CHAPTER-3

Synthesis of some new derivatives of Coumarinyloxy acetamide and their antimicrobial & anticancer evaluation.

3. Synthesis of some new derivatives of Coumarinyloxy acetamide and their antimicrobial & anticancer evaluation.

3.1 Introduction

The numbers of incidences of bacterial and fungal infections have increased significantly in the past 50 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*), *Streptococus pneumoniae* and *Enterococci* are resistant to Methicillin (MRSA), 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. Cancer is a fatal disease in terms of morbidity and mortality affecting human health worldwide.[3]

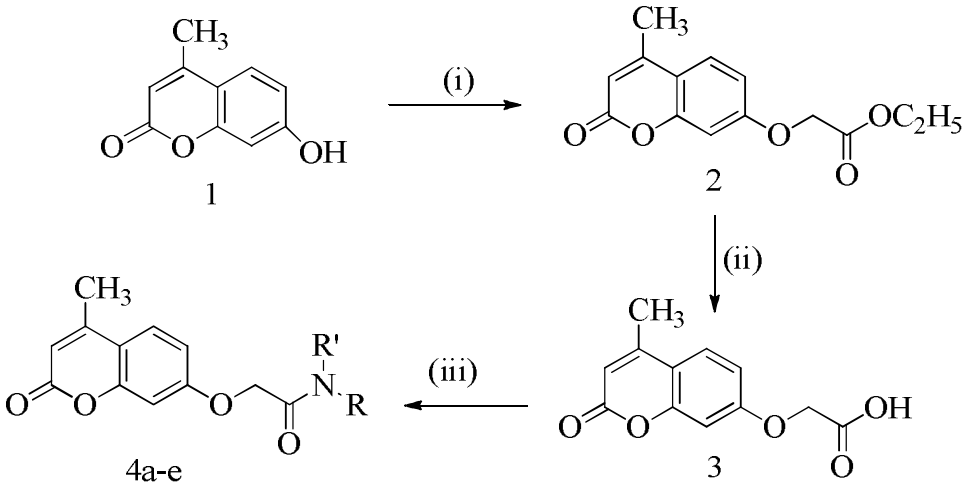
In order to overcome the threat of wide spread multi drug resistance in Gram positive and Gram negative bacterial strains as well as fungi and cancer, there is ongoing demand for new antimicrobial and anti cancer agents.

Coumarin is well known plant derived natural product which is extensively used as a biologically active compound .Natural and synthetic coumarin have been verified to have antimicrobial[4,5].anticoagulation[6],antiallergic[7],anticancer[8],antioxidant[9,10],Calcium channel blocking activity[11] Because of variety of biological activities, Ismail et al reported that anticancer activity of coumarins having topoisomerase-I inhibition.[12] The coumarin containing antibiotic is active agent against gram positive bacteria as potent inhibitor of DNA replication[13].S.S.Soman et al have reported the work on coumarin amide derivatives of benzodifuran -2-carboxylic acid [14] as potent antimicrobial agents and some coumarin derivatives as potent anticancer agents[15].In continuation of work on synthesis of antimicrobial agents[16, 17] we report herein synthesis , antimicrobial and anticancer evaluation of some of the derivatives of coumarinyloxy acetamide.

3.2 Result and discussion

3.2.1 Chemistry

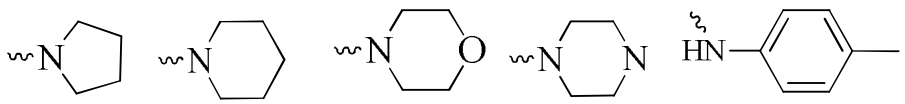
7-hydroxy-4-methyl-benzopyran-2[H]-one (**1**) was prepared by Pechmann condensation of resorcinol with ethylacetoacetate using concentrated sulfuric acid. The compound 7-Hydroxy-4-methyl coumarin (**1**), thus obtained was crystallized from methanol on reaction with ethylchloroacetate in presence of anhydrous potassium carbonate in dimethylformamide (DMF) gave 2-(4-methyl)-2-oxo-2[H]-chromen-7-yloxy) acetate **2**. The compound 2 on hydrolysis with 15% KOH in ethanol gives corresponding compound **3.** Compound 3 upon reaction with oxylyl chloride gave corresponding acetyl chloride derivative which on reaction with various aromatic or aliphatic primary or secondary amines in situ gave the desire compounds **4(a-e)**.



Scheme-1 Synthesis of coumarinyloxy acetamide derivatives.

Reagents & Conditions: (i) $ClCH_2COOC_2H_5$, K_2CO_3, DMF reflux; (ii) Ethanolic KOH (15 %), reflux; (iii) (1) ClCOCOCl, DMC at 0-5°C, 30 min, RT,4 h; (2) RR'NH, TEA, DCM.

Where in R, R'=



4a 4b 4c 4d 4e

The structure of compound 2 was confirmed from its IR, ^{1}H-NMR and ^{13}C-NMR spectral techniques. Moreover its single crystal was developed by slow evaporation technique and proved the structure of compound **2** by X-ray Single Crystal as methyl ester. Its CCDC no. is 1000266817.

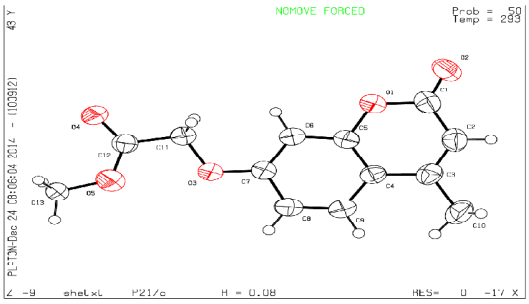


Figure 1: X-ray crystal structure of compound 2 (ORTAP Diagram)

Empirical formula	$C_{13}H_{12}O_5$
Formula Weight	248.23
Temperature/K	293(2)
Crystal system	Monoclinic
Space group	$P2_1/c$
a/ Å	10.0839(5)
b/ Å	14.5821(5)
c/ Å	8.4610(4)
α/°	90.00
β/°	112.403(6)
γ/°	90.00
Volume/Å3	1150.23/(9)
Z	4
$p_{Calc}^{mg/mm3}$	1.433
2Θ range for data collection	9.48 to 146.28°
Index range	$-11 \leq h \leq 12$, $-18 \leq k \leq 16$, $-10 \leq i \leq 10$
Reflections collected	5597
Independent reflections	2296[R(int)=0.0218]
Peak and hole e $Å^{-3}$	0.58/-0.49

Table-1 Crystal data and structure refinement parameter for compound 2.

The IR spectrum of ester compound **2** presented in Figure-2 on (Page No.83) showed characteristic bands at 1640-1680 cm^{-1} and 1700-1740 cm^{-1} for amide and lactone carbonyl groap respectively. The ^{1}H NMR spectrum of compound -**2** was presented in Figure – 3 (Page No.84) From Figure-3, a triplet observed at δ 1.33 to 1.35 and quartret at δ 4.27 to 4.33 confirms the ethyl group of ester. A singlet observed at a chemical shift δ 4.62 corresponds to methylene group attached at the carbonyl carbon. Another singlet at δ 2.4 and δ 6.17 represents the methyl group attached to pyrone ring and the 3rd position proton respectively.

The three aromatic protons on the benzene ring appeared at δ 7.36, 6.94 and 6.78 respectively.^{13}C NMR spectrum of compound-2 was recorded and presented in Figure-4 (Page No.85).The data is in agreement with the structure. After confirming the structure of compound-**2**, the ester thus formed was hydrolysed by using 15% aq KOH to get 2-[4-methyl-2-oxo-2H chromen-7-yl oxy] acetic acid-i.e compound -**3** in 90 % yield. The obtained compound was recrystallized from ethanol to get the pure compound as colourless crystals. The pure compound thus obtained was then analysed by advanced spectroscopic techniques. The ^{1}H NMR spectrum and ^{13} C NMR spectrum of compound -3 was recorded and presented in Figure-5 and Figure-6 (Page No.-86 & 87) respectively. From Figure-5, the disappearance of triplet and quartet clearly indicates the formation of compound-3. Then compound-3 was converted to corresponding acid chloride in situ by stirring with oxylyl chloride for two hours and then treated with various substituted amines (aliphatic/aromatic) to give the corresponding 2-(4-methyl-2-oxo-2H-chromen-7-yl-oxy) acetamide derivatives **(4a-4e)** at good to moderate yield. All the synthesized compounds **(4a-4e)** were recrystallized and characterized by advanced spectroscopic techniques.

Melting points were measured by open capillary method and were uncorrected. In general, the formation of Coumarinyloxy acetamide was confirmed by IR, ^{1}H ^{13} C NMR, Mass spectroscopy, and elemental analysis. The formation of amide group was confirmed by the amide –NH stretching characteristic peak at 3340-3360cm^{-1} for all the synthesized compounds and the peak at 1700-1740 cm^{-1} corresponds to the lactone carbonyl of coumarin for all the synthesized compounds. The carbonyl stretching of the amide group was observed at 1649-1680 cm^{-1}.From the ^{1}H NMR spectrum it was confirmed that –NH proton was appeared at chemical shift δ 6.40-8.12 and δ 9.50-10.50 for aliphatic and aromatic amide respectively. Figure - 7, 8, 9 & 10 on the (Page no 88, 89, 90 & 91) represents IR, ^{1}H NMR, ^{13}C NMR and Mass spectra of the compound-**4a**. The ^{1}H NMR spectrum of compound **4b,** shown in Figure-11 (Page No. 92), represents methyl group attached at 4-position of pyrone ring appeared at δ 2.38 as singlet and the olefinic proton observed at δ 6.13. A chemical shift at δ 4.77 represents the $–CH_2$ group attached at amide carbonyl. The remaining protons at δ 1.55-1.664 (m, 6H), 3.43-3.57 (m, 4H) corresponds to the five methylene groups of piperidin ring. Aromatic protons appeared at δ 6.82 (d, 1H, *J* =2.5Hz), 6.94 (d/d, 1H, *J* =2.5Hz) and 7.50(d, 1H, *J* =8.7Hz) confirmed the formation of compound **4b.** From the ^{13}C NMR spectrum data of Figure-12(Page No. 93), chemical shifts at 164.98, 161.07 & 102.01 represents amide carbonyl, lactone carbonyl and olefinic carbon of pyrone ring respectively confirmed the formation of compound **4b.** Figure – 13 and Figure-14 represents ^{1}H NMR, and ^{13}C NMR spectra of the compound-**4c** (Page No 94, 95). Figure –15, 16 & 17 represents IR, ^{1}H NMR, and ^{13}C NMR spectrum on (Page No 96, 97 & 98) of the compound-**4d** respectively. Figure – 18, 19 and 20 (Page No.99, 100 & 101) represents, ^{1}H NMR, ^{13}C NMR and Mass spectrum of the compound **4e** respectively.

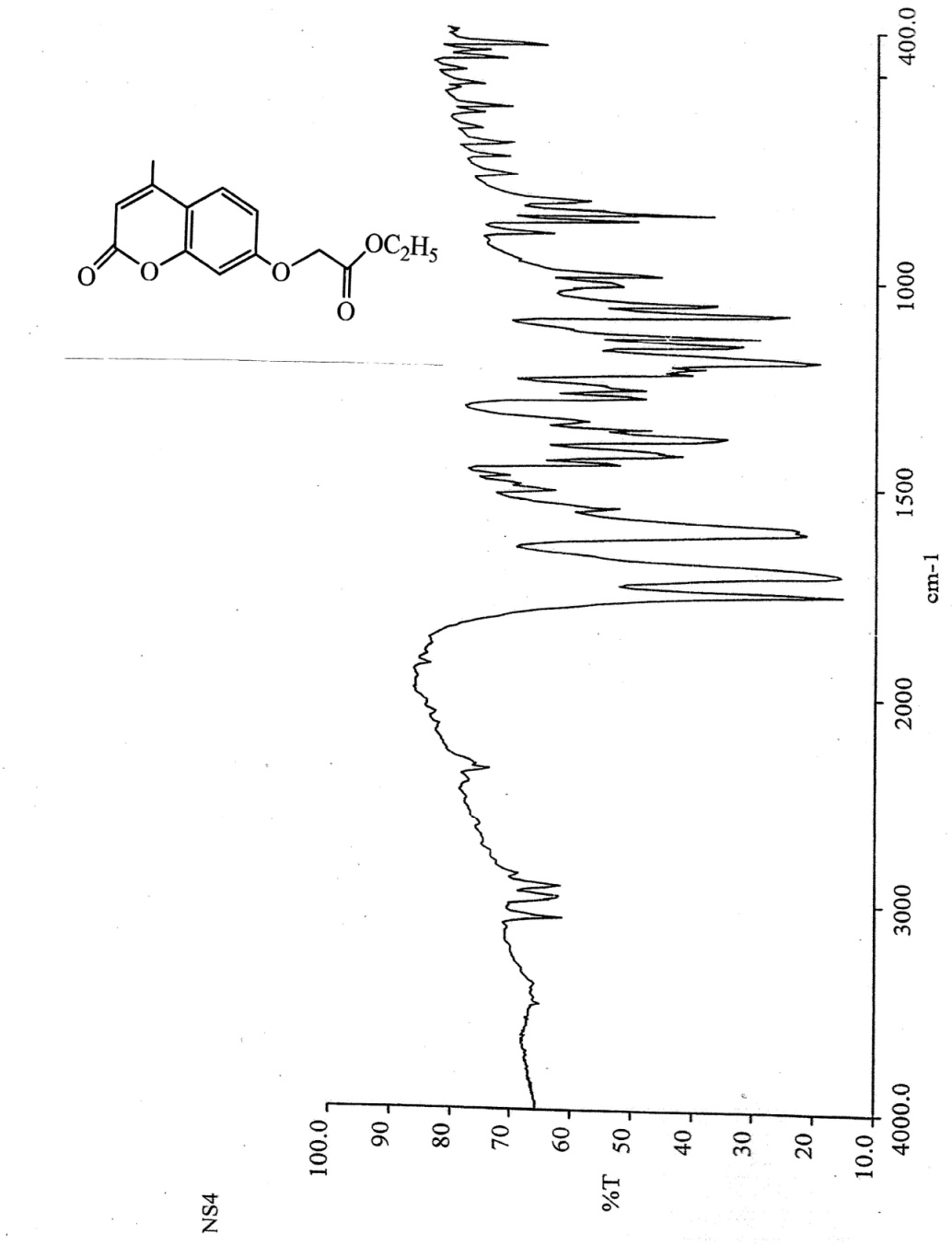


Figure-2 IR Spectrum of Ethyl-2-(4-methyl-2-oxo-2H-chromen-7-yl) oxy] acetate i.e compound- 2

