Electronic Supplementary Material (ESI) for New Journal of Chemistry.

A novel target and biomarker benzothiazolyl-naphthalimide probes for precisely and selective detection of serum albumin and anticancer activity

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1. Experimental details

1.1 Chemistry

Commercially grade chemicals and solvents were used for the synthesis and purchased from Sigma-Aldrich, TCI, and Spectrochem. Reactions were monitored by TLC (thin layer chromatography) using silica coated plate GF-254 and compounds were further purified by silica gel based column chromatography having mesh size of 60-120. Melting points were determined using micro melting apparatus and were uncorrected. ^{1}H NMR and ^{13}C NMR were recorded on Joel ECS-400MHz spectrometer, using $CDCl_3$ and DMSO-d_6 as solvents. TMS is used as internal reference. Chemical shifts and *J* values were expressed in delta (ppm) and Hertz, respectively. HSA and BSA studies were performed using Shimadzu UV- 2600 and Cary eclipse fluorescence spectrophotometer.

Synthesis of 5-bromo-1,2-dihydroacenaphthylene (2)

A solution of *N*-bromosuccinimide (11.2 g, 64.8 mmol) in DMF (15 mL) was added to a stirred solution of acenaphthene (10 g, 64.8 mmol) in DMF (15 mL) at room temperature. The reaction mixture was continuously stirred for 3 h at room temperature. After the completion of reaction, the solution was poured into 100 mL of cold water, resulted in separation of yellow colored precipitates. The solution was filtered and vacuum dried. The crude product was recrystallized in ethanol and gave pure product with 97% yield (m.pt. = 55 ^{o}C).

Synthesis of 6-bromo-1*H*,3*H*-benzo[*de*]isochromene-1,3-dione (3)

To a solution of 5-bromo-1,2-dihydroacenaphthylene (5 g, 21.4 mmol) in glacial acetic acid (100 mL), potassium dichromate (21 g, 71.38 mmol) was added slowly at 0 ^{o}C for 30 min. The reaction was conducted at 110 ^{o}C for 4 h. On the completion of reaction, the mixture was allowed to cool and 500 mL of cold water was added into it. White colored crude was obtained and the residual chromium salt was removed with boiling water. The resulting solid was recrystallized in acetic acid to give needle shaped crystals.

Synthesis of 2-(benzo[*d*]thiazolyl-2-yl-6-bromo-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione (4)

In a sealed tube, 6-bromo-1*H*,3*H*-benzo[*de*]isochromene-1,3-dione (**2**) (2 g, 7.2 mmol) and 2-amino benzothiazole (**3**) (1.09 g, 7.2 mmol) in the presence of zinc acetate (1.32 g, 7.2 mmol) and excess of pyridine were irradiated in microwave for 5 min. After completion, the resulting brown solution was poured into cold water; solid was separated out and filtered. The crude product was purified by column chromatography on silica gel with 1:20 ethylacetate: hexane as eluents. yellow solid; yield: 75%; mp: 248-250 ^{o}C; ^{1}H NMR (400 MHz, $CDCl_3$): δ(ppm) 8.76 (dd, ^{2}J = 7.32 Hz, ^{3}J = 0.92 Hz, 1H, ArH), 8.71 (dd, ^{2}J = 8.68 Hz, ^{3}J = 0.92 Hz, 1H, ArH), 8.51 (d, J = 7.80 Hz, 1H, ArH), 8.13 (t, J = 7.80 Hz, 2H, ArH), 7.98-7.90 (m, 2H, ArH), 7.59-7.49 (m, 2H, ArH); ^{13}C NMR (100 MHz, $CDCl_3$) 155.3 (C=O), 155.3

(C=O), 150.4, 136.1, 134.4, 132.9, 132.0, 131.6, 131.4, 131.0, 128.2, 126.4, 126.2, 124.2, 122.5, 122.5, 121.8, 121.6 (ArC).

Synthesis of (benzo[*d*]thiazolyl-2-yl-6-substituted aryl-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione (5-16)

2-(Benzo[*d*]thiazolyl-2-yl-6-bromo-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione (200 mg, 0.4 mmol) was reacted with various substituted aryl boronic acids (0.4 mmol) in the presence of potassium carbonate (101 mg, 0.73 mmol) and $Pd(PPh_3)_4$ (5 mol %) in acetonitrile under inert atmosphere. The reaction was conducted under reflux condition for 24 h. Then, the solvent was vacuum distilled and the product was extracted with chloroform and water. The organic layer was collected and dried over Na_2SO_4. The product was isolated through column chromatography using hexane and ethyl acetate as eluents.

2-(Benzo[*d*]thiazol-2-yl)-6-phenyl-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione (5):

yellow solid; yield: 75%; mp: 240-246 ^oC; ^1H NMR (400 MHz, $CDCl_3$): δ(ppm) 8.74-8.70 (m, 2H, ArH), 8.39 (dd, ^2J = 8.68 Hz, ^3J = 0.92 Hz, 1H, ArH), 8.17 (d, J = 8.24Hz, 1H, ArH), 7.98 (dd, ^2J = 7.32 Hz, ^3J = 0.92 Hz, 1H, ArH), 7.78-7.74 (m, 2H, ArH), 7.59-7.46 (m, 6H, ArH); ^{13}C NMR (100 MHz, $CDCl_3$): δ(ppm) 159.9 (C=O), 159.9 (C=O), 134.8, 134.3, 134.2, 133.3, 132.1, 132.0, 131.9, 131.7, 131.0, 129.7, 128.6, 128.5, 128.4, 128.3, 127.9, 119.3, 118.3 (ArC); MS (EI) : m/z 407.1 (M^++1). Anal. Calc. for $C_{25}H_{14}N_2O_2S$: C, 73.88; H, 3.47; N, 6.89; S, 7.89; Found: C, 73.69; H, 3.61; N, 7.10; S, 7.76.

2-(Benzo[*d*]thiazol-2-yl)-6-(4-ethylphenyl)-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione (6):

yellow solid; yield: 60%; mp: 260-265 ^oC; ^1H NMR (400 MHz, $CDCl_3$): δ(ppm) 8.73-8.70 (m, 2H, ArH), 8.43 (dd, ^2J = 8.68 Hz, ^3J = 0.92 Hz, 1H, ArH), 8.17 (d, J = 8.24 Hz, 1H, ArH), 7.99 (dd, ^2J = 8.24 Hz, ^3J = 0.92 Hz, 1H, ArH), 7.78-7.74 (m, 2H, ArH), 7.59-7.40 (m, 6H, ArH), 2.82 (q, J = 7.8 Hz, 2H, CH_2), 1.37 (t, J = 7.8 Hz, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ(ppm) 163.88 (C=O), 163.66 (C=O), 155.8, 150.5, 148.3, 145.0, 136.8, 135.8, 134.0, 132.0, 131.7, 130.4, 129.9, 128.2, 128.0, 126.9, 126.3, 126.1, 124.2, 122.2, 121.3, 120.8 (ArC), 28.7 (CH_2-ethyl), 15.5 (CH_3-ethyl); MS (EI) : m/z 435.1 (M^++1); Anal. Calc. for $C_{27}H_{18}N_2O_2S$: C, 74.63; H, 4.18; N, 6.45; S, 7.38; Found: C, 74.39; H, 4.44; N, 7.52; S, 7.17.

2-(Benzo[*d*]thiazol-2-yl)-6-(*m*-tolyl)-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione (7):

yellow solid; yield: 80%; mp: 248-252 ^oC; ^1H NMR (400 MHz, $CDCl_3$): δ(ppm) 8.73-8.70 (m, 2H, ArH), 8.40 (dd, ^2J = 8.24 Hz, ^3J = 1.36 Hz, 1H, ArH), 8.17 (dd, ^2J = 8.24 Hz, ^3J = 1.36 Hz, 1H, ArH), 7.98 (dd, ^2J = 8.28 Hz, ^3J = 1.34 Hz, 1H, ArH), 7.78-7.74 (m, 2H, ArH), 7.59-7.45 (m, 3H, ArH), 7.36-7.33 (m, 3H, ArH), 2.4 (s, 3H, meth-CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ(ppm) 163.8 (C=O), 163.6 (C=O), 155.8,

150.8, 148.3, 138.5, 138.5, 136.8, 133.9, 132.0, 131.8, 130.5, 129.4, 129.2, 128.6, 128.0, 127.0, 126.9, 126.3, 126.1, 124.3, 122.2, 121.8, 121.0 (ArC), 21.5 (CH_3-methyl); MS (EI) : m/z 421.1 (M^++1)); Anal. Calc. for $C_{26}H_{16}N_2O_2S$: C, 74.27; H, 3.84; N, 6.66; S, 7.62; Found: C, 74.26; H, 3.69; N, 6.88; S, 7.81.

