CHAPTER-4

Synthesis, antimicrobial and anticancer evaluation of various amide derivatives from 3-amino methyl pyridine.

4 Synthesis, antimicrobial and anticancer evaluation of various amide derivatives from 3-amino methyl pyridine.

4.1 Introduction

Many microorganisms show drug resistance[1] hence research to find new antimicrobial agents have attracted attention. Various microorganisms pose serious challenges to medicinal chemist which led to the search of novel antibacterial agents.[2]

Review of literature indicates that N-containing heterocycles have significant place in the development of pharmacologically important molecules [3]. N-containing six member Pyridine and its derivatives4 abundantly exist in nature and they have various applications. Substituted pyridines are an important class of compounds in organic synthesis [5] .Pyridine and its derivatives have been reported to show various biological activities such as antimicrobial [6-7], anticancer[8], anticonvulsant [9], antiviral [10], anti HIV[11], antifungal activity[12] various substitution on pyridine nucleus are reported as antimicrobial agents such as 2-picolylamine has been used in the synthesis of many biologically active compounds. For example, there are many reports where picolylamine derivatives were used to form metal complexes as models that mimic both the structure and reactivity of metal ion sites in complex biological systems and it possess a broad spectrum of biological activities [13-16] but among them 3-amino methyl pyridine i.e 3-picolyl amine is rarely discussed. In recent years, 3-substituted pyridine heterocyclic compounds have received considerable attention due to their wide range of useful pharmacological properties, including antimicrobial activity. The variation in reactivity of the various reported analogues indicates the importance of the group in position 3rd of the pyridine moiety of the molecule.[17-20]. Martina S D et al reported Etricoxib having pyridine moiety (developed by Merck Figure-1) as a new class of NSAID (non steroidal anti inflammatory

drug) showing selectivity for COX-2 over COX-1 (Cyclooxygenese -1 and 2) and so it was expected that it will not display any long term side effects such as gastric ulceration[21]. 3-Amino pyridine and its polymer showed good antimicrobial activity against *staphylococcus aureus* .[22]

Cancer is a fatal disease in terms of morbidity and mortality affecting human health worldwide[23] .Ongoing research in this area has wide scope. To get effective treatment for cancer is a need of day because of limited and expensive medicines, hence wide research is going on to discover anticancer drug with good potency, safety and selectivity [24]. Sangani C B et al reported some pyridine- quinoline hybrid compounds as antimicrobial and anti cancer agents.[25] Grguric -Sipka ,S. et al reported complexes of ruthenium with thiosemicarbezone of 2-acetyl pyridine as a first water-soluble anti proliferative agent against SK-BR-3 breast cancer cell line and ovarian carcinoma cell line [26].

A search for new antimicrobial compounds with a more selectivity and lower toxicity is a need of today. It is therefore important to find out newer, safer and more effective antimicrobial agent with broad spectrum of activity.

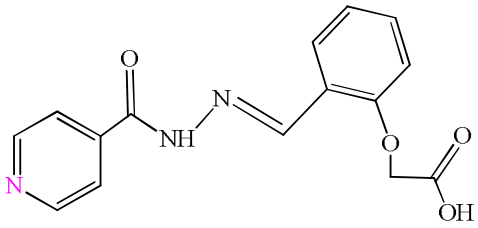
4.1.1 Therapeutic importance of pyridine:

Pyridine ring plays vital role in fundamental metabolism in two ways:

(1) As in reaction of amino acids, including racemization, decarboxylation, trasmination and elimination or replacement of substitution on the β –and γ-carbon atoms.

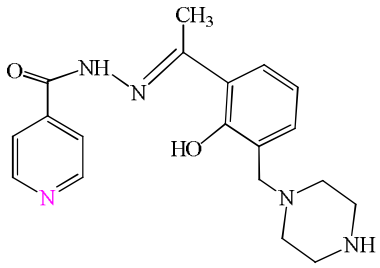
(2) As a coenzyme, nicotinamide adenine dinucleotide, it is playing roll in biological redox reactions.

Some examples of pyridine based new drugs **A** and **B** as anti tuberculosis[27].



A

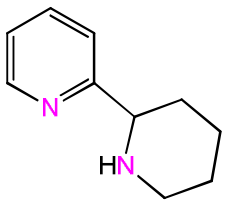
Aconazide



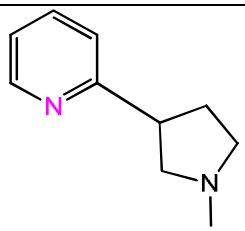
B

Substituted Ciprofloxacin

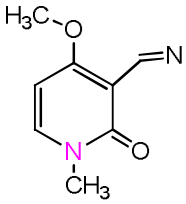
4.1.2 Some Commercially Available class of drugs having Pyridine moiety:



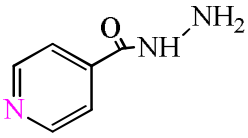
Anabasine



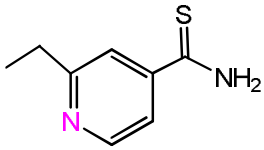
Nicotine



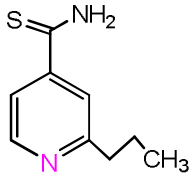
Ricinine



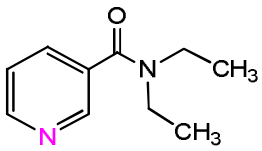
Isoniazide



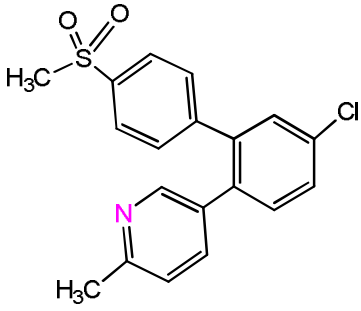
Ethionamide



Prothionamide



Nikethamide



Etricoxib

Figure-1

Since various Pyridine third position substituted derivatives are used as drugs in market and in search of new antimicrobial agents, we have synthesized some new 3-amino methyl pyridine substituted derivatives and studied their antimicrobial and anticancer activity.

Structures of the newly synthesized compounds were confirmed by IR, ^{1}H NMR, ^{13}C NMR, mass spectral studies and Purity of the compounds was checked by TLC and by C, H, and N elemental analyses. The melting points of all the synthesized compounds were taken in open capillary tube and were uncorrected.

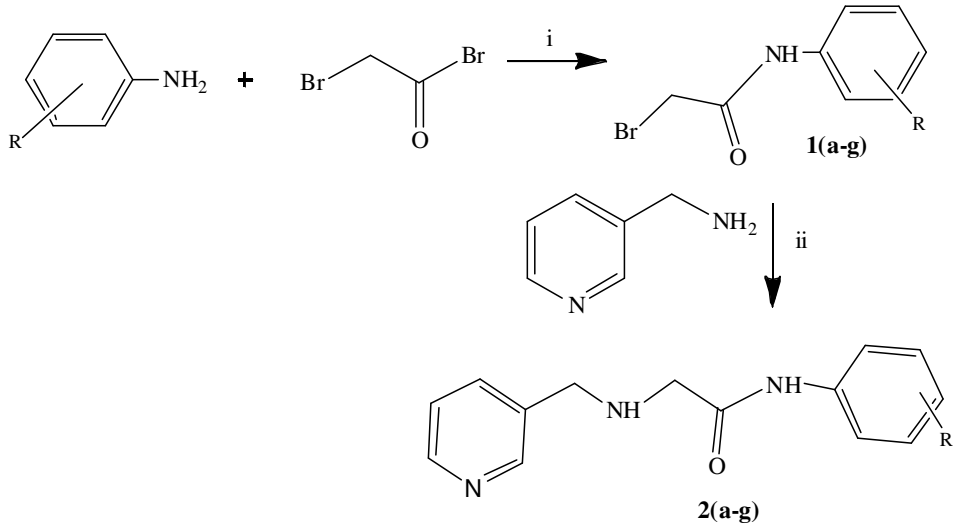
The newly synthesized compounds were tested for their antimicrobial activity against gram positive and gram negative bacteria. Some of the synthesized compounds were found to exhibit potent activity. Anticancer activity studies of all the synthesized compounds had also carried out.

4.2 Result and Discussion

4.2.1 Chemistry

Bromoacetyl bromide on reaction with various amines in dichloromethane(DCM) gave 2-bromo-N-substituted phenylacetamide **1(a-g)**.The reaction of **1(a-g)** with 3-picolylamine **2** in dimethylformamide (DMF) in presence of triethylamine (TEA) gave various N-substituted phenyl-2(pyridine-3-yl-methyl)acetamide **2(a-g)** .The structure of all compounds were confirmed by their IR, ^{1}H NMR,^{13}C NMR, elemental analysis and ESI-Mass.

Reaction Scheme for the synthesis of 3-amino methyl pyridine derivatives



Where in R=

Compound	-R
2a	4-Cl
2b	4-F
2c	3-Cl
2d	4-CH_3
2e	3-F
2f	3-NO_2
2g	4-$COCH_3$

Scheme-1: Synthesis of *N*-substituted phenyl-2-[(pyridin-3-ylmethyl) amino] acetamide

Reagents & conditions: (i)TEA, Stirring at 0- 5 °C 30 min, RT, 2 h ,DCM ,(ii)TEA, RT Stirring 8h, DMF.

The IR spectrum Figure-2, (Page No. 119) of compound 2a exhibited strong band at 3383 cm^{-1} for the characteristic –NH stretching vibrations. Another strong band at 1691 cm^{-1} for the –CO group stretching of amide and another strong band exhibited for at 2710 cm^{-1} for –CH group stretching and 1612 cm^{-1} for C=N stretching.

In ^{1}H NMR spectrum of **2a** Figure-3, (Page No. 120) the two $–CH_2$ groups were observed as singlet for the two protons at δ 4.0 and 4.4 for $-COCH_2$ and $-CH_2NH_2$ respectively. All aromatic protons observed between δ7.20 – 8.40.The amide proton was observed as a singlet at downfield to the aromatic protons i.e at δ 10.5 to 11.In the ^{13}C NMR spectrum of compound **2a** Figure-4,(Page No-121). Two carbons for methylene groups observed at δ 47 and 48.The characteristic –CO carbon was observed at δ 164. All aromatic carbons observed at δ120 to 170 .The ESI mass spectrum Figure-5,(page No-122) of compound **2a** showed M^+ peak at 291.Simmilarly in the IR spectrum of compound **2b** Figure -6,(Page No -123) exhibited strong band at 3398 cm^{-1} for the characteristic –NH stretching vibrations. Another strong band at 1689 cm^{-1} for the –CO group stretching of amide and another strong band exhibited for at 2710 cm^{-1} for –CH group stretching and 1620 cm^{-1} for -CN stretching. In ^{1}H NMR spectrum of **2b** Figure-7, (Page No. 124) the two $–CH_2$ groups were observed as singlet for the two protons at δ 4.03 and 4.46 for $-COCH_2$ and $-CH_2NH_2$ respectively. All aromatic protons observed between δ7.20 – 8.40.The amide proton was observed as a singlet at downfield to the aromatic protons i.e at δ 10.5 to 11. In general for all **2(a-g)**, the IR spectra showed characteristic band at δ 3380 cm^{-1} for the NH stretching, Strong band at 1650-1690 cm^{-1} for amide ,Carbonyl starching and band at 1612 cm^{-1} for >C=N stretching. In ^{1}H NMR of all **2(a-g)**, two methylene protons observed around δ 4.0-4.5 as two singlets. All aromatic protons were observed at δ 7.20 -8.2.The –NH proton observed at δ 10-11.

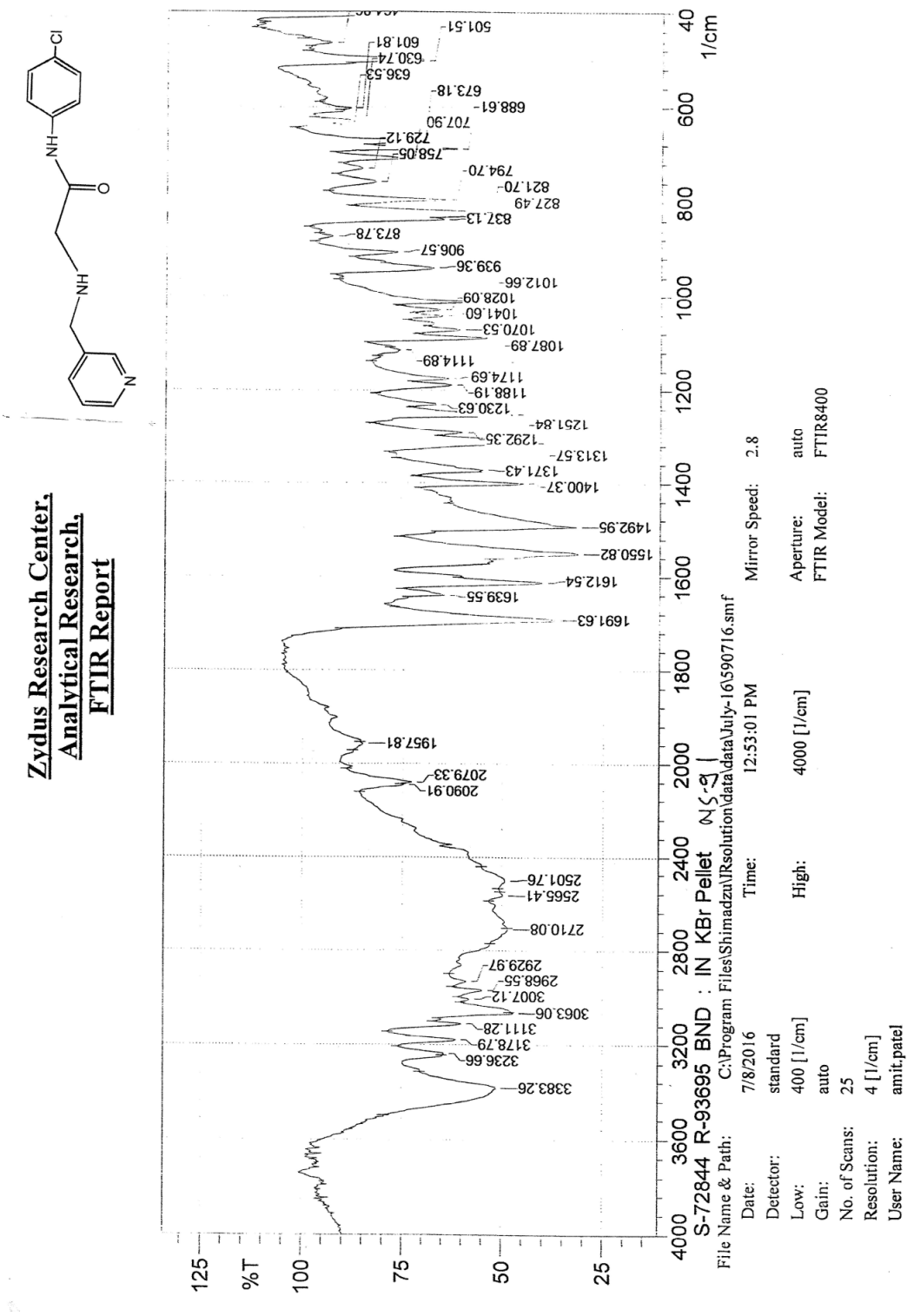


Figure-1 IR Spectrum of N-(4-chloro phenyl)-2-[(pyridin-3-ylmethyl) amino] acetamide i.e **2a**

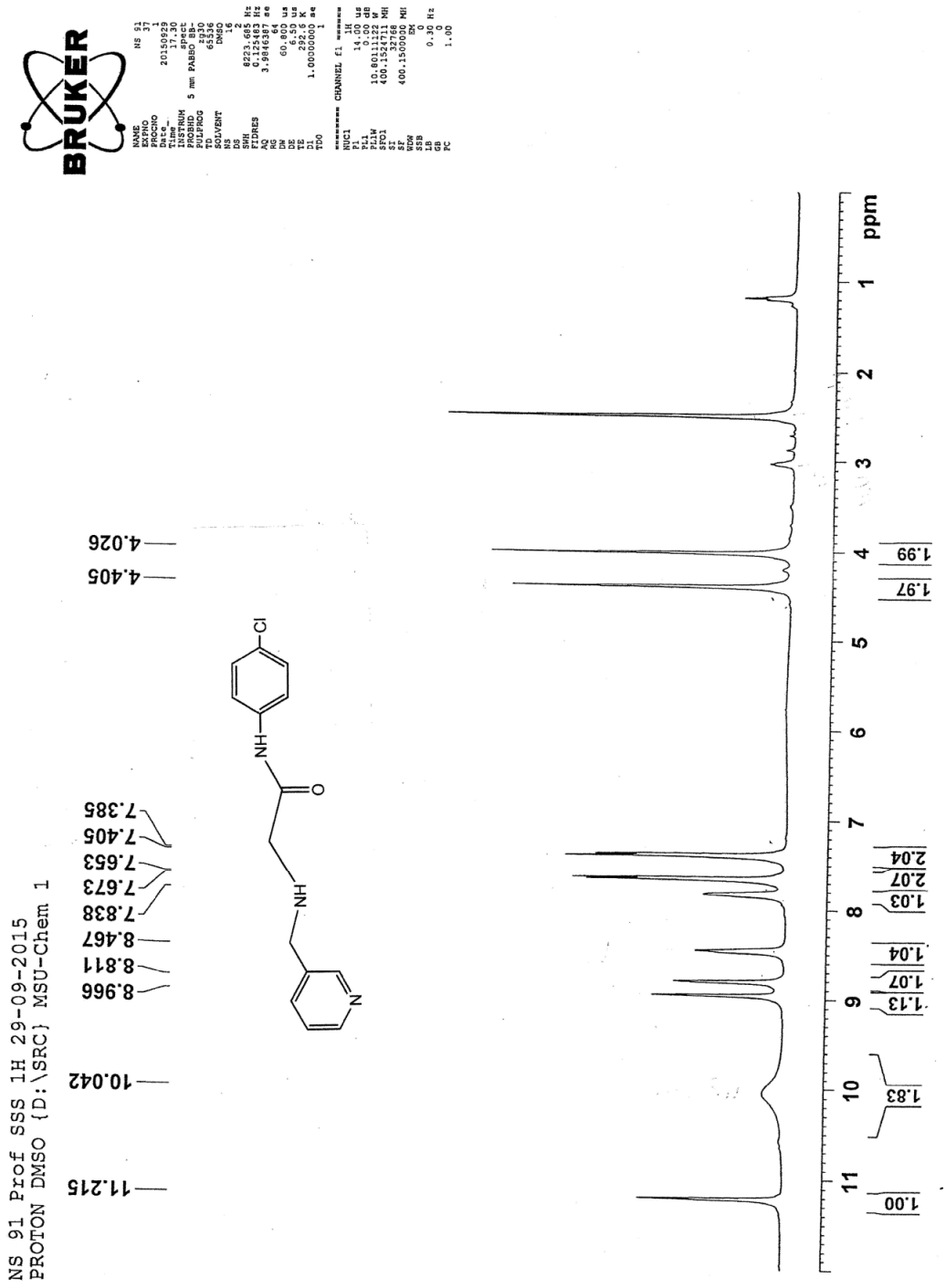


Figure-2 ^{1}H NMR Spectrum of N-(4-chloro phenyl)-2-[(pyridin-3-ylmethyl) amino] acetamide i.e **2a**

