Chapter 3

Anti-cancer application of quinuclidinone derivatives

Chapter 3A

Synthesis characterization and cytotoxicity evaluation of 2-arylidine quinuclidinones.

Part A:

3A.1 Introduction

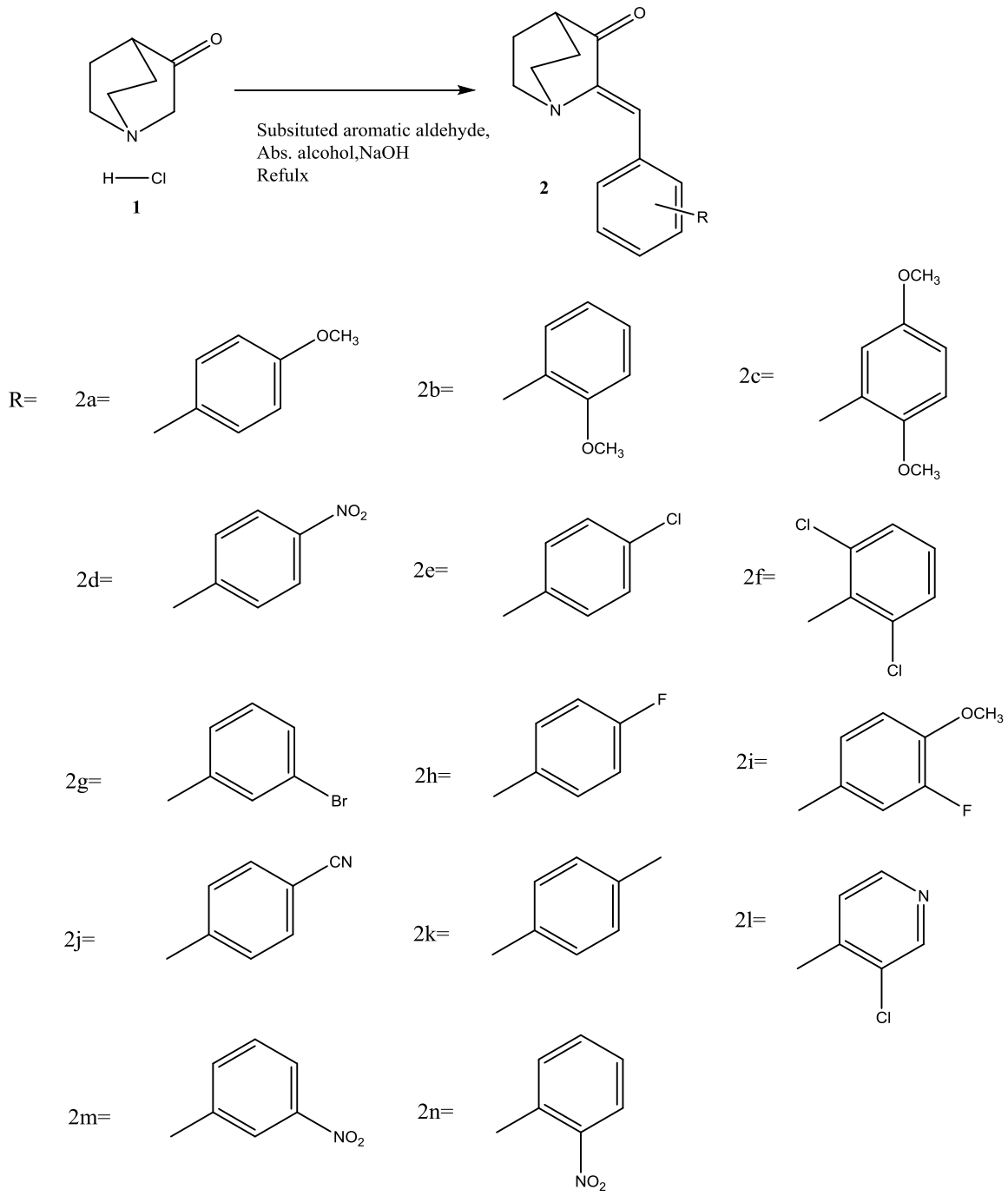
2-arylidine quinuclidinone is key motif for the preparation of various classes of compounds. 2-arylidine quinuclidinone derivatives are known to in the literature for the preparation of various biologically important derivatives. Many derivatives having this core scaffold are known to possess human α7 and α4β2 nicotinic receptors inhibitory activity.[1]

This class of compound also find important position in the field of cosmetics. Stein et al., from Merck GmbH had filed a patent in which they had used these derivatives can be used for the preparation of sunscreen, hair styling gels etc.[2] These derivatives as it has good antioxidant, anti-allergic property and they can also be used as for the protection of harmful ultraviolet sunrays as these derivatives have outstanding UVA property. Stein et al., had also mentioned that these analogues can be used for the treatment of cancer as they possess good antioxidant property.[2] We had synthesised a series of 2-arylidine quinuclidinone and screen it for its anticancer property.

3A.2 Result and discussion

3A.2.1 Chemistry

3-quinuclidinone hydrochloride **1** was refluxed with various substituted aromatic aldehyde in the presence of sodium hydroxide in absolute alcohol to give title compounds **2a-n** as shown in **Scheme 1**.



Scheme 1: Synthetic route for compounds 2a-n

Reagent and conditions: substituted aromatic aldehyde NaOH, EtOH, reflux.

The IR spectrum of compound **2b** (Figure 1) exhibited bands at 2837 and 1706 cm^{-1} indicated presence of methylene and ketone carbonyl group respectively. In ^{1}H NMR of compound **2b** (Figure 2) multiplets at δ 2.02, 2.64, 2.98 and 3.15 for four, one, two

and two protons respectively confirmed the methyl and methylene protons of quinuclidine ring. Singlet at δ 3.84 for three protons indicated presence of methoxy group. All aromatic protons and vinylic proton observed between δ 6.90 to 7.81 confirmed the formation of compound **2b**. The ^{13}C NMR spectrum of compound **2b** (Figure 3) showed peak at δ 206.5 indicate ketone carbon. The presence of 14 peaks is in accordance with structure of compound **2b**. The mass spectrum of compound **2b** (Figure 4) showed *m/z* value at 244.2 $[M+1]^+$in ESI/MS confirmed formation of **2b**.

The IR spectrum of compound **2c** (Figure 6) exhibited bands at 2835 and 1698 cm^{-1} indicated presence of methylene and ketone carbonyl group respectively. In ^{1}H NMR of compound **2c** (Figure 7) multiplets at δ 1.93, 2.54, 2.88 and 3.04 for four, one, two and two protons respectively confirmed the methyl and methylene protons of quinuclidine ring. Singlet at δ 3.71 and 3.74 for three protons each indicated presence of two methoxy group. All aromatic protons and vinylic proton observed between δ 6.72 to 8.23 confirmed the formation of compound **2c**. The ^{13}C NMR spectrum of compound **2c** (Figure 8) showed peak at δ 206.3 indicate ketone carbon. The presence of 14 peaks is in accordance with structure of compound **2c**. The mass spectrum of compound **2c** (Figure 9) showed *m/z* value at 274.3 $[M+1]^+$in ESI/MS confirmed formation of **2c**.

The IR spectrum of compound **2f** (Figure 10) exhibited bands at 2835 and 1698 cm^{-1} indicated presence of methylene and ketone carbonyl group respectively. In ^{1}H NMR of compound **2f** (Figure 11) multiplets at δ 1.94, 2.67 and 3.02 for four, one and four protons respectively confirmed the methyl and methylene protons of quinuclidine ring. Singlet at δ 3.71 and 3.74 for three protons each indicated presence of two methoxy group. All aromatic protons and vinylic proton observed between δ 7.03 to 7.31 confirmed the formation of compound **2f**. The ^{13}C NMR spectrum of compound

2f (Figure 12) showed peak at δ 204.8 indicated ketone carbon. The presence of 11 peaks is in accordance with structure of compound **2f**. The mass spectrum of compound **2f** (Figure 13) showed *m/z* value at 282.2 $[M]^+$ in ESI/MS confirmed formation of **2f**.

The IR spectrum of compound **2h** (Figure 15) exhibited bands at 3060 and 1698 cm^{-1} indicated presence of methylene and ketone carbonyl group respectively. In ^1H NMR of compound **2h** (Figure 16) multiplets at δ 2.02, 2.64, 2.95 and 3.13 for four, one, two and two protons respectively confirmed the methyl and methylene protons of quinuclidine ring. All aromatic protons and vinylic proton observed between δ 6.98 to 8.05 confirmed the formation of compound **2h**. The ^{13}C NMR spectrum of compound **2h** (Figure 17) showed peak at δ 206.3 indicate ketone carbon. The presence of 15 peaks is in accordance with structure of compound **2h**. The mass spectrum of compound **2h** (Figure 18) showed *m/z* value at 222.2 $[M+1]^+$ in ESI/MS confirmed formation of **2h**.

The IR spectrum of compound **2m** (Figure 20) exhibited bands at 3062 and 1708 cm^{-1} indicated presence of methylene and ketone carbonyl group respectively. In ^1H NMR of compound **2m** (Figure 21) multiplets at δ 1.98, 2.60, 2.89 and 3.10 for four, one, two and two protons respectively confirmed the methyl and methylene protons of quinuclidine ring. All aromatic protons and vinylic proton observed between δ 6.95 to 8.10 confirmed the formation of compound **2m**. The ^{13}C NMR spectrum of compound **2m** (Figure 22) showed peak at δ 205.8 indicate ketone carbon. The presence of 9 peaks is in accordance with structure of compound **2m**. The mass spectrum of compound **2m** (Figure 23) showed *m/z* value at 259.3 $[M+1]^+$ in ESI/MS confirmed formation of **2m**.

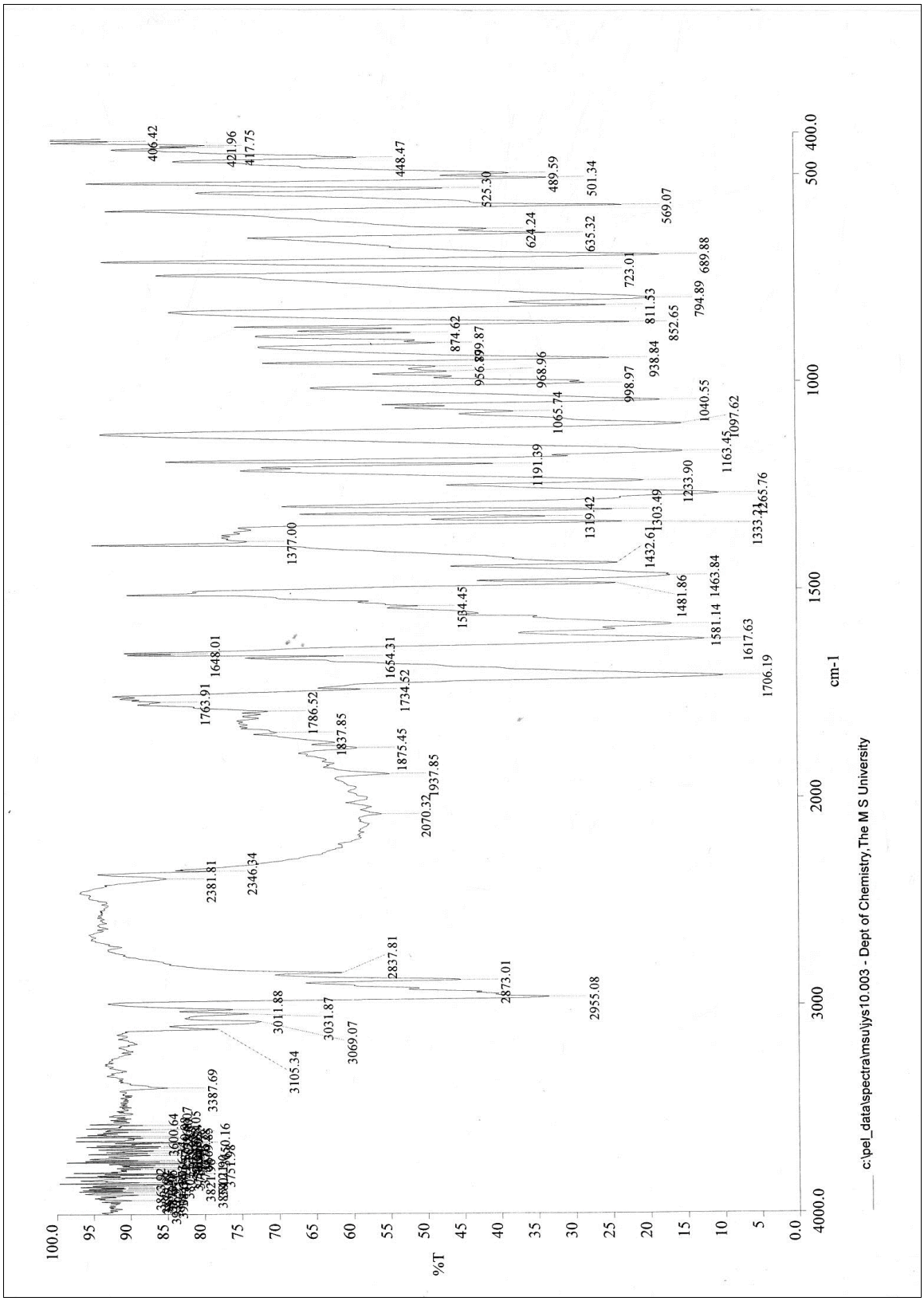


Figure1: IR spectra of (Z)-2-(2-methoxybenzylidene)quinuclidin-3-one 2b

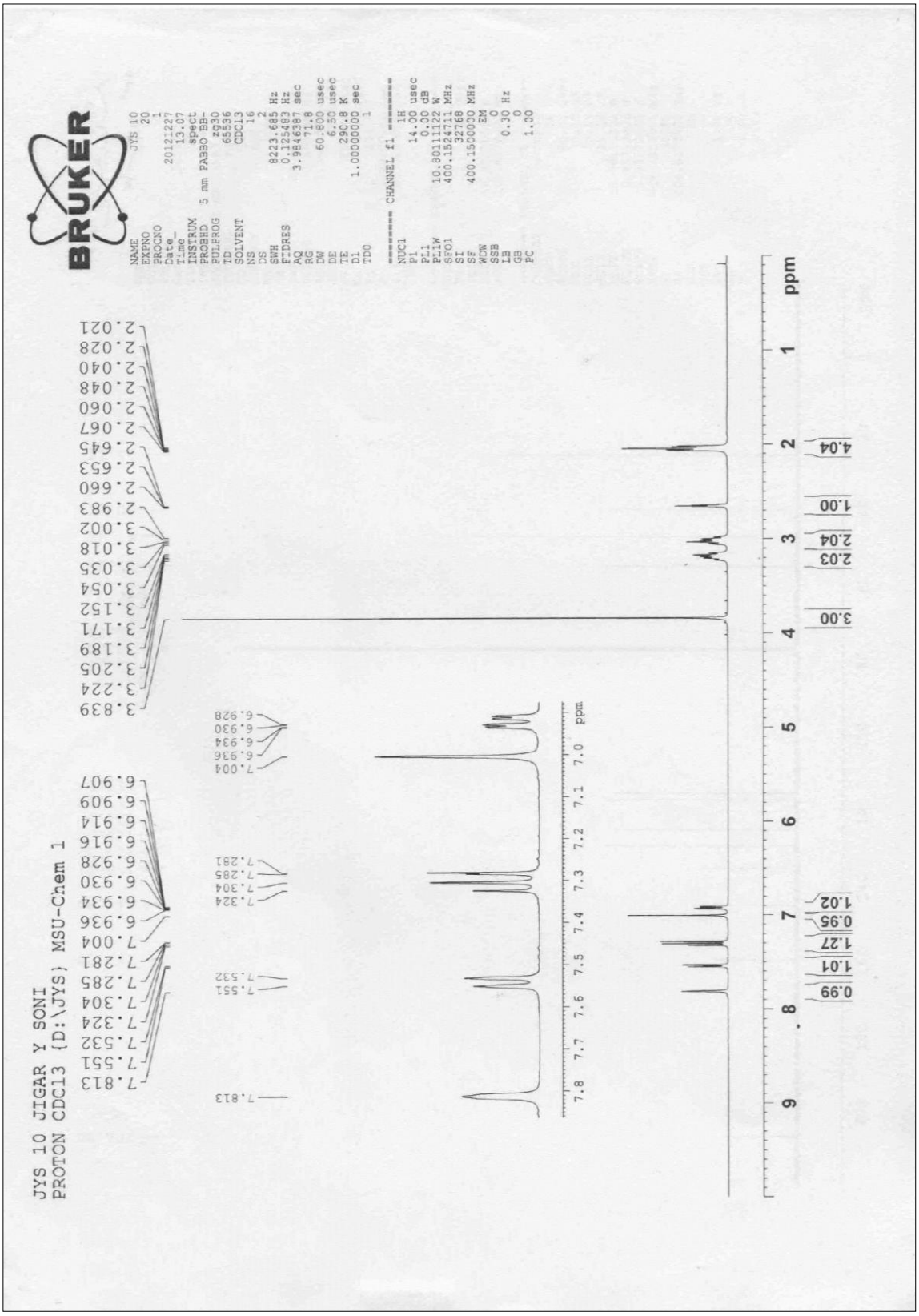


Figure 2: ^{1}H NMR spectra of (Z)-2-(2-methoxybenzylidene)quinuclidin-3-one 2b

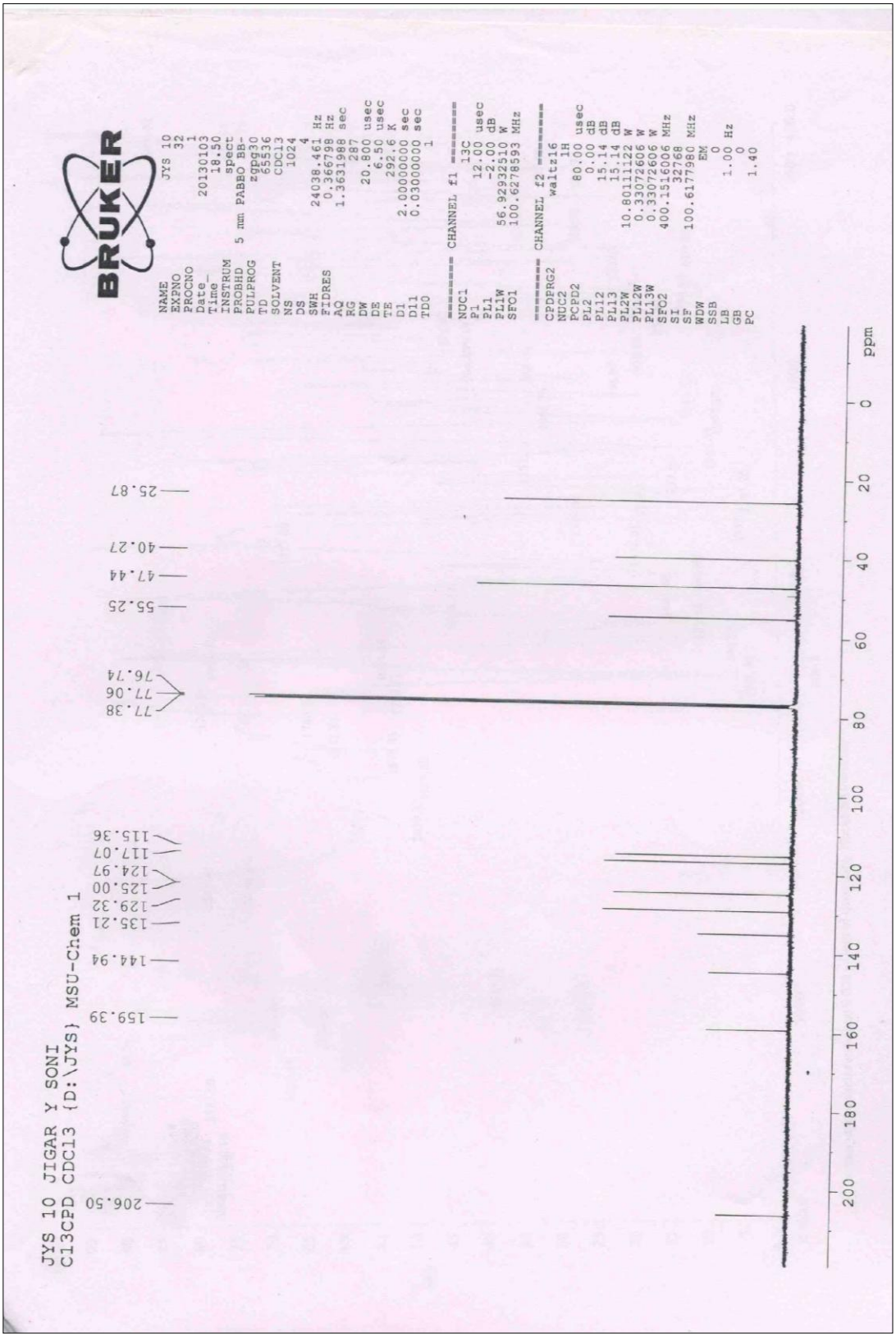


Figure 3: ^{13}C NMR spectra of (Z)-2-(2-methoxybenzylidene)quinuclidin-3-one 2b

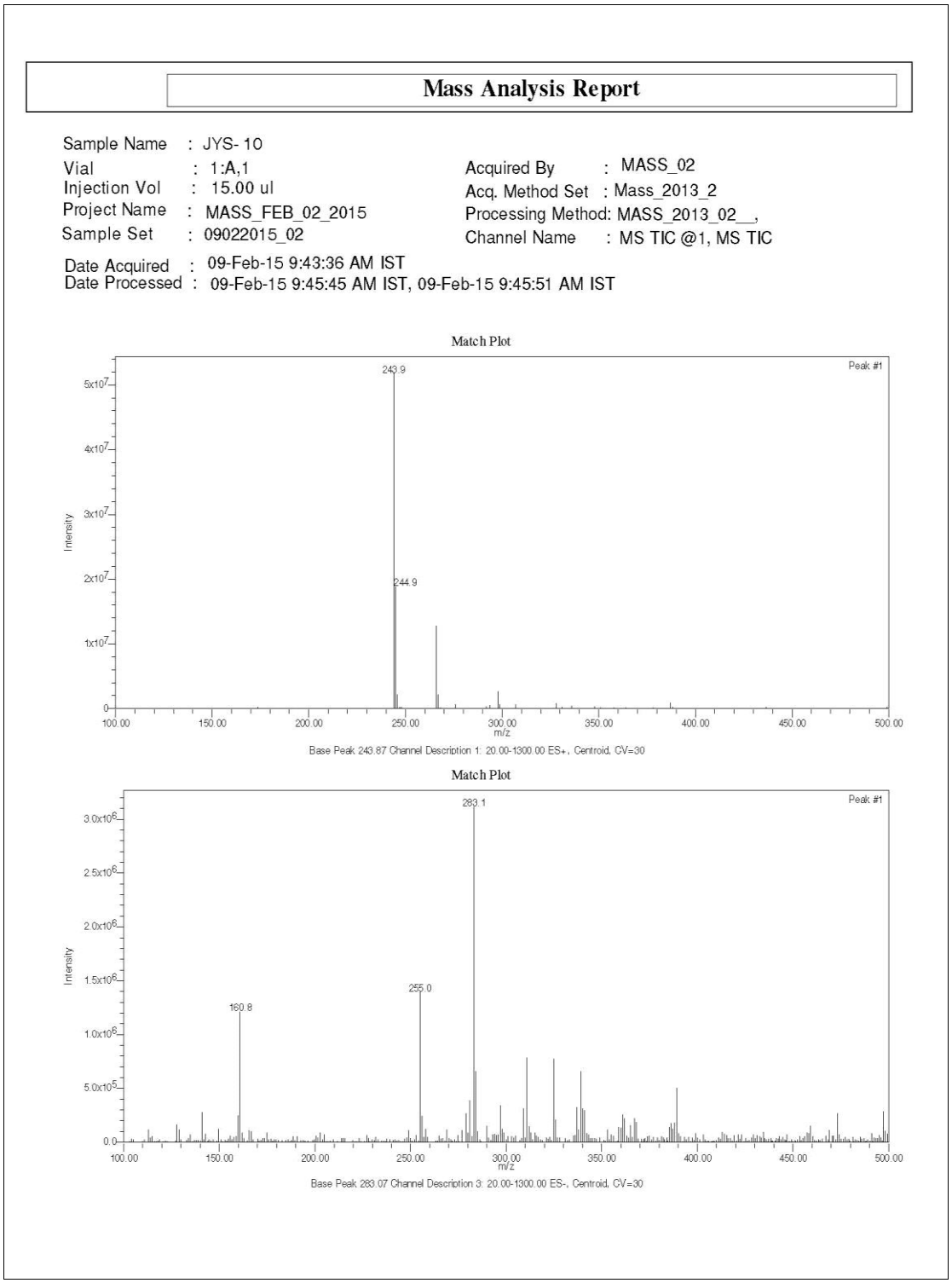


Figure 4: Mass spectra of (Z)-2-(2-methoxybenzylidene)quinuclidin-3-one 2b

LCMS Analysis Report

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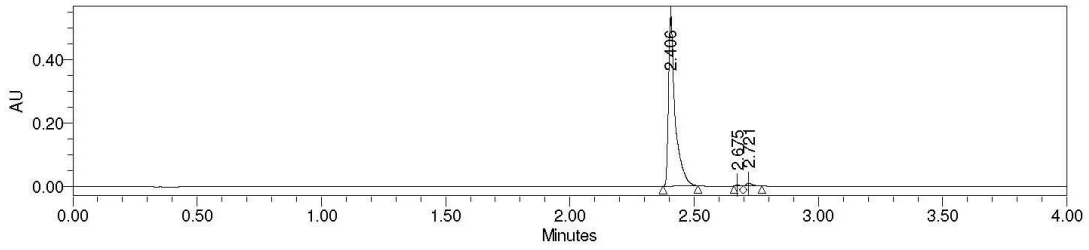
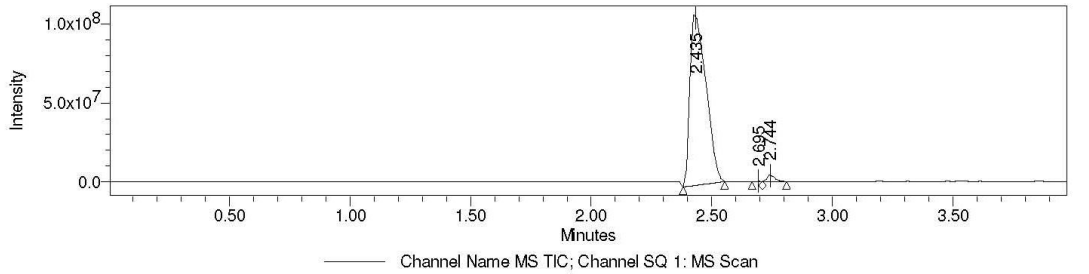
Sample Type:	Unknown	Acquired By:	LCMS_03
Vial:	1:B,4	Sample Set Name:	09022015_UCH59_ASD_SB
Injection #:	1	Acq. Method Set:	METHOD_C
Injection Volume:	1.00 ul	Processing Method:	METHOD_C1, Channel Name: MS TIC, 293.0nm
Run Time:	4.0 Minutes		
Project Name	LCMS-03_FEB_2016_WEEK_02	Proc. Chnl. Descr.:	SQ 1: MS Scan MS TIC,
Date Acquired:	2/9/2015 18:32 IST		
Date Processed:	2/9/2015 18:48 IST, 2/9/2015 18:49 IST		





Peak Results
Channel: PDA Spectrum

	Retention Time (min)	Base Peak (m/z)	Height (µV)	Area (µV*sec)	% Area	Channel	Channel Name
1	2.406		541539	993702	98.13	PDA Spectrum	293.0nm
2	2.675		3557	4449	0.44	PDA Spectrum	293.0nm
3	2.721		8800	14499	1.43	PDA Spectrum	293.0nm

Peak Results
Channel: SQ 1: MS Scan

	Retention Time (min)	Base Peak (m/z)	Height (µV)	Area (µV*sec)	% Area	Channel	Channel Name
1	2.435	244.26	110940258	500984743	97.96	SQ 1: MS Scan	MS TIC
2	2.695	250.02	459822	539015	0.11	SQ 1: MS Scan	MS TIC
3	2.744	294.12	3663611	9868535	1.93	SQ 1: MS Scan	MS TIC

Figure 5: LCMS spectra of (Z)-2-(2-methoxybenzylidene)quinuclidin-3-one 2b

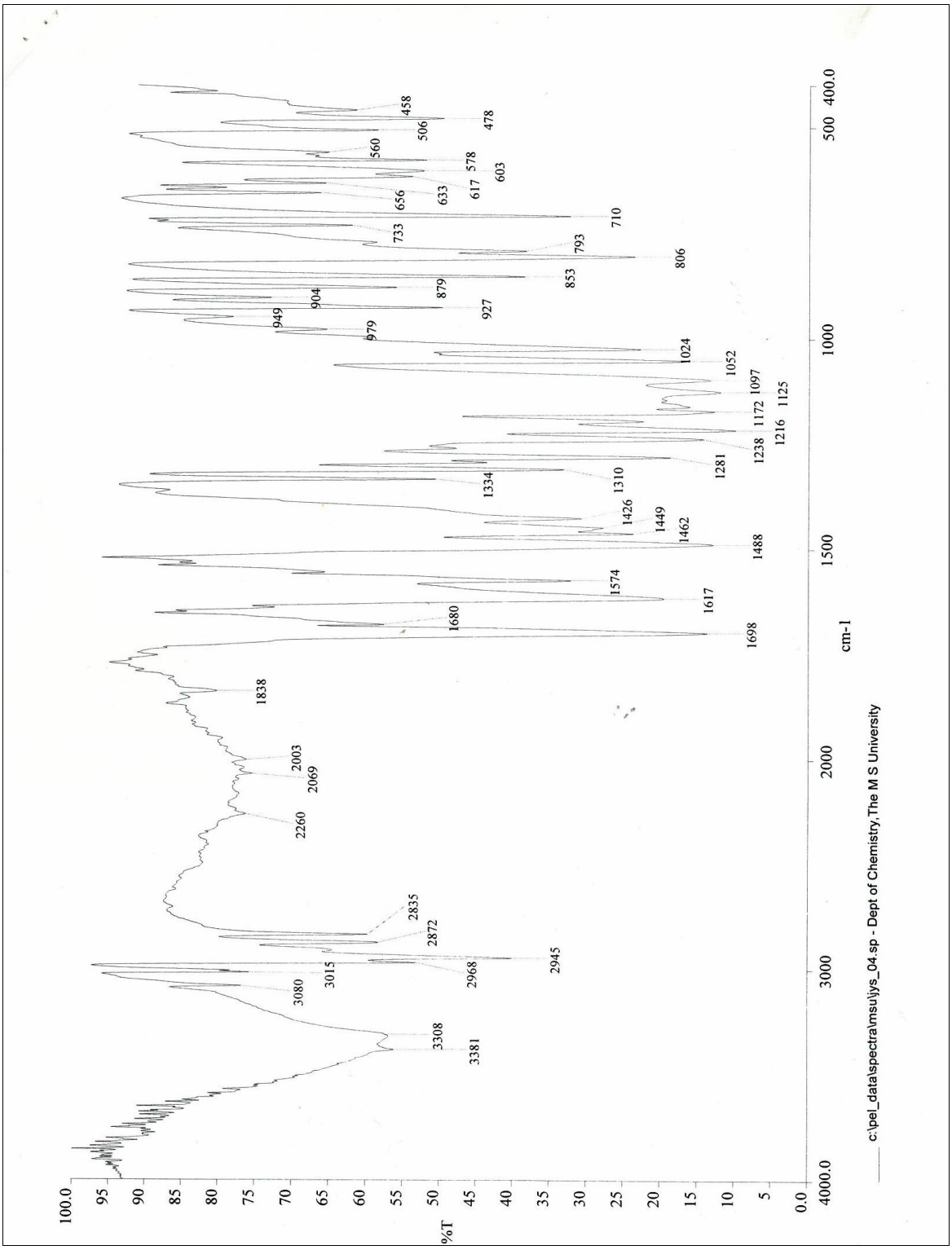


Figure 6: IR spectra of (Z)-2-(2,5-dimethoxybenzylidene)quinuclidin-3-one 2c

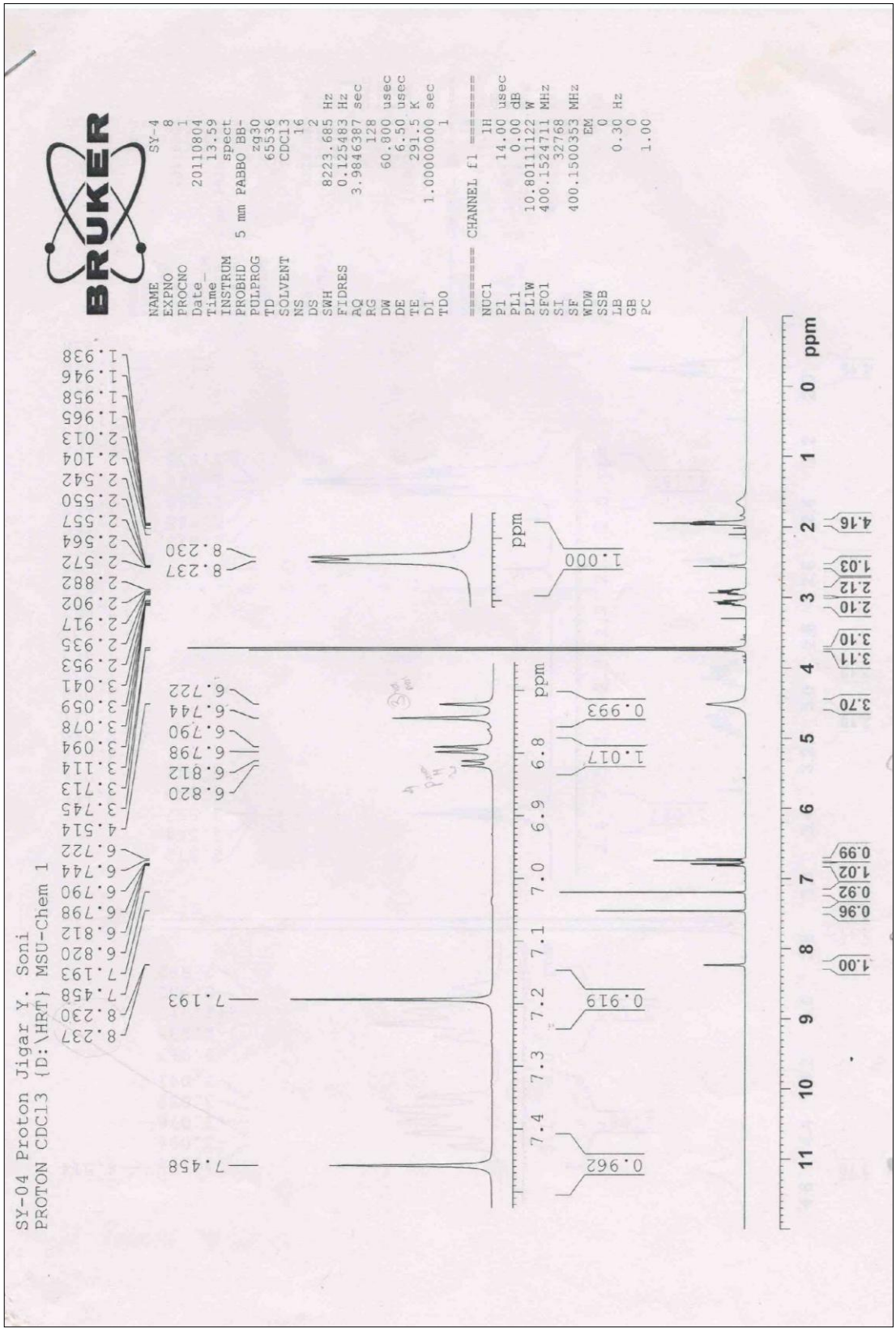


Figure 7: ^{1}H spectra NMR of (Z)-2-(2,5-dimethoxybenzylidene)quinuclidin-3-one 2c

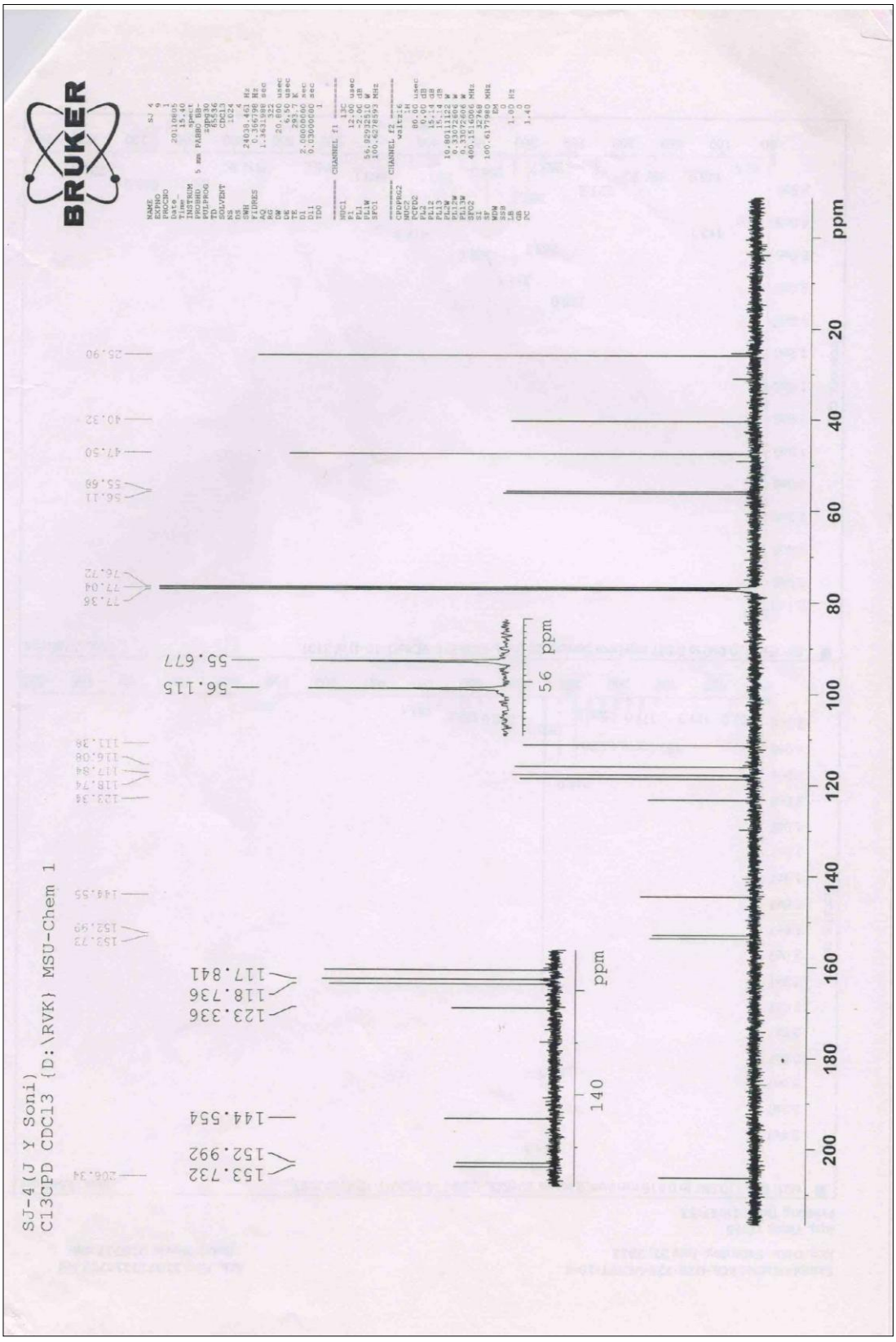

BRUKER
SJ-4(J Y Soni)
C13CPD CDCl3 {D:\RVK} MSU-Chem 1
ppm
56 ppm
140 ppm

Figure 8: ^{13}C spectra NMR of (Z)-2-(2,5-dimethoxybenzylidene)quinuclidin-3-one 2c

