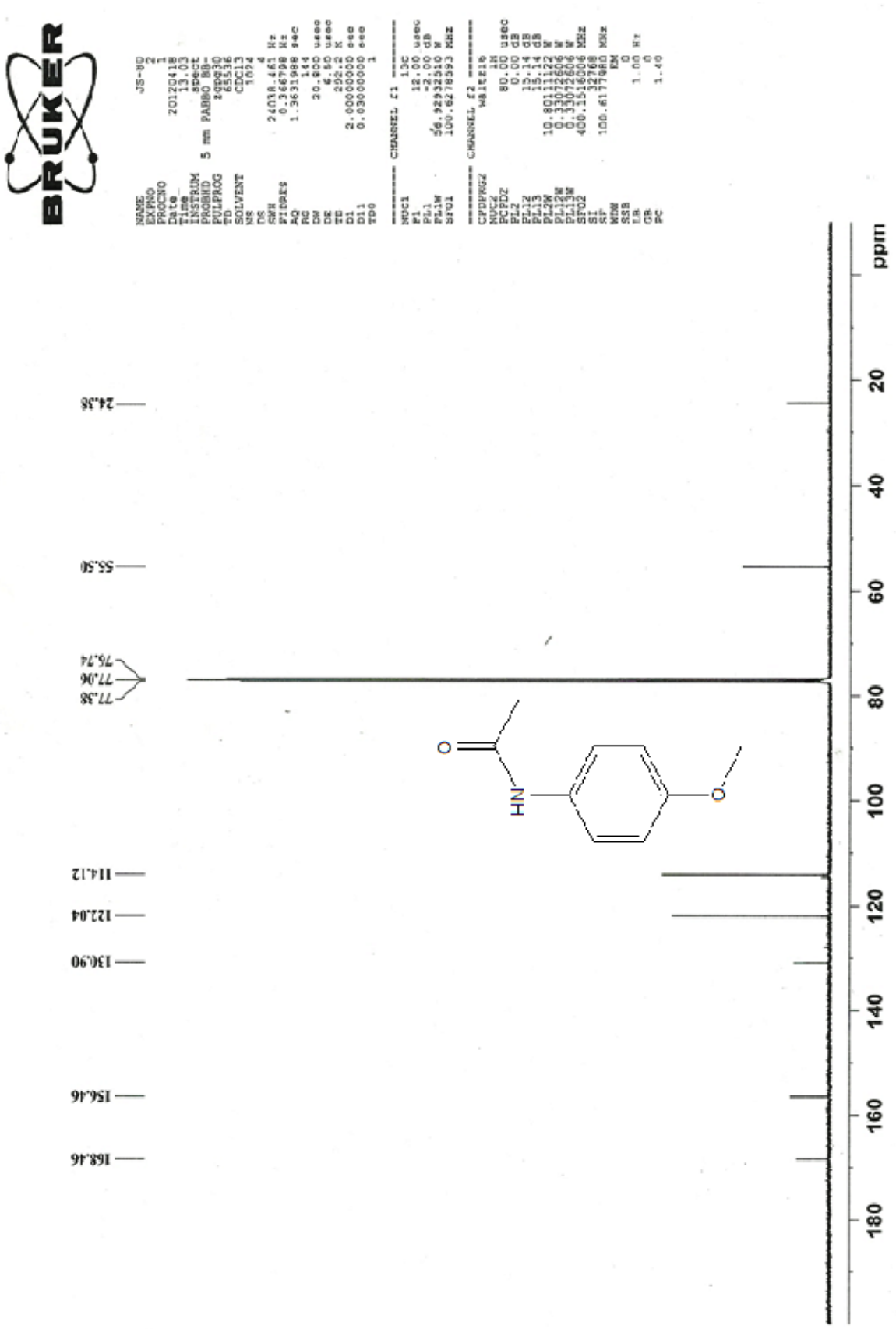


Figure 11: ^{13}C NMR of N-(4-methoxyphenyl)acetamide **5f**

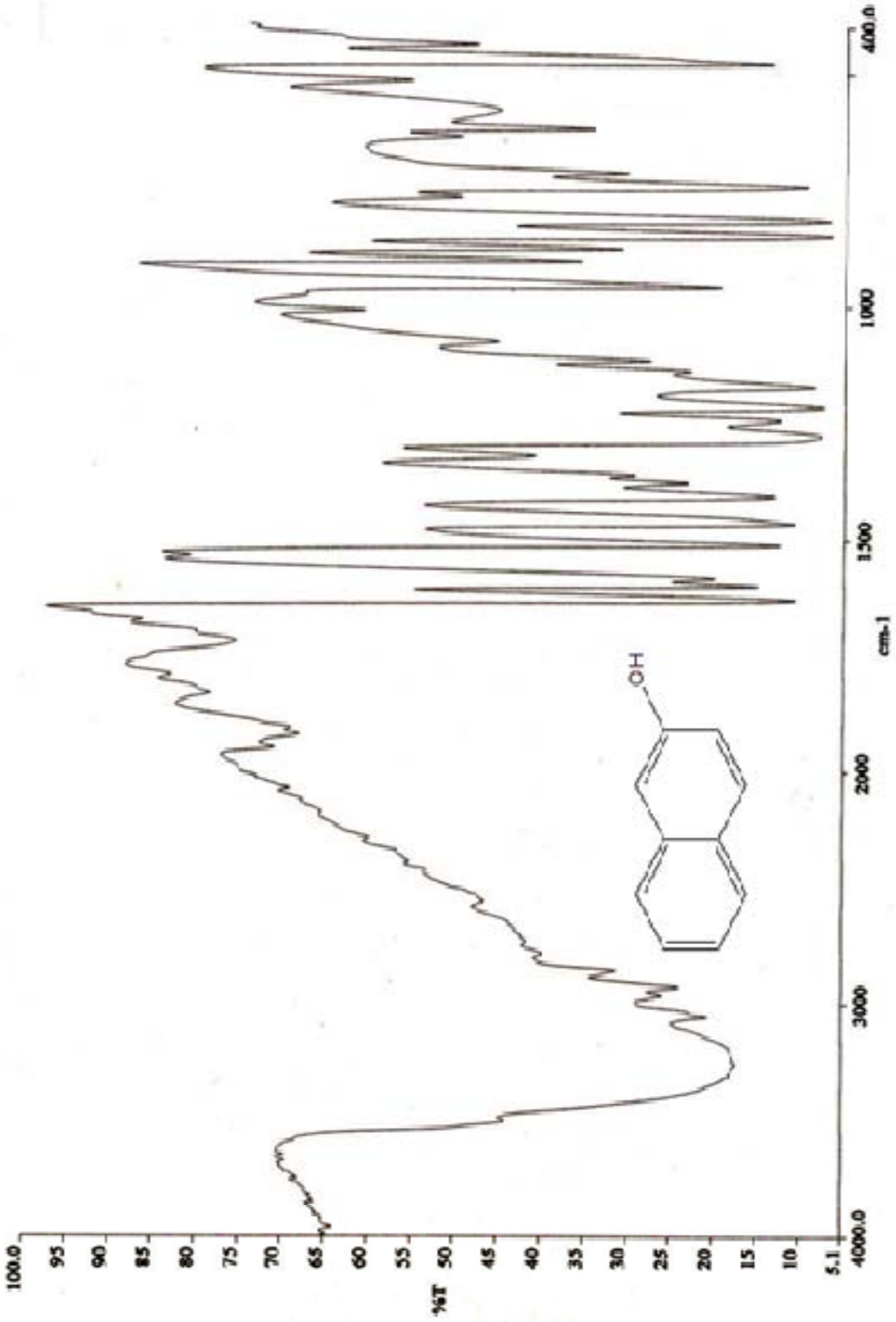


Figure 12: IR of 2-naphthol **6**

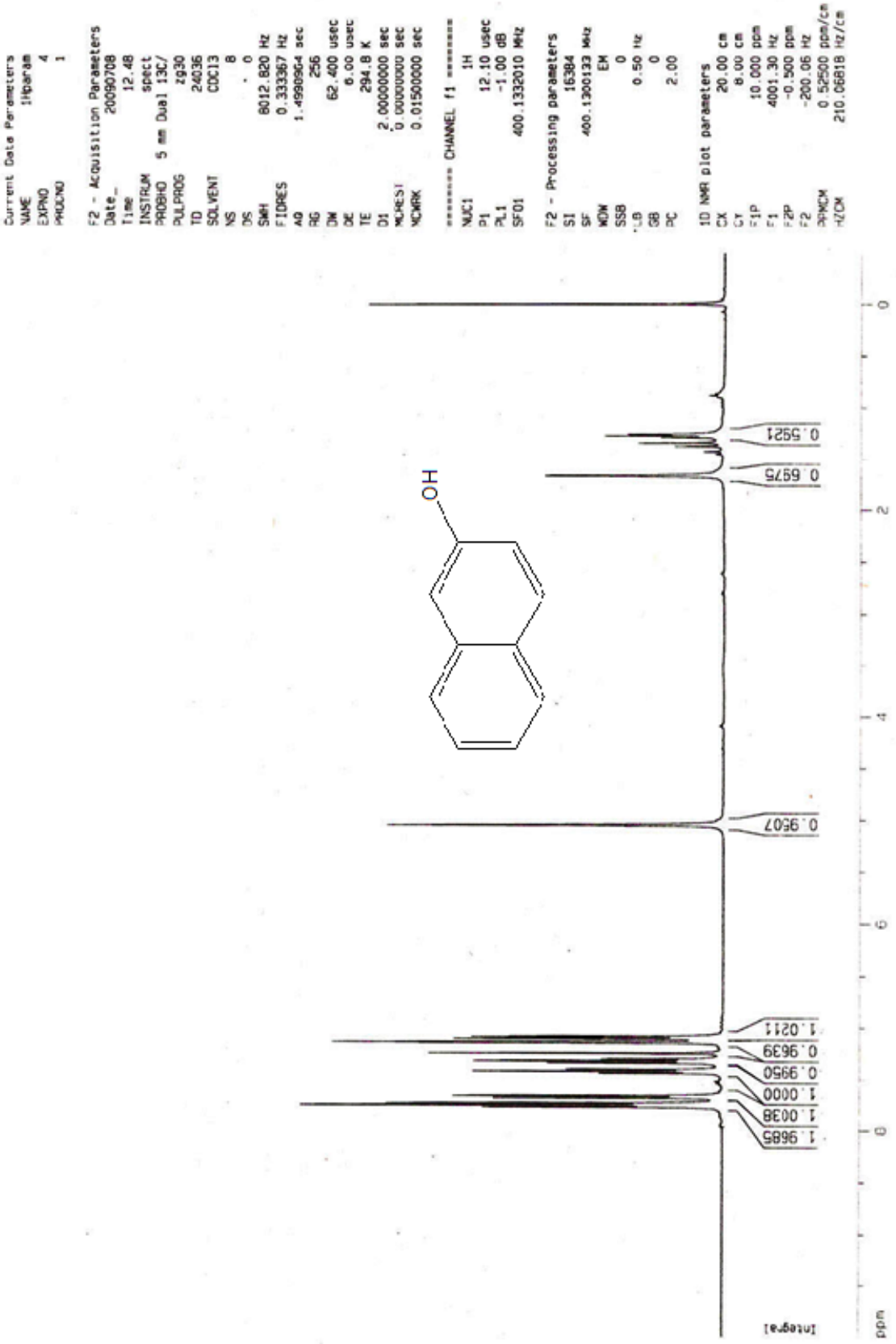


Figure 13: ^{1}H NMR of 2-naphthol **6**

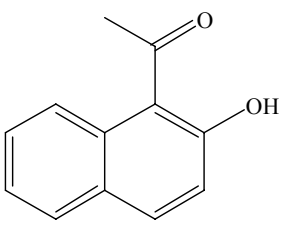
4b.3 Conclusion

We have observed that formation of Schiff base **4** had not occurred from 1-acetyl 2-naphthol **2**, but we have presented the unusual novel retro Friedel Crafts reaction and obtained N-acetylated **5a-f** products formation during reaction of 1-acetyl 2-naphthol **2** with different primary aromatic amines **5a-f** when refluxed in absolute ethanol with catalytic amount of acetic acid. With cyclic secondary amines, retro Friedel Crafts reaction has occurred and 2-naphthol **6** was obtained as major product and cyclic secondary amines were formed after deacetylation of N-acetylated cyclic secondary amines. Tertiary amines didn't react with 1-acetyl 2-naphthol **2**. We have observed that hydroxyl group at ortho position of acetyl group played role in this retro Friedel Crafts reaction. We have also proposed the mechanism for that.

4b.4 Experimental

4b.4.1 Chemistry

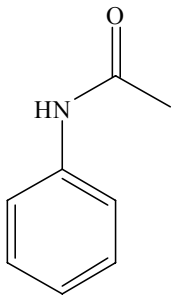
Reagent grade chemicals and solvents were purchased from commercial supplier and used without purification. TLC was performed on silica gel F254 plates (Merck). Acme's silica gel (60-120 mesh) was used for column chromatographic purification. Melting points are uncorrected and were measured in open capillary tubes, using a Rolex melting point apparatus. IR spectra were recorded as KBr pellets on Perkin Elmer RX 1 spectrometer. ^{1}H NMR and ^{13}C NMR spectral data were recorded on Bruker Advance 400 spectrometer (400 MHz) with $CDCl_3$ or DMSO-d_6 as solvent and TMS as internal standard. *J* values are in Hz. CHN elemental analyses were recorded on Thermosinnigan Flash 11-12 series EA.



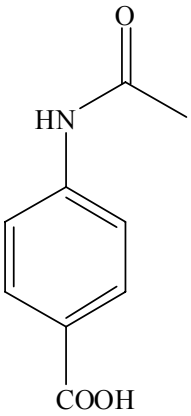
1-(2-hydroxynaphthalen-1-yl)ethanone 2: Anhydrous zinc chloride (0.1688 mol, 2.1 eq.) was dissolved in 30 ml glacial acetic acid and 2-naphthol **1** (0.0694 mol, 1 eq.) was added

portion wise. Acetic anhydride (0.1588 mol, 2.3 eq.) was added in the reaction flask. Reaction mass was heated at 100°C for 30 minutes. It was poured in ice- HCl to get solid. Crude product was purified by column chromatography using petroleum ether (60-80°C) as eluent gave white solid. Yield: 82%; mp 63-65°C (Lit. 63-65°C)[17]; IR (KBr) (Figure 1): 3431, 2926, 1755 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) (Figure 2): δ 3.03 (3H, s, CH_3), 7.41 – 7.43 (1H, d, *J*= 9.6 Hz, ArH), 7.55-7.59 (1H, t, *J*= 7.28, 7.48 Hz, ArH), 7.64-7.68 (1H, t, *J*= 7.32, 7.6 Hz, ArH), 7.91-7.93 (1H, d, *J*= 7.92 Hz, ArH), 7.97-7.99 (1H, d, *J*= 8.92 Hz, ArH), 8.42-8.44 (1H, d, *J*= 8.68 Hz, ArH); Ele. Anal. Calcd. for $C_{12}H_{10}O_2$; Requires (Found) %: C, 77.40 (77.58); H, 5.41 (5.35).

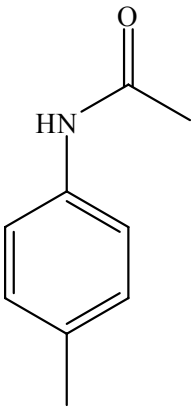
General procedure for 5a-f: 1-acetyl 2-Naphthol **1** (0.02685 mol, 1 eq.) was dissolved in 10 ml absolute ethanol. Primary aromatic amines **3a-f** (0.0268 mol, 1.0 eq.) and glacial Acetic acid (2-3 drops) were added in the flask and refluxed for 5-6 hours. Reaction completion was judged by TLC. Reaction mass was poured into ice: water (1:1) to get solid. Compound was purified by column chromatography using petroleum ether (60-80°C): ethyl acetate 9:1 as eluent.



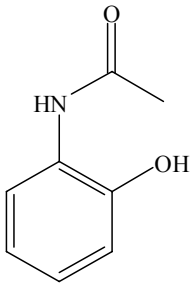
N-phenylacetamide 5a: This compound was obtained as light brown solid. Yield: 37%; mp 115-117°C (Lit. 113-115°C) [21]; IR (KBr): 3293, 3136, 3061, 2926, 1600 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.17 (3H, s, CH_3), 7.08-7.12 (1H, t, *J*= 7.32, 7.36 Hz, ArH), 7.26-7.33 (2H, q, ArH), 7.49-7.51 (2H, d, *J*= 7.92 Hz, ArH); ^{13}C NMR (400 MHz, $CDCl_3$): δ 24.5, 120.0, 124.4, 129.0, 137.9, 168.7; Ele. Anal. Calcd. for C_8H_9NO; Requires (Found) %: C, 71.09 (71.26); H, 6.71 (6.53); N, 10.36 (10.49).



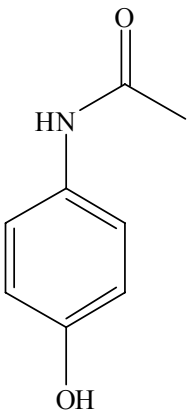
4-acetamidobenzoic acid 5b: This compound was obtained as white solid. Yield: 37%; mp >250°C (Lit. 259-261°C) [21]; IR (KBr) (Figure 3): 3306, 2828, 1681 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) (Figure 4): δ 2.13 (3H, s, CH_3), 7.73-7.75 (2H, d, *J*= 8.64 Hz, ArH), 7.92-7.94 (2H, d, *J*= 8.64 Hz, ArH), 10.30 (1H, s, NH), 12.74 (1H, s, COOH); ^{13}C NMR (400 MHz, DMSO-d_6) (Figure 5): δ 24.6, 118.6, 125.3, 130.8, 143.8, 167.4, 169.3; Ele. Anal. Calcd. for $C_9H_9NO_3$; Requires (Found) %: C, 60.33 (60.46); H, 5.06 (5.18); N, 7.82 (7.64).



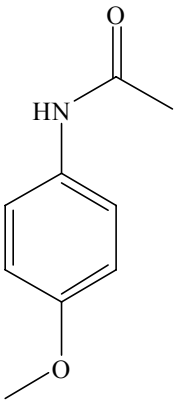
N-p-tolylacetamide 5c: This compound was obtained as light brown solid. Yield: 47%; mp 149- 151°C (Lit. 149- 151°C) [21]; IR (KBr): 3302, 3193, 3128, 3071, 2924, 1666 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.16 (3H, s, CH_3), 2.31 (3H, s, CH_3), 7.11-7.12 (2H, d, *J*= 8.04 Hz, ArH), 7.26 (1H, s, NH), 7.36-7.38 (2H, d, *J*= 8.16 Hz, ArH); ^{13}C NMR (400 MHz, $CDCl_3$): δ 20.9, 24.4, 120.2, 129.4, 134.0, 135.3, 168.6; Ele. Anal. Calcd. for $C_9H_{11}NO$; Requires (Found) %: C, 72.46 (72.31); H, 7.43 (7.57); N, 9.39 (9.25).



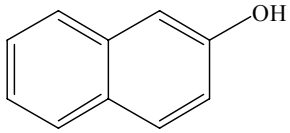
N-(2-hydroxyphenyl)acetamide 5d: This compound was obtained as Light brown solid. Yield: 42%; mp 229-231°C (Lit. 205-210°C) [21]; IR (KBr): 3403, 3084, 1658 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.13 (3H, s, CH_3), 6.85-6.89 (1H, t, *J*= 1.28, 6.72 Hz, ArH), 6.96-7.04 (2H, m, ArH), 7.12-7.18 (1H, t, *J*= 7.0, 8.4 Hz, ArH), 7.38- 7.47 (1H, s, NH), 8.66 (1H, s, OH); ^{13}C NMR (400MHz, $CDCl_3$): δ 23.8, 120.1, 120.5, 122.1, 127.4, 170.5; Ele. Anal. Calcd. for $C_8H_9NO_2$; Requires (Found) %: C, 63.56 (63.69); H, 6.00 (5.84); N, 9.27 (9.42).



N-(4-hydroxyphenyl)acetamide 5e: This compound was obtained as black solid. Yield: 29%; mp 165-170°C (Lit. 168-172°C) [21]; IR (KBr) (Figure 6): 3326, 3161, 1657 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) (Figure 7): δ 1.98 (3H, s, CH_3), 6.66-6.68 (2H, d, *J*= 6.8 Hz, ArH), 7.32-7.34 (2H, d, *J*= 6.8 Hz, ArH), 9.16 (1H, s, NH), 9.66 (1H, s, OH); ^{13}C NMR (400 MHz, DMSO-d_6) (Figure 8): δ 24.2, 115.4, 121.3, 131.5, 153.6, 168.0; Ele. Anal. Calcd. for $C_8H_9NO_2$; Requires (Found) %: C, 63.56 (63.72); H, 6.00 (5.88); N, 9.27 (9.38).



N-(4-methoxyphenyl)acetamide 5f: This compound was obtained as grey solid. Yield: 9%; mp 139-142°C (Lit. 129-132°C) [21]; IR (KBr) (Figure 9): 3191, 3130, 3071, 1650 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) (Figure 10): δ 2.17 (3H, s, CH_3), 3.79 (3H, s, OCH_3), 6.85-6.87 (2H, d, *J*= 8 Hz, ArH), 7.40-7.42 (2H, d, *J*= 8 Hz, ArH), 7.53 (1H, s, NH); ^{13}C NMR (400 MHz, $CDCl_3$) (Figure 11): δ 24.4, 55.5, 114.1, 122.0, 130.9, 156.5, 168.5; Ele. Anal. Calcd. for $C_9H_{11}NO_2$; Requires (Found) %: C, 65.44 (65.59); H, 6.71 (6.57); N, 8.48 (8.38).



2-naphthol 6: This compound was obtained as light pink solid. Yield: >50% in all cases; mp 120-124°C (121-125°C) [21]; IR (KBr) (Figure 12): 3254, 2924 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) (Figure 13): δ 5.04 (1H, s, OH), 7.08-7.11 (1H, dd, *J*= 2.48, 8.8 Hz, ArH), 7.14-7.15 (1H, d, *J*= 2.44 Hz, ArH), 7.30-7.34 (1H, m, ArH), 7.41-7.45 (1H, m, ArH), 7.62-7.69 (1H, d, *J*= 8.2 Hz, ArH), 7.74-7.78 (2H, t, *J*= 6.6, 8.4 Hz, ArH); Ele. Anal. Calcd. for $C_{10}H_8O$; Requires (Found) %: C, 83.31 (83.54); H, 5.59 (5.49).

4b.5 References

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Chapter 5a

Novel Retro Knoevenagel reaction of Substituted Coumarin-3-carboxylate

5a. Novel Retro Knoevenagel reaction of Substituted Coumarin-3-carboxylate

5a.1 Introduction

Coumarin-3-carboxylate derivatives possess wide range of activities[1-5]. Various amide derivatives of coumarin-3-caboxylate showed anti-Helicobacter pylori activity,[1, 6] while various 3-carbonyl, acyl and hydrazide derivatives of coumarin-3-caboxylate have been reported to show human monoamine oxidase inhibition.[2] The ester and amide derivatives of 6-chloromethyl coumarin-3-carboxylic acid had showed α-Chymotrypsin inhibitory activity.[3] Various amide and ester derivatives of coumarin-3-carboxylate have been reported to show anticancer activity[5] or inhibition activity of enzyme gGAPDH.[4]

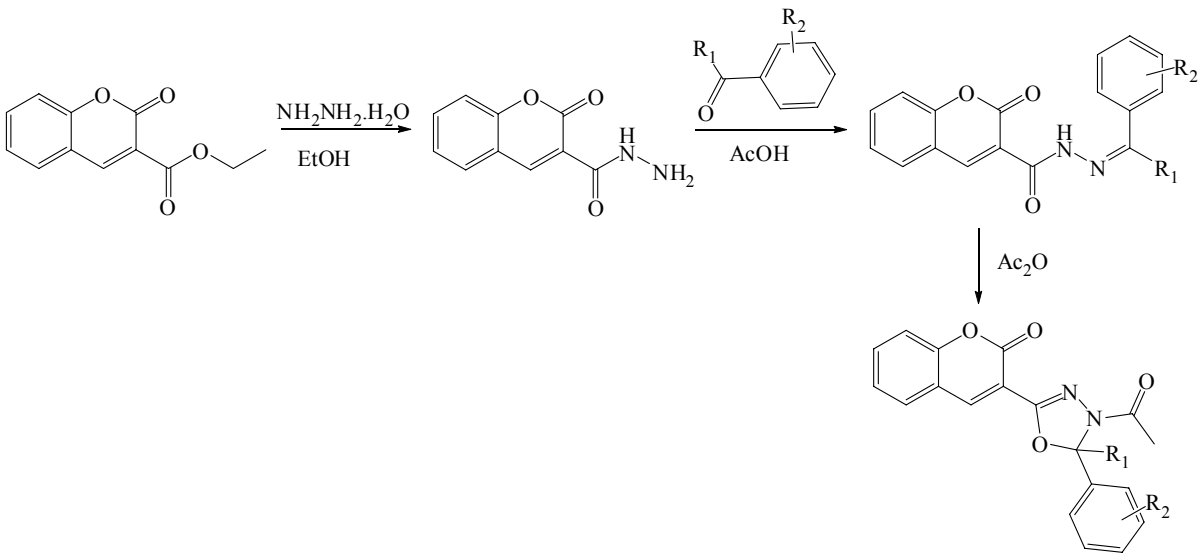
Coumarin 3-carbohydrazide[7, 8] is an important class of compound, from which synthesis of variety of heterocyclic rings have been reported to get variety of amide derivatives of coumarin-3-carboxylate having antimicrobial,[7, 9-11] anticonvulsant activity[8] and analgesic activity.[12] Oxazadiazole derivatives from naphthocoumarin-3-carboxylate[13] were reported as strong fluorescent brightners.[13]

In continuation of our work on 3-substituted coumarins[14, 15] and their activity, we have synthesized various coumarin-3-carboxylate **2a-d**. In our attempt to synthesize various hydrazide derivatives of coumarin -3-carboxylate using reported procedures[8, 9, 12] we report herein formation of novel products, the structure of which were proved by IR, ^{1}H NMR, ^{13}C NMR and X-ray Single Crystal analyses.

Recent work

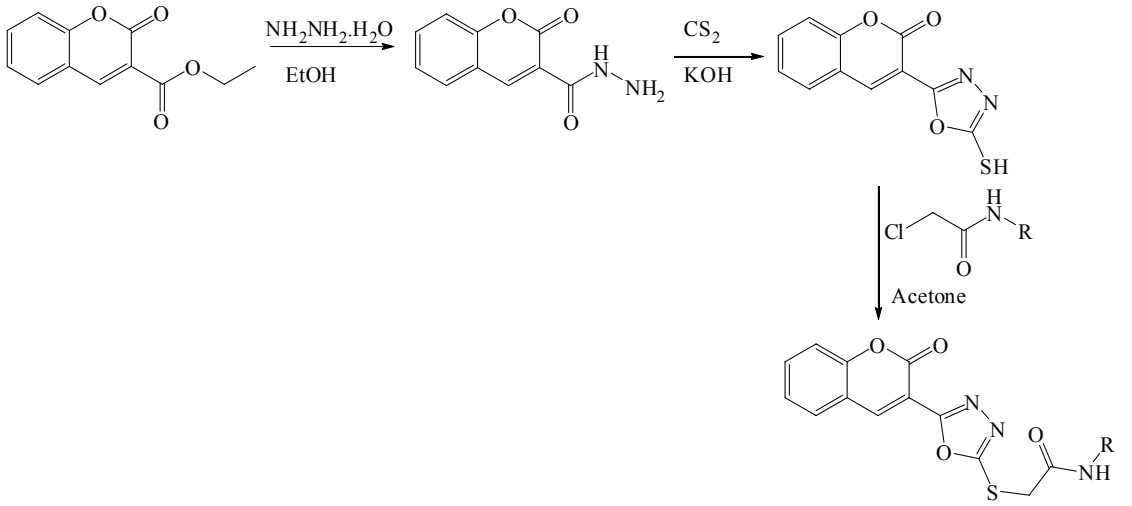
Bhat M. A. and coworkers[8] have synthesized 3-(4-acetyl -5H/Methyl-5-substituted phenyl-4,5-dihydro-1,3,4-oxadiazol-2-yl)-2H-chromen-2-ones as shown in Scheme 1.

Scheme 1:



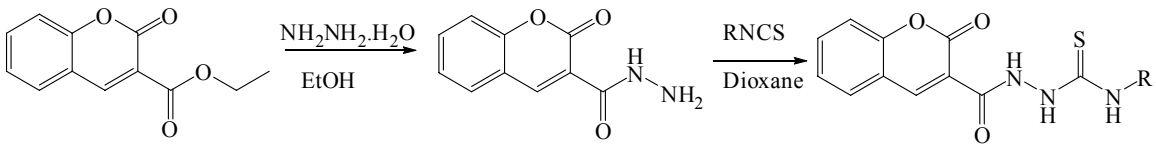
Patel R. V and coworkers[10] have synthesized coumarin based 1,3,4-oxadiazol-2-ylthio-N-phenyl/ benzothiazolyl acetamide derivatives as shown in Scheme 2.

Scheme 2:



Bhat M. A. and coworkers[12] have synthesized coumarin based thiosemicarbazide derivatives as shown in Scheme 3.

Scheme 3:

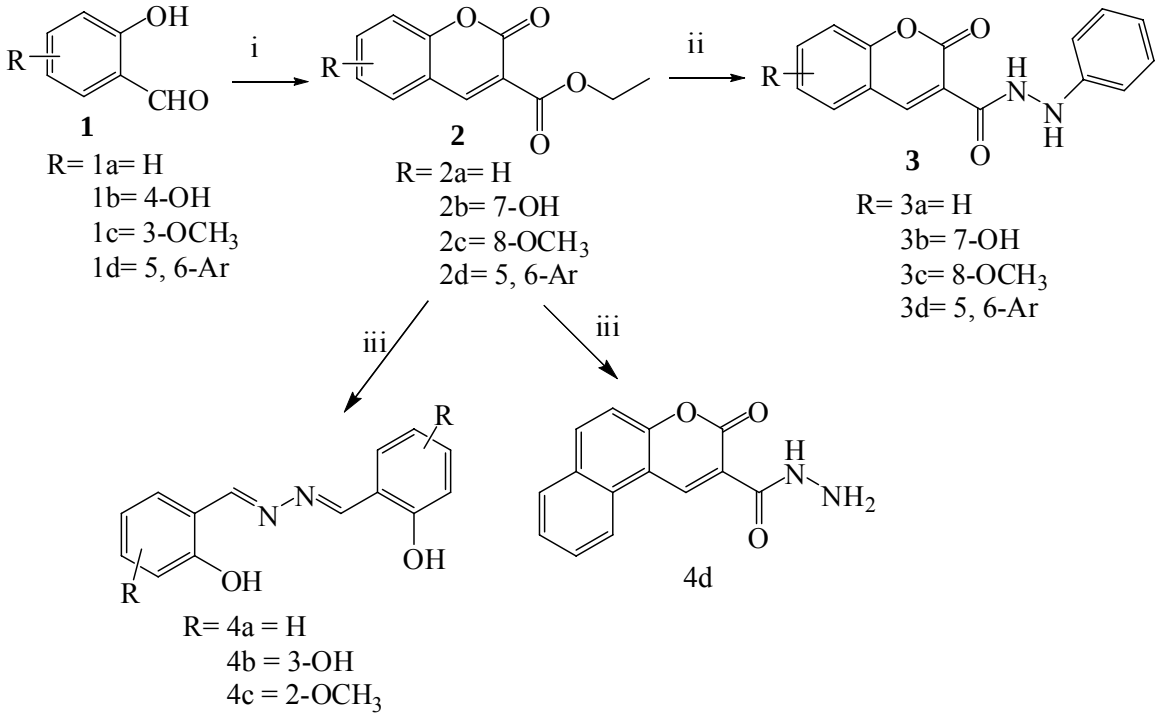


5a.2 Result and discussion

5a.2.1 Chemistry

Knoevenagel reaction of various substituted salicylaldehyde derivatives **1a-d** with diethylmalonate using pyridine and catalytic amount of piperidine gave corresponding substituted coumarin-3-carboxylate derivatives[9] **2a-d** as shown in Scheme 4.

Scheme 4:



Reagents and conditions: (i) Diethylmalonate, Pyridine, Piperidine, 6 h; (ii) Phenyl hydrazine, ethanol, reflux, 8 h; (iii) Hydrazine hydrate, ethanol, reflux, 8 h

The IR spectrum of compound **2a** (Figure 1) exhibited band at 1767 cm^{-1} for lactone carbonyl and ester carbonyl group. In ^{1}H NMR of compound **2a** in $CDCl_3$ (Figure 2) triplet observed at δ 1.42 for three protons and quartet observed at δ 4.42 for two protons confirmed presence of ethoxy group. Four aromatic protons were appeared between δ 7.34 to 7.69. Singlet at δ 8.56 for one proton indicated proton at C-4 position. The ^{13}C NMR spectrum of compound **2a** in $CDCl_3$ (Figure 3) showed presence of 12 peaks which is in accordance with structure of compound **2a**. The *m/z* value at 219.1 for

$[M+1]^+$ and base peak at *m/z* 173.2 indicated fragment for $[M\text{-}OCH_2CH_3]^+$ in ESI/MS (Figure 4) confirmed formation of compound **2a**.

Figure 5 shows IR spectrum of compound **2d** (Figure 5). In ^1H NMR of compound **2d** in $CDCl_3$ (Figure 6) triplet observed at δ 1.46 for three protons and quartet observed at δ 4.48 for two protons confirmed presence of ethoxy group. Six aromatic protons observed between δ 7.49 to 8.35. Singlet at δ 9.35 for one proton indicated proton at C-4 position. The ^{13}C NMR spectrum of compound **2d** in $CDCl_3$ (Figure 7) showed presence of 16 peaks which is in accordance with the structure of compound **2d**. The *m/z* value at 268.9 for $[M+1]^+$ and base peak at *m/z* 222.9 indicated fragment for $[M\text{-}OCH_2CH_3]^+$ in ESI/MS (Figure 8) confirmed formation of compound **2d**.

Refluxing of various coumarin-3-carboxylate derivatives **2a-d** with phenyl hydrazine in ethanol gave corresponding phenyl hydrazide derivatives[2, 6] **3a-d**. The IR, ^1H NMR and ^{13}C NMR of **3a** are shown in figure 9, 10 and 11 respectively. The IR spectrum of compound **3b** (Figure 12) exhibited band at 3484 cm^{-1} for –OH group, band at 3402 cm^{-1} for two -NH groups, band at 1670 cm^{-1} for lactone carbonyl group and band at 1629 cm^{-1} for hydrazide carbonyl group. In ^1H NMR of compound **3b** in DMSO-d_6 (Figure 13) absence of triplet and quartet confirmed absence of ethoxy group and confirmed formation of phenyl hydrazide. All aromatic protons were appeared between δ 6.30 to 7.29. Singlet at δ 8.04 for one proton indicated proton at C-4 position. Three singlets at δ 9.74, 10.14 and 10.75 indicated presence of –CONH, -NH and –OH protons respectively which disappear on D_2O exchange (Figure 13 a). The ^{13}C NMR spectrum of compound **3b** in DMSO-d_6 (Figure 14) showed presence of 11 peaks which is in accordance with structure of compound **3b** and confirmed formation of compound **3b**.

The IR spectrum of compound **3c** (Figure 15) exhibited band at 3324 cm^{-1} for two -NH groups, band at 1722 cm^{-1} for lactone carbonyl group and band at 1654 cm^{-1} for

hydrazide carbonyl group. In ^{1}H NMR of compound **3c** in $CDCl_3$ (Figure 16) absence of triplet and quartet confirmed absence of ethoxy group and formation of phenyl hydrazide. Singlet at δ 4.03 for three protons confirmed presence of $–OCH_3$ group at C-8 position. All aromatic protons along with NH proton appeared between δ 6.93 to 7.37 except one proton. The singlet at δ 8.91 indicated proton at C-4 and another singlet at δ 10.41 indicated presence of -NH proton. The ^{13}C NMR spectrum of compound **3c** in $CDCl_3$ (Figure 17) showed presence of 11 peaks which is in accordance with structure of compound **3c** and confirmed formation of compound **3c**.

Refluxing of various coumarin-3-carboxylate **2a-d** with hydrazine hydrate in ethanol by reported procedures,[7-12] expected to give corresponding hydrazide derivatives, but to our surprise, the melting points of the product obtained by this reaction were not matching with the reported ones. The spectral data were not exactly matching. The attempt of further reaction of hydrazide of **2a** obtained, with carbon disulfide was also failed. Hence single crystals of **4a**, **4c** and **4d** (CCDC No. 968039, CCDC No. 968041 and CCDC No. 968040 respectively) were developed and X-ray analysis of single crystal indicated formation of unexpected schiff base **4a-c** (Figure 18 (**4a**) and Figure 19 (**4c**)). Now the IR, ^{1}H NMR and ^{13}C NMR spectra were also consistent with the structures **4a-c**.

The IR, ^{1}H NMR, ^{13}C NMR and ESI/MS of compound **4a** are shown in figure 20, 21, 22 and 23 respectively. The IR spectrum of compound **4c** (Figure 24) exhibited band at 3390 cm^{-1} for -OH group and band at 1627 cm^{-1} for C=N group. In ^{1}H NMR of compound **4c** in $CDCl_3$ (Figure 25) singlet at δ 4.03 for three protons confirmed presence of $–OCH_3$ group at C-2 position. Three aromatic protons appeared between δ 6.92 to 7.04. Two singlets at δ 8.73 and 11.61 indicated presence of –CH=N and -OH protons respectively. The ^{13}C NMR spectrum of compound **4c** in $CDCl_3$ (Figure 26) showed

presence of 8 peaks which is in accordance with structure of compound **4c**. The *m/z* value at 301.0 for $[M+1]^+$ in ESI/MS (Figure 27) confirmed the formation of compound **4c**.

In reaction of compound **2d** with hydrazine hydrate, the product obtained was found to be hydrazide compound **4d**, which was confirmed by its IR, ^{1}H NMR, ^{13}C NMR and its single crystal analysis.

The IR spectrum of compound **4d** (Figure 28) exhibited band at 3323 cm^{-1} and 3282 cm^{-1} for -NH and $–NH_2$ group respectively while band at 1708 cm^{-1} indicated lactone carbonyl group and another band at 1680 cm^{-1} indicated –CONH group. In ^{1}H NMR of compound **4d** in DMSO-d_6 (Figure 29) singlet at δ 4.79 for two protons indicated presence of $–NH_2$ group of hydrazide. Six aromatic protons were appeared between δ 7.66 to 8.64. Two singlets at δ 9.43 and 9.70 for one proton each indicated presence of proton at C-4 position and -NH proton of hydrazide respectively. The ^{13}C NMR spectrum of compound **4d** in DMSO-d_6 (Figure 30) showed presence of 11 peaks which is consistent with structure of compound **4d**. The *m/z* value at 254.9 for $[M+1]^+$ in ESI/MS (Figure 31) further confirmed the formation and structure of compound **4d**.

To prove the formation of hydrazide in case of compound **4d**, the single crystal was developed in methanol: acetone system and its X-ray single crystal analysis was carried out. In single crystal analysis of compound **4d**, the formation of hydrazide was observed and the solvent acetone molecule also observed in it and found to form Schiff base with free $–NH_2$ group of compound **4d**. The free $–NH_2$ group of hydrazide formed schiff base with acetone during crystallization. ORTEP diagram and Crystal packing diagram of compound **4d** given in Figure 32. Crystal structure data and structure refinement parameters for single crystals **4a**, **4c** and **4d** are given in Tables, Table 1 to Table 18.

