Chapter 3. Selectivity enhancement for ME3 and in vivo preclinical evaluation

In Chapter 2, using computational tools several new compounds were designed, synthesized and screened in order to establish structure activity relationship (SAR) around compound **A.** The SAR study comprised of varying substitutions on ring-A and ring-B, bioisosteric replacement of phenolic hydroxyl group, replacements of piperazine ring and modifications of the linker. Some critical structural attributes were identified for a potent inhibition of ME3 which culminated in to a novel series of ME3 inhibitors *viz.* **3** and **7**. Further attempts to optimize these molecules were targeted towards enhancing their selectivity for ME3 over other ME isoforms.

3.1 Heterocyclic modifications to enhance selectivity for ME3

Gaining selectivity for ME3 over other ME isoforms was very important for preferential growth inhibition of *SMAD4/ME2* null PDAC cells over normal cells where ME2 is operative. In this regard, replacement of the phenyl ring (ring-A) containing phenolic hydroxyl group with diverse nitrogen containing heterocyclic rings was considered and compounds **28** to **32** were designed (**Figure 3.1**).

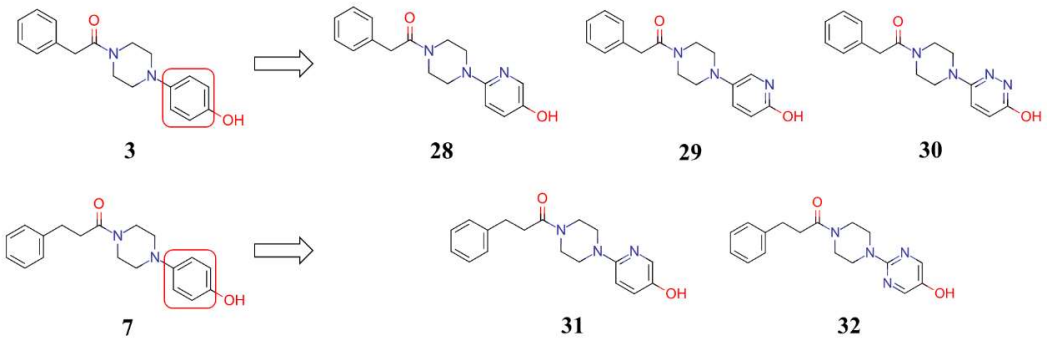


Figure 3.1. Heterocyclic modification of ring-A in compound 3 and 7

Synthesis of these compounds (**28-31)** was undertaken. For their synthesis**,** the maneuvers began with protection of the hydroxyl group of 2-chloro-5-hydroxypyridine with benzyl group to form 5-benzyloxy-2-chloropyridine **V.** The palladium catalyzed cross coupling reaction *viz.* the Buchwald-Hartwig amination (BHA) reaction between **V** and *N*-Boc piperazine yielded the intermediate **VI,** which was deprotected to give the key intermediate **VIII.** Intermediate **VIII**

was condensed with 2-phenylacetic acid and 3-phenylpropionic acid to produce compounds **28** and **31** respectively**.**

While 2-phenylacetic acid was condensed with 5-piperazin-1-ylpyridin-2-ol and 6-piperazin-1-ylpyridazin-3-ol to produce compounds **29** and **30** respectively, 3-phenylpropionic acid was coupled with 2-piperazin-1-ylpyrimidin-5-ol to afford compound **32** (**Refer Section 3.7**).

All synthesized compounds were screened *in vitro* for their inhibitory activity in all ME isoforms and BxPC-3 cells (**Table 3.1)**.

Table 3.1. Effect of heterocyclic modification of ring-A on selectivity for ME3.

Compound No.	**ME3 IC_{50} μM**	**ME2 IC_{50} μM**	**ME1 IC_{50} μM**	**BxPC-3 IC_{50} μM**
3	**0.10**	**0.19**	**0.18**	**6**
28	0.20	0.98	1.60	7.5
29	Inactive	Inactive	Inactive	-
30	Inactive	Inactive	Inactive	-
7	**0.10**	**0.27**	**0.27**	**3.6**
31	0.23	1.72	1.50	5.10
32	Inactive	Inactive	Inactive	-

The results of this study indicated that replacement of 4-hydroxy phenyl ring (compound **3**) with either 2-hydroxy-5-pyridyl (compound **29**) or 3-hydroxy-6-pyridazinyl ring (compound **30**) resulted in complete loss of activity. However, replacement with 3-hydroxy-6-pyridyl ring (compound **28**) retained the activity on ME3 and exhibited 5-fold and 8-fold selectivity over ME2 and ME3 respectively. Similarly, 5-hydroxy-2-pyrimidinyl analogue (compound **32**) of compound **7** was found to be inactive on ME3 while 3-hydroxy-6-pyridyl analogue (compound **31**) retained the potency on ME3 and showed ~7-fold selectivity over ME2 and ME1. Based on inhibitory activity of ME3 enzyme, selectivity over other ME isoforms and BxPC-3 cell growth inhibition, compound **31** was chosen as a new lead for further optimization.

3.2 Lipophilic modification of ring-B in compound 31 to improve ME3 potency

Compound **31** was subjected to docking study with ME3 enzyme and binding mode of compound **31** in malate binding pocket of ME3 showed that ring-B was aligned towards

hydrophobic pocket of ME3 enzyme and phenolic hydroxyl group undergoes H-bond donor-acceptor interactions with Asp304 and Asn489 (**Figure 3.2**).

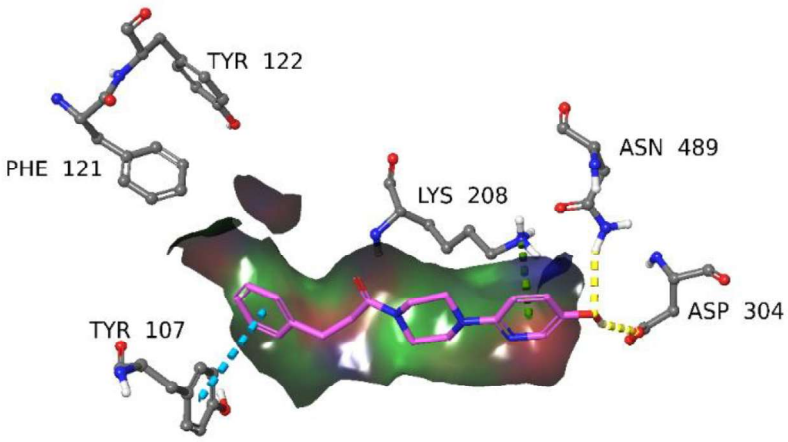
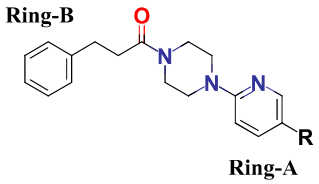


Figure 3.2. Binding mode of compound 31 in malate binding pocket of ME3

Before further optimization of compound **31,** two analogues of compound **31** were designed and synthesized to investigate importance of phenolic hydroxyl group where hydroxy group was replaced with fluoro (compound **31a**) and methoxy (compound **31b**). *in silico* analysis of these compounds revealed that these compounds lacked H-bonding with Asp304/Asn489 of the ME3 enzyme. Synthesized compounds **31a** and **31b** were screened *in vitro* for ME3 inhibition and BxPC-3 cell viability studies (**Table 3.2**).

Table 3.2. The effect of phenolic hydroxyl group replacement in compound **31**



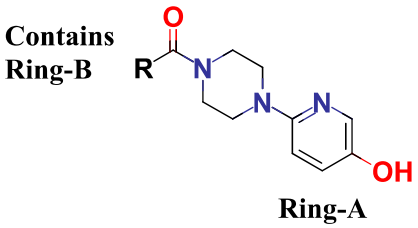
Compound No.	R	% ME3 inhibition (1 µM)	% ME3 inhibition (10 µM)	%BxPC-3 inhibition (10 µM)	BxPC-3 IC_{50} µM
31	**-OH**	**97**	**100**	**92**	**5.10**
31a	-F	00	27	13	-
31b	-OMe	10	39	00	>30

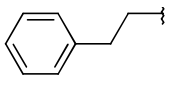
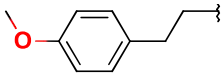
As was expected, replacement of phenolic hydroxyl group in compound **31** with fluoro group (**31a**) and methoxy group (**31b**) resulted in complete loss of activity on ME3 enzyme. Also when screened for BxPC-3 cell growth inhibition, they exhibited a loss of activity, suggesting that the target engagement with ME3 is essential for the inhibitory activity on BxPC-3 cells.

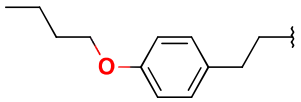
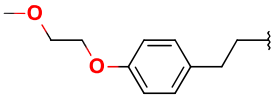
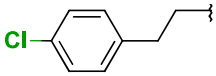
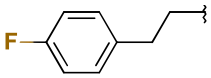
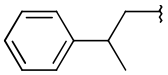
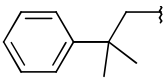
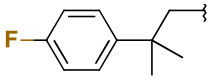
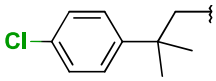
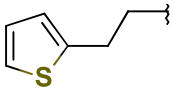
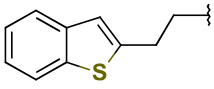
With an intent to improve the potency of compound **31,** various analogues with modifications at ring-B were designed and docked in ME3. Among them compounds with consistent binding modes with ME3 (compounds **33** to **44)** were selected for synthesis. Ring-B of compound **31** which was aligned towards lipophilic pocket of ME3 (**Figure 3.2**) was substituted with lipophilic substituents at *p*- position in designed compounds **33-37** to investigate effect of substitution on ME3 inhibition. Similarly, in **38-41** lipophilic methyl substitutions were incorporated in the linker part of the designed compounds. Phenyl ring (Ring-B) in compound **31** was replaced with its bioisostere thiophene ring to design compound **42** along with the other diversified thiophene analogues **43** and **44**. Appropriately substituted acids were condensed with the key intermediate **VIII** to produce compounds **33-44** (**Refer Section 3.7**).

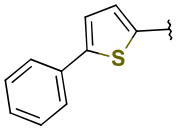
These compounds were screened *in vitro* on ME isoforms and BxPC-3 cells (**Table 3.3**).

Table 3.3. The effect of lipophilic modification of ring-B on ME3 potency



Compound No.	R	ME3 IC_{50} (μM)	ME2 IC_{50} (μM)	ME1 IC_{50} (μM)	% BxPC-3 cell growth inhibition at 10 μM	BxPC-3 IC_{50} (μM)
31		0.23	1.72	1.50	90	5.10
33		0.47	2.50	1.90	13	-

Compound No.	R	ME3 IC_{50} (μM)	ME2 IC_{50} (μM)	ME1 IC_{50} (μM)	% BxPC-3 cell growth inhibition at 10 μM	BxPC-3 IC_{50} (μM)
34		0.39	3.90	1.31	07	15
35		0.58	2.60	3.50	03	-
36		0.14	1.59	1.02	71	6.30
37		0.41	1.66	1.74	48	-
38		0.17	1.49	0.97	71	4.60
39		0.15	1.37	2.37	99	3.50
40		0.31	1.11	1.30	100	5.90
41		0.36	2.80	1.50	97	5.40
42		0.16	1.29	1.32	76	4.60
43		0.24	1.63	1.20	46	-

Compound No.	R	ME3 IC_{50} (μM)	ME2 IC_{50} (μM)	ME1 IC_{50} (μM)	% BxPC-3 cell growth inhibition at 10 μM	BxPC-3 IC_{50} (μM)
44		0.25	1.97	1.80	37	-

The results of *in vitro* screening indicated that compounds with methoxy- (**33**), butyloxy- (**34**), 2-methoxyethoxy- (**35**) and fluoro- (**37**) substitution at para position of ring B failed to exhibit any significant advantage, while chloro substitution (**36**) resulted in an enhanced potency on ME3 compared to compound **31.** Incorporating –methyl or gem-dimethyl groups at benzylic position of ring-B (*cf.* compounds **38** and **39**) resulted in enhanced potency and selectivity for ME3; however, further substitutions at -*para* position of ring B with fluorine or chlorine (compounds **40** and **41**) led to a decreased potency. Bioisosteric replacement of phenyl ring B with a thiophene ring (compound **42**) improved the potency on ME3, while replacement with either benzothiophene or 5-phenylthiophene (compounds **43** and **44**) showed the activity on ME3 similar to that of compound **31**.

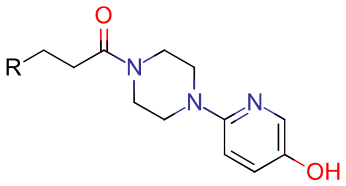
3.3 Investigating role of logP to improve cellular permeation in BxPC-3 cells.

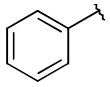
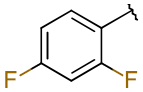
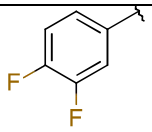
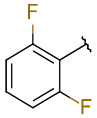
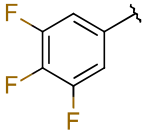
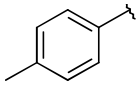
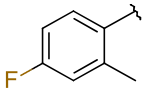
Lipophilicity is also known as hydrophobicity and it plays a crucial role in cellular permeation. It is the ability of a molecule to cross cell membrane and enter cells. It is often estimated by calculating QPlogPo/w **(**predicted octanol/water partition coefficient) using QikProp software. Higher QPlogPo/w (commonly referred as logP) value for a molecule increases its chance to interact with and cross the lipid bilayer of the cell more easily and thereby will enhance its cellular penetration.

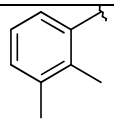
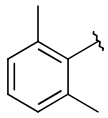
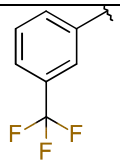
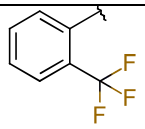
It was aimed to investigate the correlation between higher logP values and cellular permeation in this series of molecules. With an intention to enhance logP and thereby to improve cytotoxicity on BxPC-3 cells, various analogues with lipophilic substituents like fluoro-, methyl- and trifluoromethyl- at different positions (*ortho*-, *meta*- or *para*-) in ring-B of compound **31** were designed (compounds **45-54**). These designed compounds were synthesized by condensation of appropriately substituted phenylpropionic acids with the key intermediate **VIII** (**Refer Section 3.7**).

These compounds were screened *in vitro* on ME isoforms and BxPC-3 cells (**Table 3.4**).

Table 3.4. ME3 inhibition and BxPC-3 cell growth inhibition data for compounds **45-54**



Compound No.	R	% ME3 inhibition		QPlogPo/w*	% growth inhibition of BxPC3 cells (10 μM)	BxPC3 cells IC_{50} (μM)
		1 μM	IC_{50} (μM)			
31		97	0.23	2.51	90	5.10
45		97	0.23	2.79	56	-
46		98	0.22	2.79	51	-
47		97	0.38	2.79	88	6.9
48		89	0.19	2.94	52	-
49		96	-	3.02	54	-
50		95	0.27	3.16	93	9.8

Compound No.	R	% ME3 inhibition		QPlogPo/w*	% growth inhibition of BxPC3 cells (10 μM)	BxPC3 cells IC_{50} (μM)
		1 μM	IC_{50} (μM)			
51		97	0.27	3.53	69	-
52		96	0.32	3.53	96	2.1
53		94	-	3.39	29	-
54		89	-	3.39	31	-

The results of *in vitro* screening revealed that none of the compounds except compound **52** (IC_{50} = 2.1 μM) has better potency on BxPC-3 cells than compound **31** (IC_{50} = 5.1 μM) although ME3 inhibitory potencies of all these newly synthesized compounds were comparable with compound **31**. It is important to note that no significant correlation is observed between increased logP values and cellular inhibition in the present cases.

3.4 Safety evaluation of selected compounds by screening on non-oncogenic cells.

Selected compounds which exhibited a good inhibitory potency on BxPC-3 cell growth were screened on non-cancerous cell lines like HCE-T (Human corneal epithelial cell- transformed) and HUVEC (Human Umbilical Vein Endothelial Cells) to evaluate safety margins (**Table 3.5**).

Table 3.5. Cell growth inhibition data for selected compounds on non-oncogenic cells and BxPC-3 cells.

Compound	Cell lines		
	Oncogenic (PDAC)	Non-oncogenic	
	BxPC-3 IC_{50} (μM)	HCE-T IC_{50} (μM)	HUVEC IC_{50} (μM)
7	3.6	>30	NA
20	5.0	>30	~30
31	5.1	>30	~30
39	3.5	>30	NA
36	6.3	>30	NA
40	5.9	>30	NA
41	5.4	>30	NA
42	4.6	~25	NA

These compounds were found to have 5-6-fold selectivity for BxPC-3 cells over HCE-T cells (**Table 3.5**). Compound **20** and **31** were screened on HUVEC and were also found to be selective toward BxPC-3 over HUVEC. These compounds showed targeted inhibition of cancerous BxPC-3 cells over non-cancerous cells hence could provide 5-6-fold safety margins in *in vivo* testing.

3.5 *in vitro* mechanism of action study and target engagement study for compound 31

At one stage of this development, Compound **31** was selected as a tool compound for *in vivo* xenograft testing based on its biochemical and cellular toxicity data. Before *in vivo* screening of compound **31**, it was essential to study its mode of inhibition and target engagement with ME3.

Keeping in view that ME3 has two substrates, i.e., L-malate and $NADP^+$, the mode of action study was performed. The results indicated that compound **31** was a competitive inhibitor referring to L-malate and was an uncompetitive inhibitor with respect to $NADP^+$. Detailed experimental procedures and analytical data on this aspect are presented in Chapter **6**.

In order to ascertain the target (ME3) engagement by compound **31**, a cell-based thermal shift assay (CETSA) was performed using the BxPC-3 cells (*ME2*$^{-/-}$ pancreatic cancer cell line).[24,25] It was observed that compound **31** improved the thermal stability of ME3 in a dose dependent manner as a consequence of binding to ME3. A *T*m shift of nearly 5 °C was observed with live cells in the presence of compound **31** (**Figure 3.3**). Details of the related experimental procedures and analytical data are provided in Chapter **6**.

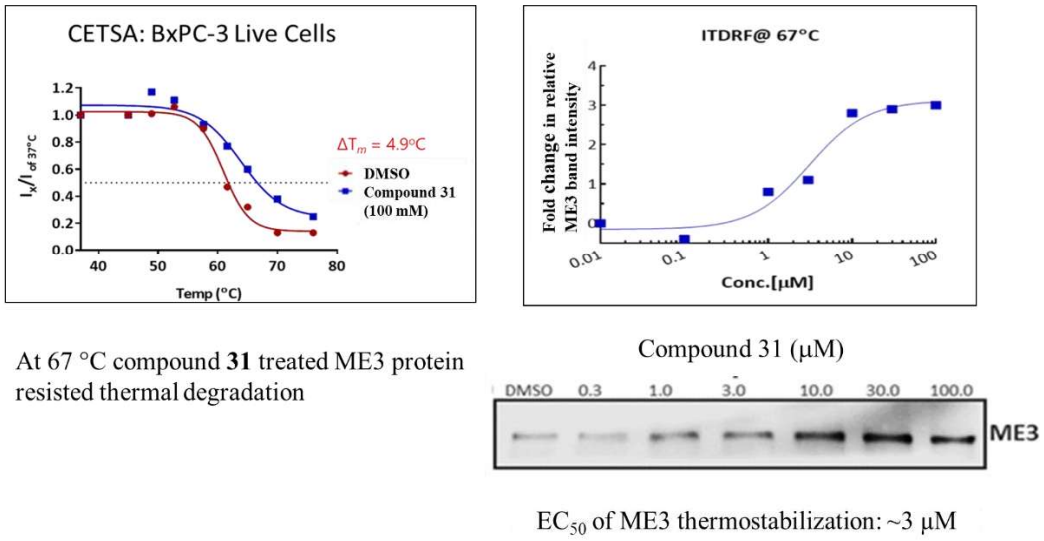


Figure 3.3. ME3 target engagement study for compound 31

3.6 *in vivo* pharmacokinetic (PK) and pharmacodynamic (PD) evaluation of compound 31

Compound **31** was selected as a tool compound for *in vivo* screening to evaluate safety and efficacy for this series of compounds. Hydrochloride salts of compound **31** were prepared (**Refer Section 3.7**) which exhibited very good water solubility. Compound **31-DH** (Dihydrochloride salt of compound **31**) showed water solubility of 50 mg/ml. When refluxed in isopropyl alcohol, compound **31-DH** got converted to its monohydrochloride salt by losing weaker HCl salt with pyridine nitrogen *viz.* compound **31-H** with a greater solubility in water (100 mg/ml). That was another reason of selecting compound **31** which helped to achieve higher dose concentrations in *in vivo* safety and efficacy studies.

3.6.1 *in vivo* pharmacokinetic (PK) profile

Before testing in *in vivo* xenograft studies, compound **31** and its dihydrochloride salt **31-DH** were investigated for *in vivo* pharmacokinetic characteristics. These compounds were administered intraperitoneally (ip) at 50 and 100 mg/kg or orally (po) at 200 mg/kg in Athymic

Nude mice. The pharmacokinetic parameters of **31** and **31-DH** are presented in **Table 3.6**. Both the molecules possessed acceptable pharmacokinetic properties.

Table 3.6. PK parameters of compound 31 and compound 31-DH in nude mice (N=3)

Compound	**Route of administration**	**Dose mg/kg**	**t_{max}(h)**	**C_{max} (ng/mL)**	**AUC_{0-t} (ng.h/mL)**	**$t_{1/2}$ (h)**
31	ip	50	0.08	13723	3873	8.5
31	ip	100	0.08	15509	6016	7.1
31	po	200	0.30	7430	3719	1.9
31-DH	po	200	0.25	12256	8338	1.4

3.6.2 *in vivo* anti-tumour activity evaluation

Compound **31** was evaluated as a tool compound in BxPC-3 subcutaneous xenograft model established in nude mice for its *in vivo* antitumor potency. It was administered at doses 50, 100 and 200 mg/kg [ip, q.d. (once a day)] for 37 days which resulted in 48%, 55% and 62% tumor growth inhibition (TGI), respectively. In comparison, current standard of care Gemcitabine, at a dose of 25 mg/kg [ip, q4d (every 4 days)] only resulted in 17% TGI. Similarly Nab Paclitaxel, at a dose of 20 mg/kg (ip, q4d) exhibited 41% TGI. Anti tumor effect of compound **31** was visualized by the tumor growth curve in **Figure 3.4**. No body weight loss was observed for tumor bearing mice used for this study, which proved good safety profile of compound **31**.

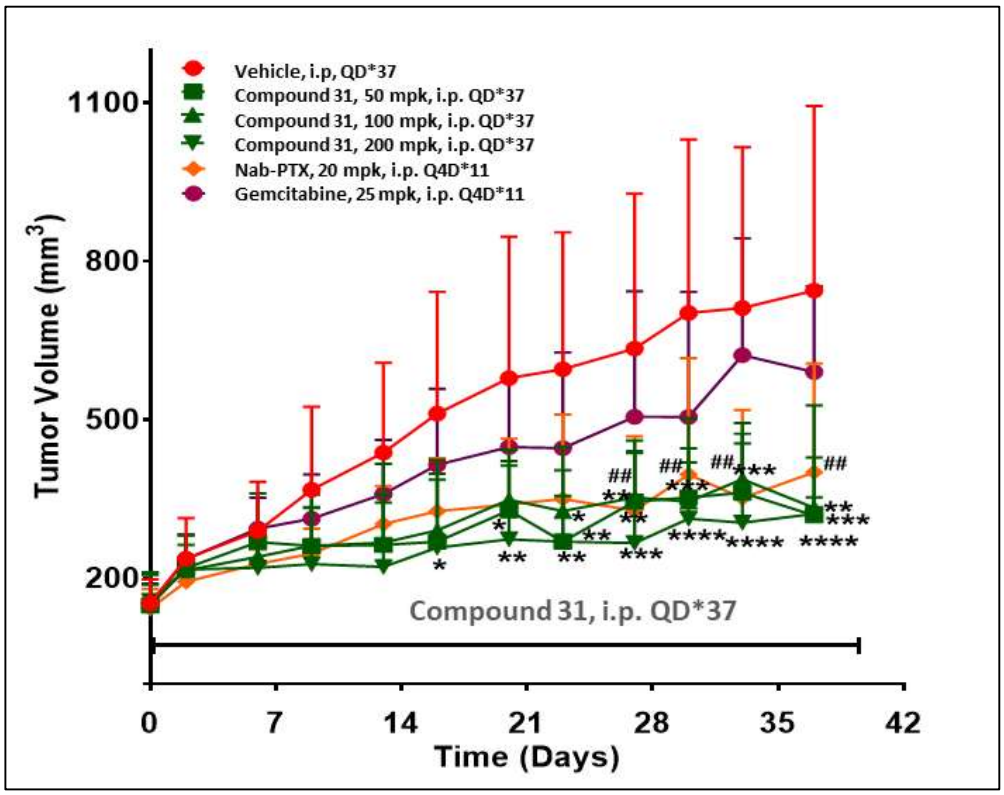


Figure 3.4. Growth inhibition of BxPC-3 xenografts in mice

3.7 Chemistry

3.7.1 General information

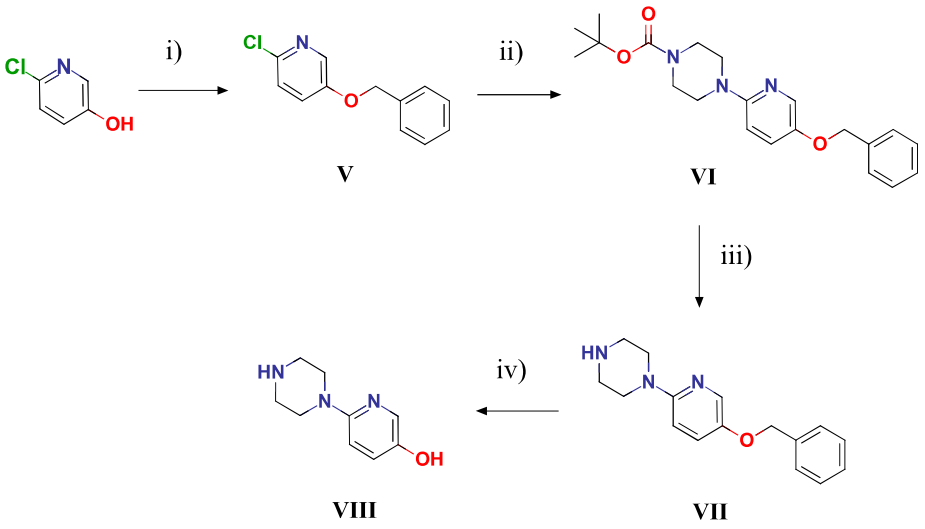
All commercial reagents and anhydrous solvents were purchased and used without further purification, unless otherwise specified. Mass spectra (MS) were obtained on a Quattro premier Waters using electrospray ionization (ESI) in positive mode unless otherwise indicated. Calculated (calcd.) mass corresponds to the exact mass. ^{1}H-NMR and ^{13}C-NMR spectra were recorded on Bruker NMR spectrometers (400 MHz and 500 MHz). Explanation of conventional multiplicity symbols is as follows: s = singlet, d = doublet, t= triplet, q= quartet, dd = doublet of doublets, m = multiplet, br = broad. ^{1}H NMR chemical shifts are expressed in parts per million (δ) downfield from tetramethylsilane as a standard. ^{13}C NMR chemical shifts are expressed in parts per million (δ) downfield from tetramethylsilane as a standard. All the compounds sent for biological tests were confirmed with purity nearby 95% or more in quantitative HPLC analysis. IR spectra were obtained on a Perkin Elmer FTIR spectrometer, with major absorption bands reported.

3.7.2 Experimental procedures and spectral data for compounds

1) Synthesis of key intermediate 6-(piperazin-1-yl)pyridin-3-ol (intermediate VIII)

These newly designed compounds were synthesized using key intermediate **VIII**. Synthesis of the same is depicted in **scheme 3.1**.

Scheme 3.1: Synthesis of key intermediate **VIII**.



Reagents and conditions: (i) N,N-Dimethylformamide, powdered K_2CO_3, benzyl bromide, 25 °C, 4 h, 90%; (ii) *N*-Boc piperazine, sodium *tert*-butoxide, *tetrakis*-(triphenylphosphine) palladium(0), s-phos, toluene, 110 °C, 3 h, 69%; (iii) 1,4-Dioxane, conc. HCl, 25 °C, 3 h, 96%; (iv) 5% Pd on carbon, H_2, Tetrahydrofuran, Methanol, 25 °C, 8 h, 88%.

Step-1: Synthesis of 5-benzyloxy-2-chloro-pyridine (V)

To a solution of 2-chloro-5-hydoroxypyridine (10 g, 77.19 mmol) in N,N-dimethylformamide (50 ml) was added potassium carbonate (15.97 g, 115.79 mmol) and benzyl bromide (9.62 ml, 81.05 mmol) drop wise and stirred at room temperature for 4 hours. On completion of reaction, reaction mixture was quenched with water (250 ml) and extracted with ethyl acetate (2 x 100 ml). Combined organic extracts were washed with water followed by brine, dried over anhydrous sodium sulfate and concentrated under reduced pressure. The residue was purified using column chromatography on silica gel using ethyl acetate - hexane mixture as eluent to afford 5-benzyloxy-2-chloro-pyridine (**V**) (15.10 g, 68.73 mmol, 90% yield) as an off white solid.

^{1}H NMR (400 MHz, DMSO-d$_6$): δ 5.24 (s, 2H), 7.40 (t, 1H, J = 7.16 *Hz*), 7.44 – 7.48 (m, 3H), 7.50 – 7.52 (m, 2H), 7.60 (dd, 1H, J_1 = 8.74 *Hz*, J_2 = 3.09 *Hz*), 8.25 (d, 1H, J = 3.02 *Hz*).

LCMS (ESI$^+$): calculated for $C_{12}H_{10}ClNO$ $[M+H]^+$ 220.05; found: *m/z* = 220.01.

Step-2: Synthesis of *tert*-butyl 4-(5-benzyloxy-2-pyridyl)piperazine-1-carboxylate (VI)

To a solution of 5-benzyloxy-2-chloro-pyridine (**V**) (10g, 45.52 mmol) in toluene (50 ml) was added 1-Boc- piperazine (8.48 g, 45.52 mmol), *tetrakis*(triphenylphosphine)palladium(0) (2.63 g, 2.27 mmol) and s-phos (1.86 g, 4.55 mmol) at room temperature. The resultant reaction mixture was heated to 50°C for 10 minutes and then was added sodium *tert*-butoxide (13.12 g, 136.57 mmol) portion wise and further heated at 110 °C for 3 hours. On completion of reaction, water (30 ml) and ethyl acetate (20 ml) was added to reaction mixture and stirred for 10 min. The mixture was passed through celite bed and bed was washed with ethyl acetate (2 x 20 ml). brine solution (30 ml) was added to filtrate and organic layer was separated. Aqueous layer was further extracted with ethyl acetate (50 ml). Combined organic extracts were washed with water followed by brine, dried over anhydrous sodium sulfate and concentrated under reduced pressure. The residue was purified using column chromatography on silica gel using ethyl acetate:hexane mixture as eluent to afford compound *tert*-butyl 4-(5-benzyloxy-2-pyridyl)piperazine-1-carboxylate (**VI**) (11.70 g, 31.66 mmol, 69% yield) as an off white solid.

^{1}H NMR (400 MHz, DMSO-d$_6$): δ 1.47 (s, 9H), 3.38 (m, 4H), 3.46 (m, 4H), 5.12 (s, 2H), 6.88 (d, 1H, *J* = 9.14 *Hz*), 7.36 – 7.41 (m, 2H), 7.44 (t, 2H, *J* = 7.43 *Hz*), 7.48 (d, 2H, *J* = 7.23 *Hz*), 7.99 (d, 1H, *J* = 2.82 *Hz*).

LCMS (ESI$^+$): calculated for $C_{21}H_{27}N_3O_3$ $[M+H]^+$ 370.21; found: *m/z* = 370.21.

Step-3: Synthesis of 1-(5-benzyloxy-2-pyridyl)piperazine (VII)

To a solution of *tert*-butyl 4-(5-benzyloxy-2-pyridyl)piperazine-1-carboxylate (**VI**) (5g, 13.53 mmol) in 1,4-dioxane (20 ml) was added conc. hydrochloric acid (20 ml) drop wise and resultant reaction mixture was further stirred for 3 hours. After completion of reaction, reaction mixture was quenched in saturated aqueous sodium bicarbonate solution (150 ml). Precipitated solid was filtered, washed with water (2 x 10 ml) and dried to afford compound 1-(5-benzyloxy-2-pyridyl)piperazine (**VII**) (3.50 g, 12.99 mmol, 96% yield) as a light brown solid.

^{1}H NMR (400 MHz, DMSO-d$_6$): δ 2.83 (m, 4H), 3.32 (m, 4H), 5.11 (s, 2H), 6.83 (br-s, 1H), 7.36 – 7.39 (m, 2H), 7.43 (t, 2H, *J* = 7.41 *Hz*), 7.48 (d, 2H, *J* = 7.21 *Hz*), 7.98 (d, 1H, *J* = 2.35 *Hz*).

LCMS (ESI$^+$): calculated for $C_{16}H_{19}N_3O$ $[M+H]^+$ 270.15; found: *m/z* = 270.12.

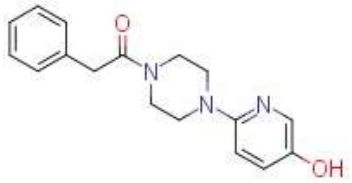
Step-4: Synthesis of 6-(piperazin-1-yl)pyridin-3-ol (VIII)

To a solution of 1-(5-benzyloxy-2-pyridyl)piperazine (**VII**) (3.5 g, 12.99 mmol) in methanol:tetrahydrofuran (1:1) (35 ml) was added palladium on activated carbon (0.35 g, 5% by weight, 50% wet) and the suspension was stirred under hydrogen pressure (2 kg) for 8 hours at room temperature. On completion of reaction, the reaction mixture was filtered off from the catalyst and the solution was evaporated under reduced pressure to afford compound 6-piperazin-1-yl-pyridin-3-ol (**VIII**) (2.10 g, 11.71 mmol, 88% yield) as a light brown solid.

^{1}H NMR (400 MHz, DMSO-d$_6$): δ 2.82 (t, 4H, *J* = 4.88 *Hz*), 3.23 (t, 4H, *J* = 4.91 *Hz*), 6.72 (d, 1H, *J* = 8.98 *Hz*), 7.10 (dd, 1H, J_1 = 8.93 *Hz*, J_2 = 2.94 *Hz*), 7.77 (d, 1H, *J* = 2.83 *Hz*), 9.09 (br-s, 1H).

LCMS (ESI$^+$): calculated for $C_9H_{13}N_3O$ $[M+H]^+$ 180.11; found: *m/z* = 180.05.