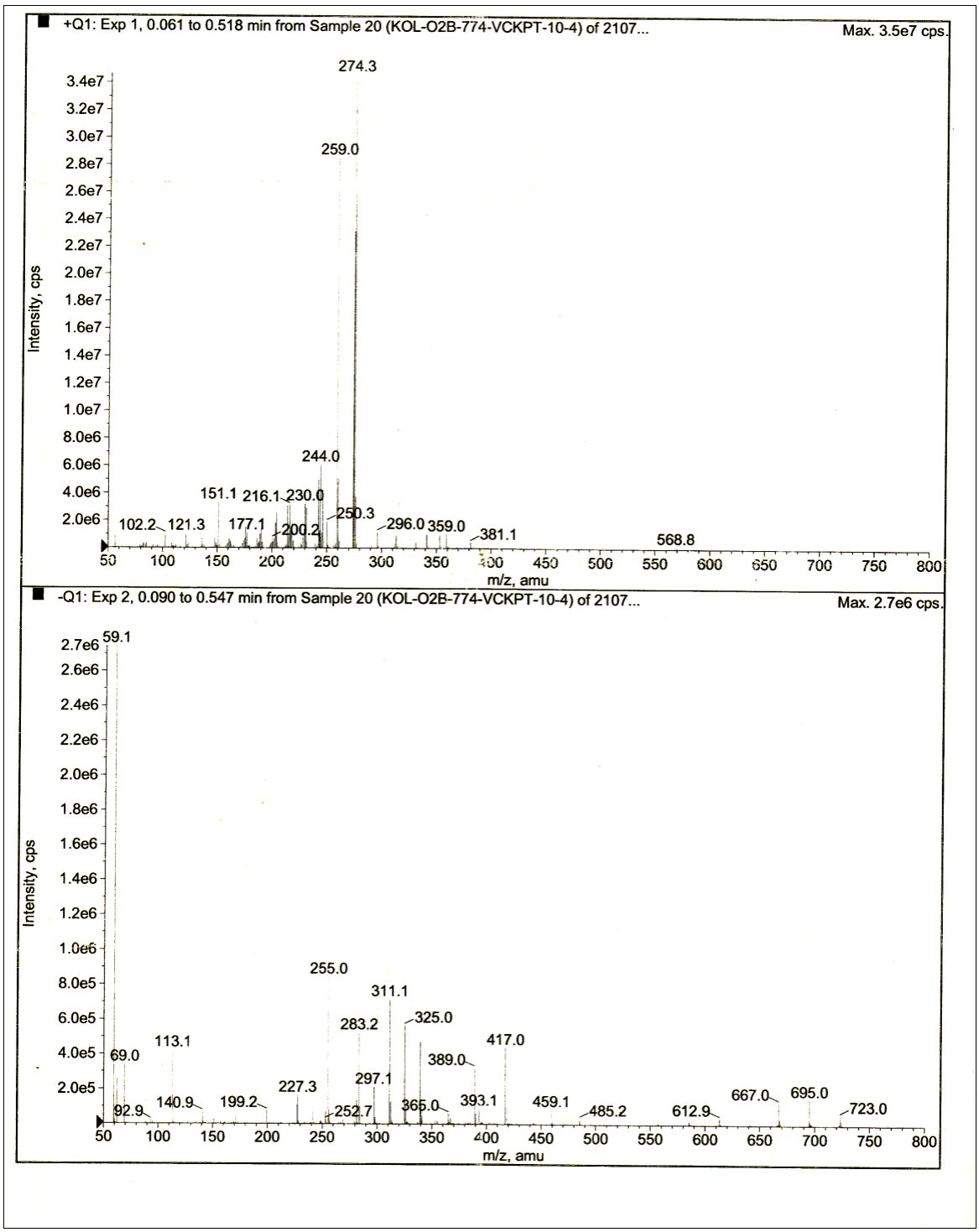


Figure 9: Mass spectra of (Z)-2-(2,5-dimethoxybenzylidene)quinuclidin-3-one 2c

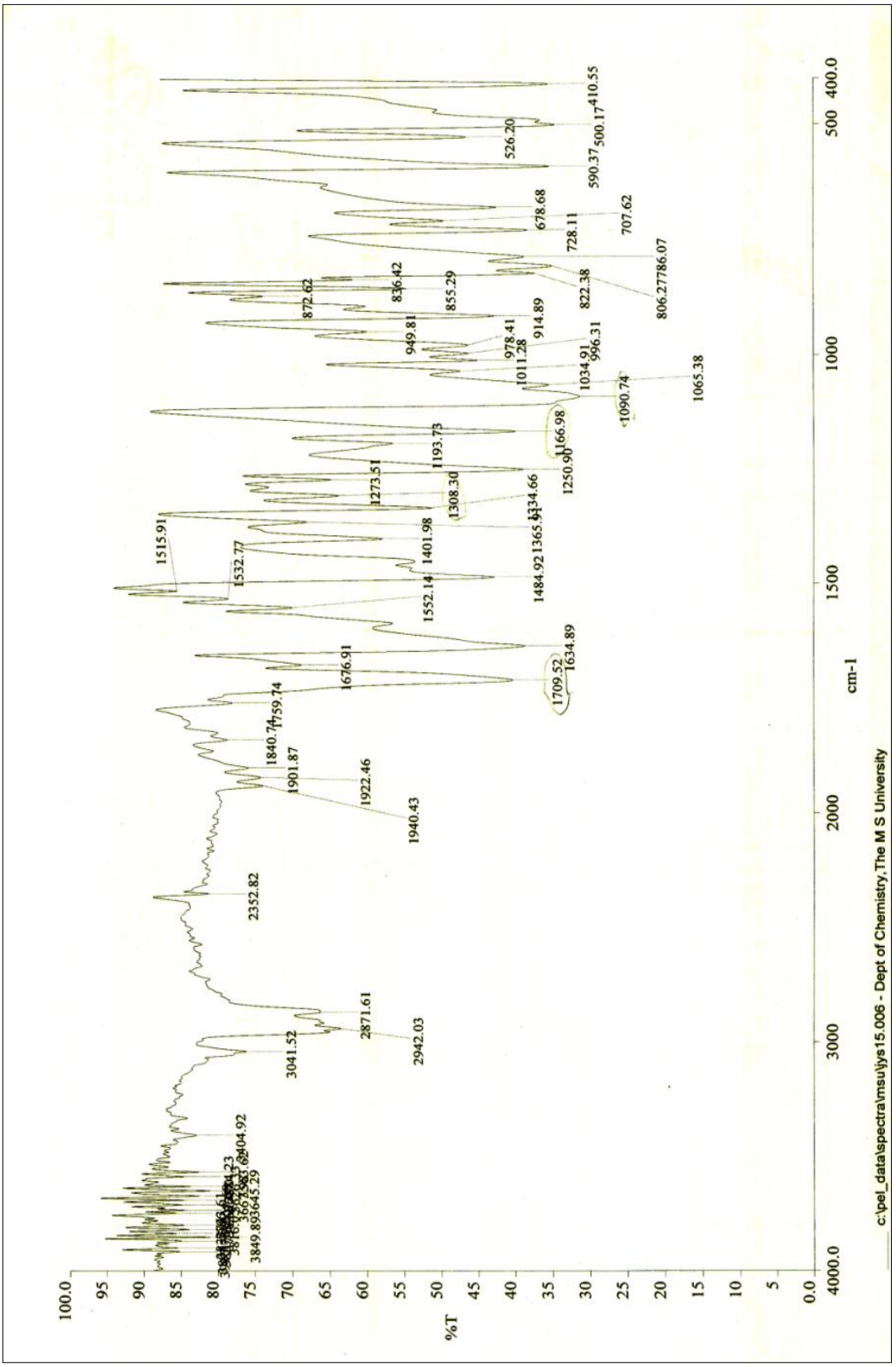


Figure 10: IR spectra of (Z)-2-(2,6-dichlorobenzylidene)quinuclidin-3-one2f

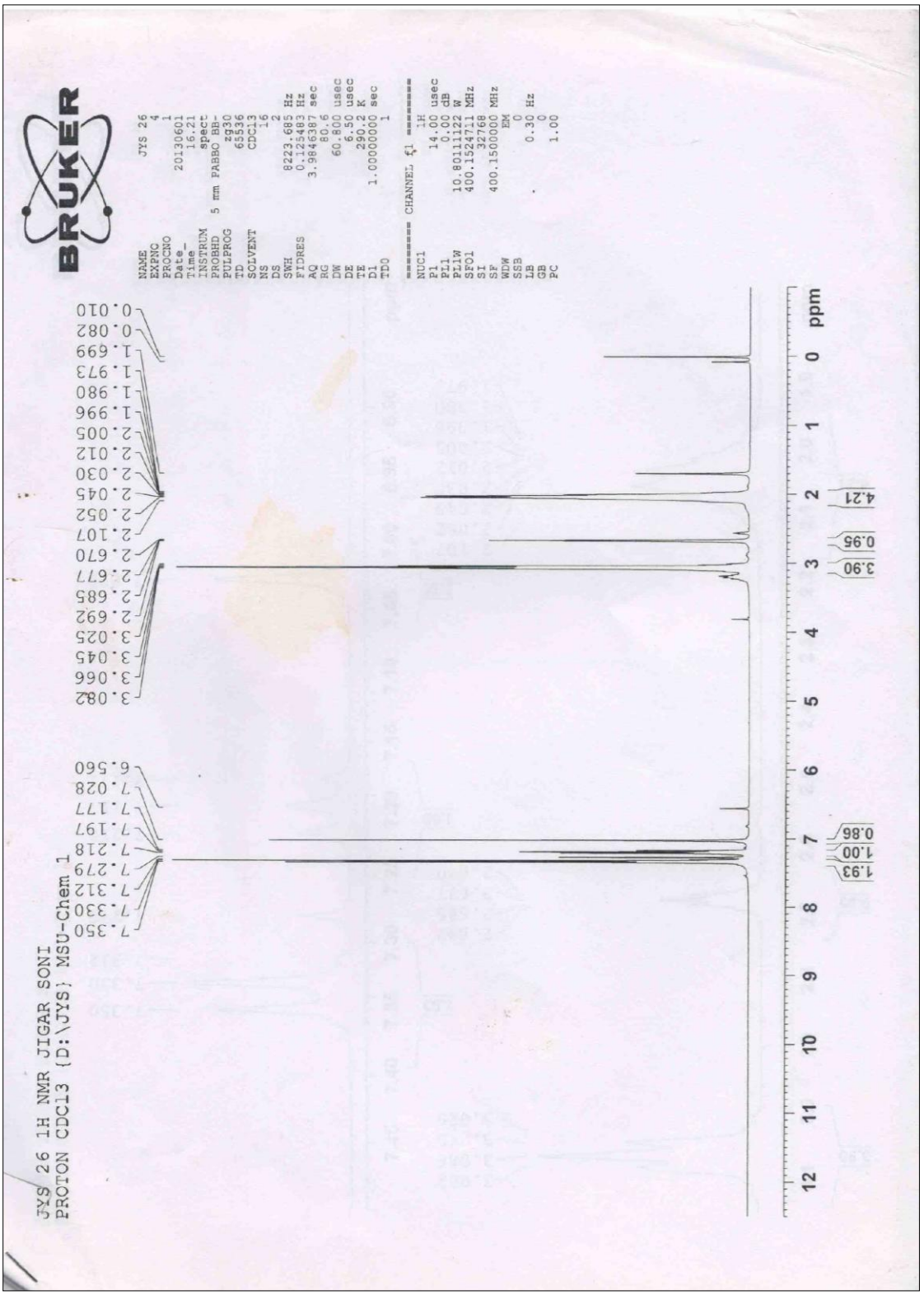


Figure 11: ^{1}H NMR spectra of (Z)-2-(2,6-dichlorobenzylidene)quinuclidin-3-one2f

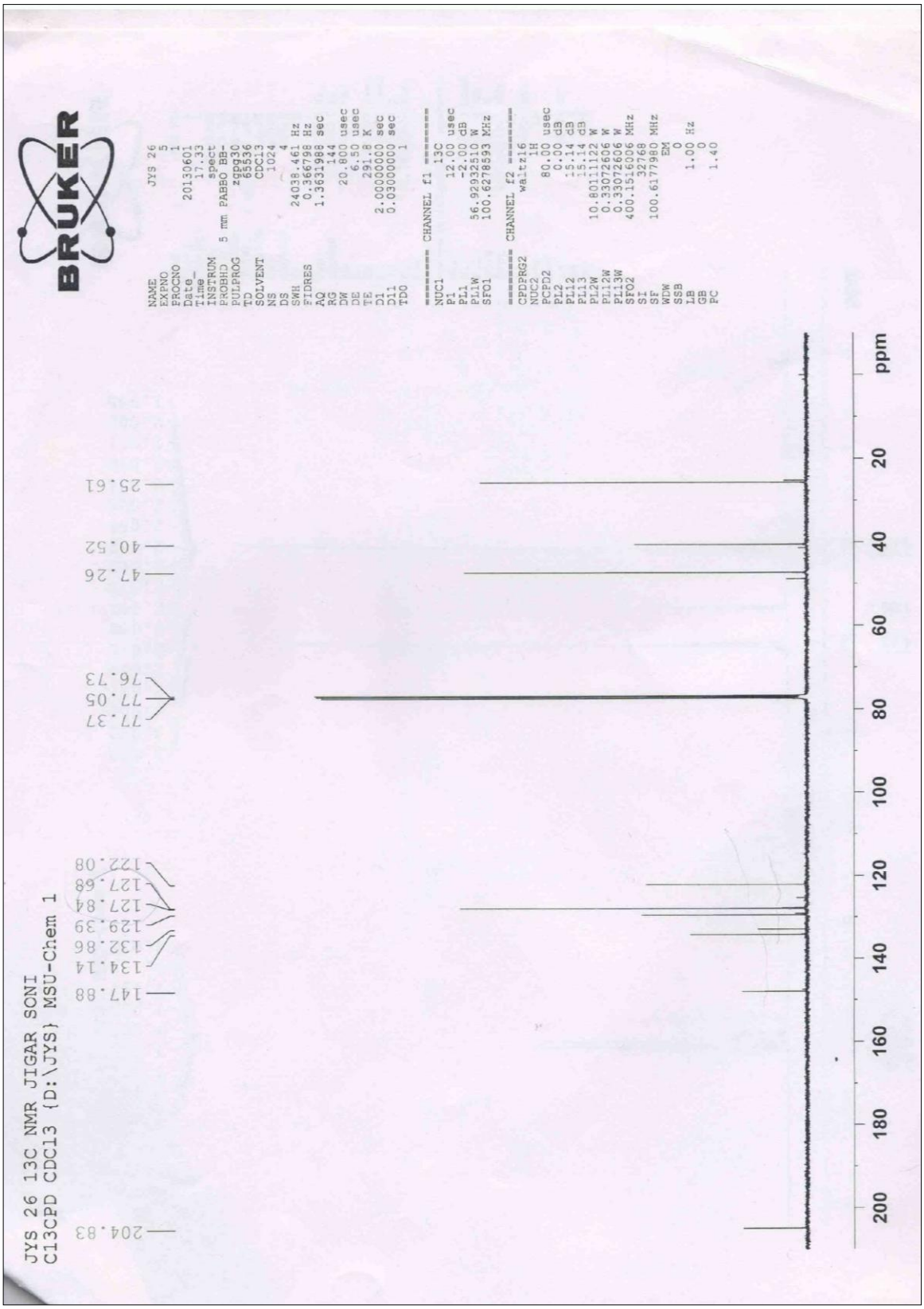


Figure 12: ^{13}C NMR spectra of (Z)-2-(2,6-dichlorobenzylidene)quinuclidin-3-one2f

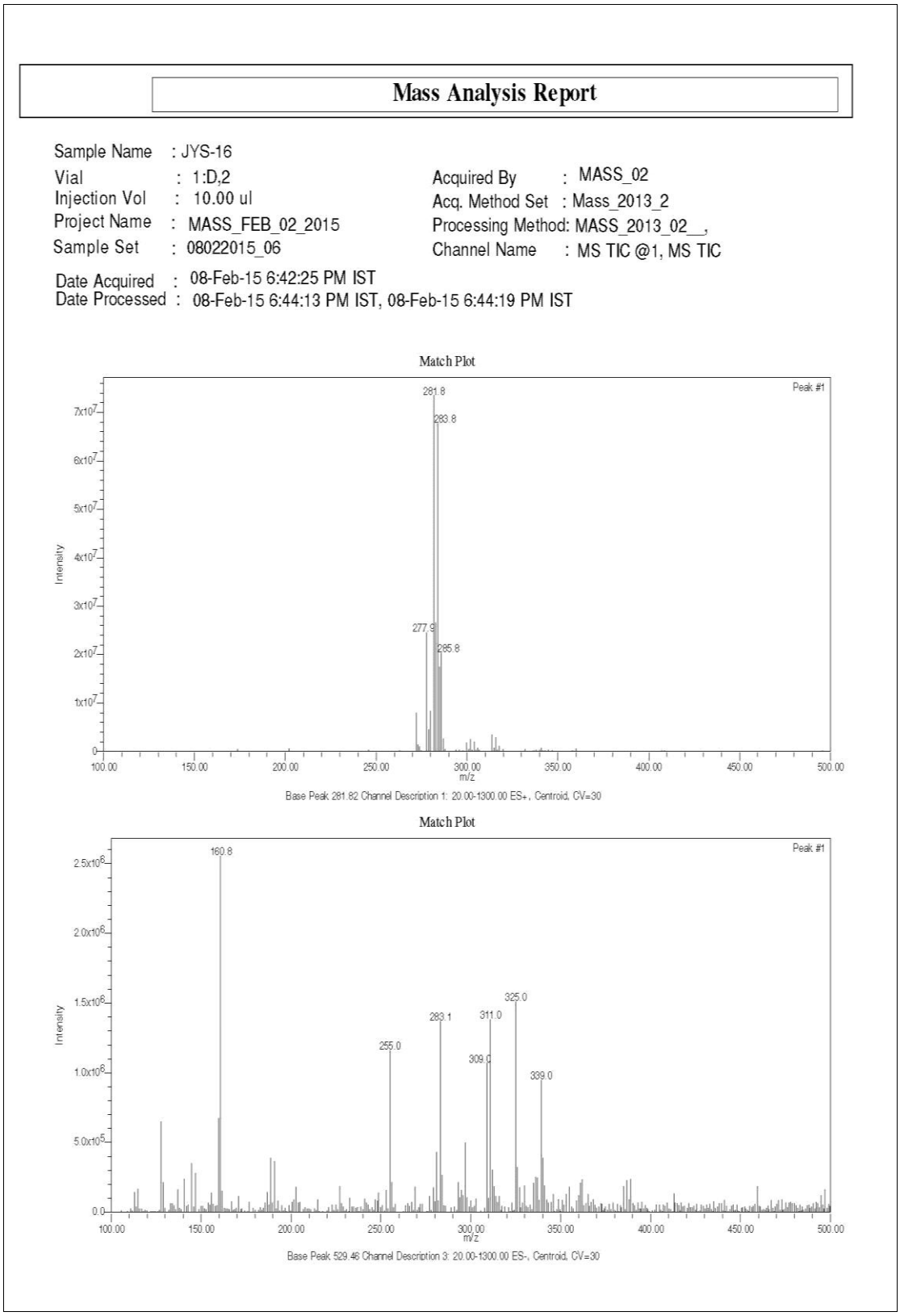


Figure 13: Mass spectra of (Z)-2-(2,6-dichlorobenzylidene)quinuclidin-3-one2f

LCMS Analysis Report

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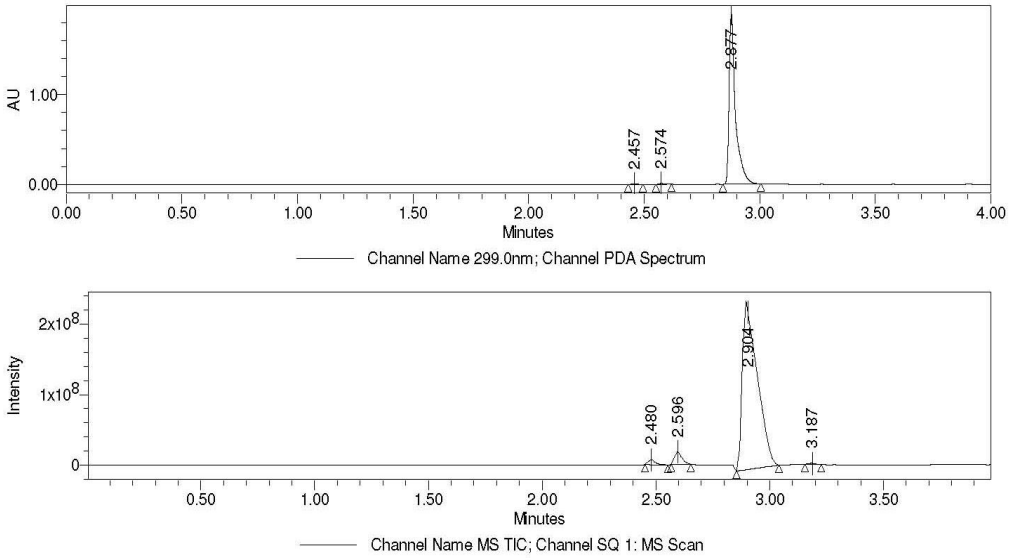
Injection Volume:	2.00 ul	Processing Method:	METHOD_C2,
Run Time:	4.0 Minutes	Channel Name:	299.0nm, MS TIC
Project Name	LCMS-03_FEB_2015_WEEK_02	Proc. Chnl. Descr.:	SQ 1: MS Scan MS TIC,
Date Acquired:	2/9/2015 18:42 IST		
Date Processed:	2/9/2015 18:57 IST, 2/9/2015 18:58 IST		



Peak Results
Channel: PDA Spectrum

	Retention Time (min)	Base Peak (m/z)	Height (µV)	Area (µV*sec)	% Area	Channel	Channel Name
1	2.457		3204	4540	0.12	PDA Spectrum	299.0nm
2	2.574		9781	14631	0.40	PDA Spectrum	299.0nm
3	2.877		1896559	3626278	99.47	PDA Spectrum	299.0nm

Peak Results
Channel: SQ 1: MS Scan

	Retention Time (min)	Base Peak (m/z)	Height (µV)	Area (µV*sec)	% Area	Channel	Channel Name
1	2.480	278.22	7473925	17236500	1.50	SQ 1: MS Scan	MS TIC
2	2.596	278.22	18385447	38945629	3.40	SQ 1: MS Scan	MS TIC
3	2.904	282.17	237940332	1085551836	94.77	SQ 1: MS Scan	MS TIC
4	3.187	468.44	1714080	3755751	0.33	SQ 1: MS Scan	MS TIC

Figure 14: LCMS spectra of (Z)-2-(2,6-dichlorobenzylidene)quinuclidin-3-one 2f

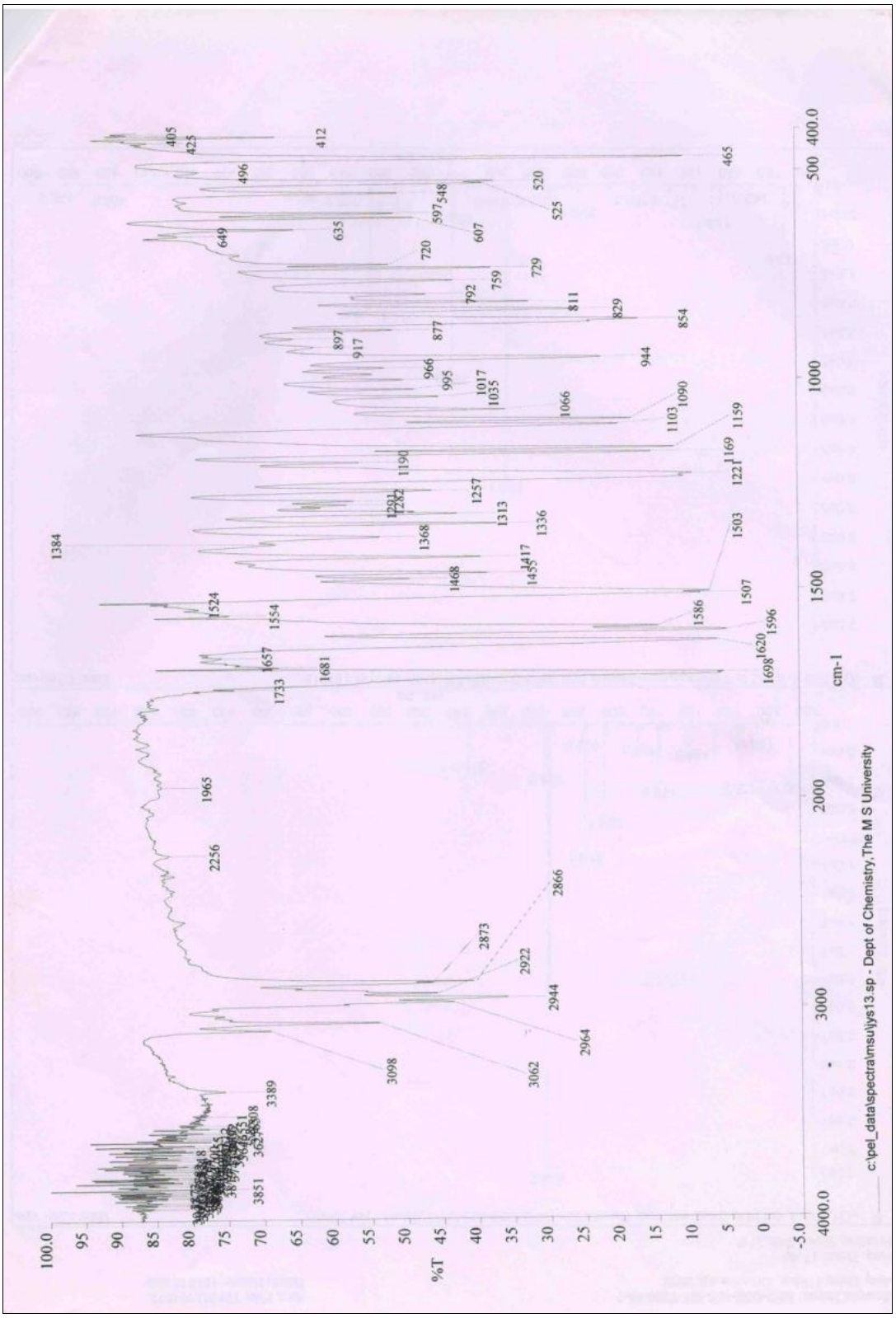


Figure 15: IR spectra of (Z)-2-(4-fluorobenzylidene)quinuclidin-3-one 2h

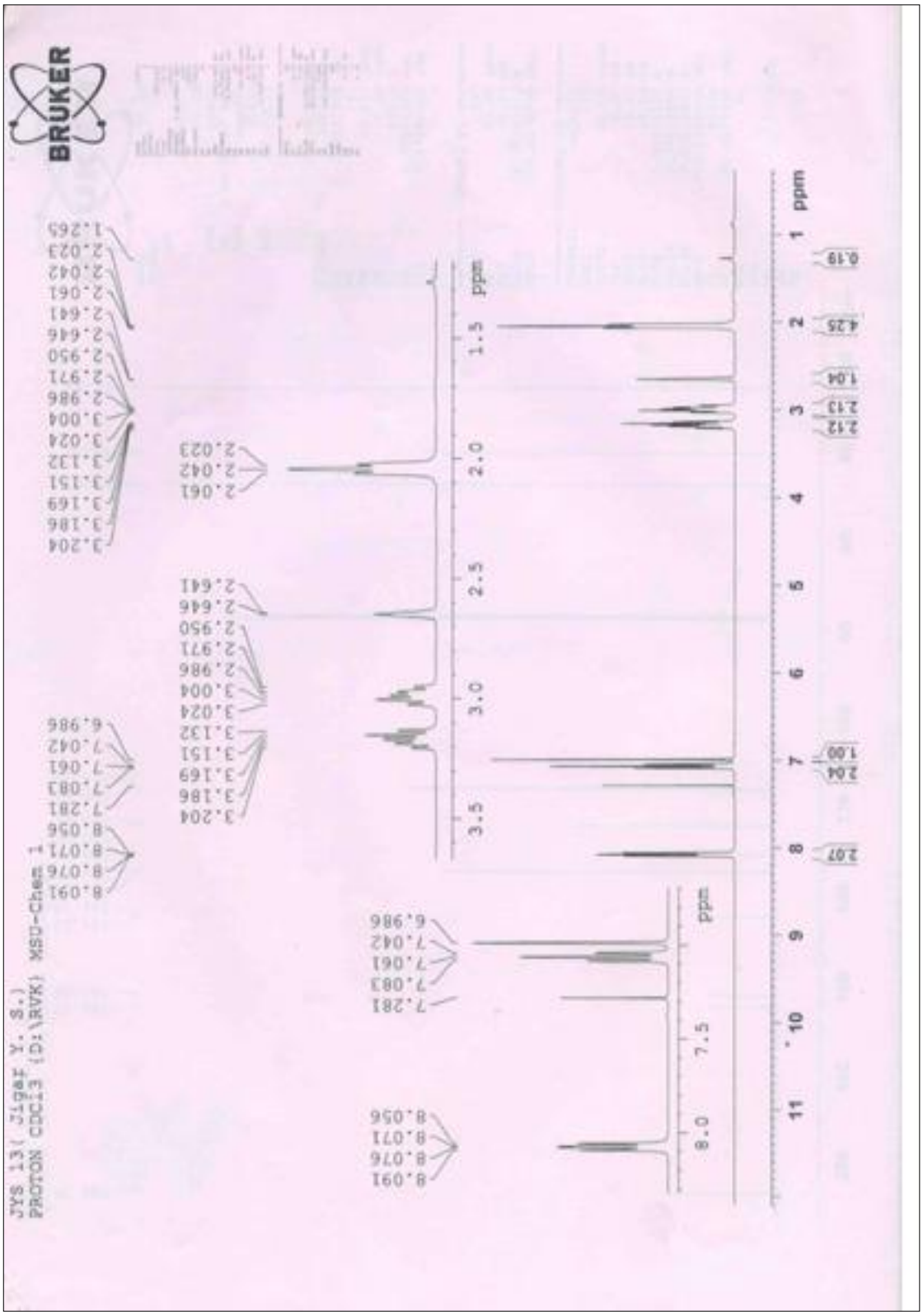


Figure 16: ^{1}H NMR spectra of (Z)-2-(4-fluorobenzylidene)quinuclidin-3-one 2h

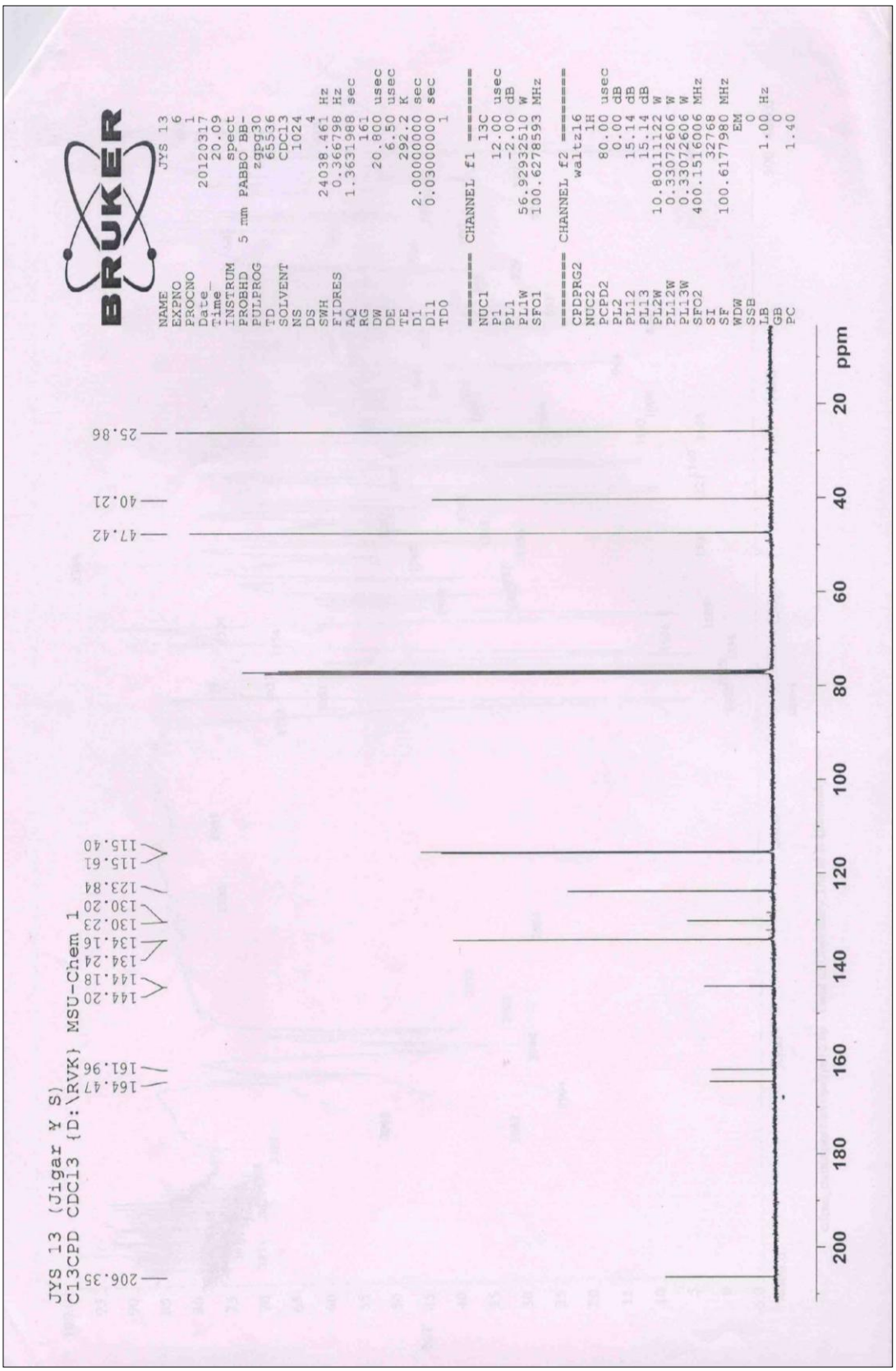


Figure 17: ^{13}C NMR spectra of (Z)-2-(4-fluorobenzylidene)quinuclidin-3-one 2h

Mass Analysis Report

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Injection Vol : 15.00 ul
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Sample Set : 09022015_02

Acquired By : MASS_02
Acq. Method Set : Mass_2013_2
Processing Method: MASS_2013_02__,
Channel Name : MS TIC @1, MS TIC

Date Acquired : 09-Feb-15 9:43:36 AM IST
Date Processed : 09-Feb-15 9:45:45 AM IST, 09-Feb-15 9:45:51 AM IST

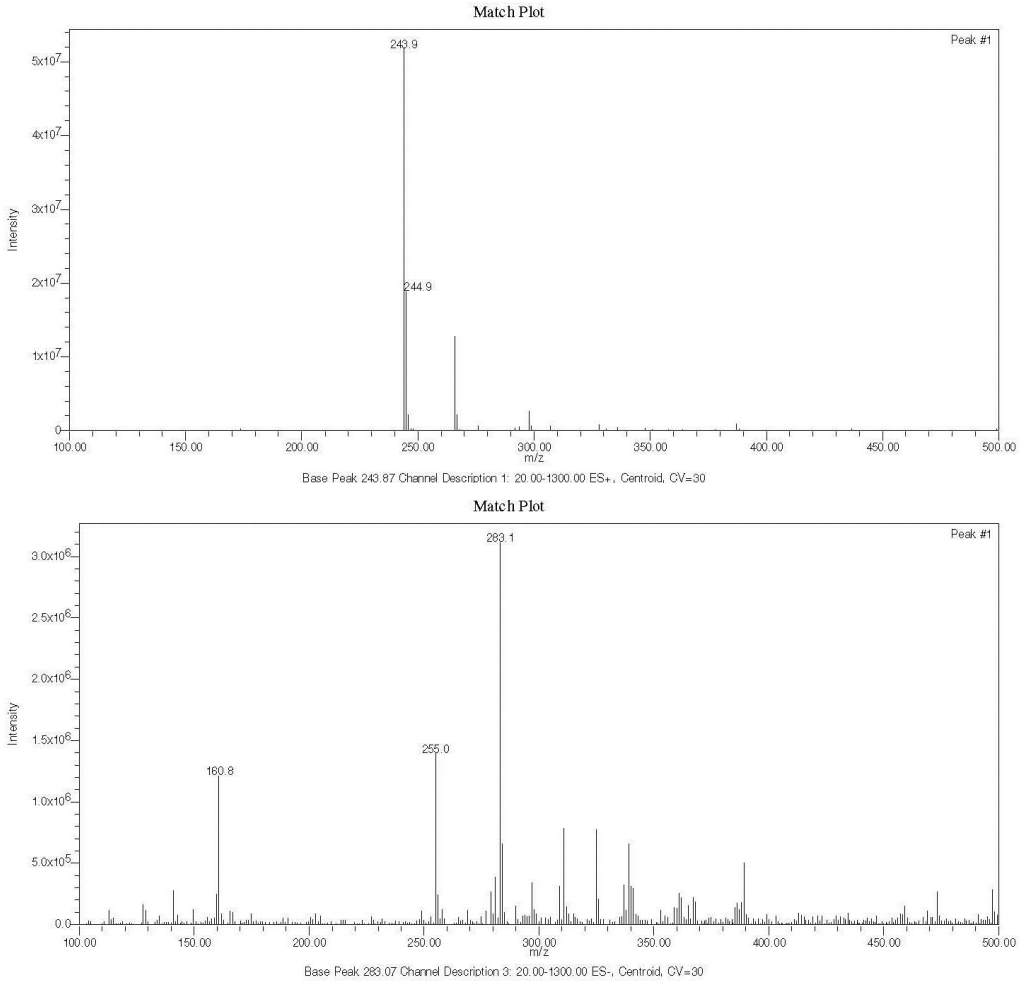
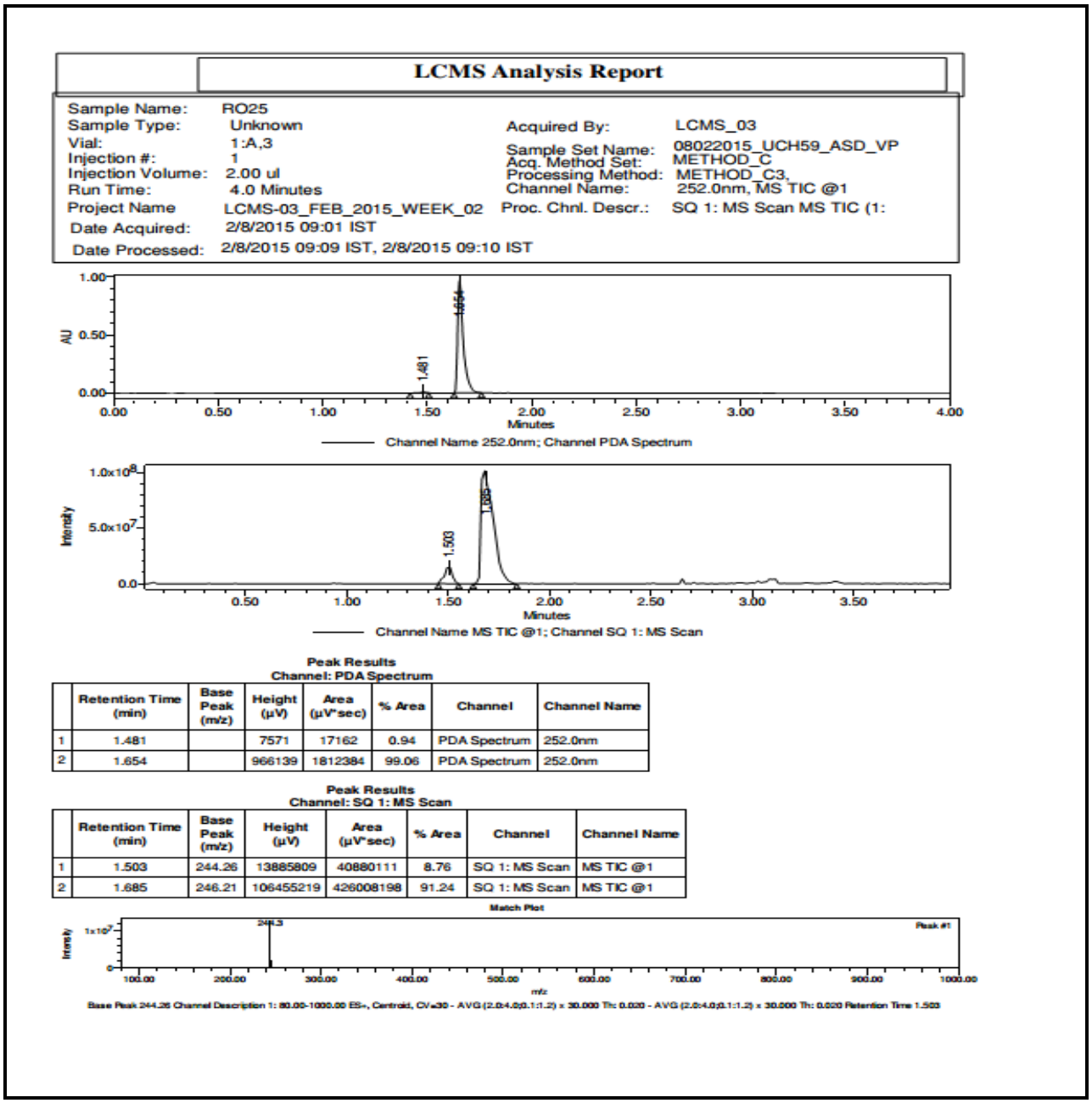


Figure 18: Mass spectra of (Z)-2-(4-fluorobenzylidene)quinuclidin-3-one 2h



	Retention Time (min)	Base Peak (m/z)	Height (µV)	Area (µV*sec)	% Area	Channel	Channel Name
1	1.481		7571	17162	0.94	PDA Spectrum	252.0nm
2	1.654		966139	1812384	99.06	PDA Spectrum	252.0nm

	Retention Time (min)	Base Peak (m/z)	Height (µV)	Area (µV*sec)	% Area	Channel	Channel Name
1	1.503	244.26	13885809	40880111	8.76	SQ 1: MS Scan	MS TIC @1
2	1.685	246.21	106455219	426008198	91.24	SQ 1: MS Scan	MS TIC @1

