CHAPTER-2

Synthesis of some new 4-aminomethyl-3[H]-benzo(f)-chromen-3-one derivatives and their antimicrobial and anticancer evaluation.

2. Synthesis of some new 4-aminomethyl-3[H]-benzo(f)-chromen-3-one derivatives and their antimicrobial and anticancer evaluation.

2.1 Introduction

Coumarins have important effect in plant biochemistry and physiology acting as antioxidants, enzyme inhibitors and toxic substances. Naphthopyrones are class of heterocyclic compounds having remarkable pharmaceutical applications. They are very versatile building blocks in organic synthesis. 4-Methylnaphthopyrone is useful as intermediates, since the stability of the ring system allows manipulation of substituent on both the rings to give functionally complex derivatives. Naphthopyrone can also be used as latent synthons in the synthesis of natural products.

The 4-H Pyran nucleus is a fertile source of biologically important molecules possessing a wide spectrum of biological and pharmacological activities, such as antimicrobial[1], antiviral[2], mutagenicity,[3] antiproliferative,[4] sex pheromone,[5] antitumor,[6] cancer therapy,[7] and central nervous system activity[8]. Some of these compounds are widely employed as cosmetics and pigments and as potential biodegradable agrochemicals.[9] therefore, the synthesis of such compounds has attracted strong interest.

In recent years, 4-functionaly substituted heterocyclic compounds have received considerable attention due to their wide range of useful biological properties, which include antimicrobial, anti-inflammatory (COX-2 inhibitors and ulcerogenic activity).

Coumarin also known as benzopyrone is class of compound found in nature with wide range of applications. It falls under the flavonoid class of plant as a secondary metabolite and found as bioactive compounds in fruits, vegetables, spices and herbs.[10-13] As coumarin is associated with low toxicity, considerable interest have been increased to find their beneficial effects on human health[14] .Coumarin naturally present in many plants

showed interesting pharmacological properties like anticoagulant, antimicrobial, antioxidant, anti-inflammatory and anti-allergic properties.[15-17]

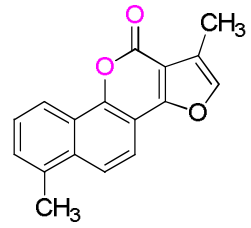
Recent studies have created interest in this class of compounds as they have showed diverse biological activities such as anti-HIV, dyslipidemic and anticancer.[18-21] Cancer is fatal disease after cardiovascular in terms of morbidity and mortality affecting human health worldwide.[22] Ongoing research in this area has developed effective routes to treat it. At the same time, drawback to get effective treatment for such a deadly disease is limited medicines. Due to this, scientific researchers and commercial bodies are trying their best to discover anticancer drugs with good potency, safety and selectivity.[23]

Lee *et al.* reported a coumarin derivative neo-tanshinlactone, (Figure 1) with inhibition for two ER+ human breast cancer cell lines with 20 fold more potency compared to Tamoxifen.[24]

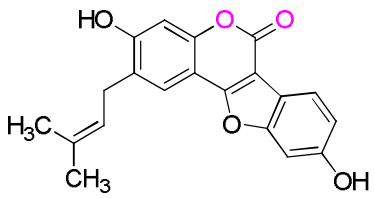
Recently, Belluti *et al.* explored stilbene-coumarin hybrid compounds as potential candidates as anticancer agents.[25] Krol *et al.* showed the anticancer effect of Psoralidin isolated from the seeds of *Psoraleacorylifolia.* This is a medicinal plant widely distributed in Southeastern Asian countries.[26] Sashidhara *et al.* reported coumarin-chalcone hybrid compounds as potential anticancer agents.[27]

Synthetic coumarin derivatives have been reported with wide range of biological activities along with beneficial effects on human health.[28-30] these new developments have encouraged us to study coumarin derivatives for their anticancer activity.

Examples of structures and applications of the similar moieties.



Neo-tanshinlactone



Psoralidin

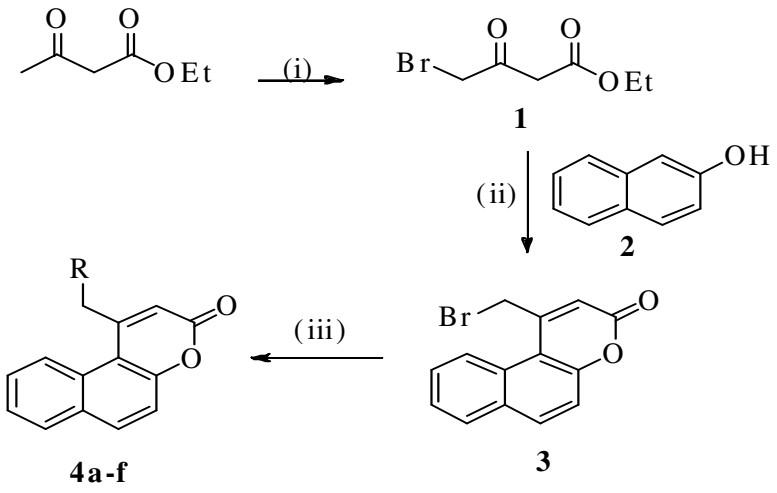
Figure 1: Some potent coumarin derivatives with anticancer activity.

In continuation of our work on synthesis of coumarin derivatives as anticancer and antimicrobial agents.[31-34], herein synthesis, characterization and anticancer as well as antimicrobial activities of naphthopyrone derivatives is reported.

2.2 Result and discussion

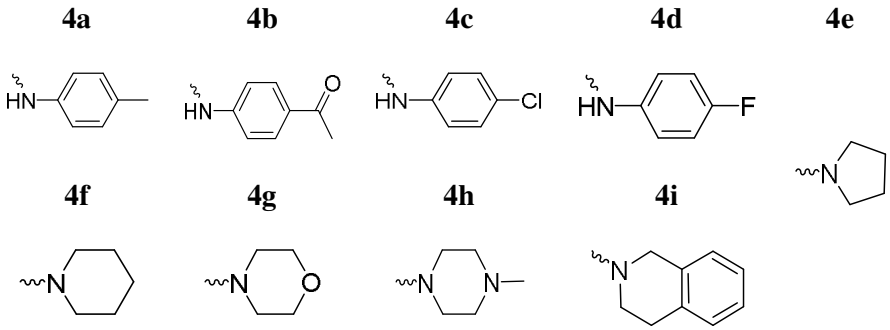
2.2.1 Chemistry

The 1-(substituted aminomethyl)-3H-benzo [f]chromen-3-one **4a-i** (Scheme1) were synthesized by substitution reaction of 1-(bromomethyl)-3H-benzo[f]chromen-3-one **3** with different amines. Ethyl 4-bromo-3-oxobutanoate**1** was obtained as red oil by bromination of ethyl acetoacetate using Br_2[34].Thus, obtained compound **1** was used as such for Pechmann reaction with β-naphthol **2** in conc. H_2SO_4 to obtain 1-(bromomethyl)-3H-benzo[f]chromen-3-one **3**.[35] The formation of compound **3** was confirmed by its melting point and its IR spectrum Figure 3 (Page no.32) which showed bands at 1725cm^{-1} and 825$cm^{-1}$for lactone carbonyl and bromine groups respectively. This compound **3** was used to carry out substitution reaction with different amines using triethylamine (TEA) in Dimethylformamide (DMF) to form substituted amino methyl naphthopyrone derivatives **4a-i**.



Scheme 1: Synthesis of substituted 4-amino methyl naphthopyron-2[H]-one derivative.

Where in R =



Reagents & Condition: (i) Br_2, 0°C to R.T stirring 18 h; (ii) Conc.H_2SO_4, 0°C to R.T 48 h.; (iii)Primary or Secondary amine, TEA, DMF, R.T stirring16h.

The IR spectrum of compound -3 is represented in Figure-3(Page No.40) .The sharp band at 1725 cm^{-1} indicated the presence of lactone carbonyl group. The ^1H NMR of compound **3** in DMSO-d_6 Figure 4(Page No.-41) showed singlet at δ 6.53 is for proton at C-2 and another singlet indicated proton at δ 4.90 at C-3.Two protons indicated $–CH_2Br$ group singlet at δ 6.66 for one proton. 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,page no.-42) showed

presence of 13 peaks which is in accordance with structure of compound **3**.The mass spectrum of compound -3 showed (M+1) ion peak at 290 Figure 6,(Page No.43) that confirms the formation of compound **3.** The IR spectrum Figure-7,(Page No.44) of compound **4a** exhibited strong band at 3355 cm^{-1} for the -NH proton, another strong band at1720$cm^{-1}$for lactone carbonyl group of coumarin ring. In the ^{1}H NMR spectrum of **4a** Figure-8, (Page No.45), all aromatic protons observed betweenδ 6.53 - 8.40. The methylene protons were observed as a singlet at δ 4.91.The methyl group is observed as singlet at δ 2.67.The NH protons was observed as singlet at δ 4.22. In ^{13}C NMR spectrum of compound 4a Figure-9, (Page No.46) the methyl and methlene carbon observed at δ 20 and δ 50 respectively. All aromatic carbons observed from δ 113-155, and the lactone carbonyl carbon observed at δ 161.

The structures of substituted amino methyl naphthopyrone **4a-i** were confirmed by different analytical techniques such as ^{1}H-NMR, ^{13}C-NMR, IR, ESI-MS and elemental analysis. For compound **4g**, single crystal was developed and studied its structure by X-ray single crystal analysis (CCDC No. 1054564). The data for single crystal is given in Table-2. The ORTEP diagram for compound **4g** is shown in (Figure-2a, page no 38).The Molecular packing in unit cell and pi-pi stacking in crystal structure is shown in (Figure-2b and Figure -2c, Page No 39). The ^{1}H NMR spectrum and ^{13} C NMR spectrum of compound -**4b** was recorded and presented in Figure-10 and Figure-11(Page no.47 & 48) respectively.

The IR, ^{1}H NMR spectrum and ^{13} C NMR spectrum of compound -**4c** was recorded and presented in Figure-12,13 and 14 (Page no.49,50 & 51) respectively. The IR, ^{13} C NMR and Mass Spectrum for the compound-**4d** is represented by Figure-15,16and 17(Page no.52, 53 and 54) respectively. The ^{1}H NMR and ^{13} C NMR spectrum for the compound-**4e,** was recorded and represented by Figure-18, 19 (Page No.55, 56) respectively. The Mass spectrum of Compound -**4g** was represented by Figure-20 (Page-

57). The IR, ^{1}H NMR and ^{13} C NMR spectrum for the compound-**4h** was represented by Figure 21, 22 and 23 (Page no, 58, 59& 60) respectively.

2.2.1.1 Single Crystal X-ray Diffraction

Compound **4g** was recrystallized 3 to 4 times from methanol by slow evaporation technique to get 99.9% purity, and compound was submitted for X-ray single crystal analysis to understand polymorphism and space grouping and experimental observations were presented in **Table**.-**1**. After refining the compounds, the data was submitted to CCDC service and the compound was numbered 1054564.

Crystals of Compound **4g** were afforded in a Centro symmetric space group *P*-1 with Z=2. The ORTEP diagram and packing diagram were represented in Figure 2 (a), 2(b) and Figure 2(c) respectively. The crystal system was found as Monoclinic and Polymorphism could not be observed in the above synthesized compounds and further studies are required to conclude this.

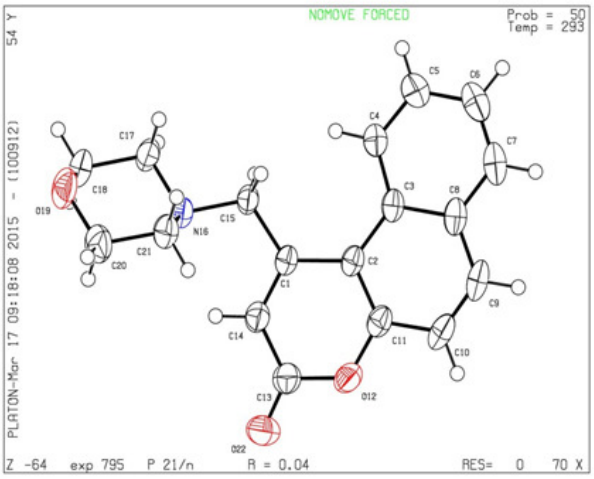


Figure 2: (a) X-ray crystal structure of compound **4g**..

Chemical formula	$C_{19}H_{22}N_3$
Molecular weight	292.40
Crystal system	Monoclinic
Space group	$P2_1/n$
a/Å	4.5843(3)
b/Å	13.6007(10)
c/Å	22.9961(15)
α/°	90.00
β/°	95.429(6)
γ/°	90.00
V/Å^3	1427.37(17)
Z	4
ρ_{calc}mg/mm^3	1.361
Θ	6.96 to 58.02°
H	-5-5
K	-10-17
L	-30-28
Total reflections	7373
Independent reflections	3237
Used no. of reflections	3237
R^a	0.0982
Absorption coefficient (m Å^{-1})	0.082
R_{int}	0.0153
Peak and hole	1.40 and -0.68 ÅÅ^3

Table 1 Crystal data and structure refinement parameters for compound **4g**

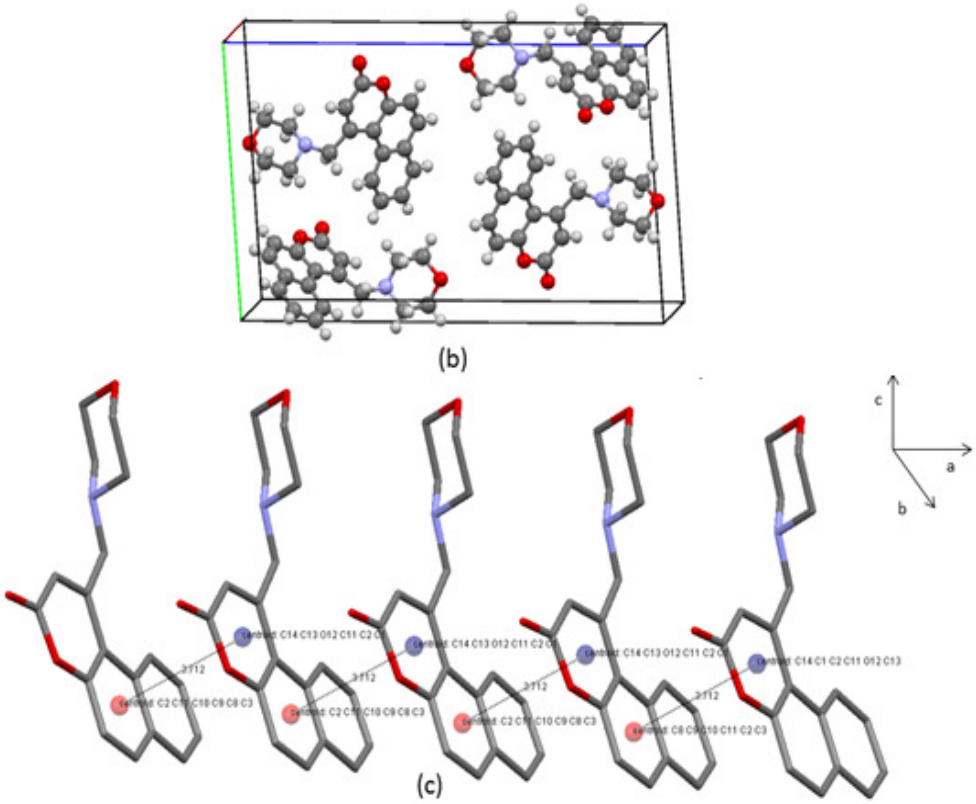


Figure 2 : (b) molecular packing in unit cell; (c) pi-pi stacking in crystal structure.

In general, the IR spectra of compounds **4a-i** exhibited one strong band in range of 1724-1711 cm^{-1} for the lactone carbonyl group of coumarin ring. In the ^{1}H NMR spectra of **4a-i**, peak for the methylene protons observed in range of δ ~3.91- 5.01depending on effect of different amine substitution on it. All these new chemical entities were subjected for their in-*vitro* studies.

