Supporting Information

Design, synthesis, anticancer activity, bioimaging, and molecular docking of novel fluorescent isatin derivatives

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Keywords:Anticancer, drug development, fluorescent, isatin, colocalization, molecular docking.

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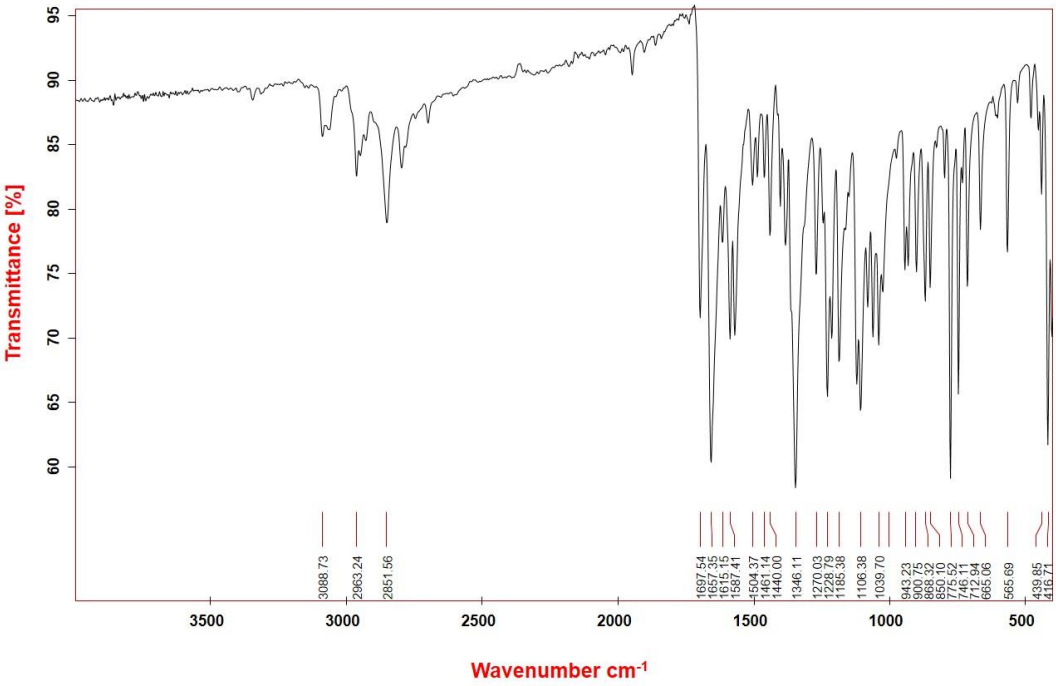
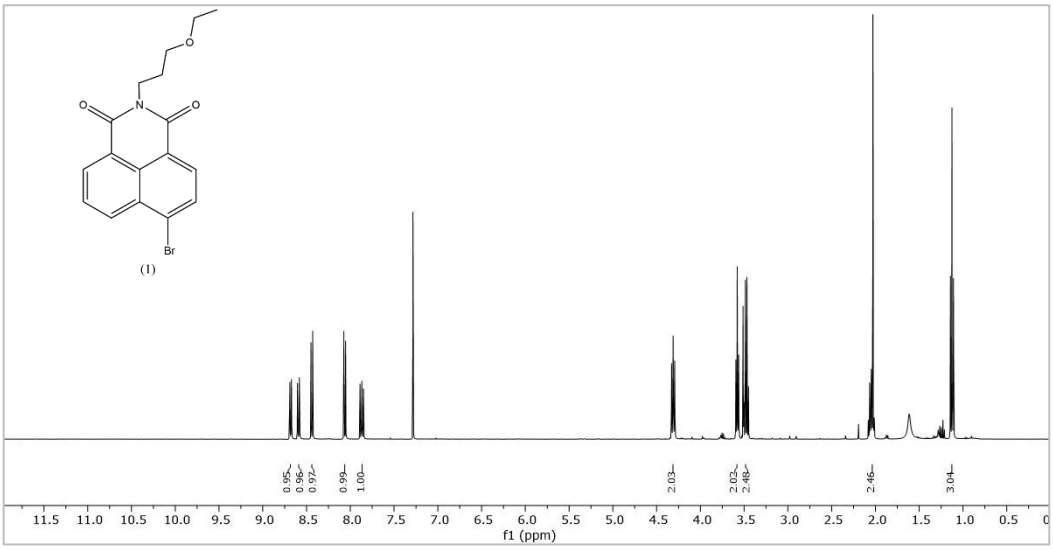
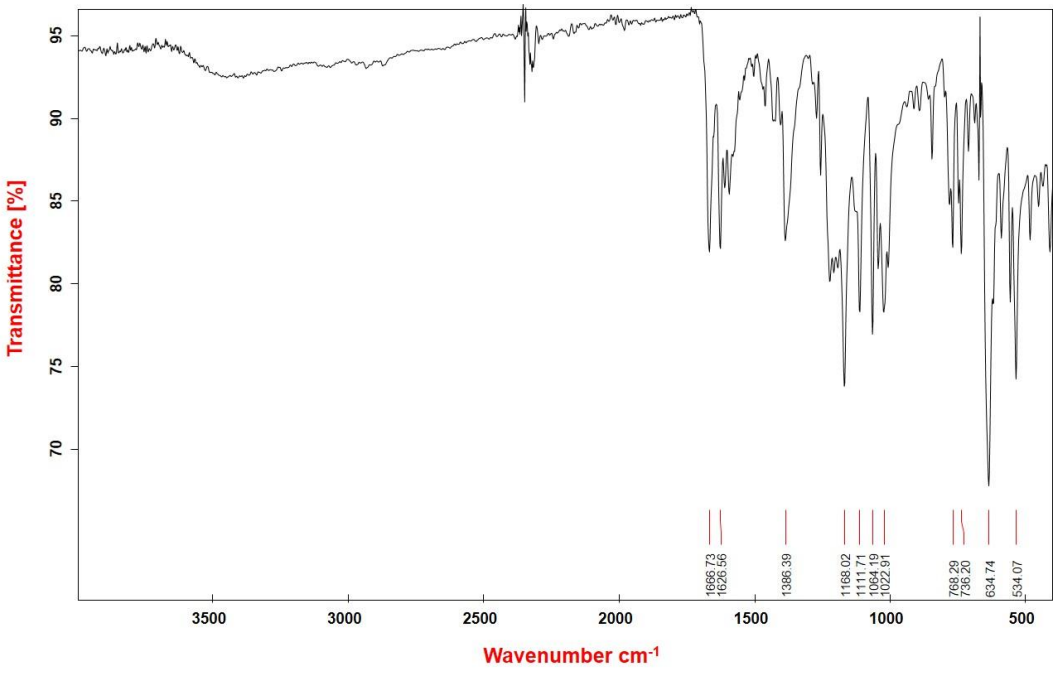
Table S1.1 List of FTIR, ^{1}H-NMR (400 MHz, CDCl3-*d*) Spectra of compound (1).

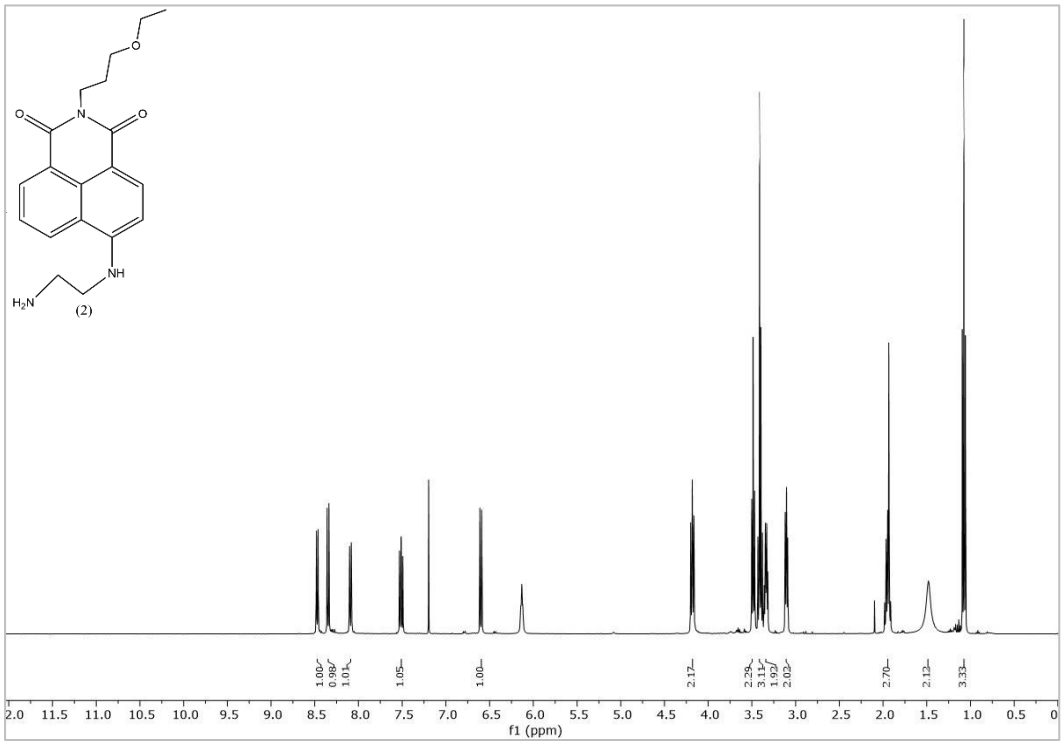




1: (%76, M.P: 186°C). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 1.12 (t, *J* = 7.0 Hz, 3H, -C*H*$_3$), 2.03 (s, 2H, -C*H*$_2$-), 3.45 – 3.52 (m, 2H, -C*H*$_2$-), 3.58 (t, *J* = 6.3 Hz, 2H, -C*H*$_2$-), 4.28 – 4.35 (m, 2H, -C*H*$_2$-), 7.86 (d, *J* = 7.3 Hz, 1H, Ar*H*$_{Nap}$), 8.05 (d, *J* = 7.9 Hz, 1H, Ar*H*$_{Nap}$), 8.44 (d, *J* = 7.9 Hz, 1H, Ar*H*$_{Nap}$), 8.59 (d, *J* = 1.1 Hz, 1H, Ar*H*$_{Nap}$), 8.69 (d, *J* = 1.1Hz, 1H, Ar*H*$_{Nap}$).

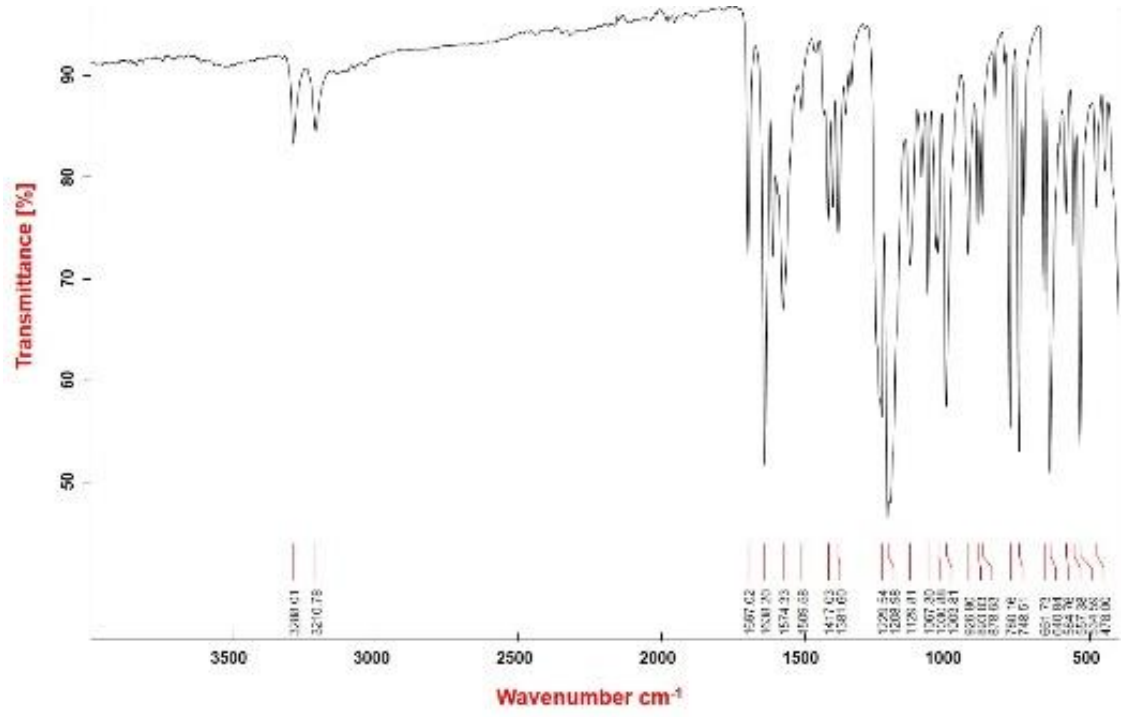
Table S1.2 List of FTIR, ^{1}H-NMR (400 MHz, CDCl3-*d*) Spectra of compound (2).

