Chapter 2. Identification of pharmacophore for ME3 inhibition and study of structure activity relationship (SAR).

2.1 Docking study of compound A in malate binding pocket of ME3

in silico binding pose of compound **A** (Glide score – 10.03 kcal/mol) in malate binding pocket of ME3 enzyme is presented in **Figure 2.1**. It revealed that **i)** the phenolic hydroxyl group in ring-A forms H-bond donor-acceptor interactions with Asp304 and Asn489 **ii)** ring A was a having cationic$\cdots\pi$ interaction with the Lys208 side chain and **iii)** the pyrrolidine-2,5-dione ring had an H-bond interaction with Tyr107. Both enantiomers (*R* and *S*) of compound **A** have identical binding modes and interactions with ME3 protein.

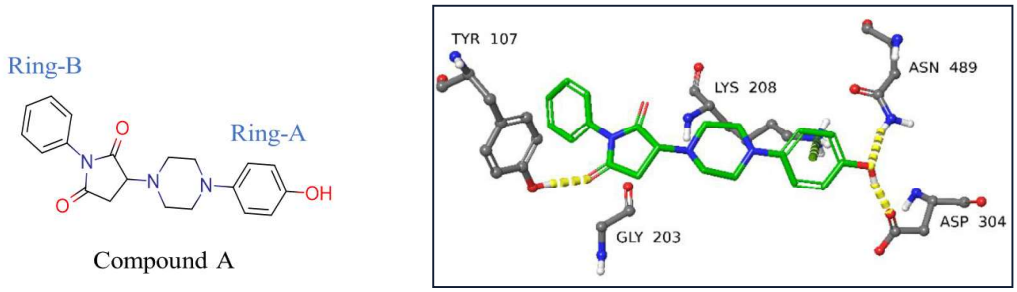


Figure 2.1: *in silico* binding pose of compound A with ME3

2.2 Design strategy – Ring opening and optimization of linker in compound A

Based on the binding mode described in **Figure 2.1**, many structures were designed by opening pyrrolidine-2,5-dione ring (compound **1**) and placing different linkers connecting ring-B and the N-aryl piperazine moiety (compound **2** to **8**) (**Figure 2.2, Chart 2.1**).

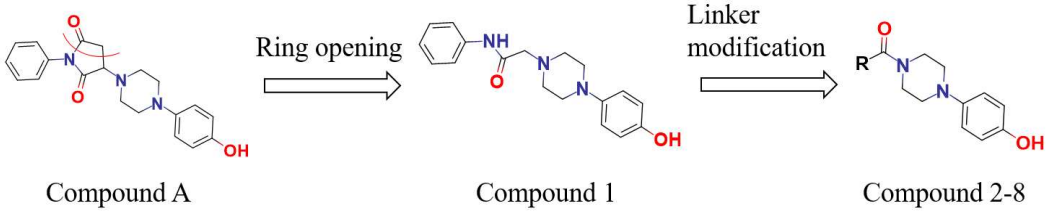
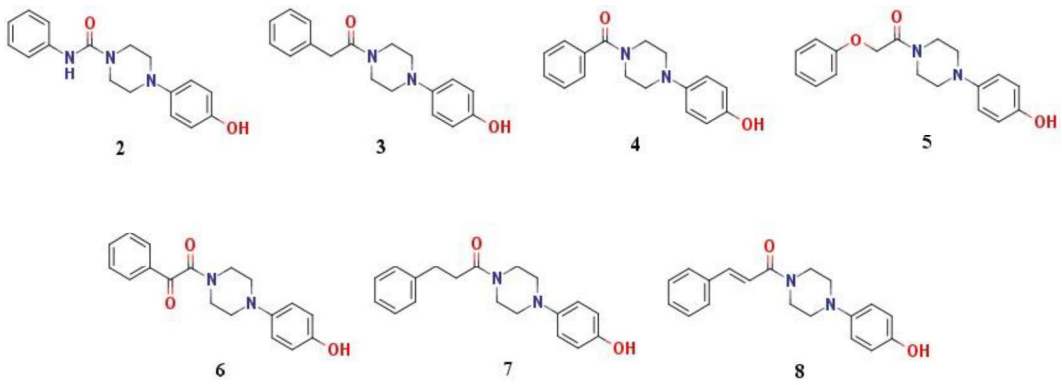


Figure 2.2: Designing strategy for compounds with different linkers

Compound **1** had acetyl linker between aniline and N-aryl piperazine moiety while compound **2** had urea linker between the same. Compound **3-8** contained amide linkers with diverse

variety and varied chain lengths connecting phenyl ring and N-aryl piperazine moiety (**Chart 2.1**).

Chart 2.1: The newly designed compounds **2-8**



The designed compounds (**Chart 2.1**) were docked using IFD grid which accommodated compound **A**. All the compounds retained critical interactions similar to compound **A**. Based on that it was predicted that inhibitory activity on ME3 would be retained. Binding mode of compound **1** with ME3 is depicted in **Figure 2.3**.

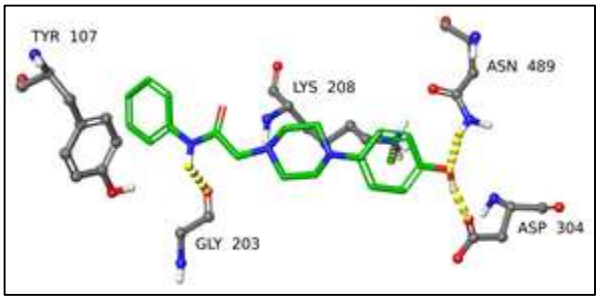
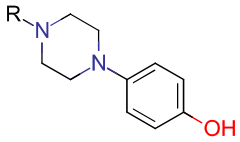


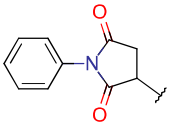
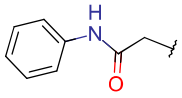
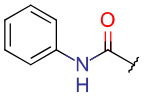
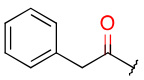
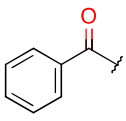
Figure 2.3: Binding pose of compound 1 with ME3

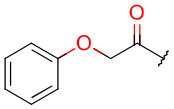
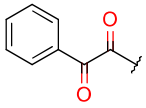
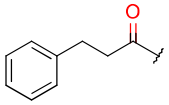
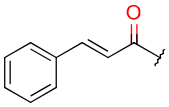
The synthesis of these compounds involved the Buchwald−Hartwig amination (BHA) reaction, the palladium catalysed cross-coupling reaction of aryl halides and primary or secondary amines yielding aryl amines, between 4-benzyloxy-1-bromobenzene (**I**) and *N*-Boc piperazine to yield the key intermediate **II**, which was double deprotected to give intermediate **IV**. Key intermediate **IV**, on condensation with appropriately substituted carboxylic acids, yielded compounds **3** to **8**. While the compounds **1** and **2** were prepared after derivatization of aniline with the needed functionalities and coupling them with **III** followed by debenzylation (**Refer Section 2.5**).

All these synthesized compounds were screened in *in vitro* for ME3 inhibitory activity along with other ME isoforms. All the compounds **1-8** exhibited promising inhibition of ME3 enzyme similar to that for compound **A (Table 2.1)**. Result indicated that they are pan inhibitors of ME isoforms. Compounds were also screened in *in vitro* for cell growth inhibition of BxPC-3 cells growth inhibition which are *SMAD4/ME2* null pancreatic cancer cell lines where ME3 takes over role of ME2 after genomic deletion of ME2. Among them compound **7** has better inhibitory activity of ME3 (IC_{50} = 0.10 μM) along with improved BxPC-3 inhibition (IC_{50} = 3.6 μM).

Table 2.1: ME isoforms and BxPC-3 cell growth inhibition data for compounds (**1-8**)



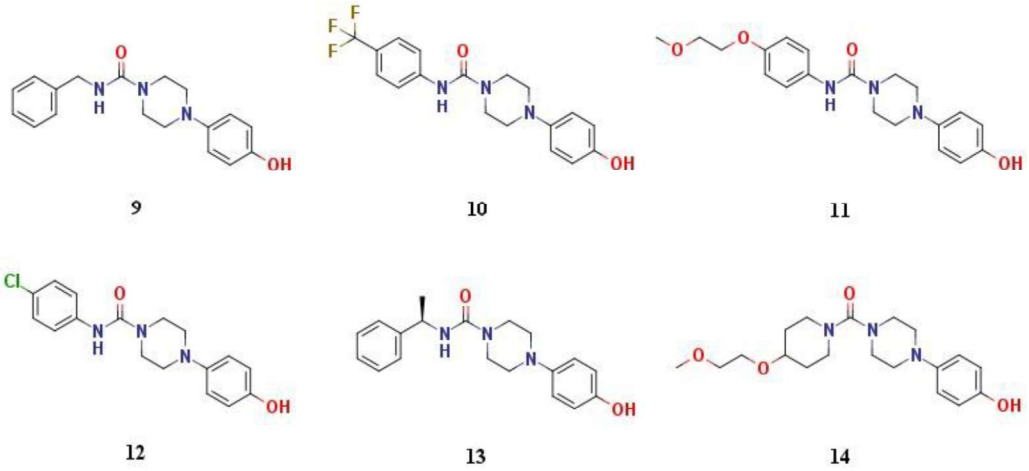
Compound No.	R	ME3 IC_{50} (μM)	ME2 IC_{50} (μM)	ME1 IC_{50} (μM)	% growth inhibition of BxPC-3 cells (10 μM)	BxPC-3 cells IC_{50} (μM)
A		**0.15**	**0.20**	**0.22**	**Inactive (IA)**	**Inactive (IA)**
1		0.11	0.36	0.34	18	-
2		0.11	0.22	0.20	28	31
3		0.10	0.19	0.18	80	6
4		0.13	-	-	60	-

Compound No.	**R**	**ME3 IC_{50} (μM)**	**ME2 IC_{50} (μM)**	**ME1 IC_{50} (μM)**	**% growth inhibition of BxPC-3 cells (10 μM)**	**BxPC-3 cells IC_{50} (μM)**
5		0.13	0.39	0.63	55	-
6		98% at 1 μM	-	-	49	-
7		0.10	0.27	0.27	93	3.6
8		0.08	0.23	0.17	29	-

In primary evaluation it was found that compound **2** having urea linkage had a good inhibitory potency for ME3 (IC_{50} = 0.11 μM) but it failed to show adequate inhibition of BxPC-3 cell growth (IC_{50} = 31 μM).

A second set of structurally diverse analogues (**9-14**) with urea linkage was designed majorly focusing on introduction of lipophilic groups to improve their cellular permeation and thereby their cellular potency (**Chart 2.2**).

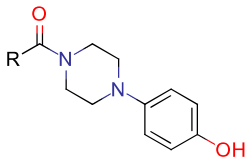
Chart 2.2: Structures of newly designed compounds **9-14**.

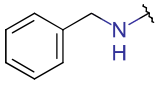
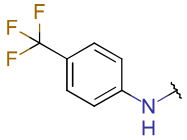


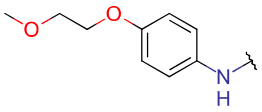
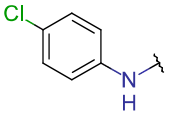
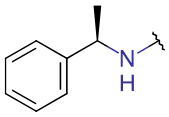
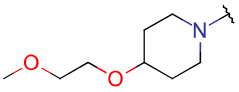
For the synthesis of these compounds, appropriately substituted aryl, aralkyl or aliphatic amines were treated with triphosgene to form carbamoyl chloride (or isocynate) intermediate which was further reacted with key intermediate **IV** to give **9-14** (Refer **Section 2.5**).

These synthesized compounds were screened on ME3 enzyme as well as on BxPC-3 cells and the results are presented in **Table 2.2**.

Table 2.2: ME3 inhibition and BxPC-3 cell growth inhibition data for urea analogues (**9-14**)



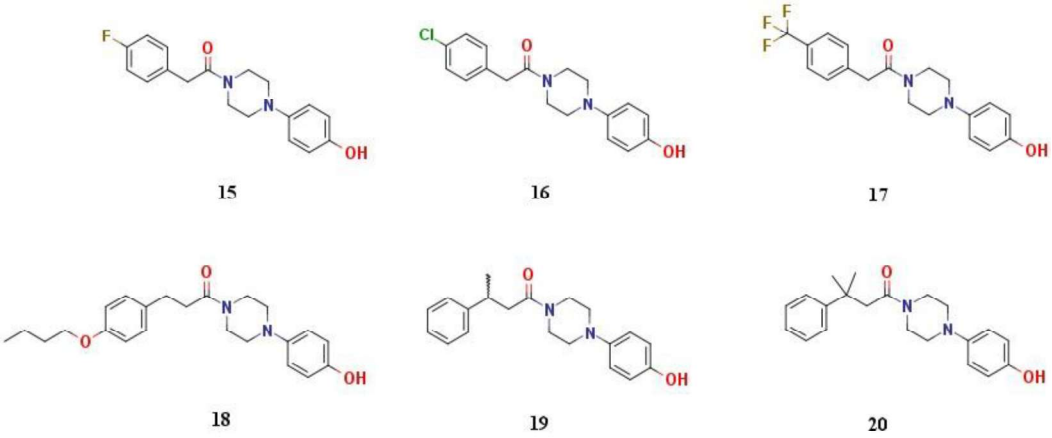
Compound No.	R	% ME3 inhibition		% growth inhibition of BxPC-3 cells (10 µM)	BxPC-3 cells IC_{50} (µM)
		1 µM	IC_{50} (µM)		
9		96	0.14	33	-
10		97	0.11	27	-

Compound No.	R	% ME3 inhibition		% growth inhibition of BxPC-3 cells	BxPC-3 cells
		1 μM	IC_{50} (μM)	(10 μM)	IC_{50} (μM)
11		95	0.07	03	-
12		98	0.10	30	`-
13		96	-	32	-
14		93	-	02	-

The compounds (**9-14**) exhibited promising inhibition of ME3 but none of the urea analogues was found to be superior compared to the amide analogues *viz.* **3** and **7** in the terms of BxPC-3 cell growth inhibition.

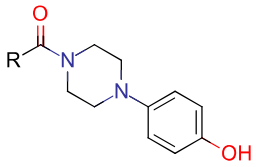
Similarly, a new set of compounds (**15-20**) (**Chart 2.3**) containing amide linker in place of urea linker was envisaged to increase lipophilicity of molecules for better cell penetration. Compound **15-18** were having lipophilic substituents at *para*- position of ring-B while in compound **19-20** methyl substituents were incorporated in linker part connection ring-B and piperazine moiety. Appropriately substituted carboxylic acids were coupled with the key intermediate **IV** to yield these compounds (refer **Section 2.5**) (**Chart 2.2**).

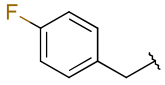
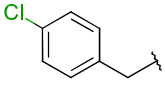
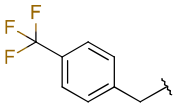
Chart 2.3: Structures of newly designed compounds **15-20**.

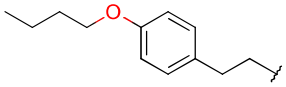
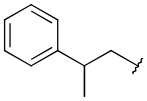
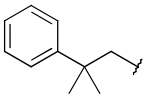


These compounds were screened on ME3 enzyme as well as on BxPC-3 cells and the results are presented in **Table 2.3**.

Table 2.3: ME3 inhibition and BxPC-3 cell growth inhibition data for amide analogues **(15-20)**



Compound No.	R	% ME3 inhibition		% growth inhibition of BxPC3 cells (10 μM)	BxPC3 cells IC_{50} (μM)
		1 μM	IC_{50} (μM)		
15		96	0.095	81	6
16		100	0.126	80	5.7
17		100	-	39	-

Compound No.	R	% ME3 inhibition		% growth inhibition of BxPC3 cells (10 µM)	BxPC3 cells IC_{50} (µM)
		1 µM	IC_{50} (µM)		
18		100	0.120	29	`-
19		98	-	81	7.4
20		100	0.120	80	5.0

On analysing the data generated for urea and amide analogues, it was revealed that the amide linkage containing compounds show better inhibition of BxPC-3 cell growth compared to the urea analogues in spite of having comparable enzymatic activity. Taking together ME3 enzyme inhibition as well as cellular potency, amide linker was selected for further optimization of the compounds.

2.3 Investigating the role of phenolic hydroxyl group on ME3 inhibition

The binding mode of amide analogue compound **3** in malate binding pocket of ME3 as depicted in **Figure 2.4** revealed that the phenolic hydroxyl group provides critical H-bond donor and acceptor interaction with Asp304 and Asn489, respectively.

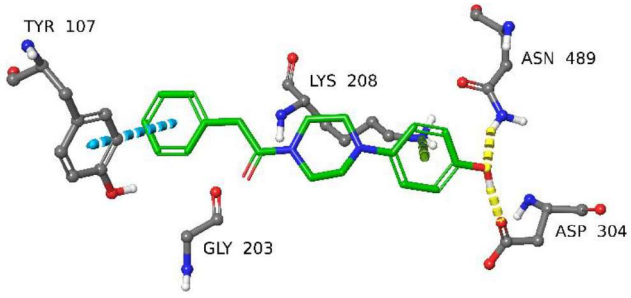


Figure 2.4: Binding pose of compound 3 with ME3

To investigate role of the phenolic hydroxyl group, next set of molecules **21** to **24** (**Figure 2.5**) were designed wherein **i**) bioisosteric replacement of phenolic hydroxyl group was carried out

with other polar functionalities bearing acidic hydrogen. (compound **21-23**); and **ii**) position of phenolic hydroxyl group was changed to -*m* with respect to the piperazine ring (compound **24**).

Ethyl 4-bromobenzoate, *N*-Boc piperazine and phenylacetic acid were employed for the synthesis of compound **21** which was further reacted with hydroxylamine hydrochloride to produce compound **22**. Compound **23** was prepared by the reaction of (4-nitrophenyl)piperazine and phenylacetic acid, followed by reduction and methylsulfonation of the resulting aniline derivative. The reaction of phenylacetic acid with (3-hydroxyphenyl)piperazine yielded compound **24** (**Refer section 2.5**).

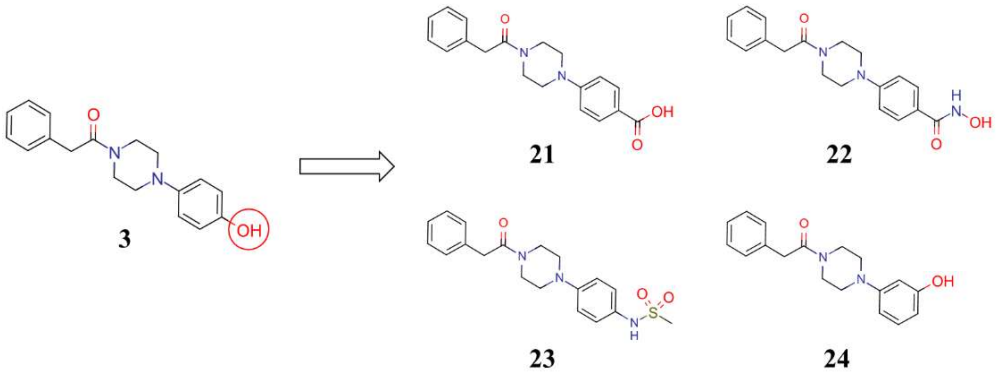


Figure 2.5: Replacement of phenolic hydroxyl group in compound 3

The compounds **21** to **24** were screened *in vitro* for ME3 enzyme inhibition and results are presented in **Table 2.4**.

Table 2.4: Effect of phenolic hydroxyl replacement on ME3 inhibition

Compound No.	% ME3 inhibition	
	1 µM	10 µM
3	**88**	**100**
21	00	19
22	00	15
23	04	32
24	06	00

The results of this study revealed that bioisosteric replacement of phenolic hydroxyl with carboxylic acid, hydroxamic acid or methyl sulphonamide in compound **21**, **22** and **23**,

respectively, resulted in loss of the activity on ME3. Changing the position of phenolic hydroxyl also abolished activity on ME3. Hence it was concluded that phenolic hydroxyl group at position-4 with respect to piperazine is critical for inhibitory potency on ME3.

2.4 Investigating the role of piperazine nitrogens on ME3 inhibition

In the binding mode of compound **3** with ME3 (**Figure 2.4**), no specific binding interaction was observed for piperazine ring. Hence to investigate the need and importance of piperazine ring, compounds **25** to **27** were designed with variants of piperazine ring (**Figure 2.6**).

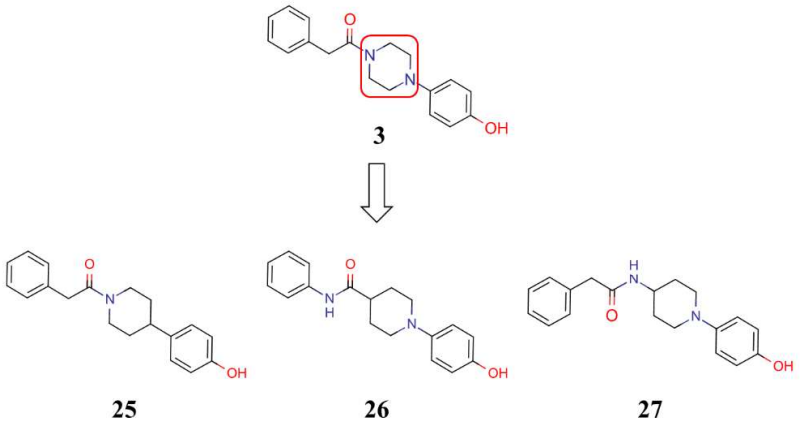


Figure 2.6: Replacement of piperazine nitrogens in compound 3

The synthesis of **25** involved coupling of phenylacetic acid with 4-(piperidin-4-yl)phenol while **26** was prepared from methyl piperidine-4-carboxylate, 1-benzyloxy-4-bromo-benzene (**I**) and aniline as starting materials and using the BHA reaction for N-arylation as a key step. Similarly, coupling of **I**, *N*-Boc piperazine and phenyl acetic acid led to the synthesis of **27** (**Refer section 2.5**).

These compounds were screened *in vitro* for ME3 enzyme inhibition as well as on BxPC-3 cell growth inhibition and results are presented in **Table 2.5**.

Table 2.5: Effect of replacement of piperazine nitrogens on ME3 inhibition

Compound No.	% ME3 inhibition			% growth inhibition of BxPC-3 cells (10 µM)	BxPC-3 cells IC_{50} (µM)
	1 µM	10 µM	IC_{50} µM		
3	**88**	**100**	**0.10**	**80**	**6**
25	13	31	-	-	-

Compound No.	% ME3 inhibition			% growth inhibition of BxPC-3 cells (10 μM)	BxPC-3 cells IC_{50} (μM)
	1 μM	10 μM	IC_{50} μM		
26	100	96	0.13	24	-
27	100	100	0.07	28	-

The results indicated that N-4 nitrogen of piperazine attached to 4-hydroxyphenol was critical for ME3 inhibition as replacement of N-4 nitrogen with carbon (compound **25**) resulted in significant loss of activity. N-1 nitrogen of piperazine, forming an amide bond, when replaced with carbon in compounds **26** (isonipecotic acid moiety) and **27** (4-aminopiperidine moiety) retained activity on ME3 enzyme. Though these variants were not superior to compound **3** in terms of BxPC-3 cell growth inhibition.

2.5 Chemistry

2.5.1 General information

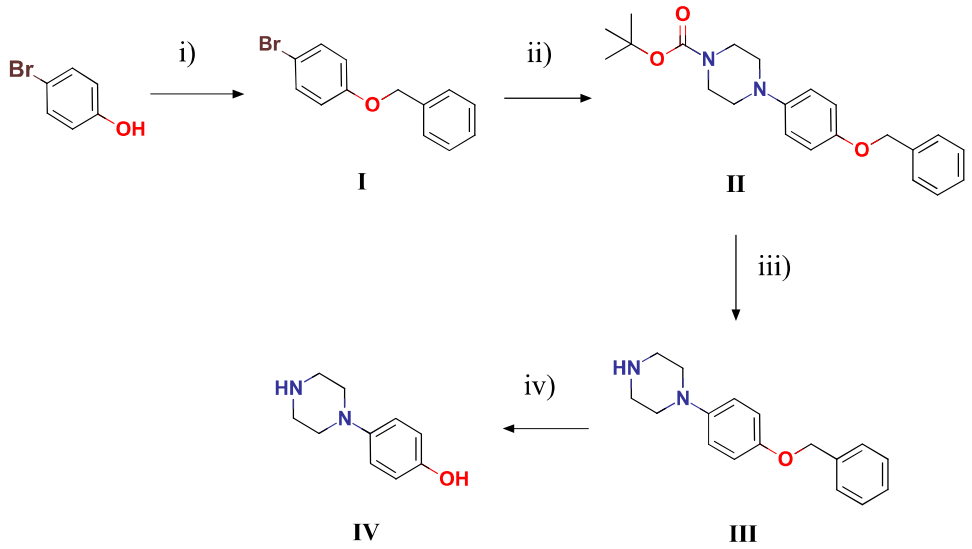
All commercial reagents and anhydrous solvents were purchased and used without further purification, unless otherwise specified. Mass spectra (MS) were obtained on a Quattro premier Waters using electrospray ionization (ESI) in positive mode unless otherwise indicated. Calculated (calcd.) mass corresponds to the exact calculated mass. ^{1}H-NMR and ^{13}C-NMR spectra were recorded on Bruker NMR spectrometers (400 MHz or 500 MHz). Conventional presentations of multiplicity are 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 compounds sent for biological tests were confirmed with purity >95% in quantitative HPLC analysis. IR spectra were obtained on a Perkin Elmer FTIR spectrometer, with major absorption bands reported. Elemental analyses were obtained on a Perkin Elmer 2400 Series II CHN S/O analyzer.

2.5.2 Experimental procedures and spectral data for compounds

1) Synthesis of 4-piperazin-1-ylphenol (intermediate IV)

For synthesis of the designed compounds, the manoeuvre began with the synthesis of the key intermediate **IV** as depicted in **Scheme 2.1**.

Scheme 2.1: Synthesis of key intermediate **IV**.



Reagents and conditions: (i) N,N-Dimethylformamide, powdered K_2CO_3, benzyl bromide, 40 °C, 16 h, 90%; (ii) *N*-Boc piperazine, sodium *tert*-butoxide, *tetrakis*-(triphenylphosphine) palladium(0), s-phos, toluene, 100 °C, 6 h, 76%; (iii) Dichloromethane, trifluoroacetic acid, 25 °C, 2 h, 90%; (iv) 5% Pd on carbon, H_2, tetrahydrofuran, methanol, 25 °C, 2 h, 90%.

Step-1: Synthesis of 1-benzyloxy-4-bromo-benzene (I)

To a solution of 4-bromophenol (2 g, 11.6 mmol) in N,N-dimethylformamide (10 ml) was added potassium carbonate (2.39 g, 17.3 mmol) and benzyl bromide (1.44 ml, 12.1 mmol). The resultant reaction mixture was stirred at 40 °C for 18 hours. The reaction was monitored by thin layer chromatography (TLC). On completion of the 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 were 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 1-benzyloxy-4-bromo-benzene (**I**) (2.73 g, 10.4 mmol, 90% yield) as an off white solid.

^{1}H NMR (400 MHz, DMSO-d$_6$): δ 5.15 (s, 2H), 7.04 (d, 2H, *J* = 8.89 *Hz*), 7.38 (t, 1H, *J* = 7.16 *Hz*), 7.44 (t, 2H, *J* = 7.41 *Hz*), 7.48 – 7.51 (m, 4H).

LCMS (ESI$^-$): calculated for $C_{13}H_{11}BrO$ $[M-H]^-$ 261.00; found: *m/z* = 260.92.

Step-2: Synthesis of *tert*-butyl 4-(4-benzyloxyphenyl)piperazine-1-carboxylate (II)

To a solution of t-butyl piperazine-1-carboxylate (1.7 g, 9.12 mmol) in toluene (40 ml) was added **I** (2.4 g, 9.12 mmol), *tetrakis*(triphenylphosphine)palladium(0) (0.53 g, 0.45 mmol) and s-phos (0.37 g, 0.91 mmol) at room temperature and heated to 50 °C for 15 minutes under nitrogen. Sodium t-butoxide (2.63 g, 2.74 mmol) was added to the reaction mixture and the reaction mixture was heated at 100 °C for 6 hours. On completion of reaction, the reaction mixture was filtered through celite bed followed by washing with ethyl acetate. The combined organic filtrates were washed with water, 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 *tert*-butyl 4-(4-benzyloxyphenyl)piperazine-1-carboxylate (**II**) (2.55 g, 6.92 mmol, 76% yield) as an off white solid.

^{1}H NMR (400 MHz, DMSO-d$_6$): δ 1.47 (s, 9H), 3.00 (t, 4H, *J* = 4.81 *Hz*), 3.49 (m, 4H), 5.08 (s, 2H), 6.95 (m, 4H), 7.36 (t, 1H, *J* = 7.18 *Hz*), 7.45 (t, 2H, *J* = 7.41 *Hz*), 7.47 (d, 2H, *J* = 7.20 *Hz*).

LCMS (ESI$^+$): calculated for $C_{22}H_{28}N_2O_3$ $[M+H]^+$ 369.21; found: *m/z* = 369.21.

Step-3: Synthesis of 1-(4-benzyloxyphenyl)piperazine (III)

A solution of **II** (2.0 g) in dichloromethane (20 ml) was treated with trifluoroacetic acid (10 ml) and stirred at room temperature for 2 hours. On completion of reaction, the residual liquid was evaporated under reduced pressure. The resultant residue was dissolved in water, basified with saturated sodium bicarbonate solution 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 to afford 1-(4-benzyloxyphenyl)piperazine (**III**) (1.31 g, 4.88 mmol, 90% yield) as an off white solid.

^{1}H NMR (400 MHz, DMSO-d$_6$): δ 2.87 (m, 4H), 2.97 (m, 4H), 5.07 (s, 2H), 6.93 (m, 4H), 7.36 (t, 1H, *J* = 7.09 *Hz*), 7.43 (t, 2H, *J* = 7.43 *Hz*), 7.47 (d, 2H, *J* = 7.43 *Hz*), 9.27 (br-s, 2H).

1H NMR of intermediate **III** was recorded as its trifluoroacetic acid salt.

LCMS (ESI$^+$): calculated for $C_{17}H_{20}N_2O$ $[M+H]^+$ 269.16; found: *m/z* = 269.16.

Step-4: Synthesis of 4-piperazin-1-ylphenol (IV)

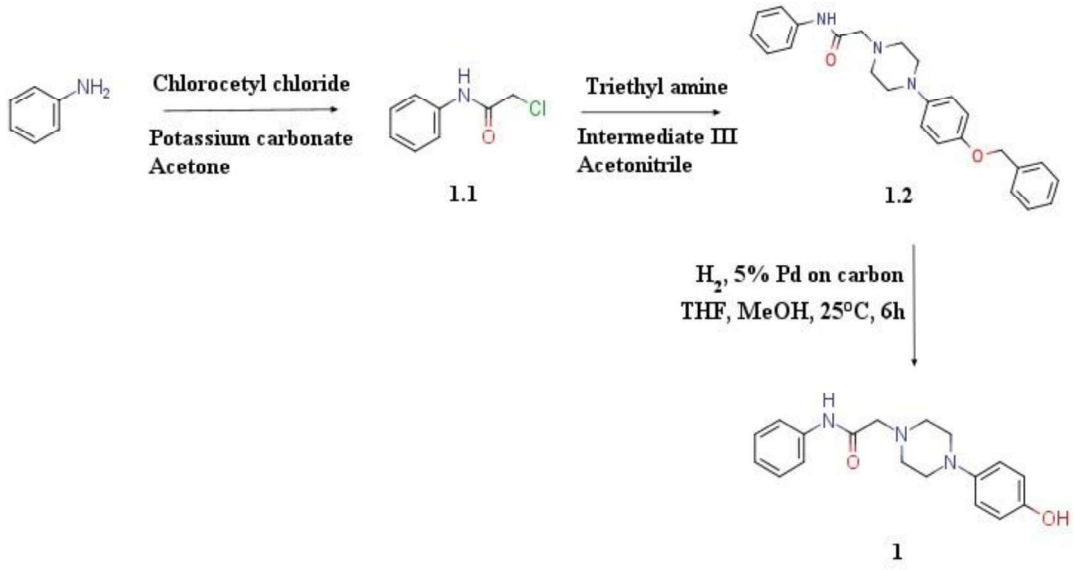
To a solution of **III** (2.0 g, 7.45 mmol) dissolved in methanol:tetrahydrofuran (1:1) (30 ml) was added 5% palladium on activated carbon (50% wet) (0.2 g) and the suspension was stirred under hydrogen atmosphere for 2 hours at room temperature. On completion of reaction, the reaction mixture was filtered off to remove the catalyst and solvents were evaporated under reduced pressure to afford 4-piperazin-1-ylphenol (**IV**) (1.19 g, 6.67 mmol, 90% yield) as pale-yellow solid.

^{1}H NMR (400 MHz, DMSO-d$_6$): δ 2.85 – 2.86 (m, 4H), 2.89 – 2.90 (m, 4H), 6.68 (d, 2H, *J* = 8.82 *Hz*), 6.80 (d, 2H, *J* = 8.83 *Hz*), 8.86 (br-s, 1H).

LCMS (ESI$^+$): calculated for $C_{10}H_{14}N_2O$ $[M+H]^+$ 179.11; found: *m/z* = 179.03.

2) Synthesis of 2-[4-(4-hydroxyphenyl)piperazin-1-yl]-N-phenylacetamide (1)

Scheme 2.2: Synthesis of compound **1**.



Step-1: Synthesis of 2-chloro-N-phenyl-acetamide (1.1)

To a solution of aniline (5.0 g, 53.69 mmol) in acetone (50 ml) was added potassium carbonate (14.81 g, 107.38 mmol). Resultant reaction mixture was cooled to 0-5 °C and added 2-chloroacetylchloride (5.12 ml, 64.43 mmol) in dropwise manner. Reaction mixture was allowed to attain room temperature and stirred for 2 hours. On completion of the reaction, reaction mixture was quenched with chilled water (0-5 °C) (100 ml) under vigorous stirring and precipitated solid was filtered and dried to afford 2-chloro-N-phenyl-acetamide (**1.1**) (4.52 g, 26.65 mmol, 49% yield) as a pale-yellow solid which was as such taken to the next step.

Step-2: Synthesis of 2-[4-[4-[(4-hydroxyphenyl)methoxy]phenyl]piperazin-1-yl]-N-phenyl-acetamide (1.2)

To a solution of intermediate **III** (0.3 g, 1.05 mmol) dissolved in acetonitrile (30.0 ml) was added compound 1.1 (0.133 g, 1.05 mmol) followed by triethylamine (0.22 ml, 2.1 mmol). Resultant reaction mixture was heated to 90 °C for 4 hours. On completion of the 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 residue was purified using column chromatography on silica gel (230 – 400 mesh) using ethyl acetate – hexane mixture as eluent to afford compound 2-[4-[4-[(4-hydroxyphenyl)methoxy]phenyl] piperazin-1-yl]-N-phenyl-acetamide (1.2) (0.25 g, 0.59 mmol, 57% yield) as an off white solid which was as such taken to the next step.