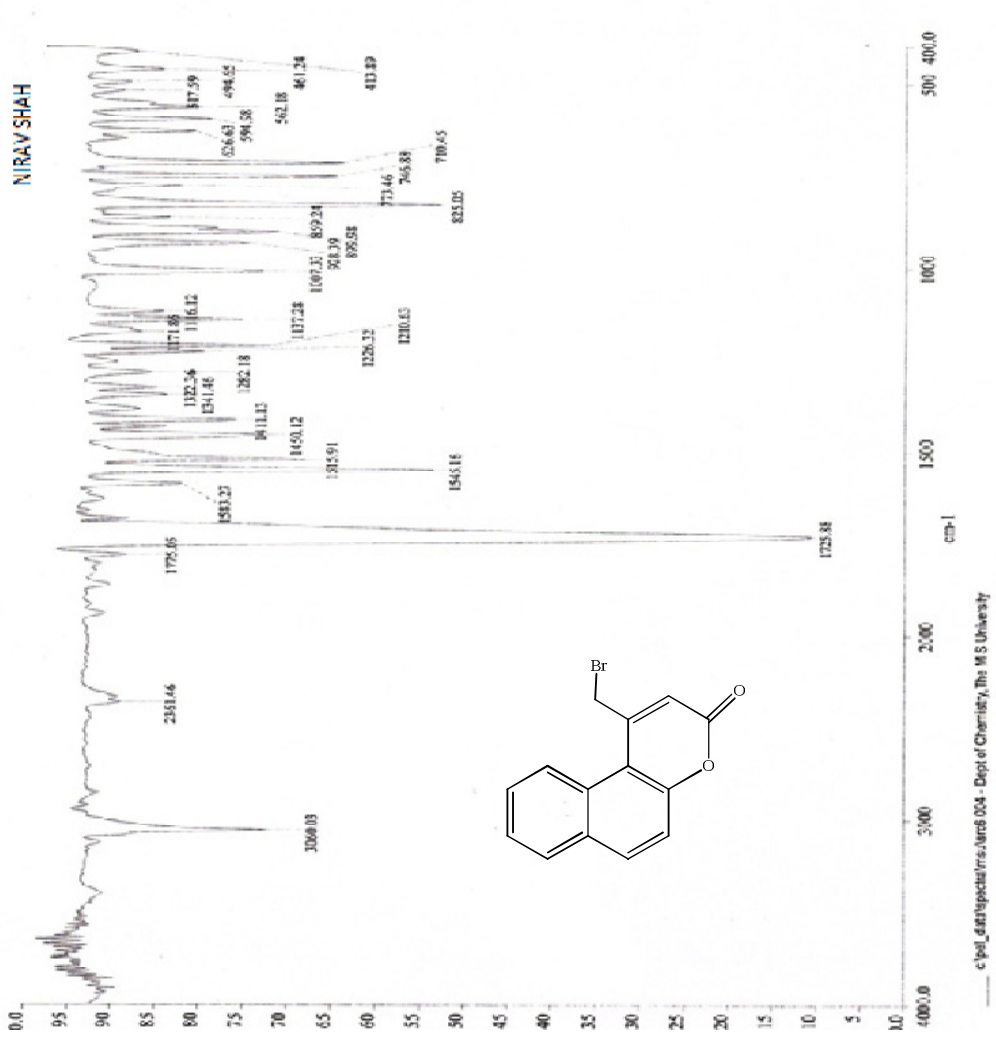


Figure-3 IR of *1-(bromomethyl)-3H-benzo[f]chromen-3-one ie compound -3*

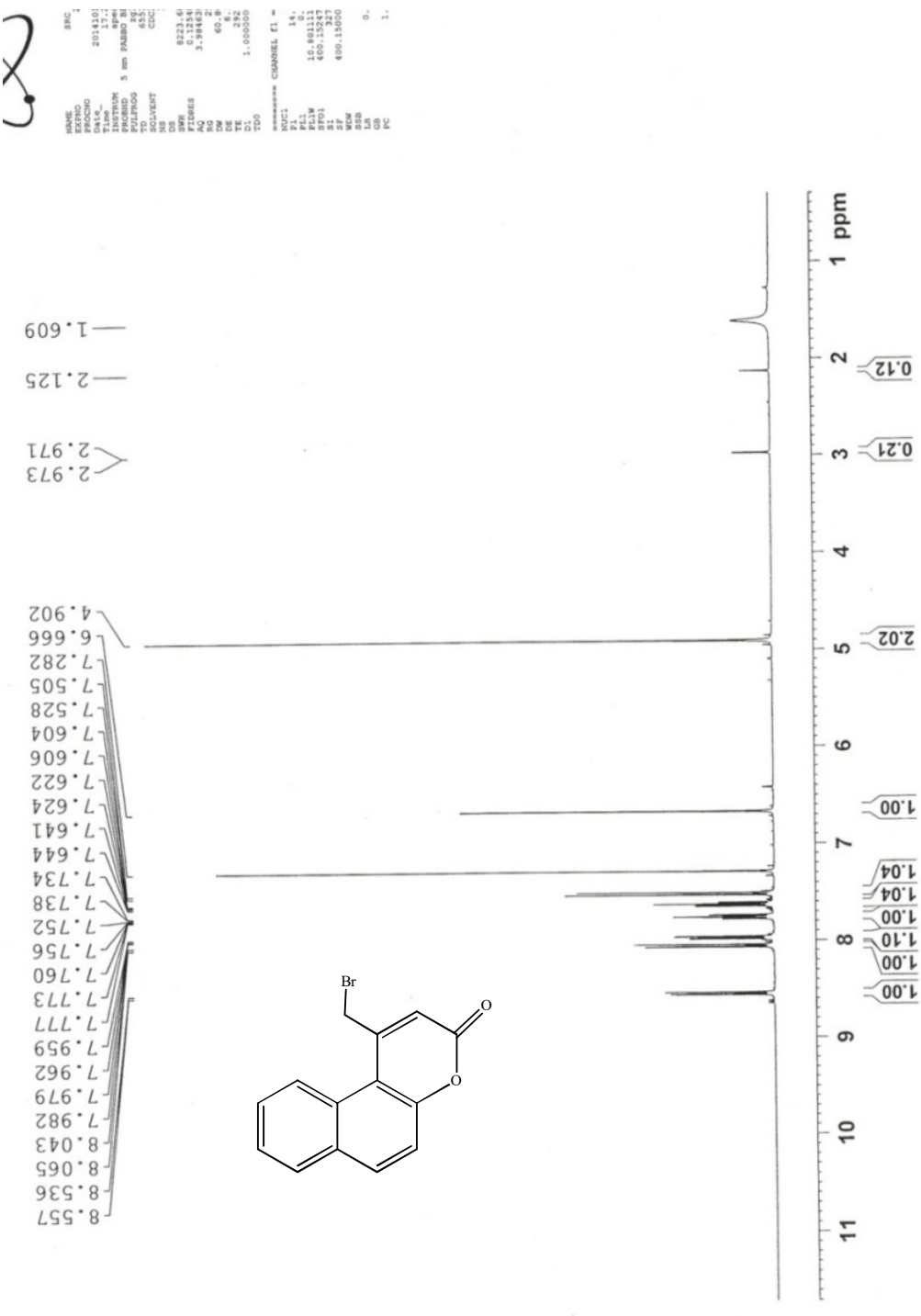


Figure-4 [1]H-NMR of *1-(bromomethyl)-3H-benzo[f]chromen-3-one i.e compound -3*

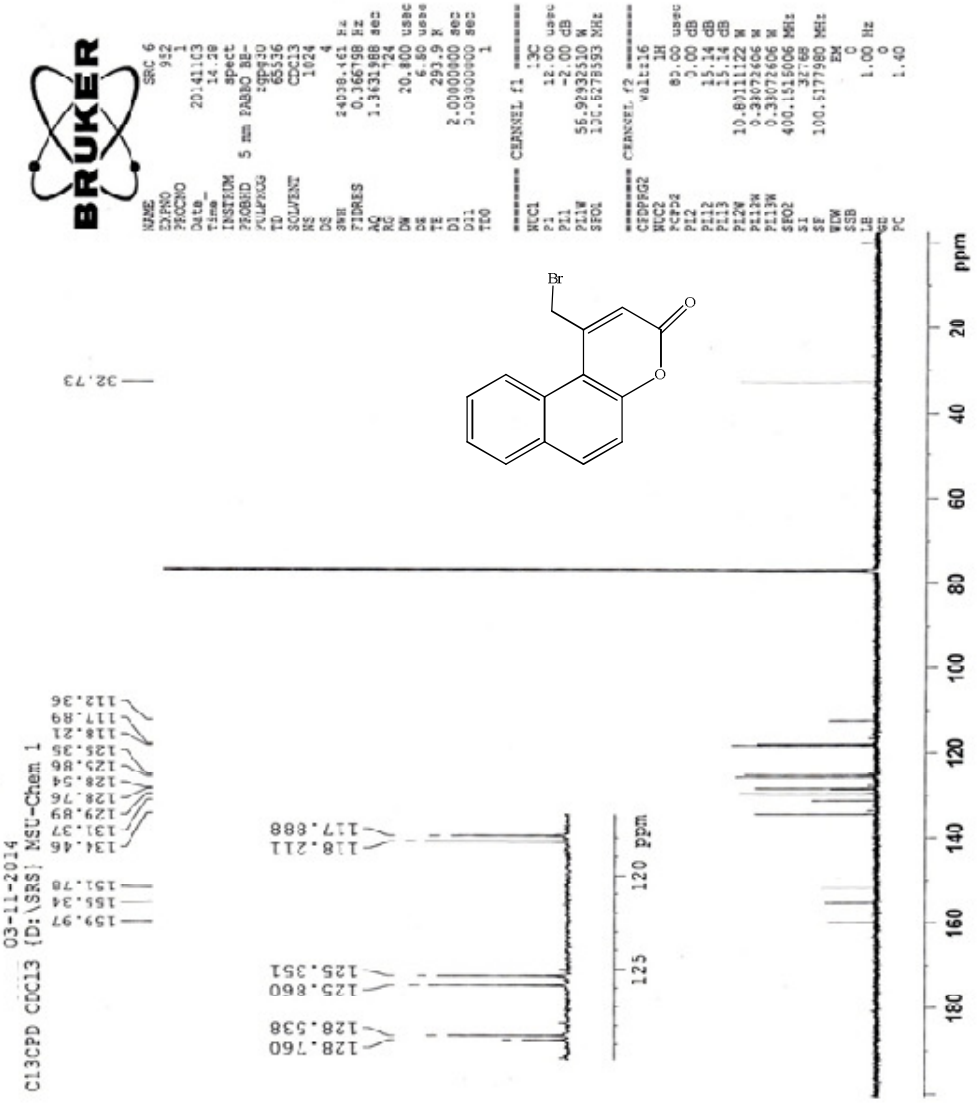


Figure-5 ^{13}C-NMR of 1-(bromomethyl)-3H-benzo[f]chromen-3-one i.e compound -3

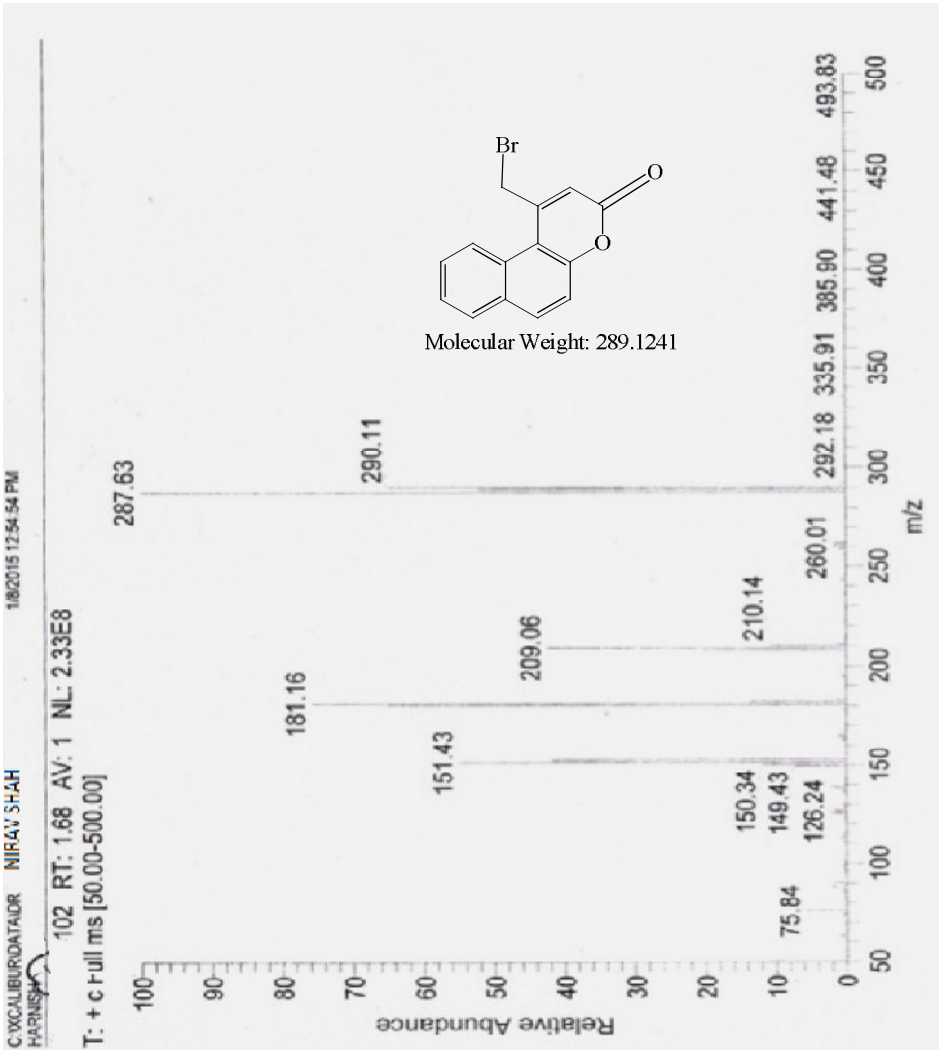


Figure-6 Mass Spectrum of *1-(bromomethyl)-3H-benzo[f]chromen-3-one i.e* compound -3

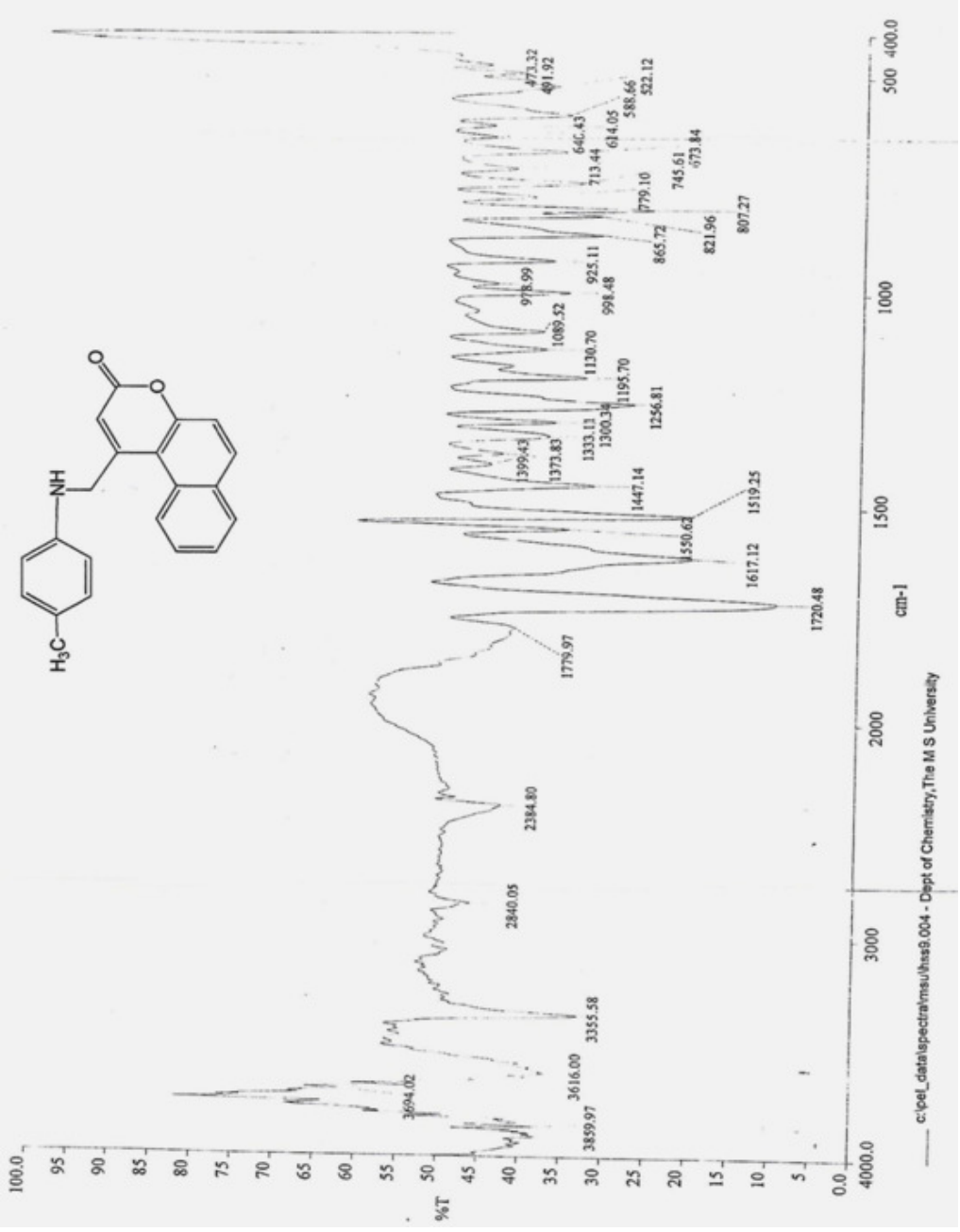


Figure-7 IR Spectrum of 1-{[4-methylphenyl) amino]methyl}-3H-benzo[*f*]chromen-3-one i.e.4a