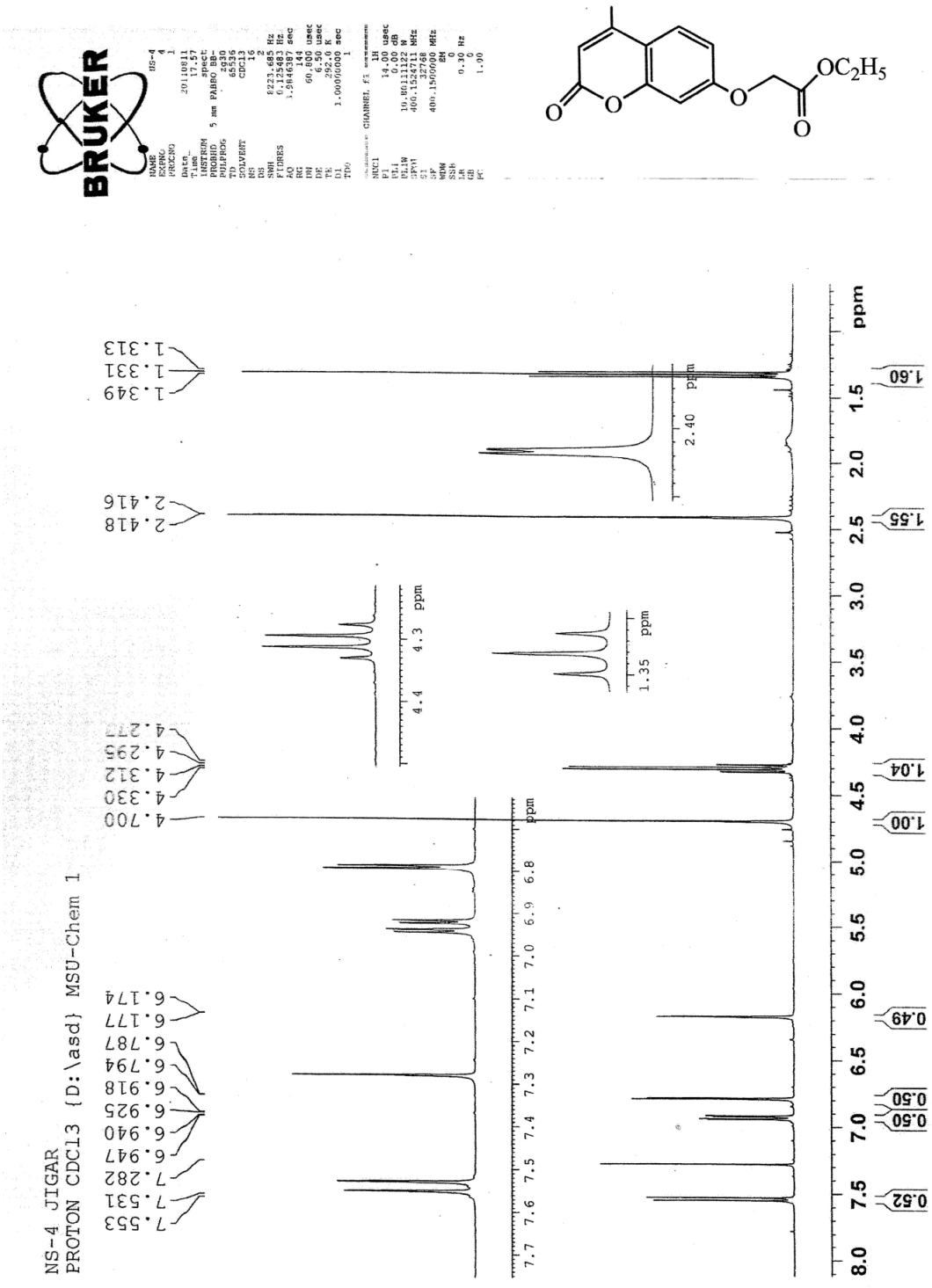


Figure-3 ^{1}H NMR Spectrum of Ethyl-2-(4-methyl-2-oxo-2H-chromen-7-yl) oxy] acetate i.e compound- 2

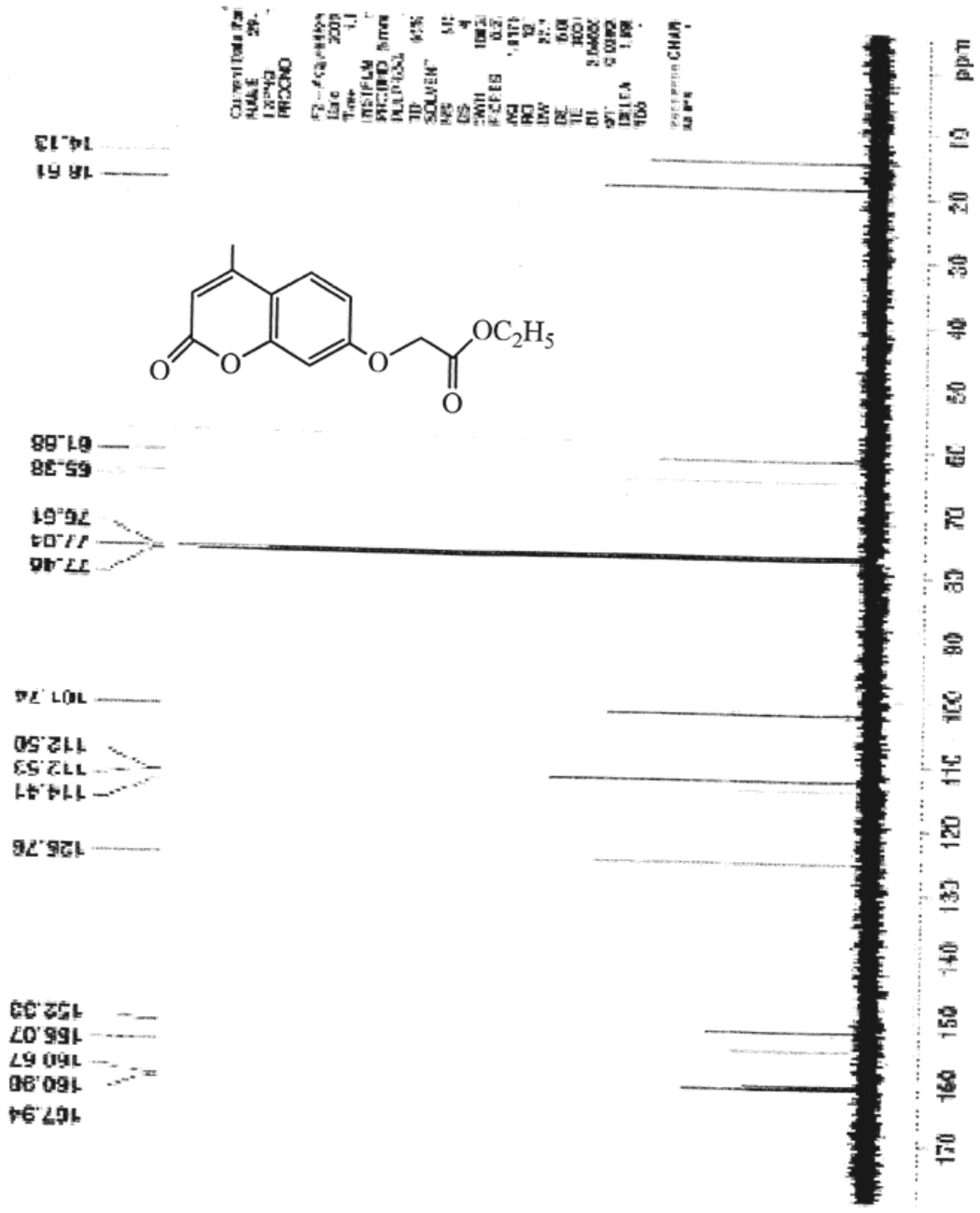


Figure-4 ^{13}C NMR Spectrum of Ethyl-2-(4-methyl-2-oxo-2H-chromen-7-yl) oxy] acetate i.e compound- 2

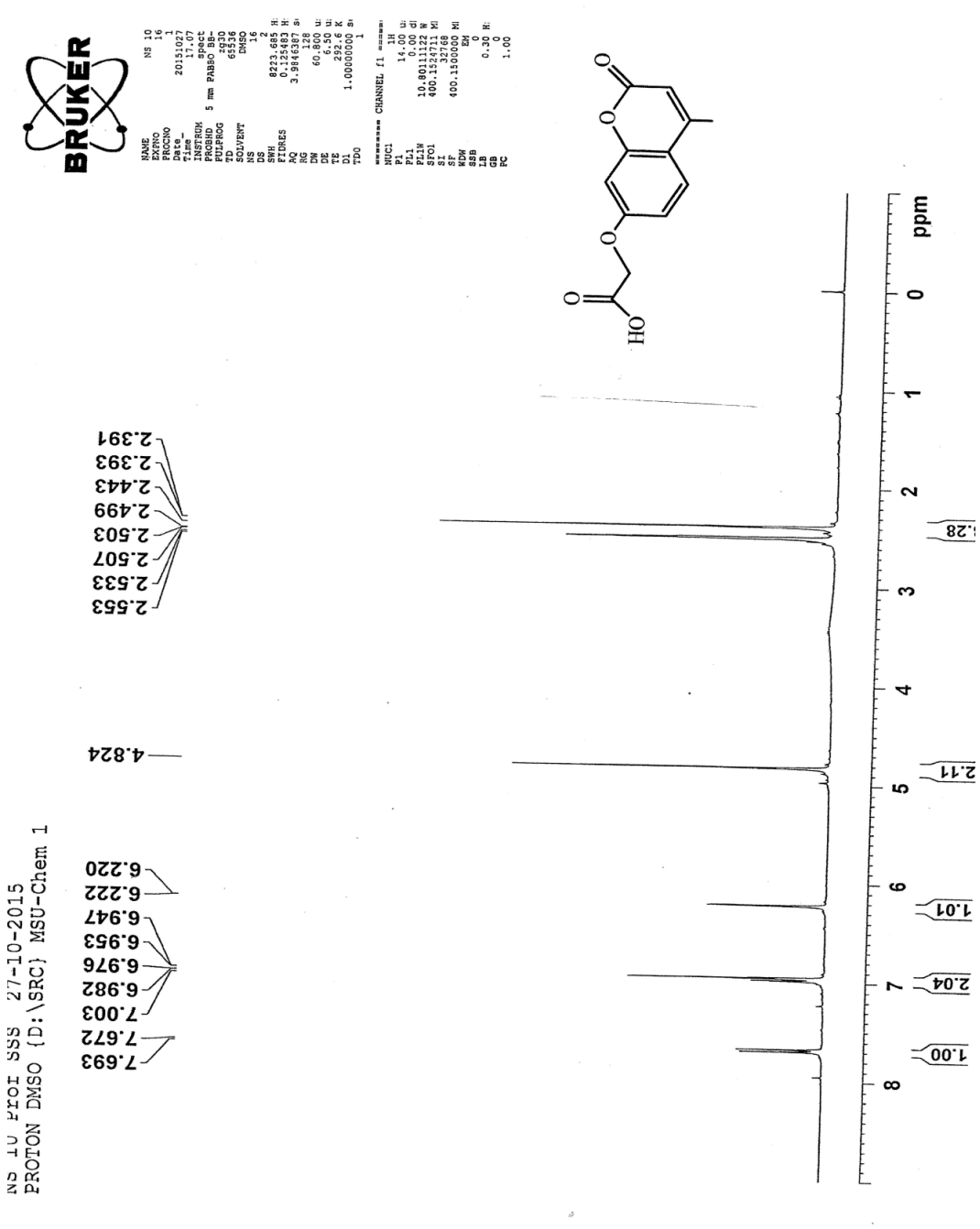


Figure-5 [1]H NMR Spectrum of 2-(4-methyl-2-oxo-2H-chromen-7-yloxy) acetic acid i.e compound- 3