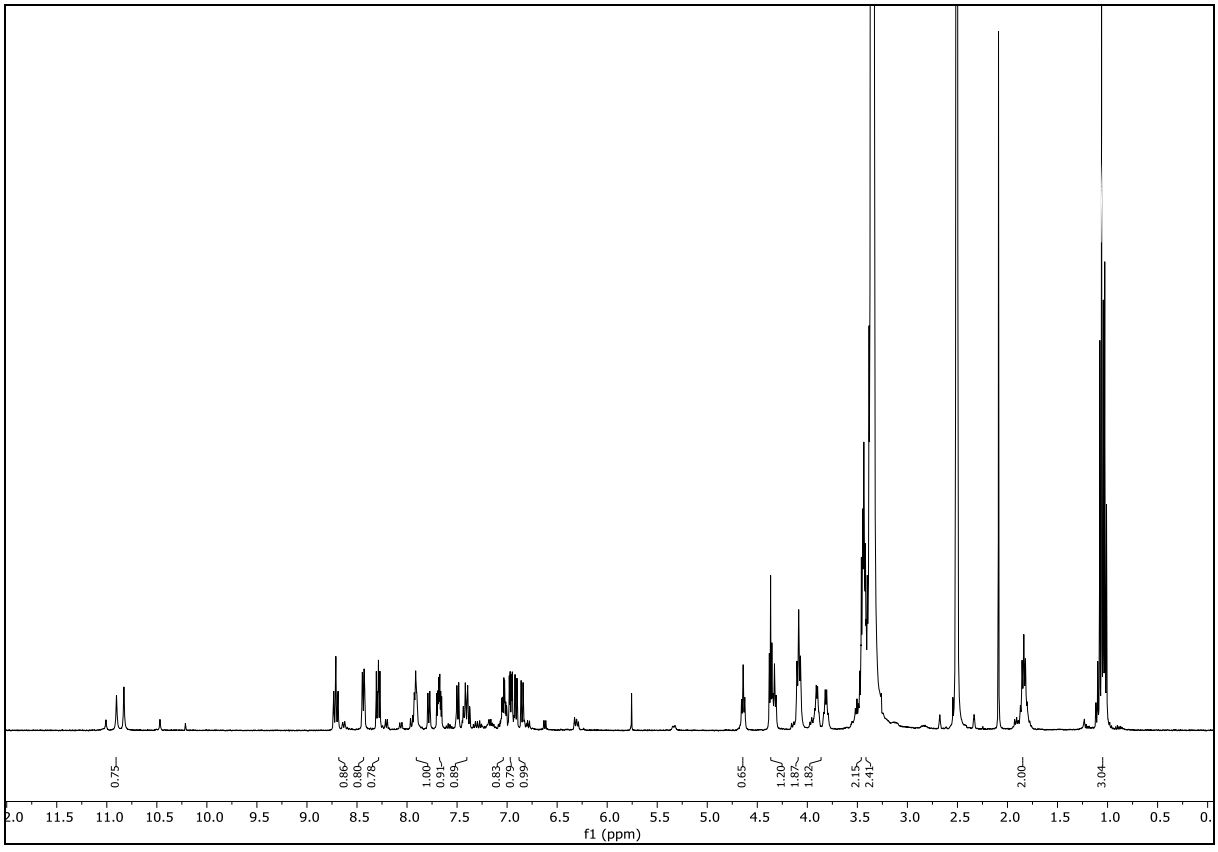


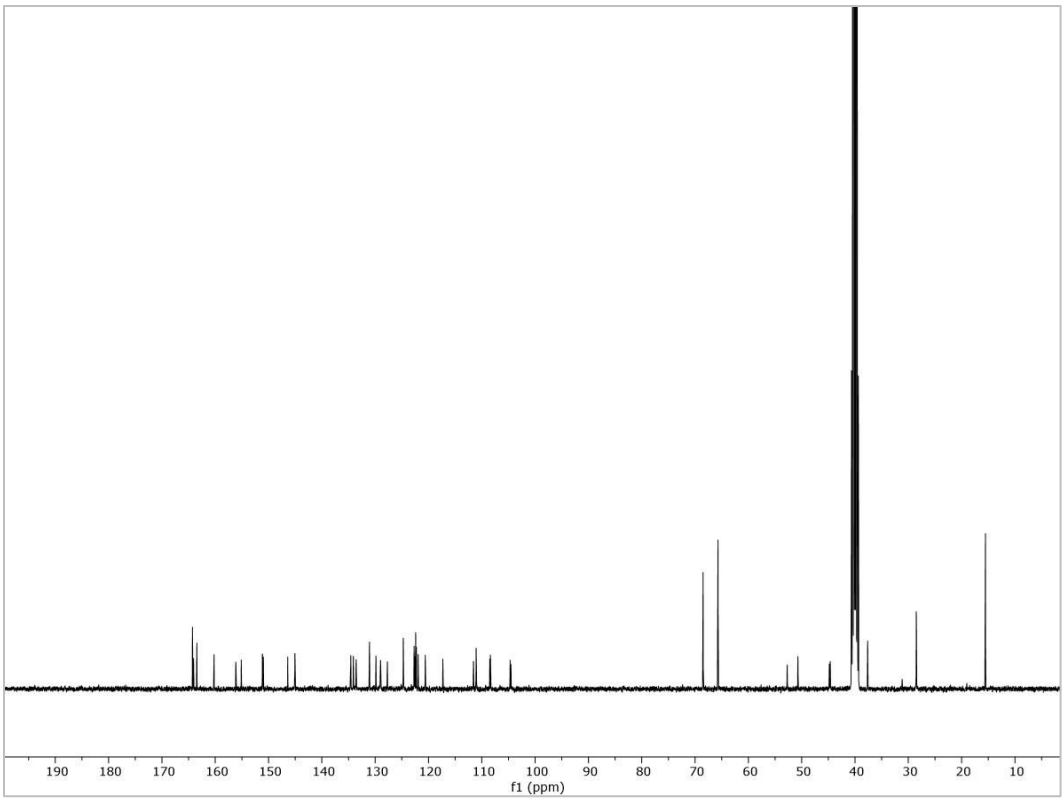


***2**: (%82, M.P: 197℃). 1H NMR (400 MHz, Chloroform-d) δ 1.08 (t, J = 7.0 Hz, 3H, -CH3), 1.91 − 1.98 (m, 2H, -CH2-), 3.08 − 3.14 (m, 2H, -CH2-), 3.34 (d, J = 4.9 Hz, 2H, -CH2-), 3.39 − 3.44 (m, 2H, -CH2-), 3.50 (t, J = 6.5 Hz, 2H, -CH2-), 4.15 − 4.21 (m, 2H, -CH2-), 6.59 (d, J = 8.4 Hz, 1H, ArHNap), 7.51 (d, J = 7.3 Hz, 1H, ArHNap), 8.09 (d, J = 1.1 Hz, 1H, ArHNap), 8.35 (d, J = 8.4 Hz, 1H, ArHNap), 8.47 (d, J = 1.1 Hz, 1H, ArHNap).*

Table S1.3 List of FTIR, ^{1}H-NMR (400 MHz, CDCl3-*d*) and ^{13}C-NMR (101 MHz, DMSO-d$_6$) Spectra of compound **3a**.

