CHAPTER-5

Synthesis and antimicrobial & anticancer evaluation of some new N-(benzo[d]thiazol-2-yl)-2-((pyridine-3-yl methyl) amino) acetamide

5. Synthesis and antimicrobial & anticancer evaluation of some new N-(benzo[d]thiazol-2-yl)-2-((pyridine-3-yl methyl) amino) acetamide.

5.1 Introduction

Heterocyclic organic compounds are widely occuring as natural products and as pharmaceutical agent. The growing prevalence of drug resistant bacteria has made the medicinal chemists to find out and discover new antimicrobial agents which are effective against pathogenic microorganisms resistant to current treatment. Therefore, it has been a growing interest pertaining to the synthesis of bioactive antimicrobial compounds in the field of organic heterocyclic chemistry.

A single molecule containing more than one pharmacophor, each with different mode of action and with different heteroatom could be beneficial for the treatment of infections. Considering these facts the development of completely new compound possessing chemical properties different than those of existing ones, is an approach warmly welcomed by a researcher now a days. Such types of heterocyclic compound combinations has several major advantages like ,slow down the rate of drug resistance, a broad antimicrobial activity and potential reduction in the dose and toxicity of each drug.

Benzothiazole ring system containing both Nitrogen and Sulphur heteroatom present in various natural compounds having useful biological activities[1-4].2-amino benzothiazole is a highly reactive compound since the endocyclic N functions and NH_2 are suitably situated to enable reactions with various reactants to form a variety of fused heterocyclic compounds. Benzothiazole and its derivatives are reported in the literature for their numerous biological activities such as, anti-inflammatory[5], analgesics[6], antitumor[7], antibacterial[8] and antiviral[9] properties.

Amides, -RCONHR' moiety is known to play pivotal role in molecular recognition. Many investigations indicated that the presence of amide linkage e.g. , -(CONH) - seems to be valuable in the structure of anti microbials[10].Over the past few decades. The amide linkage –NHCO- with delocalized electrons is a key determinant of antimicrobial activity, which would be greatly influenced by the nature of adjoining substituent.

A number of synthetic heterocyclic compounds have been discovered and used in clinical practice such as penicillines, ketoconazole, sulphonamides and micanazoles. In spite of availability of potent antimicrobial agents, problems against microbial infections caused by *S.aureus*, *B.subtilis, E.coli*, and *C.albicans* remained unresolved due to their potency, toxicity and resistance development.[11] Recently benzothiazole-acetamide containing compound was identified which plays essential role to furnish promising antimicrobial activities[12]. These findings pave a way for research to be carried out in this area and prompted us to continue our investigation towards synthesis of amide bearing benzothiazole ring system as potent antimicrobial as well as anticancer agent.

Both pyridine and benzothiazole are an important pharmacophor and exhibits outstanding biological activities. Encouraged by these observations and to affiliate multiple bioactivities in a single compact compound, a series of various N-(substituted enzo[d] thiazol-2-yl)-2-bromo acetamide coupled with 3-amino methyl pyridine were synthesized and tested *In Vitro* for their efficacy as antimicrobial agent against four bacteria *(staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Bacillus subtili)* and one *fungi(Candida albicans)*.Some of the synthesized compounds were also tested for the anticancer activity against A549(Lung cancer cell line).

Structures of the newly synthesized compounds were confirmed by IR, ^{1}H NMR, ^{13}C NMR, Mass spectral studies and by elemental analysis.

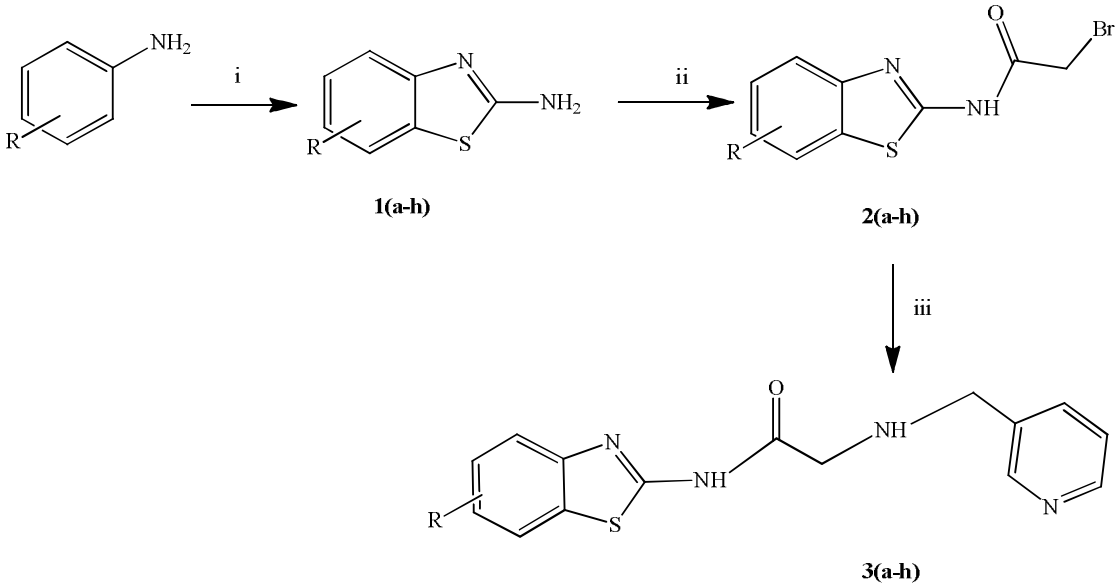
5.2 Result and Discussion

Various substituted amide derivatives of benzothiazole have been synthesized, starting from various substituted aniline derivatives. Substituted anilines on reaction with potassium thiocyanate in presence of bromine in acetic acid.[13] gave 2-aminobenzothiazole derivatives**1 (a-h) (Scheme-1).**The structure of compound **1(a-h)** were confirmed by their IR, ^{1}H NMR, ^{13}C NMR, and Mass spectra**.** In IR spectrum of compound **1b** Figure-6 (Page No.171) showed two bands at 3433 cm^{-1} and 3285 cm^{-1} indicated free NH_2 group. ^{1}H NMR of compound **1b** Figure-**7 (**Page No.172) **s**howed singlet at δ 2.42 for three protons indicates $–CH_3$ group. Multiplates at 6.88, 7.01 and 7.40 for three protons indicated all three aromatic protons. Singlet at δ 7.45 for two protons indicated $–NH_2$ protons thus confirmed the structure of compound **1b**.The ^{13}C NMR of compound **1b** is represented in Figure-8(Page No.173)**.** The stirring of **1b** with bromo acetyl bromide at room temperature gave compound **2b.** The structure of compound **2b** was also confirmed by its IR, ^{1}H NMR, ^{13}C NMR, and Mass spectra. In the IR spectrum of compound **2b**, Figure-9(Page No.174) showed one band at 3187 cm^{-1} indicated –NH stretching frequency .A sharp band at 1661 cm^{-1} indicated –CO stretching frequency of amide. In ^{1}H NMR of compound **2b ,**Figure-10 (Page No.176) showed singlet at δ 2.53 for three protons indicated $–CH_3$ group. Another singlet at δ 4.19 for two protons indicated $–CH_2$ group. The multiplet for two protons at δ 7.16 -7.23 indicated two aromatic protons and another doublet at δ 7.74 for one proton indicated third aromatic proton. Broad singlet at δ 12.83 indicated –NH proton. Further ^{13}C NMR of compound **2b,** Figure-11(Page No.176) showed two carbons at δ 17.86 and 28.36 indicated two aliphatic carbons of $–CH_3$ and $–CH_2$ groups respectively. In aromatic region presence of seven carbons at δ 118.96, 123.64, 126.58, 129.99, 131.14, 147.50, and 156.63 and carbonyl carbon at δ 165.80 confirmed the structure of compound **2b.** The reaction of compound **2b** with 3-

amino methyl pyridine in DMF at room temperature stirring gave compound **3b.** The IR spectrum of compound **3b** Figure-12(Page No.177) showed sharp singlet at 3320 cm^{-1} indicated –NH stretching band. A sharp stretching band at 1691cm^{-1} indicated carbonyl stretching frequency of amide group. The ^{1}H NMR spectrum of compound **3b,** Figure-13(Page No.178) showed singlet at δ 2.36 for three proton of $–CH_3$ group. Two singlets at δ 4.16 and δ 4.48 for two protons each indicated $-CH_2N=$ and $–CH_2-CO-N=$ protons respectively. Six signals in aromatic region for one proton each indicated six aromatic protons. One broad singlet and one sharp singlet at δ 10.15 and 10.98 for one proton each indicated two,-NH protons. In ^{13}C NMR spectrum of compound **3b** Figure-14(Page No.179) showed three aliphatic carbons at δ 19.42, 46.92 and 4.79 for one $–CH_3$ and two $–CH_2$ carbons. The remaining eleven carbons in aromatic region at δ 123.13, 124.94, 125.57, 126.74, 131.26, 140.25, 140.93, 143.97, 145.57, 146.54, 146.60 and one carbonyl carbon at δ 164.20 confirmed the formation of compound **3b.** Further mass spectrum of compound **3b** Figure-15(Page No.180) showed M^+ peak at 312 confirmed the structure of compound **3b**. Similarly Figure-16(Page No.181), Figure-17(Page No.182) and Figure-18(Page No.183) showed IR, ^{1}H NMR and ^{13}C NMR spectrum of compound **1c.** Figure-19(Page No.184) showed ^{13}C NMR spectrum of compound **2c.** Figure-23(Page No.188) showed ^{1}H NMR and Figure-24(Page No.189) showed ^{13}C NMR spectrum of compound **2d.** Figure-30(Page No.195) showed mass spectrum of compound **2e.** Figure-21(Page No.186) showed ^{13}C NMR spectrum of compound **3c.** Figure-38 (Page No.203) showed mass spectrum of compound **3f.** IR, ^{1}H NMR, ^{13}C NMR , and Mass spectrum of compound **3h** is shown in Figure-40(Page No.205) , Figure-41(Page No.206), Figure-42(Page No.207) and Figure-43(Page No.208) respectively. The reaction outlined in **scheme -1** (Page No. 165) was used for the synthesis of intermediates and title compounds **3(a-h).**

5.2.1 Chemistry

The synthesis of the compounds is shown in the **scheme -1** as shown below.



Scheme-1: Synthesis of various N-(benzo[d] thiazol-2-yl) -2-{(pyridine-3-ylmethyl) amino} acetamide derivatives.

Where in R=

Compound	-R	Compound	-R
3a	H	**3e**	6-F
3b	4-CH_3	**3f**	6-OC_2H_5
3c	6-Cl	**3g**	6-Br
3d	4-Cl	**3h**	4-CH_3-6- NO_2

Reagents and Conditions: (i) KSCN, Br_2 in Acetic acid, 0-5°C, RT string, R.T 8-10 hrs. Liq. NH_3 (25%). (ii) $BrCOCH_2Br$, TEA, DCM, string at 0-5°C; 30min, R.T. string 10 hrs.(iii) TEA, DMF, 3-amino methyl pyridine, R.T. String 12 hrs.

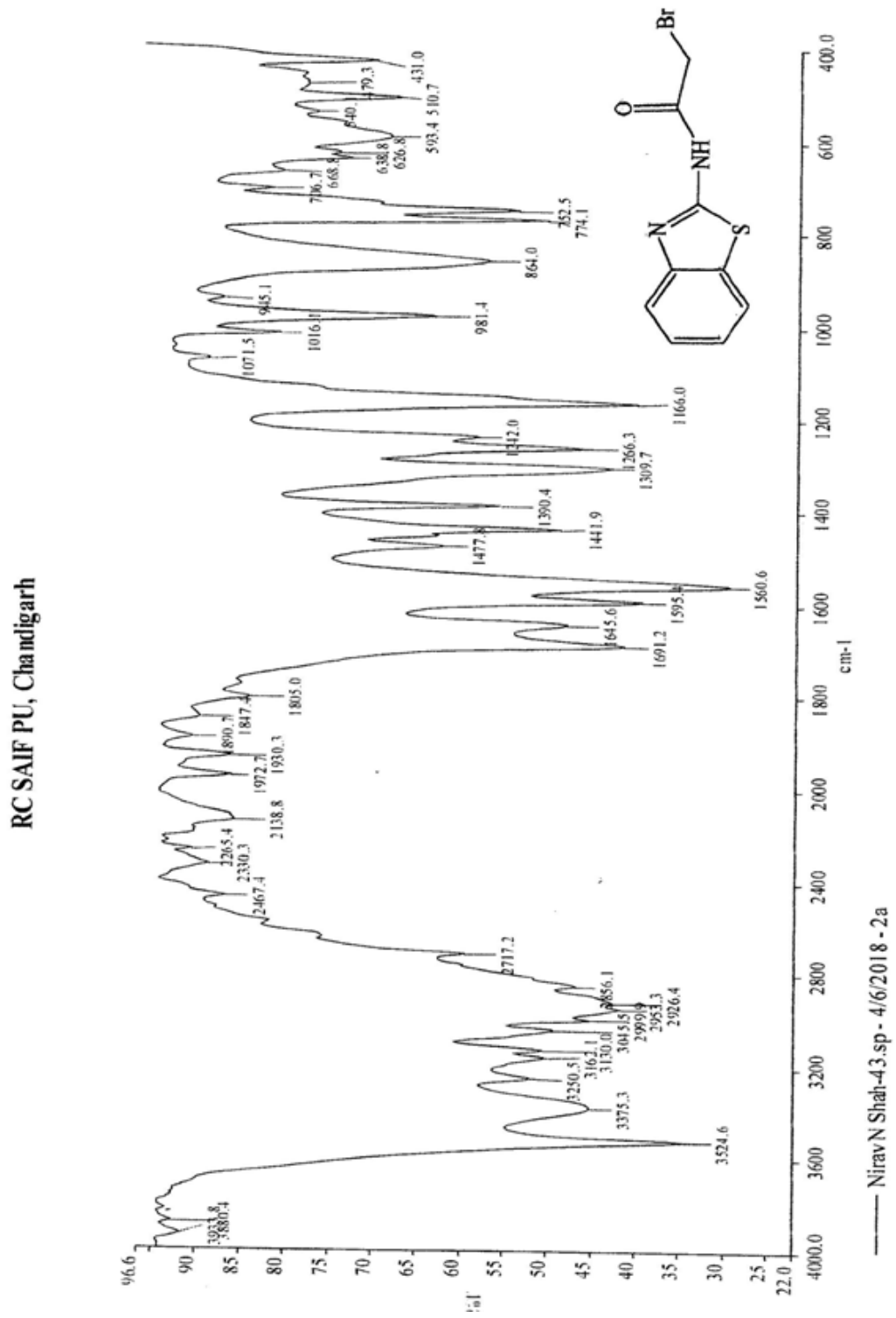


Figure-1 IR Spectrum of N-(benzo[d]thiazol-2-yl)-2-bromo acetamide i.e.**2a**

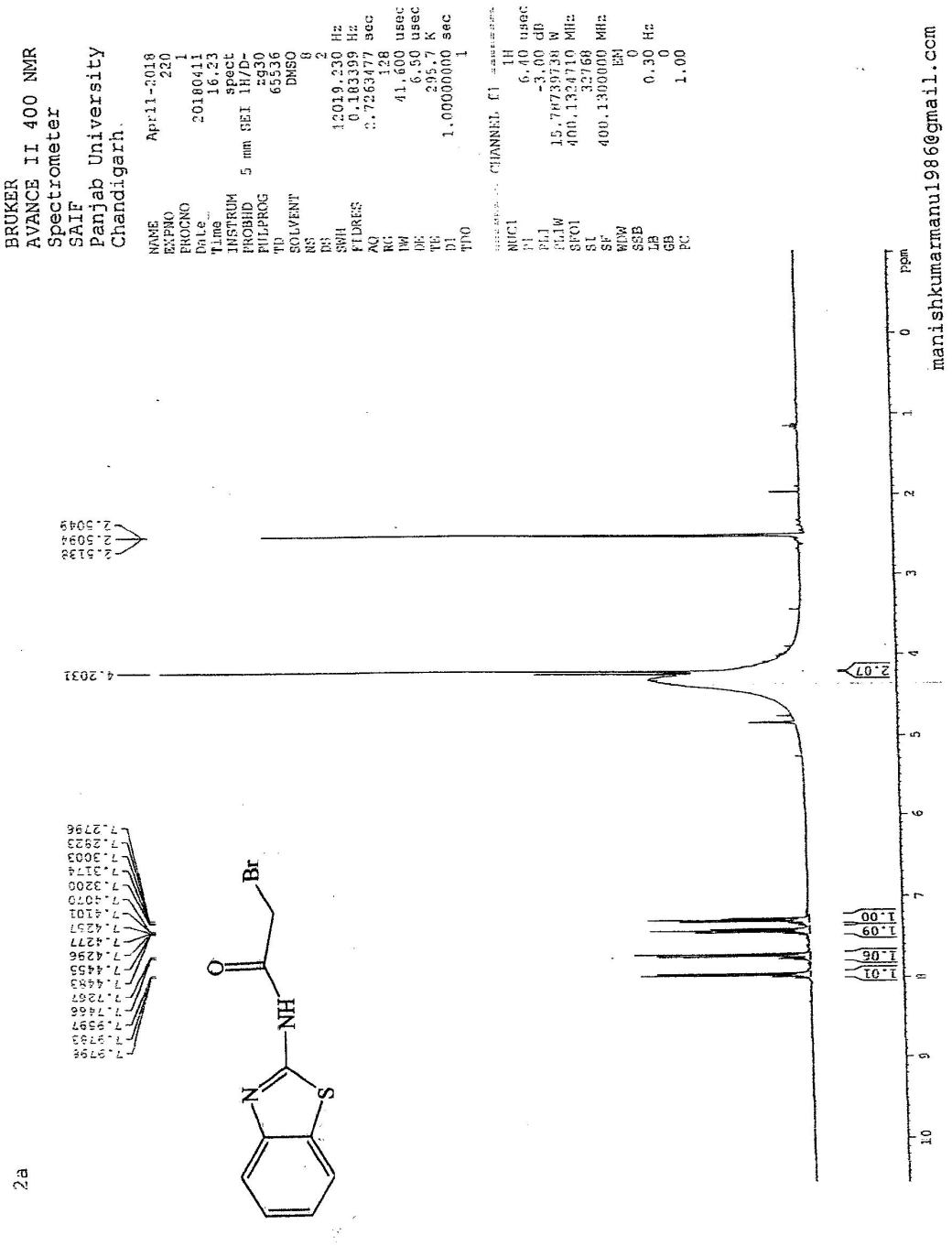


Figure-2 ^{1}H NMR spectrum of N-(benzo[d]thiazol-2-yl)-2-bromo acetamide i.e.**2a**

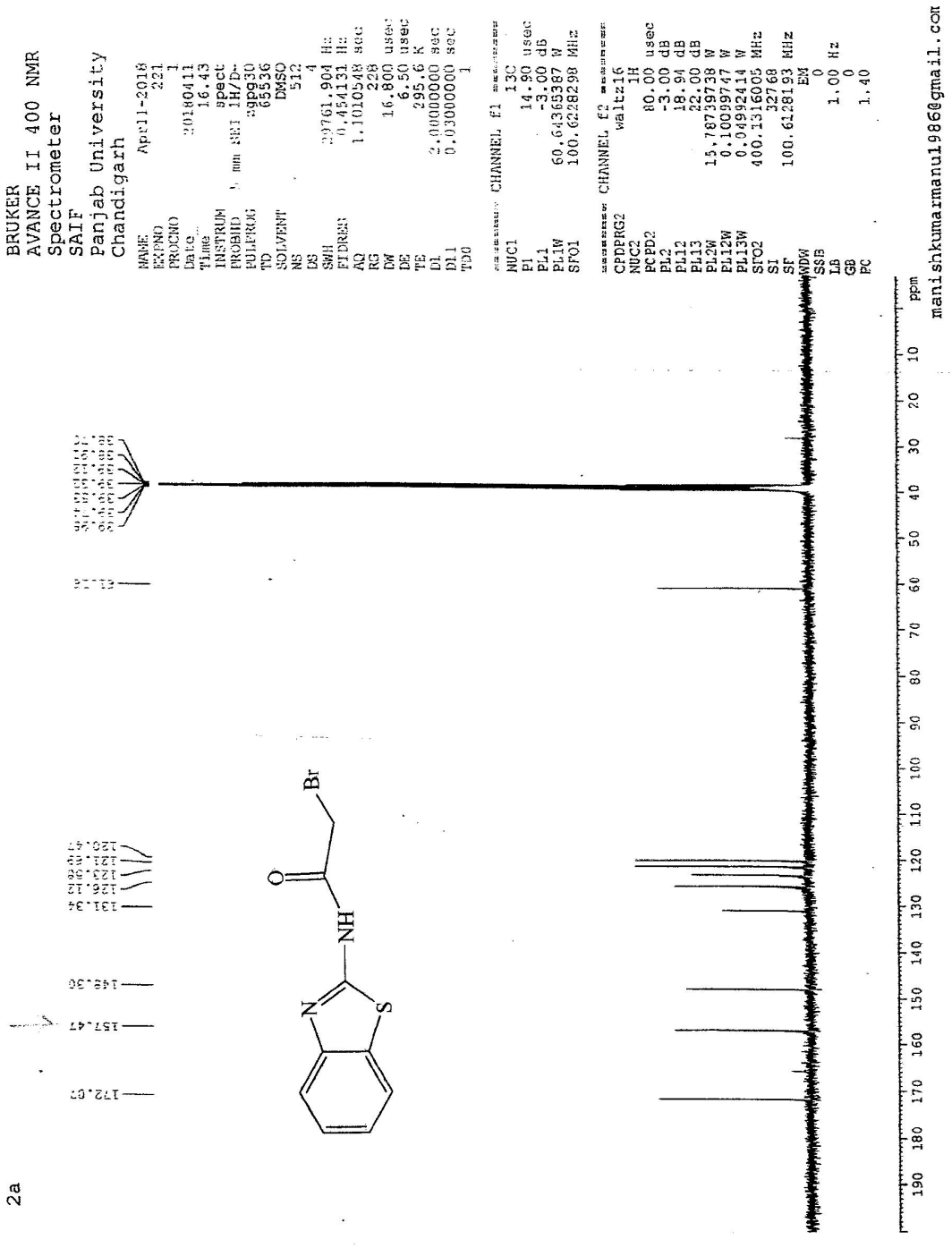


Figure-3 ^{13}C NMR spectrum of N-(benzo[d]thiazol-2-yl)-2-bromo acetamide i.e.**2a**

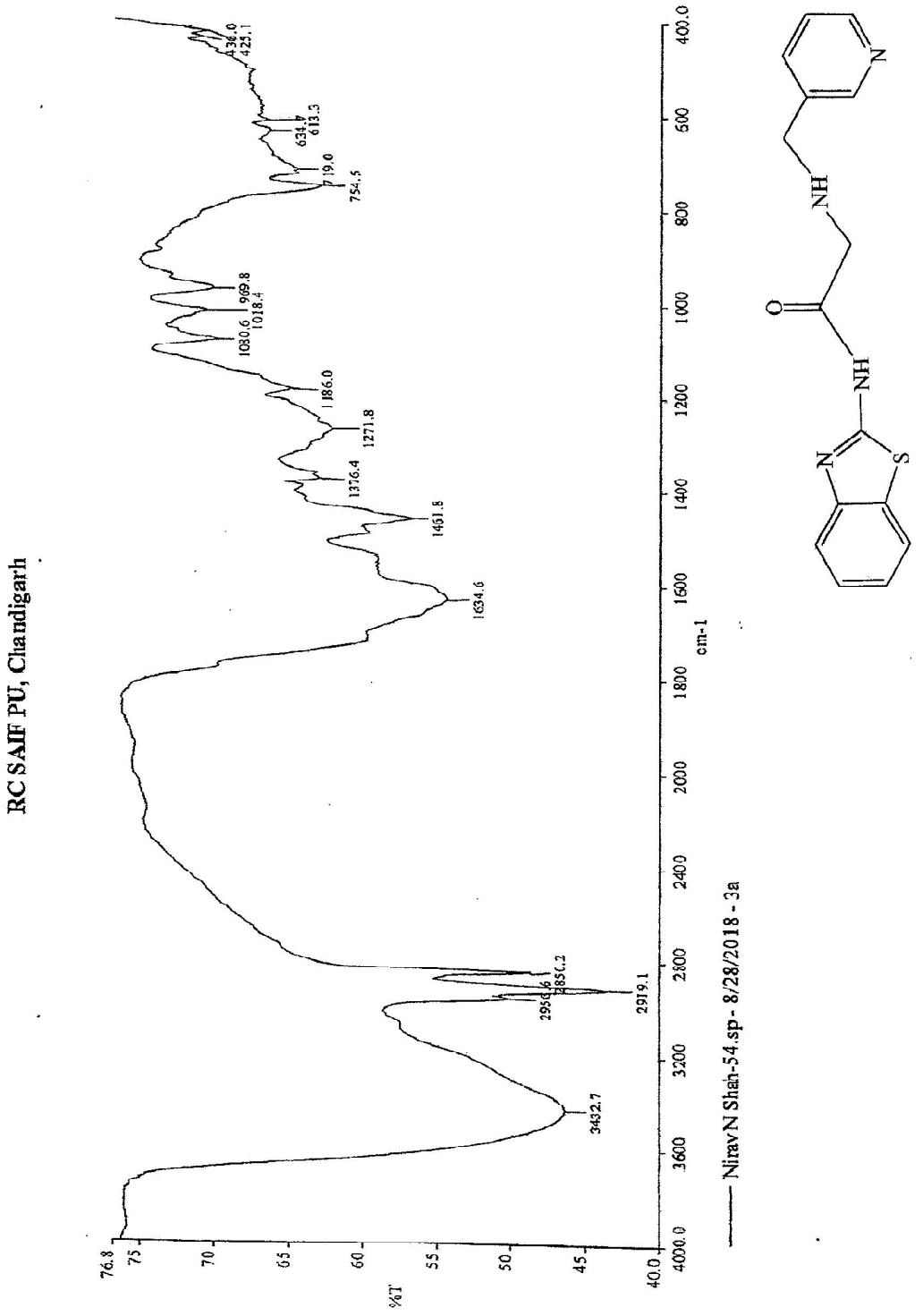


Figure-4 IR Spectrum of N-(4-methyl benzo[d]thiazol-2-((Pyridin-3yl methyl) amino) acetamide. i.e **3a**

