Supporting Information

Design, Synthesis, Modeling studies and biological Evaluation of Pyrazole-linked Aloe Emodin derivatives as potential anticancer agents

Guddeti Dileep Kumar[a,c], Bandi Siva[a], Sravanthi Vadlamudi[b], Surendar Reddy Bathula[b#], Hashnu Dutta[d], K. Suresh Babu[a]*

[a]Centre for Natural Products & Traditional Knowledge, CSIR-Indian Institute of Chemical Technology, Hyderabad 500007, India.

[b]Department of Organic Synthesis and process Chemistry, CSIR- Indian Institute of Chemical Technology, Hyderabad 500007, India.

[c]Academy of Scientific and Innovative Research (AcSIR), Ghaziabad - 201002, India.

[d]Department of Applied Biology, CSIR-Indian Institute of Chemical Technology, Hyderabad-500 007, India.

[#]Deceased

*Email: suresh@iict.res.in

Table of Contents

1. Materials and Methods

1.1 General

Diethyl/methyl but-2-ynedioates were procured from Sigma-Aldrich. Phenylhydrazine hydrochlorides and solvents were obtained from local suppliers. Reactions were monitored by thin layer chromatography (TLC) on precoated silica gel 60 F_{254} (mesh); spots were visualized under UV light and sprayed with 10% H_2SO_4 in MeOH followed by heating. Column chromatographic separations were carried out on silica gel (60-120 mesh). An IR spectrum was recorded with Nicolet-740 spectrometer with KBr pellets. The NMR spectra were recorded on a Bruker FT-300 MHz spectrometer at 300 MHz for ^1H and 75 MHz for ^{13}C, using TMS as internal standard. The chemical shifts are expressed as δ values in parts per million (ppm) and the coupling constants (*J*) are given in hertz (Hz). HRMS were carried out on Agilent 6510 Q-TOF LC/MS instrument.

1.2 Plant material

*Rheum emodi*Wall ex Meissn (Polygonaceae) rhizomes were collected from their natural habitat in the Himalayan region at Uttaranchal, India. Collected specimen were shade dried, powdered and used for solvent extraction. Voucher specimen were maintained at our laboratory for future reference.

1.3. Extraction and isolation

Rhizome powder was extracted with chloroform using a Soxhlet apparatus in a ratio of 1:6 (powder (in grams): solvent (in milliliters)). The resulted extract was evaporated to dryness at 40 ºC under reduced pressure (chloroform: 120 mbar in a rotary evaporator, Buchi, Switzerland[1].

The crude extract was then subjected to column chromatography on a silica gel column to afford several anthraquinones along with aloe emodin (1), which was isolated in major quantity. Aloe emodinwas obtained as orange solid and its structure was confirmed by ^1H and ^{13}C NMR and mass spectral analysis.

1.3.1. 1,8-dihydroxy-3-(hydroxymethyl)anthracene-9,10-dione (**1**) : Orange colour solid ; m.p : 226–228°C ; IR (KBr) ν_{max}: 3131,2367, 1672, 1628, 1572, 1400, 1288 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 12.10 (1H, s), 12.09 (1H, s), 7.86–7.80 (2H, m), 7.69 (1H, t, *J* = 7.9, 15.8 Hz), 7.37–7.30 (3H, m), 4.84 (2H, s) ; ^{13}C NMR (101 MHz, DMSO-d_6) : δ 191.51, 181.35, 161.60, 161.31, 153.65, 137.33, 133.21, 133.00, 124.40, 120.67, 119.34, 117.08, 115.76, 114.33, 62.05 ppm ; HRMS-ESI (*m/z*) : $[M+H]^+$ calculated for $C_{15}H_{11}O_5$; 271.0606, found 271.0617.

*1.4 Experimental procedure for the synthesis of **2***

2.90 g (1.50 mmol) of pyridiniumchlorochromate (PCC) was added in 200 ml of dichloromethane containing 1.62 g of compound (**1**) (1.0 mmol). The yielded mixture was stirred at room temperature 6 h till the reaction was accomplished. After the completion of reaction, the reaction mixture was washed with water in the separatory funnel. The dichloromethane layer was separated and the aqueous layer was extracted twice with dichloromethane. The combined dichloromethane solutions were dried for eight hours over anhydrous sodium sulfate and then the dichloromethane was distilled off to afford a yellow solid. Recrystallization of the yellow solid from methanol gave compound (**2**) as yellow needle.

1.4.1. 4,5-Dihydroxy-9,10-dioxo-9,10-dihydroanthracene-2-carbaldehyde (**2**) :

Yield 56%; mp 220–224°C; IR (KBr) ν_{max}: 3131, 1703, 1672, 1626,1401, 1267 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.95 (2H, s), 10.13 (1H, s), 8.14 (1H, d, J = 1.5 Hz), 7.88–7.82 (2H, m), 7.79– 7.75 (1H, m), 7.43 (1H, dd, J = 1.1, 8.3 Hz) ; ^{13}C NMR (101 MHz, DMSO-d_6) : δ 192.21, 191.35, 180.98, 161.36, 161.36, 141.39, 137.57, 134.29, 133.21, 124.59, 124.21, 119.58, 119.39, 118.02, 116.27 ppm ; HRMS-ESI (*m/z*) : $[M+H]^+$ calculated for $C_{15}H_9O_5$; 269.0450, found 269.0457.

*1.5 General procedure for the preparation of substituted (E)-1,8-dihydroxy-3-((2-phenylhydrazineylidene)methyl)anthracene-9,10-dione (**4**)*

*1.5.1. Synthesis of (E)-1,8-dihydroxy-3-((2-phenylhydrazineylidene) methyl)anthracene-9,10-dione(**4a**)*

Phenylhydrazine hydrochloride **3a** (0.029 g, 1.1 mmol) in mixtureof H_2O and AcOH was added to a stirred solution of 4,5-Dihydroxy-9,10-dioxo-9,10-dihydroanthracene-2-carbaldehyde (**2**) (0.050 g, 1.0 mmol) in methanol atroom temperature and the reaction was continued for 6 hours[2]. The reaction was monitored by TLC and an orange redprecipitate formation was observed. After completion of the reaction,the reaction mixture was cooled to room temperature, precipitate wasfiltered off, and the resulted precipitate was dissolved in ethyl acetate and washed with cold water to remove the acetic acid. The organic layer was separated and dried over Na_2SO_4, solventwas removed under reduced pressure afforded *(E)-1,8-dihydroxy-3-((2-phenylhydrazineylidene)methyl)anthracene-9,10-dione* (**4a**, 0.275 g) as orange color sold in 80 % yield; ^{1}H NMR (400 MHz, $CDCl_3$): δ 12.18 (1H, s), 12.14 (1H, s), 8.11 (1H, s), 8.08 (1H, s),

7.86 (1H, d, *J* = 8.4 Hz), 7.72–7.66 (2H, m), 7.52 (1H, s), 7.35–7.30 (2H, m), 7.20 (3H, d, *J* = 7.51 Hz), 6.98–6.95 (1H, m) ; ^{13}C NMR (151 MHz, DMSO-d_6) : δ 191.99, 190.23, 162.99, 162.91, 144.37, 137.94, 136.94, 134.30, 129.46, 128.31, 128.20, 125.11, 124.68, 124.31, 124.06, 121.53, 120.54, 120.03, 119.89, 118.02, 113.30 ppm ; HRMS-ESI (*m/z*) : $[M+H]^+$ calculated for $C_{21}H_{15}N_2O_4$; 359.1032, found 359.1047.

*1.6. General procedure for the synthesis of substituted aloe emodinpyrazole carboxylates (**5**)*

*1.6.1. Synthesis of diethyl 3-(4,5-dihydroxy-9,10-dioxo-9,10-dihydroanthracen-2-yl)-1-phenyl-1H-pyrazole-4,5-dicarboxylate (**5a**)* [3]

The compound (E)-1,8-dihydroxy-3-((2-phenylhydrazineylidene) methyl)anthracene-9,10-dione(**4a**, 0.03 g, 1 mmol) and diethyl but-2-ynedioate (0.187 g, 1.1 mmol) were heated to 130°C for 9 h. After completion of reaction (TLC), the reaction was cooled to room temperature and extracted with ethyl acetate (2×30 mL), washed with water and brine solution. The organic layer was separated and dried over Na_2SO_4, the solvent was removed under reduced pressure, the crude product was purified by column chromatography afforded diethyl 3-(4,5-dihydroxy-9,10-dioxo-9,10-dihydroanthracen-2-yl)-1-phenyl-1H-pyrazole-4,5-dicarboxylate (**5a**) as yellow colour solid.

*Diethyl 3-(4,5-dihydroxy-9,10-dioxo-9,10-dihydroanthracen-2-yl)-1-phenyl-1H-pyrazole-4-5-dicarboxylate (**5a**):* Yield: 82%; Yellow solid; ^{1}H NMR (400 MHz, $CDCl_3$): δ 12.11 (1H, s), 12.07 (1H, s), 8.28 (1H, d, *J* = 1.3 Hz), 7.87 (1H, d, *J* = 6.7 Hz), 7.78 (1H, d, *J* = 1.4 Hz), 7.70 (1H, t, *J* = 15.8, 8.0 Hz), 7.57 – 7.49 (5H, m), 7.32 (1H, d, *J* = 8.4 Hz), 4.38–4.32 (4H, m), 0.90 – 0.86 (6H, m); ^{13}C NMR (101 MHz, $CDCl_3$) δ 192.6, 181.4, 162.5, 162.3, 162.2, 159.8, 149.2, 140.4, 138.8, 137.2, 133.6, 133.3, 129.4, 129.2, 129.2, 124.6, 124.6, 124.2, 123.9, 123.4, 120.8, 120.0, 116.4, 115.6, 114.7, 62.6, 61.6, 13.9, 13.7 ppm. HRMS-ESI (*m/z*) : $[M+H]^+$ Calculated for $C_{29}H_{23}N_2O_8$; 527.1454, found 527.1450.

*1.6.2. Diethyl 3-(4,5-dihydroxy-9,10-dioxo-9,10-dihydroanthracen-2-yl)-1-(3,4-dimethylphenyl)-1H-pyrazole-4,5-dicarboxylate (**5b**):* Yield: 70%; Yellow solid; IR (KBr) ν_{max}: 3018, 2930, 2860, 1735, 1461 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 12.12 (1H, s), 12.06 (1H, s), 8.28 (1H, d, *J* = 1.7 Hz), 7.86 (1H, dd, *J* = 7.4, 1.1 Hz), 7.77 (1H, d, *J* = 1.5 Hz), 7.70 (1H, t, *J* = 15.8, 8.1 Hz), 7.35 – 7.34 (1H, m), 7.33 - 7.30 (1H, dd, *J*= 8.4, 1.1 Hz), 7.25 - 7.23 (2H, m), 4.39 - 4.31 (4H, m), 2.33 (6H, s), 1.35 – 1.26 (6H, m) ; ^{13}C NMR (126 MHz, $CDCl_3$) δ 192.6, 181.4, 162.5, 162.3, 162.2, 160.0, 149.0, 149.6, 140.6, 138.2, 137.9, 137.1, 136.4, 133.6, 133.2, 130.1, 125.5,

124.5, 124.2, 121.6, 120.9, 120.0, 115.9, 115.5, 114.2, 62.6, 61.4, 19.8, 19.5, 13.9, 13.8 ppm.HRMS-ESI (*m/z*) : $[M+H]^+$ Calculated for $C_{31}H_{27}N_2O_8$; 555.1767, found 555.1768.

*1.6.3. Diethyl 3-(4,5-dihydroxy-9,10-dioxo-9,10-dihydroanthracen-2-yl)-1-(o-tolyl)-1H-pyrazole-4,5-dicarboxylate (**5c**)* : Yield: 69%; Yellow solid; IR (KBr) ν_{max}: 3023, 2929, 1728, 1643, 1466 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 12.11 (1H, s), 12.06 (1H, s), 8.26 (1H, d, *J* = 1.5 Hz), 7.86 (1H, dd, *J* = 7.4, 1.1 Hz), 7.77 (1H, d, *J* = 1.7 Hz), 7.70 (1H, t, *J* = 15.8, 8.3 Hz), 7.47 – 7.40 (1H, m), 7.39–7.30 (4H, m), 4.41(2H, m) , 4.21 (2H, m), 2.18 (3H, s), 1.37 (3H, t, *J* = 14.3, 7.0 Hz), 1.13(3H, t, *J* = 14.1, 7.0 Hz); ^{13}C NMR (101 MHz, $CDCl_3$) δ 192.6, 181.4, 162.8, 162.5, 162.4, 158.7, 148.4, 140.4, 139.2, 138.0, 137.2, 135.6, 133.6, 133.4, 130.9, 127.1, 126.4, 124.6, 123.7, 120.3, 120.0, 115.9, 115.5, 114.8, 114.0, 62.2, 61.8, 17.3, 14.1, 13.9 ppm; HRMS-ESI (*m/z*) : $[M+H]^+$ Calculated for $C_{30}H_{25}N_2O_8$; 541.1611, found 541.1615.

*1.6.4. Diethyl 3-(4,5-dihydroxy-9,10-dioxo-9,10-dihydroanthracen-2-yl)-1-(4-(trifluoromethoxy)Phenyl)-1H-pyrazole-4,5-dicarboxylate (**5d**):* Yield: 79%; Yellow solid; m.p: 210-212; IR (KBr) ν_{max}: 3023, 1729, 1625, 1216 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 12.09 (1H, s), 12.06 (1H, s), 8.26 (1H, d, *J* = 1.5 Hz), 7.86 (1H, d, *J* = 7.3 Hz), 7.76 (1H, d, *J* = 1.5 Hz), 7.70 (1H, t, *J* = 15.8, 8.0 Hz), 7.61 (2H, dd, *J* =20.1, 8.8 Hz), 7.38–7.31 (3H, m), 4.38 (2H, q, *J* = 14.3, 7.1 Hz), 4.35 (2H, q, *J* =13.5, 6.4 Hz), 1.35 (3H, t, *J* =14.1, 7.0 Hz), 1.27 (3H, t, *J* = 14.1, 7.0 Hz); ^{13}C NMR (101 MHz, $CDCl_3$) δ 192.6, 181.3, 162.5, 162.4, 162.2, 159.4, 149.5, 149.2, 140.0, 137.5, 137.2, 137.1, 133.6, 133.4, 126.3, 126.3, 124.6, 124.0, 121.5, 121.5, 120.5, 120.1, 115.9, 115.7, 115.5, 62.8, 61.8, 29.7, 13.9, 13.7 ppm. HRMS-ESI (*m/z*) : $[M+H]^+$ Calculated for $C_{30}H_{22}N_2O_9F_3$; 611.1277, found 611.1281.

*1.6.5. Diethyl 3-(4,5-dihydroxy-9,10-dioxo-9,10-dihydroanthracen-2-yl)-1-(4-isopropylphenyl)-1H-pyrazole-4,5-dicarboxylate (**5e**) :*Yield: 84%; Yellow solid; m.p: 210-212; IR (KBr) ν_{max}: 3024, 2401, 1425, 1216 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 12.11 (1H, s), 12.06 (1H, s), 8.27 (1H, d, *J* = 1.5 Hz), 7.86 (1H, dd, *J* = 7.4,1.1 Hz), 7.77 (1H, d, *J* = 1.7 Hz), 7.70 (1H, t, *J* = 16.0, 8.3 Hz), 7.49–7.44 (2H, m), 7.37–7.30 (3H, m), 4.40–4.30 (4H, m), 3.04–2.95 (1H, m), 1.33 (3H, t, *J* =14.3, 7.2 Hz), 1.30 (3H, s), 1.28(3H,s), 1.25(3H, t, *J* =14.3,7.2); ^{13}C NMR (126 MHz, $CDCl_3$) δ 192.6, 181.4, 162.5, 162.3, 162.3, 159.8, 150.4, 149.0, 140.5, 137.8, 137.1, 136.5, 133.6, 133.3, 127.2, 127.2, 124.5, 124.4, 124.4, 124.1, 120.8, 120.0, 115.9, 115.5, 114.5, 62.5, 61.5, 33.9, 23.8, 23.8, 13.9, 13.7 ppm; HRMS-ESI (*m/z*) : $[M+H]^+$ Calculated for $C_{32}H_{29}N_2O_8$; 569.1924, found 569.1923.

1.6.6. Diethyl 1-(4-chlorophenyl)-3-(4,5-dihydroxy-9,10-dioxo-9,10-dihydroanthracen-2-yl)-1H-pyrazole-4,5-dicarboxylate ***(5f)***: Yield: 83%; Yellow solid; IR (KBr) ν_{max}: 3025, 2402, 1730, 1629, 1506, 754 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 12.10 (1H, s), 12.06 (1H, s), 8.25 (1H, d, *J* = 1.6 Hz), 7.86 (1H, dd, *J* = 7.4,1.0 Hz), 7.76 (1H, d, *J* = 1.6 Hz), 7.70 (1H, t, *J* = 16.0, 8.2 Hz), 7.54–7.47 (4H, m), 7.32 (1H, dd, *J* = 8.3, 1.0 Hz), 4.41–4.32 (4H, m), 1.34 (3H, t, *J* = 14.3, 7.1 Hz), 1.29 (3H, t, *J* = 14.3,7.1); ^{13}C NMR (101 MHz, $CDCl_3$): δ 192.6, 181.3, 162.5, 162.3, 162.2, 159.5, 149.2, 140.1, 137.5, 137.2, 135.4, 133.6, 133.4, 129.8, 129.4, 129.4, 125.9, 125.9, 124.6, 124.0, 120.5, 120.1, 115.9, 115.6, 115.3, 62.8, 61.7, 13.9, 13.8 ppm; HRMS-ESI (*m/z*) : $[M+H]^+$ Calculated for $C_{29}H_{22}N_2O_8Cl$; 561.1065, found 561.1080.

1.6.7. Diethyl 3-(4,5-dihydroxy-9,10-dioxo-9,10-dihydroanthracen-2-yl)-1-(m-tolyl)-1H-pyrazole-4,5-dicarboxylate ***(5g)****:* Yield: 72%; Yellow solid; IR (KBr) ν_{max}: 3021, 2404, 1432, 1215 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 12.12 (1H, s), 12.07 (1H, s), 8.28 (1H, d, *J* = 1.7 Hz), 7.86 (1H, dd, *J* = 7.4,1.1 Hz), 7.78 (1H, d, *J* = 1.5 Hz), 7.70 (1H, t, *J* = 15.8, 8.1 Hz), 7.41–7.28 (5H, m), 4.40–4.31 (4H, m), 2.44 (3H, m), 1.35–1.27 (6H, m); ^{13}C NMR (126 MHz, $CDCl_3$) δ 192.7, 181.4, 162.5, 162.3, 162.2, 159.9, 149.2, 140.5, 139.5, 138.6, 138.0, 137.2, 133.6, 133.3, 130.1, 128.9, 125.1, 124.6, 124.3, 121.4, 120.8, 120.1, 115.9, 115.5, 114.4, 62.6, 61.5, 21.3, 13.9, 13.7 ppm. HRMS-ESI (*m/z*) : $[M+H]^+$ Calculated for $C_{30}H_{25}N_2O_8$; 541.1611, found 541.1620.

1.6.8. Diethyl 1-(4-bromophenyl)-3-(4,5-dihydroxy-9,10-dioxo-9,10-dihydroanthracen-2-yl)-1H-pyrazole-4,5-dicarboxylate ***(5h)*** : Yield: 70%; Yellow solid; IR (KBr) ν_{max}: 3021, 2400, 1738, 1625, 757 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 12.10 (1H, s), 12.07 (1H, s), 8.25 (1H, d, *J* = 1.5 Hz), 7.87 (1H, dd, *J* = 7.4,0.9 Hz), 7.76 (1H, d, *J* = 1.7 Hz), 7.71 (1H, t, *J* = 15.8, 8.1 Hz), 7.67–7.63 (2H, m), 7.47–7.43 (2H, m), 7.33(1H, dd, *J* = 8.5, 1.1 Hz), 4.41–4.32 (4H, m), 1.36–1.27 (6H, m); ^{13}C NMR (101 MHz, $CDCl_3$) δ 192.6, 181.4, 162.5, 162.3, 162.2, 159.5, 149.3, 140.1, 137.8, 137.5, 137.2, 133.6, 133.4, 132.4, 132.4, 127.8, 126.5, 126.1, 124.6, 124.0, 120.5, 120.1, 115.9, 115.6, 115.3, 62.8, 61.7, 14.1, 13.8 ppm; HRMS-ESI (*m/z*) : $[M+H]^+$ Calculated for $C_{29}H_{22}N_2O_8Br$; 605.0461, found 605.0578.

1.6.9. Diethyl 3-(4,5-dihydroxy-9,10-dioxo-9,10-dihydroanthracen-2-yl)-1-(3-fluorophenyl)-1H-pyrazole-4,5-dicarboxylate ***(5i)***: Yield: 70%; Yellow solid; IR (KBr) ν_{max}: 3078, 3018, 1737, 1625, 1283 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 12.10 (1H, s), 12.07 (1H, s), 8.26 (1H, d, *J* = 1.7 Hz), 7.87 (1H, dd, *J* = 7.4,1.1 Hz), 7.76 (1H, d, *J* = 1.7 Hz), 7.71 (1H, t, *J* = 15.8, 8.1 Hz), 7.52–7.45 (1H, m), 7.39–7.31 (3H, m), 7.24–7.18(1H, m), 4.41–4.34 (4H, m), 1.36–1.28 (6H,

m); ^{13}C NMR (101 MHz, $CDCl_3$): δ 192.6, 181.3, 162.5, 162.3, 162.0, 159.6, 149.4, 140.1, 137.8, 137.2, 133.6, 133.4, 130.5, 130.5, 124.6, 124.1, 120.6, 120.1,120.1, 116.5, 116.3, 115.9, 115.7, 112.5, 112.2, 62.8, 61.7, 13.9, 13.7 ppm; HRMS-ESI (*m/z*) : $[M+H]^+$ Calculated for $C_{29}H_{22}N_2O_8F$; 544.1315, found 545.1380.

*1.6.10. Diethyl 1-(4-cyanophenyl)-3-(4,5-dihydroxy-9,10-dioxo-9,10-dihydroanthracen-2-yl)-1H-pyrazole-4,5-dicarboxylate (**5j**)*:Yield: 72%;^{1}H NMR (500 MHz, $CDCl_3$): δ 12.08 (2H, s, overlap), 8.25(1H, d, *J* = 1.6 Hz), 7.89–7.80 (3H, m), 7.76–7.68 (4H, m), 7.36–7.30 (1H, m), 4.43–4.35 (4H, m), 1.39–1.29 (6H, m); ^{13}C NMR (101 MHz, $CDCl_3$): δ 192.6, 181.3, 162.5, 162.3, 162.0, 159.3, 149.8, 142.0, 139.6, 137.3, 137.2, 133.5, 133.5, 133.2, 133.2, 125.1, 125.1, 124.7, 123.9, 120.3, 120.1, 115.8, 115.8, 115.7, 113.2, 113.0, 63.0, 61.9, 13.9, 13.8 ppm; HRMS-ESI (*m/z*) : $[M+H]^+$ Calculated for $C_{30}H_{22}N_3O_8$; 551.1362, found 552.1430.