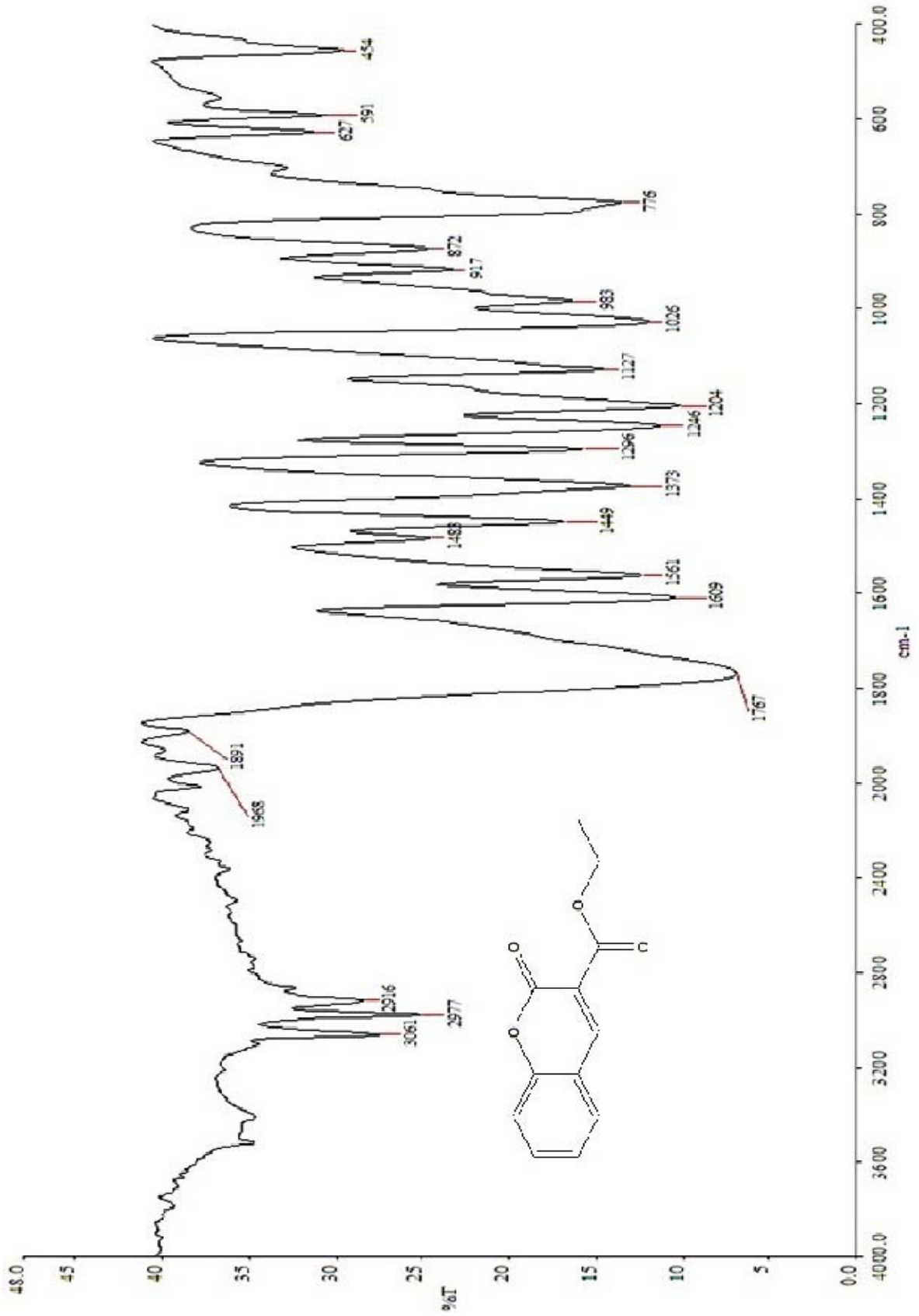


Figure 1: IR of Ethyl 2-oxo-2H-chromene-3-carboxylate **2a**

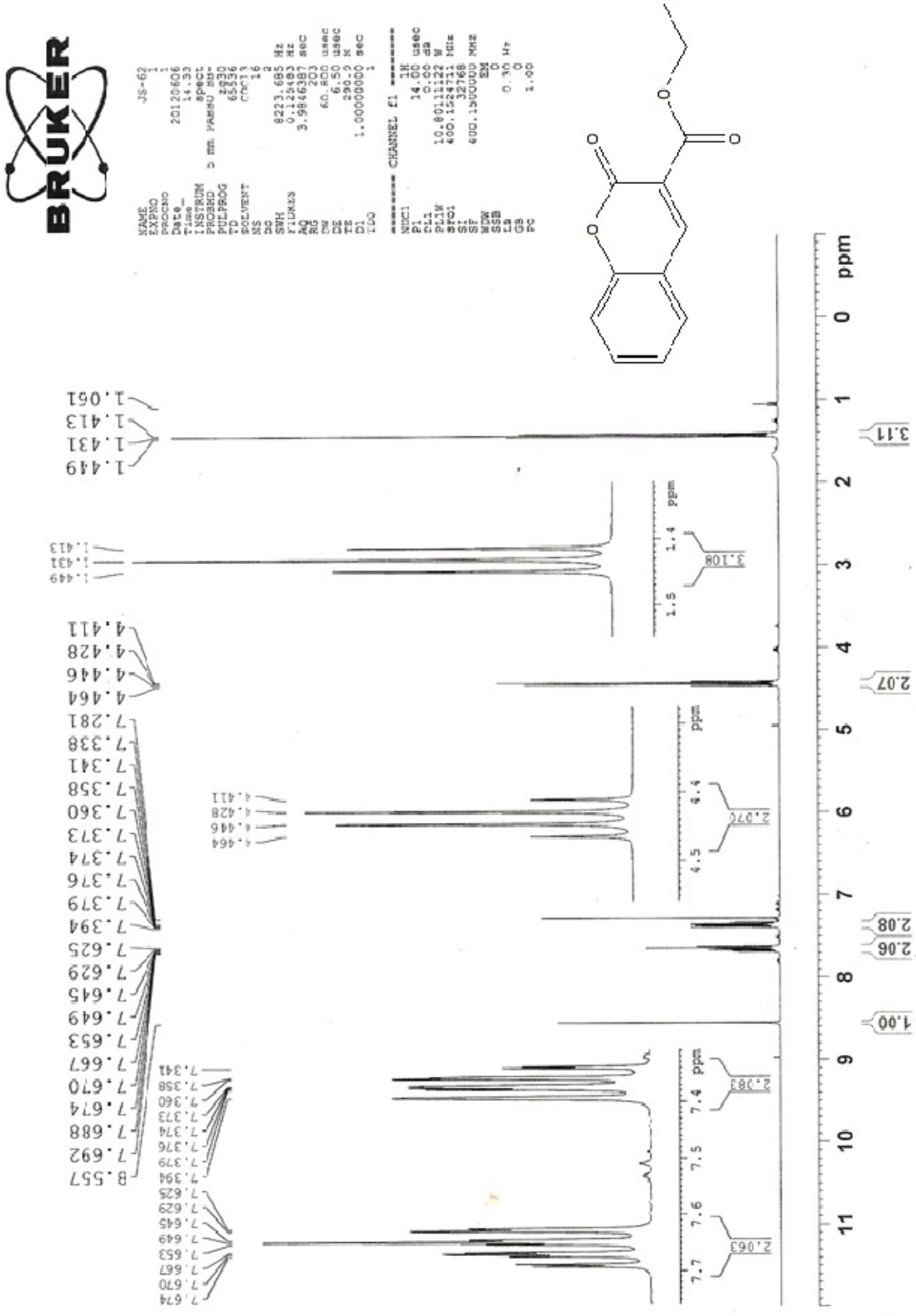


Figure 2: ^{1}H NMR of Ethyl 2-oxo-2H-chromene-3-carboxylate **2a**

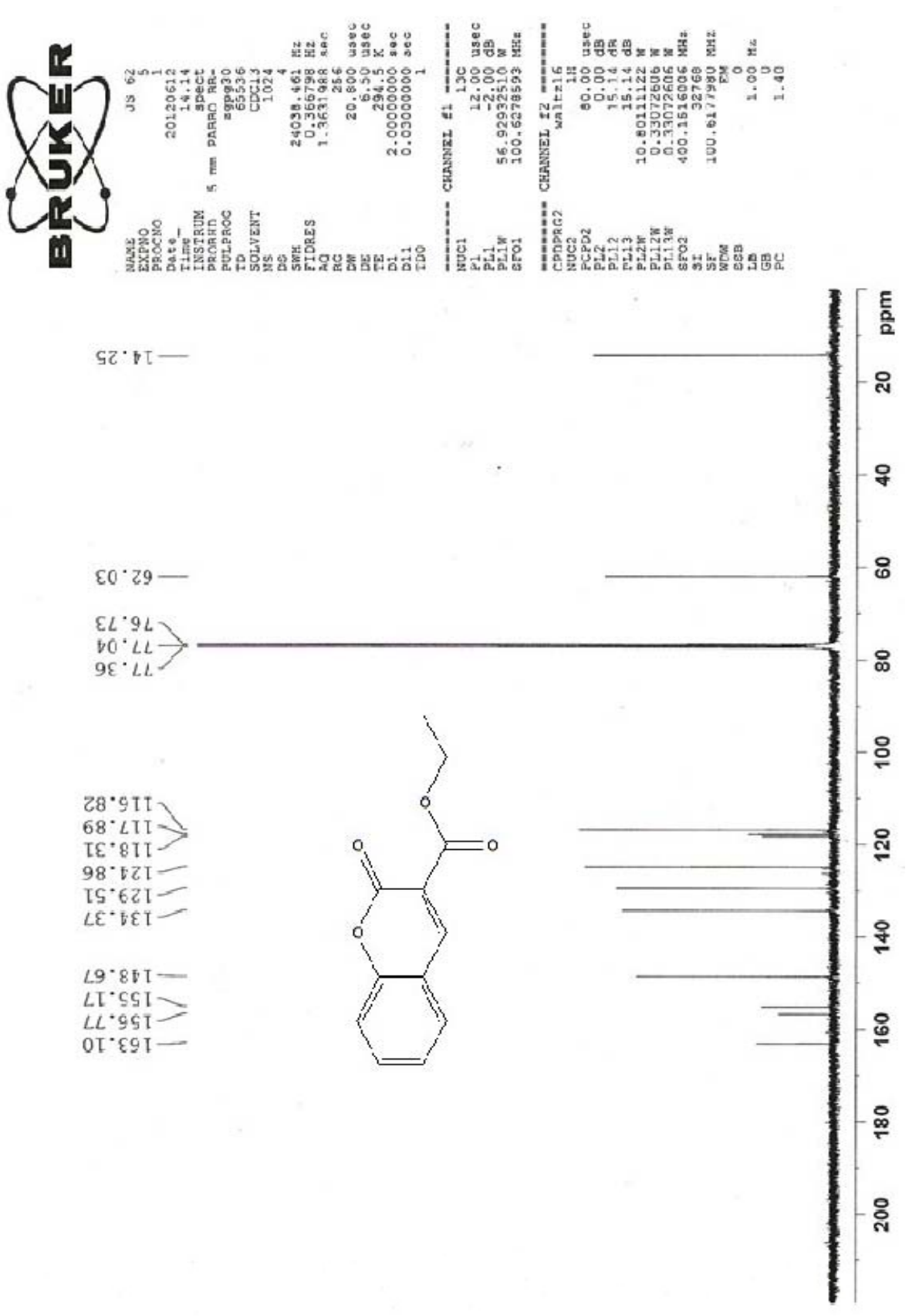


Figure 3: ^{13}C NMR of Ethyl 2-oxo-2H-chromene-3-carboxylate **2a**

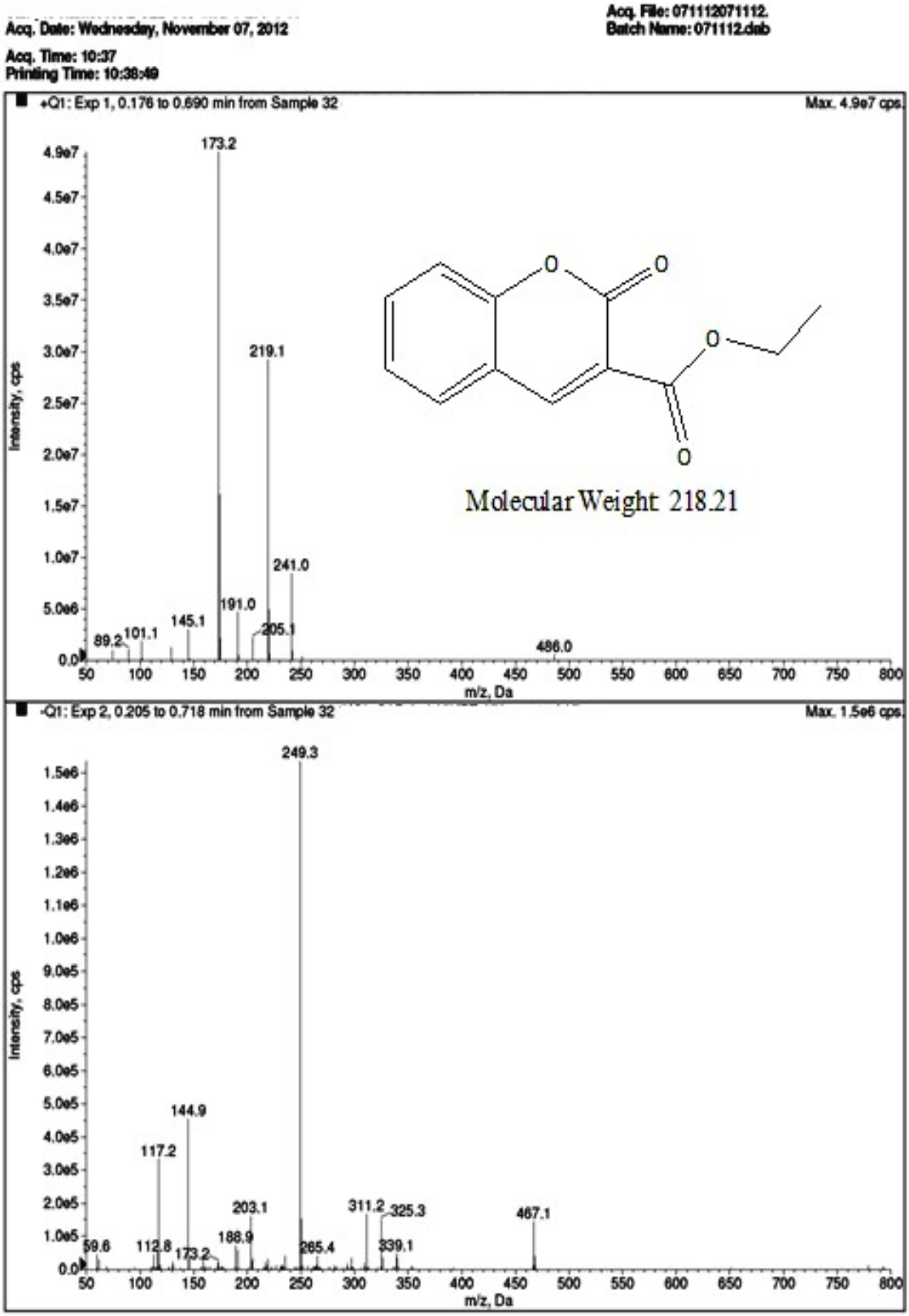


Figure 4: ESI/MS of Ethyl 2-oxo-2H-chromene-3-carboxylate **2a**

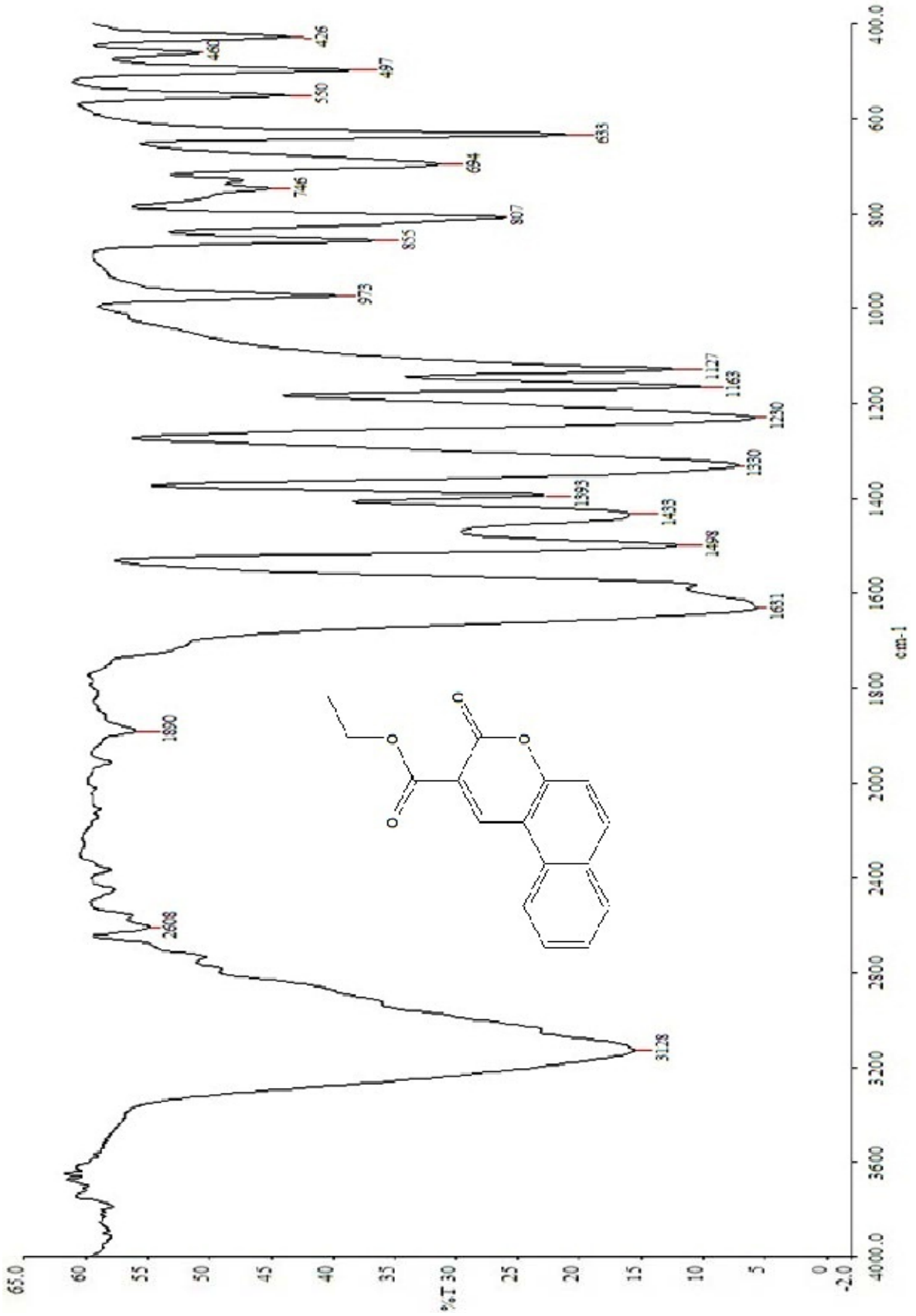


Figure 5: IR of Ethyl 3-oxo-3H-benzo[f]chromene-2-carboxylate **2d**

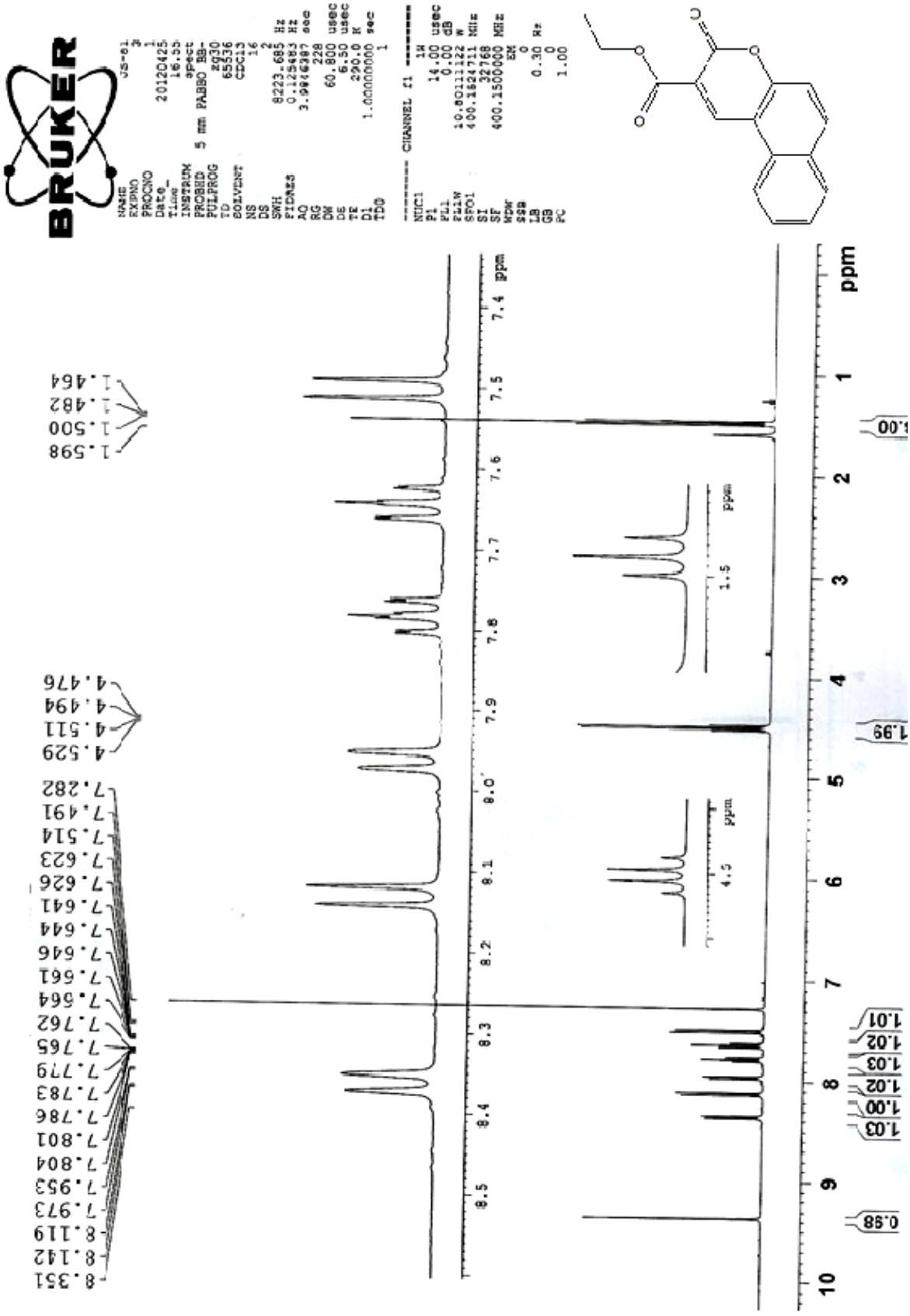


Figure 6: [1]H NMR of Ethyl 3-oxo-3H-benzo[f]chromene-2-carboxylate **2d**

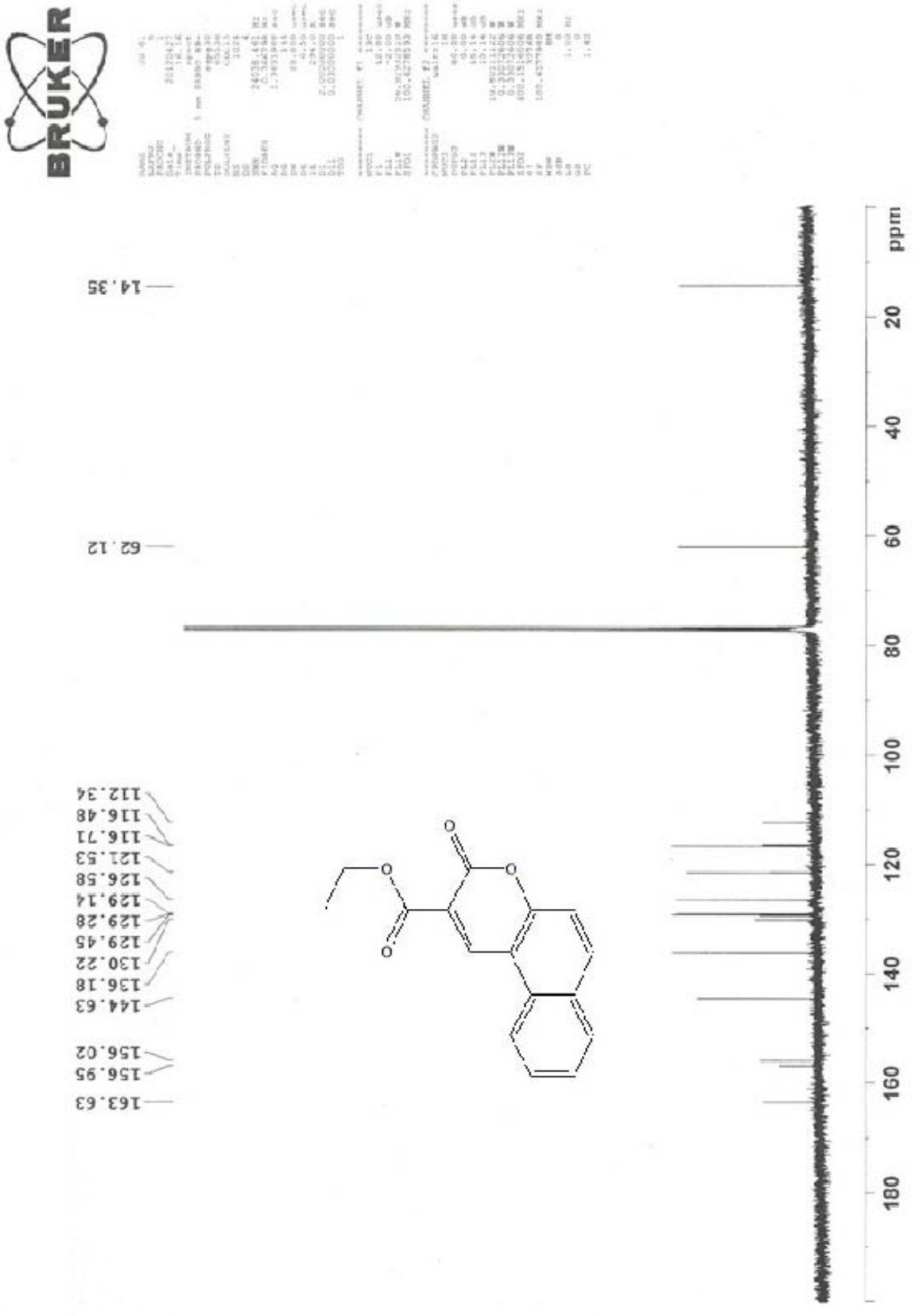


Figure 7: ^{13}C NMR of Ethyl 3-oxo-3H-benzo[f]chromene-2-carboxylate **2d**

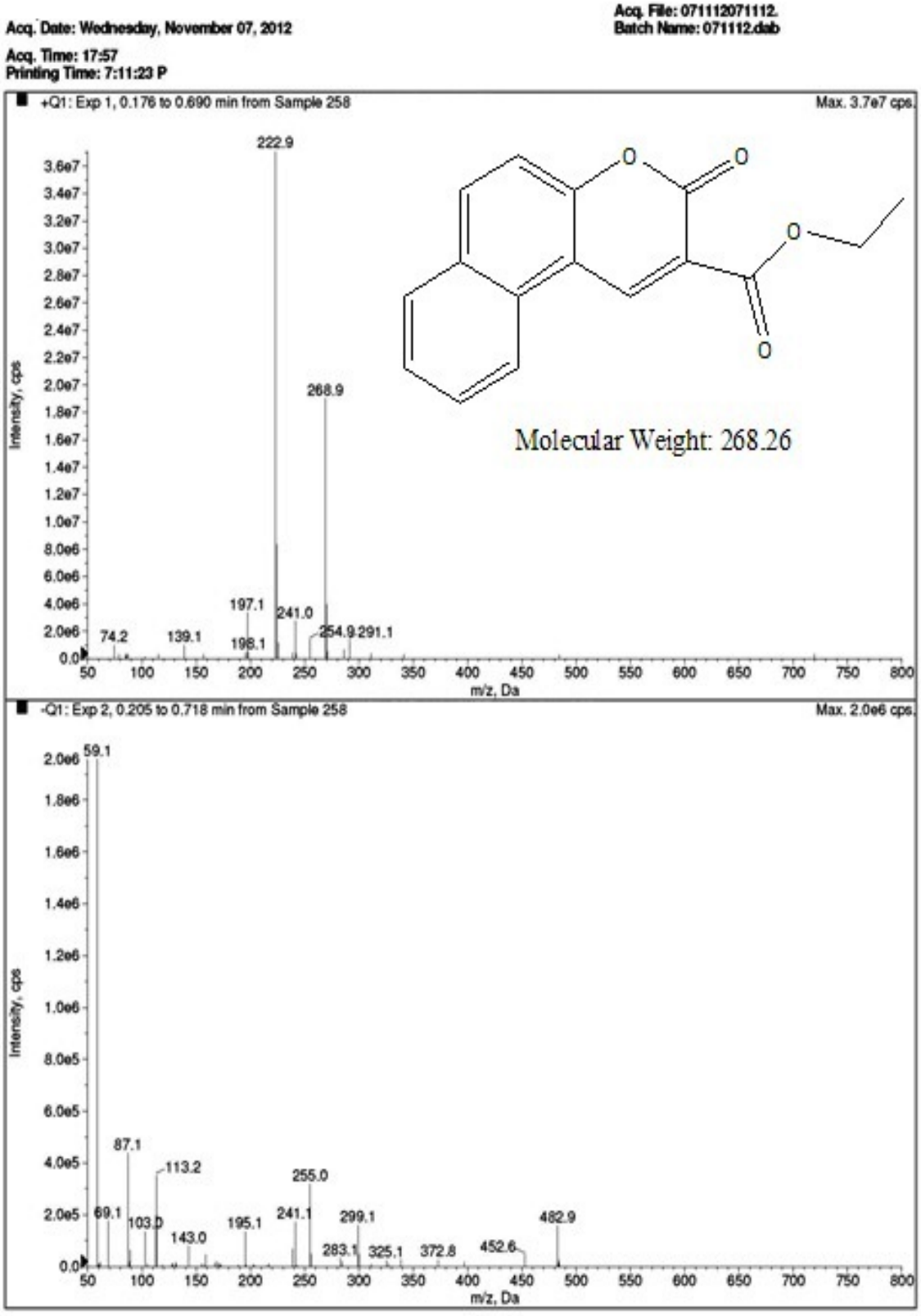


Figure 8: ESI/MS of Ethyl 3-oxo-3H-benzo[f]chromene-2-carboxylate **2d**

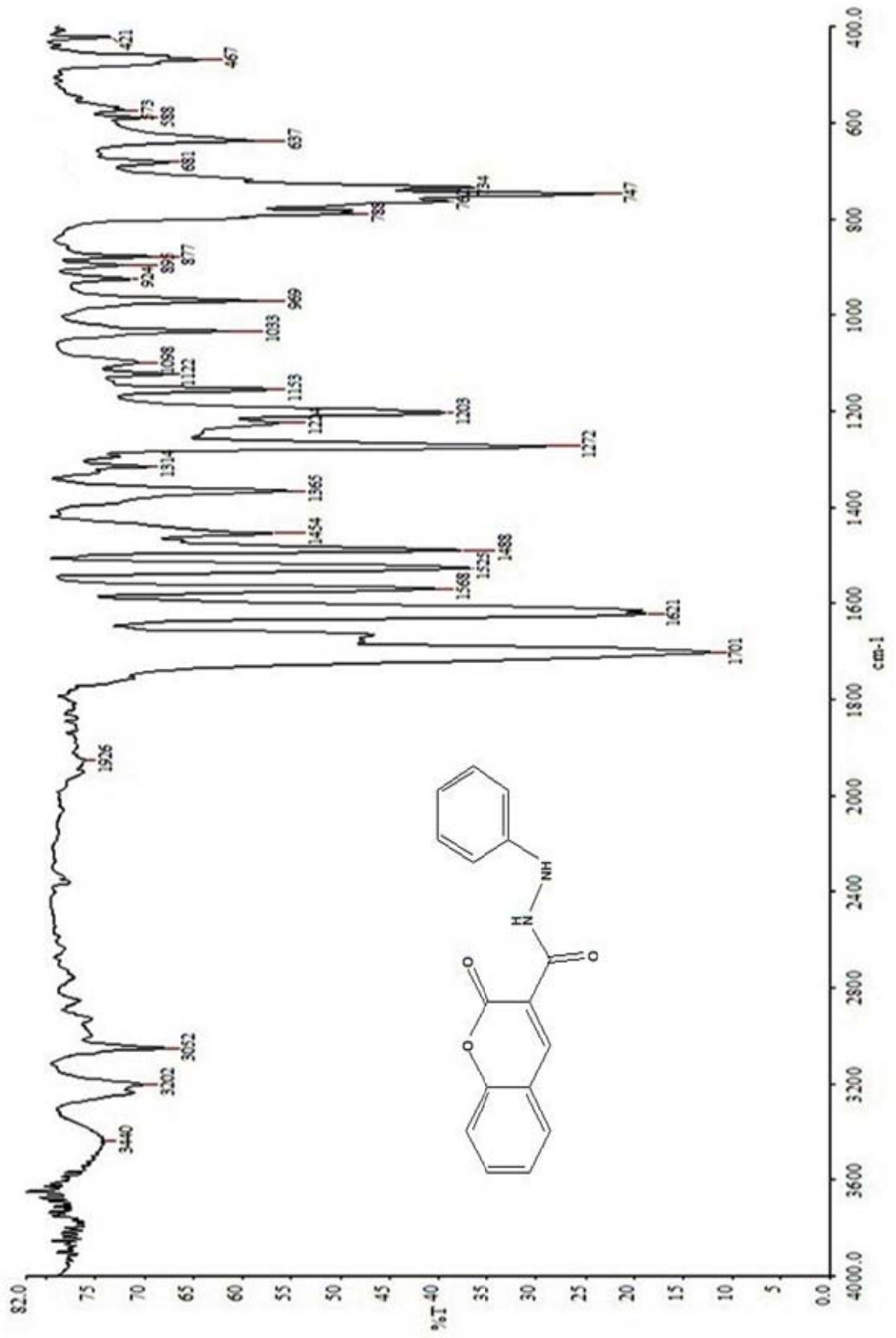


Figure 9: IR of 2-oxo-N'-phenyl-2H-chromene-3-carbohydrazide **3a**

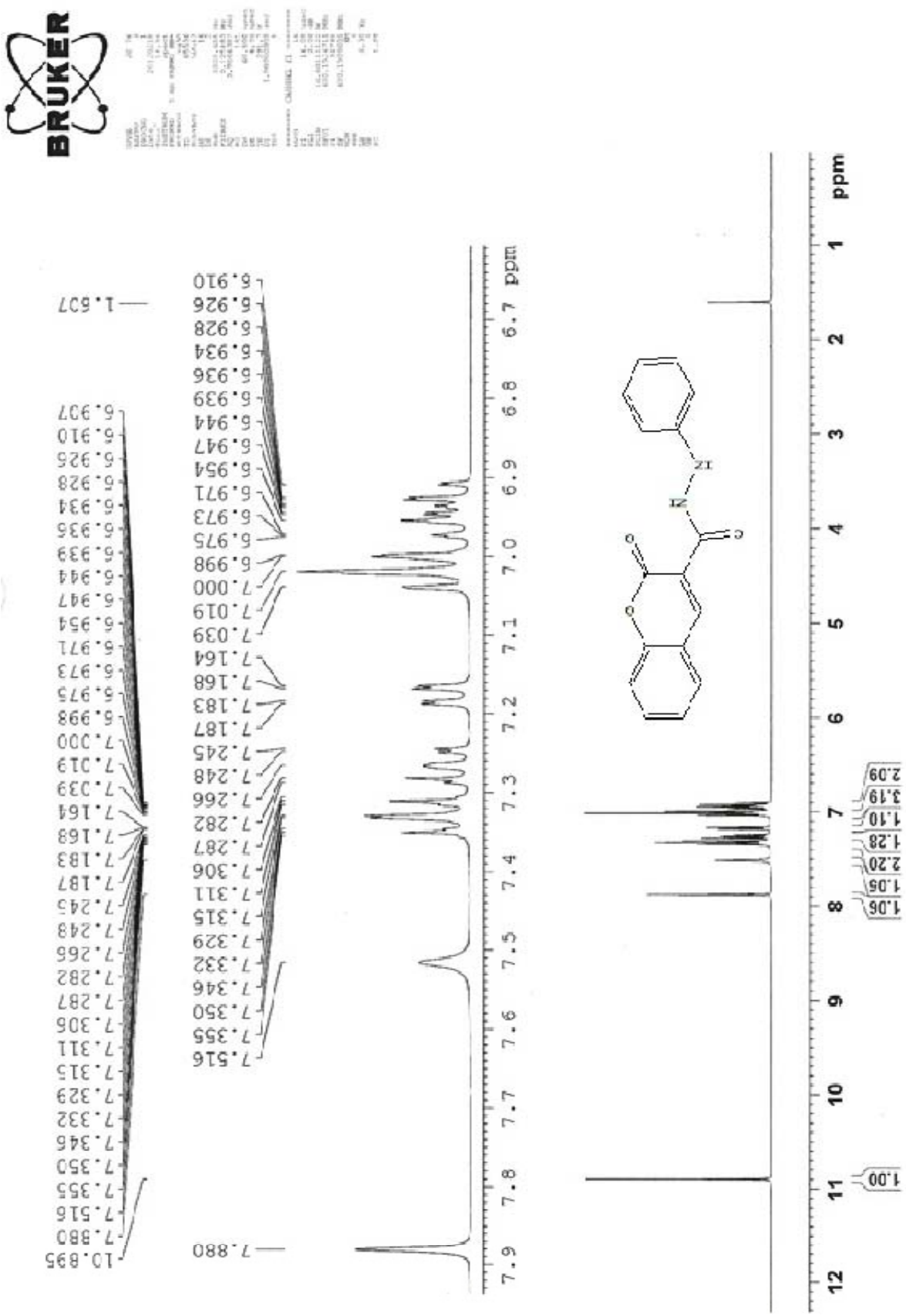


Figure 10: ^{1}H NMR of 2-oxo-N'-phenyl-2H-chromene-3-carbohydrazide **3a**

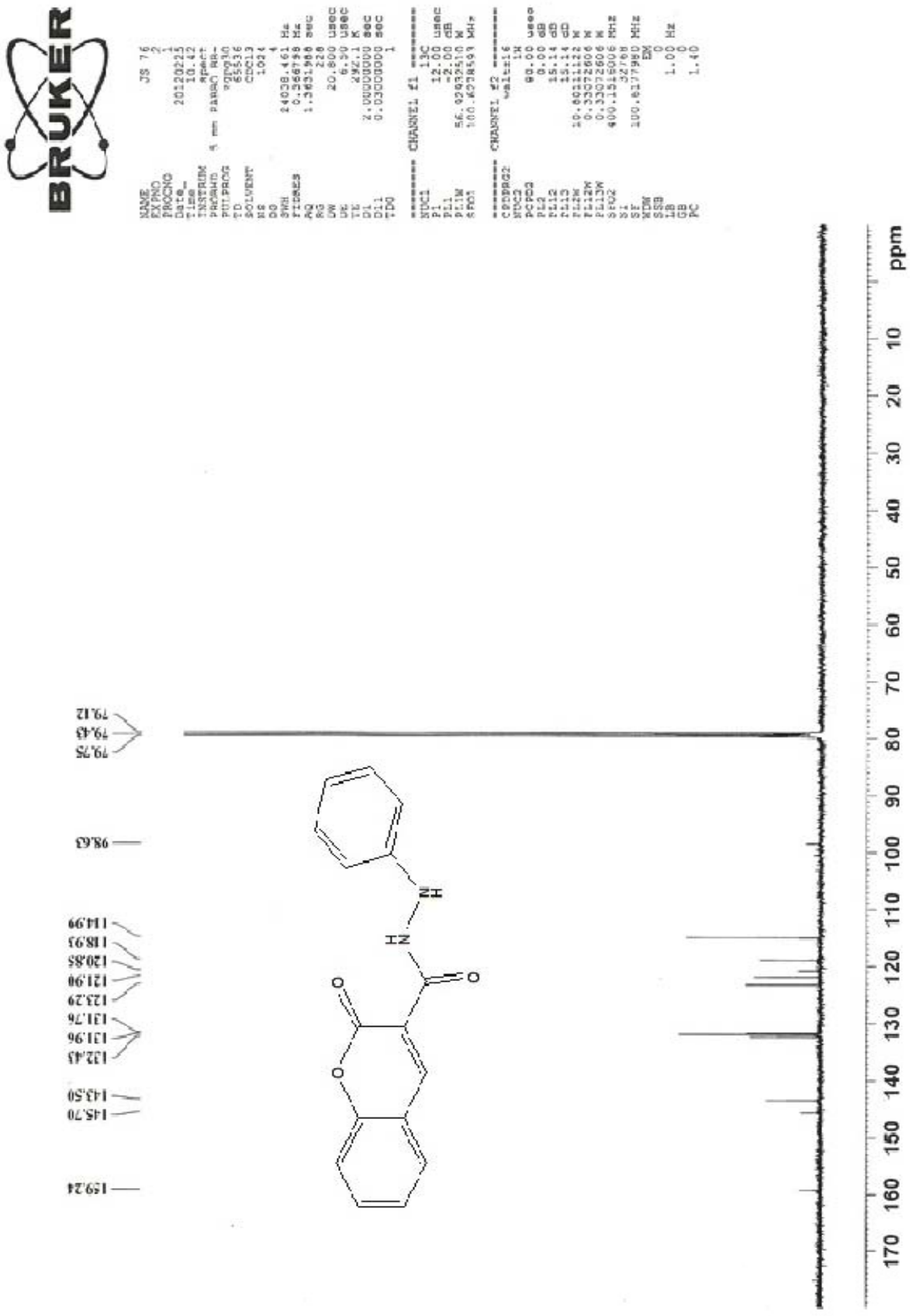


Figure 11: ^{13}C NMR of 2-oxo-N'-phenyl-2H-chromene-3-carbohydrazide **3a**

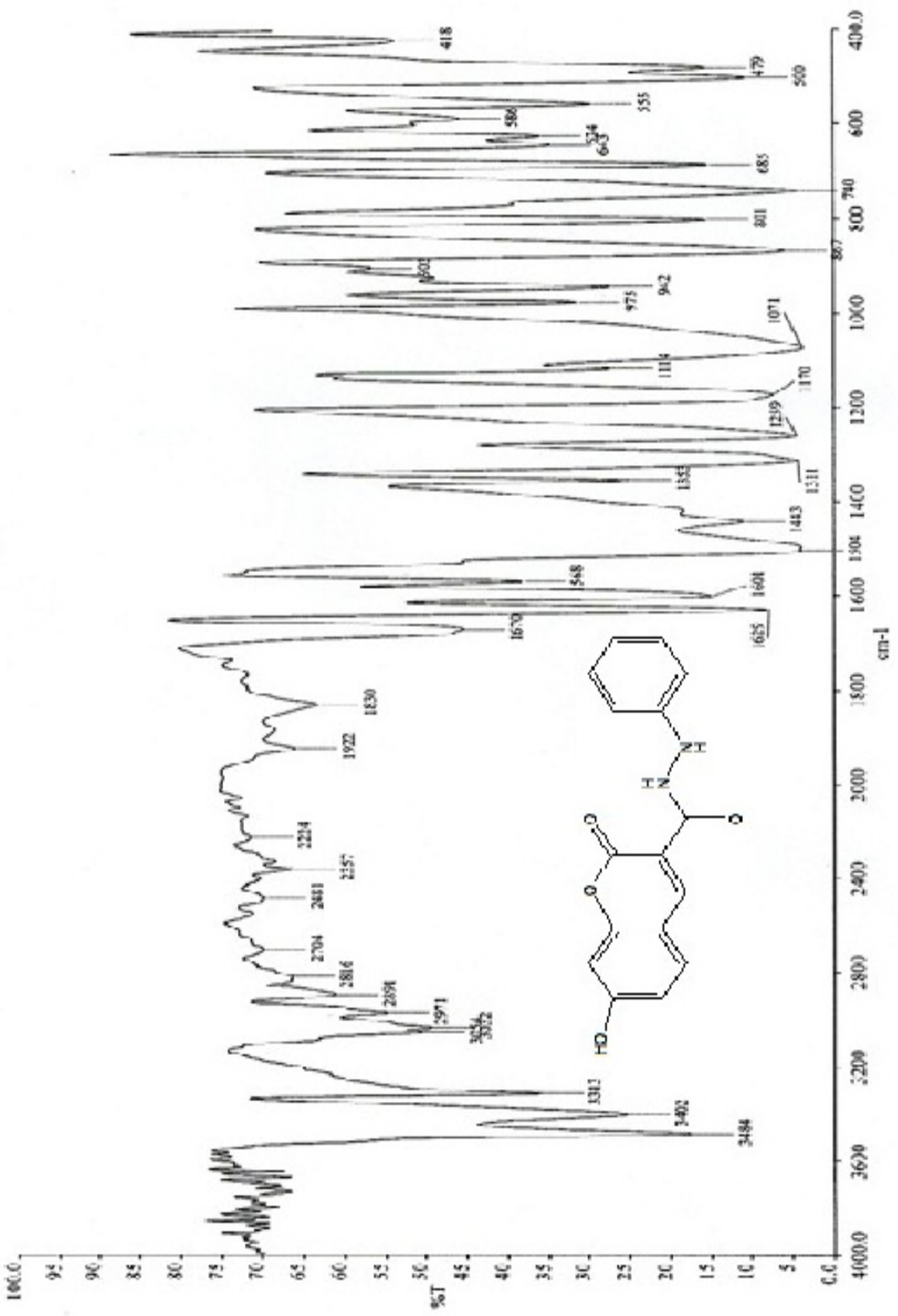


Figure 12: IR of 7-hydroxy-2-oxo-N'-phenyl-2H-chromene-3-carbohydrazide **3b**

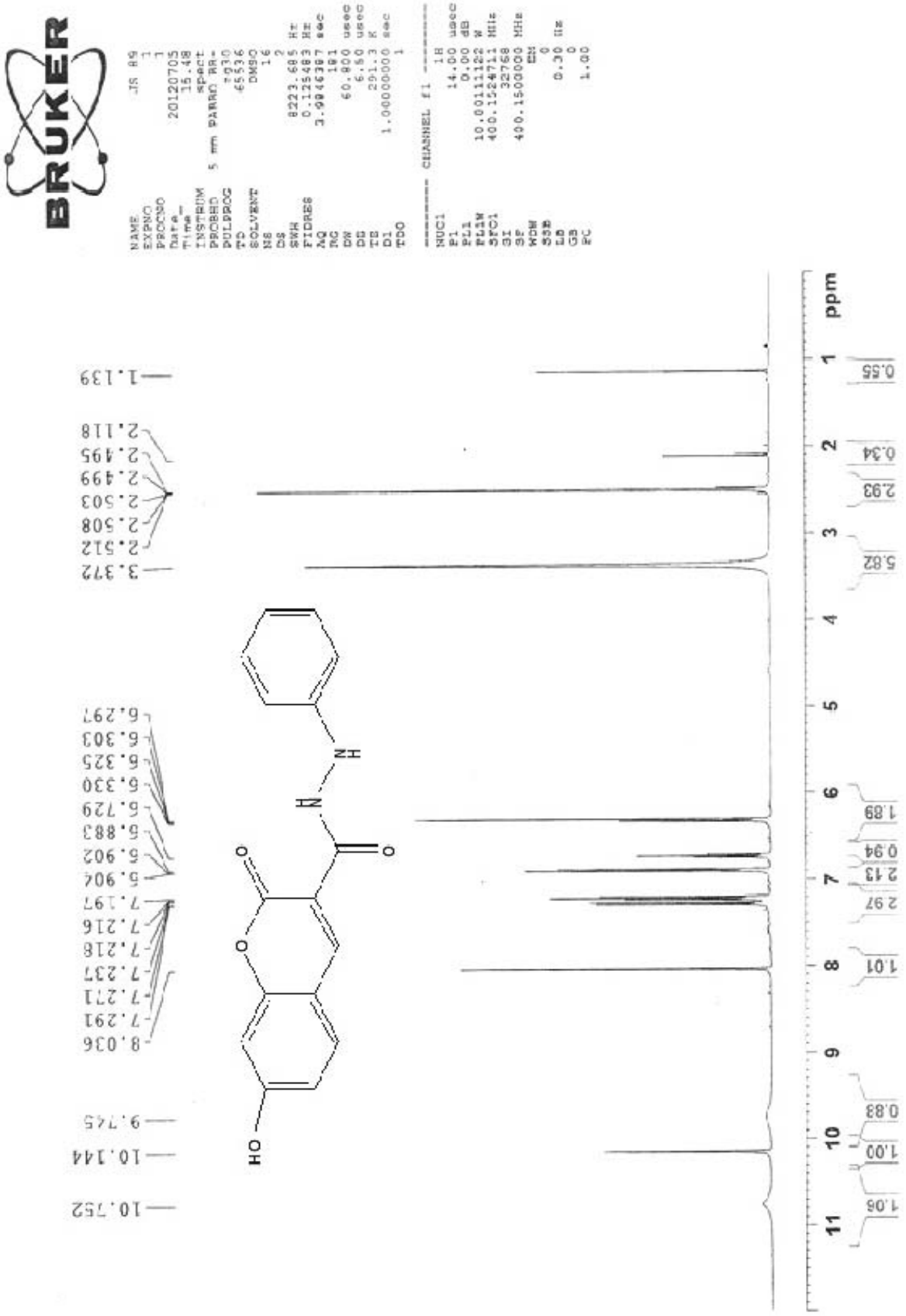


Figure 13: ^{1}H NMR of 7-hydroxy-2-oxo-N'-phenyl-2H-chromene-3-carbohydrazide **3b**

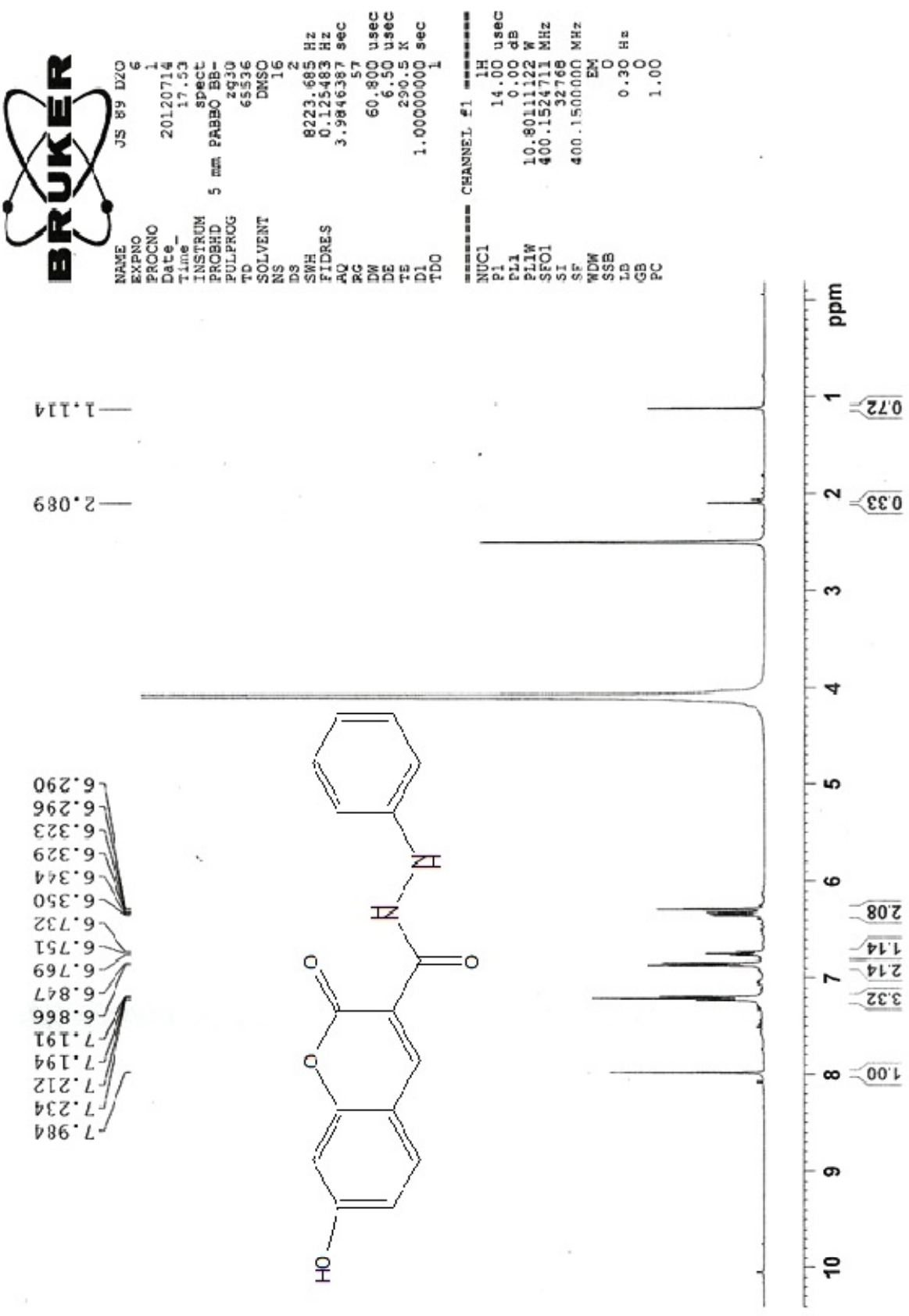


Figure 13 a: D_2O exchange of 7-hydroxy-2-oxo-N'-phenyl-2H-chromene-3-carbohydrazide **3b**

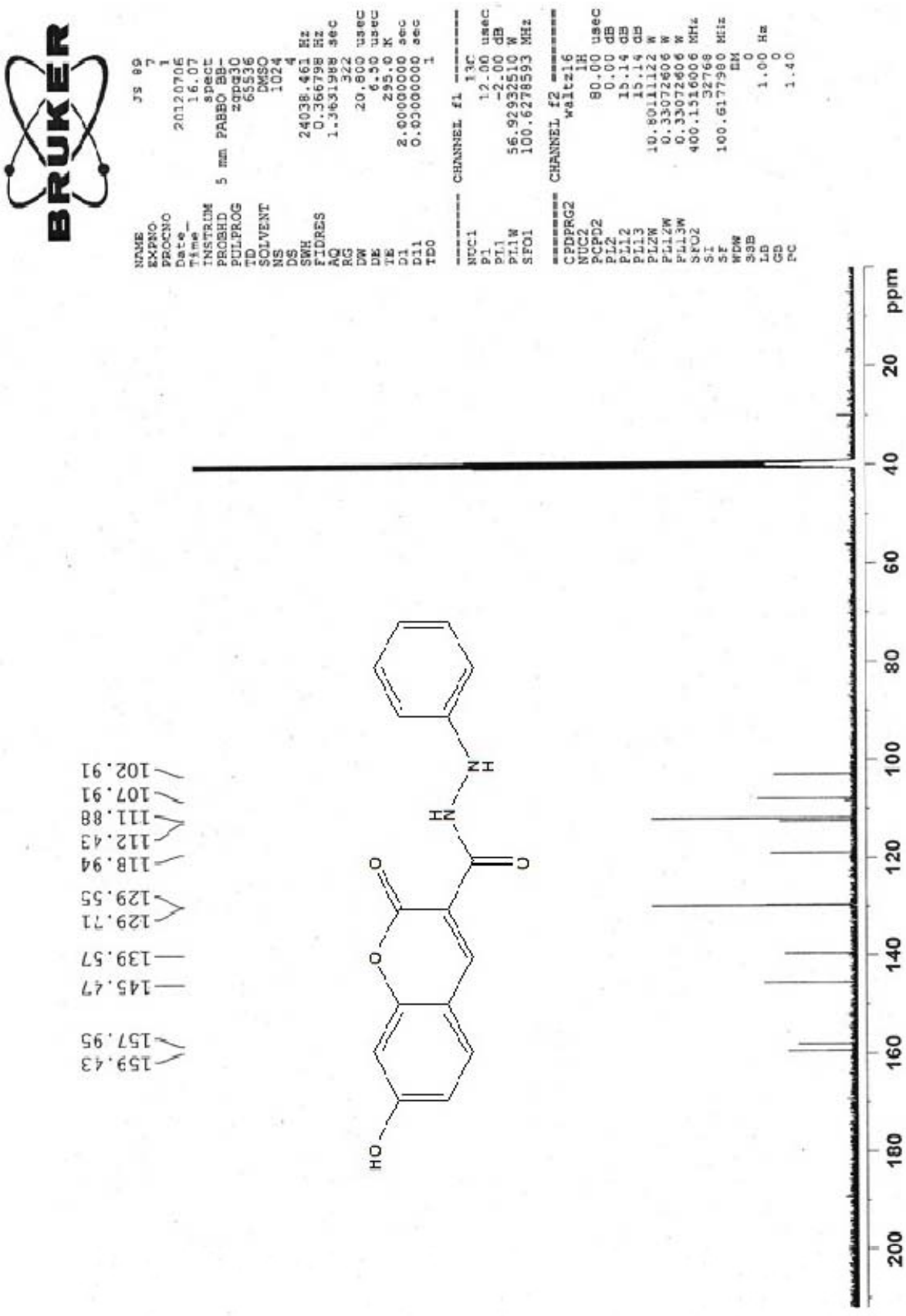


Figure 14: ^{13}C NMR of 7-hydroxy-2-oxo-N'-phenyl-2H-chromene-3-carbohydrazide **3b**