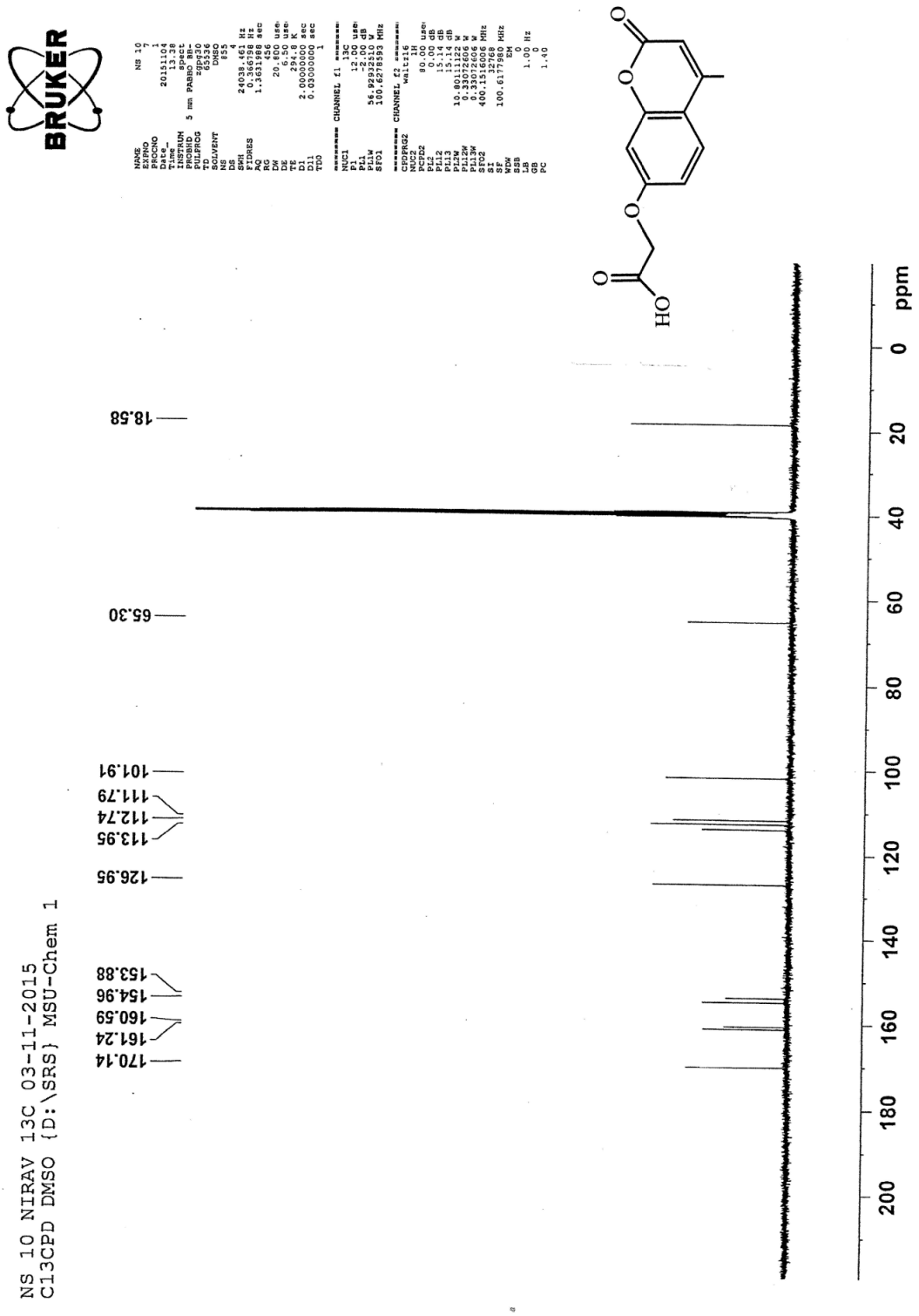


Figure-6 ^{13}C NMR Spectrum of 2-(4-methyl-2-oxo-2H-chromen-7-yloxy) acetic acid i.e compound- 3

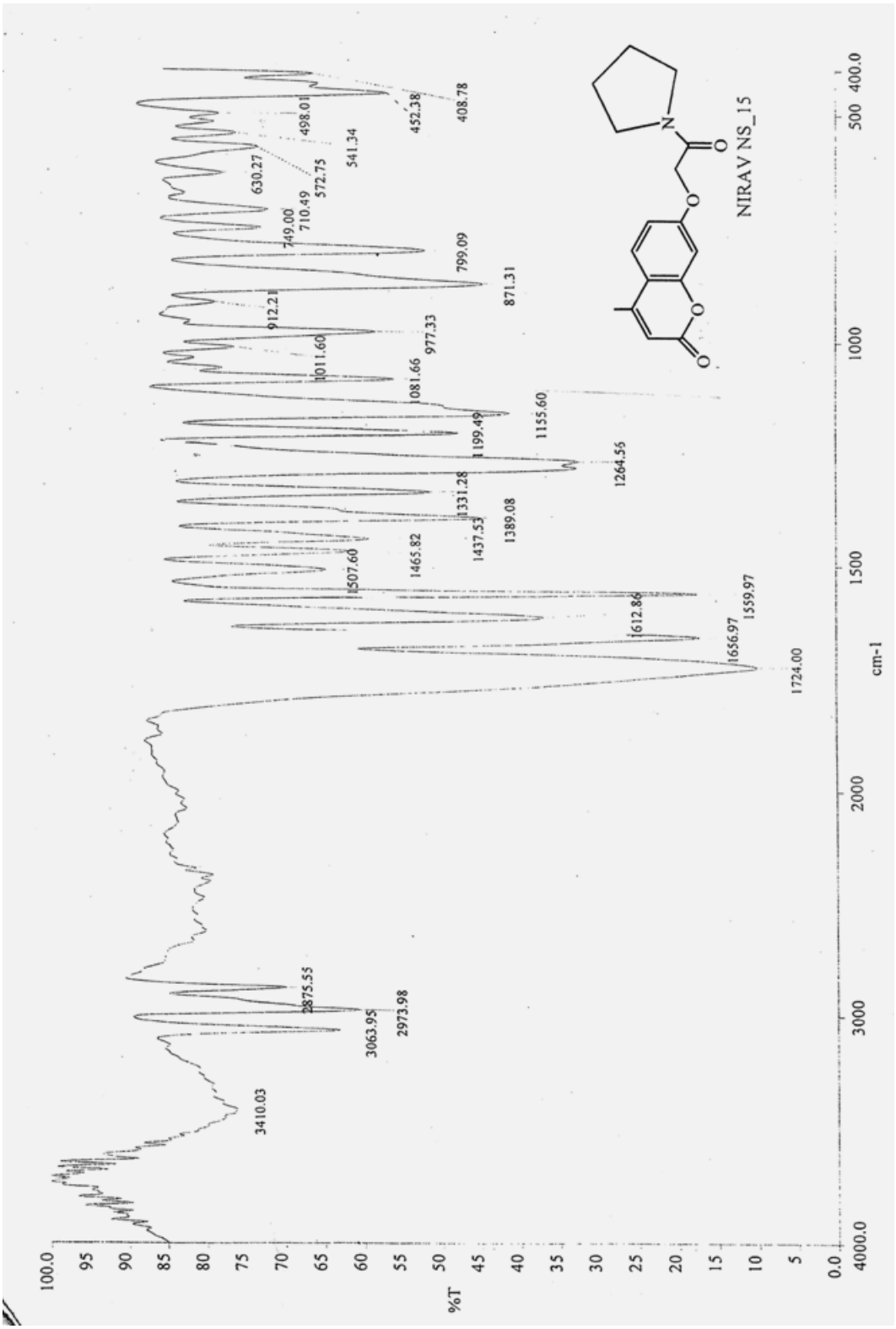


Figure-7 IR Spectrum of 4-methyl-7-(2-oxo-2-pyrrolidin-1-ylethoxy)-2*H*-chromen-2-one i.e 4a

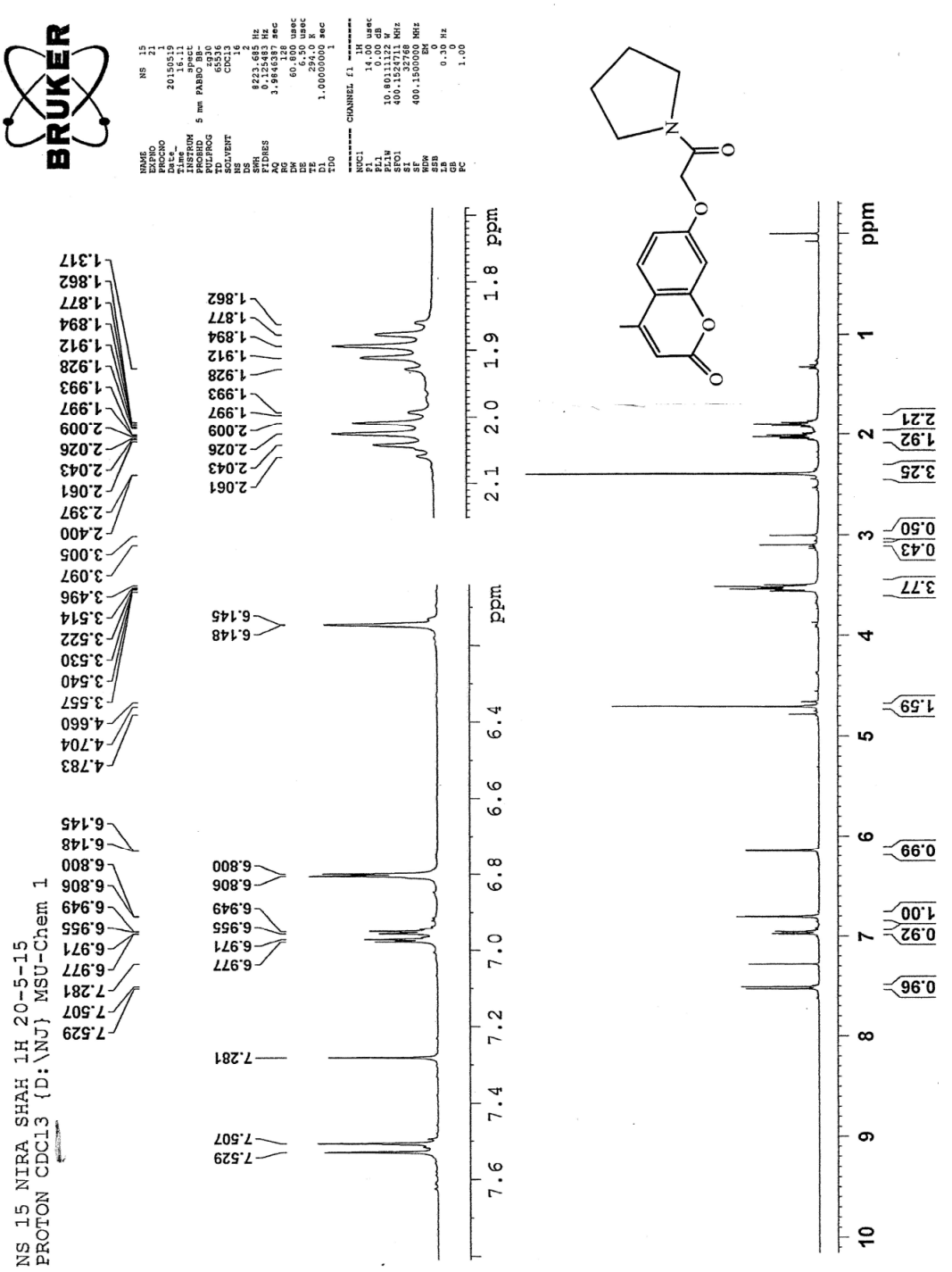


Figure-8 [1]H NMR Spectrum of 4-methyl-7-(2-oxo-2-pyrrolidin-1-ylethoxy)-2*H*-chromen-2-one i.e 4a