2) Synthesis of 1-[4-(5-hydroxypyridin-2-yl)piperazin-1-yl]-2-phenyl ethan-1-one (28)



28

To a solution of phenylacetic acid (0.25 g, 1.83 mmol) in N,N-dimethylacetamide (10 ml) was added 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC·HCl) (0.52 g, 2.75 mmol). The reaction mixture was stirred for 10 minutes before adding the key intermediate **VIII** (0.33 g, 1.83 mmol). To the resultant reaction mixture, was added 4-(dimethylamino)pyridine (0.022 g, 0.18 mmol) and was further stirred at room temperature for 3 hours. On completion of reaction, the reaction mixture was quenched with water and extracted with ethyl acetate. The combined organic extracts were washed with water followed by brine, dried over anhydrous sodium sulfate and concentrated under reduced pressure. The resultant residue was purified using column chromatography on silica gel (230–400 mesh) using ethyl acetate – hexane mixture as eluent to afford compound **28** (0.3 g, 1.00 mmol, 54% yield) as an off white solid.

Melting point: 145-148 °C. **LC purity (UV 245 nm):** 99.64%.

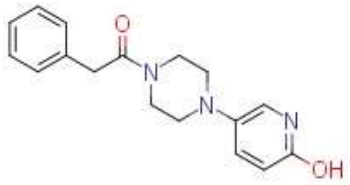
^{1}H NMR (400 MHz, DMSO-d$_6$): δ 3.25 (m, 2H), 3.30 (m, 2H), 3.62 (d, 4H, *J* = 3.16 Hz), 3.81 (s, 2H), 7.10 (d, 1H, *J* = 2.43 Hz), 7.12 (d, 1H, *J* = 2.46 Hz), 7.29 (t, 3H, *J* = 7.32 Hz) , 7.36 (t, 2H , *J* = 7.25 Hz), 7.78 (d, 1H, *J* = 2.27 Hz), 9.09 (s, 1H).

^{13}C NMR (100 MHz, DMSO-d$_6$): δ 40.5 (CH_2), 41.3 (CH_2), 45.5 (CH_2), 46.4 (CH_2), 46.7 (CH_2), 109.1 (CH), 125.8 (CH), 126.8 (CH), 128.8 (2 x CH), 129.4 (2 x CH), 134.6 (CH), 136.3 (C), 147.2 (C), 153.6 (C), 169.3 (C).

IR (ATR) / cm^{-1}: 3301, 1639, 1578, 1487, 1429, 1262, 1233, 1163, 1035, 810, 735, 700, 643.

LCMS (ESI$^+$): calculated for $C_{17}H_{19}N_3O_2$ $[M+H]^+$ 298.15; found: *m/z* = 298.12.

3) Synthesis of 1-[4-(6-hydroxy-3-pyridyl)piperazin-1-yl]-2-phenyl-ethanone (29)



29

Phenylacetic acid (0.15 g, 1.10 mmol) was reacted with commercially available 5-piperazin-1-ylpyridin-2-ol (0.19 g, 1.10 mmol) as per procedure described for compound **28** to afford compound **29** (0.20 g, 0.67 mmol, 61% yield) as an off white solid.

Melting point: 173-176 °C. **LC purity (UV 245 nm):** 99.57%.

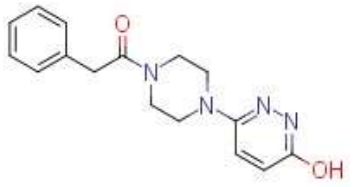
^{1}H NMR (400 MHz, DMSO-d$_6$): δ 2.96 - 2.98 (m, 4H), 3.68 (m, 4H), 3.81 (s, 2H), 6.69 (d, 1H, *J* = 9.7 *Hz*), 7.21 (s, 1H), 7.26 -7.30 (m, 3H), 7.35 – 7.38 (m, 2H), 7.78 (dd, 1H, J_1 = 9.73 *Hz*, J_2 = 3.00 *Hz)*.

^{13}C NMR (100 MHz, DMSO-d$_6$): δ 39.4 (CH_2), 39.6 (CH_2), 43.9 (CH_2), 49.2 (CH_2), 49.7 (CH_2), 116.34 (CH), 123.20 (C), 125.9 (CH), 127.8 (2 x CH), 128.5 (2 x CH), 135.2 (C), 136.5 (2 x CH), 156.9 (C), 168.5 (C).

IR (ATR) / cm^{-1}: 2319, 1686, 1644, 1604, 1457, 1443, 1416, 1248, 1235, 1203, 1024, 1002, 887, 869, 852, 729, 697.

LCMS (ESI$^+$): calculated for $C_{17}H_{19}N_3O_2$ $[M+H]^+$ 298.1555; found: m/z = 298.1274.

4) Synthesis of 1-[4-(6-hydroxypyridazin-3-yl)piperazin-1-yl]-2-phenyl-ethanone (30)



30

Phenylacetic acid (0.15 g, 1.10 mmol) was reacted with commercially available 6-piperazin-1-ylpyridazin-3-ol (0.20 g, 1.10 mmol) as per procedure described for compound **28** to afford compound **30** (0.21 g, 0.70 mmol, 63% yield) as a pale-yellow solid.

Melting point: 169-172 °C. **LC purity (UV 245 nm):** 99.59%.

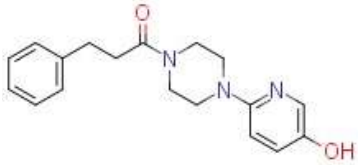
^{1}H NMR (400 MHz, DMSO-d$_6$): δ 3.13 (s, 2H), 3.19 (s, 2H), 3.62 - 3.63 (m, 4H), 3.81 (s, 2H), 6.84 (d, 1H, *J* = 10.10 *Hz*), 7.25-7.30 (m, 3H), 7.34-7.38 (m, 2H), 7.56 (d, 1H, *J* = 10.13 *Hz*), 12.22 (s, 1H).

^{13}C NMR (100 MHz, DMSO-d$_6$): δ 39.4 (CH_2), 40.1 (CH_2), 44.3 (CH_2), 45.7 (CH_2), 45.9 (CH_2), 125.9 (CH), 126.7 (CH), 127.8 (2 x CH), 128.5 (2 x CH), 130.6 (CH), 135.3 (C), 148.5 (C), 158.4 (C), 168.4 (C).

IR (ATR) / cm^{-1}: 2831, 1670, 1650, 1588, 1546, 1443, 1425, 1409, 1233, 1201, 1160, 1035, 1003, 945, 915, 843, 739, 729, 700.

LCMS (ESI$^+$): calculated for $C_{16}H_{18}N_4O_2$ $[M+H]^+$ 299.14; found: *m/z* = 299.16.

5) Synthesis of 1-[4-(5-hydroxy-pyridin-2-yl)-piperazin-1-yl]-3-phenyl-propan-1-one (31)



31

To a solution of 3-phenylpropionic acid (1 g, 6.65 mmol) in N,N-dimethylacetamide (10 ml) was added 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC·HCl) (1.91 g, 9.98 mmol). The reaction mixture was stirred for 10 minutes before adding intermediate **VIII** (1.19 g, 6.65 mmol). The resultant reaction mixture was added 4-(dimethylamino)pyridine (0.081 g, 0.66 mmol) and further stirred at room temperature for 3 hours. On completion of reaction, the reaction mixture was quenched with water and extracted with ethyl acetate. The combined organic extracts were washed with water followed by brine,

dried over anhydrous sodium sulfate and concentrated under reduced pressure. The resultant residue was purified using column chromatography on silica gel (230–400 mesh) using ethyl acetate – hexane mixture as eluent to afford compound **31** (1.1 g, 3.53 mmol, 53% yield) as an off white solid.

Melting point: 134-137 °C. **LC purity (UV 245 nm):** 98.50%.

^{1}H NMR (400 MHz, DMSO-d$_6$): δ 2.71 (t, 2H, *J* = 7.75 *Hz*), 2.88 (t, 2H, *J* = 7.70 *Hz*), 3.29 (m, 4H), 3.55 – 3.60 (m, 4H), 6.78 (d, 1H, *J* = 8.98 *Hz*), 7.12 (dd, 1H, J_1 = 8.93 *Hz*, J_2 = 2.86 *Hz*), 7.20 - 7.34 (m, 5H), 7.79 (d, 1H, *J* = 2.75 *Hz*), 9.08 (s, 1H).

^{13}C NMR (100 MHz, DMSO-d$_6$): δ 31.2 (CH_2), 34.4 (CH_2), 41.1 (CH_2), 44.9 (CH_2), 46.4 (CH_2), 46.7 (CH_2), 109.0 (CH), 125.8 (CH), 126.2 (CH), 128.6 (2 x CH), 128.8 (2 x CH), 134.62 (CH), 141.8 (C), 147.1 (C), 153.6 (C), 170.3 (C).

IR (ATR) / cm^{-1}: 3282, 2818, 1620, 1487, 1435, 1263, 1232, 1219, 1162, 1024, 924, 827, 696, 675.

LCMS (ESI$^+$): calcd for $C_{18}H_{21}N_3O_2$ $[M+H]^+$ 312.16; found: *m/z* = 312.14.

6) Synthesis of 1-[4-(5-hydroxy-pyrimidin-2-yl)-piperazin-1-yl]-3-phenyl-propan-1-one (32)

32

3-Phenylpropionic acid (0.10 g, 0.66 mmol) was reacted with commercially available 2-piperazin-1-ylpyrimidin-5-ol (0.12 g, 0.66 mmol) as per procedure described for compound **31** to afford compound **32** (0.11 g, 0.35 mmol, 53% yield) as a white solid.

Melting point: 153-156 °C. **LC purity (UV 245 nm):** 99.40%.

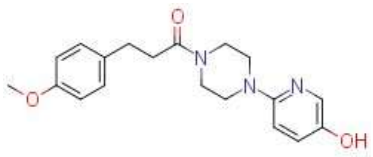
^{1}H NMR (400 MHz, DMSO-d$_6$): δ 2.65 (t, 2H, *J* = 7.43 *Hz*), 2.82 (t, 2H, *J* = 7.13 *Hz*), 3.48 - 3.52 (m, 8H), 7.17 - 7.27 (m, 5H), 8.03 (s, 2H), 9.29 (s, 1H).

^{13}C NMR (100 MHz, DMSO-d$_6$): δ 31.2 (CH_2), 34.5 (CH_2), 41.1 (CH_2), 44.5 (CH_2), 44.8 (CH_2), 44.9 (CH_2), 126.3 (CH), 128.6 (2 x CH), 128.8 (2 x CH), 141.8 (C), 144.5 (C), 145.6 (2 x CH), 156.8 (C), 170.5 (C).

IR (ATR) / cm^{-1}: 3203, 2911, 1606, 1553, 1489, 1462, 1445, 1421, 1392, 1261, 1224, 1015, 927, 845, 784, 698, 665, 649, 520.

LCMS (ESI$^+$): calculated for $C_{17}H_{20}N_4O_2$ $[M+H]^+$ 313.16; found: *m/z* = 313.06.

7) Synthesis of 3-(4-methoxy-phenyl)-1-[4-(5-hydroxy-pyridin-2-yl)-piperazin-1-yl]-propan-1-one (33)



33

3-(4-Methoxyphenyl)propanoic acid (0.20 g, 1.10 mmol) was reacted with 6-piperazin-1-yl-pyridin-3-ol (**VIII**) (0.19 g, 1.10 mmol) as per procedure described for compound **31** to afford freebase of **33** as thick colourless liquid. It was dissolved and stirred with methanolic HCl (3N, 10ml) for 30 min. Solvent was distilled out and residue was stirred with diethyl ether (20 ml). Resulted suspension was stirred, filtered and dried to afford di-HCl salt of compound **33** (0.25 g, 0.73 mmol, 66% yield) as an off white solid.

Melting point: 165-168 °C. **LC purity (UV 245 nm):** 98.54%.

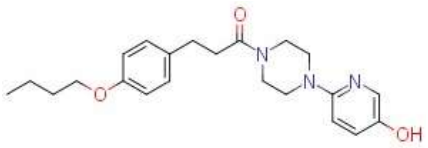
^{1}H NMR (500 MHz, DMSO-d$_6$): δ 2.68 (t, 2H, *J* = 7.62 *Hz*), 2.81 (t, 2H, *J* = 7.58 *Hz*), 3.59 (m, 4H), 3.65 (m, 4H), 3.75 (s, 3H), 6.87 (d, 2H, *J* = 8.27 *Hz*), 7.19 (d, 2H, *J* = 8.3 *Hz*), 7.34 (d, 1H, *J* = 9.63 *Hz*), 7.63 (d, 1H, *J* = 2.38 *Hz*), 7.75 (d, 1H, *J* = 8.95 *Hz*), 10.38 (br-s, 1H).

^{13}C NMR (100 MHz, DMSO-d$_6$): δ 30.1 (CH_2), 34.5 (CH_2), 43.9 (2 x CH_2), 46.5 (CH_2), 46.6 (CH_2), 55.4 (CH_3), 114.0 (2 x CH), 114.5 (CH), 121.1 (CH), 129.8 (2 x CH), 133.5 (C), 136.0 (CH), 146.7 (C), 148.0 (C), 157.9 (C), 170.8 (C).

IR (ATR) / cm^{-1}: 3024, 2927, 2800, 1643, 1611, 1543, 1511, 1452, 1420, 1326, 1296, 1239, 1164, 1028, 825, 691.

LCMS (ESI$^+$): calculated for $C_{19}H_{23}N_3O_3$ $[M+H]^+$ 342.17; found: m/z = 342.28.

8) Synthesis of 3-(4-butoxy-phenyl)-1-[4-(5-hydroxy-pyridin-2-yl)-piperazin-1-yl]-propan-1-one (34)



34

3-(4-Butoxyphenyl)propanoic acid (0.10 g, 0.44 mmol) was reacted with 6-piperazin-1-yl-pyridin-3-ol (**VIII**) (0.08 g, 0.44 mmol) as per procedure described for compound **31** to afford freebase of **34** as thick colourless liquid. It was dissolved and stirred with methanolic HCl (3N, 10ml) for 30 min. Solvent was distilled out and residue was stirred with diethyl ether (20 ml). Resulted suspension was stirred, filtered and dried to afford di-HCl salt of compound **34** (0.10 g, 0.26 mmol, 59% yield) as an off white solid.

Melting point: 170-173 °C. **LC purity (UV 245 nm):** 99.72%.

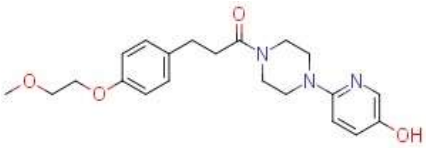
^{1}H NMR (500 MHz, DMSO-d$_6$): δ 0.97 (t, 3H, *J* = 7.36 *Hz*), 1.42 – 1.48 (m, 2H), 1.68 – 1.74 (m, 2H), 2.68 (t, 2H, *J* = 7.37 *Hz*), 2.81 (t, 2H, *J* = 7.47 *Hz*), 3.53 (m, 4H), 3.65 (m, 4H), 3.95 (t, 2H, *J* = 6.42 *Hz*), 6.87 (d, 2H, *J* = 8.32 *Hz*), 7.19 (d, 2H, *J* = 8.27 *Hz*), 7.28 (m, 1H), 7.64 (m, 2H), 10.20 (br-s, 1H).

^{13}C NMR (100 MHz, DMSO-d$_6$): δ 14.1 (CH_3), 19.1 (CH_2), 30.2 (CH_2), 31.2 (2 x CH_2), 34.5 (CH_2), 44.0 (CH_2), 46.5 (CH_2), 46.6 (CH_2), 67.4 (CH_2), 114.5 (CH), 114.6 (2 x CH), 121.1 (CH), 129.8 (2 x CH), 133.4 (C), 135.9 (CH), 146.8 (C), 148.0 (C), 157.3 (C), 170.8 (C).

IR (ATR) / cm^{-1}: 3657, 2981, 2972, 2889, 2816, 2665, 1641, 1614, 1546, 1511, 1449, 1420, 1393, 1379, 1323, 1295, 1281, 1244, 1226, 1166, 1155, 950, 831, 817.

LCMS (ESI$^+$): calculated for $C_{22}H_{29}N_3O_3$ $[M+H]^+$ 384.22; found: m/z = 384.06.

9) Synthesis of 1-[4-(5-hydroxy-pyridin-2-yl)-piperazin-1-yl]-3-[4-(2-methoxy-ethoxy)-phenyl]-propan-1-one (35)



35

3-[4-(2-Methoxyethoxy)phenyl]propanoic acid (0.20 g, 0.89 mmol) was reacted with 6-piperazin-1-yl-pyridin-3-ol (**VIII**) (0.16 g, 0.89 mmol) as per procedure described for

compound **31** to afford freebase of **35** as thick colourless liquid. It was dissolved and stirred with methanolic HCl (3N, 10ml) for 30 min. Solvent was distilled out and residue was stirred with diethyl ether (20 ml). Resulted suspension was stirred, filtered and dried to afford di-HCl salt of compound **35** (0.25 g, 0.64 mmol, 72% yield) as an off white solid.

Melting point: 161-164 °C. **LC purity (UV 245 nm):** 99.59%.

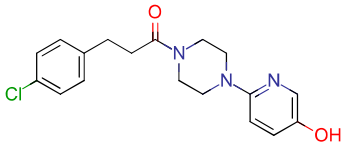
^{1}H NMR (500 MHz, DMSO-d$_6$): δ 2.63 (t, 2H, *J* = 7.58 *Hz*), 2.76 (t, 2H, *J* = 7.55 *Hz*), 3.29 (s, 3H), 3.54 - 3.60 (m, 4H), 3.61 - 3.63 (m, 6H), 4.02 (t, 2H, *J* = 4.47 *Hz*), 6.84 (d, 2H, *J* = 8.32 *Hz*), 7.15 (d, 2H, *J* = 8.32 *Hz*), 7.29 (d, 1H, *J* = 9.66 *Hz*), 7.59 (d, 1H, *J* = 2.44 *Hz*), 7.70 (d, 1H, *J* = 9.04 *Hz*), 10.36 (br-s, 1H).

^{13}C NMR (100 MHz, DMSO-d$_6$, δ): 29.2 (CH_2), 33.6 (CH_2), 43.1 (2 x CH_2), 45.5 (CH_2), 45.7 (CH_2), 57.7 (CH_3), 66.3 (CH_2), 69.9 (CH_2), 113.7 (2 x CH), 120.4 (CH), 128.9 (2 x CH), 132.7 (C), 135.0 (2 x CH), 145.9 (C), 147.2 (C), 156.2 (C), 169.9 (C).

IR (ATR) / cm^{-1}: 3150, 2981, 1640, 1615, 1543, 1509, 1451, 1425, 1326, 1297, 1244, 1167, 1127, 826, 714.

LCMS (ESI$^+$): calculated for $C_{21}H_{27}N_3O_4$ $[M+H]^+$ 386.20; found: *m/z* = 385.89.

10) Synthesis of 3-(4-chloro-phenyl)-1-[4-(5-hydroxy-pyridin-2-yl)-piperazin-1-yl]-propan-1-one (36)



36

3-(4-Chlorophenyl)propanoic acid (0.15 g, 0.81 mmol) was reacted with 6-piperazin-1-yl-pyridin-3-ol (**VIII**) (0.14 g, 0.81 mmol) as per procedure described for compound **31** to afford compound **36** (0.16 g, 0.46 mmol, 56% yield) as an off white solid.

Melting point: 145-148 °C. **LC purity (UV 245 nm):** 96.02%.

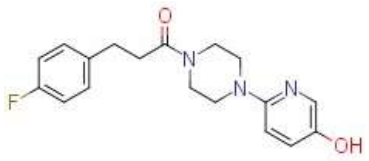
^{1}H NMR (500 MHz, DMSO-d$_6$): δ 2.71 (t, 2H, *J* = 7.64 *Hz*), 2.87 (t, 2H, *J* = 7.61 *Hz*), 3.28 – 3.34 (m, 4H), 3.56 (t, 2H, *J* = 5.01 *Hz*), 3.59 (t, 2H, *J* = 5.1 *Hz*), 6.79 (d, 1H, *J* = 8.83 *Hz*), 7.12 (dd, 1H, J_1 = 8.96 *Hz*, J_2 = 3.00 *Hz*), 7.31 – 7.34 (m, 2H), 7.36 – 7.38 (m, 2H), 7.79 (d, 1H, *J* = 2.55 *Hz*), 9.08 (s, 1H).

^{13}C NMR (100 MHz, DMSO-d$_6$): δ 30.4 (CH_2), 34.1 (CH_2), 41.1 (CH_2), 44.9 (CH_2), 46.4 (CH_2), 46.7 (CH_2), 109.0 (CH), 125.8 (CH), 128.5 (2 x CH), 130.7 (2 x CH), 130.9 (C), 134.5 (CH), 140.8 (C), 147.1 (C), 153.6 (C), 170.2 (C).

IR (ATR) / cm^{-1}: 3285, 2834, 1614, 1489, 1467, 1439, 1263, 1236, 1218, 1163, 1027, 1014, 922, 826, 811, 713, 668, 645.

LCMS (ESI$^+$): calculated for $C_{18}H_{20}ClN_3O_2$ $[M+H]^+$ 346.12; found: *m/z* = 346.34.

11) Synthesis of 3-(4-fluoro-phenyl)-1-[4-(5-hydroxy-pyridin-2-yl)-piperazin-1-yl]-propan-1-one (37)



37

3-(4-Fluorophenyl)propanoic acid (0.15 g, 0.89 mmol) was reacted with 6-piperazin-1-yl-pyridin-3-ol (**VIII**) (0.16 g, 0.89 mmol) as per procedure described for compound **31** to afford compound **37** (0.18 g, 0.54 mmol, 60% yield) as an off white solid.

Melting point: 148-151 °C. **LC purity (UV 245 nm):** 99.19%.

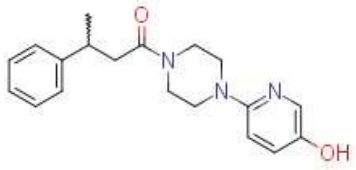
^{1}H NMR (500 MHz, DMSO-d$_6$): δ 2.65 (t, 2H, *J* = 7.61 *Hz*), 2.81 (t, 2H, *J* = 7.57 *Hz*), 3.23 (m, 4H), 3.53 (m, 4H), 6.73 (d, 1H, *J* = 8.9 *Hz*), 7.06 (m, 3H), 7.28 (m, 2H), 7.73 (d, 1H, *J* = 2.64 *Hz*), 9.04 (s, 1H).

^{13}C NMR (100 MHz, DMSO-d$_6$): δ 30.3 (CH_2), 34.4 (CH_2), 41.1 (CH_2), 44.9 (CH_2), 46.4 (CH_2), 46.7 (CH_2), 109.0 (CH), 115.2 (2 x CH, d, $J_{C\text{-}C\text{-}F}$ = 20.89 *Hz*), 125.8 (CH), 130.6 (2 x CH, d, $J_{C\text{-}C\text{-}C\text{-}F}$ = 7.85 *Hz*), 134.5 (CH), 137.9 (C), 147.1 (C), 153.6 (C), 161.0 (C, d, $J_{C\text{-}F}$ = 241.14 *Hz*), 170.3 (C).

IR (ATR) / cm^{-1}: 3277, 1626, 1509, 1489, 1439, 1276, 1264, 1235, 1216, 1163, 1153, 1025, 921, 825, 712, 680.

LCMS (ESI$^+$): calculated for $C_{18}H_{20}FN_3O_2$ $[M+H]^+$ 330.15; found: *m/z* = 329.82.

12) Synthesis of 1-[4-(5-hydroxypyridin-2-yl)piperazin-1-yl]-3-phenylbutan-1-one (38)



38

3-Phenylbutanoic acid (0.25 g, 1.52 mmol) was reacted with 6-piperazin-1-yl-pyridin-3-ol (**VIII**) (0.27 g, 1.52 mmol) as per procedure described for compound **31** to afford compound **38** (0.32 g, 0.98 mmol, 64% yield) as an off white solid.

Melting point: 138-140 °C. **LC purity (UV 245 nm):** 98.97%.

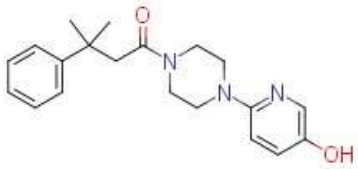
^{1}H NMR (500 MHz, DMSO-d$_6$): 1.27 (d, 3H, *J* = 6.88 *Hz*), 2.66 – 2.76 (m, 2H), 3.22 – 3.29 (m, 1H), 3.43 - 3.49 (m, 2H), 3.57 - 3.70 (m, 6H), 7.20 – 7.23 (m, 1H), 7.30 – 7.33 (m, 5H), 7.66 (s, 1H), 7.72 (d, 1H, *J* = 8.96 *Hz*), 10.35 (s, 1H).

^{13}C NMR (100 MHz, DMSO-d$_6$): δ 22.4 (CH_3), 36.3 (CH), 40.3 (CH_2), 40.5 (CH_2), 44.2 (CH_2), 46.4 (CH_2), 46.6 (CH_2), 114.2 (CH), 122.1 (CH), 126.3 (CH), 127.3 (2 x CH), 128.6 (2 x CH), 135.4 (CH), 146.7 (C), 146.9 (C), 148.2 (C), 170.2 (C).

IR (ATR) / cm^{-1}: 2925, 1604, 1543, 1426, 1323, 1287, 1256, 1219, 1161, 830, 700.

LCMS (ESI$^+$): calculated for $C_{19}H_{23}N_3O_2$ $[M+H]^+$ 326.18; found: *m/z* = 325.85.

13) Synthesis of 1-[4-(5-hydroxy-pyridin-2-yl)-piperazin-1-yl]-3-methyl-3-phenyl-butan-1-one (39)



39

3-Methyl-3-phenyl-butanoic acid (1.00 g, 5.61 mmol) was reacted with 6-piperazin-1-yl-pyridin-3-ol (**VIII**) (1.00 g, 5.61 mmol) as per procedure described for compound **31** to afford compound **39** (1.13 g, 3.32 mmol, 59% yield) as an off white solid.

Melting point: 142-145 °C. **LC purity (UV 245 nm):** 99.81%.

^{1}H NMR (500 MHz, DMSO-d$_6$): δ 1.46 (s, 6H), 2.73 (m, 2H), 3.09 (m, 2H), 3.18 (m, 2H), 3.36 (m,2H), 3.48 (m, 2H), 6.73 (d, 1H, *J* = 8.9 *Hz*), 7.10 (dd, 1H, J_1= 8.9 *Hz*, J_2 = 2.6 *Hz*),

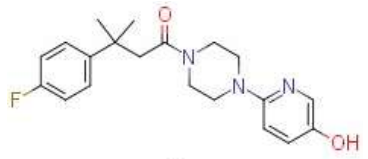
7.19 (t, 1H, *J* = 7.21 *Hz*), 7.32 (t, 2H, *J* = 7.4 *Hz*), 7.43 (d, 2H, *J* = 7.94 *Hz*), 7.77 (d, 1H, *J* = 2.59 *Hz*), 9.08 (s, 1H).

^{13}C NMR (100 MHz, DMSO-d$_6$): δ 29.5 (2 x CH_3), 37.6 (C), 40.8 (CH_2), 44.7 (CH_2), 45.5 (CH_2), 46.3 (CH_2), 46.4 (CH_2), 108.9 (CH), 125.7 (CH), 125.8 (CH), 126.0 (2 x CH), 128.2 (2 x CH), 134.5 (CH), 147.0 (C), 149.2 (C), 153.5 (C), 169.2 (C).

IR (ATR) / cm^{-1}: 3652, 3232, 2980, 2885, 1601, 1489, 1470, 1435, 1422, 1386, 1363, 1257, 1234, 1209, 1182, 1163, 1138, 970, 811, 788, 698, 686.

LCMS (ESI$^+$): calculated for $C_{20}H_{25}N_3O_2$ $[M+H]^+$ 340.19; found: *m/z* = 339.87

14) Synthesis of 3-(4-fluoro-phenyl)-1-[4-(5-hydroxy-pyridin-2-yl)-piperazin-1-yl]-propan-1-one (40)



40

3-(4-Fluorophenyl)propanoic acid (0.15 g, 0.89 mmol) was reacted with 6-piperazin-1-yl-pyridin-3-ol (**VIII**) (0.16 g, 0.89 mmol) as per procedure described for compound **31** to afford compound **40** (0.18 g, 0.54 mmol, 60% yield) as an off white solid.

Melting point: 146-148 °C. **LC purity (UV 245 nm):** 99.19%.

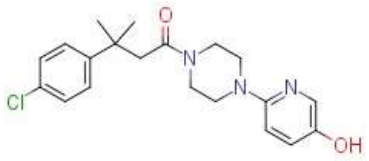
^{1}H NMR (500 MHz, DMSO-d$_6$): δ 2.65 (t, 2H, *J* = 7.61 *Hz*), 2.81 (t, 2H, *J* = 7.57 *Hz*), 3.23 (m, 4H), 3.53 (m, 4H), 6.73 (d, 1H, *J* = 8.9 *Hz*), 7.06 (m, 3H), 7.28 (m, 2H), 7.73 (d, 1H, *J* = 2.64 *Hz*), 9.04 (s, 1H).

^{13}C NMR (100 MHz, DMSO-d$_6$): δ 30.3 (CH_2), 34.4 (CH_2), 41.1 (CH_2), 44.9 (CH_2), 46.4 (CH_2), 46.7 (CH_2), 109.0 (CH), 115.2 (2 x CH, d, $J_{C\text{-}C\text{-}F}$ = 20.89 *Hz*), 125.8 (CH), 130.6 (2 x CH, d, $J_{C\text{-}C\text{-}C\text{-}F}$ = 7.85 *Hz*), 134.5 (CH), 137.9 (C), 147.1 (C), 153.6 (C), 161.0 (C, d, $J_{C\text{-}F}$ = 241.14 *Hz*), 170.3 (C).

IR (ATR) / cm^{-1}: 3277, 1626, 1509, 1489, 1439, 1276, 1264, 1235, 1216, 1163, 1153, 1025, 921, 825, 712, 680.

LCMS (ESI$^+$): calculated for $C_{18}H_{20}FN_3O_2$ $[M+H]^+$ 330.15; found: *m/z* = 329.82.

15) Synthesis of 3-(4-chloro-phenyl)-1-[4-(5-hydroxy-pyridin-2-yl)-piperazin-1-yl]-propan-1-one (41)



41

3-(4-Chlorophenyl)propanoic acid (0.15 g, 0.81 mmol) was reacted with 6-piperazin-1-yl-pyridin-3-ol (**VIII**) (0.14 g, 0.81 mmol) as per procedure described for compound **31** to afford compound **41** (0.16 g, 0.46 mmol, 56% yield) as an off white solid.

Melting point: 144-147 °C. **LC purity (UV 245 nm):** 96.02%.

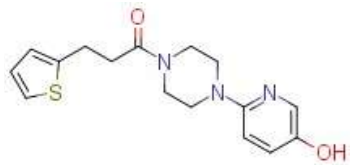
^{1}H NMR (500 MHz, DMSO-d$_6$): δ 2.71 (t, 2H, *J* = 7.64 *Hz*), 2.87 (t, 2H, *J* = 7.61 *Hz*), 3.28 – 3.34 (m, 4H), 3.56 (t, 2H, *J* = 5.01 *Hz*), 3.59 (t, 2H, *J* = 5.1 *Hz*), 6.79 (d, 1H, *J* = 8.83 *Hz*), 7.12 (dd, 1H, J_1 = 8.96 *Hz*, J_2 = 3.00 *Hz*), 7.31 – 7.34 (m, 2H), 7.36 – 7.38 (m, 2H), 7.79 (d, 1H, *J* = 2.55 *Hz*), 9.08 (s, 1H).

^{13}C NMR (100 MHz, DMSO-d$_6$): δ 30.4 (CH_2), 34.1 (CH_2), 41.1 (CH_2), 44.9 (CH_2), 46.4 (CH_2), 46.7 (CH_2), 109.0 (CH), 125.8 (CH), 128.5 (2 x CH), 130.7 (2 x CH), 130.9 (C), 134.5 (CH), 140.8 (C), 147.1 (C), 153.6 (C), 170.2 (C).

IR (ATR) / cm^{-1}: 3285, 2834, 1614, 1489, 1467, 1439, 1263, 1236, 1218, 1163, 1027, 1014, 922, 826, 811, 713, 668, 645.

LCMS (ESI$^+$): calculated for $C_{18}H_{20}ClN_3O_2$ $[M+H]^+$ 346.12; found: *m/z* = 346.34.

16) Synthesis of 1-[4-(5-hydroxy-pyridin-2-yl)-piperazin-1-yl]-3-thiophen-2-yl-propan-1-one (42)



42

3-(2-Thienyl)propanoic acid (0.10 g, 0.64 mmol) was reacted with 6-piperazin-1-yl-pyridin-3-ol (**VIII**) (0.11 g, 0.64 mmol) as per procedure described for compound **31** to afford compound **42** (0.12 g, 0.37 mmol, 57% yield) as an off white solid.

Melting point: 172-174 °C. **LC purity (UV 245 nm):** 99.53%.

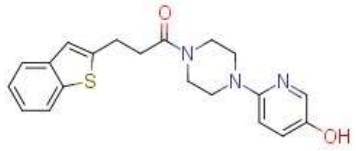
^{1}H NMR (500 MHz, DMSO-d$_6$): δ 2.70 (t, 2H, *J* = 6.99 *Hz*), 3.04 (t, 2H, *J* = 6.81 *Hz*), 3.25 - 3.27 (m, 4H), 3.25 - 3.27 (m, 4H), 6.74 (d, 1H, *J* = 8.78 *Hz*), 6.89-6.92 (m, 2H), 7.07 (d, 1H, *J* = 7.95 *Hz*), 7.29 (d, 1H, *J* = 4.33 *Hz*), 7.74 (s, 1H), 9.03 (s, 1H).

^{13}C NMR (100 MHz, DMSO-d$_6$,): δ 24.9 (CH_2), 34.1 (CH_2), 40.8 (CH_2), 44.5 (CH_2), 45.9 (CH_2), 46.3 (CH_2), 108.7 (CH), 123.7 (CH), 124.8 (CH), 125.4 (CH), 126.8 (CH), 134.1 (CH), 143.8 (C), 146.7 (C), 153.2 (C), 169.5 (C).

IR (ATR) / cm^{-1}: 3258, 2854, 1622, 1501, 1490, 1477, 1436, 1263, 1233, 1210, 1162, 946, 810, 706, 691.

LCMS (ESI$^+$): calculated for $C_{16}H_{19}N_3O_2S$ $[M+H]^+$ 318.1276; found: *m/z* = 318.1192.

17) Synthesis of 3-benzo[b]thiophen-2-yl-1-[4-(5-hydroxy-pyridin-2-yl)-piperazin-1-yl]-propan-1-one (43)



43

3-(Benzothiophen-2-yl)propanoic acid (0.20 g, 0.96 mmol) was reacted with 6-piperazin-1-yl-pyridin-3-ol (**VIII**) (0.17 g, 0.96 mmol) as per procedure described for compound **31** to afford compound **43** (0.20 g, 0.54 mmol, 56% yield) as a white solid.

Melting point: 168-171 °C. **LC purity (UV 245 nm):** 98.85%.