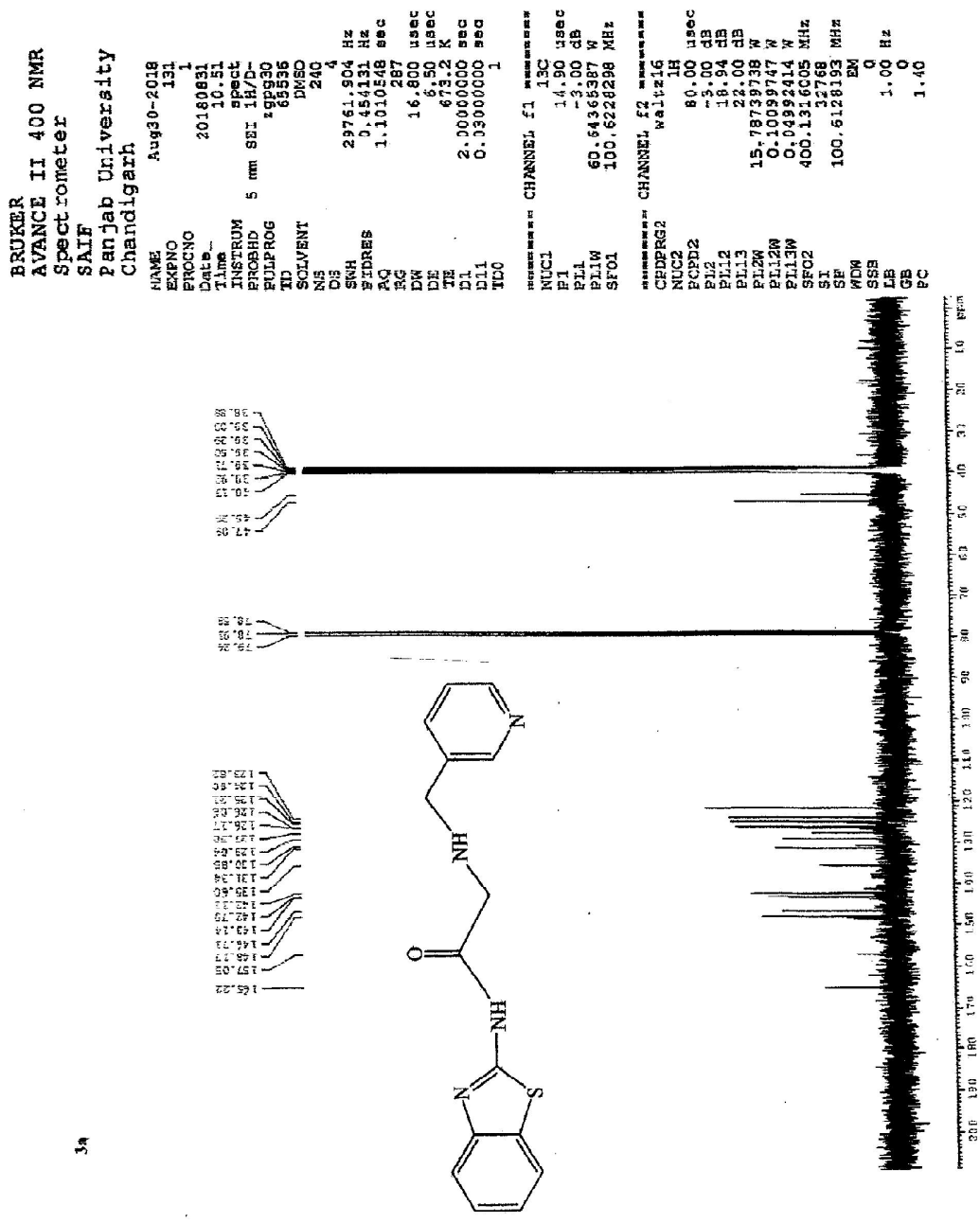


Figure-5 ^{13}C NMR Spectrum of N-(4-methyl benzo[d]thiazol-2-((Pyridin-3yl methyl) amino) acetamide. i.e **3a**

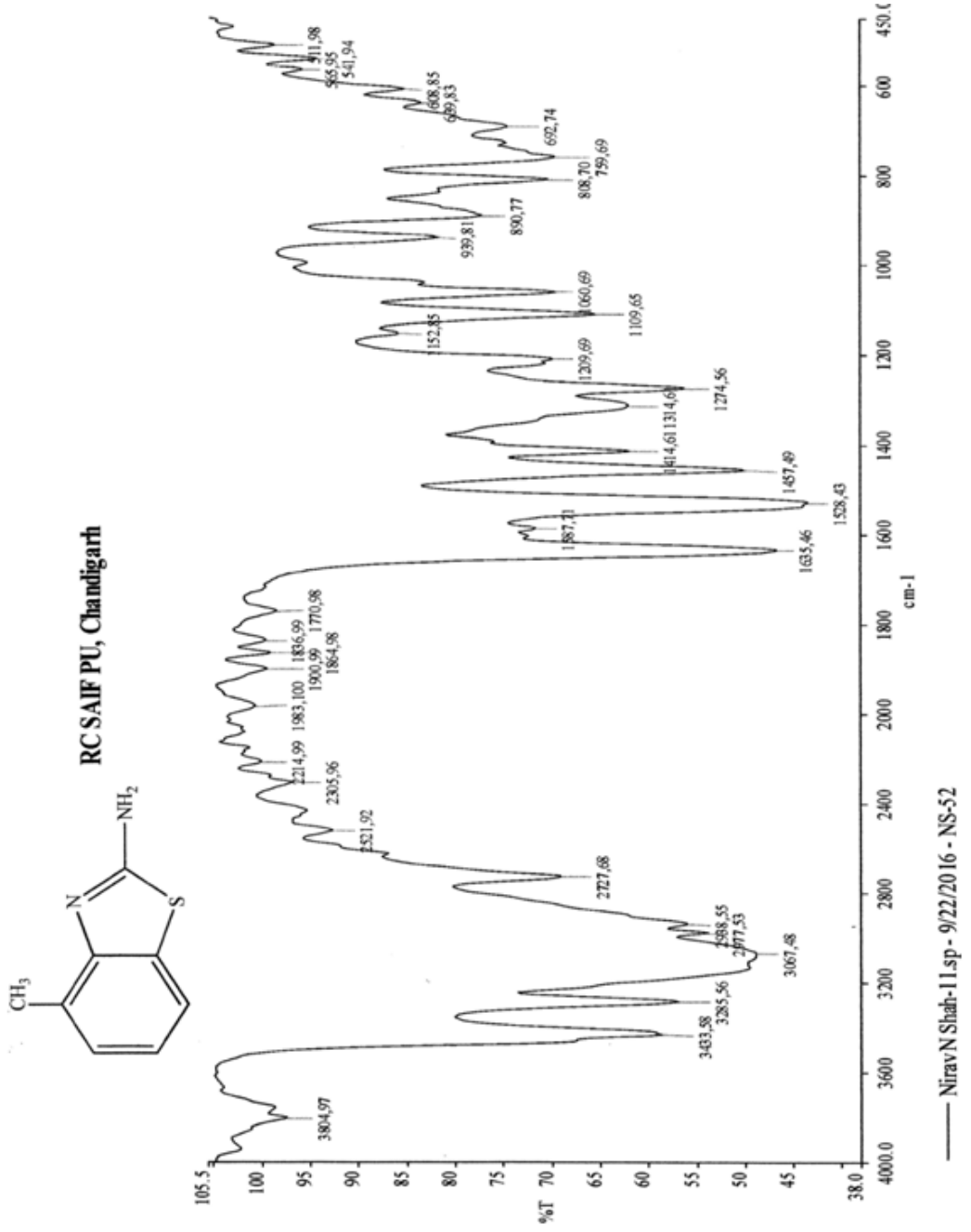


Figure-6 IR Spectrum of 4-methyl benzo[*d*] thiazol-2-amine i.e **1b**

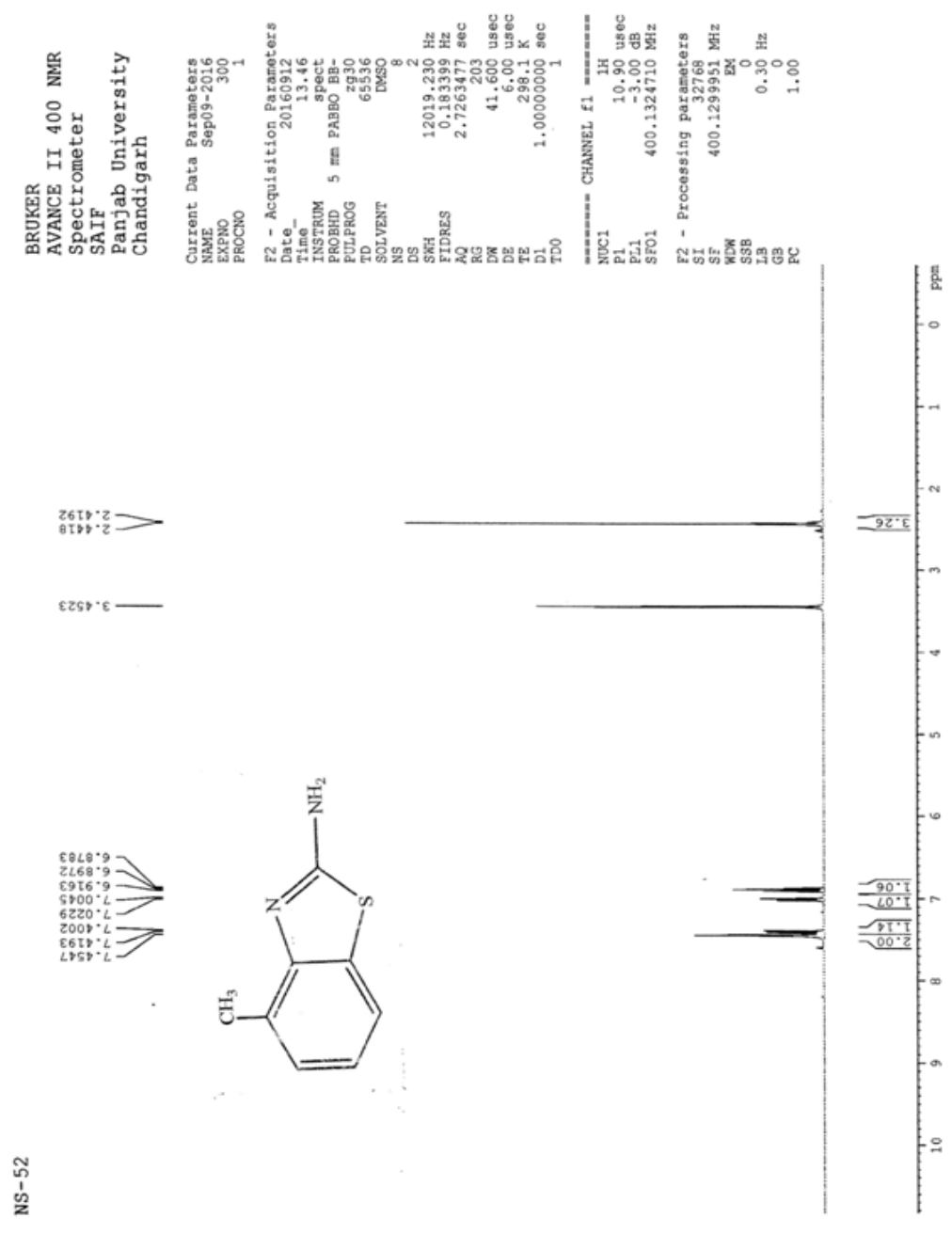


Figure-7 ^{1}H NMR Spectrum of 4-methyl benzo[*d*] thiazol-2-amine i.e **1b**

Figure-8 ^{13}C NMR spectrum of 4-methyl benzo[*d*] thiazol-2-amine i.e **1b**

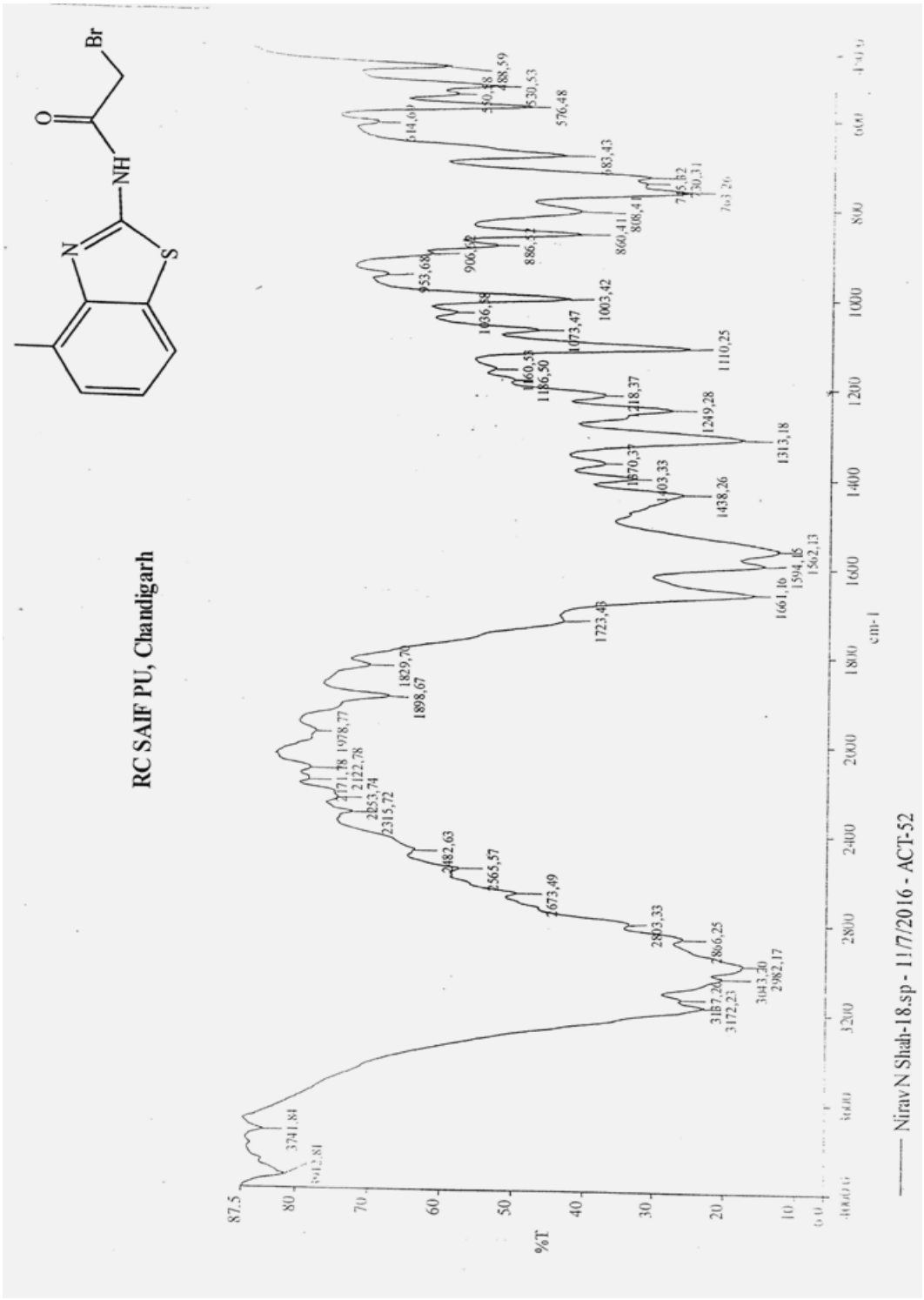


Figure-9 IR Spectrum of N-(4-Methyl benzo[d]thiazol-2-yl)-2-bromo acetamide i.e.**2b**

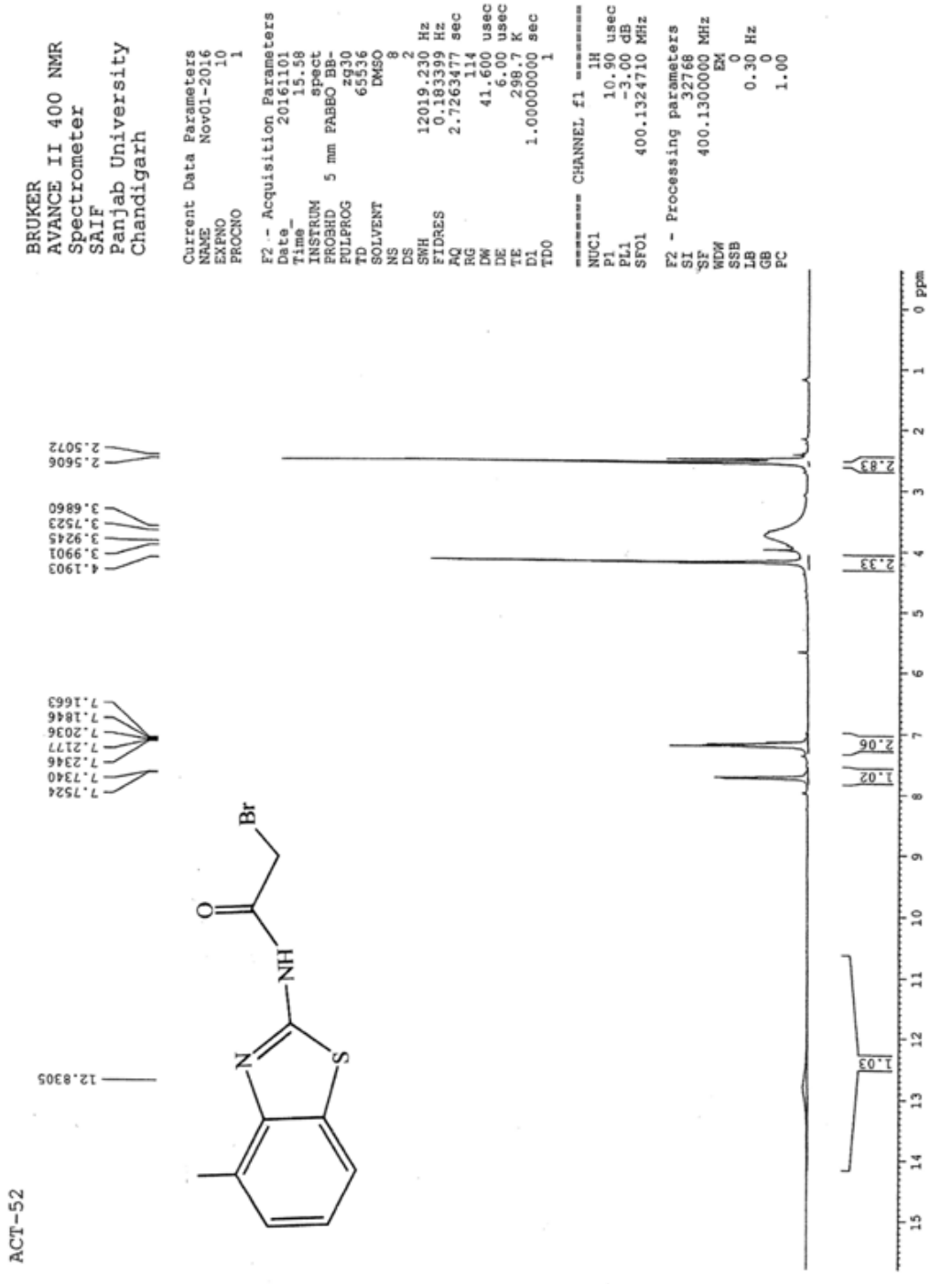


Figure-10 [1]HNMR Spectrum of N-(4-Methyl benzo[d]thiazol-2-yl)-2-bromo acetamide i.e.**2b**

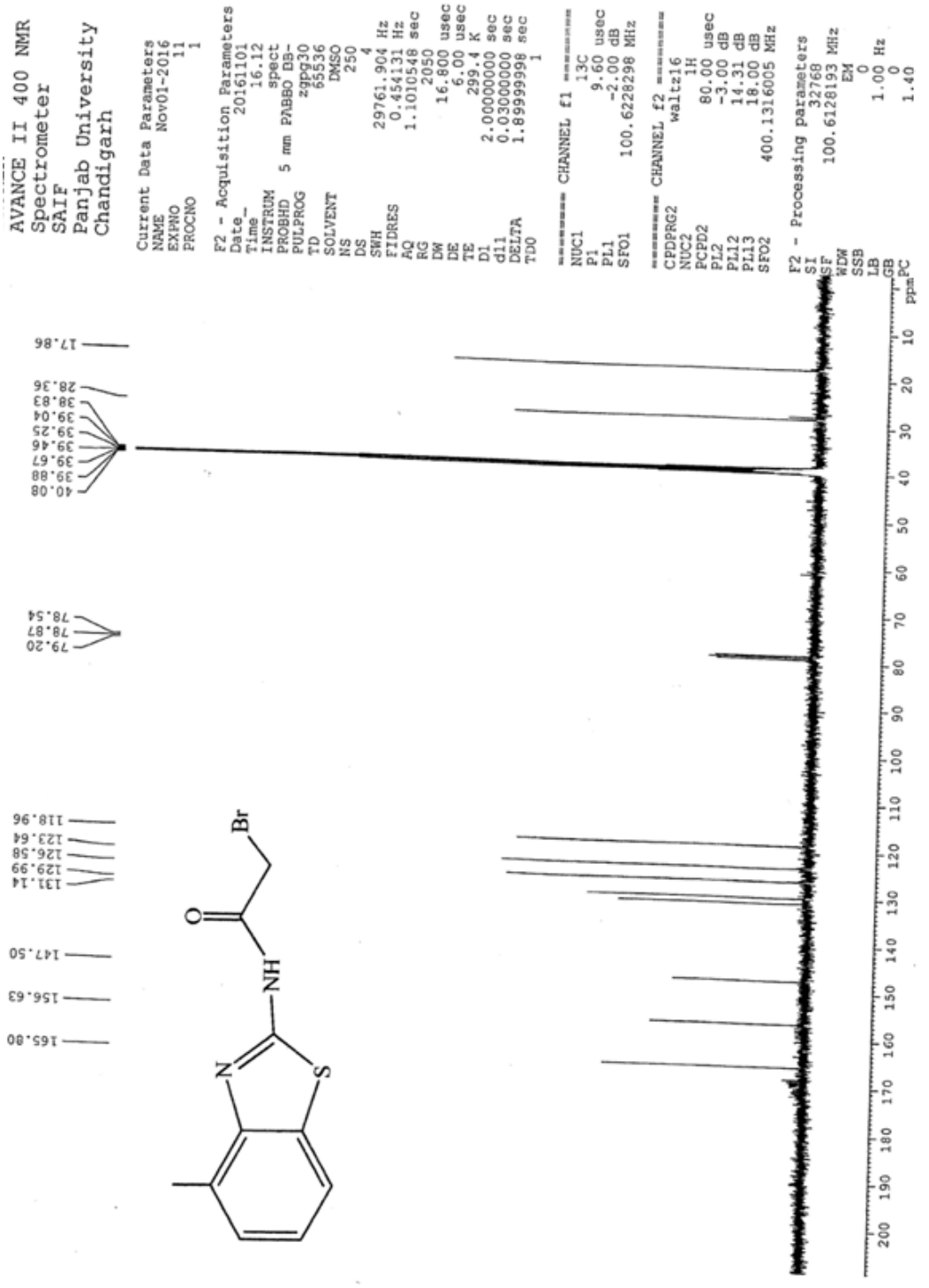


Figure-11 [13] C NMR Spectrum of N-(4-Methyl benzo[d]thiazol-2-yl)-2-bromo acetamide i.e.**2b**

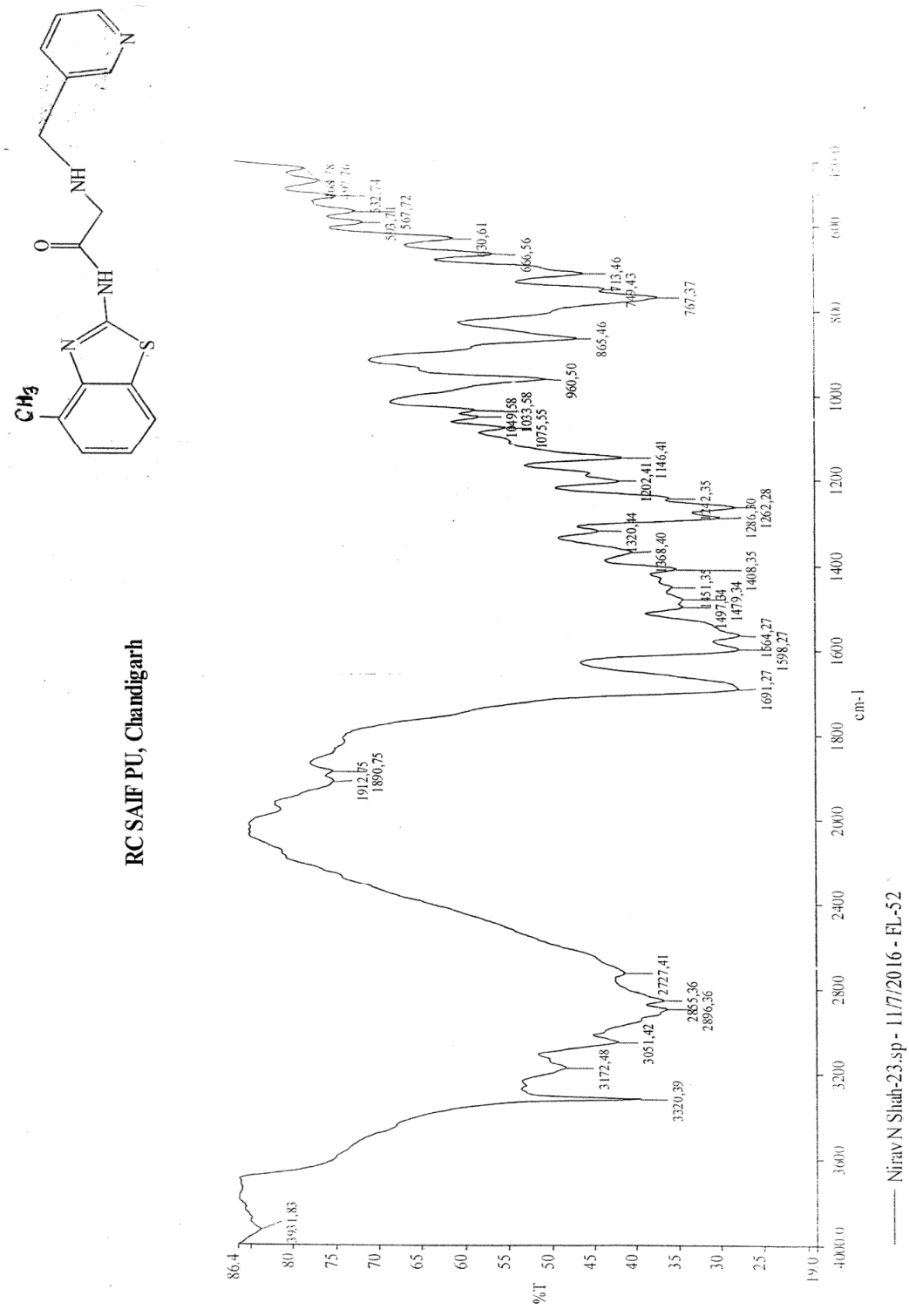


Figure- 12 IR Spectrum of N-(4-methyl benzo[d]thiazol-2-((Pyridin-3yl methyl) amino) acetamide i.e. **3b**

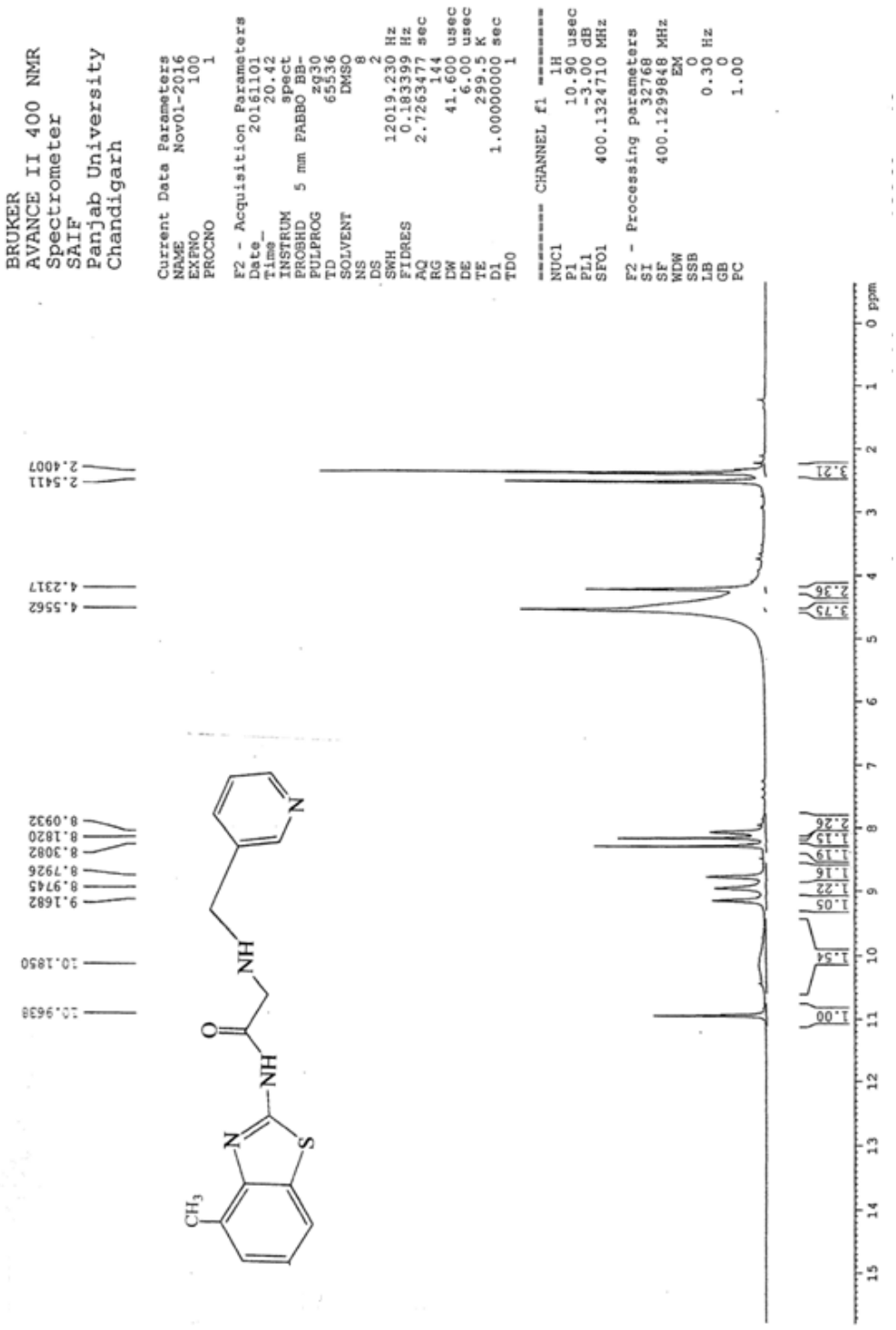


Figure- 13 ^{1}H NMR Spectrum of N-(4-methyl benzo[d]thiazol-2-((Pyridin-3yl methyl) amino) acetamide i.e. **3b**