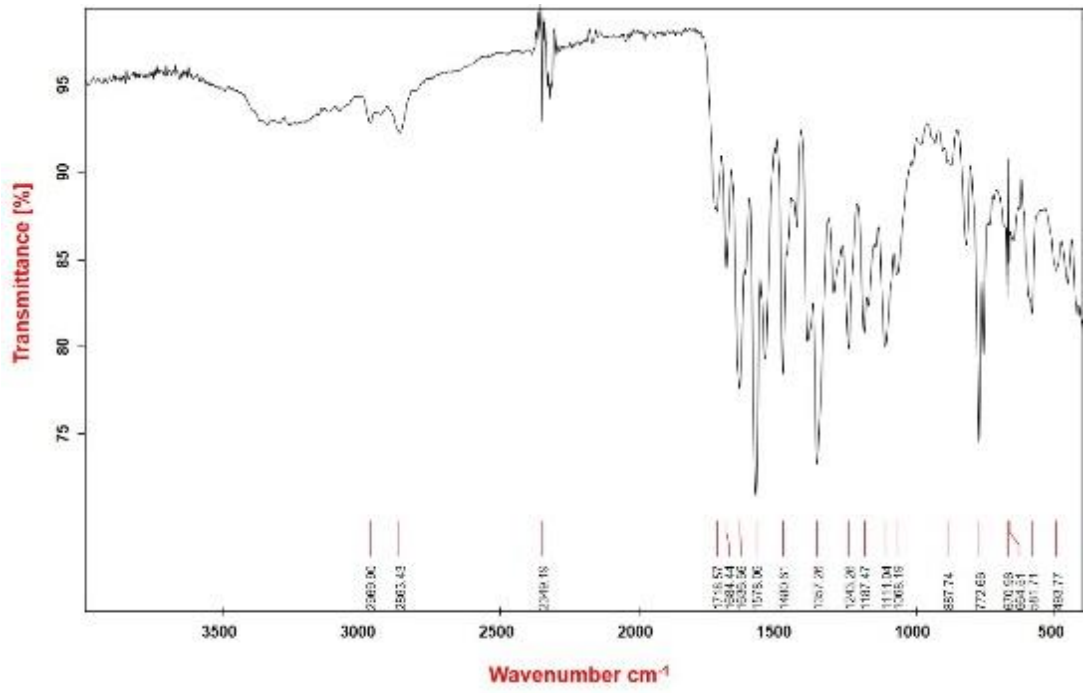


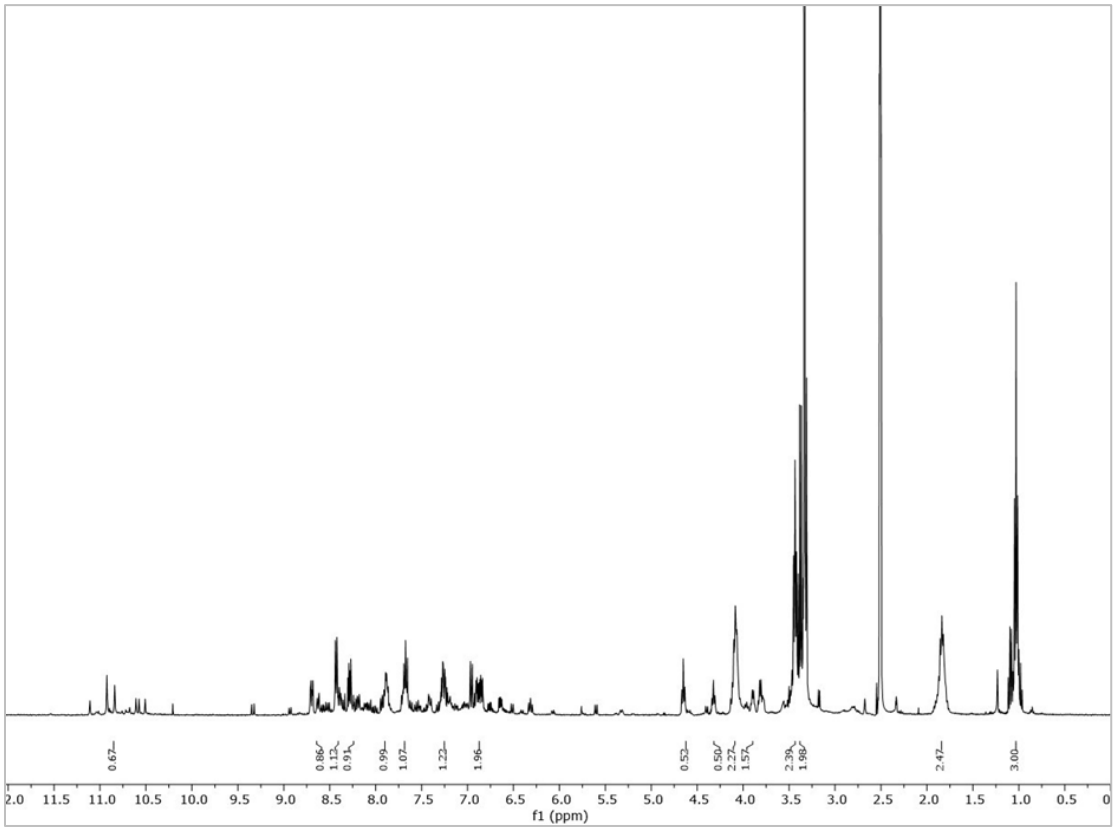


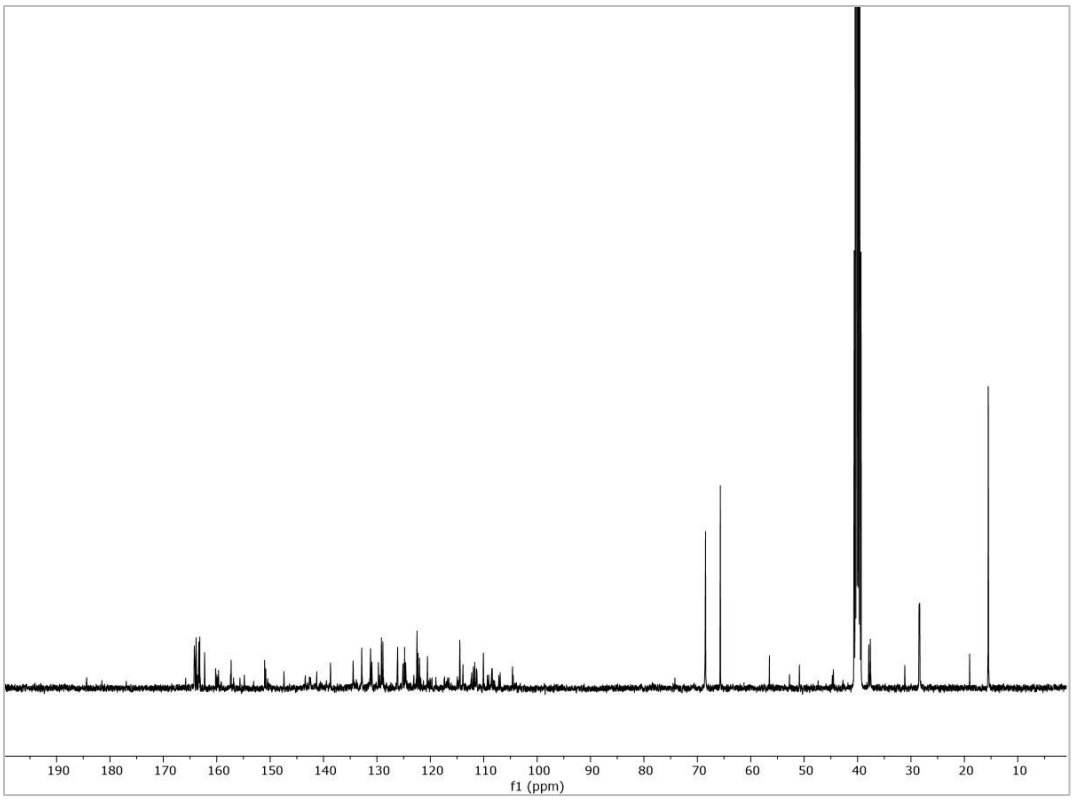


3a: %89, M.P: 235°C. ^{1}H NMR (400 MHz, Chloroform-*d*) δ1.05 (d, *J* = 7.0 Hz, 3H, -C*H*$_3$), 1.84 (p, *J* = 6.8 Hz, 2H, -C*H*$_2$-), 3.42 (d, *J* = 4.0 Hz, 2H, -C*H*$_2$-), 3.46 (d, *J* = 2.6 Hz, 2H, -C*H*$_2$-), 3.86 (d, *J* = 5.8 Hz, 2H, -C*H*$_2$-), 4.09 (t, *J* = 7.3 Hz, 2H, -C*H*$_2$-), 4.38 (t, *J* = 5.1 Hz, 1H, -C*H*-), 4.65 (t, *J* = 6.5 Hz, 1H, -C*H*-), 6.82 – 6.94 (m, 1H, Ar*H*$_{IS}$), 6.97 (d, *J* = 3.6, 8.6 Hz, 1H, Ar*H*$_{IS}$), 7.04 (d, *J* = 1.0 Hz, 1H, Ar*H*$_{IS}$), 7.40 (d, *J* = 1.2 Hz, 1H, Ar*H*$_{IS}$), 7.68 (d, *J* = 3.8 Hz, 1H, Ar*H*$_{Nap}$), 7.83 – 7.99 (m, 1H, Ar*H*$_{Nap}$), 8.28 (d, *J* = 5.9 Hz, 1H, Ar*H*$_{Nap}$), 8.44 (d, *J* = 1.0 Hz, 1H, Ar*H*$_{Nap}$), 8.61 – 8.75 (m, 1H, Ar*H*$_{Nap}$), 10.76 – 11.06 (m, 1H, -N*H*-). ^{13}C NMR (101 MHz, DMSO-$_{d6}$) δ 15.45, 28.66, 37.83, 44.82, 50.55, 65.78, 68.61, 120.42, 120.81, 121.86, 122.47, 124.55, 127.53, 128.84, 129.21, 130.89, 131.24, 133.95, 134.24, 144.98, 145.13, 150.90, 151.08, 155.03, 156.26, 163.56, 164.15, 164.35.

Table S1.4 List of FTIR, ^{1}H-NMR (400 MHz, CDCl3-*d*) and ^{13}C-NMR (101 MHz, DMSO-d_6) Spectra of compound **3b**.