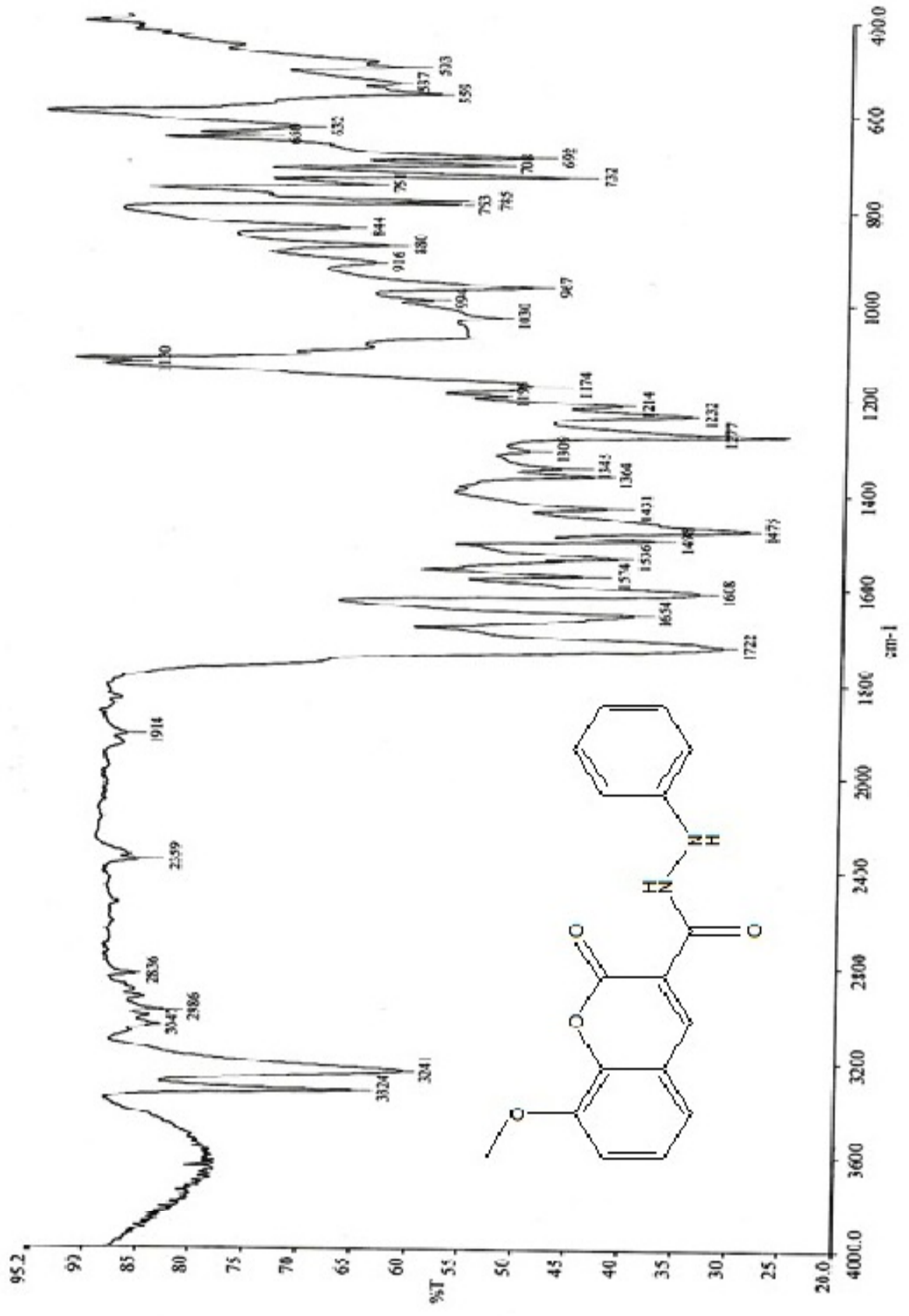


Figure 15: IR of 8-methoxy-2-oxo-N'-phenyl-2H-chromene-3-carbohydrazide **3c**

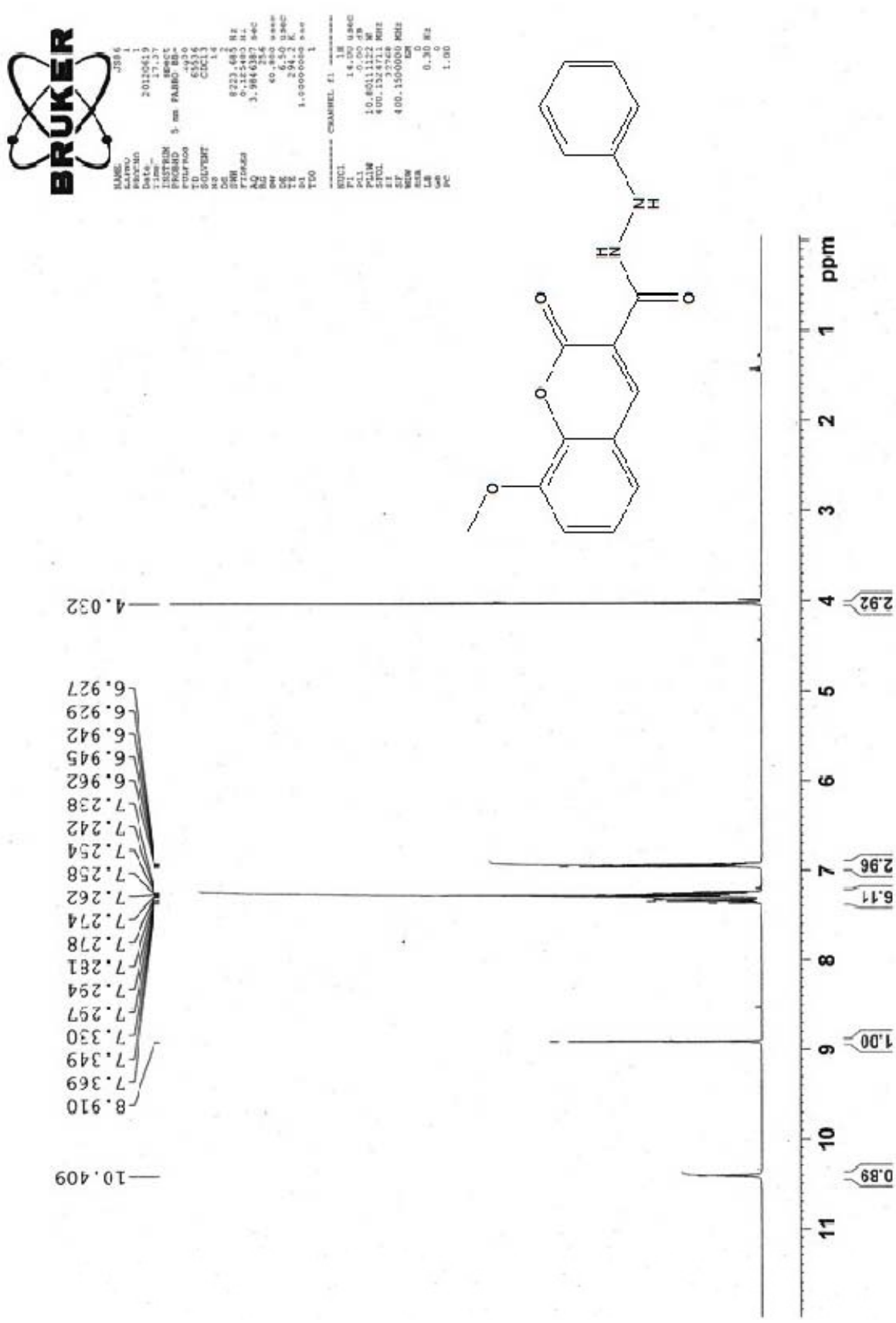


Figure 16: ^{1}H NMR of 8-methoxy-2-oxo-N'-phenyl-2H-chromene-3-carbohydrazide **3c**

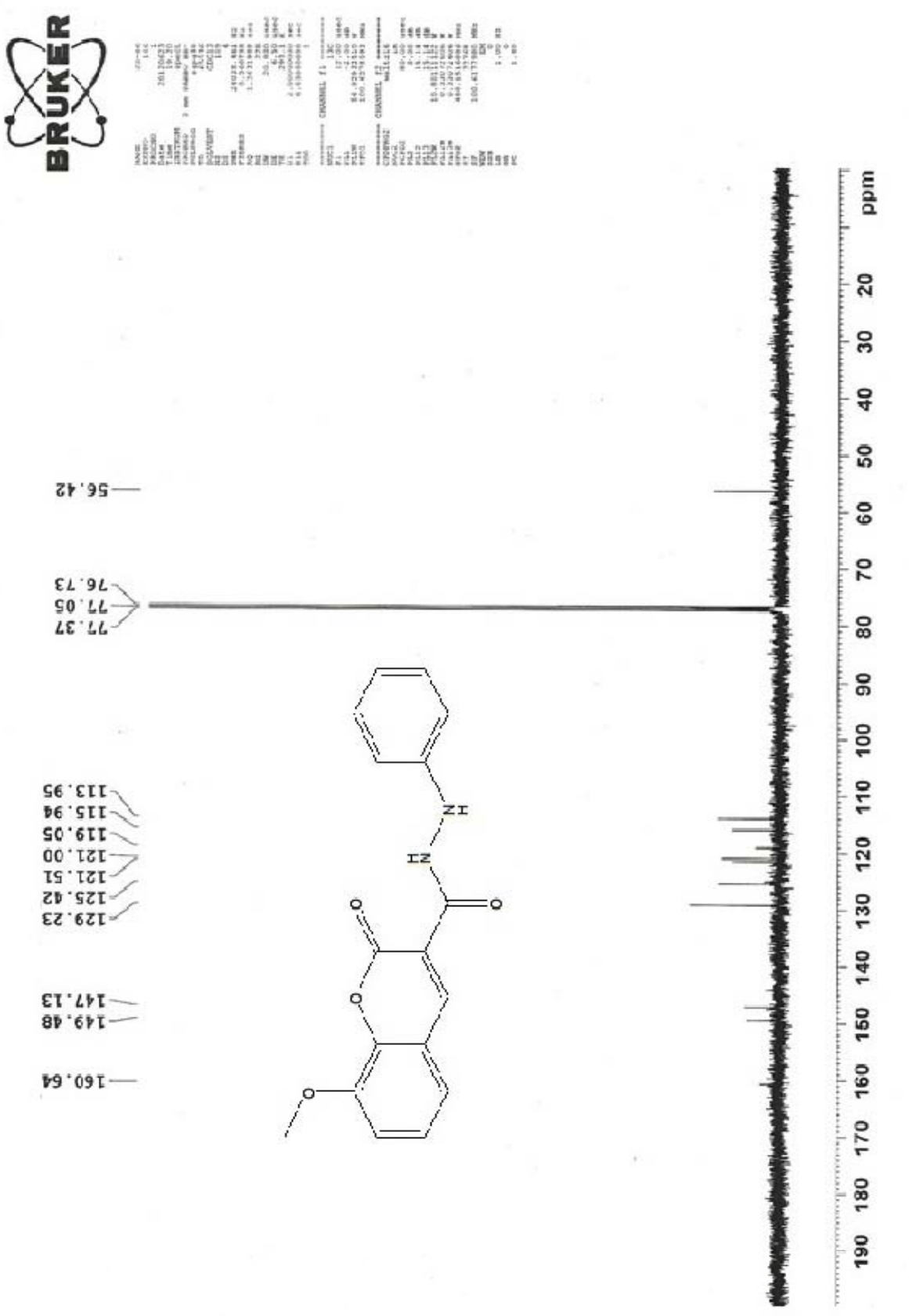


Figure 17: ^{13}C NMR of 8-methoxy-2-oxo-N'-phenyl-2H-chromene-3-carbohydrazide **3c**

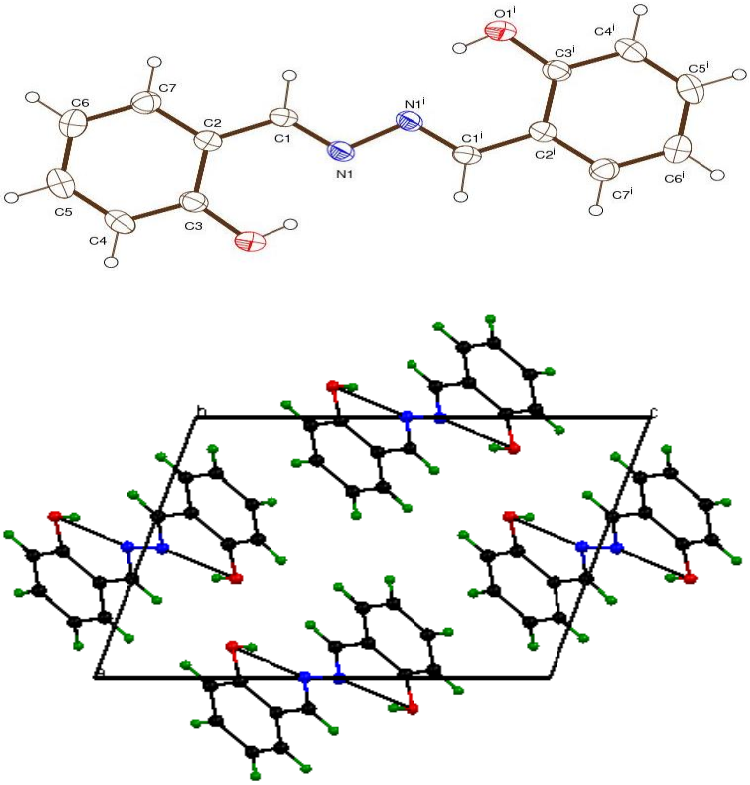


Figure 18: ORTEP diagram and Crystal packing diagram of 2, 2'-(1E,1'E)-hydrazine-1,2-diylidenebis(methan-1-yl-1-ylidene)diphenol **4a**

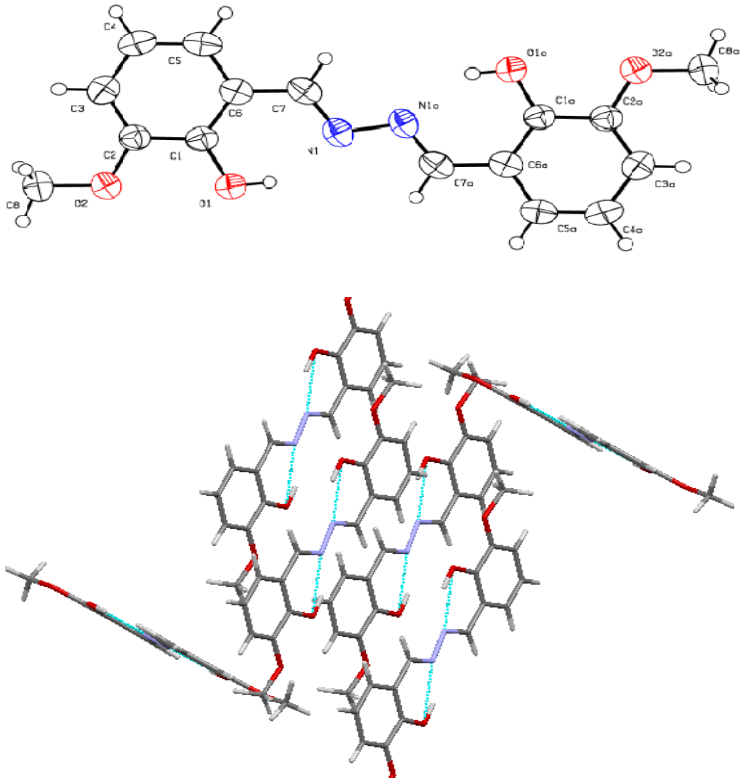


Figure 19: ORTEP diagram and Crystal packing diagram of 6, 6'-(1E, 1'E)-hydrazine-1, 2-diylidenebis(methan-1-yl-1-ylidene)bis(2-methoxy phenol) **4c**

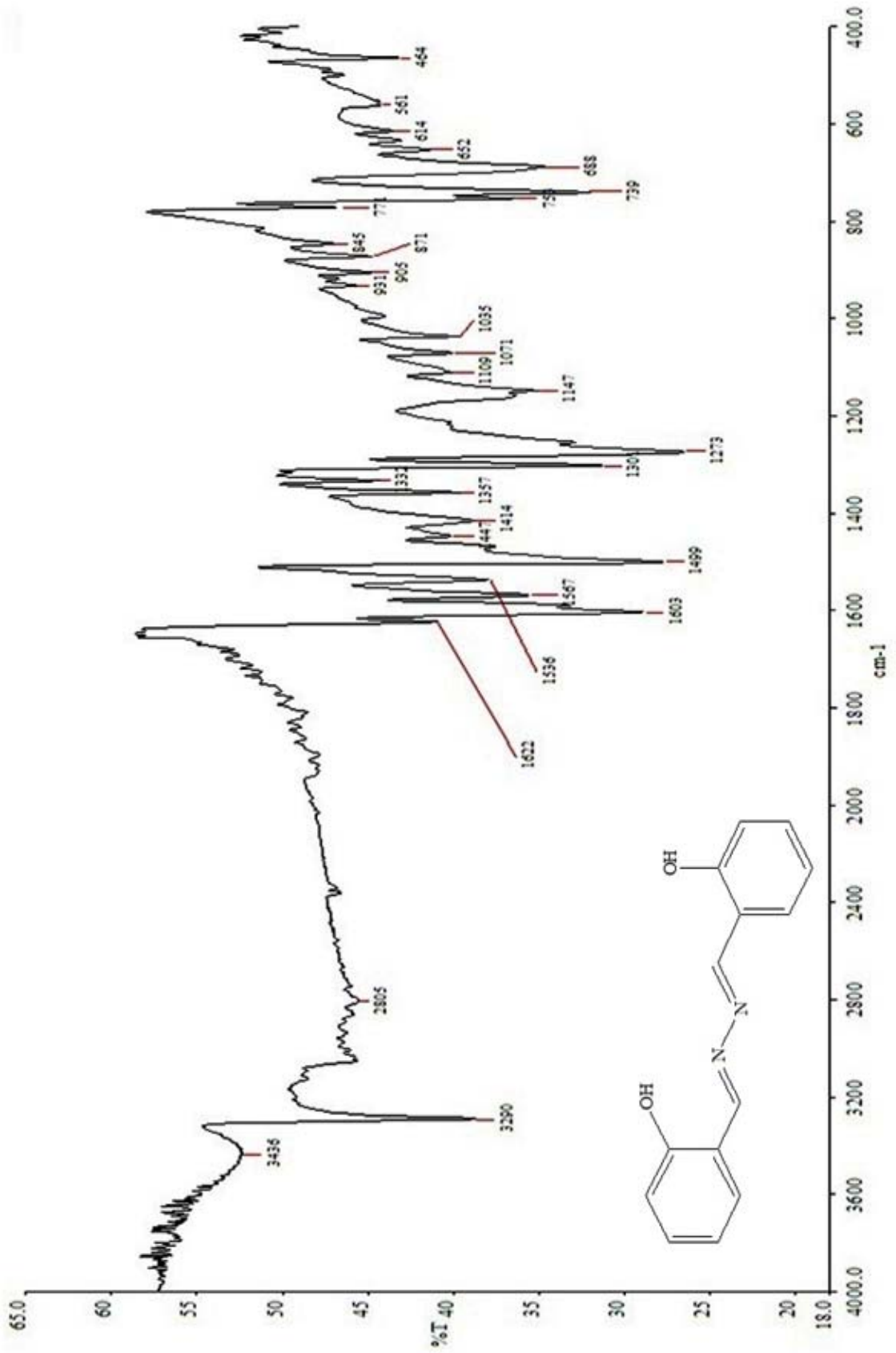


Figure 20: IR of 2, 2'-(1E,1'E)-hydrazine-1,2-diylidenebis(methan-1-yl-1-ylidene)diphenol **4a**

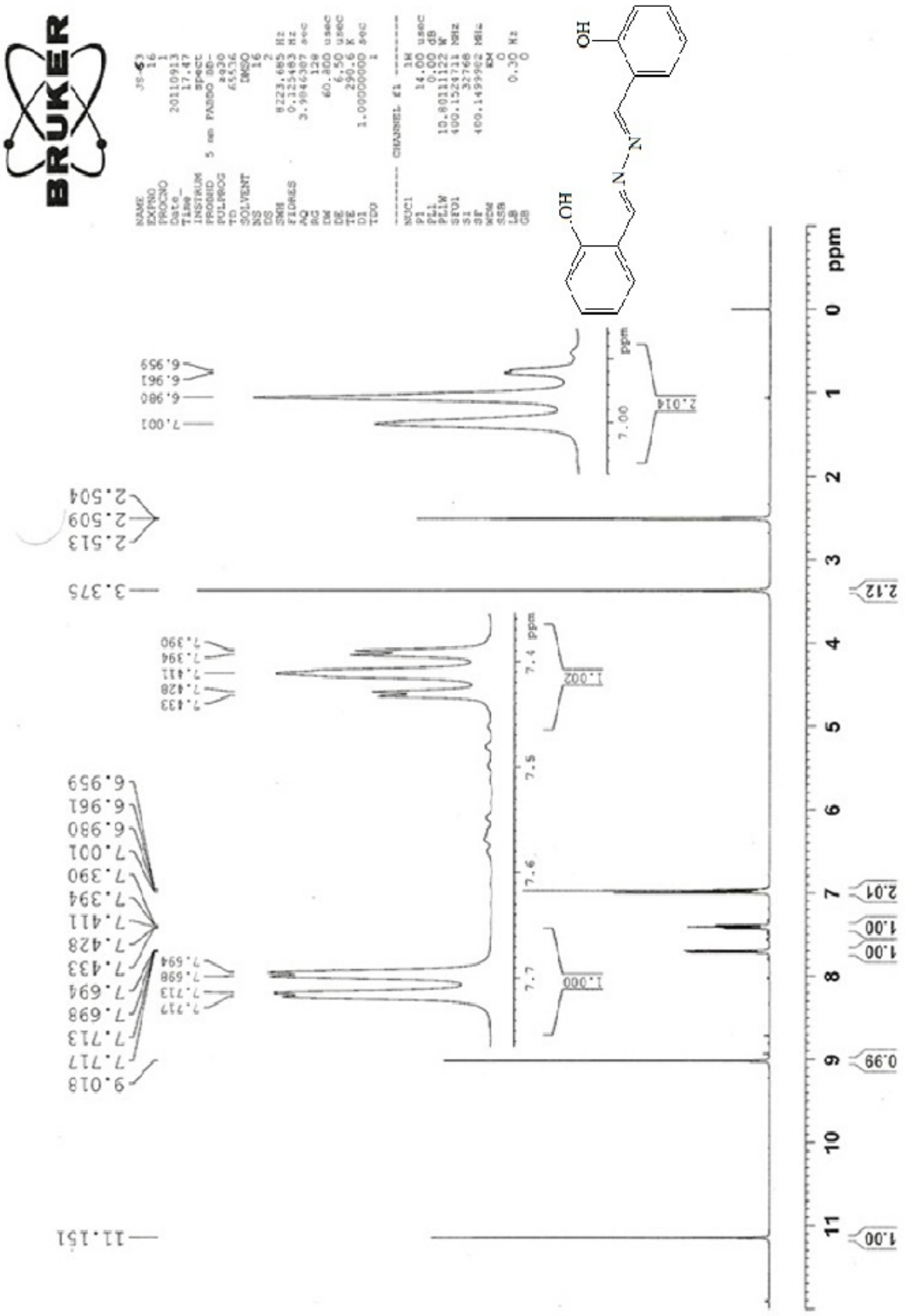


Figure 21: ^{1}H NMR of 2, 2'-(1E,1'E)-hydrazine-1,2-diylidenebis(methan-1-yl-1-ylidene)diphenol **4a**

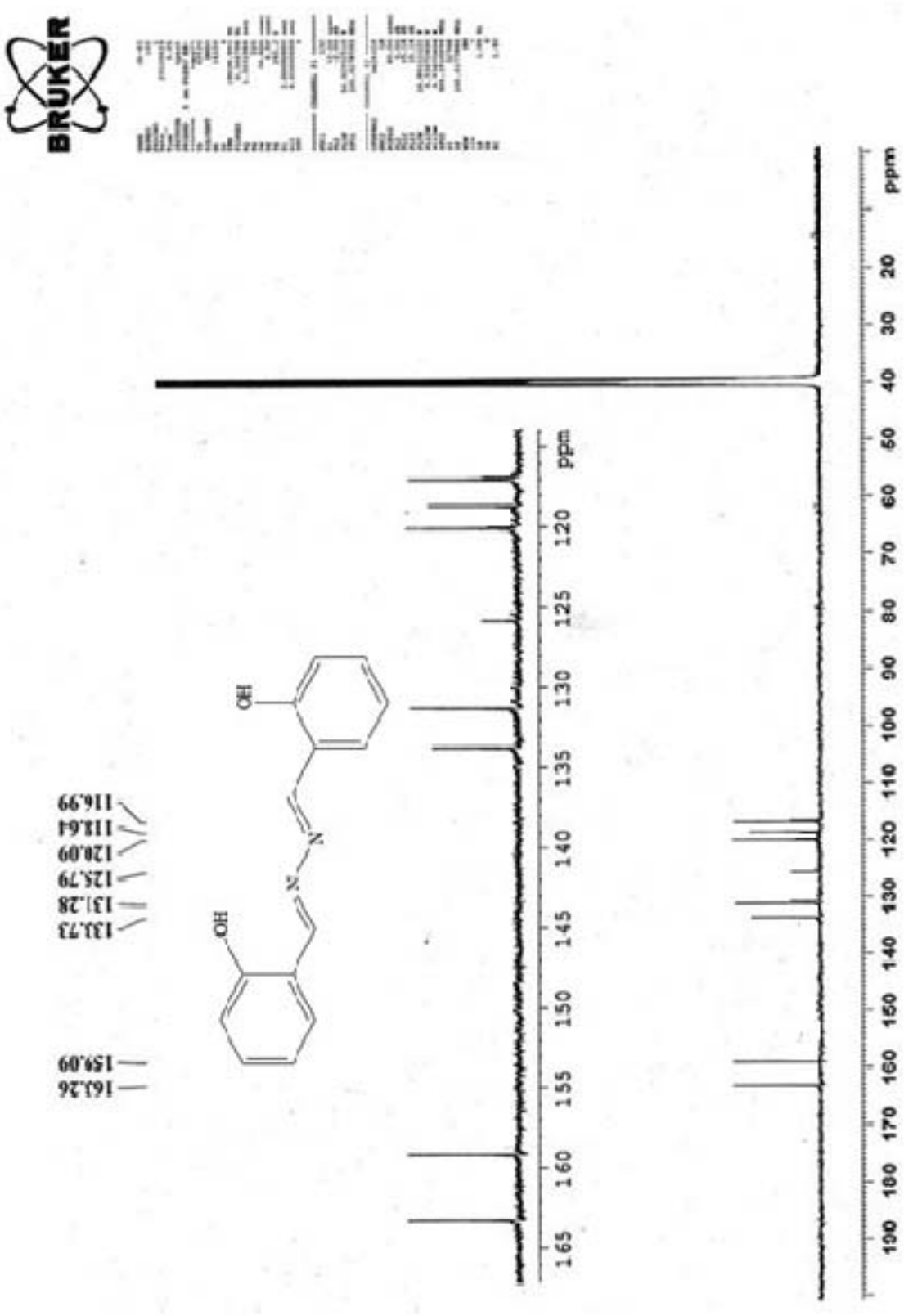


Figure 22: ^{13}C NMR of 2, 2'-(1E,1'E)-hydrazine-1,2-diylidenebis(methan-1-yl-1-ylidene)diphenol **4a**

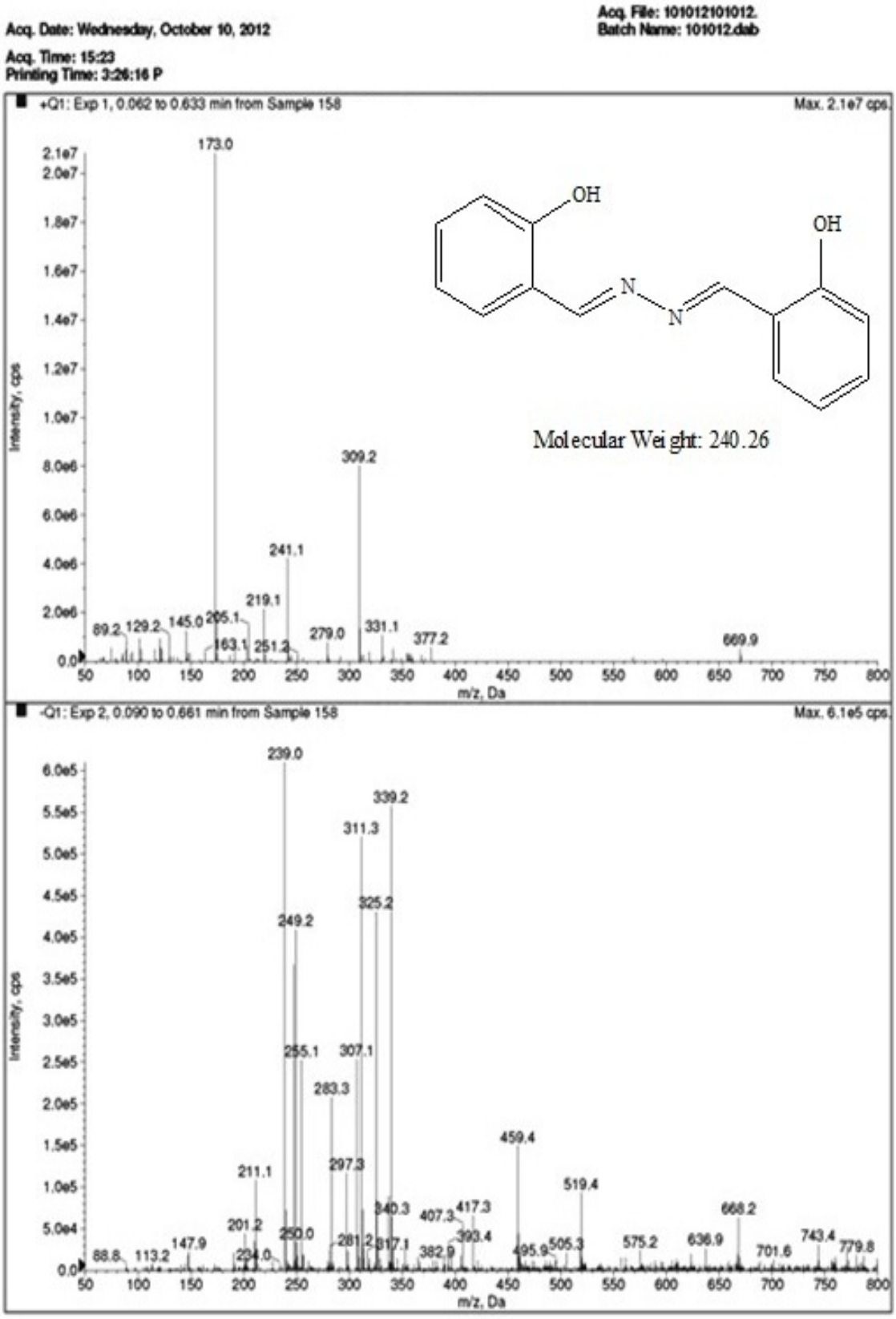


Figure 23: ESI/MS of 2, 2'-(1E,1'E)-hydrazine-1,2-diylidenebis(methan-1-yl-1-ylidene)diphenol **4a**

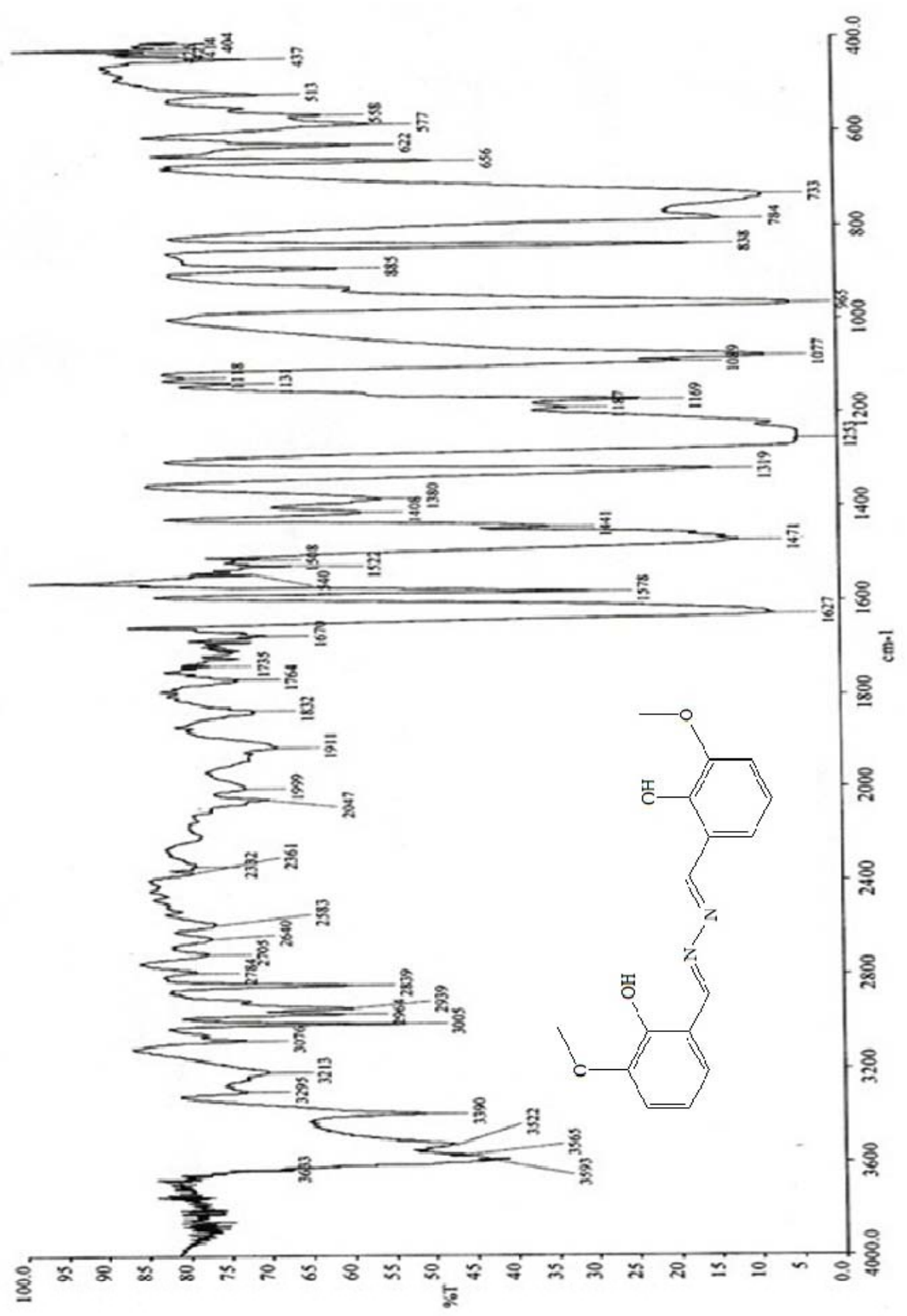


Figure 24: IR of 6, 6'-(1E, 1'E)-hydrazine-1, 2-diylidenebis(methan-1-yl-1-ylidene)bis(2-methoxyphenol)

4c

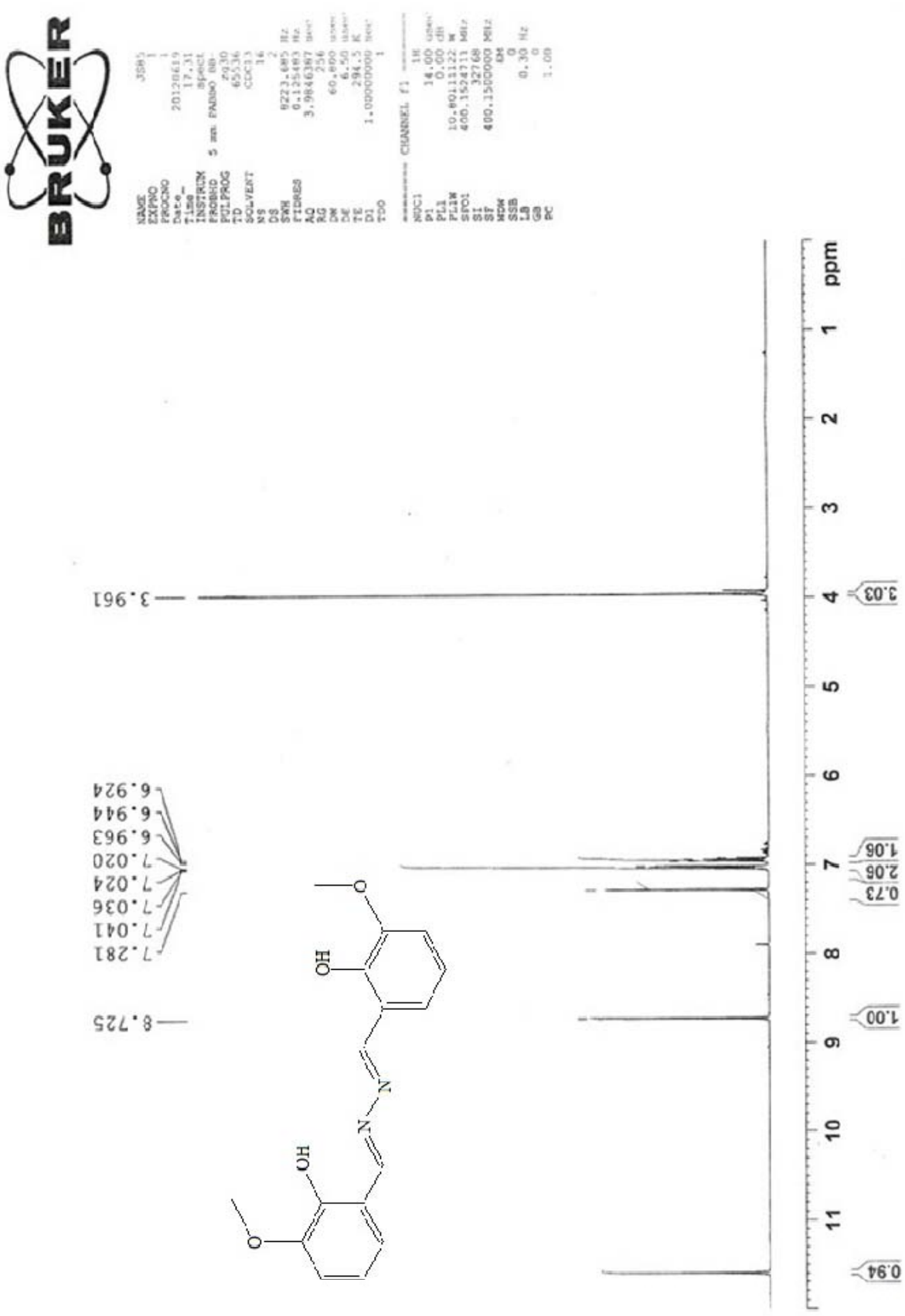


Figure 25: ^{1}H NMR of 6, 6'-(1E, 1'E)-hydrazine-1, 2-diylidenebis(methan-1-yl-1-ylidene)bis(2-methoxyphenol) **4c**

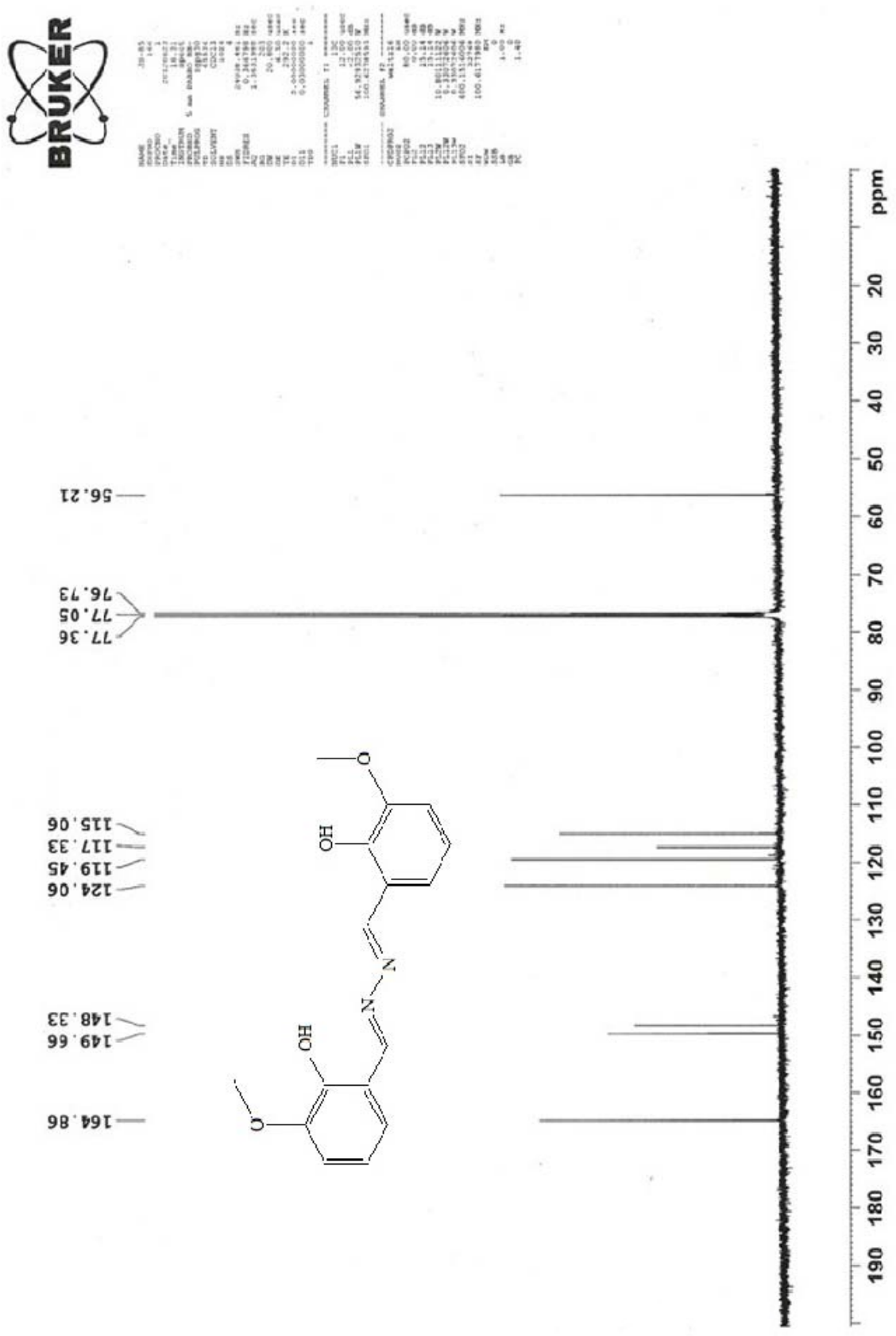


Figure 26: ^{13}C NMR of 6, 6'-(1E, 1'E)-hydrazine-1, 2-diylidenebis(methan-1-yl-1-ylidene)bis(2-methoxyphenol) **4c**

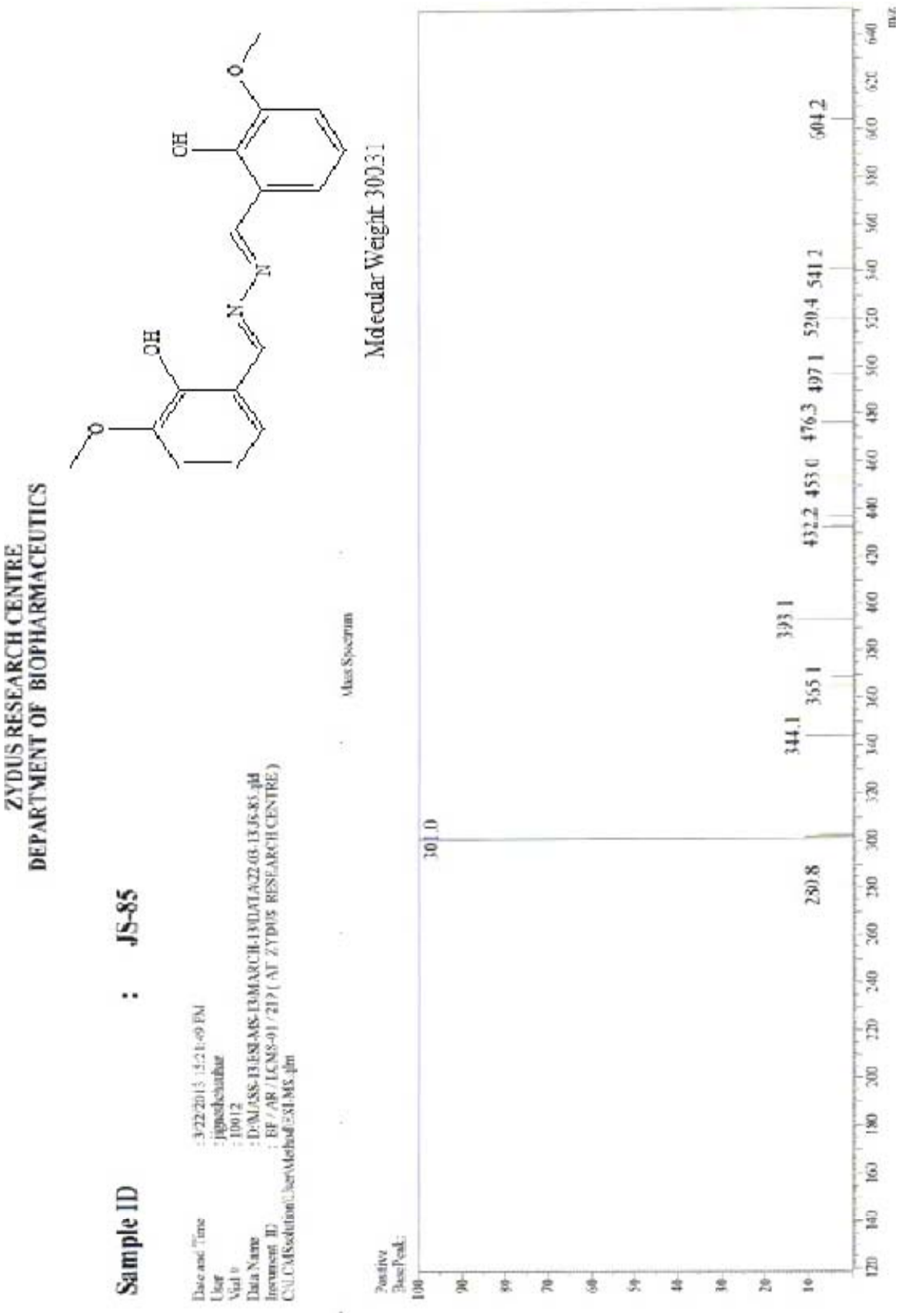


Figure 27: ESI/MS of 6, 6'-(1E, 1'E)-hydrazine-1, 2-diylidenebis(methan-1-yl-1-ylidene)bis(2-methoxyphenol) **4c**