*1.6.11. Diethyl 3-(4,5-dihydroxy-9,10-dioxo-9,10-dihydroanthracen-2-yl)-1-(4-methoxyphenyl)-1H-pyrazole-4,5-dicarboxylate (**5k**)* : Yield: 80%; Yellow solid; m.p: 210-212; IR (KBr) ν_{max}: 3021, 2928, 1732, 1627, 1463 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ 12.12 (1H, s), 12.06 (1H, s), 8.27 (1H, d, *J* = 1.7 Hz), 7.86 (1H, dd, *J* = 7.4,1.1 Hz), 7.77 (1H, d, *J* = 1.7 Hz), 7.70 (1H, t, *J* = 15.8, 8.1 Hz), 7.50–7.45 (2H, m), 7.32 (1H, dd, *J* = 8.3, 1.1 Hz), 7.02–6.97(2H, m), 4.40–4.29 (4H, m), 3.87 (3H, s), 1.33 (3H, t, *J* = 14.3, 7.2 Hz), 1.27 (3H, t, *J* = 14.3,7.2); ^{13}C NMR (101 MHz, $CDCl_3$): δ 192.6, 181.4, 162.5, 162.3, 160.2, 160.1, 159.8, 148.8, 140.4, 137.8, 137.2, 133.6, 133.3, 131.7, 126.1, 126.1, 124.5, 124.1, 120.7, 120.0, 115.9, 115.5, 114.4, 114.2, 114.2, 62.6, 61.5, 55.6, 13.9, 13.8 ppm; HRMS-ESI (*m/z*) : $[M+H]^+$ Calculated for $C_{30}H_{25}N_2O_9$; 557.1560, found 557.1599.

*1.7. General experimental procedure for preparation of compound **6a-6e***[3]

The compound (E)-1,8-dihydroxy-3-((2-phenylhydrazineylidene)methyl)anthracene-9,10-dione(**4a**, 0.3 g, 1 mmol) and dimethyl but-2-ynedioate (0.187 g, 1.1 mmol) were heated to 130°C for 12 h. After completion of reaction (TLC), the reaction was cooled to room temperature and extracted with ethyl acetate (2×30 mL), washed with water and brine solution. The organic layer was separated and dried over Na_2SO_4, the solvent was removed under reduced pressure, the crude product was purified by column chromatography afforded Dimethyl 3-(4,5-dihydroxy-9,10-dioxo-9,10-dihydroanthracen-2-yl)-1-phenyl-1H-pyrazole-4,5-dicarboxylate (**6a**) as yellow colour solid.

*1.7.1. Dimethyl 3-(4,5-dihydroxy-9,10-dioxo-9,10-dihydroanthracen-2-yl)-1-phenyl-1H-pyrazole-4,5-dicarboxylate (**6a**):* Yield: 73%; ^{1}H NMR (400 MHz, $CDCl_3$): δ 12.11 (1H, s), 12.07 (1H,s), 8.26 (1H, d, *J* = 1.7 Hz), 7.86 (1H, dd, *J* = 7.5, 1.7 Hz), 7.77 (1H, d, *J* = 1.7 Hz), 7.70 (1H, t, *J* =15.8, 8.1 Hz), 7.58 – 7.51 (5H, m), 7.32 (1H, dd, *J* = 0.9, 8.4 Hz), 3.89 (3H, s), 3.88 (3H, s); ^{13}C NMR (101 MHz, $CDCl_3$): δ 192.6, 181.4, 162.6, 162.5, 162.3, 160.3, 149.3, 140.2, 138.6, 137.5, 137.2, 133.5, 133.3, 129.4, 129.3, 129.3, 124.6, 124.3, 124.3, 124.1, 120.6, 120.0, 115.9, 115.6, 114.5, 53.3, 52.5 ppm; HRMS-ESI (*m/z*) : $[M+H]^+$ Calculated for $C_{27}H_{19}N_2O_8$; 499.1141, found 499.1132.

*1.7.2. Dimethyl 3-(4,5-dihydroxy-9,10-dioxo-9,10-dihydroanthracen-2-yl)-1-(3,4-dimethylphenyl)-1H-pyrazole-4,5-dicarboxylate (**6b**):* Yield: 73%; ^{1}H NMR (400 MHz, $CDCl_3$): δ 12.12(1H, s), 12.07 (1H, s), 8.27 (1H, d, *J* = 1.6 Hz), 7.86 (1H, dd, *J* = 7.5, 1.1 Hz), 7.76 (1H, d, *J* = 1.6 Hz), 7.70 (1H, t, *J* =15.9, 8.2 Hz), 7.35 (1H, s), 7.32 (1H, dd, *J* = 8.4, 1.1 Hz), 7.24 (2H, s), 3.89 (3H, s), 3.88(3H, s), 2.34 (3H, s), 2.33 (3H,s); ^{13}C NMR (101 MHz, $CDCl_3$): δ 192.6, 181.4, 162.7, 162.5, 162.3, 160.5, 149.1, 140.4, 138.3, 138.0, 137.5, 137.2, 136.4, 133.6, 133.3, 130.2, 125.4, 124.6, 124.2, 121.4, 120.7, 120.0, 115.9, 115.5, 114.1, 53.2, 52.4, 19.8, 19.5 ppm; HRMS-ESI (*m/z*) : $[M+H]^+$ Calculated for $C_{29}H_{23}N_2O_8$; 527.1454, found 527.1458.

*1.7.3. Dimethyl 3-(4,5-dihydroxy-9,10-dioxo-9,10-dihydroanthracen-2-yl)-1-(o-tolyl)-1H-pyrazole-4,5-dicarboxylate (**6c**):* Yield: 77%; ^{1}H NMR (400 MHz, $CDCl_3$): δ 12.11 (1H, s), 12.06 (1H, s), 8.25 (1H, d, *J* = 1.7 Hz), 7.86 (1H, dd, *J* = 7.5, 1.2 Hz), 7.75 (1H, d, *J* = 1.7 Hz), 7.70 (1H, t, *J* = 15.9, 8.2 Hz), 7.46–7.42 (1H, m), 7.38–7.36 (1H, m), 7.34–7.31 (3H, m), 3.94 (3H, s), 3.78 (3H, s), 2.18 (3H, s); ^{13}C NMR (101 MHz, $CDCl_3$): δ 192.6, 181.4, 162.5, 162.5, 163.2, 159.2, 148.5, 140.2, 137.9, 137.4, 137.2, 135.5, 133.6, 133.5, 131.0, 130.2, 127.0, 126.5, 124.6, 123.6, 120.2, 120.1, 115.9, 115.6, 114.6, 53.0, 52.6, 17.3 ppm; HRMS-ESI (*m/z*) : $[M+H]^+$ Calculated for $C_{28}H_{21}N_2O_8$; 513.1298, found 513.1288.

*1.7.4. Dimethyl 3-(4,5-dihydroxy-9,10-dioxo-9,10-dihydroanthracen-2-yl)-1-(4-(trifluoromethoxy)phenyl)-1H-pyrazole-4,5-dicarboxylate (**6d**):* Yield: 71%; ^{1}H NMR (400 MHz, $CDCl_3$): δ 12.09 (1H, s), 12.07 (1H, s), 8.25 (1H, d, *J* = 1.5 Hz), 7.87(1H, dd, *J* = 7.5, 0.8 Hz), 7.75 (1H, d, *J* = 1.5 Hz), 7.71 (1H, t, *J* = 15.8, 8.0 Hz), 7.61 (2H, d, *J* = 8.9 Hz), 7.39–7.31 (3H, m), 3.91 (3H, s), 3.90 (3H, s); ^{13}C NMR (101 MHz, $CDCl_3$): δ 192.6, 181.4, 162.6, 162.6, 162.4, 159.9, 149.5, 149.4, 139.8, 137.3, 137.2, 137.0, 133.6, 133.5, 126.2, 126.2, 124.7, 123.9, 121.5,

121.5, 121.0, 120.4, 120.1, 115.9, 115.7, 115.3, 53.4, 52.6 ppm; HRMS-ESI (*m/z*) : $[M+H]^+$ Calculated for $C_{28}H_{18}N_2O_9F_3$; 583.0964, found 583.0958.

*1.7.5. Dimethyl 3-(4,5-dihydroxy-9,10-dioxo-9,10-dihydroanthracen-2-yl)-1-(4-isopropylphenyl)-1H-pyrazole-4,5-dicarboxylate (**6e**):* Yield: 78%; Yellow solid; IR (KBr) ν_{max}: 3084, 2926, 1738, 1627, 1218 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 12.11 (1H, s), 12.06 (1H, s), 8.26 (1H, d, *J* = 1.7 Hz), 7.86(1H, dd, *J* = 7.4, 1.1 Hz), 7.76 (1H, d, *J* = 1.5 Hz), 7.70 (1H, t, *J* = 16.0, 8.3 Hz), 7.46 (2H, d, *J* = 8.5 Hz), 7.37–7.30 (3H, m), 3.89 (6H, s), 2.99 (1H, m), 1.30 (3H, s), 1.29(3H, s); ^{13}C NMR (101 MHz, $CDCl_3$): δ 192.6, 181.4, 162.7, 162.5, 162.4, 160.4, 150.4, 149.1, 140.3, 137.5, 137.2, 136.4, 133.6, 133.4, 127.3, 127.3, 124.6, 124.3, 124.3, 124.1, 120.7, 120.1, 115.9, 115.6, 114.3, 53.3, 52.4, 33.9, 23.8, 23.8 ppm; HRMS-ESI (*m/z*) : $[M+H]^+$ Calculated for $C_{30}H_{25}N_2O_8$; 541.1611, found 541.1588.

1.8. Biology

1.8.1. Cell Culture

MDA-MB-231, MCF-7, HepG2, B16F10and HEK-293 cell lines were used in the present study was obtained from ATCC (American Type Culture Collection, USA) and were routinely maintained in RPMI (Sigma Aldrich) supplemented with 10% FBS (Merck) and 1% Antibiotic/Antimycotic Solution (Merck) at 37 ˚C in a humidified incubator with 5% CO_2. All stock solution of compounds was prepared in cell culture grade DMSO and stored in -20°C. Compounds were diluted in culture media prior to use in experiments. Annexin V FITC Apoptosis Detection Kit was purchased from Calbiochem. MTT dye Kit was procured from Sigma Aldrich. Absorbance was recorded using Eliza Plate Reader. Flow cytometry was performed using a FACScan (Becton Dickinson, Mountain View, CA) flow cytometer.

1.8.2. Cell viability assay

Cell viability of the treated cells was analyzed by using an MTT assay. In brief, cells (4 x 10^3 cells /well) were cultivated in 96 well tissue culture plates and treated with 2.5, 5, 10 and 20µM concentrations of all aloe emodin derivatives for 48 h. 10µL of MTT (10mg/mL) was added to the wells after 48h and incubated for further 3h. Absorbance was recorded at 540 nm using Eliza Plate Reader. All the experiments were performed three times independently. IC_{50} of all compounds are listed in **Table-1**.

1.8.3. Lactate Dehydrogenase (LDH) assay

LDH assay is an "indicator of cell membrane injury", was measured using LDH assay kit purchased from Sigma Aldrich[4].In Brief, cells (4 x 10^3 cells /well) were cultivated in 96 well tissue culture plates and for 48 h cells were treated with different concentrations of compounds. About 20 µL of culture supernatants from the different treatment was taken for the activity analysis of extracellular LDH. The absorbance was recorded at 450 nm wavelength and the results were expressed as the percentage of LDH leakage from treated cells vs. control cells.

1.8.4. Apoptosis studies

The effect of compounds for inducing apoptosis[5] in MDA-MB-231 cells was analysed using Annexin V/PI staining assay based on the manufacturer's instructions (Calbiochem). The MDA-MB 231 cells (5×10^5 cells / well) were cultured in 6 well plates and treated with 5, 10 and 20µM of compound 6b& 6e for 24h. At the end of incubations, cells were prepared as suspension in 500µL of cold PBS, centrifuged for 5 min. at 1000 x g, resuspended in 500µL PBS and then 1.25µL of Annexin V FITC and10µL of media binding reagent was added. After centrifugation at 1000x*g*, the pelleted cells were suspended in 500 µl of cold 1x binding buffer and about 10 µl of PI was added to the cell suspension, the mixture was kept in ice for flow cytometry analysis. Flow cytometry was performed using a FACScan (Becton Dickinson, Mountain View, CA) flow cytometer, equipped with a single 488-nm argon laser. Annexin V-FITC were analyzed using excitation and emission of 488 nm and 535 nm (FL-1 channel) for PI, 488 nm and 610 nm (FL-2 channel) respectively. Debris and clumps were gated out using forward and orthogonal light scatter.

1.8.5. Cell cycle analysis

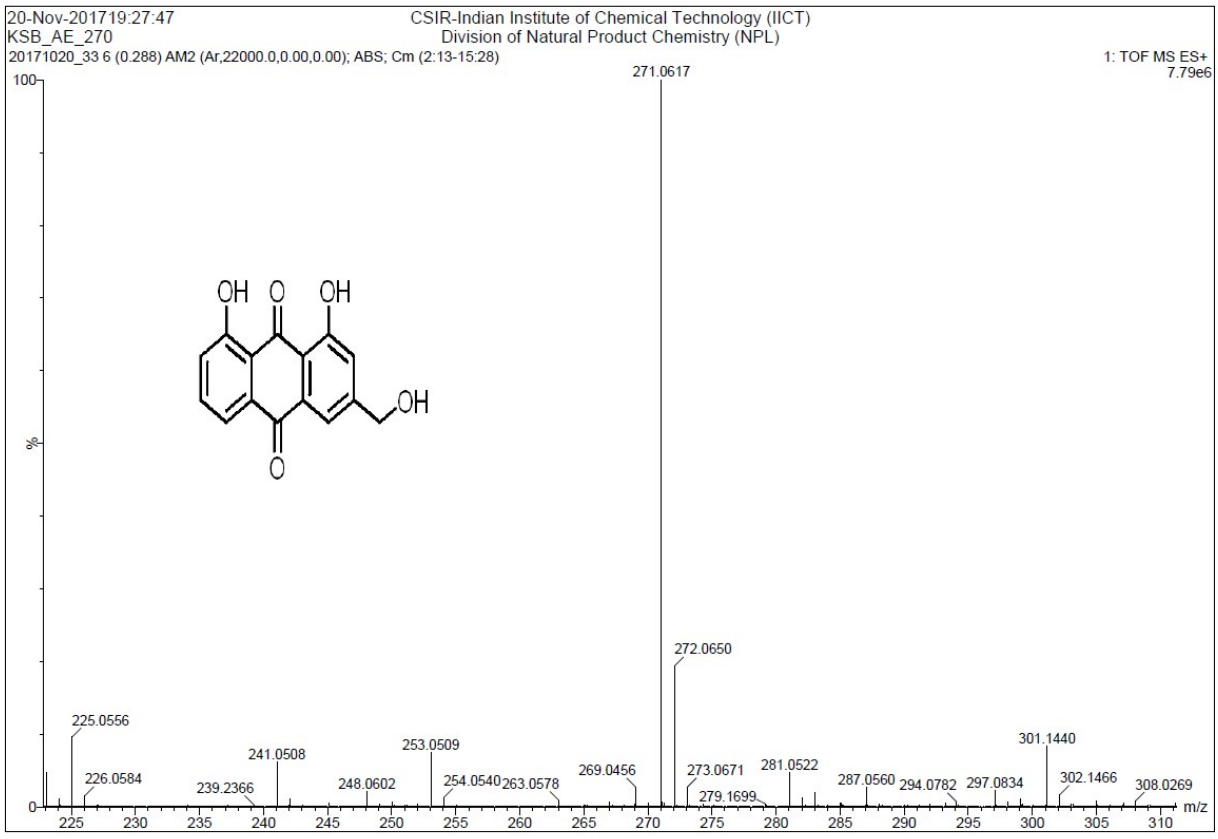
The effect of compound to inhibit the cell cycle [6] analysis in MDA-MB-231 cells was measured using flow cytometry analysis. The cells (5×10^5 cells / well) were cultured in 6 well plates and treated with 5, 10 and 20µM of compound 6b & 6e for 24 h. After the treatment, the cells were fixed with 70% ethanol and incubated overnight at -20˚C. After fixation, the cells were centrifuged at 1000x for 5 min and the 500 µL PBS with ribonuclease A (100 µg/mL) and 1% of TritonX 100 was added. After incubation for 30 mins at RT, the cells were stained with PI (50 µg/mL) and incubated further for 30 mins in dark before flow cytometry analysis. Data from 10^5 cells were collected and analyzed with FACScan flow cytometer (Becton Dickinson, Mountain View, CA) equipped with an argon laser to give 488 nm light.

1.8.6. Assay of caspase activity

A quantitative caspase enzymatic activity was carried out using ApoTarget caspase colorimetric protease assay kit (Invitrogen) by following the manufacturer's instructions. The 24 h cultured MDA-MB 231 cells were treated with compound 6b, 6e at 0, 5, 10 and 20μM concentrations for 48 h. After treatment, the cells lysed using cell lysis buffer and the 50 µL of cells and the 50 µL of reaction buffer containing 10 mM DTT were aliquoted into 96-well microtiter plate. Subsequently, the caspase substrate (5 µL; final concentration 4 mM) was added into each well and incubated for 2 h at 37 °C. Finally, the samples were read at 405 nm in a microplate reader. The selective substrates used were: VDVAD-pNA (substrate for caspase-2), DEVD-pNA (substrate for caspase-3), VEID-pNA (substrate for caspase-6), IETD-pNA (substrate for caspase-8), LEHDpNA (substrate for caspase-9).

1.8.7. Statistical Analysis

Each experiment was performed in triplicate. Data presentation was done as mean± SD and compared using student's t-test. *$p<0.05$, **$p<0.001$ or less was considered to be statistically significant.



Elemental Composition Report **Page 1**

Single Mass Analysis
Tolerance = 10.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
19 formula(e) evaluated with 1 results within limits (up to 10 closest results for each mass)
Elements Used:
C: 0-17 H: 0-75 O: 0-7

Minimum:				-1.5				
Maximum:		5.0	10.0	50.0				
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
271.0617	271.0606	1.1	4.1	10.5	873.4	n/a	n/a	C15 H11 O5

Figure S1: HRESIMS SPECTRUM OF COMPOUND **1**

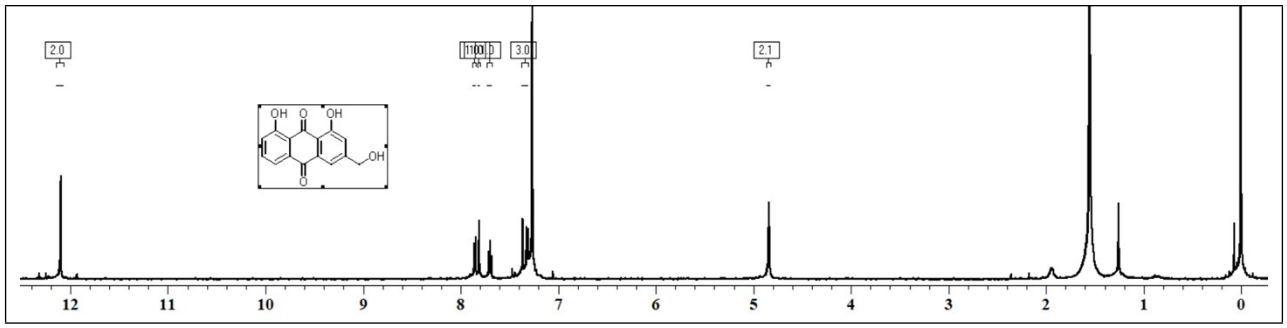


Figure:S2: ^{1}H NMR SPECTRUM OF COMPOUND **1**(500 MHz, $CDCl_3$)