2-(Benzo[*d*]thiazol-2-yl)-6-(4-methoxyphenyl)-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione (8):

yellow solid; yield: 70%; mp: 260-265 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ(ppm) 8.72 (d, *J* = 7.32, 2H, ArH), 8.43 (dd, ^2J = 8.72 Hz, ^3J = 0.92 Hz 1H, ArH), 8.17 (d, *J* = 7.8 Hz, 1H, ArH), 7.98 (d, *J* = 8.24 Hz, 1H, ArH), 7.78-7.73 (m, 2H, ArH), 7.59-7.48 (m, 4H, ArH), 7.12 (d, *J* = 8.72, 2H, ArH), 3.9 (s, 3H, OCH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ(ppm) 168.6 (C=O), 163.6 (C=O), 160.1, 147.9, 133.9, 132.0 131.7, 131.1, 130.7, 130.4, 127.9, 126.3, 126.12, 124.2, 122.1, 121.8, 114.0 (ArC), 55.2 (OCH_3); MS (EI) : m/z 437.1 (M^++1); Anal. Calc. for $C_{26}H_{16}N_2O_3S$: C, 71.55; H, 3.69; N, 6.42; S, 7.35; Found: C, 71.44; H, 3.91; N, 6.17; S, 7.52.

2-(Benzo[*d*]thiazol-2-yl)-6-(2-methoxyphenyl)-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione (9):

yellow solid; yield: 60%; mp: 255-260 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ(ppm) 8.73 (d, *J* = 7.36 Hz, 1H, ArH), 8.69 (dd, ^2J = 7.32 Hz, ^3J = 0.92 Hz, 1H, ArH), 8.17 (d, *J* = 7.80 Hz, 1H, ArH), 8.07 (dd, ^2J = 7.68 Hz, ^3J = 1.36 Hz, 1H, ArH), 7.97 (dd, ^2J = 7.76 Hz, ^3J = 0.92 Hz, 1H, ArH), 7.74-7.68 (m, 2H, ArH), 7.58-7.48 (m, 3H, ArH), 7.34 (dd, ^2J = 7.32 Hz, ^3J = 1.84 Hz, 1H, ArH), 7.17-7.09 (m, 2H, ArH), 3.72 (s, 3H, OCH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ(ppm) 164.1 (C=O), 163.6 (C=O), 156.7, 155.8, 150.70, 145.0, 136.8, 134.2, 131.9, 131.6, 131.4, 130.9, 130.6, 130.3, 128.7, 128.6, 127.2, 126.6, 126.3, 126.0, 124.2, 122.0, 121.8, 121.0, 120.8 (ArC); MS (EI) : m/z 437.1 (M^++1); Anal. Calc. for $C_{26}H_{16}N_2O_3S$: C, 71.55; H, 3.69; N, 6.42; S, 7.35; Found: C, 71.32; H, 3.85; N, 6.68; S, 7.49.

2-(Benzo[*d*]thiazol-2-yl)-6-(4-flourophenyl)-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione (10):

yellow solid; yield: 65%; mp: 265-270 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ(ppm) 8.73 (d, *J* = 7.36 Hz, 2H, ArH), 8.34 (dd, ^2J = 8.72 Hz, ^3J = 0.92 Hz, 1H, ArH), 8.17 (d, *J* = 8.24 Hz, 1H, ArH), 7.98 (d, *J* =7.36 Hz, 1H, ArH), 7.80-7.73 (m, 2H, ArH), 7.59-7.49 (m, 4H, ArH), 7.30-7.28 (m, 2H, ArH); ^{13}C NMR (100 MHz, $CDCl_3$): δ(ppm) 163.8 (C=O), 163.4 (C=O), 155.9, 150.8, 146.9, 136.8, 133.5, 132.1, 131.6, 131.5, 128.1, 127.2, 126.4, 126.1, 124.3, 121.8, 121.2, 116.0, 115.8 (ArC); MS (EI) : m/z 425.1 (M^++1); Anal. Calc. for $C_{25}H_{13}FN_2O_2S$: C, 70.74; H, 3.09; N, 6.60; S, 7.55; Found: C, 70.39; H, 3.28; N, 6.38; S, 7.44.

2-(Benzo[*d*]thiazol-2-yl)-6-(4-chlorophenyl)-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione (11):

yellow solid; yield: 55%; mp: 260-263 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ(ppm) 7.81 (d, *J* = 7.80 Hz, 1H, ArH), 7.72 (d, *J* = 8.28 Hz, 1H, ArH), 7.56 (d, *J* = 7.80 Hz, 2H, ArH), 7.51 (d, *J* = 7.80 Hz, 2H, ArH), 7.42 (t, *J* = 7.32 Hz, 1H, ArH), 7.31-7.24 (m, 4H, ArH), 7.11 (t, *J* = 7.32 Hz, 2H, ArH); ^{13}C NMR (100 MHz, $CDCl_3$): δ(ppm) 163.7 (C=O), 163.5 (C=O), 155.7, 155.3, 150.4, 146.8, 136.7, 134.4, 134.4, 133.5, 132.1, 131.6, 130.4, 129.1, 128.1, 127.0, 126.3, 126.1, 126.1, 124.2, 122.2, 121.8, 121.1, 115.9, 115.7 (ArC); MS (EI) : m/z 441.05 (M^++1); Anal. Calc. for $C_{25}H_{13}ClN_2O_2S$: C, 68.10; H, 2.97; N, 6.35; S, 7.27; Found: C, 67.95; H, 3.33; N, 6.12; S, 7.48.

2-(Benzo[*d*]thiazol-2-yl)-6-(2-flourophenyl)-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione (12):

yellow solid; yield: 70%; mp: 268-273 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ(ppm) 8.76-8.66 (m, 2H, ArH), 8.15-8.11 (m, 2H, ArH), 7.96 (dd, ^2J = 8.24Hz, ^3J = 0.92 Hz, 1H, ArH), 7.78-7.73 (m, 2H, ArH), 7.51-7.42 (m, 4H, ArH), 7.36-7.24 (m, 2H, ArH); ^{13}C NMR (100 MHz, $CDCl_3$): δ(ppm) 163.7 (C=O), 163.5 (C=O), 160.6, 158.6, 155.7, 150.5, 141.8, 133.5, 132.1,131.9, 131.5, 128.9, 127.2, 126.3, 126.1, 124.3, 121.8, 116.2, 116.1 (ArC); MS (EI) : m/z 425.1 (M^++1); Anal. Calc. for $C_{25}H_{13}FN_2O_2S$: C, 70.74; H, 3.09; N, 6.60; S, 7.55; Found: C, 70.42; H, 3.27; N, 6.36; S, 7.76.

6-(4-Acetylphenyl)-2-(benzo[*d*]thiazol-2-yl)-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione (13):

yellow solid; yield: 45%; mp: 275-280 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ(ppm) 8.75-8.72 (m, 2H, ArH), 8.31 (dd, ^2J = 8.24Hz, ^3J = 0.92 Hz, 1H, ArH), 8.18-8.15 (m, 3H, ArH), 7.98 (dd, ^2J = 7.80 Hz, ^3J = 1.36 Hz, 1H, ArH), 7.81-7.77 (m, 2H, ArH), 7.67 (d, *J* = 8.24, 2H, ArH), 7.60-7.49 (m, 2H, ArH), 2.72 (s, 3H, acetyl-CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ(ppm) 197.5 (C=O), 163.6 (C=O), 163.4 (C=O), 155.6, 150.4, 146.6, 143.1, 137.0, 136.7, 133.2, 131.5, 130.1, 130.0, 129.1, 128.7, 128.0, 127.3, 126.4, 126.1, 124.2, 122.3, 121.8, 121.6 (ArC), 26.8 (CH_3); MS (EI): m/z 449.1 (M^++1). Anal. Calc. for $C_{27}H_{16}N_2O_3S$: C, 72.31; H, 3.60; N, 6.25; S, 7.15; Found: C, 74.78; H, 5.77; N, 9.36; S, 7.10.