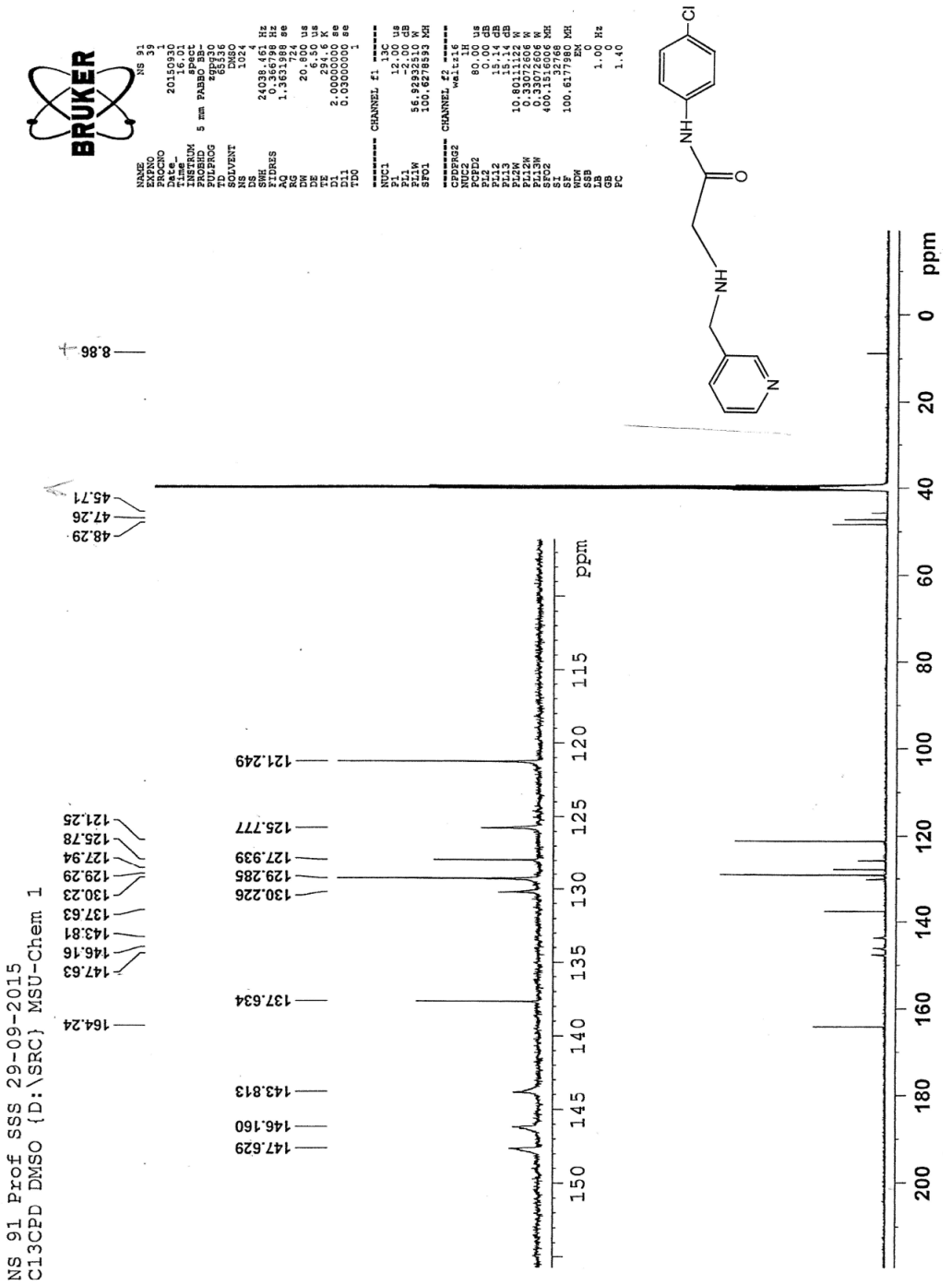


Figure-3 ^{13}C NMR Spectrum of N-(4-chloro phenyl)-2-[(pyridin-3-ylmethyl) amino] acetamide i.e **2a**

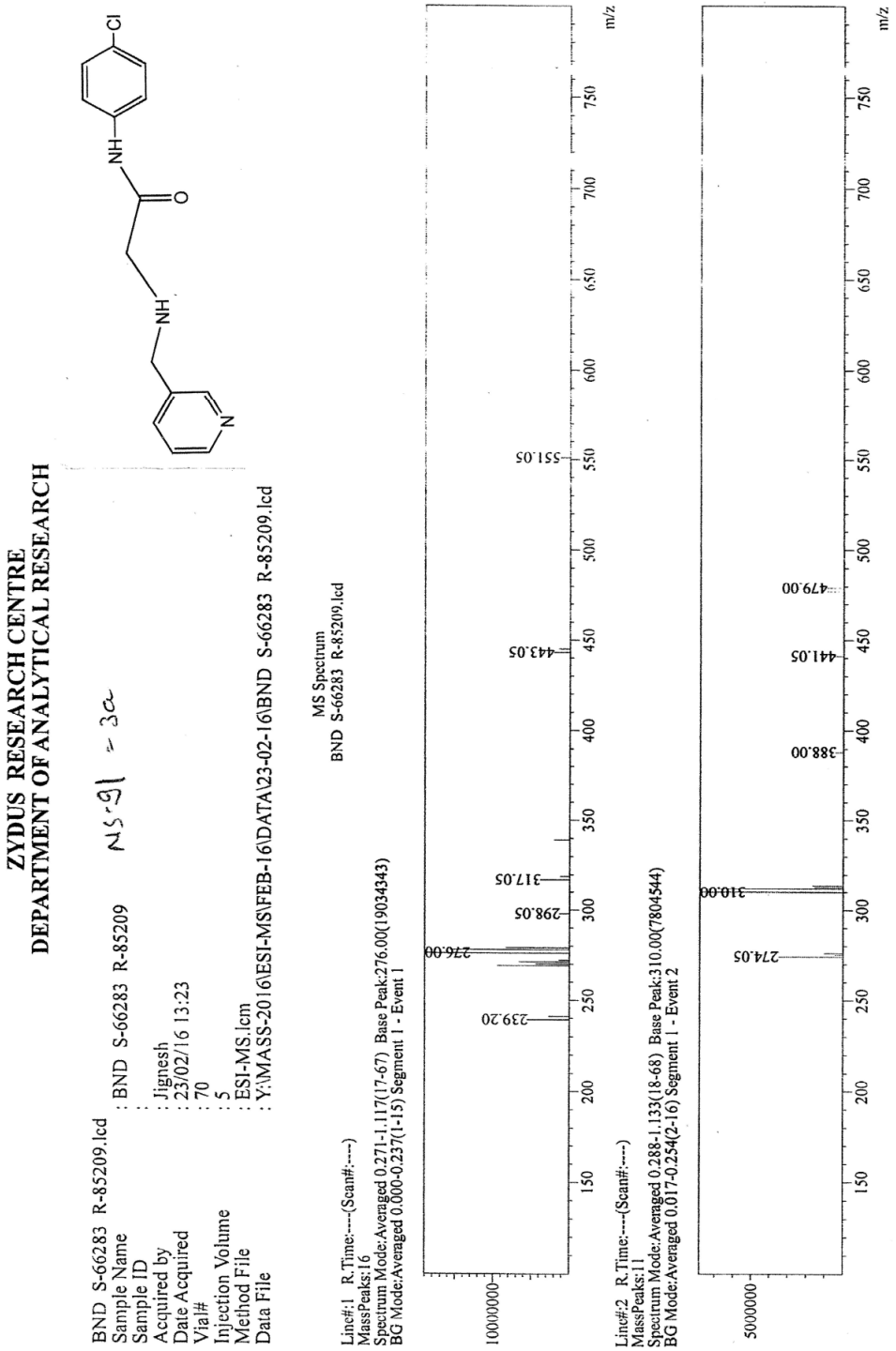


Figure-4 Mass Spectrum of N-(4-chloro phenyl)-2-[(pyridin-3-ylmethyl) amino] acetamide i.e **2a**

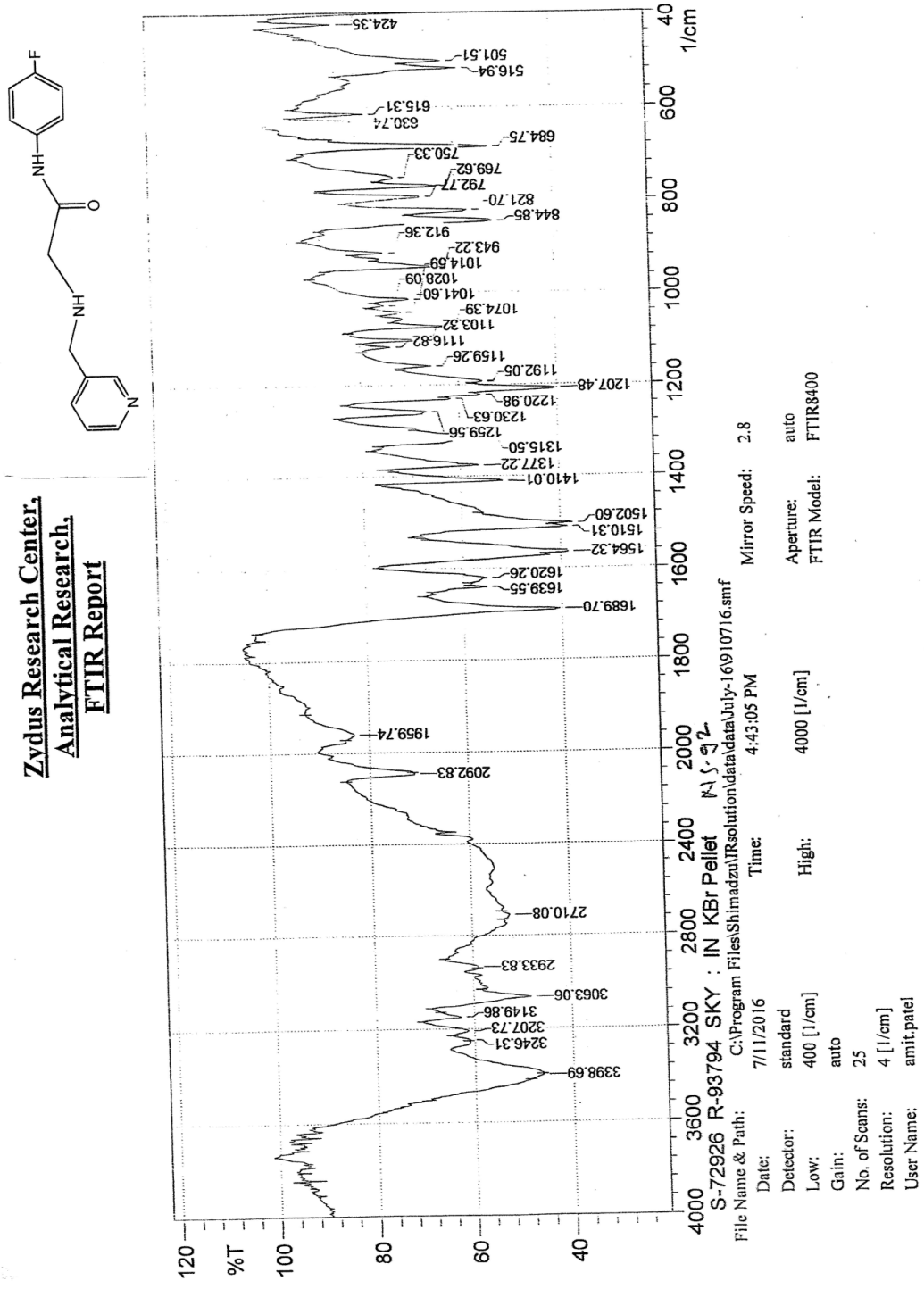


Figure-5 IR Spectrum of N-(4-Flouro phenyl)-2-[(pyridin-3-ylmethyl) amino] acetamide i.e **2b**

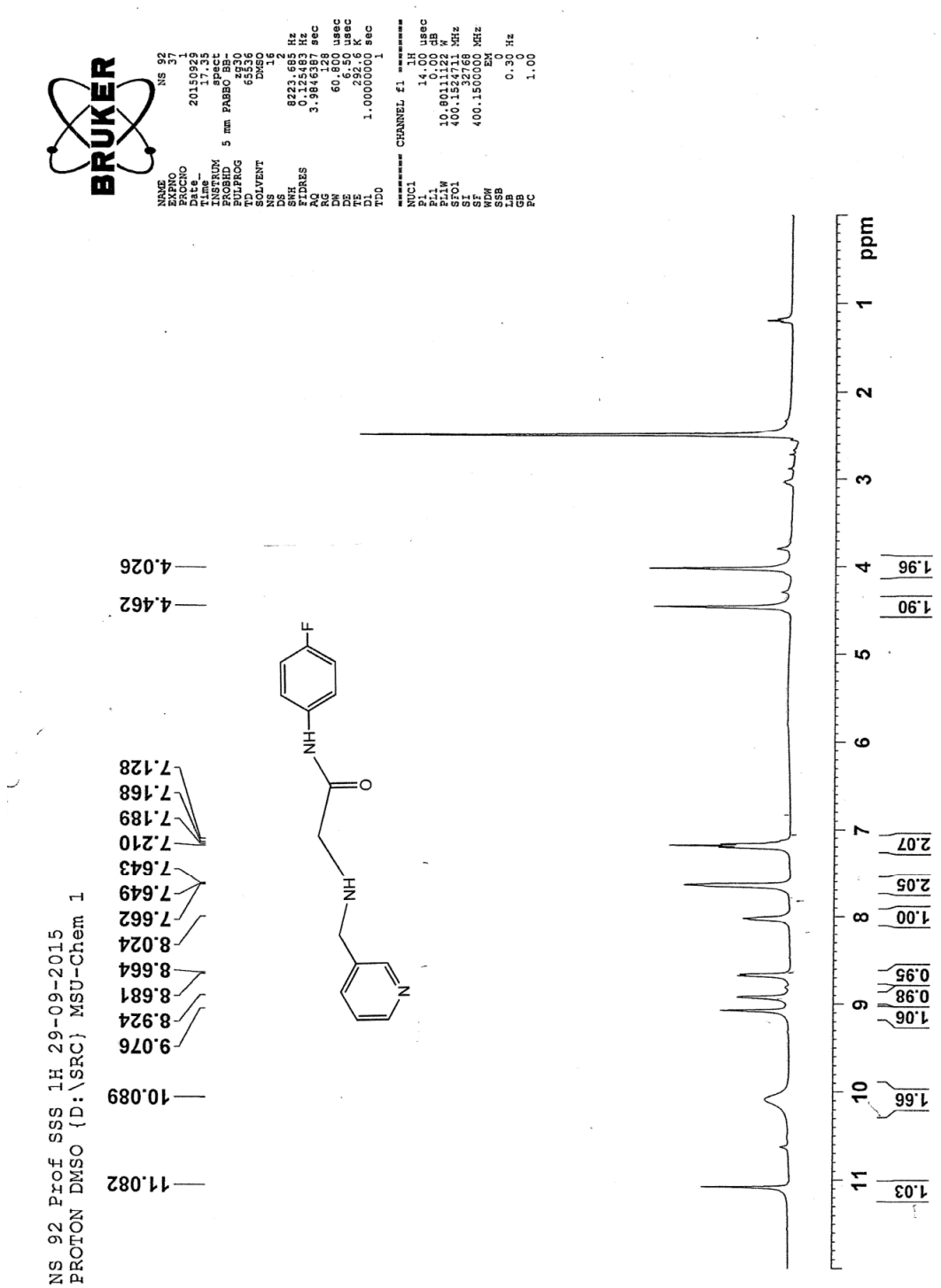


Figure-6 ^{1}H NMR Spectrum of N-(4-Flouro phenyl)-2-[(pyridin-3-ylmethyl) amino] acetamide i.e **2b**