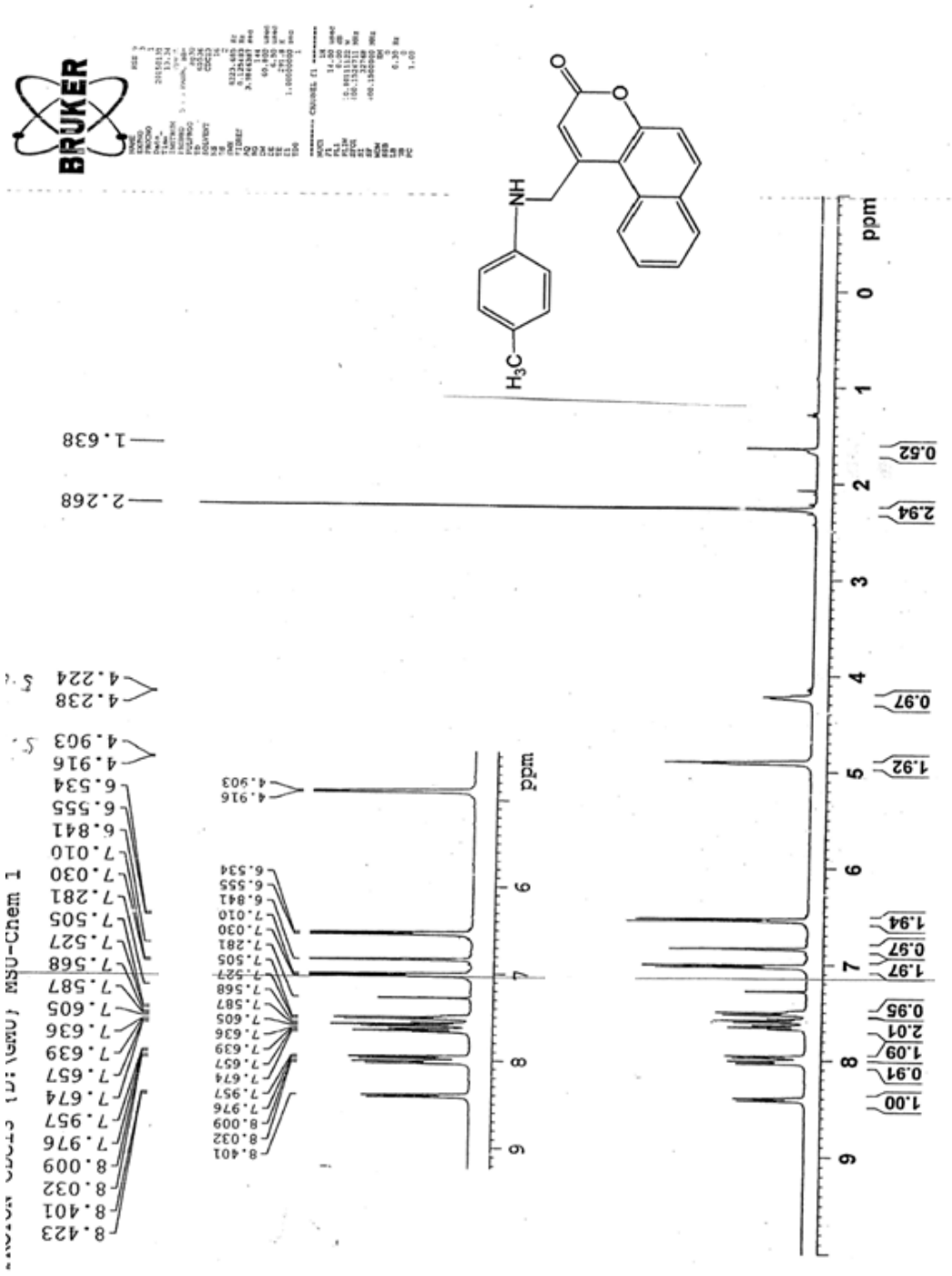


Figure-8 ^{1}H NMR spectrum of 1-{[(4-methyl phenyl) amino] methyl}-3*H*-benzo[*f*]chromen-3-one i.e 4a

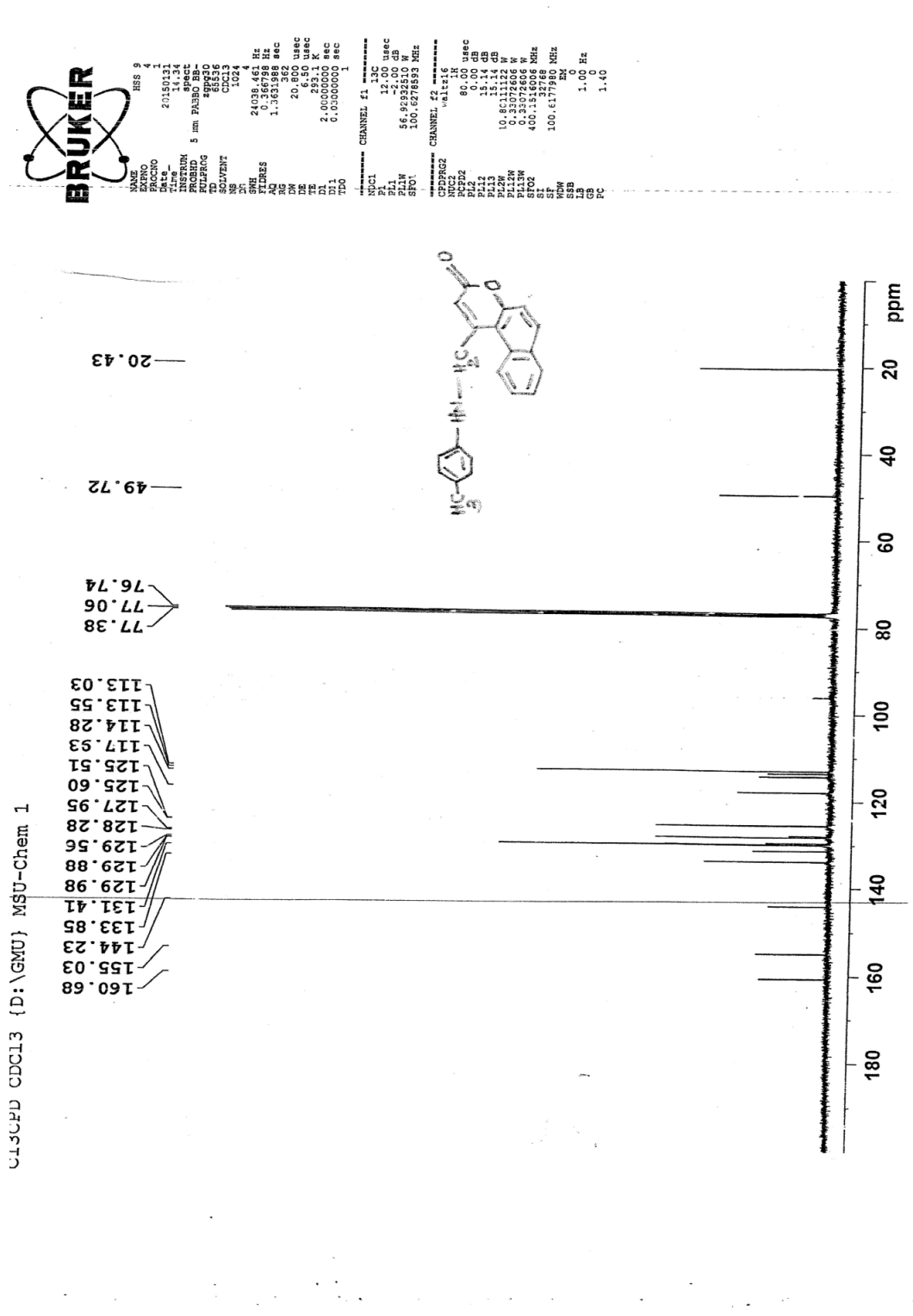


Figure-9 ^{13}C NMR spectrum of 1-{[(4-methyl phenyl) amino] methyl}-3*H*-benzo[*f*]chromen-3-one i.e 4a

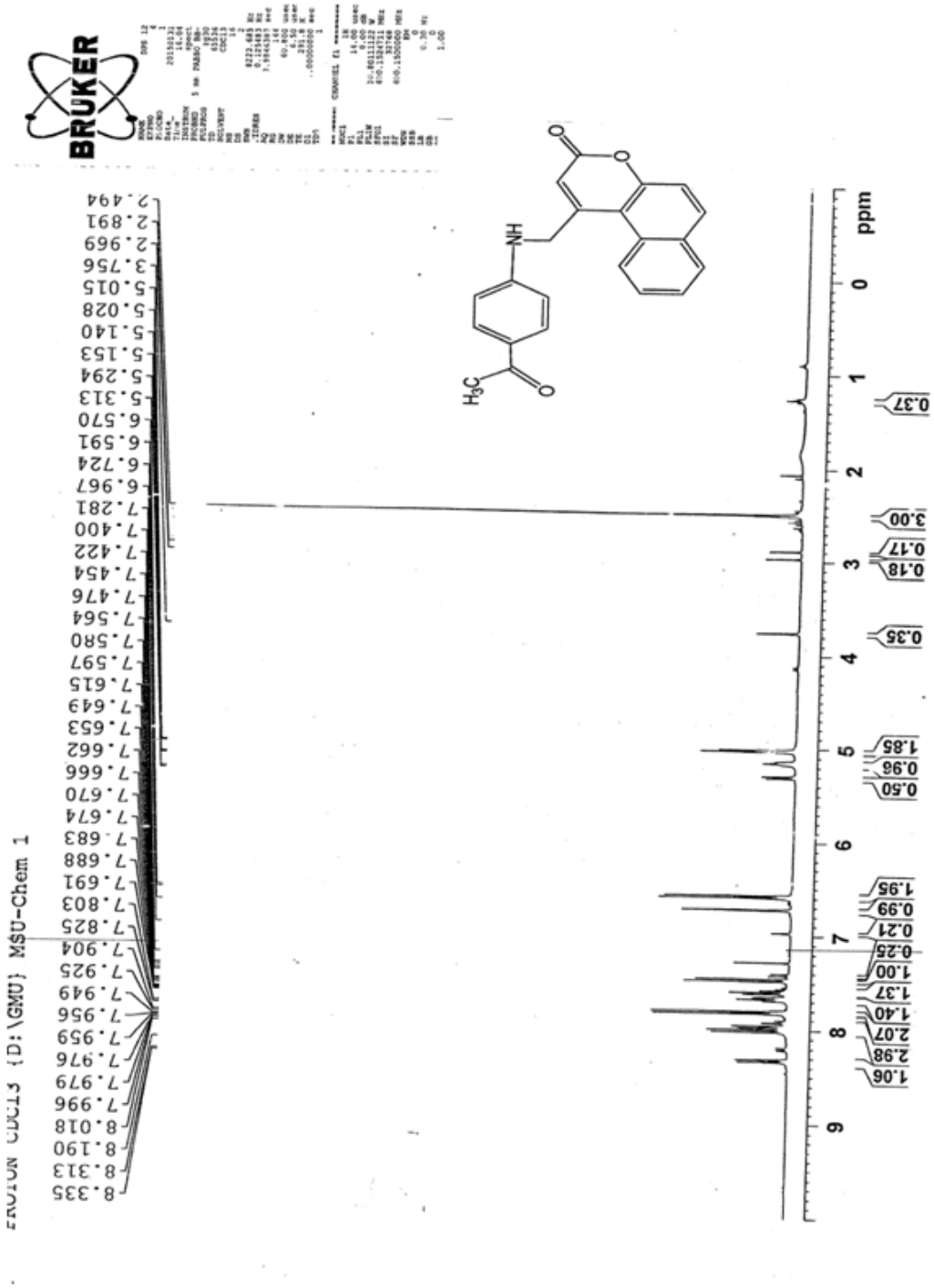


Figure-10 ^{1}H NMR spectrum of 1-{[(4-acetyl phenyl) amino] methyl}-3*H*-benzo[*f*]chromen-3-one i.e 4b

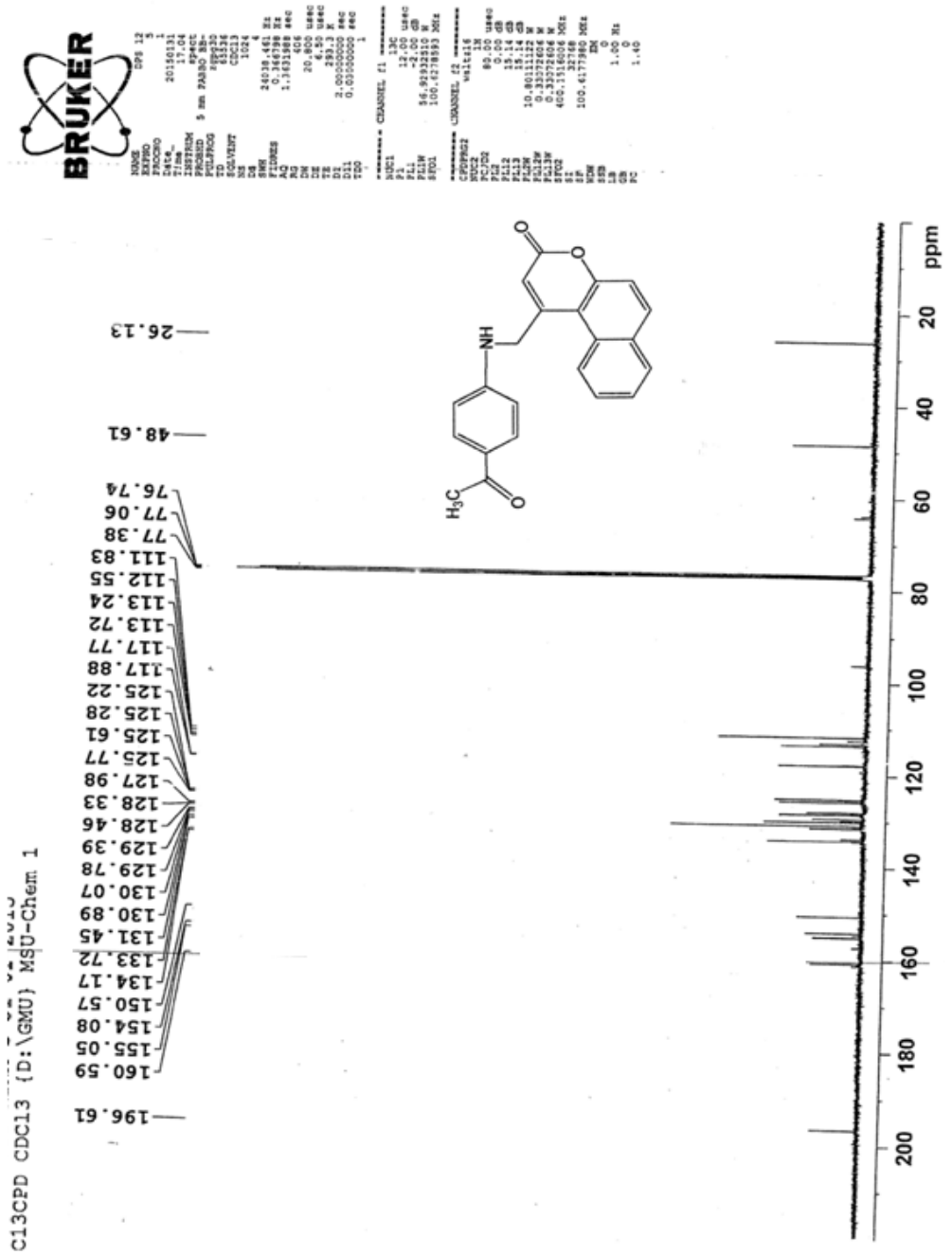


Figure-11 ^{13}C NMR spectrum of 1-{[(4-acetyl phenyl) amino] methyl}-3*H*-benzo[*f*]chromen-3-one i.e 4b