2-(Benzo[*d*]thiazol-2-yl)-6-(naphthalen-1-yl)-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione (14):

yellow solid; yield: 60%; mp: 268-272 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ(ppm) 8.79 (d, *J* = 7.44 Hz, 1H, ArH), 8.69 (d, *J* = 7.20 Hz, 1H, ArH), 8.17 (d, *J* = 8.08 Hz, 1H, ArH), 8.04-7.95 (m, 3H, ArH), 7.88 (d, *J* = 8.40 Hz, 1H, ArH), 7.84 (d, *J* = 7.44 Hz, 1H, ArH),7.66-7.48 (m, 6H, ArH), 7.38-7.24 (m, 2H, ArH); ^{13}C NMR (100 MHz, $CDCl_3$): δ(ppm) 163.8 (C=O), 163.6 (C=O), 156.0, 150.4, 146.5, 136.7, 136.0, 134.1, 133.5, 132.2, 132.1, 131.6, 131.6, 129.1, 129.1, 128.5, 127.8, 127.0, 126.8, 126.4, 126.3, 126.1,

125.7, 125.3, 124.3, 122.2, 121.8, 121.6; MS (EI) : m/z 457.10 (M^++1); Anal. Calc. for $C_{29}H_{16}N_2O_2S$: C, 76.30; H, 3.53; N, 6.14; S, 7.02; Found: C, 76.57; H, 3.67; N, 6.35; S, 6.90.

2-(Benzo[*d*]thiazol-2-yl)-6-(thiophen-3-yl)-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione (15):

yellow solid; yield: 45%; mp: 258-260 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ(ppm) 8.76-8.72 (m, 2H, ArH), 8.70 (dd, *J* = 7.36 Hz, 1H, ArH), 8.17 (dd, ^2J = 8.24 Hz, ^3J = 1.36 Hz, 1H, ArH), 7.98 (dd, ^2J = 7.32 Hz, ^3J = 0.92 Hz, 1H, ArH), 7.90 (d, *J* = 7.80Hz, 1H,ArH), 7.85-7.81 (m, 1H, ArH), 7.61-7.49 (m, 3H, ArH), 7.44-7.40 (m, 1H, ArH), 7.30-7.28 (m, 1H, ArH); ^{13}C NMR (100 MHz, $CDCl_3$): δ(ppm) 163.7 (C=O), 163.4 (C=O), 155.5, 150.4, 140.3, 139.3, 136.8, 133.5, 132.2, 131.5, 130.2, 129.2, 128.7, 128.0, 127.3, 126.4, 126.1, 124.1, 122.2, 121.9, 121.1 (ArC); MS (EI) : m/z 413.1 (M^++1); Anal. Calc. for $C_{23}H_{12}N_2O_2S_2$: C, 66.97; H, 2.93; N, 6.79; S, 15.54; Found: C, 67.30; H, 2.75; N, 6.98; S, 15.37.

2-(Benzo[*d*]thiazol-2-yl)-6-(3-(triflouromethyl)phenyl)-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione (16):

yellow solid; yield: 55%; mp: 255-258 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ(ppm) 8.76-8.73 (m, 2H, ArH), 8.27 (dd, ^2J = 8.24Hz, ^3J = 1.36 Hz, 1H, ArH), 8.17 (dd, ^2J = 7.32 Hz, ^3J = 0.92 Hz, 1H, ArH), 7.98 (dd, ^2J = 7.32 Hz, ^3J = 0.92 Hz, 1H, ArH), 7.83-7.72 (m, 6H, ArH), 7.60-7.50 (m, 2H, ArH); ^{13}C NMR (100 MHz, $CDCl_3$): δ(ppm) 163.6 (C=O), 163.4 (C=O), 155.5, 150.4, 146.0, 139.2, 136.7, 133.0, 132.2, 131.5, 130.1, 129.3, 129.1, 128.2, 127.5, 126.5, 126.4, 126.1, 124.2, 122.4, 122.3, 121.8, 121.7 (ArC); MS (EI) : m/z 475.1 (M^++1); Anal. Calc. for $C_{26}H_{13}F_3N_2O_2S$: C, 65.82; H, 2.76; N, 5.90; S, 6.76; Found: C, 65.98; H, 2.51; N, 6.19; S, 6.59.

Synthesis of (benzo[*d*]thiazolyl-2-yl-6- substituted amine -1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione (17-18)

2-(Benzo[*d*]thiazolyl-2-yl-6-bromo-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione (200 mg, 0.4 mmol) and amine (0.6 mmol) were refluxed in the presence of $PdCl_2(PPh_3)_2$ (5 mol %) and K_2CO_3 (101 mg, 0.73 mmol) in CH_3CN (5 ml) for 12 h. On completion of the reaction, CH_3CN was vacuum distilled. The product was extracted with ethyl acetate. The extract was then dried over anhydrous sodium sulfate, filtered and concentrated to get the crude product. The residue was purified using column chromatography on silica gel to obtain the desired product.

2-(Benzo[*d*]thiazol-2-yl)-6-piperdin-1-yl-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione (17):

yellow solid; yield: 55%; mp: 290-294 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ(ppm) 8.65 (dd, , ^2J = 7.32 Hz, ^3J = 1.40 Hz 1H, ArH), 8.57 (d, *J* = 8.24Hz, 1H, ArH), 8.48 (dd, ^2J = 8.24 Hz, ^3J = 1.40 Hz, 1H, ArH), 8.15 (dd, , ^2J = 7.80 Hz, ^3J = 1.84 Hz, 1H, ArH), 7.95 (dd, ^2J = 7.32 Hz, ^3J = 0.92 Hz, 1H, ArH), 7.74-

7.70 (m, 1H, ArH), 7.57-7.46 (m, 2H, ArH), 7.22 (d, J = 8.28, 1H, ArH), 3.31 (t, J = 5.04, 4H, pip-CH_2), 1.94-1.92 (m, 4H, pip-CH_2), 1.78-1.72 (m, 2H, pip- CH_2); ^{13}C NMR (100 MHz, $CDCl_3$): δ(ppm) 164.1(C=O), 163.9 (C=O), 158.4, 150.5, 155.5, 136.7, 133.5, 131.9, 131.8, 126.2, 125.9, 125.4, 124.1, 121.7, 114.8 (ArC), 54.5 (pip-NCH_2), 26.1 (pip-CH_2), 24.2 (pip-CH_2); MS (EI) : m/z 414.1 (M^++1); Anal. Calc. for $C_{24}H_{19}N_3O_2S$: C, 69.71; H, 4.63; N, 10.16; S, 7.75; Found: C, 69.88; H, 4.38; N, 10.01; S, 7.95.

2-(Benzo[*d*]thiazol-2-yl)-6-morpholino-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione (18):

yellow solid; yield: 60%; mp: 285-290 ^{o}C; ^{1}H NMR (400 MHz, $CDCl_3$): δ(ppm) 8.76 (dd, ^2J = 7.36 Hz, ^3J = 0.92 Hz 1H, ArH), 8.60 (d, J = 7.76 Hz, 1H, ArH), 8.52 (dd, ^2J = 8.72 Hz, ^3J = 0.92 Hz 1H, ArH), 8.15 (d, J = 8.24 Hz, 1H, ArH), 7.96 (dd, ^2J = 8.72 Hz, ^3J = 1.36 Hz, 1H, ArH), 7.78-7.74 (m, 1H, ArH), 7.57-7.47 (m, 2H, ArH), 7.29 (d, J = 7.8, 1H, ArH), 4.06 (t, J = 4.06 Hz, 4H, mor-CH_2), 3.34 (t, J = 4.60 Hz, 4H, mor-CH_2); ^{13}C NMR (100 MHz, DMSO-d_6): δ(ppm) 168.7 (C=O), 168.1 (C=O), 162.1, 161.5, 155.2, 141.8, 137.9, 136.8, 135.3, 131.6, 131.4, 131.3, 131.2, 128.5, 128.0, 127.9, 127.6, 120.4, 120.4 (Ar-C), 71.3 (CH_2-O morph), 58.2 (CH_2-N morph); MS (EI) : m/z 416.13 (M^++1). Anal. Calc. for $C_{23}H_{17}N_3O_3S$: C, 66.49; H, 4.12; N, 10.11; S, 7.72; Found: C, 66.64; H, 4.27; N, 9.96; S, 7.78.

1.2 Materials and Methods

1.2.1. Procedure for *in vitro* evaluation of cytotoxicity

The human cancerous and normal cell line screening panel is grown in RPMI 1640 medium containing 5% fetal bovine serum and 2 mM L-glutamine. Cells are inoculated into 96 well microtiter plates in 100 ml at plating densities ranging from 5,000 to 40,000 cells/well depending on the doubling time of individual cell lines. The microtiter plates are then incubated at 37 ˚C, 5% CO_2, 95% air and 100% relative humidity for 24 h.

