EXPERIMENTAL WORK

4.1. 2-AMINO-6-NITROBENZOTHIAZOLE DERIVED EXTENDED HYDRAZONES [BTA-1 to BTA-30]

4.1.1. Synthesis

4.1.1.1. Chemicals and reagents

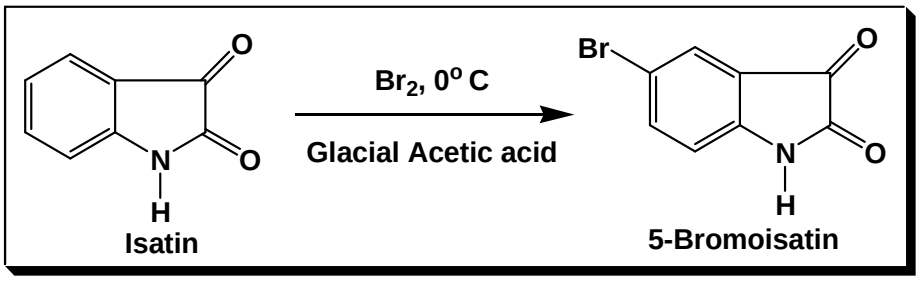
All the chemicals and reagents used for this work were of commercial quality of laboratory grade procured from Sigma-Aldrich (U.S.A.), Merck (Germany), SD Fine (Mumbai) and Qualigens (Mumbai).

4.1.1.2. Synthetic protocol

4.1.1.2.1. Synthesis of heterocyclic fragments [Da Silva *et al.* 2001]

4.1.1.2.1.1. Synthesis of 5-bromoisatin

To a solution of isatin (0.1 mol) in glacial acetic acid at 0 °C in a conical flask, bromine solution was added from the burette, until the colour of it gets lost. The reaction mixture was then allowed to stand for 10 – 15 minutes. Ice-cold water was added to the reaction mixture. The precipitate separated out was filtered, dried and recrystallized from glacial acetic acid to obtain 5-bromoisatin (**Scheme 4.1.**).



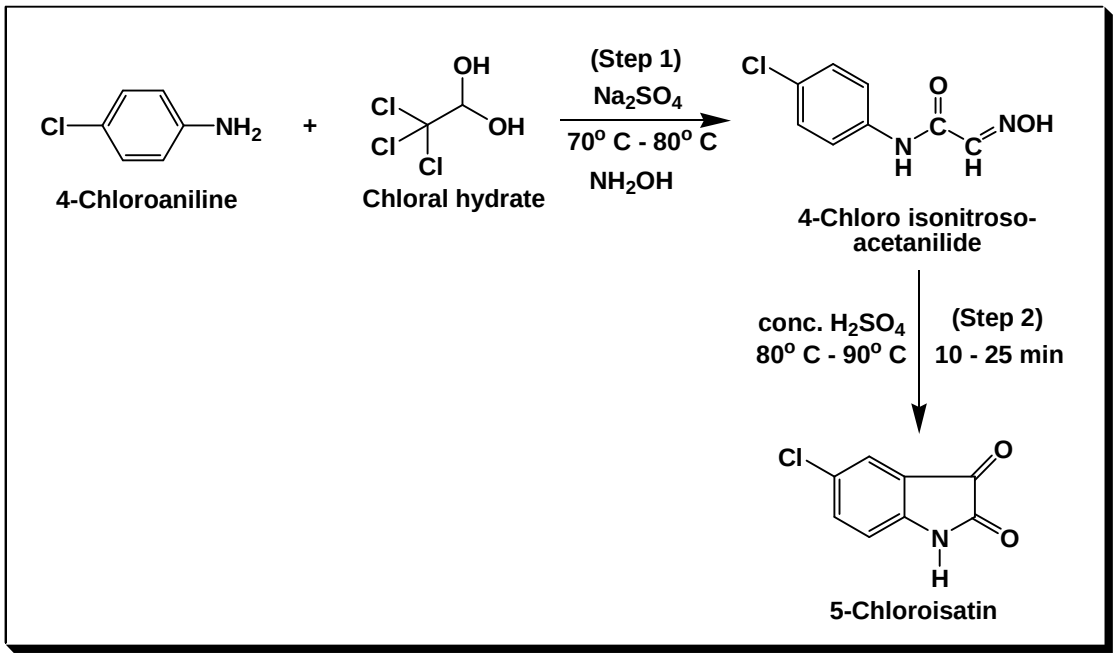
Scheme 4.1. Synthesis of 5-bromoisatin (**ISN-1**)

4.1.1.2.1.2. Synthesis of 5-chloroisatin

Step 1. Synthesis of 4-chloroisonitrosoacetanilide

Chloral hydrate (0.11 mol) was dissolved in distilled water (250 mL) and sodium sulphate (120 g) was added successively to it. Then the solution of 4-chloroaniline (0.01 mol) in HCl (5.5 mL) was added to the above reaction mixture followed by the addition of a solution of hydroxylamine hydrochloride (0.33 mol) in water. This reaction mixture was then heated for about 10 minutes at the temperature sufficient to boil it vigorously.

Thereafter, the reaction mixture was cooled to room temperature which resulted in the formation of yellow coloured needle shaped crystals which was then filtered, dried and recrystallized from ethanol to yield 4-chloro isonitrosoacetanilide (**Scheme 4.2.**).

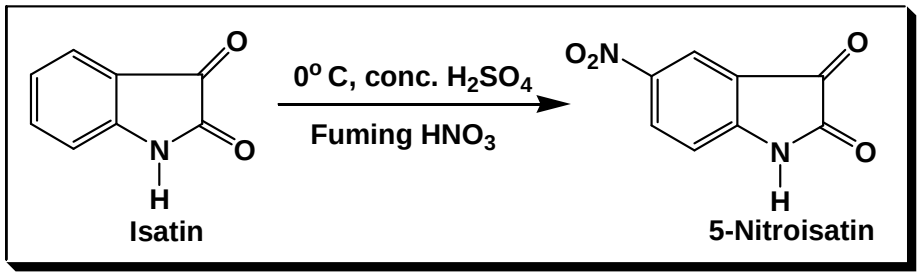


Scheme 4.2. Synthesis of 5-chloroisatin (**ISN-2**)

Step 2. Cyclization

4-Chloroisonitrosoacetanilide was added slowly to the concentrated sulphuric acid (50 mL) over the period of 30 min, with continuous stirring. Then the mixture was heated at 80 °C – 90 °C for 10 – 25 min and then poured into crushed ice. The precipitate formed was filtered, dried and recrystallized from glacial acetic acid (**Scheme 4.2.**).

4.1.1.2.1.3. Synthesis of 5-nitroisatin

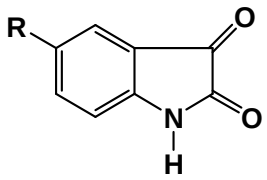


Scheme 4.3. Synthesis of 5-nitroisatin (**ISN-3**)

Isatin (0.1 mol) was mixed with conc. H_2SO_4 (6.6 g, 3.6 mL) at 0 °C. Fuming HNO_3 (6.3 g, 4.2 mL) was added dropwise to this reaction mixture and mixed properly. This was

then allowed to stand for 30 min and then crushed ice was added to it. The precipitate obtained was filtered, dried and recrystallized from ethanol to yield 5-nitroisatin (**Scheme 4.3.**).

Table 4.1. Structural data of heterocyclic fragments (**ISN-1** to **ISN-3**)



Code	Compound name	R	Mol. Formula	Mol. Wt. (g/mol)
ISN-1	5-Bromoisatin	Br	$C_9H_6BrNO_2$	240.05
ISN-2	5-Chloroisatin	Cl	$C_9H_6ClNO_2$	195.6
ISN-3	5-Nitroisatin	NO_2	$C_9H_6N_2O_4$	206.15

4.1.1.2.2. Synthesis of 2-amino-6-nitrobenzothiazole derived extended hydrazones [Cakir *et al.* 1999; Ienascu *et al.* 2009]

2-Amino-6-nitrobenzothiazole derived extended hydrazones (**BTA-1** to **BTA-30**) were synthesized by the method outlined in **Scheme 4.4.**

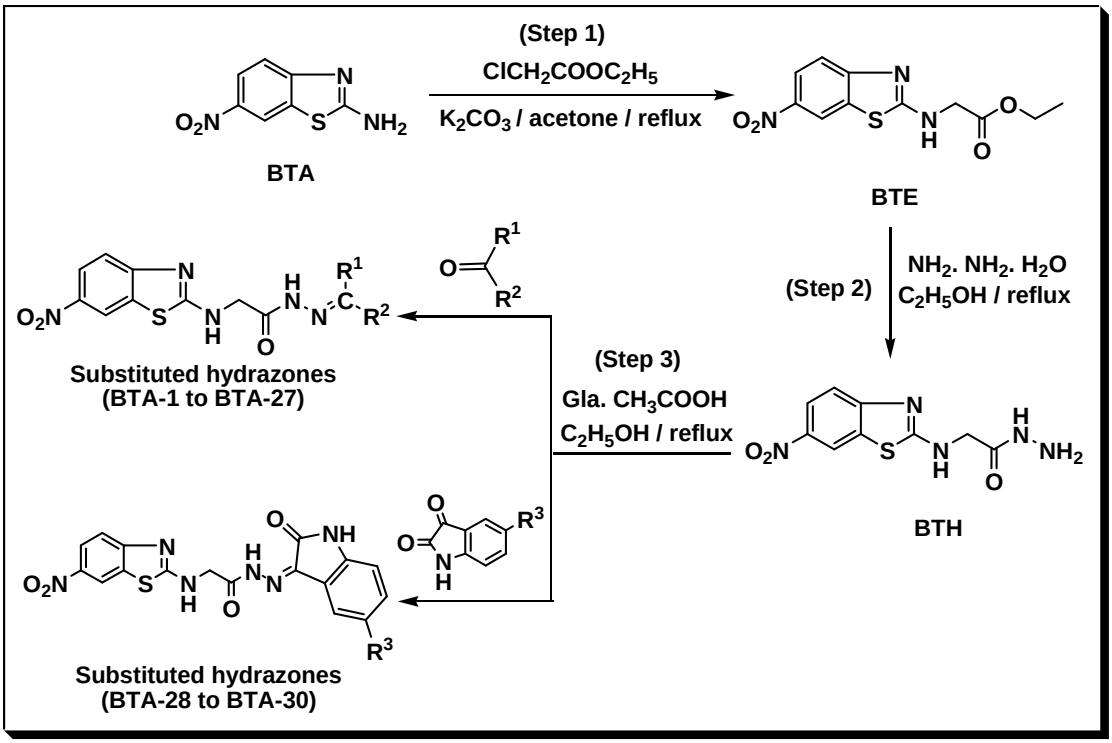
Step 1. Synthesis of ethyl-2-(6-nitrobenzothiazol-2-ylamino)acetate (BTE)

To the solution of 2-amino-6-nitrobenzothiazole (**BTA**, 0.030 mol) in acetone, ethyl chloroacetate (0.033 mol, 2 eq.) and K_2CO_3 (0.030 mol) was added, and the mixture was refluxed for 40-42 h. The reaction mixture was filtered off and the solvent was evaporated to afford an ester i.e. ethyl-2-(6-nitrobenzothiazol-2-ylamino)acetate (**BTE**). The crude product thus obtained was then recrystallized from 95% ethanol. Purity of compound was checked by TLC analysis using the solvent system (Chloroform: Methanol: Toluene (7:2:1), R_f: 0.56).

Step 2. Synthesis of 2-(6-nitrobenzothiazol-2-ylamino)acetohydrazide (BTH)

To a solution of **BTE** (0.03 mol) in ethanol, was added a solution of hydrazine hydrate (0.03 mol, 2 eq.) in small portions and stirred properly. After refluxing for 35-36 h, the reaction mixture was cooled. The solvent was evaporated to dryness and the residue

obtained was recrystallized from 95% ethanol to afford 2-(6-nitrobenzothiazol-2-ylamino)acetohydrazide (**BTH**). Purity of compound was checked by TLC analysis using the solvent system (Chloroform: Methanol: Toluene (7:2:1), R_f: 0.52).



Scheme 4.4. Synthesis of 2-amino-6-nitrobenzothiazole derived extended hydrazones (**BTA-1** to **BTA-30**)

Step 3. Synthesis of extended hydrazones (BTA-1 to BTA-30)

The final compounds **BTA-1** to **BTA-30** (substituted monoaryl/diaryl/5-(un)substituted isatinyl hydrazones) were synthesized by the reaction of **BTH** (0.003 mol) with an equimolar quantity of appropriately substituted aldehyde or ketone or 5-(un)substituted isatin (0.003 mol). The pH of the reaction mixture was adjusted to 5-6 by adding glacial acetic acid and refluxed for 25-50 h. The solvent was either evaporated or quenched in ice cold water and filtered to get the crude product, which was then recrystallized from 95% ethanol to produce the final products **BTA-1** to **BTA-30**.

4.1.1.3. Reaction mechanism

It is a nucleophilic addition reaction in which amine acts as nucleophile, attacks on the carbocation of aldehyde or ketone and intermediate carbinolamine is formed. Then proton

transfer followed by loss of water results in the formation of final compounds (**Figure 4.1.**).

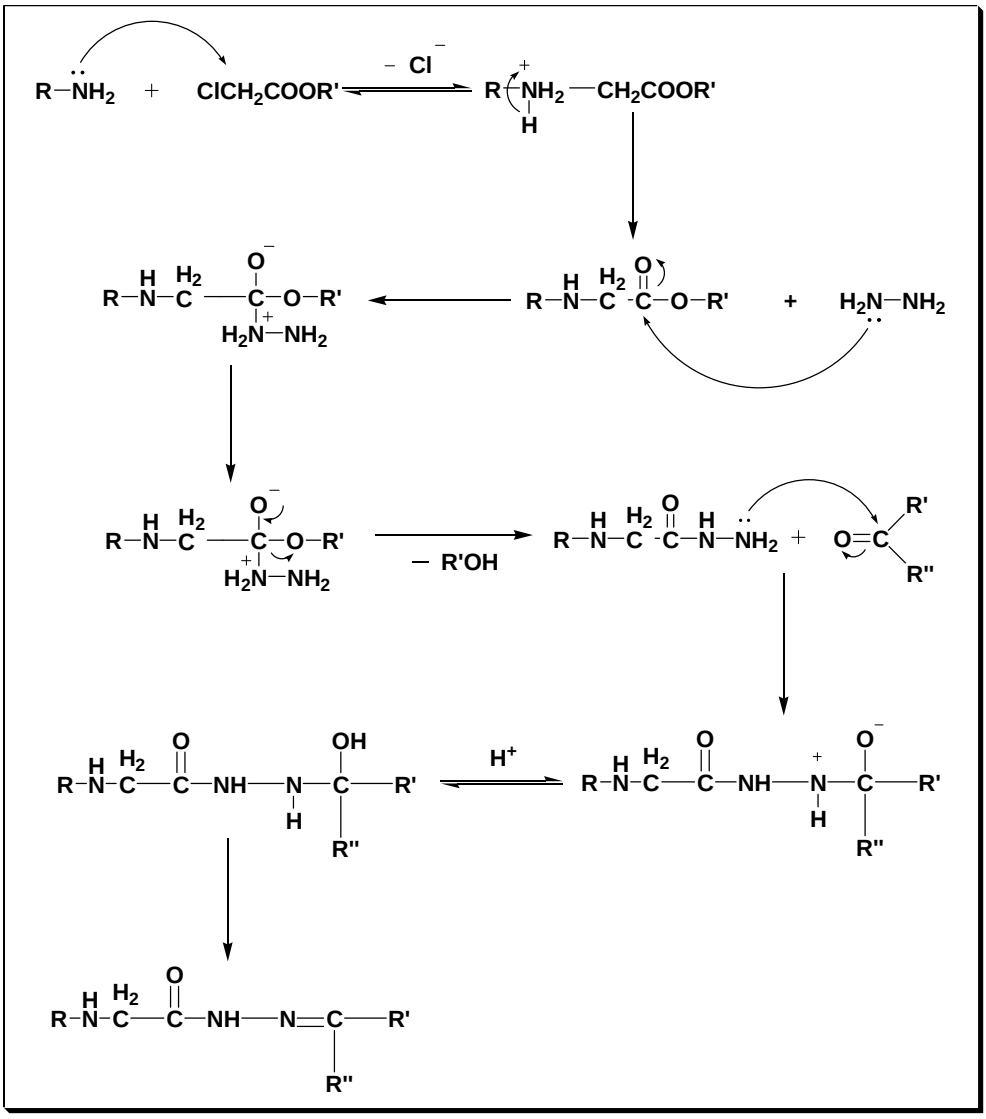
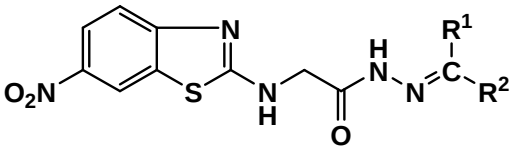
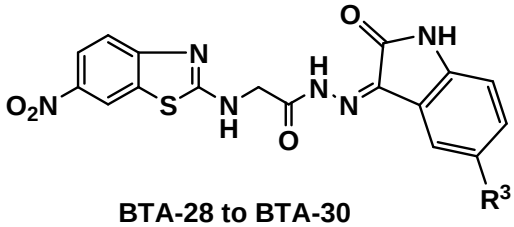


Figure 4.1. Reaction mechanism for synthesis of extended hydrazones

Table 4.2. Structural data of 2-amino-6-nitrobenzothiazole derived extended hydrazones (**BTA-1 to BTA-30**)



BTA-1 to BTA-27



BTA-28 to BTA-30

Code	R^1	R^2	R^3	Mol. Formula	Mol. Wt. (g/mol)
BTA-1	CH_3	CH_3	-	$C_{12}H_{13}N_5O_3S$	307.33
BTA-2	CH_3	C_6H_5	-	$C_{17}H_{15}N_5O_3S$	369.4
BTA-3	CH_3	4-Br C_6H_4	-	$C_{17}H_{14}BrN_5O_3S$	448.29
BTA-4	CH_3	4-Cl C_6H_4	-	$C_{17}H_{14}ClN_5O_3S$	403.84
BTA-5	CH_3	4-F C_6H_4	-	$C_{17}H_{14}FN_5O_3S$	387.39
BTA-6	CH_3	4-OH C_6H_4	-	$C_{17}H_{15}N_5O_4S$	385.40
BTA-7	CH_3	4-NO_2 C_6H_4	-	$C_{17}H_{14}N_6O_5S$	414.40
BTA-8	H	C_6H_5	-	$C_{16}H_{13}N_5O_3S$	355.37
BTA-9	H	2-OH C_6H_4	-	$C_{16}H_{13}N_5O_4S$	371.37
BTA-10	H	4-Br C_6H_4	-	$C_{16}H_{12}BrN_5O_3S$	434.27
BTA-11	H	4-$N(CH_3)_2$ C_6H_4	-	$C_{18}H_{18}N_6O_3S$	398.44
BTA-12	H	4-OH C_6H_4	-	$C_{16}H_{13}N_5O_4S$	371.37
BTA-13	H	4-OCH_3 C_6H_4	-	$C_{17}H_{15}N_5O_4S$	385.4
BTA-14	H	4-NO_2 C_6H_4	-	$C_{16}H_{12}N_6O_5S$	400.37
BTA-15	H	2,3-Cl_2 C_6H_3	-	$C_{16}H_{11}Cl_2N_5O_3S$	424.26
BTA-16	H	2,4-Cl_2 C_6H_3	-	$C_{16}H_{11}Cl_2N_5O_3S$	424.26
BTA-17	H	2,6-Cl_2 C_6H_3	-	$C_{16}H_{11}Cl_2N_5O_3S$	424.26
BTA-18	H	2,5-$(OCH_3)_2$ C_6H_3	-	$C_{18}H_{17}N_5O_5S$	415.42
BTA-19	H	3,4-$(OCH_3)_2$ C_6H_3	-	$C_{18}H_{17}N_5O_5S$	415.42
BTA-20	H	3,4,5-$(OCH_3)_3$ C_6H_2	-	$C_{19}H_{19}N_5O_6S$	445.45
BTA-21	H	4-Cl-C_6H_4-CH_2-O-C_6H_4	-	$C_{23}H_{18}ClN_5O_4S$	495.08
BTA-22	C_6H_5	C_6H_5	-	$C_{22}H_{17}N_5O_3S$	431.47

BTA-23	C_6H_5	4-Cl C_6H_4	-	$C_{22}H_{16}ClN_5O_3S$	465.91
BTA-24	C_6H_5	4-OH C_6H_4	-	$C_{22}H_{17}N_5O_4S$	447.47
BTA-25	4-Cl C_6H_4	4-Cl C_6H_4	-	$C_{22}H_{15}Cl_2N_5O_3S$	500.36
BTA-26	CH_2 Br	4-Br C_6H_4	-	$C_{17}H_{13}Br_2N_5O_3S$	527.19
BTA-27	Cyclohexyl		-	$C_{15}H_{17}N_5O_3S$	347.39
BTA-28	-	-	H	$C_{17}H_{12}N_6O_4S$	396.38
BTA-29	-	-	Cl	$C_{17}H_{11}ClN_6O_4S$	430.83
BTA-30	-	-	NO_2	$C_{17}H_{11}N_7O_6S$	441.38

4.1.2. Characterization

The identification and characterization of the synthesized compounds (**BTA-1** to **BTA-30**) were carried out by the following procedure so as to ascertain the chemical structure of compounds. The complete procedures of all the characterization methods followed and the instruments used are mentioned below.

4.1.2.1. Physicochemical characterization

4.1.2.1.1. Melting point (MP) determination

The purity of the intermediates and final compounds was confirmed by their melting points, determined in one end open capillary tubes using Sonar melting point apparatus.

4.1.2.1.2. Solubility determination

The solubility of synthesized compounds was determined in different polar (water, ethanol, methanol, DMF, DMSO), semi-polar (chloroform, dichloromethane, ethyl acetate) and non-polar solvents (benzene, hexane, toluene) at room temperature.

4.1.2.1.3. Thin layer chromatography (TLC)

TLC is one of the most widely used analytical methods by which mixtures of compounds are separated by their differential migration using stationary and mobile phase.

TLC was performed for all intermediates and final products to monitor the reaction and to confirm the purity of compounds. A single distinct spot was observed that indicated the purity of the final product in each reaction by comparing with the reactant spot, as the R_f value of the product was different from that of the reactant.

The R_f values were calculated by the following formula

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

Adsorbent layer	Silica gel G
Layer thickness	0.25 mm
Size of plates	2.5 x 7.5
Plate preparation technique	Pouring method
Development technique	Ascending
Chamber saturation state	The chamber was lined on three sides by filter paper and saturated for 30 minutes.
Length of run (Solvent Front)	5 – 6.5 cm
Solvent composition	Chloroform: Methanol: Toluene (7:1:2, 7:2:1)
Sample preparation	The sample was dissolved in a suitable solvent
Detection	Iodine chamber

4.1.2.1.4. Determination of partition coefficient (LogP)

The octanol/water partition coefficient ($LogP_{o/w}$) constitutes a quantitative, and easily accessible, hydrophobicity measurement. P is defined as the ratio of the equilibrium concentration of a substance dissolved in a two-phase system, formed by two immiscible solvents.

$$\mathbf{P_{o/w} = C_{octanol}/C_{water}}$$

Where, $C_{octanol}$ = Concentration in the octanol phase

C_{water} = Concentration in the aqueous phase

The common and standard procedure adopted for experimental LogP estimation is the Shake flask method, used to determine the hydrophobicity of compounds with LogP values ranging from -2 to 4. LogP > 0 characterize hydrophobic substances soluble in the lipid phase, while LogP < 0 typifies polar compounds soluble in the water phase.

4.1.2.1.4.1. Shake flask method

The partition coefficient between octanol and phosphate buffer was determined at room temperature [Farrar *et al.*, 1993]. About 10 ml of octanol and 10 ml of phosphate buffer were taken in a glass stoppered graduated flask and 5 mg of accurately weighed compound was added. The mixture was then shaken with the help of mechanical shaker for 24 h at room temperature and then transferred to a separating funnel and allowed to dynamic equilibrate for 6 h. The aqueous and octanol phase were separated and filtered through membrane filter and the compound content in aqueous phase was analyzed by UV spectroscopy.

Table 4.3. Physicochemical characterization data of **ISN-1** to **ISN-3**, **BTE** and **BTH**

Code	MP (°C)	Yield (%)	Colour	R_f[a]	LogP[b]	Expt. LogP[c]
ISN-1	224-228	89.43	Orange	0.54	1.21	0.86
ISN-2	250-252	91.60	Orange	0.51	0.94	-
ISN-3	256-260	75.25	Light Brown	0.32	0.16	-
BTE	260 (Charred)	65.48	Yellow	0.56	2.19	-
BTH	219-221	60.74	Yellow	0.52	0.59	-

*All the compounds were soluble in chloroform (except **BTE** and **BTH**), methanol, ethanol, DMF and DMSO; [a]Solvent system: Chloroform: Methanol (8:2, 9:1); [b]MarvinSketch generated; [c]Shake flask method; '-' indicates 'not tested'.

The physicochemical characterization data of initial fragments **ISN-1** to **ISN-3**, **BTE**, **BTH** and final compounds **BTA-1** to **BTA-30** are presented in **Table 4.3** and **Table 4.4** respectively.

Table 4.4. Physicochemical characterization data of **BTA-1** to **BTA-30**

Code	MP (°C)	Yield (%)	Colour	R_f[a]	LogP[b]	Expt. LogP[c]
BTA-1	204-206	52.07	Yellow	0.48	1.44	-
BTA-2	198-200	49.42	Yellow	0.66	2.87	2.1

BTA-3	232-234	69.87	Yellow	0.57	3.63	2.9
BTA-4	163-165	48.33	Yellow	0.53	3.47	2.8
BTA-5	185-187	68.84	Yellow	0.62	3.01	2.2
BTA-6	238-240	51.18	Yellow	0.64	2.56	-
BTA-7	206-208	72.40	Yellow	0.69	2.81	-
BTA-8	203-205	48.59	Yellow	0.49	3.02	-
BTA-9	190-192	65.99	Yellow	0.66	2.72	2.1
BTA-10	210-212	56.83	Yellow	0.64	3.79	2.9
BTA-11	176-178	52.63	Yellow	0.67	3.13	-
BTA-12	192-194	50.31	Yellow	0.63	2.72	1.7
BTA-13	167-169	72.66	Yellow	0.57	2.86	-
BTA-14	208-210	79.95	Yellow	0.53	2.96	-
BTA-15	239-241	64.28	Yellow	0.60	4.23	3.1
BTA-16	222-224	62.79	Yellow	0.58	4.23	2.9
BTA-17	200-202	71.54	Yellow	0.68	4.23	3.3
BTA-18	242-244	73.12	Yellow	0.54	2.71	1.9
BTA-19	198-200	75.99	Yellow	0.55	2.71	-
BTA-20	137-139	48.57	Yellow	0.51	2.55	-
BTA-21	157-159	67.78	Yellow	0.62	5.19	3.9
BTA-22	178-180	46.08	Yellow	0.48	4.77	3.2
BTA-23	228-230	36.72	Yellow	0.59	5.37	3.5
BTA-24	215-217	58.82	Yellow	0.51	4.46	3.3
BTA-25	161-163	85.83	Reddish brown	0.65	5.98	3.6
BTA-26	186-188	23.98	Maroon	0.54	4.36	3.1

BTA-27	202-204	54.70	Maroon	0.57	2.82	-
BTA-28	210-212	66.43	Maroon	0.59	2.29	1.7
BTA-29	206-208	68.45	Dark brown	0.46	2.89	1.5
BTA-30	178-180	57.40	Yellow	0.59	2.23	1.2

*All the compounds were soluble in methanol, ethanol, DMF and DMSO; [a]Solvent system: Chloroform: Methanol: Toluene (7:1:2, 7:2:1); [b]MarvinSketch generated; [c]Shake flask method; '-' indicates 'not tested'.

4.1.2.2. Spectral characterization and elemental analysis

4.1.2.2.1. UV spectroscopy

This is one of the premier instrumental analytical techniques used for the characterization of organic compounds possessing conjugated system or chromophore in their structures. This technique involves absorption of UV radiation (200 – 400 nm) and results in the transition of electrons from lower energy level to higher energy level.

The UV spectra were obtained for all the synthesized compounds by using SHIMADZU Model 1700 UV/VIS spectrophotometer, at the Department of Pharmaceutics, IIT (BHU), Varanasi, using DMSO as solvent. The λ_{max} value, the wavelength at which maximum absorption occurs, characteristic for each compound, was found and correlated with the conjugated groups present in the structure.

4.1.2.2.2. Infrared (IR) spectroscopy

Infrared (IR) spectroscopy is the technique used for the identification of different functional groups in the organic compounds. The FT-IR spectrum was recorded on SHIMADZU FT-IR 8400S infrared spectrophotometer in the frequency range of 400 to 4000 cm^{-1} at the Department of Pharmaceutics, IIT (BHU), Varanasi, using KBr pellet method for sample preparation. The characteristic absorption bands were identified from the spectra whereby the specific functional groups of each structure were confirmed. The spectral data obtained is expressed in cm^{-1}.

4.1.2.2.3. Nuclear Magnetic Resonance (^{1}H and ^{13}C NMR) spectroscopy

NMR spectroscopy is the most powerful tool for the structural determination of new organic molecules. ^{1}H and ^{13}C NMR spectra were recorded at a frequency of 300 MHz and 75 MHz respectively on a JEOL AL300 FT-NMR spectrometer at ambient temperature using deuterated DMSO ([D_6]DMSO) as a solvent, at the Department of

Chemistry, Faculty of Science, Banaras Hindu University, Varanasi. Chemical shifts were expressed as δ units (ppm) relative to tetramethylsilane (TMS). The exchangeable protons were confirmed by the addition of D_2O. The splitting patterns are designated as follows: s, singlet; d, doublet; t, triplet; dd, doublet of doublet; m, multiplet.

4.1.2.2.4. Mass spectrometry

Mass spectrometry is the most accurate method for determining the molecular mass of a compound. The mass spectrum of a compound helps to establish the structure of a new compound in different ways *viz.* either by providing the exact molecular mass or by revealing the presence of certain structural units in a molecule. The mass spectrum is a graph of the number of particles detected as a function of mass-to-charge ratio. This analysis was done by Electrospray Ionization (ESI) technique. Mass spectra were recorded on a Thermo LCQ Advantage Max ion trap mass spectrometer at SAIF, CDRI, Lucknow.

4.1.2.2.5. Elemental analysis

Elemental analysis is an experiment that determines the amount (typically a weight percent) of an element in a compound. The most common type of elemental analysis is for carbon, hydrogen, and nitrogen (CHN analysis). The elemental analysis of a compound is particularly useful in determining the empirical formula of the compound. Elemental analysis has been performed on Exeter Analytical Inc., USA, Model CE-440 CHN Analyzer, at the Department of Pharmaceutics, IIT (BHU), Varanasi. The results of C, H and N analysis for all the compounds were within ± 0.4% of the theoretical values.

4.1.2.2.6. X-Ray Powder Diffraction (XR-PD)

The powder X-ray diffraction analysis was carried out to confirm the crystallinity of compounds. The compounds were crushed to a uniform fine powder and subjected to powder X-ray diffraction (XRD). The XRD pattern of the compound was recorded on Rigaku MiniFlex II Desktop X-ray diffractometer, at the Department of Ceramic Engineering, IIT (BHU), Varanasi. The intensity versus 2θ was recorded by varying 2θ from 10° to 80° at a rate of 5°/min. The sharp and well defined peaks indicate the good crystalline nature of the sample while broad peaks indicate the amorphous nature of the sample.

All the synthesized compounds (**BTA-1** to **BTA-30**) were subjected to UV, IR, ^{1}H NMR and ^{13}C NMR spectral and elemental analysis and the results are presented below in

section **4.1.2.3.** In addition, compounds **BTA-26** and **BTA-28** were analyzed for the mass spectrometry and the $[M+1]^+$ peak of these compounds is presented below (**Figure 4.25.** and **Figure 4.29.**). Moreover, **BTA-17** was subjected to X-ray powder diffraction and the diffraction pattern is shown in **Figure 4.15.**

4.1.2.3. Spectral characterization and elemental analysis data of intermediates (BTE and BTH) and final compounds (BTA-1 to BTA-30)

Ethyl-2-(6-nitrobenzothiazol-2-ylamino)acetate (BTE): IR (KBr): υ = 3605.08, 3298.38 (N-H str), 3180.72 (C-H str), 3095.85 (aromatic C-H str), 2945.40 (CH_2 str), 1730.21 (C=O str), 1627.97 (C=N str), 1602.90 (C=C str), 1523.82, 1336.71 (NO_2 str), 1296.21 (C-N str), 1130.32 (C-O str); **^{1}H NMR ([D_6]DMSO):** δ = 2.21 (s, 3H, CH_3), 3.37 (s, 2H, CH_2), 5.10 (s, 2H, CH_2), 7.35 (s, 1H, NH), 8.18 (d, 1H, benzothiazole C-5), 8.25 (s, 1H, benzothiazole C-7), 8.38 ppm (d, 1H, benzothiazole C-4); **^{13}C NMR ([D_6]DMSO):** δ = 14.69 (CH_3), 44.73 (CH_2), 68.39 (O-CH_2), 116.29 (benzothiazole C-7), 117.63 (benzothiazole C-5), 121.64 (benzothiazole C-4), 128.52 (benzothiazole C-7a), 145.78 (benzothiazole C-6), 155.42 (benzothiazole C-3a), 158.75 (C=O), 171.86 ppm (benzothiazole C-2); **Elemental analysis for $C_{11}H_{11}N_3O_4S$: calcd:** C 46.97, H 3.94, N 14.94, **found:** C 47.01, H 3.97, N 14.92.

2-(6-nitrobenzothiazol-2-ylamino)acetohydrazide (BTH): IR (KBr): υ = 3402.54, 3284.88 (N-H str), 3093.92 (aromatic C-H str), 2947.33 (CH_2 str), 1647.62 (C=O str), 1595.18 (C=N str), 1572.04 (C=C str), 1519.96, 1327.07 (NO_2 str), 1290.42 (C-N str); **^{1}H NMR ([D_6]DMSO):** δ = 1.98 (s, 2H, NH_2), 3.97 (s, 2H, CH_2), 7.38 (s, 1H, NH), 9.76 (s, 1H, CONH), 8.17 (d, 1H, benzothiazole C-5), 8.23 (s, 1H, benzothiazole C-7), 8.42 ppm (d, 1H, benzothiazole C-4); **^{13}C NMR ([D_6]DMSO):** δ = 79.23 (CH_2), 116.71 (benzothiazole C-7), 117.49 (benzothiazole C-5), 121.25 (benzothiazole C-4), 128.64 (benzothiazole C-7a), 145.38 (benzothiazole C-6), 155.95 (benzothiazole C-3a), 158.92 (C=O), 171.62 ppm (benzothiazole C-2); **Elemental analysis for $C_9H_9N_5O_3S$: calcd:** C 40.45, H 3.39, N 26.20, **found:** C 40.48, H 3.36, N 26.18.

2-(6-nitrobenzothiazol-2-ylamino)-N'-(propan-2-ylidene)acetohydrazide (BTA-1): λ_{max}: 265.40, 374.40 nm; **IR (KBr):** υ = 3525.99, 3340.82 (N-H str), 3156.43 (C-H str), 3097.78 (aromatic C-H str), 2966.62 (CH_2 str), 1647.26 (C=O str), 1597.11 (C=N str),

1570.11 (C=C str), 1531.53, 1330.93 (NO_2 str), 1126.47 (C-N str); **^{1}H NMR ([D_6]DMSO):** δ = 2.16 (s, 6H, CH_3), 4.01 (s, 2H, CH_2), 7.48 (s, 1H, NH), 8.10 (d, 1H, benzothiazole C-5), 8.36 (d, 1H, benzothiazole C-4), 8.66 (s, 1H, CONH), 8.75 ppm (s, 1H, benzothiazole C-7); **^{13}C NMR ([D_6]DMSO):** δ = 14.70, 23.35 (CH_3), 79.54 (CH_2), 116.27 (benzothiazole C-7), 117.66 (benzothiazole C-5), 121.34 (benzothiazole C-4), 128.73 (benzothiazole C-7a), 145.73 (benzothiazole C-6), 153.26 (C=N), 155.52 (benzothiazole C-3a), 158.36 (C=O), 171.56 ppm (benzothiazole C-2); **Elemental analysis for $C_{12}H_{13}N_5O_3S$: calcd:** C 46.90, H 4.26, N 22.79, **found:** C 46.93, H 4.25, N 22.82.

2-(6-nitrobenzothiazol-2-ylamino)-N'-(1-phenylethylidene)acetohydrazide (BTA-2): λ_{max}: 270.60, 375.40 nm; **IR (KBr):** υ = 3406.40, 3294.53 (N-H str), 3090.07 (aromatic C-H str), 2962.76 (CH_2 str), 1678.13 (C=O str), 1627.97 (C=N str), 1570.11 (C=C str), 1527.67, 1327.07 (NO_2 str), 1288.49 (C-N str); **^{1}H NMR ([D_6]DMSO):** δ = 2.25 (s, 3H, CH_3); 4.12 (s, 2H, CH_2), 7.43 (s, 1H, NH), 7.79 (dd, 3H, Ar C-3, Ar C-4, Ar C-5), 7.93 (d, 2H, Ar C-2, Ar C-6), 8.31 (d, 1H, benzothiazole C-5), 8.37 (s, 1H, CONH), 8.41 (d, 1H, benzothiazole C-4), 8.73 ppm (s, 1H, benzothiazole C-7); **^{13}C NMR ([D_6]DMSO):** δ = 14.82 (CH_3), 79.48 (CH_2), 116.81 (benzothiazole C-7), 117.76 (benzothiazole C-5), 121.38 (benzothiazole C-4), 128.65 (benzothiazole C-7a), 129. 79 (Ar C-3, Ar C-5), 130.46 (Ar C-2, Ar C-6), 131.61 (Ar C-4), 133.43 (Ar C-1), 145.51 (benzothiazole C-6), 155.52 (benzothiazole C-3a), 158.96 (C=O), 160.68 (C=N), 171.18 ppm (benzothiazole C-2); **Elemental analysis for $C_{17}H_{15}N_5O_3S$: calcd:** C 55.27, H 4.09, N 18.96, **found:** C 55.30, H 4.12, N 18.92.

N'-(1-(4-bromophenyl)ethylidene)-2-(6-nitrobenzothiazol-2-ylamino)acetohydrazide (BTA-3): λ_{max}: 267.00, 374.80 nm; **IR (KBr):** υ = 3462.34, 3292.60 (N-H str), 3084.28 (aromatic C-H str), 2949.26 (CH_2 str), 1654.98 (C=O str), 1595.18 (C=N str), 1570.11 (C=C str), 1531.53, 1327.07 (NO_2 str), 1301.99 (C-N str), 752.26 (C-Br str); **^{1}H NMR ([D_6]DMSO):** δ = 2.24 (s, 3H, CH_3); 3.36 (s, 2H, CH_2), 6.85 (s, 1H, NH), 7.06 (d, 2H, Ar C-3, Ar C-5), 7.38 (d, 2H, Ar C-2, Ar C-6), 7.83 (d, 1H, benzothiazole C-5), 8.05 (d, 1H, benzothiazole C-4), 8.24 (s, 1H, benzothiazole C-7), 8.65 ppm (s, 1H, CONH); **^{13}C NMR ([D_6]DMSO):** δ = 14.99 (CH_3), 79.17 (CH_2), 116.80 (benzothiazole C-7), 117.65

(benzothiazole C-5), 121.95 (benzothiazole C-4), 128.52 (benzothiazole C-7a), 131.38 (Ar C-2, Ar C-6), 131.56 (Ar C-3, Ar C-5), 136.68 (Ar C-4), 137.25 (Ar C-1), 145.65 (benzothiazole C-6), 155.06 (benzothiazole C-3a), 158.57 (C=O), 160.33 (C=N), 171.77 ppm (benzothiazole C-2); **Elemental analysis for $C_{17}H_{14}BrN_5O_3S$: calcd:** C 45.55, H 3.15, N 15.62, **found:** C 45.58, H 3.11, N 15.65.

N'-(1-(4-chlorophenyl)ethylidene)-2-(6-nitrobenzothiazol-2-ylamino)acetohydrazide (BTA-4): λ_{max}: 272.40, 376.40 nm; **IR (KBr):** υ = 3525.99, 3367.82 (N-H str), 3070.78 (aromatic C-H str), 2968.36 (CH_2 str), 1647.26 (C=O str), 1593.25 (C=N str), 1570.11 (C=C str), 1527.67, 1327.07 (NO_2 str), 1292.35 (C-N str), 829.42 (C-Br str); **^{1}H NMR ([D_6]DMSO):** δ = 2.26 (s, 3H, CH_3), 3.91 (s, 2H, CH_2), 6.87 (s, 1H, NH), 7.54 (d, 2H, Ar C-3, Ar C-5), 7.72 (d, 2H, Ar C-2, Ar C-6), 8.23 (s, 1H, benzothiazole C-7), 8.51 (d, 1H, benzothiazole C-5), 8.69 (s, 1H, CONH), 8.73 ppm (d, 1H, benzothiazole C-4); **^{13}C NMR ([D_6]DMSO):** δ = 14.56 (CH_3), 79.38 (CH_2), 116.74 (benzothiazole C-7), 117.83 (benzothiazole C-5), 121.99 (benzothiazole C-4), 128.33 (benzothiazole C-7a), 129.60 (Ar C-3, Ar C-5), 130.87 (Ar C-2, Ar C-6), 132.21 (Ar C-1), 136.73 (Ar C-4), 145.67 (benzothiazole C-6), 155.68 (benzothiazole C-3a), 158.90 (C=O), 160.62 (C=N), 171.57 ppm (benzothiazole C-2); **Elemental analysis for $C_{17}H_{14}ClN_5O_3S$: calcd:** C 50.56, H 3.49, N 17.34, **found:** C 50.52, H 3.51, N 17.31.

N'-(1-(4-fluorophenyl)ethylidene)-2-(6-nitrobenzothiazol-2-ylamino)acetohydrazide (BTA-5): λ_{max}: 268.20, 368.60 nm; **IR (KBr):** υ = 3406.40, 3290.67 (N-H str), 3090.07 (aromatic C-H str), 2958.90 (CH_2 str), 1674.27 (C=O str), 1624.12 (C=N str), 1597.11 (C=C str), 1523.82, 1330.93 (NO_2 str), 1292.35 (C-N str), 1126.47 (C-F str); **^{1}H NMR ([D_6]DMSO):** δ = 2.21 (s, 3H, CH_3), 3.85 (s, 2H, CH_2), 6.99 (s, 1H, NH), 7.70 (d, 2H, Ar C-2, Ar C-6), 7.14 (d, 2H, Ar C-3, Ar C-5), 8.22 (s, 1H, benzothiazole C-7), 8.56 (d, 1H, benzothiazole C-5), 8.63 (s, 1H, CONH), 8.71 ppm (d, 1H, benzothiazole C-4); **^{13}C NMR ([D_6]DMSO):** δ = 14.53 (CH_3), 79.71 (CH_2), 115.52 (Ar C-3, Ar C-5), 116.58 (benzothiazole C-7), 117.74 (benzothiazole C-5), 121.36 (benzothiazole C-4), 128.61 (Ar C-1), 128.63 (benzothiazole C-7a), 131.37 (Ar C-2, Ar C-6), 145.87 (benzothiazole C-6), 155.54 (benzothiazole C-3a), 158.41 (C=O), 160.49 (C=N), 165.59 (Ar C-4), 171.19 ppm

(benzothiazole C-2); **Elemental analysis for $C_{17}H_{14}FN_5O_3S$: calcd:** C 52.71, H 3.64, N 18.08, **found:** C 52.73, H 3.67, N 18.11.

N'-(1-(4-hydroxyphenyl)ethylidene)-2-(6-nitrobenzothiazol-2-ylamino)acetohydrazide (BTA-6): λ_{max}: 273.40, 372.00 nm; **IR (KBr):** υ = 3463.72 (O-H str), 3294.33 (N-H str), 3056.86 (aromatic C-H str), 2952.16 (CH_2 str), 1657.35 (C=O str), 1597.45 (C=N str), 1570.11 (C=C str), 1527.30, 1327.07 (NO_2 str), 1292.29 (C-N str); **^{1}H NMR ([D_6]DMSO):** δ = 2.31 (s, 3H, CH_3), 3.85 (s, 2H, CH_2), 6.32 (s, 1H, NH), 6.93 (d, 2H, Ar C-3, Ar C-5), 7.55 (d, 2H, Ar C-2, Ar C-6), 8.51 (d, 1H, benzothiazole C-5), 8.68 (s, 1H, CONH), 8.82 (d, 1H, benzothiazole C-4), 8.98 ppm (s, 1H, benzothiazole C-7); **^{13}C NMR ([D_6]DMSO):** δ = 13.98 (CH_3), 79.69 (CH_2), 116.31 (benzothiazole C-7), 116.39 (Ar C-3, Ar C-5), 118.26 (benzothiazole C-5), 123.51 (benzothiazole C-4), 125.42 (Ar C-1), 128.29 (benzothiazole C-7a), 131.65 (Ar C-2, Ar C-6), 145.62 (benzothiazole C-6), 155.23 (benzothiazole C-3a), 160.35 (Ar C-4), 162.56 (C=N), 158.73 (C=O), 171.45 ppm (benzothiazole C-2); **Elemental analysis for $C_{17}H_{15}N_5O_3S$: calcd:** C 52.98, H 3.92, N 18.17, **found:** C C 53.02, H 3.95, N 18.14.

2-(6-nitrobenzothiazol-2-ylamino)-N'-(1-(4-nitrophenyl)ethylidene)acetohydrazide (BTA-7): λ_{max}: 276.80, 383.80 nm; **IR (KBr):** υ = 3460.41, 3298.38 (N-H str), 3063.06 (aromatic C-H str), 2947.33 (CH_2 str), 1654.98 (C=O str), 1593.25 (C=N str), 1570.11 (C=C str), 1535.39, 1330.93 (NO_2 str), 1300.07 (C-N str); **^{1}H NMR ([D_6]DMSO, D_2O exchange):** δ = 2.33 (s, 3H, CH_3), 3.36 (s, 2H, CH_2), 7.43 (s, 1H, NH), 8.07 (d, 2H, Ar C-2, Ar C-6), 8.10 (d, 2H, Ar C-3, Ar C-5), 8.15 (s, 1H, benzothiazole C-7), 8.18 (d, 1H, benzothiazole C-5), 8.29 (d, 1H, benzothiazole C-4), 8.68 ppm (s, 1H, CONH); **^{13}C NMR ([D_6]DMSO):** δ = 14.99 (CH_3), 79.17 (CH_2), 116.80 (benzothiazole C-7), 117.62 (benzothiazole C-5), 121.94 (Ar C-3, Ar C-5), 123.58 (benzothiazole C-4), 127.80 (benzothiazole C-7a), 129.49 (Ar C-2, Ar C-6), 131.55 (Ar C-1), 140.65 (benzothiazole C-6), 143.27 (Ar C-4), 148.05 (benzothiazole C-3a), 156.06 (C=N), 158.55 (C=O), 171.74 ppm (benzothiazole C-2); **Elemental analysis for $C_{17}H_{14}N_6O_5S$: calcd:** C 49.27, H 3.41, N 20.28, **found:** C 49.32, H 3.43, N 20.25.

N'-benzylidene-2-(6-nitrobenzothiazol-2-ylamino)acetohydrazide (BTA-8): λ_{max}: 273.40, 364.20 nm; **IR (KBr):** υ = 3522.13, 3294.53 (N-H str), 3093.92 (aromatic C-H

str), 2955.04 (CH_2 str), 1647.26 (C=O str), 1597.26 (C=N str), 1570.11 (C=C str), 1527.67, 1330.93 (NO_2 str), 1296.21 (C-N str); **^{1}H NMR ([D_6]DMSO):** δ = 3.38 (s, 2H, CH_2), 7.39 (s, 1H, NH), 7.51 (dd, 3H, Ar C-3, Ar C-4, Ar C-5), 7.89 (d, 2H, Ar C-2, Ar C-6), 8.36 (s, 1H, C-H), 8.53 (d, 1H, benzothiazole C-5), 8.64 (s, 1H, CONH), 8.79 (d, 1H, benzothiazole C-4), 8.91 ppm (s, 1H, benzothiazole C-7); **^{13}C NMR ([D_6]DMSO):** δ = 79.81 (CH_2), 116.87 (benzothiazole C-7), 117.20 (benzothiazole C-5), 123.19 (benzothiazole C-4), 126.31 (benzothiazole C-7a), 128.54 (Ar C-3, Ar C-5), 129.27 (Ar C-2, Ar C-6), 132.03 (Ar C-4), 133.36 (Ar C-1), 143.71 (C=N), 145.57 (benzothiazole C-6), 155.12 (benzothiazole C-3a), 158.96 (C=O), 171.50 ppm (benzothiazole C-2); **Elemental analysis for $C_{16}H_{13}N_5O_3S$: calcd:** C 54.08, H 3.69, N 19.71, **found:** C 54.05, H 3.72, N 19.69.

N'-(2-hydroxybenzylidene)-2-(6-nitrobenzothiazol-2-ylamino)acetohydrazide (BTA-9): λ_{max}: 273.20, 371.40 nm; **IR (KBr):** υ = 3542.24 (OH str), 3356.25, 3290.67 (N-H str), 3090.07 (aromatic C-H str), 2943.47 (CH_2 str), 1647.26 (C=O str), 1620.26 (C=N str), 1573.97 (C=C str), 1527.67, 1327.07 (NO_2 str), 1288.48 (C-N str); **^{1}H NMR ([D_6]DMSO):** δ = 4.11 (s, 2H, CH_2), 5.08 (s, 1H, OH), 6.91 (d, 2H, Ar C-3, Ar C-5), 7.36 (s, 1H, NH), 7.26 (d, 2H, Ar C-2, Ar C-4), 8.26 (s, 1H, C-H), 8.36 (s, 1H, C-H), 8.50 (d, 1H, benzothiazole C-5), 8.65 (s, 1H, CONH), 8.79 (d, 1H, benzothiazole C-4), 8.96 ppm (s, 1H, benzothiazole C-7); **^{13}C NMR ([D_6]DMSO):** δ = 79.29 (CH_2), 116.37 (Ar C-3), 116.80 (benzothiazole C-7), 117.56 (benzothiazole C-5), 119.24 (Ar C-1), 120.14 (Ar C-6), 122.68 (Ar C-5), 122.72 (benzothiazole C-4), 125.99 (benzothiazole C-7a), 133.53 (Ar C-4), 143.60 (C=N), 145.69 (benzothiazole C-6), 155.48 (benzothiazole C-3a), 158.92 (C=O), 161.11 (Ar C-2), 172.02 ppm (benzothiazole C-2); **Elemental analysis for $C_{16}H_{13}N_5O_4S$: calcd:** C 51.75, H 3.53, N 18.86, **found:** C 51.72, H 3.55, N 18.84.

N'-(4-bromobenzylidene)-2-(6-nitrobenzothiazol-2-ylamino)acetohydrazide (BTA-10): λ_{max}: 267.60, 358.00 nm; **IR (KBr):** υ = 3460.41, 3298.38 (N-H str), 3093.92 (aromatic C-H str), 2947.33 (CH_2 str), 1651.12 (C=O str), 1593.25 (C=N str), 1570.11 (C=C str), 1531.53, 1330.93 (NO_2 str), 1296.21 (C-N str), 748.41 (C-Br str); **^{1}H NMR ([D_6]DMSO):** δ = 3.45 (s, 2H, CH_2), 6.96 (s, 1H, NH), 7.24 (d, 2H, Ar C-3, Ar C-5), 7.41 (d, 2H, Ar C-2, Ar C-6), 7.76 (d, 1H, benzothiazole C-5), 8.06 (d, 1H, benzothiazole C-

4), 8.21 (s, 1H, benzothiazole C-7), 8.45 (s, 1H, C-H), 8.65 ppm (s, 1H, CONH); **^{13}C NMR ([D$_6$]DMSO):** δ = 79.15 (CH_2), 116.87 (benzothiazole C-7), 117.69 (benzothiazole C-5), 122.00 (benzothiazole C-4), 126.70 (benzothiazole C-7a), 131.58 (Ar C-4), 132.02 (Ar C-2, Ar C-6), 136.72 (Ar C-3, Ar C-5), 140.69 (Ar C-1), 143.86 (C=N), 146.60 (benzothiazole C-6), 155.83 (benzothiazole C-3a), 158.58 (C=O), 171.78 ppm (benzothiazole C-2); **Elemental analysis for $C_{16}H_{12}BrN_5O_3S$: calcd:** C 44.25, H 2.79, N 16.13, **found:** C 44.23, H 2.77, N 16.16.

N'-(4-(dimethylamino)benzylidene)-2-(6-nitrobenzothiazol-2-ylamino)acetohydrazide (BTA-11): λ_{max}: 264.60, 378.80 nm; **IR (KBr):** υ = 3525.99, 3336.96 (N-H str), 3172.65 (C-H str), 3093.92 (aromatic C-H str), 2916.47 (CH_2 str), 1651.12 (C=O str), 1604.83 (C=N str), 1570.11 (C=C str), 1527.67, 1330.93 (NO_2 str), 1300.07 (C-N str); **^{1}H NMR ([D$_6$]DMSO):** δ = 2.91 (s, 6H, CH_3), 3.78 (s, 2H, CH_2), 6.83 (d, 2H, Ar C-3, Ar C-5), 7.26 (s, 1H, NH), 7.56 (d, 2H, Ar C-2, Ar C-6), 7.98 (s, 1H, C-H), 8.60 (s, 1H, CONH), 8.67 (d, 1H, benzothiazole C-5), 8.99 (d, 1H, benzothiazole C-4), 9.23 ppm (s, 1H, benzothiazole C-7); **^{13}C NMR ([D$_6$]DMSO):** δ = 23.49 (CH_3), 79.17 (CH_2), 112.63 (Ar C-3, Ar C-5), 116.75 (benzothiazole C-7), 121.66 (benzothiazole C-4), 123.24 (Ar C-1), 126.69 (benzothiazole C-7a), 130.71 (Ar C-2, Ar C-6), 143.28 (C=N), 145.30 (benzothiazole C-6), 150.41 (Ar C-4), 156.25 (benzothiazole C-3a), 158.20 (C=O), 171.27 (benzothiazole C-5), 171.62 ppm (benzothiazole C-2); **Elemental analysis for $C_{18}H_{18}N_6O_3S$: calcd:** C 54.26, H 4.55, N 21.09, **found:** C 54.22, H 4.57, N 21.12.