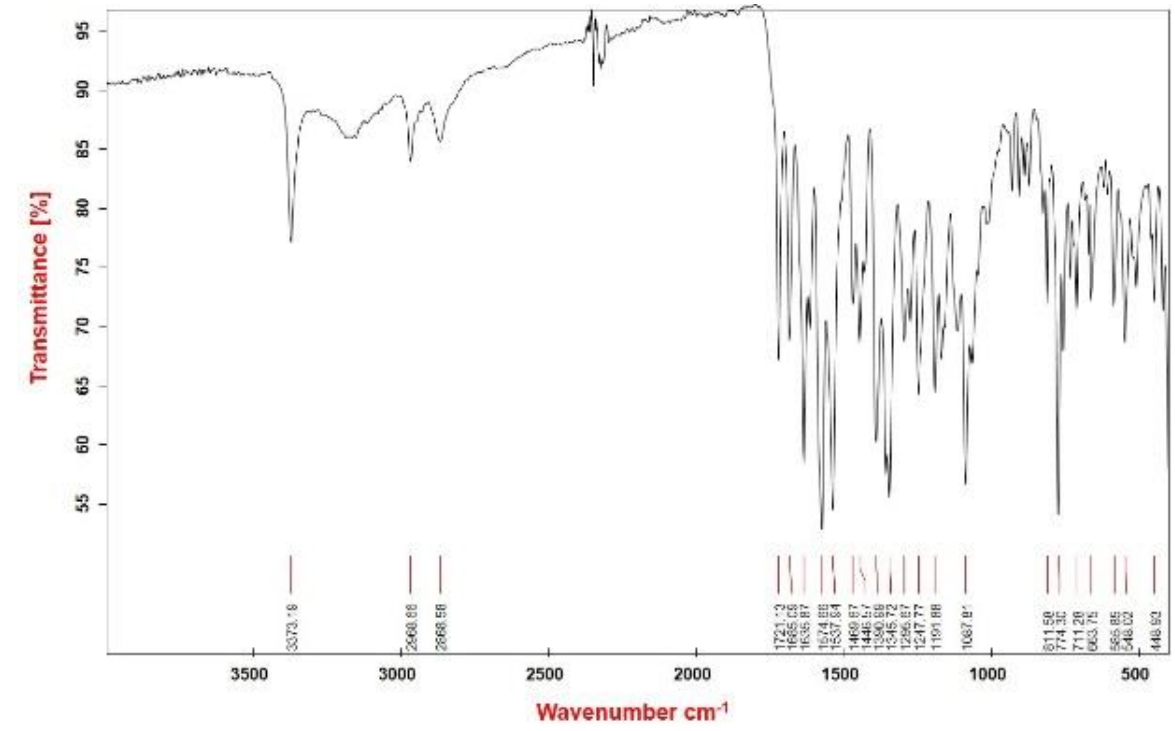


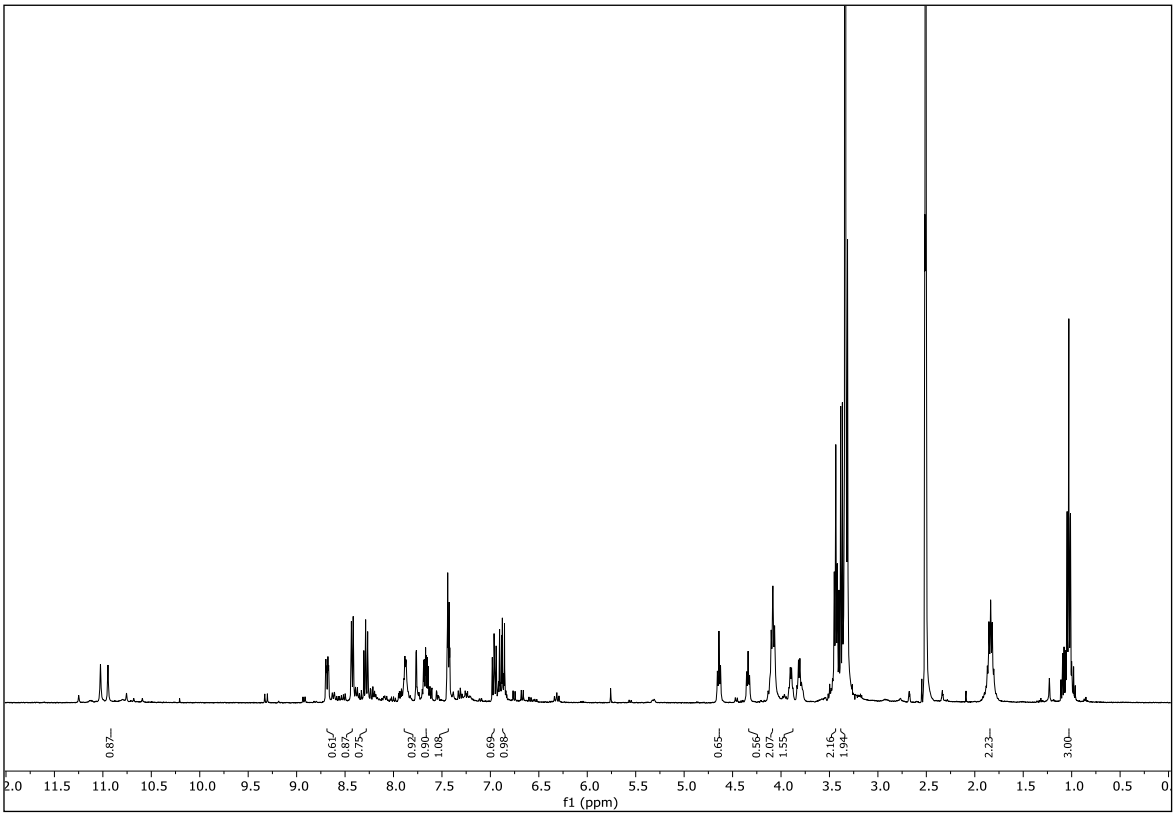


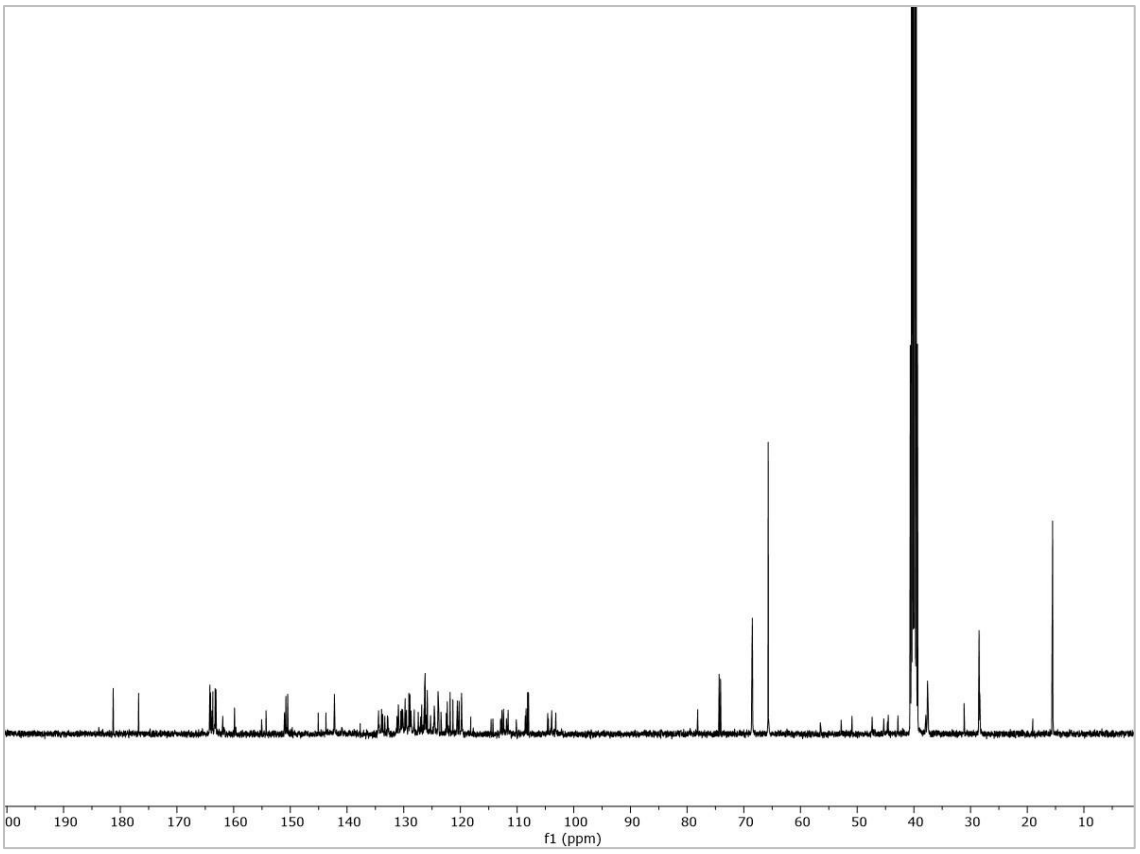


3b: %81, M.P: 231℃. 1H NMR (400 MHz, DMSO-d6) δ 1.04 (t, J = 4.5, 3H, -CH3), 1.48-1.52 (m, 2H, -NH), 1.82 (q, J = 6.4 Hz, 2H, -CH2-), 3.36 – 3.41 (m, 2H, -CH2-), 3.41 – 3.46 (m, 2H, -CH2-), 3.74 – 4.05 (m, 2H, -CH2-), 4.02 – 4.16 (m, 2H, -CH2-), 4.32 (m, 1H, -CH-), 4.64 (t, J = 6.5 Hz, 1H, -CH-), 6.69 – 7.05 (m, 2H, ArHIS), 7.18 – 7.33 (m, 1H, ArHIS), 7.63 – 7.74 (m, 1H, ArHNap), 7.80 – 8.00 (m, 1H, ArHNap), 8.17 – 8.31 (m, 1H, ArHNap), 8.34 – 8.48 (m, 1H, ArHNap), 8.52 – 8.76 (m, 1H, ArHNap), 10.64 –11.06 (m, 1H, -NH-). 13C NMR (101 MHz, DMSO-d6) δ 15.38, 19.09, 28.26, 37.76, 56.56, 65.82, 68.73, 120.66, 122.12, 124.45, 125.17, 126.28, 129.58, 130.63, 131.41, 133.03, 134.18, 138.52, 141.14, 143.87, 147.28, 150.75, 155.52, 157.20, 159.56, 162.42, 163.96, 164.29.

Table S1.5 List of FTIR, ^{1}H-NMR (400 MHz, CDCl3-*d*) and ^{13}C-NMR (101 MHz, DMSO-d_6) Spectra of compound **3c**.

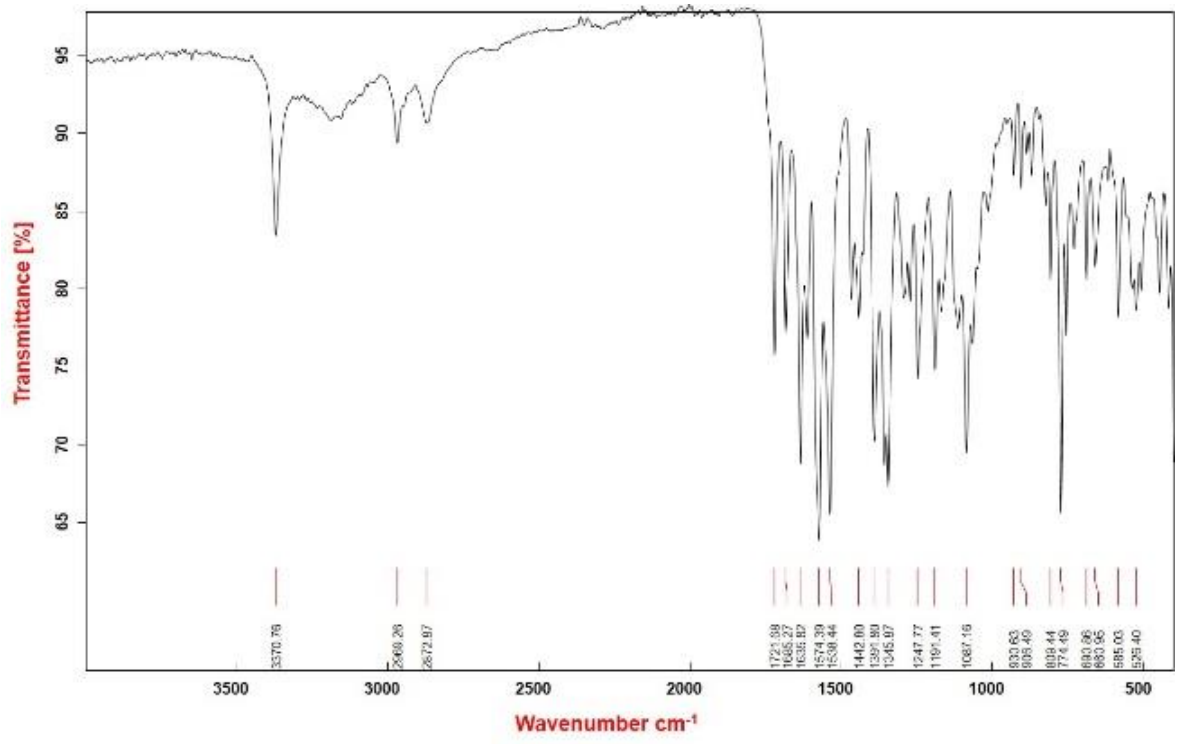


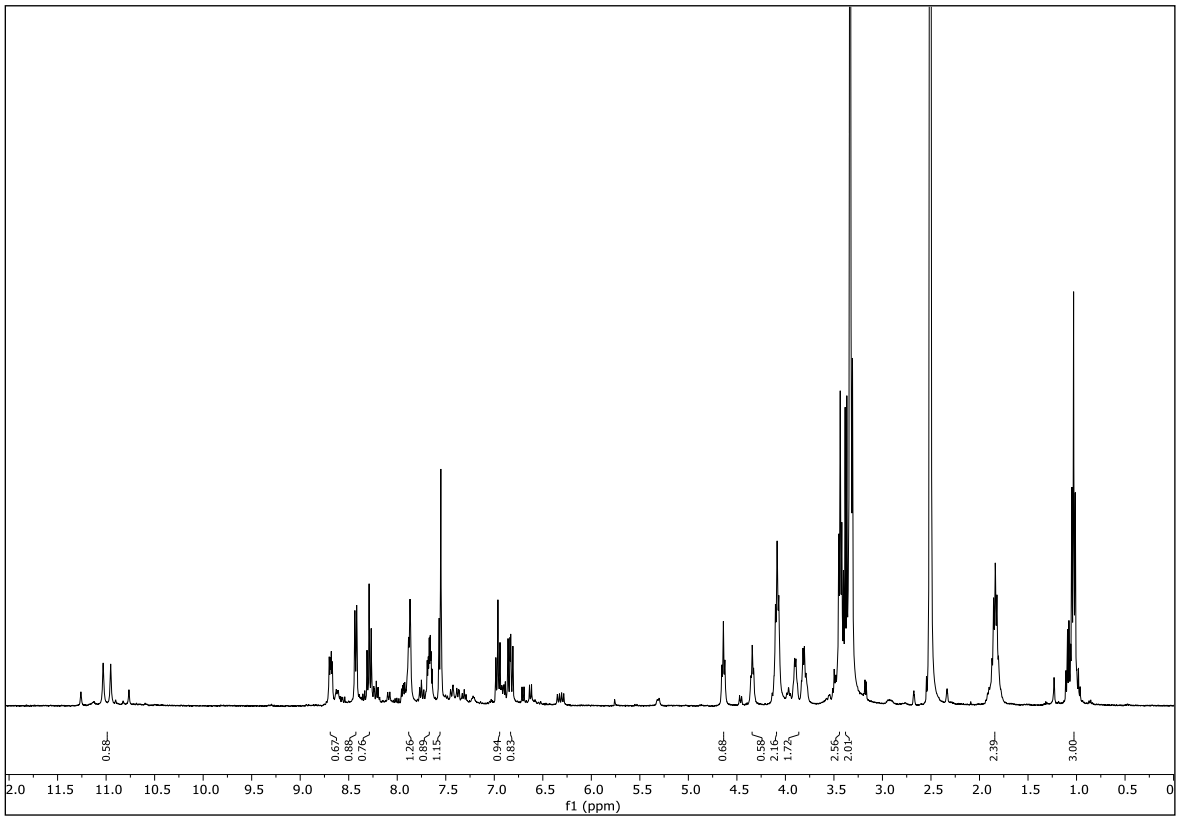


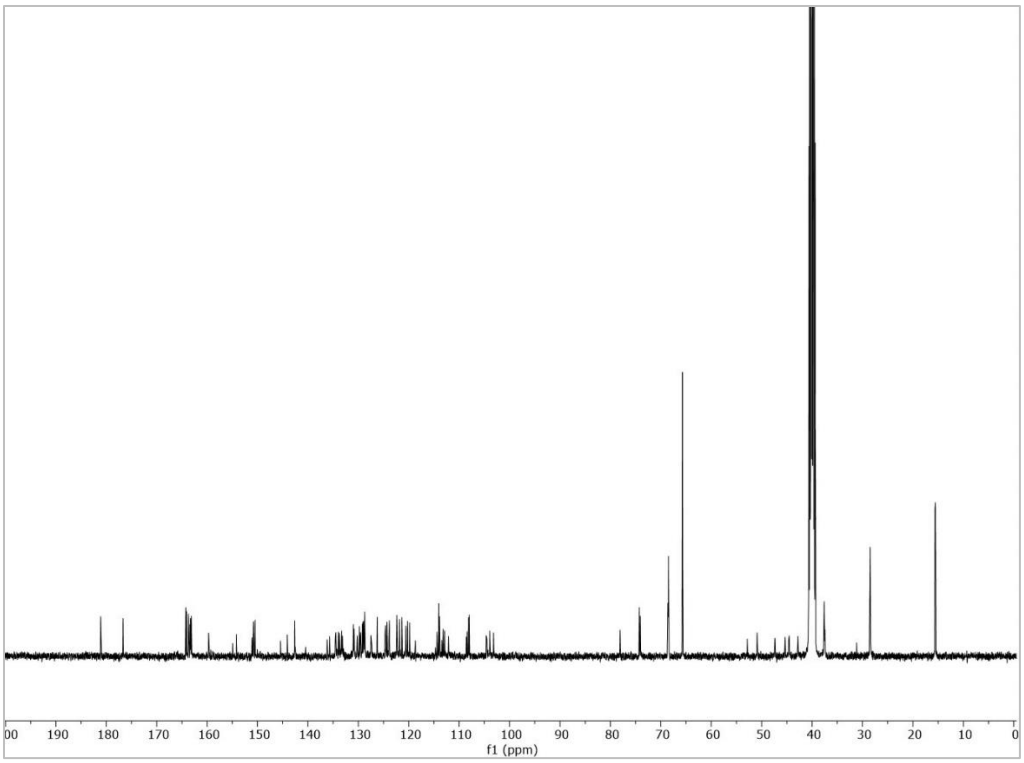


3c: %96, M.P: 222°C. ^{1}H NMR (400 MHz, DMSO-$_{d6}$) δ 0.98 – 1.07 (m, 3H, -CH_3), 1.76 – 1.93 (m, 2H, -CH_2-), 3.38 (t, J = 6.9 Hz, 2H, -CH_2-), 3.44 (t, J = 6.4 Hz, 2H, -CH_2-), 3.74 – 4.01 (m, 2H, -CH_2-), 4.03 (t, J = 7.2 Hz, 2H, -CH_2-), 4.32 (t, J = 6.1 Hz, 1H, -CH-), 4.63 (t, J = 6.4 Hz, 1H, -CH-), 6.82 – 6.93 (m, 1H, ArH_{IS}), 6.96 (d, J = 7.2 Hz, 1H, ArH_{IS}), 7.41 – 7.46 (m, 1H, ArH_{IS}), 7.62 – 7.71 (m, 1H, ArH_{Nap}), 7.83 – 7.95 (m, 1H, ArH_{Nap}), 8.24 – 8.32 (m, 1H, ArH_{Nap}), 8.42 (d, J = 1.3 Hz, 1H, ArH_{Nap}), 8.69 (d, J = 1.1 Hz, 1H, ArH_{Nap}), 10.66 – 11.18 (m, 1H, -NH-). ^{13}C NMR (101 MHz, DMSO-$_{d6}$) δ 15.38, 28.85, 38.18, 45.66, 68.46, 74.19, 78.35, 120.10, 121.21, 122.14, 123.54, 124.35, 125.51, 126.33, 127.22, 128.34, 129.29, 130.51, 131.29 132.60, 133.47, 134.13, 142.13, 150.36, 159.75, 164.17, 176.67, 181.34.