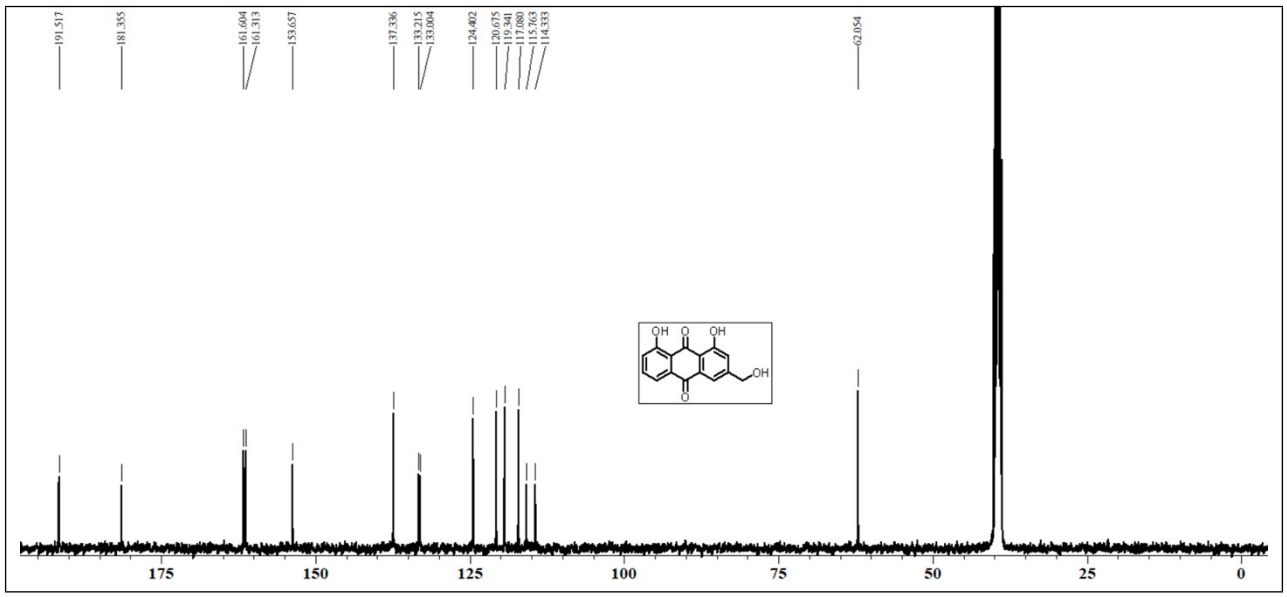
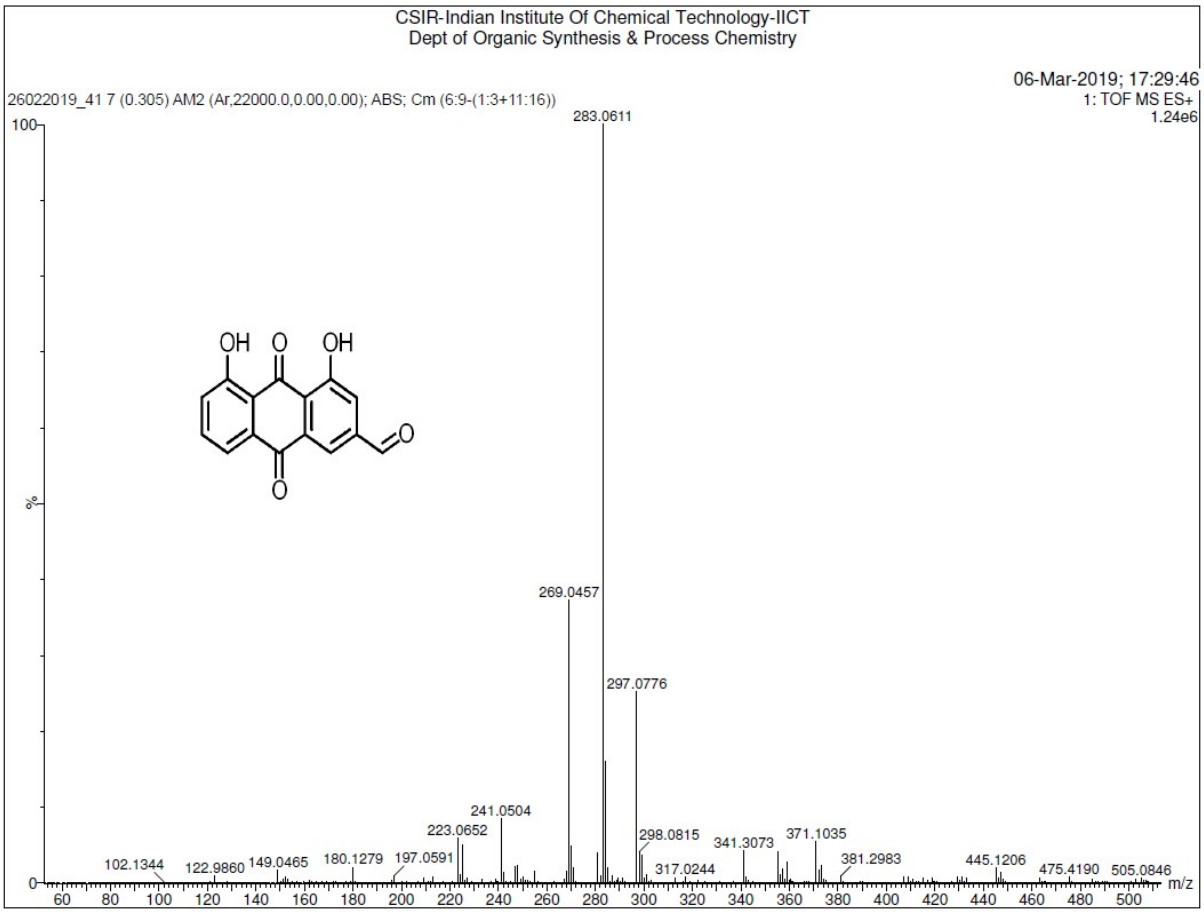


Figure:S3: ^{13}C NMR SPECTRUM OF COMPOUND **1**(101 MHz, DMSO-d_6)



Elemental Composition Report

Single Mass Analysis

Tolerance = 5.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Odd and Even Electron Ions
10 formula(e) evaluated with 1 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 0-15 H: 0-10 O: 0-5 Br: 0-1

Minimum:				-1.5				
Maximum:		5.0	5.0	50.0				
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
269.0457	269.0450	0.7	2.6	11.5	266.5	n/a	n/a	C15 H9 O5

Figure S4: HRESIMS SPECTRUM OF COMPOUND **2**

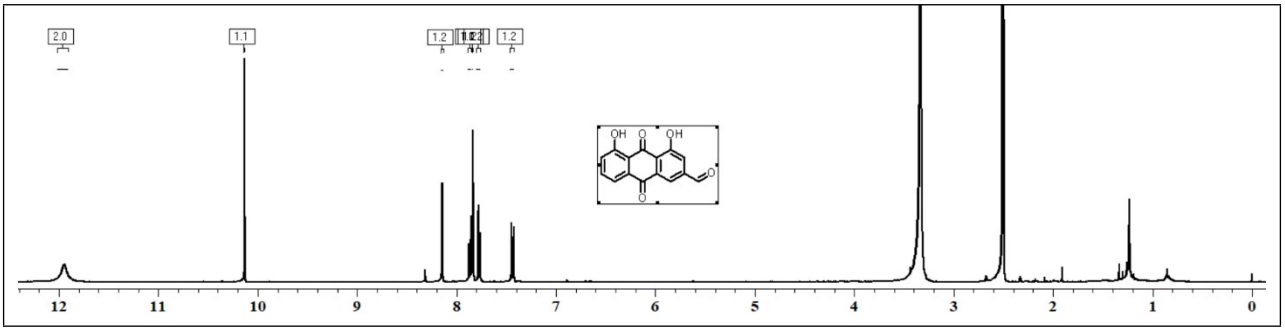


Figure: S5: ^{1}H NMR SPECTRUM OF COMPOUND **2**(400 MHz, DMSO-d_6)

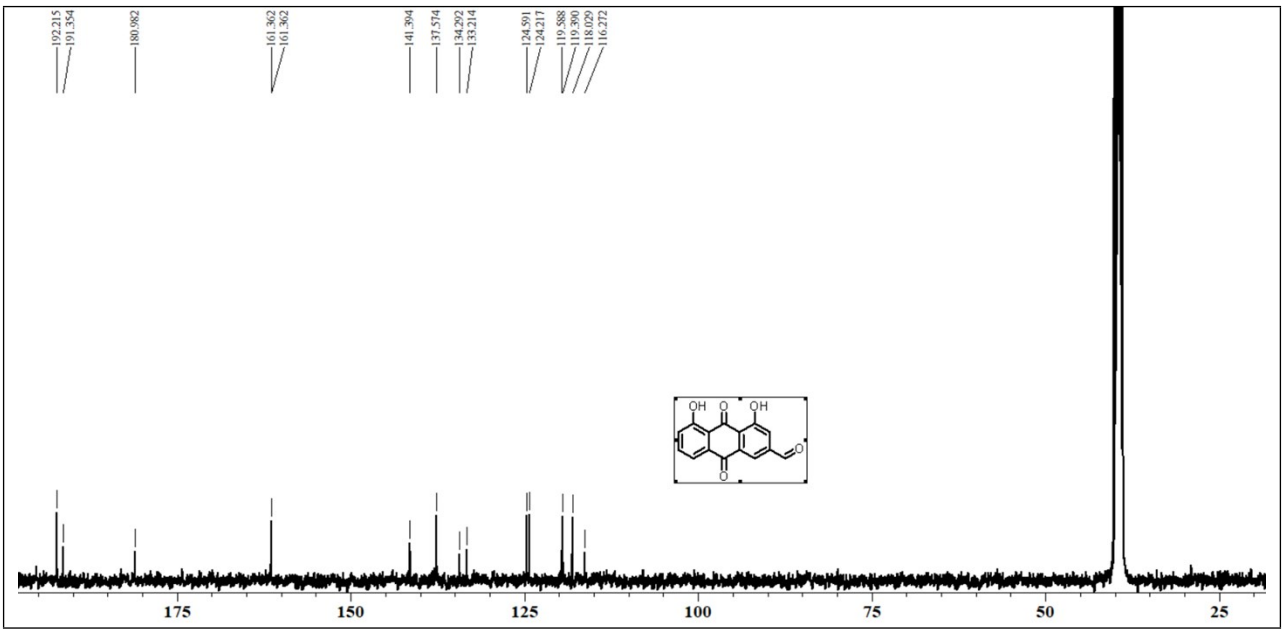
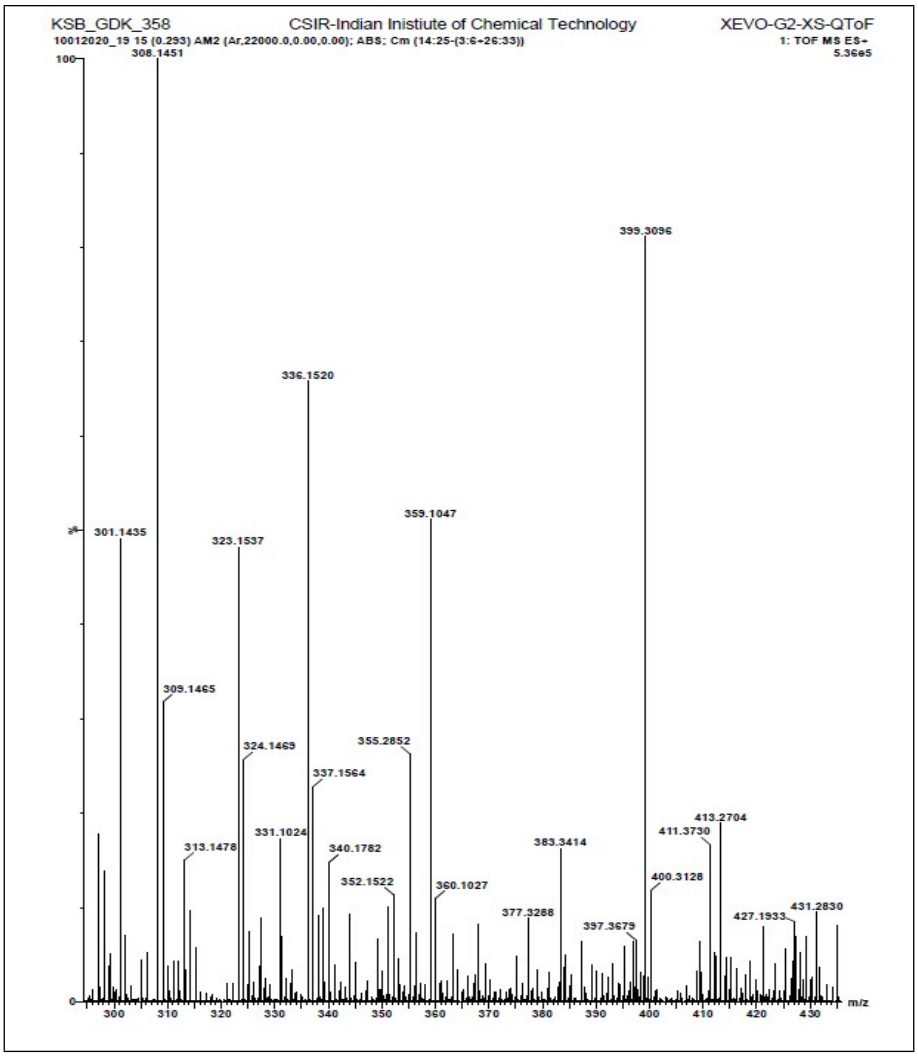


Figure: S6: ^{13}C NMR SPECTRUM OF COMPOUND **2** (101 MHz, DMSO-d_6)

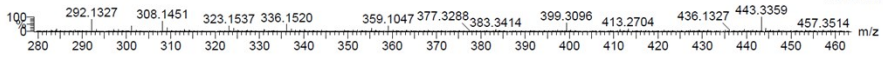


Elemental Composition Report **Page 1**

Single Mass Analysis
Tolerance = 5000.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
10 formula(e) evaluated with 1 results within limits (up to 50 closest results for each mass)
Elements Used:
C: 0-21 H: 0-15 N: 0-2 O: 0-4

KSB_GDK_358 CSIR-Indian Inistiute of Chemical Technology XEVO-G2-XS-QToF
10012020_19 15 (0.293) AM2 (Ar,22000.0,0.00,0.00); ABS; Cm (14:25-(3:6+26:33)) 1: TOF MS ES+
7.51e+005



Minimum:				-1.5				
Maximum:		5.0	5000.0	50.0				
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
359.1047	359.1032	1.5	4.2	15.5	482.9	n/a	n/a	C21 H15 N2 O4

Figure: S7: HRESIMS SPECTRUM OF COMPOUND **4a**

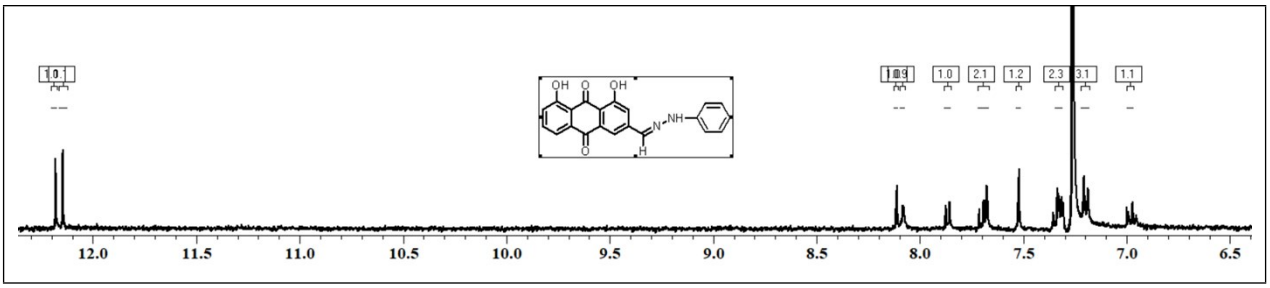


Figure: S8: ^{1}H NMR SPECTRUM OF COMPOUND **4a**(400 MHz, $CDCl_3$)

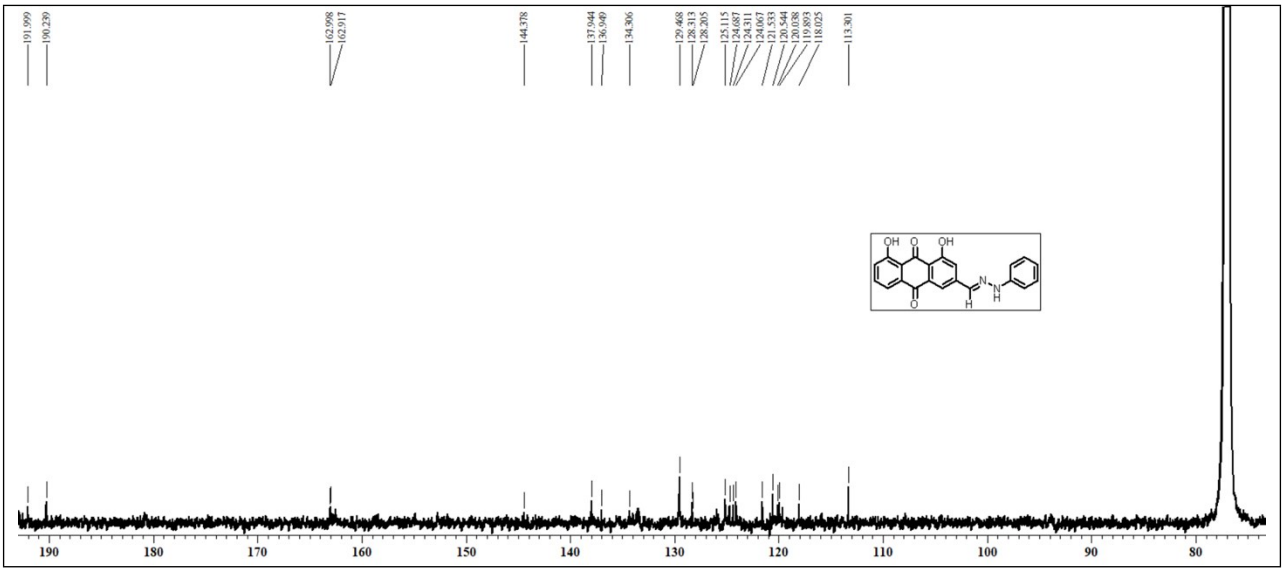
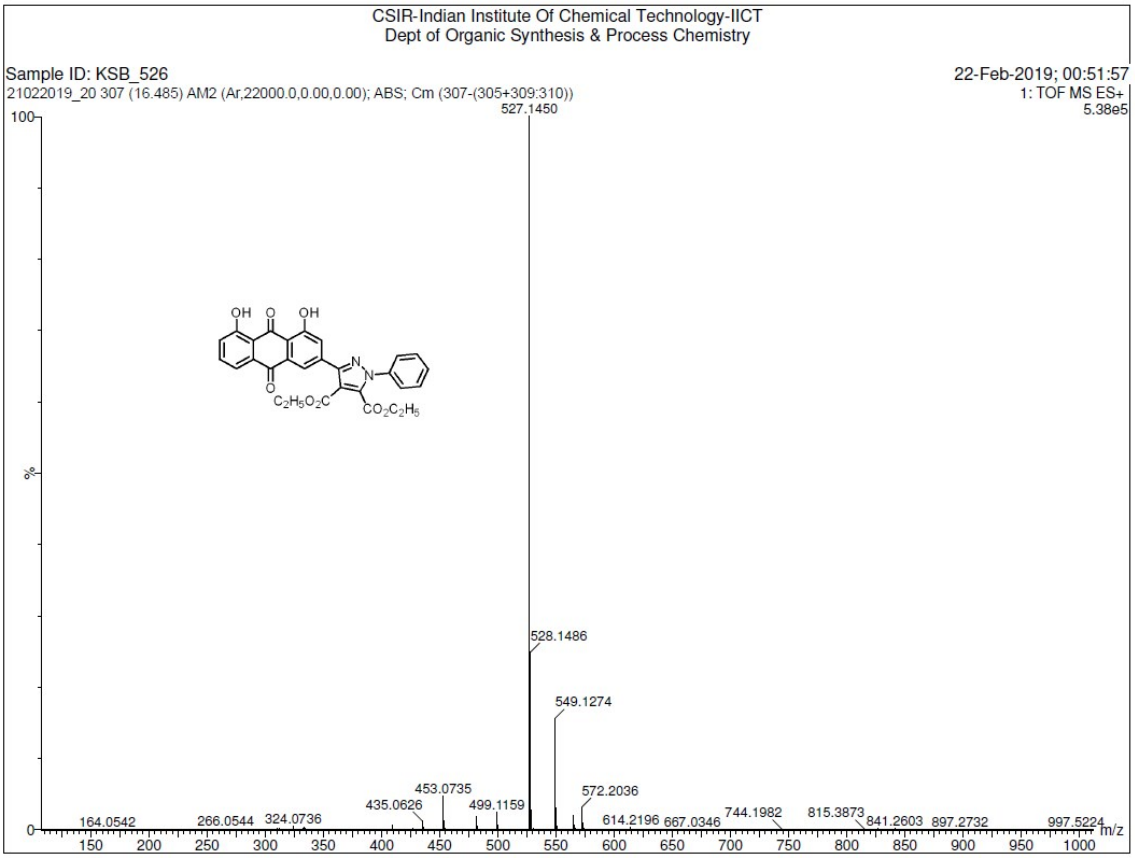


Figure: S9: ^{13}C NMR SPECTRUM OF COMPOUND **4a**(151 MHz, $CDCl_3$)



Elemental Composition Report

Single Mass Analysis

Tolerance = 5.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
38 formula(e) evaluated with 1 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 0-29 H: 0-23 N: 0-2 O: 0-8 Na: 0-1

Minimum:				-1.5				
Maximum:		5.0	5.0	50.0				
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
527.1450	527.1454	-0.4	-0.8	19.5	297.6	n/a	n/a	C29 H23 N2 O8

Figure: S10: HRESIMS SPECTRUM OF COMPOUND **5a**

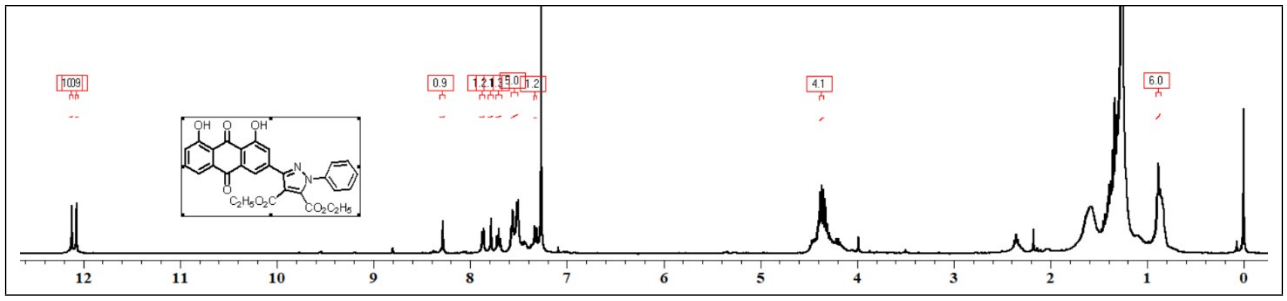


Figure: S11: ^{1}H NMR SPECTRUM OF COMPOUND**5a**(400 MHz, $CDCl_3$)