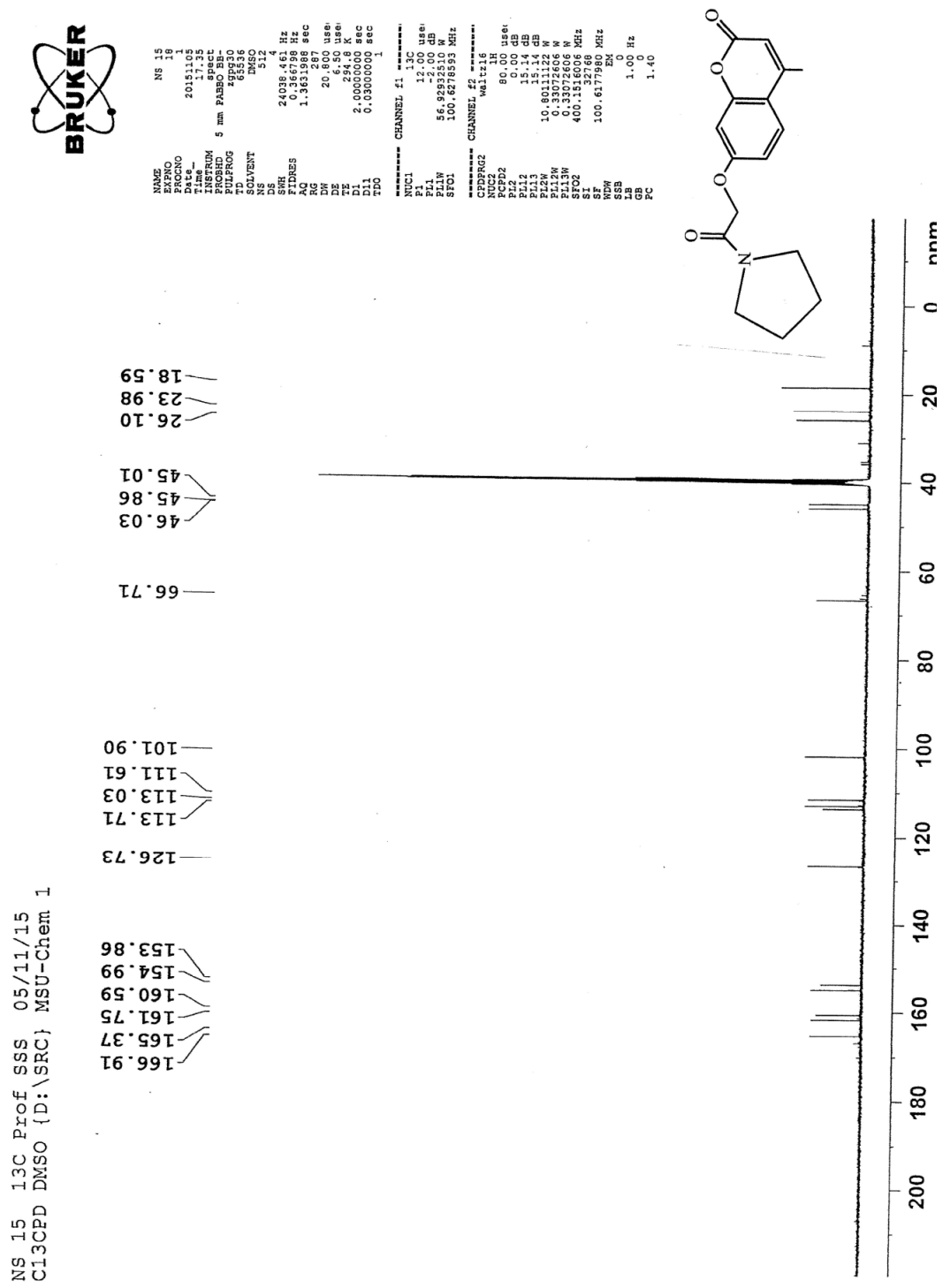


Figure-9 ^{13}C NMR Spectrum of 4-methyl-7-(2-oxo-2-pyrrolidin-1-ylethoxy)-2*H*-chromen-2-one i.e 4a

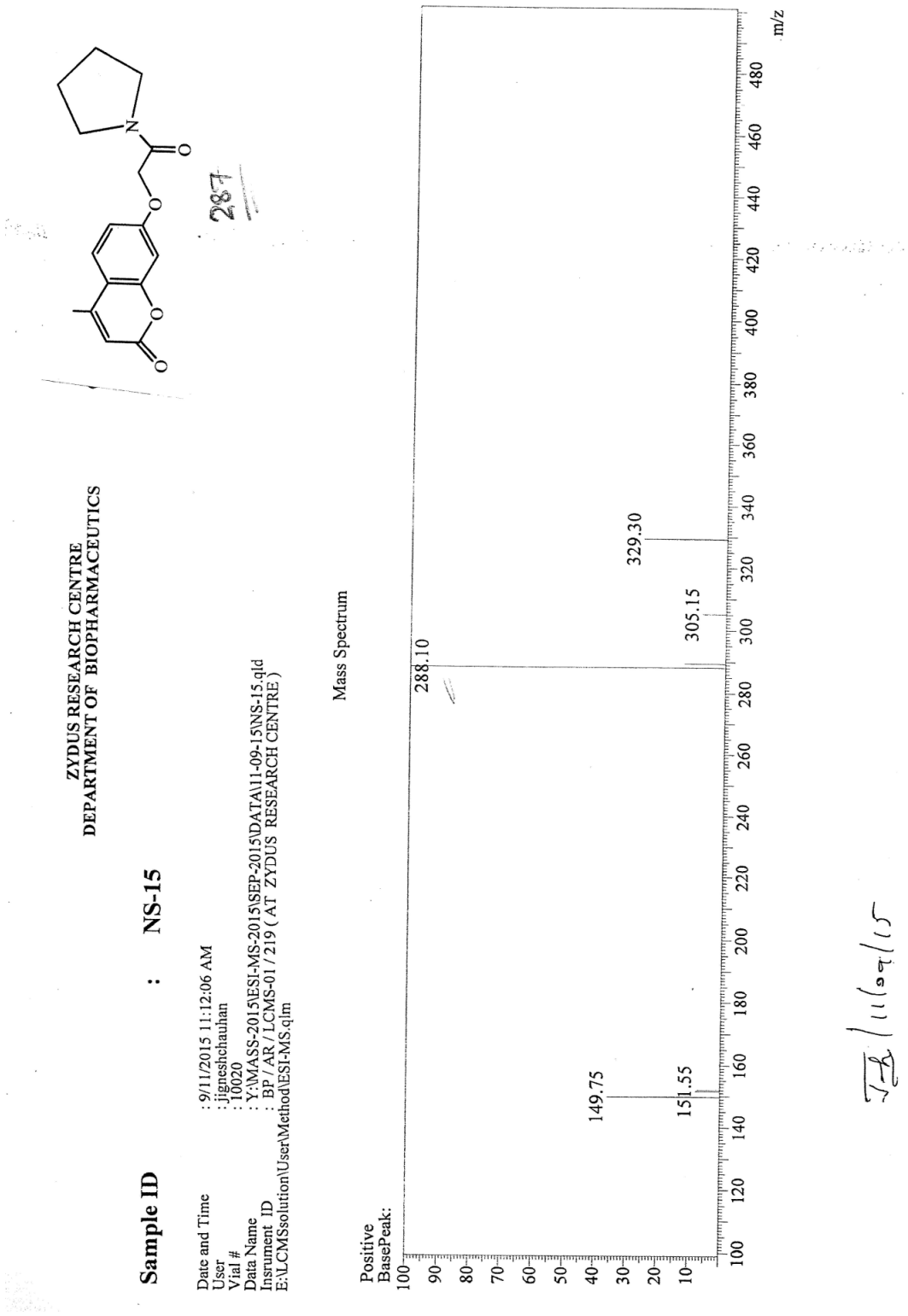


Figure-10 Mass Spectrum of 4-methyl-7-(2-oxo-2-pyrrolidin-1-ylethoxy)-2*H*-chromen-2-one i.e 4a

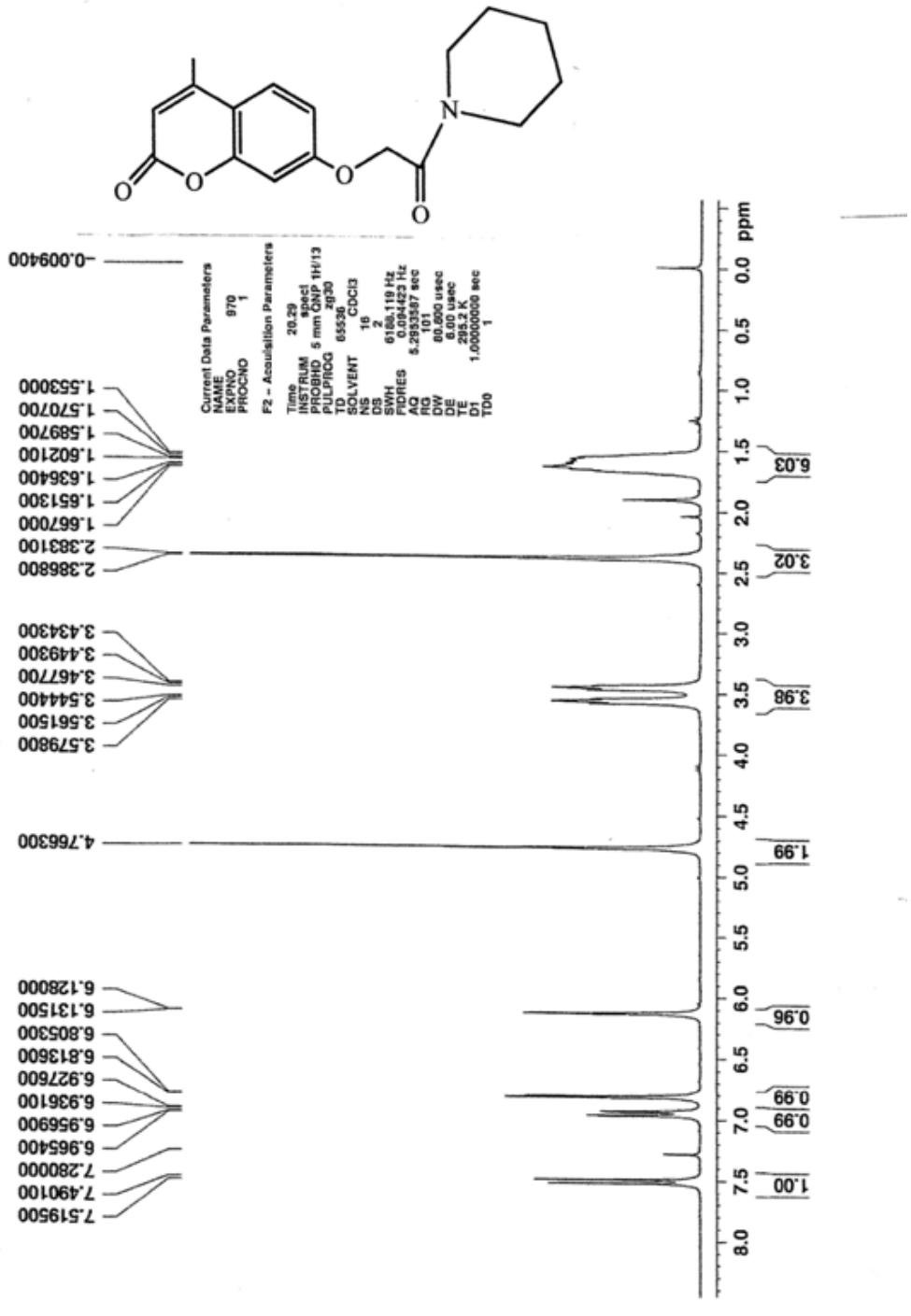


Figure-11 ^{1}H NMR Spectrum of 4-methyl-7-(2-oxo-2-piperidin-1-ylethoxy)-2*H*-chromen-2-one i.e 4b