Table S1.6 List of FTIR, ^{1}H-NMR (400 MHz, CDCl3-*d*) and ^{13}C-NMR (101 MHz, DMSO-d_6) Spectra of compound **3d**.

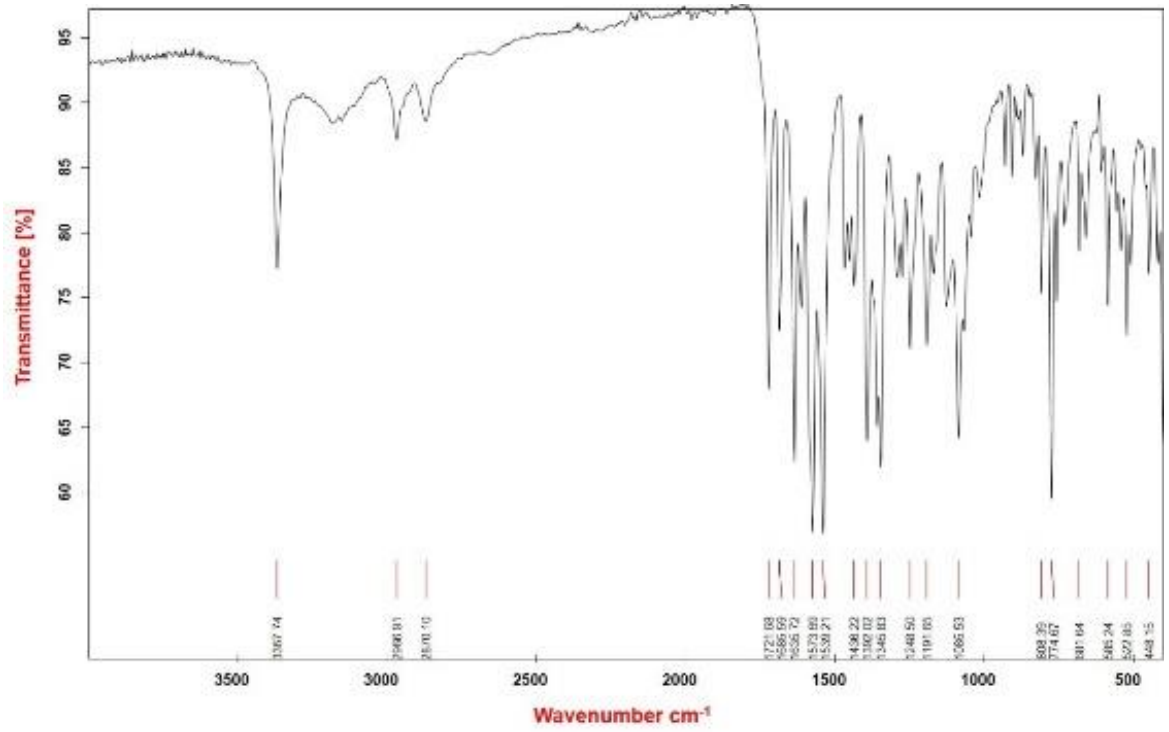


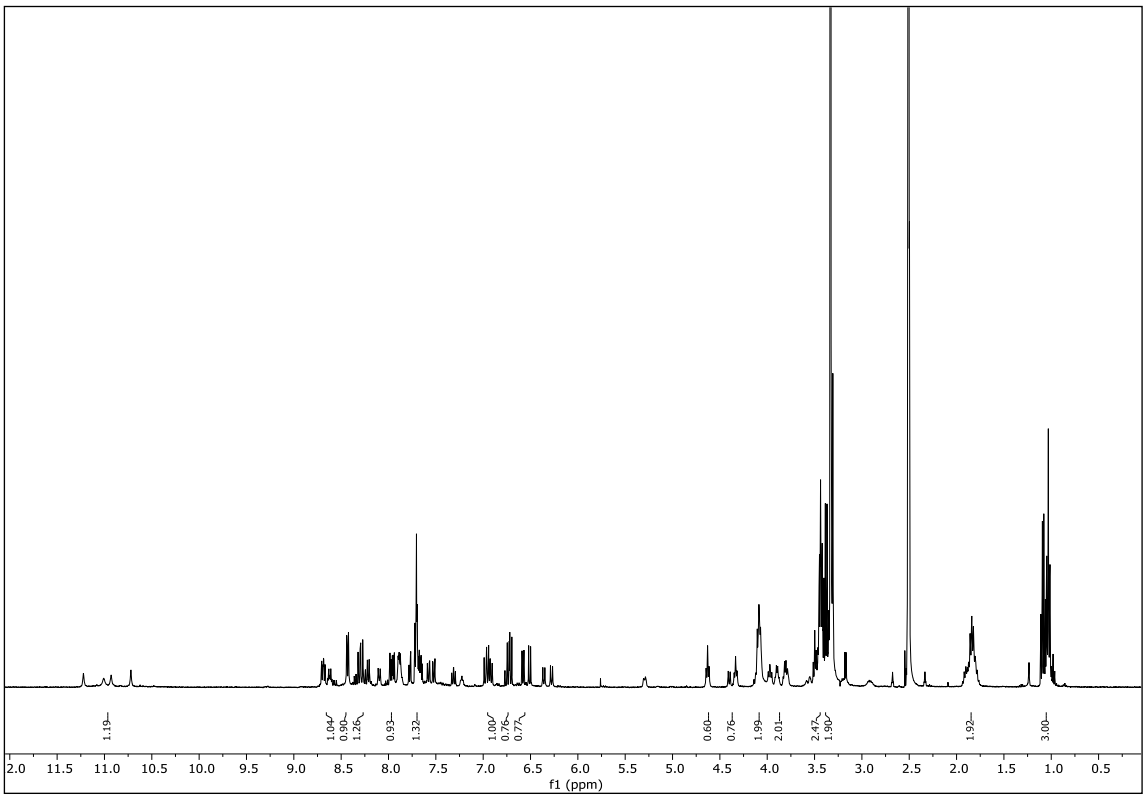


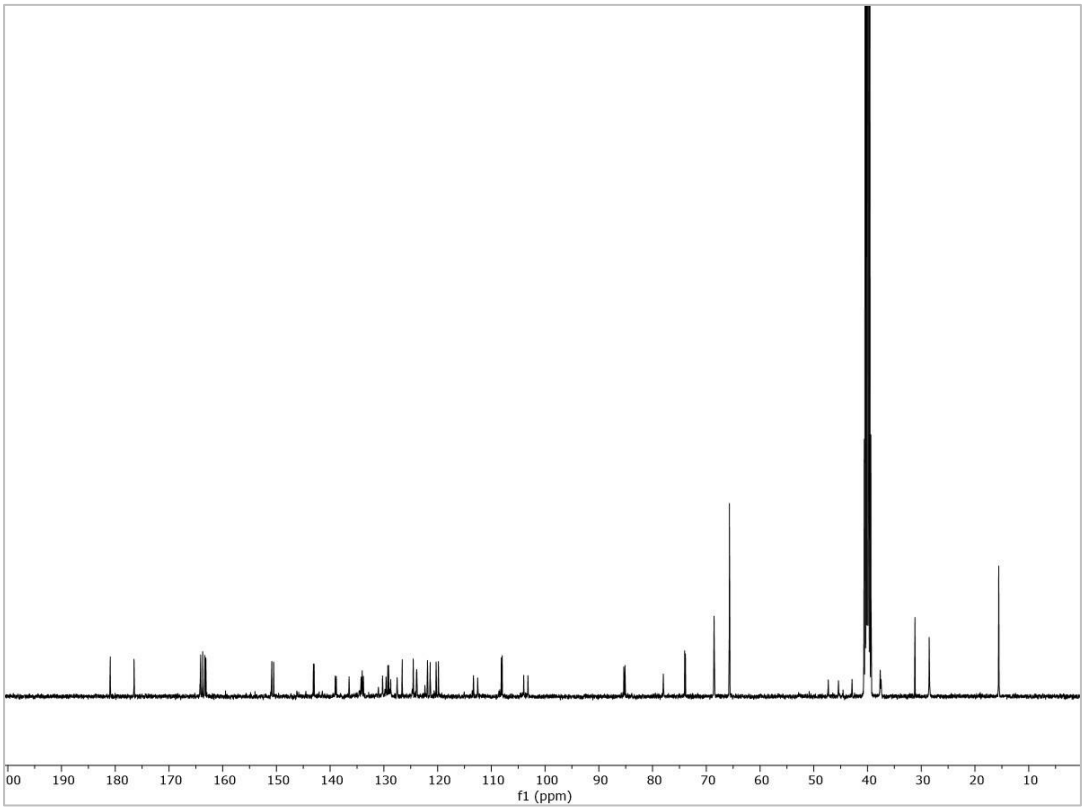


3d: %95, M.P: 215°C. ^{1}H NMR (400 MHz, DMSO-$_{d6}$) δ 1.03 (t, J = 1.1 Hz, 3H, -CH_3), 1.84 (p, J = 6.6 Hz, 2H, -CH_2-), 3.36 – 3.40 (m, 2H, -CH_2-), 3.43 (t, J = 6.4 Hz, 2H, -CH_2-), 3.75 – 3.98 (m, 2H, -CH_2-), 4.04 – 4.15 (m, 2H, -CH_2-), 4.34 (t, J = 5.8 Hz, 1H, -CH-), 4.63 (t, J = 6.4 Hz, 1H, -CH-), 6.78 – 6.88 (m, 1H, ArH_{IS}), 6.88 – 7.00 (m, 1H, ArH_{IS}), 7.53 – 7.59 (m, 1H, ArH_{IS}), 7.67 (t, J = 4.3 Hz, 1H, ArH_{Nap}), 7.82 – 7.94 (m, 1H, ArH_{Nap}), 8.29 (t, J = 8.7 Hz, 1H, ArH_{Nap}), 8.43 (d, J = 1.3 Hz, 1H, ArH_{Nap}), 8.69 (d, J = 4.3 Hz, 1H, ArH_{Nap}), 10.92 – 11.06 (m, 1H, -NH-). ^{13}C NMR (101 MHz, DMSO-$_{d6}$) δ 15.42, 28.89, 50.71, 65.54, 68.72, 74.18, 77.83, 121.48, 123.46, 124.87, 126.12, 127.27, 128.46, 128.99, 129.70, 130.68, 132.92, 133.13, 134.20, 134.65, 135.88, 136.42, 142.41, 142.78, 150.68, 164.08, 176.56, 181.22.

Table S1.7 List of FTIR, ^{1}H-NMR (400 MHz, CDCl3-*d*) and ^{13}C-NMR (101 MHz, DMSO-d_6) Spectra of compound **3e**.