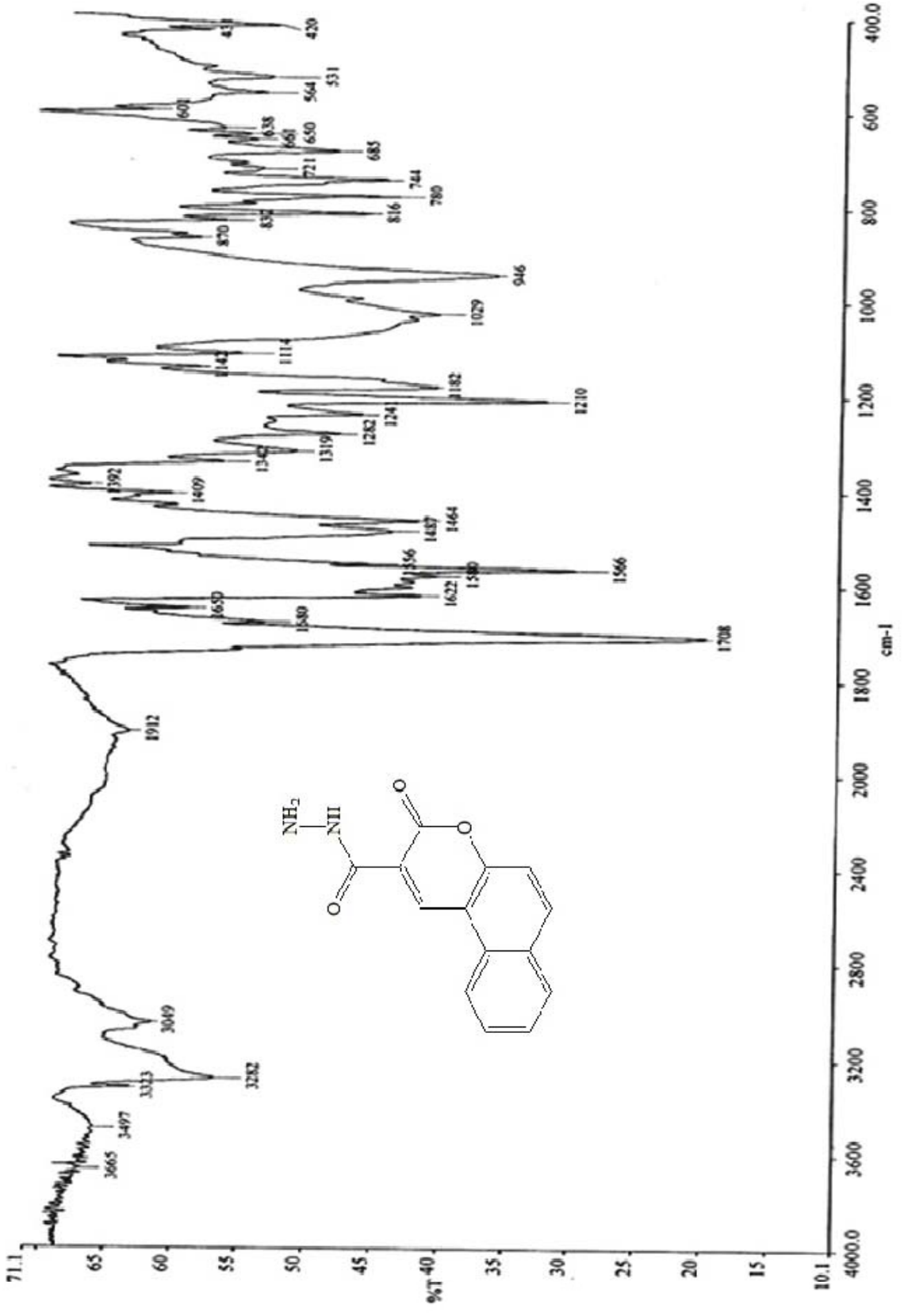


Figure 28: IR of 3-oxo-3H-benzo[f]chromene-2-carbohydrazide **4d**

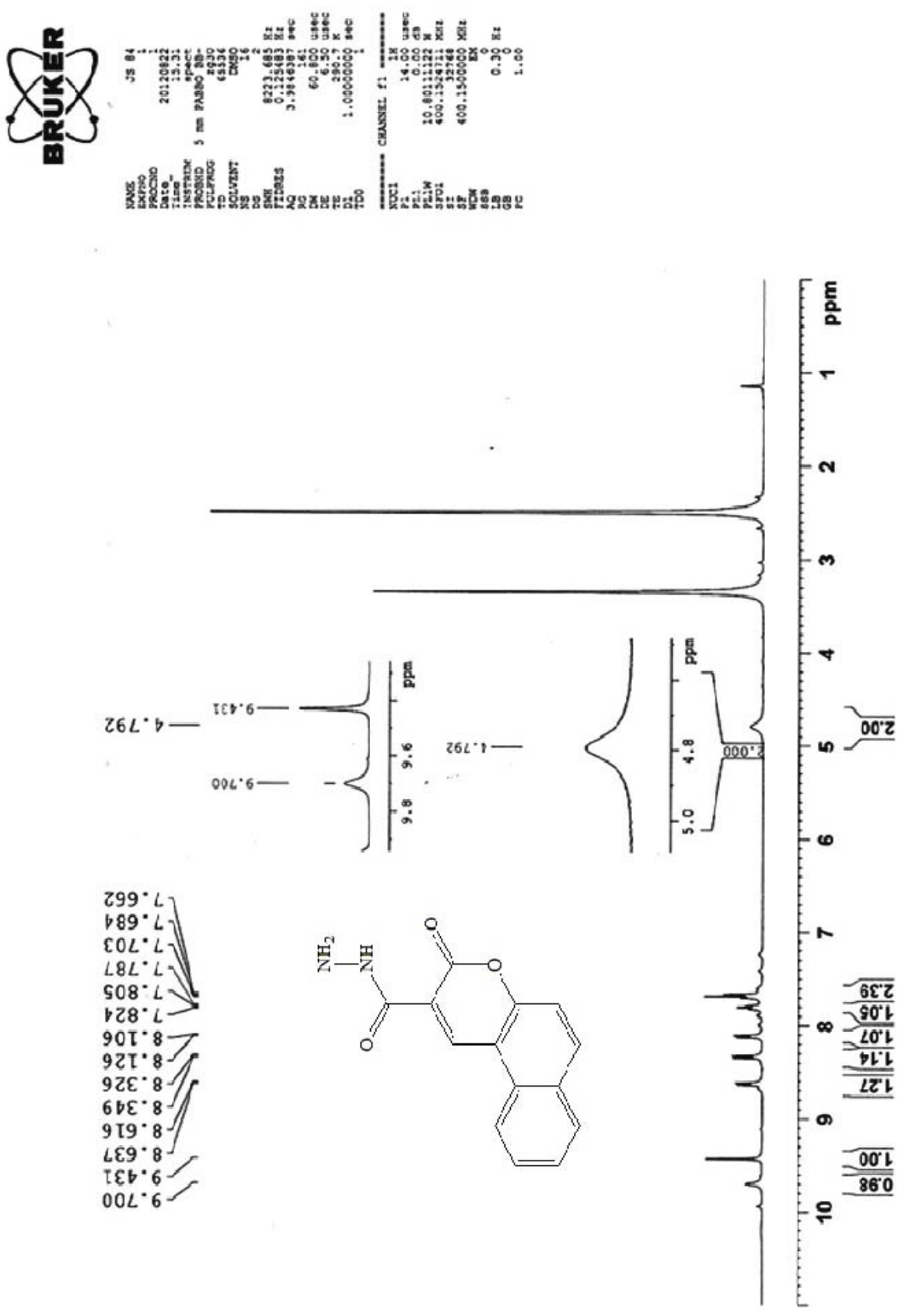


Figure 29: ^{1}H NMR of 3-oxo-3H-benzo[f]chromene-2-carbohydrazide **4d**

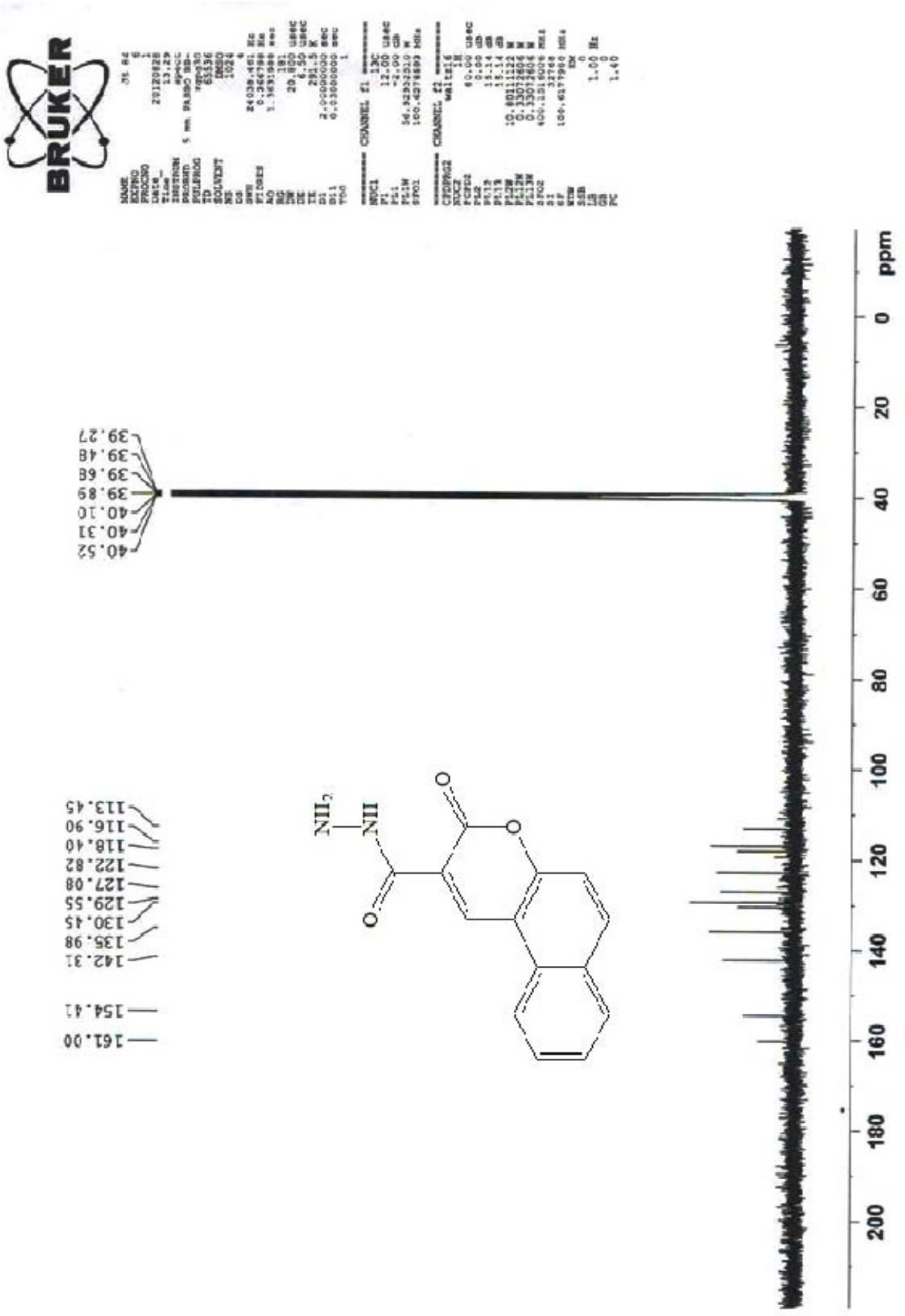


Figure 30: ^{13}C NMR of 3-oxo-3H-benzo[f]chromene-2-carbohydrazide **4d**

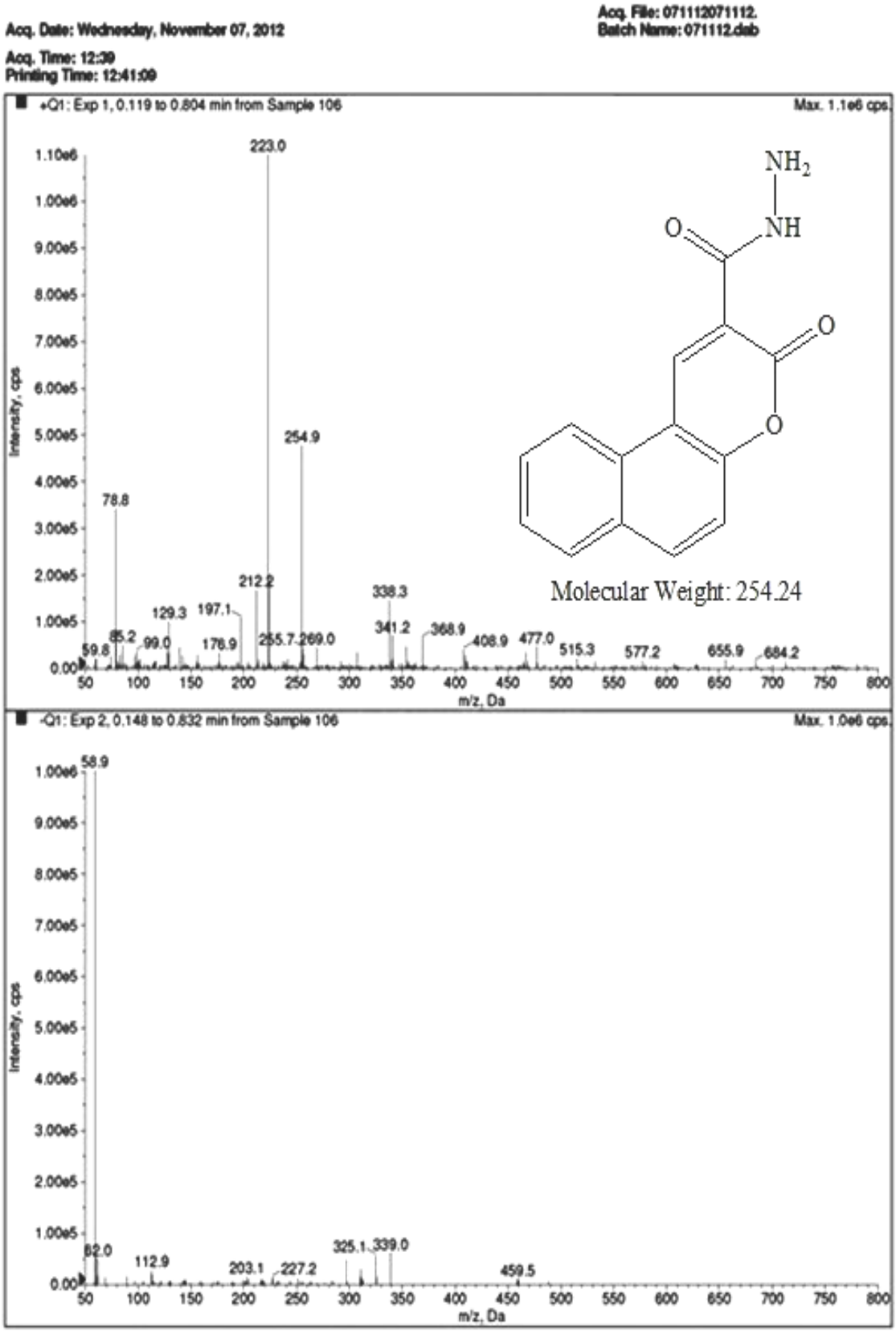


Figure 31: ESI/MS of 3-oxo-3H-benzo[f]chromene-2-carbohydrazide **4d**

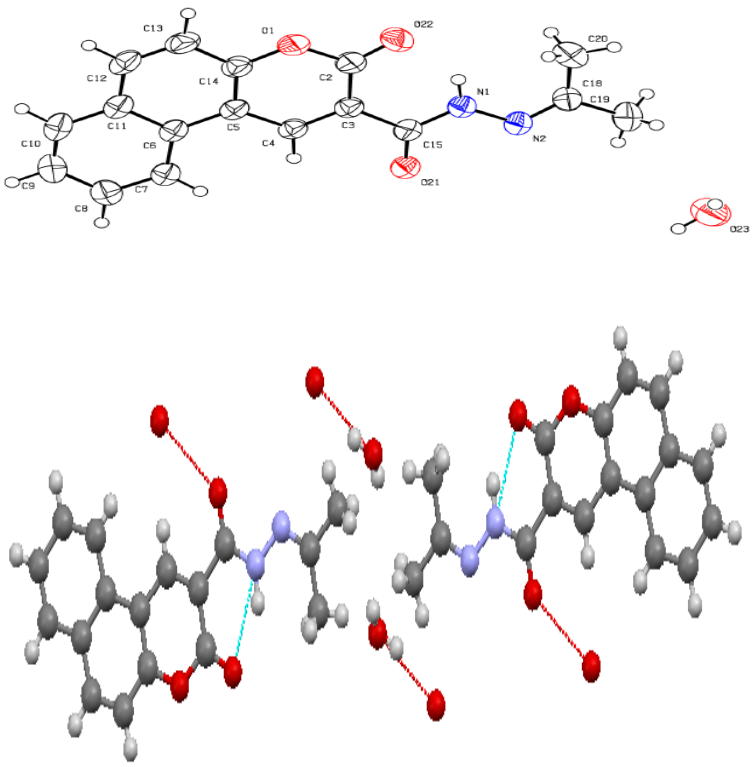
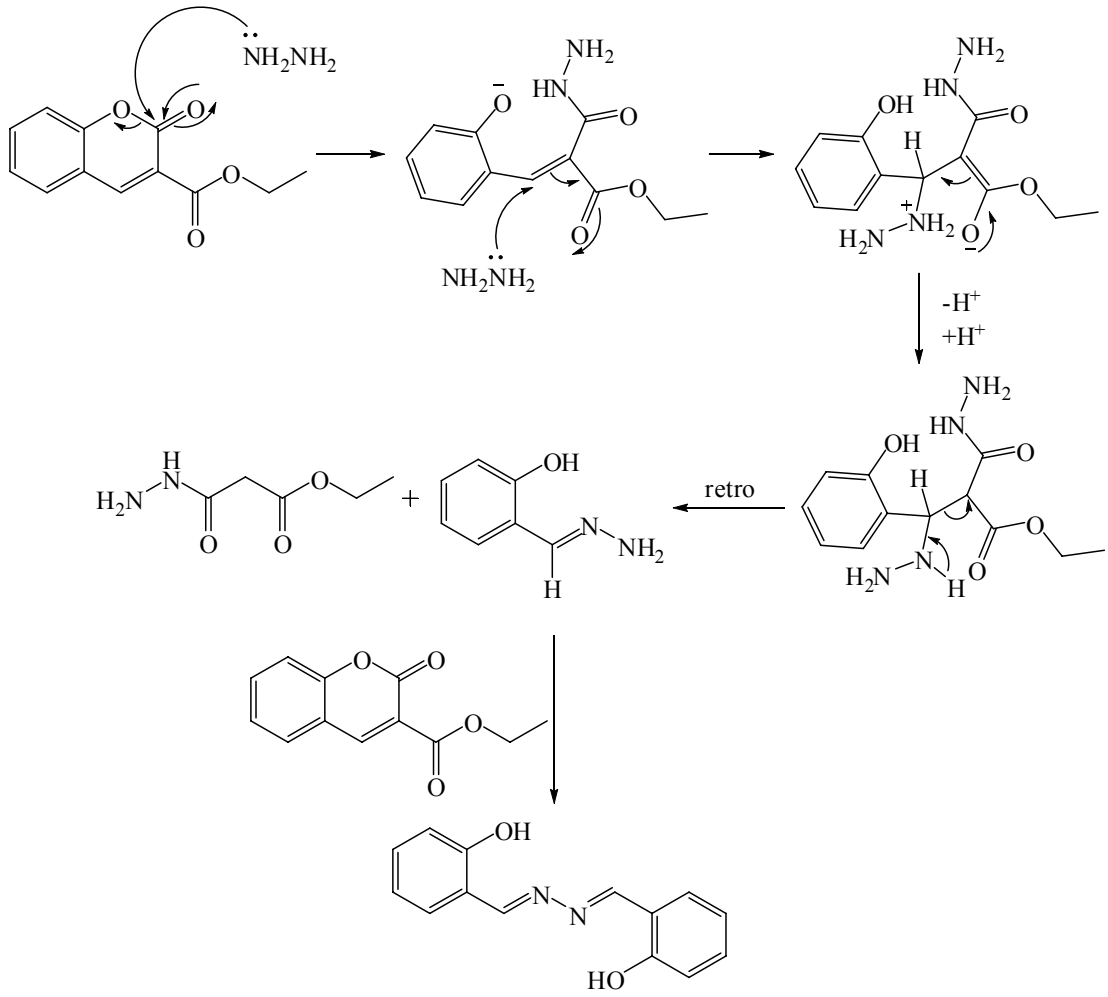


Figure 32: ORTEP diagram and Crystal packing diagram of 3-oxo-N'-(propan-2-ylidene)-3H-benzo[f]chromene-2-carbohydrazide **4d**

The formation of novel schiff bases of **2a**, **2c** and **2d** with hydrazine hydrate can be possible only if retro Knoevenagel reaction occurs. So the possible mechanism for it is suggested in Scheme 5. In case of **2a**, **2b** and **2c**, hydrazine hydrate attacks on β carbon of carbonyl instead of ester carbonyl, hence lactone ring opens up and retro Knoevenagel reaction occurs.

Scheme 5: Plausible mechanism of retro Knoevenagel reaction



While in case of **2d**, due to presence of naphthalene ring the attack on β carbon to carbonyl is sterically hindered, hence hydrazine hydrate attacks on ester carbonyl, thus gives hydrazide formation **4d**. The formations of phenyl hydrazides **3a-d** by reaction of **2a-d** with phenyl hydrazine also support the mechanism. Because of presence of phenyl ring (steric hindrance and less basicity) in phenyl hydrazine, it attacks only on ester carbonyl and not on β carbon of carbonyl, hence give phenyl hydrazides.

5a.2.2 Crystallographic Data

Table 1: Crystal structure data & structure refinement parameters

	4a	**4c**	**4d**
Empirical formula	$C_{14}H_{12}N_2O_2$	$C_{16}H_{16}N_2O_4$	$C_{17}H_{14}N_2O_3.\ H_2O$

Formula weight	240.26	300.31	312.33
Temperature (K)	293(2)	293(2)	293(2)
Wavelength (Å)	0.71073	0.71073	1.54180
Crystal system	Monoclinic	Monoclinic	Triclinic
Space group	P21/n	P21/n	P-1
a (Å)	8.5070(3)	5.98503(15)	7.9556(3)
b (Å)	6.2955(2)	18.6664(5)	10.0729(4)
c (Å)	11.7908(4)	6.86260(17)	10.6217(5)
α (°)	90.00	90.00	79.607(4)
β (°)	107.941(2)	106.399(3)	73.525(4)
γ (°)	90.00	90.00	67.657(4)
Volume ($Å^3$)	600.76(4)	735.49(3)	752.42(6)
Z	2	3	2
Density (mg/m^3)	1.328	1.3560	1.3785
Absorption coefficient (mm^{-1})	0.091	0.099	0.824
F(000)	252.0	316.2	329.1
Theta range for data collection (°)	5.22 to 50	6.56 to 54.7	12.38 to 143.14
Index ranges	-10<=h<=10, -7<=k<=7, -13<=l<=14	-7<=h<=7, -23<=k<=24, -8<=l<=8	-9<=h<=6, -12<=k<=12, -12<=l<=12
Reflections collected	5170	14668	5455

Independent reflections	1058 [R (int) = 0.0235]	1612 [R (int) = 0.0230]	2874 [R (int) = 0.0110]
Absorption correction	Semi-empirical from equivalents	Semi-empirical from equivalents	Semi-empirical from equivalents
Refinement method	Full-matrix least-squares on F^2	Full-matrix least-squares on F^2	Full-matrix least-squares on F^2
Data / restraints / parameters	1058 / 0 / 86	1612 / 0 / 132	2874/0/245
Goodness-of-fit on F^2	1.031	2.347	1.071
Final R indices [I > 2 sigma (I)]	R1= 0.0344	R1= 0.0329	R1= 0.0417
R indices (all data)	R1 = 0.0455, wR2 = 0.0942	R1= 0.0378, wR2= 0.0826	R1= 0.0455, wR2= 0.1317
Largest diff. peak and hole (eA^{-3})	0.13/-0.14	0.15/-0.11	0.21/-0.15

Crystal 1: 2, 2'-(1E,1'E)-hydrazine-1,2-diylidenebis(methan-1-yl-1-ylidene)diphenol **4a**, CCDC No. 968039

Table 2: Fractional Atomic Coordinates ($\times 10^4$) and Equivalent Isotropic Displacement Parameters ($Å^2 \times 10^3$). U_{eq} is defined as 1/3 of the trace of the orthogonalised U_{IJ} tensor.

Atom	*x*	*Y*	*z*	U(eq)
C1	3810.6(16)	2876(2)	24.7(11)	38.3(3)
C2	3664.8(15)	1032(2)	708.7(11)	37.5(3)
C3	4863.7(16)	523(2)	1789.6(12)	43.8(4)
C4	4687.6(19)	-1292(3)	2397.1(13)	53.7(4)
C5	3348(2)	-2581(3)	1967.9(15)	56.1(4)
C6	2142(2)	-2097(2)	916.7(15)	55.8(4)
C7	2314.5(17)	-320(2)	293.0(13)	47.4(4)
N1	5027.1(13)	4152.9(17)	387.4(9)	39.8(3)
O1	6197.6(13)	1754(2)	2254.8(9)	67.6(4)

Table 3: Anisotropic Displacement Parameters ($Å^2×10^3$). The Anisotropic displacement factor exponent takes the form: $-2\pi^2[h^2a^{*2}U_{11}+...+2hka×b×U_{12}]$

Atom	U_{11}	U_{22}	U_{33}	U_{23}	U_{13}	U_{12}
C1	40.2(7)	40.2(8)	30.7(7)	0.1(6)	5.3(5)	4.7(6)
C2	40.7(7)	37.7(7)	34.3(7)	-0.9(5)	12.1(5)	4.1(5)
C3	44.0(7)	49.3(8)	36.9(7)	4.9(6)	10.8(6)	1.2(6)
C4	61.0(9)	56.3(9)	44.0(8)	15.8(7)	16.7(7)	8.9(8)
C5	73.9(11)	43.9(9)	61.9(10)	10.6(7)	37.7(9)	4.7(8)
C6	61.0(9)	45.6(9)	66.2(10)	-5.4(8)	27.7(8)	-9.6(7)
C7	47.2(8)	47.1(9)	45.7(8)	-3.7(7)	10.9(6)	-1.9(6)
N1	47.8(6)	37.4(6)	31.6(6)	3.8(4)	8.2(5)	1.8(5)
O1	59.4(7)	78.8(8)	47.4(6)	21.6(6)	-8.8(5)	-18.3(6)

Table 4: Bond Lengths.

Atom	Atom	Length/Å	Atom	Atom	Length/Å
C1	C2	1.4405(18)	C3	O1	1.3437(16)
C1	N1	1.2756(17)	C4	C5	1.363(2)
C2	C3	1.4022(18)	C5	C6	1.378(2)
C2	C7	1.3921(18)	C6	C7	1.371(2)
C3	C4	1.381(2)	N1	N1[1]	1.396(2)

[1]1-X,1-Y,-Z

Table 5: Bond Angles.

Atom	Atom	Atom	Angle/˚	Atom	Atom	Atom	Angle/˚
N1	C1	C2	121.39(12)	O1	C3	C4	118.57(12)
C3	C2	C1	121.95(12)	C5	C4	C3	120.78(14)
C7	C2	C1	119.99(12)	C4	C5	C6	120.54(14)
C7	C2	C3	118.06(13)	C7	C6	C5	119.32(14)
C4	C3	C2	119.73(13)	C6	C7	C2	121.55(14)
O1	C3	C2	121.70(12)	C1	N1	N1[1]	113.71(12)

[1]1-X,1-Y,-Z

Table 6: Hydrogen Bonds.

D	H	A	d(D-H)/Å	d(H-A)/Å	d(D-A)/Å	D-H-A/°
O1	H2	N1	0.82	1.88	2.6019(14)	146.8

Table 7: Torsion Angles.

A	B	C	D	Angle/˚	A	B	C	D	Angle/˚
C1	C2	C3	C4	-178.38(12)	C4	C5	C6	C7	1.1(2)
C1	C2	C3	O1	1.3(2)	C5	C6	C7	C2	-1.3(2)
C1	C2	C7	C6	179.56(12)	C7	C2	C3	C4	0.8(2)
C2	C1	N1	N1[1]	179.81(12)	C7	C2	C3	O1	-179.57(13)
C2	C3	C4	C5	-1.0(2)	N1	C1	C2	C3	-1.4(2)
C3	C2	C7	C6	0.4(2)	N1	C1	C2	C7	179.45(13)
C3	C4	C5	C6	0.0(2)	O1	C3	C4	C5	179.35(14)

[1]1-X,1-Y,-Z

Table 8: Hydrogen Atom Coordinates ($Å×10^4$) and Isotropic Displacement Parameters ($Å^2×10^3$).

Atom	*x*	*y*	*z*	U(eq)
H4	5493	-1639	3108	64

H5	3248	-3797	2388	67
H6	1220	-2966	633	67
H7	1511	-12	-425	57
H2	6163	2775	1816	101
H1	2968(16)	3120(20)	-711(13)	43(4)

Crystal 2: 6, 6'-(1E, 1'E)-hydrazine-1, 2-diylidenebis(methan-1-yl-1-ylidene)bis(2-methoxy phenol) **4c**, CCDC No. 968041

Table 9: Fractional Atomic Coordinates ($\times 10^4$) and Equivalent Isotropic Displacement Parameters ($Å^2 \times 10^3$). U_{eq} is defined as 1/3 of of the trace of the orthogonalised U_{IJ} tensor.

Atom	*x*	*y*	*z*	U(eq)
O1	2561.5(13)	3207.2(4)	960.8(11)	59.9(3)
O2	6270.1(14)	4011.5(4)	1572.8(11)	56.7(2)
N3	9034.7(14)	4792.0(4)	24.6(14)	50.8(3)
C4	4997.1(17)	3912.0(5)	-373.2(15)	43.5(3)
C5	5634.2(17)	4204.8(5)	-2024.7(15)	45.2(3)
C6	4211(2)	4082.9(6)	-4000.0(17)	54.4(3)
C7	2989.4(17)	3491.3(5)	-733.7(15)	45.8(3)
C8	7724.5(19)	4629.5(6)	-1737.1(17)	49.1(3)
C9	1594(2)	3393.9(6)	-2691.9(17)	53.1(3)
C10	2205(2)	3695.4(7)	-4323.0(18)	58.3(3)
C11	657(2)	2723.6(8)	645(2)	64.8(4)

Table 10: Anisotropic Displacement Parameters ($Å^2 \times 10^3$). The Anisotropic displacement factor exponent takes the form: $-2\pi^2[h^2a^{*2}U_{11}+...+2hka\times b\times U_{12}]$

Atom	U_{11}	U_{22}	U_{33}	U_{12}	U_{13}	U_{23}
O1	56.6(5)	74.6(6)	49.3(5)	-19.2(4)	16.5(4)	1.2(4)
O2	53.5(5)	73.7(6)	42.4(4)	-15.1(4)	12.6(3)	-0.9(4)
N3	47.5(5)	50.2(5)	59.5(6)	-1.4(4)	23.1(4)	2.8(4)
C4	43.3(5)	46.0(6)	41.3(5)	4.2(4)	12.3(4)	-1.5(4)
C5	48.2(6)	42.7(5)	46.9(6)	7.4(4)	16.8(5)	2.8(4)
C6	65.6(7)	53.4(6)	45.4(6)	7.8(5)	18.0(5)	6.1(5)
C7	46.1(6)	47.5(6)	45.4(6)	1.8(4)	15.3(5)	-0.9(4)
C8	51.9(6)	46.8(6)	53.6(7)	6.6(5)	22.8(5)	7.3(5)
C9	48.0(6)	55.3(7)	53.5(7)	-1.0(5)	10.3(5)	-4.8(5)
C10	62.8(7)	63.2(7)	43.5(6)	4.4(6)	5.9(5)	-2.2(5)
C11	57.8(8)	70.5(9)	65.5(8)	-16.8(6)	16.6(6)	4.5(7)

Table 11: Bond Lengths.

Atom	Atom	Length/Å	Atom	Atom	Length/Å
O1	C7	1.3666(12)	C4	C7	1.3978(14)
O1	C11	1.4220(14)	C5	C6	1.4010(15)
O2	C4	1.3506(12)	C5	C8	1.4468(15)
N3	N3[1]	1.4004(16)	C6	C10	1.3654(17)
N3	C8	1.2780(14)	C7	C9	1.3794(15)
C4	C5	1.4045(14)	C9	C10	1.3909(17)

[1]2-X,1-Y,-Z

Table 12: Bond Angles.

Atom	Atom	Atom	Angle/˚	Atom	Atom	Atom	Angle/˚
C11	O1	C7	116.85(9)	C10	C6	C5	120.64(10)
C5	C4	O2	122.58(9)	C4	C7	O1	115.20(8)
C7	C4	O2	117.98(9)	C9	C7	O1	124.78(9)
C7	C4	C5	119.44(9)	C9	C7	C4	120.02(9)
C6	C5	C4	119.22(10)	C5	C8	N3	122.35(9)
C8	C5	C4	121.63(9)	C10	C9	C7	120.41(11)
C8	C5	C6	119.14(9)	C9	C10	C6	120.18(11)

Table 13: Hydrogen Atom Coordinates (Å×10^4) and Isotropic Displacement Parameters (Å^2×10^3).

Atom	*x*	*y*	*z*	U(eq)
H9	210(20)	3111(6)	-2938(17)	56(3)
H10	1200(20)	3627(6)	-5690(20)	70(3)
H11a	730(20)	2548(7)	1990(20)	76(4)
H8	8122(18)	4793(6)	-2989(17)	57(3)
H2	7470(30)	4305(8)	1540(20)	84(4)
H11b	840(20)	2326(8)	-280(20)	85(4)
H6	4730(20)	4278(6)	-5130(20)	67(3)
H11c	-830(20)	2983(7)	60(20)	79(4)

Crystal 3: Adduct of acetone and 3-oxo-N'-(propan-2-ylidene)-3H-benzo[f]chromene-2-carbohydrazide **4d**, CCDC No. 968040

Table 14: Fractional Atomic Coordinates (×10^4) and Equivalent Isotropic Displacement Parameters (Å^2×10^3). U_{eq} is defined as 1/3 of of the trace of the orthogonalised U_{IJ} tensor.

Atom	*x*	*y*	*z*	U(eq)
O21	1960.3(16)	3444.7(10)	563.8(10)	56.4(3)
O22	3702.4(17)	-284.3(11)	3102.5(11)	65.7(3)
O1	3466.5(14)	-1605.1(9)	1778.5(9)	52.5(3)
N1	2803.6(16)	2568.4(12)	2497.2(11)	46.7(3)
N2	2453.7(18)	3925.7(12)	2846.2(12)	51.4(3)
C15	2477.4(17)	2428.3(13)	1362.5(13)	42.2(3)
C2	3326.4(19)	-292.5(14)	2078.5(13)	46.7(3)
C3	2757.6(17)	926.1(13)	1129.1(12)	40.3(3)
C4	2402.9(17)	721.9(13)	18.7(13)	39.5(3)
C11	2318.9(18)	-2347.9(14)	-1551.3(14)	46.1(3)
C14	3075.4(18)	-1793.8(14)	660.3(13)	44.4(3)
C5	2565.1(16)	-657.6(13)	-264.5(12)	39.3(3)
C6	2179.2(16)	-925.6(13)	-1418.8(13)	40.9(3)
C12	2821(2)	-3449.3(14)	-542.5(16)	54.2(4)
C7	1658(2)	153.9(15)	-2423.6(14)	49.7(3)
C13	3201(2)	-3193.0(14)	534.9(16)	55.3(4)
C10	1942(2)	-2633.9(17)	-2675.6(16)	56.0(4)
C9	1453(2)	-1572.2(18)	-3626.1(17)	63.0(4)
C18	2785(2)	3975.7(16)	3938.2(14)	53.1(4)
C20	3517(3)	2725(2)	4868.3(16)	70.0(5)

C8	1303(2)	-164.5(18)	-3500.0(16)	61.1(4)
C19	2360(3)	5451(2)	4330.1(19)	80.2(6)
O23	8894.6(19)	6079.7(13)	2020.2(12)	75.2(4)

Table 15: Anisotropic Displacement Parameters ($Å^2×10^3$). The Anisotropic displacement factor exponent takes the form: $-2\pi^2[h^2a^{*2}U_{11}+...+2hka×b×U_{12}]$

Atom	U_{11}	U_{22}	U_{33}	U_{12}	U_{13}	U_{23}
O21	86.4(7)	37.0(5)	53.4(6)	-22.3(5)	-29.1(5)	-0.0(4)
O22	98.4(9)	49.2(6)	55.3(6)	-21.1(6)	-38.0(6)	2.7(4)
O1	71.7(6)	35.3(5)	49.6(6)	-16.1(4)	-20.5(5)	2.5(4)
N1	59.3(7)	38.2(6)	46.7(6)	-17.3(5)	-17.7(5)	-4.2(5)
N2	68.9(7)	41.0(6)	49.5(6)	-20.6(5)	-17.3(5)	-7.7(5)
C15	46.4(7)	38.0(6)	43.7(7)	-15.7(5)	-10.4(5)	-4.8(5)
C2	54.9(7)	38.6(7)	46.0(7)	-14.4(6)	-14.3(6)	-2.1(5)
C3	42.0(6)	34.9(6)	42.8(6)	-13.1(5)	-8.4(5)	-3.4(5)
C4	42.2(6)	32.4(6)	42.8(6)	-12.7(5)	-9.5(5)	-2.1(5)
C11	42.8(6)	40.1(7)	55.8(8)	-17.3(5)	-3.8(5)	-11.5(6)
C14	48.7(7)	35.5(6)	46.7(7)	-14.2(5)	-8.4(5)	-3.5(5)
C5	38.7(6)	33.2(6)	44.2(7)	-13.4(5)	-5.2(5)	-4.3(5)
C6	38.8(6)	36.7(6)	47.3(7)	-14.5(5)	-5.1(5)	-8.3(5)
C12	61.3(8)	34.1(7)	67.5(9)	-19.8(6)	-8.0(7)	-9.3(6)
C7	59.8(8)	42.5(7)	52.3(8)	-20.9(6)	-17.0(6)	-4.7(6)
C13	68.8(9)	33.4(7)	59.7(9)	-17.6(6)	-13.0(7)	2.0(6)
C10	57.5(8)	49.6(8)	67.9(9)	-23.6(6)	-9.0(7)	-20.7(7)
C9	72.2(10)	66.4(10)	62.3(9)	-26.9(8)	-22.0(8)	-18.4(8)
C18	63.6(8)	56.9(8)	46.5(7)	-28.1(7)	-11.3(6)	-10.0(6)
C20	88.0(12)	74.9(11)	55.0(9)	-31.1(9)	-27.0(8)	-2.7(8)
C8	75.7(10)	58.9(9)	57.5(9)	-26.2(8)	-26.2(8)	-3.7(7)
C19	122.5(16)	67.8(11)	66(1)	-41.8(11)	-25.3(10)	-19.6(8)
O23	89.6(9)	61.3(7)	64.0(7)	-9.0(6)	-23.3(6)	-9.6(5)

Table 16: Bond Lengths.

Atom	Atom	Length/Å	Atom	Atom	Length/Å
O21	C15	1.2253(16)	C11	C12	1.420(2)
O22	C2	1.2092(17)	C11	C10	1.411(2)
O1	C2	1.3719(16)	C14	C5	1.3809(18)
O1	C14	1.3710(17)	C14	C13	1.4026(19)
N1	N2	1.3853(15)	C5	C6	1.4375(18)
N1	C15	1.3441(17)	C6	C7	1.4081(19)
N2	C18	1.2735(18)	C12	C13	1.353(2)
C15	C3	1.4994(17)	C7	C8	1.369(2)
C2	C3	1.4555(18)	C10	C9	1.353(2)
C3	C4	1.3541(18)	C9	C8	1.405(2)
C4	C5	1.4249(17)	C18	C20	1.491(2)
C11	C6	1.4239(17)	C18	C19	1.503(2)

Table 17: Bond Angles.

Atom	Atom	Atom	Angle/˚	Atom	Atom	Atom	Angle/˚
C14	O1	C2	123.14(10)	C13	C14	O1	115.87(12)
C15	N1	N2	119.83(11)	C13	C14	C5	122.90(13)

C18	N2	N1	116.33(12)	C14	C5	C4	117.02(12)
N1	C15	O21	123.45(12)	C6	C5	C4	124.38(11)
C3	C15	O21	120.99(12)	C6	C5	C14	118.58(11)
C3	C15	N1	115.54(11)	C5	C6	C11	118.55(12)
O1	C2	O22	115.98(12)	C7	C6	C11	118.32(12)
C3	C2	O22	127.43(12)	C7	C6	C5	123.13(11)
C3	C2	O1	116.59(12)	C13	C12	C11	121.89(12)
C2	C3	C15	121.97(12)	C8	C7	C6	120.54(13)
C4	C3	C15	118.29(11)	C12	C13	C14	118.82(13)
C4	C3	C2	119.70(11)	C9	C10	C11	120.68(13)
C5	C4	C3	122.30(12)	C8	C9	C10	120.20(14)
C12	C11	C6	119.25(13)	C20	C18	N2	126.61(13)
C10	C11	C6	119.48(13)	C19	C18	N2	116.23(14)
C10	C11	C12	121.27(12)	C19	C18	C20	117.16(14)
C5	C14	O1	121.22(11)	C9	C8	C7	120.78(15)

Table 18: Hydrogen Atom Coordinates (Å×10^4) and Isotropic Displacement Parameters (Å^2×10^3).

Atom	***x***	***y***	***z***	**U(eq)**
H4	2030(20)	1553(17)	-580(16)	50(4)
H1	3210(20)	1741(19)	3002(18)	67(5)
H12	2900(30)	-4410(20)	-602(18)	73(5)
H7	1550(20)	1102(18)	-2388(15)	53(4)
H10	2020(20)	-3610(20)	-2717(18)	71(5)
H8	930(30)	600(20)	-4190(20)	77(5)
H9	1180(30)	-1750(20)	-4380(20)	79(6)
H13	3500(30)	-3900(20)	1227(19)	73(5)
H23a	8720(30)	6050(20)	1275(11)	112.8(6)
H23b	9988(14)	5510(20)	2070(20)	112.8(6)
H20a	2550(6)	2340(10)	5315(10)	105.0(7)
H20b	4561(12)	1996(7)	4387(2)	105.0(7)
H20c	3913(18)	3035(4)	5503(9)	105.0(7)
H19a	3508(3)	5584(7)	4288(15)	120.3(9)
H19b	1700(20)	6165(2)	3740(10)	120.3(9)
H19c	1598(19)	5547(6)	5213(6)	120.3(9)

5a.3 Conclusion

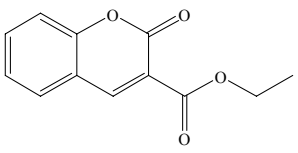
We have observed formation of novel schiff bases **4a**, **4b** and **4c** during reaction of **2a**, **2b** and **2c** with hydrazine hydrate. The formation of hydrazide **4d** was observed from **2d**, may be due to steric hindrance of naphthalene ring which prevent attack on β carbon of carbonyl group and hence attack occur on ester carbonyl to give hydrazide. The plausible mechanism for retro Knoevenagel reaction is proposed. Steric hindrance by naphthalene ring or phenyl ring, gives corresponding hydrazides only.

5a.4 Experimental

5a.4.1 Chemistry

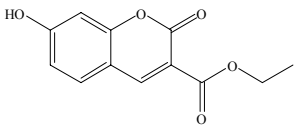
Reagent grade chemicals and solvents were purchased from commercial supplier and used without purification. TLC was performed on silica gel F254 plates (Merck). Acme's silica gel (60-120 mesh) was used for column chromatographic purification. Melting points are uncorrected and were measured in open capillary tubes, using a Rolex melting point apparatus. IR spectra were recorded as KBr pellets on Perkin Elmer RX 1 spectrometer. ^{1}H NMR and ^{13}C NMR spectral data were recorded on Advance Bruker 400 spectrometer (400 MHz) with $CDCl_3$ or DMSO-d_6 as solvent and TMS as internal standard. *J* values are in Hz. Mass spectra were determined by ESI/MS, using a Shimadzu LCMS 2020 apparatus. Elemental analyses were recorded on Thermosinnigan Flash 11-12 series EA. X-ray diffraction data were collected using Cu X-ray source (λ= 0.71073 A°) radiation on Xcalibur, Eos, Gemini diffractometer equipped with a CCD area detector. Data collection, data reduction, structure solution/refinement were carried out using the software package of OLEX 1.2.2. Graphics were generated using MERCURY 2.2.

General procedure for 2a-d: Aldehydes **1a-d** (5 g, 1 eq.) dissolved in mixture of pyridine (10 ml), piperidine (0.5 ml) and diethylmalonate (1.1 eq.) was added in the reaction flask and reaction mixtur heated at 60-70ºC for 6 hours in water bath. Reaction mass poured into crushed ice containing concentrated HCl (25 ml) which gave solid product. It was filtered, dried and crystallized using ethanol afforded pure products **2a-d**.

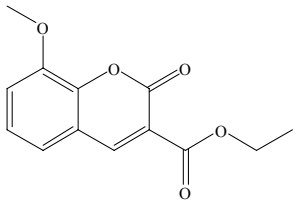


Ethyl 2-oxo-2H-chromene-3-carboxylate 2a: This compound obtained as white solid. Yield 45 %; mp 91-93°C (Lit. 93°C[16]); IR (KBr) (Figure 1): 3061, 2977, 2916, 1767, 1609 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) (Figure 2): δ 1.41-1.45 (3H, t, CH_3), 4.41-4.46 (2H, q, -OCH_2) 7.34-7.39 (2H, m, ArH),

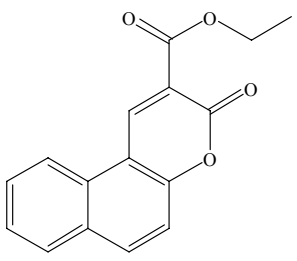
7.63-7.69 (2H, m, ArH), 8.56 (1H, s, C-4 H); ^{13}C NMR (400 MHz, $CDCl_3$) (Figure 3): δ 14.2, 62.0, 116.8, 117.9, 118.3, 124.9, 129.5, 134.4, 148.7, 155.2, 156.8, 163.1; MS (ESI, *m/z*) (Figure 4) 219.1 $[M+1]^+$ and base peak at *m/z* 173.2 for M-OCH_2CH_3 fragment calculated for $C_{12}H_{10}O_4$.



Ethyl 7-hydroxy-2-oxo-2H-chromene-3-carboxylate 2b: This compound obtained as brown solid. Yield 42 %; mp 155-160°C (Lit. 166-167°C[16]); IR (KBr): 3542, 3471, 1728, 1621 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.27-1.30 (3H, t, -CH_3), 4.22-4.28 (2H, q, -OCH_2), 6.72-6.73 (1H, d, *J*= 2.0 Hz, ArH), 6.82-6.85 (1H, dd, *J*= 2.4, 8.8 Hz, ArH), 7.74-7.76 (1H, d, *J*= 8.8 Hz, ArH), 8.67 (1H, s, C-4 H), 11.12 (1H, s, -OH); ^{13}C NMR (400 MHz, DMSO-d_6): δ 19.3, 66.1, 107.0, 115.6, 117.2, 119.2, 137.3, 154.7, 161.7, 162.3, 168.2, 169.3; MS (ESI, *m/z*) 235.2 $[M+1]^+$, base peak at *m/z* 189.1 for M-OCH_2CH_3 fragment and 233.3 $[M-1]^+$ calculated for $C_{12}H_{10}O_5$.



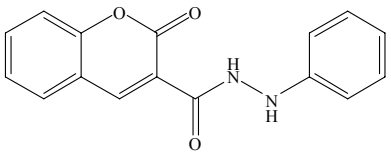
Ethyl 8-methoxy-2-oxo-2H-chromene-3-carboxylate 2c: This compound obtained as white solid. Yield 47 %; mp 90-95°C; IR (KBr): 3053, 2996, 2978, 2949, 2847, 1755, 1731 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.40-1.44 (3H, t, CH_3), 3.98 (3H, s, -OCH_3), 4.40-4.45 (2H, q, -OCH_2), 7.18-7.20 (2H, m, *J*= 1.2, 7.6 Hz, ArH), 7.27-7.30 (1H, m, ArH), 8.52 (1H, s, C-4 H); ^{13}C NMR (400 MHz, $CDCl_3$): δ 14.2, 56.3, 62.0, 115.8, 118.4, 118.5, 120.6, 124.7, 144.9, 147.1, 148.9, 156.2, 163.1; Mol. Formula: $C_{13}H_{12}O_4$.



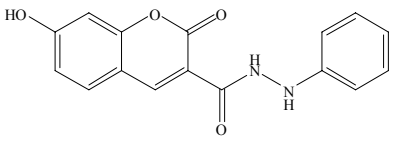
Ethyl 3-oxo-3H-benzo[f]chromene-2-carboxylate 2d: This compound obtained as light yellow solid. Yield 46 %; mp 110-115°C (Lit. 117 °C[13]); IR (KBr) (Figure 5): 3128, 1631, 973, 855, 807, 746 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) (Figure 6): δ 1.46-1.50 (3H, t, -CH_3), 4.48-4.53 (2H, q, -OCH_2), 7.49-7.51 (1H, d, *J*= 9.2 Hz, ArH),

7.62-7.66 (1H, m, ArH), 7.76-7.80 (1H, m, ArH), 7.95-7.97 (1H, d, *J*= 8.0 Hz, ArH), 8.12-8.14 (1H, d, *J*= 9.2 Hz, ArH), 8.35-8.37 (1H, d, *J*= 8.0 Hz, ArH) 9.35 (1H, s, C-4 H); ^{13}C NMR (400 MHz, $CDCl_3$) (Figure 7): δ 14.3, 62.1, 112.3, 116.5, 116.7, 121.5, 126.6, 129.1, 129.3, 129.4, 130.2, 136.2, 144.6, 156.0, 156.9, 163.6; MS (ESI, *m/z*) (Figure 8) 268.9 $[M+1]^+$ and base peak at *m/z* 222.9 for M-OCH_2CH_3 fragment calculated for $C_{16}H_{12}O_4$.

General procedure for 3a-d: A solution of **2a-d** (1 g, 1 eq.) and phenyl hydrazine (1.1 eq.) in ethanol (50 ml) were refluxed for 8 hours in water bath. Solvent distilled under reduced pressure and reaction mass poured into ice. Crude product extracted in ethyl acetate, dried over sodium sulphate and concentrated under reduced pressure. Crude product purified by column chromatography using petroleum ether (60-80°C): ethyl acetate (8:2) as eluent.

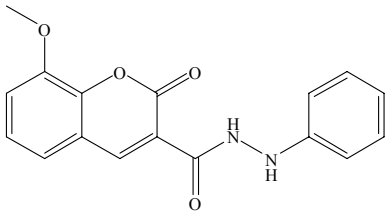


2-oxo-N'-phenyl-2H-chromene-3-carbohydrazide 3a: This compound obtained as light brown solid. Yield 49 %; mp 142-145°C; IR (KBr) (Figure 9): 3440, 3202, 3052, 1701, 1621 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) (Figure 10): δ 6.91-6.97 (2H, m, ArH), 7.00-7.04 (3H, m, ArH), 7.16-7.19 (1H, dd, J= 1.6, 7.6 Hz, ArH), 7.24-7.29 (1H, m, ArH), 7.31-7.35 (2H, m, ArH), 7.52 (1H, s, -NH), 7.88 (1H, s, C-4 H), 10.89 (1H, s, -NH); ^{13}C NMR (400 MHz, $CDCl_3$) (Figure 11): δ 98.6, 115.0, 118.9, 120.8, 121.9, 123.3, 131.8, 132.0, 132.4, 143.5, 145.7, 159.2; Ele. Anal. Calcd. for $C_{16}H_{12}N_2O_3$; Requires (Found) %: C, 68.56 (68.37); H, 4.32 (4.60); N, 9.99 (10.27).

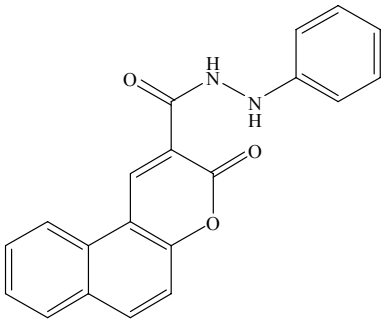


7-hydroxy-2-oxo-N'-phenyl-2H-chromene-3-carbohydrazide 3b: This compound obtained as brown solid. Yield 49 %; mp 158-160°C; IR (KBr) (Figure 12): 3484, 3402, 3313, 3054, 3032, 2971, 2891, 2816, 1670, 1629 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) (Figure 13): δ 6.30-

6.33 (2H, m, ArH), 6.73 (1H, s, ArH), 6.88-6.90 (2H, m, *J*= 0.8, 7.6 Hz, ArH), 7.20-7.29 (3H, m, ArH), 8.04 (1H, s, C-4 H), 9.74 (1H, s, -NH, D_2O exch.), 10.14 (1H, s, -OH, D_2O exch.), 10.75 (1H, s, -NH, D_2O exch.); ^{13}C NMR (400 MHz, DMSO-d_6) (Figure 14): δ 102.9, 107.9, 111.9, 112.4, 118.9, 129.5, 129,7, 139.6, 145.5, 157.9, 159.4; Ele. Anal. Calcd. for $C_{16}H_{12}N_2O_4$; Requires (Found) %: C, 64.86 (64.62); H, 4.08 (3.81); N, 9.46 (9.73).



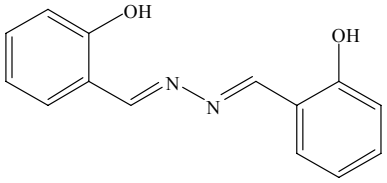
8-methoxy-2-oxo-N'-phenyl-2H-chromene-3-carbohydrazide 3c: This compound obtained as light brown solid. Yield 45 %; mp 236-240°C; IR (KBr) (Figure 15): 3324, 3214, 1722, 1654, 1608 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) (Figure 16): δ 4.03 (3H, s, OCH_3), 6.93-6.96 (3H, m, ArH), 7.24-7.37 (6H, m, ArH and NH proton), 8.91 (1H, s, C-4 H), 10.41 (1H, s, NH, D_2O exch.); ^{13}C NMR (400 MHz, $CDCl_3$) (Figure 17): δ 56.4, 113.9, 115.9, 119.1, 121.0, 121.5, 125.4, 129.2, 147.1, 149.5, 160.6; Ele. Anal. Calcd. for $C_{17}H_{14}N_2O_4$; Requires (Found) %: C, 65.80 (66.03); H, 4.55 (4.29); N, 9.03 (9.33).



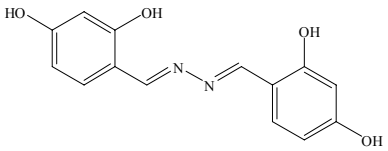
3-oxo-N'-phenyl-3H-benzo[f]chromene-2-carbohydrazide 3d: This compound obtained as light brown solid. Yield 52 %; mp 242-244°C; IR (KBr): 3334, 3294, 3051, 1708, 1680 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 6.94-7.00 (3H, m, ArH and NH proton), 7.29-7.31 (3H, d, *J*= 8.4 Hz, ArH), 7.56-7.58 (1H, d, *J*= 8.8 Hz, ArH), 7.65-7.69 (1H, t, *J*= 7.2, 7.6 Hz, ArH), 7.78-7.82 (1H, t, *J*= 7.2, 7.6 Hz, ArH), 7.98-8.00 (1H, d, *J*= 8 Hz, ArH), 8.17-8.20 (1H, d, *J*= 8.8 Hz, ArH), 8.43-8.45 (1H, d, *J*= 8.4 Hz, ArH), 9.71 (1H, s, C-4 H), 10.48 (1H, s, NH, D_2O Exch.); ^{13}C NMR (400 MHz, $CDCl_3$): δ 113.3, 114.0, 116.4, 121.6, 122.0, 127.0, 129.2, 129.3, 129.4, 129.5, 130.4, 136.5, 144.8, 155.3; ESI/MS *m/z*

332.1 $[M+2]^+$ calculated for $C_{20}H_{14}N_2O_3$; Ele. Anal. Calcd. for $C_{20}H_{14}N_2O_3$; Requires (Found) %: C, 72.72 (72.86); H, 4.27 (4.11); N, 8.48 (8.68).