After 24 h, two plates of each cell line are fixed in situ with TCA, to represent a measurement of the cell population for each cell line. Experimental drugs are solubilized in DMSO at 400-fold the desired final maximum test concentration and stored frozen prior to use. At the time of drug addition, an aliquot of frozen concentrate is thawed and diluted to twice the desired final maximum test concentration with complete medium containing 50 µg/ml gentamicin. Additional four, 10-fold or ½ log serial dilutions are made to provide a total of five drug concentrations plus control. Aliquots of 100 µL of these different drug dilutions are added to the appropriate microtiter wells, resulting in the required final drug concentrations. Following drug addition, the plates are incubated for an additional 48 h at 37 ˚C, 5% CO_2, 95% air, and 100% relative humidity. For adherent cells, the assay is terminated by the addition of cold TCA. Cells are fixed in situ by the gentle addition of 50 µL of cold 50% (w/v) TCA and incubated for 60

min at 4 ˚C. The supernatant is discarded, and the plates are washed five times with tap water and air dried. Sulforhodamine B (SRB) solution (100 μL) at 0.4% (w/v) in 1% acetic acid is added to each well, and plates are incubated for 10 min at room temperature. After staining, unbound dye is removed by washing five times with 1% acetic acid and the plates are air dried and then subsequently solubilized with 10 mM trizma base, and the absorbance is read on an automated plate reader at a wavelength of 515 nm. Using the seven absorbance measurements [time zero (T_z), control growth (C), and test growth in the presence of drug at the five concentration levels (T_i)], the percentage growth is calculated at each of the drug concentration levels. Percentage growth inhibition is calculated as:

[(Ti -Tz)/(C - Tz)] × 100 for concentrations for which $T_i \geq Tz$; [(Ti -Tz)/Tz] × 100 for concentrations for which Ti < Tz.

2. NMR data

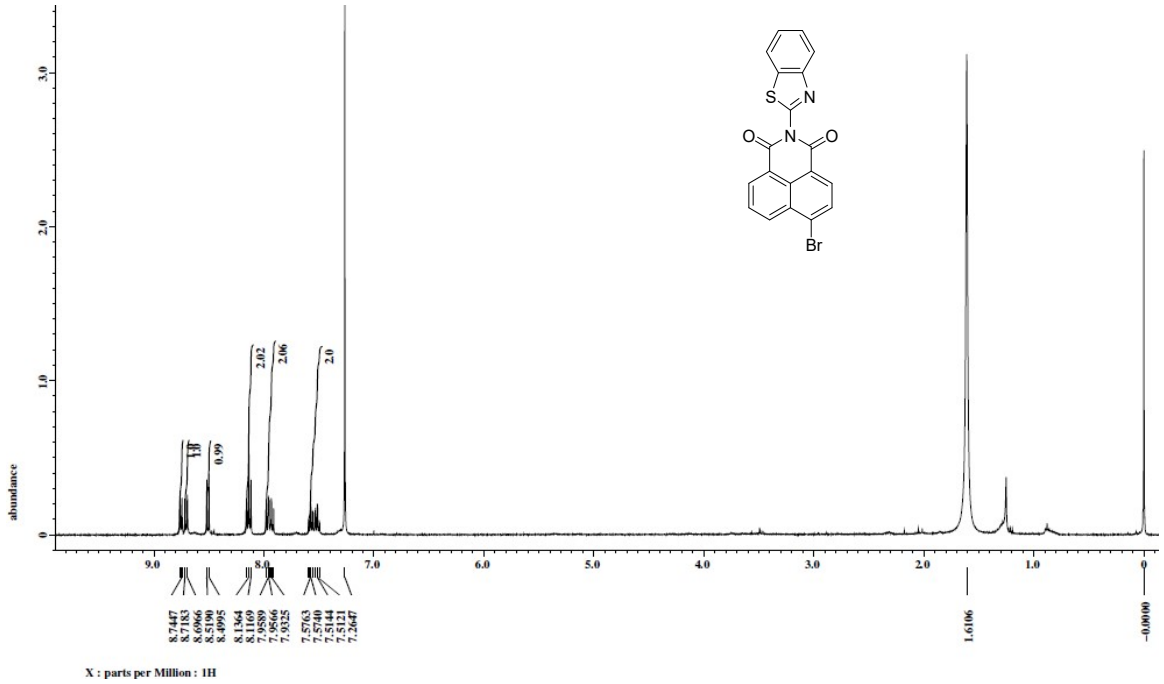


Figure S1: ^{1}H NMR spectrum of 2-(benzo[*d*]thiazolyl-2-yl-6-bromo-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione (**4**)

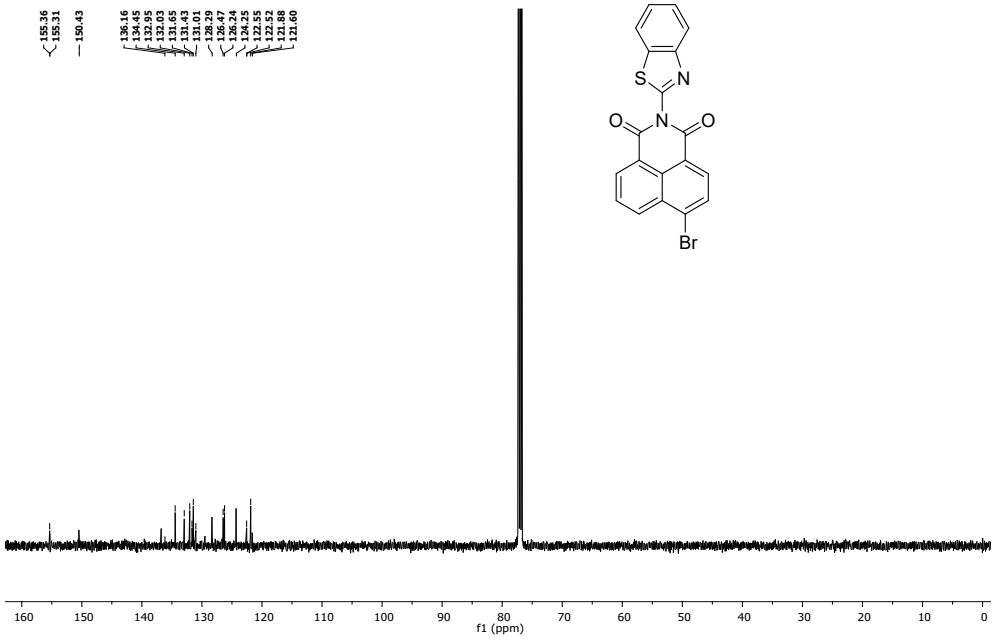


Figure S2: ^{13}C NMR spectrum of 2-(benzo[*d*]thiazolyl-2-yl-6-bromo-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione (**4**)

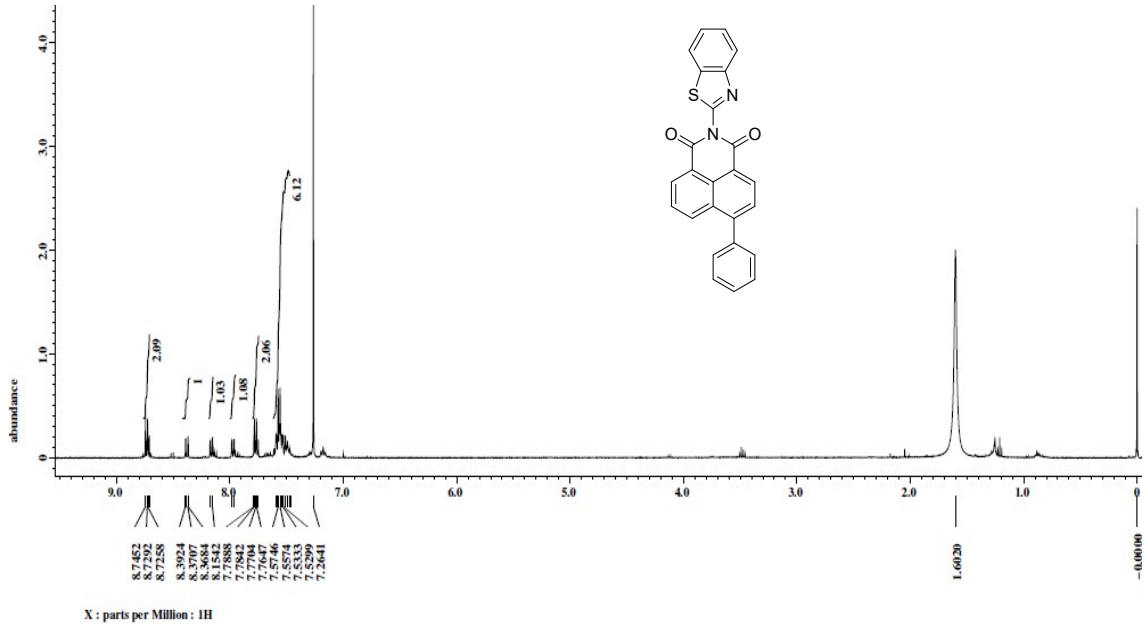


Figure S3: ^{1}H NMR spectrum of 2-(benzo[*d*]thiazol-2-yl)-6-phenyl-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione (**5**)