Step-3: Synthesis of 2-[4-(4-hydroxy-phenyl)-piperazin-1-yl]-N-phenyl-acetamide (1)

A solution of compound 1.2 (0.25 g, 0.59 mmol) in methanol:tetrahydrofuran (1:1) (20 ml) was added 10% palladium on activated carbon (0.25 g) and the suspension was stirred under hydrogen atmosphere for 6 hours at room temperature. On completion of the reaction, the reaction mixture was filtered off from the catalyst and the solution was evaporated under reduced pressure. The crude product obtained was purified by column chromatography on silica gel (230–400 mesh) to afford compound **1** (0.14 g, 0.44 mmol, 64% yield) as an off white solid.

Melting point: 216-219 °C. **LC purity (UV 245 nm):** 97.36%

^{1}H NMR (500 MHz, DMSO-d$_6$): δ 2.69 - 2.71 (m, 4H), 3.08 (m, 4H), 3.23 (s, 2H), 6.70 (d, 2H, *J* = 8.74 *Hz*), 6.84 (d, 2H, *J* = 8.78 *Hz*), 7.11 (t, 1H, *J* = 7.25 *Hz*), 7.36 (t, 2H, *J* = 7.76 *Hz*), 7.69 (d, 2H, *J* = 8.11 *Hz*), 8.85 (s,1H), 9.80 (s, 1H).

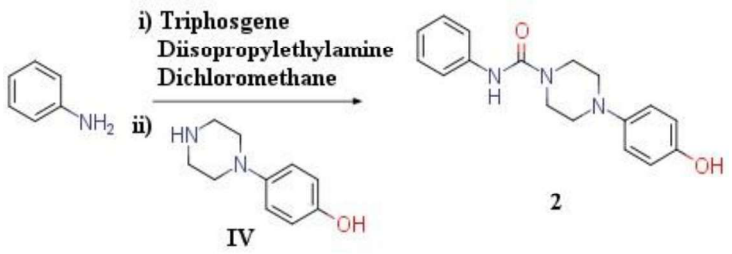
^{13}C NMR (125 MHz, DMSO-d$_6$): δ 50.3 (2 x CH_2), 53.3 (2 x CH_2), 62.1(CH_2), 115.8 (2 x CH), 118.2 (2 x CH), 119.8 (2 x CH), 123.8 (CH), 129.0 (2 x CH), 139.0 (C), 144.5 (C), 151.4 (C), 168.6 (C).

IR (ATR) / cm^{-1}: 3170, 2823, 1661, 1594, 1535, 1513, 1448, 1441, 1378, 1368, 1278, 1231, 1214, 1136, 1019, 938, 745, 731, 715, 686.

LCMS (ESI$^+$): calculated for $C_{18}H_{21}N_3O_2$ $[M+H]^+$ 312.16; found: *m/z* = 311.94.

3) Synthesis of 4-(4-hydroxyphenyl)-N-phenylpiperazine-1-carboxamide (2) - representative example for the synthesis of urea analogues

Scheme 2.3: Synthesis of compound **2**.



To a solution of aniline (1 g, 10.73 mmol) in dichloromethane (20 ml) was added triphosgene (1.05 g, 3.54 mmol) followed by diisopropylethylamine (3.74 ml, 21.47 mmol) at room temperature in dropwise manner. The resultant reaction mixture was stirred for 2h before adding intermediate IV (1.91 g, 10.73 mmol). The resultant reaction mixture was further stirred to for 3h. On completion of reaction, reaction mixture was washed with water (3 x 50 ml). The organic layer was washed with saturated sodium chloride solution, 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 **2** (1.4 g, 4.70 mmol, 44% yield) as an off white solid.

Melting point: 195-197 °C. **LC purity (UV 245 nm):** 98.34%

^{1}H NMR (500 MHz, DMSO-d$_6$): δ 3.02 (t, 4H, *J* = 4.91 *Hz*), 3.62 (t, 4H, *J* = 4.91 *Hz*), 6.72 (d, 2H, *J* = 8.86 *Hz*), 6.89 (d, 2H, *J* = 8.89 *Hz*), 6.98 (t, 1H, *J* = 7.33 *Hz*), 7.28 (t, 2H, *J* = 7.91 *Hz*), 7.52 (d, 2H, *J* = 7.66 *Hz*), 8.64 (s, 1H), 8.93 (s, 1H).

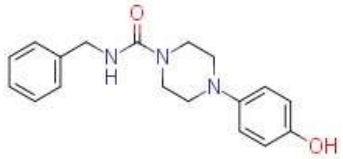
^{13}C NMR (125 MHz, DMSO-d$_6$): δ 44.4 (2 x CH_2), 50.8 (2 x CH_2), 115.9 (2 x CH), 118.8 (2 x CH), 120.1 (2 x CH), 122.2 (CH), 128.8 (2 x CH), 140.9 (C), 144.5 (C), 151.8 (C), 155.5 (C).

IR (ATR) / cm^{-1}: 3285, 2971, 2809, 1645, 1595, 1511, 1439, 1266, 1224, 1153, 831, 752, 695.

LCMS (ESI$^+$): calculated for $C_{17}H_{19}N_3O_2$ $[M+H]^+$ 298.15; found: *m/z* = 298.30.

The following 4-piperazin-1-ylphenol urea analogues (**9-14**) were prepared using 4-piperazin-1-ylphenol and commercially available amines in analogous fashion as described for representative example for the synthesis of compound **2**:

4) Synthesis of N-benzyl-4-(4-hydroxyphenyl)piperazine-1-carboxamide (9)



9

Benzylamine (0.50 g, 4.66 mmol) was reacted with 4-piperazin-1-ylphenol (IV) (0.83 g, 4.66 mmol) as per procedure described for compound **2** to afford compound **9** (0.62 g, 1.99 mmol, 42% yield) as an off white solid.

Melting point: 203-206 °C. **LC purity (UV 245 nm):** 96.78%.

^{1}H NMR (500 MHz, DMSO-d$_6$): δ 2.96 (t, 4H, *J* = 4.84 *Hz*), 3.50 (t, 4H, *J* = 4.84 *Hz*), 4.30 (d, 2H, *J* = 5.73 *Hz*), 6.70 (d, 2H, *J* = 8.85 *Hz*), 6.86 (d, 2H, *J* = 8.87 *Hz*), 7.21 – 7.27 (m, 1H), 7.30 – 7.32 (m, 1H), 7.34 – 7.37 (m, 4H), 8.92 (s, 1H).

^{13}C NMR (125 MHz, DMSO-d$_6$): δ 43.9 (CH_2), 44.1 (2 x CH_2), 50.4 (2 x CH_2), 115.9 (2 x CH), 118.8 (2 x CH), 126.9 (CH), 127.5 (2 x CH), 128.6 (2 x CH), 141.4 (C), 144.6 (C), 151.7 (C), 157.9 (C).

IR (ATR) / cm^{-1}: 3335, 1601, 1584, 1544, 1509, 1454, 1403, 1382, 1265, 1230, 1183, 1156, 813, 756, 697, 627, 522.

LCMS (ESI$^+$): calculated for $C_{18}H_{21}N_3O_2$ $[M+H]^+$ 312.16; found: *m/z* = 311.83.

5) Synthesis of 4-(4-hydroxyphenyl)-N-[4-(trifluoromethyl)phenyl]piperazine-1-carboxamide (10)

10

4-(Trifluoromethyl)aniline (0.50 g, 3.10 mmol) was reacted with 4-piperazin-1-ylphenol (IV) (0.55 g, 3.10 mmol) as per procedure described for compound **2** to afford compound **10** (0.52 g, 1.42 mmol, 45% yield) as an off white solid.

Melting point: 212-215 °C. **LC purity (UV 245 nm):** 98.37%.

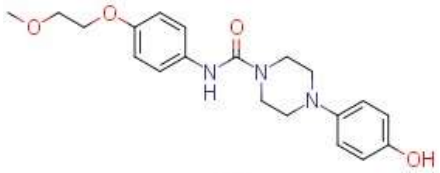
^{1}H NMR (500 MHz, DMSO-d$_6$): δ 3.03 (t, 4H, *J* = 4.88 Hz), 3.65 (t, 4H, *J* = 4.89 Hz), 6.72 (d, 2H, *J* = 8.87 Hz), 6.89 (d, 2H, *J* = 8.88 Hz), 7.64 (d, 2H, *J* = 8.72 Hz), 7.76 (d, 2H, *J* = 8.60 Hz), 8.94 (s, 1H), 9.04 (s, 1H).

^{13}C NMR (125 MHz, DMSO-d$_6$): δ 44.3 (2 x CH_2), 50.7 (2 x CH_2), 115.9 (2 x CH), 118.8 (2 x CH), 119.3 (2 x CH), 121.9 (C), 123.7 (C), 126.00 (CH), 126.04 (CH), 144.4 (C), 144.8 (C), 154.9 (C), 151.7 (C).

IR (ATR) / cm^{-1}: 3347, 1642, 1513, 1448, 1416, 1324, 1307, 1251, 1229, 1162, 1111, 1101, 1063, 828, 535.

LCMS (ESI$^+$): calculated for $C_{18}H_{18}F_3N_3O_2$ $[M+H]^+$ 366.14; found: *m/z* = 365.80.

6) Synthesis of 4-(4-hydroxyphenyl)-N-[4-(2-methoxyethoxy)phenyl]piperazine-1-carbox amide (11)



11

4-(2-Methoxyethoxy)aniline (0.50 g, 2.99 mmol) was reacted with 4-piperazin-1-ylphenol (IV) (0.53 g, 2.99 mmol) as per procedure described for compound **2** to afford compound **11** (0.42 g, 1.13 mmol, 37% yield) as an off white solid.

Melting point: 232-235 °C. **LC purity (UV 245 nm):** 97.34%.

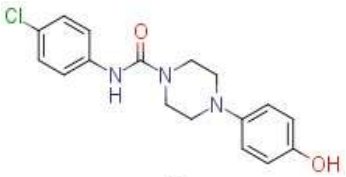
^{1}H NMR (500 MHz, DMSO-d$_6$): δ 3.01 (m, 4H), 3.35 (s, 3H), 3.60 (m, 4H), 3.68 (t, 2H, *J* = 4.32 Hz), 4.08 (t, 2H, *J* = 4.14 Hz), 6.72 (d, 2H, *J* = 8.71 Hz), 6.88 (m, 4H), 7.39 (d, 2H, *J* = 8.85 Hz), 8.47 (s, 1H), 8.91 (s, 1H).

^{13}C NMR (125 MHz, DMSO-d$_6$): δ 44.2 (2 x CH_2), 50.7 (2 x CH_2), 58.5 (CH_3), 67.3 (CH_2), 70.8 (CH_2), 114.5 (2 x CH), 115.8 (2 x CH), 118.7 (2 x CH), 121.9 (2 x CH), 133.9 (C), 144.5 (C), 151.6 (C), 154.0 (C), 155.6 (C).

IR (ATR) / cm^{-1}: 3282, 2981, 1625, 1609, 1532, 1510, 1478, 1449, 1423, 1399, 1222, 1146, 1116, 1061, 993, 836, 818, 522.

LCMS (ESI$^+$): calculated for $C_{20}H_{25}N_3O_4$ $[M+H]^+$ 372.18; found: *m/z* = 371.74.

7) Synthesis of 4-(4-hydroxyphenyl)-N-[4-(2-methoxyethoxy)phenyl]piperazine-1-carboxamide (12)



12

4-Chloroaniline (0.50 g, 3.91 mmol) was reacted with 4-piperazin-1-ylphenol (IV) (0.69 g, 3.91 mmol) as per procedure described for compound **2** to afford compound **12** (0.82 g, 2.74 mmol, 70% yield) as an off white solid.

Melting point: 212-215 °C. **LC purity (UV 245 nm):** 96.02%.

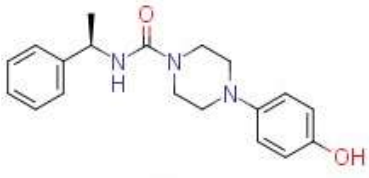
^{1}H NMR (500 MHz, DMSO-d$_6$): δ 3.01 (t, 4H), 3.62 (t, 4H), 6.72 (d, 2H, *J* = 8.71 Hz), 6.88 (d, 2H, *J* = 8.72 Hz), 7.33 (d, 2H, *J* = 8.75 Hz), 7.57 (d, 2H, *J* = 8.77 Hz),), 8.77 (s, 1H), 8.93 (s, 1H).

^{13}C NMR (125 MHz, DMSO-d$_6$): δ 44.3 (2 x CH_2), 50.7 (2 x CH_2), 115.9 (2 x CH), 118.8 (2 x CH), 121.4 (2 x CH), 125.7 (C), 128.6 (2 x CH), 140.0 (C), 144.5 (C), 151.7 (C), 155.2 (C).

IR (ATR) / cm^{-1}: 3359, 2981, 1643, 1511, 1491, 1444, 1404, 1382, 1303, 1250, 1227, 1200, 1150, 1084, 1002, 822, 537, 495.

LCMS (ESI$^+$): calculated for $C_{17}H_{18}ClN_3O_2$ $[M+H]^+$ 332.11; found: *m/z* = 331.77.

8) Synthesis of 4-(4-hydroxyphenyl)-N-[(1R)-1-phenylethyl]piperazine-1-carboxamide (13)



13

(1R)-1-Phenylethan-1-amine (0.45 g, 3.71 mmol) was reacted with 4-piperazin-1-ylphenol (IV) (0.66 g, 3.71 mmol) as per procedure described for compound **2** to afford compound **13** (0.54 g, 1.65 mmol, 44% yield) as an off white solid.

Melting point: 230-233 °C. **LC purity (UV 245 nm):** 99.27%.

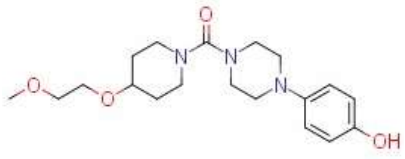
^{1}H NMR (500 MHz, DMSO-d_6): δ 1.41 (d, 3H, *J* = 7.06 Hz), 2.94 (t, 4H, *J* = 4.75 Hz), 3.49 (t, 4H, *J* = 4.75 Hz), 4.27 - 4.93 (m, 1H), 6.70 (d, 2H, *J* = 8.83 Hz) , 6.86 (d, 2H, *J* = 8.86 Hz), 6.91 (d, 1H, *J* = 7.87 Hz), 7.23 (t, 1H, *J* = 6.85 Hz), 7.33 - 7.38 (m, 4H), 8.90 (s, 1H).

^{13}C NMR (125 MHz, DMSO-d_6): δ 23.3 (CH_3), 44.1 (2 x CH_2), 49.8 (CH), 50.7 (2 x CH_2), 115.9 (2 x CH), 118.7 (2 x CH), 126.4 (2 x CH), 126.7 (CH), 128.5 (2 x CH), 144.6 (C), 146.5 (C), 151.7 (C), 157.2 (C).

IR (ATR) / cm^{-1}: 3144, 2981, 1628, 1524, 1509, 1445, 1376, 1261, 1224, 1155, 992, 829, 757, 698, 531.

LCMS (ESI$^+$): calculated for $C_{19}H_{23}N_3O_2$ $[M+H]^+$ 326.18; found: *m/z* = 325.79.

9) Synthesis of 4-{4-[4-(2-methoxyethoxy)piperidine-1-carbonyl] piperazin-1-yl}phenol (14)



14

4-(2-Methoxyethoxy)piperidine (0.50 g, 3.14 mmol) was reacted with 4-piperazin-1-ylphenol (IV) (0.55 g, 3.14 mmol) as per procedure described for compound **2** to afford compound **14** (0.47 g, 1.29 mmol, 41% yield) as an off white solid.

Melting point: 223-226 °C. **LC purity (UV 245 nm):** 96.15%.

^{1}H NMR (500 MHz, DMSO-d_6): δ 1.42 - 1.44 (m, 2H), 1.66 - 1.86 (m, 2H), 2.97 (m, 1H), 3.30 (m, 5H), 3.34 (m, 7H), 3.59 (m, 5H), 3.66 (m, 2H), 6.70 (d, 2H, *J* = 8.72 Hz), 6.84 (d, 2H, *J* = 8.74 Hz), 8.91 (s, 1H).

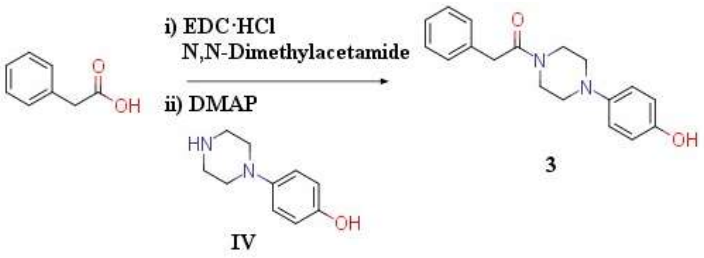
^{13}C NMR (125 MHz, DMSO-d_6): δ 31.2 (2 x CH_2), 44.1 (2 x CH_2), 44.3 (CH_2), 54.5 (CH_2), 58.4 (CH_3), 66.8 (2 x CH_2), 71.9 (2 x CH_2), 74.5 (CH), 104.0 (CH), 115.4 (CH), 116.4 (2 x CH), 122.9 (C), 157.7 (C), 162.6 (C).

IR (ATR) / cm^{-1}: 3428, 2388, 1628, 1603, 1518, 1439, 1421, 1382, 1289, 1262, 1245, 1219, 1108, 1091, 1067, 1023, 1013, 1006, 847, 548.

LCMS (ESI$^+$): calculated for $C_{19}H_{29}N_3O_{24}$ $[M+H]^+$ 364.22; found: *m/z* = 363.86.

10) Synthesis of 1-[4-(4-hydroxyphenyl)piperazin-1-yl]-2-phenylethanone (3) - representative example for the synthesis of amide analogues

Scheme 2.3: Synthesis of compound **3**.



To a solution phenylacetic acid (1.0 g, 7.34 mmol) dissolved in N,N-dimethylacetamide (10 ml) was added 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC·HCl) (2.11 g, 11.01 mmol). The reaction mixture was stirred for 10 minutes before adding intermediate IV (1.30 g, 7.34 mmol). To the resultant reaction mixture was added 4-(dimethylamino)pyridine (0.089 g, 0.73 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 **3** (0.96 g, 3.23 mmol, 58% yield) as an off white solid.

Melting point: 192-195 °C. **LC purity (UV 245 nm):** 98.48%.

^{1}H NMR (500 MHz, DMSO-d$_6$): δ 2.88 (t, 2H, *J* = 4.47 *Hz*), 2.93 (t, 2H, *J* = 4.68 *Hz*), 3.65 (t, 4H, *J* = 4.91 *Hz*), 3.80 (s, 2H), 6.69 (d, 2H, *J* = 8.77 *Hz*), 6.82 (d, 2H, *J* = 8.78 *Hz*), 7.26 – 7.30 (m, 3H), 7.36 (t, 2H, *J* = 7.51 *Hz*), 8.93 (s, 1H).

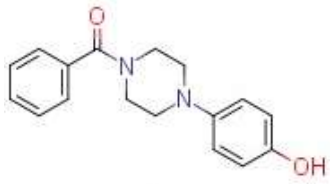
^{13}C NMR (125 MHz, DMSO-d$_6$): δ 39.9 (CH_2), 41.7 (CH_2), 46.0 (CH_2), 50.5 (CH_2), 51.0 (CH_2), 115.9 (2 x CH), 118.8 (2 x CH), 126.8 (CH), 128.7 (2 x CH), 129.4 (2 x CH), 136.3 (C), 144.3 (C), 151.8 (C), 169.2 (C).

IR (ATR) / cm^{-1}: 3284, 1645, 1591, 1513, 1438, 1269, 1221, 1152, 1033, 828, 817, 738, 701, 687.

LCMS (ESI$^+$): calcd for $C_{18}H_{20}N_2O_2$ $[M+H]^+$ 297.16; found: *m/z* = 297.33.

The following 4-piperazin-1-ylphenol amide analogues (**4-8 and 15-20**) were prepared using 4-piperazin-1-ylphenol and commercially available carboxylic acids in analogous fashion as described for representative example for the synthesis of compound **3**:

11) Synthesis of 4-(4-benzoylpiperazin-1-yl)phenol (4)



4

Benzoic acid (0.5 g, 4.09 mmol) was reacted with 4-piperazin-1-ylphenol (**IV**) (0.73 g, 4.09 mmol) as per the procedure described for compound **3** to afford compound **4** (0.80 g, 2.83 mmol, 69% yield) as an off white solid.

Melting point: 197-200 °C. **LC purity (UV 245 nm):** 96.76%.

^{1}H NMR (500 MHz, DMSO-d$_6$): δ 2.98 – 3.06 (m, 4H), 3.49 (m, 2H), 3.80 (m, 2H), 6.71 (d, 2H, *J* = 8.90 *Hz*), 6.86 (d, 2H, *J* = 8.90 *Hz*), 7.46 – 7.50 (m, 2H), 7.51 – 7.52 (m, 3H), 8.94 (s, 1H).

^{13}C NMR (125 MHz, DMSO-d$_6$): δ 42.0 (CH_2), 47.5 (CH_2), 50.8 (2 x CH_2), 115.8 (2 x CH), 118.9 (2 x CH), 127.3 (2 x CH), 128.8 (2 x CH), 129.9 (CH), 136.2 (C), 144.2 (C), 151.8 (C), 169.3 (C).

IR (ATR) / cm^{-1}: 3239, 2820, 1661, 1596, 1512, 1459, 1441, 1366, 1223, 1011, 728, 714, 700, 687, 544.

LCMS (ESI$^+$): calculated for $C_{17}H_{18}N_2O_2$ $[M+H]^+$ 283.14; found: *m/z* = 283.30.

12) Synthesis of 1-[4-(4-Hydroxy-phenyl)-piperazin-1-yl]-2-phenoxy-ethanone (5)

5

2-Phenoxyacetic acid (0.15 g, 0.98 mmol) was reacted with 4-piperazin-1-ylphenol (**IV**) (0.17 g, 0.98 mmol) as per procedure described for compound **3** to afford compound **5** (0.20 g, 0.64 mmol, 65% yield) as an off white solid.

Melting point: 227-230 °C. **LC purity (UV 245 nm):** 94.42%.

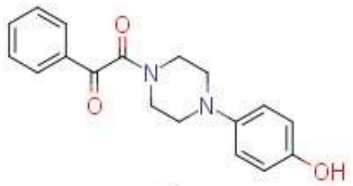
^{1}H NMR (500 MHz, DMSO-d$_6$): δ 2.96 (m, 2H), 3.04 (m, 2H), 3.64 (m, 4H), 4.90 (s, 2H), 6.71 (d, 2H, *J* = 8.81 *Hz*), 6.86 (d, 2H, *J* = 8.82 *Hz*) , 6.98 (t, 3H , *J* = 7.83 *Hz*), 7.33 (t, 2H, *J* =7.93 *Hz*), 8.96 (s, 1H).

^{13}C NMR (125 MHz, DMSO-d$_6$): δ 41.8 (CH_2), 44.8 (CH_2), 50.6 (CH_2), 51.0 (CH_2), 66.3 (CH_2), 115.0 (2 x CH), 115.9 (2 x CH), 118.9 (2 x CH), 121.32 (CH), 129.8 (2 x CH), 144.3 (C), 151.9 (C), 158.4 (C), 166.3 (C).

IR (ATR) / cm^{-1}: 3121, 2971, 2799, 1633, 1587, 1512, 1485, 1438, 1368, 1314, 1268, 1211, 1176, 1153, 1084, 1031, 1023, 816, 752, 688.

LCMS (ESI$^+$): calculated for $C_{18}H_{20}N_2O_3$ $[M+H]^+$ 313.15; found: *m/z* = 313.32.

13) Synthesis of 1-[4-(4-hydroxyphenyl)piperazin-1-yl]-2-phenylethane-1,2-dione (6)



6

2-Oxo-2-phenylacetic acid (0.40 g, 2.66 mmol) was reacted with 4-piperazin-1-ylphenol (**IV**) (0.47 g, 2.66 mmol) as per procedure described for compound **3** to afford compound **6** (0.25 g, 0.80 mmol, 30% yield) as an off white solid.

Melting point: 185-188 °C. **LC purity (UV 245 nm):** 99.62%.

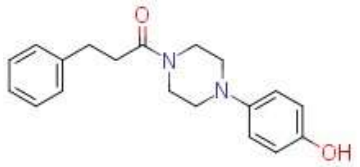
^{1}H NMR (500 MHz, DMSO-d$_6$): δ 2.95 (t, 2H, *J* = 5.04 *Hz*), 3.13 (t, 2H, *J* = 5.14 *Hz*), 3.45 (t, 2H, *J* = 5.01 *Hz*), 3.82 (t, 2H, *J* = 5.14 *Hz*), 6.72 (d, 2H, *J* = 8.92 *Hz*), 6.86 (d, 2H, *J* = 8.93 *Hz*), 7.69 (t, 2H, *J* = 7.80 *Hz*), 7.82 – 7.85 (m, 1H), 7.98 (dd, 2H, J_1 = 8.32 *Hz*, J_2 = 1.19 *Hz*), 8.98 (s, 1H)

^{13}C NMR (125 MHz, DMSO-d$_6$): δ 41.1 (CH_2), 45.8 (CH_2), 50.6 (CH_2), 51.0 (CH_2), 115.8 (2 x CH), 119.1 (2 x CH), 129.7 (2 x CH), 129.8 (2 x CH), 133.0 (C), 135.6 (CH), 144.0 (C), 151.9 (C), 165.1 (C), 192.16 (C).

IR (ATR) / cm^{-1}: 2974, 2820, 1677, 1637, 1593, 1512, 1438, 1275, 1220, 1207, 1174, 1132, 1112, 981, 845, 800, 717, 691, 678, 653, 548.

LCMS (ESI$^+$): calculated for $C_{18}H_{18}N_2O_3$ $[M+H]^+$ 311.13; found: *m/z* = 311.25.

14) Synthesis of 1-[4-(4-hydroxyphenyl)piperazin-1-yl]-3-phenylpropan-1-one (7)



7

3-Phenylpropanoic acid (0.15 g, 0.99 mmol) was reacted with 4-piperazin-1-ylphenol (**IV**) (0.18 g, 0.99 mmol) as per procedure described for compound **3** to afford compound **7** (0.22 g, 0.70 mmol, 70% yield) as an off white solid.

Melting point: 243-246 °C. **LC purity (UV 245 nm):** 99.67%.

^{1}H NMR (500 MHz, DMSO-d$_6$): δ 2.70 (t, 2H, *J* = 7.76 *Hz*), 2.88 (t, 2H, *J* = 7.91 *Hz*), 2.91 (m, 4H), 3.58 (t, 2H), 3.62 (t, 2H), 6.70 (d, 2H, *J* = 8.88 *Hz*), 6.83 (d, 2H, *J* = 8.89 *Hz*), 7.21 – 7.24 (m, 1H), 7.29 – 7.34 (m, 4H), 8.92 (s, 1H).

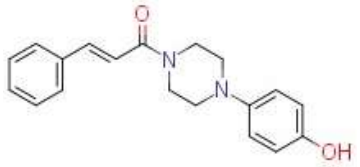
^{13}C NMR (125 MHz, DMSO-d$_6$): δ 31.2 (CH_2), 34.3 (CH_2), 41.6 (CH_2), 45.4 (CH_2), 50.7 (CH_2), 51.1 (CH_2), 115.9 (2 x CH), 118.9 (2 x CH), 126.3 (CH), 128.7 (2 x CH), 128.9 (2 x CH), 141.8 (C), 144.4 (C), 151.8 (C), 170.3 (C).

IR (ATR, cm^{-1}): 3299, 2815, 1623, 1589, 1452, 1440, 1365, 1336, 1280, 1265, 1219, 1180, 1156, 1027, 830, 762, 707.

LCMS (ESI^{+}): calculated for $C_{19}H_{22}N_2O_2$ $[M+H]^+$ 311.17; found: *m/z* = 311.21.

Elemental analysis calcd. (%) for $C_{19}H_{22}N_2O_2$: C 73.52, H 7.14, N 9.03; found: C 73.59, H 7.55, N 8.93.

15) Synthesis of (2E)-1-[4-(4-hydroxyphenyl)piperazin-1-yl]-3-phenylprop-2-en-1-one (8)



8

Cinnamic acid (0.15 g, 1.01 mmol) was reacted with 4-piperazin-1-ylphenol (**IV**) (0.18 g, 1.01 mmol) as per procedure described for compound **3** to afford compound **8** (0.18 g, 0.58 mmol, 57% yield) as an off white solid.

Melting point: 185-189 °C. **LC purity (UV 245 nm):** 97.83%.

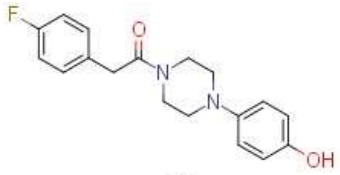
^{1}H NMR (500 MHz, DMSO-d_6): δ 3.02 (m, 4H), 3.75 (m, 2H), 3.89 (m, 2H), 6.72 (d, 2H, *J* = 8.80 *Hz*), 6.88 (d, 2H, *J* = 8.83 *Hz*), 7.36 (d, 1H, *J* = 15.41 *Hz*), 7.41 – 7.52 (m, 3H), 7.57 (d, 1H, *J* = 15.40 *Hz*), 7.78 (d, 2H, *J* = 6.95 *Hz*), 8.94 (s, 1H).

^{13}C NMR (125 MHz, DMSO-d_6): δ 42.2 (CH_2), 45.6 (CH_2), 50.7 (CH_2), 51.5 (CH_2), 115.9 (2 x CH), 118.6 (CH), 118.9 (2 x CH), 128.5 (2 x CH), 129.2 (2 x CH), 130.0 (CH), 135.6 (C), 142.0 (CH), 144.4 (C), 151.8 (C), 164.8 (C).

IR (ATR, cm^{-1}): 3300, 2936, 1639, 1542, 1509, 1442, 1357, 1324, 1232, 1178, 1124, 1037, 1004, 827, 812, 762, 701.

LCMS (ESI$^+$): calculated for $C_{19}H_{20}N_2O_2$ $[M+H]^+$ 309.15; found: *m/z* = 309.21.

16) Synthesis of 2-(4-fluorophenyl)-1-[4-(4-hydroxyphenyl)piperazin-1-yl]ethan-1-one (15)



15

2-(4-Fluorophenyl)acetic acid (0.15 g, 0.97 mmol) was reacted with 4-piperazin-1-ylphenol (**IV**) (0.17 g, 0.97 mmol) as per procedure described for compound **3** to afford compound **15** (0.19 g, 0.60 mmol, 61% yield) as an off white solid.

Melting point: 233-236 °C. **LC purity (UV 245 nm):** 99.14%.

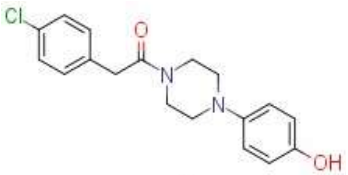
^{1}H NMR (500 MHz, DMSO-d_6): δ 2.91 – 2.94 (m, 4H), 3.63 – 3.67 (m, 4H), 3.80 (s, 2H), 6.70 (d, 2H, *J* = 8.81 *Hz*), 6.83 (d, 2H, *J* = 8.84 *Hz*), 7.18 (t, 2H, *J* = 8.82 *Hz*), 7.32 (dd, 2H, J_1 = 8.34 *Hz*, J_2 = 5.73 *Hz*), 8.94 (s, 1H).

^{13}C NMR (125 MHz, DMSO-d_6): δ 38.9 (CH_2), 41.8 (CH_2), 45.9 (CH_2), 50.6 (CH_2), 51.0 (CH_2), 115.4 (2 x CH, d, $J_{C\text{-}C\text{-}F}$ = 21.03 *Hz*), 115.9 (2 x CH), 118.8 (2 x CH), 131.4 (2 x CH, d, $J_{C\text{-}C\text{-}C\text{-}F}$ = 8.09 *Hz*), 132.5 (C, d, $J_{C\text{-}C\text{-}C\text{-}C\text{-}F}$ = 3.29 *Hz*), 144.3 (C), 151.8 (C), 161.4 (C, d, $J_{C\text{-}F}$ = 241.95 *Hz*), 169.2 (C).

IR (ATR, cm^{-1}): 3276, 2895, 2828, 1624, 1590, 1512, 1443, 1268, 1218, 1157, 1034, 831, 794, 707.

LCMS (ESI$^+$): calculated for $C_{18}H_{19}FN_2O_2$ $[M+H]^+$ 315.14; found: *m/z* = 315.19.

17) Synthesis of 2-(4-chlorophenyl)-1-[4-(4-hydroxyphenyl)piperazin-1-yl]ethan-1-one (16)



16

2-(4-Chlorophenyl)acetic acid (0.20 g, 1.17 mmol) was reacted with 4-piperazin-1-ylphenol (**IV**) (0.21 g, 1.17 mmol) as per procedure described for compound **3** to afford compound **16** (0.25 g, 0.75 mmol, 64% yield) as an off white solid.

Melting point: 205-208 °C. **LC purity (UV 245 nm):** 97.85%.

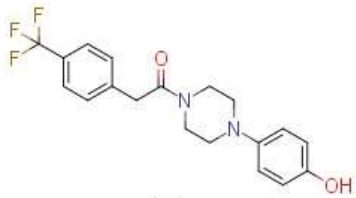
^{1}H NMR (500 MHz, DMSO-d$_6$): δ 2.93 – 2.95 (m, 4H), 3.63 – 3.67 (m, 4H), 3.81 (s, 2H), 6.70 (d, 2H, *J* = 8.83 *Hz*), 6.84 (d, 2H, *J* = 8.86 *Hz*), 7.31 (d, 2H, *J* = 8.33 *Hz*), 7.41 (d, 2H, *J* = 8.36 *Hz*), 8.93 (s, 1H).

^{13}C NMR (125 MHz, DMSO-d$_6$): δ 31.2 (CH_2), 41.8 (CH_2), 45.9 (CH_2), 50.6 (CH_2), 51.1 (CH_2), 115.9 (2 x CH), 118.8 (2 x CH), 128.6 (2 x CH), 131.5 (2 x CH), 131.5 (C), 135.5 (C), 144.3 (C), 151.8 (C), 168.9 (C).

IR (ATR) / cm^{-1}: 3366, 2853, 1636, 1511, 1491, 1437, 1412, 1264, 1247, 1227, 1205, 1154, 1032, 825, 777.

LCMS (ESI$^+$): calculated for $C_{18}H_{19}ClN_2O_2$ $[M+H]^+$ 331.11; found: *m/z* = 331.18. LC purity (UV 245 nm): 97.85%.

18) Synthesis of 1-[4-(4-hydroxyphenyl)piperazin-1-yl]-2-[4-(trifluoromethyl)phenyl] ethan-1-one (17)



17

2-[4-(Trifluoromethyl)phenyl]acetic acid (0.20 g, 0.97 mmol) was reacted with 4-piperazin-1-ylphenol (**IV**) (0.17 g, 0.97 mmol) as per procedure described for compound **3** to afford compound **17** (0.18 g, 0.49 mmol, 50% yield) as an off white solid.

Melting point: 190-193 °C. **LC purity (UV 245 nm):** 96.38%.

