Supporting information

Green method synthesis of 3-methyl-4-(hetero)aryl methylene isoxazole-5(4*H*)-ones using WEOFPA/glycerol: Evaluation of anticancer and electrochemical behaviour properties

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1. Material and method

1.1. General

The reagents used in this work for the synthesis purchased from Avra and used directly without further purification. FT-IR spectra Thermo-Fischer scientific in KBr pellet method data collected, ^{1}H and ^{13}C-NMR spectra recorded on a Bruker-Avance 300MHz spectrometer, chemical shifts are reported in ppm (δ) and it relative to the tetramethylsilane as internal standard. LC-MS spectra are recorded in waters synapt G2 high detection mass spectrometry. The progress of the reaction was monitored by TLC using mobile phase ($CHCl_3$: MeOH, 9.5:0.5). The melting point determined in open capillaries and are uncorrected. The electrochemical behavior studies were evaluated using CHI6005E Electrochemical Analyzer.

1.2. Preparation of WEOFPA

We are adopted recently reported by our group procedure for the preparation of WEOFPA, Briefly, the peel is collected from the local market, washed with distilled water and dried under sunlight for about 4-5 days. After thorough drying, the shell was directly burnt to obtain ash by Bunsen flame. Taken 10 gm of the ash, suspended in 100 mL of double-distilled water, and stirred at room temperature for about 1 h, filtered, and the brown colored filtrate obtained is named WEOFPA.

1.3. General synthetic procedure

In a round-bottomed flask WEOFPA 5 mL, glycerol (0.4 mL) is added and heated with constant stirring in an oil bath for about 5 min to become a eutectic mixture at 60 ^{o}C. To this added, substituted aromatic or heteroaromatic aldehyde **(1)** (1 mmol), ethyl acetoacetate **(2)** (1 mmol) and hydroxylamine hydrochloride **(3)** (1 mmol). The stirring and temperature are controlled and continued until the reaction's completion. The progress of the reaction was confirmed by

the separation of the reactant and product spots indication by the TLC. After reaction completion, poured reaction mixture into cold water, and the solid product separated was filtered, washed with cold water and recrystallized by ethanol gave pure product and was characterized for its homogeneity by FT-IR, ^{1}H-,^{13}C-NMR and LC-MS spectrometry.

1.4. Spectral data of the selected derivatives

*1.4.1. 4-((1H-Indol-3-yl)methylene)-3-methylisoxazol-5(4H)-one **(4h)**:*

White powder; FT- IR: 3016 (CH stretching), 2897, 1704 (C=O stretching), 1661 (C=N stretching), 1557 (N-O stretching), 1408, 1347, 1374 cm^{-1}; ^{1}H-NMR: δ 8.31(s, 1H, NH), 8.14 (s, 1H, CH), 7.88 (s, 1H, H), 7.79 (d, 1H, CH), 7.56 (s, 1H, CH), 7.46 (t, CH, 1H), 7.23 (s, 1H, CH), 1.25 (s,3H, CH_3); ^{13}C-NMR: 170, 164, 150, 145, 136, 131, 130, 125, 122, 119, 111, 110, 20 ppm; LC-MS: m/z Calcd.: 226.07 Da; m/z (Obs.): 227.09 Da $[M + H]^+$.

1.4.2. 3-Methyl-4-((2-phenyl-1H-indol-3-yl)methylene)isoxazol-5(4H)-one **(4i)**:

Yellow powder; FT-IR: ν 3072 (CH stretching), 2888, 1747 (C=O stretching), 1645 (C=N stretching), 1511 (N-O stretching), 1456 cm^{-1}; ^{1}H-NMR: δ 9.35(s, 1H, NH), 8.78 (s, 1H, H), 7.54 (d, 1H, CH), 7.96 (s, 1H, CH), 7.85 (s, 1H, CH), 7.78 (t, 1H, CH,), 7.69 (d, 1H, CH) 7.59 (s, 1H, CH), 7.44 (d, 1H, CH), 7.18 (s, 1H, CH), 2.46 (d, 3H, CH_3); ^{13}C-NMR: 165, 162, 150, 135, 133, 133, 130, 129, 127, 127, 127, 126, 122, 121, 119, 110, 100, 21 ppm; LC-MS : m/z (Calcd.): 302.11 Da; m/z Obs.: 303.12.09 Da $[M + H]^+$.

*1.4.3. 4-((2-Chloro-1H-indol-3-yl)methylene)-3-methylisoxazol-5(4H)-one **(4j)***

White powder; FT-IR: ν 3072 (CH stretching), 2888, 1747 (C=O stretching), 1645 (C=N stretching), 1569 (N-O stretching), 1465 cm^{-1}; ^{1}H-NMR: δ 8.31(s, 1H, NH), 8.00 (d, 1H, H), 7.95 (s, 1H, CH), 7.81 (d, 1H, CH), 7.58 (d, 1H, CH), 7.24 (s, CH, 1H), 1.69 (t, 3H, CH_3); ^{13}C-NMR: 165, 163, 149, 143, 138, 133, 127, 123, 121, 119, 117, 113, 20 ppm; LC-MS : m/z (Calcd.): 260.04 Da; m/z Obs.: 261.12 Da $[M + H]^+$.

*1.4.4. 4-((2-Hydroxynaphthalen-1-yl)methylene)-3-methylisoxazol-5(4H)-one **(4k)***

Light yellow powder; FT-IR: ν 2922 (CH stretching), 1700 (C=O stretching), 1653 (C=N stretching), 1556 (N-O stretching), 1496 cm^{-1}; ^{1}H-NMR: δ 8.09 (s, 1H, CH), 7.86 (s, 1H, H), 7.49 (d, 1H, CH), 7.40 (d, 1H, CH), 7.26 (s, 1H, CH), 6.85 (s, CH, 1H), 4.77(s, 1H, OH), 2.67 (t, 3H, CH_3); ^{13}C-NMR: 176, 164, 164, 141, 138, 131, 130, 129, 128, 128, 127, 126, 120, 120, 21 ppm; LC-MS : m/z (Calcd.): 253.07 Da; m/z Obs.: 254.10 Da $[M + H]^+$.

*1.4.5. 4-((1H-Pyrrol-2-yl)methylene)-3-methylisoxazol-5(4H)-one **(4l)***

Light brown powder; FT-IR: 3058 (CH stretching), 2951, 1774 (C=O stretching), 1685 (C=N

stretching), 1567 (N-O stretching), 1422 cm^{-1}; ^{1}H-NMR: δ 8.24 (s, 1H, NH), 7.98 (d, 1H, H), 7.51 (d, 1H, CH), 7.02 (d, 1H, CH), 6.85 (t, 1H, CH), 2.16 (d, 3H, CH_3); ^{13}C-NMR: 160, 142, 136, 128, 127, 122, 109, 102, 29 ppm; LC-MS : m/z (Calcd.): 176.06 Da; m/z Obs.: 177.07 Da $[M + H]^+$.

1.4.6. 3-Methyl-4-(thiophen-2-ylmethyl)isoxazol-5(4H)-one ***(4m)***

Brown powder; FT-IR: ν 3016 (CH stretching), 2897, 1661 (C=O stretching), 1601 (C=N stretching), 1557 (N-O stretching), 1493 cm^{-1}; ^{1}H-NMR: δ 7.47 (s, 1H, CH), 7.24 (s, 1H, CH), 6.98 (t, 1H, CH), 3.24 (s, 2H, CH_2), 2.46 (s, 1H, CH), 1.21 (s, 3H, CH_3); ^{13}C-NMR: 163, 143, 137, 127, 118, 115, 60, 34, 19 ppm; LC-MS : m/z (Calcd.): 195.04 Da; m/z Obs.: 194.03 Da $[M + H]^+$.

1.4.7. 4-(3,4-Dimethoxybenzylidene)-3-methylisoxazol-5(4H)-one ***(4n)***

Yellow powder; FT-IR: ν 3056 (CH stretching), 2931, 1744 (C=O stretching), 1685 (C=N stretching), 1567 (N-O stretching), 1423 cm^{-1}; ^{1}H-NMR: δ 7.17 (s, 1H, H), 6.78 (s, 1H, CH), 6.63 (s, 1H, CH), 6.50 (t, 1H, CH), 3.83 (t, 1H, CH), 3.74 (t, 3H, CH_3); ^{13}C-NMR: 166, 164, 159, 151, 141, 132, 130, 121, 114, 113, 60, 55, 21 ppm; LC-MS : m/z (Calcd.): 247.08 Da; m/z Obs.: 248.10 Da $[M + H]^+$.

1.4.8. 4-((1,4-Diphenyl-1H-pyrrol-3-yl)methyl)-3-methylisoxazol-5(4H)-one ***(4o)***

Light orange powder; FT-IR: ν 3047 (CH stretching), 1784 (C=O stretching), 1633 (C=N stretching), 1544 (N-O stretching), 1472 cm^{-1}; ^{1}H-NMR: δ 7.94 (s, 1H, CH) 7.86 (s, 1H, CH), 7.66 (d, 1H, CH), 7.61(s, 1H, CH), 7.58 (d, 1H, CH), 7.50 (s, 1H, CH), 7.49 (s, 1H, CH), 7.37 (s, 1H, CH), 7.32 (s, 1H, CH), 7.22 (s, 1H, CH), 6.95 (s, 1H, CH), 6.88 (d, 1H, CH), 3.16 (t, 2H, CH_2), 2.46 (s, 1H, CH), 1.20 (s 3H, CH), 2.22 (s, 3H); ^{13}C-NMR: 163, 160, 158, 148, 142, 133, 131, 128, 127, 126, 126, 121, 120, 119, 118, 110, 59, 22, 14, ppm; LC-MS : m/z (Calcd.): 330.78 Da; m/z Obs.: 330.14 Da $[M + H]^+$.

1.5. Electrochemical measurements

Cyclic voltammetry is a simple, rapid, and most commonly influenced technique for the electrochemical investigation of electrode surfaces and electroactive catalysts. Oxidation reaction data are obtained from the cathodic parameters, whereas reduction data are obtained from anodic parameters. The cyclic voltametric experiments were carried out using the three-electrode system with a standard set-up consisting of a glassy carbon electrode (GCE) having a diameter of 3mm as the working electrode, Ag/AgCl as reference electrode and Pt wire as a counter electrode used for the studies.

1.6. In vitro Anticancer Evaluation

The anticancer activity of the derivatives **4h-4o** was evaluated against A549 cell line using MTT (3-(4,5-imethylthiazol-2-yl)2,5-diphenyltetrazolium bromide) method. The evaluation process was described elsewhere and adopted in this study [40-42]. Briefly, each compound was dissolved in DMSO at a concentration of 500μM, then diluted series with DMSO for eight different concentrations (500μM, 50μM, 5μM, 500nM, 50nM, 5nM, 500pM and 50pM respectively) as a stock solution.

The cells were seeded on a 96-well plate-bottom microplate and maintained at 37ºC in 95% humidity and 5% CO_2 overnight. Different concentration (100, 50, 25, 12.5, 6.25, 3.125 μg/mL) of samples prepared were added. The cells were incubated for another 48 hrs, after the wells were washed twice with PBS and 20 μL of the MTT staining solution was added to each well and the plate was incubated at 37ºC. After 4h, 100 μL of DMSO was added to each well to dissolve the formazan crystals, and absorbance was recorded 570 nm using microplate reader.

The formula used for the calculation of % inhibition, is as follows

Surviving cells (%) = Mean OD of test compound /Mean OD of negative control ×100

Using graph Pad Prism Version5.1, we calculate the IC_{50} value of the compounds.