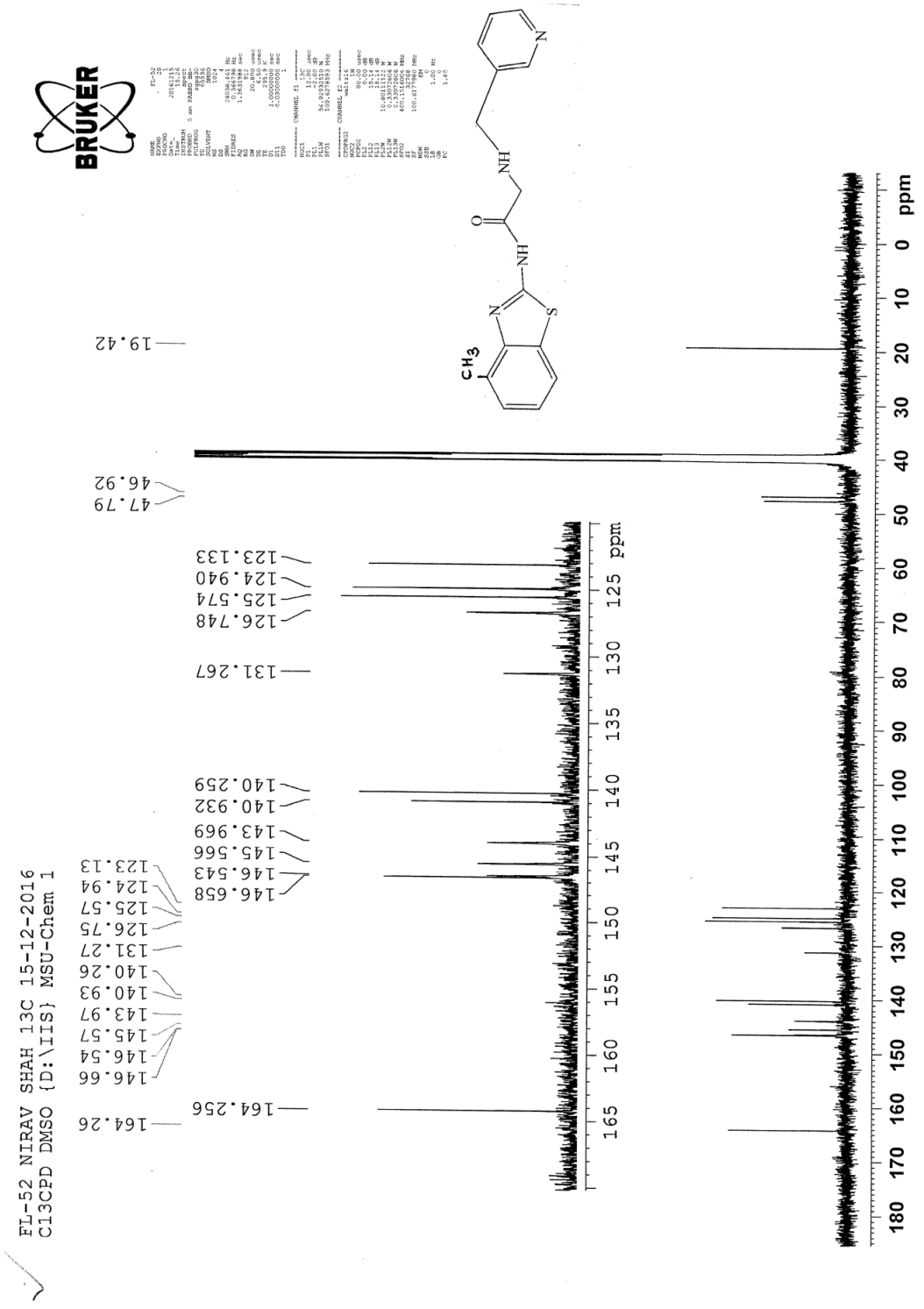
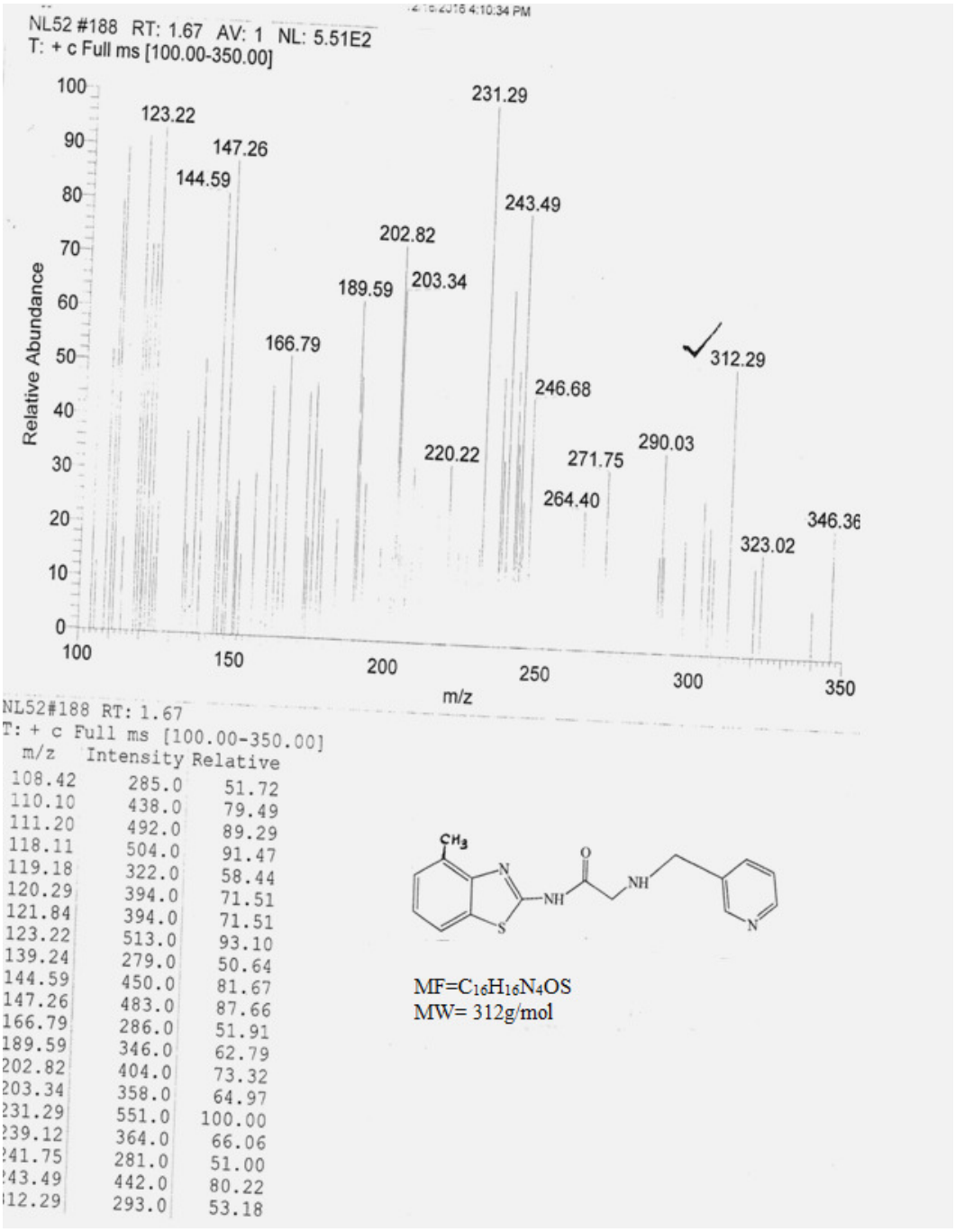


Figure-14 ^{13}C NMR Spectrum of N-(4-methyl benzo[d]thiazol-2-((Pyridin-3yl methyl) amino) acetamide. i.e **3b**



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108.42	285.0	51.72
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111.20	492.0	89.29
118.11	504.0	91.47
119.18	322.0	58.44
120.29	394.0	71.51
121.84	394.0	71.51
123.22	513.0	93.10
139.24	279.0	50.64
144.59	450.0	81.67
147.26	483.0	87.66
166.79	286.0	51.91
189.59	346.0	62.79
202.82	404.0	73.32
203.34	358.0	64.97
231.29	551.0	100.00
239.12	364.0	66.06
241.75	281.0	51.00
243.49	442.0	80.22
312.29	293.0	53.18

Figure-15 Mass Spectrum of N-(4-methyl benzo[d]thiazol-2-((Pyridin-3yl methyl) amino) acetamide i.e. **3b**

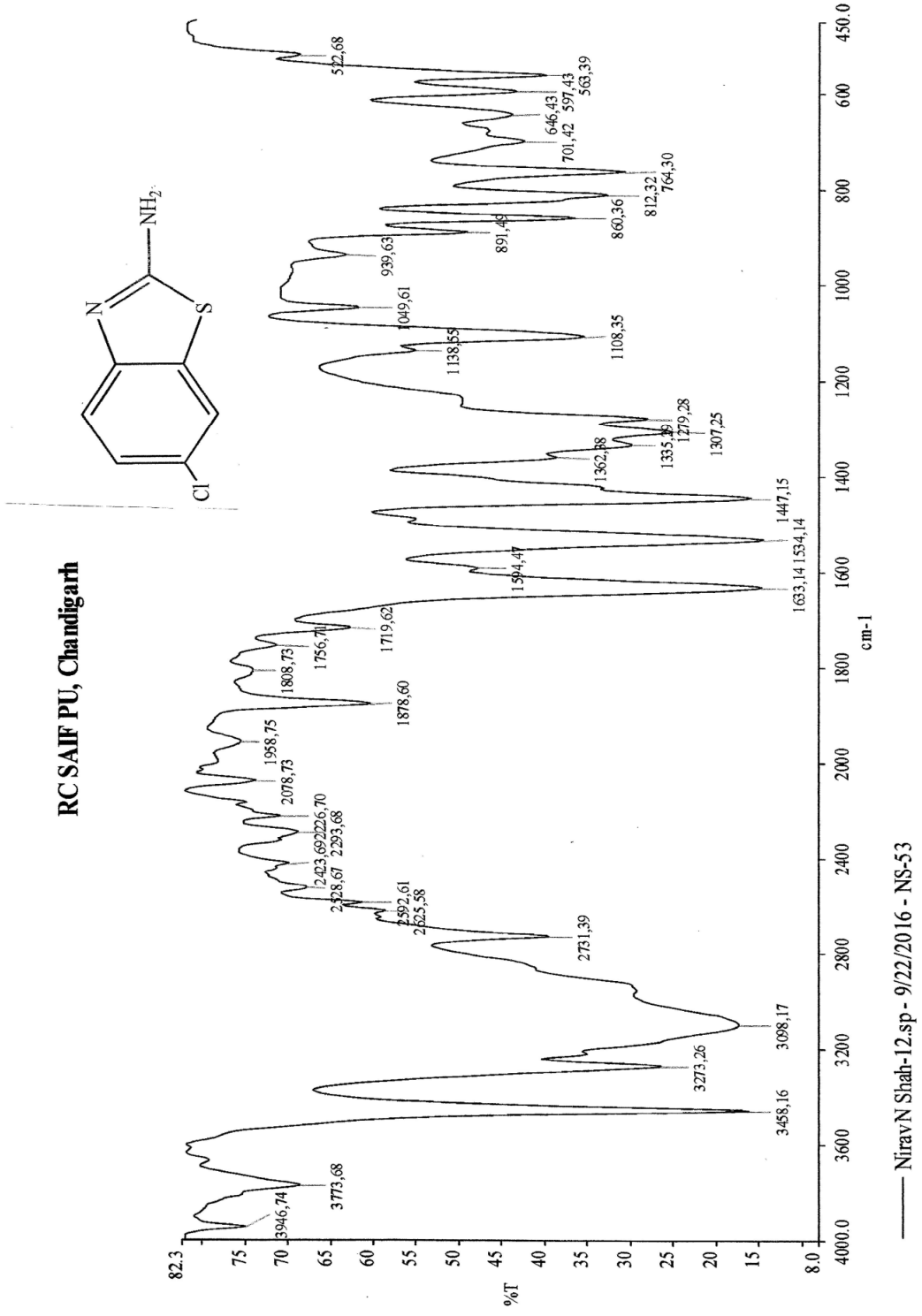


Figure-16 IR Spectrum of 6-Chloro benzo[*d*] thiazol-2-amine i.e **1c**

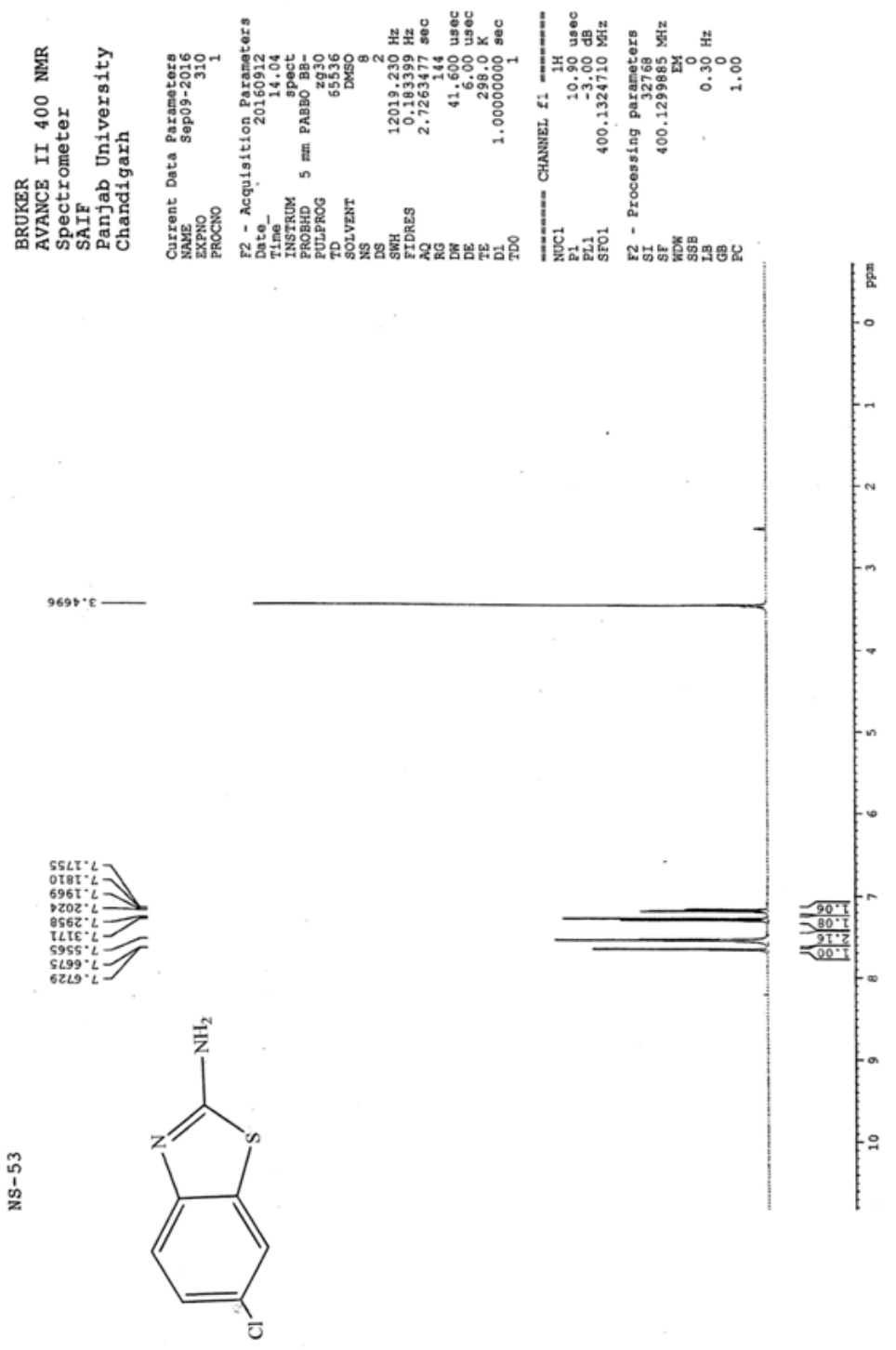


Figure-17 ^{1}H NMR Spectrum of 6-Chloro benzo[*d*] thiazol-2-amine i.e **1c**

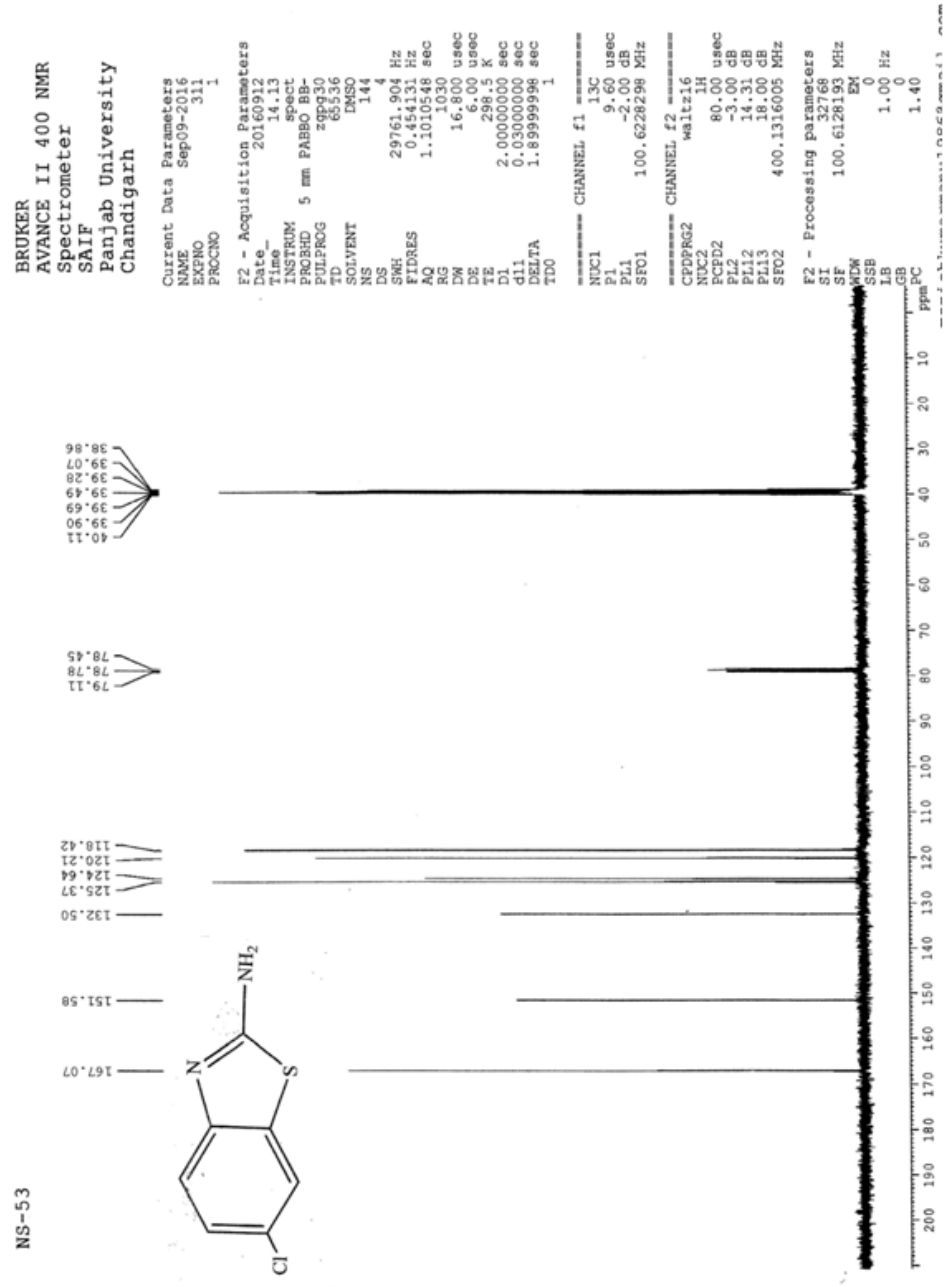


Figure-18 ^{13}C NMR Spectrum of 6-Chloro benzo[*d*] thiazol-2-amine i.e **1c**

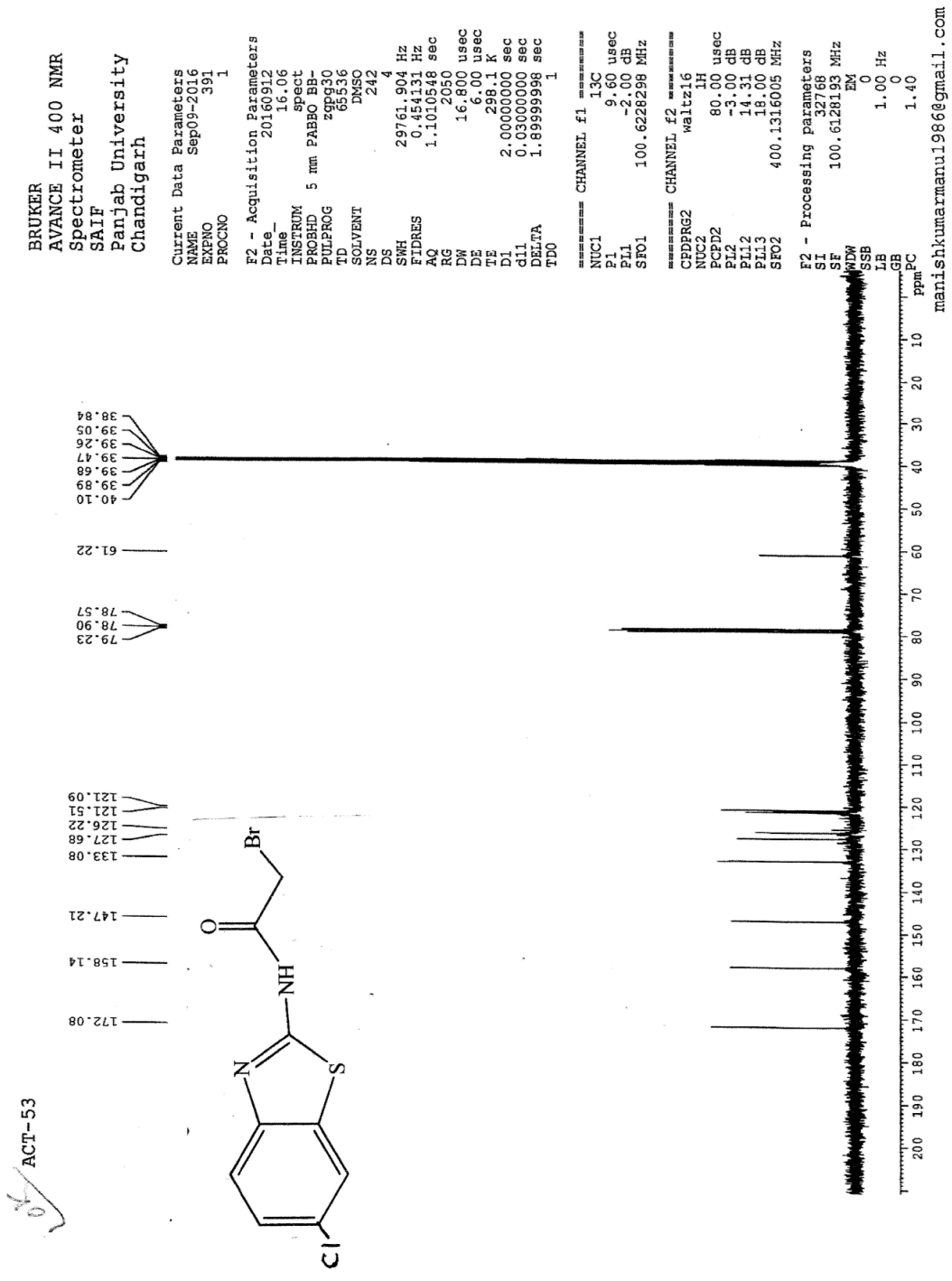


Figure-19 ^{13}C Spectrum of N-(6-Chloro benzo[d]thiazol-2-yl)-2-bromo acetamide i.e.**2c**

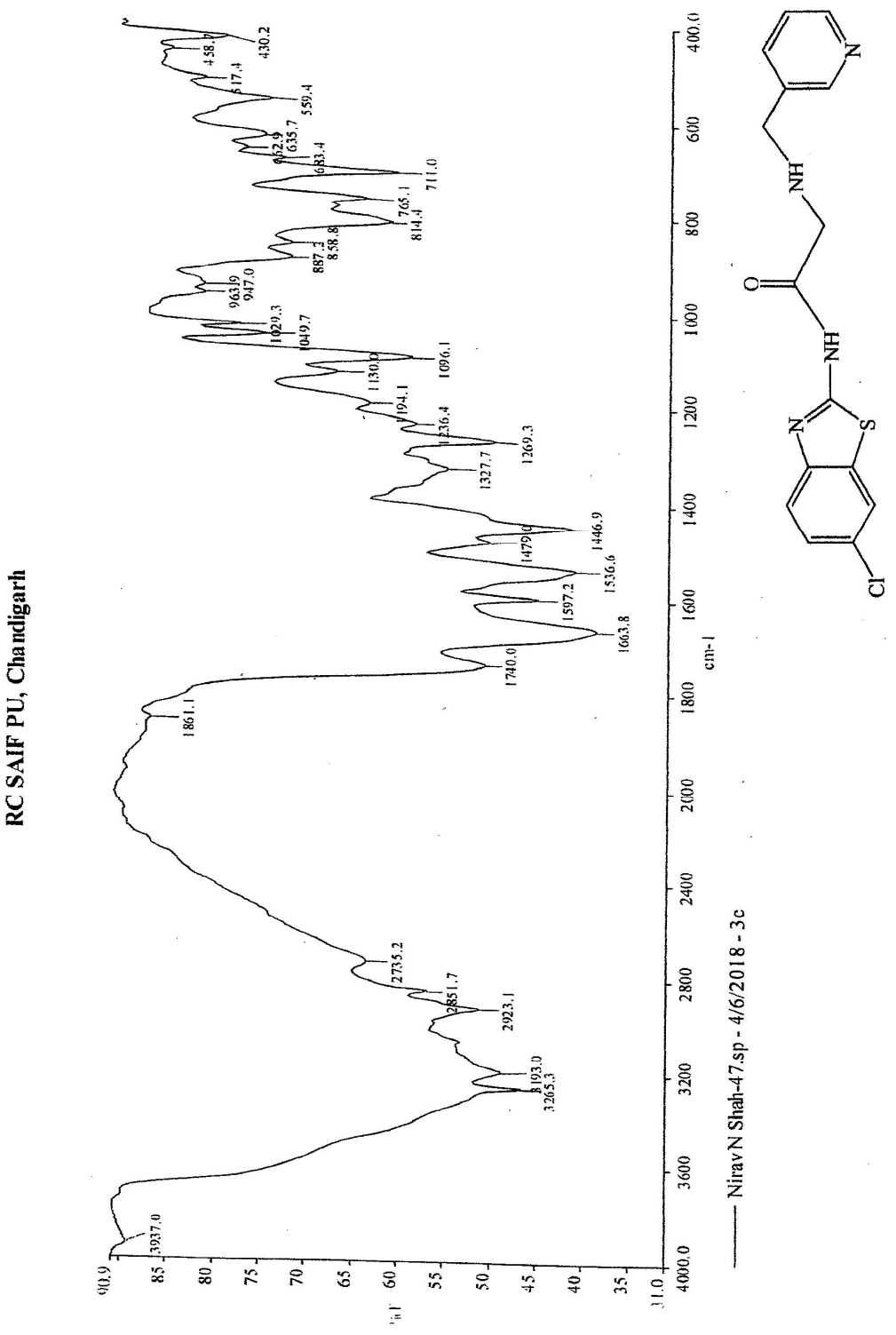


Figure-20 IR Spectrum of N-(6-chloro benzo[d]thiazol-2-((Pyridin-3yl-methyl) amino) acetamide i.e **3C**