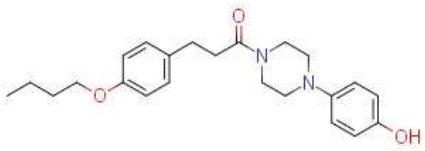
^{1}H NMR (500 MHz, DMSO-d_6): δ 2.95 (m, 4H), 3.65 – 3.68 (m, 4H), 3.94 (s, 2H), 6.70 (d, 2H, *J* = 8.79 *Hz*), 6.84 (d, 2H, *J* = 8.81 *Hz*), 7.51 (d, 2H, *J* = 7.97 *Hz*), 7.72 (d, 2H, *J* = 8.01 *Hz*), 8.94 (s, 1H).

^{13}C NMR (125 MHz, DMSO-d_6): δ 39.5 (CH_2), 41.8 (CH_2), 45.9 (CH_2), 50.6 (CH_2), 51.1 (CH_2), 115.9 (2 x CH), 118.9 (2 x CH), 125.4 (2 x CH, d, $J_{C\text{-}C\text{-}C\text{-}F}$ = 3.67 *Hz*), 125.4 (C, d, $J_{C\text{-}C\text{-}F}$ = 10.88 *Hz*), 127.5 (C, q, *J* = 31.63 *Hz*), 130.6 (2 x CH), 141.4 (C), 144.3 (C), 151.8 (C), 168.7 (C).

IR (ATR) / cm^{-1}: 3370, 2806, 1638, 1513, 1460, 1448, 1321, 1266, 1229, 1213, 1204, 1167, 1137, 1065, 1033, 1018, 826.

LCMS (ESI^{+}): calculated for $C_{19}H_{19}F_3N_2O_2$ $[M+H]^+$ 365.14; found: *m/z* = 365.25.

19) Synthesis of 3-(4-butoxyphenyl)-1-[4-(4-hydroxyphenyl)piperazin-1-yl]propan-1-one (18)



18

3-(4-Butoxyphenyl)propanoic acid (0.10 g, 0.44 mmol) was reacted with 4-piperazin-1-ylphenol (**IV**) (0.08 g, 0.44 mmol) as per procedure described for compound **3** to afford compound **18** (0.09 g, 0.23 mmol, 52% yield) as an off white solid.

Melting point: 178-181 °C. **LC purity (UV 245 nm):** 99.85%.

^{1}H NMR (400 MHz, DMSO-d_6): δ 0.97 (t, 3H, *J* = 7.39 *Hz*), 1.42 - 1.50 (m, 2H), 1.68 - 1.74 (m, 2H), 2.65 (t, 2H, *J* = 7.59 *Hz*), 2.80 (t, 2H, *J* = 7.60 *Hz*), 2.86 (t, 2H, *J* = 4.81 *Hz*), 2.90 (t, 2H, *J* = 4.99 *Hz*), 3.56 (t, 2H, *J* = 4.81 *Hz*), 3.61 (t, 2H, *J* = 4.94 *Hz*), 3.94 (t, 2H, *J* = 6.49 *Hz*), 6.70 (d, 2H, *J* = 8.93 *Hz*), 6.83 (d, 2H, *J* = 8.94 *Hz*), 6.87 (d, 2H, *J* = 8.60 *Hz*), 7.18 (d, 2H, *J* = 8.61 *Hz*), 8.93 (s, 1H).

^{13}C NMR (100 MHz, DMSO-d_6): δ 13.22 (CH_3), 18.3 (CH_2), 29.5 (CH_2), 30.3 (CH_2), 33.7 (CH_2), 40.6 (CH_2), 44.5 (CH_2), 49.8 (CH_2), 50.1 (CH_2), 66.5 (CH_2), 113.7 (2 x CH), 114.9 (2 x CH), 117.9 (2 x CH), 128.9 (2 x CH), 132.5 (C), 143.4 (C), 150.9 (C), 156.5 (C), 169.5 (C).

IR (ATR) / cm^{-1}: 2958, 1597, 1582, 1512, 1439, 1271, 1253, 1229, 1208, 1153, 1097, 1040, 814.

LCMS (ESI$^+$): calculated for $C_{23}H_{30}N_2O_3$ $[M+H]^+$ 383.22; found: *m/z* = 383.05.

20) Synthesis of 1-[4-(4-hydroxyphenyl)piperazin-1-yl]-3-phenylbutan-1-one (19)

19

3-Phenylbutanoic acid (0.50 g, 3.04 mmol) was reacted with 4-piperazin-1-ylphenol (**IV**) (0.54 g, 3.04 mmol) as per procedure described for compound **3** to afford compound **19** (0.62 g, 1.91 mmol, 62% yield) as an off white solid.

Melting point: 195-198 °C. **LC purity (UV 245 nm):** 99.73%.

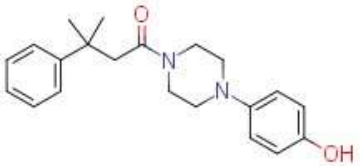
^{1}H NMR (400 MHz, DMSO-d$_6$): δ 1.27 (d, 3H, *J* = 6.93 *Hz*), 2.63 (dd, 1H, J_1 = 15.12 *Hz*, J_2 = 7.81 *Hz*), 2.71 (dd, 1H, J_1 = 15.16 *Hz*, J_2 = 6.67 *Hz*), 2.76 - 2.81 (m, 2H), 2.91 (m, 2H), 3.22-3.27 (m, 1H), 3.56 - 3.59 (m, 4H), 6.69 (d, 2H, *J* = 8.82 *Hz*), 6.82 (d, 2H, *J* = 8.80 *Hz*), 7.21-7.23 (m, 1H), 7.32 – 7.33 (m, 4H), 8.93 (s, 1H).

^{13}C NMR (100 MHz, DMSO-d$_6$): δ 21.5 (CH_3), 35.6 (CH), 39.7 (CH_2), 40.6 (CH_2), 44.6 (CH_2), 49.8 (CH_2), 50.1 (CH_2), 114.9 (2 x CH), 117.9 (2 x CH), 125.5 (CH), 126.4 (2 x CH), 127.7 (2 x CH), 143.4 (C), 146.0 (C), 150.8 (C), 168.8 (C).

IR (ATR) / cm^{-1}: 3061, 2981, 2962, 1597, 1585, 1511, 1489, 1462, 1445, 1375, 1272, 1241, 1229, 1218, 1189, 1181, 1153, 1084, 1037, 1029, 983, 902, 834, 763, 696, 623, 618, 527.

LCMS (ESI$^+$): calculated for $C_{20}H_{24}N_2O_2$ $[M+H]^+$ 325.19; found: *m/z* = 325.06.

21) Synthesis of 1-[4-(4-hydroxyphenyl)piperazin-1-yl]-3-methyl-3-phenylbutan-1-one (20)



20

3-Methyl-3-phenyl-butanoic acid (1.00 g, 5.61 mmol) was reacted with 4-piperazin-1-ylphenol (**IV**) (1.00 g, 5.61 mmol) as per procedure described for compound **3** to afford compound **20** (1.26 g, 3.72 mmol, 66% yield) as white solid.

Melting point: 205-208 °C. **LC purity (UV 245 nm):** 99.91%.

^{1}H NMR (400 MHz, DMSO-d$_6$): δ 1.46 (s, 6H), 2.69 (m, 2H), 2.72 (s, 2H), 2.81 (m, 2H), 3.38 - 3.51 (m, 4H), 6.68 (d, 2H, *J* = 8.81 *Hz*), 6.78 (d, 2H, *J* = 8.84 *Hz*), 7.20 (t, 1H, *J* = 7.21 *Hz*), 7.33 (t, 2H, *J* = 7.66 *Hz*), 7.43 (d, 2H, *J* = 7.87 *Hz*), 8.93 (s, 1H).

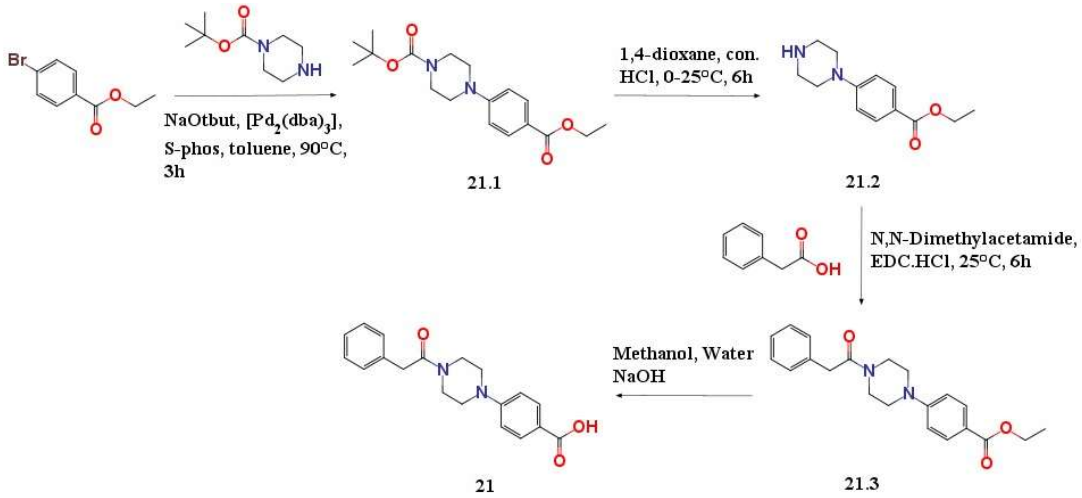
^{13}C NMR (100 MHz, DMSO-d$_6$): δ 29.5 (2 x CH_3), 37.7 (C), 41.2 (CH_2), 44.7 (CH_2), 45.9 (CH_2), 50.6 (CH_2), 50.8 (CH_2), 115.8 (2 x CH), 118.8 (2 x CH), 125.9 (C), 126.1 (2 x CH), 128.3 (2 x CH), 144.3 (C), 149.2 (C), 151.7 (C), 169.1 (C).

IR (ATR) / cm^{-1}: 3326, 2971, 2946, 2805, 1626, 1596, 1512, 1463, 1446, 1242, 1220, 1201, 1180, 1149, 1038, 836, 763, 698.

LCMS (ESI$^+$): calculated for $C_{21}H_{26}N_2O_2$ $[M+H]^+$ 339.20; found: *m/z* = 338.90.

22) Synthesis of 4-[4-(2-phenylacetyl)piperazin-1-yl]benzoic acid (21)

Scheme 2.4: Synthesis of compound **21**



Step-1: *tert*-Butyl 4-(4-ethoxycarbonylphenyl)piperazine-1-carboxylate (21.1)

To a solution of ethyl 4-bromobenzoate (2.15 g, 9.38 mmol) in toluene (50 ml) was added 1-Boc-piperazine (5.58 g, 30 mmol), tris-(dibenzylideneacetone)dipalladium(0) (0.45 g, 0.49 mmol) and s-phos (0.41 g, 1 mmol) at room temperature. The resultant reaction mixture was heated to 50°C for 10 minutes and then was added sodium *tert*-butoxide (2.88 g, 30 mmol) portion wise and further stirred at 90 °C for 3 hours. On completion of reaction, water (30 ml) and ethyl acetate (20 ml) were added to the 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 *tert*-butyl 4-(4-ethoxycarbonylphenyl)piperazine-1-carboxylate (**21.1**) (2.70 g, 8.42 mmol, 84% yield) as a colourless oil.

^{1}H NMR (400 MHz, DMSO-d$_6$): δ 1.34 (t, 3H, *J* = 7.08 *Hz*), 1.47 (s, 9H), 3.36 (t, 4H, *J* = 4.96 *Hz*), 3.50 (m, 4H), 4.30 (q, 2H, *J* = 7.07 *Hz*), 7.03 (d, 2H, *J* = 8.93 *Hz*), 7.84 (d, 2H, *J* = 8.87 *Hz*).

Step-2: Ethyl 4-piperazin-1-yl-benzoate (21.2)

To a solution of *tert*-butyl 4-(4-ethoxycarbonylphenyl)piperazine-1-carboxylate (**21.1**) (2.70 g, 8.07 mmol) in 1,4-dioxane (27 ml) was added hydrochloric acid (27 ml) drop wise and the resultant reaction mixture was stirred for 6 hours after completion of addition. After completion of the reaction, solvents were distilled out from reaction mixture and was quenched with saturated sodium bicarbonate solution in water (250 ml). Reaction mixture was 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 to afford ethyl 4-piperazin-1-yl-benzoate (**21.2**) (1.78 g, 7.94 mmol, 96% yield as colourless oil.

^{1}H NMR (400 MHz, DMSO-d$_6$): δ 1.34 (t, 3H, *J* = 7.08 *Hz*), 3.08 (t, 4H, *J* = 4.91 *Hz*), 3.43 (t, 4H, *J* = 4.94 *Hz*), 4.30 (q, 2H, *J* = 7.06 *Hz*), 7.05 (d, 2H, *J* = 8.92 *Hz*), 7.85 (d, 2H, *J* = 8.86 *Hz*).

Step-3: Ethyl 4-[4-(2-phenylacetyl)piperazin-1-yl]benzoate (21.3)

To a solution of phenylacetic acid (1.0 g, 7.34 mmol) dissolved in N,N-dimethylacetamide (10 ml) was added 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC·HCl) (2.11 g, 11.01 mmol). The reaction mixture was stirred for 10 minutes before adding **21.2** (1.72 g, 7.34 mmol). To the resultant reaction mixture was added 4-(dimethylamino)pyridine (0.089 g, 0.73 mmol) and was further stirred at room temperature for 6 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 ethyl 4-[4-(2-phenylacetyl)piperazin-1-yl]benzoate (**21.3**) (1.10 g, 3.25 mmol, 71% yield) as an off white solid.

^{1}H NMR (400 MHz, DMSO-d$_6$): δ 1.34 (t, 3H, *J* = 7.08 *Hz*), 3.32 (m, 2H), 3.36 (m, 2H), 3.67-3.68 (m, 4H), 3.83 (s, 2H), 4.29 (q, 2H, *J* = 7.07 *Hz*), 7.02 (d, 2H, *J* = 8.94 *Hz*), 7.26-7.30 (m, 3H), 7.35-7.38 (m, 2H), 7.84 (d, 2H, *J* = 8.89 *Hz*)

Step-4: 4-[4-(2-Phenylacetyl)piperazin-1-yl]benzoic acid (21)

To a solution of ethyl 4-[4-(2-phenylacetyl)piperazin-1-yl]benzoate (**21.3**) (0.55 g, 1.56 mmol) in methanol (10 ml) was added 1N sodium hydroxide (10 ml). Resultant reaction mixture was stirred overnight at room temperature. After completion of the reaction, solvent was distilled out from reaction mixture using rotary evaporator. Further it was acidified to pH 5 with 1N HCl and extracted with ethyl acetate (2 x 50 ml). The combined organic extracts were washed with 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 dichloromethane:methanol mixture as eluent to afford compound **21** (0.40 g, 1.25 mmol, 83% yield) as an off white solid.

Melting point: 201-205 °C. **LC purity (UV 245 nm):** 99.95%.

^{1}H NMR (500 MHz, DMSO-d$_6$): δ 3.29 - 3.35 (m, 4H), 3.66 - 3.70 (m, 4H), 3.83 (s, 2H), 6.99 (d, 2H, *J* = 9.00 *Hz*), 7.26 - 7.30 (m, 3H), 7.35 - 7.38 (m, 2H), 7.82 (d, 2H, *J* = 8.92 *Hz*), 12.33 (s, 1H).

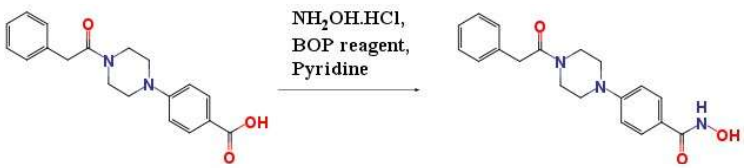
^{13}C NMR (125 MHz, DMSO-d$_6$): δ 40.6 (CH_2), 41.2 (CH_2), 45.3 (CH_2), 46.9 (CH_2), 47.3 (CH_2), 113.9 (CH), 114.0 (CH), 120.2 (CH), 126.8 (CH), 128.7 (CH), 128.8 (CH), 129.5 (2 x CH), 131.2 (C), 131.4 (CH), 136.2 (C), 153.8 (C), 167.7 (C), 169.5 (C).

IR (ATR) / cm^{-1}: 2843, 2533, 1655, 1594, 1521, 1416, 1388, 1320, 1299, 1230, 1189, 1163, 1129, 1029, 962, 944, 906, 832, 776, 735, 698, 632, 555.

LCMS (ESI$^+$): calculated for $C_{19}H_{20}N_2O_3$ $[M+H]^+$ 325.15; found: *m/z* = 325.05.

23) Synthesis of N-hydroxy-4-[4-(2-phenylacetyl)piperazin-1-yl] benzamide (22)

Scheme 2.5: Synthesis of compound **22**



21 22

To a solution of 4-[4-(2-phenylacetyl)piperazin-1-yl]benzoic acid (**21**) (0.20 g, 0.61 mmol) in pyridine (6 ml) was added hydroxylamine hydrochloride (0.047 g, 0.67 mmol) followed by BOP (benzotriazol-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate) reagent (0.299 g, 0.67 mmol). The resultant reaction mixture was stirred at room temperature for 6 hours. On completion of the reaction, pH of reaction mixture was set to 5.0 using dilute HCl and extracted with ethyl acetate. The combined organic extracts were washed with 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 **22** (0.10 g, 0.209 mmol, 47% yield) as an off white solid.

Melting point: 183-187 °C. **LC purity (UV 245 nm):** 96.63%.

^{1}H NMR (500 MHz, DMSO-d$_6$): δ 3.29 - 3.35 (m, 4H), 3.66 - 3.70 (m, 4H), 3.83 (s, 2H), 6.97 (d, 2H, *J* = 8.90 *Hz*), 7.26 - 7.30 (m, 3H), 7.35 - 7.38 (m, 2H), 7.87 (d, 2H, *J* = 8.87 *Hz*), 8.86 (s, 1H), 10.97 (s, 1H).

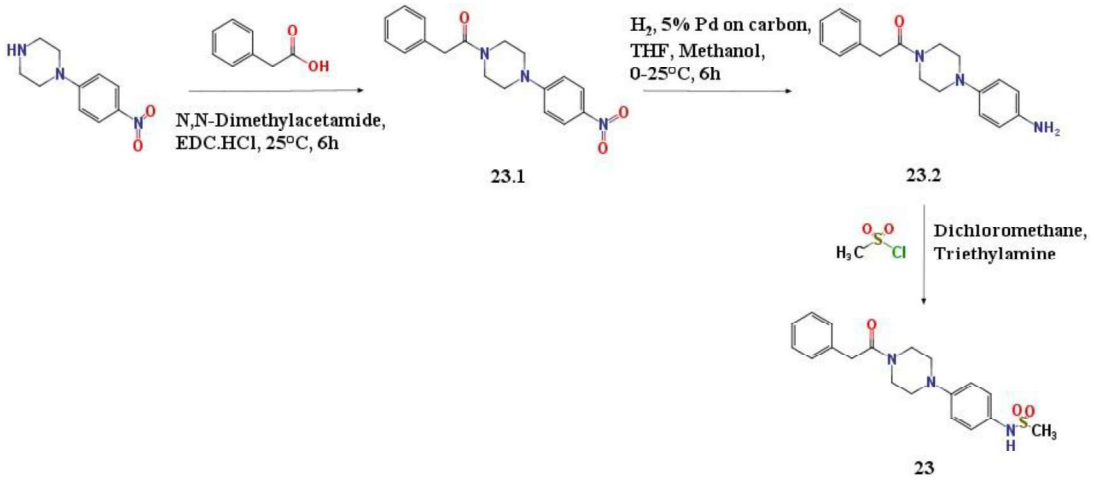
^{13}C NMR (125 MHz, DMSO-d$_6$): δ 40.3 (CH_2), 41.2 (CH_2), 45.3 (CH_2), 47.3 (CH_2), 47.7 (CH_2), 114.3 (2 x CH), 126.7 (2 x CH), 128.4 (CH), 128.70 (2 x CH), 129.22 (C), 129.3 (2 x CH), 136.2 (C), 152.7 (C), 167.9 (C), 169.3 (C).

IR (ATR) / cm^{-1}: 3195, 3001, 2845, 1625, 1603, 1506, 1471, 1442, 1385, 1329, 1234, 1156, 1026, 892, 830, 736, 700, 652, 630, 594.

LCMS (ESI$^+$): calculated for $C_{19}H_{21}N_3O_3$ $[M+Na]^+$ 362.14; found: *m/z* = 362.13.

24) Synthesis of N-{4-[4-(2-phenylacetyl)piperazin-1-yl]phenyl} methane sulphonamide (23)

Scheme 2.6: Synthesis of compound **23**



Step-1: 1-[4-(4-Nitrophenyl)piperazin-1-yl]-2-phenyl-ethanone (23.1)

To a solution of phenylacetic acid (1 g, 7.34 mmol) in N,N-dimethylacetamide (10 ml) was added 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC·HCl) (2.11 g, 11.01 mmol). The reaction mixture was stirred for 10 minutes before adding 1-(4-nitrophenyl)piperazine (1.52 g, 7.34 mmol). The resultant reaction mixture was added 4-(dimethylamino)pyridine (0.089 g, 0.73 mmol) and further stirred at room temperature for 6 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 1-[4-(4-nitrophenyl)piperazin-1-yl]-2-phenyl-ethanone (23.1) (1.40 g, 4.30 mmol, 89% yield) as yellow solid which was as such taken to the next step.

Step-2: 1-[4-(4-Aminophenyl)piperazin-1-yl]-2-phenyl-ethanone (23.2)

A solution of compound 1-[4-(4-nitrophenyl)piperazin-1-yl]-2-phenyl-ethanone (**23.1**) (1.40 g, 4.30 mmol) in methanol:tetrahydrofuran (1:1) (20 ml) was added 10% palladium on activated carbon (0.25 g) and the suspension was stirred under hydrogen atmosphere for 6 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. The crude product obtained was purified by column chromatography on silica gel (230–400 mesh) to afford 1-[4-(4-aminophenyl)piperazin-1-yl]-2-phenyl-ethanone (**23.2**) (1.05 g, 3.55 mmol, 82% yield) as an off white solid which was as such taken to the next step.

Step-3: N-[4-(4-Phenylacetyl-piperazin-1-yl)-phenyl]-methanesulfonamide (23)

A solution of compound 1-[4-(4-aminophenyl)piperazin-1-yl]-2-phenyl-ethanone (**23.2**) (1.05 g, 3.55 mmol) and triethylamine (0.54 ml, 3.91 mmol) in dichloromethane (5 ml) at 0-5°C was added a solution of methanesulphonyl chloride (0.29 ml, 3.73 mmol) in dichloromethane (1 ml) in dropwise manner. Resultant reaction mixture was further stirred at 0-5°C for 2 hours. On completion of reaction, the reaction mixture was diluted with dichloromethane (25 ml) and washed with water (2 x 25 ml) followed by saturated sodium chloride solution. Organic layer was 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 **23** (0.45 g, 1.20 mmol, 34% yield) as an off white solid.

Melting point: 156-159 °C. **LC purity (UV 245 nm):** 98.62%.

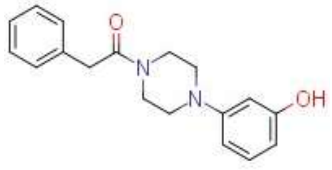
^{1}H NMR (500 MHz, DMSO-d$_6$): δ 2.91 (s, 3H), 3.04 - 3.09 (m, 4H), 3.66 - 3.67 (m, 4H), 3.82 (s, 2H), 6.97 (d, 2H, *J* = 8.79 *Hz*), 7.13 (d, 2H, *J* = 8.75 *Hz*), 7.24 - 7.30 (m, 3H), 7.35 - 7.38 (m, 2H), 9.33 (s, 1H).

^{13}C NMR (125 MHz, DMSO-d$_6$): δ 38.9 (CH_3), 39.9 (CH_2), 41.4 (CH_2), 45.6 (CH_2), 48.8 (CH_2), 49.2 (CH_2), 116.9 (2 x CH), 123.2 (2 x CH), 126.7 (CH), 128.70 (2 x CH), 129.3 (2 x CH), 130.3 (C), 136.2 (C), 148.4 (C), 169.2 (C).

IR (ATR) / cm^{-1}: 3205, 2823, 1637, 1511, 1466, 1449, 1397, 1329, 1305, 1273, 1230, 1213, 1150, 1033, 981, 963, 909, 820, 802, 776, 739, 700, 673, 583, 544, 511, 498.

LCMS (ESI$^-$): calculated for $C_{19}H_{23}N_3O_3S$ [M-H]$^-$ 372.15; found: *m/z* = 372.06.

25) Synthesis of 1-[4-(3-hydroxyphenyl)piperazin-1-yl]-2-phenylethan-1-one (24)



24

Phenylacetic acid (0.1 g, 0.73 mmol) was reacted with 3-(piperazin-1-yl)phenol (0.13 g, 0.73 mmol) as per procedure described for compound **3** to afford compound **24** (0.14 g, 0.47 mmol, 64% yield) as an off white solid.

Melting point: 194-197 °C. **LC purity (UV 245 nm):** 97.60%.

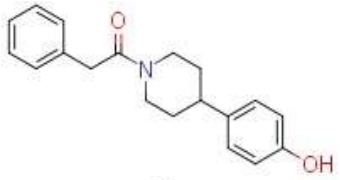
^{1}H NMR (500 MHz, DMSO-d$_6$): δ 3.01 – 3.07 (m, 4H), 3.64 – 3.65 (m, 4H), 3.80 (s, 2H), 6.27 (d, 1H, *J* = 7.91 *Hz*), 6.33 (s, 1H), 6.41 (d, 1H, *J* = 8.16 *Hz*), 7.03 (t, 1H, *J* = 8.07 *Hz*), 7.26 – 7.30 (m, 3H), 7.36 (t, 2H, *J* = 7.49 *Hz*), 9.27 (s, 1H).

^{13}C NMR (125 MHz, DMSO-d$_6$): δ 39.4 (CH_2), 41.4 (CH_2), 45.6 (CH_2), 48.6 (CH_2), 48.9 (CH_2), 103.2 (CH), 106.9 (CH), 107.3 (CH), 126.7 (CH), 128.7 (2 x CH), 129.3 (2 x CH), 129.9 (CH), 136.2 (C), 152.5 (C), 158.5 (C), 169.2 (C).

IR (ATR) / cm^{-1}: 3227, 2827, 1614, 1575, 1497, 1444, 1415, 1388, 1366, 1347, 1239, 1207, 1194, 1159, 973, 766, 735, 698, 689.

LCMS (ESI$^+$): calculated for $C_{18}H_{20}N_2O_2$ $[M+H]^+$ 297.15; found: *m/z* = 297.12.

26) Synthesis of 1-[4-(4-Hydroxy-phenyl)-piperidin-1-yl]-2-phenyl-ethanone (25)



25

To a solution of phenylacetic acid (1.0 g, 7.34 mmol) in N,N-dimethylacetamide (10 ml) was added 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC·HCl) (2.11 g, 11.01 mmol). The reaction mixture was stirred for 10 minutes before adding 4-(4-piperidyl)phenol (1.30 g, 7.34 mmol). The resultant reaction mixture was added 4-(dimethylamino)pyridine (0.089 g, 0.73 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 sulphate 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 **25** (0.85 g, 2.87 mmol, 39% yield) as an off white solid.

Melting point: 215-218 °C. **LC purity (UV 245 nm):** 95.01%.

^{1}H NMR (500 MHz, DMSO-d$_6$): δ 1.21 – 1.42 (m, 2H), 1.70 (d, 1H, *J* = 12.80 *Hz*), 1.76 (d, 1H, *J* = 12.86 *Hz*), 2.61 – 2.69 (m, 2H), 3.08 (t, 1H, *J* = 12.00 *Hz*), 3.79 (s, 2H), 4.07 (d, 1H, *J* = 13.28 *Hz*), 4.58 (d, 1H, *J* = 12.97 *Hz*), 6.71 (d, 2H, *J* = 8.42 *Hz*), 7.00 (d, 2H, *J* = 8.42 *Hz*), 7.26 – 7.33 (m, 3H), 7.35 – 7.38 (m, 2H), 9.22 (s, 1H).

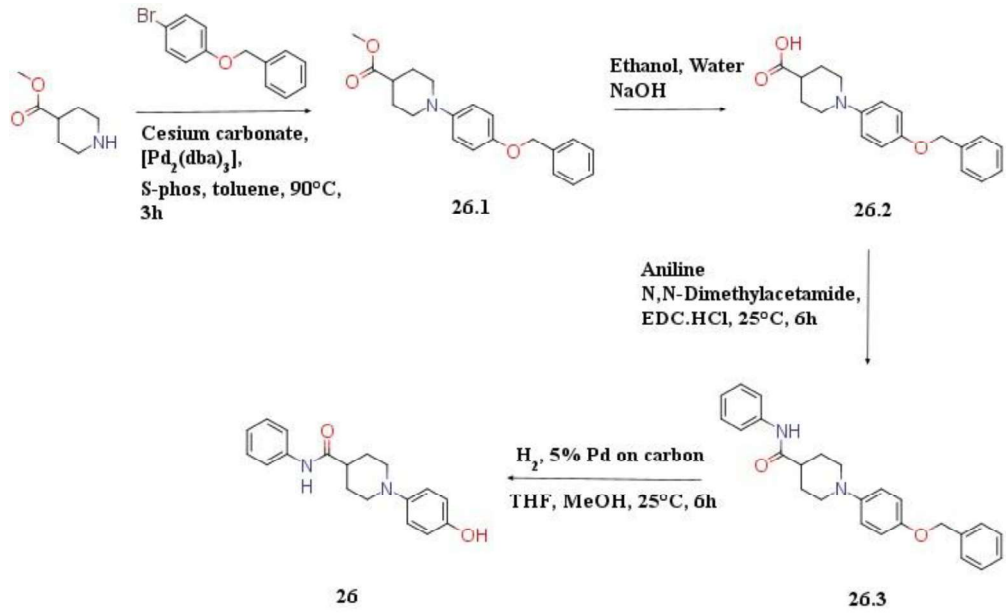
^{13}C NMR (100 MHz, DMSO-d$_6$): δ 33.3 (CH_2), 33.9 (CH_2), 40.0 (CH_2), 41.2 (CH), 42.2 (CH_2), 46.5 (CH_2), 115.4 (2 x CH), 126.72 (CH), 127.8 (2 x CH), 128.7 (2 x CH), 129.3 (2 x CH), 136.42 (C), 136.4 (C), 156.0 (C), 168.9 (C).

IR (ATR) / cm^{-1}: 2926, 1577, 1515, 1481, 1453, 1368, 1275, 1251, 1231, 1176, 1008, 826, 714, 703, 624, 606, 592, 538.

LCMS (ESI$^+$): calculated for $C_{19}H_{21}NO_2$ $[M+H]^+$ 296.16; found: *m/z* = 296.07.

27) Synthesis of 1-(4-hydroxyphenyl)-N-phenylpiperidine-4-carboxamide (26)

Scheme 2.7: Synthesis of compound **26**



Step-1: Synthesis of methyl 1-(4-benzyloxyphenyl)piperidine-4-carboxylate (26.1)

To a solution of methyl isonipecotate (10 g, 69.8 mmol) in toluene (100 ml) was added intermediate **I** (18.37 g, 69.8 mmol), $Pd_2(dba)_3$ (3.19 g, 3.49 mmol) and s-phos (2.86 g, 6.98 mmol) at room temperature. Resultant reaction mixture was heated to 50 °C for 15 minutes under nitrogen and added cesium carbonate (68.26 g, 209.5 mmol). Reaction mixture was further heated at 90 °C for 3 hours. On completion of reaction, reaction mixture was filtered through celite bed and washed 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 methyl 1-(4-

benzyloxyphenyl)piperidine-4-carboxylate (**26.1**) (8.1 g, 24.89 mmol, 36% yield) as colourless oil.

^{1}H NMR (400 MHz, DMSO-d$_6$): δ 1.67 – 1.77 (m, 2H), 1.94 – 1.96 (m, 2H), 2.46 – 2.53 (m, 1H), 2.65 – 2.72 (m, 2H), 3.48 – 3.51 (m, 2H), 3.67 (s, 3H), 5.07 (s, 2H), 6.93 (s, 4H), 7.35 – 7.49 (m, 5H).

Step-2: 1-(4-Benzyloxyphenyl)piperidine-4-carboxylic acid (26.2)

To a solution of methyl 1-(4-benzyloxyphenyl)piperidine-4-carboxylate (**26.1**) (8.1 g, 24.89 mmol) in ethanol (80 ml) was added 1N sodium hydroxide (80 ml). Resultant reaction mixture was stirred overnight at room temperature. After completion of the reaction, solvent was distilled out from reaction mixture. Further reaction mixture was acidified to pH 5 with 1N HCl and extracted with ethyl acetate (2 x 50 ml). The combined organic extracts were washed with brine, dried over anhydrous sodium sulfate and concentrated under reduced pressure to afford 1-(4-benzyloxyphenyl)piperidine-4-carboxylic acid (**26.2**) (7.0 g, 22.48 mmol, 91% yield) as colourless oil.

^{1}H NMR (400 MHz, DMSO-d$_6$): δ 1.65 – 1.75 (m, 2H), 1.93 – 1.96 (m, 2H), 2.34 - 2.41 (m, 1H), 2.64 – 2.71 (m, 2H), 3.47 – 3.50 (m, 2H), 5.07 (s, 2H), 6.93 (s, 4H), 7.35 – 7.49 (m, 5H), 12.26 (s, 1H).

Step-3: 1-(4-Benzyloxyphenyl)-N-phenyl-piperidine-4-carboxamide (26.3)

To a solution of 1-(4-benzyloxyphenyl)piperidine-4-carboxylic acid (**26.2**) (2.0 g, 6.42 mmol) in N,N-dimethylacetamide (10 ml) was added 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide Hydrochloride (EDC·HCl) (1.84 g, 9.63 mmol). The reaction mixture was stirred for 10 minutes before adding aniline (0.6 g, 6.42 mmol). The resultant reaction mixture was added 4-(dimethylamino)pyridine (0.078 g, 0.64 mmol) and further stirred at room temperature for 6 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 1-(4-benzyloxyphenyl)-N-phenyl-piperidine-4-carboxamide (**26.3**) (1.20 g, 3.10 mmol, 48% yield) as an off white solid which was as such taken to the next step.

Step-4: 1-(4-Hydroxyphenyl)-N-phenyl-piperidine-4-carboxamide (26)

To 1-(4-benzyloxyphenyl)-N-phenyl-piperidine-4-carboxamide (**26.3**) (1.20 g, 3.10 mmol) dissolved in methanol:tetrahydrofuran (1:1) (20 ml) was added 10% palladium on activated carbon (0.20 g) and the suspension was stirred under hydrogen atmosphere for 6 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. The crude product obtained was purified by column chromatography on silica gel (230–400 mesh) to afford compound **26** (0.63 g, 2.12 mmol, 68% yield) as an off white solid.

Melting point: 205-208 °C. **LC purity (UV 245 nm):** 98.76%.

