EXPERIMENTAL – PART – 1

(SECTION – A)

4A.1. Synthesis of *N*3 aryl/heteroaryl substituted 2–(2–chlorostyryl)–6,7–dimethoxy–quinazolin–4(3*H*)–ones (Series-I)

4A.1.1. Chemical and Reagents

All the chemicals and reagents used in the study were of analytical grade and were procured from Sigma–Aldrich (India), Merck (Germany) and SD fine Chemicals (India).

4A.1.2. Method and Preparation

The synthetic route and mechanism of reaction of *N*3–aryl/heteroaryl substituted 2–(2–chlorostyryl)–6,7–dimethoxy–quinazolin–4(3*H*)–ones are illustrated in Figure 4A.1. and Figure 4A.2 respectively. The compounds were synthesized using procedures mentioned in the literatures (Al–Obaid *et al.*, 2009, Ukrainets *et al.*, 1994).

4A.1.2.1. Procedure for synthesis of *N*3–aryl/heteroaryl substituted 2–(2–chlorostyryl)–6,7–dimethoxy–quinazolin–4(3*H*)–ones (5a-5l)

- **2–(3–(2–Chlorophenyl) acrylamido)–4,5–dimethoxybenzoic acid (3):**
  2–chlorocinnamoyl chloride (2) (0.05 mol) was added to a solution of anthranilic acid (1) (0.05 mol) in pyridine (50 mL) and the reaction mixture was stirred at room temperature for 3 h. The mixture was then poured into 10% cold dilute HCl solution (50 mL). The solid obtained was then filtered followed by washing several times with cold water. The product 2–(3–(2–chlorophenyl) acrylamido)–4,5–dimethoxybenzoic acid (3) was dried and crystallized from absolute ethanol.
- **2–(2–Chlorostyryl)–6,7–dimethoxy–4*H*–benzo[*d*][1,3]oxazin–4–one (4)**
  A mixture of 2–(3–(2–chlorophenyl)acrylamido)–4,5–dimethoxybenzoic acid (**3**) (0.03 mol) and acetic anhydride (0.3 mol) was heated under reflux in an oil bath at 180 °C for 4 h. Upon completion, the reaction mixture was subsequently washed with water and then extracted with chloroform, dried over Na_2SO_4, and evaporated to yield a crude product, 2–(2–chlorostyryl)–6,7–dimethoxy–4*H*–benzo[*d*][1,3]oxazin–4–one (**4**). The residue so obtained was triturated with petroleum ether (40–60), dried and crystallized from toluene.

- **General procedure for the synthesis of compounds 5a–5l**

  A mixture of 2–(2–chlorostyryl)–6,7–dimethoxy–4*H*–benzo[*d*][1,3]oxazin–4–one (0.01 mol) (**4**) and an appropriate aryl/heteroaryl amine (0.01 mol) in glacial acetic acid (10 mL) was refluxed at 210 °C in an oil bath for 3 h. The reaction was quenched by pouring the mixture obtained into crushed ice and left overnight. The solid which separated out was filtered, washed thoroughly with cold water, dried and then crystallized from absolute ethanol to obtain the product (**5a–5l**).

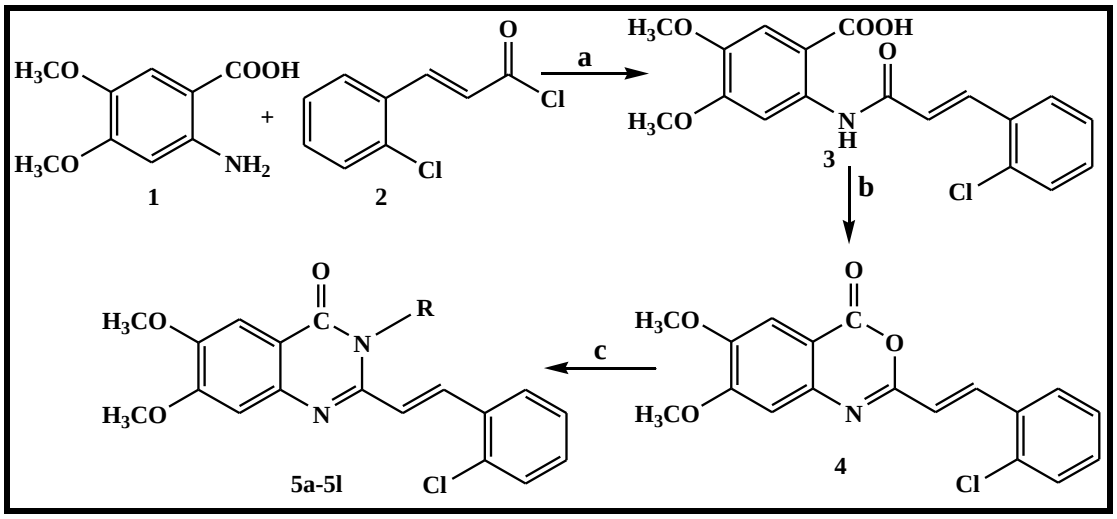

Figure 4A.1. General scheme for the synthesis of compounds **5a-5l**. Reagents and conditions: (a) Pyridine, stir, r.t., 3h; (b) Ac_2O, reflux, 180 °C, 4h; (c) Substituted aryl/heteroaryl amine, glacial acetic acid, reflux, 210 °C, 3h.

- **Reaction mechanism (Series I and Series II)**

  All the compounds were synthesized by three–step method. The initial two steps involve amidation followed by intramolecular cyclization using acetic anhydride which converts the hydroxyl group of the carboxylic acid group into a good leaving group to form the benzo[*d*][1,3]oxazin–4–one ring (Shariat *et al.*, 2013). Thereafter, fusion of various substituted aryl/heteroarylamines with 2–(2–chlorostyryl)–6,7–dimethoxy–4*H*–benzo[*d*][1,3]oxazin–4–one (4) *via* formation of amidine salt (Ukrainets *et al.*, 1994) followed by cyclodehydration (Figure 4A.2.) yielded the title compounds.

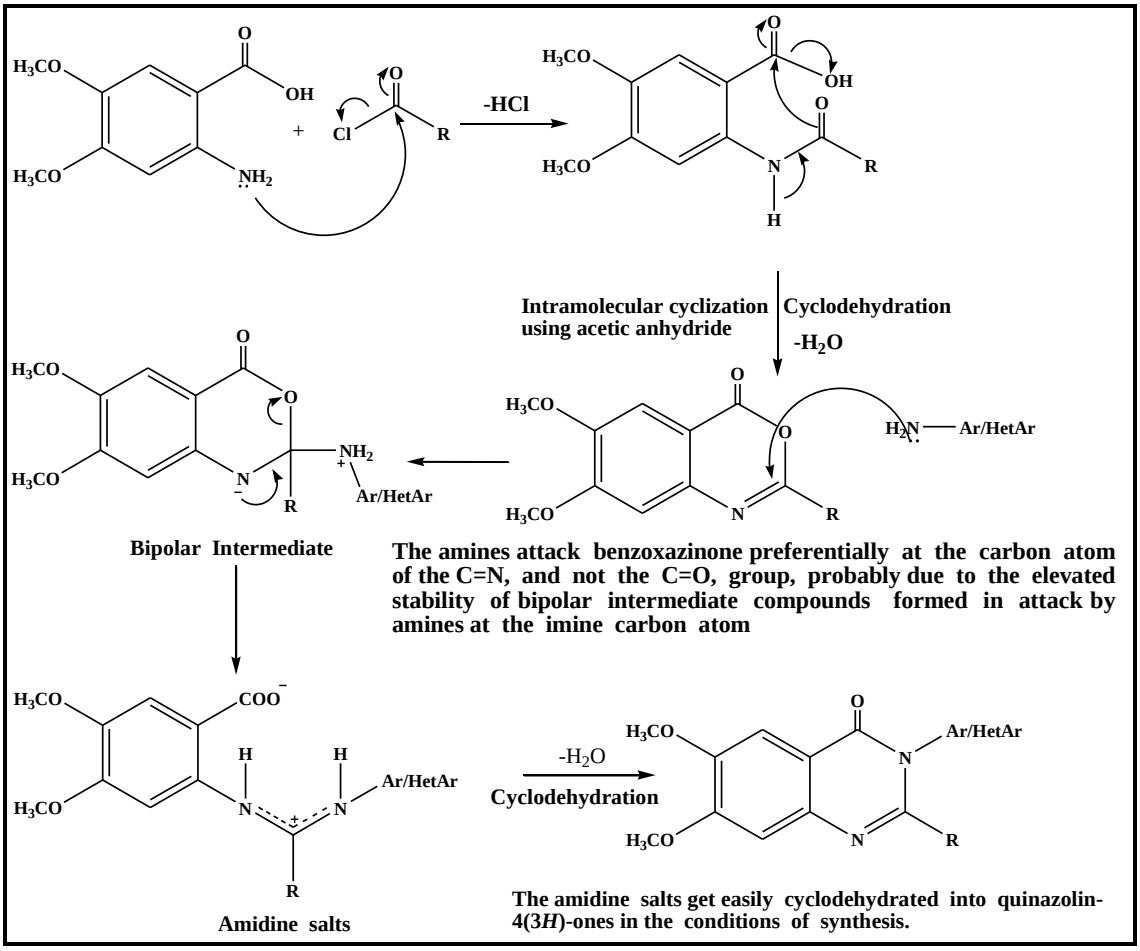

Figure 4A.2. Possible mechanism of reaction for the synthesis of quinazolin–4(3*H*) ones

4A.2. Characterization of synthesized *N*3–aryl/heteroaryl substituted 2–(2–chlorostyryl)–6,7–dimethoxy–quinazolin–4(3*H*)–ones

The following procedures were employed to ascertain the structures of the synthesized compounds.

4A.2.1. Physicochemical characterization

Physicochemical characterization of synthesized compounds includes (Table 4A.1–4A.2.):

- **Melting point determination**

  Melting point signifies the relationship between structure and properties and hence different compounds tends to have different melting points. It is one of the important criteria and gives an indication of purity. The Melting points were

determined in open capillaries using Stuart SMP10 (Barloworld Scientific Ltd., UK), electrothermal melting point apparatus and were uncorrected.

- **Solubility determination**

  The solubility of synthesized compounds was assessed in water, ethanol, ethylacetate, chloroform and hexane.

- **Thin layer chromatograghy (TLC) analysis (R_f value)**

  The TLC is an important technique and it can be used to qualitatively monitor the progress of a reaction and it also indicates the purity of a substance. The R_f value is a characteristic property of a given compound in a given solvent and it was calculated by using the equation given below:

$$\mathbf{R_f} = \frac{\textbf{Distance travelled by solute}}{\textbf{Distance travelled by solvent}}$$

  The reactions were monitored by TLC with ethyl acetate/hexane (2:3) as the mobile phase on precoated Merck silica gel 60 F254 aluminium sheets (Merck, Germany) of dimension 7.5 cm long and 2.5 cm width. The chromatograms were developed by ascending technique, where solvent front was allowed to travel to an appropriate distance and the plates were taken out and dried. The locations of spots were inspected by using the ultraviolet cabinet and iodine chamber.

- **Log P value determination**

  The partition coefficient quantifies the ratio of the amount of a substance distributed between buffer (pH 7.4) and n–octanol. The logarithm of the concentration of the unionized solute in the solvents is called LogP. The partition coefficient between n–octanol and aqueous phase (buffer) (37±1°C) were determined by shake flask method. The n–octanol and the buffer solution were co-saturated with each other for 24 h at 37 °C before use. n–octanol (5mL) was shaken with 5mL aqueous buffer solution containing sample (1mg/ml) for 8 h at 37±1°C. The mixture was then centrifuged at 2000 rpm for 10 min and the sample concentration was determined by comparison to a calibration curve constructed with 6 points chosen in the same range of concentration as the measured one. Owing to the low aqueous solubility of the solutes, the solution used to build up the calibration curve was prepared by dissolving a known

amount of solute in methanol. This solution was first diluted with methanol/water (50:50). Then the latter solution was diluted with pure water. The partition coefficient (P) was calculated from the equation:

$$P = \frac{Va}{V_0}\left[\frac{C_1}{C2} - 1\right]$$

Where

Va is the volume of aqueous phase
V_0 is the volume of n–octanol
C_1 is the concentration of drug in the aqueous phase before extraction
C_2 is the concentration of drug in aqueous phase after extraction

Since **Va = V_0 =** 5mL

Hence,

$$P = \left[\frac{C_1}{C2} - 1\right]$$

Table 4A.1. List of synthesized compounds and physicochemical data (**5a–5l**)

Sl. No.	Code	R	Melting Point (°C)	Yield (%)	R_f^*
1	5a	phenyl	172–174	75.45	0.57
2	5b	2,4–dimethylphenyl	158–160	74.81	0.64
3	5c	*m*–tolyl	162–164	71.52	0.61
4	5d	2–methoxyphenyl	166–168	67.34	0.63
5	5e	3–methoxyphenyl	210–212	74.63	0.68
6	5f	benzyl	178–180	65.76	0.66
7	5g	4–nitrophenyl	223–225	69.20	0.63
8	5h	2–nitrophenyl	219–221	71.23	0.69
9	5i	4–bromophenyl	175–177	66.85	0.61
10	5j	pyrimidin–2–yl	226–228	73.57	0.63
11	5k	pyridin–4–yl	232–234	72.41	0.59
12	5l	thiazol–2–yl	229–231	68.26	0.57

*__Solvent system:__ ethyl acetate/hexane (2:3)

Table 4A.2. Partition coefficient and solubility of synthesized compounds (**5a–5l**)

Code	Calculated Log P	Log P	Water	Ethanol	Ethylacetate	Chloroform	Hexane
5a	4.99	2.14	++	++	++	+++	–
5b	4.57	2.68	–	++	++	+++	++
5c	4.59	2.43	–	++	++	+++	–
5d	4.40	2.61	–	++	++	+++	–
5e	4.99	2.50	–	++	++	+++	++
5f	4.69	2.23	–	–	–	+++	–
5g	4.21	2.75	–	++	++	+++	++
5h	4.71	2.56	++	++	+++	+++	–
5i	4.54	2.78	++	++	++	+++	–
5j	3.68	2.54	–	++	++	+++	–
5k	4.27	2.81	–	–	++	+++	++
5l	3.99	2.47	–	–	++	+++	++

++Sparingly soluble, +++ soluble, –insoluble

4A.2.2. Spectral characterization and elemental analysis

Spectral characterization and elemental analysis were performed by using following instruments:

- **FT–IR Spectroscopy**

  FTIR spectra were recorded on a Shimadzu FT–IR 8400S spectrophotometer at the scanning range of 400–4000 cm^{-1} at the Department of Pharmaceutics, Indian Institute of Technology (Banaras Hindu University), Varanasi.

- **NMR and Mass Spectroscopy**

  ^{1}H NMR spectra were recorded on a JEOL AL 300 FT–NMR spectrometer in $CDCl_3$ at the Department of Chemistry, Faculty of Science, Banaras Hindu University, Varanasi and ^{13}C NMR using Bruker AvIII HD–300 NMR spectrometer at SAIF, CDRI, Lucknow. Tetramethylsilane (TMS) was used as an internal standard. Chemical shift values were expressed in parts per million (δ). The coupling constant, *J* is expressed in Hertz (Hz). The mass spectra of few representative compounds were recorded on a Jeol SX–102 electron impact mass spectrometer at 70 eV ionising beam and using a direct insertion probe spectrometer at SAIF, CDRI, Lucknow.

- **Elemental analysis**

  Elemental analysis was performed using Exeter CE-440 elemental analyzer at the Department of Pharmaceutics, Indian Institute of Technology (Banaras

Hindu University), Varanasi. The elemental analysis results were within ± 0.4 % of the theoretical values.

4A.2.2.1. Spectral characterization and elemental analysis

2–(3–(2–Chlorophenyl) acrylamido)–4,5–dimethoxybenzoic acid (3)

IR (KBr, cm^{-1}): 3437 (–NH str); 3074 (–OH str –COOH); 2931 (methoxy C–H str); 1732 (–C=O str –COOH); 1685 (amide –C=O str); 1608 (aryl substituted –C=C str); 1037 (–C–O–C str). ^{1}H NMR (300 MHz, $CDCl_3$): δ 11.34 (s, 1H, –COOH); δ 8.14 (s, 1H, NHC=O, D_2O exchangeable); δ 7.30–7.71 (m, 4H, Ar–H); δ 7.26 (s, 1H, 1–Ar–H); δ 7.06 (s, 1H, Ar–H); δ 6.59 (d, *J*=15.6, 1H, –ethylene); δ 6.46 (d, *J*=14.7, 1H, –ethylene); δ 4.02 (s, 3H, $–OCH_3$); δ 3.92 (s, 3H, $–OCH_3$). Elemental analysis, for $C_{18}H_{16}ClNO_5$, cacld. (%): 59.76 C, 4.46 H, 3.87 N; Found (%): 59.83 C, 4.47 H, 3.85 N.

2–(2–Chlorostyryl)–6,7–dimethoxy–4*H*–benzo[*d*][1,3]oxazin–4–one (4)

IR (KBr, cm^{-1}): 2928 (methoxy C–H str); 1735 (–C=O str cyclic ester); 1597 (–C=N str); 1037 (–C–O–C str). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.30–7.69 (m, 4H, Ar–H); δ 7.26 (s, 1H, Ar–H); δ 7.05 (s, 1H, Ar–H); δ 6.76 (d, *J*=15.9, 1H, –ethylene); δ 6.53 (d, *J*=15.9, 1H, –ethylene); δ 4.20 (s, 3H, $–OCH_3$); δ 3.99 (s, 3H, $–OCH_3$). Elemental analysis, for $C_{18}H_{14}ClNO_4$, calcd. (%): 62.89 C, 4.10 H, 4.07 N; Found (%): 63.03 C, 4.09 H, 4.08 N.

2–(2–Chlorostyryl)–6,7–dimethoxy–3–phenylquinazolin–4(3*H*)–one (5a)

IR (KBr, cm^{-1}): 2941 (methoxy C–H str); 1674 (–C=O); 1606 (–C=N str); 1218 (C–N str). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.38–7.67 (m, 4H, Ar–H); δ 7.15–7.30 (m, 5H, Ar–H); δ 7.13 (s, 1H, Ar–H); δ 7.05 (s, 1H, Ar–H); δ 6.58 (d, *J*=15.3, 1H, –ethylene); δ 6.38 (d, *J*=15.6, 1H, –ethylene); δ 4.06 (s, 3H, $–OCH_3$). δ 3.91 (s, 3H, $–OCH_3$). ^{13}C NMR (75 MHz, $CDCl_3$): 56.41, 56.52, 107.92, 108.06, 109.75, 121.62, 126.95, 127.40, 128.74, 130.12, 133.11, 134.90, 136.85, 144.02, 156.58, 160.28. MS (m/z): 419 (M+1). Elemental analysis, for $C_{24}H_{19}ClN_2O_3$, calcd. (%): 68.82 C, 4.57 H, 6.69 N; Found (%): 68.64 C, 4.55 H, 6.71 N.

2–(2–Chlorostyryl)–6,7–dimethoxy–3–(2,4–dimethylphenyl)quinazolin–4(3*H*)–one (5b)

IR (KBr, cm^{-1}): 2928 (methoxy C–H str); 1670 (–C=O str); 1608 (–C=N str); 1213 (C–N str). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.79–7.86 (m, 4H, Ar–H), δ 7.25–7.63 (m, 3H, Ar–H); δ 7.21 (s, 1H, Ar–H); δ 7.09 (s, 1H, Ar–H); δ 6.57 (d, *J*=11.7, 1H, –ethylene); δ 6.36 (d, *J*=15.3, 1H, –ethylene); 4.06 (s, 3H, $–OCH_3$); δ 3.90 (s, 3H, $–OCH_3$); δ 2.11 (s, 3H, $–CH_3$); δ 2.42 (s, 3H, $–CH_3$).^{13}C NMR (75 MHz, $CDCl_3$): 17.54, 21.48, 56.31, 56.54, 108.28, 114.91, 122.52, 124.91, 127.68, 130.31, 133.72 135.39, 144.41, 149.46, 155.46, 161.44. MS (m/z): 447 (M+1). Elemental analysis, for $C_{26}H_{23}ClN_2O_3$, calcd. (%): 69.87 C, 5.19 H, 6.27 N; Found (%): 69.65 C, 5.17 H, 6.29 N.

2–(2–Chlorostyryl)–6,7–dimethoxy–3–*m*–tolylquinazolin–4(3*H*)–one (5c)

IR (KBr, cm^{-1}): 2924 (methoxy C–H str); 1658 (–C=O str), 1606 (–C=N str); 1211 (C–N str). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.38–7.67 (m, 4H, Ar–H); δ 7.21–7.38 (m, 4H, Ar–H); δ 7.16–7.17 (m, 1H, Ar–H); δ 7.09 (s, 1H, Ar–H); δ 6.58 (d, *J*=15.6, 1H, –ethylene); δ 6.48 (d, *J*=15.6, 1H, –ethylene); δ 4.03 (s, 3H, $–OCH_3$); δ 3.94 (s, 3H, $–OCH_3$), δ 2.32 (s, 3H, $–CH_3$). ^{13}C NMR (75 MHz, $CDCl_3$): 23.49, 56.26, 56.52, 109.30, 114.26, 122.31, 124.80, 127.29, 130.45, 133.50, 135.63, 144.35, 149.53, 155.55, 161.36. Elemental analysis, for $C_{25}H_{21}ClN_2O_3$, calcd. (%): 69.36 C, 4.89 H, 6.47 N; Found (%): 69.45 C, 4.88 H, 6.48 N.

2–(2–Chlorostyryl)–6,7–dimethoxy–3–(2–methoxyphenyl)quinazolin–4(3*H*)–one (5d)

IR (KBr, cm^{-1}): 2939 (methoxy C–H str); 1672 (–C=O); 1604 (–C=N str); 1211 (C–N str). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.43–7.68 (m, 4H Ar–H); δ 7.04–7.25 (m, 4H, Ar–H); δ 6.96 (s, 1H, Ar–H); δ 6.93 (s, 1H, Ar–H); δ 6.60 (d, *J*=15.6, 1H, –ethylene); δ 6.41 (d, *J*=15.3, 1H, –ethylene); δ 4.06 (s, 3H, $–OCH_3$); δ 3.93 (s, 3H, $–OCH_3$), δ 3.77 (s, 3H, $–OCH_3$). ^{13}C NMR (75 MHz, $CDCl_3$): 54.24, 55.10, 55.85, 107.11, 109.20, 113.53, 124.14, 126.05, 129.34, 130.27, 134.82, 137.26, 143.36, 152.73, 160.57, 162.43. Elemental analysis, for $C_{25}H_{21}ClN_2O_4$, calcd. (%): 66.89 C, 4.72 H, 6.24 N; Found (%): 67.03 C, 4.73 H, 6.23 N.

2–(2–Chlorostyryl)–6,7–dimethoxy–3–(3–methoxyphenyl)quinazolin–4(3*H*)–one (5e)

IR (KBr, cm^{-1}): 2933 (methoxy C–H str); 1656 (–C=O str); 1606 (–C=N str); 1213 (C–N str). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.39–7.66 (m, 4H, Ar–H); δ 6.90–7.29 (m, 4H, Ar–H); δ 6.73 (s, 1H, Ar–H); δ 6.76 (s, 1H, Ar–H); δ 6.57 (d, *J*=15.6, 1H, –ethylene); δ 6.42 (d, *J*=15.6, 1H, –ethylene); δ 4.07 (s, 3H, $–OCH_3$); δ 4.00 (s, 3H, $–OCH_3$); 3.84 (s, 3H, $–OCH_3$). ^{13}C NMR (75 MHz, $CDCl_3$): 54.64, 55.30, 55.80, 104.35, 106.21, 109.10, 112.45, 123.84, 126.25, 129.14, 129.36, 134.91, 137.89, 143.75, 152.02, 159.57, 162.65. MS (m/z): 449 (M+1). Elemental analysis, for $C_{25}H_{21}ClN_2O_4$, calcd. (%): 66.89 C, 4.72 H, 6.24 N; Found (%): 67.13 C, 4.71 H, 6.21 N.

2–(2–Chlorostyryl)–3–benzyl–6,7–dimethoxyquinazolin–4(3*H*)–one (5f)

IR (KBr, cm^{-1}): 2935 (methoxy C–H str); 2835 (–CH str methylene); 1658 (–C=O str); 1604 (–C=N str); 1211 (C–N str). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.35–7.66 (m, 5H, Ar–H); δ 7.02–7.32 (m, 4H, Ar–H); δ 6.97 (s, 1H, Ar–H); δ 6.94 (s, 1H, Ar–H); δ 6.76 (d, *J*=15.9, 1H, –ethylene); δ 6.61 (d, *J*=15.6, 1H, –ethylene); δ 4.34 (s, 2H, $–CH_2$); δ 4.03 (s, 3H, $–OCH_3$); δ 3.88 (s, 3H, $–OCH_3$). ^{13}C NMR (75 MHz, $CDCl_3$): 41.35, 56.24, 56.49, 112.14, 118.33, 125.52, 127.22, 130.13, 132.50, 133.49, 135.62 137.15, 138.52, 142.36, 148.73, 152.95 157.83, 163.26. Elemental analysis, for $C_{25}H_{21}ClN_2O_3$, calcd. (%): 69.36 C, 4.89 H, 6.47 N; Found (%): 69.17 C, 4.87 H, 6.48 N.

2–(2–Chlorostyryl)–6,7–dimethoxy–3–(4–nitrophenyl)quinazolin–4(3*H*)–one (5g)

IR (KBr, cm^{-1}): 2924 (methoxy C–H str); 1631 (–C=O str); 1595 (–C=N str); 1506, 1379 (–N=O str); 1212 (C–N str). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.55–8.22 (m, 4H, Ar–H); δ 7.29–7.45 (m, 4H, Ar–H); δ 7.26 (s, 1H, Ar–H); δ 7.04 (s, 1H, Ar–H); δ 6.76 (d, *J*=15.9, 1H, –ethylene); δ 6.57 (d, *J*=13.2, 1H, –ethylene); δ 4.02 (s, 3H, $–OCH_3$); δ 3.89 (s, 3H, $–OCH_3$). ^{13}C NMR (75 MHz, $CDCl_3$): 56.63, 56.67, 108.07, 109.87, 120.24, 121.72, 125.23, 127.34, 130.45, 131.03, 133.20, 135.04, 136.91, 143.41, 150.01, 156.58, 159.26. MS (m/z): 464 (M+1). Elemental analysis, for $C_{24}H_{18}ClN_3O_5$, calcd. (%): 62.14 C, 3.91 H, 9.06 N; Found (%): 61.98 C, 3.92 H, 9.08 N.

2–(2–Chlorostyryl)–6,7–dimethoxy–3–(2–nitrophenyl)quinazolin–4(3*H*)–one (5h)

IR (KBr, cm^{-1}): 2933 (methoxy C–H str); 1603 (–C=N str); 1632 (–C=O str); 1504, 1373 (–N=O str); 1210 (C–N str). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.58–8.23 (m, 4H,

Ar–H); δ 7.25–7.47 (m, 4H, Ar–H); δ 7.23 (s, 1H, Ar–H); δ 7.06 (s, 1H, Ar–H); δ 6.76 (d, *J*=15.9, 1H, –ethylene); δ 6.57 (d, *J*=11.4, 1H, –ethylene); δ 4.02 (s, 3H, $–OCH_3$); δ 3.88 (s, 3H, $–OCH_3$). ^{13}C NMR (75 MHz, $CDCl_3$): 56.62, 56.66, 108.08, 109.76, 120.27, 121.77, 126.45, 127.56, 130.69, 131.75, 133.56, 135.25, 137.85, 143.54, 150.57, 156.61, 159.72. Elemental analysis, for $C_{24}H_{18}ClN_3O_5$, calcd. (%): 62.14 C, 3.91 H, 9.06 N; Found (%): 62.21 C, 3.90 H, 9.05 N.

2–(2–Chlorostyryl)–3–(4–bromophenyl)–6,7–dimethoxyquinazolin–4(3*H*)–one (5i)

IR (KBr, cm^{-1}): 2935 (methoxy C–H str), 1664 (–C=O str); 1608 (–C=N str); 1209 (C–N str). ^1H NMR (300 MHz, $CDCl_3$): δ 7.55–8.22 (m, 4H, Ar–H); δ 7.25–7.52 (m, 4H, Ar–H); δ 7.04 (s, 1H, Ar–H); δ 7.02 (s, 1H, Ar–H); δ 6.76 (d, *J*=16.5, 1H, –ethylene); δ 6.55 (d, *J*=15.6, 1H, –ethylene); δ 4.06 (s, 3H, $–OCH_3$); δ 3.89 (s, 3H, $–OCH_3$). ^{13}C NMR (75 MHz, $CDCl_3$): 56.31, 56.69, 106.29, 108.19, 109.99, 121.89, 122.91, 127.37, 130.51, 131.05,133.35, 135.60, 134.75, 136.97, 137.05, 150.14, 155.67, 156.78, 159.29. MS (m/z): 499 (M+2). Elemental analysis, for $C_{24}H_{18}BrClN_2O_3$, calcd. (%): 57.91 C, 3.64 H, 5.63 N; Found (%): 58.13 C, 3.63 H, 5.65 N.

2–(2–Chlorostyryl)–6,7–dimethoxy–3–(pyrimidin–2–yl)quinazolin–4(3*H*)–one (5j)

IR (KBr, cm^{-1}): 2937 (methoxy C–H str); 1629 (–C=O str); 1597 (–C=N str); 1209 (C–N str). ^1H NMR (300 MHz, $CDCl_3$): δ 7.67–7.70 (m, 3H, pyrimidine); δ 7.29–7.55 (m, 4H, Ar–H); δ 7.25 (s, 1H, Ar–H); δ 7.04 (s, 1H, Ar–H); δ 6.76 (d, *J*=16.2, 1H, –ethylene); δ 6.56 (d, *J*=16.5, 1H, –ethylene); δ 4.02 (s, 3H, $–OCH_3$); δ 4.00 (s, 3H, $–OCH_3$). ^{13}C NMR (75 MHz, $CDCl_3$): 56.51, 56.64, 106.26, 108.14, 109.81, 121.78, 122.51, 125.08, 127.60, 129.29, 130.46, 133.30, 135.09, 136.97, 137.84, 156.64, 161.84. Elemental analysis, for $C_{22}H_{17}ClN_4O_3$, calcd. (%): 62.79 C, 4.07 H, 13.31 N; Found (%): 62.64 C, 4.06 H, 13.33 N.

2–(2–Chlorostyryl)–6,7–dimethoxy–3–(pyridin–4–yl)quinazolin–4(3*H*)–one (5k)

IR (KBr, cm^{-1}): 2939 (methoxy C–H str); 1631 (–C=O str); 1600 (–C=N str); 1200 (C–N str). ^1H NMR (300 MHz, $CDCl_3$): δ 7.55–7.69 (m, 4H, pyridine); δ 7.29–7.45 (m, 4H, Ar–H); δ 7.26 (s, 1H, Ar–H); δ 7.04 (s, 1H, Ar–H); δ 6.76 (d, *J*=16.2, 1H, –ethylene); δ 6.55 (d, *J*=13.2, 1H, –ethylene); δ 4.02 (s, 3H, $–OCH_3$), δ 4.00 (s, 3H, $–OCH_3$). ^{13}C NMR (75 MHz, $CDCl_3$): 56.21, 56.69, 107.99, 108.96, 109.98, 121.86, 127.37, 130.50, 131.04, 133.33, 135.09, 137.00, 143.49, 150.10, 156.66, 159.26.

Elemental analysis, for $C_{23}H_{18}ClN_3O_3$, calcd. (%): 65.79 C, 4.32 H, 10.01 N; Found (%): 66.01 C, 4.33 H, 10.04 N.

2–(2–Chlorostyryl)–6,7–dimethoxy–3–(thiazol–2–yl)quinazolin–4(3*H*)–one (5l)

IR (KBr, cm^{-1}): 2939 (methoxy C–H str); 1631 (–C=O str); 1599 (–C=N str); 1212 (C–N str); 1138 (C–S). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.68 (s, 1H, thiazole methine); δ 7.66 (s, 1H, thiazole methine); δ 7.29–7.55 (m, 4H, Ar–H); δ 7.25 (s, 1H, Ar–H); 7.04 δ (s, 1H, Ar–H); δ 6.76 (d, *J*=15.9, 1H, –ethylene); δ 6.58 (d, *J*=15.3, 1H, –ethylene); δ 4.02 (s, 3H, $–OCH_3$); δ 4.00 (s, 3H, $–OCH_3$). ^{13}C NMR (75 MHz, $CDCl_3$): 56.32, 56.71, 108.02, 110.00, 121.89, 127.38, 130.52, 131.06, 133.36, 135.11, 137.04, 143.52, 150.12, 156.68, 159.29. Elemental analysis, for $C_{21}H_{16}ClN_3O_3S$, calcd. (%): 59.22 C, 3.79 H, 9.87 N; Found (%): 58.99 C, 3.80 H, 9.89 N.

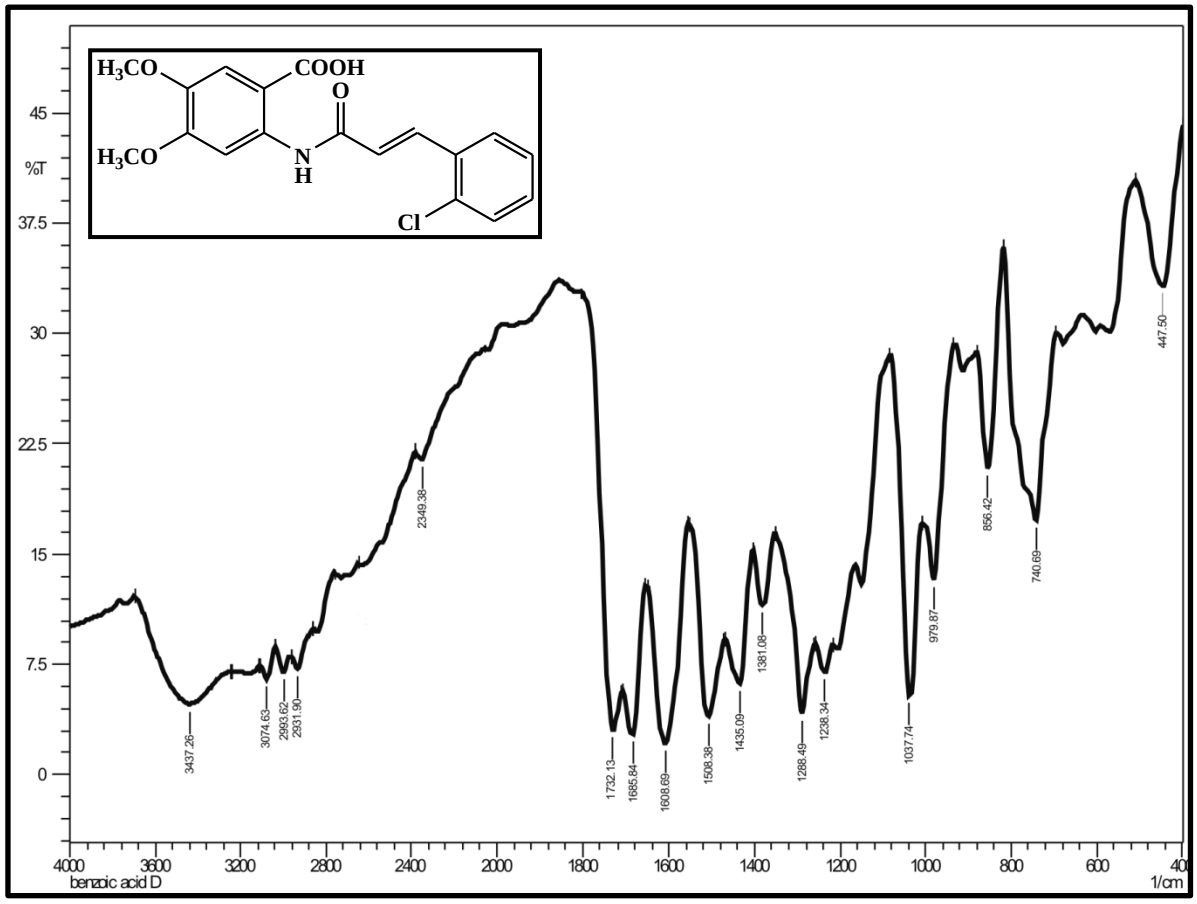

Figure 4A.3. FT–IR spectrum of 2–(3–(2–chlorophenyl) acrylamido)–4,5 dimethoxybenzoic acid (**3**)

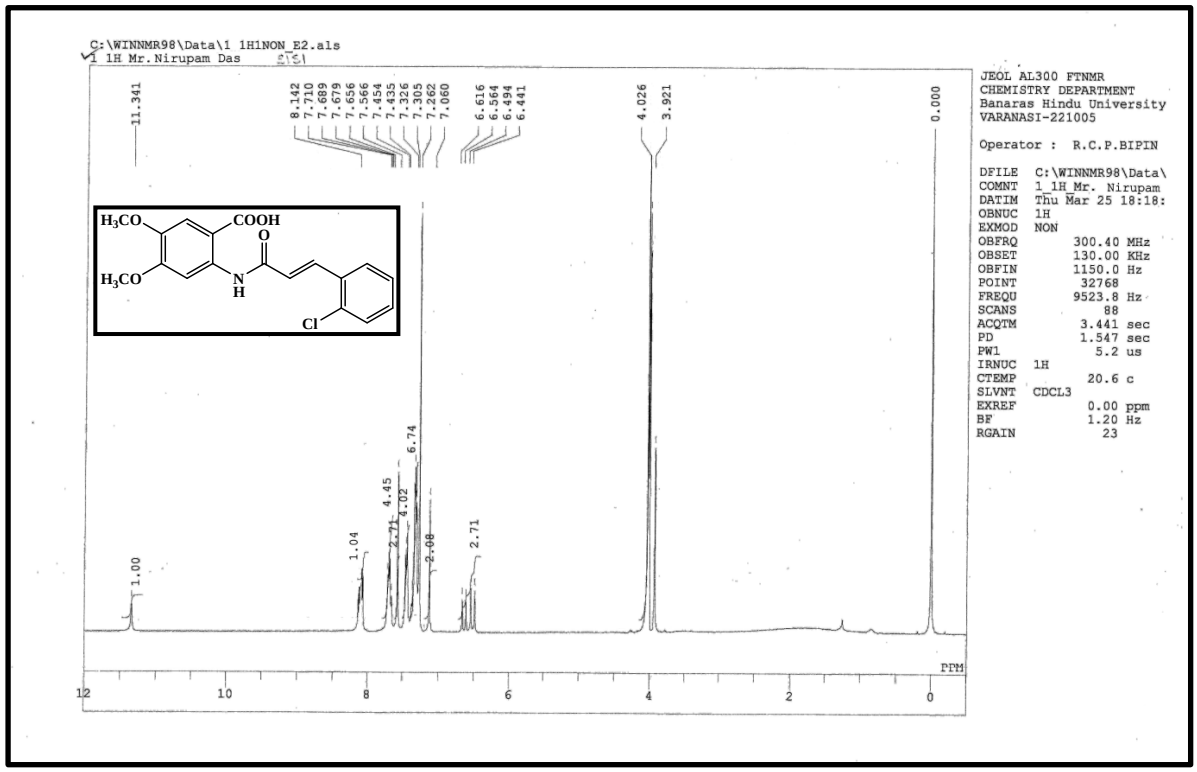

Figure 4A.4. ^{1}H NMR spectrum of 2–(3–(2–chlorophenyl) acrylamido)–4,5 dimethoxybenzoic acid (**3**)