^{1}H NMR (500 MHz, DMSO-d$_6$): δ 2.87 (t, 2H, *J* = 7.22 *Hz*), 3.20 (t, 2H, *J* = 7.12 *Hz*), 3.32 - 3.35 (m, 4H), 3.62 - 3.63 (m, 4H), 6.79 (d, 1H, *J* = 8.96 *Hz*), 7.11 - 7.13 (m, 1H), 7.26 (s, 1H), 7.32 (t, 1H, *J* = 7.40 *Hz*), 7.37 (t, 1H, *J* = 7.35 *Hz*), 7.77 (d, 1H, *J* = 9.89 *Hz*), 7.79 (d, 1H, *J* = 2.06 *Hz*), 7.91 (d, 1H, *J* = 7.83 *Hz*), 9.10 (s, 1H).

^{13}C NMR (125 MHz, DMSO-d$_6$): δ 25.6 (CH_2), 33.4 (CH_2), 40.9 (CH_2), 44.5 (CH_2), 45.9 (CH_2), 46.3 (CH_2), 108.6 (CH), 121.0 (CH), 122.1 (CH), 122.7 (CH), 123.6 (CH), 124.2 (CH), 125.4 (CH), 134.2 (CH), 138.7 (C), 139.8 (C), 145.3 (C), 146.7 (C), 153.2 (C), 169.3 (C).

IR (ATR) / cm^{-1}: 3293, 1618, 1488, 1439, 1263, 1236, 1219, 1164, 1026, 922, 826, 744, 724, 674, 645.

LCMS (ESI$^+$): calculated for $C_{20}H_{21}N_3O_2S$ $[M+H]^+$ 368.14; found: *m/z* = 367.83.

18) Synthesis of [4-(5-hydroxy-pyridin-2-yl)-piperazin-1-yl]-(5-phenyl-thiophen-2-yl)-methanone (44)

44

5-Phenylthiophene-2-carboxylic acid (0.20 g, 0.97 mmol) was reacted with 6-piperazin-1-yl-pyridin-3-ol (**VIII**) (0.17 g, 0.97 mmol) as per procedure described for compound **31** to afford compound **44** (0.21 g, 0.57 mmol, 58% yield) as an off white solid.

Melting point: 180-183 °C. **LC purity (UV 245 nm):** 97.91%.

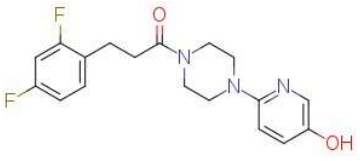
^{1}H NMR (500 MHz, DMSO-d$_6$): δ 3.46 – 3.47 (m, 4H), 3.83 (m, 4H), 6.82 (d, 1H, *J* = 8.95 Hz), 7.14 - 7.16 (m, 1H), 7.42 (t, 1H, *J* = 7.30 Hz), 7.49 -7.53 (m, 3H), 7.58 (d, 1H, *J* = 3.62 Hz), 7.77 (d, 2H, *J* = 7.66 Hz), 7.83 (d, 1H, *J* = 2.29 Hz), 9.12 (s, 1H).

^{13}C NMR (125 MHz, DMSO-d$_6$): δ 46.1 (4 x CH_2), 108.6 (CH), 123.6 (CH), 125.4 (CH), 125.7 (2 x CH), 128.5 (CH), 129.2 (2 x CH), 130.5 (CH), 132.9 (C), 134.2 (CH), 136.3 (C), 146.4 (C), 146.7 (C), 153.1 (C), 161.9 (C).

IR (ATR) / cm^{-1}: 2857, 1606, 1565, 1493, 1447, 1422, 1391, 1366, 1262, 1233, 1155, 1002, 819, 763, 737, 713, 693.

LCMS (ESI$^+$): calculated for $C_{20}H_{19}N_3O_2S$ $[M+H]^+$ 366.12; found: *m/z* = 365.83.

19) Synthesis of 3-(2,4-difluorophenyl)-1-[4-(5-hydroxypyridin-2-yl)piperazin-1-yl] propan-1-one (45)



45

3-(2,4-Difluorophenyl)propanoic acid (0.25 g, 1.34 mmol) was reacted with 6-piperazin-1-yl-pyridin-3-ol (**VIII**) (0.24 g, 1.34 mmol) as per procedure described for compound **31** to afford compound **45** (0.18 g, 0.51 mmol, 38% yield) as an off white solid.

Melting point: 140-142 °C. **LC purity (UV 245 nm):** 99.26%.

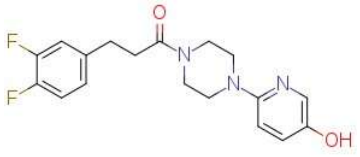
^{1}H NMR (500 MHz, DMSO-d$_6$): δ 2.70 (t, 2H, *J* = 7.56 Hz), 2.88 (t, 2H, *J* = 7.74 Hz), 3.20 - 3.34 (m, 4H), 3.42 - 3.61 (m, 4H), 6.79 (d, 1H, *J* = 8.96 Hz), 7.04 -7.08 (m, 1H), 7.12 (dd, 1H, J_1 = 8.9 Hz, J_2 = 3.00 Hz), 7.19 - 7.25 (m, 1H), 7.42 - 7.48 (m, 1H), 7.8 (d, 1H, *J* = 2.80 Hz), 9.08 (s, 1H)

^{13}C NMR (125 MHz, DMSO-d$_6$): δ 23.7 (CH_2), 32.8 (CH_2), 41.1 (CH_2), 44.8 (CH_2), 46.3 (CH_2), 46.6 (CH_2), 103.9 (CH), 109.0 (CH), 111.6 (CH), 124.6 (C), 125.8 (CH), 132.2 (CH), 134.5 (CH), 147.1 (C), 153.6 (C), 160.0 (C), 162.3 (C), 169.9 (C).

IR (ATR) / cm^{-1}: 3282, 2837, 1668, 1504, 1489, 1438, 1264, 1237, 1222, 1132, 960, 828

LCMS (ESI$^+$): calculated for $C_{18}H_{19}F_2N_3O_2$ $[M+H]^+$ 348.1445; found *m/z* = 348.0847.

20) Synthesis of 3-(3,4-difluorophenyl)-1-[4-(5-hydroxypyridin-2-yl)piperazin-1-yl] propan-1-one (46)



46

3-(3,4-Difluorophenyl)propanoic acid (0.20 g, 1.07 mmol) was reacted with 6-piperazin-1-yl-pyridin-3-ol (**VIII**) (0.19 g, 1.07 mmol) as per procedure described for compound **31** to afford compound **46** (0.15 g, 0.43 mmol, 40% yield) as an off white solid.

Melting point: 158-160 °C. **LC purity (UV 245 nm):** 97.53%.

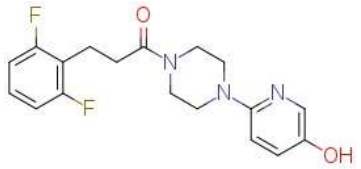
^{1}H NMR (500 MHz, DMSO-d$_6$): δ 2.73 (t, 2H, *J* = 7.56 Hz), 2.88 (t, 2H, *J* = 7.58 Hz), 3.28 – 3.33 (m, 4H), 3.56 – 3.61 (t, 4H), 6.80 (d, 1H, *J* = 9.0 Hz), 7.12 -7.15 (m, 2H), 7.33 - 7.43 (m, 2H), 7.8 (d, 1H, *J* = 2.72 Hz), 9.10 (s, 1H).

^{13}C NMR (125 MHz, DMSO-d$_6$): δ 30.9 (CH_2), 34.7 (CH_2), 41.9 (CH_2), 45.6 (CH_2), 47.2 (CH_2), 47.5 (CH_2), 109.9 (CH), 118.1 (CH), 118.5 (CH), 126.4 (CH), 126.7 (CH), 135.1 (CH), 140.5 (C), 147.9 (C), 149.0 (C), 151.0 (C), 154.3 (C), 170.9 (C).

IR (ATR) / cm^{-1}: 3284, 1623, 1592, 1517, 1488, 1464, 1434, 1265, 1234, 1220, 1206, 1162, 1114, 1025, 943, 922, 825, 771, 674, 642, 620, 527.

LCMS (ESI$^+$): calculated for $C_{18}H_{19}F_2N_3O_2$ $[M+H]^+$ 348.1445; found *m/z* = 348.0614.

21) Synthesis of 3-(2,6-difluorophenyl)-1-[4-(5-hydroxypyridin-2-yl)piperazin-1-yl] propan-1-one (47)



47

3-(2,6-Difluorophenyl)propanoic acid (0.35 g, 1.88 mmol) was reacted with 6-piperazin-1-yl-pyridin-3-ol (**VIII**) (0.33 g, 1.88 mmol) as per procedure described for compound **31** to afford compound **47** (0.92 g, 0.43 mmol, 48% yield) as an off white solid.

Melting point: 157-159 °C. **LC purity (UV 245 nm):** 99.96%.

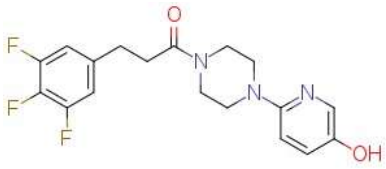
^{1}H NMR (500 MHz, DMSO-d$_6$): δ 2.67 (t, 2H, *J* = 7.94 Hz), 2.91 (t, 2H, *J* = 7.90 Hz), 3.32 - 3.38 (m, 4H), 3.55 (t, 2H, *J* = 4.98 Hz), 3.60 (t, 2H, *J* = 5.06 Hz), 6.79 (d, 1H, *J* = 8.96 Hz), 7.08 - 7.14 (m, 3H), 7.32 - 7.39 (m, 1H), 7.79 (d, 1H, *J* = 2.88 Hz), 9.08 (s, 1H).

^{13}C NMR (125 MHz, DMSO-d$_6$): δ 18.4 (CH_2), 32.5 (CH_2), 41.2 (CH_2), 45.0 (CH_2), 46.4 (CH_2), 46.7 (CH_2), 109.2 (CH), 111.8 (CH), 112.0 (CH), 116.7 (C), 125.9 (CH), 128.9 (CH), 134.6 (CH), 147.2 (C), 153.7 (C), 160.2 (C), 162.7 (C), 169.8 (C).

IR (ATR) / cm^{-1}: 3294, 2981, 1606, 1565, 1483, 1469, 1440, 1305, 1277, 1263, 1232, 1195, 1160, 1026, 982, 922, 813, 786, 726.

LCMS (ESI$^+$): calculated for $C_{18}H_{19}F_2N_3O_2$ $[M+H]^+$ 348.1445; found *m/z* = 348.1208.

22) Synthesis of 1-[4-(5-hydroxypyridin-2-yl)piperazin-1-yl]-3-(3,4,5-trifluorophenyl) propan-1-one (48)



48

3-(3,4,5-Trifluorophenyl)propanoic acid (0.25 g, 1.22 mmol) was reacted with 6-piperazin-1-yl-pyridin-3-ol (**VIII**) (0.22 g, 1.22 mmol) as per procedure described for compound **31** to afford compound **48** (0.32 g, 0.87 mmol, 71% yield) as an off white solid.

Melting point: 162-165 °C. **LC purity (UV 245 nm):** 96.28%.

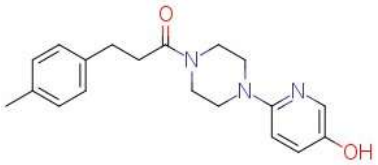
^{1}H NMR (500 MHz, DMSO-d$_6$): δ 2.75 (t, 2H, *J* = 7.58 Hz), 2.87 (t, 2H, *J* = 7.63 Hz), 3.29 (t, 2H, *J* = 5.17 Hz), 3.34 (t, 2H, *J* = 5.12 Hz), 3.57 – 3.61 (m, 4H), 6.80 (d, 1H, *J* = 8.95 Hz), 7.13 (dd, 1H, J_1 = 8.96 Hz, J_2 = 3.00 Hz), 7.29 – 7.33 (m, 2H), 7.79 (d, 1H, *J* = 2.55 Hz), 9.09 (s, 1H).

^{13}C NMR (125 MHz, DMSO-d$_6$): δ 31.0 (CH_2), 34.3 (CH_2), 41.9 (CH_2), 45.6 (CH_2), 47.2 (CH_2), 47.5 (CH_2), 109.8 (CH), 114.1 (CH), 114.2 (CH), 126.6 (CH), 135.3 (CH), 138.1 (C), 140.2 (C), 147.9 (C), 149.9 (C), 152.3 (C), 154.4 (C), 170.7 (C).

IR (ATR) / cm^{-1}: 3294, 1623, 1527, 1492, 1469, 1440, 1355, 1271, 1258, 1236, 1214, 1164, 1042, 1026, 1007, 944, 923, 818, 667, 579.

LCMS (ESI$^+$): calculated for $C_{18}H_{18}F_3N_3O_2$ $[M+H]^+$ 366.1351; found *m/z* = 366.1906.

23) Synthesis of 1-[4-(5-hydroxypyridin-2-yl)piperazin-1-yl]-3-(4-methylphenyl)propan-1-one (49)



49

3-(4-Methylphenyl)propanoic acid (0.25 g, 1.52 mmol) was reacted with 6-piperazin-1-yl-pyridin-3-ol (**VIII**) (0.27 g, 1.52 mmol) as per procedure described for compound **31** to afford compound **49** (0.28 g, 0.86 mmol, 56% yield) as an off white solid.

Melting point: 148-150 °C. **LC purity (UV 245 nm):** 99.35%.

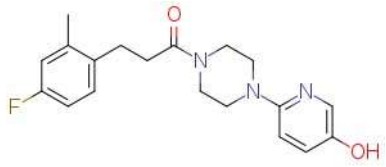
^{1}H NMR (500 MHz, DMSO-d$_6$): δ 2.23 (s, 3H), 2.61 (t, 2H, *J* = 7.71 *Hz*), 2.77 (t, 2H, *J* = 7.68 *Hz*), 3.22 – 3.23 (m, 4H), 3.49 – 3.54 (m, 4H), 6.72 (d, 1H, *J* = 8.98 *Hz*), 7.05 – 7.07 (m, 3H), 7.12 (d, 2H, *J* = 7.77 *Hz*), 7.73 (d, 1H, *J* = 2.76 *Hz*), 9.03 (s, 1H)

^{13}C NMR (125 MHz, DMSO-d$_6$): δ 21.1 (CH_3), 30.9 (CH_2), 34.6 (CH_2), 41.2 (CH_2), 45.0 (CH_2), 46.5 (CH_2), 46.8 (CH_2), 109.7 (CH), 125.9 (CH), 128.8 (2 x CH), 129.3 (2 x CH), 134.6 (CH), 135.2 (C), 138.7 (C), 147.2 (C), 153.7 (C), 170.5 (C).

IR (ATR) / cm^{-1}: 3291, 1615, 1489, 1439, 1264, 1237, 1220, 1163, 1027, 938, 921, 827, 713, 673, 540.

LCMS (ESI$^+$): calculated for $C_{19}H_{23}N_3O_2$ $[M+H]^+$ 326.17; found *m/z* = 326.30.

24) Synthesis of 3-(4-fluoro-2-methylphenyl)-1-[4-(5-hydroxypyridin-2-yl)piperazin-1-yl] propan-1-one (50)



50

3-(4-Fluoro-2-methylphenyl)propanoic acid (0.30 g, 1.64 mmol) was reacted with 6-piperazin-1-yl-pyridin-3-ol (**VIII**) (0.29 g, 1.64 mmol) as per procedure described for compound **31** to afford compound **50** (0.41 g, 1.19 mmol, 72% yield) as an off white solid.

Melting point: 152-155 °C. **LC purity (UV 245 nm):** 97.99%.

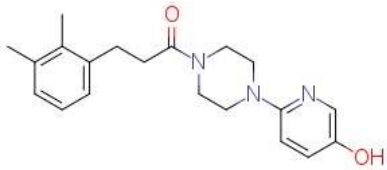
^{1}H NMR (500 MHz, DMSO-d$_6$): δ 2.34 (s, 3H), 2.64 (t, 2H, *J* = 7.78 *Hz*), 2.84 (t, 2H, *J* = 7.76 *Hz*), 3.30 (t, 4H, *J* = 5.12 *Hz*), 3.54 – 3.60 (m, 4H), 6.78 (d, 1H, *J* = 8.96 *Hz*), 6.96 (td, 1H, J_1 = 14.4 *Hz*, J_2 = 4.3 *Hz*), 7.04 (dd, 1H, J_1 = 10.06 *Hz*, J_2 = 2.66 *Hz*), 7.13 (dd, 1H, J_1 = 8.96 *Hz*, J_2 = 3.00 *Hz*), 7.25 (dd, 1H, J_1 = 8.4 *Hz*, J_2 = 6.2 *Hz*), 7.80 (d, 1H, *J* = 2.92 *Hz*), 9.08 (s, 1H)

^{13}C NMR (125 MHz, DMSO-d$_6$): δ 18.9 (CH_3), 27.3 (CH_2), 32.7 (CH_2), 40.7 (CH_2), 44.5 (CH_2), 45.9 (CH_2), 46.2 (CH_2), 108.6 (CH), 112.2 (CH), 116.2 (CH), 125.4 (CH), 130.3 (CH), 134.1 (CH), 135.6 (C), 138.4 (C), 146.7 (C), 153.1 (C), 160.4 (C), 170.0 (C).

IR (ATR) / cm^{-1}: 3229, 1630, 1493, 1439, 1424, 1262, 1240, 1218, 1179, 1160, 1027, 945, 898, 809, 706, 693.

LCMS (ESI$^+$): calculated for $C_{19}H_{22}FN_3O_2$ $[M+H]^+$ 344.16; found *m/z* = 344.31.

25) Synthesis of 3-(2,3-dimethylphenyl)-1-[4-(5-hydroxypyridin-2-yl)piperazin-1-yl] propan-1-one (51)



51

3-(2,3-Dimethylphenyl)propanoic acid (0.25 g, 1.40 mmol) was reacted with 6-piperazin-1-yl-pyridin-3-ol (**VIII**) (0.25 g, 1.40 mmol) as per procedure described for compound **31** to afford compound **51** (0.38 g, 1.11 mmol, 79% yield) as an off white solid.

Melting point: 153-155 °C. **LC purity (UV 245 nm):** 98.80%.

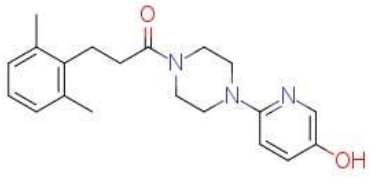
^{1}H NMR (500 MHz, DMSO-d$_6$): δ 2.22 (s, 3H), 2.27 (s, 3H), 2.61 (t, 2H, *J* = 7.9 *Hz*), 2.88 (t, 2H, *J* = 7.9 *Hz*), 3.28 - 3.31 (m, 4H), 3.53 (t, 2H, *J* = 4.96 *Hz*), 3.60 (t, 2H, *J* = 5.06 *Hz*), 6.78 (d, 1H, *J* = 8.96 *Hz*), 7.02 – 7.08 (m, 3H), 7.13 (dd, 1H, J_1 = 8.96 *Hz,* J_2 = 3.00 *Hz*), 7.79 (d, 1H, *J* = 2.68 *Hz*), 9.08 (s, 1H)

^{13}C NMR (125 MHz, DMSO-d$_6$): δ 15.2 (CH_3), 20.9 (CH_3), 29.4 (CH_2), 33.7 (CH_2), 41.2 (CH_2), 45.1 (CH_2), 46.5 (CH_2), 46.7 (CH_2), 109.1 (CH), 125.7 (CH), 125.9 (CH), 127.3 (CH), 128.1 (CH), 134.63 (C), 134.67 (CH), 136.7 (C), 139.7 (C), 147.2 (C), 153.7 (C), 170.6 (C).

IR (ATR) / cm^{-1}: 3210, 2981, 1612, 1487, 1417, 1385, 1263, 1238, 1211, 1163, 1145, 986, 926, 827, 782, 718.

LCMS (ESI$^+$): calculated for $C_{20}H_{25}N_3O_2$ $[M+H]^+$ 340.1947; found *m/z* = 340.0687.

26) Synthesis of 3-(2,6-dimethylphenyl)-1-[4-(5-hydroxypyridin-2-yl)piperazin-1-yl] propan-1-one (52)



52

3-(2,6-Dimethylphenyl)propanoic acid (0.25 g, 1.40 mmol) was reacted with 6-piperazin-1-yl-pyridin-3-ol (**VIII**) (0.25 g, 1.40 mmol) as per procedure described for compound **31** to afford compound **52** (0.35 g, 1.03 mmol, 73% yield) as an off white solid.

Melting point: 160-162 °C. **LC purity (UV 245 nm):** 99.71%.

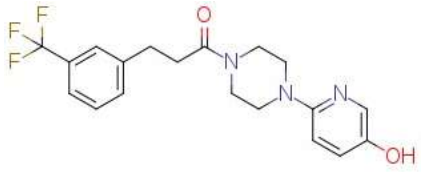
^{1}H NMR (500 MHz, DMSO-d$_6$): δ 2.33 (s, 6H), 2.52 – 2.53 (m, 2H), 2.88 (t, 2H, *J* = 8.26 *Hz*), 3.30 – 3.33 (m, 4H), 3.41 - 3.63 (m, 4H), 6.78 (d, 1H, *J* = 9.00 *Hz*), 7.03 (m, 3H), 7.13 (dd, 1H, J_1 = 8.96 *Hz,* J_2 = 3.00 *Hz*), 7.79 (d, 1H, *J* = 2.84 *Hz*), 9.06 (s, 1H)

^{13}C NMR (125 MHz, DMSO-d$_6$): δ 20.6 (2 x CH_3), 26.1 (CH_2), 32.6 (CH_2), 41.9 (CH_2), 45.8 (CH_2), 47.1 (CH_2), 47.4 (CH_2), 109.8 (CH), 126.6 (CH), 126.9 (CH), 129.1 (2 x CH), 135.3 (CH), 137.0 (2 x C), 139.1 (C), 147.8 (C), 154.3 (C), 171.4 (C).

IR (ATR) / cm^{-1}: 3134, 1613, 1566, 1488, 1459, 1440, 1301, 1266, 1233, 1200, 1156, 982, 818, 779.

LCMS (ESI$^+$): calculated for $C_{20}H_{25}N_3O_2$ $[M+H]^+$ 340.1947; found *m/z* = 340.2096.

27) Synthesis of 1-[4-(5-hydroxypyridin-2-yl)piperazin-1-yl]-3-[3-(trifluoromethyl) phenyl]propan-1-one (53)



53

3-[3-(Trifluoromethyl)phenyl]propanoic acid (0.50 g, 2.29 mmol) was reacted with 6-piperazin-1-yl-pyridin-3-ol (**VIII**) (0.41 g, 2.29 mmol) as per procedure described for compound **31** to afford compound **53** (0.42 g, 1.10 mmol, 48% yield) as an off white solid.

Melting point: 160-163 °C. **LC purity (UV 245 nm):** 99.70%.

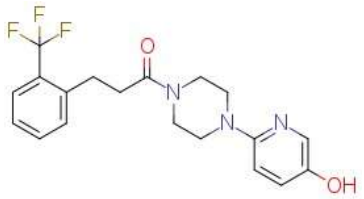
^{1}H NMR (500 MHz, DMSO-d$_6$): δ 2.78 (t, 2H, *J* = 7.65 *Hz*), 2.98 (t, 2H, *J* = 7.61 *Hz*), 3.28 – 3.31 (m, 4H), 3.56 – 3.61 (m, 4H), 6.78 (d, 1H, *J* = 8.99 *Hz*), 7.12 (dd, 1H, J_1 = 8.96 *Hz*, J_2 = 2.99 *Hz*), 7.54 – 7.59 (m, 2H), 7.63 (d, 1H, *J* = 7.10 *Hz*), 7.68 (s, 1H), 7.79 (d, 1H, *J* = 2.90 *Hz*), 9.09 (s, 1H).

^{13}C NMR (125 MHz, DMSO-d$_6$): δ 30.9 (CH_2), 34.0 (CH_2), 41.3 (CH_2), 45.0 (CH_2), 46.5 (CH_2), 46.8 (CH_2), 109.1 (CH), 123.5 (CH), 124.3 (C), 125.9 (CH), 126.2 (CH), 129.3 (C), 129.7 (CH), 133.3 (CH), 134.6 (CH), 143.4 (C), 147.2 (C), 153.7 (C), 170.2 (C).

IR (ATR) / cm^{-1}: 3284, 2823, 1623, 1489, 1439, 1334, 1263, 1235, 1219, 1200, 1151, 1111, 1094, 1070, 1024, 921, 823, 704, 676.

LCMS (ESI$^+$): calculated for $C_{19}H_{20}F_3N_3O_2$ $[M+H]^+$ 380.15; found *m/z* = 380.29.

28) Synthesis of 1-[4-(5-hydroxypyridin-2-yl)piperazin-1-yl]-3-[2-(trifluoromethyl) phenyl]propan-1-one (54)



54

3-[2-(Trifluoromethyl)phenyl]propanoic acid (0.50 g, 2.29 mmol) was reacted with 6-piperazin-1-yl-pyridin-3-ol (**VIII**) (0.41 g, 2.29 mmol) as per procedure described for compound **31** to afford compound **54** (0.55 g, 1.44 mmol, 62% yield) as an off white solid.

Melting point: 161-163 °C. **LC purity (UV 245 nm):** 99.47%.

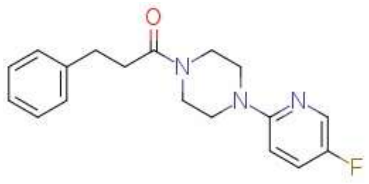
^{1}H NMR (500 MHz, DMSO-d$_6$): δ 2.73 (t, 2H, *J* = 7.98 *Hz*), 3.05 (t, 2H, *J* = 7.93 *Hz*), 3.32 - 3.33 (m, 4H), 3.56 (t, 2H, *J* = 4.47 *Hz*), 3.62 (t, 2H, *J* = 4.71 *Hz*), 6.8 (d, 1H, *J* = 8.9 *Hz*), 7.13 (dd, 1H, J_1 = 8.92 *Hz,* J_2 = 2.69 *Hz*), 7.47 (t, 1H, *J* = 7.57 *Hz*), 7.60 (d, 1H, *J* = 7.69 *Hz*), 7.67 (t, 1H, *J* = 7.52 *Hz*), 7.73 (d, 1H, *J* = 7.88 *Hz*), 7.79 (d, 1H, *J* = 2.62 *Hz*), 9.1 (s, 1H).

^{13}C NMR (125 MHz, DMSO-d$_6$): δ 27.8 (CH_2), 34.3 (CH_2), 41.3 (CH_2), 44.9 (CH_2), 46.5 (CH_2), 46.7 (CH_2), 109.1 (CH), 125.9 (CH), 126.0 (CH), 126.2 (C), 127.1 (CH), 127.3 (C), 131.9 (CH), 133.0 (CH), 134.6 (CH), 140.4 (C), 147.2 (C), 153.7 (C), 169.9 (C).

IR (ATR) / cm^{-1}: 3208, 1614, 1488, 1474, 1436, 1308, 1265, 1237, 1226, 1213, 1152, 1113, 1036, 1016, 921, 820, 775.

LCMS (ESI$^+$): calculated for $C_{19}H_{20}F_3N_3O_2$ $[M+H]^+$ 380.15; found *m/z* = 380.29.

29) Synthesis of 1-[4-(5-fluoropyridin-2-yl)piperazin-1-yl]-3-phenylpropan-1-one (31a)



31a

3-Phenylpropionic acid (0.40 g, 2.66 mmol) was reacted with commercially available 1-(5-fluoro-2-pyridyl)piperazine (0.48 g, 2.66 mmol) as per procedure described for compound **31** to afford compound **31a** (0.51 g, 1.62 mmol, 61% yield) as an off white solid.

Melting point: 112-115 °C. **LC purity (UV 245 nm):** 98.61%.

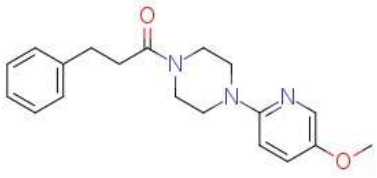
^{1}H NMR (400 MHz, DMSO-d$_6$): δ 2.72 (t, 2H, *J* = 7.66 *Hz*), 2.88 (t, 2H, *J* = 7.64 *Hz*), 3.53 – 3.59 (m, 4H), 3.61 – 3.63 (m, 4H), 7.10 (dd, 1H, J_1 = 9.52 *Hz,* J_2 = 3.60 *Hz*), 7.20 – 7.26 (m, 1H), 7.29 – 7.34 (m, 4H), 7.73 – 7.78 (m, 1H), 8.18 (d, 1H, *J* = 2.76 *Hz*).

^{13}C NMR (100 MHz, DMSO-d$_6$): δ 32.9 (CH_2), 36.1 (CH_2), 42.4 (CH_2), 46.0 (CH_2), 48.1 (CH_2), 48.3 (CH_2), 114.6 (CH), 128.1 (CH), 129.8 (CH, d, $J_{C\text{-}C\text{-}F}$ = 29.55 *Hz*), 130.5 (2 x CH), 130.7 (2 x CH), 133.0 (CH, d, $J_{C\text{-}C\text{-}F}$ = 21.34 *Hz*), 143.6 (C), 154.2 (C, d, $J_{C\text{-}F}$ = 239.24 *Hz*), 154.6 (C), 172.6 (C).

IR (ATR) / cm^{-1}: 3075, 2582, 1632, 1603, 1548, 1444, 1415, 1351, 1286, 1269, 1253, 1204, 1034, 810, 757, 701.

LCMS (ESI$^+$): calculated for $C_{18}H_{20}FN_3O$ $[M+H]^+$ 314.16; found: *m/z* = 314.35.

30) Synthesis of 1-[4-(5-methoxypyridin-2-yl)piperazin-1-yl]-3-phenylpropan-1-one (31b)



31b

3-Phenylpropionic acid (0.10 g, 0.66 mmol) was reacted with commercially available 1-(5-methoxy-2-pyridyl)piperazine (0.13 g, 0.66 mmol) as per procedure described for compound **31** to afford compound **31b** (0.13 g, 0.39 mmol, 59% yield) as an off white solid.

Melting point: 125-127 °C. **LC purity (UV 245 nm):** 99.59%.

^{1}H NMR (400 MHz, DMSO-d$_6$): δ 2.68 (t, 2H, *J* = 7.76 *Hz*), 2.83 (t, 2H, *J* = 7.68 *Hz*), 3.50 (m, 4H), 3.59 (m, 4H), 3.78 (s, 3H), 7.17 (t, 2H, *J* = 6.6 *Hz*), 7.24 - 7.29 (m, 4H), 7.62 (d, 1H, *J* = 5.86 *Hz*), 7.71 (s, 1H).

^{13}C NMR (100 MHz, DMSO-d$_6$): δ 31.0 (CH_2), 34.2 (CH_2), 44.1 (2 x CH_2), 46.5 (CH_2), 46.6 (CH_2), 56.9 (CH_3), 113.7 (CH), 121.5 (CH), 126.2 (CH), 128.6 (2 x CH), 128.8 (2 x CH), 134.1 (CH), 141.7 (C), 148.4 (C), 149.9 (C), 170.7 (C).

IR (ATR) / cm^{-1}: 3392, 2574, 1634, 1602, 1542, 1466, 1420, 1349, 1257, 1223, 1145, 1020, 836, 816, 750, 696.

LCMS (ESI$^+$): calculated for $C_{19}H_{23}N_3O_2$ $[M+H]^+$ 326.18; found: *m/z* = 326.31.

31) Synthesis of 1-[4-(5-hydroxy-pyridin-2-yl)-piperazin-1-yl]-3-phenyl-propan-1-one di-hydrochloride salt (31-DH)

31-DH

Compound **31** (1 g, 3.21 mmol) was dissolved in 10 ml 4N HCl solution in 1,4-dioxane and stirred for 10 minutes. Distilled out the solvents under vacuum and stirred the residue with 10 ml acetone. Filtered the precipitated solid under nitrogen, washed with 1 ml acetone and dried

under vacuum to afford compound **31-DH** (1.1 g, 2.86 mmol, 89% yield) as a pale-yellow powder.

^{1}H NMR (400 MHz, DMSO-d$_6$): δ 2.71 (t, 2H, *J* = 7.68 *Hz*), 2.88 (t, 2H, *J* = 7.68 *Hz*), 3.30 (t, 4H, *J* = 4.68 *Hz*), 3.56 (t, 2H, *J* = 4.92 *Hz*), 3.60 (t, 2H, *J* = 5.00 *Hz*), 6.80 (d, 1H, *J* = 9.00 *Hz*), 7.15 (dd, 1H, J_1 = 8.93 *Hz*, J_2 = 2.86 *Hz*), 7.20 - 7.37 (m, 5H), 7.79 (d, 1H, *J* = 2.75 *Hz*).

Chloride content [assay by titrimetry (Silver nitrate)] : 21.27 % (Theoritical chloride content for dihydrochloride salt : 18.50 %).

32) Synthesis of 1-[4-(5-hydroxy-pyridin-2-yl)-piperazin-1-yl]-3-phenyl-propan-1-one mono hydrochloride salt (31-H)

31-H

Compound **31-DH** (1 g, 2.67 mmol) was suspended in 15 ml isopropyl alcohol and heated to 90 °C. Resultant clear solution was refluxed for 1h and further stirred at room temp for 60 min. Precipitated solid was filtered under nitrogen and washed with 2 ml isopropyl alcohol. Filtered solid was dried under vacuum to afford compound **31-H** (0.8 g, 2.29 mmol, 85% yield) as a pale-yellow powder.

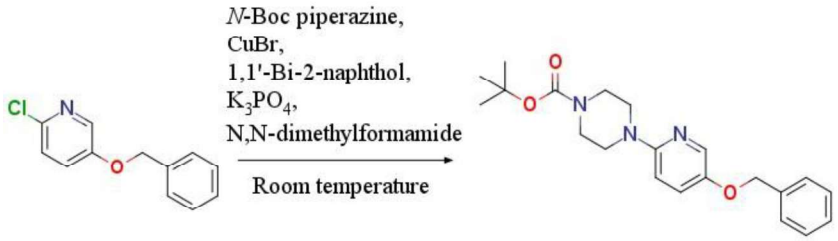
^{1}H NMR (400 MHz, DMSO-d$_6$): δ 2.74 (t, 2H, *J* = 7.68 *Hz*), 2.86 (t, 2H, *J* = 7.68 *Hz*), 3.59 (m, 4H), 3.66 (m, 4H), 7.21-7.25 (m, 1H), 7.26-7.35 (m, 5H), 7.66 (d, 1H, *J* = 2.76 *Hz*), 7.73 (d, 1H, *J* = 9.56 *Hz*), 10.36 (br-s, 1H).

Chloride content [assay by titrimetry (Silver nitrate)] : 10.34 % (Theoritical chloride content for mono hydrochloride salt : 10.20 %).

33) Novel copper-catalysed synthesis of *tert*-butyl 4-(5-benzyloxy-2-pyridyl)piperazine-1-carboxylate (VI)

For cost effective scale up of key intermediate **VIII** to produce required quantities of compound **31** for *in vivo* studies, a convenient copper-catalysed synthesis of intermediate **VII** was developed using cuprous bromide (CuBr) as the catalyst, 1,1′-bi-2-naphthol as the ligand and K_3PO_4 as the base.