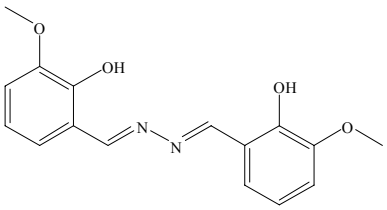
General procedure for 4a-d: A solution of **2a-d** (5 g, 1 eq.) in ethanol (50 ml) and hydrazine hydrate (1.1 eq.) were refluxed for 8 hours in water bath. Solvent removed under reduced pressure and reaction mass poured into crushed ice to obtain solid. Crude product filtered, dried and crystallized using ethanol to give products.



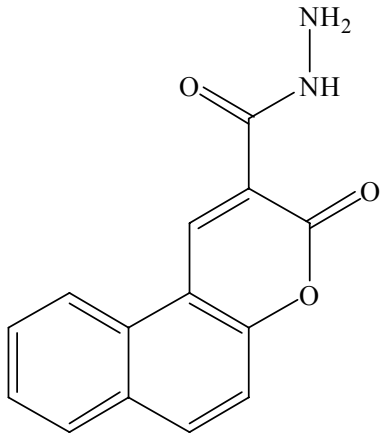
2, 2'-(1E,1'E)-hydrazine-1,2-diylidenebis(methan-1-yl-1-ylidene)diphenol 4a: This compound obtained as yellow solid. Yield 40 %; mp 210-215°C; IR (KBr) (Figure 20): 3436, 3290, 1622, 1603 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) (Figure 21): δ 6.96-7.00 (2H, m, ArH), 7.39-7.43 (1H, m, ArH), 7.69-7.72 (1H, dd, *J*= 1.6, 7.6 Hz ArH), 9.02 (1H, s, CH=N), 11.15 (1H, s, OH); ^{13}C NMR (400 MHz, DMSO-d_6) (Figure 22): δ 117.0, 118.6, 120.1, 125.8, 131.3, 133.7, 159.1, 163.3; MS (ESI, *m/z*) (Figure 23) 241.1 $[M+1]^+$ and 239.0 $[M-1]^+$ calculated for $C_{14}H_{12}N_2O_2$; Ele. Anal. Calcd. for $C_{14}H_{12}N_2O_2$; Requires (Found) %: C, 69.99 (69.96); H, 5.03 (4.55); N, 11.66 (11.69).



4, 4'-(1E, 1'E)-hydrazine-1, 2-diylidenebis(methan-1-yl-1-ylidene)dibenzene-1, 3-diol 4b: This compound obtained as yellow solid. Yield 54 %; mp 220-240°C (dec.); IR (KBr): 3467, 3223, 1634, 1615 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ 6.32-6.33 (1H, d, *J*= 2.0 Hz, ArH), 6.38-6.41 (1H, dd, J= 2.0, 8.4 Hz, ArH), 7.41-7.43 (1H, d, *J*= 8.8 Hz, ArH), 8.77 (1H, s, CH=N), 10.29 (1H, s, -OH, D_2O exch.), 11.42 (1H, s, -OH, D_2O exch.); ^{13}C NMR (400 MHz, DMSO-d_6): δ 102.9, 108.7, 110.7, 133.4, 161.1, 162.3, 162.5; MS (ESI, *m/z*) 273.2 $[M+1]^+$ and 271.1 $[M-1]^+$ calculated for $C_{14}H_{12}N_2O_4$.



6, 6'-(1E, 1'E)-hydrazine-1, 2-diylidenebis(methan-1-yl-1-ylidene)bis(2-methoxyphenol) 4c: This compound obtained as yellow solid. Yield 43 %; mp 152-155°C; IR (KBr) (Figure 24): 3390, 3295, 3213, 3076, 3005, 2963, 2939, 2839, 1627, 1578 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) (Figure 25): δ 3.96 (3H, s, $-OCH_3$), 6.92-6.96 (1H, t, *J*= 7.6, 8.0 Hz, ArH), 7.02-7.04 (2H, m, ArH), 8.73 (1H, s, CH=N), 11.61 (1H, s, -OH, D_2O exch.); ^{13}C NMR (400 MHz, $CDCl_3$) (Figure 26): δ 56.2, 115.1, 117.3, 119.4, 124.1, 148.3, 149.7, 164.9; MS (ESI, *m/z*) (Figure 27) 301.0 $[M+1]^+$ calculated for $C_{16}H_{16}N_2O_4$.



3-oxo-3H-benzo[f]chromene-2-carbohydrazide 4d: This compound obtained as yellow solid. Yield 16 %; mp 280-282°C; IR (KBr) (Figure 28): 3323, 3282, 3019, 1708, 1622, 1580 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) (Figure 29): δ 4.79 (2H, s, NH_2), 7.66-7.70 (2H, t, *J*= 7.6, 8.8 Hz, ArH), 7.79-7.82 (1H, m, *J*= 7.6 Hz, ArH), 8.11-8.13 (1H, d, *J*= 8.0 Hz, ArH), 8.33-8.35 (1H, d, *J*= 8.8 Hz, ArH), 8.62-8.64 (1H, d, *J*= 8.4 Hz, ArH), 9.43 (1H, s, C-4 H), 9.70 (1H, s, NH); ^{13}C NMR (400 MHz, DMSO-d_6) (Figure 30): δ 113.4, 116.9, 118.4, 122.8, 127.1, 129.5, 130.4, 136.0, 142.3, 154.4, 161.0; MS (ESI, *m/z*) (Figure 31) 254.9 $[M+1]^+$ calculated for $C_{14}H_{10}N_2O_3$.

5a.4.2 Single crystal X-ray diffraction

X-ray quality single crystals of 2, 2'-(1E,1'E)-hydrazine-1,2-diylidenebis(methan-1-yl-1-ylidene)diphenol **4a,** 6, 6'-(1E, 1'E)-hydrazine-1, 2-diylidenebis(methan-1-yl-1-ylidene)bis(2-methoxyphenol) **4c** and 3-oxo-3H-benzo[f]chromene-2-carbohydrazide **4d** were grown in a slow evaporation condition at room temperature. Crystals were obtained from a mixture of methanol and acetone (1:1). The structure was solved by direct

methods & refined in a routine manner. All hydrogen atoms were geometrically fixed and refined.

5a.5 References

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17. Crystallographic data (excluding structure factors) for the structures in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication nos. CCDC. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK.

Chapter 5b

Synthesis and anticancer activity of 4-hydroxy Naphtho coumarin derivatives and naphtho coumestans

5b. Synthesis and anticancer activity of 4-hydroxy Naphtho coumarin derivatives and naphtho coumestans

5b.1 Introduction

Coumestan ring system is present in number of natural products like coumestrol, psoralidine, pterocarsin,[1] lucernol[2] and wedelolactone[3]. Coumestans represent an important class of natural oxygenated aromatic products responsible for medicinal effects. Eclipta alba[4] and wedelia calendulacea[5] are the plant sources of nor-wedelolactone and wedelolactone. Both of them show medicinal effects such as antihepatotoxic, anti hypertensive, antitumor, antiphospholipase A_2 and antidote activities against snake venome.[6-9]

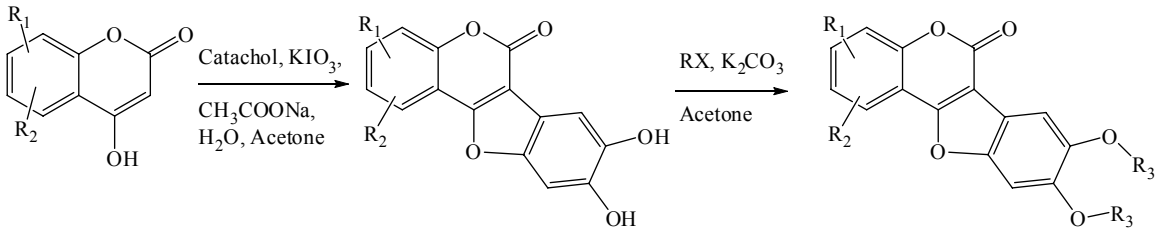
Coumestans belongs to the flavonoids category of phytoestrogens, which have diverse pharmacological properties such as anti hemorrhagic, antiproteolytic, antihepatotoxic,[10, 6] antiphospholipase and antimyotoxic activity.[11] In traditional Chinese medicine, coumestans are used in the treatment of septic shock and in Indian Ayurvedic medicine as a treatment for liver diseases,[12] skin disorders and viral infections. Coumestans have also been shown to reduce cancer risk[13] due to their structural similarity to phytoestrogens.

A series of coumestan derivatives were recently reported as HCVNS5B polymerase inhibitors[14] and they also found to inhibit binding to the $GABA_A$ receptors from the rat brain.[11] Wedelolactone has been shown to inhibit the NF-κB mediated gene transcription in cells by blocking the phosphorylation and degradation,[15] selective 5-lipoxygenase-inhibitor[15] and LPS- induced caspase-11 expression inhibitor.[15] Coumestrol has been reported to have strong estrogenic activity.[16] Coumestan derivatives have been reported to show inhibitory effect of lipid peroxidation[17] and Na^+, K^+ ATPase activity.[18,19]

Recent Work

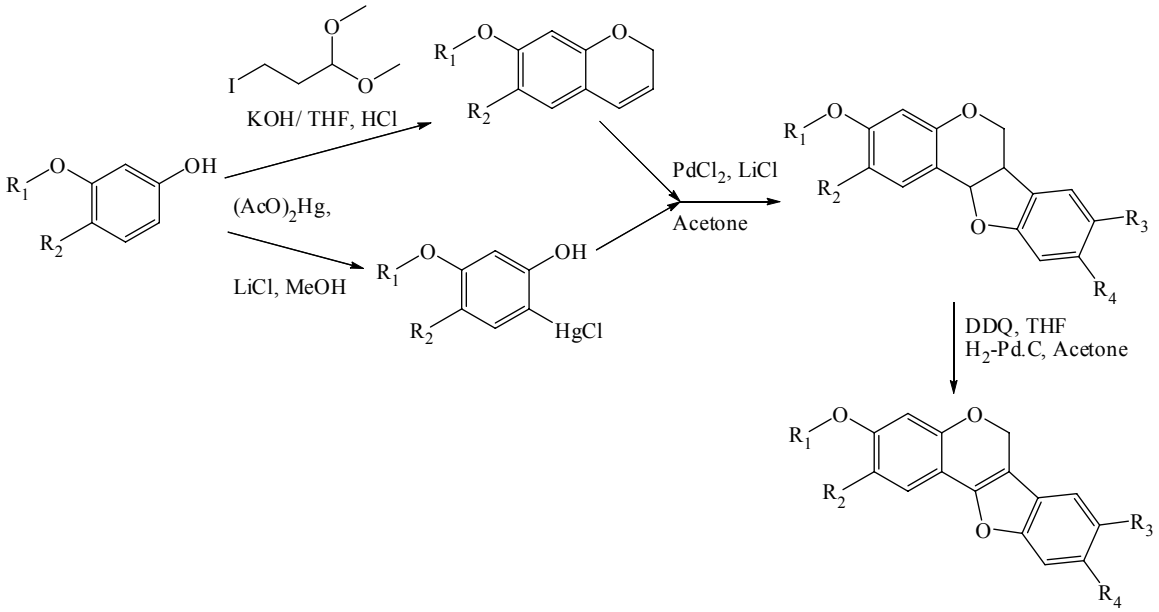
Soman S. S. and Trivedi K. N.[20] have synthesized benzofurocoumestan and difurocoumestan derivatives as shown in Scheme 1.

Scheme 1:



da Silva A. J. M and co workers[11] has synthesized various coumestan derivatives as shown in Scheme 2.

Scheme 2:



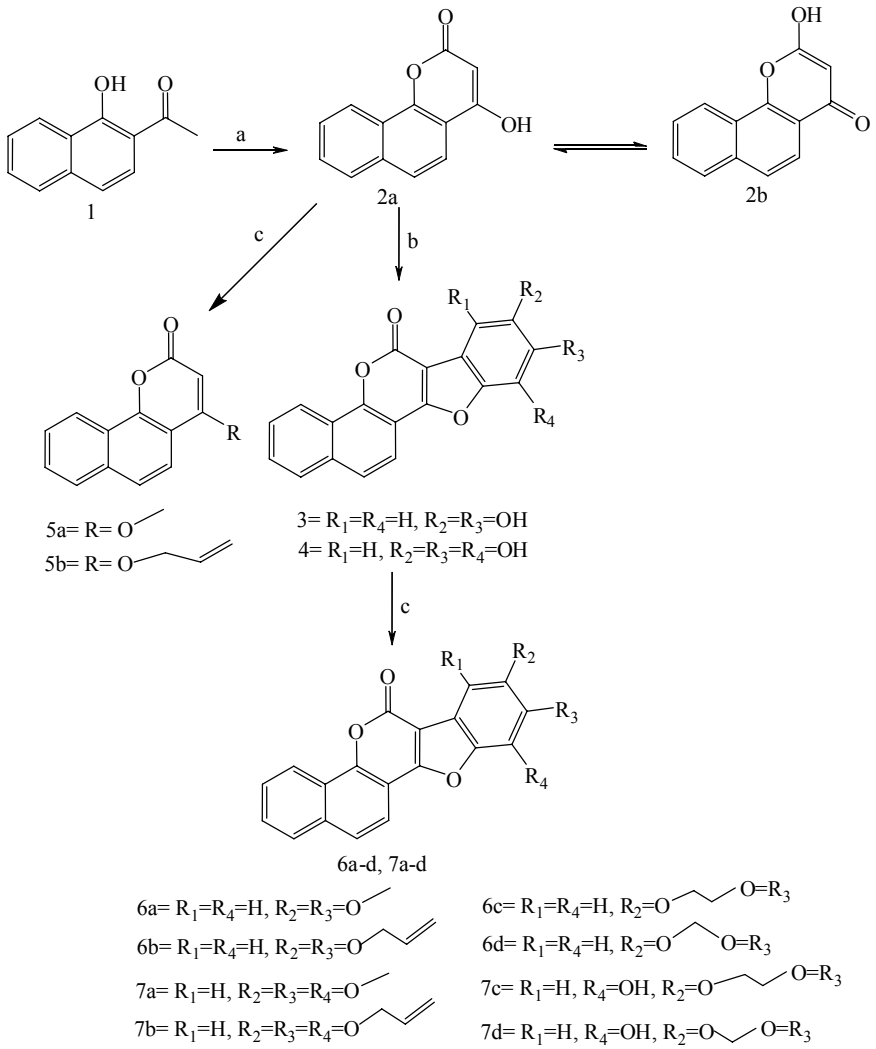
5b.2 Result and discussion

5b.2.1 Chemistry

2-Acetyl 1-naphthol **1** on Hoesch reaction[21] with diethyl carbonate in presence of pulverized sodium gave 4-hydroxy-2H-benzo[h]chromen-2-one **2** (Scheme 3).[22] Oxidative cyclization[22] of **2** with catechol and pyrogallol in presence of sodium acetate and potassium iodate gave corresponding coumestan derivatives 8, 9-dihydroxy-6H-

benzo[h] benzofuro[3, 2-c]chromen-6-one **3** and 8, 9, 10-trihydroxy-6H-benzo[h]benzofuro [3, 2-c] chromen-6-one **4**. Reaction of 4-hydroxy-2H-benzo[h]chromen-2-one **2** and coumestan derivatives **3** and **4** with dimethyl sulphate and various mono or dihaloalkanes in presence of base like anhydrous K_2CO_3[22] gave corresponding alkyl derivatives of coumestans **5a-b**, **6a-d** and **7a-d** as shown in Scheme 3.

Scheme 3:



Reagents and conditions: (a) pulverized sodium, diethyl carbonate, 30 min; (b) Catechol for **3** and pyrogallol for **4**, CH_3COONa, KIO_3, Acetone, water, RT, 30 min; (c) anhydrous K_2CO_3, Dry acetone, dimethyl sulphate or mono or di substituted alkyl halide, reflux, 10 h

Synthesis of compound **2** (page no. 33) is already discussed in chapter 2 and its IR spectrum, ^{1}H NMR spectrum and ^{13}C NMR spectrum were given in Chapter 2 also given in this chapter (Figure 1, Figure 2 and Figure 3).

The IR spectrum of compound **5a** (Figure 4) showed disappearance of band at 3423 cm^{-1} and appearance of band at 1734 cm^{-1} which confirmed presence of lactone ring. The ^{1}H NMR spectrum of compound **5a** in $CDCl_3$ (Figure 5) showed singlet at δ 4.07 for three protons indicated methoxy group, singlet at δ 5.81 indicated proton at C-3 position and all aromatic protons were observed between δ 7.64-8.6 confirmed the formation of compound **5a**. The ^{13}C NMR spectrum of compound **5a** in $CDCl_3$ (Figure 6) showed presence of 14 peaks which is in accordance with structure of compound **5a**.

The IR spectrum of compound **5b** (Figure 7) showed disappearance of band at 3423 cm^{-1} and appearance of band at 1726 cm^{-1} which confirmed presence of lactone ring. The ^{1}H NMR spectrum of compound **5a** in $CDCl_3$ (Figure 8) showed doublet of doublet at δ 4.73 for two protons indicated $–OCH_2$ group, doublet of doublet at δ 5.45-5.48 for one proton, another doublet of doublet at δ 5.53-5.58 for one proton indicated vinyl protons ($=CH_2$). Singlet at δ 5.79 for one proton indicated C-3 proton and multiplet at δ 6.10-6.17 for one proton indicated (CH=). All aromatic protons were observed between δ 7.64-8.57 confirmed formation of compound **5b**.The ^{13}C NMR spectrum of compound **5b** in $CDCl_3$ (Figure 9) showed presence of 16 peaks which is in accordance with structure of compound **5b**.

The IR spectrum of compound **3** (Figure 10) showed band at 3416 and 1702 cm^{-1} for hydroxyl group and lactone carbonyl group respectively. The IR spectrum of compound **6a** (Figure 11) showed disappearance of band at 3416 cm^{-1} and appearance of band at 1731 cm^{-1} which confirmed presence of lactone ring. The ^{1}H NMR of compound **6a** in DMSO-d_6 (Figure 12) showed two singlets at δ 3.92 and 3.93 for six protons

indicated two $-OCH_3$ groups. All aromatic protons were observed between δ 7.31-8.51 confirmed formation of compound **6a**. The IR spectrum of compound **6b** (Figure 13) showed disappearance of band at 3416 cm^{-1} and appearance of band at 1726 cm^{-1} which confirmed presence of lactone ring. The ^1H NMR of compound **6b** in DMSO-d_6 (Figure 14) showed doublet at δ 4.64-4.65 for two protons indicated $-OCH_2$ group, doublet of doublet at δ 5.25-5.29 for two protons, another doublet of doublet at δ 5.42-5.47 for two protons indicated two vinyl protons ($=CH_2$). One multiplet at δ 6.08 for two protons indicated one vinyl proton (=CH) each thus confirmed the presence of two allyloxy groups and all aromatic protons were observed between δ 7.30-8.50 confirmed formation of compound **6b**.

The IR spectrum of compound **4** (Figure 15) showed band at 3843 cm^{-1} for hydroxyl group and band at 1734 cm^{-1} for lactone carbonyl group confirmed formation of compound **4**. The IR spectrum of compound **7a** (Figure 16) showed disappearance of band at 3843 cm^{-1} for hydroxyl group and presence of bands at 3210, 3075, 2946, 2924 and 2851 cm^{-1} and band at 1733 cm^{-1} for lactone group confirmed formation of compound **7a**. The IR spectrum of compound **7c** (Figure 17) showed band at 3614 cm^{-1} indicated presence of one free –OH group and presence of band at 1734 cm^{-1} for lactone group confirmed formation of compound **7c**.

The analyses of coumestan derivatives **3**, **4**, **6a-d** and **7a-d** using ^1H NMR was not possible except two compounds **6a** and **6b** which were soluble in DMSO-d_6 and rest of all compounds were insoluble in DMSO-d_6. ^1H NMR in other deuterated solvents like DMF-d_7, acetic acid-d_4, triflouro acetic acid-d_1 and pyridine-d_5 of these compounds were also tried but the samples were insoluble. The melting point of these compounds were also very high (>250ºC) hence TG/DTA and DSC analyses of all coumestan derivatives carried out. The DSC of compound **3** (Figure 18) showed degradation at 390ºC

temperature. The TG/DTA of compound **6a** (Figure 19) showed maximum degradation at 328ºC temperature. The TG/DTA of compound **6b** (Figure 20) showed phase transition at 158ºC and 215ºC and maximum degradation at 331ºC temperature. TG graph of **6b** showed continuous weight loss from 210ºC. The DSC of compound **4** (Figure 21) showed maximum degradation at 342ºC temperature. The DSC of compound **7a** (Figure 22) showed maximum degradation at 367ºC temperature. The TG/DTA of compound **7c** (Figure 23) showed maximum degradation at 177-186ºC temperature.