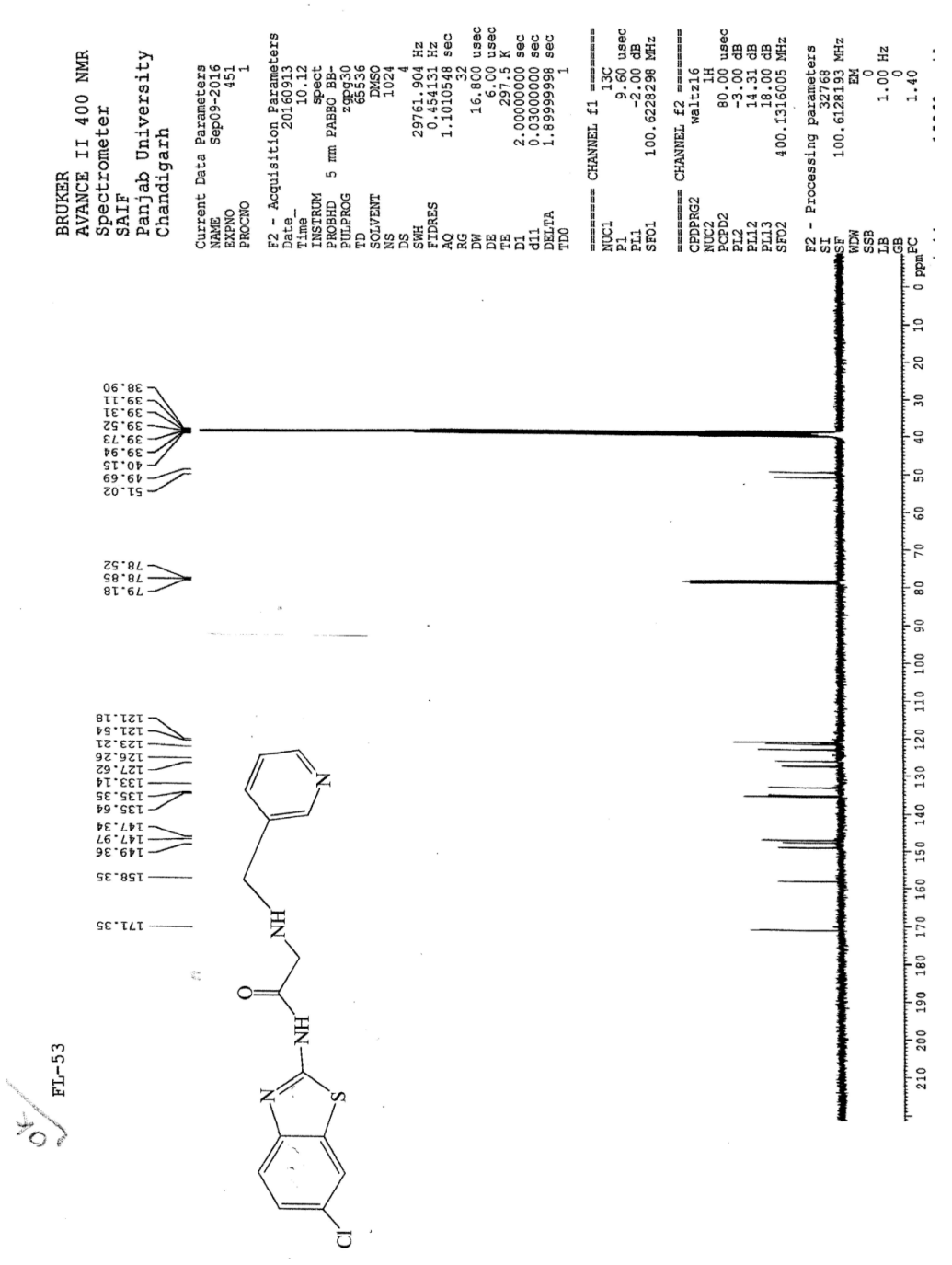


Figure-21 [13] C NMR Spectrum of N-(6-Chloro benzo[d]thiazol-2-((Pyridin-3yl methyl) amino) acetamide i.e **3C**

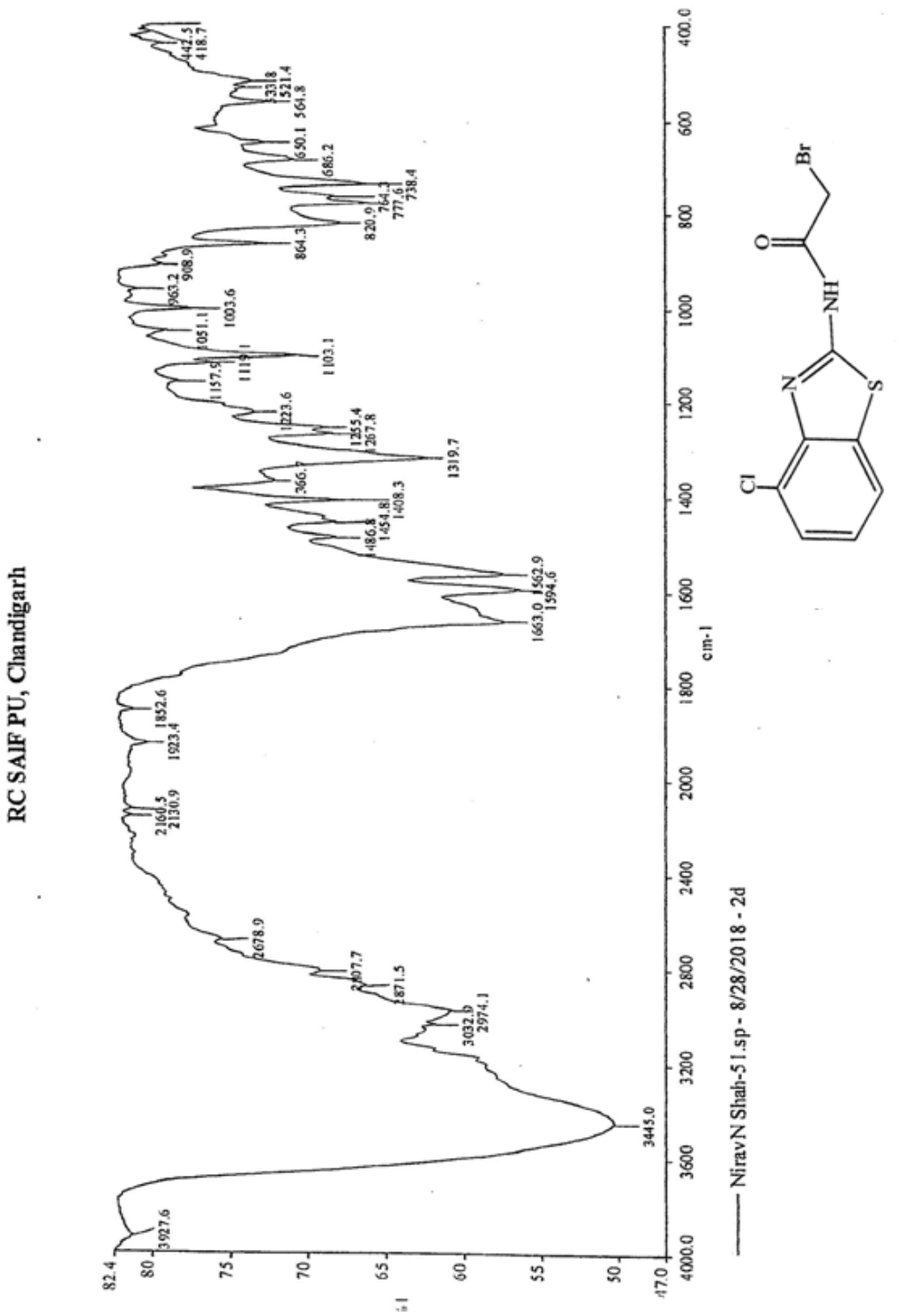


Figure-22 ^{1}H NMR Spectrum of N-(4-Chloro benzo[d]thiazol-2-yl)-2-bromo acetamide i.e.**2d**

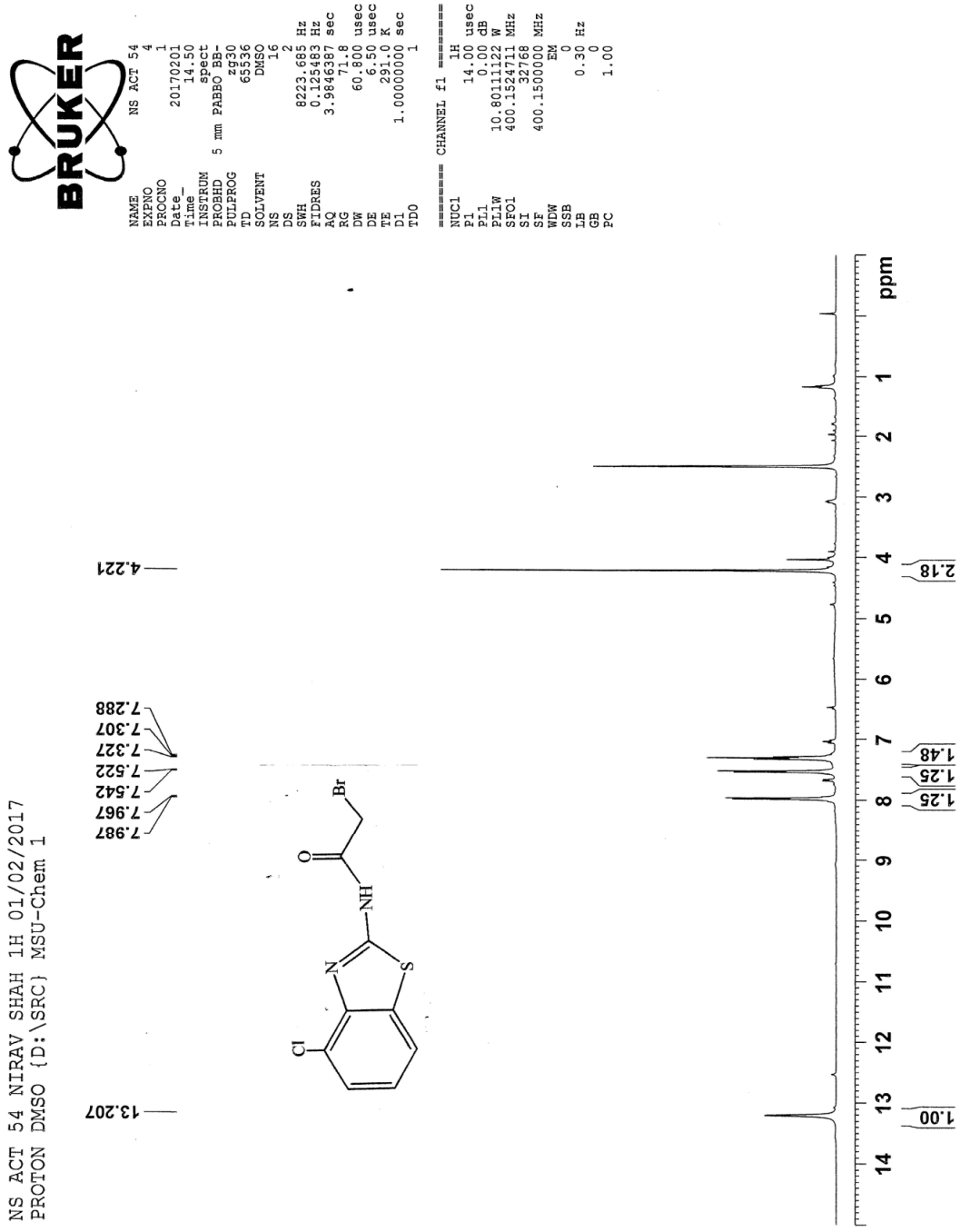


Figure-23 ^{1}H NMR Spectrum of N-(4-Chloro benzo[d]thiazol-2-yl)-2-bromo acetamide i.e.**2d**

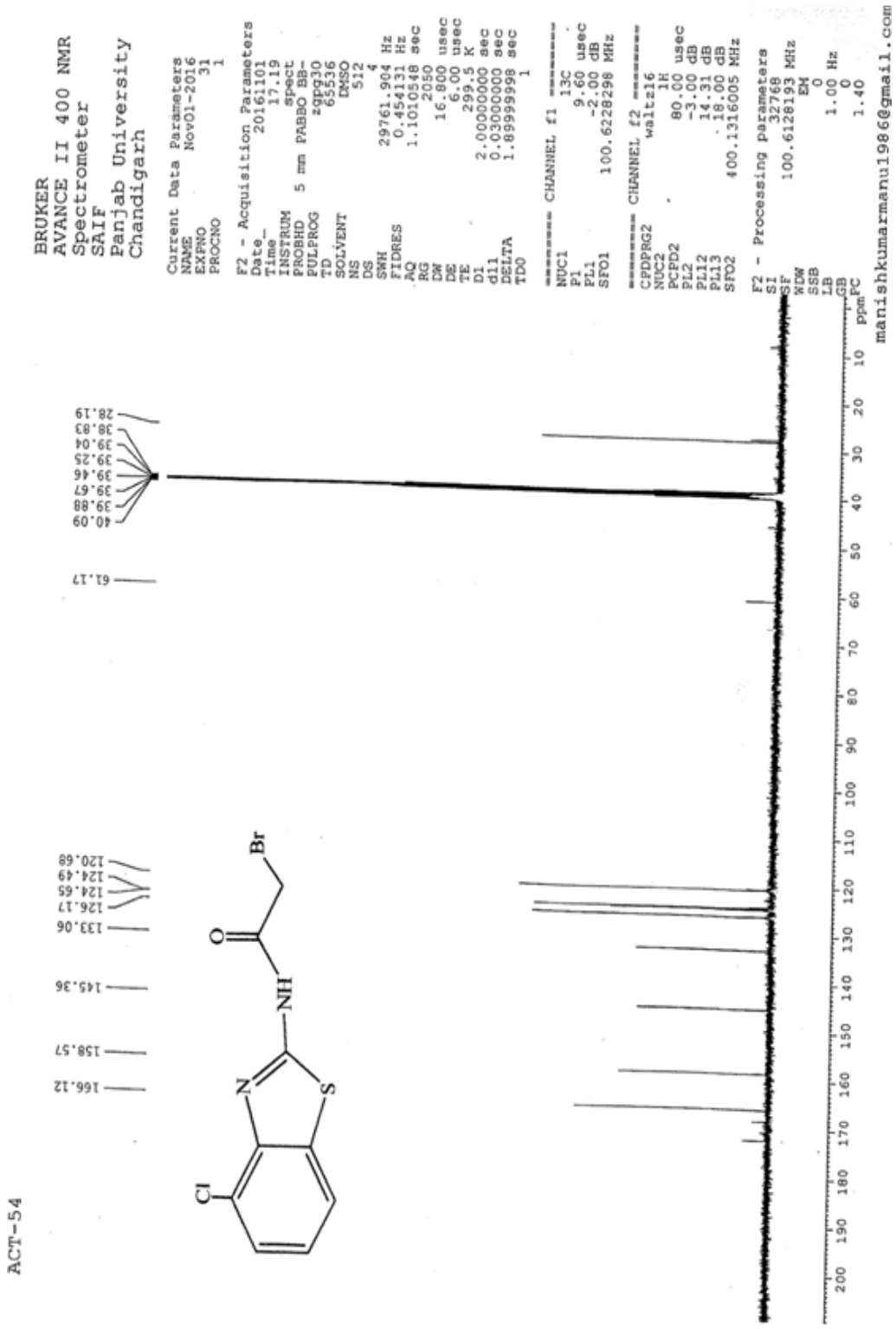


Figure-24 ^{13}C NMR Spectrum of N-(4-Chloro benzo[d]thiazol-2-yl)-2-bromo acetamide i.e.**2d**

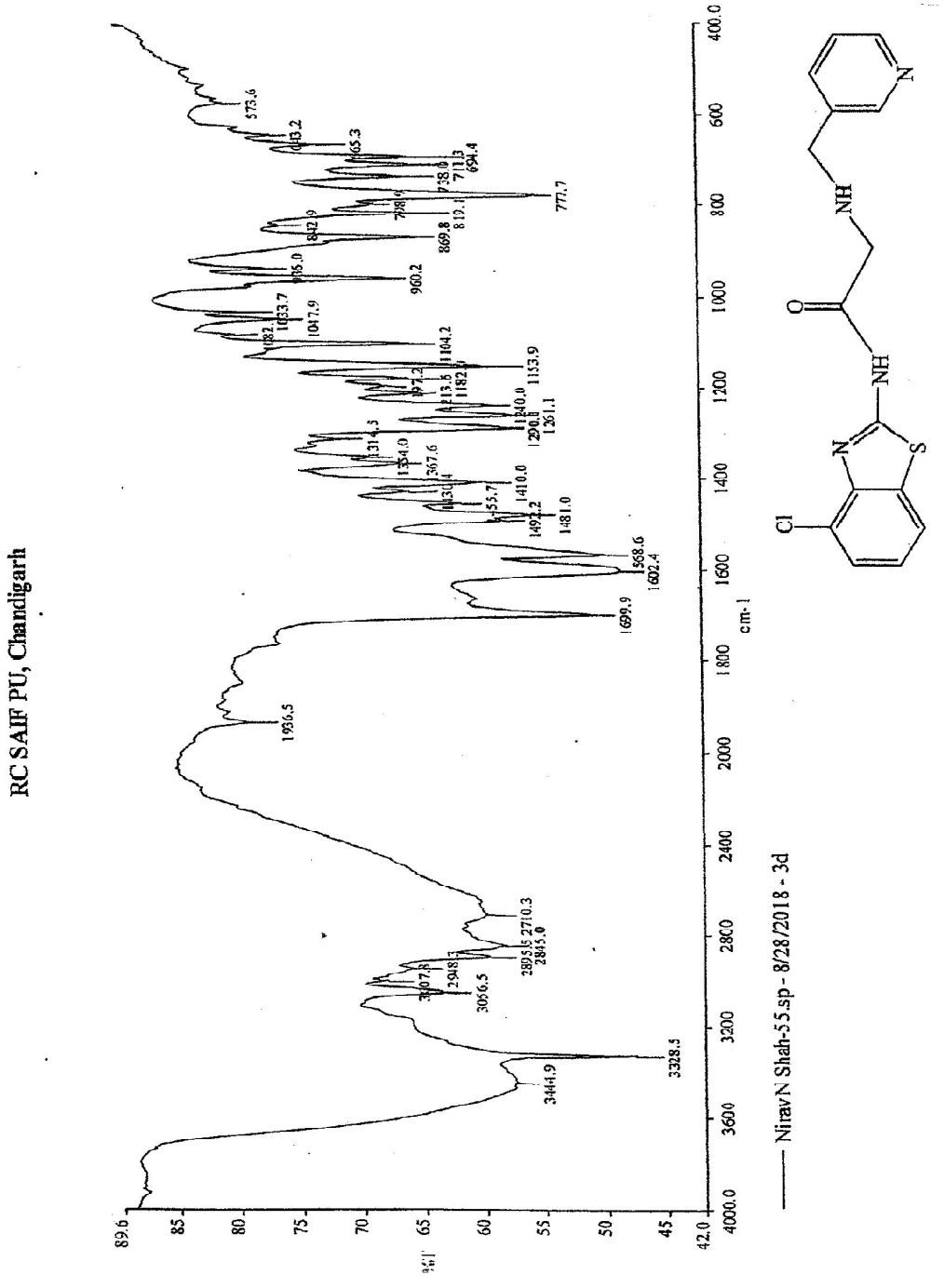


Figure-25 IR Spectrum of N-(4-Chloro benzo[d]thiazol-2-((Pyridin-3yl-methyl) amino) acetamide i.e **3d**

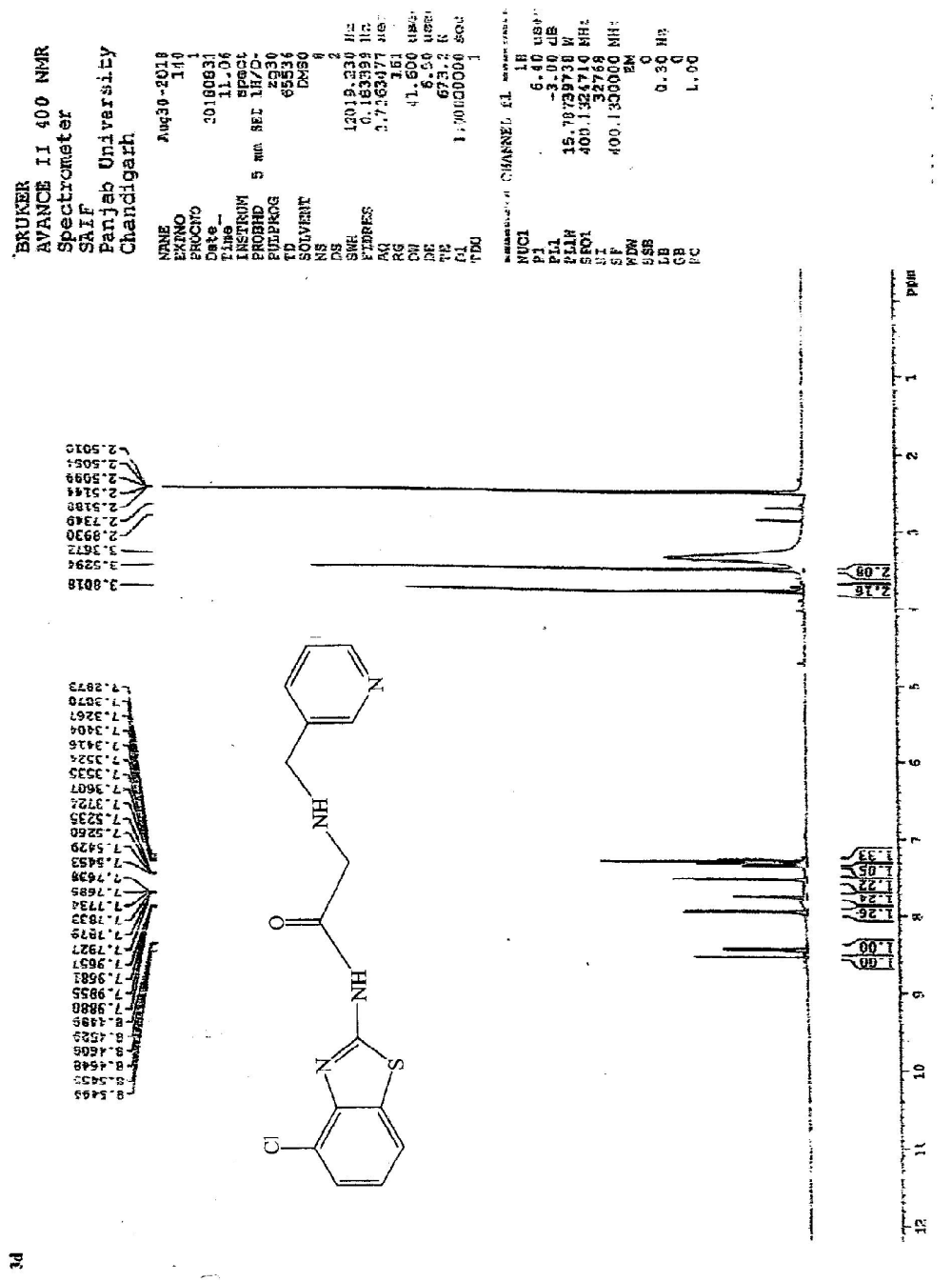


Figure-26 ^{1}H NMR Spectrum of N-(4-Chloro benzo[d]thiazol-2-((Pyridin-3yl-methyl) amino) acetamide i.e **3d**

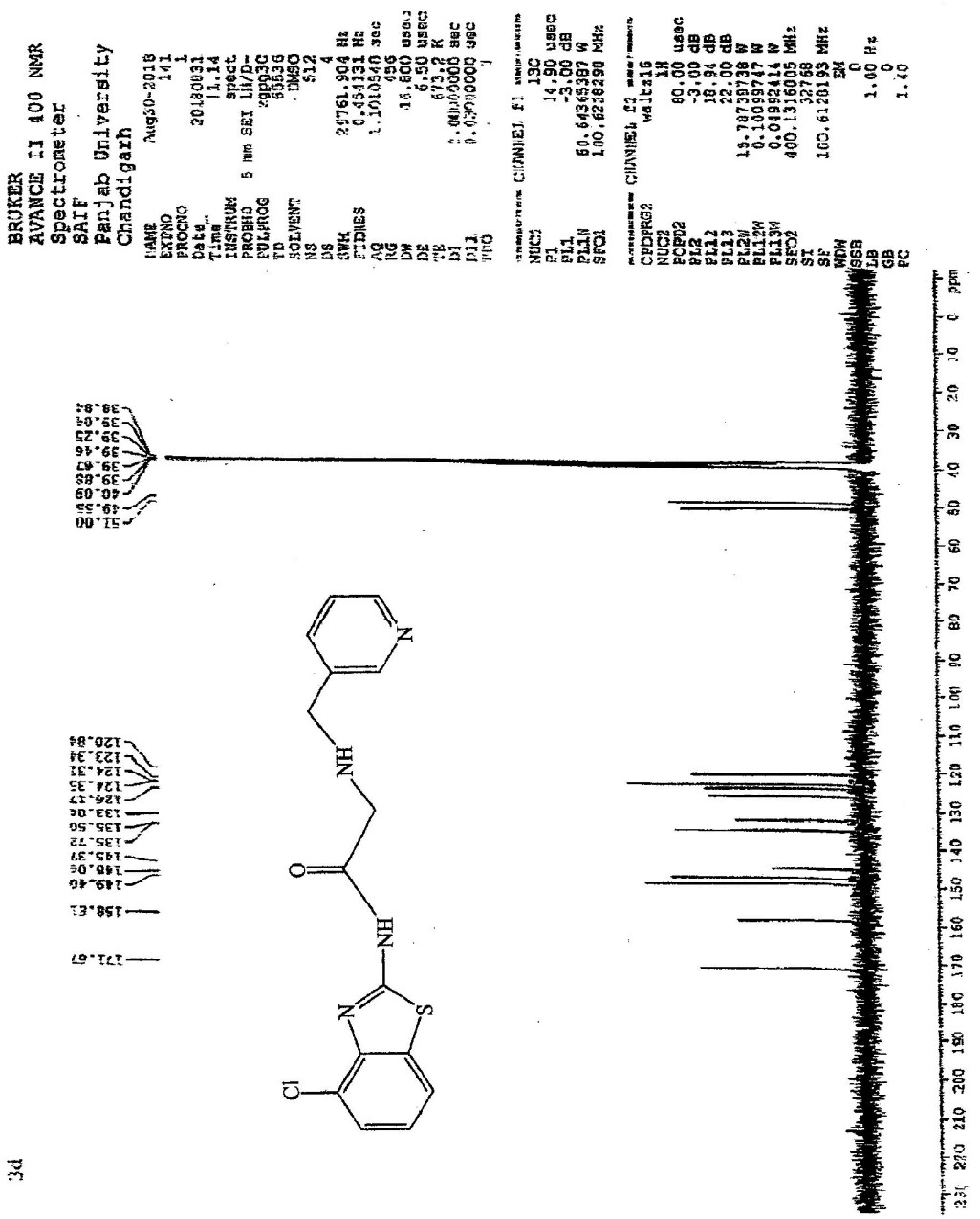


Figure-27 [13] C NMR Spectrum of N-(4-Chloro benzo[d]thiazol-2-((Pyridin-3yl-methyl) amino) acetamide i.e **3d**

