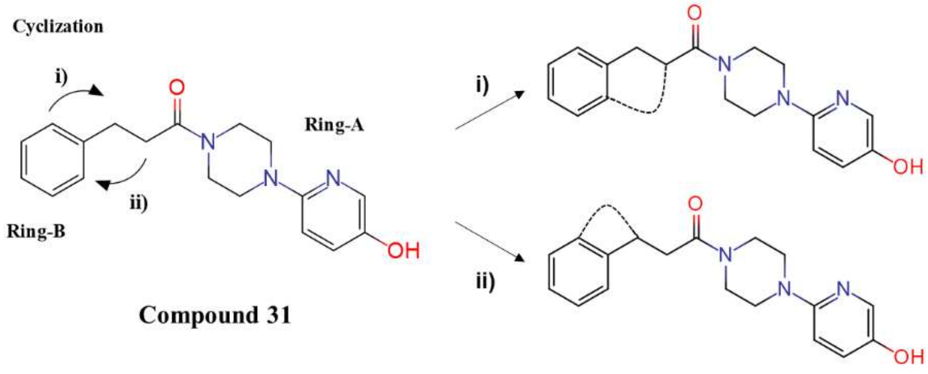
Chapter 4. Design and synthesis of indole-piperazine carboxamide series and *in vitro* evaluation of tool compound in combination with trametinib

As presented in the earlier chapters, through systematic structure activity relationship (SAR), critical structural features were identified for potent inhibition of ME3 and hit molecule **A** was optimized in to potent ME3 inhibitors like compound **3** and **7** which were also effective in terms of cell growth inhibition if BxPC-3 PDAC cells. Further, through heterocyclic modification of ring-A, a novel series of ME3 inhibitors *viz.* compound **31** was identified with a promising activity and marginal selectivity for ME3 over other ME isoforms. Mechanism of ME3 enzyme inhibition was studied for compound **31** which confirmed that it is a malate competitive inhibitor of ME3. Further, its target engagement in cells with ME3 was also demonstrated using cellular thermal shift assay (CETSA). Compound **31** was screened *in vivo* as a tool compound where it showed a very good PK profile and also exhibited a good efficacy in animal xenograft model. Further endeavours were pursued for improvement of biochemical potency and cellular inhibition for the discovered compounds as described in the following chapters.

4.1 Structural diversification by incorporating rigidity

With an aim to further improve biochemical potency and cellular inhibition achieved so far, several new molecules were designed, synthesized and screened for their biological activity. The first step towards optimization was to incorporate structural rigidity by reducing the number of rotatable bonds. Several structures were designed by **1**) building of bicyclic ring systems through cyclization at α- or β-carbon of the three-carbon linker in compound **31**; and **2**) incorporation of small rings between the carbonyl group and ring-B (**Figure 4.1**).

1) **Inclusion of bicyclic systems**



2) Introducing small rings as spacer

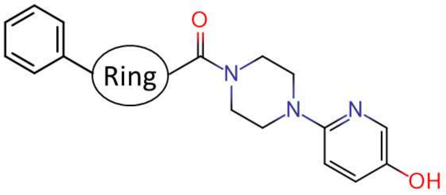
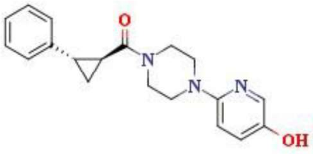


Figure 4.1. Designing strategy for incorporating structural rigidity in the molecules

For getting the insight of molecular interactions with the target enzyme, the designed structures were docked in the malate binding pocket of ME3. The *in silico* study predicted that among them, compounds **55** to **59** would retain ME3 inhibitory activity and hence their synthesis was undertaken. The key intermediate **VIII** was coupled with suitably substituted acids to produce compounds **55 – 59** (**refer section 4.5**) (**Chart 4.1**).

Chart 4.1: Structures of designed compounds **55-59**



55

56

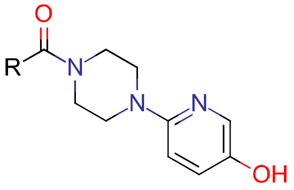
57

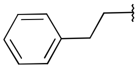
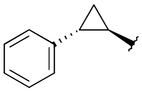
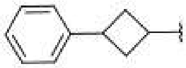
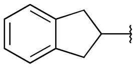
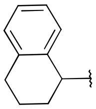
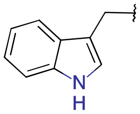
58

59

These synthesized compounds were screened *in vitro* in ME3 enzyme and on BxPC-3 cells (**Table 4.1**).

Table 4.1. ME3 inhibition and BxPC-3 cell growth inhibition data for compounds **55-59**.



Compound	R	% inhibition of ME3			ME3 IC_{50} (µM)	% inhibition of BxPC-3 cells (10 µM)	BxPC-3 IC_{50} (µM)
		0.1 µM	1 µM	10 µM			
31		27	97	100	0.23	92	5.2
55		26	91	100	-	18	-
56		2	93	100	-	21	-
57		4	94	100	-	22	-
58		7	92	100	-	22	-
59		20	93	100	0.25	78	3.7

The *in vitro* screening results obtained were very encouraging as compounds **55** - **59** retained both potency and selectivity for ME3, similar to that for compound **31**. Among them compound **59** with the indolyl moiety was most potent in terms of *ME2*-null BxPC-3 cells (IC_{50} = 3.7 µM) inhibition. After considering the inhibitory potential of compound **59** in biochemical as well as in cell viability assays together, it was selected as a new lead for further development.

4.2 Lead optimization of indole-piperazine carboxamides

Docking study of compound **59** showed a couple of vital H-bond interactions: **a)** interaction of phenolic hydroxyl group with acid side chain of Asp304; **b)** interaction of indolyl –NH with carbonyl oxygen of val136 main chain (**Figure 4.2**).

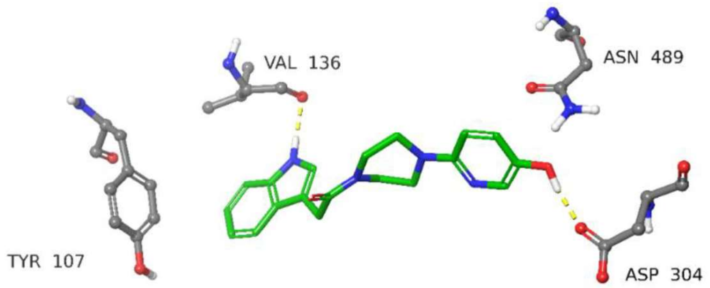
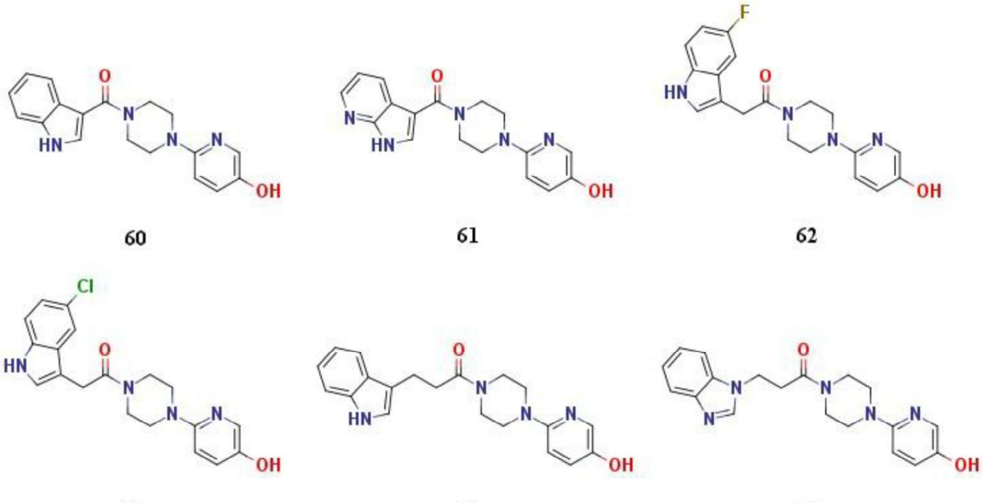


Figure 4.2. *in silico* binding mode of compounds 59 in the malate binding pocket of the ME3 enzyme.

For further optimization of compound **59**, a few analogues with varying linker length and the compounds with substituents containing halogen were designed and docked in ME3. The structures exhibiting consistent binding modes (compounds **60** - **65**) were taken up for their synthesis. The key intermediate **VIII** was coupled with suitably substituted acids to produce compounds **60** - **65** (**refer section 4.5**) (**Chart 4.2**).

Analogues **60** and **61** have one methylene carbon less while **64** has one methylene more compared to compound **59**. Compounds **62** and **63** have halogen substituents on indole ring at the position-5 which is metabolically susceptible for hydroxylation when unsubstituted. To investigate the role of nitrogen attached hydrogen in the indole ring, In compound **65**, indole ring was replaced with benzimidazole ring to study the importance of -NH of indole ring.

Chart 4.2: Structures of designed compounds **60-65**

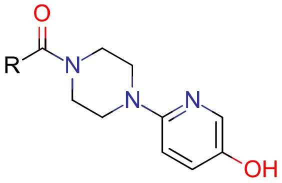


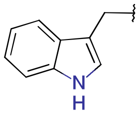
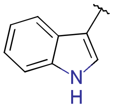
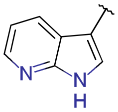
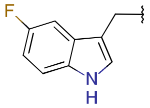
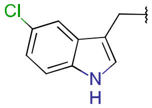
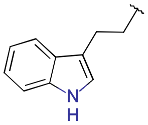
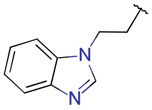
60 61 62

63 64 65

These synthesized compounds **60** - **65** were screened *in vitro* on ME isoforms for biochemical inhibition and in BxPC-3 cells for growth inhibition (**Table 4.2**).

Table 4.2. ME3 inhibition and BxPC-3 cell growth inhibition data for compounds **60-65**.

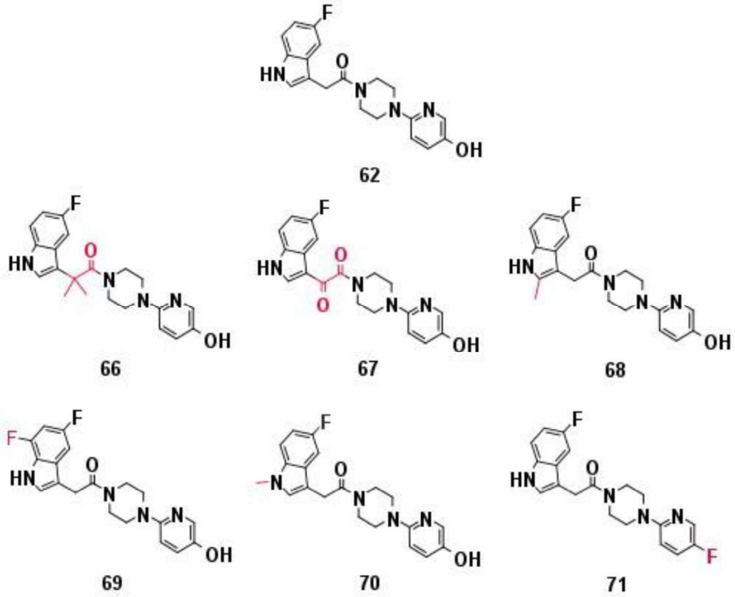


Compound	**R**	**% inhibition of ME3**			**ME3 IC₅₀ (µM)**	**% inhibition of BxPC-3 cells (10 µM)**	**BxPC-3 IC₅₀ (µM)**
		0.1 µM	**1 µM**	**10 µM**			
59		20	93	100	0.25	78	3.7
60		27	97	100	-	45	-
61		07	90	100	-	16	-
62		15	96	100	0.21	63	4.8
63		24	97	100	0.21	100	5.4
64		31	96	100	0.25	58	7.7
65		0	67	100	-	0	-

The *in vitro* screening data for these compounds revealed that compounds **60**, **61** and **64** were equipotent either on ME3 potency or on the growth inhibition of BxPC-3 cells compared to compound **59.** As anticipated, Compound **65** which is devoid of the critical H-bond interaction (N-H···O) with Val136 lost activity both in the biochemical and cellular assays on ME3 enzyme and on BxPC-3 cells respectively. Compound **62** and **63** with 5-halo substitution on indole retained the activity on ME3 as well as on the BxPC-3 cells and this might be advantageous in terms of metabolic stability over compound **59**. Hence, 5-fluoro and 5-chloro indolylacetic acid constructs were fixed in the molecules designed for the next round of SAR.

To develop further insight into critical structural features in compound **62**, several designs were made without changing the basic molecular skeleton (**66-71**) as depicted in **chart 4.3**.

Chart 4.3. Structures of compound **62** analogues (variations highlighted in red)



Docking studies were carried out for these modifications with ME3 where it was found that the critical interactions observed for compound **62** with Asp304 and Val136 of ME3 enzyme (**Figure 4.3**) were retained in compounds **66** to **69.** Methyl substitution on indole nitrogen (compound **70**) and replacement of the phenolic hydroxyl group with -F (compound **71**) resulted in loss of the essential H-bond interactions with the amino acids as was expected.

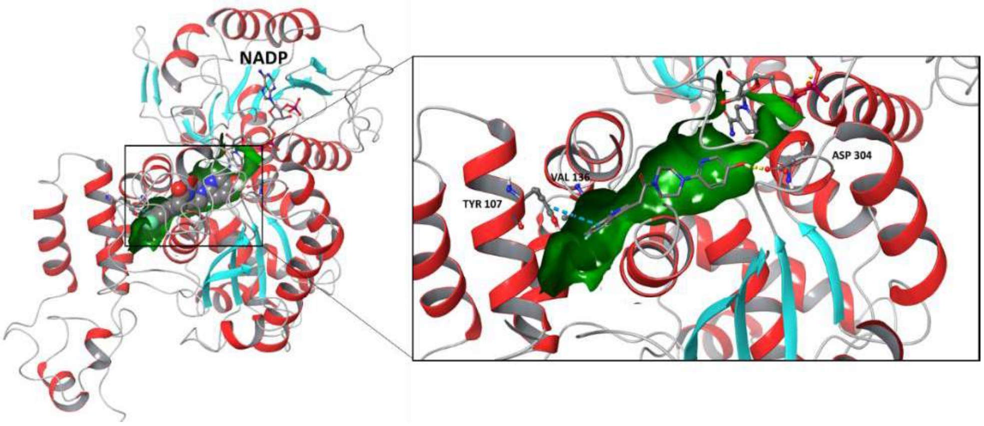
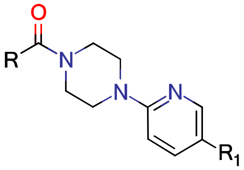
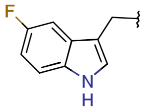
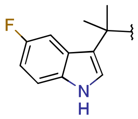
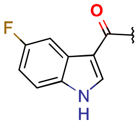
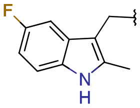
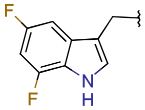
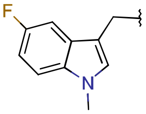
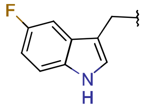


Figure 4.3. Structure of ME3 protein and *in silico* binding mode of compound 62 in malate binding pocket of ME3

These newly designed analogues were synthesized (**Refer section 4.5**) and were screened on ME3 enzyme and BxPC-3 cells. The results are presented in **Table 4.3**.

Table 4.3. ME3 inhibition and BxPC-3 cell growth inhibition data for compounds **66-71**.

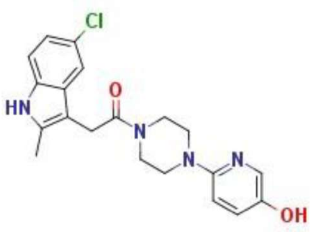


Compound	R	R_1	ME3 IC_{50} (µM)	BxPC-3 IC_{50} (µM)
62		-OH	0.21	4.8
66		-OH	0.42	4.1
67		-OH	0.38	3.0
68		-OH	0.42	4.3
69		-OH	0.32	4.5
70		-OH	0.41	62 % at 10 µM
71		-F	32 % at 10 µM	ND

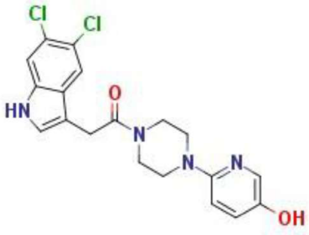
Compounds **66** to **71** exhibited inhibitory potency on ME3 similar to that exhibited by compound **62.** In concurrence with the *in silic*o predictions, replacement of the hydroxyl group with fluorine (compound **71**) resulted in loss of the inhibitory activity in the ME3 enzyme assay, attributable to the absence of the critical H-bond interaction with Asp304. however, *N*-methylation of indole ring (compound **70**) retained the inhibitory activity on ME3 enzyme which was unexpected. Replacement of amide with an oxoamide (compound **67**) as linker led to enhanced cell growth inhibition of BxPC-3 cells.

To investigate the effect of increasing logP on cell growth inhibition, analogues of compound **63** (compound **72 - 74**) were designed wherein lipophilic groups such as methyl and chloro were introduced at the positions 2 and 6 of the indole moiety (**Chart 4.4**).

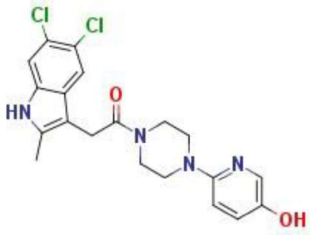
Chart 4.4: Structures of designed compounds **72-74**



72



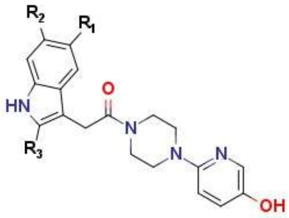
73



74

Compounds **72 - 73** were synthesized by coupling key intermediate **VIII** with suitably substituted acids (**Refer section 4.5**). These compounds were screened *in vitro* on ME isoforms for biochemical inhibition and in BxPC-3 cells for growth inhibition (**Table 4.4**).

Table 4.4. ME3 and BxPC-3 cell growth inhibition data for compounds **72-74**.



Compound	R1	R2	R3	ME3 IC50 (μM)	QPlog Po/w*	BxPC-3 IC50 (μM)
63	- Cl	- H	- H	0.210	3.20	5.4
72	- Cl	- H	- Me	0.280	3.53	4.9

Compound	R_1	R_2	R_3	ME3 IC_{50} (μM)	QPlog Po/w*	BxPC-3 IC_{50} (μM)
73	- Cl	- Cl	- H	0.280	3.59	2.9
74	- Cl	- Cl	- Me	0.320	3.95	2.2

* *logP prediction was performed using QikProp*

In biochemical screen for ME3 inhibition, they were found to retain inhibitory potency similar to that of compound **63**. Gradual increase in logP was correlated with the inhibition of cell viability in BxPC3 cells; compound **74** having the highest logP being the most potent (**Table 4.4**).

4.3 *in vitro* screening of selected compounds in Hs766T PDAC cells

BxPC-3 is a *ME2*$^{-/-}$ PDAC cell line with the wild-type *RAS* genotype while Hs766T PDAC cell line carries *KRAS*, *TP53* and *P16* mutations in addition to *SMAD4/ME2* deletions. In addition, Hs766T cells carrying *KRAS*^{Q61H} mutation also exhibit a high level of activated RAS.[26] Due to these reasons, Hs766T cells are clinically more relevant with regard to metastatic PDAC. Therefore, the selected compounds exhibiting potent inhibition on BxPC-3 were also screened in Hs766T cells with varying incubation periods viz. short term (96 hours) and long term (2 weeks). In the short- term assay, except for compound **62** (IC_{50} = 11.8 μM), none of the other compounds were found to be active. Similarly, in the long-term assay, compound **62** showed the best potency among these compounds on Hs766T cells with an IC_{50} of 0.8 μM **(Table 4.5)**.

Table 4.5. Antiproliferative effect of selected compounds on Hs766T cells

Compound No.		31	59	62	63	66	67	68	72	73	74
Hs766T cells (IC_{50}) μM	2 weeks assay	3.5	2.1	0.8	1.8	2.8	3.0	2.0	2.0	0.9	2.9
	96 hours assay	>30	>50	11.8	>50	>50	>50	>50	>50	>50	>50

4.4 Combination study of compound 62 and trametinib on Hs766T cells

Combination of drugs is a common practice for clinical management of complex cancers like PDAC. Through comprehensive *in vivo* screening of 57 drug conditions (16 as a single-agent and 41 as two-drug combinations) in genetically engineered KPC mice model, Grbovic-Huezo *et. al.* demonstrated that a combination of trametinib (MEKi) and PU-H71 (HSP90i) resulted in prolonged survival.[27]

Taking cue from these findings, it was sought to investigate the combination effect of trametinib with the most potent of the compounds designed and studied in the current research viz. compound **62** on Hs766T cell growth inhibition in *in vitro*. The combination of compound **62** and trametinib was thought to be synergistic in PDAC because it targets two different pathways that are essential for cancer cell survival. By inhibiting ME3, compound **62** can disrupt cancer cell metabolism and by inhibiting MEK, trametinib blocks the *KRAS* mediated RAS-RAF-MEK pathway. The combination effects of trametinib and compound **62** on Hs766T cells were assessed in *in vitro* using isobologram analysis as it is one of the mathematically proven techniques to evaluate synergistic effects in biological studies (**Figure 4.4**).

To investigate synergistic drug combinations, level of synergism is typically measured and quantified by the drug combination index (CI) such as Chou and Talalay's CI from experimental dose-response data.[28] CI for the combination of compound **62** and trametinib was found to be < 1 indicating clear synergism between these two compounds (**Figure 4.4**). Detailed experimental procedures and analytical data on this aspect are presented in Chapter **6**.

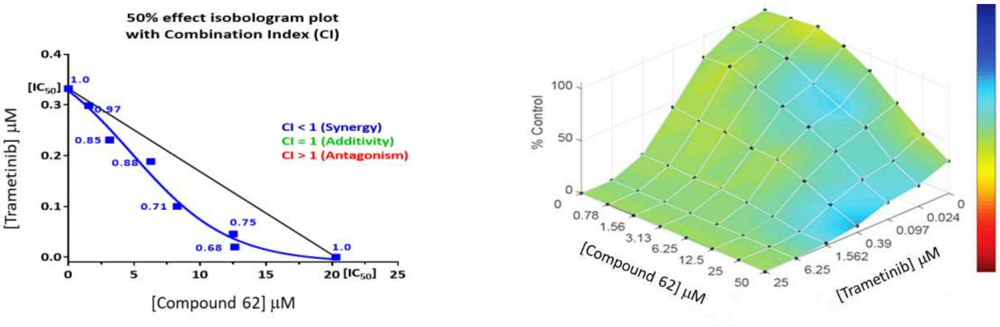


Figure 4.4. Combination index for compound 62 and trametinib

4.5 Chemistry

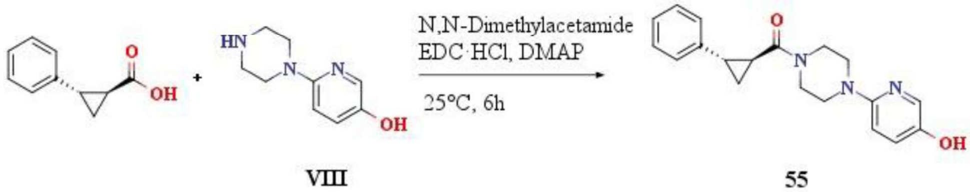
4.5.1 General information

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

4.5.2 Experimental procedures and spectral data for compounds (55-74)

1) Synthesis of 6-{4-[(1S,2S)-2-phenylcyclopropanecarbonyl]piperazin-1-yl}pyridin-3-ol (55)

Scheme 4.1: Synthesis of compound **55**.