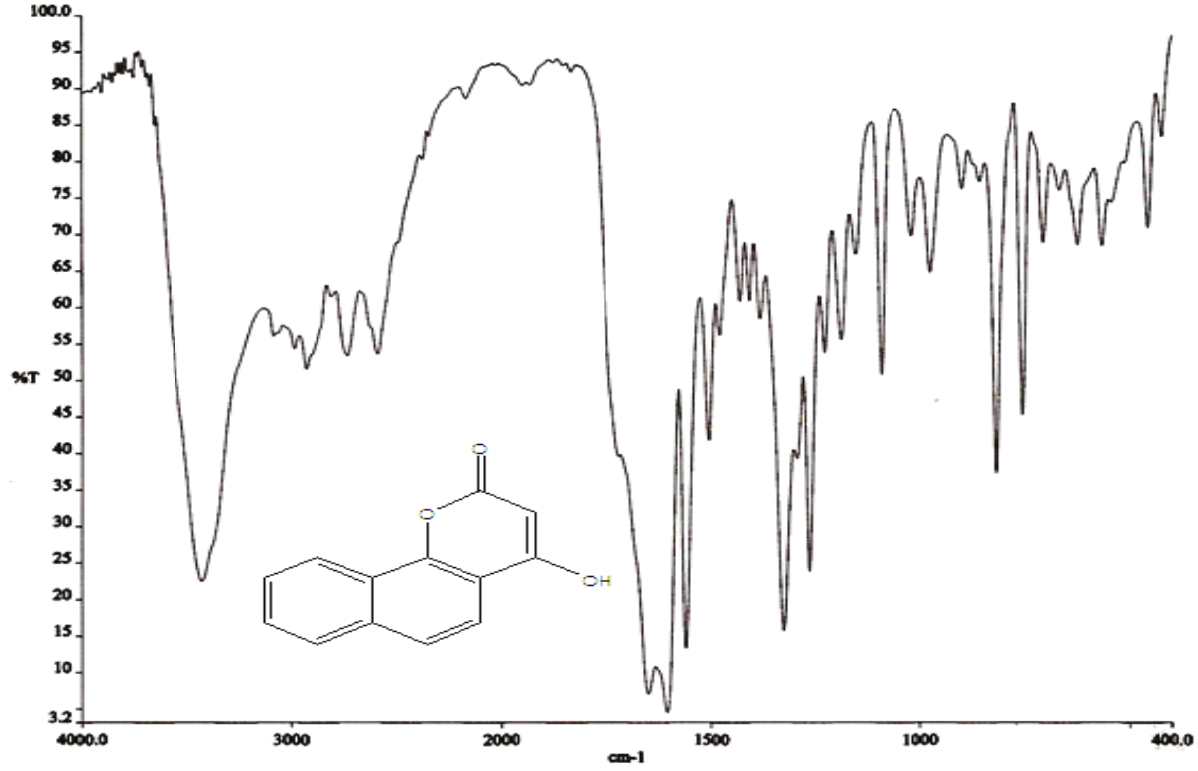


Figure 1: IR of 4-hydroxy-2H-benzo[h]chromen-2-one **2**

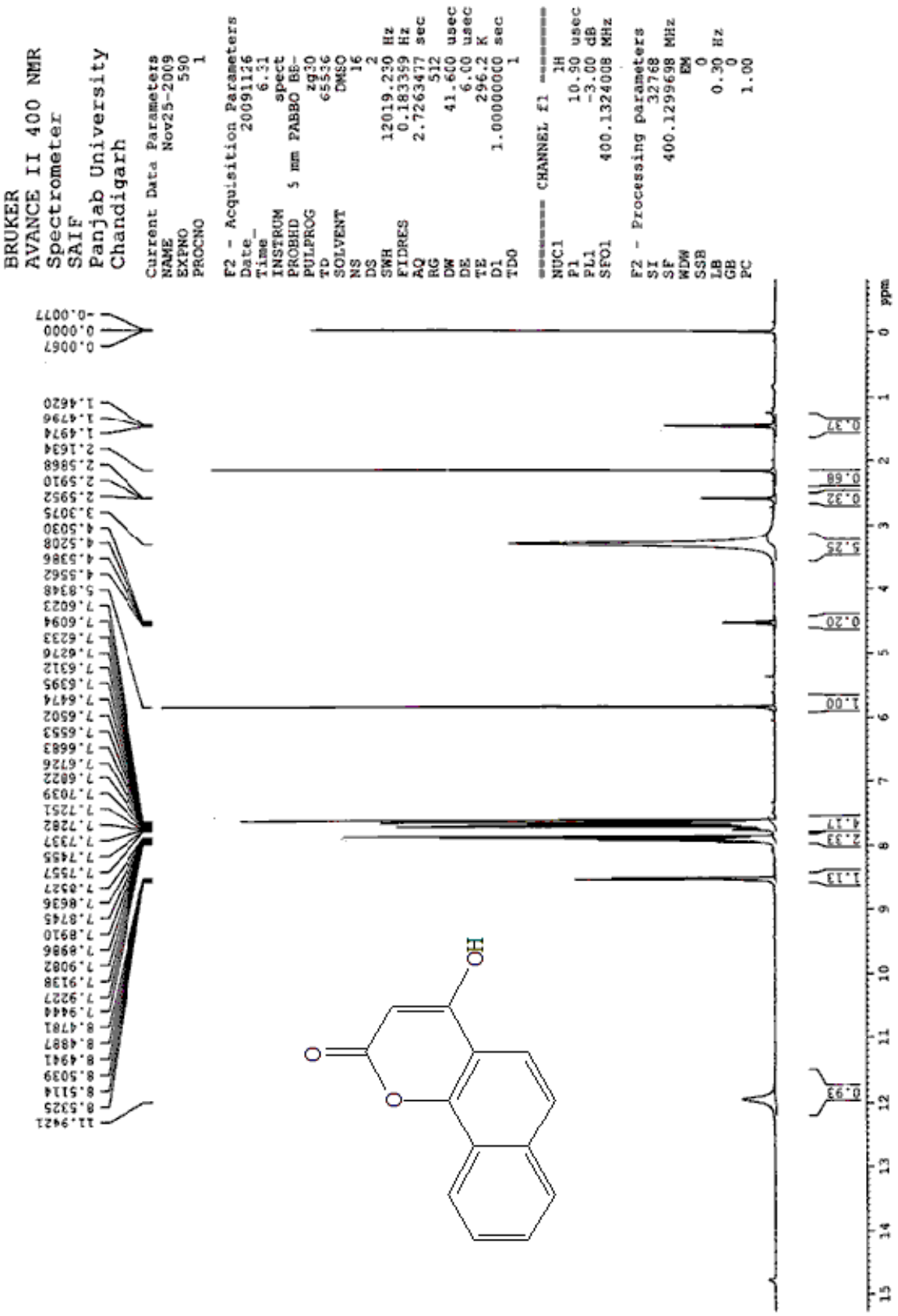


Figure 2: ^{1}H NMR of 4-hydroxy-2H-benzo[h]chromen-2-one **2**

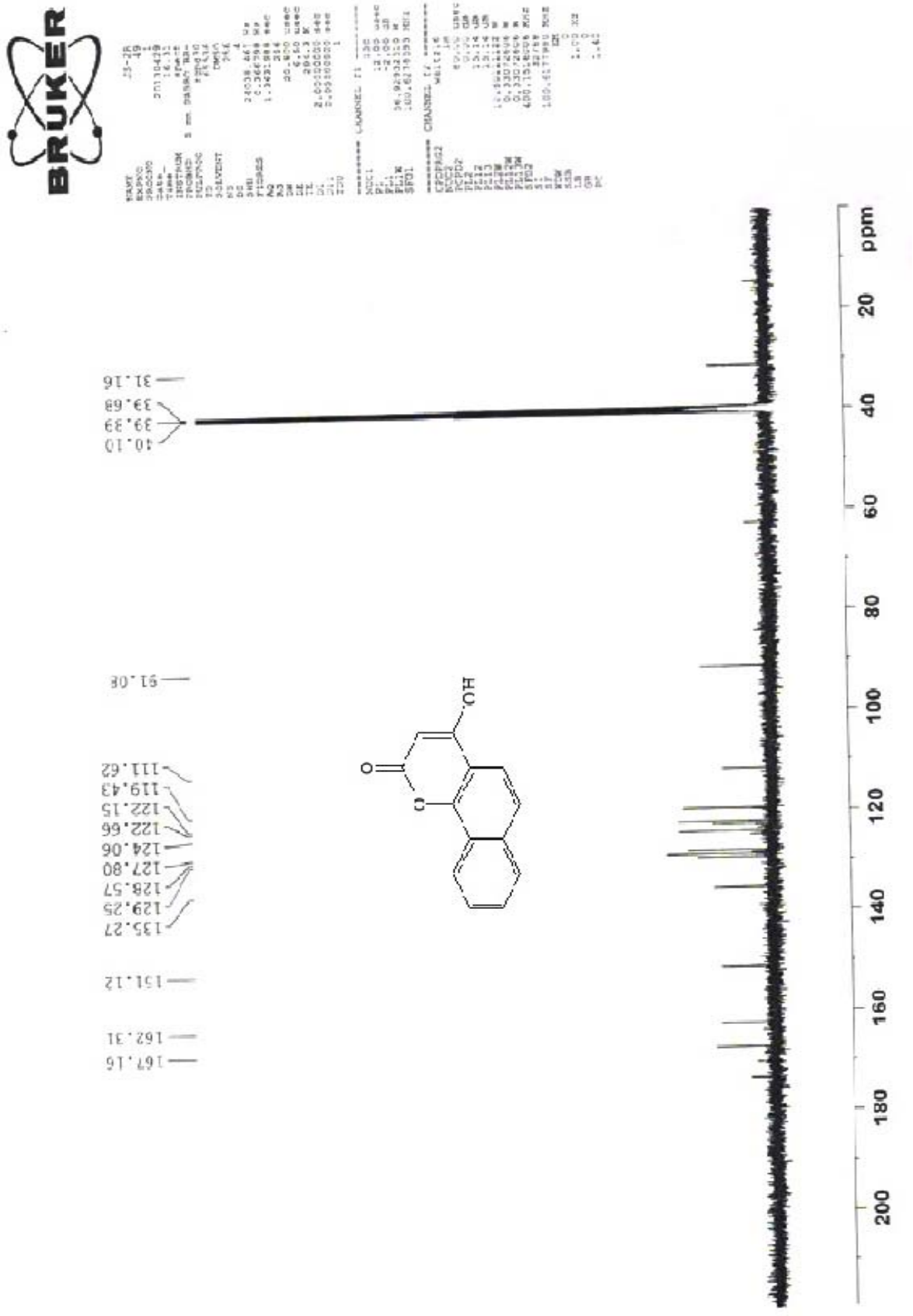


Figure 3: ^{13}C NMR of 4-hydroxy-2H-benzo[h]chromen-2-one **2**

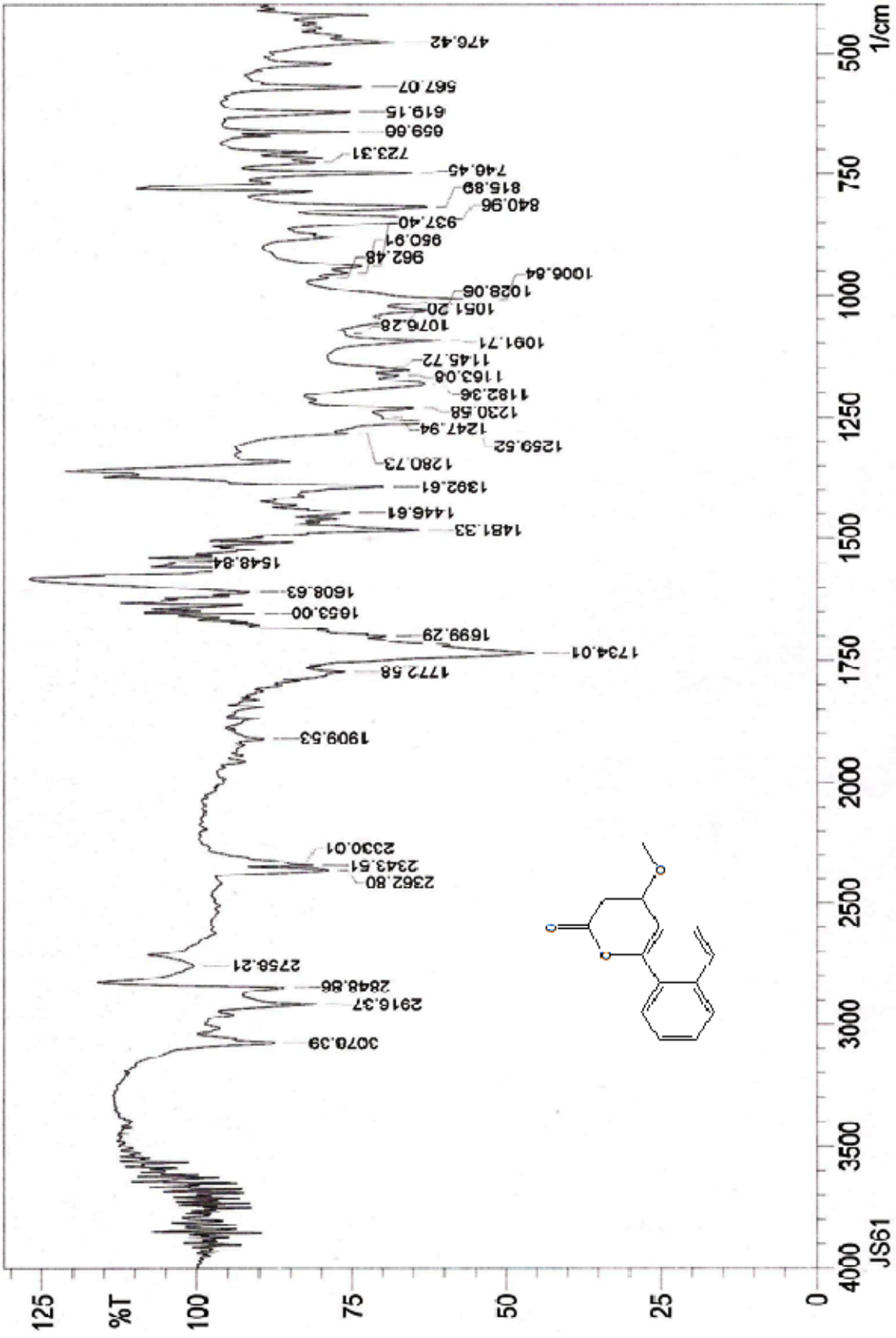


Figure 4: IR of 4-methoxy-2H-benzo[h]chromen-2-one **5a**

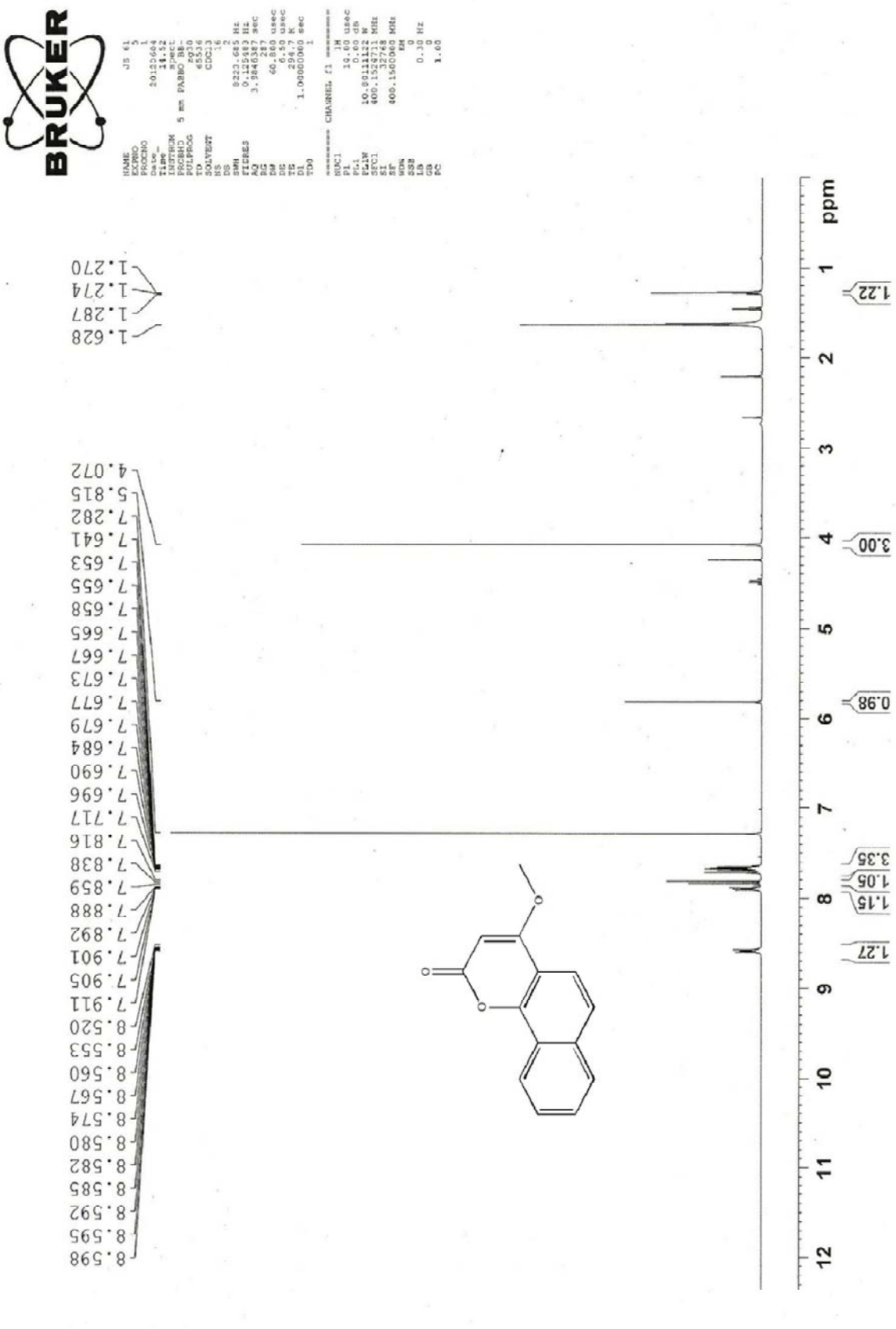


Figure 5: ^{1}H NMR of 4-methoxy-2H-benzo[h]chromen-2-one **5a**

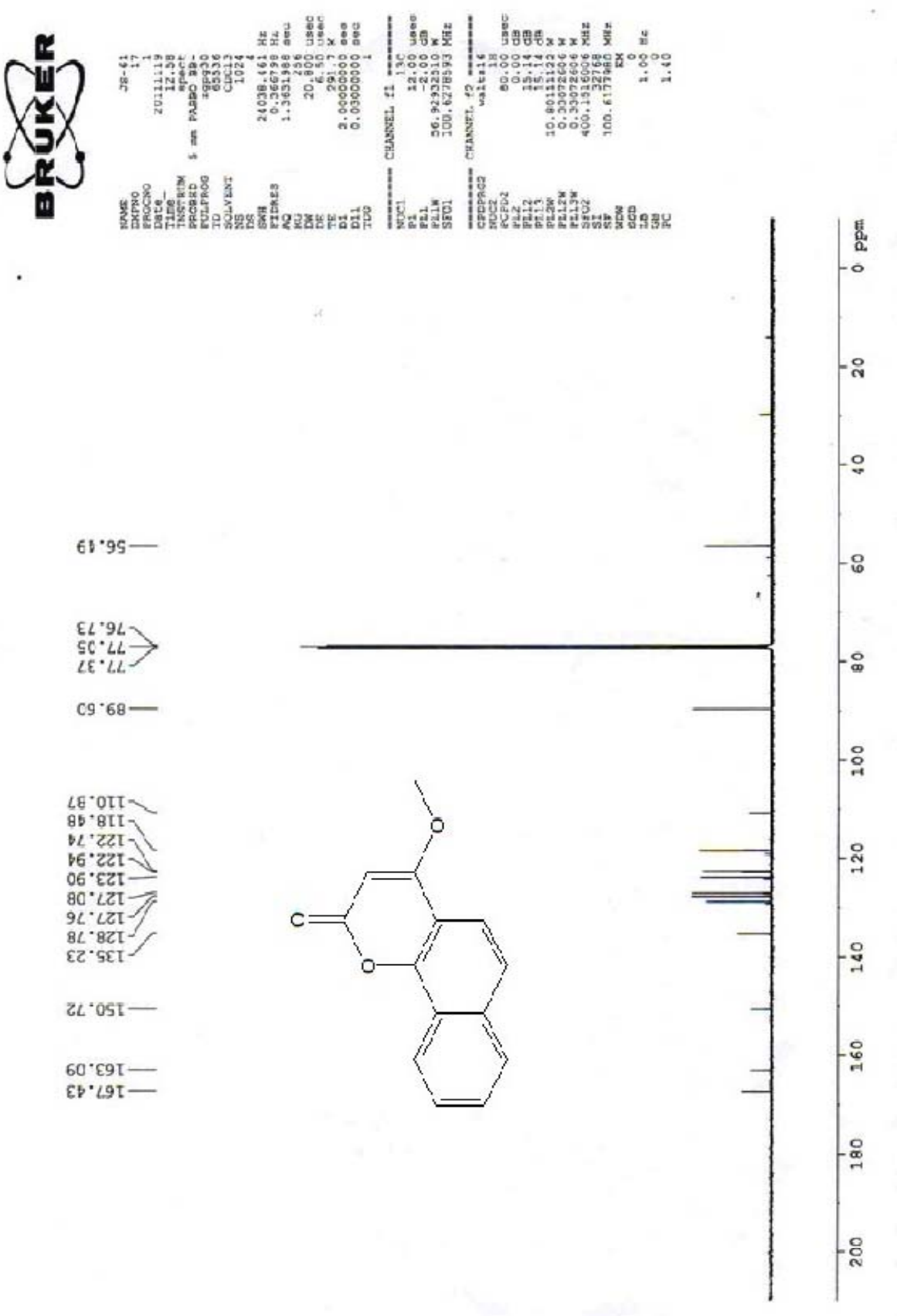


Figure 6: ^{13}C NMR of 4-methoxy-2H-benzo[h]chromen-2-one **5a**

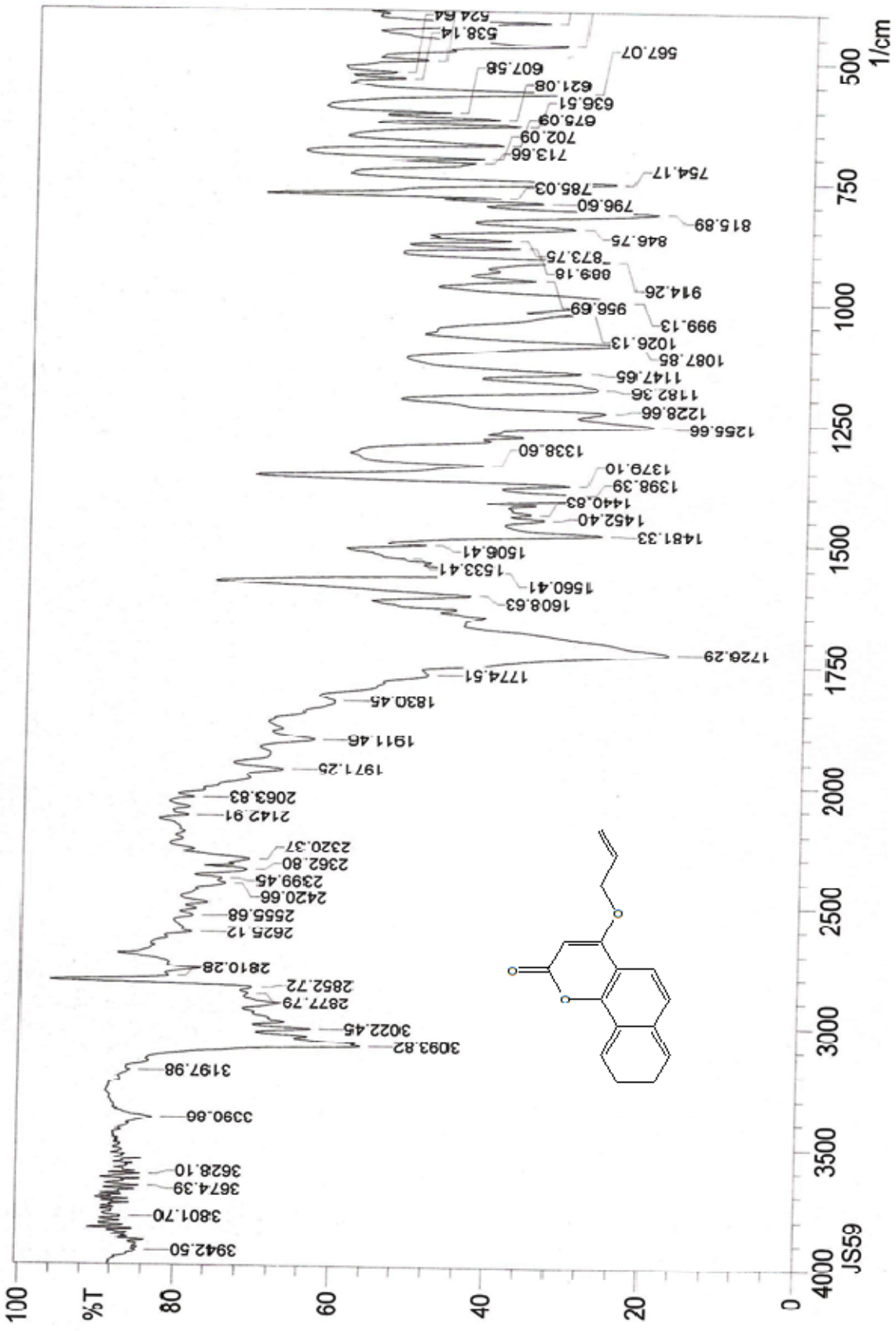


Figure 7: IR of 4-allyloxy-2H-benzo[h]chromen-2-one **5b**

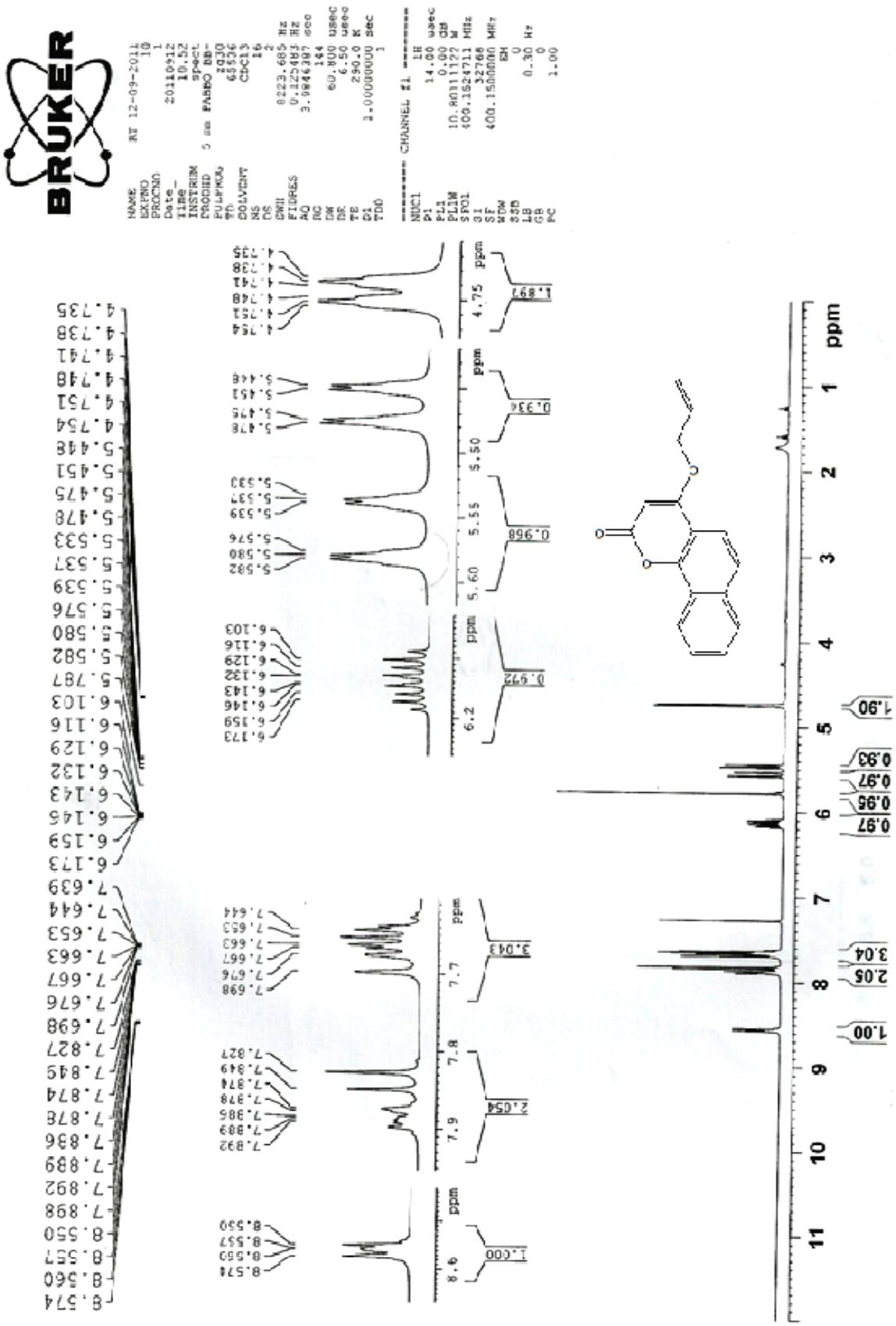


Figure 8: ^{1}H NMR of 4-allyloxy-2H-benzo[h]chromen-2-one **5b**

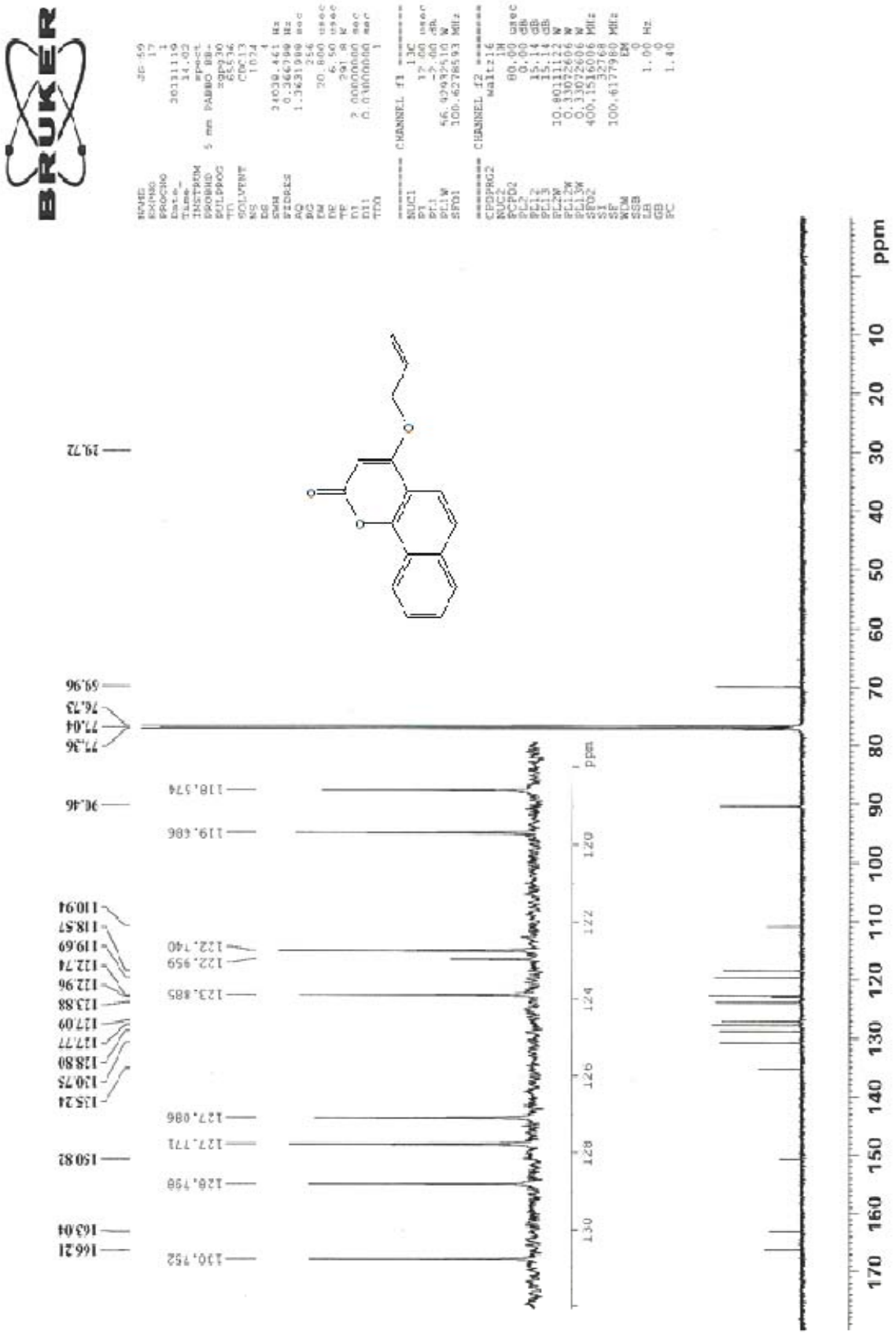


Figure 9: ^{13}C NMR of 4-allyloxy-2H-benzo[h]chromen-2-one **5b**

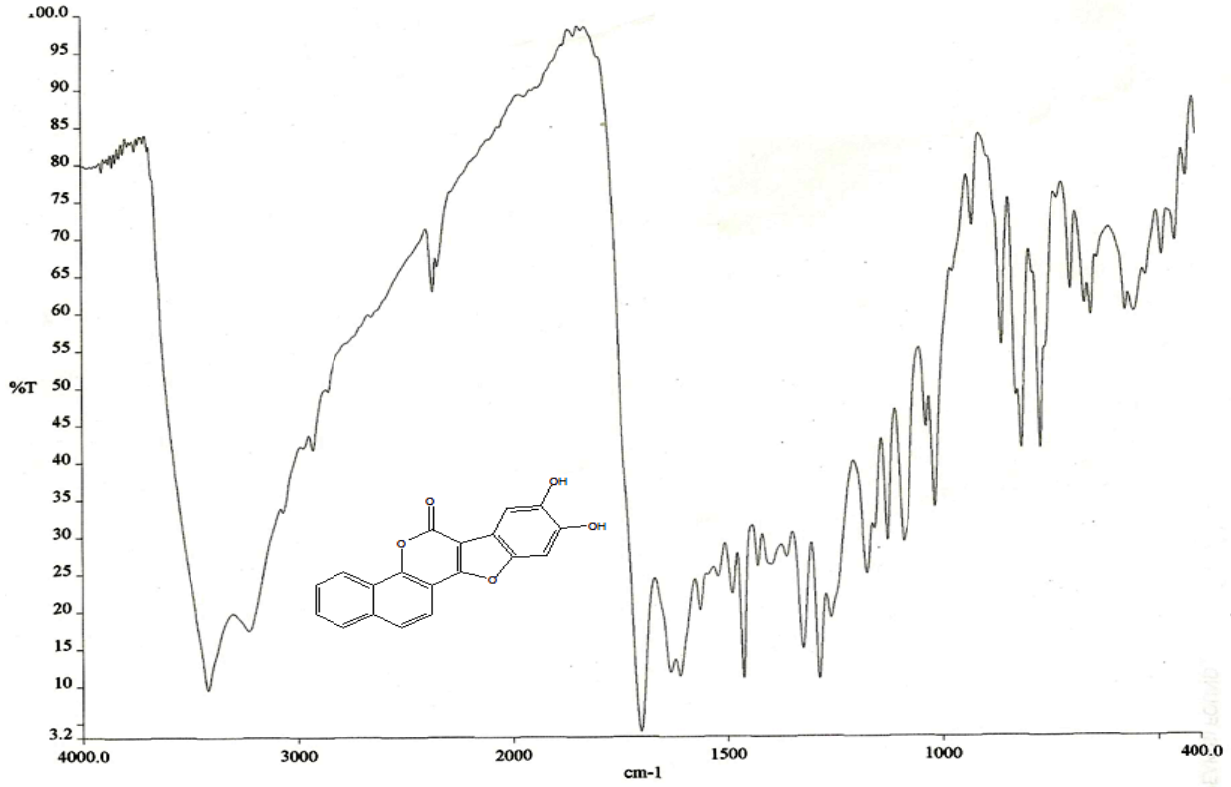


Figure 10: IR of 8, 9-dihydroxy-6H-benzo[h]benzofuro[3,2-c]chromen-6-one **3**

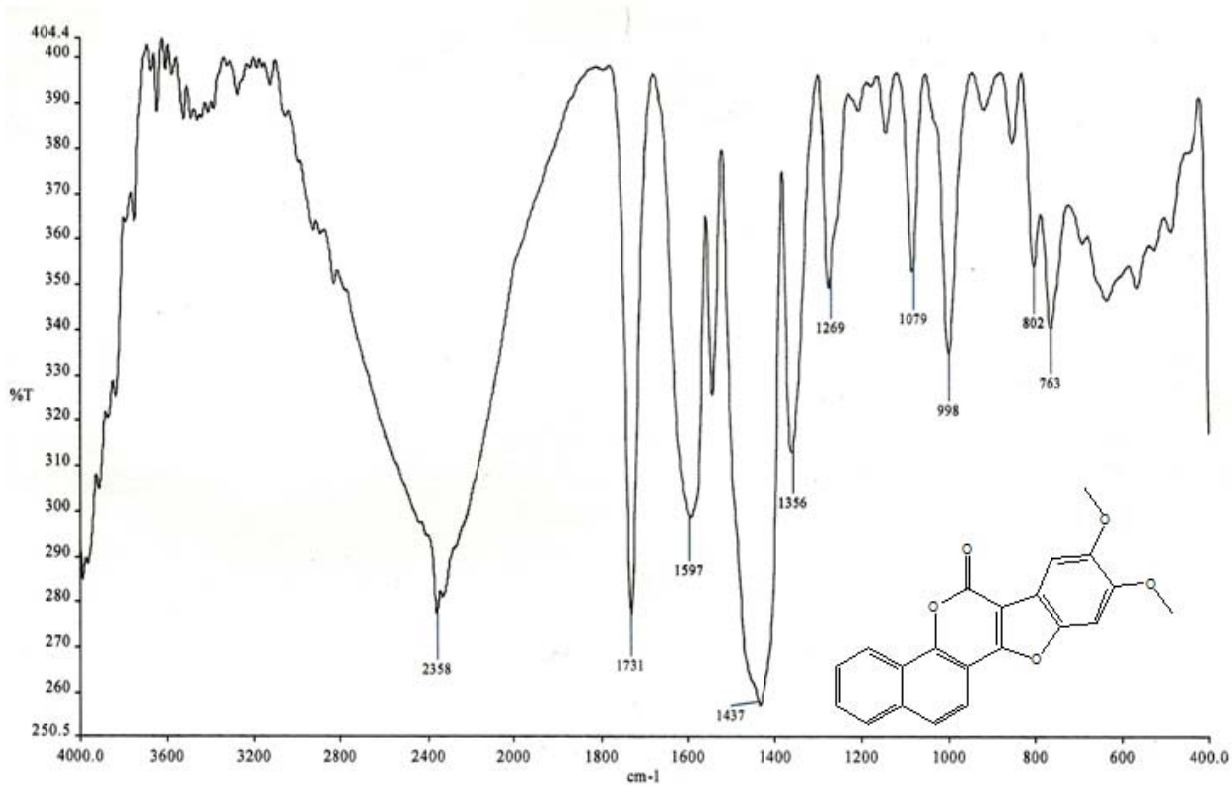


Figure 11: IR of 8,9-dimethoxy-6H-benzo[h]benzofuro[3,2-c]chromen-6-one **6a**

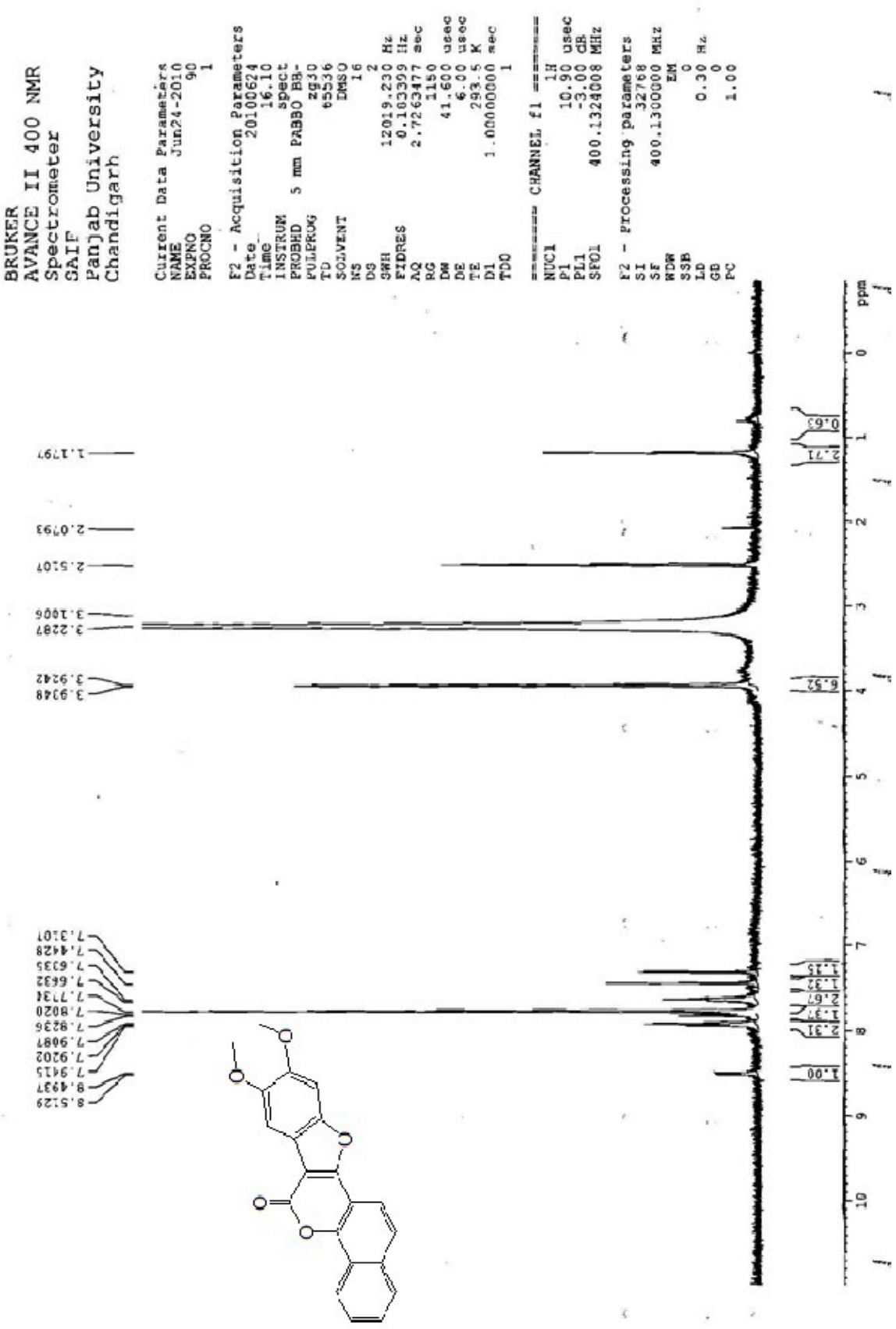


Figure 12: ^{1}H NMR of 8,9-dimethoxy-6H-benzo[h]benzofuro[3,2-c]chromen-6-one **6a**

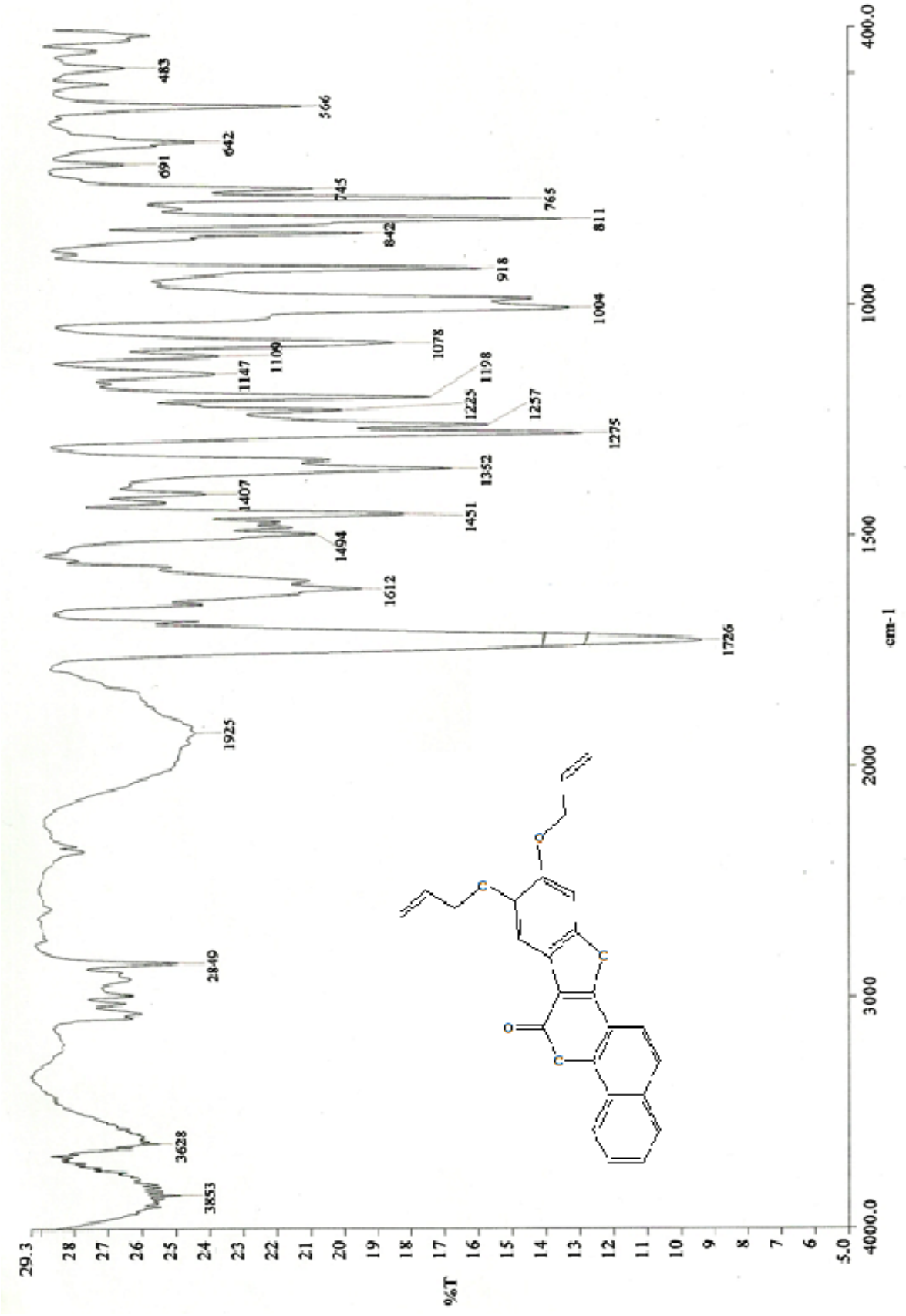


Figure 13: IR of 8, 9-bis(allyloxy)-6H-benzo[h]benzofuro[3,2-c]chromen-6-one **6b**

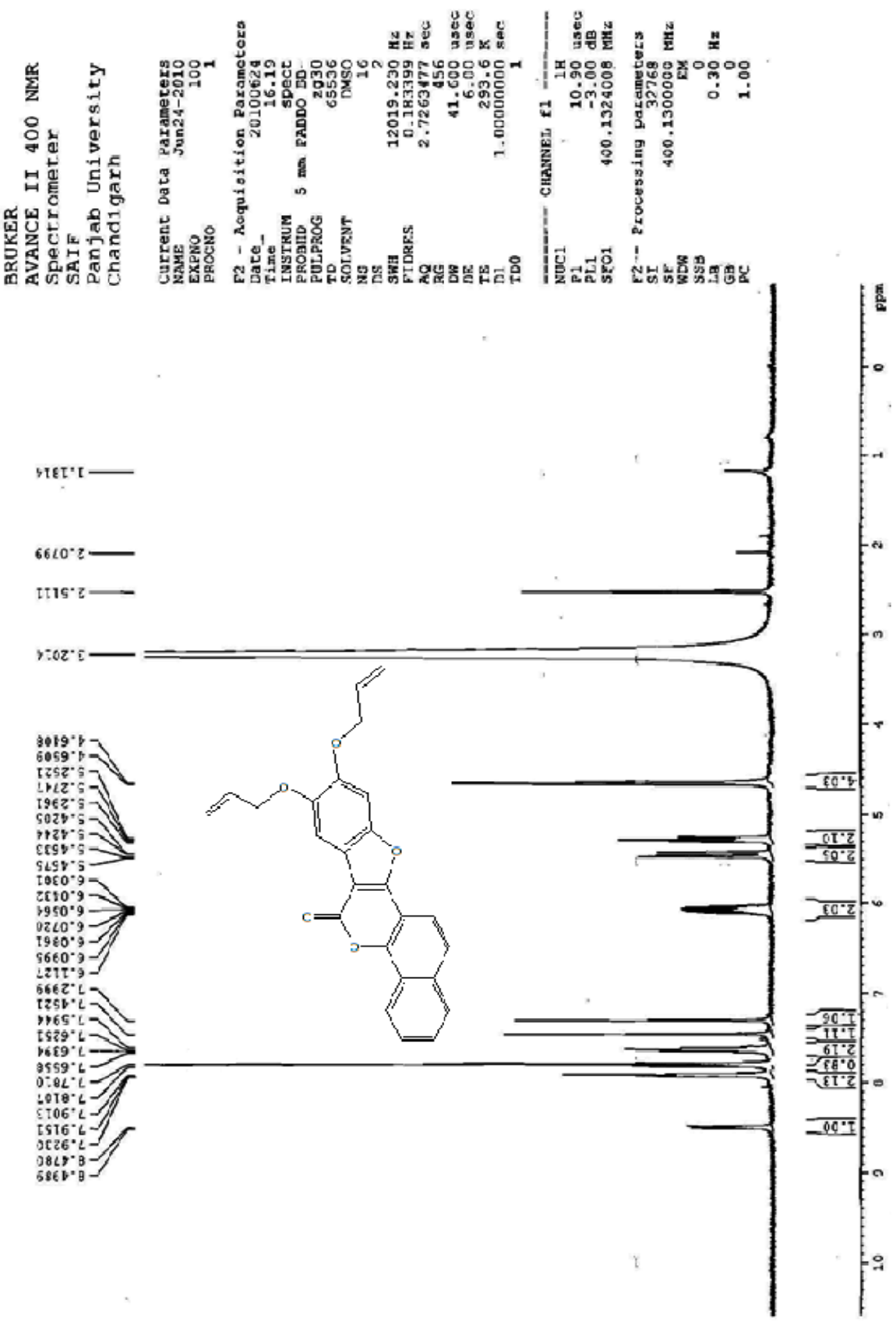


Figure 14: ^{1}H NMR of 8, 9-bis(allyloxy)-6H-benzo[h]benzofuro[3,2-c]chromen-6-one **6b**

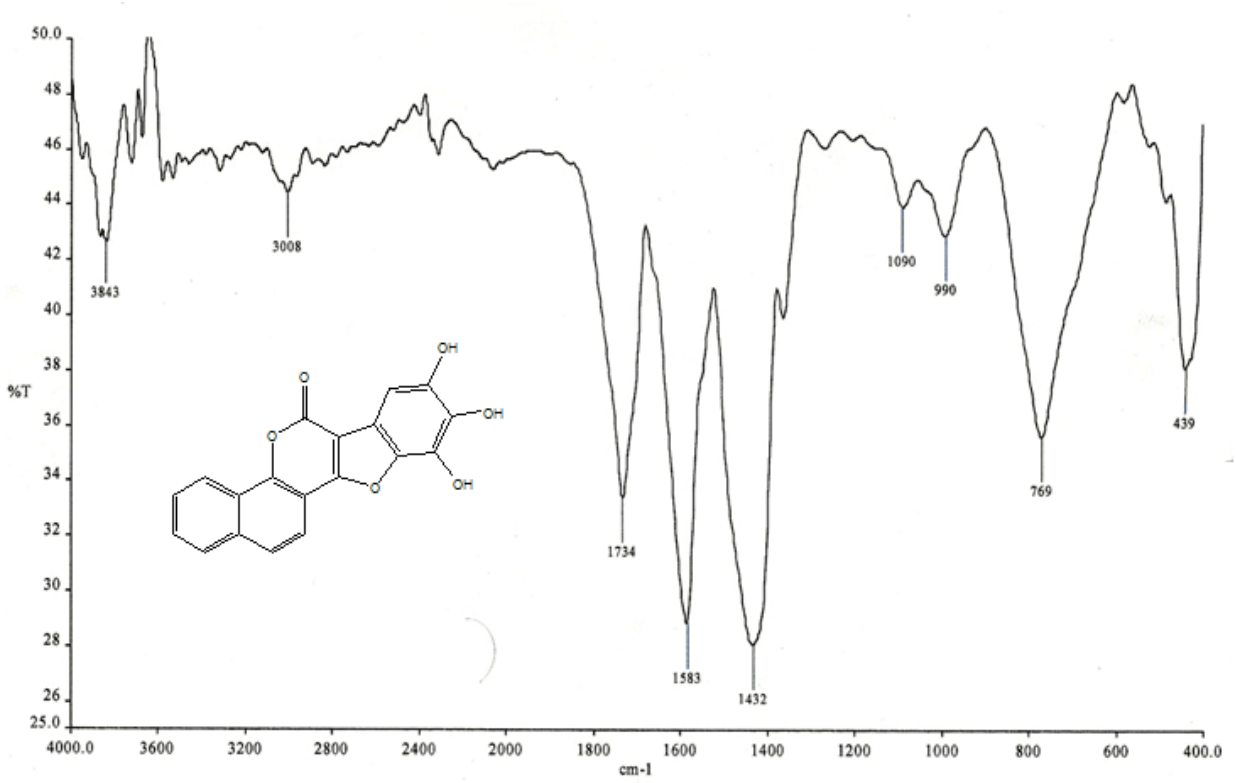


Figure 15: IR of 8, 9, 10-trihydroxy-6H-benzo[h]benzofuro[3,2-c]chromen-6-one **4**

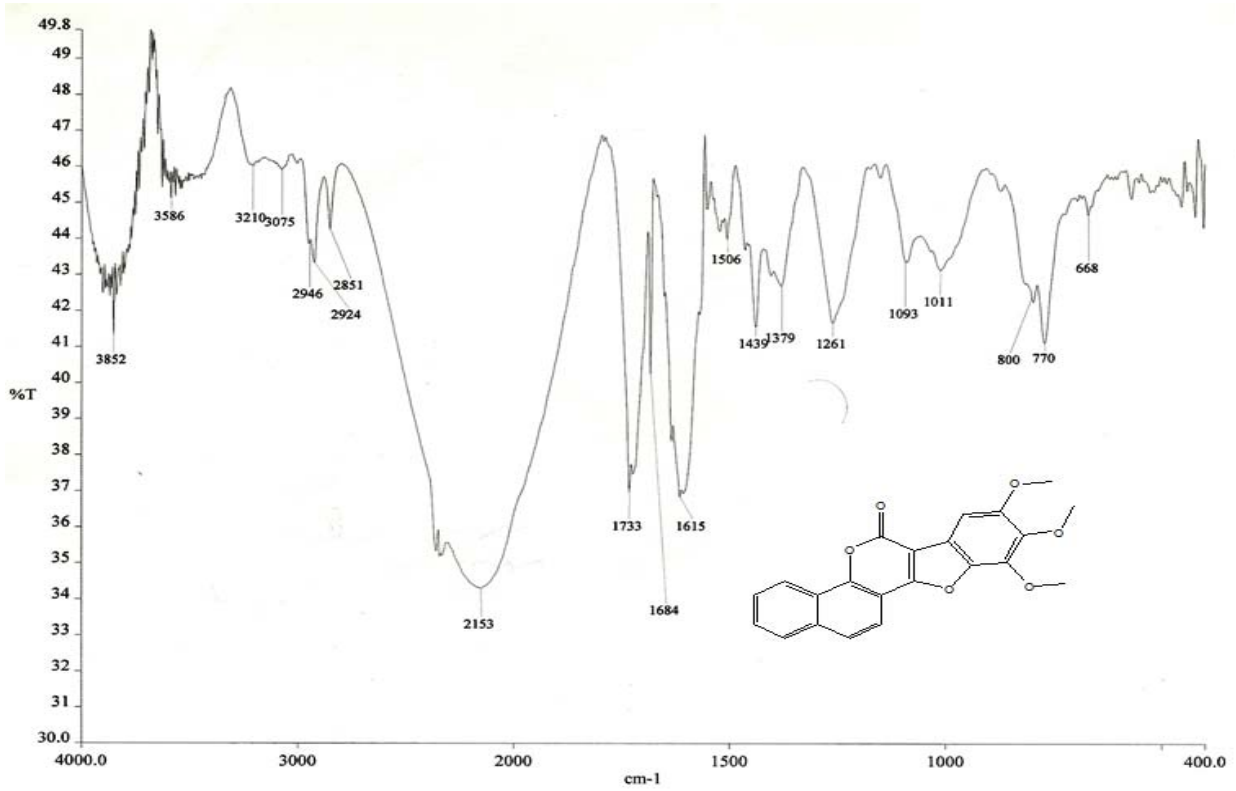


Figure 16: IR of 8, 9, 10-trimethoxy-6H-benzo[h]benzofuro[3,2-c]chromen-6-one **7a**

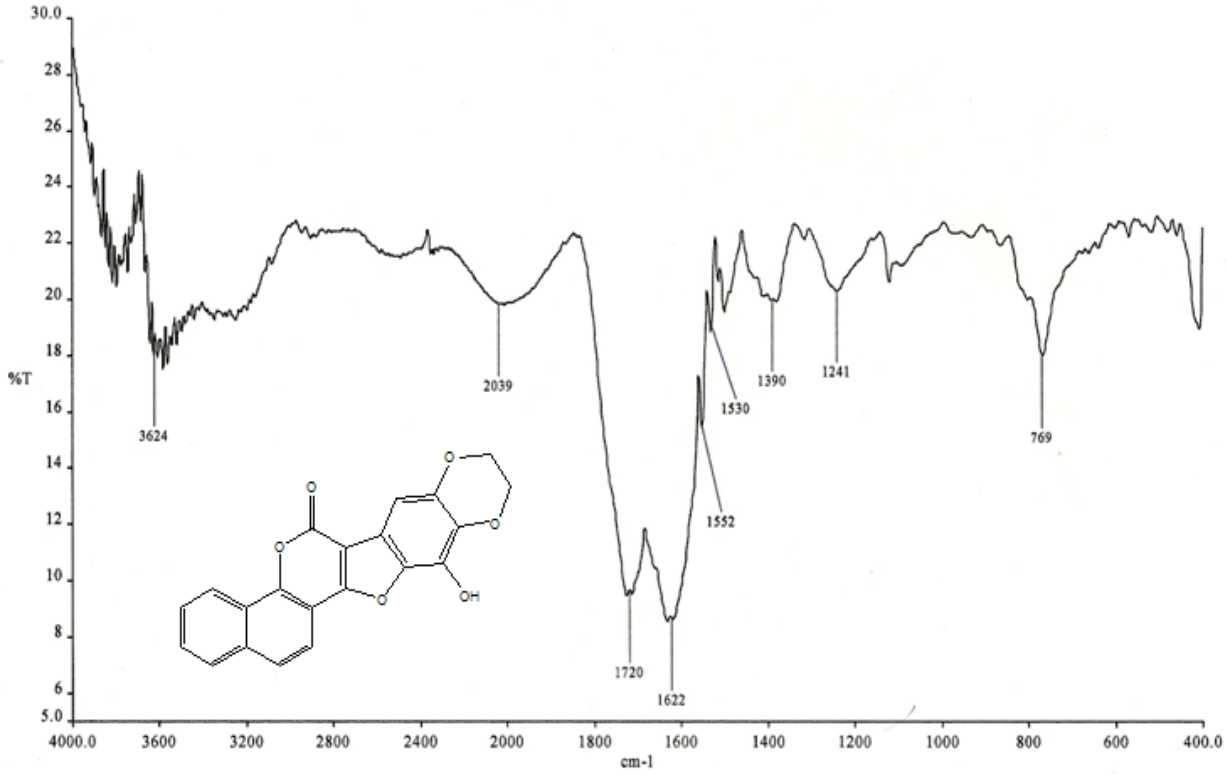


Figure 17: IR of 8, 9-ethylenedioxy, 10-hydroxy-6H-benzo[h]benzofuro[3,2-c]chromen-6-one **7c**

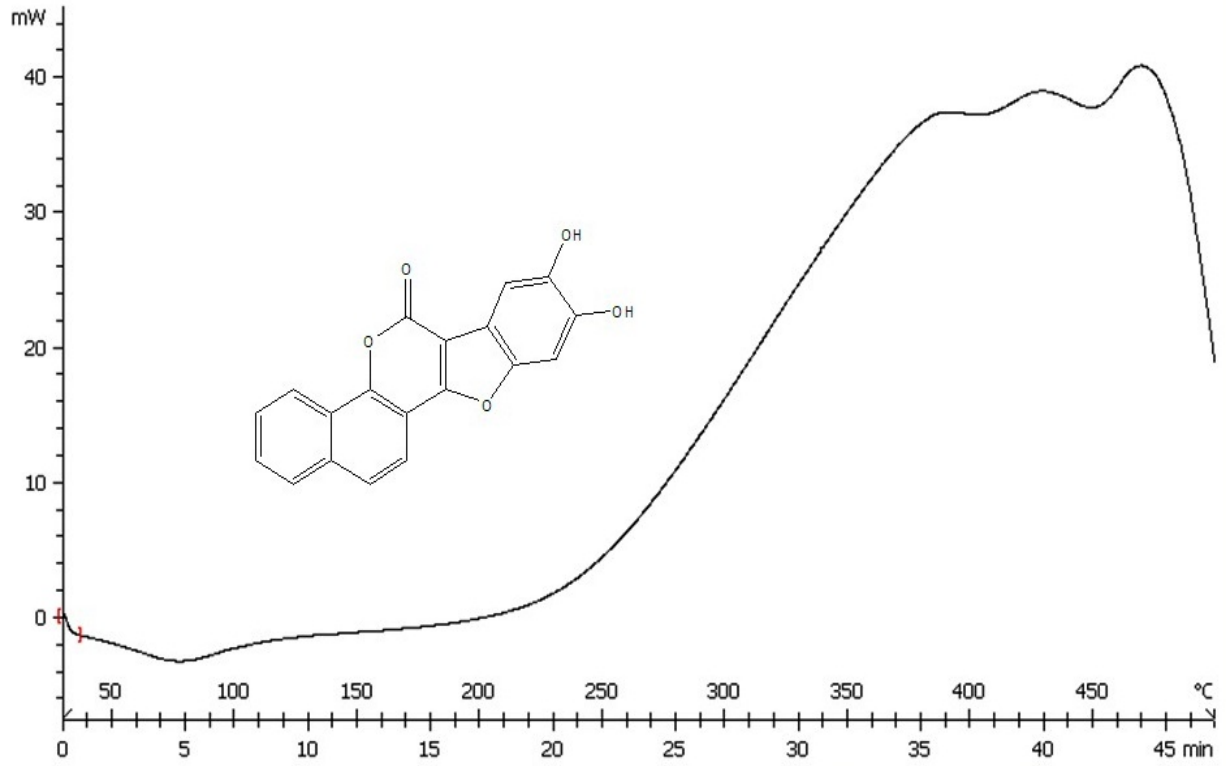


Figure 18: DSC of 8, 9-dihydroxy-6H-benzo[h]benzofuro[3,2-c]chromen-6-one **3**

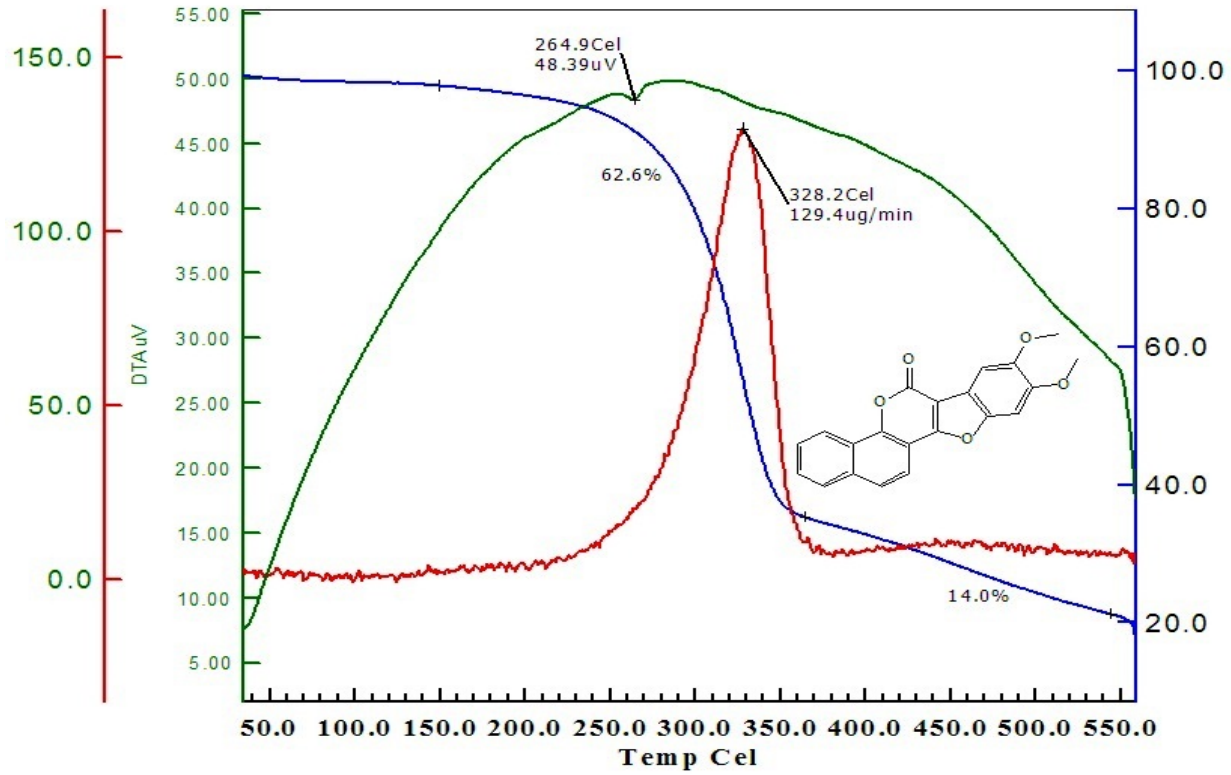


Figure 19: TG/DTA of 8,9-dimethoxy-6H-benzo[h]benzofuro[3,2-c]chromen-6-one **6a**

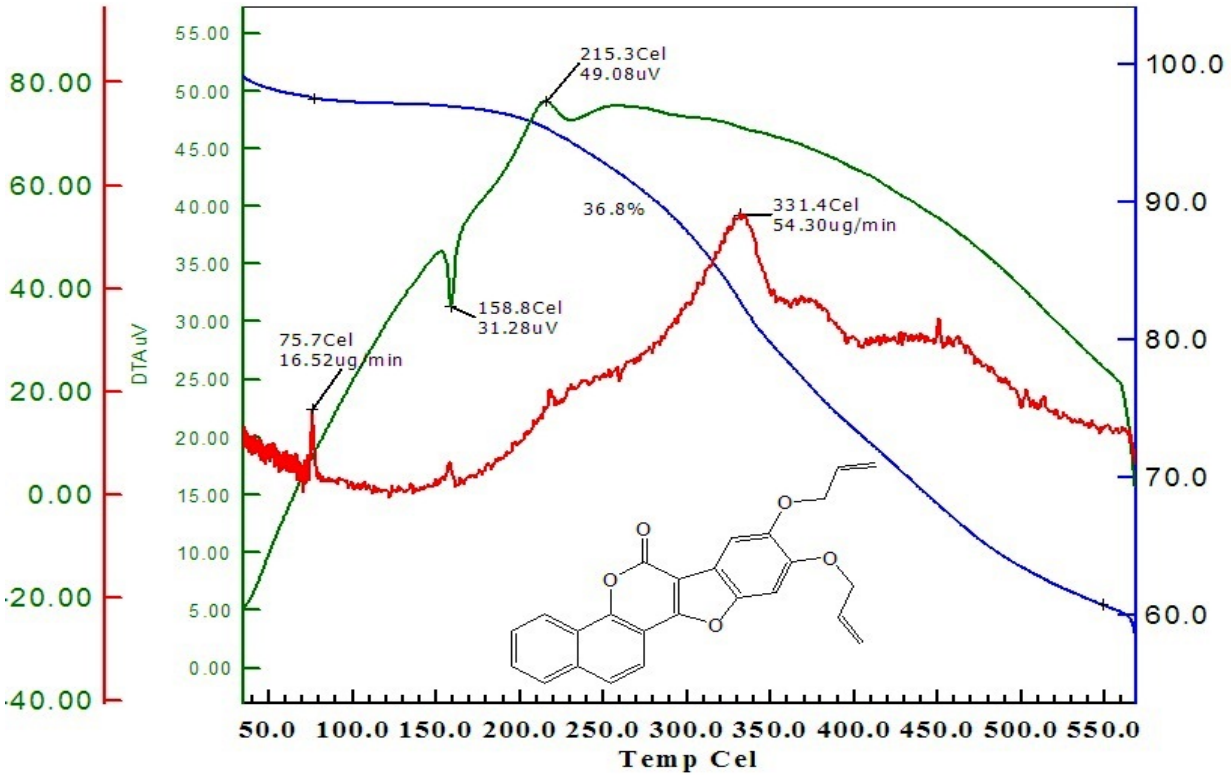


Figure 20: TG/DTA of 8, 9-bis(allyloxy)-6H-benzo[h]benzofuro[3,2-c]chromen-6-one **6b**

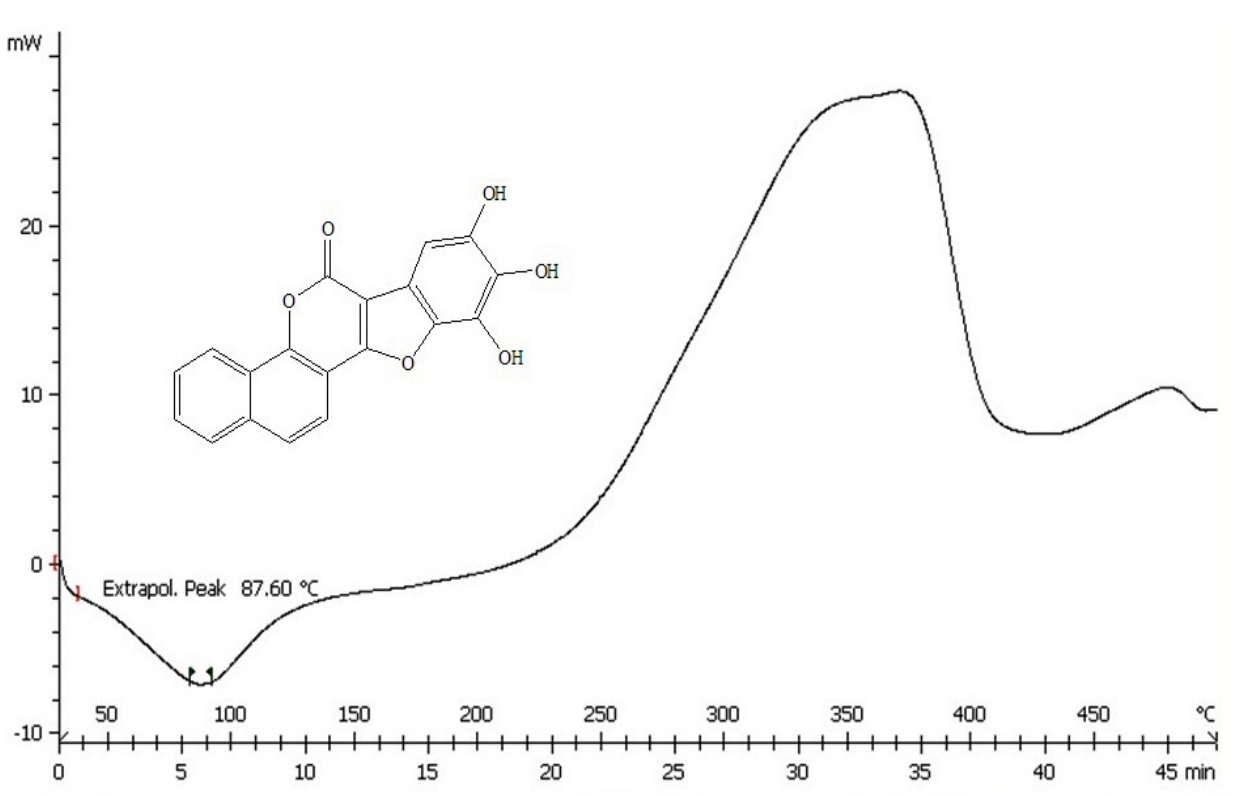


Figure 21: DSC of 8, 9, 10-trihydroxy-6H-benzo[h]benzofuro[3,2-c]chromen-6-one **4**

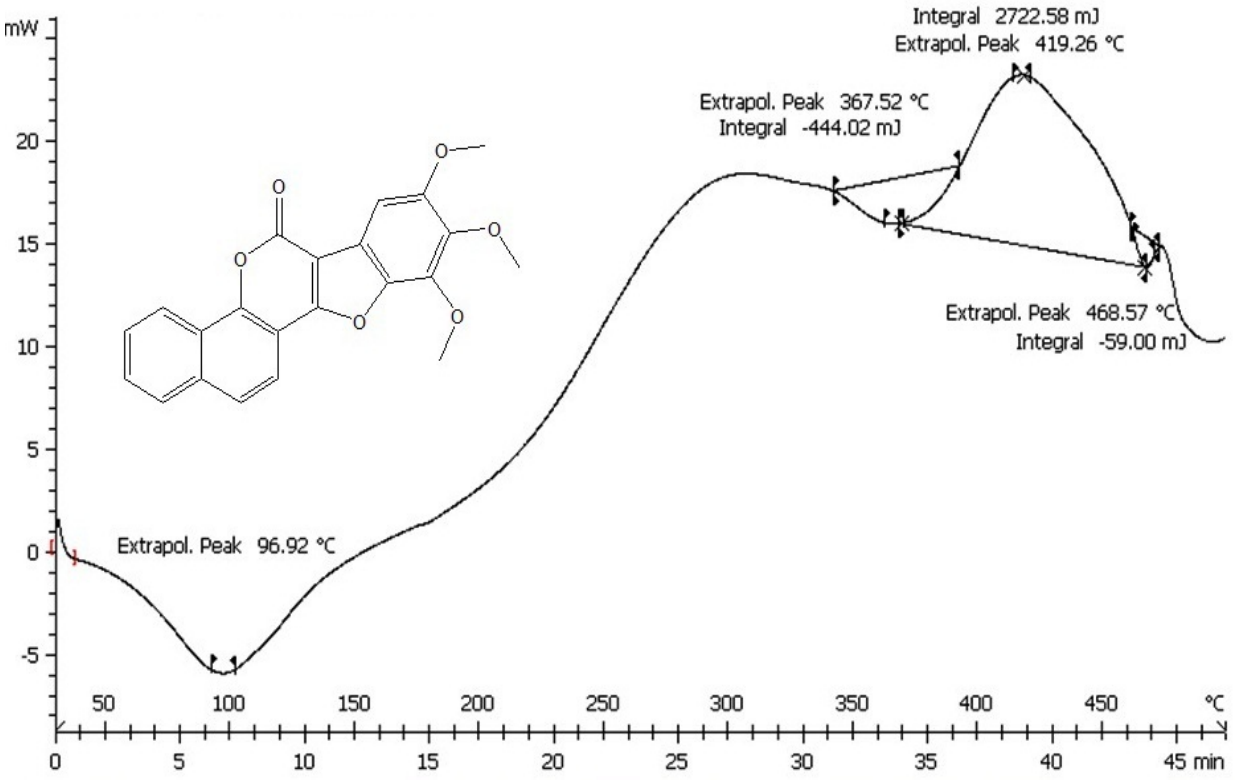


Figure 22: DSC of 8, 9, 10-trimethoxy-6H-benzo[h]benzofuro[3,2-c]chromen-6-one **7a**

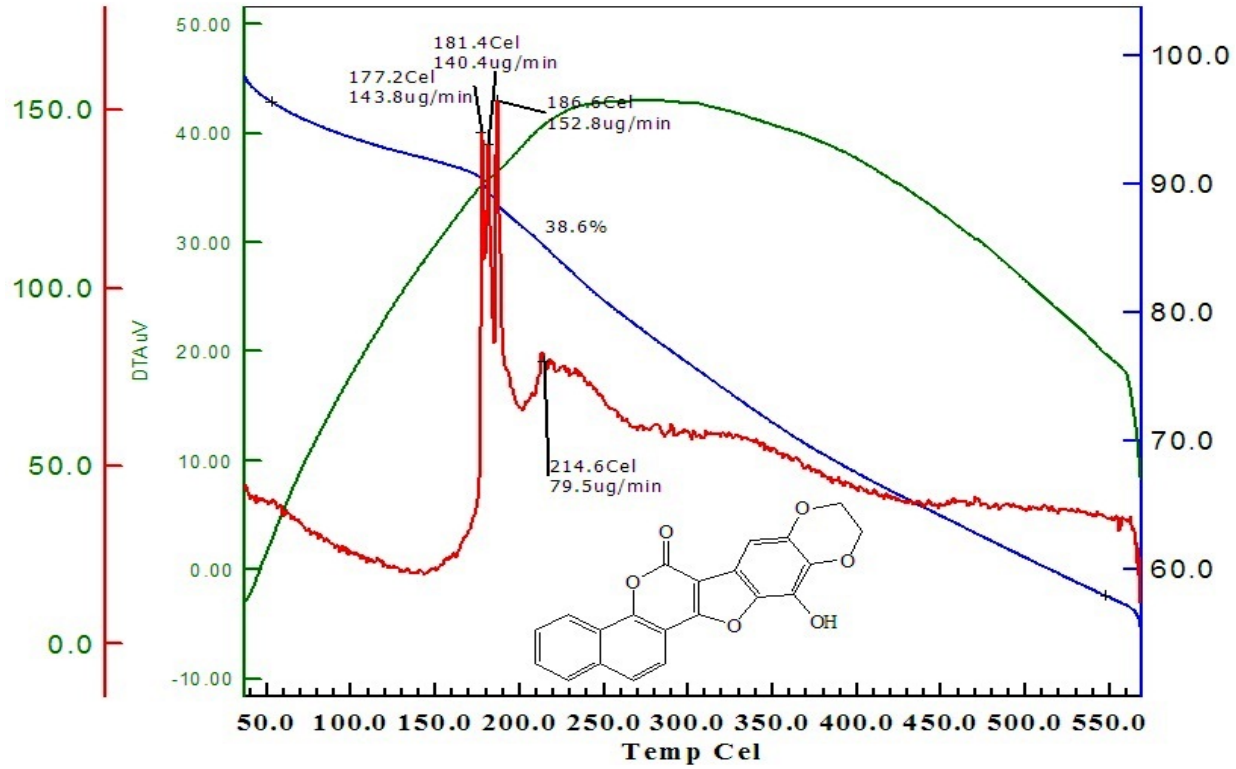
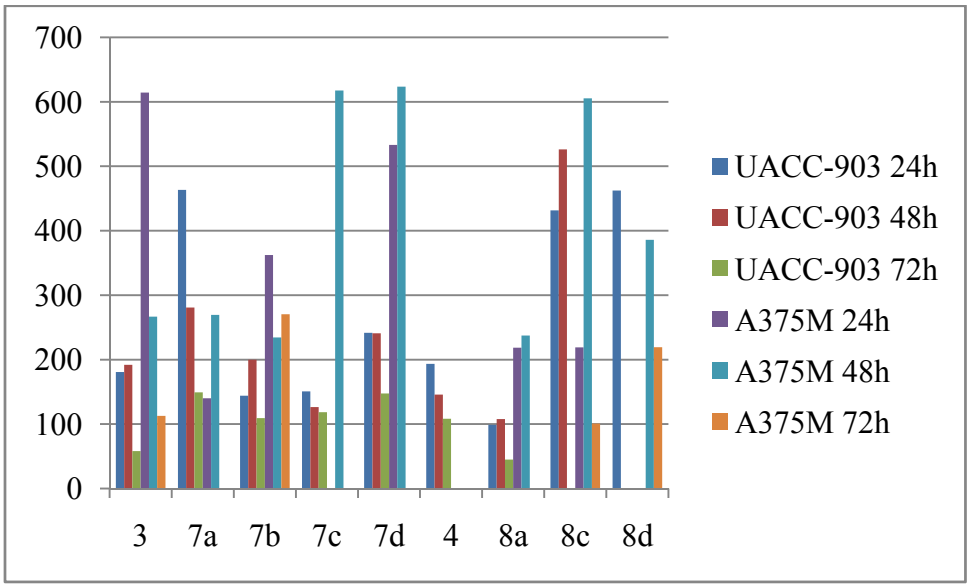


Figure 23: TG/DTA of 8, 9-ethylenedioxy, 10-hydroxy-6H-benzo[h]benzofuro[3,2-c]chromen-6-one **7c**

5b.2.2 Biological Evaluation

All synthesized coumestan derivatives were screened against two melanoma cancer cell lines UACC-903 and A375M, one breast cancer cell line MCF-7 and fibroblast (FF2441-Precursors of normal cells) to determine IC_{50} values of synthesized compounds by MTS Assay method.[23] The results are shown in Figure 13, Figure 14 and Figure 15 respectively. Details of MTS Assay method is already given in Chapter 2 page no. 74.

