Chapter 5. Design and synthesis of dual ME3-tubulin inhibitors for the treatment of PDAC

5.1 Designing dual ME3-tubulin inhibitors for enhanced cell growth inhibition of PDAC cell lines.

Microtubules play a crucial role in multiple cellular functions such as cell division, mitosis, signal transmission and angiogenesis.[29] Hence, the drugs targeting microtubule are highly successful as chemotherapeutic agents. Albumin bound paclitaxel (nab-PTX) in combination with gemcitabine is the current standard of care for PDAC as a first line therapy. It works with an impact on the stabilization of microtubule.

In literature, a couple of molecules presented in **Figure 5.1** are described as small molecule tubulin inhibitors.[30,31] There is a remarkable structural similarity between these molecules and the molecules we have investigated and identified as ME3 inhibitors. Both of them contain similar piperazine carboxamide moiety. Taking a cue from their structural features and our identified ME3 inhibitors, a series of piperazine carboxamides was designed as dual tubulin - ME3 inhibitors by combining the key structural features of both the series *viz.* compounds **75-78** (**Figure 5.1**). The idea was that ME3 and microtubule dual inhibition would have superior cytotoxic effects on PDAC cells.

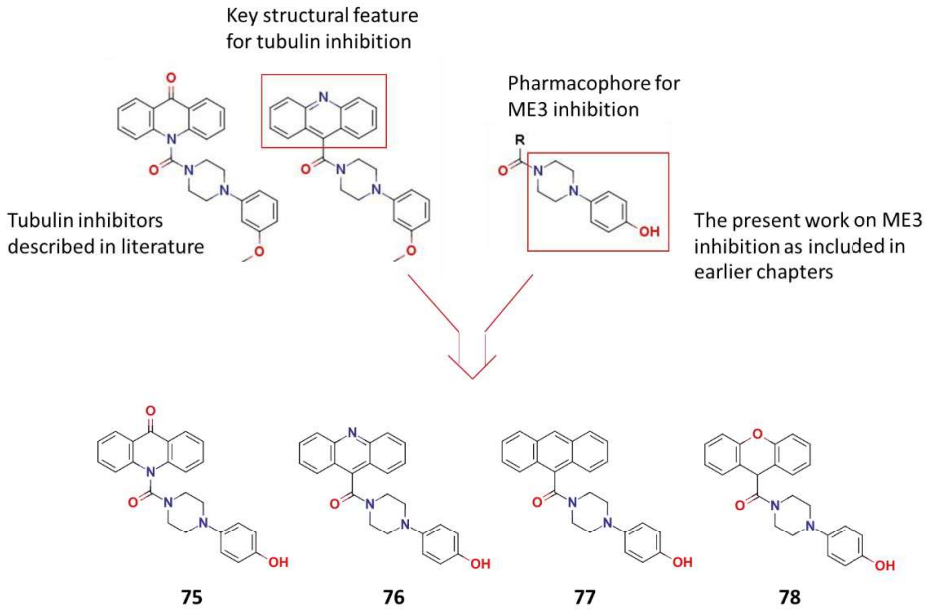
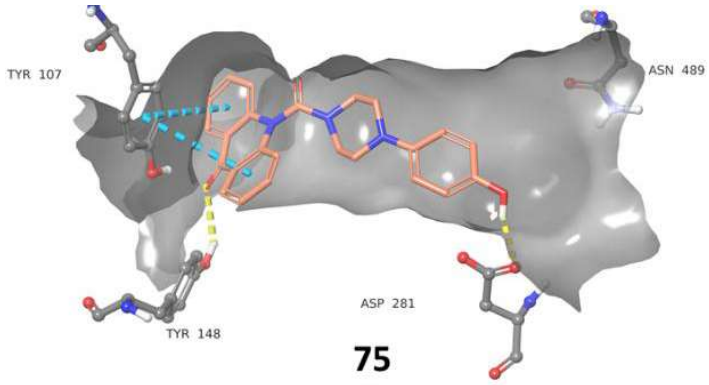


Figure 5.1. Designing of dual Tubulin - ME3 inhibitors

5.2 Docking studies of compounds 75 and 76 with ME3 and tubulin.

To perceive the prospective activity of the designed molecules, the ME3 crystal structure which was resolved in house as oxalate (resolution: 1.8 Å) was used for the docking studies on compounds **75** and **76** in malate binding pocket of ME3. *in silico* docking poses for these molecules (**Figure 5.2**), revealed that they possessed a very strong and consistent binding with ME3 protein. Vital interactions observed for compound **75** are: **a)** the phenolic hydroxyl group in phenyl ring (ring-A) of compound **75** undergoes H-bond acceptor interaction with carbonyl oxygen of acid group of Asp281 side chain; **b)** acridone ring had π-π stacking interaction with phenyl ring of the side chain of Tyr107; **c)** oxygen atom of the keto group situated in acridone ring had H-bond acceptor interaction with phenolic hydroxyl group of the side chain of Tyr148. Vital interactions observed for compound **76** are: **a)** the phenolic hydroxyl group in phenyl ring (ring-A) of compound **76** undergoes H-bond acceptor interaction with carbonyl oxygen of acid group of Asp281 side chain; **b)** ring-A exhibited π-π stacking interaction with phenyl ring of the side chain of Tyr137; **c)** acridine ring had π-π stacking interaction with phenyl ring of the side chain of Tyr107; **d)** nitrogen atom situated in acridine ring experienced H-bind acceptor interaction with phenolic hydroxyl group of Tyr107 side chain.



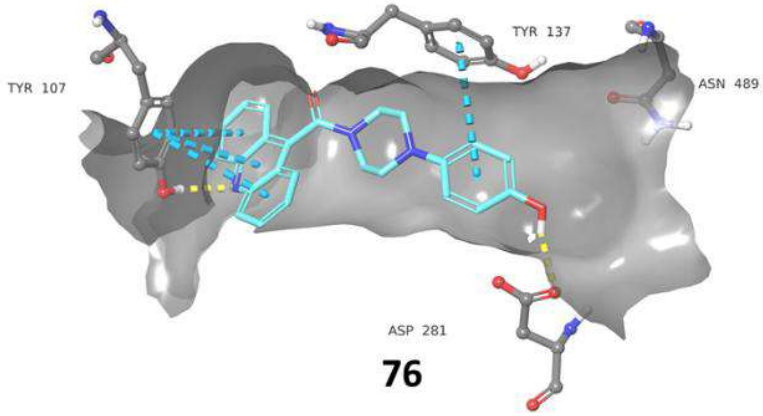


Figure 5.2: Binding poses for compound 75 and 76 in malate binding pocket of ME3.

Compound **75** and **76** were also docked in tubulin (PDB ID - 4O2B). *in silico* docking poses for these molecules revealed that they occupied colchicine binding site of tubulin protein where phenolic hydroxyl group underwent vital H-bond donor interaction with carbonyl oxygen of Asn349 backbone (**Figure 5.3**).

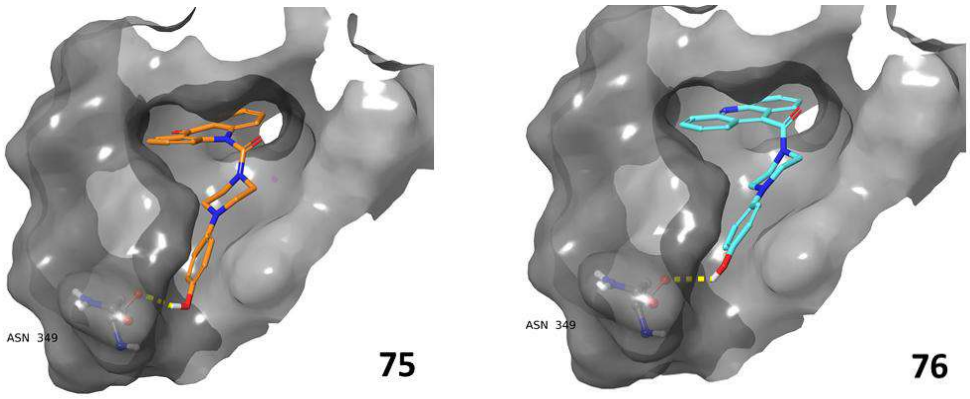


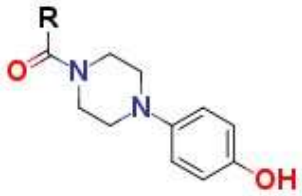
Figure 5.3: Binding poses for compound 75 and 76 in colchicine binding pocket of tubulin.

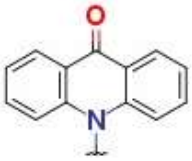
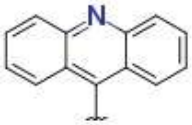
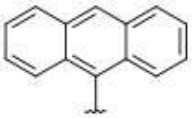
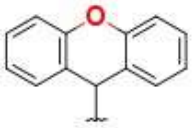
5.3 Synthesis and biological evaluation of the designed compounds and related SAR.

For synthesis of the designed compound **76**, the manoeuvre began with Suzuki coupling reaction between 2-aminobenzonitrile and (2-bromophenyl)boronic acid to get substituted benzophenone intermediate **75.1** which was condensed with 1-[4-(benzyloxy)phenyl] piperazine (**III**) in the presence of triphosgene to get the urea intermediate **75.2** which was converted in to the cyclized intermediate **75.3** using palladium catalysed intramolecular Buchwald-Hartwig amination reaction. Debenzylation of the intermediate **75.3** yielded compound **75**. Appropriately substituted carboxylic acids were coupled with the key intermediate **IV** to synthesize these compounds **77-78**. (**refer Section 5.4**) (**Figure 5.1**).

These newly synthesized compounds **76-78** were screened for ME3 enzyme inhibition and BxPC-3 cell growth inhibition assays (**Table 5.1**).

Table 5.1. ME3 inhibition and BxPC-3 cell growth inhibition data for compounds **75-78**.

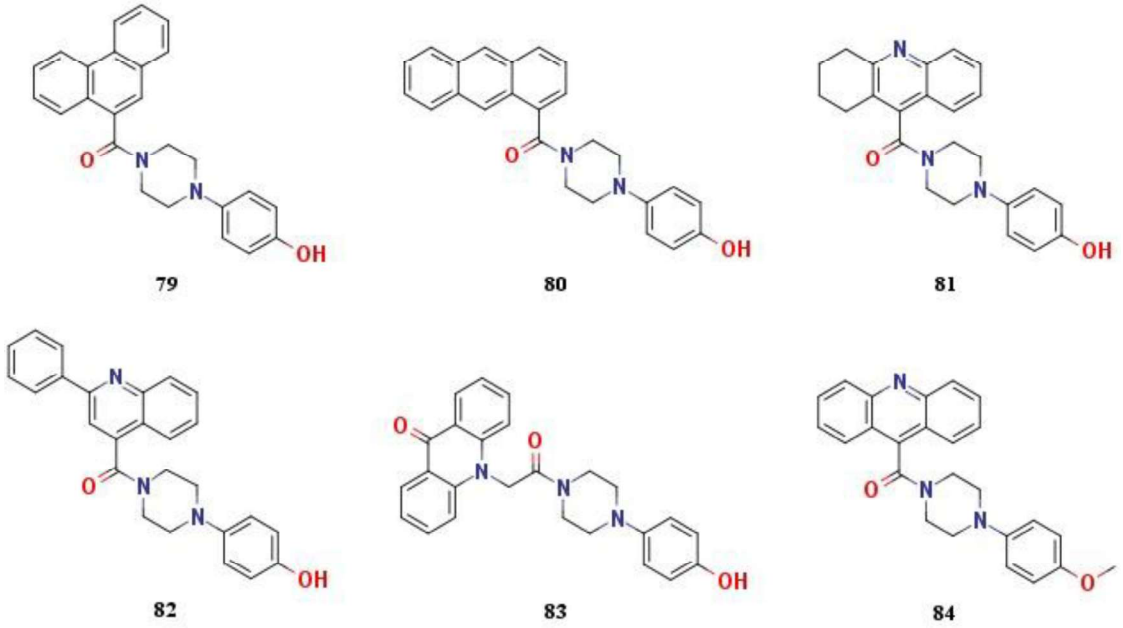


Compound	R	ME3 IC_{50} (µM)	BxPC-3 IC_{50} (µM)
75		0.150	0.320
76		0.095	0.106
77		0.152	1.1
78		40% at 0.1 µM	56% at 10 µM

Compounds **75** and **76** exhibited potent inhibition of ME3 and gained potency in terms of BxPC-3 cell growth inhibition having sub nanomolar IC_{50} values. This transition of BxPC-3 IC_{50} values from micromolar to sub nanomolar range were supposed to come from engaging tubulin as a target. This proved that these compounds work by dual mechanism of action engaging ME3 as well as tubulin as targets. Compound **77** and **78** exhibited a comparable ME3 inhibitory potency but were inferior in terms of BxPC-3 cell growth inhibition.

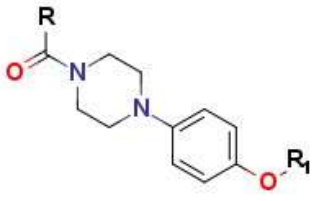
Compounds (**79-84**) listed in **Chart 5.1** were designed to generate SAR around compounds **75-77**.