To a solution of *trans*-2-phenylcyclopropane-1-carboxylic acid (1 g, 6.16 mmol) in N,N-dimethylacetamide (10 ml) was added 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC·HCl) (1.77 g, 9.24 mmol). The reaction mixture was stirred for 10 minutes before adding the key intermediate **VIII** (1.10 g, 6.16 mmol). To the resultant reaction mixture was added 4-(dimethylamino)pyridine (0.150 g, 0.61 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 compound **55** (1.1 g, 3.40 mmol, 55% yield) as an off white solid.

Melting point: 130-133 °C. **LC purity (UV 245 nm):** 99.00%.

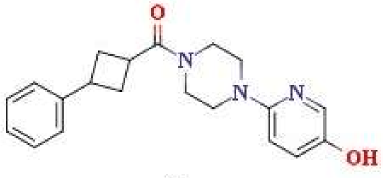
^{1}H NMR (400 MHz, DMSO-d$_6$): δ 1.27 (br-s, 1H), 1.48 (br-s, 1H), 2.38 (d, 2H, *J* = 5.04 *Hz*), 3.65 (m, 4H), 3.76 (br-s, 2H), 3.80 (br-s, 2H), 6.80 (d, 1H, *J* = 8.92 *Hz*), 7.12 (d, 1H, *J* = 6.05 *Hz*), 7.24 (m, 3H), 7.31 - 7.34 (m, 2H), 7.80 (s, 1H), 9.09 (s, 1H).

^{13}C NMR (100 MHz, DMSO-d$_6$): δ 16.7 (CH_2), 22.6 (CH), 25.0 (CH), 41.8 (CH_2), 45.0 (CH_2), 46.3 (CH_2), 46.8 (CH_2), 109.0 (CH), 125.8 (CH), 126.0 (CH), 126.4 (2 x CH), 128.7 (2 x CH), 134.5 (CH), 141.3 (C), 147.1 (C), 153.5 (C), 170.0 (C).

IR (ATR) / cm^{-1}: 3129, 1601, 1583, 1487, 1434, 1384, 1267, 1233, 1163, 934, 817, 748, 697.

LCMS (ESI$^+$): calculated for $C_{19}H_{21}N_3O_2$ $[M+H]^+$ 324.1712; found: *m/z* = 324.0182.

2) Synthesis of 6-[4-(3-phenylcyclobutanecarbonyl)piperazin-1-yl]pyridin-3-ol (56)



56

3-Phenylcyclobutane-1-carboxylic acid (0.10 g, 0.56 mmol) was reacted with 6-piperazin-1-yl-pyridin-3-ol (**VIII**) (0.10 g, 0.56 mmol) as per the procedure described for compound **55** to afford compound **56** (0.11 g, 0.32 mmol, 57% yield) as an off white solid.

Melting point: 123-126 °C. **LC purity (UV 245 nm):** 95.04%.

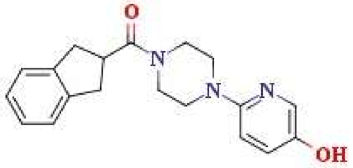
^{1}H NMR (400 MHz, DMSO-d$_6$): δ 2.27 - 2.35 (m, 2H), 2.58 (br-s, 1H), 3.31 - 3.38 (m, 1H), 3.41 - 3.45 (m, 2H), 3.46 - 3.48 (m, 1H), 3.50 - 3.52 (m, 3H), 3.55 - 3.60 (m, 4H), 6.80 (d, 1H, *J* = 8.92 *Hz*), 7.13 (d, 1H, *J* = 6.90 *Hz*), 7.23 - 7.27 (m, 2H), 7.33 - 7.37 (m, 3H), 7.80 (s, 1H), 9.13 (s, 1H).

^{13}C NMR (100 MHz, DMSO-d$_6$): δ 31.5 (CH_2), 32.5 (CH), 32.7 (CH_2), 35.1 (CH), 41.3 (CH_2), 44.6 (CH_2), 46.4 (CH_2), 46.9 (CH_2), 109.1 (CH), 125.8 (CH), 126.3 (CH), 126.7 (2 x CH), 128.6 (2 x CH), 134.5 (CH), 145.4 (C), 147.1 (C), 153.6 (C), 172.0 (C).

IR (ATR) / cm^{-1}: 3272, 1615, 1487, 1429, 1278, 1262, 1238, 1212, 1160, 1014, 922, 823, 758, 698, 644, 523.

LCMS (ESI$^+$): calculated for $C_{20}H_{23}N_3O_2$ $[M+H]^+$ 338.1868; found: *m/z* = 338.0286.

3) Synthesis of 6-[4-(2,3-dihydro-1H-indene-2-carbonyl)piperazin-1-yl]pyridin-3-ol (57)



57

Indan-2-carboxylic acid (0.10 g, 0.61 mmol) was reacted with 6-piperazin-1-yl-pyridin-3-ol (**VIII**) (0.10 g, 0.61 mmol) as per procedure described for compound **55** to afford compound **57** (0.11 g, 0.34 mmol, 55% yield) as an off white solid.

Melting point: 137-140 °C. **LC purity (UV 245 nm):** 99.94%.

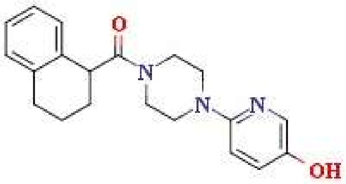
^{1}H NMR (400 MHz, DMSO-d$_6$): δ 3.18 (d, 4H, *J* = 8.32 *Hz*), 3.34 (t, 2H, *J* = 5.00 *Hz*), 3.42 (t, 2H, *J* = 4.98 *Hz*), 3.65 (t, 2H, *J* = 5.00 *Hz*), 3.69 – 3.78 (m, 3H), 6.83 (d, 1H, *J* = 8.96 *Hz*), 7.13 – 7.20 (m, 3H), 7.23 – 7.26 (m, 2H), 7.81 (d, 1H, *J* = 2.92 *Hz*), 9.16 (s, 1H).

^{13}C NMR (100 MHz, DMSO-d$_6$): δ 36.4 (2 x CH$_2$), 40.3 (CH), 41.5 (CH$_2$), 45.1 (CH$_2$), 46.5 (CH$_2$), 47.0 (CH$_2$), 109.1 (CH), 124.5 (2 x CH), 125.8 (CH), 126.6 (2 x CH), 134.5 (CH), 142.2 (2 x C), 147.1 (C), 153.6 (C), 172.8 (C).

IR (ATR) / cm^{-1}: 3307, 1621, 1486, 1457, 1440, 1273, 1228, 1199, 811, 756, 737, 646.

LCMS (ESI$^+$): calculated for $C_{19}H_{21}N_3O_2$ [M+H]$^+$ 324.1712; found: *m/z* = 324.0029.

4) Synthesis of 6-[4-(1,2,3,4-tetrahydronaphthalene-1-carbonyl)piperazin-1-yl]pyridin-3-ol (58)



58

1,2,3,4-Tetrahydronaphthalene-1-carboxylic acid (0.15 g, 0.85 mmol) was reacted with 6-piperazin-1-yl-pyridin-3-ol (**VIII**) (0.15 g, 0.85 mmol) as the per procedure described for compound **55** to afford compound **58** (0.17 g, 0.50 mmol, 58% yield) as an off white solid.

Melting point: 145-148 °C. **LC purity (UV 245 nm):** 96.60%.

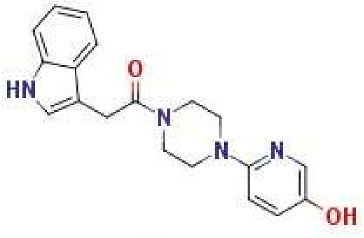
^{1}H NMR (400 MHz, DMSO-d$_6$): δ 1.73 - 1.75 (m, 1H), 1.83 - 1.87 (m, 1H), 1.91 - 1.96 (m, 1H), 1.98 – 2.01 (m, 1H), 2.72 – 2.81 (m, 2H), 3.37 (m, 2H), 3.41 (m, 2H), 3.68 - 3.87 (m, 4H), 4.30 (t, 1H, *J* = 6.66 *Hz*), 6.83 (d, 1H, *J* = 8.96 *Hz*), 6.95 (d, 1H, *J* = 7.0 *Hz*), 7.14 (m, 4H), 7.81 (s, 1H), 9.11 (s, 1H).

^{13}C NMR (100 MHz, DMSO-d$_6$): δ 20.9 (CH$_2$), 27.1 (CH$_2$), 29.1 (CH$_2$), 41.2 (CH), 41.5 (CH$_2$), 45.6 (CH$_2$), 46.6 (CH$_2$), 47.1 (CH$_2$), 109.1 (CH), 125.8 (CH), 125.9 (CH), 126.3 (CH), 128.8 (CH), 129.2 (CH), 134.5 (CH), 136.2 (C), 137.5 (C), 147.1 (C), 153.6 (C), 173.2 (C).

IR (ATR) / cm^{-1}: 2913, 1633, 1493, 1429, 1268, 1225, 1203, 1158, 1028, 829, 749, 726.

LCMS (ESI$^+$): calculated for $C_{20}H_{23}N_3O_2$ [M+H]$^+$ 338.1868; found: *m/z* = 338.0080.

5) Synthesis of 1-[4-(5-hydroxypyridin-2-yl)piperazin-1-yl]-2-(1H-indol-3-yl)ethan-1-one (59)



59

2-(1H-Indol-3-yl)acetic acid (0.10 g, 0.57 mmol) was reacted with 6-piperazin-1-yl-pyridin-3-ol (**VIII**) (0.10 g, 0.57 mmol) as per the procedure described for compound **55** to afford compound **59** (0.15 g, 0.44 mmol, 77% yield) as a light brown solid.

Melting point: 184-187 °C. **LC purity (UV 245 nm):** 98.01%.

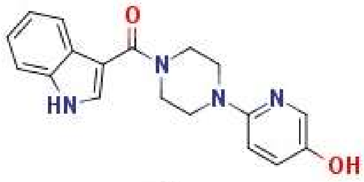
^{1}H NMR (400 MHz, DMSO-d$_6$): δ 3.20 (t, 2H, *J* = 4.94 *Hz*), 3.27 (t, 2H, *J* = 5.12 *Hz*), 3.62 (t, 2H, *J* = 5.05 *Hz*), 3.66 (t, 2H, *J* = 4.93 *Hz*), 3.86 (s, 2H), 6.76 (d, 1H, *J* = 8.90 *Hz*), 7.00 – 7.03 (m, 1H), 7.09 – 7.13 (m, 2H), 7.27 (d, 1H, *J* = 2.27 *Hz*), 7.38 (d, 1H, *J* = 8.08 *Hz*), 7.62 (d, 1H, *J* = 7.90 *Hz*), 7.77 (d, 1H, *J* = 2.84 *Hz*), 9.08 (s, 1H), 10.95 (s, 1H).

^{13}C NMR (100 MHz, DMSO-d$_6$): δ 31.2 (CH$_2$), 41.2 (CH$_2$), 45.6 (CH$_2$), 46.4 (CH$_2$), 46.6 (CH$_2$), 108.5 (C), 109.0 (CH), 111.7 (CH), 118.7 (CH), 119.1 (CH), 121.4 (CH), 123.8 (C), 125.7 (CH), 127.5 (CH), 134.4 (CH), 136.5 (C), 147.0 (C), 153.5 (C), 169.6 (C).

IR (ATR) / cm^{-1}: 3318, 3113, 2833, 1606, 1487, 1433, 1341, 1238, 1160, 960, 794, 763, 698, 678, 594.

LCMS (ESI$^+$): calculated for $C_{19}H_{20}N_4O_2$ $[M+H]^+$ 337.1664; found: *m/z* = 337.0322.

6) Synthesis of 6-[4-(1H-indole-3-carbonyl)piperazin-1-yl]pyridin-3-ol (60)



60

1H-Indole-3-carboxylic acid (0.15 g, 0.93 mmol) was reacted with 6-piperazin-1-yl-pyridin-3-ol (**VIII**) (0.16 g, 0.93 mmol) as per the procedure described for compound **55** to afford compound **60** (0.10 g, 0.31 mmol, 33% yield) as a light brown solid.

Melting point: 168-171 °C. **LC purity (UV 245 nm):** 98.18%.

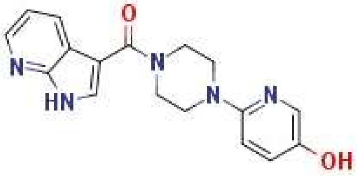
^{1}H NMR (400 MHz, DMSO-d$_6$): δ 3.42 (t, 4H, *J* = 4.84 *Hz*), 3.78 (t, 4H, *J* = 4.89 *Hz*), 6.81 (d, 1H, *J* = 8.97 *Hz*), 7.13 - 7.17 (m, 2H), 7.21 (t, 1H, *J* = 7.50 *Hz*), 7.49 (d, 1H, *J* = 8.06 *Hz*), 7.75 (d, 1H, *J* = 7.89 *Hz*), 7.80 (dd, 2H, J_1 = 14.37 *Hz*, J_2 = 2.66 *Hz*), 9.10 (s, 1H), 11.66 (s, 1H).

^{13}C NMR (100 MHz, DMSO-d$_6$): δ 44.8 (2 x CH$_2$), 46.8 (2 x CH$_2$), 109.0 (C), 110.1 (CH), 112.3 (CH), 120.5 (CH), 120.6 (CH), 122.2 (CH), 125.8 (CH), 126.3 (C), 128.4 (CH), 134.5 (CH), 136.0 (C), 147.1 (C), 153.7 (C), 166.0 (C).

IR (ATR) / cm^{-1}: 3189, 1608, 1559, 1537, 1508, 1457, 1445, 1433, 1225, 1203, 1186, 1005, 830, 756, 745, 735, 546.

LCMS (ESI$^+$): calculated for $C_{18}H_{18}N_4O_2$ [M+H]$^+$ 323.1508; found: *m/z* = 323.0135.

7) Synthesis of 6-(4-{1H-pyrrolo[2,3-b]pyridine-3-carbonyl}piperazin-1-yl)pyridin-3-ol (61)



61

7-Azaindole-3-carboxylic acid (0.20 g, 1.23 mmol) was reacted with 6-piperazin-1-yl-pyridin-3-ol (**VIII**) (0.22 g, 1.23 mmol) as per the procedure described for compound **55** to afford compound **61** (0.31 g, 0.95 mmol, 77% yield) as an off white solid.

Melting point: 175-178 °C. **LC purity (UV 245 nm):** 98.32%.

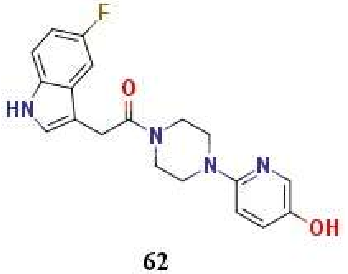
^{1}H NMR (400 MHz, DMSO-d$_6$): δ 3.43 (t, 4H, *J* = 4.83 *Hz*), 3.80 (t, 4H, *J* = 5.05 *Hz*), 6.81 (d, 1H, *J* = 8.99 *Hz*), 7.14 (dd, 1H, J_1 = 8.95 *Hz*, J_2 = 2.98 *Hz*), 7.22 (dd, 1H, J_1 = 7.91 *Hz*, J_2 = 4.64 *Hz*), 7.81 (d, 1H, *J* = 2.90 *Hz*), 7.93 (s, 1H), 8.16 (dd, 1H, J_1 = 7.91 *Hz*, J_2 = 1.48 *Hz*), 8.34 (dd, 1H, J_1 = 4.62 *Hz*, J_2 = 1.47 *Hz*), 9.11 (s, 1H), 12.25 (s, 1H).

^{13}C NMR (100 MHz, DMSO-d$_6$): δ 45.5 (2 x CH$_2$), 47.5 (2 x CH$_2$), 109.6 (CH), 109.8 (C), 117.9 (CH), 119.7 (C), 126.6 (CH), 129.7 (CH), 129.8 (CH), 135.4 (CH), 144.7 (CH), 147.9 (C), 149.2 (C), 154.4 (C), 165.9 (C).

IR (ATR) / cm^{-1}: 3098, 2826, 1607, 1497, 1442, 1418, 1292, 1260, 1233, 1194, 992, 814, 801, 776, 740, 634.

LCMS (ESI$^+$): calculated for $C_{17}H_{17}N_5O_2$ $[M+H]^+$ 324.1460; found: *m/z* = 323.9622.

8) Synthesis of 2-(5-fluoro-1H-indol-3-yl)-1-[4-(5-hydroxypyridin-2-yl)piperazin-1-yl] ethan-1-one (62)



62

5-Fluoroindole-3-acetic acid (0.15 g, 0.77 mmol) was reacted with 6-piperazin-1-yl-pyridin-3-ol (**VIII**) (0.14 g, 0.77 mmol) as per the procedure described for compound **55** to afford compound **62** (0.10 g, 0.28 mmol, 36% yield) as an off white solid.

Melting point: 202-205 °C. **LC purity (UV 245 nm):** 99.56%.

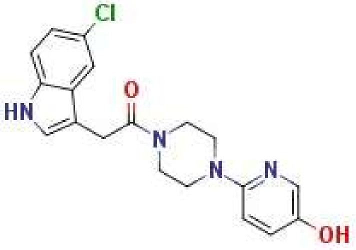
^{1}H NMR (400 MHz, DMSO-d$_6$): δ 3.22 (t, 2H, J = 4.6 *Hz*), 3.27 (t, 2H, J = 5.16 *Hz*), 3.62 – 3.66 (m, 4H), 3.84 (s, 2H), 6.76 (d, 1H, J = 9.0 *Hz*), 6.95 (td, 1H, J_1 = 15.8 *Hz*, J_2 = 4.6 *Hz*), 7.11 (dd, 1H, J_1 = 8.9 *Hz*, J_2 = 3 *Hz*), 7.37 (m, 3H), 7.77 (d, 1H, J = 2.8 *Hz*), 9.08 (s, 1H), 11.05 (s, 1H).

^{13}C NMR (100 MHz, DMSO-d$_6$): δ 31.0 (CH_2), 41.2 (CH_2), 45.6 (CH_2), 46.4 (CH_2), 46.6 (CH_2), 103.8 (CH, d, $J_{C\text{-}C\text{-}F}$ = 23.2 *Hz*), 108.8 (C, d, $J_{C\text{-}C\text{-}C\text{-}C\text{-}F}$ = 4.7 *Hz*), 109.0 (CH), 109.4 (CH), 109.6 (CH), 112.6 (CH, d, $J_{C\text{-}C\text{-}C\text{-}F}$ = 9.77 *Hz*), 125.9 (CH, d, $J_{C\text{-}C\text{-}F}$ = 26.3 Hz), 127.8 (C, d, $J_{C\text{-}C\text{-}C\text{-}F}$ = 10.05 *Hz*), 133.2 (C), 134.5 (CH), 147.1 (C), 153.5 (C), 157.0 (C, d, $J_{C\text{-}F}$ = 230.86 *Hz*), 169.4 (C).

IR (ATR) / cm^{-1}: 3406, 2901, 1614, 1573, 1492, 1442, 1408, 1355, 1308, 1282, 1274, 1230, 1198, 1158, 1018, 922, 818, 782, 703, 596.

LCMS (ESI$^+$): calculated for $C_{19}H_{19}FN_4O_2$ $[M+H]^+$ 355.1570; found: *m/z* = 355.0299.

9) Synthesis of 2-(5-chloro-1H-indol-3-yl)-1-[4-(5-hydroxypyridin-2-yl)piperazin-1-yl] ethan-1-one (63)



63

5-Chloroindole-3-acetic acid (0.10 g, 0.47 mmol) was reacted with 6-piperazin-1-yl-pyridin-3-ol (**VIII**) (0.085 g, 0.47 mmol) as per the procedure described for compound **55** to afford compound **63** (0.10 g, 0.27 mmol, 57% yield) as a light brown solid.

Melting point: 186-189 °C. **LC purity (UV 245 nm):** 94.95%.

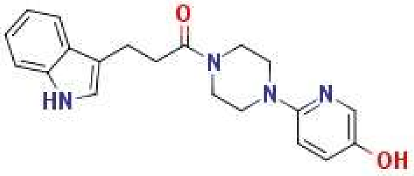
^{1}H NMR (400 MHz, DMSO-d$_6$): δ 3.23 - 3.28 (m, 4H), 3.62 - 3.67 (m, 4H), 3.86 (s, 2H), 6.78 (d, 1H, J = 8.96 *Hz*), 7.09 - 7.12 (m, 2H), 7.37 - 7.41 (m, 2H), 7.66 (d, 1H, J = 2.04 *Hz),* 7.77 (d, 1H, J = 2.76 *Hz),* 9.09 (s, 1H), 11.16 (s, 1H).

^{13}C NMR (100 MHz, DMSO-d$_6$): δ 32.7 (CH$_2$), 43.1 (CH$_2$), 47.4 (CH$_2$), 48.3 (CH$_2$), 48.5 (CH$_2$), 110.4 (CH), 110.9 (C), 115.1 (CH), 120.4 (CH), 123.2 (CH), 125.3 (C), 127.6 (2 x CH), 130.6 (C), 136.3 (CH), 136.8 (C), 148.9 (C), 155.4 (C), 171.2 (C).

IR (ATR) / cm^{-1}: 3149, 2861, 1615, 1494, 1444, 1353, 1312, 1282, 1233, 1199, 1162, 1033, 953, 888, 821, 783, 739, 707, 586.

LCMS (ESI$^+$): calculated for $C_{19}H_{19}ClN_4O_2$ $[M+H]^+$ 371.1274; found: *m/z* = 371.0545.

10) Synthesis of 1-[4-(5-hydroxypyridin-2-yl)piperazin-1-yl]-3-(1H-indol-3-yl)propan-1-one (64)



64

3-(1H-Indol-3-yl)propanoic acid (0.15 g, 0.79 mmol) was reacted with 6-piperazin-1-yl-pyridin-3-ol (**VIII**) (0.14 g, 0.79 mmol) as per the procedure described for compound **55** to afford compound **64** (0.12 g, 0.34 mmol, 43% yield) as an off white solid.

Melting point: 193-195 °C. **LC purity (UV 245 nm):** 97.89%.

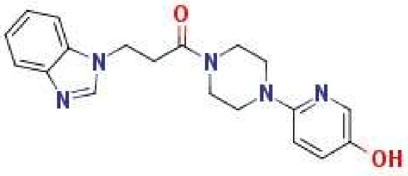
^{1}H NMR (400 MHz, DMSO-d$_6$): δ 2.76 (t, 2H, *J* = 7.74 *Hz*), 2.99 (t, 2H, *J* = 7.66 *Hz*), 3.26 – 3.29 (m, 4H), 3.55 (t, 2H, *J* = 4.88 *Hz*), 3.61 (t, 2H, *J* = 4.97 *Hz*), 6.77 (d, 1H, *J* = 9.00 *Hz*), 7.02 (t, 1H, *J* = 7.04 *Hz*), 7.09 – 7.13 (m, 2H), 7.20 (d, 1H, *J* = 2.06 *Hz*), 7.37 (d, 1H, *J* = 8.04 *Hz*), 7.57 (d, 1H, *J* = 7.81 *Hz*), 7.79 (d, 1H, *J* = 2.89 *Hz*), 9.10 (s, 1H), 10.84 (s, 1H).

^{13}C NMR (100 MHz, DMSO-d$_6$): δ 21.0 (CH$_2$), 33.6 (CH$_2$), 41.1 (CH$_2$), 45.0 (CH$_2$), 46.3 (CH$_2$), 46.6 (CH$_2$), 109.0 (CH), 111.7 (CH), 114.1 (C), 118.5 (CH), 118.6 (CH), 121.2 (CH), 122.8 (CH), 125.8 (CH), 127.4 (C), 134.5 (CH), 136.6 (C), 147.0 (C), 153.5 (C), 170.9 (C).