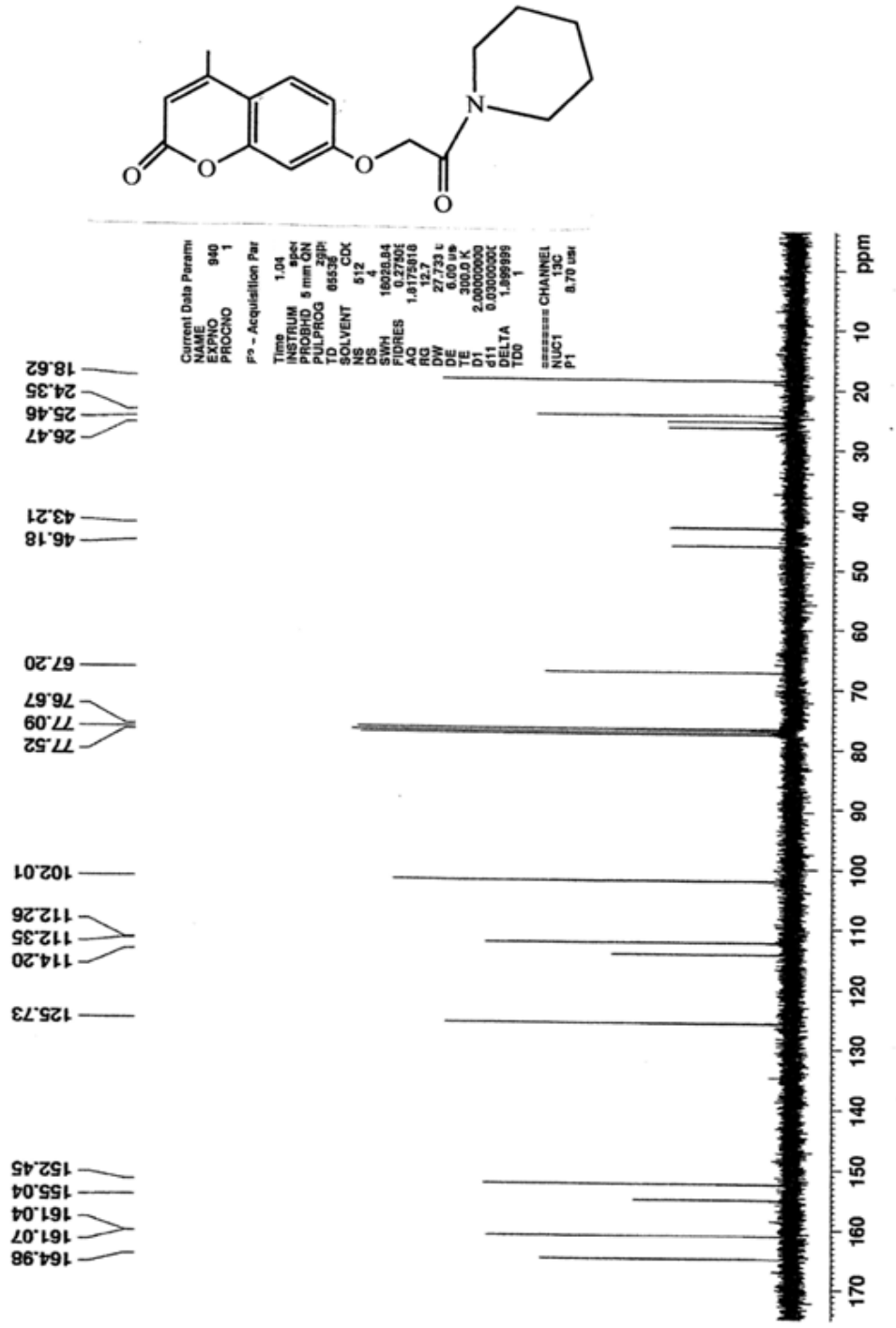


Figure-12 ^{13}C NMR Spectrum of 4-methyl-7-(2-oxo-2-piperidin-1-ylethoxy)-2*H*-chromen-2-one i.e 4b

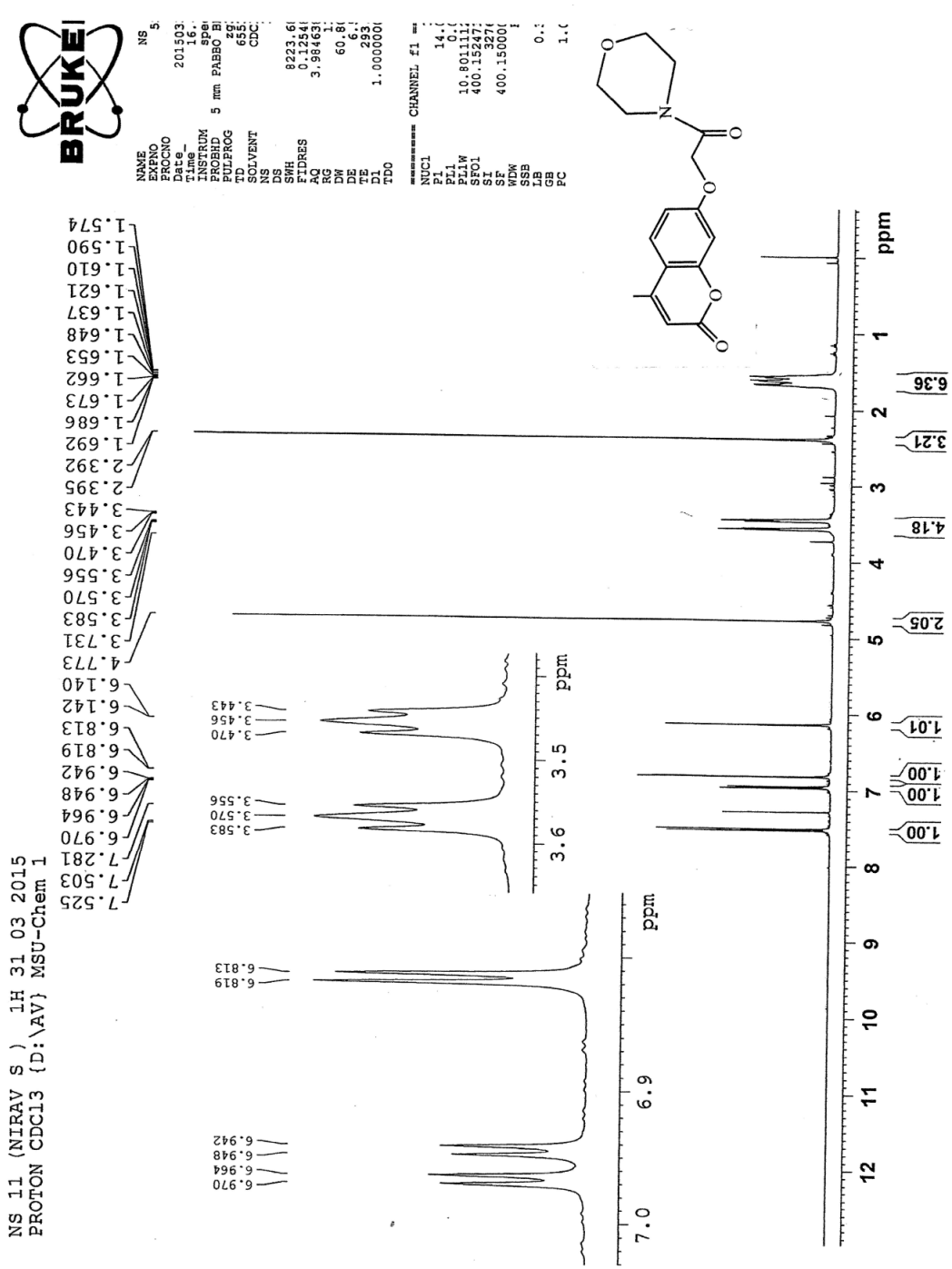


Figure-13 ^{1}H NMR Spectrum of 4-methyl-7-(2-oxo-2-Morpholin-1-ylethoxy)-2*H*-chromen-2-one i.e 4c

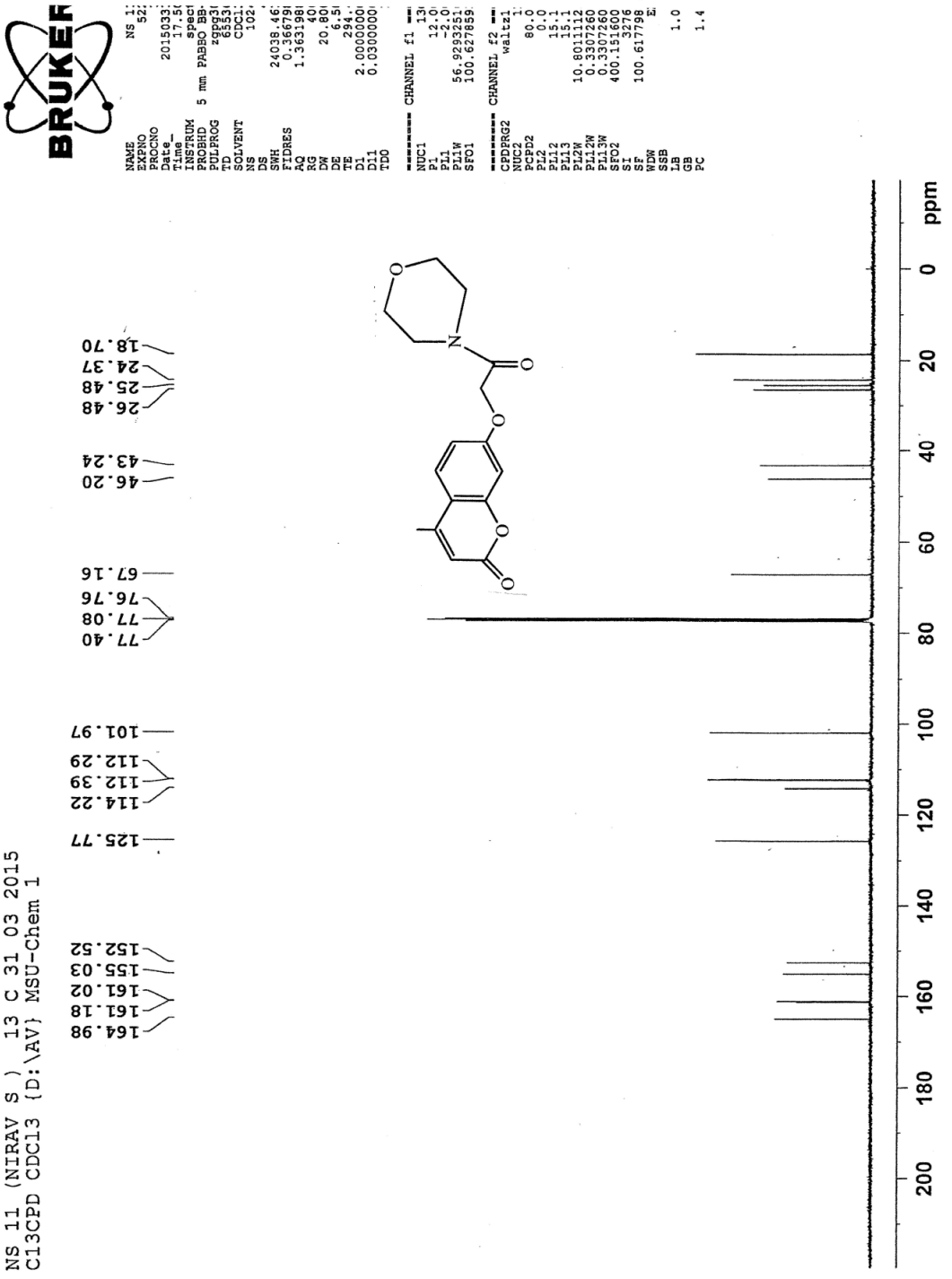


Figure-14 ^{13}C NMR Spectrum of 4-methyl-7-(2-oxo-2-Morpholin-1-ylethoxy)-2*H*-chromen-2-one i.e 4c