N'-(4-hydroxybenzylidene)-2-(6-nitrobenzothiazol-2-ylamino)acetohydrazide (BTA-12): λ_{max}: 265.20, 341.20 nm; **IR (KBr):** υ = 3510.56 (O-H str), 3441.12, 3298.38 (N-H str), 3086.21 (aromatic C-H str), 2943.47 (CH_2 str), 1647.26 (C=O str), 1608.69 (C=N str), 1573.97 (C=C str), 1510.96, 1330.93 (NO_2 str), 1296.21 (C-N str); **^{1}H NMR ([D$_6$]DMSO):** δ = 3.71 (s, 2H, CH_2), 6.88 (s, 1H, NH), 7.40 (d, 2H, Ar C-3, Ar C-5), 7.68 (d, 2H, Ar C-2, Ar C-6), 8.07 (d, 1H, benzothiazole C-5), 8.12 (s, 1H, C-H), 8.24 (s, 1H, benzothiazole C-7), 8.67 (d, 1H, benzothiazole C-4), 8.85 ppm (s, 1H, CONH); **^{13}C NMR ([D$_6$]DMSO):** δ = 100.07 (CH_2), 115.72 (Ar C-3, Ar C-5), 116.82 (benzothiazole C-7), 117.67 (benzothiazole C-5), 121.96 (benzothiazole C-4), 125.08 (benzothiazole C-7a), 130.05 (Ar C-1), 131.55 (Ar C-2, Ar C-6), 140.65 (C=N), 145.24 (benzothiazole C-

6), 156.62 (benzothiazole C-3a), 158.57 (C=O), 160.33 (Ar C-4), 171.76 ppm (benzothiazole C-2); **Elemental analysis for $C_{16}H_{13}N_5O_4S$: calcd:** C 51.75, H 3.53, N 18.86, **found:** C 51.78, H 3.50, N 18.90.

N'-(4-methoxybenzylidene)-2-(6-nitrobenzothiazol-2-ylamino)acetohydrazide (BTA-13): λ_{max}: 270.60, 369.60 nm; **IR (KBr):** υ = 3448.68, 3294.53 (N-H str), 3097.78 (aromatic C-H str), 2966.62 (CH_2 str), 1647.31 (C=O str), 1604.83 (C=N str), 1531.53 (C=C str), 1508.38, 1330.93 (NO_2 str), 1300.07 (C-N str), 1253.77 (C-O str); **^{1}H NMR ([D_6]DMSO):** δ = 3.87 (s, 3H, OCH_3), 4.12 (s, 2H, CH_2), 6.98 (s, 1H, NH), 7.12 (d, 2H, Ar C-3, Ar C-5), 7.71 (d, 2H, Ar C-2, Ar C-6), 8.15 (s, 1H, C-H), 8.37 (d, 1H, benzothiazole C-5), 8.51 (s, 1H, CONH), 8.67 (d, 1H, benzothiazole C-4), 8.83 ppm (s, 1H, benzothiazole C-7); **^{13}C NMR ([D_6]DMSO):** δ = 56.95 (OCH_3), 79.61 (CH_2), 114.37 (Ar C-3, Ar C-5), 116.11 (benzothiazole C-7), 117.90 (benzothiazole C-5), 121.72 (benzothiazole C-4), 125.46 (benzothiazole C-7a), 126.79 (Ar C-1), 131.02 (Ar C-2, Ar C-6), 144.51 (C=N), 145.74 (benzothiazole C-6), 155.19 (benzothiazole C-3a), 158.63 (C=O), 161.18 (Ar C-4), 171.15 ppm (benzothiazole C-2); **Elemental analysis for $C_{17}H_{15}N_5O_4S$: calcd:** C 52.98, H 3.92, N 18.17, **found:** C 52.95, H 3.90, N 18.20.

N'-(4-nitrobenzylidene)-2-(6-nitrobenzothiazol-2-ylamino)acetohydrazide (BTA-14): λ_{max}: 271.40, 356.00 nm; **IR (KBr):** υ = 3510.56, 3333.10 (N-H str), 3090.07 (aromatic C-H str), 2958.90 (CH_2 str), 1647.26 (C=O str), 1593.25 (C=N str), 1570.11 (C=C str), 1523.82, 1330.93 (NO_2 str), 1296.21 (C-N str); **^{1}H NMR ([D_6]DMSO):** δ = 3.34 (s, 2H, CH_2), 6.85 (s, 1H, NH), 7.12 (d, 2H, Ar C-2, Ar C-6), 7.40 (d, 2H, Ar C-3, Ar C-5), 7.68 (s, 1H, C-H), 8.08 (d, 1H, benzothiazole C-5), 8.09 (d, 1H, benzothiazole C-4), 8.23 (s, 1H, benzothiazole C-7), 8.67 ppm (s, 1H, CONH); **^{13}C NMR ([D_6]DMSO):** δ = 79.15 (CH_2), 116.80 (benzothiazole C-7), 117.64 (benzothiazole C-5), 121.94 (Ar C-3, Ar C-5), 125.37 (benzothiazole C-4), 130.56 (benzothiazole C-7a), 131.54 (Ar C-2, Ar C-6), 137.06 (Ar C-1), 140.65 (C=N), 145.07 (benzothiazole C-6), 150.19 (Ar C-4), 155.08 (benzothiazole C-3a), 158.55 (C=O), 171.73 ppm (benzothiazole C-2); **Elemental analysis for $C_{16}H_{12}N_6O_5S$: calcd:** C 48.00, H 3.02, N 20.99, **found:** C 48.03, H 2.99, N 21.01.

N'-(2,3-dichlorobenzylidene)-2-(6-nitrobenzothiazol-2-ylamino)acetohydrazide (BTA-15): λ_{max}: 273.20, 271.80 nm; **IR (KBr):** υ = 3404.47, 3294.53 (N-H str), 3176.87 (aromatic C-H str), 2928.04 (CH_2 str), 1674.27 (C=O str), 1593.25 (C=N str), 1570.11 (C=C str), 1523.82, 1327.07 (NO_2 str), 1290.42 (C-N str), 748.41 (C-Cl str); **^{1}H NMR ([D_6]DMSO):** δ = 3.37 (s, 2H, CH_2), 6.68 (s, 1H, NH), 7.52 (dd, 1H, Ar C-5), 7.74 (d, 1H, Ar C-4), 7.91 (d, 1H, Ar C-5), 8.05 (s, 1H, C-H), 8.25 (s, 1H, benzothiazole C-7), 8.45 (d, 1H, benzothiazole C-5), 8.67 (d, 1H, benzothiazole C-4), 8.69 ppm (s, 1H, CONH); **^{13}C NMR ([D_6]DMSO):** δ = 79.27 (CH_2), 116.31 (benzothiazole C-7), 117.73 (benzothiazole C-5), 125.41 (benzothiazole C-4), 126.21 (benzothiazole C-7a), 128.77 (Ar C-5), 128.98 (Ar C-6), 131.23 (Ar C-2), 132.52 (Ar C-4), 133.60 (Ar C-3), 135.43 (Ar C-1), 140.84 (C=N), 145.48 (benzothiazole C-6), 155.59 (benzothiazole C-3a), 158.57 (C=O), 171.35 ppm (benzothiazole C-2); **Elemental analysis for $C_{16}H_{11}Cl_2N_5O_3S$: calcd:** C 45.30, H 2.61, N 16.51, **found:** C 45.34, H 2.63, N 16.49.

N'-(2,4-dichlorobenzylidene)-2-(6-nitrobenzothiazol-2-ylamino)acetohydrazide (BTA-16): λ_{max}: 266.40, 372.80 nm; **IR (KBr):** υ = 3512.49, 3342.75 (N-H str), 3090.07 (aromatic C-H str), 2949.25 (CH_2 str), 1647.26 (C=O str), 1597.11 (C=N str), 1570.11 (C=C str), 1529.60, 1327.07 (NO_2 str), 1298.14 (C-N str), 752.26 (C-Cl str); **^{1}H NMR ([D_6]DMSO):** δ = 3.34 (s, 2H, CH_2), 6.69 (s, 1H, NH), 6.93 (s, 1H, Ar C-3), 7.13 (d, 1H, Ar C-5), 7.38 (d, 1H, Ar C-6), 7.66 (s, 1H, C-H), 8.04 (d, 1H, benzothiazole C-5), 8.07 (d, 1H, benzothiazole C-4), 8.24 (s, 1H, benzothiazole C-7), 8.65 ppm (s, 1H, CONH); **^{13}C NMR ([D_6]DMSO):** δ = 79.16 (CH_2), 116.81 (benzothiazole C-7), 117.66 (benzothiazole C-5), 121.95 (benzothiazole C-4), 125.22 (benzothiazole C-7a), 130.50 (Ar C-5), 131.56 (Ar C-3), 136.81 (Ar C-1), 137.64 (Ar C-6), 139.66 (Ar C-2), 140.64 (Ar C-4), 143.01 (C=N), 146.03 (benzothiazole C-6), 155.57 (benzothiazole C-3a), 158.58 (C=O), 171.76 ppm (benzothiazole C-2); **Elemental analysis for $C_{16}H_{11}Cl_2N_5O_3S$: calcd:** C 45.30, H 2.61, N 16.51, **found:** C 45.27, H 2.64, N 16.53.

N'-(2,6-dichlorobenzylidene)-2-(6-nitrobenzothiazol-2-ylamino)acetohydrazide (BTA-17): λ_{max}: 273.40, 359.20 nm; **IR (KBr):** υ = 3460.41 (N-H str), 3298.38 (N-H str), 3074.63 (aromatic C-H str), 2947.33 (CH_2 str), 1651.12 (C=O str), 1597.11 (C=N str), 1570.11 (C=C str), 1527.67, 1330.93 (NO_2 str), 1292.35 (C-N str), 779.27, 752.26

(C-Cl str); **^{1}H NMR ([D$_6$]DMSO):** δ = 3.43 (s, 2H, CH_2), 7.06 (m, 3H, Ar C-3, Ar C-4, Ar C-5), 7.37 (s, 1H, NH), 7.40 (s, 1H, C-H), 7.87 (d, 1H, benzothiazole C-5), 8.06 (d, 1H, benzothiazole C-4), 8.24 (s, 1H, benzothiazole C-7), 8.65 ppm (s, 1H, CONH); **^{13}C NMR ([D$_6$]DMSO):** δ = 79.41 (CH_2), 121.94 (benzothiazole C-4), 117.64 (benzothiazole C-5), 116.79 (benzothiazole C-7), 124.00 (benzothiazole C-7a), 129.80 (Ar C-3, Ar C-5), 131.56 (Ar C-1), 135.25 (Ar C-4), 135.90 (Ar C-2, Ar C-6), 140.63 (C=N), 145.06 (benzothiazole C-6), 155.35 (benzothiazole C-3a), 158.56 (C=O), 171.77 ppm (benzothiazole C-2); **Elemental analysis for $C_{16}H_{11}Cl_2N_5O_3S$: calcd:** C 45.30, H 2.61, N 16.51, **found:** C 45.33, H 2.59, N 16.55.

N'-(2,5-dimethoxybenzylidene)-2-(6-nitrobenzothiazol-2-ylamino)acetohydrazide (BTA-18): λ_{max}: 265.20, 375.60 nm; **IR (KBr):** υ = 3452.34, 3296.46 (N-H str), 3076.55 (aromatic C-H str), 2949.26 (CH_2 str), 1680.05 (C=O str), 1599.04 (C=N str), 1570.11 (C=C str), 1529.60, 1327.07 (NO_2 str), 1298.14 (C-N str), 1124.54 (C-O); **^{1}H NMR ([D$_6$]DMSO):** δ = 3.35 (s, 2H, CH_2), 3.89 (s, 6H, OCH_3), 7.05 (s, 1H, Ar C-6), 7.23 (s, 1H, NH), 7.37 (d, 2H, Ar C-3, Ar C-4), 7.75 (s, 1H, C-H), 8.03 (d, 1H, benzothiazole C-5), 8.06 (d, 1H, benzothiazole C-4), 8.24 (s, 1H, benzothiazole C-7), 8.65 ppm (s, 1H, CONH); **^{13}C NMR ([D$_6$]DMSO):** δ = 56.05 (OCH_3), 76.77 (CH_2), 171.76 (benzothiazole C-2), 156.66 (benzothiazole C-3a), 130.07 (benzothiazole C-4), 125.38 (benzothiazole C-5), 145.56 (benzothiazole C-6), 117.63 (benzothiazole C-7), 131.52 (benzothiazole C-7a), 121.94 (Ar C-1), 151.00 (Ar C-2), 110.22 (Ar C-6), 115.59 (Ar C-3), 153.52 (Ar C-5), 116.80 (Ar C-4), 140.65 (C=N), 158.56 ppm (C=O); **Elemental analysis for $C_{18}H_{17}N_5O_5S$: calcd:** C 52.04, H 4.12, N 16.86, **found:** C 52.07, H 4.17, N 16.82.

N'-(3,4-dimethoxybenzylidene)-2-(6-nitrobenzothiazol-2-ylamino)acetohydrazide (BTA-19): λ_{max}: 268.00, 364.80 nm; **IR (KBr):** υ = 3456.69, 3293.58 (N-H str), 3068.76 (aromatic C-H str), 2954.43 (CH_2 str), 1657.69 (C=O str), 1599.11 (C=N str), 1564.32 (C=C str), 1530.71, 1330.91 (NO_2 str), 1300.07 (C-N str), 1129.16 (C-O); **^{1}H NMR ([D$_6$]DMSO):** δ = 3.75 (s, 2H, CH_2), 3.91 (s, 6H, OCH_3), 6.54 (d, 1H, Ar C-5), 7.13 (s, 1H, Ar C-2), 7.34 (s, 1H, NH), 7.52 (d, 1H, Ar C-6), 8.12 (s, 1H, C-H), 8.23 (s, 1H, benzothiazole C-7), 8.50 (d, 1H, benzothiazole C-5), 8.63 (s, 1H, CONH), 8.76 ppm (d, 1H, benzothiazole C-4); **^{13}C NMR ([D$_6$]DMSO):** δ = 56.83 (OCH_3), 76.88 (CH_2), 114.43

(Ar C-2), 115.44 (Ar C-5), 117.10 (benzothiazole C-7), 123.38 (Ar C-6), 125.46 (benzothiazole C-5), 127.72 (Ar C-1), 130.14 (benzothiazole C-4), 131.33 (benzothiazole C-7a), 143.69 (C=N), 145.81 (benzothiazole C-6), 150.01 (Ar C-3), 152.31 (Ar C-4), 156.61 (benzothiazole C-3a), 158.89 (C=O), 171.47 ppm (benzothiazole C-2); **Elemental analysis for $C_{18}H_{17}N_5O_5S$: calcd:** C 52.04, H 4.12, N 16.86, **found:** C 52.01, H 4.15, N 16.89.

N'-(3,4,5-trimethoxybenzylidene)-2-(6-nitrobenzothiazol-2-ylamino)acetohydrazide (BTA-20): λ_{max}: 271.60, 375.00 nm; **IR (KBr):** υ = 3452.70, 3294.53 (N-H str), 3093.92 (aromatic C-H str), 2947.33 (CH_2 str), 1681.68.69 (C=O str), 1627.97 (C=N str), 1523.82 (C=C str), 1504.53, 1334.78 (NO_2 str), 1292.35 (C-N str), 1126.47 (C-O); **^{1}H NMR ([D_6]DMSO):** δ = 3.39 (s, 2H, CH_2), 3.89 (s, 9H, OCH_3), 6.92 (s, 2H, Ar C-2, Ar C-6), 7.28 (s, 1H, NH), 8.22 (s, 1H, C-H), 8.32 (s, 1H, benzothiazole C-7), 8.42 (d, 1H, benzothiazole C-5), 8.62 (s, 1H, CONH), 8.72 ppm (d, 1H, benzothiazole C-4); **^{13}C NMR ([D_6]DMSO):** δ = 56.90 (OCH_3), 76.21 (CH_2), 107.52 (Ar C-2, Ar C-6), 117.29 (benzothiazole C-7), 125.41 (benzothiazole C-5), 128.91 (Ar C-1), 130.09 (benzothiazole C-4), 131.44 (benzothiazole C-7a), 142.25 (Ar C-4), 143.69 (C=N), 145.32 (benzothiazole C-6), 150.11 (Ar C-3, Ar C-5), 155.94 (benzothiazole C-3a), 158.89 (C=O), 171.59 ppm (benzothiazole C-2); **Elemental analysis for $C_{19}H_{19}N_5O_6S$: calcd:** C 51.23, H 4.30, N 15.72, **found:** C 51.20, H 4.34, N 15.69.

N'-(4-(4-chlorobenzyloxy)benzylidene)-2-(6-nitrobenzo[d]thiazol-2-ylamino)acetohydrazide (BTA-21): λ_{max}: 268.40, 371.20 nm; **IR (KBr):** υ = 3525.99 (N-H str), 3340.82 (N-H str), 3097.78 (aromatic C-H str), 2970.48 (CH_2 str), 1651.12 (C=O str), 1595.18 (C=N str), 1570.11 (C=C str), 1531.53, 1330.93 (NO_2 str), 1300.07 (C-N str), 1126.47 (C-N str), 752.26 (C-Cl str); **^{1}H NMR ([D_6]DMSO):** δ = 3.36 (s, 2H, CH_2), 5.18 (s, 2H, O-CH_2), 7.07 (d, 2H, Ar' C-2, Ar' C-6), 7.40 (s, 1H, NH), 7.43 (d, 2H, Ar' C-3, Ar' C-5), 7.66 (s, 1H, C-H), 7.88 (d, 2H, Ar C-3, Ar C-5), 8.09 (d, 2H, Ar C-2, Ar C-6), 8.25 (s, 1H, benzothiazole C-7), 8.68 (s, 1H, CONH), 9.08 (d, 1H, benzothiazole C-5), 9.48 ppm (d, 1H, benzothiazole C-4); **^{13}C NMR ([D_6]DMSO):** δ = 68.56 (O-CH_2), 78.96 (CH_2), 114.58 (Ar C-3, Ar C-5), 115.25 (benzothiazole C-7), 116.81 (benzothiazole C-5), 117.65 (benzothiazole C-4), 121.94 (benzothiazole C-7a), 123.28 (Ar C-1), 128.44 (Ar C-2, Ar

C-6), 129.54 (Ar' C-2, Ar' C-6), 131.31 (Ar' C-3, Ar' C-5), 131.55 (Ar' C-4), 131.74 (Ar' C-1), 132.54 (benzothiazole C-6), 135.55 (benzothiazole C-3a), 140.65 (C=N), 158.55 (C=O), 161.69 (Ar C-4), 171.74 ppm (benzothiazole C-2); **Elemental analysis for $C_{23}H_{18}ClN_5O_4S$: calcd:** C 55.70, H 3.66, N 14.12, **found:** C 55.73, H 3.69, N 14.10.

N'-(diphenylmethylene)-2-(6-nitrobenzothiazol-2-ylamino)acetohydrazide (BTA-22): λ_{max}: 268.20, 381.00 nm; **IR (KBr):** υ = 3402.54 (N-H str), 3294.53 (N-H str), 3057.27 (aromatic C-H str), 2998.31 (CH_2 str), 1656.91 (C=O str), 1589.40 (C=N str), 1564.32 (C=C str), 1525.74, 1321.28 (NO_2 str), 1292.35 (C-N str); **^{1}H NMR ([D_6]DMSO):** δ = 3.82 (s, 2H, CH_2), 7.13 (m, 3H, Ar C-3, Ar C-4, Ar C-5), 7.31 (m, 3H, Ar' C-3, Ar' C-4, Ar' C-5), 7.45 (s, 1H, NH), 7.54 (d, 2H, Ar C-2, Ar C-6), 8.04 (d, 2H, Ar' C-2, Ar' C-6), 8.26 (s, 1H, benzothiazole C-7), 8.41 (s, 1H, CONH), 8.71 (d, 1H, benzothiazole C-5), 8.92 ppm (d, 1H, benzothiazole C-4); **^{13}C NMR ([D_6]DMSO):** δ = 79.91 (CH_2), 116.28 (benzothiazole C-7), 117.38 (benzothiazole C-5), 121.72 (benzothiazole C-4), 127.81 (benzothiazole C-7a), 128.45 (Ar C-3, Ar C-5, Ar' C-3, Ar' C-5), 129.67 (Ar C-2, Ar C-6, Ar' C-2, Ar' C-6), 131.76 (Ar C-4, Ar' C-4), 132.79 (Ar C-1, Ar' C-1), 145.19 (benzothiazole C-6), 154.16 (C=N), 155.57 (benzothiazole C-3a), 158.68 (C=O), 171.70 ppm (benzothiazole C-2); **Elemental analysis for $C_{22}H_{17}N_5O_3S$: calcd:** C 61.24, H 3.97, N 16.23, **found:** C 61.28, H 3.99, N 16.25.

N'-((4-chlorophenyl)(phenyl)methylene)-2-(6-nitrobenzothiazol-2-ylamino)acetohydrazide (BTA-23): λ_{max}: 265.80, 375.60 nm; **IR (KBr):** υ = 3514.42, 3338.89 (N-H str), 3074.63 (aromatic C-H str), 2960.83 (CH_2 str), 1647.26 (C=O str), 1595.18 (C=N str), 1570.11 (C=C str), 1527.67, 1327.07 (NO_2 str), 1296.21 (C-N str), 750.33 (C-Cl str); **^{1}H NMR ([D_6]DMSO):** δ = 3.98 (s, 2H, CH_2), 7.32 (s, 1H, NH), 7.55 (d, 2H, Ar C-3, Ar C-5), 7.63 (m, 3H, Ar' C-3, Ar' C-4, Ar' C-5), 7.78 (d, 2H, Ar C-2, Ar C-6), 8.12 (s, 1H, benzothiazole C-7), 8.43 (s, 1H, CONH), 8.51 (d, 2H, Ar' C-2, Ar' C-6), 8.73 (d, 1H, benzothiazole C-5), 8.89 ppm (d, 1H, benzothiazole C-4); **^{13}C NMR ([D_6]DMSO):** δ = 79.40 (CH_2), 116.73 (benzothiazole C-7), 117.12 (benzothiazole C-5), 121.86 (benzothiazole C-4), 128.76 (benzothiazole C-7a), 129.10 (Ar' C-3, Ar' C-5), 129.26 (Ar C-3, Ar C-5), 130.13 (Ar C-2, Ar C-6), 131.56 (Ar C-1), 131.72 (Ar' C-4), 133.06 (Ar' C-1), 134.18 (Ar' C-2, Ar' C-6), 135.98 (Ar C-4), 145.31 (benzothiazole C-6), 155.46

(benzothiazole C-3a), 156.27 (C=N), 158.93 ppm (C=O), 171.13 (benzothiazole C-2); **Elemental analysis for $C_{22}H_{16}ClN_5O_3S$: calcd:** C 56.71, H 3.46, N 15.03, **found:** C 56.68, H 3.49, N 15.01.

N'-((4-hydroxyphenyl)(phenyl)methylene)-2-(6-nitrobenzothiazol-2-ylamino)acetohydrazide (BTA-24): λ_{max}: 268.40, 363.20 nm; **IR (KBr):** υ = 3615.06 (O-H str), 3502.51, 3201.94 (N-H str), 3068.85 (aromatic C-H str), 2987.56 (CH_2 str), 1658.76 (C=O str), 1602.90 (C=N str), 1564.32 (C=C str), 1512.24, 1323.21 (NO_2 str), 1288.49 (C-N str); **^{1}H NMR ([D_6]DMSO):** δ = 3.92 (s, 2H, CH_2), 5.12 (s, 1H, OH), 6.91 (d, 2H, Ar C-3, Ar C-5), 7.32 (m, 3H, Ar' C-3, Ar' C-4, Ar' C-5), 7.34 (s, 1H, NH), 7.61 (d, 2H, Ar C-2, Ar C-6), 8.21 (s, 1H, benzothiazole C-7), 8.45 (d, 2H, Ar' C-2, Ar' C-6), 8.62 (d, 1H, benzothiazole C-5), 8.65 (s, 1H, CONH), 8.91 ppm (d, 1H, benzothiazole C-4); **^{13}C NMR ([D_6]DMSO):** δ = 79.86 (CH_2), 116.33 (benzothiazole C-7), 116.45 (Ar C-3, Ar C-5), 117.59 (benzothiazole C-5), 121.62 (benzothiazole C-4), 125.60 (Ar C-1), 128.53 (benzothiazole C-7a), 129.09 (Ar' C-3, Ar' C-5), 130.73 (Ar C-2, Ar C-6), 131.20 (Ar' C-2, Ar' C-6), 132.41 (Ar' C-4), 132.91 (Ar' C-1), 145.38 (benzothiazole C-6), 155.71 (benzothiazole C-3a), 156.12 (C=N), 158.26 (C=O), 160.16 (Ar C-4), 171.49 ppm (benzothiazole C-2); **Elemental analysis for $C_{22}H_{17}N_5O_4S$: calcd:** C 59.05, H 3.83, N 15.65, **found:** C 59.01, H 3.86, N 15.63.

N'-(bis(4-chlorophenyl)methylene)-2-(6-nitrobenzothiazol-2-ylamino)acetohydrazide (BTA-25): λ_{max}: 275.40, 371.60 nm; **IR (KBr):** υ = 3525.99 (N-H str), 3340.82.89 (N-H str), 3097.78 (aromatic C-H str), 2966.62 (CH_2 str), 1654.98 (C=O str), 1589.40 (C=N str), 1531.53 (C=C str), 1496.81, 1330.93 (NO_2 str), 1303.92 (C-N str), 833.26, 752.26 (C-Cl str); **^{1}H NMR ([D_6]DMSO):** δ = 3.34 (s, 2H, CH_2), 7.33 (s, 1H, NH), 7.38 (d, 4H, Ar C-3, Ar C-5, Ar' C-3, Ar' C-5), 7.60 (d, 4H, Ar C-2, Ar C-6, Ar' C-2, Ar' C-6), 7.72 (d, 1H, benzothiazole C-5), 8.06 (d, 1H, benzothiazole C-4), 8.21 (s, 1H, benzothiazole C-7), 8.65 ppm (s, 1H, CONH); **^{13}C NMR ([D_6]DMSO):** δ = 79.93 (CH_2), 116.81 (benzothiazole C-7), 117.63 (benzothiazole C-5), 121.94 (benzothiazole C-4), 128.73 (benzothiazole C-7a), 131.43 (Ar C-1, Ar' C-1), 131.55 (Ar C-2, Ar C-6, Ar' C-2, Ar' C-6), 135.33 (Ar C-3, Ar C-5, Ar' C-3, Ar' C-5), 137.78 (Ar C-4, Ar' C-4), 145.66 (benzothiazole C-6), 155.34 (benzothiazole C-3a), 155.90 (C=N), 158.55 (C=O), 171.74

ppm (benzothiazole C-2); **Elemental analysis for $C_{22}H_{15}Cl_2N_5O_3S$: calcd:** C 52.81, H 3.02, N 14.00, **found:** C 52.83, H 3.00, N 14.03.

N'-(2-bromo-1-(4-bromophenyl)ethylidene)-2-(6-nitrobenzothiazol-2-ylamino)aceto-hydrazide (BTA-26): λ_{max}: 265.00, 364.20 nm; **IR (KBr):** υ = 3525.99, 3336.96 (N-H str), 3097.78 (aromatic C-H str), 2974.33 (CH_2 str), 1651.12 (C=O str), 1570.11 (C=N str), 1531.53 (C=C str), 1496.81, 1330.93 (NO_2 str), 1300.07 (C-N str), 825.96 (C-Br str); **^{1}H NMR ([D_6]DMSO):** δ = 3.35 (s, 2H, CH_2); 3.97 (s, 2H, CH_2), 7.40 (d, 2H, Ar C-3, Ar C-5), 7.75 (s, 1H, NH), 7.77 (d, 1H, benzothiazole C-5), 8.08 (d, 1H, benzothiazole C-4), 8.24 (s, 1H, benzothiazole C-7), 8.68 (s, 1H, CONH), 8.69 ppm (d, 2H, Ar C-2, Ar C-6); **^{13}C NMR ([D_6]DMSO):** δ = 67.40 (CH_2), 79.91 (CH_2), 116.81 (benzothiazole C-7), 117.66 (benzothiazole C-5), 121.95 (benzothiazole C-4), 126.26 (benzothiazole C-7a), 127.15 (Ar C-4), 131.55 (Ar C-2, Ar C-6), 135.53 (Ar C-3, Ar C-5), 135.93 (Ar C-1), 145.64 (benzothiazole C-6), 155.05 (benzothiazole C-3a), 155.91 (C=N), 158.56 (C=O), 171.74 ppm (benzothiazole C-2); **MS:** *m/z*=528.42 $[M+1]^+$; **Elemental analysis for $C_{17}H_{13}Br_2N_5O_3S$: calcd:** C 38.73, H 2.49, N 13.28, **found:** C 38.69, H 2.47, N 13.30.

N'-cyclohexylidene-2-(6-nitrobenzothiazol-2-ylamino)acetohydrazide (BTA-27): λ_{max}: 265.60, 376.80 nm; **IR (KBr):** υ = 3525.99, 3340.82 (N-H str), 3097.78 (aromatic C-H str), 2943.47 (CH_2 str), 1647.26 (C=O str), 1570.11 (C=N str), 1531.53 (C=C str), 1496.81, 1330.93 (NO_2 str), 1300.07 (C-N str); **^{1}H NMR ([D_6]DMSO):** δ = 1.21 (m, 2H, cyclohexyl C-4), 1.47 (m, 8H, cyclohexyl C-2, cyclohexyl C-3, cyclohexyl C-5, cyclohexyl C-6), 3.39 (s, 2H, CH_2), 7.21 (s, 1H, NH), 8.05 (d, 1H, benzothiazole C-5), 8.12 (d, 1H, benzothiazole C-4), 8.27 (s, 1H, benzothiazole C-7), 8.68 ppm (s, 1H, CONH); **^{13}C NMR ([D_6]DMSO):** δ = 23.48 (cyclohexyl C-3, cyclohexyl C-5), 26.94 (cyclohexyl C-4), 29.38 (cyclohexyl C-2, cyclohexyl C-6), 79.23 (CH_2), 116.80 (benzothiazole C-7), 122.11 (benzothiazole C-5), 125.63 (benzothiazole C-4), 126.44 (benzothiazole C-7a), 145.76 (benzothiazole C-6), 155.30 (benzothiazole C-3a), 158.31 (C=O), 160.58 (C=N), 170.99 ppm (benzothiazole C-2), **Elemental analysis for $C_{15}H_{17}N_5O_3S$: calcd:** C 51.86, H 4.93, N 20.16, **found:** C 51.83, H 4.97, N 20.17.

2-(6-nitrobenzothiazol-2-ylamino)-N'-(2-oxoindolin-3-ylidene)acetohydrazide (BTA-28): λ_{max}: 264.60, 372.40 nm; **IR (KBr):** υ = 3514.42, 3336.96 (N-H str), 3097.78

(aromatic C-H str), 2947.33 (CH_2 str), 1720.56, 1651.12 (C=O str), 1620.26 (C=N str), 1572.04 (C=C str), 1529.60, 1329.00 (NO_2 str), 1298.14 (C-N str); **^{1}H NMR ([D_6]DMSO):** δ = 3.34 (s, 2H, CH_2), 6.35 (m, 1H, isatinyl C-5), 6.41 (m, 1H, isatinyl C-6), 6.91 (d, 1H, isatinyl C-7), 7.02 (s, 1H, NH), 7.40 (d, 1H, isatinyl C-4), 8.06 (d, 1H, benzothiazole C-5), 8.09 (d, 1H, benzothiazole C-4), 8.23 (s, 1H, benzothiazole C-7), 8.67 (s, 1H, CONH), 10.99 ppm (s, 1H, isatinyl NH); **^{13}C NMR ([D_6]DMSO):** δ = 79.90 (CH_2), 116.82 (benzothiazole C-7), 117.67 (isatinyl C-3a), 121.96 (benzothiazole C-5), 126.52 (isatinyl C-7), 127.38 (benzothiazole C-4), 131.55 (isatinyl C-5), 135.53 (benzothiazole C-7a), 136.25 (isatinyl C-6), 138.45 (isatinyl C-4), 140.50 (C=N), 145.36 (benzothiazole C-6), 146.55 (isatinyl C-7a), 155.17 (benzothiazole C-3a), 158.56 (C=O), 167.72 (isatinyl C=O), 171.75 ppm (benzothiazole C-2); **MS:** *m/z*=397.65 $[M+1]^+$; **Elemental analysis for $C_{17}H_{12}N_6O_4S$: calcd:** C 51.51, H 3.05, N 21.20, **found:** C 51.53, H 3.08, N 21.18.

N'-(5-chloro-2-oxoindolin-3-ylidene)-2-(6-nitrobenzothiazol-2-ylamino)acetohydrazide (BTA-29): λ_{max}: 264.20, 344.40 nm; **IR (KBr):** υ = 3311.89, 3200.01 (N-H str), 3172.54 (aromatic C-H str), 2982.05 (CH_2 str), 1720.56, 1703.20 (C=O str), 1618.33 (C=N str), 1570.04 (C=C str), 1523.82, 1330.93 (NO_2 str), 1292.28 (C-N str); **^{1}H NMR ([D_6]DMSO):** δ = 3.41 (s, 2H, CH_2), 7.03 (s, 1H, NH), 7.26 (d, 1H, isatinyl C-6), 7.65 (d, 1H, isatinyl C-7), 7.71 (s, 1H, isatinyl C-4), 8.12 (d, 1H, benzothiazole C-5), 8.24 (s, 1H, benzothiazole C-7), 8.32 (d, 1H, benzothiazole C-4), 8.67 (s, 1H, CONH), 10.45 ppm (s, 1H, isatinyl NH); **^{13}C NMR ([D_6]DMSO):** δ = 79.76 (CH_2), 116.93 (benzothiazole C-7), 119.32 (isatinyl C-3a), 121.37 (benzothiazole C-5), 126.97 (benzothiazole C-4), 127.46 (isatinyl C-7), 134.42 (isatinyl C-5), 135.49 (benzothiazole C-7a), 136.17 (isatinyl C-6), 138.26 (isatinyl C-4), 140.66 (C=N), 144.39 (isatinyl C-7a), 145.81 (benzothiazole C-6), 155.39 (benzothiazole C-3a), 158.79 (C=O), 167.83 (isatinyl C=O), 171.62 ppm (benzothiazole C-2); **Elemental analysis for $C_{17}H_{11}ClN_6O_4S$: calcd:** C 47.39, H 2.57, N 19.51, **found:** C 47.36, H 2.59, N 19.54.

N'-(5-nitro-2-oxoindolin-3-ylidene)-2-(6-nitrobenzothiazol-2-ylamino)acetohydrazide (BTA-30): λ_{max}: 262.80, 364.60 nm; **IR (KBr):** υ = 3525.99, 3336.96 (N-H str), 3097.78 (aromatic C-H str), 2978.19 (CH_2 str), 1716.70, 1701.27 (C=O str), 1624.12

(C=N str), 1573.97 (C=C str), 1527.67, 1334.78 (NO_2 str), 1296.21 (C-N str); **^{1}H NMR ([D_6]DMSO):** δ = 3.42 (s, 2H, CH_2); 7.11 (s, 1H, NH), 7.54 (d, 1H, isatinyl C-7), 7.89 (d, 1H, isatinyl C-6), 8.11 (d, 1H, benzothiazole C-5), 8.23 (d, 1H, benzothiazole C-4), 8.29 (s, 1H, benzothiazole C-7), 8.78 (s, 1H, CONH), 10.33 ppm (s, 1H, isatinyl NH); **^{13}C NMR ([D_6]DMSO):** δ = 79.91 (CH_2), 116.29 (benzothiazole C-7), 121.31 (benzothiazole C-5), 123.35 (isatinyl C-3a), 124.32 (isatinyl C-4), 125.11 (isatinyl C-6), 126.07 (benzothiazole C-4), 127.30 (isatinyl C-7), 135.58 (benzothiazole C-7a), 141.05 (C=N), 144.57 (isatinyl C-5), 145.19 (benzothiazole C-6), 145.42 (isatinyl C-7a), 155.56 (benzothiazole C-3a), 158.55 (C=O), 167.59 (isatinyl C=O), 171.47 ppm (benzothiazole C-2); **Elemental analysis for $C_{17}H_{11}N_7O_6S$: calcd:** C 46.26, H 2.51, N 22.21, **found:** C 46.22, H 2.53, N 22.19.

The IR, ^{1}H NMR, ^{13}C NMR, mass and X-RD spectra of compounds **BTA-3**, **BTA-7**, **BTA-10**, **BTA-17**, **BTA-21**, **BTA-25**, **BTA-26** and **BTA-28** are presented in **Figure 4.2** to **Figure 4.29.**