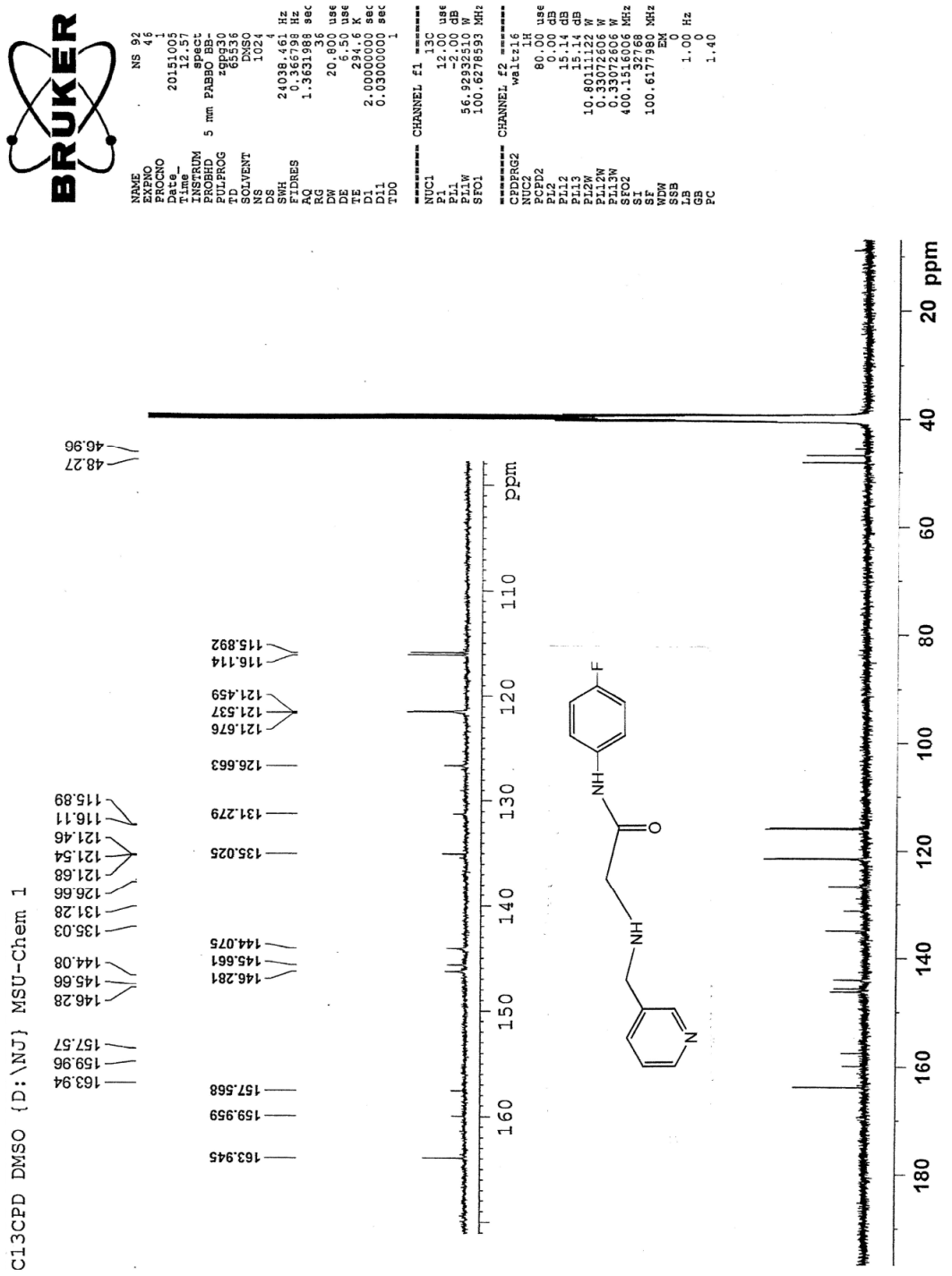


Figure-7 ^{13}C NMR Spectrum of N-(4-Flouro phenyl)-2-[(pyridin-3-ylmethyl) amino] acetamide i.e **2b**

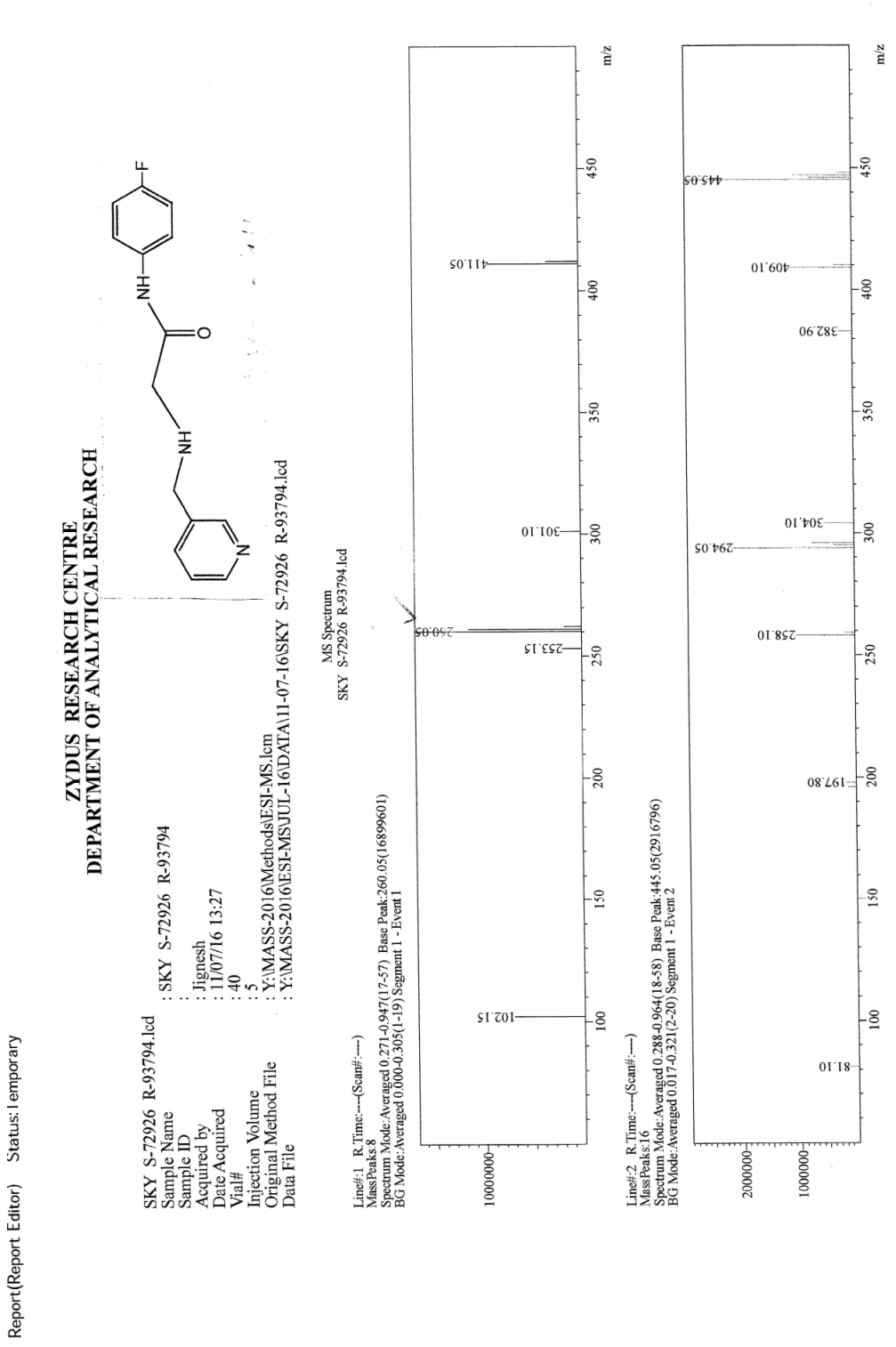


Figure-8 Mass Spectrum of N-(4-Flouro phenyl)-2-[(pyridin-3-ylmethyl) amino] acetamide i.e **2b**

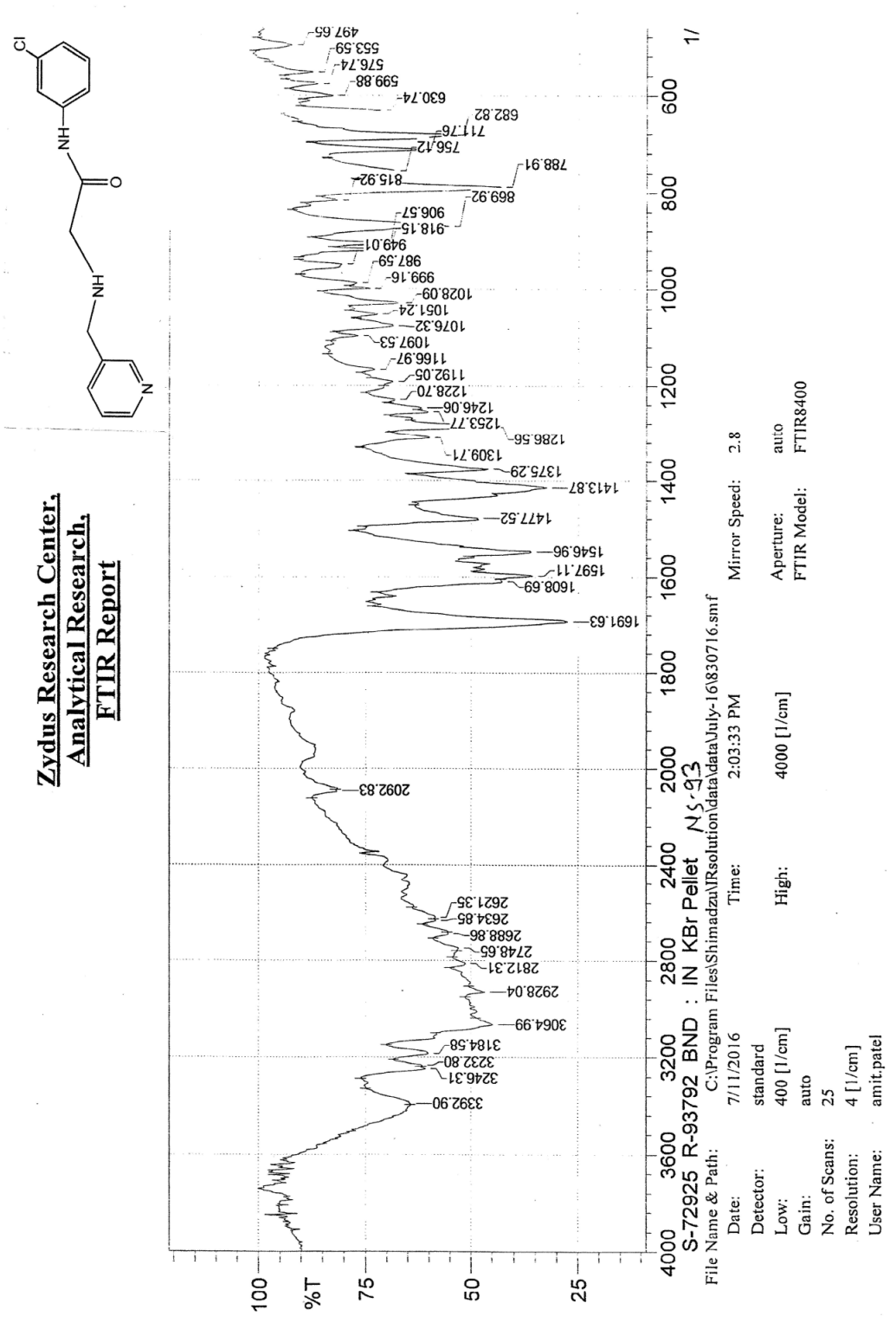


Figure-9 IR Spectrum of N-(3-chlorophenyl)-2-[(pyridin-3-ylmethyl) amino] acetamide i.e **2c**

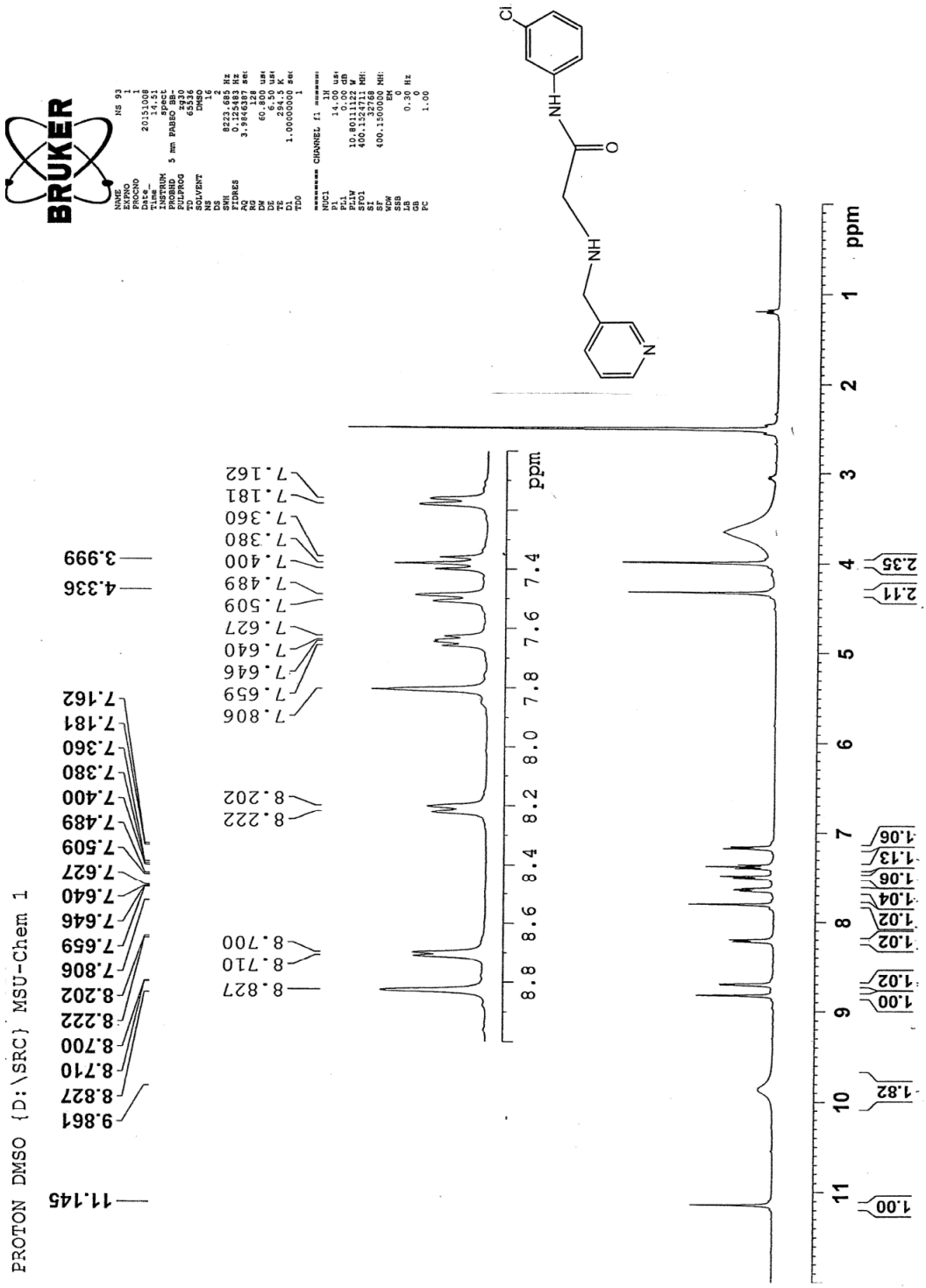


Figure-10 ^{1}H NMR Spectrum of N-(3-chlorophenyl)-2-[(pyridin-3-ylmethyl) amino] acetamide i.e **2c**

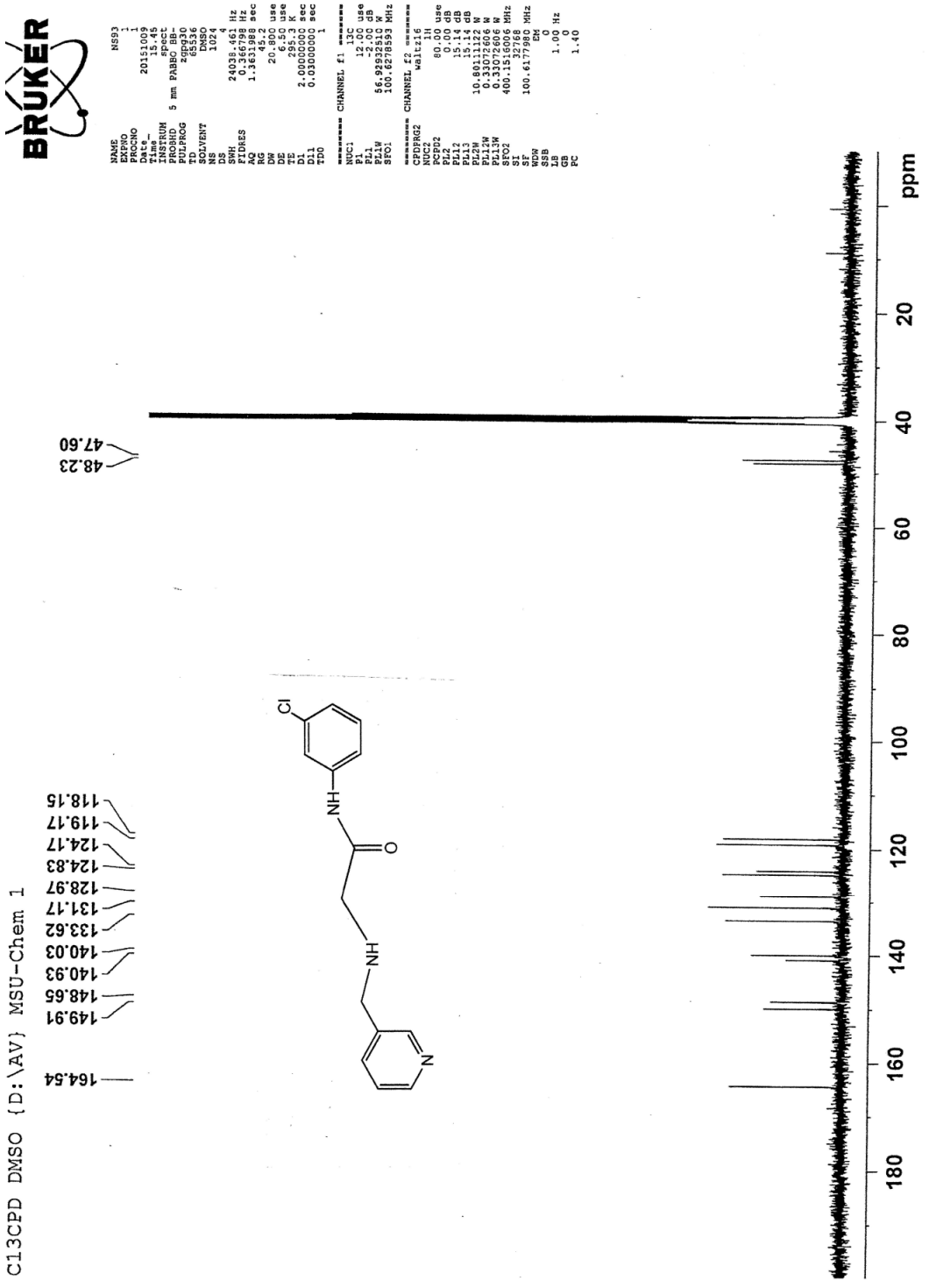


Figure-11 ^{13}C NMR Spectrum of N-(3-chlorophenyl)-2-[(pyridin-3-ylmethyl) amino] acetamide i.e **2c**