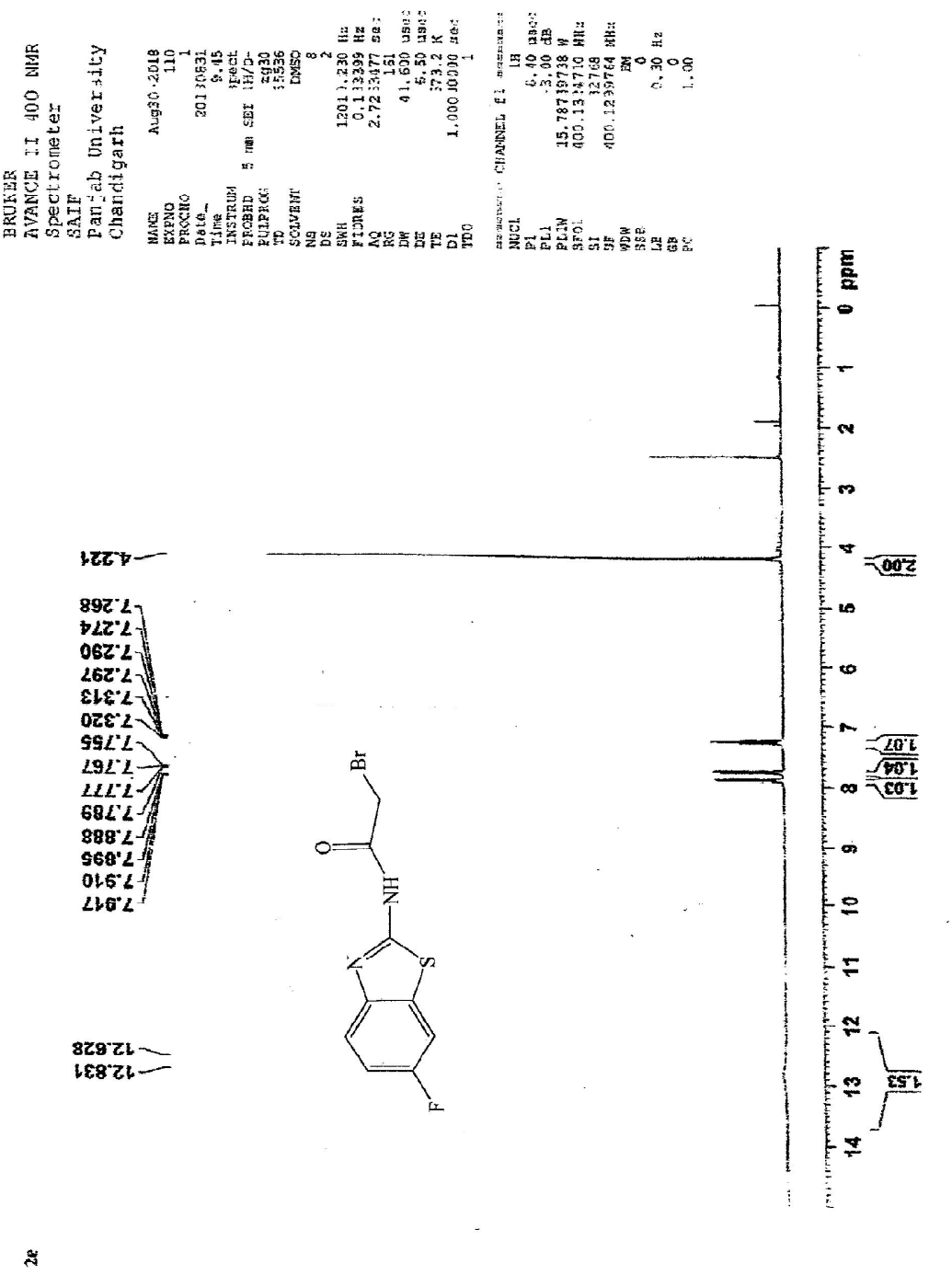


Figure- 28 ^{1}H NMR Spectrum of N-(6-Flouro benzo[d]thiazol-2-yl)-2-bromo acetamide i.e. **2e**

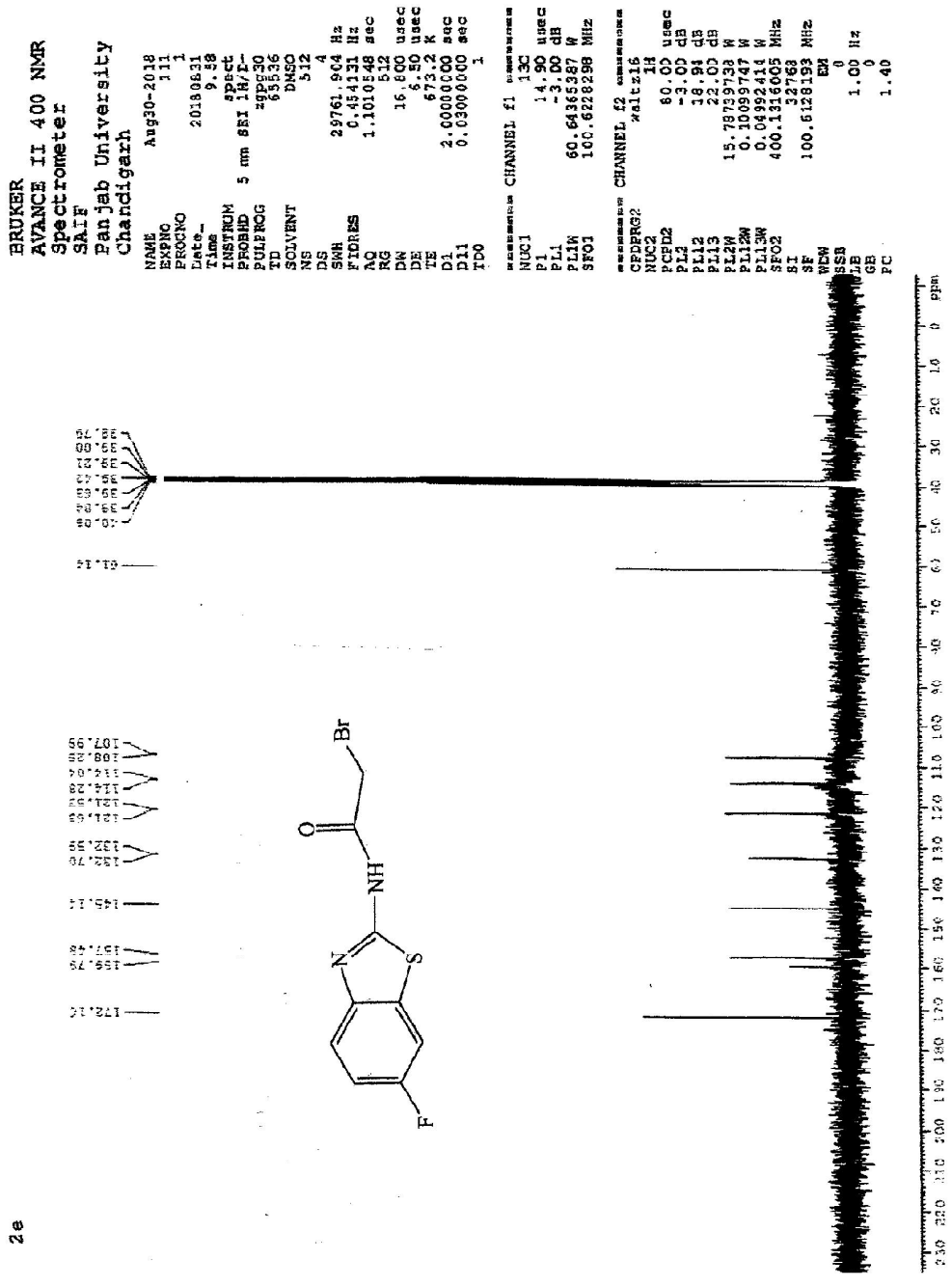
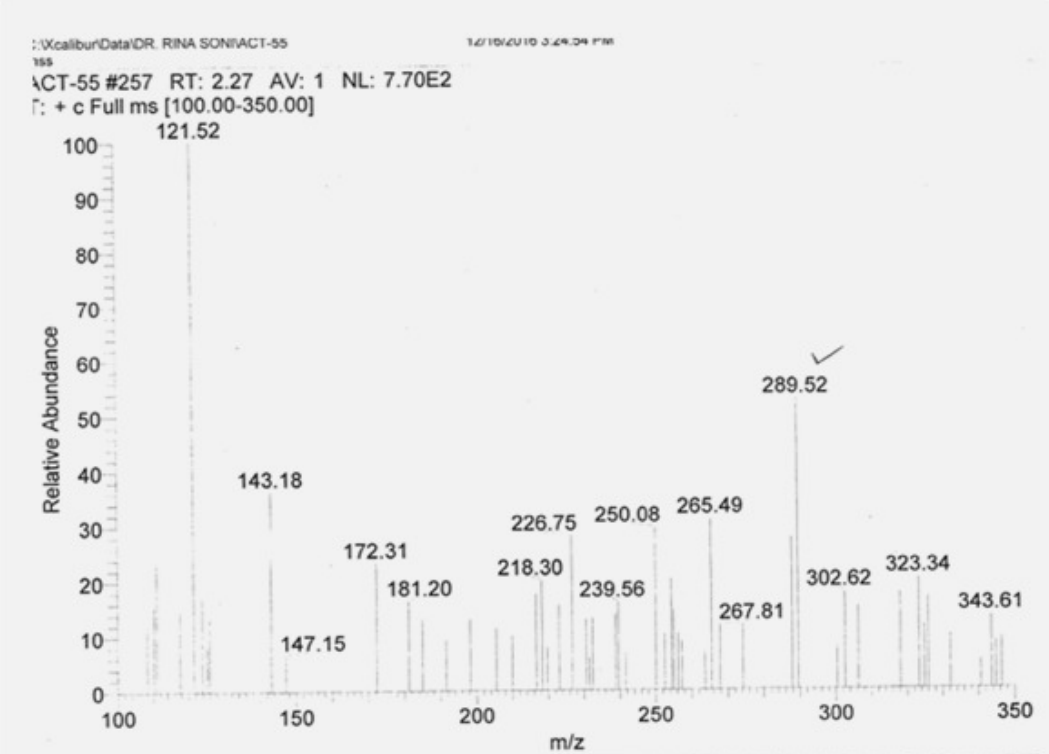


Figure- 29 ^{13}C NMR Spectrum of N-(6-Flouro benzo[d]thiazol-2-yl)-2-bromo acetamide i.e. **2e**



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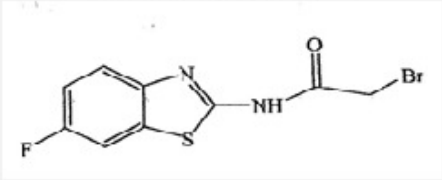
123.87	129.0	16.75
143.18	279.0	36.23
172.31	179.0	23.25
181.20	126.0	16.36
216.66	135.0	17.53
218.30	154.0	20.00
223.10	120.0	15.58
226.75	216.0	28.05
239.56	123.0	15.97
250.08	227.0	29.48
254.41	156.0	20.26
265.49	239.0	31.04
287.87	212.0	27.53
289.52	405.0	52.60
302.62	136.0	17.66
318.08	135.0	17.53
323.34	155.0	20.13
326.01	129.0	16.75



MF = $C_9H_6BrFN_2OS$

MW = 289 g/mol

Figure- 30 Mass Spectrum of N-(6-Flouro benzo[d]thiazol-2-yl)-2-bromo acetamide i.e. **2e**

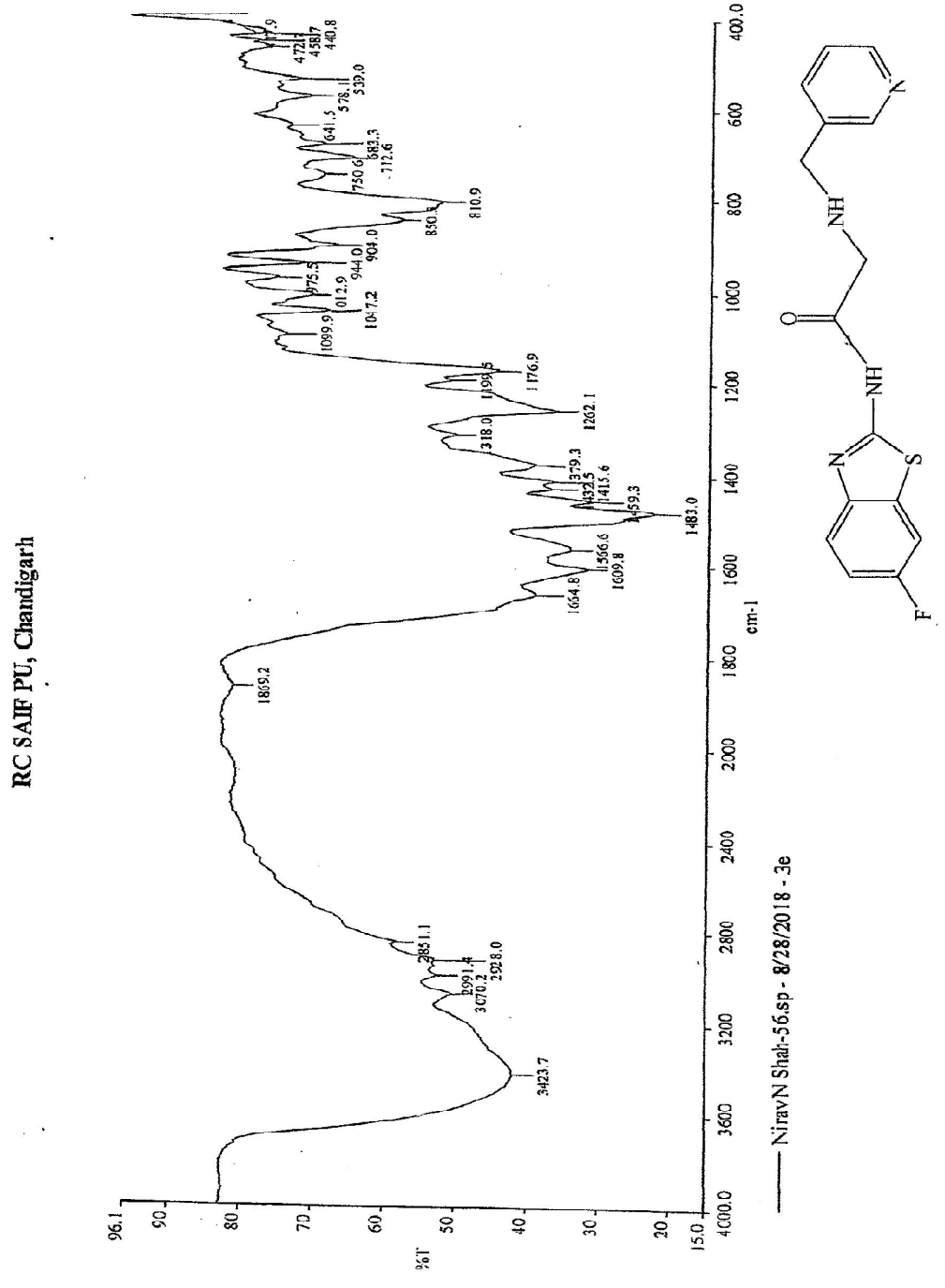


Figure-31 IR Spectrum of N-(6-Flouro benzo[d]thiazol-2-((Pyridin-3yl methyl) amino) acetamide i.e **3e**

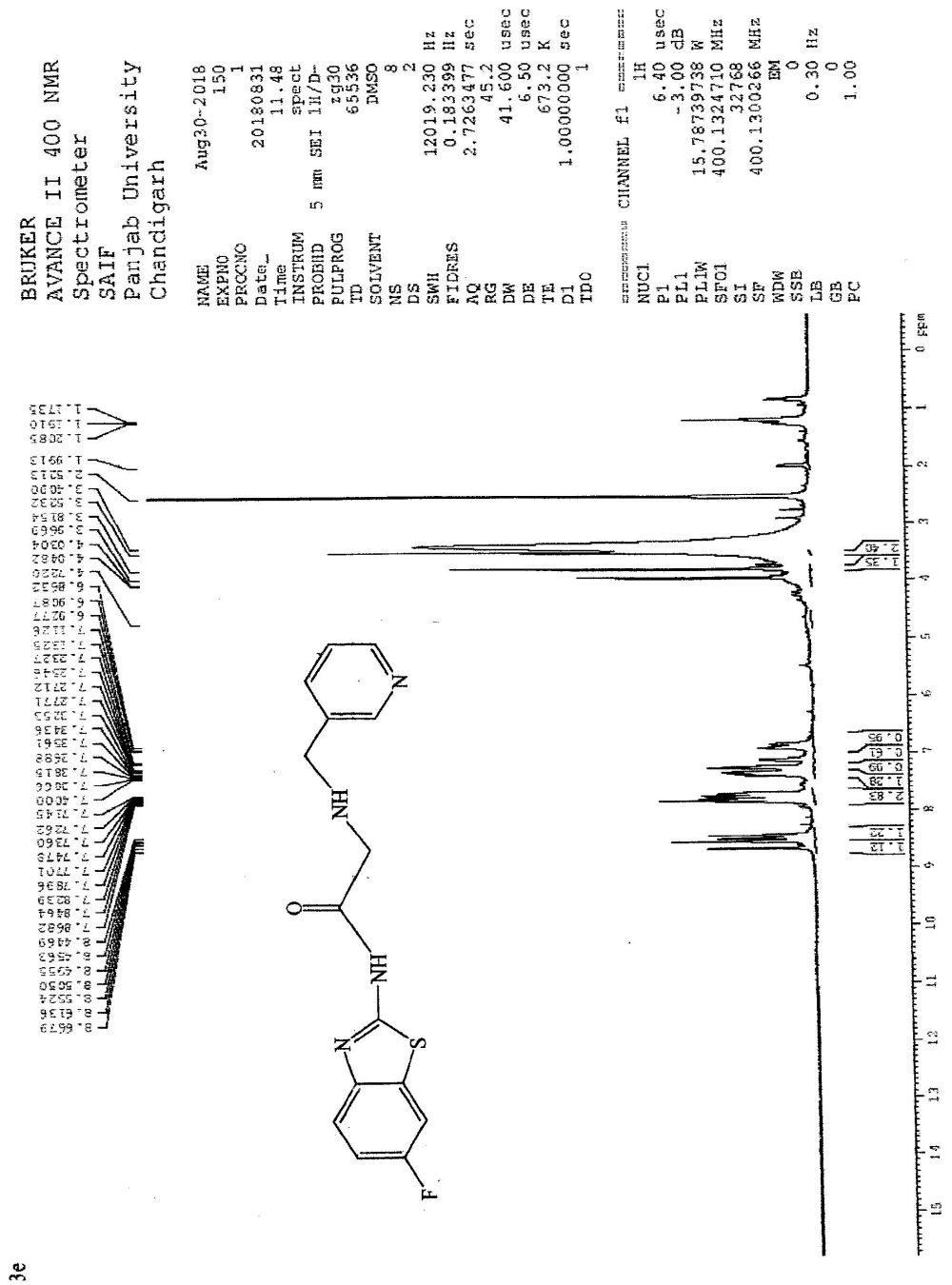


Figure-32 ^{1}H NMR Spectrum of N-(6-Flouro benzo[d]thiazol-2-((Pyridin-3yl methyl) amino) acetamide i.e **3e**

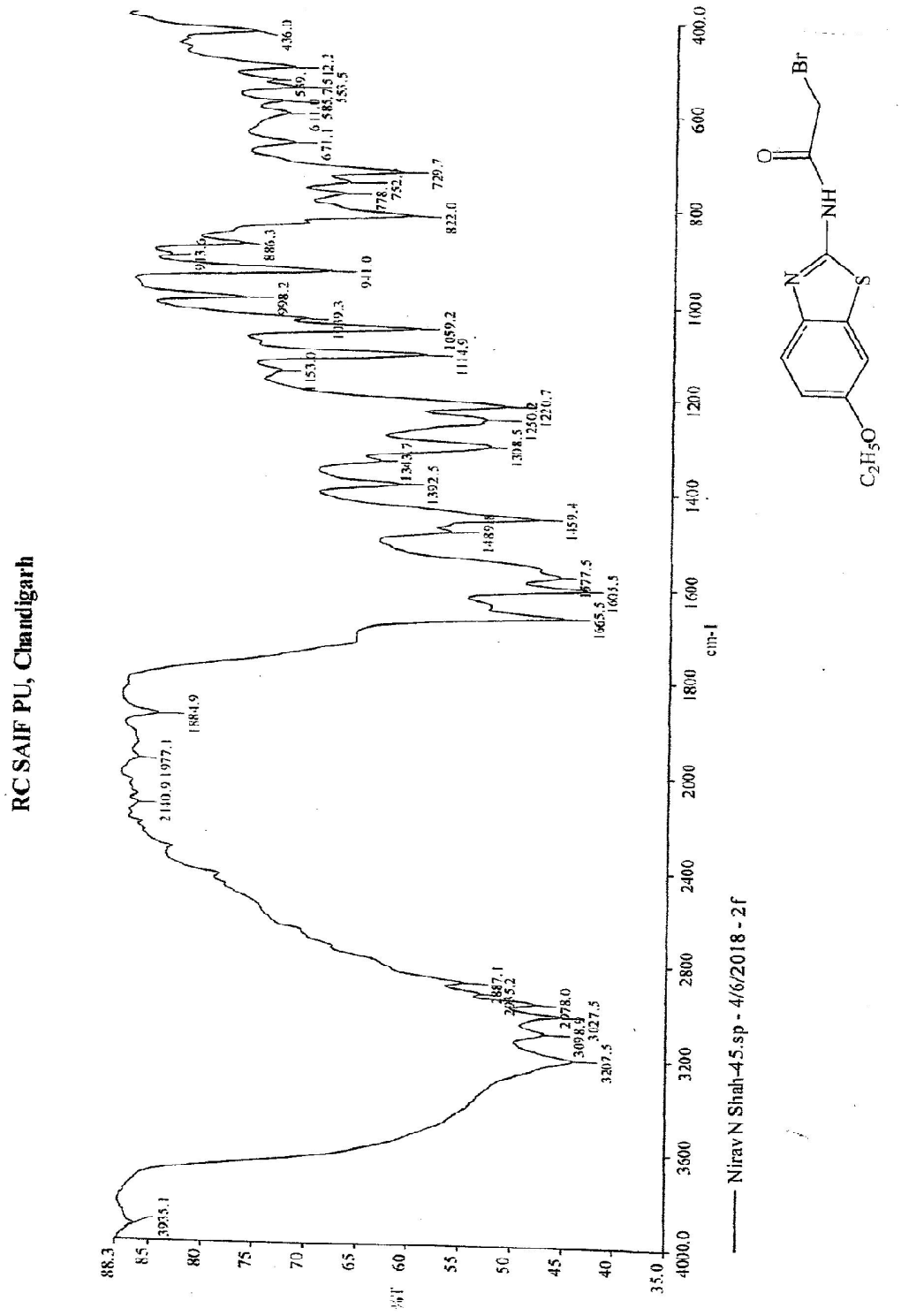


Figure- 33 IR Spectrum of N-(6-Ethoxy benzo[d]thiazol-2-yl)-2-bromo acetamide i.e. **2f**

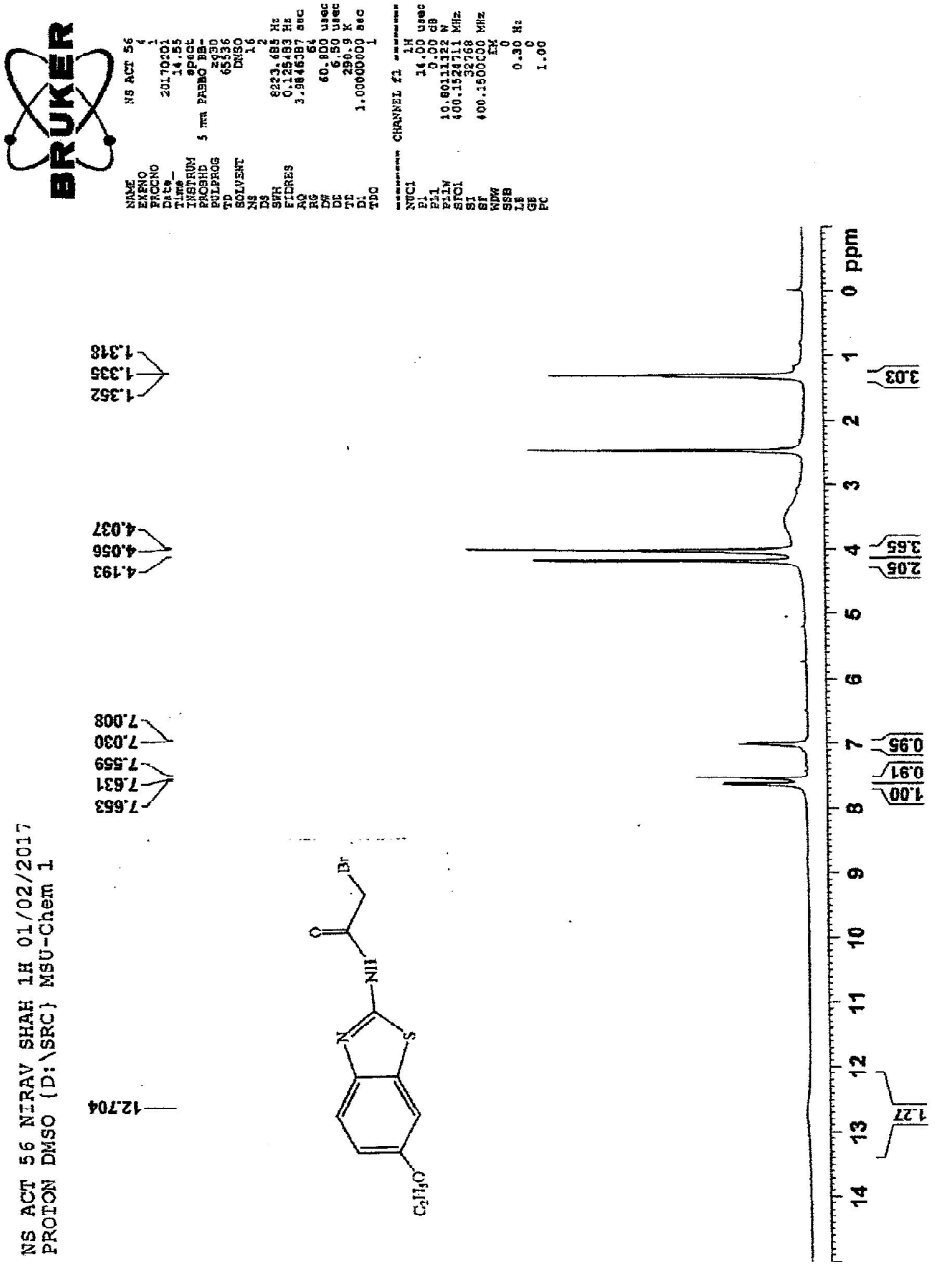


Figure- 34 ^{1}H NMR Spectrum of N-(6-Ethoxy benzo[d]thiazol-2-yl)-2-bromo acetamide i.e. **2f**