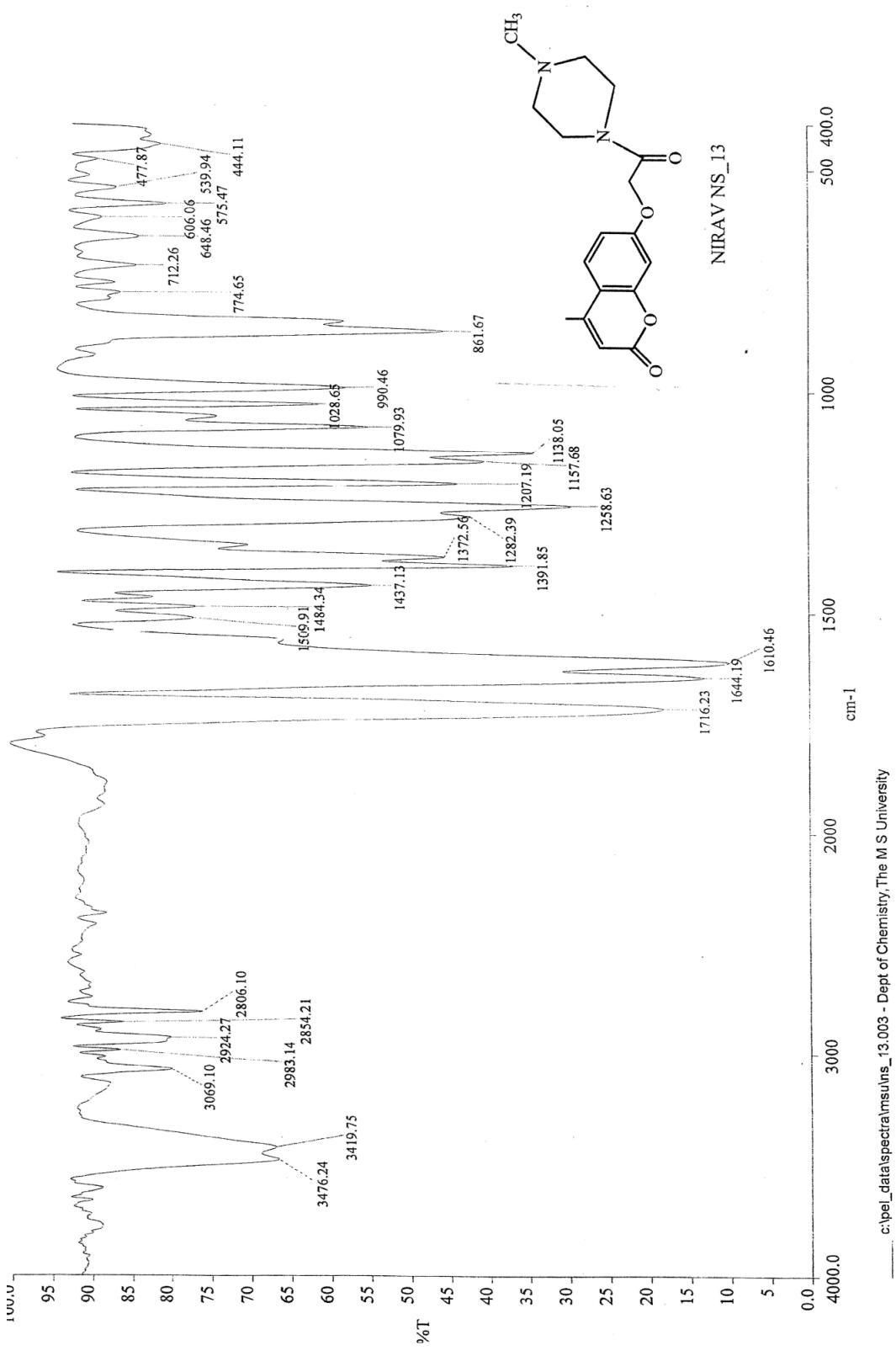


Figure-15 IR Spectrum of 4-methyl-7-[2-(4-methylpiperazin-1-yl)-2-oxoethoxy]-2*H*-chromen-2 one i.e 4d

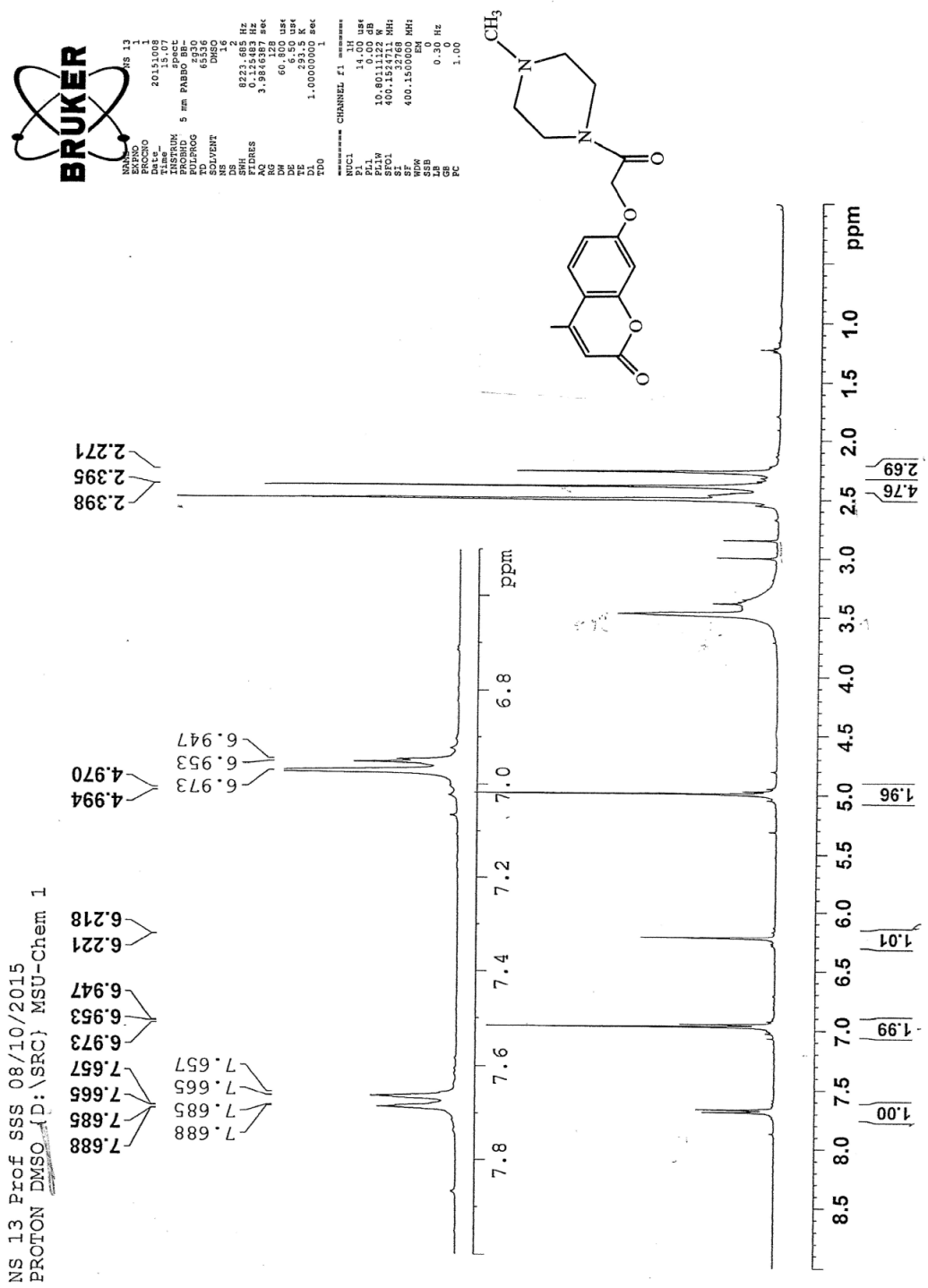


Figure-16 ^{1}H NMR Spectrum of 4-methyl-7-[2-(4-methylpiperazin-1-yl)-2-oxoethoxy]-2*H*-chromen-2 one i.e 4d

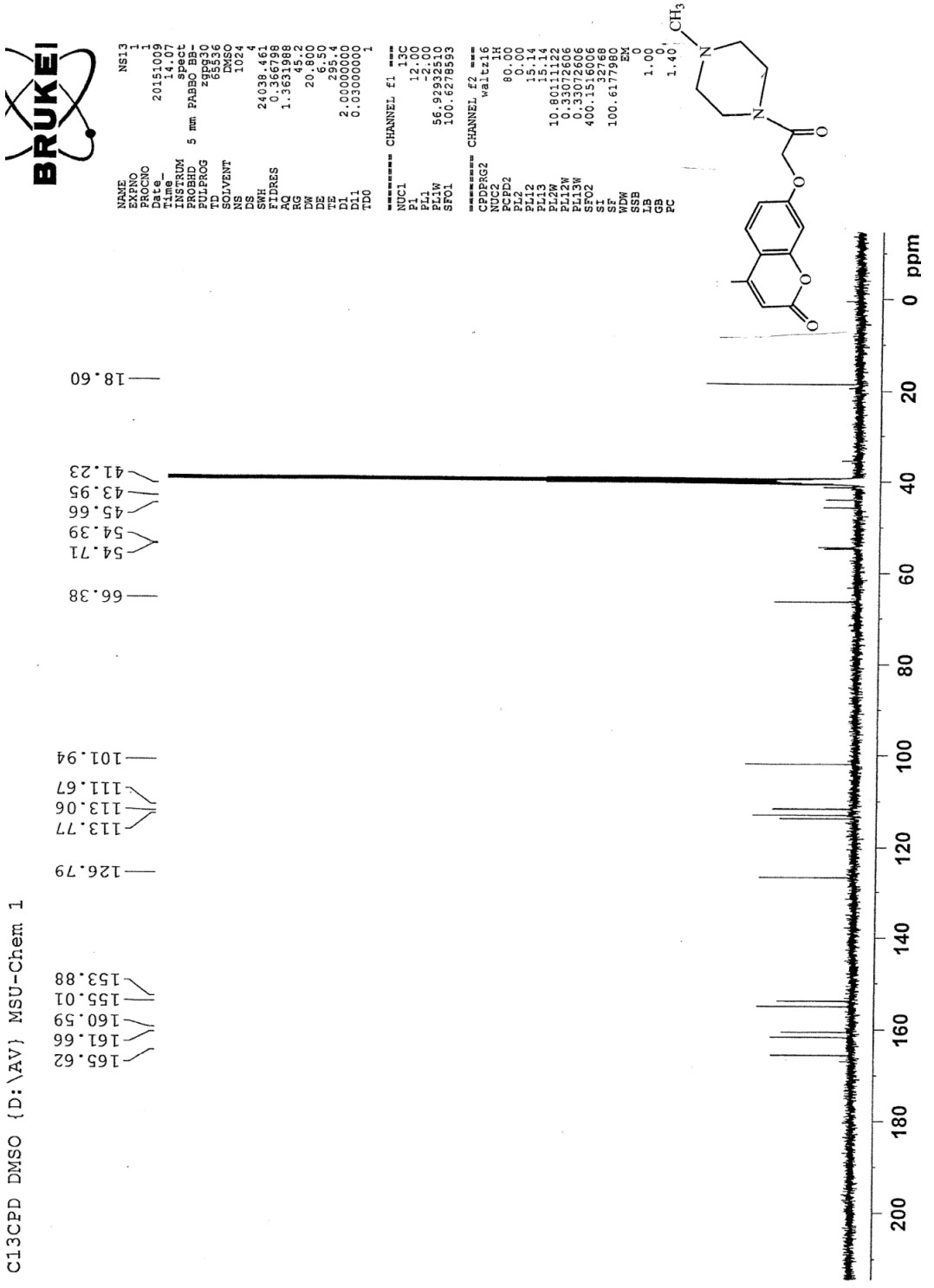


Figure-17 ^{13}C NMR Spectrum of 4-methyl-7-[2-(4-methylpiperazin-1-yl)-2-oxoethoxy]-2*H*-chromen-2 one i.e 4d

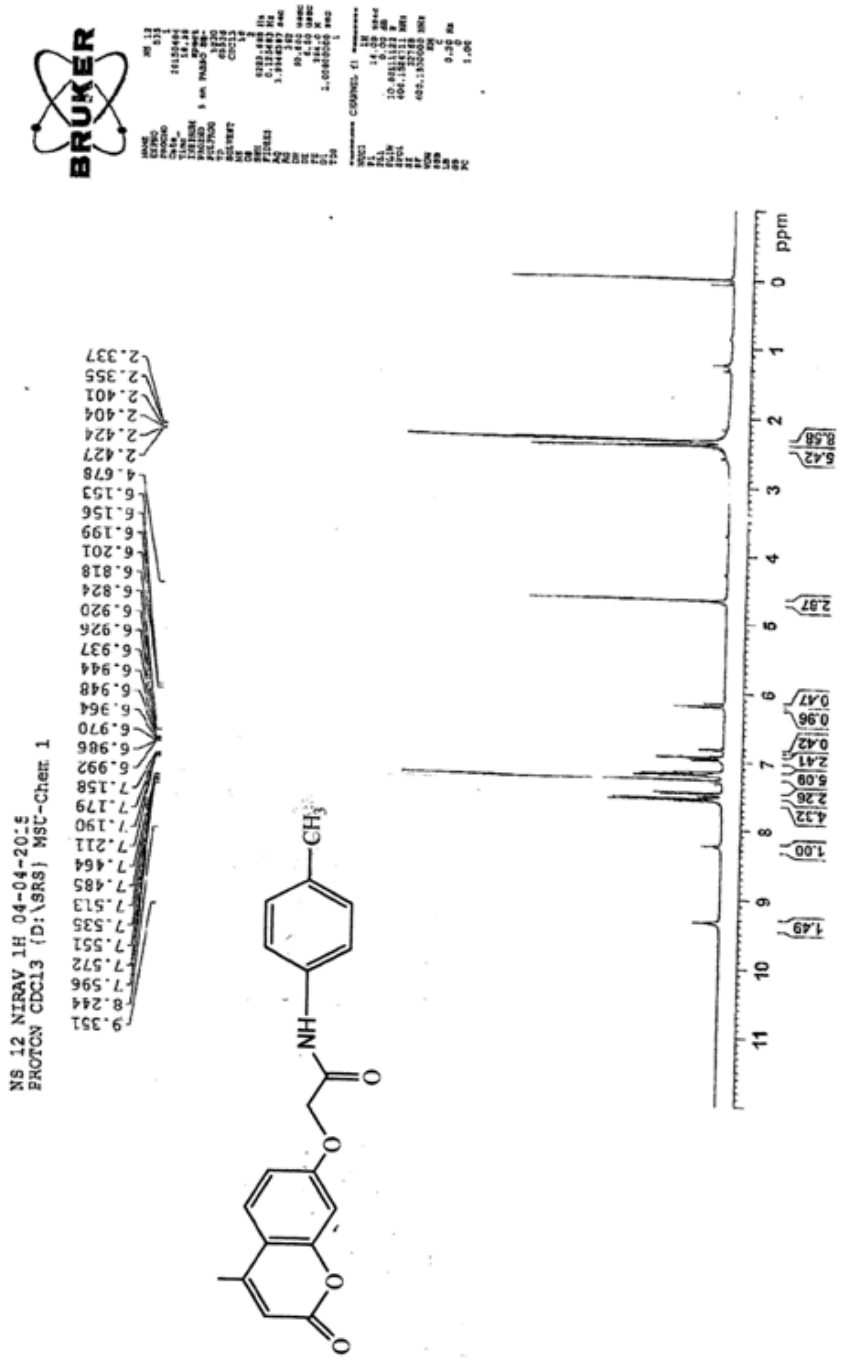


Figure-18 ^{1}H NMR spectrum of *N*-(4-methylphenyl)-2-[(4-methyl-2-oxo-2*H*-chromen-7-yl) oxy] acetamide i.e 4e