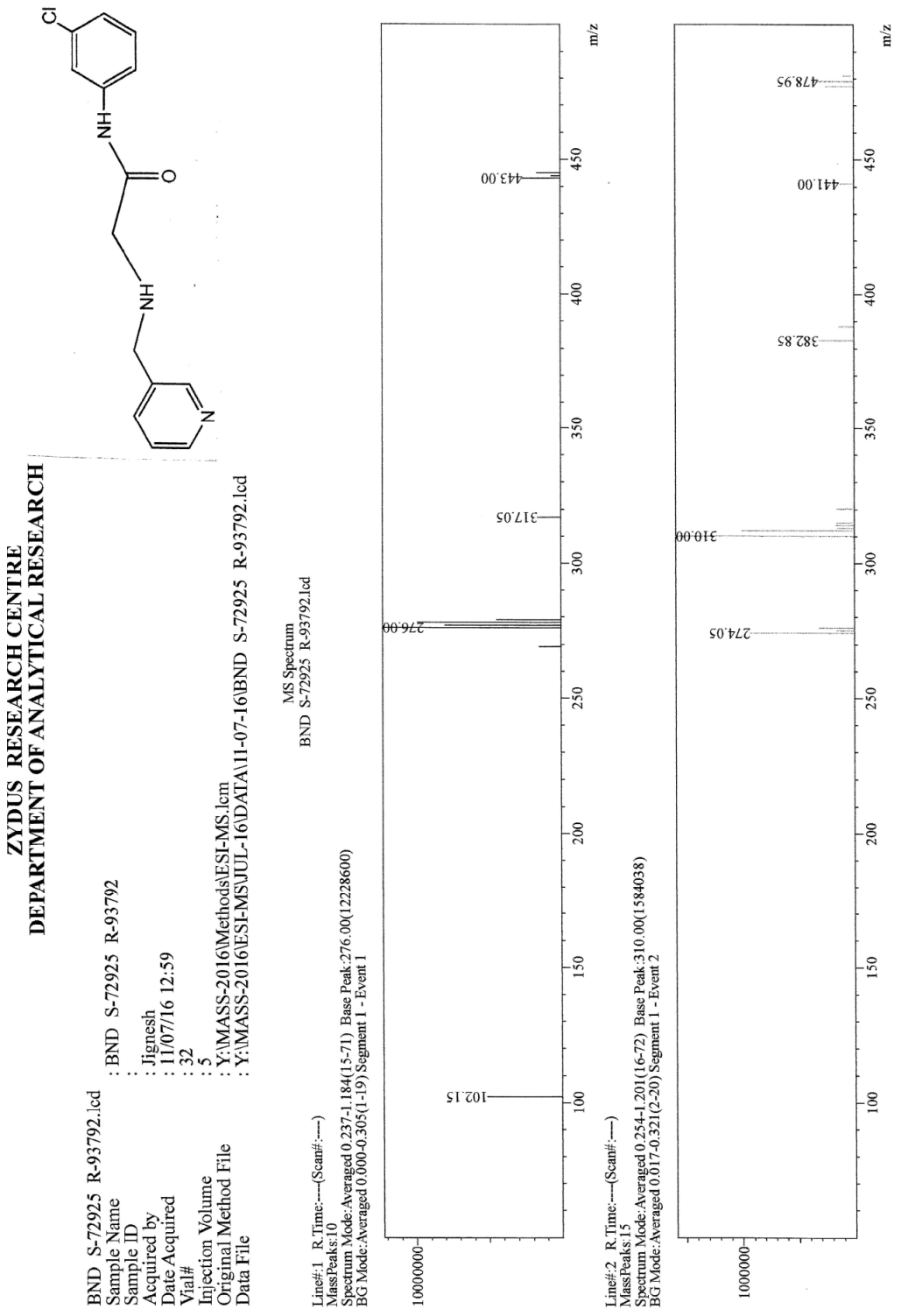


Figure-12 Mass Spectrum of N-(3-chlorophenyl)-2-[(pyridin-3-ylmethyl) amino] acetamide i.e **2c**

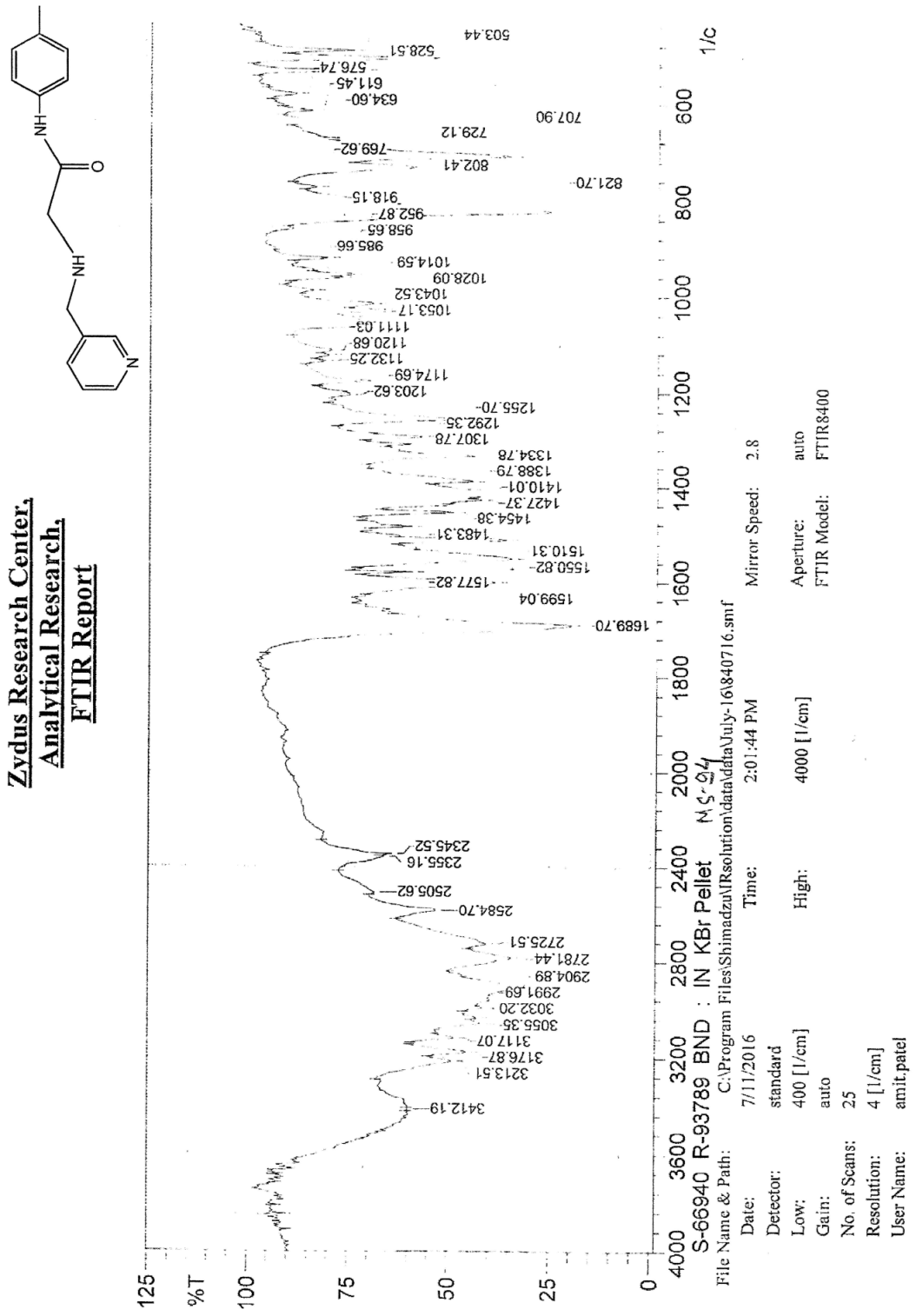


Figure-13 IR Spectrum of N-(4-Methyl phenyl)-2-[(pyridin-3-ylmethyl) amino] acetamide i.e **2d**

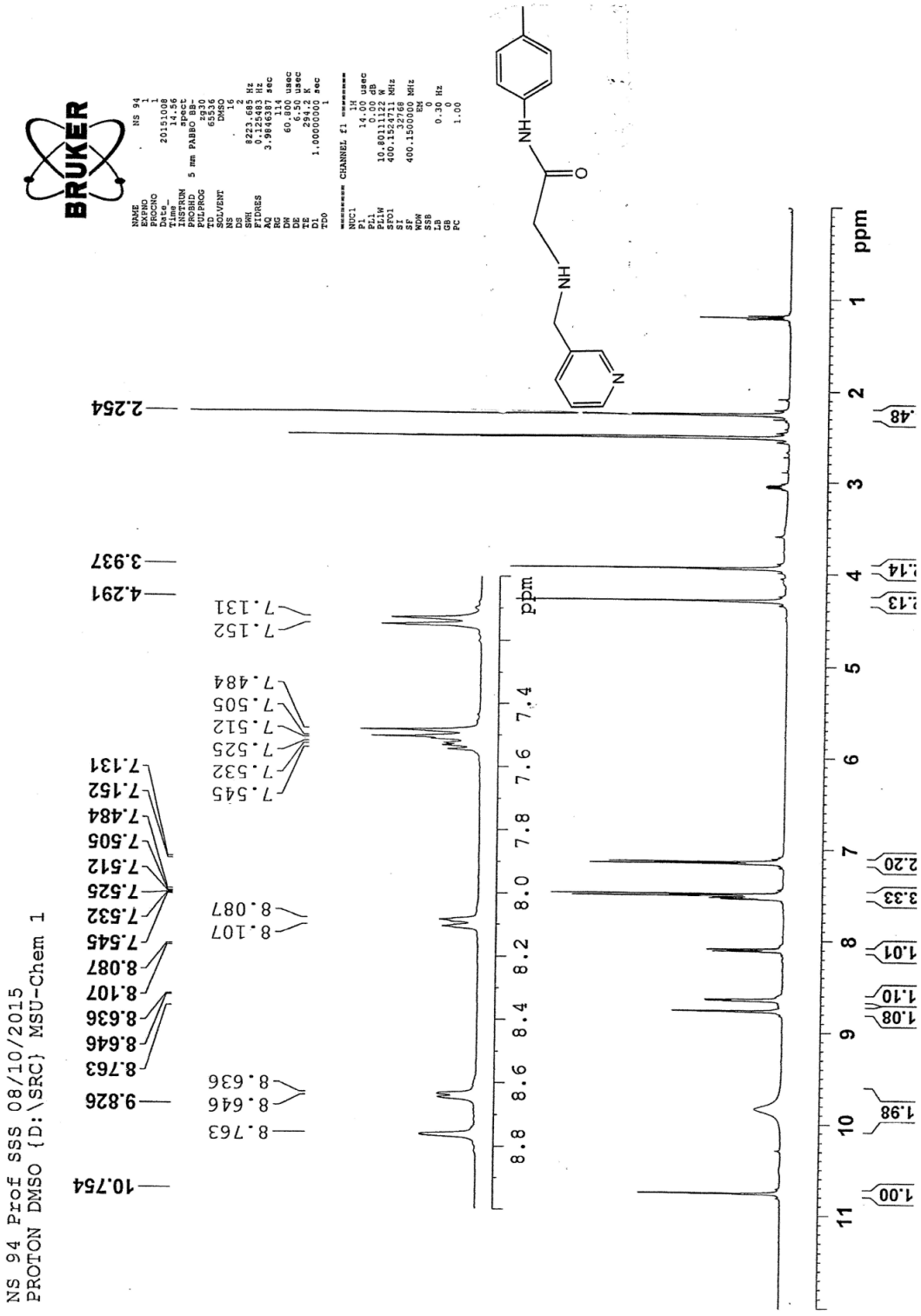


Figure-14 ^{1}H NMR Spectrum of N-(4-Methyl phenyl)-2-[(pyridin-3-ylmethyl) amino] acetamide i.e **2d**

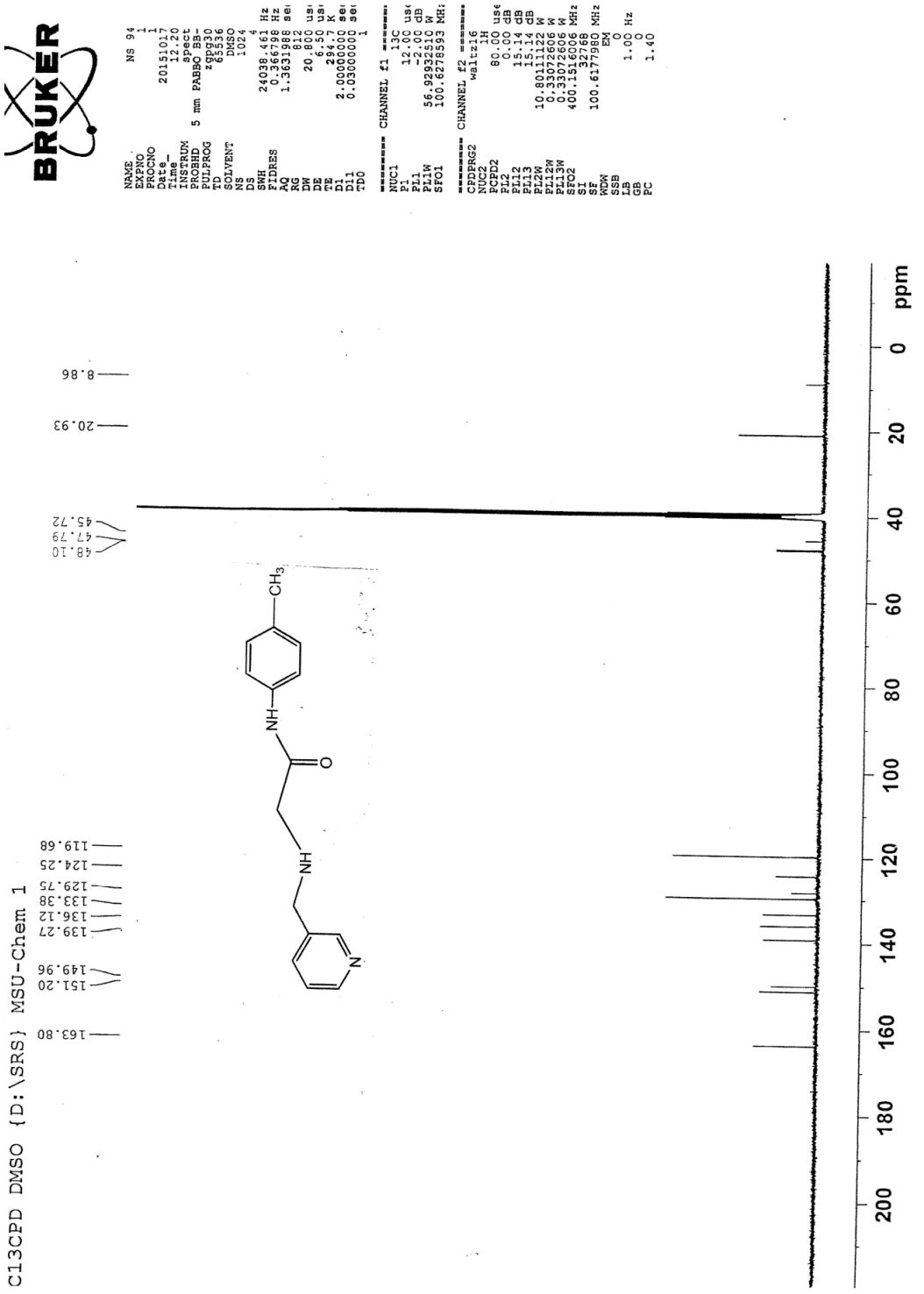


Figure-15 ^{13}C NMR Spectrum of N-(4-Methyl phenyl)-2-[(pyridin-3-ylmethyl) amino] acetamide i.e **2d**

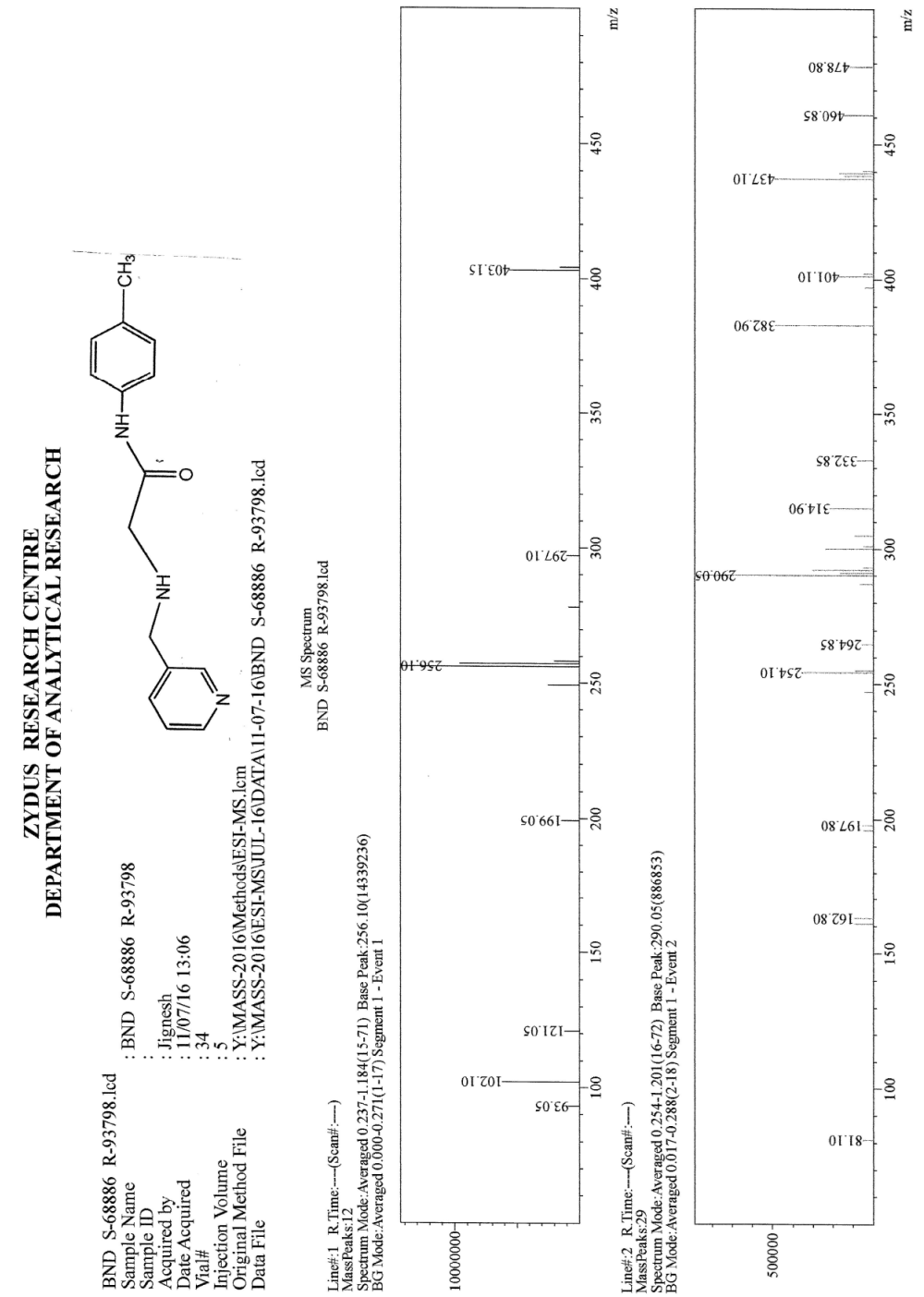


Figure-16 Mass Spectrum of N-(4-Methyl phenyl)-2-[(pyridin-3-ylmethyl) amino] acetamide i.e **2d**