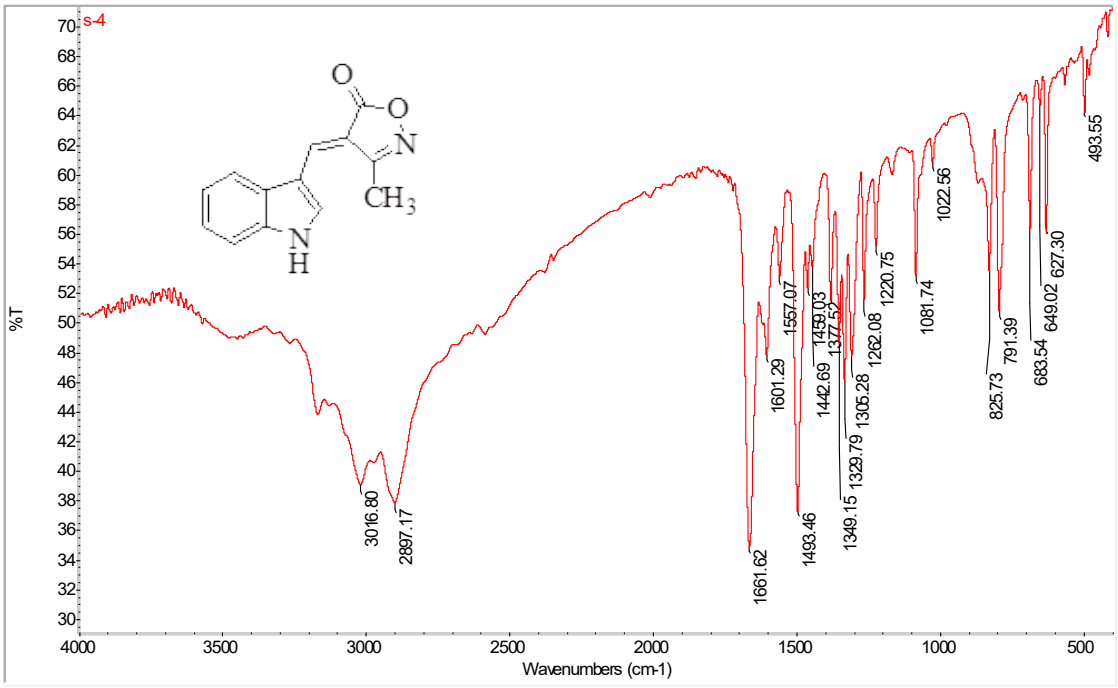

Figure: S1: FT-IR SPECTRUM OF COMPOUND **4h**

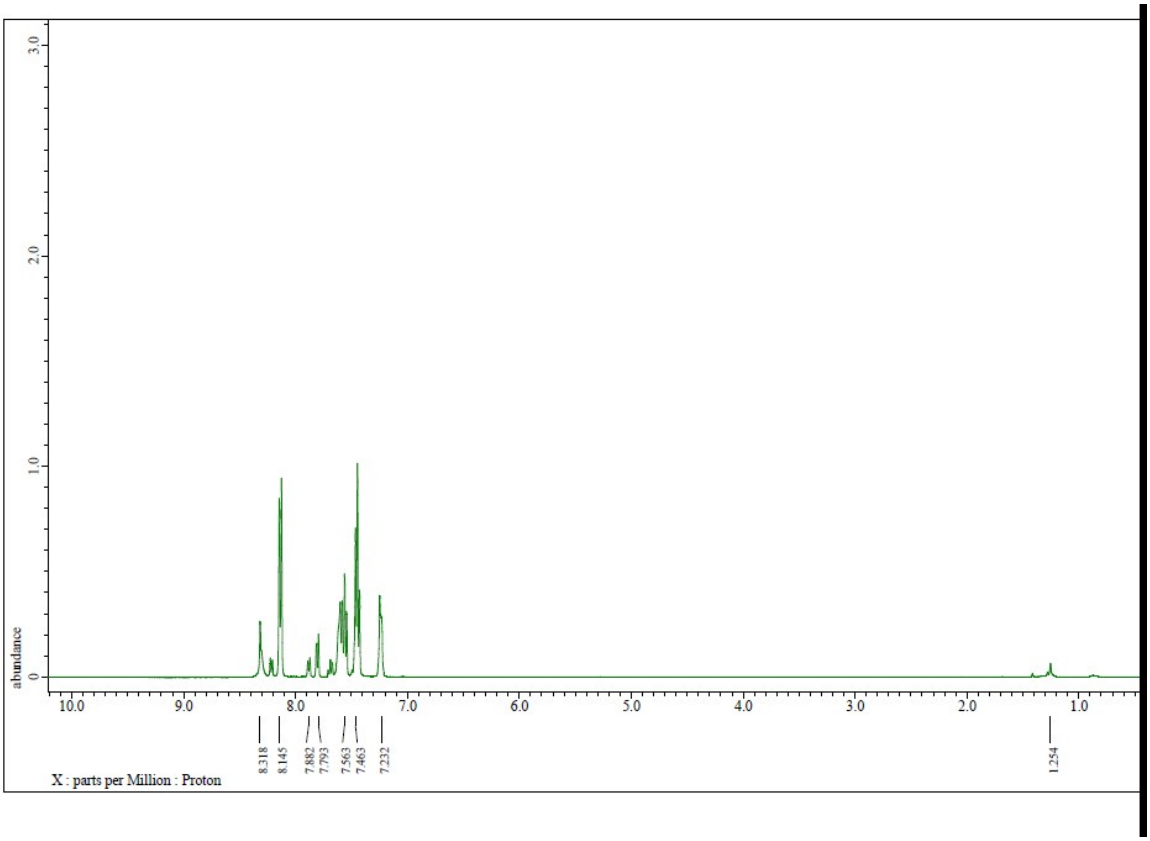

Figure: S2: ^{1}H-NMR SPECTRUM OF COMPOUND **4h**

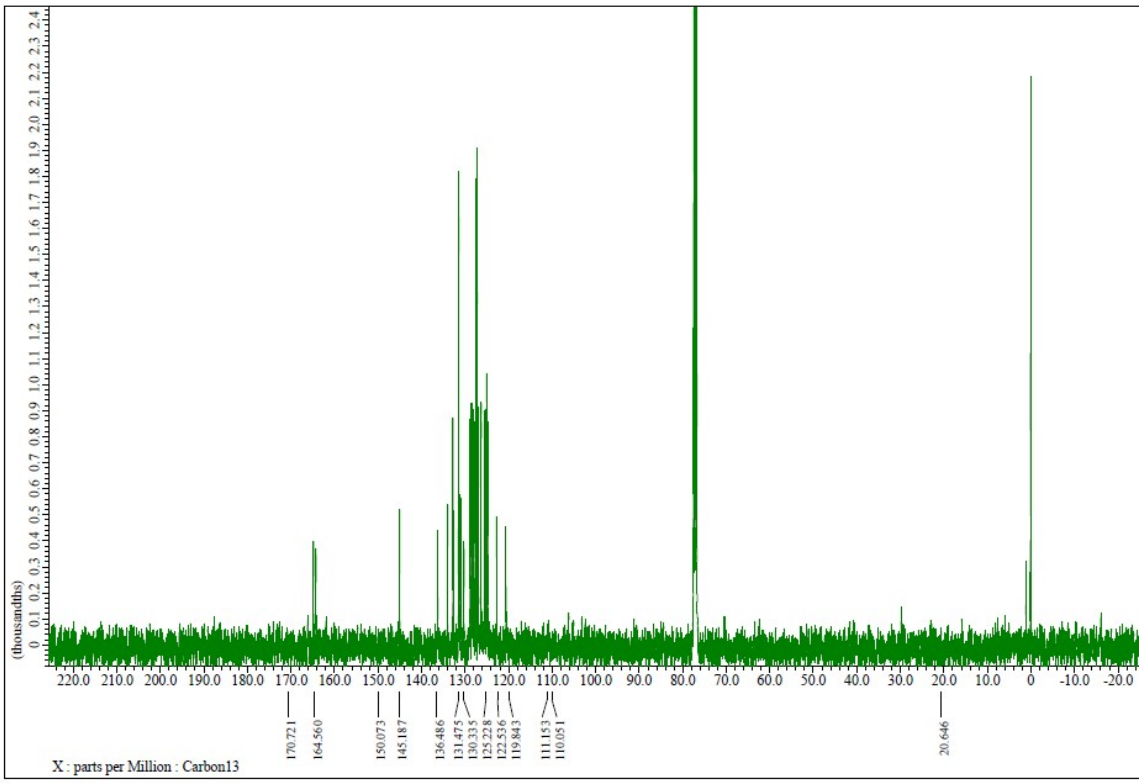

Figure: S3: ^{13}C-NMR spectrum of SPECTRUM OF COMPOUND **4h**

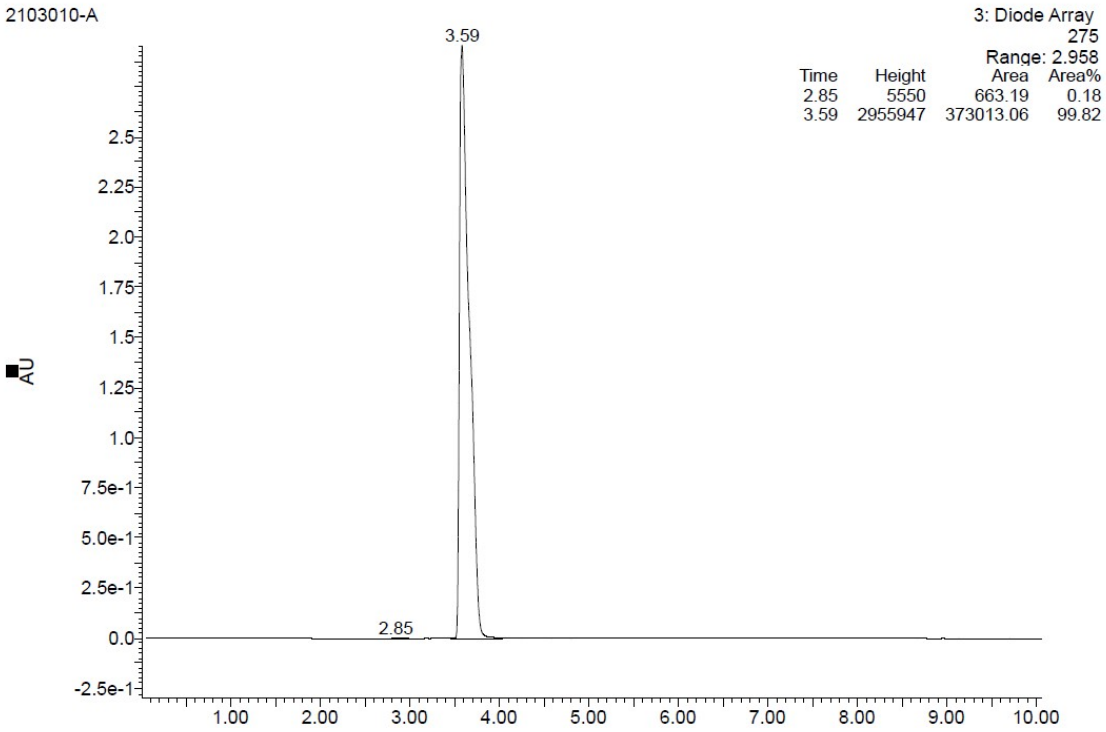

Figure: S4: LC PROFILE OF COMPOUND **4h**

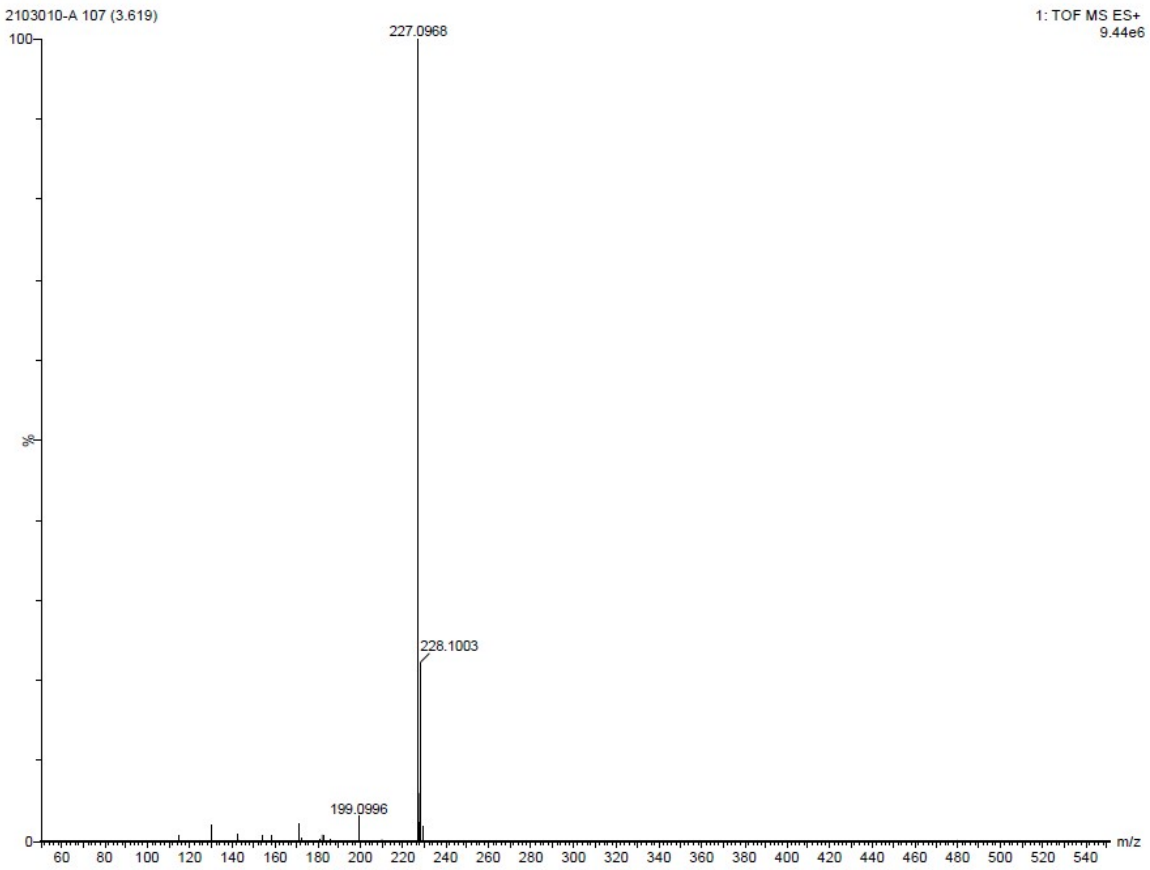

Figure: S5: LC-MS SPECTRUM OF COMPOUND **4h**

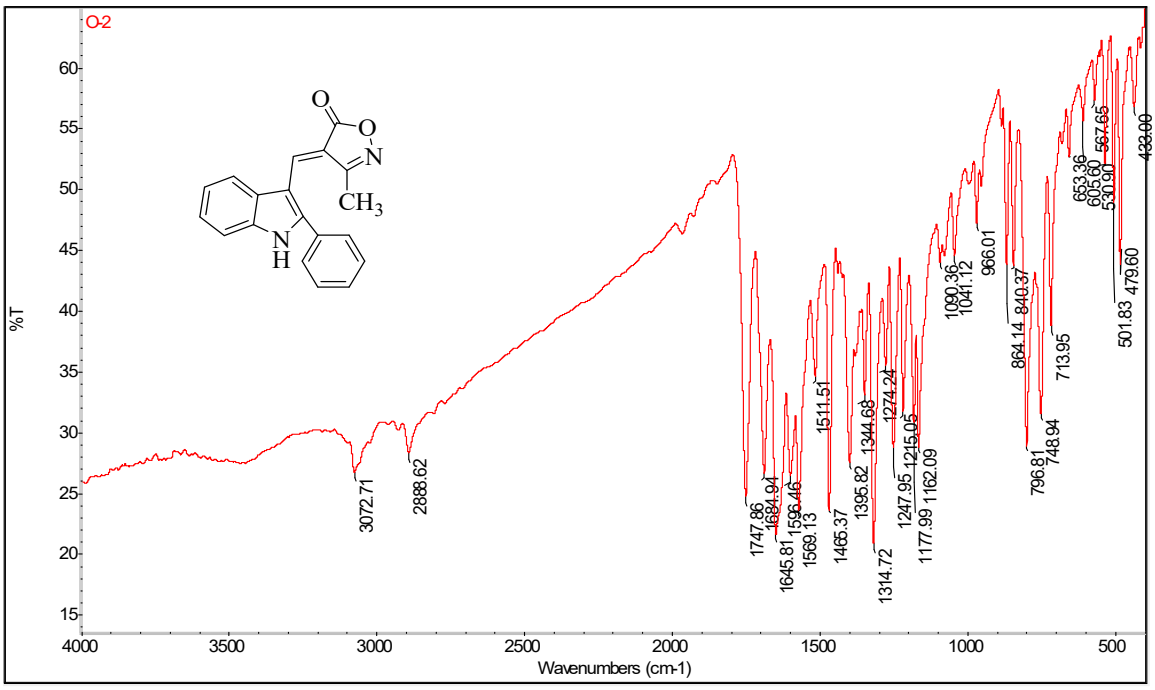

Figure: S6: FT-IR SPECTRUM OF COMPOUND **4i**

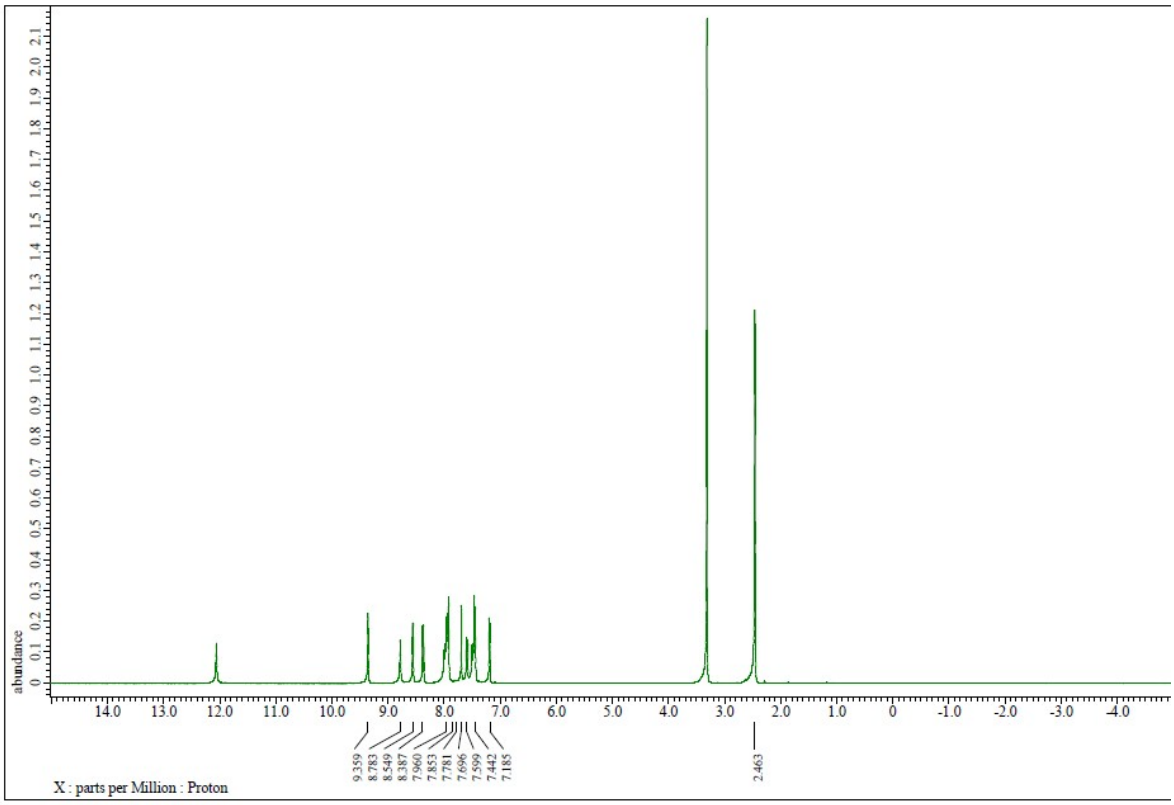

Figure: S7. ^{1}H-NMR SPECTRUM OF COMPOUND **4i**

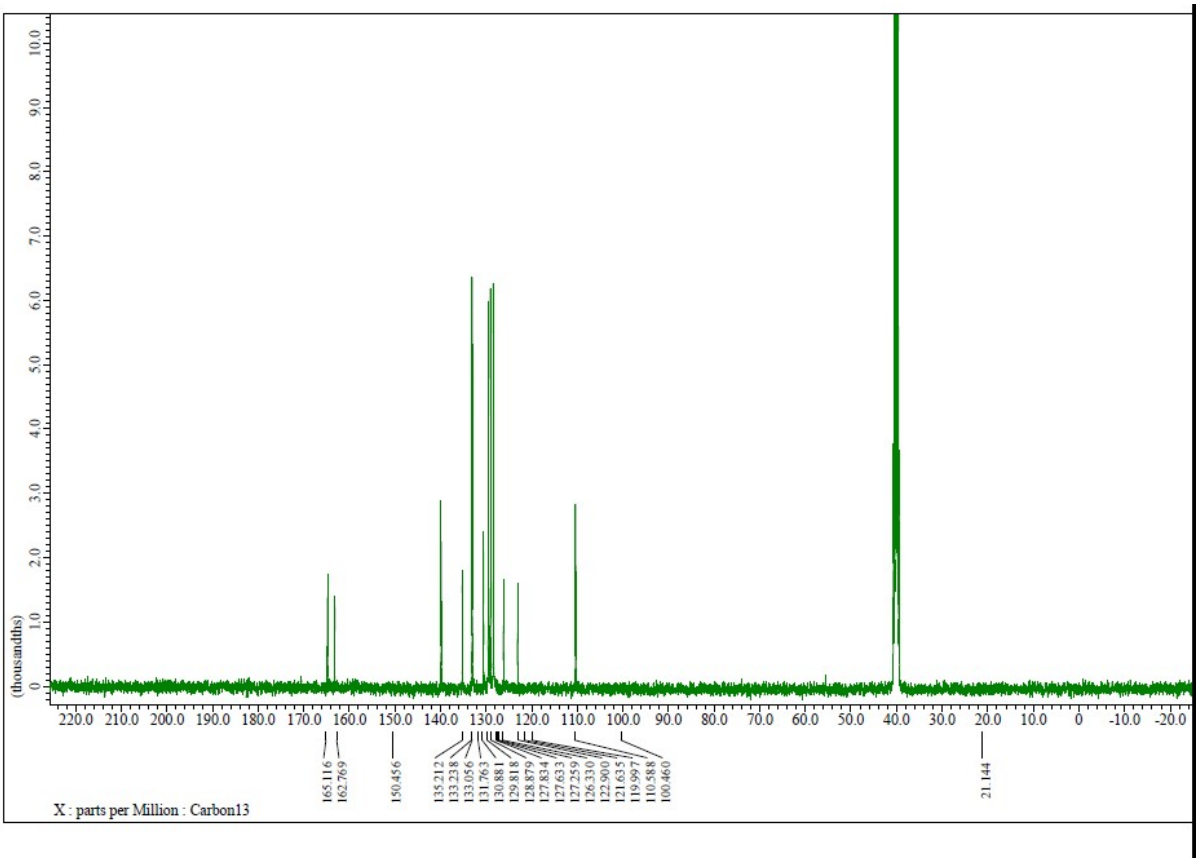

Figure: S8. ^{13}C-NMR SPECTRUM OF COMPOUND **4i**

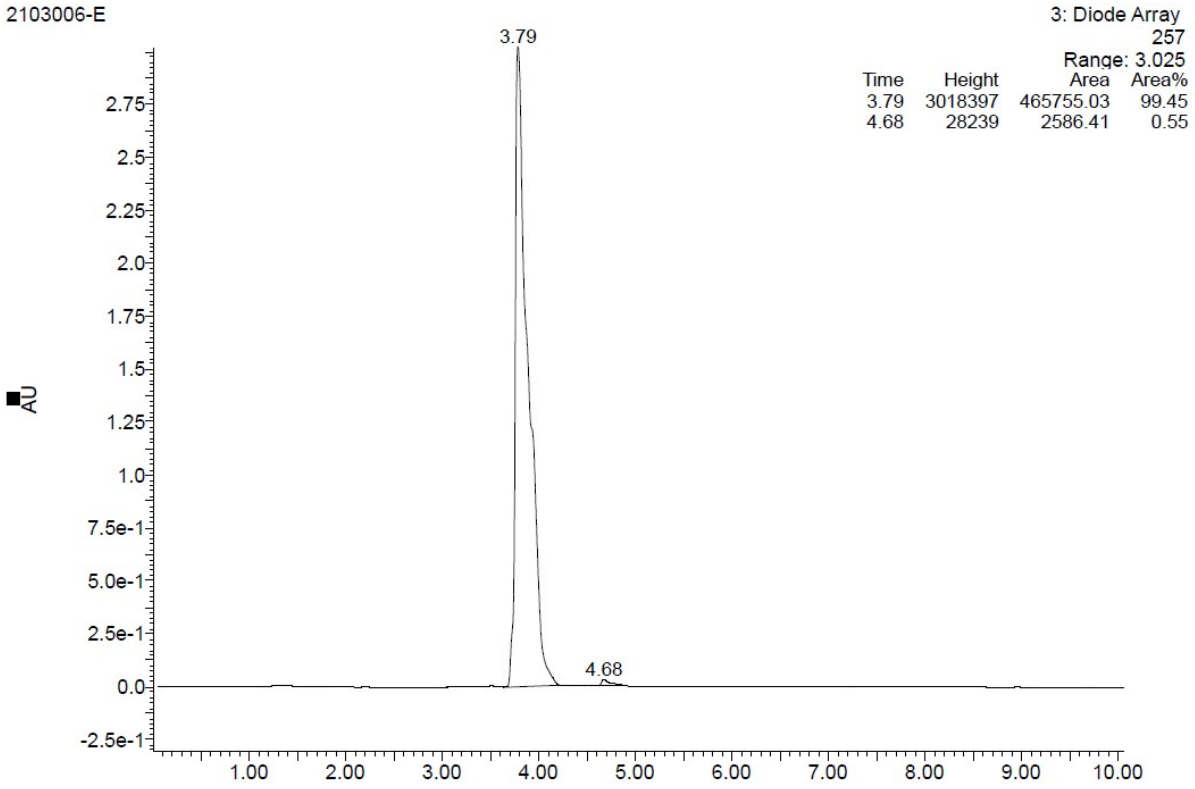