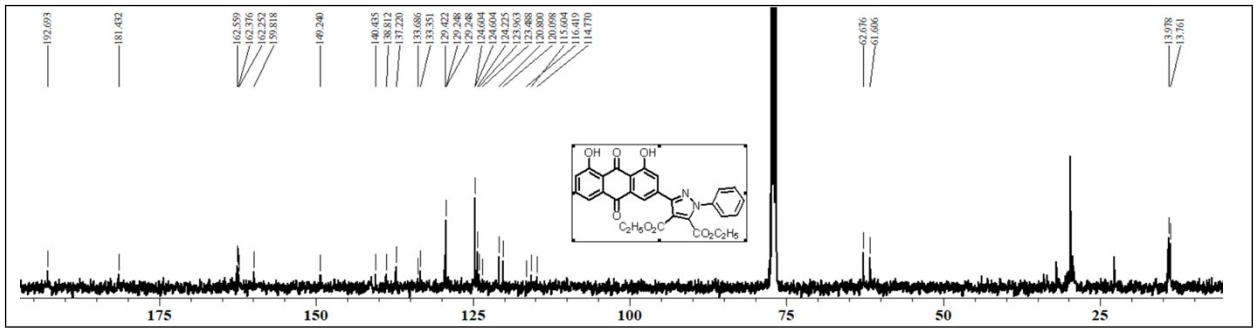
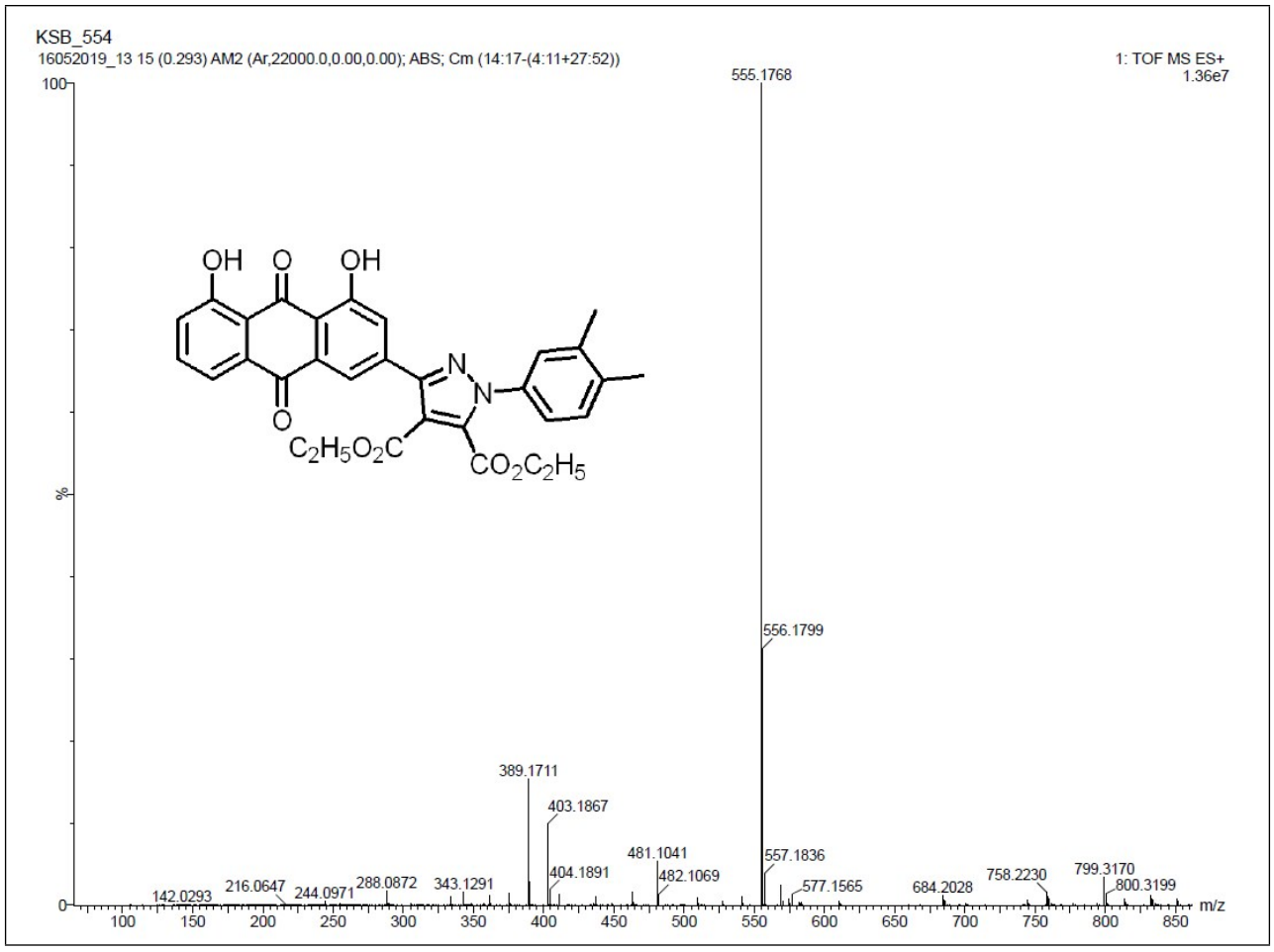


Figure: S12: ^{13}C NMR SPECTRUM OF COMPOUND**5a**(101 MHz, $CDCl_3$)

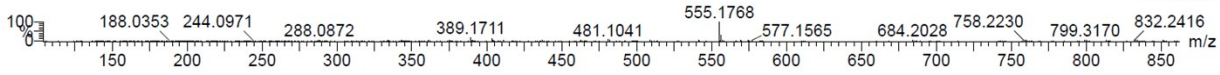


Elemental Composition Report **Page 1**

Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
19 formula(e) evaluated with 1 results within limits (up to 50 best isotopic matches for each mass)
Elements Used:
C: 0-31 H: 0-27 N: 0-2 O: 0-8

KSB_554
16052019_13 15 (0.293) AM2 (Ar,22000.0,0.00,0.00); ABS; Cm (14:17-(4:11+27:52))
1: TOF MS ES+
1.36e+007



Minimum:				-1.5				
Maximum:		5.0	5.0	50.0				
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
555.1768	555.1767	0.1	0.2	19.5	120.9	n/a	n/a	C31 H27 N2 O8

Figure: S13: HRESIMS SPECTRUM OF COMPOUND **5b**

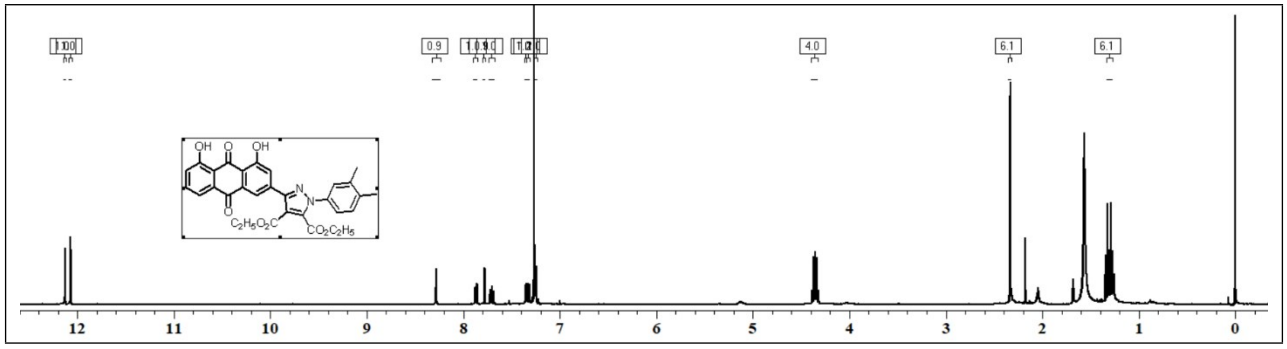


Figure: S14:[1]H NMR SPECTRUM OF COMPOUND**5b** (400 MHz, $CDCl_3$)

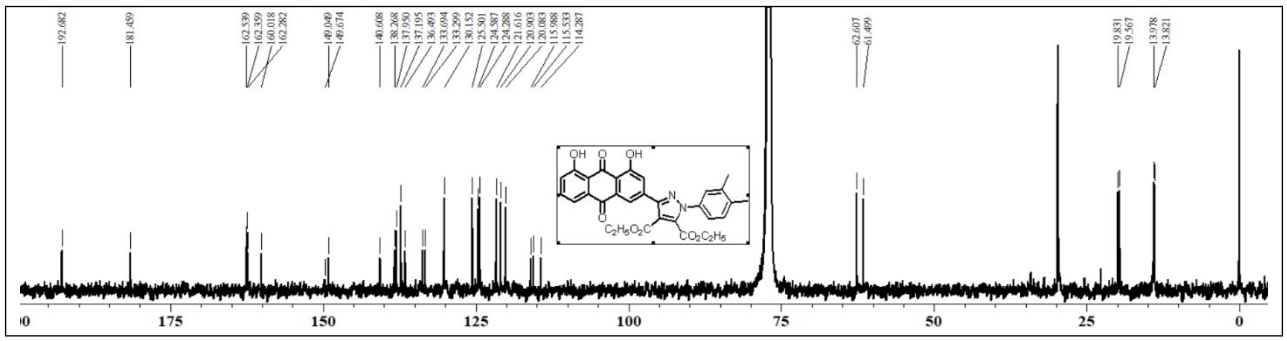
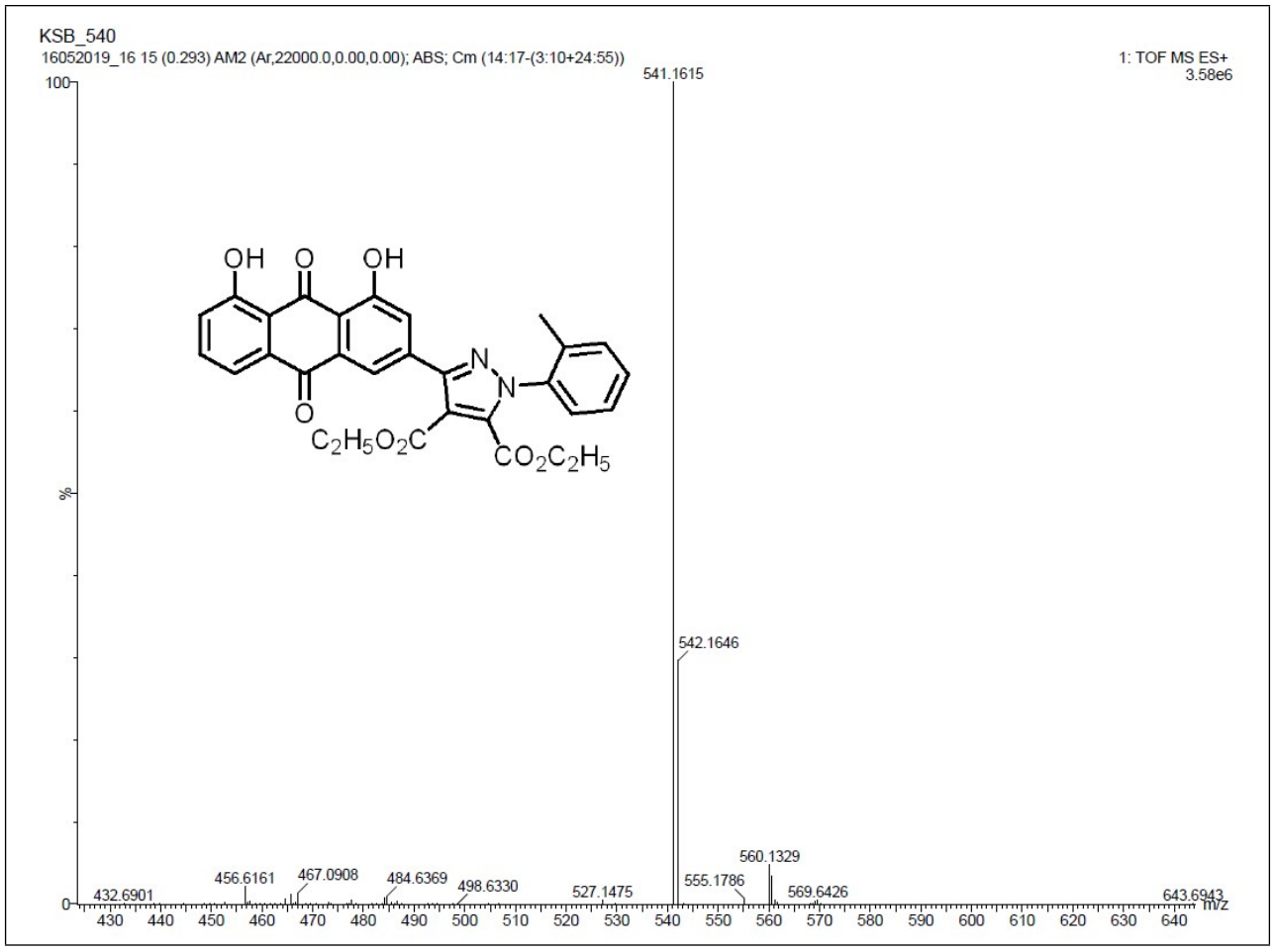


Figure: S15: [13]C NMR SPECTRUM OF COMPOUND **5b**(151 MHz, $CDCl_3$)

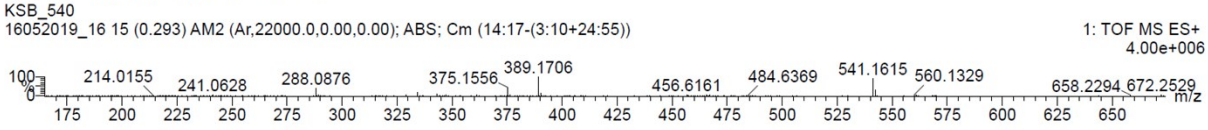


Elemental Composition Report

Single Mass Analysis

Tolerance = 5.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
19 formula(e) evaluated with 1 results within limits (up to 50 best isotopic matches for each mass)
Elements Used:
C: 0-31 H: 0-27 N: 0-2 O: 0-8



Minimum:				-1.5				
Maximum:		5.0	5.0	50.0				
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
541.1615	541.1611	0.4	0.7	19.5	39.3	n/a	n/a	C30 H25 N2 O8

Figure: S16: HRESIMS SPECTRUM OF COMPOUND **5c**

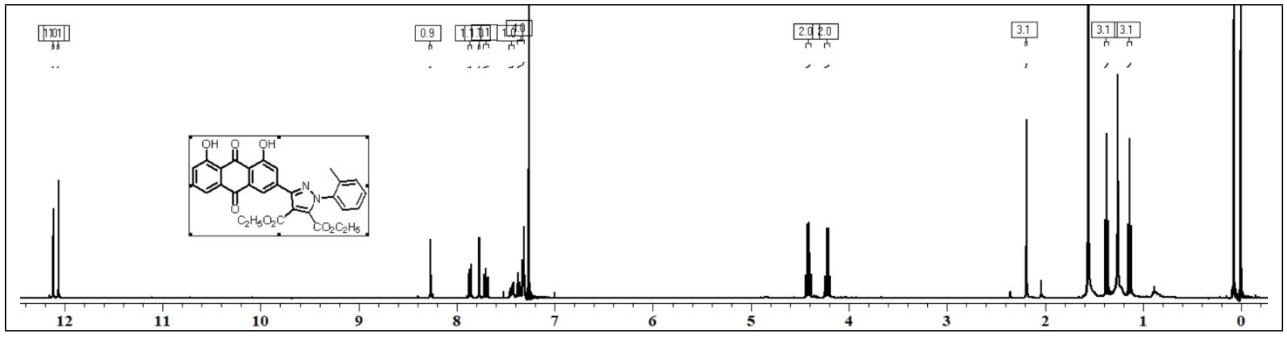


Figure: S17: ^{1}H NMR SPECTRUM OF COMPOUND **5c**(400 MHz, $CDCl_3$)

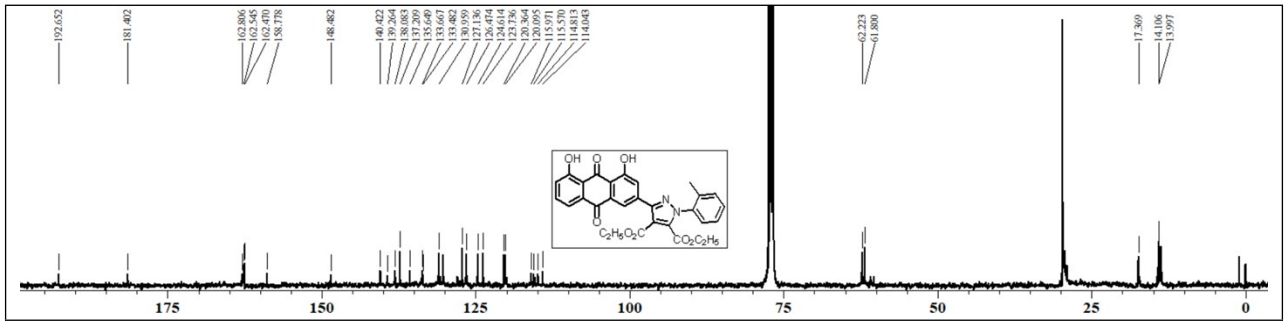
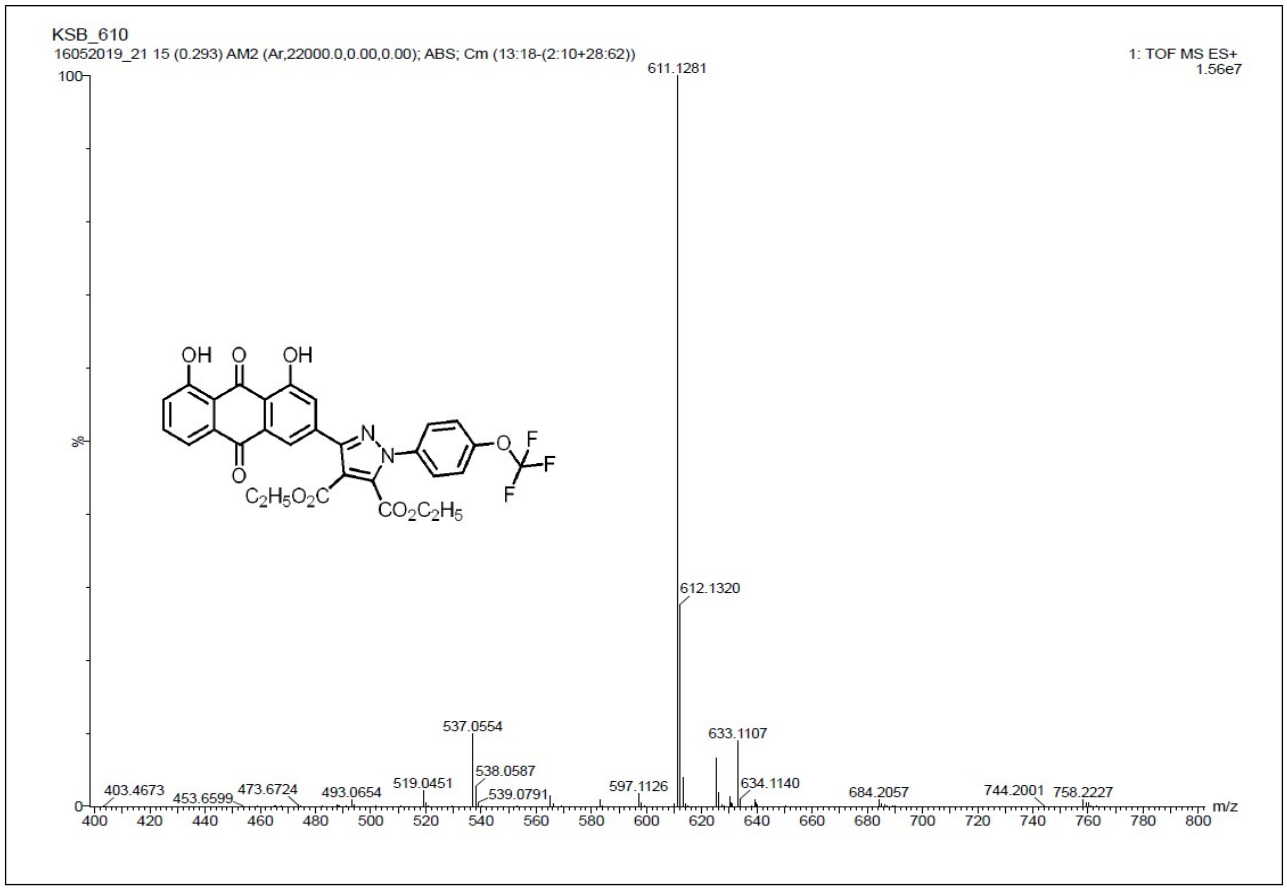


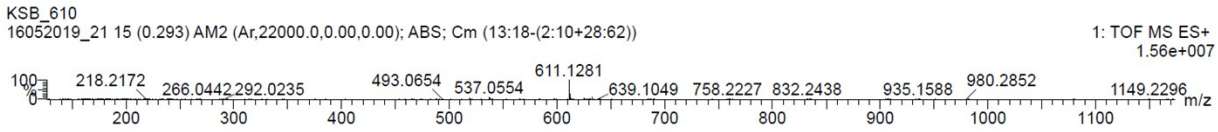
Figure: S18: ^{13}C NMR SPECTRUM OF COMPOUND **5c**(101 MHz, $CDCl_3$)



Elemental Composition Report **Page 1**

Single Mass Analysis
Tolerance = 1000.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
106 formula(e) evaluated with 1 results within limits (up to 50 best isotopic matches for each mass)
Elements Used:
C: 0-30 H: 0-23 N: 0-2 O: 0-9 F: 0-3



Minimum:				-1.5				
Maximum:		5.0	1000.0	50.0				
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
611.1281	611.1277	0.4	0.7	19.5	100.2	n/a	n/a	C30 H22 N2 O9 F3

Figure: S19: HRESIMS SPECTRUM OF COMPOUND **5d**

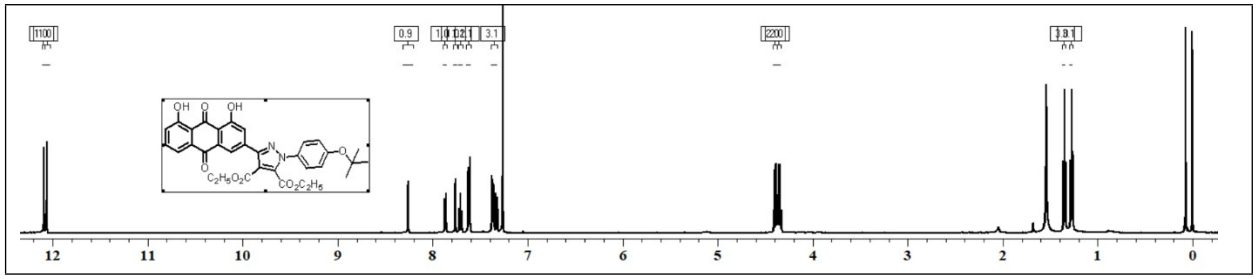


Figure: S20: ^{1}H NMR SPECTRUM OF COMPOUND**5d** (500 MHz, $CDCl_3$)