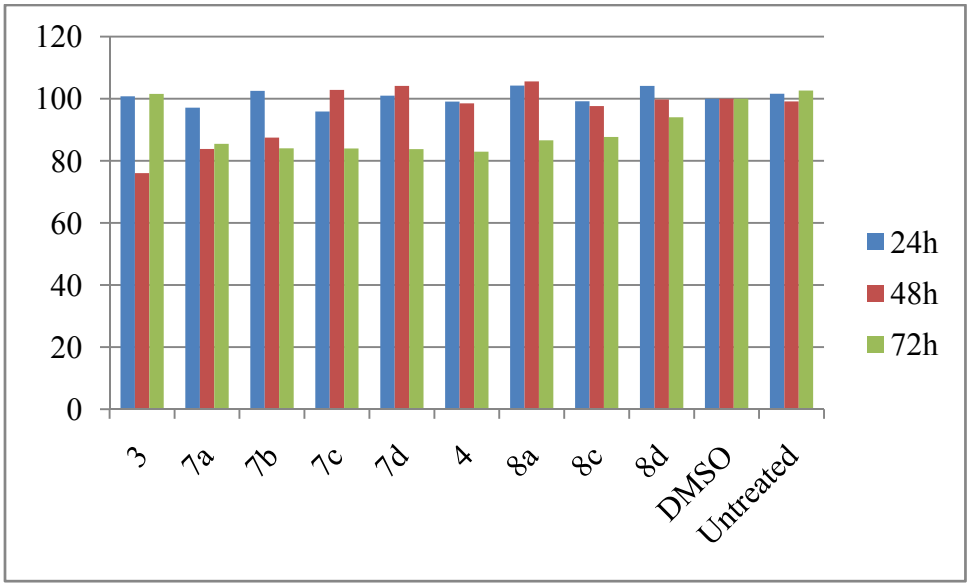
Figure 13: IC_{50} of compounds against melanoma cell lines UACC903 and A375M



Compound **3** show no significant activity till 48 h then show 69% inhibition up to 72 h against UACC903 cell line while 57% and 58% inhibition is observed after 48h and 72 h respectively against A375M cell line. Compound **7a** showed 39% and 47% inhibition after 48 h and 72 h respectively against UACC903 cell line while 192% cell growth up to 48 h against A375M cell line was observed. Compound **7b** showed 138% cell growth up to 48 h then show 45% inhibition against UACC7 cell line while 35% inhibition observed up to 48 h and then no significant change up to 72 h against A375M cell line. Compound **7c** showed 16% inhibition after 48 h then decreasing activity to 6% after 72 h against UACC703 cell line. Compound **7d** showed no significant activity up to 48 h but then show 39% inhibition up to 72 h against UACC903 cell line. Compound **4** showed 25% and 26% inhibition after 48 h and 72 h respectively against UACC903 cell line. Compound **8a** showed no significant activity up to 48 h but then show 58% inhibition after 72 h against UACC903 cell line. Compound **8c** showed 276% cell growth up to 48 h and then 83% inhibition up to 72 h against A375M cell line. Compound **8d** showed no significant activity up to 48 h then 43% inhibition observed against A375M cell line. Compound **8c** and **8d** were inactive up to 72 h against UACC903 cell line while

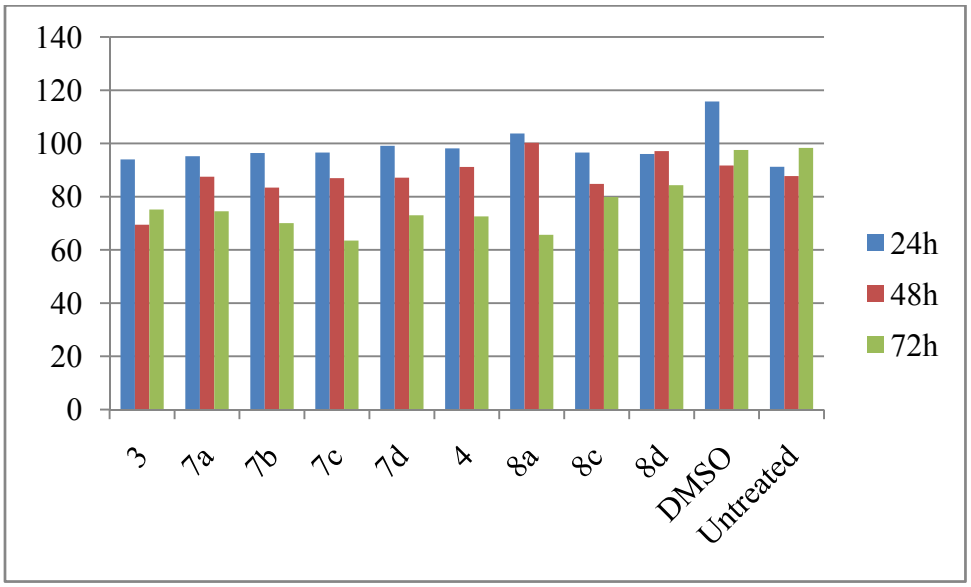
compound **7c**, **7d**, **4** and **8a** were inactive up to 72h against A375M cell line as shown in Figure 13.

Figure 14: IC_{50} of compounds against Breast Cancer cell line MCF-7



All Synthesized compounds showed moderate activity against breast cancer cell line MCF-7 in 5 µM concentration as shown in Figure 14. All compounds did not show activity up to 24 h. Compound **3**, **7a** and **7b** showed 23%, 15% and 12% inhibition up to 48 h but then no significant change in activity up to 72 h for compound **3** but compound **7a** and **7b** showed 17%, and 18% inhibition respectively. While compounds **7c**, **7d**, **4**, **8a**, **8c** and **8d** showed no significant change in activity up to 48 h but then showed 19%, 19%, 19%, 16%, 16% and 8% inhibition up to 72 h respectively.

Figure 15: IC_{50} of compounds against Fibroblast (FF2441-precursors of normal cells)



All Synthesized compounds show moderate activity against fibroblast FF2441 in 6 µM concentration as shown in Figure 15. All compounds were not showing activity up to 24 h. Compound **3** showed 18% inhibition up to 48 h and 23% inhibition up to 72 h. Compounds **7a**, **7b**, **7c**, **7d**, **4**, **8a**, **8c** and **8d** showed no significant change in activity up to 48 h but then showed 24%, 28%, 35%, 25%, 26%, 33%, 17% and 14% inhibition up to 72 h respectively.

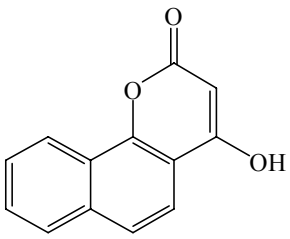
5b.3 Conclusion

All coumestan derivatives showed moderate activity. Methoxy and allyloxy coumestan derivatives were more active than methylenedioxy and ethylenedioxy coumestan derivatives. Methoxy derivatives showed better activity than allyloxy derivatives. Compound **3** showed better activity than other synthesized compounds against melanoma cancer cell lines, Breast cancer cell line and Fibroblast. Some compounds showed activity up to 48 h while some compounds were not so active till 48 h but then showed activity up to 72 h.

5b.4 Experimental

5b.4.1 Chemistry

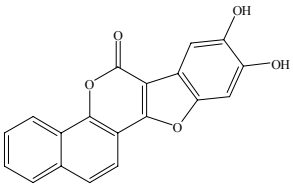
Reagent grade chemicals and solvents were purchased from commercial supplier and used without purification. TLC was performed on silica gel F254 plates (Merck). Acme's silica gel (60-120 mesh) was used for column chromatographic purification. Melting points are uncorrected and were measured in open capillary tubes, using a Rolex melting point apparatus. IR spectra were recorded as KBr pellets on Perkin Elmer RX 1 spectrometer. ^{1}H NMR and ^{13}C NMR spectral data were recorded on Bruker Advance 400 spectrometer (400 MHz) and Bruker Advance 300 spectrometer (300 MHz) with $CDCl_3$ or DMSO-d_6 as solvent and TMS as internal standard. *J* values are in Hz. CHN elemental analyses were recorded on Thermosinnigan Flash 11-12 series EA.



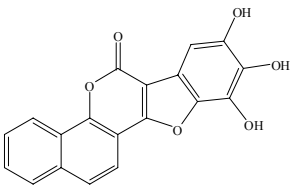
4-hydroxy-2H-benzo[h]chromen-2-one 2: A solution of 2-acetyl 1-napthtol **1** (0.00107 mol) in diethyl carbonate (30 ml) was slowly added to pulverized sodium (0.01739 mol) under anhydrous conditions. Highly exothermic reaction was observed. It was then allowed to cool to room temperature. Ethanol (50 ml) was added to decompose the unreacted sodium. The reaction mass was then poured into water (250 ml) and the aqueous layer washed twice with petroleum ether (50 ml). Concentrated hydrochloric acid was slowly added to the aqueous layer until pH 2 and the solid obtained was collected by filtration. The crude product was crystallized from ethanol to give 4-hydroxy-2H-benzo[h]chromen-2-one **2** as light yellow solid. Yield: 96%; mp 283-285°C (Lit. 284°C[24]); IR (KBr) (Figure 1): 3423, 2926, 1604, 1561 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) (Figure 2): δ 5.83 (1H, s, C-3 proton), 7.60-7.76 (3H, m, ArH), 7.86-7.94 (2H, m, ArH), 8.48-8.53 (1H, m, ArH), 11.94 (1H, s, OH); ^{13}C NMR (400 MHz, DMSO-d_6)

(Figure 3): δ 91.1, 111.6, 119.3, 122.1, 122.7, 124.1, 127.8, 128.6, 129.2, 135.3, 151.1, 162.3, 167.2; Molecular formula $C_{13}H_8O_3$.

General procedure for synthesis of 3 and 4: In a solution of **2** (3 gm, 1.415 mmol) in 1:1 water: acetone (50 ml), sodium acetate (4 gm, 4.878 mmol) and catechol or pyrogallol (1.964 mmol) were added. Reaction mixture was stirred at room temperature for 10 minutes. Mixture of KIO_3 (9 gm, 4.2056 mmol) and sodium acetate (4 gm, 4.878 mmol) in hot water (50 ml) was added slowly in the reaction flask in period of 20 minutes and stirred at room temperature for 30 minutes. Solid product separated was filtered and washed with hot water. Crude product washed with hot ethanol (3×50 ml) and hot petroleum ether (3×50 ml) and dried.



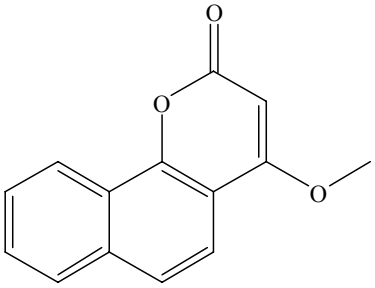
8,9-dihydroxy-6H-benzo[h]benzofuro[3,2-c]chromen-6-one 3: Yield: 89%; mp: >300°C; IR (KBr) (Figure 10): 3416, 2363, 1702, 1609, 1487, 1462, 1323, 1285, 1174, 1125, 1086, 1012, 922, 855, 811, 767, 690, 644 cm^{-1}; DSC (Figure 18) degradation at 390ºC; Mol. Formula: $C_{19}H_{10}O_5$.



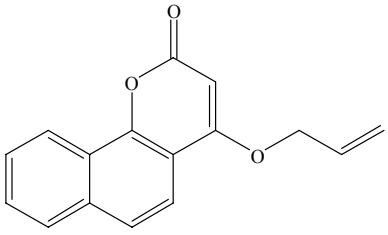
8,9,10-trihydroxy-6H-benzo[h]benzofuro[3,2-c]chromen-6-one 4: Yield: 84%; mp: >300°C; IR (KBr) (Figure 15): 3843, 3008, 1734, 1583, 1432, 1090, 990, 769 cm^{-1}; DSC (Figure 21) degradation at 342ºC; Mol. Formula: $C_{19}H_{10}O_6$.

General Procedure for synthesis of 5a-b, 6a-b and 7a-b: 2, 3 and **4 (**1 gm, 1 eq.) dissolved in 20 ml dry acetone. Freshly fused K_2CO_3 (3.5 eq.) and dimethyl sulphate or allyl bromide was added (1.1 eq.) in a reaction flask and it was refluxed for 10 hours. Reaction mass was poured in ice-water, solid crude product obtained was filtered and washed with water. Crude product was washed with hot ethanol (3×50 ml) and hot

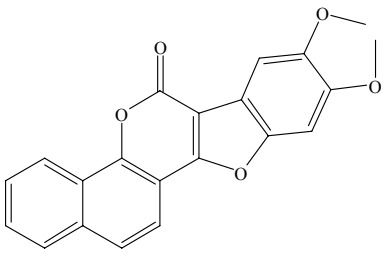
petroleum ether (3×50 ml) and dried. Compound **5a-b** was purified by column chromatography using 5% Ethyl acetate/ petroleum ether.



4-methoxy-2H-benzo[h]chromen-2-one 5a: Yield: 10%; mp: 154-156°C; IR (KBr) (Figure 4): 3078, 2916, 2846, 1734, 1699, 1653, 1608, 1548, 1481, 1446, 1392, 1247, 1230, 1182, 1091, 1051, 1028, 937, 840, 815, 746 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) (Figure 5): δ 4.07 (3H, s, $-OCH_3$), 5.81 (1H, s, C-3 proton), 7.64-7.72 (3H, m, ArH), 7.82-7.91 (2H, m, ArH), 8.52-8.6 (1H, m, ArH); ^{13}C NMR (400 MHz, $CDCl_3$) (Figure 6): δ 56.5, 89.6, 110.9, 118.5, 122.7, 122.9, 123.9, 127.1, 127.8, 128.8, 135.2, 150.7, 163.1, 167.4; Ele. Anal. Calcd. for $C_{14}H_{10}O_3$; Requires (Found) %: C, 74.33 (74.46); H, 4.46 (4.18).

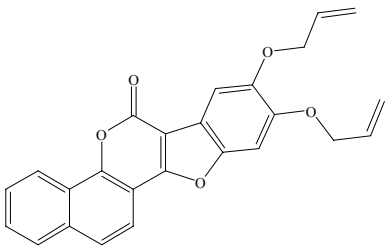


4-allyloxy-2H-benzo[h]chromen-2-one 5b: Yield: 9%; mp: 200-205°C; IR (KBr) (Figure 7): 3092, 3022, 1726, 1481, 1255, 1228, 1182, 1147, 1087, 1026, 999, 956, 914, 846, 815, 785, 754 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) (Figure 8): δ 4.73 (2H, dd, $-OCH_2$), 5.45-5.48 (1H, dd, vinyl proton), 5.53- 5.58 (1H, dd, vinyl proton), 5.79 (1H, s, C-3 proton), 6.10- 6.17 (1H, m, vinyl proton), 7.64-7.70 (3H, m, ArH), 7.83-7.89 (2H, m, ArH), 8.55-8.57 (1H, m, ArH); ^{13}C NMR (400 MHz, $CDCl_3$) (Figure 9): 69.9, 90.5, 110.9, 118.6, 119.7, 122.7, 122.9, 123.9, 127.1, 127.8, 128.8, 130.7, 135.2, 150.8, 163.0, 166.2; Ele. Anal. Calcd. for $C_{16}H_{12}O_3$; Requires (Found) %: C, 76.18 (76.43); H, 4.79 (4.53).

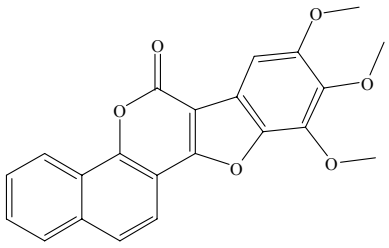


8,9-dimethoxy-6H-benzo[h]benzofuro[3,2-c]chromen-6-one 6a: Yield: 22%; mp: 259-261°C; IR (KBr) (Figure 11): 3066, 2935, 2358, 1731, 1597, 1437, 1356, 1296, 1079, 998, 802, 763 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6)

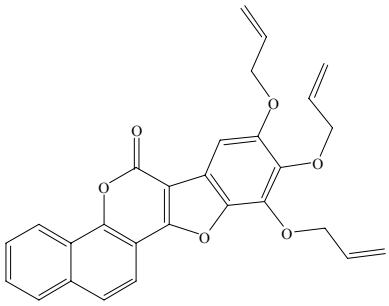
(Figure 12): δ 3.92-3.93 (6H, d, CH_3), 7.31 (1H, s, ArH), 7.44 (1H, s, ArH), 7.63-7.66 (2H, m, ArH), 7.77-7.82 (1H, m, ArH), 7.91-7.94 (2H, m, ArH), 8.49-8.51 (1H, d, ArH); TG/DTA (Figure 19) 264ºC (mp), degradation at 328ºC; Ele. Anal. Calcd. for $C_{21}H_{14}O_5$; Requires (Found) %: C, 72.83 (72.57); H, 4.07 (3.82).



8,9-bis(allyloxy)-6H-benzo[h]benzofuro[3,2-c] chromen-6-one 6b: Yield: 44%; mp: >300°C; IR (KBr) (Figure 13): 3020, 2849, 1726, 1612, 1494, 1451, 1407, 1352, 1275, 1257, 1225, 1198, 1147, 1109, 1078, 1004, 918, 842, 811, 765, 745 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) (Figure 14): δ 4.64- 4.65 (4H, d, -OCH_2 protons), 5.25- 5.30 (2H, dd, vinyl protons), 5.42- 5.47 (2H, dd, vinyl protons), 6.03- 6.11 (2H, m, vinyl protons), 7.30 (1H, s, ArH), 7.45 (1H, s, ArH), 7.59-7.66 (2H, m, ArH), 7.78-7.81 (1H, d, ArH), 7.90-7.92 (2H, m, ArH), 8.48-8.50 (1H, d, ArH); TG/DTA (Figure 20) degradation at 331ºC; Ele. Anal. Calcd. for $C_{25}H_{18}O_5$; Requires (Found) %: C, 75.37 (75.76); H, 4.55 (3.72).

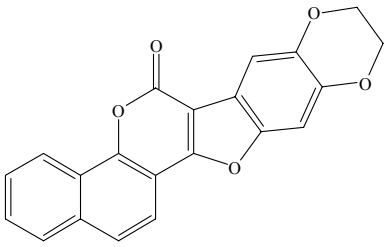


8,9,10-trimethoxy-6H-benzo[h]benzofuro[3,2-c] chromen-6-one 7a: Yield: 41%; mp: >300°C; IR (KBr) (Figure 16): 3210, 3075, 2946, 2924, 2851, 2153, 1733, 1684, 1615, 1506, 1439, 1379, 1261, 1093, 1011, 800, 770 cm^{-1}; DSC (Figure 22) degradation at 331ºC; Mol. Formula: $C_{22}H_{16}O_6$.

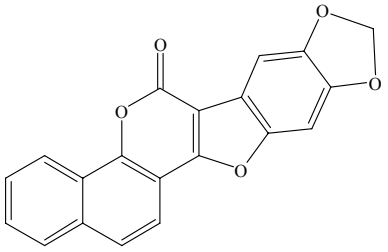


8,9,10-tris(allyloxy)-6H-benzo[h]benzofuro[3,2-c] chromen-6-one 7b: Yield: 32%; mp: >300°C; IR (KBr): 2918, 2851, 1675, 1625, 1381, 1118, 864 cm^{-1}; TG/DTA degradation at 289ºC; Mol. Formula: $C_{28}H_{22}O_6$.

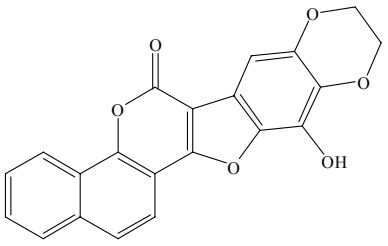
General Procedure for synthesis of 6c-d and 7c-d: Compound **3** or **4 (**1 gm, 1 eq.) dissolved in 20 ml dry acetone in a round bottom flask. Freshly fused K_2CO_3 (3.5 eq.) and di substituted bromo alkane were added (0.6 eq.) in a reaction flask and it was refluxed for 10 hours. Reaction mass was poured in ice-water, solid crude product obtained was filtered and washed with water. Crude product was refluxed with hot ethanol (3×50 ml) and hot petroleum ether (3×50 ml), filtered and dried.



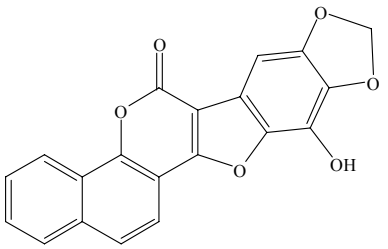
8,9-ethylenedioxy-6H-benzo[h]benzofuro[3,2-c] chromen-6-one 6c: Yield: 54%; mp: >300°C; IR (KBr): 1717, 1608, 1541, 1493, 1359, 1255, 1180, 1110, 1030, 763 cm^{-1}; DSC degradation at 368ºC; Mol. Formula: $C_{21}H_{12}O_5$.



8,9-methylenedioxy-6H-benzo[h]benzofuro[3,2-c] chromen-6-one 6d: Yield: 27%; mp: >300°C; IR (KBr): 1731, 1636, 1546, 1490, 1362, 1278, 1085, 1001, 811, 769 cm^{-1}; DSC degradation at 382ºC; Mol. Formula: $C_{20}H_{10}O_5$.



8,9-ethylenedioxy-10-hydroxy-6H-benzo[h]benzofuro [3,2-c]chromen-6-one 7c: Yield: 37%; mp: 177-186°C; IR (KBr) (Figure 17): 3624, 2039, 1720, 1622, 1552, 1530, 1390, 1241, 1090, 1050, 769 cm^{-1}; TG/DTA (Figure 23) degradation at 177-186ºC; Mol. Formula: $C_{21}H_{12}O_6$.



8,9-methylenedioxy-10-hydroxy-6H-benzo[h] benzofuro[3,2-c]chromen-6-one 7d: Yield: 53%; mp: >300°C; IR (KBr): 3473, 1731, 1622, 1496, 1376, 1247, 1120, 970, 920, 830, 763 cm^{-1}; TG/DTA degradation at

198-213ºC; Mol. Formula: $C_{20}H_{10}O_6$.

5b.5 References

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List of research paper published

1. Novel deacetylation of 1-acetyl 2-naphthol in facile manner published in Indian Journal of Heterocyclic Chemistry, 2013, 22, 377-382
2. Synthesis of amide and ester derivatives of Naphthopyrone carboxylic acid accepted in Journal of Heterocyclic Chemistry, dated 30th Sep 2013, DOI: 10.1002/jhet.2136
3. Synthesis and anticancer activity of 4-hydroxy Naphtho coumarin derivatives and naphtho coumestans published in Der Pharma Chemica, 2013, 5(6), 201-207

List of research papers in communication

1. Synthesis of New Naphthoisoxazole Derivatives and their Biological evaluations
2. Synthesis and antimicrobial evaluation of amide derivatives of Benzodifuran-2-carboxylic acid
3. Novel Retero Knovenagel reaction of Substituted Coumarin-3-carboxylate

Papers Presented at National and International Conferences

1. Modern Trends in Chemistry 2013, Department of Chemistry, Faculty of Science, The M. S. University of Baroda, 22-23rd February 2013

   Poster Presentation: Synthesis of various amides of difuran 2-carboxylic acid
2. International Conference on Emerging Trends in Chemical Sciences, School of Chemical Sciences, Central University of Gujarat, Gandhinagar, 14-15th March 2013

   Oral Presentation: Synthesis of amide and ester derivatives of Naphthopyrone 2-carboxylic acid
3. 1st International Science congress by International Science Congress Association, Maharaja Ranjitsingh College of professional Sciences, Indore 24-25th December 2011

   Oral presentation: Synthesis of acid derivatives of naphthopyrones

4. 47th Annual Convention of Chemist 2010 and International Conference on Recent advances in Chemical Sciences, Indian Chemical Society, Pandit RaviShankar Shukla University, Raipur, Chhattisgarh, 23 -27th December 2010

   Oral Presentation: Synthesis of some Coumestan derivatives

5. 6th International Symposium on Innovations in Pharmaceutical Sciences and Technology, B. V. Patel PERD Centre, Ahmedabad, Gujarat, 26-28th November, 2010.

   Poster Presentation: Studies in the Synthesis and Biological activity of Isoxazole derivatives

6. 6th All Gujarat Research Scholar Meet conducted by Indian Chemical Society, Vadodara Chapter, Department of Chemistry, The M. S. University of Baroda, 31st January 2010

   Oral Presentation: Synthesis of Naphthoisoxazoles

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NOVEL DEACETYLATION OF 1-ACETYL 2-NAPHTHOL IN FACILE MANNER

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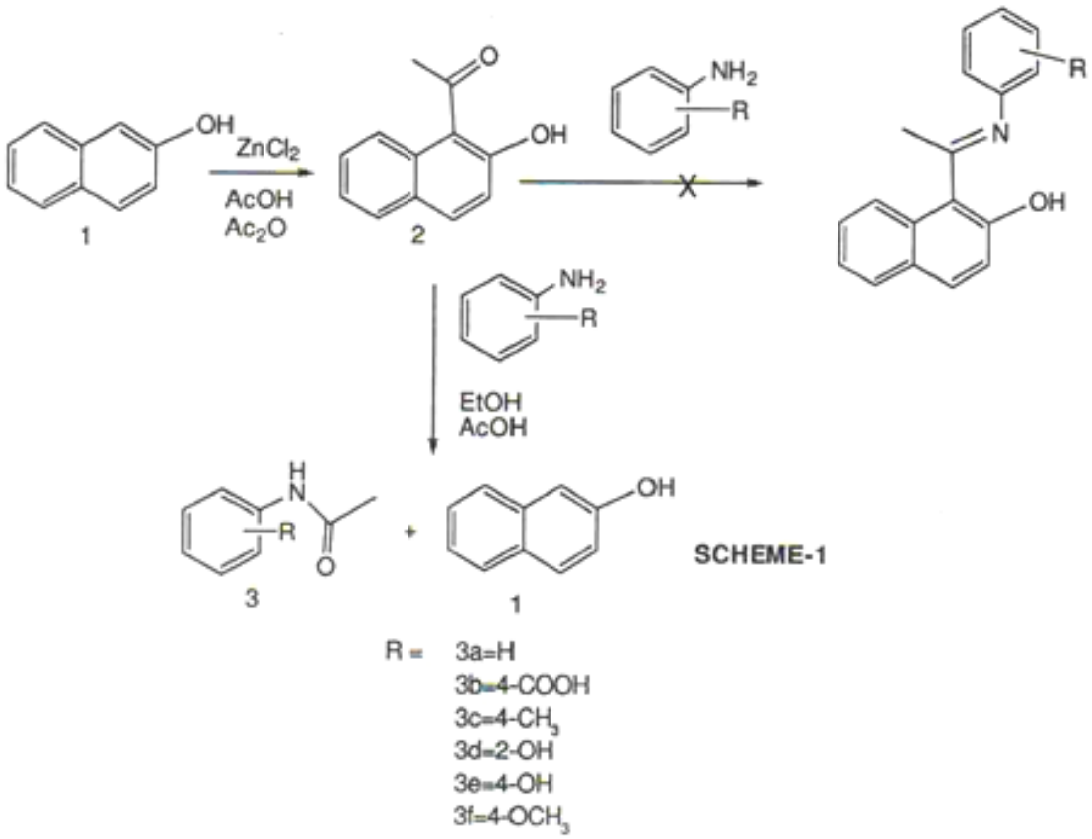
We have observed a novel deacylation reaction during the attempted Schiff base reaction of 1-acetyl 2-naphthol **2** with various primary aromatic amines in presence of acetic acid in absolute ethanol. The product formation was confirmed by spectral analyses and comparison with reported melting point. The mechanism is proposed to explain its formation.

Formation of chalcones[1,2] or Schiff bases[3-6] from *o*-hydroxy ketones is one of the most frequently used reaction in organic synthesis because various heterocyclic rings can be formed further from it. Chalcones, chromenes, imidazoles and other heterocyclic rings show various biological activities[7-10].

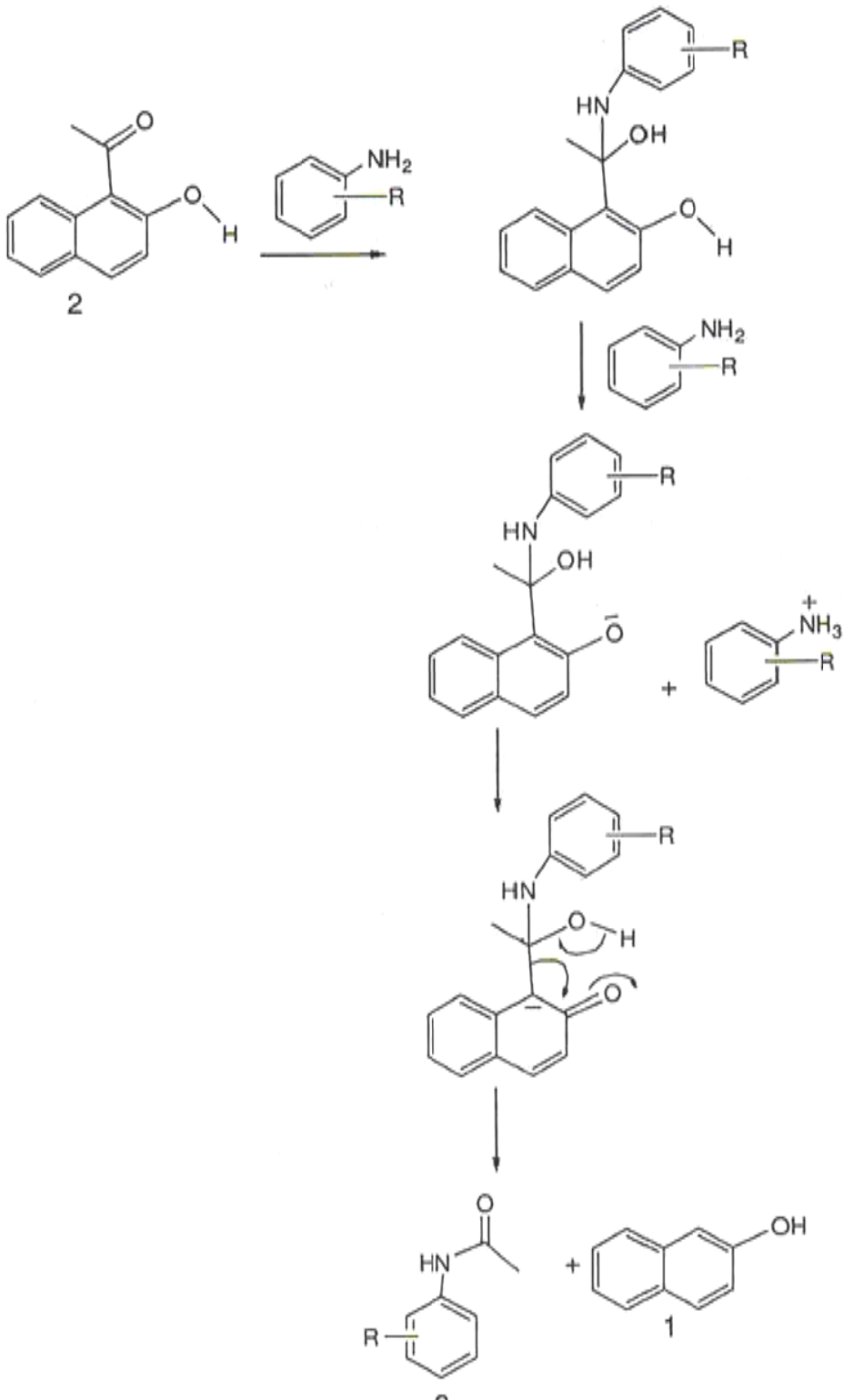
We have already reported formation of chalcones[11] from various substituted *o*-hydroxy acetophenone derivatives[12] and further formation of flavones[11] from it.



SCHEME-1

R = 3a=H
3b=4-COOH
3c=4-CH_3
3d=2-OH
3e=4-OH
3f=4-OCH_3

This paper is dedicated to Prof. S.K. Paknikar, retired Professor and Head of Goa University, Goa.



SCHEME-2

Hence we were keen to form Schiff bases from 1-acetyl 2-naphthol **2** and then further formation of heterocyclic ring. Here we report novel retero Friedel Crafts reaction of 1-acetyl 2-naphthol **2** when we have tried to prepare Schiff bases from 1-acetyl 2-naphthol **2**.

When 1-acetyl 2-naphthol **2** was refluxed in absolute ethanol with primary aromatic amines and 2-3 drops of acetic acid, we observed formation of two products on TLC, different from starting material. The products were separated by column chromatography and were found to be N-acetylated anilines **3a**-f and 2-naphthol **1** (Scheme-1).

We carried out reaction with different primary aromatic amines with electron releasing and electron withdrawing groups and in all cases with electron releasing groups on aniline, the formation of N-acetylated product was observed. When electron withdrawing nitro group was present, the reaction doesn't take place, while with carboxylic acid group on aniline the reaction was observed with less yield. When methoxy group was there on aniline the yield was very poor.

Since with primary aromatic amines, N-acetylated aniline product formation was observed, we have extended the reaction with secondary and tertiary amines. When the reaction was carried out with cyclic saturated secondary amines e.g. pyrrolidine and morpholine, 2-naphthol was obtained as major product and small amount of secondary amine was obtained back while in case of aromatic secondary amines e.g. indole and benzotriazole, the reaction doesn't take place and starting material **2** was recovered. The formation of 2-naphthol was more than 50% in all cases as expected from molar ratios.

There were several other and easy methods available for formation of N-acetylated products of aromatic amines[13-16]. Though our purpose was not to form N-acetylated amines, this is found to be new method for formation of N-acetylated amines.

The proposed mechanism for formation of N-acetylated product can be explained by the fact that aromatic amines reacts with acetyl group and then instead of dehydration, since hydroxyl group is there at ortho position of acetyl group, it participates in the reaction and due to which retero Friedel Crafts reaction has occurred. One can call it as reverse Claisen rearrangement. In case of cyclic secondary amines, retero Friedel Crafts reaction occur and then acetyl group is removed as acetic acid from N-acetyl pyrrolidine or N-acetyl morpholine.

The involvement of hydroxyl group at ortho position of acetyl group in this reaction is supported by the fact that when reaction was carried out with 3-acetyl naphthopyrone where no hydroxyl group was there at ortho position of acetyl group, under the same conditions, the starting material remained as such i.e. retero Friedel Crafts reaction has not been observed, also Schiff base formation was not observed. In case of ortho hydroxyl aniline the product obtained was highly stabilized by hydrogen bonding[17] hence the reaction yield is good (Scheme-2).

Reaction of acetic anhydride with 2-naphthol **1** in acetic acid in presence of zinc chloride gave 1-acetyl 2-naphthol[18] **2**, which was further refluxed with different amines in absolute ethanol using 2-3 drops of acetic acid as catalyst to get respective anilide products **3a**-f. In IR of **2** band at 1755 cm^{-1} confirmed presence of acetyl group. In IR of **3a** bands at 3293 and 1600 and in ^1H NMR singlet at 2.17 for methyl protons and multiplet between δ 7.08 to 7.51 for five aromatic protons ccnfirmed formation of **3a**. All compounds obtained from primary aromatic amines show similar results as described and their structures were confirmed by melting points and mix melting points, IR, ^1H NMR, ^{13}C NMR and CHN elemental analyses.

Experimental

Melting points (uncorrected) were determined using a scientific capillary melting point apparatus. Reaction was monitored by Merck TLC Silica gel 60F 254 mesh using UV/iodine vapour as a visualizing agent and Acme's silica gel (60-120 mesh) was used for column chromatographic purification. IR spectra were recorded on a Perkin-Elmer FT-IR spectrometer (spectrum RXI) using potassium bromide optics. NMR spectra were recorded on a Bruker 400 MHz spectrophotometer in $(CD_3)_2SO$ (compounds **3b**, **3e**) or $CDCl_3$ (compounds **2**, **3a**, **3c**, **3d**, **3f**, **4**). Chemical shifts are relative to tetramethylsilane; relative peak areas were in agreement with all assignments. CHN elemental analyses were recorded on Thermosinnigan Flash 11-12 series EA.

1-(2-Hydroxynaphthalen-1-yl) ethanone (2)

Dry zinc chloride (20g, 0.1467 mol) was dissolved in gl acetic acid (30 ml) and 2-naphthol (**1**) (10g, 0.0694 mol) was added portionwise. Acetic anhyd (15 ml, 0.1588 mol) was added in the reaction flask. Reaction mass was heated at 100° for 30 minutes. It was poured in ice-HCl to get solid. Crude product was purified by column chromatography using Pet. Ether (60-80°) as eluent. White solid: yield 82%, m.p. 63-65° (lit. 63-65°C[18]); IR (KBr): 3431 (OH), 2926 (CH stretching), 1755 (CO); ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 3.03 (s, 3H, CH_3), 7.41-7.43 (d, J=9.6 Hz, 1H, ArH), 7.55-7.59 (t, J=7.28,. 7.48 Hz, 1H ArH), 7.64-7.68 (t, J=7.32, 7.6 Hz, 1H, ArH), 7.91-7.93 (d, J=7.92 Hz, 1H, ArH), 7.97-7.99 (d, J=8.92 Hz, 1H, ArH), 8.42-8.44 (d, J=8.68 Hz, 1H, ArH). [Found : C, 77.58, H, 5.35 $C_{12}H_{10}O_2$ requires C, 77.40, H, 5.41%].

Synthesis of anilide derivatives 3a-f : General procedure

1-Acetyl 2-naphthol (**2**) (5g, 0.02685 mol) was dissolved in 10 ml absolute ethanol. Primary aromatic amines (0.0268 mol) and glacial acetic acid (2-3 drops) were added in the flask and refluxed for 5-6 hr. Reaction completion was judged by TLC. Reaction mass was poured into ice: water (1:1) to get solid. Compound was purified by column chromatography using ethyl acetate : pet. ether (60-80°) 9:1 as eluent.

N-Phenylacetamide (3a)

Yield 37%, m.p. 115-117° (Lit 113-115°[19]); IR (KBr, cm^{-1}): 3293 (NH), 1600 (CO); ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 2.17 (s, 3H, CH_3), 7.08-7.12 (1H, t, J=7.32, 7.36 Hz, ArH), 7.26-7.33 (q, 2H, ArH), 7.49-7.51 (d, J=7.92 Hz, 2H, ArH); ^{13}C NMR (400, $CDCl_3$): 24.5, 120.0, 124.4, 129.0, 137.9, 168.7. [Found : C, 71.26, H, 6.53, N, 10.49 C_8H_9NO requires C, 71.09, H, 6.71, N, 10.36%].

4-Acetamidobenzoic acid (3b)

Yield 37%, m.p. >250° (Lit 259-261°[19]); IR (KBr): 3306 (NH), 2828 (CH stretching), 1681 (CO); ^{1}H NMR (400 MHz, DMSO-d_6): 2.13 (s, 3H, CH_3), 7.73-7.75 (d, J=8.64 Hz, 2H, ArH), 7.92-7.94 (d, J=8.64 Hz, 2H, ArH), 10.30 (s, 1H, NH), 12.74 (s, 1H, COOH); NMR (400 MHz, DMSO-d_6): 24.6, 118.6, 125.3, 130.8, 143.8, 167.4, 169.3. [Found : C, 60.46, H, 5.18, N, 7.64 $C_9H_9NO_3$ requires C, 60.33, H, 5.06, N, 7.82%].

N-*p*-Tolylacetamide (3c):

Yield 47%; m.p. 149-151° (Lit. 149-151°[19]); IR (KBr): 3302 (NH), 3193, 3128 (CH stretching), 1666 (CO); ^{1}H NMR (400 MHz, $CDCl_3$): 2.16 (s, 3H, CH_3), 2.31 (s, 3H, CH_3), 7.11-7.12 (d, J=8.04 Hz, 2H, ArH), 7.26 (s, 1H, NH), 7.36-7.38 (d, J=8.16 Hz, 2H, ArH); ^{13}C NMR (400 MHz, $CDCl_3$): 20.9, 24.4, 120.2, 129.4, 134.0, 135.3, 168.6. [Found : C, 72.31, H, 7.57, N, 9.25 $C_9H_{11}NO$ requires C, 72.46, H, 7.43, N, 9.39%].

N-(2-Hydroxyphenyl) acetamide (3d):

Yield 42%, m.p. 229-231° (Lit. 205-210[19]); IR (KBr): 3403 (NH), 3084 (CH stretching), 1658 (C=O); ^{1}H NMR (400 MHz, $CDCl_3$): 2.13 (s, 3H, CH_3), 6.85-6.89 (t, J=6.72, 1.28 Hz, 1H, ArH), 6.96-7.04 (dd, 2H,

ArH), 7.12-7.18 (t, J=7, 8.4 Hz, 1H, ArH), 7.38-7.47 (s, 1H, NH), 8.66 (s, 1H, OH); ^{13}C NMR (400 MHz, $CDCl_3$): 23.8, 120.1, 120.5, 122.1, 127.4, 170.5. [Found : C, 63.69, H, 5.84, N, 9.42 $C_8H_9NO_2$ requires C, 63.56, H, 6.00, N, 9.27%].

N-(4-Hydroxyphenyl) acetamide (3e)

Yield 29%; m.p. 165-170^0 (Lit. 168-172^{019}); IR (KBr): 3326 (NH), 3161 (OH), 1657 (CO); ^{1}H NMR (400 MHz, DMSO-d_6): 1.98 (s, 3H, CH_3), 6.66-6.68 (q, 2H, ArH), 7.32-7.34 (q, 2H, ArH), 9.56 (s, 1H, NH), 9.65 (s, 1H, OH); ^{13}C NMR (400 MHz, DMSO-d_6): 24.2, 115.4, 121.3, 131.5, 153.6, 168.0. [Found : C, 63.72, H, 5.88, N, 9.38 $C_8H_9NO_2$ requires C, 63.56, H, 6.00, N, 9.27%].

N-(4-Methoxyphenyl) acetamide (3f)

Yield 9%; m.p. 139-142^0 (Lit 129-132^{019}); IR (KBr): 3191 (NH), 3130, 3071 (CH stretching), 1650 (CO); ^{1}H NMR (400 MHz, $CDCl_3$): 2.17 (s, 3H, CH_3), 3.79 (s, 3H, OCH_3), 6.85-6.87 (d, J=8 Hz, 2H, ArH), 7.40-7.42 (d, J=8Hz, 2H, ArH), 7.53 (s, 1H, NH); ^{13}C NMR (400 MHz, $CDCl_3$): 24.4, 55.5, 114.1, 122.0, 130.9, 156.5, 168.5. [Found : C, 65.59, H, 6.57, N, 8.38 $C_9H_{11}NO_2$ requires C, 65.44, H, 6.71, N, 8.48%].

2-Naphthol (1)

Yield > 50% in all cases ; m.p. 120-124^0 (Lit. 121-125^{019}); IR (KBr): 3254 (OH), 2924 (CH stretching); ^{1}H NMR (400 MHz, $CDCl_3$): 5.04 (s, 1H, OH), 7.08-7.11 (dd, 1H, ArH), 7.14-7.15 (d, J=2.44 Hz, 1H, ArH), 7.30-7.34 (m, 1H, ArH), 7.41-7.45 (m, 1H, ArH), 7.62-7.69 (d, J=8.2 Hz, 1H, ArH), 7.74-7.78 (t, J=6.6, 8.4 Hz, 2H, ArH). [Found : C, 83.54, H, 5.49 $C_{10}H_8O$ requires C, 83.31, H, 5.59%].

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Synthesis of Amide and Ester Derivatives of Naphthopyrone Carboxylic Acid

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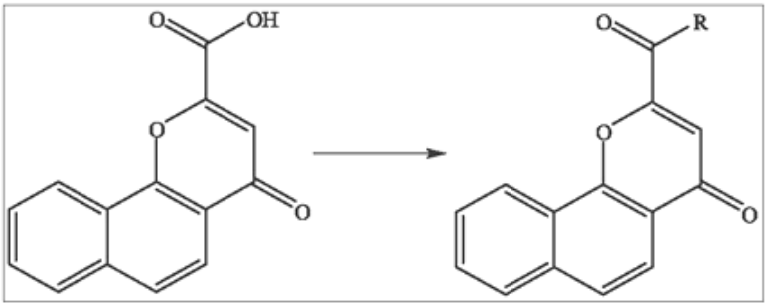

The synthesis of various amide and ester derivatives of naphthopyrone-2-carboxylic acid has been carried out by reaction of 1-naphthol with dimethyl acetylenedicarboxylate, which gave a mixture of *E* and *Z* isomers of naphthoxy diester. The diester on hydrolysis with KOH gave corresponding diacid, which was a mixture of *E* and *Z* isomers. The *E* and *Z* isomers were difficult to separate, which were subjected to cyclization in sulfuric acid to get cyclized naphthopyrone carboxylic acid. This acid is converted into titled compounds.

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INTRODUCTION

Chromones (benzopyran-4-ones) are one of the most abundant groups of naturally occurring heterocyclic compounds [1]. Chromones have remarkable biological properties such as antituberculosis [2,3]. Chromone scaffold has been recognized as a pharmacophore of a number of bioactive molecules. Disodium cromoglycate, the sodium salt of cromoglycic acid, is a clinically useful antiallergic agent, particularly for bronchial asthma, and reported to inhibit the release of mediators such as histamine, several kinins, and so on, of immediate hypersensitivity reactions [4], which contain a chromone ring. Heterocyclic analogues of ritonavir, HIV-1 protease inhibitor are amide derivatives of chromone-2-carboxylic acid [5]. Chromone-3-carboxamide derivatives have been reported as monoamine oxidase-B (MAO-B) inhibitors [6,7], whereas chromone-2-carboxylic acid derivatives are reported as melanin-concentrating hormone receptor 1 antagonists [8] or adenosine receptor ligands [9]. Photodimerization [10] reactions of chromone-2-carboxylic esters and microwave-assisted synthesis of various amide derivatives of chromone-2-carboxylic acids [11] have been reported. Vercauteren *et al.* [12] have reported the use of activated alkynes for condensing it with tryptamine Pictel–Spengler synthesis of tertahydro-β-carbolines, which involves acid-catalyzed ring closure.

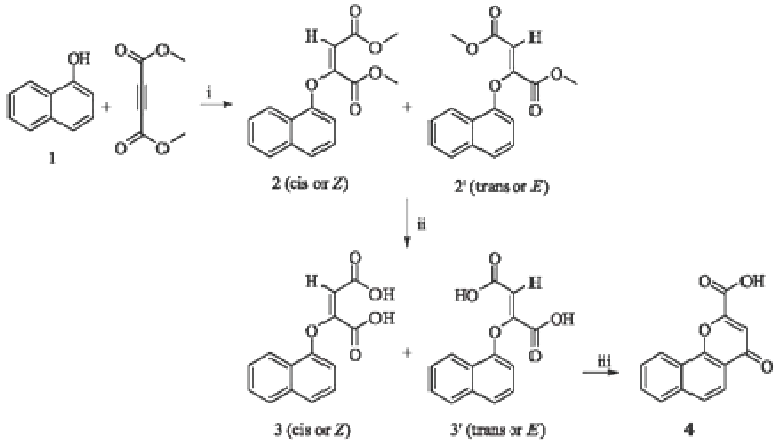
RESULTS AND DISCUSSION

In view of these findings, we have synthesized various amide and ester derivatives of naphthopyrone-2-carboxylic acid, expecting to show monoamine oxidase inhibition activity and antiallergic activity. Synthesis of chromone-2-carboxylic acid was reported from our laboratory earlier [13]. No one has reported angular amide and ester derivatives of naphthopyrone-2-carboxylic acid. Present investigation reports synthesis of a new class of various amides using two different methods and ester derivatives of naphthopyrone-2-carboxylic acid.