Figure 19: LCMS spectra of (Z)-2-(4-fluorobenzylidene)quinuclidin-3-one 2h

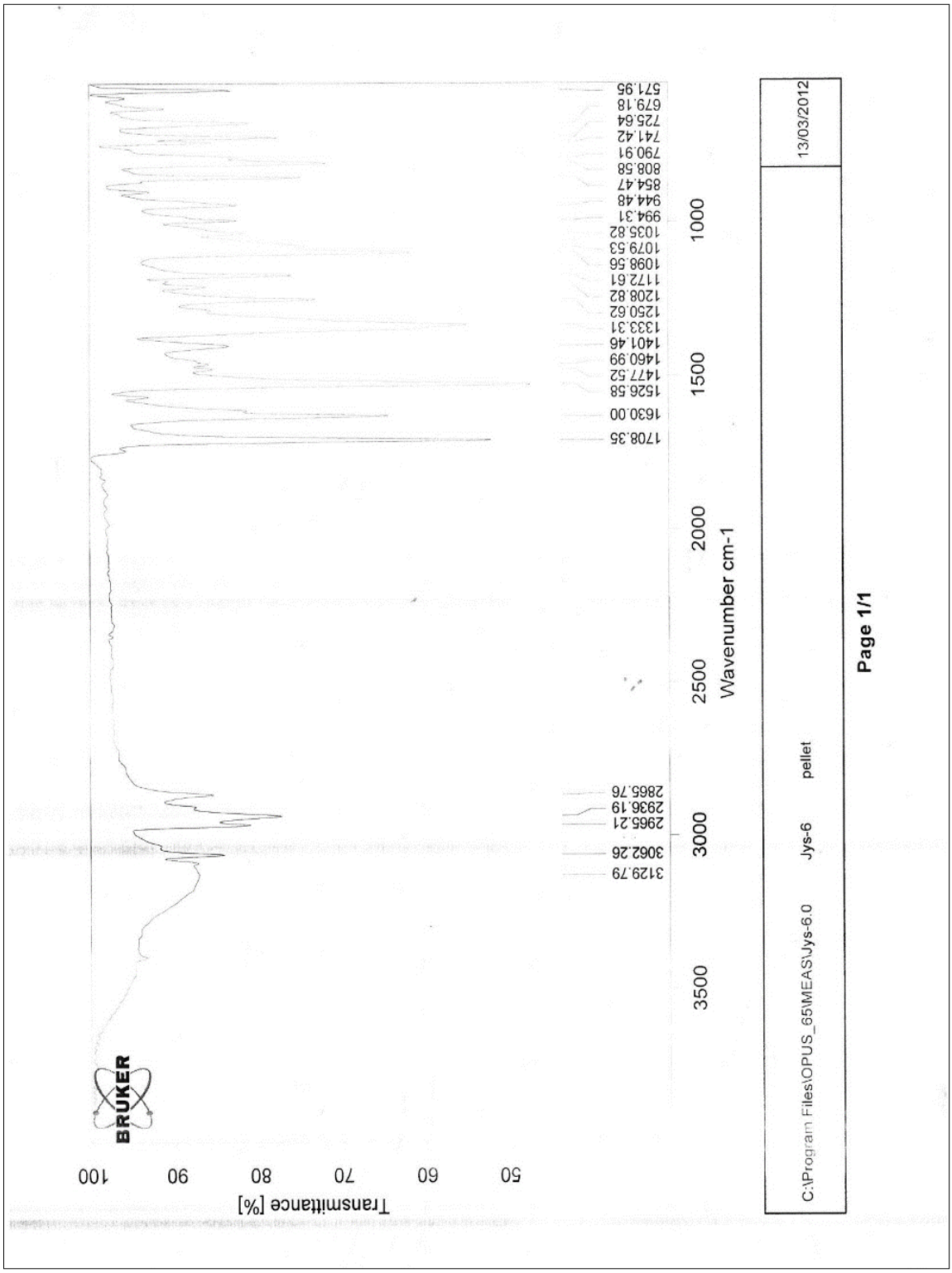


Figure 20: IR spectra of (Z)-2-(3-nitrobenzylidene)quinuclidin-3-one 2m

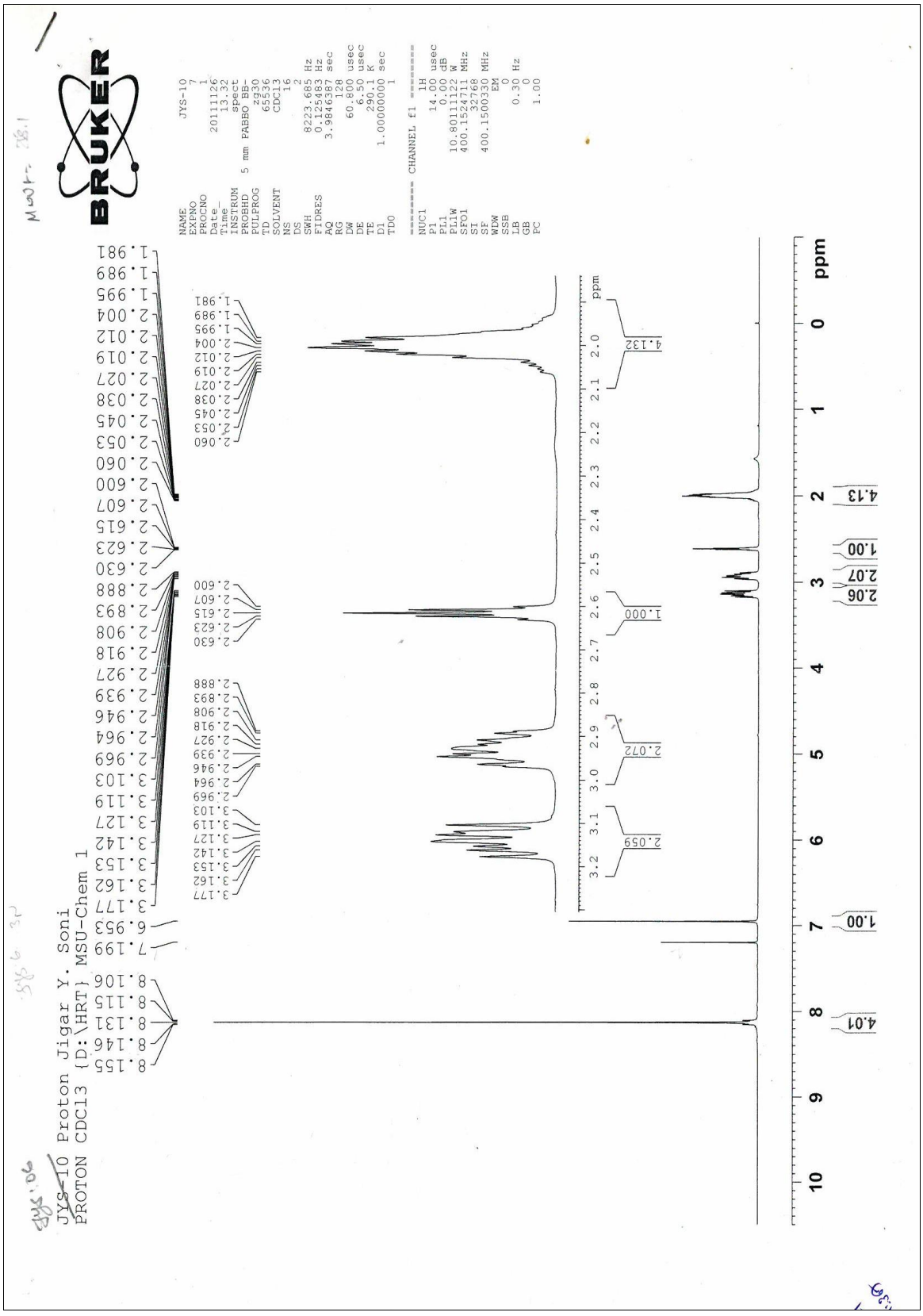


Figure 21: ^{1}H NMR spectra of (Z)-2-(3-nitrobenzylidene)quinuclidin-3-one 2m

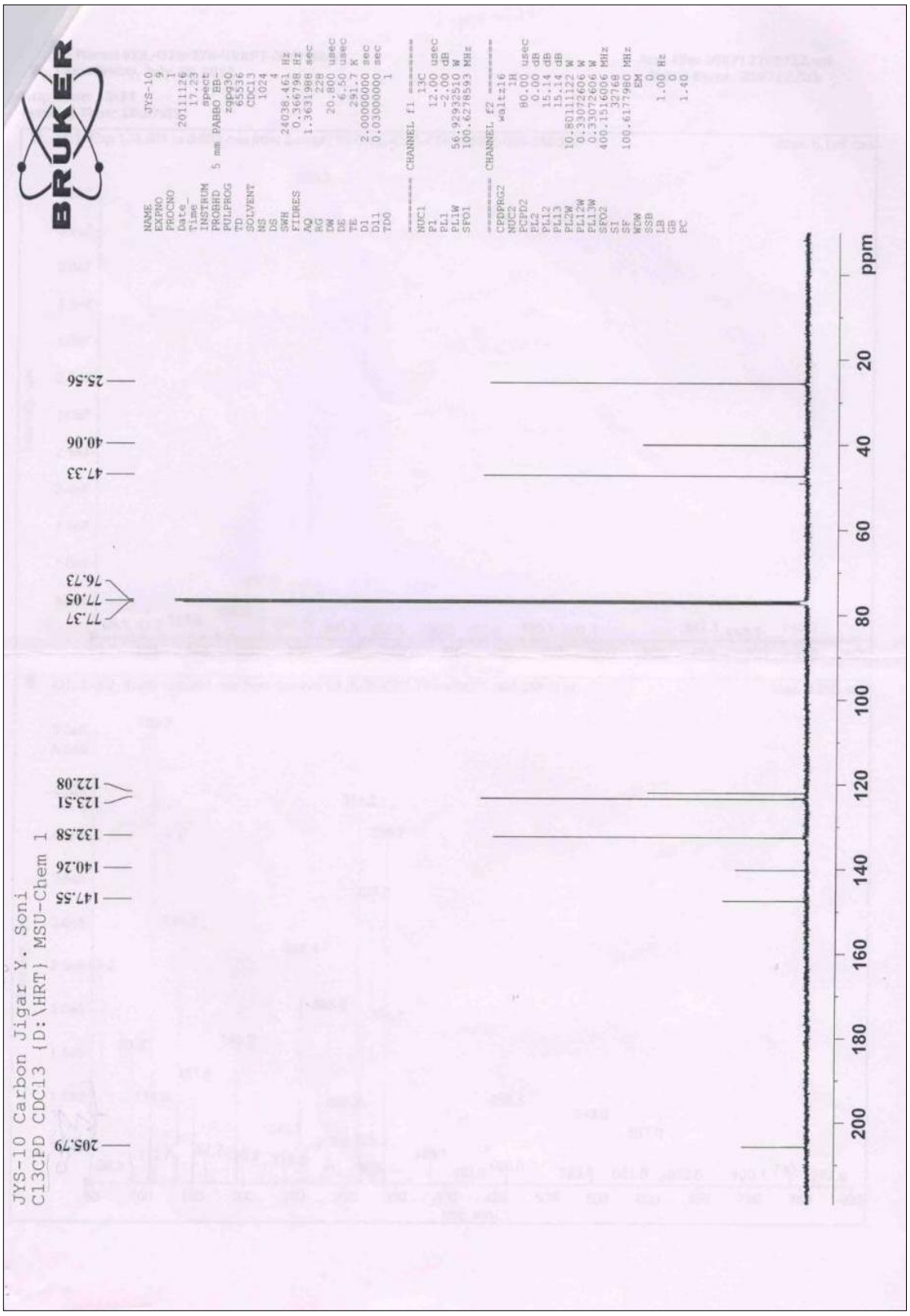


Figure 22: ^{13}C NMR spectra of (Z)-2-(3-nitrobenzylidene)quinuclidin-3-one 2m

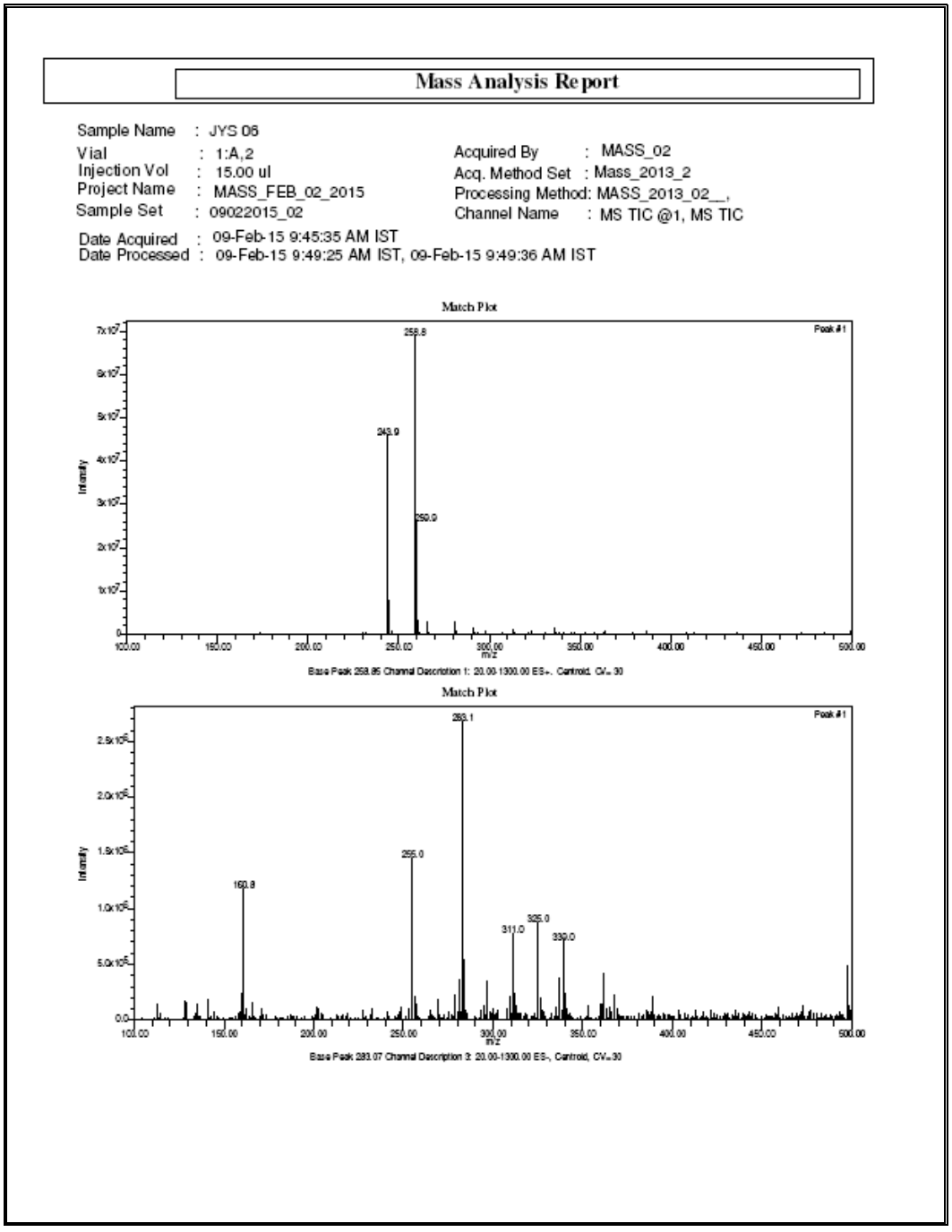


Figure 23: Mass spectra of (Z)-2-(3-nitrobenzylidene)quinuclidin-3-one 2m

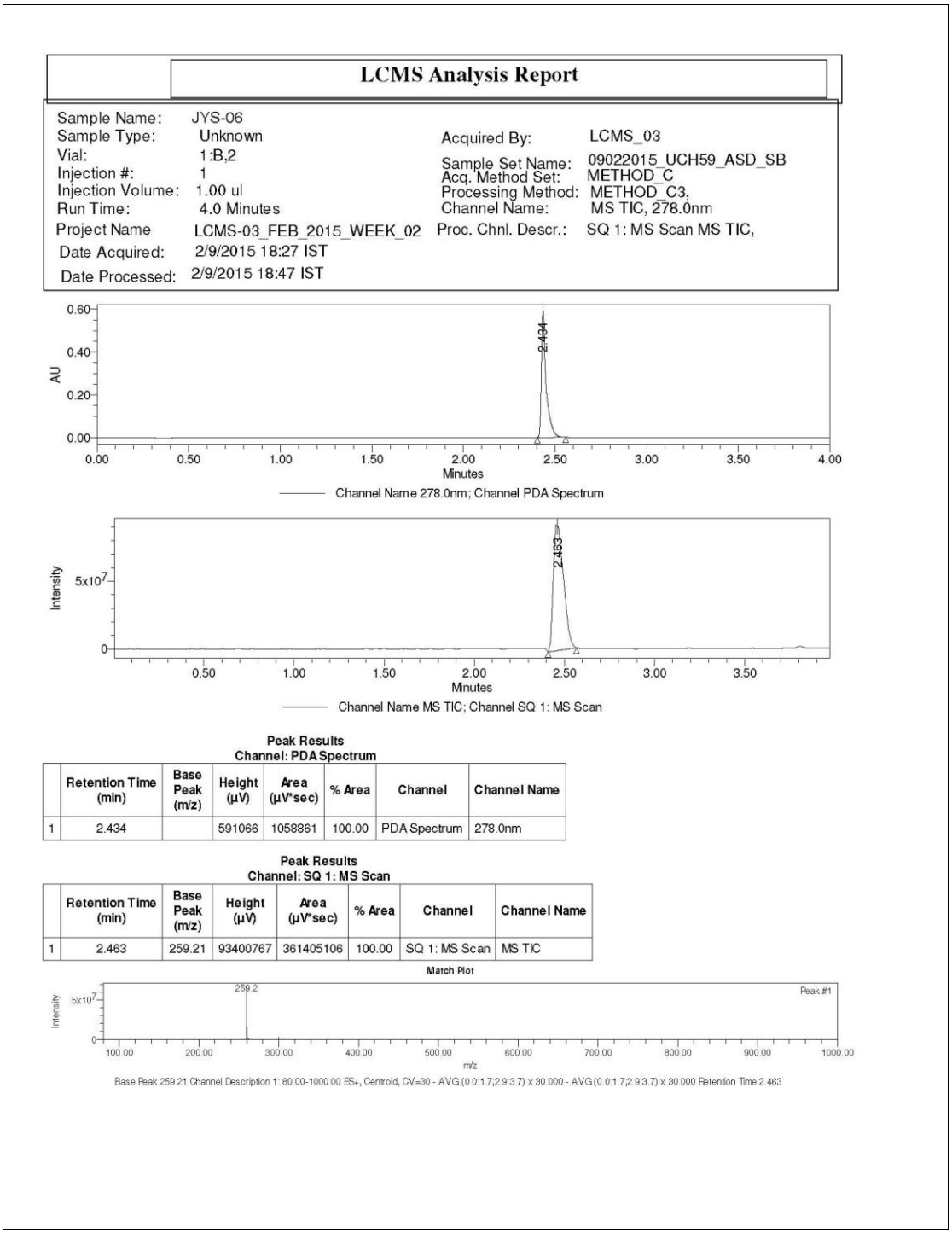
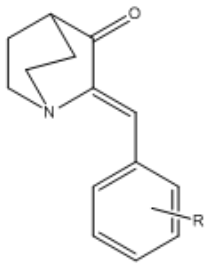


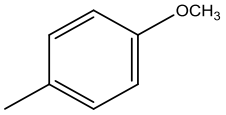
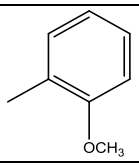
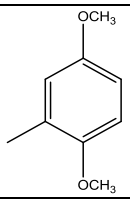
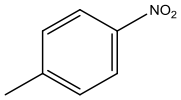
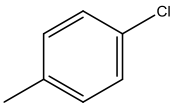
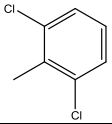
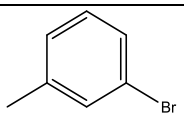
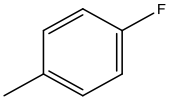
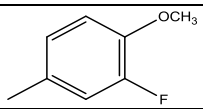
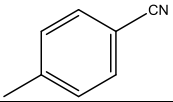
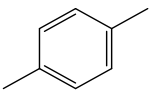
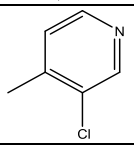
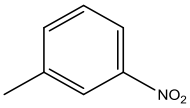
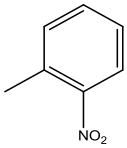
Figure 24: LCMS spectra of (Z)-2-(3-nitrobenzylidene)quinuclidin-3-one 2m

3A.2 Biological activity

The synthesized compounds were screened for MTT assay. All compounds were screened at 1, 5, 10 and 20 µM concentrations. The results obtained in a cytotoxicity assay for quinuclidinone and its arylidinone quinuclidinone analogues accounted for decreased cell viability in a dose dependent manner. The results also suggest that the synthesized compounds showed specificity for cancer cells over normal lung cells. Cancer cells were stained with fluorescent stains to gain a deeper insight into the mechanism of cell death. Induction of apoptosis is a key event and a preferred pathway for induction of cell death by a test compound.[3] Hence, a fluorescent probe (AO/EB) was used to gather qualitative evidence on apoptosis. It has been reported that the viable cells show green fluorescence and late apoptotic cells show orange to red fluorescence with condensed chromatin.[4]

Table 1: IC_{50} values of the compounds 4a-f and 5a-e



Compound	Structure	IC_{50} (µM)	Compound	Structure	IC_{50} (µM)
2a		3.79	2b		--
2c		--	2d		1.66
2e		3.96	2f		1.38
2g		0.01	2h		1.16
2i		--	2j		2.04
2k		1.16	2l		2.74
2m		0.07	2n		1.55

3A.2.1 Structure Activity Relationship (SAR)

Derivative with *para*methoxy group **2a** was found to least active compound where as derivative with tolyl group **2k** was increase the activity. While derivative having cyano group **2j** is found to be moderately active. Compound having nitro group on *meta* position **2m** was found to be most active compound where as nitro group on *ortho* and *para* position **2n** and **2d** decrease the activity. The derivative having bromo group **2g** was found to be most active where as derivative having chloro and fluoro **2e** and **2h** groups on was found to be moderately active. Compound having chloropyridine ring **2l** decrease the activity. In conclusion among all compounds 3-nitro and 3-Bromo were found to be more potent while compound 4-chloro was least potent.

3A.3.Experimental

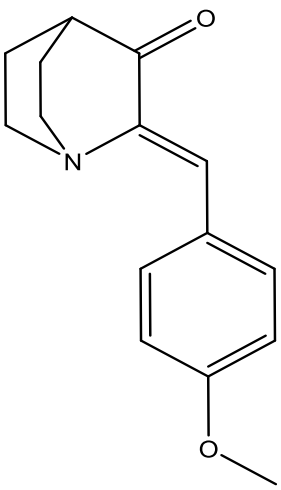
3A.3.1Chemistry

Commercial grade solvents and reagents (alcohols and amines) were purchased from Sigma Aldrich or Alfa aesar or Spectrochem Mumbai India and used without further purification. Quinuclidinone Hydrochloride was prepared as described in literature.[5,6] Melting points were measured using a (Buchi B-545) melting point apparatus and were uncorrected. Infrared spectra were recorded on a Perkin-Elmer RX 1 spectrometer. Elemental analyses were recorded on Thermo finnigan Flash 11-12 series EA.^{1}H and ^{13}C NMR spectra were recorded on an Advance Bruker (400 MHz) spectrometer in suitable deuterated solvents. ^{1}H NMR data were recorded as follows: chemical shift measured in parts per million (ppm) downfield from TMS multiplicity, observed coupling constant (*J*) in Hertz (Hz), proton count. Multiplicities are reported as singlet (s), broad singlet (br s), doublet (d), triplet (t), quartet (q) and multiplet (m).

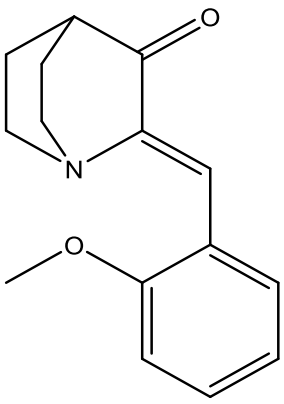
^{13}C NMR chemical shifts are reported in ppm downfield from TMS. Solvents and reagents were purified by literature methods. Mass spectra were determined by LC/MS, using Shimadzu LCMS 2020 and AB Sciex 3200 QTRAP. The reaction progress was monitored by TLC in ultraviolet light as well as with iodine vapour.

General procedure for synthesis of 2-arylidine quinuclidinone

3-quinuclidinone hydrochloride was refluxed with substituted various aromatic aldehyde in the presence of sodium hydroxide by using absolute alcohol as a solvent to give title compound as a yellow-yellowish brown product as shown in **Scheme 1.**

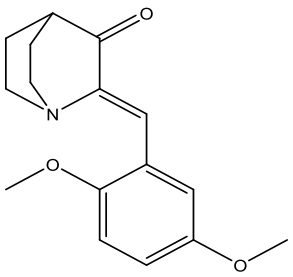


(Z)-2-(4-methoxybenzylidene)quinuclidin-3-one2a: Yellow solid, mp 105-108ºC (rep 106-108ºC)[7]; yield 78%. IR (KBr,cm^{-1}): 3061, 3024, 2950, 2835, 1699, 1255. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.99-2.04 (4H, m, CH_2); 2.61-2.63 (1H, m, CH); 2.94-3.02 (2H, m, CH_2); 3.12-3.19 (2H, m, CH_2); 3.84 (3H, s, OCH_3); 6.90 (2H, d, *J*= 8.8 Hz, ArH); 6.99 (1H, s, vinylic proton); 8.03 (2H, d, *J*= 9.2 Hz, ArH). ^{13}C NMR (100 MHz, $CDCl_3$): δ 26.0, 40.3, 47.5, 55.3, 55.4, 93.3, 113.9, 114.2, 114.5, 125.1, 126.7, 129.1, 130.1, 134.0, 134.2, 142.7, 150.1, 160.7, 206.5. ESI/MS 244.2 $[M+1]^+$ calculated for $C_{15}H_{17}NO_2$. Anal.Calcd. for $C_{15}H_{17}NO_2$: C, 74.05; H, 7.04; N, 5.76; Found: C, 74.29; H, 6.83; N, 5.61.

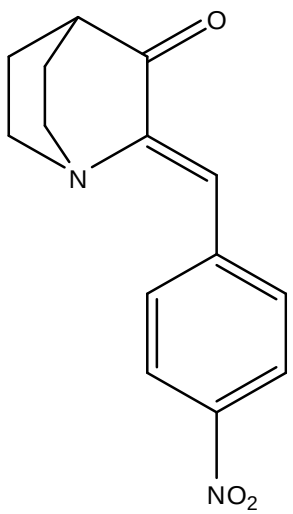


(Z)-2-(2-methoxybenzylidene)quinuclidin-3-one2b: Yellow solid, mp 112-114ºC; yield 82%. IR (KBr,cm^{-1}) (Figure 1): 3069, 3031, 3011, 2955, 2837, 1706, 1040. ^{1}H NMR (400 MHz, $CDCl_3$) (Figure 2): δ 2.02-2.07 (4H, m, CH_2); 2.64-2.66 (1H, m, CH); 2.98-3.05 (2H, m, CH_2); 3.15-3.22 (2H, m,

CH_2); 3.84 (3H, s, OCH_3); 6.93 (1H, dd, *J*= 3.2 Hz, ArH) 7.00 (1H, s, vinylic proton); 7.28 (1H, t, J= 1.6 Hz, ArH); 7.54 (1H, d, *J*= 7.6 Hz, ArH); 7.81 (1H, s, ArH). ^{13}C NMR (100 MHz, $CDCl_3$) (Figure 3): δ 25.8, 40.3, 47.4, 55.2, 115.4, 117.0, 124.9, 125.0, 129.3, 135.2, 145.0, 159.4, 159.9, 206.5. ESI/MS (Figure 4) 244.2 [M+1]+ calculated for $C_{15}H_{17}NO_2$. Anal.Calcd. for $C_{15}H_{17}NO_2$: C, 74.05; H, 7.04; N, 5.76; Found: C, 73.89; H, 7.28; N, 5.89.

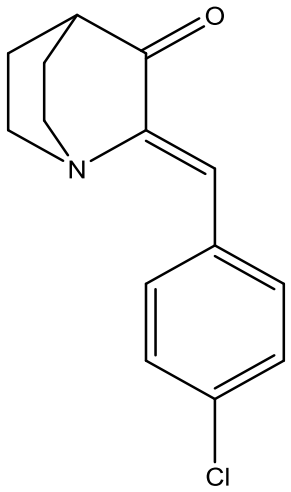


(**Z)-2-(2,5-dimethoxybenzylidene)quinuclidin-3-one 2c:** Yellow solid, mp 128-130ºC; yield 73%. IR (KBr, cm^{-1}) (Figure 6): 3308, 3080, 3015, 2945, 2835, 1698, 979. ^1H NMR (400 MHz, $CDCl_3$) (Figure 7): δ 1.93-1.96 (4H, m, CH_2); 2.54-2.57 (1H, m, CH); 2.88-2.95 (2H, m, CH_2); 3.04-3.11 (2H, m, CH_2); 3.71 (3H, s, OCH_3); 3.74 (3H, s, OCH_3); 6.73 (1H, d, *J*= 8.8 Hz, ArH); 6.80 (1H, d, *J*= 12 Hz, ArH); 7.19 (1H, s, vinylic proton); 7.45 (1H, s, ArH) 8.23 (1H, d, *J*= 2.8 Hz, ArH). ^{13}C NMR (100 MHz, $CDCl_3$) (Figure 8): δ 25.9, 40.3, 47.5, 55.7, 56.1, 111.4, 116.1, 117.8, 118.7, 123.3, 144.5, 153.0, 153.7, 206.3. ESI/MS (Figure 9) 274.3 $[M+1]^+$ calculated for $C_{16}H_{19}NO_3$. Anal.Calcd. for $C_{16}H_{19}NO_3$: C, 70.31; H, 7.01; N, 5.12; Found: C, 70.12; H, 7.23; N, 4.99.

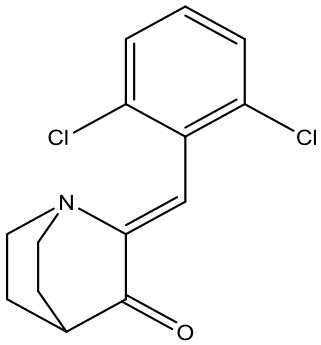


(**Z)-2-(4-nitrobenzylidene)quinuclidin-3-one 2d:** Yellow solid, mp 127-129ºC (rep 126-128ºC)[8]; yield 48%. IR (KBr, cm^{-1}): 3310, 3062, 1708, 1526, 1333, 1098, 994, 854, 679. ^1H NMR (400 MHz, $CDCl_3$): δ 1.98-2.06 (4H, m, CH_2); 2.60-2.63 (1H, m, CH); 2.88-2.96 (2H, m, CH_2); 3.10-3.17 (2H, m, CH_2); 6.95 (1H, s, vinylic proton); 8.10-8.15 (4H, s, ArH). ^{13}C NMR (100 MHz, $CDCl_3$): δ 32.8, 34.2, 46.4, 100.5, 124.1, 126.0, 139.6, 147.1, 149.1, 150.7. ESI/MS 259.3 $[M+1]^+$ calculated for

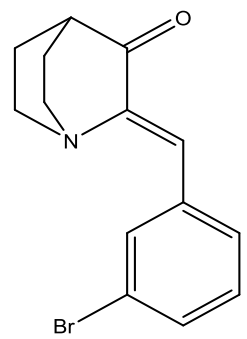
$C_{14}H_{14}N_2O_3$. Anal.Calcd. for $C_{14}H_{14}N_2O_3$: C, 65.11; H, 5.46; N, 10.85; Found: C, 65.35; H, 5.29; N, 10.68.



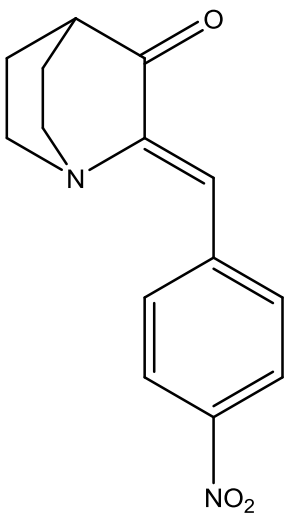
(**Z)-2-(4-chlorobenzylidene)quinuclidin-3-one 2e:** Yellow solid, mp 114-116ºC (rep 112.5-114.5ºC)[9]; yield 83%. IR (KBr, cm^{-1}): 1709, 1308, 1166, 1096. ^1H NMR (400 MHz, $CDCl_3$): δ 2.06-2.08 (4H, m, CH_2); 2.64-2.67 (1H, m, CH); 2.94-3.03 (2H, m, CH_2); 3.13-3.21 (2H, m, CH_2); 6.97 (1H, s, vinylic proton); 7.33-7.36 (2H, m, ArH); 7.98-8.02 (2H, m, ArH). ^{13}C NMR (100 MHz, $CDCl_3$): δ 25.8, 40.2, 47.4, 123.7, 128.7, 133.3, 135.4, 145.0, 206.2. ESI/MS 247.9 $[M]^+$, 249.2 $[M+2]^+$ calculated for $C_{14}H_{14}NOCl$. Anal.Calcd. for $C_{14}H_{14}NOCl$: C, 67.88; H, 5.70; N, 5.65; Found: C, 68.13; H, 5.47; N, 5.43.



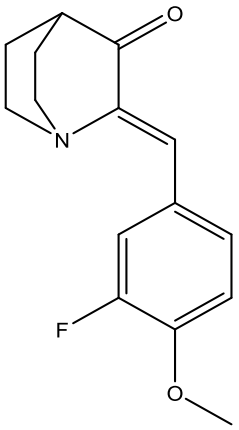
(Z)-2-(2,6-dichlorobenzylidene)quinuclidin-3-one2f: Yellow solid, mp121-123ºC (rep 118-120ºC)[10]; yield 81%. IR (KBr, cm^{-1}) (Figure 10): 3055, 3023, 1713, 1103, 979, 851. ^1H NMR (400 MHz, $CDCl_3$) (Figure 11): δ 1.94-2.11 (4H, m, CH_2); 2.67-2.69 (1H, m, CH); 3.02-3.08 (4H, m, CH_2); 7.03 (1H, s, vinylic proton); 7.20 (1H, dd, *J*= 8.4, 8.0 Hz, ArH); 7.33 (2H, d, *J*= 8.0 Hz, ArH). ^{13}C NMR (100 MHz, $CDCl_3$) (Figure 12): δ 25.6, 40.5, 47.3, 122.1, 127.7, 127.8, 129.4, 132.9, 134.1, 147.9 204.8. ESI/MS (Figure 13) 282.2 $[M]^+$, 284.2 $[M+2]^+$calculated for $C_{14}H_{13}NOCl_2$. Anal.Calcd. for $C_{14}H_{13}NOCl_2$: C, 59.59; H, 4.64; N, 4.96; Found: C, 59.73; H, 4.52; N, 4.82.



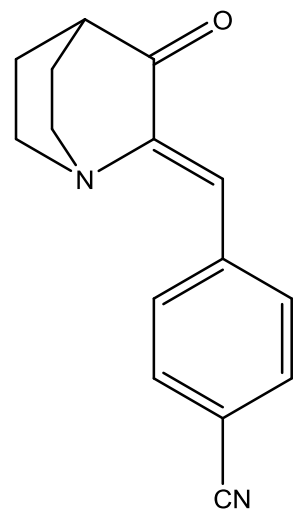
(Z)-2-(3-bromobenzylidene)quinuclidin-3-one 2g: Yellow solid, mp 138-140ºC; yield 79%. IR (KBr, cm^{-1}): 3083, 3043, 2964, 2938, 1701, 978. ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.02-2.07 (4H, m, CH_2); 2.65-2.67 (1H, m, CH); 2.98-3.03 (2H, m, CH_2); 3.15-3.23 (2H, m, CH_2); 6.98 (1H, s, vinylic proton); 7.04 (1H, t, *J*= 2.8, 2, 0.8 Hz, ArH); 7.30-7.36 (1H, m, ArH); 7.60 (1H, d, *J*= 8 Hz, ArH); 8.08 (1H, t, *J*= 8.4 Hz, ArH). ^{13}C NMR (100 MHz, $CDCl_3$): δ 25.7, 40.1, 47.4, 116.4, 116.7, 118.1, 118.3, 112.7, 123.7, 128.1, 128.2, 129.7, 129.8, 135.9, 136.0, 145.6, 161.4, 163.8, 206.2. ESI/MS 292 $[M]^+$, 294 $[M+2]^+$calculated for $C_{14}H_{14}NBrO$. Anal.Calcd. for $C_{14}H_{14}NBrO$: C, 57.55; H, 4.83; N, 4.79; Found: C, 57.32; H, 4.95; N, 4.64.



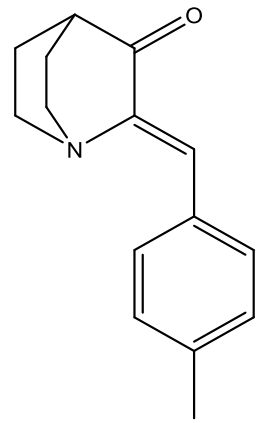
(**Z)-2-(4-fluorobenzylidene)quinuclidin-3-one 2h:** Yellow solid, mp 117-118ºC (rep 118-120ºC)[5]; yield 81%. IR (KBr, cm^{-1}) (Figure 15): 3098, 3060, 1698, 1257, 1221, 1035, 995. ^{1}H NMR (400 MHz, $CDCl_3$) (Figure 16): δ 2.02-2.06 (4H, m, CH_2); 2.64-2.65 (1H, m, CH); 2.95-3.02 (2H, m, CH_2); 3.13-3.20 (2H, m, CH_2); 6.98 (1H, s, vinylic proton); 7.04-7.08 (2H, m, ArH); 8.07 (2H, q, *J*= 12, ArH). ^{13}C NMR (100 MHz, $CDCl_3$) (Figure 17): δ 25.8, 40.2, 47.8, 115.4, 115.6, 123.8, 130.2, 130.2, 134.2, 134.2, 144.1, 144.2, 162.0, 164.5, 206.3. ESI/MS (Figure 18) 232.2 [M+1]+ calculated for $C_{14}H_{14}NOF$. Anal.Calcd. for $C_{14}H_{14}NOF$: C, 72.71; H, 6.10; N, 6.06; Found: C, 72.94; H, 5.93; N, 5.93.



(**Z)-2-(3-fluoro-4-methoxybenzylidene)quinuclidin-3-one 2i:** Yellow solid, mp 108-110ºC; yield 64%; IR (KBr, cm^{-1}): 3093, 3075, 1700, 1304, 1169, 686, 518. ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.01-2.05 (4H, m, CH_2); 2.62-2.64 (1H, m, CH); 2.95-3.00 (2H, m, CH_2); 3.01-3.19 (2H, m, CH_2); 3.92 (3H, S, OCH_3) 6.91-6.95 (2H, m, ArH + vinylic proton); 7.51 (1H, d, *J*= 8.0 Hz, ArH); 8.27 (1H, dd, *J*= 1.6, 2 Hz, ArH). ^{13}C NMR (100 MHz, $CDCl_3$): δ 25.9, 40.2, 47.4, 56.1, 112.5, 118.8, 119, 123.8, 127.28, 127.36, 129.4, 143.8, 148.8, 148.9, 150.6, 153.11. ESI/MS 262.1 [M+1]+ calculated for $C_{15}H_{16}NO_2F$.Anal.Calcd. for $C_{15}H_{16}NO_2F$: C, 68.95; H, 6.17; N, 5.36; Found: C, 68.81; H, 6.39; N, 5.59.

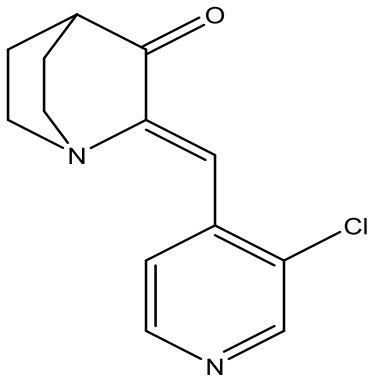


(**Z)-4-((3-oxoquinuclidin-2-ylidene)methyl)benzonitrile 2j:** Yellow solid, mp 157-159ºC[11]; yield 79%. IR (KBr, cm^{-1}): 3083, 3043, 2964, 2938, 2265, 2229, 1701. ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.05-2.07 (4H, m, CH_2); 2.66-2.67 (1H, m, CH); 2.96-3.02 (2H, m, CH_2); 3.15-3.23 (2H, m, CH_2); 6.97 (1H, s, vinylic proton); 7.64 (2H, d, *J*= 8.4 Hz, ArH); 8.13 (2H, d, *J*= 8.4 Hz, ArH). ^{13}C NMR (100 MHz, $CDCl_3$): δ 25.6, 40.0, 47.3, 112.3, 118.8, 122.6, 127.4, 132.0, 132.2, 138.3, 147.1 205.8. ESI/MS 239 [M+1]+ calculated for $C_{15}H_{14}N_2O$. Anal.Calcd. for $C_{15}H_{14}N_2O$: C, 75.61; H, 5.92; N, 11.76; Found: C, 75.79; H, 5.77; N, 11.58.

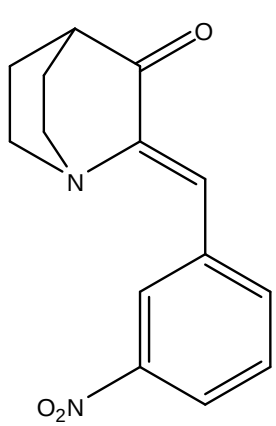


(**Z)-2-(4-methylbenzylidene)quinuclidin-3-one 2k:** Yellow solid, mp 115-117ºC (rep 118-120ºC)[12]; yield 82%. IR (KBr,

cm^{-1}): 3083, 3043, 2964, 2938, 1701.^{1}H NMR (400 MHz, $CDCl_3$): δ 2.03-2.09 (4H, m, CH_2); 2.39 (3H, S, CH_3) 2.67-2.68 (1H, m, CH); 2.96-3.06 (2H, m, CH_2); 3.16-3.24 (2H, m, CH2); 7.04 (1H, s, vinylic proton); 7.20 (2H, dd, *J*= 8.0 Hz, ArH); 7.94 (2H, d, *J*= 8.4 Hz, ArH). ^{13}C NMR (100 MHz, $CDCl_3$): δ 21.6, 25.8, 40.2, 47.6, 124.7, 125.4, 129.2, 132.1, 140.1 206.4. ESI/MS 228.2 $[M+1]^+$ calculated for $C_{15}H_{17}NO$. Anal.Calcd. for $C_{15}H_{17}NO$: C, 79.26; H, 7.54; N, 6.16; Found: C, 79.01; H, 7.69; N, 6.30.

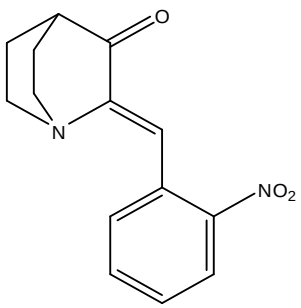


(**Z)-2-((3-chloropyridine-4-yl)methylene) quinuclidin-3-one 2l:** Brown solid, mp 120-122ºC; yield 46%. IR (KBr, cm^{-1}): 3080, 3010, 1709, 1600, 1430. ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.02-2.09 (4H, m, CH_2); 2.67-2.68 (1H, m, CH); 2.96-3.04 (2H, m, CH_2); 3.16-3.24 (2H, m, CH_2); 6.92 (1H, s, vinylic proton); 7.84 (1H, dd, *J*= 7.2, 1.6 Hz, ArH); 8.62 (2H, d, *J*= 4.4, 1.6 Hz, ArH). ^{13}C NMR (100 MHz, $CDCl_3$): δ 25.5, 40.0, 47.3, 122.1, 125.4, 140.9, 148.4, 150.1, 205.7. ESI/MS 249 $[M]^+$, 251$[M+2]^+$ calculated for $C_{13}H_{13}N_2OCl$.Anal.Calcd. for $C_{13}H_{13}N_2OCl$: C, 62.78; H, 5.27; N, 11.26; Found: C, 62.99; H, 5.00; N, 11.41.



(**Z)-2-(3-nitrobenzylidene)quinuclidin-3-one 2m:** Yellow solid, mp 123-125ºC; yield 44%.IR (KBr, cm^{-1}) (Figure 20): 3062, 1708, 1526, 1338, 1098, 994, 854, 679. ^{1}H NMR (400 MHz, $CDCl_3$) (Figure 21): δ 1.98-2.06 (4H, m, CH_2); 2.60-2.63 (1H, m, CH); 2.89-2.96 (2H, m, CH_2); 3.10-3.17 (2H, m, CH_2); 6.95 (1H, s, vinylic proton); 8.10-8.15 (4H, s, ArH). ^{13}C NMR (100 MHz, $CDCl_3$) (Figure 22): δ 25.5, 40.1, 47.3, 122.1, 123.5, 132.6,

140.3, 147.5, 205.8. ESI/MS (Figure 23) 259.3 $[M+1]^+$calculated for $C_{14}H_{14}N_2O_3$. Anal.Calcd. for $C_{14}H_{14}N_2O_3$: C, 65.11; H, 5.46; N, 10.85; Found: C, 64.89; H, 5.59; N, 10.99.



(Z)-2-(2-nitrobenzylidene)quinuclidin-3-one 2n: Yellow solid, mp 120-122ºC; yield 38%. IR (KBr, cm^{-1}): 3318, 3042, 2940, 2921, 2862, 1556, 1702,1319, 893, 705.^{1}H NMR (400 MHz, $CDCl_3$): δ 2.02-2.07 (4H, m, CH_2); 2.66-2.69 (1H, m, CH); 2.90-2.98 (2H, m, CH_2); 3.06-3.13 (2H, m, CH_2); 7.38 (1H, s, vinylic proton); 7.46-7.50 (1H, m, ArH); 7.60-7.65 (1H, m, ArH); 7.90 (1H, dd, *J*= 8.0, 1.2 Hz, ArH); 7.98-8.00 (1H, dd, *J*= 8.0, 1.2 Hz, ArH). ^{13}C NMR (100 MHz, $CDCl_3$): δ 25.4, 40.1, 47.5, 121.3, 124.4, 128.9, 129.3, 132.5, 132.7, 146.2, 149.2, 205.1. ESI/MS 259.3$[M+1]^+$ calculated for $C_{14}H_{14}N_2O_3$.Anal.Calcd. for$C_{14}H_{14}N_2O_3$: C, 65.11; H, 5.46; N, 10.85; Found: C, 65.36; H, 5.19; N, 10.71.

3A.3.2 Biological assay

3A.3.2.1 Cell line and culture

The A549 and L132 cell lines were obtained from the National Center for Cell Sciences, Pune whereas Dubecoos Modified Essential Medium (DMEM), Fetus Bovine Serum (FBS) and antimycotic–antibiotic solution were obtained from HiMedia. The human cell lines A549 and L132 were seeded in T-25 flask with DMEM, 10% FBS and 1% antimycotic-antibody solution in a humidified atmosphere supplied with 5% CO_2 at 37^0C. Cells were subsequently sub-cultured every third day by trypsinization with 0.25% Trypsin versus glucose solution. Both the cell lines were utilized to examine the antitumor activity of testing compound at varying concentration.

3A.3.2.2 Cell viability assay

The IC_{50} values of cell proliferation were determined using MTT assay. Quinuclidinone and its derivatives were dissolved in 0.5% dimethyl sulfoxide and subsequent doses were prepared in the media. A549 cells were seeded in 96 well culture plates and were treated with different concentrations of the compounds for 24 h. The positive control cells were treated with quinuclidinone in culture medium at subsequent doses. At the end of the incubation period 100µl of 3-(4, 5-dimethylthiazol-2-yl) -2, 5-diphenyl tetrazolium bromide (MTT; $1mgml^{-1}$) was added to the wells and plates were incubated at 37^0C for 4 h. Later, culture medium was discarded and 150µl DMSO was added. Absorbance was read at 540nm in ELX800 Universal Microplate Reader.

3A.4 Conclusion

2-arylidinone quinuclidinone compounds were Synthesized and screened for their anti-cancer activity by using A 549 (Lung carcinoma cell) and normal cell (L132). IC_{50} values were determined and SAR was established. Among the synthesized compounds compound having 3 nitro-2-arylidine quinucliione and 3-Bromo-2-arylidinone quinuclidinone were found to be more potent while compound p-chloro-2-arylidine quinuclidinone was least potent among the all compound.

References

[1] Horenstein, N. A., Leonik, F. A., Papke, R. L., Mol. Pharmacol., 2008, 74, 6, 1496.

[2] Merck GmbH Patent EP0576974B1.

[3] Thakore, S., Valodkar, M., Soni, J. Y., Vyas, K., Jadeja, R. N., Devkar, R. V., Rathore, P. S., Bioorg. Chem., 2013, 46, 26.

[4] Valodkar, M., Jadeja, R. N., Thounaojam, M. C., Devkar, R. V., Thakore, S., Mater Chem. Phys., 2011, 128, 83.

[5] Soni J. Y., Premasagar, V, Thakore, S. I., Lett. Org. Chem., 2015, 15, 277.

[6] Soni, J. Y., Sanghvi, S., Devkar, R. V., Thakore, S. I., RSC Advances, 2015, 5, 82112, 18.

[7] Mazurov, A., Klucik, J., Miao, L,. Phillips, T. Y., Seamans, A., Schmitt, J. D., Hauser, T. A., Johnson Jr., R. T., Miller, C., Bioorg. Med. Chem. Lett., 2005, 15, 2073.

[8] Gorbyleva, O. I., Filipenko, T. Y., Mikhlina, E. E., Turchin, K. F., Sheinker, Y. N., Yakhontov, L. N., Chem. Heterocycl. Compd., 1982, 18, 601.

[9] Warawa, E. J., Mueller, N. J., Gylys, J. A., J. Med. Chem.,1975, 18, 71.

[10] Coffen, D. L., Katonak, D. A., Wong, F. J., Am. Chem. Soc., 1974, 96, 3966.

[11] Davis, B. N., Abelt, C. J., J. Phys. Chem. A., 2005, 109, 1295.

[12] Sonar, V. N., Venkataraj, M., Parkin, Crooks, S. P., Acta Crystallogr. Sect. E Struct. Reports Online, 2006, 62, 05576.

Chapter 3B

Synthesis, characterization and evaluation of (Z)-4-((3-Oxoquinuclidin-2-yldine)methyl) benzoic acid derivatives as anti-proliferative agents

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Synthesis and evaluation of novel quinuclidinone derivatives as potential anti-proliferative agents†

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In this study a new series of substituted (*Z*)-4-(3-oxoquinuclidin-2-ylidene) benzamides and substituted (*Z*)-4-((3-oxoquinuclidin-2-ylidene)methyl)benzoates have been designed and synthesised as potential anti-cancer agents. This set of compounds were prepared by using a common intermediate (*Z*)-4-((3-oxoquinuclidin-2-ylidene)methyl)benzoic acid. They were well characterized by various spectroscopic techniques as well as a crystallographic study and screened for anti-cancer activity. A cell viability assay using MTT was performed on A549 & L132 cell lines and IC_{50} values were determined. Analogues **4c** and **5e** exhibited the most potent anti-cancer activity among all the analogues synthesized in this present study. A haemolytic assay using normal human erythrocytes was performed to study the blood compatibility of the compounds. Acridine orange/ethidium bromide (AO/EB) staining also showed cell death. To get a better insight into the mechanism of cell death DAPI (4′,6-diamido-2-phenylindol nuclear staining) and DNA fragmentation studies were performed. A Structure Activity Relationship (SAR) was explored to facilitate further development of this new class of compounds.