^{1}H NMR (400 MHz, DMSO-d$_6$): δ 1.78 – 1.86 (m, 2H), 1.90 – 1.92 (m, 2H), 2.44 – 2.52 (m, 1H), 2.60 – 2.62 (m, 2H), 3.53 – 3.56 (m, 2H), 6.70 (d, 2H, *J* = 8.80 *Hz*), 6.86 (d, 2H, *J* = 8.84 *Hz*), 7.07 (t, 1H, *J* = 7.37 *Hz*), 7.34 (t, 2H, *J* = 7.84 *Hz*), 7.66 (d, 2H, *J* = 7.92 *Hz*), 8.86 (s, 1H), 9.95 (s, 1H).

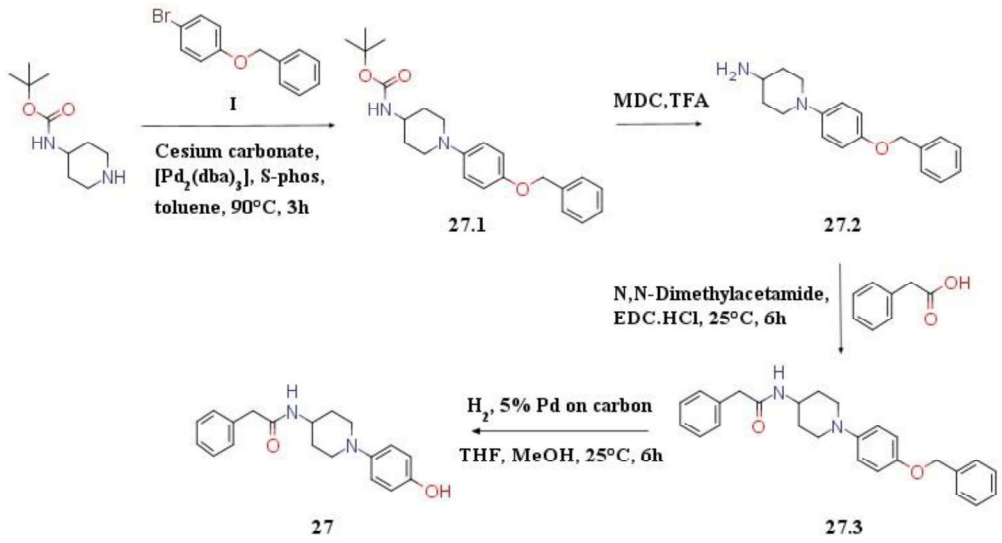
^{13}C NMR (100 MHz, DMSO-d$_6$): δ 29.7 (2 x CH_2), 43.9 (CH), 51.6 (2 x CH_2), 116.6 (2 x CH), 119.7 (2 x CH), 120.3 (2 x CH), 124.22 (CH), 129.9 (2 x CH), 140.6 (C), 145.8 (C), 152.1 (C), 174.7 (C).

IR (ATR) / cm^{-1}: 3314, 1655, 1597, 1526, 1510, 1441, 1383, 1340, 1323, 1240, 1220, 1182, 1144, 1108, 966, 820, 749, 689, 592, 535, 508.

LCMS (ESI$^+$): calculated for $C_{18}H_{20}N_2O_2$ $[M+H]^+$ 297.15; found: *m/z* = 297.19.

28) Synthesis of N-[1-(4-hydroxyphenyl)piperidin-4-yl]-2-phenyl acetamide (27)

Scheme 2.8: Synthesis of compound **27**



Step-1: Synthesis of *tert*-butyl N-[1-(4-benzyloxyphenyl)-4-piperidyl]carbamate (27.1)

To a solution of 4-(*N*-Boc-amino)piperidine (10 g, 49.93 mmol) in toluene (100 ml) was added intermediate **I** (13.13 g, 49.93 mmol), tris-(dibenzylideneacetone)dipalladium(0) (2.28 g, 2.49 mmol) and s-phos (2.04 g, 4.99 mmol) at room temperature. Resultant reaction mixture was heated to 50 °C for 15 minutes under nitrogen and added cesium carbonate (48.80 g, 149.8 mmol). Reaction mixture was further heated at 90 °C for 3 hours. On completion of the reaction, reaction mixture was filtered through celite bed and washed 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 *tert*-butyl N-[1-(4-benzyloxyphenyl)-4-piperidyl]carbamate (**27.1**) (12.5 g, 32.67 mmol, 65% yield) as colourless oil which was as such taken to the next step.

Step-2: 1-(4-Benzyloxyphenyl)piperidin-4-amine (27.2)

To a solution of *tert*-butyl N-[1-(4-benzyloxyphenyl)-4-piperidyl]carbamate (**27.1**) (12.5 g, 32.67 mmol) in dichloromethane (30 ml) was added trifluoroacetic acid (30 ml) drop wise and resultant reaction mixture was stirred for 3 hours after completion of addition. After completion of reaction, solvents were distilled out from reaction mixture and it was quenched with saturated sodium bicarbonate solution in water (250 ml). Reaction mixture was 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 to afford

1-(4-benzyloxyphenyl)piperidin-4-amine (**27.2**) (8.34 g, 29.53 mmol, 90% yield) as an off white solid.

^{1}H NMR (400 MHz, DMSO-d$_6$): δ 1.40 – 1.42 (m, 1H), 1.52 - 1.56 (m, 1H), 1.86 (m, 2H), 2.64 – 2.69 (m, 1H), 3.49 (m, 4H), 5.07 (s, 2H), 6.92 (s, 4H), 7.36 (t, 1H, *J* = 7.10 *Hz*), 7.43 (t, 2H, *J* = 7.42 *Hz*), 7.47 (d, 2H, *J* = 7.24 *Hz*).

Step-3: N-[1-(4-Benzyloxyphenyl)-4-piperidyl]-2-phenyl-acetamide (27.3)

To a solution of phenylacetic acid (1 g, 7.34 mmol) in N,N-dimethylacetamide (10 ml) was added 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC·HCl) (2.11 g, 11.01 mmol). The reaction mixture was stirred for 10 minutes before adding **27.2** (2.07 g, 7.34 mmol). The resultant reaction mixture was added 4-(dimethylamino)pyridine (0.089 g, 0.73 mmol) and further stirred at room temperature for 6 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 N-[1-(4-benzyloxyphenyl)-4-piperidyl]-2-phenyl-acetamide (**27.3**) (1.35 g, 3.37 mmol, 47% yield) as an off white solid which was as such taken to the next step.

Step-4: N-[1-(4-Hydroxy-phenyl)-piperidin-4-yl]-2-phenyl-acetamide (27)

N-[1-(4-Benzyloxyphenyl)-4-piperidyl]-2-phenyl-acetamide (**27.3**) (1.35 g, 3.37 mmol) dissolved in methanol : tetrahydrofuran (1:1) (20 ml) was added 10% Palladium on activated carbon (0.20 g) and the suspension was stirred under hydrogen atmosphere for 6 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. The crude product obtained was purified by column chromatography on silica gel (230–400 mesh) to afford compound **27** (0.53 g, 1.70 mmol, 51% yield) as an off white solid.

Melting point: 172-175 °C. **LC purity (UV 245 nm):** 99.55%.

^{1}H NMR (500 MHz, DMSO-d$_6$): δ 1.56 (d, 2H, *J* = 8.87 *Hz*), 1.84 (d, 2H, *J* = 11.02 *Hz*), 2.66 (t, 2H, *J* = 11.85 *Hz*), 3.42 (m, 2H), 3.44 (s, 2H), 3.65 - 3.68 (m, 1H), 6.68 (d, 2H, *J* = 8.83 *Hz*), 6.83 (d, 2H, *J* = 8.86 *Hz*), 7.28 (t, 1H, *J* = 7.07 *Hz*) , 7.30 - 7.36 (m, 4H), 8.11 (d, 1H, *J* = 7.53 *Hz*), 8.85 (s, 1H).

^{13}C NMR (100 MHz, DMSO-d$_6$): δ 31.5 (2 x CH_2), 42.4 (CH_2), 45.8 (CH), 49.5 (2 x CH_2), 115.4 (2 x CH), 118.4 (2 x CH), 126.2 (CH), 128.2 (2 x CH), 128.9 (2 x CH), 136.6 (C), 144.3 (C), 150.8 (C), 169.3 (C).

IR (ATR) / cm^{-1}: 3288, 3029, 2946, 2787, 1638, 1541, 1510, 1451, 1384, 1352, 1294, 1246, 1231, 1219, 1179, 1145, 1126, 819, 719, 691, 596, 531.

LCMS (ESI$^+$): calculated for $C_{19}H_{22}N_2O_2$ $[M+H]^+$ 311.17; found: *m/z* = 310.97.

2.6 Biological evaluation of compounds

2.6.1 ME isoforms inhibition data for selected compounds:

Among the synthesized compounds (**1**-**27**) 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 2.7**.

Table 2.7: ME isoforms inhibition data for selected compounds.

Compound	ME3 IC_{50} (μM)	ME2 IC_{50} (μM)	ME1 IC_{50} (μM)
2	0.11	0.22	0.20
3	0.10	0.19	0.18
7	0.10	0.27	0.27
11	0.07	0.19	0.15
15	0.09	0.20	0.12
16	0.12	0.14	0.15
18	0.12	0.16	0.15
26	0.13	0.26	0.23
27	0.07	0.19	0.24

in vitro screening data of selected compounds suggested that they are pan inhibitors of ME isoforms.

2.7 Conclusion

In summary, compound **A** was identified as a hit molecule and was optimized for enzymatic activity and cellular potency which led to potent and cell active ME3 enzyme inhibitors

compound **3** and **7** with amide linker. Among these two, compound **7** consisting of the propanamide linker exhibited superior growth inhibition of BxPC-3 cells compared to compound **3** with acetamide linker. The structural elements critical for ME3 inhibition that have been identified are depicted in **Figure 2.7**. These learnings have been utilized for further optimization of molecules as included in the following chapters.

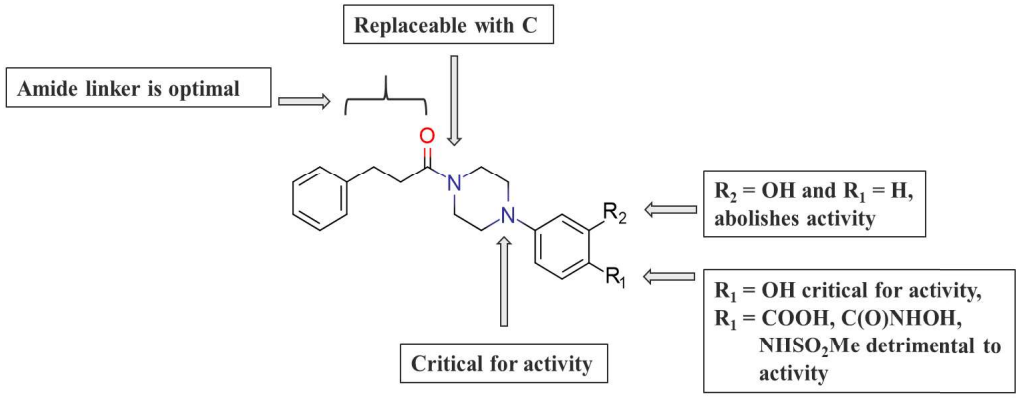
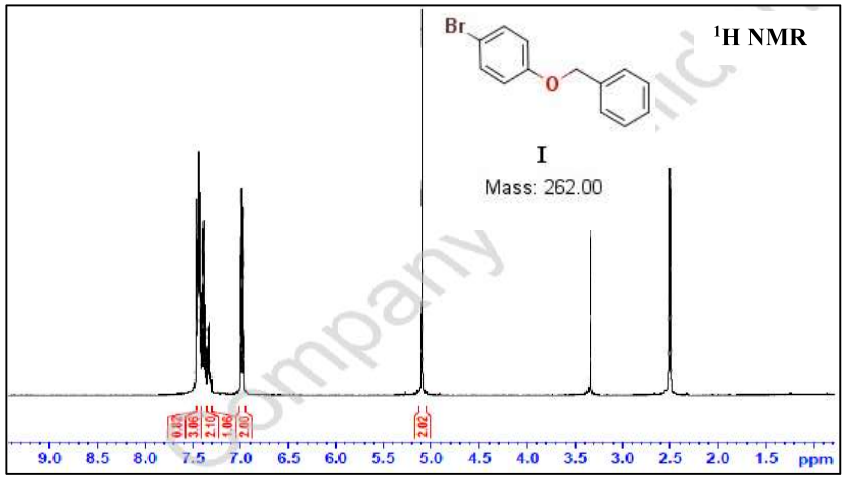
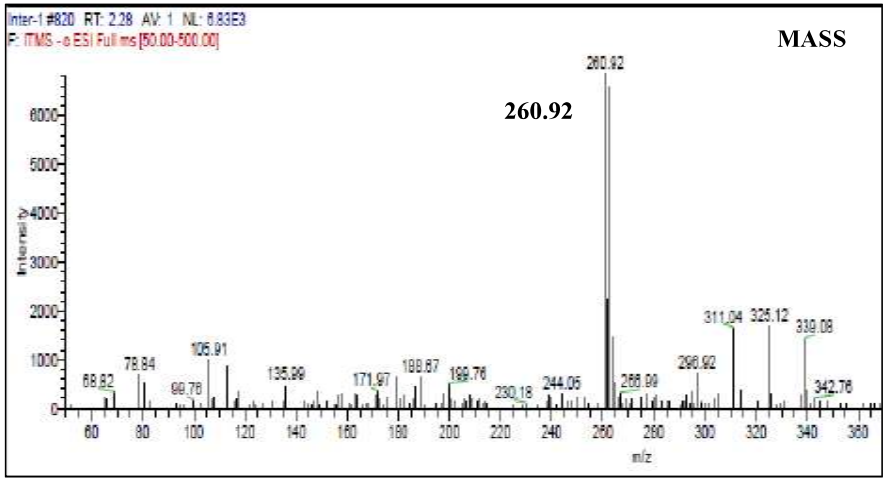


Figure 2.7. Summary of SAR for ME3 inhibition (chapter 2)