IR (ATR) / cm^{-1}: 3143, 2917, 1580, 1489, 1428, 1382, 1360, 1278, 1261, 1236, 1210, 1197, 1151, 815, 749, 713.

LCMS (ESI$^+$): calculated for $C_{20}H_{22}N_4O_2$ $[M+H]^+$ 351.1821; found: *m/z* = 351.0847.

11) Synthesis of 3-(1H-1,3-benzodiazol-1-yl)-1-[4-(5-hydroxypyridin-2-yl)piperazin-1-yl] propan-1-one (65)



65

3-(1H-1,3-Benzodiazol-1-yl)propanoic acid (0.20 g, 1.05 mmol) was reacted with 6-piperazin-1-yl-pyridin-3-ol (**VIII**) (0.18 g, 1.05 mmol) as per the procedure described for compound **55** to afford compound **65** (0.18 g, 0.51 mmol, 48% yield) as an off white solid.

Melting point: 212-215 °C. **LC purity (UV 245 nm):** 95.59%.

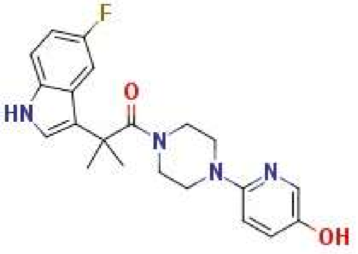
^{1}H NMR (400 MHz, DMSO-d$_6$): δ 3.02 (t, 2H, *J* = 6.78 *Hz*), 3.22 – 3.27 (m, 4H), 3.47 – 3.58 (m, 4H), 4.54 (t, 2H, *J* = 6.78 *Hz*), 6.76 (d, 1H, *J* = 9.01 *Hz*), 7.12 (dd, 1H, J_1 = 8.96 *Hz*, J_2 = 3.00 *Hz*), 7.24 (t, 1H, *J* = 7.02 *Hz*), 7.30 (t, 1H, *J* = 7.08 *Hz*), 7.68 (dd, 2H, J_1 = 7.88 *Hz*, J_2 = 2.92 *Hz*), 7.78 (d, 1H, *J* = 2.84 *Hz*), 8.27 (s, 1H), 9.08 (s, 1H).

^{13}C NMR (100 MHz, DMSO-d$_6$): δ 33.0 (CH$_2$), 40.9 (CH$_2$), 41.2 (CH$_2$), 44.8 (CH$_2$), 46.3 (CH$_2$), 46.6 (CH$_2$), 109.1 (CH), 111.0 (CH), 119.8 (CH), 121.9 (CH), 122.7 (CH), 125.9 (CH), 134.2 (C), 134.6 (CH), 143.8 (C), 144.8 (CH), 147.2 (C), 153.6 (C), 170.9 (C).

IR (ATR) / cm^{-1}: 3296, 1621, 1488, 1436, 1269, 1233, 1163, 1029, 925, 829, 745, 671, 625.

LCMS (ESI$^+$): calculated for $C_{19}H_{21}N_5O_2$ [M+H]$^+$ 352.1773; found: *m/z* = 352.0700.

12) Synthesis of 2-(5-fluoro-1H-indol-3-yl)-1-[4-(5-hydroxypyridin-2-yl)piperazin-1-yl]-2-methylpropan-1-one (66)



66

2-(5-Fluoro-1H-indol-3-yl)-2-methylpropanoic acid (0.35 g, 1.58 mmol) was reacted with 6-piperazin-1-yl-pyridin-3-ol (**VIII**) (0.283 g, 1.58 mmol) as per the procedure described for compound **55** to afford compound **66** (0.30 g, 0.78 mmol, 49% yield) as an off white solid.

Melting point: 217-220 °C. **LC purity (UV 245 nm):** 99.68%.

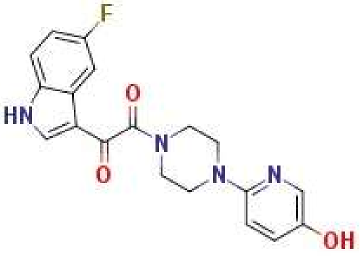
^{1}H NMR (400 MHz, DMSO-d$_6$): δ 1.59 (s, 6H), 2.60 (m, 4H), 3.42 (m, 4H), 6.59 (d, 1H, *J* = 8.98 *Hz*), 6.92 (t, 1H, *J* = 9.06 *Hz*), 6.98 – 7.03 (m, 2H), 7.38 – 7.41 (m, 2H), 7.68 (d, 1H, *J* = 2.8 *Hz*), 9.01 (s, 1H), 11.16 (s, 1H).

^{13}C NMR (100 MHz, DMSO-d$_6$): δ 28.8 (2 x CH_3), 40.0 (C), 42.2 (2 x CH_2), 46.9 (2 x CH_2), 104.3 (CH, d, $J_{C\text{-}C\text{-}F}$ = 23.55 *Hz*), 109.6 (CH), 110.4 (CH, d, $J_{C\text{-}C\text{-}F}$ = 25.98 *Hz*), 113.9 (CH, d, $J_{C\text{-}C\text{-}C\text{-}F}$ = 9.86 *Hz*), 121.6 (C), 123.4 (CH), 126.2 (C, d, $J_{C\text{-}C\text{-}C\text{-}F}$ = 9.79 *Hz*), 126.5 (CH), 134.5 (CH), 135.2 (C), 147.8 (C), 154.1 (C), 156.4 (C, d, $J_{C\text{-}F}$ = 224.70 *Hz*), 174.7 (C).

IR (ATR) / cm^{-1}: 3281, 2982, 1605, 1493, 1428, 1279, 1263, 1237, 1142, 1038, 1020, 933, 809.

LCMS (ESI$^+$): calculated for $C_{21}H_{23}FN_4O_2$ [M+H]$^+$ 383.1883; found: *m/z* = 383.0271.

13) Synthesis of 1-(5-fluoro-1H-indol-3-yl)-2-[4-(5-hydroxypyridin-2-yl)piperazin-1-yl] ethane-1,2-dione (67)



67

To a solution of 6-piperazin-1-yl-pyridin-3-ol (**VIII**) (0.397 g, 2.21 mmol) in N,N-dimethylacetamide (5 ml) was added triethylamine (0.61 ml, 4.42 mmol). The reaction mixture was cooled to 10 °C and was added 2-(5-fluoro-1H-indol-3-yl)-2-oxoacetyl chloride (0.5 g, 2.21 mmol). The resultant reaction mixture was stirred at room temperature for 4 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 was 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 **67** (0.7 g, 1.90 mmol, 85% yield) as a yellow solid.

Melting point: 245-248 °C. **LC purity (UV 245 nm):** 97.61%.

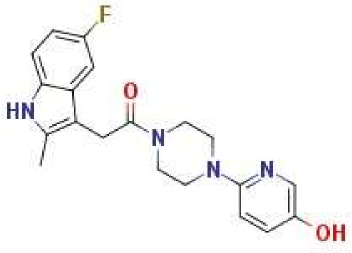
^{1}H NMR (400 MHz, DMSO-d$_6$): δ 3.32 – 3.34 (m, 2H), 3.49 – 3.51 (m, 4H), 3.76 – 3.77 (m, 2H), 6.80 (d, 1H, J = 8.98 *Hz*), 7.14 (dd, 1H, J_1 = 8.94 *Hz*, J_2 = 2.89 *Hz*), 7.19 – 7.23 (m, 1H), 7.59 – 7.62 (m, 1H), 7.80 (d, 1H, J = 2.79 *Hz*), 7.85 (d, 1H, J = 7.64 *Hz*), 8.32 (s, 1H), 9.11 (s, 1H), 12.46 (s, 1H).

^{13}C NMR (100 MHz, DMSO-d$_6$): δ 41.5 (CH$_2$), 46.4 (CH$_2$), 47.0 (CH$_2$), 47.6 (CH$_2$), 110.0 (CH), 107.1 (CH, d, $J_{C\text{-}C\text{-}F}$ = 24.75 *Hz*), 113.0 (CH, d, $J_{C\text{-}C\text{-}F}$ = 25.90 *Hz*), 114.4 (C), 115.3 (CH, d, $J_{C\text{-}C\text{-}C\text{-}F}$ = 9.86 *Hz*), 126.6 (CH), 126.8 (C), 134.7 (C), 135.3 (CH), 139.8 (CH), 148.0 (C), 151.1 (C), 160.0 (C, d, $J_{C\text{-}F}$ = 235.92 *Hz*), 166.8 (C), 187.2 (C).

IR (ATR) / cm^{-1}: 3180, 1607, 1522, 1488, 1427, 1264, 1234, 1179, 1025, 935, 803, 771, 728, 665, 623.

LCMS (ESI$^+$): calculated for $C_{19}H_{17}FN_4O_3$ [M+H]$^+$ 369.1362; found: *m/z* = 369.0342.

14) Synthesis of 2-(5-fluoro-2-methyl-1H-indol-3-yl)-1-[4-(5-hydroxypyridin-2-yl) piperazin-1-yl]ethan-1-one (68)



68

2-(5-Fluoro-2-methyl-1H-indol-3-yl)acetic acid (0.15 g, 0.72 mmol) was reacted with 6-piperazin-1-yl-pyridin-3-ol (**VIII**) (0.129 g, 0.72 mmol) as per the procedure described for compound **55** to afford compound **68** (0.12 g, 0.32 mmol, 44% yield) as an off white solid.

Melting point: 218-221 °C. **LC purity (UV 245 nm):** 99.90%.

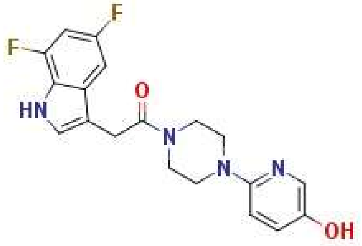
^{1}H NMR (400 MHz, DMSO-d$_6$): δ 2.38 (s, 3H), 3.16 (m, 2H), 3.25 (m, 2H), 3.60 (t, 4H, *J* = 5.01 *Hz*), 3.77 (s, 2H), 6.75 (d, 1H, *J* = 8.99 *Hz*), 6.82 - 6.86 (m, 1H), 7.10 (dd, 1H, J_1 = 8.96 *Hz*, J_2 = 2.96 *Hz*), 7.23 – 7.26 (m, 2H), 7.77 (d, 1H, *J* = 2.87 *Hz*), 9.09 (s, 1H), 10.97 (s, 1H).

^{13}C NMR (100 MHz, DMSO-d$_6$): δ 12.8 (CH_3), 30.8 (CH_2), 42.2 (CH_2), 46.2 (CH_2), 47.3 (2 x CH_2), 103.9 (CH, d, $J_{C\text{-}C\text{-}F}$ = 23.24 *Hz*), 105.7 (C, d, $J_{C\text{-}C\text{-}C\text{-}C\text{-}F}$ = 4.39 *Hz*), 108.9 (CH, d, $J_{C\text{-}C\text{-}F}$ = 25.73 *Hz*), 109.8 (CH), 112.2 (CH, d, $J_{C\text{-}C\text{-}C\text{-}F}$ = 9.66 *Hz*), 126.6 (CH), 129.8 (C, d, $J_{C\text{-}C\text{-}C\text{-}F}$ = 9.99 *Hz*), 132.9 (C), 135.3 (CH), 136.1 (C), 147.8 (C), 154.3 (C), 157.8 (C, d, $J_{C\text{-}F}$ = 230.09 *Hz*), 170.4 (C).

IR (ATR) / cm^{-1}: 3106, 1618, 1493, 1445, 1315, 1284, 1235, 1199, 1161, 1094, 1034, 919, 854, 823, 781, 585.

LCMS (ESI$^+$): calculated for $C_{20}H_{21}FN_4O_2$ $[M+H]^+$ 369.1726; found: *m/z* = 369.0964.

15) Synthesis of 2-(5,7-difluoro-1H-indol-3-yl)-1-[4-(5-hydroxypyridin-2-yl)piperazin-1-yl]ethan-1-one (69)



69

2-(5,7-Difluoro-1H-indol-3-yl)acetic acid (0.15 g, 0.71 mmol) was reacted with 6-piperazin-1-yl-pyridin-3-ol (**VIII**) (0.127 g, 0.71 mmol) as per the procedure described for compound **55** to afford compound **69** (0.21 g, 0.56 mmol, 78% yield) as a light brown solid.

Melting point: 212-215 °C. **LC purity (UV 245 nm):** 95.26%.

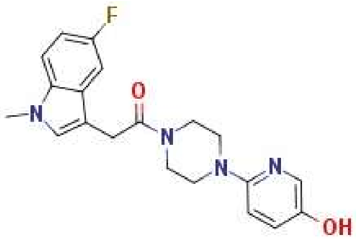
^{1}H NMR (400 MHz, DMSO-d$_6$): δ 3.24 – 3.28 (m, 4H), 3.61 (m, 2H), 3.67 (m, 2H), 3.85 (m, 2H), 6.77 (d, 1H, *J* = 8.98 Hz), 6.97 - 7.02 (m, 1H), 7.11 (dd, 1H, J_1 = 8.94 Hz, J_2 = 2.92 Hz), 7.26 (dd, 1H, J_1 = 9.58 Hz, J_2 = 1.90 Hz), 7.44 (d, 1H, *J* = 1.88 Hz), 7.78 (d, 1H, *J* = 2.84 Hz), 9.09 (s, 1H), 11.59 (s, 1H).

^{13}C NMR (100 MHz, DMSO-d$_6$): δ 31.5 (CH$_2$), 42.1 (CH$_2$), 46.3 (CH$_2$), 47.2 (CH$_2$), 47.5 (CH$_2$), 97.3 (CH, dd, $J_{1\ C\text{-}C\text{-}F}$ = 30.38 *Hz*, $J_{2\ C\text{-}C\text{-}F}$ = 20.64 *Hz*), 101.1 (CH, d, $J_{C\text{-}C\text{-}F}$ = 23.44 *Hz*), 109.9 (CH), 111.1 (C, d, $J_{C\text{-}C\text{-}C\text{-}C\text{-}F}$ = 2.81 *Hz*), 121.9 (C, d, $J_{C\text{-}C\text{-}F}$ = 13.13 *Hz*), 126.6 (CH), 127.8 (CH), 131.2 (C, dd, $J_{1\ C\text{-}C\text{-}C\text{-}F}$ = 11.43 *Hz*, $J_{2\ C\text{-}C\text{-}C\text{-}F}$ = 7.18 *Hz*), 135.3 (CH), 147.9 (C), 149.3 (C, dd, $J_{1\ C\text{-}F}$ = 245.89 *Hz*, $J_{2\ C\text{-}F}$ = 14.75 *Hz*), 154.3 (C), 156.6 (C, dd, $J_{1\ C\text{-}F}$ = 233.37 *Hz*, $J_{2\ C\text{-}F}$ = 9.95 *Hz*), 170.0 (C).

IR (ATR) / cm^{-1}: 3010, 1616, 1571, 1498, 1445, 1411, 1310, 1280, 1232, 1199, 1159, 1090, 1019, 951, 818, 797, 741.

LCMS (ESI$^+$): calculated for $C_{19}H_{18}F_2N_4O_2$ $[M+H]^+$ 373.1476; found: *m/z* = 373.0294.

16) Synthesis of 2-(5-fluoro-1-methyl-1H-indol-3-yl)-1-[4-(5-hydroxypyridin-2-yl) piperazin-1-yl]ethan-1-one (70)



70

2-(5-Fluoro-1-methyl-1H-indol-3-yl)acetic acid (0.20 g, 0.96 mmol) was reacted with 6-piperazin-1-yl-pyridin-3-ol (**VIII**) (0.172 g, 0.96 mmol) as per the procedure described for compound **55** to afford compound **70** (0.15 g, 0.40 mmol, 41% yield) as an off white solid.

Melting point: 205-208 °C. **LC purity (UV 245 nm):** 96.16%.

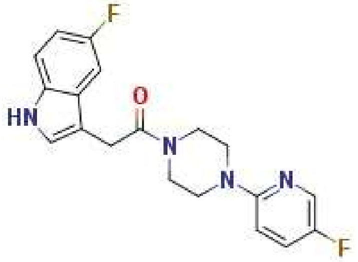
^{1}H NMR (400 MHz, DMSO-d$_6$): δ 3.27 – 3.31 (m, 4H), 3.61 – 3.66 (m, 4H), 3.83 (s, 3H), 3.96 (s, 2H), 6.78 (d, 1H, *J* = 8.99 Hz), 7.03 (td, 1H, J_1 = 15.79 Hz, J_2 = 4.59 Hz), 7.11 (dd, 1H, J_1 = 8.96 Hz, J_2 = 3.00 Hz), 7.34 (s, 1H), 7.39 (dd, 1H, J_1 = 10.08 Hz, J_2 = 2.48 Hz), 7.45 (dd, 1H, J_1 = 8.87 Hz, J_2 = 4.42 Hz), 7.78 (d, 1H, *J* = 2.87 Hz), 9.07 (s, 1H).

^{13}C NMR (100 MHz, DMSO-d$_6$): δ 31.4 (CH$_2$), 33.8 (CH$_3$), 42.0 (CH$_2$), 46.4 (CH$_2$), 47.1 (CH$_2$), 47.5 (CH$_2$), 104.9 (CH, d, $J_{C\text{-}C\text{-}F}$ = 23.41 *Hz*), 108.7 (C, d, $J_{C\text{-}C\text{-}C\text{-}C\text{-}F}$ = 4.79 *Hz*), 109.8 (CH), 110.3 (CH, d, $J_{C\text{-}C\text{-}F}$ = 26.14 *Hz*), 111.8 (CH, d, $J_{C\text{-}C\text{-}C\text{-}F}$ = 9.79 Hz), 126.6 (CH), 128.8 (C, d, $J_{C\text{-}C\text{-}C\text{-}F}$ = 10.08 *Hz*), 130.9 (CH), 134.5 (CH), 135.3 (C), 147.9 (C), 154.3 (C), 158.0 (C, d, $J_{C\text{-}F}$ = 231.30 *Hz*), 170.2 (C).

IR (ATR) / cm^{-1}: 3259, 2830, 1622, 1488, 1462, 1427, 1264, 1232, 1209, 1161, 1034, 904, 840, 810, 788, 687, 666, 597, 587.

LCMS (ESI$^+$): calculated for $C_{20}H_{21}FN_4O_2$ [M+H]$^+$ 369.1726; found: *m/z* = 369.0542.

17) Synthesis of 2-(5-fluoro-1H-indol-3-yl)-1-[4-(5-fluoropyridin-2-yl)piperazin-1-yl] ethan-1-one (71)



71

2-(5-Fluoro-1H-indol-3-yl)acetic acid (0.50 g, 2.58 mmol) was reacted with commercially available 1-(5-fluoropyridin-2-yl)piperazine (0.47 g, 2.58 mmol) in lieu of 6-piperazin-1-yl-pyridin-3-ol as per the procedure described for compound **55** to afford compound **71** (0.38 g, 1.06 mmol, 41% yield) as an off white solid.

Melting point: 126-128 °C. **LC purity (UV 245 nm):** 99.07%.

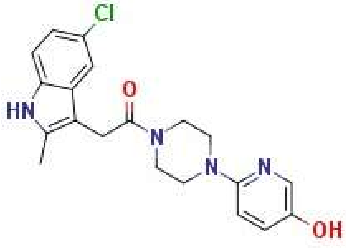
^{1}H NMR (400 MHz, DMSO-d$_6$): δ 3.38 (m, 2H), 3.43 (m, 2H), 3.63 (m, 2H), 3.68 (m, 2H), 3.85 (s, 2H), 6.90 – 6.98 (m, 2H), 7.37 – 7.39 (m, 3H), 7.56 (td, 1H, J_1 = 14.5 *Hz*, J_2 = 4.45 *Hz*), 8.14 (d, 1H, J = 2.85 *Hz*), 11.06 (s, 1H).

^{13}C NMR (100 MHz, DMSO-d$_6$): δ 31.7 (CH_2), 41.9 (CH_2), 46.2 (CH_2), 46.3 (CH_2), 46.6 (CH_2), 104.7 (CH, d, $J_{C\text{-}C\text{-}F}$ = 23.33 *Hz*), 109.5 (CH), 109.6 (C), 110.3 (CH, d, $J_{C\text{-}C\text{-}F}$ = 26.12 *Hz*), 113.4 (CH, d, $J_{C\text{-}C\text{-}C\text{-}F}$ = 9.81 Hz), 126.5 (CH, d, $J_{C\text{-}C\text{-}F}$ = 20.09 *Hz*), 126.8 (CH), 128.5 (C, d, $J_{C\text{-}C\text{-}C\text{-}F}$ = 10.06 *Hz*), 134.0 (C), 135.1 (CH, d, $J_{C\text{-}C\text{-}F}$ = 24.11 *Hz*), 154.3 (C, d, $J_{C\text{-}F}$ = 241.42 *Hz*), 157.1 (C), 157.8 (C, d, $J_{C\text{-}F}$ = 230.82 *Hz*), 170.3 (C).

IR (ATR) / cm^{-1}: 3202, 1621, 1489, 1473, 1454, 1436, 1401, 1224, 1187, 1158, 924, 807, 791, 654.

LCMS (ESI$^+$): calculated for $C_{19}H_{18}F_2N_4O$ [M+H]$^+$ 357.1526; found: *m/z* = 356.9905.

18) Synthesis of 2-(5-chloro-2-methyl-1H-indol-3-yl)-1-[4-(5-hydroxypyridin-2-yl) piperazin-1-yl]ethan-1-one (72)



72

2-(5-Chloro-2-methyl-1H-indol-3-yl)acetic acid (0.20 g, 0.89 mmol) was reacted with 6-piperazin-1-yl-pyridin-3-ol (**VIII**) (0.16 g, 0.89 mmol) as per the procedure described for compound **55** to afford compound **72** (0.28 g, 0.72 mmol, 80% yield) as a light brown solid.

Melting point: 270-273 °C. **LC purity (UV 245 nm):** 95.96%.

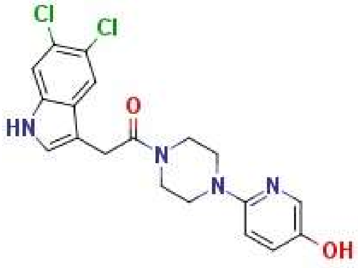
^{1}H NMR (400 MHz, DMSO-d$_6$): δ 2.38 (s, 3H), 3.19 (m, 2H), 3.26 (m, 2H), 3.61 (m, 4H), 3.79 (s, 2H), 6.77 (d, 1H, *J* = 9.01 *Hz*), 7.01 (dd, 1H, *J*$_1$ = 8.47 *Hz, J*$_2$ = 1.84 *Hz*), 7.10 (dd, 1H, *J*$_1$ = 8.93 *Hz, J*$_2$ = 2.92 *Hz*), 7.28 (d, 1H, *J* = 8.49 *Hz*), 7.52 (d, 1H, *J* = 1.51 *Hz*), 7.77 (d, 1H, *J* = 2.83 *Hz*), 9.07 (s, 1H), 11.07 (s, 1H).

^{13}C NMR (100 MHz, DMSO-d$_6$): δ 12.7 (CH_3), 30.6 (CH_2), 42.2 (CH_2), 46.2 (CH_2), 47.29 (CH_2), 47.30 (CH_2), 105.4 (C), 109.8 (CH), 112.9 (CH), 118.4 (CH), 120.9 (CH), 124.0 (C), 126.6 (CH), 130.7 (C), 134.7 (C), 135.3 (C), 135.9 (CH), 147.8 (C), 154.3 (C), 170.3 (C).

IR (ATR) / cm^{-1}: 3135, 2858, 1615, 1573, 1493, 1443, 1409, 1312, 1283, 1235, 1199, 1159, 1035, 894, 823, 788, 734, 555.

LCMS (ESI$^+$): calculated for $C_{20}H_{21}ClN_4O_2$ $[M+H]^+$ 385.1431; found: *m/z* = 385.0328.