1 Introduction

Cancer, a serious health problem, is one of the main causes of mortality in the developing as well as developed countries.[1] Different theories have been proposed for the cause of cancer and several strategies have been formulated and examined for combating the disease. Survival rates for five years of some cancers have significantly improved in the past two decades while those of other cancers, such as lung and pancreatic cancer remain low.[2] The major form of cancer treatment is chemotherapy, alone or combined with radiation. However, the majority of cancers develop resistance to chemotherapy during treatment. As a result, the design and discovery of non-traditional, efficient and safe classes of chemical agents are the prime targets in contemporary medicinal chemistry.[3]

The molecule 3-quinuclidinone hydrochloride possesses variety of biological spectrum and is a part of many existing drugs such as azasetron, palonosetron, solifenacin, cevimeline, quinupramine (Fig. 1). Literature survey revealed that several derivatives of quinuclidines have been reported to show wide range of biological activity such as Alzheimer's disease[4] chronic obstructive pulmonary disease,[5] antihistamine-bronchodilating agents,[6] α7 and α4β2 nicotinic receptors inhibitory activity.[7] According to Malki *et al.* analogs of quinuclidinone can provide an excellent scaffold for novel anti-cancer agents with improved safety profile.[8] They used lung carcinoma cells for study and observed that in more potent derivatives, the carbonyl group of quinuclidinone was intact.[8] Further in another study they observed that quinuclidinone derivatives induce apoptosis in human breast cancer cells *via* reduced expression level of Bcl-2, Bcl-XL and increased mitochondrial apoptotic pathways by activating the release of cytochrome C.[9] The derivatives of this molecule may have a selective mode of action as they are structurally unique, and yet have a great deal of known chemistry upon which to prepare analogs. In search of novel more potent anti-cancer compounds with greater affinity for cancer cells than healthy normal cells, we decided to explore the anti-cancer activity of some novel quinuclidinone derivatives. We have recently reported the synthesis of quinuclidinone hydrochloride from isonipecotic acid.[10] In the present article we report the synthesis of some quinuclidinone based ester and amide derivatives with cytotoxicity and apoptosis-inducing property in lung cancer cells.

2 Result and discussion

2.1 Synthesis

In the first step 3-quinuclidinone hydrochloride (**1**) was refluxed with 4-formyl benzoic acid (**2**) in the presence of sodium hydroxide, using absolute ethanol as solvent to give 4-(3-oxo-1-aza-bicyclo[2.2.2]oct-2-ylidenemethyl)-benzoic acid (**3**) (Scheme 1).

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† Electronic supplementary information (ESI) available. CCDC 1025491 and 1051487 contains the crystallographic data for the compounds **4c** and **5c** respectively. For ESI and crystallographic data in CIF or other electronic format see DOI: 10.1039/c5ra15127a

Part: B

3B.1 Introduction

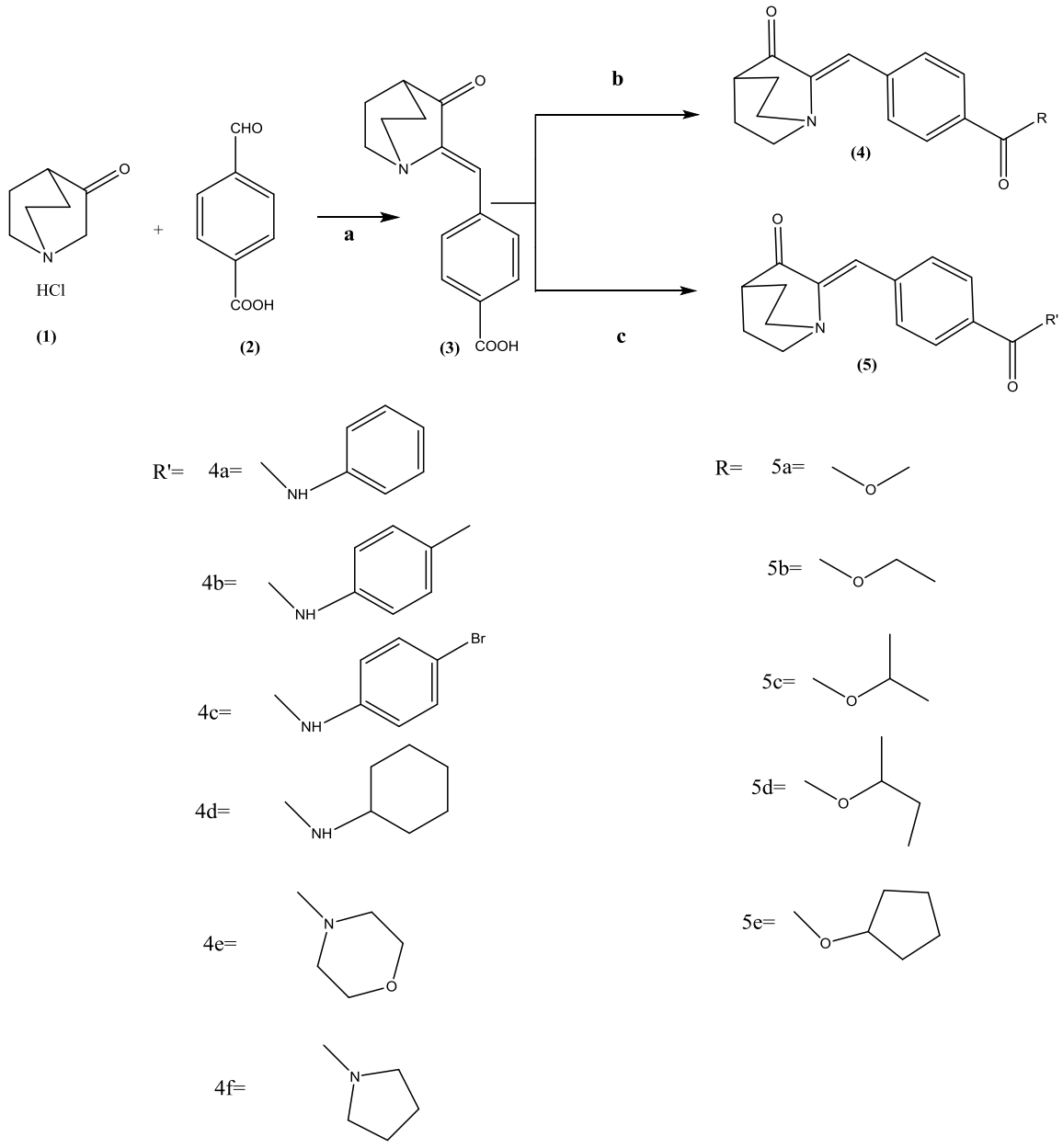
Literature survey revealed that several derivatives of quinuclidines have been reported to show wide range of biological activity such as Alzheimer's disease[1] chronic obstructive pulmonary disease,[2] Antihistamine-Bronchodilating Agents,[3] α7 and α4β2 nicotinic receptors inhibitory activity.[4] Analogs of quinuclidinone can provide an excellent scaffold for novel anti-cancer agents with improved safety profile.[5] They used lung carcinoma cells for study and observed that in more potent derivatives, the carbonyl group of quinuclidinone was intact.[5] Further in another study they observed that quinuclidinone derivatives induce apoptosis in human breast cancer cells via reduced expression level of Bcl-2, Bcl-XL and increased mitochondrial apoptotic pathways by activating the release of cytochrome C.[6] The derivatives of this molecule may have a selective mode of action as they are structurally unique and yet have a great deal of known chemistry upon which to prepare analogs. In search of novel more potent anti-cancer compounds with greater affinity for cancer cells than healthy normal cells, we decided to explore the anti-cancer activity of some novel quinuclidinone derivatives. We have recently reported the synthesis of quinuclidinone hydrochloride from isonepecotic acid.[7] In the present article we report the synthesis of some quinuclidinone based ester and amide derivatives with cytotoxicity and apoptosis-inducing property in lung cancer cells.

3B.2 Result and discussion

3B.2.1 Chemistry

In the first step 3-quinuclidinone hydrochloride **1** was refluxed with 4-formyl benzoic acid **2** in the presence of sodium hydroxide, using absolute ethanol as solvent to give

4-(3-oxo-1-aza-bicyclo[2.2.2]oct-2-ylidenemethyl)-benzoic acid **3** as shown in Scheme 1.



Scheme 1: Synthetic route for compounds 4a-f and 5a-e

Reagent and conditions: (a) NaOH, EtOH, reflux; (b) (1) MDC, TEA, $SOCl_2$, reflux; (2) R–NH_2, ACN, K_2CO_3, reflux; (c) (1) MDC, TEA, $SOCl_2$, reflux; (2) R–OH reflux.

The IR spectrum of compound **3** (Figure 1) exhibited bands at 3396 and 1706 cm^{-1} indicated presence of NH and ketone carbonyl group respectively. In ^1H NMR of compound **3** (Figure 2) multiplets at δ 2.06, 2.67, 2.99 and 3.17 for four, one, two and

two protons respectively confirmed the methyl and methylene protons of quinuclidine ring. Pick at δ 13.1 proton confirmed the presence of acid group. All aromatic protons and vinylic proton observed between δ 6.95 to 8.16 confirmed the formation of compound **3**. The ^{13}C NMR spectrum of compound **3** (Figure 3) showed peak at δ 205.4 indicated ketone carbon. The presence of 9 peaks is in accordance with structure of compound **3**. The DEPT-135 spectrum of compound **3** showed 6 peaks (Figure 4) confirmed formation of **3**.

The IR spectrum of compound **4b** (Figure 5) exhibited bands at 3295 and 1703 cm^{-1} indicated presence of NH and ketone carbonyl group respectively. In ^{1}H NMR of compound **4b** (Figure 6-7) multiplets at δ 2.06, 2.67, 2.99 and 3.17 for four, one, two and two protons respectively confirmed the methyl and methylene protons of quinuclidine ring. Aromatic Methyl proton observed at δ 2.36. All aromatic protons and vinylic proton observed between δ 7.04 to 8.13 confirmed the formation of compound **4b**. The ^{13}C NMR spectrum of compound **4b** (Figure 8) showed peak at δ 206.2 indicated ketone carbon. The presence of 15 peaks is in accordance with structure of compound **4b**. The mass spectrum of compound **4b** (Figure 9) showed *m/z* value at 347.2 $[M+1]^{+}$ in ESI/MS confirmed formation of **4b**.

The IR spectrum of compound **4d** (Figure 10) exhibited bands at 3341 and 1703 cm^{-1} indicated presence of NH and ketone carbonyl group respectively. In ^{1}H NMR of compound **4d** (Figure 11) multiplets at δ 1.11, 1.24, 1.59, 1.72, 1.86, 1.98, 2.83, 3.12, 3.73 for one, four, one, two, two, two, two, two and one protons respectively confirmed the methyl and methylene protons of quinuclidine ring and cyclo hexyl ring. All aromatic protons and vinylic proton observed between δ 6.94 to 8.22 and NH proton observed at δ 8.22 confirmed the formation of compound **4d**. The ^{13}C NMR spectrum of compound **4d** (Figure 13) showed peak at δ 205.4 indicated ketone

carbon. The presence of 12 peaks is in accordance with structure of compound **4d**. The mass spectrum of compound **4d** (Figure 14) showed *m/z* value at 338.3 $[M+1]^+$ in ESI/MS confirmed formation of **4d**.

The IR spectrum of compound **4e** (Figure 16) exhibited bands at 3444 and 1707 cm^{-1} indicated presence of NH and ketone carbonyl group respectively. In ^1H NMR of compound **4e** (Figure 17) multiplets at δ 2.02, 2.65, 2.96 and 3.15 for four, one, two and two protons respectively confirmed the methyl and methylene protons of quinuclidine ring. All aromatic protons and vinylic proton observed between δ 7.07 to 8.07 confirmed the formation of compound **4e**. The ^{13}C NMR spectrum of compound **4e** (Figure 19) showed peak at δ 206.2 indicated ketone carbon. The presence of 13 peaks is in accordance with structure of compound **4e**. The mass spectrum of compound **4e** (Figure 20) showed *m/z* value at 327.3 $[M+1]^+$ in ESI/MS confirmed formation of **4e**.

IR spectrum of compound **4f** (Figure 21) exhibited bands at 3444 and 1707 cm^{-1} indicated presence of NH and ketone carbonyl group respectively. In ^1H NMR of compound **4f** (Figure 22) multiplets at δ 2.01, 2.64, 2.96 and 3.14 for four, one, two and two protons respectively confirmed the methyl and methylene protons of quinuclidine ring. All aromatic protons and vinylic proton observed between δ 7.00 to 8.07 confirmed the formation of compound **4f**. The ^{13}C NMR spectrum of compound **4f** (Figure24) showed peak at δ 206.3 indicated ketone carbon. The presence of 15 peaks is in accordance with structure of compound **4f**. The mass spectrum of compound **4f** (Figure 25) showed *m/z* value at 311.4 $[M+1]^+$ in ESI/MS confirmed formation of **4f**.

The IR spectrum of compound **5b** (Figure 27) exhibited bands at 1754 and 1701 cm^{-1} indicated presence of ester and ketone carbonyl group respectively. In ^1H NMR of

compound **5b** (Figure 28) multiplets at δ 2.04, 2.65, 2.99 and 3.14 for four, one, two and two protons respectively confirmed the methyl and methylene protons of quinuclidine ring. All aromatic protons and vinylic proton observed between δ 7.04 to 8.02 and triplet observed at δ 1.39 and quartet δ 2.99 confirmed the presence of ethyl group of compound **5b**. The ^{13}C NMR spectrum of compound **5b** (Figure 30) showed peak at δ 206 indicated ketone carbon. The presence of 17 peaks is in accordance with structure of compound **5b**. The mass spectrum of compound **5b** (Figure 31) showed *m/z* value at 286.2 $[M+1]^+$ in ESI/MS confirmed formation of **5b**.

The IR spectrum of compound **5c** (Figure 32) exhibited bands at 1758 and 1707 cm^{-1} indicated presence of ester and ketone carbonyl group respectively. In ^{1}H NMR of compound **5c** (Figure 33) multiplets at δ 2.04, 2.66, 2.99 and 3.16 for four, one, two and two protons respectively confirmed the methyl and methylene protons of quinuclidine ring. All aromatic protons and vinylic proton observed between δ 7.04 to 8.10 and doublet observed at δ 1.37 and downfield singlet δ 5.23 confirmed the presence of isopropyl group of compound **5c**. The ^{13}C NMR spectrum of compound **5c** (Figure 35) showed peak at δ 206.2 indicated ketone carbon. The presence of 12 peaks is in accordance with structure of compound **5c**. The mass spectrum of compound **5c** (Figure 36) showed *m/z* value at 300.1 $[M+1]^+$ in ESI/MS confirmed formation of **5c**.

The IR spectrum of compound **5e** (Figure 37) exhibited bands at 1755 and 1708 cm^{-1} indicated presence of ester and ketone carbonyl group respectively. In ^{1}H NMR of compound **5e** (Figure 38) multiplets at δ 2.04, 2.66, 2.97 and 3.22 for four, one, two and two protons respectively confirmed the methyl and methylene protons of quinuclidine ring. All aromatic protons and vinylic proton observed between δ 7.04 to 8.13 and multiplates observed at δ 1.47, 1.56, 1.78 and 1.92 confirmed the presence of

cyclopentyl group of compound **5e**. The ^{13}C NMR spectrum of compound **5e** (Figure 39) showed peak at δ 206.2 indicated ketone carbon. The presence of 16 peaks is in accordance with structure of compound **5e**. The mass spectrum of compound **5e** showed *m/z* value at 325.2 $[M+1]^+$ in ESI/MS confirmed formation of **5e**.

The crystal structures of compound **4c** and **5c** shows the presence of double bond with Z geometry. This is well in agreement with previous report.[8]

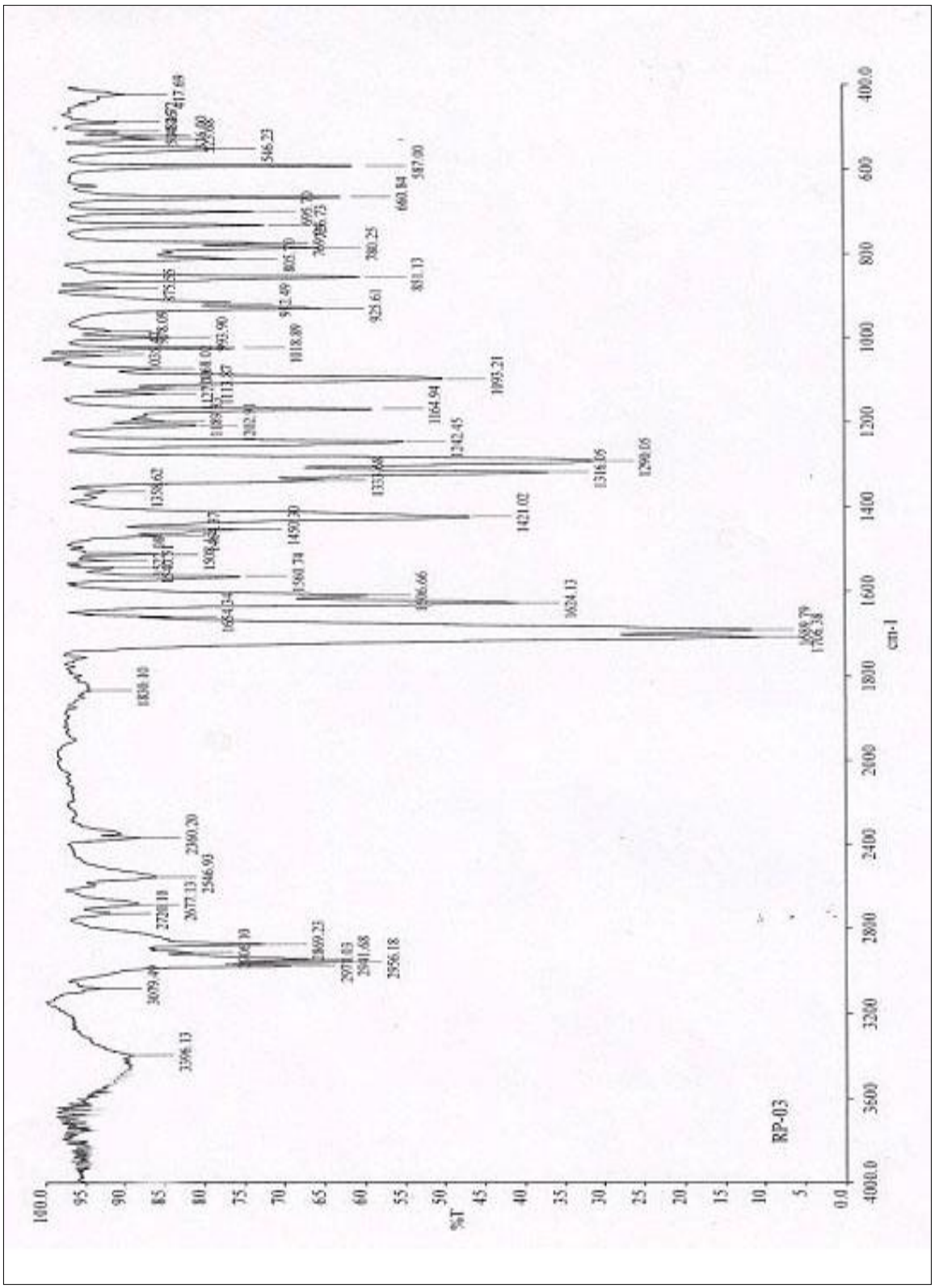


Figure 1: IR of (Z)-4-((3-oxoquinuclidin-2-ylidene)methyl)benzoic acid (3)

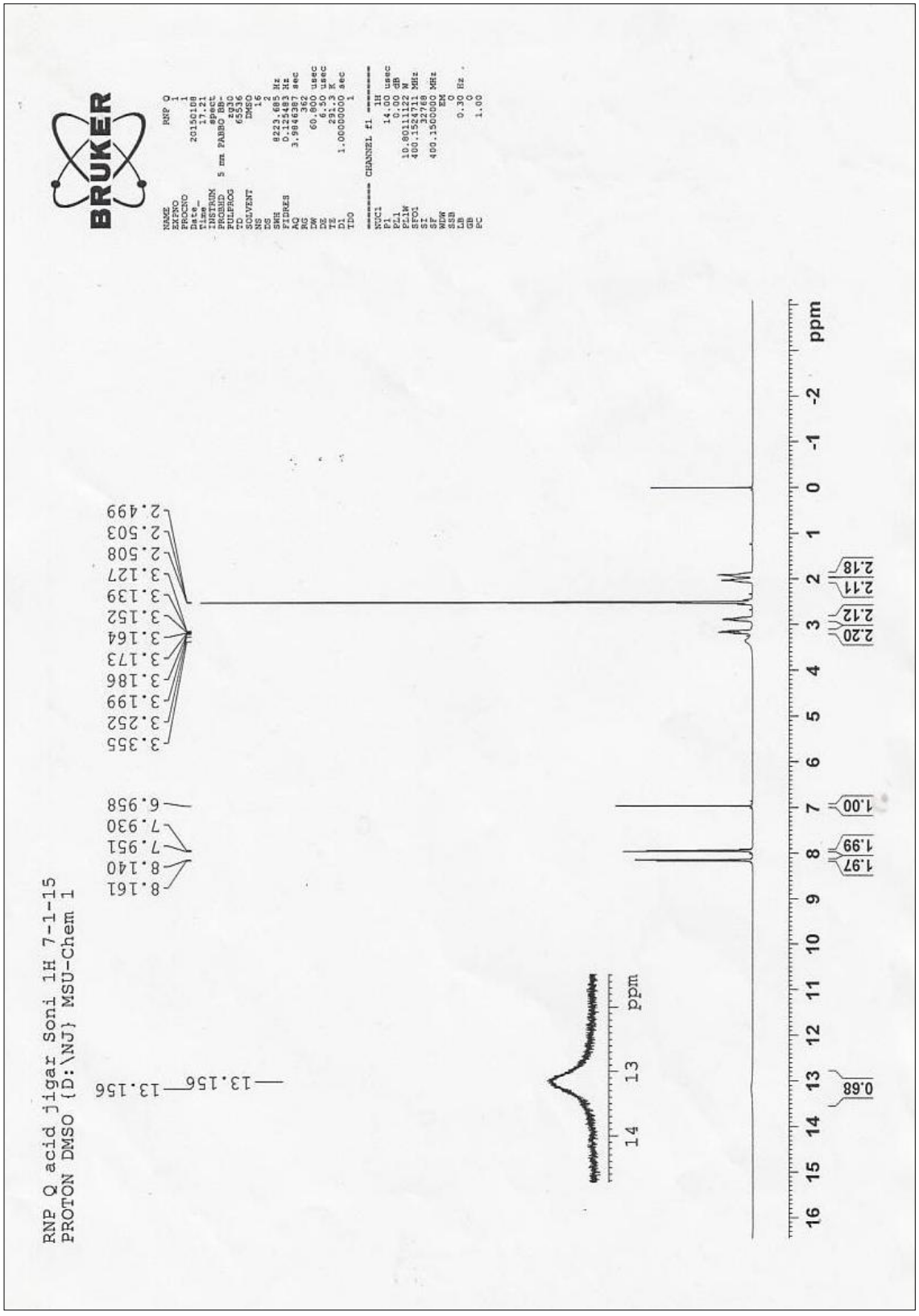


Figure 2: ^{1}H NMR of (Z)-4-((3-oxoquinuclidin-2-ylidene)methyl)benzoic acid (3)

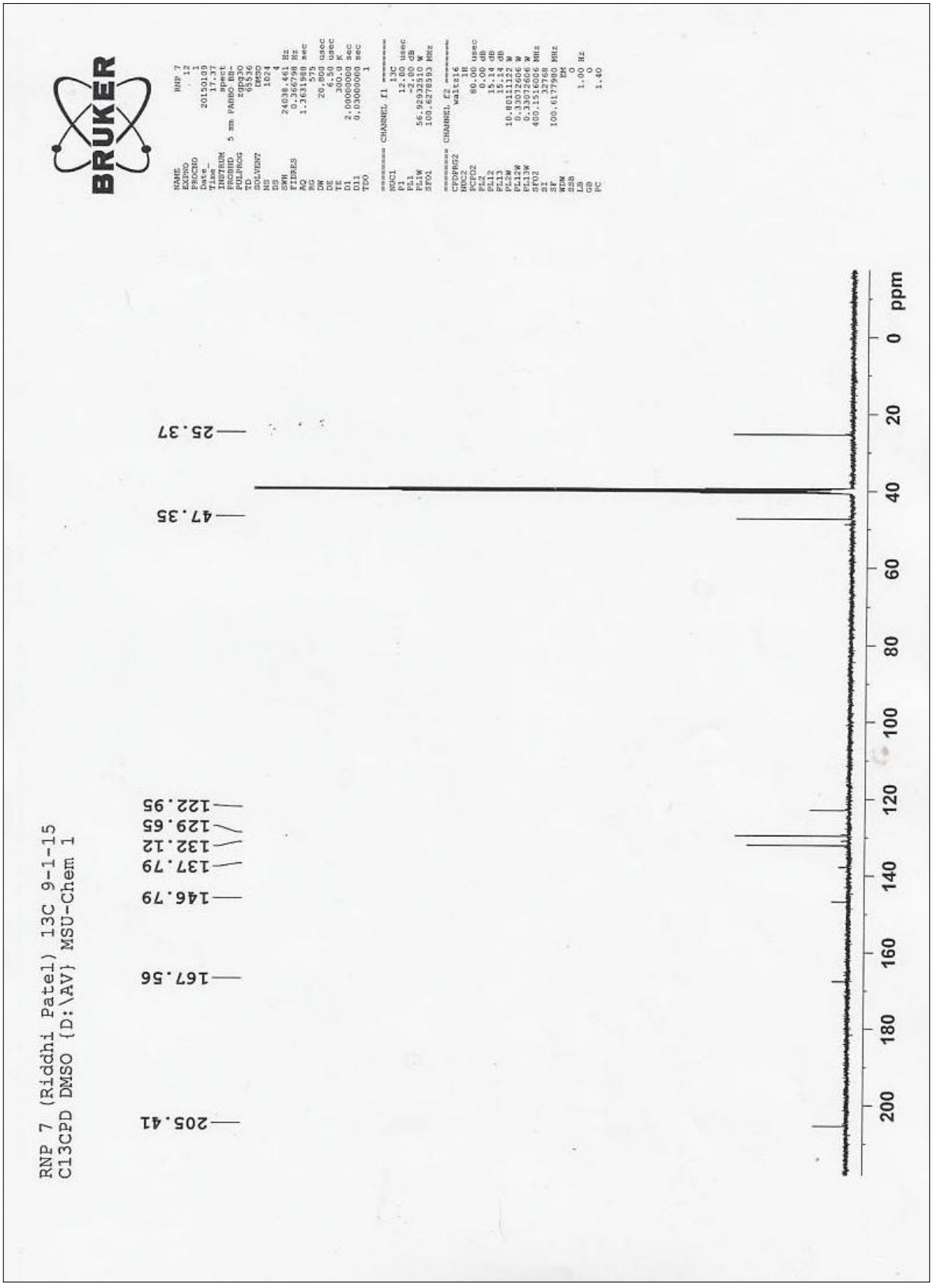


Figure 3: ^{13}C NMR of (Z)-4-((3-oxoquinuclidin-2-ylidene)methyl)benzoic acid (3)

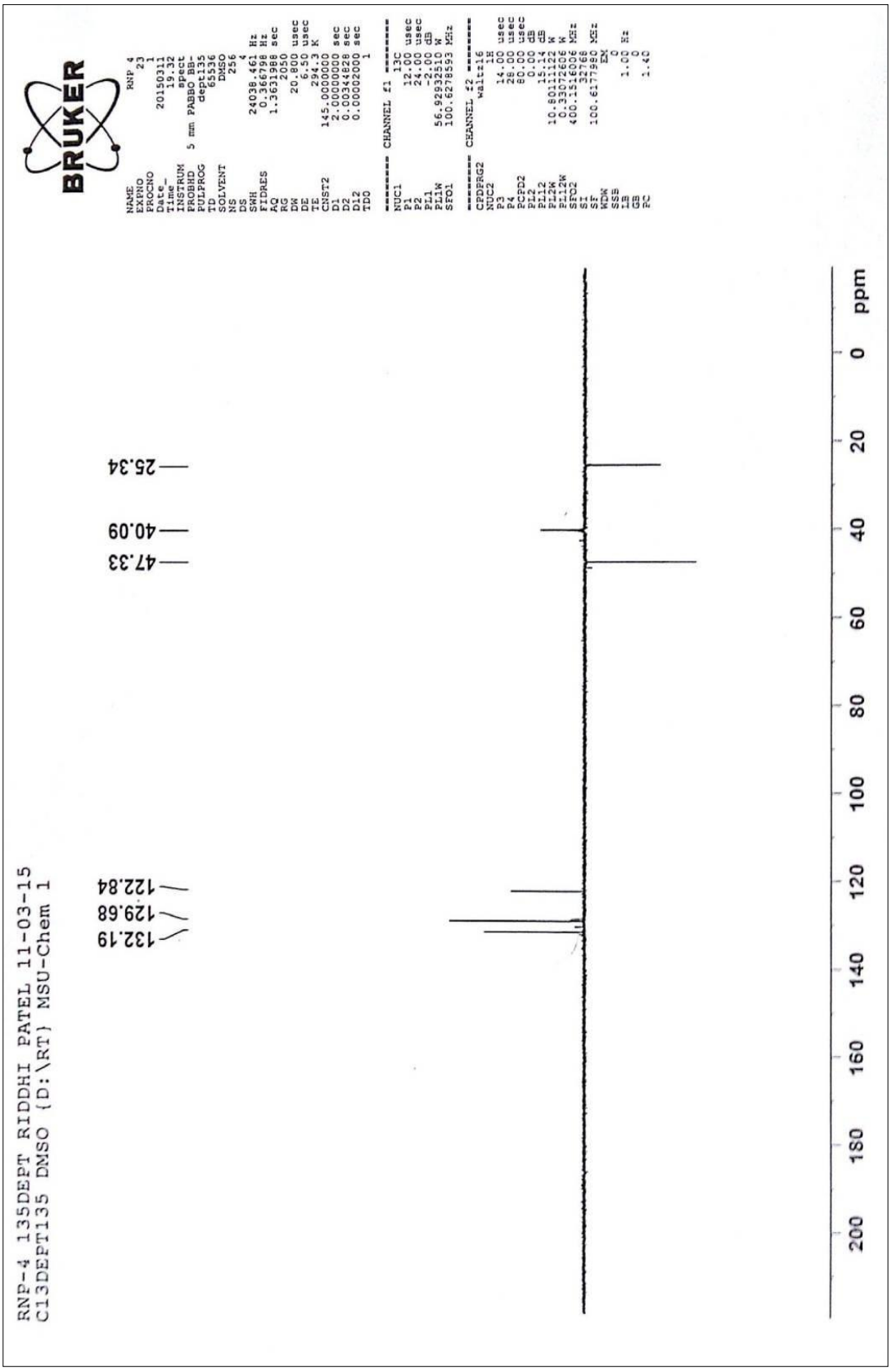


Figure 4: DEPT 135 of (Z)-4-((3-oxoquinuclidin-2-ylidene)methyl)benzoic acid 3

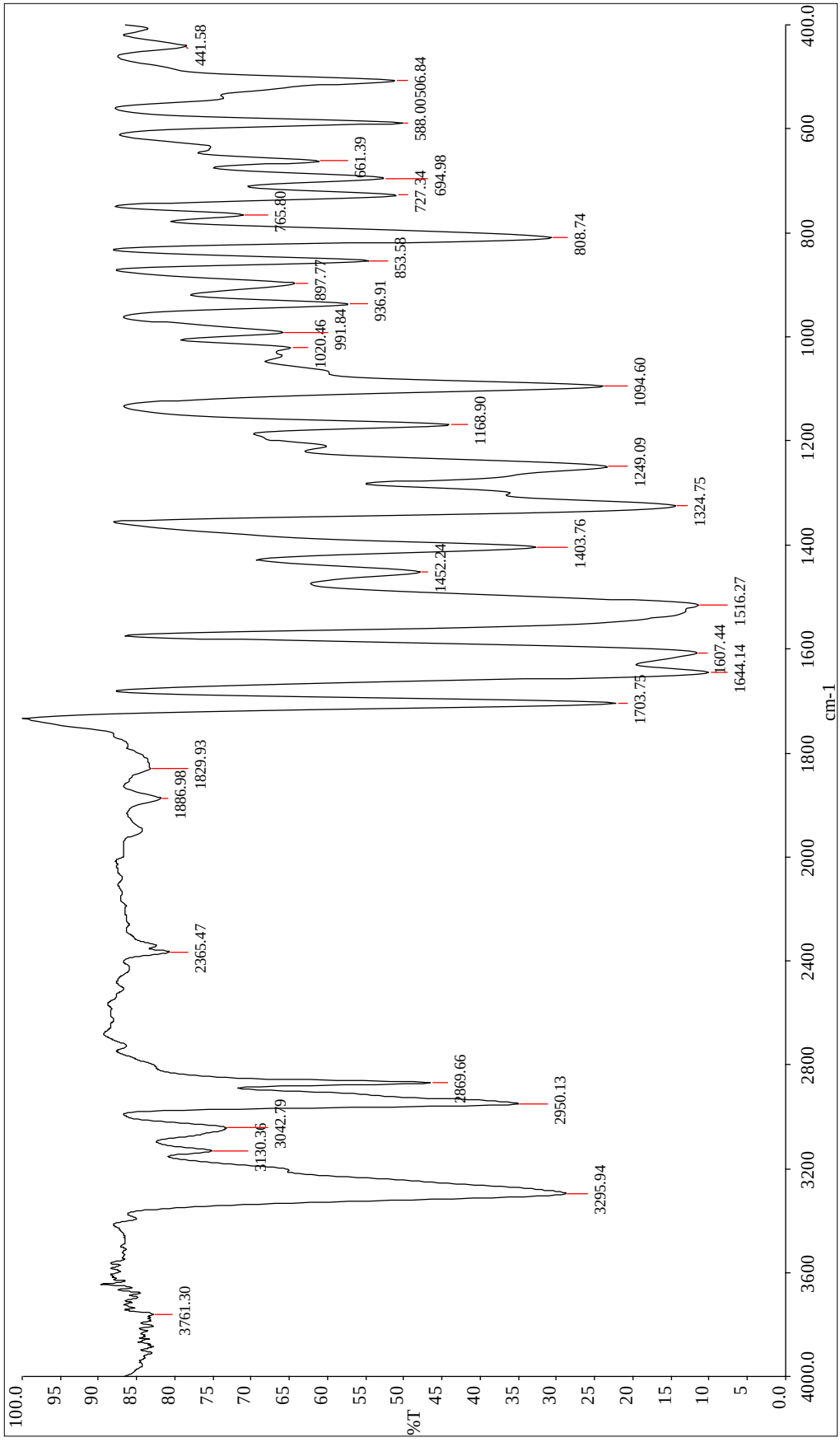


Figure 5: IR Spectra of (Z)-4-((3-oxoquinuclidin-2-ylidene)methyl)-N-(p-tolyl)benzamide 4b

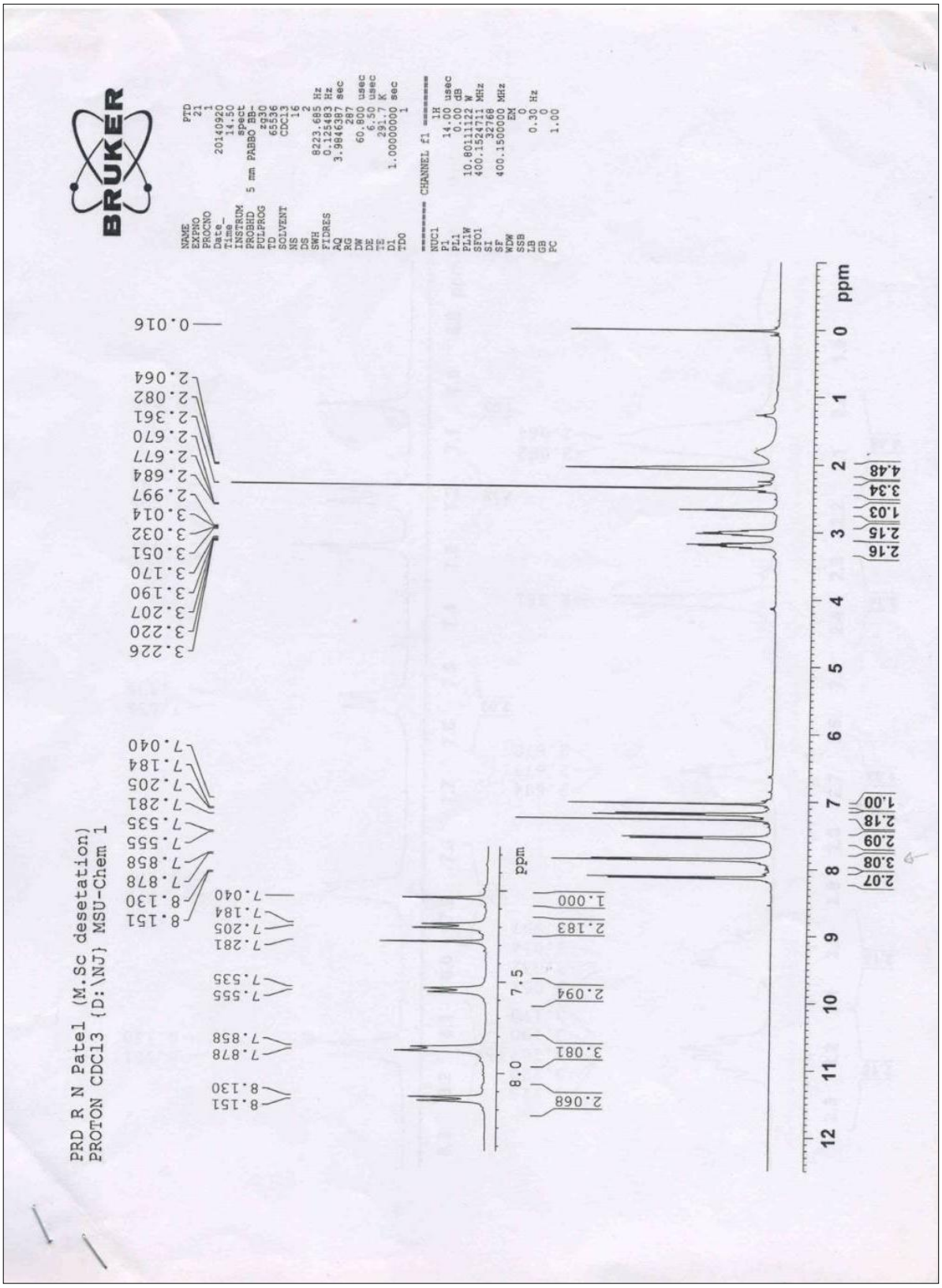


Figure 6: ^{1}H NMR (Z)-4-((3-oxoquinuclidin-2-ylidene)methyl)-N-(p-tolyl)benzamide 4b

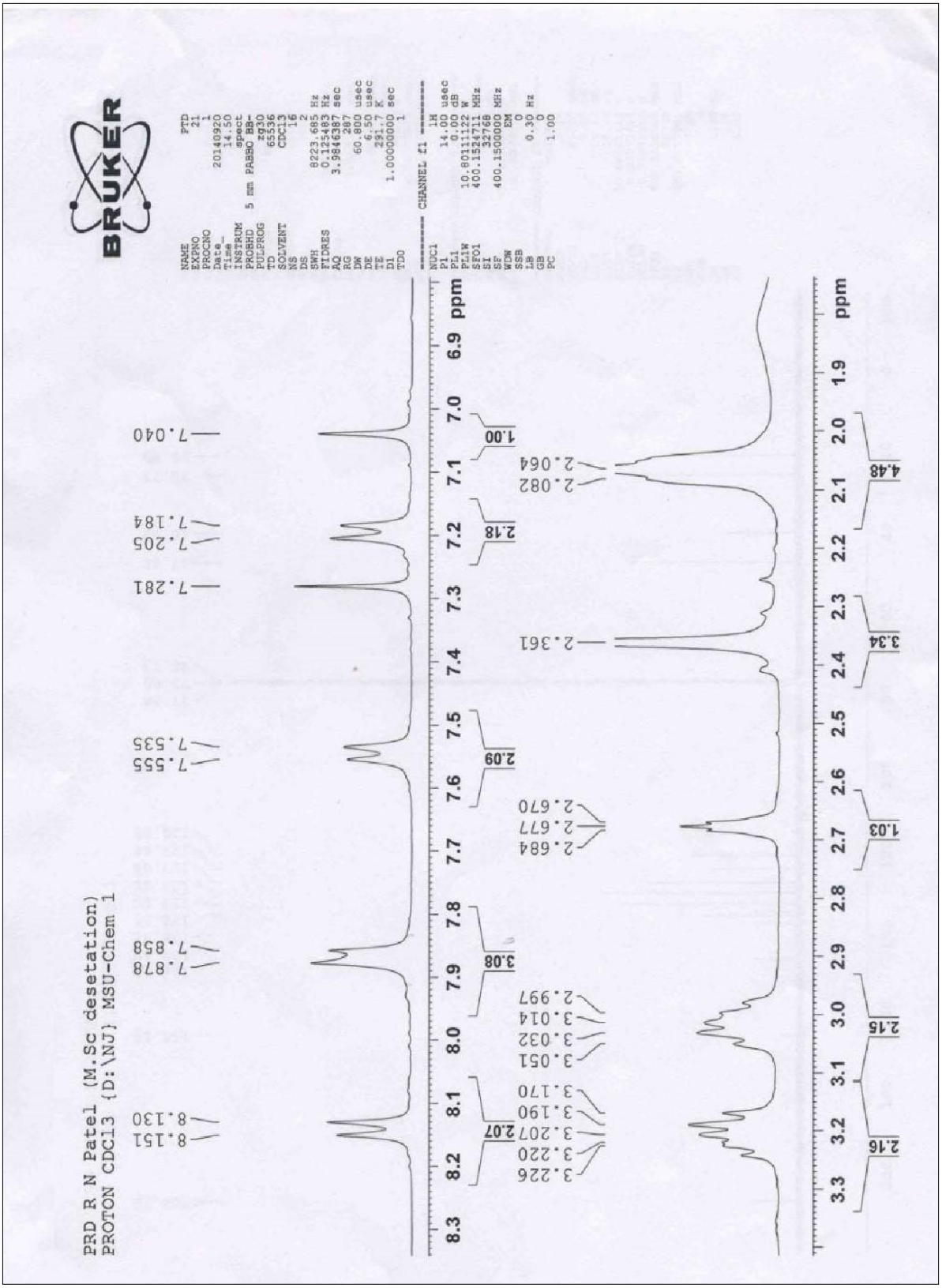


Figure 7: ^{1}H NMR (Z)-4-((3-oxoquinuclidin-2-ylidene)methyl)-N-(p-tolyl)benzamide 4b

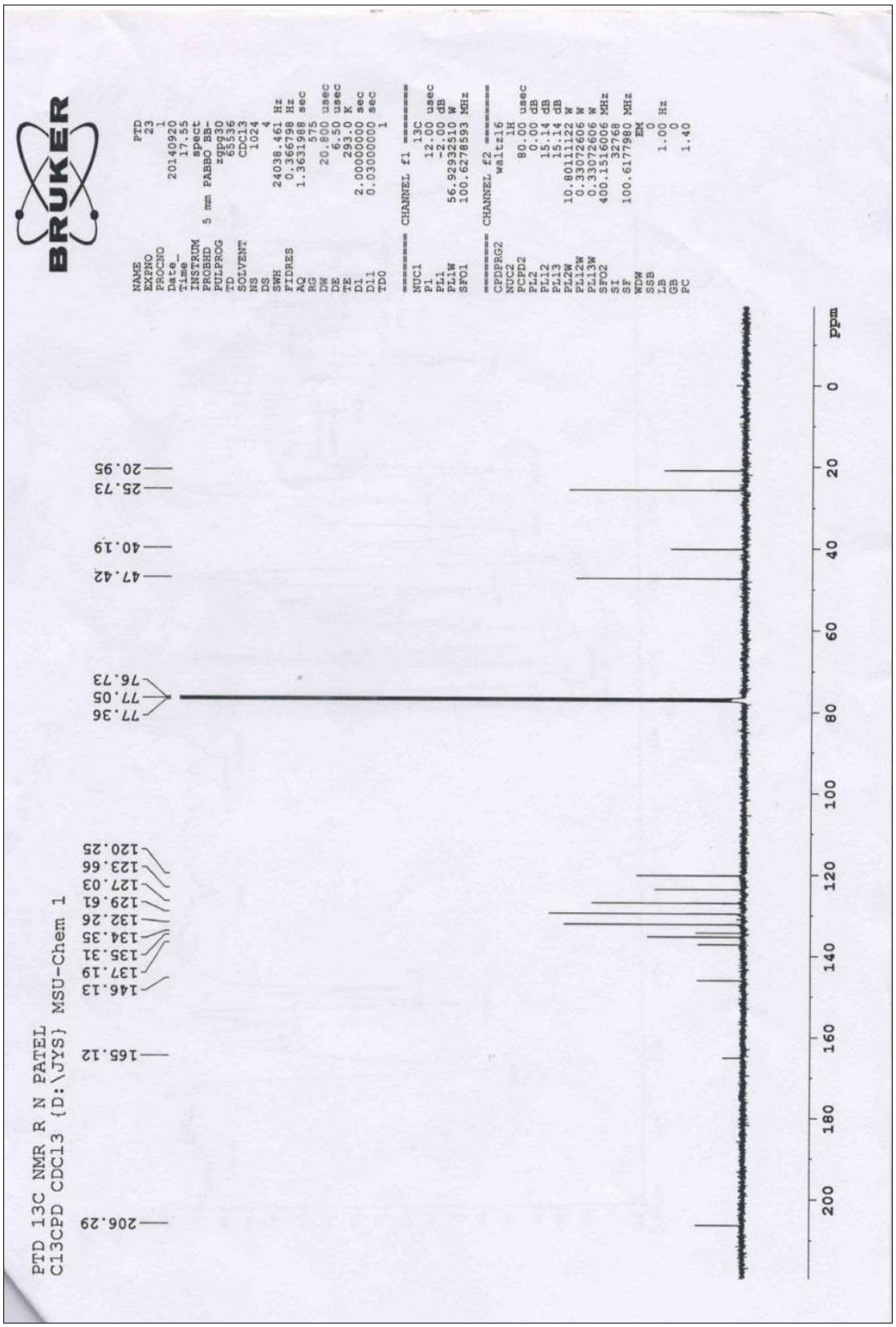


Figure 8: ^{13}C NMR (Z)-4-((3-oxoquinuclidin-2-ylidene)methyl)-N-(p-tolyl)benzamide 4b