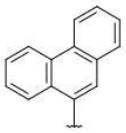
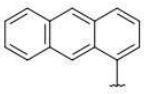
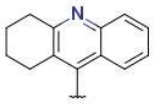
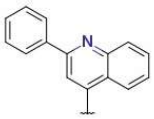
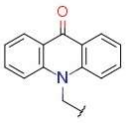
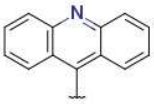
Chart 5.1: Structures of designed compounds **79-84**



In compound **79**, linearly fused anthracene ring in compound **77** was replaced with angularly fused phenanthrene ring. Position of the carboxamide bond in compound **77** was changed from position-9 to position-1 of anthracene ring in compound **80** to evaluate its positional importance. To assess the significance of linearly fused tricyclic ring, acridine ring in compound **76** was replaced with a 1,2,3,4-tetrahydroacridine ring and 2-phenylquinoline ring in compound **81** and **82** respectively. Compound **83** was designed by introducing one carbon spacer between acridone ring and the carbonyl group of compound **75** to assess the importance of rigidity. Phenolic hydroxyl group in compound **76** was replaced with methoxy group (compound **84**) to estimate how much contribution of BxPC-3 cell growth inhibition comes from ME3 depletion. Designed compounds were synthesized (**Refer section 5.4**) and screened in ME3 enzyme inhibition and BxPC-3 cell growth inhibition assays (**Table 5.2**).

Table 5.2. ME3 inhibition and BxPC-3 cell growth inhibition data for compounds **79-84**.



Compound	R	R_1	% inhibition of ME3			ME3 IC_{50} (µM)	BxPC-3 IC_{50} (µM)
			0.1 µM	1 µM	10 µM		
79		-H	57	100	100	-	53% at 10 µM
80		-H	35	99	100		24% at 10 µM
81		-H	18	98	100	-	57% at 10 µM
82		-H	67	100	100	0.138	21% at 10 µM
83		-H	28	100	100	0.220	5% at 10 µM
84		$-CH_3$	0	0	0	-	1.4

The results of *in vitro* study data revealed that compounds **79**-**83** retained activity on ME3 but failed to show sub nanomolar inhibition of BxPC-3 cell growth. This could be attributed to probable loss of activity on tubulin front. *in vitro* data for compound **79** and **80** showed that linearly fused tricyclic ring attached from 9-position is critical in terms of tubulin inhibition and subsequent inhibitory potency on BxPC-3 cells. Analogues of compound **76** wherein: i) saturation of one ring in tricyclic acridine ring was carried out (compound **81)**; and ii) acridine ring was replaced with 2-phenylquinoline (compound **82**), resulted in a loss of the activity on BxPC-3 cells. Flexible analogue of compound **75** with one more carbon spacer between acridone ring and carbonyl group (compound **83**) also resulted in a loss of the activity on BxPC-3 cells. These findings suggested that these modifications were not favourable in the terms of tubulin inhibition and related impact on BxPC-3 cells.

Compound **84** lost its activity on ME3 enzyme and a steep decrease was observed in the inhibitory potential on BxPC-3, from sub nanomolar to micromolar. This proved that sub

nanomolar activity on BxPC-3 for compound **76** was attributed to its capability to engage two different targets (ME3 and tubulin).

Compounds **75** and **76** showing a potent inhibition of BxPC-3 cells were also screened in the other pancreatic cell lines where they exhibited potent impact on cell viability (**Table 5.3**).

Table 5.3. PDAC cell lines growth inhibition data for compounds **75** and **76**.

Compound	ME3 IC_{50} (µM)	Pancreatic cancer cell lines			
		BxPC-3 IC_{50} (µM)	Hs766T IC_{50} (µM)	Panc05.04 IC_{50} (µM)	Panc-1 IC_{50} (µM)
75	0.150	0.320	3.6	0.94	1.5
76	0.095	0.106	0.62	0.28	0.44

The results of *in vitro* study data revealed that compounds **75** and **76** exhibited a strong inhibition of cell viability across all four pancreatic cancer cell lines taken in to the study.

5.4 Chemistry

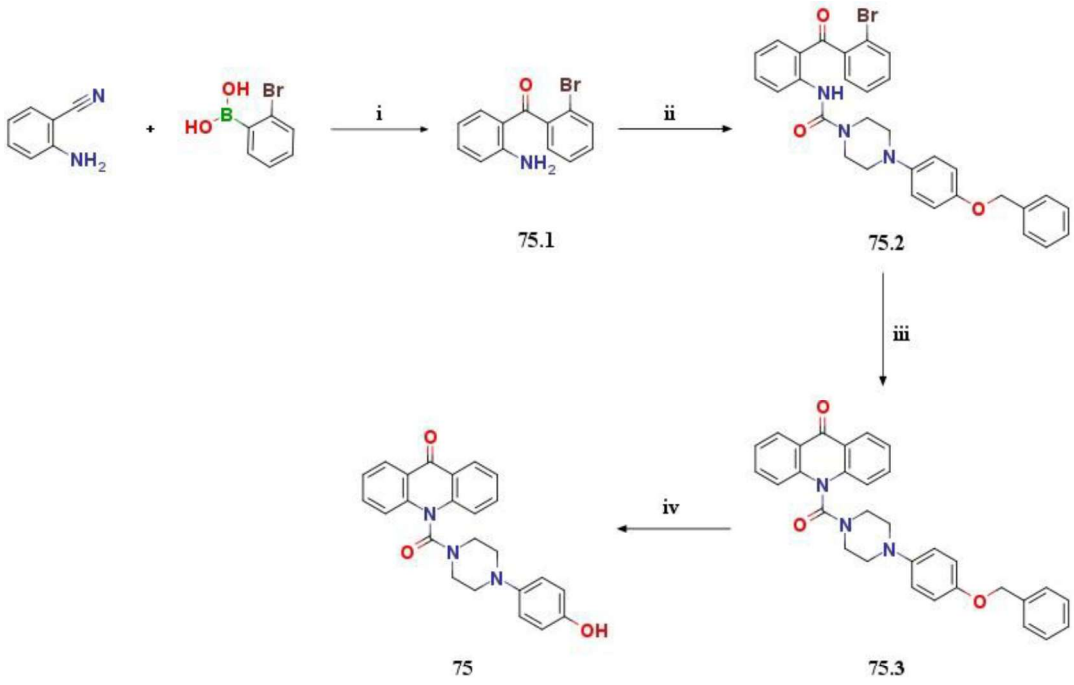
5.4.1 General information

All commercial reagents and anhydrous solvents were purchased and used without further purification, unless otherwise specified. Mass spectra (MS) were obtained on a Quattro premier Waters using electrospray ionization (ESI) in positive mode unless otherwise indicated. Calculated (calcd.) mass corresponds to the exact mass. ^{1}H-NMR and ^{13}C-NMR spectra were recorded on Bruker NMR spectrometers (400 MHz and 500 MHz). 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 recorded on a Perkin Elmer FTIR spectrometer, with major bands reported.

5.4.2 Experimental procedures and spectral data for compounds

1) Synthesis of 10-[4-(4-hydroxyphenyl)piperazine-1-carbonyl]-9,10-dihydroacridin-9-one (75)

Scheme 5.1: Synthesis of compound **75**



Reagents and conditions: (i) 2-Aminobenzonitrile, 2-bromophenylboronic acid, 2-methyl tetrahydro furan, palladium(II) trifluoroacetate, 5,5'-dimethyl-2,2'-bipyridyl, methanesulfonic acid, water, 80 °C, 36 h, 38%; (ii) 2-(2-Bromobenzoyl)aniline, triphosgene, 1,2-dichloroethane, pyridine, 90 °C, 5 h, 40%; (iii) Tris (dibenzylideneacetone)dipalladium(0), xantphos, cesium carbonate, 1,4-dioxane, 110 °C, 3 h, 40%; (iv) 5% Pd on carbon, H_2, tetrahydrofuran, methanol, 25 °C, 2 h, 69%.

Step-1: Synthesis of 2-(2-bromobenzoyl)aniline (75.1)

2-Aminobenzonitrile (2.0 g, 16.9 mmol) was dissolved in 2-methyltetrahydrofuran (20 ml) and was added 2-bromophenylboronic acid (6.79 g, 33.85 mmol), palladium(II) trifluoroacetate (0.28 g, 0.84 mmol), 5,5'-dimethyl-2,2'-bipyridyl (0.31 g, 1.7 mmol), methanesulfonic acid (10.8 ml, 166.4 mmol) followed by water (10 ml). The resultant reaction mixture was heated to 80 °C for 36 h. After completion of the reaction it was allowed to cool to room temperature and product was extracted with ethyl acetate (3 × 50 ml). Combined organic extracts were washed with water followed by brine and dried over Na_2SO_4. Solvent was distilled off and crude product was purified by column chromatography using 10% ethyl acetate in hexane as an eluent to afford **75.1** (1.8 g, 6.51 mmol, 38% yield) as a yellow oil.

^{1}H NMR (400 MHz, DMSO-d$_6$): δ 6.49 (t, 1H, *J* = 7.50 *Hz*), 6.90 (d, 1H, *J* = 8.53 *Hz*), 6.97 (d, 1H, *J* = 7.89 *Hz*), 7.32 – 7.38 (m, 1H), 7.42 (d, 1H, *J* = 7.23 *Hz*), 7.45 - 7.50 (m, 1H), 7.54 - 7.57 (m, 3H), 7.77 (d, 1H, *J* = 7.90 *Hz*).

Step-2: Synthesis of 4-[4-(benzyloxy)phenyl]-N-[2-(2-bromobenzoyl)phenyl]piperazine-1-carboxamide (75.2)

2-(2-Bromobenzoyl)aniline (**75.1**) (1.8 g, 6.5 mmol) was dissolved in 1,2-dichloroethane (30 ml) and was added triphosgene (1.16 g, 3.9 mmol) followed by pyridine (1.57 ml, 19.55 mmol). The resultant reaction mixture was heated to 90 °C and stirred for 1h before adding 1-[4-(benzyloxy)phenyl]piperazine (intermediate **III)** (1.74 g, 6.5 mmol). Reaction mixture was further stirred for 4h at 90 °C. After completion of the reaction, it was diluted with 30 ml dichloromethane and washed with water (2 x 20 ml) and brine (1 x 10 ml). Organic layer was dried over Na_2SO_4 and distilled under reduced pressure. Obtained crude product was purified by column chromatography using 50% ethyl acetate in hexane as an eluent to afford **75.2** (1.5 g, 2,62 mmol, 40%) as a pale yellow solid.

Step-3: Synthesis of 10-{4-[4-(benzyloxy)phenyl]piperazine-1-carbonyl}-9,10-dihydro acridin-9-one (75.3)

To a solution of **75.2** (1.5 g, 2.62 mmol) in 1,4-dioxane (30 ml) was added tris (dibenzylideneacetone)dipalladium(0) (0.36 g, 0.39 mmol), xantphos (0.45 g, 0.78 mmol) and cesium carbonate (4.56 g, 7.88 mmol) at room temperature and heated to 110 °C for 3h. On completion of reaction, the reaction mixture was filtered through celite bed and washed with ethyl acetate. The combined organic layers were washed with water, dried over anhydrous sodium sulfate and concentrated under reduced pressure. The obtained crude was purified using column chromatography on silica gel (230–400 mesh) using ethyl acetate:hexane mixture as eluent to afford **75.3** (1.1 g, 2.24 mmol, 85% yield) as a pale yellow solid which was as such taken to the next step.

Step-4: Synthesis of 10-[4-(4-hydroxyphenyl)piperazine-1-carbonyl]-9,10-dihydro acridin-9-one (75)

To a solution of **75.3** (1.1 g, 2.24 mmol) in methanol:tetrahydrofuran (1:1) (50 ml) was added 5% palladium on activated carbon (50% wet) (0.2 g) and the suspension was stirred under hydrogen atmosphere for 2h at room temperature. On completion of reaction, the reaction mixture was filtered off from the catalyst and the solution was evaporated under reduced pressure to afford crude product. Crude product was stirred with 10 ml diethyl ether for 10 minutes. Resultant yellow suspension was filtered and washed with 5 ml diethyl ether to get pure compound **75** (0.62 g, 1.55 mmol, 69% yield) as a pale-yellow solid.

Melting point: 195-197 °C. **LC purity (UV 245 nm):** 97.20%

^{1}H NMR (400 MHz, DMSO-d$_6$): δ 2.87 (t, 2H, *J* = 4.6 *Hz*), 3.32 (m, 4H), 4.10 (t, 2H, *J* = 4.8 *Hz*), 6.70 (d, 2H, *J* = 8.83 *Hz*), 6.84 (d, 2H, *J* = 8.86 *Hz*), 7.46 - 7.51 (m, 4H), 7.88 – 7.91 (m, 2H), 8.41 (d, 2H, *J* = 7.08 *Hz*), 8.95 (s, 1H).

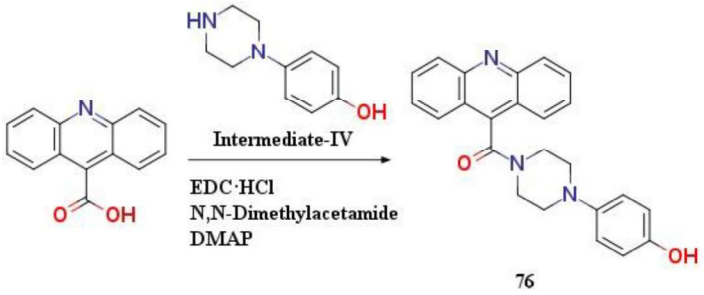
^{13}C NMR (100 MHz, DMSO-d$_6$): δ 44.2 (CH_2), 46.3 (CH_2), 50.2 (CH_2), 50.7 (CH_2), 115.9 (2 x CH), 116.1 (2 x CH), 118.9 (2 x CH), 121.1 (2 x C), 123.1 (2 x CH), 127.2 (2 x CH), 135.1 (2 x CH), 138.9 (2 x C), 143.8 (C), 151.3 (C), 151.9 (C), 176.8 (C)

IR (ATR) / cm^{-1}: 3250, 3065, 1703, 1595, 1515, 1488, 1463, 1440, 1228, 1170, 1036, 823.