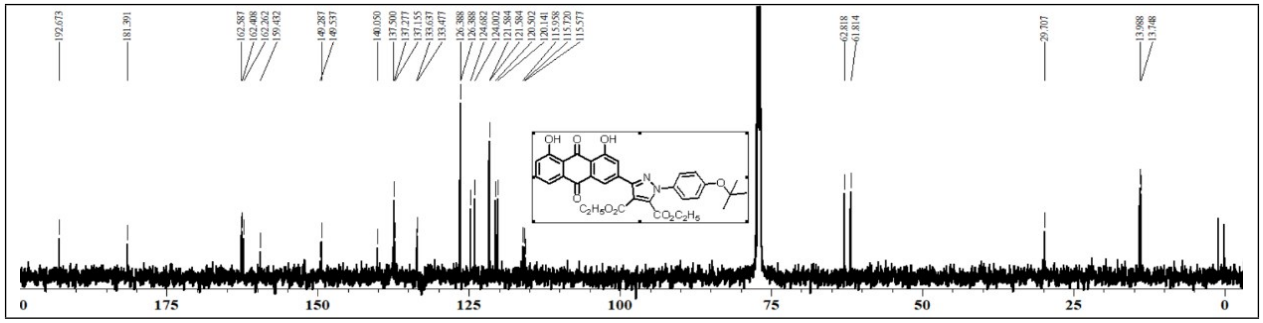
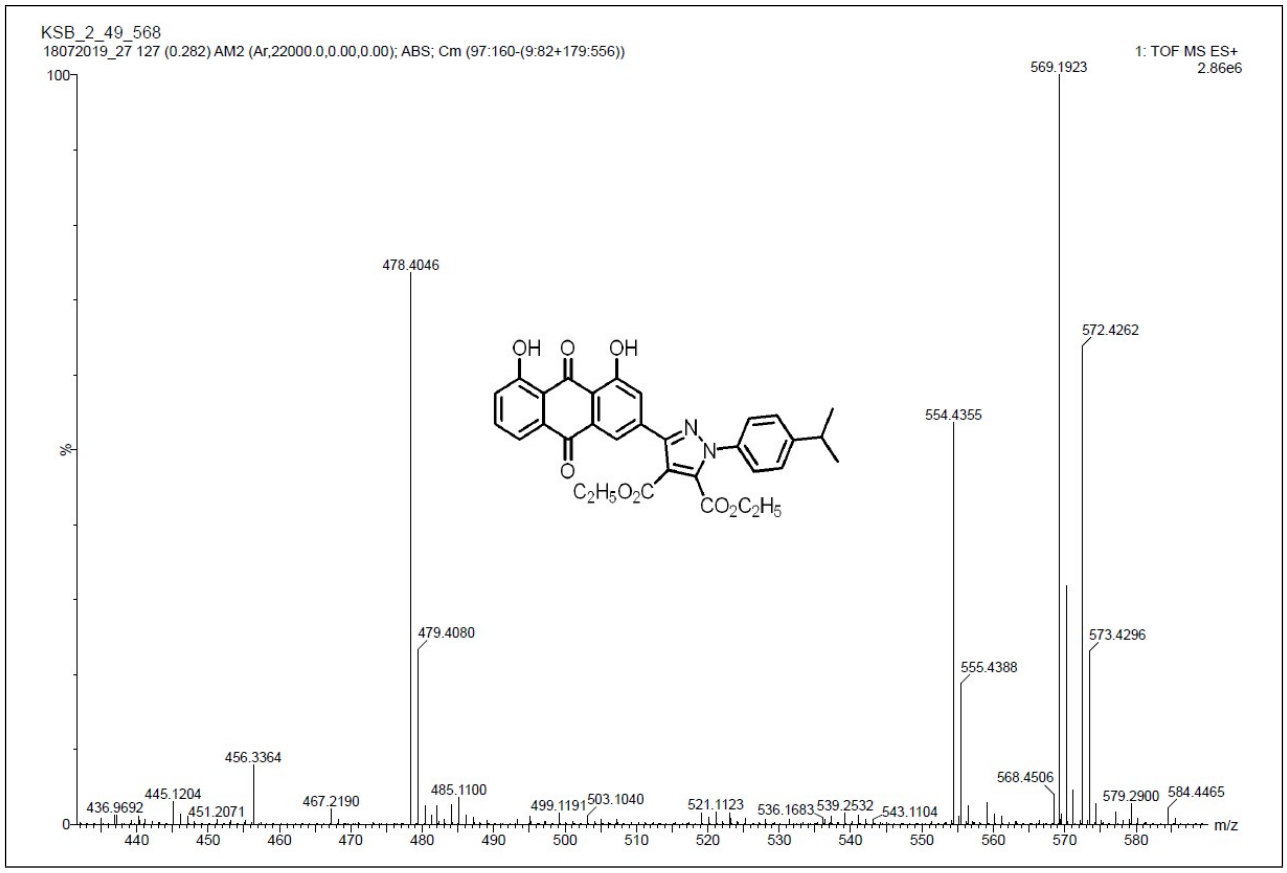


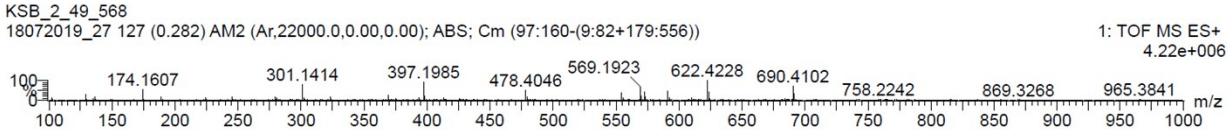
Figure: S21: ^{13}C NMR SPECTRUM OF COMPOUND**5d**(101 MHz, $CDCl_3$)



Elemental Composition Report **Page 1**

Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Odd and Even Electron Ions
71 formula(e) evaluated with 1 results within limits (up to 50 best isotopic matches for each mass)
Elements Used:
C: 0-32 H: 0-29 N: 0-2 O: 0-8 I: 0-1



Minimum:				-1.5				
Maximum:		5.0	5.0	50.0				
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
569.1923	569.1924	-0.1	-0.2	19.5	214.7	n/a	n/a	C32 H29 N2 O8

Figure: S22: HRESIMS SPECTRUM OF COMPOUND **5e**

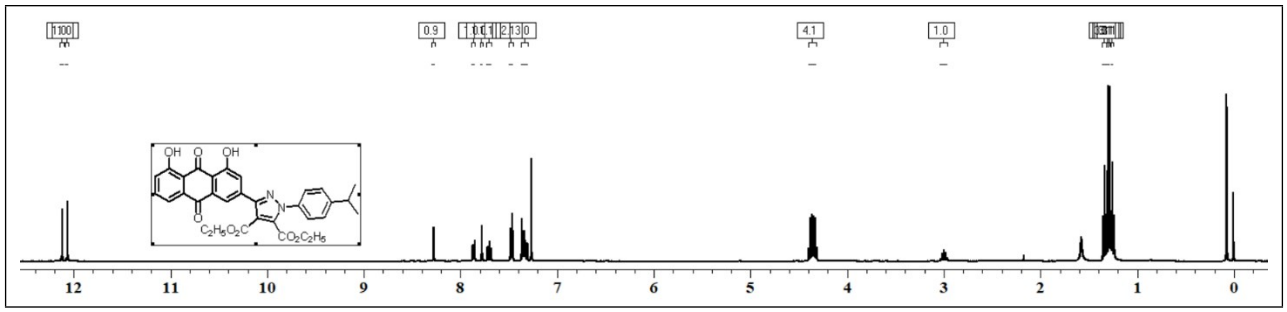


Figure: S23: ^{1}H NMR SPECTRUM OF COMPOUND**5e**(500 MHz, $CDCl_3$)

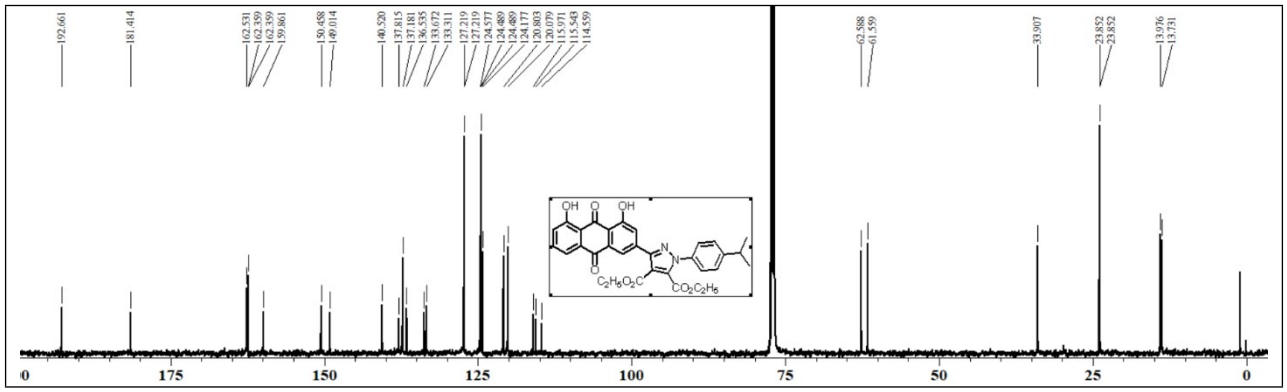
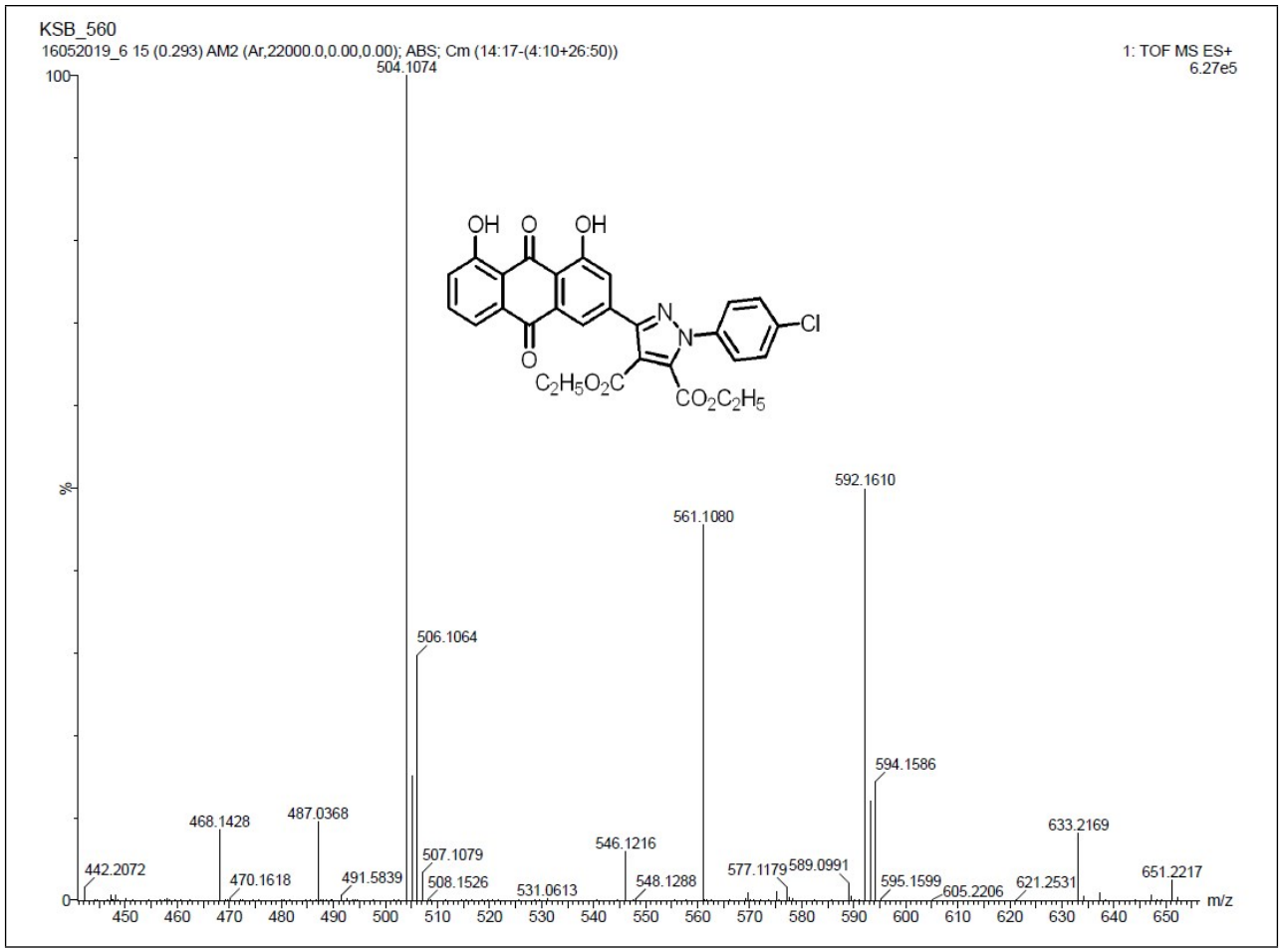


Figure: S24: ^{13}C NMR SPECTRUM OF COMPOUND**5e**(126 MHz, $CDCl_3$)

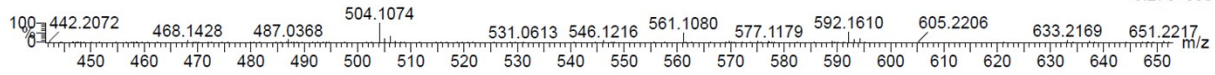


Elemental Composition Report **Page 1**

Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
44 formula(e) evaluated with 1 results within limits (up to 50 best isotopic matches for each mass)
Elements Used:
C: 0-29 H: 0-22 N: 0-2 O: 0-8 Cl: 0-1

KSB_560
16052019_6 15 (0.293) AM2 (Ar,22000.0,0.00,0.00); ABS; Cm (14:17-(4:10+26:50))
1: TOF MS ES+
6.27e+005



Minimum:				-1.5				
Maximum:		5.0	5.0	50.0				
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
561.1080	561.1065	1.5	2.7	19.5	67.6	n/a	n/a	C29 H22 N2 O8 Cl

Figure: S25: HRESIMS SPECTRUM OF COMPOUND **5f**

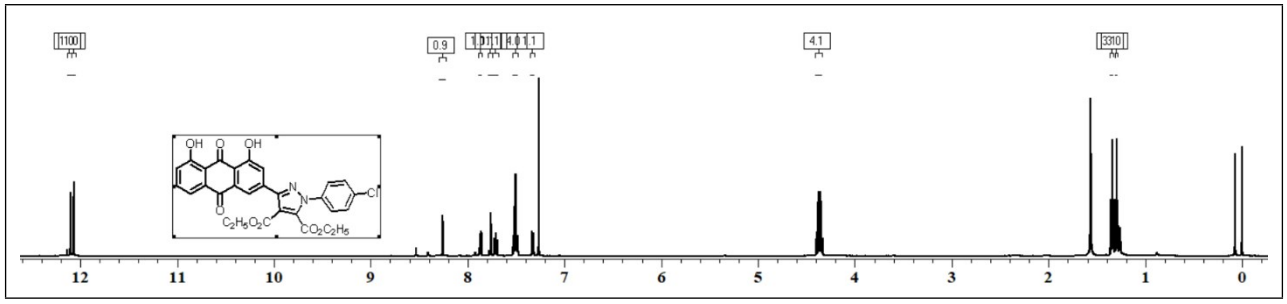


Figure: S26: ^{1}H NMR SPECTRUM OF COMPOUND **5f**(500 MHz, $CDCl_3$)

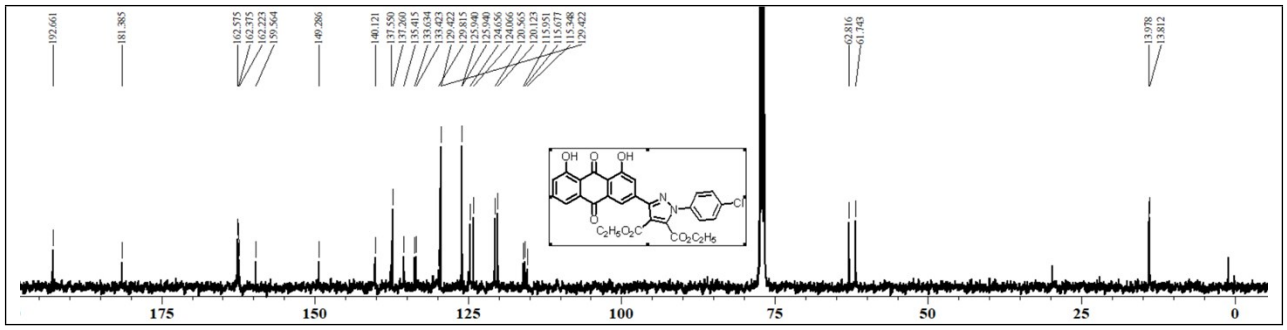
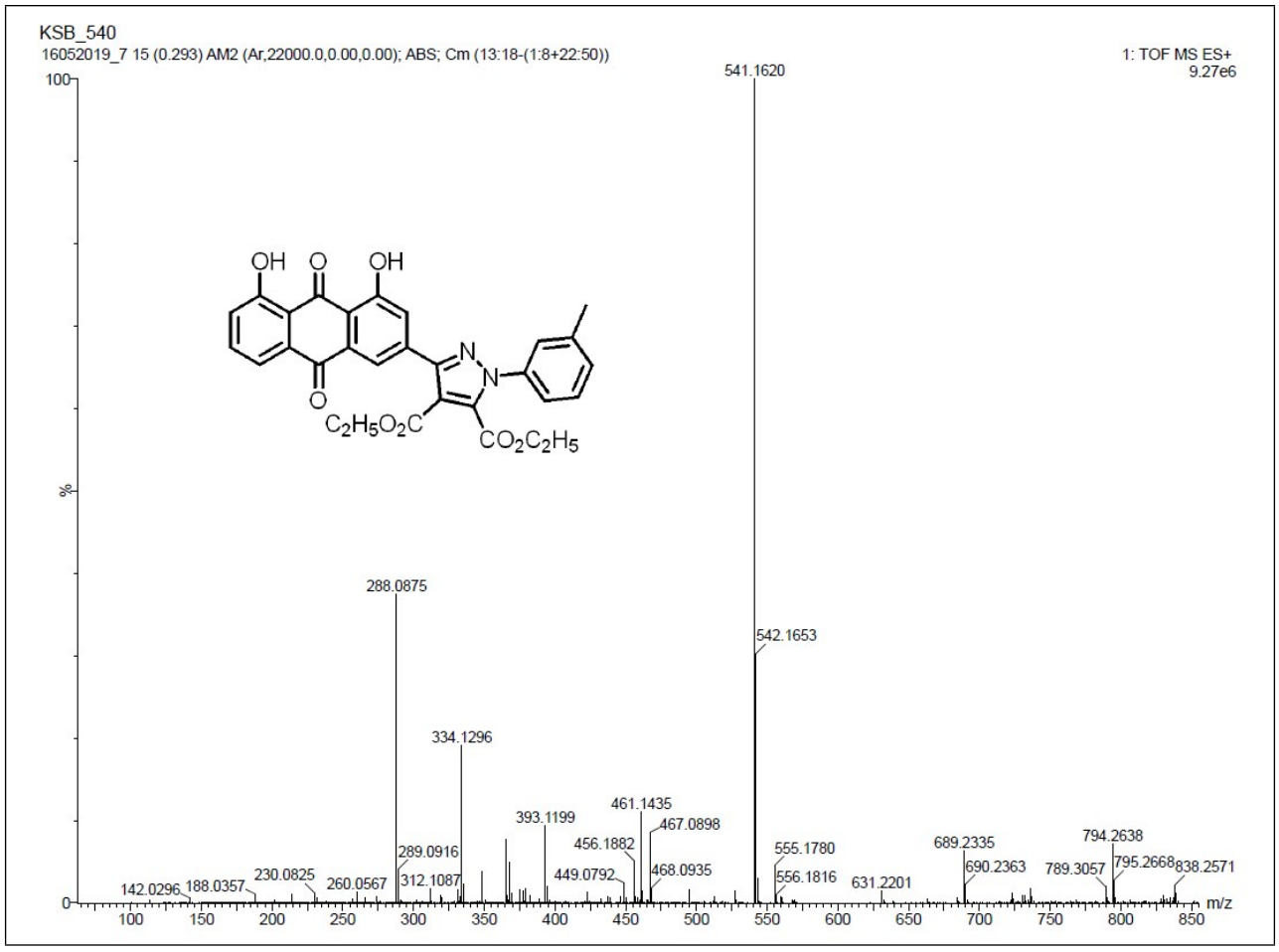


Figure: S27: ^{13}C NMR SPECTRUM OF COMPOUND **5f**(101 MHz, $CDCl_3$)

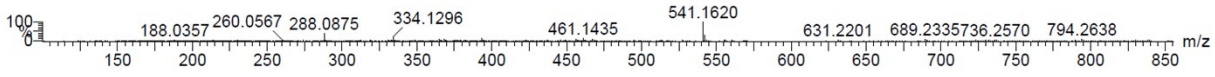


Elemental Composition Report **Page 1**

Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
40 formula(e) evaluated with 1 results within limits (up to 50 best isotopic matches for each mass)
Elements Used:
C: 0-30 H: 0-25 N: 0-2 O: 0-8 Cl: 0-1

KSB_540
16052019_7 15 (0.293) AM2 (Ar,22000.0,0.00,0.00); ABS; Cm (13:18-(1:8+22:50))
1: TOF MS ES+
9.27e+006



Minimum:				-1.5				
Maximum:		5.0	5.0	50.0				
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
541.1620	541.1611	0.9	1.7	19.5	114.2	n/a	n/a	C30 H25 N2 O8

Figure: S28: HRESIMS SPECTRUM OF COMPOUND **5g**

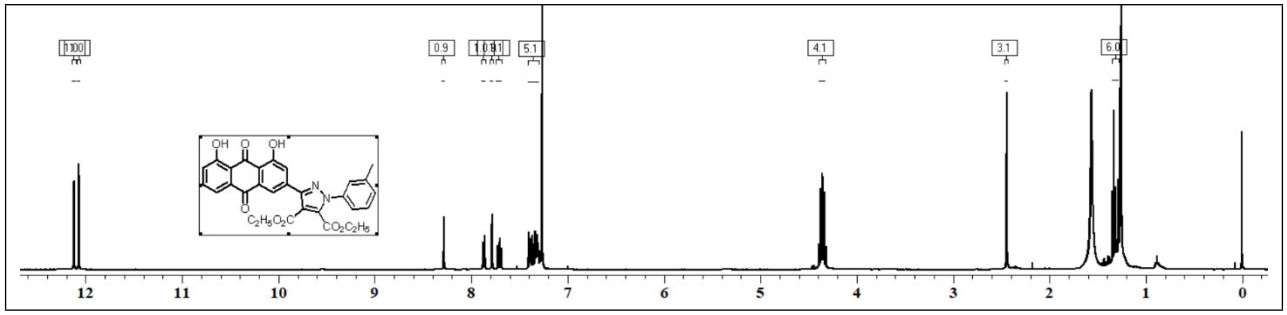


Figure: S29: ^{1}H NMR SPECTRUM OF COMPOUND **5g**(500 MHz, $CDCl_3$)