Scheme 3.2: Copper-catalysed synthesis of intermediate **VII**.



To a solution of 5-benzyloxy-2-chloro-pyridine (**V**) (2 g, 9.10 mmol) in N,N-dimethylformamide (10 ml) was added copper (I) bromide (0.26 g, 1.82 mmol) and 1,1′-bi-2-naphthol (0.39 g, 1.35 mmol). Resultant reaction mixture was stirred at room temperature for 10 min before adding *N*-Boc piperazine (1.69 g, 9.10 mmol) and K_3PO_4 (3.86 g, 2.27 mmol). The resultant reaction mixture was stirred at room temp. for 2h. On completion of the reaction, water (50 ml) and ethyl acetate (30 ml) was added to reaction mixture and stirred for 10 min. The mixture was passed through celite bed and the bed was washed with ethyl acetate (2 x 20 ml). Brine solution (30 ml) was added to filtrate and organic layer was separated. Aqueous layer was further extracted with ethyl acetate (30 ml). Combined organic extracts were washed with water followed by brine, dried over anhydrous sodium sulfate and were concentrated under reduced pressure. The residue was purified using column chromatography on silica gel using ethyl acetate:hexane mixture as eluent to afford compound *tert*-butyl 4-(5-benzyloxy-2-pyridyl)piperazine-1-carboxylate (**VI**) (2.10 g, 5.68 mmol, 62% yield) as an off white solid.

This reaction was also performed using *N,N′*-dimethylethylenediamine (DMEDA) and *N,N, N′,N′*-tetramethyl ethylenediamine (TMEDA) as ligands in lieu of 1,1′-bi-2-naphthol but it was unsuccessful as no product formation was observed.

3.8 Biological evaluation of compounds

3.8.1 ME2 and ME1 inhibition data for selected compounds

Among the synthesized compounds (**28-54**) presented in this chapter, some compounds were selected based on their ME3 inhibitory potency and BxPC-3 cell growth inhibition and screened in vitro on other ME isoforms to evaluate their selectivity for ME3. The results are presented in **Table 3.7**.

Table 3.7: ME2 and ME1 inhibition data for selected compounds.

Compound	% inhibition of ME2			ME2 IC_{50} (µM)	% inhibition of ME1			ME1 IC_{50} (µM)
	0.1 µM	1 µM	10 µM		0.1 µM	1 µM	10 µM	
28	12	47	90	0.98	3	31	98	1.60
31	0	31	73	1.72	4	24	97	1.50
34	0	30	100	-	0	0	86	-
37	0	16	99	1.66	15	40	94	1.74
38	0	34	90	1.49	0	80	99	0.97
39	1	38	98	1.37	2	33	92	2.37
40	0	47	100	1.11	0	34	96	1.30
41	8	59	100	-	1	17	92	-
42	1	40	90	1.29	7	24	98	1.32
43	0	12	91	1.63	11	27	94	1.20
44	0	29	79	1.97	3	20	89	1.80
45	0	42	98	1.50	9	31	97	2.66
46	14	37	99	1.43	0	29	97	1.68
47	0	40	100	1.53	0	10	97	1.50
48	0	30	99	-	0	11	97	-
50	0	28	99	1.33	1	07	98	3.08
51	0	34	99	1.47	4	25	94	3.32
52	0	42	97	1.62	0	20	98	2.00
53	5	23	75	-	3	6	93	-
54	0	25	95	-	15	31	95	-

in vitro screening data of selected compounds suggested that they have certain degree of selectivity towards ME3 over other ME isoforms.

3.9 Conclusion

In summary, total **54** compounds were prepared so far to establish structure activity relationship (SAR) for potent and selective inhibition of ME3. The structural elements that are critical for

selective ME3 inhibition were identified (**Figure 3.5**). Potent and cell-active ME3 inhibitors that show selectivity over the other ME isoforms were discovered for the first time. Systematic SAR study led to compound **31** which showed improved isoform selectivity, favourable PK profile and a good efficacy in animal xenograft model. Overall pre-clinical data suggest that the development of a potent and selective ME3 inhibitor like compound **31** could be a viable therapeutic option for the safe and effective treatment of PDAC.

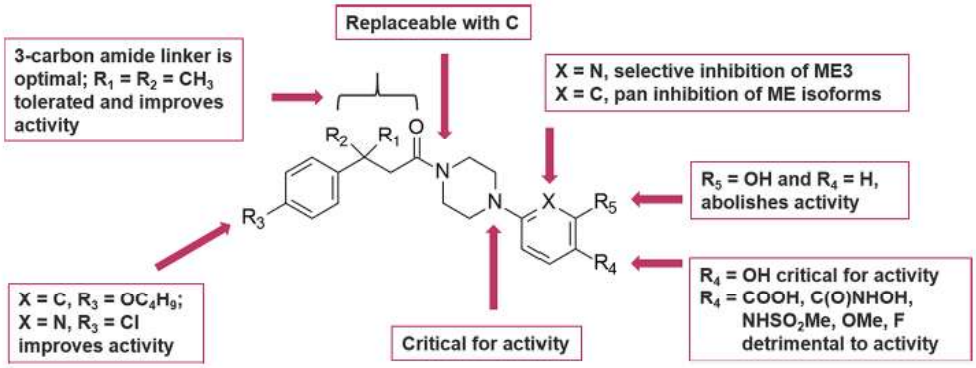
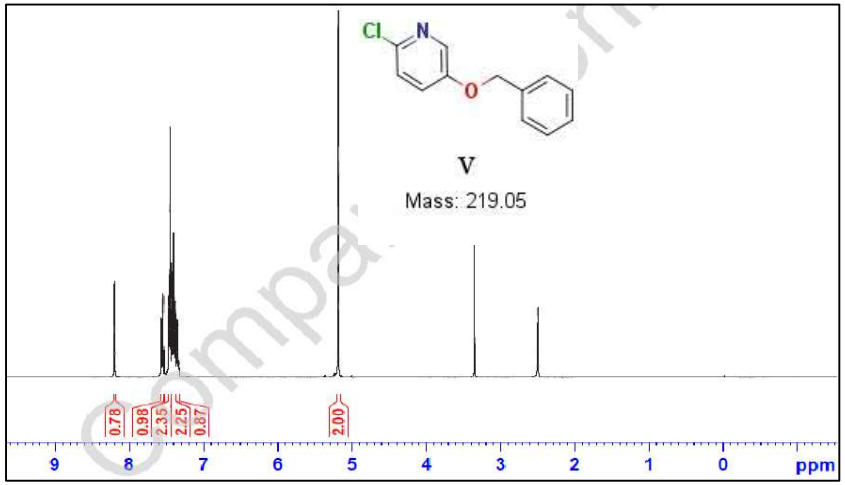
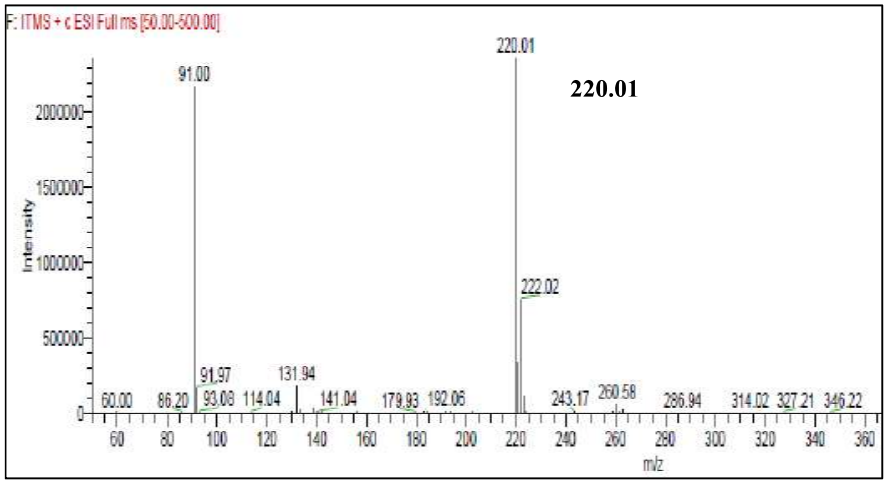


Figure 3.5. SAR summary for ME3 enzyme inhibition (chapter 3)