LCMS (ESI$^+$): calculated for $C_{24}H_{21}N_3O_3$ $[M+H]^+$ 400.1583; found: *m/z* = 400.2678.

2) Synthesis of 4-[4-(acridine-9-carbonyl)piperazin-1-yl]phenol (76)

Scheme 5.2: Synthesis of compound **76**



To a solution acridine-9-carboxylic acid (0.5 g, 2.23 mmol) in *N,N*-dimethylacetamide (10 ml) was added 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC·HCl) (0.64 g, 3.35 mmol). The reaction mixture was stirred for 10 minutes before adding 4-piperazin-1-ylphenol (**IV**) (0.4 g, 2.23 mmol). The resultant reaction mixture was added 4-(dimethylamino)pyridine (0.027 g, 0.22 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 layers 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 **76** (0.48 g, 1.25 mmol, 56% yield) as a yellow solid.

Melting point: 170-173 °C. **LC purity (UV 245 nm):** 99.44%

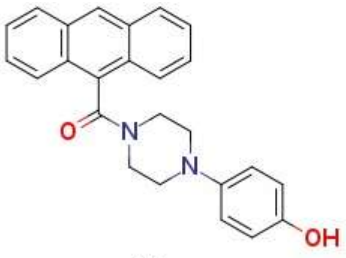
^{1}H NMR (400 MHz, DMSO-d$_6$): δ 2.94 (s, 2H), 3.12 (s, 2H), 3.53 (s, 2H), 4.16 (s, 2H), 6.69 (d, 2H, *J* = 8.81*Hz*), 6.83 (d, 2H, *J* = 8.85*Hz*), 7.76 (t, 2H, *J* = 7.69*Hz*), 7.97 (d, 2H, *J* = 7.76*Hz*), 8.01 (d, 2H, *J* = 7.90 *Hz*), 8.27 (d, 2H, *J* = 6.72 *Hz*), 8.95 (s, 1H).

^{13}C NMR (100 MHz, DMSO-d$_6$): δ 39.2 (CH_2), 43.8 (CH_2), 53.7 (CH_2), 54.4 (CH_2), 116.4 (2 x CH), 122.4 (2 x C), 122.5 (2 x CH), 122.7 (2 x CH), 126.7 (2 x CH), 129.3 (2 x CH), 135.1 (C), 136.4 (2 x CH), 142.1 (2 x C), 148.3 (C), 157.8 (C), 163.5 (C).

IR (ATR) / cm^{-1}: 3204, 2555, 1650, 1469, 1437, 1276, 1238, 978, 839, 762, 540.

LCMS (ESI$^+$): calculated for $C_{24}H_{21}N_3O_2$ $[M+H]^+$ 384.16; found: *m/z* = 384.16.

3) Synthesis of 4-[4-(anthracene-9-carbonyl)piperazin-1-yl]phenol (77)



77

Anthracene-9-carboxylic acid (0.3 g, 1.34 mmol) was reacted with 4-piperazin-1-ylphenol (**IV**) (0.24 g, 1.34 mmol) as per the procedure described for compound **76** to afford compound **77** (0.28 g, 0.73 mmol, 54% yield) as an off white solid.

Melting point: 192-195 °C. **LC purity (UV 245 nm):** 97.67%

^{1}H NMR (400 MHz, DMSO-d$_6$): δ 2.77 (t, 2H, *J* = 4.56 *Hz*), 3.11 (t, 2H, *J* = 4.73 *Hz*), 3.27 (t, 2H, *J* = 4.78 *Hz*), 4.15 (t, 2H, *J* = 4.75 *Hz*), 6.69 (d, 2H, *J* = 8.75 *Hz*), 6.82 (d, 2H, *J* = 8.80 *Hz*), 7.61 – 7.68 (m, 4H), 7.94 (d, 2H, *J* = 8.39 *Hz*), 8.22 (d, 2H, *J* = 8.17 *Hz*), 8.75 (s, 1H), 8.94 (s, 1H).

^{13}C NMR (100 MHz, DMSO-d$_6$): δ 41.5 (CH_2), 46.6 (CH_2), 50.9 (CH_2), 51.1 (CH_2), 115.8 (2 x CH), 118.8 (2 x CH), 125.1 (2 x CH), 126.1 (2 x CH), 127.2 (2 x C), 127.3 (2 x CH), 127.7 (CH), 129.0 (2 x CH), 131.0 (2 x C), 131.1 (C), 144.0 (C), 151.7 (C), 167.3 (C).

IR (ATR) / cm^{-1}: 3271, 1611, 1510, 1493, 1451, 1248, 1229, 1209, 991, 824, 740, 692, 654, 535.

LCMS (ESI$^+$): calculated for $C_{25}H_{22}N_2O_2$ $[M+H]^+$ 383.16; found: *m/z* = 383.14.

4) Synthesis of 4-[4-(9H-xanthene-9-carbonyl)piperazin-1-yl]phenol (78)

78

9H-Xanthene-9-carboxylic acid (0.3 g, 1.32 mmol) was reacted with 4-piperazin-1-ylphenol (**IV**) (0.23 g, 1.32 mmol) as per the procedure described for compound **76** to afford compound **78** (0.25 g, 0.64 mmol, 48% yield) as an off white solid.

Melting point: 123-125 °C. **LC purity (UV 245 nm):** 97.94%

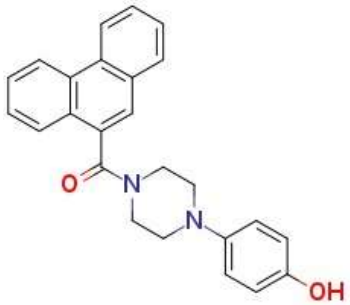
^{1}H NMR (400 MHz, DMSO-d$_6$): δ 2.94 (s, 2H), 3.12 (s, 2H), 3.53 (s, 2H), 4.16 (s, 2H), 5.82 (s, 1H), 6.71 (d, 2H, *J* = 8.92*Hz*), 6.88 (d, 2H, *J* = 8.89*Hz*), 7.15 (t, 2H, *J* = 5.92*Hz*), 7.20 (d, 2H, *J* = 7.89*Hz*), 7.53 (d, 2H, *J* = 7.90 *Hz*), 7.35 (t, 2H, *J* = 6.72 *Hz*), 8.95 (s, 1H).

^{13}C NMR (100 MHz, DMSO-d$_6$): δ 42.3 (CH_2), 46.4 (CH_2), 50.5 (CH_2), 51.4 (CH_2), 55.2 (CH), 115.8 (2 x CH), 116.8 (2 x CH), 118.7 (2 x CH), 120.9 (2 x C), 123.7 (2 x CH), 128.8 (2 x CH), 128.9 (CH), 144.1 (C), 151.3 (2 x C), 151.7 (2 x C), 170.5 (C).

IR (ATR) / cm^{-1}: 3266, 2564, 1607, 1592, 1514, 1481, 1453, 1440, 1255, 1242, 1212, 832, 755, 745, 541.

LCMS (ESI^{+}): calculated for $C_{24}H_{22}N_2O_3$ $[M+H]^+$ 387.1630; found: *m/z* = 386.96.

5) Synthesis of 4-[4-(phenanthrene-9-carbonyl)piperazin-1-yl]phenol (79)



79

Phenanthrene-9-carboxylic acid (0.5 g, 2.25 mmol) was reacted with 4-piperazin-1-ylphenol (**IV**) (0.4 g, 2.25 mmol) as per the procedure described for compound **76** to afford compound **79** (0.38 g, 0.99 mmol, 44% yield) as an off white solid.

Melting point: 195-198 °C. **LC purity (UV 245 nm):** 98.26%.

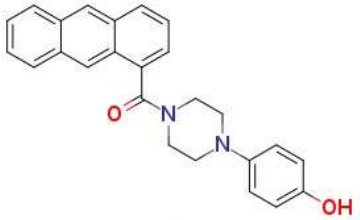
^{1}H NMR (400 MHz, DMSO-d$_6$): δ 2.81-2.82 (m, 1H), 2.95 (m, 1H), 3.19 (m, 2H), 3.33 (m, 2H), 3.93 – 3.95 (m, 1H), 4.05 – 4.07 (m, 1H), 6.70 (d, 2H, *J* = 8.68 *Hz*), 6.84 (d, 2H, *J* = 8.72 *Hz*), 7.74 – 7.78 (m, 2H), 7.81 (t, 2H, *J* = 7.49 *Hz*), 7.91 (d, 1H, *J* = 7.67 *Hz*), 7.92 (s, 1H), 8.11 (d, 1H, *J* = 7.83 *Hz*), 8.93 (d, 1H, *J* = 8.26 *Hz*), 8.97 (s, 1H), 8.98 (d, 1H, *J* = 8.43 *Hz*).

^{13}C NMR (100 MHz, DMSO-d$_6$): δ 41.6 (CH_2), 47.1 (CH_2), 50.8 (CH_2), 51.0 (CH_2), 115.8 (2 x CH), 118.8 (2 x CH), 123.3 (CH), 123.8 (CH), 124.8 (CH), 125.8 (CH), 127.6 (CH), 127.7 (CH), 127.8 (CH), 128.0 (CH), 128.3 (C), 129.3 (CH), 130.0 (C), 130.2 (C), 130.7 (C), 133.4 (C), 144.1 (C), 151.8 (C), 168.0 (C).

IR (ATR) / cm^{-1}: 3265, 2817, 1631, 1508, 1481, 1440, 1267, 1240, 1198, 1145, 992, 915, 823, 761.

LCMS (ESI$^+$): calculated for $C_{25}H_{22}N_2O_2$ $[M+H]^+$ 383.16; found: *m/z* = 383.20.

6) Synthesis of 4-[4-(anthracene-1-carbonyl)piperazin-1-yl]phenol (80)



80

Anthracene-1-carboxylic acid (0.3 g, 1.34 mmol) was reacted with 4-piperazin-1-ylphenol (**IV**) (0.24 g, 1.34 mmol) as per the procedure described for compound **76** to afford compound **80** (0.40 g, 1.04 mmol, 77% yield) as an off white solid.

Melting point: 193-197 °C. **LC purity (UV 245 nm):** 98.90%.

^{1}H NMR (400 MHz, DMSO-d$_6$): δ 2.80 (m, 1H), 2.91 (m, 1H), 3.22 - 3.33 (m, 4H), 3.98 (m, 1H), 4.10 (m, 1H), 6.69 (d, 2H, *J* = 8.79 *Hz*), 6.84 (d, 2H, *J* = 8.78 *Hz*), 7.54 (d, 1H, *J* = 6.59 *Hz*), 7.58 – 7.63 (m, 3H), 8.17 (d, 1H, *J* = 7.83 *Hz*), 8.22 - 8.23 (m, 2H), 8.50 (s, 1H), 8.73 (s, 1H), 8.94 (s, 1H)

^{13}C NMR (100 MHz, DMSO-d$_6$): δ 41.7 (CH_2), 47.1 (CH_2), 50.8 (CH_2), 51.0 (CH_2), 115.8 (2 x CH), 118.8 (2 x CH), 123.7 (CH), 123.8 (CH), 125.0 (CH), 126.4 (2 x CH), 127.2 (CH), 127.6 (C), 128.2 (CH), 128.6 (CH), 129.5 (CH), 131.2 (C), 131.6 (C), 131.9 (C), 134.6 (C), 144.1 (C), 151.7 (C), 168.2 (C).

IR (ATR) / cm^{-1}: 3238, 2808, 1602, 1591, 1509, 1471, 1439, 1266, 1215, 1178, 1147, 1029, 879, 835, 733, 545, 475.

LCMS (ESI$^+$): calculated for $C_{25}H_{22}N_2O_2$ $[M+H]^+$ 383.16; found: *m/z* = 383.20.

7) Synthesis of 4-[4-(1,2,3,4-tetrahydroacridine-9-carbonyl)piperazin-1-yl]phenol (81)

81

1,2,3,4-Tetrahydroacridine-9-carboxylic acid (0.3 g, 1.32 mmol) was reacted with 4-piperazin-1-ylphenol (**IV**) (0.23 g, 1.32 mmol) as per the procedure described for compound **76** to afford compound **81** (0.28 g, 0.72 mmol, 54% yield) as an off white solid.

Melting point: 167-170 °C. **LC purity (UV 245 nm):** 99.29%.

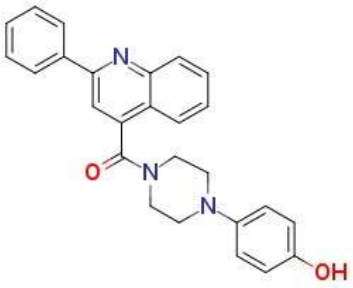
^{1}H NMR (400 MHz, DMSO-d$_6$): δ 1.86 - 2.00 (m, 4H), 2.79 - 2.93 (m, 4H), 3.09 - 3.28 (m, 6H), 4.04 - 4.09 (m, 2H), 6.70 (d, 2H, *J* = 8.93 *Hz*), 6.84 (d, 2H, *J* = 8.96 *Hz*), 7.58 - 7.62 (m, 1H), 7.68 (d, 1H, *J* = 8.26 *Hz*), 7.73 - 7.77 (m, 1H), 7.99 (d, 1H, *J* = 8.45 *Hz*), 8.95 (s, 1H).