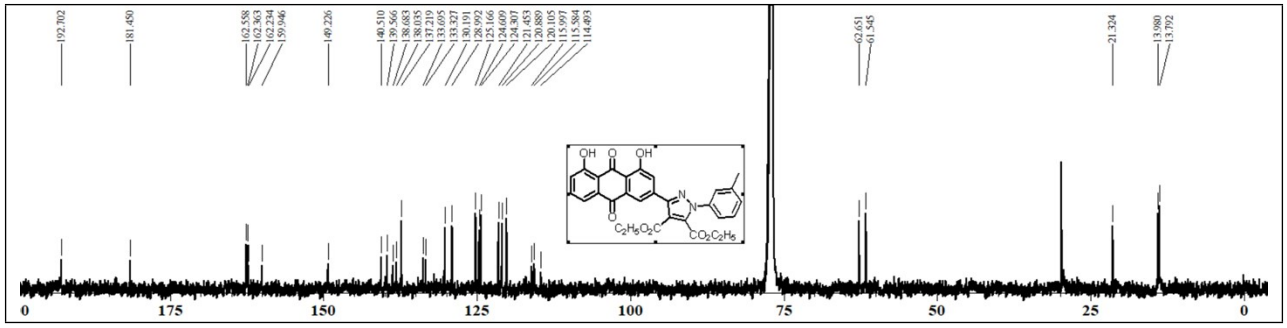


Figure: S30: ^{13}C NMR SPECTRUM OF COMPOUND**5g**(126 MHz, $CDCl_3$)

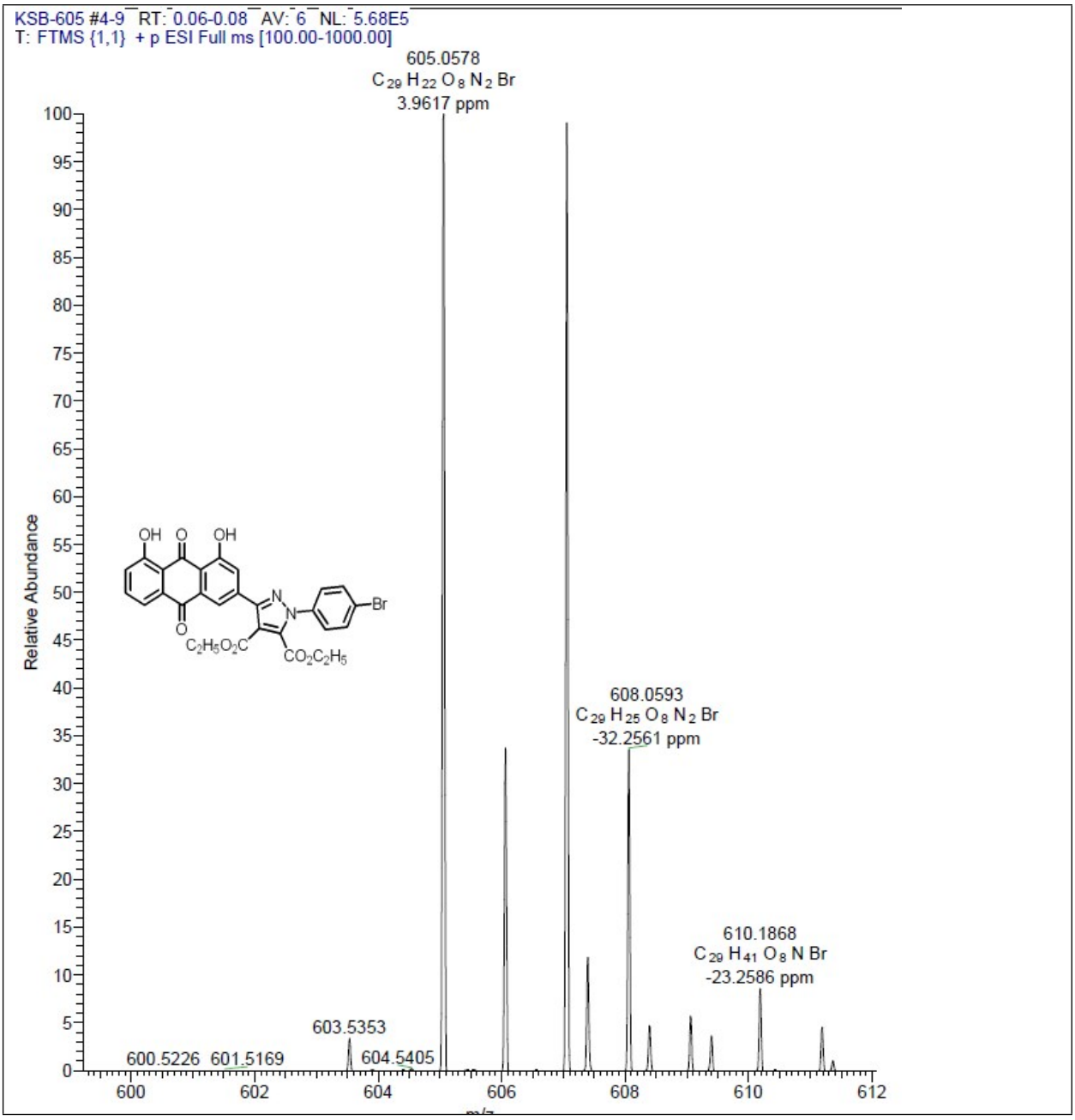


Figure: S31: HRESIMS SPECTRUM OF COMPOUND **5h**

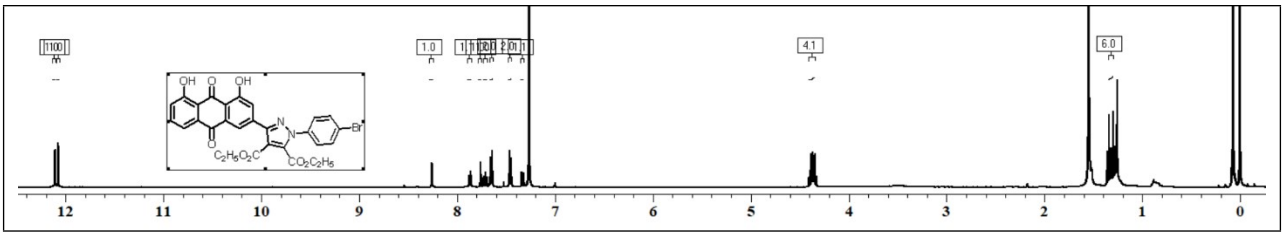


Figure: S32: ^{1}H NMR SPECTRUM OF COMPOUND **5h**(500 MHz, $CDCl_3$)

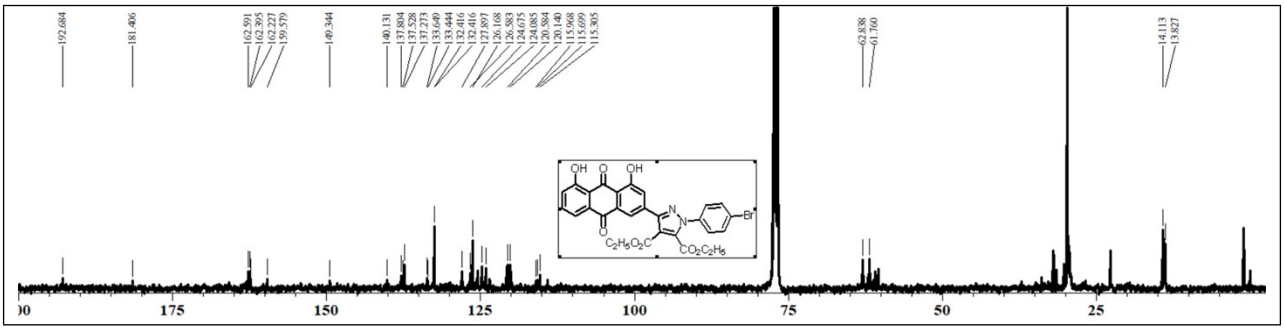


Figure: S33: ^{13}C NMR SPECTRUM OF COMPOUND **5h**(101 MHZ, $CDCl_3$)

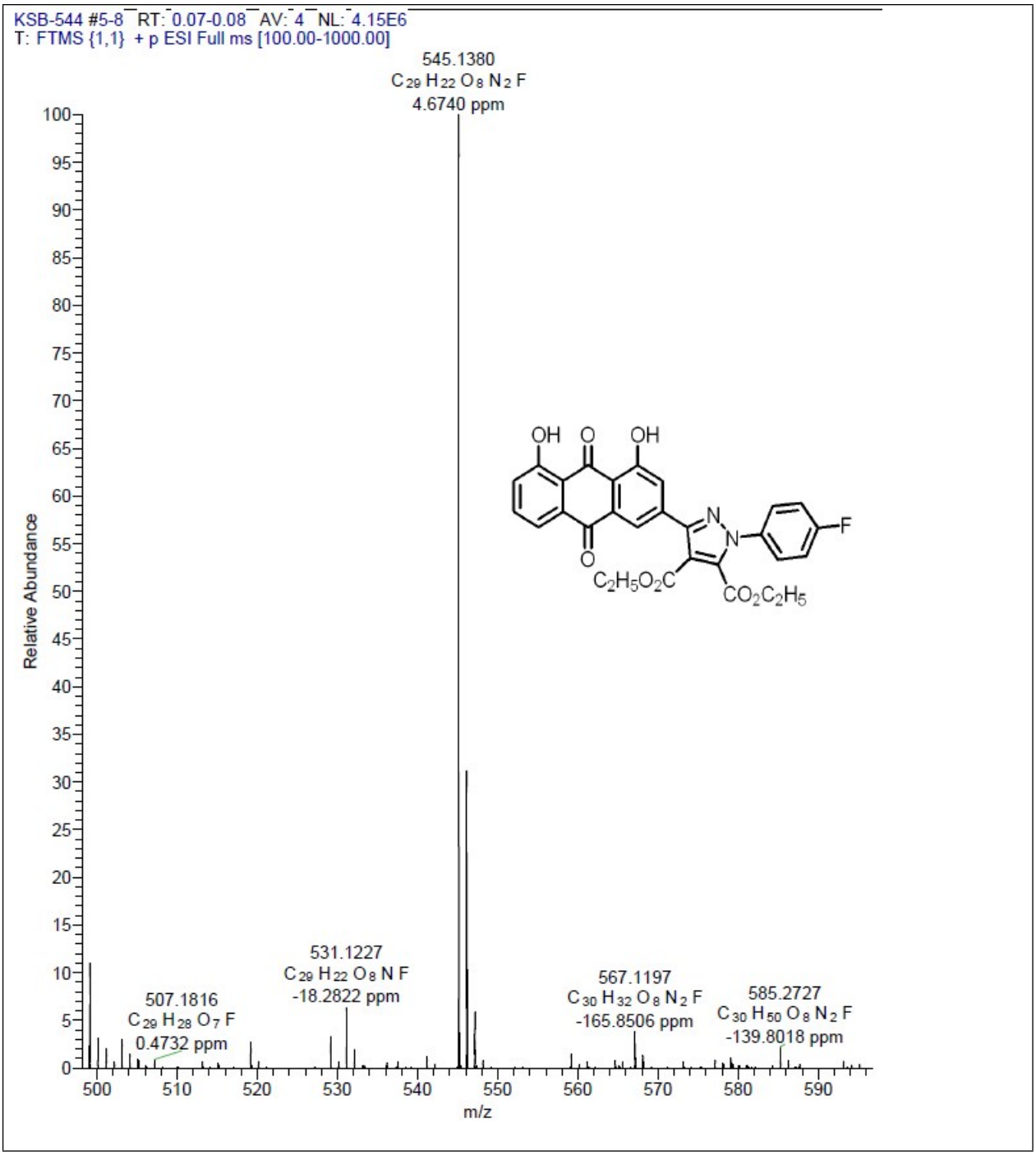


Figure: S34: HRESIMS SPECTRUM OF COMPOUND **5i**

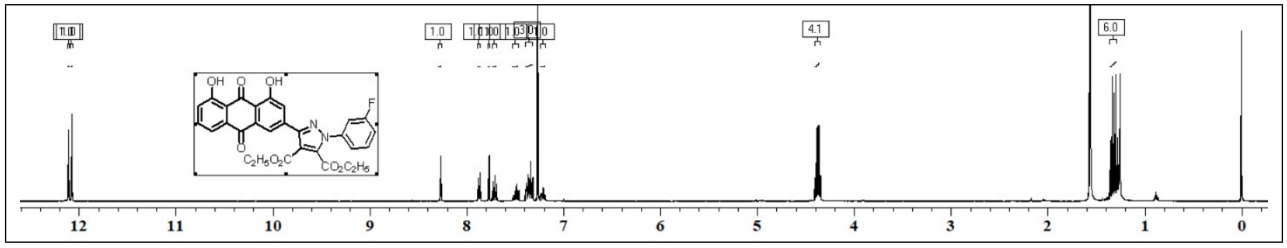


Figure: S35: ^{1}H NMR SPECTRUM OF COMPOUND**5i**(500 MHz, $CDCl_3$)

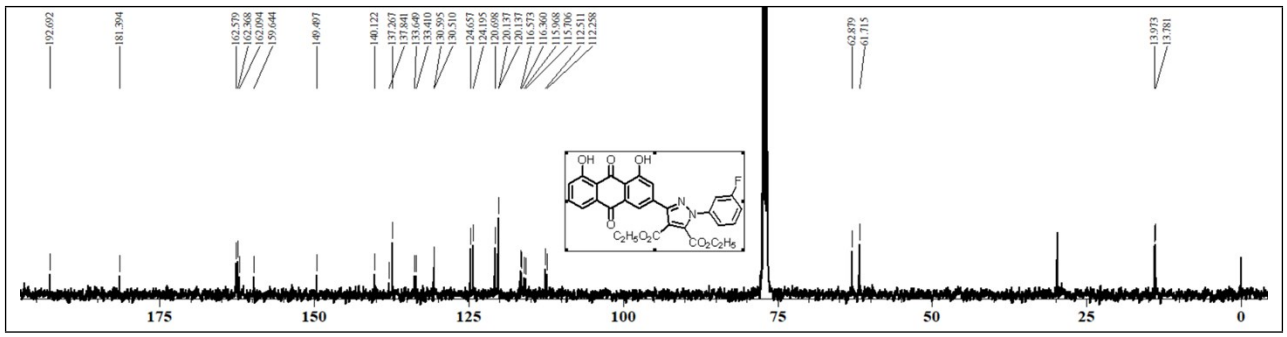


Figure: S36: ^{13}C NMR SPECTRUM OF COMPOUND **5i**(101 MHz, $CDCl_3$)

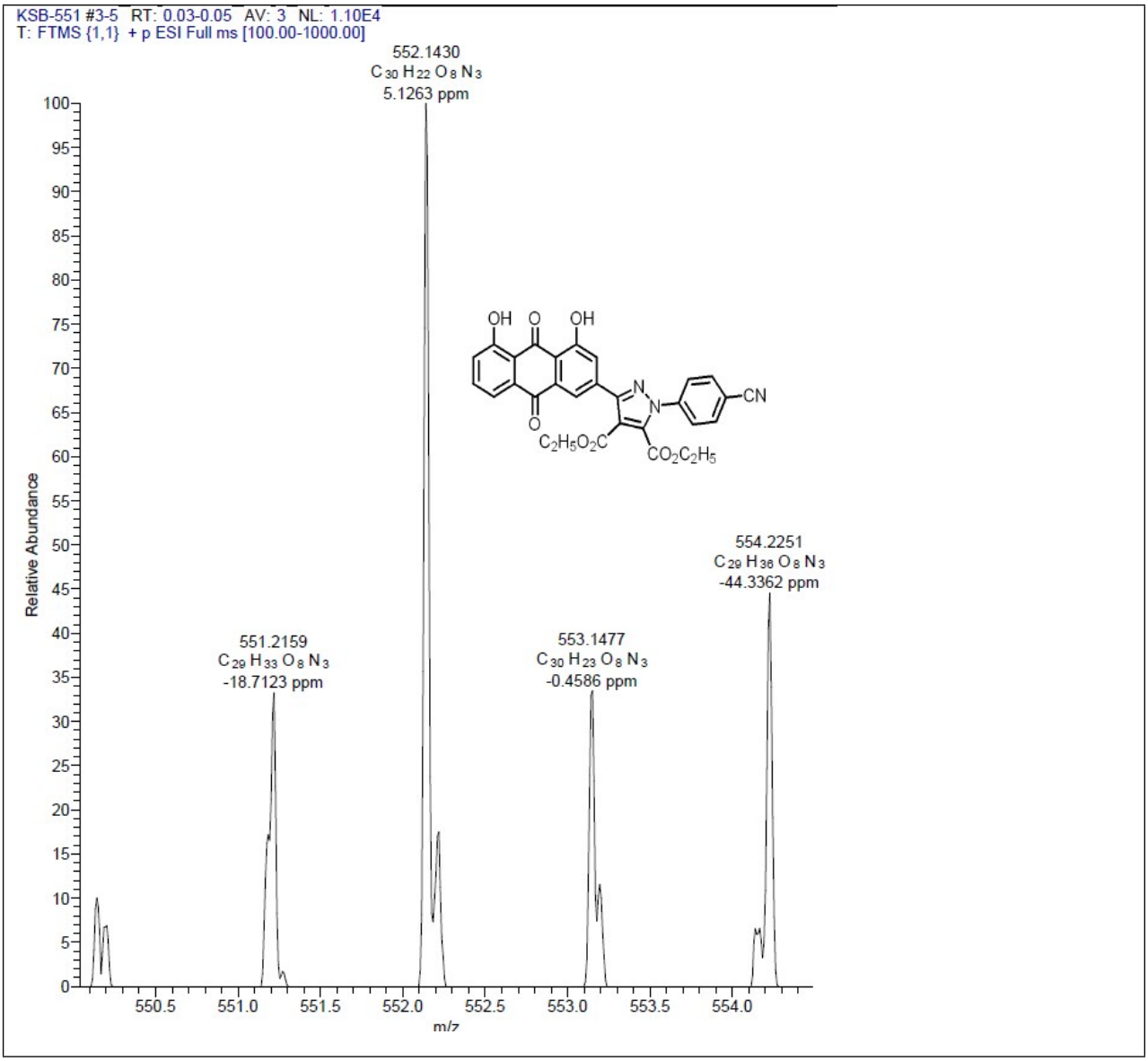


Figure: S37: HRESIMS SPECTRUM OF COMPOUND **5j**

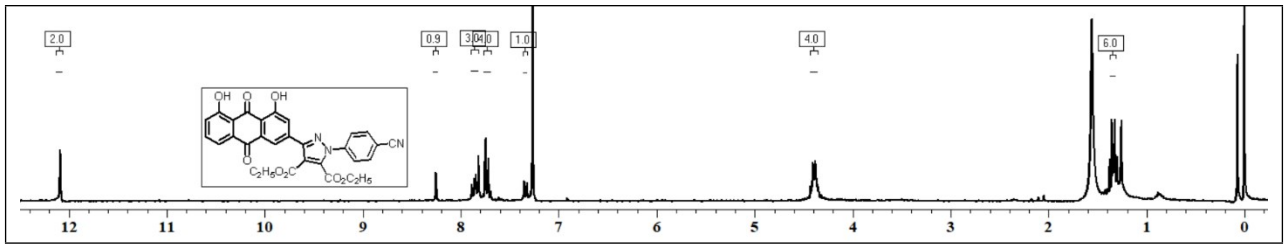


Figure: S38: ^{1}H NMR SPECTRUM OF COMPOUND **5j**(500 MHz, $CDCl_3$)

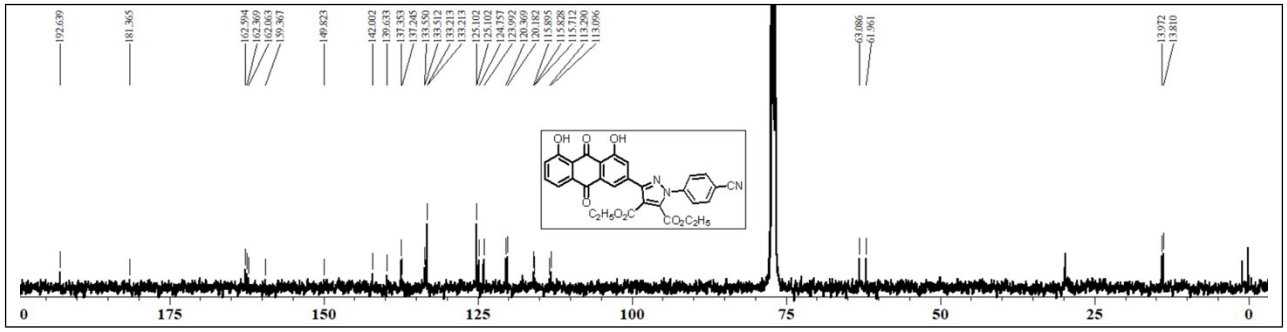
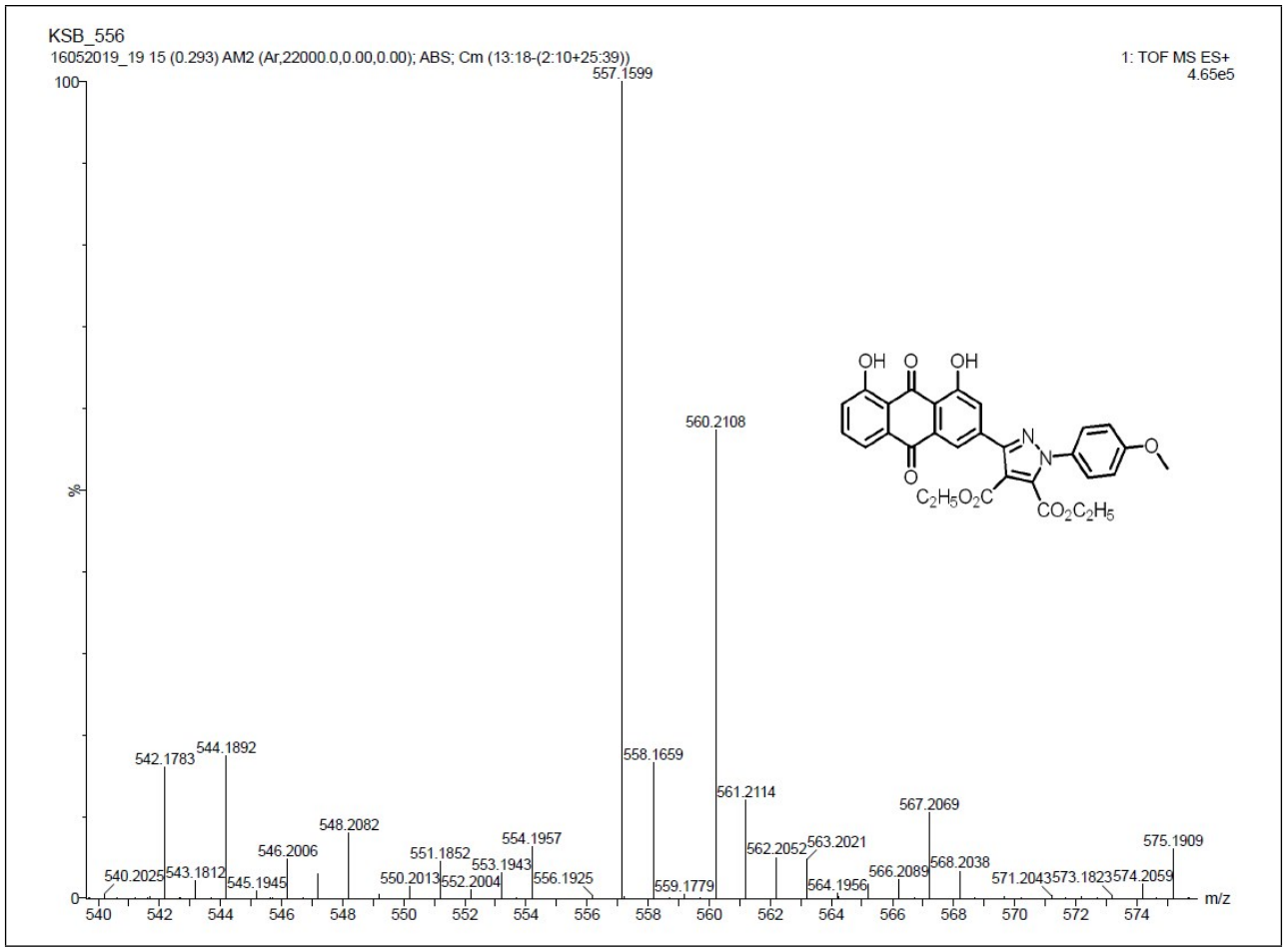