Figure: S9. LC PROFILE OF COMPOUND **4i**

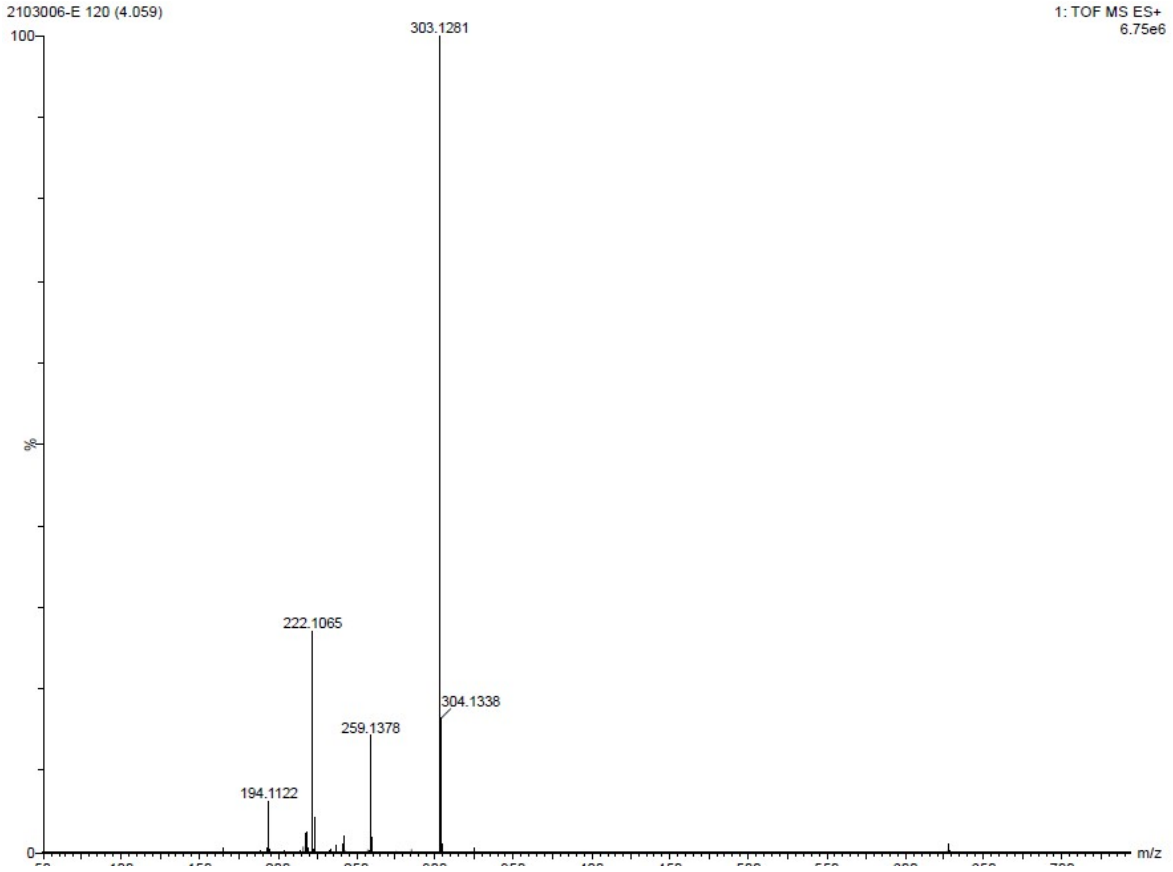

Figure: S10: LC-MS SPECTRUM OF COMPOUND **4i**

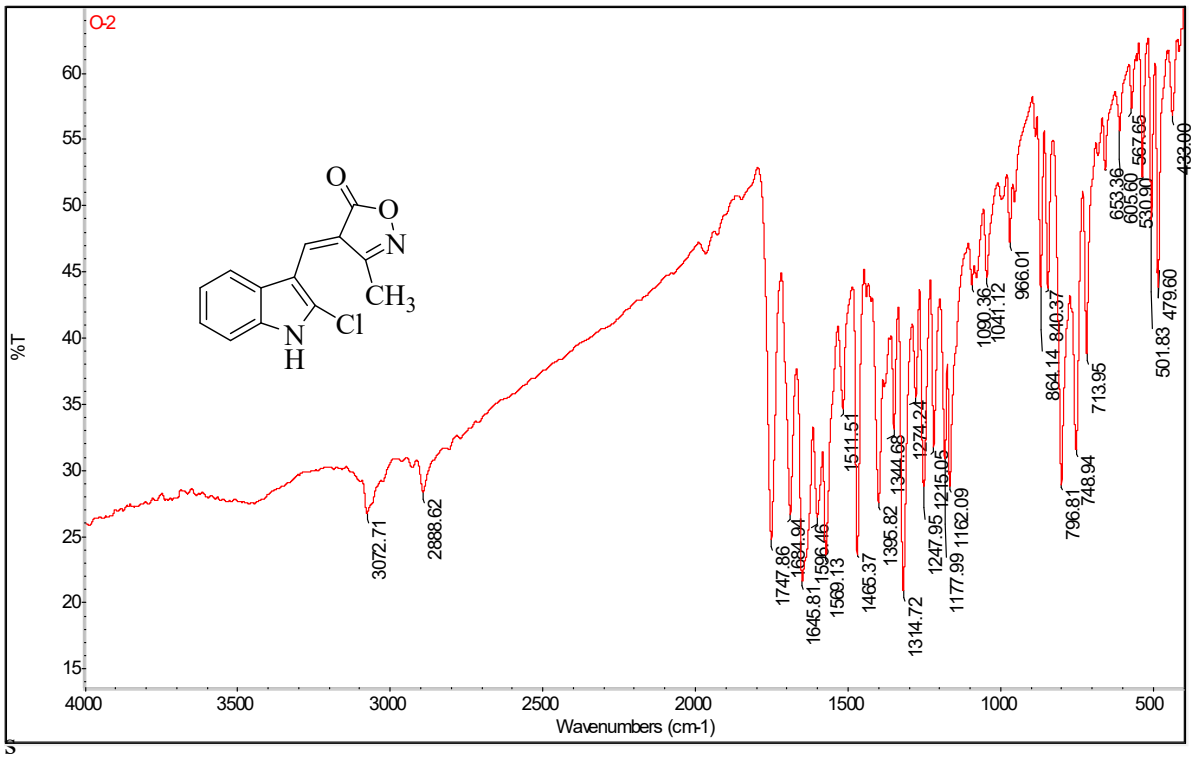

Figure: S11: FT-IR SPECTRUM OF COMPOUND **4j**

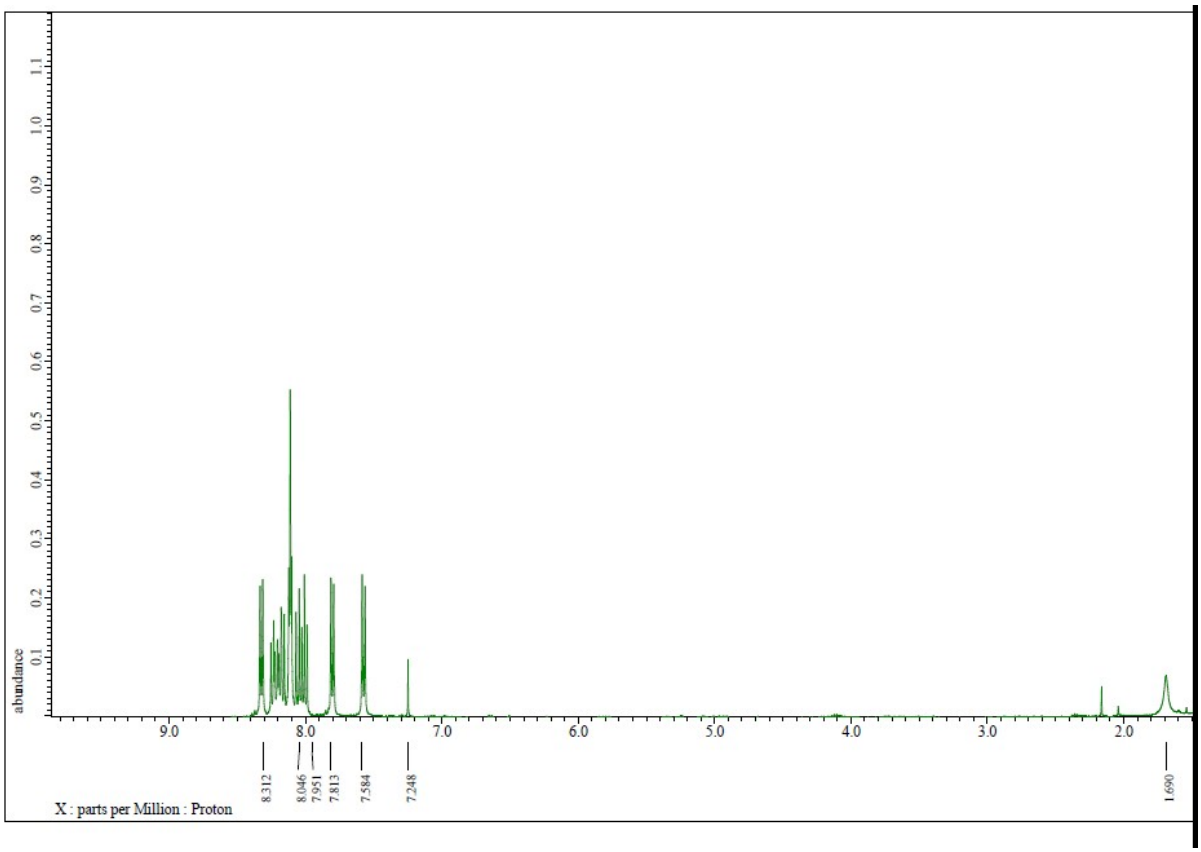

Figure: S12: ^{1}H-NMR SPECTRUM OF COMPOUND **4j**

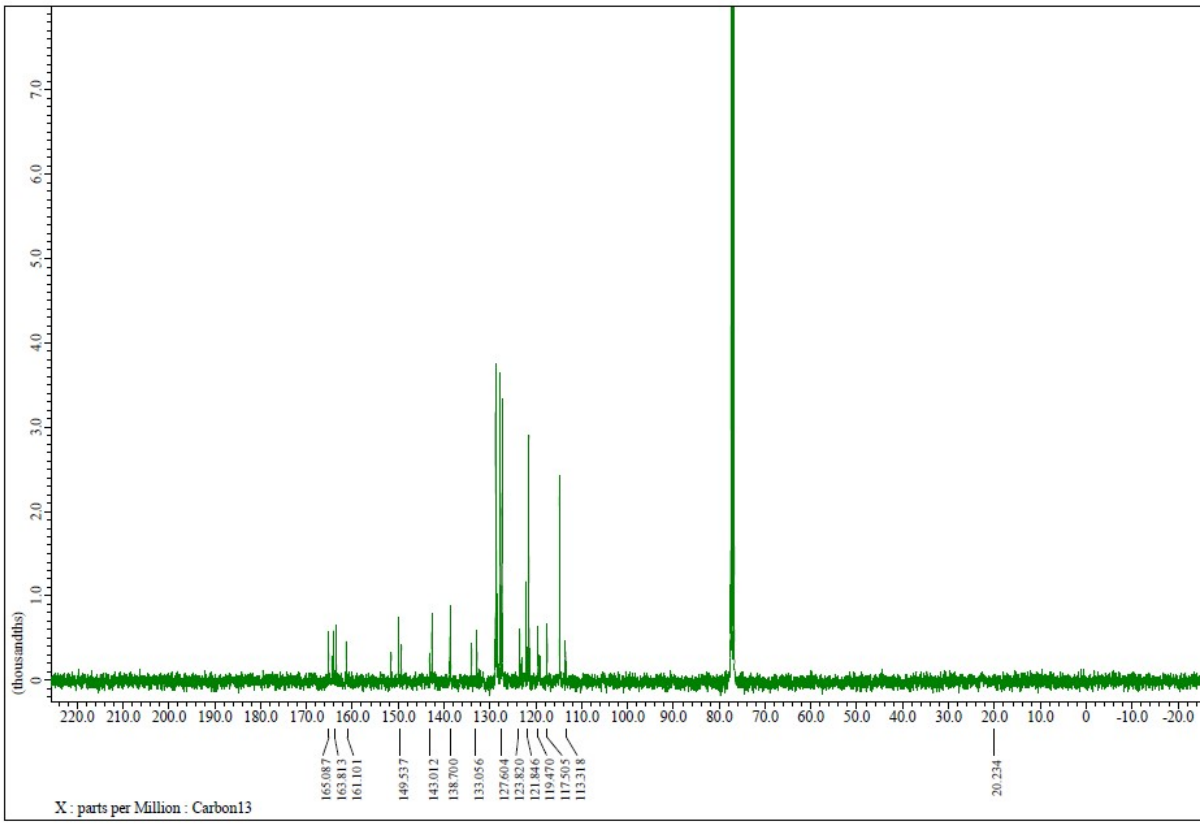

Figure: S13. ^{13}C-NMR SPECTRUM OF COMPOUND **4j**

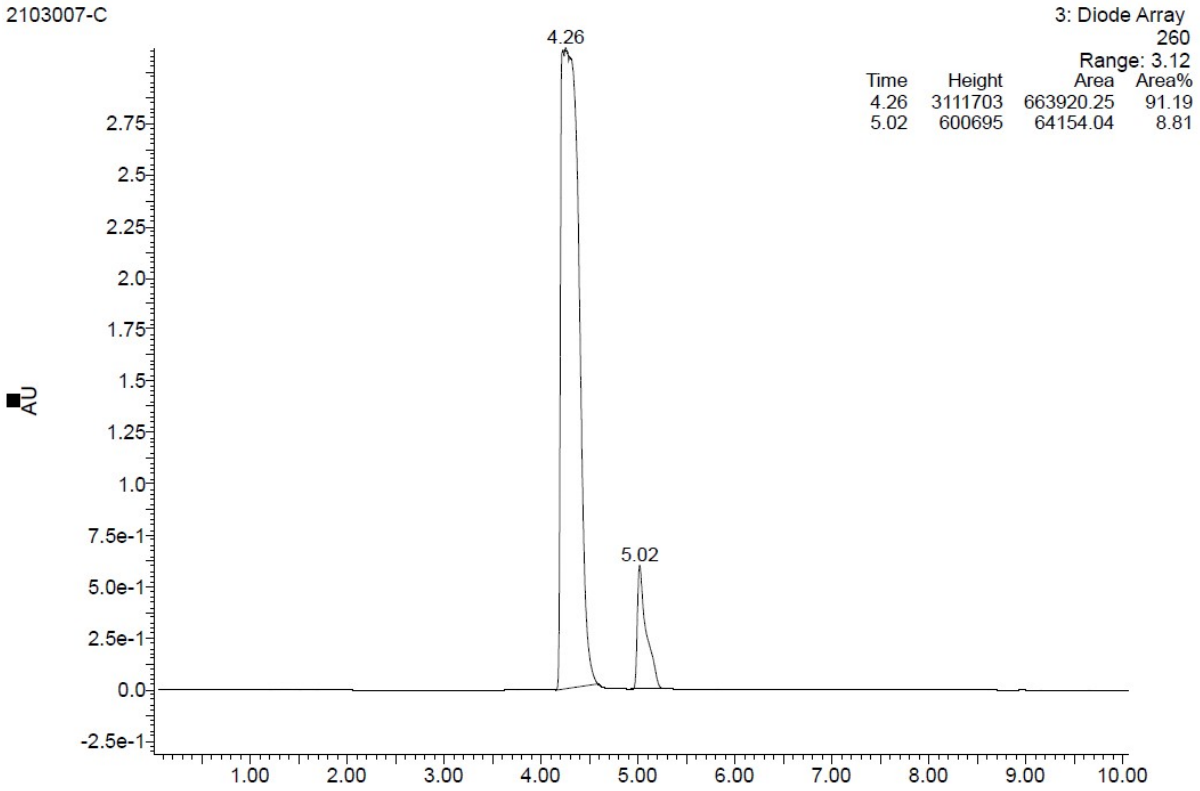

Figure: S14. LC PROFILE OF COMPOUND **4j**

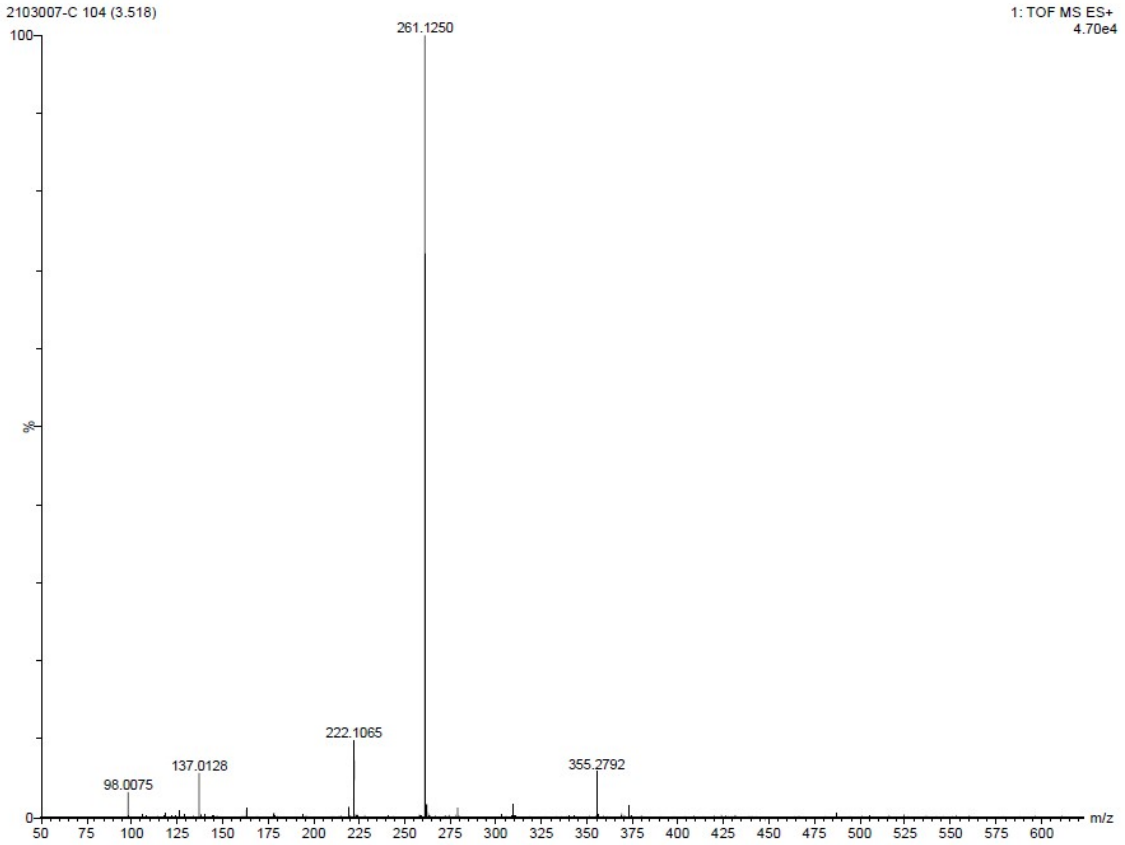

Figure: S15: LC-MS SPECTRUM OF COMPOUND **4j**

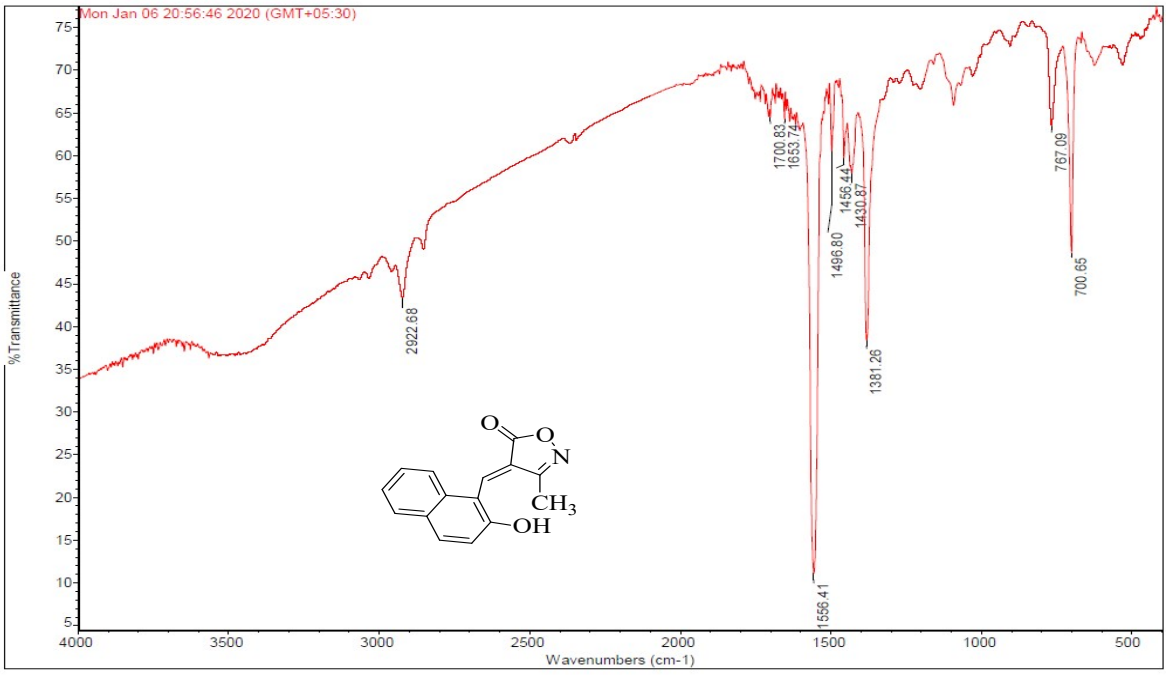

Figure: S16: FT-IR SPECTRUM OF COMPOUND **4k**

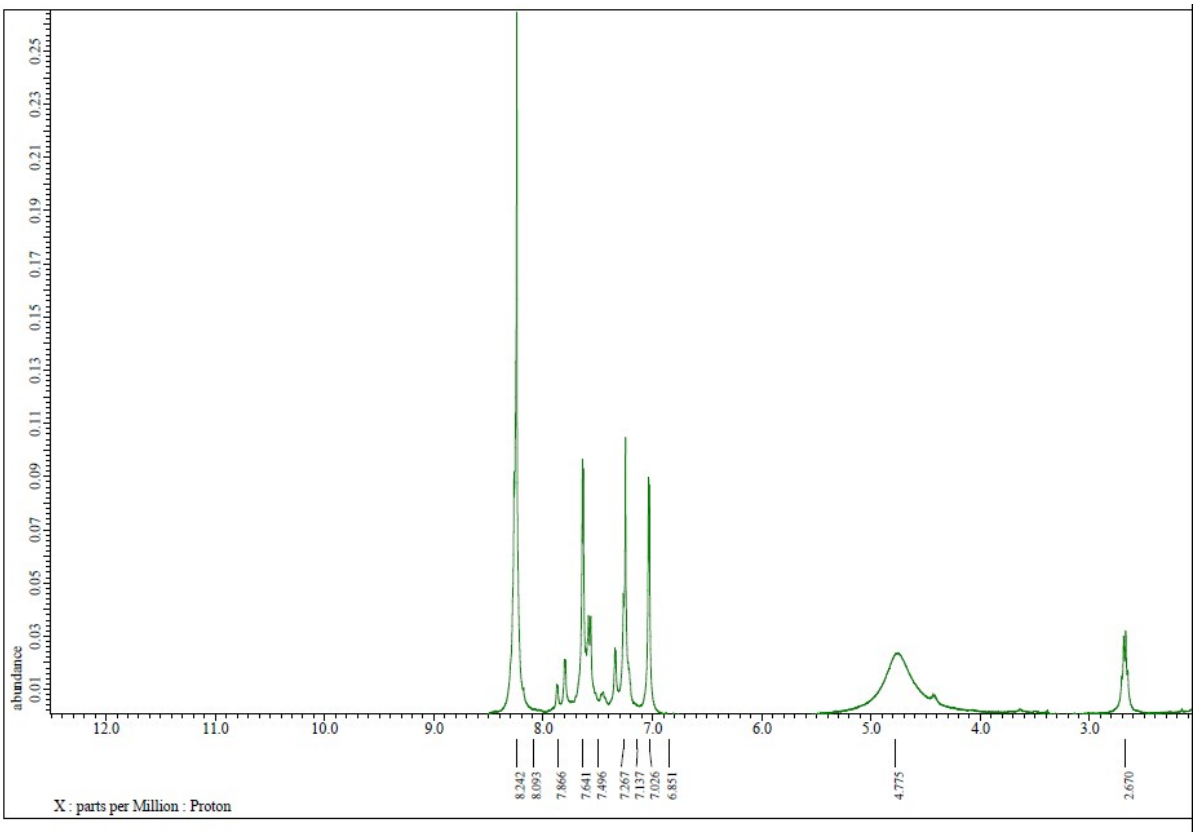

Figure: S17: ^{1}H-NMR SPECTRUM OF COMPOUND **4k**