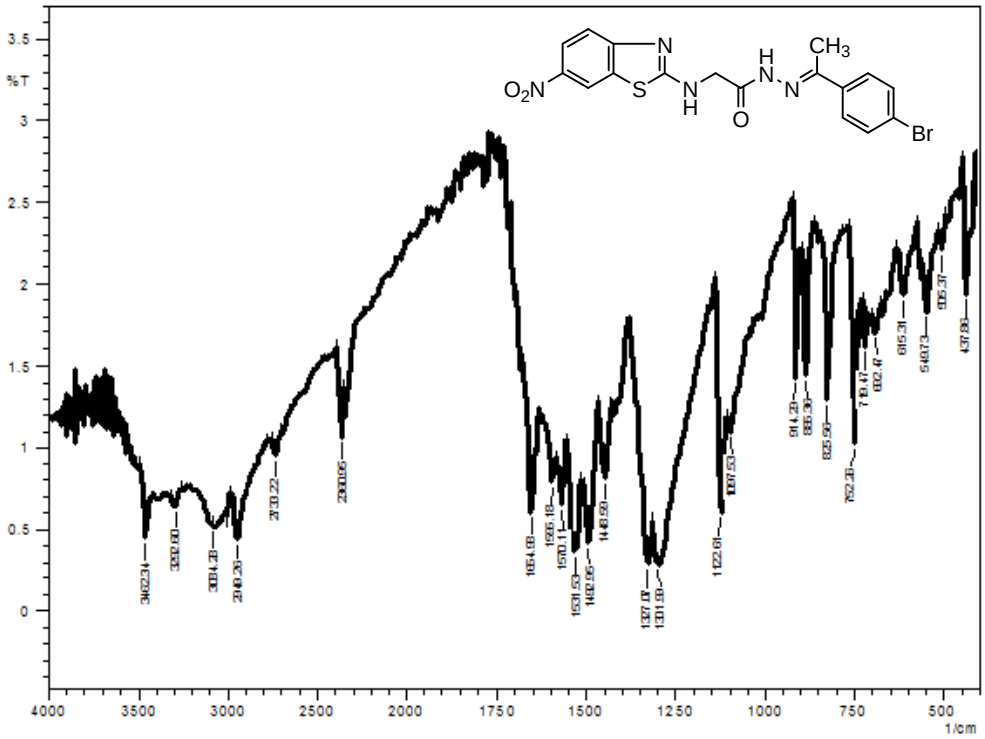


Figure 4.2. IR spectrum of **BTA-3**

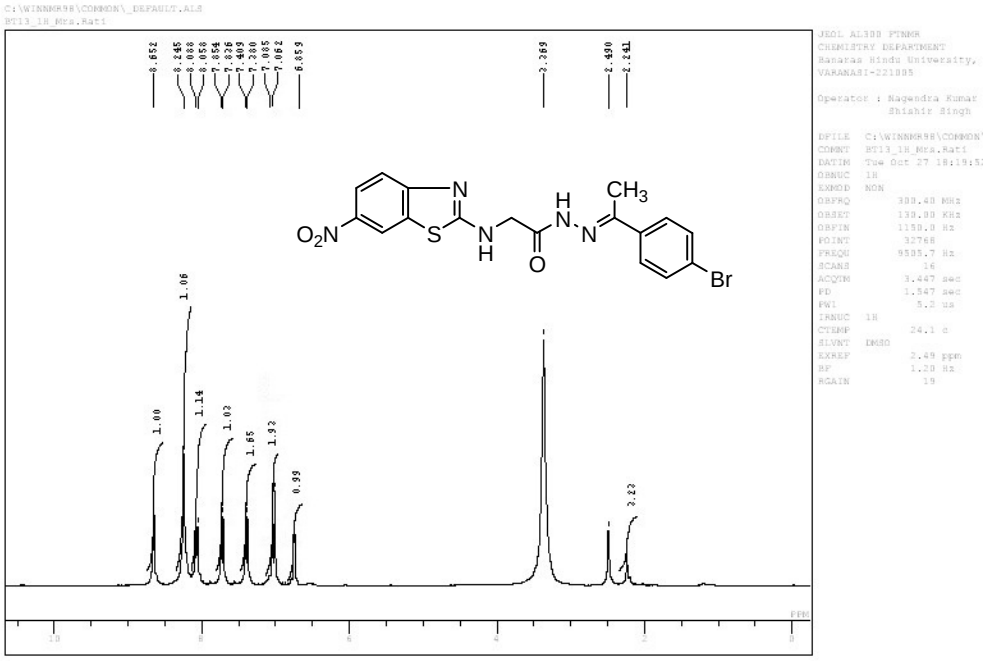


Figure 4.3. ^{1}H NMR spectrum of **BTA-3**

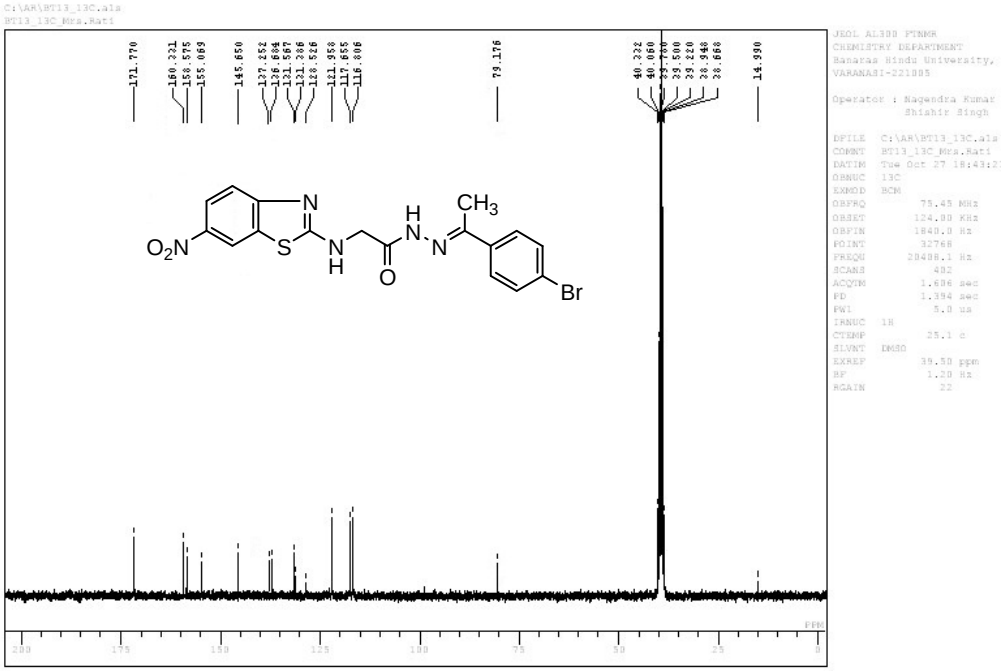


Figure 4.4. ^{13}C NMR spectrum of **BTA-3**

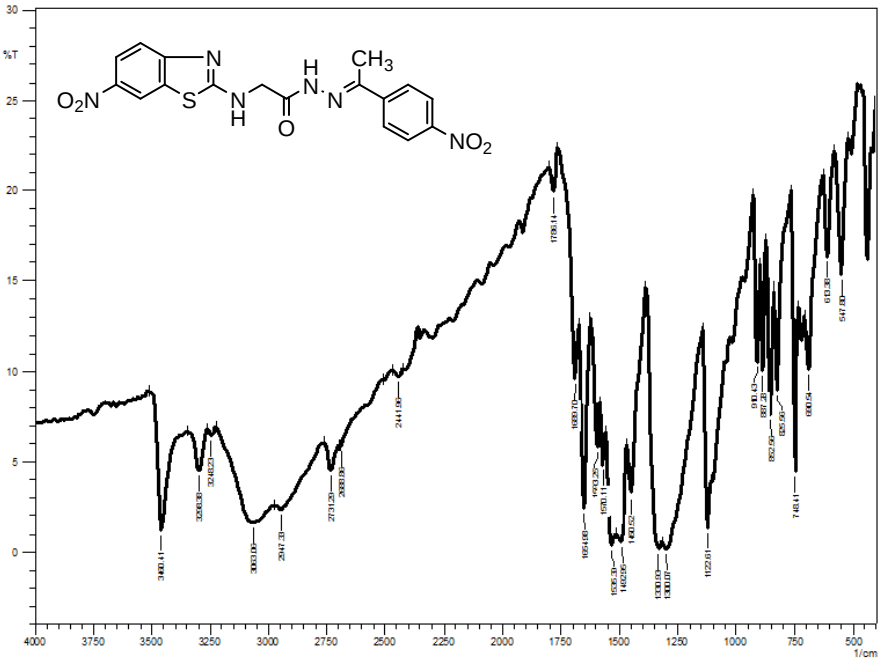


Figure 4.5. IR spectrum of **BTA-7**

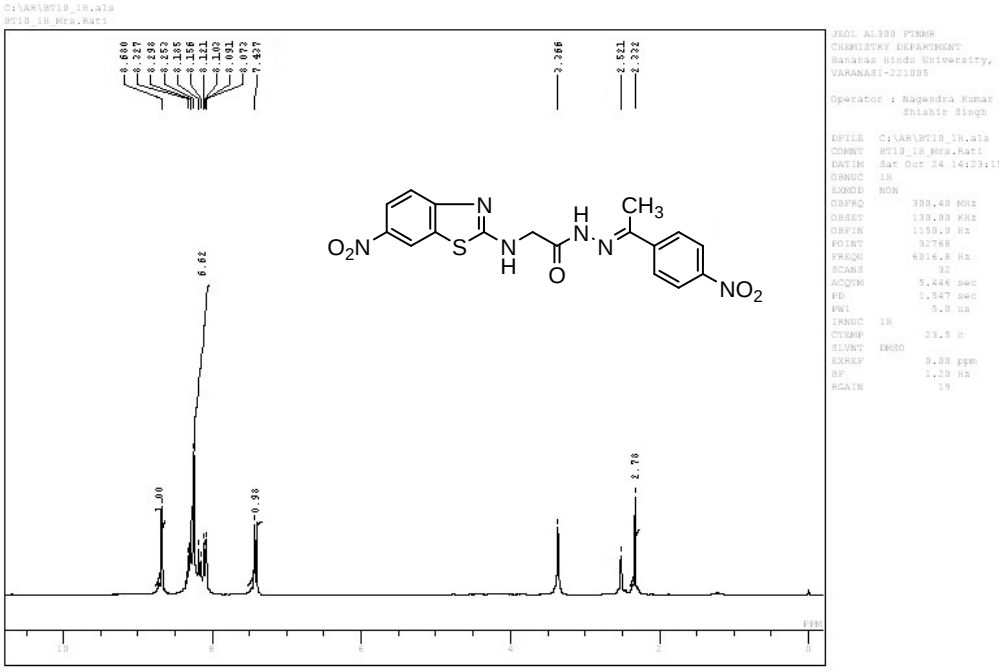


Figure 4.6. ^{1}H NMR spectrum of **BTA-7**

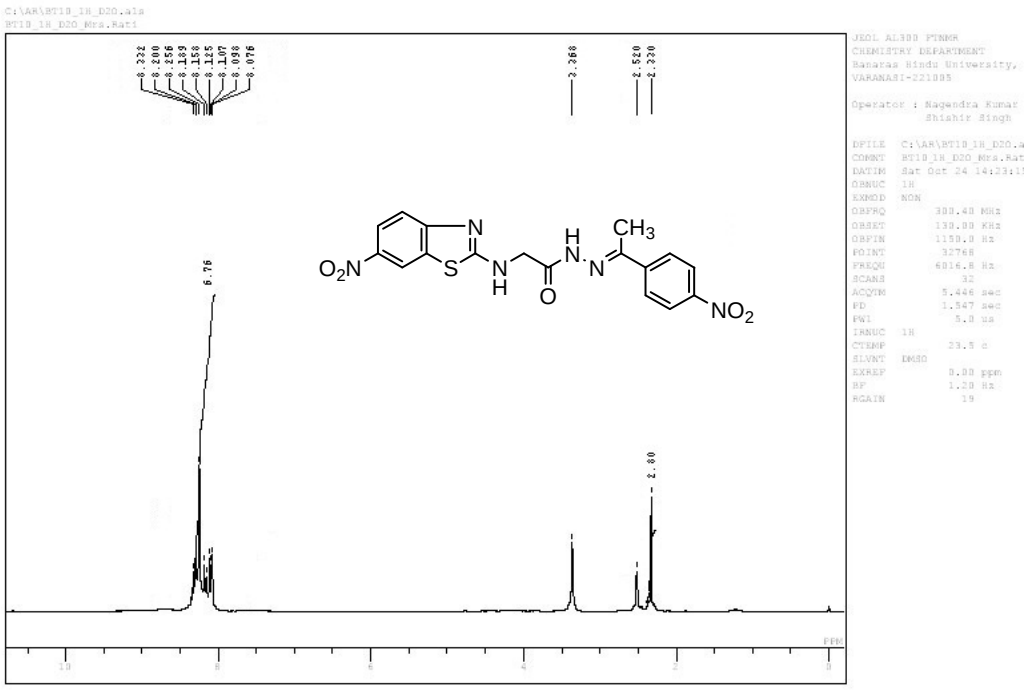


Figure 4.7. ^{1}H (D_2O exchange) spectrum of **BTA-7**

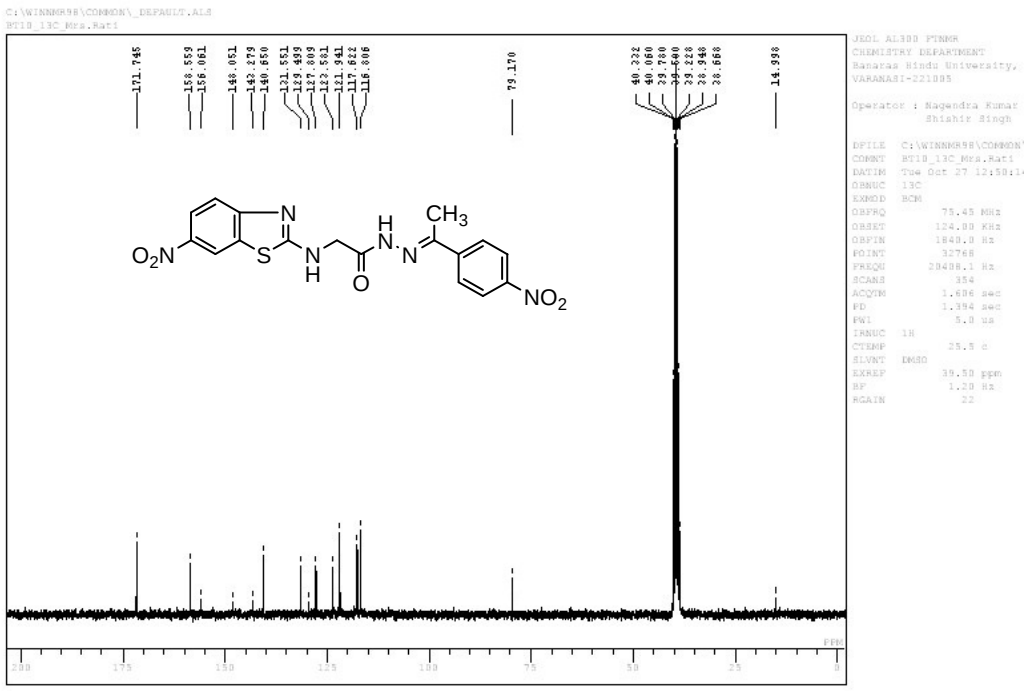


Figure 4.8. ^{13}C spectrum of **BTA-7**

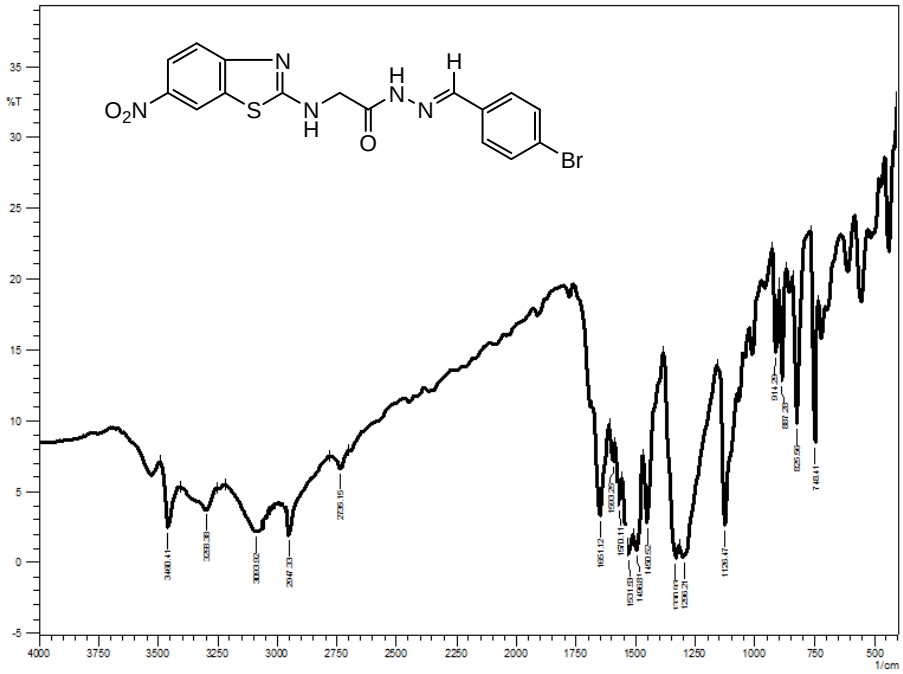


Figure 4.9. IR spectrum of **BTA-10**

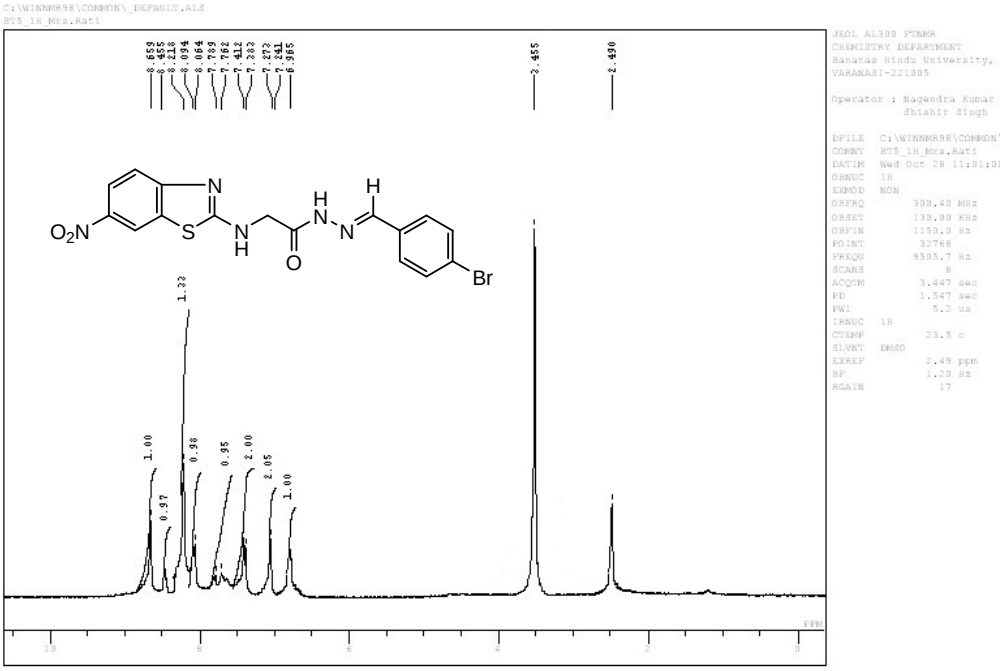


Figure 4.10. ^{1}H NMR spectrum of **BTA-10**

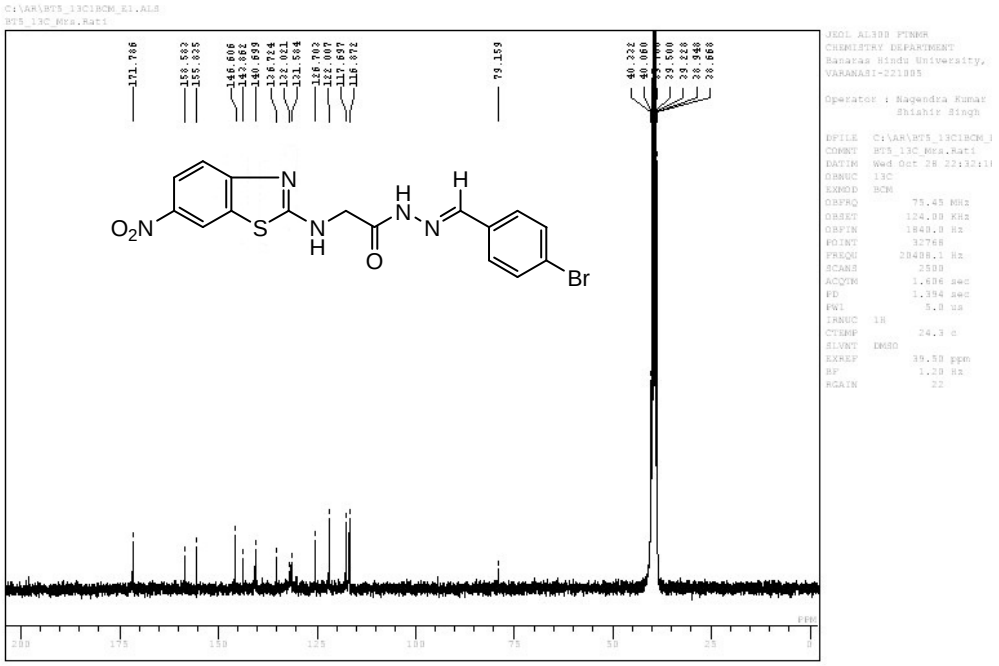


Figure 4.11. ^{13}C NMR spectrum of **BTA-10**

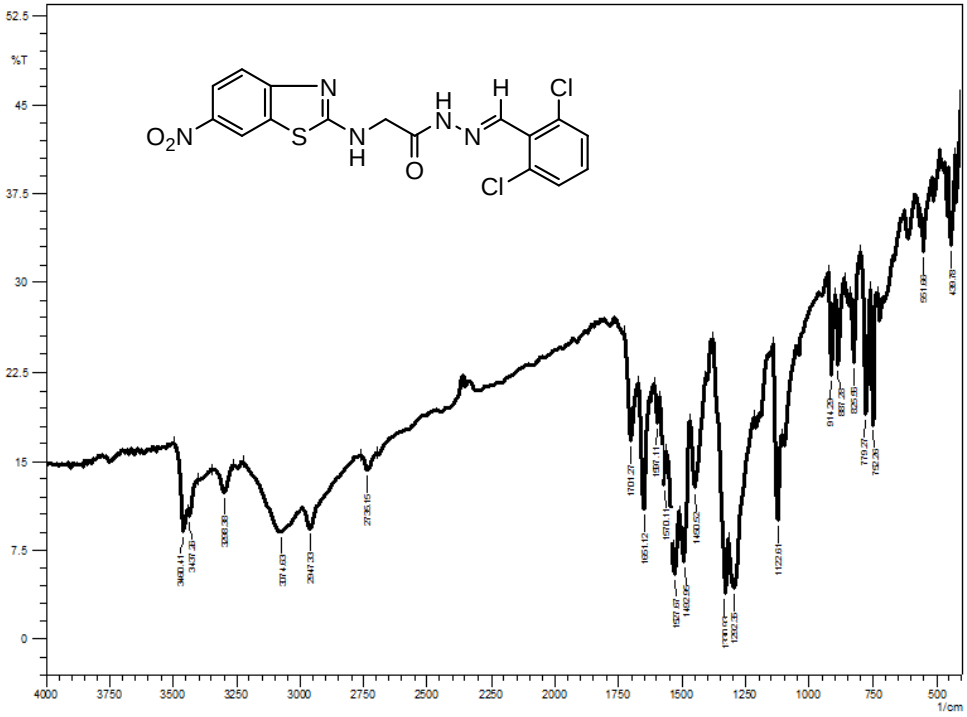


Figure 4.12. IR spectrum of **BTA-17**

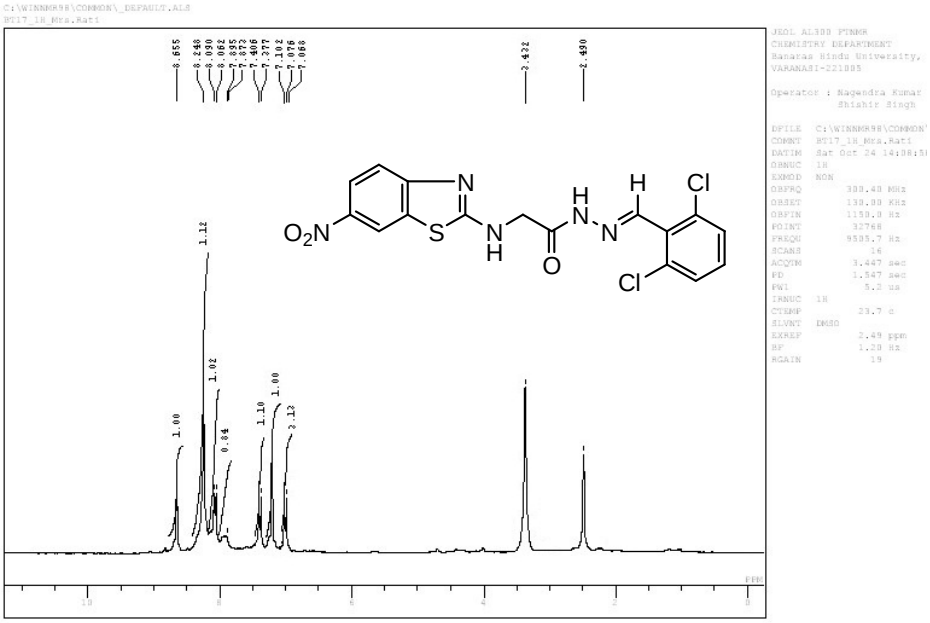


Figure 4.13. ^{1}H NMR spectrum of **BTA-17**

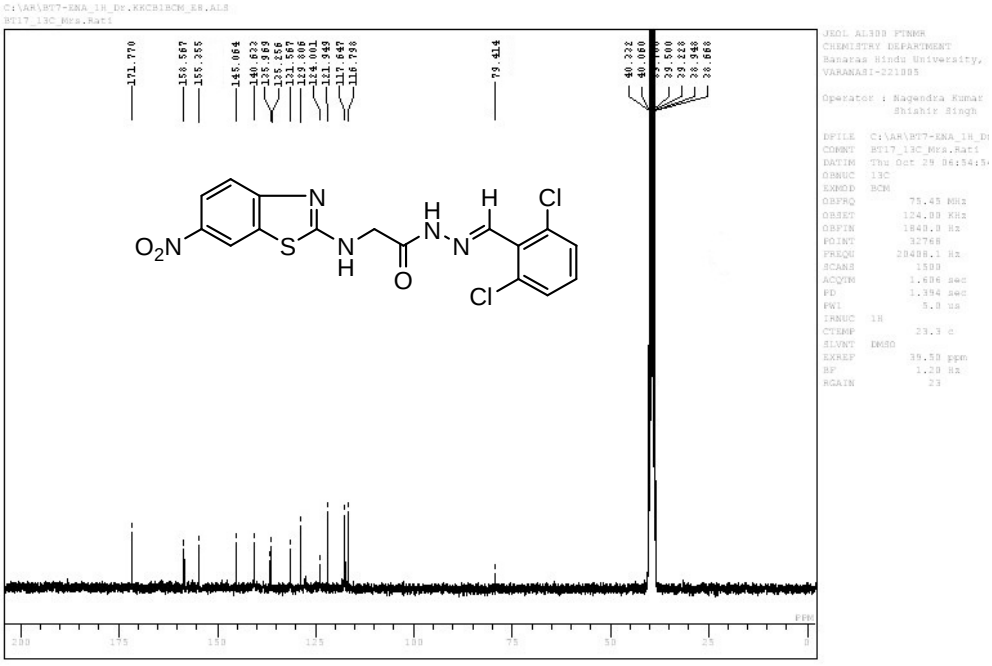


Figure 4.14. ^{13}C NMR spectrum of **BTA-17**

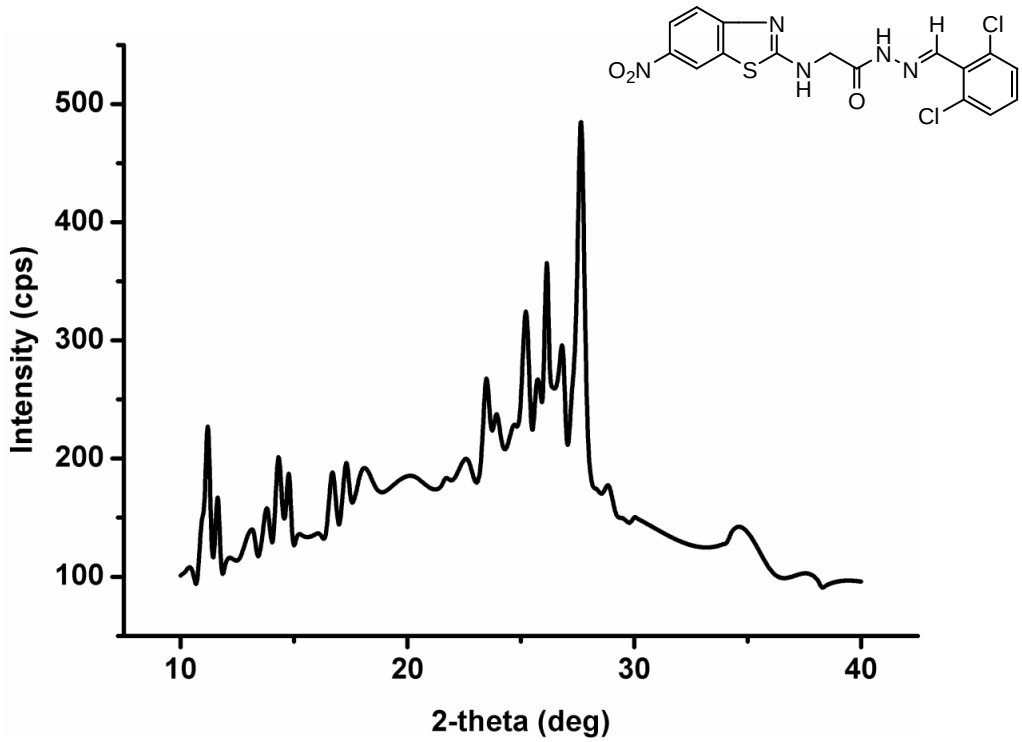


Figure 4.15. XR-PD spectrum of **BTA-17**

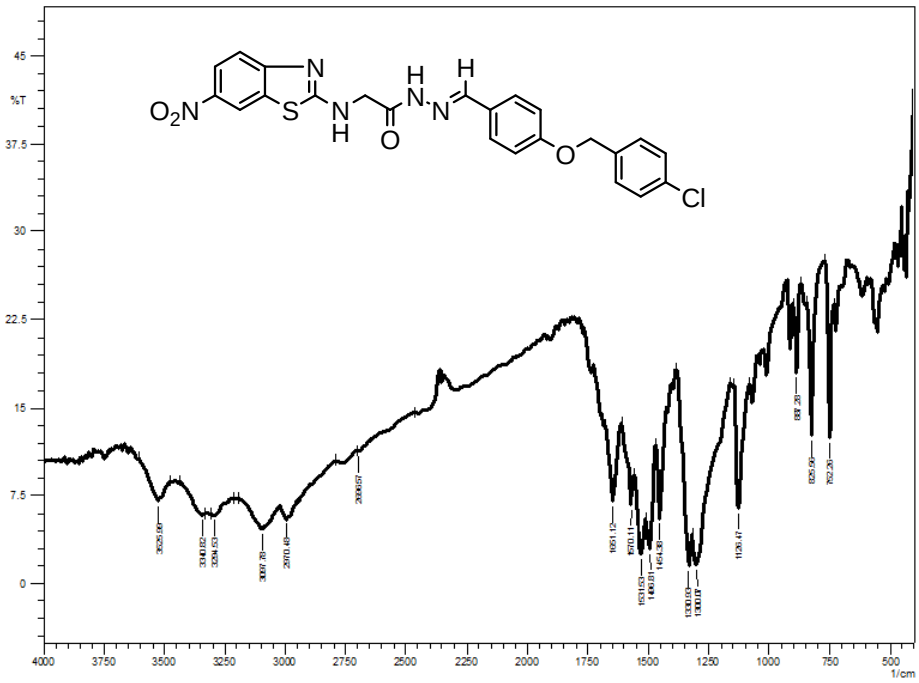


Figure 4.16. IR spectrum of **BTA-21**

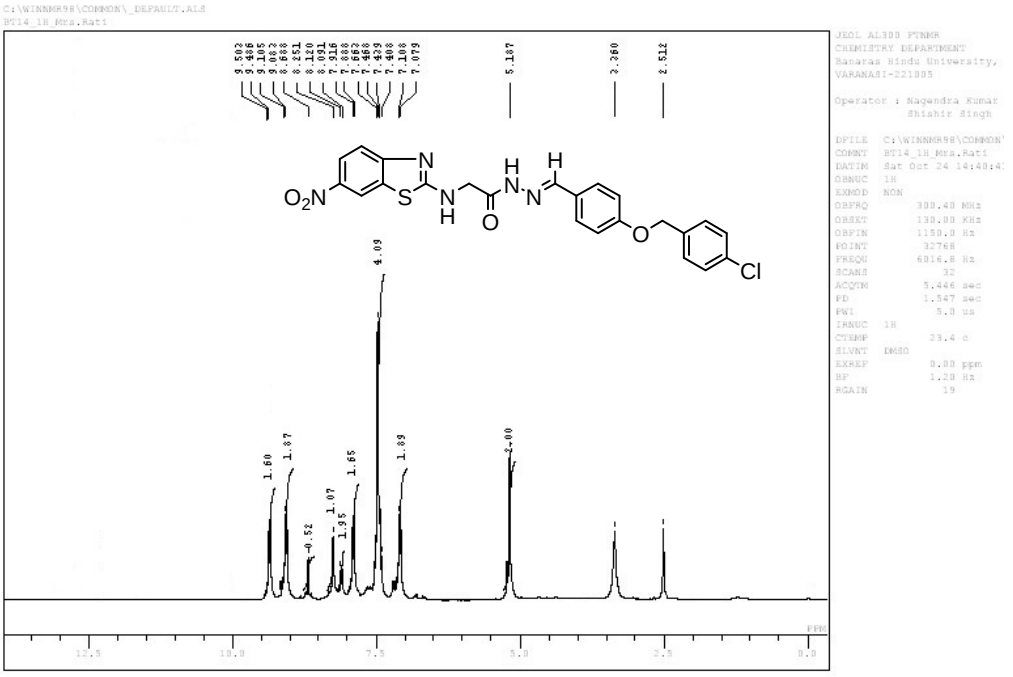


Figure 4.17. ^{1}H NMR spectrum of **BTA-21**

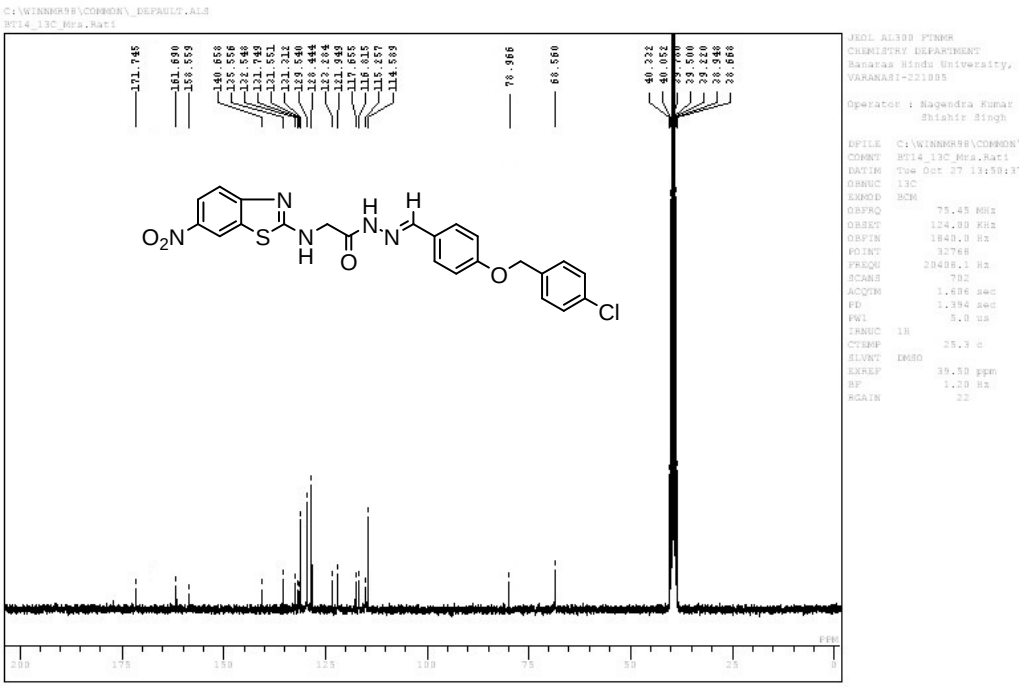


Figure 4.18. ^{13}C NMR spectrum of **BTA-21**

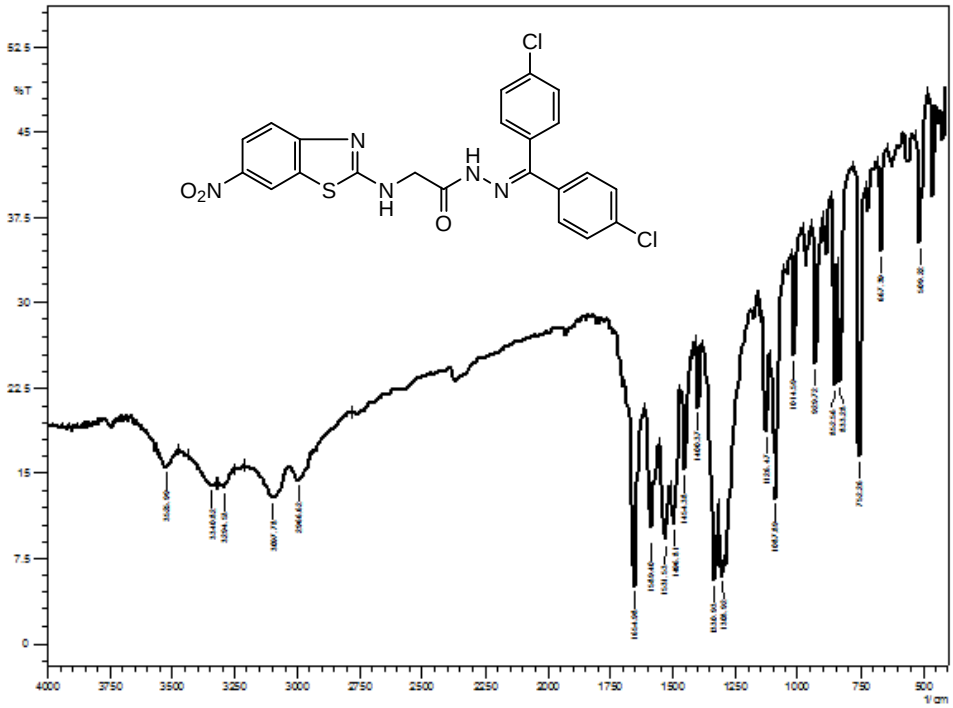


Figure 4.19. IR spectrum of **BTA-25**

Figure 4.20. ^{1}H NMR spectrum of **BTA-25**

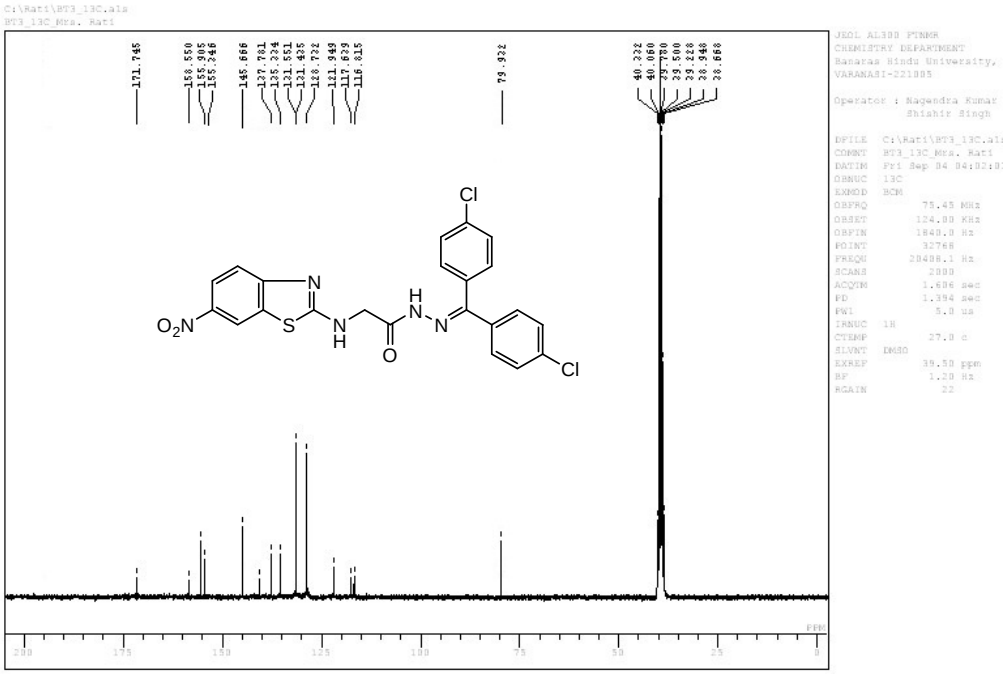


Figure 4.21. ^{13}C NMR spectrum of **BTA-25**

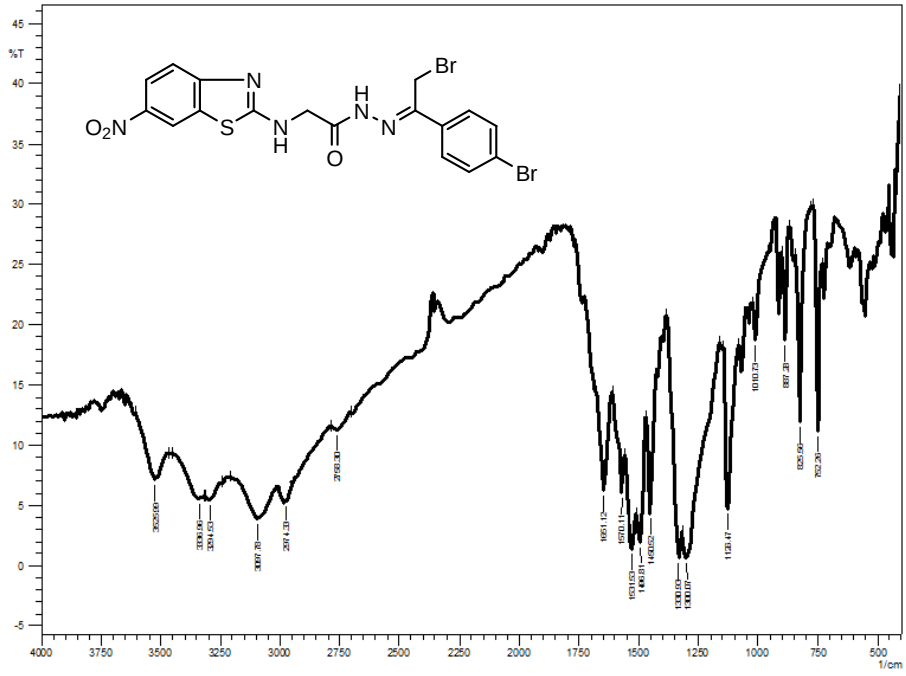


Figure 4.22. IR spectrum of **BTA-26**

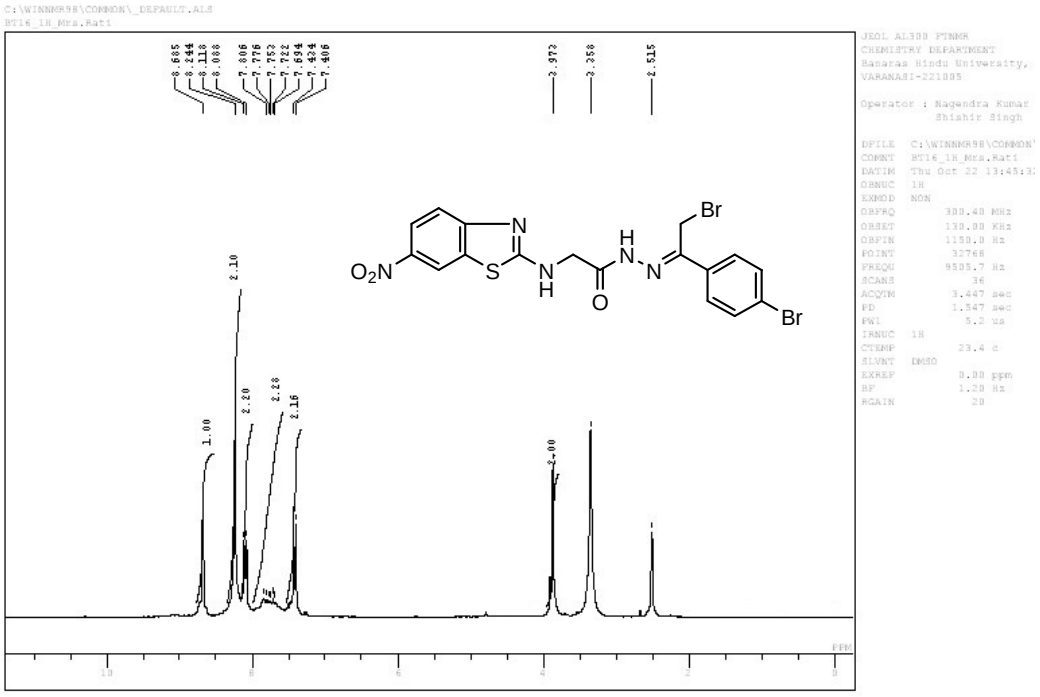


Figure 4.23. ^{1}H NMR spectrum of **BTA-26**

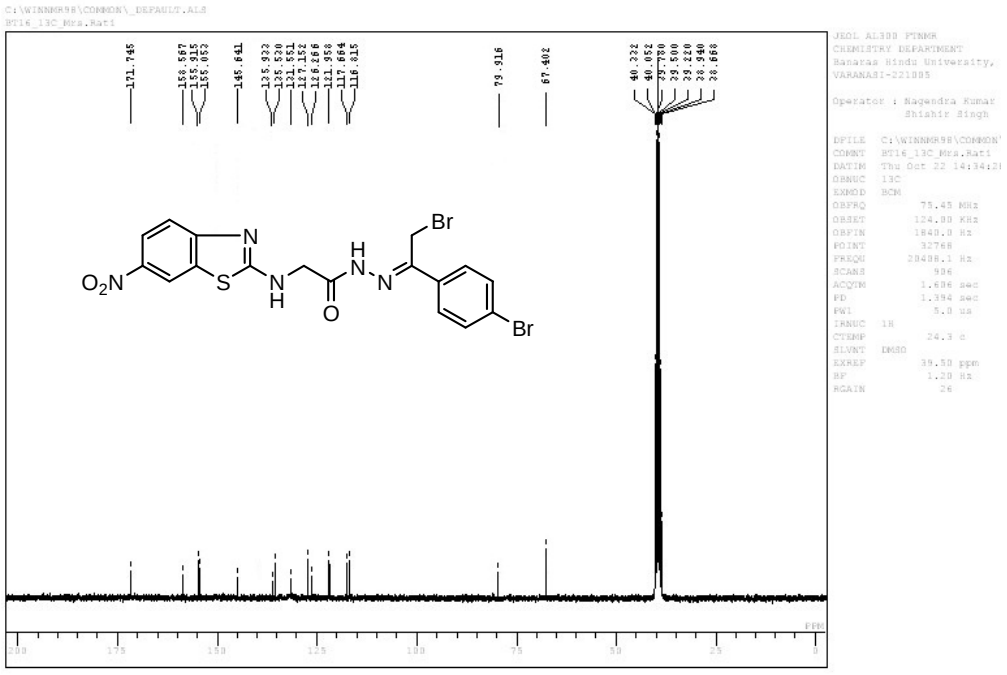


Figure 4.24. ^{13}C NMR spectrum of **BTA-26**

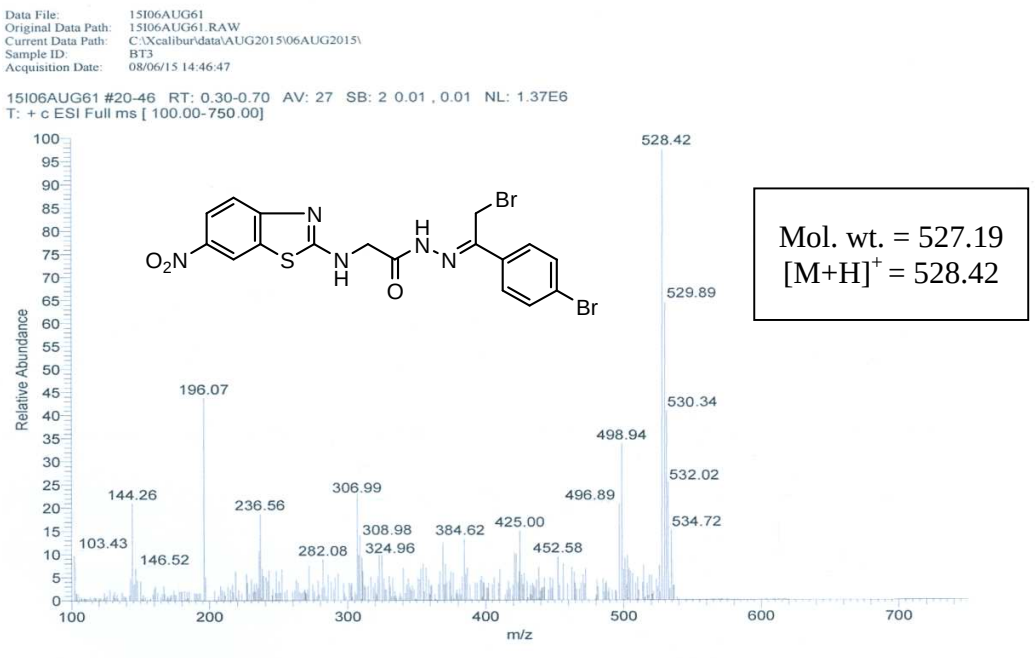


Figure 4.25. Mass spectrum of **BTA-26**

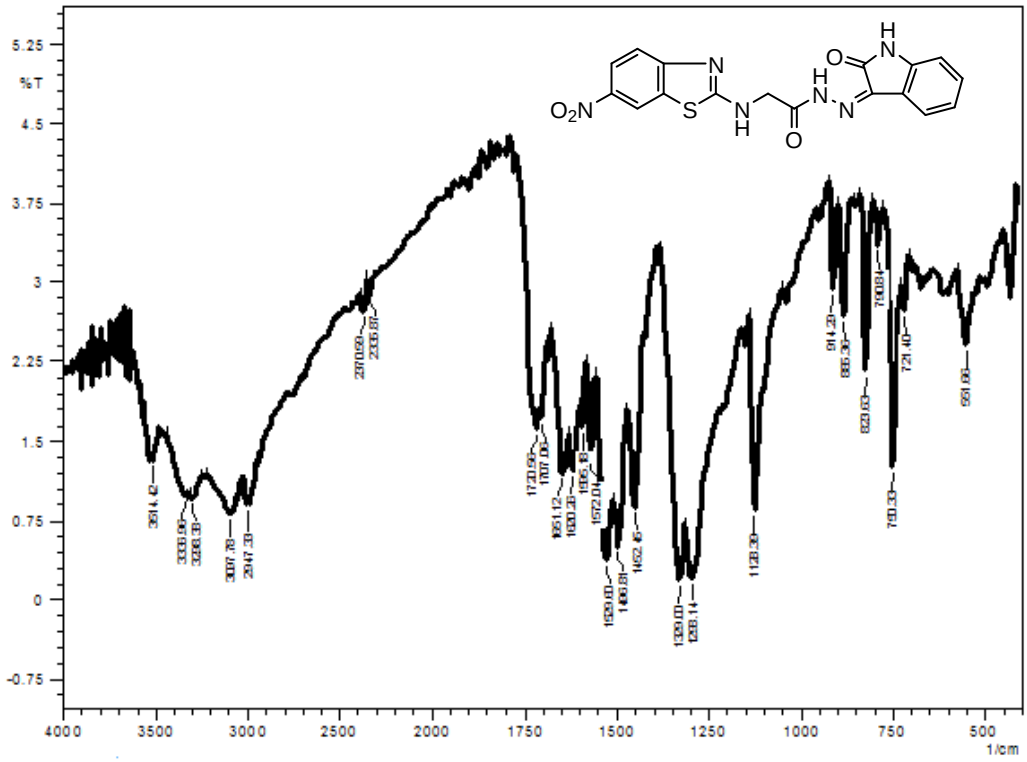


Figure 4.26. IR spectrum of **BTA-28**

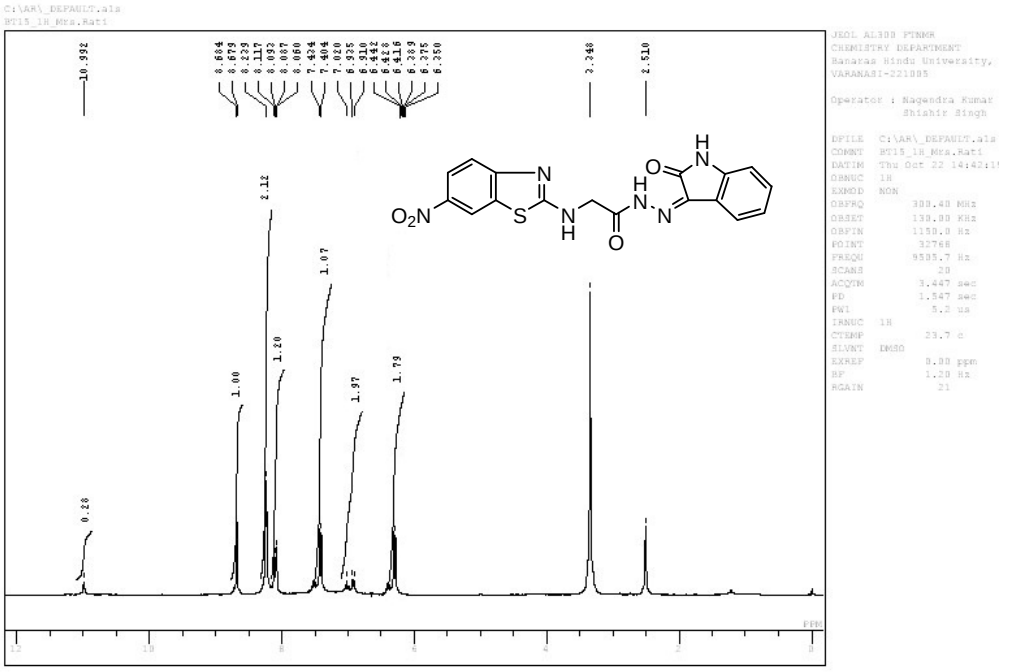


Figure 4.27. ^{1}H NMR spectrum of **BTA-28**

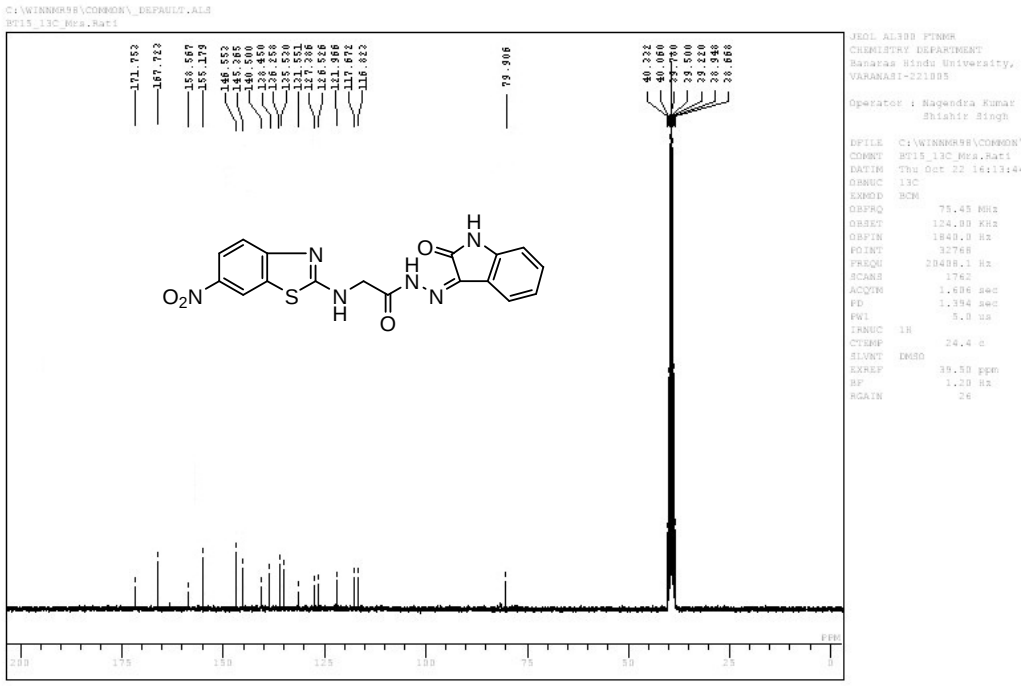


Figure 4.28. ^{13}C NMR spectrum of **BTA-28**

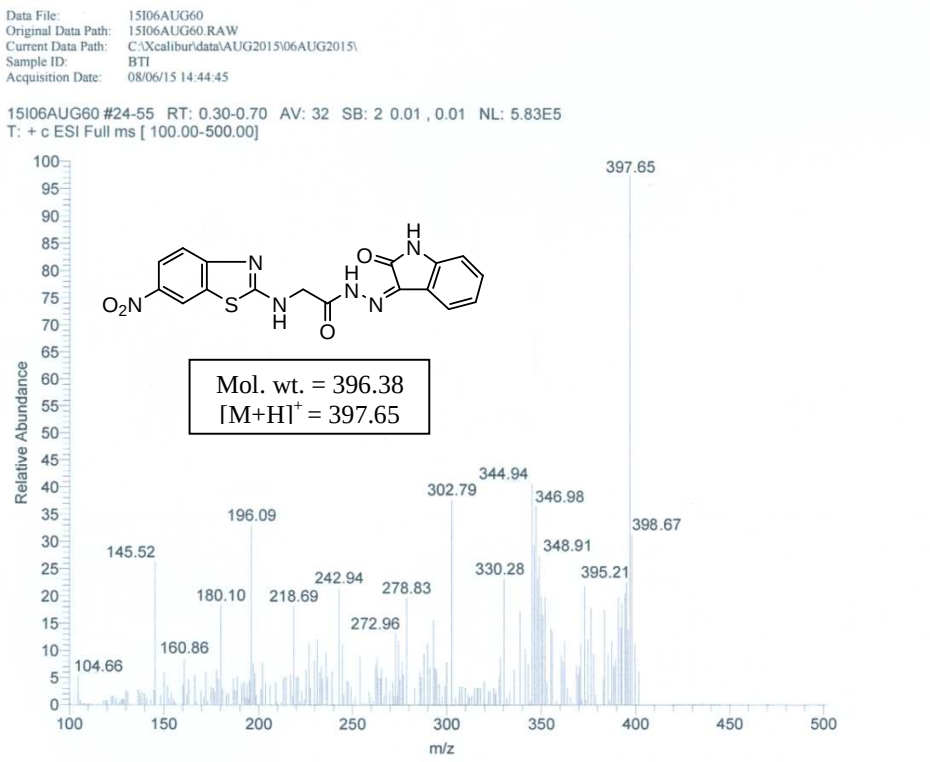


Figure 4.29. Mass spectrum of **BTA-28**

4.1.3. Biological evaluation

Animal ethical approval

The Ethics Committee of Laboratory Animals at Banaras Hindu University, Varanasi (India), approved the animal experimentation reported herein. Albino Wistar rats weighing between 200 and 220 g and Swiss albino mice weighing between 20 and 25 g were obtained from Central Animal House, Institute of Medical Sciences, Banaras Hindu University (Registration No. Dean/12-13/CAEC/23).

The procedures followed for the *in-vitro* and *in-vivo* biological screening studies of the synthesized compounds (**BTA-1** to **BTA-30**) are summarized below.

4.1.3.1. Monoamine oxidase (MAO) enzyme inhibition studies

4.1.3.1.1. *In-vitro* MAO inhibition assay

The inhibitory activities of the final compounds (**BTA-1** to **BTA-30**) were investigated against MAO-A and MAO-B isozymes by measuring the effects of each compound on the production of 5-hydroxyindole acetic acid from serotonin (5-HT) for MAO-A activity

and benzaldehdye from benzylamine for MAO-B activity, using the UV based spectrophotometric MAO enzyme inhibition assay. The assay offers advantages like rapid, versatile, quantitative, reproducible and adaptable to large-scale screening.

4.1.3.1.1.1. Materials

5-HT (serotonin) for MAO-A assay, benzylamine for MAO-B assay and the reference inhibitors clorgyline (MAO-A) and selegiline (MAO-B) were purchased from Sigma-Aldrich (U.S.A.). Tris-HCl, sodium phosphate and zinc sulfate were obtained from Merck (Germany and SD Fine (Mumbai).

4.1.3.1.1.2. Isolation of rat brain mitochondria [Reinhart *et al.* 1982]

Rat brain mitochondria were used as a source of MAO isoforms. All operations were carried out at 4 °C. Male and female adult Wistar rats weighing 200-220 g were decapitated. All brains were rapidly removed and homogenized with a Potter-Elvehjem homogenizer in cold 0.32 M sucrose and 50 mM Tris-hydrochIoride, pH 8.2 (10:1, *v/w*). The homogenate was centrifuged twice at 1000 g for 5 min at 4 °C. The resulting supernatant was centrifuged at 20,000 g for 20 min. The mitochondrial pellet obtained was suspended in 100 mM sodium phosphate buffer, pH 7.4 (4:1, *v/w*), fractionated in plastic vials to 500 µl samples and stored at -80 °C. Before use, mitochondria were diluted with 100 mM sodium phosphate buffer to give a working solution of 0.84 mg of protein per millilitre.

4.1.3.1.1.3. Protocol for MAO assay

The experiments were carried out under all the suitable laboratory conditions. The final compounds (**BTA-1** to **BTA-30**) were evaluated for their *in vitro* MAO-A inhibitory activity according to the method reported by Sjoerdsma *et al.* [Sjoerdsma *et al.* 1955] described for the metabolism of serotonin and by the UV spectrophotometric method described by Udenfriend *et al.* [Udenfriend *et al.* 1955] which was applied for the determination of serotonin. Inhibitory activity of MAO-B was measured using benzylamine as a substrate according to the procedure reported by Tabor *et al.* [Tabor *et al.* 1954] with necessary modifications. The test compounds were dissolved in dimethyl sulphoxide (DMSO) and added to the buffered incubation mixture such that the final DMSO concentration was 4% which caused no MAO inhibition. Clorgyline, selegiline and isatin were taken as reference inhibitors for the determination of MAO-A and MAO-B activity respectively.

An aliquot was made to contain mixture of 55 µl mitochondrial suspension (0.84 mg of protein/ml), 90 µl 50 mM tris-HCl buffer, pH 8.2 and 30 µl solubilising solution (control or inhibitor solution at five different concentrations). The reaction was initiated by adding 25 µl of serotonin (4 mM, substrate for MAO-A) for evaluation of MAO-A activity and 25 µl benzylamine (0.1 M, substrate for MAO-B) for the determination of MAO-B activity. The mixture was incubated at 37°C for 30 minutes, and the reaction was stopped by the addition of 50 µl of 1N HCl. The absorbance was taken at 280 nm (due to formation of 5-hydroxyindolacetic acid) for MAO-A estimation and at 250 nm (due to the formation of benzaldehyde) for MAO-B estimation. All the assays were performed in duplicate and were repeated twice. Control experiments were carried out without inhibitor and blanks were run without mitochondrial suspension [Park and Choi 1983].

4.1.3.1.1.4. Protein estimation

The rat brain mitochondrial protein content was determined according to Lowry *et al.* [Lowry *et al.* 1951] with bovine serum albumin as the standard.

4.1.3.1.1.5. Statistical analysis

IC_{50} values were calculated with the 95% confidence limits by using the GraphPad Prism Software (version 5.0), from plots of inhibition percentages (calculated in relation to a sample of the enzyme treated under the same conditions without inhibitors) versus the logarithm of the inhibitor concentration.

4.1.3.1.2. Kinetic analysis of MAO-A and MAO-B inhibition [Strydom *et al.* 2010]

In order to perform the kinetic characterization of MAO, the most active test MAO-A and MAO-B inhibitors, were preincubated with the mitochondrial suspension, Tris-HCl buffer and substrate (serotonin for MAO-A and benzylamine for MAO-B) at 37 °C for 30 min. Kinetic characterization of the hydrolysis of serotonin catalyzed by MAO-A and benzylamine catalyzed by MAO-B was recorded spectrophotometrically at 280 nm and 250 nm respectively. A parallel control was made for an assay solution with no inhibitor. The plots were assessed by a weighed least square analysis that assumed the variance of V to be a constant percentage of V for the entire data set. The Lineweaver-Burk plots were plotted as a function of the concentrations of the inhibitors in a weighted analysis.

4.1.3.1.3. Reversibility and irreversibility experiments

To investigate whether the observed enzyme inhibition is reversible or irreversible, time-dependant inhibition study was carried with the representative MAO-A and MAO-B inhibitors, using the slightly modified method described by Legoabe *et al*. [Legoabe *et al.* 2012]. The compounds were preincubated with the mitochondrial working solution for various periods of time (0, 15, 30, 60 min) at 37 °C in tris-HCl buffer (50 mM, pH 8.2). For this purpose, the concentration of inhibitors was equal to two-fold the measured IC_{50} value for the inhibition of MAO-A and MAO-B. The reactions were subsequently diluted two-fold by the addition of 4 mM serotonin and 0.1 M benzylamine to yield a final enzyme concentration equal to half the concentration of mitochondrial working solution and the concentration of the inhibitors equal to the IC_{50} values. The reactions were further incubated at 37 °C for a further 15 min and the residual enzyme activities were measured and the bar graphs were constructed. All measurements were carried out in triplicate and are expressed as mean ± SEM.

4.1.3.1.4. Molecular modeling studies

4.1.3.1.4.1. Platform

A computational approach through docking calculations was used to obtain insights into the possible binding mode and interactions of the test inhibitors within the active sites of MAO-A and MAO-B. The molecular docking studies were carried out in the PC based machines running Windows 7 (x86) as operating system.

4.1.3.1.4.2. Software

The molecular modelling software included MGL tools 1.5.4 based AutoDock 4.2 (www.scripps.edu) which uses Python 2.7 language - Cygwin C:\ program (www.cygwin.com) and Python 2.5 (www.python.com). Discovery Studio Visualizer 3.1 (www.accelrys.com) was employed for visualizing the docked molecules.

4.1.3.1.4.3. Receptor data set preparation

The X-ray crystallographic structures of human MAO-A co-crystallized with harmine (PDB entry: 2Z5X, resolution = 2.20 Å) [Son *et al.* 2008] and human MAO-B co-crystallized with safinamide (PDB entry: 2V5Z, resolution = 1.6 Å) [Binda *et al.* 2004] were retrieved from the Brookhaven Protein Data Bank (http://www.rcsb.org/pdb) and used for performing molecular docking studies.

4.1.3.1.4.4. Coordinate file preparation

Computational studies were carried out on only one subunit of the MAO isozymes. The pdb files of MAO isozymes obtained from Protein Data Bank were edited and β – chain was removed together with the complexed inhibitor except FAD from the active site. All the water molecules and all non-interacting ions were also removed. This refinement in the crystal structure of both MAO isozymes was carried out with the help of Discovery Studio Visualizer. This was then loaded on AutoDock Tools. Addition of missing hydrogen atoms was carried out after assigning the bond orders. Then the Gasteiger-Marsili charges and Kollman charges were calculated, non-polar hydrogens were merged and rotatable bonds were assigned. The file was then saved to pdbqt format [Madeswaran *et al.* 2011].

4.1.3.1.4.5. Ligand data set preparation

The structures of the test ligands (**BTA-1** to **BTA-30**) and reference ligand molecules (harmine for MAO-A and safinamide for MAO-B) were built with the aid of the MarvinSketch 5.6 module of Chemaxon tools (www.chemaxon.com) and optimized using "Prepare Ligands" in the AutoDock 4.2 and saved in PDB format. The structures of the ligands were then loaded in ADT Tools and were converted to pdbqt file format [Goodsell *et al.* 1996].

4.1.3.1.4.6. Docking methodology

Lamarckian genetic algorithm (LGA), a hybrid of a genetic algorithm and a local search algorithm, was employed for performing molecular docking [Goodsell *et al.* 1996].

In all docking, a grid of definite size points was built in x, y, and z directions, and the maps were centered on the N5 atom of the flavin (FAD) in the catalytic site of the protein. A grid spacing of 0.375 Å (approximately one fourth of the length of a carbon-carbon covalent bond) and a distance-dependent function of the dielectric constant were used for the calculation of the energetic map. Furthermore, an electrostatic map and a desolvation map were calculated [Konc *et al.* 2011]. Rapid energy evaluation was achieved by pre-calculating atomic affinity potentials for each atom in the ligand molecule. In the Auto Grid procedure, the respective receptor was embedded on a three-dimensional grid point [Madeswaran *et al.* 2011] and the energy of interaction for each atom in the ligand was encountered. The grid maps were calculated by Auto Grid, one for each atom type present in the ligand being docked. Thus, the grid parameter file

(receptor.gpf) was generated and saved. This is followed by the generation of docking parameter file (ligand.dpf).

The various important docking parameters selected for LGA includes population size of 150 individuals, 2.5 million energy evaluations, maximum of 27000 generations, maximum number of top individuals to automatically survive to next generation of 1, gene mutation rate of 0.02, crossover rate of 0.8, 10 docking runs and random initial positions and conformations. The probability of performing local search on an individual in the population was set to 0.06.

AutoDock was run several times to get various docked conformations and used to analyze the predicted docking energy. The binding sites for these molecules were selected based on the ligand binding pocket of the templates. For each ligand, 10 best poses were generated and scored using AutoDock 4.2 scoring functions [Madeswaran *et al.* 2011]. At the end of docking, ligands with the most favourable free energy of binding were selected as the resultant complex structure. The same above procedure was repeated separately for all the ligands. The docking solutions were ranked according to their respective dock score values.

Finally, the docking results generated were directly loaded into the Discovery Studio Visualizer and hydrogen bonding and hydrophobic interactions between docked ligands and macromolecule were analyzed. AutoDock tools provide various methods to analyze the results of docking simulations, like conformational similarity, visualizing the binding site and its energy, in addition to other parameters such as intermolecular energy and inhibition constant.