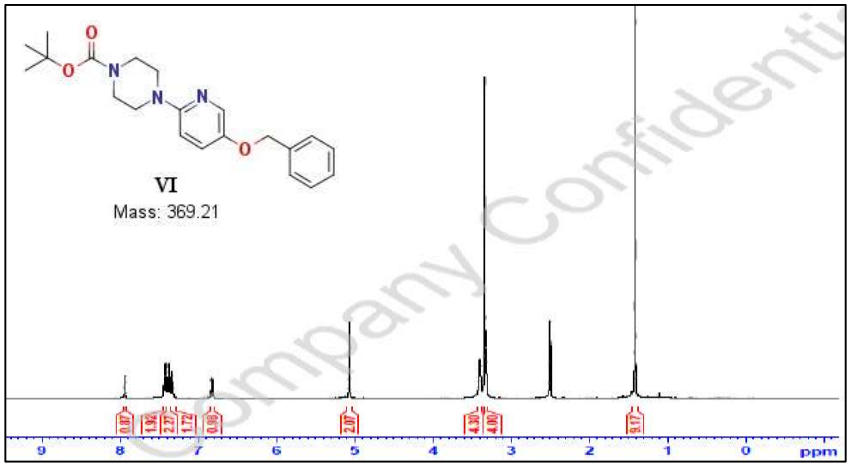
3.10 Spectral data

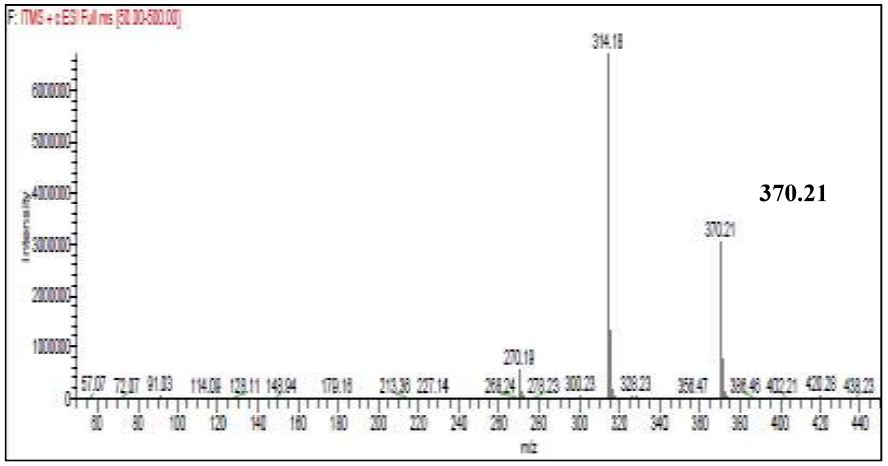
Spectral data of **Intermediate-V**



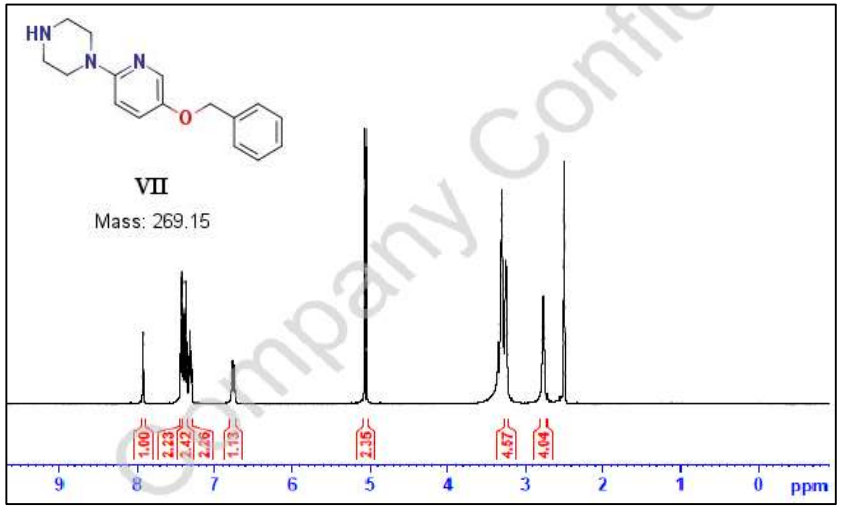


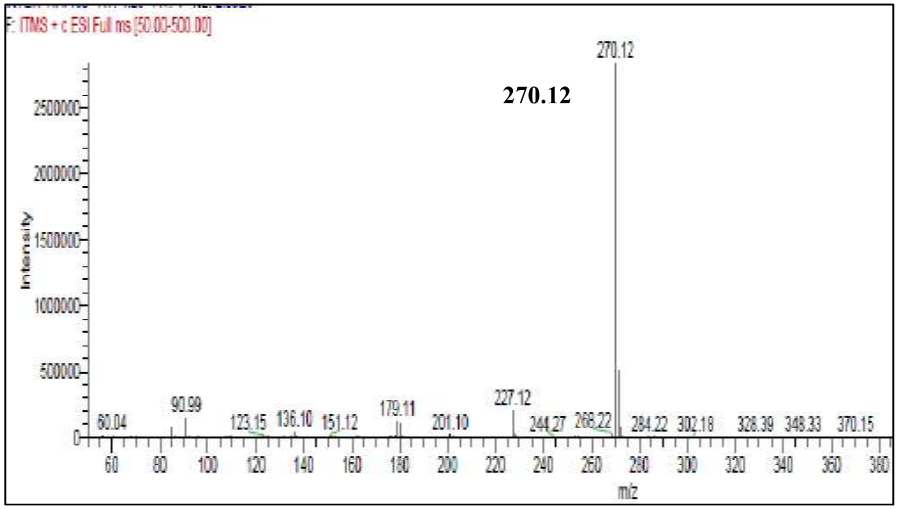
Spectral data of **Intermediate-VI**



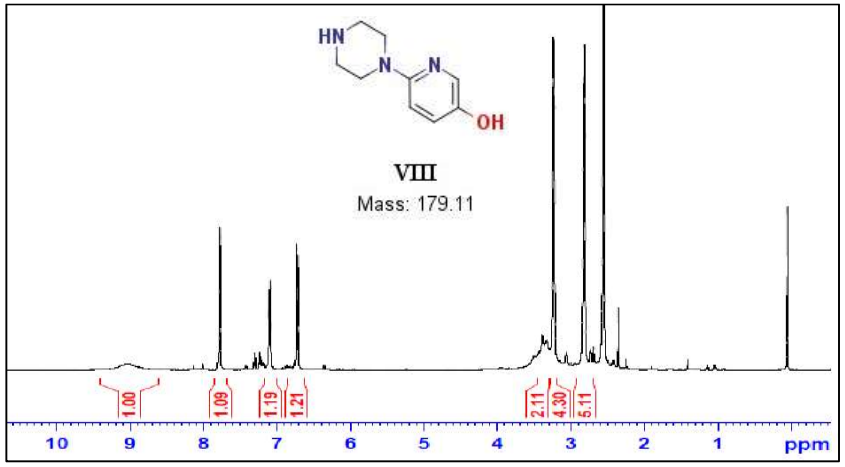


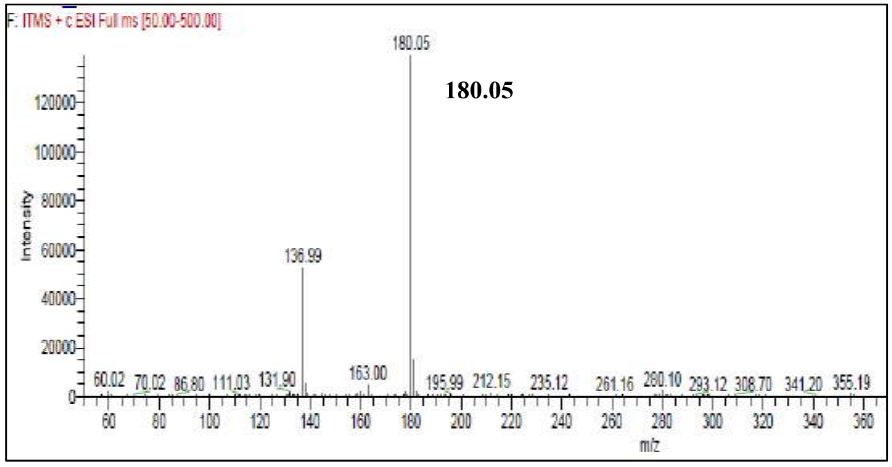
Spectral data of **Intermediate-VII**



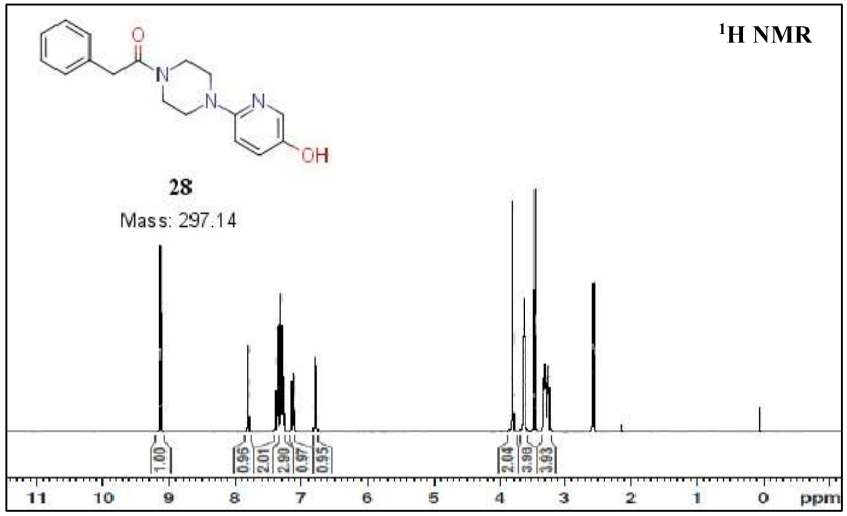


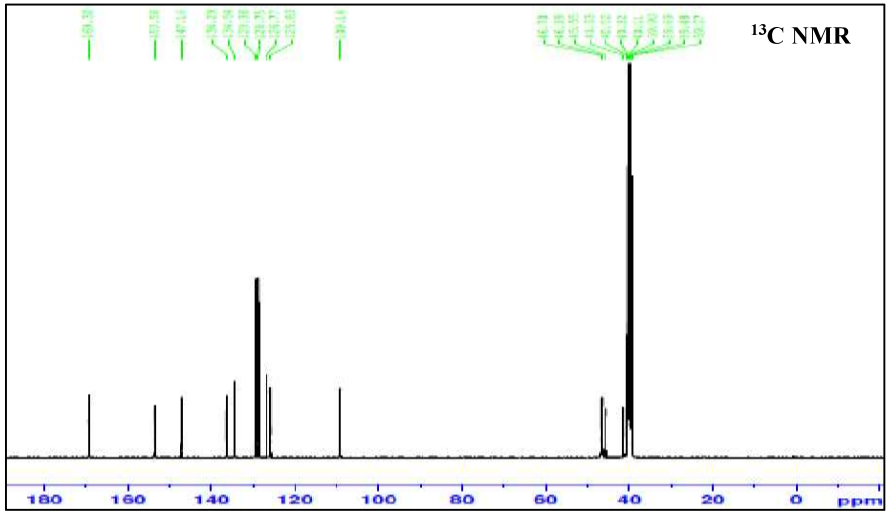
Spectral data of **Intermediate-VIII**

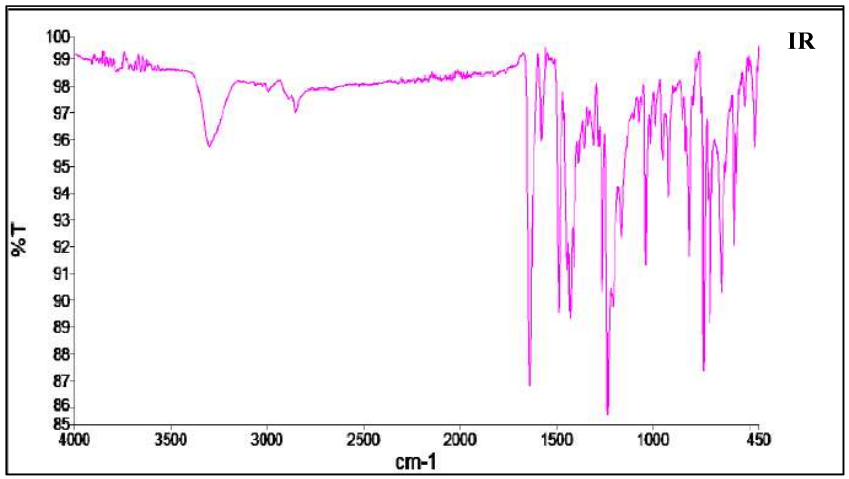


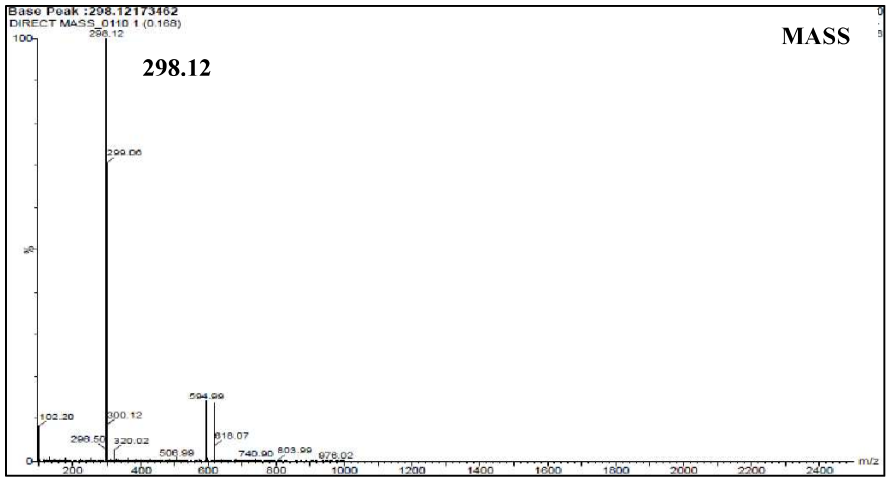


Spectral data of **28**

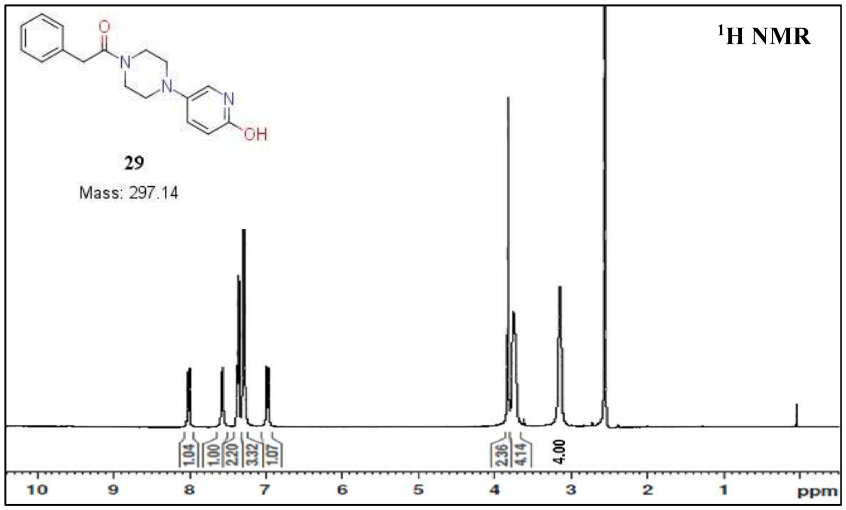


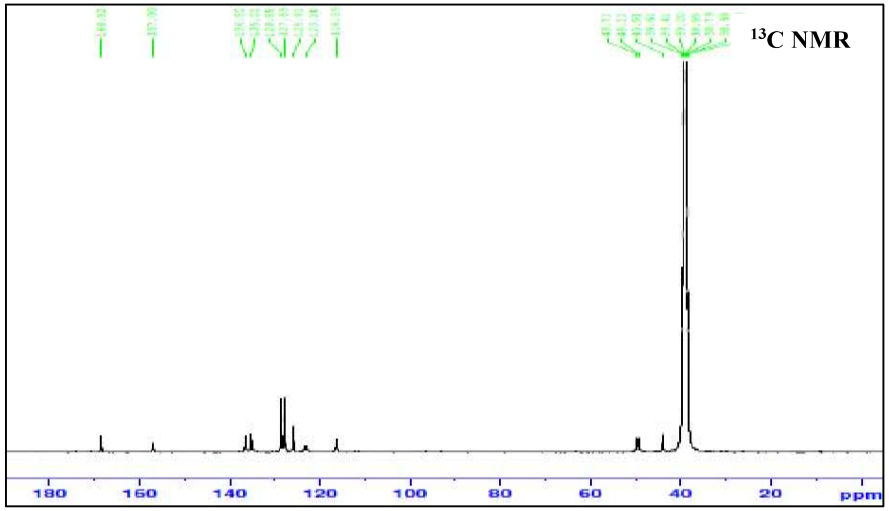


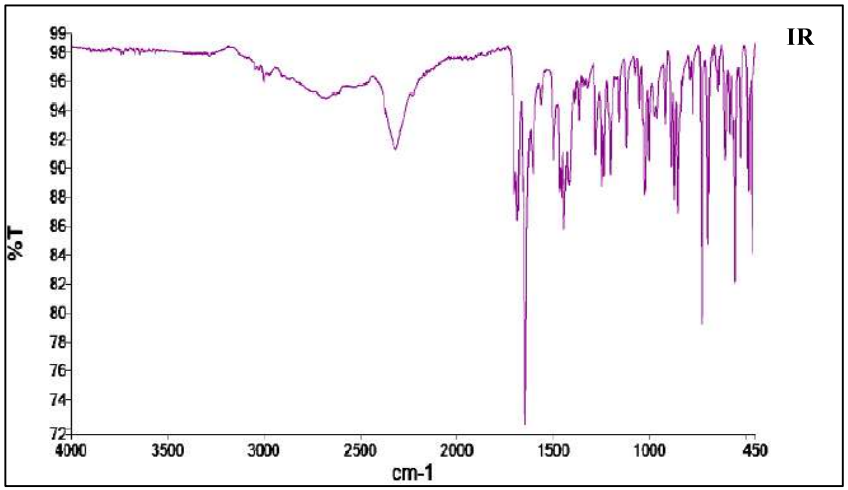


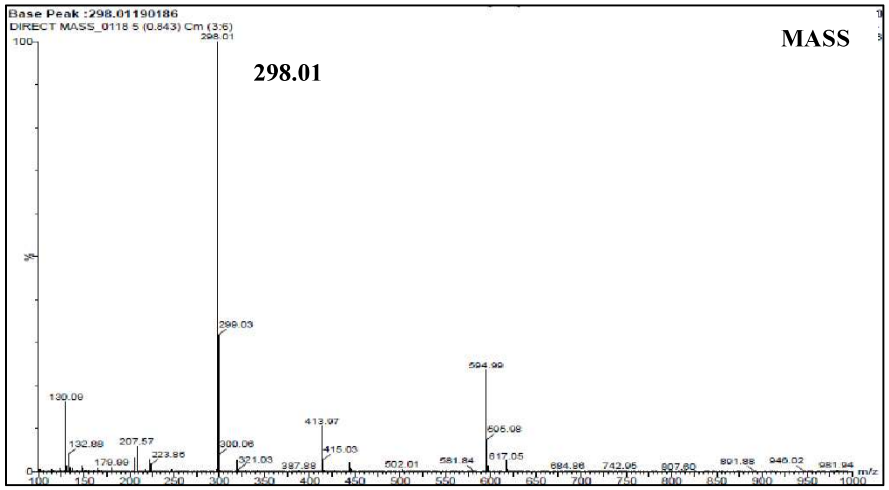


Spectral data of **29**

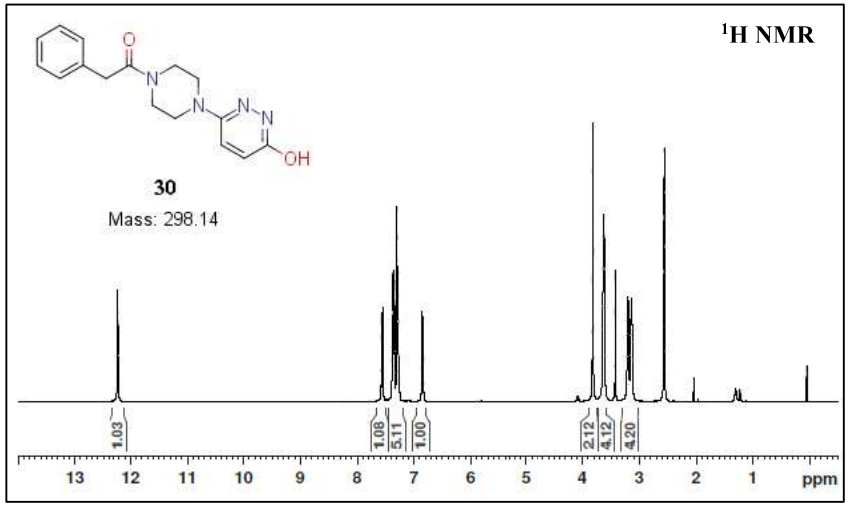


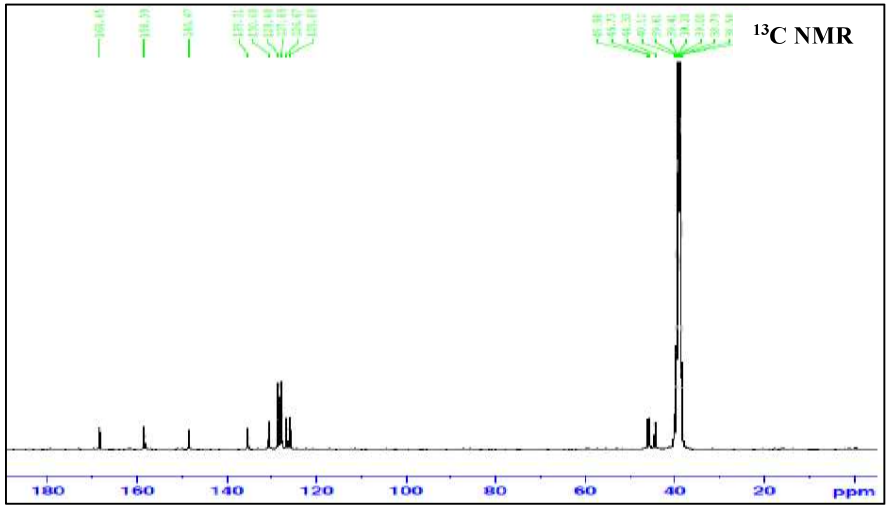


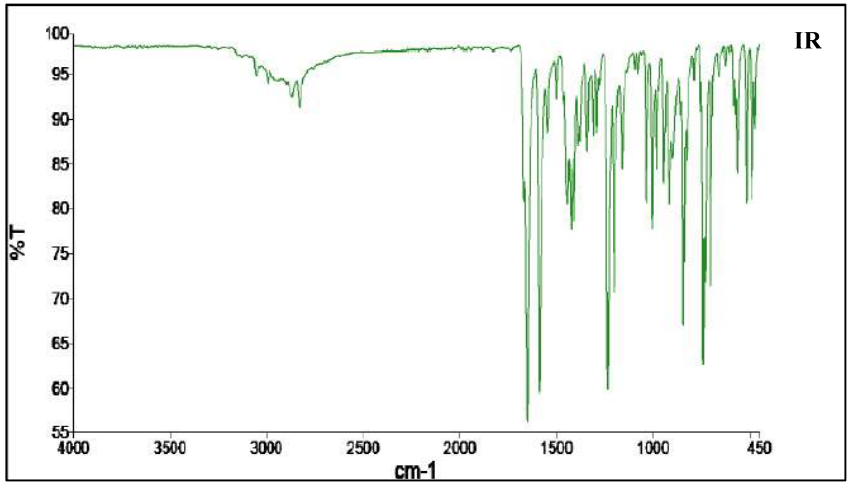


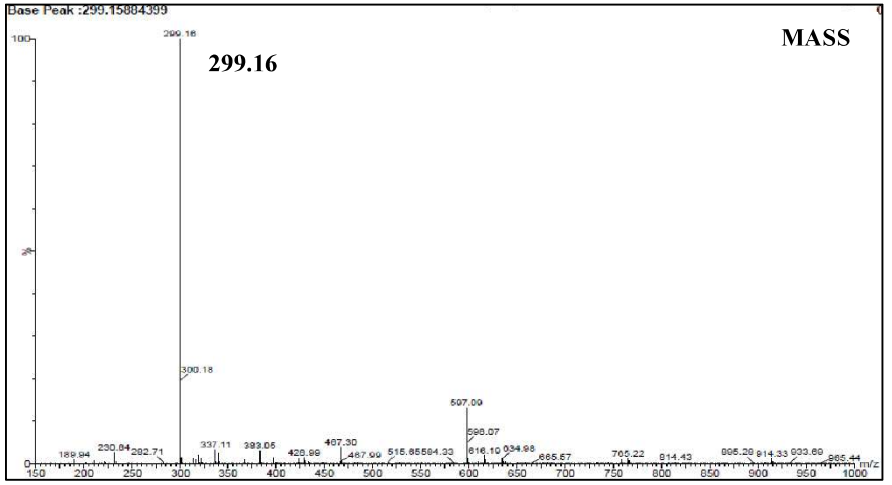


Spectral data of **30**

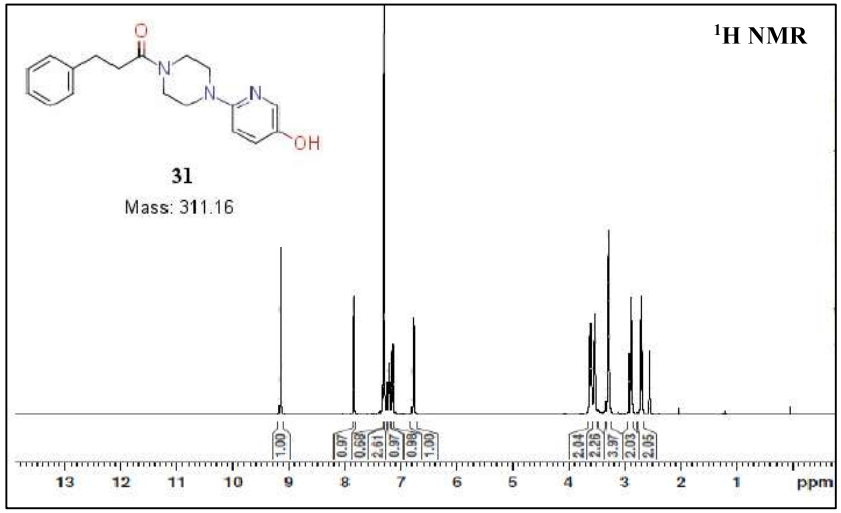


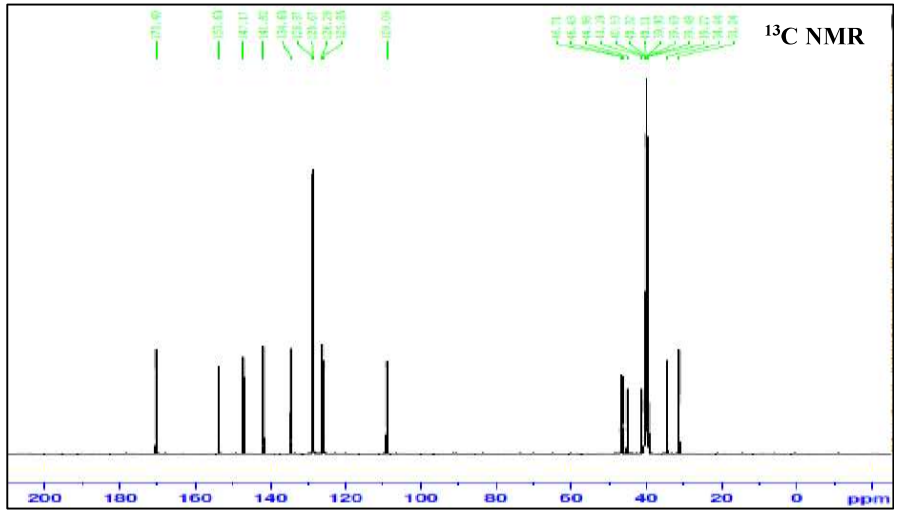


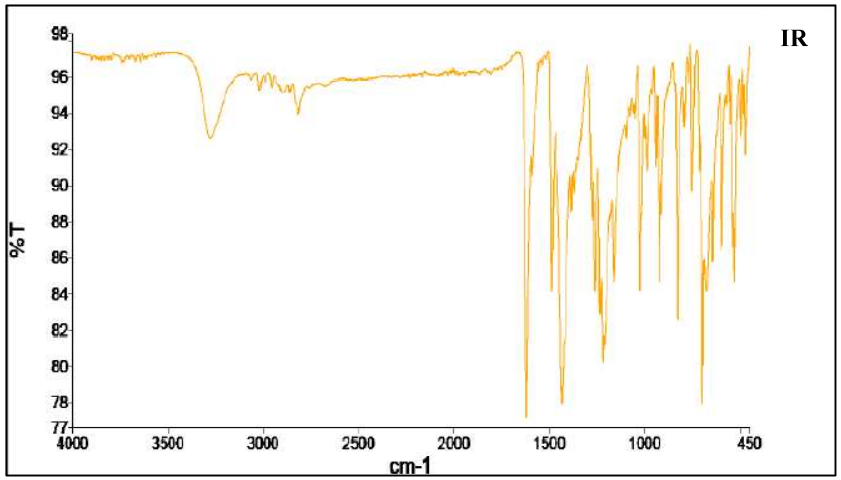


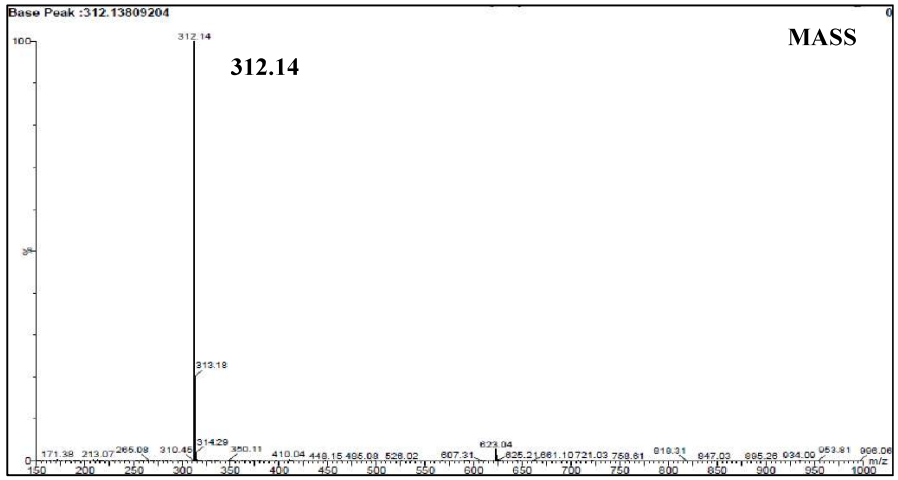


Spectral data of **31**

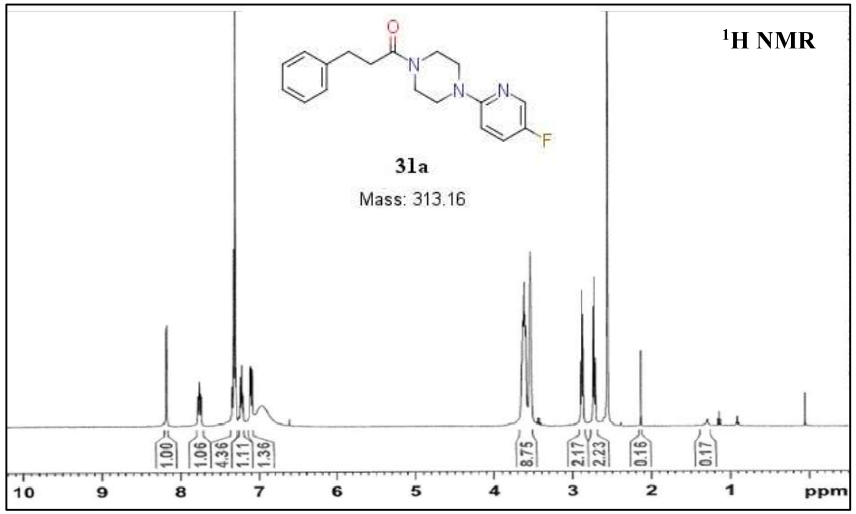


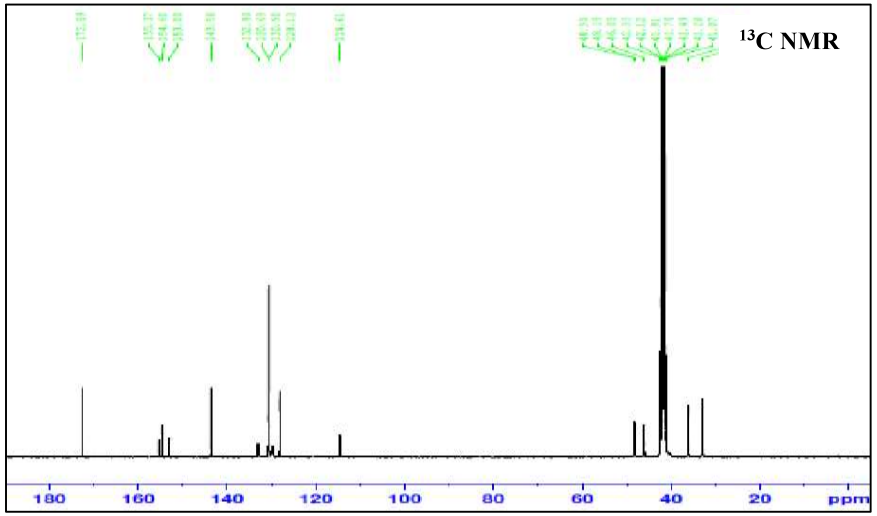


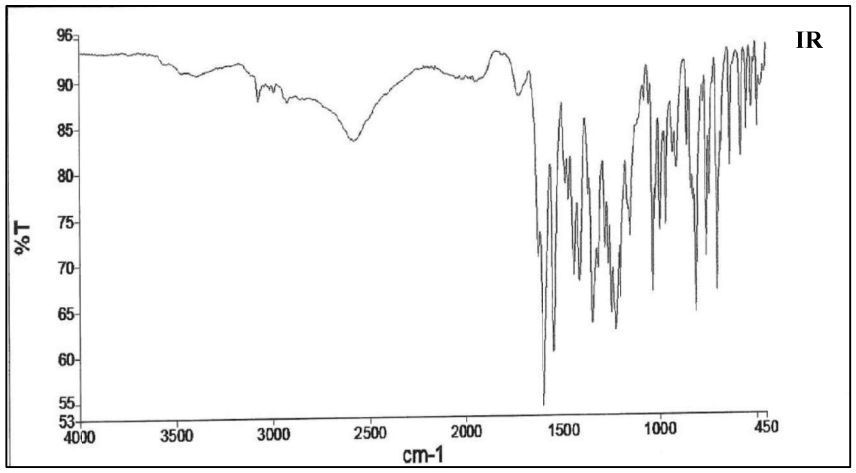


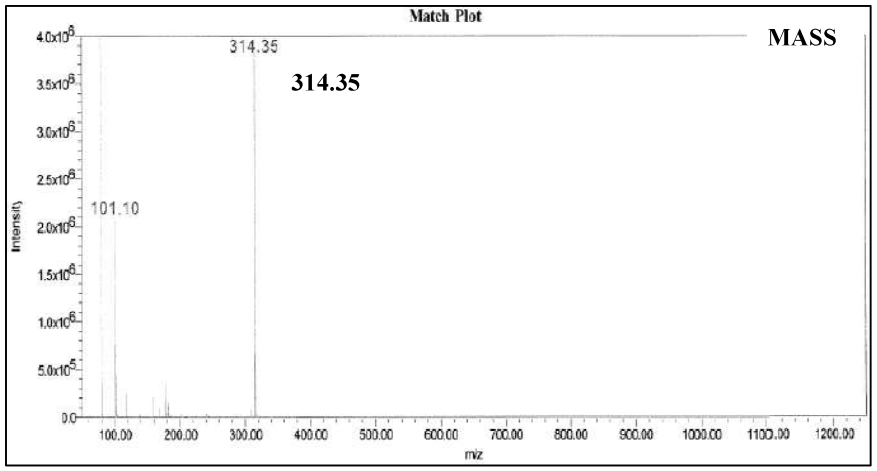


Spectral data of **31a**

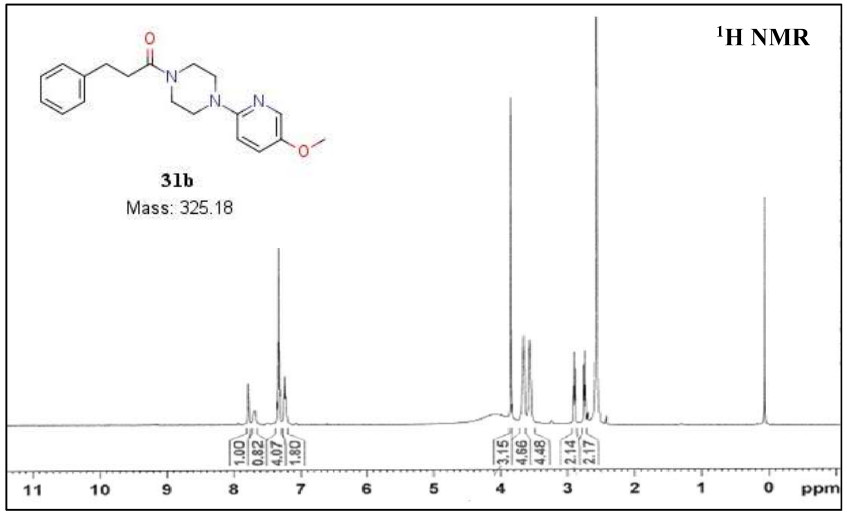


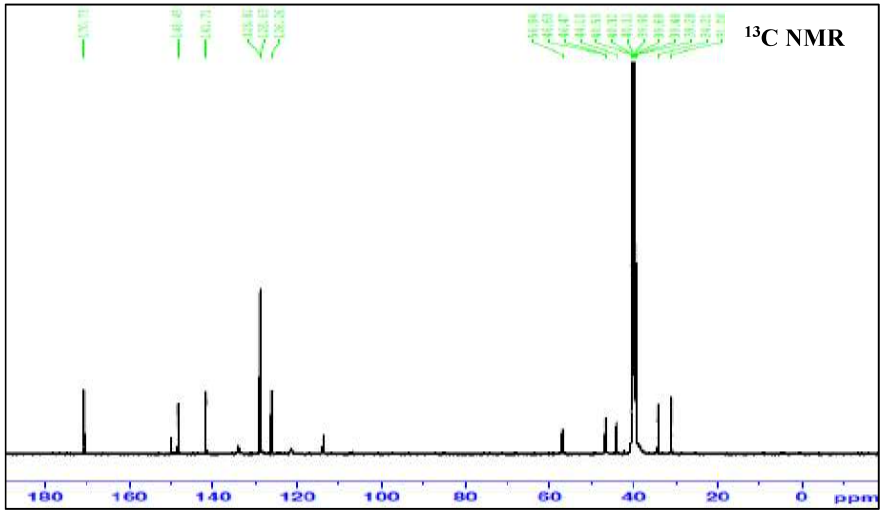


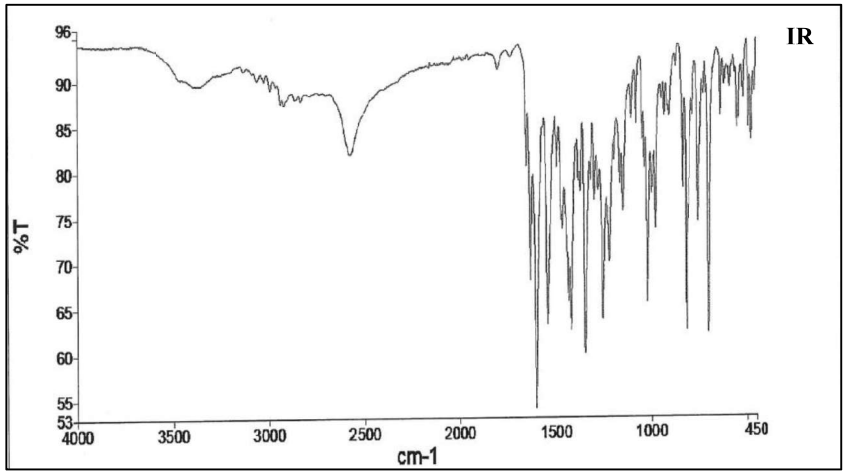


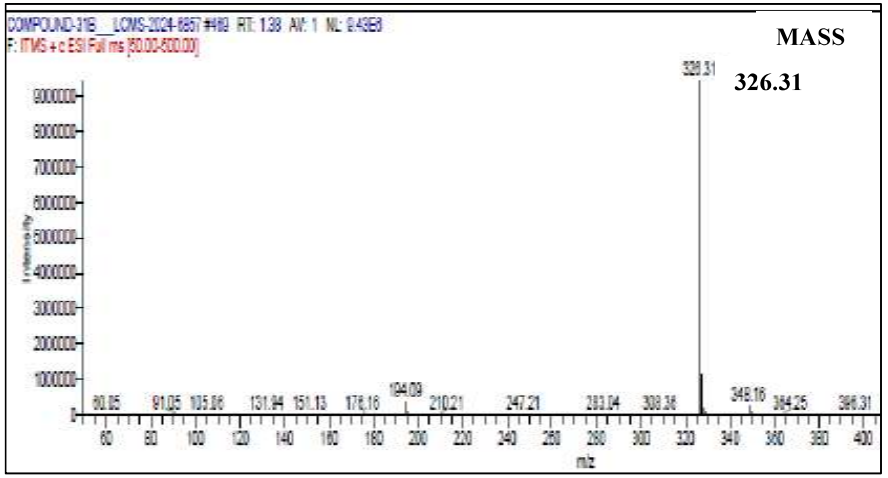


Spectral data of **31b**

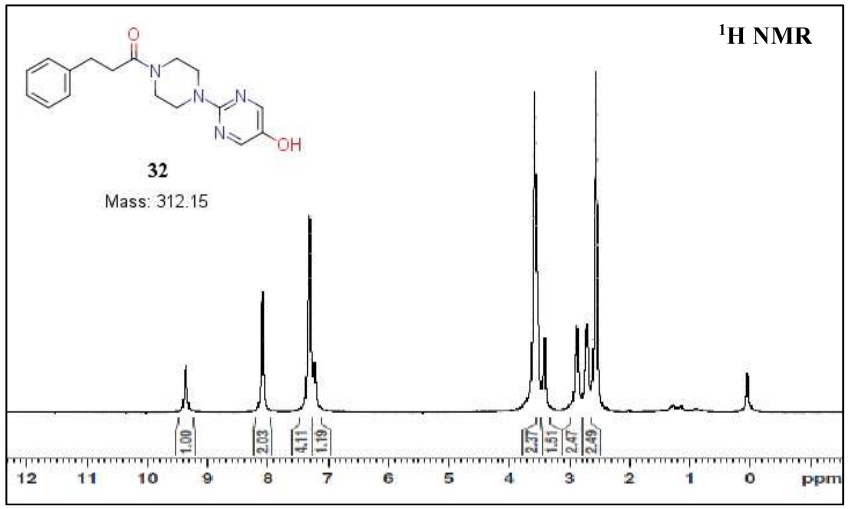


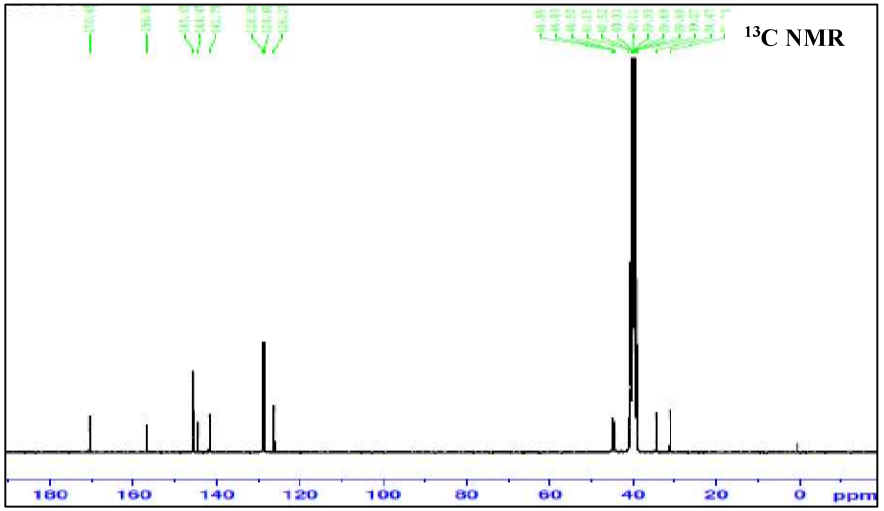


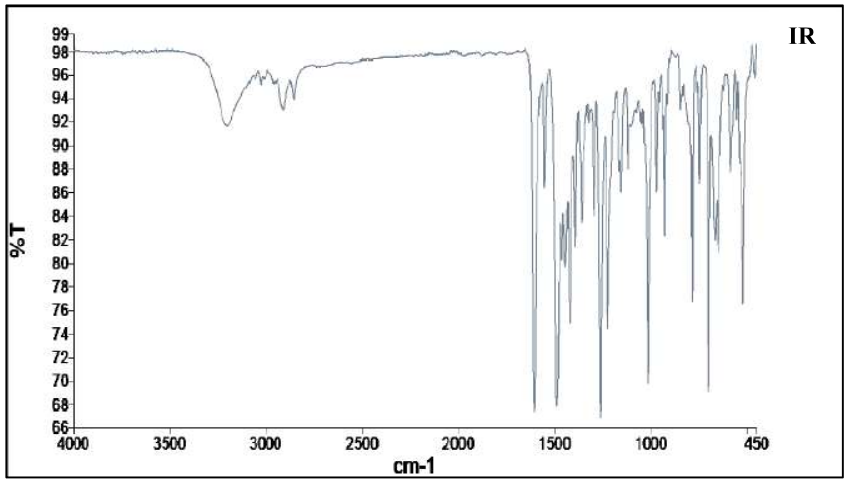


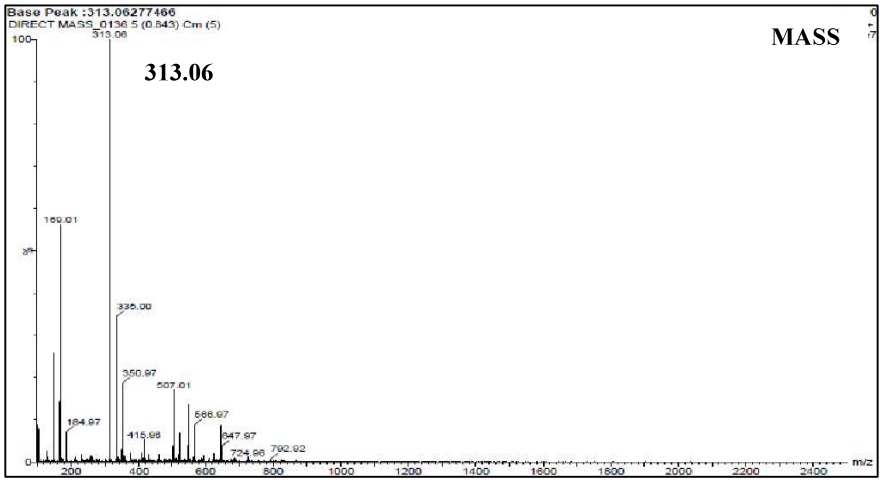


Spectral data of **32**









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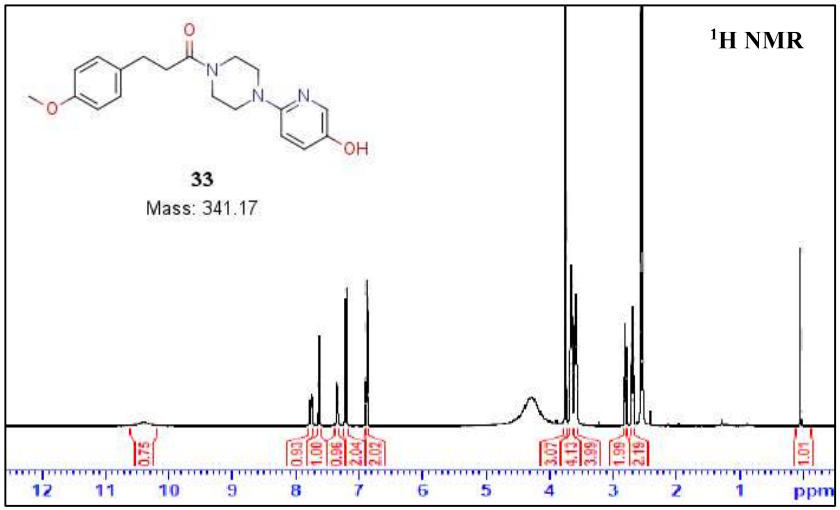
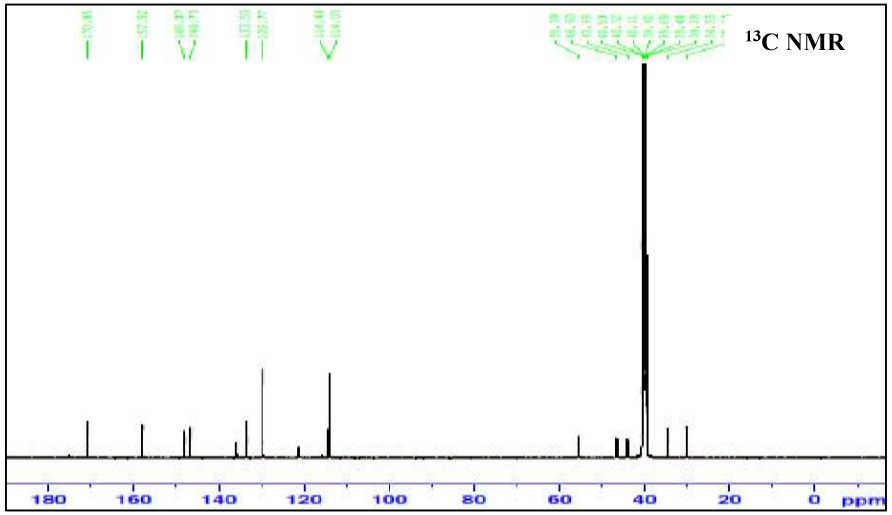
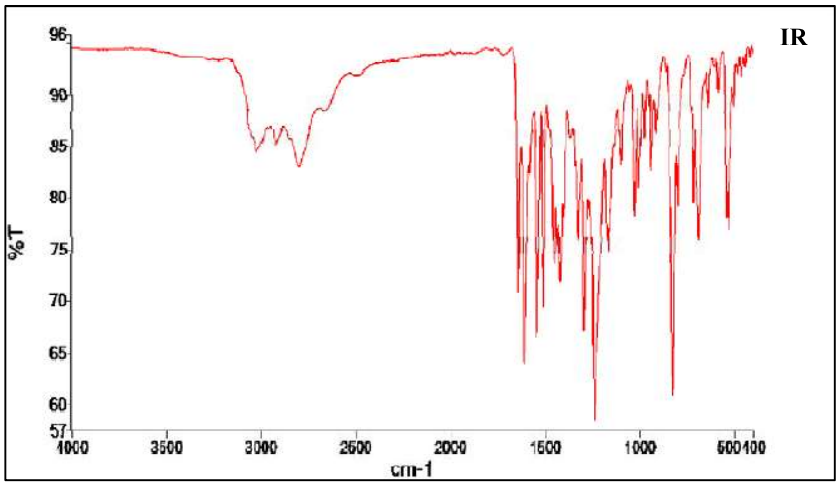
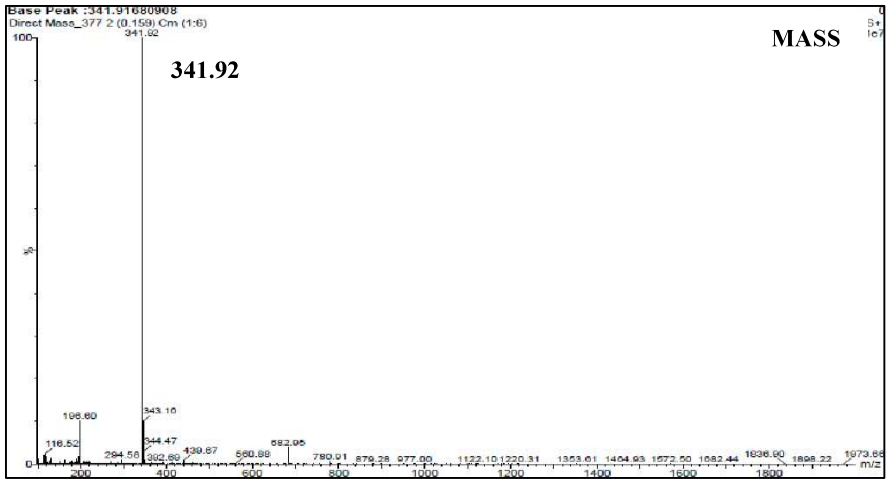
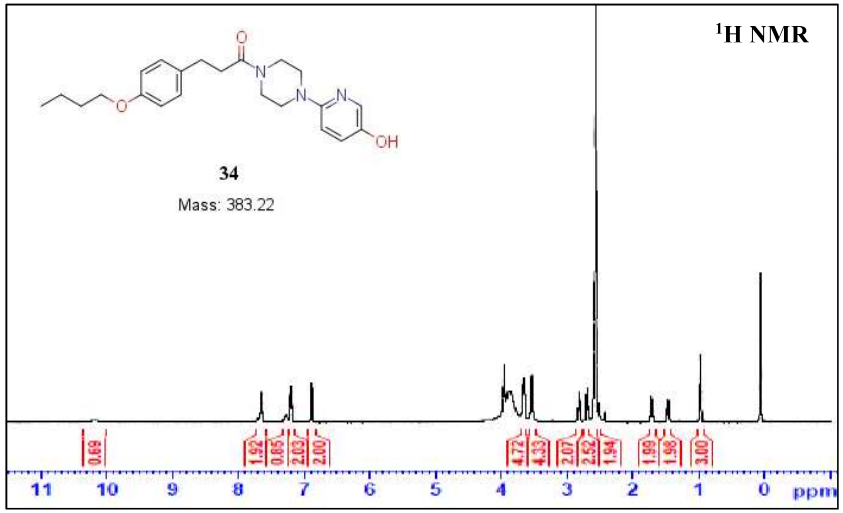


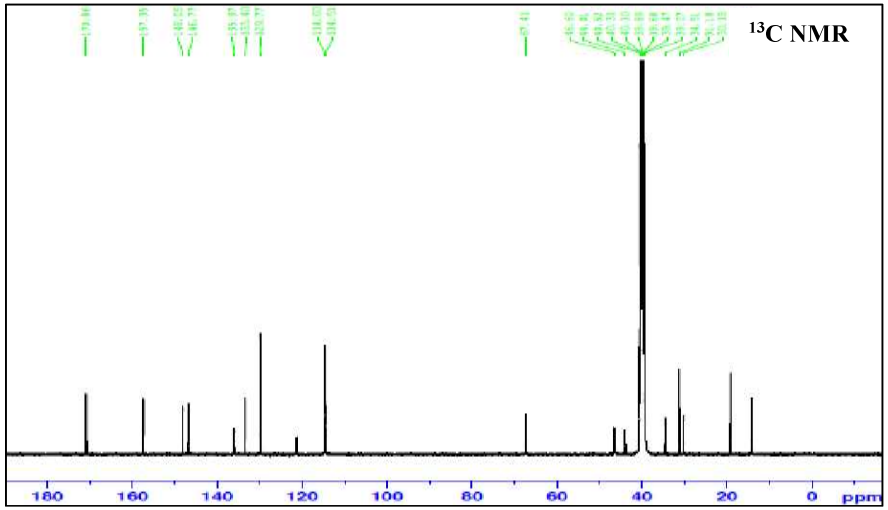


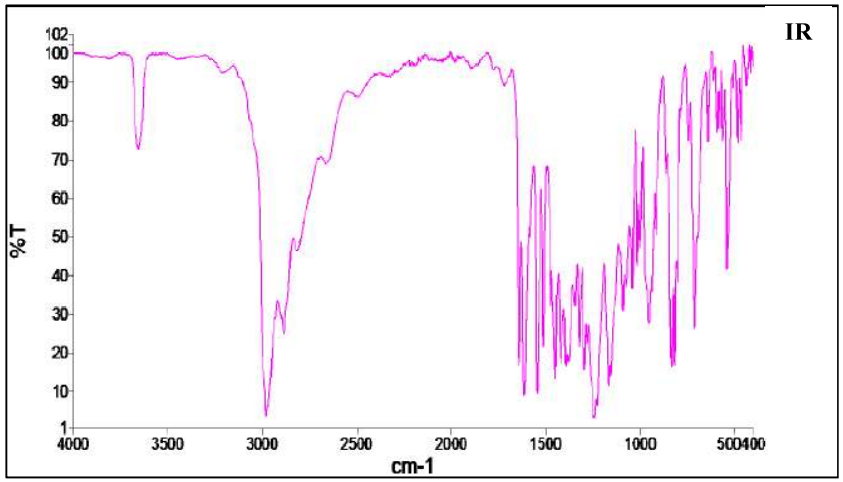


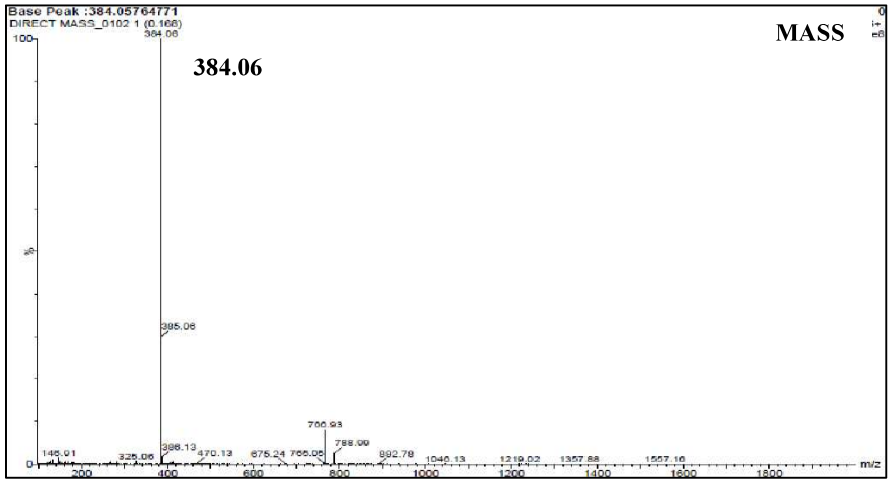


Spectral data of **34**









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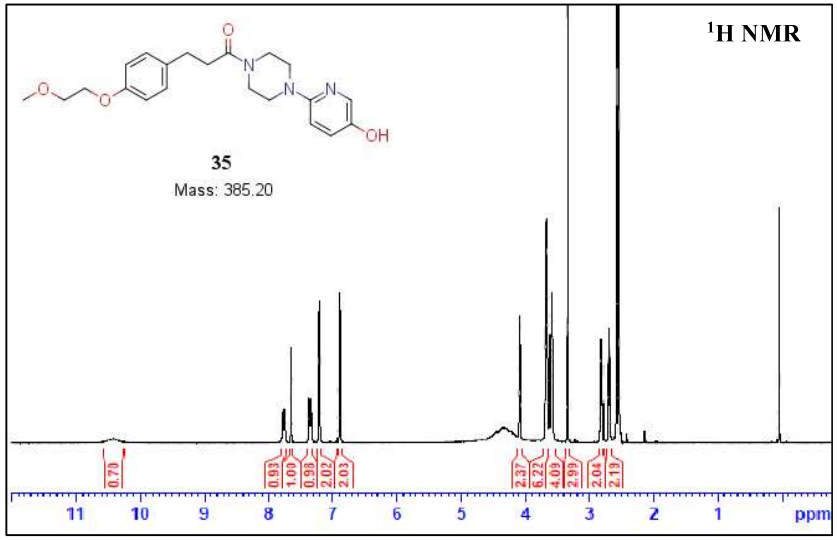
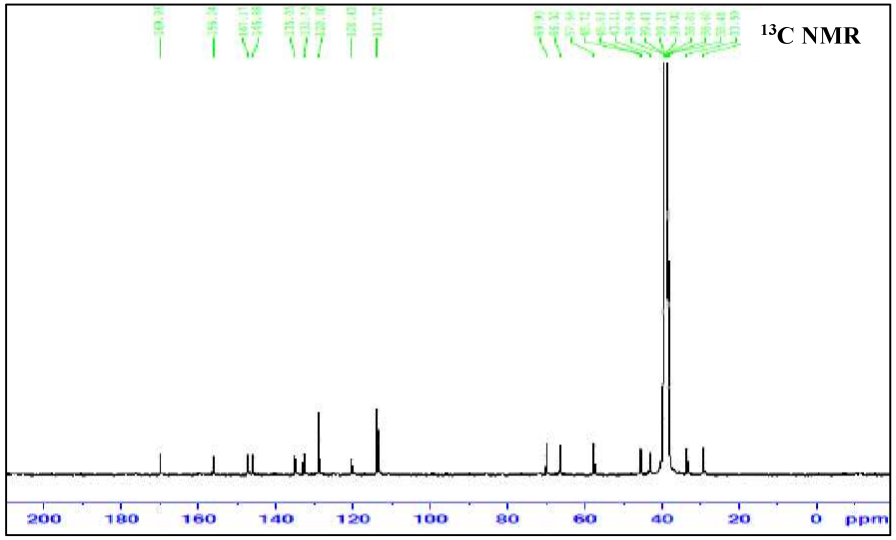
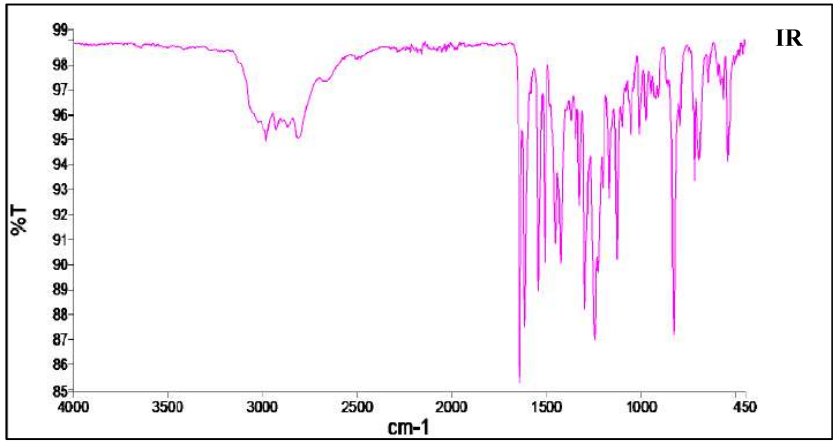
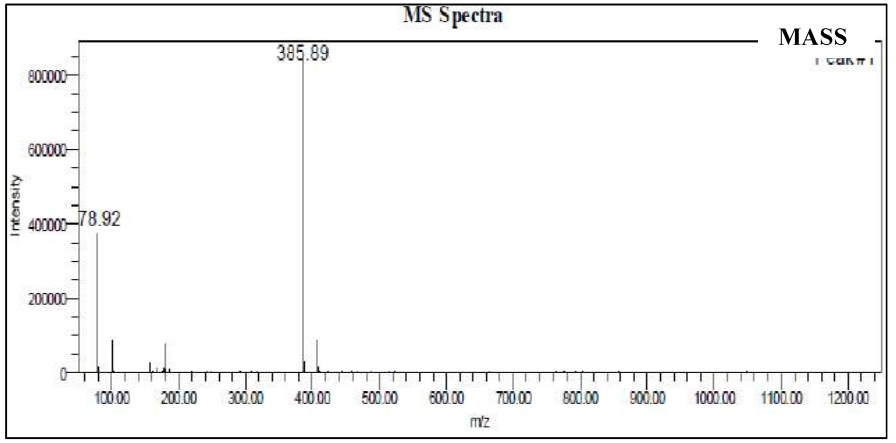
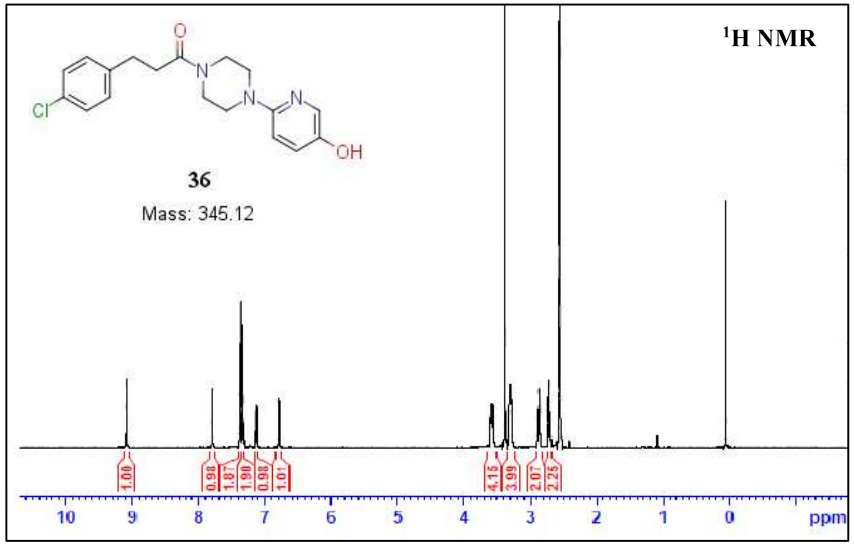