Condensation [13] of 1-naphthol **1** with dimethyl acetylenedicarboxylate (DMAD) in the presence of anhydrous K_2CO_3 and dry acetone gave *Z* or cis and *E* or trans mixture of dimethyl 2-(naphthalen-1-yloxy)maleate **2**. It was not possible to separate the *Z* and *E* mixture either by column chromatography or by TLC method (Scheme 1). Earlier, S. S. Soman [13] and J. K. Lynch [8] could not separate the *Z* and *E* isomer of adducts obtained by the condensation of DMAD with coumarin and phenol, respectively. The IR spectrum of **2/2′** exhibited band at 1735 cm^{-1} for ester group. In the ^{1}H-NMR spectrum of **2/2′**, four singlets at δ 3.65, 3.68, 3.70, and 4.03 for methoxy group clearly indicated that it is a *Z* and *E* mixture. The singlet at δ 5.01 for one proton indicated *Z* or cis vinylic proton, whereas the singlet at δ 6.72 indicated *E* or trans vinylic proton. All aromatic protons appeared between δ 6.75 and 8.34 confirmed the formation of **2** as cis–trans mixture. The ratio of *E* and *Z* isomer of **2** was found to be 57:43 from ^{1}H-NMR.

Mild hydrolysis of **2/2′** in aqueous KOH (1%) at room temperature gave a mixture of *Z* and *E* or cis and trans 2-(naphthalen-1-yloxy)maleic acid **3/3′**, which also could not be separated into *Z* and *E* isomer. The IR spectrum of

Scheme 1. Condition: (i) 1-Naphthol, Dimethylacetylenedicarboxylate, K_2CO_3, dry acetone, reflux, 8 h (ii) 1% aqueous KOH, RT, 12 h (iii) H_2SO_4, 60–70°C, 2 h.



3/3′ showed bands at 1713 cm^{-1} and broad band at 2500–3053 cm^{-1} for > C=O and –OH of carboxylic acid. Now, the absence of singlet for methoxy group in ^{1}H-NMR spectrum of **3/3′** confirmed the hydrolysis of esters. The presence of singlet for one proton each at δ 4.95 and 6.66 indicated cis and trans vinylic protons confirming it as *Z* and *E* mixture. Compound **3/3′**on cyclization with concentrated sulfuric acid gave 4-oxo-4H-benzo[h]chromene-2-carboxylic acid **4.** The IR spectrum of **4** showed bands at 1725 cm^{-1}, and broad band from 2500–3500 cm^{-1} indicated presence of carboxylic acid group. The other sharp band at 1641 cm^{-1} indicated presence of carbonyl group of chromone. In ^{1}H-NMR spectrum of **4** in DMSO-d_6, the disappearance of two singlets at δ 4.95 and 6.66 and the appearance of one singlet at δ 7.07 indicated aromatic proton at C-3, thus confirmed the cyclization. The multiplet from δ 7.81 to 8.53 for six protons indicated remaining aromatic protons (Scheme 1).

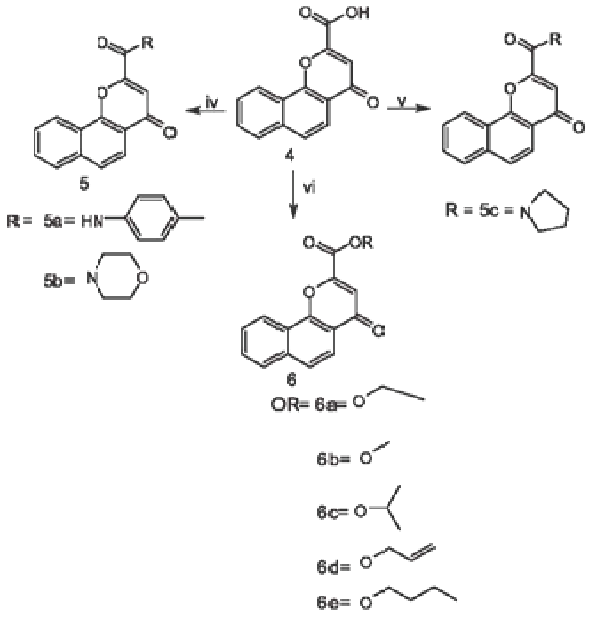
This acid **4** is converted into corresponding amide **5** using two different methods [14]. Formation of amide **5a–b** using 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC.HCl), 4, 4′-dimethylaminopyridine (DMAP) and amine gave very low yield, hence, we have used oxalylchloride and prepared acid chloride of **4** and then its reaction with pyrolidine gave **5c** (Scheme 2).

We have synthesized various esters [15] **6a–e** of naphthopyrone-2-carboxylic acid by using various alcohols and passing dry HCl gas as shown in Scheme 2. The structures of all compounds were confirmed by their IR, ^{1}H-NMR, ^{13}C-NMR, mass spectra, and elemental analyses.

CONCLUSION

We have synthesized naphthopyrone-2-carboxylic acid from corresponding naphthyloxy diacid. The diester **2** obtained by reaction of 1-naphthol with DMAD was found to be inseparable cis and trans mixture. From ^{1}H-NMR, the trans isomer was found to be a major product than cis isomer. The corresponding diacid **3** obtained by hydrolysis of diester **2** with aqueous KOH was found to be cis and trans mixture. Here, also trans isomer was found to be a major product. Hence, the yield of naphthopyrone-2-carboxylic acid was found to be less. The cyclized naphthopyrone-2-carboxylic acid was converted into corresponding amides using EDC.HCl. Because the yields were very

Scheme 2. Condition: (iv) EDC.HCl, DMAP, Triethylamine, Amine, dry THF, RT, 8 h (v) Oxalyl chloride, DMF, CH_2Cl_2, 2 h (vi) Amine, TEA, CH_2Cl_2, 8 h (vii) Alcohol, Dry HCl gas, 2 h.



low, the naphthopyrone-2-carboxylic acid was converted into the corresponding acid chloride and then treated with amine to get the corresponding amide. Naphthopyrone-2-carboxylates were prepared by using various alcohols and passing dry HCl gas.

EXPERIMENTAL

Reagent grade chemicals and solvents were purchased from a commercial supplier and used without purification. TLC was performed on silica gel F254 plates (Merck, India). Acme's (India) silica gel (60–120 mesh) was used for column chromatographic purification. Melting points are uncorrected and were measured in open capillary tubes, using a Rolex melting point apparatus (India). IR spectra were recorded as KBr pellets on PerkinElmer RX 1 spectrometer. ^{1}H-NMR and ^{13}C-NMR spectral data were recorded on Bruker Advance 400 spectrometer (400 MHz) (India) with TMS as internal standard. *J* values are in hertz. Elemental analyses were recorded on Thermosinnigan Flash 11-12 series EA. Mass spectra were determined by ESI/MS, using a Shimadzu LCMS 2020 apparatus (India).

Dimethyl 2-(naphthalen-1-yloxy)maleate (2/2′). To the solution of 1-naphthol **1** (5 g, 3.47 mmol) in dry acetone (50 mL), anhydrous potassium carbonate (16.77 g, 12.13 mmol), a solution of dimethyl acetylenedicarboxylate (4.26 mL, 3.47 mmol) in dry acetone (100 mL), was added very slowly. The reaction mixture was refluxed for 8 h in water bath. The reaction mass was poured in crushed ice and extracted with ethyl acetate, which gave a viscous liquid after the removal of ethyl acetate, which was purified by column chromatography using 5% ethyl acetate in petroleum ether, which gave a brown liquid. (7.2 g, 72.5%); IR (KBr): υ 3053, 2880, 1735 (>C=O) cm^{-1};^{1}H-NMR (400 MHz, $CDCl_3$): δ 3.65 (3H, s), 3.68 (3H, s), 3.70 (3H, s), 4.03 (3H, s), 5.01 (1H, s, cis vinylic proton), 6.72 (1H, s, trans vinylic proton), 6.75–6.77 (1H, dd, *J* = 8.0 Hz, Ar H), 7.26–7.29 (1H, m, Ar H), 7.31–7.35 (1H, t, *J* = 7.6, 8.4 Hz, Ar H), 7.47–7.51 (1H, t, *J* = 7.6, 8.4 Hz, Ar H), 7.55–7.60 (5H, m, Ar H), 7.79–7.81 (1H, d, *J* = 8.4 Hz, Ar H), 7.84–7.87 (1H, m, Ar H), 7.91–7.93 (1H, m, Ar H), 8.00–8.04 (1H, m, Ar H), 8.34–8.36 (1H, m, Ar H).

2-(Naphthalen-1-yloxy)maleic acid (3/3′). The adduct **2/2′** (7.2 g, 2.51 mmol) was stirred with 1% aqueous solution of KOH (350 mL) for 3 h and then left at room temperature for 12 h. The reaction mixture was extracted with dichloromethane (50 mL) thrice, and the aqueous solution was acidified with concentrated hydrochloric acid till pH 2; the solid obtained was filtered, the crude product dissolved in saturated sodium bicarbonate, filtered, and reprecipitated using concentrated hydrochloric acid to give a yellow solid. (6 g, 92.4%); mp: 165–170°C; IR (KBr): υ 3053, 2880, 1713 (>C=O) cm^{-1}; ^{1}H-NMR (400 MHz, DMSO-d_6): δ 4.95 (1H, s, cis vinylic proton), 6.66 (1H, s, trans vinylic proton), 6.76–0.678 (1H, d, *J* = 7.6 Hz, Ar H), 7.38–7.42 (2H, m, Ar H), 7.54–7.64 (6H, m, Ar H), 7.87–8.04 (4H, m, Ar H), 8.17–8.19 (1H, m, Ar H).

4-Oxo-4H-benzo[h]chromene-2-carboxylic acid (4). A mixture of **3/3′** (1 g, 0.38 mmol) and concentrated sulfuric acid (10 mL) was heated at 60–70C for 2 h. The reaction mixture was poured into ice. The solid product was filtered, washed with plenty of water, and dried, which gave a yellow solid. (0.2 g, 21.5%); mp: 148–150°C; IR (KBr): υ 3445 (OH), 3084, 2984, 1724 (>C=O), 1641 (>C=O) cm^{-1}; ^{1}H-NMR (400 MHz, DMSO-d_6): δ 7.07 (1H, s, Ar H), 7.81–7.87 (2H, m Ar H), 7.97 (2H, s, Ar H), 8.12–8.15 (1H, m, Ar H), 8.48–8.53 (1H, m, Ar H).

General method for 5a–5b. In a solution of **4** (0.5 g, 0.21 mmol) in dry THF (50 mL) EDC.HCl (0.4 g, 0.21 mmol), DMAP (0.05 g, 10%), triethylamine (0.61 mL, 0.43 mmol), and p-toluidine/morpholine (0.21 mmol) were added. The reaction mass was stirred at room temperature for 8 h, and then poured into ice and extracted with ethyl acetate. After removal of solvent, it gave a brown liquid. The crude product was purified by column chromatography using 5% ethyl acetate in petroleum ether.

4-Oxo-N-p-tolyl-4H-benzo[h]chromene-2-carboxamide (5a). This compound was obtained as a brown solid. (0.125 g, 14%); mp: 205–210°C; IR (KBr): υ 3348 (NH), 3063, 2919, 2855, 1694 (>C=O), 1651 (>C=O) cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 2.41 (3H, s, CH_3), 7.27–7.29 (2H, d, *J* = 8 Hz, Ar H), 7.41 (1H, s, Ar H), 7.66–7.68 (2H, d, *J* = 8.4 Hz, Ar H), 7.78–7.82 (2H, m, Ar H), 7.85–7.88 (1H, d, *J* = 8.8 Hz, Ar H), 7.99–8.02 (1H, m, Ar H), 8.17–8.20 (1H, d, *J* = 8.4 Hz, Ar H), 8.46–8.48 (1H, dd, *J* = 3.6, 6.0 Hz, Ar H), 8.57 (1H, s, NH); ^{13}C-NMR (400 MHz, $CDCl_3$): δ 21.05, 113.90, 120.56, 120.78, 121.06, 121.70, 123.48, 126.44, 127.71, 128.68, 129.87, 129.91, 133.75, 135.73, 136.36, 152.62, 154.29, 156.97, 177.79; ms: *m/z* 330.0 $[M+1]^+$; Anal. Calcd for $C_{21}H_{15}NO_3$: C, 76.58; H, 4.59; N, 4.25. Found: C, 76.45; H, 4.69; N, 4.41.

2-(Morpholine-4-carbonyl)-4H-benzo[h]chromen-4-one (5b). This compound was obtained as a brown solid. (0.09 g, 12%); mp: 96–100°C; IR (KBr): υ 3099, 2961, 2921, 2851, 1743 (>C=O), 1645 (>C=O) cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 2.03–2.06 (4H, m, -N- CH_2), 3.67–3.70 (2H, t, -O- CH_2), 4.51–4.54 (2H, t, -O- CH_2), 7.30 (1H, s, Ar H), 7.73–7.80 (2H, m, Ar H), 7.84–7.86 (1H, d, *J* = 8.8 Hz, Ar H), 7.97–7.99 (1H, dd, *J* = 1.6, 6.8 Hz, Ar H), 8.14–8.16 (1H, d, *J* = 8.8 Hz, Ar H), 8.64–8.66 (1H, dd, *J* = 1.6, 7.6 Hz); Anal. Calcd for $C_{18}H_{15}NO_4$: C, 69.89; H, 4.89; N, 4.53. Found: C, 70.15; H, 4.63; N, 4.71.

2-(Pyrrolidine-1-carbonyl)-4H-benzo[h]chromen-4-one (5c). Compound **4** (0.5 g, 0.21 mmol) dissolved in dichloromethane (50 mL) and dimethylformamide (2 to 3 drops), oxalyl chloride (0.79 mL, 0.83 mmol) was added slowly to the reaction flask and stirred at room temperature for 2 h. the solvent was removed under reduced pressure. The acid chloride obtained was dissolved in dichloromethane (50 mL), triethylamine (0.56 mL, 0.40 mmol), and pyrrolidine (0.17 mL, 0.21 mmol) were added and stirred at room temperature for 8 h. The reaction mixture was poured into water and extracted with dichloromethane (50 mL) thrice. The solvent was removed under vacuum and the compound purified by column chromatography using 5% ethyl acetate in petroleum ether, which gave a yellow solid. (0.16 g, 23%); mp: 105–110°C; IR (KBr): υ 2963, 2919, 2875, 1629 (>C=O) cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 2.05–2.08 (4H, m, CH_2), 3.74–3.77 (2H, t, CH_2), 3.84–3.88 (2H, t, CH_2), 6.95 (1H, s, Ar H), 7.69–7.78 (2H, m, Ar H), 7.83–7.85 (1H, d, *J* = 8.4 Hz, Ar H), 7.97–7.99 (1H, dd, *J* = 8.0 Hz, Ar H), 8.17–8.19 (1H, d, *J* = 8.4 Hz, Ar H), 8.50–8.52 (1H, dd, *J* = 8.0 Hz, Ar H); Anal. Calcd for $C_{18}H_{15}NO_3$: C, 73.71; H, 5.15; N, 4.78. Found: C, 73.45; H, 5.33; N, 4.91.

General procedure for 6a–6e. Compound **4** (0.1 g, 0.04 mmol) dissolved in different alcohols and dry HCl gas was purged for 2 h. Different alcohols were removed under vacuum, poured into ice, and then extracted with dichloromethane; after removal of the solvent, it gave a brown liquid. The crude compound was purified by column chromatography using 5% ethyl acetate in petroleum ether to obtain corresponding ester derivatives.

Ethyl 4-oxo-4H-benzo[h]chromene-2-carboxylate (6a). This compound was obtained as a yellow solid. (0.03 g, 27%); mp: 126–128°C; IR (KBr): υ 2994, 2904, 1739 (>C=O), 1651 (>C=O) cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 1.49–1.53 (3H, t, CH_3), 4.53–4.55 (2H, q, CH_2), 7.29 (1H, s, Ar H), 7.73–7.77 (2H, m, Ar H), 7.81–7.83 (1H, d, J = 8.8 Hz, Ar H), 7.94–7.97 (1H, dd, J = 1.6, 7.2 Hz, Ar H), 8.12–8.14 (1H, d, J = 8.4 Hz, Ar H), 8.63–8.65 (1H, dd, J = 1.6, 7.6 Hz, Ar H); ^{13}C-NMR (400 MHz, $CDCl_3$): δ 14.18, 63.02, 116.09, 120.34, 121.03, 122.89, 124.04, 126.20, 127.53, 128.11, 129.92, 136.23, 151.65, 153.65, 160.49, 178.18; ms: *m/z* 269.0 $[M+1]^+$; Anal. Calcd for $C_{16}H_{12}O_4$: C, 71.64; H, 4.51. Found: C, 71.35; H, 4.72.

Methyl 4-oxo-4H-benzo[h]chromene-2-carboxylate (6b). This compound was obtained as a yellow solid. (0.025 g, 24%); mp: 148–150°C; IR (KBr): υ 3072, 2956, 2922, 2853, 1746 (>C=O), 1655 (>C=O) cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 4.01 (3H, s, CH_3), 7.29 (1H, d, J = 1.2 Hz, Ar H), 7.72–7.79 (2H, m, Ar H), 7.82–7.84 (1H, d, J = 8.8 Hz, Ar H), 7.95–7.98 (1H, dd, J = 1.6, 7.2Hz, Ar H), 8.13–8.15 (1H, d, J = 8.8 Hz, Ar H), 8.64–8.65 (1H, s, Ar H); ^{13}C-NMR (400 MHz, $CDCl_3$): δ 53.60, 116.21, 120.30, 121.00, 122.85, 123.95, 126.17, 127.52, 128.09, 129.92, 136.20, 151.34, 153.57, 160.98, 178.02; Anal. Calcd for $C_{15}H_{10}O_4$: C, 70.86; H, 3.96. Found: C, 70.55; H, 4.29.

Isopropyl 4-oxo-4H-benzo[h]chromene-2-carboxylate (6c). This compound was obtained as a yellow solid. (0.026 g, 23%); mp: 122–124°C; IR (KBr): υ 3059, 2885, 2834, 1732 (>C=O), 1683 (>C=O) cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 1.48 (3H, s, CH_3), 1.49 (3H, s, CH_3), 5.32–5.40 (1H, m, CH), 7.73 (1H, s, Ar H), 7.74–7.78 (2H, m, Ar H), 7.83–7.85 (1H, d, Ar H), 7.96–7.99 (1H, dd, J = 1.6, 8.8 Hz, Ar H), 8.14–8.16 (1H, d, J = 8.8 Hz, Ar H), 8.65–8.74 (1H, d, J = 7.6 Hz, Ar H); ^{13}C-NMR (400 MHz, $CDCl_3$): δ 21.79, 29.72, 71.24, 115.96, 120.36, 121.03, 122.91, 124.08, 126.18, 127.52, 128.11, 129.91, 136.25, 151.95, 153.69, 159.98, 178.30; ms: *m/z* 282.9 $[M+1]^+$; Anal. Calcd for $C_{17}H_{14}O_4$: C, 72.76; H, 4.51. Found: C, 72.33; H, 5.00.

Allyl 4-oxo-4H-benzo[h]chromene-2-carboxylate (6d). This compound was obtained as a yellow solid. (0.04 g, 34%); mp: 96–98°C; IR (KBr): υ 3059, 2885, 2829, 1732 (>C=O), 1683 (>C=O) cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 4.96–4.97 (2H, t, $-OCH_2$), 5.41–5.44 (1H, dd, vinylic terminal proton), 5.51–5.56 (1H, m, vinylic terminal proton), 6.02–6.13 (1H, m, vinylic proton), 7.30 (1H, s, Ar H), 7.72–7.76 (2H, m, Ar H), 7.80–7.82 (1H, d, J = 8.8 Hz, Ar H), 7.94–7.96 (1H, d, J = 7.6 Hz, Ar H), 8.11–8.13 (1H, d, J = 8.8 Hz, Ar H), 8.61–8.63 (1H, dd, J = 1.6, 7.6 Hz, Ar H); ^{13}C-NMR (400 MHz, $CDCl_3$): δ 67.26, 116.29, 119.86, 120.33, 121.05, 122.86, 124.00, 126.25, 127.56, 128.12, 129.95, 130.80, 136.24, 151.40, 153.64, 160.20, 178.08; ms: *m/z* 281.0 $[M+1]^+$; Anal. Calcd for $C_{17}H_{12}O_4$: C, 72.85; H, 4.32. Found: C, 72.63; H, 4.50.

Butyl 4-oxo-4H-benzo[h]chromene-2-carboxylate (6e). This compound was obtained as a yellow solid. (0.04 g, 32%); mp: 84–86°C; IR (KBr): υ 3099, 3056, 2959, 2872, 1744 (>C=O), 1651 (>C=O) cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 1.02–1.06 (3H, t, CH_3), 1.52–1.57 (2H, q, CH_2), 1.83–1.86 (2H, m, CH_2), 4.46–4.49 (2H, t, $-OCH_2$), 7.26 (1H, s, Ar H), 7.71–7.75 (2H, m, Ar H), 7.77–7.81 (1H, d, J = 8.8 Hz, Ar H), 7.92–7.95 (1H, dd, J = 1.2, 8.8 Hz, Ar H), 8.09–8.11 (1H, d, J = 8.8 Hz, Ar H), 8.59–8.62 (1H, dd, J = 1.2, 8.8 Hz, Ar H); ^{13}C-NMR (400 MHz, $CDCl_3$): δ 13.73, 19.17, 30.50, 66.76, 116.06, 120.33, 121.02, 122.86, 124.03, 126.20, 127.53, 128.11, 129.91, 136.23, 151.64, 153.65, 160.52, 178.19; ms: *m/z* 296.9 $[M+1]^+$; Anal. Calcd for $C_{18}H_{16}O_4$: C, 72.96; H, 5.44. Found: C, 72.67; H, 5.61.

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Synthesis and anticancer activity of 4-hydroxy naphtho coumarin derivatives and naphtho coumestans

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ABSTRACT

We have synthesized various coumestan derivatives from 4-hydroxy-2H-benzo[h]chromen-2-one ***2****. Oxidative cyclization of 4-hydroxy-2H-benzo[h]chromen-2-one with catechol and pyrogallol in presence of sodium acetate and potassium iodate gave 8, 9-dihydroxy-6H-benzo[h]benzofuro[3,2-c]chromen-6-one* ***3*** *and 8, 9, 10-trihydroxy-6H-benzo[h]benzofuro[3,2-c]chromen-6-one* ***4*** *respectively. These coumestan derivatives and 4-hydroxy-2H-benzo[h]chromen-2-one were condensed with dimethyl sulphate and different mono and/or di alkyl halides in presence of base like anhydrous K_2CO_3 and dry acetone gave corresponding condensed or cyclized coumestan* ***6a-d****,* ***7a-d*** *and 4-hydroxy-2H-benzo[h]chromen-2-one* ***5a-b*** *derivatives.*

Keywords: naphthocoumarin, naphthocoumestan, anticancer activity

INTRODUCTION

Coumestan ring system is present in number of natural products like coumestrol, psoralidine, pterocarsin [1], lucernol [2] and wedelolactone [3]. Coumestans represent an important class of natural oxygenated aromatic products responsible for medicinal effects. Eclipta alba [4] and wedelia calendulacea [5] are the plant sources of nor-wedelolactone and wedelolactone. Both of them show medicinal effects such as antihepatotoxic, antihypertensive, antitumor, antiphospholipase A_2 and antidote activities against snake venome [6-9].

Coumestans belongs to the flavonoids category of phytoestrogens, which have diverse pharmacological properties such as antihemorrhagic, antiproteolytic, antihepatotoxic [10, 6], antiphospholipase and antimyotoxic activity [11]. In traditional Chinese medicine, coumestans are used in the treatment of septic shock and in Indian Ayurvedic medicine as a treatment for liver diseases [12], skin disorders and viral infections. Coumestans have also been shown to reduce cancer risk [13] due to their structural similarity to phytoestrogens. A series of coumestan derivatives were recently reported as HCVNS5B polymerase inhibitors [14] and they also found to inhibit binding to the $GABA_A$ receptors from the rat brain [11]. Wedelolactone has been shown to inhibit the NF-κB mediated gene transcription in cells by blocking the phosphorylation and degradation [15] selective 5-lipoxygenase-inhibitor [15] and LPS- induced caspase-11 expression inhibitor [15]. Coumestrol has been reported to have strong estrogenic activity [16]. Coumestan derivatives have been reported to show inhibitory effect of lipid peroxidation [17] and Na^+, K^+ ATPase activity [18, 19].

MATERIALS AND METHODS

Chemistry

Melting points are uncorrected and were measured in open capillary tubes, using a Rolex melting point apparatus. IR

spectra were recorded as KBr discs on Perkin Elmer RX 1 spectrometer. ^{1}H NMR and ^{13}C NMR spectral data were recorded from a Bruker Advance 300 spectrometer (300 MHz) and Advance 400 spectrometer (400 MHz). TLC was performed on silica gel F254 plates (Merck). CHN elemental analyses were recorded on Thermofinnigan Flash 11-12 series EA.

Synthesis of 4-hydroxy 2H-benzo[h]chromen-2-one (2)

A solution of 2-acetyl 1-naphthol **1** (0.00107 mol) in diethyl carbonate (30 ml) was added slowly to pulverized sodium (0.01739 mol) under anhydrous conditions. Highly exothermic reaction was observed. It was then allowed to cool to room temperature. Ethanol (50 ml) was added to decompose the unreacted sodium. The reaction mass was then poured into water (250 ml) and the aqueous layer washed twice with petroleum ether (50 ml). Concentrated hydrochloric acid was added slowly to the aqueous layer until pH 2 and the solid obtained was collected by filtration. The crude product crystallized from ethanol to give 4-hydroxy-2H-benzo[h]chromen-2-one **2** as light-yellow solid. Yield 96%; mp 283-285°C (Lit. 284°C [4]); IR (KBr, cm^{-1}): 3423, 2926, 1604, 1561; ^{1}H NMR (DMSO, d_6, δ ppm): 5.83 (s, 1H, C-3 proton), 7.60-7.76 (m, 3H, ArH), 7.86-7.94 (m, 2H, ArH), 8.48-8.53 (m, 1H, ArH), 11.94 (s, 1H, OH); ^{13}C NMR (DMSO, d_6, δ ppm): 91.08, 111.62, 119.43, 122.15, 122.66, 124.06, 127.80, 128.57, 129.25, 135.27, 151.12, 162.31, 167.16; Elemental Analysis for $C_{13}H_8O_3$: Calculated, %: 73.58; H, 3.80; Found, %: C 73.75; H 3.68.

General procedure for synthesis of 3 and 4

In a solution of **2** (3 gm, 1.415 mmol) in 1:1 water: acetone (50 ml), sodium acetate (4 gm, 4.878 mmol) and catechol or pyrogallol (1.964 mmol) were added. Reaction mixture was stirred at room temperature for 10 minutes. Mixture of KIO_3 (9 gm, 4.2053 mmol) and sodium acetate (4 gm, 4.878 mmol) in hot water (50 ml) was added slowly in the reaction flask in period of 20 minutes and stirred at room temperature for 30 minutes. Solid product separated was filtered and washed with hot water. Crude product washed with hot ethanol (3×50 ml) and hot petroleum ether (3×50 ml) and dried.

Spectral Data:

8, 9-dihydroxy-6H-benzo[h]benzofuro[3,2-c]chromen-6-one (3)

Yield: 89%; mp: >300°C; IR (KBr, cm^{-1}): 3416, 1702, 1609; Mol. Formula: $C_{19}H_{10}O_5$.

8, 9, 10-trihydroxy-6H-benzo[h]benzofuro[3,2-c]chromen-6-one (4)

Yield: 84%; mp: >300°C; IR (KBr, cm^{-1}): 3843, 3308, 1734, 1583; Mol. Formula: $C_{19}H_{10}O_6$.

General Procedure for synthesis of 5a-b, 6a-b and 7a-b

2, 3 and **4** (1 gm, 1 eq.) dissolved in 20 ml dry acetone. Freshly fused K_2CO_3 (3.5 eq.) and dimethyl sulphate or allyl bromide was added (1.1 eq.) in a reaction flask and it was refluxed for 16 hours. Reaction mass was poured in ice-water, solid crude product obtained was filtered and washed with water. Crude product was washed with hot ethanol (3×50 ml) and hot petroleum ether (3×50 ml) and dried. Compound **5a-b** was purified by column chromatography using 5% Ethyl acetate/ petroleum ether.

Spectral Data:

4-methoxy-2H-benzo[h]chromen-2-one (5a)

Yield: 10%; mp: 154-156°C; IR (KBr, cm^{-1}): 3078, 2916, 2846, 1734; ^{1}H NMR ($CDCl_3$, d_6, δ ppm): 4.07 (3H, s, -OCH_3), 5.81 (1H, s, C-3 proton), 7.64-7.72 (3H, m, ArH), 7.82-7.91 (2H, m, ArH), 8.52-8.6 (1H, m, ArH); ^{13}C NMR ($CDCl_3$, d_6, δ ppm): 56.5, 89.6, 110.9, 118.5, 122.7, 122.9, 123.9, 127.1, 127.8, 128.8, 135.2, 150.7, 163.1, 167.4; Elemental Analysis for $C_{14}H_{10}O_3$: Calculated, %: C 74.33; H 4.46, Found, %: C 74.46; H 4.18.

4-allyloxy-2H-benzo[h]chromen-2-one (5b)

Yield: 9%; mp: 200-205°C; IR (KBr, cm^{-1}): 3092, 3022, 1726; ^{1}H NMR ($CDCl_3$, d_6, δ ppm): 4.73 (2H, dd, -OCH_2), 5.45-5.48 (1H, dd, vinyl proton), 5.53-5.58 (1H, dd, vinyl proton), 5.78 (1H, s, C-3 proton), 6.10-6.17 (1H, m, vinyl proton), 7.64-7.70 (3H, m, ArH), 7.83-7.89 (2H, m, ArH), 8.55-8.57 (1H, m, ArH); ^{13}C NMR ($CDCl_3$, d_6, δ ppm): 69.9, 90.5, 110.9, 118.6, 119.7, 122.7, 122.9, 123.9, 127.1, 127.8, 128.8, 130.7, 135.2, 150.8, 163.0, 166.2; Elemental Analysis for $C_{16}H_{12}O_3$: Calculated, %: C 76.18; H 4.79, Found, %: C 76.43; H 4.53.

8, 9-dimethoxy-6H-benzo[h]benzofuro[3,2-c]chromen-6-one (6a)

Yield: 22%; mp: 259-261°C; IR (KBr, cm^{-1}): 1731, 1266, 1079, 998; ^{1}H NMR (DMSO, d_6, δ ppm): 3.92-3.93 (6H, d, CH_3), 7.31 (1H, s, ArH), 7.44 (1H, s, ArH), 7.63-7.66 (2H, m, ArH), 7.77-7.82 (1H, m, ArH), 7.91-7.94 (2H, m, ArH), 8.49-8.51 (1H, d, ArH); Elemental Analysis for $C_{21}H_{14}O_5$ %: Calculated, %: C 72.83; H 4.07, Found, %: C 72.57; H 3.82.

8, 9-bis(allyloxy)-6H-benzo[h]benzofuro[3,2-c]chromen-6-one (6b)
Yield: 44%; mp: >300°C; IR (KBr, cm^{-1}): 3020, 2849, 1726; ^1H NMR (DMSO, d_6, δ ppm): 4.64- 4.65 (4H, d, $-OCH_2$ protons), 5.25- 5.30 (2H, dd, vinyl protons), 5.42- 5.47 (2H, dd, vinyl protons), 6.03- 6.11 (2H, m, vinyl protons), 7.30 (1H, s, ArH), 7.45 (1H, s, ArH), 7.59-7.66 (2H, m, ArH), 7.78-7.81 (1H, d, ArH), 7.90-7.92 (2H, m, ArH), 8.48-8.50 (1H, d, ArH); Elemental Analysis for $C_{25}H_{18}O_5$: Calculated, %: C 75.37; H 4.55, Found, %: C 75.76; H 4.72.

8, 9, 10-trimethoxy-6H-benzo[h]benzofuro[3,2-c]chromen-6-one (7a)
Yield: 41%; mp: >300°C; IR (KBr, cm^{-1}): 3210, 3075, 2946, 2924, 2851, 1733, 1684; Mol. Formula: $C_{22}H_{16}O_6$.

8, 9, 10-tris(allyloxy)-6H-benzo[h]benzofuro[3,2-c]chromen-6-one (7b)
Yield: 32%; mp: >300°C; IR (KBr, cm^{-1}): 2918, 2851, 1675; Mol. Formula: $C_{28}H_{22}O_6$.

General Procedure for synthesis of 6c-d and 7c-d
Compound **3** or **4** (1 gm, 1 eq.) dissolved in 20 ml dry acetone in a round bottom flask. Freshly fused K_2CO_3 (3.5 eq.) and di substituted bromo alkane were added (0.6 eq.) in a reaction flask and it was refluxed for 10 hours. Reaction mass poured in ice-water, solid crude product obtained. It was filtered and washed with water. Crude product was refluxed with hot ethanol (3×50 ml) and hot petroleum ether (3×50 ml), filtered and dried.

Spectral Data:
8, 9-ethylenedioxy-6H-benzo[h]benzofuro[3,2-c]chromen-6-one (6c)
Yield: 54%; mp: >300°C; IR (KBr, cm^{-1}): 1717, 1359, 1255, 1180, 1030, 763; Mol. Formula: $C_{21}H_{12}O_5$.

8, 9-methylenedioxy-6H-benzo[h]benzofuro[3,2-c]chromen-6-one (6d)
Yield: 27%; mp: >300°C; IR (KBr, cm^{-1}): 1731, 1362, 1278, 1085, 1001, 769; Mol. Formula: $C_{20}H_{10}O_5$.

8, 9-ethylenedioxy, 10-hydroxy-6H-benzo[h]benzofuro[3,2-c]chromen-6-one (7c)
Yield: 37%; mp: >300°C; IR (KBr, cm^{-1}): 3624, 1720, 1552, 1530, 1241, 1090, 1050, 769; Mol. Formula: $C_{21}H_{12}O_6$.

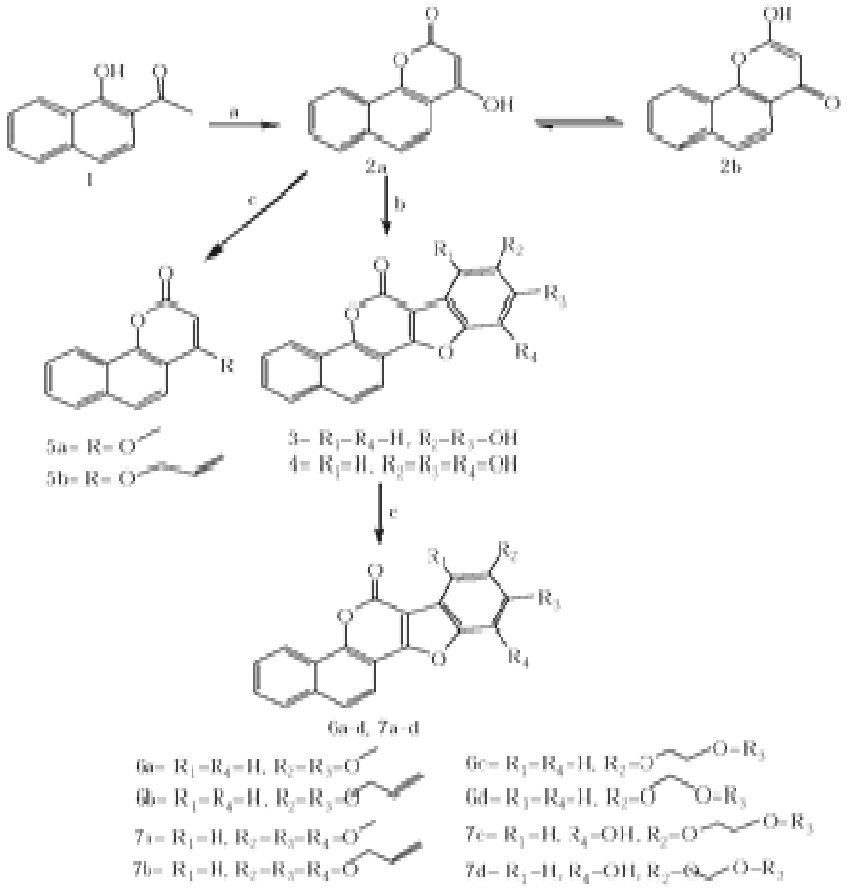
8, 9-methylenedioxy, 10-hydroxy-6H-benzo[h]benzofuro[3,2-c]chromen-6-one (7d)
Yield: 53%; mp: >300°C; IR (KBr, cm^{-1}): 3473, 1731, 1376, 1247, 1120, 970, 920, 830, 753; Mol. Formula: $C_{20}H_{10}O_6$.

Anticancer activity: Procedure to assess the effect of the coumestan derivatives on melanoma cell survival using the MTS Method
96 well plates were plated with 100µl Media (DMEM + 10% Fetal bovine serum and L-Glutamine) containing 5000 cells/well. Stock solution of 20 mM was prepared for compounds to get a series of concentration ranging from 50 µM to 0.625 µM. 100 µl of these compounds were added to the 96 well plates. These 96 well plates were incubated at 37°C in humidified incubator under 5% CO_2 atmosphere for 24, 48 and 72 hours.

In vitro inhibitory efficacy of cancer cell lines representing different cancer types following treatment with compounds was measured using the 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) assay (Promega, Madison, WI). In brief, 5×10^3 cells per well in 100 µL of DMEM containing 10% FBS were grown in a 96-well plate for 24 h and treated with either control DMSO vehicle or increasing concentrations (0.625-50 µM) of these compounds for 24, 48 and 72 h. The proportion of viable cells compared to control DMSO treated cells were determined using MTS assay and IC50 values calculated using GraphPad Prism, version 4.01 (GraphPad software, San Diego, CA). The IC_{50} value for each compound was determined by at least three independent experiments and represented with a standard error. IC_{50} values in µM concentration of all compounds were given in Table 1, Figure 1 and Figure 2

RESULTS AND DISCUSSION

Chemistry
2-Acetyl 1-naphthol **1** on Hoesch reaction [20] with diethyl carbonate in presence of pulverized sodium gave 4-hydroxy-2H-benzo[h]chromen-2-one **2** [21]. Oxidative cyclization [22] of **2** with catechol and pyrogallol in presence of sodium acetate and potassium iodate gave corresponding coumestan derivatives 8,9-dihydroxy-*6H*-benzo[*h*]benzofuro[3,2-c]chromen-6-one **3** and 8,9,10-trihydroxy-*6H*-benzo[*h*]benzofuro[3,2-c]chromen-6-one **4**. Reaction of 4-hydroxy-2H-benzo[h]chromen-2-one **2** and coumestan derivatives **3** and **4** with dimethyl sulphate and various mono or dihaloalkanes in presence of base like anhydrous K_2CO_3 [22] gave corresponding alkyl derivatives of coumestans **5a-b**, **6a-d** and **7a-d** as shown in Scheme 1.



Scheme 1: Reagents and conditions: (a) pulverized sodium, diethyl carbonate, 10 min; (b) Catechol 3 and pyrogallol 4, CH_3COONa, KIO_3, Acetone, water, 30 min. (c) K_2CO_3, Dry acetone, dimethyl sulphate or mono or di substituted halide, 10 h

The IR spectrum of compound **2** showed band at 3423 cm^{-1} for hydroxyl group and band at 1604 cm^{-1} for carbonyl group which exist in **2a** and **2b** form. In ^1H NMR of **2**, singlet at δ 5.83 indicated proton at C-3, broad peak at δ 11.94 indicated hydroxy proton at C-4 and all aromatic protons were observed between δ 7.50-8.53 thus confirmed the formation of **2**.

The IR spectrum of compound **5a** showed disappearance of band at 3423 cm^{-1} and appearance of banc at 1734 cm^{-1} which confirmed presence of lactone ring. In ^1H NMR of **5a** singlet at δ 4.07 for three protons indicated methoxy group, singlet at δ 5.81 indicated proton at C-3 and all aromatic protons were observed between δ 7.64-8.6 confirmed the formation of **5a**. In ^1H NMR of **5b** multiplet at δ 4.73-4.75 for two protons indicated $-OCH_2$ protons, two doublet of doublets at δ 5.45-5.48 and δ 5.53-5.58 each indicated presence of vinyl protons while all aromatic protons were observed between δ 7.64-8.57 thus confirmed formation of **5b**.

The IR spectrum of compound **3** showed band at 3416 and 1702 cm^{-1} for hydroxyl group and lactone carbonyl group respectively. The ^1H NMR of **6a** showed doublet at δ 3.92-3.93 for two methoxy groups and all aromatic protons were observed between δ 7.31-8.51 confirmed formation of **6a**. The ^1H NMR of **6b** showed doublet at δ 4.64-4.65 for two protons indicated $-OCH_2$ group, doublet of doublet at δ 5.25-5.29 for two protons, another doublet of doublet at δ 5.42-5.47 for two protons indicated two vinyl protons ($=CH_2$). One multiplet at δ 6.08 for two protons indicated one vinyl proton ($=CH$) each thus confirmed the presence of two allyloxy groups and all aromatic protons were observed between δ 7.30-8.50 confirmed formation of **6b**. The IR spectrum of compound **6c** showed disappearance of band at 3416 cm^{-1} for hydroxyl group and presence of band at 1717 cm^{-1} for lactone group confirmed formation of **6c**. The IR spectrum of compound **6d** showed disappearance of band at 3416 cm^{-1} for hydroxyl group and presence of band at 1731 cm^{-1} for lactone group indicated formation of **6d**.

The IR spectrum of compound **4** showed band at 3843 cm^{-1} for hydroxyl group and band at 1734 cm^{-1} for lactone carbonyl group confirmed formation of **4**. The IR spectrum of compound **7a** showed disappearance of band at 3843 cm^{-1} for hydroxyl group and presence of bands at 3210, 3075, 2946, 2924 and 2851 cm^{-1} and band at 1733 cm^{-1} for lactone group confirmed formation of **7a**. The IR spectrum of compound **7b** showed disappearance of band at 3843 cm^{-1}, while presence of bands at 2918 and 2851 cm^{-1} and band at 1675 for carbonyl group confirmed formation of **7b**. The IR spectrum of compound **7c** showed disappearance of band at 3843 $cm^{-1}$and presence of band at 1734 cm^{-1} for lactone group confirmed formation of **7c**. The IR spectrum of compound **7d** showed disappearance of band at 3843 cm^{-1} while presence of band at 1720 cm^{-1} for lactone group confirmed formation of **7d**.

Anticancer activity

All synthesized coumestan derivatives were screened against two melanoma cancer cell lines UACC-903 and A375M, one breast cancer cell line MCF-7 and fibroblast (FF2441-Precursors of normal cells) to determine IC_{50} values of synthesized compounds by MTS Assay method [23]. The results are shown in Table-1, Figure-1 and Figure-2 respectively.

Table 1: IC_{50} of compounds against melanoma cell lines UACC903 and A375M

Cell lines	UACC-903			A375M		
	24h	48h	72h	24h	48h	72h
JS-20 (3)	180.8	191.9	58.01	514.3	266.8	112.7
JS-21 (3a)	463.3	283.8	149.3	140.1	269.3	DNC
JS-22 (3b)	144.1	280	109.3	362.3	234.4	270.5
JS-23 (3c)	150.8	125.5	118.5	DNC	617.5	DNC
JS-24 (3d)	241.5	240.9	147.4	533.2	623.5	DNC
JS-25 (4)	193.5	145.7	108.2	DNC	DNC	DNC
JS-26 (4a)	98.92	107.8	44.95	218.6	237.4	DNC
JS-28 (4c)	431.6	526.3	DNC	219.0	605.4	180.9
JS-29 (4d)	462.3	DNC	DNC	DNC	386	249.3

DNC: Does not calculate

Compound **3** showed no significant activity till 48 h then show 69% inhibition up to 72 h against UACC903 cell line while 57% and 58% inhibition observed after 48h and 72 h respectively against A375M cell line. Compound **7a** showed 39% and 47% inhibition after 48 h and 72 h respectively against UACC903 cell line while 192% cell growth up to 48 h against A375M cell line was observed. Compound **7b** showed 138% cell growth up to 48 h then show 45% inhibition against UACC7 cell line while 35% inhibition observed up to 48 h and then no significant change up to 72 h against A375M cell line. Compound **7c** showed 16% inhibition after 48 h then decreasing activity to 9% after 72 h against UACC703 cell line. Compound **7d** showed no significant activity up to 48 h but then show 39% inhibition up to 72 h against UACC903 cell line. Compound **4** showed 25% and 26% inhibition after 48 h and 72 h respectively against UACC903 cell line. Compound **8a** showed no significant activity up to 48 h but then show 58% inhibition after 72 h against UACC903 cell line. Compound **8c** showed 270% cell growth up to 48 h and then 83% inhibition up to 72 h against A375M cell line. Compound **8d** showed no significant activity up to 48 h then 43% inhibition observed against A375M cell line. Compound **8c** and **8d** were inactive up to 72 h against UACC903 cell line while compound **7c**, **7d**, **4** and **8a** were inactive up to 72h against A375M cell line as shown in Table 1.

All Synthesized compounds showed moderate activity against breast cancer cell line MCF-7 in 5 µM concentration as shown in Figure 1. All compounds did not show activity up to 24 h. Compound **3**, **7a** and **7b** showed 23%, 15% and 12% inhibition up to 48 h but then no significant change in activity up to 72 h for compound **3** but compound **7a** and **7b** showed 17%, and 18% inhibition respectively. While compounds **7c**, **7d**, **4**, **8a**, **8c** and **8d** showed no significant change in activity up to 48 h but then showed 19%, 19%, 19%, 16%, 16% and 8% inhibition up to 72 h respectively.

All Synthesized compounds showed moderate activity against fibroblast FF2441 in 6 µM concentration as shown in Figure 2. All compounds were not showing activity up to 24 h. Compound **3** showed 18% inhibition up to 48 h and 23% inhibition up to 72 h. Compounds **7a**, **7b**, **7c**, **7d**, **4**, **8a**, **8c** and **8d** showed no significant change in activity up to 48 h but then showed 24%, 28%, 35%, 25%, 26%, 33%, 17% and 14% inhibition up to 72 h respectively.

Figure 1: IC_{50} of compounds against Breast Cancer cellHines MCF-7

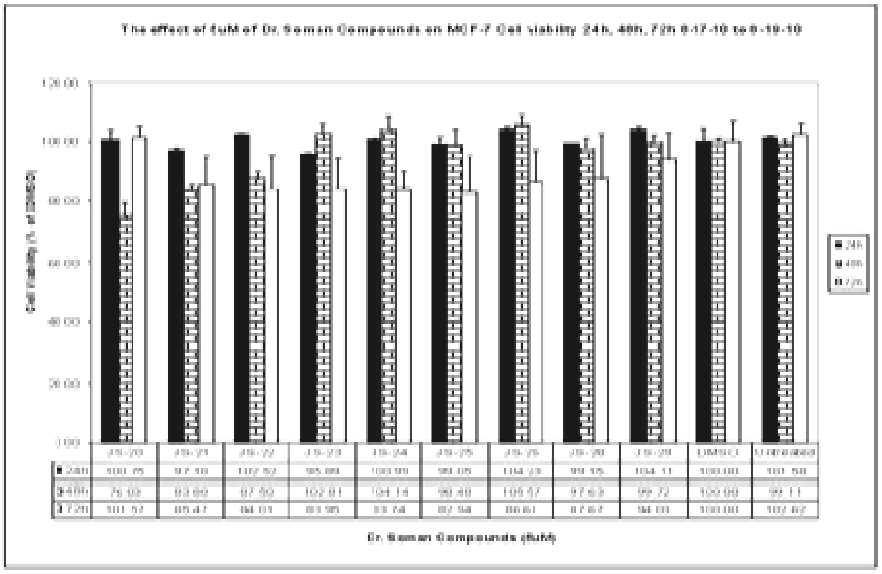
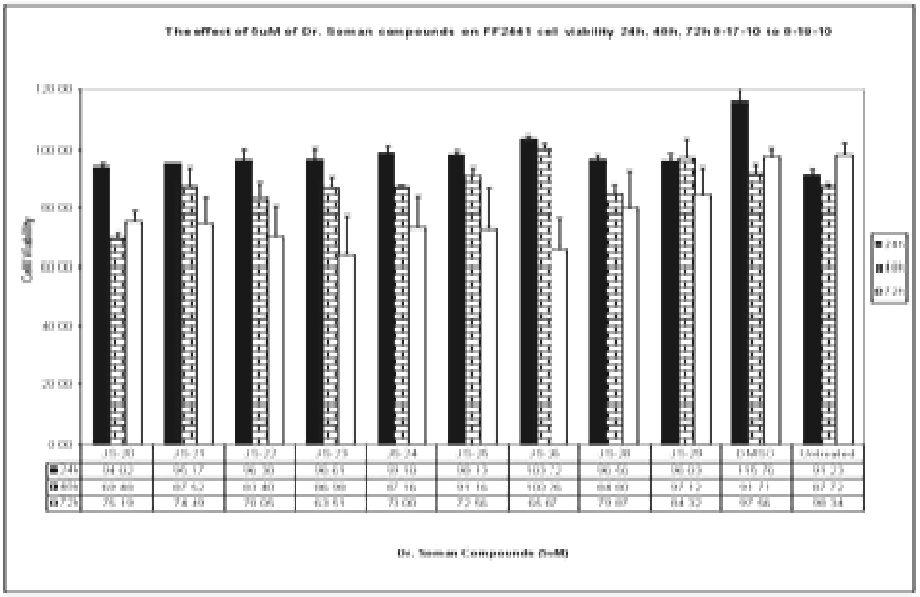


Figure 2: IC_{50} of compounds against Fibroblast (FF2441 precursors of normal cells)



CONCLUSION

All coumestan derivatives showed moderate activity. Methoxy and allyloxy coumestan derivatives were more active than methylenedioxy and ethylenedioxy coumestan derivatives. Methoxy derivatives showed better activity than allyloxy derivatives. Compound **3** showed better activity than other synthesized compounds against melanoma cancer cell lines, Breast cancer cell line and Fibroblast. Some compounds showed activity up to 48 h while some compounds were not so active till 48 h but then show activity up to 72 h.

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