19) Synthesis of 2-(5,6-dichloro-1H-indol-3-yl)-1-[4-(5-hydroxypyridin-2-yl)piperazin-1-yl]ethan-1-one (73)



73

2-(5,6-Dichloro-1H-indol-3-yl)acetic acid (0.20 g, 0.81 mmol) was reacted with 6-piperazin-1-yl-pyridin-3-ol (**VIII**) (0.146 g, 0.81 mmol) as per the procedure described for compound **55** to afford compound **73** (0.25 g, 0.61 mmol, 75% yield) as a light brown solid.

Melting point: 265-268 °C. **LC purity (UV 245 nm):** 98.13%.

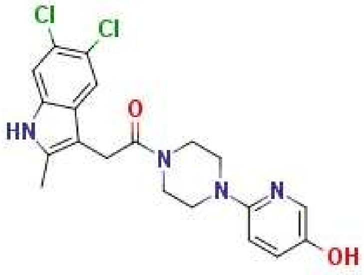
^{1}H NMR (400 MHz, DMSO-d$_6$): δ 3.25 - 3.28 (m, 4H), 3.61 - 3.67 (m, 4H), 3.87 (s, 2H), 6.78 (d, 1H, $J = 8.98$ *Hz*), 7.10 (dd, 1H, $J_1 = 8.93$ *Hz*, $J_2 = 2.88$ *Hz*), 7.42 (s, 1H), 7.64 (s, 1H), 7.78 (d, 1H, $J = 2.79$ *Hz*), 7.86 (s, 1H), 9.09 (s, 1H), 11.24 (s, 1H).

^{13}C NMR (100 MHz, DMSO-d$_6$): δ 31.3 (CH$_2$), 41.3 (CH$_2$), 46.3 (CH$_2$), 47.2 (CH$_2$), 47.4 (CH$_2$), 109.6 (CH), 109.9 (C), 114.0 (CH), 121.3 (CH), 122.1 (C), 124.4 (C), 126.6 (CH), 127.7 (CH), 128.5 (C), 135.3 (CH), 136.2 (C), 147.9 (C), 154.3 (C), 170.0 (C).

IR (ATR) / cm^{-1}: 3196, 2859, 1623, 1494, 1443, 1408, 1308, 1282, 1271, 1235, 1200, 1162, 1033, 951, 867, 812, 795, 655, 527.

LCMS (ESI$^+$): calculated for $C_{19}H_{18}Cl_2N_4O_2$ $[M+H]^+$ 405.0885; found: *m/z* = 404.9021.

20) Synthesis of 2-(5,6-dichloro-2-methyl-1H-indol-3-yl)-1-[4-(5-hydroxypyridin-2-yl) piperazin-1-yl]ethan-1-one (74)



74

2-(5,6-Dichloro-2-methyl-1H-indol-3-yl)acetic acid (0.25 g, 0.96 mmol) was reacted with 6-piperazin-1-yl-pyridin-3-ol (**VIII**) (0.17 g, 0.96 mmol) as per the procedure described for compound **55** to afford compound **74** (0.15 g, 0.36 mmol, 37% yield) as a light brown solid.

Melting point: 295-298 °C. **LC purity (UV 245 nm):** 94.90%.

^{1}H NMR (400 MHz, DMSO-d$_6$): δ 2.37 (s, 3H), 3.22 (m, 2H), 3.27 (m, 2H), 3.61 (m, 4H), 3.80 (s, 2H), 6.77 (d, 1H, $J = 8.98$ *Hz*), 7.11 (dd, 1H, $J_1 = 8.92$ *Hz*, $J_2 = 2.86$ *Hz*), 7.50 (s, 1H), 7.72 (s, 1H), 7.77 (d, 1H, $J = 2.75$ *Hz*), 9.08 (s, 1H), 11.21 (s, 1H).

^{13}C NMR (100 MHz, DMSO-d$_6$): δ 11.8 (CH_3), 29.4 (CH_2), 41.7 (CH_2), 45.2 (CH_2), 46.7 (CH_2), 47.0 (CH_2), 106.1 (C), 109.2 (CH), 111.2 (CH), 121.5 (C), 121.6 (C), 121.8 (CH), 125.9 (C), 126.8 (CH), 134.7 (C), 135.3 (CH), 137.2 (C), 147.3 (C), 153.8 (C), 169.9 (C).

IR (ATR) / cm-1: 3205, 2926, 1616, 1573, 1489, 1438, 1424, 1313, 1261, 1233, 1160, 1021, 824, 812, 706.

LCMS (ESI$^+$): calculated for $C_{20}H_{20}Cl_2N_4O_2$ $[M+H]^+$ 419.1041; found: *m/z* = 418.8765.

4.6 Biological evaluation of the prepared compounds

4.6.1 ME2 and ME1 inhibition data for selected compounds

Table 4.6. ME2 and ME1 inhibition data for synthesized compounds **55-74**.

Compound	% inhibition of ME2			ME2 IC$_{50}$ (µM)	% inhibition of ME1			ME1 IC$_{50}$ (µM)
	0.1 µM	1 µM	10 µM		0.1 µM	1 µM	10 µM	
31	0	31	73	1.72	4	24	97	1.50
55	4	46	98	-	0	21	95	-
56	8	51	97	-	0	12	95	-
57	0	9	68	-	0	20	93	-
58	10	35	98	-	0	14	80	-
59	28	40	83	1.39	4	13	86	1.84
60	9	23	66	-	6	16	98	-
61	4	14	78	-	1	7	83	-
62	0	47	99	1.38	0	21	97	1.52
63	0	49	100	-	0	33	98	-
64	17	36	80	1.27	10	22	87	1.79
65	22	19	96	-	3	16	84	-
66	12	48	99	-	0	32	98	-
67	18	54	100	-	0	16	99	-
68	10	25	100	-	1	31	98	-
69	12	14	100	-	0	18	99	-
70	0	17	98	-	0	12	98	-
71	0	0	15	-	0	0	13	-
72	2	49	99	-	1	21	91	-

Compound	**% inhibition of ME2**			**ME2 IC_{50} (µM)**	**% inhibition of ME1**			**ME1 IC_{50} (µM)**
	0.1 µM	**1 µM**	**10 µM**		**0.1 µM**	**1 µM**	**10 µM**	
73	18	62	99	-	0	44	91	-
74	1	45	95	-	0	24	90	-

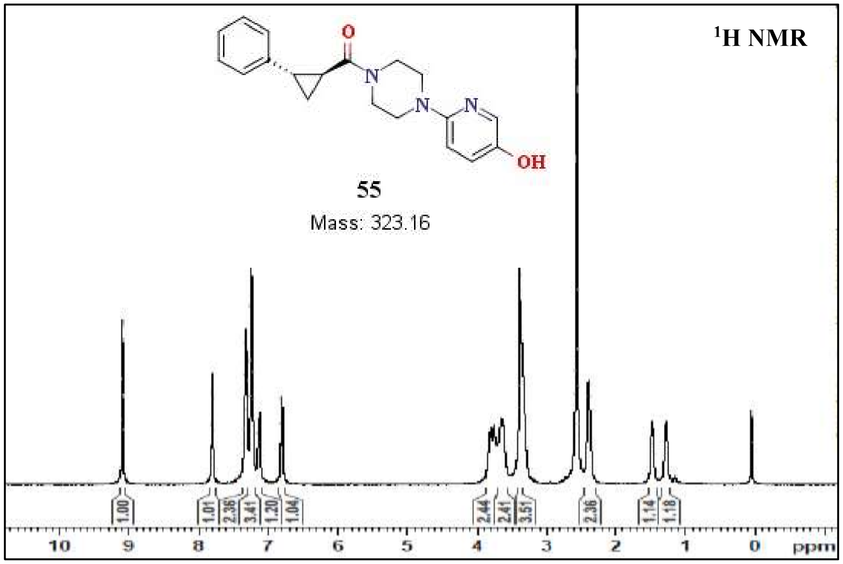
in vitro screening data of compounds **55**-**74** suggested that they have certain degree of selectivity towards ME3 over other ME isoforms.

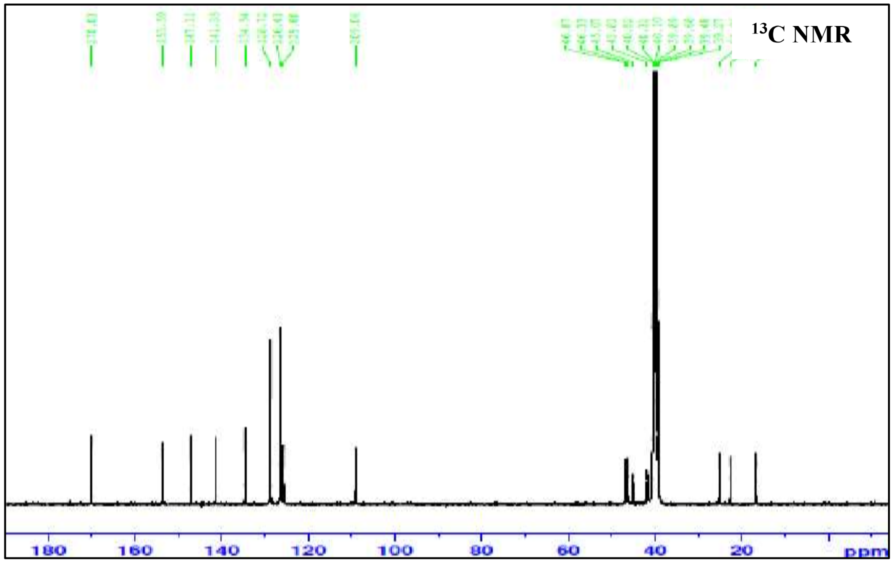
4.7 Conclusion

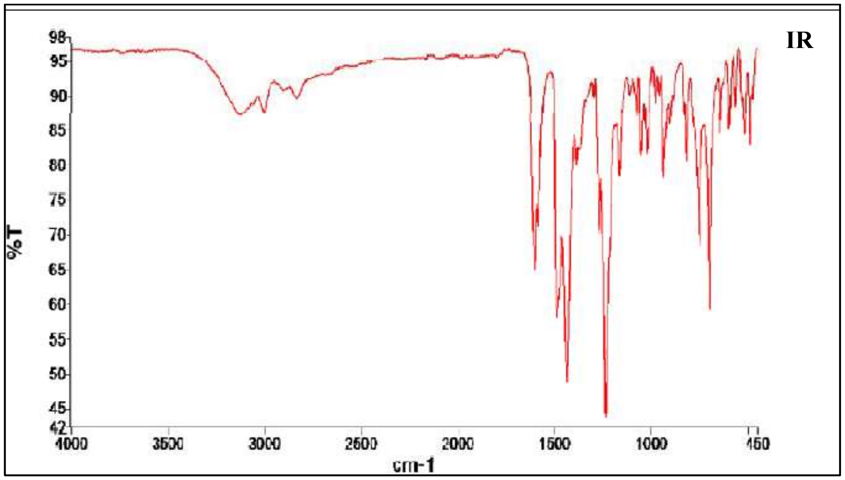
Optimization of compound **31** led to a new series of compounds containing indole-piperazine carboxamides. Among them compound **62** exhibited very good inhibitory potency in ME3 (IC_{50} = 0.21 µM) and retained a marginal 6-fold and 7-fold selectivity over ME2 and ME1 respectively. Compound **62** showed superior growth inhibitory effects on BxPC-3 as well as on Hs766T PDAC cell lines compared to compound **31**. When combined with the MEK inhibitor trametinib, compound **62** showed an encouraging synergistic effect on Hs766T cell growth inhibition. This combination could be more effective at killing the cancer cells than either of the drugs alone.

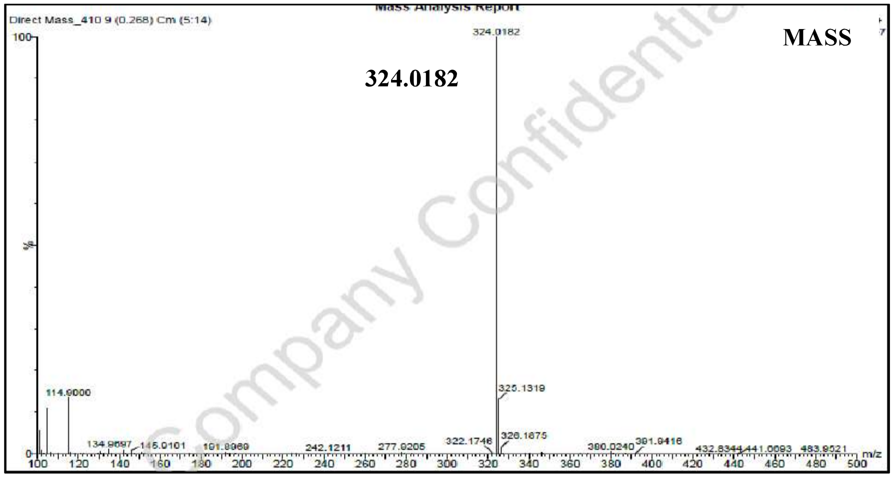
4.8 Spectral data

Spectral data of **55**









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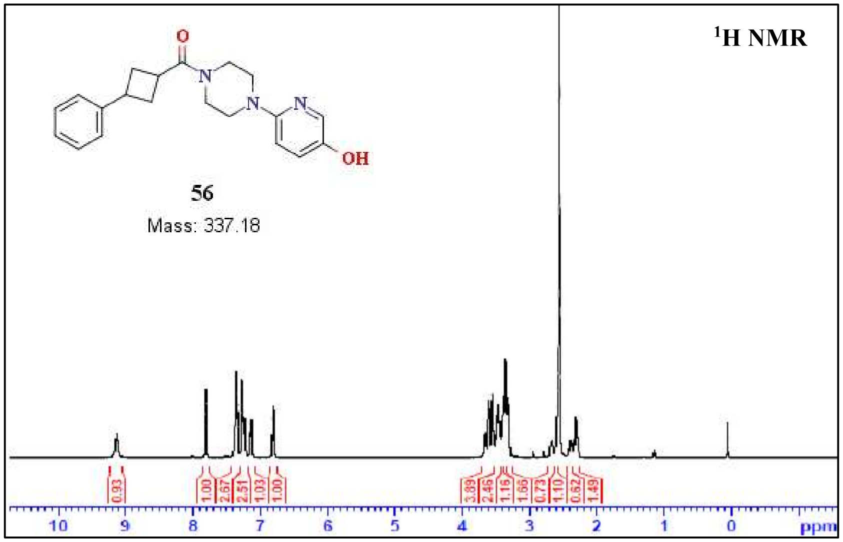

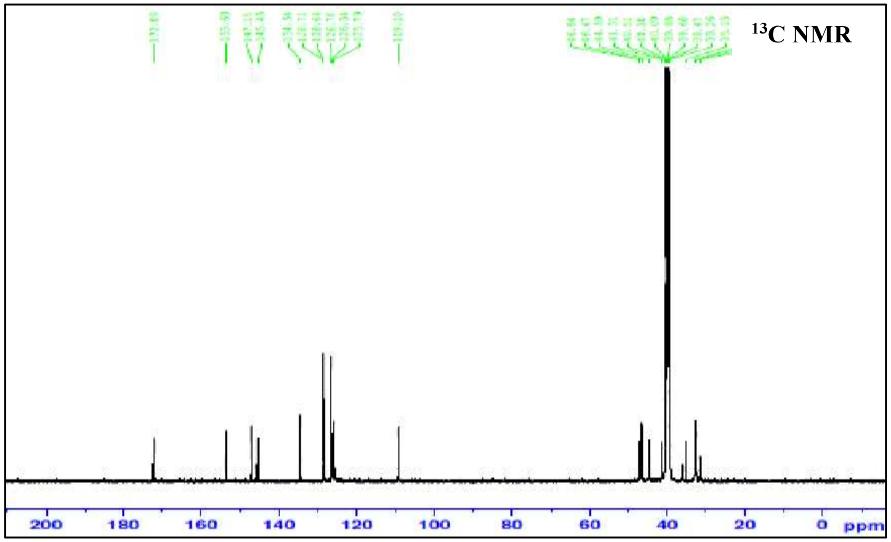

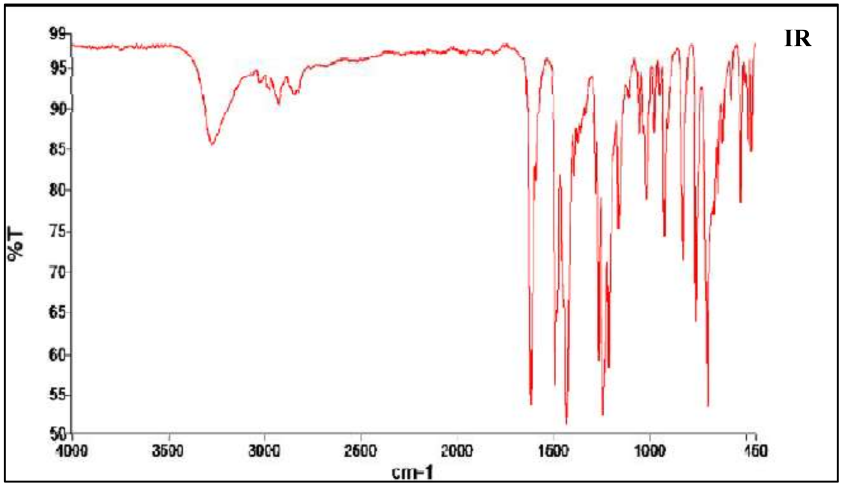

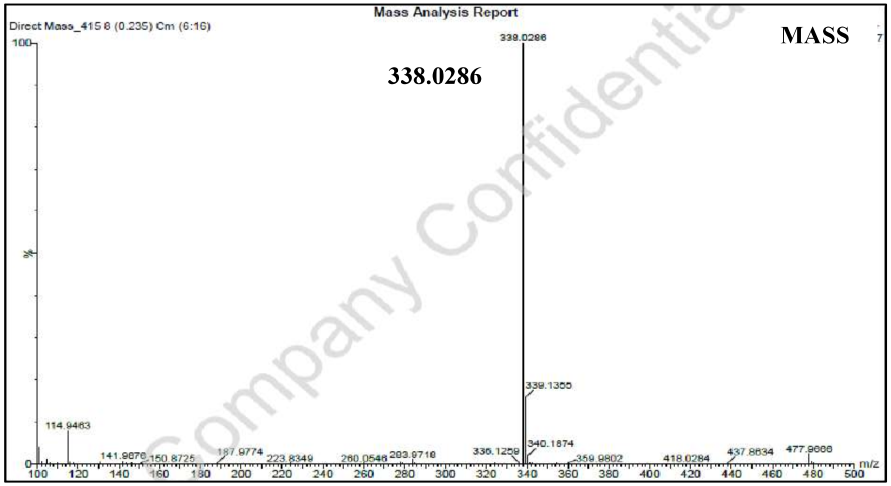

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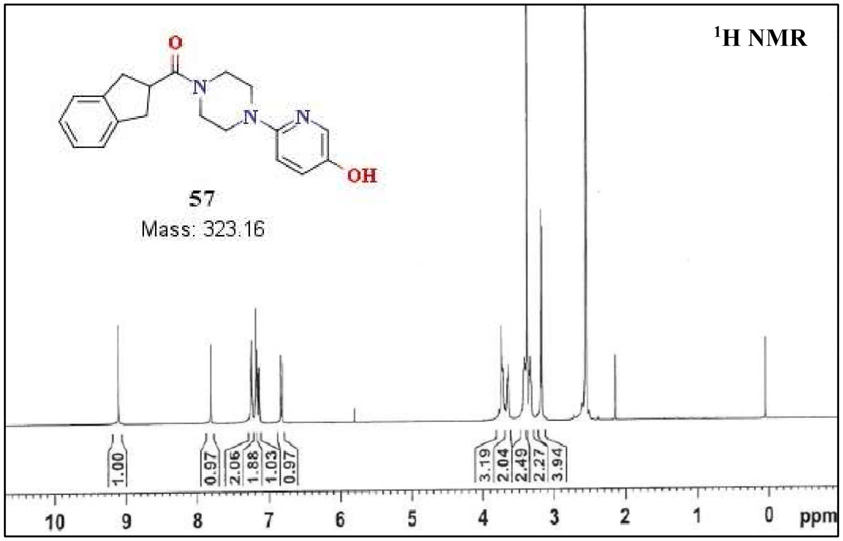

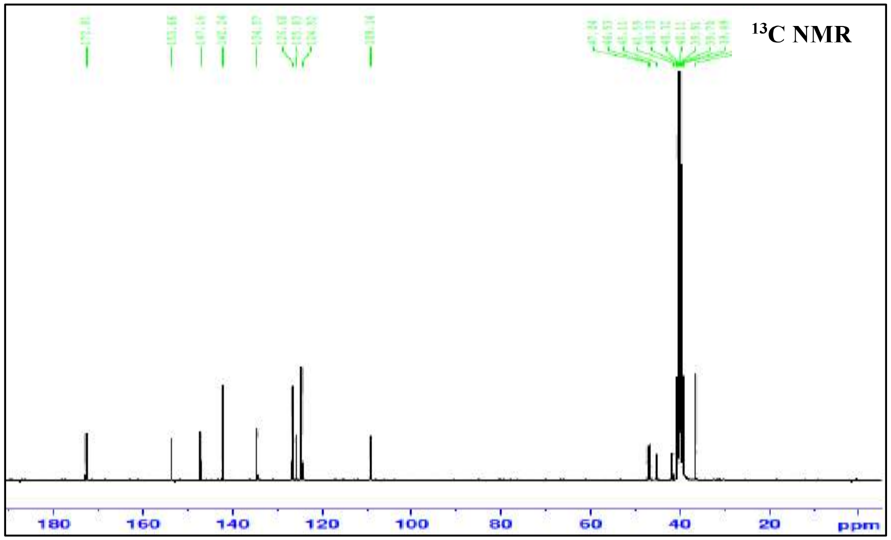

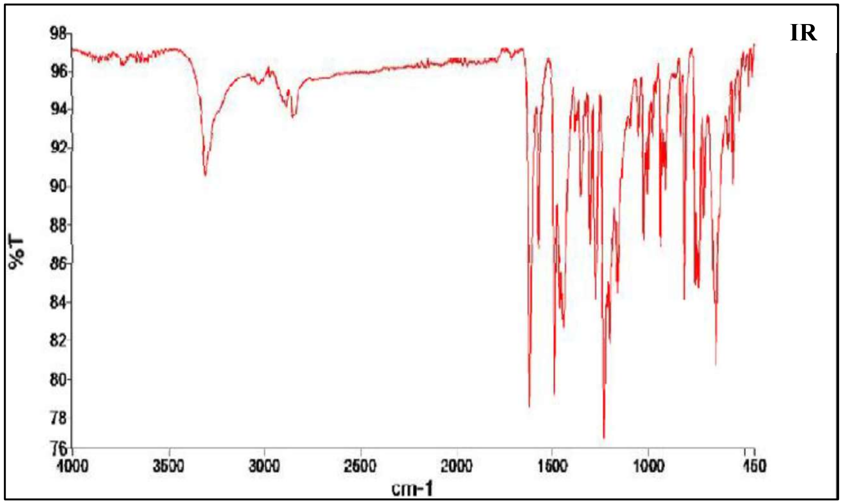

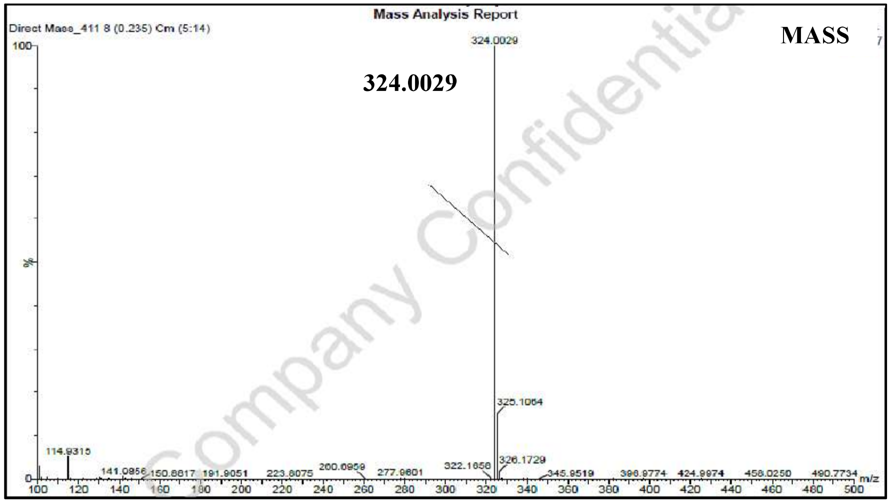

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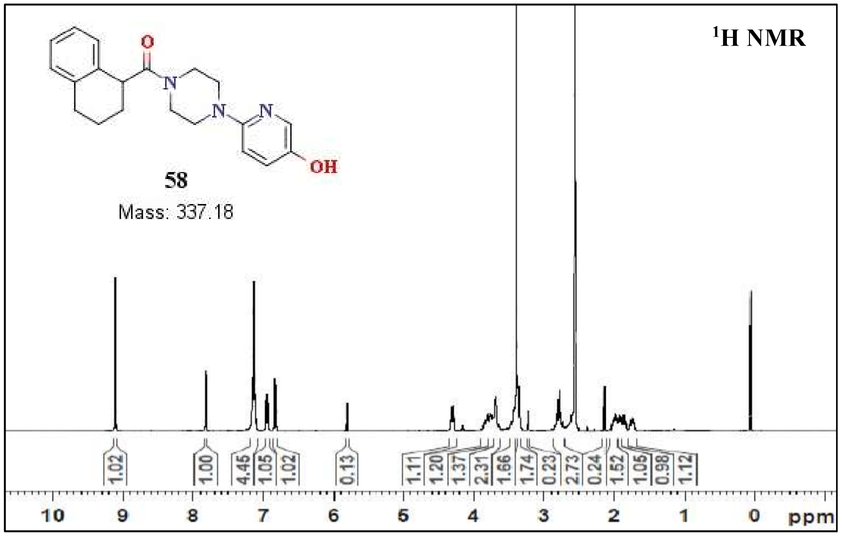