^{13}C NMR (100 MHz, DMSO-d$_6$): δ 21.9 (CH_2), 22.3 (CH_2), 26.0 (CH_2), 33.3 (CH_2), 40.8 (CH_2), 45.8 (CH_2), 50.4 (CH_2), 50.8 (CH_2), 115.5 (2 x CH), 118.5 (2 x CH), 122.5 (C), 124.1 (CH), 126.0 (CH), 126.6 (CH), 128.5 (CH), 129.1 (C), 140.2 (C), 143.6 (C), 145.7 (C), 151.5 (C), 159.0 (C), 165.3 (C).

IR (ATR) / cm^{-1}: 3018, 2810, 1627, 1514, 1438, 1338, 1284, 1262, 1226, 1155, 993, 939, 821, 797, 759, 702, 624, 533.

LCMS (ESI$^+$): calculated for $C_{24}H_{25}N_3O_2$ $[M+H]^+$ 388.19; found: *m/z* = 388.18.

8) Synthesis of 4-[4-(2-phenylquinoline-4-carbonyl)piperazin-1-yl]phenol (82)



82

2-Phenylquinoline-4-carboxylic acid (0.4 g, 1.60 mmol) was reacted with 4-piperazin-1-ylphenol (**IV**) (0.28 g, 1.60 mmol) as per the procedure described for compound **76** to afford compound **82** (0.35 g, 0.85 mmol, 53% yield) as an off white solid.

Melting point: 181-185 °C. **LC purity (UV 245 nm):** 99.69%.

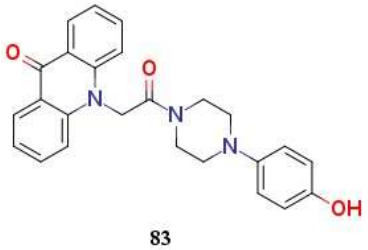
^{1}H NMR (400 MHz, DMSO-d$_6$): δ 2.94 (s, 2H), 3.12 (s, 4H), 3.98 (s, 2H), 6.72 (d, 2H, *J* = 8.93*Hz*), 6.92 (d, 2H, *J* = 8.57*Hz*), 7.56-7.67(m, 3H), 7.72 (t, 1H, *J* = 5.92*Hz*), 7.89 (d, 2H, *J* = 7.83*Hz*), 8.19-8.23 (m, 2H), 7.35 (d, 2H, *J* = 7.25 *Hz*), 8.95 (s, 1H).

^{13}C NMR (100 MHz, DMSO-d$_6$): δ 41.8 (CH_2), 47.2 (CH_2), 50.8 (CH_2), 51.1 (CH_2), 116.00 (2 x CH), 116.05 (CH), 119.0 (2 x CH), 123.4 (C), 125.2 (CH), 127.8 (2 x CH), 128.0 (CH), 129.4 (2 x CH), 130.2 (CH), 130.5 (CH), 131.0 (CH), 138.5 (C), 143.9 (C), 144.2 (C), 148.1 (C), 151.9 (C), 156.4 (C), 166.2 (C).

IR (ATR) / cm^{-1}: 2980, 1618, 1510, 1441, 1382, 1227, 1152, 995, 788, 694, 636, 541.

LCMS (ESI$^+$): calculated for $C_{26}H_{23}N_3O_2$ $[M+H]^+$ 410.17; found: *m/z* = 410.21.

9) Synthesis of 10-{2-[4-(4-hydroxyphenyl)piperazin-1-yl]-2-oxoethyl}-9,10-dihydro acridin-9-one (83)



83

2-(9-Oxo-9,10-dihydroacridin-10-yl)acetic acid (0.25 g, 0.98 mmol) was reacted with 4-piperazin-1-ylphenol (**IV**) (0.17 g, 0.98 mmol) as per the procedure described for compound **76** to afford compound **83** (0.35 g, 0.84 mmol, 85% yield) as a pale yellow solid.

Melting point: 181-185 °C. **LC purity (UV 245 nm):** 98.75%.

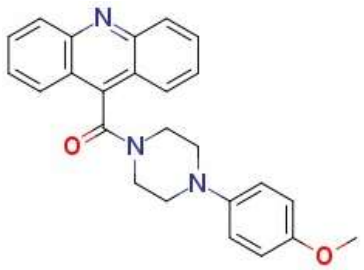
^{1}H NMR (400 MHz, DMSO-d$_6$): δ 3.06 (m, 2H), 3.26 (m, 2H), 3.69 (m, 2H), 3.93 (m, 2H), 5.61 (s, 2H), 6.76 (d, 2H, *J* = 8.74 *Hz*), 6.94 (d, 2H, *J* = 8.76 *Hz*), 7.39 (t, 2H, *J* = 7.41 *Hz*), 7.63 (d, 2H, *J* = 8.75 *Hz*), 7.85 (t, 2H, *J* = 7.20 *Hz*), 8.41 (d, 2H, *J* = 6.94 *Hz*), 8.97 (s, 1H).

^{13}C NMR (100 MHz, DMSO-d$_6$): δ 42.3 (CH_2), 45.0 (CH_2), 47.9 (CH_2), 50.7 (CH_2), 51.2 (CH_2), 115.9 (2 x CH), 116.7 (2 x CH), 119.0 (2 x CH), 121.8 (2 x CH), 122.1 (2 x CH), 126.9 (2 x CH), 134.5 (2 x C), 143.0 (2 x C), 144.4 (C), 151.9 (C), 165.3 (C), 177.2 (C).

IR (ATR) / cm^{-1}: 3156, 2981, 1663, 1608, 1596, 1562, 1514, 1500, 1461, 1438, 1267, 1224, 1185, 1153, 1029, 818, 755, 691, 676, 559.

LCMS (ESI$^+$): calculated for $C_{25}H_{23}N_3O_3$ $[M+H]^+$ 414.17; found: *m/z* = 414.20.

10) Synthesis of 9-[4-(4-methoxyphenyl)piperazine-1-carbonyl]acridine (84)



84

Acridine-9-carboxylic acid (0.50 g, 2.23 mmol) was reacted with commercially available 1-(4-methoxyphenyl)piperazine (0.43 g, 2.23 mmol) as per the procedure described for compound **76** to afford compound **84** (0.81 g, 2.03 mmol, 91% yield) as a pale yellow solid.

Melting point: 173-176 °C. **LC purity (UV 245 nm):** 99.15%.

^{1}H NMR (400 MHz, DMSO-d$_6$): δ 2.85 (t, 2H, *J* = 4.73 *Hz*), 3.16 (t, 2H, *J* = 4.73 *Hz*), 3.36 (t, 2H, *J* = 4.76 *Hz*), 3.72 (s, 3H), 4.16 (t, 2H, *J* = 4.89 *Hz*), 6.86 (d, 2H, *J* = 9.02 *Hz*), 6.94 (d, 2H, *J* = 9.04 *Hz*), 7.76 (t, 2H, *J* = 7.58 *Hz*), 7.97 (t, 2H, *J* = 7.65 *Hz*), 8.02 (d, 2H, *J* = 8.59 *Hz*), 8.28 (d, 2H, *J* = 8.75 *Hz*).

^{13}C NMR (100 MHz, DMSO-d$_6$): δ 43.5 (CH_2), 48.6 (CH_2), 52.4 (CH_2), 52.6 (CH_2), 57.5 (CH_3), 116.6 (2 x CH), 120.5 (2 x CH), 123.8 (2 x C), 127.6 (2 x CH), 129.7 (2 x CH), 131.9 (2 x CH), 133.3 (2 x CH), 142.9 (C), 147.1 (C), 150.5 (2 x C), 155.7 (C), 167.1 (C).

IR (ATR) / cm^{-1}: 1637, 1509, 1438, 1405, 1278, 1240, 1225, 1175, 1152, 1035, 819, 787, 762, 693, 656, 633, 536.

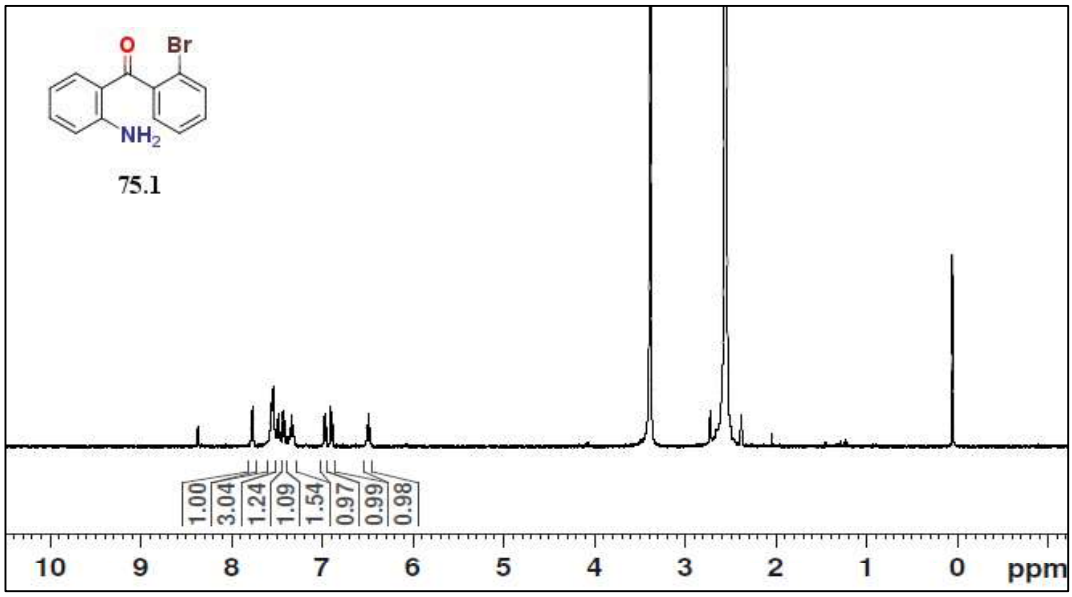
LCMS (ESI$^+$): calculated for $C_{25}H_{23}N_3O_2$ $[M+H]^+$ 398.17; found: *m/z* = 398.21.

5.5 Conclusion

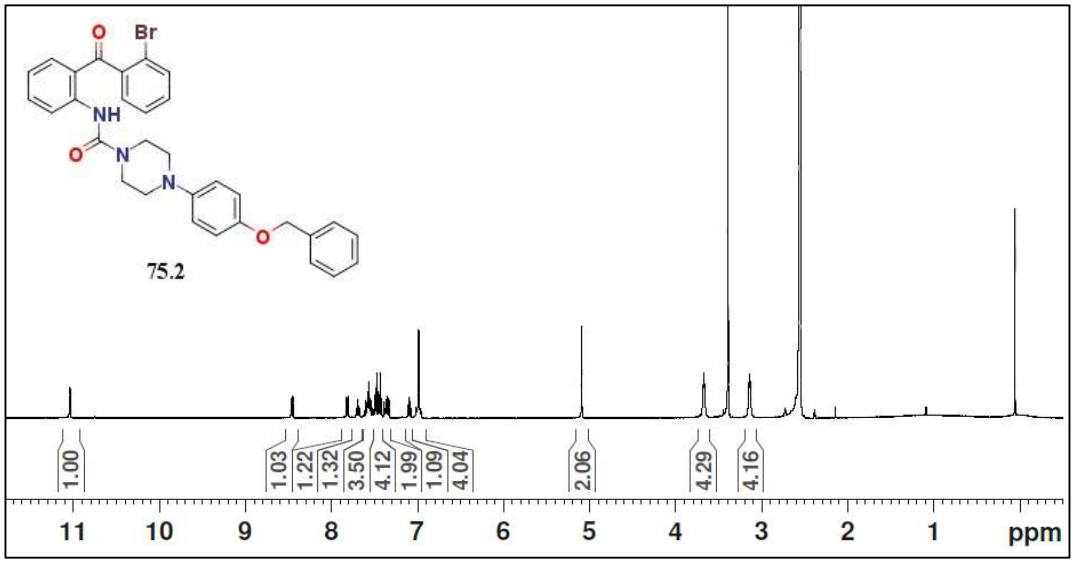
Diverse analogues with tricyclic aromatic rings were designed, synthesized and evaluated which led to compound **76** as potential dual ME3-tubulin inhibitor. Further this compound exhibited sub nanomolar activity on the pancreatic cancer cell lines studied. However, these analogues have to be assessed comprehensively for their risk vs benefit ratio in *in vivo* context.