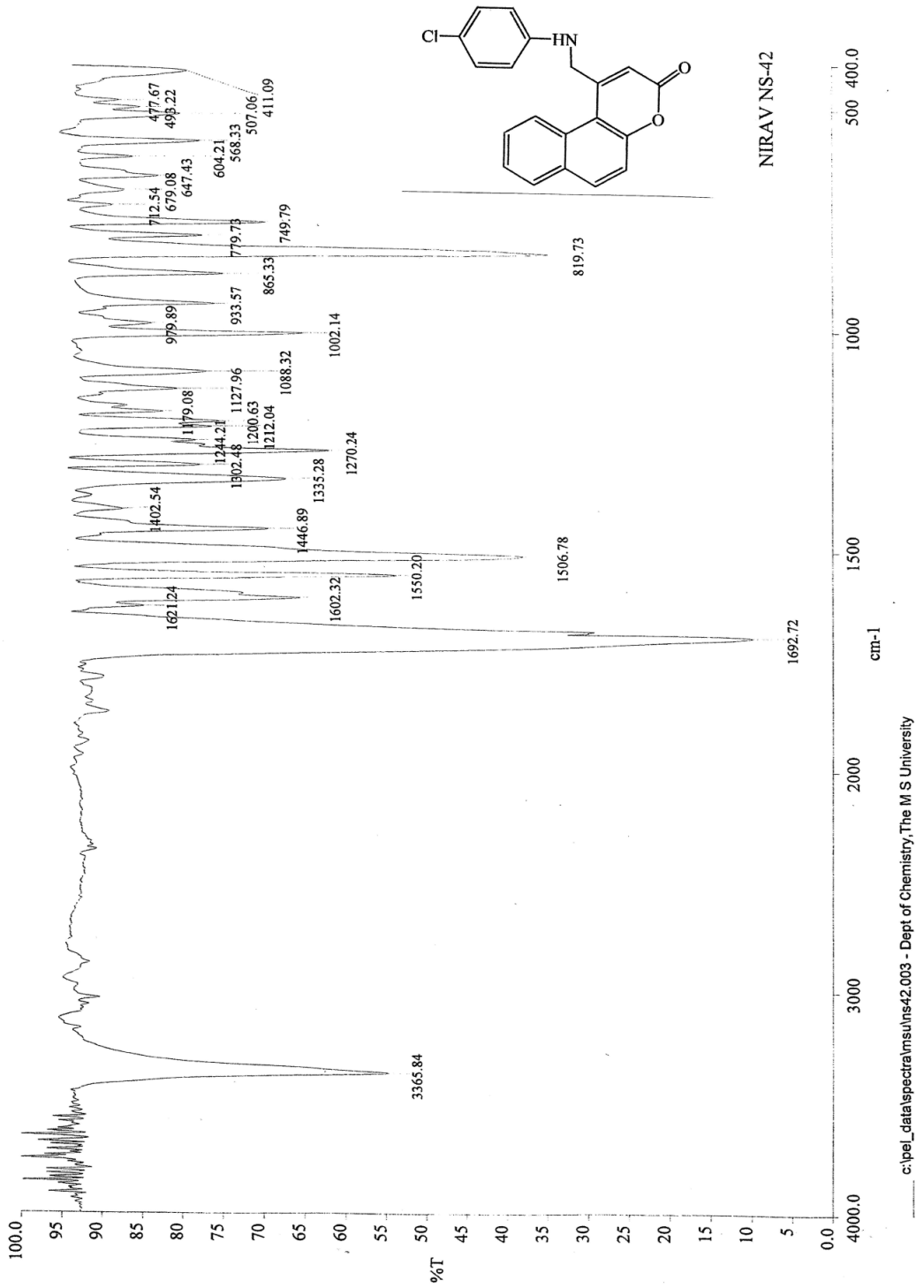


Figure-12 IR spectrum of 1-{[(4-Chloro phenyl) amino] methyl}-3*H*-benzo[*f*]chromen-3-one i.e 4c

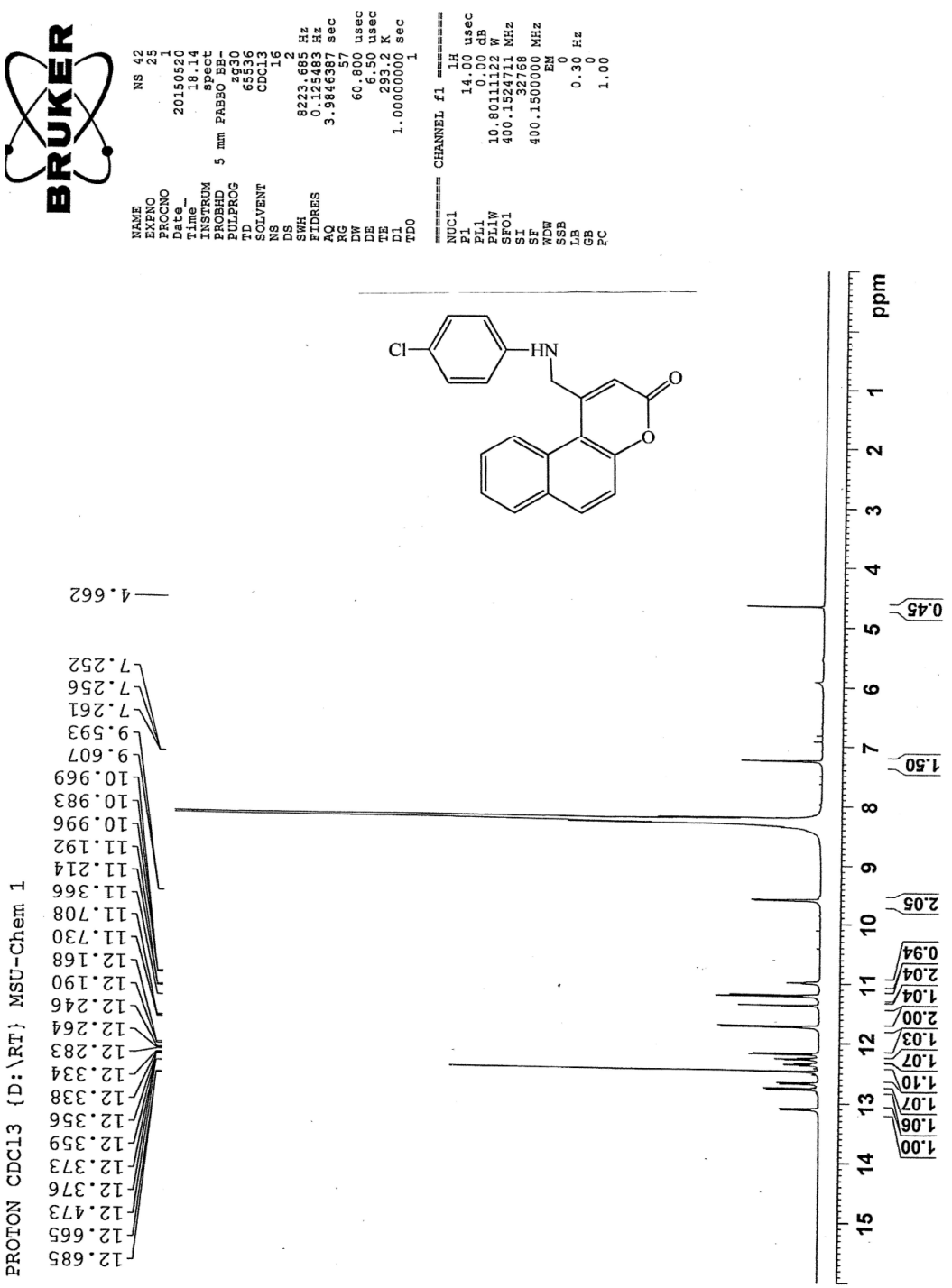


Figure-13 [1] H NMR spectrum of 1-{[(4-Chloro phenyl) amino] methyl}-3*H*-benzo[*f*]chromen-3-one i.e 4c

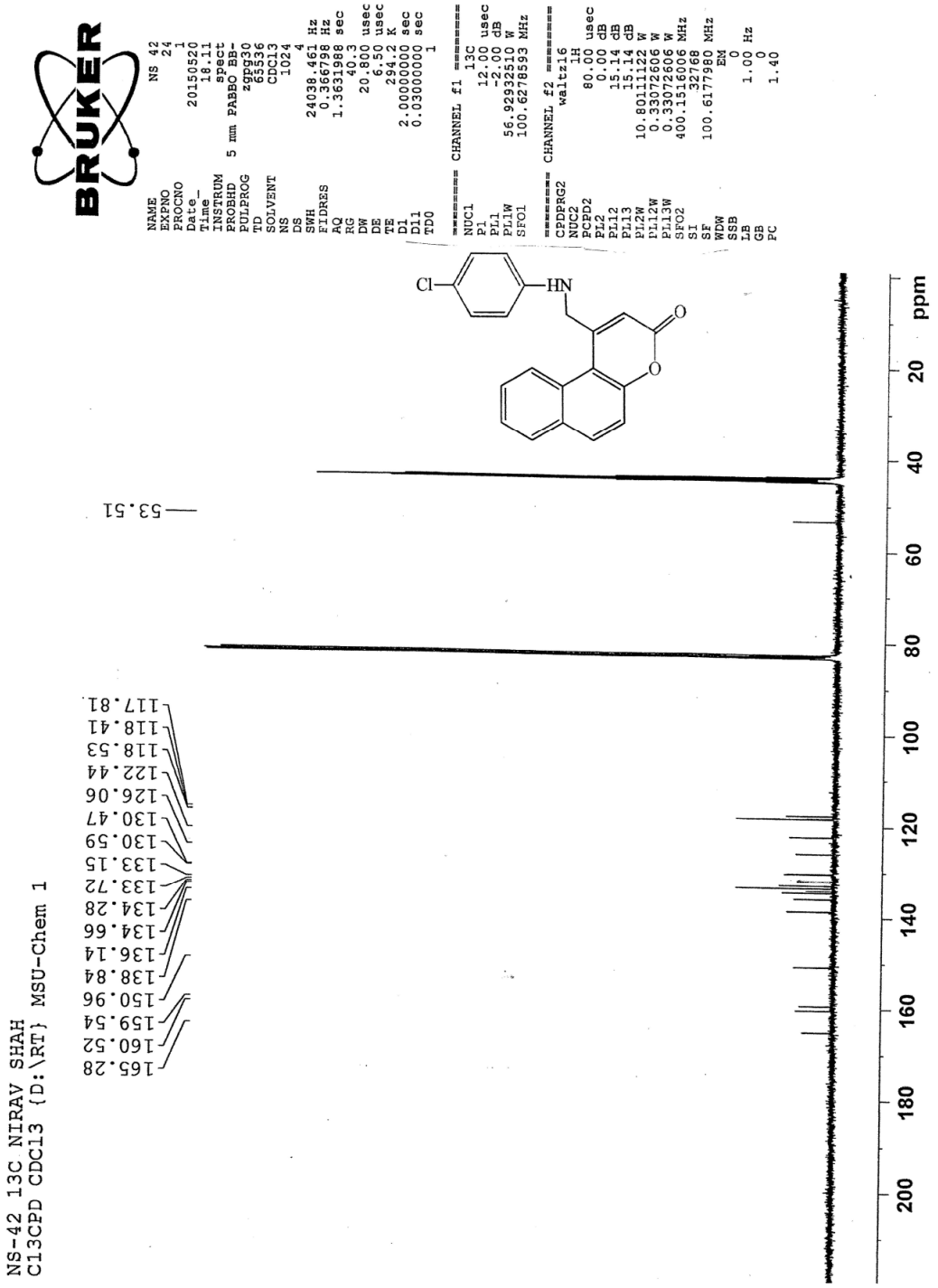


Figure-14 [13] C NMR spectrum of 1-{[(4-Chloro phenyl) amino] methyl}-3*H*-benzo[*f*]chromen-3-one i.e 4c

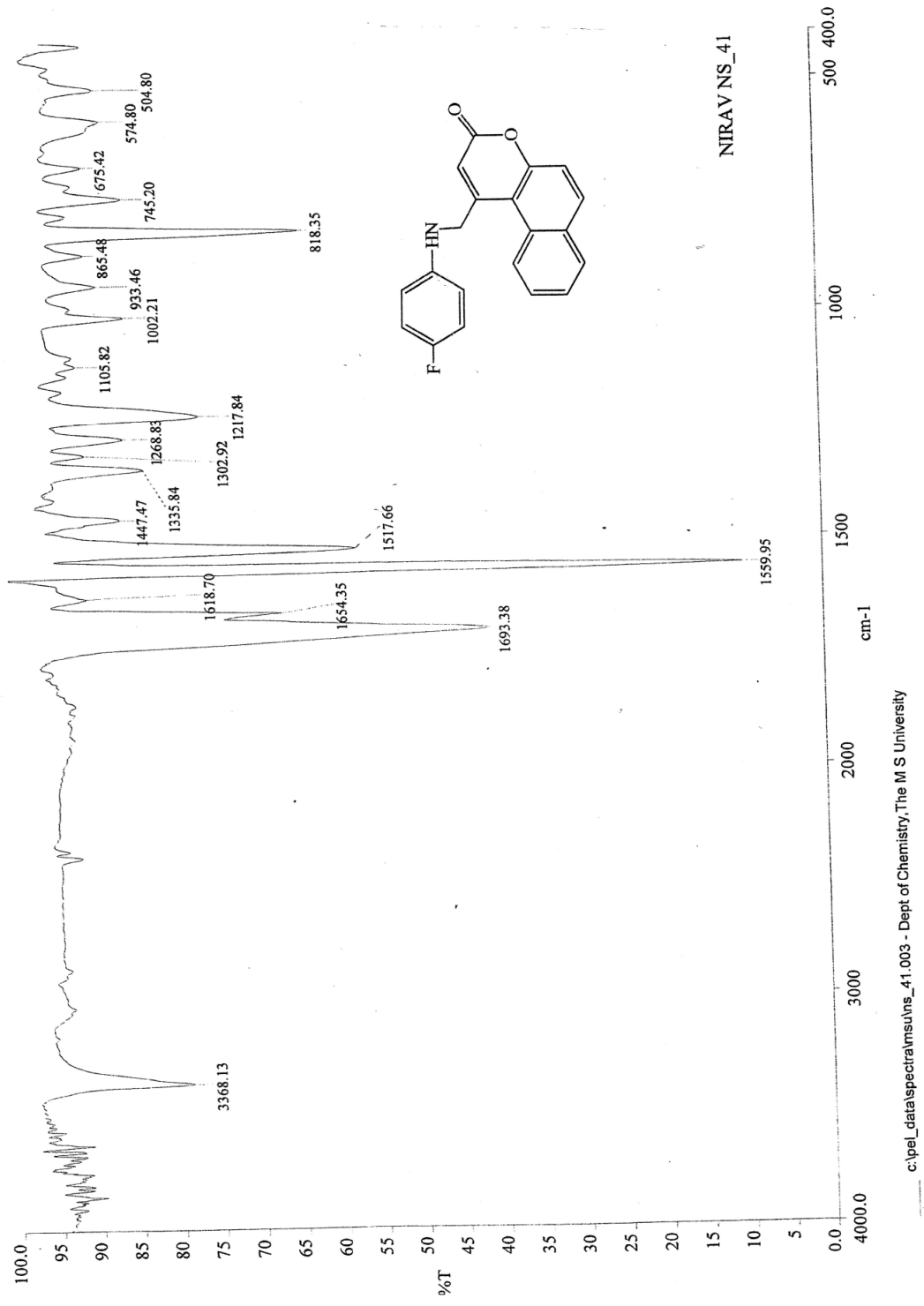


Figure-15 IR spectrum of 1-{[(4-Flouro phenyl) amino] methyl}-3*H*-benzo[*f*]chromen-3-one i.e 4d