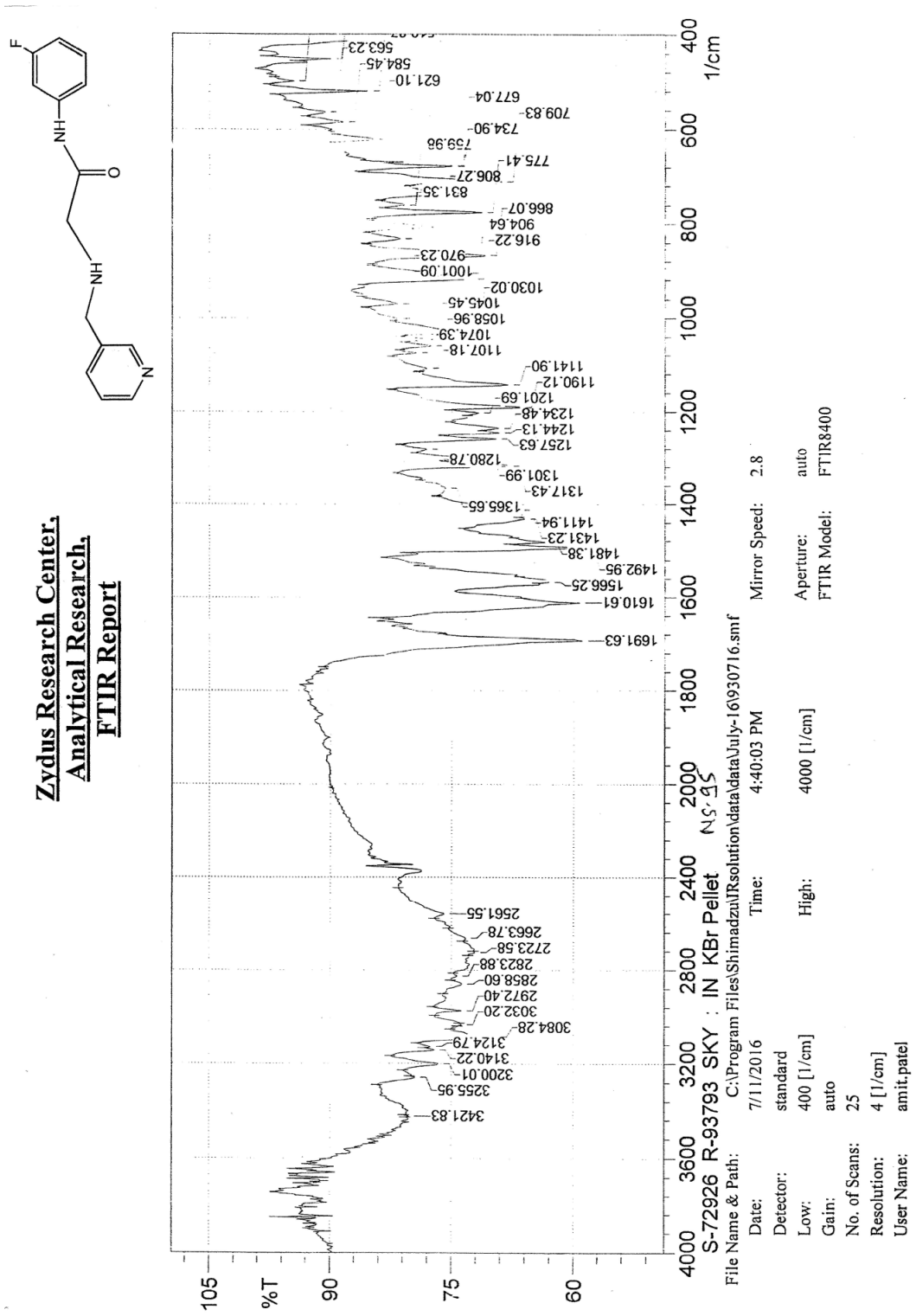


Figure-17 IR Spectrum of N-(3-Flouro phenyl)-2-[(pyridin-3-ylmethyl) amino] acetamide i.e **2e**

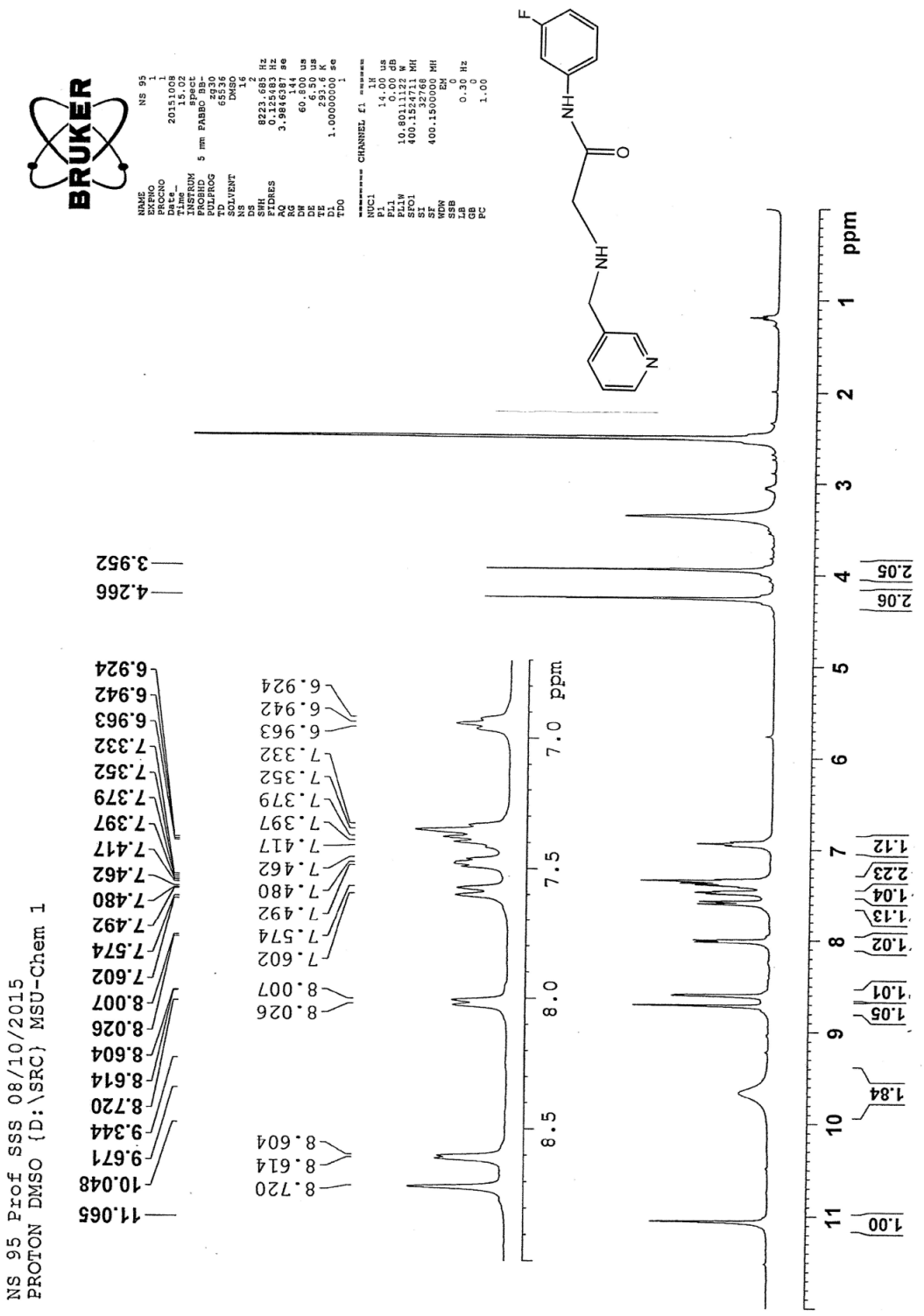


Figure-18 ^{1}H NMR Spectrum of N-(3-Flouro phenyl)-2-[(pyridin-3-ylmethyl) amino] acetamide i.e **2e**

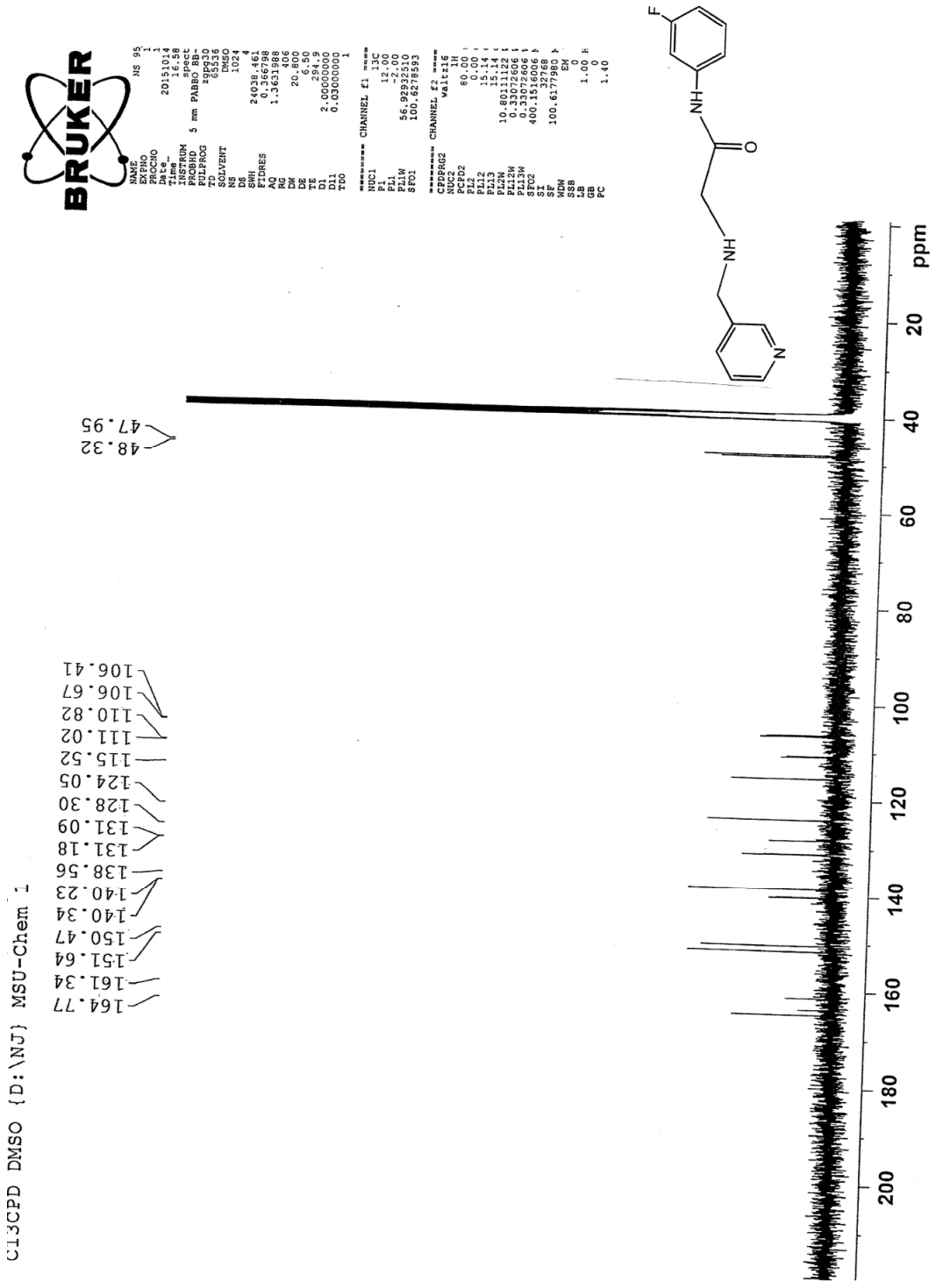


Figure-19 ^{13}C NMR Spectrum of N-(3-Flouro phenyl)-2-[(pyridin-3-ylmethyl) amino] acetamide i.e **2e**

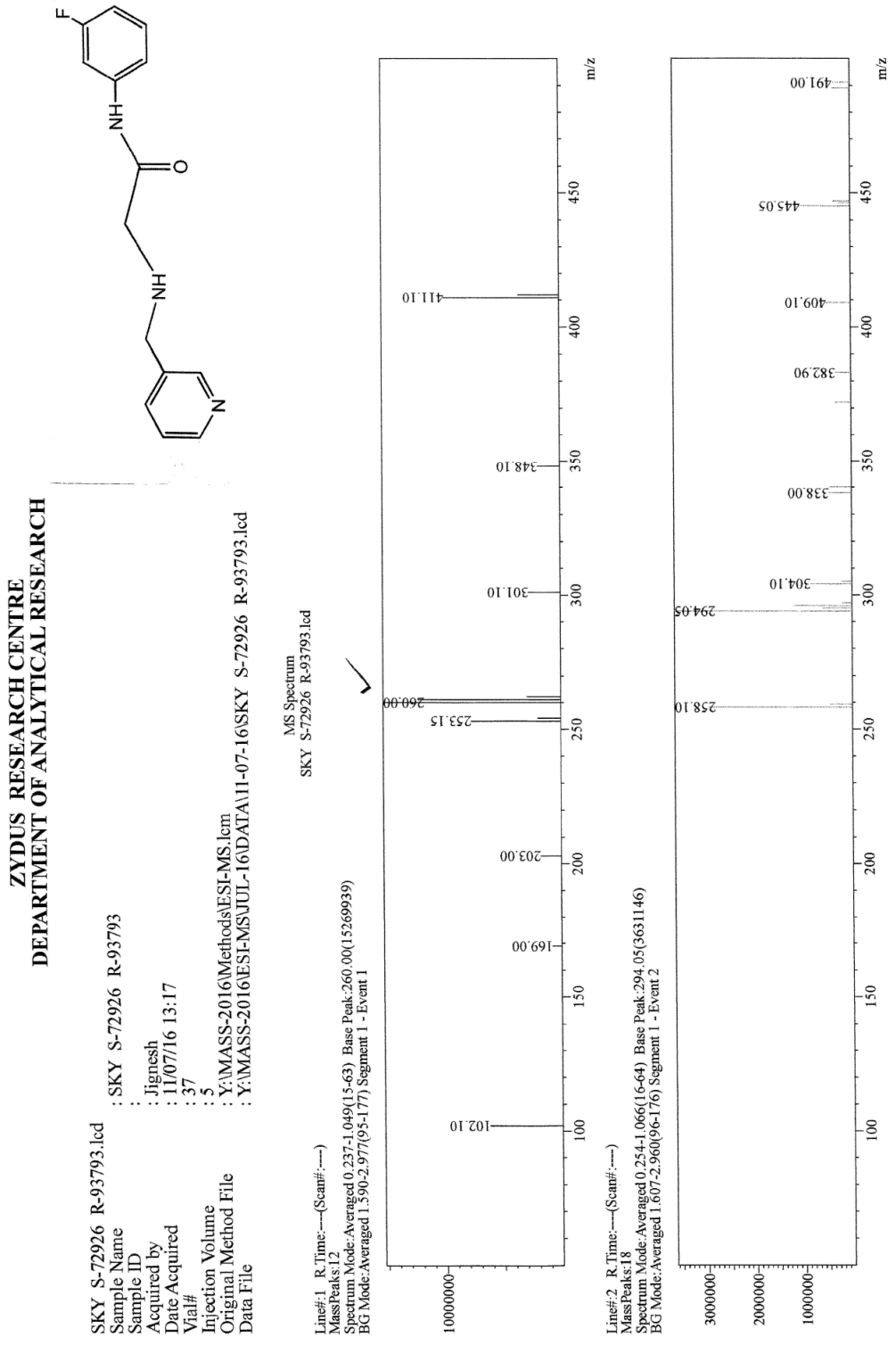


Figure-20 Mass Spectrum of N-(3-Flouro phenyl)-2-[(pyridin-3-ylmethyl) amino] acetamide i.e **2e**