4.1.3.2. Acetylcholinesterase (AChE) enzyme inhibition studies

4.1.3.2.1. *In-vitro* AChE inhibition assay

The *in-vitro* AChE inhibition of the synthesized compounds against rat AChE was performed according to the slightly modified spectrophotometric method of Ellman *et al.*, 1961 [Ellman *et al.* 1961]. The assay is based on the reaction of released thiocholine to give the coloured product with a chromogenic reagent 5,5'-dithio-bis(2-nitrobenzoic acid) (DTNB). The chemical principle of the reaction is depicted in **Figure 4.30.**

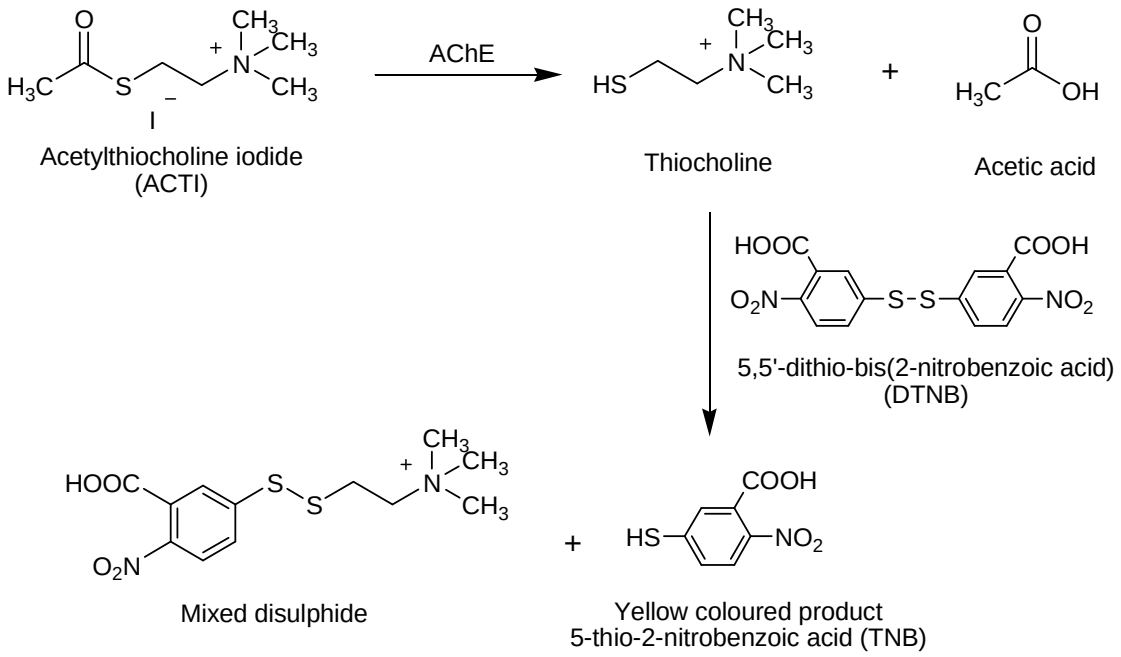


Figure 4.30. Principle of Ellman's assay

4.1.3.2.1.1. Materials

Acetylthiocholine iodide (ACTI), 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB) and the reference inhibitors donepezil hydrochloride and tacrine hydrochloride were procured from Sigma-Aldrich (U.S.A.). Tris-HCl and sodium phosphate were obtained from Merck (Germany) and SD Fine (Mumbai).

4.1.3.2.1.2. Isolation of rat brain AChE

All the experiments were carried out under the optimal laboratory conditions. Test compounds were dissolved in DMSO. Acetylthiocholine iodide (ACTI) was used as a substrate and 5,5'-Dithiobis[2-nitrobenzoic acid] (DTNB) was used for the measurement of AChE activity. Rat brain was used as a source of AChE enzyme. Rats were separately decapitated, the brains quickly removed, weighed and homogenized in cold 10 mM Tris-HCl buffer, pH 7.2, containing 160 mM sucrose. The homogenates were centrifuged at 10,000 g for 10 min at 4 °C, and the resulting clear supernatants were used as enzyme sources that were divided into aliquots and stored at -20 °C.

4.1.3.2.1.3. Protocol for AChE assay

About 20 µl of enzyme sample and 60 µl of 20 mM sodium phosphate buffer, pH 7.4 were incubated in the presence of 20 µl of 10 mM DTNB solution with different concentrations of the test compounds. The enzyme reaction was initiated by the addition

of 20 µl of 0.8 mM acetylthiocholine iodide (ACTI). The mixtures were incubated for 15 min at 25 °C. The hydrolysis of acetylthiocholine was monitored by the formation of yellow coloured enzymatic product 5-thio-2-nitrobenzoate anion which was determined by the variation in absorbance measured at 415 nm, with 96-well plate reader (BioTek, ELx800 TM, Instruments Inc., Winooski, VT, USA). Control experiments were carried out without inhibitor, and blanks were run without AChE enzyme. Donepezil and tacrine were taken as reference inhibitors for the determination of AChE activity. All the assays were performed in triplicate.

4.1.3.2.1.4. Statistical analysis

IC_{50} values were determined using the GraphPad Prism 5.0 (San Diego, CA, USA), from the plots of inhibition percentages (calculated in relation to a sample of the enzyme treated under the same conditions without inhibitor) versus the logarithm of the inhibitor concentration and the results were expressed as mean ± standard error mean (SEM).

4.1.3.2.2. Kinetic analysis of AChE inhibition

In order to perform the kinetic characterization of AChE, the most active test inhibitors were preincubated with the enzyme, sodium phosphate buffer, DTNB solution and substrate (ACTI) at 25 °C for 15 min. Kinetic characterization of the hydrolysis of ACTI catalyzed by AChE was recorded colorimetrically at 415 nm. A parallel control was made for an assay solution with no inhibitor. The plots were assessed by a weighed least square analysis that assumed the variance of V to be a constant percentage of V for the entire data set. The Lineweaver-Burk plots (reciprocal plot slopes) were plotted as a function of the concentrations of the inhibitors in a weighted analysis.

4.1.3.2.3. Reversibility and irreversibility experiments

Time-dependant inhibition study was performed with the most active inhibitor in order to investigate the reversibility of the observed enzyme inhibition. The test inhibitor was preincubated with the AChE enzyme for various periods of time *viz.* 0, 15, 30, 60 min at 25 °C in sodium phosphate buffer (20 mM, pH 7.4) and DTNB solution (10 mM). For this purpose, the concentration of test inhibitor was equal to two-fold the measured IC_{50} value for the inhibition of AChE. The reaction was subsequently diluted two-fold by the addition of 0.8 mM ACTI such that the final concentration of the test inhibitor was equal to its IC_{50} value. The reaction was further incubated at 25 °C for further 15 min and the

residual enzyme activities were measured and the bar graphs were constructed. All measurements were carried out in triplicate and expressed as mean ± SEM.

4.1.3.2.4. Molecular modeling studies

A computational approach through molecular docking simulations was used to shed some light on the possible binding orientation and interactions between the virtual AChE-inhibitor complexes.

4.1.3.2.4.1. Receptor data set preparation

The simulation system was built on the X-ray crystal structure of the human recombinant Donepezil – AChE complex (PDB entry 4EY7, resolution = 2.35 Å) [Cheung *et al.* 2012], which was retrieved from the Brookhaven Protein Data Bank (http://www.rcsb.org/pdb). Computational studies were performed only on one subunit of the enzyme. The original ligand, heteroatoms, co-crystallized solvent if any was removed using Discovery Studio Visualizer. This was then loaded on AutoDock Tools (ADT; version 1.5.4). Proper bonds and bond orders were assigned and the missing hydrogen atoms were added. Partial charges for proteins were added using Gasteiger-Marsili charges and Kollman charges, non-polar hydrogen were merged and rotatable bonds were assigned. The file was then saved to pdbqt file format for further analysis.

4.1.3.2.4.2. Ligand data set preparation

The 3D structures of all the ligands (**BTA-1** to **BTA-30**) and reference inhibitor (donepezil) were generated using the Marvin Sketch 5.6 module of Chemaxon tools [www.chemaxon.com] and optimized using "Prepare Ligands" in the AutoDock 4.2 and saved in PDB format. The structures of the ligands were then loaded in ADT Tools and flexible torsions were assigned with AutoTors module, and the acyclic dihedral angles were allowed to rotate freely. The file was then saved as pdbqt file format for further analysis [Goodsell *et al.* 1996]

4.1.3.2.4.3. Docking Methodology

The docking methodology was performed using the refined receptor molecule, 4EY7 for rhAChE according to the protocol described in section **4.1.3.1.4.6.**

4.1.3.3. Behavioural studies

4.1.3.3.1. Antidepressant activity (Porsolt's forced swim test)

The forced swim test (FST) is the most widely used pharmacological *in-vivo* model for assessing antidepressant activity. The synthesized compounds (**BTA-1** to **BTA-30**) were

evaluated for their antidepressant activity using Porsolt's forced swim pool method [Porsolt *et al.* 1978]. The Wistar mice weighing 20 and 25 g were used in the experiment. The animals were randomly allocated into three groups (standard, control, and test) of six animals each and were fasted for 24 h before the experiment with free access to water. The animals were placed in a plexiglass cylinder (diameter: 12 cm and height: 20 cm) containing water upto a height of 15 cm at 25 ± 2 °C. Two swim sessions were conducted, an initial pre-test session for 15 min followed by the test session of 6 min after 24 h of pre-test session, in which the duration of immobility was recorded for the last 5 min. Control group received only saline treatment. Citalopram (CIT) was used as a reference drug. The test compounds and the reference drug were administered at a dose of 30 mg/kg i.p. as suspension in 1% (w/v) carboxymethylcellulose in water. After 60 min of injection of the test compounds and the standard drug, the animals were allowed to swim inside the cylinder and the duration of immobility (the period between when the mouse was immersed and when no further attempts to escape was made apart from the movements necessary to keep its head above the water) was recorded using the tracking software and analyzed.

4.1.3.3.2. Anxiolytic activity (Elevated plus maze test)

The anxiolytic activity of the synthesized compounds (**BTA-1** to **BTA-30**) was examined in mice using the elevated plus maze model [Pellow *et al.* 1985]. The apparatus consisted of a plus shaped maze elevated 38.5 cm above the room floor with two oppositely positioned closed arms (30 cm x 5 cm), two oppositely positioned open arms (30 cm x 5 cm), and the central area (5 cm x 5 cm). The Wistar mice weighing 20-25 g were used in the experiment. The animals were randomly allocated into three groups (standard, control and test) of six animals each and were fasted for 24 h before the experiment with free access to water. The synthesized compounds and the reference drug diazepam (DZM) were administered at a dose of 30 mg/kg i.p. and 10 mg/kg i.p respectively as suspension of 1% (w/v) carboxymethylcellulose in water one hour prior to the experiment. Control group received only saline treatment. Each animal was placed in the centre of the maze facing one of the open arms and the time spent in open and closed arms was recorded for 5 min. [Trullas 1993; Lister 1990; 1987]. The movement of animals across the arms was calculated by interruption of beams which was analyzed by Maze tracking software (M/s Columbus Instruments, USA). After each test, the maze was carefully cleaned up with

10% ethanol solution. The preference for being in open arms over closed arms expressed as either the percentage of entries in open arm and/or percentage of time spent in the open arms was interpreted as the level of anxiety.

4.1.3.3.3. Sedative-hypnotic activity (Pentobarbitone potentiation test)

The potentiation or antagonism of the synthesized compounds (**BTA-1** to **BTA-30**) with respect to the pentobarbitone (PB) induced narcosis in the mice was evaluated using the righting reflex method [Porter *et al*. 1985]. The Wistar mice weighing 20-25 g were used in the experiment. The animals were randomly allocated into three groups (standard, control and test) of six animals each and were fasted for 24 h before the experiment with free access to water. The test groups were administered at a dose of 30 mg/kg i.p. and control with vehicle (1% Tween 80 in water). Thirty minutes later, pentobarbitone (30 mg/kg i.p.) was administered to each mouse to induce sleep. The animals were observed for the duration of sleep which depicts the time between the loss and recovery of righting reflex [Ramirez *et al.* 1998]

4.1.3.4. Neurotoxicity screening (Rotarod test)

Rotarod test is commonly used for the evaluation of neurotoxicity or neurological deficit in mice treated with the synthesized test compounds **(BTA-1** to **BTA-30)** and reference drug (phenytoin) used in the study [Dunham and Miya 1957]. The neurotoxicity screening was undertaken according to the method followed by the National Institute of Health, using their reported procedures. Male albino mice (CF-1 strain or Swiss, 20-25 g) were used as experimental animals. The animals were housed in metabolic cages, and allowed free access to food and water. The test compounds were suspended in 0.5% methylcellulose/water mixture or in polyethylene glycol (PEG 200).

The mice were trained to stay on an accelerating rotarod that rotates at six revolutions per minute. The rod diameter was 3.2 cm. Animals were trained on the rotarod for duration of 2 min per trial, with three trials per day for two days. On the third day, trained animals were given i.p. injection of the selected inhibitors at a dose of 30 mg/kg. Neurotoxicity was indicated by the inability of the animal to maintain equilibrium on the rod for at least 1 minute in each of the three trials. Results are expressed as number of animals exhibiting toxicity/number of animals tested.

4.1.3.5. Antioxidant activity (DPPH radical scavenging assay)

The basis of the DPPH assay method is the reduction of DPPH which is a stable free radical. DPPH· possesses an odd electron which confers a maximum absorption at 517 nm (purple colour). The reaction of DPPH· with an antioxidant results in the pairing off DPPH· in the presence of a hydrogen donor (i.e. a free radical-scavenging antioxidant) and is reduced to the DPPHH and as a result the absorbance is reduced from the DPPH. DPPH· to the DPPH-H conversion results in decolorization (yellow colour). More the decolorization more is the reducing ability. When a solution of DPPH is mixed with the compound which can donate a hydrogen atom, it gives rise to the reduced form viz. Diphenylpicrylhydrazine (a non radical) with the loss of this violet colour (**Figure 4.31.**).

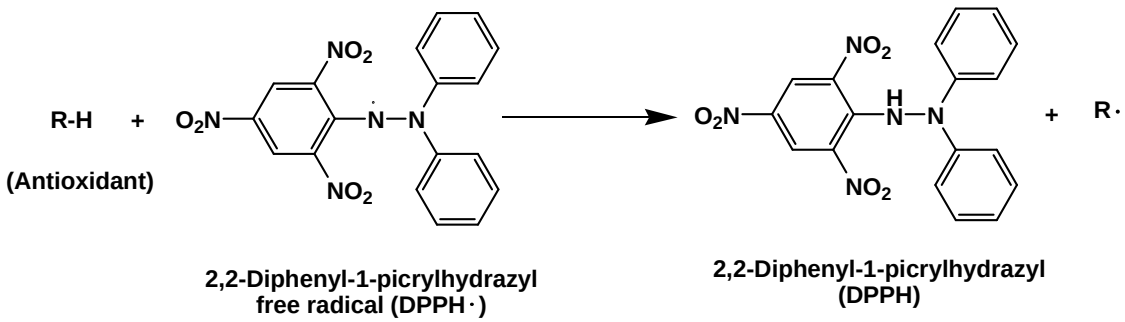


Figure 4.31. Conversion of DPPH free radical to DPPH by an antioxidant

Antioxidant activity of the synthesized compounds (10 mg/ ml) was evaluated using the radical scavenging capability against the stable free radical, 2,2'-diphenyl-1-picryl hydrazyl (DPPH·). The DPPH· scavenging capability was determined using UV based spectrophotometric assay [Rajesh and Natvar, 2011]. 200 µl of the test sample solution (100 µg/ml) was added to 4 ml of 100 µM methanolic DPPH. The sample tubes were wrapped with aluminium foil and kept in dark for 30 min at room temperature and the absorbance was measured at 517 nm using UV–Vis spectrophotometer with solvent and DPPH as blank. Ascorbic acid (100 µg/ml) was used as standard. The percentage inhibition of DPPH activity was estimated using the formula

$$\text{Percent (\%) inhibition of DPPH activity} = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

Where $A_{control}$ is the absorbance of DPPH· in methanol without an antioxidant and A_{sample} is the absorbance of DPPH· in the presence of an antioxidant

4.1.3.6. Assessment of liver function

Enzyme estimation and histopathological studies of some of the selected compounds were done to check the magnitude of liver toxicity [Siddiqui *et al.* 2007]

4.1.3.6.1. Liver toxicity tests

Compounds were investigated for their influence on the biochemical parameters such as serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), [Reitman and Frankel 1957] alkaline phosphatase (ALP), [King *et al.* 1934; Reinhold 1953], total protein and total albumin [Varley 1988].

4.1.3.6.2. Liver histopathological studies

The histopathological studies were carried according to the previously reported method [Luna 1968]. The animals were randomly allocated into three groups (positive control, negative control and test) of six animals each with free access to water. The rats were administered with the test compounds (dose = 30 mg/kg i.p.) while the positive control was treated with normal saline while the negative control was treated with chloroform for 15 days. The rats were scarified under light ether anaesthesia after 24 h of last dosage; the livers were removed and washed with normal saline. Small pieces of liver tissue were processed and embedded in paraffin wax. Sections of 5 – 6 µm in thickness were cut, stained with haematoxylin and eosin and then studied under an electron microscope.

4.1.4. *In-silico* molecular property analysis and ADMET prediction studies

Unfavourable pharmacokinetic properties have been identified as the major cause of failure for new drug entities moving towards higher phases of drug development. [Yu and Adedoyin, 2003] Therefore, with the objective of increasing the success rate of compounds reaching development, *in-silico* pharmacokinetic prediction studies of the synthesized compounds were performed using online computational softwares. Chemical structures, SMILES notations and mol files of the titled compounds were generated using Marvin Sketch module of Chemaxon Marvin Beans 5.6.0.2 (www.chemaxon.com). SMILES notations of the synthesized compounds were then fed in the online Molinspiration software 2015 (www.molinspiration.com) to calculate various molecular properties. [Molinspiration Cheminformatics, Bratislava, Slovak Republic, Available from: molinspiration.com/services/properties.html] The molecular properties such as molecular weight (MW), partition coefficient (MiLog P), topological polar surface area (TPSA), number of hydrogen bond acceptors (HBA) and number of hydrogen bond

donors (HBD), and violations of Lipinski's rule of five and molecular volume were calculated to evaluate the drug likeness of the synthesized compounds [Lipinski *et al.* 2001].

For *in-silico* ADMET profiling, the mol files of compounds were uploaded on the PreADMET online server (http://www.preadmet.bmdrc.org) [www.preadmet.bmdrc.org]. The server calculates parameters such as human intestinal absorption (HIA), *in-vitro* Caco2 cell and MDCK (Maden Darby Canine Kidney) cell permeability, *in-vitro* skin permeability, *in-vitro* plasma protein binding (PPB), *in-vivo* blood brain barrier (BBB) penetration and toxicity profiles such as mutagenicity, carcinogenicity (mouse and rat) [Chikhale *et al.* 2012] and cardiotoxicity (hERG inhibition) [Thomas and Karle 2006].

4.2. 2-AMINO-5-NITROTHIAZOLE DERIVED SEMICARBAZONES [NTA-1 to NTA-18]

4.2.1. Synthesis

4.2.1.1. Chemicals and reagents

All the chemicals and reagents used for this work were of analytical grade and obtained from Sigma-Aldrich (U.S.A.), Merck (Germany), SD Fine (Mumbai) and Qualigens (Mumbai).

4.2.1.2. Synthetic protocol [Siddique *et al.* 2007]

2-Amino-5-nitrothiazole derived semicarbazones (**NTA-1** to **NTA-18**) were synthesized through the synthetic route illustrated in **Scheme 4.5.**

Step 1. Synthesis of 1-(5-nitrothiazol-2-yl)urea (NTU)

2-Amino-5-nitrothiazole (**NTA**, 0.030 mol) was dissolved in glacial acetic acid (30 mL) with continuous stirring on a magnetic stirrer. To this, a warm solution of sodium cyanate (0.031 mol, 2.0 equiv) in H_2O (60 mL) was added with vigorous stirring. The mixture was stirred for 5 h before being left to stand overnight. The resultant solid was collected by filtration, washed with ice cold H_2O to remove excess glacial acetic acid and dried. The reddish-brown coloured crude product 1-(5-nitrothiazol-2-yl)urea (**NTU**) obtained was then recrystallized from 95% ethanol. Purity of compound was checked by TLC analysis using the solvent system (Chloroform: Methanol: Toluene (7:2:1), R_f: 0.49).

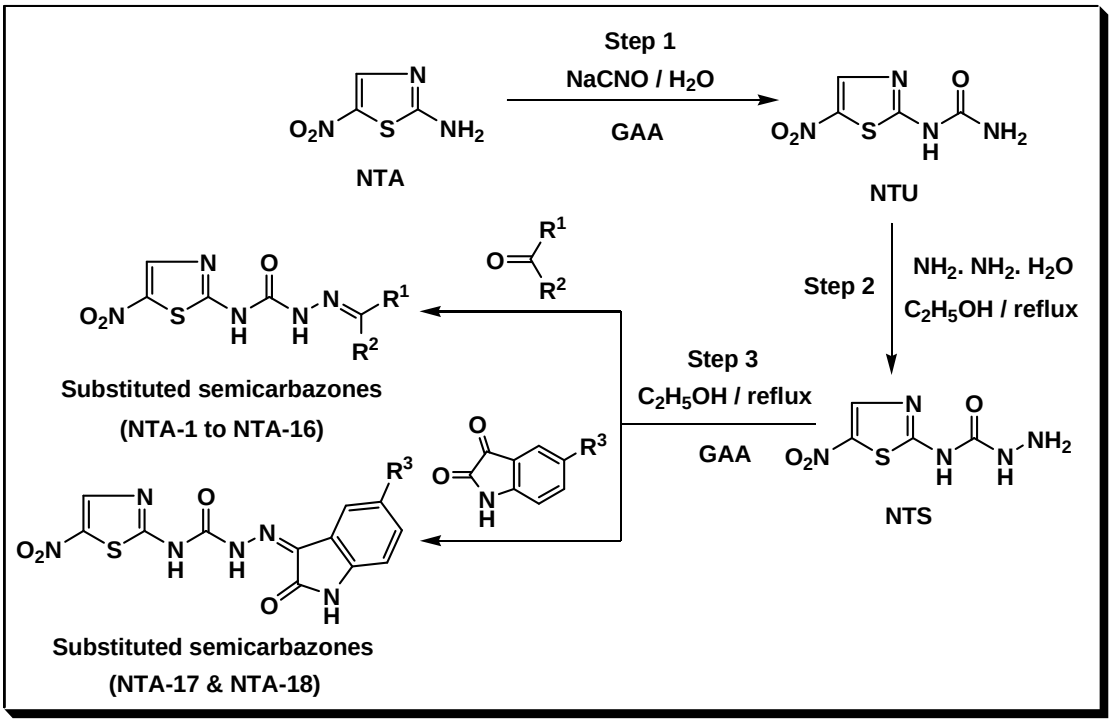
Step 2. Synthesis of 4-(5-nitrothiazol-2-yl)semicarbazide (NTS)

In a RB flask containing **NTU (**0.03 mol) dissolved in ethanol (30 mL), hydrazine hydrate (0.03 mol, 2.0 eq.) was added and the reaction mixture was refluxed for about 18 h. Solvent was evaporated, and the resultant residue obtained was recrystallized from 95% ethanol to produce 4-(5-nitrothiazol-2-yl)semicarbazide (**NTS**). Purity of compound was checked by TLC analysis using the solvent system (Chloroform: Methanol: Toluene (7:2:1), R_f: 0.41).

Step 3. Synthesis of semicarbazones (NTA-1 to NTA-18)

The final compounds **NTA-1** to **NTA-18** (substituted semicarbazones) were synthesized by the reaction of **NTS** (0.003 mol) with appropriately substituted aldehydes or ketones or 5-(un)substituted isatin (0.003 mol). The reaction mixture was adjusted to pH 5-6 by adding few drops of glacial acetic acid and held at reflux for 29-80 h. The solvent was either evaporated or the contents of the flask was quenched in ice cold water and the

precipitate obtained was filtered, dried and recrystallized from 95% ethanol to produce final products **NTA-1** to **NTA-18**.



Scheme 4.5. Synthesis of 2-amino-5-nitrothiazole derived semicarbazones (**NTA-1** to **NTA-18**)

4.2.1.3. Reaction mechanism

The reaction mechanism for semicarbazone formation is illustrated in **Figure 4.32.**

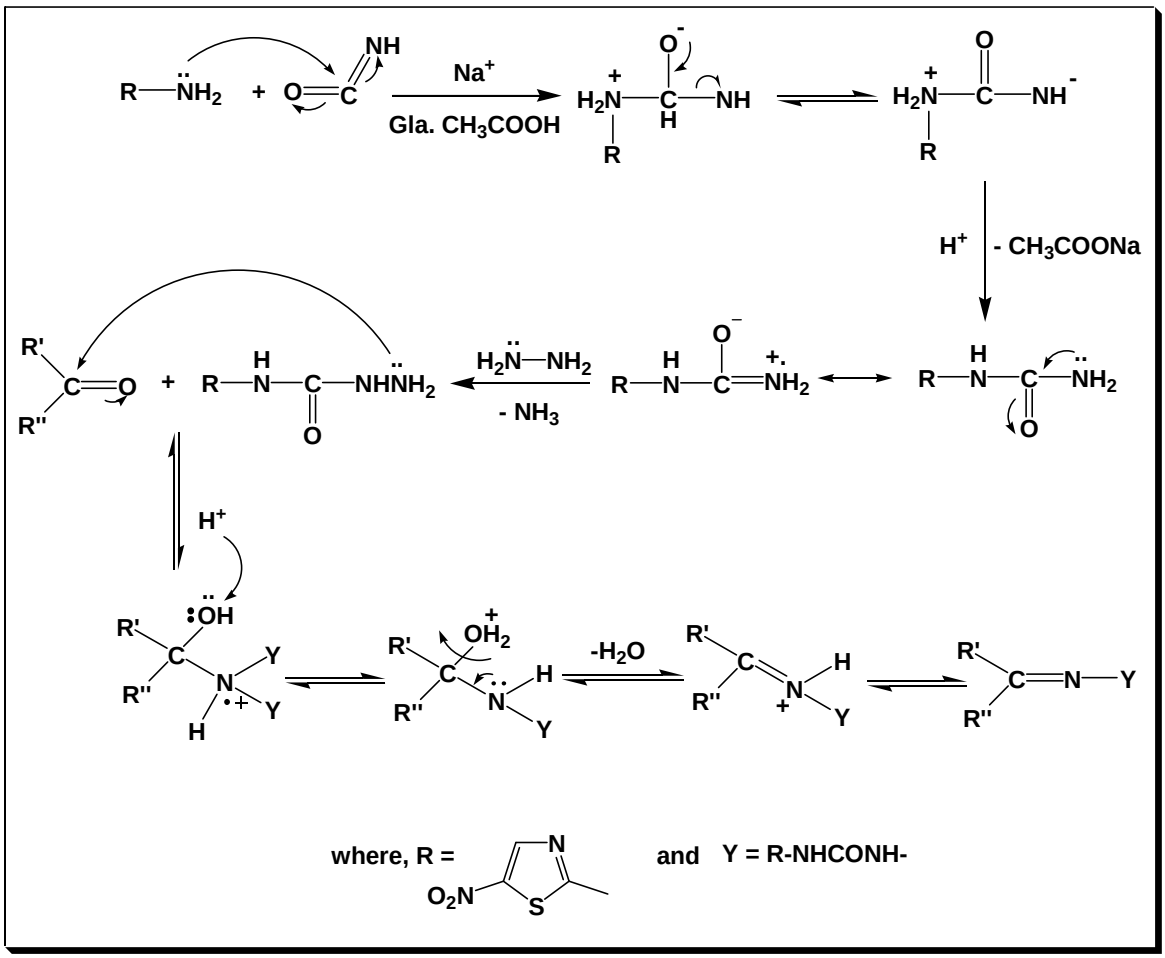
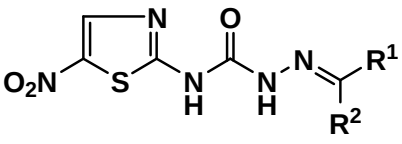
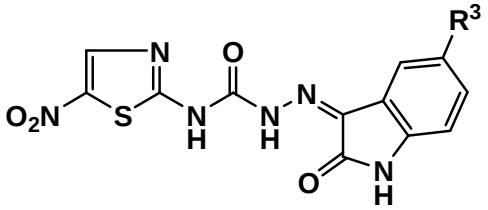


Figure 4.32. Reaction mechanism for synthesis of semicarbazones

Table 4.5. Structural data of 2-amino-5-nitrothiazole derived semicarbazones (**NTA-1** to **NTA-18**)



NTA-1 to NTA-16



NTA-17 and NTA-18

Code	R^1	R^2	R^3	Mol. Formula	Mol. Wt. (g/mol)
NTA-1	CH_3	4-Br C_6H_4	-	$C_{12}H_{10}BrN_5O_3S$	384.21
NTA-2	CH_3	4-Cl C_6H_4	-	$C_{12}H_{10}ClN_5O_3S$	339.76

NTA-3	CH_3	4-F C_6H_4	-	$C_{12}H_{10}FN_5O_3S$	323.3
NTA-4	CH_3	4-OH C_6H_4	-	$C_{12}H_{11}N_5O_4S$	321.31
NTA-5	CH_3	4-NO_2 C_6H_4	-	$C_{12}H_{10}N_6O_5S$	350.31
NTA-6	CH_2 Br	4-Br C_6H_4	-	$C_{12}H_9Br_2N_5O_3S$	463.1
NTA-7	H	4-Br C_6H_4	-	$C_{11}H_8BrN_5O_3S$	370.18
NTA-8	H	4-OH C_6H_4	-	$C_{11}H_9N_5O_4S$	307.29
NTA-9	H	2,3-Cl_2 C_6H_3	-	$C_{11}H_7Cl_2N_5O_3S$	360.18
NTA-10	H	2,4-Cl_2 C_6H_3	-	$C_{11}H_7Cl_2N_5O_3S$	360.18
NTA-11	H	2,6-Cl_2 C_6H_3	-	$C_{11}H_7Cl_2N_5O_3S$	360.18
NTA-12	H	2,5-$(OCH_3)_2$ C_6H_3	-	$C_{13}H_{13}N_5O_5S$	351.34
NTA-13	C_6H_5	C_6H_5	-	$C_{17}H_{13}N_5O_3S$	367.38
NTA-14	C_6H_5	4-Cl C_6H_4	-	$C_{17}H_{12}ClN_5O_3S$	401.83
NTA-15	C_6H_5	4-OH C_6H_4	-	$C_{17}H_{13}N_5O_4S$	383.38
NTA-16	4-Cl C_6H_4	4-Cl C_6H_4	-	$C_{17}H_{11}Cl_2N_5O_3S$	436.27
NTA-17	-	-	H	$C_{12}H_8N_6O_4S$	332.29
NTA-18	-	-	Br	$C_{12}H_7BrN_6O_4S$	411.19

4.2.2. Characterization

The physicochemical and spectral characterizations of the synthesized compounds (**NTA-1** to **NTA-18**) were performed so as to ascertain the chemical structure of compounds. The complete procedures of all the characterization methods followed and the instruments used are stated in **Section 4.1.2.**

4.2.2.1. Physicochemical characterization

The physicochemical characterization data of synthesized compounds (**NTA-1** to **NTA-18**) are listed in **Table 4.6.**

Table 4.6. Physicochemical characterization data of **NTA-1** to **NTA-18**

Code	MP (°C)	Yield (%)	Colour	R_f[a]	LogP[b]	Expt. LogP[c]
NTA-1	245-247	59.42	Maroon	0.51	3.08	2.2
NTA-2	144-146	65.84	Brown	0.49	2.91	2.1
NTA-3	267-269	63.92	Brown	0.47	2.45	1.3

NTA-4	193-195	25.61	Brown	0.53	2.01	1.2
NTA-5	292-294	71.25	Brown	0.46	2.25	1.4
NTA-6	123-125	38.23	Maroon	0.52	3.80	-
NTA-7	287-289	56.71	Maroon	0.48	3.23	2.5
NTA-8	148-150	49.56	Brown	0.45	2.16	1.6
NTA-9	>300	61.32	Brown	0.52	3.67	2.6
NTA-10	232-234	64.51	Brown	0.56	3.67	2.5
NTA-11	100-102	33.27	Brown	0.48	3.67	3.1
NTA-12	276-278	59.42	Brown	0.43	2.15	-
NTA-13	Charred at 286	51.54	Brown	0.41	4.21	3.4
NTA-14	182-184	48.86	Brown	0.44	4.82	3.6
NTA-15	113-115	29.75	Brown	0.49	3.91	2.9
NTA-16	>300	58.67	Brown	0.54	5.42	3.9
NTA-17	158-160	79.58	Dark brown	0.57	1.73	1.1
NTA-18	297-299	75.46	Dark brown	0.49	2.50	1.9

*All the compounds were soluble in methanol, ethanol, DMF and DMSO; [a]Solvent system: (a) Chloroform: Methanol: Toluene (7:1:2, 7:2:1) (b) Benzene: Acetone (8:2, 9:1); [b]Marvinsketch generated; [c]Determined using Shake flask method

4.2.2.2. Spectral characterization and elemental analysis

All the synthesized compounds (**NTA-1** to **NTA-14**) were subjected to UV, IR, ^{1}H NMR, ^{13}C NMR and elemental analysis and the results are presented below in section **4.2.2.3.** In addition, mass spectrum was measured for compounds **NTA-5**, **NTA-10** and **NTA-17** and the $[M+1]^{+}$ peak of these compounds is presented below (**Figure 4.36.**, **Figure 4.41.** and **Figure 4.49.**). Moreover, compound **NTA-5** was subjected to X-ray powder diffraction analysis and the diffraction pattern is shown in **Figure 4.37.**

4.2.2.3. Spectral characterization and elemental analysis data of intermediates (NTU and NTS) and final compounds (NTA-1 to NTA-18)

1-(5-Nitrothiazol-2-yl)urea (NTU): IR (KBr): υ = 3402.54, 3254.02 (N-H str), 1705.13 (C=O str), 1622.19 (C=N str), 1489.10, 1371.3 (NO_2 str), 1201.69 (C-N str); **^{1}H NMR ([D_6]DMSO):** δ = 8.42 (s, 1H, thiazole C-H), 8.59 (s, 1H, NH), 9.02 ppm (s, 2H, NH_2);

^{13}C NMR ([D$_6$]DMSO): δ = 107.70 (thiazole C-5), 143.92 (thiazole C-4), 158.93 (C=O), 160.51 ppm (thiazole C-2); **Elemental analysis for $C_4H_4N_4O_3S$: calcd:** C 25.53, H 2.14, N 29.78, **found:** C 25.50, H 2.18, N 29.73.

4-(5-Nitrothiazol-2-yl)semicarbazide (NTS): IR (KBr): υ = 3313.82, 3178.79 (N-H str), 1662.69 (C=O str), 1541.18 (C=N str), 1508.38, 1357.93 (NO_2 str), 1276.92 (C-N str); **^{1}H NMR ([D$_6$]DMSO):** δ = 1.95 (s, 2H, NH_2), 8.49 (s, 1H, thiazole C-H), 9.132 (s, 1H, NH), 10.63 ppm (s, 1H, CONH); **^{13}C NMR ([D$_6$]DMSO):** δ = 107.95 (thiazole C-5), 142.06 (thiazole C-4), 158.91 (C=O), 162.84 ppm (thiazole C-2); **Elemental analysis for $C_4H_5N_5O_3S$: calcd:** C 23.65, H 2.48, N 34.47, **found:** C 23.60, H 2.51, N 34.44.

1-(1-(4-Bromophenyl)ethylidene)-4-(5-nitrothiazol-2-yl)semicarbazide (NTA-1): λ_{max}: 271.00, 305.00 nm; **IR (KBr):** υ = 3410.26, 3156 (N-H str), 3082.35 (aromatic C-H str), 1683.93 (C=O str), 1624.42 (C=N str), 1586.25, 1381.08 (NO_2 str), 1300.07 (C-N str), 713.89 (C-Br str); **^{1}H NMR ([D$_6$]DMSO):** δ = 1.14 (s, 3H, CH_3), 7.50 (d, J = 6.3 Hz, 2H, Ar C-3, Ar C-5), 7.78 (d, J = 5.7 Hz, 2H, Ar C-2, Ar C-6), 8.81 (s, 1H, thiazole C-H), 9.17 (s, 1H, NH), 10.48 ppm (s, 1H, CONH); **^{13}C NMR ([D$_6$]DMSO):** δ = 19.14 (CH_3), 107.85 (thiazole C-5), 128.05 (Ar C-4), 129.58 (Ar C-2, Ar C-6), 130.90 (Ar C-3, Ar C-5), 133.33 (Ar C-1), 140.88 (thiazole C-4), 156.11 (C=O), 164.25 (thiazole C-2), 168.95 ppm (C=N); **Elemental analysis for $C_{12}H_{10}BrN_5O_3S$: calcd:** C 37.51, H 2.62, N 18.23, **found:** C 37.49, H 2.65, N 18.27.

1-(1-(4-Chlorophenyl)ethylidene)-4-(5-nitrothiazol-2-yl)semicarbazide (NTA-2): λ_{max}: 269.40, 302.40 nm; **IR (KBr):** υ = 3431.48, 3313.82 (N-H str), 3090.07 (aromatic C-H str), 1670.41 (C=O str), 1606.76 (C=N str), 1531.30, 1400.27 (NO_2 str), 1161.39 (C-N str), 831.35 (C-Cl str); **^{1}H NMR ([D$_6$]DMSO):** δ = 1.13 (s, 3H, CH_3), 7.54 (d, J = 6.6 Hz, 2H, Ar C-3, Ar C-5), 7.85 (d, J = 6.6 Hz, 2H, Ar C-2, Ar C-6), 8.29 (s, 1H, thiazole C-H), 9.08 (s, 1H, NH), 10.33 ppm (s, 1H, CONH); **^{13}C NMR ([D$_6$]DMSO):** δ = 19.12 (CH_3), 109.17 (thiazole C-5), 129.92 (Ar C-3, Ar C-5), 131.54 (Ar C-2, Ar C-6), 132.15 (Ar C-1), 134.13 (Ar C-4), 140.65 (thiazole C-4), 158.54 (C=O), 163.93 (thiazole C-2), 171.72 ppm (C=N); **Elemental analysis for $C_{12}H_{10}ClN_5O_3S$: calcd:** C 42.42, H 2.97, N 20.61, **found:** C 42.38, H 3.01, N 20.58.

1-(1-(4-Fluorophenyl)ethylidene)-4-(5-nitrothiazol-2-yl)semicarbazide (NTA-3): λ_{max}: 252.40, 294.20 nm; **IR (KBr):** υ = 3419.90, 3306.10 (N-H str), 3064.99 (aromatic C-H str), 1699.77 (C=O str), 1581.68 (C=N str), 1558.54, 1410.01(NO_2 str), 1232.55 (C-N str), 1012.66 (C-F str); **^{1}H NMR ([D$_6$]DMSO):** δ = 1.12 (s, 3H, CH_3), 7.28 (d, J = 6.6 Hz, 2H, Ar C-3, Ar C-5), 7.92 (d, J = 5.7 Hz, 2H, Ar C-2, Ar C-6), 8.39 (s, 1H, thiazole C-H), 8.98 (s, 1H, NH), 10.14 ppm (s, 1H, CONH); **^{13}C NMR ([D$_6$]DMSO):** δ = 19.16 (CH_3), 109.85 (thiazole C-5), 116.51 (Ar C-3, Ar C-5), 127.62 (Ar C-1), 133.03 (Ar C-2, Ar C-6), 140.63 (thiazole C-4), 156.94 (C=O), 164.75 (thiazole C-2), 166.25 (Ar C-4), 168.78 ppm (C=N); **Elemental analysis for $C_{12}H_{10}FN_5O_3S$: calcd:** C 44.58, H 3.12, N 21.66, **found:** C 44.53, H 3.17, N 21.61.

1-(1-(4-Hydroxyphenyl)ethylidene)-4-(5-nitrothiazol-2-yl)semicarbazide (NTA-4): **λ_{max}:** 273.00, 310.90 nm; **IR (KBr):** υ = 3520.21 (O-H str), 3356.25, 3159.51 (N-H str), 2997.48 (aromatic C-H str), 1670.41 (C=O str), 1606.76 (C=N str), 1550.82, 1442.80 (NO_2 str), 1224.84 (C-N str); **^{1}H NMR ([D$_6$]DMSO):** δ = 1.03 (s, 3H, CH_3), 4.99 (s, 1H, OH), 7.04 (d, J = 5.1 Hz, 2H, Ar C-3, Ar C-5), 7.68 (d, J = 5.7 Hz, 2H, Ar C-2, Ar C-6), 8.43 (s, 1H, thiazole C-H), 9.09 (s, 1H, NH), 10.25 ppm (s, 1H, CONH); **^{13}C NMR ([D$_6$]DMSO):** δ = 19.31 (CH_3), 109.77 (thiazole C-5), 117.63 (Ar C-3, Ar C-5), 128.92 (Ar C-1), 132.30 (Ar C-2, Ar C-6), 142.39 (thiazole C-4), 156.75 (C=O), 161.86 (Ar C-4), 164.36 (thiazole C-2), 168.71 ppm (C=N); **Elemental analysis for $C_{12}H_{11}N_5O_4S$: calcd:** C 44.86, H 3.45, N 21.80, **found:** C 44.82, H 3.48, N 21.76.

1-(1-(4-Nitrophenyl)ethylidene)-4-(5-nitrothiazol-2-yl)semicarbazide (NTA-5): λ_{max}: 267.40, 321.80 nm; **IR (KBr):** υ = 3487.42, 3331.18 (N-H str), 3111.28 (aromatic C-H str), 1695.49 (C=O str), 1585.54 (C=N str), 1508.38, 1338.64 (NO_2 str), 1107.18 (C-N str); **^{1}H NMR ([D$_6$]DMSO):** δ = 2.22 (s, 3H, CH_3), 8.28 (d, J = 8.1 Hz, 2H, Ar C-3, Ar C-5), 8.14 (d, J = 8.2 Hz, 2H, Ar C-2, Ar C-6), 8.85 (s, 1H, thiazole C-H), 9.34 (s, 1H, NH), 9.75 ppm (s, 1H, CONH); **^{13}C NMR ([D$_6$]DMSO):** δ = 19.03 (CH_3), 107.94 (thiazole C-5), 127.85 (Ar C-3, Ar C-5), 129.52 (Ar C-2, Ar C-6), 140.76 (thiazole C-4), 143.30 (Ar C-1), 148.08 (Ar C-4), 156.04 (C=O), 164.24 (thiazole C-2), 169.60 ppm (C=N); **MS:** *m/z*=351.39 $[M+1]^+$; **Elemental analysis for $C_{12}H_{10}N_6O_5S$: calcd:** C 41.14, H 2.88, N 23.99, **found:** C 41.17, H 2.86, N 24.02.

1-(2-Bromo-1-(4-bromophenyl)ethylidene)-4-(5-nitrothiazol-2-yl)semicarbazide (NTA-6): λ_{max}: 262.80, 304.40 nm; **IR (KBr):** υ = 3410.26, 3240.52 (N-H str), 3005.20 (aromatic C-H str), 2895.20 (CH_2 str), 1697.41 (C=O str), 1587.47 (C=N str), 1508.38, 1458.23 (NO_2 str), 1232.55 (C-N str), 831.35 (C-Br str); **^{1}H NMR ([D$_6$]DMSO):** δ = 3.56 (s, 2H, CH_2), 8.14 (d, J = 7.2 Hz, 2H, Ar C-2, Ar C-6), 8.19 (s, 1H, thiazole C-H), 8.28 (d, J = 8.1 Hz, 2H, Ar C-3, Ar C-5), 9.27 (s, 1H, NH), 10.08 ppm (s, 1H, CONH); **^{13}C NMR ([D$_6$]DMSO):** δ = 74.77 (CH_2), 109.85 (thiazole C-5), 116.51 (Ar C-3, Ar C-5), 127.62 (Ar C-1), 133.03 (Ar C-2, Ar C-6), 140.63 (thiazole C-4), 156.94 (C=O), 166.25 (Ar C-4), 164.75 (thiazole C-2), 168.78 ppm (C=N); **Elemental analysis for $C_{12}H_9Br_2N_5O_3S$: calcd:** C 31.12, H 1.96, N 15.12, **found:** C 31.09, H 1.93, N 15.17.

1-(4-Bromobenzylidene)-4-(5-nitrothiazol-2-yl)semicarbazide (NTA-7): λ_{max}: 260.20, 305.90 nm; **IR (KBr):** υ = 3443.05, 3281.02 (N-H str), 3086.25 (aromatic C-H str), 1662.69 (C=O str), 1591.33 (C=N str), 1556.61, 1410.01 (NO_2 str), 1273.06 (C-N str), 798.56 (C-Br str); **^{1}H NMR ([D$_6$]DMSO):** δ = 7.67 (d, J = 7.2 Hz, 2H, Ar C-2, Ar C-6), 7.85 (d, J = 7.5 Hz, 2H, Ar C-3, Ar C-5), 8.19 (s, 1H, CH), 8.26 (s, 1H, thiazole C-H), 9.68 (s, 1H, NH), 10.05 ppm (s, 1H, CONH); **^{13}C NMR ([D$_6$]DMSO):** δ = 109.50 (thiazole C-5), 124.55 (Ar C-4), 128.77 (Ar C-2, Ar C-6), 129.81 (Ar C-3, Ar C-5), 132.63 (Ar C-1), 141.02 (thiazole C-4), 156.71 (C=N), 158.77 (C=O), 164.22 ppm (thiazole C-2); **Elemental analysis for $C_{11}H_8BrN_5O_3S$: calcd:** C 35.69, H 2.18, N 18.92, **found:** C 35.65, H 2.23, N 18.95.

1-(4-Hydroxybenzylidene)-4-(5-nitrothiazol-2-yl)semicarbazide (NTA-8): λ_{max}: 280.80, 310.40 nm; **IR (KBr):** υ = 3419.66 (OH str), 3325.39, 3192.30 (N-H str), 2931.90 (aromatic C-H str), 1670.41 (C=O str), 1599.04 (C=N str), 1458.23, 1388.79 (NO_2 str), 1161.19 (C-N str); **^{1}H NMR ([D$_6$]DMSO):** δ = 4.96 (s, 1H, OH), 6.85 (d, J = 8.4 Hz, 2H, Ar C-3, Ar C-5), 7.42 (d, J = 7.5 Hz, 2H, Ar C-2, Ar C-6), 8.09 (s, 1H, CH), 8.58 (s, 1H, thiazole C-H), 9.68 (s, 1H, NH), 10.08 ppm (s, 1H, CONH); **^{13}C NMR ([D$_6$]DMSO):** δ = 109.52 (thiazole C-5), 115.75 (Ar C-3, Ar C-5), 121.12 (Ar C-1), 122.54 (Ar C-2, Ar C-6), 141.07 (thiazole C-4), 144.66 (C=N), 158.33 (C=O), 161.09 (Ar C-4), 164.26 ppm (thiazole C-2); **Elemental analysis for $C_{11}H_9N_5O_4S$: calcd:** C 43.00, H 2.95, N 22.79, **found:** C 43.05, H 2.99, N 22.74.

1-(2,3-Dichlorobenzylidene)-4-(5-nitrothiazol-2-yl)semicarbazide (NTA-9): λ_{max}: 256.60, 305.60 nm; IR (KBr): υ=3448.84, 3252.09 (N-H str), 2995.55 (aromatic C-H str), 1654.98 (C=O str), 1560.46 (C=N str), 1469.66, 1383.64 (NO_2 str), 1101.39 (C-N str), 742.62 (C-Cl str); **^{1}H NMR ([D$_6$]DMSO):** δ = 7.49 (dd, J = 7.2, 6.5 Hz, 1H, Ar C-5), 7.61 (d, J = 6.6 Hz, 1H, Ar C-4), 7.86 (d, J = 6.9 Hz, 1H, Ar C-6), 8.30 (s, 1H, CH), 8.85 (s, 1H, thiazole C-H), 9.21 (s, 1H, NH), 10.25 ppm (s, 1H, CONH); **^{13}C NMR ([D$_6$]DMSO):** δ = 107.89 (thiazole C-5), 111.73 (Ar C-5), 128.70 (Ar C-6), 128.83 (Ar C-4), 133.79 (Ar C-3), 134.80 (Ar C-2), 136.87 (Ar C-1), 142.59 (thiazole C-4), 146.90 (C=N), 158.80 (C=O), 167.88 ppm (thiazole C-2); **Elemental analysis for $C_{11}H_7Cl_2N_5O_3S$: calcd:** C 36.68, H 1.96, N 19.44, **found:** C 36.62, H 1.99, N 19.49.

1-(2,4-Dichlorobenzylidene)-4-(5-nitrothiazol-2-yl)semicarbazide (NTA-10): λ_{max}: 260.70, 304.40 nm; **IR (KBr):** υ = 3400.09, 3346.61 (N-H str), 3088.14 (aromatic C-H str), 1680.05 (C=O str), 1585.54 (C=N str), 1467.88, 1381.08 (NO_2 str), 1199.76 (C-N str), 823.63 (C-Cl str); **^{1}H NMR ([D$_6$]DMSO):** δ = 6.92 (d, J = 7.5 Hz, 1H, Ar C-5), 7.02 (s, 1H, Ar C-3), 7.41 (d, J = 6.0 Hz, 1H, Ar C-6), 8.11 (s, 1H, CH), 8.68 (s, 1H, thiazole C-H), 9.23 (s, 1H, NH), 10.99 ppm (s, 1H, CONH); **^{13}C NMR ([D$_6$]DMSO):** δ = 109.16 (thiazole C-5), 129.92 (Ar C-5), 130.23 (Ar C-3), 130.94 (Ar C-1), 131.08 (Ar C-6), 137.32 (Ar C-2), 139.15 (Ar C-4), 139.71 (thiazole C-4), 148.51 (C=N), 158.65 (C=O), 162.98 ppm (thiazole C-2); **MS:** *m/z*=361.14 $[M+1]^+$; **Elemental analysis for $C_{11}H_7Cl_2N_5O_3S$: calcd:** C 36.68, H 1.96, N 19.44, **found:** C 36.65, H 1.98, N 19.47.

1-(2,6-Dichlorobenzylidene)-4-(5-nitrothiazol-2-yl)semicarbazide (NTA-11): λ_{max}: 261.20, 302.60 nm; IR (KBr): υ=3446.91, 3369.75 (N-H str), 3064.99 (aromatic C-H str), 1658.84 (C=O str), 1624.12 (C=N str), 1556.61, 1427.37 (NO_2 str), 1190.12 (C-N str), 777.34 (C-Cl str); **^{1}H NMR ([D$_6$]DMSO):** δ = 7.47 (d, J = 6.6 Hz, 3H, Ar C-3, Ar C-4, Ar C-5), 8.22 (s, 1H, CH), 8.58 (s, 1H, thiazole C-H), 9.17 (s, 1H, NH), 10.05 ppm (s, 1H, CONH); **^{13}C NMR ([D$_6$]DMSO):** δ = 109.25 (thiazole C-5), 129.11 (Ar C-3, Ar C-5), 130.71 (Ar C-1), 135.70 (Ar C-4), 138.48 (Ar C-2, Ar C-6), 141.29 (thiazole C-4), 144.35 (C=N), 158.72 (C=O), 162.97 ppm (thiazole C-2); **Elemental analysis for $C_{11}H_7Cl_2N_5O_3S$: calcd:** C 36.68, H 1.96, N 19.44, **found:** C 36.71, H 2.01, N 19.39.

1-(2,5-Dimethoxybenzylidene)-4-(5-nitrothiazol-2-yl)semicarbazide (NTA-12): λ_{max}: 287.40, 310.80 nm; **IR (KBr):** υ = 3524.06, 3342.75 (N-H str), 3091.99 (aromatic C-H str), 1660.77 (C=O str), 1564.32 (C=N str), 1413.87, 1373.36 (NO_2 str), 1149.61 (C-N str); 1116.82 (C-O-C str); **^{1}H NMR ([D_6]DMSO):** δ = 3.36 (s, 6H, OCH_3), 6.85 (d, J = 7.5 Hz, 2H, Ar C-3, Ar C-5), 7.26 (s, 1H, Ar C-6), 8.19 (s, 1H, CH), 8.36 (s, 1H, thiazole C-H), 9.90 (s, 1H, NH), 10.69 ppm (s, 1H, CONH); **^{13}C NMR ([D_6]DMSO):** δ = 59.93 (OCH_3), 109.16 (thiazole C-5), 117.45 (Ar C-4), 118.26 (Ar C-6), 119.76 (Ar C-3), 121.54 (Ar C-1), 142.12 (thiazole C-4), 145.65 (C=N), 148.53 (Ar C-2), 149.81 (Ar C-5), 158.31 (C=O), 163.23 ppm (thiazole C-2); **Elemental analysis for $C_{13}H_{13}N_5O_5S$: calcd:** C 44.44, H 3.73, N 19.93, **found:** C 44.49, H 3.69, N 19.98.

1-(Diphenylmethylene)-4-(5-nitrothiazol-2-yl)semicarbazide (NTA-13): λ_{max}: 267.60, 312.60 nm; **IR (KBr):** υ = 3428.19, 3358.70 (N-H str), 3078.49 (aromatic C-H str), 1660.81 (C=O str), 1589.47 (C=N str), 1556.52, 1431.89 (NO_2 str); **^{1}H NMR ([D_6]DMSO):** δ = 6.85 (dd, J = 7.2, 6.7 Hz, 3H, Ar C-3, Ar C-4, Ar C-5), 7.02 (dd, J = 6.9, 5.6 Hz, 3H, Ar' C-3, Ar' C-4, Ar' C-5), 7.24 (d, J = 7.2 Hz, 2H, Ar' C-2, Ar' C-6), 7.94 (d, J = 6.9 Hz, 2H, Ar C-2, Ar C-6), 8.39 (s, 1H, thiazole C-H), 9.88 (s, 1H, NH), 10.19 ppm (s, 1H, CONH); **^{13}C NMR ([D_6]DMSO):** δ = 110.24 (thiazole C-5), 126.31 (Ar C-3, Ar C-5),130.29 (Ar C-2, Ar C-6), 132.63 (Ar C-1), 133.11 (Ar C-4), 140.11 (thiazole C-4), 156.45 (C=N), 158.43 (C=O), 163.33 ppm (thiazole C-2); **Elemental analysis for $C_{17}H_{13}N_5O_3S$: calcd:** C 55.58, H 3.57, N 19.06, **found:** C 55.52, H 3.61, N 19.11.