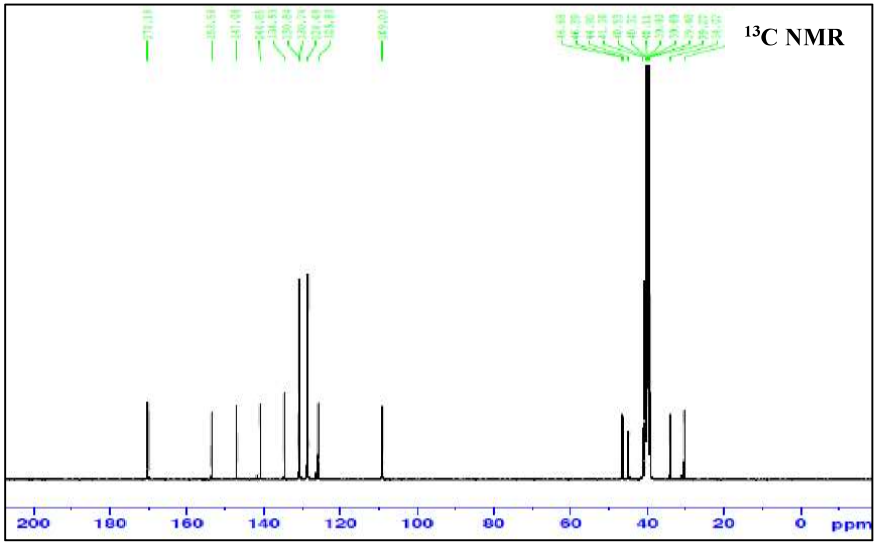


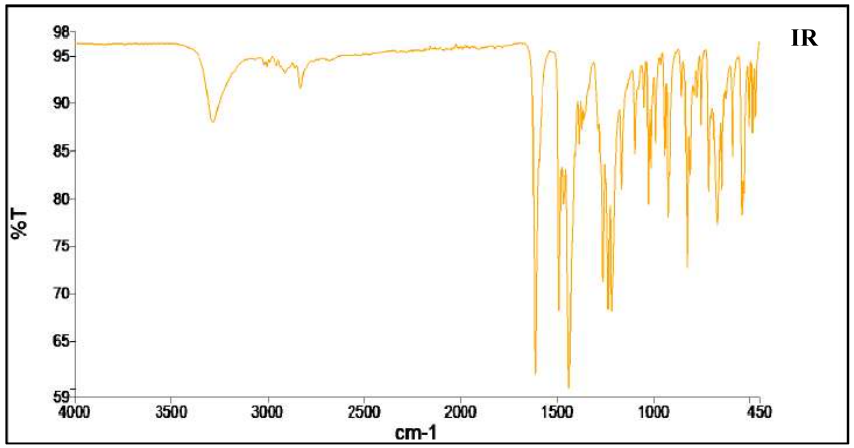


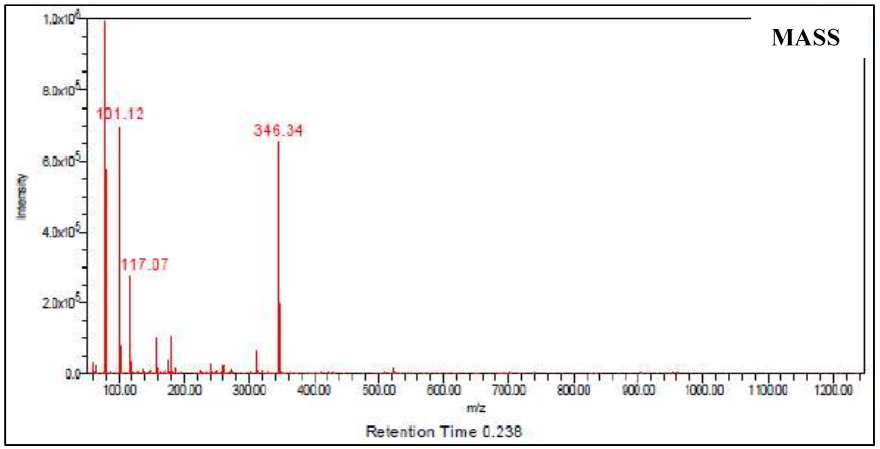


Spectral data of **36**









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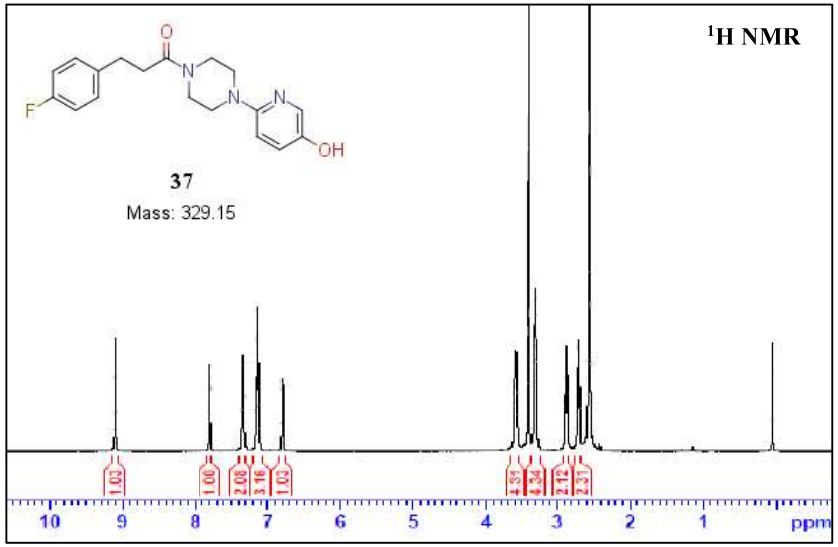
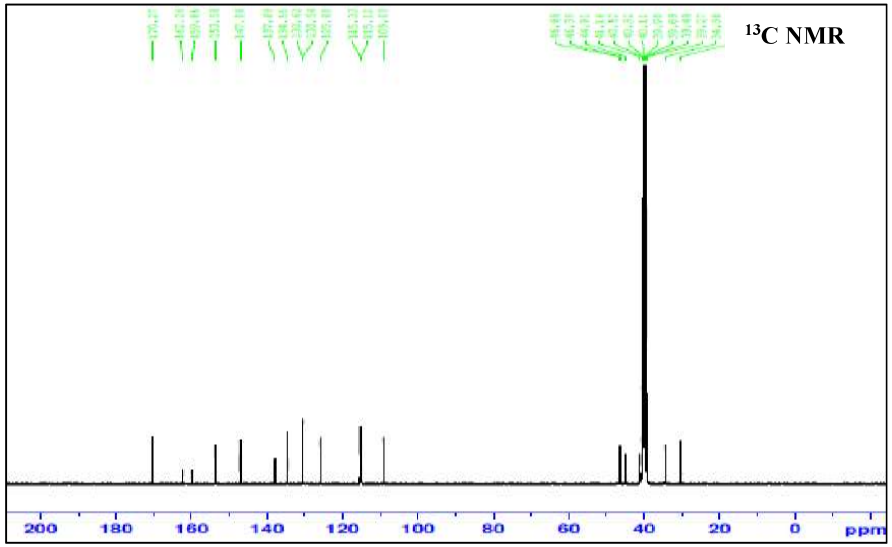
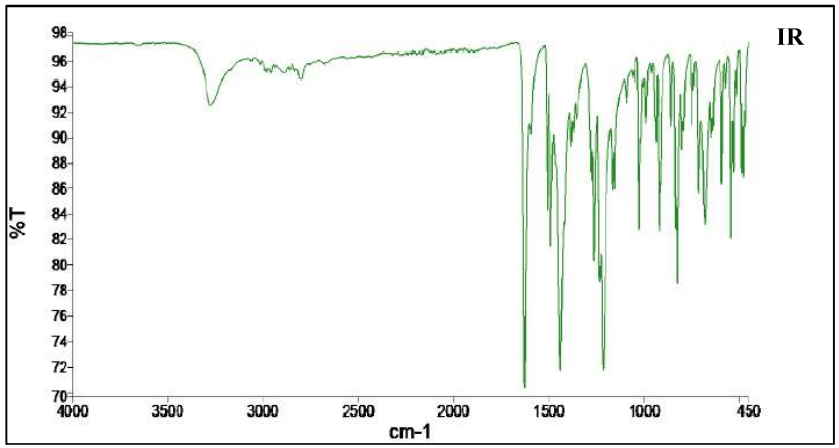
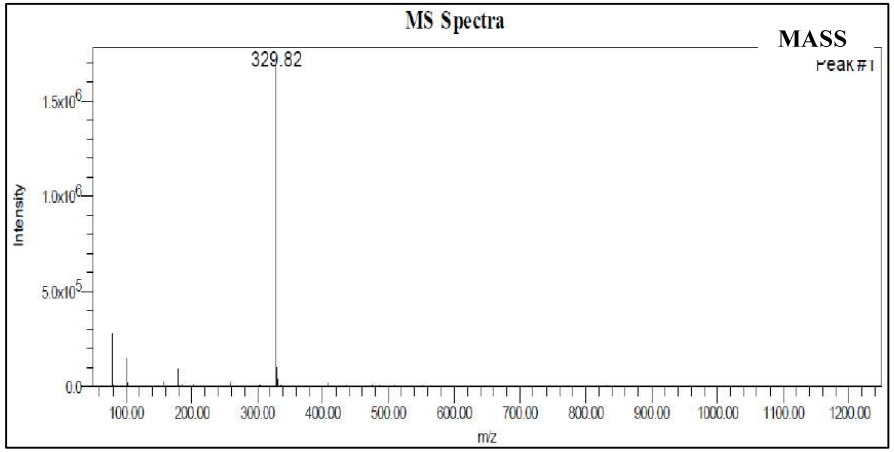
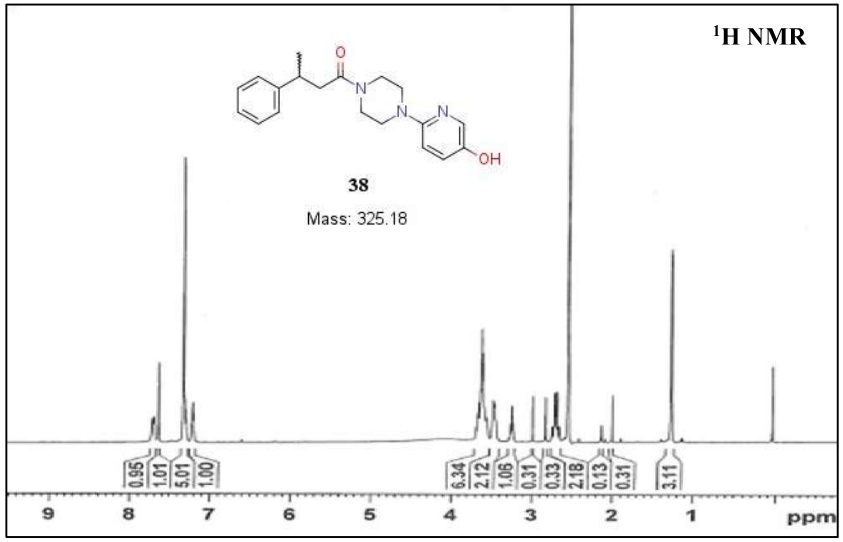


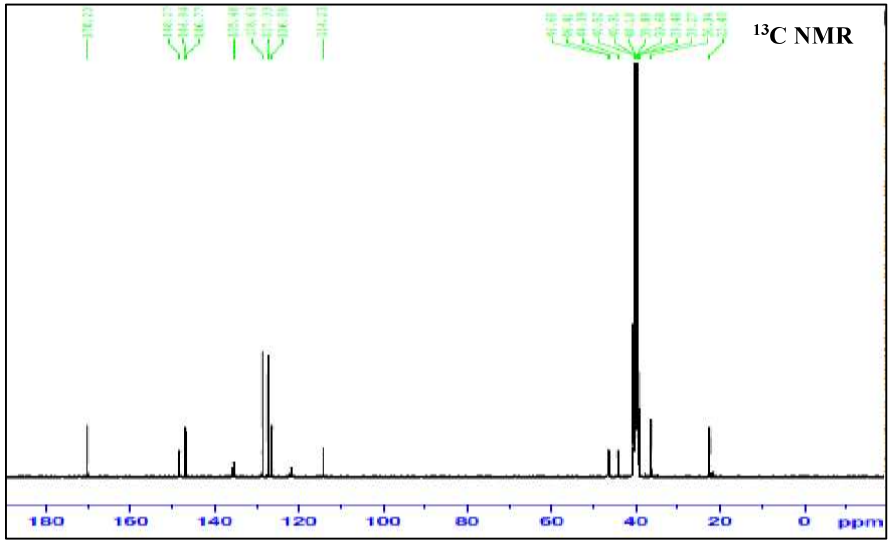


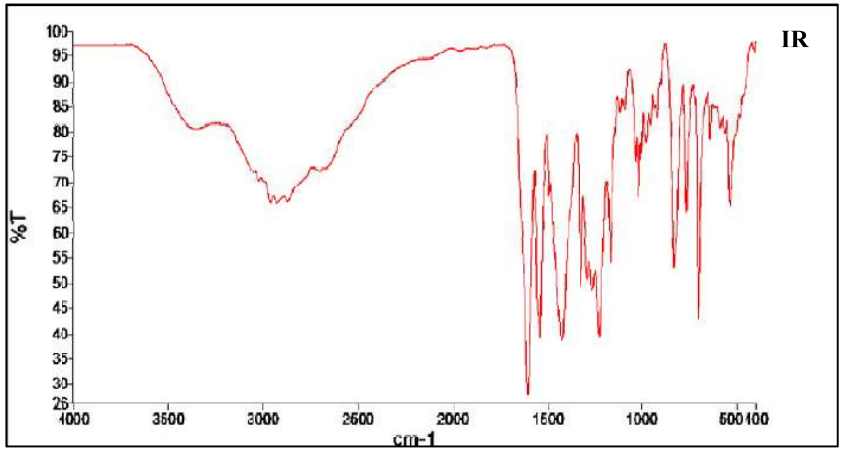


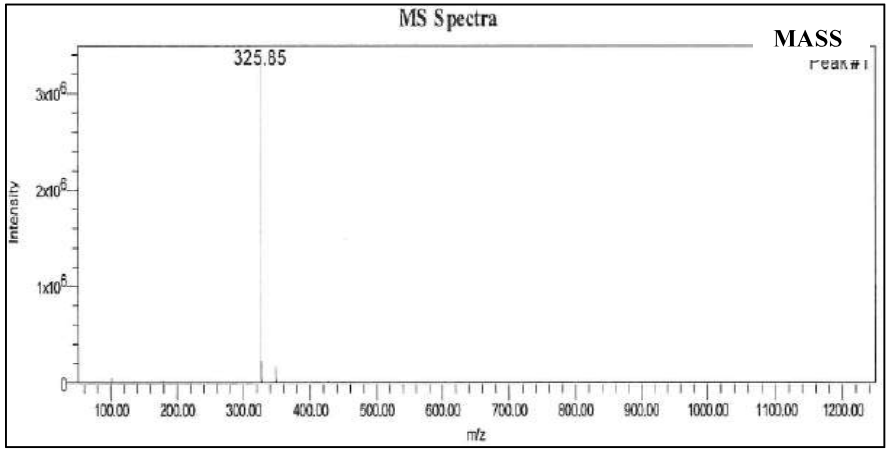


Spectral data of **38**









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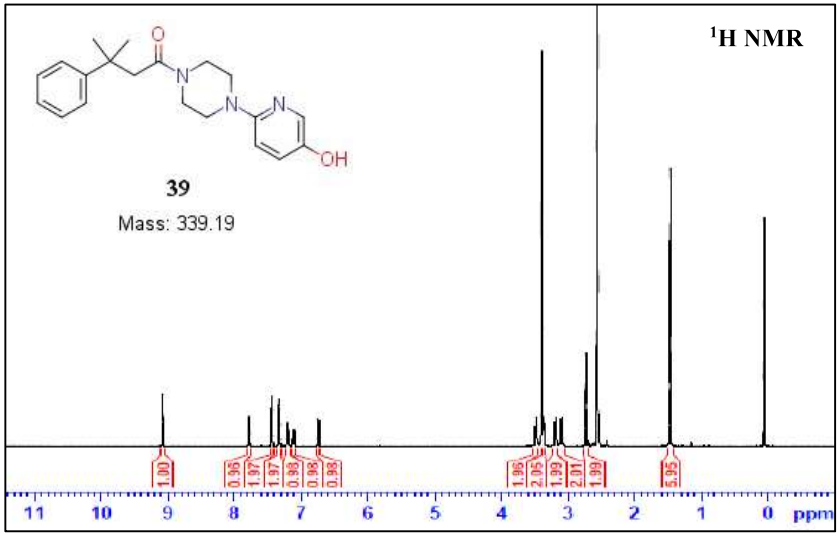
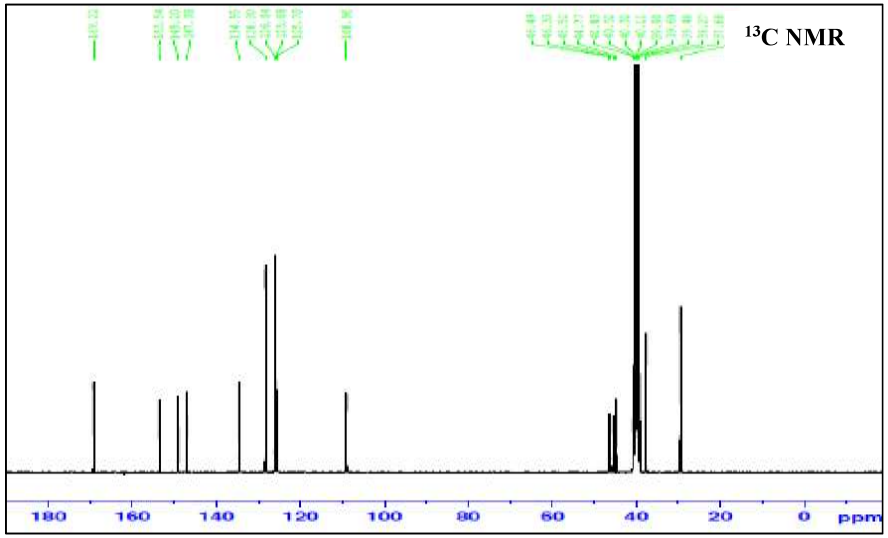
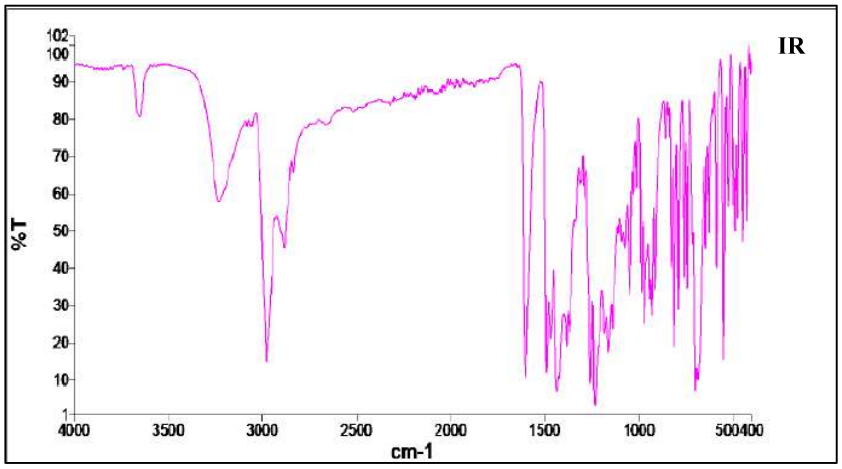
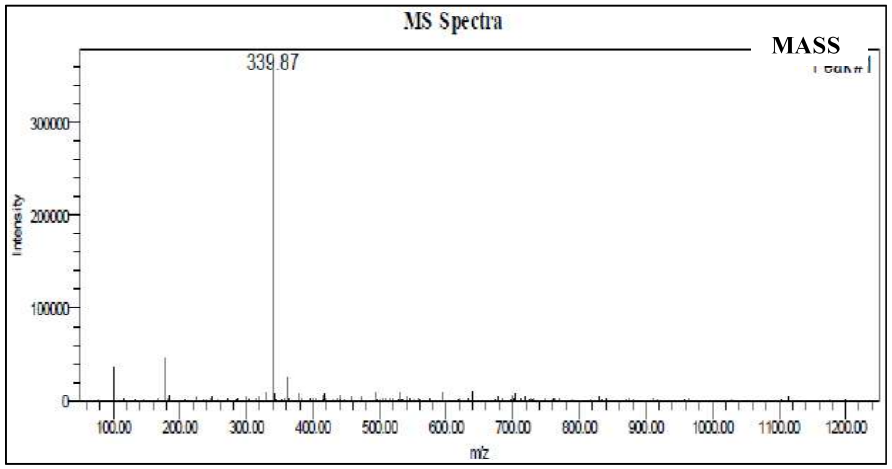
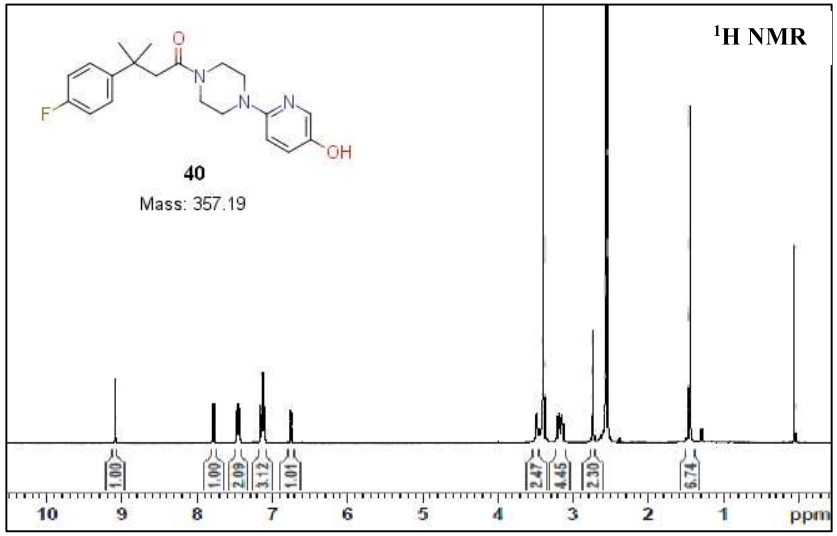