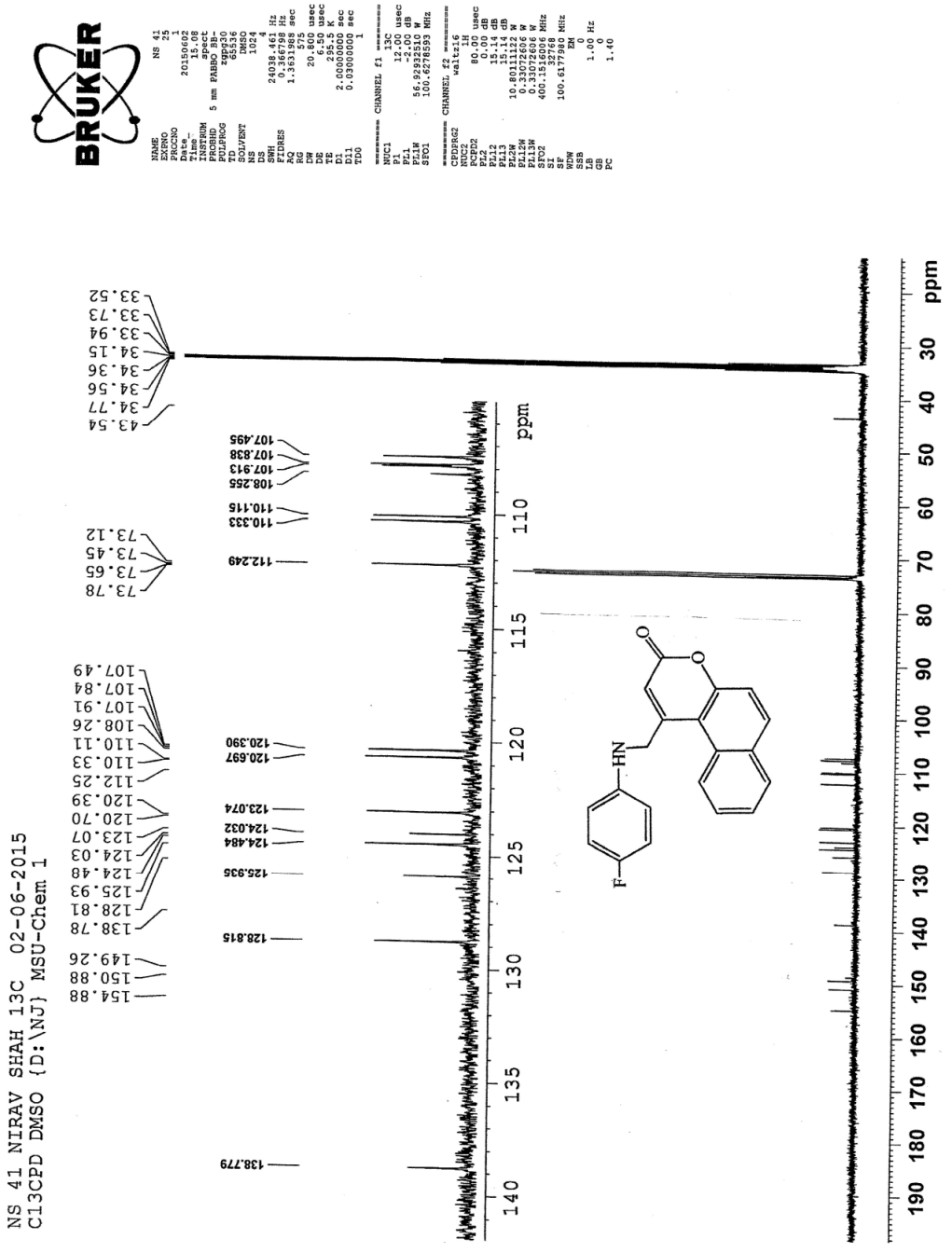


Figure-16 [13] C NMR spectrum of 1-{[(4-flouro phenyl) amino] methyl}-3*H*-benzo[*f*]chromen-3-one i.e 4d

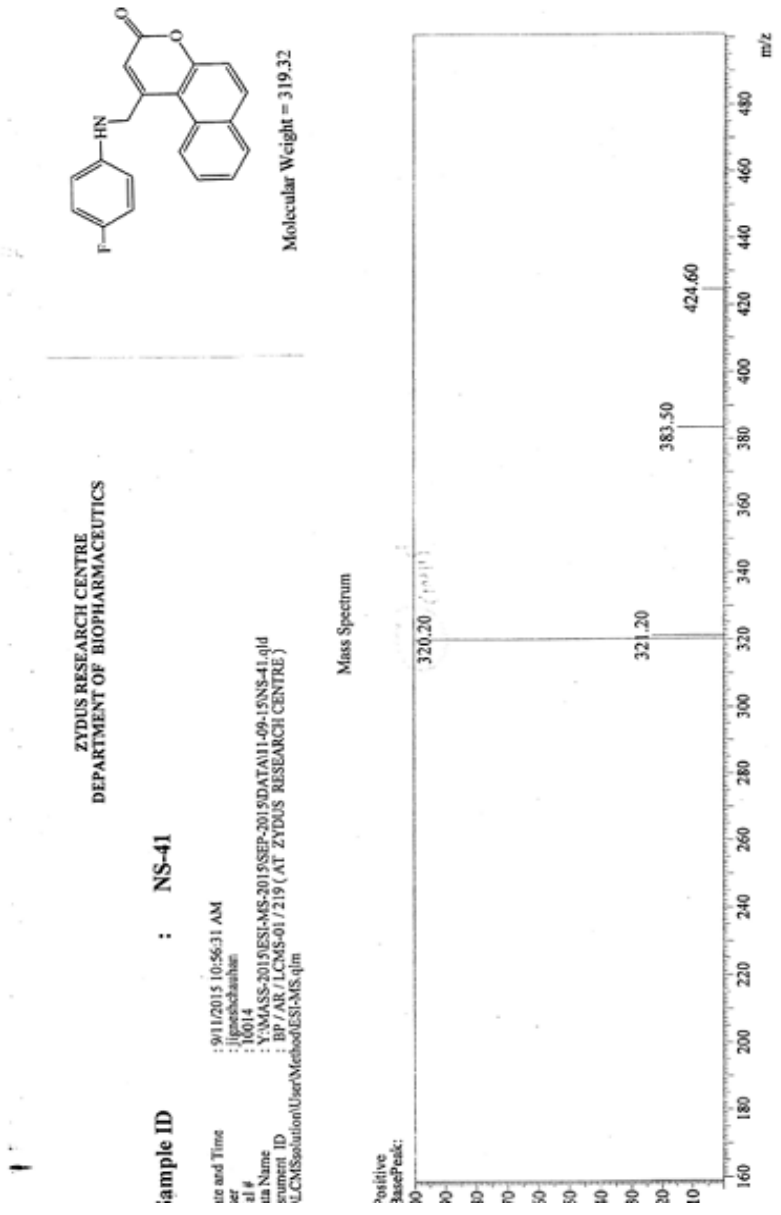


Figure-17 Mass spectrum of 1-{[(4-Flouro phenyl) amino] methyl}-3*H*-benzo[*f*]chromen-3-one i.e 4d

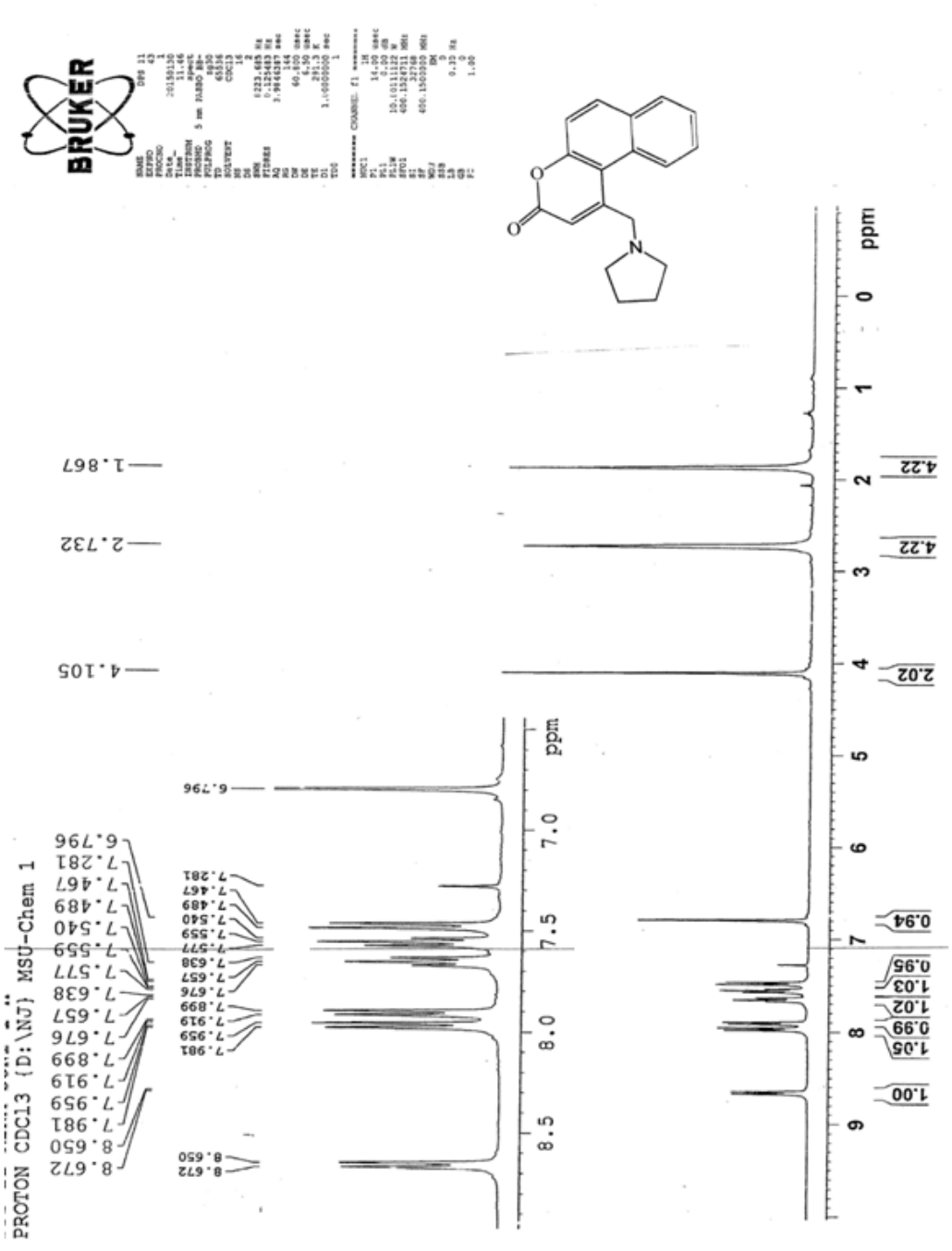


Figure-18 ^{1}H NMR spectrum of 1-(Pyrrolidin-1-ylmethyl)-3H-benzo[f]chromen-3-one,i.e 4e

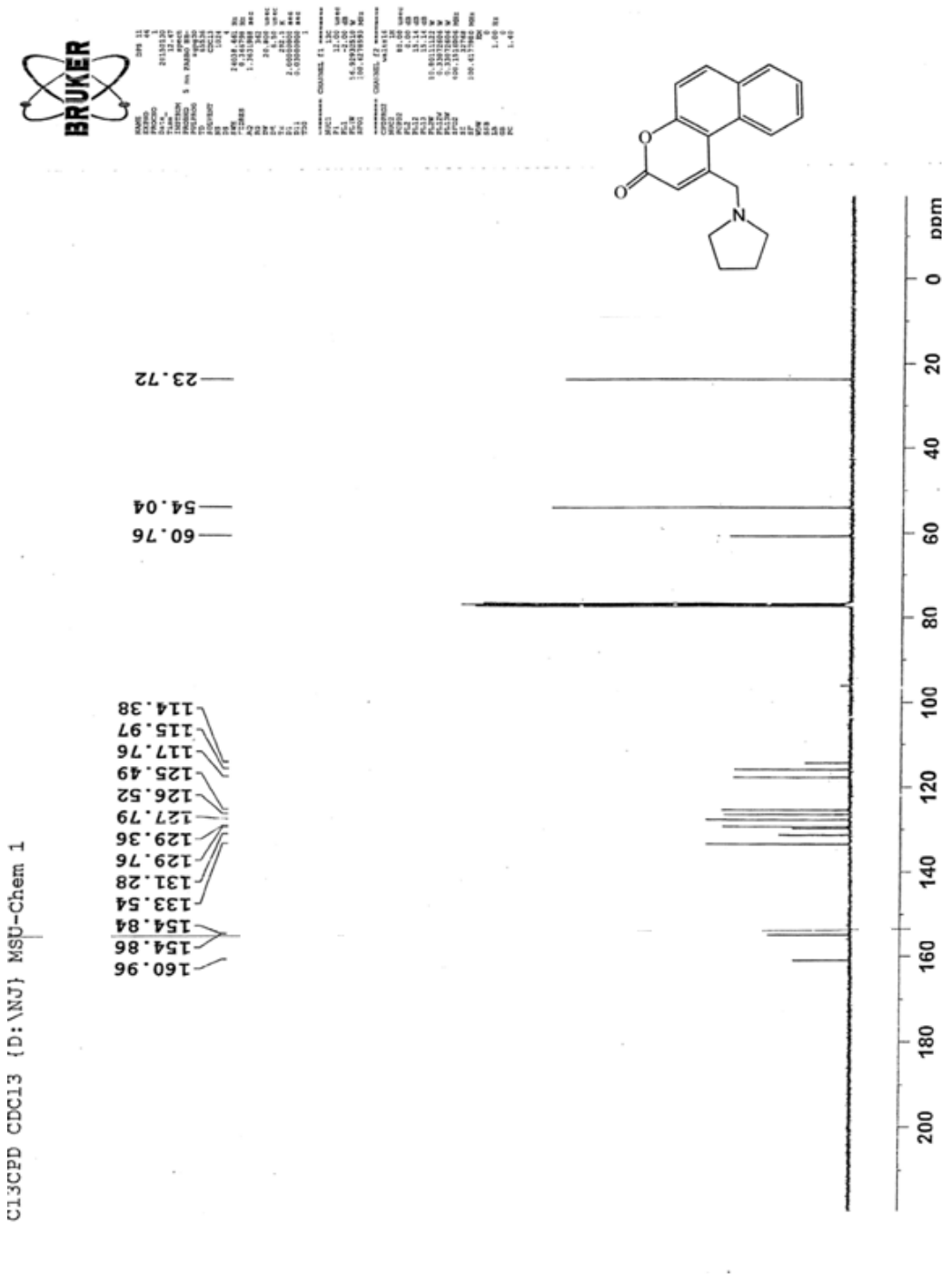


Figure-19 [13] C NMR spectrum of 1-(Pyrrolidin-1-ylmethyl)-3H-benzo[f]chromen-3-one,i.e 4e