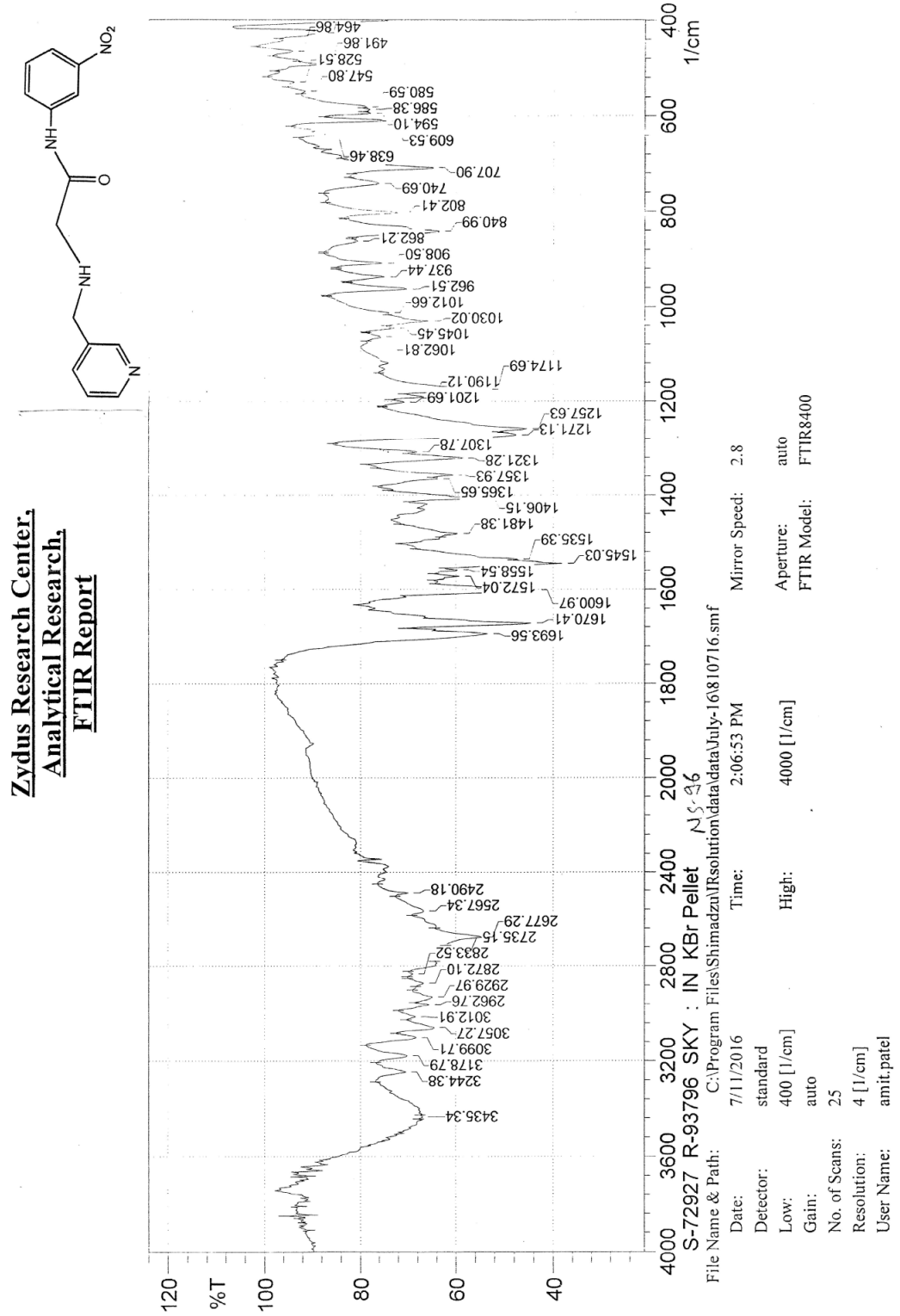


Figure-21 IR Spectrum of N-(3-Nitro phenyl)-2-[(pyridin-3-ylmethyl) amino] acetamide i.e **2f**

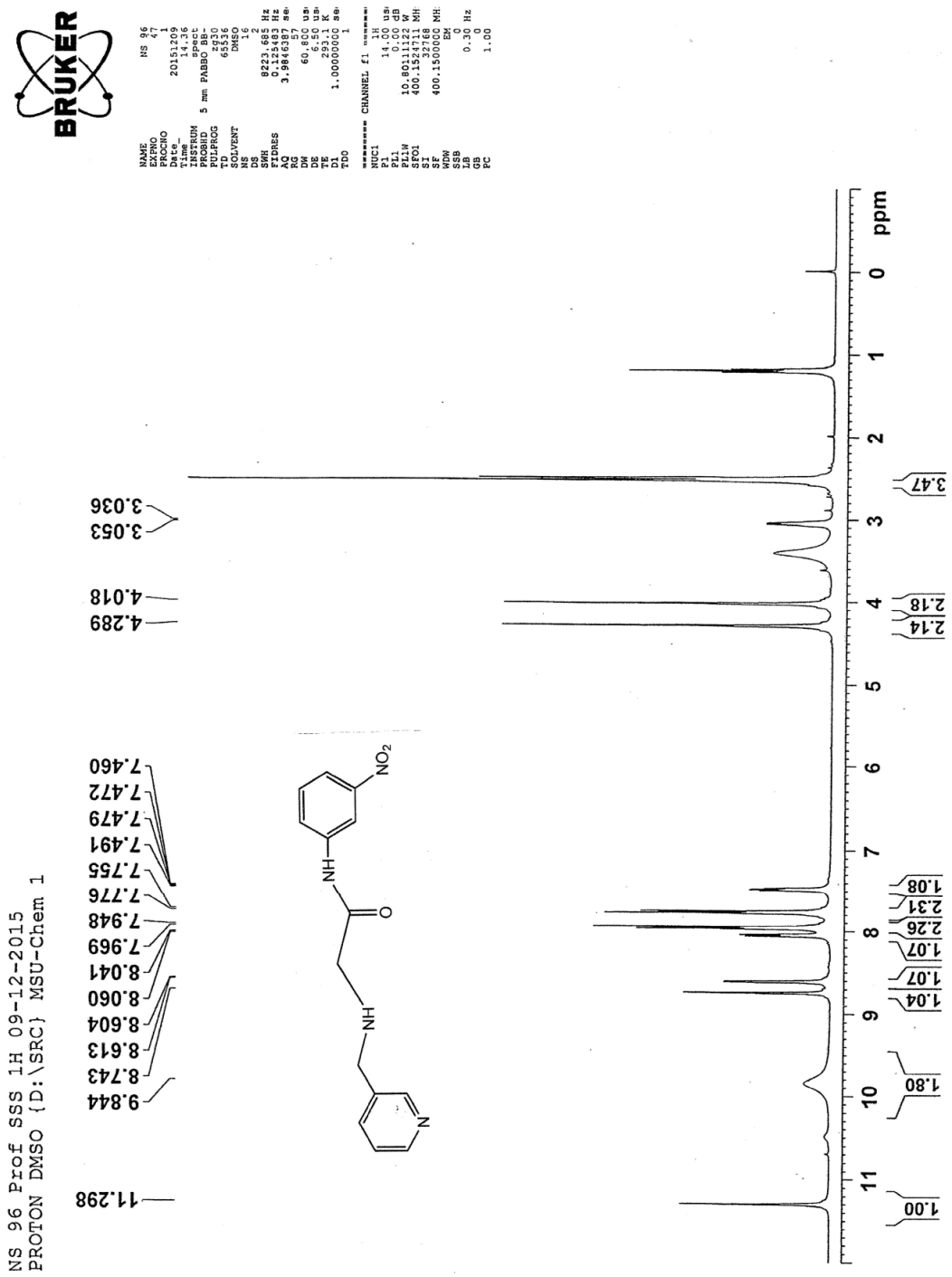


Figure-22 ^{1}H NMR Spectrum of N-(3-Nitro phenyl)-2-[(pyridin-3-ylmethyl) amino] acetamide i.e **2f**

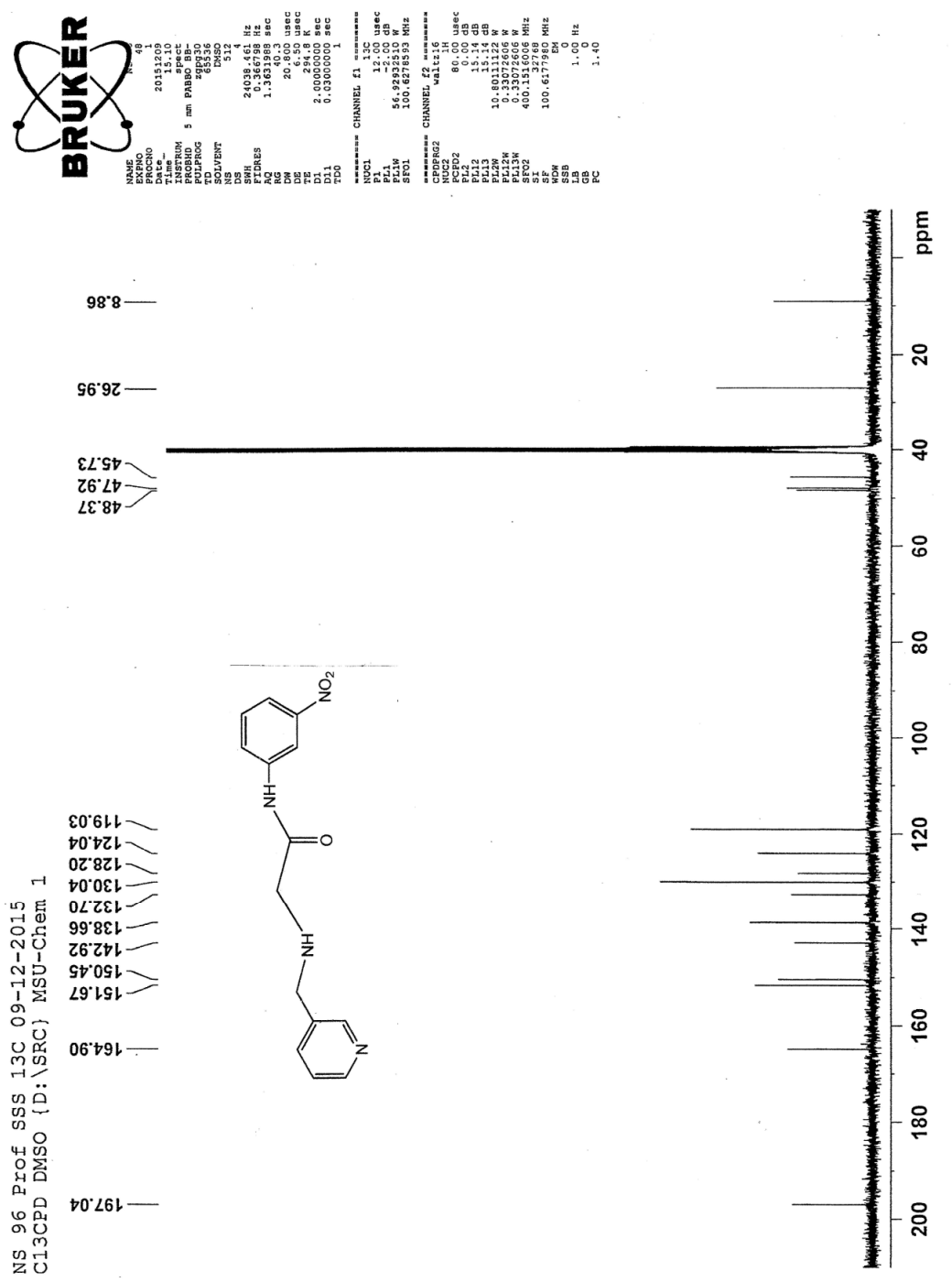


Figure-23 ^{13}C NMR Spectrum of N-(3-Nitro phenyl)-2-[(pyridin-3-ylmethyl) amino] acetamide i.e **2f**

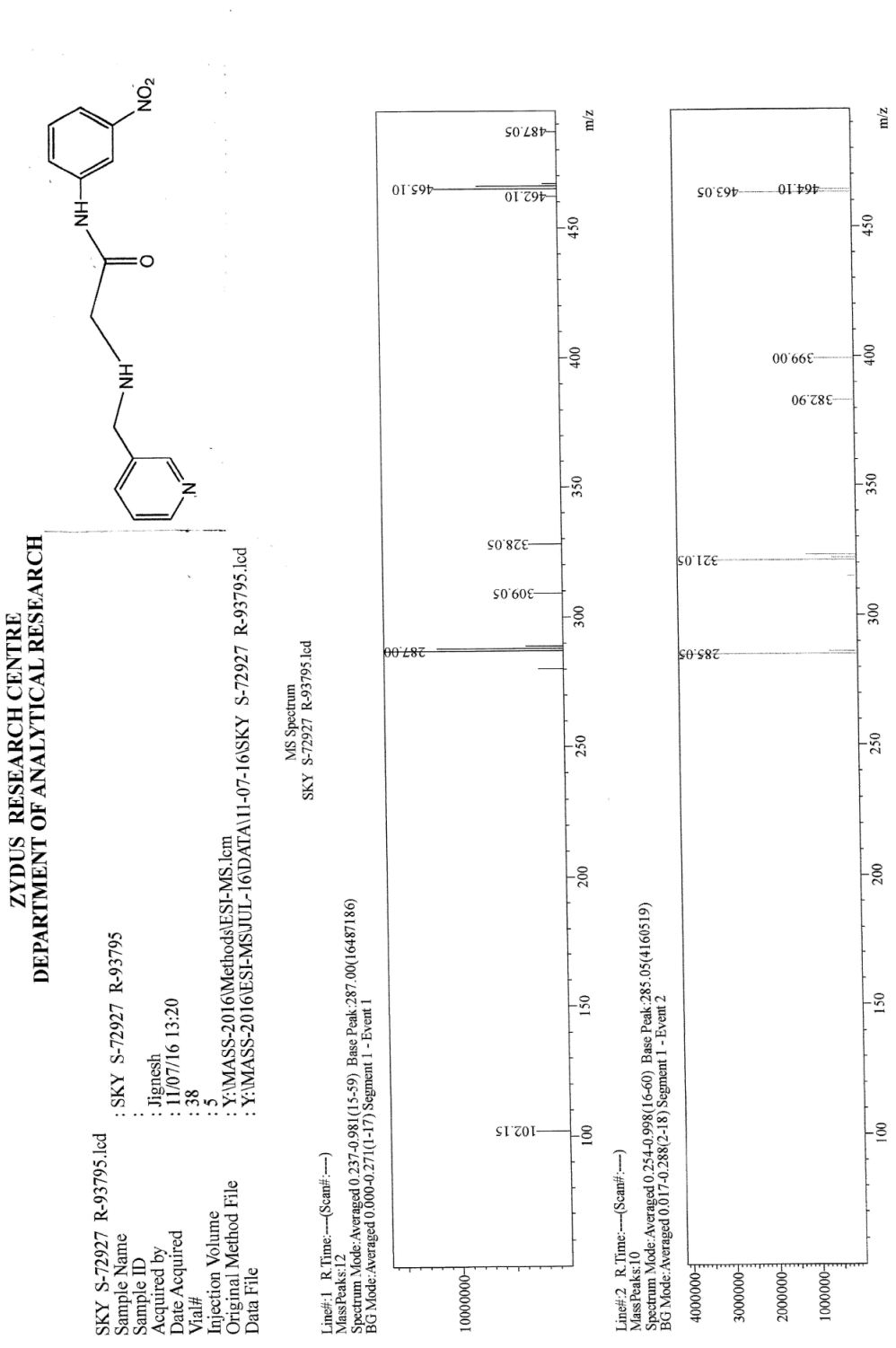


Figure-24 Mass Spectrum of N-(3-Nitro phenyl)-2-[(pyridin-3-ylmethyl) amino] acetamide i.e **2f**

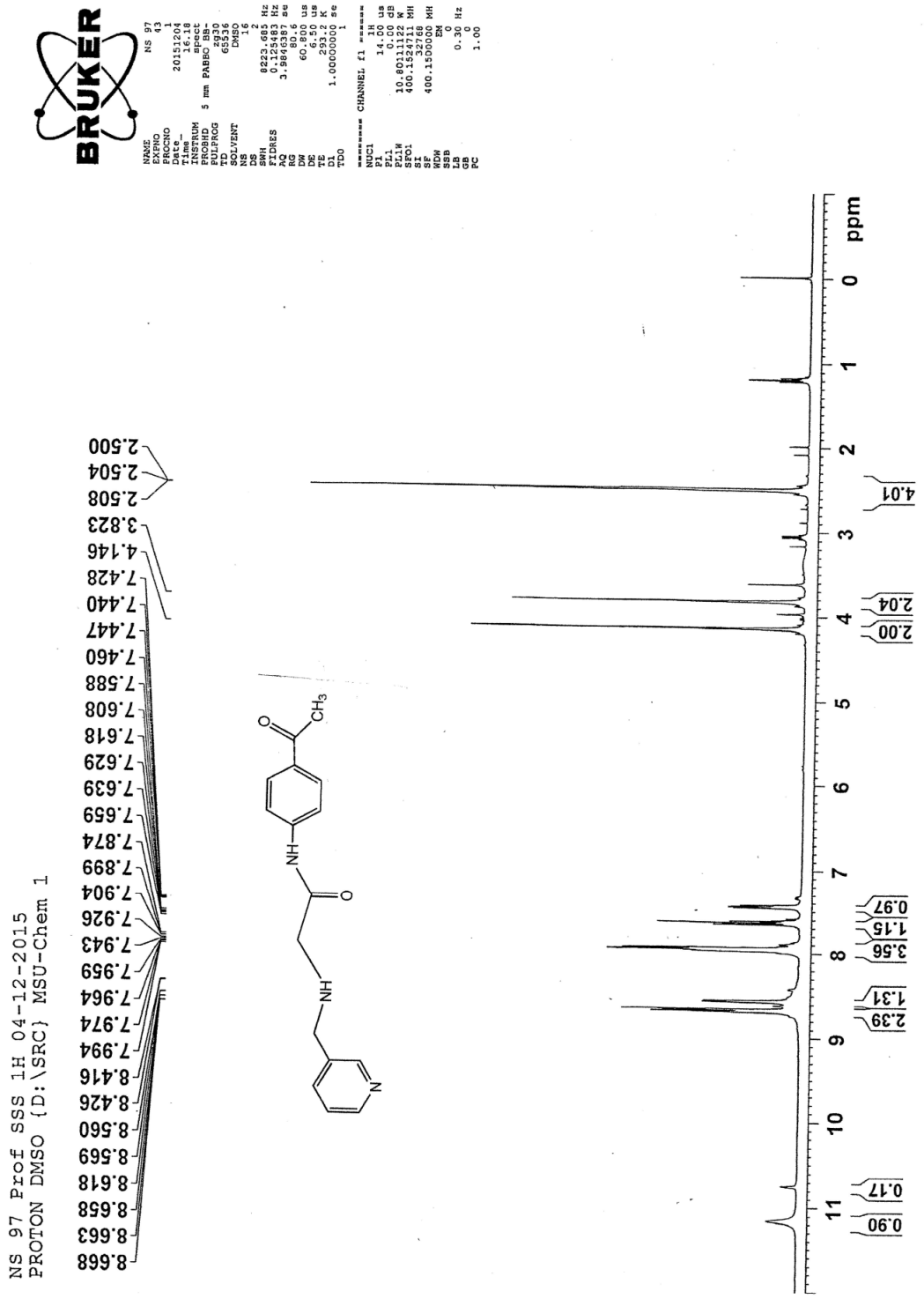


Figure-25 ^{1}H NMR Spectrum of N-(4-Acetyl phenyl)-2-[(pyridin-3-ylmethyl) amino] acetamide i.e **2g**