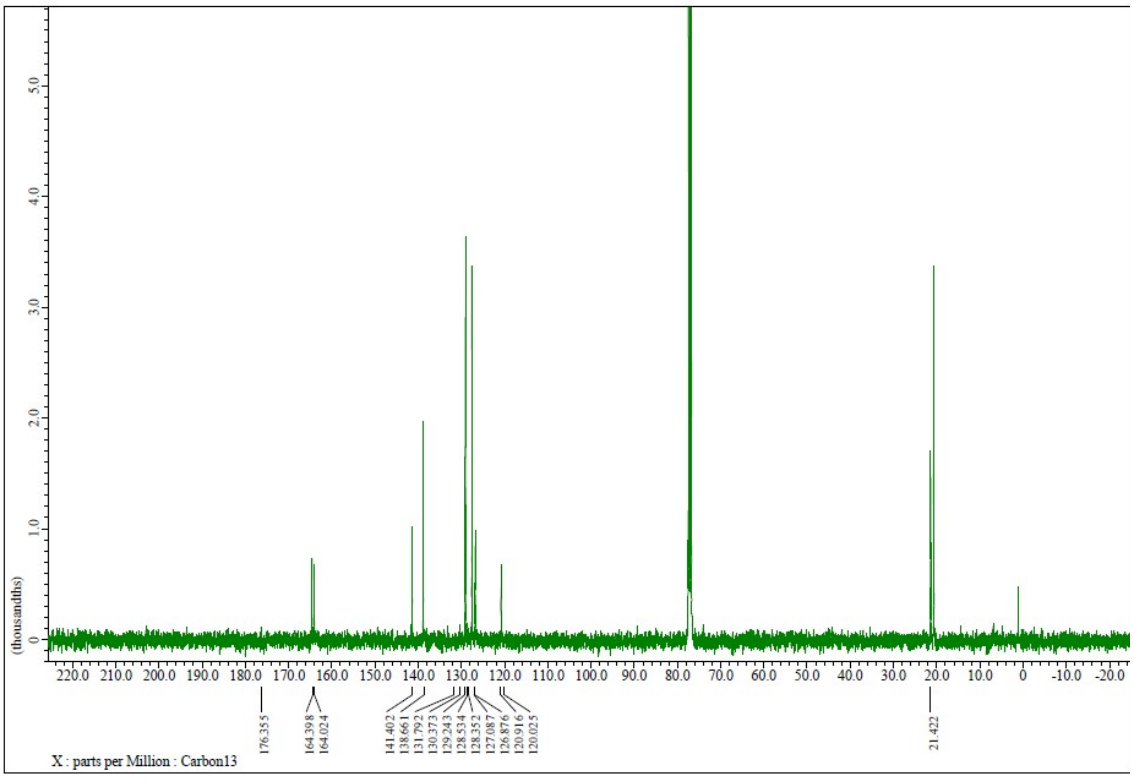

Figure: S18: ^{13}C-NMR SPECTRUM OF COMPOUND **4k**

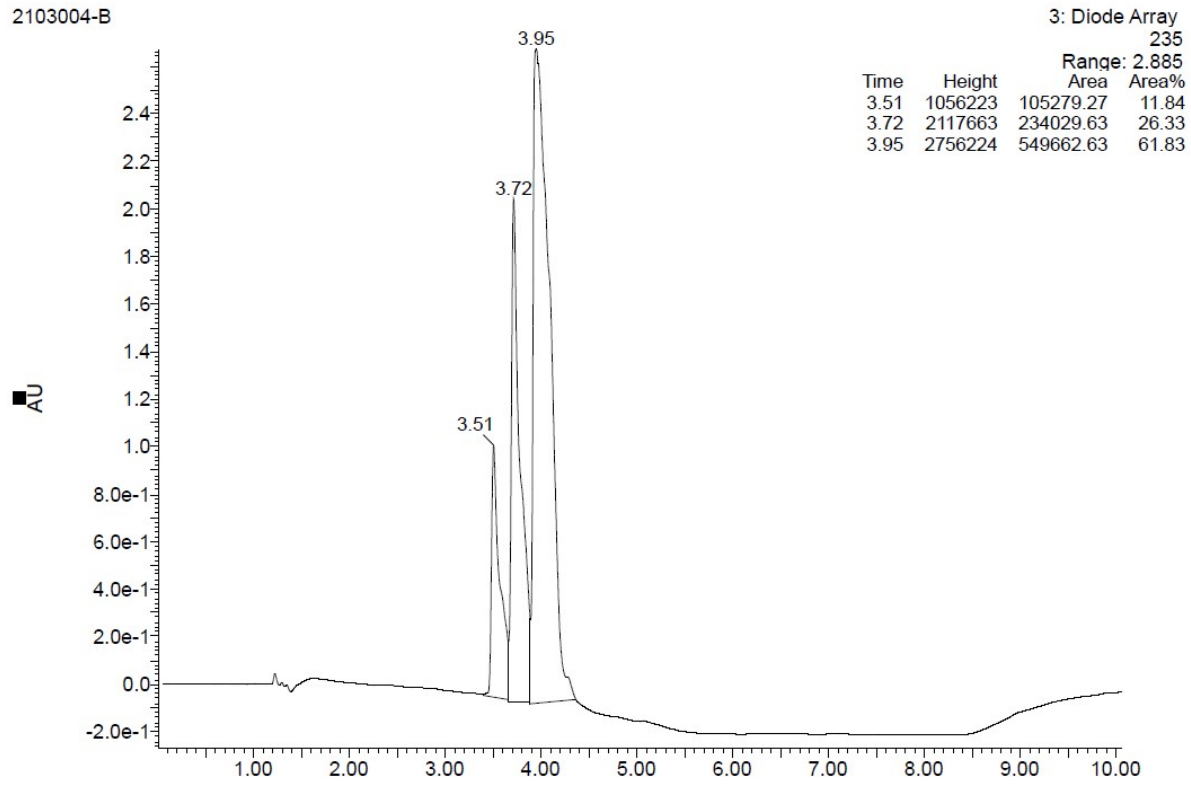

Figure: S19: LC profile of COMPOUND **(4k)**

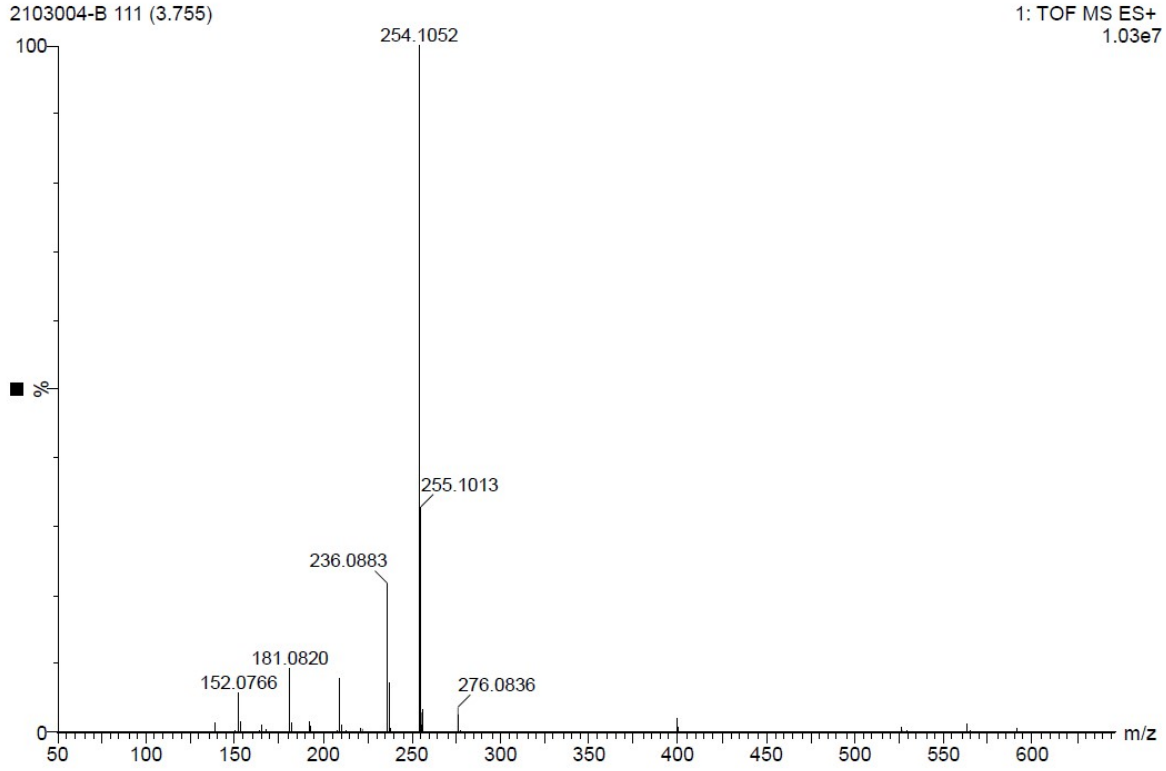

Figure: S20: LC-MS SPECTRUM OF COMPOUND **4k**

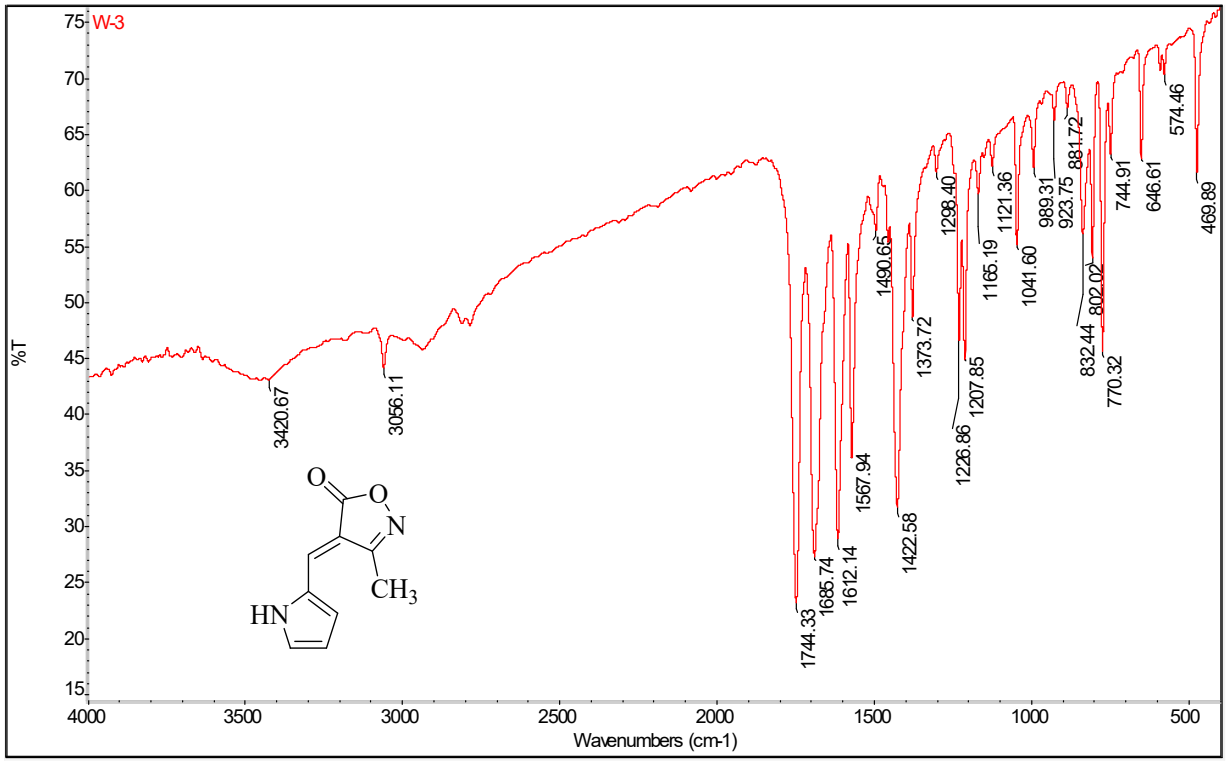

Figure: S21: FT-IR SPECTRUM OF COMPOUND **4l**

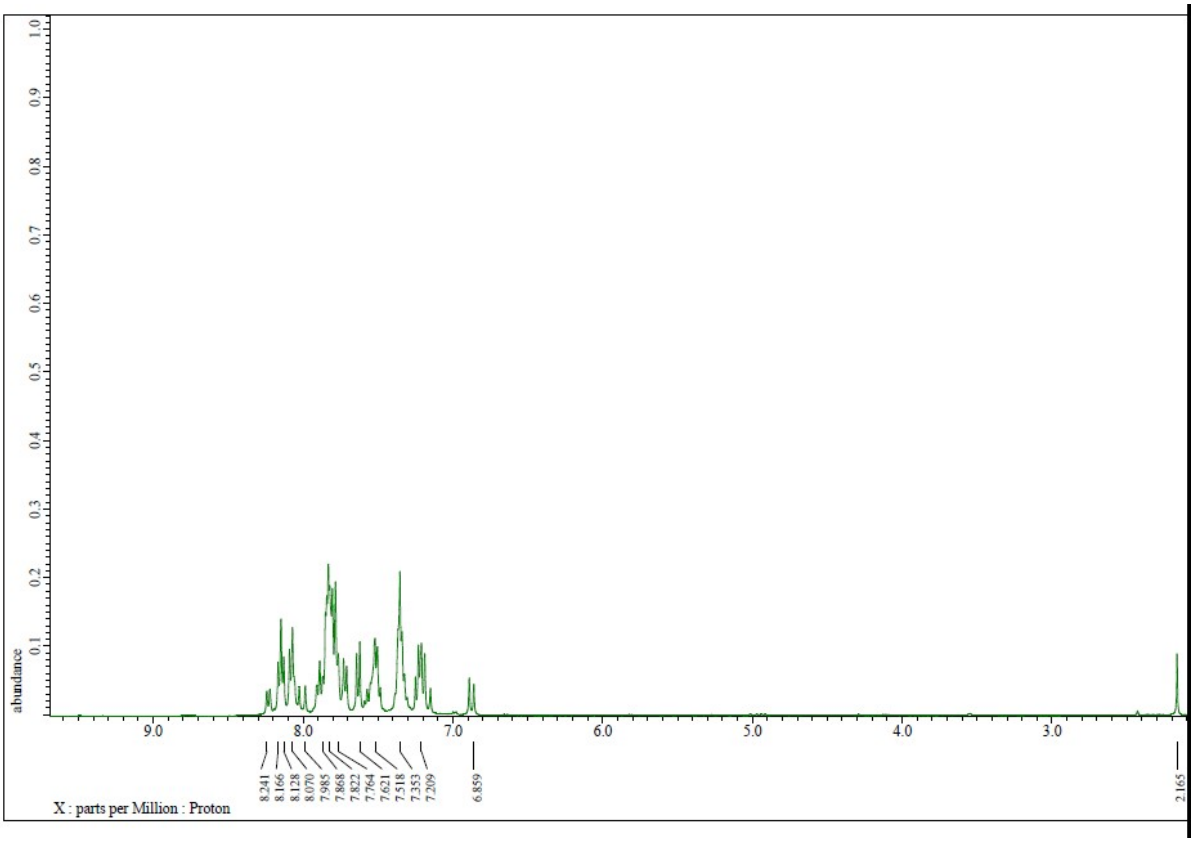

Figure: S22: [1]H-NMR SPECTRUM OF COMPOUND **4l**

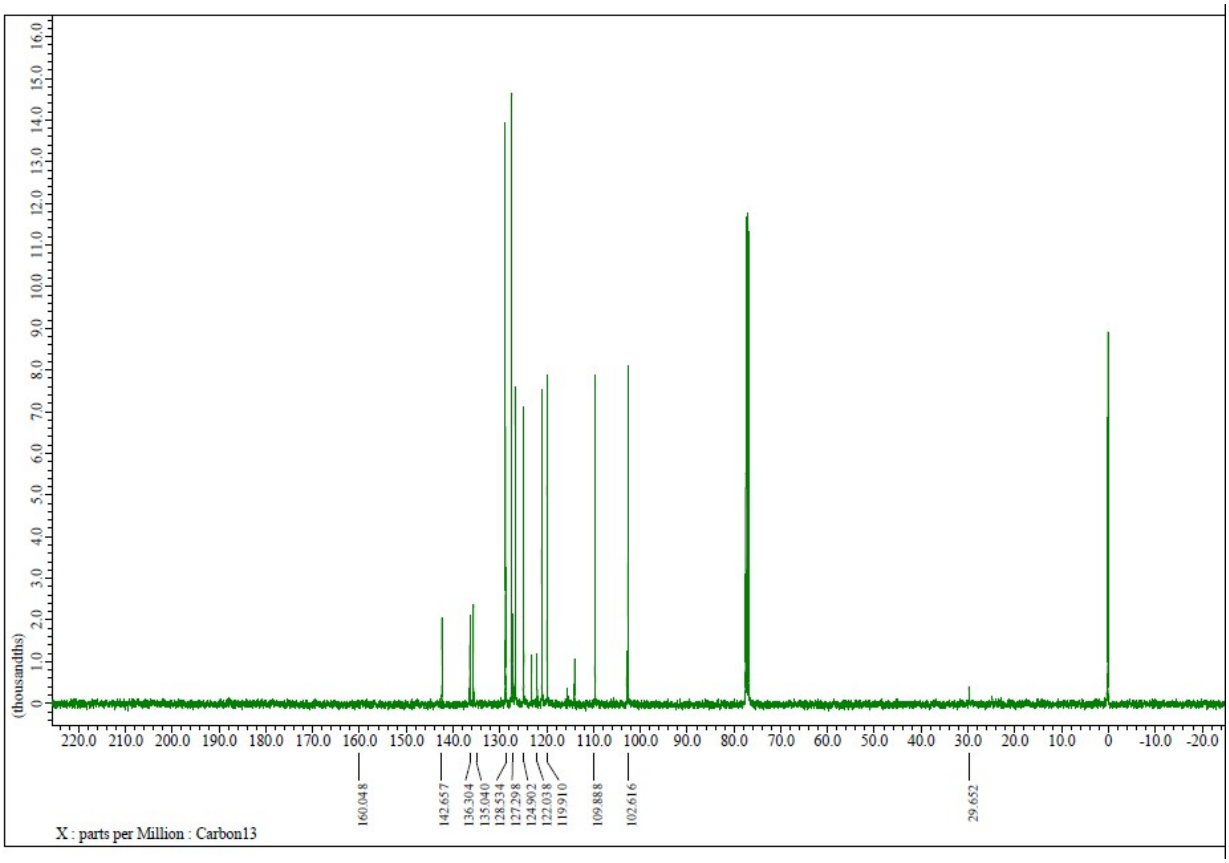

Figure: S23: ^{13}C-NMR SPECTRUM OF COMPOUND **4l**

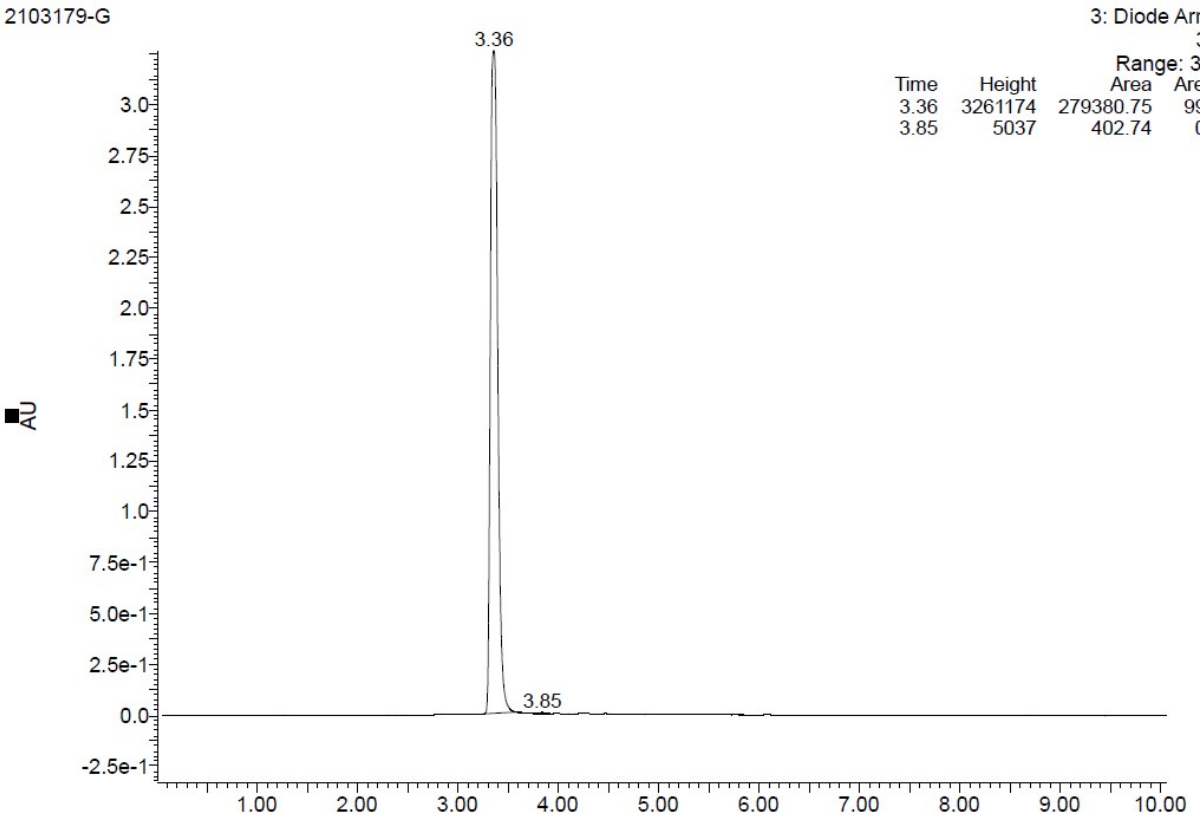

Figure: S24: LC profile of COMPOUND **4l**

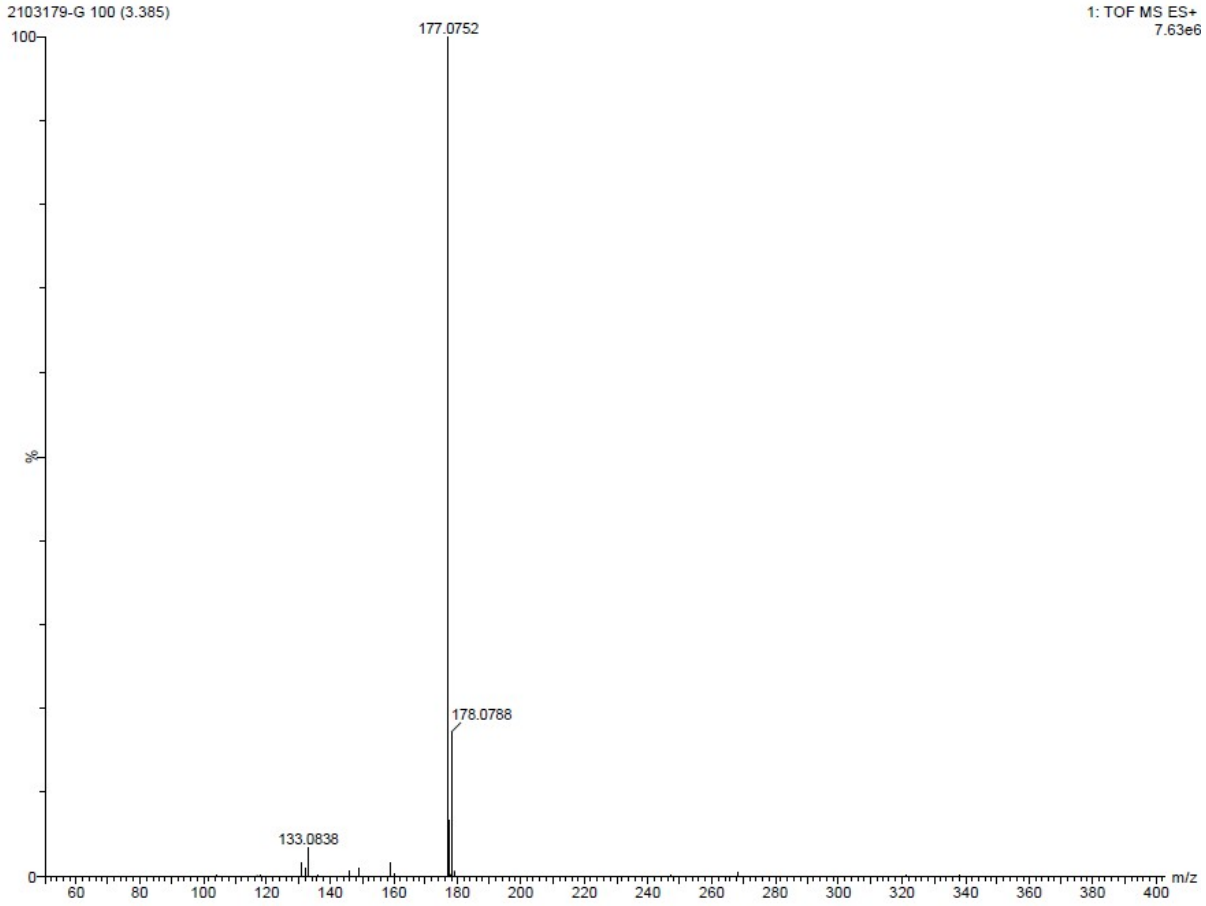

Figure: S25: LC-MS SPECTRUM OF COMPOUND **4l**