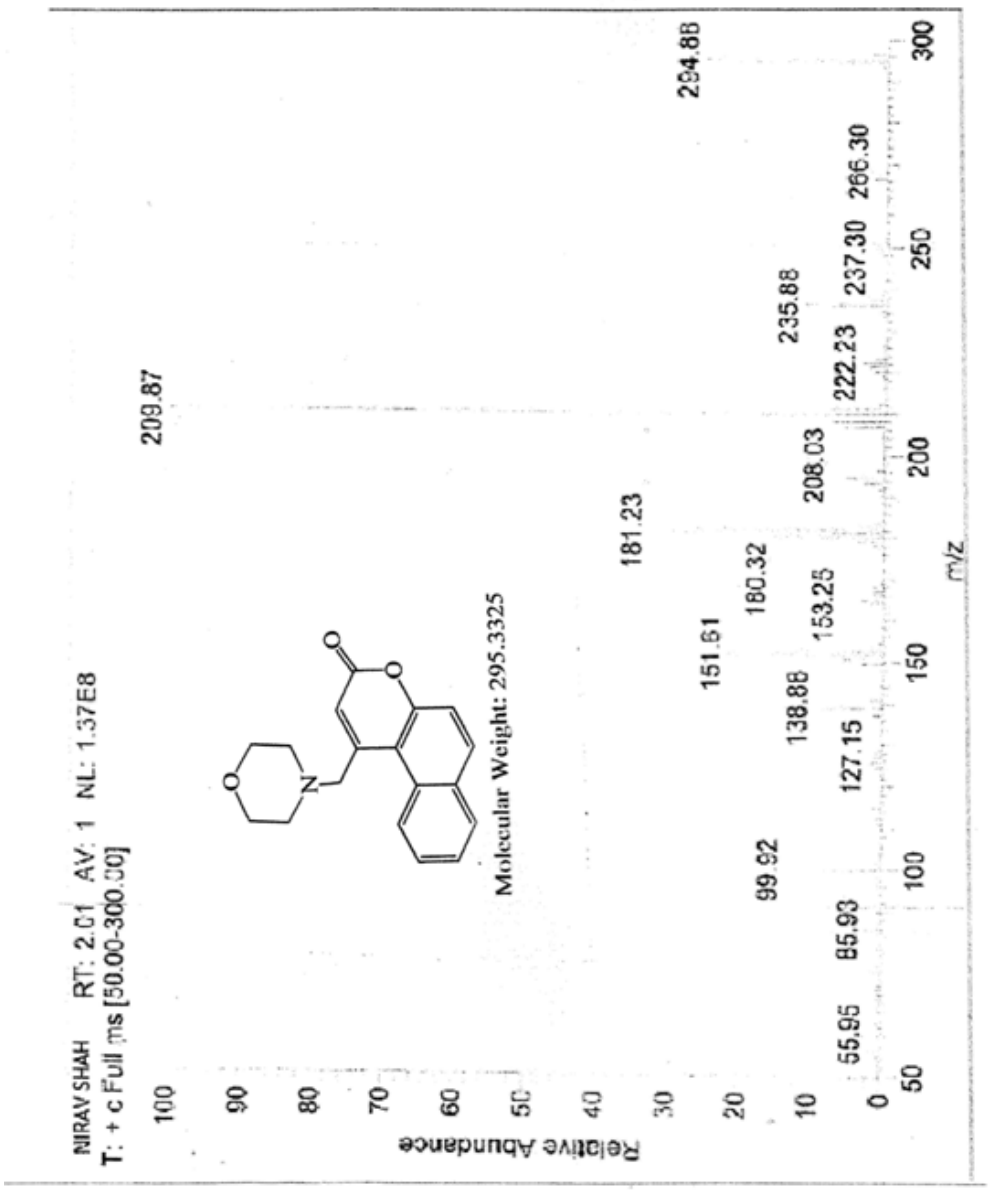


Figure-20 Mass spectrum of 1-(Morpholinomethyl)-3H-benzo[f]chromen-3-one i.e 4g.

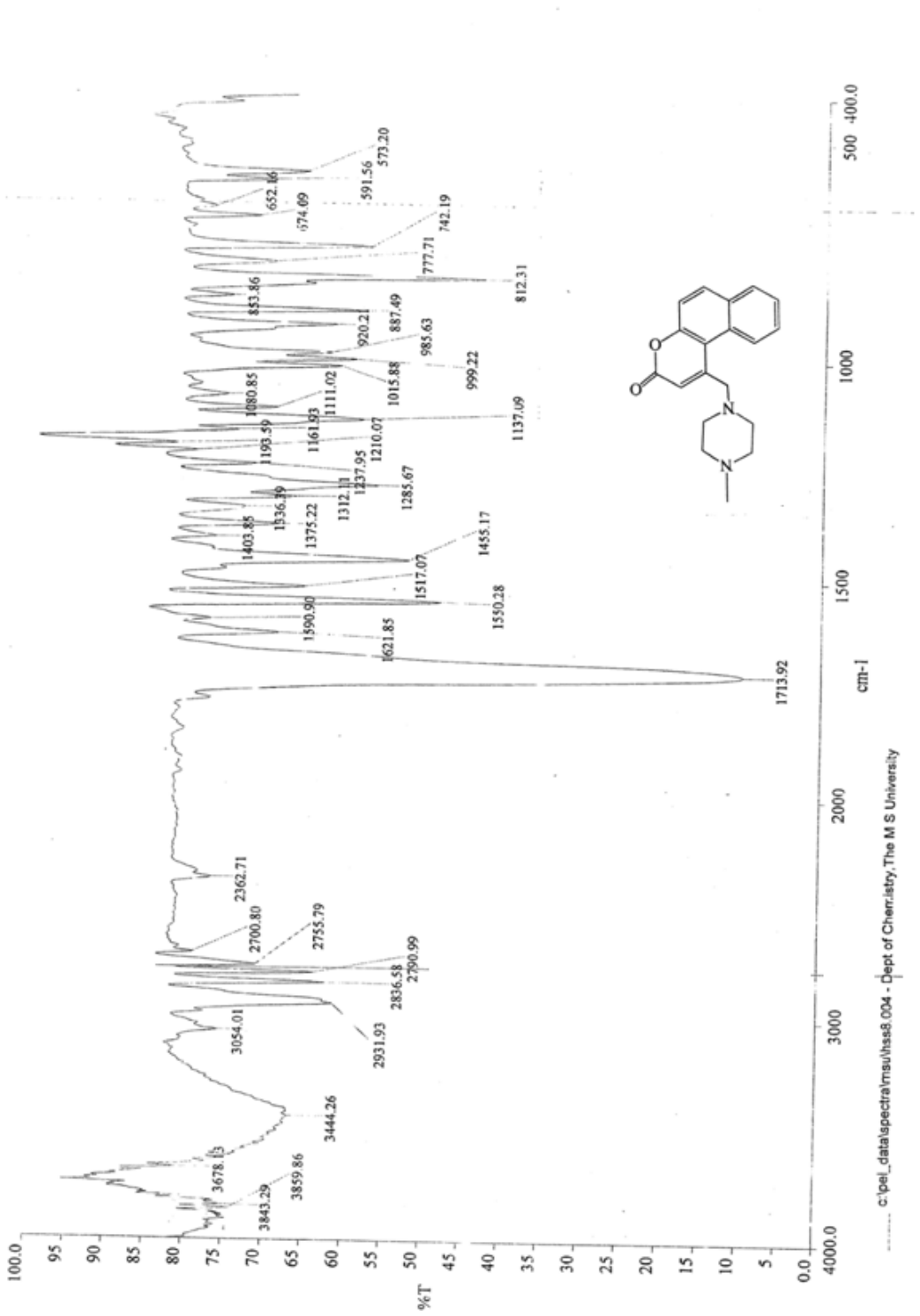


Figure-21 IR spectrum of 1-((4-methylpiperazin-1-yl) methyl)-3H-benzo[f]chromen-3-one i.e 4h

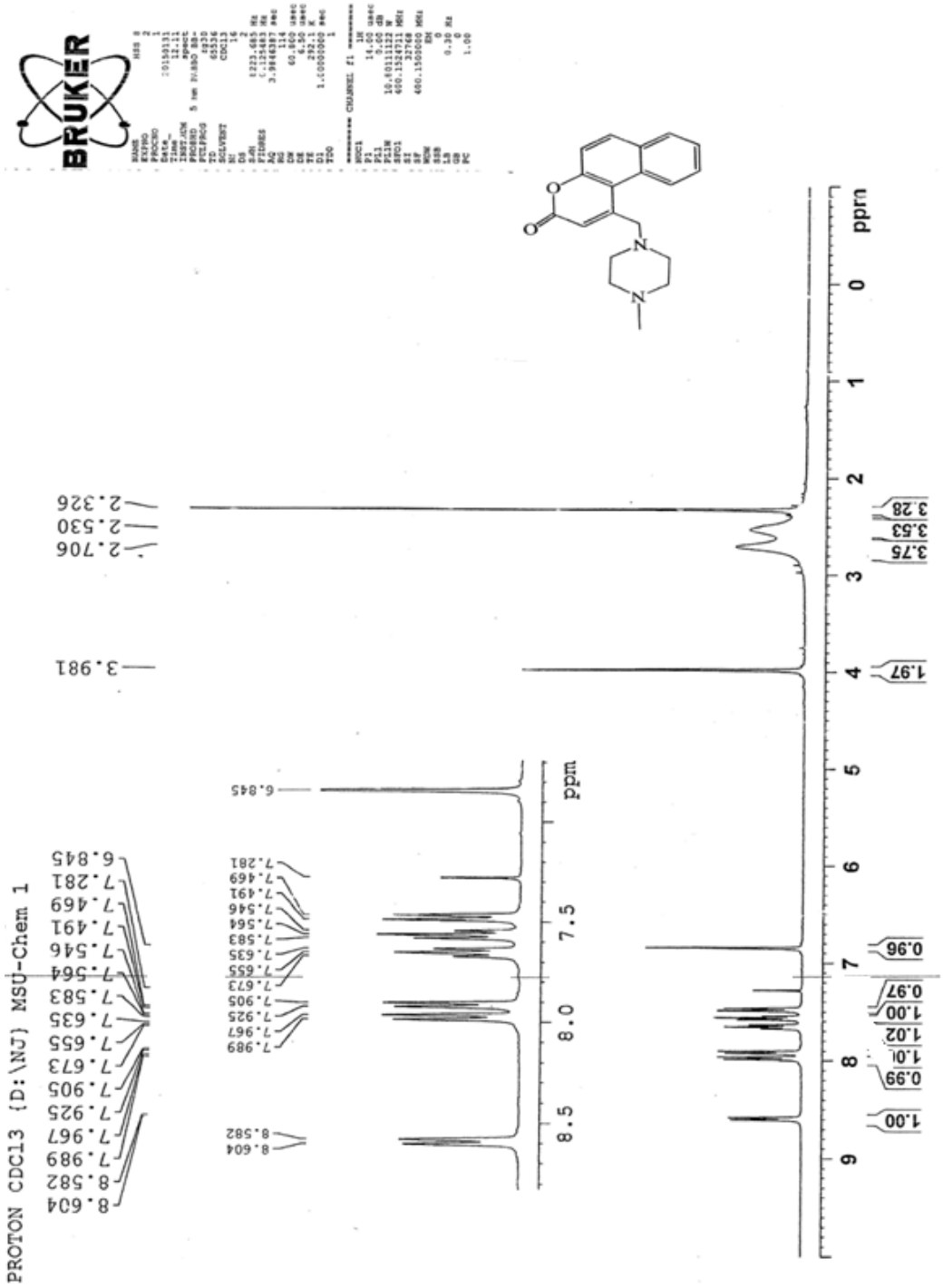


Figure-22 ^{1}H NMR spectrum of 1-((4-methylpiperazin-1-yl) methyl)-3H-benzo[f]chromen-3-one i.e 4h

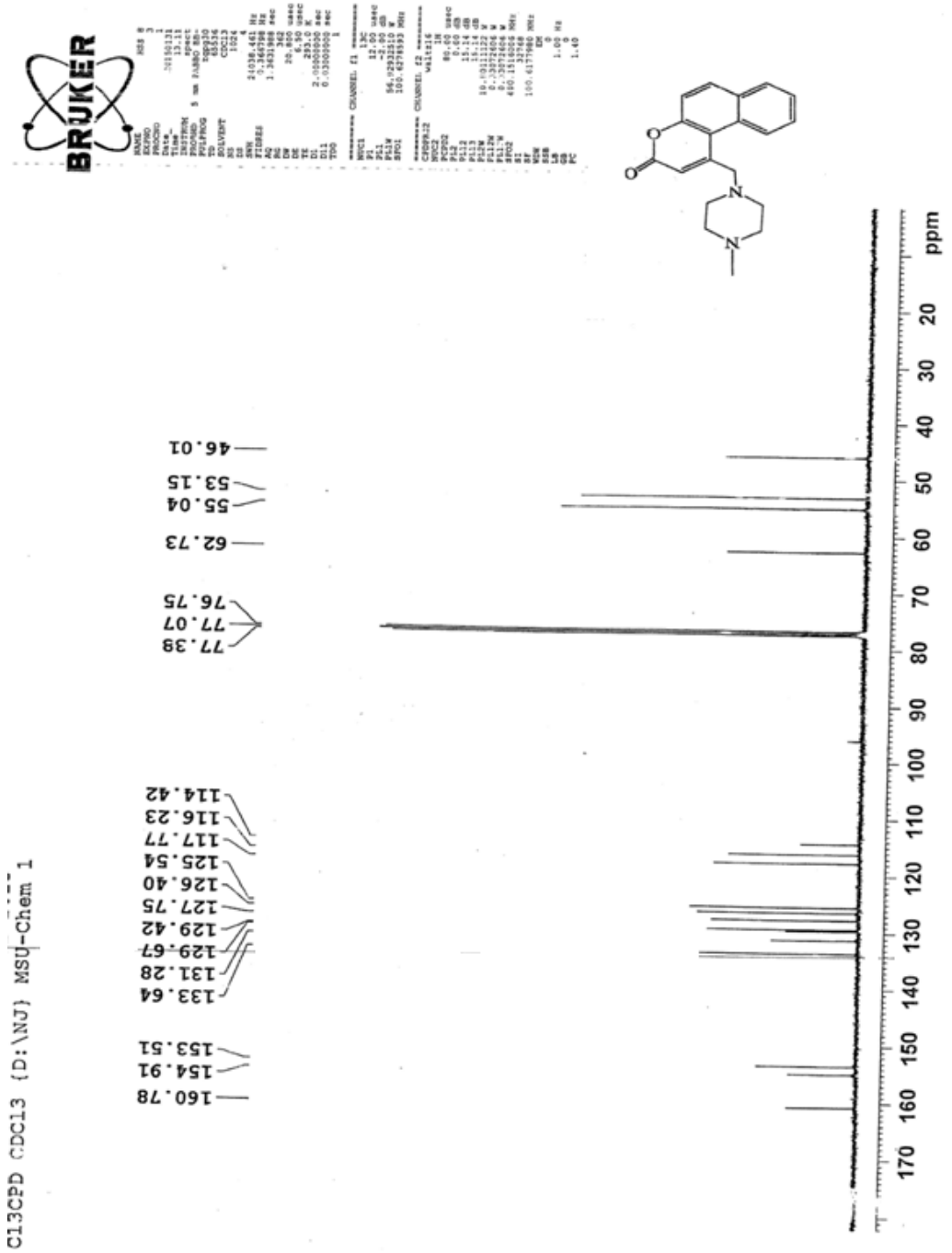


Figure-23 [13] C NMR spectrum of 1-((4-methylpiperazin-1-yl) methyl)-3H-benzo[f]chromen-3-one i.e 4h

2.2.2 Biological Evaluation

2.2.2.1 Antibacterial and antifungal activity

The antimicrobial and antifungal activity of Compounds **4a**, **4e**, **4f** and **4g** were screened by cup plate method[36] for two Gram positive bacteria *S. aureus* and *B. subtilis* two Gram negative bacteria *E. coli* and *P. aeruginosa* and one fungus *C. albicans* respectively Concentrations of compounds were ranging from 40 µg to 320 µg. The lowest concentration of compounds that prevented visible growth is given in Table 2. It was determined that the solvent has no antibacterial or antifungal activities against any of the test organisms. Ampicillin 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 2.