1-((4-Chlorophenyl)(phenyl)methylene)-4-(5-nitrothiazol-2-yl)semicarbazide (NTA-14): λ_{max}: 264.20, 304.20 nm; **IR (KBr):** υ = 3443.05, 3289.25 (N-H str), 3054.89 (aromatic C-H str), 1647.26 (C=O str), 1590.61 (C=N str), 1560.46, 1437.02 (NO_2 str), 1195.30 (C-N str), 781.20 (C-Cl str); **^{1}H NMR ([D_6]DMSO):** δ = 7.67 (d, J = 6.9 Hz, 2H, Ar C-3, Ar C-5), 7.78 (d, J = 7.2 Hz, 2H, Ar' C-2, Ar' C-6), 7.94 (d, J = 7.5 Hz, 2H, Ar C-2, Ar C-6), 8.17 (dd, J = 7.2, 5.8 Hz, 3H, Ar' C-3, Ar' C-4, Ar' C-5), 8.89 (s, 1H, thiazole C-H), 9.16 (s, 1H, NH), 10.28 ppm (s, 1H, CONH); **^{13}C NMR ([D_6]DMSO):** δ = 110.58 (thiazole C-5), 126.78 (Ar' C-3, Ar' C-5), 128.32 (Ar C-3, Ar C-5), 129.13 (Ar' C-2, Ar' C-6), 131.56 (Ar C-2, Ar C-6), 132.11 (Ar C-1), 133.53 (Ar' C-4), 135.32 (Ar'

C-1), 137.13 (Ar C-4), 138.66 (thiazole C-4), 156.61 (C=N), 158.30 (C=O), 163.12 ppm (thiazole C-2); **Elemental analysis for $C_{17}H_{12}ClN_5O_3S$: calcd:** C 50.81, H 3.01, N 17.43, **found:** C 50.85, H 3.05, N 17.40.

1-((4-Hydroxyphenyl)(phenyl)methylene)-4-(5-nitrothiazol-2-yl)semicarbazide (NTA-15): λ_{max}: 273.20, 320.20 nm; IR (KBr): υ=3427.62 (OH str), 3300.31, 3167.22 (N-H str), 2955.04 (aromatic C-H str), 1654.84 (C=O str), 1600.97 (C=N str), 1560.46, 1444.73 (NO_2 str), 1288.49 (C-N str); **^{1}H NMR ([D_6]DMSO):** δ = 4.92 (s, 1H, OH), 7.59 (d, J = 6.6 Hz, 2H, Ar' C-2, Ar' C-6), 7.83 (d, J = 8.7 Hz, 2H, Ar C-2, Ar C-6), 7.97 (d, J = 7.5 Hz, 2H, Ar C-3, Ar C-5), 8.09 (dd, J = 8.4, 6.3 Hz, 3H, Ar' C-3, Ar' C-4, Ar' C-5), 8.85 (s, 1H, thiazole C-H), 9.66 (s, 1H, NH), 10.31 ppm (s, 1H, CONH); **^{13}C NMR ([D_6]DMSO):** δ = 110.25 (thiazole C-5), 117.31 (Ar C-3, Ar C-5), 124.13 (Ar C-1), 127.56 (Ar' C-3, Ar' C-5),130.82 (Ar' C-2, Ar' C-6), 131.51 (Ar C-2, Ar C-6), 132.28 (Ar' C-4), 133.92 (Ar' C-1), 140.71 (thiazole C-4), 156.39 (C=N), 158.43 (C=O), 160.82 (Ar C-4), 163.21 ppm (thiazole C-2); **Elemental analysis for $C_{17}H_{13}N_5O_4S$: calcd:** C 53.26, H 3.42, N 18.27, **found:** C 53.29, H 3.47, N 18.23.

1-(Bis(4-chlorophenyl)methylene)-4-(5-nitrothiazol-2-yl)semicarbazide (NTA-16): λ_{max}: 262.60, 305.20 nm; **IR (KBr):** υ = 3468.13, 3215.44 (N-H str), 2997.48 (aromatic C-H str), 1654.98 (C=O str), 1587.47 (C=N str), 1485.24, 1398.44 (NO_2 str), 1286.56 (C-N str), 754.19 (C-Cl str); **^{1}H NMR ([D_6]DMSO, D_2O exchange):** δ = 7.39 (d, J = 8.7 Hz, 4H, Ar C-3, Ar C-5, Ar' C-3, Ar' C-5), 8.07 (d, J = 7.5 Hz, 4H, Ar C-2, Ar C-6, Ar' C-2, Ar' C-6), 8.60 (s, 1H, thiazole C-H), 9.72 (s, 1H, NH), 10.24 ppm (s, 1H, CONH; **^{13}C NMR ([D_6]DMSO):** δ = 109.85 (thiazole C-5), 122.65 (Ar C-3, Ar C-5, Ar' C-3, Ar' C-5), 128.26 (Ar C-2, Ar C-6, Ar' C-2, Ar' C-6), 129.54 (Ar C-1, Ar' C-1), 129.81 (Ar C-4, Ar' C-4), 142.15 (thiazole C-4), 156.29 (C=N), 159.93 (C=O), 164.77 ppm (thiazole C-2); **Elemental analysis for $C_{17}H_{11}Cl_2N_5O_3S$: calcd:** C 46.80, H 2.54, N 16.05, **found:** C 46.83, H 2.58, N 16.01.

4-(5-Nitrothiazol-2-yl)-1-(2-oxoindolin-3-ylidene)semicarbazide (NTA-17): λ_{max}: 286.20, 320.60 nm; IR (KBr): υ=3431.48, 3335.03, 3219.30 (N-H str), 3132.20 (aromatic C-H str), 1692.69 (isatinyl C=O str), 1652.19 (C=O str), 1600.97 (C=N str), 1464.02, 1338.64 (NO_2 str), 1234.48 (C-N str); **^{1}H NMR ([D_6]DMSO):** δ = 6.92 (dd, J = 8.1, 7.1

Hz, 1H, isatinyl C-5), 6.97 (d, J = 8.1 Hz, 1H, isatinyl C-7), 7.32 (d, J = 7.5 Hz, 1H, isatinyl C-4), 7.38 (dd, J = 8.7, 7.6 Hz, 1H, isatinyl C-6), 8.13 (s, 1H, thiazole C-H), 9.05 (s, 1H, NH), 9.77 (s, 1H, isatinyl N-H), 10.86 ppm (s, 1H, CONH); **^{13}C NMR ([D_6]DMSO):** δ = 109.92 (thiazole C-5), 128.15 (isatinyl C-3a), 129.24 (isatinyl C-7), 132.58 (isatinyl C-5), 133.30 (isatinyl C-4), 134.38 (isatinyl C-6), 144.06 (thiazole C-4), 144.73 (C=N), 145.17 (isatinyl C-7a), 154.45 (C=O), 160.52 (thiazole C-2), 168.96 ppm (isatinyl C=O); **MS:** *m/z*=333.10 $[M+1]^+$; **Elemental analysis for $C_{12}H_8N_6O_4S$: calcd:** C 43.37, H 2.43, N 25.29, **found:** C 43.32, H 2.46, N 25.32.

1-(5-Bromo-2-oxoindolin-3-ylidene)-4-(5-nitrothiazol-2-yl)semicarbazide (NTA-18): **λ_{max}:** 265.40, 305.20 nm; **IR (KBr):** υ = 3433.41, 3302.24, 3144.07 (N-H str), 2914.54 (aromatic C-H str), 1699.34 (isatinyl C=O str), 1668.48 (C=O str), 1577.82 (C=N str), 1554.68, 1408.08 (NO_2 str), 1238.34 (C-N str); 623.93 (C-Br str); **^{1}H NMR ([D_6]DMSO):** δ = 7.08 (d, J = 8.7 Hz, 1H, isatinyl C-6), 7.40 (d, J = 9.3 Hz, 1H, isatinyl C-7), 7.88 (s, 1H, isatinyl C-4), 8.36 (s, 1H, isatinyl N-H), 8.58 (s, 1H, thiazole C-H), 9.07 (s, 1H, NH), 10.19 ppm (s, 1H, CONH); **^{13}C NMR ([D_6]DMSO):** δ = 107.63 (thiazole C-5), 121.94 (isatinyl C-5), 128.73 (isatinyl C-3a), 131.43 (isatinyl C-7), 131.55 (isatinyl C-4), 135.33 (isatinyl C-6), 137.78 (isatinyl C-7a), 140.66 (C=N), 143.59 (thiazole C-4), 158.55 (C=O), 160.81 (thiazole C-2), 171.74 ppm (isatinyl C=O); **Elemental analysis for $C_{12}H_7BrN_6O_4S$: calcd:** C 35.05, H 1.72, N 20.44, **found:** C 35.01, H 1.78, N 20.49.

The IR, ^{1}H NMR, ^{13}C NMR, mass and X-RD spectra of compounds **NTA-5**, **NTA-10**, **NTA-16**, **NTA-17** and **NTA-18** are presented in **Figure 4.33.** to **Figure 4.52.**

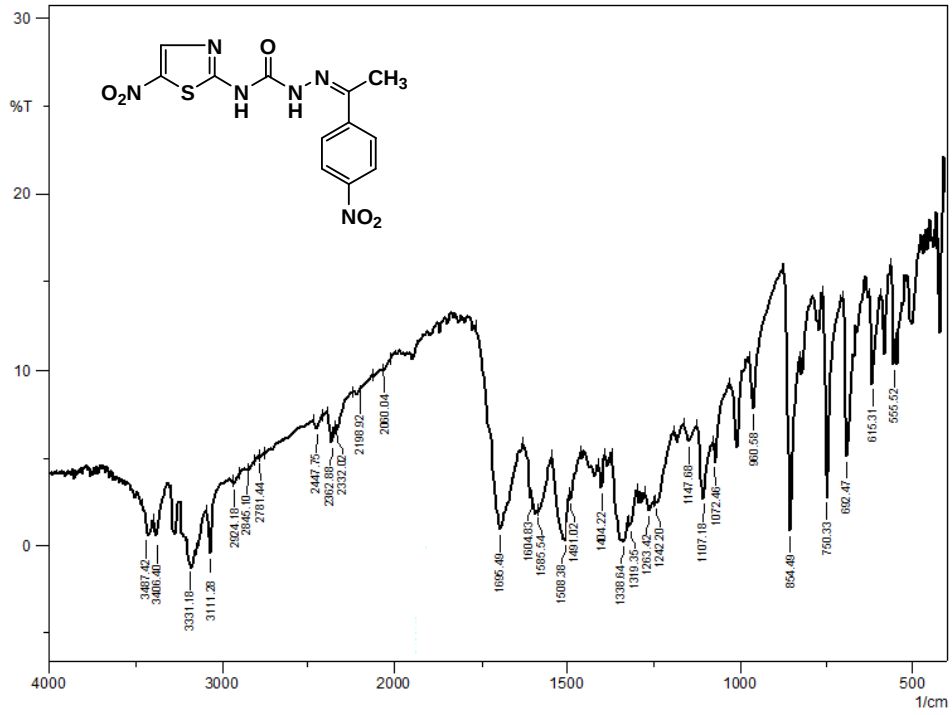


Figure 4.33. IR spectrum of **NTA-5**

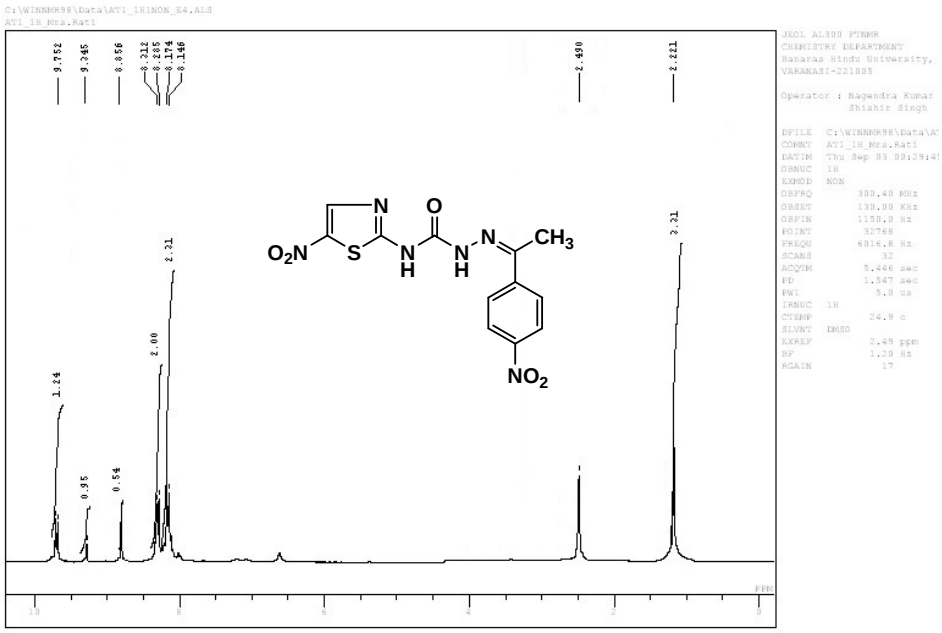


Figure 4.34. ^{1}H NMR spectrum of **NTA-5**

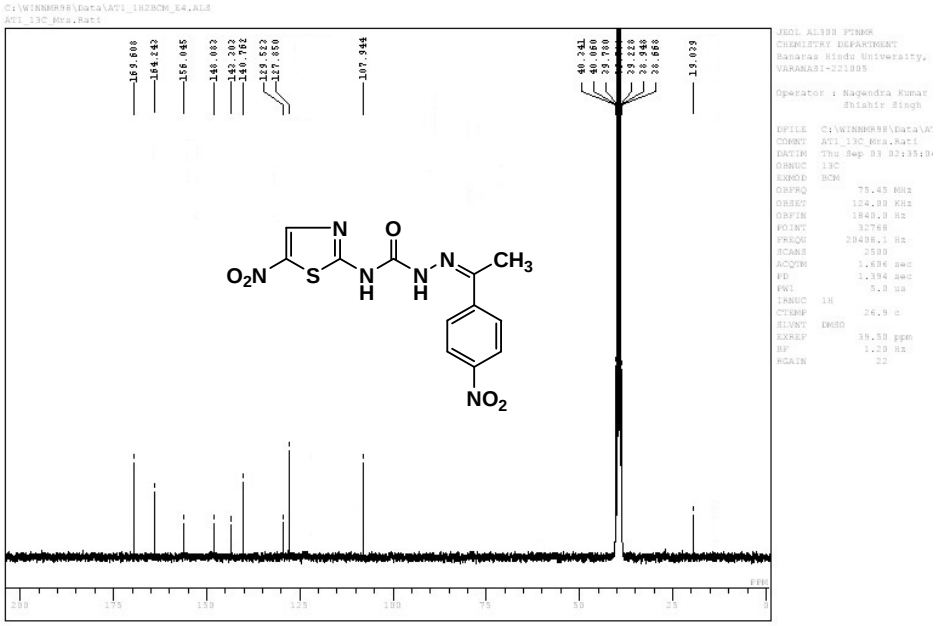


Figure 4.35. ^{13}C NMR spectrum of **NTA-5**

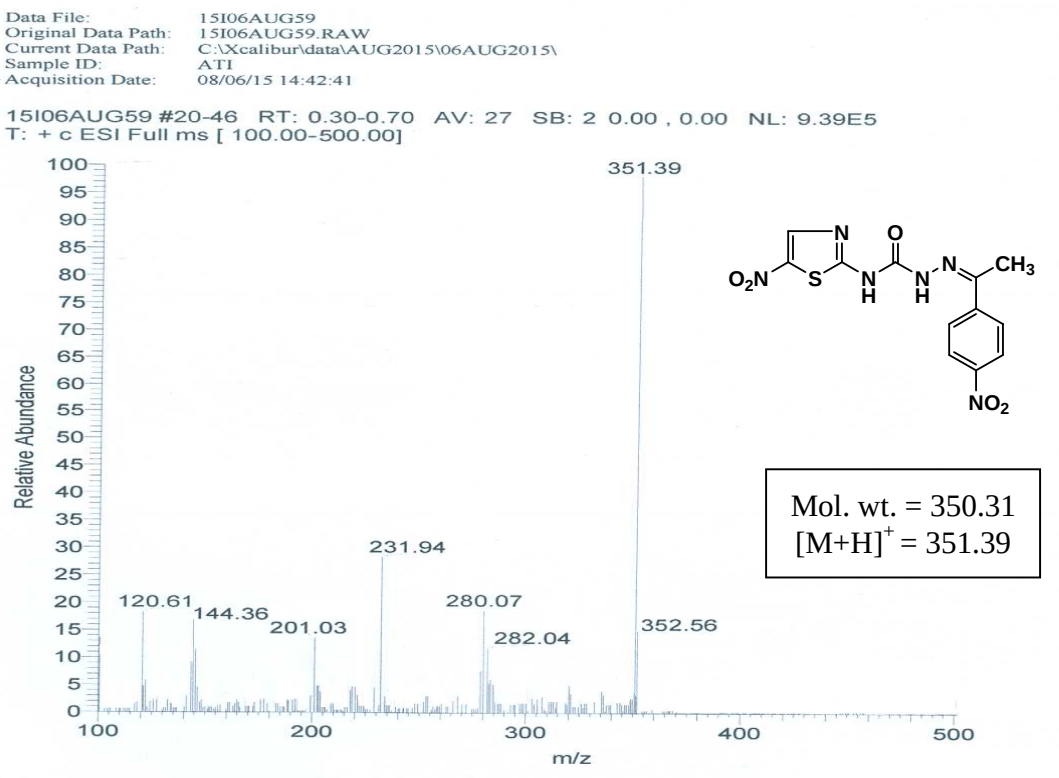


Figure 4.36. Mass spectrum of **NTA-5**

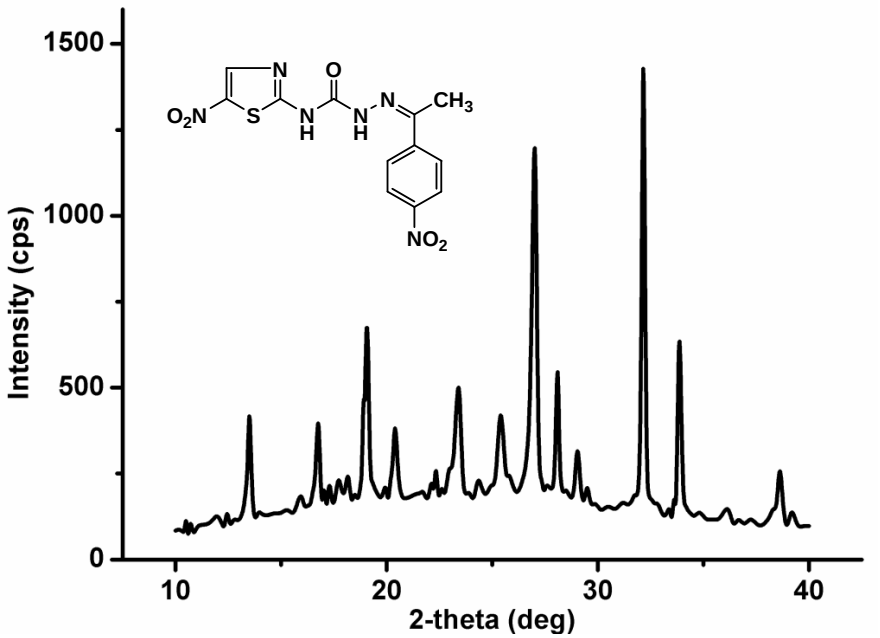


Figure 4.37. XR-PD spectrum of **NTA-5**

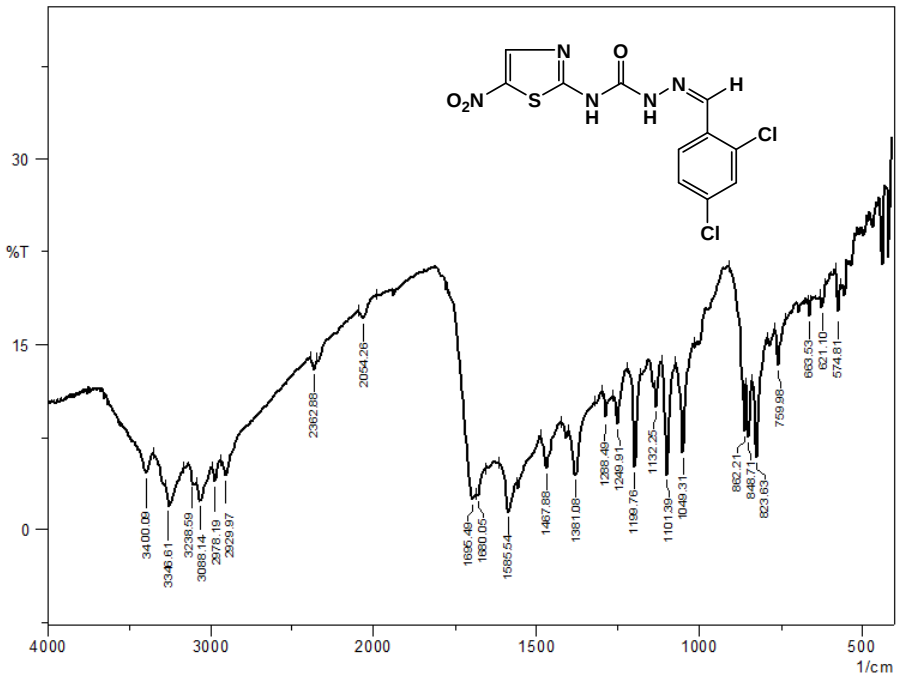


Figure 4.38. IR spectrum **NTA-10**

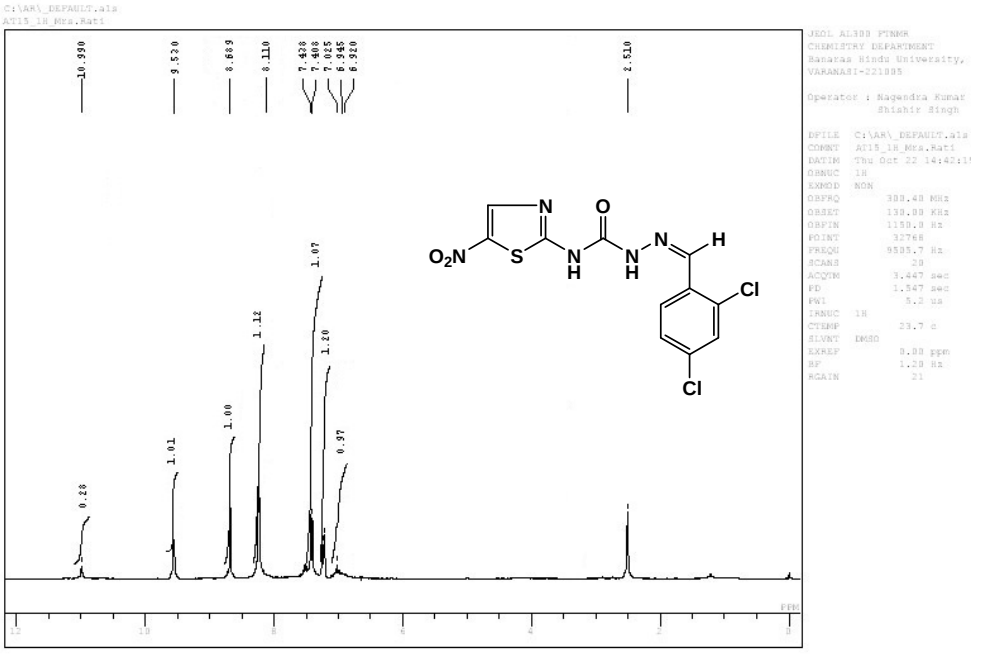


Figure 4.39. ^{1}H NMR spectrum of **NTA-10**

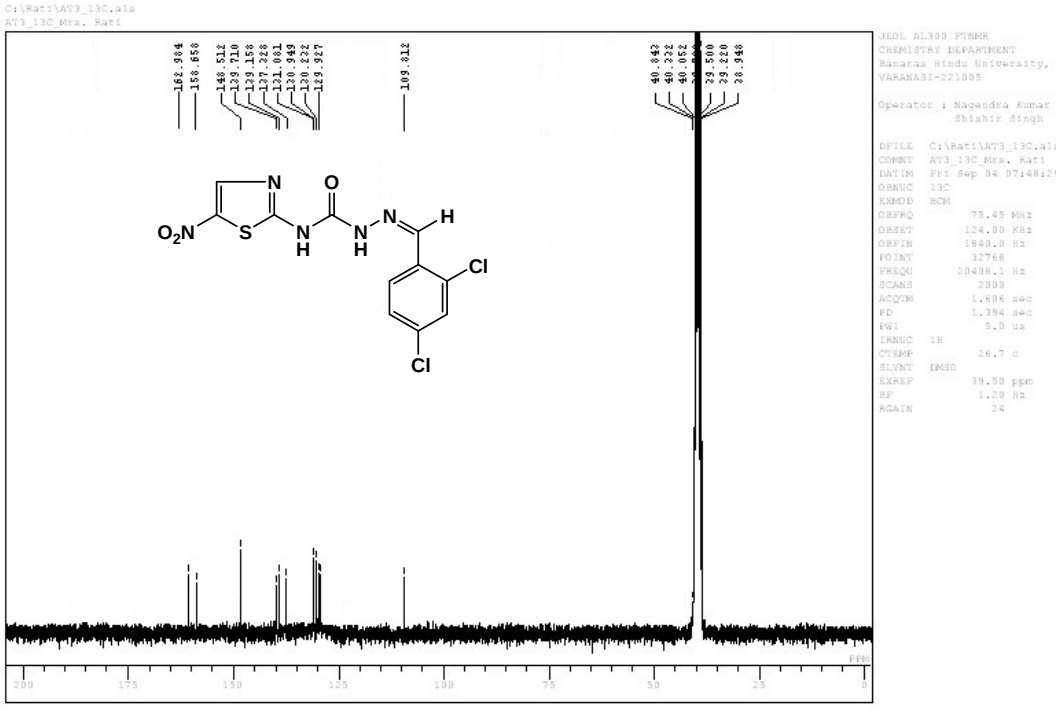


Figure 4.40. ^{13}C NMR spectrum of **NTA-10**

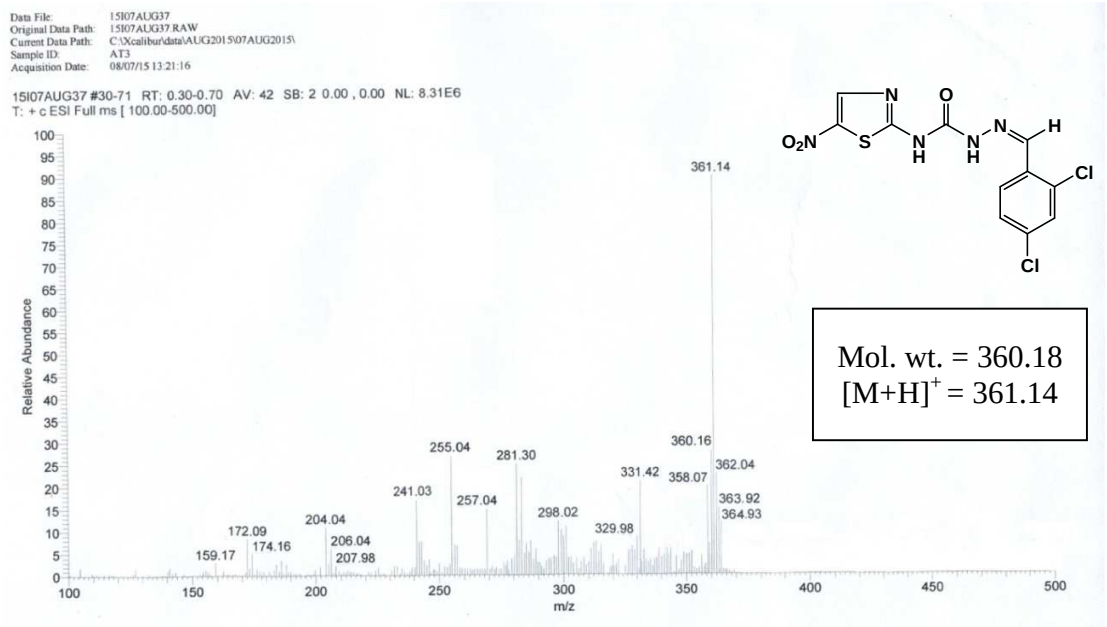


Figure 4.41. Mass spectrum of **NTA-10**

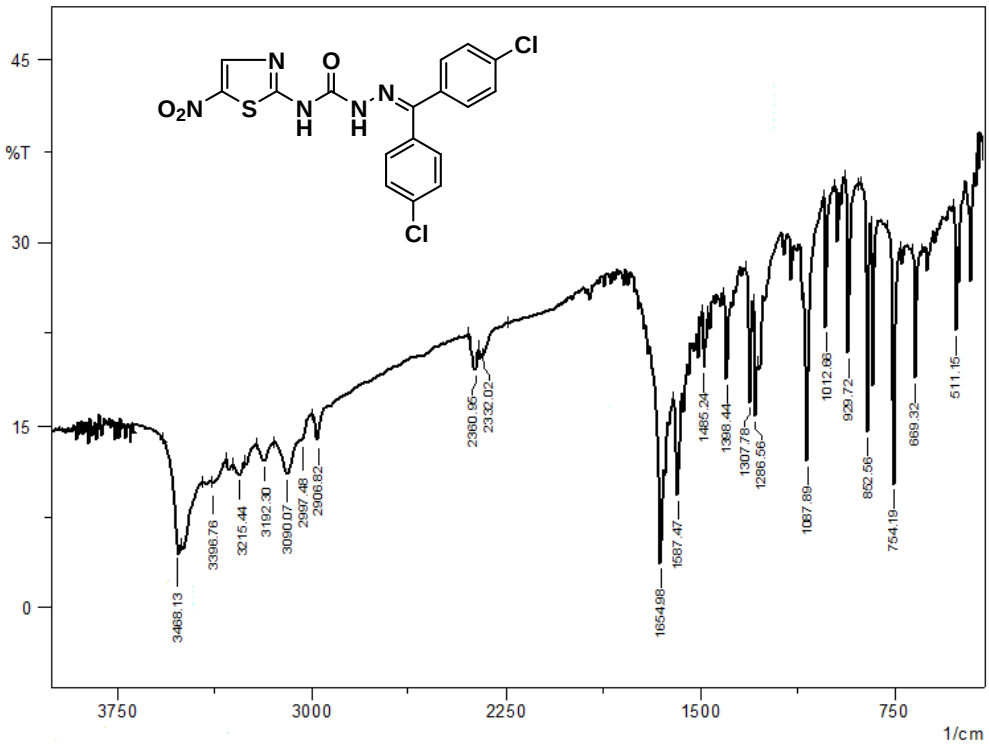


Figure 4.42. IR spectrum of **NTA-16**

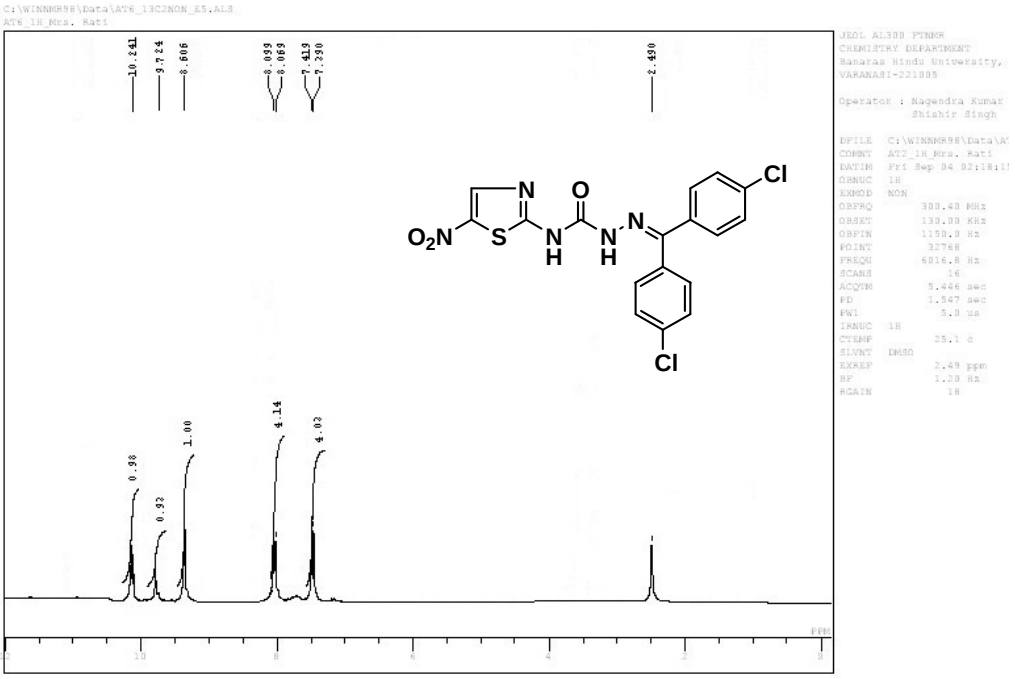


Figure 4.43. ^{1}H NMR spectrum of **NTA-16**

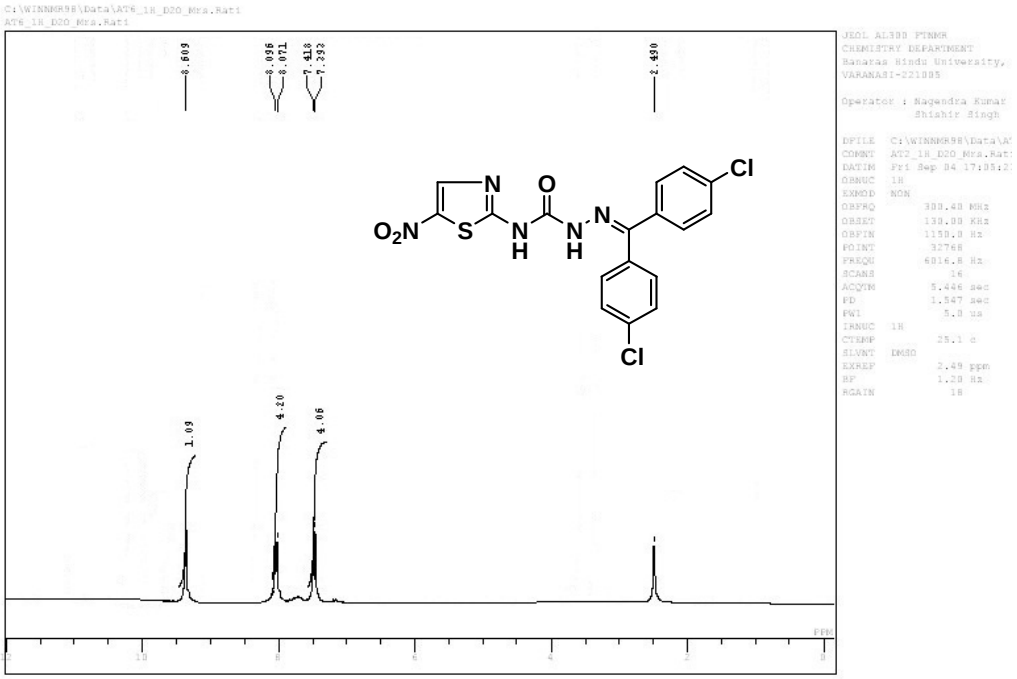


Figure 4.44. ^{1}H (D_2O exchange) NMR spectrum of **NTA-16**

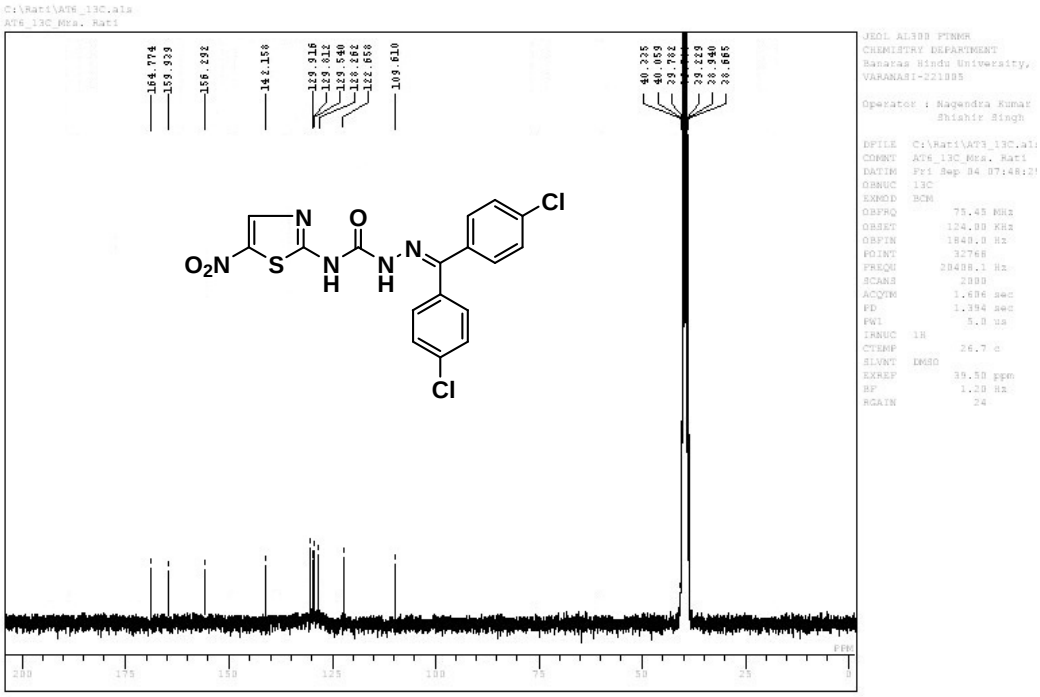


Figure 4.45. ^{13}C NMR spectrum of **NTA-16**

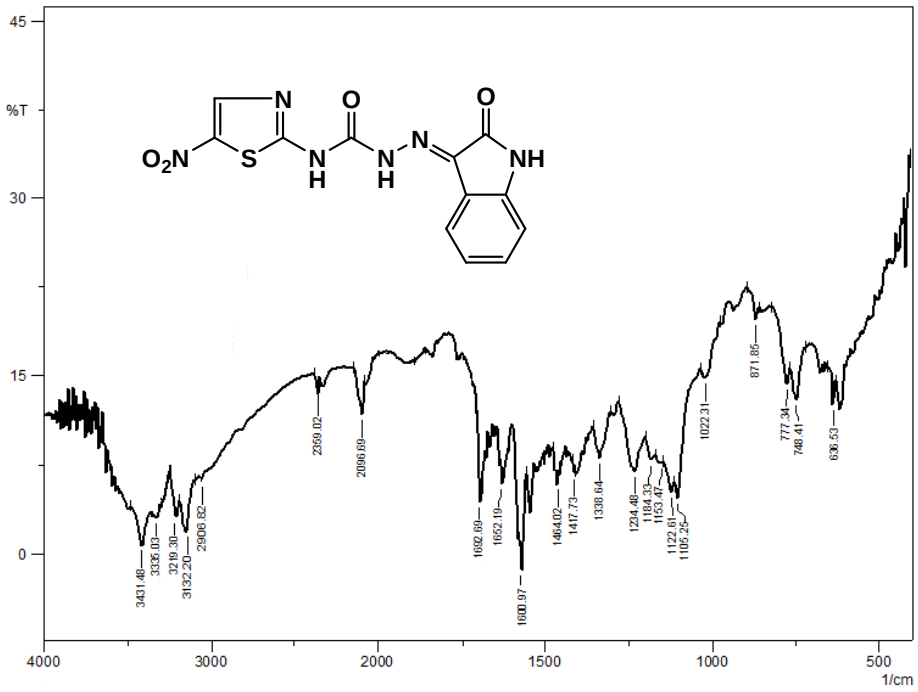


Figure 4.46. IR spectrum of **NTA-17**

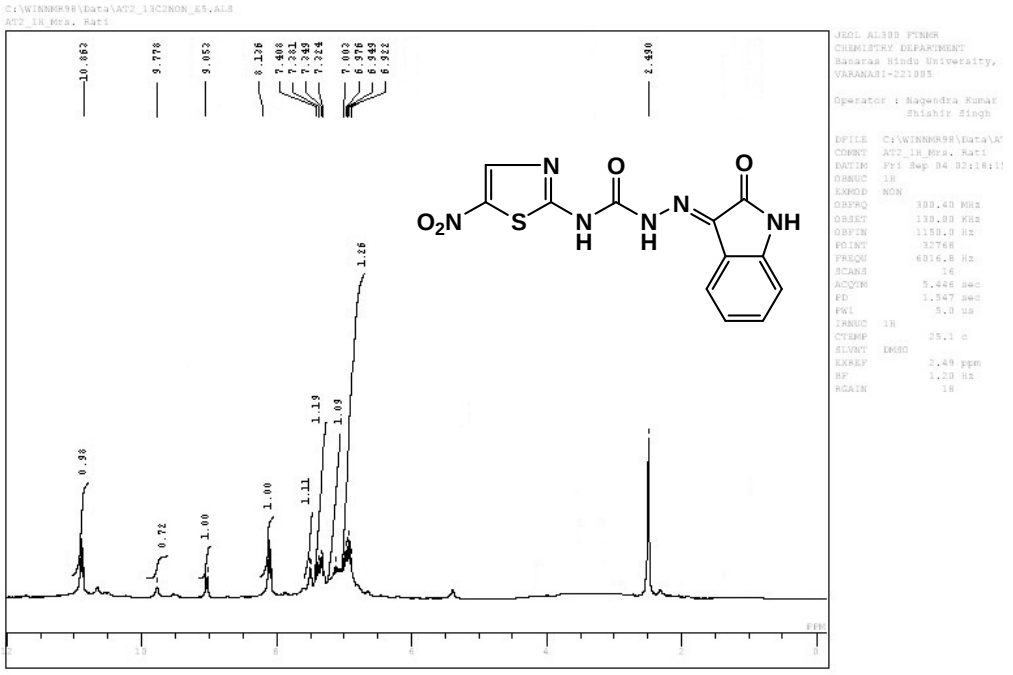


Figure 4.47. ^{1}H NMR spectrum of **NTA-17**

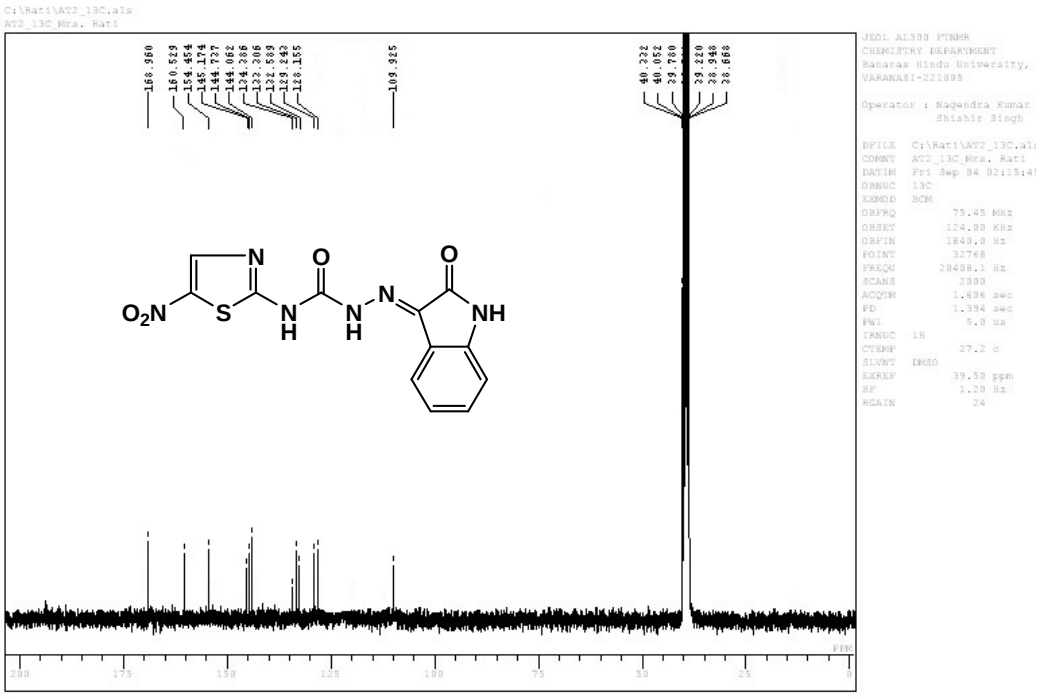


Figure 4.48. ^{13}C NMR spectrum of **NTA-17**

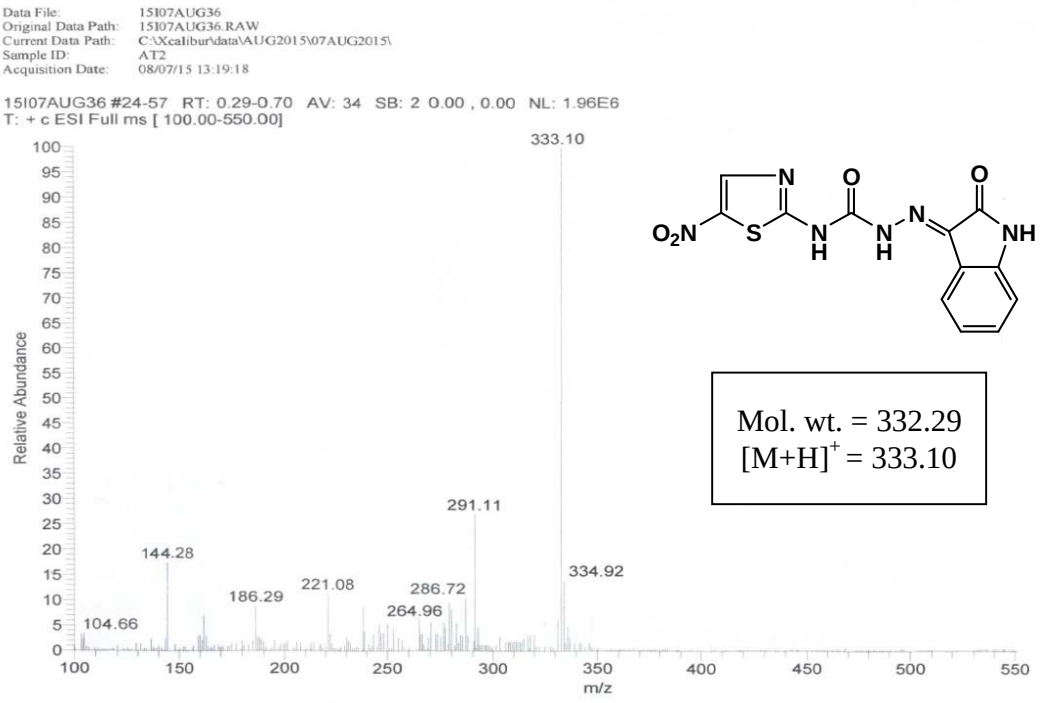


Figure 4.49. Mass spectrum of **NTA-17**

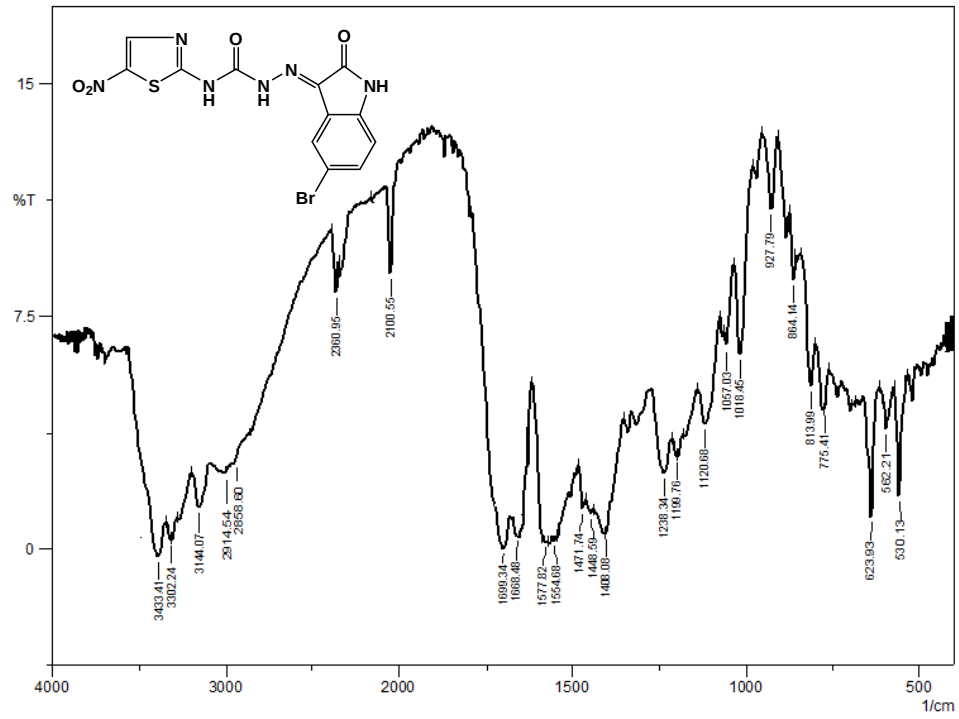


Figure 4.50. IR spectrum of **NTA-18**

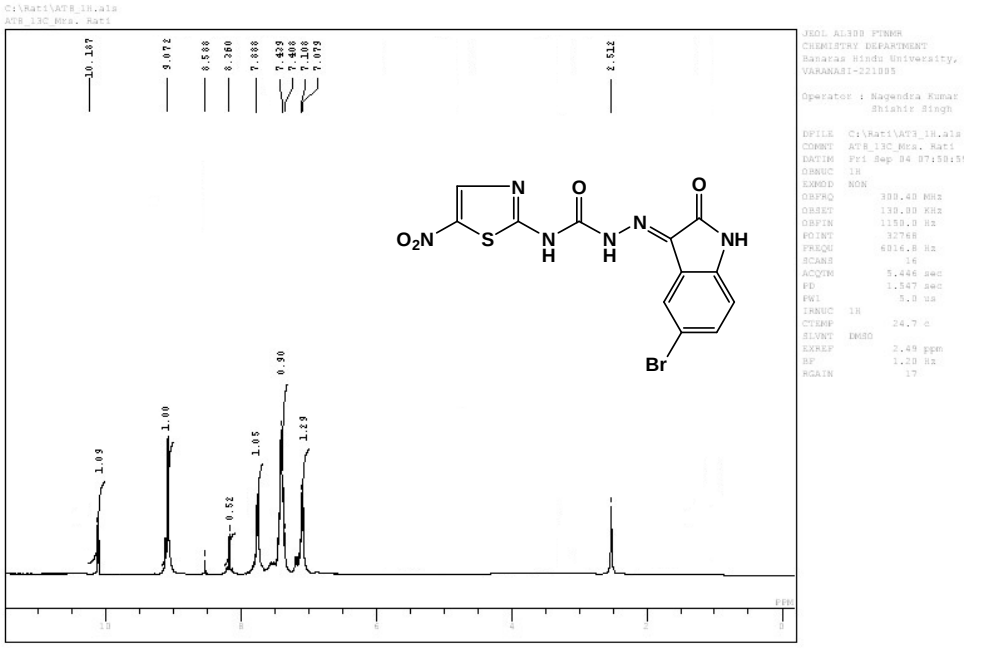


Figure 4.51. ^{1}H NMR spectrum of **NTA-18**

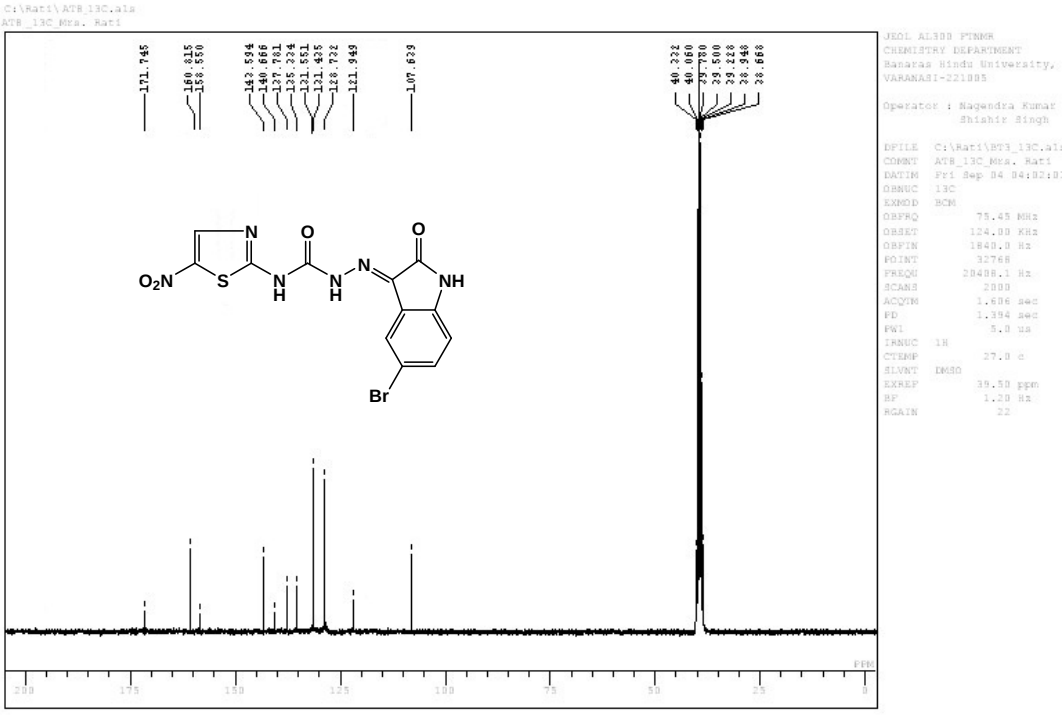


Figure 4.52. ^{13}C NMR spectrum of **NTA-18**

4.2.3. Biological evaluation

The procedures followed for *in-vitro* and *in-vivo* biological screening of the synthesized compounds (**NTA-1** to **NTA-18**) are summarized in **Section 4.1.3.**

4.2.4. *In-silico* molecular property analysis and ADMET prediction studies

The procedures followed for *in-silico* molecular property analysis and ADMET prediction studies of all the synthesized compounds (**NTA-1** to **NTA-18**) are described in **Section 4.1.4.**

4.3. 3,4-(METHYLENEDIOXY)ANILINE DERIVED SEMICARBAZONES [MDA-1 to MDA-14]

4.3.1. Synthesis

4.3.1.1. Chemicals and reagents

All the chemicals and solvents used for this work were reagent grade and were procured from Sigma-Aldrich (U.S.A.), Merck (Germany), SD Fine (Mumbai) and Qualigens (Mumbai).