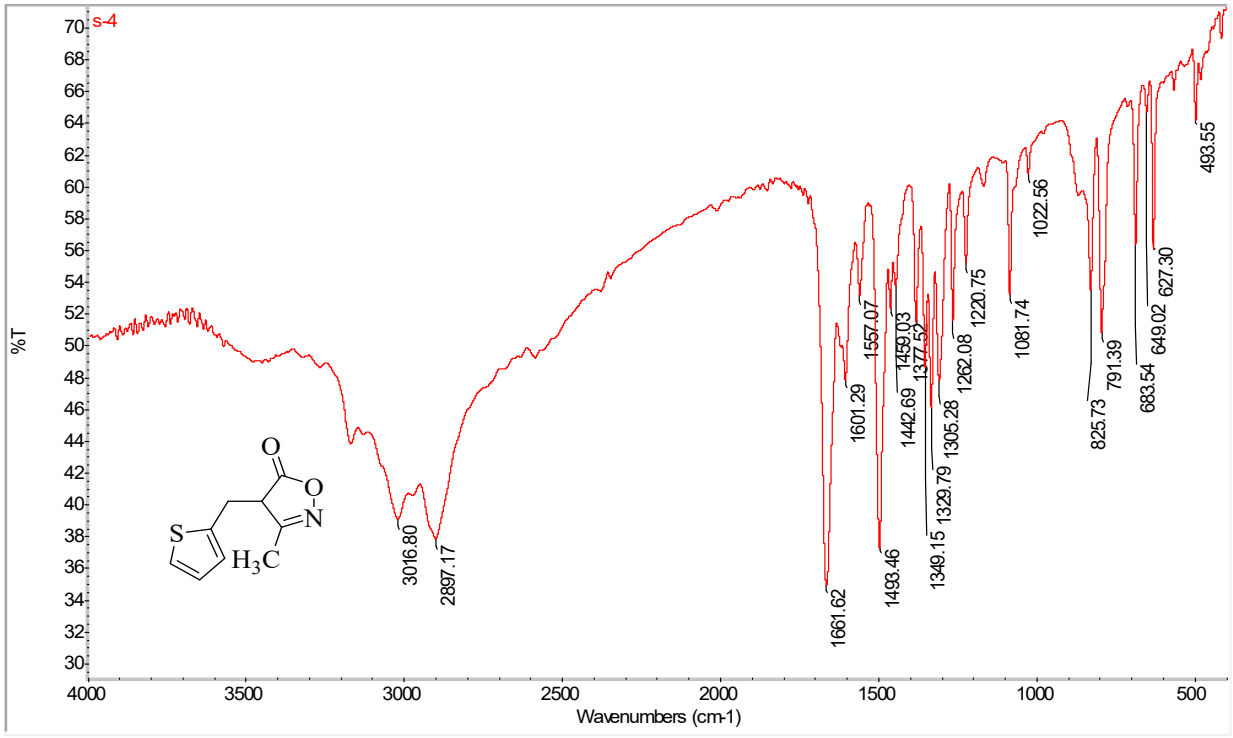

Figure: S26: FT-IR SPECTRUM OF COMPOUND **4m**

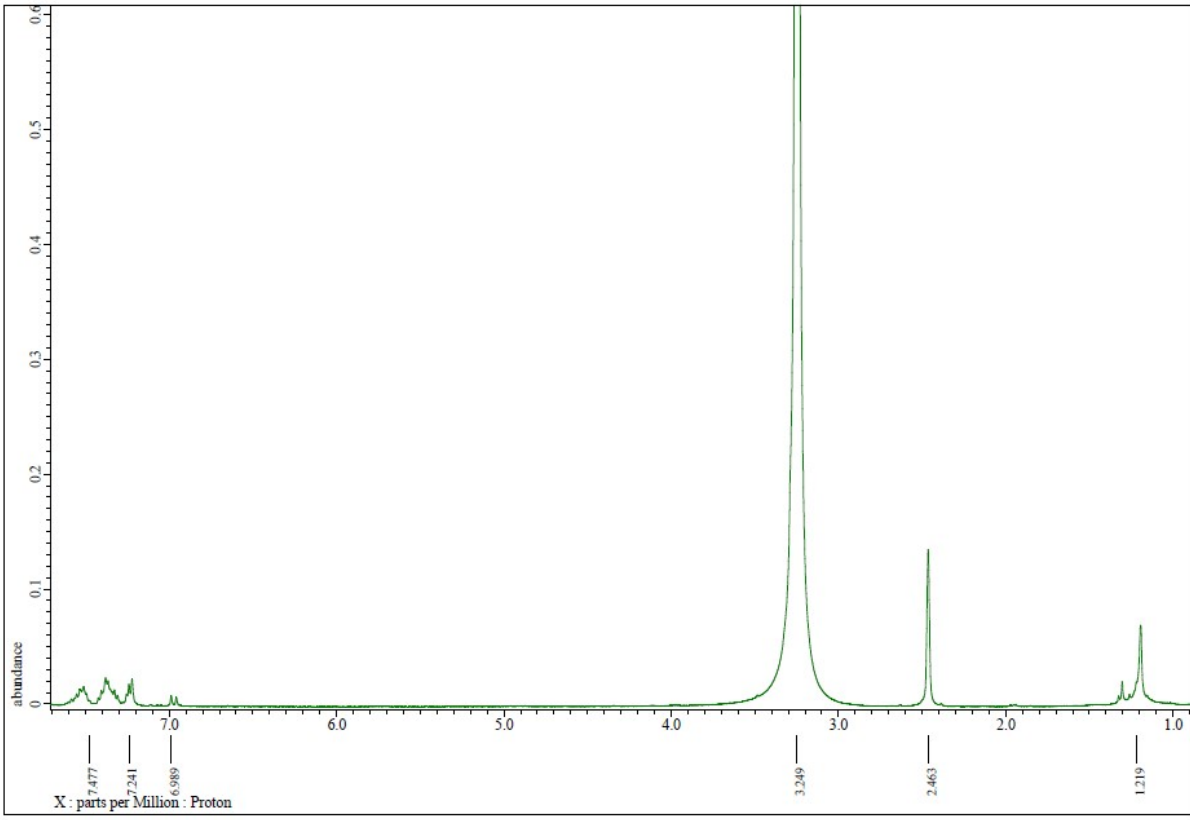

Figure: S27: [1]H-NMR SPECTRUM OF COMPOUND **4m**

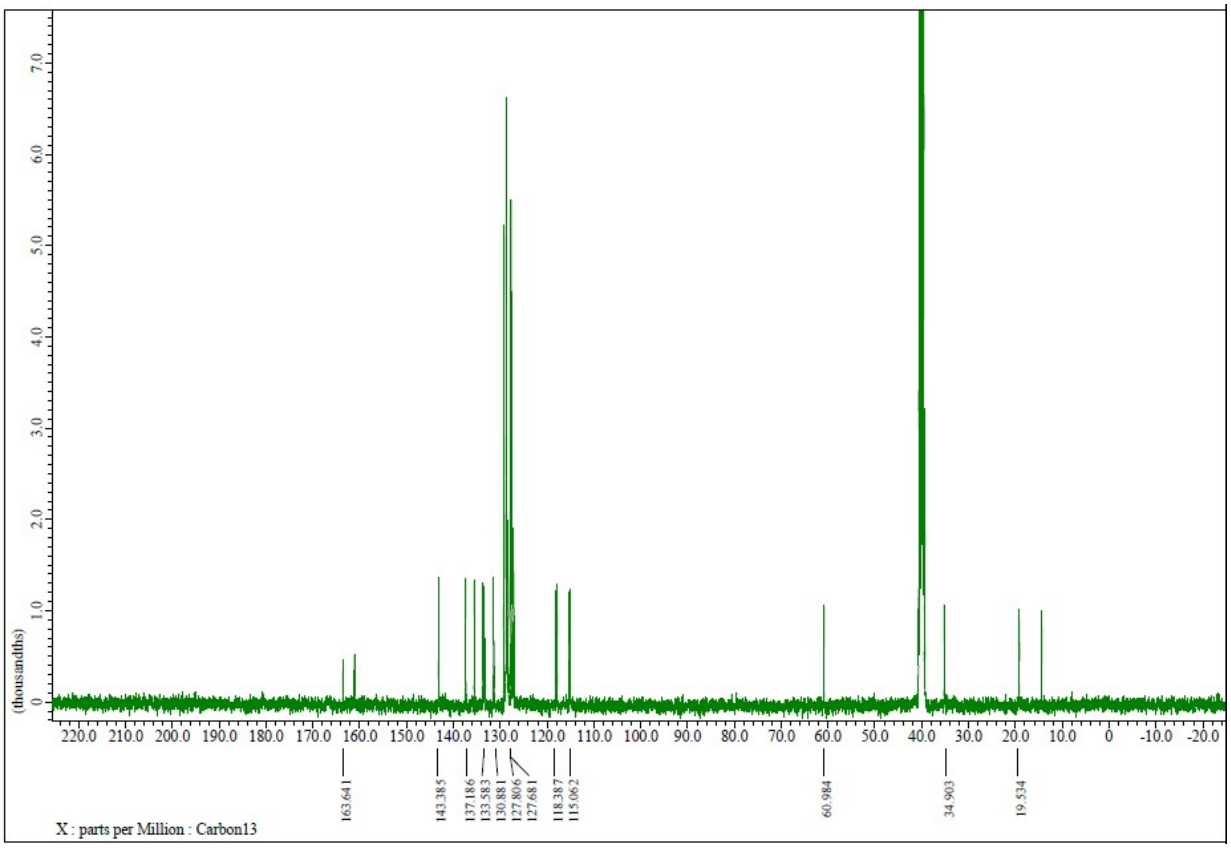

Figure: S28: [13]C-NMR SPECTRUM OF COMPOUND **4m**

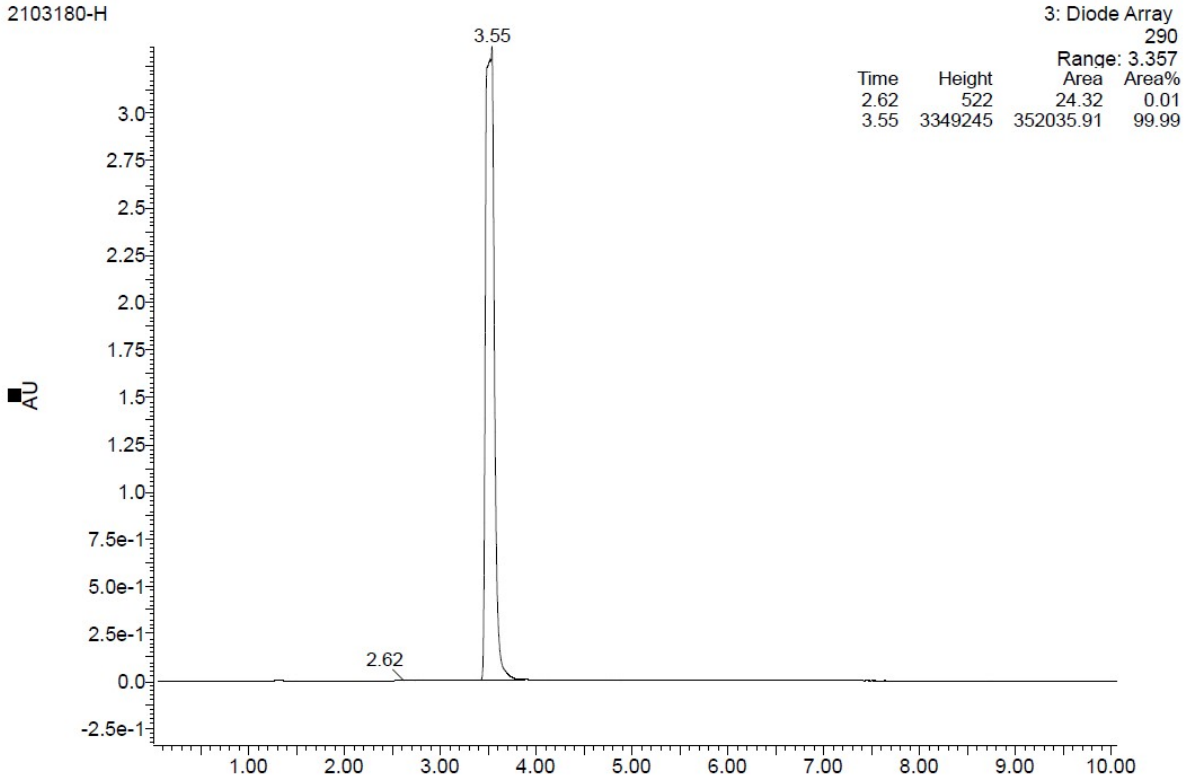

Figure: S29: LC PROFILE OF COMPOUND **4m**

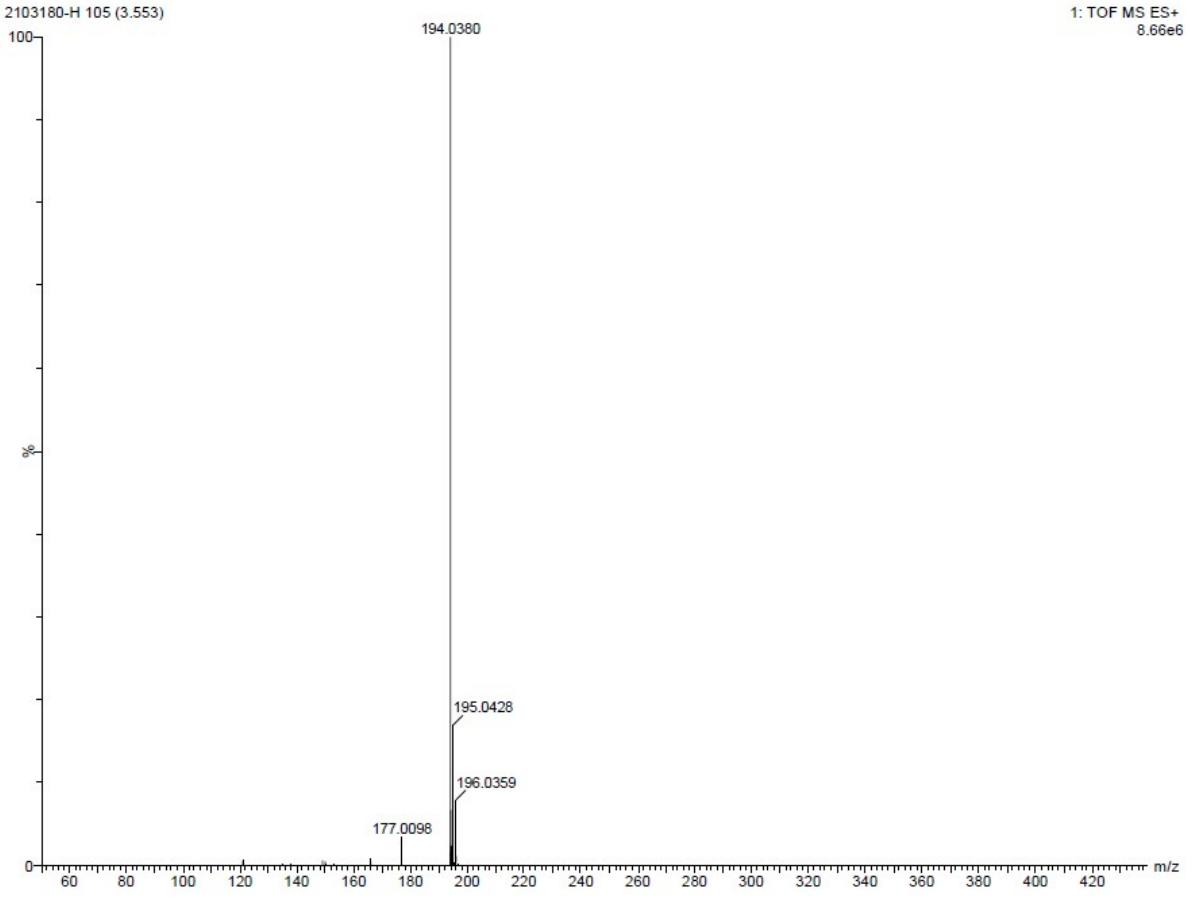

Figure: S30: LC-MS SPECTRUM OF COMPOUND **4m**

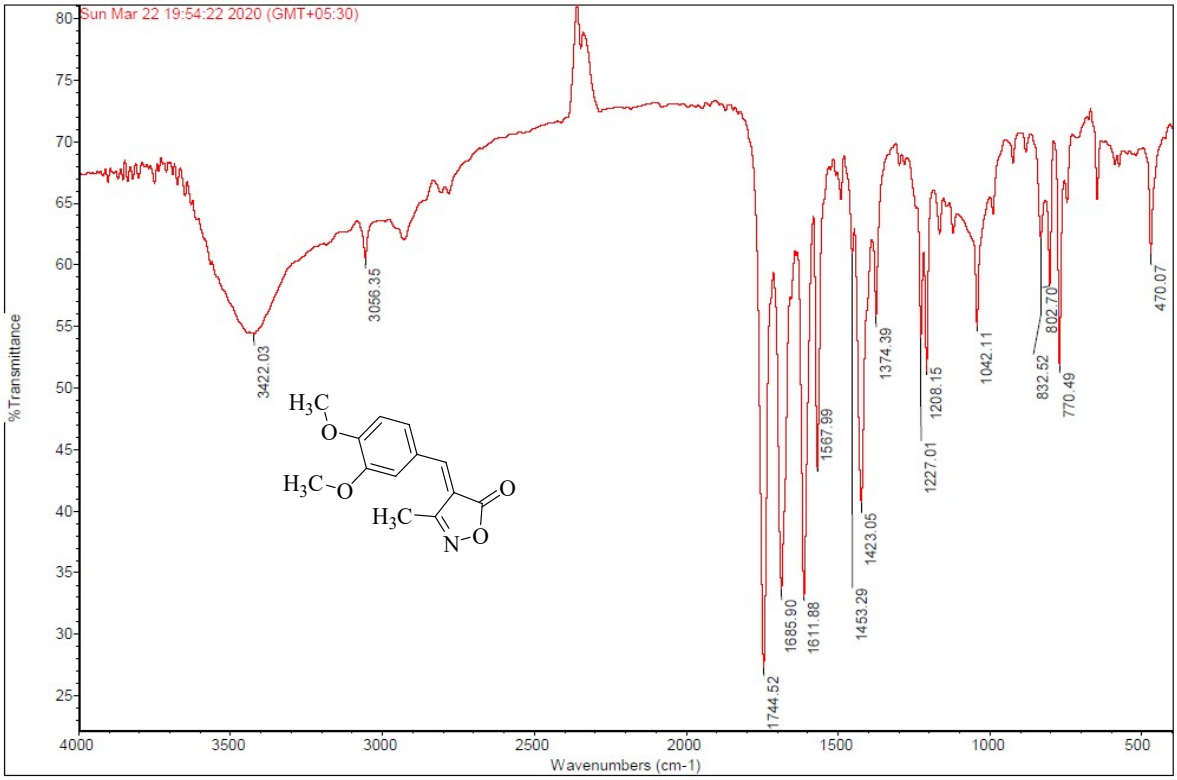

Figure: S31: FT-IR SPECTRUM OF COMPOUND **4n**

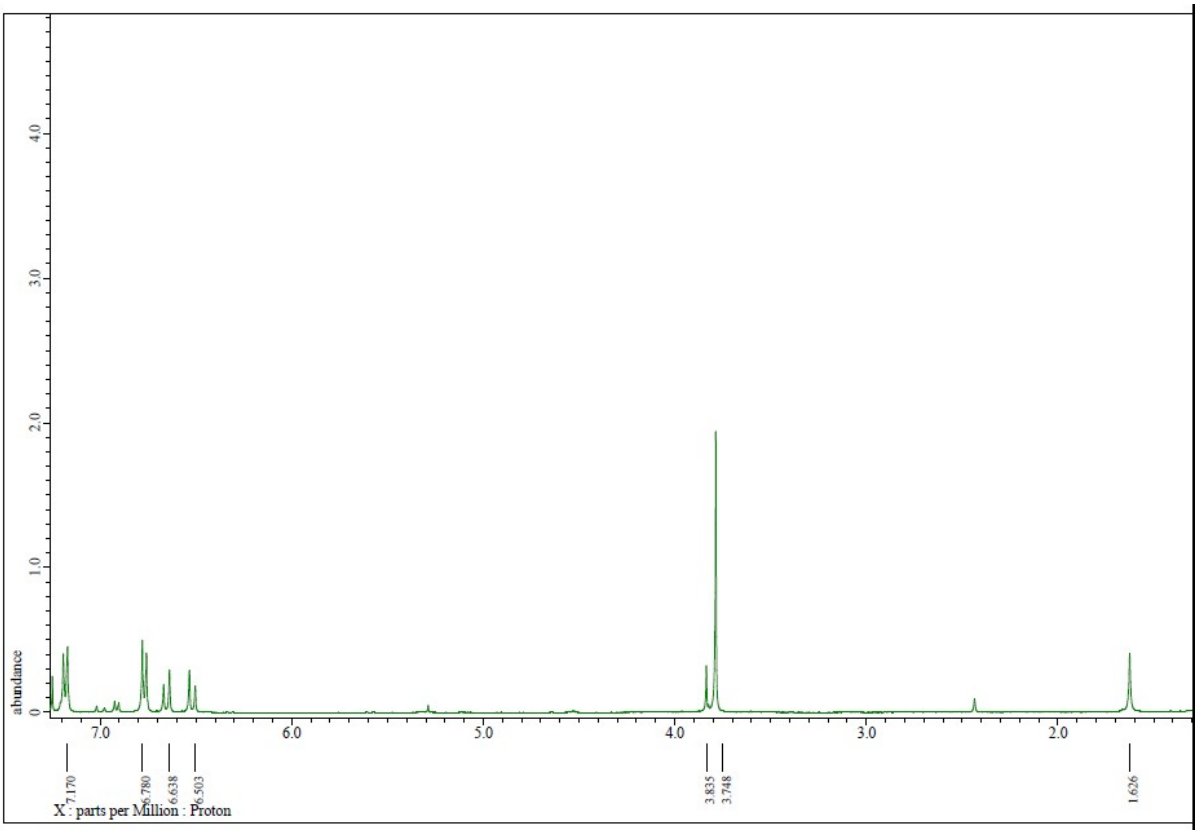

Figure: S32: ^{1}H-NMR SPECTRUM OF COMPOUND **4n**

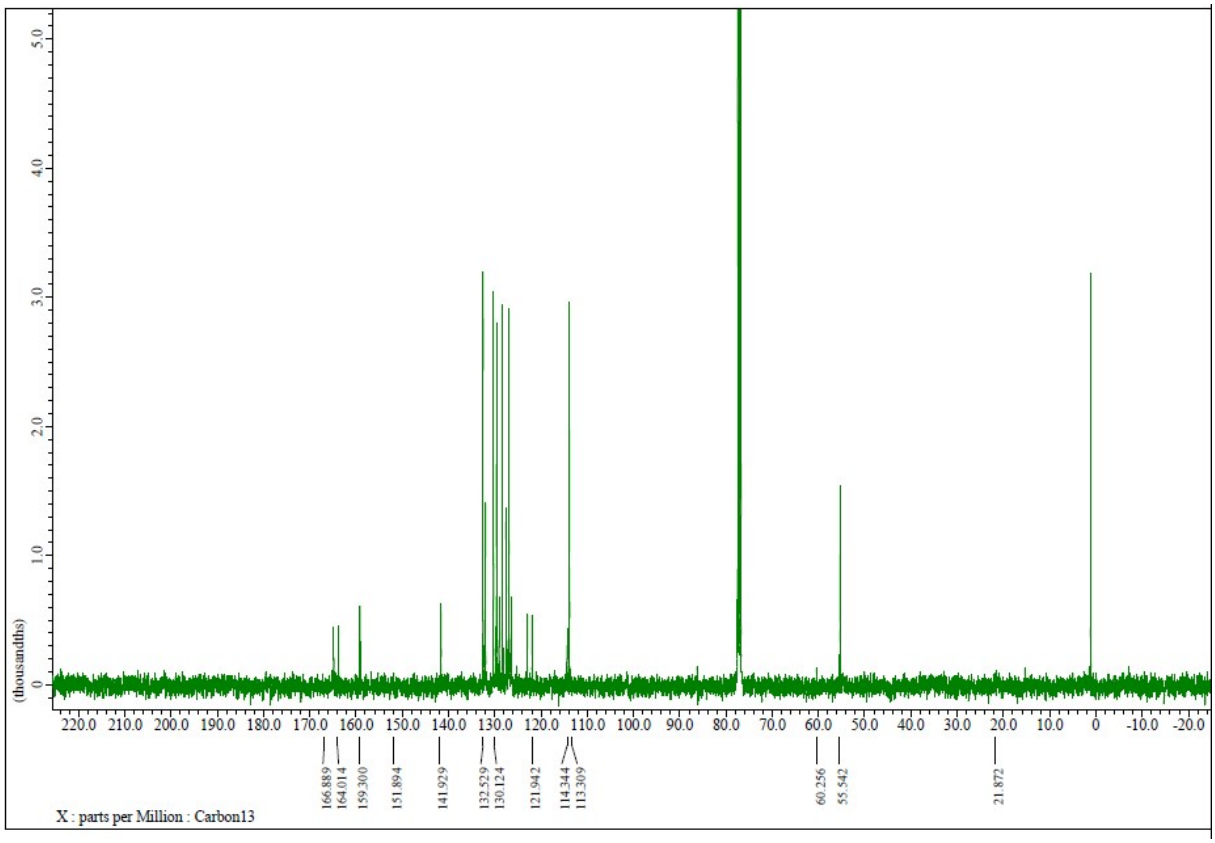

Figure: S33: ^{13}C-NMR SPECTRUM OF COMPOUND **4n**

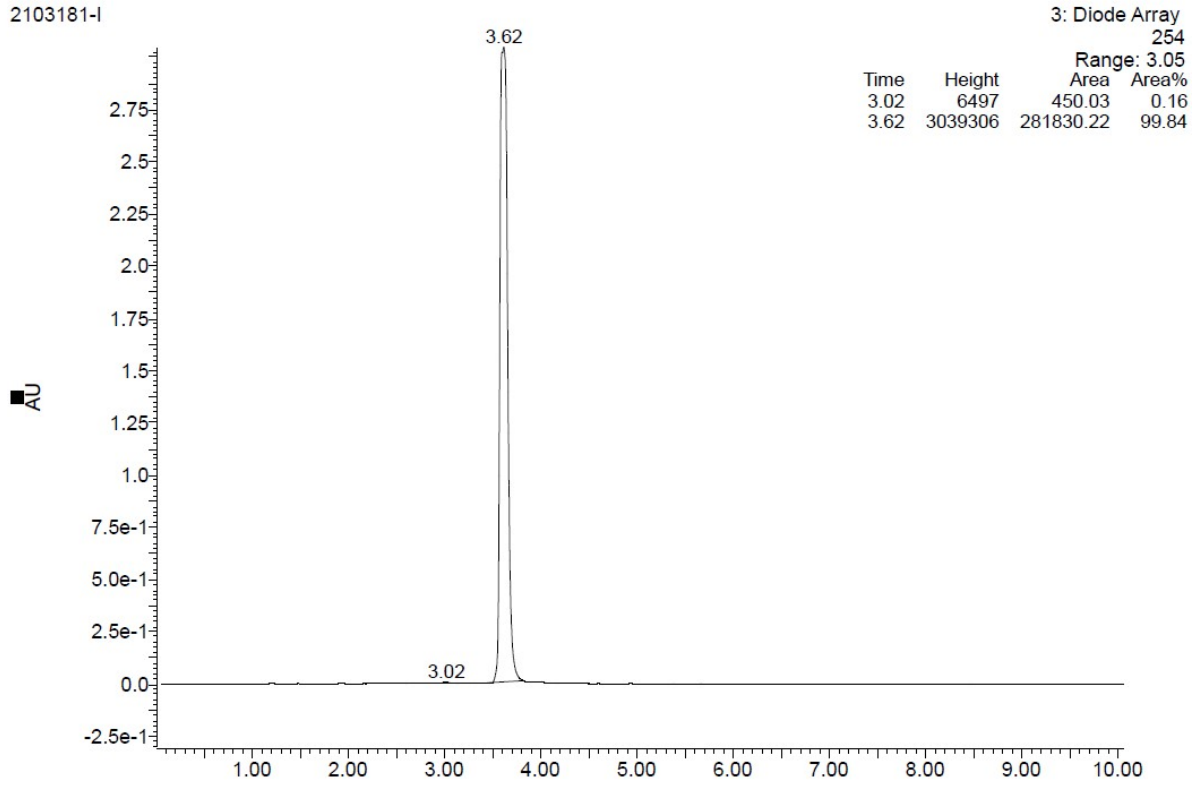