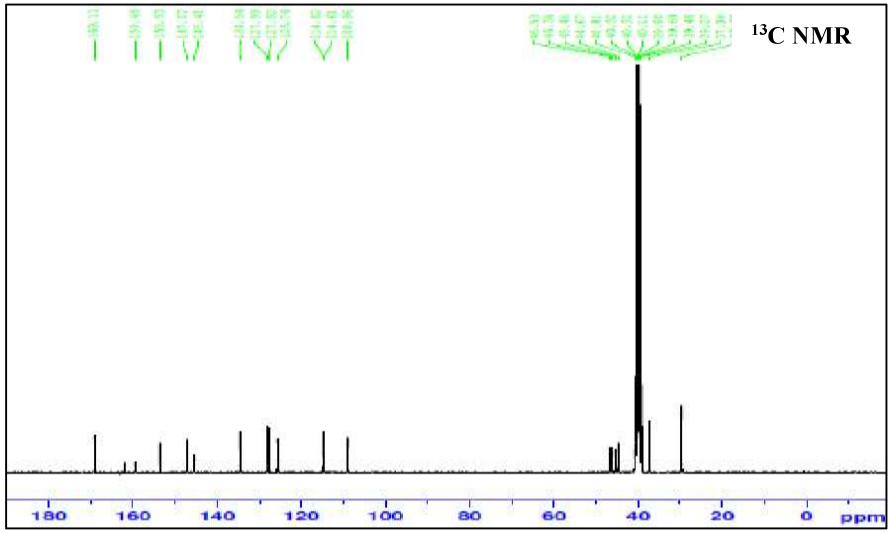


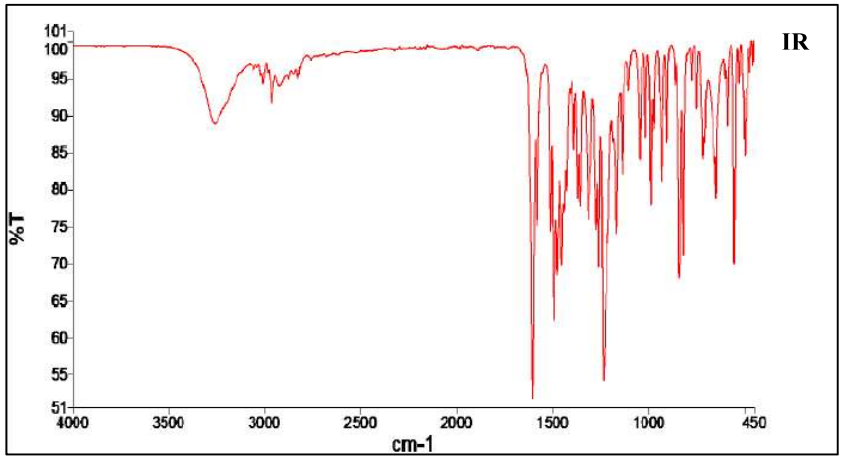


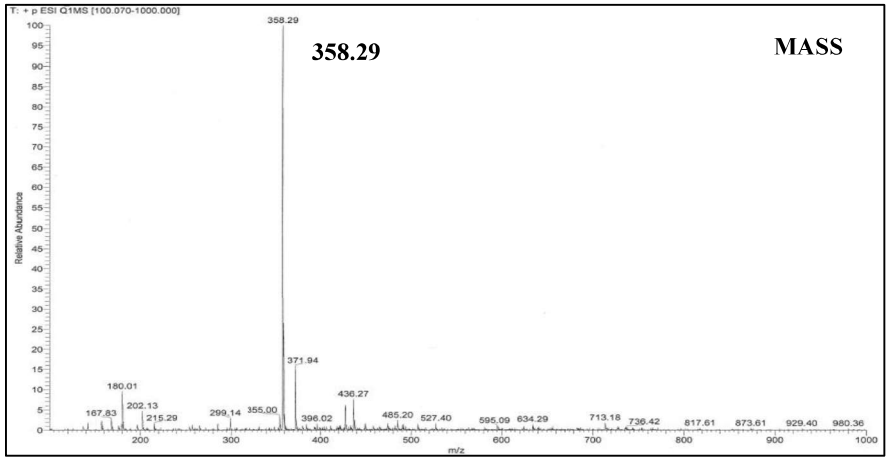


Spectral data of **40**

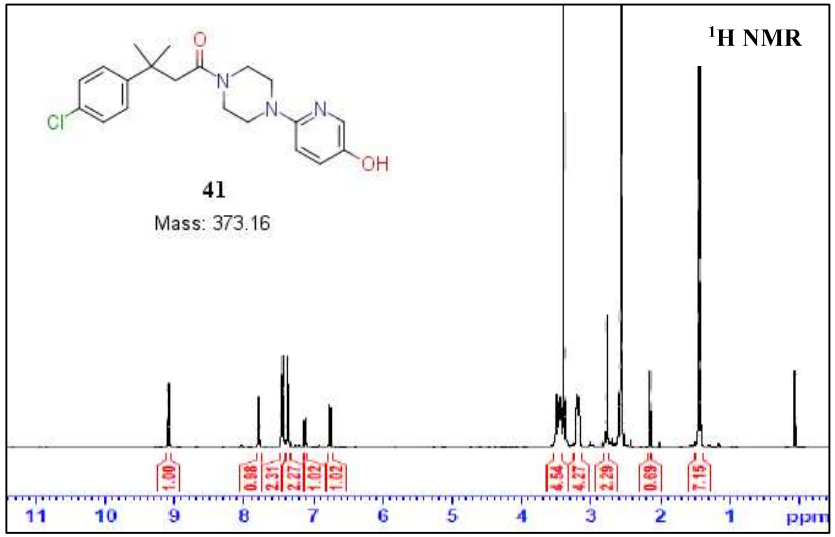


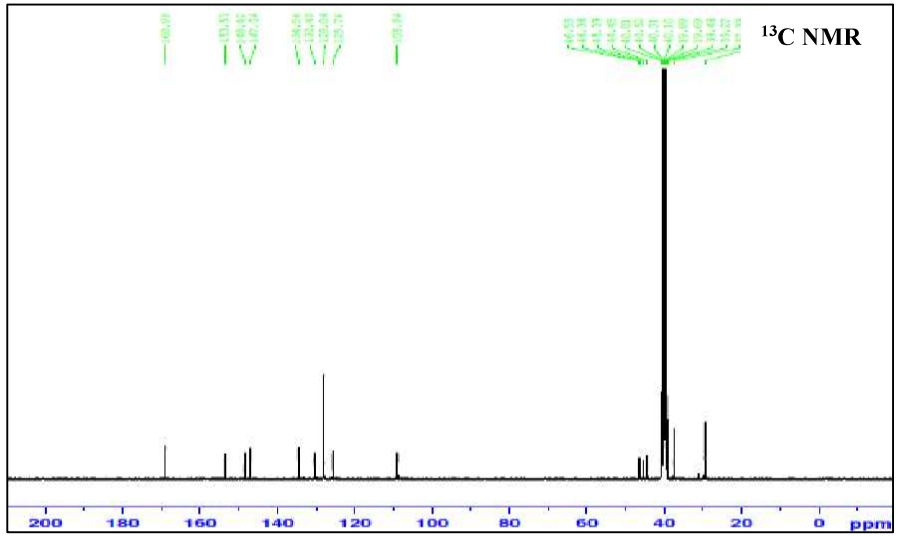


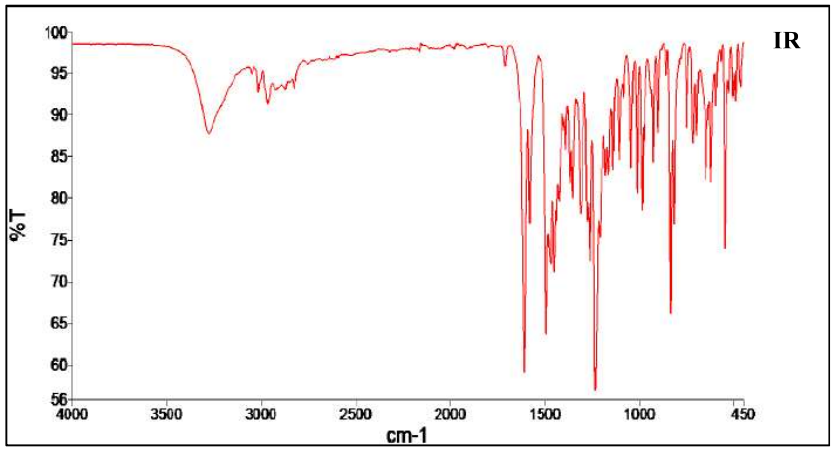


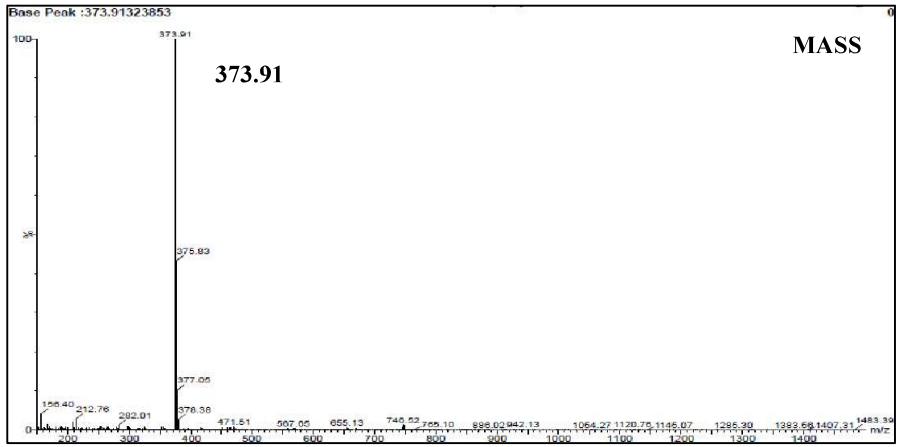


Spectral data of **41**

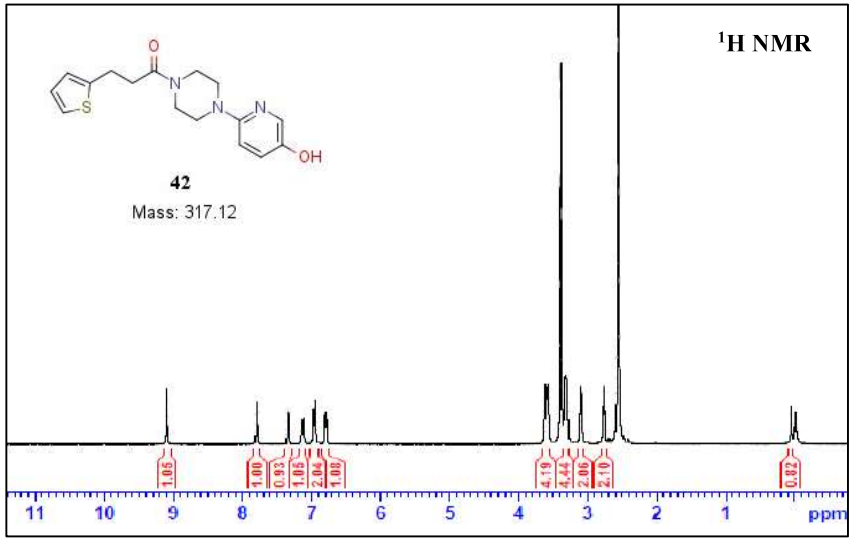


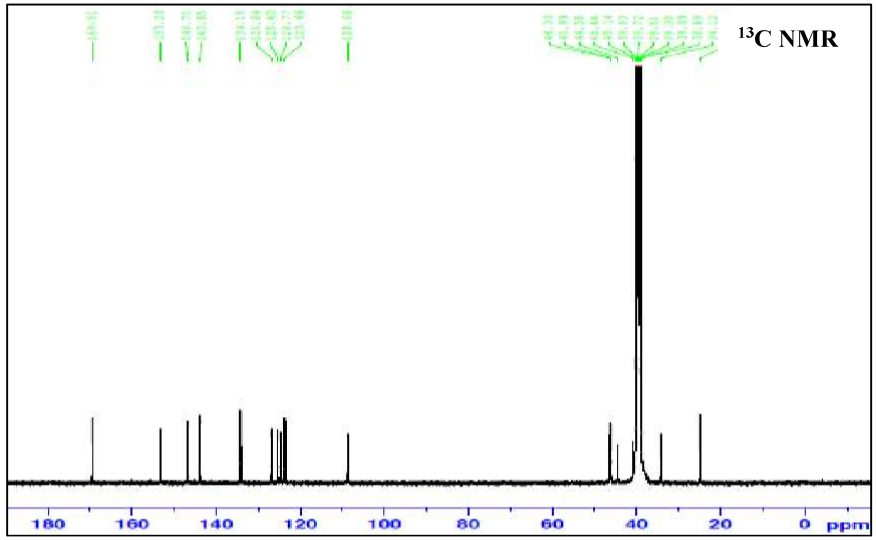


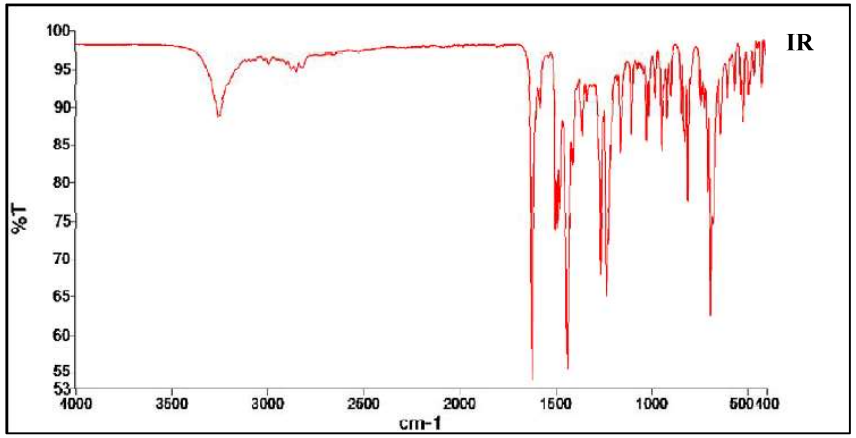


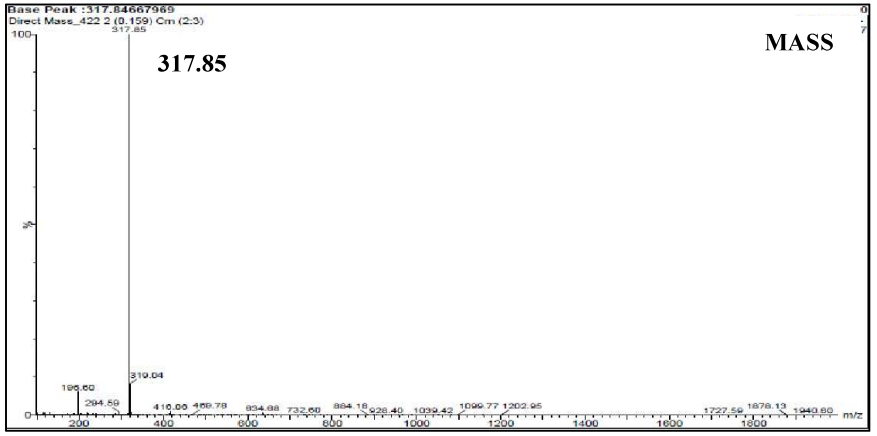


Spectral data of **42**

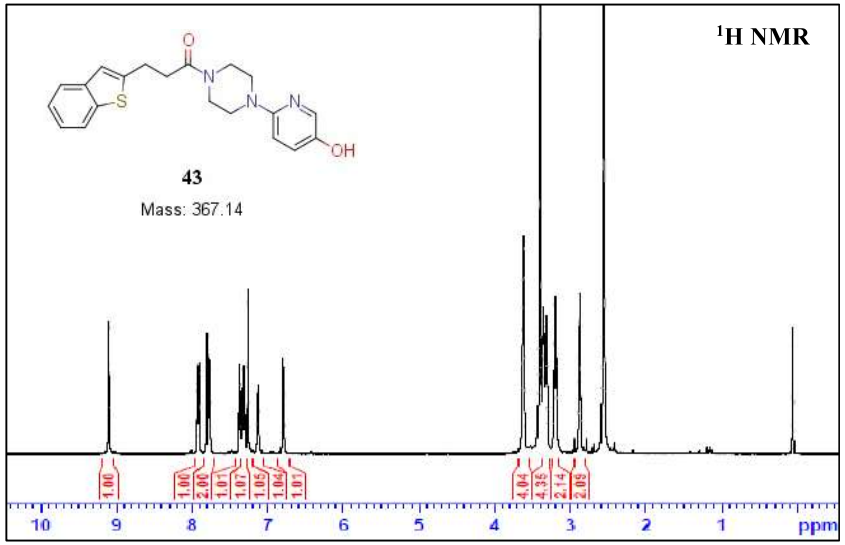


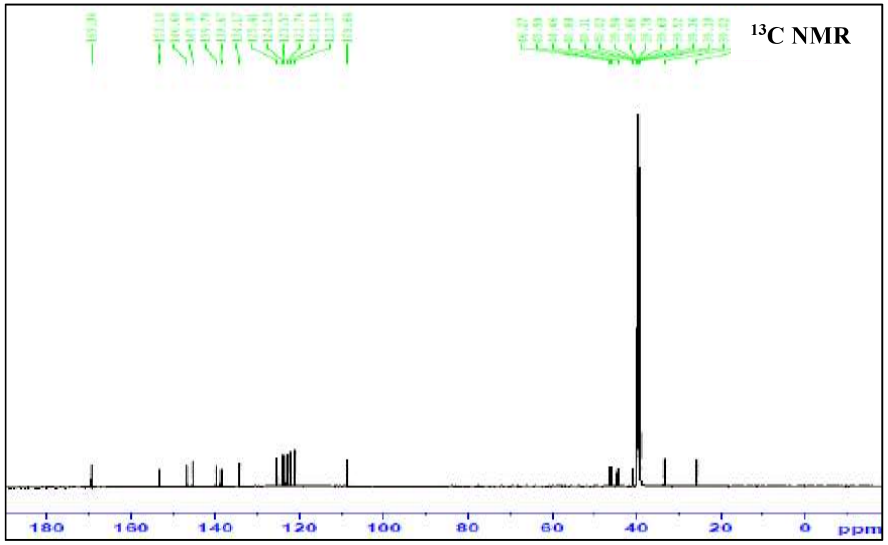


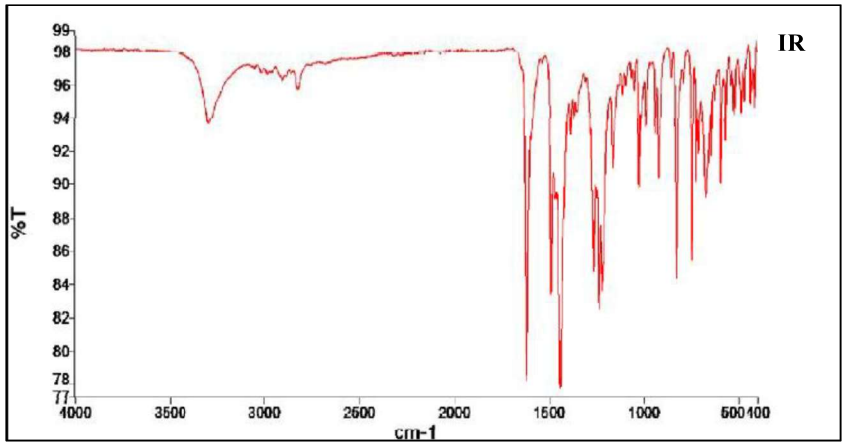


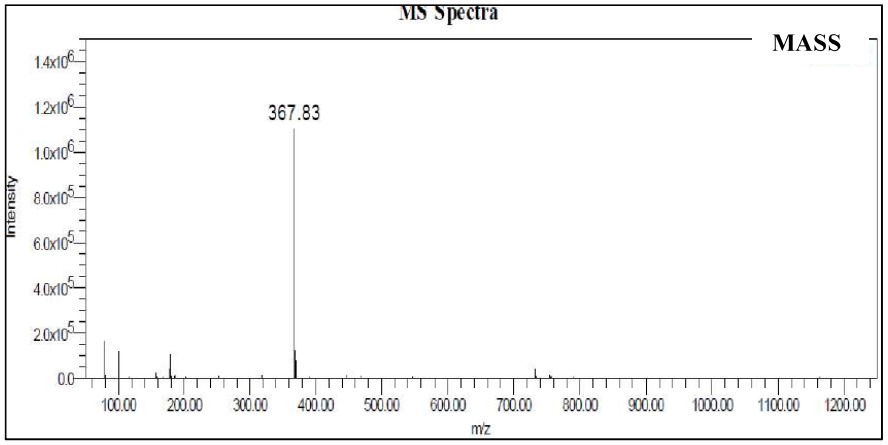


Spectral data of **43**

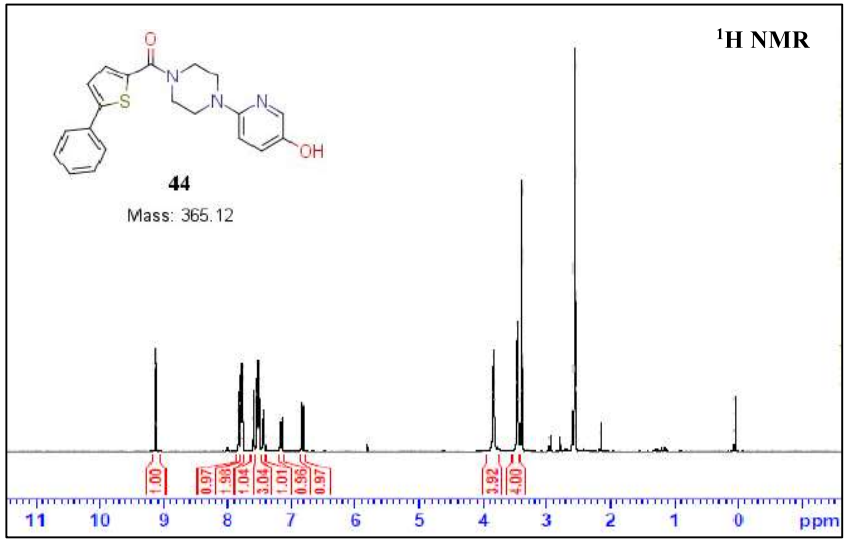


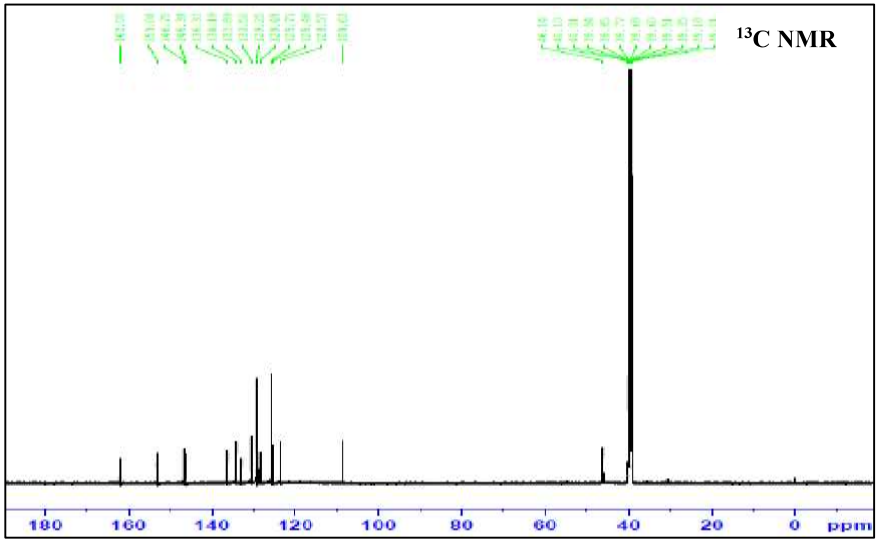


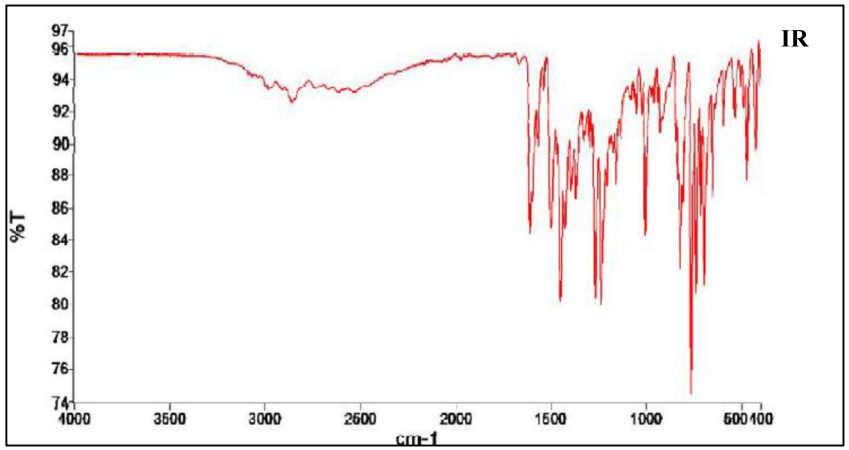


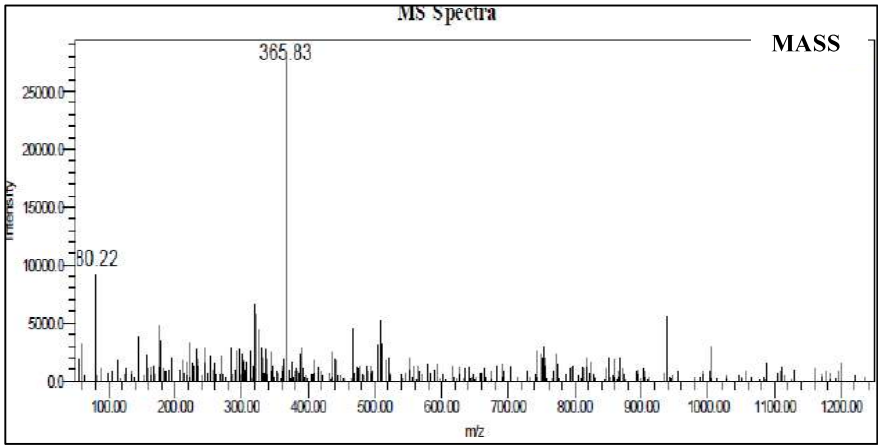


Spectral data of **44**

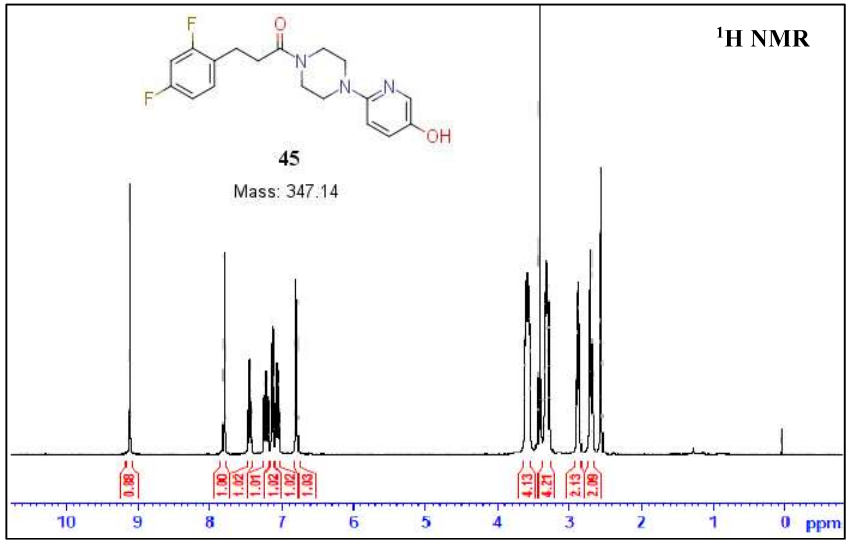


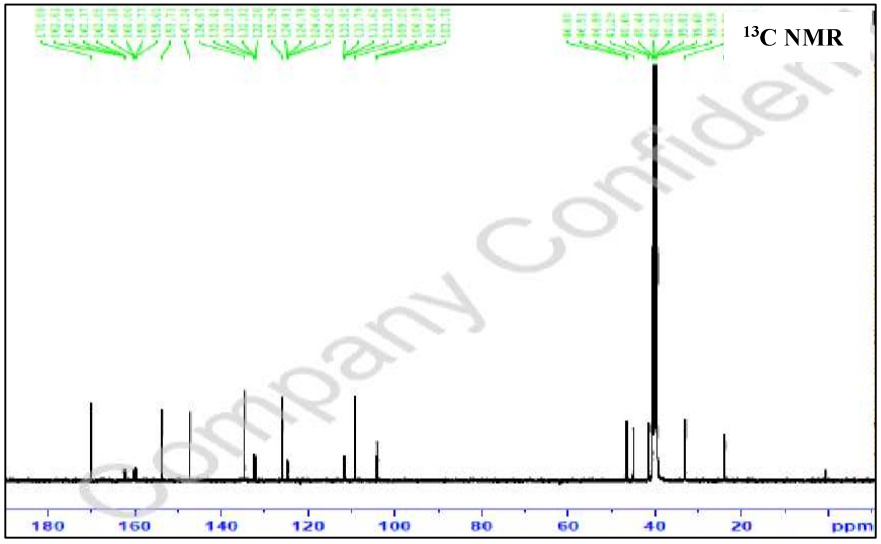


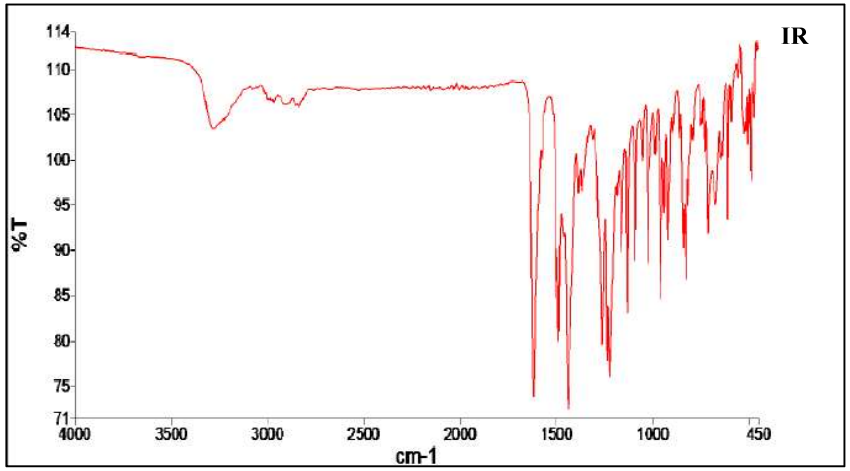


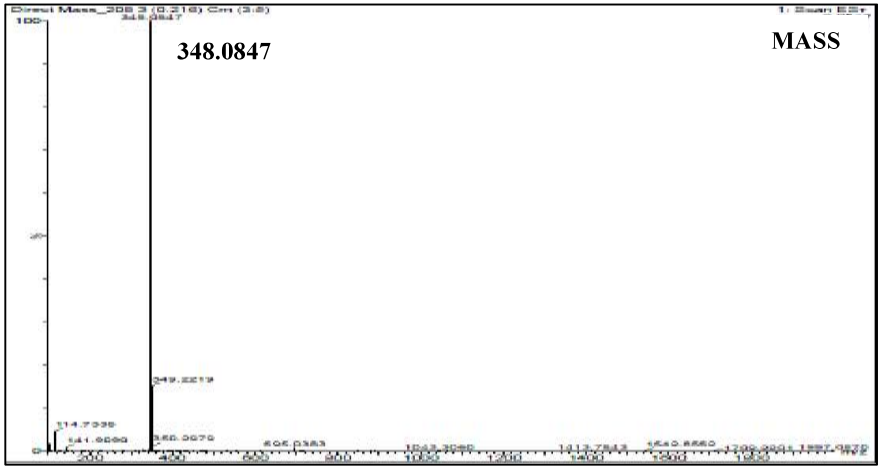


Spectral data of **45**

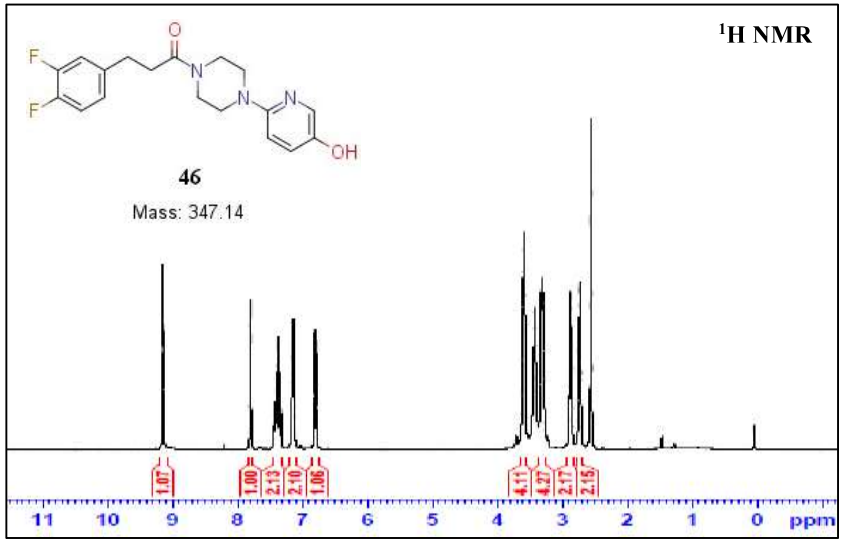


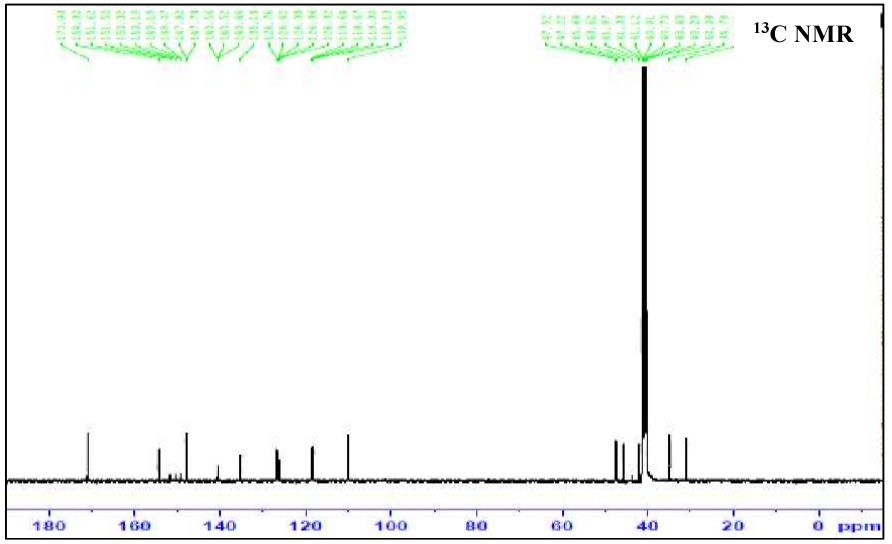


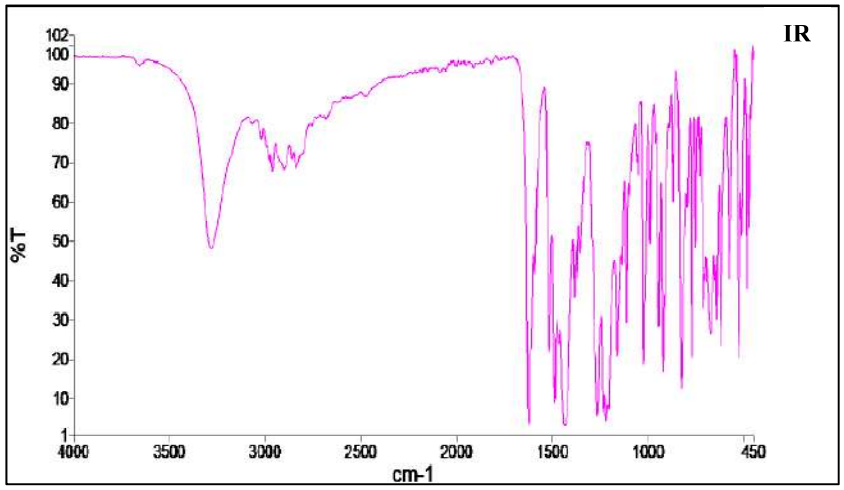


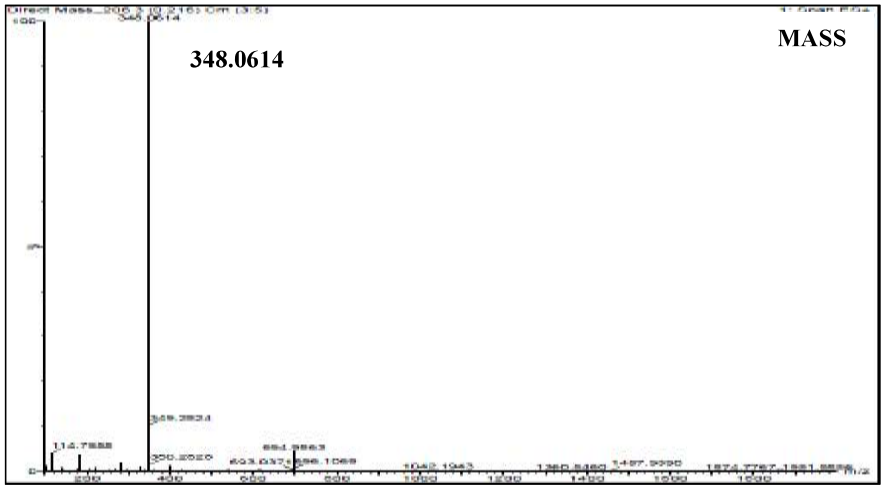


Spectral data of **46**

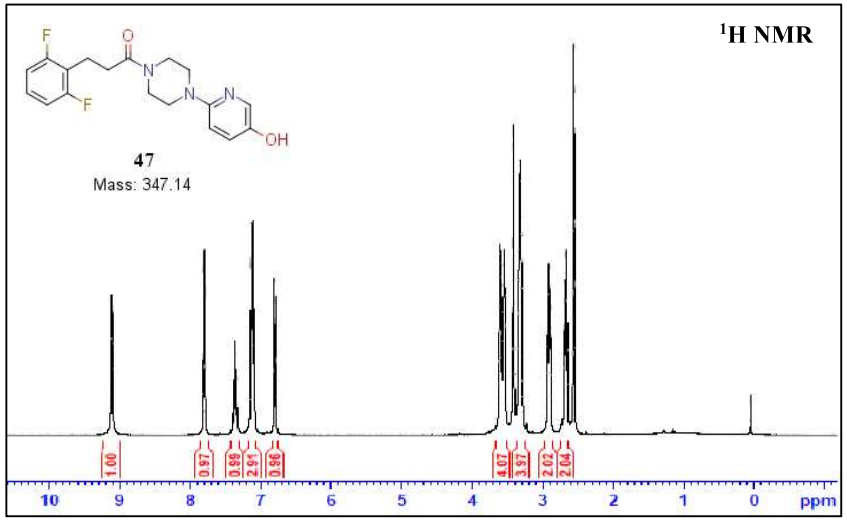