4.3.1.2. Synthetic protocol

3,4-(Methylenedioxy)aniline derived semicarbazones (**MDA-1** to **MDA-14**) were synthesized by the method illustrated in **Scheme 4.6.**

Step 1. Synthesis of 3,4-(methylenedioxy)phenyl urea (MDU)

In a 50 ml flask containing glacial acetic acid (10 mL) was added 3,4-(methylenedioxy)aniline (**MDA**, 0.003 mol) at 0 °C. To this was added sodium cyanate (0.0036 mol) with stirring. The cold bath was removed and the mixture was stirred at room temperature for 24 h before being left to stand overnight. The excess solvent was removed and the resulting residue was neutralized with 2N NaOH. The mixture was extracted with chloroform (30 mL). The combined organic phase was washed with water and brine, dried over sodium sulphate. The solvent was removed and the resulting product was dried, recrystallized from chloroform to yield compound **MDU**. Purity of compound was checked by TLC analysis using the solvent system (Dichloromethane : CH_3OH (9.8 : 0.2), R_f: 0.52).

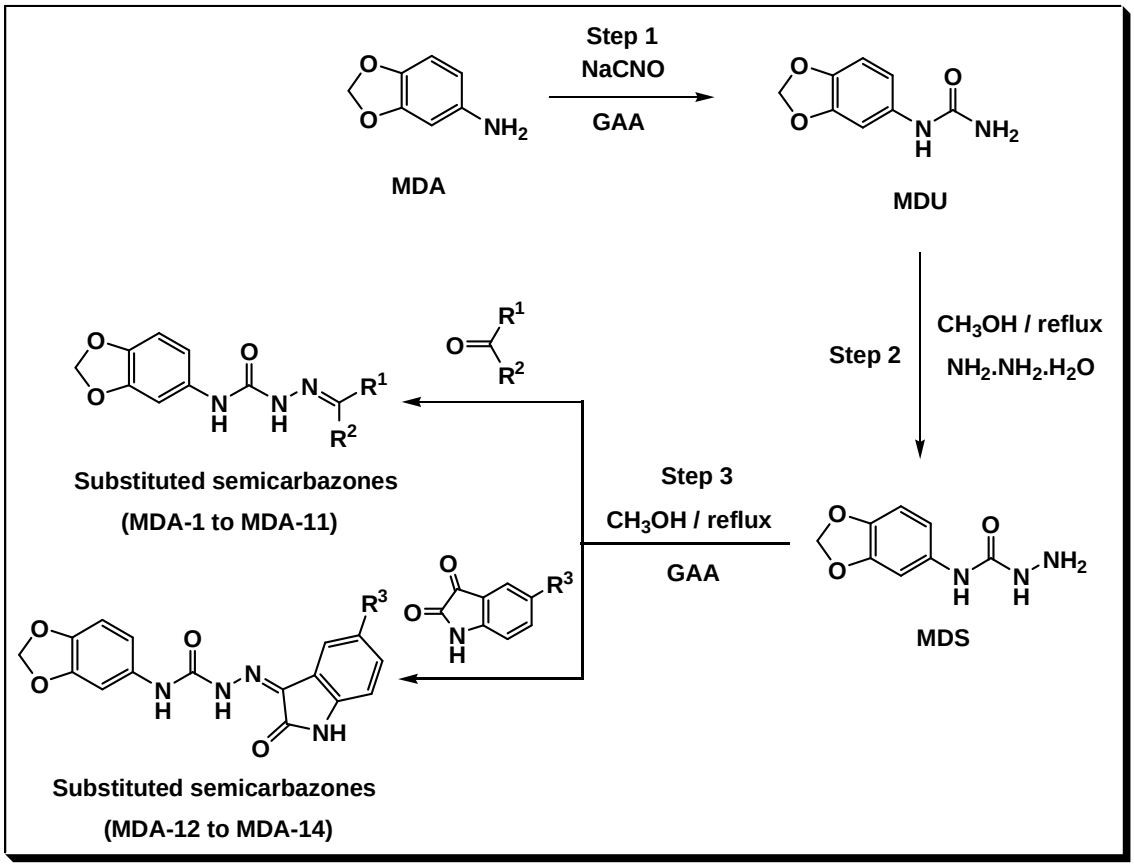
Step 2. Synthesis of 3,4-(methylenedioxy)phenyl semicarbazide (MDS)

In a RB flask containing **MDU** (0.003 mol) dissolved in methanol (20 mL), hydrazine hydrate (99%, 0.29 mL, 2.0 eq.) was added and the reaction mixture was refluxed for about 10 h. Solvent was evaporated, and the resultant product (**MDS**) obtained was recrystallized from methanol. Purity of compound was checked by TLC analysis using the solvent system (Dichloromethane: CH_3OH (9.8 : 0.2), R_f: 0.44).

Step 3. Synthesis of semicarbazones (MDA-1 to MDA-14)

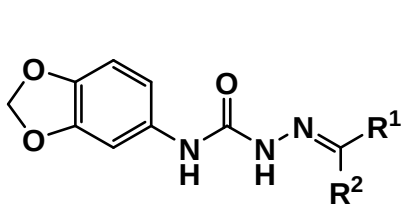
The final compounds **MDA-1** to **MDA-14** (substituted semicarbazones) were synthesized by the reaction of **MDS** (0.003 mol) with appropriate aldehydes or ketones or 5-(un)substituted isatin (0.003 mol). The reaction mixture was adjusted to pH 5-6 by adding

few drops of glacial acetic acid and refluxed for 5-10 h. The solvent was evaporated, and the crude product was filtered and recrystallized from methanol.

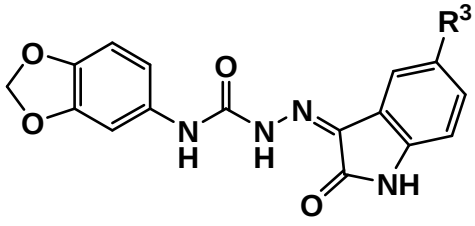


Scheme 4.6. Synthesis of 3,4-(methylenedioxy)aniline derived semicarbazones (**MDA-1** to **MDA-14**)

Table 4.7. Structural data of 3,4-(methylenedioxy)aniline derived semicarbazones (**MDA-1** to **MDA-14**)



MDA-1 to MDA-11



MDA-12 to MDA-14

Code	R^1	R^2	R^3	Mol. Formula	Mol. Wt. (g/mol)
MDA-1	CH_3	C_6H_5	-	$C_{16}H_{15}N_3O_3$	297.31
MDA-2	CH_3	4-Cl C_6H_4	-	$C_{16}H_{14}ClN_3O_3$	331.75
MDA-3	CH_3	4-F C_6H_4	-	$C_{16}H_{14}FN_3O_3$	315.3
MDA-4	CH_3	4-OH C_6H_4	-	$C_{16}H_{15}N_3O_4$	313.31
MDA-5	H	2,3-Cl_2 C_6H_3	-	$C_{15}H_{11}Cl_2N_3O_3$	352.17
MDA-6	H	2,4-Cl_2 C_6H_3	-	$C_{15}H_{11}Cl_2N_3O_3$	352.17
MDA-7	H	2,6-Cl_2 C_6H_3	-	$C_{15}H_{11}Cl_2N_3O_3$	352.17
MDA-8	H	2,5-$(OCH_3)_2$ C_6H_3	-	$C_{17}H_{17}N_3O_5$	343.33
MDA-9	C_6H_5	C_6H_5	-	$C_{21}H_{17}N_3O_3$	359.38
MDA-10	C_6H_5	4-Cl C_6H_4	-	$C_{21}H_{16}ClN_3O_3$	393.82
MDA-11	4-Cl C_6H_4	4-Cl C_6H_4	-	$C_{21}H_{15}Cl_2N_3O_3$	428.27
MDA-12	-	-	H	$C_{16}H_{12}N_4O_4$	324.29
MDA-13	-	-	Cl	$C_{16}H_{11}ClN_4O_4$	358.74
MDA-14	-	-	NO_2	$C_{16}H_{11}N_5O_6$	369.29

4.3.2. Characterization

The physicochemical and spectral characterization of the synthesized compounds (**MDA-1** to **MDA-14**) was performed so as to ascertain the chemical structure of compounds. The complete procedures of all the characterization methods followed and the instruments used are described in **Section 4.1.2.**

4.3.2.1. Physicochemical characterization

The physicochemical characterization data of synthesized compounds (**MDA-1** to **MDA-14**) are presented in **Table 4.8.**

Table 4.8. Physicochemical characterization data of **MDA-1** to **MDA-14**

Code	MP (°C)	Yield (%)	Colour	R_f[a]	LogP[b]	Expt. LogP[c]
MDA-1	Charred at 180	47.8	Black	0.53	2.38	-
MDA-2	110-112	59.6	Black	0.49	2.94	2.1

MDA-3	90-92	60.4	Black	0.51	2.54	1.8
MDA-4	153-155	39.31	Black	0.46	2.34	-
MDA-5	134-136	45.3	Black	0.48	3.93	2.9
MDA-6	172-175	44.1	Black	0.54	3.93	3.2
MDA-7	92-94	46.9	Black	0.52	2.56	2.1
MDA-8	126-128	40.4	Black	0.55	2.56	1.8
MDA-9	168-170	43.9	Black	0.51	4.28	3.3
MDA-10	192-194	53.72	Black	0.46	4.84	3.5
MDA-11	203-205	41.5	Black	0.49	5.59	-
MDA-12	158-160	42.3	Reddish black	0.50	1.38	0.9
MDA-13	70-72	55.6	Reddish black	0.49	1.93	1.1
MDA-14	186-188	57.2	Reddish black	0.54	0.36	-

*All the compounds were soluble in chloroform, methanol, ethanol, dichloromethane, DMF and DMSO; [a]Solvent system: Dichloromethane: CH_3OH (9.8:0.2); [b]MarvinSketch generated ; [c]Determined using Shake flask method; '-' indicates 'not tested'

4.3.2.2. Spectral characterization and elemental analysis

All the synthesized compounds (**MDA-1** to **MDA-14**) were subjected to UV, IR, ^{1}H NMR and ^{13}C NMR spectral and elemental analysis and the results are presented below in section **4.3.2.2.1.** In addition, mass spectrum was measured for compounds **MDA-3** and **MDA-8** and the $[M+1]^+$ peak of these compounds is presented below (**Figure 4.60.** and **Figure 4.67.**). Moreover, compounds **MDA-2** was subjected to X-ray powder diffraction analysis and the diffraction pattern is shown in **Figure 4.56.**

4.3.2.3. Spectral characterization and elemental analysis data of intermediates (MDU and MDS) and final compounds (MDA-1 to MDA-14)

3,4-(Methylenedioxy)phenyl urea (MDU): IR (KBr): υ = 3417.98, 3215.44 (N-H str), 3082.35 (aromatic C-H str), 2910.68 (CH_2 str), 1649.19 (C=O str), 1487.17 (C=C str), 1197.83 (C-O-C str); **^{1}H NMR ([D$_6$]DMSO):** δ = 3.38 (s, 2H, CH_2), 6.83-7.34 (m, 3H, benzene C-H), 7.51 (s, 1H, NH), 9.46 ppm (s, 2H, NH_2); **^{13}C NMR ([D$_6$]DMSO):** δ = 101.47 (C-2), 108.52 (C-4), 111.66 (C-6), 112.51 (C-7), 127.89 (C-5), 143.24 (C-7a), 147.91 (C-3a), 160.39 ppm (C=O); **Elemental analysis for $C_8H_8N_2O_3$: calcd:** C 53.33, H 4.48, N 15.55, **found:** C 53.30, H 4.52, N 15.58.

3,4-(Methylenedioxy)phenyl semicarbazide (MDS): IR (KBr): υ = 3327.32, 3215.44 (N-H str), 3090.07 (aromatic C-H str), 2906.82 (CH_2 str), 1660.77 (C=O str), 1546.96 (C=N str), 1504.53 (C=C str), 1238.34 (C-O-C str); **^{1}H NMR ([D_6]DMSO):** δ = 1.99 (s, 2H, NH_2), 3.33 (s, 2H, CH_2), 5.95 (s, 1H, NH), 6.83-7.27 (m, 3H, benzene C-H), 9.81 ppm (s, 1H, CONH); **^{13}C NMR ([D_6]DMSO):** δ = 101.40 (C-2), 108.50 (C-4), 111.50 (C-6), 112.63 (C-7), 127.99 (C-5), 147.44 (C-7a), 148.91 (C-3a), 160.01 ppm (C=O); **Elemental analysis for $C_8H_9N_3O_3$: calcd:** C 49.23, H 4.65, N 21.53, **found:** C 49.25, H 4.60, N 21.58.

4-(Benzo[1,3]dioxol-5-yl)-1-(1-phenylethylidene)semicarbazide (MDA-1): λ_{max}: 263.70, 310.90 nm; **IR (KBr):** υ = 3444.98, 3423.76 (N-H str), 3032.20 (aromatic C-H str), 2916.47 (CH_2 str), 1639.55 (C=O str), 1591.33 (C=N str), 1485.24 (C=C str), 1289.82 (C-O-C str); **^{1}H NMR ([D_6]DMSO):** δ = 1.96 (s, 3H, CH_3), 3.46 (s, 2H, CH_2), 5.96 (s, 1H, NH), 6.83-7.21 (m, 3H, benzene C-H), 7.48-8.00 (m, 5H, Ar C-H), 9.81 ppm (s, 1H, CONH); **^{13}C NMR ([D_6]DMSO):** δ = 23.82 (-CH_3), 101.24 (C-2), 107.89 (C-4), 111.71 (C-6), 115.23 (C-7), 127.88 (Ar C-3, Ar C-5), 128.40 (C-5), 128.63 (Ar C-2, Ar C-6), 131.80 (Ar C-4), 134.79 (Ar C-1),142.53 (C-7a), 146.94 (C-3a), 157.10 (C=O), 167.85 ppm (C=N); **Elemental analysis for $C_{16}H_{15}N_3O_3$: calcd:** C 64.64, H 5.09, N 14.13, **found:** C 64.59, H 5.11, N 14.17.

4-(Benzo[1,3]dioxol-5-yl)-1-(1-(4-chlorophenyl)ethylidene)semicarbazide (MDA-2): λ_{max}: 259.40, 303.80 nm; **IR (KBr):** υ = 3325.39, 3211.59 (N-H str), 3097.78 (aromatic C-H str), 2914.54 (CH_2 str), 1664.62 (C=O str), 1550.82 (C=N str), 1483.31 (C=C str), 1240.27 (C-O-C str), 825.56 (C-Cl str); **^{1}H NMR ([D_6]DMSO):** δ = 1.98 (s, 3H, CH_3), 3.47 (s, 2H, CH_2), 5.95 (s, 1H, NH), 6.78-7.27 (m, 3H, benzene C-H), 7.49-7.90 (m, 4H, Ar C-2, Ar C-3, Ar C-5, Ar C-6), 9.82 ppm (s, 1H, CONH); **^{13}C NMR ([D_6]DMSO):** δ = 23.84 (-CH_3), 101.23 (C-2), 107.93 (C-4), 111.70 (C-6), 127.76 (C-7), 128.29 (Ar C-3, Ar C-5), 128.46 (C-5), 133.75 (Ar C-2, Ar C-6), 134.58 (Ar C-1), 136.55 (Ar C-4), 142.61 (C-7a), 146.93 (C-3a), 156.91 (C=O), 167.84 ppm (C=N); **Elemental analysis for $C_{16}H_{14}ClN_3O_3$: calcd:** C 57.93, H 4.25, N 12.67, **found:** C 57.97, H 4.22, N 12.62.

4-(Benzo[1,3]dioxol-5-yl)-1-(1-(4-fluorophenyl)ethylidene)semicarbazide (MDA-3): λ_{max}: 264.00, 300.20 nm; **IR (KBr):** υ = 3441.12, 3329.25 (N-H str), 2999.41 (aromatic

C-H str), 2906.82 (CH_2 str), 1662.69 (C=O str), 1558.54 (C=N str), 1506.46 (C=C str), 1236.41 (C-O-C str), 1039.67 (C-F str); **^{1}H NMR ([D_6]DMSO):** δ = 1.98 (s, 3H, CH_3), 3.39 (s, 2H, CH_2), 5.94 (s, 1H, NH), 6.28-7.95 (m, 3H, benzene C-H), 6.92-8.25 (m, 4H, Ar C-2, Ar C-3, Ar C-5, Ar C-6), 9.98 ppm (s, 1H, CONH); **^{13}C NMR ([D_6]DMSO):** δ = 23.83 (-CH_3), 101.24 (C-2), 107.93 (C-4), 111.72 (C-6), 115.17 (C-7), 115.46 (Ar C-3, Ar C-5), 128.74 (C-5), 128.85 (Ar C-1), 133.84 (Ar C-2, Ar C-6), 142.58 (C-7a), 146.91 (C-3a), 156.72 (C=O), 164.98 (Ar C-4), 167.90 ppm (C=N); **MS:** *m/z*=316.58 $[M+1]^+$; **Elemental analysis for $C_{16}H_{14}FN_3O_3$: calcd:** C 60.95, H 4.48, N 13.33, **found:** C 60.90, H 4.51, N 13.30.

1-(4-Hydroxybenzylidene)-4-(benzo[1,3]dioxol-5-yl)semicarbazide (MDA-4): λ_{max}: 265.90, 320.60 nm; **IR (KBr):** υ = 3478.26 (OH str), 3381.72, 3299.29 (N-H str), 3095.27 (aromatic C-H str), 2916.58 (CH_2 str), 1654.36 (C=O str), 1570.48 (C=N str), 1502.61 (C=C str), 1259.83 (C-O-C str); **^{1}H NMR ([D_6]DMSO):** δ = 3.35 (s, 2H, CH_2), 4.91 (s, 1H, OH), 5.98 (s, 1H, NH), 6.89-7.43 (m, 3H, benzene C-H), 7.04-7.87 (m, 4H, Ar C-2, Ar C-3, Ar C-5, Ar C-6), 7.98 (s, 1H, C-H), 9.83 ppm (s, 1H, CONH); **^{13}C NMR ([D_6]DMSO):** δ = 101.22 (C-2), 107.52 (C-4),111.57 (C-6), 115.61 (C-7), 116.52 (Ar C-3, Ar C-5), 127.33 (Ar C-1), 129.41 (C-5), 131.20 (Ar C-2, Ar C-6), 144.31 (C=N), 142.54 (C-7a), 146.83 (C-3a), 156.74 (C=O), 160.75 ppm (Ar C-4); **Elemental analysis for $C_{15}H_{13}N_3O_4$: calcd:** C 60.20, H 4.38, N 14.04, **found:** C 60.23, H 4.39, N 14.09.

1-(2,3-Dichlorobenzylidene)-4-(benzo[1,3]dioxol-5-yl)semicarbazide (MDA-5): λ_{max}: 248.80, 292.20 nm; **IR (KBr):** υ = 3327.32, 3173.01 (N-H str), 3064.99 (aromatic C-H str), 2902.96 (CH_2 str), 1664.62 (C=O str), 1545.03 (C=N str), 1506.46 (C=C str), 1240.27 (C-O-C str), 813.99 (C-Cl str); **^{1}H NMR ([D_6]DMSO):** δ = 3.62 (s, 2H, CH_2), 6.01 (s, 1H, NH), 6.60-7.18 (m, 3H, benzene C-H), 7.35-7.72 (m, 3H, Ar C-4, Ar C-5, Ar C-6), 8.12 (s, 1H, C-H), 9.98 ppm (s, 1H, CONH); **^{13}C NMR ([D_6]DMSO):** δ = 101.78 (C-2), 107.51 (C-4), 111.48 (C-6), 115.52 (C-7), 127.38 (Ar C-5), 127.85 (Ar C-6), 128.94 (C-5), 131.25 (Ar C-2), 133.44 (Ar C-4), 134.35 (Ar C-3),135.73 (Ar C-1), 144.58 (C=N), 142.43 (C-7a), 146.70 (C-3a), 156.28 ppm (C=O); **Elemental analysis for $C_{15}H_{11}Cl_2N_3O_3$: calcd:** C 51.16, H 3.15, N 11.93, **found:** C 51.21, H 3.18, N 11.89.

1-(2,4-Dichlorobenzylidene)-4-(benzo[1,3]dioxol-5-yl)semicarbazide (MDA-6): λ_{max}: 253.30, 298.70 nm; **IR (KBr):** υ = 3190.37 (N-H str), 3088.14 (aromatic C-H str), 2955.04 (CH_2 str), 1708.99 (C=O str), 1600.97 (C=N str), 1465.95 (C=C str), 1101.39 (C-O-C str), 866.07 (C-Cl str); **^{1}H NMR ([D$_6$]DMSO):** δ = 3.32 (s, 2H, CH_2), 6.05 (s, 1H, NH), 7.48-7.89 (m, 3H, benzene C-H), 8.37-8.71 (m, 1H, Ar C-3, Ar C-5, Ar C-6), 8.80 (s, 1H, C-H), 9.93 ppm (s, 1H, CONH); **^{13}C NMR ([D$_6$]DMSO):** δ = 101.86 (C-2), 107.35 (C-4), 111.57 (C-6), 116.30 (C-7), 127.66 (Ar C-5), 127.94 (C-5), 129.35 (Ar C-3), 130.23 (Ar C-1), 133.63 (Ar C-6), 134.90 (Ar C-2),140.03 (Ar C-4), 143.66 (C=N), 144.83 (C-7a), 149.90 (C-3a), 165.10 ppm (C=O); **Elemental analysis for $C_{15}H_{11}Cl_2N_3O_3$: calcd:** C 51.16, H 3.15, N 11.93, **found:** C 51.18, H 3.10, N 11.99.

1-(2,6-Dichlorobenzylidene)-4-(benzo[1,3]dioxol-5-yl)semicarbazide (MDA-7): λ_{max}: 249.80, 300.60 nm; **IR (KBr):** υ = 3327.32, 3308.03 (N-H str), 3090.07 (aromatic C-H str), 2995.55 (CH_2 str), 1705.13 (C=O str), 1560.46 (C=N str), 1487.17 (C=C str), 1242.20 (C-O-C str), 781.20 (C-Cl str); **^{1}H NMR ([D$_6$]DMSO):** δ = 3.62 (s, 2H, CH_2), 5.94 (s, 1H, NH), 6.73-7.56 (m, 3H, benzene C-H), 7.26-7.31 (m, 3H, Ar C-3, Ar C-4, Ar C-5), 8.52 (s, 1H, C-H), 9.81 ppm (s, 1H, CONH); **^{13}C NMR ([D$_6$]DMSO):** δ = 101.27 (C-2), 107.97 (C-4), 110.96 (C-6), 111.74 (C-7), 129.10 (Ar C-3, Ar C-5), 133.75 (C-5), 134.18 (Ar C-1), 142.65 (Ar C-4), 146.34 (Ar C-2, Ar C-6), 146.96 (C=N), 147.17 (C-7a), 152.80 (C-3a), 167.89 ppm (C=O); **Elemental analysis for $C_{15}H_{11}Cl_2N_3O_3$: calcd:** C 51.16, H 3.15, N 11.93, **found:** C 51.23, H 3.20, N 11.88.

1-(2,5-Dimethoxybenzylidene)-4-(benzo[1,3]dioxol-5-yl)semicarbazide (MDA-8): λ_{max}: 263.40, 299.80 nm; **IR (KBr):** υ = 3311.89, 3184.58 (N-H str), 3080.42 (aromatic C-H str), 2982.05 (CH_2 str), 1720.56 (C=O str), 1545.03 (C=N str), 1500.67 (C=C str), 1242.20 (C-O-C str); **^{1}H NMR ([D$_6$]DMSO):** δ = 3.32 (s, 2H, CH_2), 3.98 (s, 6H, -OCH_3), 6.05 (s, 1H, NH), 6.29-7.41 (m, 3H, benzene C-H), 7.36-7.38 (m, 3H, Ar C-3, Ar C-4, Ar C-6), 7.55 (s, 1H, C-H), 9.93 ppm (s, 1H, CONH); **^{13}C NMR ([D$_6$]DMSO):** δ = 57.51 (OCH_3), 101.32 (C-2), 107.84 (C-4), 110.98 (Ar C-6), 111.53 (C-6), 115.33 (Ar C-3), 115.42 (C-7), 117.80 (Ar C-4), 118.20 (Ar C-1), 130.20 (C-5), 142.52 (C-7a), 143.23 (C=N), 146.76 (C-3a), 153.12 (Ar C-2), 153.76 (Ar C-5), 167.40 ppm (C=O); **MS:**

m/z=344.98 $[M+1]^+$; **Elemental analysis for $C_{17}H_{17}N_3O_5$: calcd:** C 59.47, H 4.99, N 12.24, **found:** C 59.41, H 5.03, N 12.27.

4-(Benzo[1,3]dioxol-5-yl)-1-(diphenylmethylene)semicarbazide (MDA-9): λ_{max}: 268.40, 311.20 nm; **IR (KBr):** υ = 3327.32, 3309.96 (N-H str), 3032.20 (aromatic C-H str), 2962.76 (CH_2 str), 1660.77 (C=O str), 1568.18 (C=N str), 1487.17 (C=C str), 1251.84 (C-O-C str); **^{1}H NMR ([D_6]DMSO):** δ = 3.52 (s, 2H, CH_2), 5.95 (s, 1H, NH), 6.80-721 (m, 3H, benzene C-H), 7.30-7.45 (m, 5H, , Ar' C-2, Ar' C-3, Ar' C-4, Ar' C-5, Ar'- C-6), 7.35-7.71 (m, 5H, Ar C-2, Ar C-3, Ar C-4, Ar C-5, Ar C-6), 9.99 ppm (s, 1H, CONH); **^{13}C NMR ([D_6]DMSO):** δ = 101.97 (C-2), 104.35 (C-4), 111.75 (C-6), 116.30 (C-7), 125.55 (Ar C-3, Ar C-5, Ar' C-3, Ar' C-5), 128.11 (C-5), 128.35 (Ar C-2, Ar C-6, Ar' C-2, Ar' C-6), 128.60 (Ar C-4, Ar' C-4), 129.60 (Ar C-1, Ar' C-1), 142.60 (C-7a), 146.79 (C-3a), 147.82 (C=N), 167.96 ppm (C=O); **Elemental analysis for $C_{21}H_{17}N_3O_3$: calcd:** C 70.18, H 4.77, N 11.69, **found:** C 70.15, H 4.80, N 11.61.

4-(Benzo[1,3]dioxol-5-yl)-1-((4-chlorophenyl)(phenyl)methylene)semicarbazide (MDA-10): λ_{max}: 260.20, 301.90 nm; **IR (KBr):** υ = 3446.91, 3369.75 (N-H str), 3064.99 (aromatic C-H str), 2895.25 (CH_2 str), 1658.84 (C=O str), 1589.40 (C=N str), 1487.17 (C=C str), 1273.06 (C-O-C str), 829.42 (C-Cl str); **^{1}H NMR ([D_6]DMSO):** δ = 3.56 (s, 2H, CH_2), 5.84 (s, 1H, NH), 6.68-7.34 (m, 3H, benzene C-H), 7.45-7.89 (m, 5H, Ar' C-2, Ar' C-3, Ar' C-4, Ar' C-5, Ar'- C-6), 7.73-8.12 (m, 4H, Ar C-2, Ar C-3, Ar C-5, Ar C-6), 9.63 ppm (s, 1H, CONH); **^{13}C NMR ([D_6]DMSO):** δ = 101.23 (C-2), 107.31 (C-4), 111.45 (C-6), 115.78 (C-7), 127.98 (Ar C-3, Ar C-5), 128.11 (Ar' C-3, Ar' C-5), 128.31 (C-5), 128.78 (Ar C-2, Ar C-6), 131.56 (Ar' C-2, Ar' C-6), 132.95 (Ar' C-1), 133.11 (Ar C-4), 133.50 (Ar C-1), 135.73 (Ar' C-4), 142.14 (C-7a), 146.67 (C-3a), 153.81 (C=N), 167.11 ppm (C=O); **Elemental analysis for $C_{21}H_{16}ClN_3O_3$: calcd:** C 64.05, H 4.09, N 10.67, **found:** C 64.01, H 4.13, N 10.62.

4-(Benzo[1,3]dioxol-5-yl)-1-(bis(4-chlorophenyl)methylene)semicarbazide (MDA-11): λ_{max}: 259.60, 298.50 nm; **IR (KBr):** υ = 3446.91, 3369.75 (N-H str), 3064.99 (aromatic C-H str), 2895.25 (CH_2 str), 1658.84 (C=O str), 1589.40 (C=N str), 1487.17 (C=C str), 1273.06 (C-O-C str), 829.42 (C-Cl str); **^{1}H NMR ([D_6]DMSO):** δ = 3.39 (s, 2H, CH_2), 5.90 (s, 1H, NH), 6.70-7.13 (m, 3H, benzene C-H), 7.55-7.89 (m, 8H, Ar C-2,

Ar C-3, Ar C-5, Ar C-6, Ar' C-2, Ar' C-3, Ar' C-5, Ar' C-6), 9.83 ppm (s, 1H, CONH); **^{13}C NMR ([D$_6$]DMSO):** δ = 101.34 (C-2), 107.56 (C-4), 111.54 (C-6), 115.83 (C-7), 128.71 (Ar C-3, Ar C-5, Ar' C-3, Ar' C-5), 128.80 (C-5), 130.96 (Ar C-2, Ar C-6, Ar' C-2, Ar' C-6), 131.11 (Ar C-1, Ar' C-1), 135.66 (Ar C-4, Ar' C-4), 142.93 (C-7a), 146.87 (C-3a), 154.65 (C=N), 166.53 ppm (C=O); **Elemental analysis for $C_{21}H_{15}Cl_2N_3O_3$: calcd:** C 58.89, H 3.53, N 9.81, **found:** C 58.85, H 3.59, N 9.84.

4-(Benzo[1,3]dioxol-5-yl)-1-(2-oxoindolin-3-ylidene)semicarbazide (MDA-12): λ_{max}: 266.20, 303.20 nm; **IR (KBr):** υ = 3402.54, 3263.66 (N-H str), 3090.07 (aromatic C-H str), 2920.32 (CH_2 str), 1699.34 (lactam C=O), 1622.19 (C=O str), 1560.46 (C=N str), 1489.10 (C=C str), 1240.27 (C-O-C str); **^{1}H NMR ([D$_6$]DMSO, D$_2$O exchange):** δ = 3.38 (s, 2H, CH_2), 5.94 (s, 1H, NH), 6.81-7.16 (m, 3H, benzene C-H), 7.07-7.52 (m, 4H, isatinyl C-H), 8.28 (s, 1H, isatinyl N-H), 9.79 ppm (s, 1H, CONH); **^{13}C NMR ([D$_6$]DMSO):** δ = 101.40 (C-2), 108.50 (C-4), 109.72 (C-6), 111.66 (C-7), 112.51 (isatinyl C-3a), 121.99 (isatinyl C-7), 127.99 (isatinyl C-5), 128.51 (C-5), 128.99 (isatinyl C-4), 131.89 (isatinyl C-6), 133.12 (C=N), 142.92 (C-7a), 147.44 (isatinyl C-7a), 148.91 (C-3a), 168.39 (C=O), 179.01 ppm (isatinyl C=O); **Elemental analysis for $C_{16}H_{12}N_4O_4$: calcd:** C 59.26, H 3.73, N 17.28, **found:** C 59.22, H 3.79, N 17.24.

4-(Benzo[1,3]dioxol-5-yl)-1-(5-chloro-2-oxoindolin-3-ylidene)semicarbazide (MDA-13): λ_{max}: 269.40, 301.60 nm; **IR (KBr):** υ = 3456.55, 3203.87 (N-H str), 3096.47 (aromatic C-H str), 2928.04 (CH_2 str), 1716.70 (lactam C=O), 1654.98 (C=O str), 1618.33 (C=N str), 1473.66 (C=C str), 1124.54 (C-O-C str), 823.63 (C-Cl str); **^{1}H NMR ([D$_6$]DMSO):** δ = 3.48 (s, 2H, CH_2), 5.90 (s, 1H, NH), 6.81-7.11 (m, 3H, benzene C-H), 7.40-7.74 (m, 3H, isatinyl C-H), 8.19 (s, 1H, isatinyl N-H), 9.76 ppm (s, 1H, CONH); **^{13}C NMR ([D$_6$]DMSO):** δ = 101.57 (C-2), 107.98 (C-4), 110.12 (C-6), 112.76 (C-7), 115.41 (isatinyl C-3a), 122.94 (isatinyl C-7), 128.32 (C-5), 128.79 (isatinyl C-4), 130.86 (isatinyl C-5), 131.30 (isatinyl C-6), 133.23 (C=N), 142.38 (isatinyl C-7a), 142.72 (C-7a), 148.64 (C-3a), 168.10 (C=O), 179.11 ppm (isatinyl C=O); **Elemental analysis for $C_{16}H_{11}ClN_4O_4$: calcd:** C 53.57, H 3.09, N 15.62, **found:** C 53.57, H 3.13, N 15.67.

4-(Benzo[1,3]dioxol-5-yl)-1-(5-nitro-2-oxoindolin-3-ylidene)semicarbazide (MDA-14): λ_{max}: 266.60, 299.40 nm; **IR (KBr):** υ = 3421.83, 3194.23 (N-H str), 3097.27

(aromatic C-H str), 2929.97 (CH_2 str), 1712.85 (lactam C=O), 1674.27 (C=O str), 1620.26 (C=N str), 1489.10 (C=C str), 1471.74, 1319.35 (NO_2 str), 1124.54 (C-O-C str); **^{1}H NMR ([D$_6$]DMSO):** δ = 3.37 (s, 2H, CH_2), 5.98 (s, 1H, NH), 6.84-7.12 (m, 3H, benzene C-H), 8.10-8.63 (m, J = 6.3 Hz, 3H, isatinyl C-H), 8.21 (s, 1H, isatinyl N-H), 9.73 ppm (s, 1H, CONH); **^{13}C NMR ([D$_6$]DMSO):** δ = 101.45 (C-2), 107.43 (C-4),110.32 (C-6), 112.56 (C-7), 115.60 (isatinyl C-3a), 121.62 (isatinyl C-7), 122.83 (isatinyl C-6), 123.48 (isatinyl C-4), 129.01 (C-5), 133.13 (C=N), 142.11 (isatinyl C-5), 142.63 (C-7a), 148.52 (C-3a), 151.25 (isatinyl C-7a), 168.53 (C=O), 179.62 ppm (isatinyl C=O); **Elemental analysis for $C_{16}H_{11}N_5O_6$: calcd:** C 52.04, H 3.00, N 18.96, **found:** C 51.97, H 3.04, N 19.02.

The IR, ^{1}H NMR, ^{13}C NMR, mass and X-RD spectra of compounds **MDA-2**, **MDA-3**, **MDA-6**, **MDA-8**, **MDA-9** and **MDA-12** are presented in **Figure 4.53.** to **Figure 4.74.**