Figure: 34: LC PROFILE OF COMPOUND **4n**

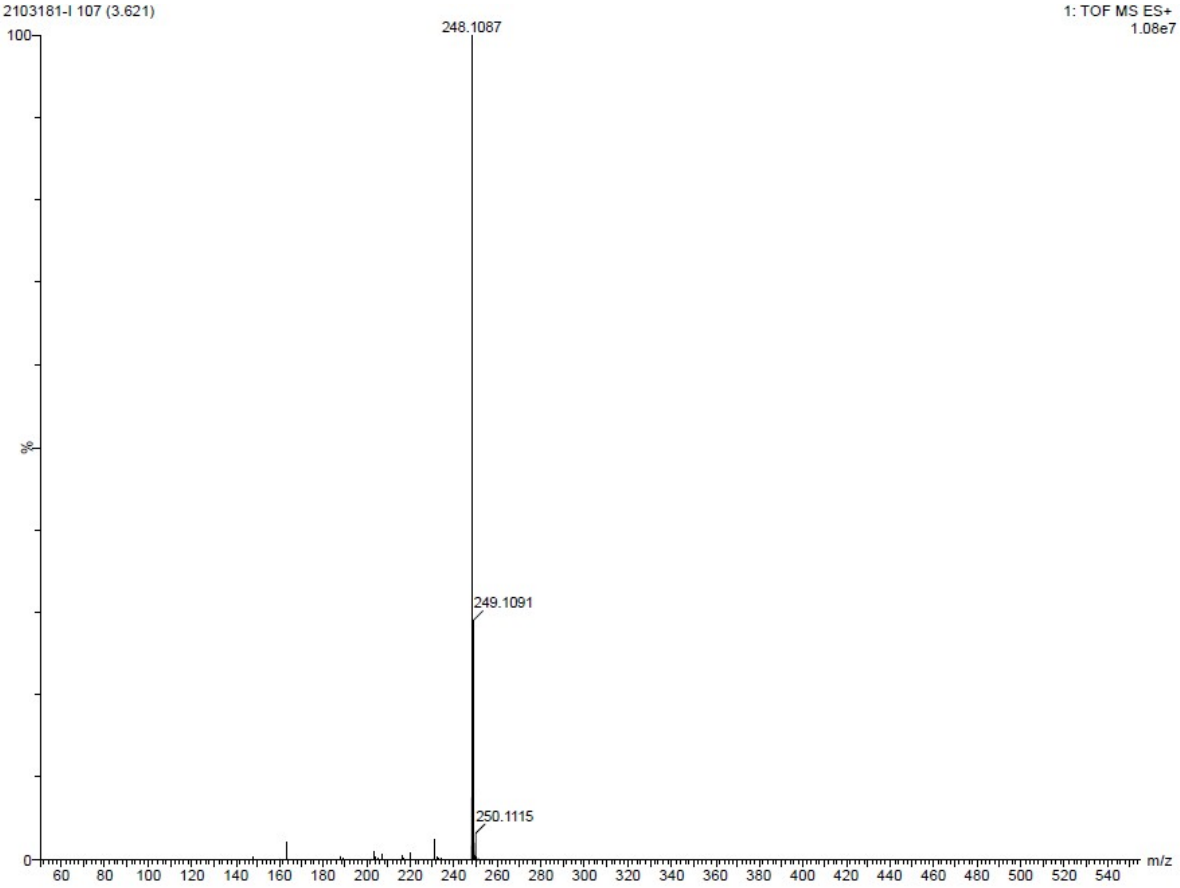

Figure: S35: LC-MS SPECTRUM OF COMPOUND **4n**

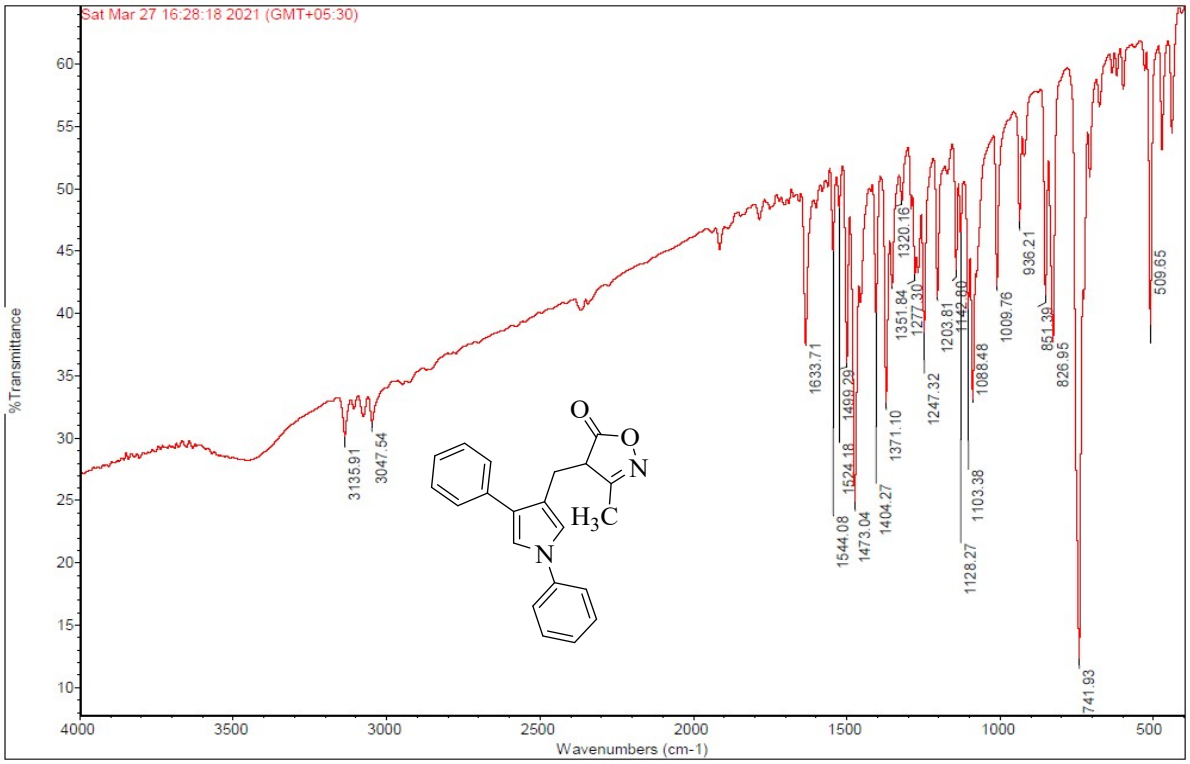

Figure: S36: FT-IR SPECTRUM OF COMPOUND **4o**

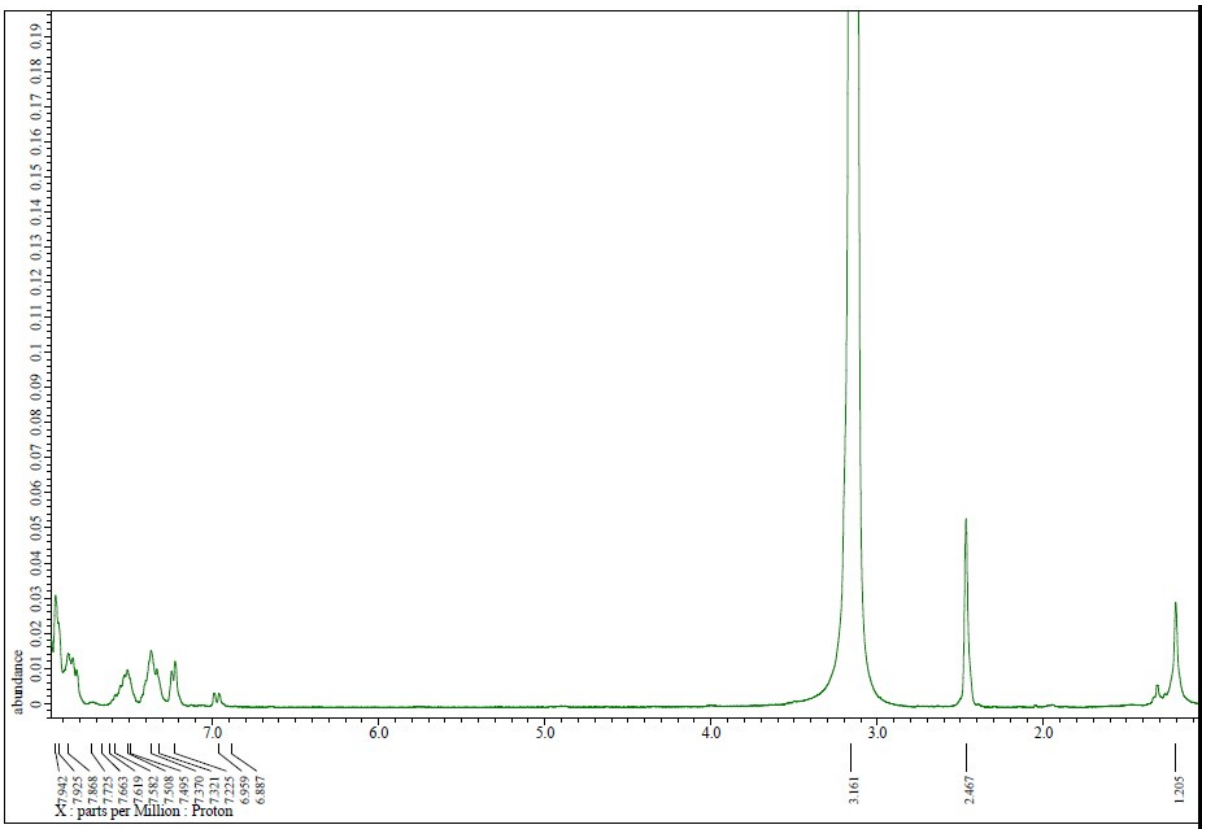

Figure: S37: [1]H-NMR SPECTRUM OF COMPOUND **4o**

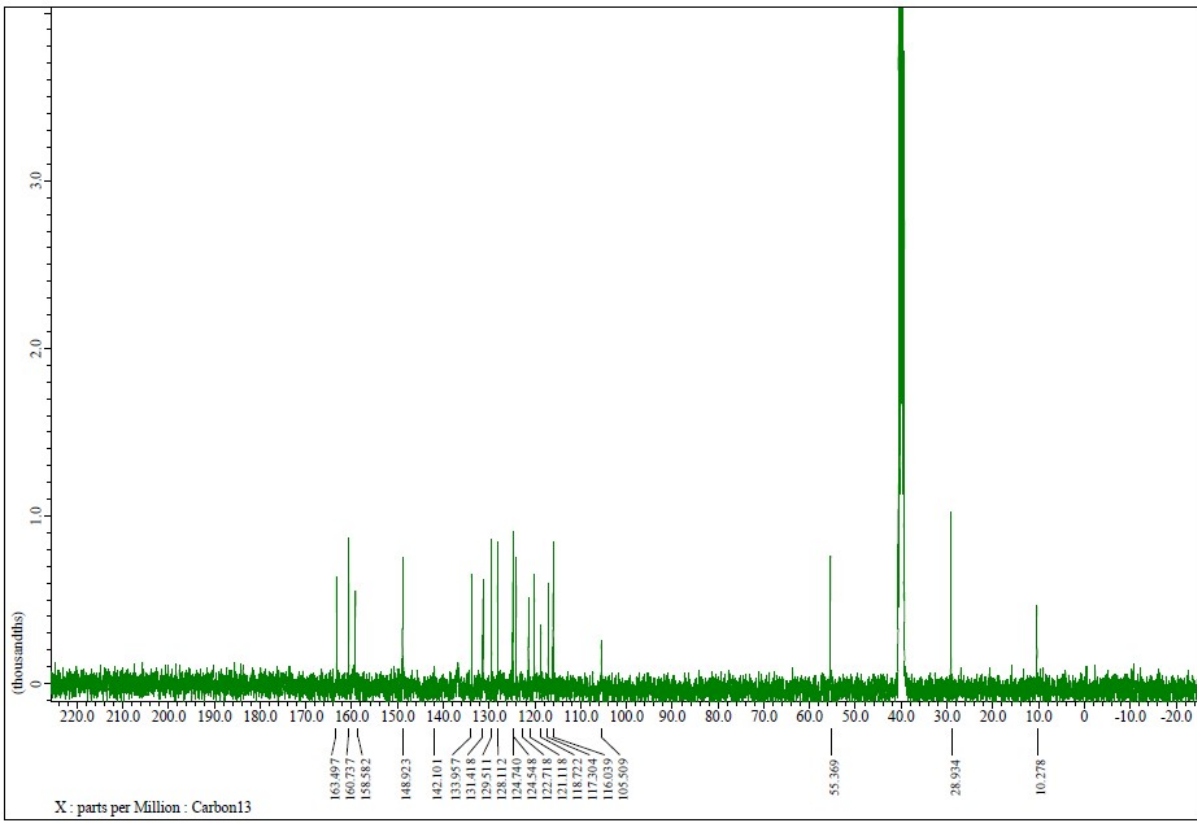

Figure: S38: [13]C-NMR SPECTRUM OF COMPOUND **4o**

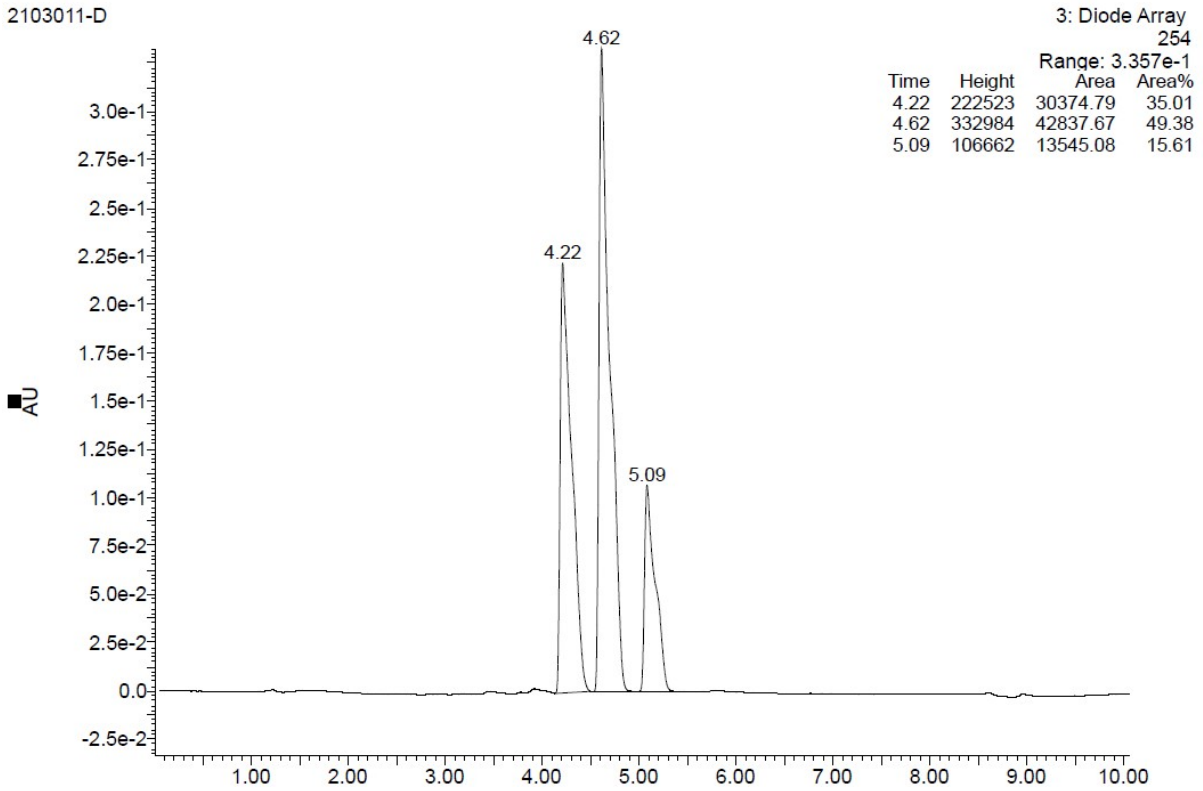

Figure: S39: LC PROFILE OF COMPOUND **4o**

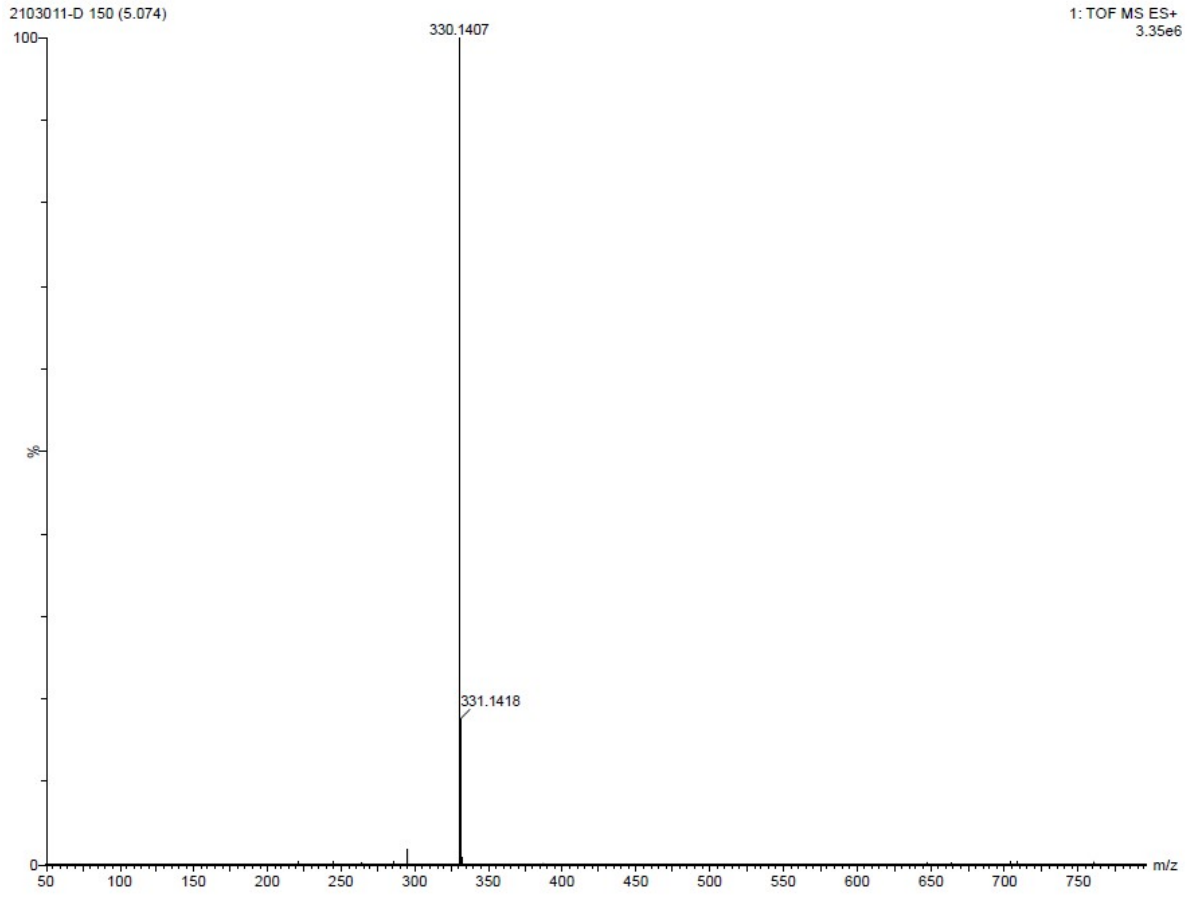

Figure: S40. LC-MS SPECTRUM OF COMPOUND **4o**

Table S1: In vitro anticancer evaluation of compounds

	Compounds	Concentration (µg/mL)						Negative control
		100	**50**	**25**	**12.5**	**6.25**	**3.125**	