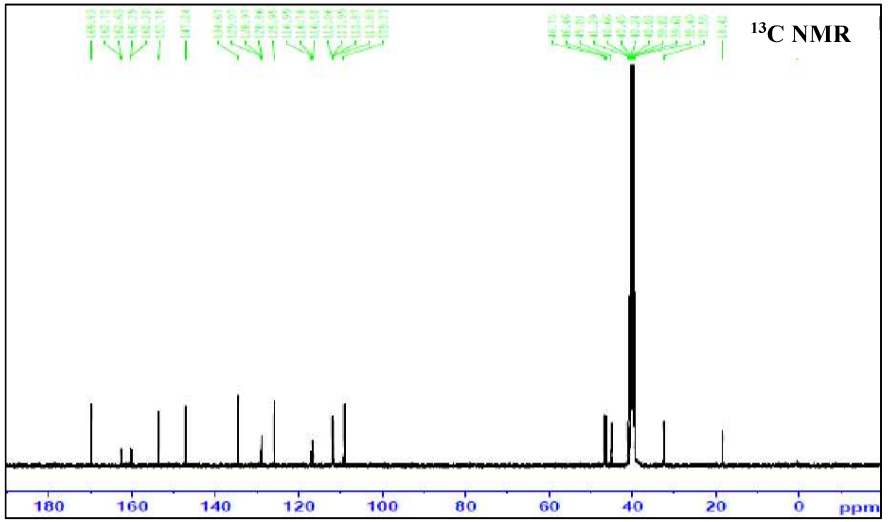


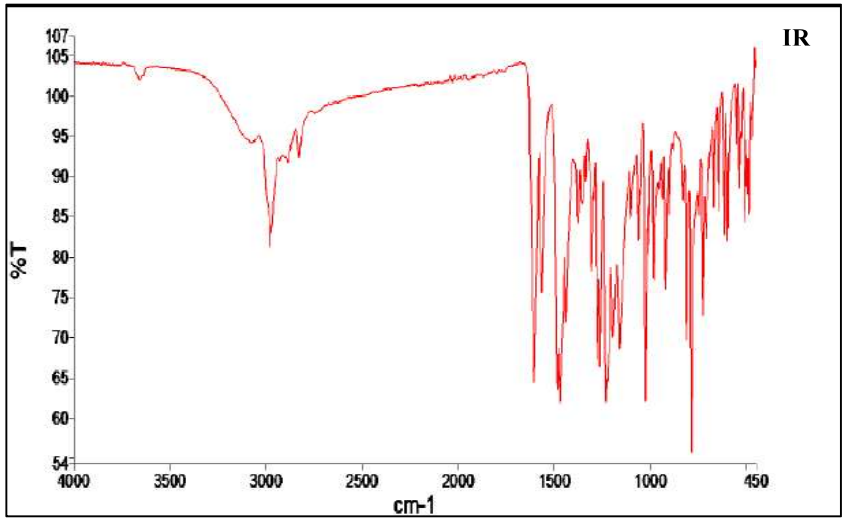


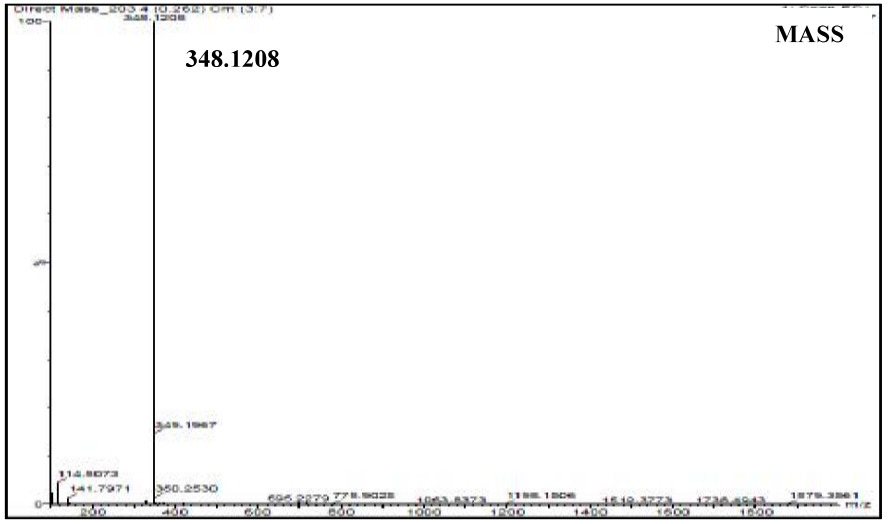


Spectral data of **47**

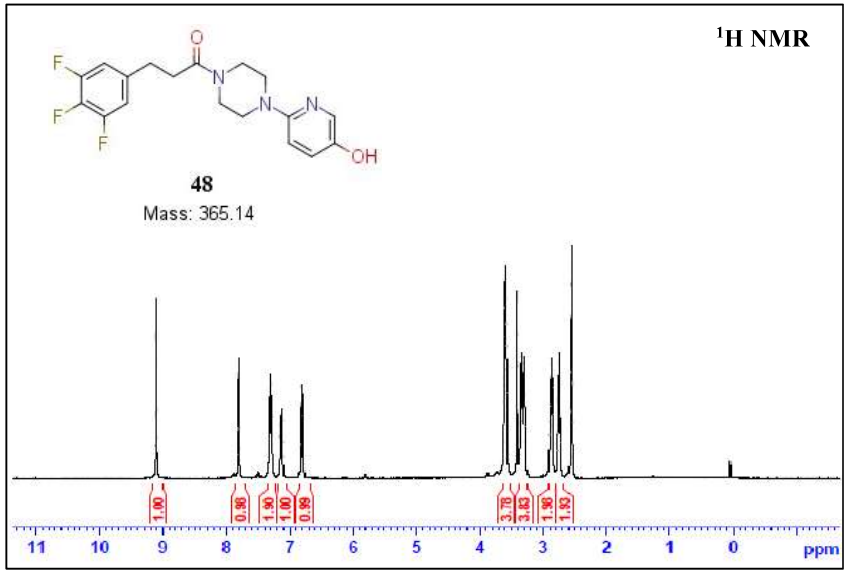


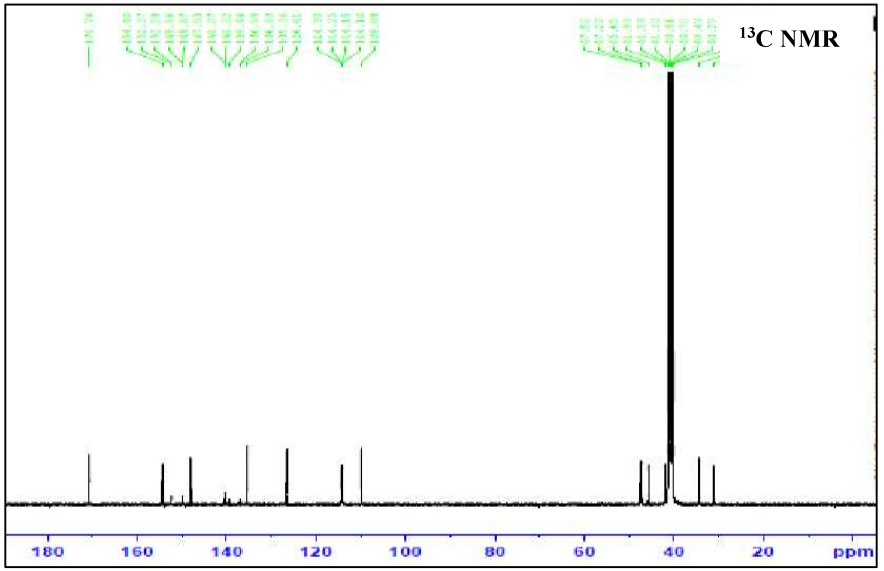


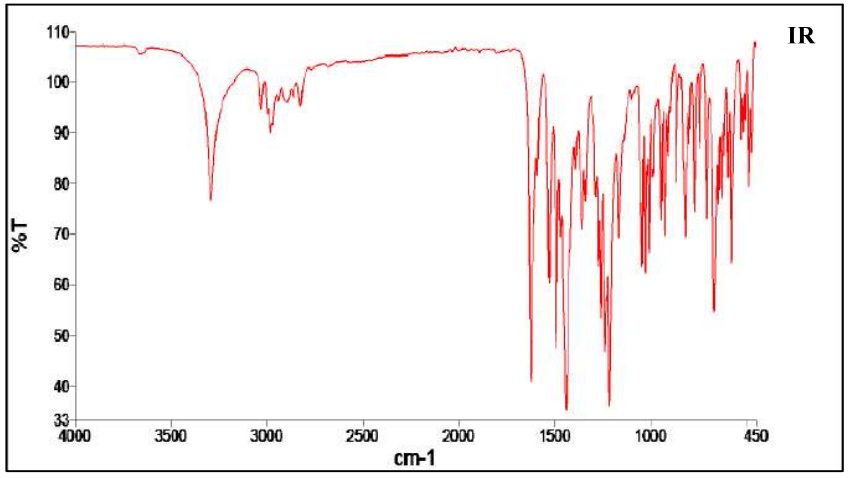


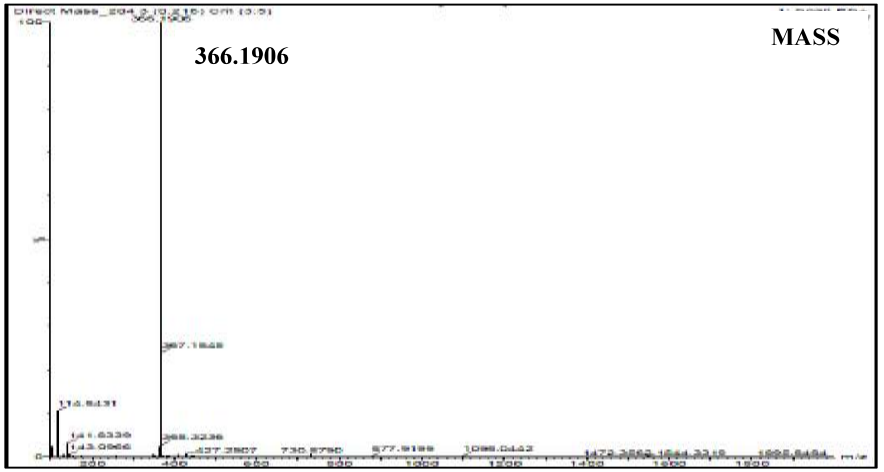


Spectral data of **48**

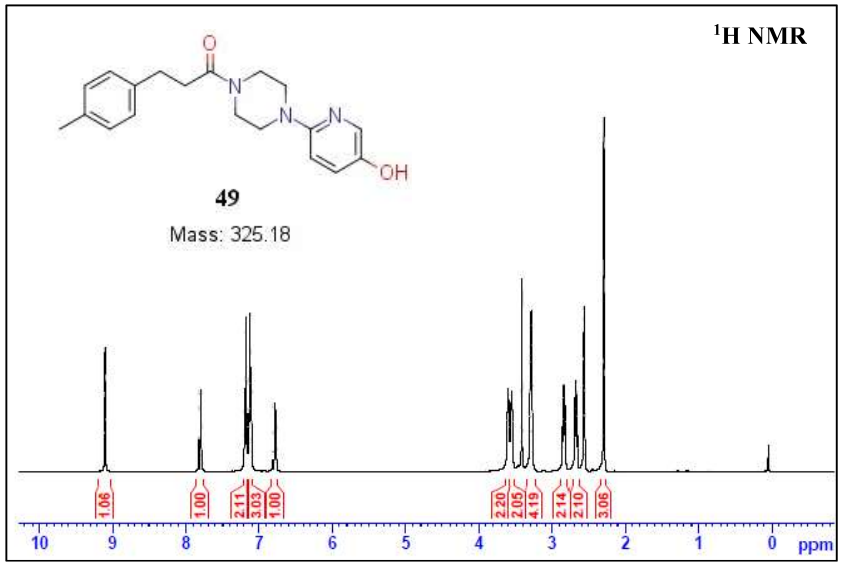


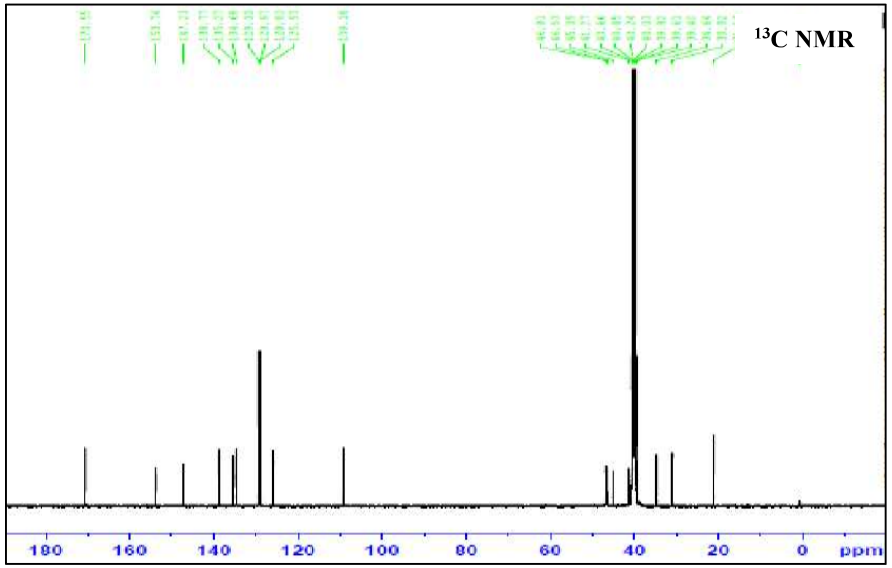


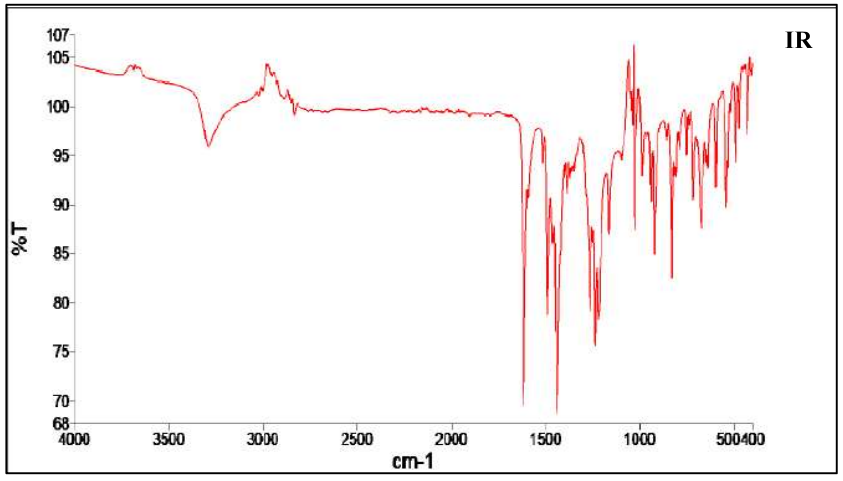


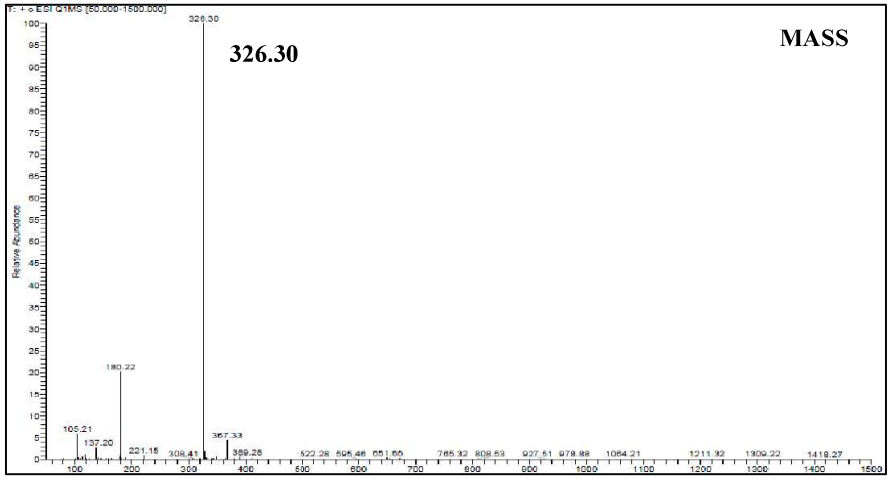


Spectral data of **49**

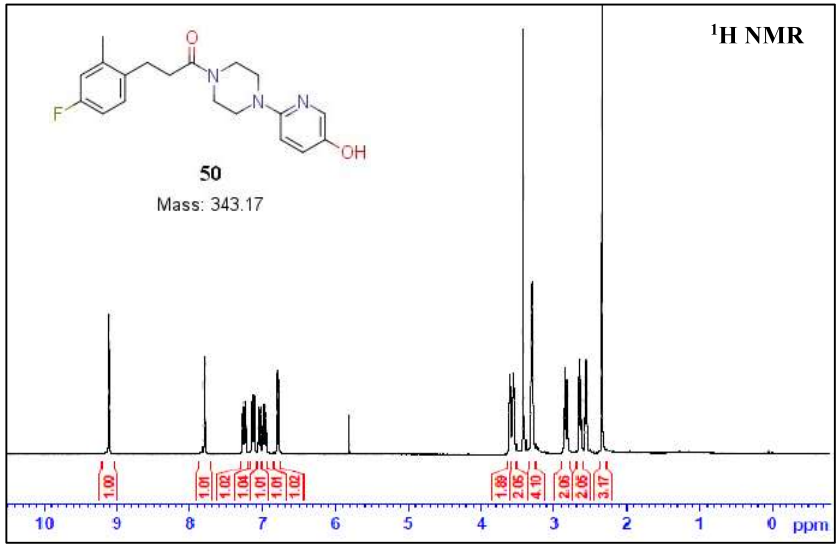


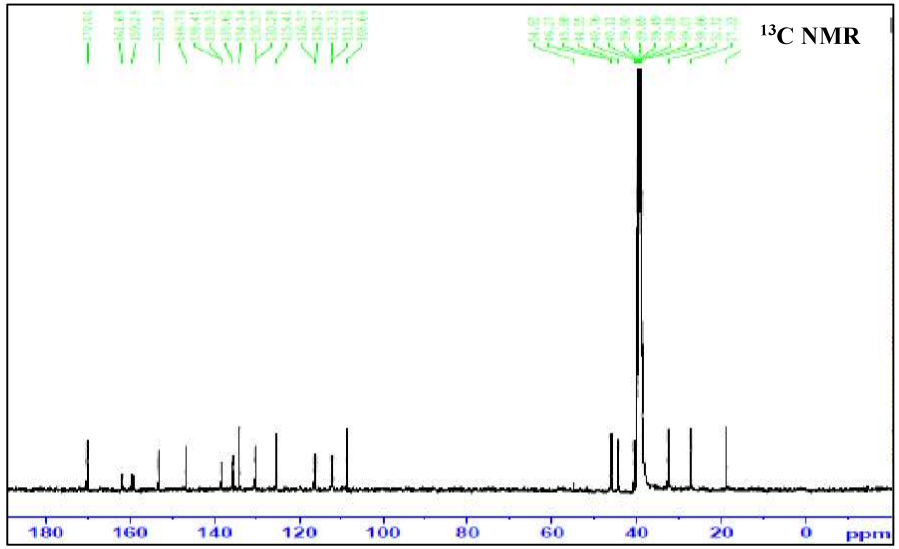


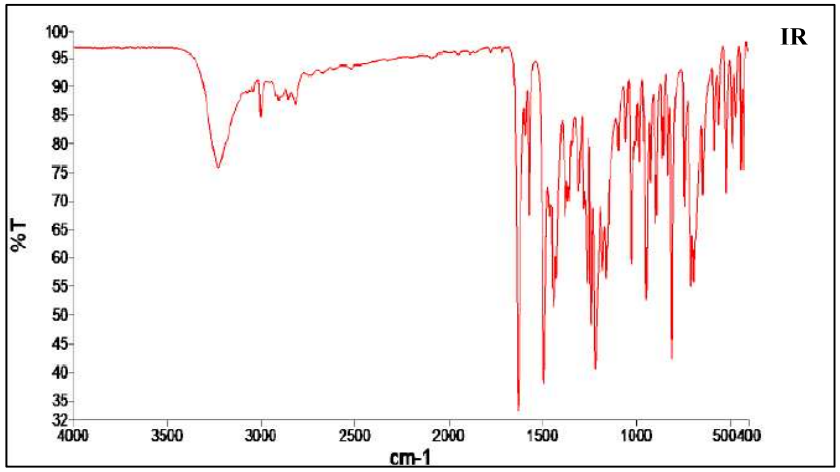


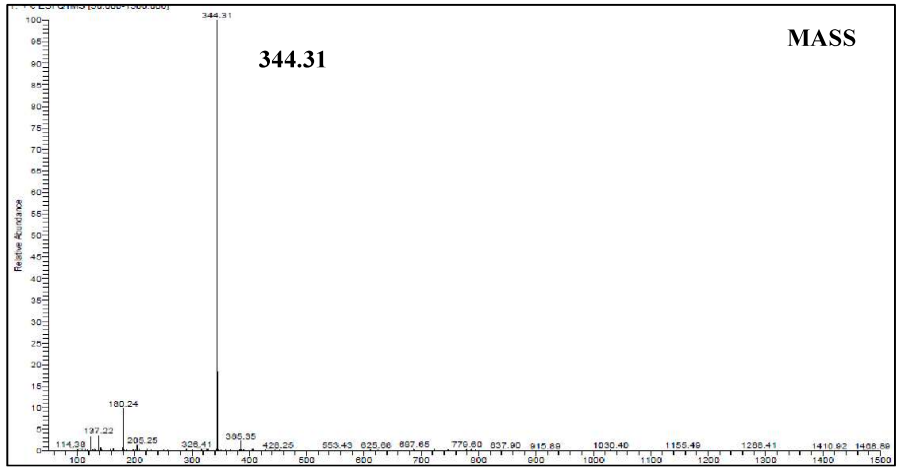


Spectral data of **50**

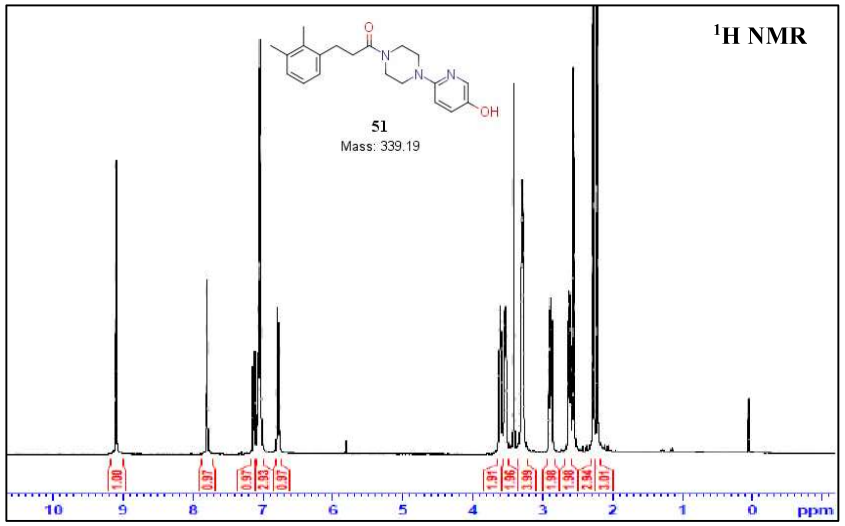


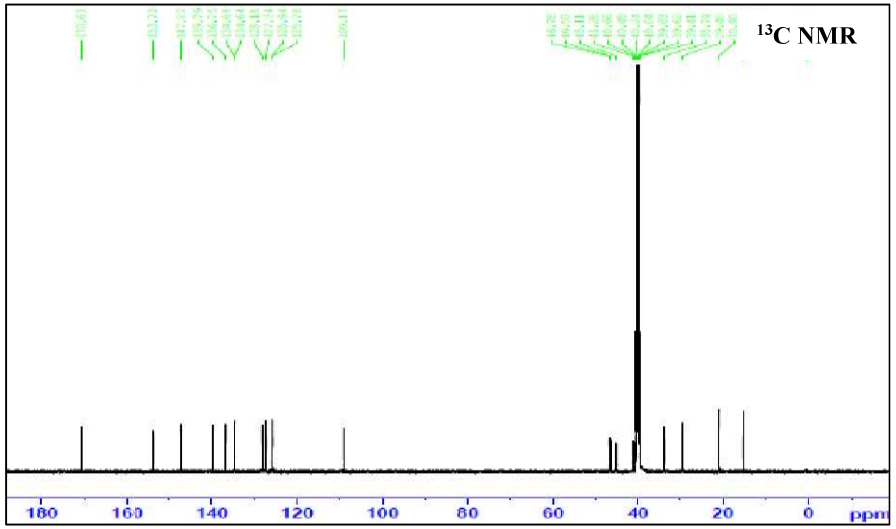


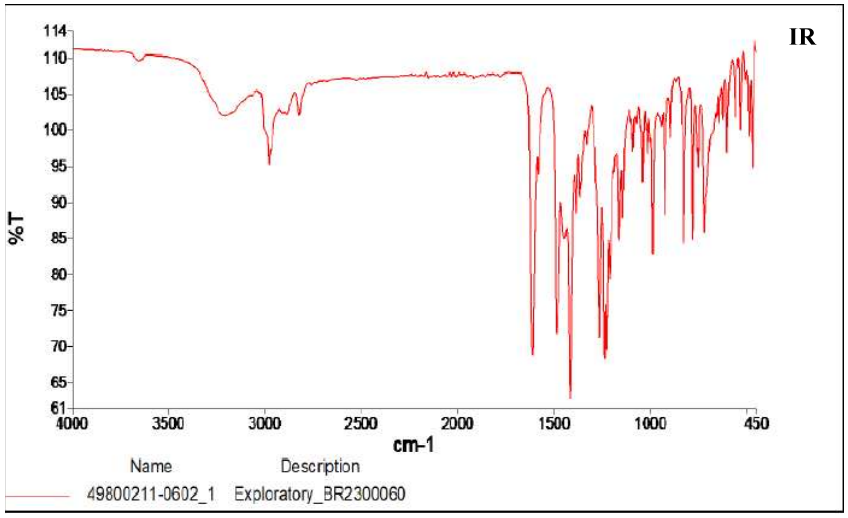


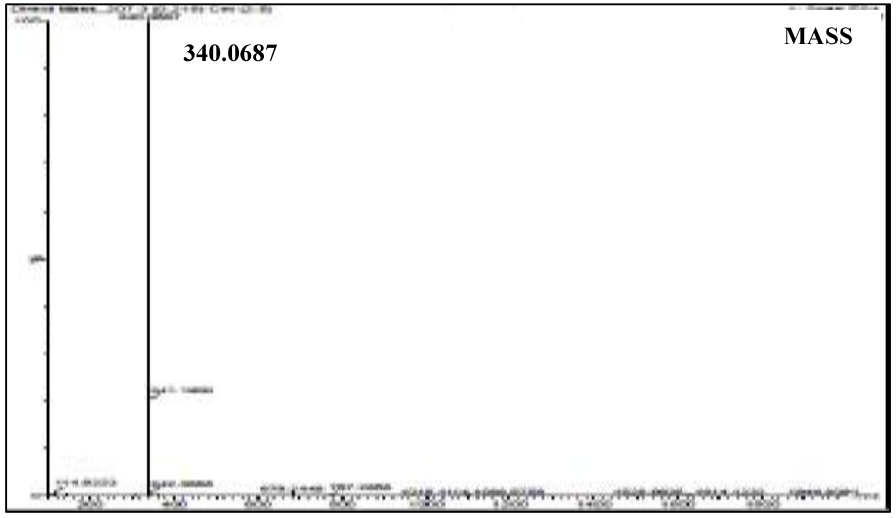


Spectral data of **51**

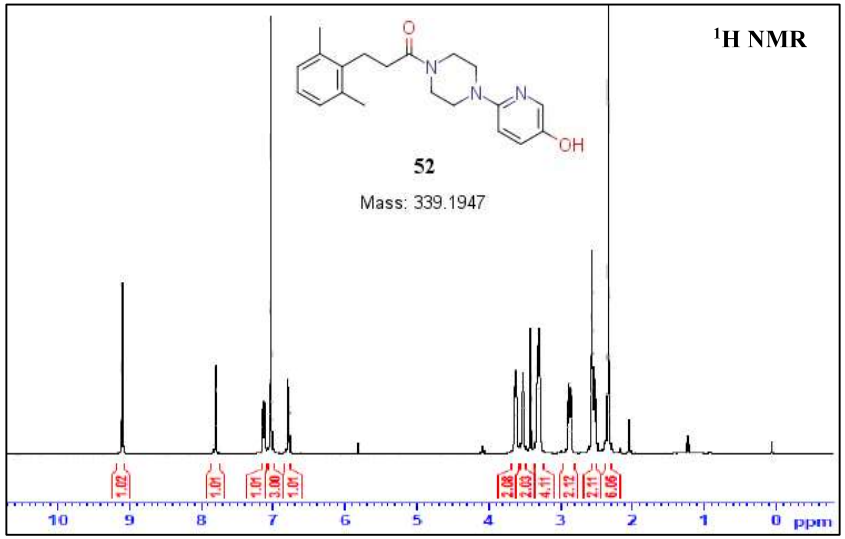


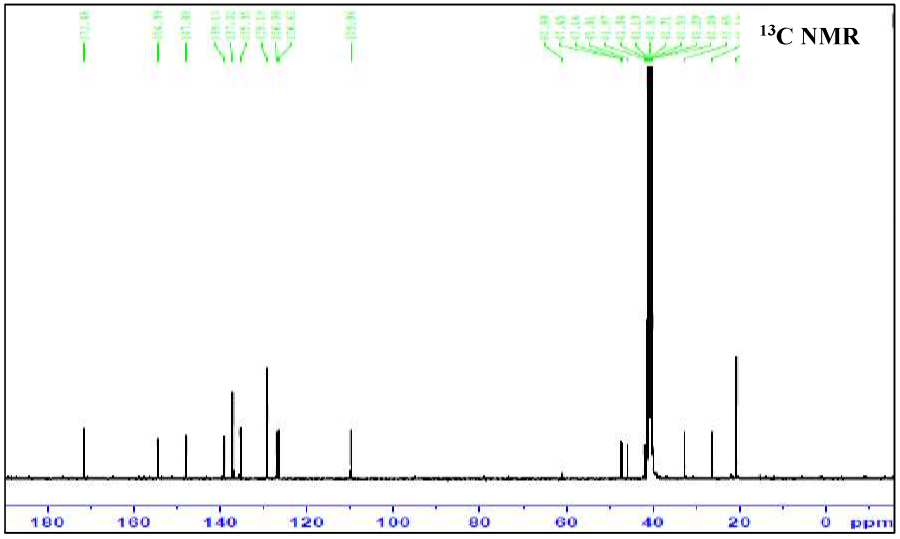


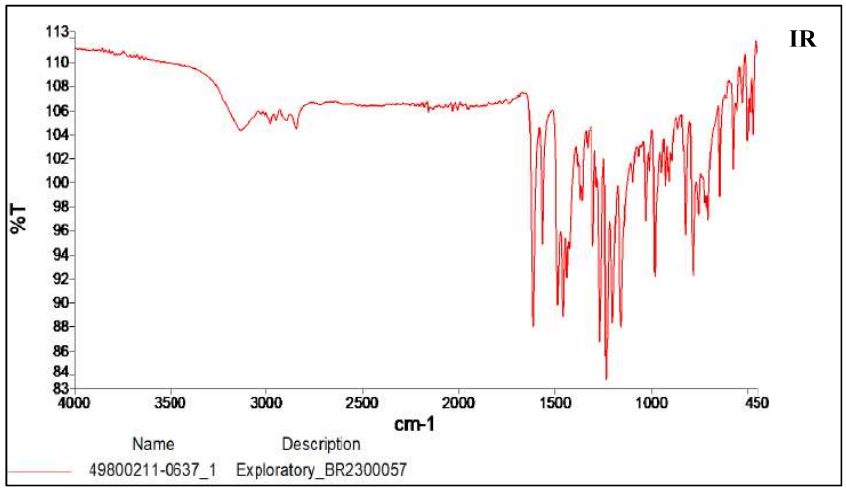


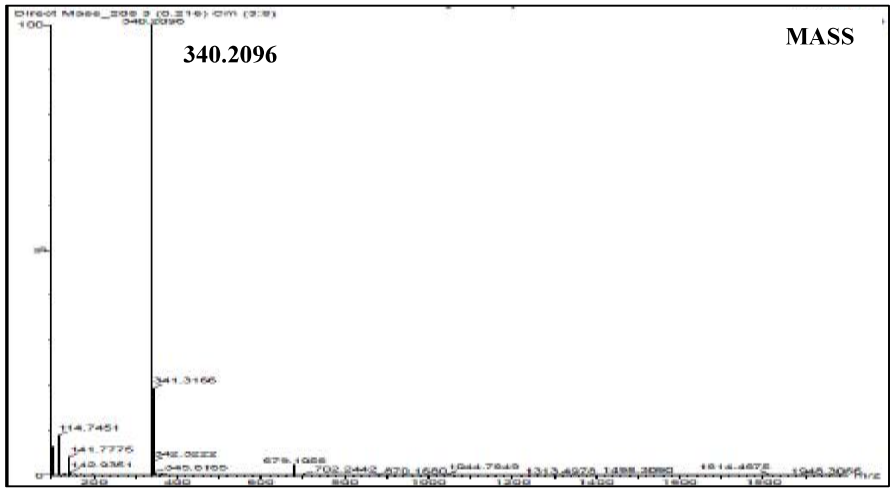


Spectral data of **52**

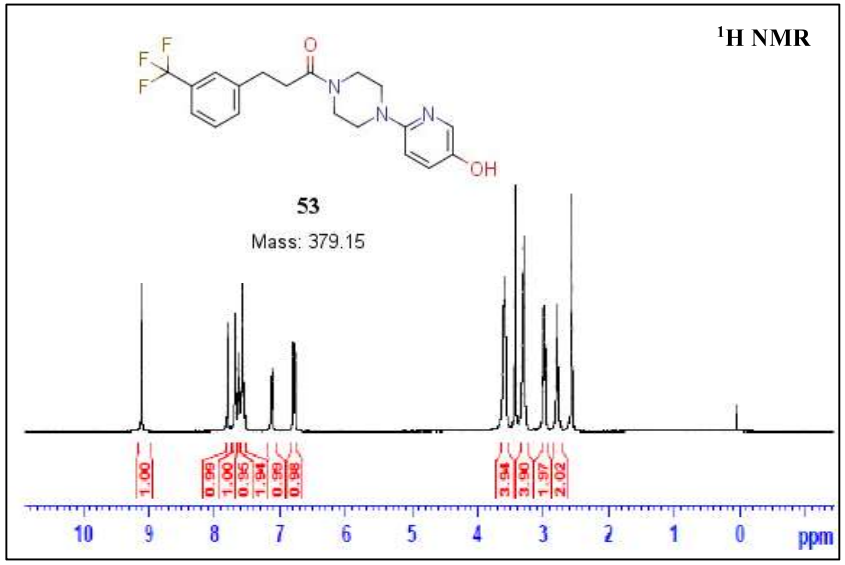


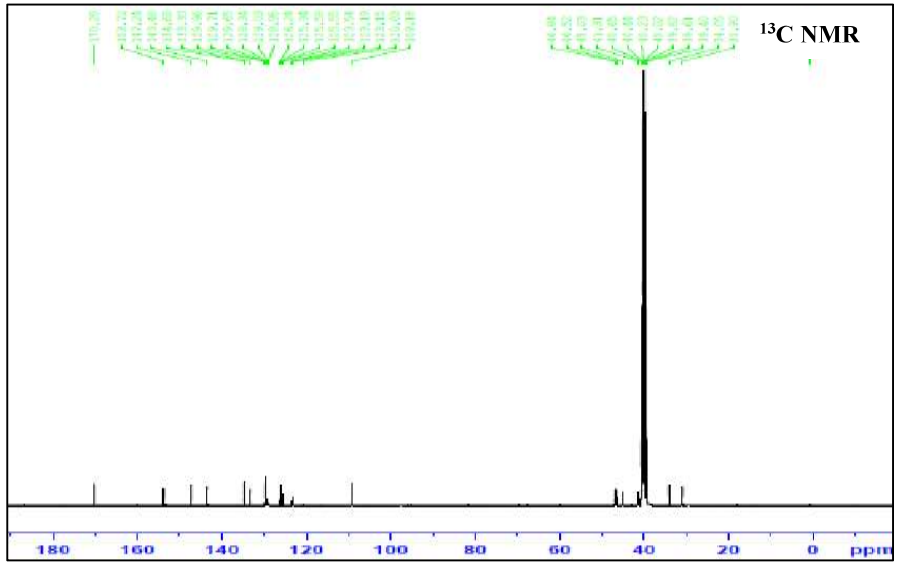


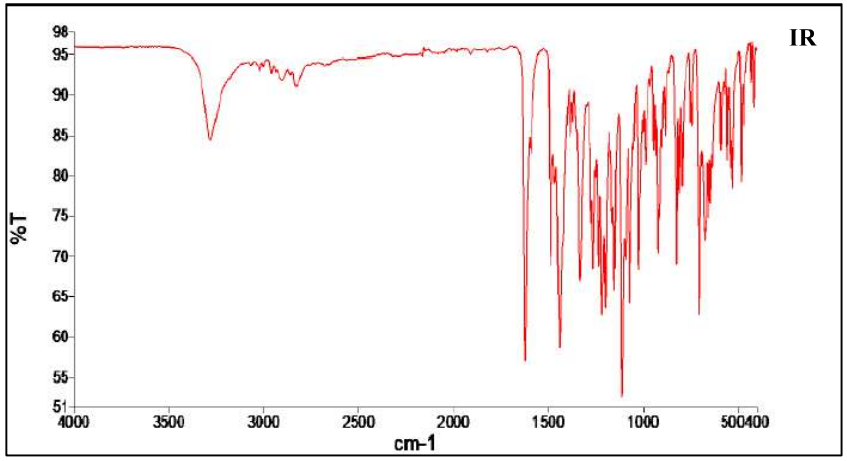


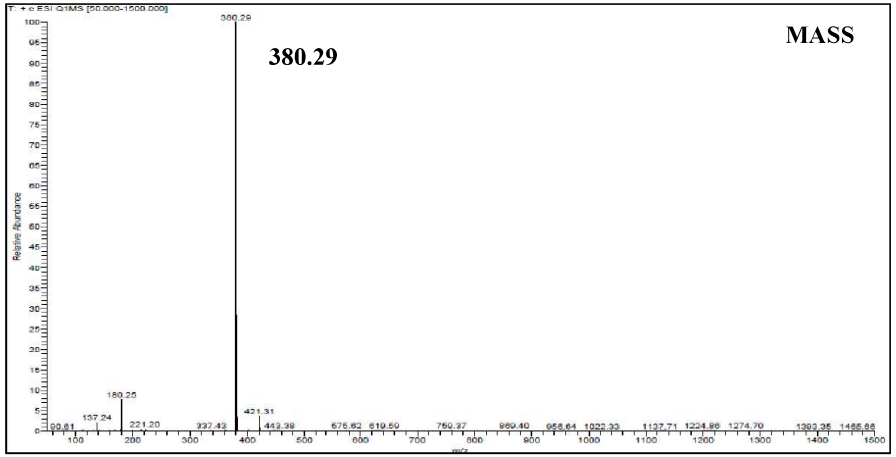


Spectral data of **53**









Spectral data of **54**