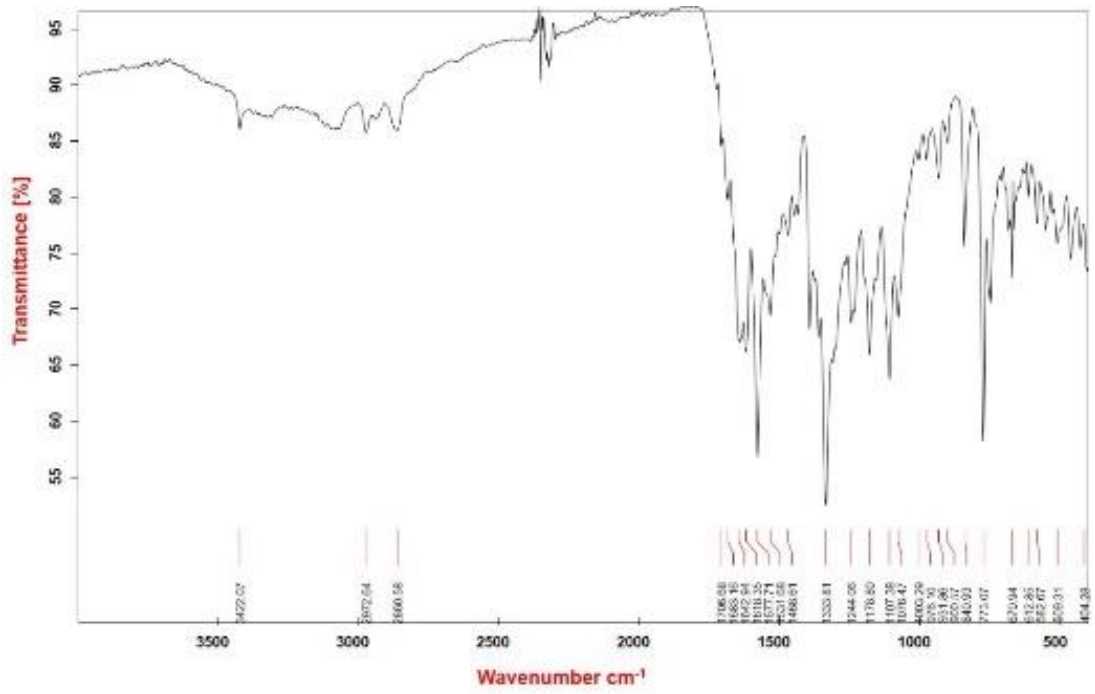


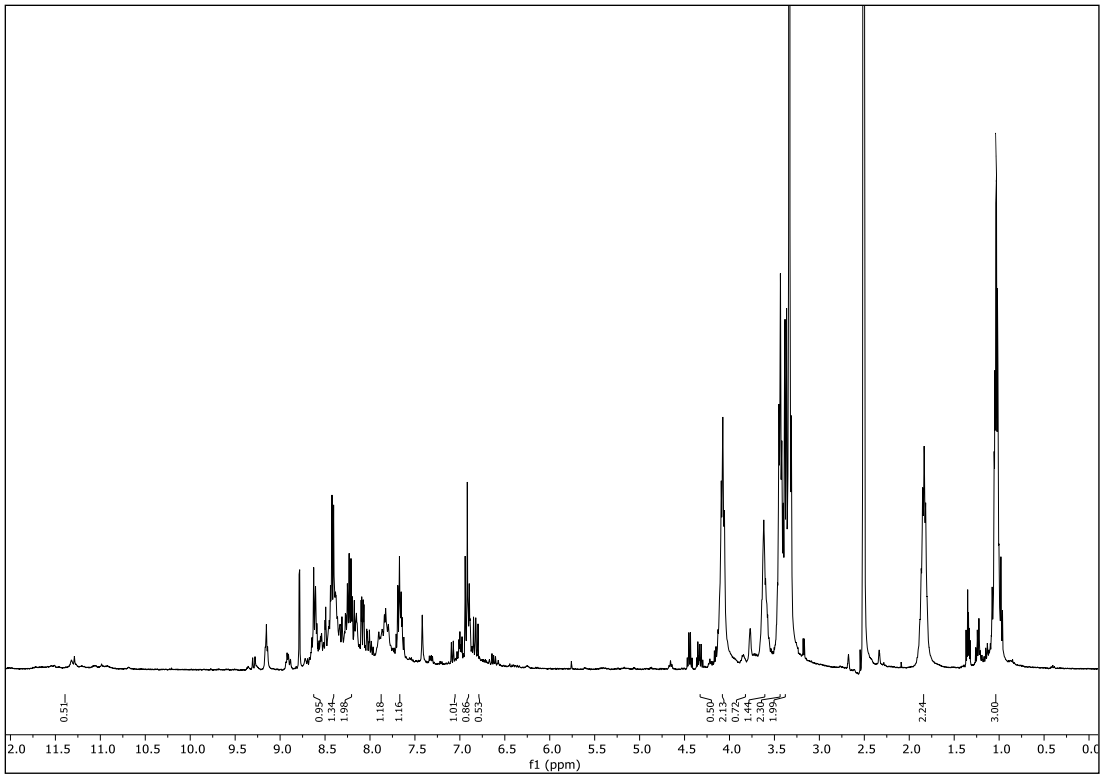


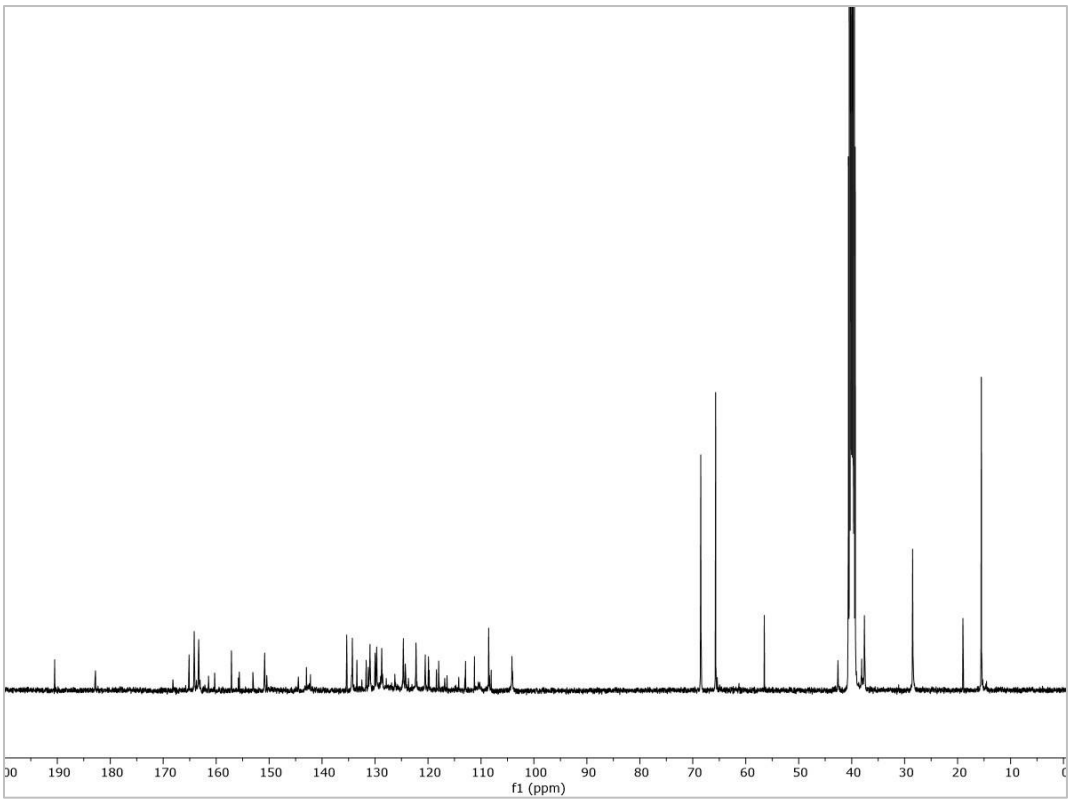


3e: %94, M.P: 213°C. 1H NMR (400 MHz, DMSO-d6) δ 1.02 – 1.08 (m, 3H, -CH3), 1.85 (q, J = 6.8 Hz, 2H, -CH2-), 3.38 (d, J = 5.9 Hz, 2H, -CH2-), 3.42 – 3.46 (m, 2H, -CH2-), 3.73 – 4.00 (m, 2H, -CH2-), 4.08 (t, J = 7.1 Hz, 2H, -CH2-), 4.30 – 4.44 (m, 1H, -CH-), 4.54 – 4.70 (m, 1H, -CH-), 6.56 (d, J = 8.2, Hz, 1H, ArHIS), 6.68 – 6.80 (m, 1H, ArHIS), 6.88 – 7.02 (m, 1H, ArHIS), 7.68 – 7.72 (m, 1H, ArHNap), 7.92 – 8.02 (m, 1H, ArHNap), 8.19 – 8.35 (m, 1H, ArHNap), 8.45 (d, J = 1.2 Hz, 1H, ArHNap), 8.59 – 8.72 (m, 1H, ArHNap), 10.68 – 11.25 (m, 1H, -NH-). 13C NMR (101 MHz, DMSO-d6) δ 15.38, 28.72, 31.23, 65.56, 68.72, 73.78, 85.24, 119.96, 120.38, 121.26 123.60, 124.19, 126.48, 127.28, 128.49, 129.39, 130.01, 133.57, 134.04, 136.24, 138.64, 139.32, 142.87, 143.05, 150.97, 164.00, 176.40, 181.02.

Table S1.8 List of FTIR, ^{1}H-NMR (400 MHz, CDCl3-*d*) and ^{13}C-NMR (101 MHz, DMSO-d_6) Spectra of compound **3f.**