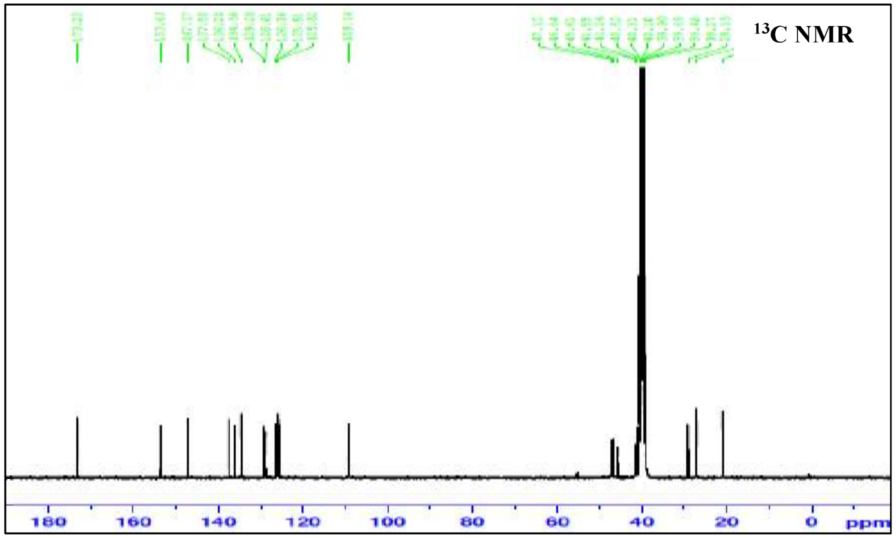

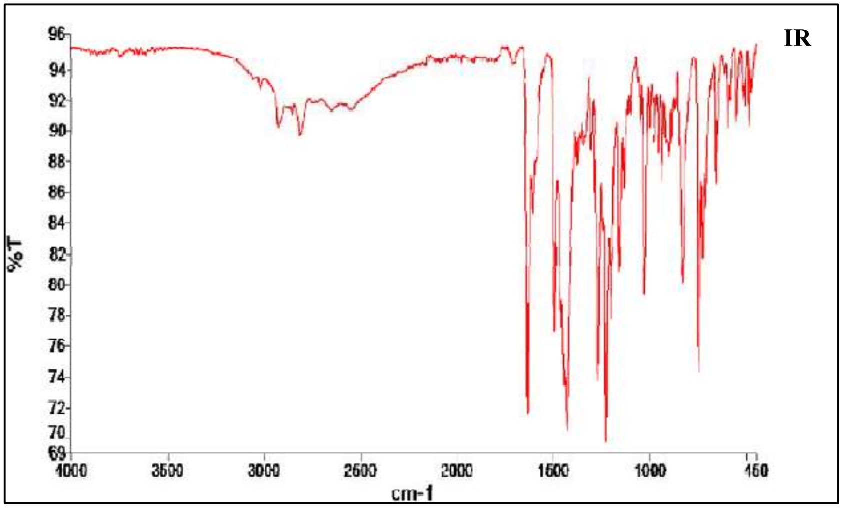

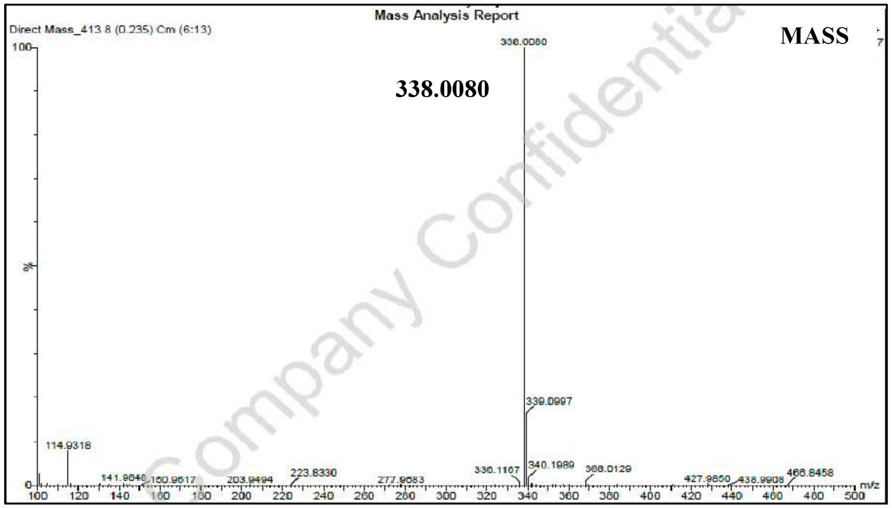

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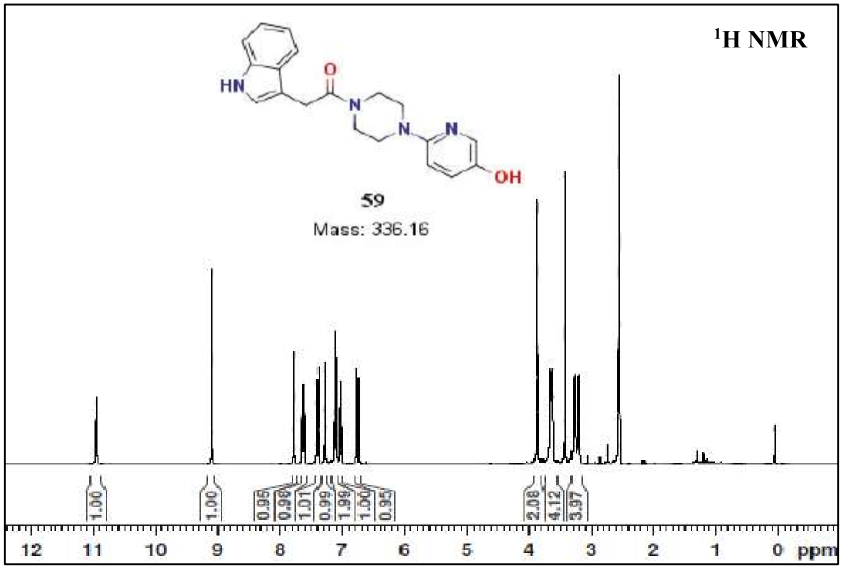

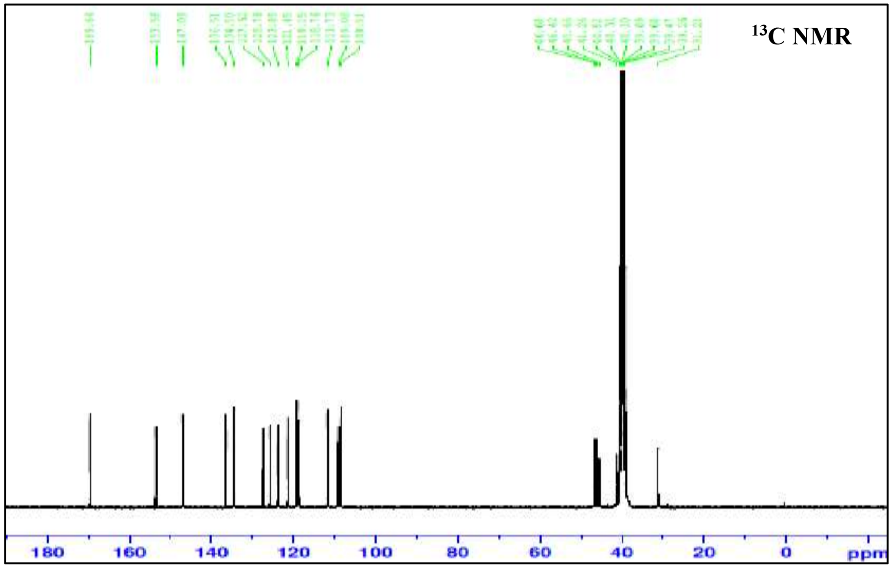

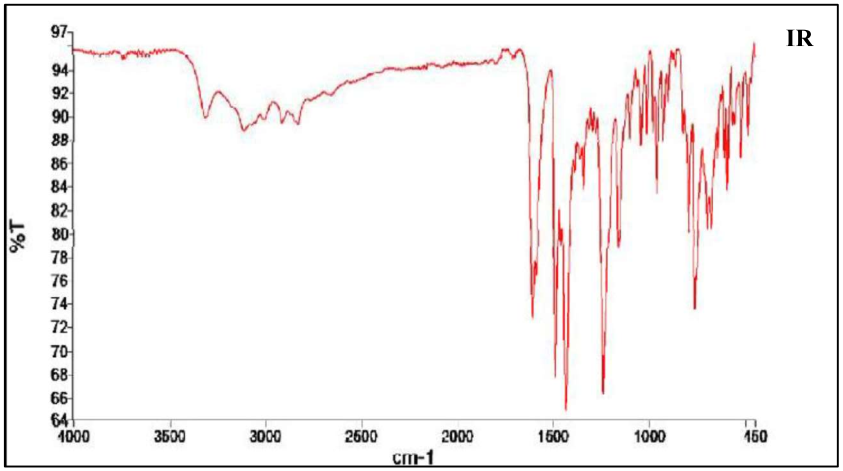

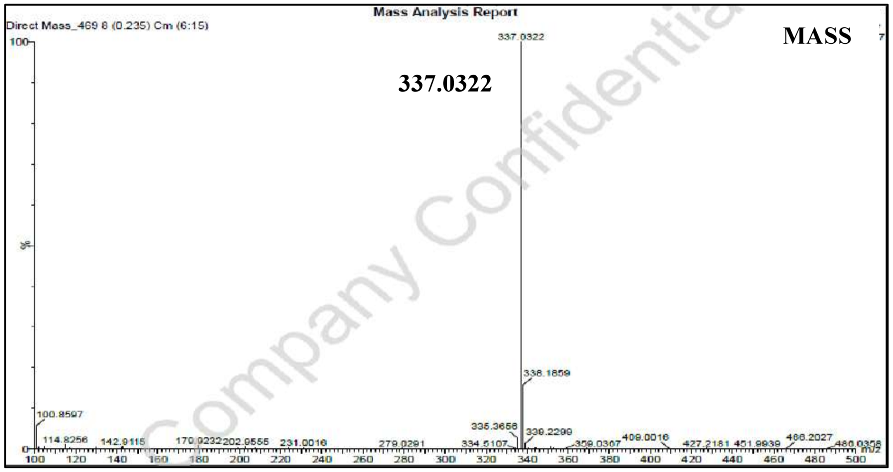

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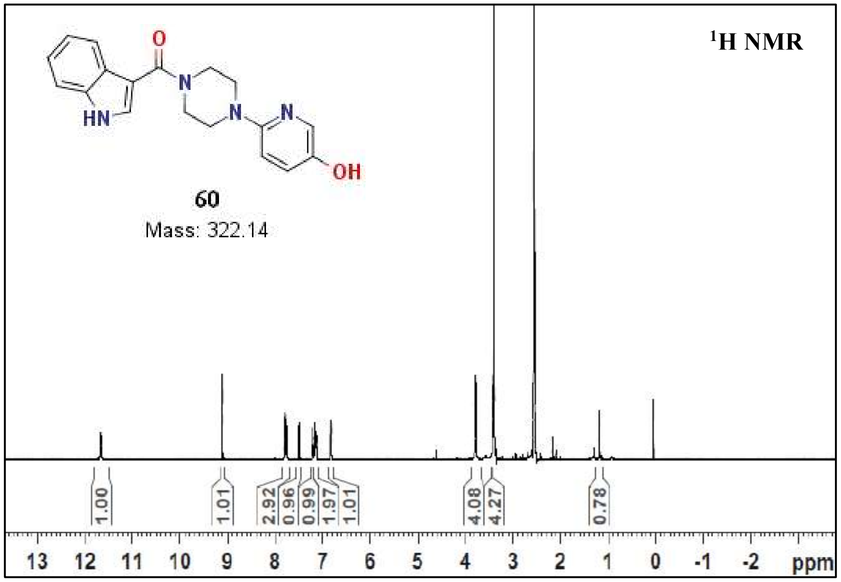

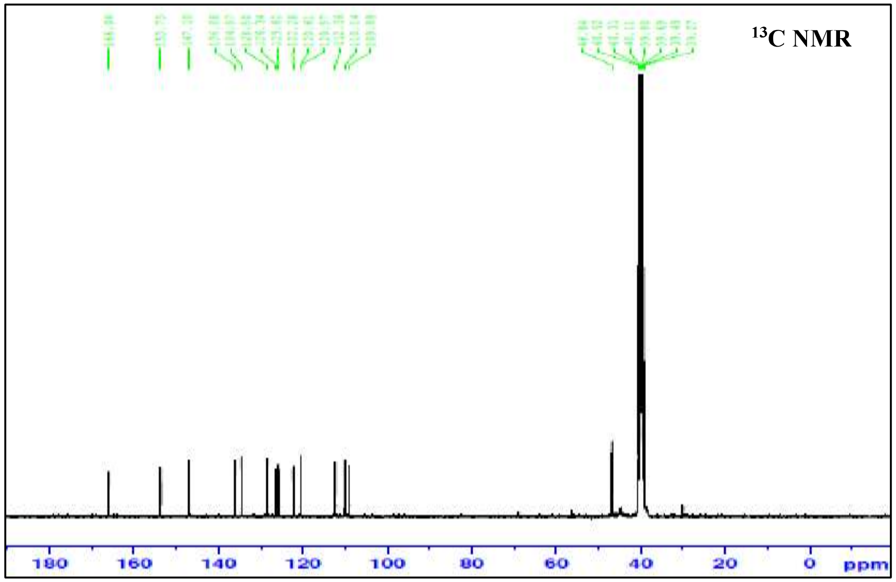

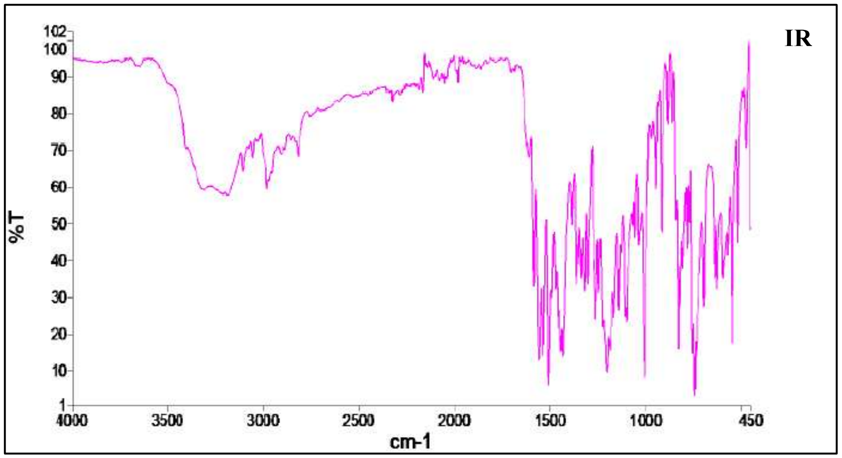

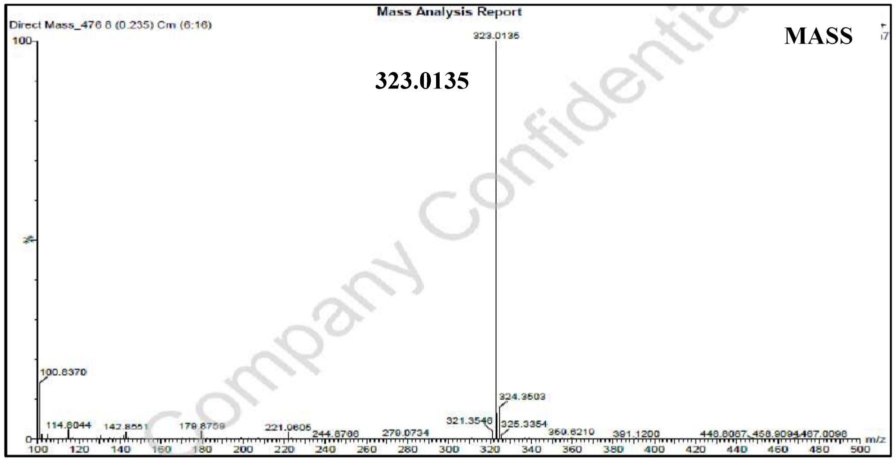

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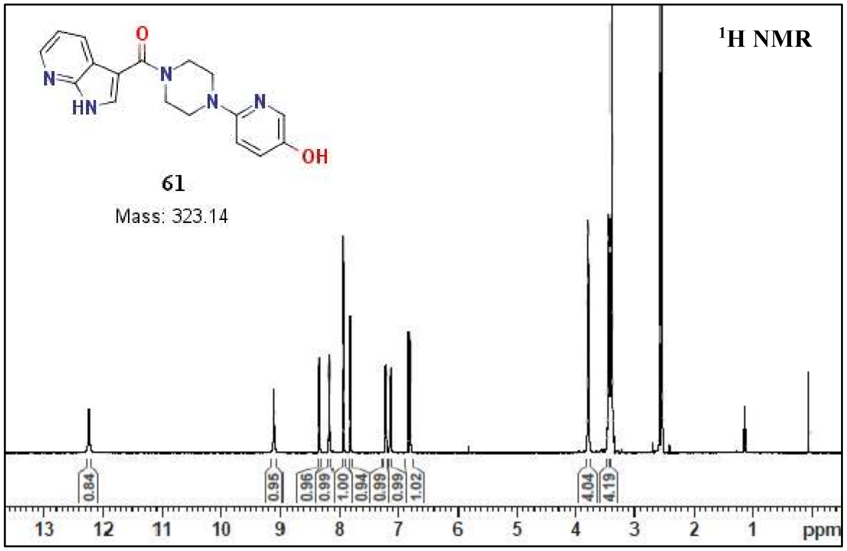

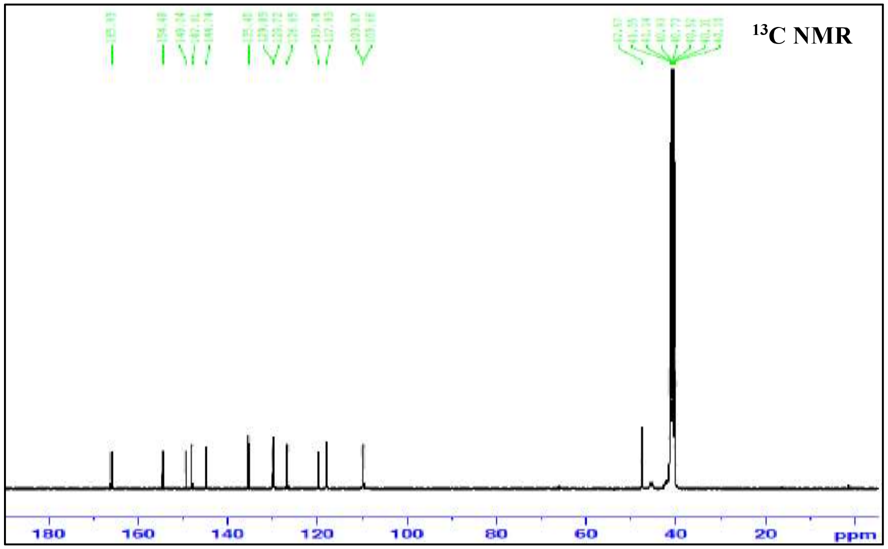

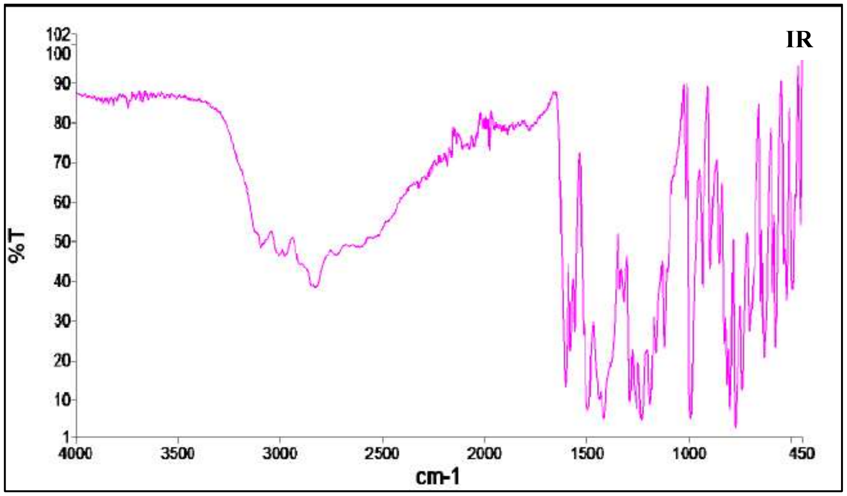

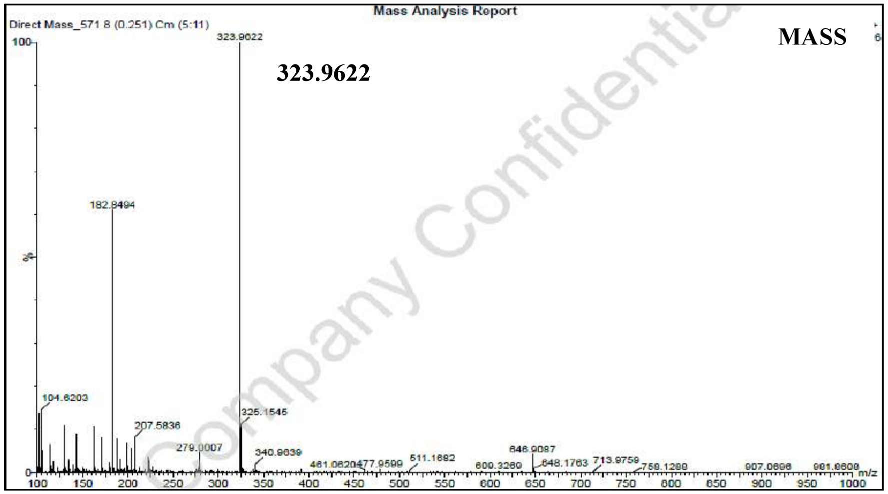

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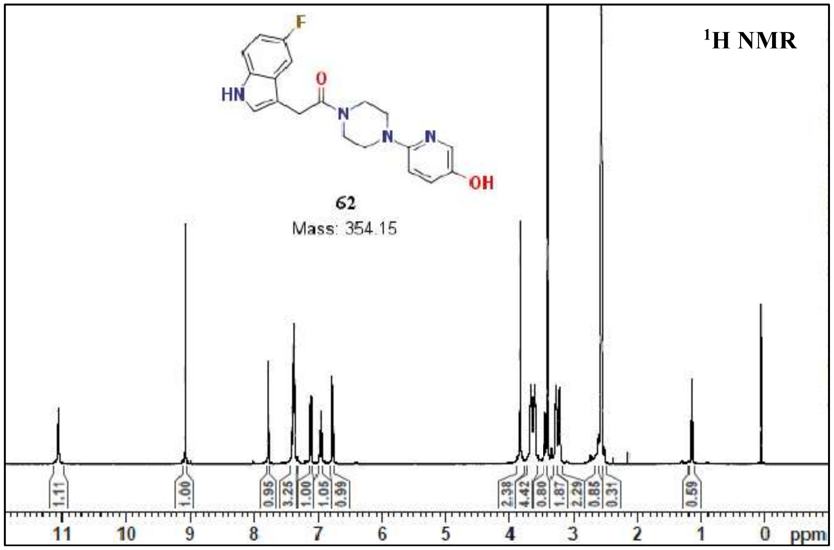