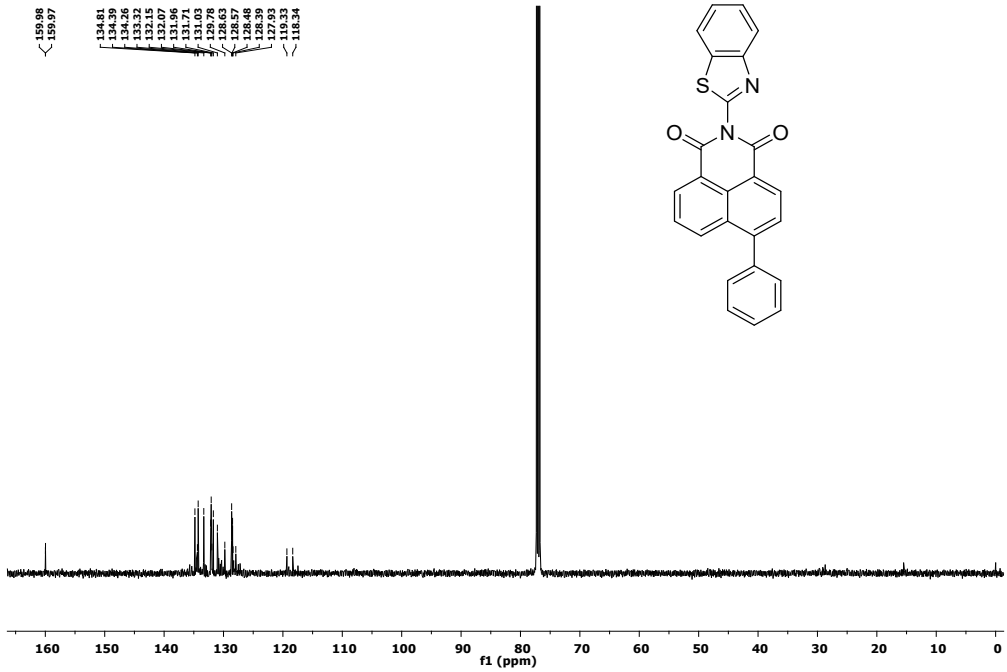


Figure S4: ^{13}C NMR spectrum of 2-(benzo[*d*]thiazol-2-yl)-6-phenyl-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione (**5**)

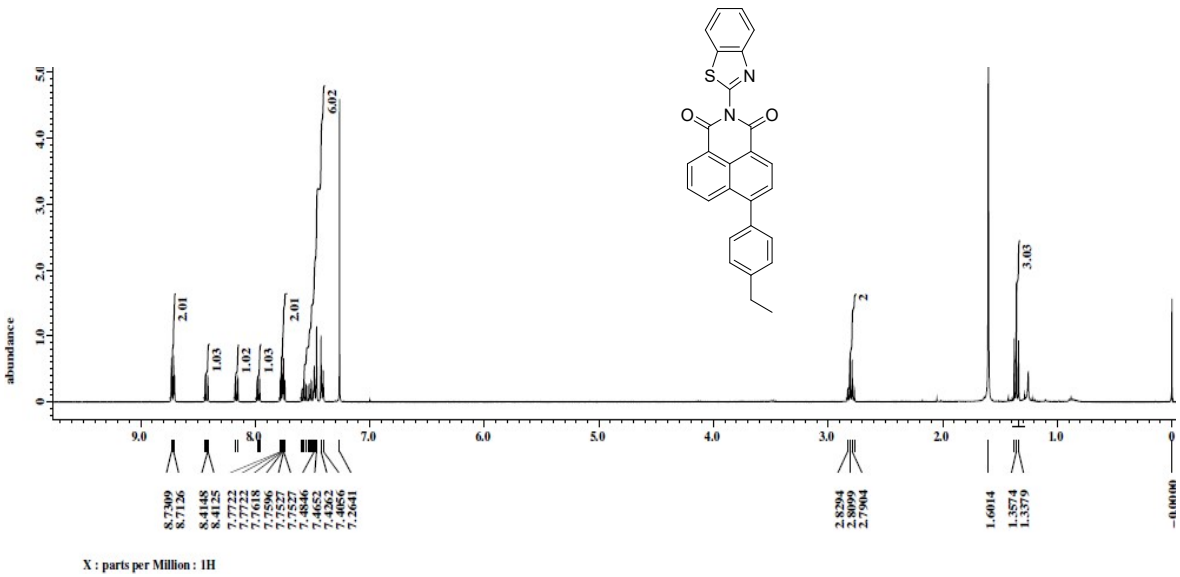


Figure S5: ^{1}H NMR spectrum of 2-(benzo[*d*]thiazol-2-yl)-6-(4-ethylphenyl)-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione (**6**)

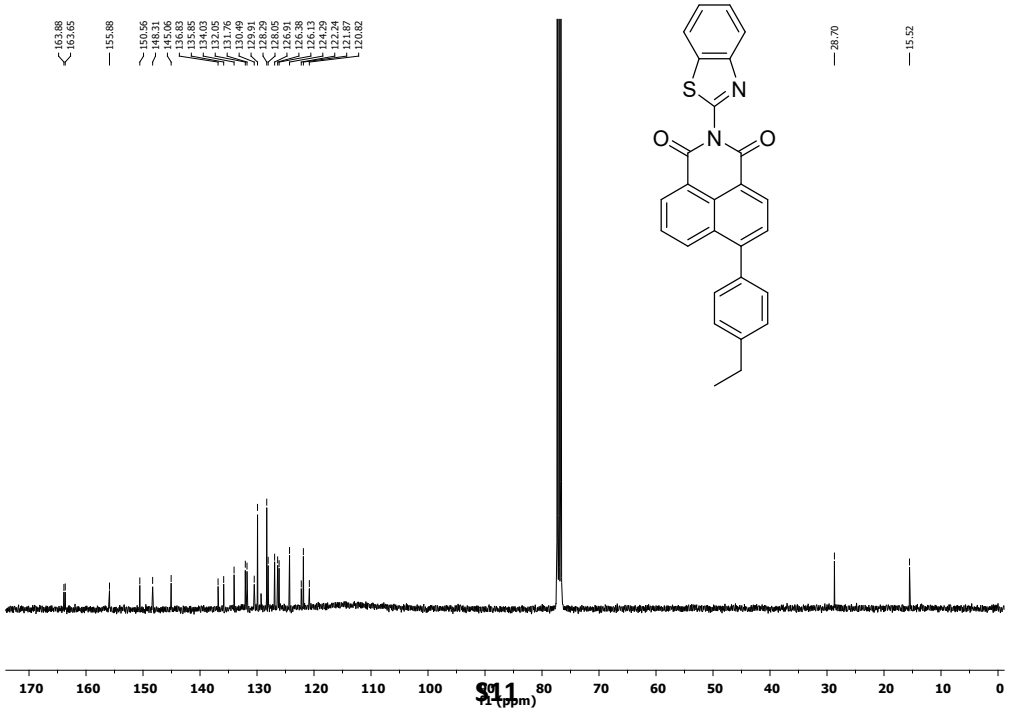


Figure S6:

^{13}C NMR spectrum of 2-(benzo[*d*]thiazol-2-yl)-6-(4-ethylphenyl)-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione (**6**)

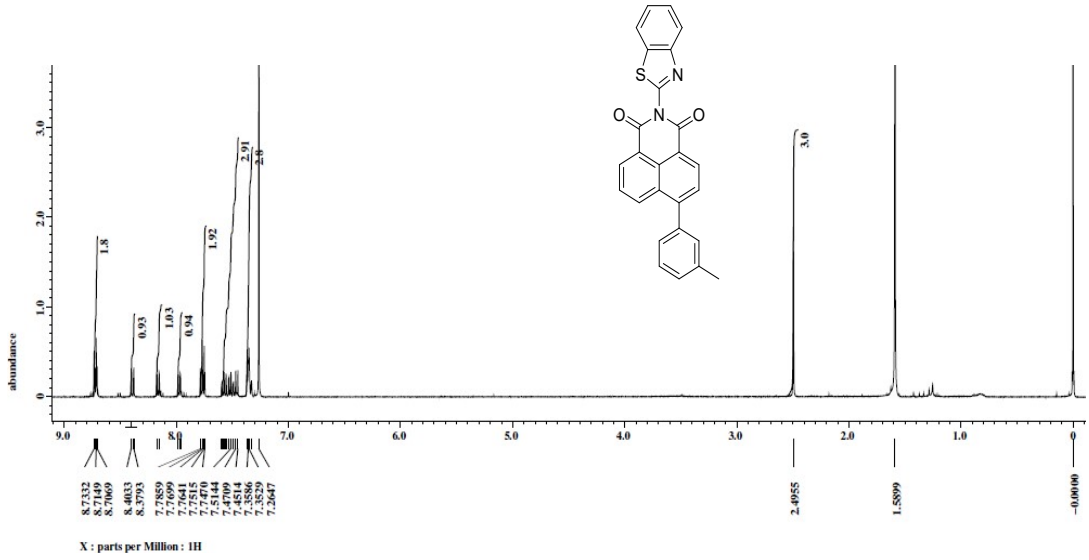


Figure S7: ^{1}H NMR spectrum of 2-(benzo[*d*]thiazol-2-yl)-6-(*m*-tolyl)-1*H*benzo[*de*]isoquinoline-1,3(2*H*)-dione (**7**)