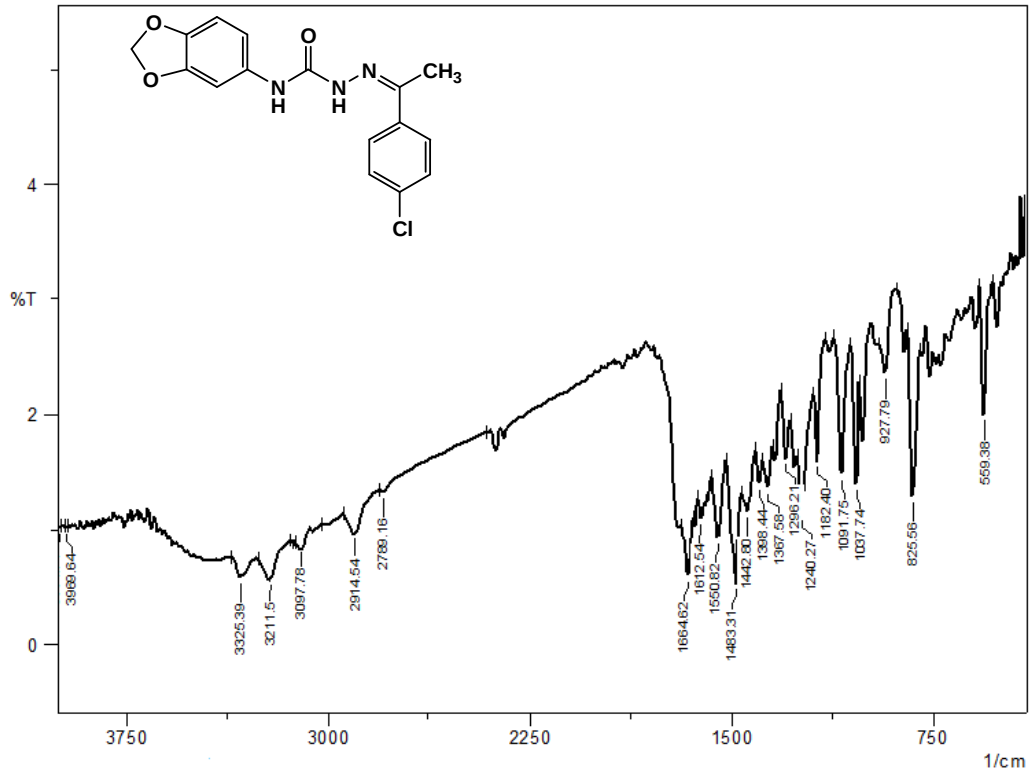


Figure 4.53. IR spectrum of **MDA-2**

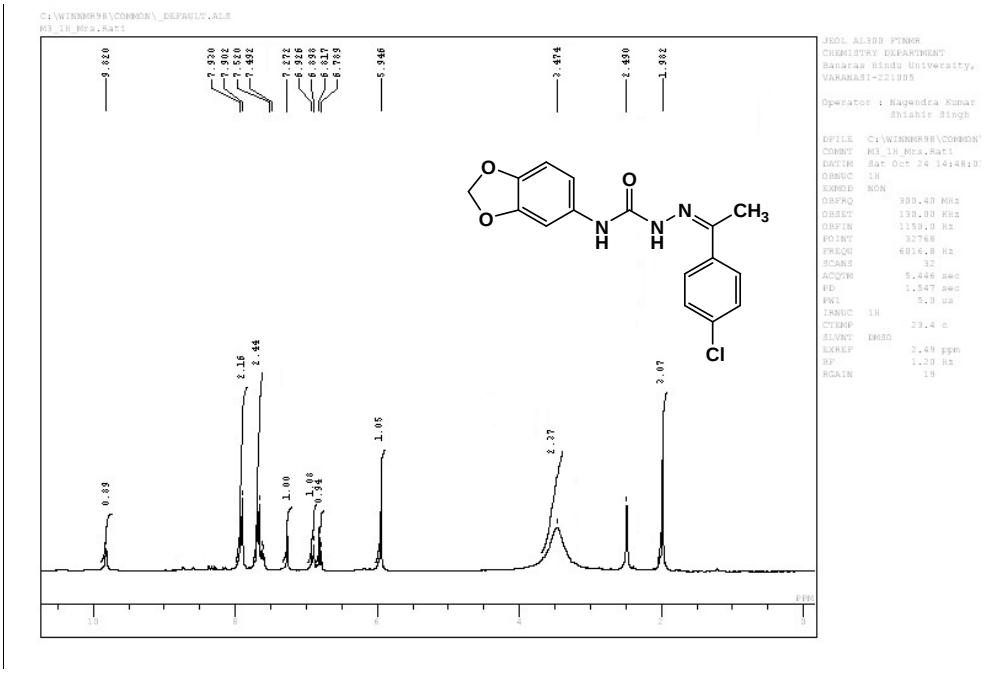


Figure 4.54. ^{1}H NMR spectrum of **MDA-2**

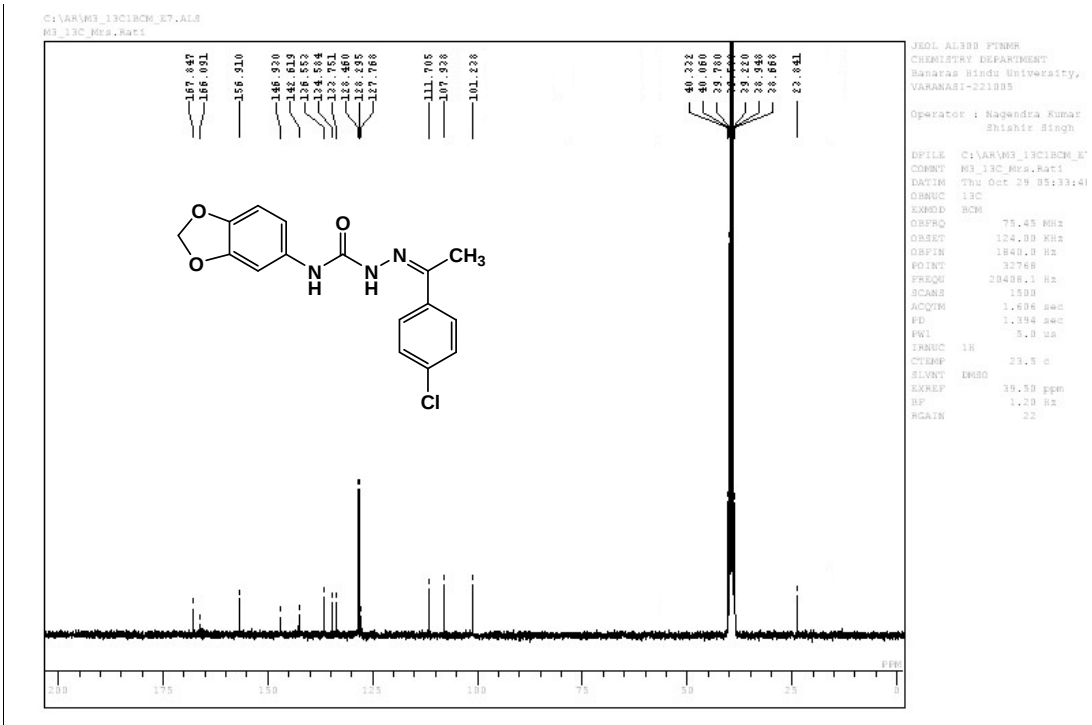


Figure 4.55. ^{13}C NMR spectrum of **MDA-2**

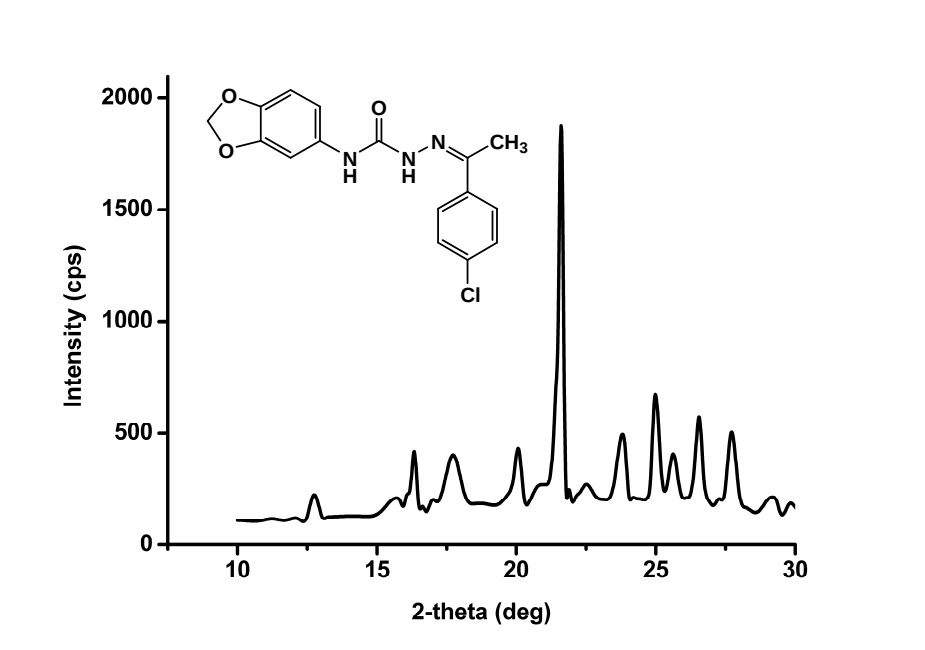


Figure 4.56. XR-PD spectrum of **MDA-2**

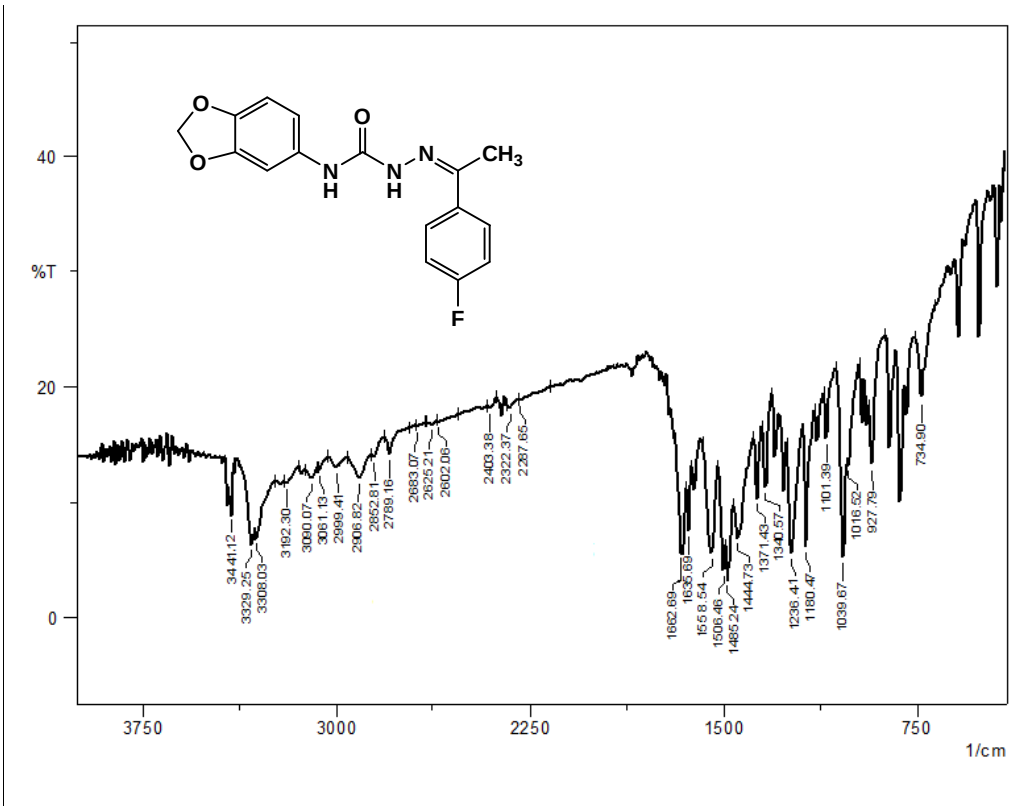


Figure 4.57. IR spectrum of **MDA-3**

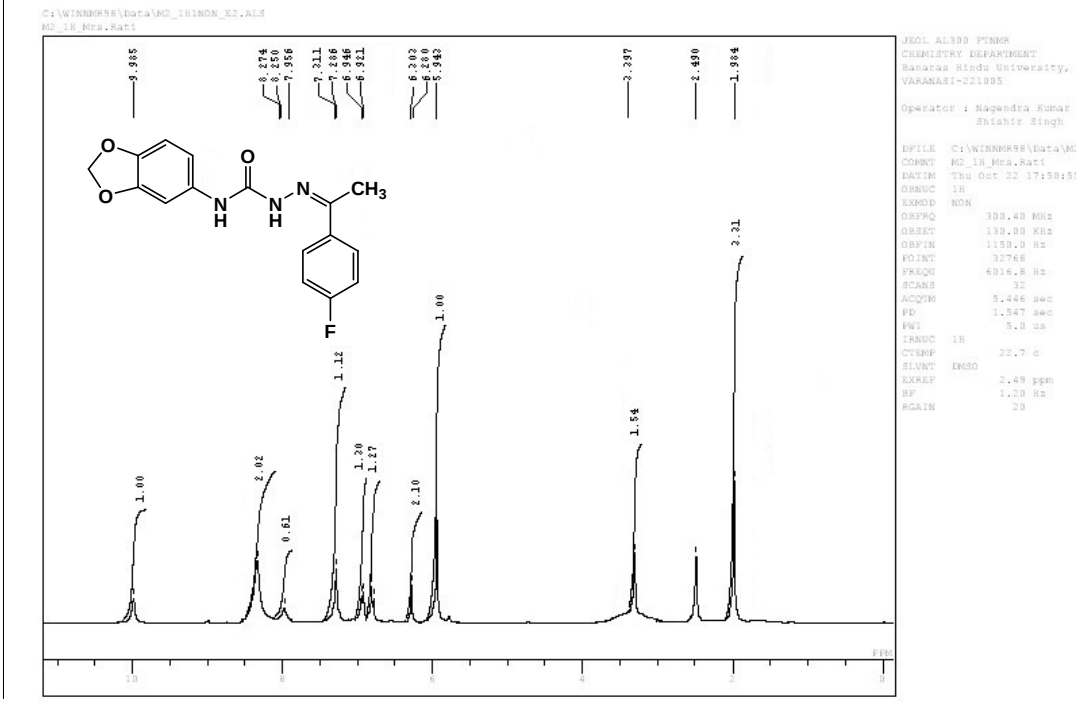


Figure 4.58. ^{1}H NMR spectrum of **MDA-3**

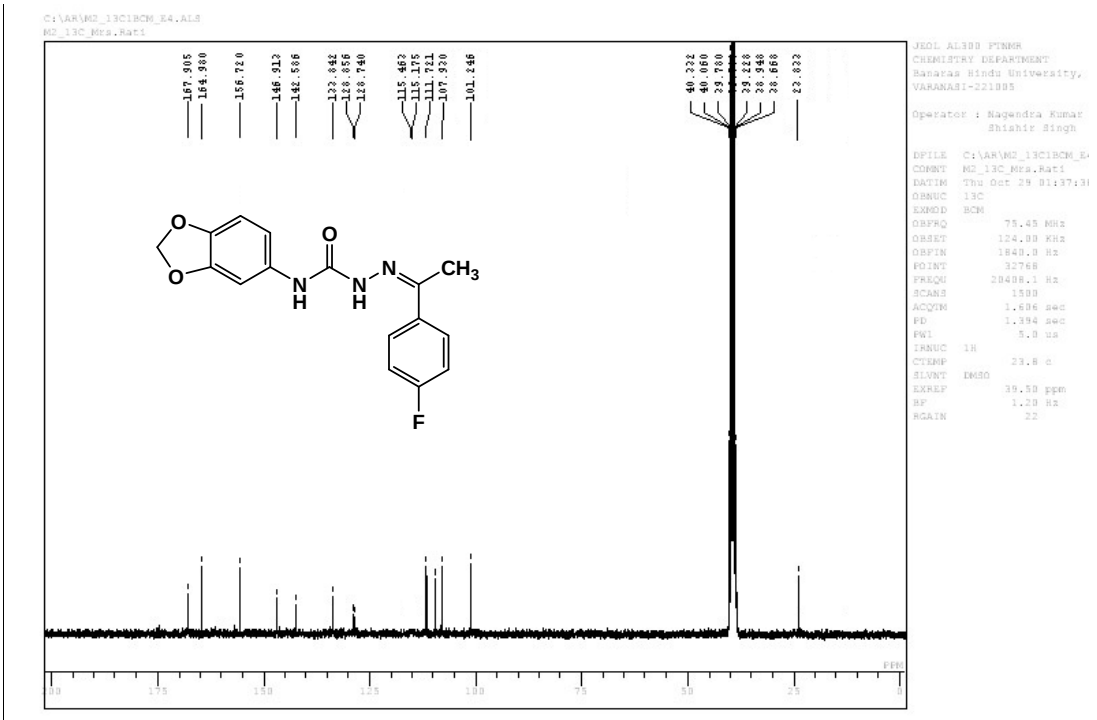


Figure 4.59. ^{13}C NMR spectrum of **MDA-3**

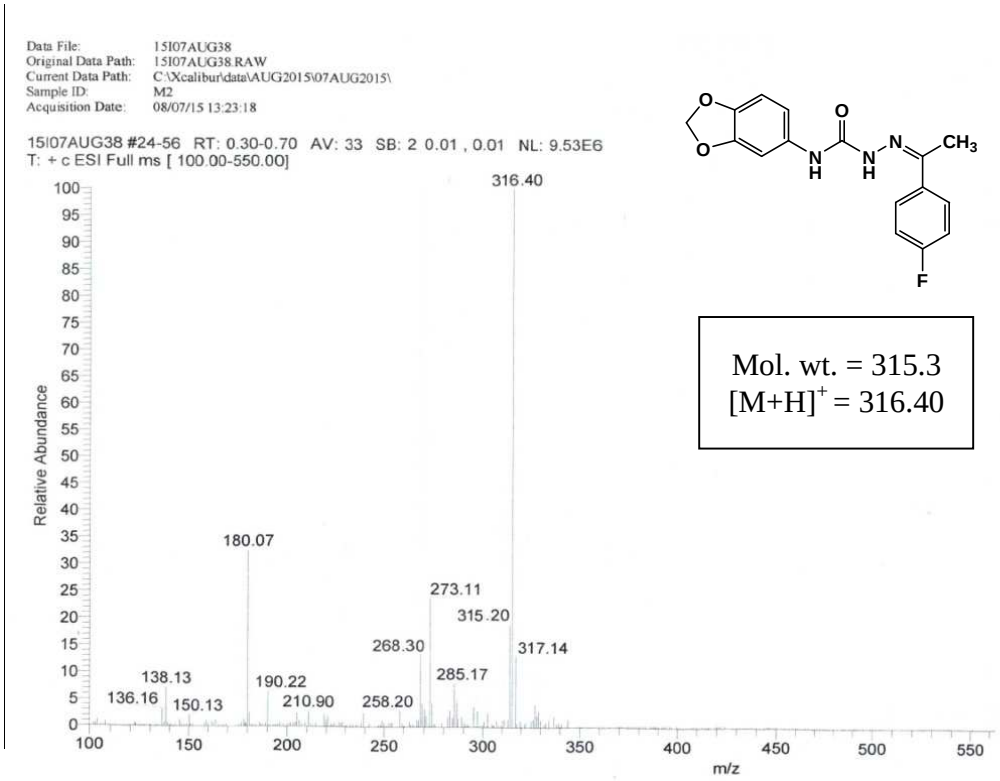


Figure 4.60. Mass spectrum of **MDA-3**

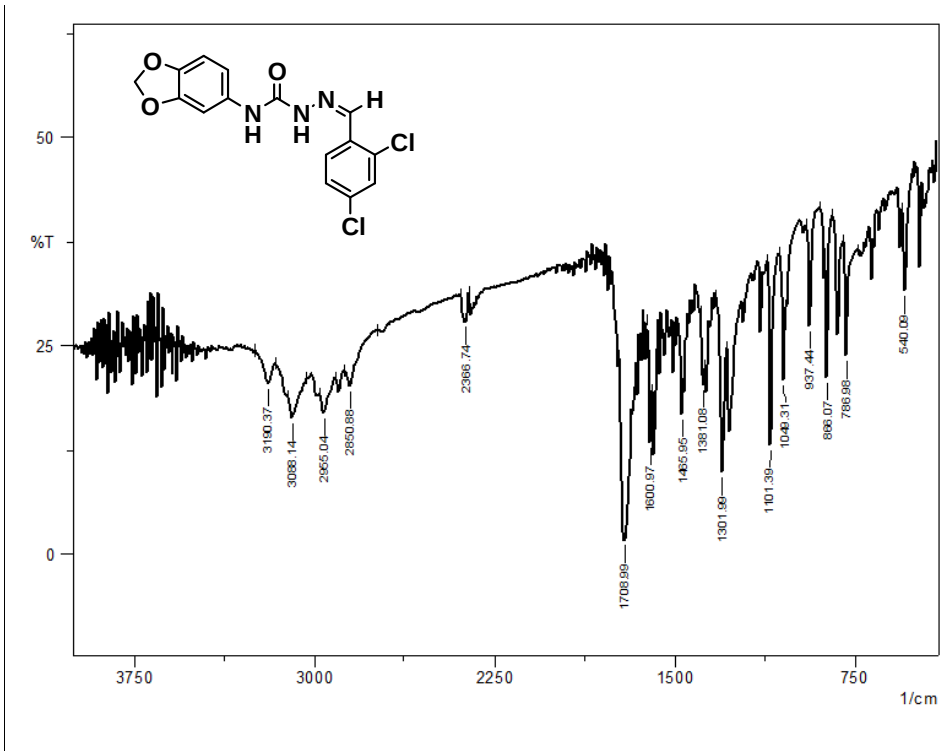


Figure 4.61. IR spectrum of **MDA-6**

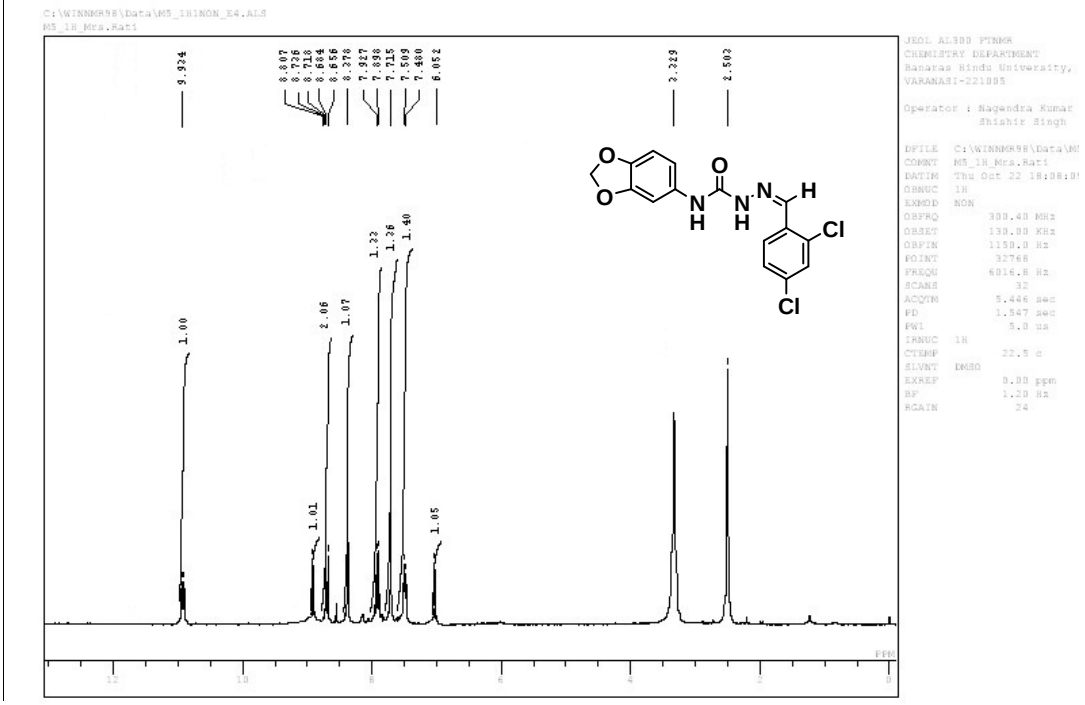


Figure 4.62. ^{1}H NMR spectrum of **MDA-6**

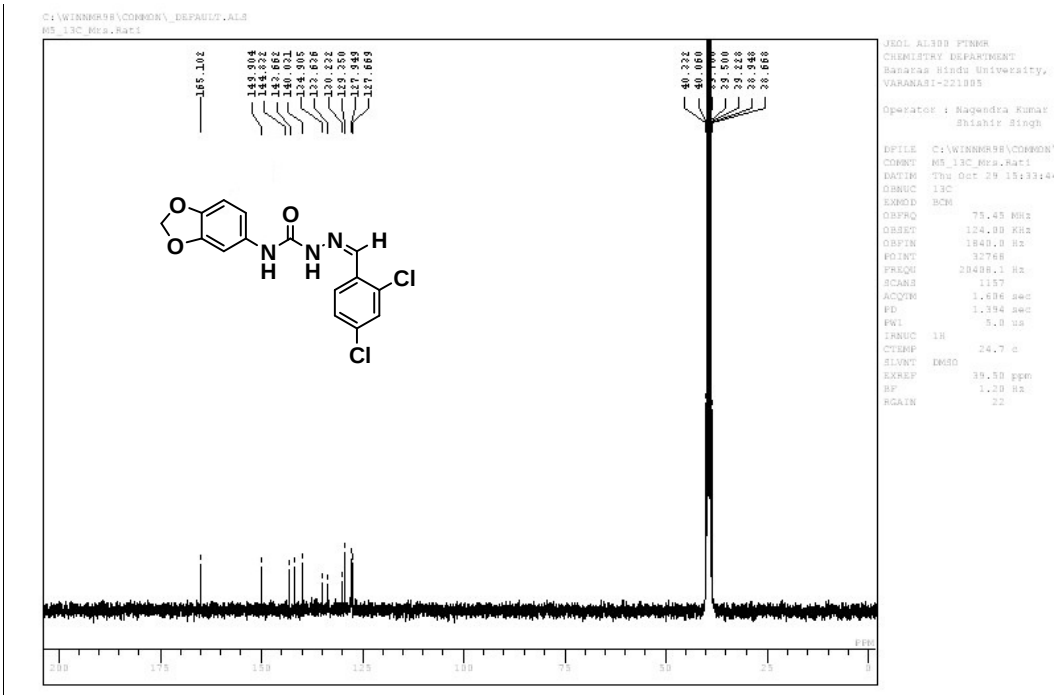


Figure 4.63. ^{13}C NMR spectrum of **MDA-6**

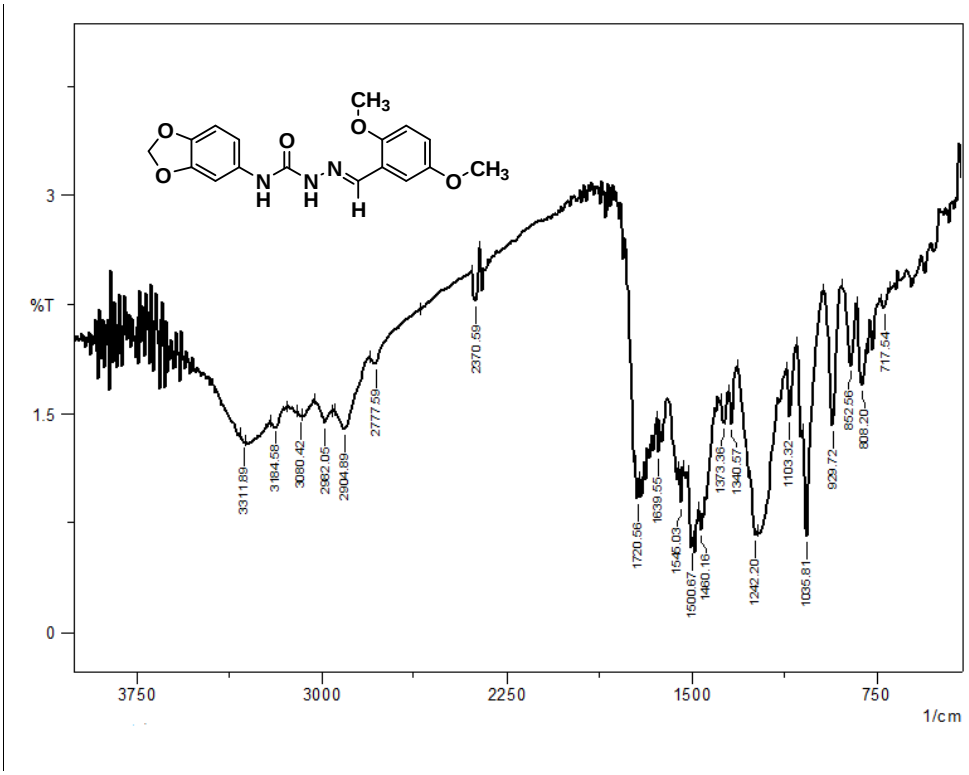


Figure 4.64. IR spectrum of **MDA-8**

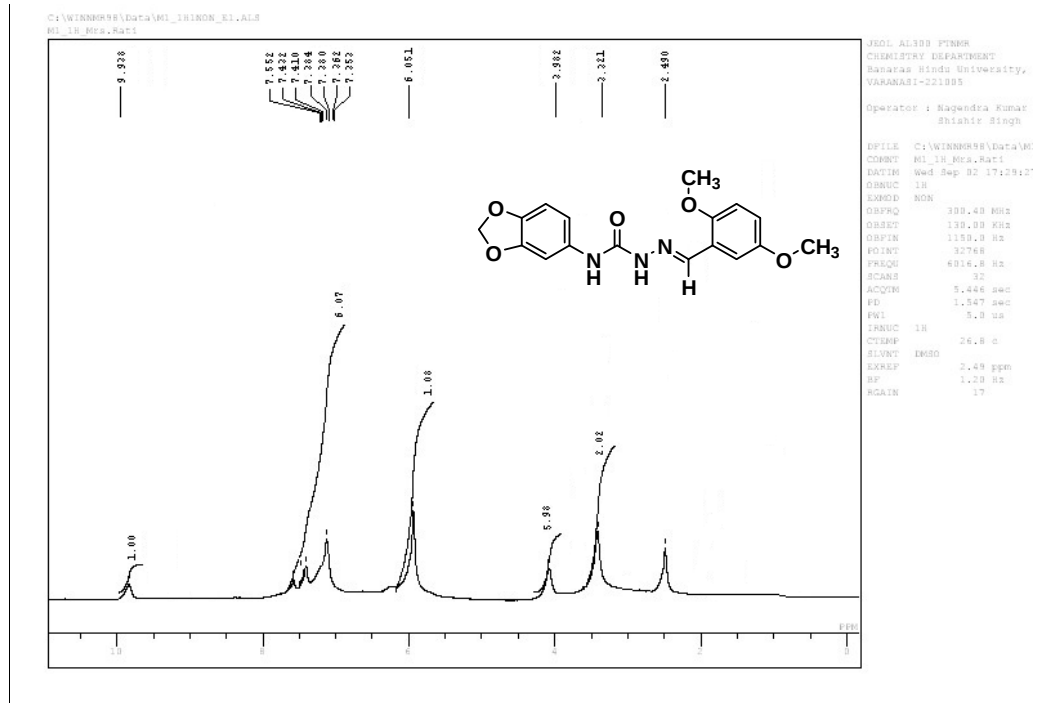


Figure 4.65. ^{1}H NMR spectrum of **MDA-8**

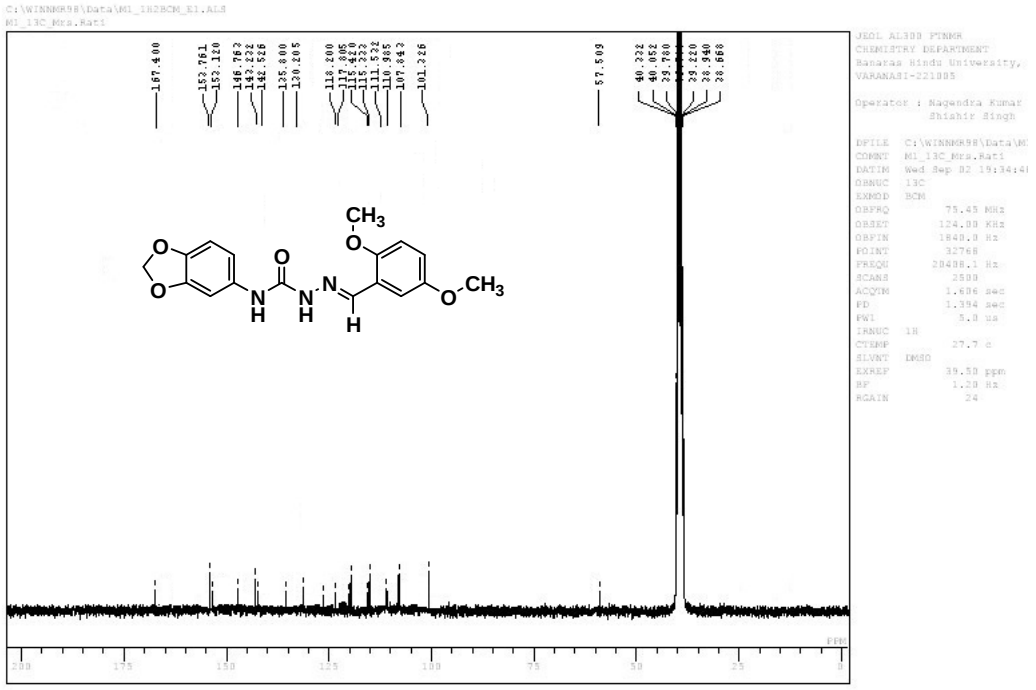


Figure 4.66. ^{13}C NMR spectrum of **MDA-8**

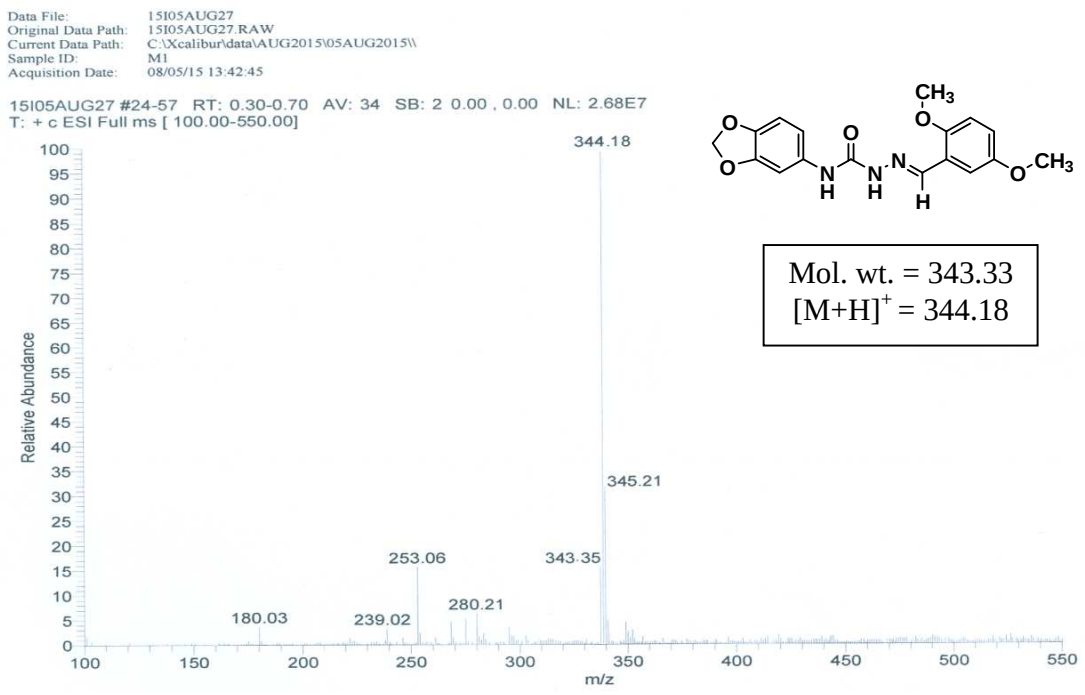


Figure 4.67. Mass spectrum of **MDA-8**

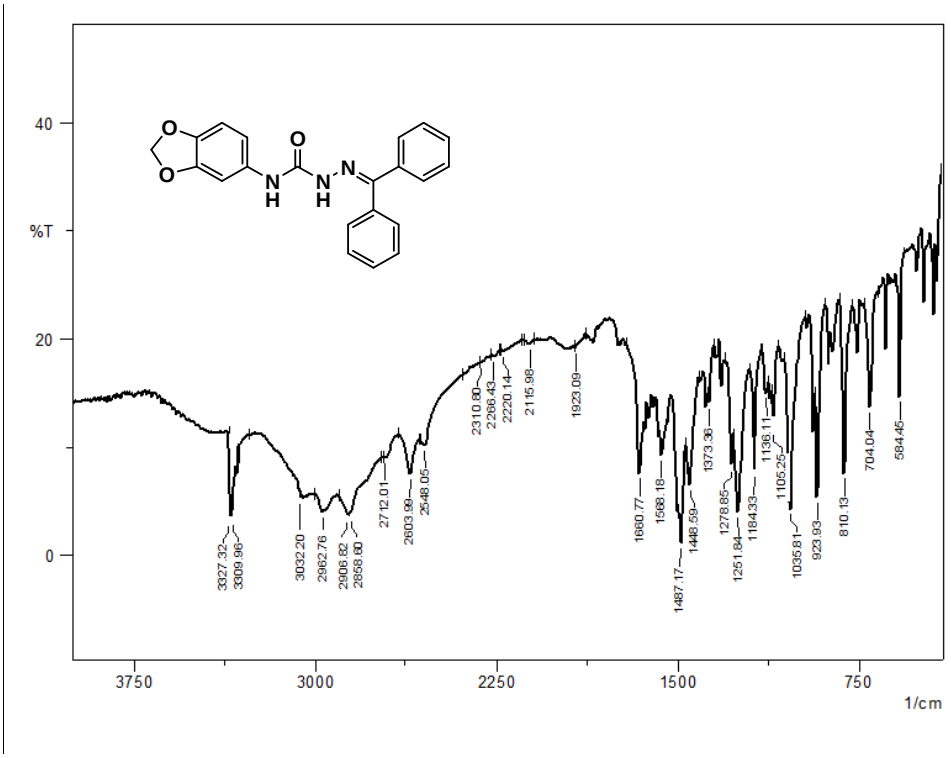


Figure 4.68. IR spectrum of **MDA-9**

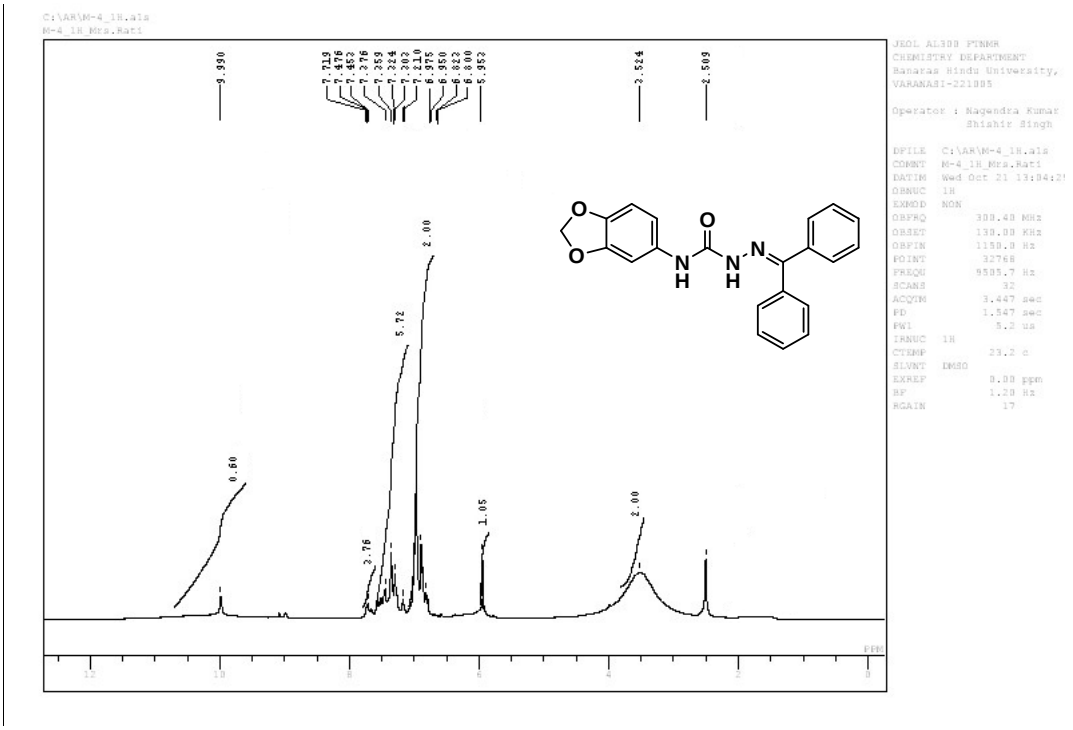


Figure 4.69. ^{1}H NMR spectrum of **MDA-9**

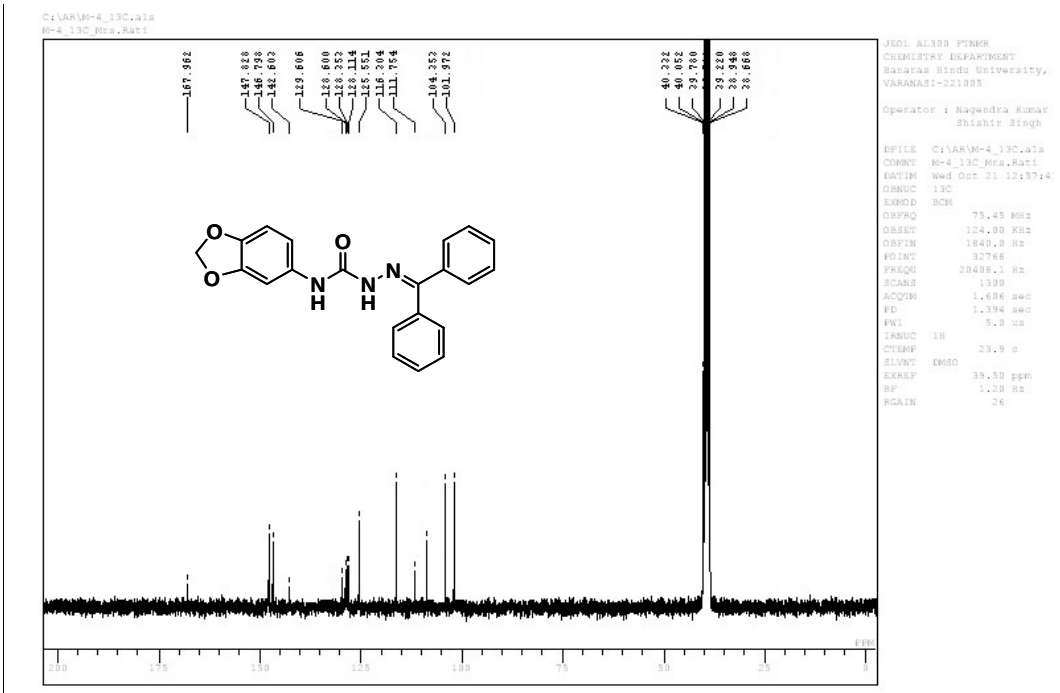


Figure 4.70. ^{13}C NMR spectrum of **MDA-9**

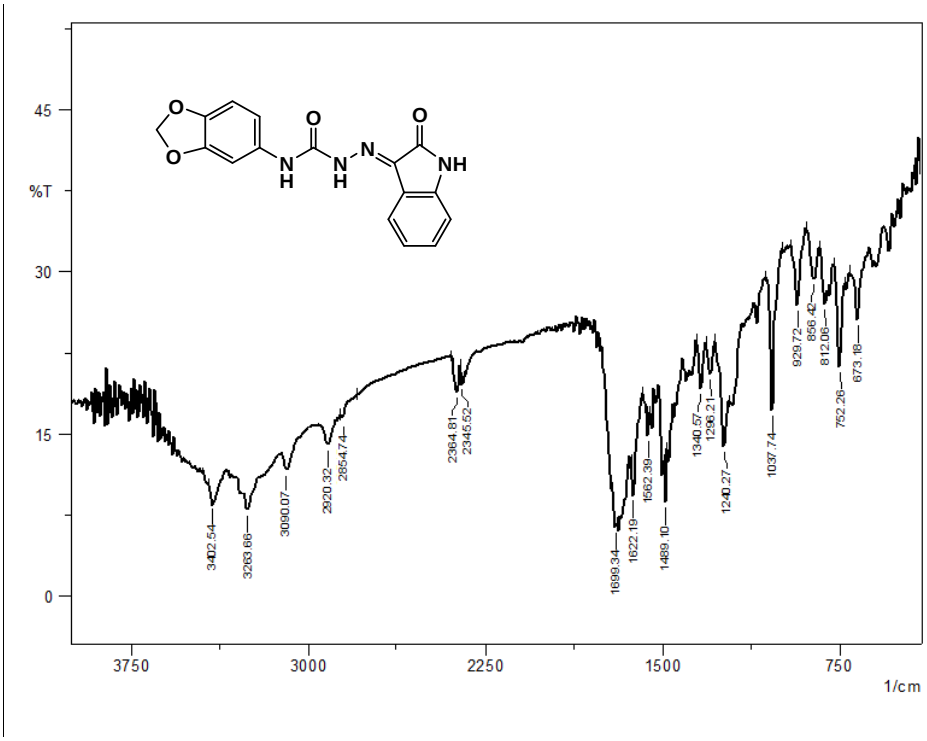


Figure 4.71. IR spectrum of **MDA-12**

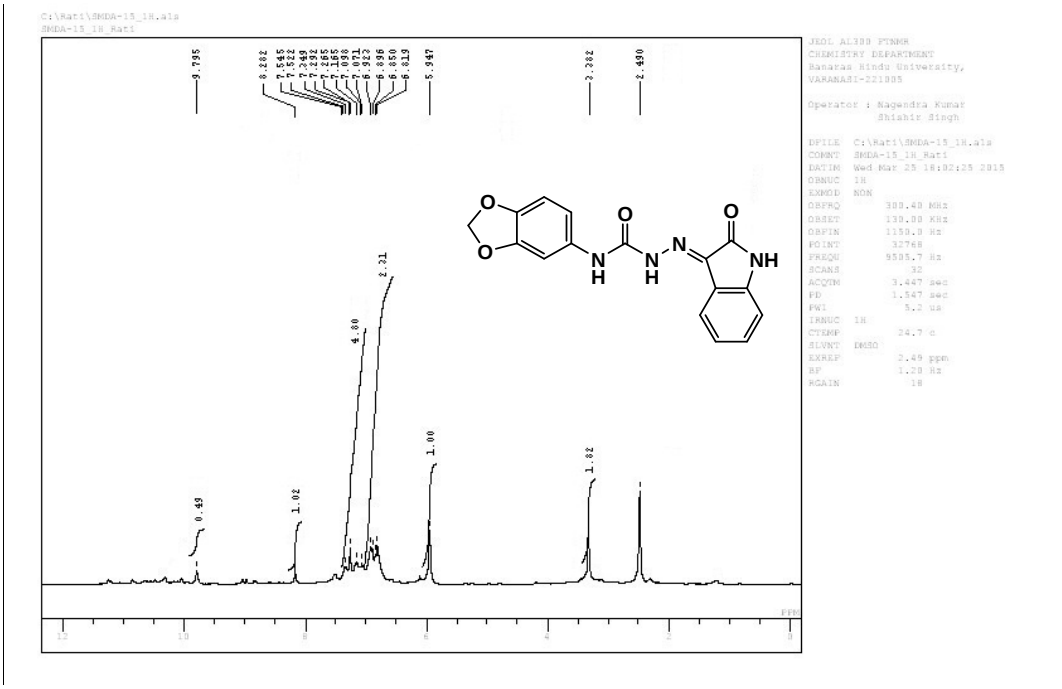


Figure 4.72. ^{1}H NMR spectrum of **MDA-12**

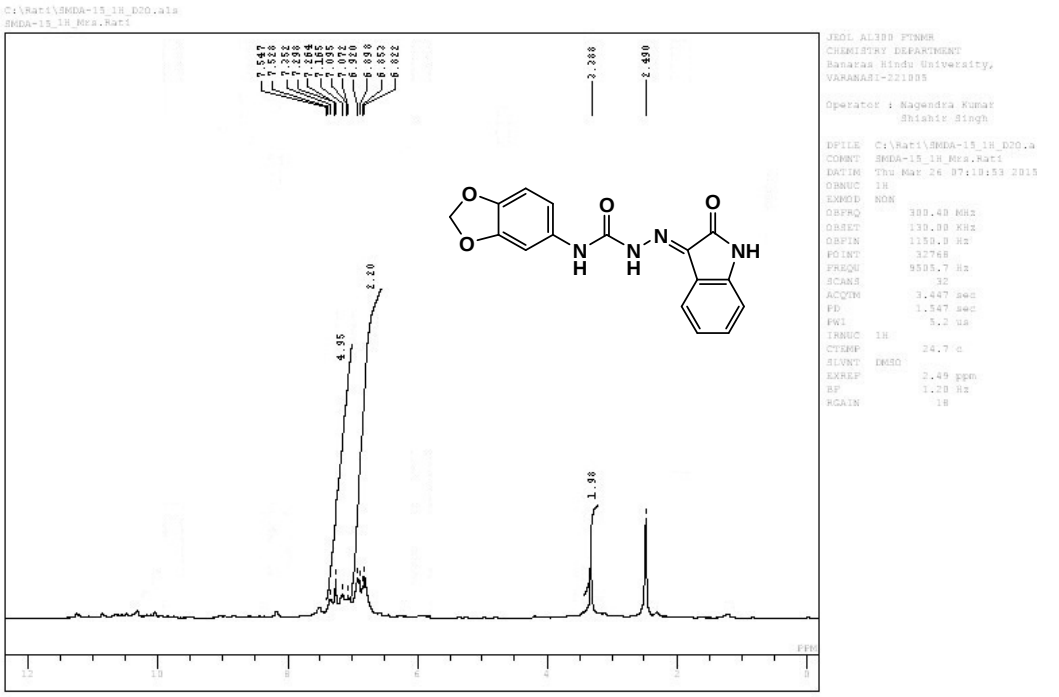


Figure 4.73. ^{1}H (D_2O exchange) NMR spectrum of **MDA-12**

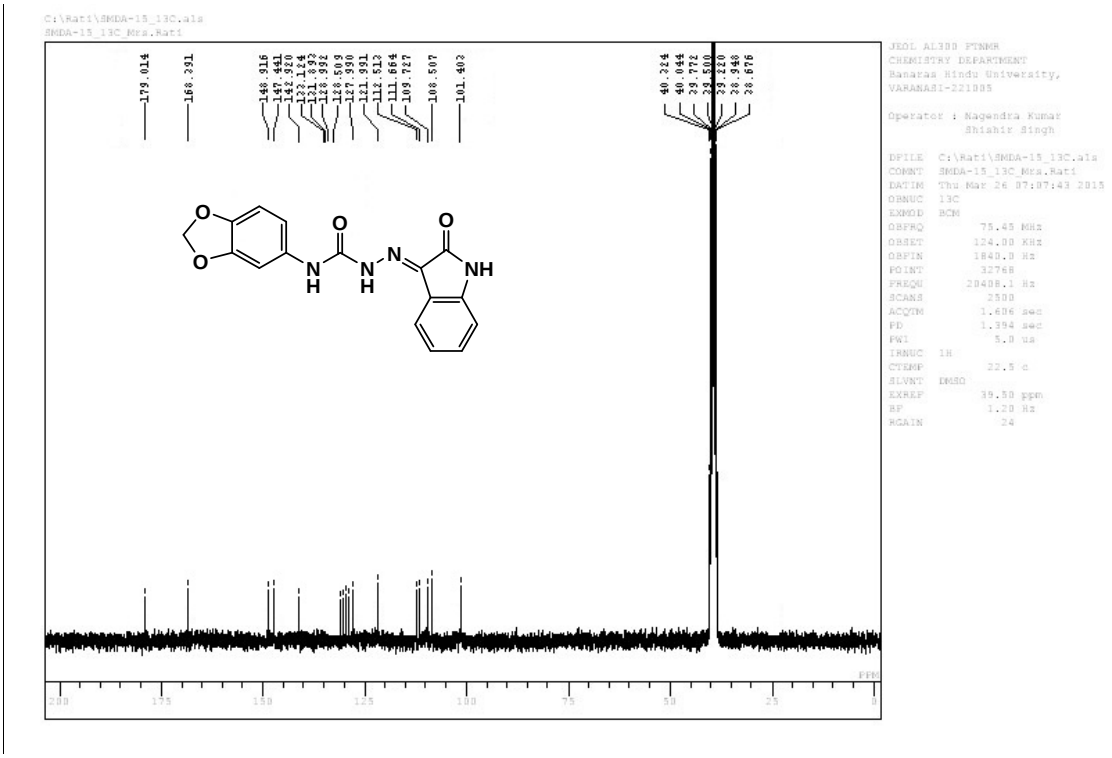


Figure 4.74. ^{13}C NMR spectrum of **MDA-12**

4.3.3. Biological evaluation

The procedures followed for the *in-vitro* and *in-vivo* biological screening of the synthesized compounds (**MDA-1** to **MDA-14**) are summarized in **Section 4.1.3.**

4.3.4. *In-silico* molecular property analysis and ADMET prediction studies

The procedures followed for *in-silico* molecular property analysis and ADMET prediction studies for all the synthesized compounds (**MDA-1** to **MDA-14**) are described in **Section 4.1.4.**

4.4. 3-HYDROXY-3-SUBSTITUTED OXINDOLE ANALOGUES OF ISATIN & ITS DERIVATIVES [HPO-1 to HPO-14]

4.4.1. Synthesis

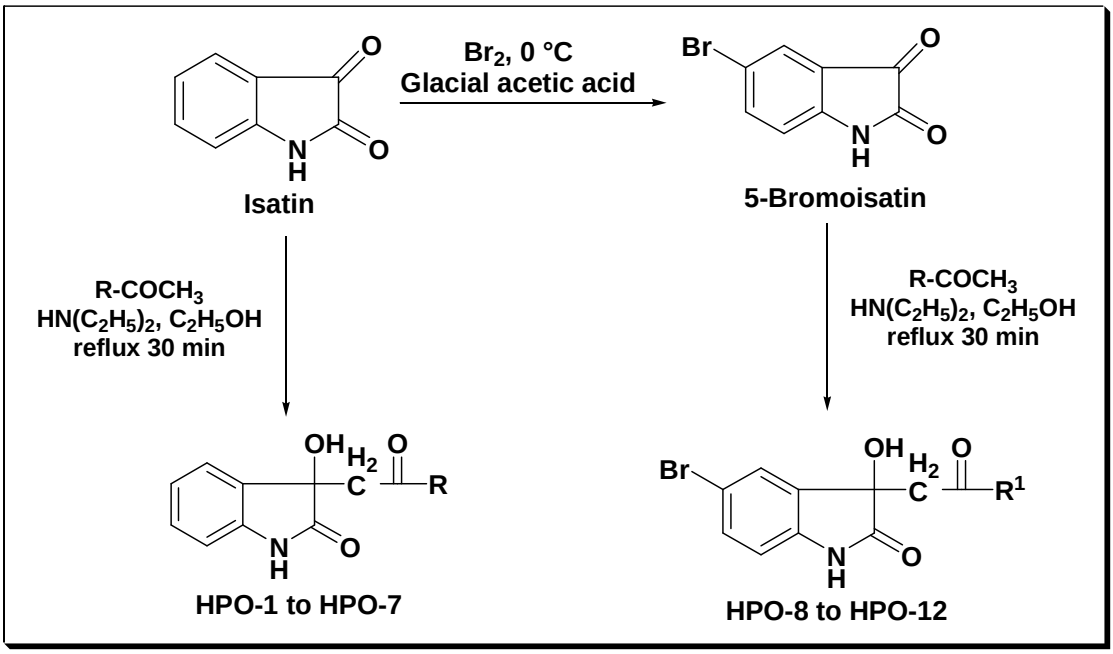
4.4.1.1. Chemicals and reagents

Unless otherwise specified, all starting materials and solvents were of commercial quality of laboratory grade purchased from Sigma-Aldrich (U.S.A.) and Merck (Germany) and were used without purification.

4.4.1.2. Synthetic protocol

The intermediates and final compounds were synthesized according to the well established procedures **[**Popp *et al.*, 1979, 1980] but with modifications wherever needed. The synthetic route followed for 3-hydroxy-3-substituted oxindole analogues of isatin and 5-bromoisatin (**HPO-1** to **HPO-14**) is outlined in **Scheme 4.7.** and **Scheme 4.8.**

3-Hydroxy-3-substituted oxindole analogues of isatin (**HPO-1** to **HPO-7**) and 5-bromoisatin (**HPO-8** to **HPO-12**) were obtained through Knoevenagel condensation of appropriate isatin with acetone or substituted acetophenones in presence of diethyl amine as basic catalyst.



Scheme 4.7. Synthesis of 3-hydroxy-3-substituted oxindole analogues of isatin and 5-bromoisatin with acetone or substituted acetophenones (**HPO-1** to **HPO-12**)

4.4.1.2.1. Synthesis of 3-hydroxy-3-phenacyloxindole analogues of isatin (HPO-1 to HPO-7) with acetophenones

Isatin (0.005 mol) and an equimolecular amount of unsubstituted or 4-substituted acetophenone (0.005 mol) were dissolved in absolute alcohol (10–12 mL) and diethyl amine (10–15 drops) was added. The mixture was heated at reflux on the steam bath for 30 minutes. After standing for 5–7 days at room temperature, the products were collected by filtration, dried and recrystallized from 95% ethanol (**Scheme 4.7.**).

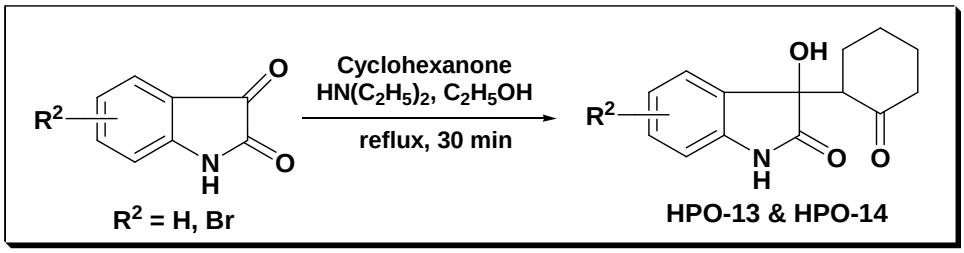
4.4.1.2.2. Synthesis of 5-bromoisatin

The procedure followed for the synthesis of 5-bromoisatin is depicted in Section **4.1.1.2.1.1.** (**Scheme 4.7.**).

4.4.1.2.3. Synthesis of 3-hydroxy-3-phenacyloxindole analogues of 5-bromoisatin (HPO-8 to HPO-12) with acetophenones

5-Bromoisatin (0.003 mol) and an equimolecular amount of acetone or 4-substituted acetophenone (0.003 mol) were dissolved in absolute alcohol (10–12 mL) and diethylamine (10–15 drops) was added. The reaction mixture was heated at reflux on the steam bath for 30 minutes and allowed to stand for 7–10 days at room temperature, whereby the products formed were collected by filtration, dried and recrystallized from 95% ethanol (**Scheme 4.7.**).

4.4.1.2.4. Synthesis of 3-hydroxy-3-substituted oxindole analogues of isatin and 5-bromoisatin with cyclohexanone (HPO-13 and HPO-14)



Scheme 4.8. Synthesis of 3-hydroxy-3-substituted oxindole analogues of isatin and 5-bromoisatin with cyclohexanone (**HPO-13** to **HPO-14**)

Isatin or 5-bromoisatin (0.005 mol) and an equimolecular amount of cyclohexanone (0.005 mol) were dissolved in absolute alcohol (10–12 mL) and diethyl amine (10–15 drops) was added. The mixture was heated at reflux on the steam bath for 30 minutes.

After standing for 5–7 days at room temperature, the products were collected by filtration, dried and recrystallized from 95% ethanol (**Scheme 4.8.**).

4.4.1.3. Reaction mechanism

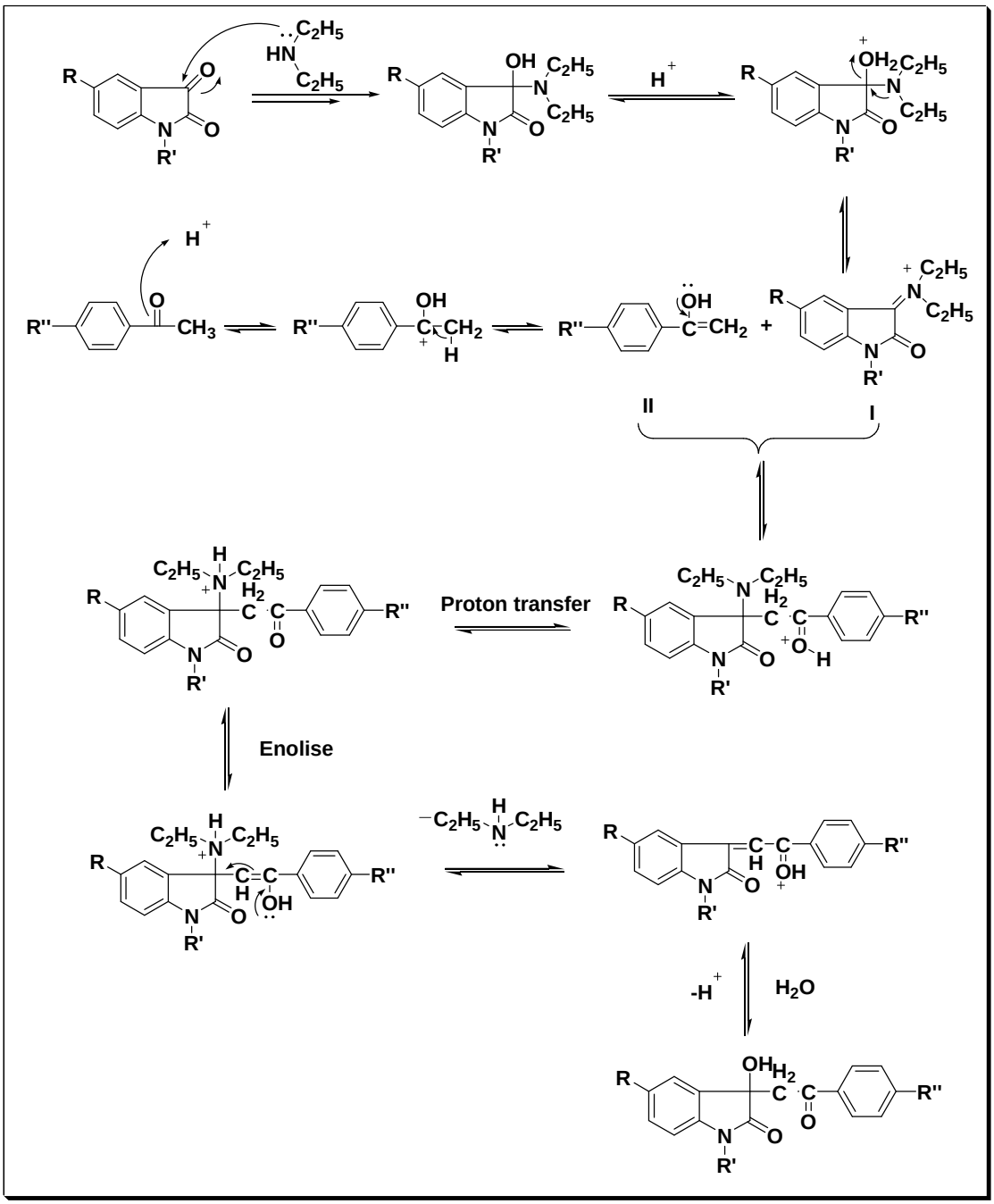
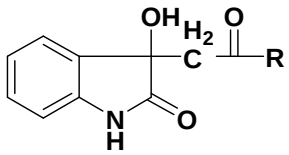


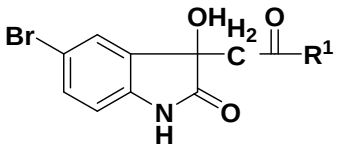
Figure 4.75. Proposed mechanism of condensation reaction between isatin and substituted acetophenones

The products were evidently the result of interaction of reactive beta-carbonyl group of isatin or substituted isatin with the methyl group of acetone or substituted acetophenone through Knoevenagel condensation. The proposed mechanism of this condensation reaction is depicted in **Figure 4.75.**

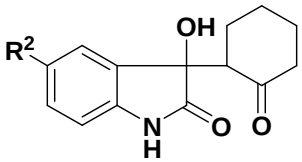
Table 4.9. Structural data of 3-hydroxy-3-substituted oxindole analogues of isatin (**HPO-1** to **HPO-14**)



(HPO-1 to HPO-7)



(HPO-8 to HPO-12)



(HPO-13 and HPO-14)

Code	R	R^1	R^2	Mol. Formula	Mol. Wt. (g/mol)
HPO-1	CH_3	-	-	$C_{11}H_{11}NO_3$	205.21
HPO-2	C_6H_5	-	-	$C_{16}H_{13}NO_3$	267.28
HPO-3	4-Br C_6H_4	-	-	$C_{16}H_{12}BrNO_3$	346.18
HPO-4	4-Cl C_6H_4	-	-	$C_{16}H_{12}ClNO_3$	301.72
HPO-5	4-F C_6H_4	-	-	$C_{16}H_{12}FNO_3$	285.27
HPO-6	4-NO_2 C_6H_4	-	-	$C_{16}H_{12}N_2O_5$	312.28
HPO-7	2',4'-Br_2 C_6H_3	-	-	$C_{16}H_{11}Br_2NO_3$	425.07
HPO-8	-	4-Br C_6H_4	-	$C_{16}H_{11}Br_2NO_3$	425.07
HPO-9	-	4-Cl C_6H_4	-	$C_{16}H_{11}BrClNO_3$	380.62
HPO-10	-	4-F C_6H_4	-	$C_{16}H_{11}BrFNO_3$	364.17
HPO-11	-	4-OH C_6H_4	-	$C_{16}H_{12}BrNO_4$	362.17
HPO-12	-	4-NO_2 C_6H_4	-	$C_{16}H_{11}BrN_2O_5$	391.17
HPO-13	-	-	H	$C_{14}H_{15}NO_3$	245.27
HPO-14	-	-	Br	$C_{14}H_{14}BrNO_3$	324.17

4.4.2. Characterization

The physicochemical and spectral characterizations of the synthesized compounds (**HPO-1** to **HPO-14**) were performed so as to ascertain the chemical structure of the compounds. The complete procedures of all the characterization methods followed and the instruments used are mentioned in **Section 4.1.2.**

4.4.2.1. Physicochemical characterization

The physicochemical characterization data of synthesized compounds (**HPO-1** to **HPO-14**) are listed in **Table 4.10.**

Table 4.10. Physicochemical characterization data of **HPO-1** to **HPO-14**

Code	MP (°C)	Yield (%)	Colour	R_f[a]	LogP[b]	Expt. LogP[c]
HPO-1	154-156	39.48	Orange	0.43	0.48	-
HPO-2	166-168	42.67	Orange	0.57	1.90	1.1
HPO-3	176-179	23.81	Maroon	0.49	2.67	1.9
HPO-4	171-174	59.86	Red	0.52	2.51	1.8
HPO-5	204-206	89.76	Red	0.56	2.04	1.2
HPO-6	158-159	32.19	Orange	0.51	1.84	1.3
HPO-7	163-165	32.35	Maroon	0.47	3.44	2.7
HPO-8	159-161	12.36	Maroon	0.42	3.44	2.4
HPO-9	116-118	19.71	Red	0.48	3.27	2.5
HPO-10	156-159	25.34	Red	0.41	2.81	2.1
HPO-11	168-170	42.81	Red	0.53	2.37	1.7
HPO-12	Charred at 188-190	13.52	Red	0.59	2.61	2.1
HPO-13	202-204	57.81	Maroon	0.56	1.70	1.1
HPO-14	220-222	61.08	Maroon	0.45	2.47	1.9

*All the compounds were soluble in methanol, ethanol, DMF and DMSO

[a]Solvent system: Chloroform: CH_3OH: Toluene (7:1:2; 7:2:1); [b]MarvinSketch generated

[c]Determined using Shake flask method; '-' indicates 'not tested'

4.4.2.2. Spectral characterization and elemental analysis

All the synthesized compounds (**HPO-1** to **HPO-14**) were subjected to UV, IR, ^{1}H NMR and ^{13}C NMR spectral and elemental analysis and the results are presented below in

section **4.4.2.2.1.** In addition, mass spectrum was measured for compounds **HPO-4** and **HPO-9** and the $[M+1]^+$ peak of these compounds is presented below (**Figure 4.104.** and **Figure 4.115.**). Moreover, compound **HPO-4** was subjected to X-ray powder diffraction analysis and the diffraction pattern is shown in **Figure 4.105.**

4.4.2.3. Spectral characterization and elemental analysis data of intermediates (ISN-1) and final compounds (HPO-1 to HPO-14)

5-Bromoisatin (ISN-1): IR (KBr): υ = 3205.80 (N-H str), 2996.55 (aromatic C-H str), 1755.28 (2 C=O str), 1710.92 (3 C=O str), 1282.71 (C-N str), 886.52 (C-Br str); **^{1}H NMR ([D$_6$]DMSO):** δ = 7.43-8.01 (m, 4H, oxindole C-H), 7.93 ppm (s, 1H, oxindole N-H); **^{13}C NMR ([D$_6$]DMSO):** δ = 121.52-145.23 (oxindole C-3a, C-4, C-5, C-6, C-7, C-7a), 160.37-175.28 (oxindole 2C=O, 3C=O).

3-Hydroxy-3-(2-oxopropyl)indolin-2-one (HPO-1): λ_{max}: 293.60, 373.80 nm; **IR (KBr):** υ = 3342.65 (O-H str), 3265.18 (N-H str), 3095.66 (aromatic C-H str), 2936.50 (CH_2 str), 1710.80 (2 C=O str), 1681.75 (C=O str), 1597.63 (C=C str), 1481.25 (CH_2 bend); **^{1}H NMR ([D$_6$]DMSO):** δ = 2.76 (s, 3H, CH_3), 3.35-3.58 (s, 2H, CH_2), 4.29 (s, 1H, O-H), 6.85-7.78 (m, 4H, oxindole C-H), 8.05 ppm (s, 1H, oxindole N-H); **^{13}C NMR ([D$_6$]DMSO):** δ = 35.81 (-CH_3), 60.52 (-CH_2), 85.91 (oxindole C-OH), 123.39-143.21 (oxindole C-3a, C-4, C-5, C-6, C-7, C-7a), 180.32 (oxindole C=O), 199.73 ppm (C=O); **Elemental analysis for $C_{11}H_{11}NO_3$: calcd:** C, 64.38; H, 5.40; N, 6.83, **found:** C, 64.32; H, 5.42; N, 6.81.

3-Hydroxy-3-phenacyloxindole (HPO-2): λ_{max}: 268.20, 376.80 nm; **IR (KBr):** υ = 3306.10 (O-H str), 3254.02 (N-H str), 3097.78 (aromatic C-H str), 2914.54 (CH_2 str), 1712.85 (2 C=O str), 1681.98 (C=O str), 1597.11 (C=C str), 1477.52 (CH_2 bend); **^{1}H NMR ([D$_6$]DMSO):** δ = 1.17-1.19 (s, 2H, CH_2), 4.23 (s, 1H, O-H), 6.03-6.80 (m, 4H, oxindole C-H), 7.34-7.75 (m, 5H, Ar'C-H), 7.85 ppm (s, 1H, oxindole N-H); **^{13}C NMR ([D$_6$]DMSO):** δ = 64.29 (-CH_2), 79.60 (oxindole C-OH), 102.57-154.85 (oxindole C-3a, C-4, C-5, C-6, C-7, C-7a), 122.38-154.32 (Ar C'-1 to Ar C'-6), 155.89 (oxindole C=O), 190.29 ppm (C=O); **Elemental analysis for $C_{16}H_{13}NO_3$: calcd:** C, 71.90; H, 4.90; N, 5.24, **found:** C, 71.95; H, 4.93; N, 5.20.