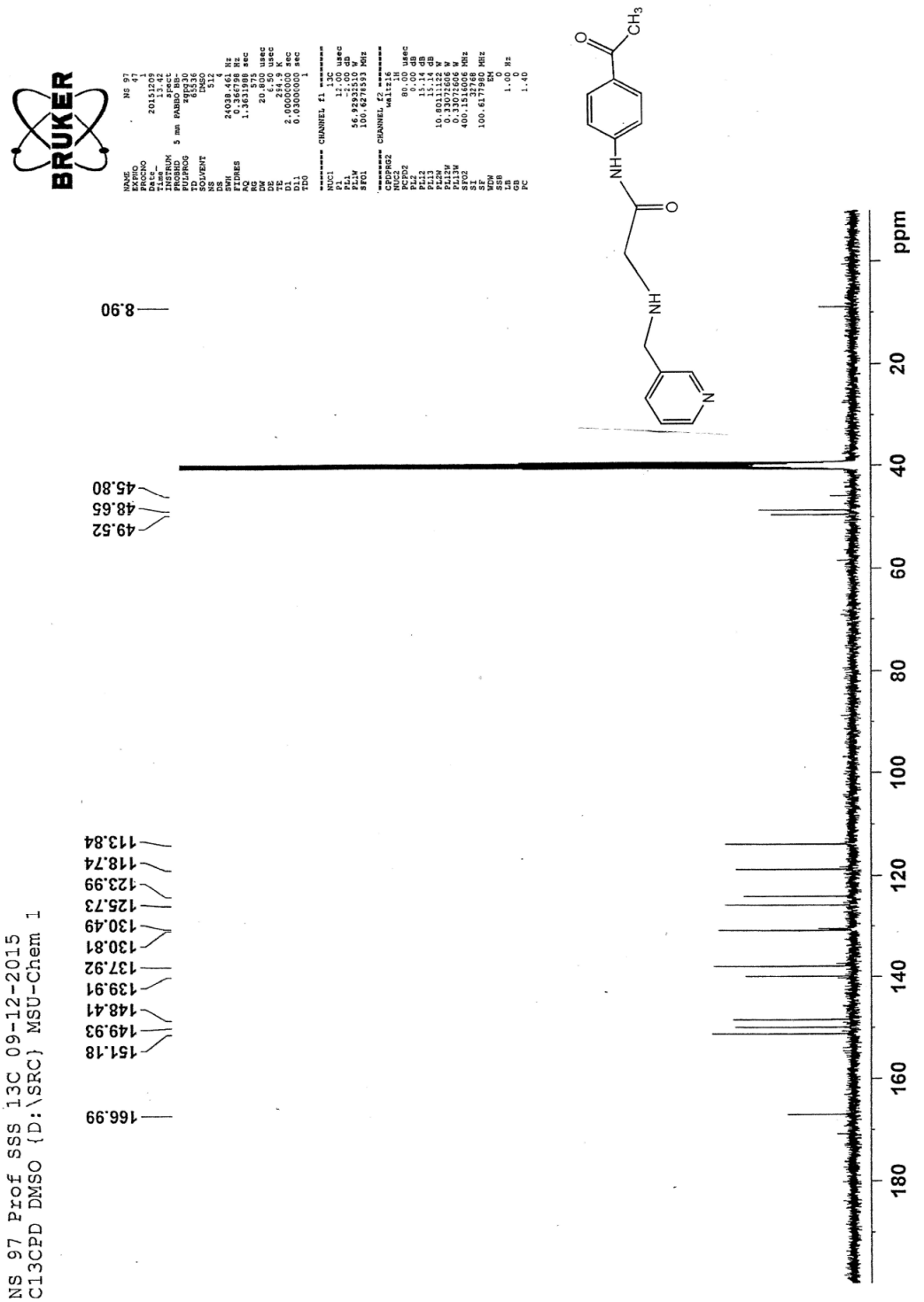


Figure-26 ^{13}C NMR Spectrum of N-(4-Acetyl phenyl)-2-[(pyridin-3-ylmethyl) amino] acetamide i.e **2g**

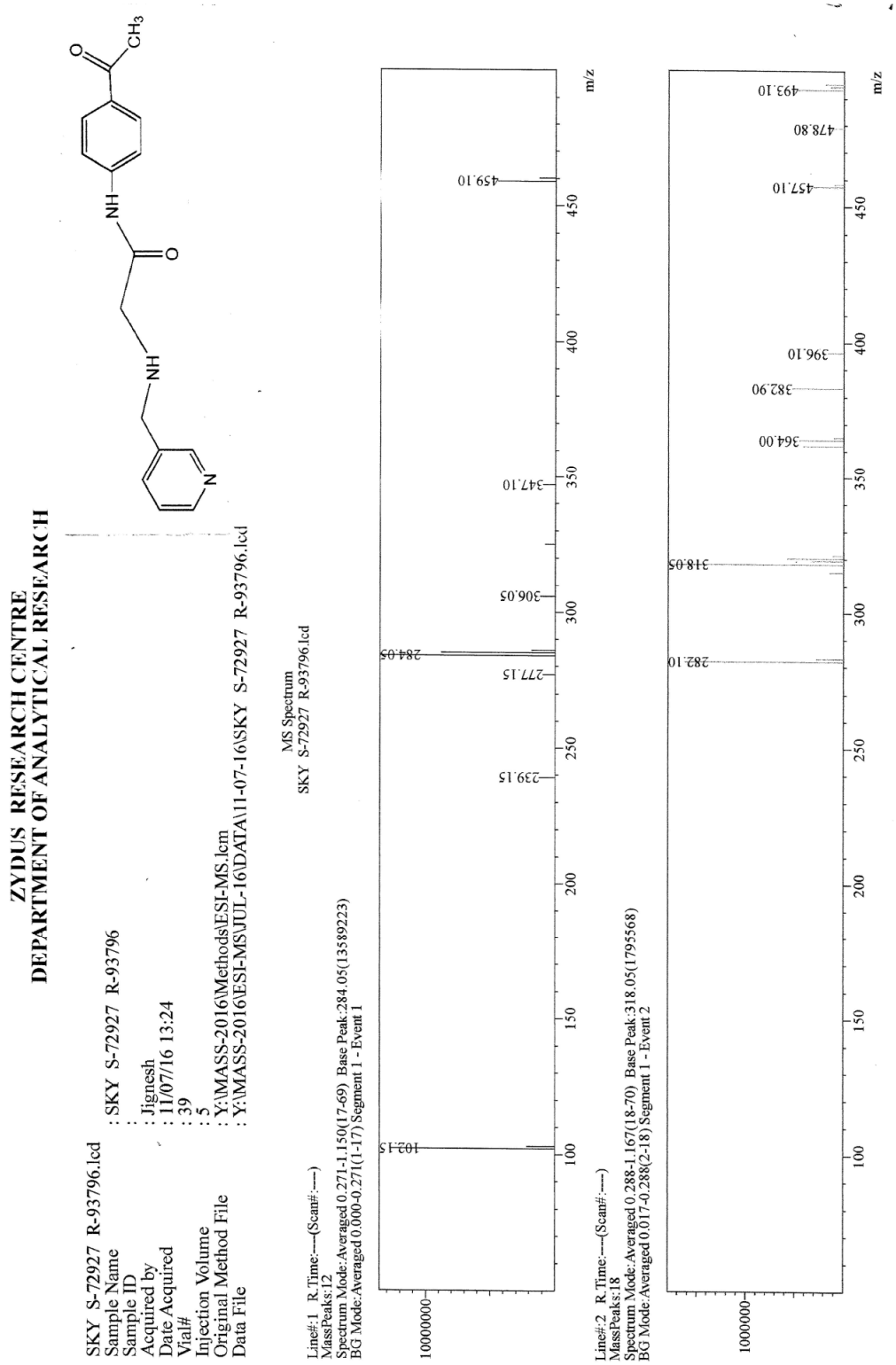


Figure-27 Mass Spectrum of N-(4-Acetyl phenyl)-2-[(pyridin-3-ylmethyl) amino] acetamide i.e **2g**

4.2.2 Biological Evaluation

4.2.2.1 Antibacterial and antifungal 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*) and antifungal activity for *C.Albicans* by serial dilution agar diffusion method [29] at 100 ppm concentration in DMF solvent. Ciprofloxacin was used as standard drug for bacteria and Flucanazole was used as standard drug for fungi.

Table-1 *In Vitro* antibacterial and antifungal activity of compounds **2(a-g)**

			MIC of antibacterial and antifungal agent (µg)				
Sr. No	Compound	-R	S. aureus	B. Subtilis	E. Coli	P. aeruginosa	C. albicans
01	2a	4-Cl	100	100	100	200	100
02	2b	4-F	200	250	100	150	100
03	2c	3-Cl	50	50	200	250	100
04	2d	4-CH_3	200	150	150	200	100
05	2e	3-F	50	50	200	>250	50
06	2f	3-NO_2	>250	150	150	150	150
07	2g	4-$COCH_3$	200	250	150	250	100

Table 1: Antimicrobial and antifungal activity of compound 2(a-g)

S. aureus = *Staphylococcus aureus,* ***B. Subtilis*** =*Bacillus Subtilis,* ***E. coli*** =*Escherichia coli,* ***P. aeruginosa*** = *pseudomonas aeruginosa,* ***C. albicans*** =*Candida albicans.*

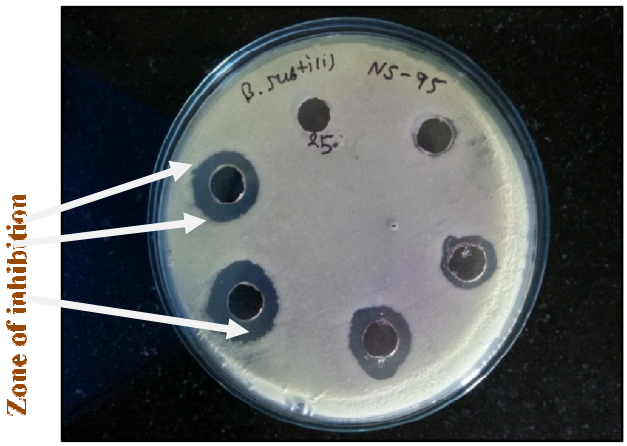


Figure-28a Zone of inhibition against Gramm +ve becteria (B. Subtilis) in **2e**

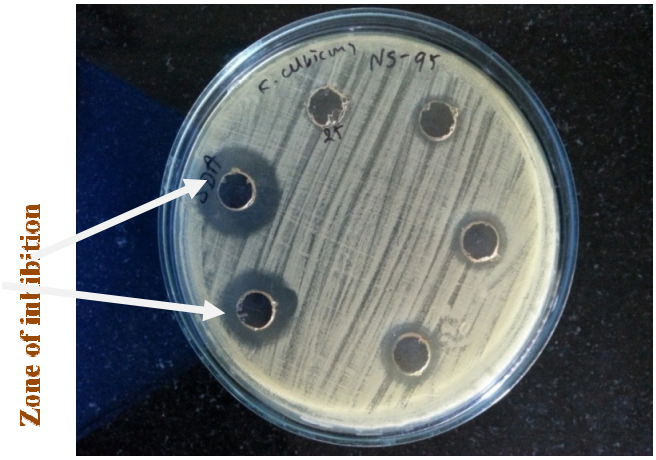


Figure-28b Zone of inhibition against fungi *C.albicans* in compound **2e**

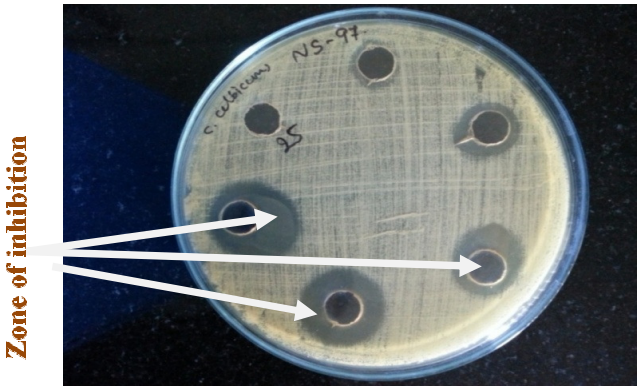


Figure-28c Zone of inhibition against fungi *C.albicans* in Compound **2g**

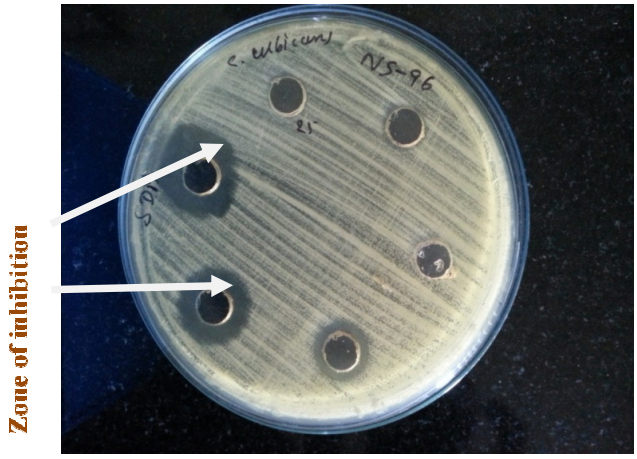


Figure-28d Zone of inhibition against Fungi *C. albicans* in **2f**

Compounds **2(a-g)** were screened for their antimicrobial and antifungal activities.Both of the Compounds **3c** and **3e** showed good activity. Compounds **2c** and **2e** showed antimicrobial activity at 50 µg concentration against tested *gram +ve* bacteria (*S.aureus and B. Subtilis).* Compound **2e** Showed encouraging antifungal activity at 50 µg concentrations against *C. Albicans (Fungi).* Compound **2f** remains inactive against tested *Gram-positivee* bacteria (*S.aureus)* but showed promising activity against fungi *C.albicans.* Compound **2e** did not show any antimicrobial activity against tested *Gram-negative* bacteria (*P. aeruginosa).* Rest of the compounds showed moderate activity.

4.2.2.2 Anticancer activity:

MTT assay

The synthesized compounds were tested for their cytotoxic potential on five types of cancer cell lines, *viz.*, A549 (lung cancer cell-line), MCF7 (breast cancer cell-line), K562 (Human Chronic Myelogenous leukaemia cell line), KG1 (Human acute Myeloid Leukaemia cells) and MOLT-3 (Human Acute Lymphoblastic Leukaemia cell line).The MTT assay was used to determine the effect of each compound on the proliferation of cancer cells.

The MTT assay was performed to screen test compounds **2(a-g)** for their activity against two of the five cancer cell lines such as lung cancer cell line A549 and breast cancer cell line MCF-07. The IC_{50} (µM) values were determined using Graph Pad prism software for the all compounds **2(a-g)** as shown in Table 2.

Table-2 Anticancer activity against A549 and MCF-07 cancer cell lines

No.	**Compounds Code**	**-R**	**Lungs cancer Cell line**	**Breast cancer Cell line**
			A549 IC_{50} **(µM)**	**MCF7** IC_{50} **(µM)**
01	2a	4-Cl	**0.2129**	**950.1**
02	2b	4-F	**1.186**	**NA**
03	2c	3-Cl	NA	**NA**
04	2d	4-CH_3	NA	**NA**
05	2e	3-F	**NA**	NA
06	2f	3-NO_2	**32.63**	**90.78**
07	2g	4-$COCH_3$	**ND**	**ND**

Table 2: Anticancer activity of synthesized amide derivatives of 3- amino methyl pyridine **2(a-g)** against two different cancer cell lines

NA=Not Active. ND=Not Done,

All the synthesized compounds 2(a-g) were also screened for their efficacy as anticancer agent against remaining three leukaemia cancer cell lines among the discussed five cancer cell lines. The IC_{50}µM values for compounds 2(a-g) are summarized in Table-**3**.

Table-3: IC_{50} (μM) values for three different Leukemia cancer cell lines

No.	Compounds	-R	Leukemia Cancer cell lines		
			K 562	**MOLT 3**	**KG 1**
			IC_{50}(μM)	IC_{50}(μM)	IC_{50}(μM)
01	2a	4-Cl	50.19	3.881	6.228
02	2b	4-F	16.32	**1.25**	9.73
03	2c	3-Cl	**2.35**	**0.51**	**0.374**
04	2d	4-CH_3	20.47	**0.14**	4.968
05	2e	3-F	55.94	14.73	**3.437**
06	2f	3-NO_2	8.304	**3.094**	**3.883**
07	2g	4-$COCH_3$	13.57	**3.64**	**4.499**

Table-3 Anticancer activity of compounds **2(a-g)** against three different Leukemia cell lines. Data are reported as IC_{50} values i.e (concentrations of complexes required to inhibit cell viability by 50%) determined by MTT assay after 48h of continuous exposure to each compound. The data represent the mean values ± SEM (standard error of mean) of at least three independent experiments.

The bar chart representation as showed in Figure-29 summarizes the anticancer effect of newly synthesised amide derivatives from 3-amino methyl pyridine **2(a-g)** on K562, KG 1 and MOLT 3 cell growth and % cell viability.