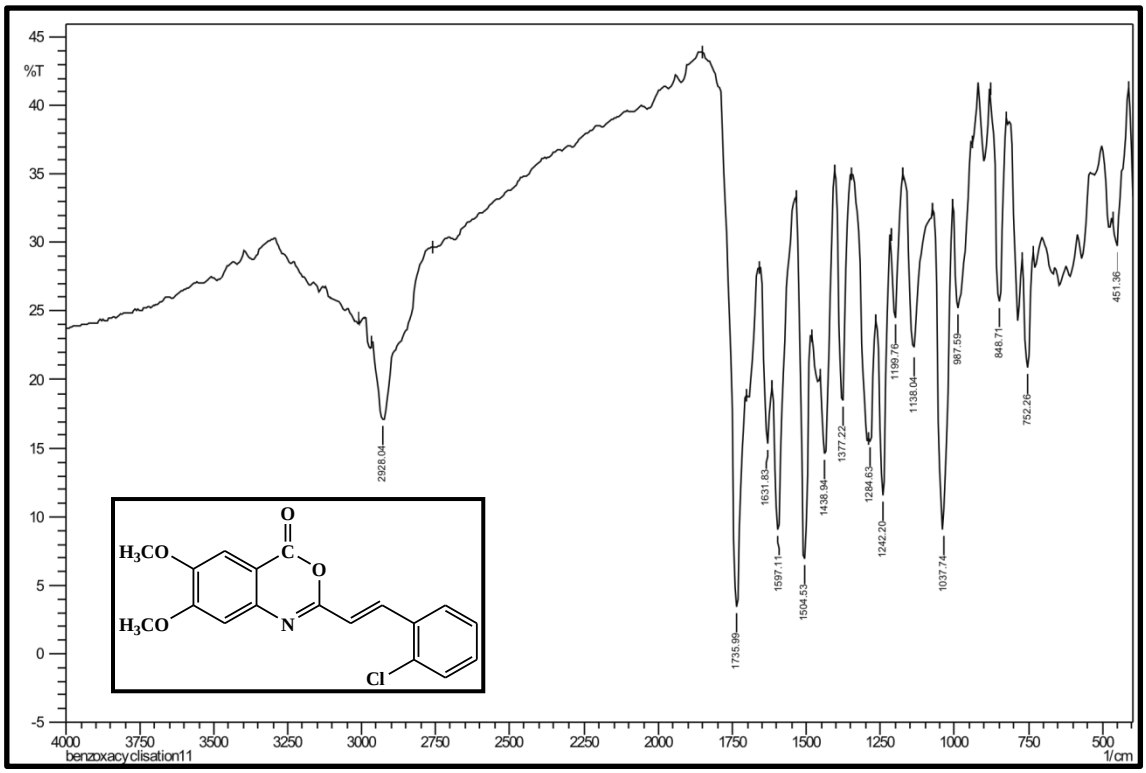

Figure 4A.5. FT–IR spectrum of 2–(2–chlorostyryl)–6,7–dimethoxy–4*H*–benzo[*d*][1,3]oxazin–4–one (**4**)

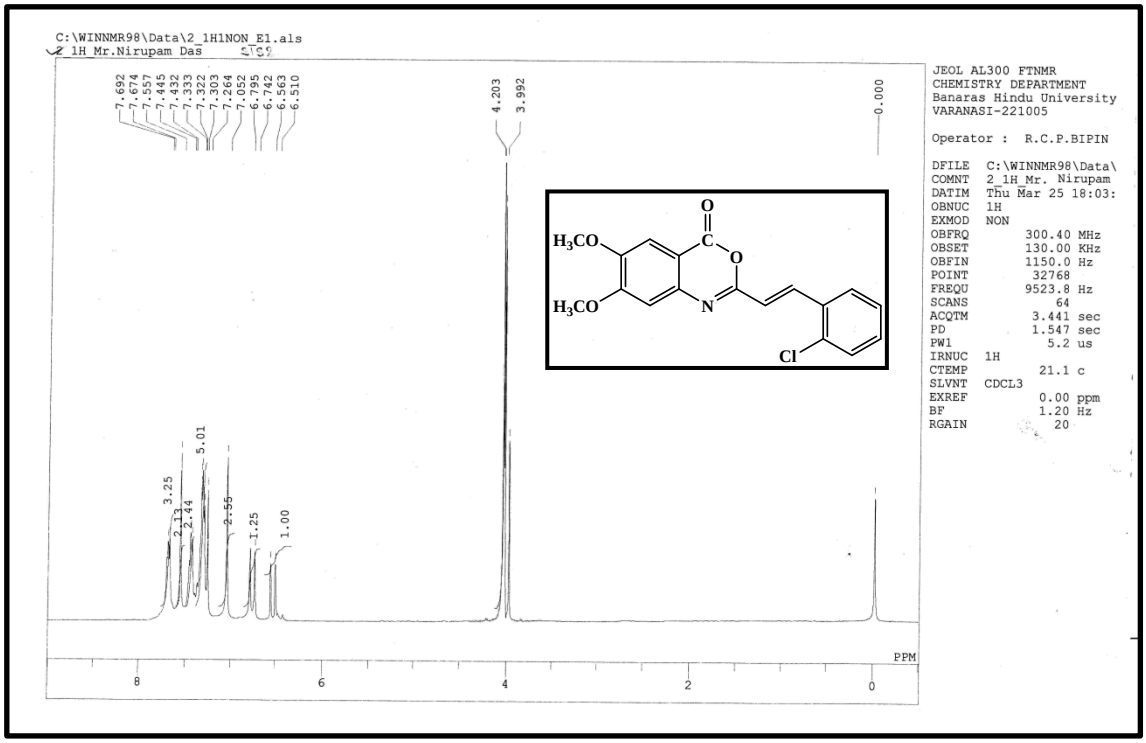

Figure 4A.6. ^{1}H NMR spectrum of 2–(2–chlorostyryl)–6,7–dimethoxy–4*H*–benzo[*d*][1,3]oxazin–4–one (**4**)

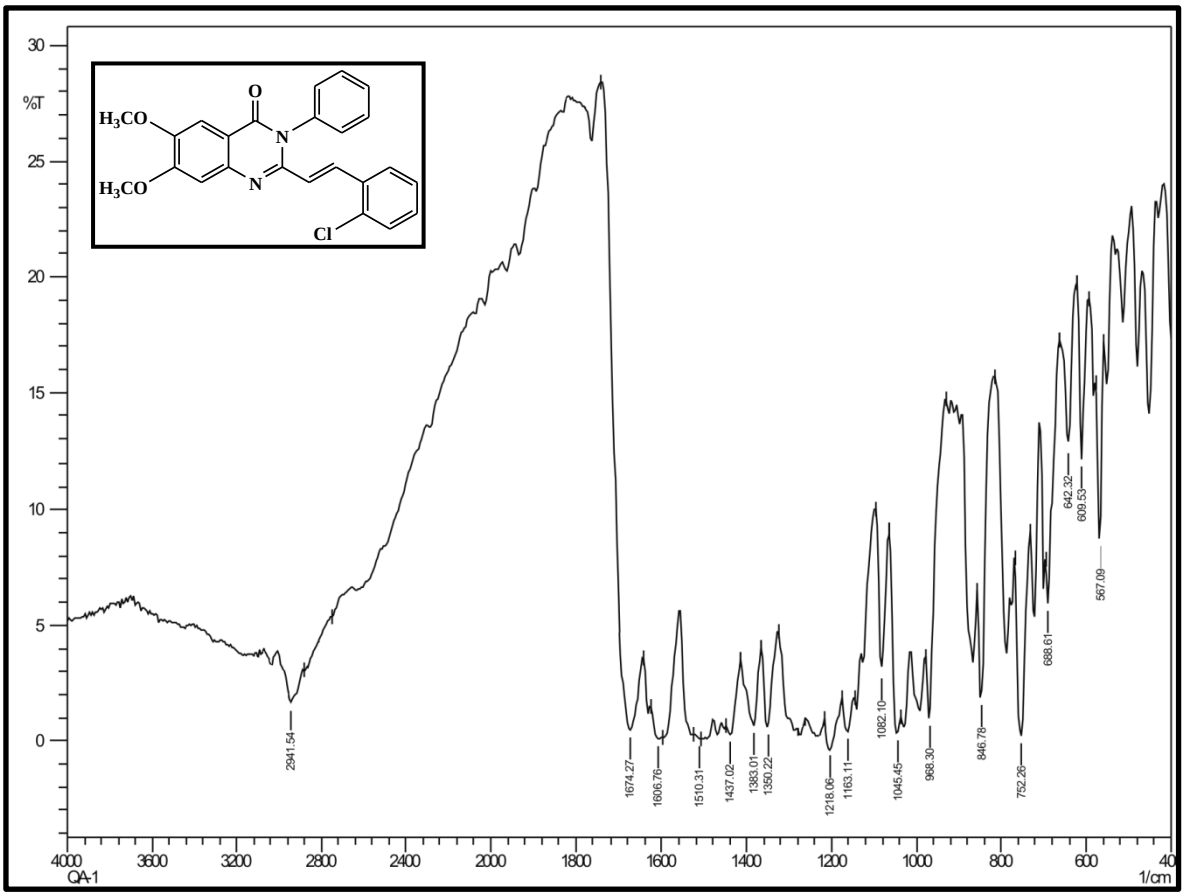

Figure 4A.7. FT–IR spectrum of 2–(2–chlorostyryl)–6,7–dimethoxy–3–phenylquinazolin–4(3*H*)–one (**5a**)

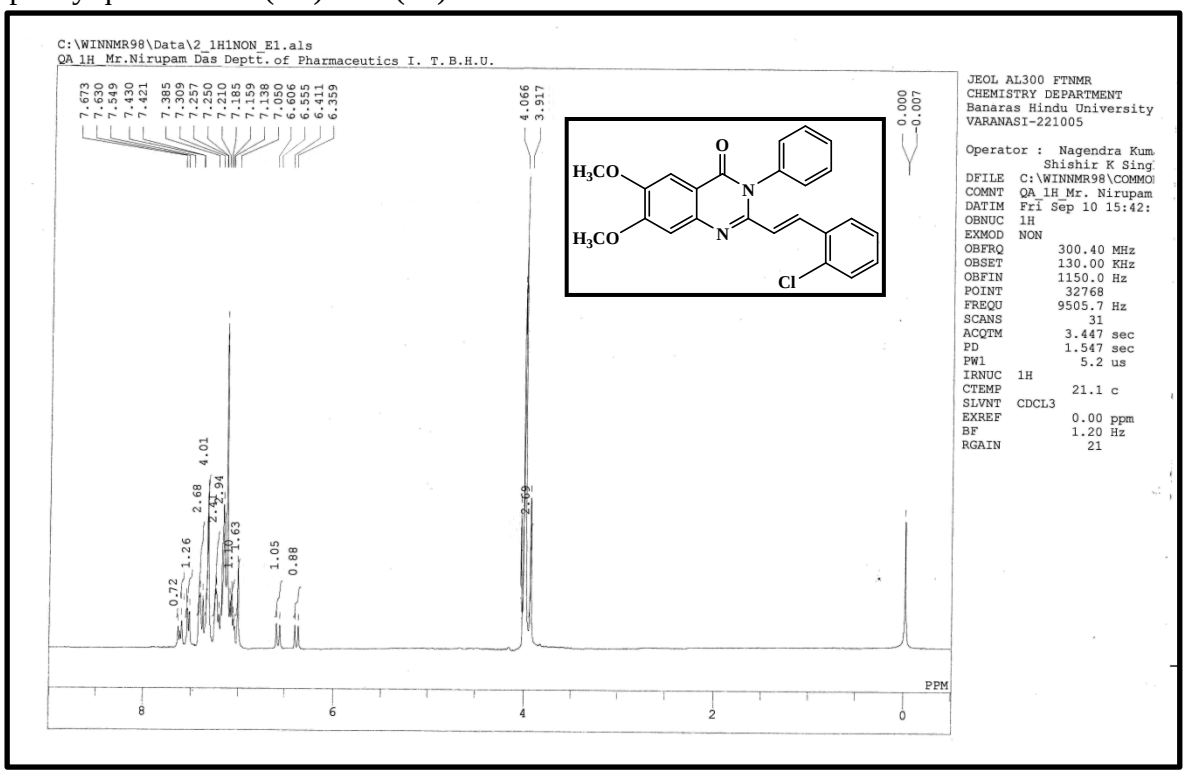

Figure 4A.8. ^{1}H NMR spectrum of 2–(2–chlorostyryl)–6,7–dimethoxy–3–phenylquinazolin–4(3*H*)–one (**5a)**

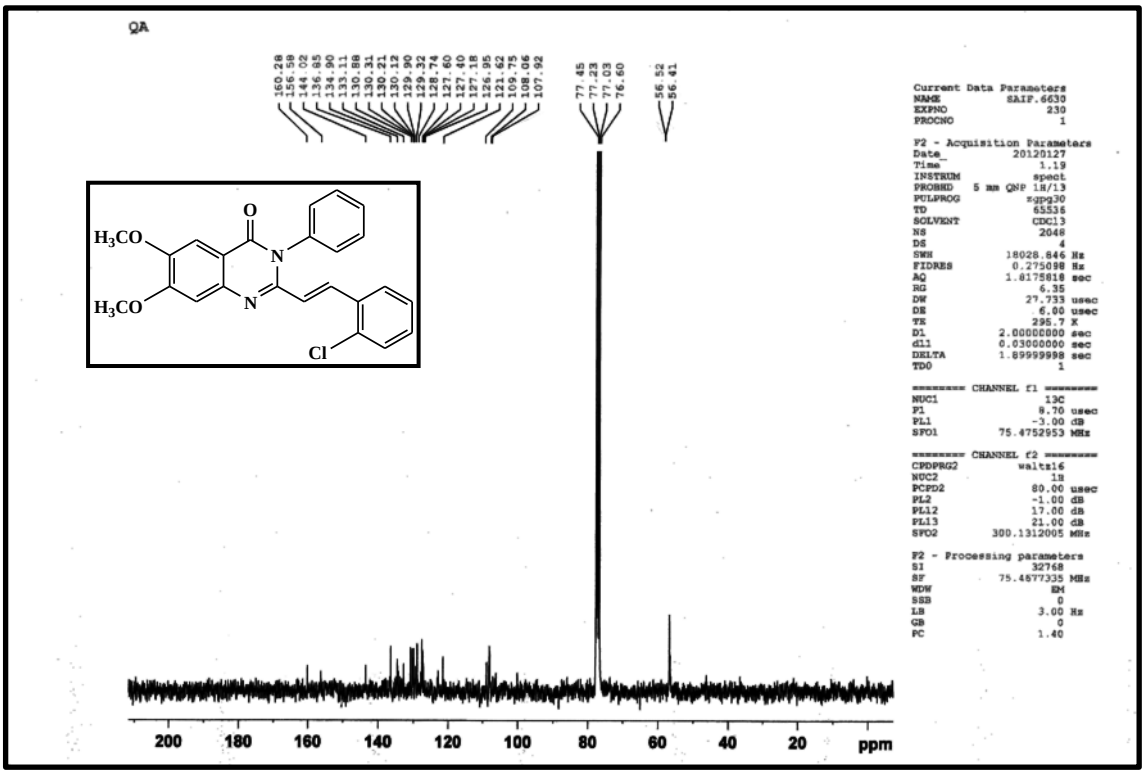

Figure 4A.9. ^{13}C NMR spectrum of 2–(2–chlorostyryl)–6,7–dimethoxy–3–phenylquinazolin–4(3*H*)–one (**5a)**

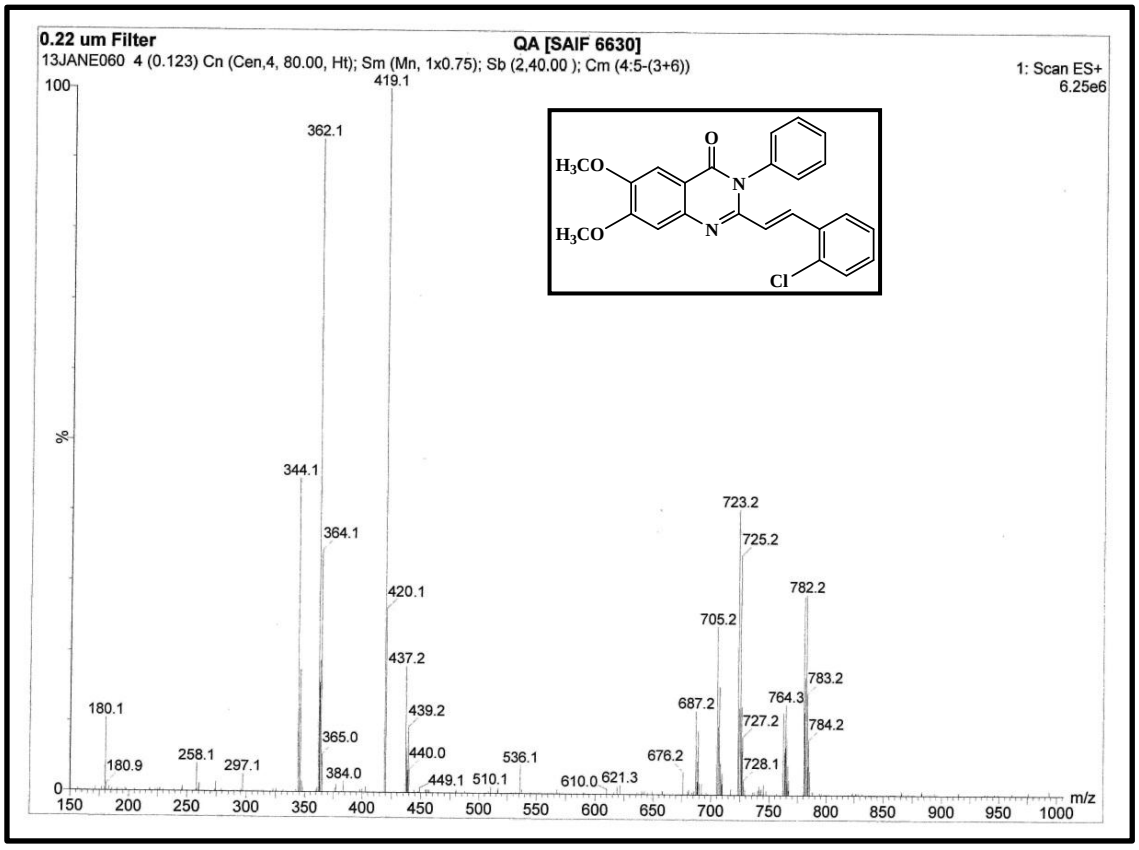

Figure 4A.10. Mass spectrum of 2–(2–chlorostyryl)–6,7–dimethoxy–3–phenylquinazolin–4(3*H*)–one (**5a**)

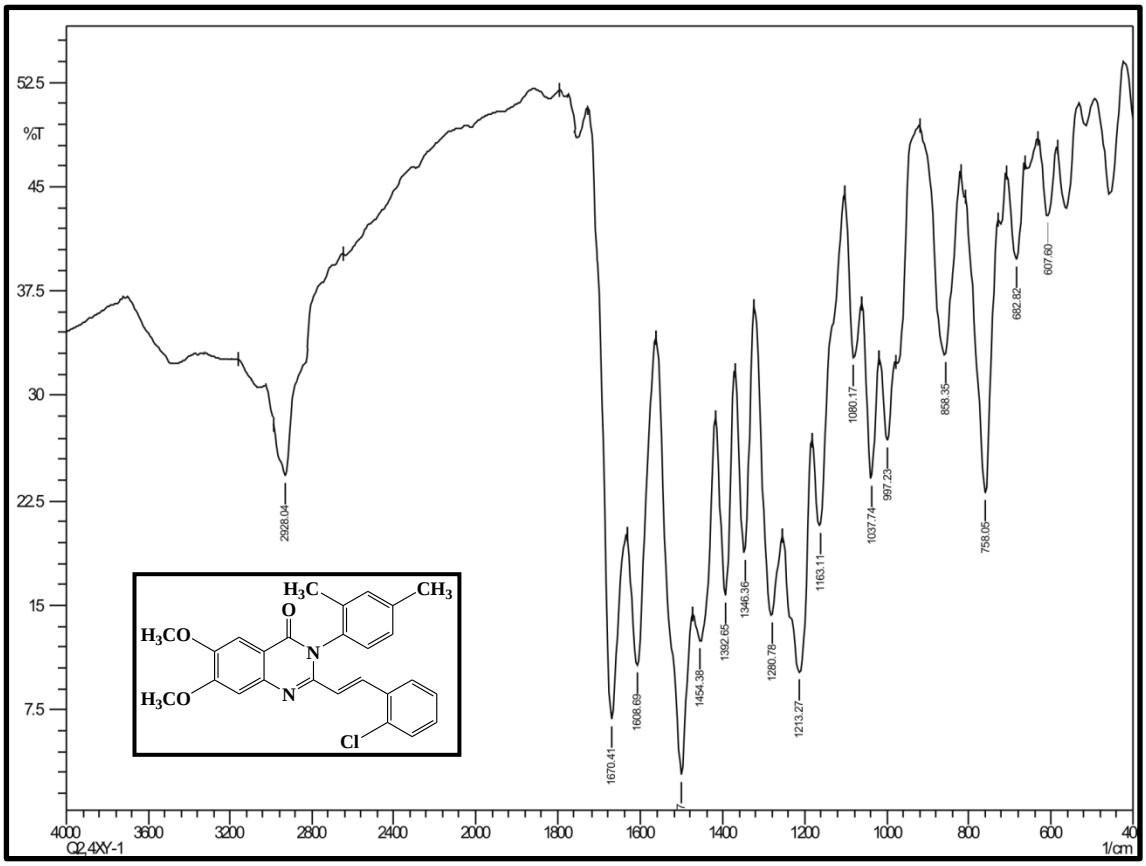

Figure 4A.11. FT–IR spectrum of 2–(2–chlorostyryl)–6,7–dimethoxy–3–(2,4–dimethylphenyl)quinazolin–4(3*H*)–one (**5b**)

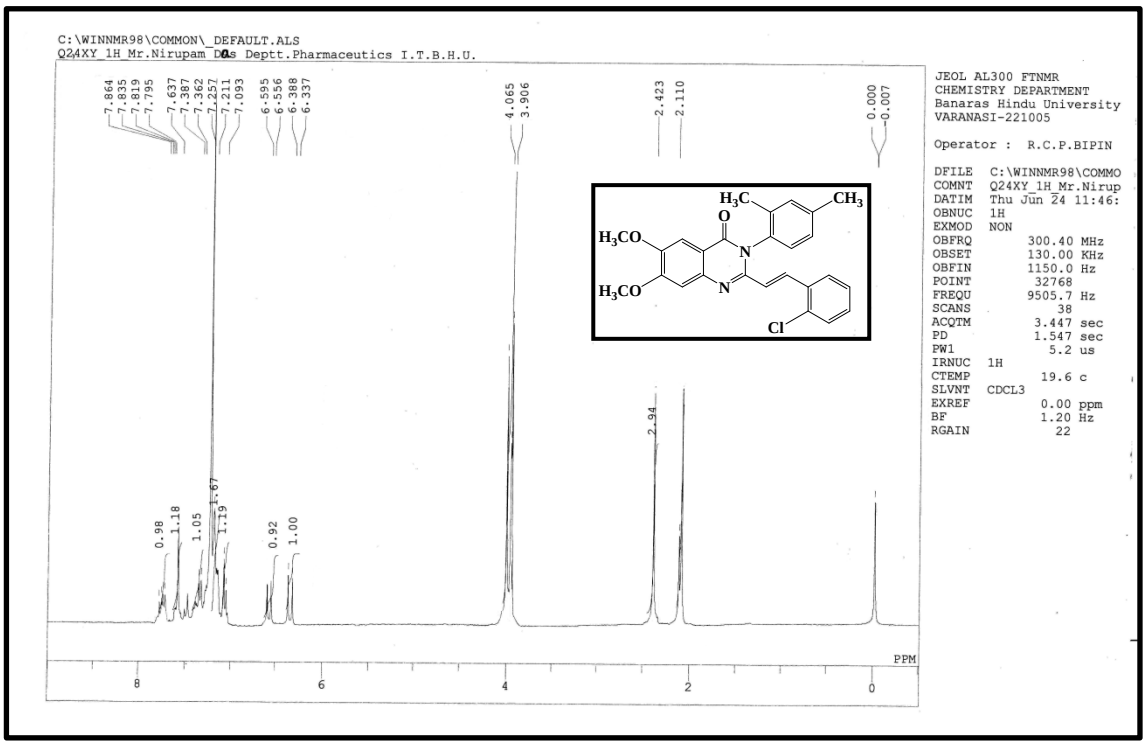

Figure 4A.12. ^{1}H NMR spectrum of 2–(2–chlorostyryl)–6,7–dimethoxy–3–(2,4–dimethylphenyl)quinazolin–4(3*H*)–one (**5b**)

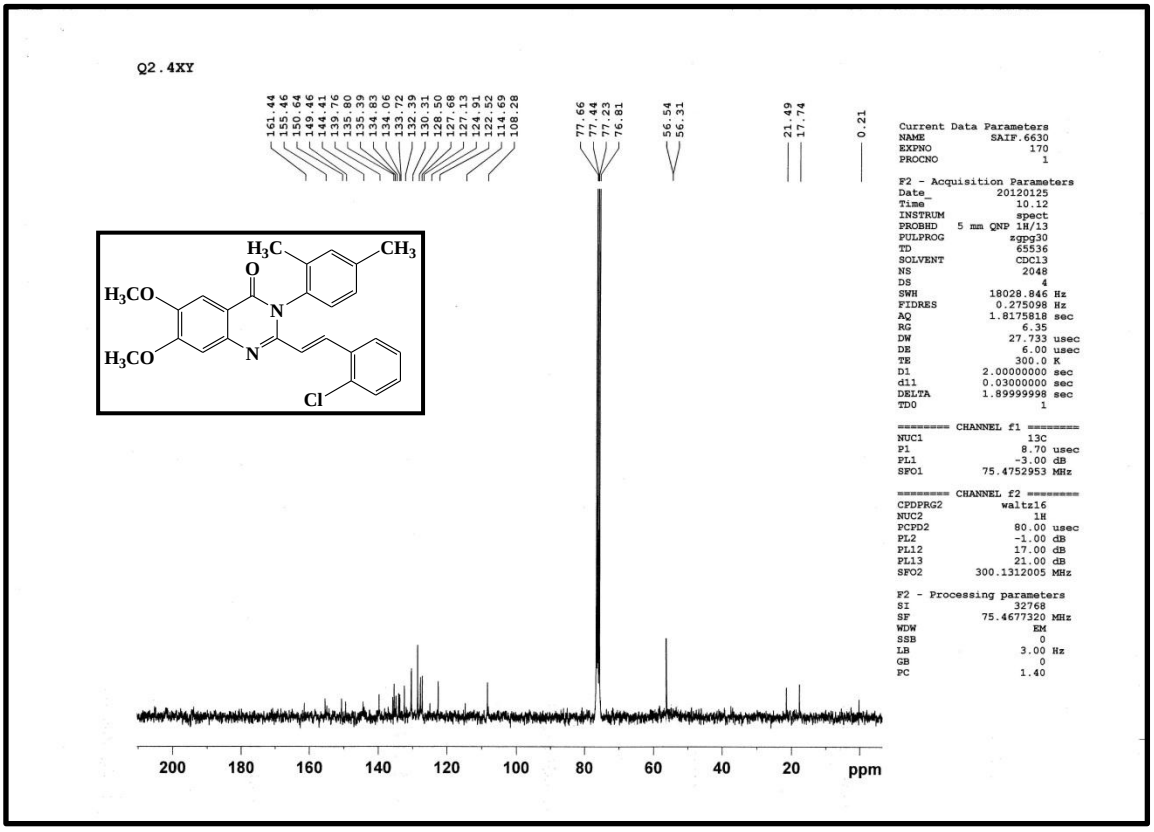

Figure 4A.13. ^{13}C NMR spectrum of 2–(2–chlorostyryl)–6,7–dimethoxy–3–(2,4–dimethylphenyl)quinazolin–4(3*H*)–one (**5b**)

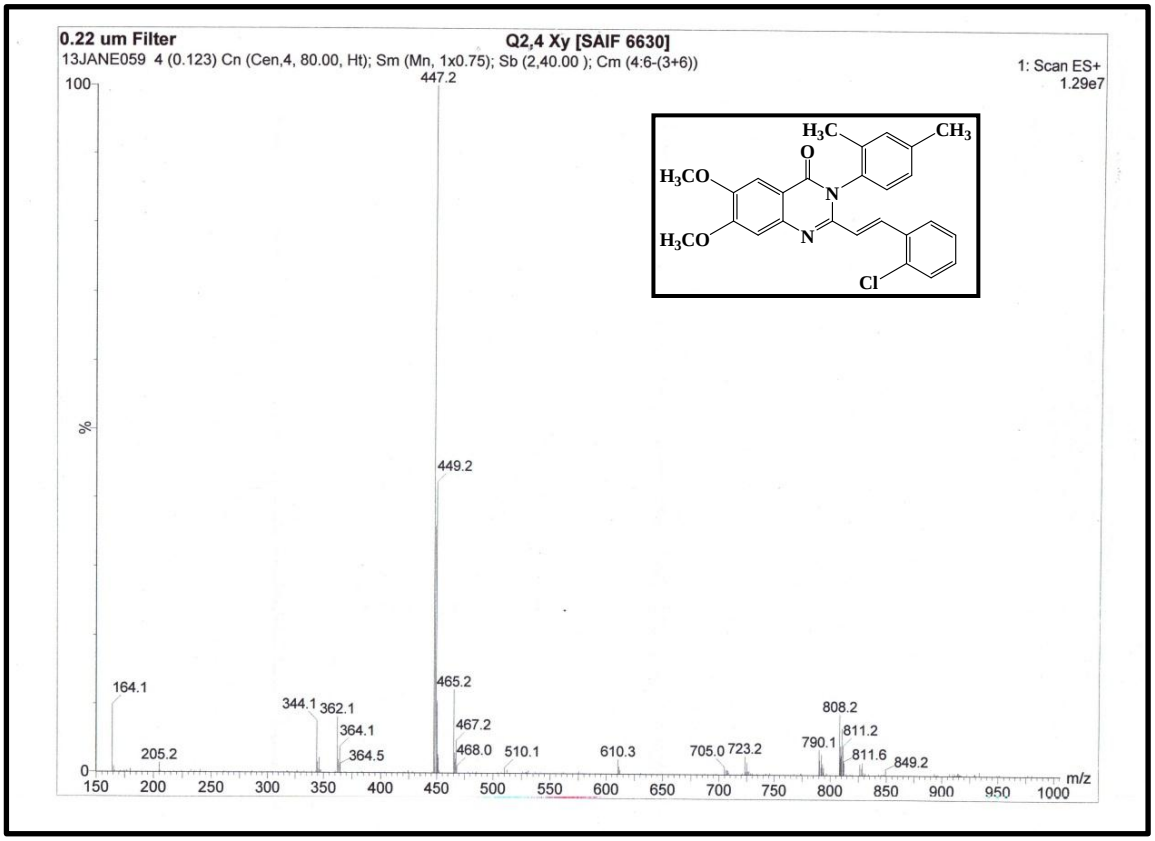

Figure 4A.14. Mass spectrum of 2–(2–chlorostyryl)–6,7–dimethoxy–3–(2,4–dimethylphenyl)quinazolin–4(3*H*)–one (**5b**)

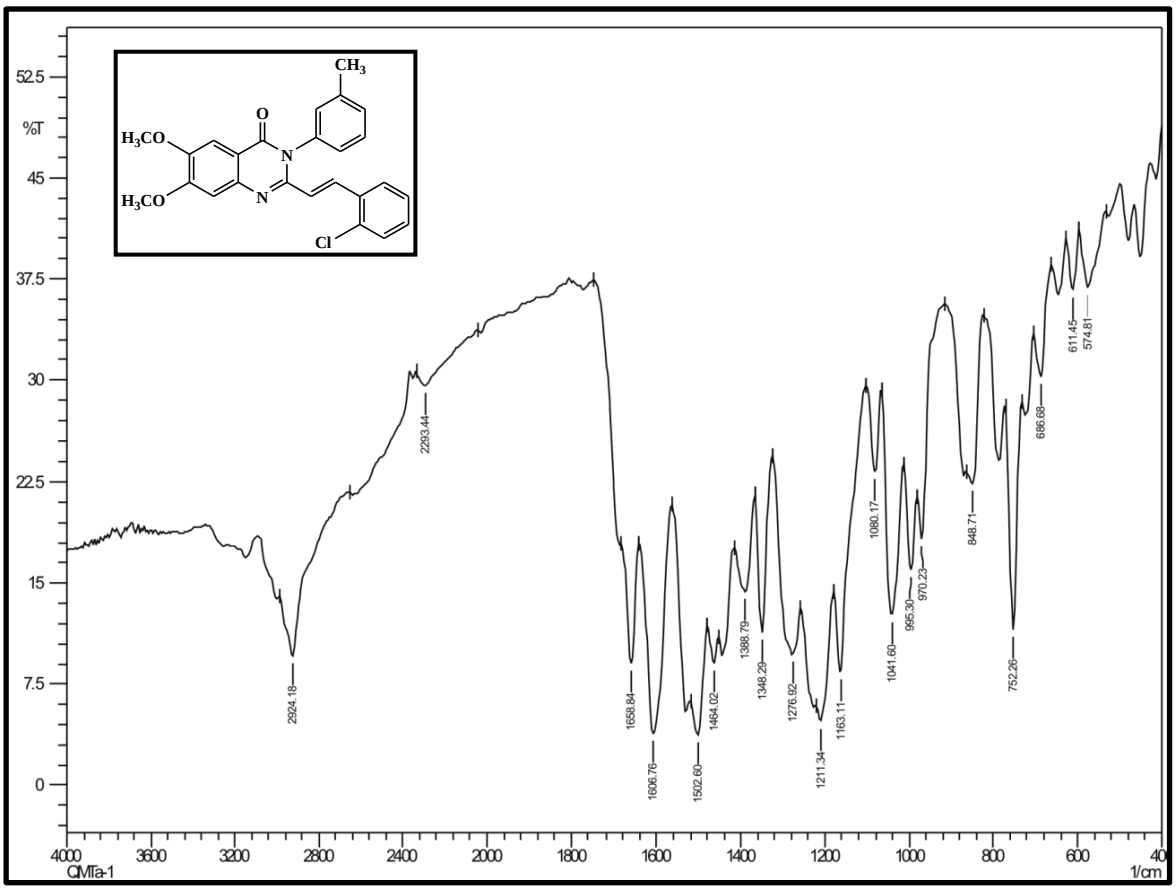

Figure 4A.15. FT–IR spectrum of 2–(2–chlorostyryl)–6,7–dimethoxy–3–*m*–tolylquinazolin–4(3*H*)–one (**5c**)

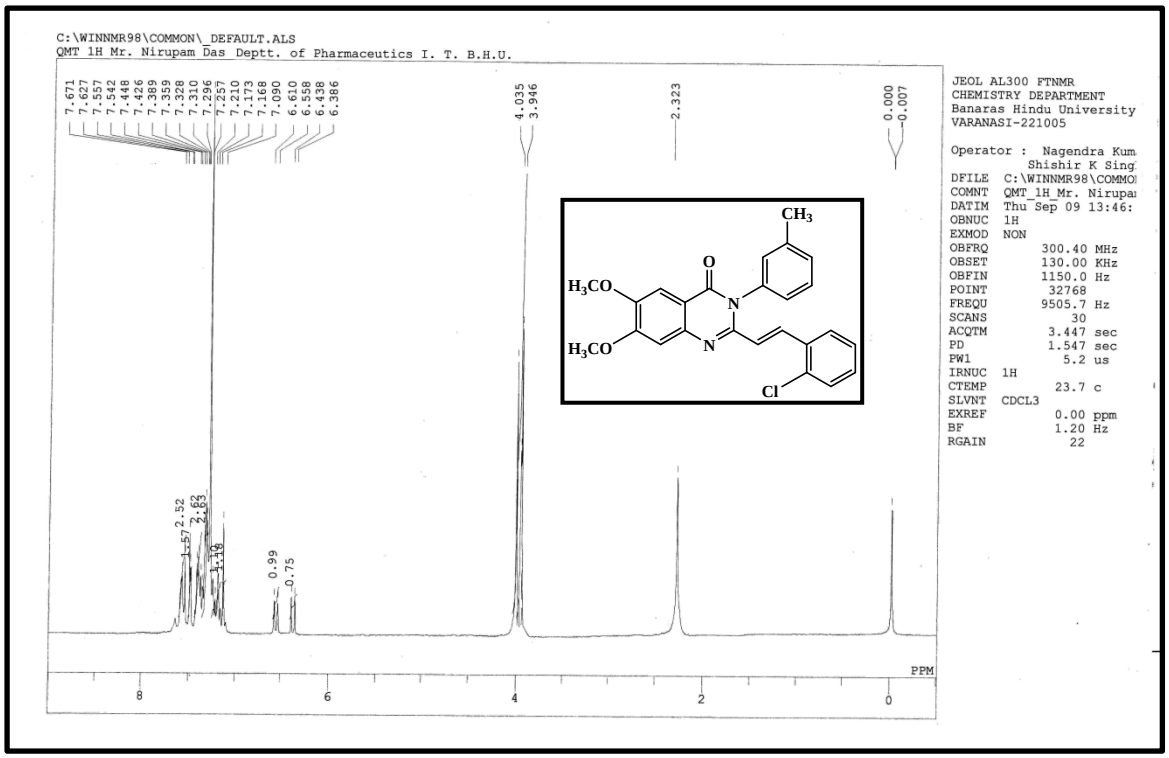

Figure 4A.16. ^{1}H NMR spectrum of 2–(2–chlorostyryl)–6,7–dimethoxy–3–*m*–tolylquinazolin–4(3*H*)–one (**5c**)

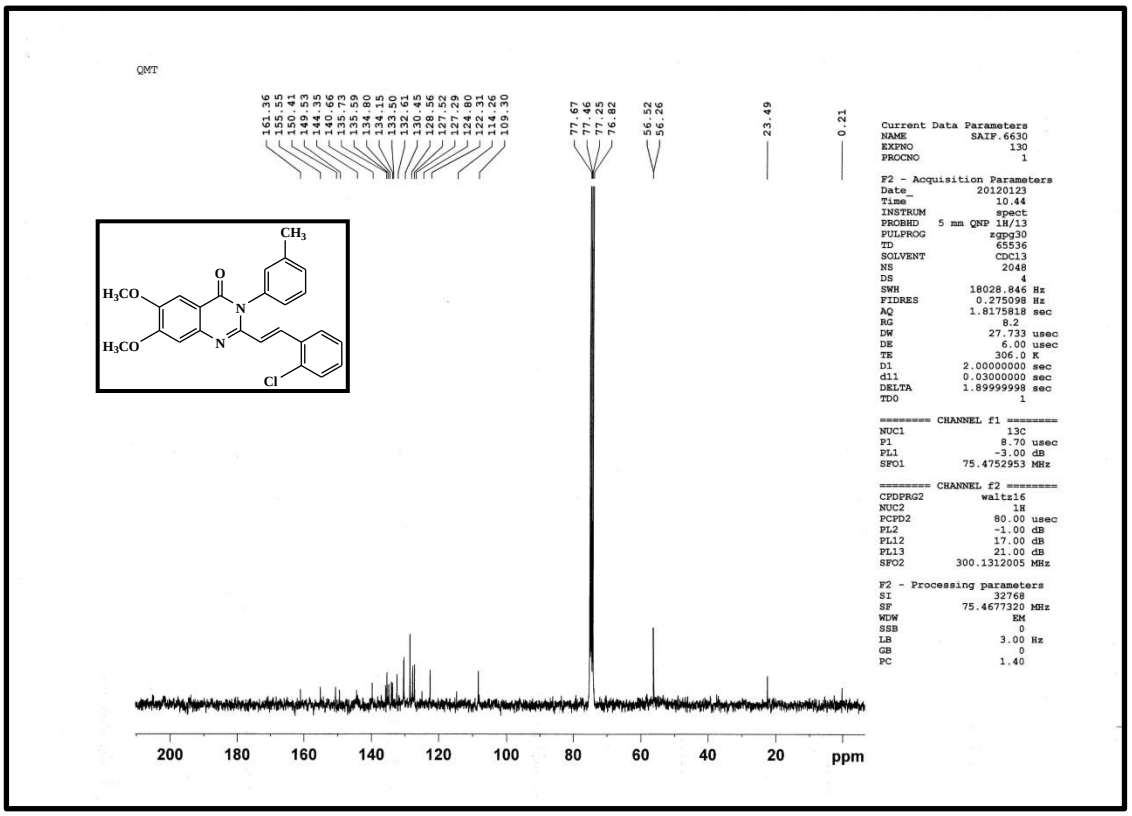

Figure 4A.17. ^{13}C NMR spectrum of 2–(2–chlorostyryl)–6,7–dimethoxy–3–*m*–tolylquinazolin–4(3*H*)–one (**5c**)