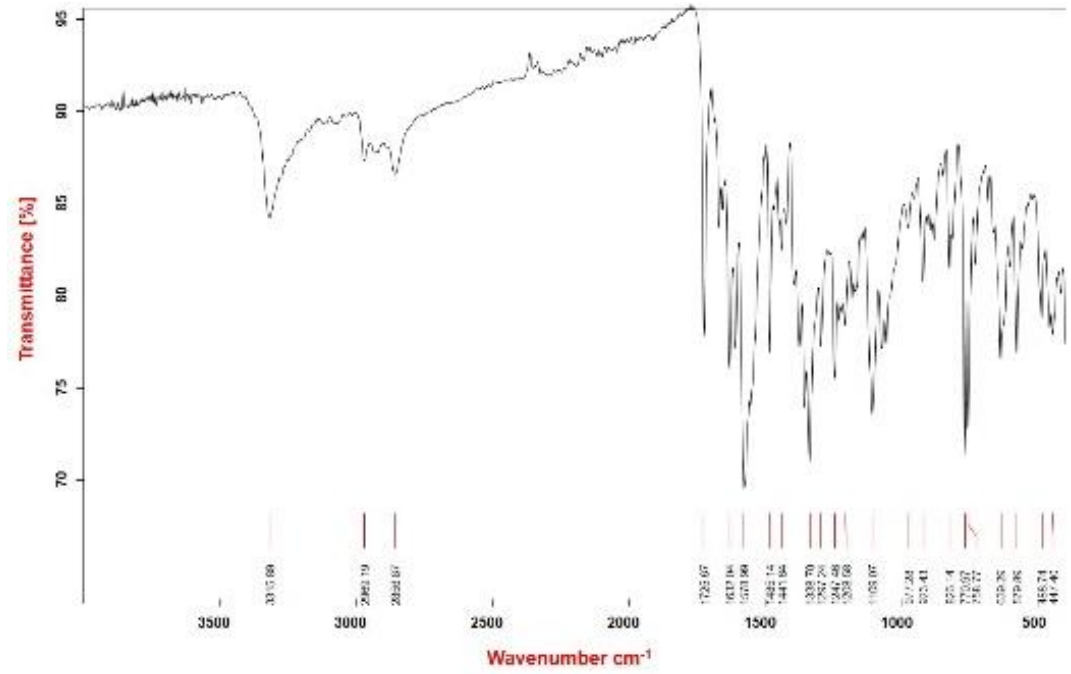


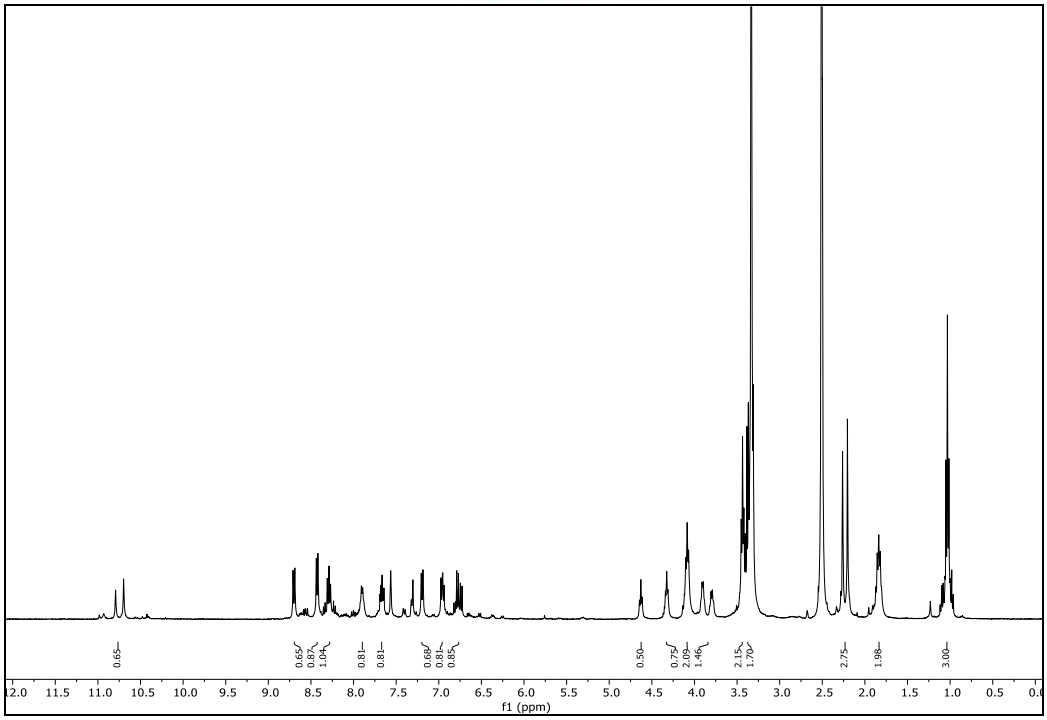


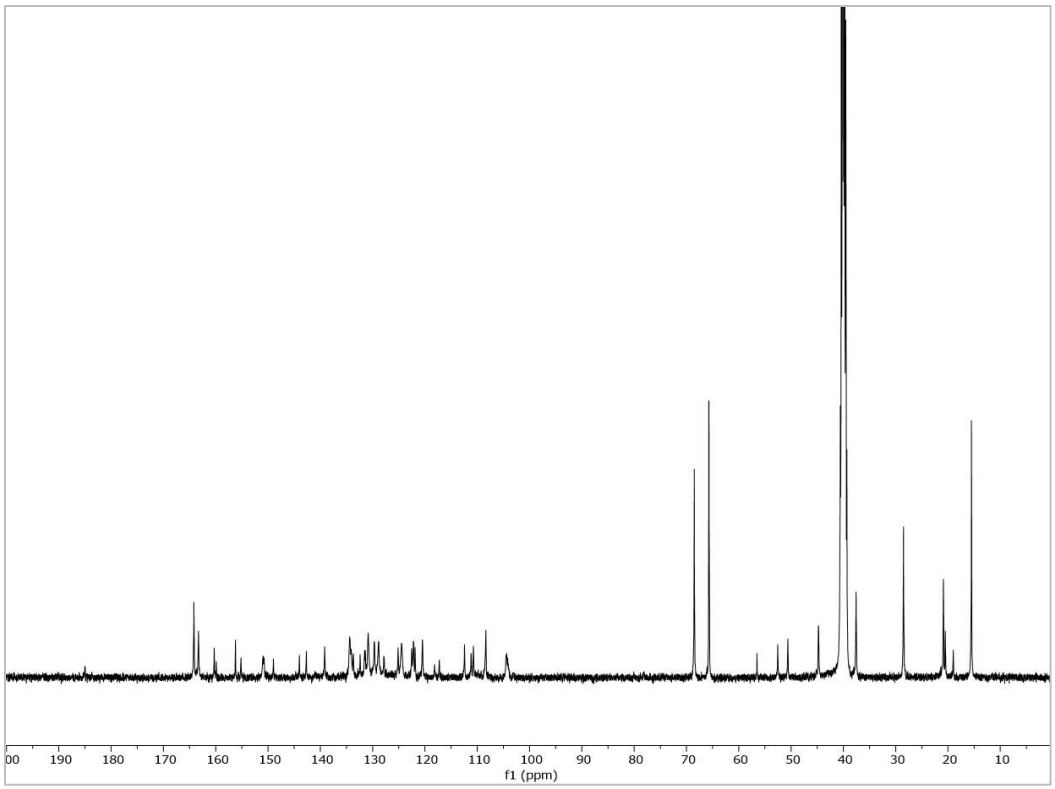


3f: %90, M.P: 209°C. 1H NMR (400 MHz, DMSO-d6) δ 1.01 – 1.06 (m, 3H, -CH3), 1.85 (q, J = 6.9 Hz, 2H, -CH2-), 3.38 (d, J = 2.3 Hz, 2H, -CH2-), 3.41 – 3.46 (m, 2H, -CH2-), 3.61 (d, J = 8.8 Hz, 1H, -CH2-), 3.68 – 3.96 (m, 1H, -CH-), 4.08 (d, J = 6.5 Hz, 2H, -CH2-), 4.12 – 4.53 (m, 1H, -CH-), 6.70 – 6.87 (m, 1H, ArHIS), 6.90 (t, J = 4.0 Hz, 1H, ArHIS), 6.91 – 7.19 (m, 1H, ArHIS), 7.57 – 7.76 (m, 1H, ArHNap), 7.78 – 7.97 (m, 1H, ArHNap), 8.09 – 8.32 (m, 2H, ArHNap), 8.36 – 8.44 (m, 1H, ArHNap), 8.54 – 8.71 (m, 1H, ArHNap), 10.74 – 12.05 (m, 1H, -NH-). 13C NMR (101 MHz, DMSO-d6) δ 15.33, 19.14, 28.74, 38.33, 56.43, 65.52, 68.69, 120.45, 121.92, 123.51, 124.16, 126.01, 128.25, 129.37, 130.45, 131.15, 132.23, 133.29, 134.54, 135.26, 142.83, 143.09, 150.59, 155.48, 157.22, 164.23, 165.02, 190.38.

Table S1.9 List of FTIR, ^{1}H-NMR (400 MHz, CDCl3-*d*) and ^{13}C-NMR (101 MHz, DMSO-d_6) Spectra of compound **3g**







3g: %84, M.P: 212°C. 1H NMR (400 MHz, DMSO-d6) δ 0.99 – 1.09 (m, 3H, -CH3), 1.84 (p, J = 6.4 Hz, 2H, -CH2-), 2.17 – 2.29 (m, 3H, -CH3), 3.38 (d, J = 7.0 Hz, 2H, -CH2-), 3.44 (t, J = 6.2 Hz, 2H, -CH2-), 3.84 (d, J = 6.2 Hz, 2H, -CH2-), 4.09 (d, J = 7.2 Hz, 2H, -CH2-), 4.29 – 4.37 (m, 1H, -CH-), 4.58 – 4.67 (t, J = 6.5 Hz, 1H, -CH-), 6.71 – 6.83 (m, 1H, ArHIS), 6.93 (d, J = 5.9 Hz, 1H, ArHIS), 7.17 – 7.23 (m, 1H, ArHIS), 7.68 (d, J = 2.8 Hz, 1H, ArHNap), 7.90 (d, J = 6.3 Hz, 1H, ArHNap), 8.20 – 8.36 (m, 1H, ArHNap), 8.42 (d, J = 1.2 Hz, 1H, ArHNap), 8.69 (d, J = 1.2 Hz, 1H, ArHNap), 10.65 – 10.88 (m, 1H, -NH-). 13C NMR (101 MHz, DMSO-d6) δ 15.45, 20.17, 28.85, 38.12, 44.24, 52.20, 65.69, 68.78, 119.61, 121.50, 123.00, 125.80, 127.39, 128.59, 129.89, 130.77, 132.09, 132.87, 134.64, 136.25, 138.44, 139.89, 142.27, 143.10, 148.95, 152.89, 162.53, 163.82, 164.77.

Table S2. It presents Annexin V–PI flow cytometry results, showing (a) FSC-A vs SSC-A for cell population gating and (b) FITC-A vs PE-A for distinguishing viable, early apoptotic, late apoptotic, and necrotic cells based on fluorescence for untreated MCF cell line (c) and (d) as same for untreated MDA-MB-231 cell line. Other treated samples are shown in panels with the same respective analyses e-i: **3a** on MCF-7 and MDA-MB-231, h-l: **3c** on MCF-7 and MDA-MB-231, m-p: **3g** on MCF-7 and MDA-MB-231 cell lines. See Table S2 and Table S3 for other compounds' results.

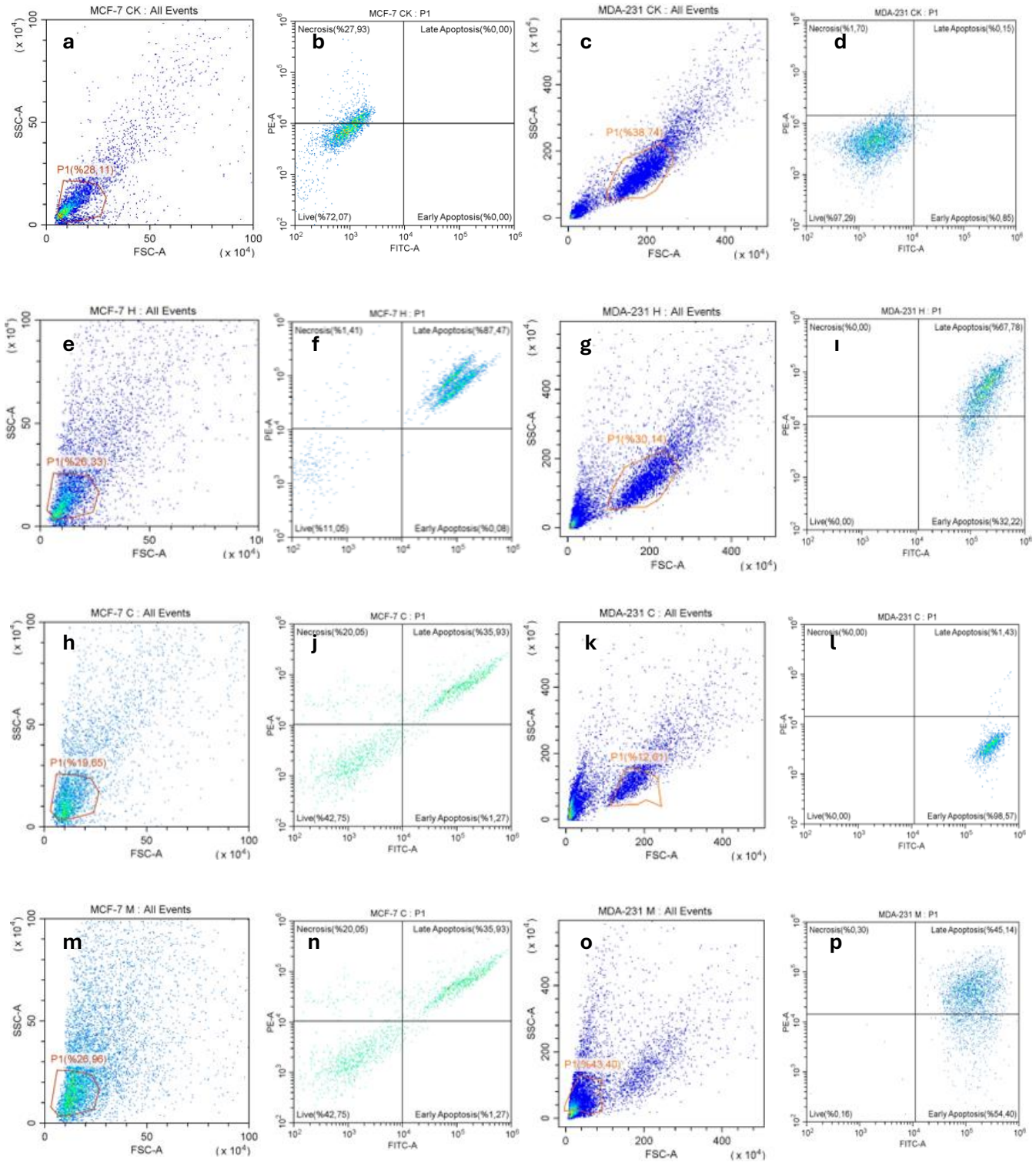


Table S3. Flowcytometry results: Annexin V / PI apoptosis necrosis analyses of compounds **3b**, **3d**, **3e**, **3f** on MCF-7 cell line