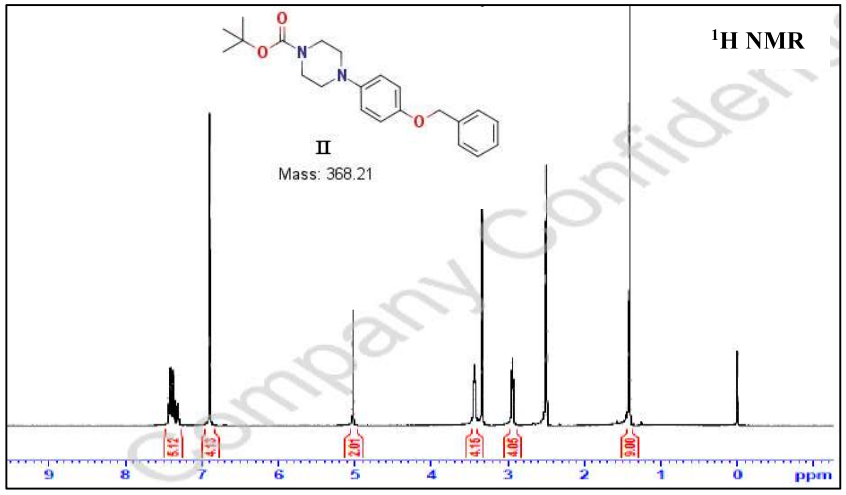
2.8 Spectral data

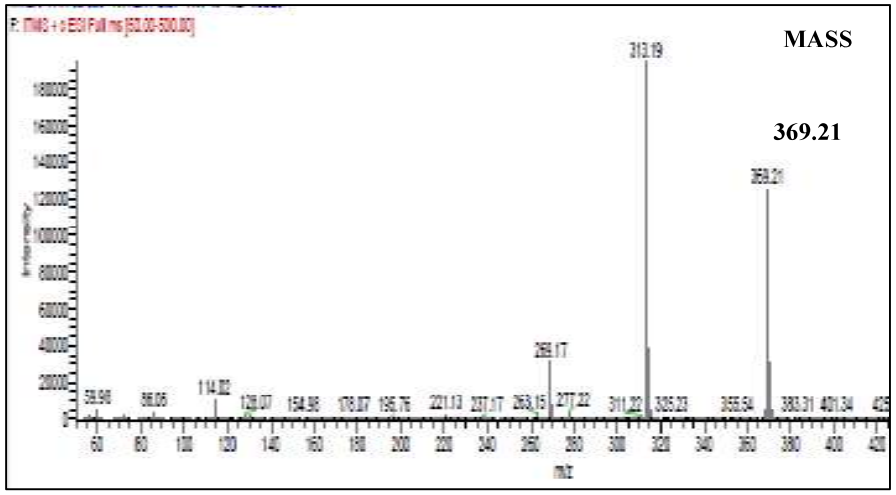
Spectral data of **Intermediate-I**



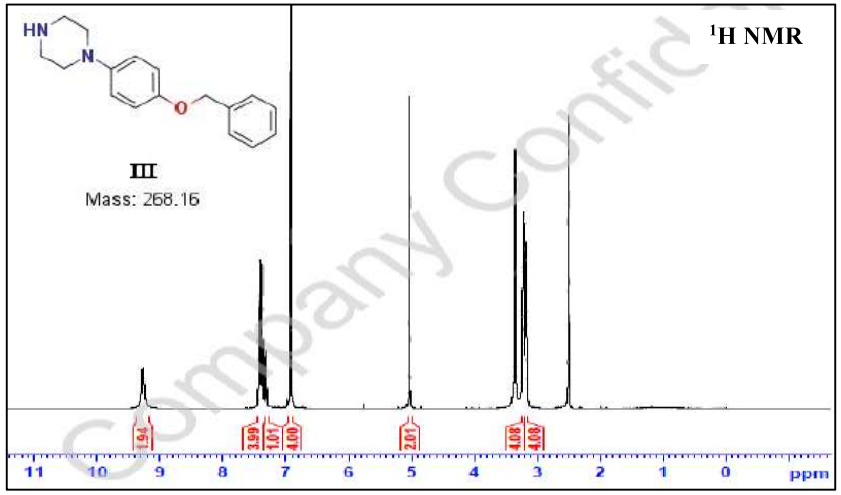


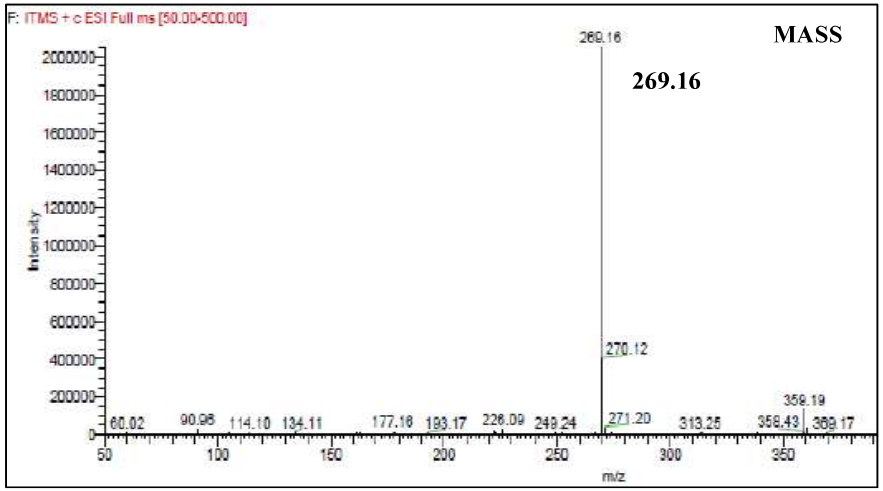
Spectral data of **Intermediate-II**



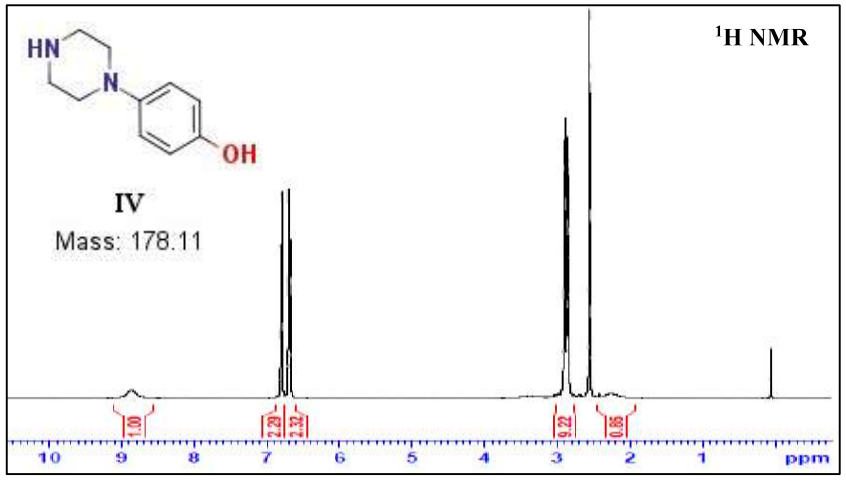


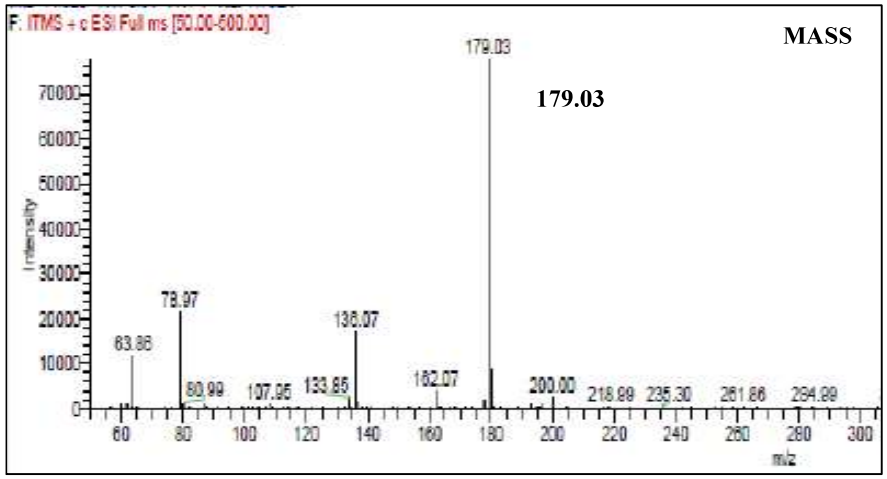
Spectral data of **Intermediate-III**



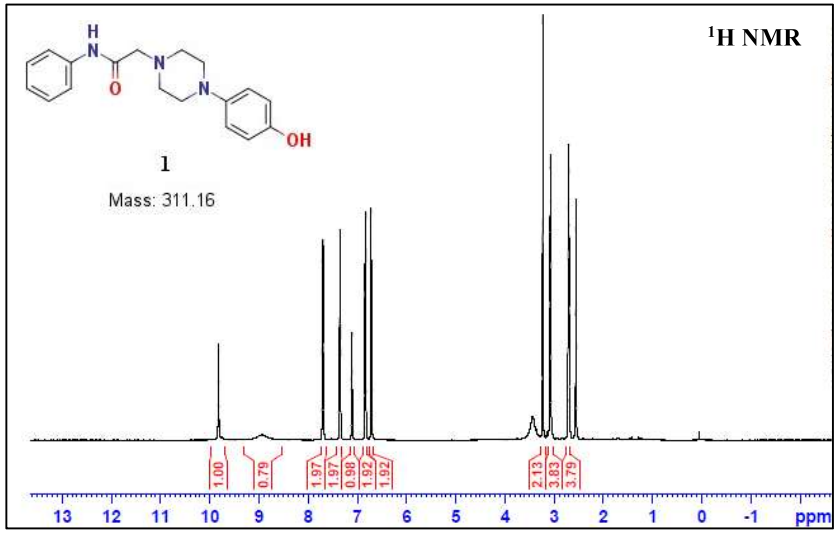


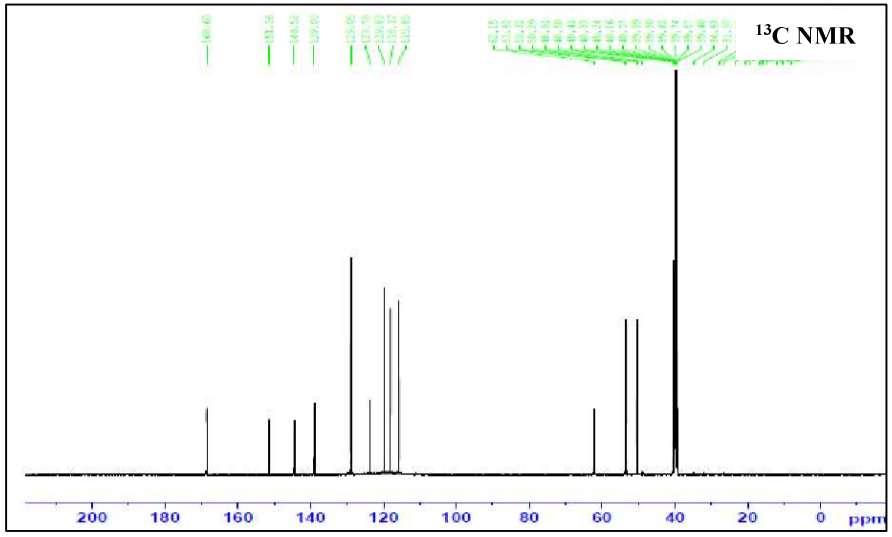
Spectral data of **Intermediate-IV**

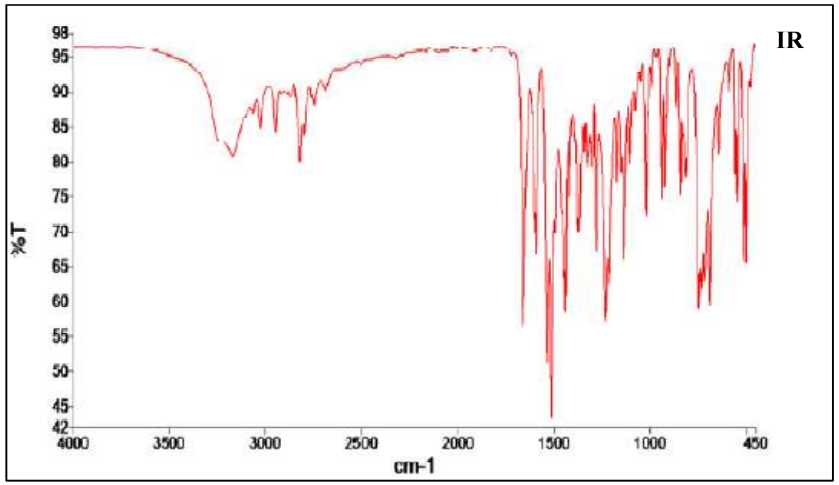


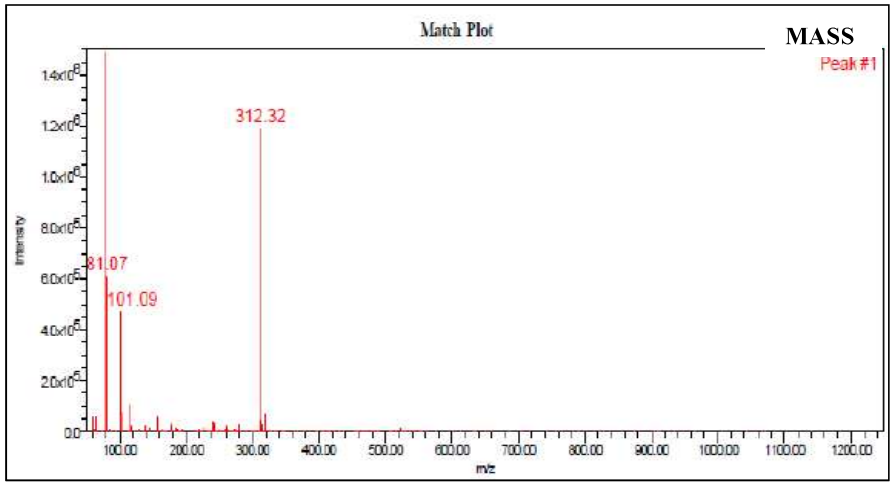


Spectral data of **1**

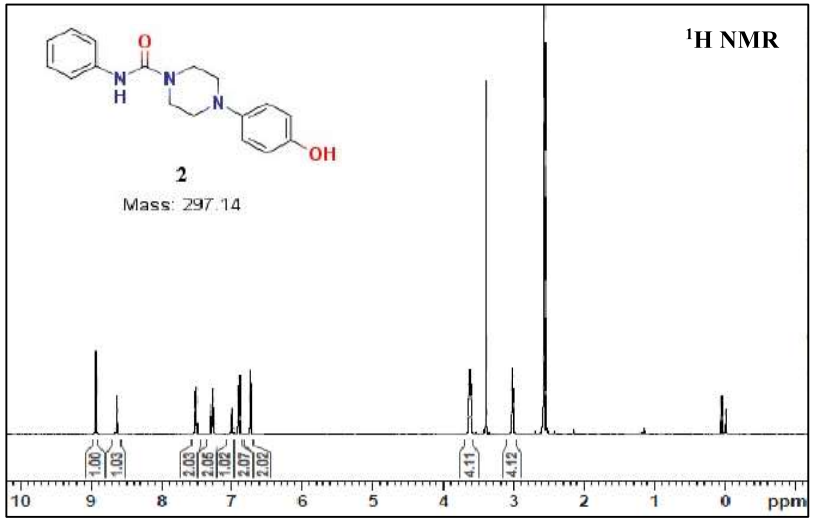


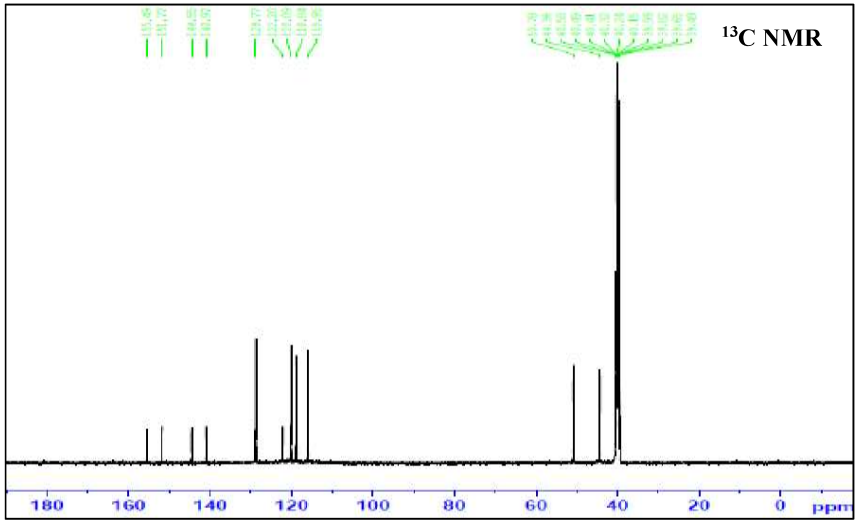


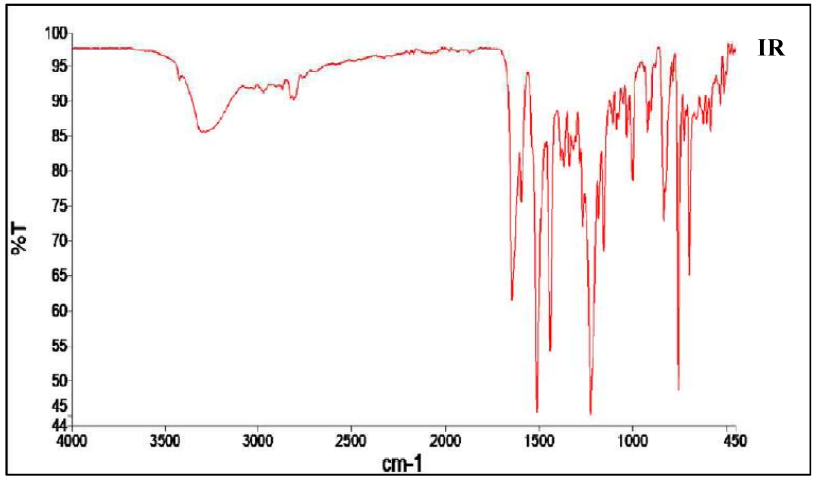


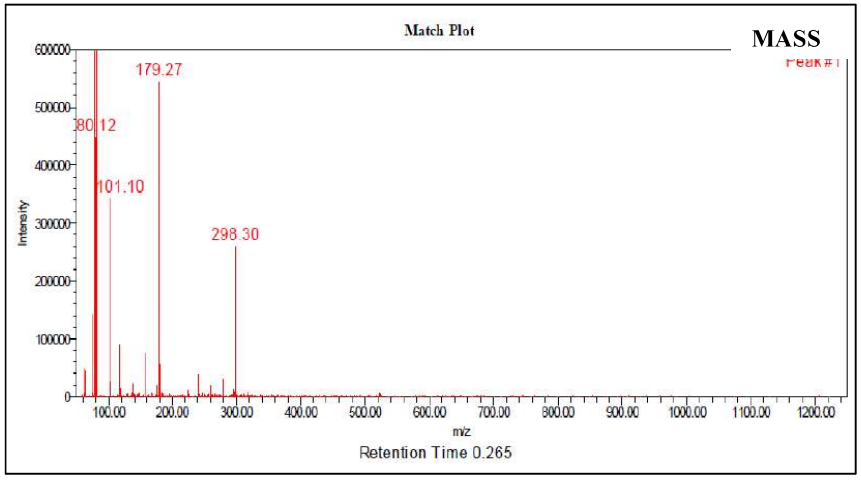


Spectral data of **2**

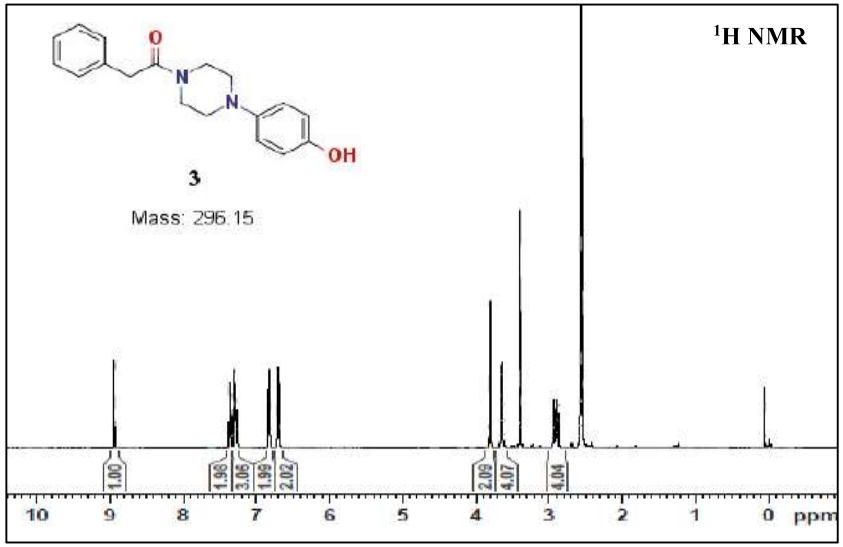


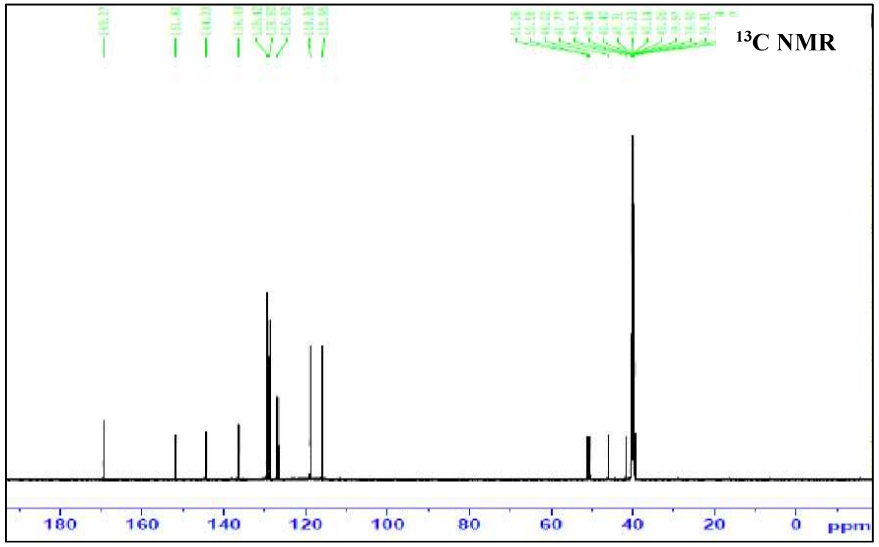


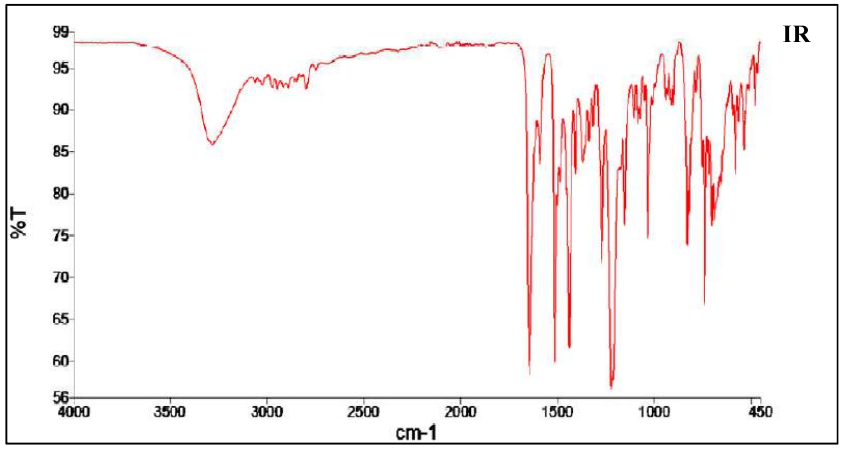


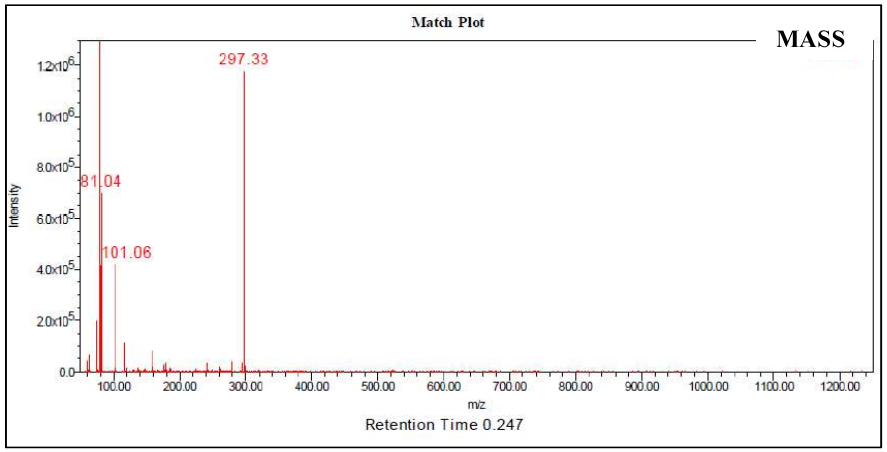


Spectral data of **3**

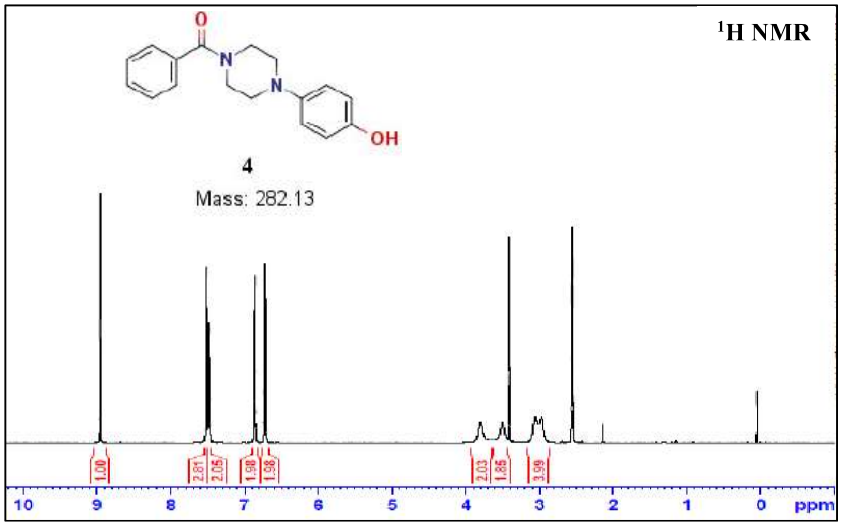


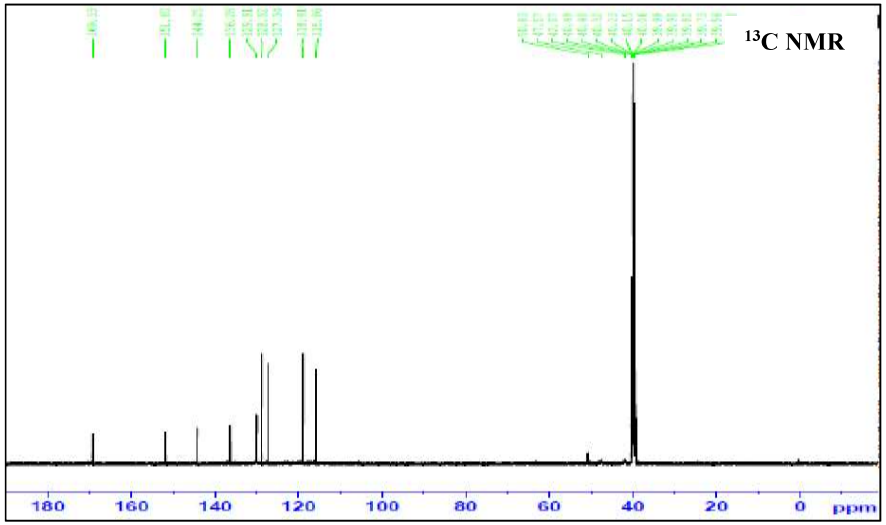


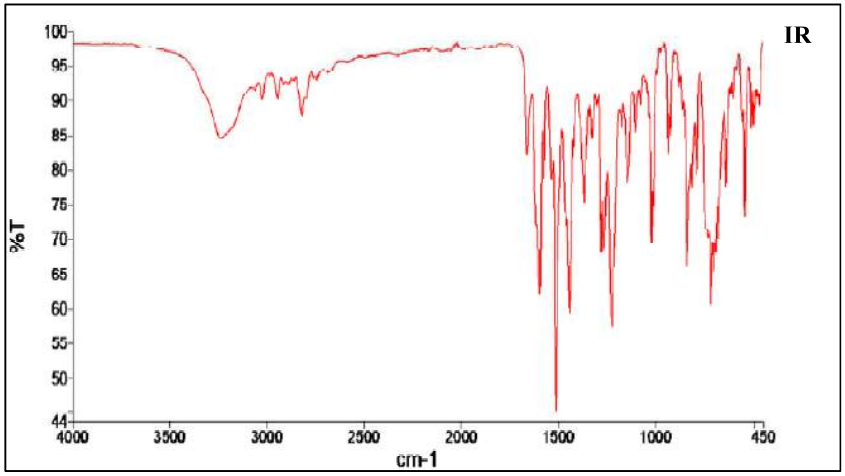


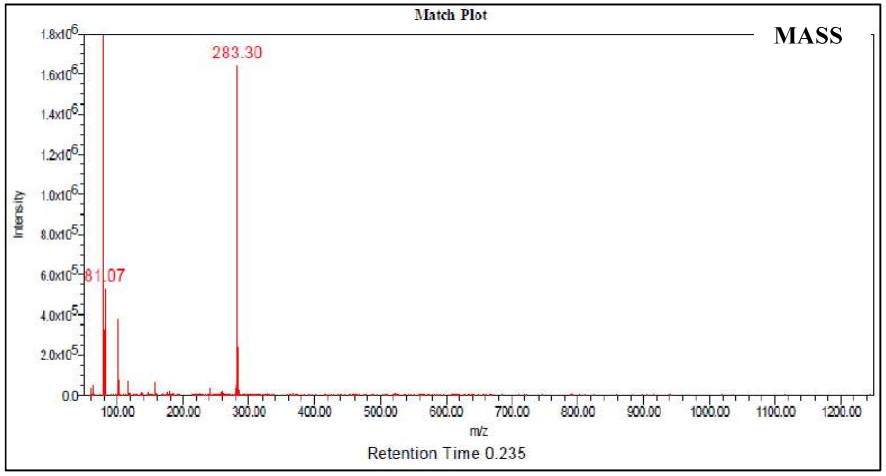


Spectral data of **4**

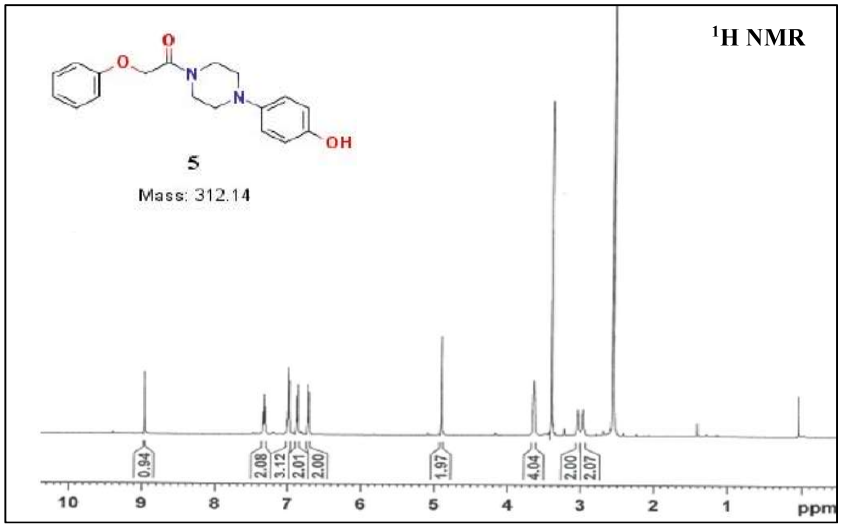


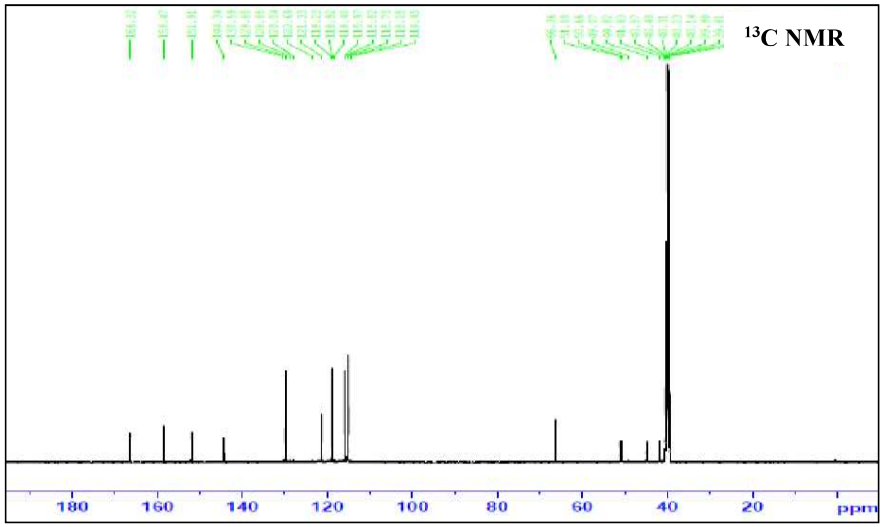


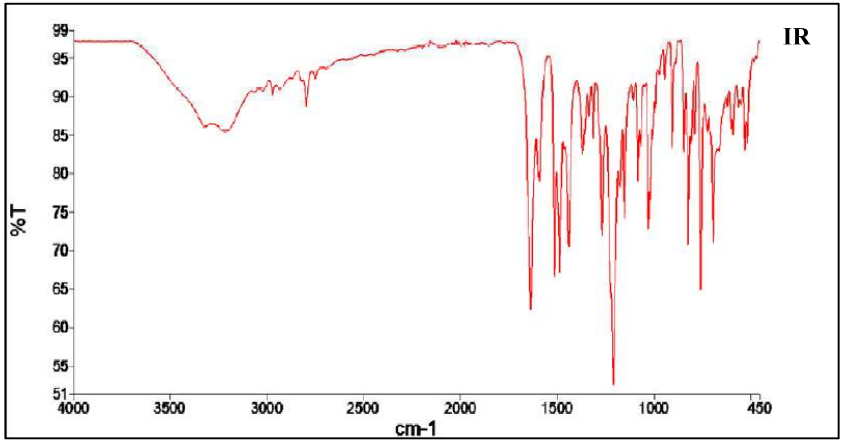


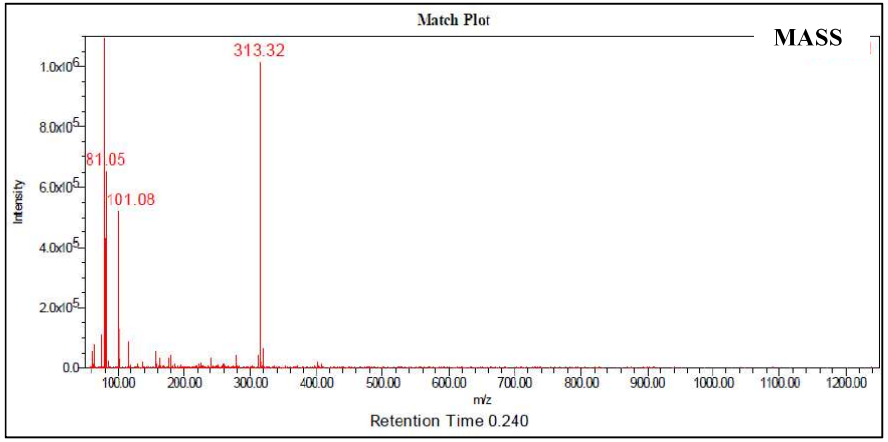


Spectral data of **5**

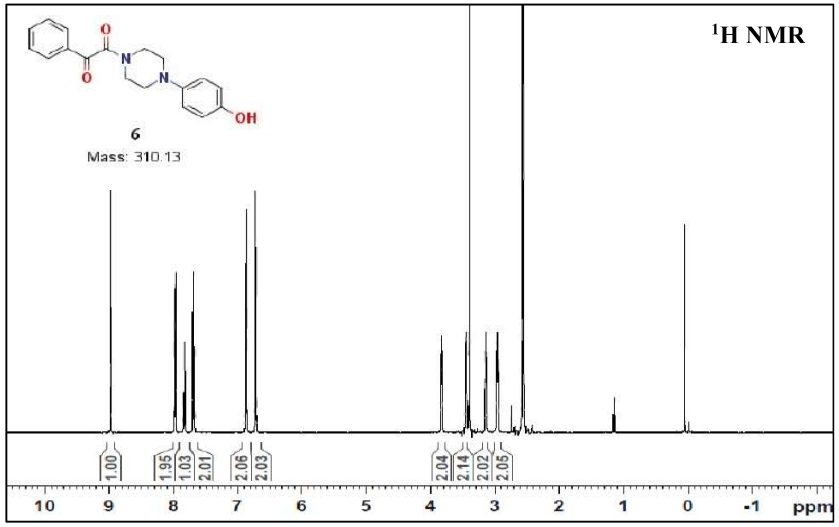


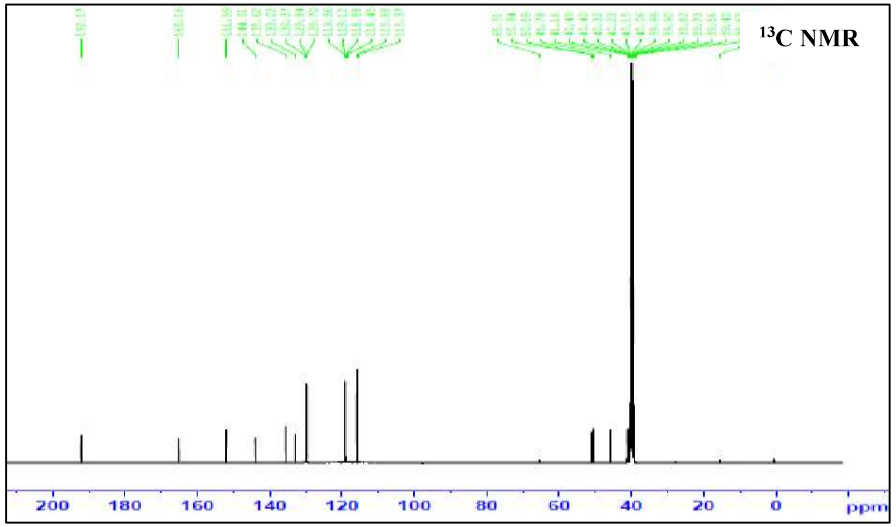