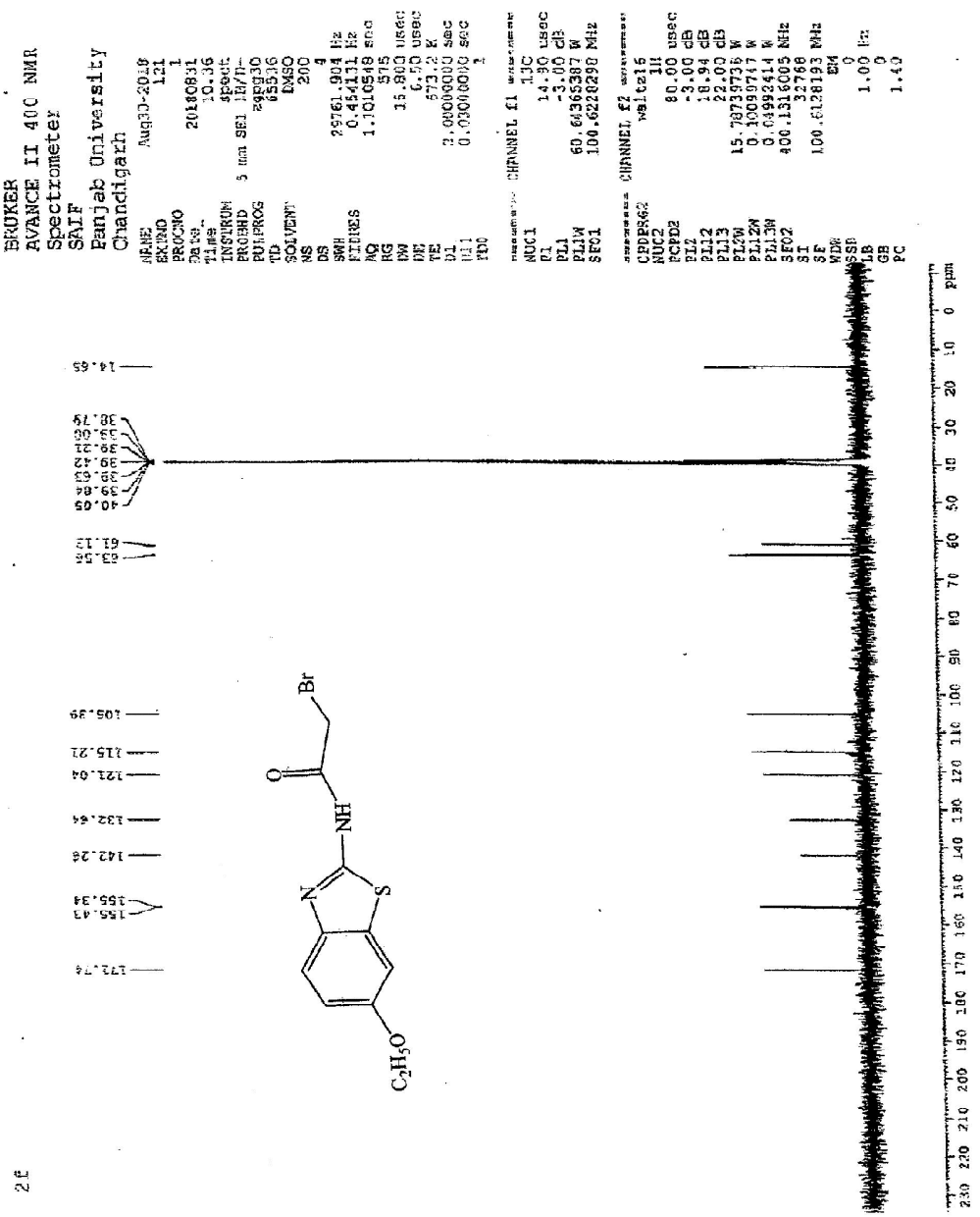
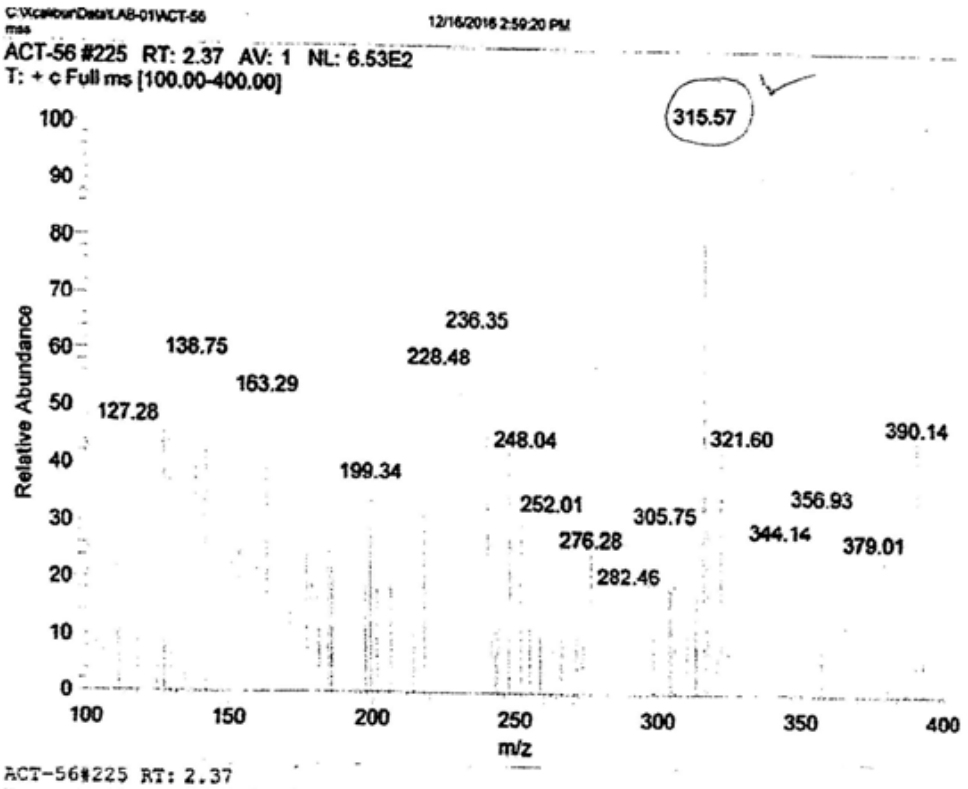
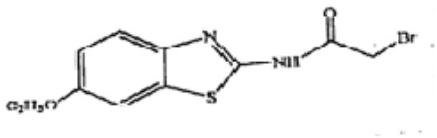


Figure- 35 ^{13}C NMR Spectrum of N-(6-Ethoxy benzo[d]thiazol-2-yl)-2-bromo acetamide i.e. **2f**



ACT-56#225 RT: 2.37
T: + c Full ms [100.00-400.00]

m/z	Intensity	Relative
100.95	354.0	54.21
115.57	225.0	34.46
127.28	302.0	46.25
128.03	254.0	38.90
129.52	364.0	55.74
138.75	379.0	58.04
142.06	277.0	42.42
163.29	336.0	51.45
176.88	222.0	34.00
199.34	237.0	36.29
217.88	214.0	32.77
228.48	370.0	56.66
236.35	414.0	63.40
240.08	407.0	62.33
248.04	277.0	42.42
315.57	653.0	100.00
316.69	226.0	34.61
321.60	281.0	43.03
356.93	214.0	32.77
390.14	295.0	45.18



MF = $C_{11}H_{11}BrN_2O_2S$
MW = 315 g/mol

Figure- 36 Mass Spectrum of N-(6-Ethoxy benzo[d]thiazol-2-yl)-2-bromo acetamide i.e. **2f**

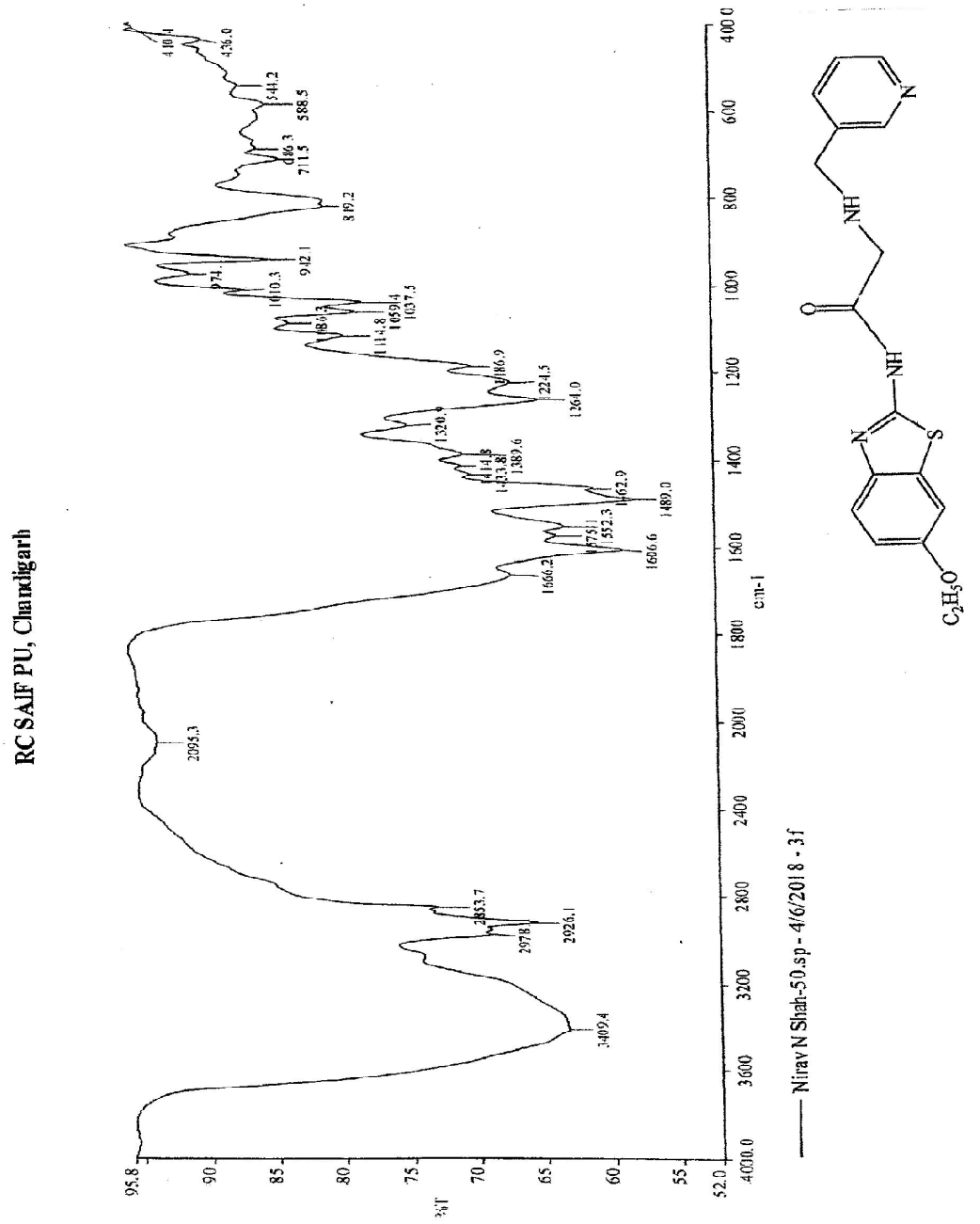
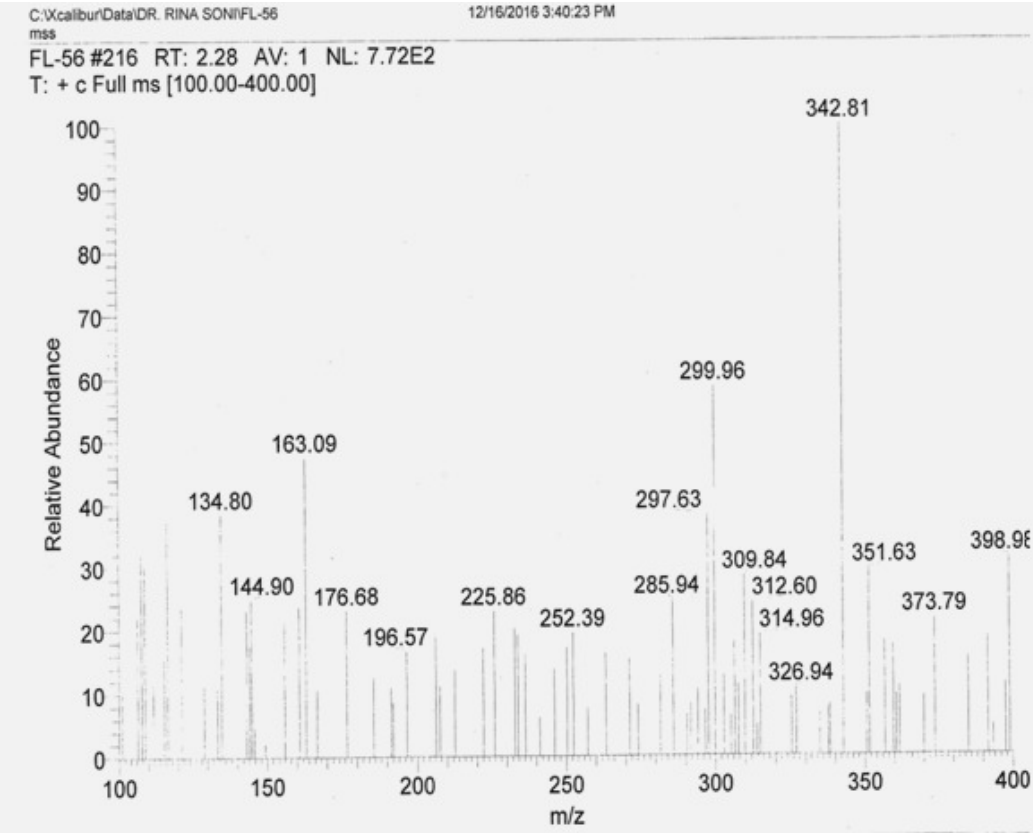
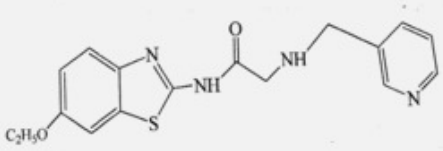


Figure-37 IR Spectrum of N-(6-Ethoxy benzo[d]thiazol-2-((Pyridin-3yl methyl) amino) acetamide i.e **3f**



FL-56#216 RT: 2.28
T: + c Full ms [100.00-400.00]

m/z	Intensity	Relative
106.35	169.0	21.89
107.70	250.0	32.38
108.76	235.0	30.44
116.57	287.0	37.18
121.22	181.0	23.45
134.80	296.0	38.34
143.29	178.0	23.06
144.90	191.0	24.74
160.84	182.0	23.58
163.09	366.0	47.41
176.68	177.0	22.93
225.86	176.0	22.80
285.94	189.0	24.48
297.63	294.0	38.08
299.96	452.0	58.55
309.84	219.0	28.37
312.60	186.0	24.09
342.81	772.0	100.00
351.63	227.0	29.40
398.98	240.0	31.09



MF=$C_{17}H_{18}N_4O_2S$
MW = 342g/mol

Figure-38 Mass Spectrum of N-(6-Ethoxy benzo[d]thiazol-2-((Pyridin-3yl methyl) amino) acetamide i.e **3f**

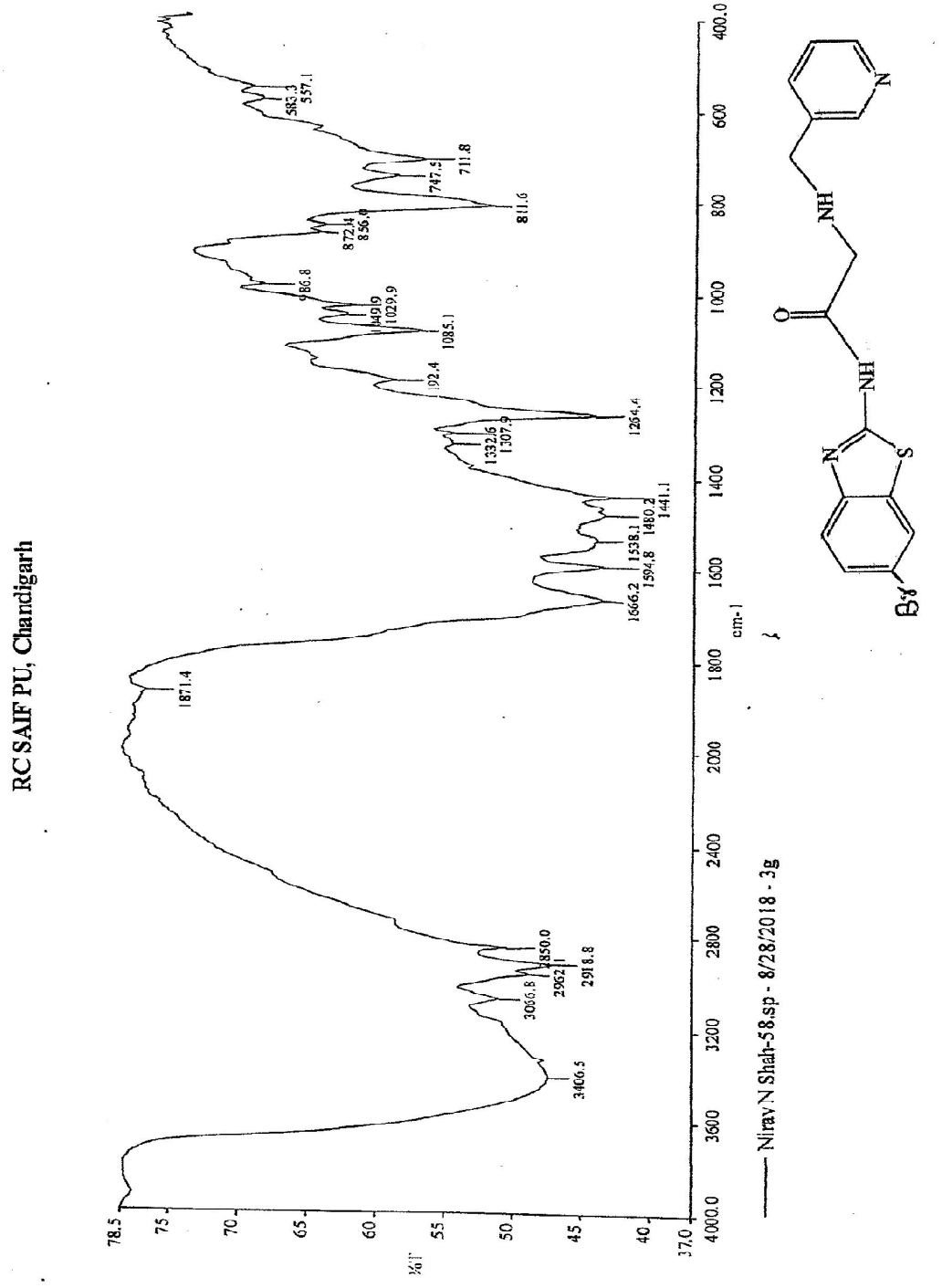


Figure- 39 IR Spectrum of N-(6-Bromo benzo[d]thiazol-2-((Pyridin-3yl methyl) amino) acetamide i.e **3g**

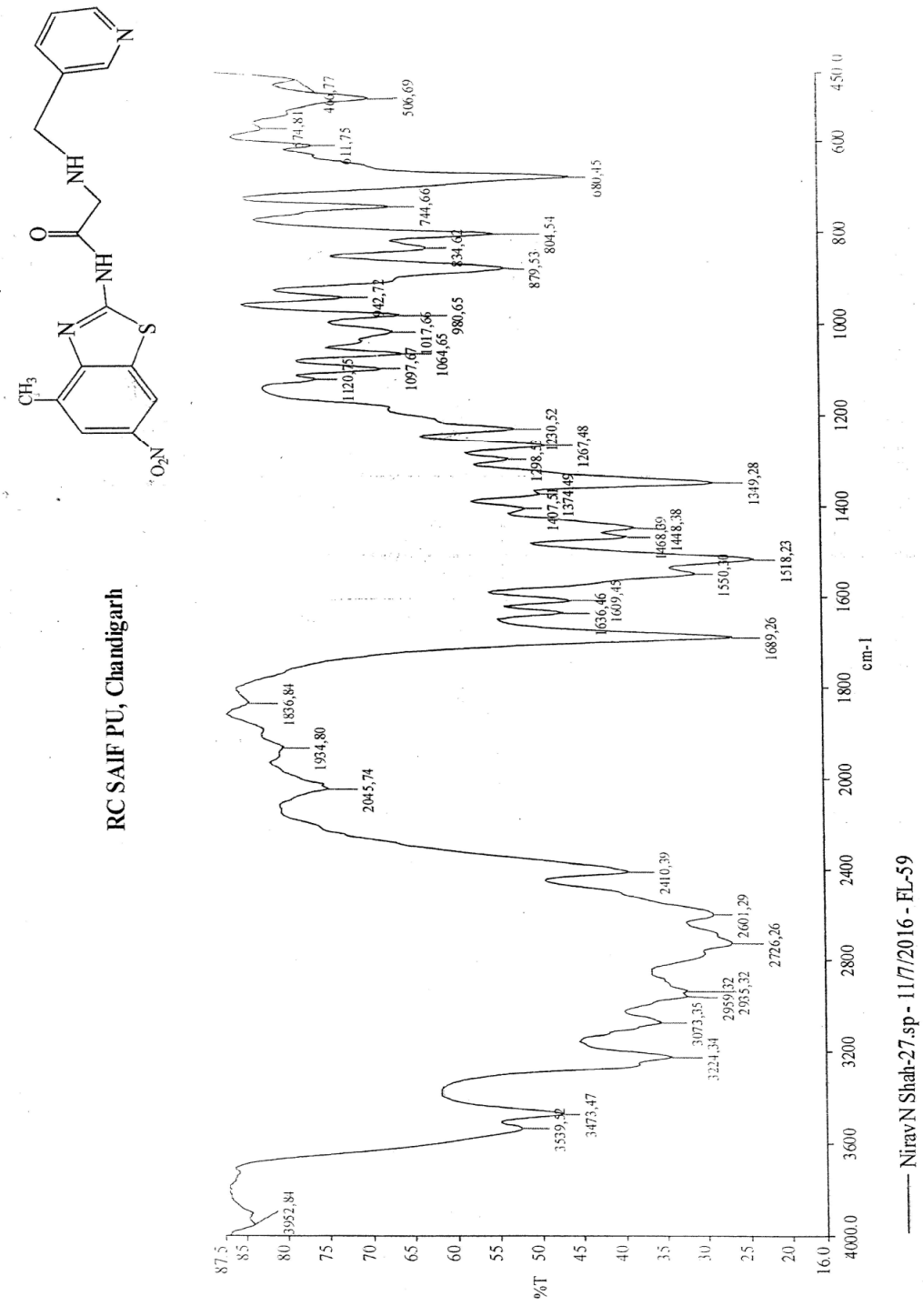


Figure- 40 IR Spectrum of N-(4-methyl -6-Nitro benzo[d]thiazol-2-((Pyridin-3yl methyl) amino) acetamide i.e. **3h**

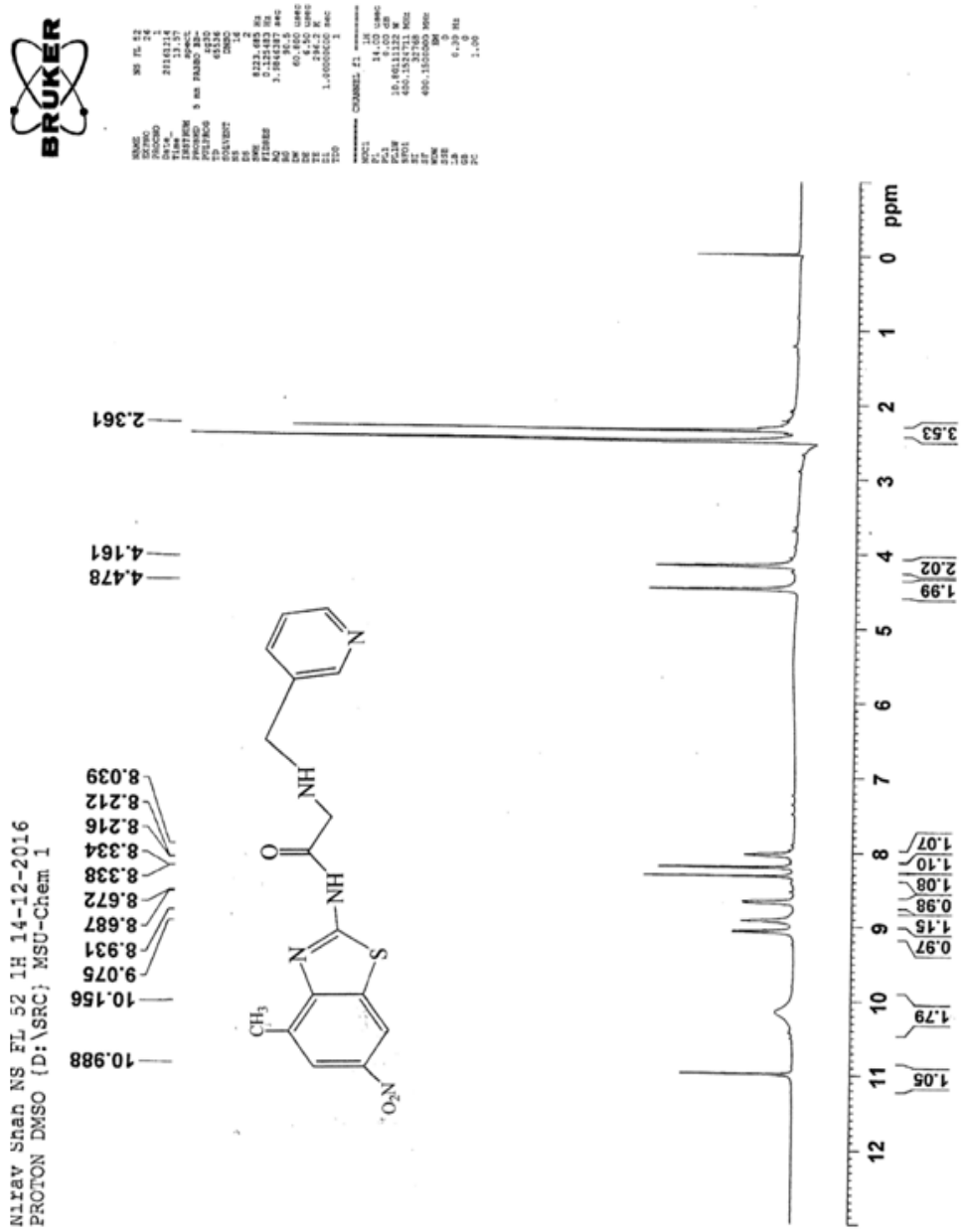


Figure-41 ^{1}H NMR Spectrum of N-(4-methyl-6-Nitro benzo[d]thiazol-2-((Pyridin-3yl methyl) amino) acetamide i.e. **3h.**