5.6 Spectral data

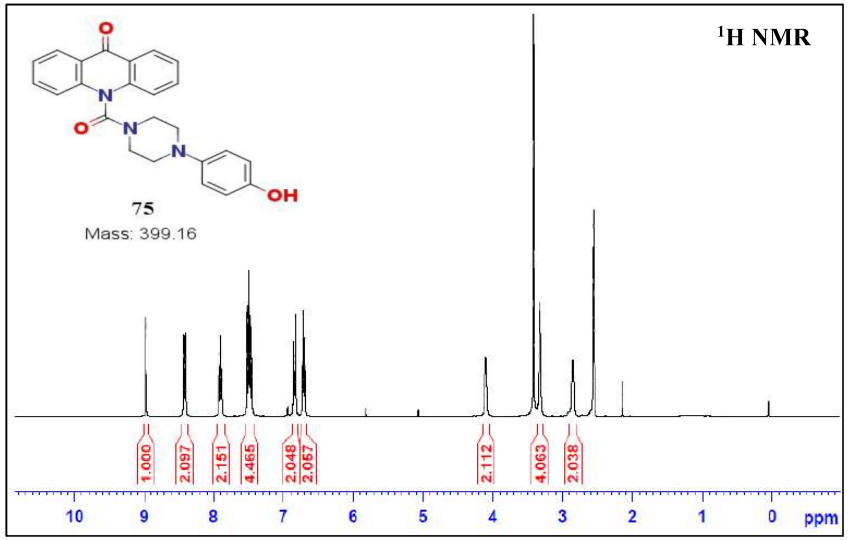
^{1}H NMR of **Intermediate-75.1**

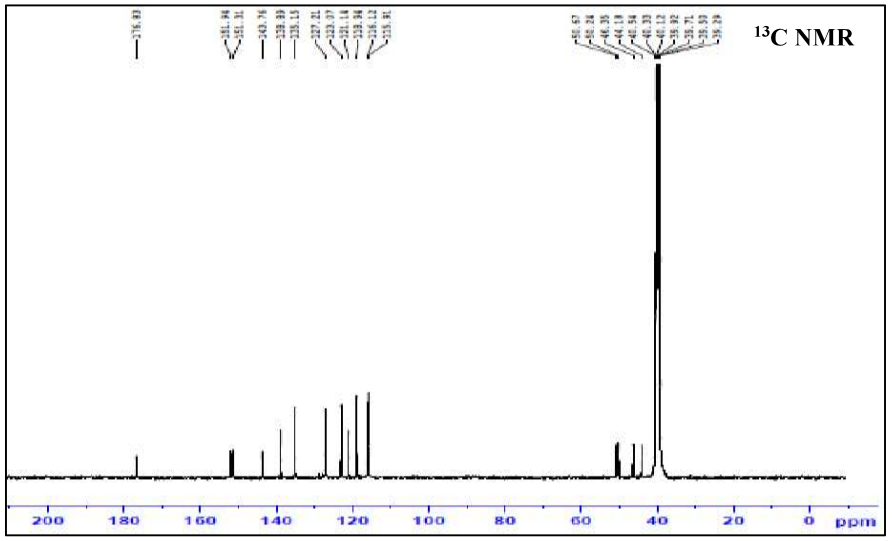


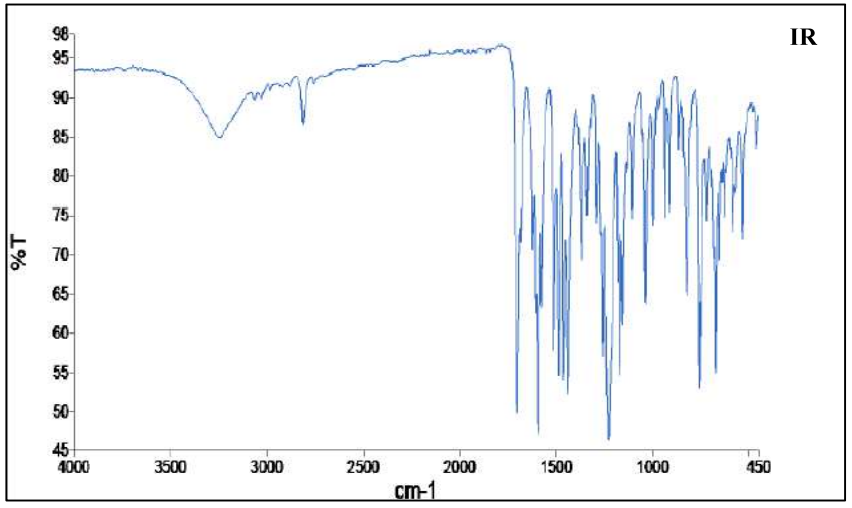
^{1}H NMR of **Intermediate-75.2**

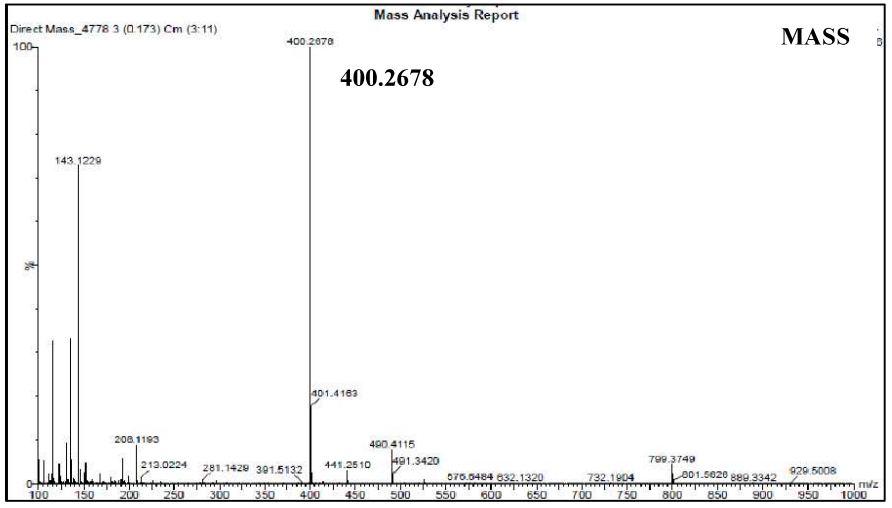


Spectral data of **75**

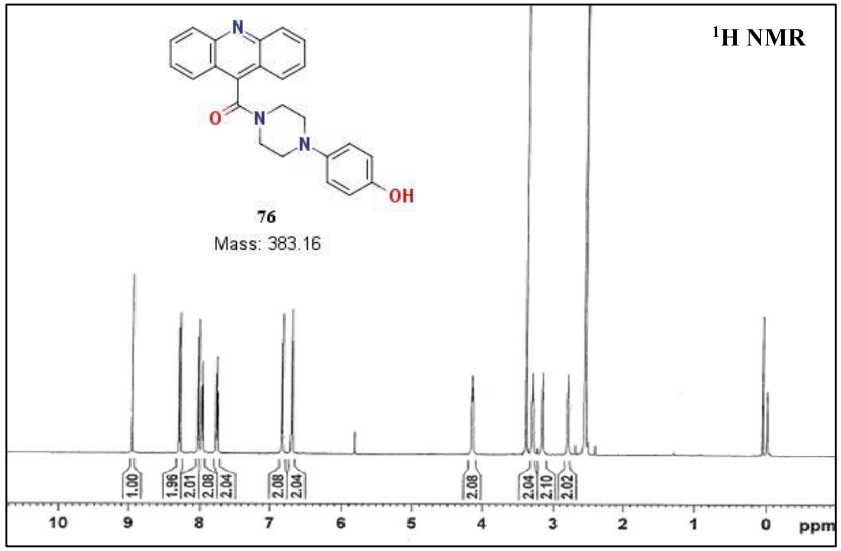


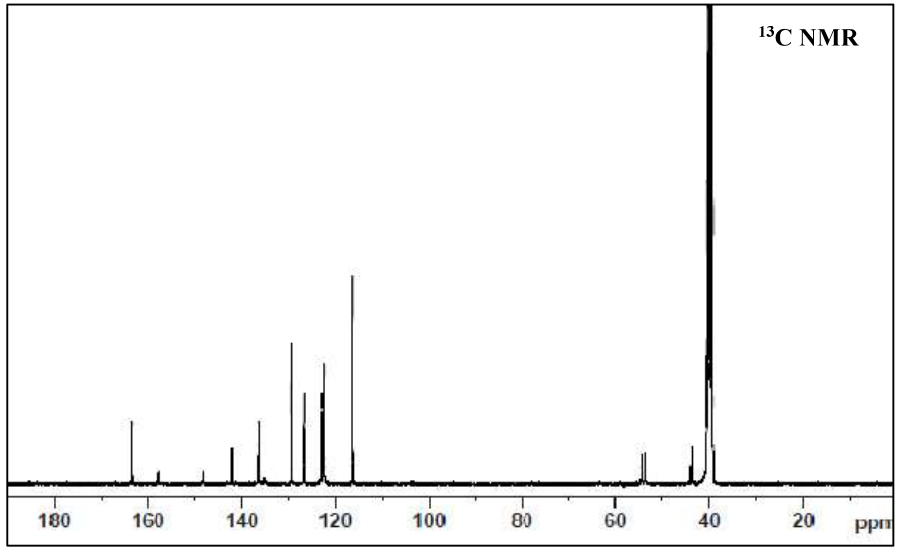


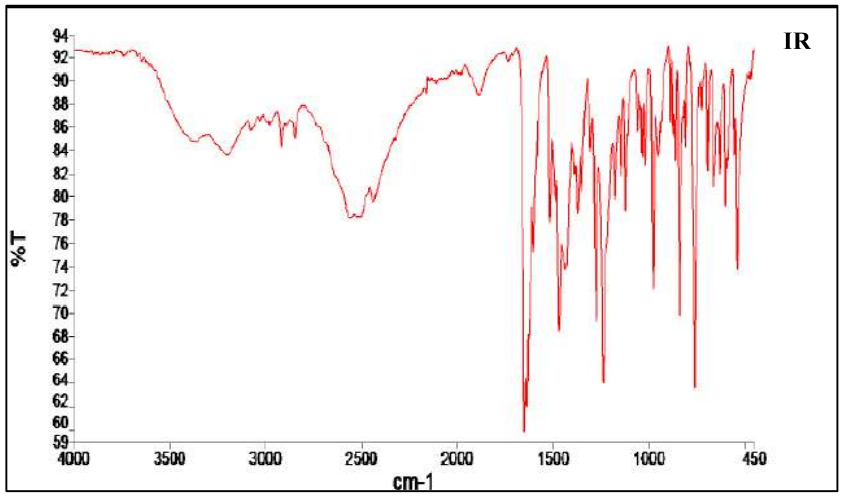


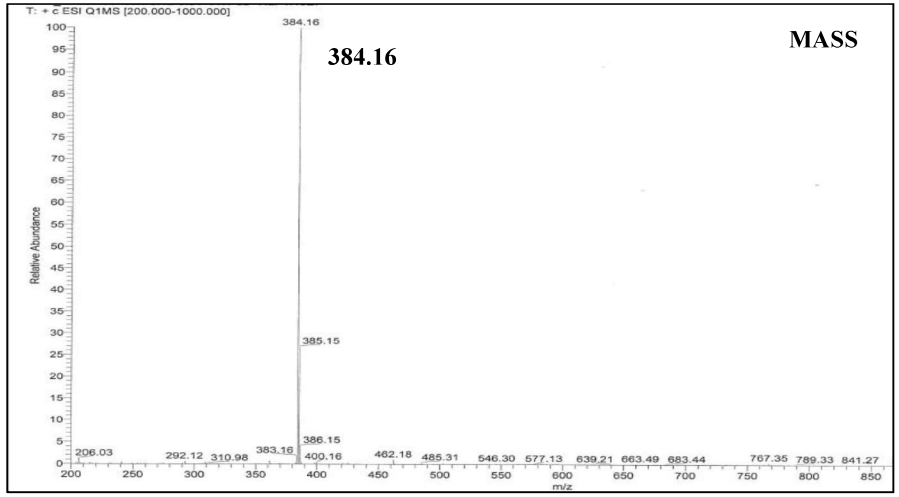


Spectral data of **76**