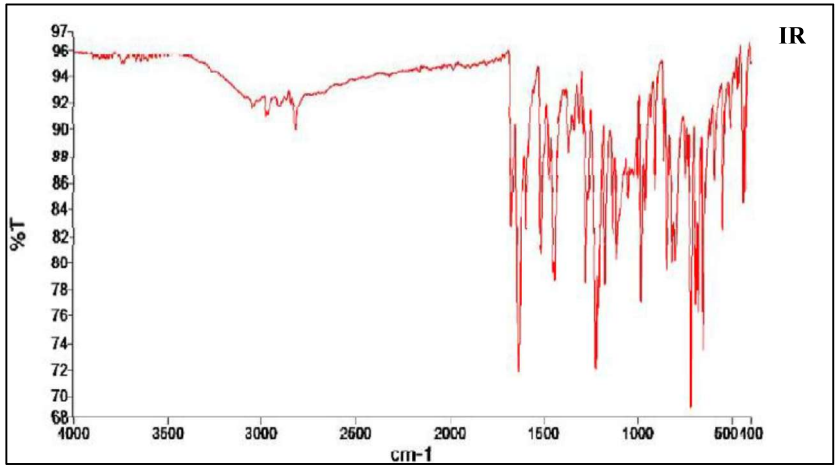


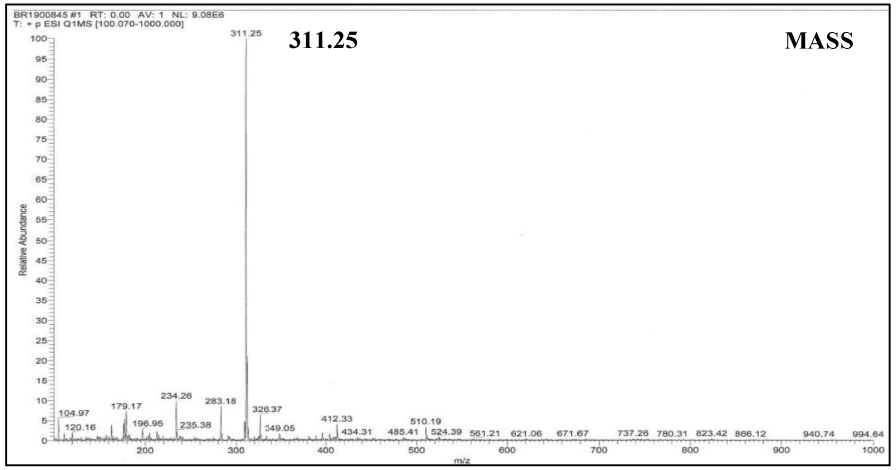


Spectral data of **6**

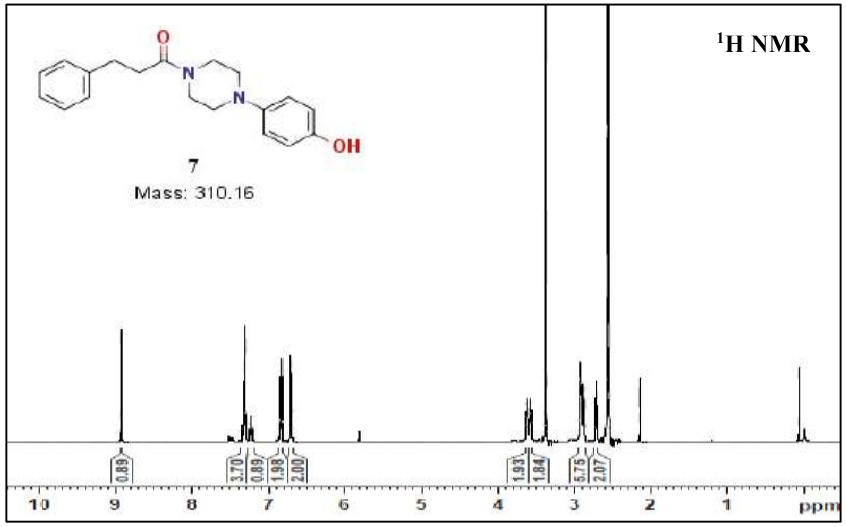


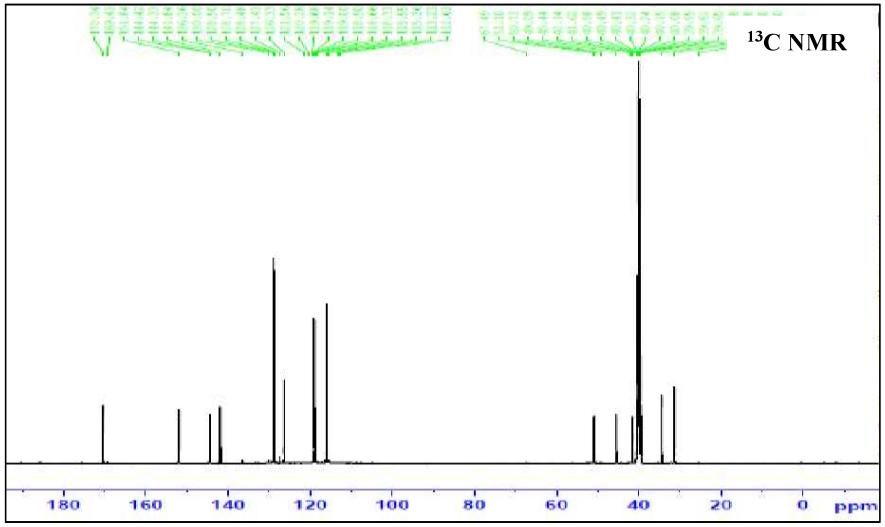


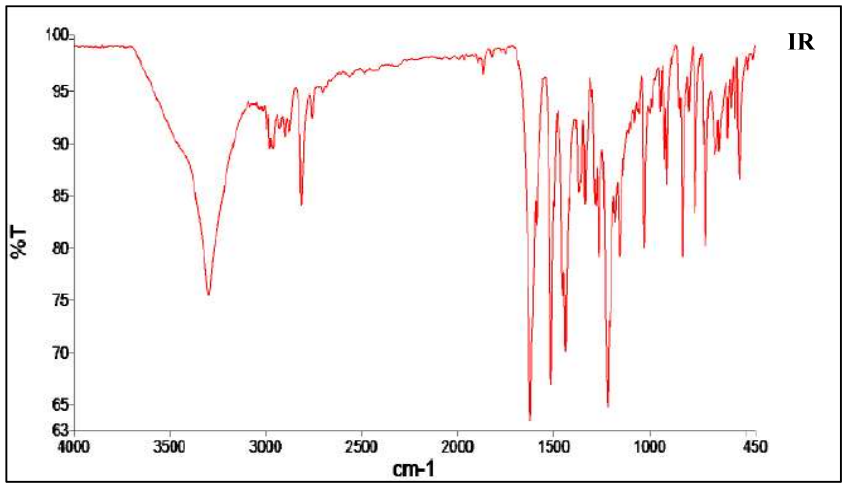


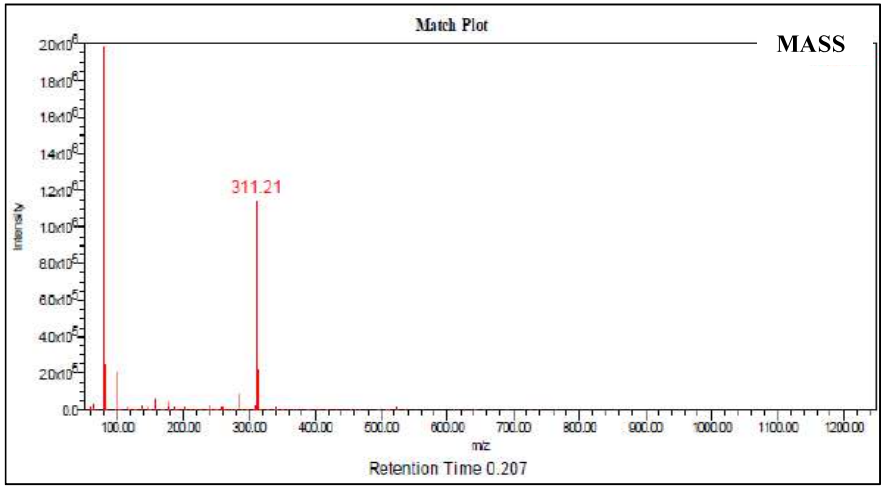


Spectral data of **7**

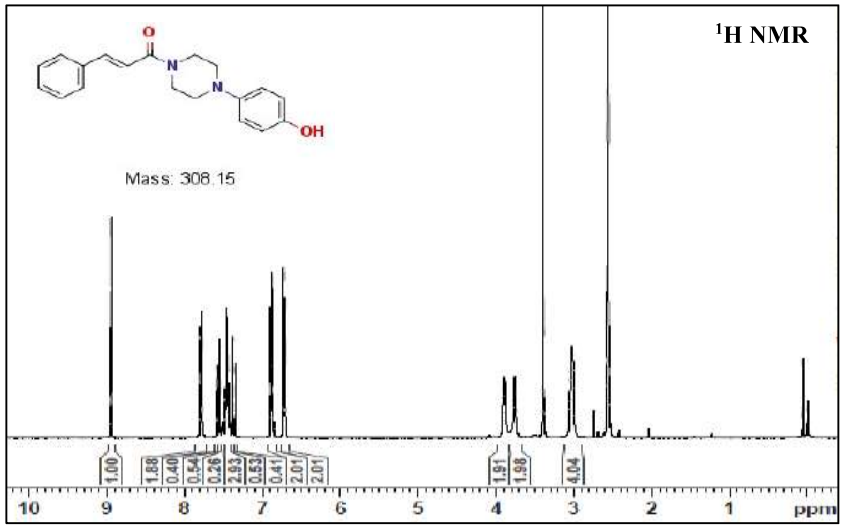


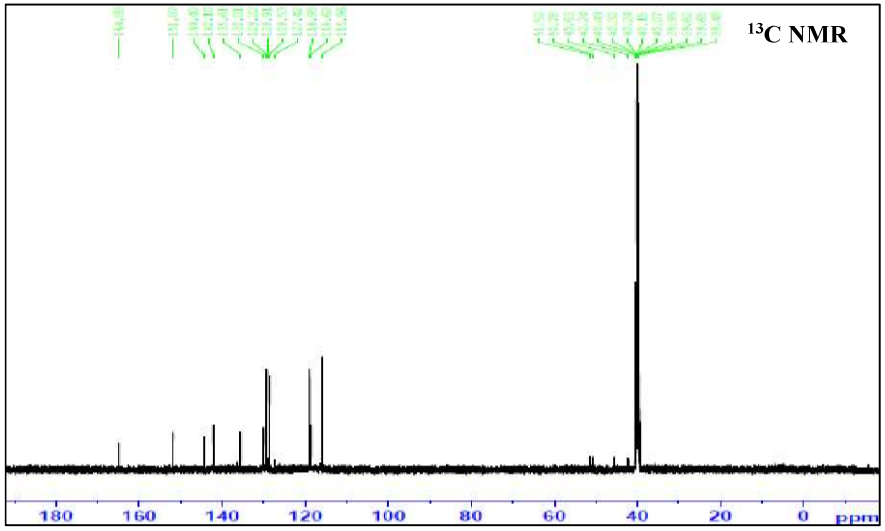


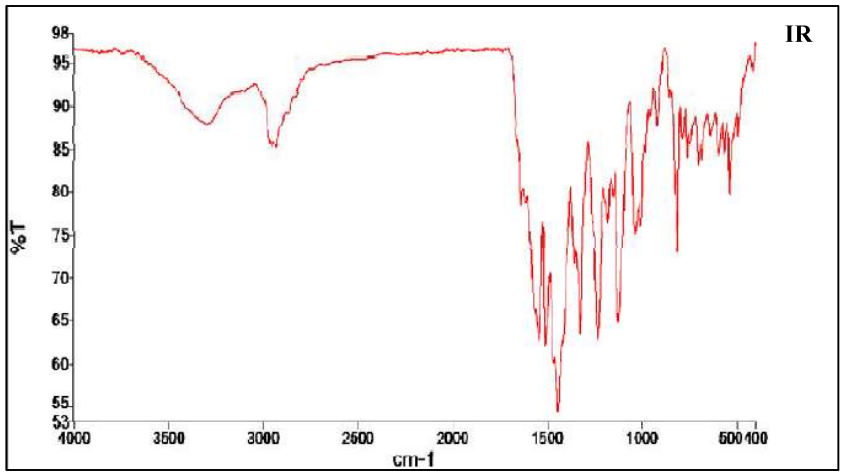


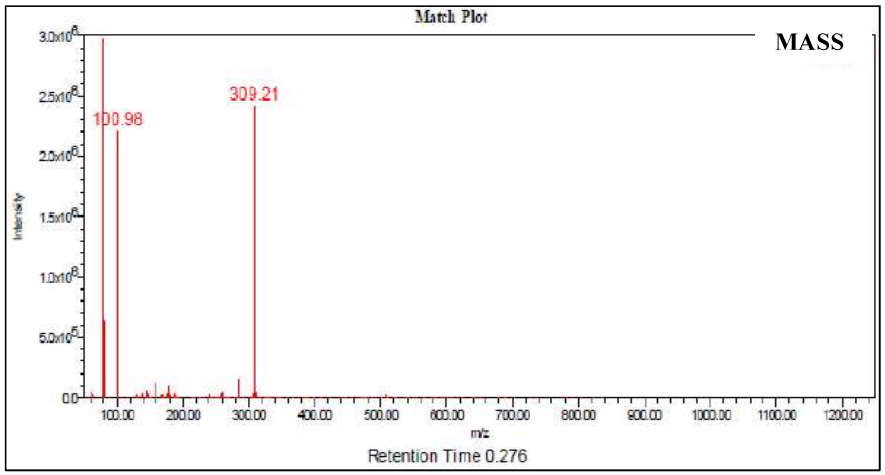


Spectral data of **8**

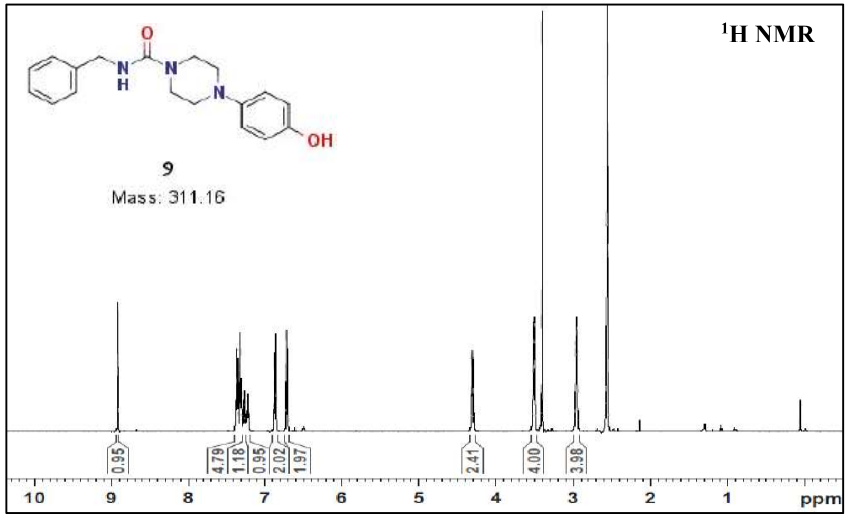


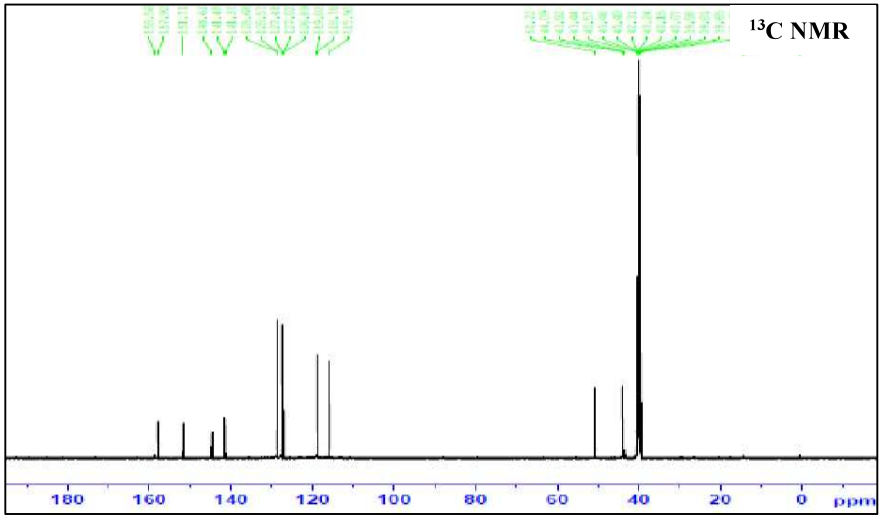


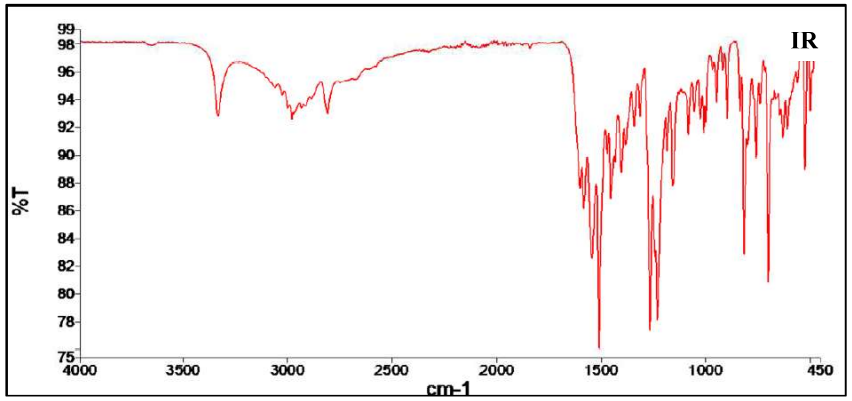


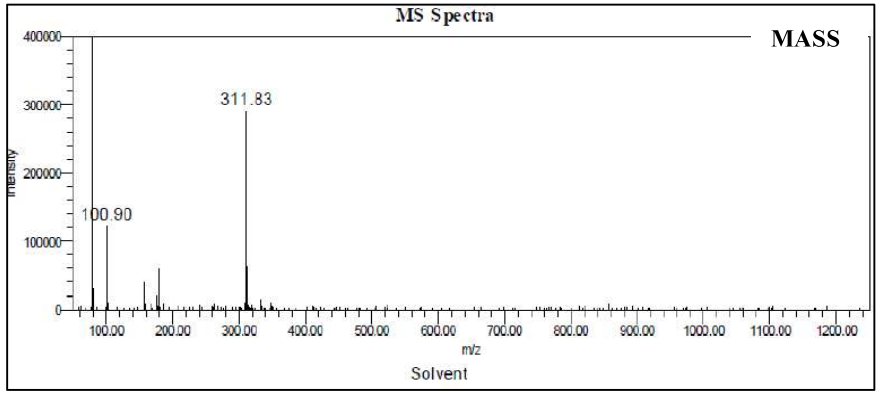


Spectral data of **9**

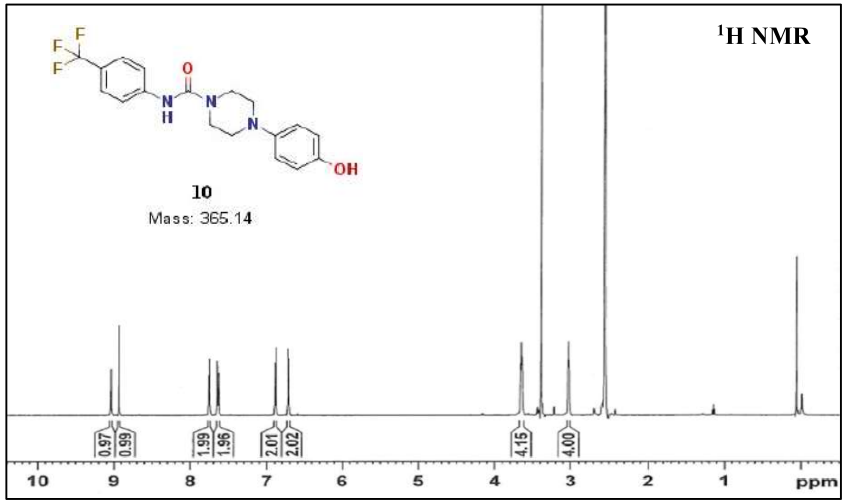


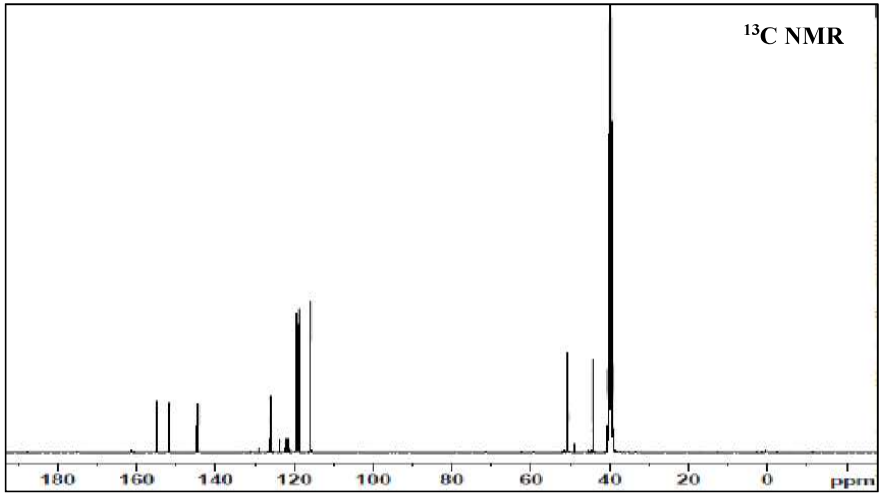


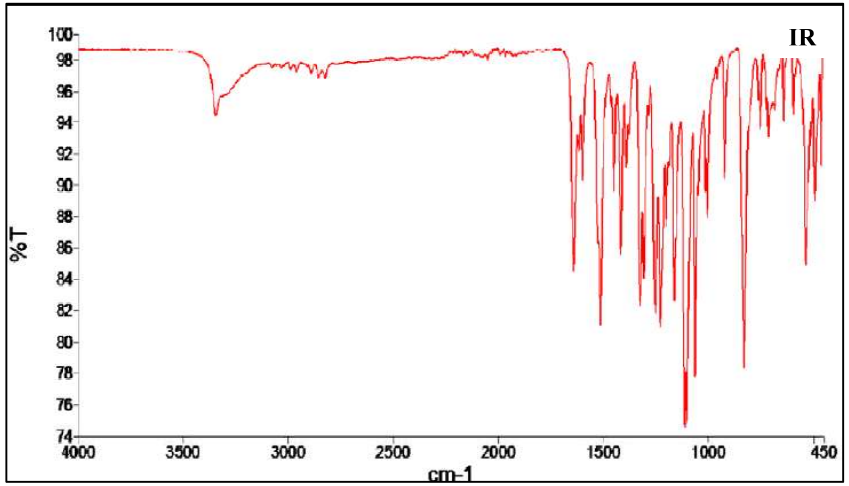


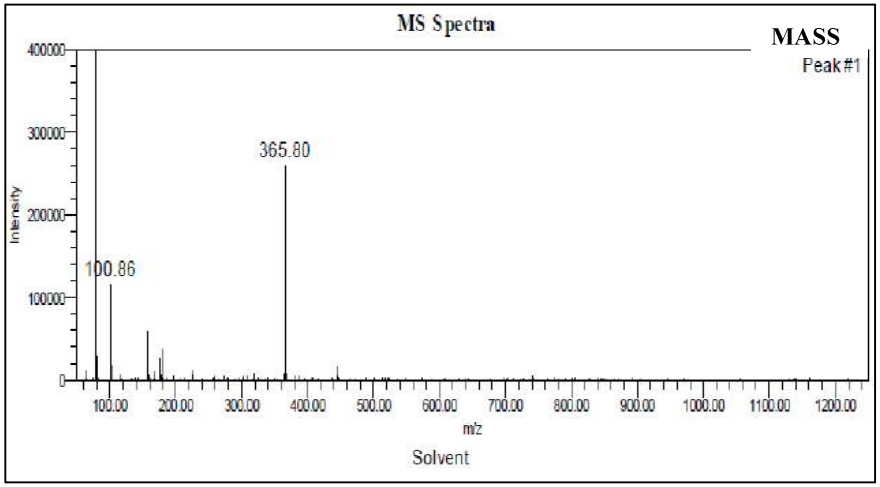


Spectral data of **10**

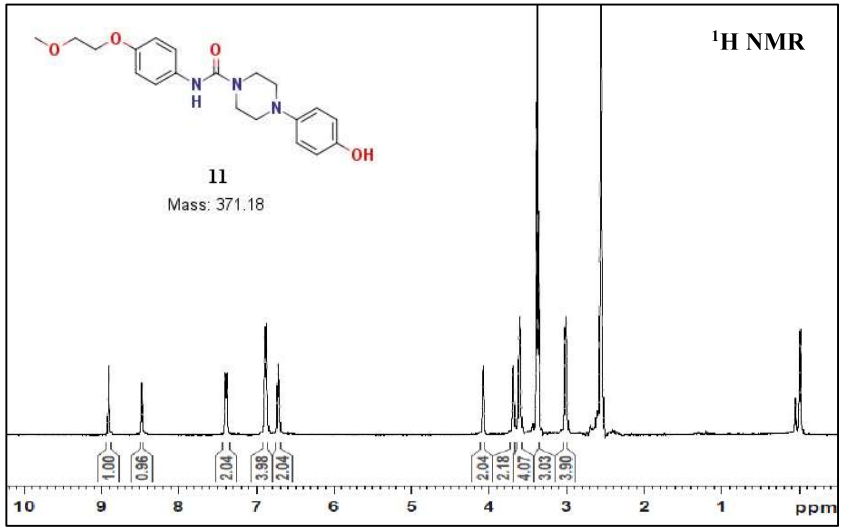


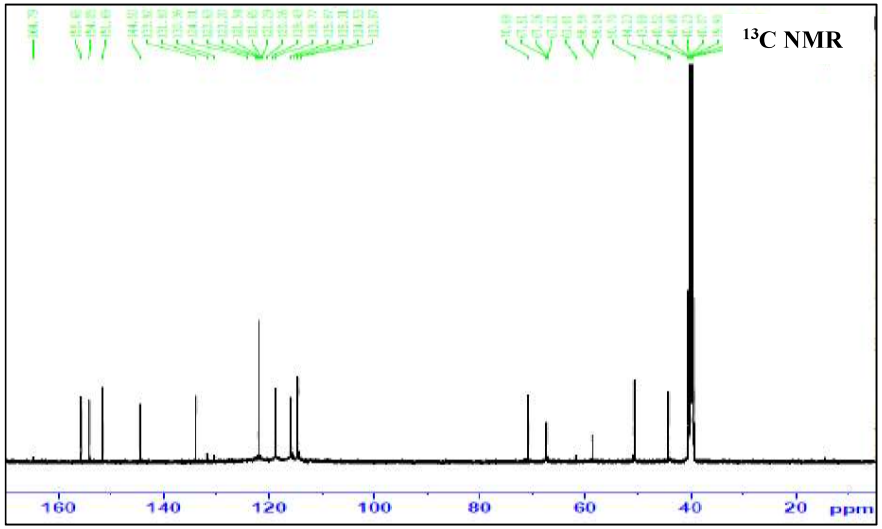


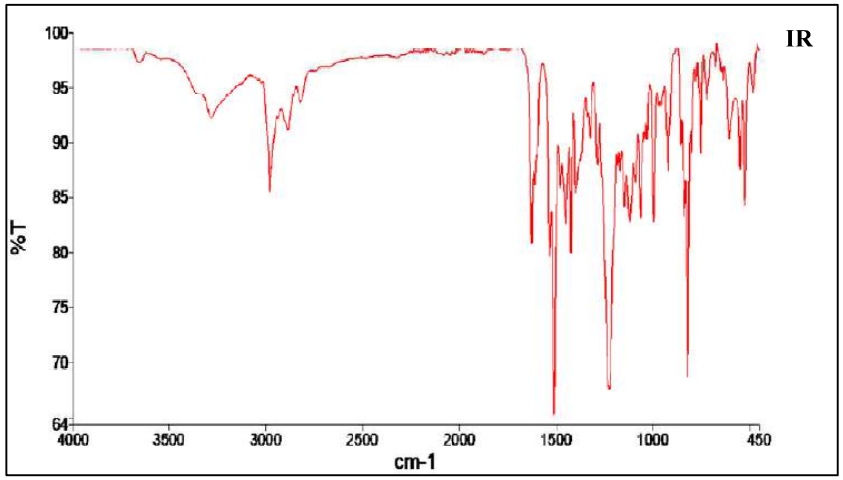


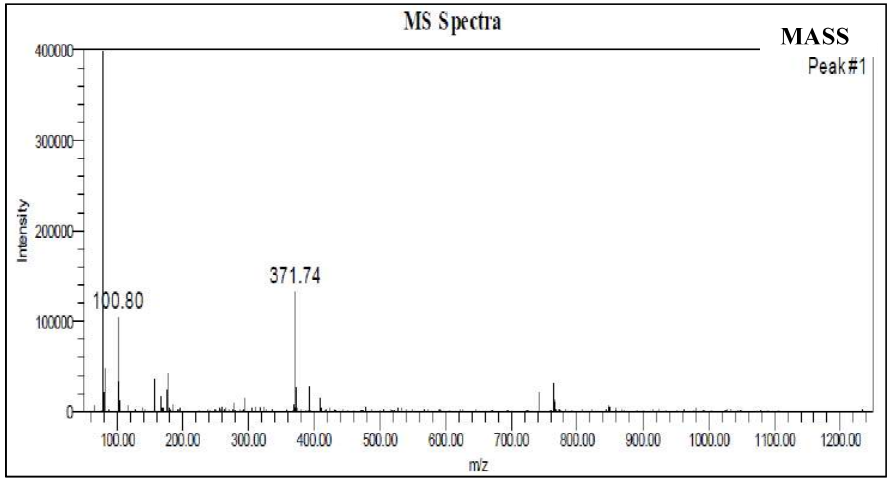


Spectral data of **11**

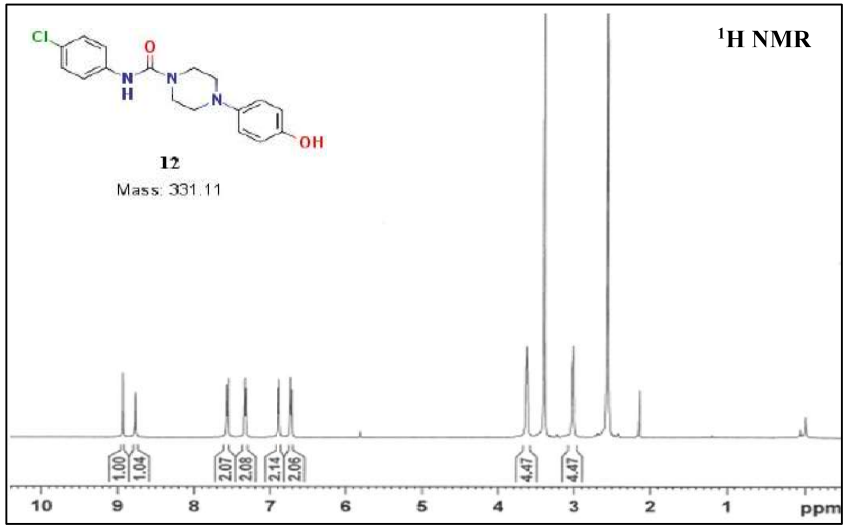


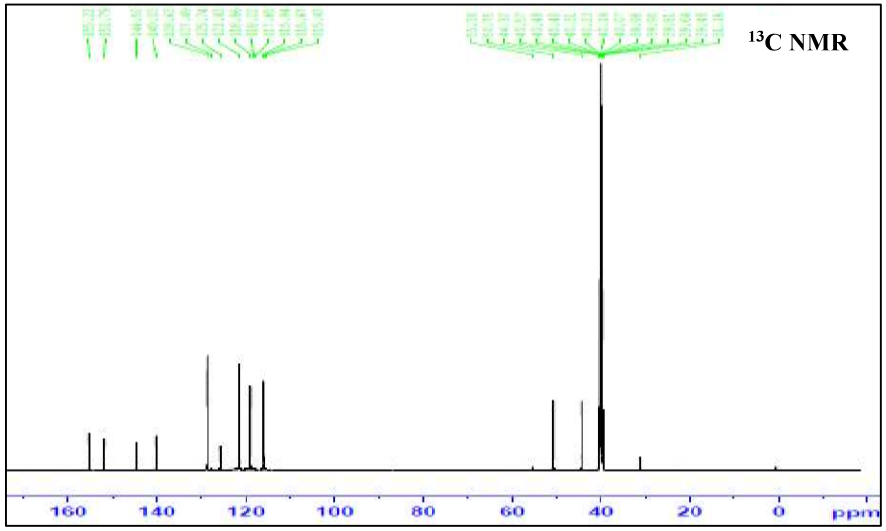


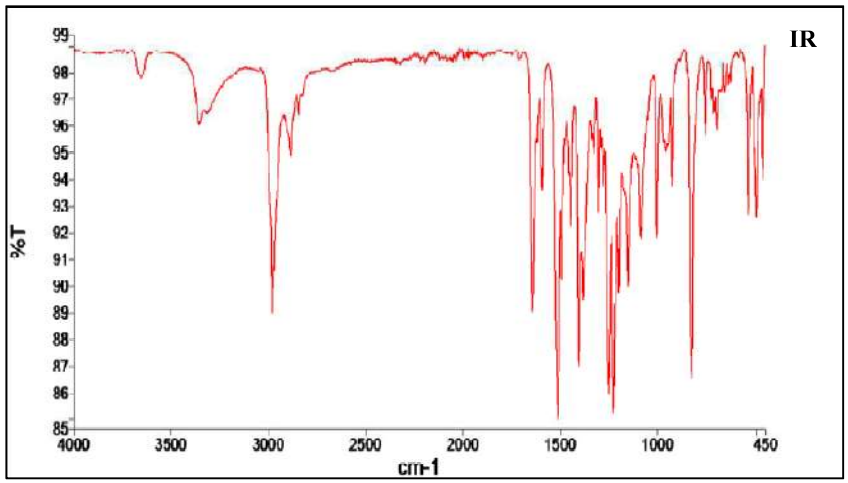


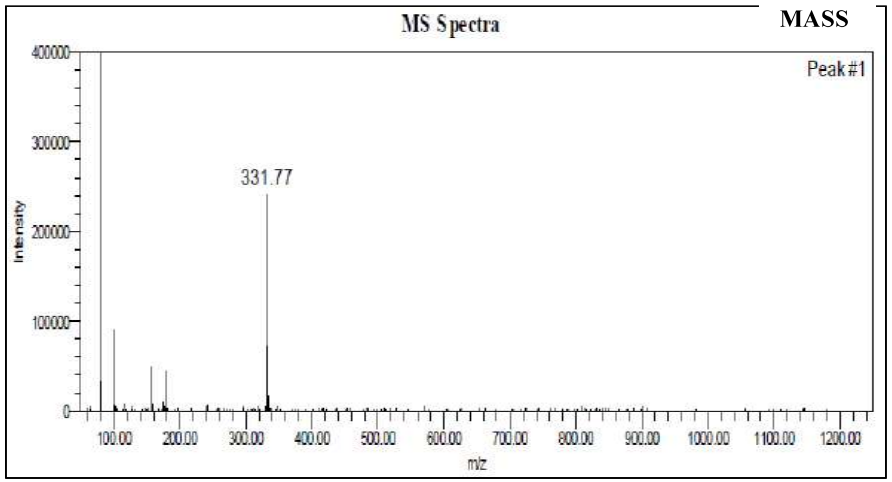


Spectral data of **12**

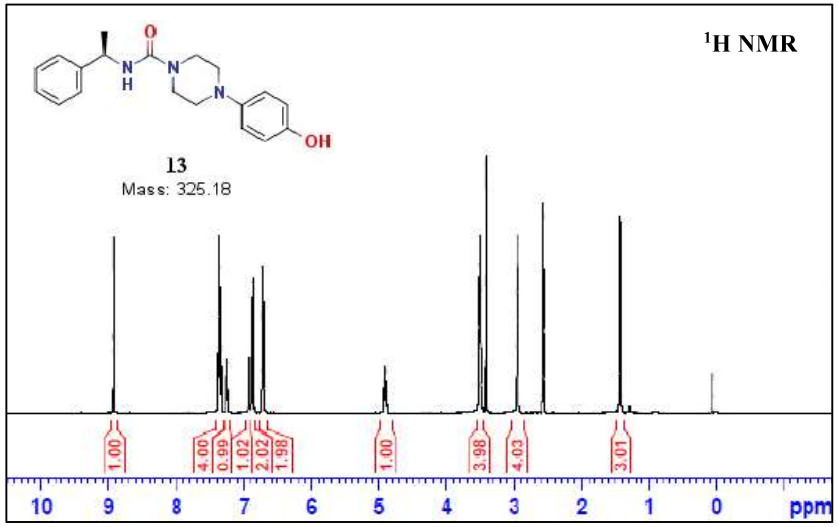


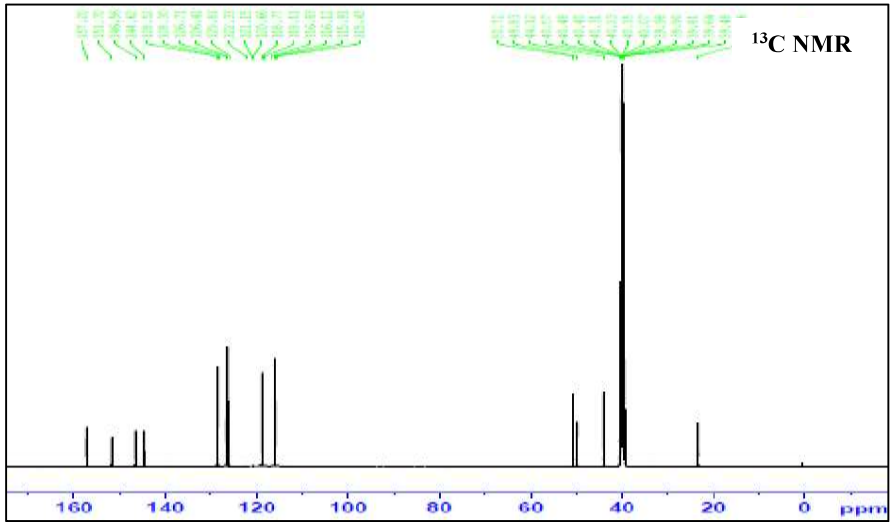


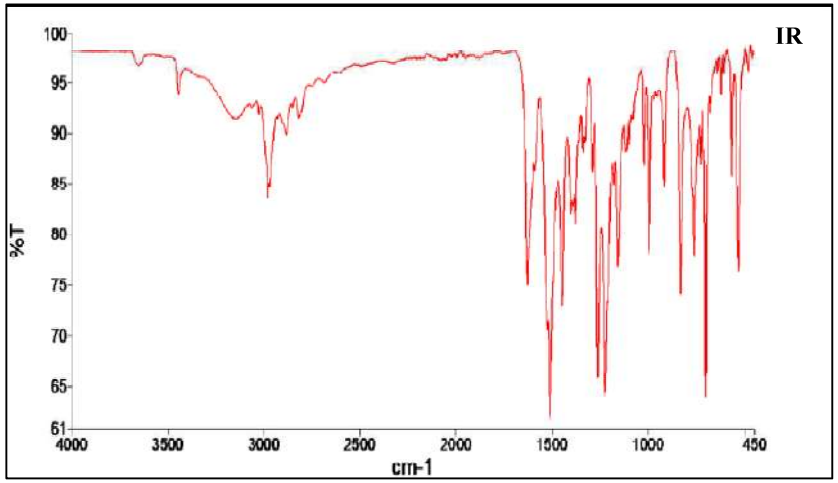


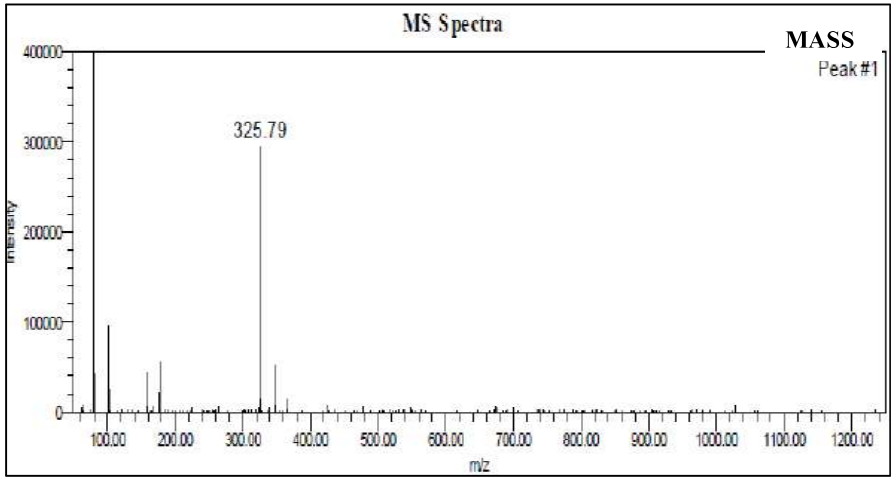


Spectral data of **13**

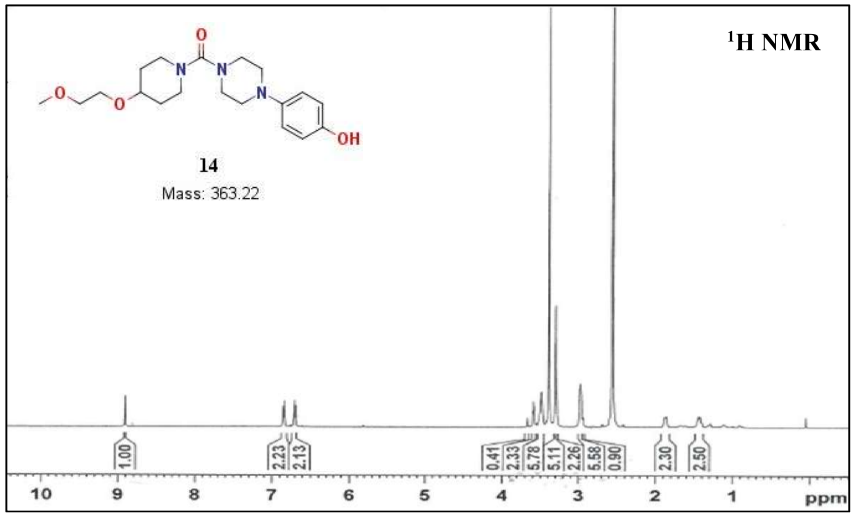