Compounds	MIC(µg)				
	Gram –Ve bacteria		Gram +ve bacteria		Fungi
	Escherichia coli	*Pseudomonas aeruginosa*	*Staphylococcus aureus*	*Bacillus Subtilis*	*Candida Albicans*
4a	>320	>320	>320	>320	>320
4e	>320	>320	>320	>320	>320
4f	>320	>320	>320	>320	>320
4g	>320	>320	320	**80**	>320
Ampiciline	5	2	15	7.5	-
Flucanazole	-	-	-	-	5

Table 2: MIC determination of antibacterial and antifungal agent (µM).

The compounds remained inactive except one 4g which showed moderate antimicrobial activity against one Gram-positive bacterium at 80 µg concentration.

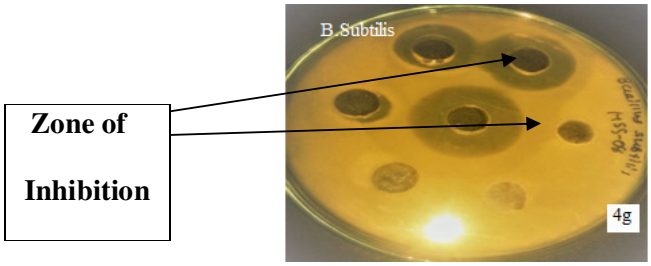


Figure-13 Zone of inhibition against Gram Positive bacteria (B.Subtilis) in compound **4g**

2.2.2.2 Anticancer activity

The MTT assay was performed to screen test compounds **4a-i** (Table 3)for their activity against three different cancer cell lines such as A549 (lung cancer cell-line), MCF7 (breast cancer cell-line) and A375 (melanoma cell-line). $IC_{50}{}^{a}$ (µM) values and $IC_{50}{}^{b}$ (µM) values were determined using Graph Pad prism software for compounds **4a-i** as shown in **Table -3** as shown bellow.

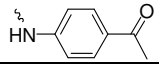
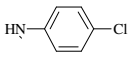
Compound	-NR^1R^2	$IC_{50}{}^{a}$			$IC_{50}{}^{b}$
		A549	MCF7	A375	A549
4a		4.29 µM	5.17 µM	9.02 µM	
4b		2.32 µM	47.80 µM	15.46 µM	
4c		NS	NS	NS	56.75 µM
4d		NS	NS	NS	59.14 µM
4e		6.20 µM	77.90 µM	84.45 µM	
4f		1.12 µM	0.83 µM	5.26 µM	
4g		NS	NS	NS	**0.32 nM**
4h		**0.74 µM**	14.24 µM	13.61 µM	
4i		NS	NS	NS	**19.98 nM**
5-Fluorouracil	---	11.13 µM	45.04 µM	28.6 µM	---

Table 3: Anticancer activity of substituted amino methyl naphthopyrone (**4a-i**).

[a]IC_{50} values were determined using Graph Pad Prism software by MTT assay using DMSO. [b]IC_{50} values were determined using Graph Pad Prism software by MTT assay using DMF. NS= Not Determined as insoluble in DMSO. NA = Not active.

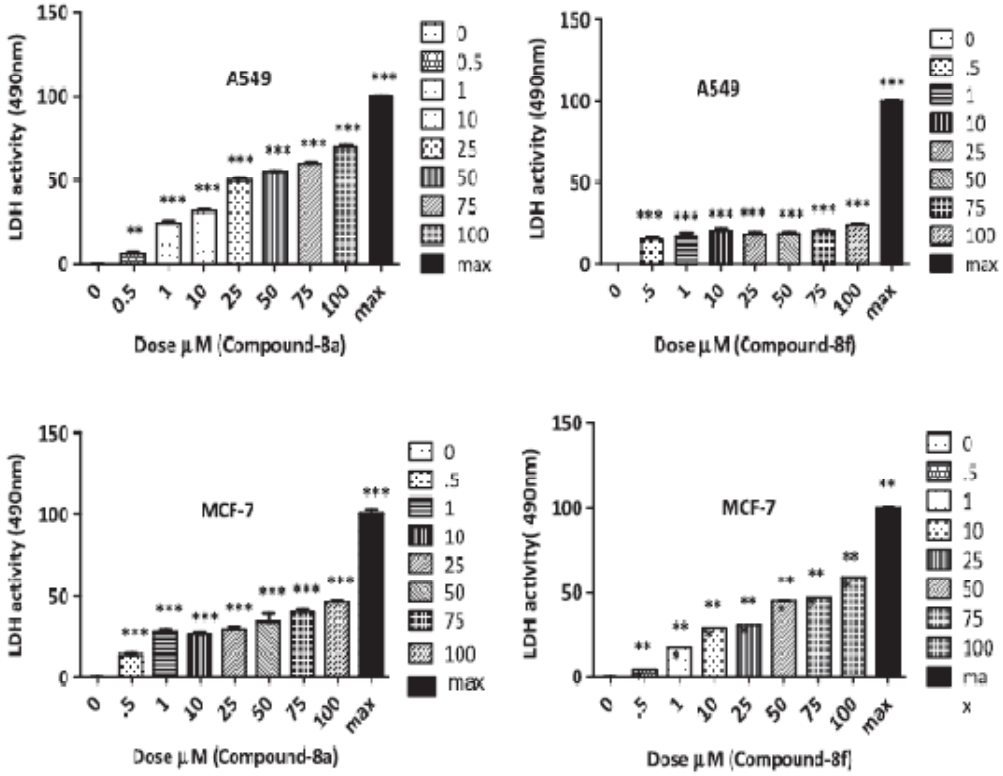
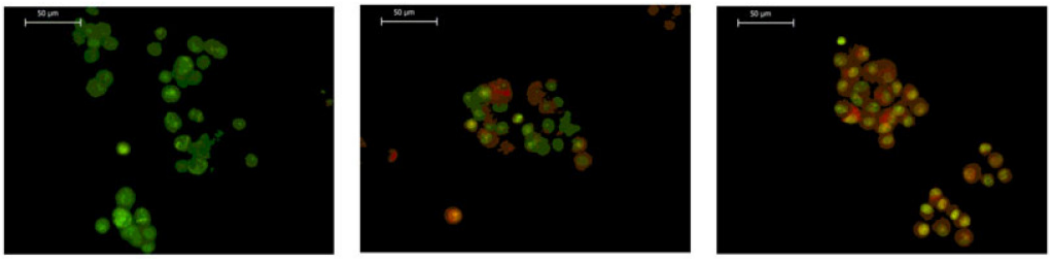
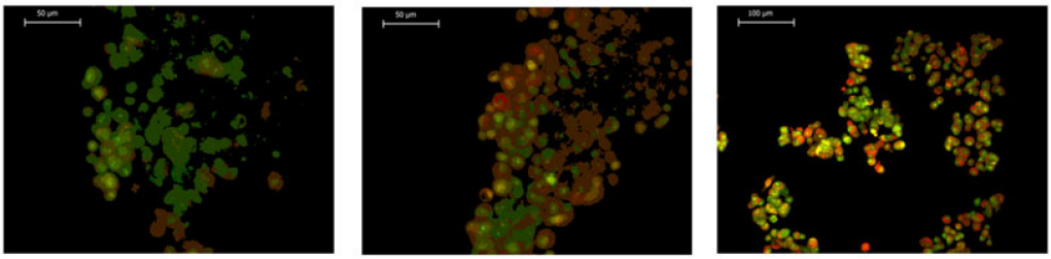


Figure-**14** (a) Representation of Cytosolic enzyme LDH in A549 cell line; activity of LDH in A549 treated with different concentration of compounds 4a and 4f Graph plotted against LDH release value dose (***$P,\leq .001$, **$p< 01$, Significance one way ANOVA (Tukey –Kramer).(b) Representation of Cytosolic enzyme LDH in MCF-7 cell line; activity of LDH in MCF-7 treated with different concentration of compounds 4a and 4f Graph plotted against LDH release value dose (***$P,\leq .001$, **$p< 01$, Significance one way ANOVA (Tukey –Kramer).ANOVA, analysis of variance; LDH, lactic dehydrogenase.



(Control-Untreated) (4a Treated) (4f-Treated)



(Control-Untreated) (4a Treated) (4f-Treated)

Figure-**15**. (a) Ethidium bromide/ acridine orange staining indication of apoptosis in A549 cell line when treated with IC_{50} (µM) concentration of compounds. (b) Ethidium bromide/ acridine orange staining indication of apoptosis in MCF-7 cell line when treated with IC_{50} (µM) concentration of compounds.

[Color figures can be viewed at wileyonlinelibrary.com]

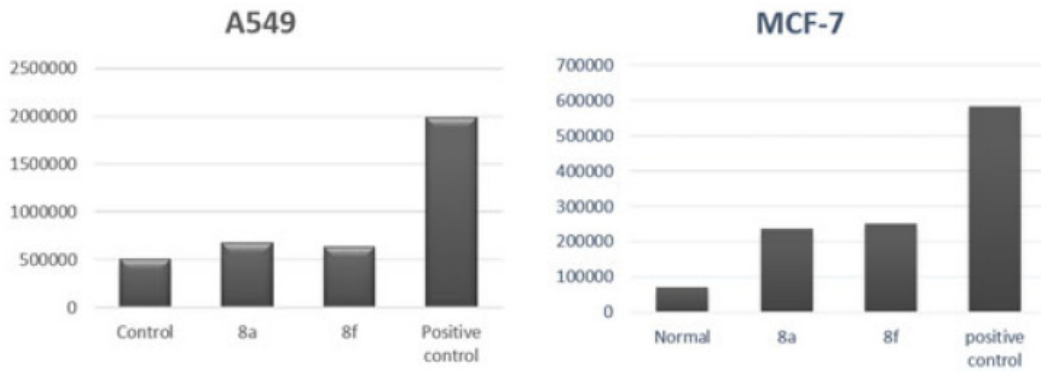


Figure-**16 :** Representation of intracellular reactive oxygen species production in treated cell lines; reactive oxygen species production was measured using 2,4 dichlorodihydrofluorescein in diacetate dye. [Color figures can be viewed at wileyonlinelibrary.com]

Results from MTT assay were used to assess the growth inhibitory effect of the various compounds on three types of cancer cells (A549, MCF7 and A375). IC_{50} values were calculated to determine the concentration of test compound at which 50% of the cells are killed.

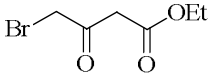
From the MTT assay, compound **4a** showed better activity against A375 with IC_{50} 4.29μM, against MCF7 with IC_{50} 5.17μM and against A549 cell line with IC_{50} 9.02μM. When methyl substituent on aromatic ring was replaced by acetyl group in compound **4b**, it resulted in loss of activity against MCF7 cell line. Replacement of methyl group with halogens, compounds **4c** with –Cl and compound **4d** with –F resulted in compounds with poor solubility in DMSO; hence these compounds were screened against A549 cell line in DMF. Both compounds **4c** and **4d** found inactive and not showed anticancer activity against A549 cell line. On replacement of aromatic amine with pyrrolidine ring as in compound **4e**, showed good activity against A549 cancer cell line with IC_{50} 6.20 μM, while no anticancer activity observed against MCF7 and A375 cell lines. Interestingly, piperidine substituted compound **4f** showed very good activity pattern against all tested cancer cell lines. Compound **4f** gave IC_{50} 1.12 μM for A549 cell line and 0.83μM for MCF7cell line. Further, replacement of piperidine with *N*-methyl piperazine in compound **4h** resulted in very good activity against A549 cancer cell line with IC_{50}0.74 μM, while good activity was observed against MCF7 and A375 cancer cell line with IC_{50}14.24 μM and 13.96 μM respectively. On the other hand replacement of piperidine moiety with morpholine in compound **4g** and tetra hydro isoquinoline in compound **4i**, gave compounds with poor solubility in DMSO, hence these compounds were screened against A549 cell line in DMF. Compound **4g** showed excellent activity withIC_{50} 0.32 nM for A549 cell line, other hand compound **4i** showed very good activity with IC_{50} 19.98 nM. Compounds **4g** and **4i** showed promising inhibitory potential with IC_{50} values falling in nM region.

2.3 Experimental

Reagent grade chemicals and solvents were purchased from commercial supplier and used after purification. TLC was performed on silica gel F254 plates (Merck). Acme's silica gel (60-120 mesh) was used for column chromatographic purification. All reactions were carried out in nitrogen atmosphere. 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 Eager Xperience.

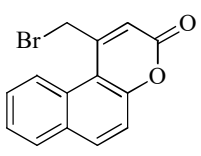
2.3.1 Chemistry

2.3.1.1 Preparation of *ethyl 4-bromo-3-oxobutanoate.* 1



To an ice-cold solution of ethyl acetoacetate (25.24 mL), liquid bromine (10.25 mL) added dropwise over a period of 10-15 min. The resulting solution stirred at 0-5 ^{o}C for 30 min and at room temperature for 24 hrs. The mixture thus obtained was diluted with ice-cold water and neutralised with saturated sodium bicarbonate (Na_2CO_3) solution in saturated sodium chloride (NaCl) solution. The organic layer separated, filtered through calcium chloride ($CaCl_2$) to give reddish brown oil. The ethyl 4-bromo-3-oxobutanoate **1** thus obtained (25 g), used directly for next step.

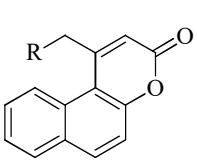
2.3.1.2 Preparation of *1-(bromomethyl)-3H-benzo[f]chromen-3-one.* 3



To an ice-cold solution of con H_2SO_4 (30mL),ethyl 4-bromo-3-oxobutanoate **1**(9mL) was added slowly followed by portion wise addition of β-naphthol **2**(8.5 g) over a period of 10-15 min. Resulting

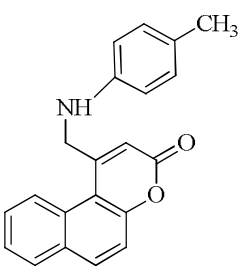
mixture was stirred at room temperature for 48 hrs. The reaction mixture poured on crushed ice. The solid obtained was filtered and recrystallized from acetic acid to obtained compound **3** as golden yellow crystals. Yield :79.81%; M.P : 180 ºC; IR (KBr): 3060, 1725, 1545, 1515, 1210, 1007, 928, 899, 825, 710 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): 4.90 (s, 2H), 6.66 (s, 1H), 7.51 (d, *J*=9.2 Hz, 1H), 7.61 (m, 1H), 7.63 (m, 1H), 7.97 (d, *J*=9.2 Hz, 1H), 8.05 (d, *J*=8.8 Hz, 1H), 8.54 (d, *J*=8.4 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): 32.73, 112.36, 117.89, 118.21,125.35, 125.86, 128.54, 128.76, 129.89, 131.37, 134.46, 151.78, 155.34, 159.97; ESI Ms: 290.11(M+2), 287.63 (M+, basepeak), 210.14, 181.16, 151.43, 75.84.

2.3.1.3 General procedure for the preparation of compounds



4-bromomethylnaphthopyrone **3**(500 mg) dissolved in DMF (20-30 mL) and substituted amine (1.1eq), along with base triethylamine (1.5 eq) was added to it. The resulting mixture was stirred at room temperature for 16 hrs and then poured into cold water. The aqueous layer thus obtained was extracted using ethyl acetate and/or dichloromethane (checked by TLC) and solvent evaporated to give crude product. The product thus, obtained was purified by column chromatography using pet ether: ethyl acetate.