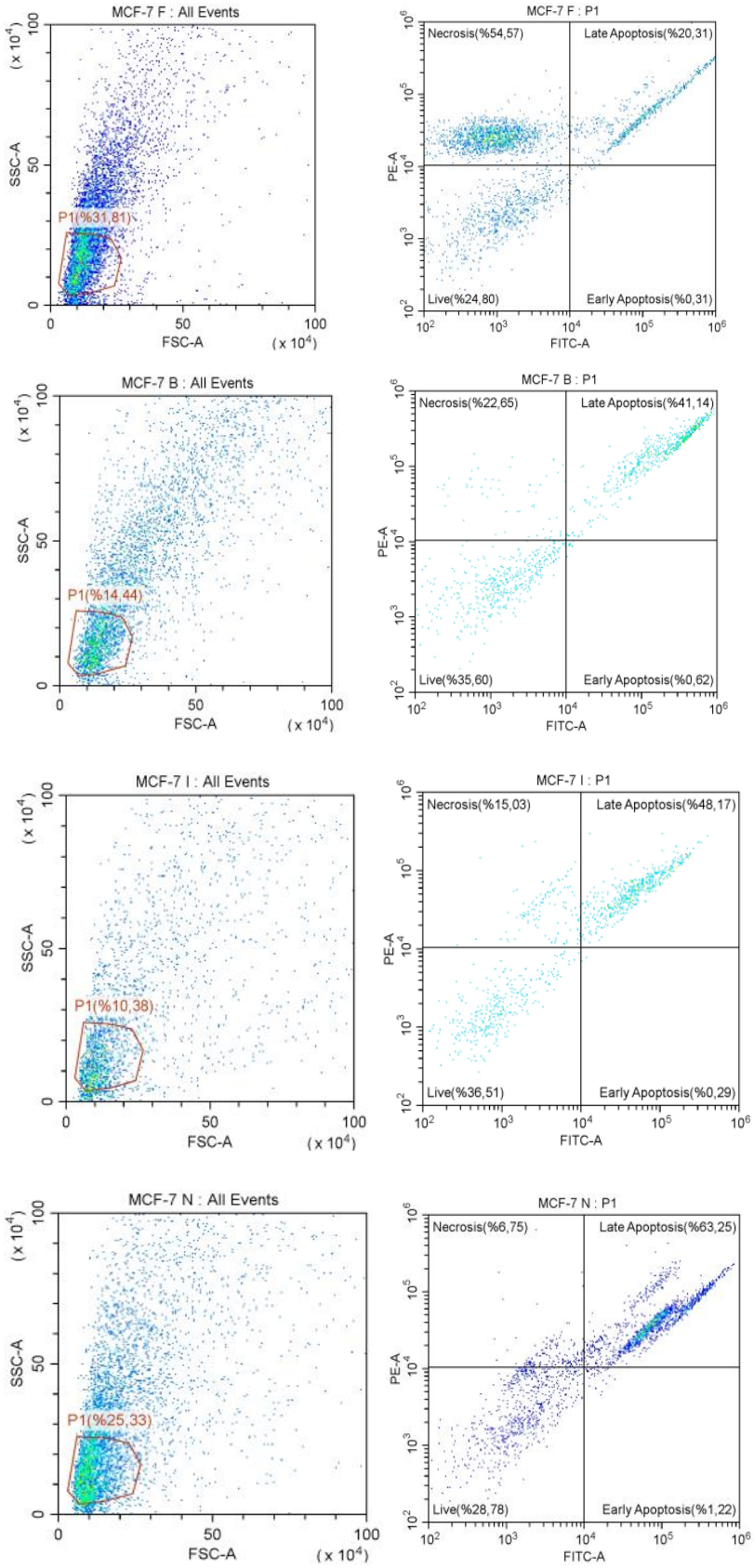


Table S4. Flowcytometry results: Annexin V / PI apoptosis necrosis analyses of compounds **3b**, **3d**, **3e**, **3f** on MDA-MB-231 cell line

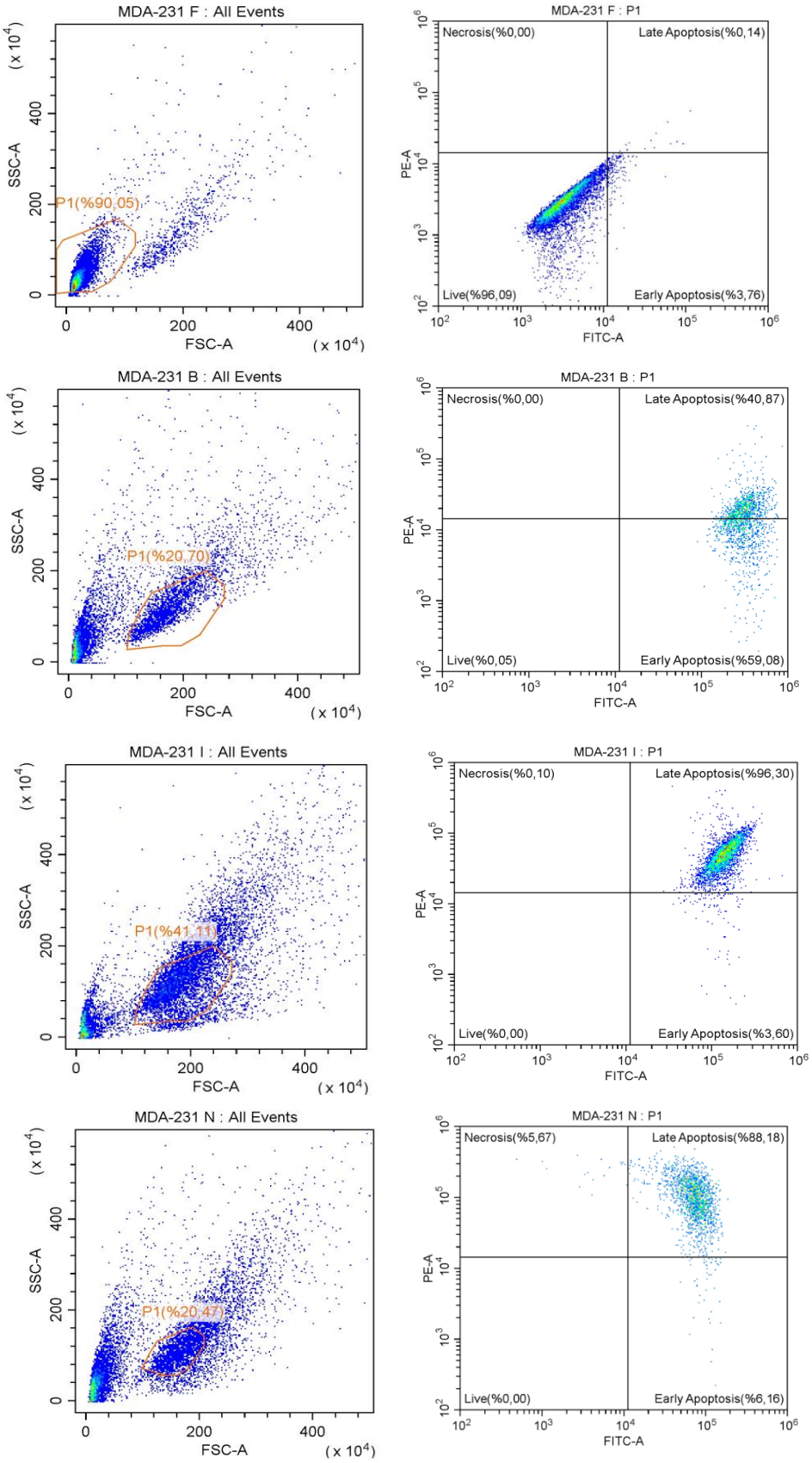
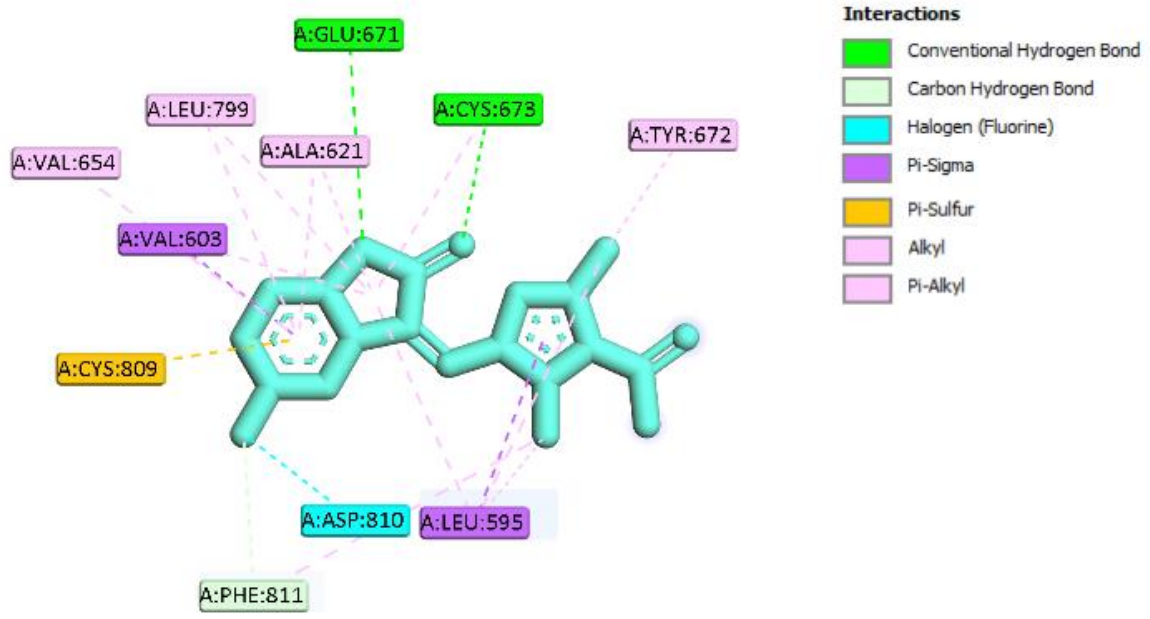


Table S5. Cytotoxicity result of compounds **3a-g** with IC_{50} values on HEK cell line.

	HEK-293 MDA-MB-231 IC_{50}	
	24th hour	**48th hour**
3a	87,12 ± 0,05	N/A
3b	92,37 ± 0,14	66,96 ± 0,10
3c	90,49 ± 0,05	67,43 ± 0,15
3d	91,17 ± 0,05	76,92 ± 0,66
3e	83,63 ± 0,06	66,39 ± 0,48
3f	90,23 ± 0,02	68,18 ± 0,34
3g	85,69 ± 0,04	73,25 ± 0,08

FigS1. Two-dimensional histogram of all ligand–protein bonds for sunitinib bound to VEGFR2 (PDB 3GEO)



FigS2. Two-dimensional histogram of all ligand–protein bonds for **3c** bound to VEGFR2 (PDB 3GEO)

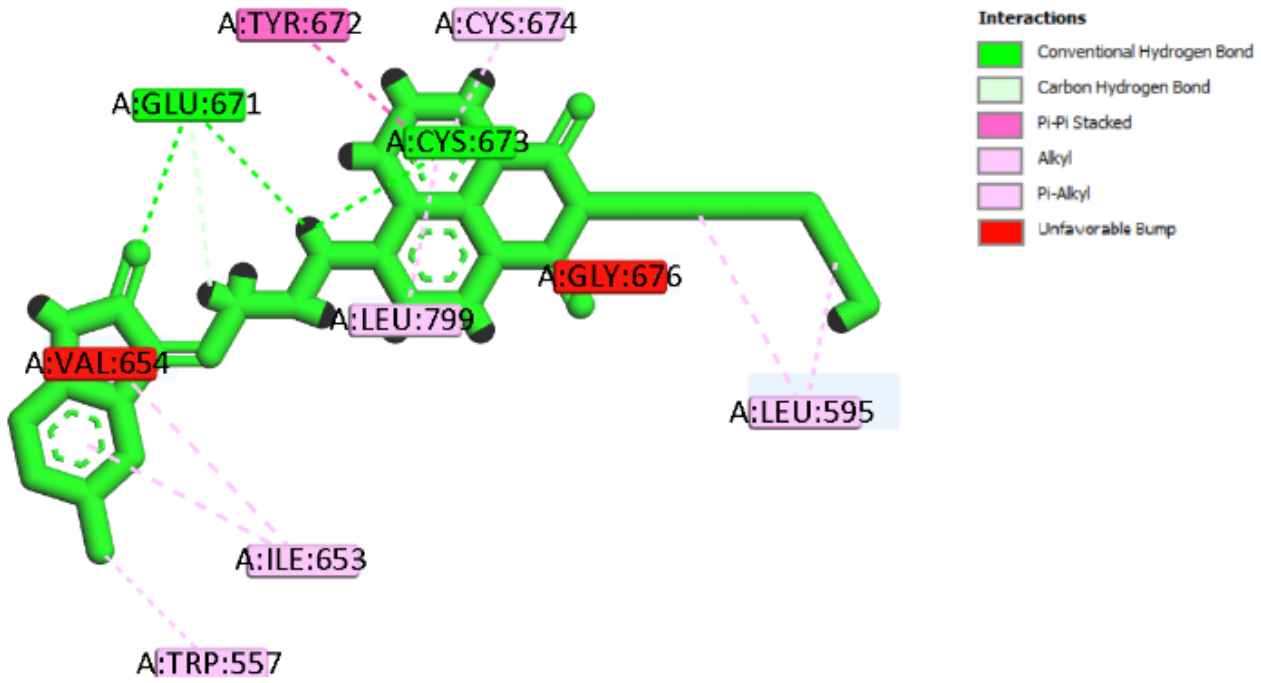


Table S6. The docking results. Mode indicates the rank of each predicted binding pose. Affinity is the predicted binding energy in kilocalories per mole (kcal/mol), with more negative values showing stronger binding. RMSD l.b. (root-mean-square deviation lower bound) and RMSD u.b. (upper bound) are both measured in angstroms (Å) and show how much each pose differs from the best pose; lower values mean more similar structures.