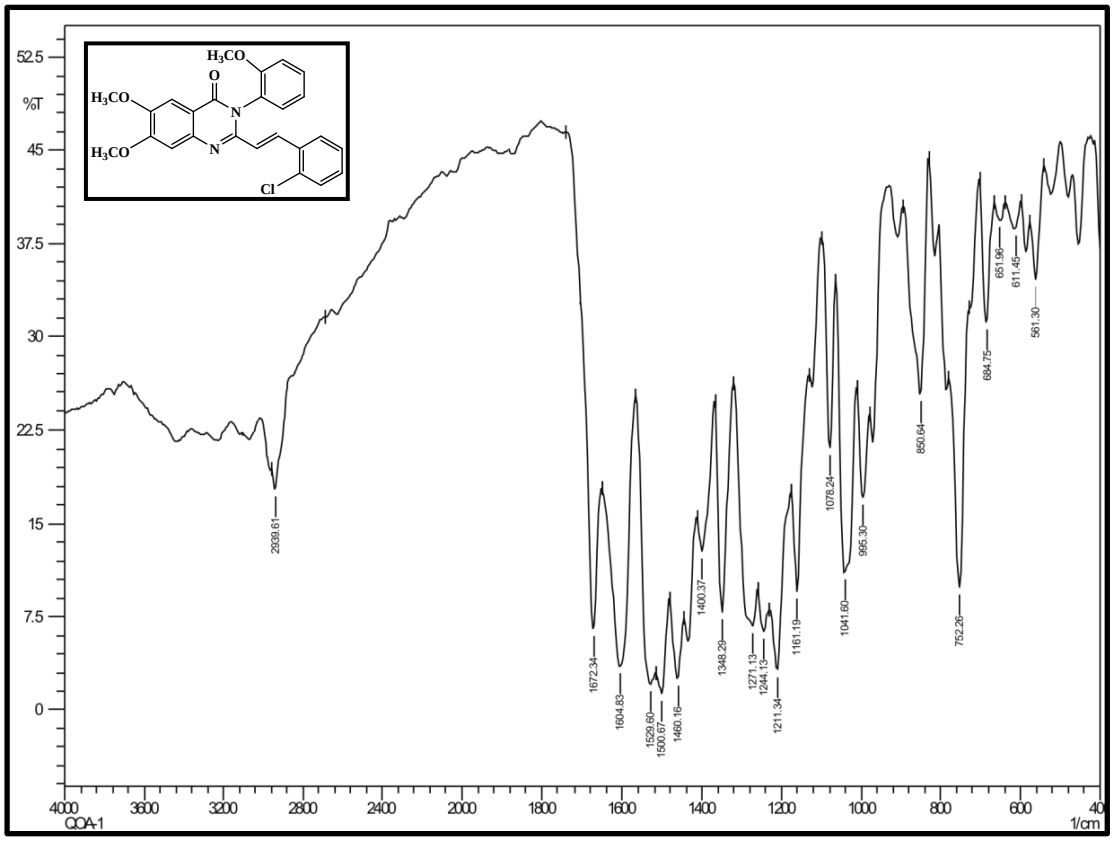

Figure 4A.18. FT–IR spectrum of 2–(2–chlorostyryl)–6,7–dimethoxy–3–(2–methoxyphenyl)quinazolin–4(3*H*)–one (**5d**)

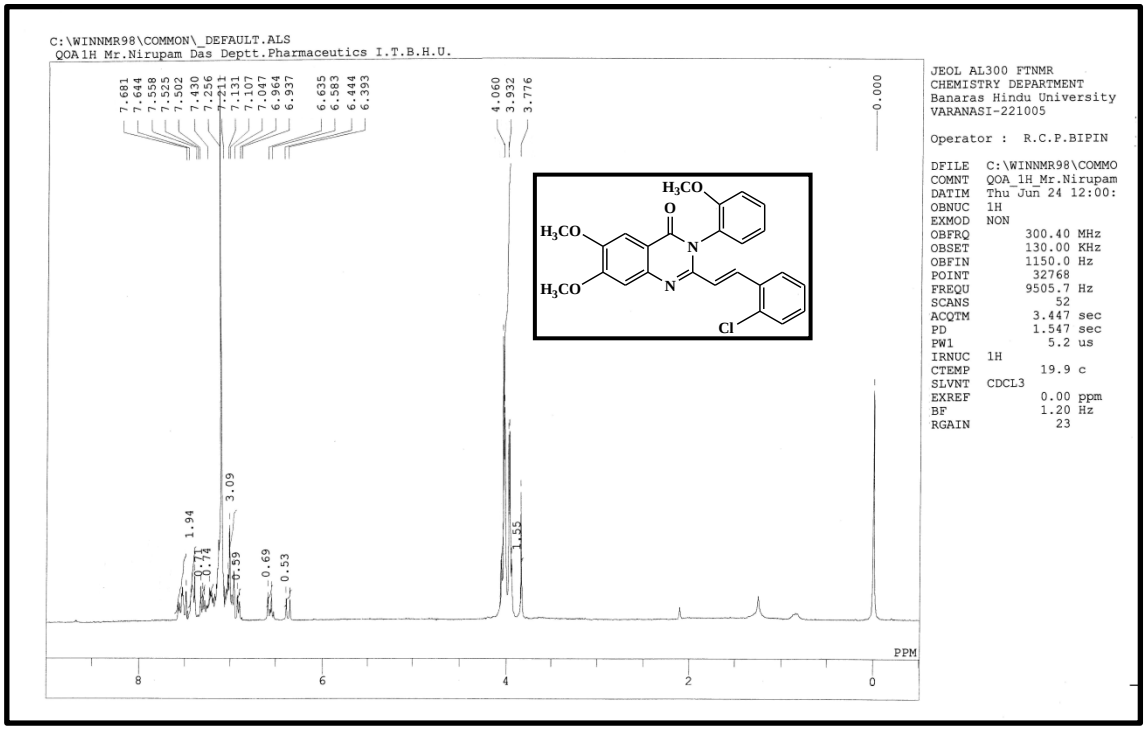

Figure 4A.19. ^{1}H NMR spectrum of 2–(2–chlorostyryl)–6,7–dimethoxy–3–(2–methoxyphenyl)quinazolin–4(3*H*)–one (**5d**)

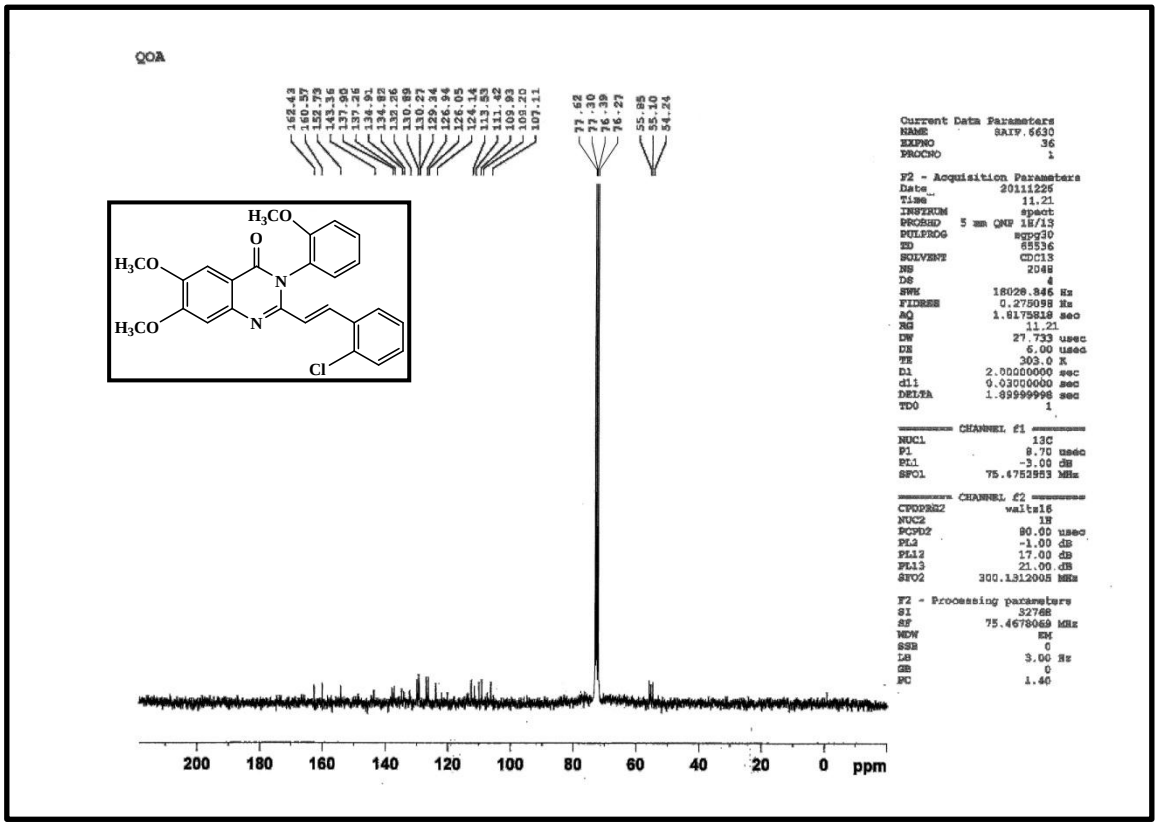

Figure 4A.20. ^{13}C NMR spectrum of 2–(2–chlorostyryl)–6,7–dimethoxy–3–(2–methoxyphenyl)quinazolin–4(3*H*)–one (**5d**)

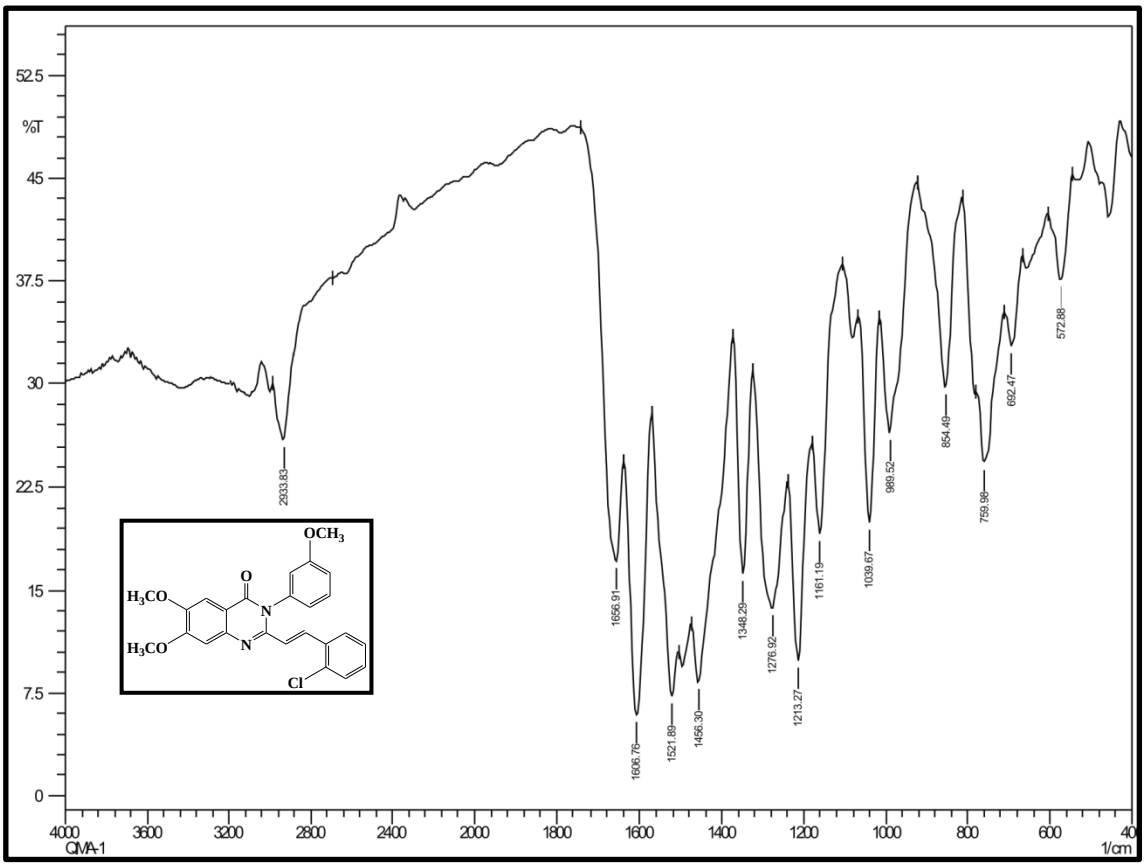

Figure 4A.21. FT–IR spectrum of 2–(2–chlorostyryl)–6,7–dimethoxy–3–(3–methoxyphenyl)quinazolin–4(3*H*)–one (**5e**)

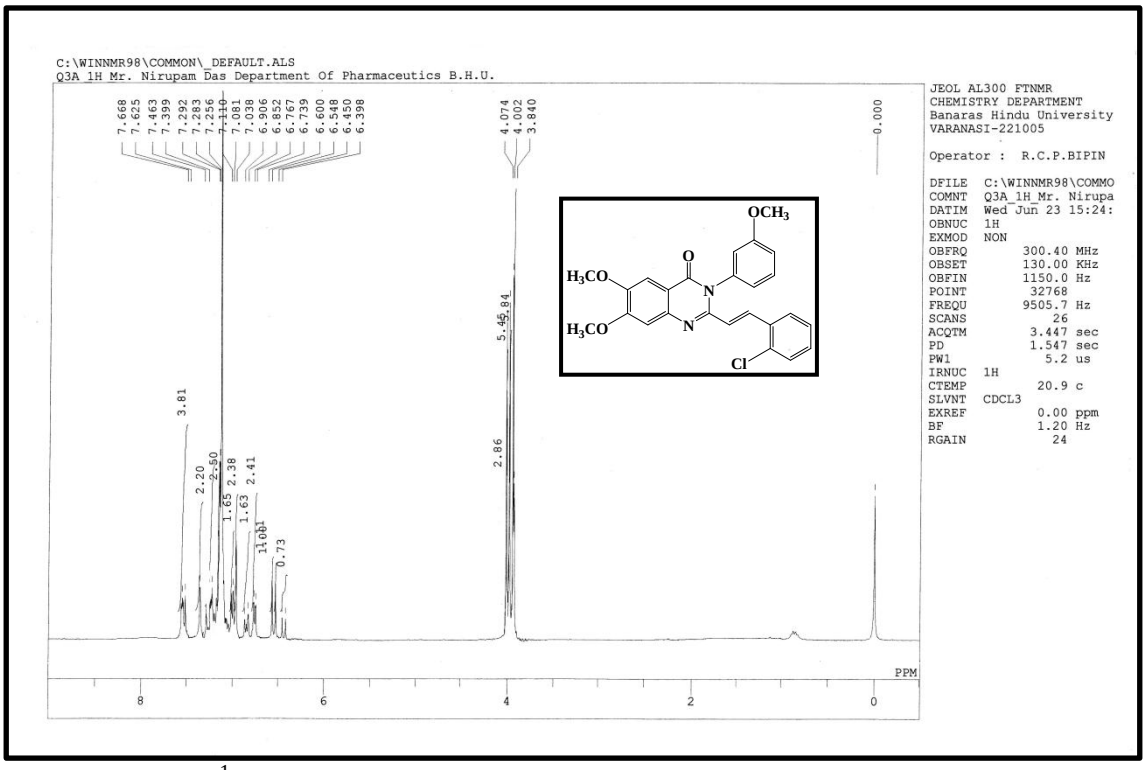

Figure 4A.22. ^{1}H NMR spectrum of 2–(2–chlorostyryl)–6,7–dimethoxy–3–(3–methoxyphenyl)quinazolin–4(3*H*)–one (**5e**)

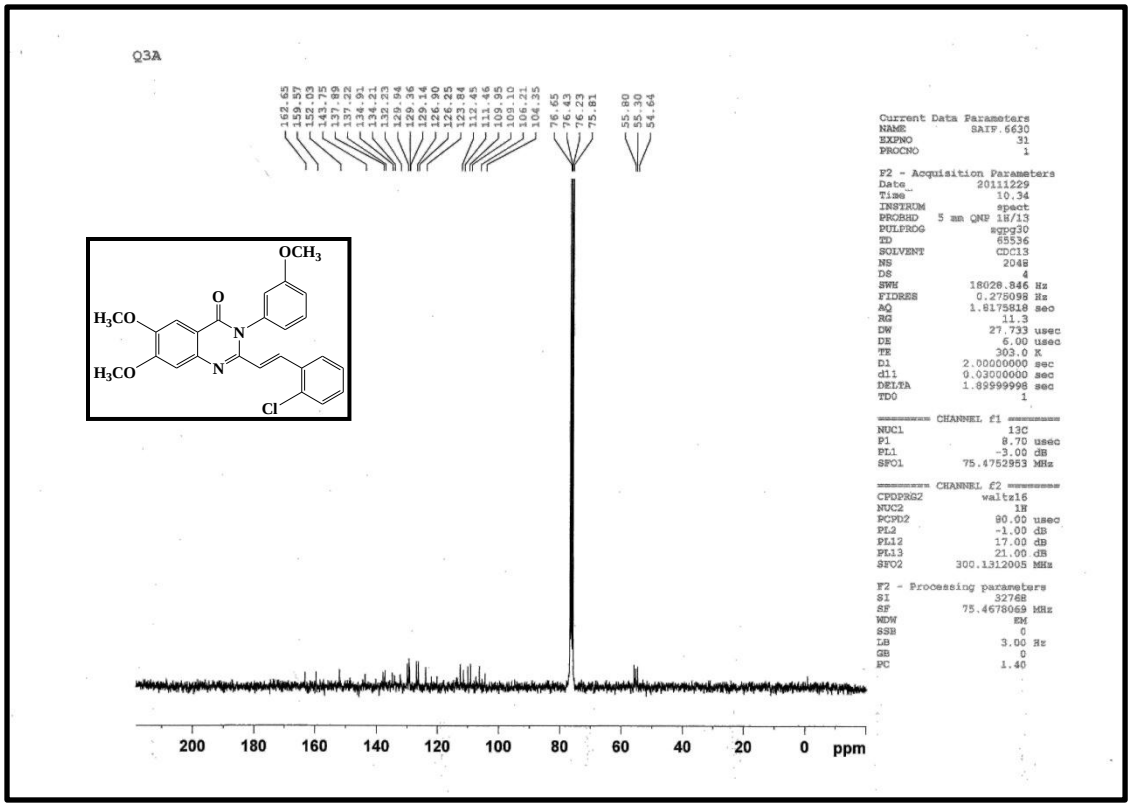

Figure 4A.23. ^{13}C NMR spectrum of 2–(2–chlorostyryl)–6,7–dimethoxy–3–(3–methoxyphenyl)quinazolin–4(3*H*)–one (**5e**)

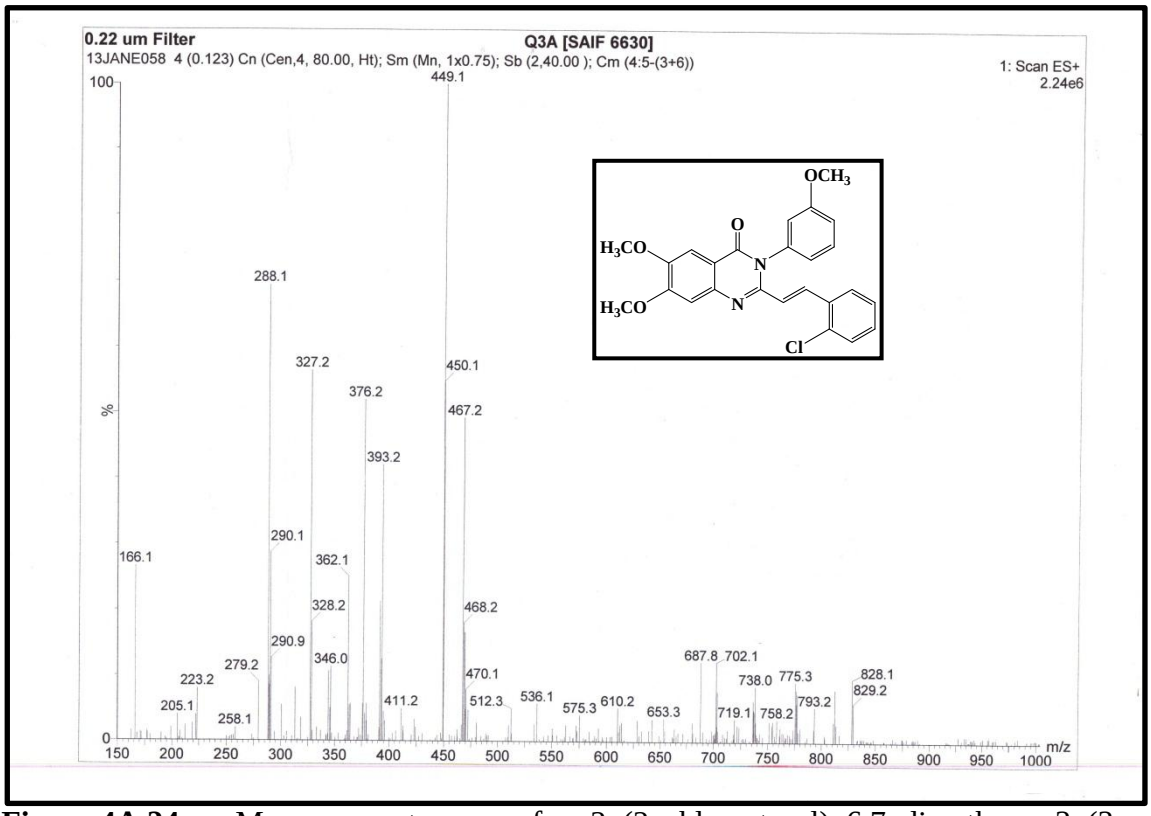

Figure 4A.24. Mass spectrum of 2–(2–chlorostyryl)–6,7–dimethoxy–3–(3–methoxyphenyl)quinazolin–4(3*H*)–one (**5e**)

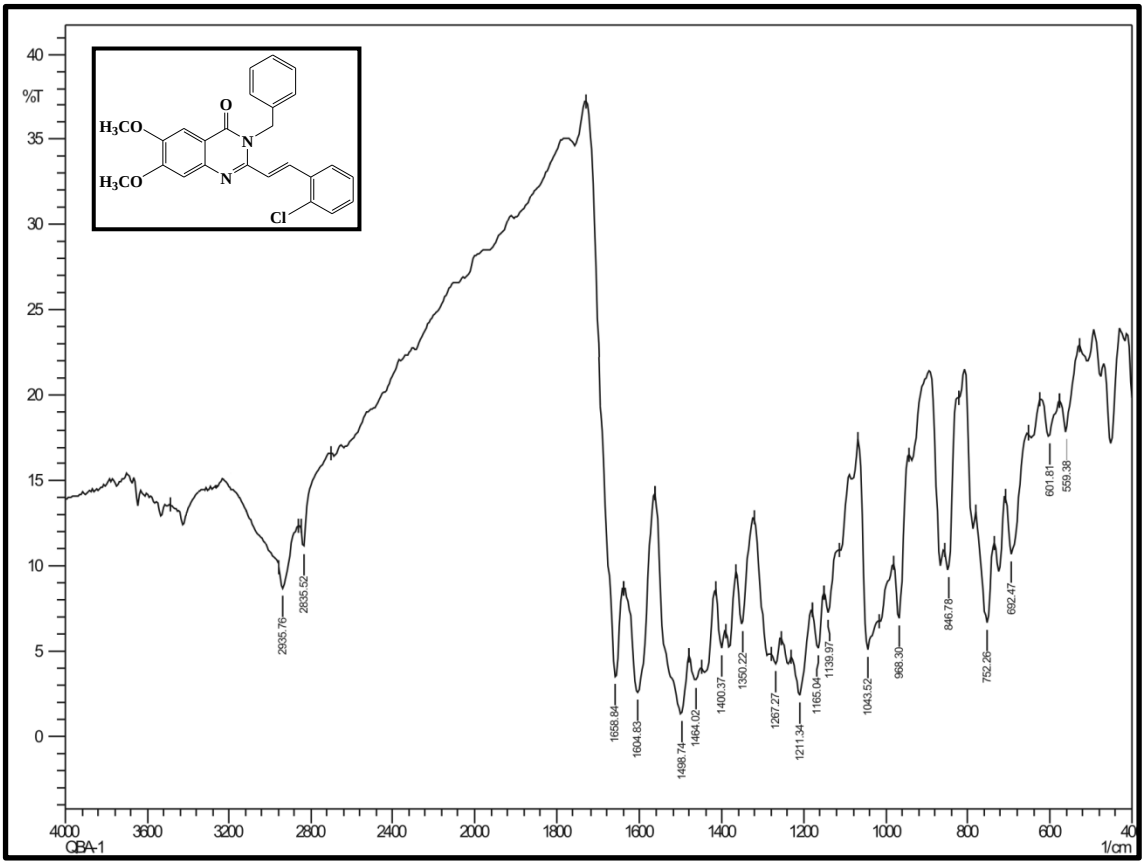

Figure 4A.25. FT–IR spectrum of 2–(2–chlorostyryl)–3–benzyl–6,7–dimethoxyquinazolin–4(3*H*)–one (**5f**)

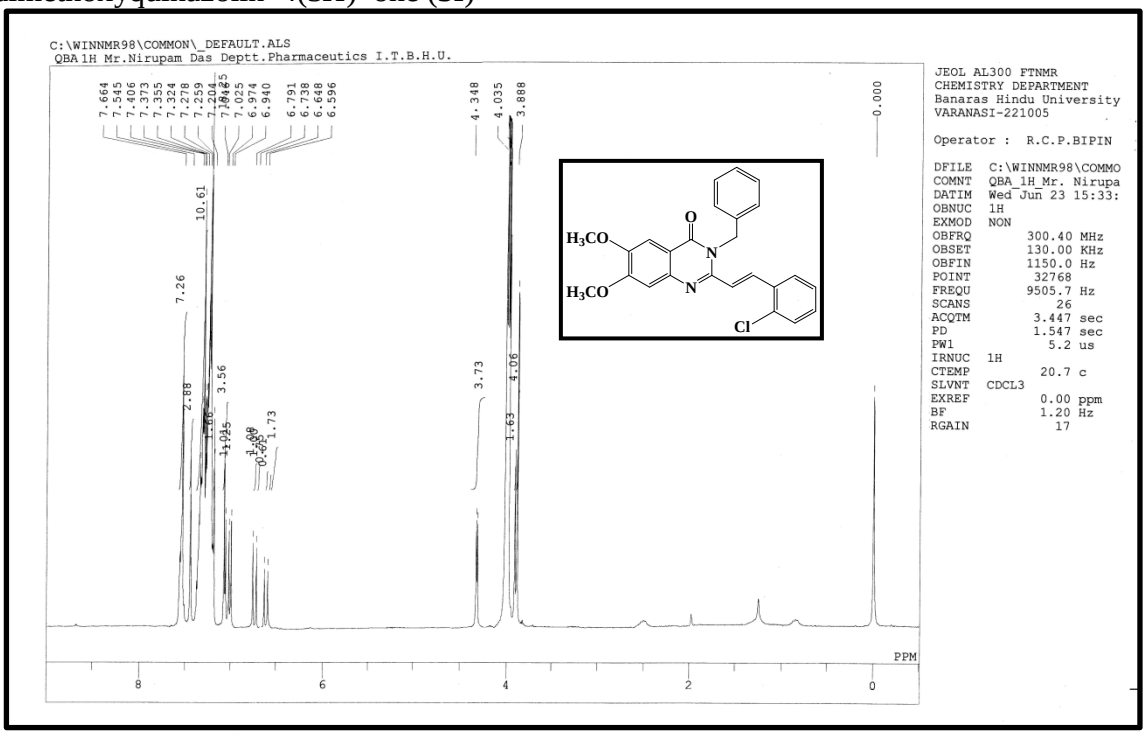

Figure 4A.26. ^{1}H NMR spectrum of 2–(2–chlorostyryl)–3–benzyl–6,7–dimethoxyquinazolin–4(3*H*)–one (**5f**)

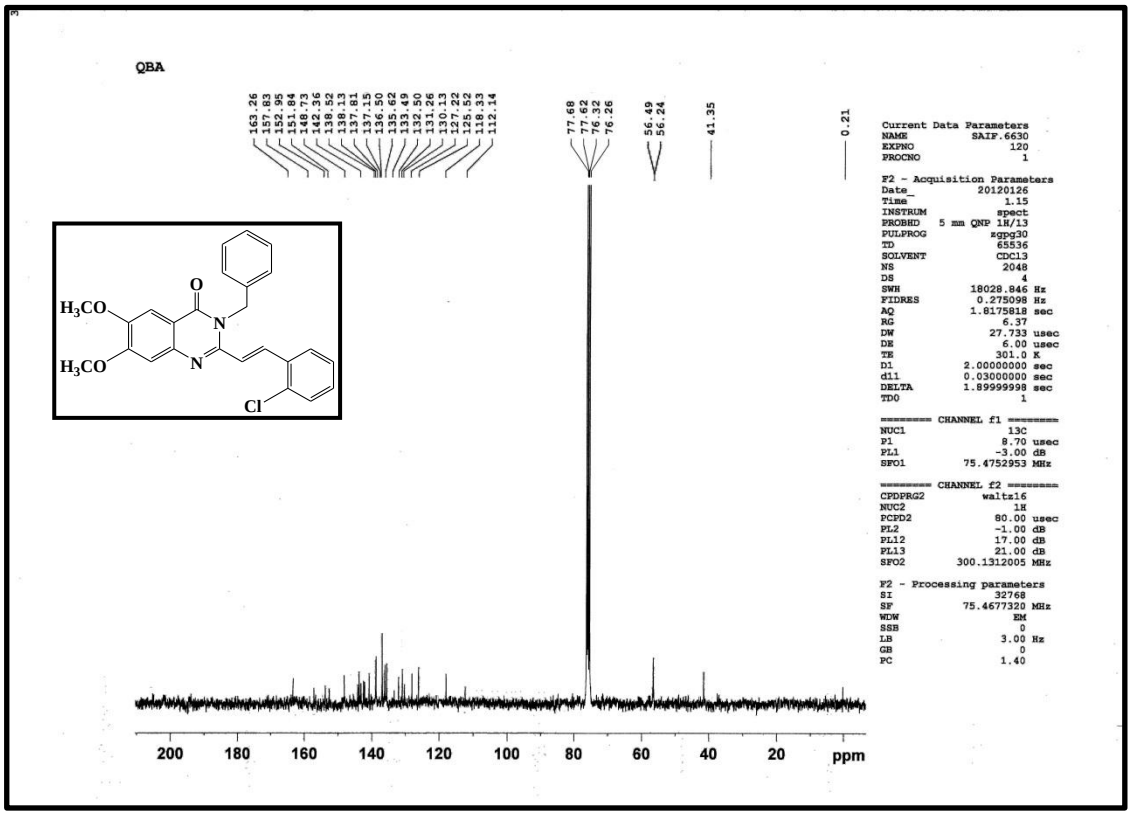

Figure 4A.27. ^{13}C NMR spectrum of 2–(2–chlorostyryl)–3–benzyl–6,7–dimethoxyquinazolin–4(3*H*)–one (**5f**)

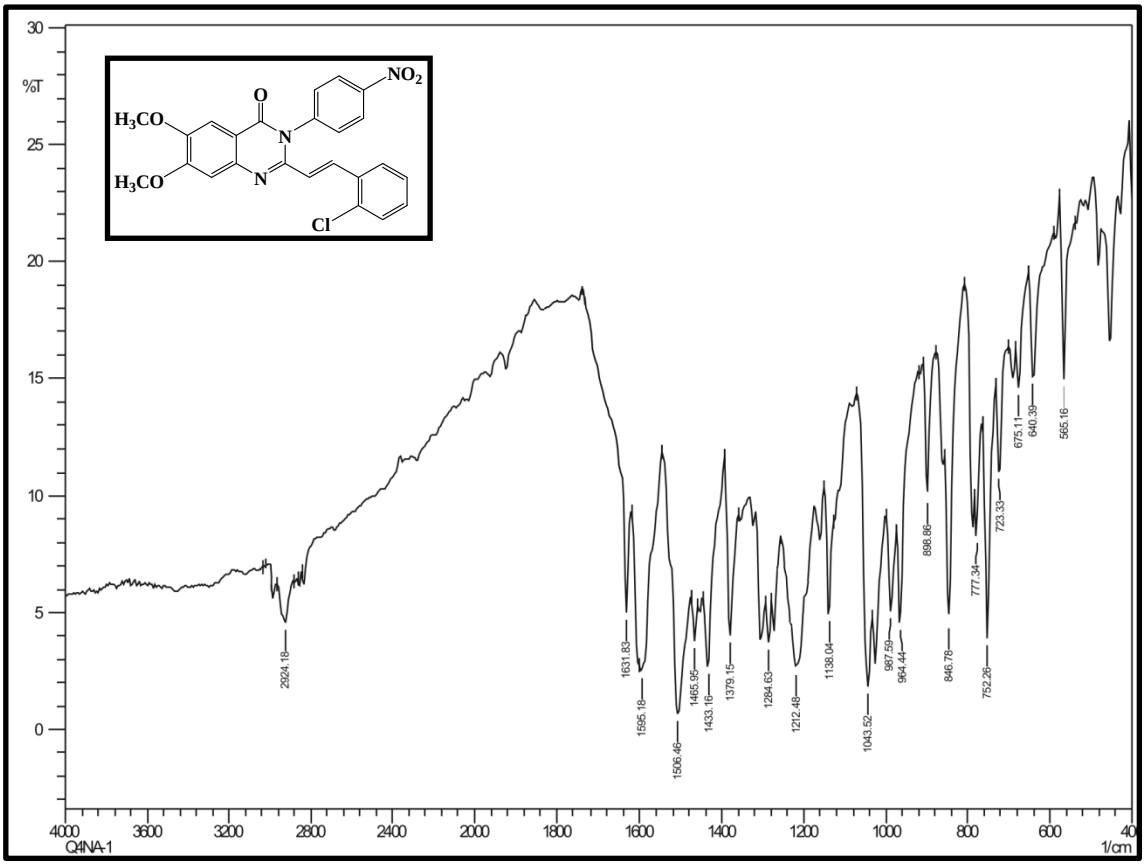

Figure 4A.28. FT–IR spectrum of 2–(2–chlorostyryl)–6,7–dimethoxy–3–(4–nitrophenyl)quinazolin–4(3*H*)–one (**5g**)

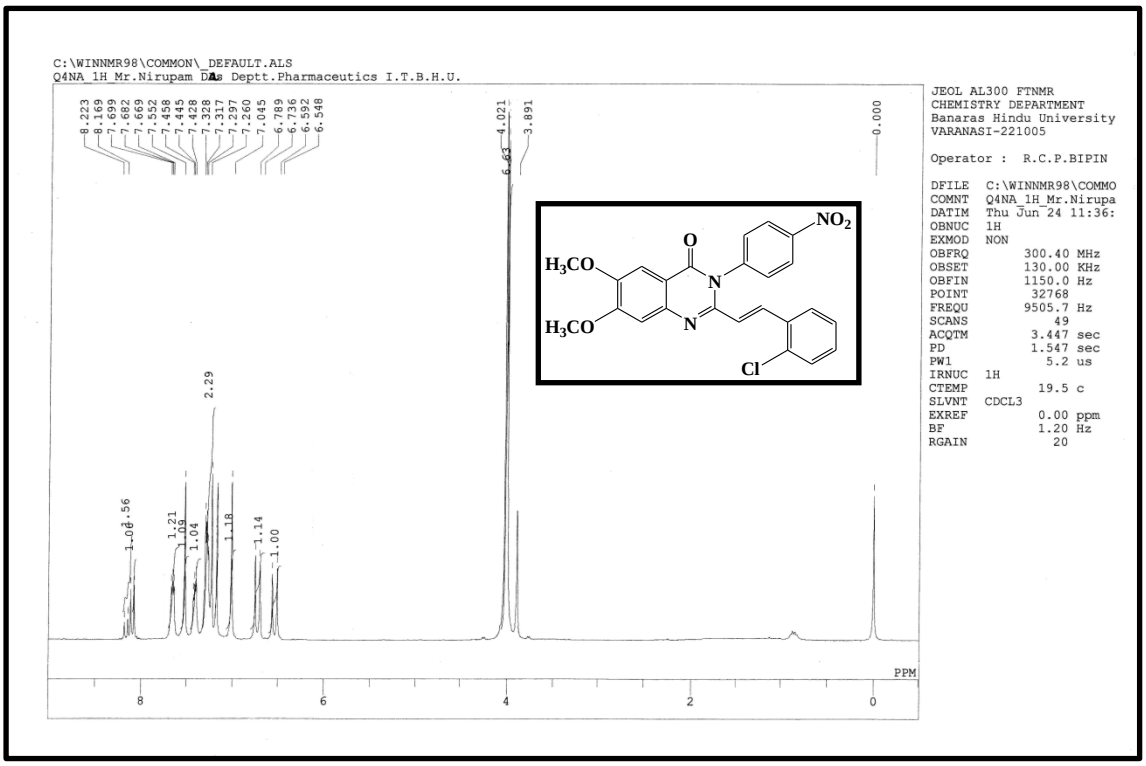

Figure 4A.29. ^{1}H NMR spectrum of 2–(2–chlorostyryl)–6,7–dimethoxy–3–(4–nitrophenyl)quinazolin–4(3*H*)–one (**5g**)

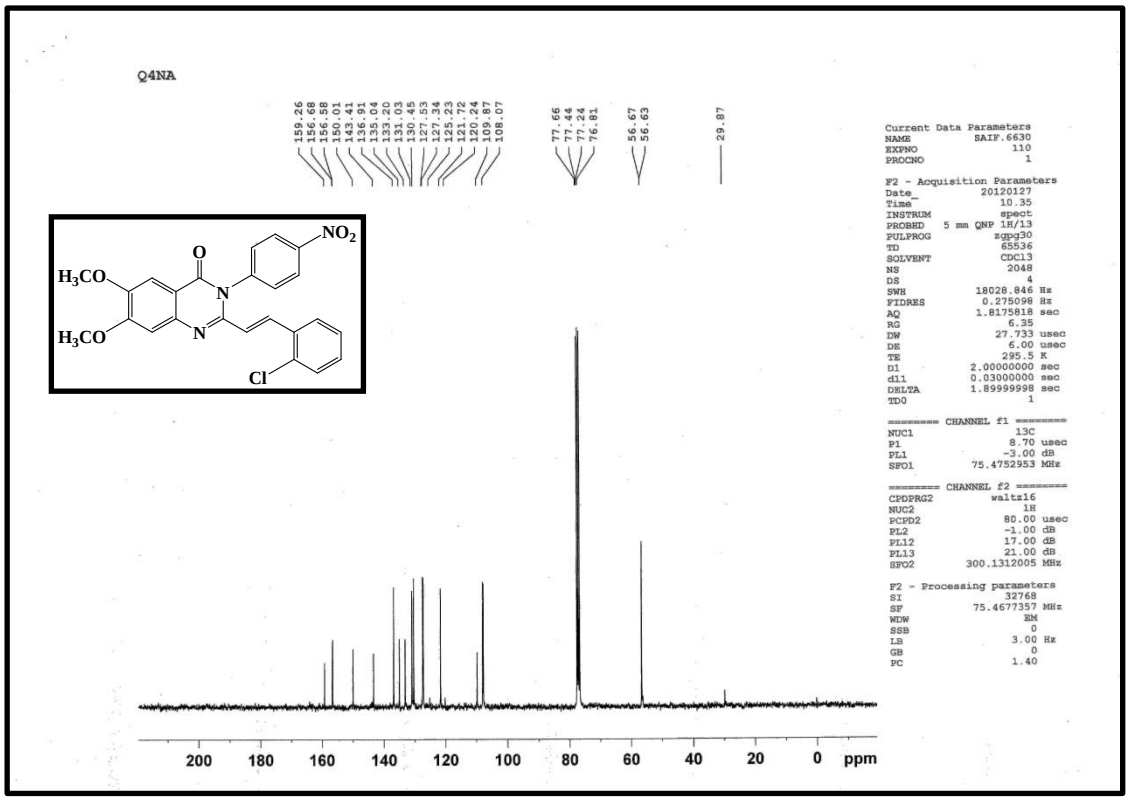

Figure 4A.30. ^{13}C NMR spectrum of 2–(2–chlorostyryl)–6,7–dimethoxy–3–(4–nitrophenyl)quinazolin–4(3*H*)–one (**5g**)