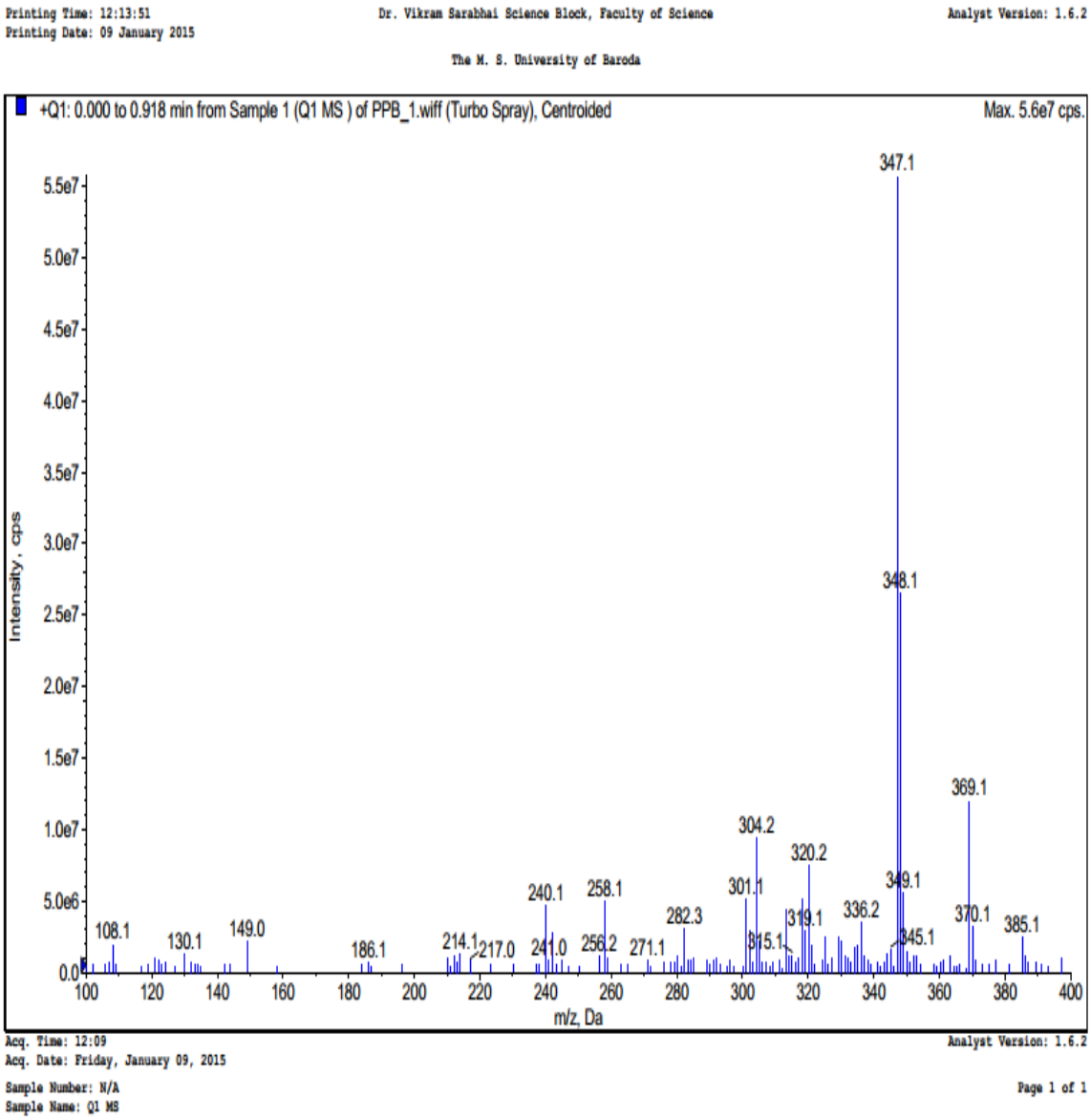


Figure 9: Mass spectra of (Z)-4-((3-oxoquinuclidin-2-ylidene)methyl)-N-(p-tolyl)benzamide 4d

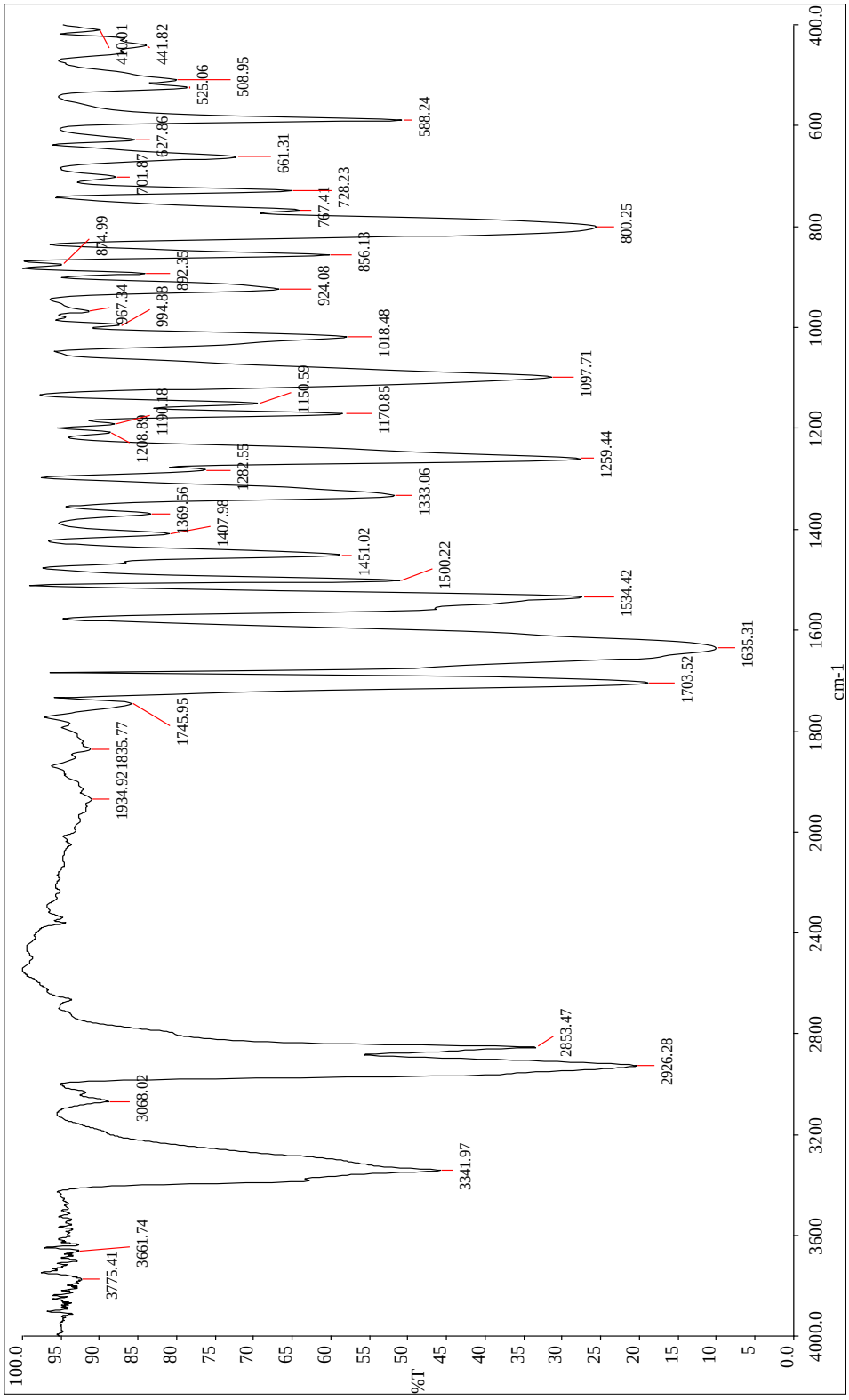


Figure10: IR of (Z)-N-cyclohexyl-4-((3-oxoquinuclidin-2-ylidene)methyl) benzamide 4d

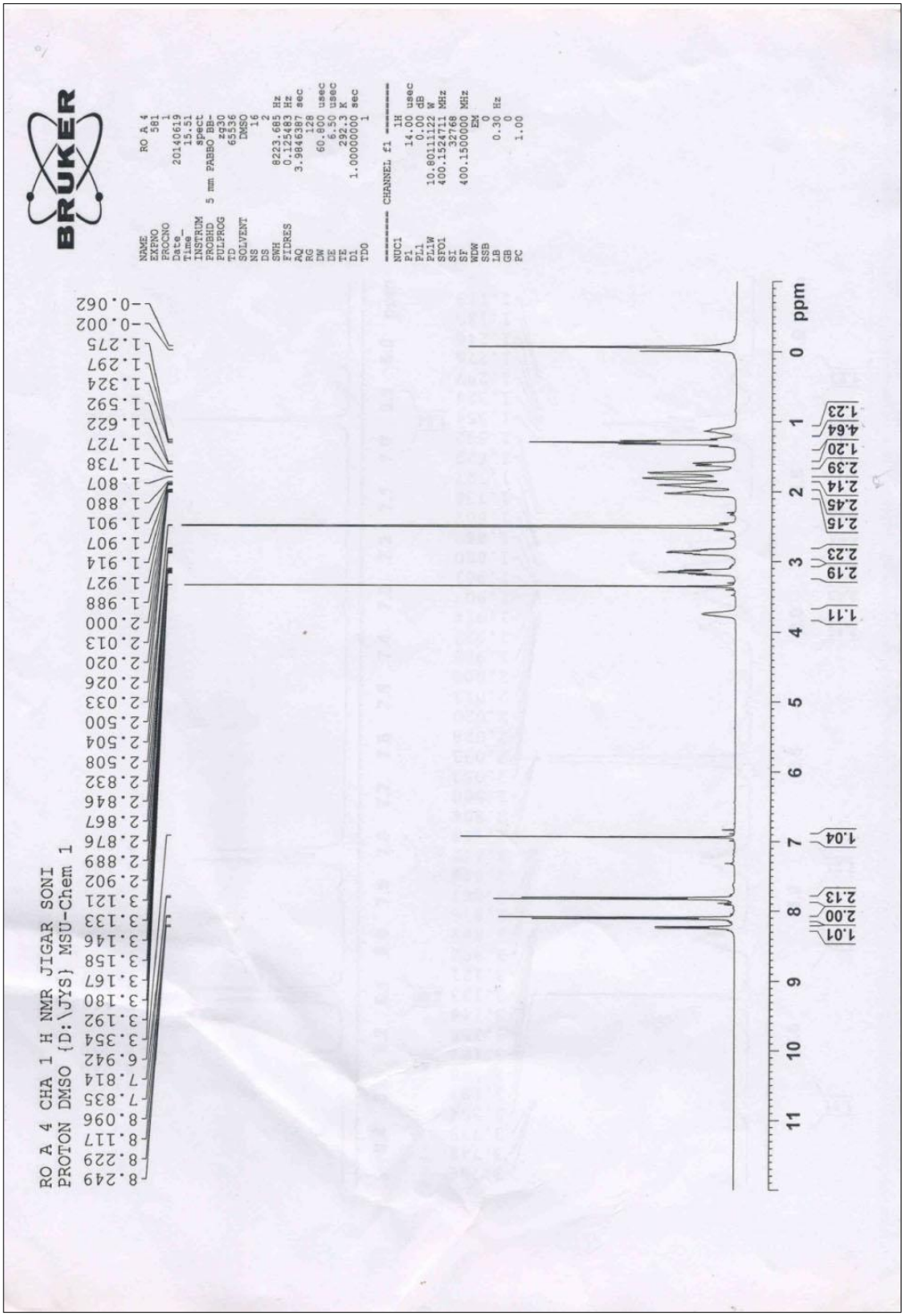


Figure 11: ^{1}H NMR of (Z)-N-cyclohexyl-4-((3-oxoquinuclidin-2-ylidene)methyl) benzamide 4d

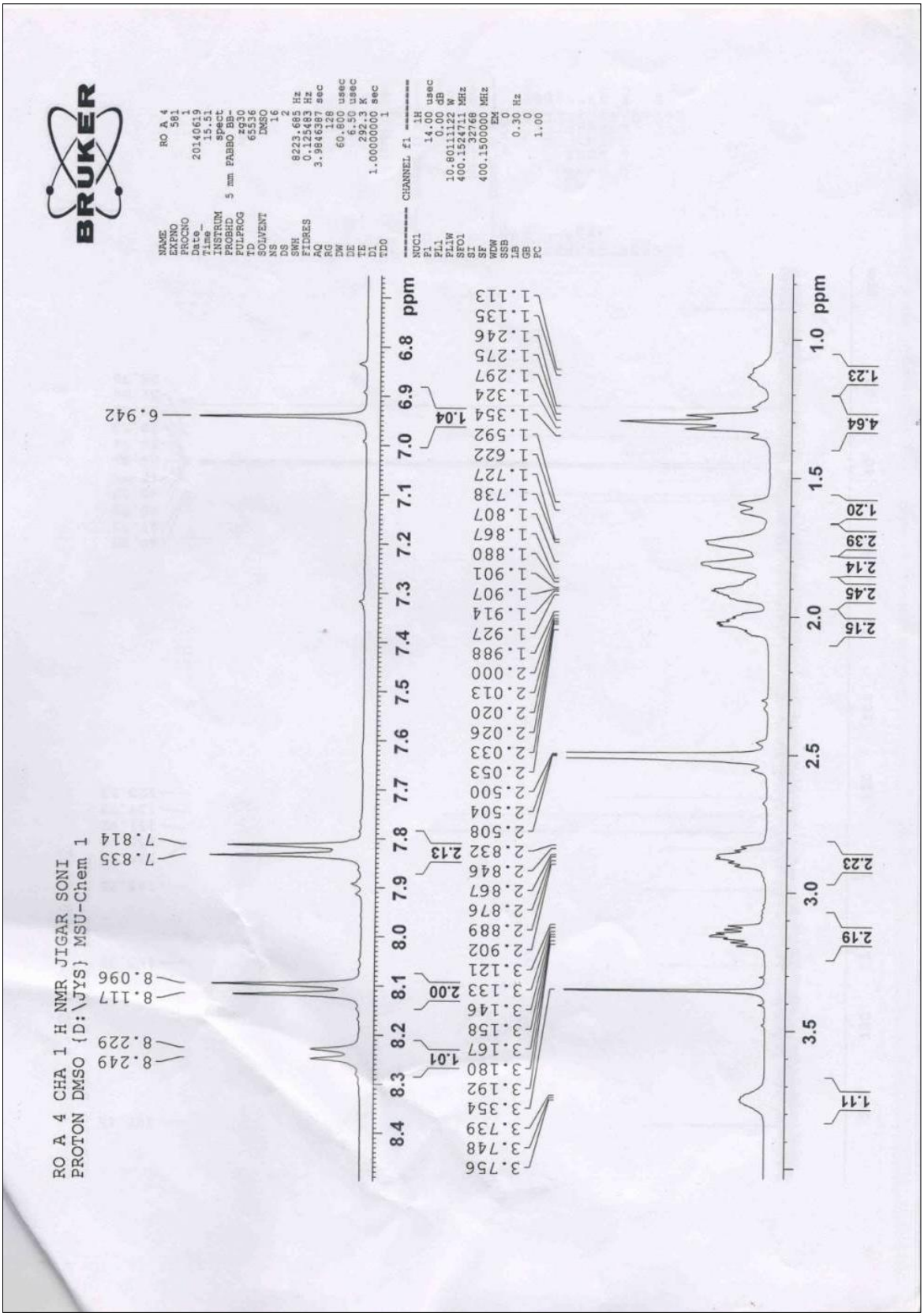


Figure 12: ^{1}H NMR of expansion of (Z)-N-cyclohexyl-4-((3-oxoquinuclidin-2-ylidene)methyl) benzamide 4d

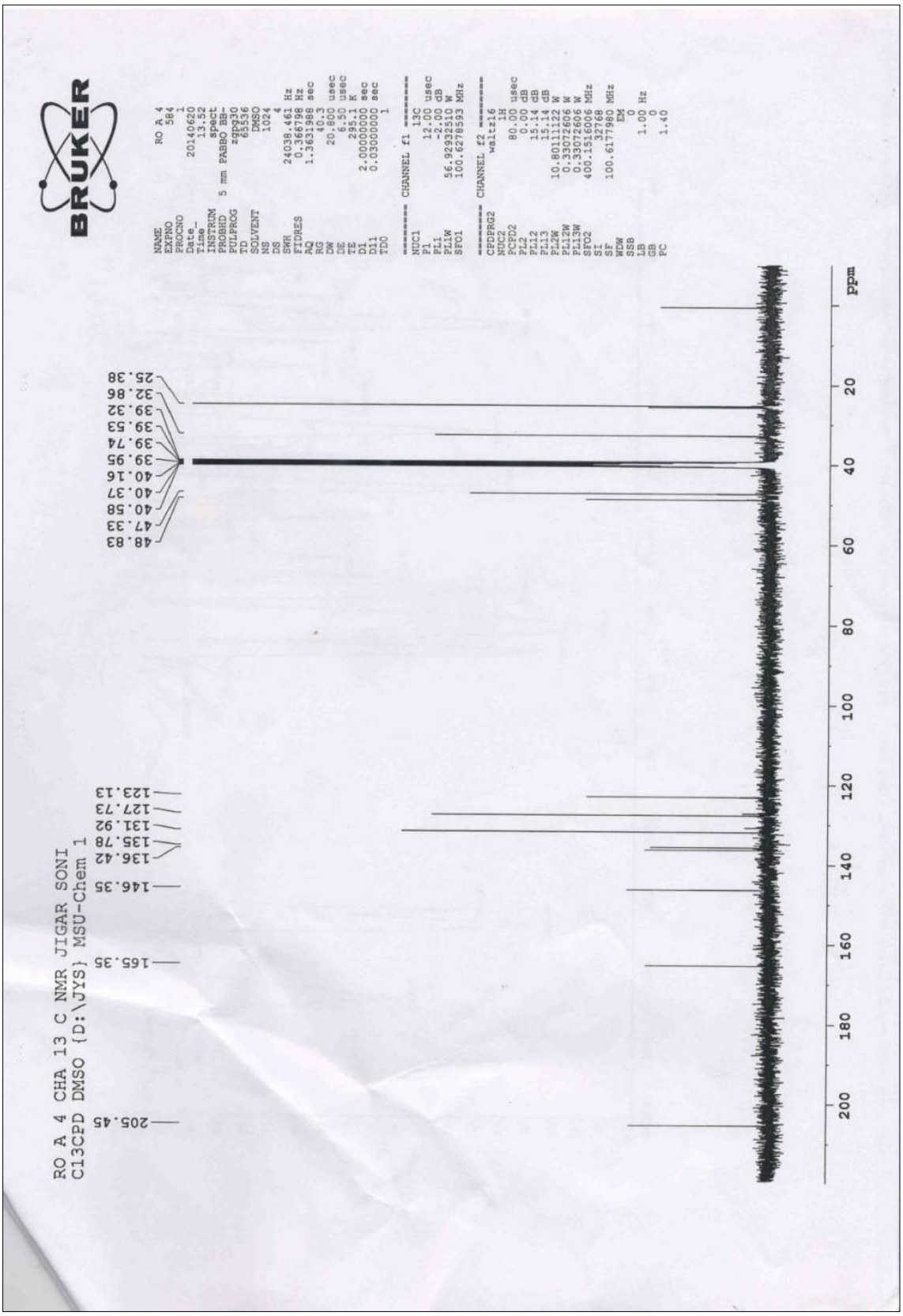


Figure 13: ^{13}C NMR of (Z)-N-cyclohexyl-4-((3-oxoquinuclidin-2-ylidene)methyl) benzamide 4d

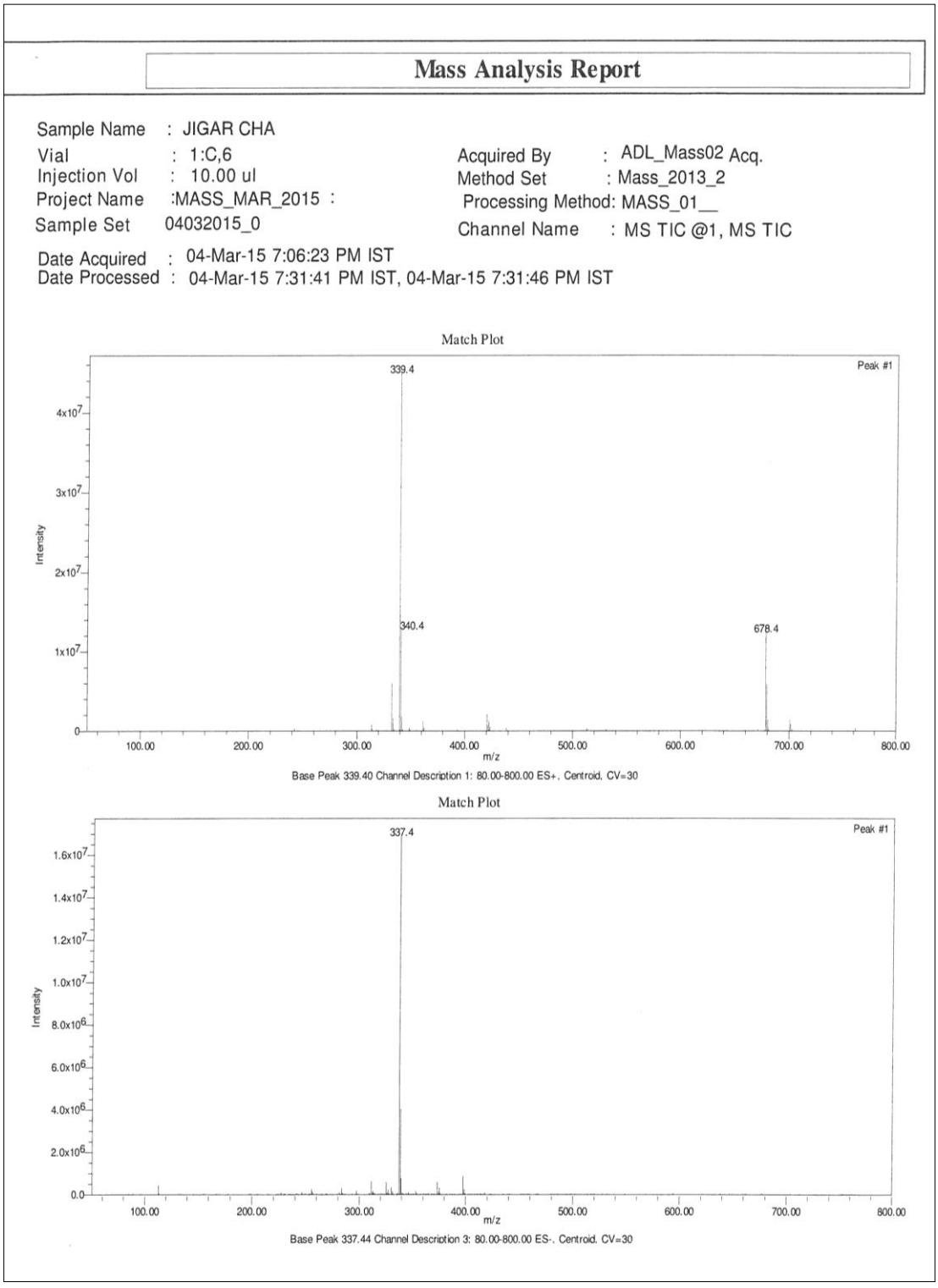
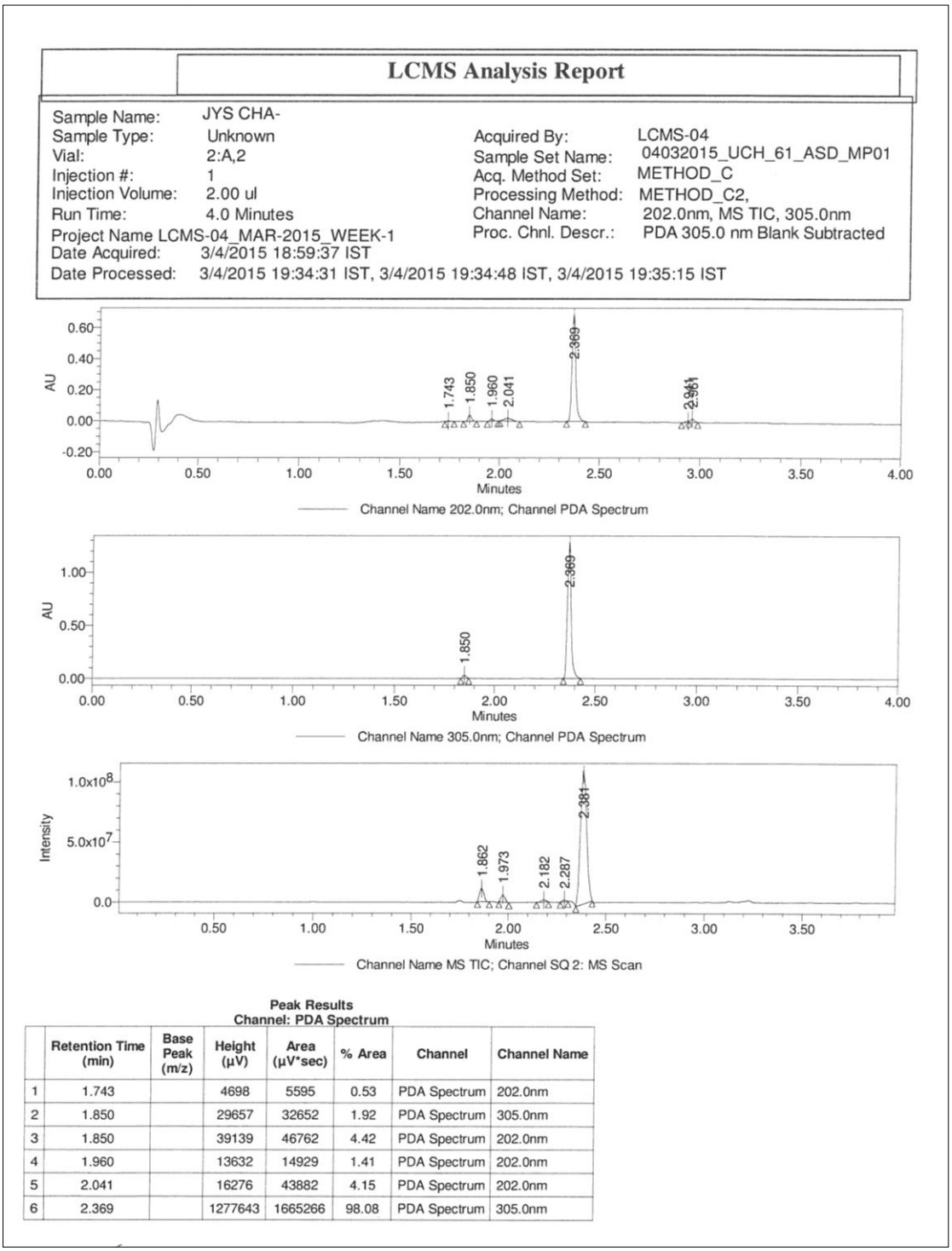


Figure 14: Mass Spectra of (Z)-N-cyclohexyl-4-((3-oxoquinuclidin-2-ylidene) methyl) benzamide 4d



Peak Results
Channel: PDA Spectrum

	Retention Time (min)	Base Peak (m/z)	Height (µV)	Area (µV*sec)	% Area	Channel	Channel Name
1	1.743		4698	5595	0.53	PDA Spectrum	202.0nm
2	1.850		29657	32652	1.92	PDA Spectrum	305.0nm
3	1.850		39139	46762	4.42	PDA Spectrum	202.0nm
4	1.960		13632	14929	1.41	PDA Spectrum	202.0nm
5	2.041		16276	43882	4.15	PDA Spectrum	202.0nm
6	2.369		1277643	1665266	98.08	PDA Spectrum	305.0nm

Figure 15: (Page-1) Lcms Spectra of (Z)-N-cyclohexyl-4-((3-oxoquinuclidin-2-ylidene)methyl) benzamide 4d

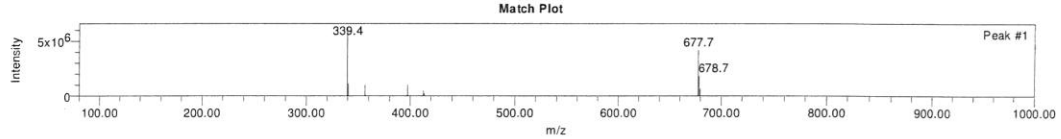
LCMS Analysis Report

Peak Results
Channel: PDA Spectrum

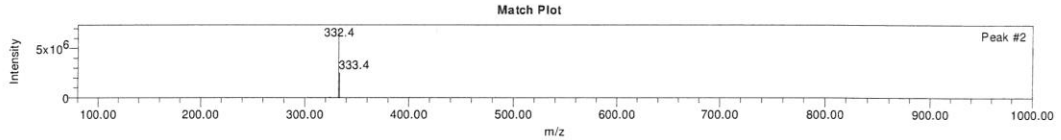
	Retention Time (min)	Base Peak (m/z)	Height (µV)	Area (µV*sec)	% Area	Channel	Channel Name
7	2.369		688459	907024	85.80	PDA Spectrum	202.0nm
8	2.941		9376	8693	0.82	PDA Spectrum	202.0nm
9	2.961		21363	30214	2.86	PDA Spectrum	202.0nm

Peak Results
Channel: SQ 2: MS Scan

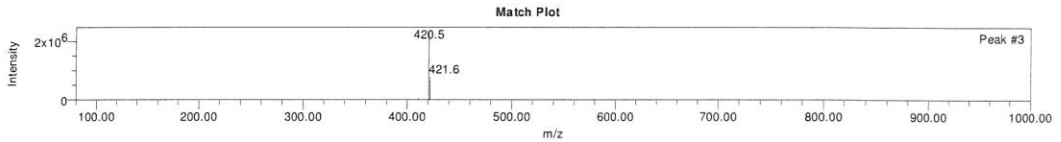
	Retention Time (min)	Base Peak (m/z)	Height (µV)	Area (µV*sec)	% Area	Channel	Channel Name
1	1.862	339.43	11438914	16611564	6.29	SQ 2: MS Scan	MS TIC
2	1.973	332.38	6356324	8606693	3.26	SQ 2: MS Scan	MS TIC
3	2.182	420.53	2089864	3095886	1.17	SQ 2: MS Scan	MS TIC
4	2.287	422.53	1661923	2129129	0.81	SQ 2: MS Scan	MS TIC
5	2.381	339.43	113649209	233444346	88.46	SQ 2: MS Scan	MS TIC



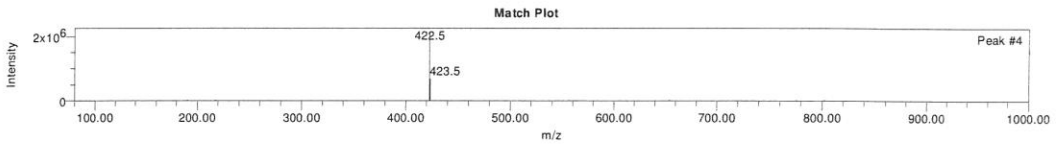
Base Peak 339.43 Channel Description 2: 80.00-1000.00 ES+, Centroid, CV=10 - AVG (0.0:1.8;2.5:4.0) x 60.000 - AVG (0.0:1.8;2.5:4.0) x 60.000 Retention Time 1.862



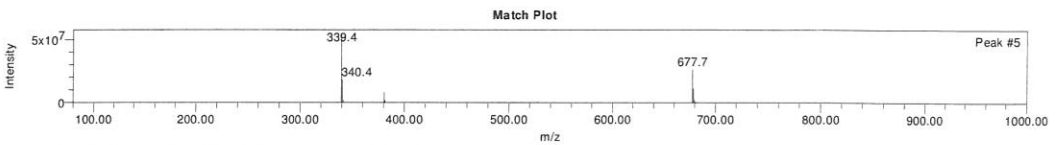
Base Peak 332.38 Channel Description 2: 80.00-1000.00 ES+, Centroid, CV=10 - AVG (0.0:1.8;2.5:4.0) x 60.000 - AVG (0.0:1.8;2.5:4.0) x 60.000 Retention Time 1.973



Base Peak 420.53 Channel Description 2: 80.00-1000.00 ES+, Centroid, CV=10 - AVG (0.0:1.8;2.5:4.0) x 60.000 - AVG (0.0:1.8;2.5:4.0) x 60.000 Retention Time 2.182



Base Peak 422.53 Channel Description 2: 80.00-1000.00 ES+, Centroid, CV=10 - AVG (0.0:1.8;2.5:4.0) x 60.000 - AVG (0.0:1.8;2.5:4.0) x 60.000 Retention Time 2.287



Base Peak 339.43 Channel Description 2: 80.00-1000.00 ES+, Centroid, CV=10 - AVG (0.0:1.8;2.5:4.0) x 60.000 - AVG (0.0:1.8;2.5:4.0) x 60.000 Retention Time 2.381

Figure 15: (Page-2) Lcms Spectra of (Z)-N-cyclohexyl-4-((3-oxoquinuclidin-2-ylidene)methyl) benzamide 4d

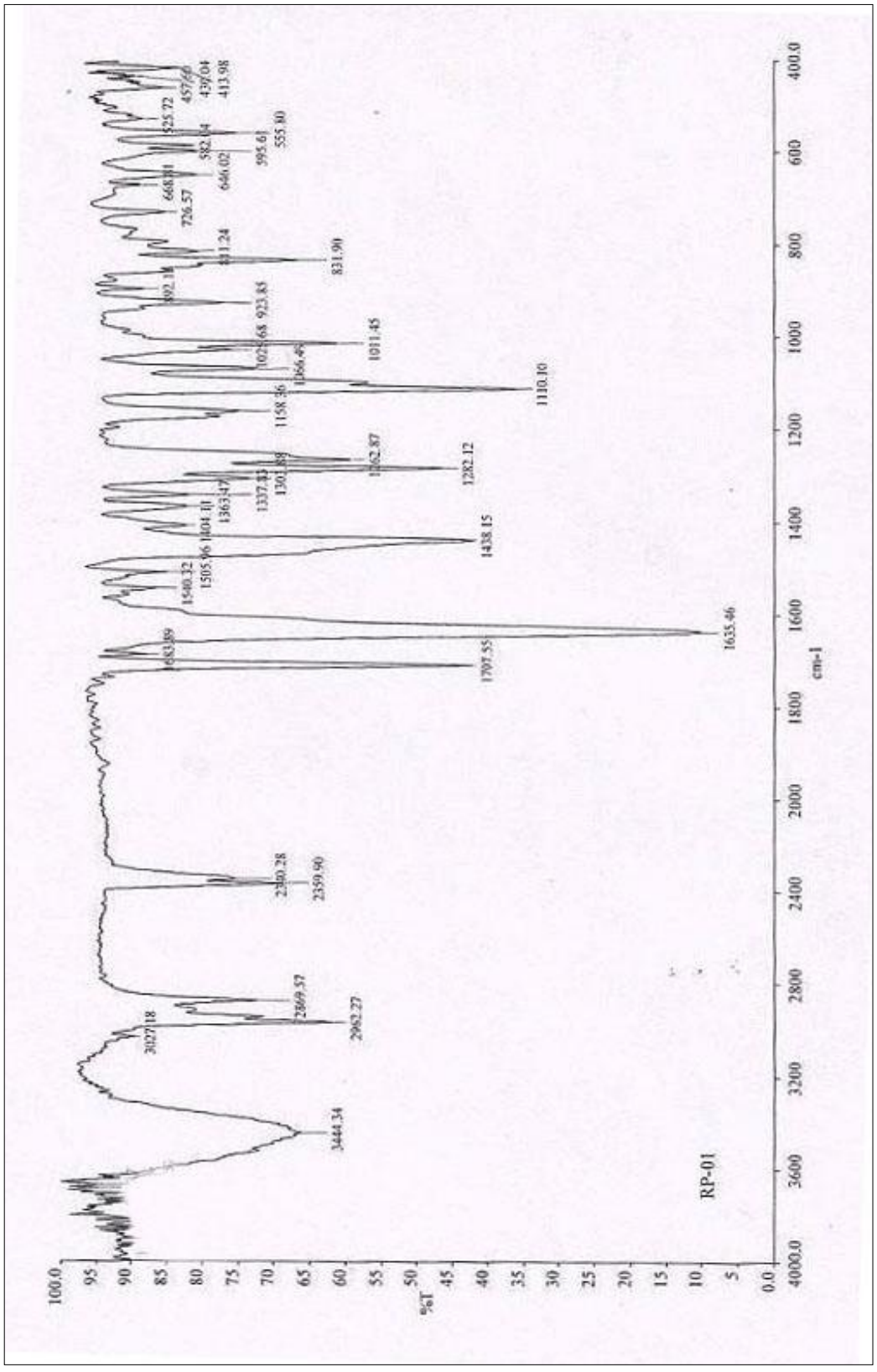


Figure 16: IR of (Z)-2-(4-(morpholine-4-carbonyl) benzylidene)quinuclidin-3-one 4e

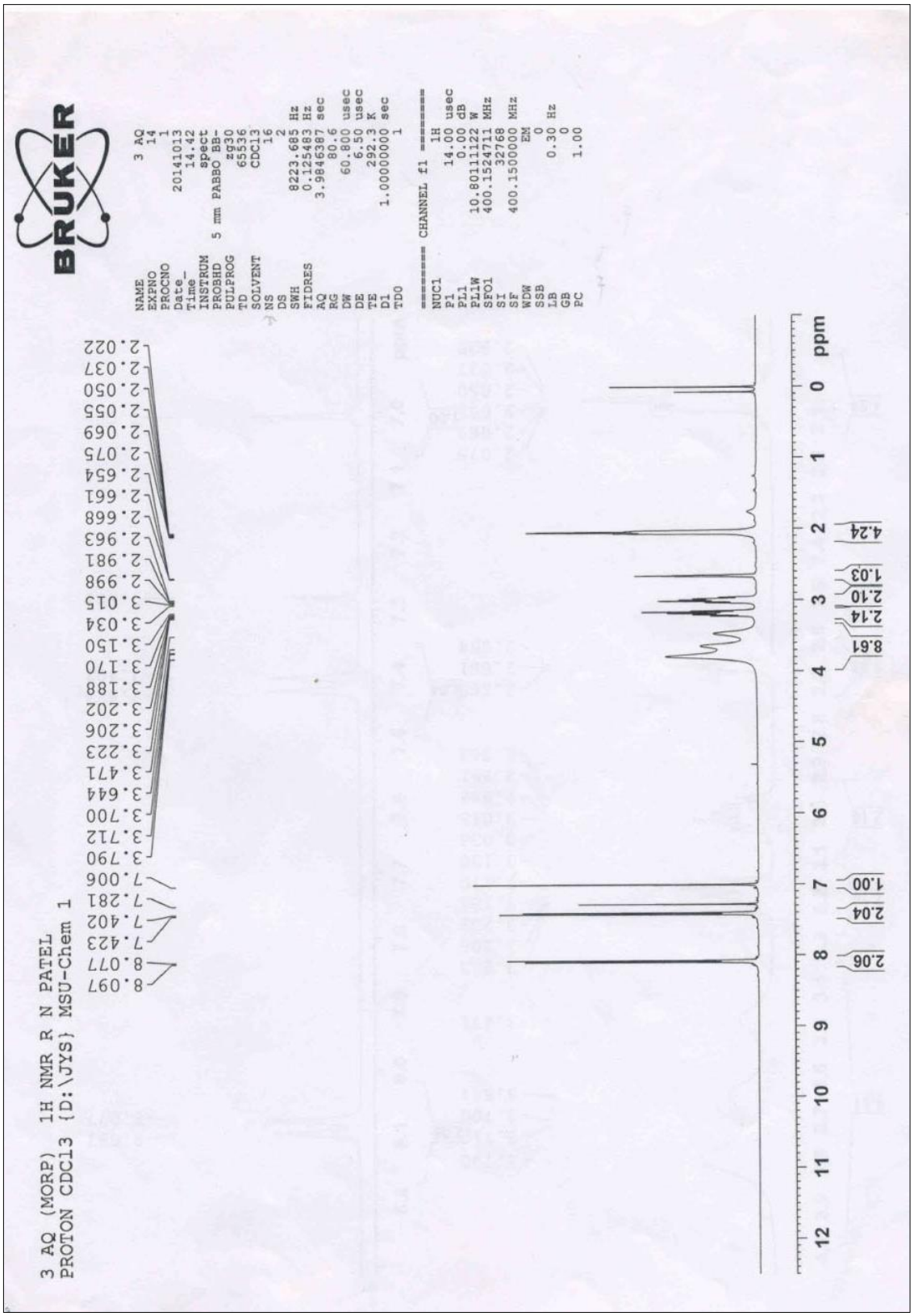


Figure 17: ^{1}H NMR of (Z)-2-(4-(morpholine-4-carbonyl) benzylidene)quinuclidin-3-one 4e

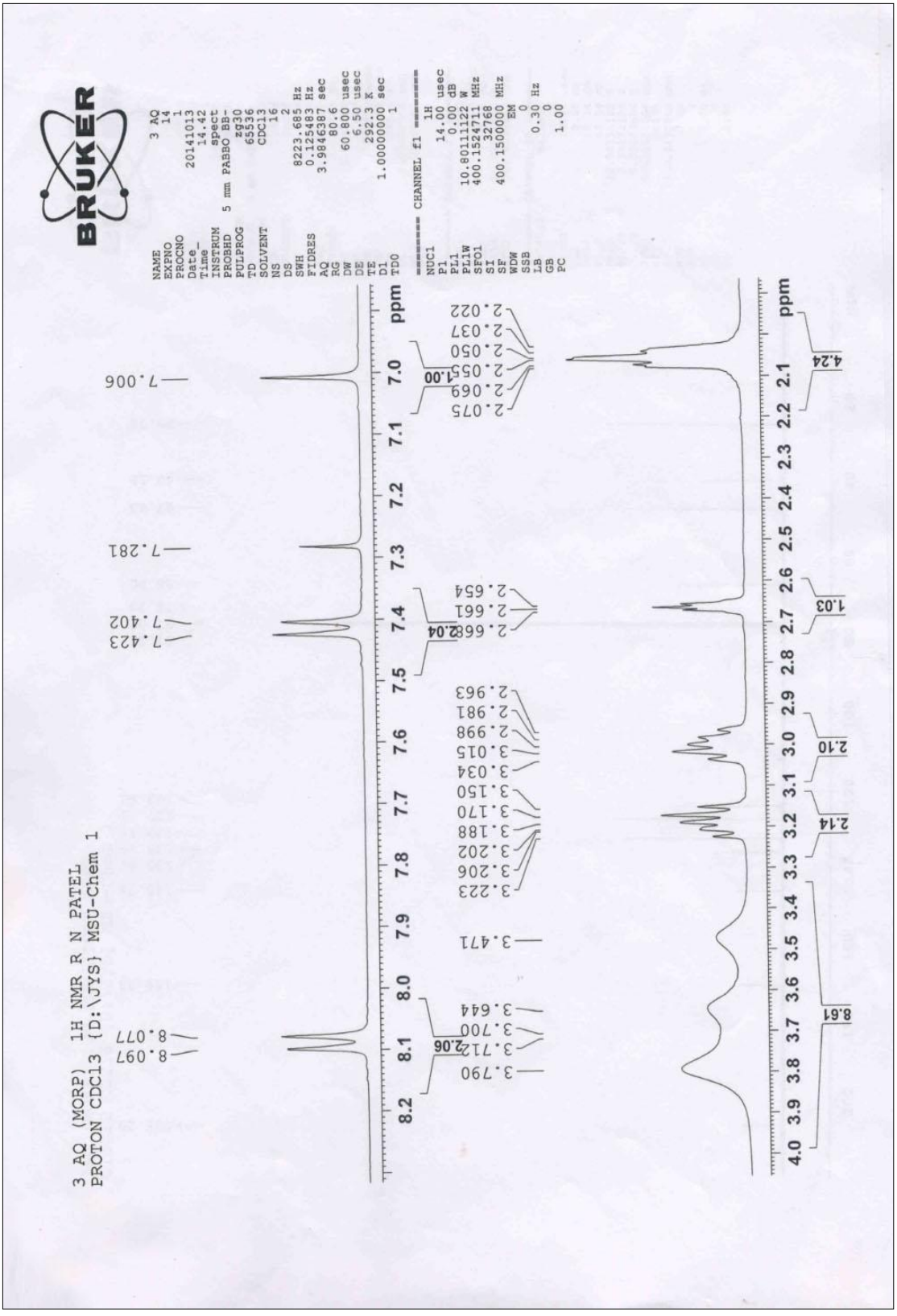


Figure 18: ^{1}H NMR Expansion of (Z)-2-(4-(morpholine-4-carbonyl) benzylidene)quinuclidin-3-one 4e