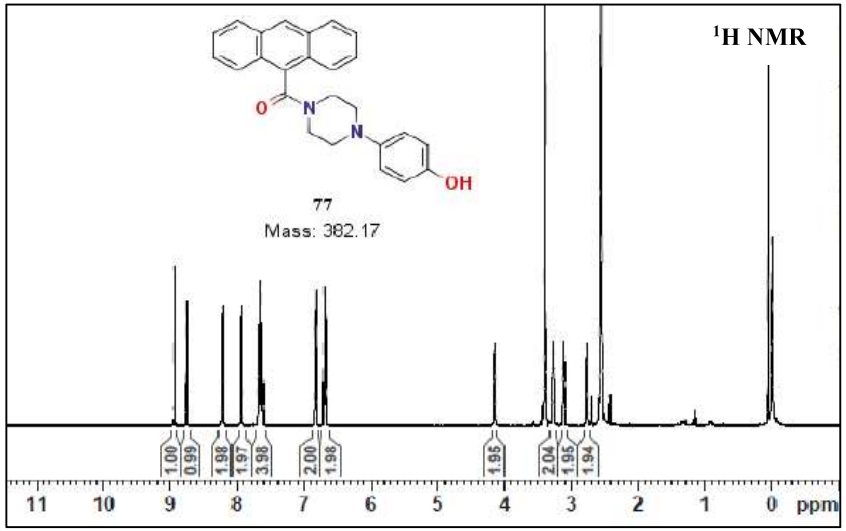


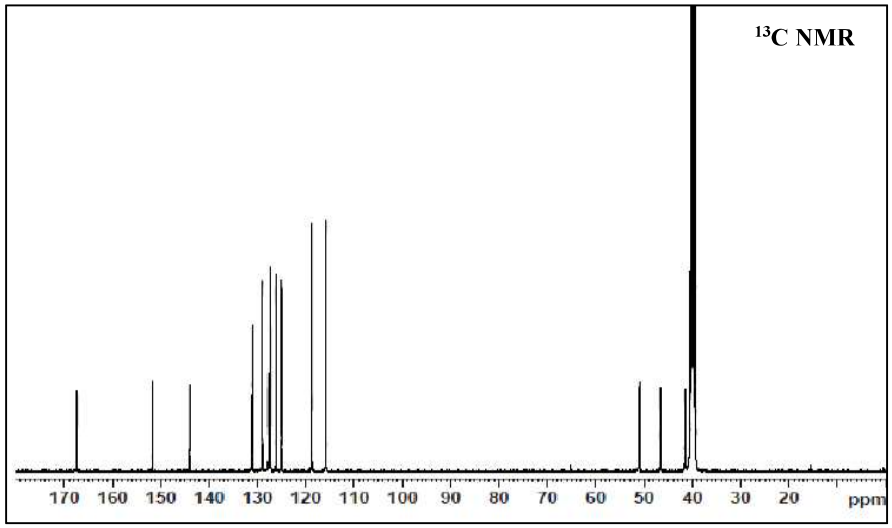


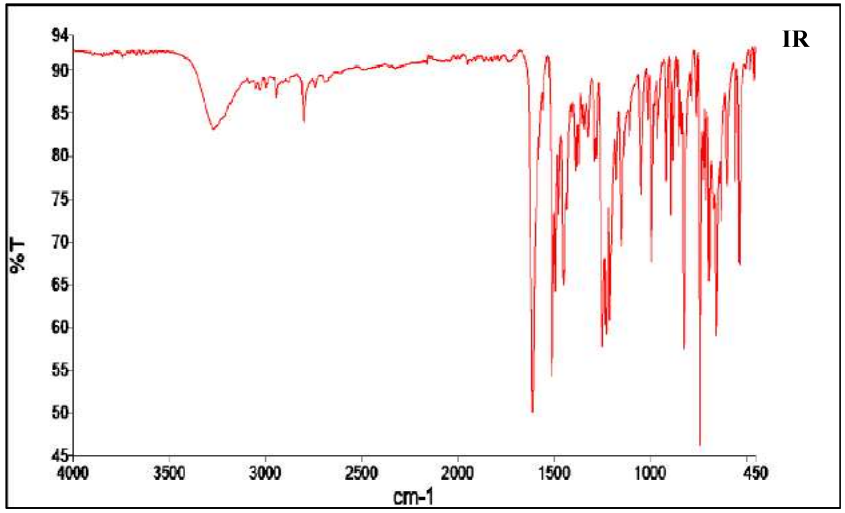


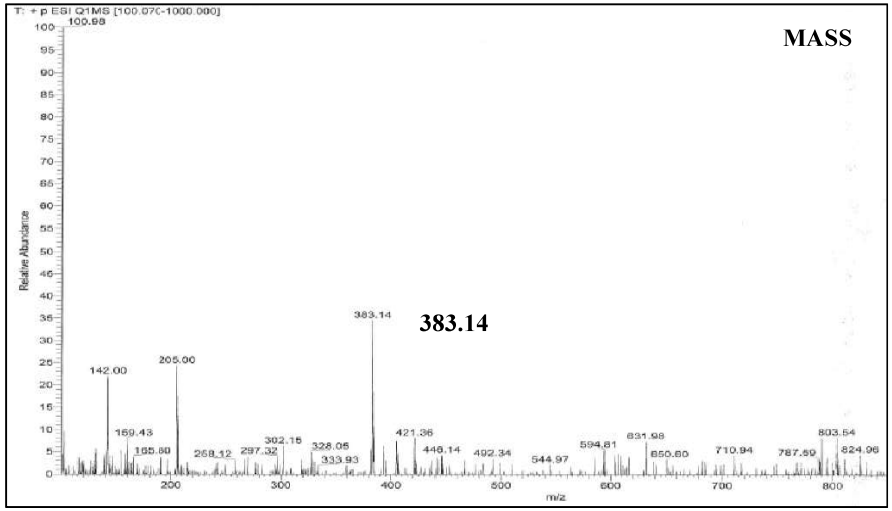


Spectral data of **77**

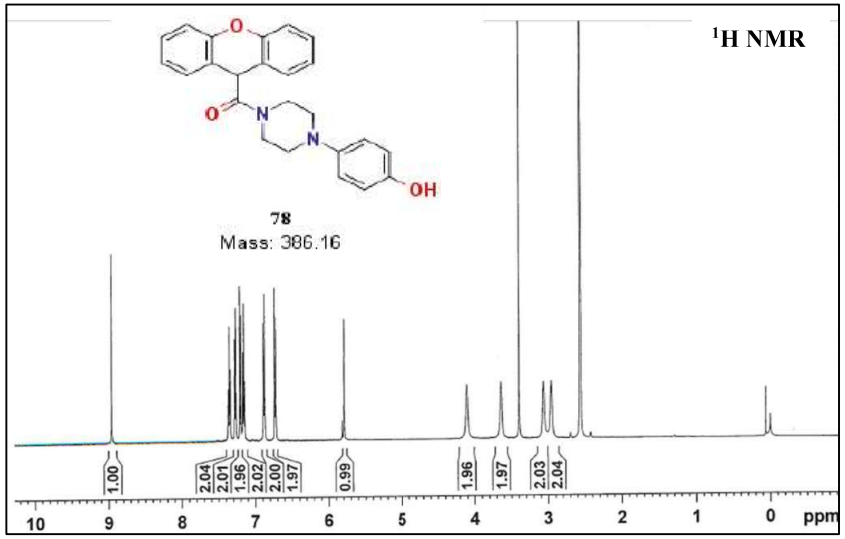


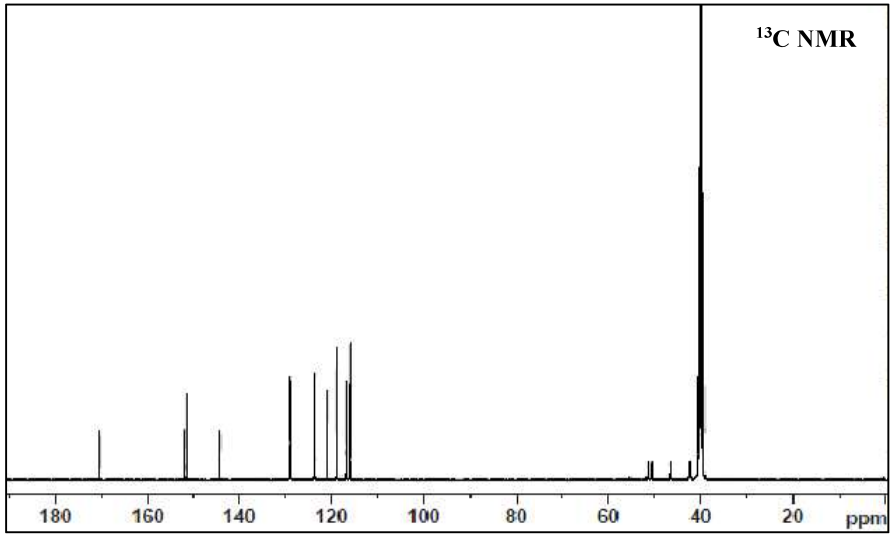


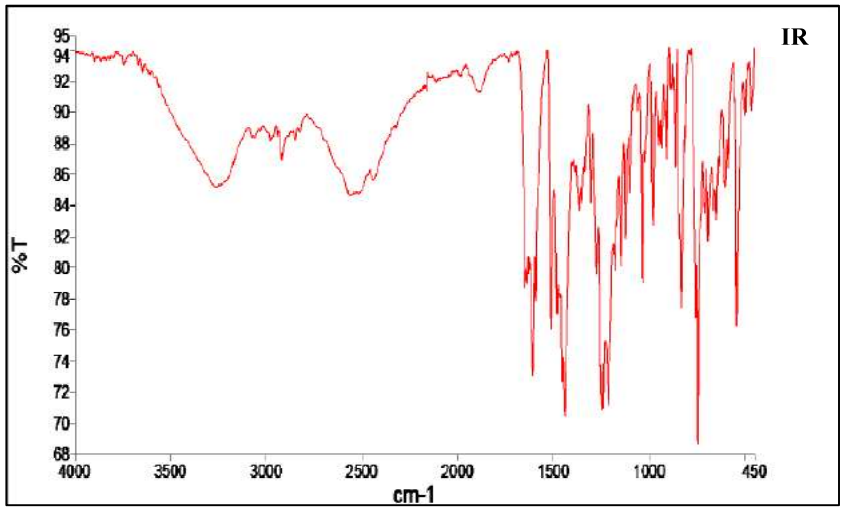


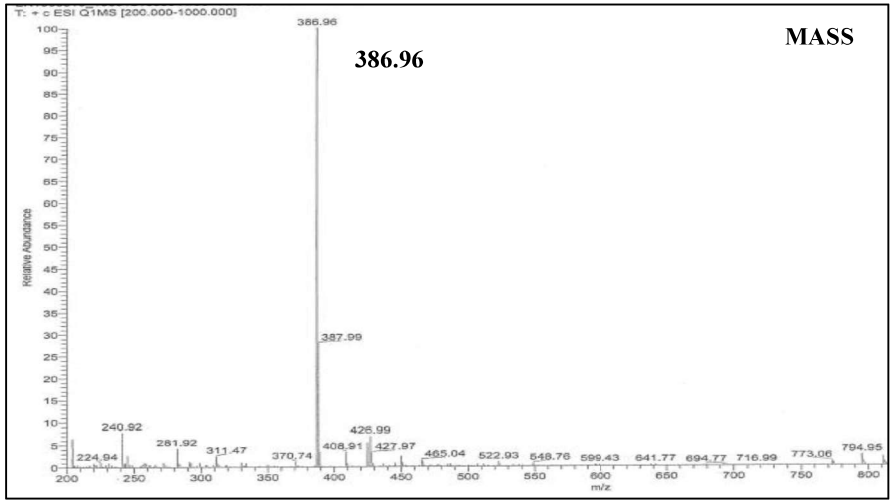


Spectral data of **78**

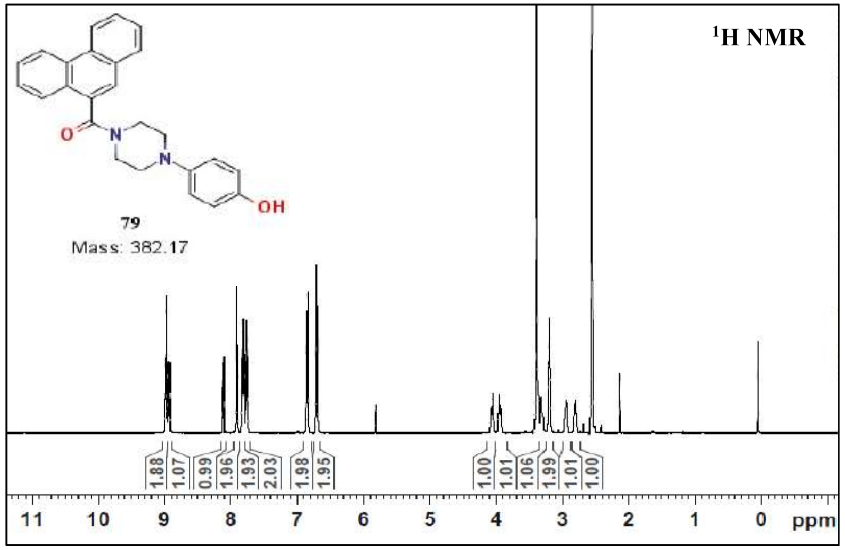


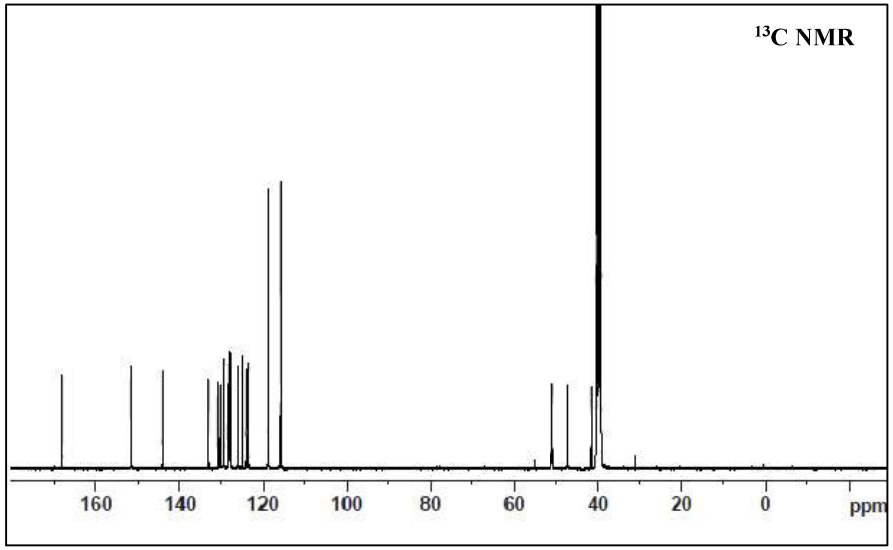