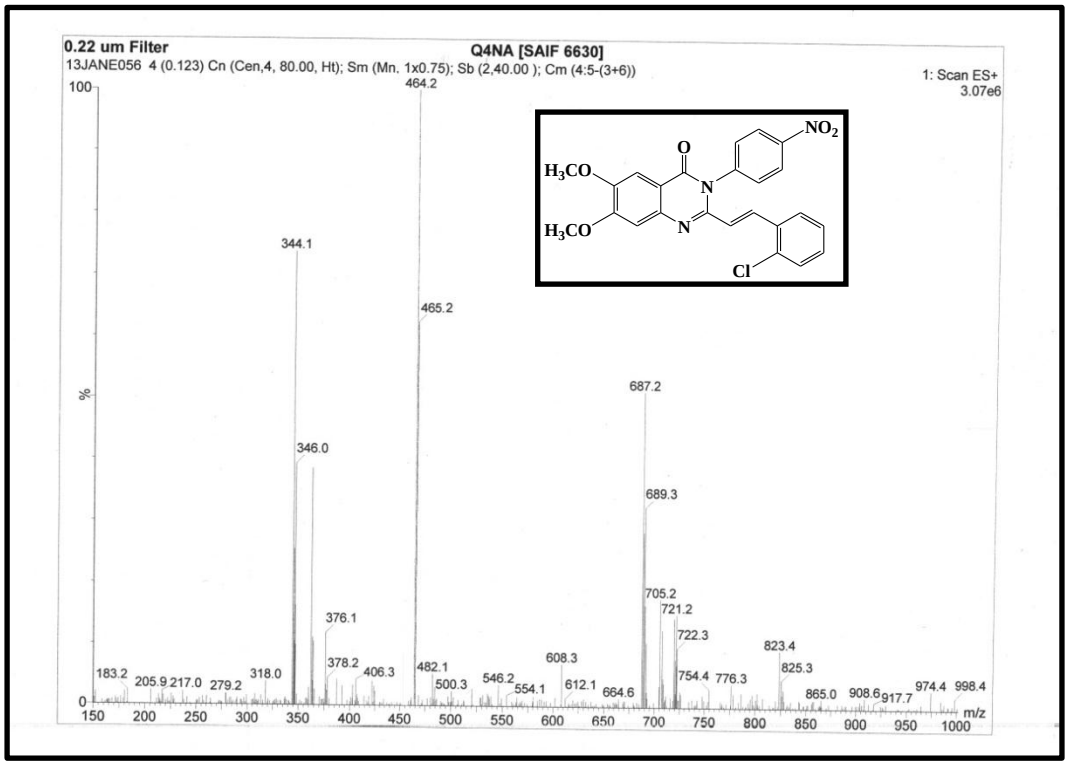

Figure 4A.31. Mass spectrum of 2–(2–chlorostyryl)–6,7–dimethoxy–3–(4–nitrophenyl)quinazolin–4(3*H*)–one (**5g**)

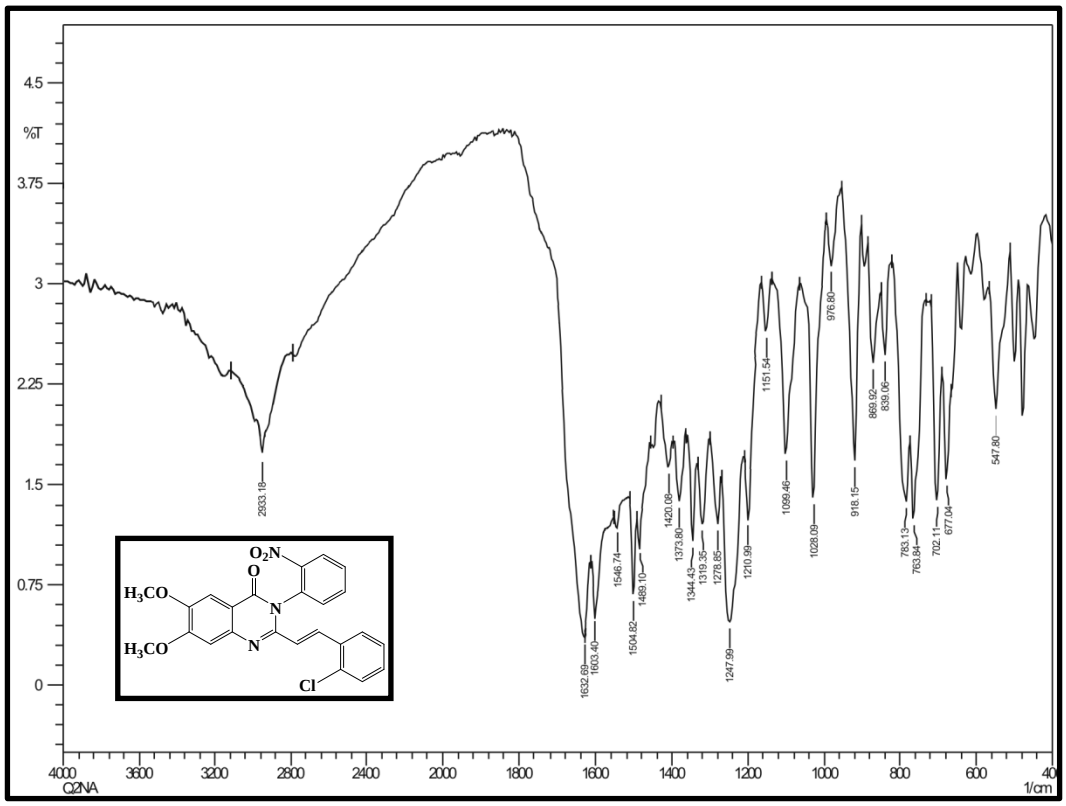

Figure 4A.32. FT–IR spectrum of 2–(2–chlorostyryl)–6,7–dimethoxy–3–(2–nitrophenyl)quinazolin–4(3*H*)–one (**5h**)

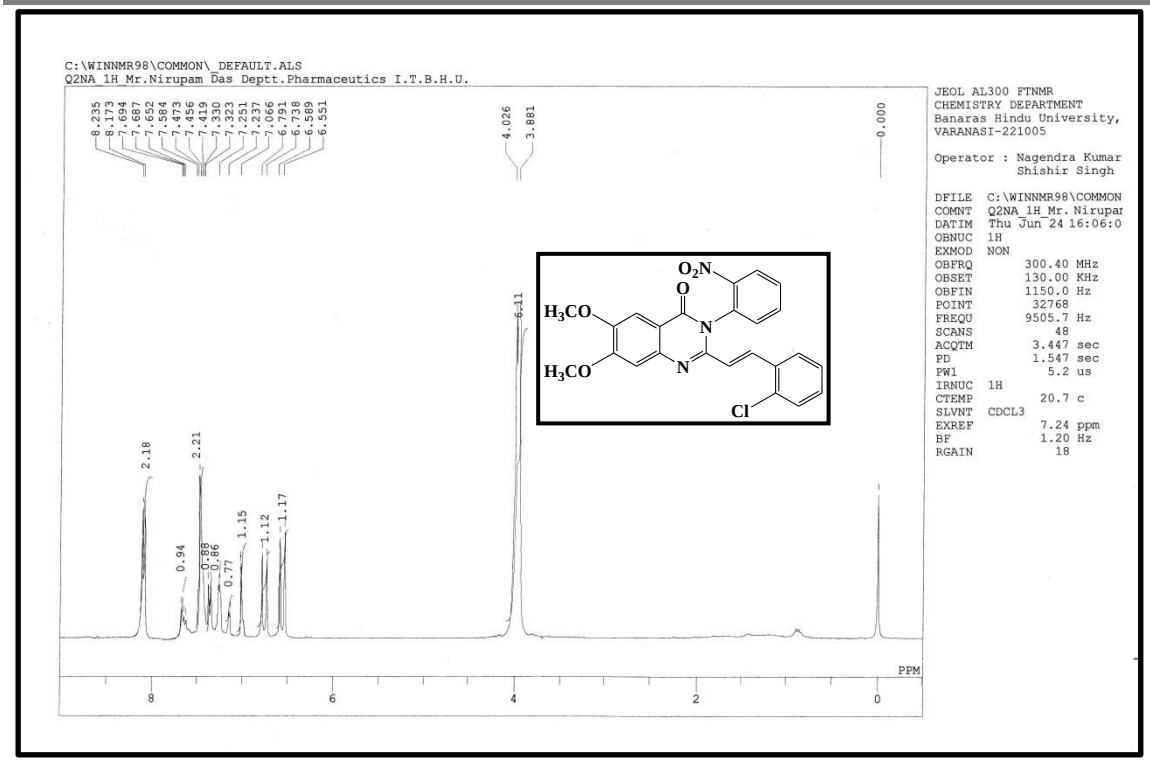

Figure 4A.33. ^{1}H NMR spectrum of 2–(2–chlorostyryl)–6,7–dimethoxy–3–(2–nitrophenyl)quinazolin–4(3*H*)–one (**5h**)

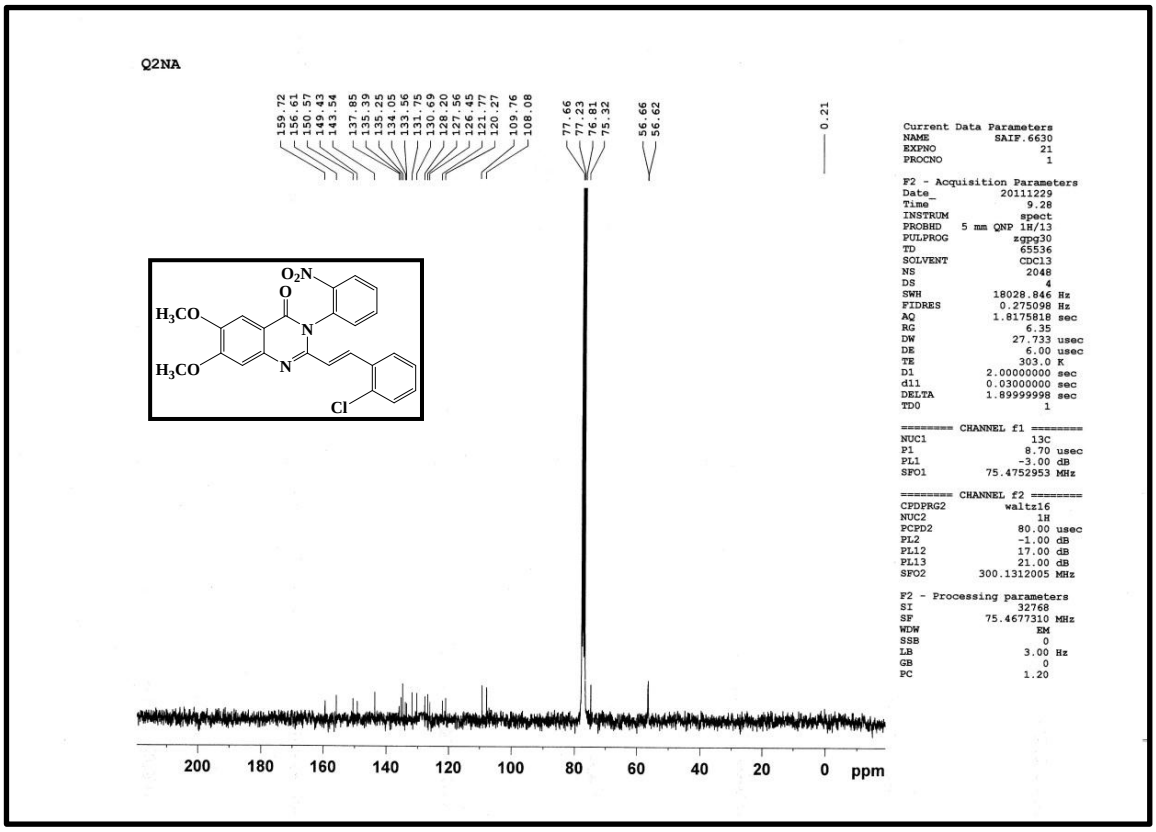

Figure 4A.34. ^{13}C NMR spectrum of 2–(2–chlorostyryl)–6,7–dimethoxy–3–(2–nitrophenyl)quinazolin–4(3*H*)–one (**5h**)

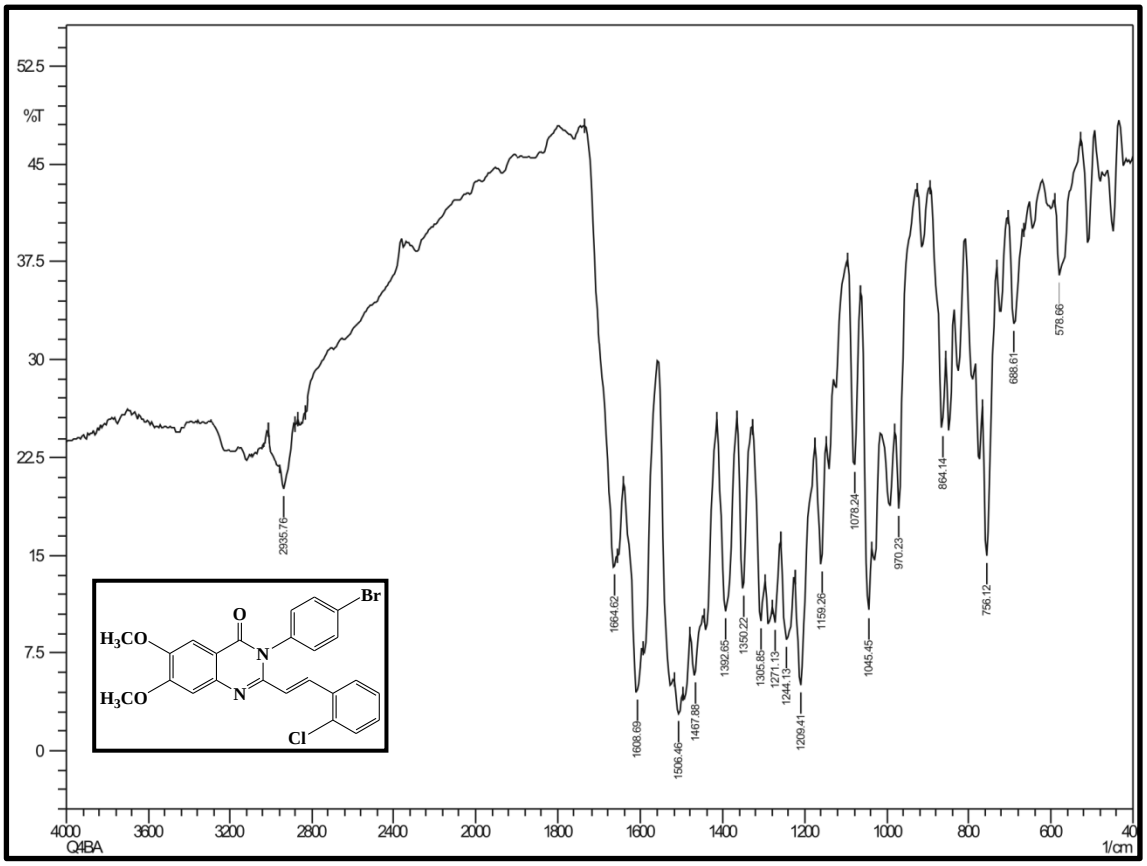

Figure 4A.35. FT–IR spectrum of 2–(2–chlorostyryl)–3–(4–bromophenyl)–6,7–dimethoxyquinazolin–4(3*H*)–one (**5i**)

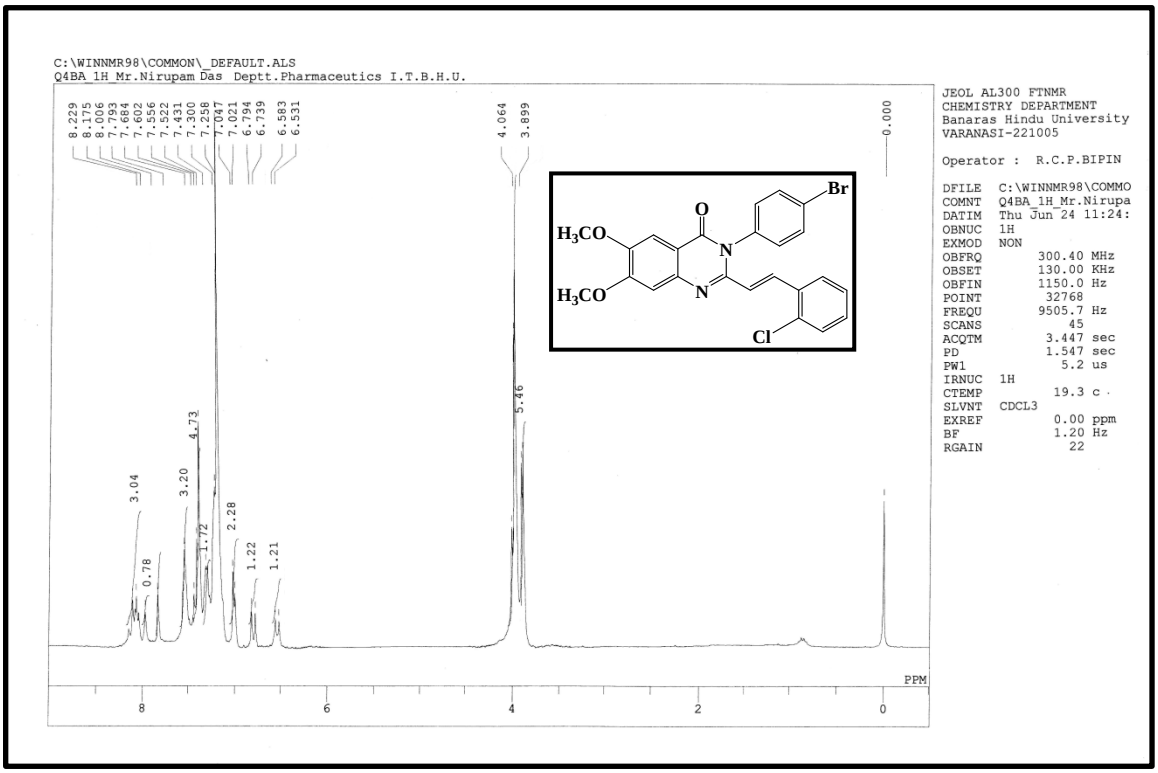

Figure 4A.36. ^{1}H NMR spectrum of 2–(2–chlorostyryl)–3–(4–bromophenyl)–6,7–dimethoxyquinazolin–4(3*H*)–one (**5i**)

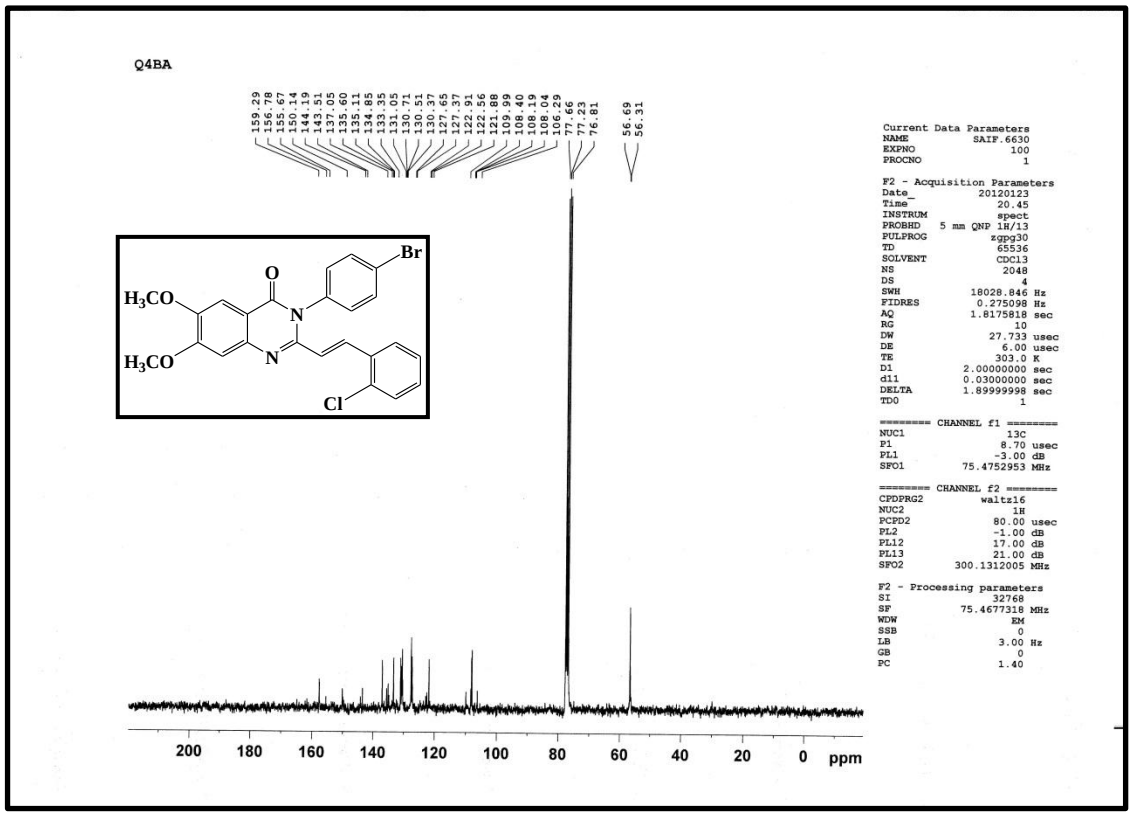

Figure 4A.37. ^{13}C NMR spectrum of 2–(2–chlorostyryl)–3–(4–bromophenyl)–6,7–dimethoxyquinazolin–4(3*H*)–one (**5i**)

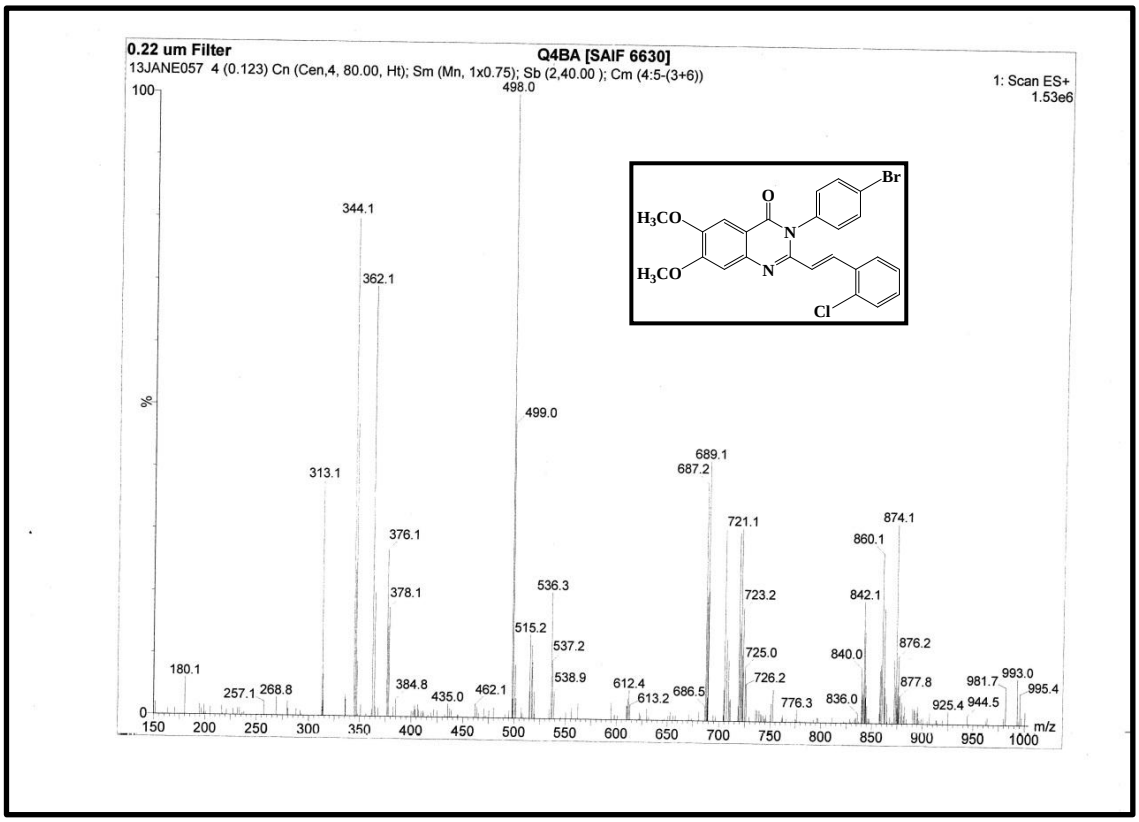

Figure 4A.38. Mass spectrum of 2–(2–chlorostyryl)–3–(4–bromophenyl)–6,7–dimethoxyquinazolin–4(3*H*)–one (**5i**)

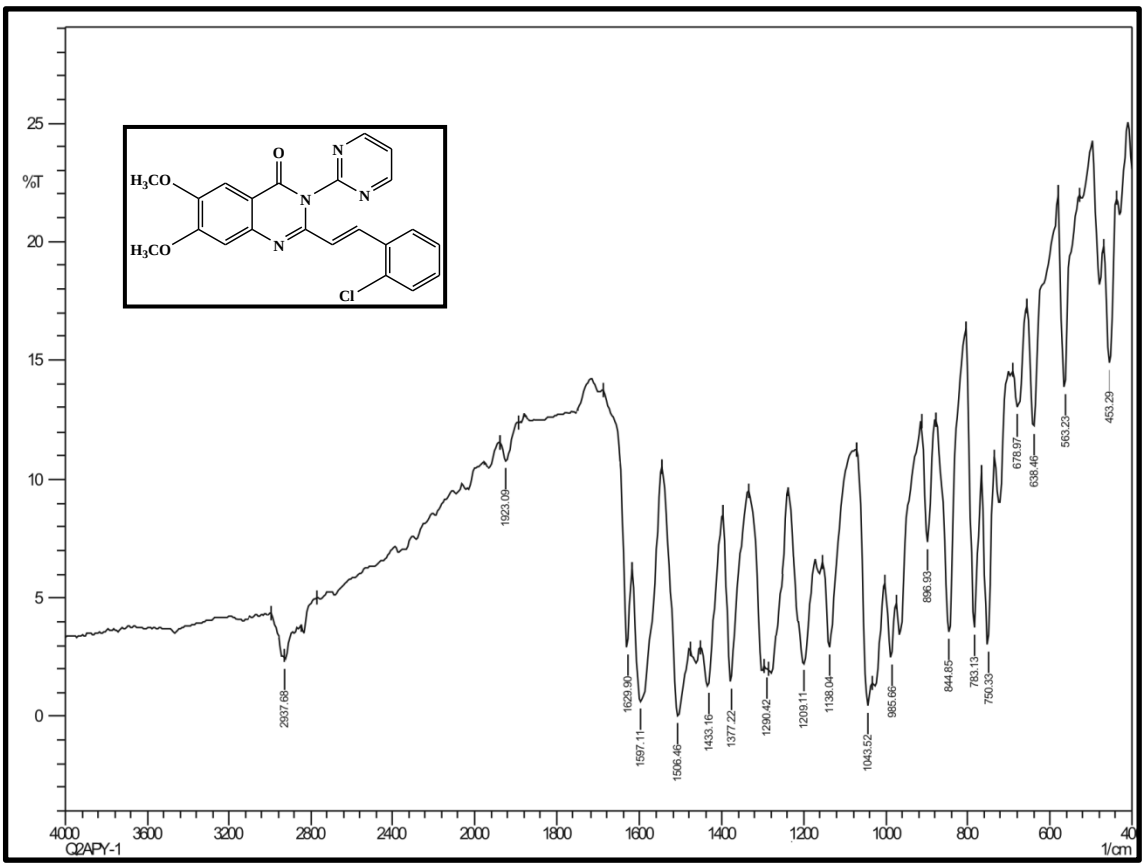

Figure 4A.39. FT–IR spectrum of 2–(2–chlorostyryl)–6,7–dimethoxy–3–(pyrimidin–2–yl)quinazolin–4(3*H*)–one (**5j**)

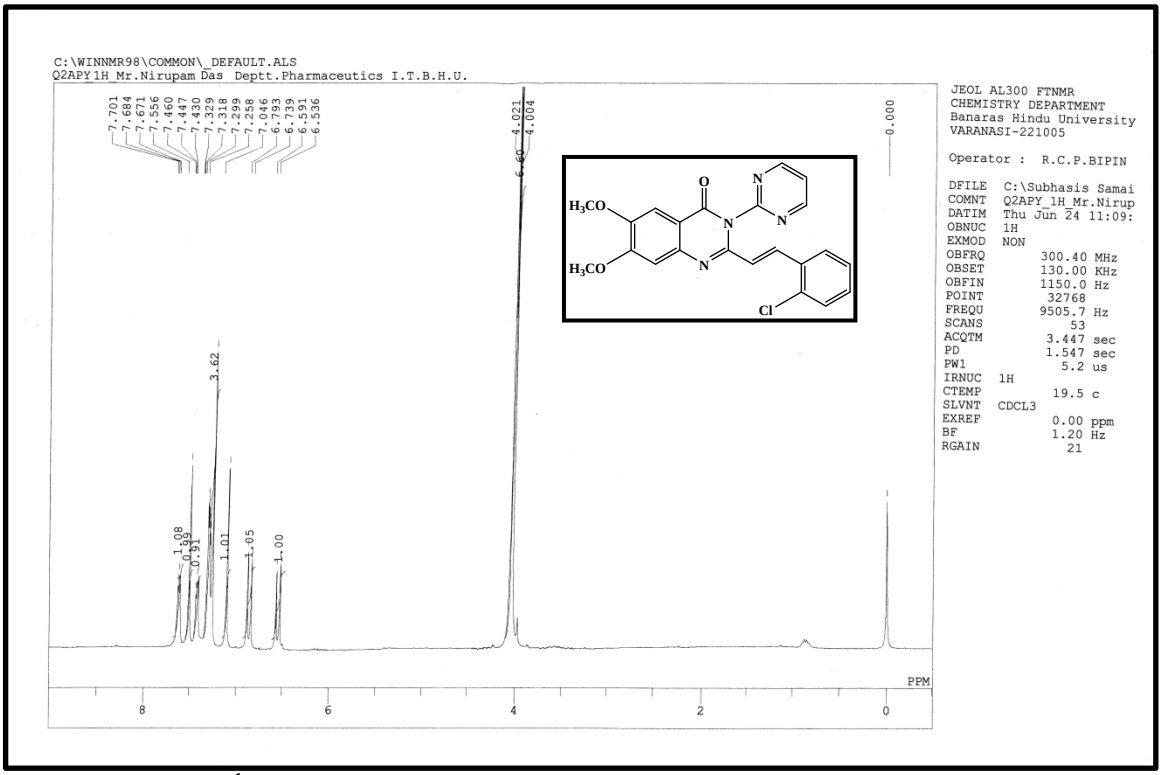

Figure 4A.40. ^{1}H NMR spectrum of 2–(2–chlorostyryl)–6,7–dimethoxy–3–(pyrimidin–2–yl)quinazolin–4(3*H*)–one (**5j**)

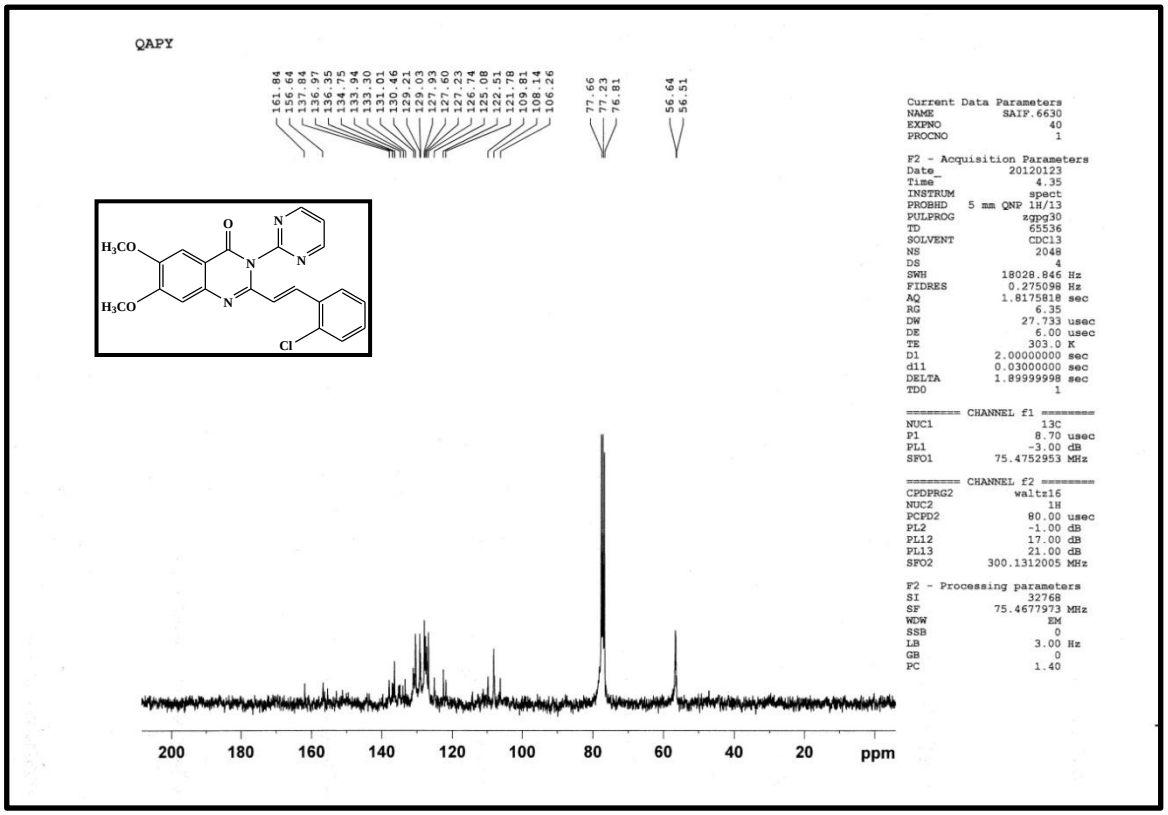

Figure 4A.41. ^{13}C NMR spectrum of 2–(2–chlorostyryl)–6,7–dimethoxy–3–(pyrimidin–2–yl)quinazolin–4(3*H*)–one (**5j**)

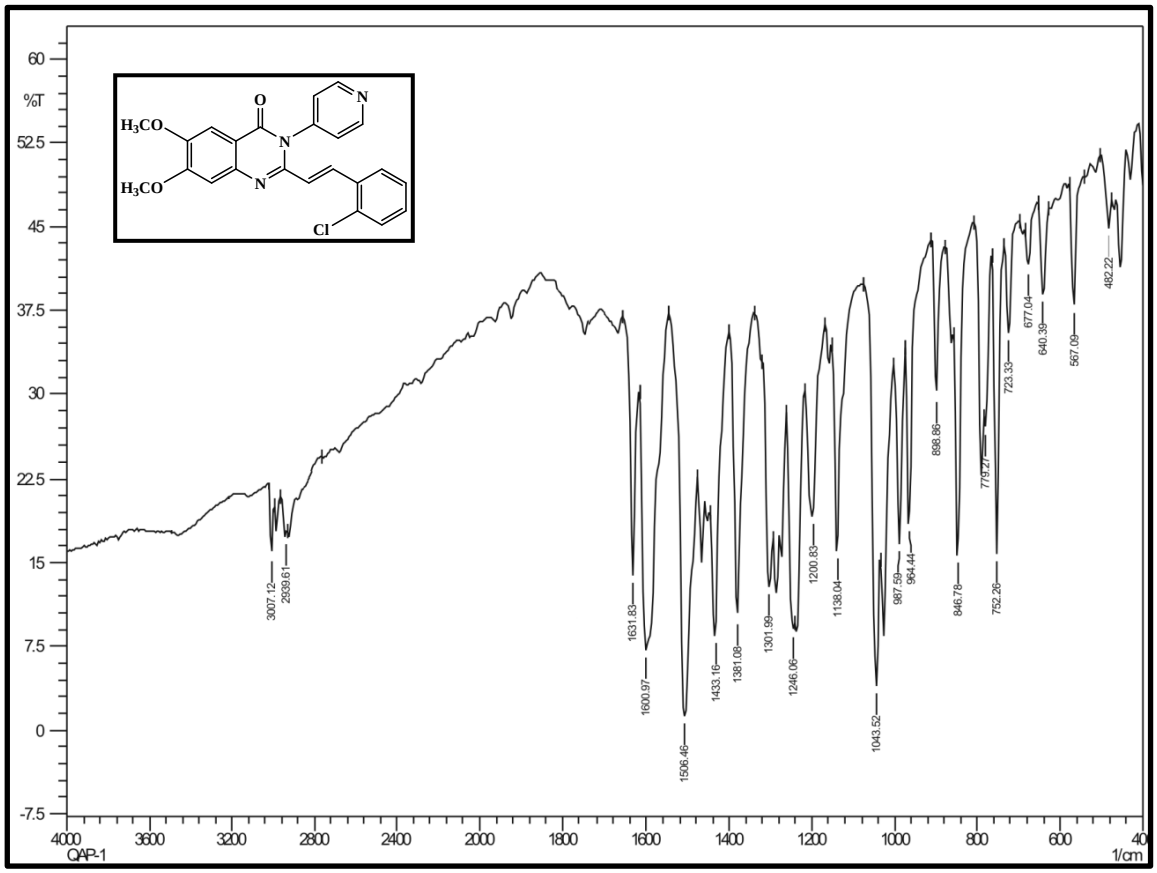

Figure 4A.42. FT–IR spectrum of 2–(2–chlorostyryl)–6,7–dimethoxy–3–(pyridin–4–yl)quinazolin–4(3*H*)–one (**5k**)