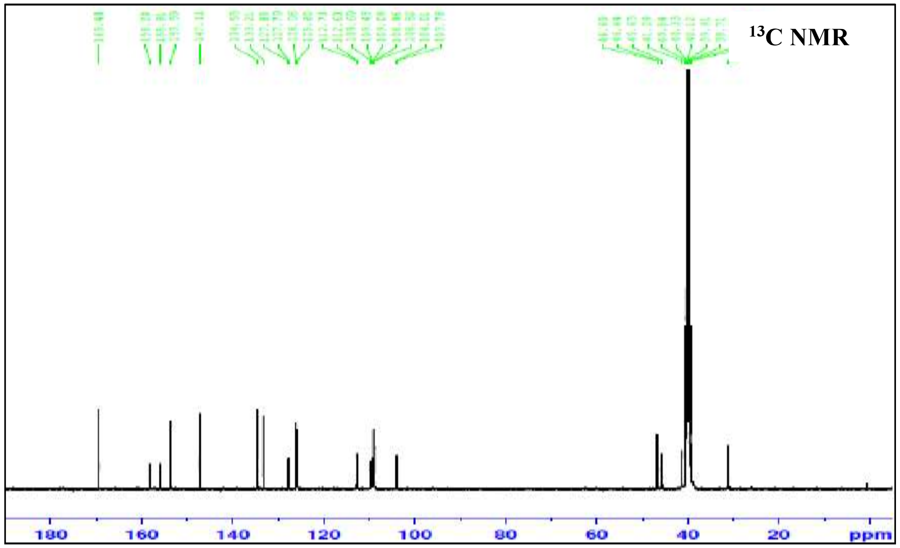

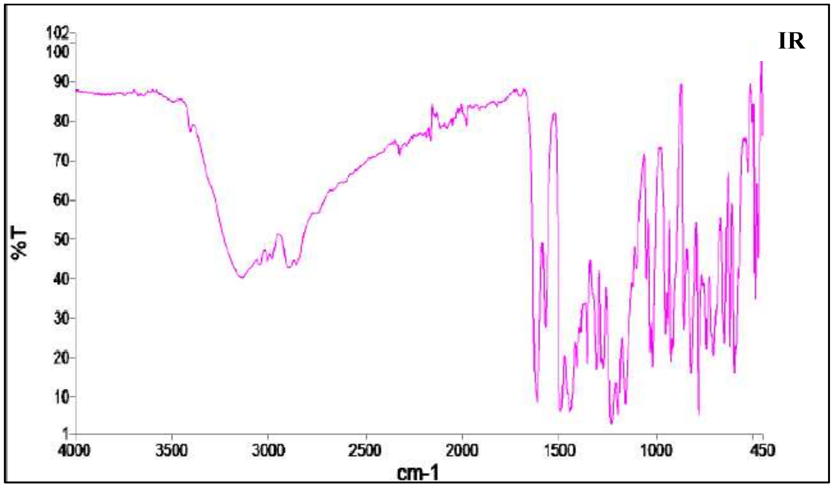

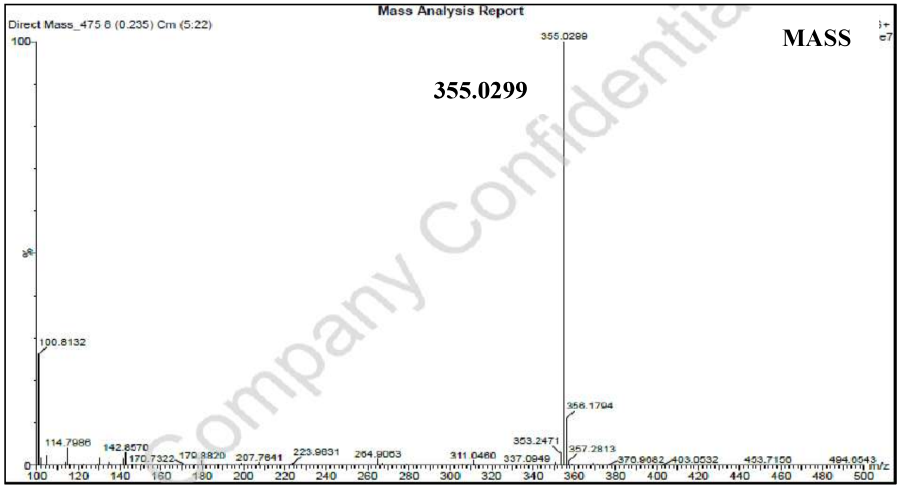

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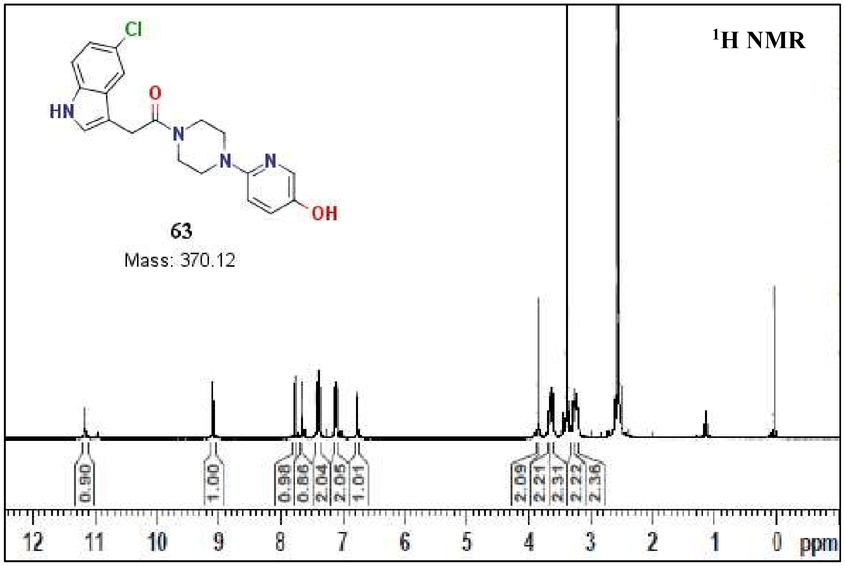

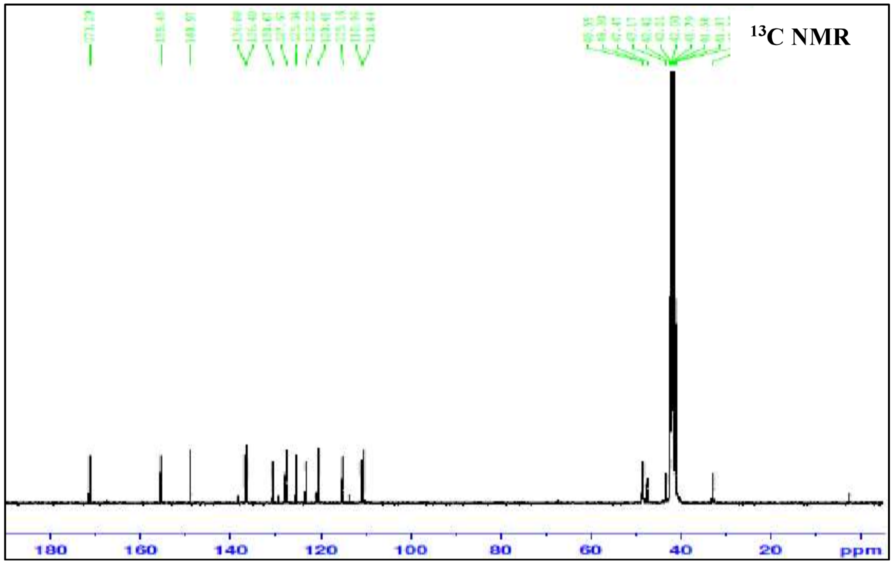

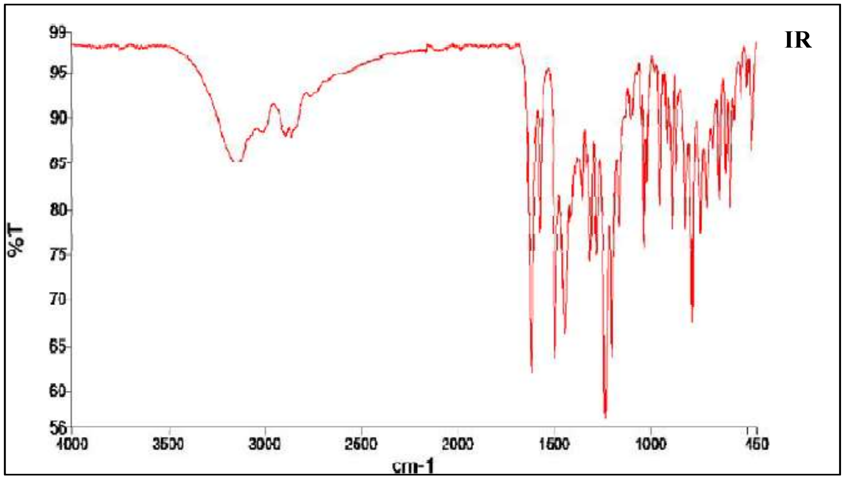

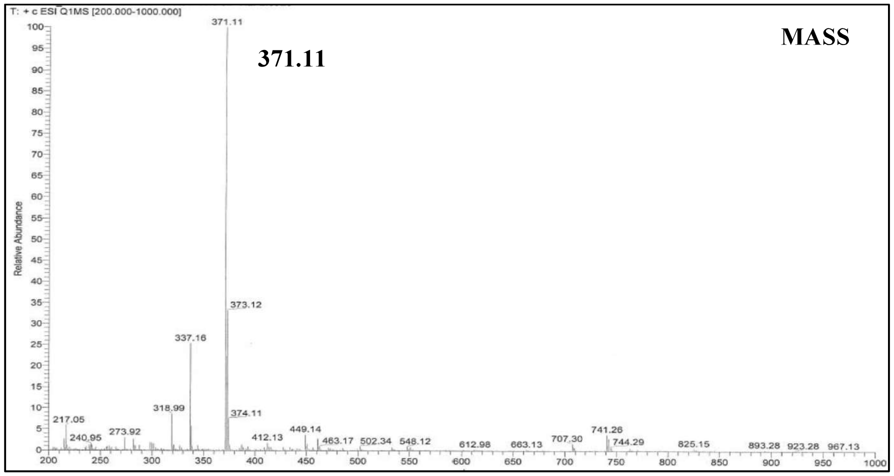

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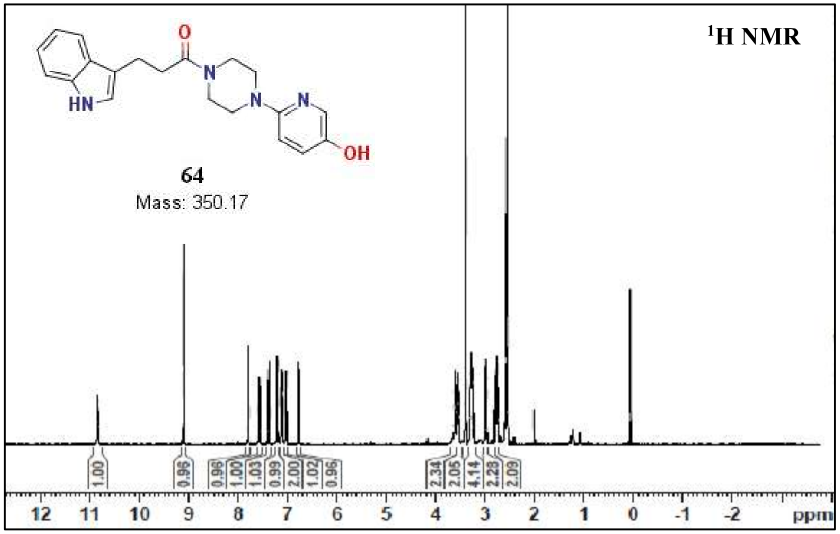

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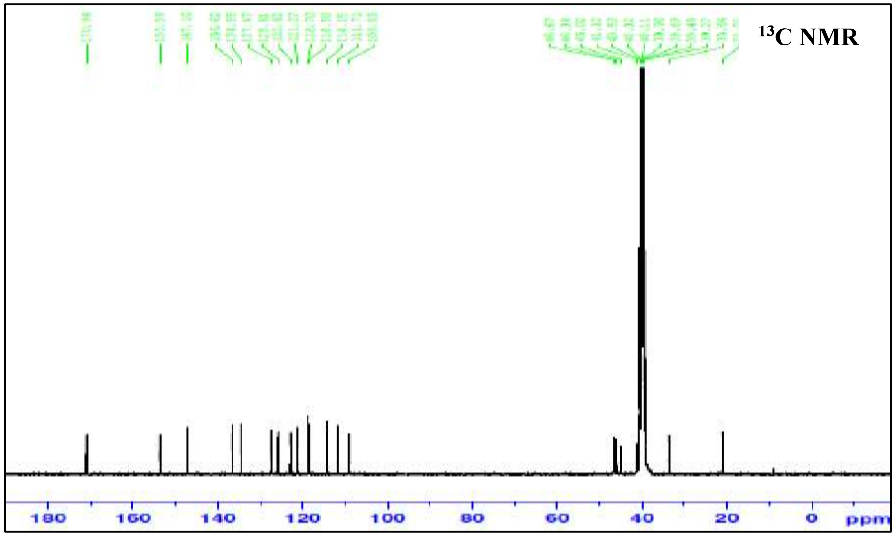

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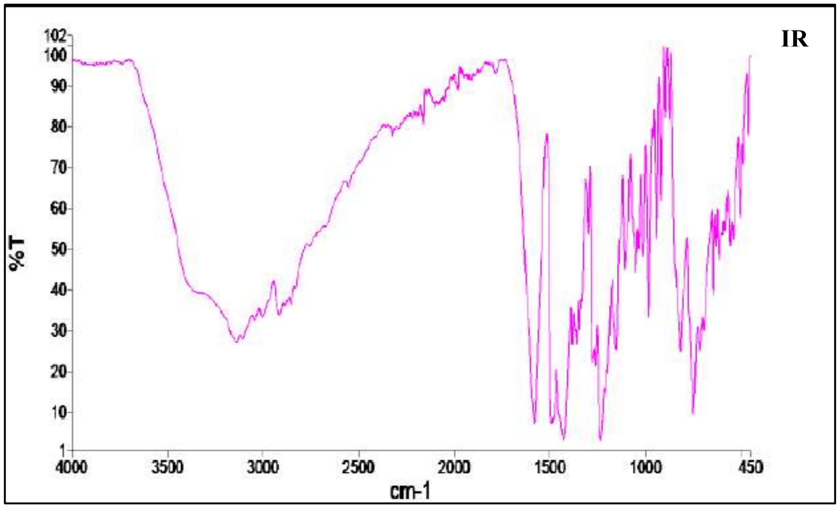

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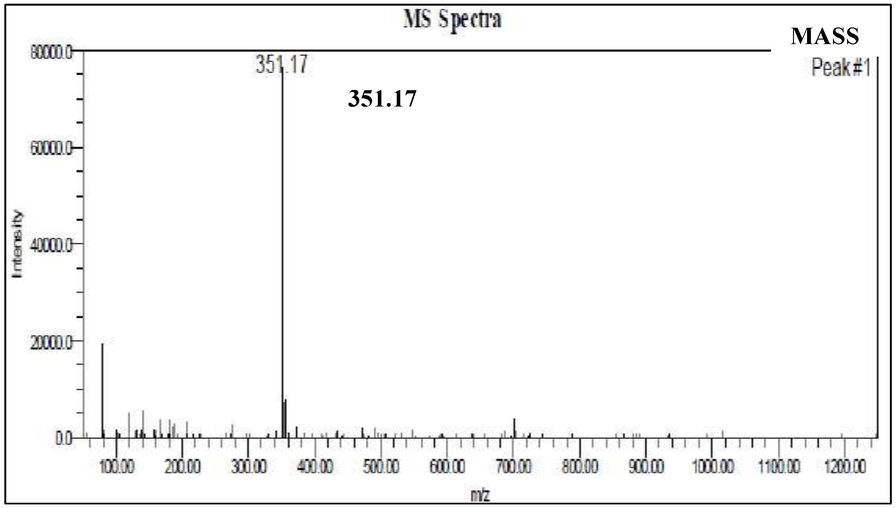

MASS

351.17

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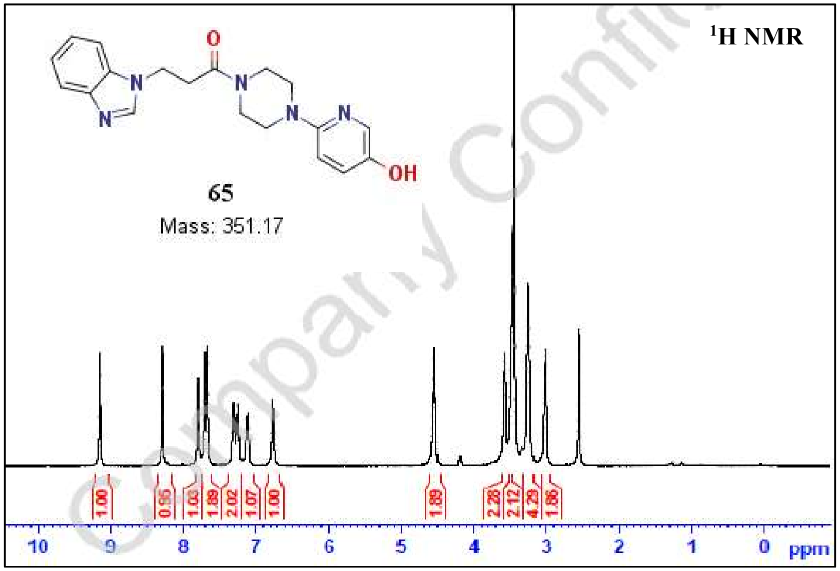

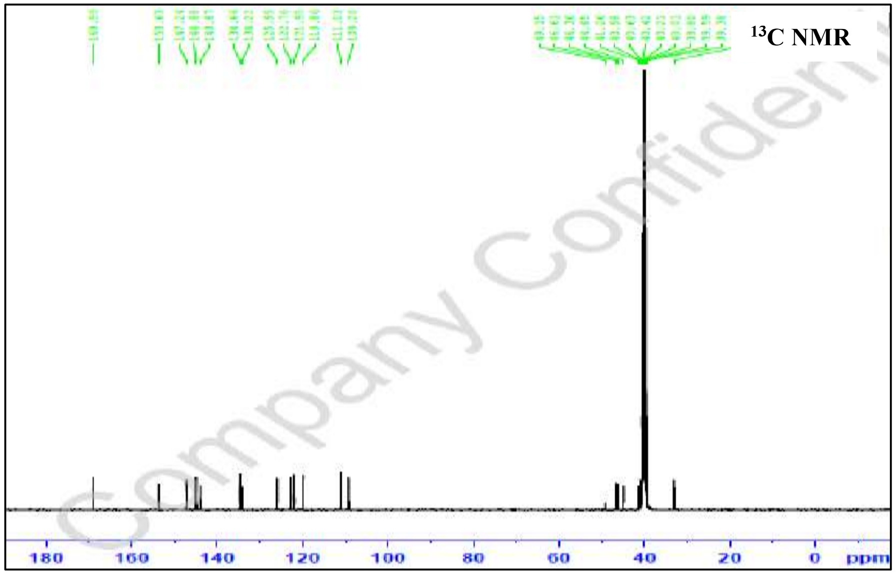

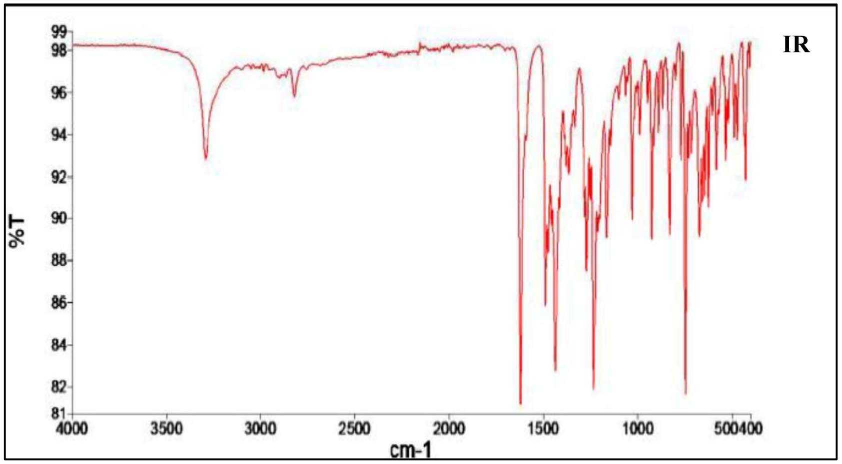

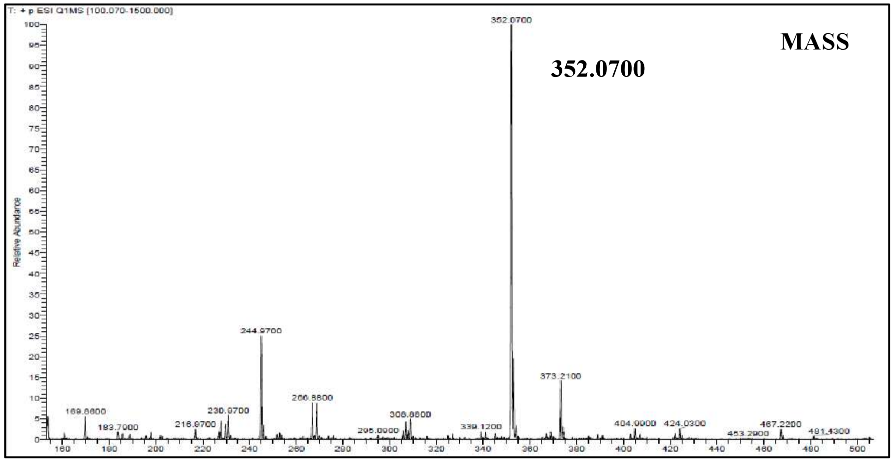

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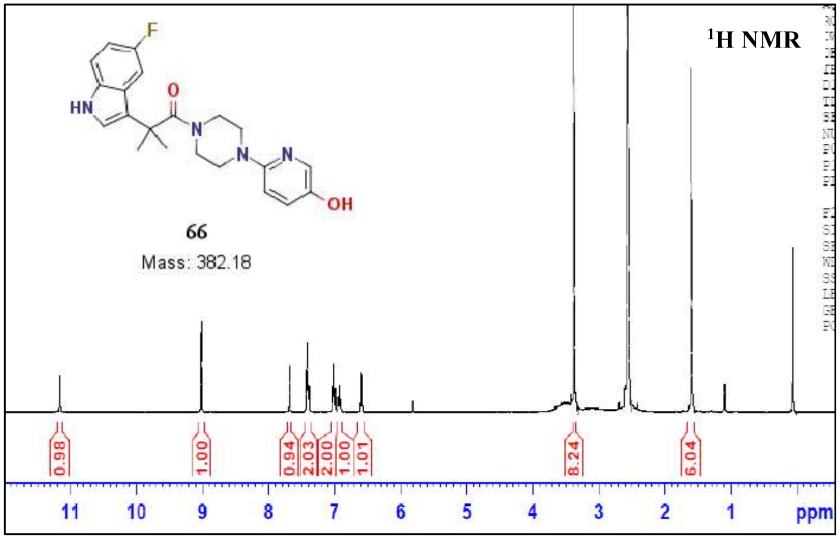