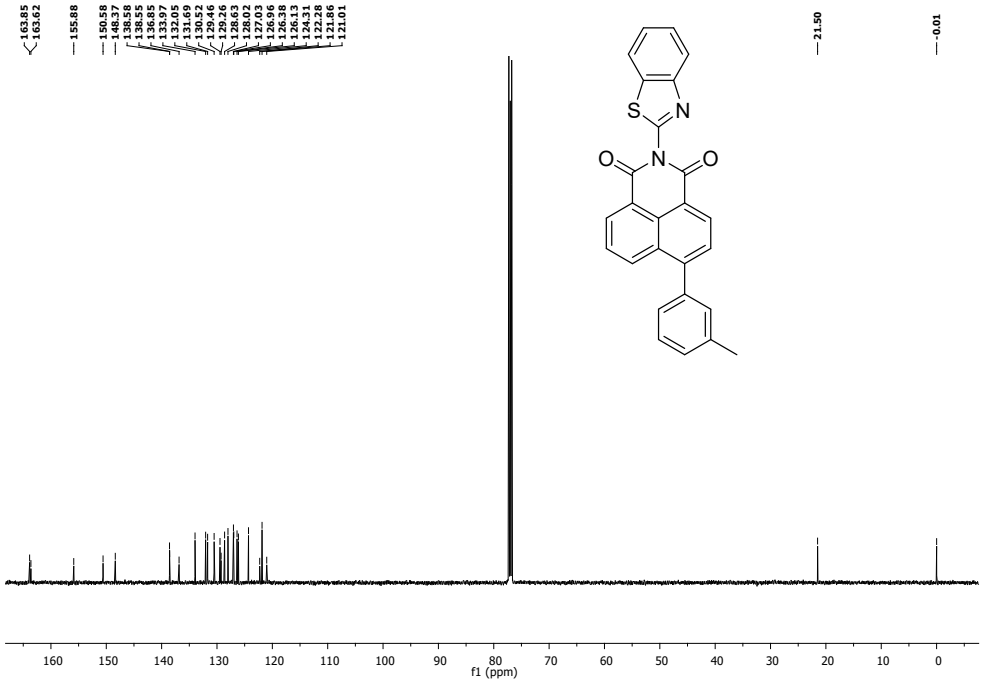


Figure S8: ^{13}C NMR spectrum of 2-(benzo[*d*]thiazol-2-yl)-6-(*m*-tolyl)-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione (**7**)

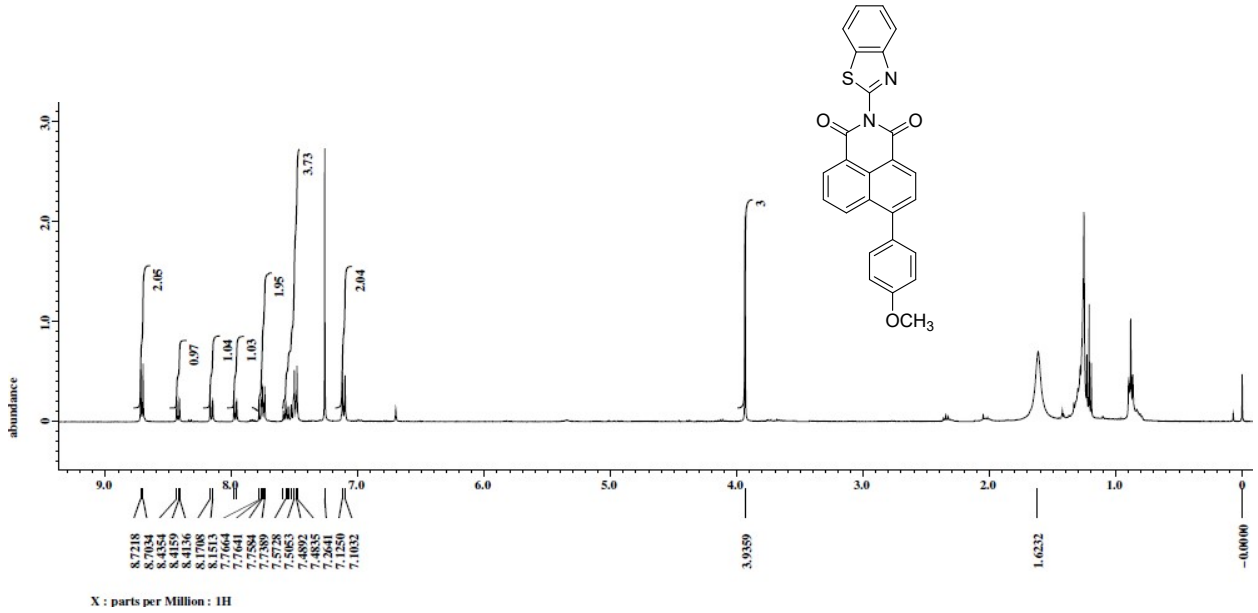


Figure S9: ^{1}H NMR spectrum of 2-(benzo[*d*]thiazol-2-yl)-6-(4-methoxyphenyl)-1*H*benzo[*de*]isoquinoline-1,3(2*H*)-dione (**8**)

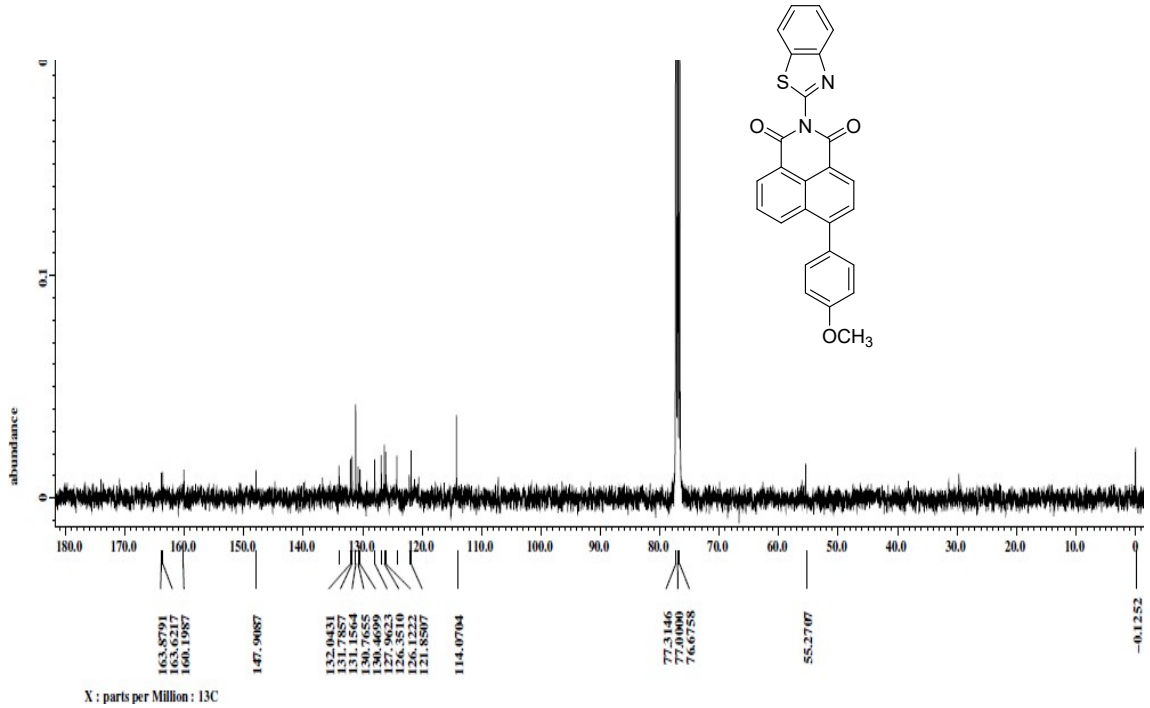


Figure S10: ^{13}C NMR spectrum of 2-(benzo[*d*]thiazol-2-yl)-6-(4-methoxyphenyl)1*H*benzo[*de*] isoquinoline-1,3(2*H*)-dione (**8**)