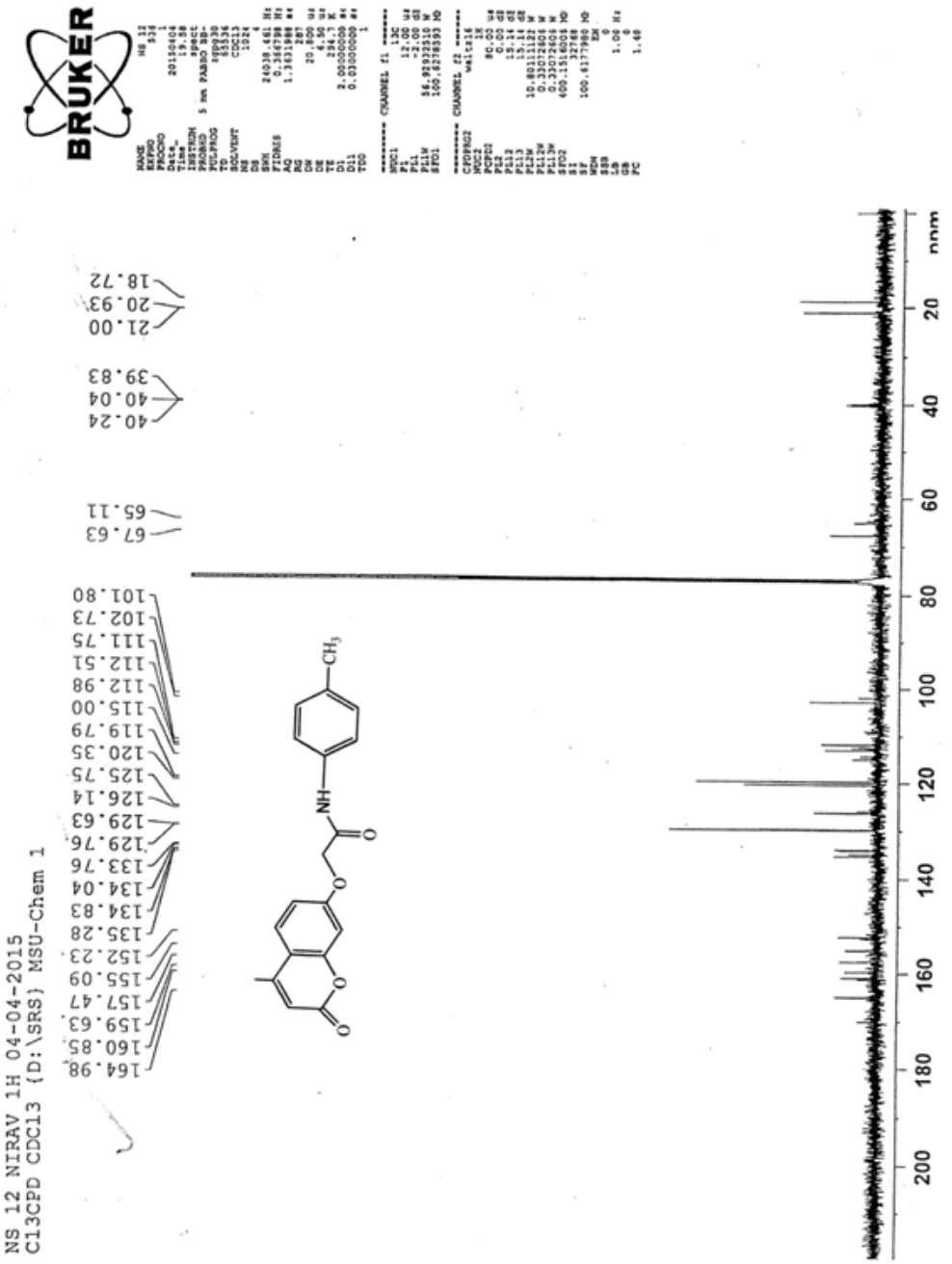


Figure-19 [13] CNMR spectrum of *N*-(4-methylphenyl)-2-[(4-methyl-2-oxo-2*H*-chromen-7-yl) oxy] acetamide i.e 4e

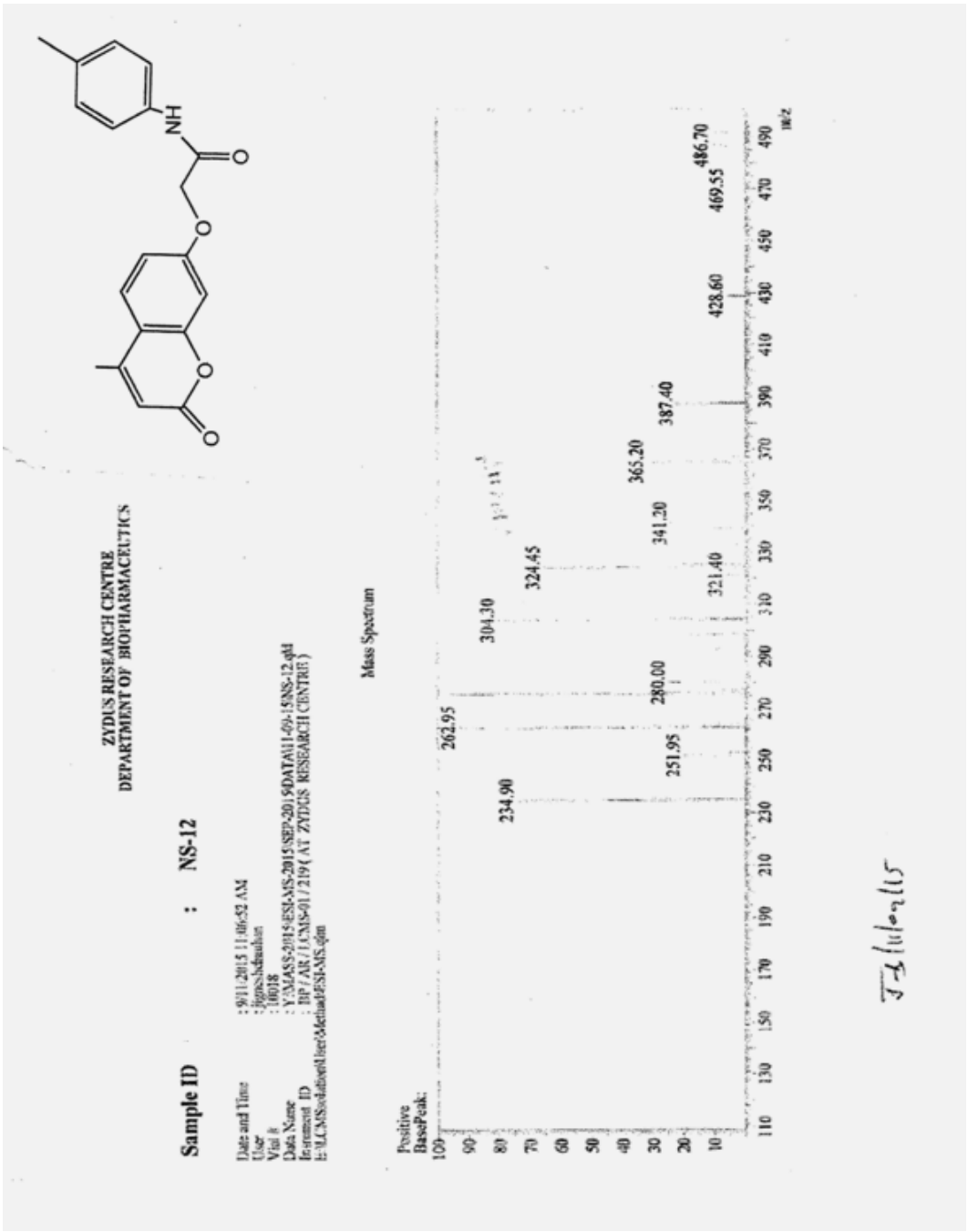


Figure-20 Mass spectrum of *N*-(4-methylphenyl)-2-[(4-methyl-2-oxo-2*H*-chromen-7-yl) oxy] acetamide i.e 4e

3.2.2 Biological evaluation

3.2.2.1 Antibacterial and antifungal activity

All the synthesized compounds were screened for their antibacterial and antifungal activity and are summarized in **Table-1** as given bellow:

Compounds	Gram –Ve bacteria		Gram +ve bacteria		Fungi
	Escherichia coli	*Pseudomonas aeruginosa*	*Staphylococcus aureus*	*Bacillus Subtilis*	*Candida Albicans*
4a	>228	>228	>228	>228	>228
4b	>228	>228	>228	>228	>228
4c	>228	>228	>228	>228	>228
4d	>228	>228	>228	>228	>228
4e	>228	>228	>228	>228	>228
Ampiciline	10	8	9	10	10

Table 1: Antimicrobial and antifungal activity of compounds **4a**-**4e**.

Compounds **4a**-**4e** did not show any antimicrobial activity against tested Gram-negative bacteria (*E. coli*, *P. aeruginosa)* and Gram-Positive bacteria (*S.aureus, B. subtilis).* Also compounds **4a**-**4e** found inactive against fungi *C.albicans*.

3.2.2.2 Anticancer activity

All the synthesized compounds were screened againstA549 (lung cancer cell-line) and one of the compound **4b, 4c, 4d** and **4e** were screened against A375 (melanoma cell-line). IC_{50} (μM) values were determined using Graph Pad prism software for compounds **4a-e** as shown in Table 2.

Table-2 Describing the anticancer evaluation of synthesized compounds

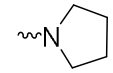
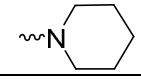
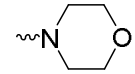
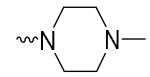
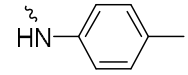
Compound	-NRR[1]	IC_{50} (µM)[a]	
		A549	A375
4a		**9.26**	ND
4b		**0.66**	NA
4c		**0.41**	NA
4d		NA	**0.041**
4e		**1.1nM**	NA

Table 2: Anticancer activity (IC_{50}, µg/mL) of substituted Coumarinyloxy acetamide **4a-i**.

[a]IC_{50} values were determined using Graph Pad Prism software.

NA= Not active, ND = not determined.

From the MTT assay, pyrrolidine compound **4a** showed better activity against A549 (Lung Cancer cell line) with IC_{50} value 9.26 µM. On replacement of pyrrolidine with piperidine in compound **4b** resulted with 14 fold higher activity against A549 with IC_{50} value 0.66 µM. Compound **4c** containing morpholine ring showed 22 fold higher activity compared to compound **4a** against A549 cell line with IC_{50} value 0.41 µM. *N*-methyl piperazine compound **4d** did not show any activity against A549 cell line, but it showed very good activity against A375 cell line with IC_{50} value 0.041µM. Interestingly, p-toluidine substituted compound **4e** showed excellent activity against A549 cell line with IC_{50} value 1.1 nM.

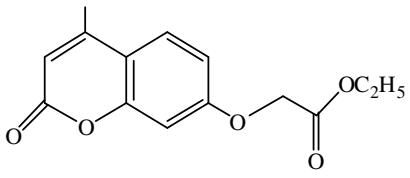
3.3 Experimental

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 Thermo sinnigan Flash 11-12 series EA.

3.3.1 Chemistry

The starting compound 7-hydroxy-4-methyl -2H-chromen-2-one was prepared by the reported method[15].