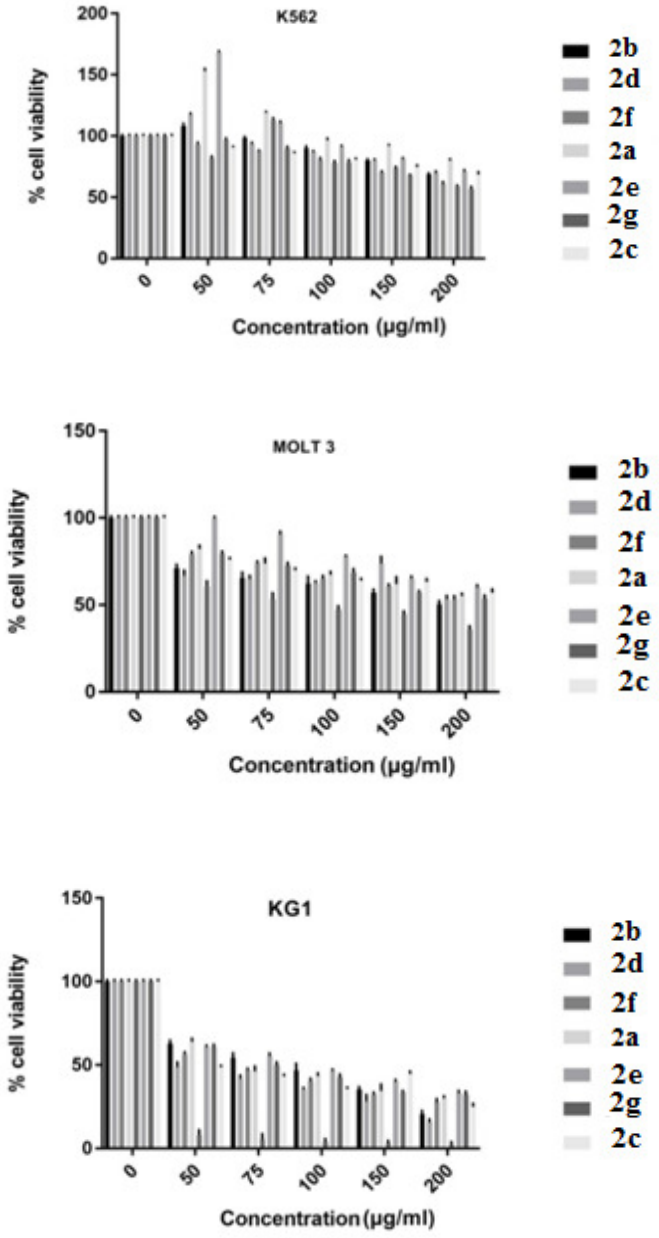


Figure-29**:** Bar chart representation for the effect of compounds **2(a-g)** on K562, KG 1 and MOLT 3 cell growth and determining % cell viability.

4.3 Experimental

Reagent grade chemicals and solvents were purchased from commercial supplier and used after purification. 3-amino methyl pyridne was purchased from M/s TCI chemicals; Japan.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 CHNS analyser.

4.3.1 Chemistry

4.3.1.1 General procedure for the Synthesis of Compounds 1(a-g) reported method[28]

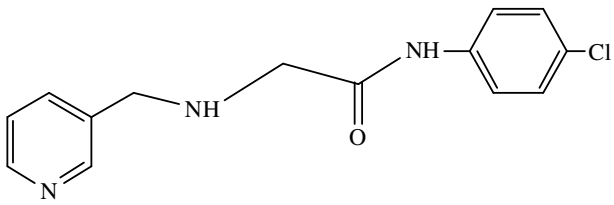
To a well stirred solution of substituted aniline 1.0eq. in dichloromethane, tri ethyl amine (2.09 mmol,1.01eq.) was added slowly and allowed to stir at 0-5°C for 30 minute. To this bromo acetyl bromide (1.0eq.) added slowly and the reaction mixture was stirred at room temperature for 6-8 hrs. The completion of the reaction monitored on TLC and then the reaction mixture was extracted with ethyl acetate. The extract was washed with water, dilute HCl again washed with water and dried over anhydrous sodium sulphate and concentrated under vacuum. The yellow precipitates obtained were crystallized from ethanol to give **1(a-g)** as off-white solid.

4.3.1.2 General Procedure for the Synthesis of Compounds 2 (a-g)

To a well stirred solution of **1(a-g)** 1.0eq. in dimethylformamide 15 mL, tri ethyl amine (2.09 mmol,1.01eq.) was added slowly and allowed to stir at 0-5°C for 30 minute. To this 3-amino methyl pyridine (1.0eq.) was added slowly and the reaction mixture was stirred at room temperature for 10-12 hrs. The completion of the reaction monitored on

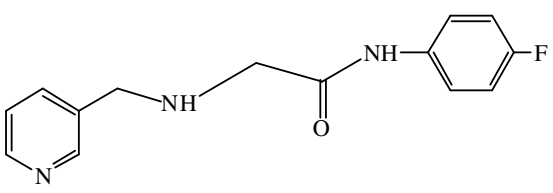
TLC and then the reaction mixture was poured on crushed ice. The solid thus obtained was filtered and washed with excess of water and extracted with ethyl acetate. The extract was washed with excess of water, and dried over anhydrous sodium sulphate and concentrated under vacuum. The precipitates obtained were crystallized from ethanol to give **2(a-g)** as off-white solid.

4.3.1.2.1 Preparation of *N-(4-chlorophenyl)-2-((pyridin-3-ylmethyl) amino) acetamide* 2a.



Yield 75%; m.p: 210-212°C; IR(KBr):3383, 3063, 2968, 2710, 1691, 1612, 1550, 1492, 1400, 1313, 1292, 1251, 1087, 939, 827, 794, 688.cm^{-1};^{1}H-NMR (DMSOd_6,400MHz) δ 4.026 (S,2H) ,4.405 (S,2H) ,7.39 (d, *J* = 8.0Hz,2H), 7.66(d, *J*=8.0Hz,2H) ,7.83 (S,1H) ,8.46 (S,1H) ,8.81(S,1H), 8.97(S,1H), 10.04(S,1H) 11.21 (s ,1H) (amidic proton) ^{13}C NMR: δ 47.26, 48.29, 121.25, 125.78,127.94, 129.28, 130.23, 137.63, 143.81, 146.16, 147.63, 164.24 Molecular weight:275.73g/mol;Mol.Formula:$C_{14}H_{14}ClN_3O$;Elementalanalysis;(C,H,N),(Cal: found.),(60.98, 5.12, 15.24: 61.00, 5.14, 15.25). EI MS: 276(m+1).

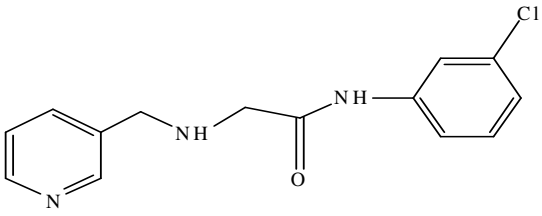
4.3.1.2.2 Preparation of N-(4-flouro phenyl)-2-((pyridin-3-ylmethyl) amino acetamide 2b



Yield 75%; m.p: 222-224°C;IR(KBr): 3383, 3063, 2968, 1689, 1564, 1510, 1502, 1410, 1377, 1315, 1259, 1220, 1192, 1116, 1014, 912, 827, 793, 685.cm^{-1};^{1}H-NMR (DMSOd_6,400MHz) δ 4.026 (S,2H), 4.462 (S,2H),7.12-7.20(m, *J*=16Hz,2H),7.64-7.66(m, *J*=7.6.Hz,2H), 7.83(S,1H), 8.024 (d, *J* = 6.8Hz , 1H) 8.67(S, *J*=6.8.Hz,1H) ,8.92 (S,1H), 9.07 (s,1H), 10.09(s,1H) 11.082

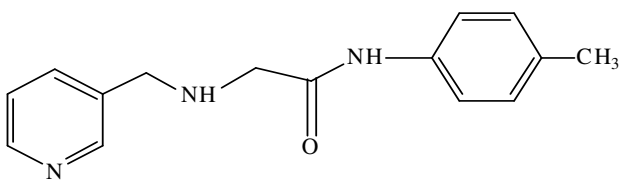
(Ss,1H) (amidic proton).^{13}CNMR:46.96, 48.27, 115.89, 116.11, 121.46(^{19}F) coupling, 121.54(^{19}F) coupling, 121.68, 125.46, 126.66, 131.68, 135.02, 144.07(^{19}F) coupling, 145.66(^{19}F) coupling, 146.28, 157.57, 159.96, 163.94., Molecular weight: 259.23 g/mol; Molecular Formula:$C_{14}H_{14}FN_3O$; Elemental analysis; (C,H,N), (Cal:Obs.), (64.85,5.44,16.21: 64.87,5.42,16.23).EI MS(m/z): 260(m+1)..

4.3.1.2.3 Preparation of N-(3-chlorophenyl)-2-((pyridin-3-yl methyl) amino) acetamide 2c.



Yield 64% ;m.p : 204 -206°C ; IR (KBr) :3392, 3246, 3065, 2928, 2812, 1691,1608,1597,1546,1477,1414,1375,1286,1246,1192,1166,1076,918,869,788,756,711,682,.cm^{-1};^{1}H-NMR(DMSO d_6, 400MHz) δ 3.99 (S, 2H) ,4.33(S,2H), 7.17 (d, *J* = 8.0 Hz , 1H) 7.37 (d, *J* = 8.0Hz,1H), 7.49(d, *J* = 8.0 Hz ,1H) ,7.64(t, *J*=13 Hz ,3H),7.80 (S,1H) ,8.21(d, *J*=8.0Hz,1H) 8.705 (d, *J*=4Hz,1H) ,8.83(S,1H), 9.86(S,1H), 11.14(S,1H) ^{13}C NMR δ:47.60, 48.23, 118.15 ,119.17, 124.17, 124.83, 128.97, 131.17, 133.62, 140.03,140.93,148.65,149.91,164.54. Molecular weight: 259.23g/mol; Mol. Formula: $C_{14}H_{14}ClN_3O$; Elemental analysis; (C, H, N), (Cal: Obs.), (60.98, 5.12, 15. 24:60.96, 5.32, 15.26),. EI mass (m/z): 275(m+1).

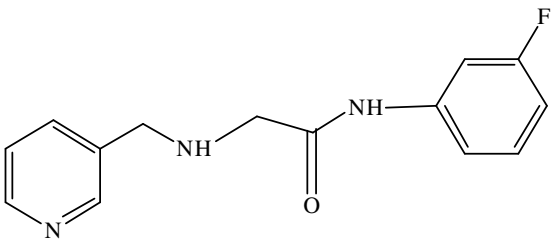
4.3.1.2.4 Preparation of 2-((pyridin-3-yl methyl) amino) –N-p-tolyl acetamide 2d.



Yield 71%; m.p:208-210°C; IR (KBr): 3412, 3213, 3176, 3055, 2991, 2781, 1690, 1577, 1510, 1483, 1427, 1410, 1388, 1334, 1307, 1292, 1255, 952, 918, 821, 802, 769, 729.cm^{-1};^{1}H-NMR (DMSO d_6, 400MHz) δ2.25(S,3H), 3.94(S, 2H), 4.29 (S,2H), 7.14 (d, *J* = 8.0 Hz , 2H) 7.48-7.54(m,3H), 8.09 (d, *J*=8.0Hz,1H), 8.63 (d, *J*=8.0Hz, 2H), 8.76 (S,1H),

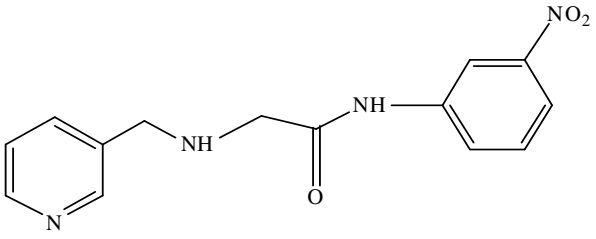
10.75(S,1H).^{13}C NMR δ:20.93,45.72, 48.10, 119.68, 124.25, 129.75, 133.38, 139.27, 149.96, 151.60, 163.80.Molecular weight; 255.14g/mol, Molecular formula;$C_{15}H_{17}N_3O$ Elemental analysis (C,H,N) (Cal: Obs.), (70.56,6.71,16.41:70.73,6.69,16.43).EI MS(m/z):256(m+1).

4.3.1.2.5 Preparation of N-(3-flouro phenyl)-2-((pyridin-3-ylmethyl) amino) acetamide 2e.



Yield 62%; m.p:199-201°C; IR (KBr):3421, 3255, 3200, 3124, 3084, 2972, 2858, 2723, 1691, 1610, 1493, 1481, 1317, 1274, 1257, 1244, 1234, 1190, 1142, 1141, 1074, 1030, 916, 866, 806, 775, 709, 677cm^{-1}; ^{1}H-NMR (DMSOd_6, 400 MHz) δ 3.95 (S,2H) ,4.27 (S,2H),6.94 (t, *J* = 16Hz , 1H) 7.46 (S,1H) , 7.33-7.41(m, J=16Hz,8Hz, 2H) 7.46-7.49 (m,1H) ,7.57-7.60 (d,1H) 8.01 (d, *J* =8Hz,1H), 8.60(d, *J*=8Hz,1H).8.72(S,1H),10.05(S,1H),11.06(S,1H),^{13}C NMR (DMSOd_6 :100MHz) δ47.95, 48.32, 106.67, 111.02, 115.52, 124.05, 128.30, 131.18, 138.56, 140.23, 140.34, 150.47, 151.64, 164.77. Molecular weight 259.27g/mol, Molecular Formula: $C_{14}H_{14}FN_3O$; Elemental analysis: (C,H,N) (Cal: Obs.), (64.85, 5.44, 16.21:64.87, 5.45, 16.23).EI Ms(m/z) : 260(M+1)

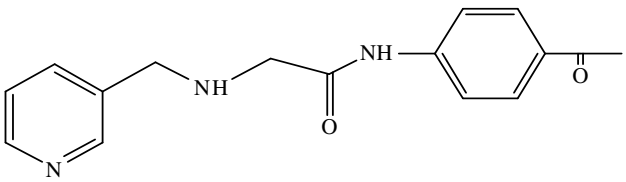
4.3.1.2.6 Preparation of N-(3-Nitro phenyl)-2-((pyridin-3-ylmethyl) amino) acetamide 2f



Yield 59 %; m.p:208-210°C; IR (KBr) : 3435, 3244, 3178, 3057, 3012, 2929, 2872, 1693, 1670, 1600, 1545, 1535, ,1481, 1365, 1357, 1321, 1271,1257, 1201, 1174, 1030, 962, 937, 908, 840, 802, 707, 609 cm^{-1}; ^{1}H-NMR

(DMSOd_6, 400MHz) δ 4.02 (S, 2H) ,4.29 (S, 2H) ,7.49 (m,1H), 7.76 (d, *J* = 8.4Hz , 2H) ,7.95(d, J=8.4 Hz, 2H), 8.05 (d, *J* = 7.6Hz ,1H), 8.61 (d, 1H), 8.74 (s, 1H), 9.84 (s,1H) 11.30 (s,1H) .^{13}C NMR (DMSOd_6,100MHz) δ:45.73, 47.92, 48.37, 119.03, 124.04, 128.20, 130.04, 132.70, 138.66, 142.92, 150.45, 151.67, 164.90, 197.04. Molecular weight: 286.28g/mol, Molecular formula: $C_{14}H_{14}N_4O_3$ Elemental analysis (C, H, N) (Cal: Obs.), (58.73, 4.93, 19.57:58.76, 4.91, 19.57).ESI MS(m/z): 287(m+1)

4.3.1.2.7 Preparation of N-(4-acetyl phenyl)-2-((pyridin-3-ylmethyl) amino) acetamide 2g.



Yield 68 %; m.p:216-218°C; IR (KBr) : 3412, 3213, 3176, 3055, 2991, 2781, 1690, 1577, 1510, 1483, 1427, 1410, 1388, 1334, 1307, 1292, 952, 918, 821, 802, 769, 729.cm^{-1};^{1}H-NMR (DMSOd_6, 400MHz) δ 2.50(S,3H), 3.82 (S,2H) ,4.15 (S,2H) ,7.42-7.61(m, J=8.0Hz,2Hz,1H),7.62-7.66 (m, J=8.0 Hz, 2Hz 1H) 7.87-7.90 (m, 3H),7.93-7.99 (m,1H), 8.41-8.42 (d, J=2Hz, 1H) 8.56-8.67(m, J=16Hz, 2Hz,2H),11.29 (S,1H).^{13}C NMR (DMSO d_6,100 MHz)δ: 8.9, 48.62, 49.52, 113.84, 118.74, 123.99, 125.73, 130.49, 130.81, 137.92, 139.91, 148.41, 149.93, 151.18, 166.99. Molecular weight: 283g/mol, Molecular formula: $C_{16}H_{17}N_3O_2$, Elemental analysis:(C,H,N)(Cal: Obs.),(67.83, 6.05, 14.83:67.86, 6.03, 14.85).ESI Ms(m/z):284(M+1)

4.3.2 Biological Activity Screening.

4.3.2.1 Procedure of Serial dilution agar method for the antimicrobial activity

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,(four bacterial species and one fungi) 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.The antimicrobial activity of tested compounds is shown in Table-**1.**

4.3.2.2 Procedure for the MTT Assay for Anticancer activity.

A549 and MCF7 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 compounds were 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.

4.4 Conclusion

In conclusion, we report here synthesis of 3-amino methyl pyridine derivatives **2(a-g)** .The structures of all the synthesized compounds were confirmed by IR, ^{1}H NMR, ^{13}C NMR, mass and elemental analysis. All newly synthesized compounds were screened for their antimicrobial activity. They showed moderate to good antibacterial and antifungal activity. Compound 3c showed good activity against gram +ve bacteria (*S.aureus and B. Subtilis) at* 50 µg concentration. Compound 3e showed promising activity at 50µg against tested gram +ve bacteria (*S.aureus and B. Subtilis).*Compound 3e also Showed encouraging activity at 50 µg against *C. Albicans (Fungi).* Compound 3f remains inactive against tested Gram-positive bacteria (*S.aureus)* but showed promising activity against fungi *C.albicans.,* Compound 3e did not show any antimicrobial activity against tested Gram-positive bacteria(*P. aeruginosa).*In general it is also concluded that when phenyl ring of amine is substituted at 3rd position plays important role in showing antibacterial as well as antifungal activity. The structure variations such as methyl and halo groups at *meta* and *para* positions of phenyl ring in amide linkage resulted in promising antibacterial and antifungal activity. Among the synthesized compounds 3(a-g), the six compounds were screened for the anticancer activity against lung cancer cell line A549 and breast cancer cell line MCF7.Compound 3a showed promising anticancer activity against lung cancer cell line A549 at 0.2129µM concentration and against breast cancer cell line MCF7 at 950.1µM concentration. Compound 3b also showed very good cytotoxic activity against lung cancer cell line A549 at 1.186µM concentration but remains inactive against breast cancer cell line MCF7.Compound 3f also showed very good activity against both the cancer cell line at 32.63 µM concentrations and 90.76 µM concentrations respectively. The remaining compounds remained inactive against both the

cancer cell lines. Compound **2c** showed very good cytotoxic potential with IC_{50} 2.351 μM against K562 cell line compared to all other compounds. Likewise, compound **2c** and **2d** also showed very good anticancer activity with IC_{50} 0.51 μM and 0.14 μM against MOLT3 cell line compared to other Compounds. Similarly, Compound **2c** showed better cytotoxic potential with IC_{50} 0.374 μM against KG1 cell line.

4.5 References

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