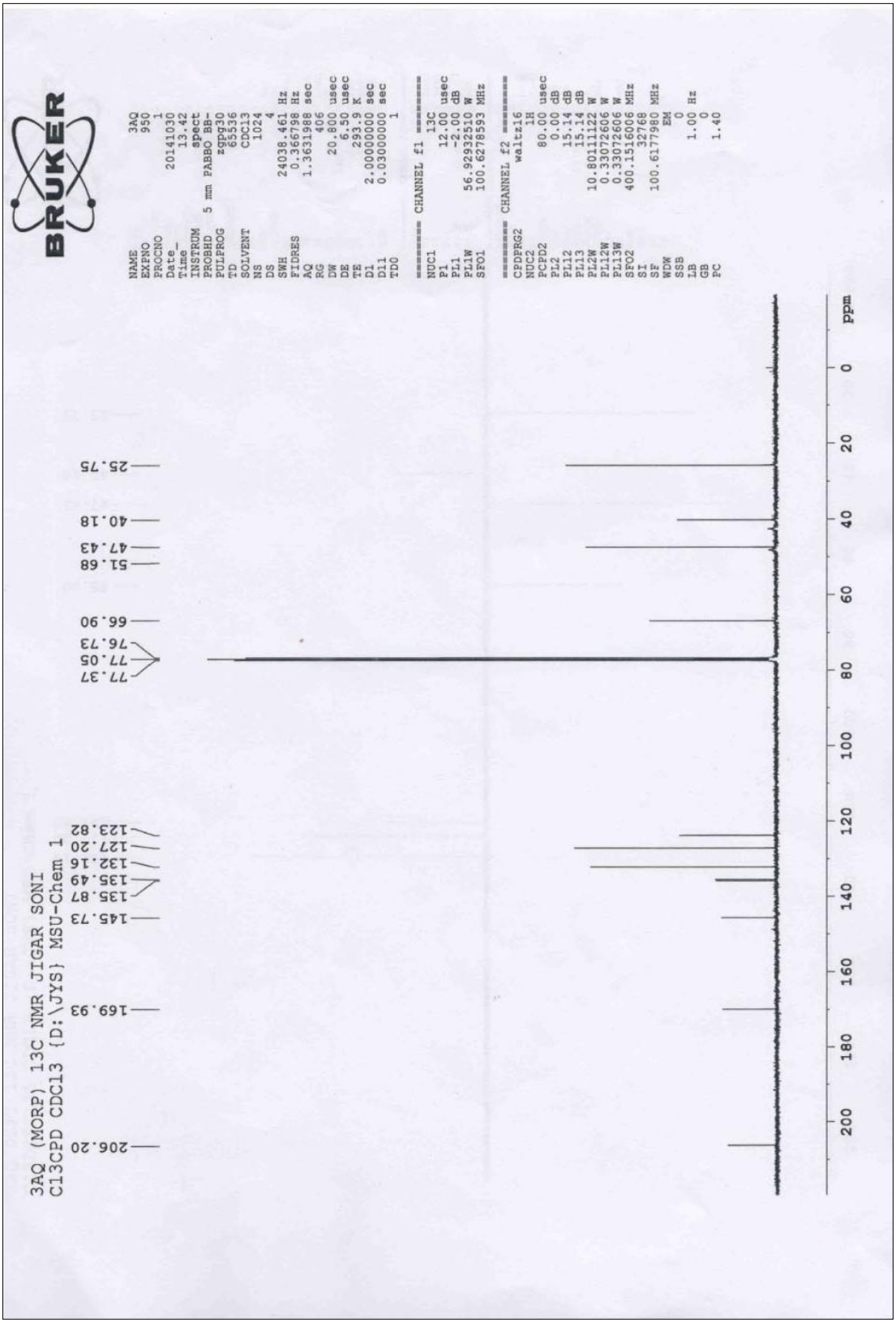
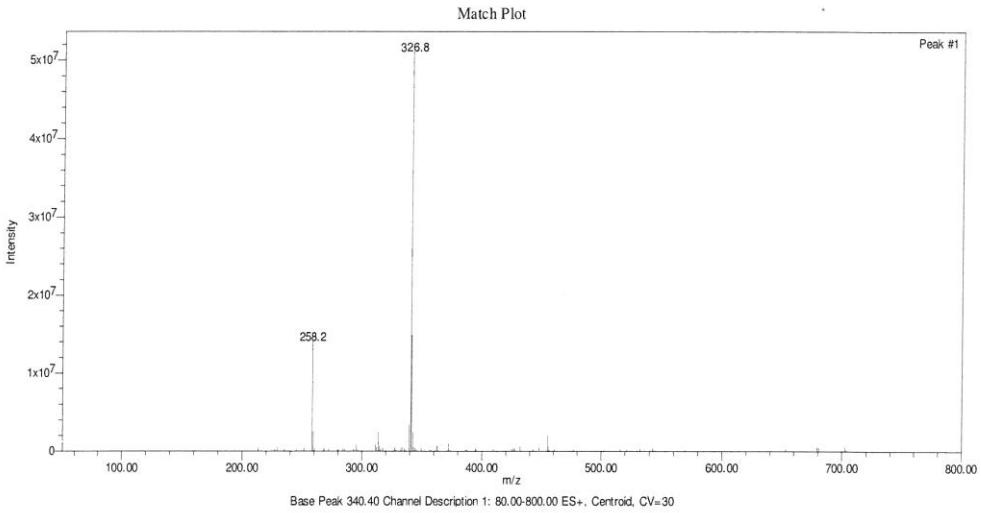


Figure 19: ^{13}C NMR of (Z)-2-(4-(morpholine-4-carbonyl) benzylidene)quinuclidin-3-one 4e

Mass Analysis Report

Sample Name : JIGAR ESTER c6
Vial : 1:C,7
Injection Vol : 10.00 ul
Project Name :MASS_MAR_2015
Sample Set : 04032015_0
Acquired By : ADL_Mass02 Acq.
Method Set : Mass_2013_2
Processing Method: MASS_01__
Channel Name : MS TIC, MS TIC @1
Date Acquired : 04-Mar-15 7:08:13 PM IST
Date Processed : 04-Mar-15 7:32:36 PM IST, 04-Mar-15 7:32:43 PM IST



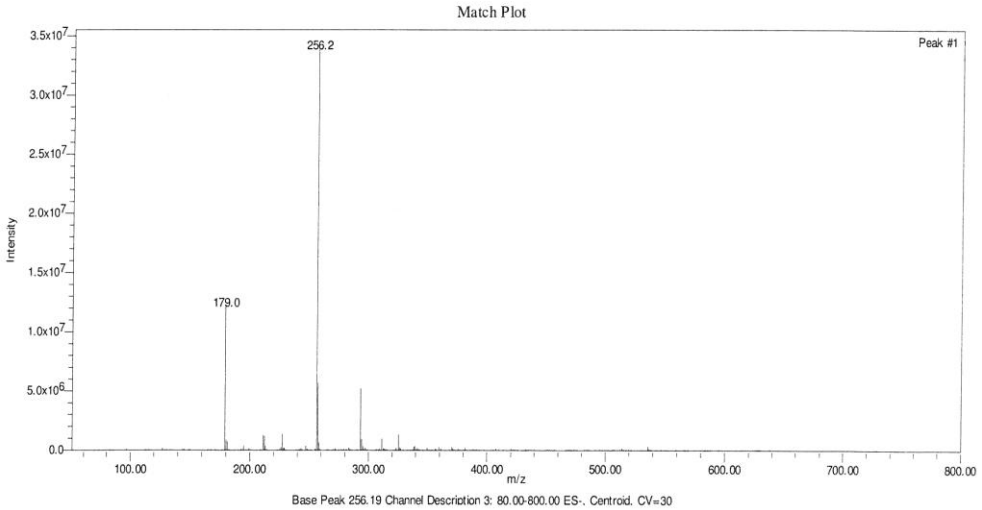


Figure 20: Mass of (Z)-2-(4-(pyrrolidine-1-carbonyl)benzylidene)quinuclidin-3-one 4e

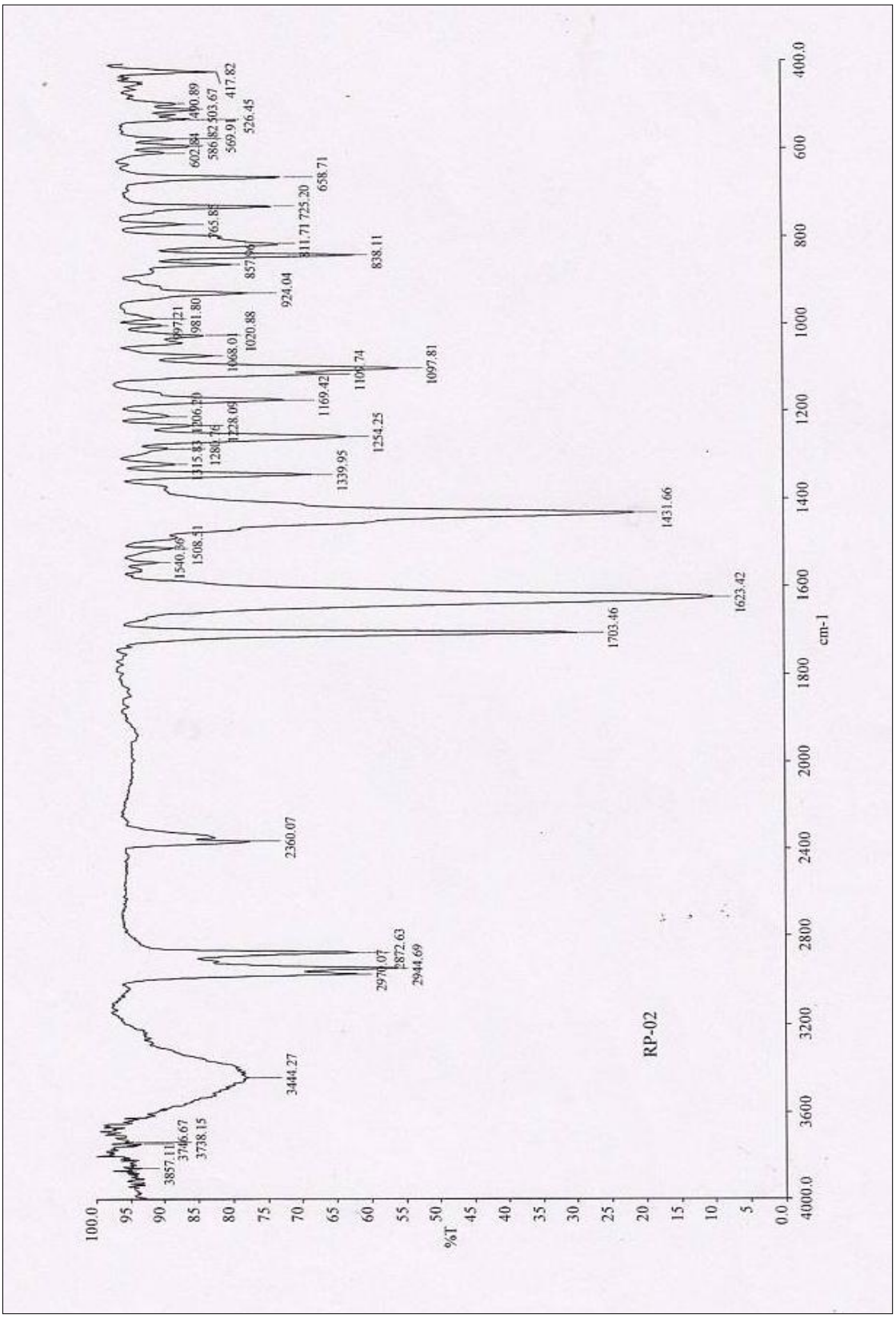


Figure 21: IR of (Z)-2-(4-(pyrrolidine-1-carbonyl)benzylidene)quinuclidin-3-one 4f

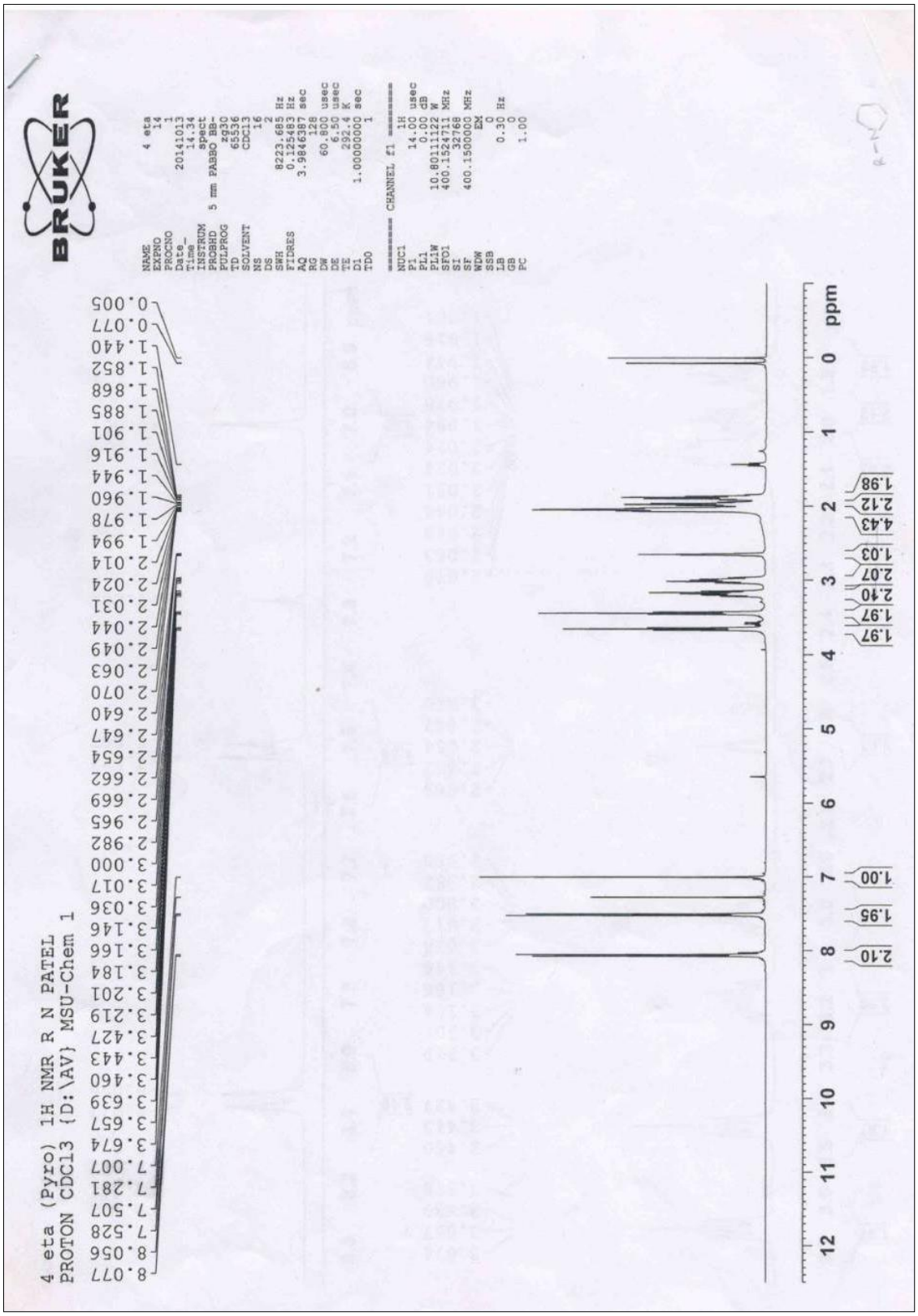


Figure 22: ^{1}H NMR of (Z)-2-(4-(pyrrolidine-1-carbonyl) benzylidene)quinuclidin-3-one 4f

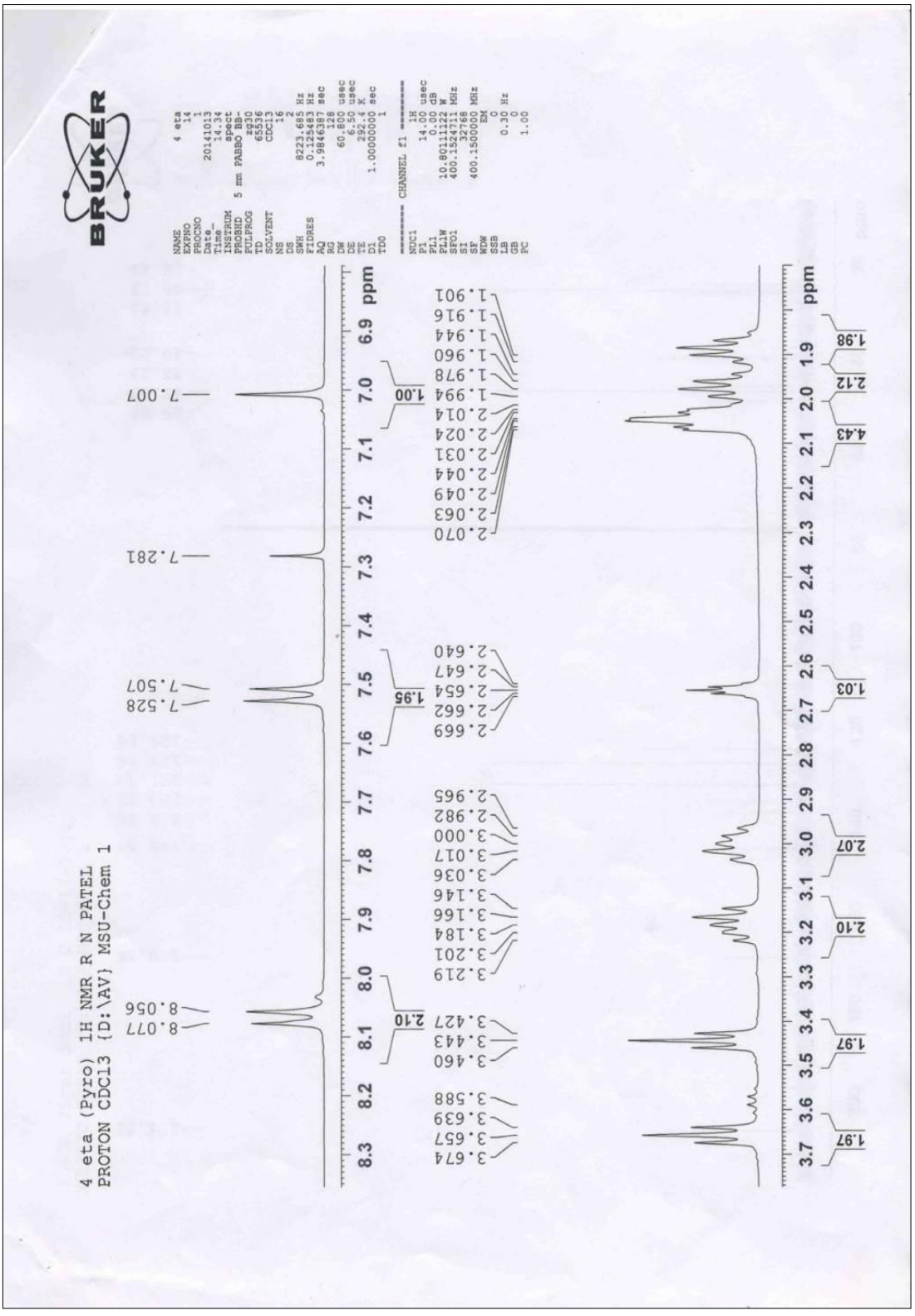


Figure 23: ^{1}H NMR expansion of (Z)-2-(4-(pyrrolidine-1-carbonyl) benzylidene)quinuclidin-3-one 4f

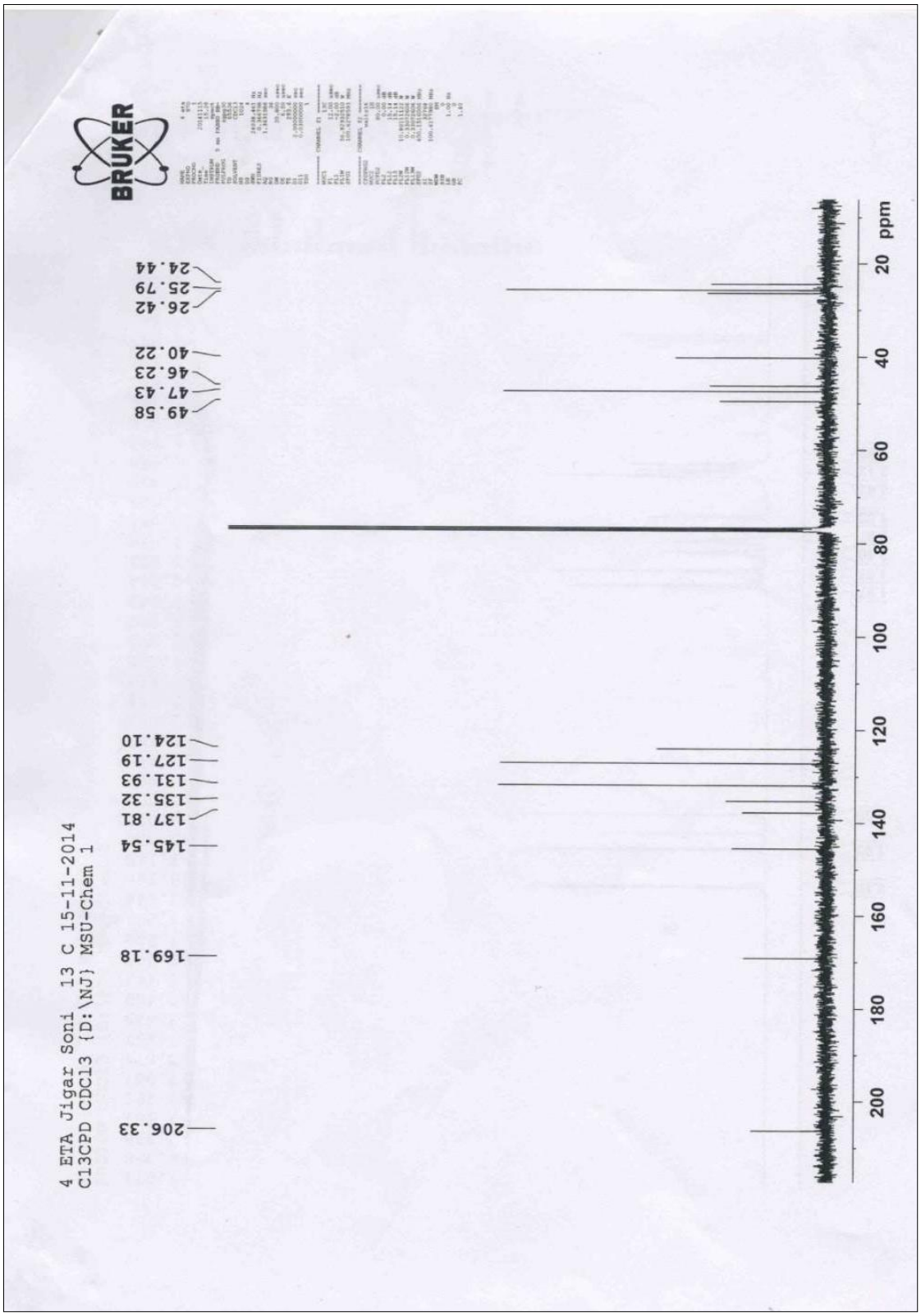


Figure 24: ^{13}C NMR of (Z)-2-(4-(pyrrolidine-1-carbonyl) benzylidene)quinuclidin-3-one 4f

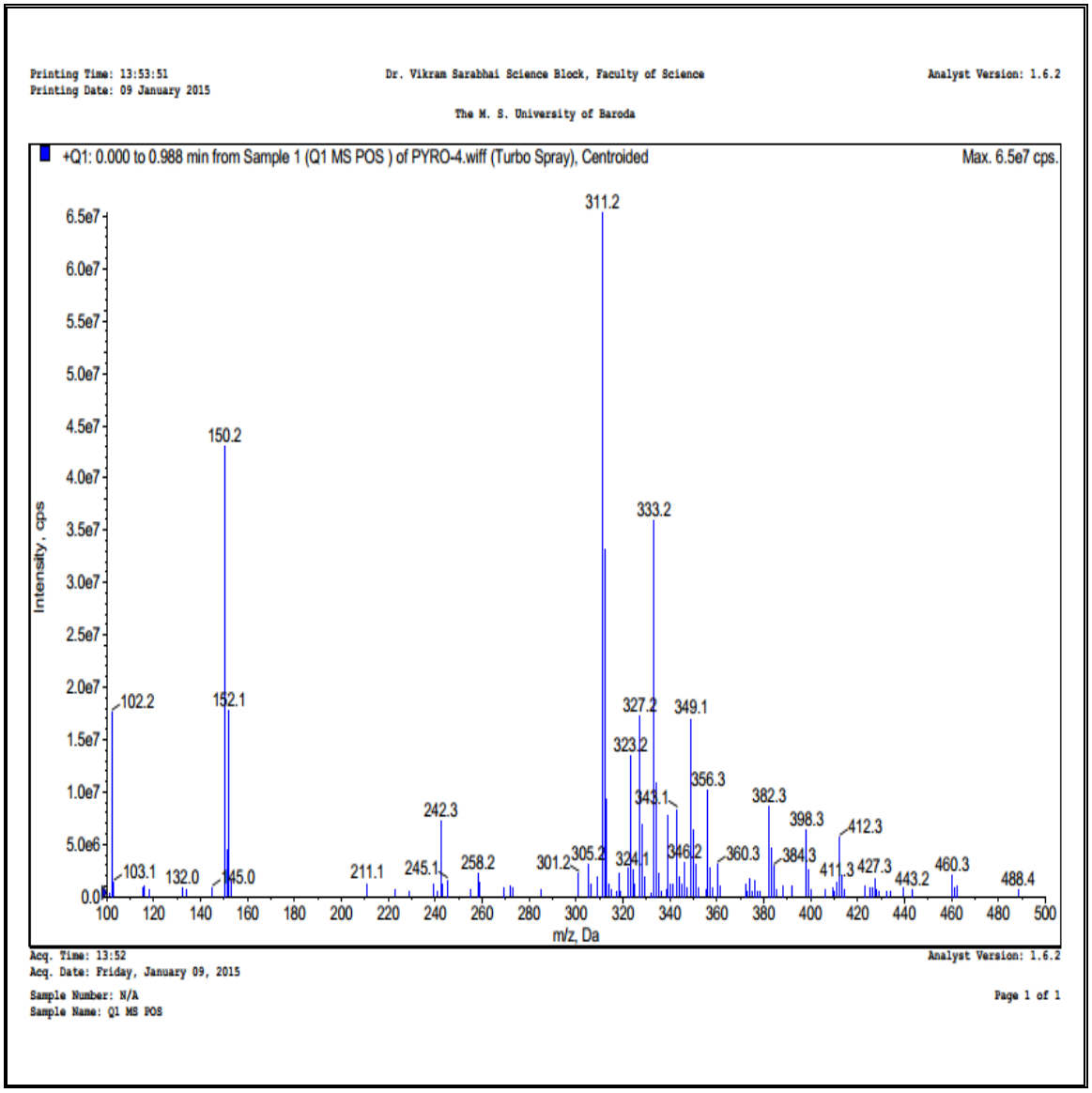


Figure 25: Mass spectra of (Z)-2-(4-(pyrrolidine-1-carbonyl) benzylidene)quinuclidin-3-one 4f

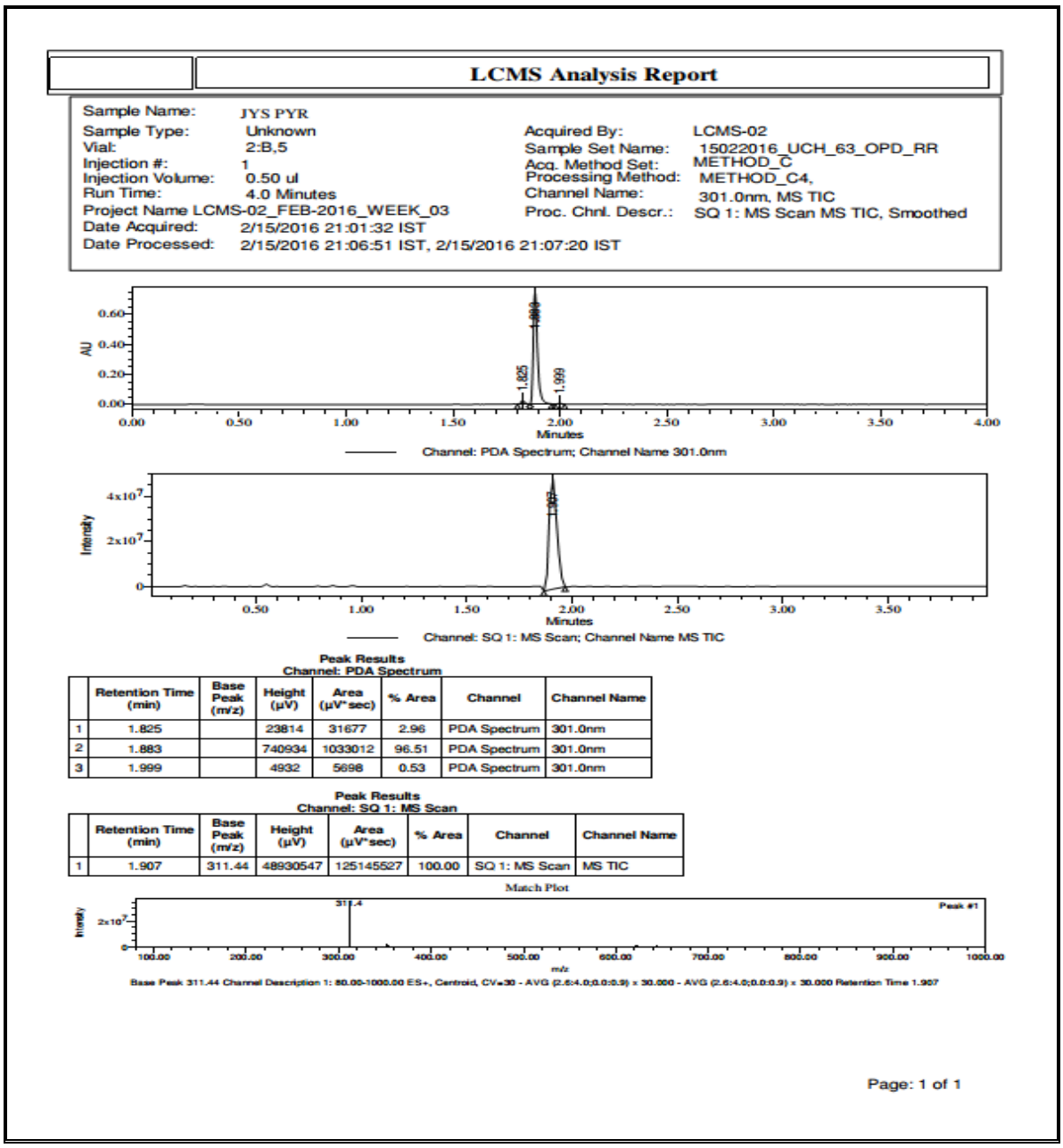


Figure 26: LCMS of (Z)-2-(4-(pyrrolidine-1-carbonyl)benzylidene)quinuclidin-3-one 4f

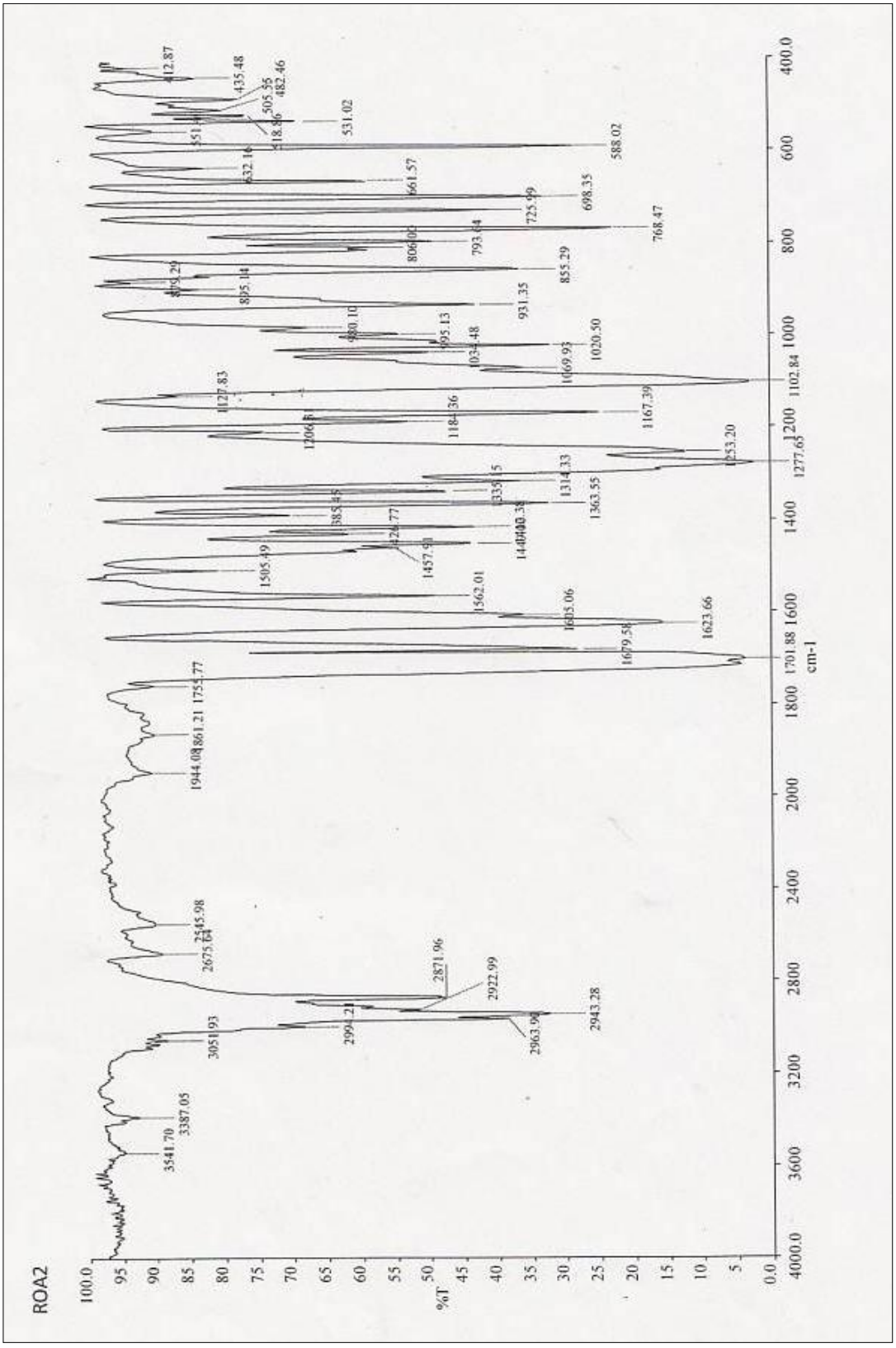


Figure 27: ^{1}H NMR of Ethyl (Z)-4-((3-oxoquinuclidin-2-ylidene) methyl)benzoate 5b

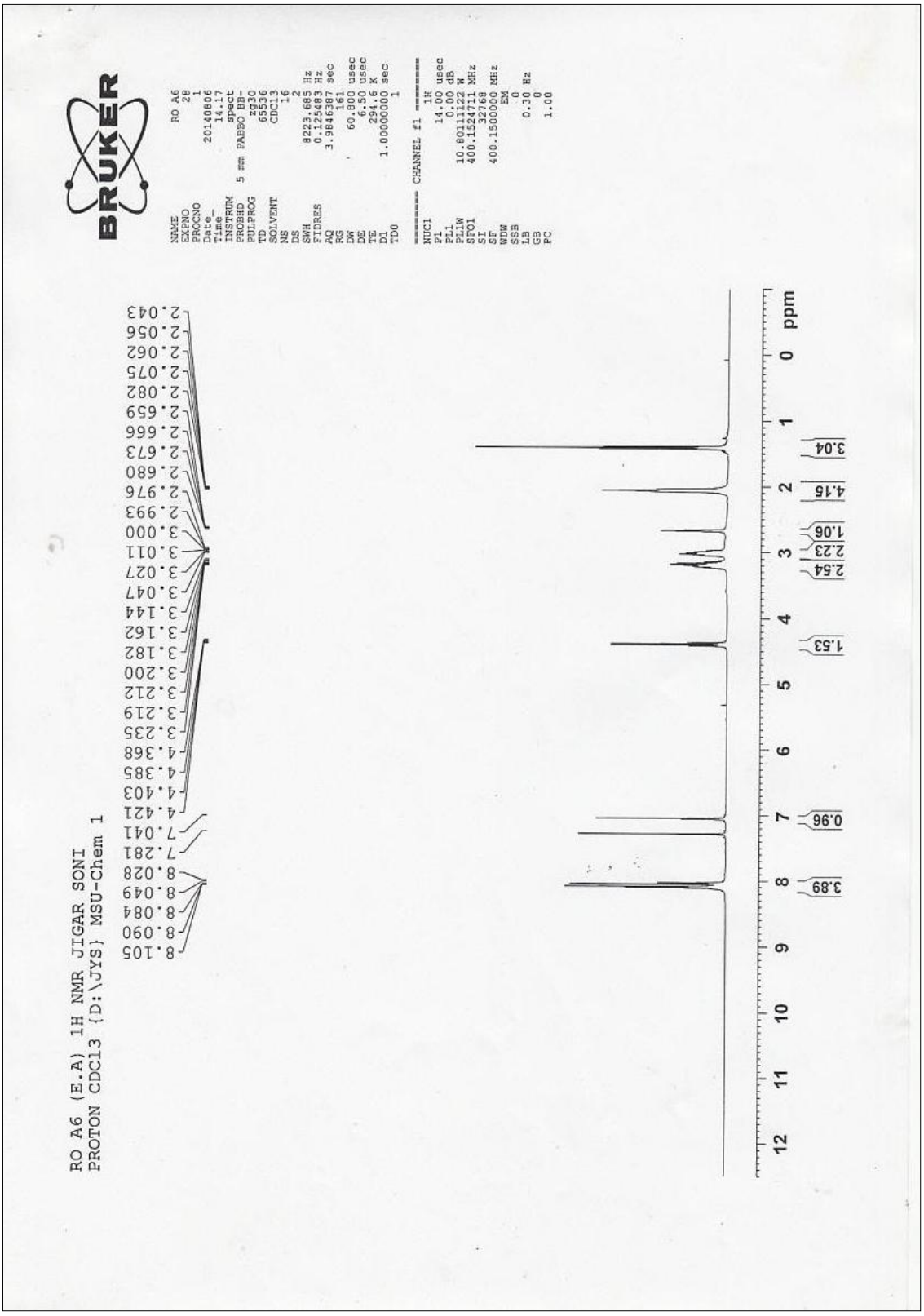


Figure 28: ^{1}H NMR of Ethyl (Z)-4-((3-oxoquinuclidin-2-ylidene)methyl) benzoate 5b

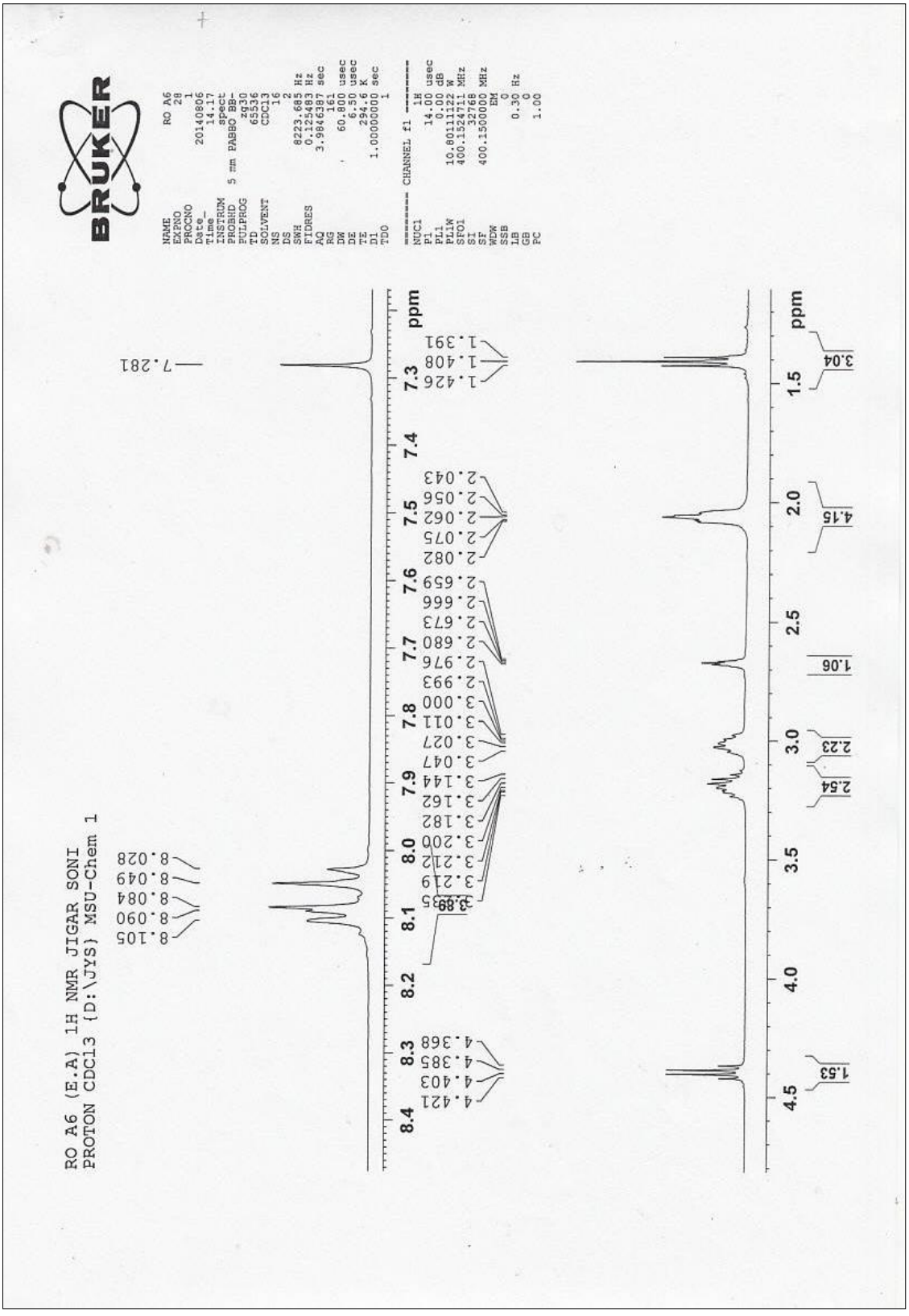


Figure 29: ^{1}H NMR expansion of Ethyl (Z)-4-((3-oxoquinuclidin-2-ylidene)methyl)benzoate 5b