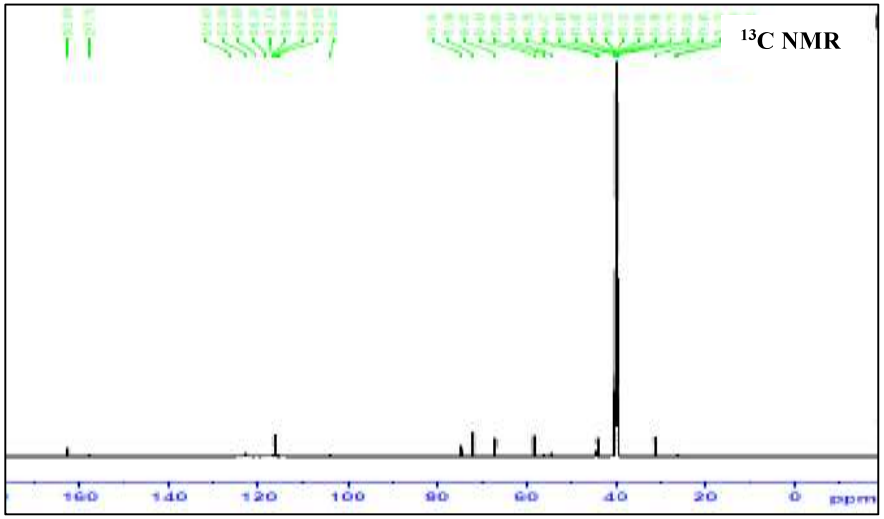


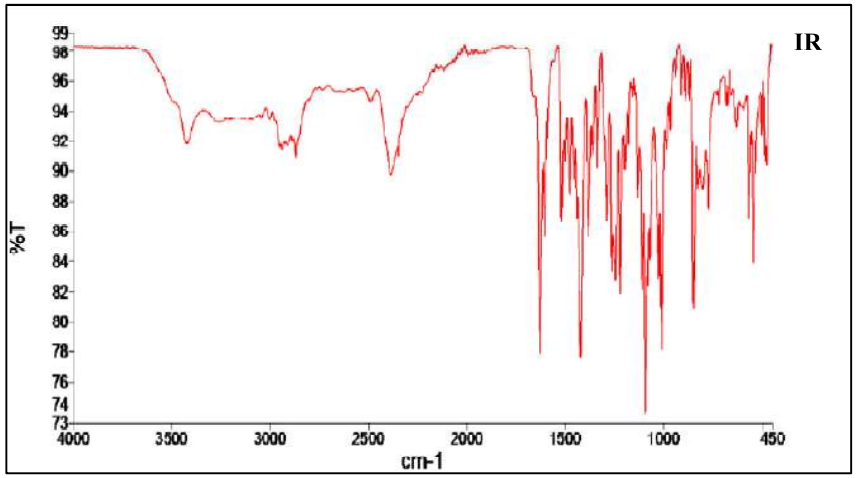


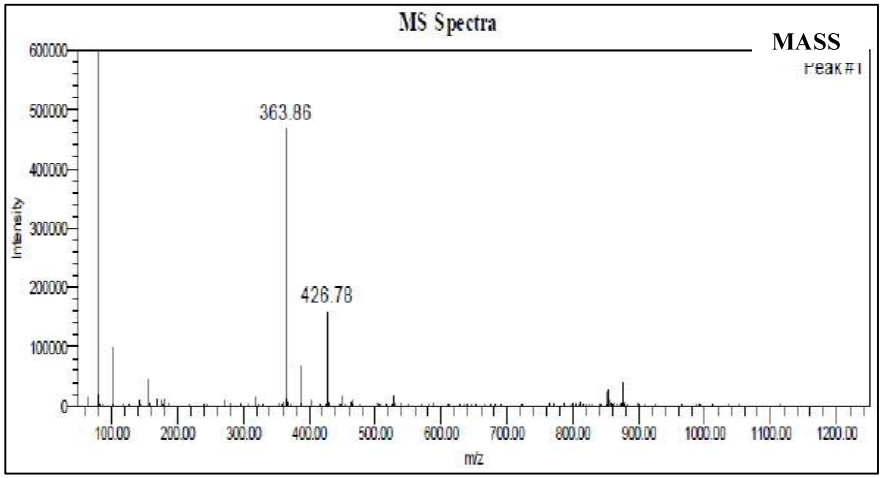


Spectral data of **14**

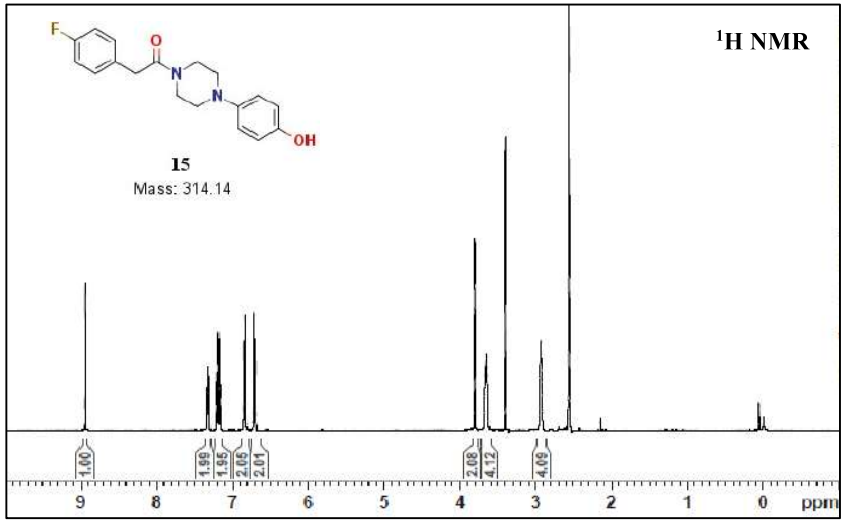


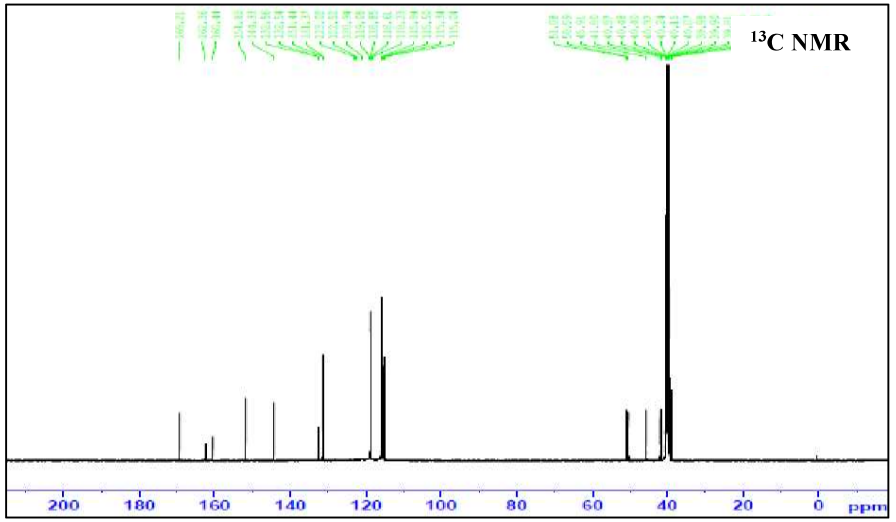


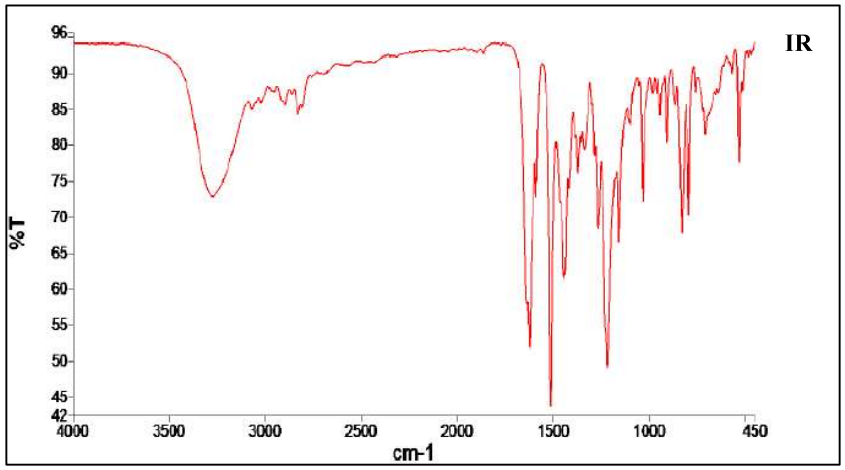


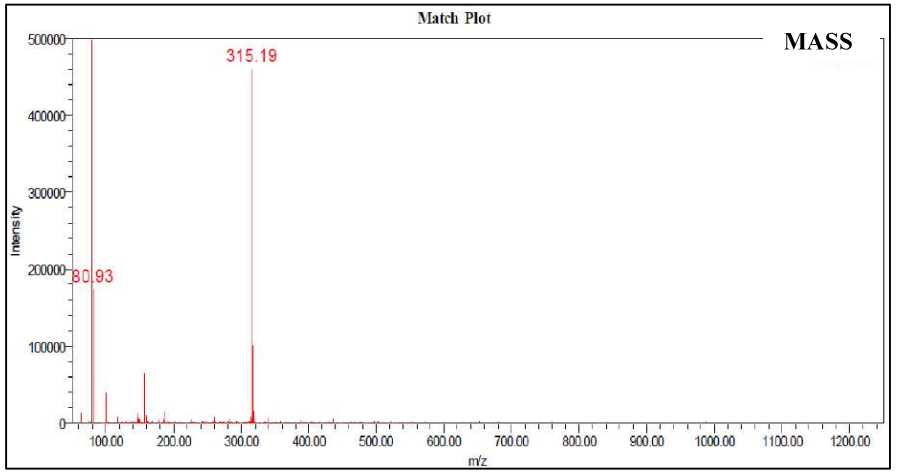


Spectral data of **15**

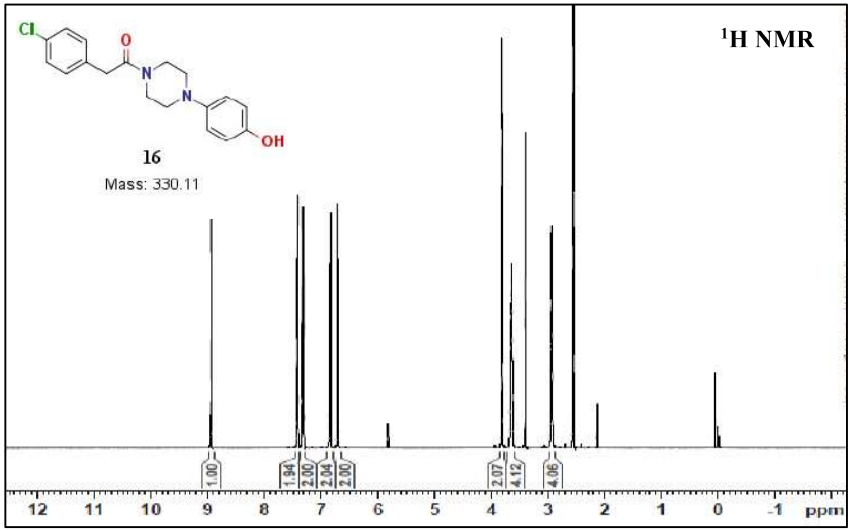


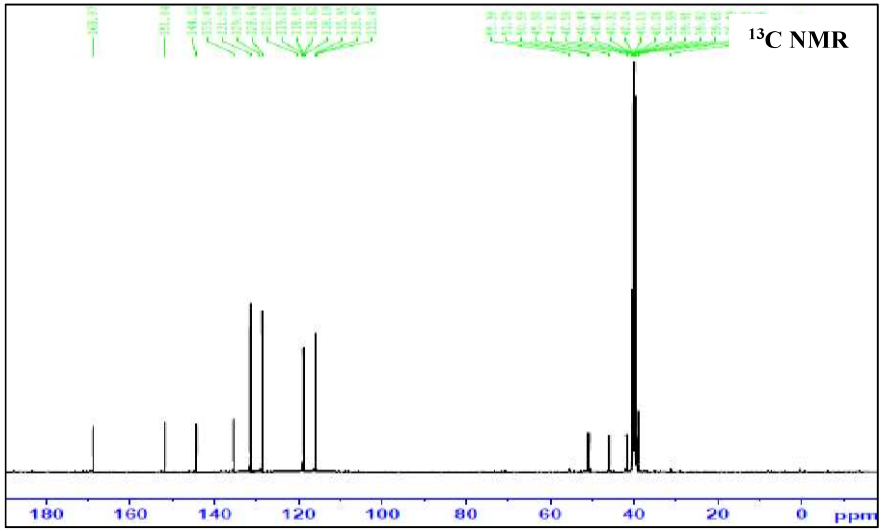


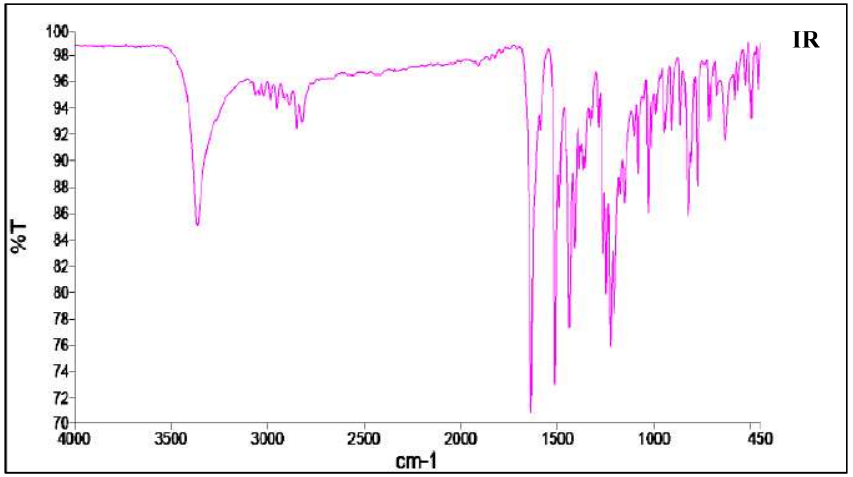


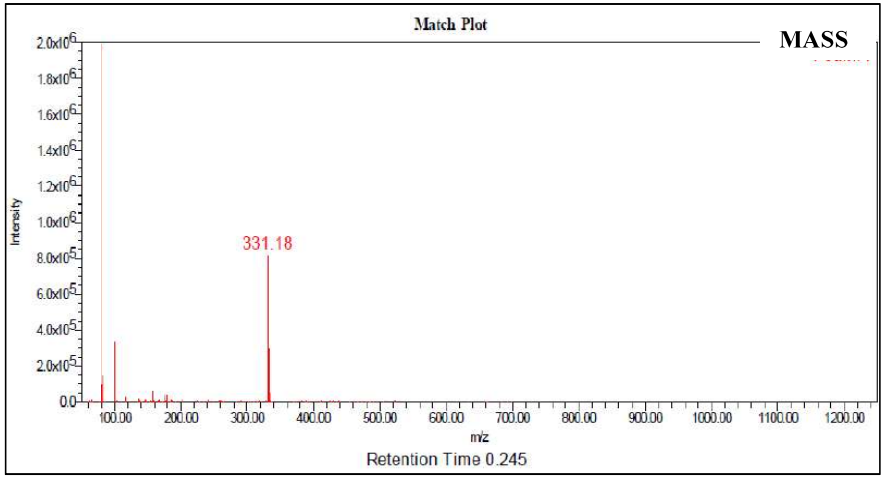


Spectral data of **16**

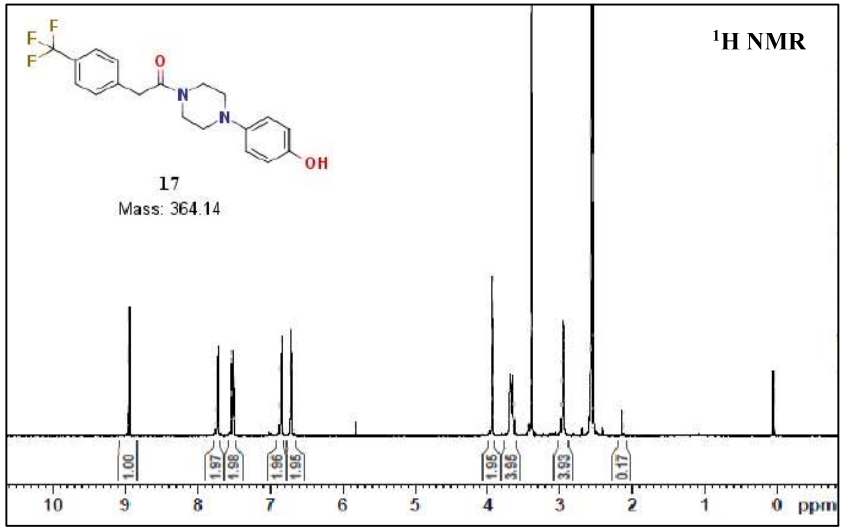


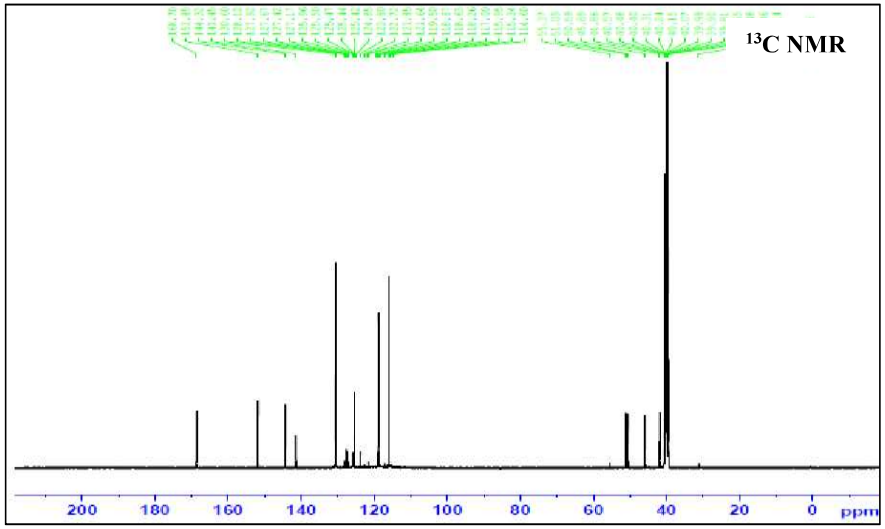


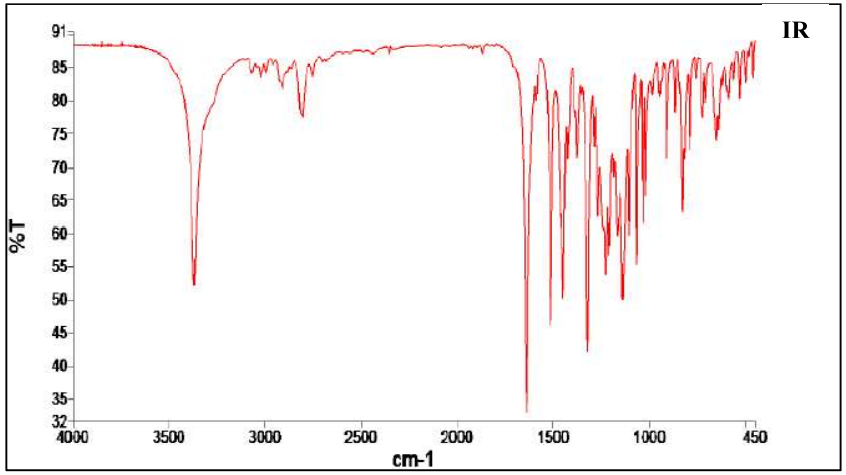


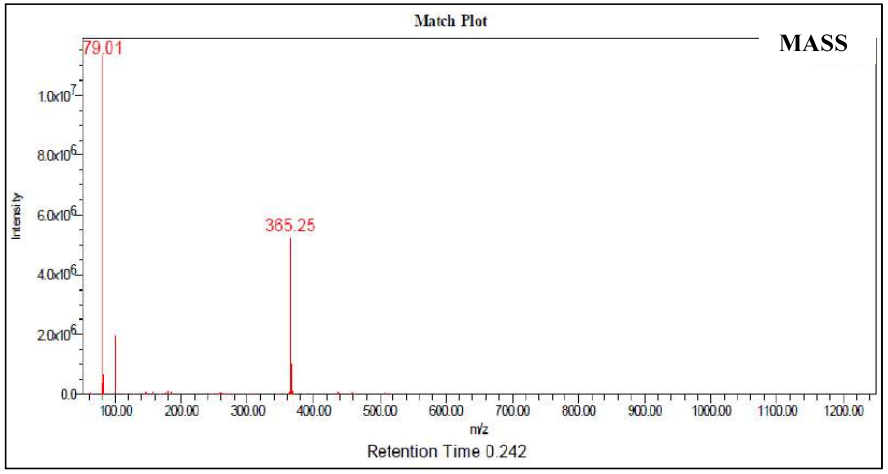


Spectral data of **17**

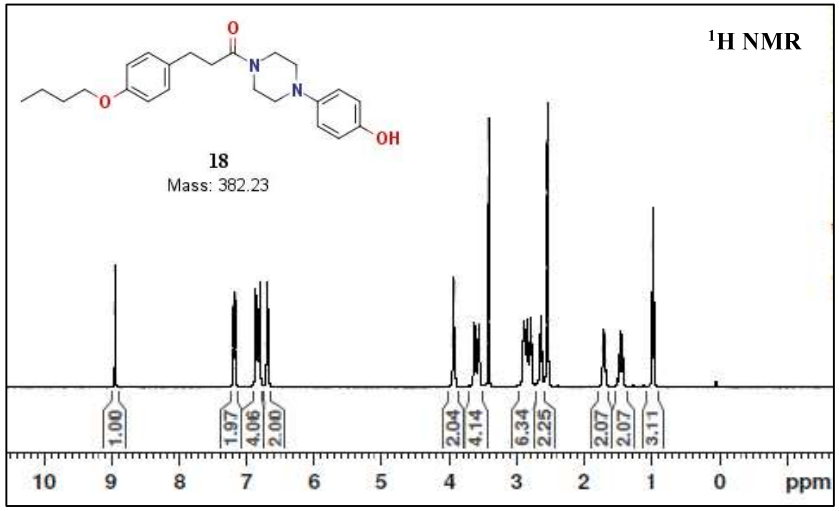


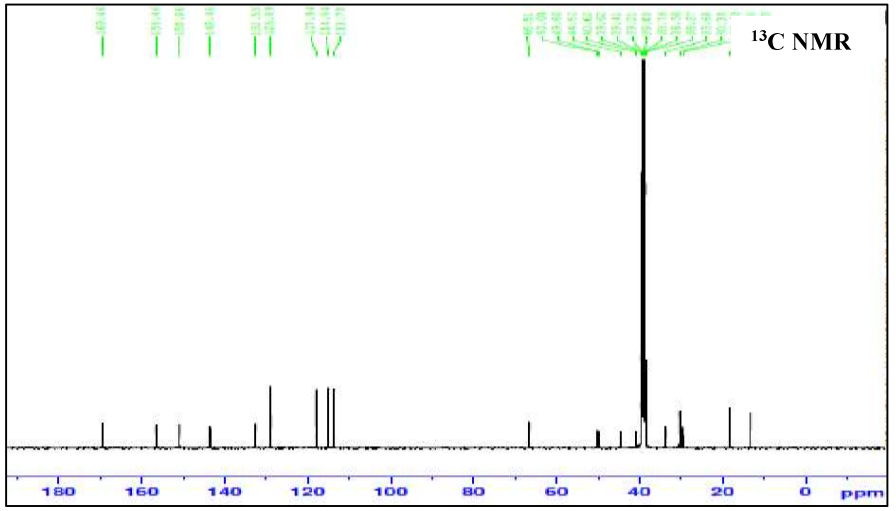


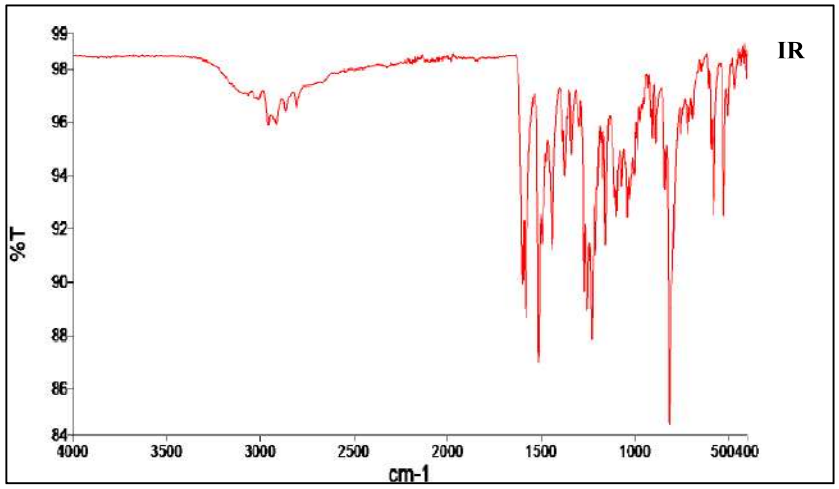


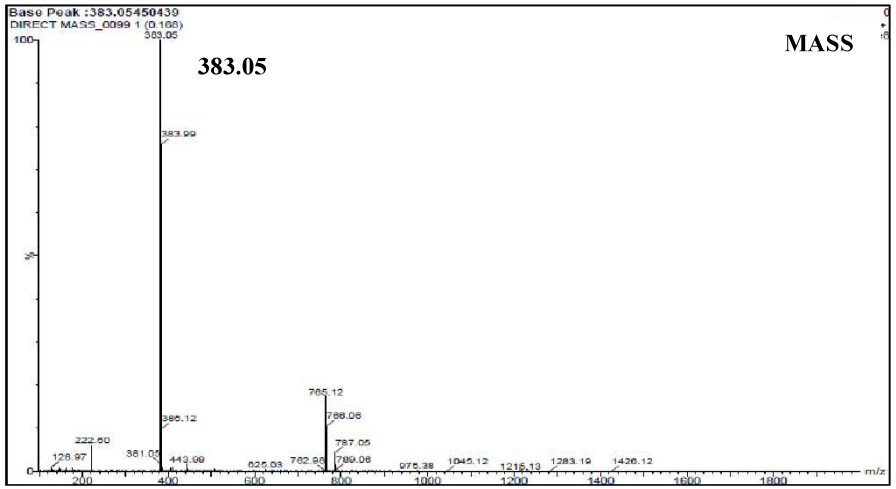


Spectral data of **18**

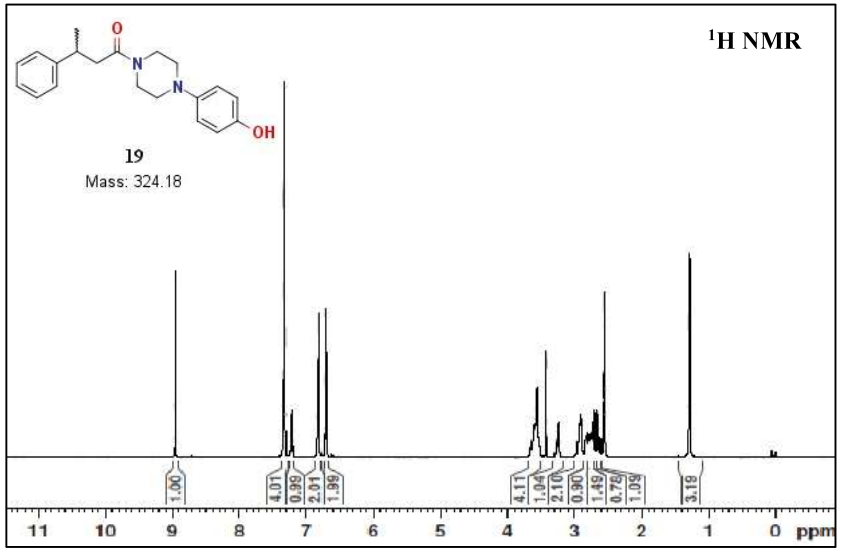


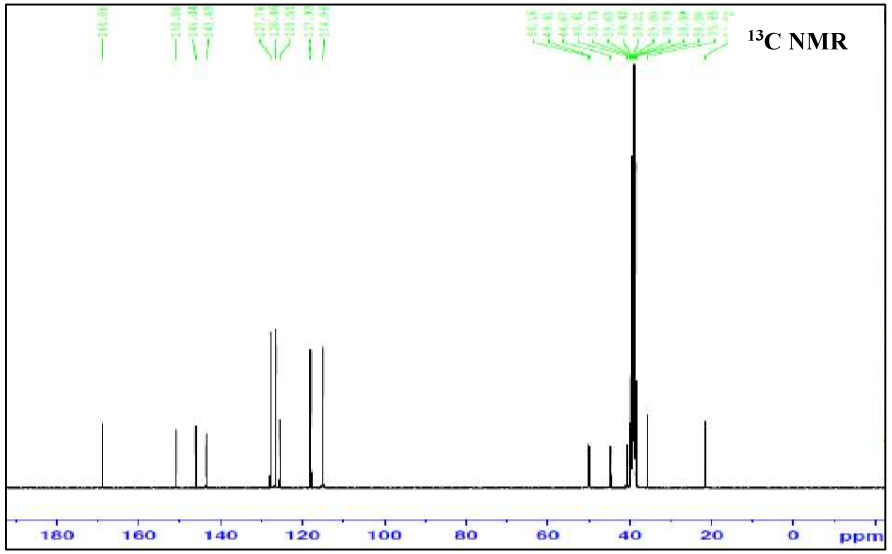


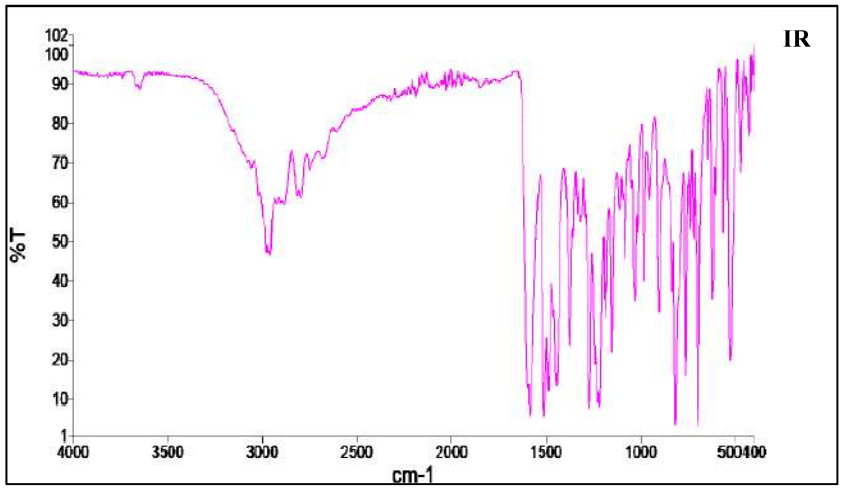


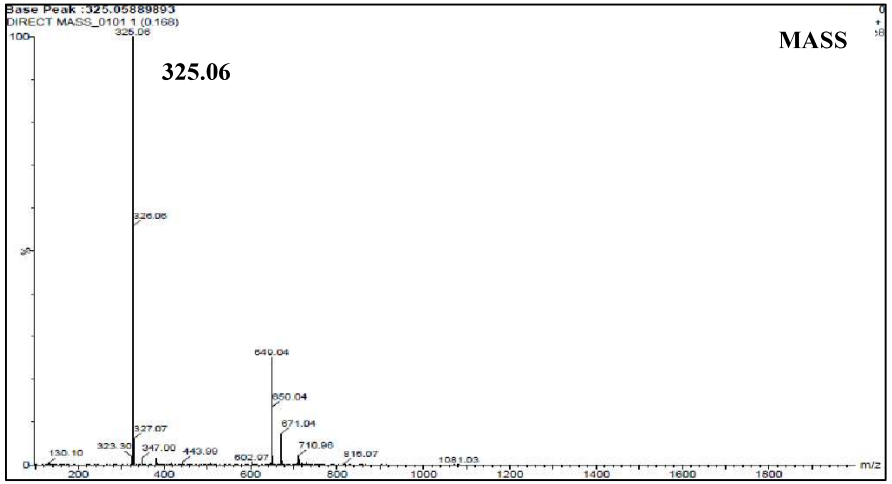


Spectral data of **19**

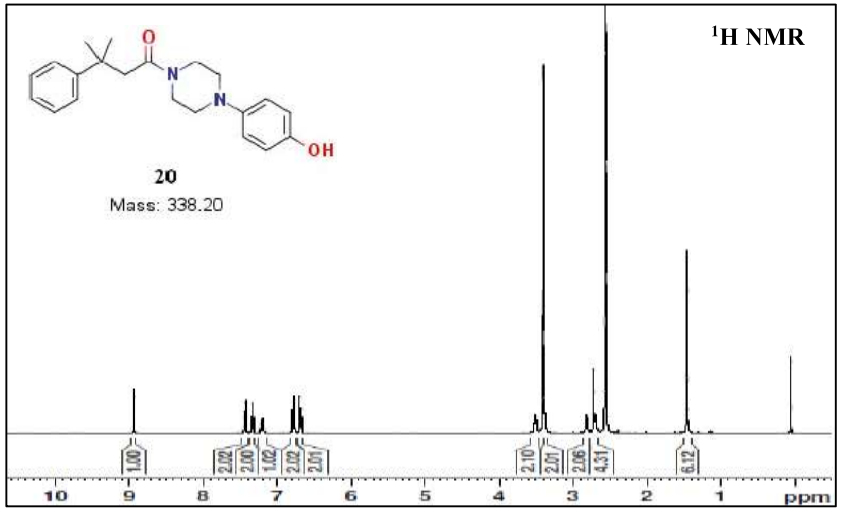


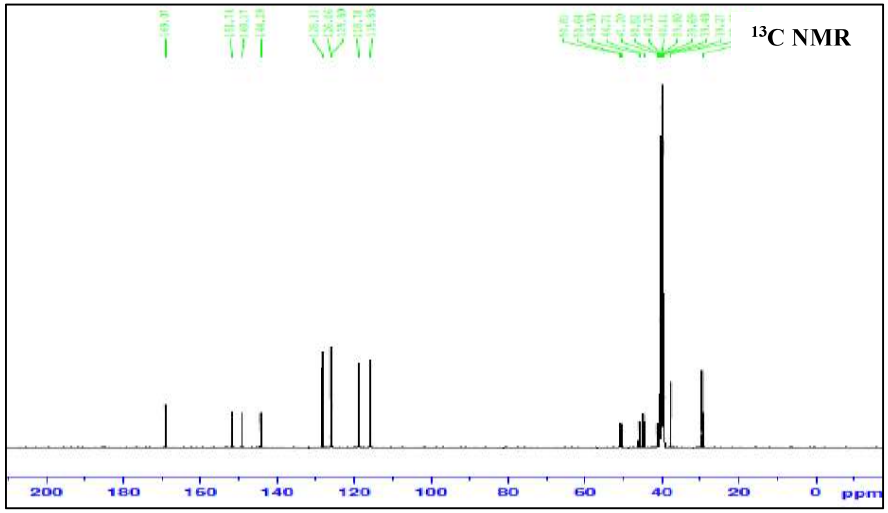


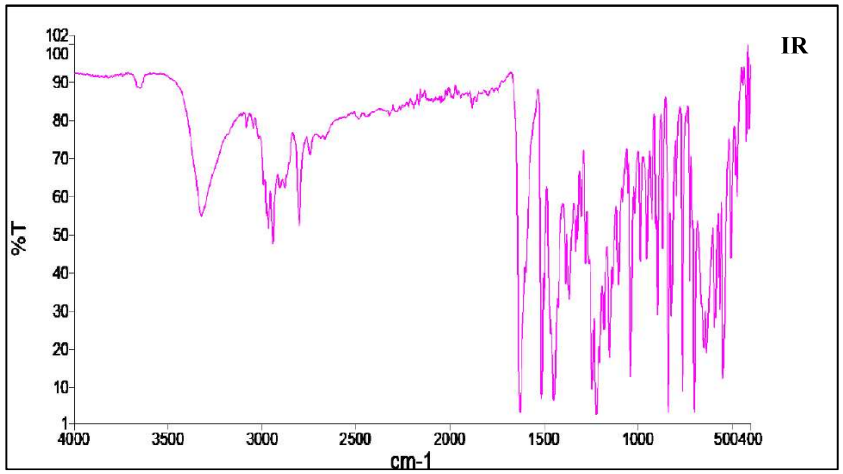


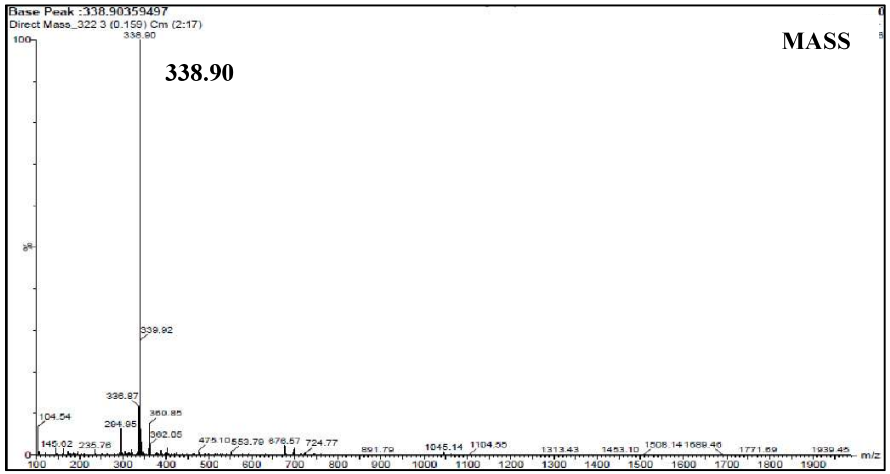


Spectral data of **20**

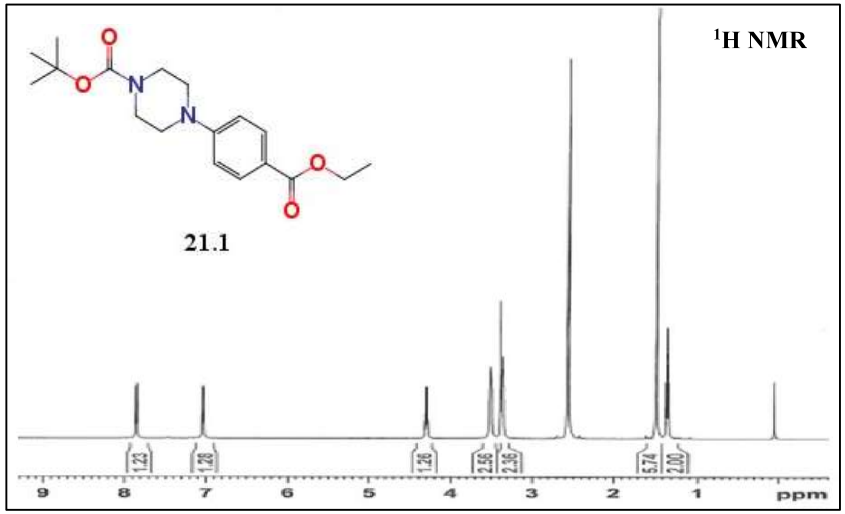




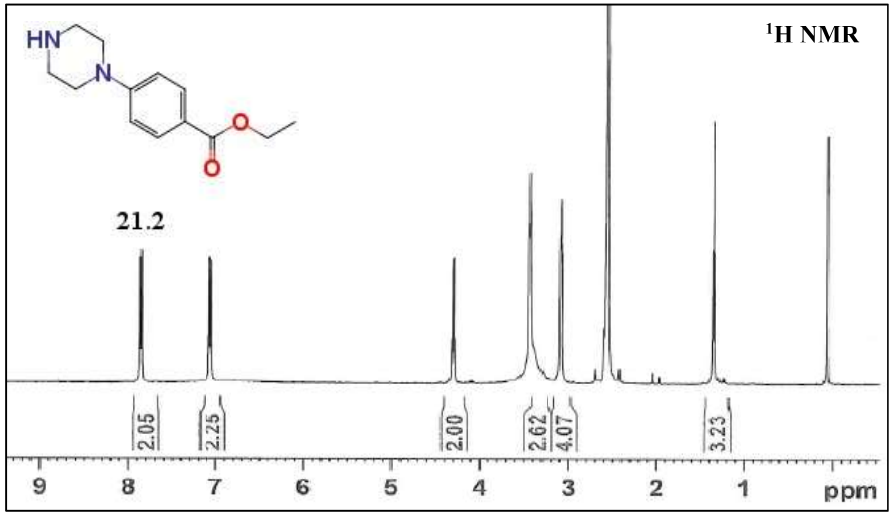




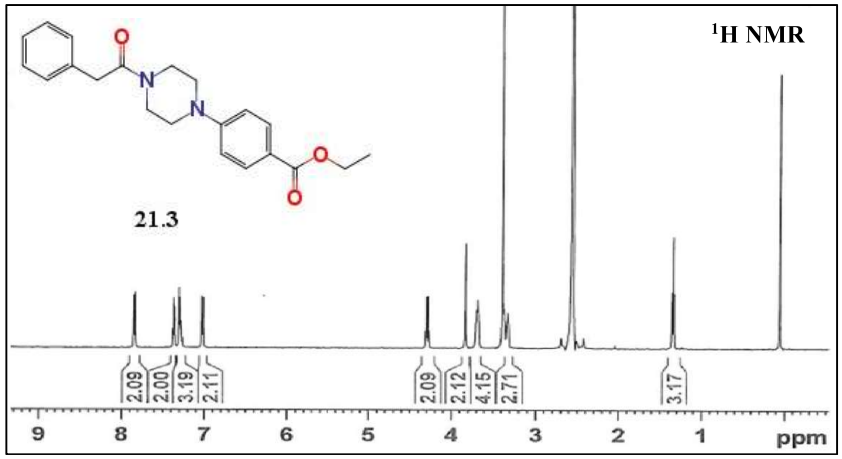
^{1}H NMR of **Intermediate-21.1**