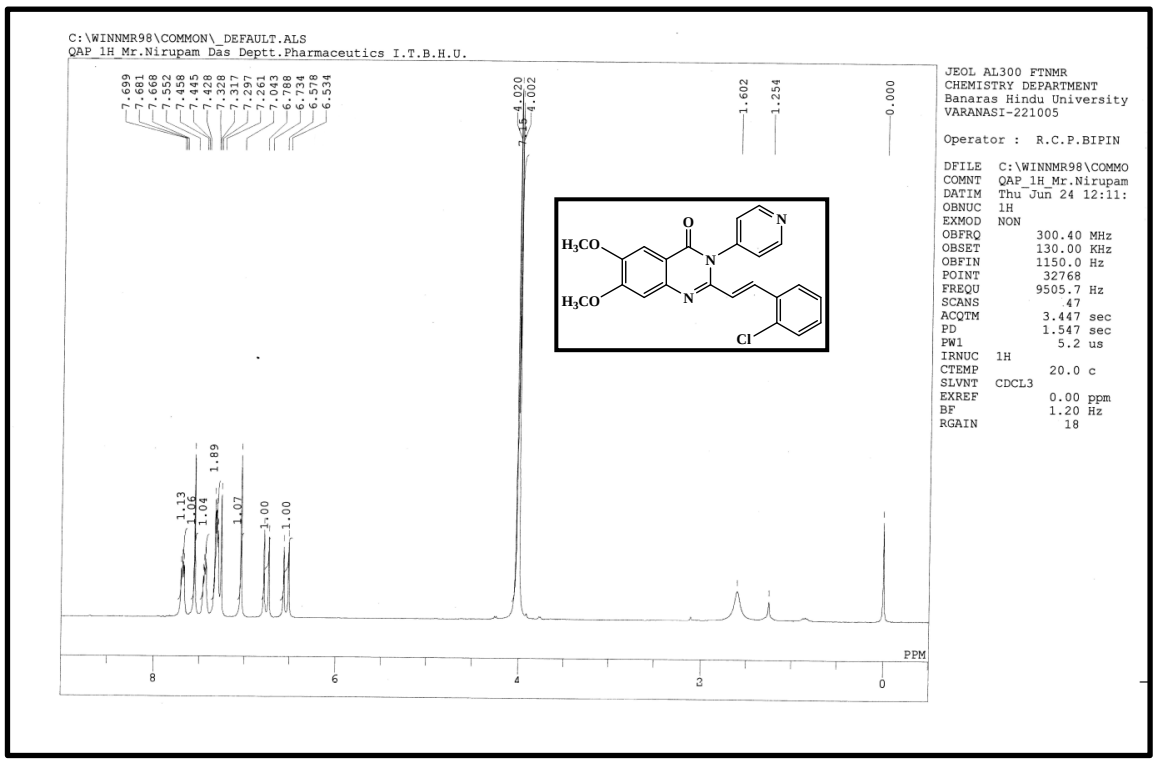

Figure 4A.43. ^{1}H NMR spectrum of 2–(2–chlorostyryl)–6,7–dimethoxy–3–(pyridin–4–yl)quinazolin–4(3*H*)–one (**5k**)

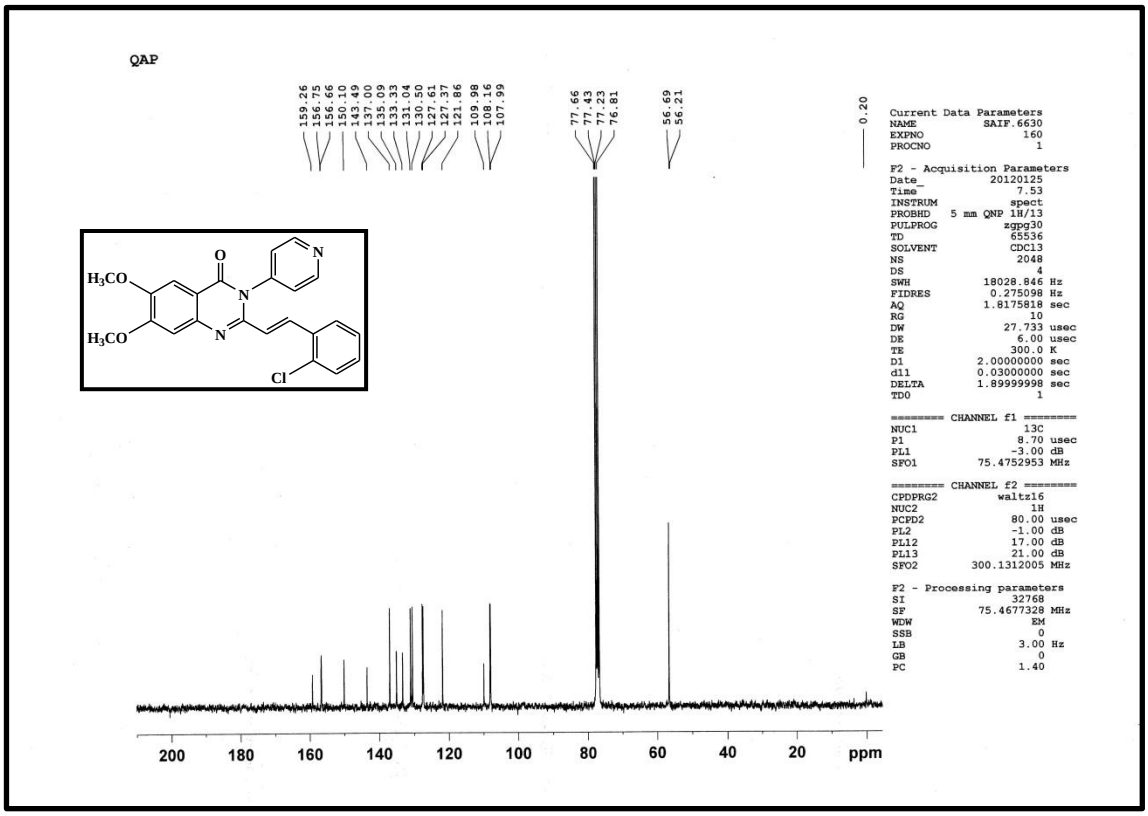

Figure 4A.44. ^{13}C NMR spectrum of 2–(2–chlorostyryl)–6,7–dimethoxy–3–(pyridin–4–yl)quinazolin–4(3*H*)–one (**5k**)

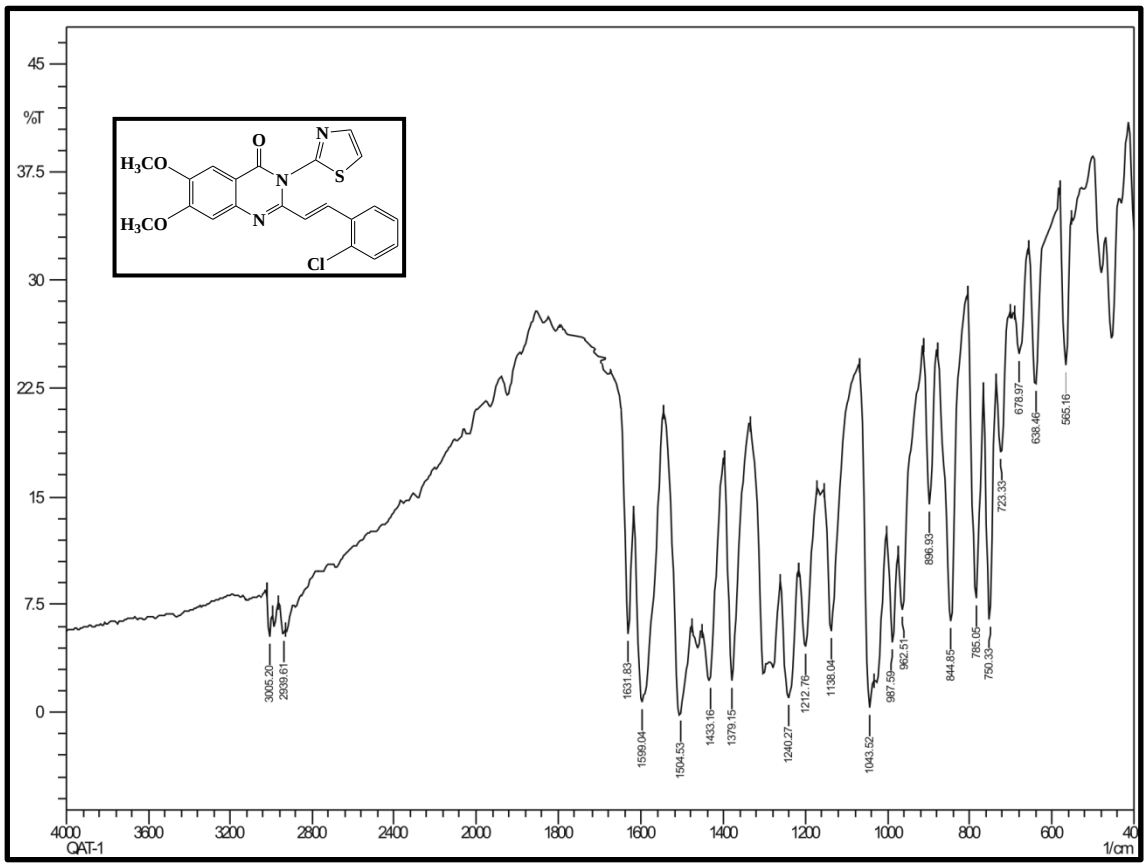

Figure 4A.45. FT–IR spectrum of 2–(2–chlorostyryl)–6,7–dimethoxy–3–(thiazol–2–yl)quinazolin–4(3*H*)–one **(5l)**

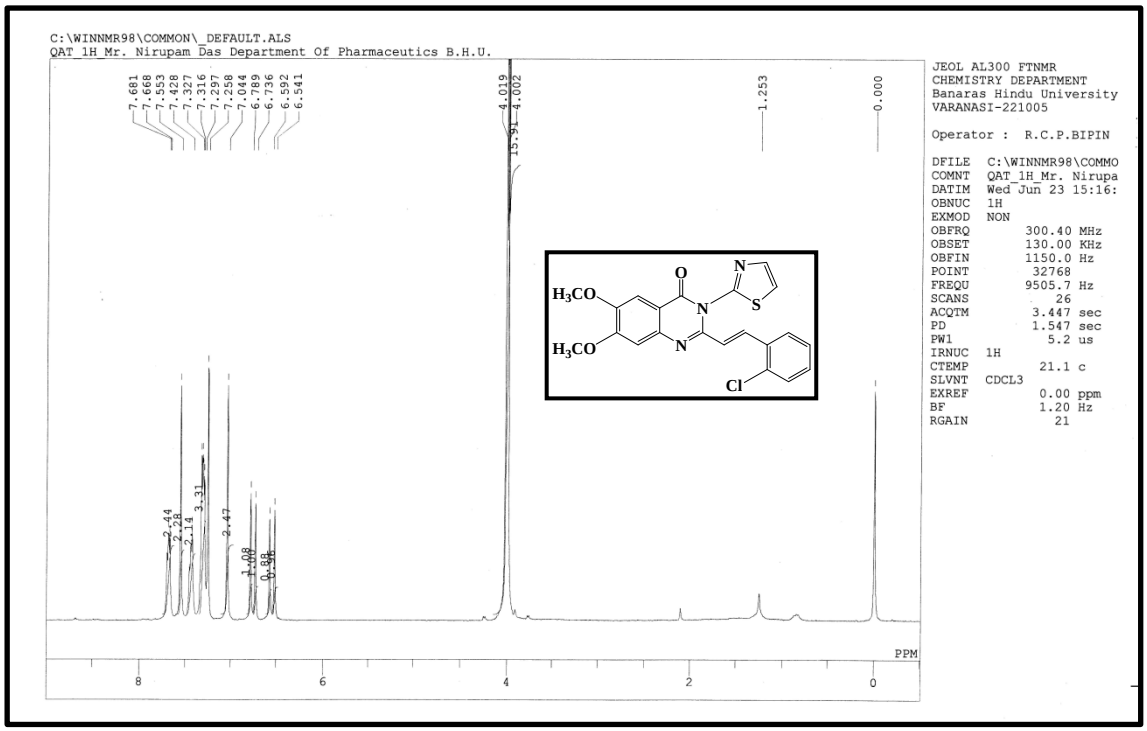

Figure 4A.46. ^{1}H NMR spectrum of 2–(2–chlorostyryl)–6,7–dimethoxy–3–(thiazol–2–yl)quinazolin–4(3*H*)–one **(5l)**

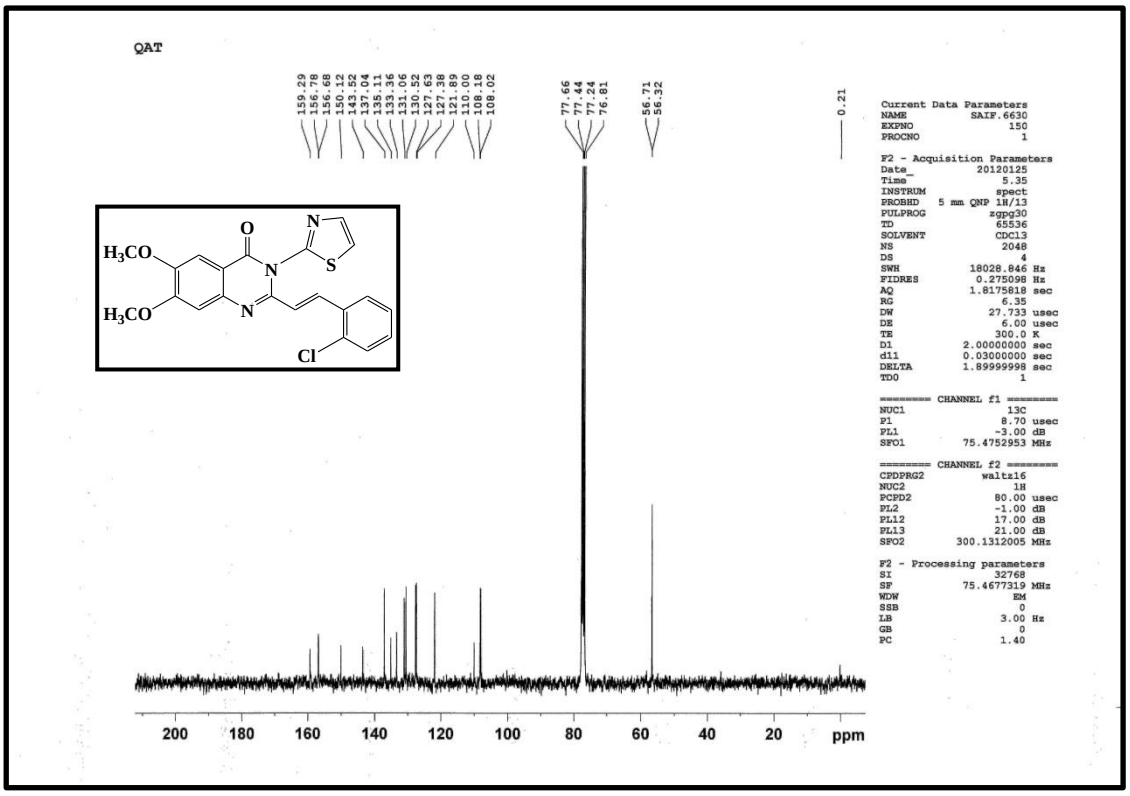

Figure 4A.47. ^{13}C NMR spectrum of 2–(2–chlorostyryl)–6,7–dimethoxy–3–(thiazol–2–yl)quinazolin–4(3*H*)–one **(5l)**

EXPERIMENTAL – PART – 1
(SECTION – B)

4B.1. Synthesis of *N*3 aryl/heteroaryl substituted 2–((benzyloxy and phenylthio) methyl) 6,7–dimethoxyquinazolin–4(3*H*)–ones (Series-II)

4B.1.1. Chemical and Reagents

All reagents and solvents used in the study were of analytical grade and were procured from Sigma–Aldrich (India), Merck (Germany) and SD fine Chemicals (India).

4B.1.2. Method and Preparation

The synthetic route and reaction mechanism of *N*3 aryl/heteroaryl substituted 2–((benzyloxy and phenylthio) methyl) 6,7–dimethoxyquinazolin–4(3*H*)–ones are illustrated in Figure 4B.1 and Figure 4A.2.

4B.1.2.1. Procedure for the synthesis of *N*3 aryl/heteroaryl substituted 2–((benzyloxy and phenylthio) methyl) 6,7–dimethoxyquinazolin–4(3*H*)–ones

- **General procedure for the synthesis of 2–(2–(benzyloxy or phenylthio)acetamido)–4,5–dimethoxybenzoic acid (4–5):**
  2–(Benzyloxy or phenylthio) acetyl chloride (**2**–**3**) (0.05 mol) were added to a solution of anthranilic acid (1) (0.05 mol) in pyridine (50 mL) and the reaction mixture was stirred at room temperature for 3 h. The mixture was then poured into 10% cold dilute hydrochloric acid solution (50 mL). The solid obtained was then filtered followed by washing several times with cold water. The product 2–(2–(benzyloxy or phenylthio)acetamido)–4,5–dimethoxybenzoic acid (**4**–**5**) were dried and crystallized from absolute ethanol.

- **General procedure for the synthesis of 2–((benzyloxy or phenylthio)methyl)–6,7–dimethoxy–4*H*–benzo[*d*][1,3]oxazin–4–one (6–7):**
  A mixture of 2–(2–(benzyloxy or phenylthio)acetamido)–4,5–dimethoxybenzoic acid (**4**–**5**) (0.03 mol) and acetic anhydride (0.3 mol) was heated under reflux in an oil bath at 180 °C for 4 h. The reaction mixture was subsequently washed with water (3X30 mL) and then extracted with chloroform (2X50 mL), dried over Na_2SO_4 and evaporated to give the crude product, 2–

((benzyloxy or phenylthio)methyl)–6,7–dimethoxy–4*H*–benzo[*d*][1,3]oxazin–4–one (**6**–**7**). The residue obtained was then triturated with petroleum ether (40–60), dried and crystallized from toluene.

- **General procedure for the synthesis of compounds 8a–8l**

  A mixture of 2–((benzyloxy or phenylthio)methyl)–6,7–dimethoxy–4*H*–benzo[*d*][1,3]oxazin–4–one (**6**–**7**) (0.01 mol) and an appropriate aryl/heteroaryl amine in glacial acetic acid (10 mL) were refluxed at 210 °C in an oil bath for 3 h. The reaction mixture obtained was then poured into crushed ice and left overnight. The solid separated out was filtered, washed thoroughly with cold water, dried and crystallized from chloroform to obtain the product (**8a**–**8l**).

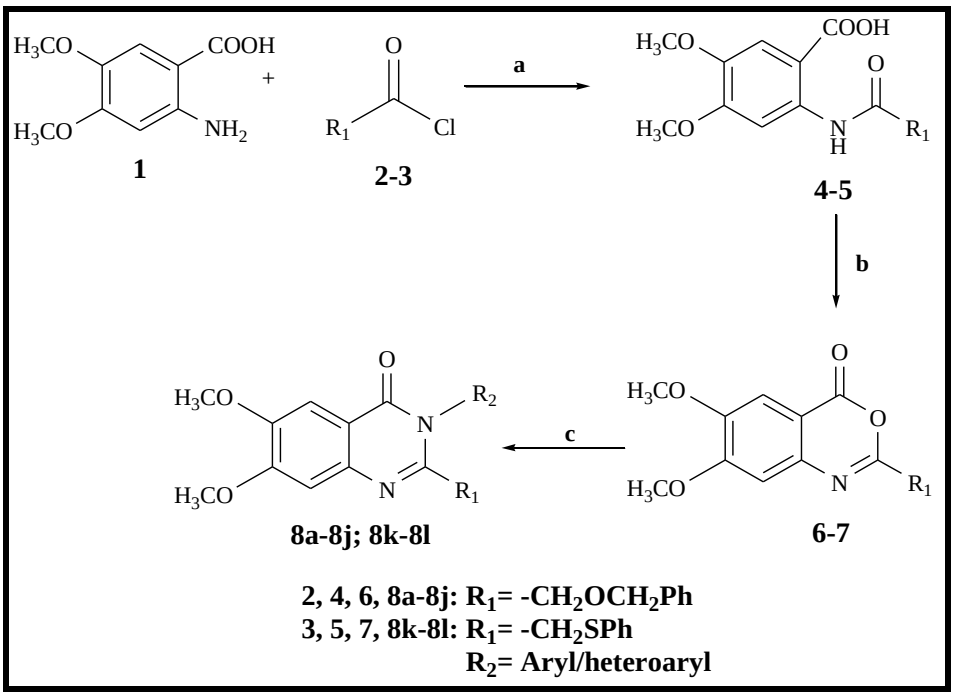

Figure 4B.1. General scheme for the synthesis of compounds **8a**–**8l**. Reagents and conditions: (a) Pyridine, stir, r. t., 3h; (b) Ac_2O, reflux, 180 °C, 4h; (c) Substituted aryl/heteroaryl amine, glacial acetic acid, 210 °C, reflux, 3h.

4B.2. Characterization of *N*3 aryl/heteroaryl substituted 2–((benzyloxy and phenylthio) methyl) 6,7–dimethoxyquinazolin–4(3*H*)–ones

The identification and characterization of the synthesized compounds were carried out by physicochemical characterization (melting point, solubility, R_f value and Log P), spectroscopic studies (FT–IR, ^{1}H NMR) and elemental analysis. Mass spectra were recorded for representative compounds.

4B.2.1. Physicochemical characterization

Table 4B.1. and Table 4B.2. presents the physicochemical data of synthesized compounds.

Table 4B.1. List of the synthesized compounds and physicochemical data (**8a–8l**)

Sl. No.	Code	R_2	Melting Point (°C)	Yield (%)	R_f^*
1	8a	*m*–tolyl	216–218	65.35	0.61
2	8b	2,4–dimethylphenyl	220–221	72.38	0.64
3	8c	3–methoxyphenyl	205–206	79.63	0.58
4	8d	2–methoxyphenyl	214–216	71.56	0.67
5	8e	benzyl	227–229	64.36	0.53
6	8f	4–nitrophenyl	211–213	73.82	0.56
7	8g	4–bromophenyl	235–236	76.51	0.51
8	8h	pyridin–4–yl	227–229	72.93	0.63
9	8i	pyrimidin–2–yl	231–233	67.55	0.66
10	8j	thiazol–2–yl	218–220	70.53	0.60
11	8k	4–nitrophenyl	231–233	66.45	0.65
12	8l	3–methoxyphenyl	223–225	68.74	0.68

*__Solvent system:__ ethyl acetate/hexane (2:3)

Table 4B.2. Partition coefficient and solubility of synthesized compounds (**8a–8l**)

Code	Calculated Log P	Log P	Water	Ethanol	Ethylacetate	Chloroform	Hexane
8a	4.21	2.80	–	–	++	+++	–
8b	4.51	2.61	–	–	++	+++	++
8c	3.92	2.64	–	++	+++	+++	–
8d	3.99	2.66	–	–	++	+++	++
8e	3.87	2.53	++	++	+++	+++	++
8f	3.24	2.24	++	++	+++	+++	++
8g	4.63	2.70	–	–	–	+++	–
8h	3.06	2.52	–	–	++	+++	–
8i	2.35	2.54	++	–	++	+++	–
8j	3.08	2.56	++	++	+++	+++	++
8k	3.68	2.83	–	–	++	+++	++
8l	3.45	2.27	–	–	++	+++	++

++Sparingly soluble, +++ soluble, –insoluble

4B.2.2. Spectral characterization and elemental analysis

2–(2–(Benzyloxy)acetamido)–4,5–dimethoxybenzoic acid (4)

IR (KBr, cm^{-1}): 3415 (–NH str); 3081 (–OH str –COOH); 2941 (methoxy C–H str); 1725 (–C=O str –COOH); 1681 (amide –C=O str); 1120 (–C–O–C str). ^{1}H NMR (300

MHz, $CDCl_3$): δ 11.34 (s, 1H, OH); δ 8.55 (s, 1H, –NH D_2O exchangeable); δ 7.59–7.31 (m, 5H, Ar–H); δ 7.28 (s, 1H, Ar–H); δ 7.25 (s, 1H, Ar–H); δ 4.71 (s, 2H, $–CH_2$); δ 4.14 (s, 2H, $–CH_2$); δ 3.98 (s, 3H, $–OCH_3$); δ 3.88 (s, 3H, $–OCH_3$). Elemental analysis, for $C_{18}H_{19}NO_6$, calcd. (%): 62.60 C, 5.55 H, 4.06 N; Found (%): 62.49 C, 5.56 H, 4.05 N.

2–(2–(Phenylthio)acetamido)–4,5–dimethoxybenzoic acid (5)

IR (KBr, cm^{-1}): 3355 (–NH str); 3087 (–OH str –COOH); 2946 (methoxy C–H str); 1718 (–C=O str –COOH); 1683 (amide –C=O str). ^1H NMR (300 MHz, $CDCl_3$): δ 11.23 (s, 1H, OH); δ 8.34 (s, 1H, –NH D_2O exchangeable); δ 7.58–7.33 (m, 5H, Ar–H); δ 7.29 (s, 1H, Ar–H); δ 7.25 (s, 1H, Ar–H); δ 3.94 (s, 3H, $–OCH_3$); δ 3.88 (s, 3H, $–OCH_3$); δ 3.69 (s, 2H, $–CH_2$). Elemental analysis, for $C_{17}H_{17}NO_5S$, calcd. (%): 58.78 C, 4.93 H, 4.03 N; Found (%): 58.91 C, 4.94 H, 4.02 N.

2–((Benzyloxy) methyl)–6,7–dimethoxy–4*H*–benzo[*d*][1,3]oxazin–4–one (6)

IR (KBr, cm^{-1}): 2941 (methoxy C–H str); 1737 (–C=O str cyclic ester); 1602 (–C=N str); 1125 (–COC– str). ^1H NMR (300 MHz, $CDCl_3$): δ 7.59–7.32 (m, 5H, Ar–H); δ 7.28 (s, 1H, Ar–H); δ 7.25 (s, 1H, Ar–H); δ 4.71 (s, 2H, $–CH_2$); δ 4.13 (s, 2H, $–CH_2$); δ 3.98 (s, 3H, $–OCH_3$); δ 3.89 (s, 3H, $–OCH_3$). Elemental analysis, for $C_{18}H_{17}NO_5$, calcd. (%): 66.05 C, 5.23 H, 4.28 N; Found (%): 66.27 C, 5.23 H, 4.27 N.

6,7–Dimethoxy–2–((phenylthio) methyl)–4*H*–benzo[*d*][1,3]oxazin–4–one (7)

IR (KBr, cm^{-1}): 2923 (methoxy C–H str); 1735 (–C=O str cyclic ester); 1606 (–C=N str). ^1H NMR (300 MHz, $CDCl_3$): δ 7.57–7.33 (m, 5H, Ar–H); δ 7.29 (s, 1H, Ar–H); δ 7.25 (s, 1H, Ar–H); δ 3.92 (s, 3H, $–OCH_3$); δ 3.88 (s, 3H, $–OCH_3$); δ 3.71 (s, 2H, $–CH_2$). Elemental analysis, for $C_{17}H_{15}NO_4S$, calcd. (%): 61.99 C, 4.59 H, 4.25 N; Found (%): 61.86 C, 4.60 H, 4.24 N.

2–((Benzyloxy)methyl)–6,7–dimethoxy–3–*m*–tolylquinazolin–4(3*H*)–one (8a)

IR (KBr, cm^{-1}): 2920 (methoxy C–H str); 1680 (–C=O str); 1607 (–C=N str); 1190 (C–N str); 1123 (C–O–C str). ^1H NMR (300 MHz, $CDCl_3$): δ 7.41–7.56 (m, 5H, Ar–H); δ 7.32–7.30 (m, 4H, Ar–H); δ 7.28 (s, 1H, Ar–H); δ 7.13 (s, 1H, Ar–H); δ 4.72 (s, 2H, $–CH_2$); δ 4.13 (s, 2H, $–CH_2$); δ 3.98 (s, 3H, $–OCH_3$); δ 3.89 (s, 3H, $–OCH_3$); δ 2.31 (s,

3H, –CH_3). Elemental analysis, for $C_{25}H_{24}N_2O_4$, calcd. (%): 72.10 C, 5.81 H, 6.73 N; Found (%): 71.87 C, 5.82 H, 6.71 N.

2–((Benzyloxy)methyl)–6,7–dimethoxy–3–(2,4–dimethylphenyl)quinazolin–4(3*H*)–one (8b)

IR (KBr, cm^{-1}): 2924 (methoxy C–H str); 1681 (–C=O str); 1610 (–C=N str); 1195 (C–N str); 1130 (C–O–C str). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.58–7.49 (m, 5H, Ar–H); δ 7.31–7.44 (m, 3H, Ar–H); δ 7.25 (s, 1H, Ar–H); δ 7.21 (s, 1H, Ar–H); δ 4.72 (s, 2H, –CH_2); δ 4.13 (s, 2H, –CH_2); δ 3.98 (s, 3H, –OCH_3); δ 3.88 (s, 3H, –OCH_3); δ 2.42 (s, 3H, –CH_3); δ 2.34 (s, 3H, –CH_3). MS (m/z): 431 (M+1). Elemental analysis, for $C_{26}H_{26}N_2O_4$, calcd. (%): 72.54 C, 6.09 H, 6.51 N; Found (%): 72.71 C, 6.11 H, 6.53 N.

2–((Benzyloxy)methyl)–6,7–dimethoxy–3–(3–methoxyphenyl)quinazolin–4(3*H*)–one (8c)

IR (KBr, cm^{-1}): 2939 (methoxy C–H str); 1674 (–C=O str); 1608 (–C=N str); 1198 (C–N str); 1126 (C–O–C str). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.67–7.39 (m, 5H, Ar–H); δ 7.36–7.33 (m, 4H, Ar–H); δ 7.29 (s, 1H, Ar–H); δ 7.21 (s, 1H, Ar–H); δ 4.75 (s, 2H, –CH_2); δ 4.16 (s, 2H, –CH_2); δ 3.97 (s, 3H, –OCH_3), δ 3.88 (s, 3H, –OCH_3), δ 3.85 (s, 3H, –OCH_3). MS (m/z): 433 (M+1). Elemental analysis, for $C_{25}H_{24}N_2O_5$, calcd. (%): 69.43 C, 5.59 H, 6.48 N; Found (%): 69.34 C, 5.61 H, 6.49 N.

2–((Benzyloxy)methyl)–6,7–dimethoxy–3–(2–methoxyphenyl)quinazolin–4(3*H*)–one (8d)

IR (KBr, cm^{-1}): 2941 (methoxy C–H str); 1676 (–C=O str); 1606 (–C=N str); 1192 (C–N str); 1124 (C–O–C str). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.69–7.49 (m, 5H, Ar–H); δ 7.47–7.35 (m, 4H, Ar–H); δ 7.33 (s, 1H, Ar–H); δ 7.24 (s, 1H, Ar–H); δ 4.69 (s, 2H, –CH_2); δ 4.12 (s, 2H, –CH_2); δ 3.96 (s, 3H, –OCH_3); δ 3.86 (s, 3H, –OCH_3); δ 3.84 (s, 3H, –OCH_3). Elemental analysis, for $C_{25}H_{24}N_2O_5$, calcd. (%): 69.43 C, 5.59 H, 6.48 N; Found (%): 69.45 C, 5.57 H, 6.46 N.

2–((Benzyloxy)methyl)–6,7–dimethoxy–3–(benzyl)quinazolin–4(3*H*)–one (8e)

IR (KBr, cm^{-1}): 2943 (methoxy C–H str); 2860 (–CH str methylene); 1681 (–C=O str); 1608 (–C=N str); 1196 (C–N str); 1122 (C–O–C str). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.68–7.34 (m, 10H, Ar–H); δ 7.32 (s, 1H, Ar–H); δ 7.25 (s, 1H, Ar–H); δ 4.71 (s, 2H, –

CH_2); δ 4.37 (s, 2H, –CH_2); δ 4.14 (s, 2H, –CH_2); δ 3.98 (s, 3H, –OCH_3); δ 3.88 (s, 3H, –OCH_3). Elemental analysis, for $C_{25}H_{24}N_2O_4$, calcd. (%): 72.10 C, 5.81 H, 6.73 N; Found (%): 72.36 C, 5.79 H, 6.75 N.

2–((Benzyloxy)methyl)–6,7–dimethoxy–3–(4–nitrophenyl)quinazolin–4(3*H*)–one (8f)

IR (KBr, cm^{-1}): 2943 (methoxy C–H str); 1683 (–C=O str); 1611 (–C=N str); 1194 (C–N str); 1125 (C–O–C str); 1342, 1531 (–N=O str). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.18–7.63 (m, 4H, Ar–H); δ 7.55–7.33 (m, 5H, Ar–H); δ 7.31 (s, 1H, Ar–H); δ 7.28 (s, 1H, Ar–H); δ 4.69 (s, 2H, –CH_2); δ 4.12 (s, 2H, –CH_2); δ 3.97 (s, 3H, –OCH_3); δ 3.86 (s, 3H, –OCH_3). MS (m/z): 448 (M+1). Elemental analysis, for $C_{24}H_{21}N_3O_6$, calcd. (%): 64.42 C, 4.73 H, 9.39 N; Found (%): 64.27 C, 4.72 H, 9.41 N.

2–(Benzyloxymethyl)–3–(4–bromophenyl)–6,7–dimethoxyquinazolin–4(3*H*)–one (8g)

IR (KBr, cm^{-1}): 2924 (methoxy C–H str); 1678 (–C=O str); 1604 (–C=N str); 1193 (C–N str); 1127 (C–O–C str). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.13–7.55 (m, 4H, Ar–H); δ 7.49–7.31 (m, 5H, Ar–H); δ 7.28 (s, 1H, Ar–H); δ 7.25 (s, 1H, Ar–H); δ 4.71 (s, 2H, –CH_2); δ 4.13 (s, 2H, –CH_2); δ 3.98 (s, 3H, –OCH_3); δ 3.86 (s, 3H, –OCH_3). Elemental analysis, for $C_{24}H_{21}BrN_2O_4$, calcd. (%): 59.89 C, 4.40 H; 5.82 N; Found (%): 59.70 C, 4.39 H, 5.83 N.

2–((Benzyloxy)methyl)–6,7–dimethoxy–3–(pyridin–4–yl)quinazolin–4(3*H*)–one (8h)

IR (KBr, cm^{-1}): 2943 (methoxy C–H str); 1681 (–C=O str); 1610 (–C=N str); 1195 (C–N str); 1131 (C–O–C str). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.78–7.63 (m, 4H, pyridine); δ 7.50–7.32 (m, 5H, Ar–H); δ 7.30 (s, 1H, Ar–H); δ 7.25 (s, 1H, Ar–H); δ 4.75 (s, 2H, –CH_2); δ 4.14 (s, 2H, –CH_2); δ 3.98 (s, 3H, –OCH_3); δ 3.87 (s, 3H, –OCH_3). Elemental analysis, for $C_{23}H_{21}N_3O_4$, calcd. (%): 68.47 C, 5.25 H, 10.42 N; Found (%): 68.64 C, 5.24 H, 10.39 N.

2–(Benzyloxymethyl)–6,7–dimethoxy–3–(pyrimidin–2–yl)quinazolin–4(3*H*)–one (8i)

IR (KBr, cm^{-1}): 2933 (methoxy C–H str); 1683 (–C=O str); 1612 (–C=N str); 1196 (C–N str); 1123 (C–O–C str). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.49–7.42 (m, 3H,

pyrimidine); δ 7.39–7.31 (m, 5H, Ar–H); δ 7.29 (s, 1H, Ar–H); δ 7.25 (s, 1H, Ar–H); δ 4.73 (s, 2H, $–CH_2$); δ 4.15 (s, 2H, $–CH_2$); δ 3.98 (s, 3H, $–OCH_3$); δ 3.88 (s, 3H, $–OCH_3$). Elemental analysis, for $C_{22}H_{20}N_4O_4$, calcd. (%): 65.34 C, 4.98 H, 13.85 N; Found (%): 65.51 C, 4.97 H, 13.81 N.

2–((Benzyloxy)methyl)–6,7–dimethoxy–3–(thiazol–2–yl)quinazolin–4(3*H*)–one (8j)

IR (KBr, cm^{-1}): 2924 (methoxy C–H str); 1674 (–C=O str); 1606 (–C=N str); 1194 (C–N str); 1125 (C–O–C str). ^1H NMR (300 MHz, $CDCl_3$): δ 7.73 (s, 1H, thiazole methine); δ 7.63 (s, 1H, thiazole methine); δ 7.62–7.21 (m, 5H, Ar–H); δ 7.09 (s, 1H, Ar–H); δ 6.94 (s, 1H, Ar–H); δ 4.71 (s, 2H, $–CH_2$); δ 4.13 (s, 2H, $–CH_2$); δ 3.98 (s, 3H, $–OCH_3$); δ 3.89 (s, 3H, $–OCH_3$). Elemental analysis, for $C_{21}H_{19}N_3O_4S$, calcd. (%): 61.60 C, 4.68 H, 10.26 N; Found (%): 61.75 C, 4.67 H, 10.28 N.

6,7–Dimethoxy–3–(4–nitrophenyl)–2–((phenylthio)methyl)quinazolin–4(3*H*)–one (8k)

IR (KBr, cm^{-1}): 2932 (methoxy C–H str); 1676 (–C=O str); 1610 (–C=N str); 1197 (C–N str); 1352, 1527 (–N=O str); 680 (thioether –C–S–C– str). ^1H NMR (300 MHz, $CDCl_3$): δ 7.67–7.48 (m, 5H, Ar–H); δ 7.46 –7.33 (m, 4H, Ar–H); δ 7.16 (s, 1H, Ar–H); δ 6.95 (s, 1H, Ar–H); δ 3.92 (s, 3H, $–OCH_3$); δ 3.89 (s, 3H, $–OCH_3$); δ 3.73 (s, 2H, $–SCH_2$). Elemental analysis, for $C_{23}H_{19}N_3O_5S$, calcd. (%): 61.46 C, 4.26 H, 9.35 N; Found (%): 61.37 C; 4.25 H; 9.33 N.

6,7–Dimethoxy–3–(3–methoxyphenyl)–2–((phenylthio)methyl)quinazolin–4(3*H*)–one (8l)

IR (KBr, cm^{-1}): 2927 (methoxy C–H str); 1678 (–C=O str); 1608 (–C=N str); 1195 (C–N str); 678 (thioether –C–S–C– str). ^1H NMR (300 MHz, $CDCl_3$): δ 7.66–7.36 (m, 5H, Ar–H); δ 7.35–7.19 (m, 4H, Ar–H); δ 7.17 (s, 1H, Ar–H); δ 7.09 (s, 1H, Ar–H); δ 3.96 (s, 3H, $–OCH_3$); δ 3.89 (s, 3H, $–OCH_3$); δ 3.84 (s, 3H, $–OCH_3$); δ 3.71 (s, 2H, $–SCH_2$). Elemental analysis, for $C_{24}H_{22}N_2O_4S$, calcd. (%): 66.34 C, 5.10 H, 6.45 N; Found (%): 66.13 C, 5.08 H, 6.46 N.