3-Hydroxy-3-(4'-bromophenacyl)oxindole (HPO-3): λ_{max}: 265.00, 373.20 nm; **IR (KBr):** υ = 3371.68 (O-H str), 3200.01 (N-H str), 3061.13 (aromatic C-H str), 2983.98 (CH_2 str), 1701.27 (2 C=O str), 1687.77 (C=O str), 1583.61 (C=C str), 1469.81 (CH_2 bend), 657.75 (C-Br str); **^{1}H NMR ([D_6]DMSO):** δ = 4.20 (s, 1H, O-H), 3.06-3.25 (s, 2H, CH_2), 6.59-7.39 (m, 4H, oxindole C-H), 7.16-7.49 (m, 4H, Ar'C-H), 7.69 ppm (s, 1H, oxindole N-H); **^{13}C NMR ([D_6]DMSO):** δ = 45.69 (-CH_2), 72.69 (oxindole C-OH), 116.86-132.92 (Ar C'-1 to Ar C'-6), 122.59-143.28 (oxindole C-3a, C-4, C-5, C-6, C-7, C-7a), 178.60 (oxindole C=O), 195.98 ppm (C=O); **Elemental analysis for $C_{16}H_{12}BrNO_3$: calcd:** C, 55.51; H, 3.49; N, 4.05, **found:** C, 55.58; H, 3.46; N, 4.09.

3-Hydroxy-3-(4'-chlorophenacyl)oxindole (HPO-4): λ_{max}: 263.20, 340.80 nm; **IR (KBr):** υ = 3375.54 (O-H str), 3196.15 (N-H str), 3059.20 (aromatic C-H str), 2902.96 (CH_2 str), 1699.34 (2 C=O str), 1683.91 (C=O str), 1587.47 (C=C str), 1471.74 (CH_2 bend), 750.33 (C-Cl str); **^{1}H NMR ([D_6]DMSO):** δ = 3.50-3.57 (s, 2H, CH_2), 4.05 (s, 1H, O-H), 6.86-7.55 (m, 4H, oxindole C-H), 7.26-7.86 (m, 4H, Ar'C-H), 7.89 ppm (s, 1H, oxindole N-H); **^{13}C NMR ([D_6]DMSO):** δ = 45.69 (-CH_2), 72.96 (oxindole C-OH), 109.37-142.79 (oxindole C-3a, C-4, C-5, C-6, C-7, C-7a), 128.91-138.28 (Ar C'-1 to Ar C'-6), 178.14 (oxindole C=O), 195.49 ppm (C=O); **MS** (m/z) = 302.78 $[M+1]^+$; **Elemental analysis for $C_{16}H_{12}ClNO_3$: calcd:** C, 63.69; H, 4.01; N, 4.64, **found:** C, 63.74; H, 4.06; N, 5.67.

3-Hydroxy-3-(4'-fluorophenacyl)oxindole (HPO-5): λ_{max}: 260.20, 348.80 nm; **IR (KBr):** υ = 3385.18 (O-H str), 3198.08 (N-H str), 3064.99 (aromatic C-H str), 2901.04 (CH_2 str), 1701.27 (2 C=O str), 1681.98 (C=O str), 1599.04 (C=C str), 1471.74 (CH_2 bend), 993.37 (C-F str); **^{1}H NMR ([D_6]DMSO):** δ = 3.51-3.57 (s, 2H, CH_2), 4.05 (s, 1H, O-H), 6.86-7.46 (m, 4H, oxindole C-H), 7.12 (s, 1H, oxindole N-H), 7.32-7.95 ppm (m, 4H, Ar'C-H); **^{13}C NMR ([D_6]DMSO):** δ = 45.66 (-CH_2), 72.97 (oxindole C-OH), 115.78-166.73 (Ar C'-1 to Ar C'-6), 121.06-142.84 (oxindole C-3a, C-4, C-5, C-6, C-7, C-7a), 178.19 (oxindole C=O), 195.04 ppm (C=O); **Elemental analysis for $C_{16}H_{12}FNO_3$: calcd:** C, 67.36; H, 4.24; N, 4.91, **found:** C, 67.42; H, 4.26; N, 4.95.

3-Hydroxy-3-(4'-nitrophenacyl)oxindole (HPO-6): λ_{max}: 279.20, 372.20 nm; **IR (KBr):** υ = 3380.68 (O-H str), 3230.26 (N-H str), 3031.57 (aromatic C-H str), 2980.12

(CH_2 str), 1701.27 (2 C=O str), 1654.98 (C=O str), 1602.90 (C=C str), 1460.16 (CH_2 bend), 1523.82, 1344.43 (C-NO_2 str); **^{1}H NMR ([D$_6$]DMSO):** δ = 3.62-3.71 (s, 2H, CH_2), 4.23 (s, 1H, O-H), 6.92-7.51 (m, 4H, oxindole C-H), 7.16 (s, 1H, oxindole N-H), 7.39-7.90 (m, 4H, Ar'C-H); **^{13}C NMR ([D$_6$]DMSO):** δ = 45.60 (-CH_2), 73.07 (oxindole C-OH), 117.83 (C'-3, C'-5), 123.29-143.34 (oxindole C-3a, C-4, C-5, C-6, C-7, C-7a), 133.71-156.70 (Ar C'-1 to Ar C'-6), 179.21 (oxindole C=O), 196.41 ppm (C=O); **Elemental analysis for $C_{16}H_{12}N_2O_5$: calcd:** C, 61.54; H, 3.87; N, 8.97, **found:** C, 61.50; H, 3.91; N, 8.94.

3-Hydroxy-3-(2',4'-dibromophenacyl)oxindole (HPO-7): λ_{max}: 248.00, 356.80 nm; **IR (KBr):** υ = 3368.54 (O-H str), 3210.14 (N-H str), 3095.85 (aromatic C-H str), 2970.48 (CH_2 str), 1732.13 (2 C=O str), 1685.84 (C=O str), 1581.68 (C=C str), 1465.95 (CH_2 bend), 648.10 (C-Br str); **^{1}H NMR ([D$_6$]DMSO):** δ = 3.34-3.90 (s, 2H, CH_2), 5.12 (s, 1H, O-H), 6.93-7.71 (m, 4H, oxindole C-H), 7.27-7.78 (m, 5H, Ar'C-H), 7.82 ppm (s, 1H, oxindole N-H); **^{13}C NMR ([D$_6$]DMSO):** δ = 60.43 (-CH_2), 63.50 (oxindole C-OH), 111.01-148.08 (oxindole C-3a, C-4, C-5, C-6, C-7, C-7a), 119.16-143.99 (Ar C'-1 to Ar C'-6), 170.64 (oxindole C=O), 190.36 ppm (C=O); **Elemental analysis for $C_{16}H_{11}Br_2NO_3$: calcd:** C, 45.21; H, 2.61; N, 3.30, **found:** C, 45.18; H, 2.63; N, 3.34.

5-Bromo-3-hydroxy-3-(4'-bromophenacyl)oxindole (HPO-8): λ_{max}: 264.20, 345.20 nm; **IR (KBr):** υ = 3471.98 (O-H str), 3365.90 (N-H str), 3082.35 (aromatic C-H str), 2983.98 (CH_2 str), 1701.56 (2 C=O str), 1685.84 (C=O str), 1587.47 (C=C str), 1483.31 (CH_2 bend), 628.81, 588.31 (C-Br str); **^{1}H NMR ([D$_6$]DMSO):** δ = 3.04-3.42 (s, 2H, CH_2), 4.04 (s, 1H, O-H), 6.69-7.56 (m, 3H, oxindole C-H), 7.32-7.98 (m, 4H, Ar'C-H), 7.97 ppm (s, 1H, oxindole N-H); **^{13}C NMR ([D$_6$]DMSO):** δ = 48.50 (-CH_2), 70.32 (oxindole OH), 112.61-142.02 (oxindole C-3a, C-4, C-5, C-6, C-7, C-7a), 128.93-138.38 (Ar C'-1 to Ar C'-6), 175.67 (oxindole C=O), 196.47 ppm (C=O); **Elemental analysis for $C_{16}H_{11}Br_2NO_3$: calcd:** C, 45.21; H, 2.61; N, 3.30, **found:** C, 45.26; H, 2.58; N, 3.26.

5-Bromo-3-hydroxy-3-(4'-chlorophenacyl)oxindole (HPO-9): λ_{max}: 268.20, 336.20 nm; **IR (KBr):** υ = 3363.97 (O-H str), 3275.24 (N-H str), 3080.42 (aromatic C-H str), 2982.05 (CH_2 str), 1703.62 (2 C=O str), 1687.77 (C=O str), 1587.47 (C=C str), 1477.52 (CH_2 bend), 817.85 (C-Cl str), 692.47 (C-Br str); **^{1}H NMR ([D$_6$]DMSO):** δ = 2.01-2.04

(s, 2H, CH_2), 4.23 (s, 1H, O-H), 6.76-7.74 (m, 3H, oxindole C-H), 7.37-7.94 (m, 4H, Ar'C-H), 7.96 ppm (s, 1H, oxindole N-H); **^{13}C NMR ([D$_6$]DMSO):** δ = 48.45 (-CH_2), 60.20 (oxindole C-OH), 102.14-152.02 (oxindole C-3a, C-4, C-5, C-6, C-7, C-7a), 128.69-138.39 (Ar C'-1 to Ar C'-6), 165.71 (oxindole C=O), 194.61 ppm (C=O); **MS** (m/z) = 381.88 $[M+1]^+$; **Elemental analysis for $C_{16}H_{11}BrClNO_3$: calcd:** C, 50.49; H, 2.91; N, 3.68, **found:** C, 50.46; H, 2.94; N, 3.70.

5-Bromo-3-hydroxy-3-(4'-fluorophenacyl)oxindole (HPO-10): λ_{max}: 272.20, 346.80 nm; **IR (KBr):** υ = 3483.56 (O-H str), 3373.61 (N-H str), 3080.42 (aromatic C-H str), 2982.05 (CH_2 str), 1718.63 (2 C=O str), 1685.84 (C=O str), 1597.11 (C=C str), 1467.88 (CH_2 bend), 1031.95 (C-F str), 590.24 (C-Br str); **^{1}H NMR ([D$_6$]DMSO):** δ = 3.28-3.31 (s, 2H, CH_2), 4.25 (s, 1H, O-H), 6.77-7.35 (m, 3H, oxindole C-H), 6.73-7.74 (m, 4H, Ar'C-H), 7.96 ppm (s, 1H, oxindole N-H); **^{13}C NMR ([D$_6$]DMSO):** δ = 45.78 (-CH_2), 73.95 (oxindole C-OH), 111.60-162.54 (Ar C'-1 to Ar C'-6), 112.19-142.63 (oxindole C-3a, C-4, C-5, C-6, C-7, C-7a), 177.37 (oxindole C=O), 194.74 ppm (C=O); **Elemental analysis for $C_{16}H_{11}BrFNO_3$: calcd:** C, 52.77; H, 3.04; N, 3.85, **found:** C, 52.81; H, 3.07; N, 3.90.

5-Bromo-3-hydroxy-3-(4'-hydroxyphenacyl)oxindole (HPO-11): λ_{max}: 277.40, 377.40 nm; **IR (KBr):** υ = 3368.54 (O-H str), 3210.14 (N-H str), 3095.85 (aromatic C-H str), 2970.48 (CH_2 str), 1732.13 (2 C=O str), 1685.84 (C=O str), 1581.68 (C=C str), 1465.95 (CH_2 bend), 648.10 (C-Br str); **^{1}H NMR ([D$_6$]DMSO):** δ = 2.96 (s, 1H, Ar'-OH), 3.46-3.52 (s, 2H, CH_2), 4.03 (s, 1H, O-H), 6.79-7.74 (m, 4H, Ar'C-H), 7.30-7.45 (m, 3H, oxindole C-H), 8.33 ppm (s, 1H, oxindole N-H); **^{13}C NMR ([D$_6$]DMSO):** δ = 45.18 (-CH_2), 73.05 (oxindole C-OH), 111.26-162.75 (Ar C'-1 to Ar C'-6), 112.72-142.31 (oxindole C-3a, C-4, C-5, C-6, C-7, C-7a), 177.97 (oxindole C=O), 194.38 ppm (C=O); **Elemental analysis for $C_{16}H_{12}BrNO_4$: calcd:** C, 53.06; H, 3.34; N, 3.87, **found:** C, 53.02; H, 3.36; N, 3.90.

5-Bromo-3-hydroxy-3-(4'-nitrophenacyl)oxindole (HPO-12): λ_{max}: 279.60, 381.60 nm; **IR (KBr):** υ = 3367.98 (O-H str), 3173.01 (N-H str), 3103.57 (aromatic C-H str), 2985.91 (CH_2 str), 1720.56 (2 C=O str), 1697.41 (C=O str), 1602.90 (C=C str), 1514.17 (CH_2 bend), 1464.02, 1346.36 (NO_2 str), 663.53 (C-Br str); **^{1}H NMR ([D$_6$]DMSO):** δ =

3.26-3.55 (s, 2H, CH_2), 4.07 (s, 1H, O-H), 7.21-7.63 (m, 3H, oxindole C-H), 7.28-7.86 (m, 4H, Ar'C-H), 7.98 ppm (s, 1H, oxindole N-H); **^{13}C NMR ([D$_6$]DMSO):** δ = 45.35 (-CH_2), 72.91 (oxindole C-OH), 120.86-150.76 (Ar C'-1 to Ar C'-6), 121.08-143.58 (oxindole C-3a, C-4, C-5, C-6, C-7, C-7a), 178.17 (oxindole C=O), 195.80 ppm (C=O); **Elemental analysis for $C_{16}H_{11}BrN_2O_5$: calcd:** C, 42.13; H, 2.83; N, 7.16, **found:** C, 42.17; H, 2.88; N, 7.21.

3-Hydroxy-3-(2-oxocyclohexyl)indolin-2-one (HPO-13): λ_{max}: 293.80, 345.20 nm; **IR (KBr):** υ = 3302.24 (O-H str), 3173.01 (N-H str), 3028.34 (aromatic C-H str), 2953.12 (CH_2 str), 1728.28 (2 C=O str), 1681.98 (C=O str), 1616.40 (C=C str), 1471.74 (CH_2 bend); **^{1}H NMR ([D$_6$]DMSO):** δ = 1.95-2.84 (m, 9H, cyclohexyl CH_2), 3.38 (s, 1H, cyclohexyl CH), 4.46 (s, 1H, O-H), 7.83-8.02 (m, 4H, oxindole C-H), 8.25 ppm (s, 1H, oxindole N-H); **^{13}C NMR ([D$_6$]DMSO):** δ = 24.56-61.53 (cyclohexyl -CH_2), 78.96 (oxindole C-OH), 120.97-141.28 (oxindole C-3a, C-4, C-5, C-6, C-7, C-7a), 179.60 (oxindole C=O), 198.90 ppm (cyclohexyl C=O); **Elemental analysis for $C_{14}H_{15}NO_3$: calcd:** C, 68.56; H, 6.16; N, 5.71, **found:** C, 68.51; H, 6.20; N, 5.68.

5-Bromo-3-hydroxy-3-(2-oxocyclohexyl)indolin-2-one (HPO-14): λ_{max}: 263.00, 344.20 nm; **IR (KBr):** υ = 3292.60 (O-H str), 3254.02 (N-H str), 3082.35 (aromatic C-H str), 2943.47 (CH_2 str), 1708.99 (2 C=O str), 1697.41 (C=O str), 1618.33 (C=C str), 1475.59 (CH_2 bend), 688.61 (C-Br str); **^{1}H NMR ([D$_6$]DMSO):** δ = 1.80-2.99 (m, 8H, cyclohexyl CH_2), 3.43 (s, 1H, cyclohexyl CH), 4.37 (s, 1H, O-H), 7.79-7.95 (m, 3H, oxindole C-H), 8.20 ppm (s, 1H, oxindole N-H); **^{13}C NMR ([D$_6$]DMSO):** δ = 21.85-59.78 (cyclohexyl -CH_2), 75.72 (oxindole C-OH), 122.69-144.11 (oxindole C-3a, C-4, C-5, C-6, C-7, C-7a), 176.83 (oxindole C=O), 198.29 ppm (cyclohexyl C=O); **Elemental analysis for $C_{14}H_{14}BrNO_3$: calcd:** C, 51.87; H, 4.35; N, 4.32, **found:** C, 51.84; H, 4.39; N, 4.29.

The IR, ^{1}H NMR, ^{13}C NMR, mass and X-RD spectra of compounds **HPO-4**, **HPO-5**, **HPO-9** and **HPO-10** are presented in **Figure 4.76.** to **Figure 4.90.**

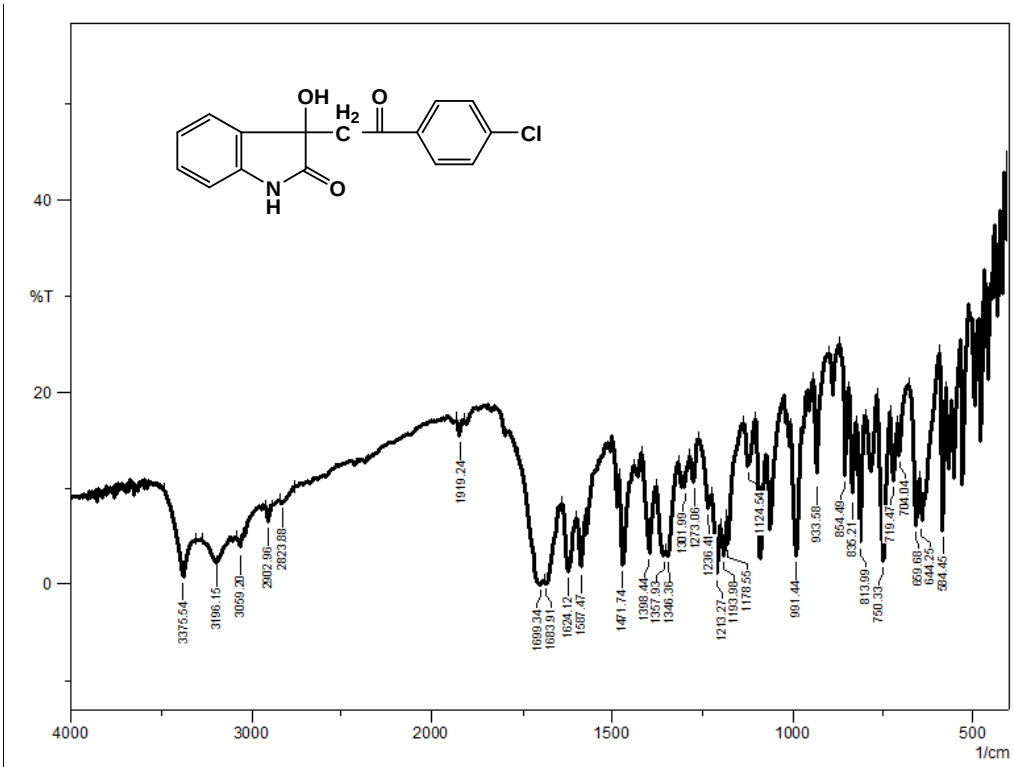


Figure 4.76. IR spectrum of **HPO-4**

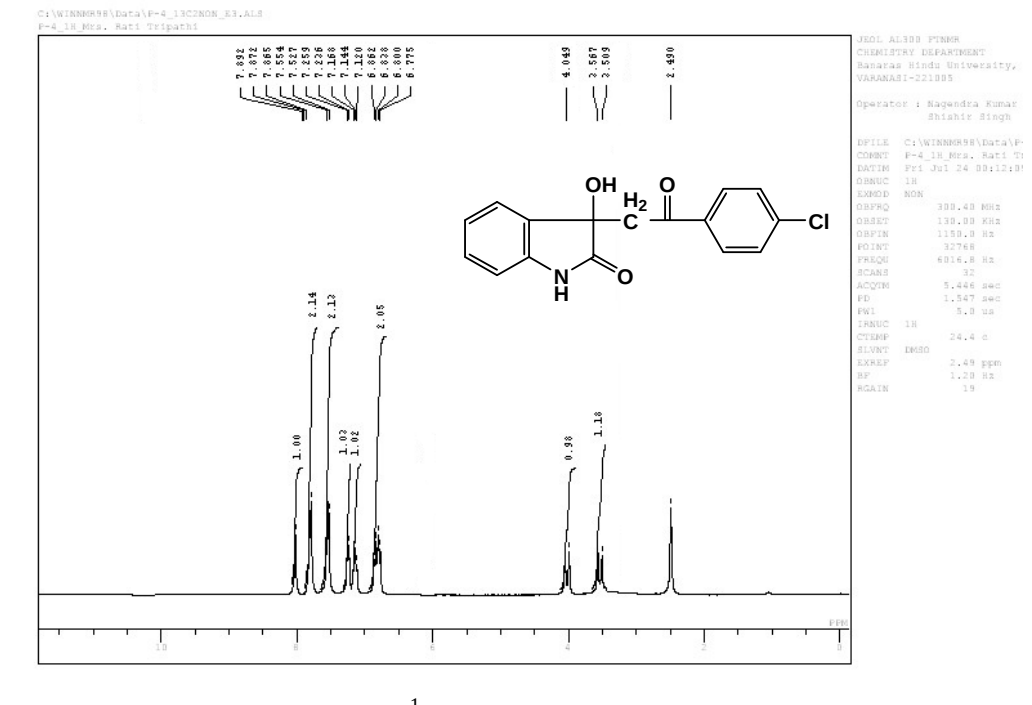


Figure 4.77. ^{1}H NMR spectrum of **HPO-4**

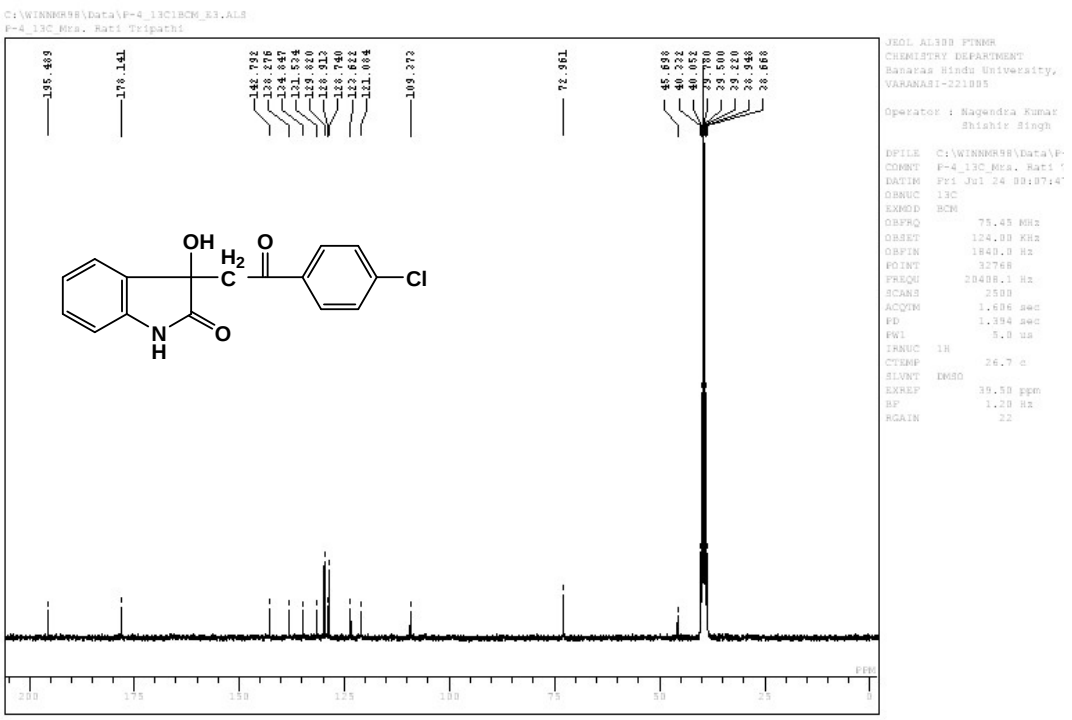


Figure 4.78. ^{13}C NMR spectrum of **HPO-4**

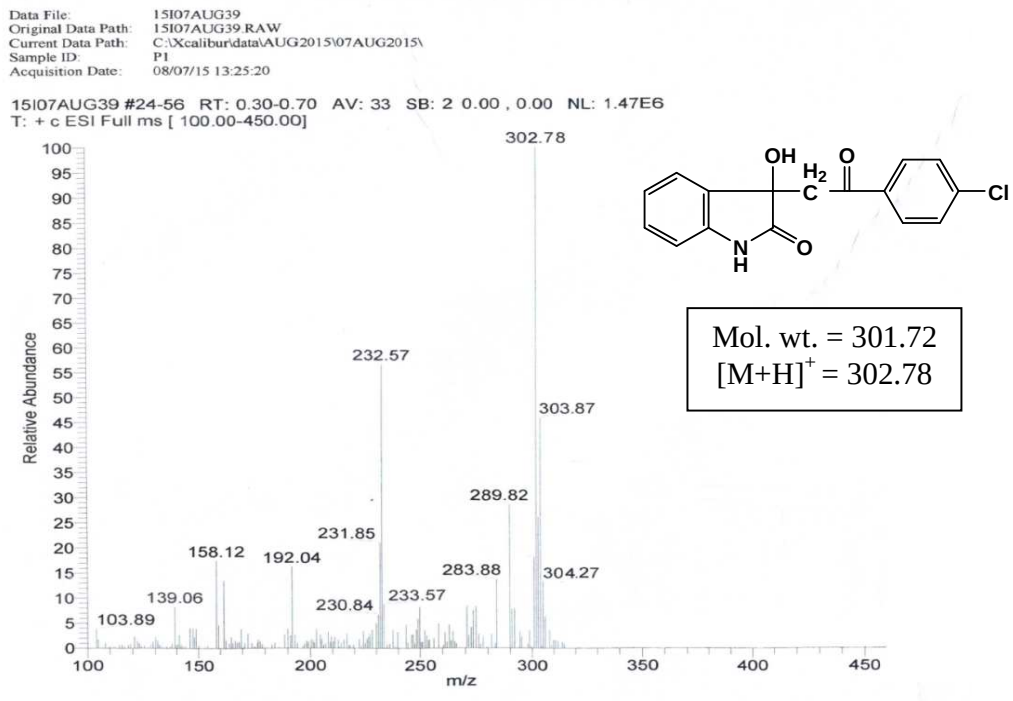


Figure 4.79. Mass spectrum of **HPO-4**

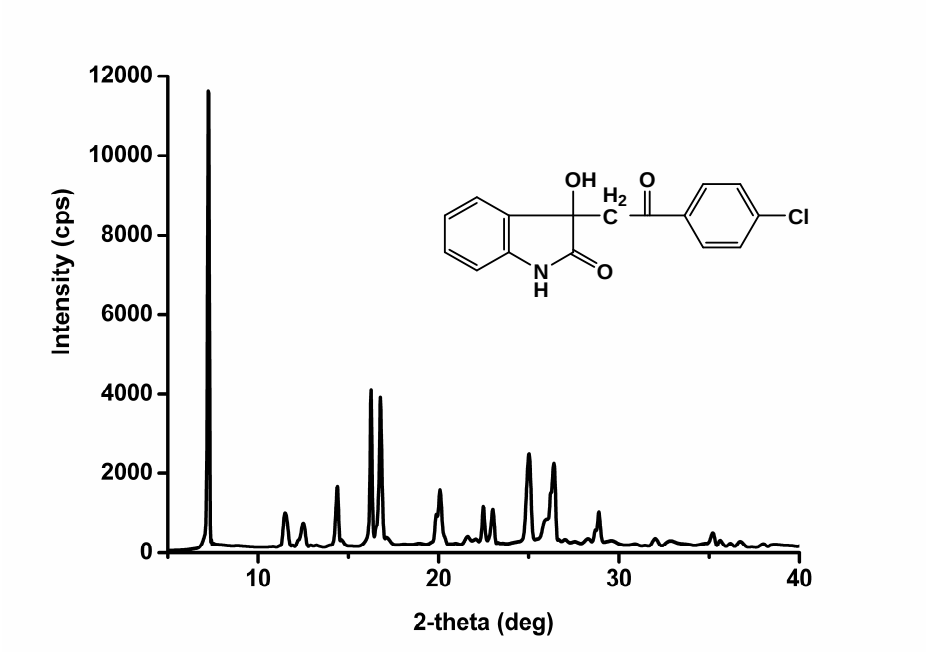


Figure 4.80. XR-PD spectrum of **HPO-4**

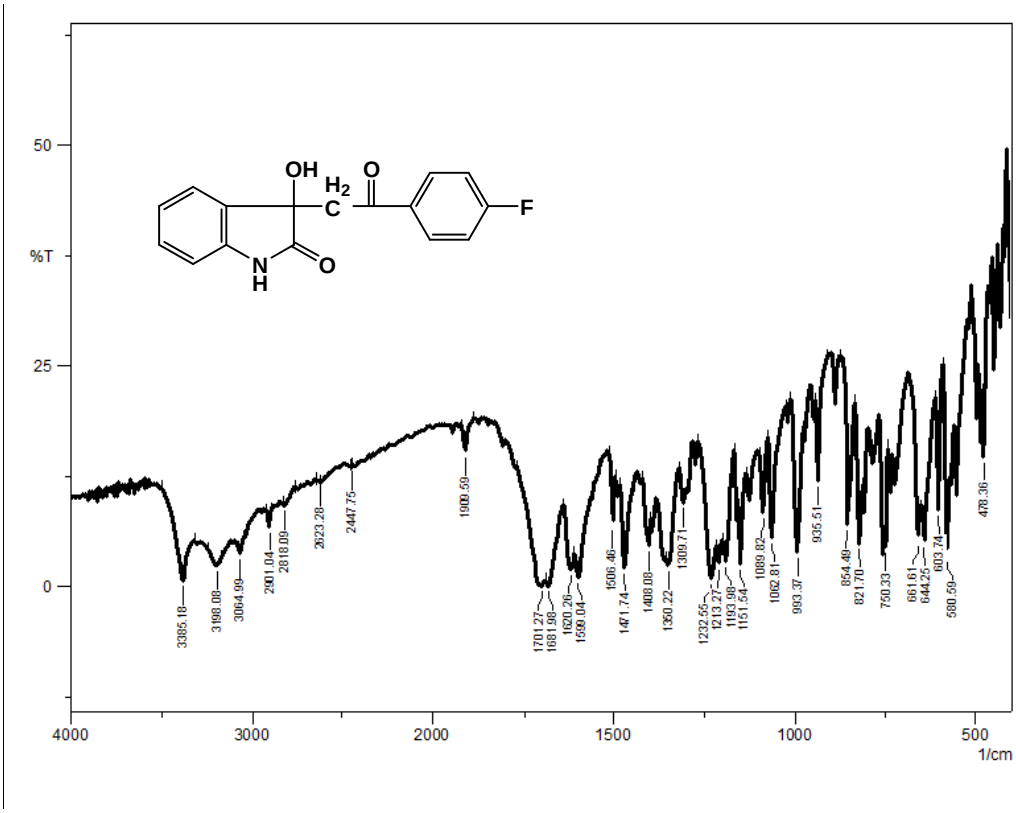


Figure 4.81. IR spectrum of **HPO-5**

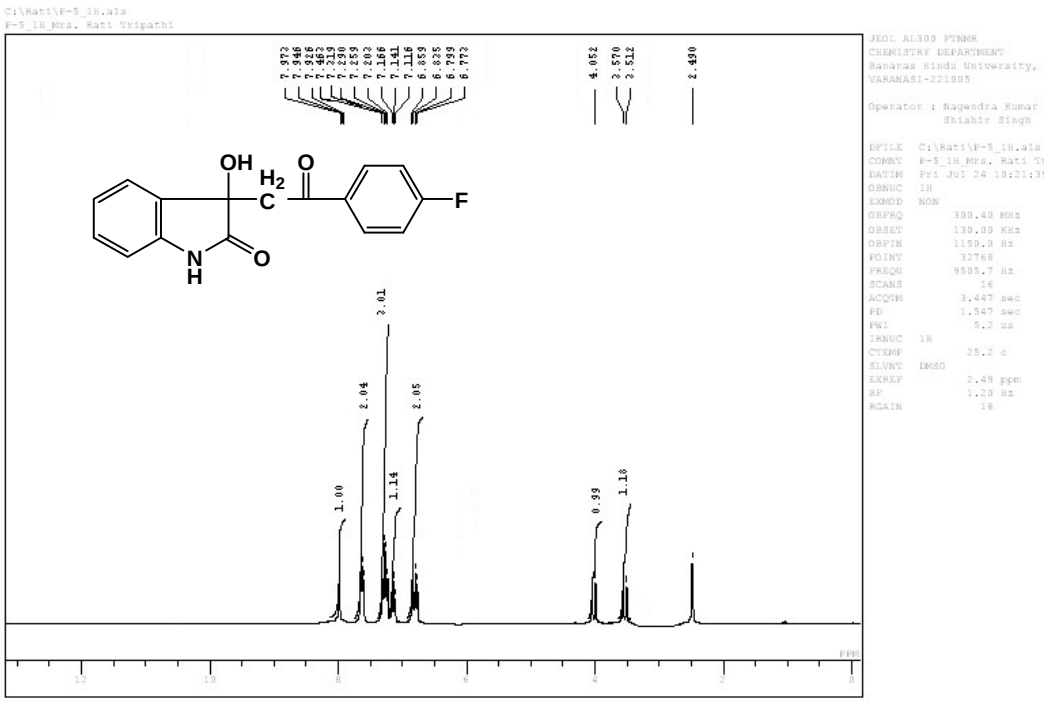


Figure 4.82. ^{1}H NMR spectrum of **HPO-5**

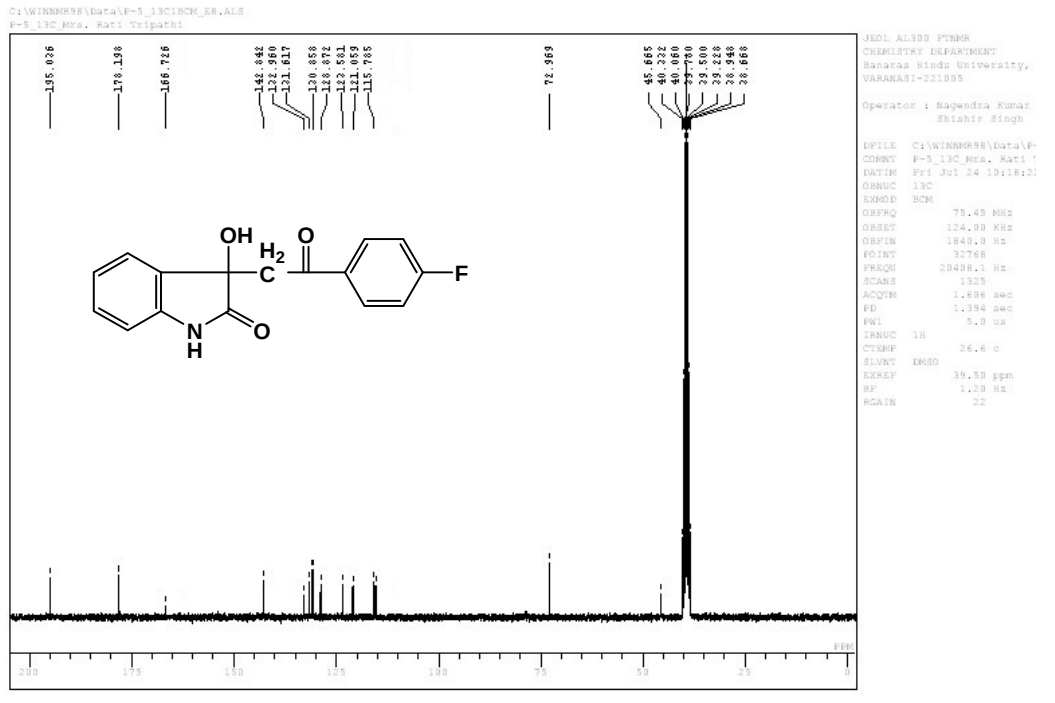


Figure 4.83. ^{13}C spectrum of **HPO-5**

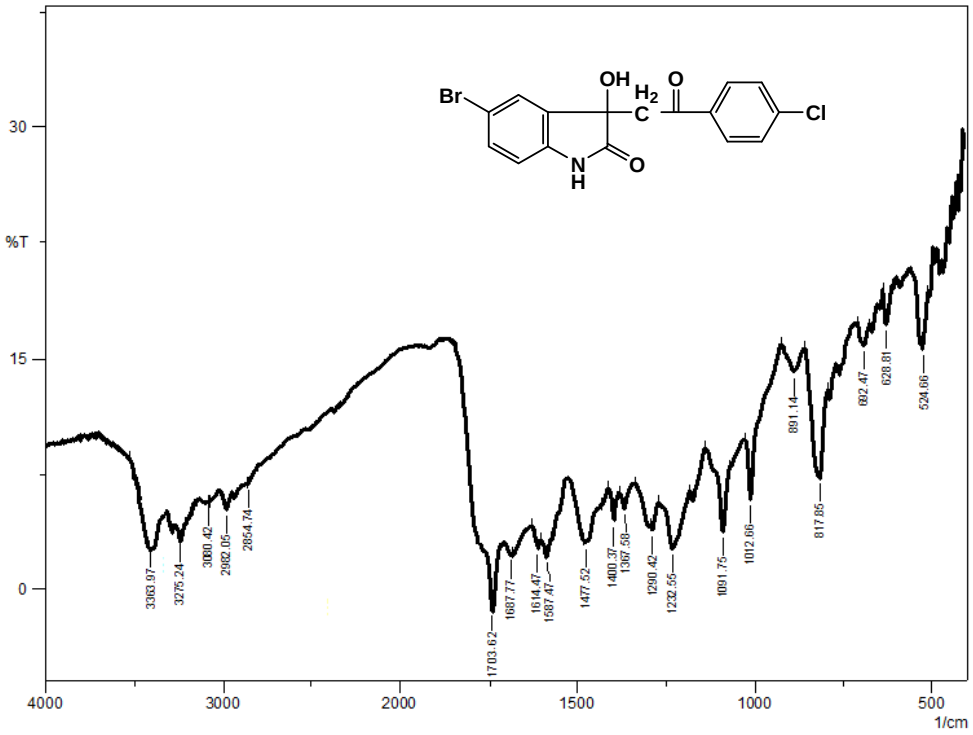


Figure 4.84. IR spectrum of **HPO-9**

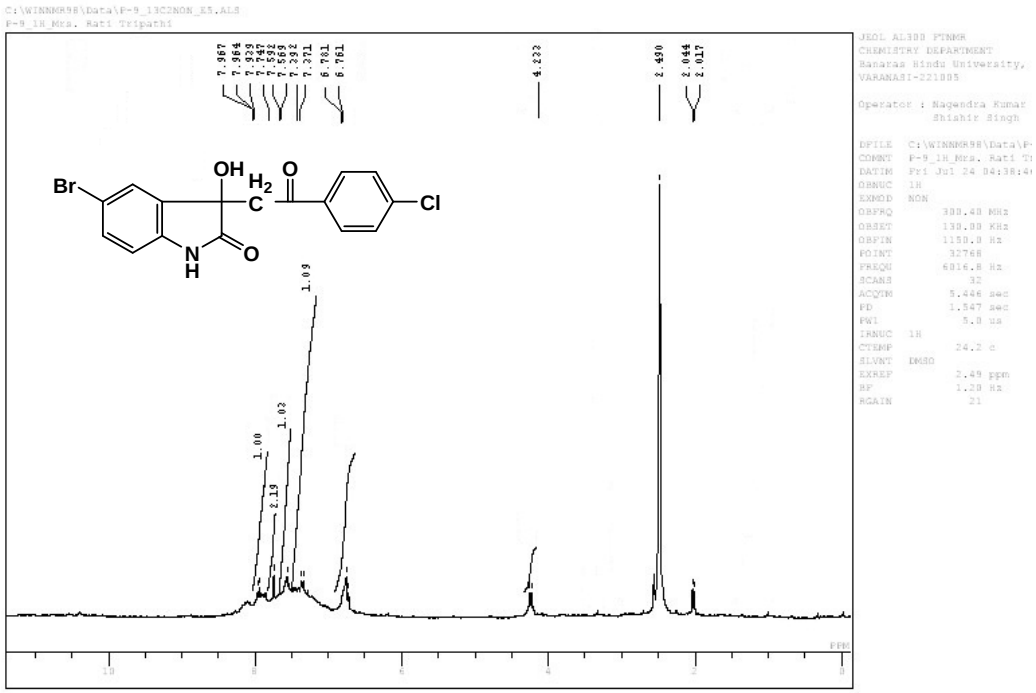


Figure 4.85. ^{1}H NMR spectrum of **HPO-9**

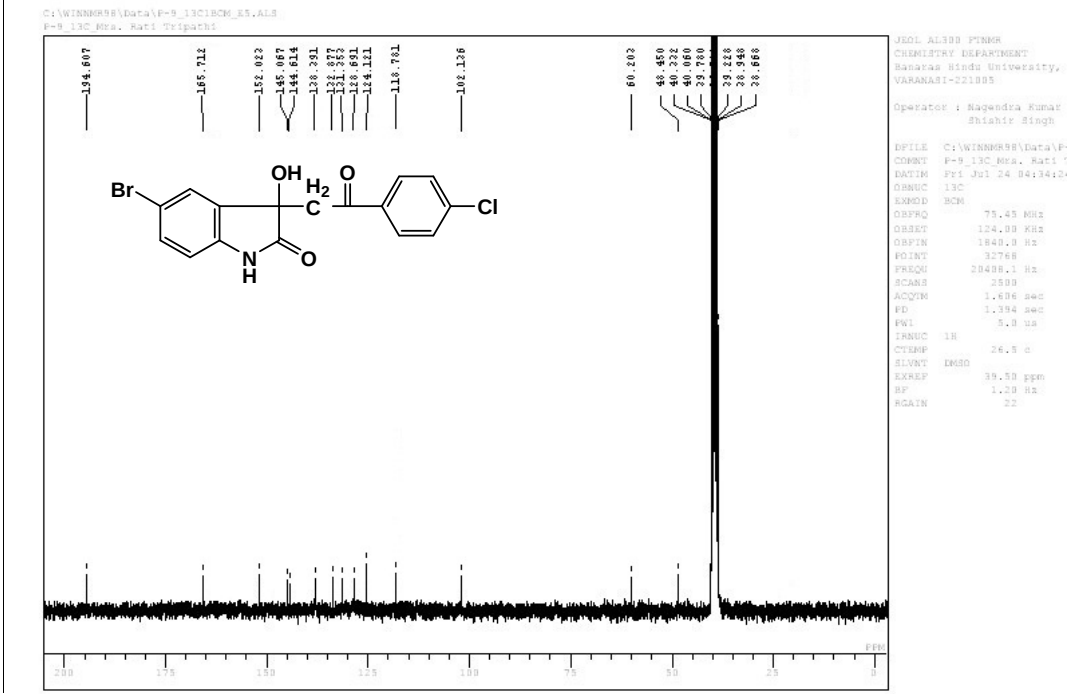


Figure 4.86. ^{13}C NMR spectrum of **HPO-9**

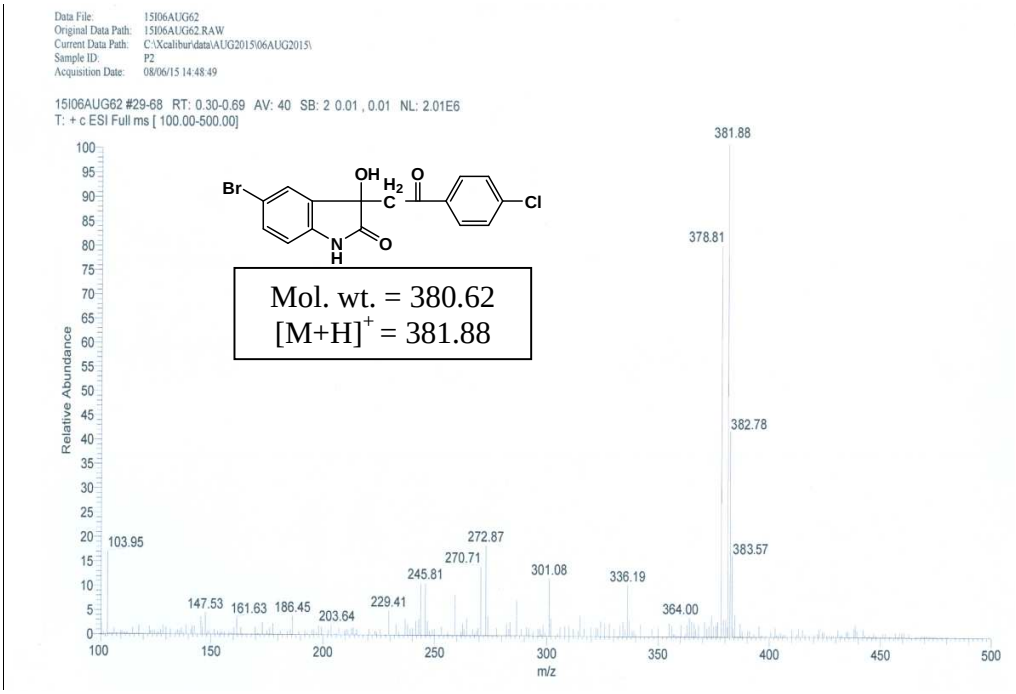


Figure 4.87. Mass spectrum of **HPO-9**

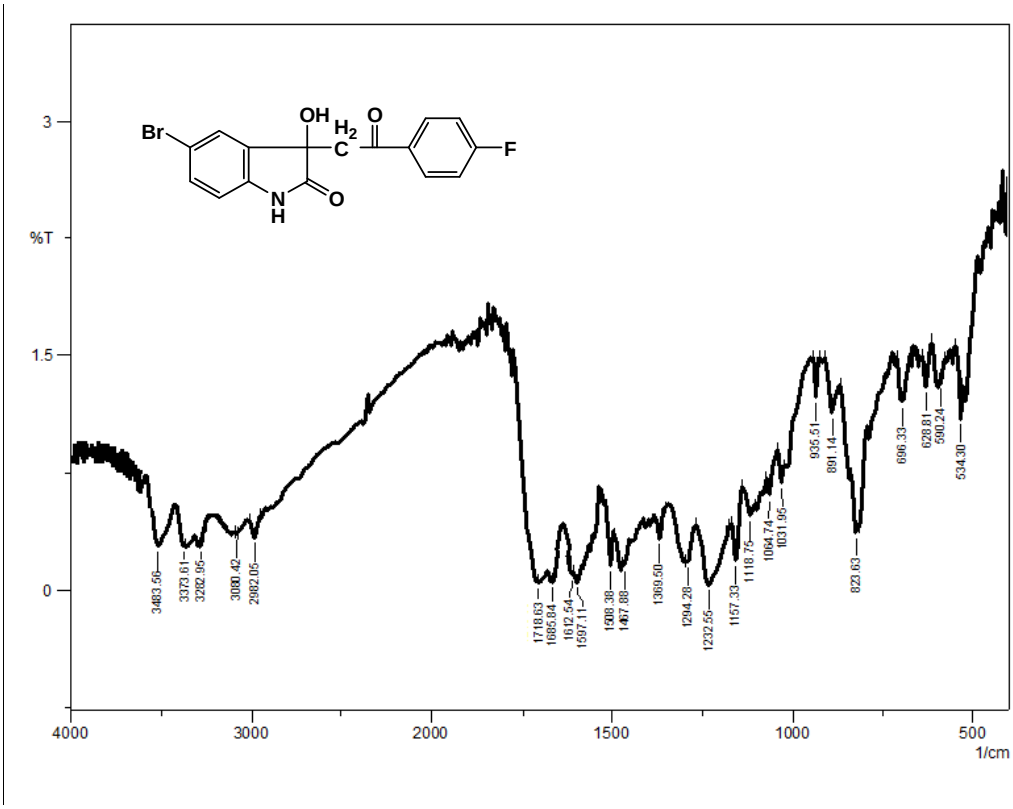


Figure 4.88. IR spectrum of **HPO-10**

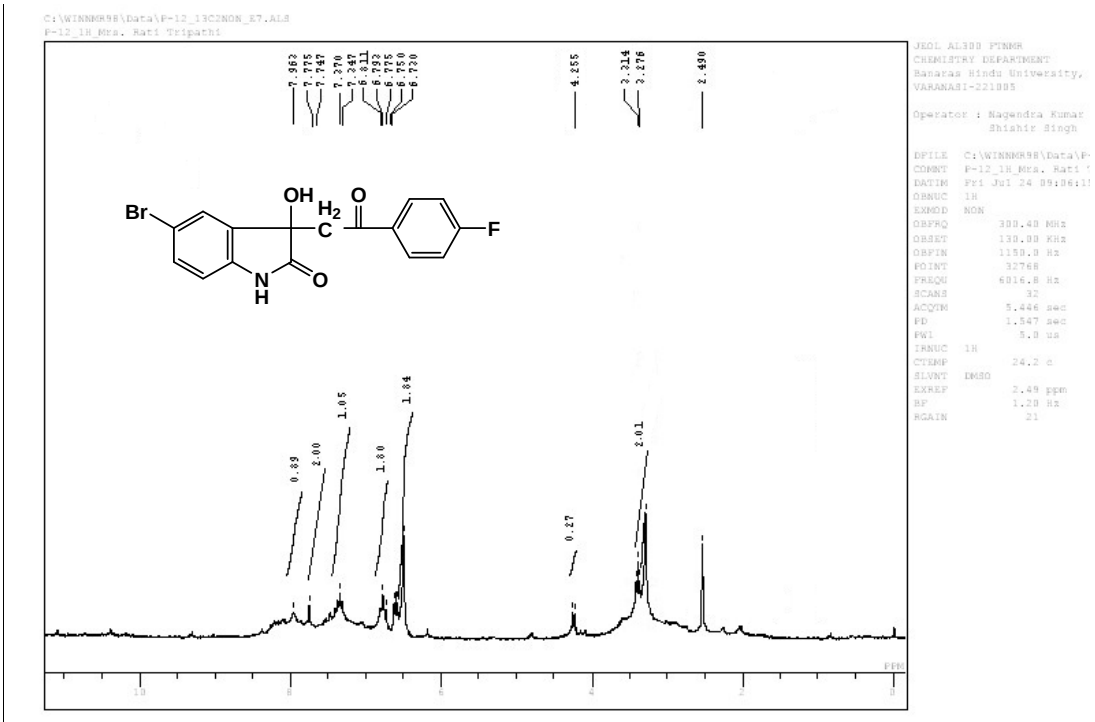


Figure 4.89. ^{1}H spectrum of **HPO-10**

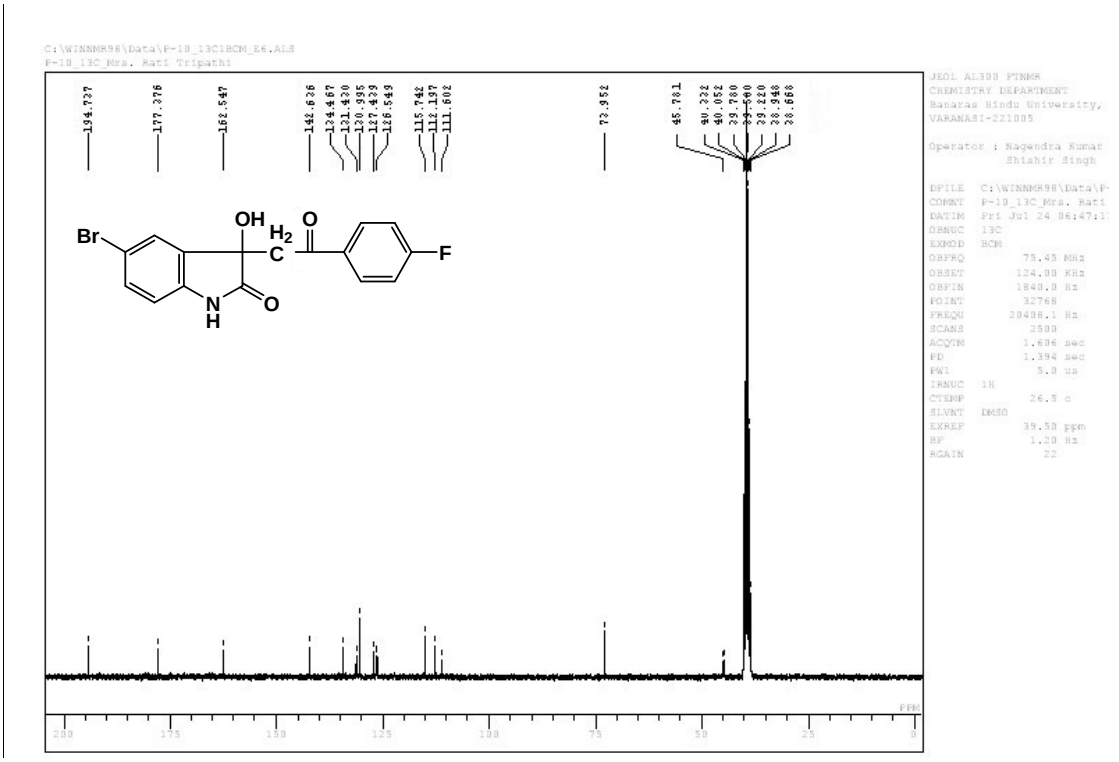


Figure 4.90. ^{13}C spectrum of **HPO-10**

4.4.3. Biological evaluation

The procedures followed for the *in-vitro* and *in-vivo* biological screening of the synthesized compounds (**HPO-1** to **HPO-14**) are summarized in **Section 4.1.3.**

During the MAO inhibition assay, following modification was done under the **Section 4.1.3.1.1.3.** *viz.* 'Protocol for MAO assay':

In case of HPO series, the reaction was stopped by the addition of 1 ml of 3% ice-cold zinc sulphate solution. Subsequently it was mixed by vortexing for 10 seconds and then centrifuged at 3000 rpm for 15 minutes. The supernatant was taken and the respective absorbance was measured for MAO-A and MAO-B estimation.

4.4.4. *In-silico* molecular property analysis and ADMET prediction studies

The procedures followed for *in-silico* molecular property analysis and ADMET prediction studies for all the synthesized compounds (**HPO-1** to **HPO-18**) are described in **Section 4.1.4.**