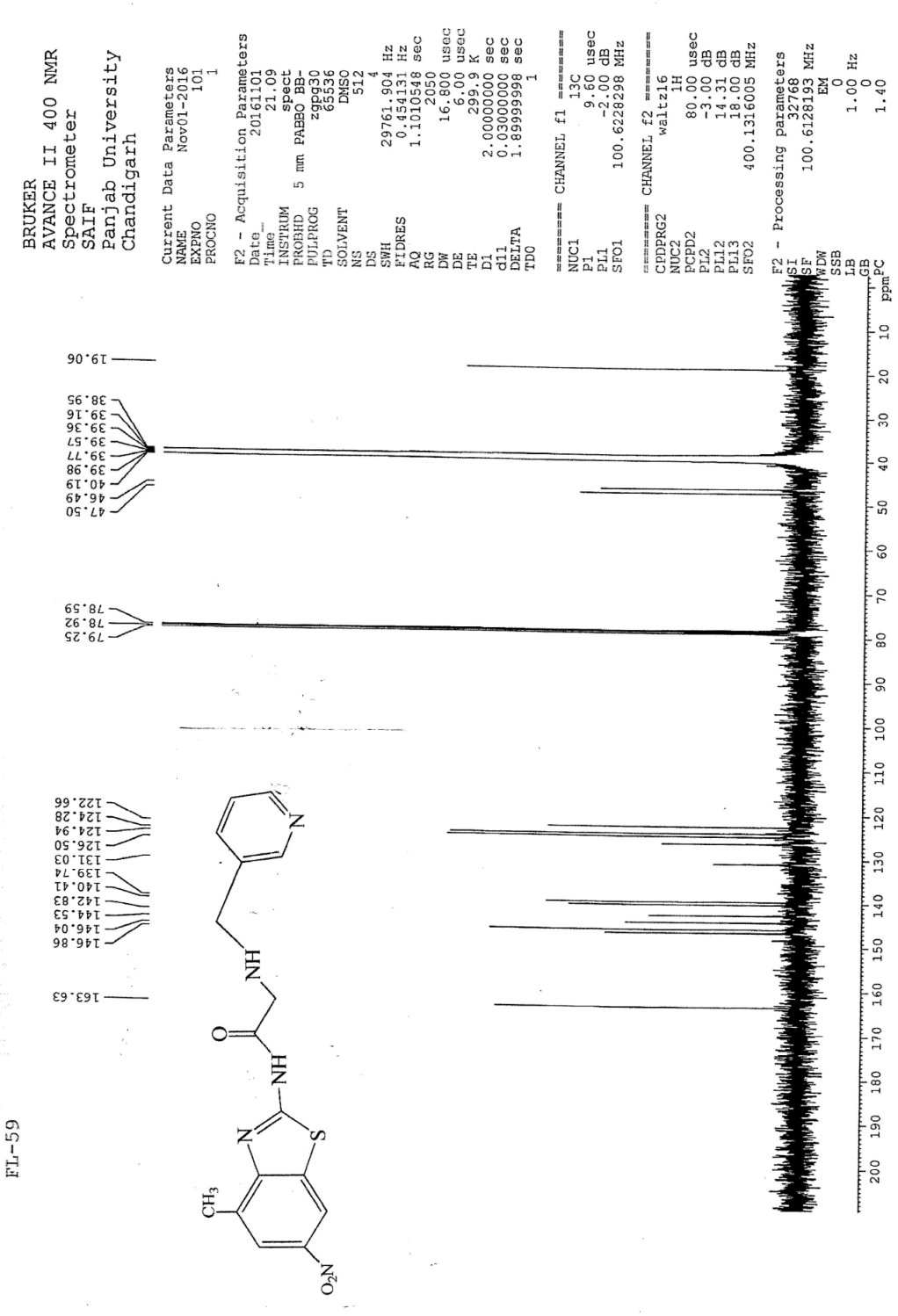
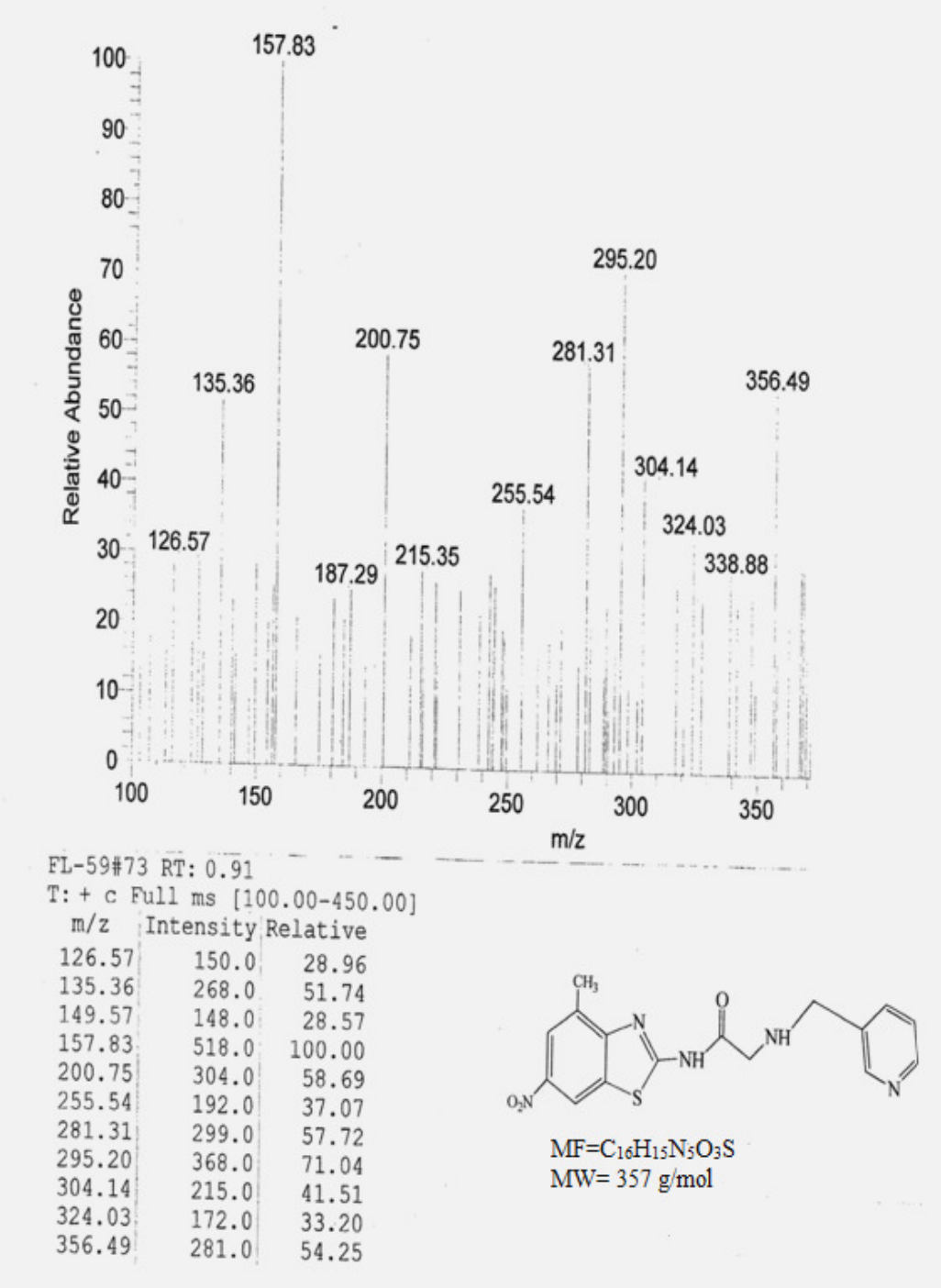


Figure-42 ^{13}C NMR Spectrum of N-(4-methyl -6-Nitro benzo[d]thiazol-2-((Pyridin-3yl methyl) amino) acetamide i.e. **3h**



FL-59#73 RT: 0.91
T: + c Full ms [100.00-450.00]

m/z	Intensity	Relative
126.57	150.0	28.96
135.36	268.0	51.74
149.57	148.0	28.57
157.83	518.0	100.00
200.75	304.0	58.69
255.54	192.0	37.07
281.31	299.0	57.72
295.20	368.0	71.04
304.14	215.0	41.51
324.03	172.0	33.20
356.49	281.0	54.25

MF=$C_{16}H_{15}N_5O_3S$
MW= 357 g/mol

Figure-43 Mass Spectrum of N-(4-methyl-6-Nitro benzo[d]thiazol-2-((Pyridin-3yl methyl) amino) acetamide i.e **3h**

5.2.2 Biological evaluation

5.2.2.1 Antibacterial and Antifungal evaluation

All the synthesized compounds were tested for their antibacterial activity against Gram negative (*Escherichia coli*, *Pseudomonas aeruginosa*) and Gram positive (*Staphylococcus aureus, Bacillus Subtilis*) bacteria by serial dilution agar diffusion method. The antifungal activity was tested against *Candida albicans.* Concentration of compounds was ranging from 10µg to 300µg.The lowest concentration of compounds that prevented visible growth is given in **Table-1**. Ciprofloxacin and Flucanazole were used as standard control drug.

Table-1

Minimum Inhibitory Concentration (MIC) of antibacterial and antifungal agent (µg/mL)

Compound	-R	*S.a.*	*B.s.*	*E.Coli*	*P.a.*	*C.a.*
3a	H	150	300	>300	>300	>300
3b	4-CH_3	**80**	300	>300	>300	>300
3c	6-Cl	**80**	150	>300	>300	>300
3d	4-Cl	>300	>300	>300	>300	>300
3e	6-F	**40**	**40**	>300	>300	**10**
3f	6-C_2H_5	**80**	150	>300	>300	300
3g	6-Br	**80**	300	>300	>300	300
3h	4-CH_3-6-NO_2	300	>300	>300	>300	>300
Ciprofloxacin	-	10	10	15	10	-
Flucanazole	-	-	-	-	-	10

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

S.a = Staphylococcus aureus,(Gram +ve);B.s= Bacillus subtilis,(Gram +ve);E.c= Escherichia coli,(Gram-ve) ;P.a = pseudomonas aeruginosa,(Gram-ve) Ca=Candida albicans(fungi) ; Ciprofloxacin and Flucanazole were used as standard control drugs.

Zone of inhibition against Gram +ve bacteria and Fungi in Compound **3e** and **3c**

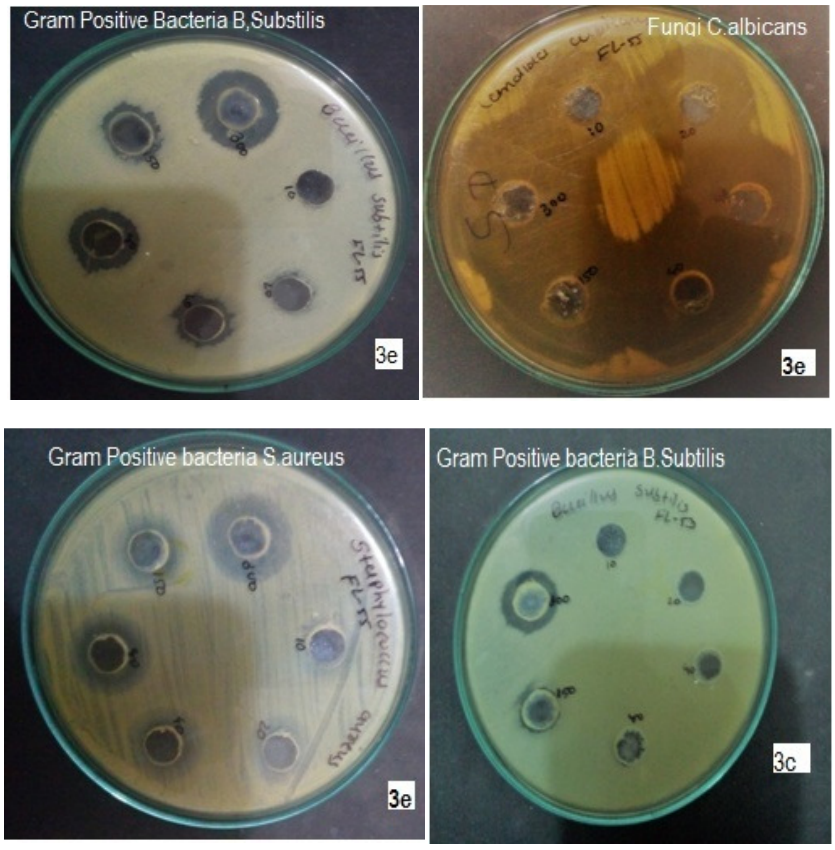


Figure**-44**

Compounds **3(a-h)** were screened for their antimicrobial and antifungal activity. Compounds **3b, 3c, 3f** and **3g** showed moderate activity against tested gram +ve bacteria *Staphylococcus aureus* at MIC 80µg while Compound **3e** showed comparatively good activity at 40µg. Rest of the compounds remain inactive against tested Gram positive bacteria *Staphylococcus aureus.* Only one compound i.e compound **3e** showed good to moderate activity against testes *Gram positive* bacteria *Bacillus subtilis*. All the other compounds remain inactive against tested *Gram positive* bacteria *Bacillus subtilis*. All the compounds were screened for antimicrobial activity against *Gram negative* bacteria i.e against *Escherichia coli, pseudomonas aeruginosa*; only one compound-**3e** showed moderate activity against *E.Coli* with MIC 40µg rest of the compounds remain inactive against both the Gram negative bacteria. All the synthesized compounds were also

screened for antifungal activity against one fungi *Candida albicans* and only one compound -**3e** showed excellent activity near to the standard drug with MIC 10µg while rest of the compounds remain inactive against tested fungi.

5.2.2.2 Anticancer evaluation

MTT assay

Some of the synthesized compounds were tested for their cytotoxic potential on four of the cancer cell lines, *viz.,* A 549 (lung cancer cell line) and three leukaemia cancer cell lines namely 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 selected compound on the proliferation of cancer cell. For the test, five different concentrations were prepared 50µg/mL, 75µg/mL, 100µg/mL, 150µg/mL and 200 µg/mL respectively in the triplicate of the four selected compound **3a**, **3c**, **3d** and **3h** using serial dilution method as shown in Figure- 45 (page No-210). The percentage inhibition was evaluated for all the tested compounds as shown in Table-2(page No-210). All the four compounds showed very enhancing and excellent anticancer activity. Compound **3a** showed very good percentage inhibition against A549 cell line with 98.81% at 50 µg/mL concentration. Compound **3c** showed almost 99.5% inhibition at 50 µg/mL concentration. Similarly compounds **3d** and **3h** also showed excellent percentage inhibition against A549 cell line with almost 99%.It was also observed that not only at 50µg/mL but also from the range of 50µg/ml to 200µg/mL concentration , all the compounds under investigation for anticancer activity showed very good to excellent percentage inhibition ranging from 94 to 99%. It may also concluded that all the compounds under investigation if further diluted and if tested for their nM concentration for the anticancer activity, then they may show very enhancing results as compared to the results found at µg/mL concentrations.

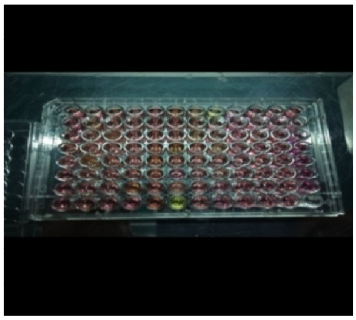


Figure-45: Serial Dilution in MTT assay for the tested compounds against A549 cell line**.**

Sr. No	Compounds	-R,R'	Percentage inhibition against of A549 at five different Concentrations (µg/mL)				
			50 µg/mL	**75** µg/mL	**100** µg/mL	**150** µg/mL	**200** µg/mL
1	**3a**	-H	98.81%	99.04%	99.34%	99.16%	97.74%
2	**3c**	6-Cl	99.46%	96.37%	90.73%	95.30%	98.57%
3	**3d**	4-Cl	97.98%	98.75%	97.74%	93.94%	98.40%
4	**3h**	4-CH_3-6NO_2	98.99%	91.15%	95.90%	98.21%	98.51 %

Table-2: Describing the percentage inhibition through MTT assay of the four tested compounds at five different concentrations µg/mL against lung cancer cell line A549.

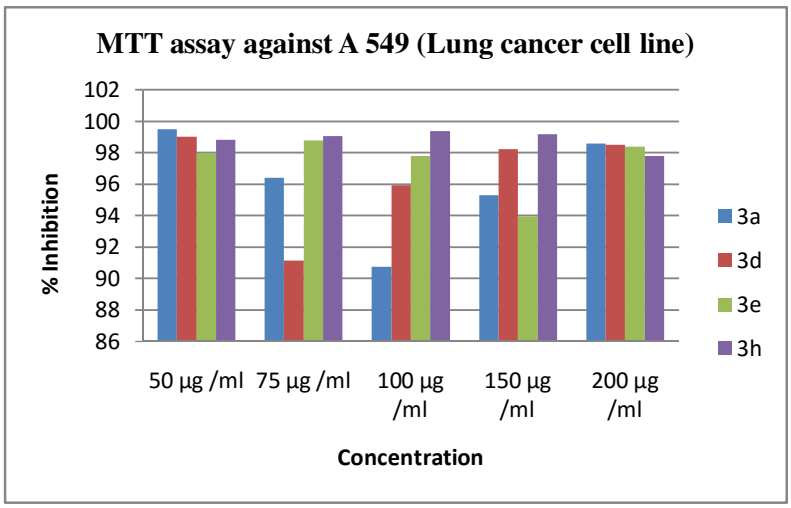


Figure**-46** Bar Chart representation of MMT assays against A 549 cancer Cell line.

Among the synthesized compounds 3(a-h) some of the compounds namely **3a**, **3c**,**3d** and **3h** were screened for their efficacy as anticancer agent against three leukaemia cancer cell lines namely K562 (Human Chronic Myelogenous leukaemia cell line), KG1 (Human acute Myeloid Leukaemia cells) and MOLT-3 (Human Acute Lymphoblastic Leukaemia cell line).

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

No.	**Compounds**	**-R**	**Cancer cell lines**		
			K 562	MOLT 3	KG 1
			IC_{50}(μM)	IC_{50}(μM)	IC_{50}(μM)
01	3a	-H	284.3	**0.73**	53.67
02	3c	6-Cl	244.2	67.68	**2.34**
03	3d	4-Cl	225.2	8.82	**0.50**
04	3h	4-CH_3-6-NO_2	225.0	96.35	20.36

Table-**3** Anticancer activity of compound**s 3a, 3c, 3d and 3h** 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-**47** summarizes the anticancer effect of newly synthesised amide derivatives 3a, 3c, 3d and 3h on leukemic cancer cell lines K562, KG 1 and MOLT 3 and showed cell growth and % cell viability.

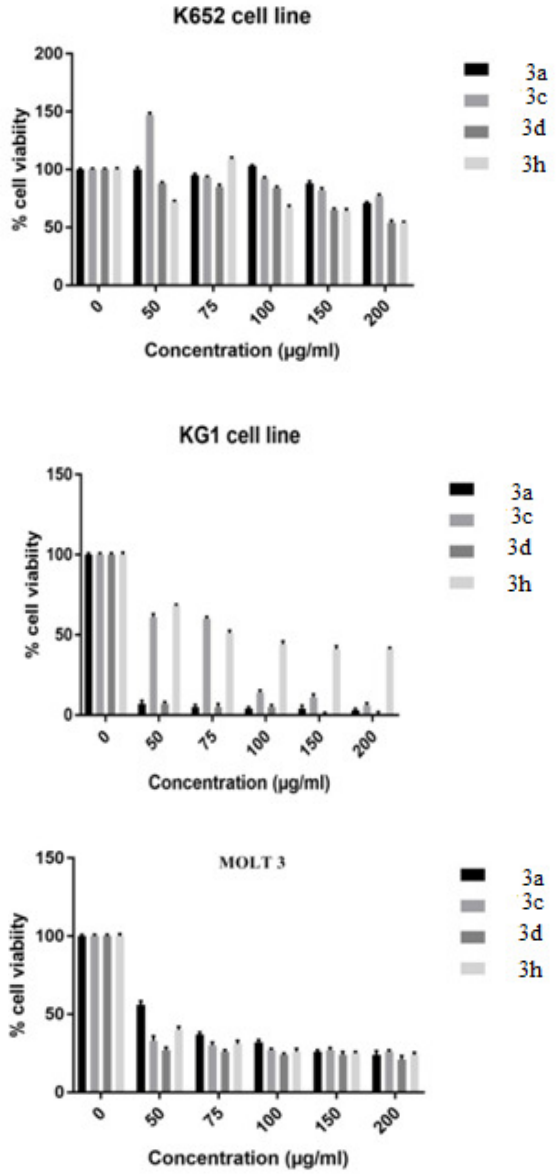


Figure-47: Effect of compounds **3a**, **3c**, **3d** and **3h** on K562, KG 1 and MOLT 3 cell growth and determining % cell viability. Cells were cultured and treated with DMSO and 5-Flouro Uracil and % viability was determined by MTT assay test, Experiments were conducted in triplicate and repeated thrice. The value represents the mean ±SD.**5.3**

5.3 Experimental

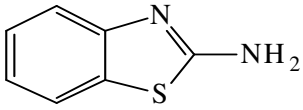
Reagent grade chemicals and solvents were purchased from commercial supplier and used after purification. TLC was performed on silica gel F254 plates (Merck). Acme's silica gel (60-120 mesh) was used for column chromatographic purification. All reactions were carried out in nitrogen atmosphere. Melting 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 Thermosinnigan Flash 11-12 series EA. All reactions were carried out under nitrogen atmosphere.

5.3.1 Chemistry

5.3.1.1 General Synthesis of substituted 1, 3 -benzothiazol-2-amine 1(a-h) [13]

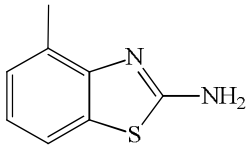
To a mixture of chilled acetic acid, 50 mL and KSCN (21.9 mmole) and various substituted anilines (7.30 mmoles) were added and freezed the mixture. The solution was stirred mechanically with drop wise addition of Br_2 in glacial acetic acid at such a rate that temperature does not rise above 5°C.The stirring was continued for an additional 3hr at 0-10°C and separated hydrochloride salt was filtered, washed with acetic acid and dried. It was dissolved in hot water and neutralized with aqueous ammonia solution (25%).The resulting precipitates filtered ,washed with water and recrystallized from ethanol to obtain pure substituted 1,3-benzothiazol-2-amine.**(1a-h).**

5.3.1.1.1 Benzo[*d*] thiazol-2-amine 1a.[14]



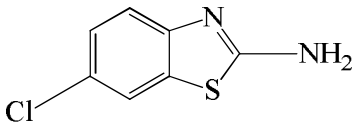
Yield 68%, mp:125-127°C(lit. mp.:126-128°C) IR (KBr cm^{-1}) :3398, 3273, 3058, 2731, 1643, 1531, 1448, 1313, 1108, 887, 741, 720 cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 6.97-7.01 (m, 1H), 7.17-7.21 (m, 1H), 7.34-7.36 (d, *J*=8 Hz, 1H), 7.42 (s, 2H), 7.58-7.60 (d, 7.9Hz, 1H); ^{13}CNMR (DMSO-d_6): δ 117.68, 120.56, 120.68, 125.22, 130.91, 152.69, 166.48 Elemental analysis: (Anal: Cal).For $C_7H_6N_2OS$: (C, H, N) C 55.99; H 4.00: N 18.66., C 55.80; H 3.94 : N 18.34 M.W=150 g/mol.

5.3.1.1.2 4-methyl benzo[*d*] thiazol-2-amine 1b.



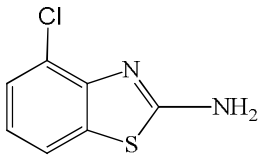
Yield: 61% mp:135-137°C; (lit.mp.:137-139°C),IR (KBr): 3433, 3285, 3067, 1635, 1528, 1457, 1414, 1274, 1109, 939, 890, 808, 760, 692 cm^{-1}; ^{1}H NMR: (DMSO-d_6): δ 2.42 (s, 3H), 6.88-6.92 (t, *J*= 7.64, 7.56 Hz, 1H), 7.00-7.02 (d, *J*= 7.36Hz, 1H), 7.40-7.42(d , *J*=7.64Hz,1H), 7.45(s,2H). ^{13}C NMR (DMSO-d_6): δ: 15.87, 118.03 120.60, 126.02, 126.96, 130.34, 151.51, 165.78. Elemental analysis :(Anal: Cal). For $C_8H_8N_2S$: (C, H, N) C 58.54: H 4.87: N 17.07, C 57.52: H 4.76: N 17.78.M.W=164 g/mol.

5.3.1.1.3 6-chloro benzo[*d*] thiazol-2-amine. 1c[15]



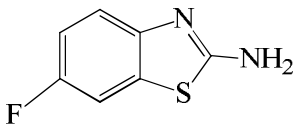
Yield : 66%, mp:198-200°C;(lit.mp.:199-201°C) IR KBr): 3458, 3273, 3098, 2731, 1878, 1719, 1633, 1534, 1447, 1307, 1108, 1049, 939, 891, 860, 812, 764, 701, 646, 597, 563.^{1}H NMR(DMSO-d_6): δ 7.17-7.20(dd, *J*= 2.2, 8.56Hz, 1H), 7.29-7.32 (d, *J*= 8.52 Hz,1H), 7.55(s, 2H), 7.67-7.69 (d,1H),^{13}C NMR (DMSO-d_6) δ: 118.42, 120.21, 124.64, 125.37, 132.50, 151.58, 167.07 Elemental analysis :(Anal. Cal). For $C_7H_5ClN_2S$: (C, H, N) C 45.49; H 2.71: N 15.16, C45.47; H 2.69: N 15.03.M.W=184.65 g/mol.

5.3.1.1.4 4-chloro benzo[*d*] thiazol-2-amine. 1d



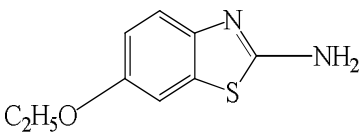
Yield: 72%, mp: 203-205°C, (lit.mp.203-205°C) IR (KBr):3469, 3277, 3064, 2722, 1644, 1541, 1454, 1416, 1305, 1276, 1110, 1058, 883, 726, 686. ^{1}HNMR: δ (DMSO-d_6): 6.96 7.00 (t, *J* = 7.92Hz, 1H) ,7.25-7.27 (dd, *J*=1Hz, 9.6Hz, 1H), 7.56-7.59 (dd, *J*= 7Hz 9.6Hz, 1H)7.88 (s, 2H), ^{13}C NMR (DMSO-d_6) δ: 119.52, 121.30, 121.36, 125.41, 132.08, 149.37, 167.37, Elemental analysis (Anal: Cal). For $C_7H_5ClN_2S$: (C, H, N) C 45.49; H 2.71: N 15.16: C 45.58; H 2.70: N 14.91.M.W=184 .65g/mol.

5.3.1.1.5 6-flouro benzo[*d*] thiazol-2-amine. 1e



Yield : 77%, mp:182-184°,(lit.mp.183-185°C) IR(KBr): 3387, 3270, 3085, 2926, 1639, 1541, 1464, 1344, 1314, 1259, 1193, 1114, 1048, 922, 848, 810, 709, 608 cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 6.95-7.01 (m, 1H),7.27-7.31(dd, *J* = 4.89, 8.76 Hz, 1H), 7.36 (s, 2H),7.44-7.46(dd, *J* = 2.72 ,8.64Hz, 1H), ^{13}C NMR (DMSO-d_6): δ 107.27, 112.92, 118, 131.87, 149.29, 155.94, 166.24. Elemental analysis :(Anal: Cal). For $C_7H_5FN_2S$: (C, H, N) C 50.01; H 2.97: N 16.65, C 49.64; H 2.98: N 15.94.M.W= 168.20 g/mol.

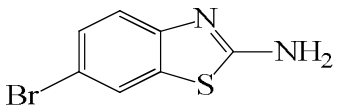
5.3.1.1.6 6-ethoxy benzo[*d*] thiazol-2-amine. 1f



Yield: 81%, Colourless crystals, mp: 164-166°C, (lit.mp.163-165°C), IR (KBr):3434, 3287, 3064, 2976, 2962, 2727, 1637, 1540, 1459, 1274, 1111, 1061, 938, 894, 808. cm^{-1}.^{1}H NMR (DMSO-d_6): δ 1.31-1.35(t, *J*= 7.2 Hz, 3H), 3.95-4.00 (q, 2H), 6.77-6.79 (dd, *J* = 2.60, 8.68 Hz,1H) 7.16(s, 2H), 7.18-7.19(d, *J* = 2.56 Hz, 1H), 7.22-7.25 (d, *J*= 4.64 Hz,1H) ^{13}C NMR ($CDCl_3$+DMSO-d_6): δ :14.68, 63.48, 105.95, 113.24,

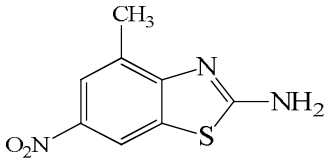
117.99, 131.84, 146.67, 153.48, 164.75 Elemental analysis :(Anal: Cal). For $C_9H_{10}N_2OS$: (C, H, N) C 55.67; H 5.15: N 14.43, C 55.60; H 5.17: N 14.39. M.W=194 g/mol.

5.3.1.1.7 6-bromo benzo[*d*] thiazol-2-amine. 1g[16]



Yield: 74%, colourless solid, mp: 215-217 °C, (lit.mp.213 - 215°C) IR, (KBr): 3453, 3273, 3097, 2729, 1632, 1529, 1443, 1306, 1278, 1109, 862, 811, 746, 695, 648, 598. cm^{-1}. ^1H NMR (DMSO-d_6): δ 7.24-7.26(d, *J* = 8.48 Hz, 1H), 7.30-7.33(dd, *J* = 2.12, 8.52 Hz, 1H), 7.58(s, 2H), 7.81-7.82 (d, *J*=20Hz, 1H) ^{13}C NMR (DMSO-d_6): δ 112.08, 118.97, 123.01, 128.13, 133.04, 151.96, and 167.08. Elemental analysis :(Anal: Cal). For $C_7H_5BrN_2S$: (C, H, N) C 36.53; H: 2.17 N 12.18., C 36.49; H 2.15: N 12.15.M.W=229.94 g/mol.