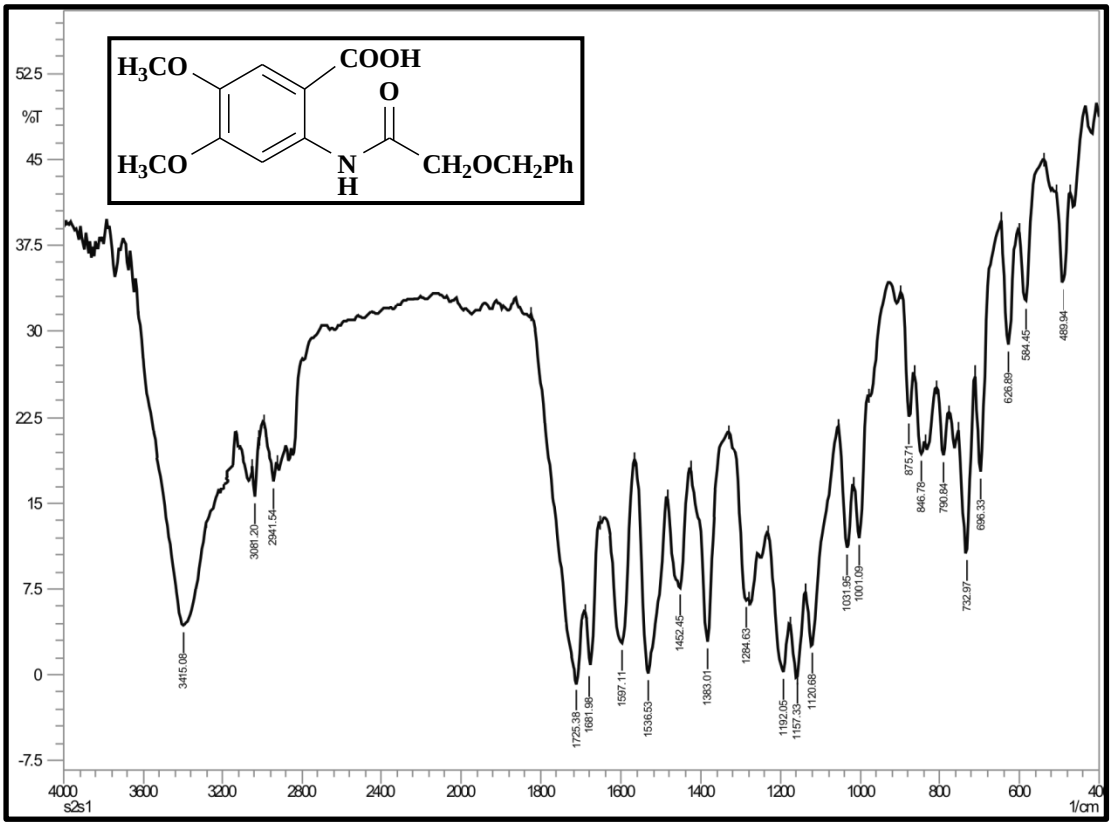

Figure 4B.2. FT–IR spectrum of 2–(2–(benzyloxy)acetamido)–4,5–dimethoxybenzoic acid (**4**)

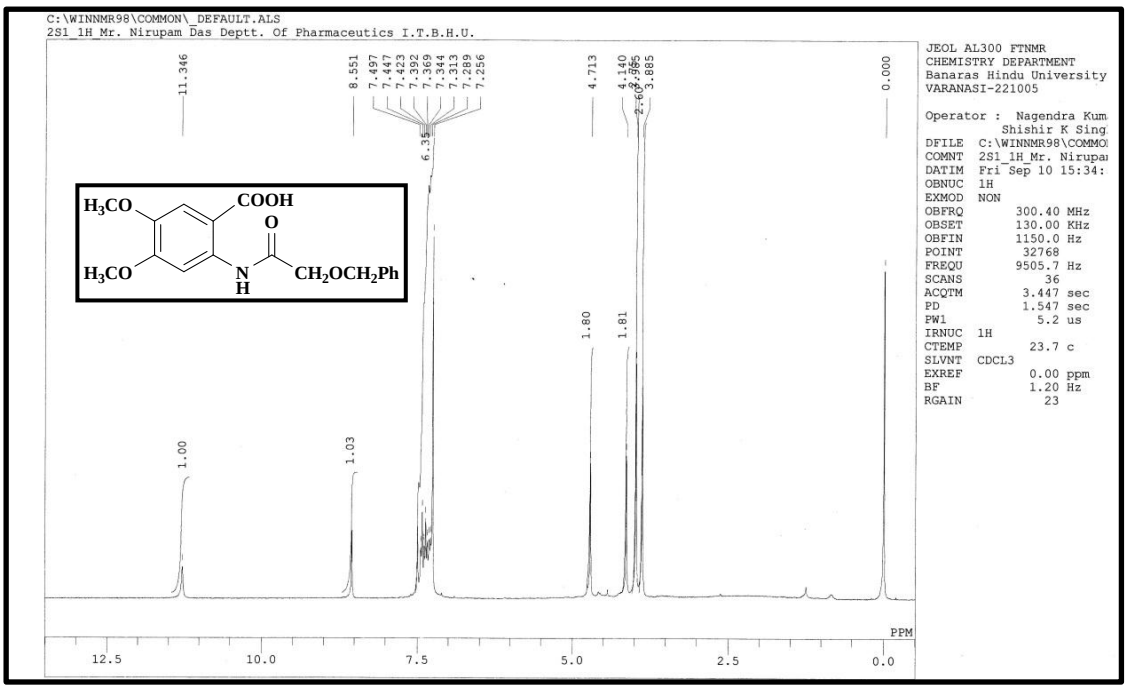

Figure 4B.3. ^{1}H NMR spectrum of 2–(2–(benzyloxy)acetamido)–4,5–dimethoxybenzoic acid (**4**)

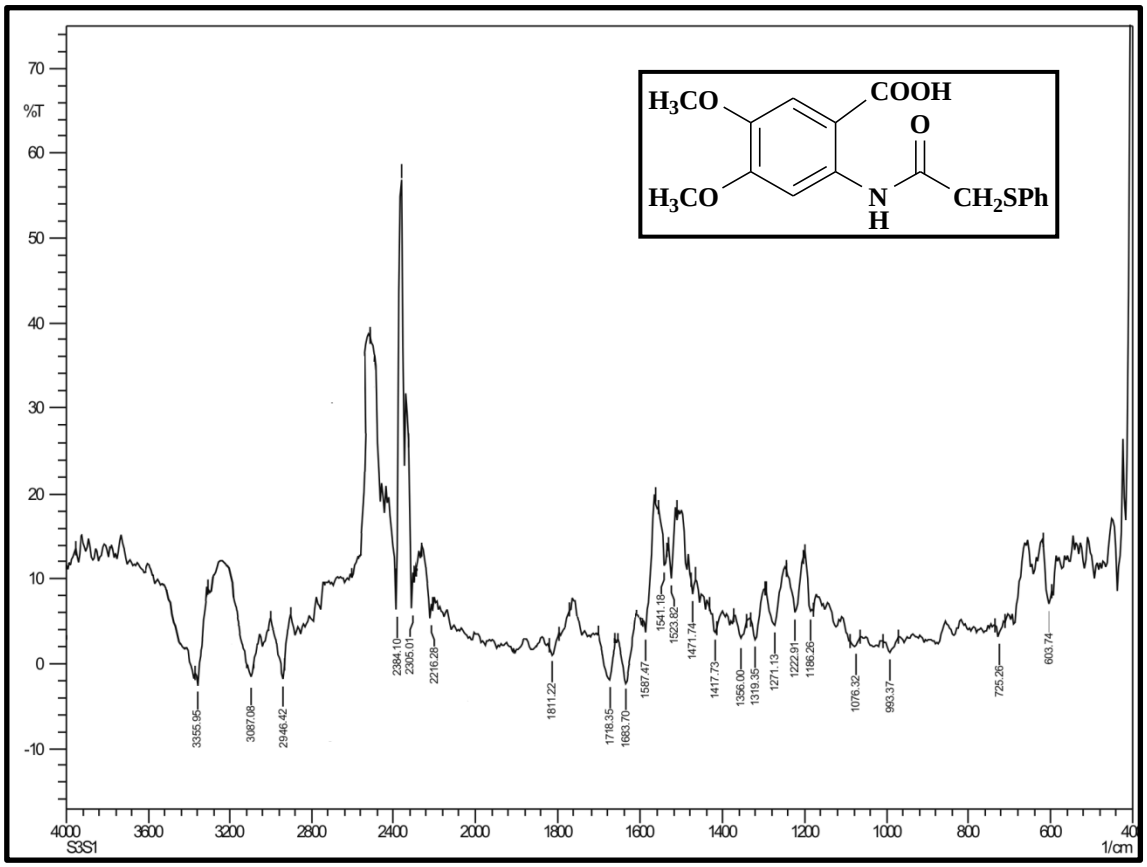

Figure 4B.4. FT–IR spectrum of 2–(2–(phenylthio)acetamido)–4,5–dimethoxybenzoic acid (**5**)

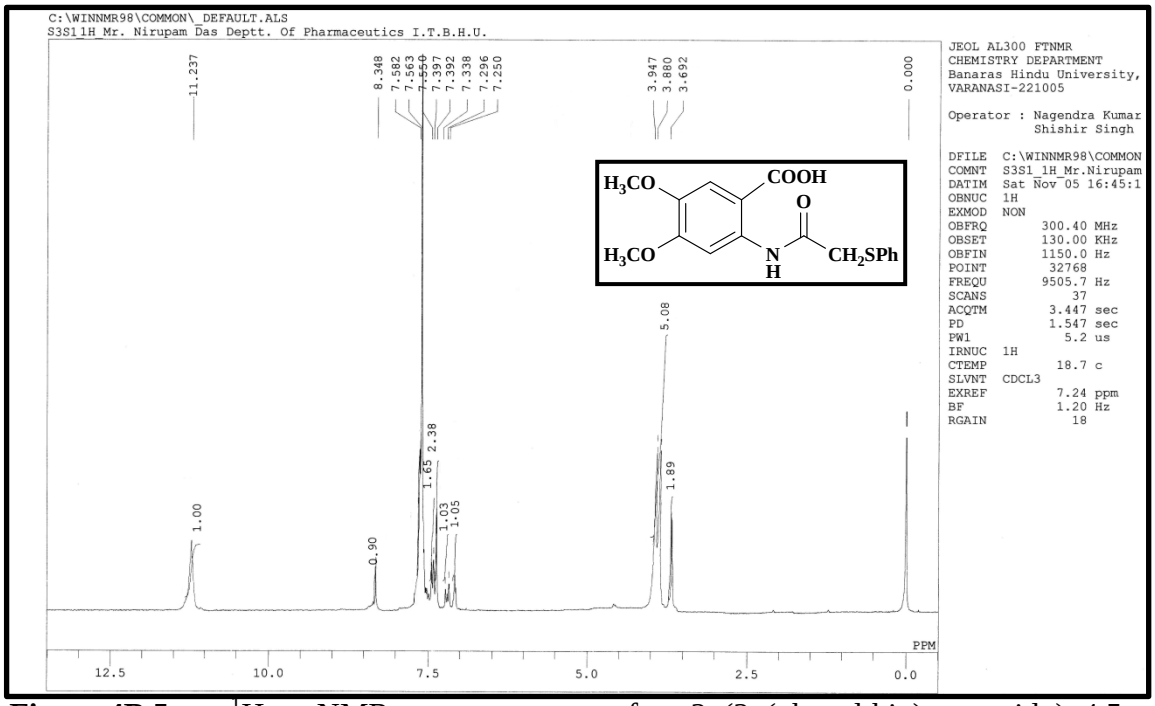

Figure 4B.5. ^{1}H NMR spectrum of 2–(2–(phenylthio)acetamido)–4,5–dimethoxybenzoic acid (**5**)

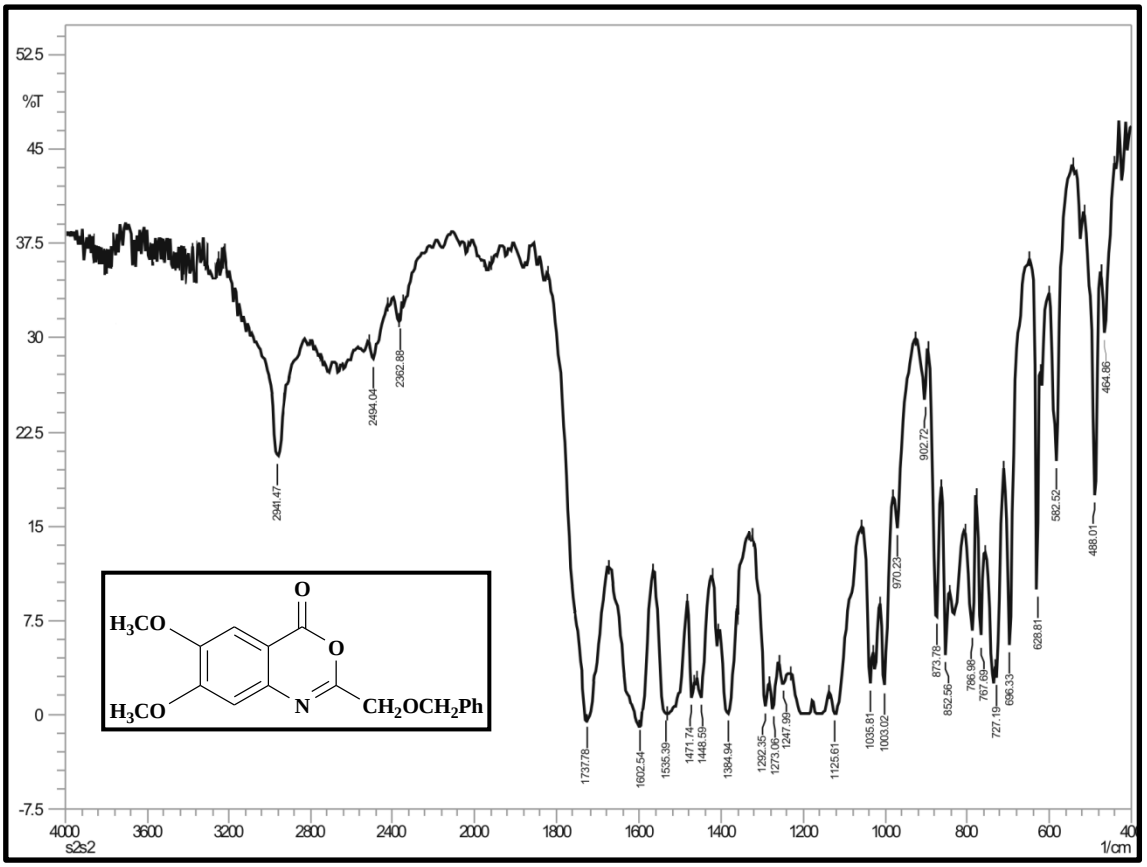

Figure 4B.6. FT–IR spectrum of 2–((benzyloxy) methyl)–6,7–dimethoxy–4*H*–benzo[*d*][1,3]oxazin–4–one (**6**)

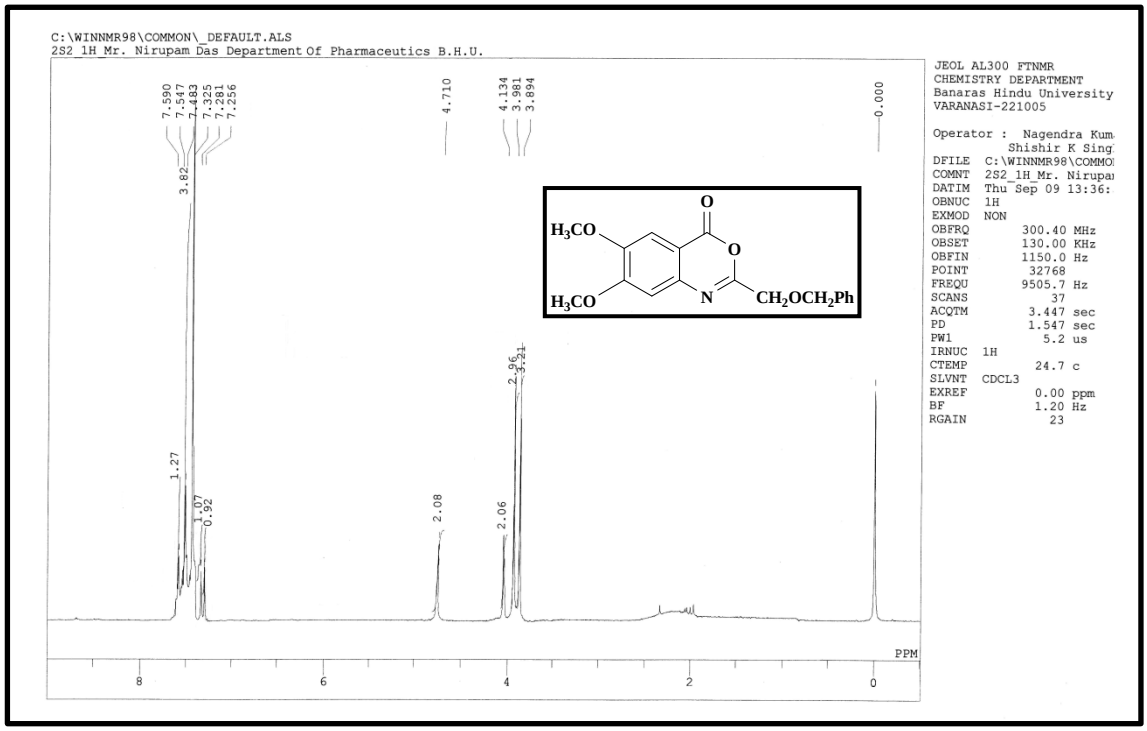

Figure 4B.7. ^{1}H NMR spectrum 2–((benzyloxy) methyl)–6,7–dimethoxy–4*H*–benzo[*d*][1,3]oxazin–4–one (**6**)

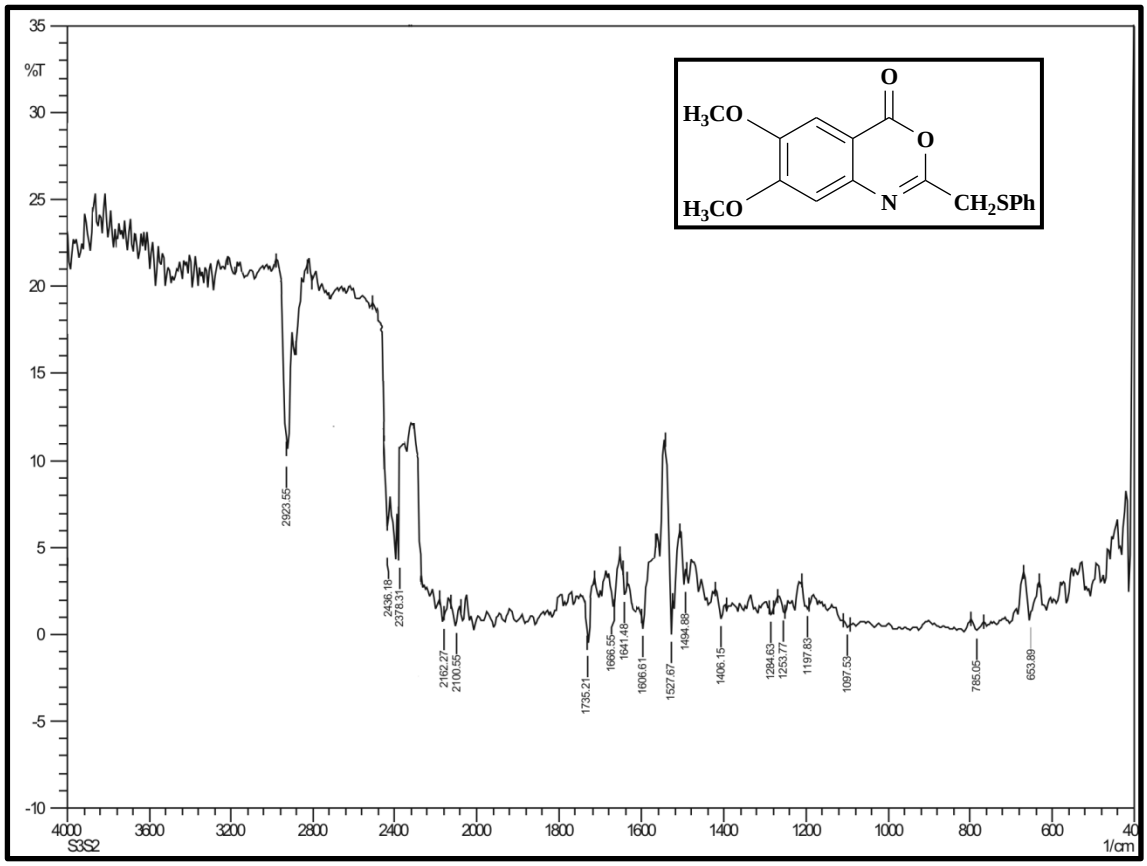

Figure 4B.8. FT–IR spectrum of 6,7–dimethoxy–2–((phenylthio) methyl)–4*H*–benzo[*d*][1,3]oxazin–4–one **(7)**

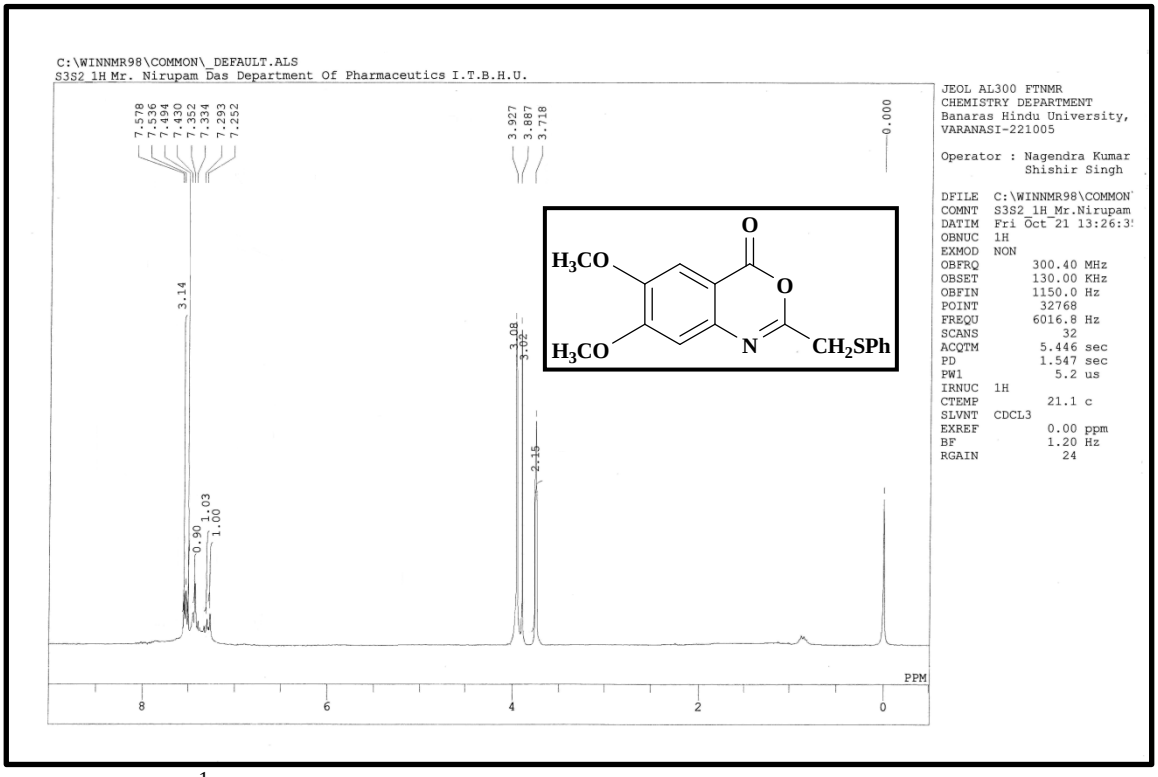

Figure 4B.9. ^{1}H NMR spectrum of 6,7–dimethoxy–2–((phenylthio) methyl)–4*H*–benzo[*d*][1,3]oxazin–4–one **(7)**

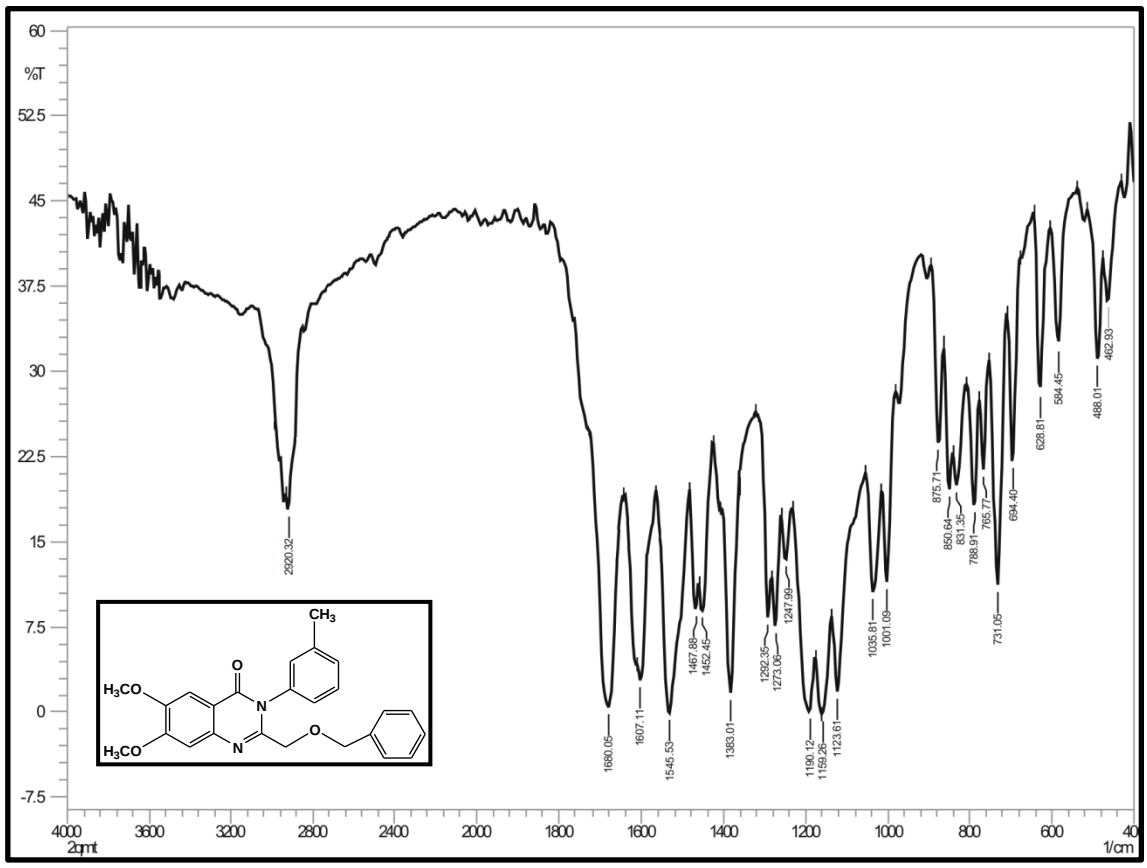

Figure 4B.10. FT–IR spectrum of 2–((benzyloxy)methyl)–6,7–dimethoxy–3–*m*–tolylquinazolin–4(3*H*)–one **(8a)**

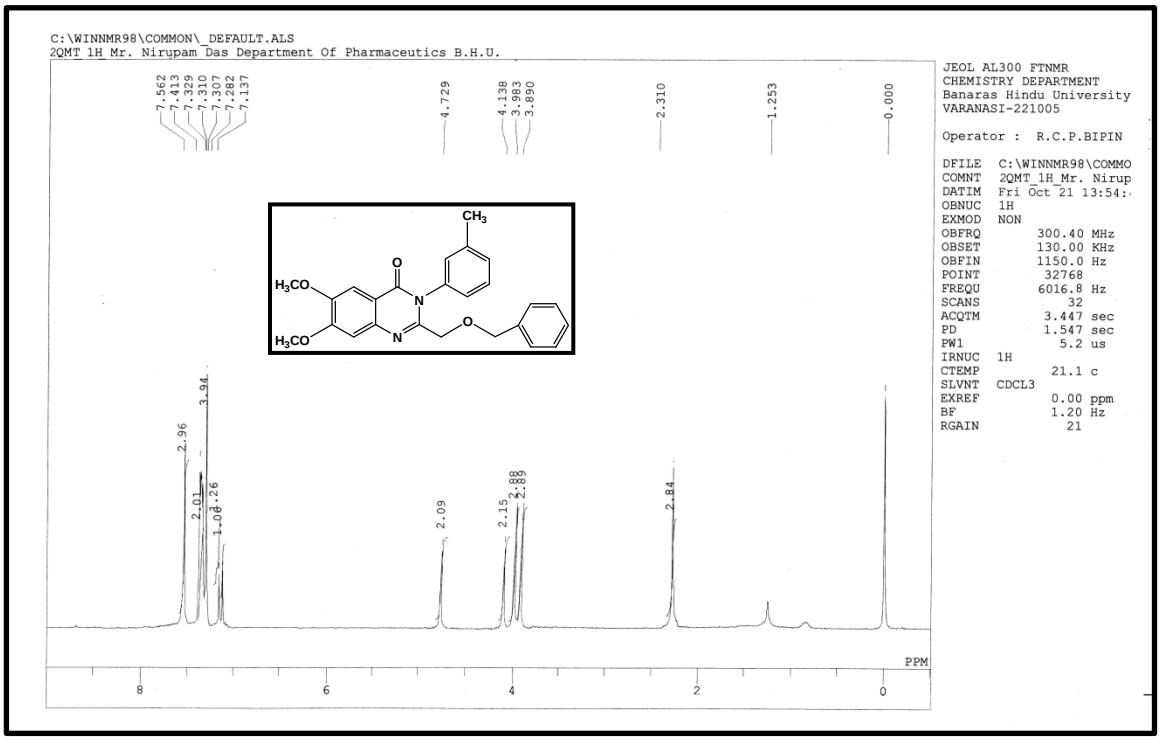

Figure 4B.11. ^{1}H NMR spectrum 2–((benzyloxy)methyl)–6,7–dimethoxy–3–*m*–tolylquinazolin–4(3*H*)–one **(8a)**

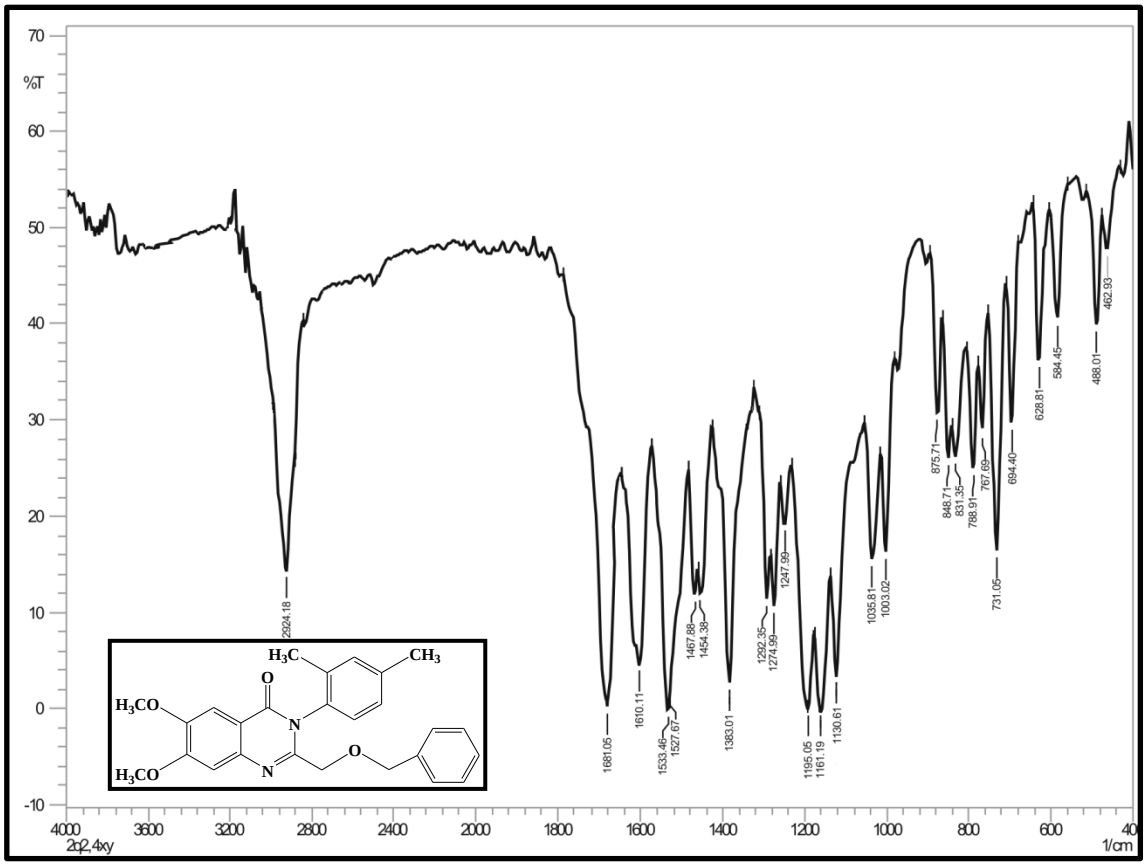

Figure 4B.12. FT–IR spectrum of 2–((benzyloxy)methyl)–6,7–dimethoxy–3–(2,4–dimethylphenyl)quinazolin–4(3*H*)–one **(8b)**

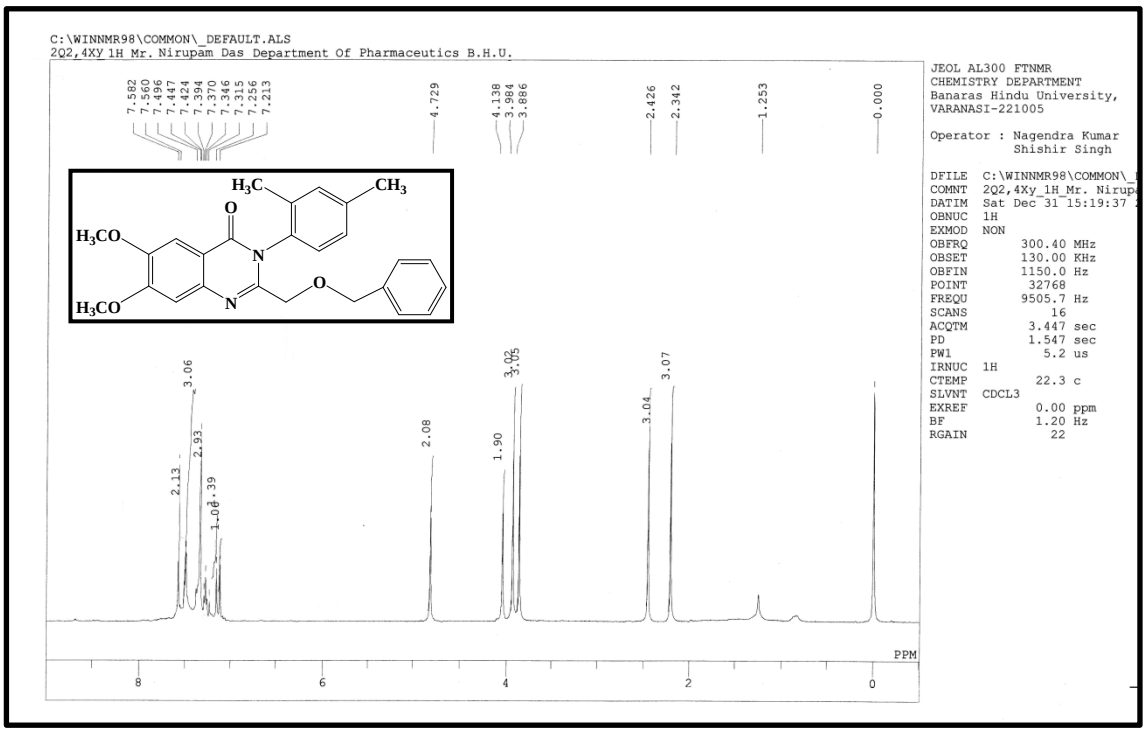

Figure 4B.13. ^{1}H NMR spectrum 2–((benzyloxy)methyl)–6,7–dimethoxy–3–(2,4–dimethylphenyl)quinazolin–4(3*H*)–one **(8b)**

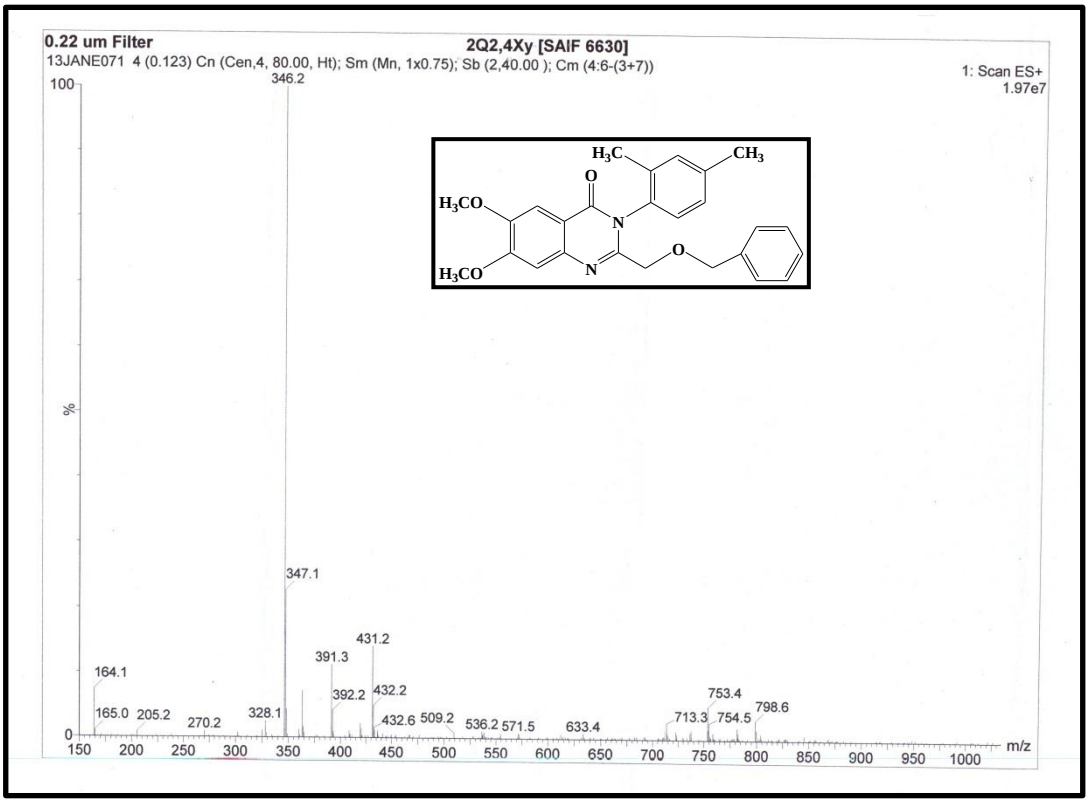

Figure 4B.14. Mass spectrum of 2–((benzyloxy)methyl)–6,7–dimethoxy–3–(2,4–dimethylphenyl)quinazolin–4(3*H*)–one **(8b)**

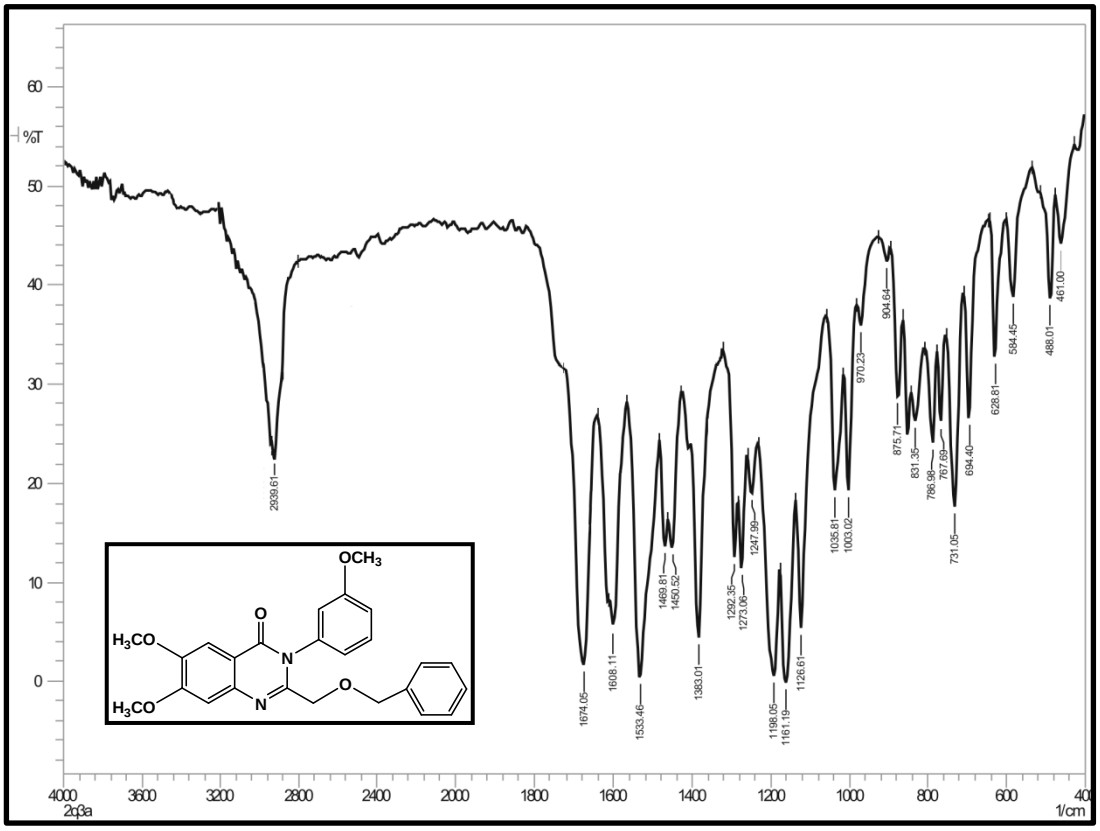

Figure 4B.15. FT–IR spectrum of 2–((benzyloxy)methyl)–6,7–dimethoxy–3–(3–methoxyphenyl)quinazolin–4(3*H*)–one **(8c)**