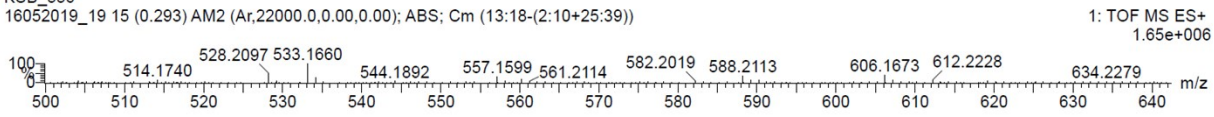
Figure: S39: ^{13}C NMR SPECTRUM OF COMPOUND **5j**(101 MHz, $CDCl_3$)



Elemental Composition Report **Page 1**

Single Mass Analysis
Tolerance = 10.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
22 formula(e) evaluated with 1 results within limits (up to 50 best isotopic matches for each mass)
Elements Used:
C: 0-31 H: 0-27 N: 0-2 O: 0-9



Minimum:				-1.5				
Maximum:		5.0	10.0	50.0				
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
557.1599	557.1560	3.9	7.0	19.5	66.2	n/a	n/a	C30 H25 N2 O9

Figure: S40: HRESIMS SPECTRUM OF COMPOUND **5k**

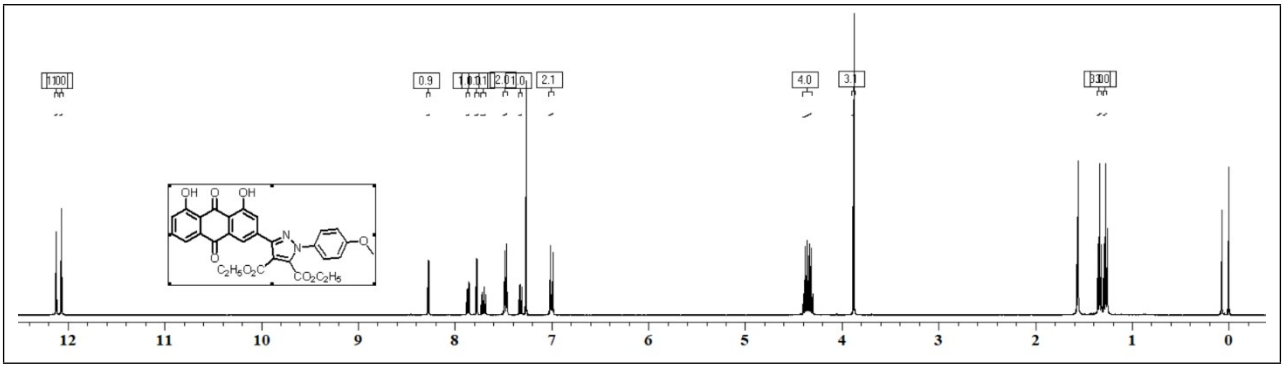


Figure: S41: ^{1}H NMR SPECTRUM OF COMPOUND **5k**(500 MHz, $CDCl_3$)

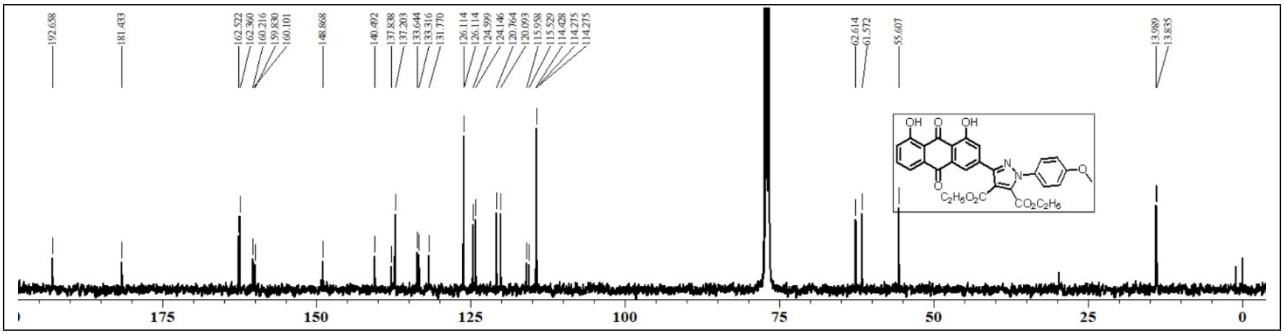
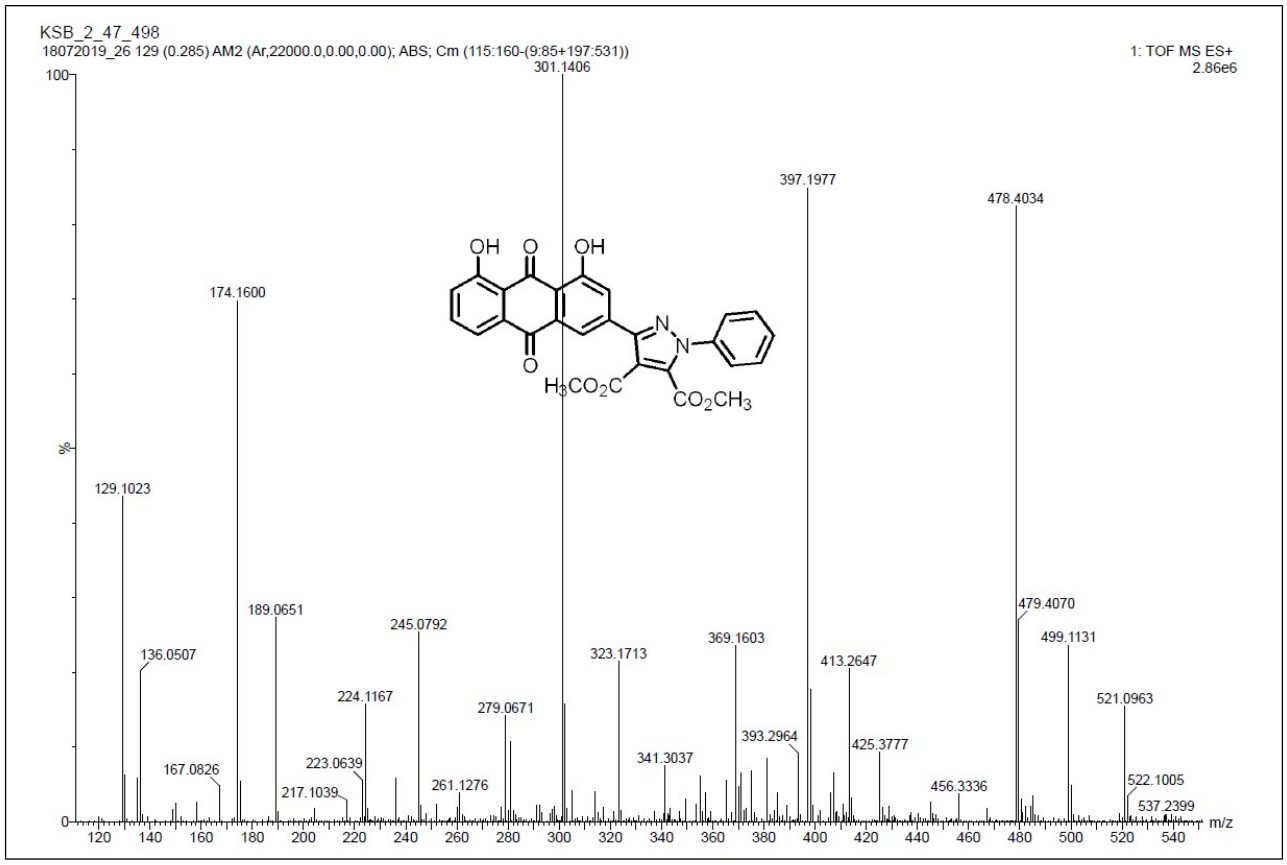


Figure: S42: ^{13}C NMR SPECTRUM OF COMPOUND **5k**(101 MHz, $CDCl_3$)

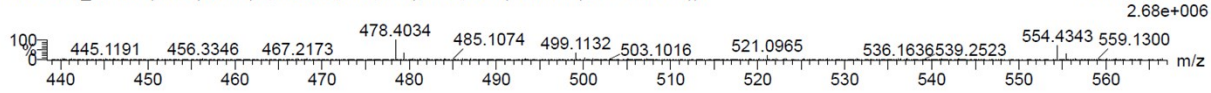


Elemental Composition Report **Page 1**

Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
57 formula(e) evaluated with 1 results within limits (up to 50 best isotopic matches for each mass)
Elements Used:
C: 0-27 H: 0-19 N: 0-2 O: 0-8 I: 0-1

KSB_2_47_498
18072019_26 129 (0.285) AM2 (Ar,22000.0,0.00,0.00); ABS; Cm (100:161-(9:71+225:496))
1: TOF MS ES+
2.68e+006



Minimum:				-1.5				
Maximum:		5.0	5.0	50.0				
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
499.1132	499.1141	-0.9	-1.8	19.5	351.1	n/a	n/a	C27 H19 N2 O8

Figure: S43: HRESIMS SPECTRUM OF COMPOUND **6a**

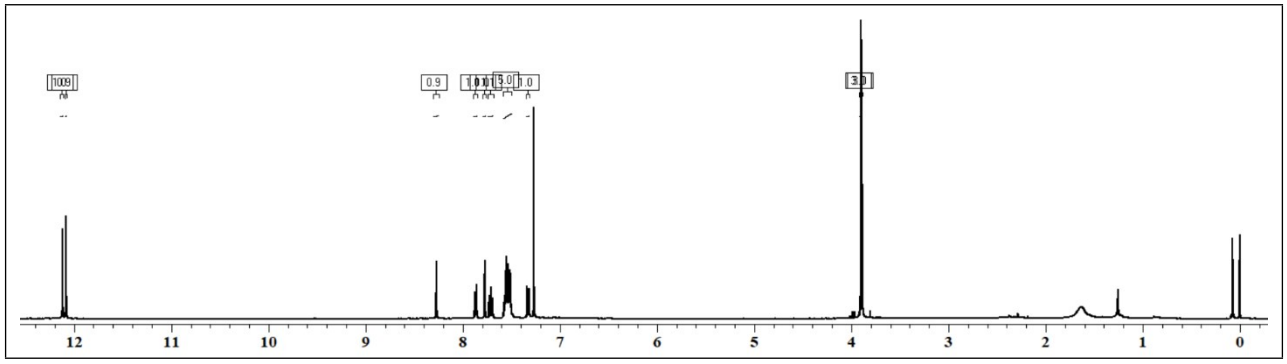


Figure: S44: ^{1}H NMR SPECTRUM OF COMPOUND **6a**(400 MHz, $CDCl_3$)

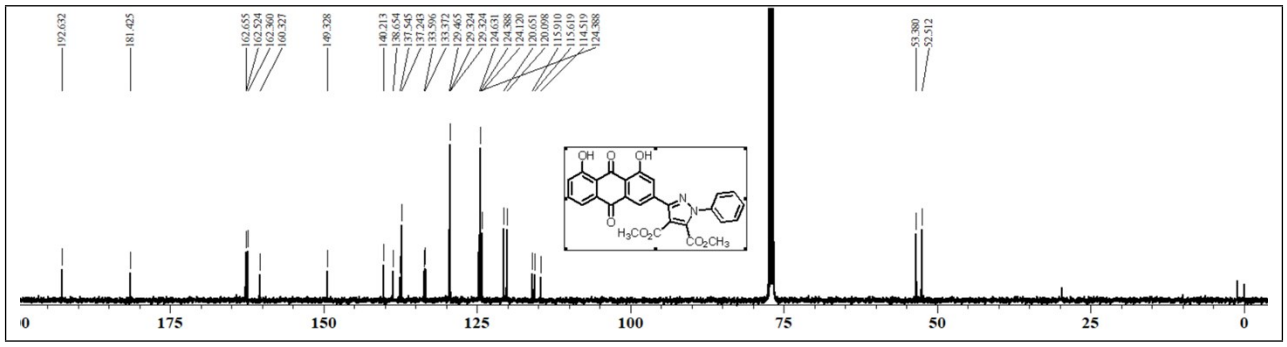
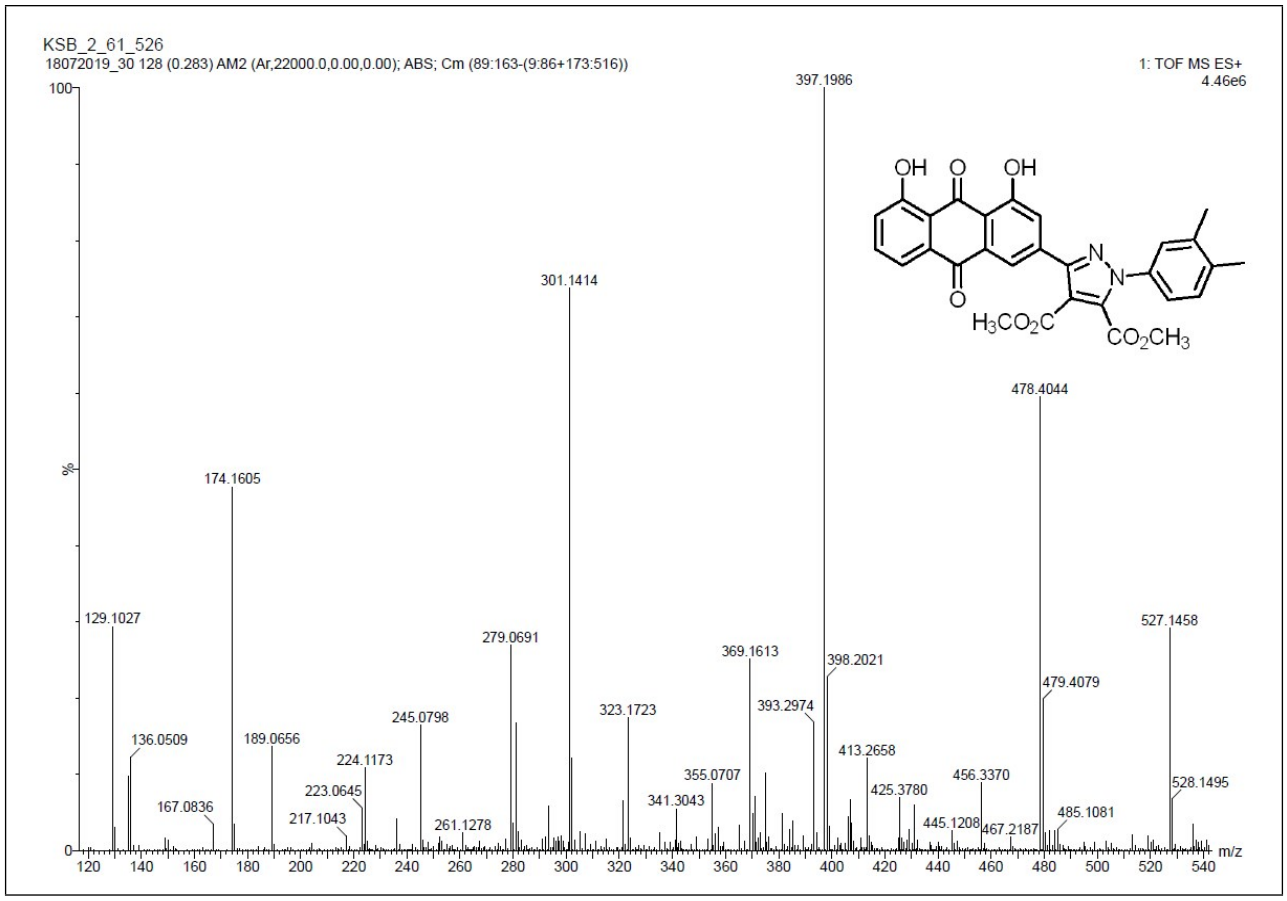


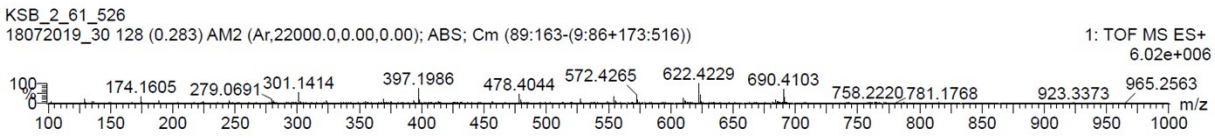
Figure: S45: ^{13}C NMR SPECTRUM OF COMPOUND **6a**(101 MHz, $CDCl_3$)



Elemental Composition Report **Page 1**

Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
63 formula(e) evaluated with 1 results within limits (up to 50 best isotopic matches for each mass)
Elements Used:
C: 0-29 H: 0-23 N: 0-2 O: 0-8 I: 0-1



Minimum:				-1.5				
Maximum:		5.0	5.0	50.0				
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
527.1458	527.1454	0.4	0.8	19.5	383.9	n/a	n/a	C29 H23 N2 O8

Figure: S46: HRESIMS SPECTRUM OF COMPOUND **6b**

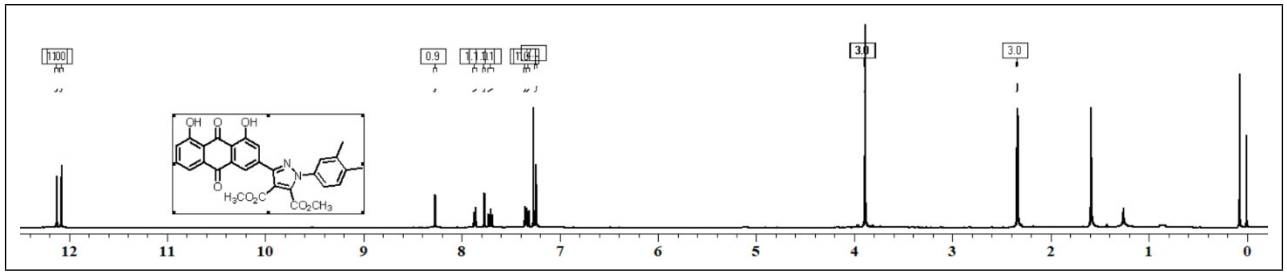


Figure: S47: ^{1}H NMR SPECTRUM OF COMPOUND **6b**(400 MHz, $CDCl_3$)

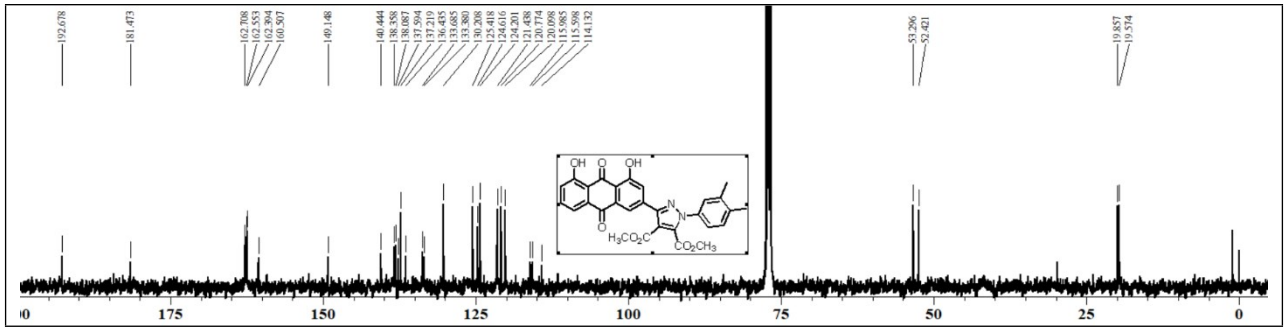
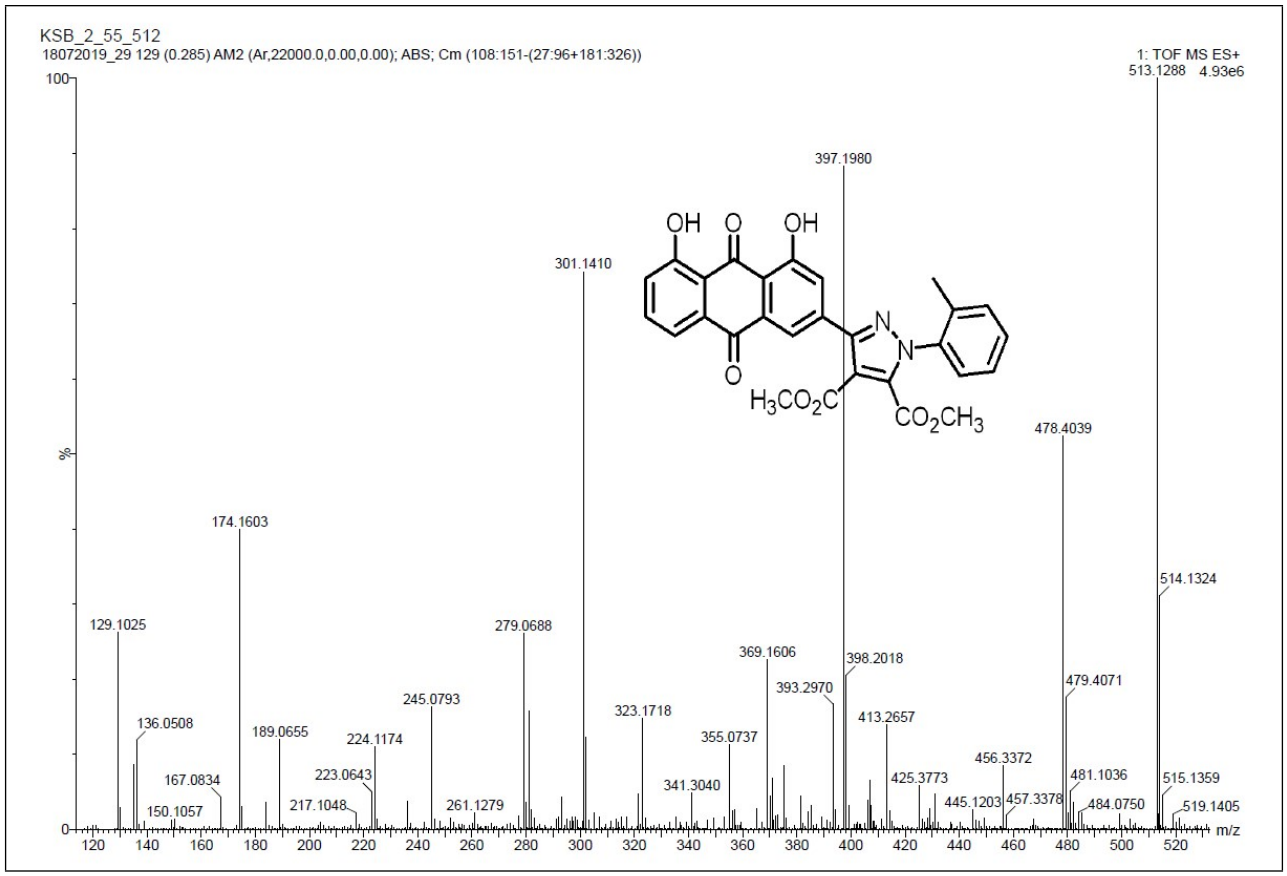