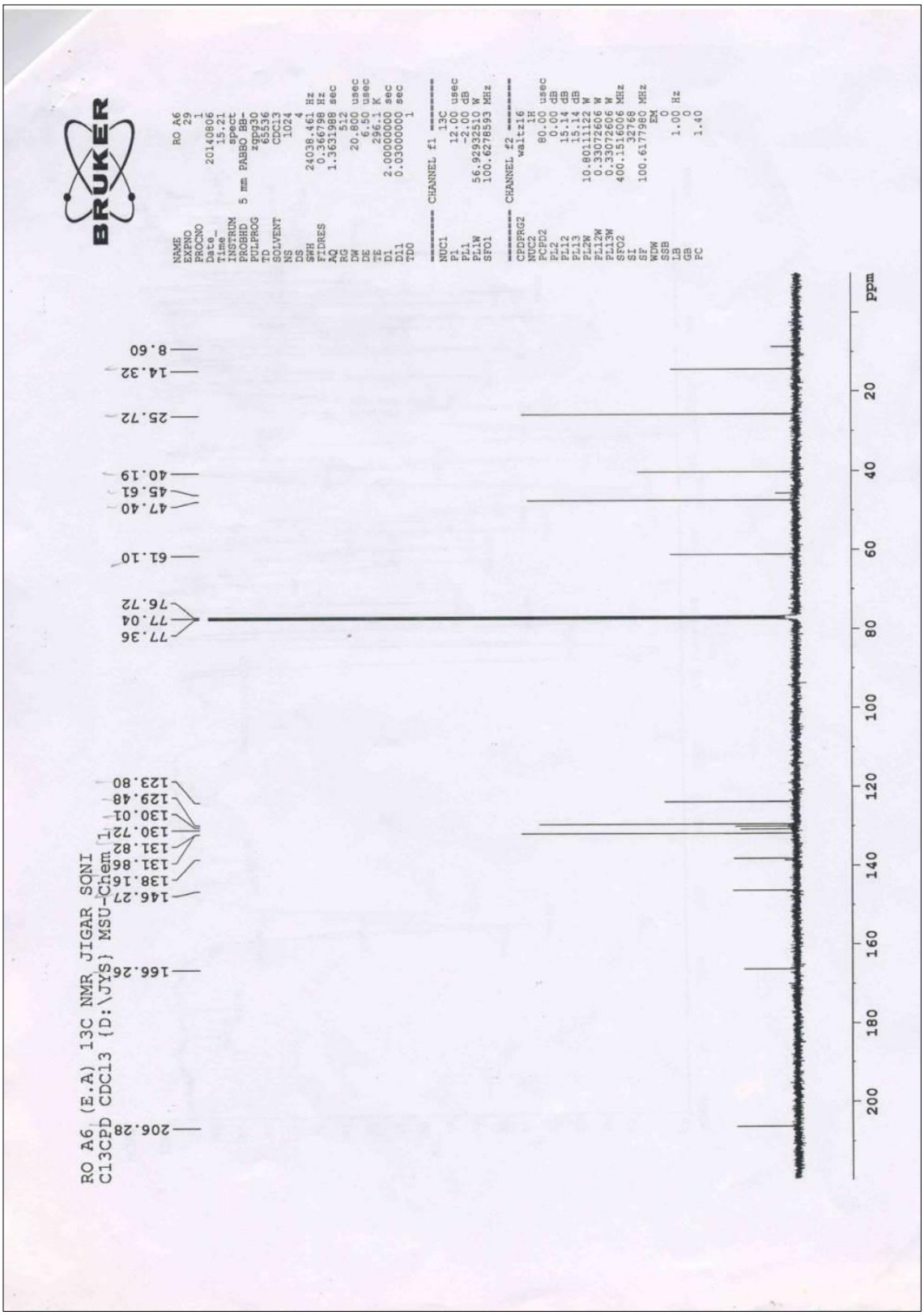


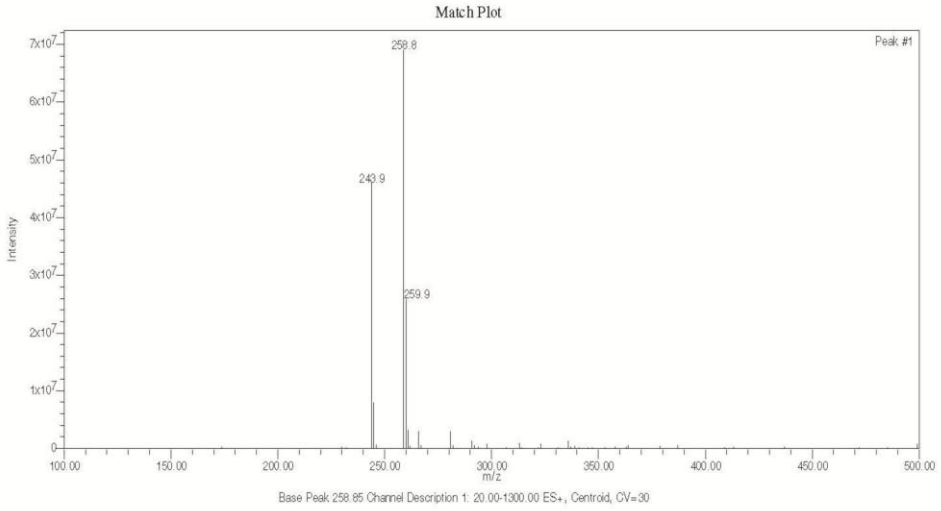
Figure 30: ^{13}C NMR of Ethyl (Z)-4-((3-oxoquinuclidin-2-ylidene) methyl)benzoate 5b

Mass Analysis Report

Sample Name : JYS 06
Vial : 1:A,2
Injection Vol : 15.00 ul
Project Name : MASS_FEB_02_2015
Sample Set : 09022015_02

Acquired By : MASS_02
Acq. Method Set : Mass_2013_2
Processing Method: MASS_2013_02__,
Channel Name : MS TIC @1, MS TIC

Date Acquired : 09-Feb-15 9:45:35 AM IST
Date Processed : 09-Feb-15 9:49:25 AM IST, 09-Feb-15 9:49:36 AM IST



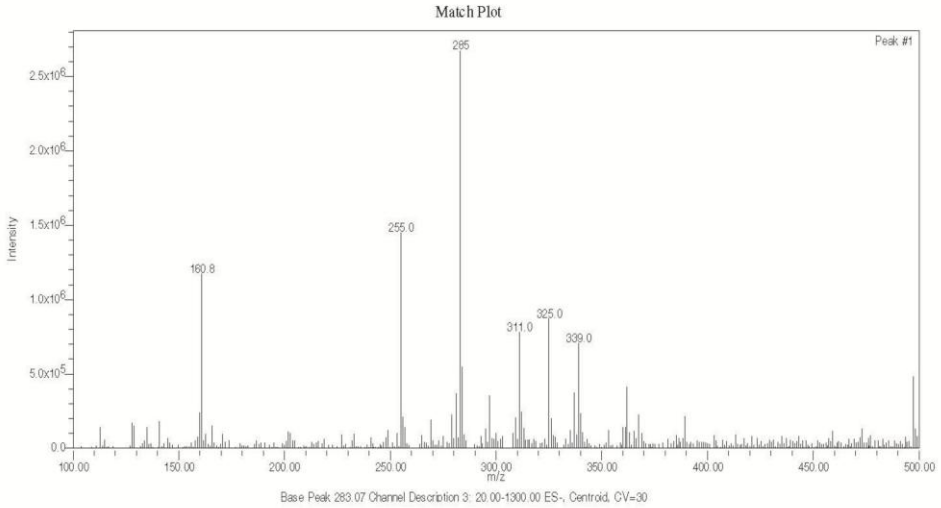


Figure 31: Mass of Ethyl (Z)-4-((3-oxoquinuclidin-2-ylidene)methyl)benzoate 5b

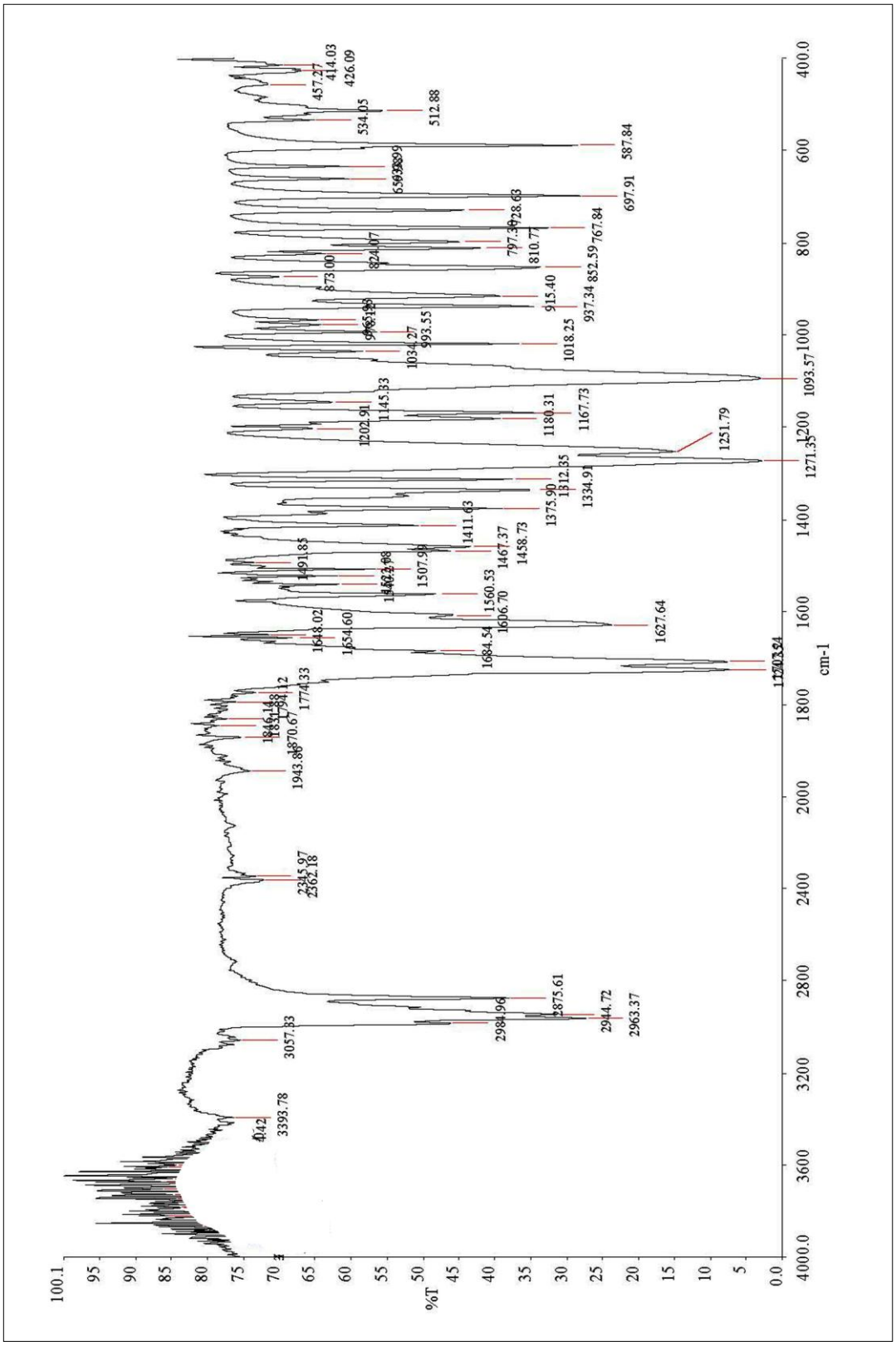


Figure 32: IR of Isopropyl (Z)-4-((3-oxoquinuclidin-2-ylidene) methyl)benzoate 5c

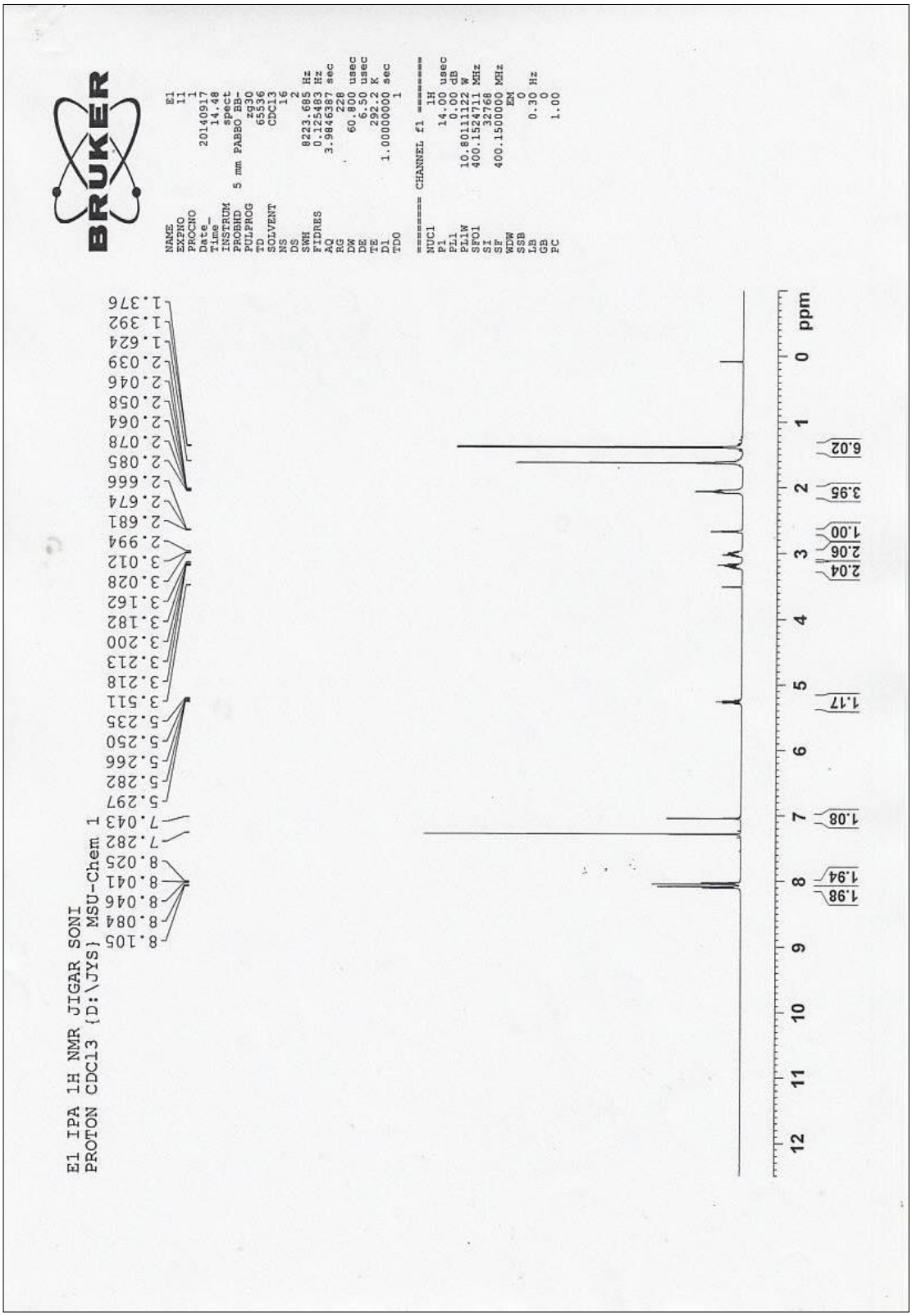


Figure 33: ^{1}H NMR of Isopropyl (Z)-4-((3-oxoquinuclidin-2-ylidene) methyl)benzoate 5c

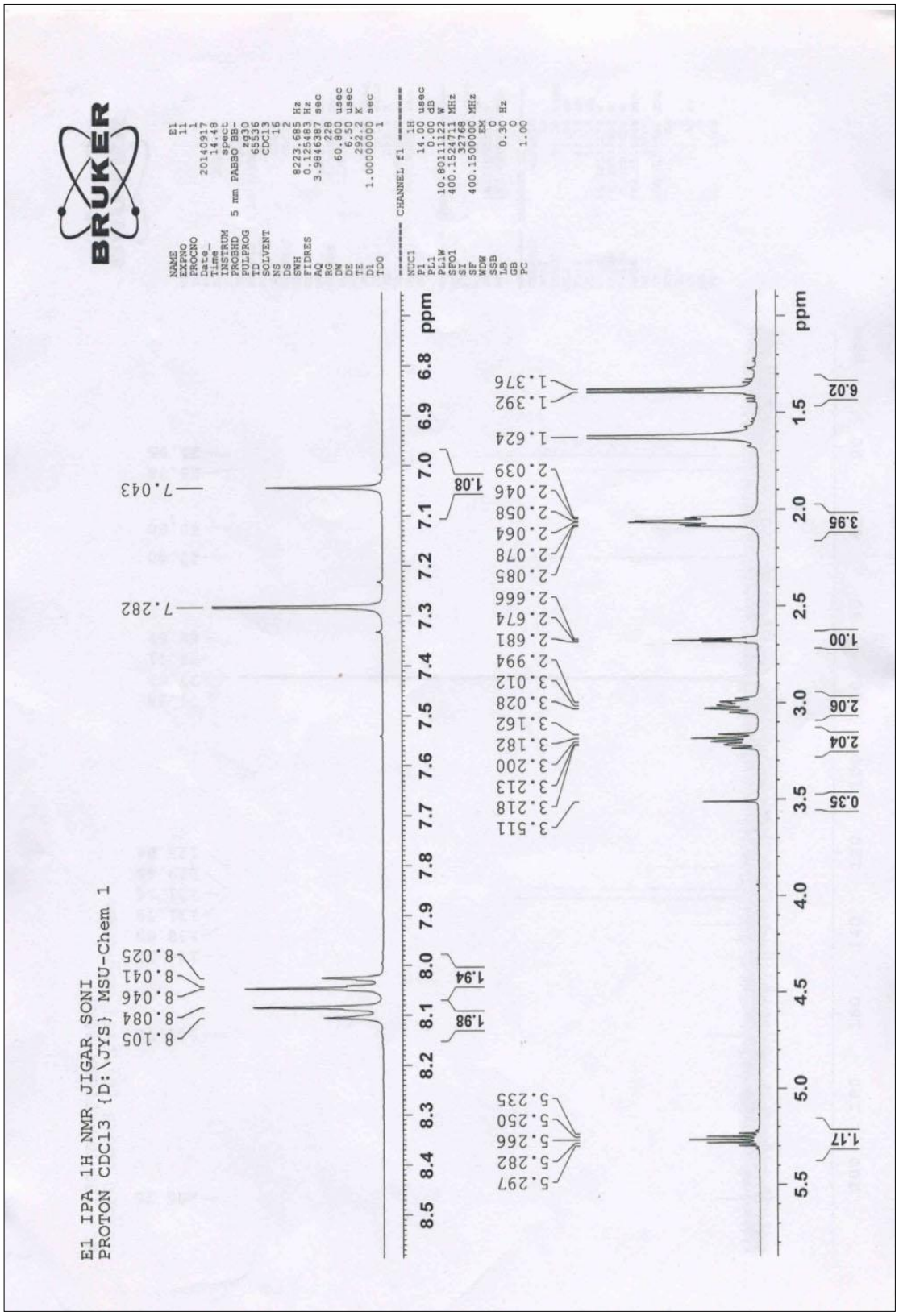


Figure 34: ^{1}H NMR Expansion of Isopropyl (Z)-4-((3-oxoquinuclidin-2-ylidene)methyl)benzoate 5c

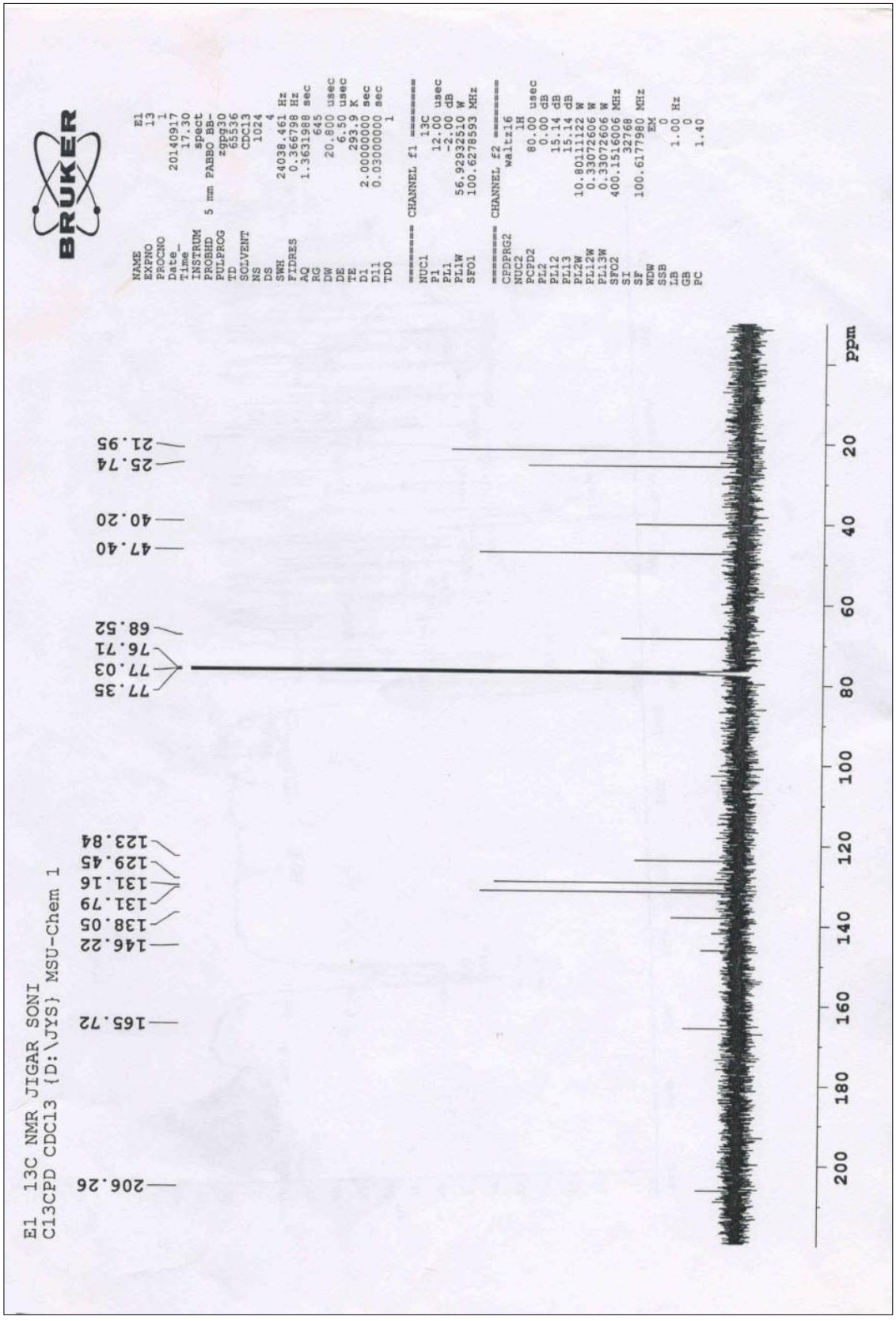


Figure 35: ^{13}C NMR of Isopropyl (Z)-4-((3-oxoquinuclidin-2-ylidene)methyl)benzoate 5c

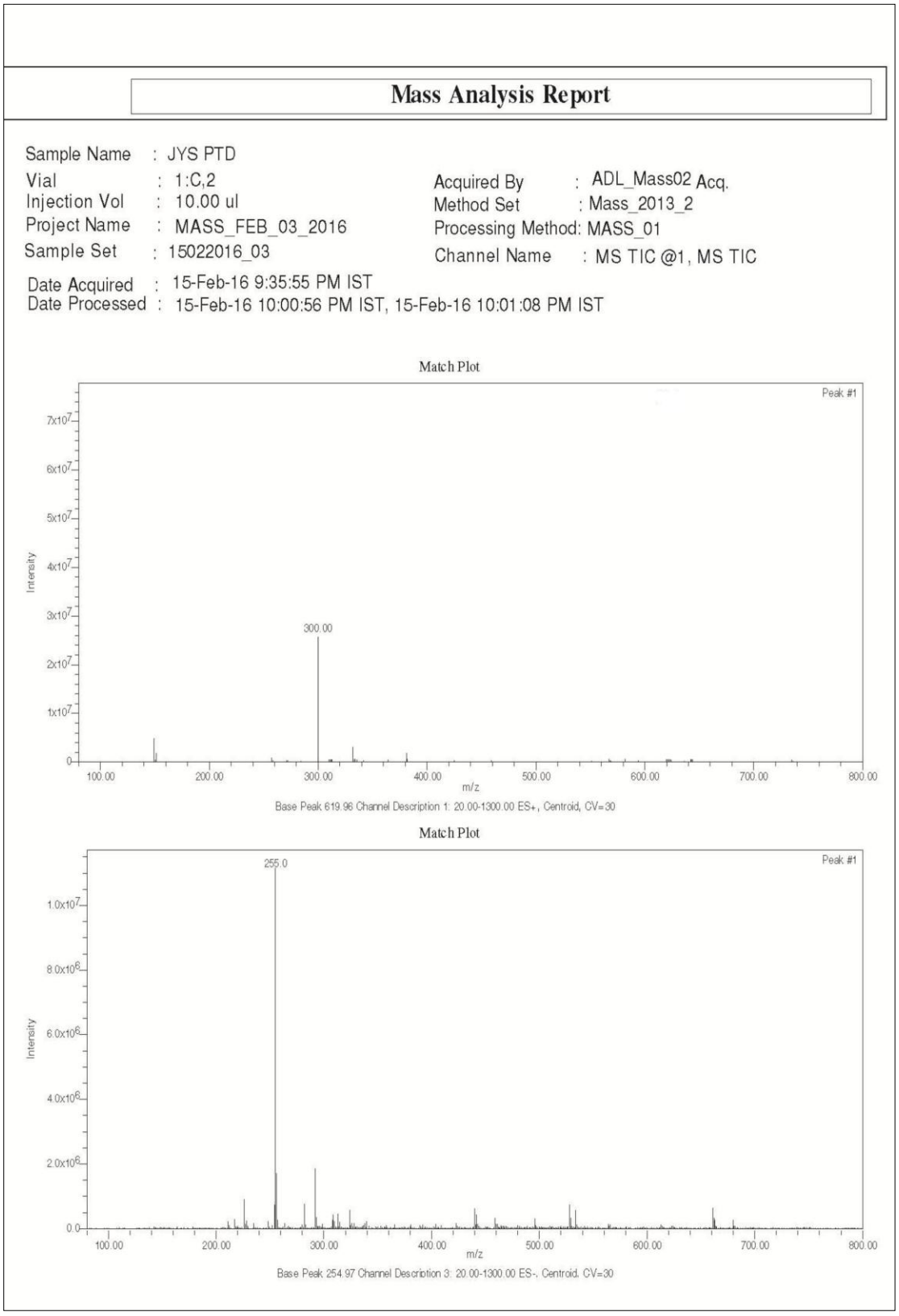


Figure 36: Mass of Isopropyl (Z)-4-((3-oxoquinuclidin-2-ylidene) methyl)benzoate 5c

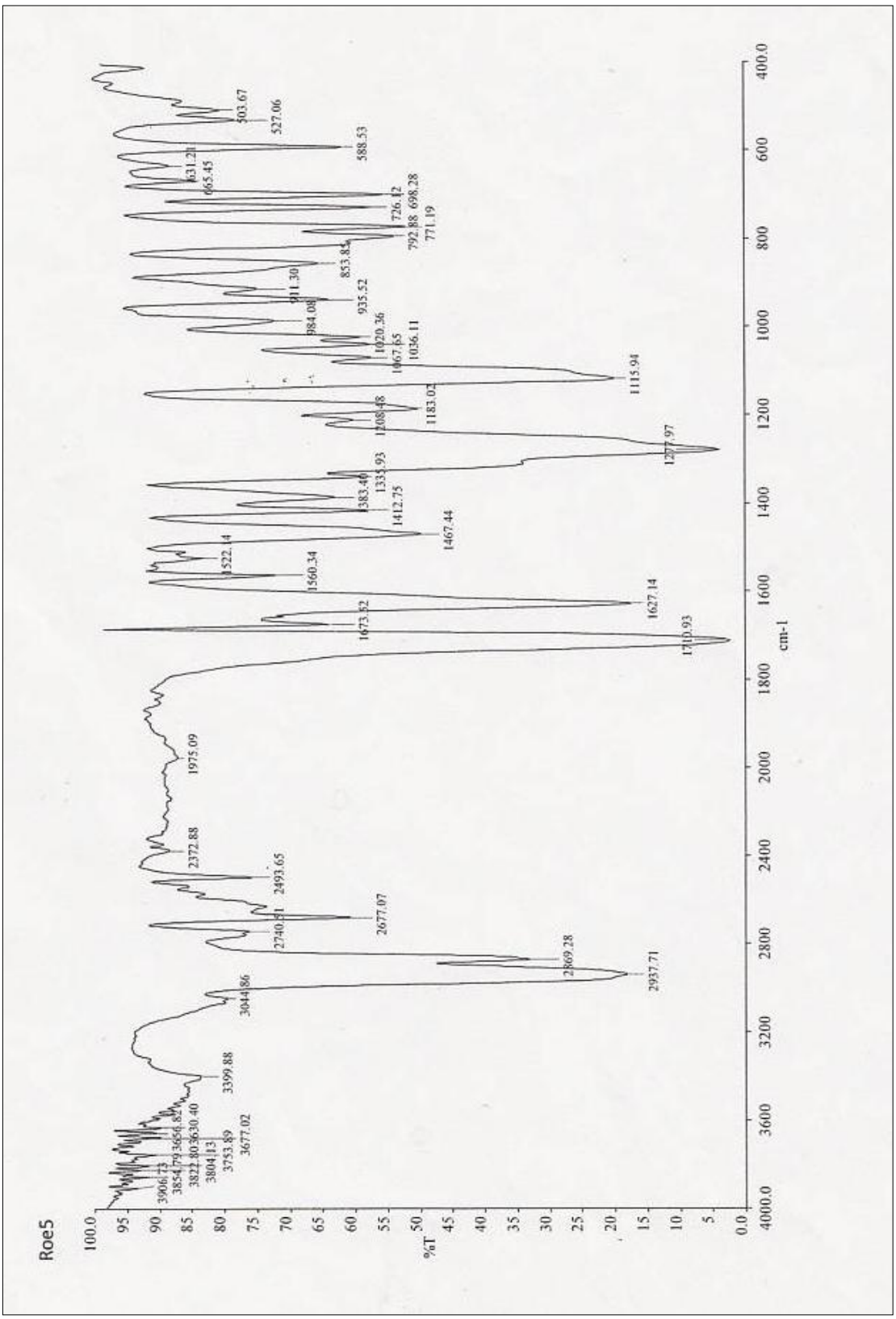


Figure 37: IR of Cyclopentyl(Z)-4-((3-oxoquinuclidin-2-ylidene)methyl)benzoate 5e

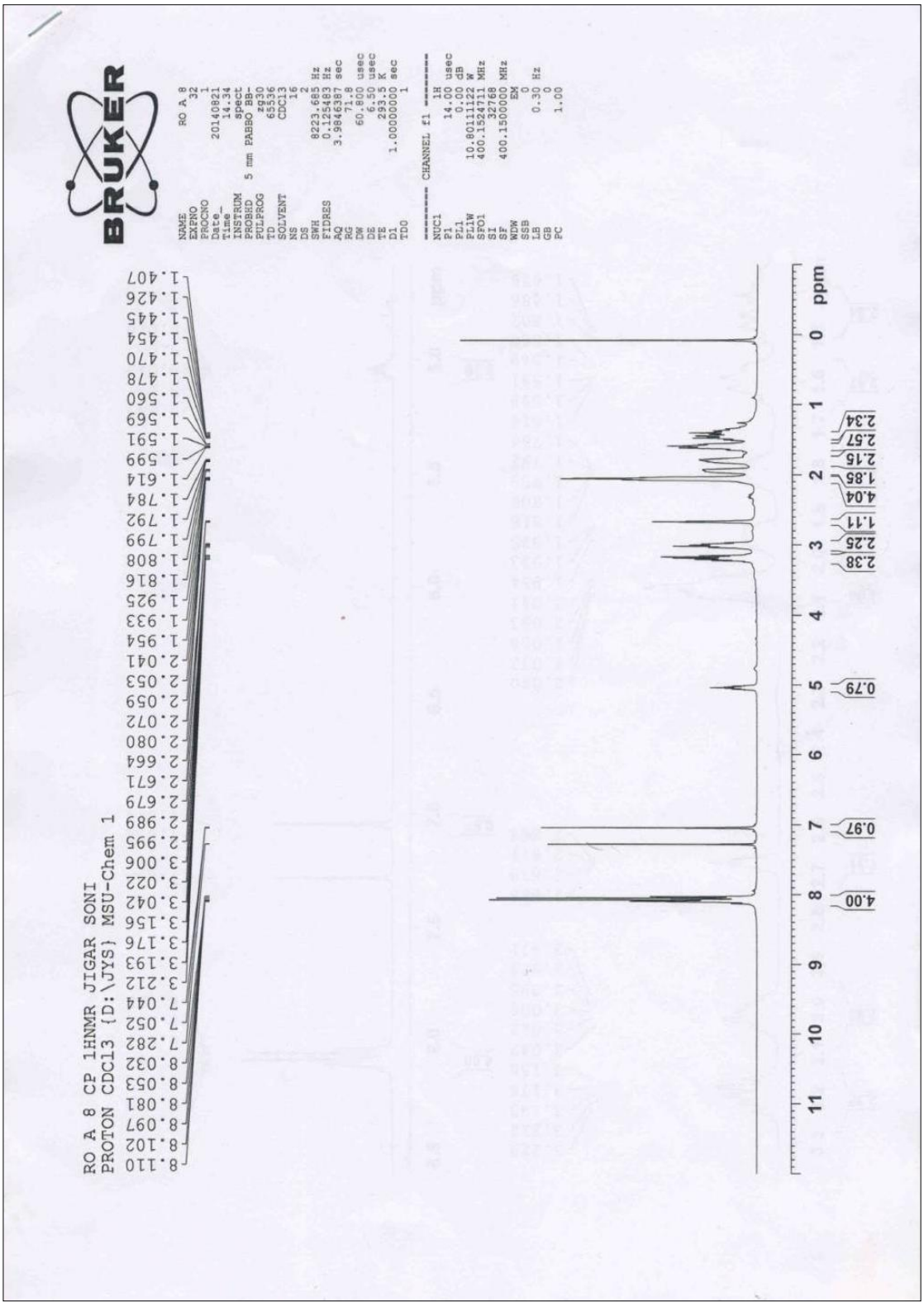


Figure 38: ^{1}H NMR of Cyclopentyl(Z)-4-((3-oxoquinuclidin-2-ylidene)methyl)benzoate 5e

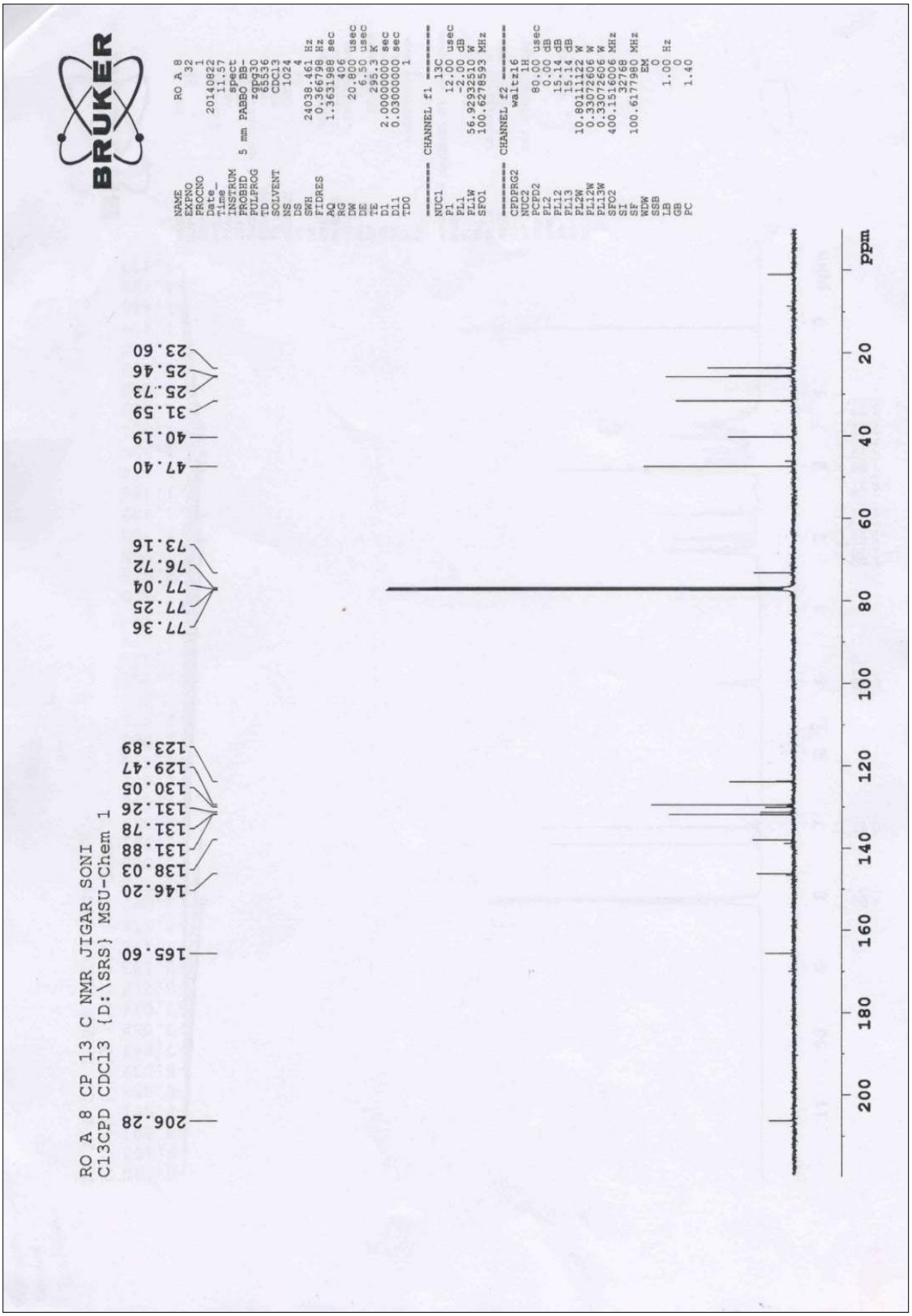


Figure 39: ^{13}C NMR of Cyclopentyl(Z)-4-((3-oxoquinuclidin-2-ylidene) methyl)benzoate 5e

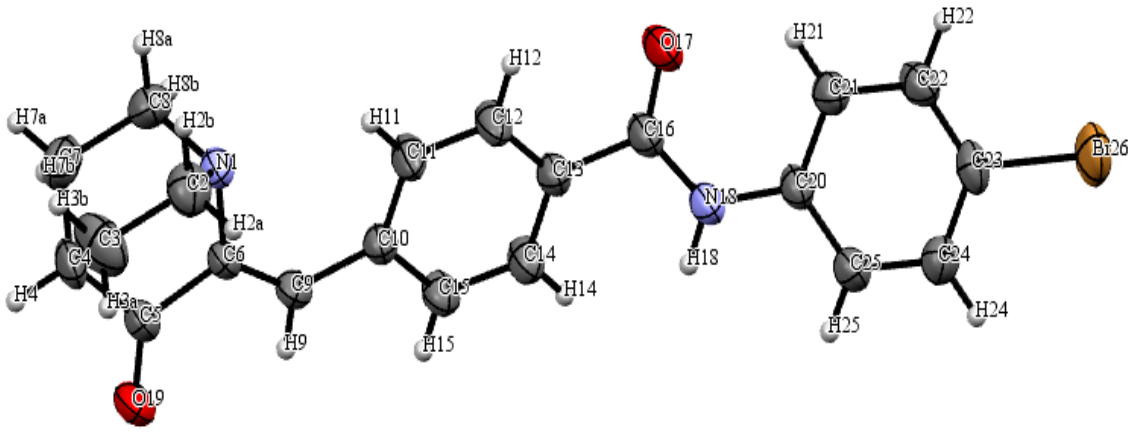


Figure 40: ORTEP diagram of compound 4c with atom numbering scheme.

Displacement ellipsoids are drawn at the 50% probability.

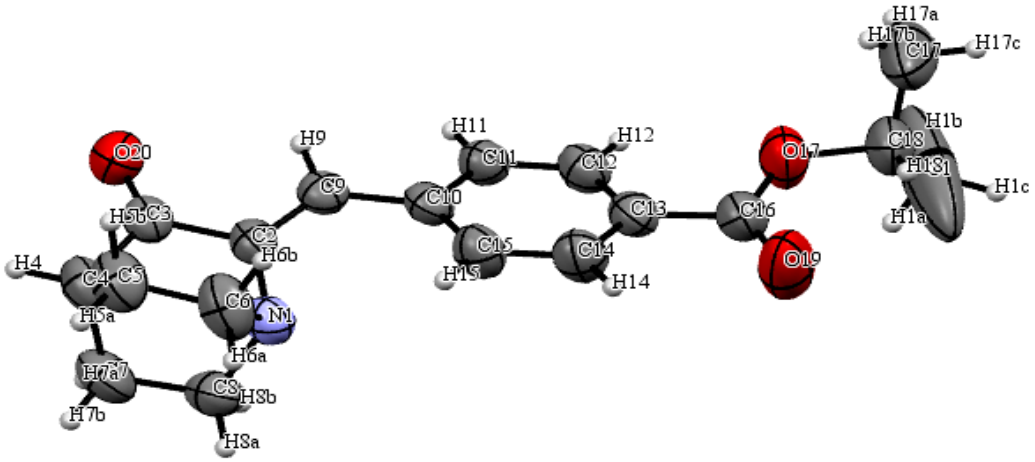


Figure 41: ORTEP diagram of compound 5c with atom numbering scheme.

Displacement ellipsoids are drawn at the 50% probability.

Table 1: Single crystal data of 4c and 5c

Identification code	**4c**	**5c**
Empirical formula	$C_{16}H_{17}NO_3$	$C_{21}H_{19}BrN_2O_2$
Formula weight	271.31	411.29
Temperature/K	293(2)	293(2)
Crystal system	monoclinic	triclinic
Space group	$P2_1$	P-1
a/Å	5.8724(3)	9.1410(4)
b/Å	8.1184(3)	9.6496(7)
c/Å	17.0501(10)	11.1631(8)
α/°	90.00	75.954(6)
β/°	94.221(5)	80.580(5)
γ/°	90.00	74.016(5)
Volume/$Å^3$	810.64(7)	913.33(10)
Z	2	2
ρ_{calc} g/cm^3	1.111	1.496
μ/mm^{-1}	0.625	3.209
F(000)	288.0	420.0
Crystal size/mm^3	0.32 × 0.25 × 0.23	0.30 × 0.28 × 0.25
Radiation	Cu Kα (λ = 1.54184)	Cu Kα (λ = 1.54184)
2Θ range for data collection/°	10.4 to 146.2	8.2 to 150.86°
Index ranges	-7 ≤ h ≤ 6, -10 ≤ k ≤ 5, -	-8 ≤ h ≤ 11, -12 ≤ k

	$21 \leq l \leq 18$	$\leq 12, -13 \leq l \leq 13$
Reflections collected	3740	6973
Independent reflections	2183 [R_{int} = 0.0305, R_{sigma} = 0.0336]	3753 [R(int) = 0.0313]
Data/restraints/parameters	2183/8/201	3753/0/235
Goodness-of-fit on F^2	1.310	1.050
Final R indexes [I>=2σ (I)]	R_1 = 0.0850, wR_2 = 0.2430	R_1 = 0.0435, wR_2 = 0.1206
Final R indexes [all data]	R_1 = 0.0878, wR_2 = 0.2507	R_1 = 0.0483, wR_2 = 0.1275
Largest diff. peak/hole / e $Å^{-3}$	0.86/-0.24	0.72/-0.53
Flack parameter	-0.2(7)	

3.B.2.2 Biological assay and Structure Activity Relationship (SAR)

The synthesized compounds were screened for MTT assay. All compounds were screened at 1, 5, 10 and 20 µM concentrations. The results obtained in a cytotoxicity assay for quinuclidinone and its ester and amide analogues accounted for decreased cell viability in a dose dependent manner. The results also suggest that the synthesized compounds showed specificity for cancer cells over normal lung cells.

The half inhibitory concentration IC_{50} values in µM concentration were determined and presented in Table 2. The results indicate that compared to esters all amide derivative exhibited reasonably good activity. Among amides the derivative having unsubstituted phenyl ring **4a** was found to be less active among all. The introduction

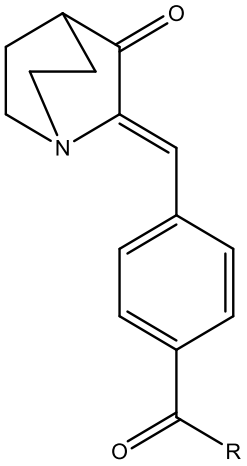
of either electron donating group (methyl) or electron withdrawing group (bromo) increases the activity. Amide derivative having *para* bromo phenyl group **4c** was found to be most potent among all aromatic amides. In order to determine if aromatic ring is essential for activity, derivatives with cyclohexyl **4d** and heterocyclic ring such as morpholine **4e** and pyrrolidine **4f** were synthesized. In all these cases an increase in the activity was observed.