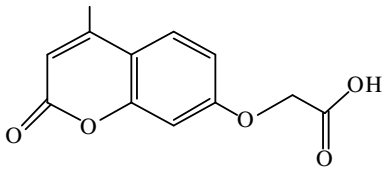
3.3.1.1 Synthesis of Ethyl-2-(4-methyl-2-oxo-2H-chromen-7-yl) oxy] acetate (2)



To the well stirred solution of **1** (1 g, 5.0mmol, 1.0 eq) in DMF, ethylchloroacetate (0.9 g, 5 mmol, 1.2 eq) and K_2CO_3 (0.9 g, 6.5 mmol, 1.3eq) were added and the resulting mixture was refluxed for 16 h. After completion of reaction (monitored by TLC, Ethyl acetate: Pet ether 60:40), reaction mixture was cooled to room temperature and poured on to crushed ice to give solid. The solid was filtered, washed with water and recrystallized from ethanol to give compound **2**. Yield85 %; m.p: 110-112°C; IR (KBr): 3085, 2950, 2880, 1750, 1705, 1615, 1375, 1210, 950 cm^{-1}; ^{1}H-NMR($CDCl_3$, 400MHz)

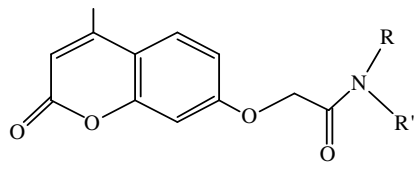
δ1.33 (3H, d, *J* = 7.2 Hz), 2.41 (3H, s), 4.30(2H, q, *J* = 7.2 Hz), 4.70 (2H, s), 6.17 (1H, d, *J* = 1.2 Hz), 6.78 (1H, d, *J* = 2.8 Hz), 6.93 (1H, dd, *J* = 8.8, 2.8 Hz), 7.54 (1H, d, *J* = 8.8 Hz); X-ray crystal data (CCDC No. 1000266817) is given in Table-1

3.3.1.2 Synthesis of 2-(4-methyl-2-oxo-2H-chromen-7-yloxy) acetic acid (3)



Compound **2** (2.62 g, 10.0 mmol, 1.0eq) was treated with KOH (5.6 g, 100.0 mmol, 10.0eq) in Ethanol (30 ml). The resulting mixture was refluxed at a temperature 100–108 °C for 15 h. After that the reaction mixture was allowed to cool up to room temperature, the resulting residue was poured into ice cold water and acidified with Con. HCl to pH 2. The resulting solid was filtered off and washed with cold water. The solid was dissolved in saturated $NaHCO_3$ solution, acidified with conc.HCl to give white solid. The solid was filtered, washed with water, dried and recrystallized from ethanol to give compound **3** as a white solid. Yield %, m.p : 246-248 °C; ^{1}H-NMR (DMSO-d_6, 400MHz) δ2.37 (3H, s), 4.82 (2H, s), 6.20 (1H, s), 6.94 (2H, s), 7.66 (1H, d, *J* = 8.4 Hz); ^{13}C-NMR (DMSO-d_6, 100 MHz)δ18.57, 65.23, 101.89, 111.80, 112.73, 113.95, 126.94, 153.85, 154.95, 160.59, 161.20, 170.11.

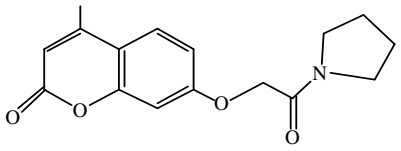
3.3.1.3 General procedure for the synthesis of amides (4a-4e):



A suspension of compound **3** (0.5 g, 2.136mmol) in dichloromethane (DCM) (25 mL) was cooled to 0-5°C. To this oxylyl chloride (0.46 mL, 5.34 mmol, 2.5 eq) was added drop wise at 0-5°C followed by a drop of DMF. The resulting solution was stirred at 0-5°C for 30 min and at RT for 3 h. The resulting solution was concentrated on rota vapour to give residue. The residue was taken in DCM and concentrated to remove traces of oxylyl chloride. The residue was dissolved in DCM (25 mL) and cooled

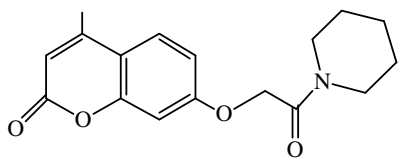
to 0-5°C. To this cold solution, different amines (1.1 eq.) were added followed by the (TEA) triethylamine (1.5 eq). The resulting mixture was stirred at 0-5°Cfor 30 min and then at RT for the 16 h. The reaction mixture was washed with water (25 mL), sat. $NaHCO_3$ (25 mL) and then brine solution (10 mL), dried over anhydrous Na_2SO_4, filtered and concentrated to give crude compound. The crude product was purified by column chromatography (60–120 mesh) using Pet. ether: Ethyl acetate (70:30 to 20:80) to give corresponding amide (**4a**-**4g**) in good to excellent yield.

3.3.1.3.1 Preparation of 4-methyl-7-(2-oxo-2-pyrrolidin-1-ylethoxy)-2*H*-chromen-2-one (4a)



Yield : 82 %; off white solid (EtOH); m.p: 160–162 °C;IR (KBr): 3063, 2973, 2875, 1724, 1656, 1612, 1559, 1437, 1331, 1264, 1199, 1155, 1081, 977, 871, 799 cm^{-1};; ^{1}H-NMR ($CDCl_3$, 400MHz)δ d 1.86–1.92 (2H, m), 2.06–1.99 (2H, m), 3.55-3.49 (4H, m), 4.70 (2H, s), 6.14 (1H, d, *J* = 1.2 Hz), 6.80 (1H, d, *J* = 2.4 Hz), 6.96 (1H, dd, *J* = 8.8, 2.4 Hz), 7.51 (1H, d, *J* = 8.8 Hz);^{13}C-NMR ($CDCl_3$, 100 MHz) δ18.68, 23.82, 26.24, 26.48, 67.36, 101.81, 101.91, 112.28, 112.51, 112.59, 114.23, 125.75, 152.52, 155.05, 161.00 161.16, 165.34; ESI Ms ; 288.10 [m+1]; Elemental Analysis for $C_{17}H_{19}NO_4$,M.W= Calculated,%:C 67.76;H 6.36;N 4.65,Found,% C 66.89;H 5.96 ;N 4.88

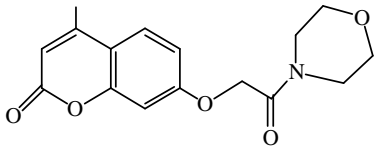
3.3.1.3.2 Preparation of 4-methyl-7-(2-oxo-2-piperidin-1-yl-ethoxy)-2*H*-chromen-2-one (4b)



Yield : 82 %; off white solid (EtOH); m.p: 172–174 °C;IR (KBr): 3069, 2993, 2939, 2859, 1725, 1663, 1610, 1502, 1450, 1429, 1390, 1255, 1196, 1084, 1012, 973, 858 cm^{-1};; ^{1}H-NMR ($CDCl_3$, 400MHz)δ1.57–1.69 (6H, m), 2.38(3H, s), 3.44–3.73 (4H, m), 4.77 (2H, s), 6.14 (1H, d, *J* = 1.2 Hz), 6.81(1H, d, *J* = 2.4 Hz), 6.94 (1H, dd, *J* = 8.8, 2.4 Hz), 7.50(1H, d, *J* = 8.8 Hz);^{13}C-NMR ($CDCl_3$, 100 MHz) δ18.70, 24.37,

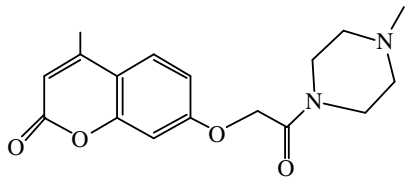
25.48, 26.48, 43.24, 46.20, 67.16, 101.97, 112.29,112.39, 114.22, 125.77, 152.52, 155.03, 161.02, 161.18, 164.98; Elemental Analysis for $C_{17}H_{19}NO_4$, M.W= Calculated,%:C 67.76;H 6.36;N 4.65,Found,% C 63.36;H 5.65;N 4.61.

3.3.1.3.3 Preparation of 4-methyl-7-(2-morpholin-4-yl-2-oxo-ethoxy)-2*H*-chromen-2-one (4c)



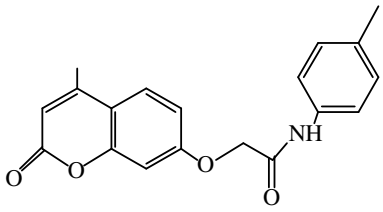
Yield: 78 %; white solid (EtOH);m.p:146–148 °C; IR (KBr): 3069, 2993, 2939, 2859, 1725, 1663, 1610, 1502, 1450, 1429, 1390, 1255, 1196, 1084, 1012, 973, 858 cm^{-1};; ^{1}H-NMR (DMSO-d_6, 400MHz)δ d2.39 (3H, S), 3.36 (4H, br s), 3.76 (4H, br s), 4.95 (2H, s), 6.22 (1H, s), 6.98 (2H, br s), 7.68 (1H, d, J = 9.2 Hz);^{13}C-NMR (DMSO-d_6, 100 MHz) δ18.58, 31.14, 52.40, 65.31, 101.99, 111.95, 112.77, 114.14, 127.00, 153.79, 154.98, 160.51, 161.00, 169.16; Elemental Analysis for $C_{16}H_{17}NO_5$, M.W= :Calculated,%:C 63.36;H 5.65;N 4.62,Found,% C 67.76;H 6.36;N 4.61.

3.3.1.3.4 Preparation of 4-methyl-7-(2-(4-methylpiperazin-1-yl)-2-oxoethoxy)-2*H*-chromen-2 one (4d)



Yield: 68 %; Off white solid (EtOH); m.p: 98-100 °C;IR (KBr):3476, 3419, 3069, 2924, 2806, 1716, 1644, 1610, 1437, 1391, 1372, 1258, 1207, 1157, 1138, 1079, 861 cm^{-1};; ^{1}H-NMR (DMSO-d_6, 400MHz)δ 2.27 (3H, s), 2.39(4H, s), 2.5 (3H, s), 3.5 (4H, S), 4.99 (2H, s), 6.21 (1H, d, J = 1.2 Hz), 6.97-6.94 (2H, m), 7.67 (1H, dd, J = 9.2, 1,2 Hz);^{13}C-NMR (DMSO-d_6, 100 MHz) δ18.60, 41.23, 43.95, 45.66, 66.38, 101.94, 111.67, 113.06, 113.77, 126.79, 153.88, 155.01, 160.59, 161.66, 165.62; Elemental Analysis for $C_7H_{20}N_2O_4$ Calculated,%:C 64.54;H 6.37;N 8.86,Found,% C 64.54;H 6.37;N 8.86.

3.3.1.3.5 Preparation of *N*-(4-methylphenyl)-2-((4-methyl-2-oxo-2*H*-chromen-7-yl) oxy) acetamide (4e)



Yield: 74%; off white solid (EtOH); m.p: 214-216 °C; IR (KBr): 3365, 3304, 3038, 2914, 1700, 1681, 1627, 1594, 1534, 1392, 1364, 1296, 1153, 1081, 887, 849, 819 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 400 MHz)δδ 2.25 (3H, s), 2.40 (3H, s), 4.82 (2H, s), 6.23 (1H, s), 7.07-7.02 (2H, m), 7.11 (2H, d, *J* = 8.4 Hz), 7.51(2H, d, *J* = 8.4 Hz), 7.72 (1H, d, *J* = 8.8, Hz), 10.09 (1H, s); ^{13}C-NMR($CDCl_3$, 100 MHz): δ 18.61, 20.92, 67.74, 102.12, 111.90, 112.86, 114.08, 120.13, 127.02, 129.61, 133.19, 136.25, 153.84, 154.98, 160.53, 161.30, 166.05; Elemental Analysis for $C_{19}H_{17}NO_4$: Calculated,%:C 70.58;H 5.30;N 4.33,Found,% C 70.58;H 5.30;N 4.33.

3.3.2 Biological activity screening

3.3.2.1 Antimicrobial activity:

All the synthesized compounds were tested for their antibacterial activity against Gram negative (*Escherichia coli*, *Pseudomonas aeruginosa*) and Gram positive (*Staphylococcus aureus, Bacillus Subtilis*) by cup plate method[27] at 100 ppm concentration in DMF solvent. Ampiciline was used as standard drug. All compounds did not show activity against all types of Gram positive and Gram negative strains.

Antibacterial activity of all the 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.

3.3.2.2 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% fetal 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^{o}C 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 microplate reader (MicrotekSigma360). Absorbance at 570nm directly correlates with cell viability. IC_{50} (µM) values were determined using Graph Pad prism software.

3.4 Conclusion

In conclusion, we have reported here synthesis of 7-coumarinyloxy acetamide derivatives (**4a** - **4e**) and evaluated for their antimicrobial and anticancer activity. Compounds **4a**-**4 e** has shown promising anticancer activity against A549 (lung cancer cell-line). Compounds **4b** and **4c** are showing very good activity against A549 (lung cancer cell-line) with IC_{50} values 0.66 and 0.41 µM respectively. Compounds **4e** showed excellent activity against A549 (lung cancer cell-line) with IC_{50} value 1.1 nM. While compound **4d** is showing very good activity against A375 (melanoma cell-line) with IC_{50} value 0.041 µM. All the synthesized compounds remain inactive against tested gram positive and gram negative bacteria.

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