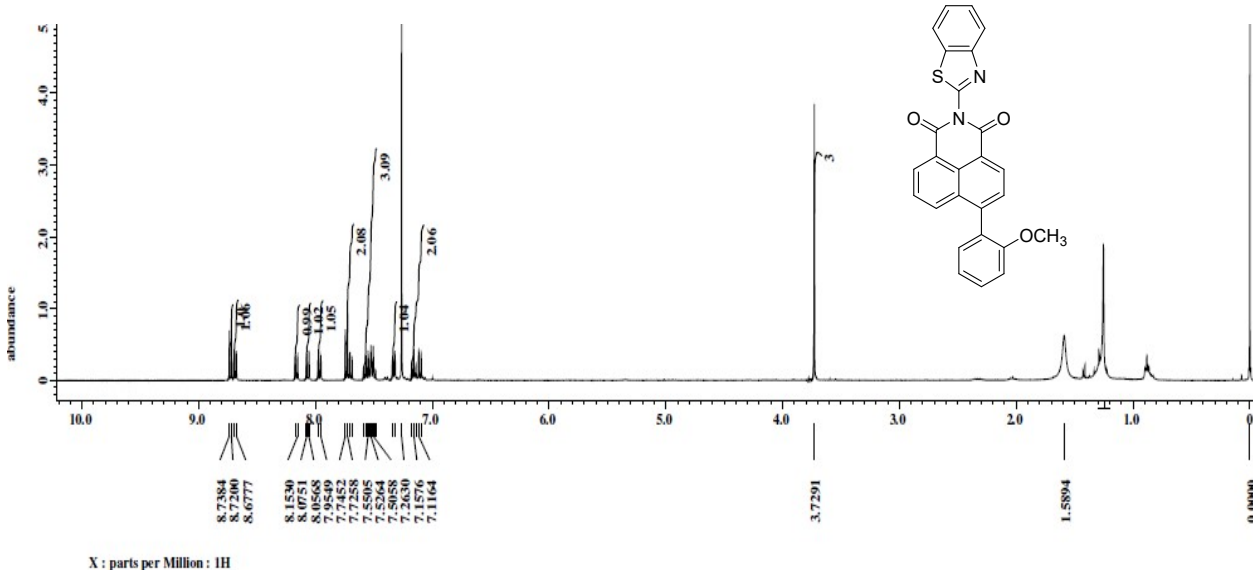


Figure S11: ^{1}H NMR spectrum of 2-(benzo[*d*]thiazol-2-yl)-6-(3-methoxyphenyl)-1*H*benzo[*de*]isoquin oline -1,3(2*H*)-dione (**9)**

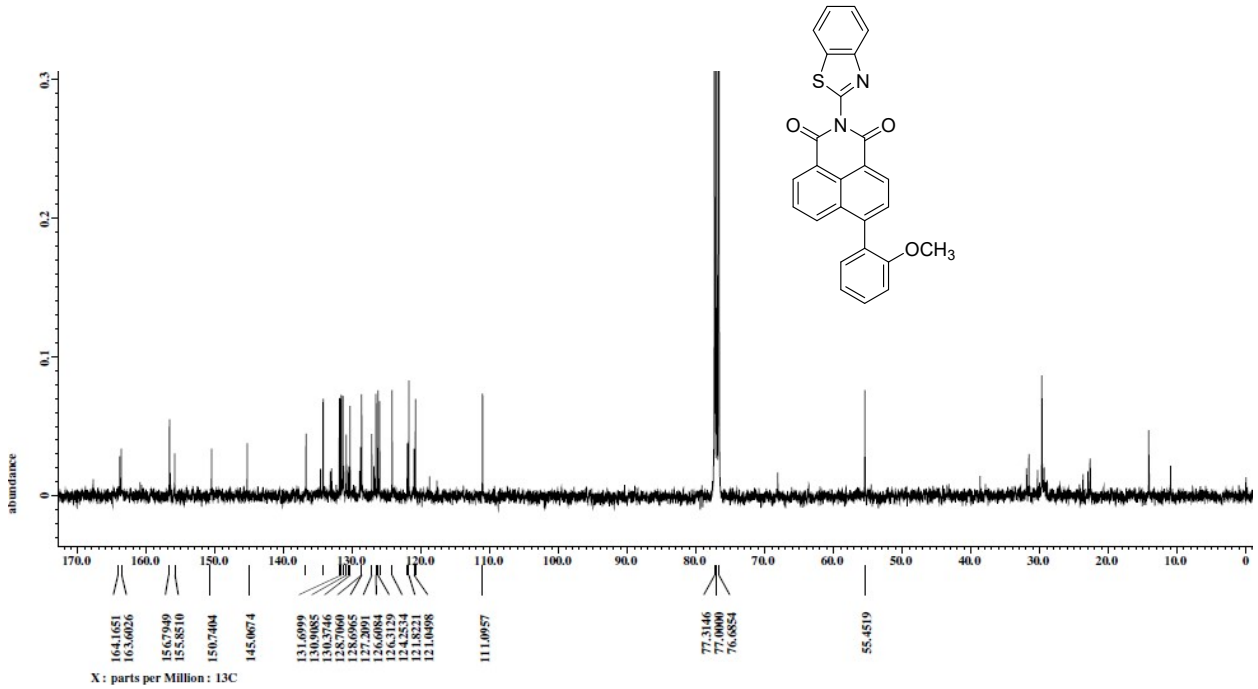


Figure S12: ^{13}C NMR spectrum of 2-(benzo[*d*]thiazol-2-yl)-6-(3methoxyphenyl)1*H*benzo[*de*]isoquino line-1,3(2*H*)-dione (**9**)

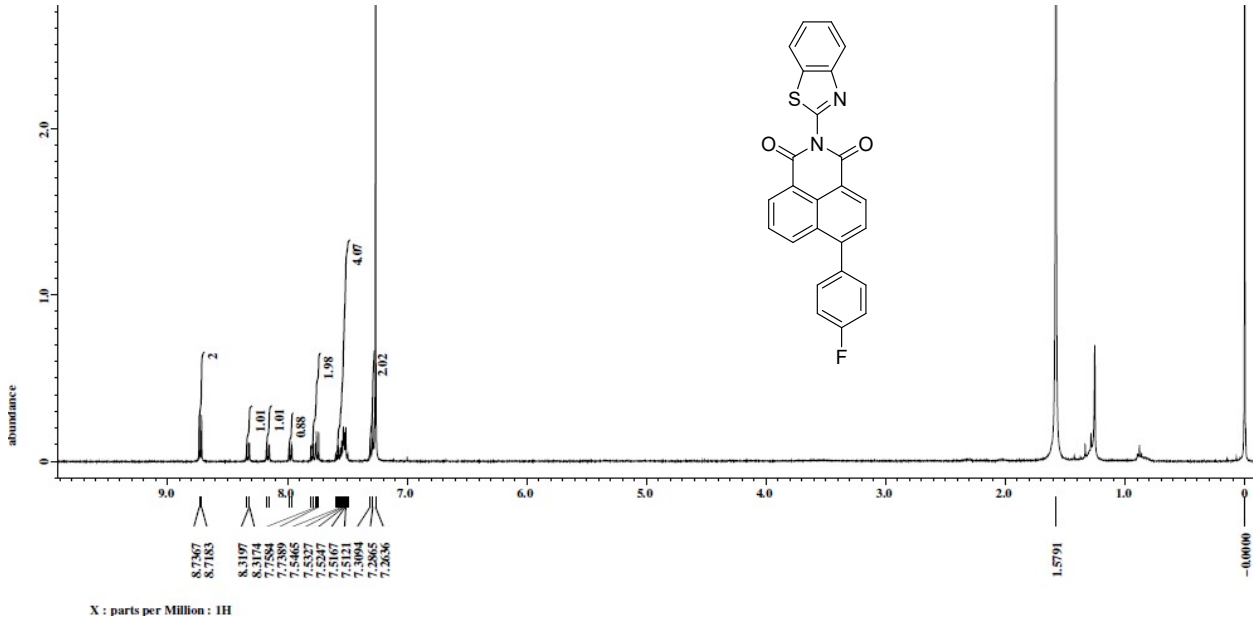


Figure S13: ^{1}H NMR spectrum of 2-(benzo[*d*]thiazol-2-yl)-6-(4-flourophenyl)-1*H*-benzo[*de*] isoquinoline-1,3(2*H*)-dione (**10**)