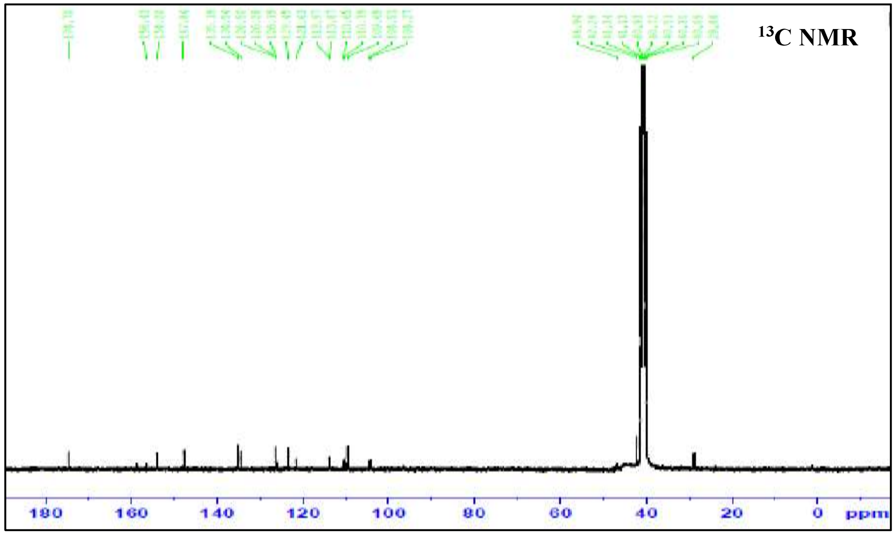

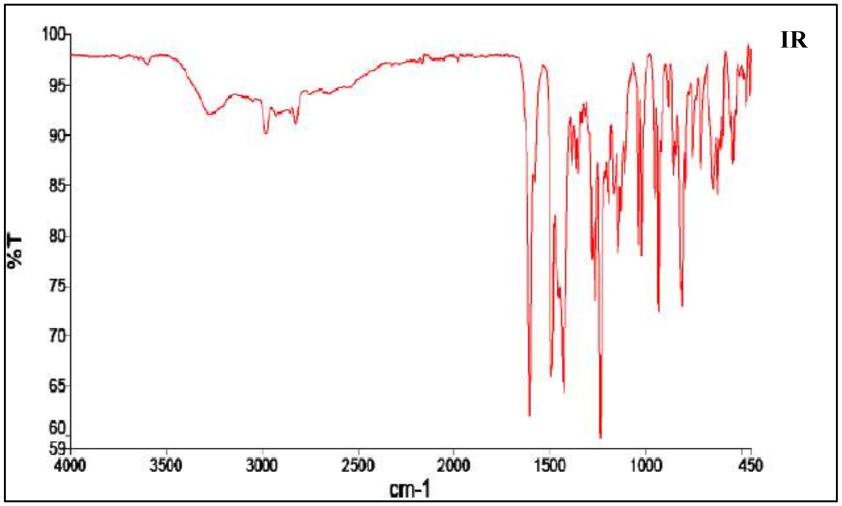

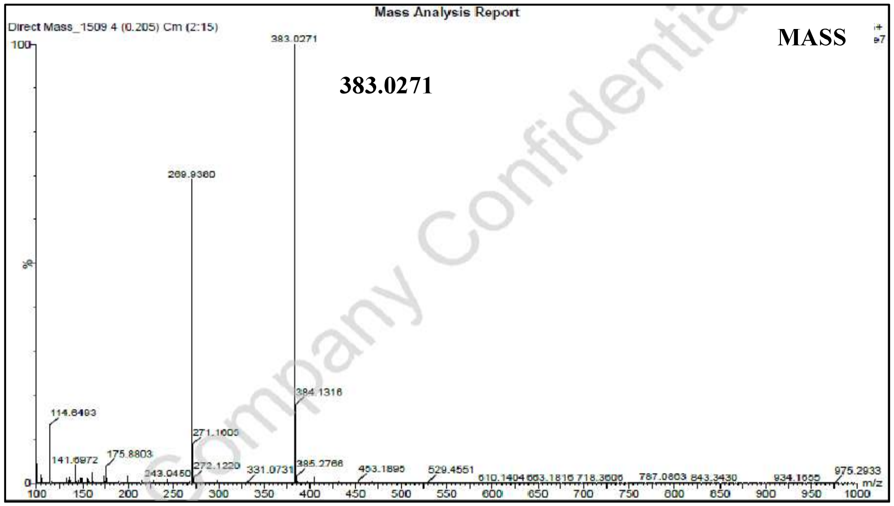

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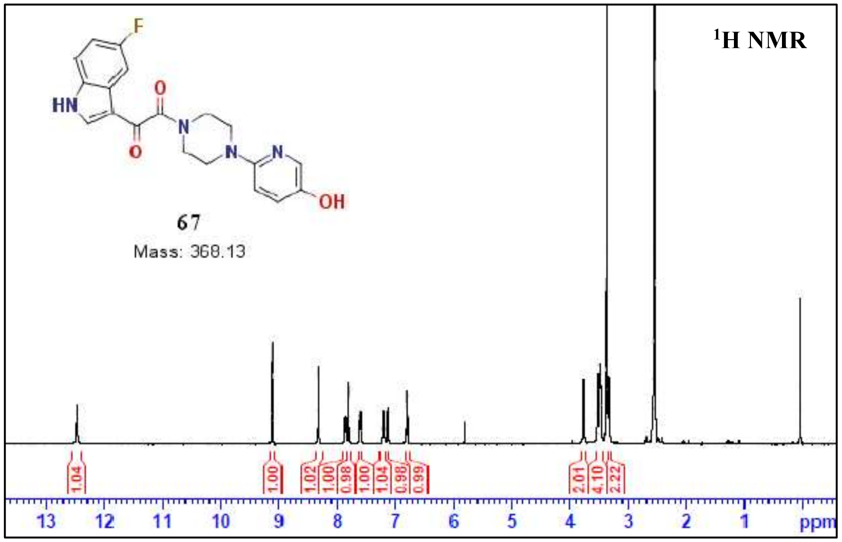

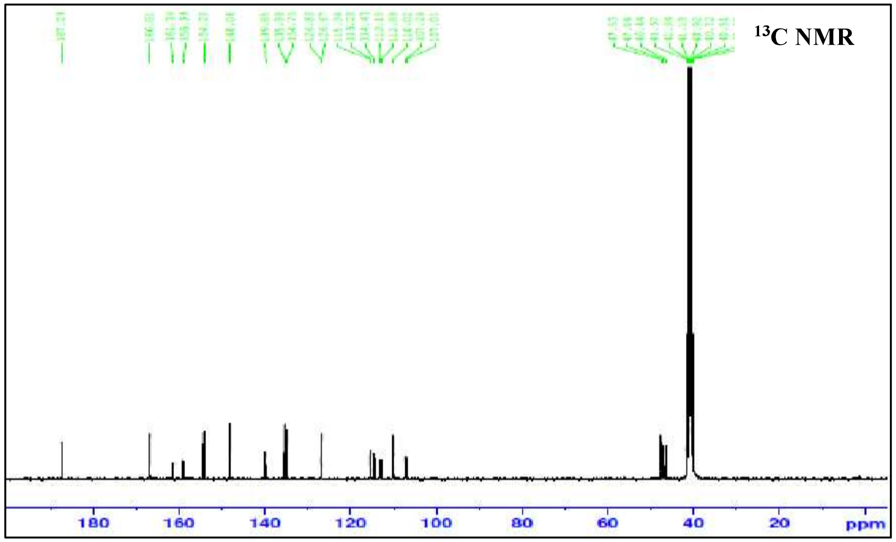

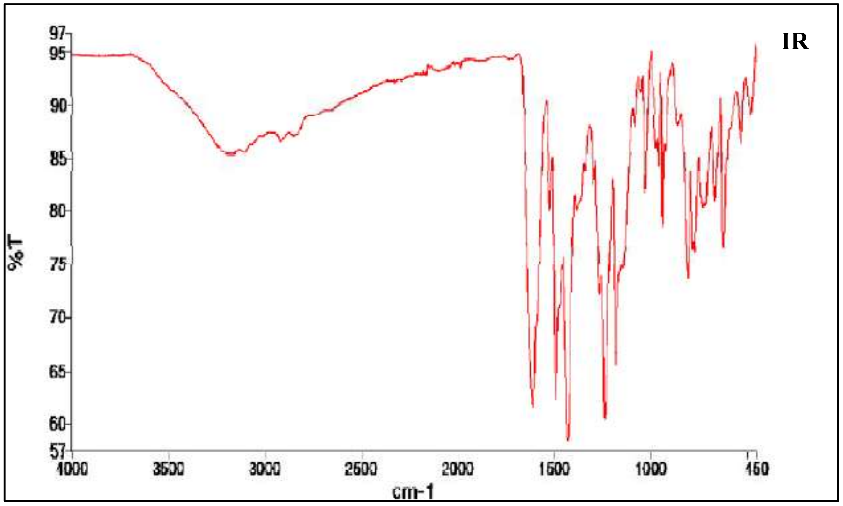

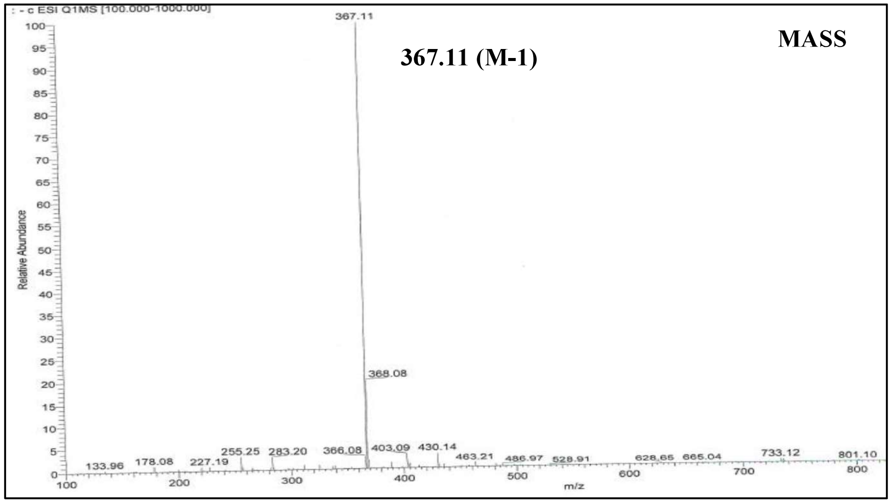

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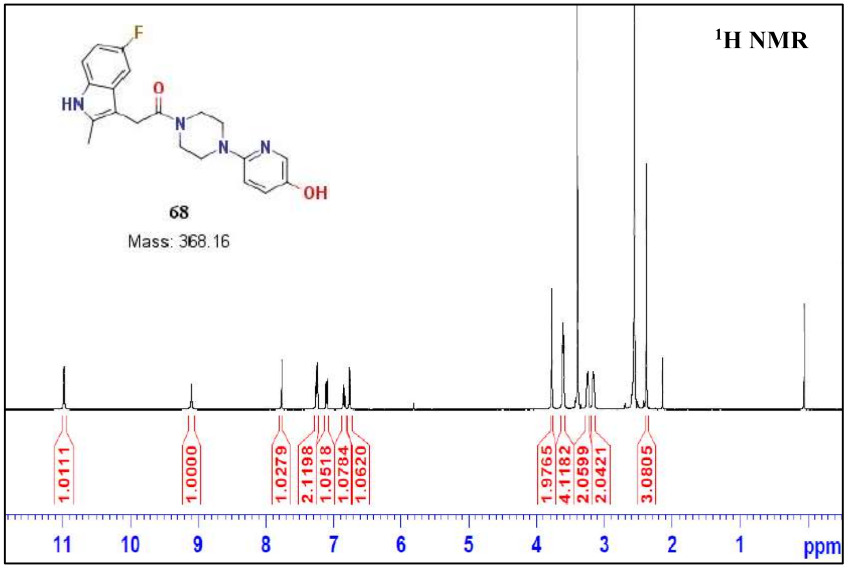

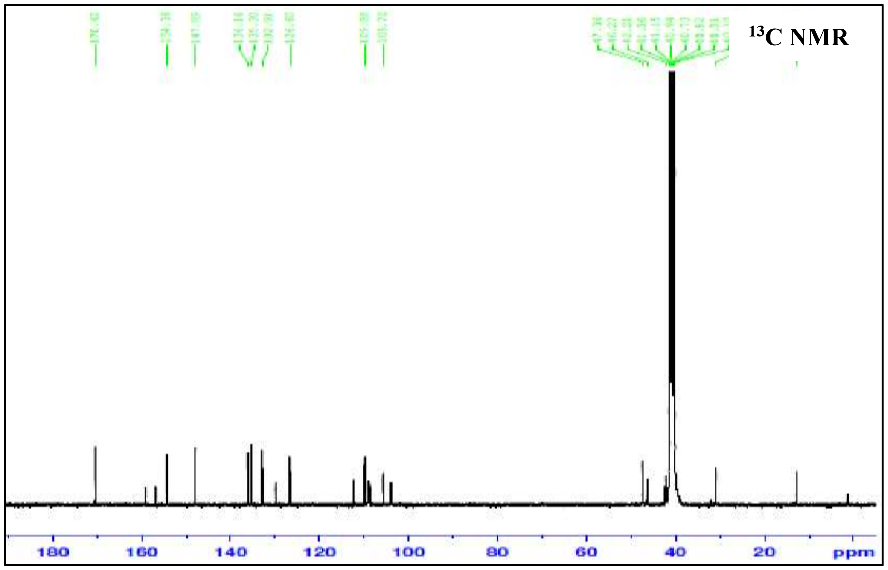

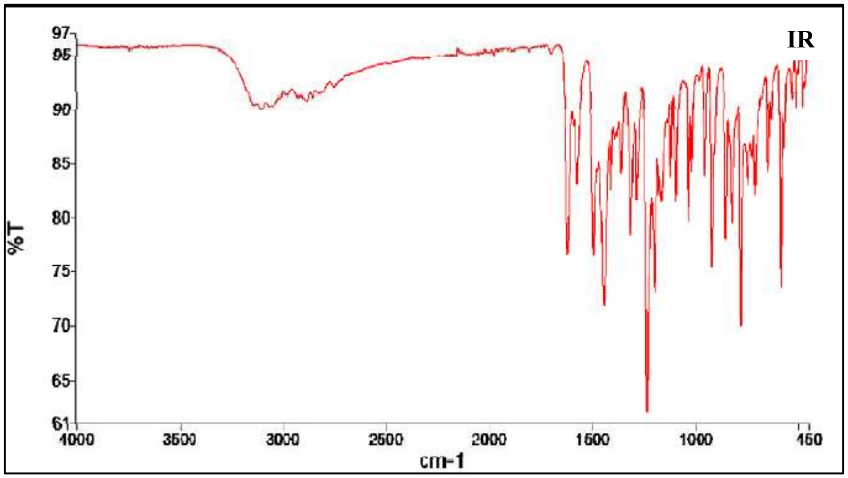

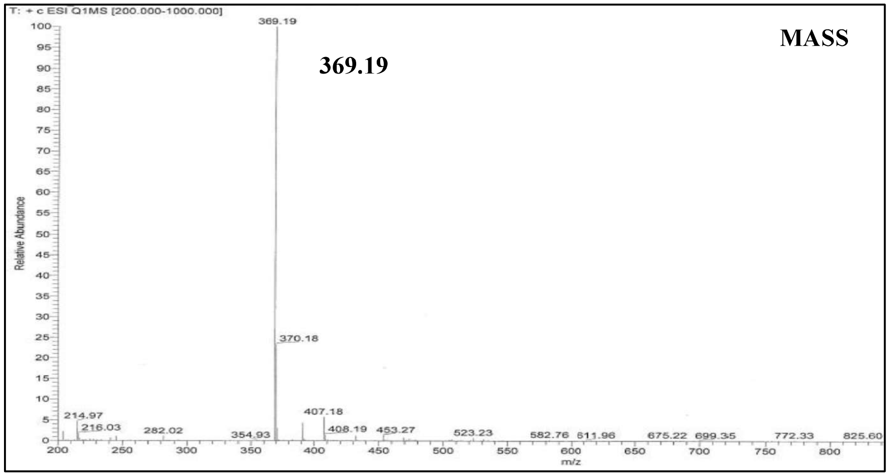

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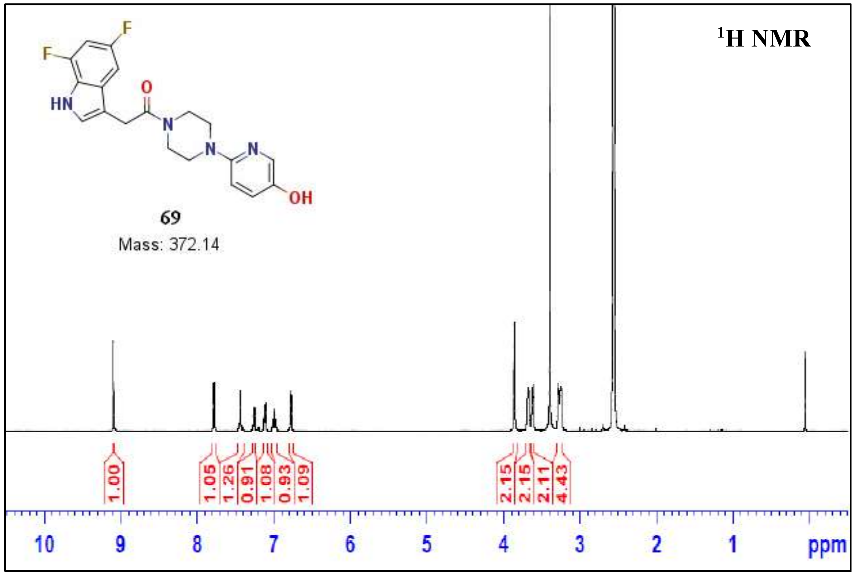

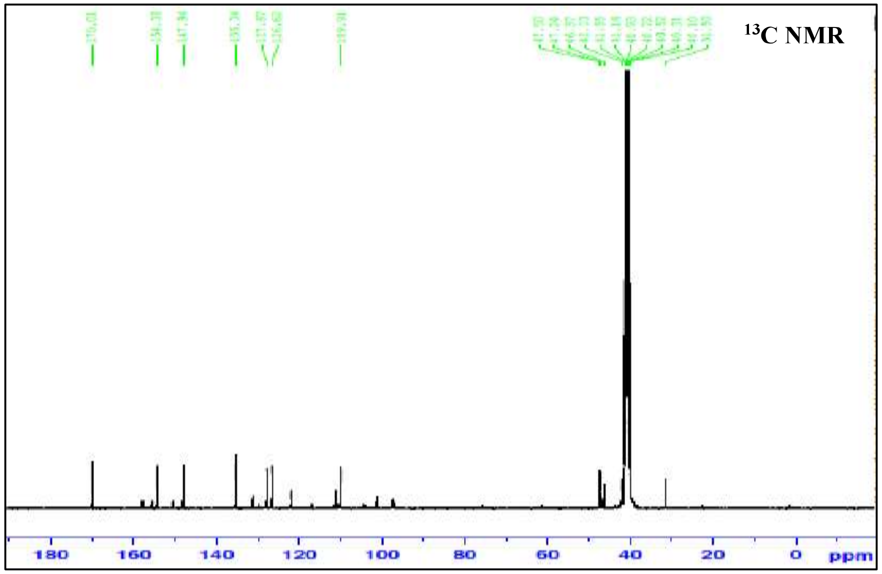

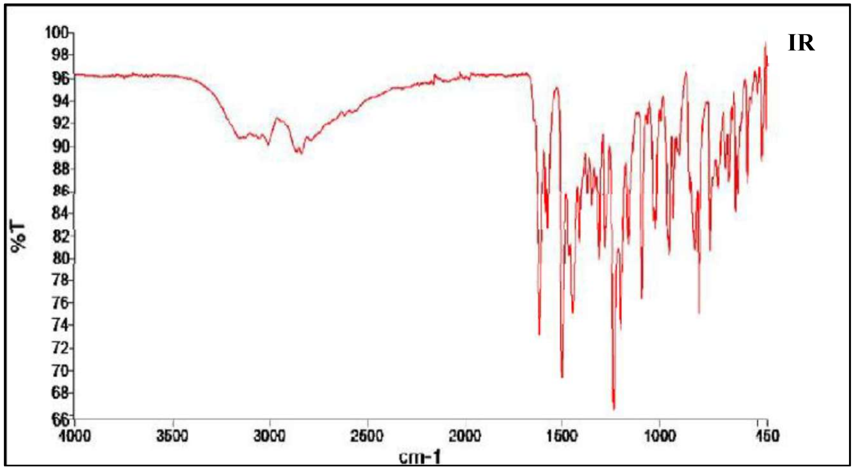

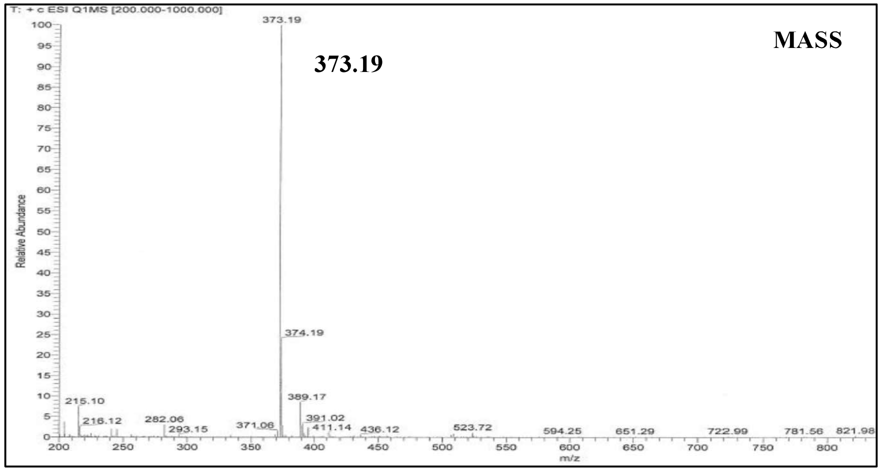

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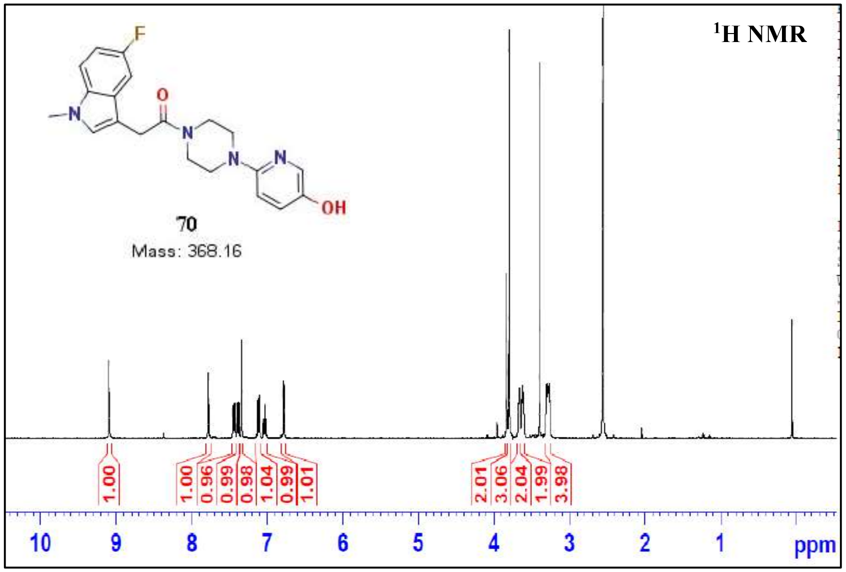