Cell Viability of A549	**4h**	59.21	64.02	80.45	68.69	93.77	97.17	100
		57.51	64.59	77.62	69.87	95.18	96.6	
		57.22	64.59	77.62	67.12	91.78	95.75	
	Average	57.98	64.4	78.56	68.56	93.57	96.50	
	4i	52.41	54.39	55.81	56.66	62.89	81.59	100
		50.71	54.67	56.09	58.36	66.01	81.3	
		51.84	53.54	54.96	60.62	63.74	77.34	
	Average	51.65	54.2	55.62	58.54	64.21	80.07	
	4j	34.56	36.26	38.81	39.94	48.16	51.27	100
		33.43	34.28	38.53	42.21	48.44	52.41	
		31.73	37.39	38.24	41.64	50.14	50.71	
	Average	33.24	35.97	38.60	41.26	48.91	51.46	
	4k	20.96	24.08	40.23	71.95	88.67	93.48	100
		19.83	24.08	37.96	66.86	88.95	92.92	
		20.68	24.65	38.24	70.54	86.97	93.2	
	Average	20.46	24.27	38.81	69.78	88.19	93.2	
	4l	64.31	81.59	87.25	92.07	93.48	96.88	100
		63.46	82.15	87.25	91.22	94.33	96.88	
		63.74	81.59	86.12	89.24	94.9	98.3	
	Average	63.83	81.77	86.87	90.84	94.23	97.35	
	4m	25.5	28.33	34.56	43.91	72.24	79.04	100
		27.2	29.18	34.56	44.19	74.22	79.04	
		26.06	30.03	35.98	39.66	70.82	79.32	
	Average	26.25	29.18	35.03	42.58	72.42	79.13	
	4n	27.2	62.89	76.2	84.14	84.7	88.95	100
		25.78	63.74	72.8	82.15	87.25	91.78	
		26.35	64.31	72.24	81.87	86.97	89.8	
	Average	26.44	63.64	73.66	82.72	86.30	90.57	
	4o	19.55	20.68	21.81	48.44	69.41	88.39	100
		19.26	20.11	21.53	47.88	65.44	89.24	
		18.7	20.4	24.08	50.14	67.14	87.54	
	Average	19.17	20.39	22.47	48.82	67.33	88.39	
	Doxorubicin	34.58	37.69	40.24	42.51	44.78	49.88	100
		34.86	37.13	41.09	41.66	43.21	51.01	
		32.44	36.56	39.96	43.64	45.34	50.16	
	Average	**33.96**	**37.12**	**40.43**	**42.60**	**44.44**	**50.35**	