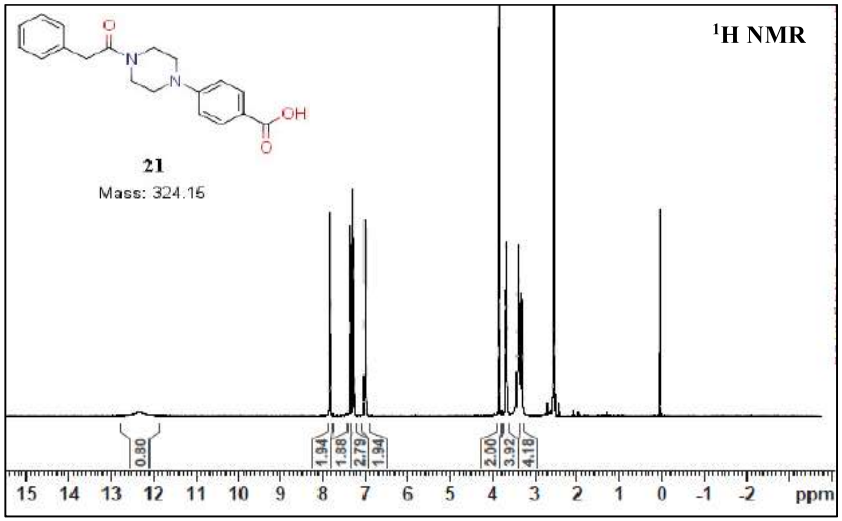
^{1}H NMR of **Intermediate-21.2**

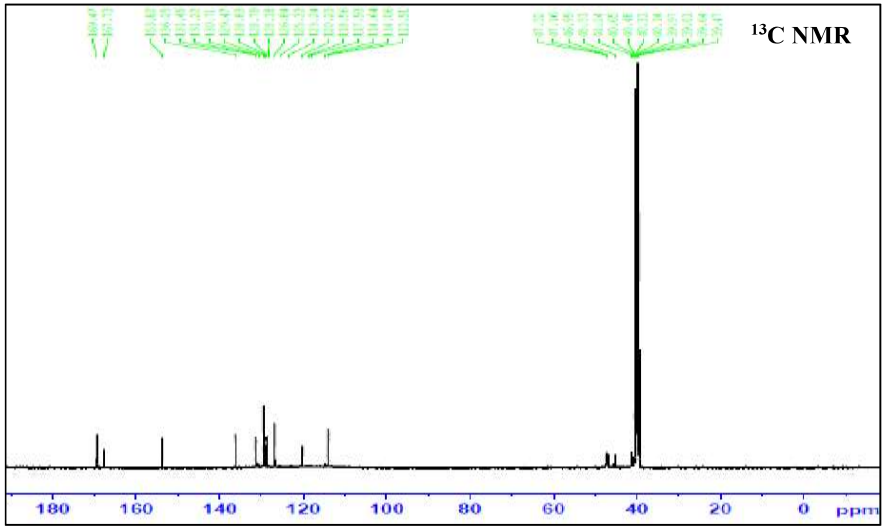


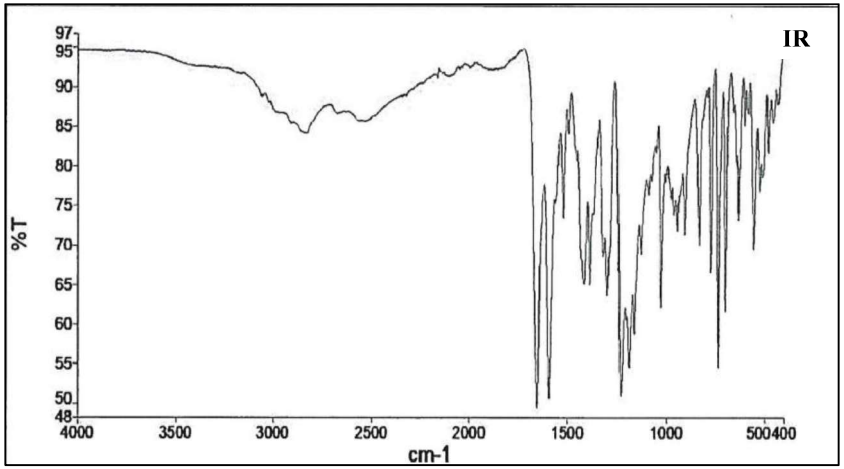
^{1}H NMR of **Intermediate-21.3**

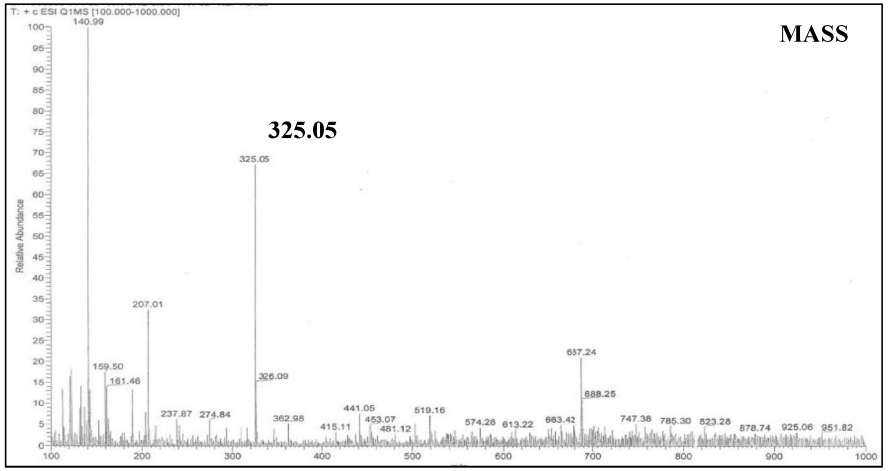


Spectral data of **21**

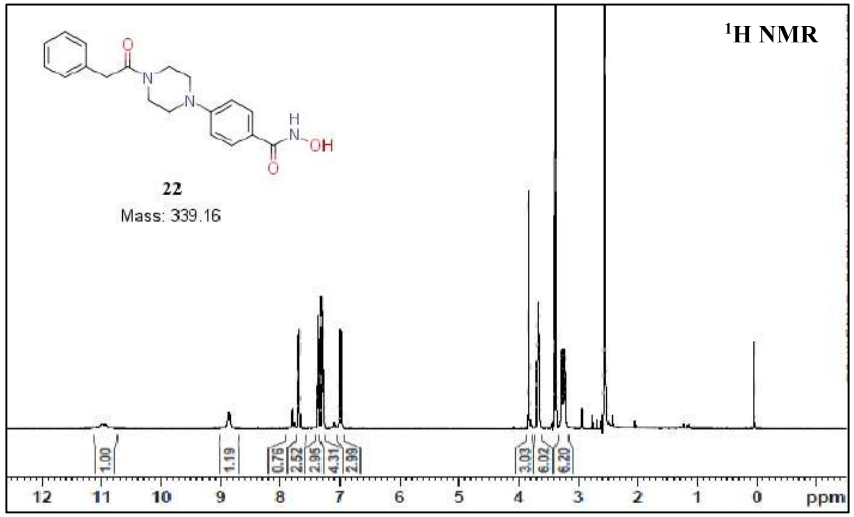


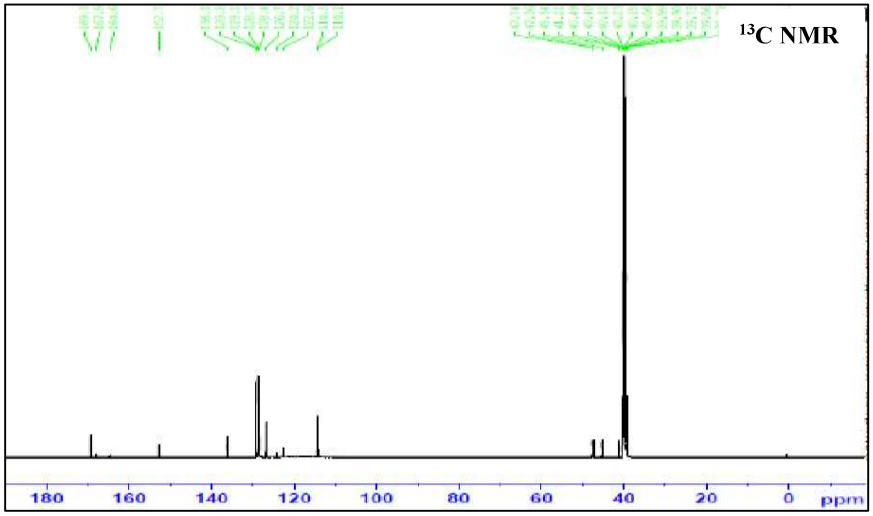


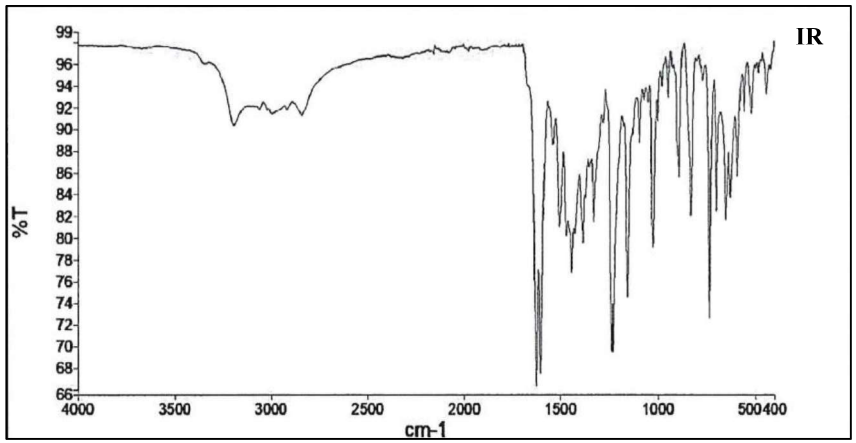


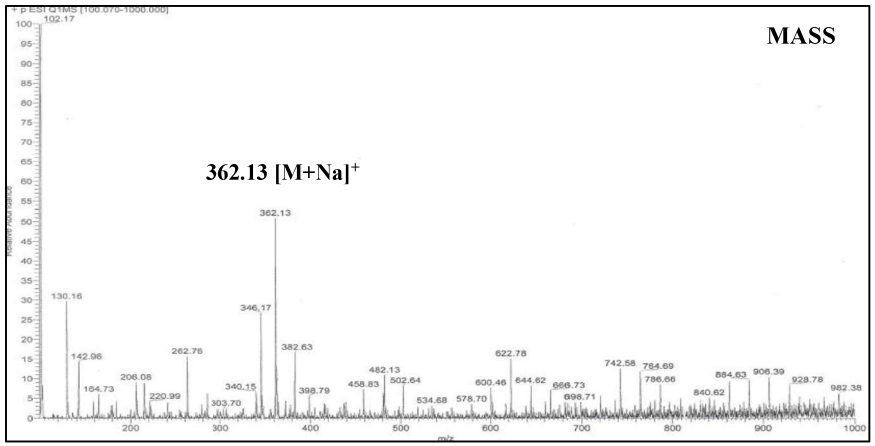


Spectral data of **22**

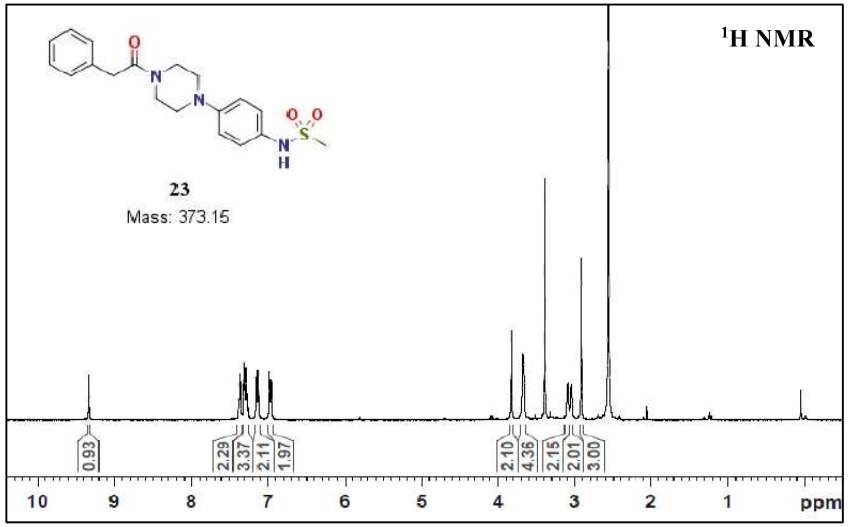


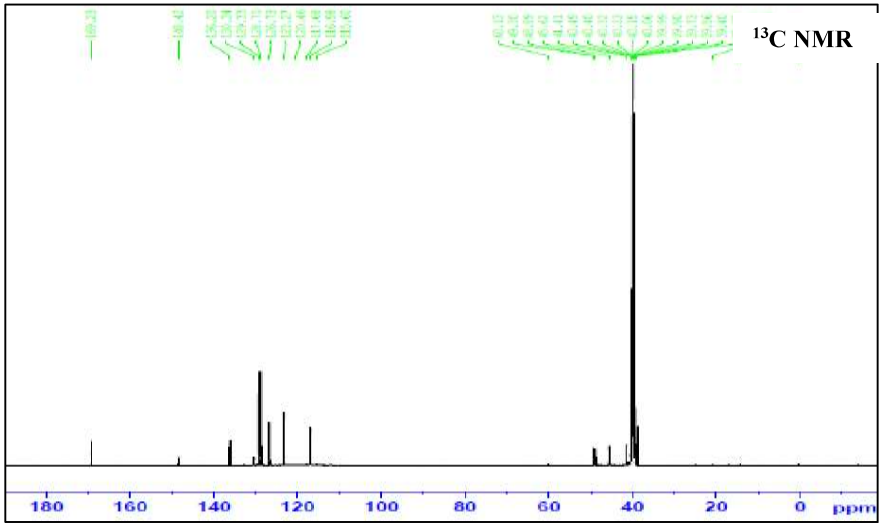


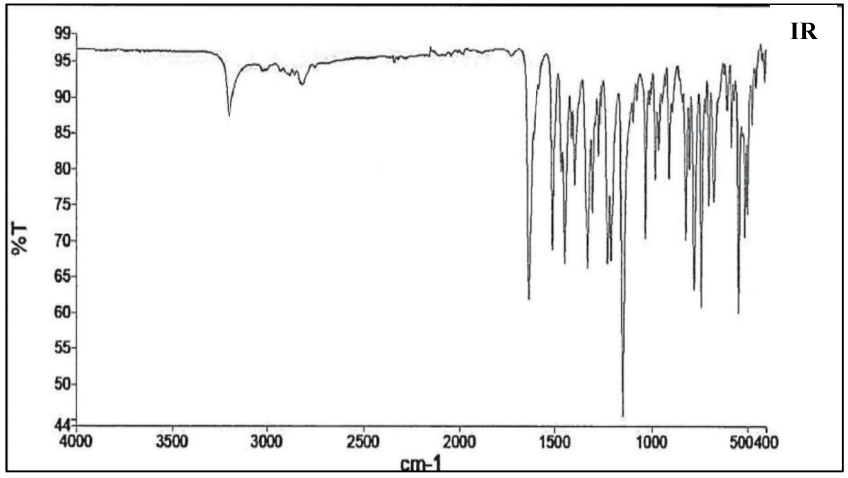


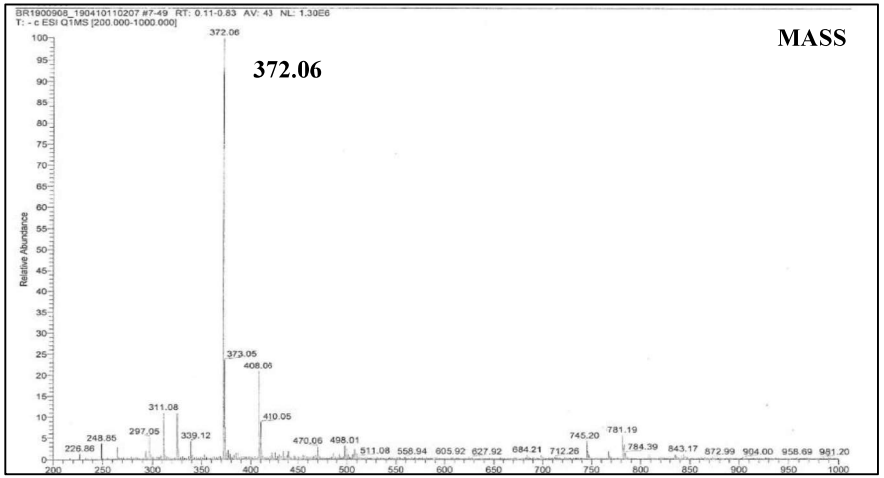


Spectral data of **23**

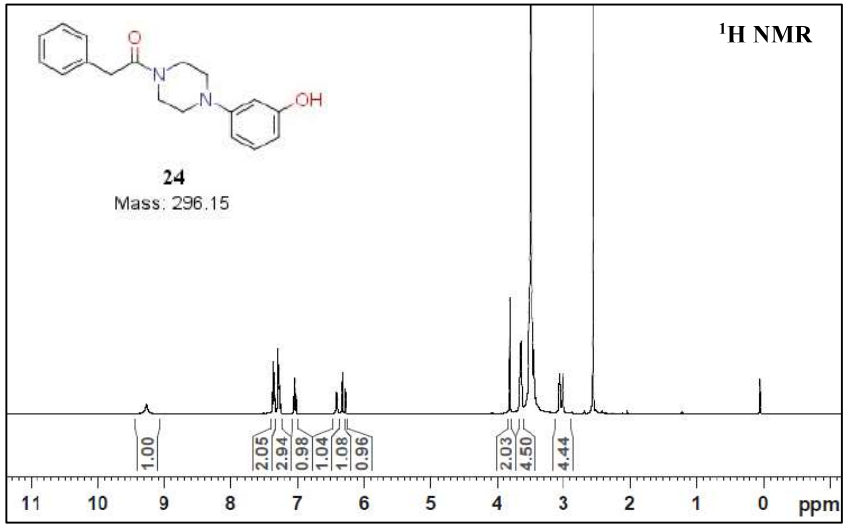


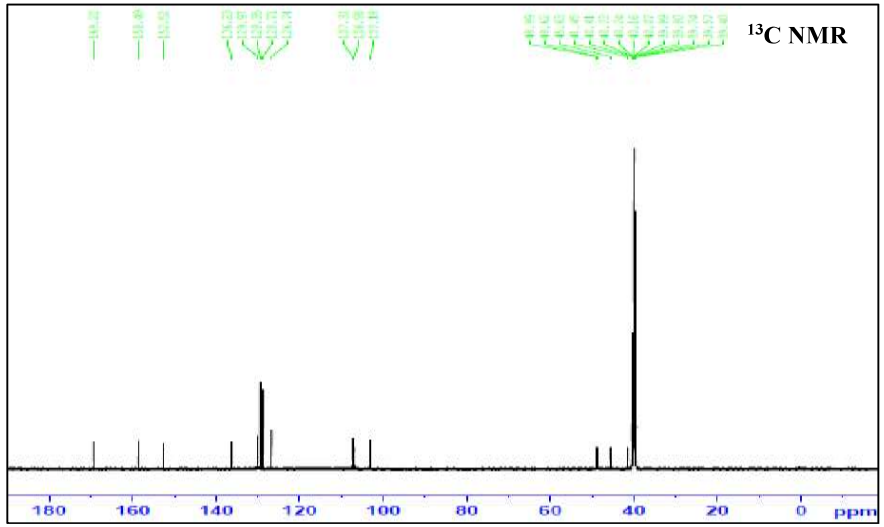


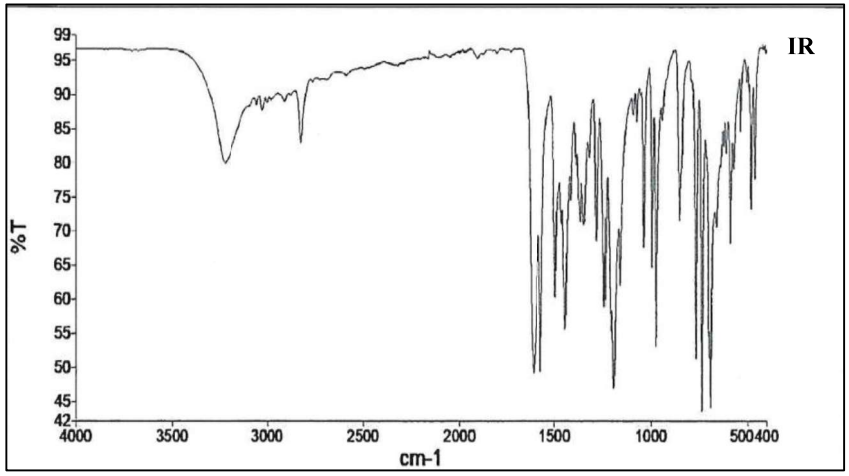


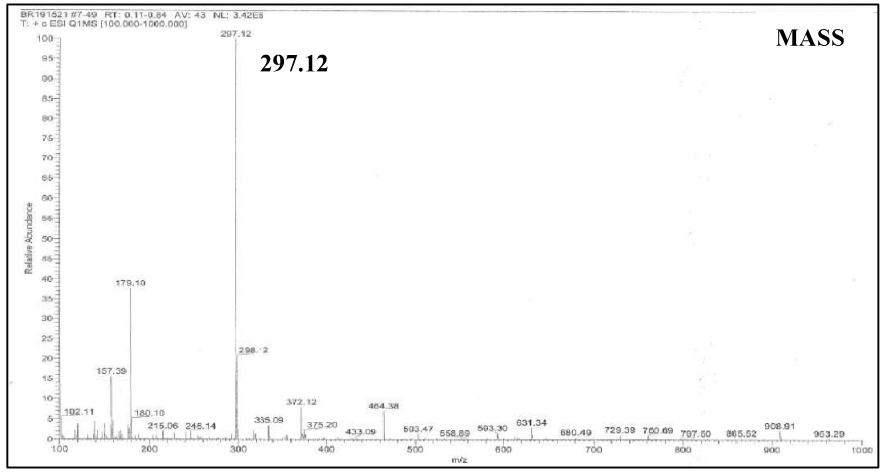


Spectral data of **24**

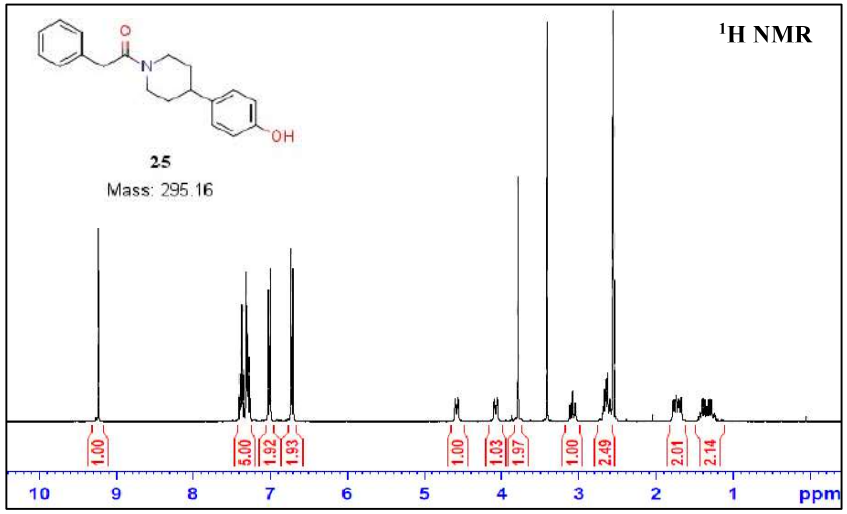


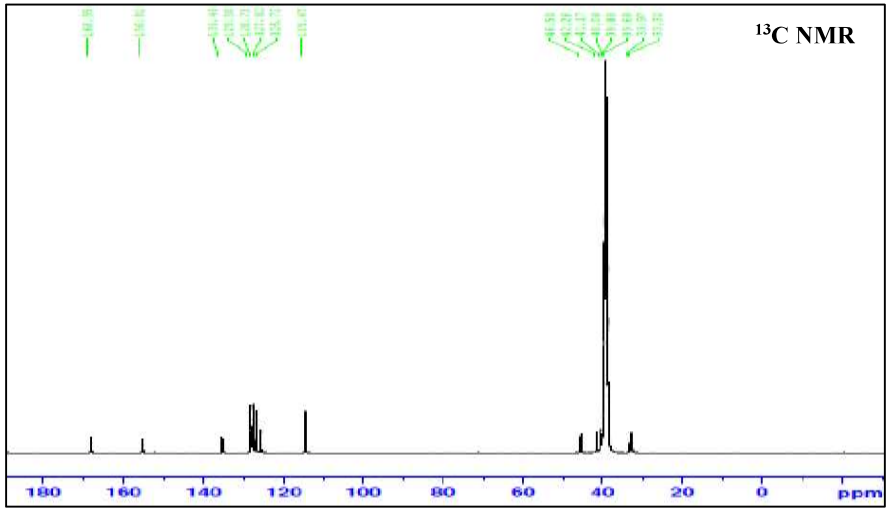


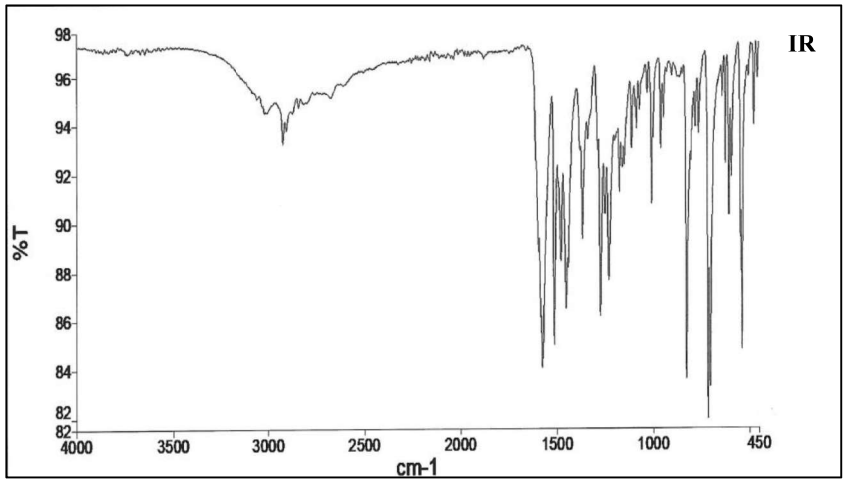


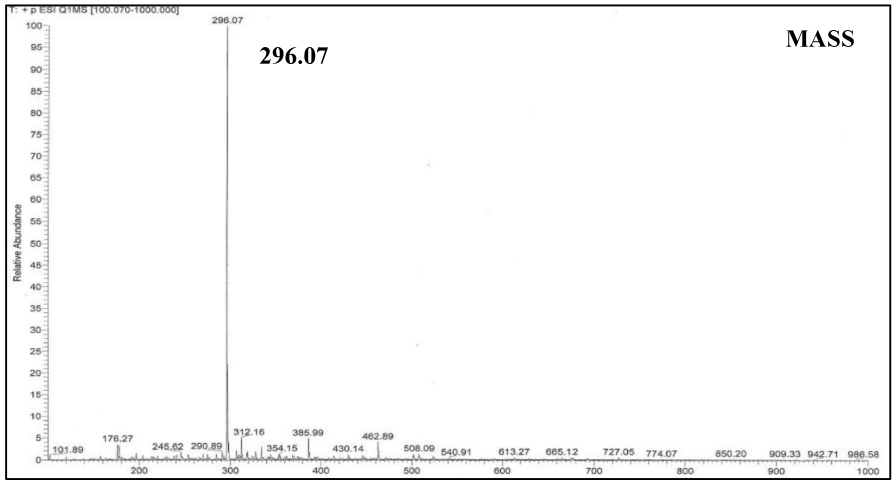


Spectral data of **25**

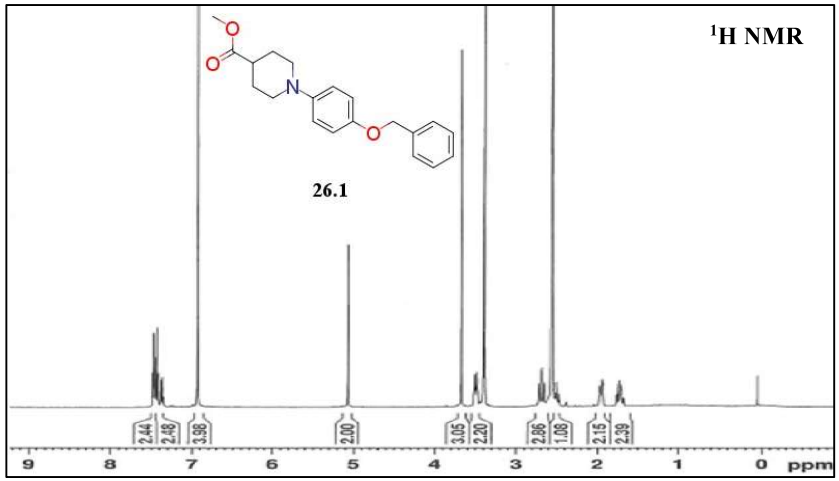




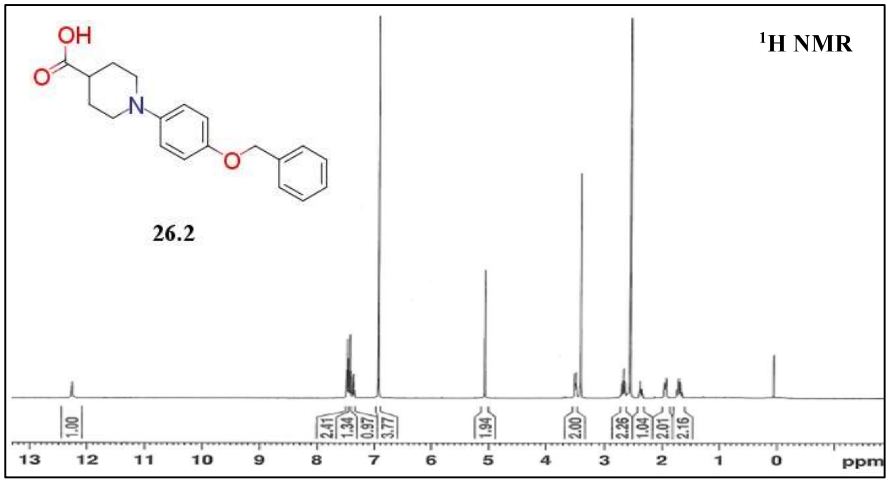




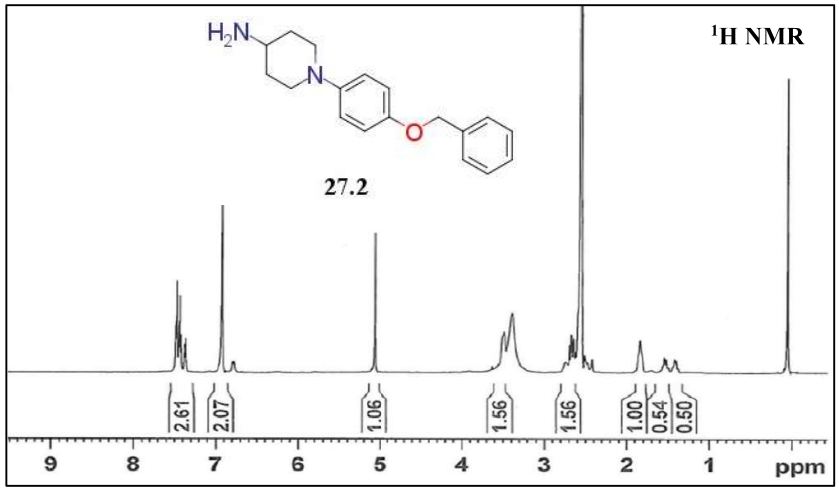
^{1}H NMR of **Intermediate-26.1**



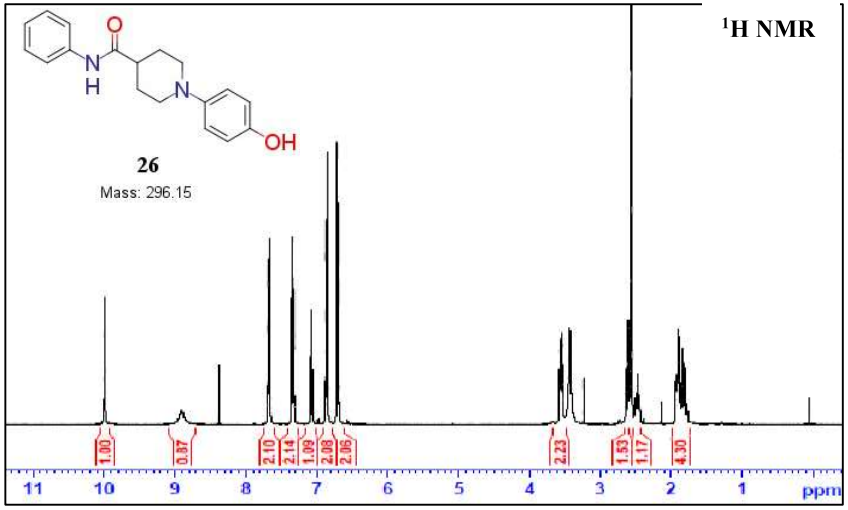
^{1}H NMR of **Intermediate-26.2**

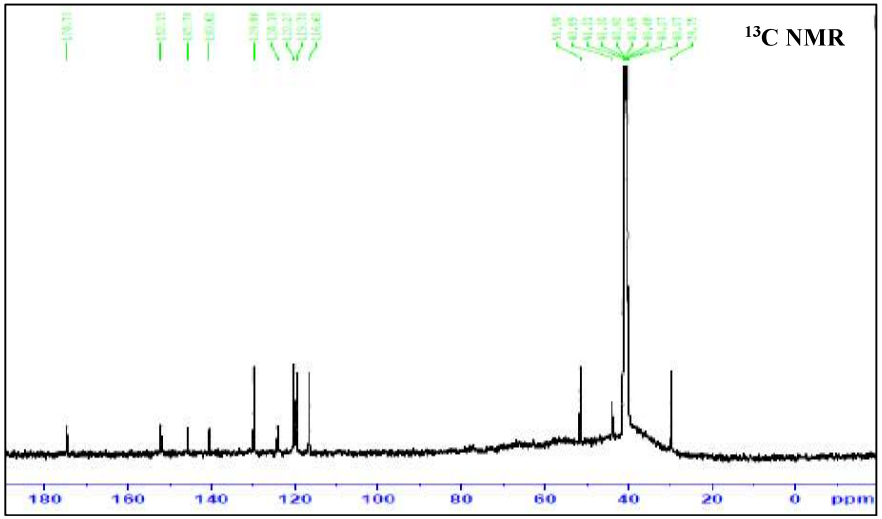


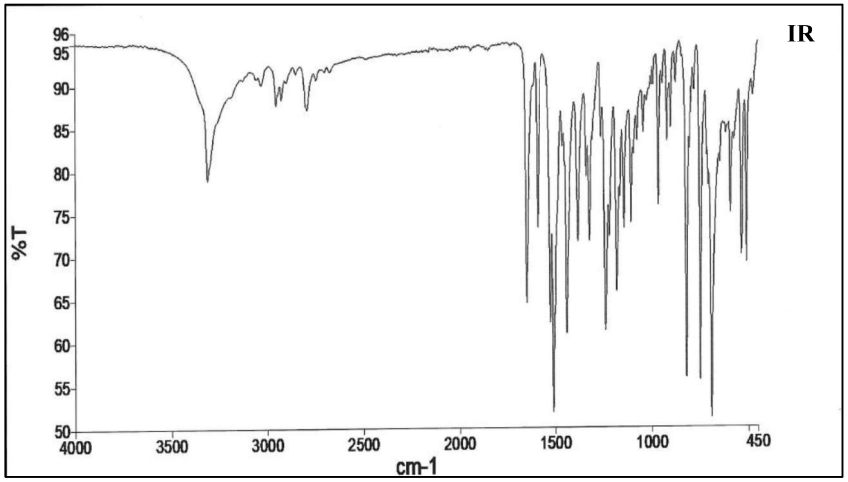
^{1}H NMR of **Intermediate-27.2**

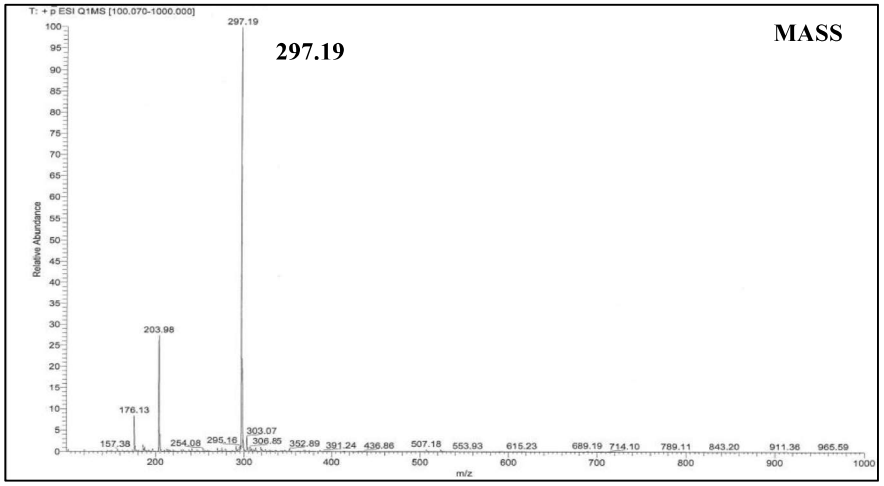


Spectral data of **26**









Spectral data of **27**