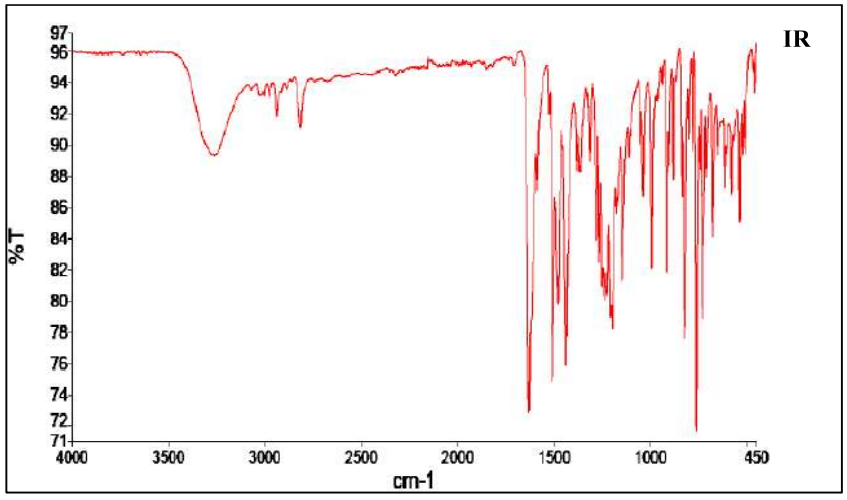


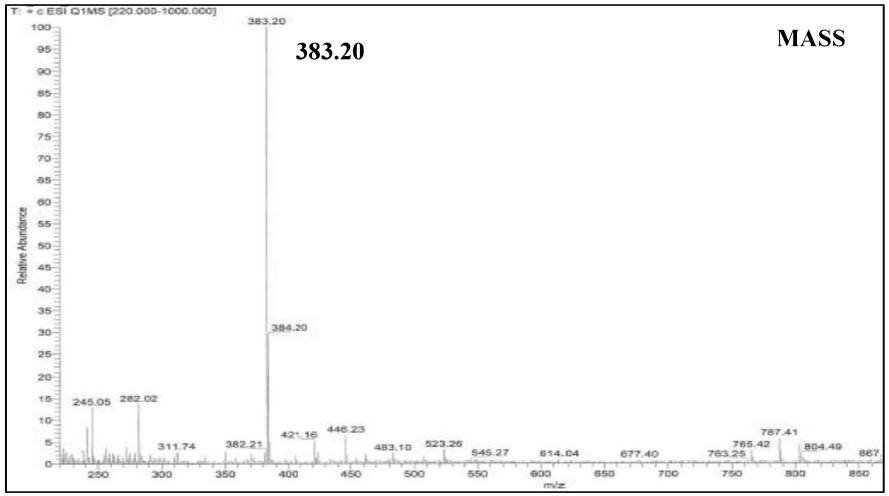


Spectral data of **79**

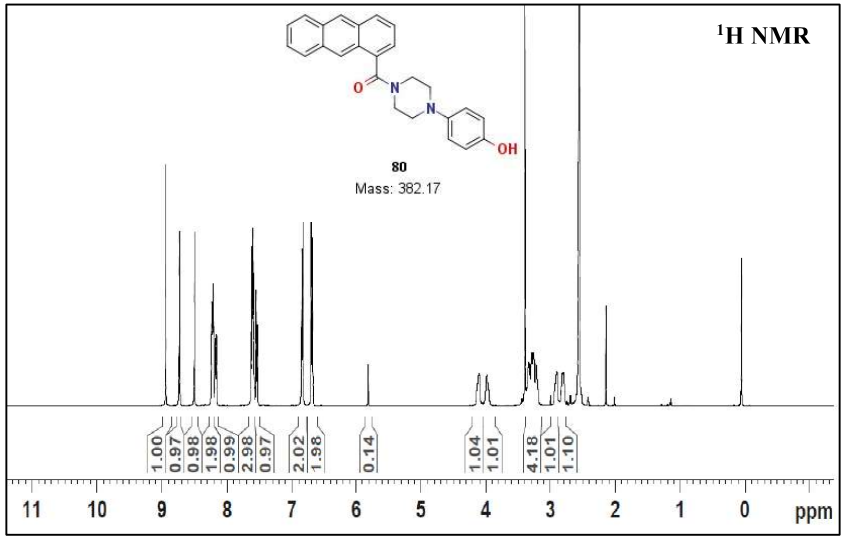


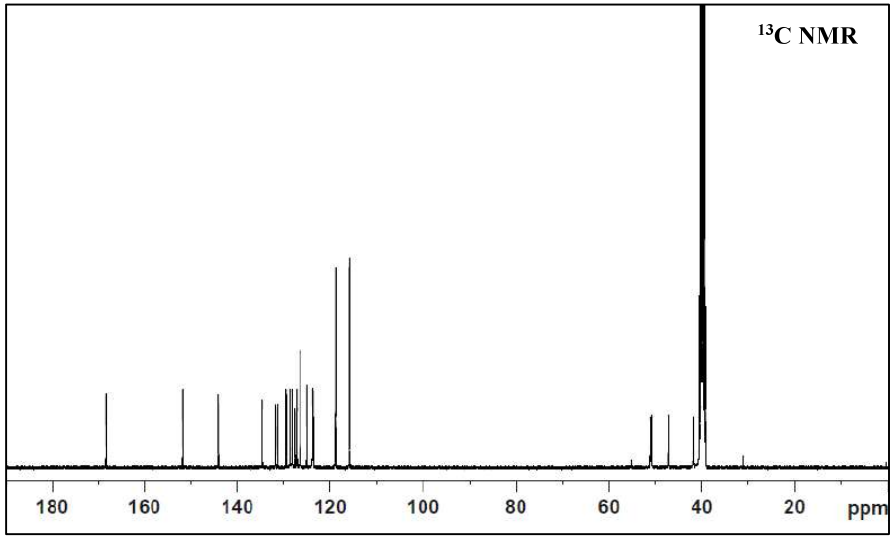


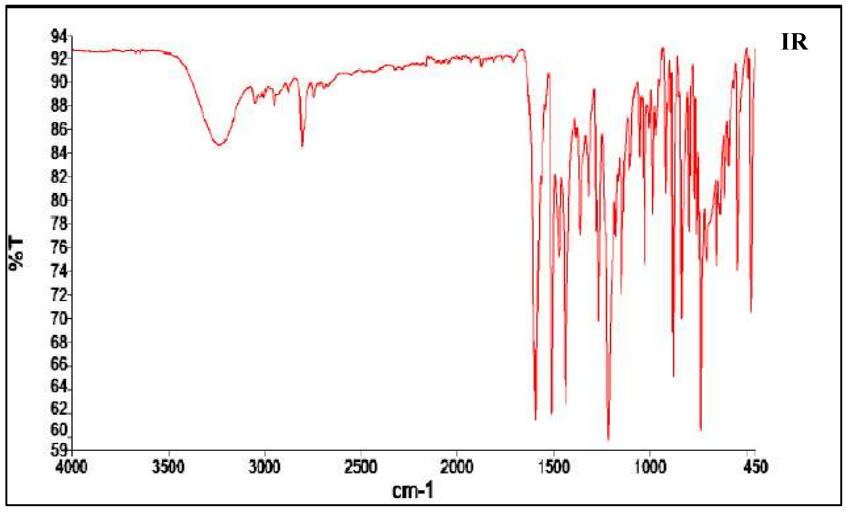


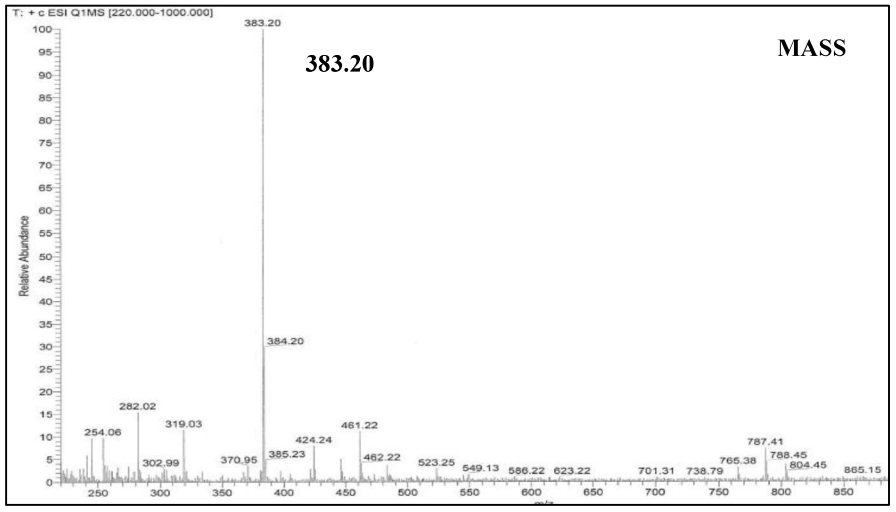


Spectral data of **80**

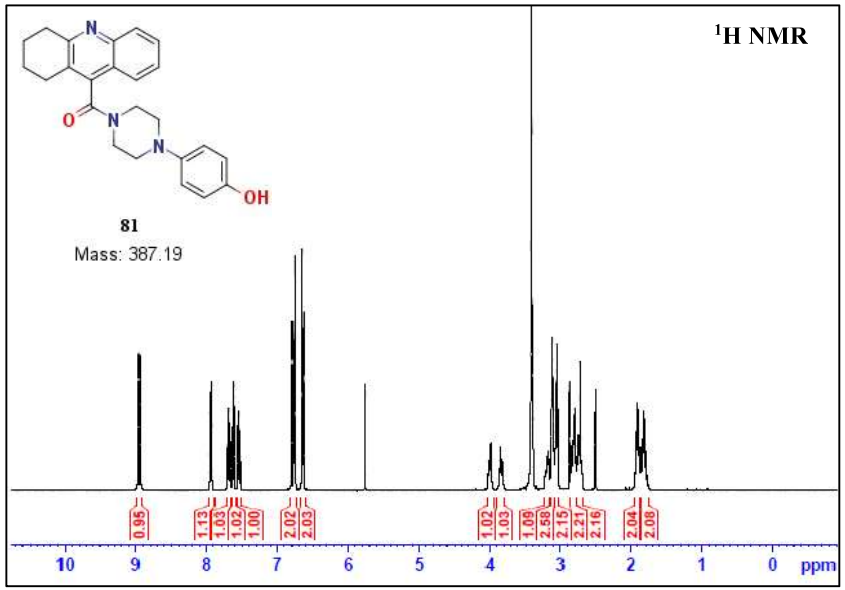


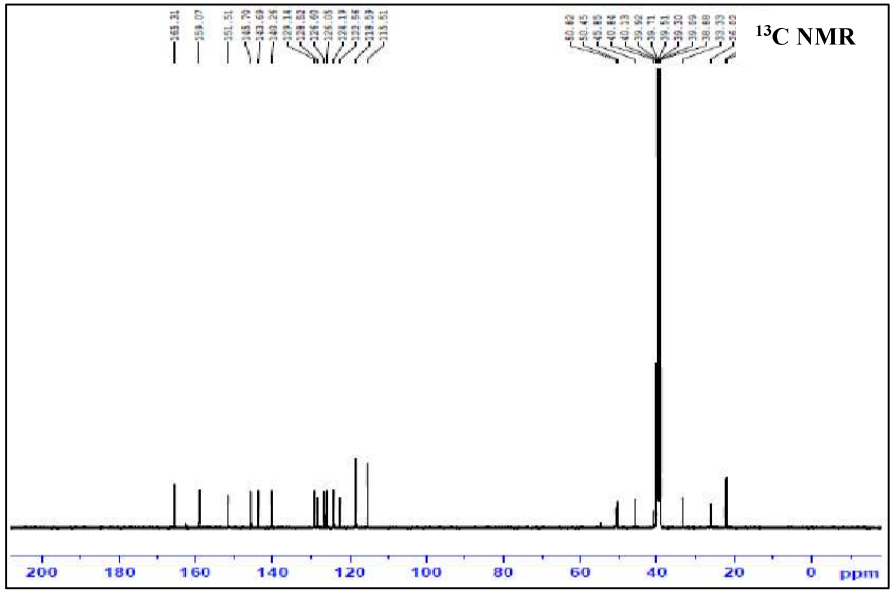


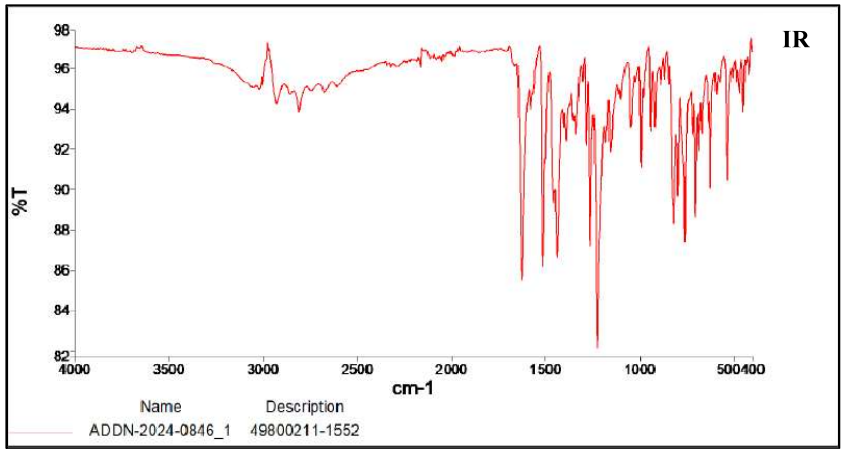