5.3.1.1.8 4- Methyl -6-Nitro benzo[*d*] thiazol-2-amine. 1h



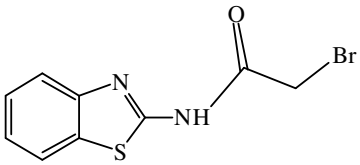
Yield 59 %, mp: 155-157 °C: IR (KBr, cm^{-1}): 3460, 3268, 2725, 1872, 1729, 1626, 1541, 1444, 1302, 1278, 1110, 863, 810, 747, 694, 651, 600; ^1H NMR (400 MHz, DMSO-d_6) δ : 2.44 (s,3H), 4.20 (s,2H), 6.48 (s,1H), 7.22 (d,1H, *J*=8 Hz), 7.63 (d,1H, *J* = 8Hz) ,7.75 (s,1H) ^{13}C NMR (75 MHz, DMSO-d_6) δ : 16.03, 117.24, 121.09, 125.48'/, 132.10, 144.54, 153.06, 166.98. Elemental analysis For $C_8H_7N_3OS$: (Anal: Cal), (C, H, N) C 45.88; H 3.34; N 20.07 , C 45.76; H 3.30:N 19.98 .Molecular Weight:209.22 g/mol.

5.3.1.2 General method of synthesis of N-(benzo[d]thiazol-2-yl)-2-bromo acetamide 2(a-h)

Bromo acetyl bromide (1.2mmol) was added dropwise to a mixture of 2-aminobenzothiazole derivatives **1(a-i)** (1mmole, 1 eq.) and triethylamine (1.2 mmol, 2.1 eq.) in dichloromethane (DCM) (15ml) at 0-5°C for 30 minutes and then at room temperature with constant stirring. The stirring was continued up to 8-10 hrs. The completion of reaction was checked by TLC and then the reaction mixture washed with

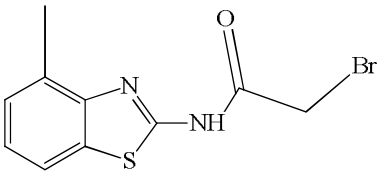
excess of water, separated in separating funnel ,dried over sodium sulphate, evaporated the solvent under vacuum, and the solid thus obtained was crystallized from ethanol.

5.3.1.2.1 N-(benzo[d]thiazol-2-yl)-2-bromoacetamide 2a.



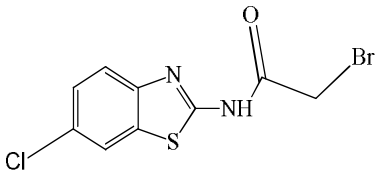
Yield 74%, colourless needles, mp: 175-177 °C IR (KBr): 3375, 3124, 2953, 2856, 1691, 1645, 1595, 1560, 1441, 1390, 1309, 1266, 1166, 981, 864, 774, 752.cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 4.20 (s,2H), 7.28-7.32 (m,1H),7.41-7.46 (m,1H), 7.73-7.95(m,1H),7.96-7.98(m,1H) ^{13}C NMR (DMSO-d_6):δ 61.36, 120.47, 121.69, 123.58, 126.12, 131.34, 148.30, 157.47, 172.07.Elemental analysis: (Anal: Cal) For $C_9H_7BrN_2OS$: (C, H, N) C 39.85; H 2.58: N 10.33., C 39.76; H 2.63: N 10.40.M.W=271 g/mol.

5.3.1.2.2 N-(4-methyl benzo[d]thiazol-2-yl)-2-bromoacetamide 2b.



Yield 77 %, colourless needles, mp: 155-157 °C: IR (KBr, cm^{-1}): 3172, 2982, 1661, 1594, 1562, 1438, 1313, 1249, 1110, 1003, 886, 860, 808, 763,= ^{1}H NMR (400 MHz, DMSO-d_6) δ : 2.53 (s,3H), 4.79 (s,2H), 7.16-7.23(m,2H), 7.74 (d,1H,*J* = 8Hz) ,12.83 (s,1H) ^{13}C NMR (75 MHz, DMSO-d_6) δ : 17.86, 28.36, 118.96, 123.64, 126.58, 129.99, 131.14, 147.50, 156.63, 165.80. Elemental analysis For $C_{10}H_6BrN_2OS$: (Anal: Cal), (C, H, N) C 42.12; H 3.18; N 9.12, C 42.10; H 3.16: N 9.14. M.W=285 g/mol.

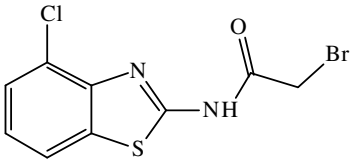
5.3.1.2.3 N-(6-Chloro benzo[d]thiazol-2-yl)-2-bromoacetamide 2c.



Yield 68 %, buff coloured solid , mp: 155-157 °C: IR (KBr, cm^{-1}): 3184, 3064, 3009, 1668, 1601, 1560, 1444, 1317, 1275, 1113, 999, 855, 813, 763; ^{1}H NMR (400 MHz, DMSO-d_6) δ : 4.21 (s,2H),7.39-7.69 (m, 1H), 7.71-7.91 (d,1H,*J* = 8Hz) ,8.03-8.05 d(*J* = 8Hz), 12.01(s, 1H).^{13}C NMR (75 MHz, DMSO-d_6) δ :61.22, 121.09, 121.51,

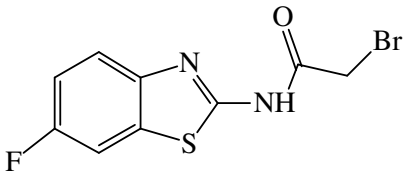
126.22, 127.68, 133.08, 147.21, 158.14.,172.08 Elemental analysis For $C_{10}H_9BrClN_2OS$: (Anal: Cal), (C, H, N) C 39.27; H 2.95; N 9.16, C 39.25; H 2.94: N 9.14. calculated for M.W= 305.58 g/mol.

5.3.1.2.4 N-(4-Chloro benzo[d]thiazol-2-yl)-2-bromoacetamide 2d.



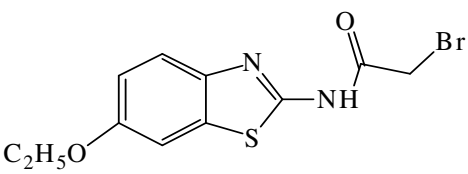
Yield 68 %, buff coloured solid , mp: 142-144°C: IR (KBr, cm^{-1}):3445, 2974, 1663, 1594, 1563, 1408, 1319, 1103, 864, 820, 777, 764, 738; ^1H NMR (400 MHz, DMSO-d_6) δ : 4.22 (s,2H), 6.47 (s,1H), 7.29-7.33(1H, t, *J* =7.6Hz,8.0Hz), 7.52-7.54(d, 1H, *J* =8.0Hz) 7.97-7.99(d,1H,*J* = 8.0Hz),13.21 (s,1H,),^{13}C NMR (75 MHz, DMSO-d_6) δ: 28.19, 121.68, 124.49, 124.65, 126.17, 133.06, 145.36, 158.57, 166.12.Elemental analysis For $C_9H_7BrN_2OSCl$: (found: Cal), (C, H, N) C 39.27; H 2.95; N 9.16, C 39.28; H 2.92: N 9.13 . M.W= 305.58 g/mol

5.3.1.2.5 N-(6-Flouro benzo[d]thiazol-2-yl)-2-bromoacetamide 2e.



Yield 64 %, buff coloured solid , mp: 197-199 °C: IR (KBr, cm^{-1}):3188, 3072, 2991, 2724, 1703, 1665, 1611, 1574, 1461, 1412, 1323, 1251, 1193, 1160, 1046, 986, 885, 757; ^1H NMR (400 MHz, DMSO-d_6) δ : 4.22 (s,2H), 7.27-7.32(m, 1H,),7.75-7.78(m,1H),7.89-7.92(m,1H),12.63(s,1H) ^{13}C NMR (75 MHz, DMSO-d_6) δ : 61.14, 108.25, 114.28, 121.63, 132.70, 145.14, 157.48, 159.79, 172.16. Elemental analysis For $C_9H_6BrN_2OSF$: (Found: Cal), (C, H, N) C 37.35; H 2.07; N 9.68, C 37.30; H 2.04: N 9.59 EI MS (m/z):289 (m^+).M.W= 289.12 g/mol.

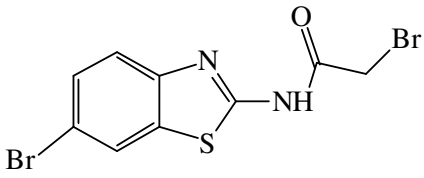
5.3.1.2.6 N-(6-ethoxy benzo[d]thiazol-2-yl)-2-bromoacetamide 2f.



Yield 64 %, buff coloured solid, mp: 202-204 °C: IR (KBr, cm^{-1}):3207, 3098, 3027, 2978, 1665, 1605, 1577, 1459, 1308, 1250, 1220,

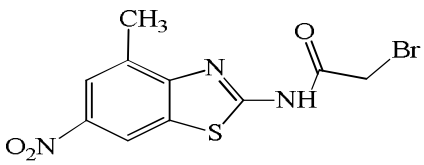
1114, 1059, 998, 941, 822, 729.; ^{1}H NMR (400 MHz, DMSO-d_6) δ :1.32-1.35(t, 3H), 4.04-4.06 (q, 2H), 4.19(s,2H), 7.01-7.03(d, *J* = 8.8 Hz 1H) , 7.56(s,1H),7.63-7.65(d, *J*= 8.8Hz, 1H),12.70(s,1H).^{13}C NMR (75 MHz, DMSO-d_6) δ: 14.65, 61.13, 63.56, 115.91, 121.81, 133.25, 142.86, 155.88, 166.21, 168.99, 172.25. Elemental analysis For $C_{11}H_{11}BrN_2O_2S$: (Anal: Cal), (C, H, N) C 41.88; H 3.49; N 8.88, C 41.97; H 3.47: N 8.8 .M.W= 315.18g/mol.

5.3.1.2.7 N-(6-bromo benzo[d]thiazol-2-yl)-2-bromoacetamide 2g.



Yield 64 %, buff coloured solid , mp: 189-191 °C: IR (KBr, cm^{-1}):3181, 3082, 3009, 1669, 1598, 1560, 1441, 1316, 1279, 1196, 1111, 1082, 999, 855, 811, 763, 742, 679.;^{1}H NMR (400 MHz, DMSO-d_6) δ : 4.22 (s,2H),7.57-7.59(m,1H),7.69-7.71(d,1H, *J*= 8.8 Hz),8.27-8.28 (d,1H,*J* = 2.0 Hz),12.88 (s,1H).^{13}C NMR (75 MHz, DMSO-d_6) δ 29.00, 116.24, 122.83, 124.86, 129.77, 134.10, 148.07, 158.90,168.98, 172.81.:Elemental analysis For $C_9H_6Br_2N_2OSF$: (Anal: Cal), (C, H, N) C 27.99; H 1.55; N 7.26, C 27.93; H 1.51: N 7.28 .M.W= 385.80 g/mol.

5.3.1.2.8 N-(4-Methyl-6-Nitro benzo[d]thiazol-2-yl)-2-bromoacetamide 2h.

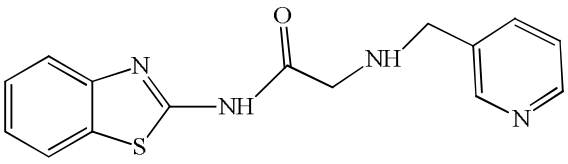


Yield 64 %, buff coloured solid , mp: 165-167 °C: IR (KBr, cm^{-1}):3185, 3098, 2951, 1704, 1614, 1571, 1525, 1340, 1276, 1185, 1127, 1046, 893, 831, 748, 720. ; ^{1}H NMR (400 MHz, DMSO-d_6) δ : 4.22 (s,2H),7.57-7.59 (m,1H),7.69-7.71(d,1H, *J*= 8.8 Hz),8.27-8.28 (d,1H,*J* = 2.0 Hz), 12.88 (s,1H).^{13}C NMR (75 MHz, DMSO-d_6) δ: 29.85, 116.11, 122.39 , 129.94, 134.58, 148.85, 158.66, 166.21, 172.77 Elemental analysis For $C_{10}H_8BrN_3O_3S$: (Anal: Cal), (C, H, N) C 36.34; H 2.42; N 12.72, C 36.30; H 2.45: N 12.74 . calculated for M.W=330.16 g/mol.

5.3.1.3 General method for the synthesis of N – (Benzo [*d*]thiazol-2-yl)-2-((pyridine-3-yl methyl) amino) acetamide derivatives 3 (a-h).

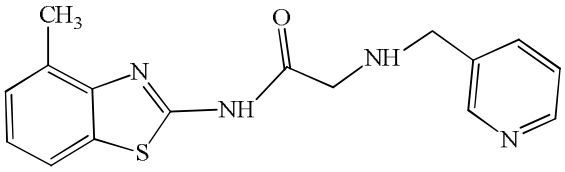
To a well stirred solution of compounds 2(a-h) in dimethylformamide (DMF) 15 mL, tri ethyl amine (TEA), (2.10 mmol, 1.01eq.) was added slowly and allowed to stir at 0-5°C for 30 minutes .To this 3-amino methyl pyridine (1.0eq.) was added slowly and the reaction mixture was stirred at room temperature for 12-14 hrs. The completion of the reaction was checked 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 water, dried over anhydrous sodium sulphate and concentrated under vacuum. The precipitates obtained were crystallized from ethanol to give 3(a-h) as light yellow solid.

5.3.1.3.1 N – (Benzo [*d*]thiazol-2-yl)-2-((pyridine-3-yl methyl) amino) acetamide. 3a



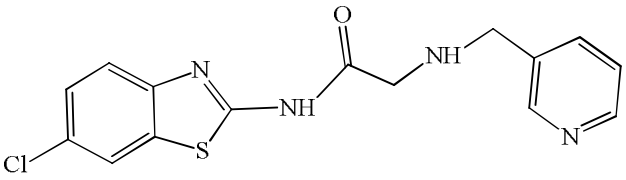
Yield 42 %, Light yellow coloured solid , mp: 95-97 °C: IR (KBr, cm^{-1}):3318, 3167, 3048, 2898, 1917, 1702, 1402, 1251,1144, 958, 863, 762, 747, 712, 665 ; ^{1}H NMR (400 MHz, DMSO-d_6) δ : 4.20(s,2H), 4.42(s, 2H), 6.99-7.18(m, 2H), 7.29-7.45(m, 2H), 7.70-7.76(m, 1H), 7.89-7.95(m, 1H), 8.38-8.50(dd, *J*=8.8Hz 1H), 8.74-8.97(d, *J*=8.8 Hz 1H). ^{13}C NMR (75 MHz, DMSO-d_6) δ : 45.39, 47.08, 121.61, 123.82, 124.80, 126.17, 129.04, 130.85, 131.34, 135.60, 142.11, 143.14, 148.17, 165.22, 172.48.Elemental analysis For $C_{15}H_{14}N_4OS$: (Anal: Cal), (C, H, N) C 60.40; H 4.69; N 18.79, C 60.42; H 4.73: N 18.74 .M.W=298 g/mol.

5.3.1.3.2 N – (4-Methyl benzo [*d*]thiazol-2-yl)-2-((pyridine-3-yl methyl) amino) acetamide. 3b



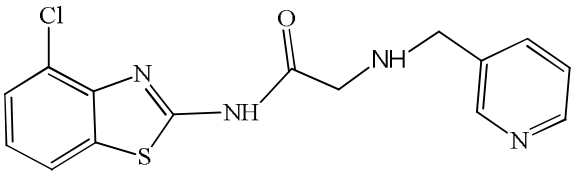
Yield 55 %, Light yellow coloured solid , mp: 146-148 °C: IR (KBr, cm^{-1}):3320, 3172, 3051, 2896, 2855, 2724, 1691, 1598, 1564, 1479, 1408, 1286, 1262, 1146, 960, 865, 767, 749, 713, 666; ^{1}H NMR (400 MHz, DMSO-d_6) δ : 2.40(s, 3H), 4.23(s, 2H), 4.55(s, 2H), 8.09(s,2H),8.18(s, 1H), 8.31(s, 1H), 8.79(s, 1H), 8.97(s, 1H), 9.17(s, 1H), 10.18(s, 1H), 10.96(s, 1H).^{13}C NMR (75 MHz, DMSO-d_6) δ : 19.06, 46.49, 47.50, 123.13, 124.94, 125.57, 126.74, 131.26, 140.26, 140.93, 143.96, 145.56, 146.54, 146.66, 164.26.Elemental analysis For $C_{16}H_{16}N_4OS$: (Anal: Cal), (C, H, N) C 61.54; H 5.13; N 17.95, C 61.58; H 5.09: N 17.99 M.W=312 g/mol.

5.3.1.3.3 N-(6-Chloro benzo [*d*]thiazol-2-yl)-2-((pyridine-3-yl methyl) amino) acetamide. 3c



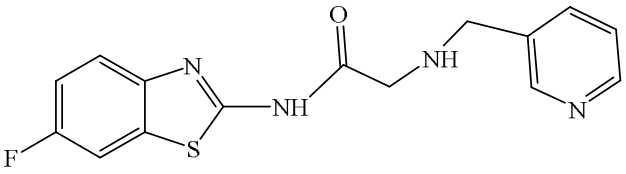
Yield 45 %, Light yellow coloured solid , mp: 176-178°C: IR (KBr, cm^{-1}):3265, 3193, 2923, 2851, 1740, 1663, 1597, 1536, 1446, 1269, 1056, 814, 765, 711 ; ^{1}H NMR (400 MHz, DMSO-d_6) δ:3.50(s,2H), 3.81(s, 2H), 7.32-7.40(m, 2H), 7.41-7.44(m, 1H), 7.68-7.78(m, 1H), 8.05(s,1H), 8.45(d, *J* =3.2 Hz, 1H), 8.55(s, 1H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 49.69, 51.02, 121.18, 123.21, 126.26, 127.62, 133.14, 135.35, 135.64, 147.97, 149.36, 147.34, 149.36, 158.35, 171.35.Elemental analysis For $C_{15}H_{13}N_4OClS$: (Anal: Cal), (C, H, N) C 61.54; H 5.13; N 17.95, C 61.58; H 5.09: N 17.99 .M.W=332 .81g/mol.

5.3.1.3.4 N-(4-Chloro benzo [*d*]thiazol-2-yl)-2-((pyridine-3-yl methyl) amino) acetamide. 3d



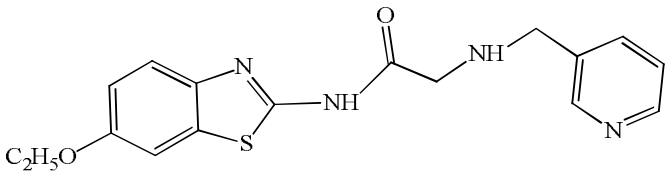
Yield 45 %, Light yellow coloured solid , mp: 176-178°C: IR (KBr,cm^{-1}) :3345, 3328, 3056, 2895, 2545, 1700, 1602, 1568, 1481, 1410, 1290, 1261, 1153, 1104, 960, 869, 819, 777, 738, 711, 694; ^{1}H NMR (400 MHz, DMSO-d_6) δ : 3.53(s, 2H), 3.80(s, 2H), 7.29-7.37(m, 2H), 7.52-7.54(m, 1H), 7.76-7.79(m, 1H).7.97-7.99(m, 1H), 8.45-8.46(d, *J*= 4Hz, 1H), 8.54(s, 1H).^{13}C NMR (75 MHz, DMSO-d_6) δ: 49.55, 51.00, 120.84, 123.39, 124.31, 124.35, 126.17, 133.04, 135.50, 135.72, 145.37, 148.04, 149.40, 158.61, 171.67. Elemental analysis For $C_{15}H_{13}N_4OClS$: (Anal: Cal), (C, H, N) C 61.54; H 5.13; N 17.95, C 61.51; H 5.10: N 17.92 . M.W=332 .81g/mol.

5.3.1.3.5 N-(6-Flouro benzo [*d*]thiazol-2-yl)-2-((pyridine-3-yl methyl) amino) acetamide. 3e



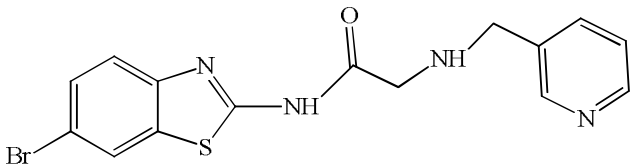
Yield 45 %, Light yellow coloured solid , mp: 168-171°C: IR (KBr,cm^{-1}) :3423, 3070, 2991, 2928, 1664, 1609, 1566, 1483, 1379, 1262, 1176, 1047, 810, 712; ^{1}H NMR (400 MHz, DMSO-d_6) δ : 3.96(s,2H), 4.03(s,2H), 6.86-6.92(m, 1H), 7.11-7.27(m, 1H), 7.32-7.40(m, 1H), 7.71-7.87(m,3H), 8.44-8.55(m, 1H), 8.61-8.66(m, 1H). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 49.70, 50.99, 108.08, 114.15, 121.53, 123.52, 132.75, 135.39, 145.16, 147.97, 148.49, 149.35, 157.53, 159.82, 171.25.EI MS (m/z):316(m^+). Elemental analysis For $C_{15}H_{13}N_4OFS$: (Anal: Cal), (C, H, N) C 56.90; H 4.11; N 17.70, C 56.87; H 4.08: N 17.72 .M.W=316 .35g/mol.

5.3.1.3.6 N-(6-ethoxy benzo [*d*]thiazol-2-yl)-2-((pyridine-3-yl methyl) amino) acetamide. 3f



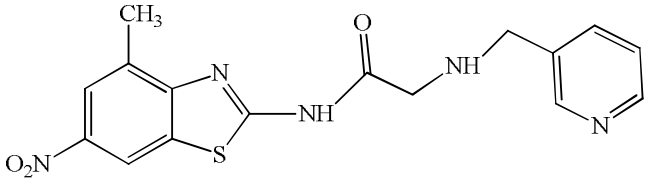
Yield 45 %, pale yellow coloured solid, mp: 168-171°C: IR (KBr,cm^{1}):3409, 2978,, 2926, 1666, 1606, 1489, 1389, 1264, 1224, 1186, 1059, 1037, 942, 819, 711; ^{1}H NMR (400 MHz, DMSO-d_6) δ :2.48(t, 3H), 4.02(q, 2H), 8.04(s, 1H),8.21-8.23 (d, *J* =1.6 Hz, 1H), 8.40-8.42 (d, *J* =1.6 Hz 1H,), 8.52-8.54 d, 1H, *J*=1.6 Hz) 8.94(s, 1H), 8.94(s,1H), 10.1(s, 1H), 12.00.(s, 1H). ^{13}C NMR δ: 19.45, 47.02, 48.19, 121.32, 123.08, 124.75, 126.21, 131.10, 140.33, 140.87, 143.90, 145.38, 146.42, 146.15, 164.85, 166.65. (75 MHz, DMSO-d_6) δ:.EI MS (m/z):342.41 (m^+). Elemental analysis For $C_{17}H_{18}N_4O_2S$: (Anal: Cal), (C, H, N) C 59.58; H 5.25; N 16.35, C 59.55; H 5.20: N 16.32 .M.W=342.41 g/mol.

5.3.1.3.7 N-(6-bromo benzo [*d*]thiazol-2-yl)-2-((pyridine-3-yl methyl) amino) acetamide. 3g



Yield 39 %, light brown coloured solid, mp: 185-187°C: IR (KBr,cm^{1}):3406, 3066, 2918, 2850, 1666, 1594, 1538, 1480, 1441, 1264, 1085, 811, 747, 711; ^{1}H NMR (400 MHz, DMSO-d_6) δ : 3.95(s, 2H), 4.73(s, 2H), 7.23-7.39(m, 2H), 7.45-7.55(m, 1H) 7.64-7.78(m, 1H), 8.15(s, 1H),8.45-8.50(m, 1H), 8.50-8.66(m, 1H).^{13}C NMR (75 MHz, DMSO-d_6) δ:49.73, 51.08, 115.49, 121.94, 123.97, 128.93, 132.49, 133.65, 135.65, 147.64, 147.68, 148.48, 149.33, 158.31, 171.34.Elemental analysis For $C_{15}H_{13}BrN_4OS$: (Anal: Cal), (C, H, N) C 47.71; H 3.44; N 14.84, C 47.69; H 3.42: N 14.81 .M.W=377.26 g/mol.

5.3.1.3.8 N-(4-methyl-6-Nitro benzo [*d*]thiazol-2-yl)-2-((pyridine-3-yl methyl) amino) acetamide. 3h



Yield 59 %, light brown coloured solid, mp: 195-197°C: IR (KBr,cm^{1}):3539, 3473, 3224, 3073, 2959, 2935, 2726, 2601, 1689, 1636, 1609, 1550, 1518, 1468, 1448, 1349, 1267, 1230, 1120, 1097, 1064, 980, 879, 804, 744, 680.; ^{1}H NMR (400 MHz, DMSO-d_6) δ : 2.36(s,3H), 4.16(s,2H), 4.47(s,2H), 8.03(s,1H), 8.21(d, *J* = 1.6Hz, 1H),8.33(d, *J*=1.6Hz, 1H), 8.67(d, *J* =6.0Hz, 1H) 8.93(s,1H), 9.07(s,1H), 10.15(s,1H), 10.99(s,1H).^{13}*C* NMR (75 MHz, DMSO-d_6) δ: 19.06, 46.49, 47.50, 122.66, 124.28, 124.94, 126.50, 131.03, 139.74, 140.41, 142.83, 144.53, 146.04, 146.86, 163.68 .EI MS (m/z):356.49 (m-1). Elemental analysis For $C_{16}H_{15}N_5O_3S$: (Anal: Cal), (C, H, N) C 47.71; H 3.44; N 14.84, C 47.69; H 3.42: N 14.81 .M.W=357 g/mol.

5.3.2 Biological Activity Screening.

5.3.2.1 Procedure of Cup-plate method for the antimicrobial activity[16]

Antibacterial activity of all the synthesized compounds were 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, 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

5.3.2.2 Procedure for the MTT Assay for Anticancer activity.

A549 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 five different concentrations of 50µg, 75 µg, 100 µg, 150 µg and 200 µg. 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 570 nm in a micro plate reader (MicrotekSigma360). Absorbance at 570nm directly correlates with cell viability. Percentage inhibition was observed through physical method.

5.4 Conclusion

In conclusion, we report here synthesis of various N-(substituted benzo [*d*]thiazol-2-yl)-2-((pyridine-3-yl methyl) amino) acetamide derivatives 3(a-h).The structures of all the synthesized compounds were elucidated and confirmed by IR,^{1}H NMR, ^{13}C NMR, Mass spectral data and purity of the compounds were checked by TLC and elemental analysis. All newly synthesized compounds were screened for their antimicrobial activity. They showed moderate to good antibacterial and antifungal activity. Compound **3b**, **3c** and **3e 3f** and **3g** showed moderate to promising activity against gram positive bacteria

(*S.aureus)at 80*µg, 80µg, 40µg *and 80*µg concentration respectively. Compound **3e** showed promising activity at 40µg against tested Gram positive bacteria (*B. Subtilis).*Compound **3e** also showed excellent antifungal activity at 10 µg against *C. Albicans (Fungi).* Rest of the compounds remains inactive against tested gram positive bacteria (*S.aureus) ,(B. Subtilis) and one* fungi *C. Albicans. ,* All of the synthesized compounds did not show any activity against tested Gram negative bacteria i.e against *(E. coli)* and *(P. aeruginosa).*

Among the synthesized compounds **3(a-h)**, the four compounds **3a**, **3c**,**3d** and **3h** were screened for the anticancer activity against lung cancer cell line A549.The MTT assay test was applied for the anticancer activity at five different concentrations wise, 50µg, 75µg, 100µg, 150µg and 200 µg respectively in the triplicate using serial dilution method as shown in Figure- 44. All the four compounds when subjected to undergo the anticancer screening against the three Leukemia cancer cell lines; they showed very promising results with IC_{50} value 0.73 µM for compound **3a** against MOLT-3 leukemia cancer cell line and IC_{50} value 0.50 µM for compound **3d** against KG-1 leukemia cancer cell line. Compound **3c** also gave enhancing activity with IC_{50} value 2.34 µM against KG-1 leukemia cancer cell line.

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 bearing amide linkage resulted in promising antibacterial and antifungal activity. Furthermore it can be concluded that the designing of amide derivatives of various aromatic amines and 2-amino benzothiazoles with 3-amino methyl pyridine gave the biologically active molecules that can lead to discovery of potential drug candidate.

5.5 Reference

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