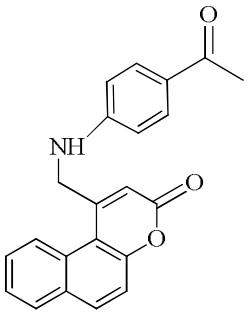
2.3.1.3.1 1-(p-tolylamino) methyl)-3H-benzo[f]chromen-3-one (4a)



Yield: 39 %; m.p:180-182 ºC; IR (KBr): 3859, 3616, 3355, 2840, 2384, 1779, 1720, 1617, 1550, 1519, 1447, 1399, 1373, 1333, 1300, 1256 , 1195, 1130, 1089, 998, 925, 865, 821, 807, 779, 745, 640, 588 cm^{-1}; ^{1}H NMR(400 MHz, $CDCl_3$): δ 2.26 (s, 3H), 4.23 (br s, 1H), 4.90 (d, *J*=5.2 Hz, 2H), 6.84 (s, 1H), 7.02 (d, *J*=8.0 Hz, 2H), 7.51 (d, *J*=8.8 Hz, 1H), 7.58 (m, 1H), 7.65 (m ,1H), 7.99 (m, 1H), 8.40 (d,1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 20.43, 49.72, 113.03, 113.55, 114.28, 117.93, 125.51, 125.60, 127.95,

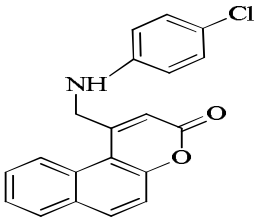
128.28, 129.56, 129.88, 129.98, 131.41, 133.85, 144.23, 155.03,160.68; MS (ESI, *m/z*): 315.8 (M^+, base peak) for $C_{21}H_{17}NO_2$, M.W=315.36g/mol

2.3.1.3.2 1-(4-Acetylphenyl) amino) methyl)-3H-benzo[f]chromen-3-one (4b)



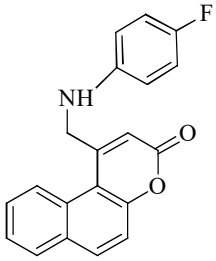
Yield: 54 %; m.p: 194-196 ºC; IR (KBr): 3069, 2969, 2784, 1724, 1676, 1560, 1549, 1458, 1342, 1302, 1269, 1236, 1190, 1149, 987, 919, 858, 825, 738 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): 2.40 (s, 3H), 5.05 (d, *J*=5.3 Hz, 2H), 6.50 (s, 1H), 6.67 (d, *J*=8.6Hz, 2H), 7.39 (t, *J*=5.3 Hz, 1H), 7.65-7.60 (m, 2H), 7.70 (d, *J*=7.2 Hz, 1H), 7.74 (d, *J*=8.6 Hz, 2H), 8.11 (d, *J*=8.0 Hz, 1H), 8.25 (d, *J*=8.8 Hz, 1H), 8.51 (d, *J*=8.8 Hz, 1H); ^{13}C NMR(100 MHz, $CDCl_3$): 26.43, 47.68, 111.77, 112.97, 113.73, 117.98, 126.14, 126.42, 126.64, 128.82, 129.51, 130.10, 131.02, 131.51, 134.65, 152.26, 154.85, 155.94, 160.00, 195.74;MS(ESI, *m/z*):342 (M-1) for $C_{22}H_{17}NO_3$, M.W=343.37.

2.3.1.3.3 1-(4-Chlorophenylamino) methyl)-3H-benzo[f]chromen-3-one (4c)



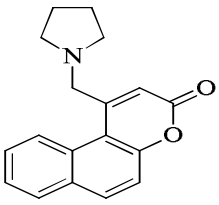
Yield: 42 %; m.p:195-197 ºC; IR (KBr): 3365, 1692, 1602, 1550, 1506, 1446, 1335, 1270, 1088, 1002, 819, 749cm^{-1}; ^1H NMR(400 MHz, $CDCl_3$): δ 4.84 (d, *J* = 5.3 Hz, 2H), 6.22 (t, *J* = 5.3 Hz 1H), 6.44 (d, *J*= 8.8 Hz, 2H), 6.61 (s, 1H), 6.96 (d, *J* = 8.8 Hz , 2H), 7.42 (d, *J*=8.8 Hz, 2H), 7.50 (t, *J*=7.4Hz, 1H), 7.14 (m, 1H), 7.91 (d, *J* = 8.0 Hz, 1H), 8.00 (d, *J* = 9.2 Hz, 1H), 8.33 (d, *J* = 8.4 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 53.51, 117.81, 118.41, 118.53, 122.44, 126.06, 130.47, 130.59, 133.15, 133.72, 134.28, 134.66, 136.14, 138.84, 150.96, 159.54, 160.52, 165.28; MS (ESI, *m/z*): 336.8 (M +1), base peak calculated for $C_{20}H_{14}ClNO_2$, M.W = 335.78 g/mol

2.3.1.3.4 1-(4-Fluorophenylamino) methyl)-3H-benzo[f]chromen-3-one (4d)



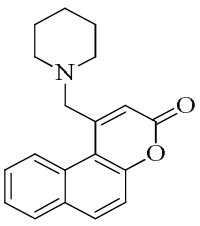
Yield: 54 %; m.p:175-177 ºC; IR (KBr): 3369, 2897, 1690, 1550, 1515, 1447, 1336, 1223, 1212, 1002, 818, 746 cm^{-1}; ^1H NMR(400 MHz, $CDCl_3$): δ 4.10 (d, *J* = 5.2 Hz, 2H),5.52 (br t, 1H), 5.75-5.76 (m, 2H), 5.85 (s, 1H), 6.04-6.08 (m, 2H), 6.73 (d, *J*=8.8 Hz, 1H), 6.80(t, *J*=7.4 Hz, 1H),6.88(t, *J*=7.4 Hz, 1H),7.24 (d, *J* = 8.0 Hz, 1H), 7.36 (d, *J* = 9.2 Hz, 1H), 7.66 (d, *J* = 8.4 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 43.54, 107.49, 107.84, 108.26, 110.11, 110.33, 112.25, 120.39, 120.70, 123.07, 124.03, 124.48, 125.93, 128.81, 138.78, 149.26, 150.88, 154.88; MS (ESI, *m/z*): 320.2 (M+1).calculated for $C_{20}H_{14}FNO_2$, M.W = 319.3g/mol

2.3.1.3.5 1-(Pyrrolidin-1-ylmethyl)-3H-benzo[f]chromen-3-one (4e)



Yield : 38 %; m.p : 128-130 ºC; IR (KBr): 3068, 2966, 2784, 1724, 1676, 1654, 1560, 1549, 1518, 1458, 1342, 1236, 1190, 1149, 1112, 987, 919, 858, 825, 738 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): 1.86 (brs, 4H), 2.73 (s , 4H), 4.10 (s, 2H), 6.79 (s, 1H), 7.47 (d, *J*=8.8 Hz, 1H), 7.55 (t, *J*=7.5 Hz, 1H), 7.65 (t, *J*=7.5 Hz, 1H), 7.90 (d, *J*=8.0 Hz, 1H), 7.97 (d, *J*=8.8 Hz, 1H), 8.66 (d, *J*=8.8 Hz, 1H); ^{13}C NMR(100 MHz, $CDCl_3$): 23.72, 54.04, 60.76, 114.38, 115.97, 117.76, 125.49, 126.52, 127.79, 129.36, 129.76, 131.28, 133.54, 154.84, 154.86, 160.96; MS (ESI, *m/z*): 278(M-1) for $C_{18}H_{17}NO_2$, M.W=279.33g/mol.

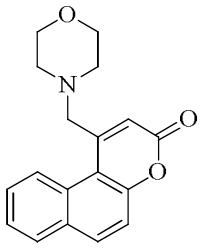
2.3.1.3.6 1-(piperidine-1-ylmethyl)-3H-benzo[f]chromen-3-one (4f)



Yield: 58 %; m.p:138-140 ºC; IR (KBr):3056, 2924, 2838, 2809, 2764, 1711, 1646, 1570, 1516, 1453, 1403, 1373, 1334, 1270, 1236, 1190, 1129, 1108, 1037, 996, 927, 885, 810, 740, 673, 609, 571; ^1H

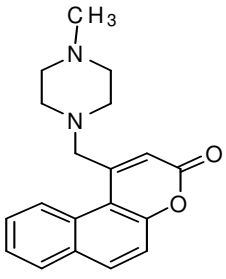
NMR(400 MHz, $CDCl_3$): δ 1.51 (br s , 2H), 1.65 (m , 4H), 2.59 (m , 4H), 3.91 (s, 2H), 6.84 (s, 1H), 7.48 (d, *J*=8.8 Hz, 1H), 7.56 (m, 1H), 7.65 (m, 1H), 7.91 (m, 1H), 7.97 (d, *J* =8.8 Hz, 1H), 8.67 (d, *J*=8.8 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ ppm 24.02, 26.02, 54.68, 63.70, 114.60, 116.15, 117.76, 125.48, 126.64, 127.65, 129.34, 129.77, 131.27, 133.50, 154.23, 154.86, 160.9; MS (ESI, *m/z*): 293.20 (M^+), 209.84 (b), 181, 151.calculated for $C_{19}H_{19}NO_2$, M.W=293.36.

2.3.1.3.7 1-(Morpholino methyl)-3H-benzo[f]chromen-3-one (4g)



Yield: 57 %; m.p: 186-188 ºC; IR (KBr): 3059, 2955, 2887, 2839, 1712, 1623, 1549, 1515, 1454, 1428, 1373, 1315, 1250, 1192, 1113, 1000, 911, 883, 861, 817, 738 cm^{-1}; ^{1}H NMR(400 MHz, $CDCl_3$): 2.67 (br s, 4H), 3.78 (br s, 4H), 3.99 (s, 2H), 6.84 (s, 1H), 7.56 (s, 1H), 7.59 (m,1H), 7.66 (m, 1H), 7.92-7.94 (m, 1H), 7.98 (d, *J*=8.8 Hz, 1H), 8.59 (d, *J*=8.8 Hz, 1H); ^{13}C NMR(100 MHz, $CDCl_3$): 53.61, 63.16, 66.91, 114.30, 116.27, 117.77, 125.59, 126.25, 127.77, 129.49, 129.61, 131.29, 133.74, 152.98, 154.96, 160.67; MS (ESI, *m/z*: 294.88 (M+),209.87(b), 181, 151 :Molecular Formula : $C_{18}H_{17}NO_3$, M.W=295.33g/mol

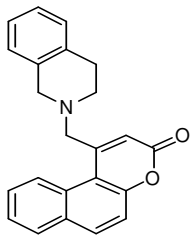
2.3.1.3.8 1-((4-methylpiperazin-1-yl) methyl)-3H-benzo[f]chromen-3-one (4h)



Yield: 42 %; m.p:124-126 ºC; IR (KBr, cm^{-1}): 3859, 3843, 3444, 3054, 2931, 2836, 2790, 2755, 2700, 2363, 1713, 1621, 1550, 1517, 1455, 1375, 1285, 1193, 1137, 1015, 999, 985, 887, 812, 777, 742, 674, 591, 573; ^{1}H 9NMR(400 MHz, $CDCl_3$): δ 2.32 (s, 3H), 2.53 (br s, 4H), 2.71 (brs, 4H), 3.98 (s, 2H), 6.84 (s, 1H), 7.48 (d, *J*=8.8 Hz, 1H), 7.54-7.58 (m, 1H), 7.65 (m, 1H); 7.91 (d, *J*=8.0Hz,1H), 7.97 (d, *J*=8.8 Hz, 1H), 8.59 (d, *J*=8.8 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 46.01, 53.015, 55.04, 62.73, 114.42, 116.23, 117.77, 125.54, 126.40, 127.75, 129.42, 129.67, 131.28, 133.64, 153.51,

154.91, 160.78; MS (ESI, *m/z*): 308.75 (M^+, basepeak) Molecular Formula:$C_{19}H_{20}N_2O_2$:M.W= 308.37g/mol

2.3.1.3.9 **1-((3,4-Dihydroisoquinolin-2(1H)-yl)methyl)-3H-benzo[f]chromen-3one (4i)**



Yield : 49 %; m.p : 172-174 ºC; IR (KBr):3022, 2908, 2828, 2799, 1713, 1645, 1570, 1552, 1532, 1452, 1426, 1336, 1268, 1194, 1130, 1095, 1054, 1000, 930, 883, 855, 813, 780, 739 cm-1; ^{1}H NMR(400 MHz, $CDCl_3$) : 2.99, s , 4H ; 3.87, s , 2H ; 4.10, s , 2H ; 6.90, s , 1H ; 7.06, m 1H ; 7.16, m , 3H ; 7.51, d , 1H(*J*=8.8 Hz) ; 7.52-7.58, m ,1H ; 7.63-7.67, m, 1H ; 7.91, d , 1H ; 8.1, d , 1H ; 8.7, d ,1H; ^{13}C NMR(100 MHz, $CDCl_3$) : 29.21, 51.01, 55.90, 62.84, 125.55, 125.82, 126.41, 126.56, 127.83, 128.73, 129.44, 129.71, 131, 133, 134.08, 134.12, 153, 155, 160.Molecular Formula:$C_{23}H_{19}NO_2$,M.W=341.40g/mol.

2.3.2 Biological activity screening

2.3.2.1 Antimicrobial activity:

Antibacterial activity of the four synthesized compounds was tested in vitro by (cup plate method)/ serial agar dilution in which bacterial strains of Gram negative (*Escherichia coli*, *Pseudomonas aeruginosa*) and Gram positive (*Staphylococcus aureus, Bacillus Subtilis*) were used, using serial agar dilution (cup plate method).The two microorganisms were cultured in petri dishes containing agar medium, cups (8 mm) were put onto the dishes and each synthesized compound dissolved in DMF (0.1 ml of 10 mg/ml) was added into the cups under aseptic condition. Then, the petri dishes were incubated at 37 ^{o}C for 24 h. The zone of inhibition of the growth of the bacteria, which were produced by diffusion of the compounds from the cup into the surrounding medium, was measured to evaluate the antibacterial activity. Each experiment was repeated twice. DMF was used as a positive control for the experiments.

2.3.2.2 MTT assay

The compounds were tested for their cytotoxic potential on three types of cancer cells, *viz.*, A549 (lung cancer cell-line), MCF7 (breast cancer cell-line) and A375 (melanoma cell-line). The MTT assay was used to determine the effect of each compound on the proliferation of cancer cells.

A549, MCF7 and A375 cultures were purchased from National Centre for Cell Science, Pune, India. All growth media, supplements and reagents were purchased from HiMedia Labs, Mumbai, India. For the assay, cells were seeded at 10^5 cells/ml in a 96-well plate in dulbecco's modified minimum essential medium (DMEM) supplemented with 10% fatal bovine serum (FBS). To each well, test compound was added at six different concentrations of 100µM, 50µM, 10µM, 5µM, 1µM and 0.5µM. Each concentration was tested in triplicates. The cells were incubated with these compounds at 37^oC under 5% CO_2 for 48 hours. Following this, 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) was added to each well at a final concentration of 0.5mg/ml. Cells were incubated with this tetrazolium dye for 4 hours. Subsequently, purple crystals of formazan were observed in each well, formed as a metabolic product of MTT. These crystals were dissolved in Isopropanol and the absorbance in each well was recorded at 570nm in a micro plate reader (MicrotekSigma360). Absorbance at 570nm directly correlates with cell viability.IC_{50} (µM) values were determined using Graph Pad prism software.

2.4 Conclusion

In conclusion, we report here synthesis of substituted amino methyl naphthopyrone derivatives **4a**-**4i.** The structures of all the synthesized compounds were elucidated by modern analytical techniques and confirmed. All the synthesized compounds were studied for their antimicrobial and anticancer activity. Compound **4g** showed promising antimicrobial activity with MIC 80 µg against Gram negative bacteria *Bacillus subtilis*. Rest of the tested compounds remains inactive against gram positive bacteria, gram negative bacteria and against fungi *Candida albicans.* Compounds **4a, 4f** and **4g** are showing promising anticancer activity against A549 (lung cancer cell-line), MCF7 (breast cancer cell-line) and A375 (melanoma cell-line). Compound **4f** is showing very good activity against A549 (lung cancer cell-line) and MCF7 (breast cancer cell-line) with IC_{50} values 1.12 and 0.83 µM respectively. Both compounds **4g** and **4i** tested against A375 (melanoma cell-line) are showing excellent activity with IC_{50} values 0.32 nM and 19.98 nM respectively. The cytotoxicity of these compounds is due to apoptosis by LDH assay method, intracellular ROS assay and ETBr/AO assay.

2.5 References

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