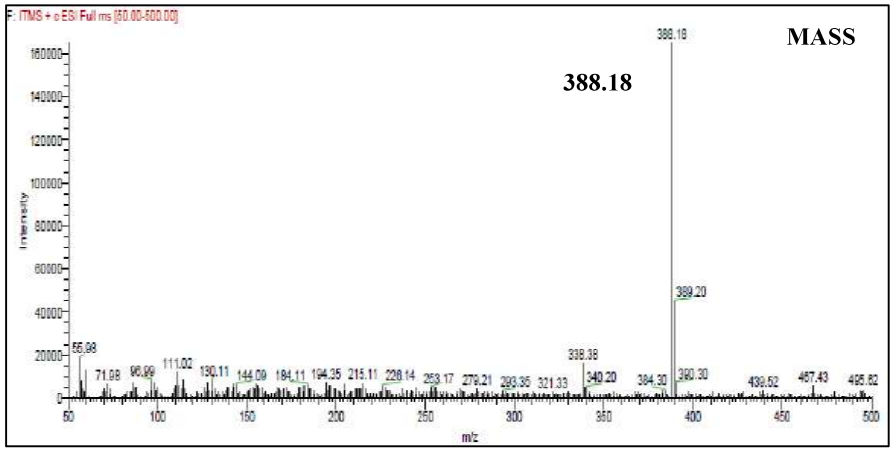


Spectral data of **81**

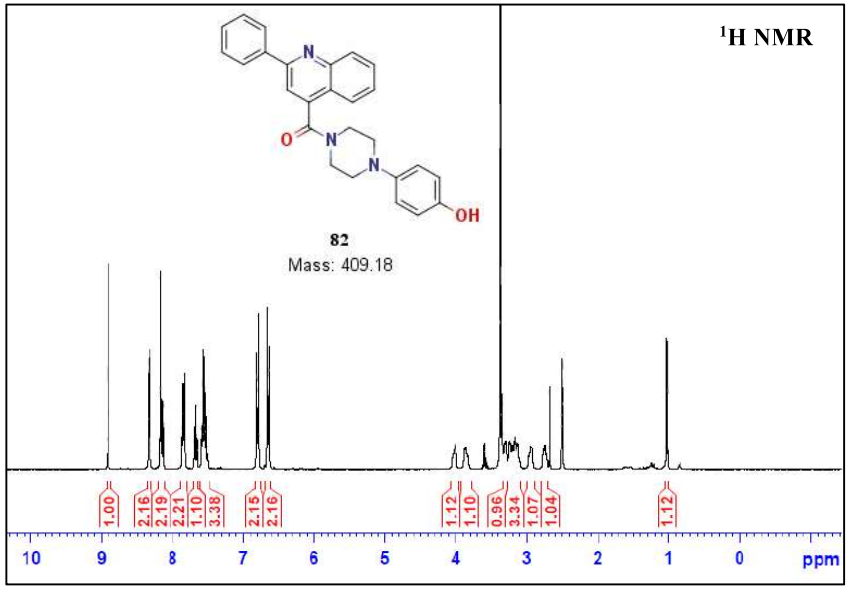


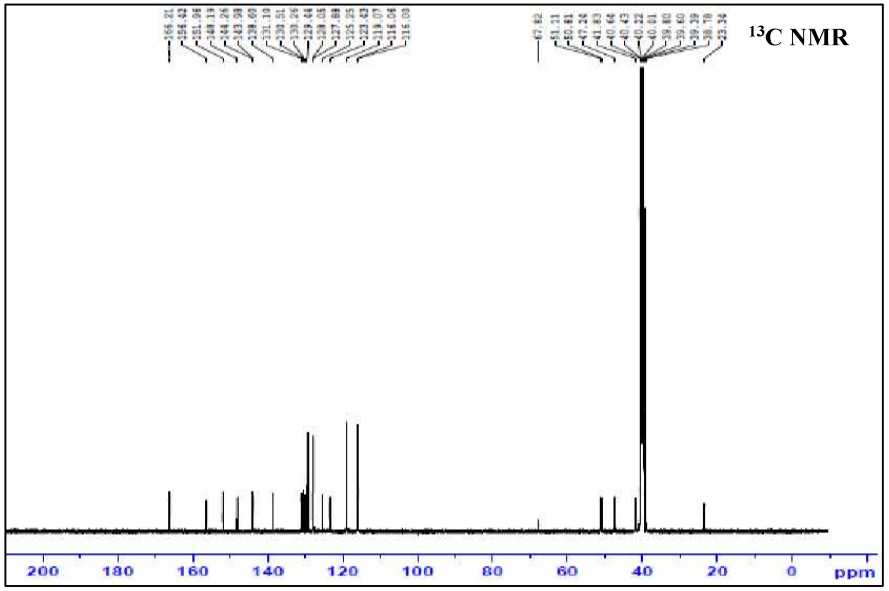


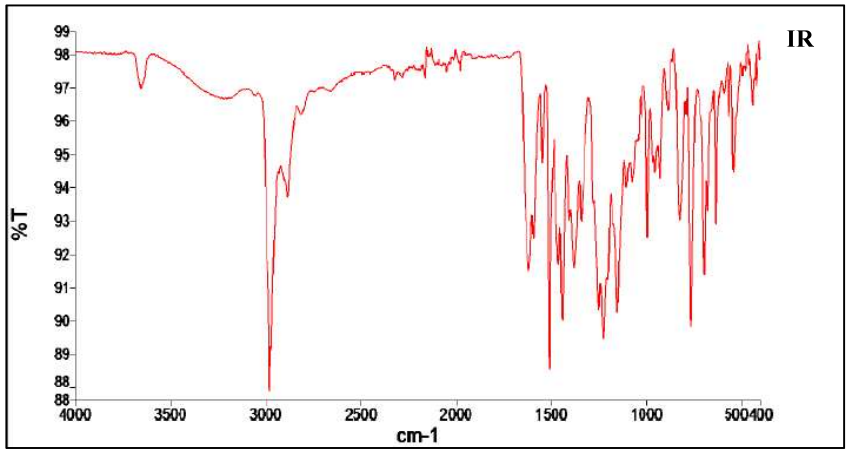


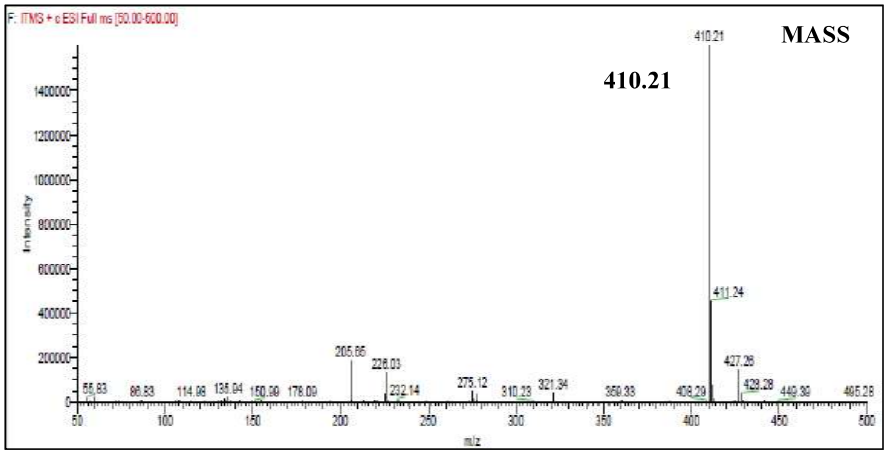


Spectral data of **82**

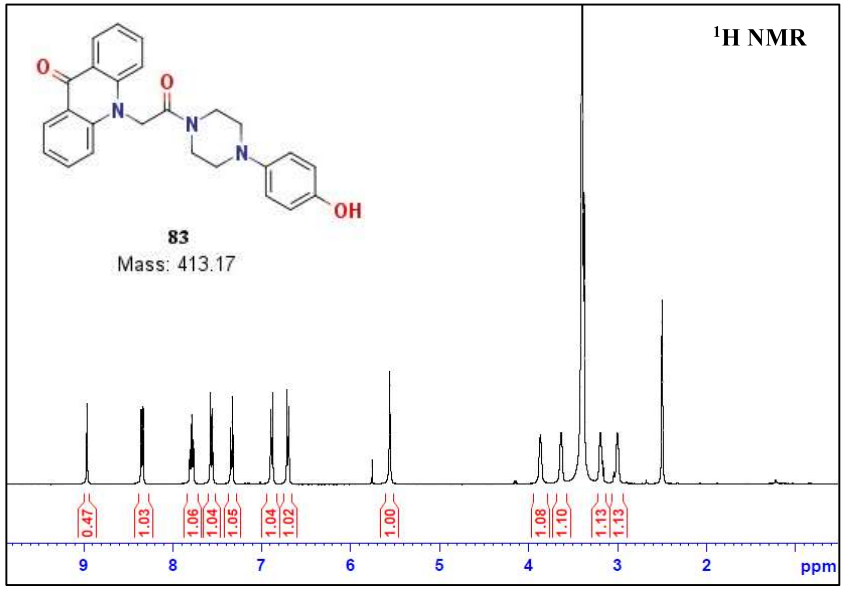


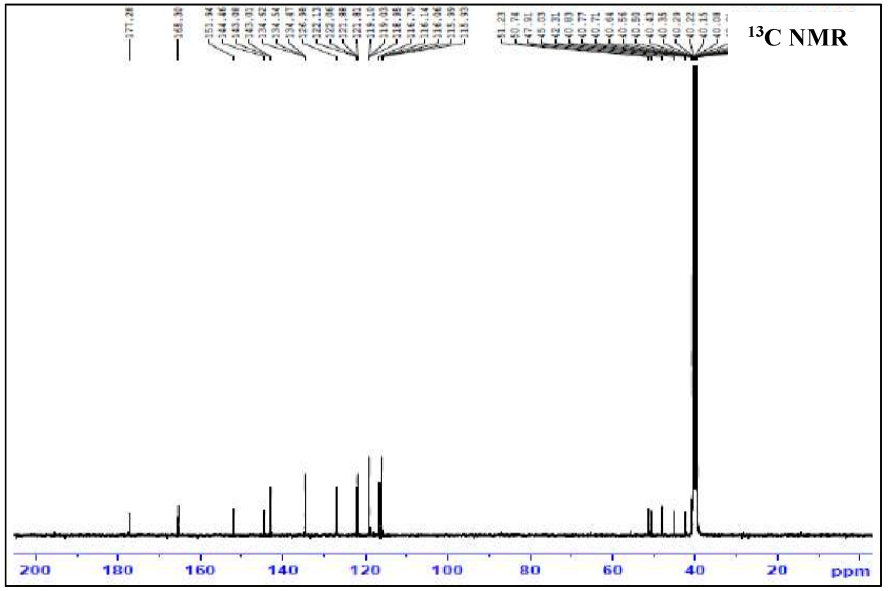


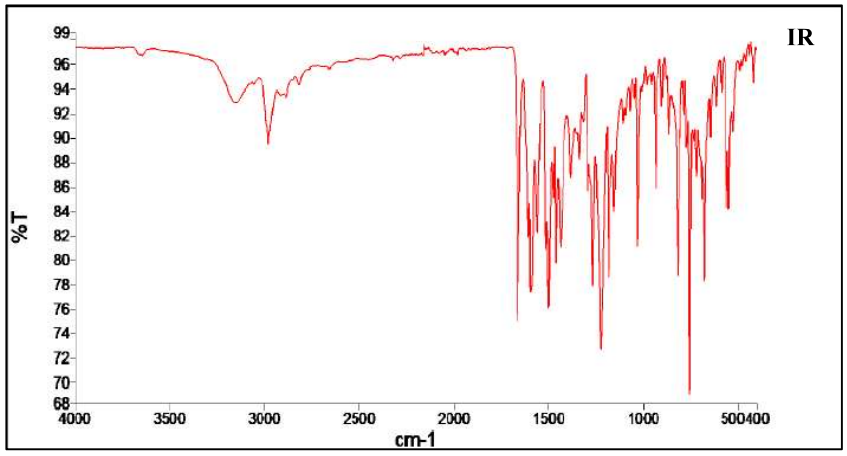


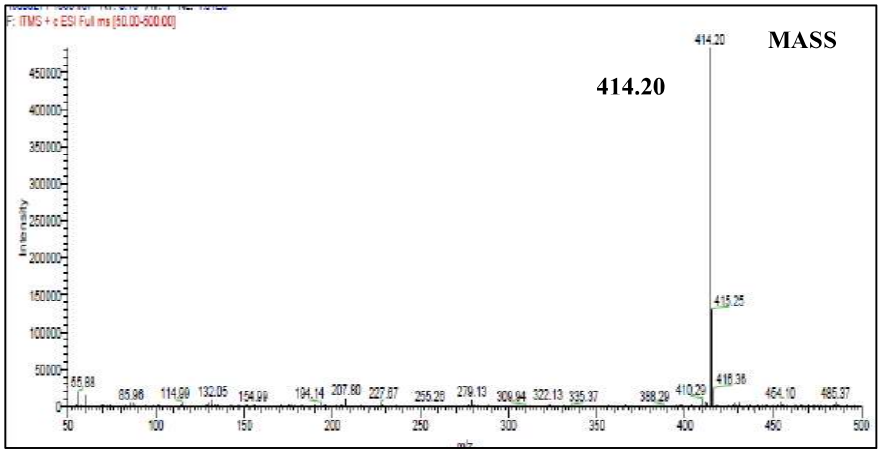


Spectral data of **83**









Spectral data of **84**