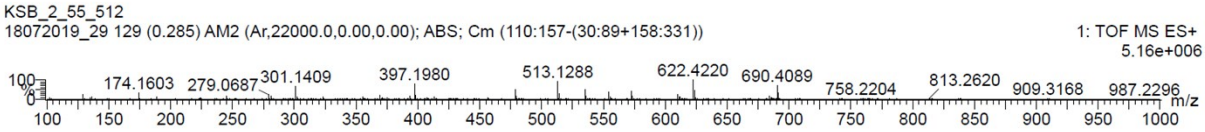
Figure: S48: ^{13}C NMR SPECTRUM OF COMPOUND **6b**(101 MHz, $CDCl_3$)



Elemental Composition Report **Page 1**

Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
59 formula(e) evaluated with 1 results within limits (up to 50 best isotopic matches for each mass)
Elements Used:
C: 0-28 H: 0-21 N: 0-2 O: 0-8 I: 0-1



Minimum:				-1.5				
Maximum:		5.0	5.0	50.0				
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
513.1288	513.1298	-1.0	-1.9	19.5	427.4	n/a	n/a	C28 H21 N2 O8

Figure: S49: HRESIMS SPECTRUM OF COMPOUND **6c**

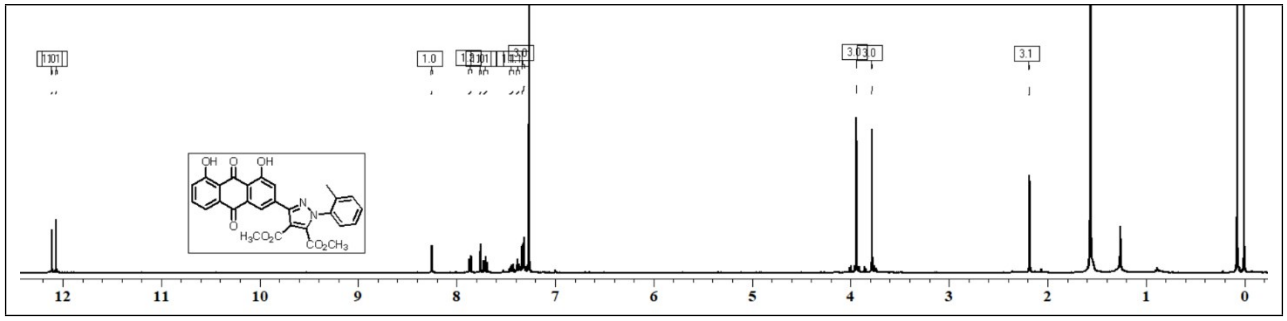


Figure: S50: ^{1}H NMR SPECTRUM OF COMPOUND **6c**(400 MHz, $CDCl_3$)

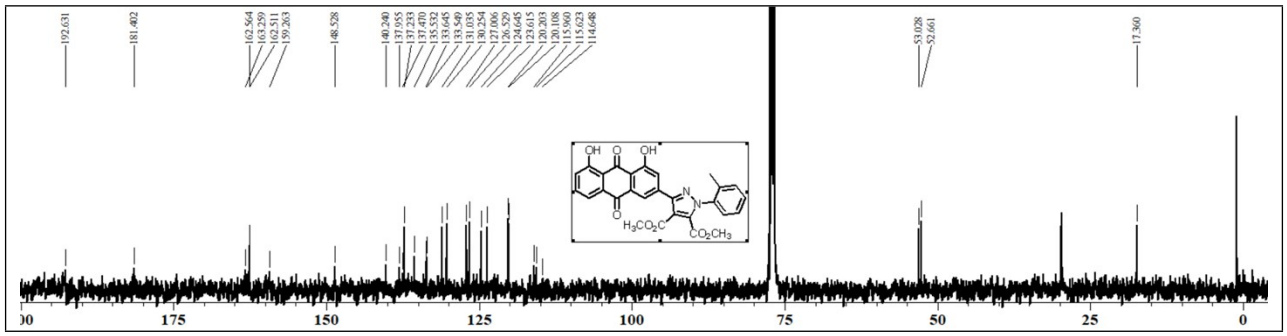
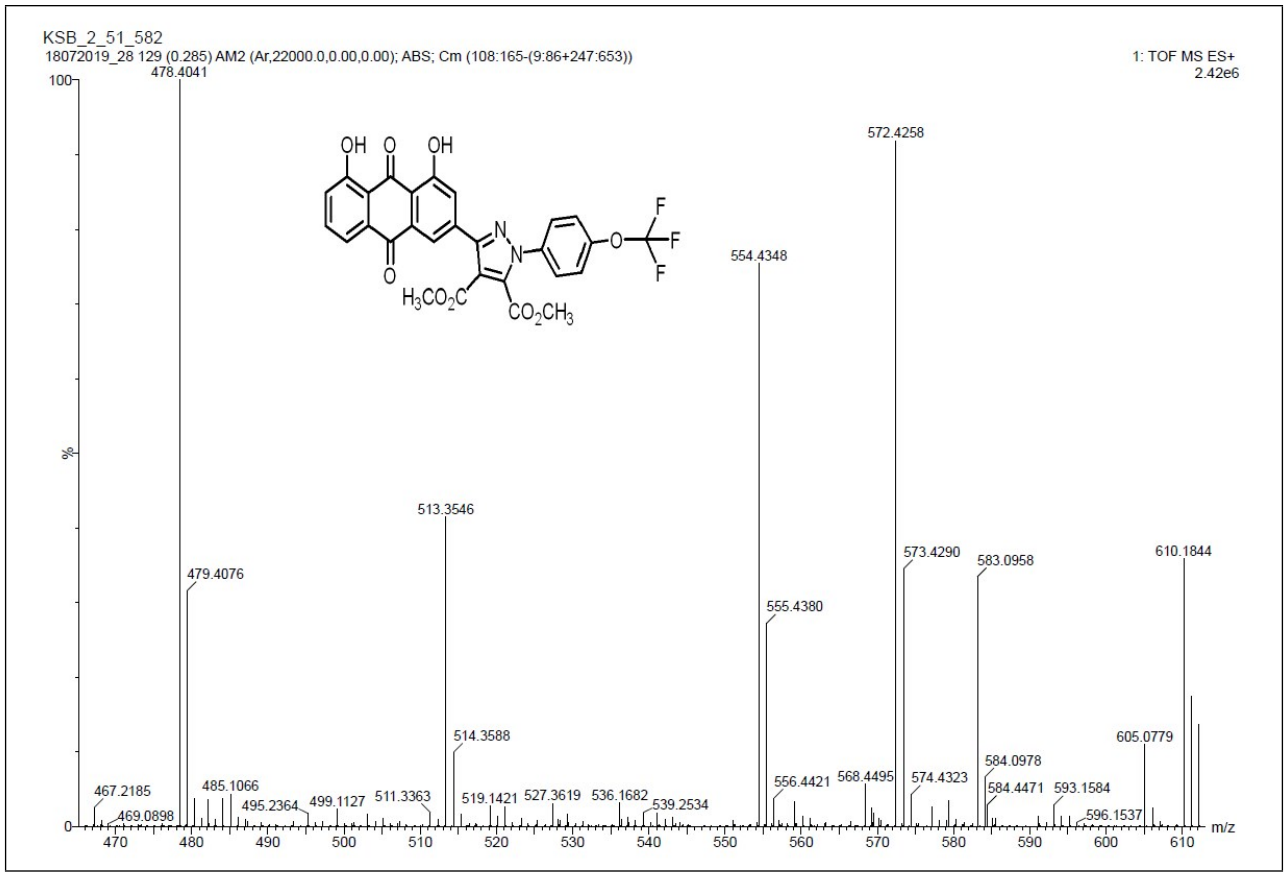


Figure: S51: ^{13}C NMR SPECTRUM OF COMPOUND **6c**(101 MHz, $CDCl_3$)

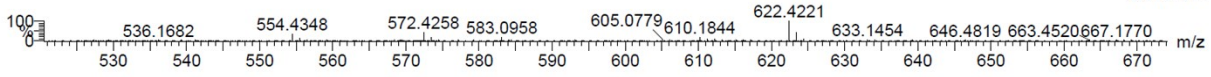


Elemental Composition Report **Page 1**

Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
236 formula(e) evaluated with 1 results within limits (up to 50 best isotopic matches for each mass)
Elements Used:
C: 0-28 H: 0-18 N: 0-2 O: 0-9 I: 0-1 F: 0-3

KSB_2_51_582
18072019_28 129 (0.285) AM2 (Ar,22000.0,0.00,0.00); ABS; Cm (108:165-(9:86+247:653))
1: TOF MS ES+
5.51e+006



Minimum:				-1.5				
Maximum:		5.0	5.0	50.0				
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
583.0958	583.0964	-0.6	-1.0	19.5	173.5	n/a	n/a	C28 H18 N2 O9 F3

Figure: S52: HRESIMS SPECTRUM OF COMPOUND **6d**

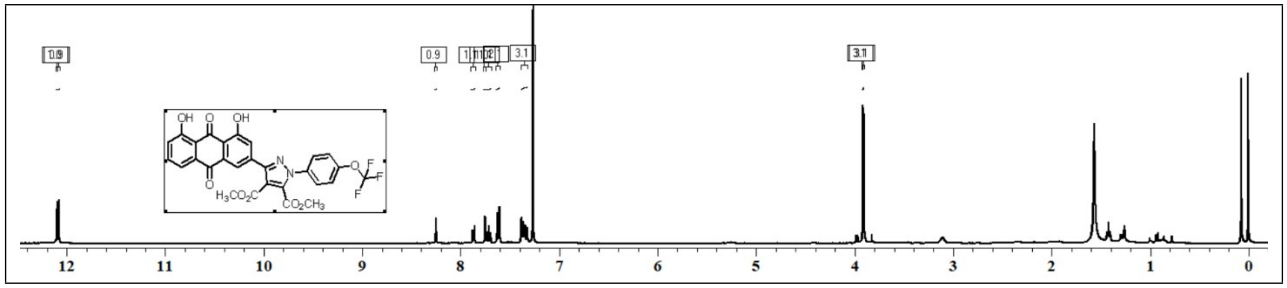


Figure: S53: ^{1}H NMR SPECTRUM OF COMPOUND **6d**(400 MHz, $CDCl_3$)

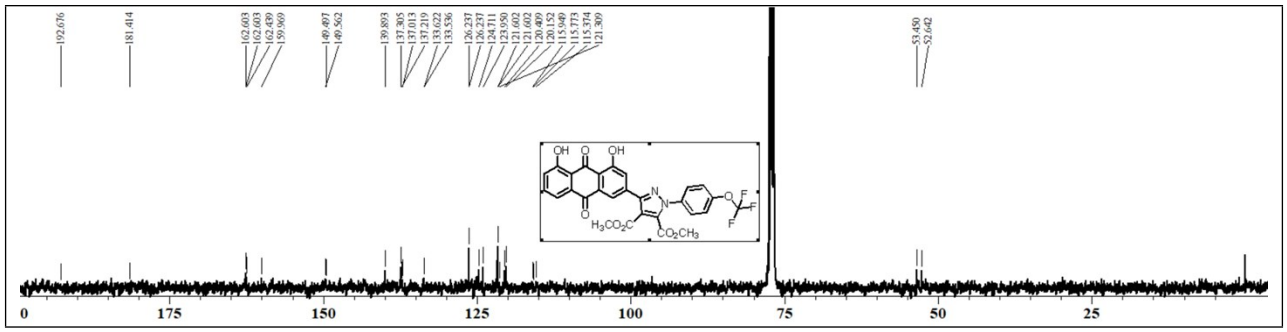
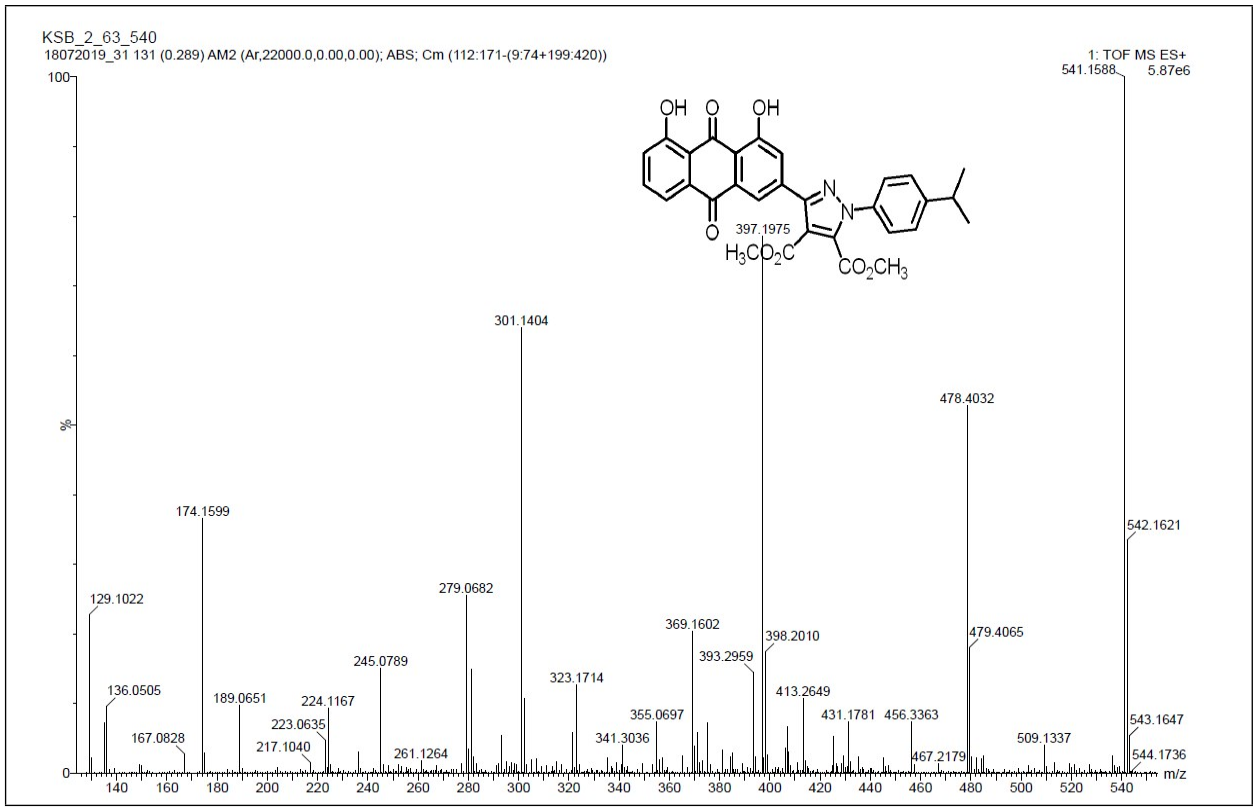


Figure: S54: ^{13}C NMR SPECTRUM OF COMPOUND **6d**(101 MHz, $CDCl_3$)

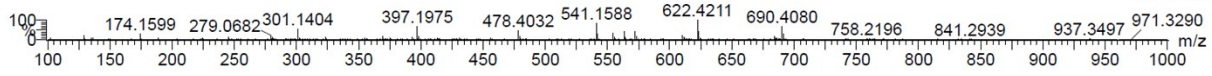


Elemental Composition Report **Page 1**

Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
65 formula(e) evaluated with 1 results within limits (up to 50 best isotopic matches for each mass)
Elements Used:
C: 0-30 H: 0-25 N: 0-2 O: 0-8 I: 0-1

KSB_2_63_540
18072019_31 131 (0.289) AM2 (Ar,22000.0,0.00,0.00); ABS; Cm (112:171-(9:74+199:420))
1: TOF MS ES+
6.87e+006



Minimum:				-1.5				
Maximum:		5.0	5.0	50.0				
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
541.1588	541.1611	-2.3	-4.3	19.5	340.4	n/a	n/a	C30 H25 N2 O8

Figure: S55: HRESIMS SPECTRUM OF COMPOUND **6e**

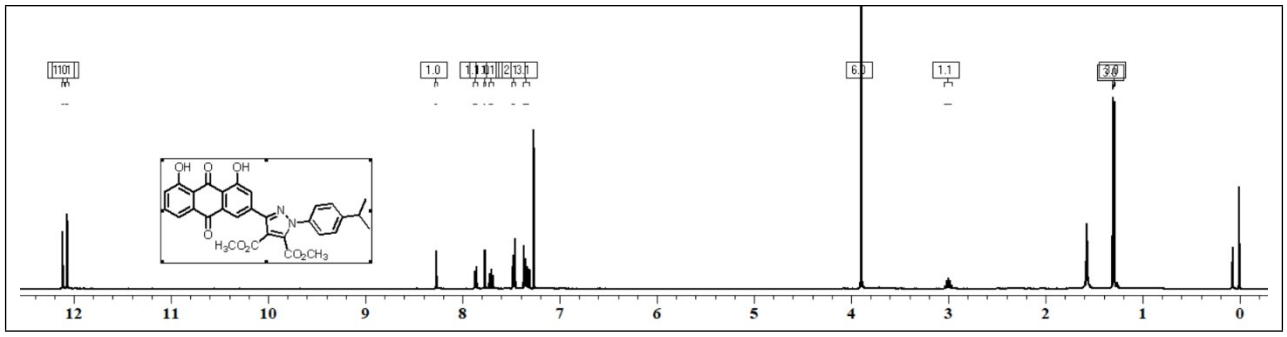


Figure: S56: ^{1}H NMR SPECTRUM OF COMPOUND **6e**(400 MHz, $CDCl_3$)

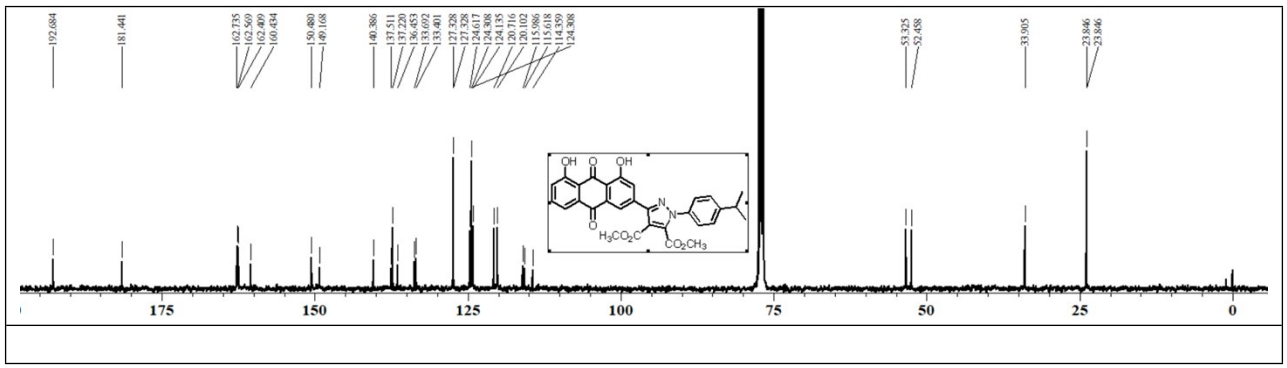


Figure: S57: ^{13}C NMR SPECTRUM OF COMPOUND**6e**(101 MHz, $CDCl_3$)

References:

1. V. Rajkumar, G. Guha and R. Ashok Kumar, *Evidence-Based Complementary and Alternative Medicine*, 2011, **2011**, 1-9.
2. I. Strakova, M. Petrova, S. Belyakov and A. Strakovs, *Chemistry of Heterocyclic Compounds*, 2003, **39**, 1608-1616.
3. J. A. Kumar, G. Saidachary, G. Mallesham, B. Sridhar, N. Jain, S. V. Kalivendi, V. J. Rao and B. C. Raju, *European Journal of Medicinal Chemistry*, 2013, **65**, 389-402.
4. K. V. Sashidhara, A. Kumar, M. Kumar, J. Sarkar and S. Sinha, *Bioorganic & Medicinal Chemistry Letters*, 2010, **20**, 7205-7211.
5. K. V. Sashidhara, S. R. Avula, K. Sharma, G. R. Palnati and S. R. Bathula, *European Journal of Medicinal Chemistry*, 2013, **60**, 120-127.
6. K. Sashidhara, G. Palnati, R. Dodda, S. Avula and P. Swami, *Synlett*, 2013, **24**, 1795-1800.