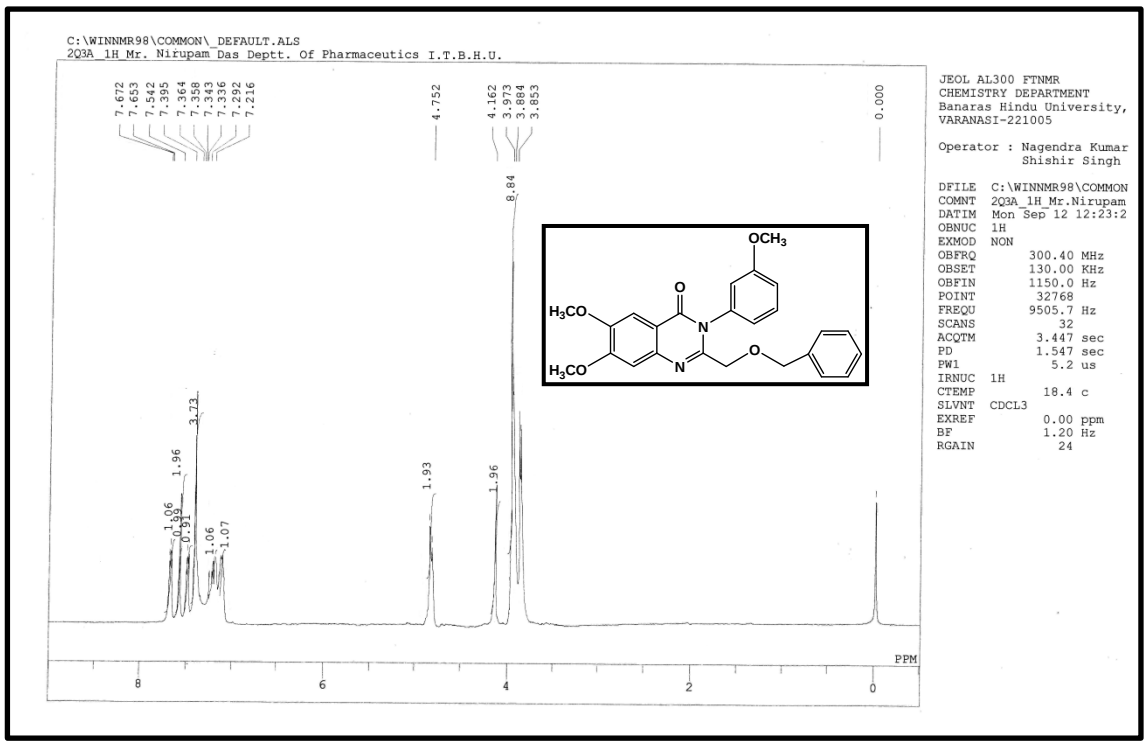

Figure 4B.16. ^{1}H NMR spectrum of 2–((benzyloxy)methyl)–6,7–dimethoxy–3–(3–methoxyphenyl)quinazolin–4(3*H*)–one **(8c)**

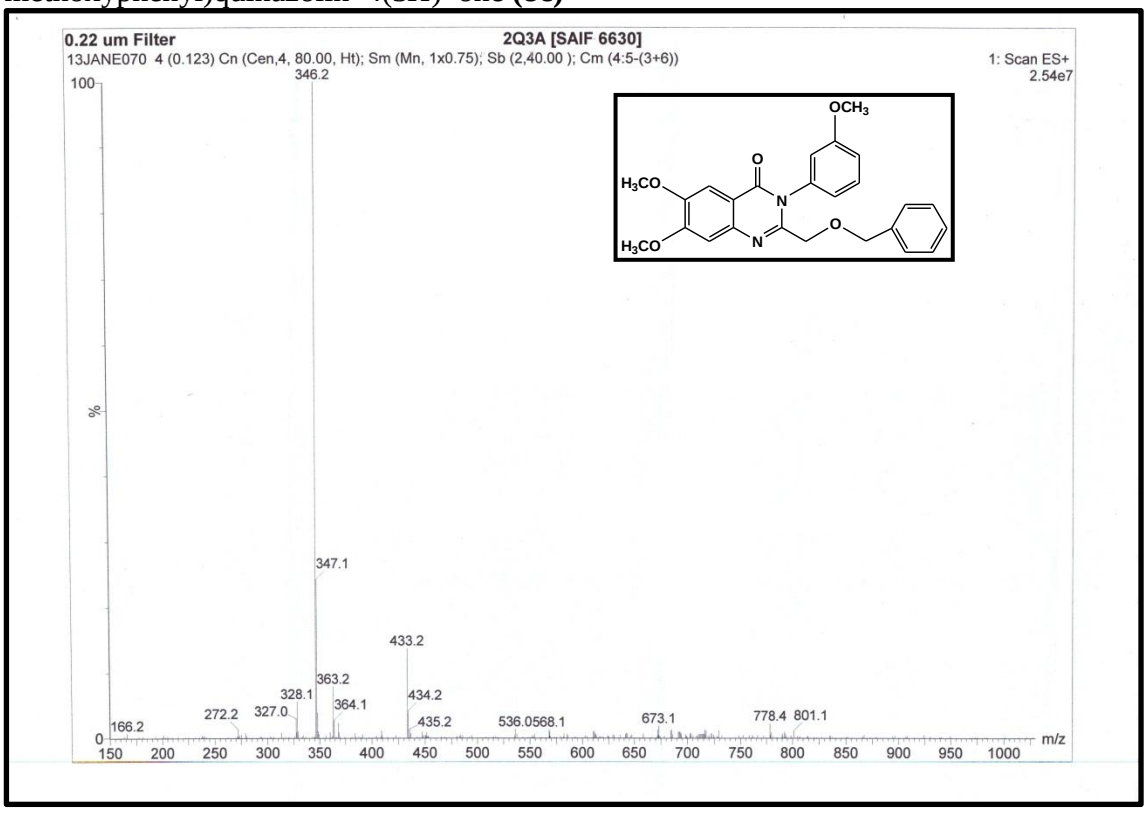

Figure 4B.17. Mass spectrum of 2–((benzyloxy)methyl)–6,7–dimethoxy–3–(3–methoxyphenyl)quinazolin–4(3*H*)–one **(8c)**

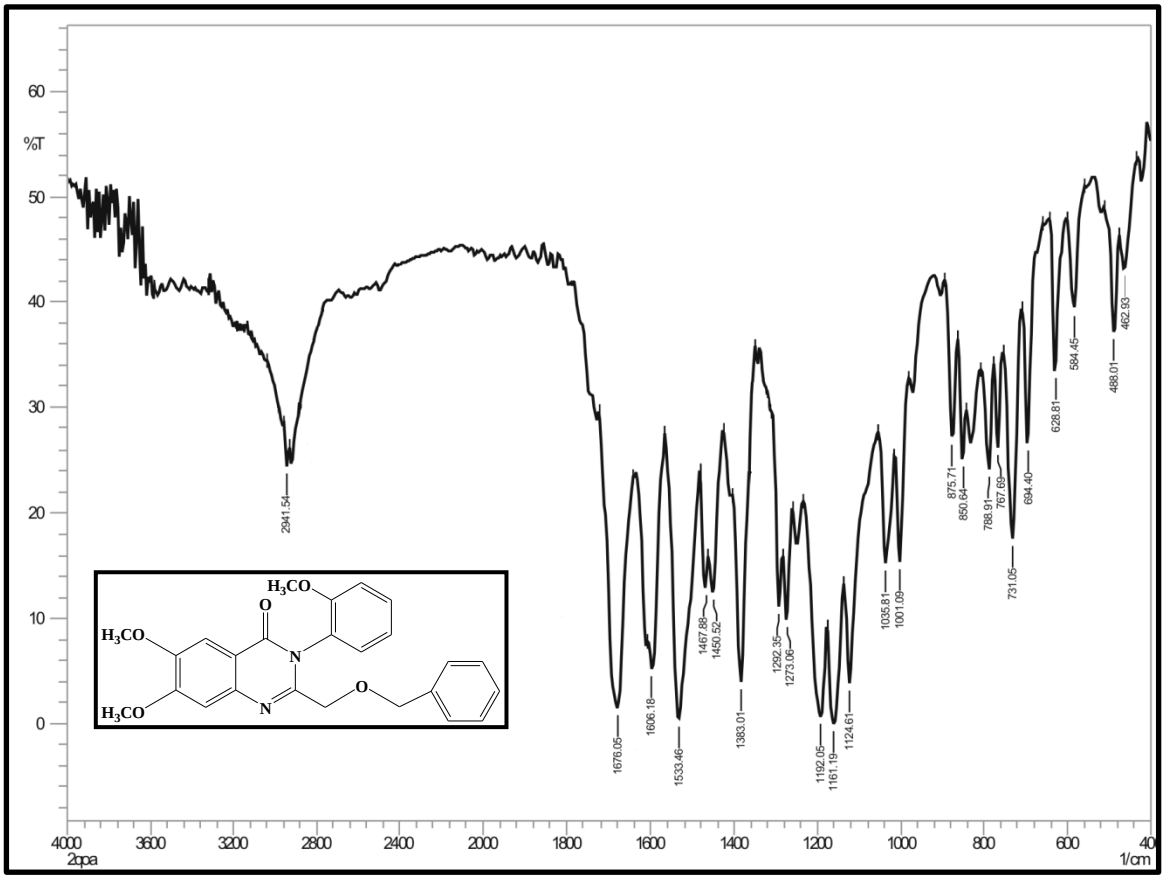

Figure 4B.18. FT–IR spectrum of 2–((benzyloxy)methyl)–6,7–dimethoxy–3–(2–methoxyphenyl)quinazolin–4(3*H*)–one **(8d)**

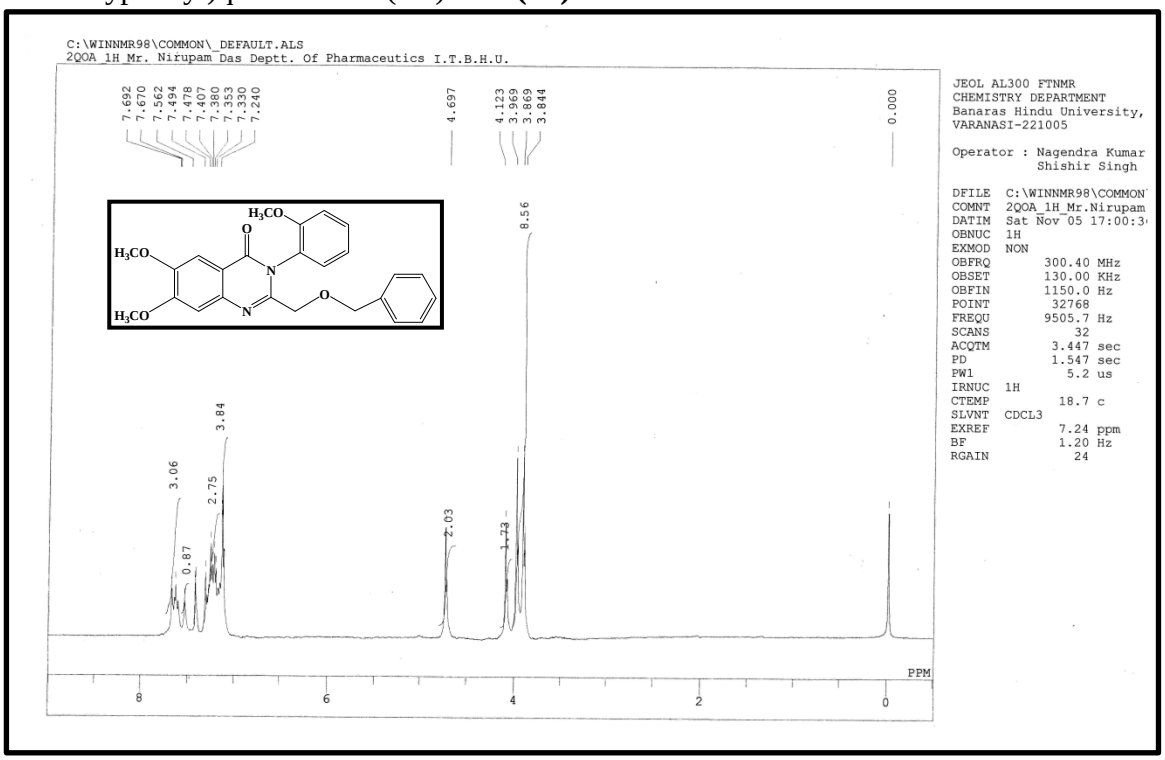

Figure 4B.19. ^{1}H NMR spectrum of 2–((benzyloxy)methyl)–6,7–dimethoxy–3–(2–methoxyphenyl)quinazolin–4(3*H*)–one **(8d)**

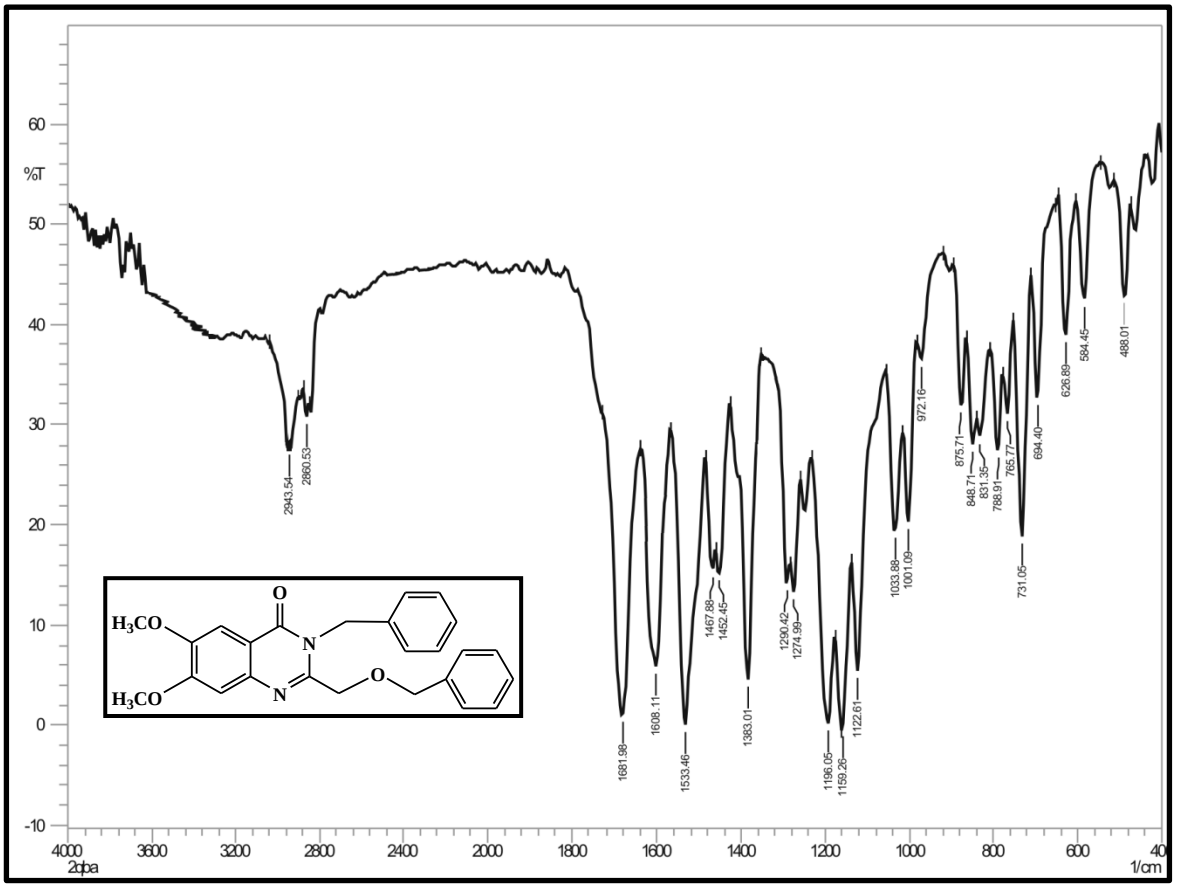

Figure 4B.20. FT–IR spectrum of 2–((benzyloxy)methyl)–6,7–dimethoxy–3–(benzyl)quinazolin–4(3*H*)–one **(8e)**

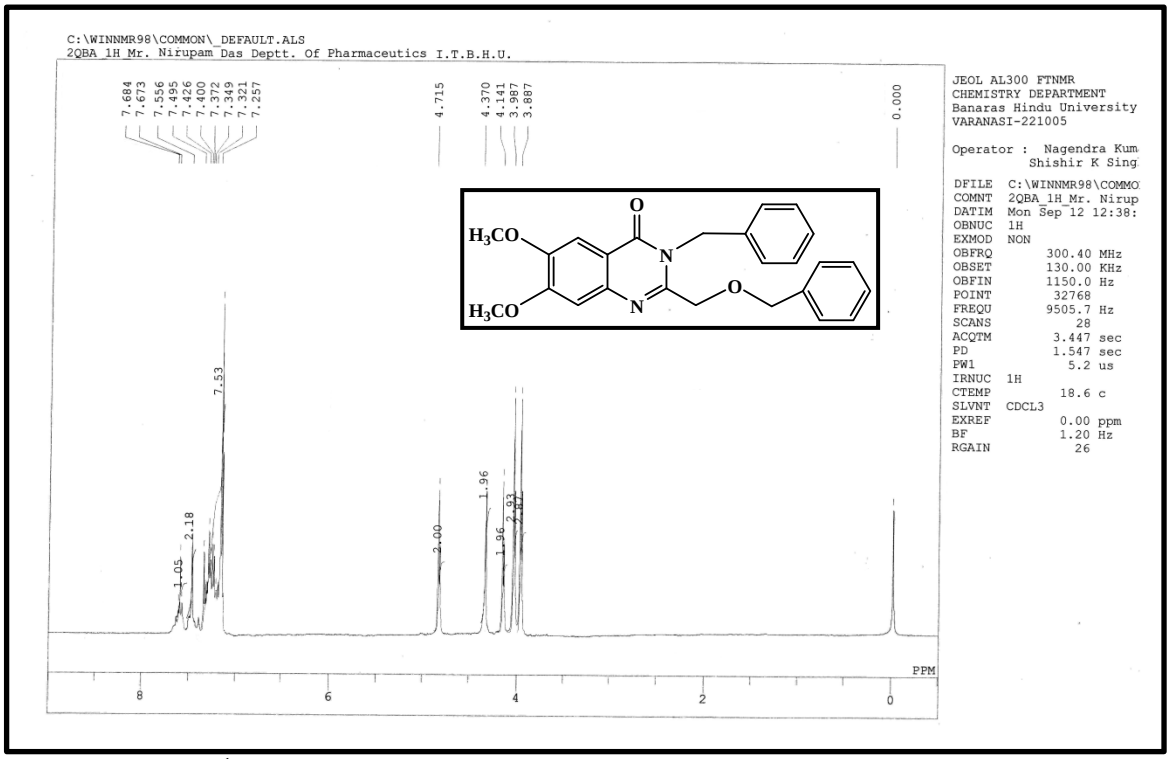

Figure 4B.21. ^{1}H NMR spectrum of 2–((benzyloxy)methyl)–6,7–dimethoxy–3–(benzyl)quinazolin–4(3*H*)–one **(8e)**

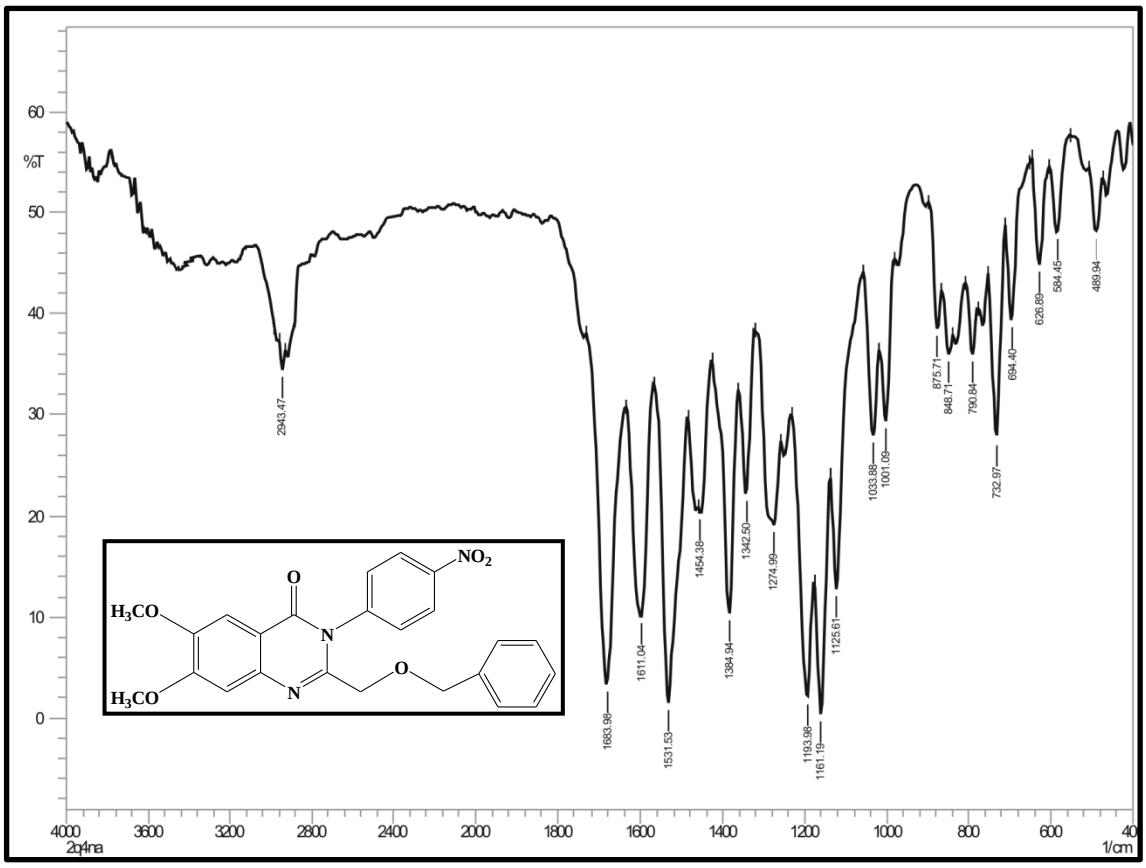

Figure 4B.22. FT–IR spectrum of 2–((benzyloxy)methyl)–6,7–dimethoxy–3–(4–nitrophenyl)quinazolin–4(3*H*)–one **(8f)**

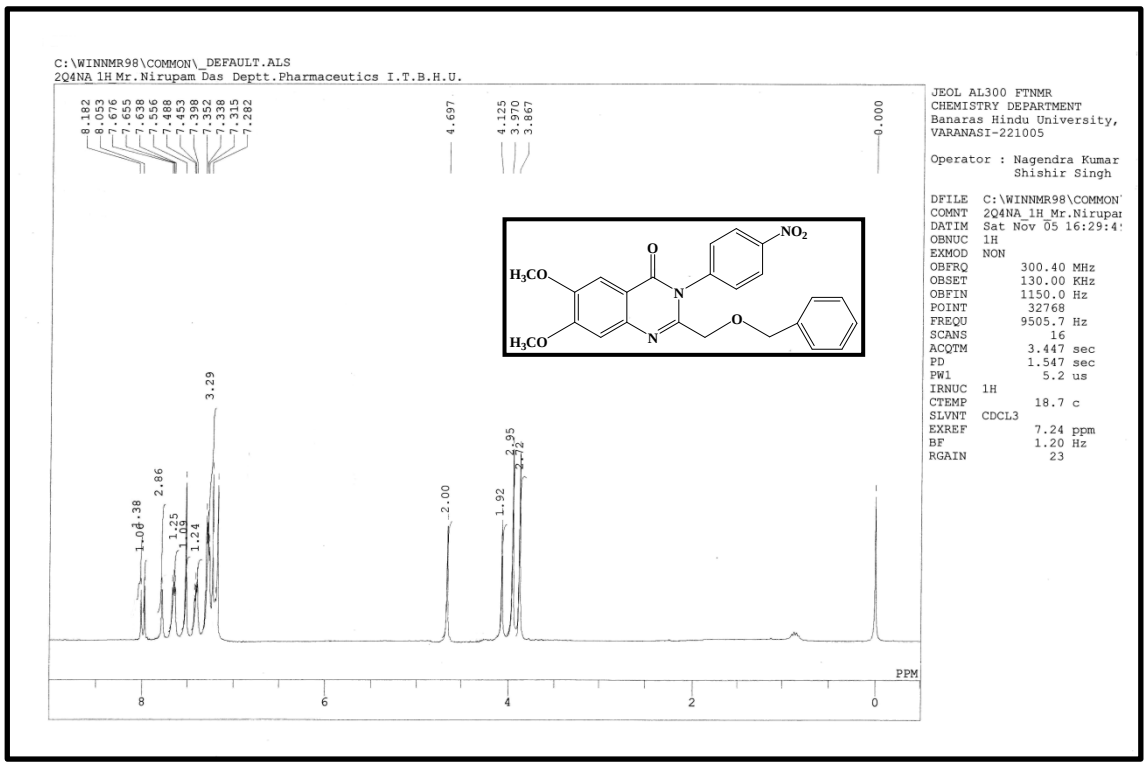

Figure 4B.23. ^{1}H NMR spectrum of 2–((benzyloxy)methyl)–6,7–dimethoxy–3–(4–nitrophenyl)quinazolin–4(3*H*)–one **(8f)**

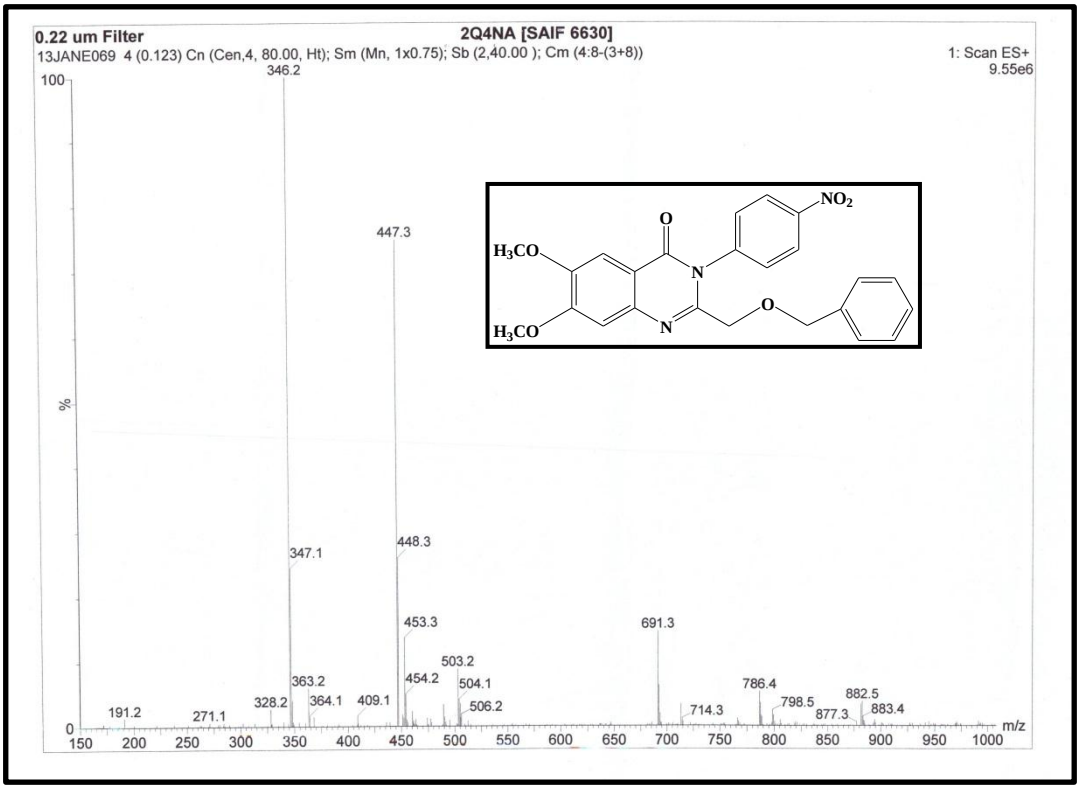

Figure 4B.24. Mass spectrum of 2–((benzyloxy)methyl)–6,7–dimethoxy–3–(4–nitrophenyl)quinazolin–4(3*H*)–one **(8f)**

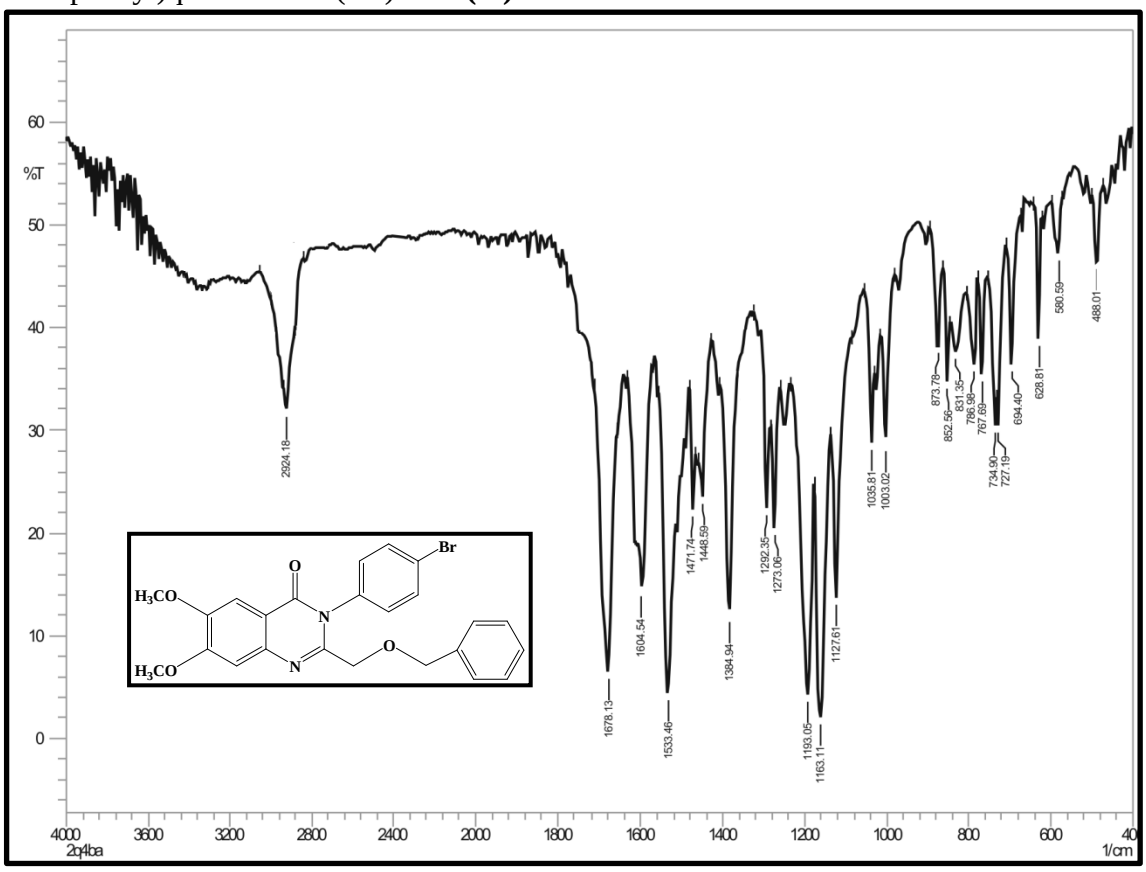

Figure 4B.25. FT–IR spectrum of 2–(benzyloxymethyl)–3–(4–bromophenyl)–6,7–dimethoxyquinazolin–4(3*H*)–one **(8g)**

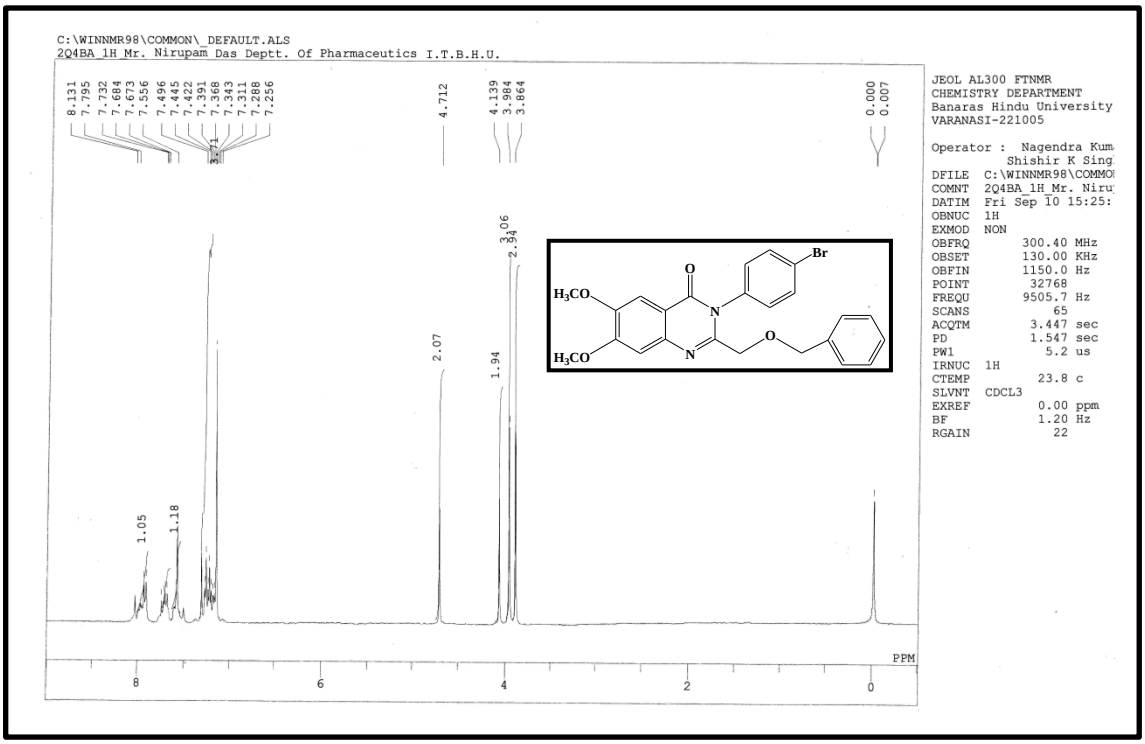

Figure 4B.26. ^{1}H NMR spectrum of 2–(benzyloxymethyl)–3–(4–bromophenyl)–6,7–dimethoxyquinazolin–4(3*H*)–one **(8g)**

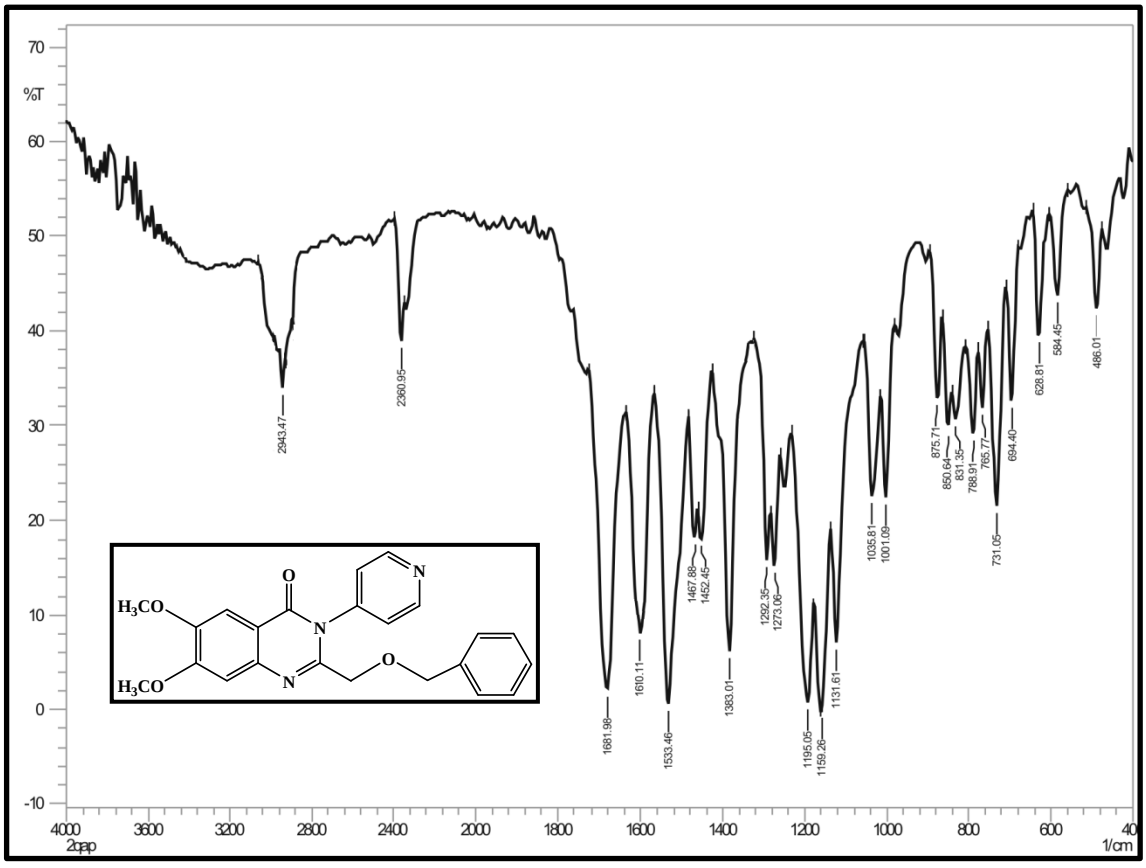

Figure 4B.27. FT–IR spectrum of 2–((benzyloxy)methyl)–6,7–dimethoxy–3–(pyridin–4–yl)quinazolin–4(3*H*)–one **(8h)**