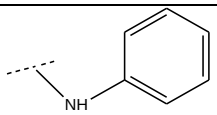
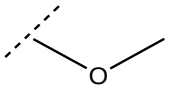
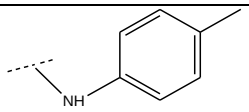
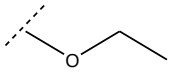
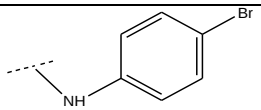
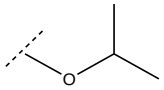
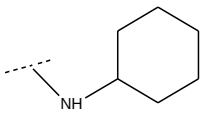
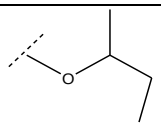
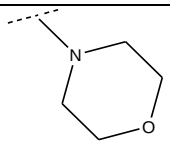
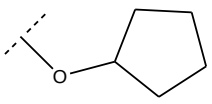
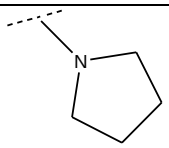
In ester series the derivatives in which the oxygen of ester group was attached to a secondary carbon exhibited better activity. Thus esters of secondary alcohols such as isopropyl alcohol, *sec*-butanol, and cyclopropyl alcohol were found to be more active. The compound **5c** bearing isopropyl group was found to be most active followed by **5d** and **5e** which were moderately active. Lowering of activity was observed when methyl **5a** and ethyl groups **5b** were introduced.

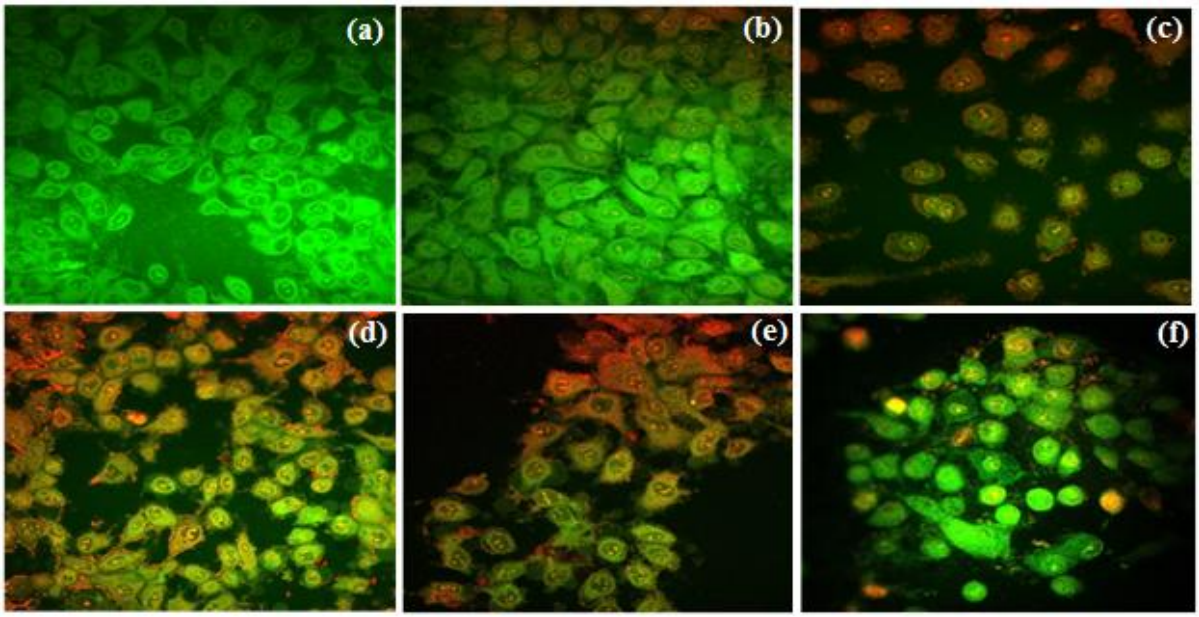
Synthesis of new chemotherapeutic agents with selective cytotoxicity towards cancer cells is always a major challenge for chemists and biotechnologists. Based upon the results obtained in the cytotoxicity assay the derivatives **4c, 4e, 5c** and **5d** were selected for a detailed scrutiny to assess the mode of cell death.

Cancer cells were stained with fluorescent stains to gain a deeper insight into the mechanism of cell death. Induction of apoptosis is a key event and a preferred pathway for induction of cell death by a test compound.[9] Hence, a fluorescent probe (AO/EB) was used to gather qualitative evidence on apoptosis. It has been reported that the viable cells show green fluorescence and late apoptotic cells show orange to red fluorescence with condensed chromatin.[10] We could observe more orange to red fluorescent cells **4c, 4e** and **5c** as compared to **5d** and quinuclidinone HCl **1** treated cells suggesting induction of apoptosis (**Figure: 42**).

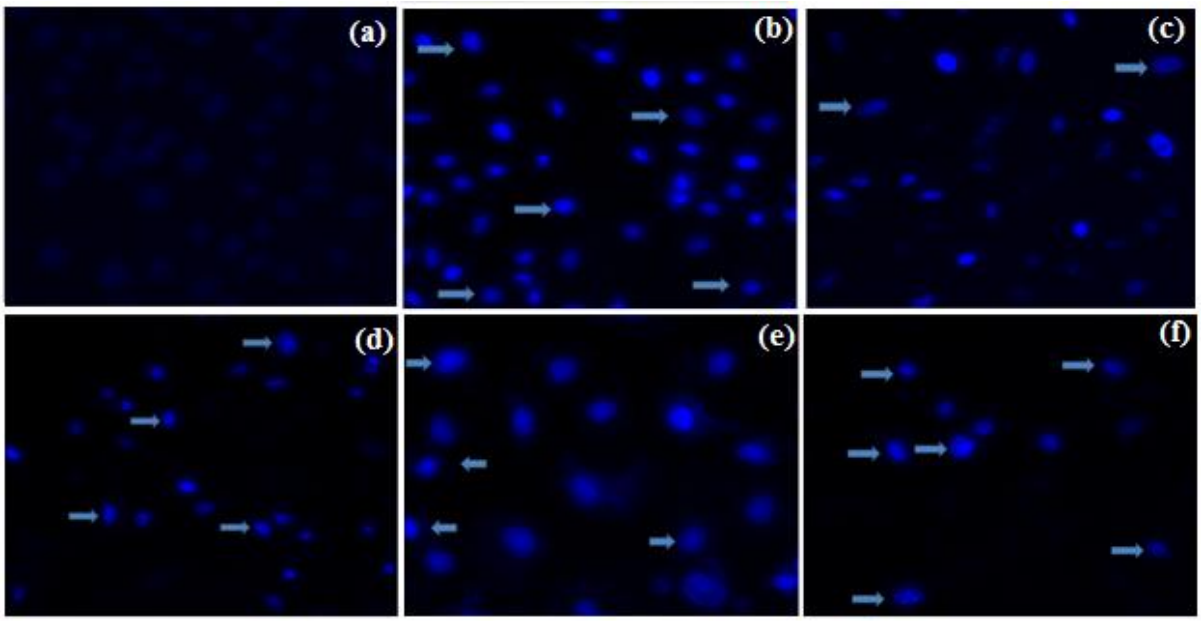
Table 2: IC_{50} values of the compounds 4a-f and 5a-e



Compound	Structure	IC_{50} (µM)	Compound	Structure	IC_{50} (µM)
4a		3.26 ± 1.4	**5a**		9.22 ± 1.2
4b		1.24 ± 0.5	**5b**		8.77 ± 0.6
4c		0.225 ± 0.7	**5c**		1.5 ± 0.9
4d		1.301 ± 0.4	**5d**		5.74 ± 0.8
4e		0.665 ± 1.4	**5e**		6.15 ± 1.0
4f		1.24 ± 0.9			



Nuclear condensation resulting due to any test compound is assessed using DAPI staining.[11] In our study, condensation/fragmentation/distortion of nuclei was evident in all treatment groups (**Figure: 43**).



Further confirmation on apoptosis was obtained from DNA ladder assay, wherein apoptosis induced DNA damage is accredited to possible induction of apoptotic

pathway.[12] In the present study, all the test compounds showed moderate to heavy ladder formation **(Figure: 46)** suggesting induction of apoptosis.

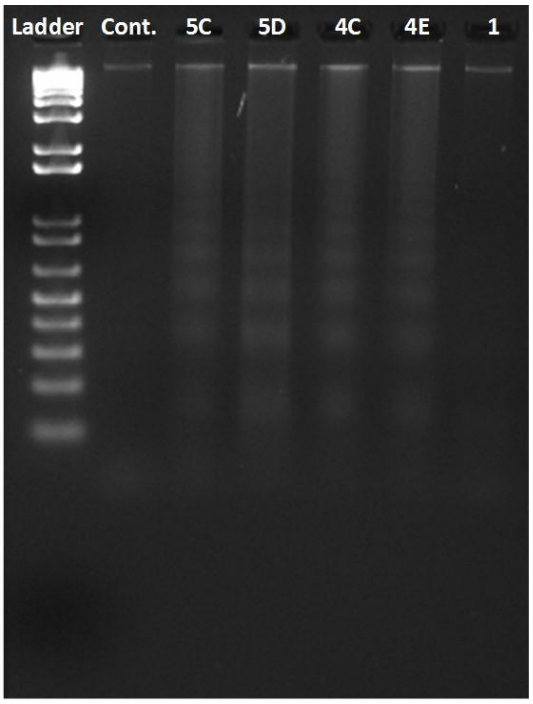


Figure: 44

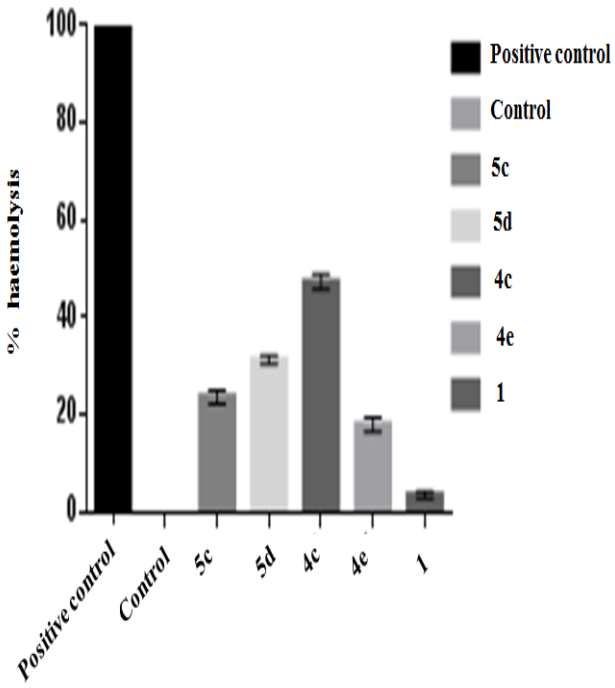


Figure :45

Novel compounds that may be potent in destruction of cancer cells often cause damage to the red blood corpuscles (RBC) and hence, haemolytic assay provides a clue on its merit in not destroying the RBC.[13] Hence, haemolytic assay is a popular tool to assess the relative impact of test compounds on RBCs.[13] In our study **4e, 5e**, **5f**, and the quinuclidinone HCl **1** accounted for moderate haemolysis indicating at their relative safety for *in vivo* use as a possible therapeutant (**Figure: 45**).

3B.3 Experimental

3B.3.1 Material and methods

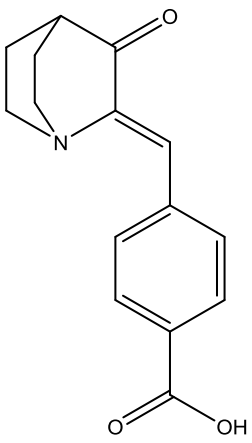
3B.3.1.1 Chemistry

Commercial grade solvents and reagents (alcohols and amines) were purchased from Sigma Aldrich or Alfa aesar or Spectrochem Mumbai India and used without further purification. Quinuclidinone Hydrochloride was prepared as described in literature.[7]

Melting points were measured using a (Buchi B-545) melting point apparatus and were uncorrected. Infrared spectra were recorded on a Perkin-Elmer RX 1 spectrometer. Elemental analyses were recorded on Thermo finnigan Flash 11-12 series EA. ^{1}H and ^{13}C NMR spectra were recorded on an Advance Bruker (400 MHz) spectrometer in suitable deuterated solvents. ^{1}H NMR data were recorded as follows: chemical shift measured in parts per million (ppm) downfield from TMS multiplicity, observed coupling constant (*J*) in Hertz (Hz), proton count. Multiplicities are reported as singlet (s), broad singlet (br s), doublet (d), triplet (t), quartet (q) and multiplet (m). ^{13}C NMR chemical shifts are reported in ppm downfield from TMS. Solvents and reagents were purified by literature methods. Mass spectra were determined by LC/MS, using Shimadzu LCMS 2020 and AB Sciex 3200 QTRAP. The reaction progress was monitored by TLC in ultraviolet light as well as with iodine vapour.

Synthesis of (Z)-4-((3-oxoquinuclidin-2-ylidene)methyl)benzoic acid (3)

Compound 1 (1 mmol) and formyl benzoic acid (1.1 mmol) were taken in absolute ethanol and the solution was refluxed with catalytic amount of sodium hydroxide for about 3-4 h. The reaction progress was monitored by TLC. After completion the reaction mixture was acidified with glacial acetic acid. Crude product precipitated was filtered and washed with water. It was dried under vacuum and recrystallized using IPA-water.

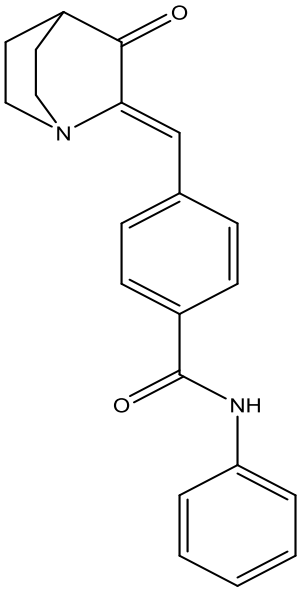


(Z)-4-((3-oxoquinuclidin-2-ylidene)methyl)benzoic acid (3): Yellow solid, mp: >250 ºC; yield 78.6%. IR (KBr, cm^{-1}): 3396, 2971, 2956, 2941, 2869, 1706, 1689, 1624, 1290, 805. ^{1}H NMR (400 MHz, DMSO): δ 1.88-1.94 (2H, m, -CH_2); 1.99-2.02 (2H, m, -CH_2); 2.49 (1H, m, -CH); 2.82-2.92 (2H, m, -CH_2); 3.12-3.25 (2H, m, -CH_2); 6.95 (1H, s,

vinylic proton); 7.94 (2H, d, *J*=8.0, -ArH); 8.15 (2H, d, *J*=8.0, -ArH); 13.15 (1H, b, Acid proton). ^{13}C NMR (100 MHz, DMSO): δ 25.3, 40, 47.3, 122.9, 129.6, 132.1, 137.7, 146.7, 167.5, 205.4.DEPT- 135 (100 MHz, DMSO): δ 25.3, 40.0, 47.3, 122.8, 129.6, 132.1. ESI/MS 258.1 $[M+1]^+$ calculated for $C_{15}H_{15}NO_3$ .Anal.Calcd. for: C, 70.02; H , 5.88; N, 5.44; O, 18.65; found C, 70.42; H, 5.90; N, 5.38.

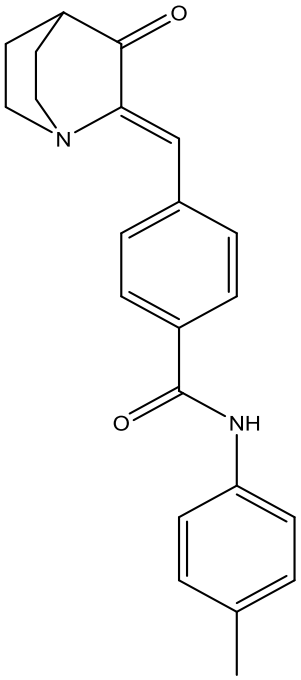
General procedure for the preparation of amides (4)

The product of step one (1 mmol) was taken in 20 ml dichloromethane and triethyl amine (3 mmol) was added. The resulting mixture was stirred at 0 °C and thionyl chloride (1.1 mmol) was added carefully. It was then warmed to room temperature and refluxed for 30 min. After solvent evaporation the unreacted thionyl chloride was removed under vacuum. The acid chloride thus obtained was taken in 20 ml acetonitrile and appropriate amine (1.12 mol) was added followed by K_2CO_3 (3 mmol). The reaction mixture was refluxed and the progress of the reaction was monitored by TLC. After completion, the alcohol was evaporated under vacuum. The residue was dissolved in ethyl acetate (50 ml) and washed with water and sat. $NaHCO_3$ solution. The organic layer was dried with sodium sulfate and solvent evaporated to give the ester as solid product. .

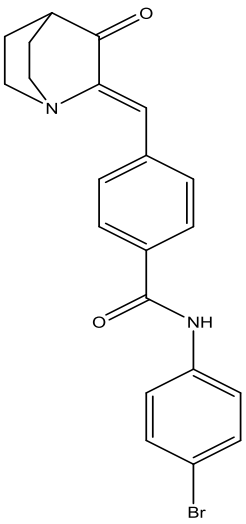


(Z)-4-((3-oxoquinuclidin-2-ylidene)methyl)-N-phenylbenzamide (4a): Yellow solid, mp: 130-133 ºC; yield 60%. IR (KBr, cm^{-1}): 3388, 2960, 2943, 2869, 1699, 1656, 1597, 685. ^{1}H NMR (400 MHz, DMSO): δ 2.00-2.03 (4H, m, 2-CH_2); 2.50-2.52 (1H, m, -CH); 2.86-2.91 (2H, m, -CH_2); 3.13-3.91 (2H, m, -CH_2); 6.98 (1H, s, vinylic proton); 7.10 (1H, t, *J*=7.2, 14.2 Hz, -ArH), 7.34 (2H, t, *J*=7.6, 15.6 Hz, -ArH); 7.78 (2H, d, *J*=8 Hz, -ArH), 7.95 (2H, d, *J*=8.4

Hz, -ArH); 8.18 (2H, d, *J*=8.0 Hz, -ArH), 10.33 (1H, s , Amide proton). ^{13}C NMR (100 MHz, DMSO): δ 25.3, 47.3, 114.3, 120.7, 123.0, 124.2, 128.1, 129.1, 129.2, 129.4, 131.5, 132.0, 135.8, 136.9, 139.5, 146.6, 165.5, 205.5 ESI/MS 333.2 $[M+1]^+$ calculated for $C_{21}H_{20}N_2O_2$ .Anal.Calcd. For: C, 75.88; H, 6.06; N, 8.43; O, 9.63; found C, 75.71; H, 6.27; N, 8.51.

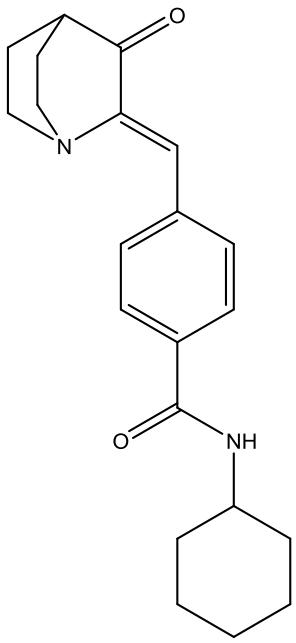


(Z)-4-((3-oxoquinuclidin-2-ylidene)methyl)-N-(p-tolyl)benzamide (4b): Yellow solid, mp: >250ºC; yield 58%. IR (KBr, cm^{-1}) (figure 5): 3295, 3130, 3042, 2950, 2869, 1703, 1644, 1607, 808. ^{1}H NMR (400 MHz, $CDCl_3$) (figure 6): δ 2.06-2.08 (4H, m, 2-CH_2); 2.36 (3H, s, -CH_3); 2.67-2.68 (1H, m, -CH), 2.99-3.05 (2H, m, -CH_2); 3.17-3.22 (2H, m, -CH_2); 7.04 (1H, s, vinylic proton); 7.18 (2H, d, *J*=8.4 Hz, -ArH); 7.54 (2H, d, *J*=8.0 Hz, -ArH); 7.86 (2H, d, *J*=8.0 Hz, -ArH), 8.16 (2H, d, *J*=8.4 Hz, -ArH). ^{13}C NMR (100 MHz, $CDCl_3$) (figure 8): δ 20.9, 25.7, 40.1, 47.4, 120.2, 123.6, 127.0, 129.6, 132.2, 134.3, 135.3, 137.1, 146.1, 165.1, 206.2. ESI/MS (figure 9) 347.2 $[M+1]^+$ calculated for $C_{22}H_{22}N_2O_2$ .Anal.Calcd. for: C, 76.28; H, 6.40; N, 8.09; O, 9.24; found C, 76.51; H, 6.26; N, 8.27.

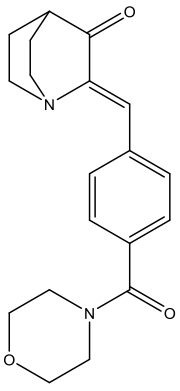


(Z)-N-(4bromophenyl)-4-((3-oxoquinuclidin-2-ylidene) methyl)benzamide (4c): Yellow solid, mp: 207-208ºC; yield 69%. IR (KBr, cm^{-1}): 3373, 3302, 3086, 2942, 2866, 1698, 1677, 1620, 1588, 1466, 1067, 1036, 854. ^{1}H NMR (400 MHz, DMSO): δ 1.91-2.07 (4H, m, 2-CH_2); 2.85-2.92 (2H, m, -CH_2); 3.13-3.20 (2H, m, -CH_2); 6.98 (1H, s, vinylic proton); 7.54 (2H, d, *J*=9.4 Hz, -ArH); 7.78 (2H, d, *J*=9.2 Hz, -ArH); 7.95 (2H, d,

J=8.4 Hz, -ArH); 8.19 (2H, d, *J*=8.4 Hz, -ArH); 10.50 (1H, s, Amide). ^{13}C NMR (100 MHz, DMSO): δ 25.3, 47.3, 115.8, 122.6, 122.9, 128.1, 129.3, 131.9, 132.0, 135.5, 137.1, 139.0, 146.6, 165.6, 205.4. ESI/MS 410.2 $[M]^+$ calculated for $C_{21}H_{19}N_2BrO_2$. Anal.Calcd. for: C, 61.33; H, 4.66; N, 6.81; O, 7.78; Br, 19.43; found C, 61.47; H, 4.45; N, 6.97.

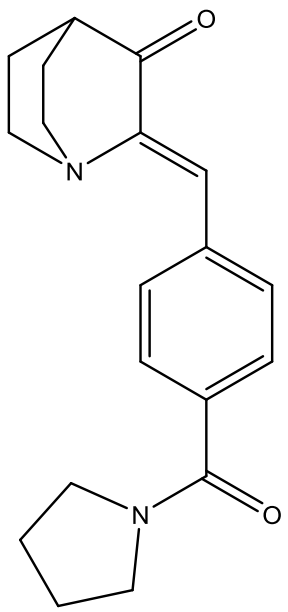


(Z)-N-cyclohexyl-4-((3-oxoquinuclidin-2-ylidene)methyl) benzamide (4d): Yellow solid, mp: >225ºC; yield 73%. IR (KBr, cm^{-1}) (figure 10): 3341, 2926, 2853, 1745, 1703, 1653, 1534, 1500, 800. ^{1}H NMR (400 MHz, DMSO) (figure 11): δ 1.11-1.13 (1H, m, -CH_2); 1.24-1.35 (4H, m, 2-CH_2); 1.59-1.62 (1H, m, -CH_2); 1.72-1.73 (2H, m, -CH_2); 1.73-1.72 (2H, m, -CH_2) ;1.86-1.92 (2H, m, -CH_2);1.98-2.03 (2H, m, -CH_2); 2.83-2.90 (2H, m, -CH_2); 3.12-3.19 (2H, m, -CH_2); 3.73-3.75 (1H, m, -CH); 6.94 (1H, s, - vinylic proton); 7.82 (2H, d, *J*=8.4 Hz, -ArH); 8.10 (2H, d, *J*=8.4 Hz, -ArH); 8.23 (1H, d, Amide). ^{13}C NMR (100 MHz, DMSO) (figure 13): δ 25.3, 32.8, 47.3, 48.8, 123.1, 127.7, 131.9, 135.7, 136.4, 146.35, 165.3, 205.4. ESI/MS (figure 14) 338.3 $[M+1]^+$ calculated for $C_{21}H_{26}N_2O_2$. Anal.Calcd. for: C, 74.53; H, 7.74; N, 8.28; O, 9.45; found C, 74.78; H, 7.51; N, 8.37.



(Z)-2-(4-(morpholine-4-carbonyl)benzylidene)quinuclidin-3-one (4e): Yellow solid, mp: 155-156 ºC; yield 55%.. IR (KBr, cm^{-1}) (figure 16): 3444, 3027, 2962, 2869, 1707, 1683, 1635, 1438, 1110. ^{1}H NMR (400 MHz, DMSO) (figure 17): δ 2.02-2.07 (4H, m, 2-CH_2); 2.65-2.66 (1H, m, -CH); 2.96-3.03 (2H, m, -CH_2); 3.15-3.22 (2H, m, -CH_2); 3.47-3.79 (8H, m, -4CH_2); 7.00 (1H, s,-

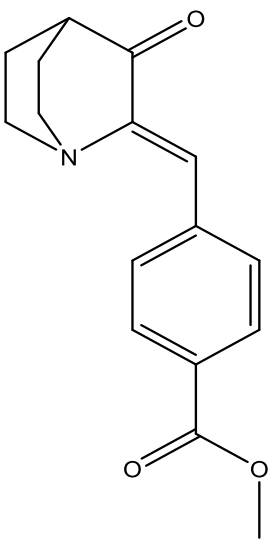
vinylic proton); 7.41 (2H, d, *J*=8.4 Hz, -ArH); 8.07 (2H, d, *J*=8 Hz, -ArH).[13]C NMR (100 MHz, $CDCl_3$) (figure 19): δ 25.3, 40.1, 47.4, 51.6, 66.9, 123.8, 127.2, 132.1, 135.5, 135.8, 145.7, 169.9, 206.2 ESI/MS (figure 20) 327.3 $[M+1]^+$ calculated for $C_{15}H_{17}NO_2$ .Anal.Calcd. for: C, 69.92; H, 6.79; N, 8.58; O, 14.71.; C, 70.14; H, 6.51; N, 8.42.



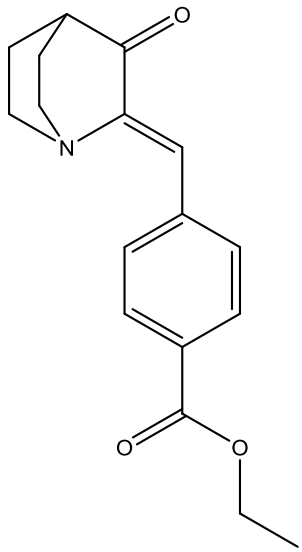
(Z)-2-(4-(pyrrolidine-1-carbonyl)benzylidene) quinuclidin-3-one (4f): Yellow solid, mp: 170-172 ºC; yield 49%. IR (KBr, cm^{-1}) (figure 21): 3444, 2970, 2944, 2872, 1703, 1623, 1431, 1097. [1]H NMR (400 MHz,$CDCl_3$) (figure 22): δ 1.90-1.96 (2H, m, -CH_2); 1.97-1.99 (2H, m, -CH_2); 2.01-2.07 (4H, m, 2-CH_2); 2.64-2.66 (1H, m, -CH); 2.96-3.03 (2H, m, -CH_2); 3.14-3.21 (2H, m, -CH_2); 3.42-3.46 (2H, t, -CH_2); 3.63-3.67 (2H, m, -CH_2); 7.00 (1H, s,-vinylic proton); 7.51 (2H, d, *J*=8.4 Hz, -ArH); 8.06 (2H, d, *J*=8.4 Hz, -ArH). [13]C NMR (100 MHz, $CDCl_3$) (figure 24): δ 24.4, 25.7, 26.4, 40.2, 46.2, 47.4, 49.5, 124.1, 127.1, 131.9, 135.3, 137.8, 145.5, 169.1, 206.3. ESI/MS (figure 25) 311.4 $[M+1]^+$ calculated for $C_{19}H_{22}N_2O_2$. Anal.Calcd. for: C, 73.52; H, 7.14; N, 9.03; O, 10.31; found C, 73.37; H, 7.29; N, 9.25.

General procedure for the preparation of esters (5)

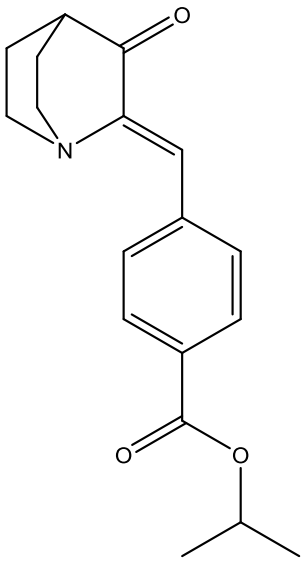
To the acid chloride obtained above, appropriate alcohol was added in excess and the mixture was refluxed. The progress of the reaction was monitored by TLC. After completion, the alcohol was evaporated under vacuum. The residue was dissolved in ethyl acetate (50 ml) and washed with water and sat. $NaHCO_3$ solution. The organic layer was dried with sodium sulfate and solvent evaporated to give the ester as solid product.



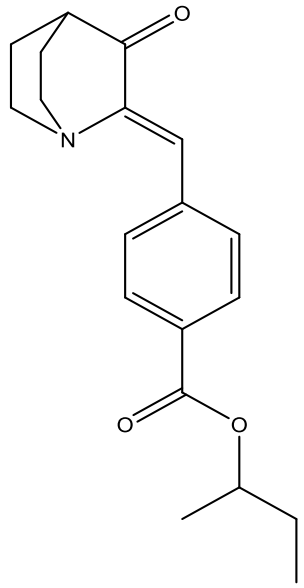
Methyl(Z)-4-((3-oxoquinuclidin-2-ylidene)methyl)benzoate (5a): Yellow solid, mp: 142-143ºC; yield 70%. IR (KBr, cm^{-1}): 3380, 3049, 2290, 2220, 2869, 1759, 1728, 1700,1620. ^1H NMR (400 MHz, $CDCl_3$): δ2.02-2.07 (4H, m, CH_2); 2.64-2.66 (1H, m, CH); 2.98-3.05 (2H, m, CH_2); 3.15-3.22 (2H, m, CH2); 3.84 (3H, s, -CH_3); 6.90-6.93 (1H, dd, J= 4.0, 0.8 Hz, ArH) 7.00 (1H, s, vinylic proton); 7.30 (1H, t, J= 8.0, 7.6 Hz, ArH); 7.54 (1H, d, *J*= 7.2 Hz, ArH); 7.81 (1H, s, ArH). ^{13}C NMR (100 MHz, $CDCl_3$): δ 25.8, 40.2, 47.4, 51.2, 115.3, 117.0, 124.9, 125.0, 129.3, 135.2, 169.3, 206.5. ESI/MS 272.2 $[M+1]^+$ calculated for $C_{16}H_{17}NO_3$ .Anal.Calcd.for $C_{15}H_{17}NO_2$: C, 70.83; H, 6.32; N, 5.16; O, 17.69 Found: C, 70.92; H, 6.28; N, 5.20.



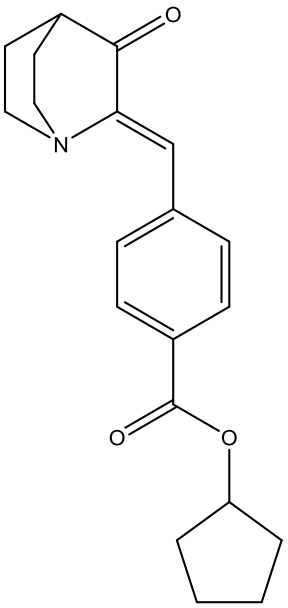
Ethyl(Z)-4-((3-oxoquinuclidin-2-ylidene)methyl)benzoate (5b): Yellow solid, mp: 112-115ºC; yield 68%. IR (KBr, cm^{-1}) (figure 27): 3387, 3051, 2994, 2963, 2943, 2922, 2871, 1754, 1701, 1679, 1623, 806. ^1H NMR (400 MHz, $CDCl_3$) (figure 28): δ 1.39-1.42 (3H, t,-CH_2); 2.04-2.08 (4H, m, CH_2); 2.65-2.68 (1H, m, CH); 2.99-3.04 (2H, m, CH_2); 3.14-3.23 (2H, m, CH_2); 4.46-4.42 (1H, q, CH_2); 7.04 (1H, s, vinylic proton); 8.02-8.10 (4H, m, ArH). ^{13}C NMR (100 MHz, $CDCl_3$) (figure 30): δ 8.60, 14.3, 25.7, 40.1, 45.6, 47.4, 61.1, 123.8, 129.4, 130.0, 130.7, 131.8, 131.9, 138.1, 146.2, 166.2, 206 Hz. ESI/MS (figure 31) 285 $[M-1]^+$ calculated for $C_{17}H_{19}NO_3$ .Anal.Calcd.for: C, 71.56 ; H,6.71: N, 4.91; O, 16.82. Found: C, 71.87; H, 6.50; N, 5.13.



Isopropyl (Z)-4-((3-oxoquinuclidin-2-ylidene)methyl) benzoate (5c): Yellow solid, mp: 96-97ºC; yield 56%. IR (KBr, cm^{-1}) (figure 32): 3057, 2984, 2944, 2964, 2875, 1758, 1724, 1707, 1606, 1458, 810. ^{1}H NMR (400 MHz, $CDCl_3$) (figure 33): δ 1.37-1.39 (6H, d, CH_3); 2.04-2.08 (4H, m, CH_2); 2.66-2.68 (1H, m, CH); 2.99-3.02 (2H, m, CH_2); 3.16-3.21 (2H, m, CH_2); 5.23-5.29 (1H, m, CH_2); 7.04 (1H, s, vinylic proton); 8.02-8.04 (2H, m, ArH). 8.09 (2H, d, *J*=8.4 Hz, ArH). ^{13}C NMR (100 MHz, $CDCl_3$) (figure 35): δ 21.0, 25.7, 40.2, 47.4, 68.5, 123.8, 129.4, 131.8, 138.0 146.2, 166.2, 206.2. ESI/MS (figure 36) 300.1 $[M+1]^+$ calculated for $C_{18}H_{21}NO_3$ .Anal.Calcd. for: C, 72.22 ; H, 7.07: N, 4.68; O, 16.03. Found: C, 72.45; H, 7.00: N, 4.93



Sec-butyl(Z)-4-((3-oxoquinuclidin-2-ylidene)methyl) benzoate (5d): Yellow solid, mp: 100-102 ºC; yield 72%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.96-1.06 (3H, t,-CH_3);1.34-1.35 (3H, d, - CH_2); 1.68-1.74 (2H, m, -CH_2); 2.04-2.90 (4H, m,-CH_2); 2.67-2.68 (1H, m, CH); 3.00-3.05 (2H, m, CH_2); 3.17-3.23 (2H, m, CH_2); 7.05 (1H, s, vinylic proton); 8.03-8.05 (2H, m, -ArH). 8.09 (2H, d, *J*=8.66 Hz, -ArH). ^{13}C NMR (100 MHz, $CDCl_3$): δ 9.7, 19.5, 25.7, 28.9, 40.1, 47.4, 123.9, 129.4, 131.8, 165.8, 206.2. ESI/MS 313.3 $[M+1]^+$ calculated for $C_{19}H_{23}NO_2$. Anal.Calcd. for: C, 72.22; H, 7.07; N, 4.68; O, 16.03. Found: C, 72.45; H, 7.00; N, 4.93.



Cyclopentyl (Z)-4-((3-oxoquinuclidin-2-ylidene)methyl) benzoate (5e): Yellow solid, mp: 140-143ºC; yield 43%. IR (KBr, cm^{-1}) (figure 37): 3392, 3056, 2942, 2869, 1755, 1708, 1675, 1624, 805. ^{1}H NMR (400 MHz, $CDCl_3$) (figure 38): δ 1.47-1.50 (2H, m, $-CH_2$); 1.56-1.64 (2H, m, $-CH_2$); 1.78-1.81 (2H, m, $-CH_2$); 1.92-1.95 (2H, m, $-CH_2$); 2.04-2.08 (4H, m, $2-CH_2$); 2.66-2.68 (1H, m, -CH); 2.97-3.04 (2H, m, $-CH_2$); 3.15-3.22 (2H, m, $-CH_2$); 5.02-5.06 (1H, m, -CH); 7.05 (1H, s, vinylic proton); 8.03-8.13 (4H, m, -ArH). ^{13}C NMR (100 MHz, $CDCl_3$) (figure 39): δ 23.6, 25.4, 25.7, 31.5, 40.1, 47.9, 123.8, 129.4, 130.0, 131.2, 131.7, 131.8, 138.0, 146.2, 165.6, 206.2 ESI/MS (figure 40) 325.2 $[M+1]^+$ calculated for $C_{20}H_{23}NO_3$. Anal.Calcd. for: C, 73.37; H, 7.70; N, 4.28; O, 14.66. C, 73.08; H, 7.91; N, 4.45

3B.3.1.2 Biology

3B.3.1.2.1 Cell line and culture

The A549 and L132 cell lines were obtained from the National Center for Cell Sciences, Pune whereas Dubecoos Modified Essential Medium (DMEM), Fetus Bovine Serum (FBS) and antimycotic–antibiotic solution were obtained from HiMedia. The human cell lines A549 and L132 were seeded in T-25 flask with DMEM, 10% FBS and 1% antimycotic-antibody solution in a humidified atmosphere supplied with 5% CO_2 at 37^0C. Cells were subsequently sub-cultured every third day by trypsinization with 0.25% Trypsin versus glucose solution. Both the cell lines were utilized to examine the antitumor activity of testing compound at varying concentration.

3B.3.1.2.2 Cell viability assay

The IC_{50} values of cell proliferation were determined using MTT assay. Quinuclidinone and its derivatives were dissolved in 0.5% dimethyl sulfoxide and subsequent doses were prepared in the media. A549 cells were seeded in 96 well culture plates and were treated with different concentrations of the compounds for 24 h. The positive control cells were treated with quinuclidinone in culture medium at subsequent doses. At the end of the incubation period 100µl of 3-(4, 5-dimethylthiazol-2-yl) -2, 5-diphenyl tetrazolium bromide (MTT; $1mgml^{-1}$) was added to the wells and plates were incubated at 37^0C for 4 h. Later, culture medium was discarded and 150µl DMSO was added. Absorbance was read at 540nm in ELX800 Universal Microplate Reader.

3B.3.1.2.3 Nuclear morphology assessment (DAPI staining)

A549 cells were seeded in 6 well plate (5×10^5) and were allowed to achieve 80% confluence. Cells were treated with IC_{50} values of the compounds for 24 h at 37^0C. Cells were washed with PBS and fixed with 1% paraformaldehyde, rewashed with PBS and incubated with DAPI for 5 min. Treated cells were examined for condensed and fragmented nuclei and photographed under Leica DMRB fluorescence microscope.

3B.3.1.2.4 Assessment of apoptosis AO/EtBr staining

Cells were grown on glass cover slip (5×10^6) and were incubated in a CO_2 incubator at 37^0C. Cells were dosed with IC_{50} concentration of compounds. After 24 h incubation, cells were washed with PBS and stained with 5µl of AO-EtBr mixture. The coverslip was placed on clean microscopic slides and photograph was taken under confocal microscope (Carl Zeiss LSM-710.)

3B.3.1.2.5 Haemolytic assay

Whole blood was collected for haemolytic assay from a healthy human volunteer after taking a written consent. Protocol was approved and experiments were performed in compliance with the relevant laws and guidelines of Indian medical association for research on human subjects at Blue cross pathology lab (IMA-BMWMC No. 1093), Vadodara, India. The blood samples were placed in vacutainer tubes coated with ethylenediamine tetra-acetic acid (EDTA) and were gently mixed and treated with IC_{50} concentration of the compounds. Control was untreated (with 0% haemolysis) and positive control was sample treated with 3% hydrogen peroxide (with 100% haemolysis). After incubation for 3 h (an adjustment of the standard ASTM F-756[14]) the tubes containing blood samples were centrifuged at 1500 rpm for 10 min to collect the plasma. The supernatant was analysed for the presence of the haemoglobin at 540 nm and percentage haemolysis calculated according to the procedure described by Shiny et al.[15]

3B.3.1.2.6. DNA ladder assay

A549 cells (3×10^6) were exposed to the IC_{50} concentration of the compounds. Cells were centrifuged and then washed with PBS, and the pellet was lysed with 400 µL hypotonic buffer solution (containing 10 mM of tris (pH 7.5), 1 mM of EDTA and 0.2% triton X-100) for 15 min at room temperature, and then centrifuged at 13000 rpm for 15 min. 350 µL of the supernatant was again lysed in 106 µL of second lysis buffer (150 mM NaCl, 10 mM Tris–HCl (pH 8.0), 40 mM EDTA, 1% SDS and 0.2 $mgmL^{-1}$ of proteinase K, at final concentration) for 4 h at 37^0C. The DNA was extracted with phenol/chloroform/isoamyl alcohol (25:25:1 v/v/v), and the pellet thus obtained was washed with ethanol and re-suspended for RNAase digestion in 15 µL

of 10 mMTris, 1 mM of EDTA (pH 8.5), and 50 µgmL^{-1} of RNAase for 1 h at 37^{0}C. The fragmented DNA was quantified on 2% agarose gel electrophoresis.[16]

3B.4 Conclusions

In conclusion a series of novel quinuclidinone based amides and esters **4a-f** and **5a-f** were synthesized. The structures of title compounds were well supported by spectroscopic data and elemental analysis. Test compounds were able to induce apoptosis of A549 lung carcinoma cells with minimal damage to the L132 normal lung cells. The most potent compounds **4c**, **4e 5c** and **5d**, were subjected to further investigations. DNA fragmentation suggests that the cytotoxic effect of the compound is selectively mediated through the induction of apoptosis. Additional experiments are required to determine the mechanism of action and for better elucidation of structure activity relationships of this class of molecules.

References

[1] Rodrı, I., Dorronsoro, A., Castro, A., Badı, A., Ban, J. E. Bioorg. Med. Chem., 2003, 11, 2263.

[2] Starck, J. P., Provins, L., Christophe, B., Gillard, M., Jadot, S., Lo Brutto, P., Quéré, P., Talaga, M., Guyaux, M. Bioorg. Med. Chem. Lett., 2008, 18, 2675.

[3] Farnell, L., Ganellin, C. R., Durant, G. J., Parsons, E., Ganellin, C. R., Port, J., Richards, W. G., Ganellin, R., Ganellin, C. R., Black, W., Pullman, H., Port, J., Kakudo, M., Aschman, D. H., Black, J. W., J. Med. Chem., 1975, 18, 666.

[4] Arias, H. R., López, J. J., Feuerbach, D., Fierro, A., Ortells, M. O., Pérez, E. G. Int. J. Biochem. Cell Biol., 2013, 45, 2420.

[5] Malki, A., Pulipaka, A. B., Evans, S. C., Bergmeier, S. C. Bioorg. Med. Chem. Lett., 2006, 16, 1156.

[6] Malki, A., Ashry, E. S. El ., J. Chemother., 2012, 24, 268.

[7] Soni, J. Y., Premasagar, V., Thakore, S., Lett. Org. Chem., 2015, 12, 277.

[8] Madadi, N. R., Parkin. S., Crooks, P. A., Acta Cryst., 2012, E68, 0730.

[9] Thakore, S., Valodkar, M., Soni, J. Y., Vyas, K., Jadeja, R. N., Devkar, R. V., Rathore, P. S., Bioorg. Chem. 2013, 46, 26.

[10] Valodkar, M., Jadeja, R. N., Thounaojam, M. C., Devkar, R. V., Thakore, S., Mater Chem. phys., 2011, 128, 83.

[11] Vyas, K. M., Jadeja, R. N., Patel, D., Devkar, R. V., Gupta, V. K., Polyhedron 2013, 65, 262.

[12] Singh, R., Jadeja, R. N., Thounaojam, M. C., Patel, T., Devkar, R. V., Chakraborty, D., Inorg. Chem. Commun., 2012, 23, 78.

[13] Hayat, F., Moseley, E., Salahuddin, A., Van Zyl, R. L., Azam, A., Eur. J. Med. Chem., 2011, 46, 1897.

[14] Kutwin, M., Sawosz, E., Jaworski, S., Kurantowicz, N., Strojny, B., Chwalibog, A. Nanoscale Res. Lett., 2014, 9, 257.

[15] Shiny, P. J., Mukherjee, A., Chandrasekaran, N. Bioprocess Biosyst. Eng., 2014, 37, 991.

[16] Nath, M., Vats, M., Roy, P., Inorganica Chim. Acta., 2014, 423, 70.