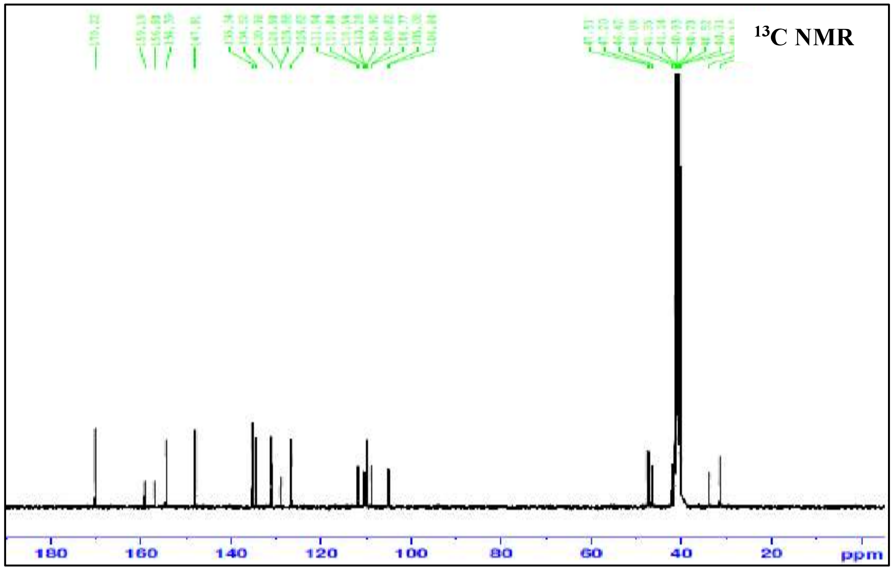

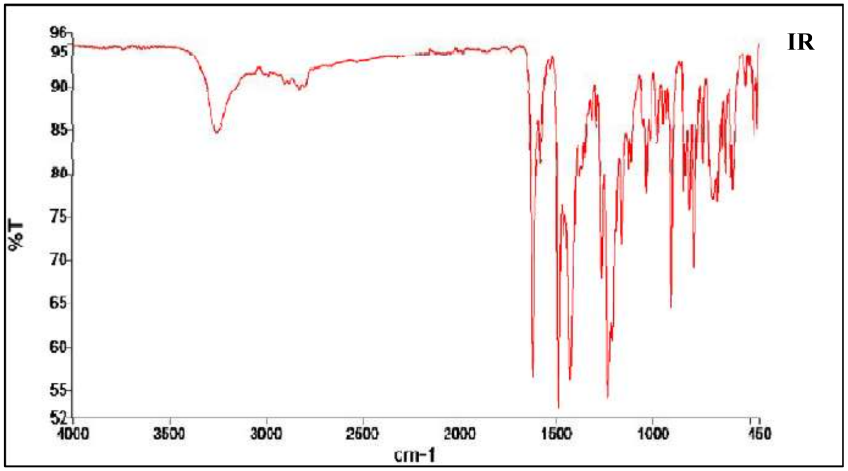

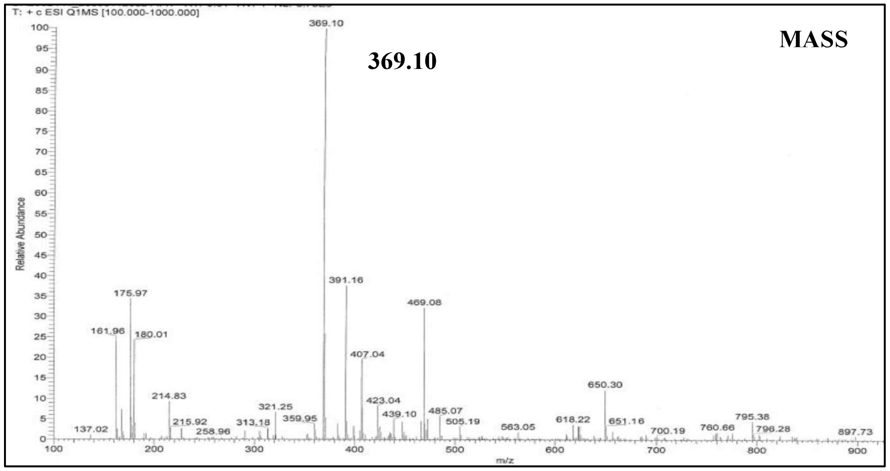

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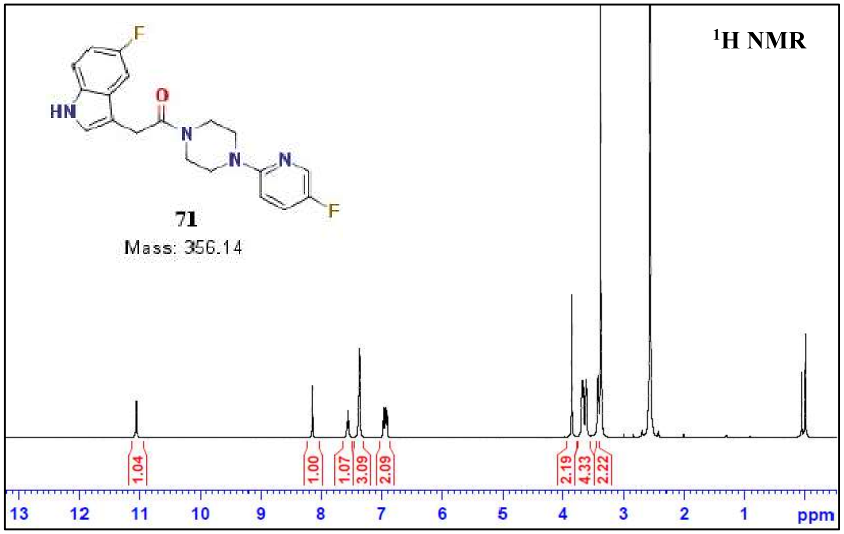

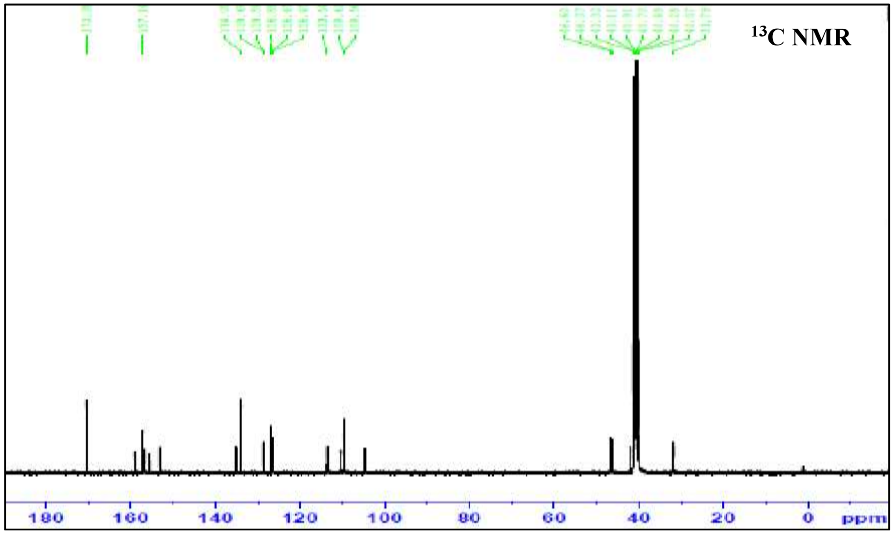

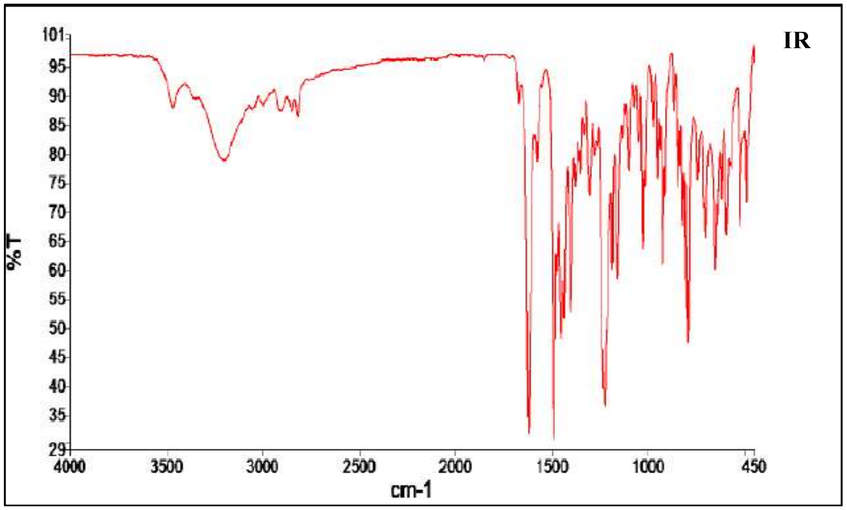

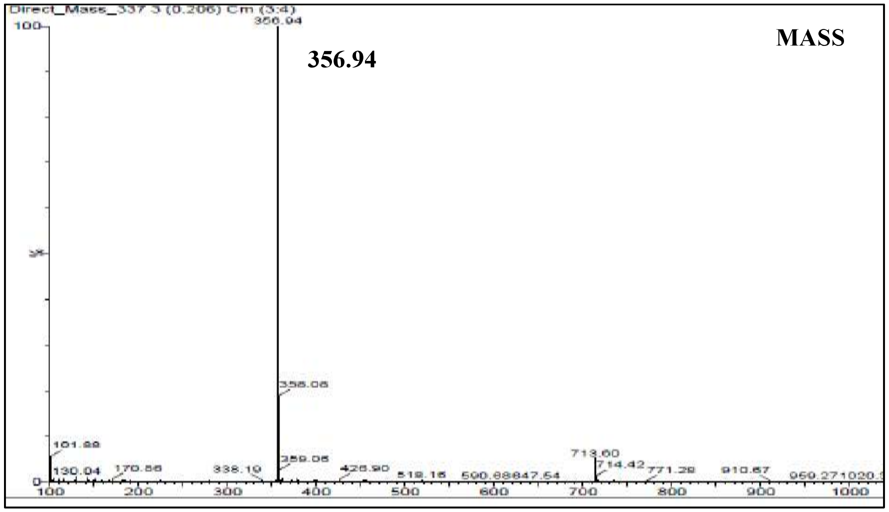

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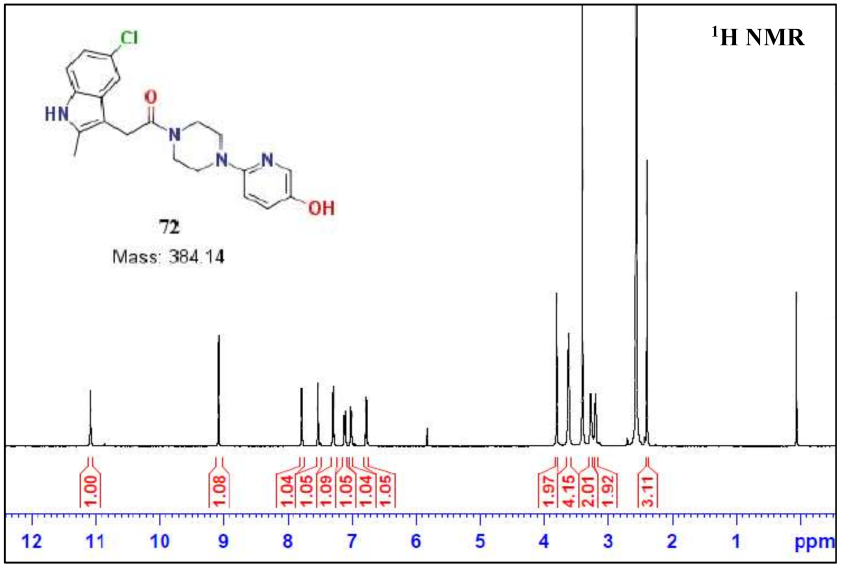
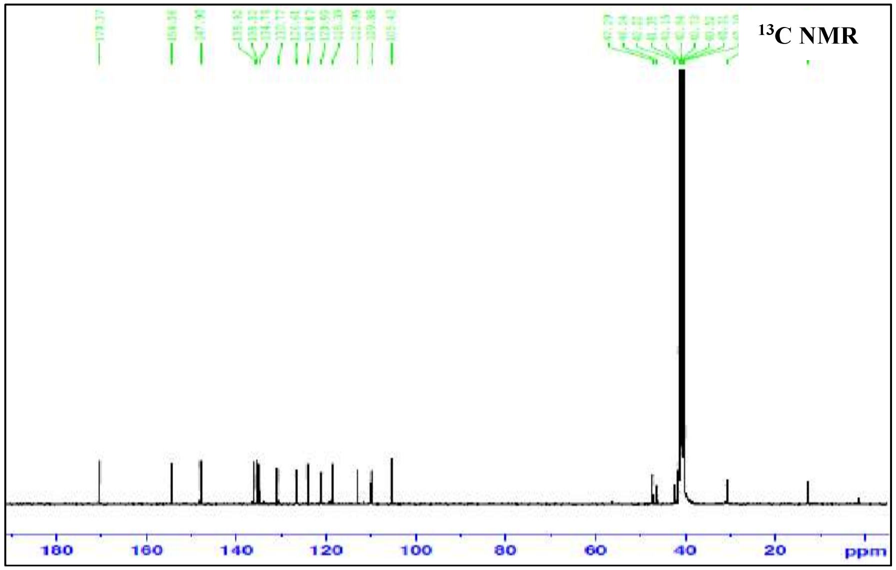
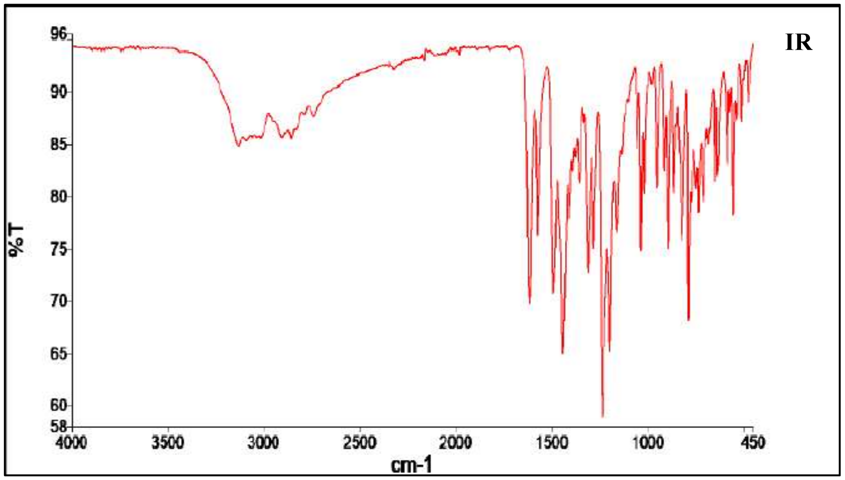
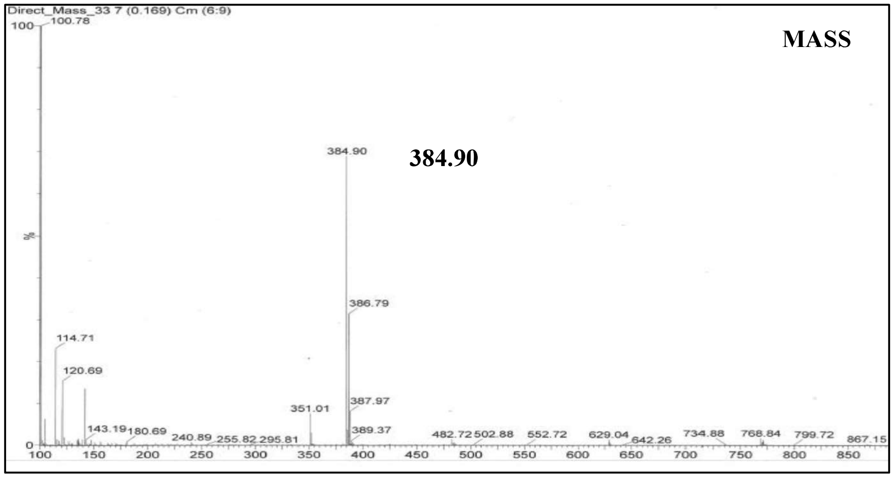
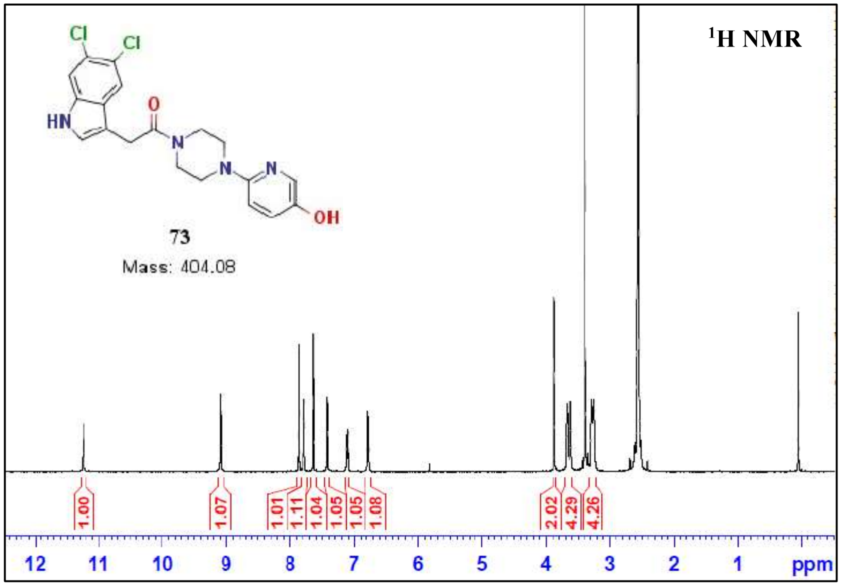


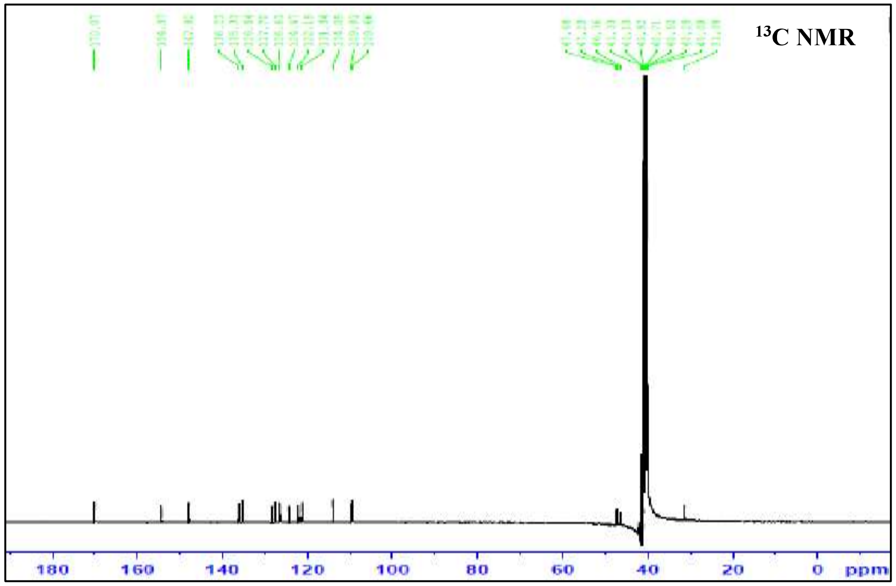


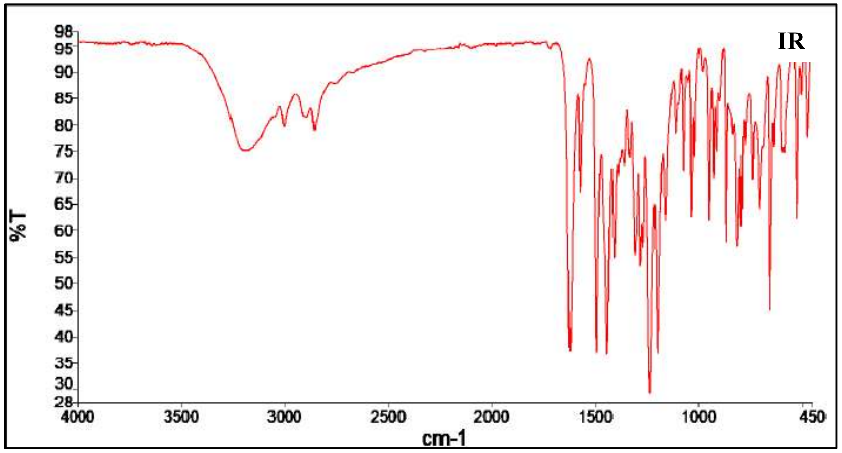


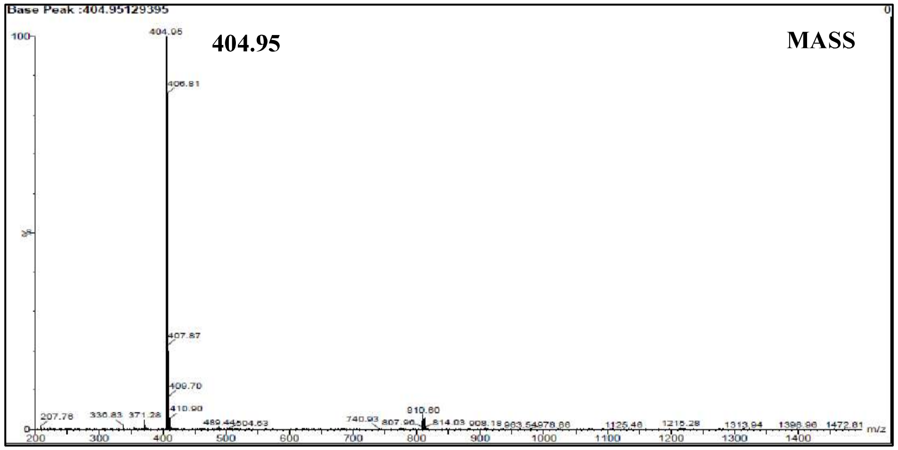


Spectral data of **73**









Spectral data of **74**