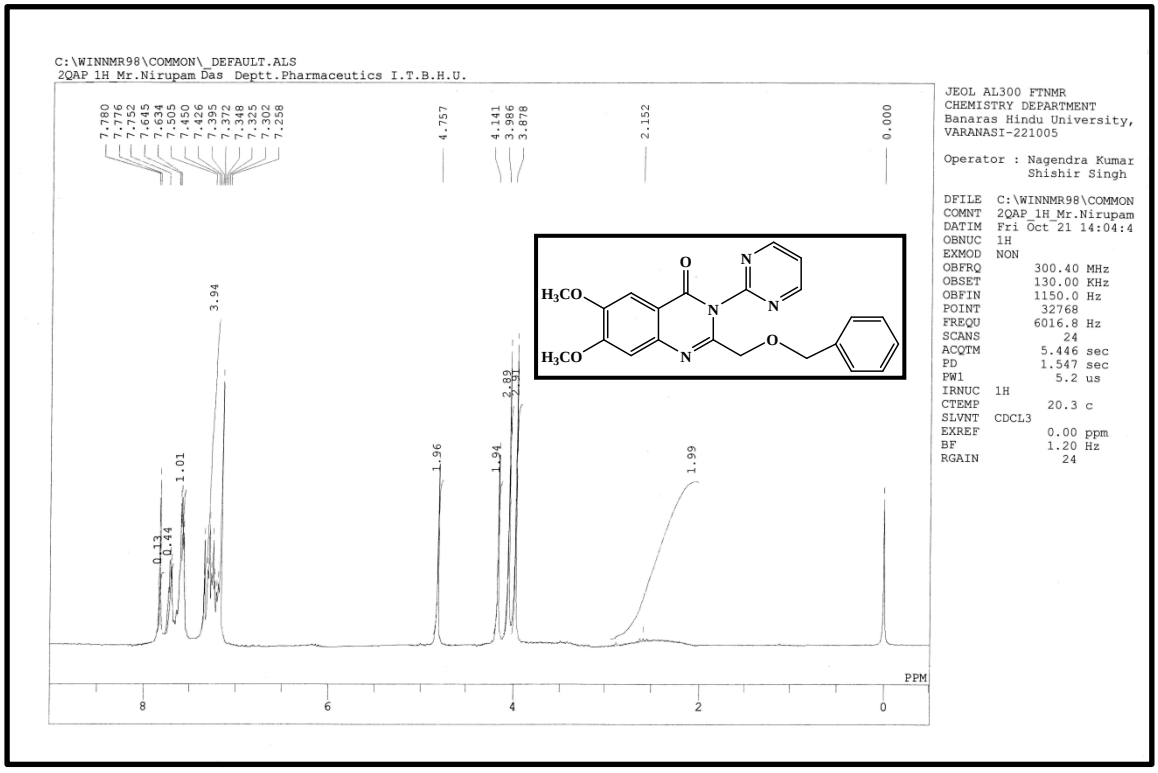

Figure 4B.28. ^{1}H NMR spectrum of 2–((benzyloxy)methyl)–6,7–dimethoxy–3–(pyridin–4–yl)quinazolin–4(3*H*)–one **(8h)**

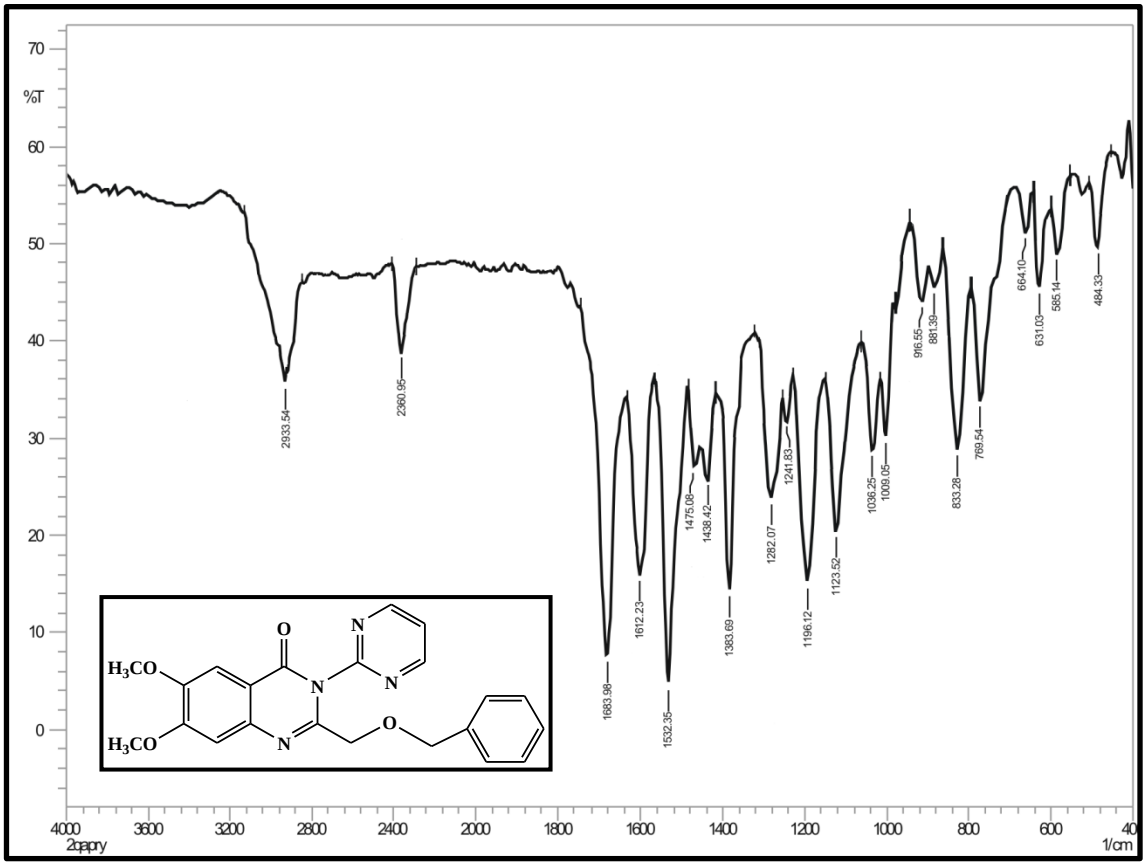

Figure 4B.29. FT–IR spectrum of 2–(benzyloxymethyl)–6,7–dimethoxy–3–(pyrimidin–2–yl)quinazolin–4(3*H*)–one **(8i)**

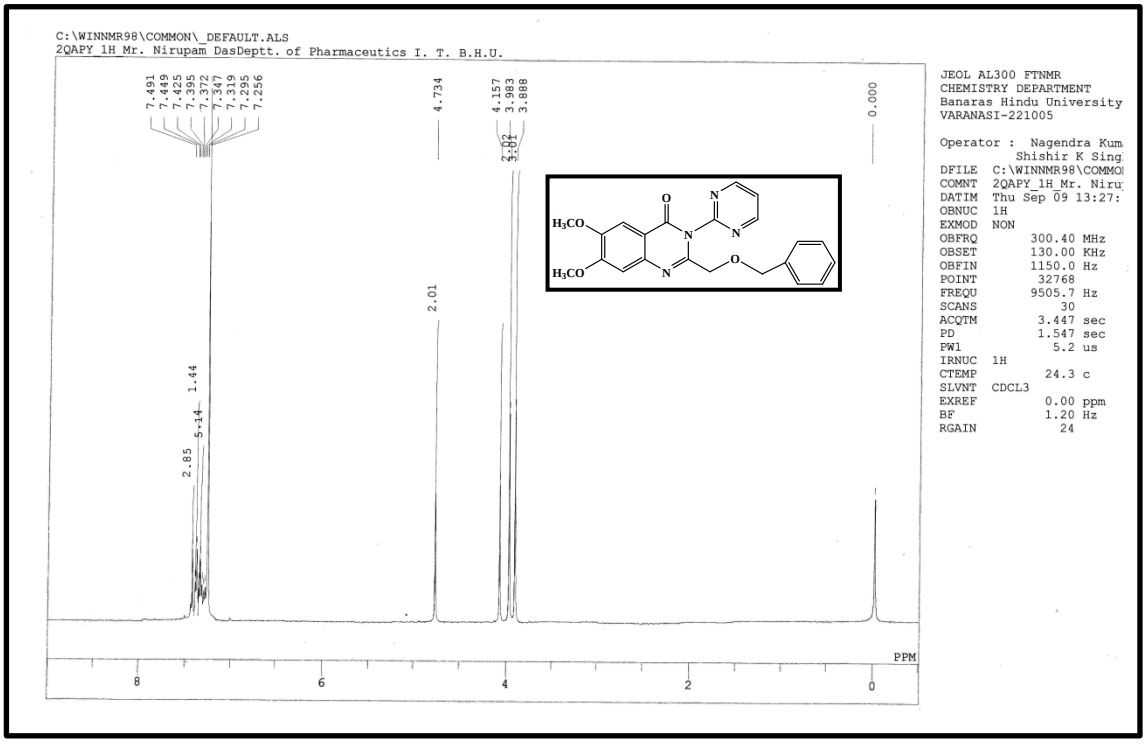

Figure 4B.30. ^{1}H NMR spectrum of 2–(benzyloxymethyl)–6,7–dimethoxy–3–(pyrimidin–2–yl)quinazolin–4(3*H*)–one **(8i)**

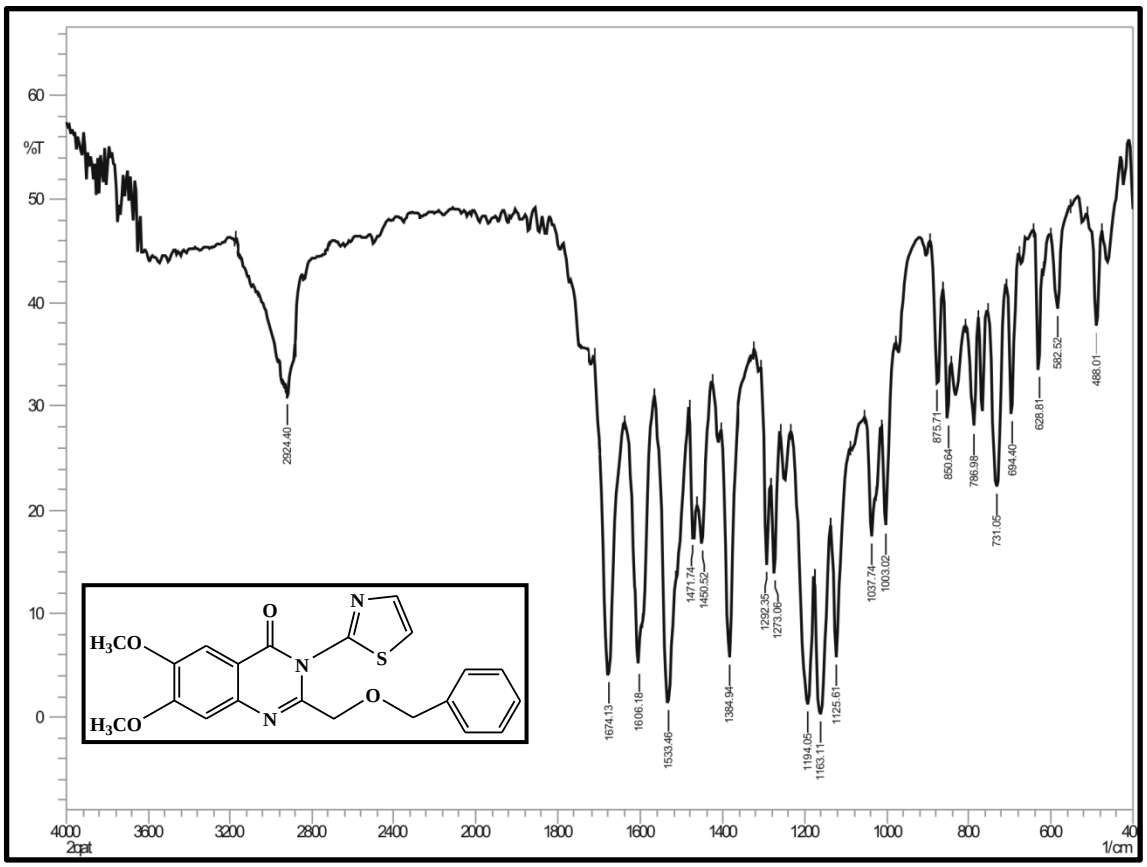

Figure 4B.31. FT–IR spectrum of 2–((benzyloxy)methyl)–6,7–dimethoxy–3–(thiazol–2–yl)quinazolin–4(3*H*)–one **(8j)**

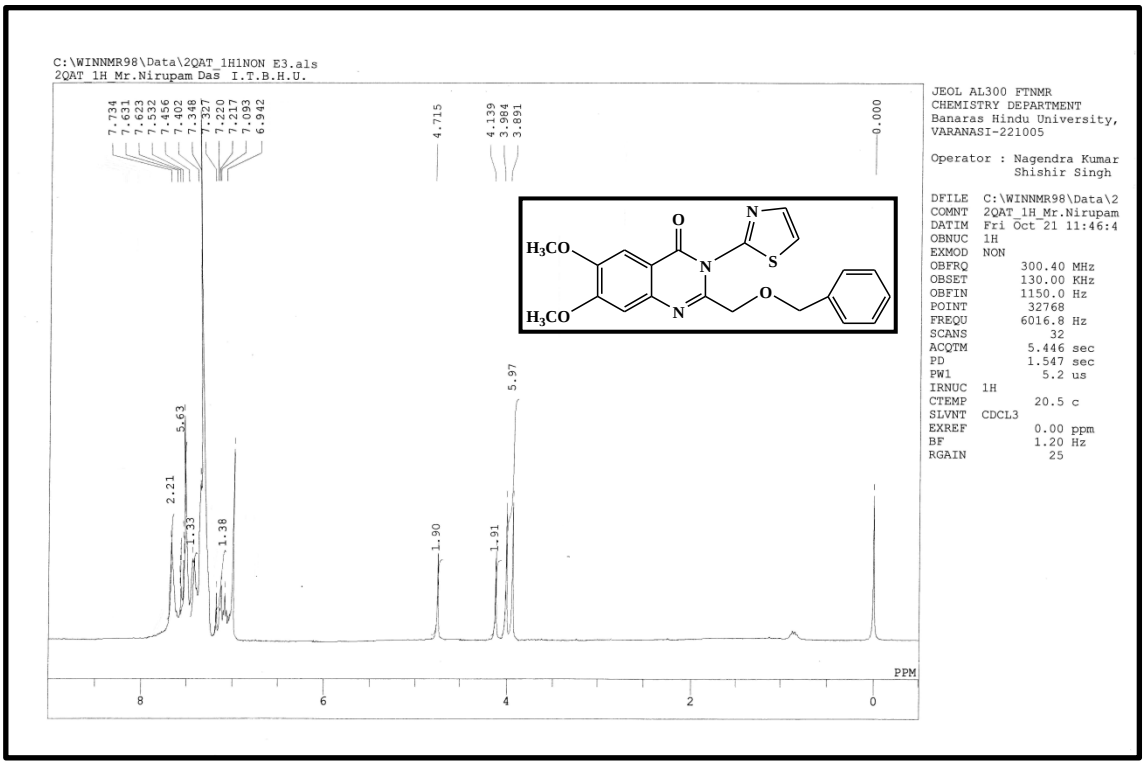

Figure 4B.32. ^{1}H NMR spectrum of 2–((benzyloxy)methyl)–6,7–dimethoxy–3–(thiazol–2–yl)quinazolin–4(3*H*)–one **(8j)**

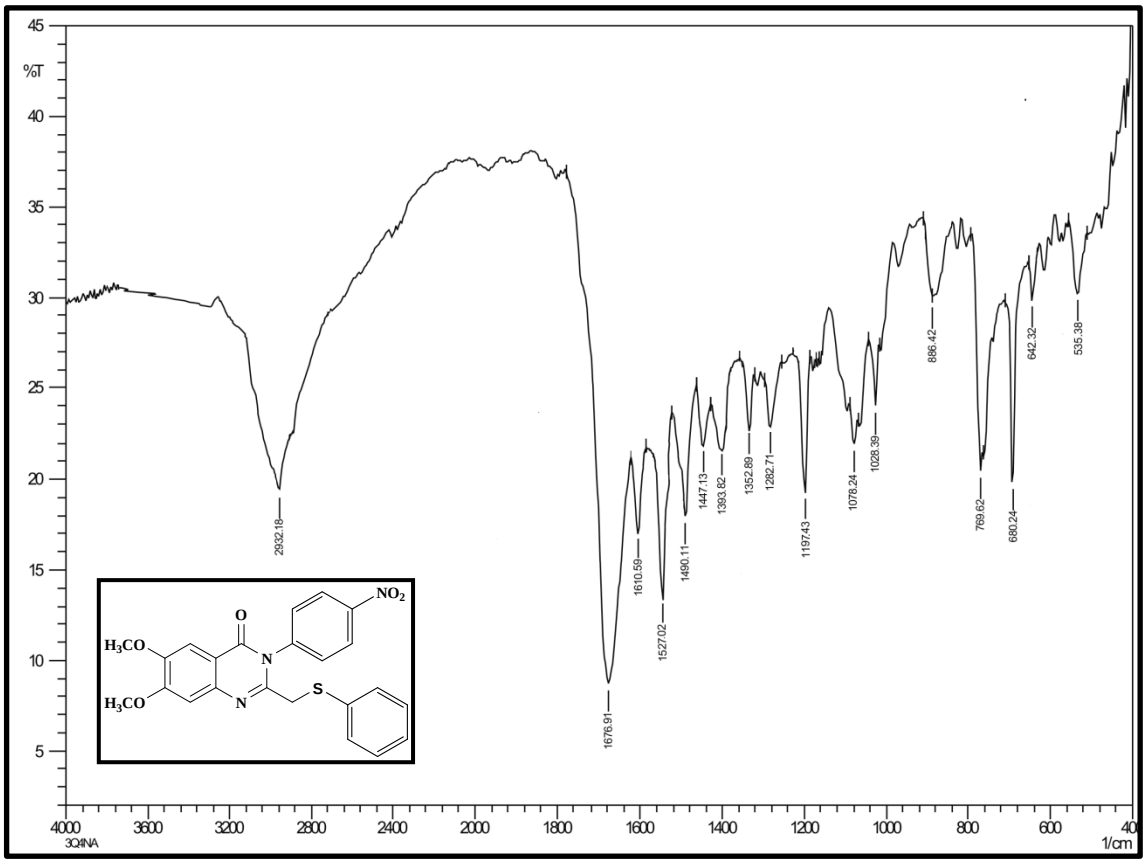

Figure 4B.33. FT–IR spectrum of 6,7–dimethoxy–3–(4–nitrophenyl)–2–((phenylthio)methyl)quinazolin–4(3*H*)–one **(8k)**

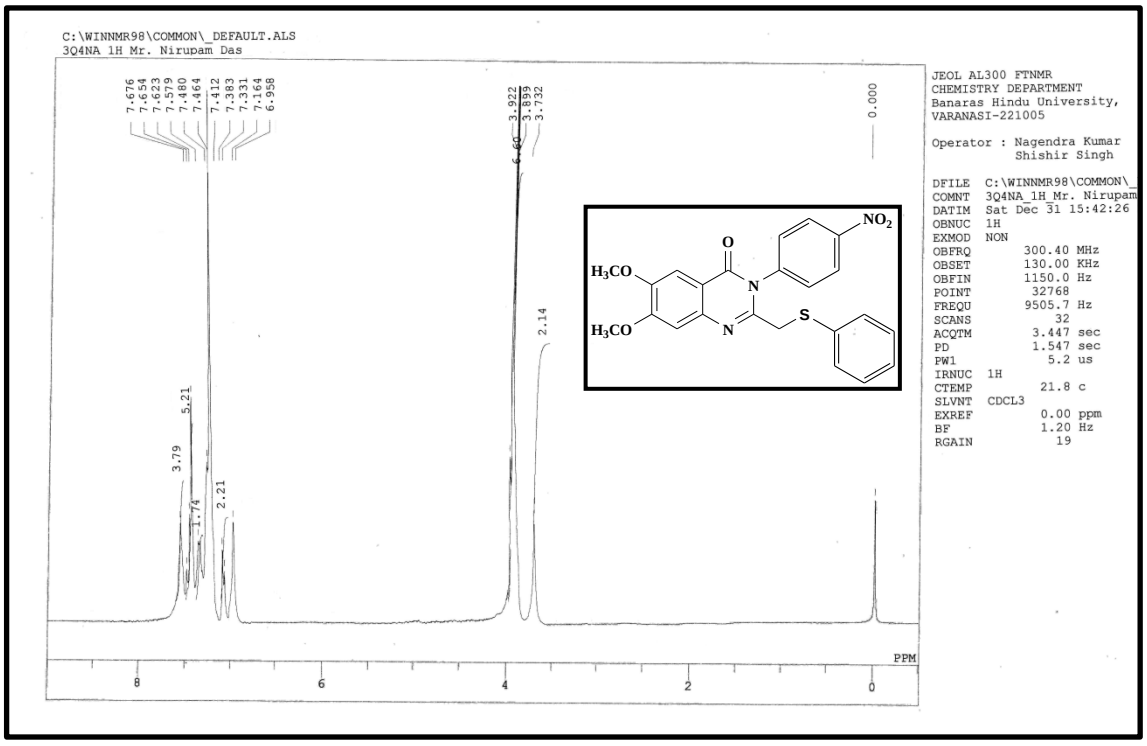

Figure 4B.34. ^{1}H NMR spectrum of 6,7–dimethoxy–3–(4–nitrophenyl)–2–((phenylthio)methyl)quinazolin–4(3*H*)–one **(8k)**

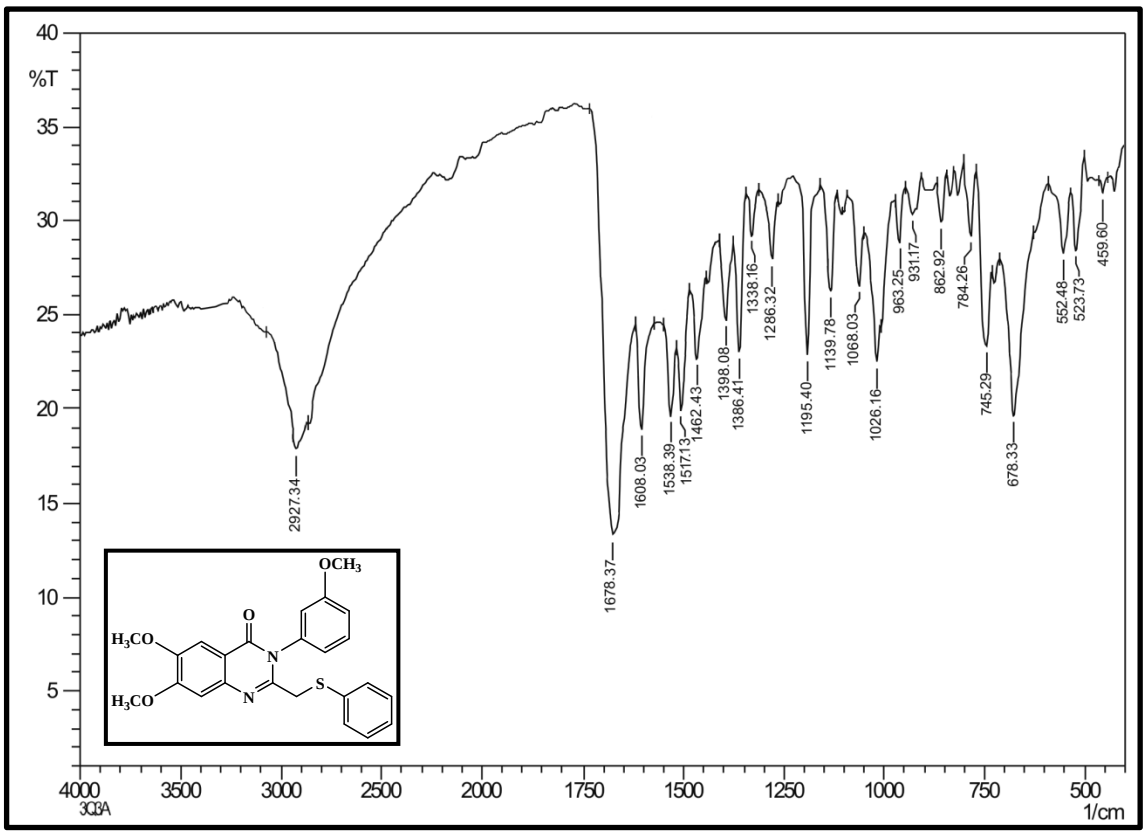

Figure 4B.35. FT–IR spectrum 6,7–dimethoxy–3–(3–methoxyphenyl)–2–((phenylthio)methyl)quinazolin–4(3*H*)–one **(8l)**

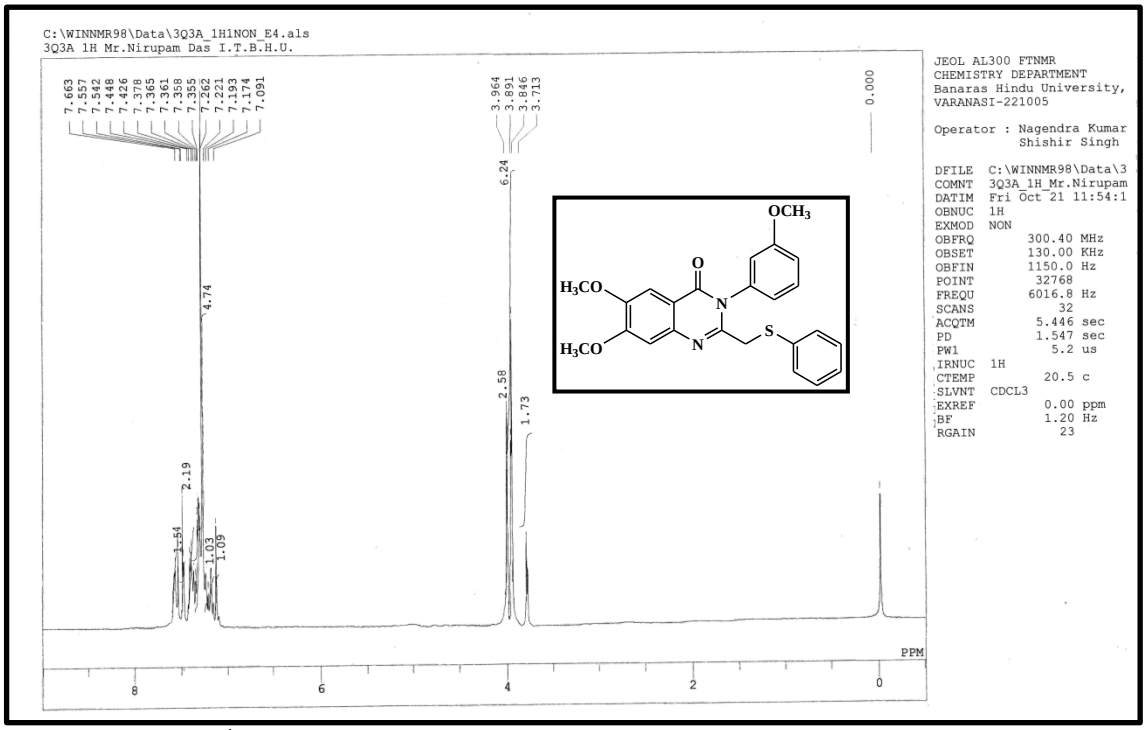

Figure 4B.36. ^{1}H NMR spectrum of 6,7–dimethoxy–3–(3–methoxyphenyl)–2–((phenylthio)methyl)quinazolin–4(3*H*)–one **(8l)**

EXPERIMENTAL – PART – 2

(SECTION – C)

4C.1. Pharmacological Evaluation

4C.1.1. Instruments

4C1.1.1. Electro convulsometer (Inco–Ambala, Model No. 100–3)

The Inco electro convulsometer provide 60 Hz alternating current stimulus for producing seizures required in the assay of anticonvulsant drugs and works on 220 A.C. The unit is supplied along with 3 pairs of corneal electrode of cup size 4, 6, 8¸ mm and a pair alligator clips as ear clip electrode as standard accessories. The application of stimulus current can be controlled by an electronic timer from 0.1 to 1 second. A light emitting diode indicates the duration of stimulus.

4C1.1.2. Quintessential Stereotaxic injector™

The Quintessential Stereotaxic Injector™ manufactured by Stoelting Co., USA, is designed for the infusion and/or withdrawal of pico/nano/microliter liter volumes *via* a Hamilton syringe (0.5–250 microliters). Injector enables the researcher to set volumes as small as 1 picoliter and flow rates from 0.05µl/min to 520µl/min. It works with syringes from 0.5ul up to 250µl.

4C1.1.3. Nikon Digital Microscope (Eclipse E200®, Tokyo, Japan)

The Nikon Digital Eclipse E200 biological Microscope is ideal for basic laboratory use and produces a sharp and clear image at any magnification. The Eclipse E200 maintains the same operational ease and rigidity. The optical system utilize CFI60 (infinity optical system) with a parfocal distance of 60mm. The magnification is in the range of 40-1000X for observation and the eyepiece tube is E2–TB binocular tube.

4C.1.2. Animals

Swiss albino mice (20–25 g) and rats (200–220 g) of either sex were procured from the Central Animals House, Institute of Medical Sciences, Banaras Hindu University (BHU). The animals were housed in polypropylene cages and kept under controlled environmental conditions at a temperature of 25±1 °C, 45–55% relative humidity and a 12:12 h light/dark cycle. The animals were provided with free access to commercial rodent feed (Doodh Dhara Pashu Ahar, India) and water *ad libitum.* The animals were the acclimatized for a week before the actual pharmacological study. The experiments

on animals were approved by the Central Animal Ethical Committee (BHU), Varanasi, India (Protocol No: Dean/10–11/282).

4C.1.3. Anticonvulsant activity, neurotoxicity study and hepatotoxicity study

The anticonvulsant activity of the synthesized compounds was evaluated on Swiss albino mice (20–25 g) of either sex. Food was withdrawn 12–15 h before commencing the experiment while water was withdrawn immediately before the experiment. All the newly synthesized compounds **(5a–5l, 8a–8l)** were tested for their anticonvulsant activity against MES–induced seizures. The compounds exhibiting protection against MES–induced seizures were selected for further evaluation against *sc*PTZ–induced seizure model in Swiss albino mice. Thereafter, all the compounds were evaluated for their possible ability to protect against AMPA–induced seizures. The rotarod test was performed to assess any probable changes in motor coordination induced by the test compounds. Few of the selected compounds were also subjected to liver function test and followed by histopathological examination of liver to assess for any probable drug–induced liver injury. Phenytoin, GYKI 52466 and talampanel were selected as the standard drugs and the synthesized compounds and standards were suspended in 30 % v/v aqueous solution of poly(ethylene glycol) (PEG 400).

4C.1.3.1. Maximal electroshock (MES) test

The synthesized compounds and the standard drugs were administered to the Swiss albino mice intraperitoneally (*ip*) in a volume of 0.5 mL per 20 g body mass at a dose of 20–500 µmol/kg body weight. The control animals received 30 % aqueous PEG 400. After 0.5 h following the drug administration, seizure was induced by transmission of an electrical stimulus (50mA at 60Hz) across the brain of 0.2 s in duration *via* a pair of ear clip electrodes. After applying the shock, the animals were observed for the type of convulsion produced and the hind limb extensor response was taken as the end point. The reduction in time or the absence of hind limb tonic extension of seizure was taken as protection against seizure. Median effective dose (ED_{50}) was calculated at different doses of the test compounds **(5a–5l, 8a–8l**), phenytoin and GYKI 52466 using probit analysis at 95% confidence limit as per the reported procedure (Castel–Branco *et al.*, 2009). The ED_{50} values indicate the dose at which tonic hind limb seizure was prevented in 50% of the test animals.

4C.1.3.2. Subcutaneous pentylenetetrazole induced seizure

PTZ dissolved in 0.9% w/v NaCl solution at a dose of 70 mg/kg in mice was injected subcutaneously and the onset and severity of convulsion was noted for the control group. The test (**5c, 5g, 5i, 5k, 8a**, **8f**, **8h** and **8k**) compounds and the standards were administered *ip* 0.5 h prior to the administration of PTZ. The activity was calculated in terms of ED_{50} at 95% confidence interval at a dose of 20–500 µmol/kg body weight in 30 % v/v aqueous PEG 400.

4C.1.3.3. AMPA–induced seizure

Mice were anaesthetized with pentobarbital sodium (2.5–3.0 mg/kg *ip*) and placed in a stereotaxic instrument (Quintessential Stereotaxic injector™, Stoelting Co., USA). For the intracerebroventricular (*icv*) injection of AMPA, a 24–gauge cannula was implanted at 0.9 mm lateral and 0.7 mm posterior to bregma, at a depth 3.0 mm below the surface of the skull. It was held in place with dental cement applied to the exposed skull surface (Yamashita *et al.*, 2004). After the implantation, the mice were housed individually to avoid damage to the injection apparatus. The test compounds (**5a–5l, 8a–8l**) and the standards were administered *ip* at various doses in the range of 20–500 µmol/kg body weight in 30 % v/v aqueous PEG 400 after a recovery period of 1 week was following implantation. After 60 min, AMPA at a dose of 1µgm/mouse was injected at a volume of 4µl. Wild running; tonic and clonic seizures were monitored for 10 min. ED_{50} values were calculated using Environmental Protection Agency, USA probit analysis program (Version 1.5).

4C.1.3.4. Acute neurotoxicity study

The rotarod test (Vogel, 2002) was carried out to assess the impairment of motor performance, ataxia, loss of skeletal muscular strength, and acute neurotoxicity produced by the synthesized compounds. Swiss albino mice weighing between 20–25 g, were trained to balance on the knurled wooden rod (3.2 cm diameter) rotating at 6 rpm. The trained animals were treated with the test compounds (**5a–5l, 8a–8l**) and the standards administered *ip* at a dose of 20–500 µmol/kg body weight in 30 % v/v aqueous PEG 400. After 0.5 h, the mice were placed onto the rotating rod for 1 min. Neurological impairment was determined as the inability of the animal to remain on the rod for 1 min. The number of mice having motor impairment were counted and the

TD_{50} (the dose which induced motor toxicity in 50% of mice) at 95% confidence intervals were calculated using probit analysis.

4C.1.3.5. Hepatotoxicity study

The rats were divided into groups of six and the control group received standard diet and vehicle. The other groups were administered the selected test drug (**8f** and **8h**) at a dose of 100 µmol/kg/day (in PEG 400) for 14 days according to the reported procedure (Tong *et al.*, 2005). The 14 days period for assessment of acute toxicity is the official guideline recommended by OECD (Organisation for Economic Co-operation and Development) 423 (17^{th} December 2001). A higher dose (100 µmol/kg/day) was selected for both the compounds to assess any possible dose–induced liver injury. After the period, blood samples were collected and analyzed for serum AST and ALT activity using a photometric auto analyzer (ERBA Chem Pro, Transasia Bio–Medicals, Mumbai, India). After collection of blood samples, the animals were sacrificed for isolation of liver tissues to observe histopathological changes, if any. The tissue samples were dissected out and were fixed in 10% formalin solution. Paraffin sections were made and stained with hematoxylin and eosin for histopathological examination using a Nikon digital microscope (Eclipse E200, Tokyo, Japan).

4C.2. Active site prediction, molecular docking and *in silico* pharmacokinetic predictions:

4C.2.1. Preparation of the ligand

The 3D structures of the selected compounds exhibiting potential activity and GYKI 52466 were built using Maestro 9.0 build panel and prepared by LigPrep 2.3 version v23118 (Schrödinger, LLC., USA). The application uses Optimized Potentials for Liquid Simulations (OPLS) 2005 force field and energy minimized with Macromodel-v97110.

4C.2.2. Preparation of the protein and prediction of active site

The templates of the synthesized molecules were based on quinazolin–4(3*H*)–one nucleus and majority of the reported quinazolin–4(3*H*)–ones elicit anticonvulsant activity at the amino terminal domain (ATD) of AMPA receptor site (Menniti *et al*., 2000; Welch *et al.*, 2001). Therefore, a docking study was carried out on the allosteric

ATD of ionotropic glutamate receptor to find out the putative binding mode. The crystal structure of ATD of ionotropic glutamate receptor structure with a resolution of 2.50 Å was retrieved from the protein data bank (PDB ID: 3SAJ) (Yao *et al.*, 2011). The structure was prepared by the protein preparation wizard within the Maestro Schrödinger® 9.0 module, and includes addition of hydrogens, assigning partial charges, assigning protonation states, energy minimization using the OPLS–2005 force field. Thereafter, the resulting structure was used to predict the possible active site. As the ATD of the AMPA receptor was devoid of associated co–crystallized ligand and therefore, the location of the primary binding site on a receptor was unknown. SiteMap® generates information on the character of binding sites using novel search and provides information of the probable active sites where the possibility of tight ligand–receptor interaction could be achieved (SiteMap®, version 2.3, Schrödinger, LLC, New York, NY, 2009). Therefore, the Sitemap® (version 2.3) module in Maestro Schrödinger® 9.0 version v23118 was utilized to detect the potential binding cavities within the ATD. The outcome of sitemap using OPLS 2005 force field resulted in the detection of five binding sites (Halgren, 2007) and the highest scored (1.046) binding site was selected for the present molecular docking (Table 4C.1.).

Table 4C.1. Top–ranked SiteMap® prediction for receptor binding sites

Sl. No	Title	Sitescore
1	Sitemap_site1	1.046
2	Sitemap_site2	0.912
3	Sitemap_site3	0.776
4	Sitemap_site4	0.748
5	Sitemap_site5	0.748

4C.2.3. Molecular docking

The centroid of the contours of sitemap_site1 (as it occupies the total volume of the cavity) was used **(Figure 4C.1.)** in the generation of the grid. The compound **5g**, **8f** and **GYKI 42466** were docked using Glide XP 5.5 at the predicted active site and the lowest energy pose was taken into consideration for each docking run.

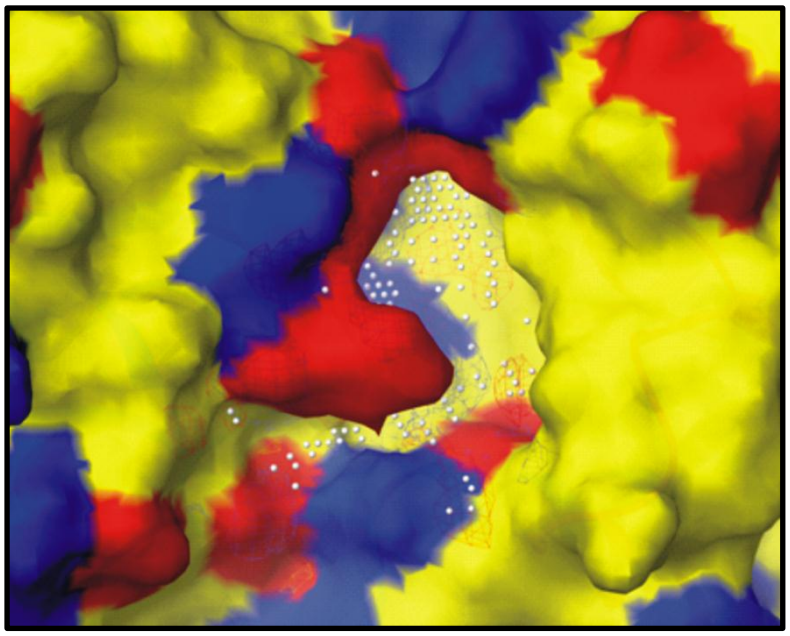

Figure 4C.1. The centroid of the sitemap_site1 used in the generation of grid (hydrophobic map: **yellow surface**; hydrogen–bond (HB) donor map: **blue surface**; HB acceptor map: **red surface**

4C.2.4. *In silico* pharmacokinetic predictions

Few of the compounds were subjected to predict the pharmacokinetic properties using the Qikprop® module of the Maestro Schrödinger. All the compounds were neutralized before being subjected to Qikprop® pharmacokinetic predictions. The program was processed in default mode and the properties predicted consist of principal descriptors (Table 4.C.2.) such as log P (octanol/water), Lipinski's rule of five violation, detection of reactive functional groups, CNS activity, predicted brain/blood partition coefficient and % human oral absorption.

Table 4C.2. Qikprop® properties and descriptors

S.No	Descriptor	Description	Recommended Range
1.	QPlogP (o/w)	Predicted octanol/water coefficient	-2.0 to 6.5
2.	Lipinski's rule of five	Lipinski's rules of five are: mol_MW < 500, QPlogPo/w < 5, donorHB ≤ 5, accptHB ≤ 10. Compounds that satisfy these rules are considered drug like. (The "five" refers to the limits, which are multiples of 5	Maximum is 4
3.	#rtvFG	This particular descriptor indicates the number of reactive functional groups. The presence of these groups can lead to decomposition, reactivity, or toxicity problems *in vivo*.	0 to 2
4.	CNS	Predictive central nervous activity on a –2 (inactive) to +2 (active) scale.	-2 to +2
5.	QPlogBB	Predicted brain/blood partition coefficient. Predictions are for orally delivered drugs.	-3.0–1.2
6.	% Human oral absorption	It predicts human oral absorption on 0 to 100% scale. The prediction is based on a quantitative multiple linear regression model. This property usually correlates well with human oral absorption.	>80% is high <25% is poor