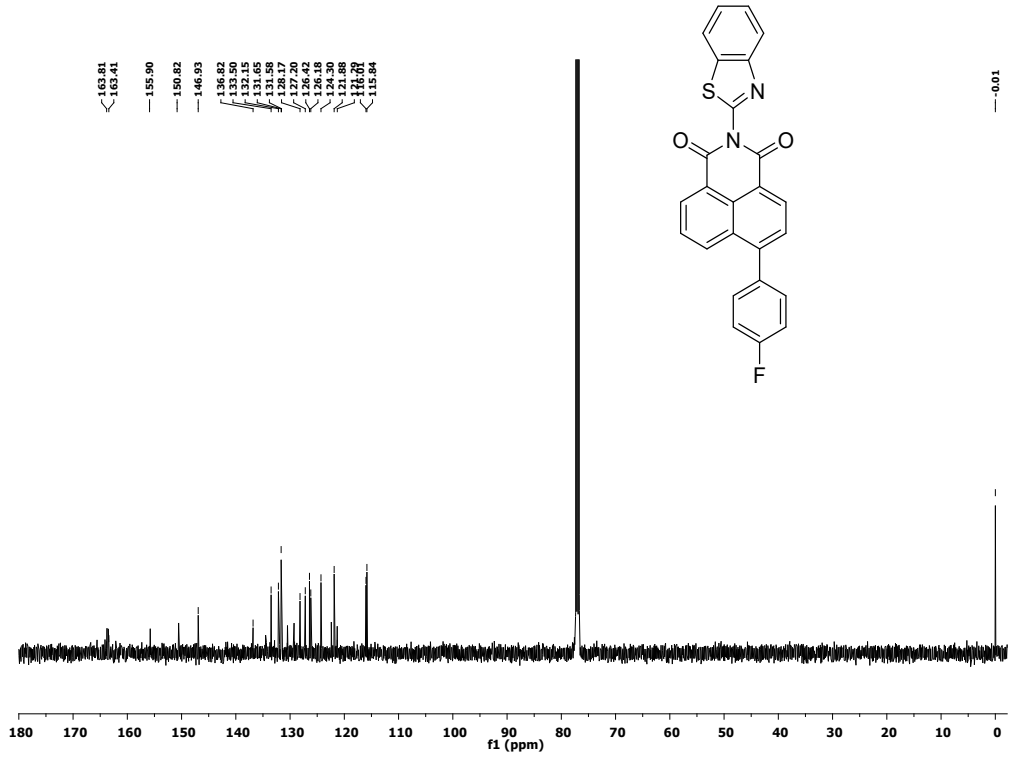


Figure S14: ^{13}C NMR spectrum of 2-(benzo[*d*]thiazol-2-yl)-6-(4-flourophenyl)-1*H*-benzo[*de*] isoquinoline-1,3(2*H*)-dione (**10**)

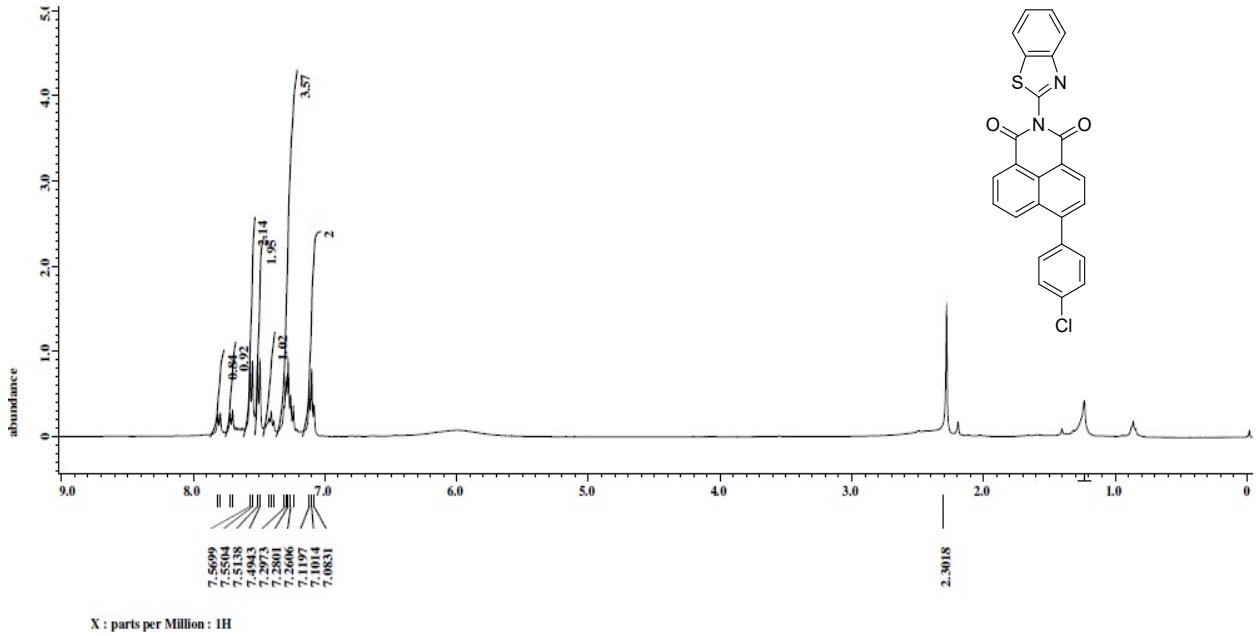


Figure S15: ^{1}H NMR spectrum of 2-(benzo[*d*]thiazol-2-yl)-6-(4-chlorophenyl)-1*H*-benzo[*de*] isoquinoline-1,3(2*H*)-dione (**11**)

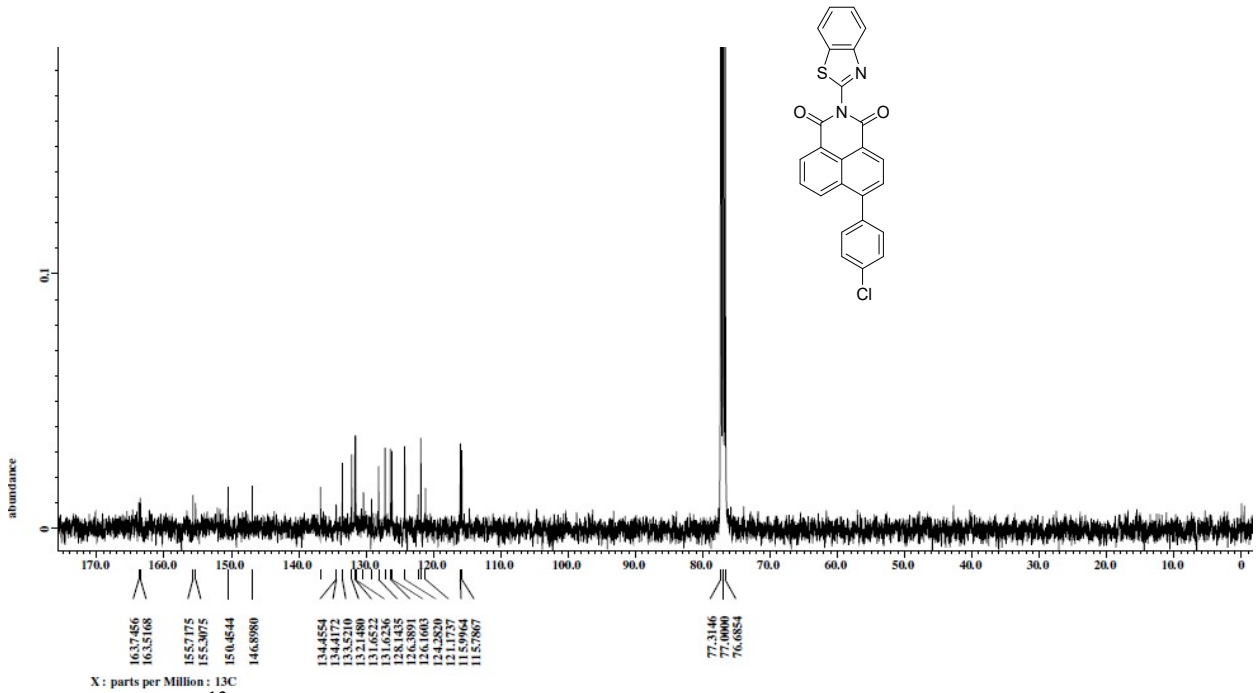


Figure S16: ^{13}C NMR spectrum of 2-(benzo[*d*]thiazol-2-yl)-6-(4-chlorophenyl)-1*H*-benzo[*de*] isoquinoline-1,3(2*H*)-dione (**11**)

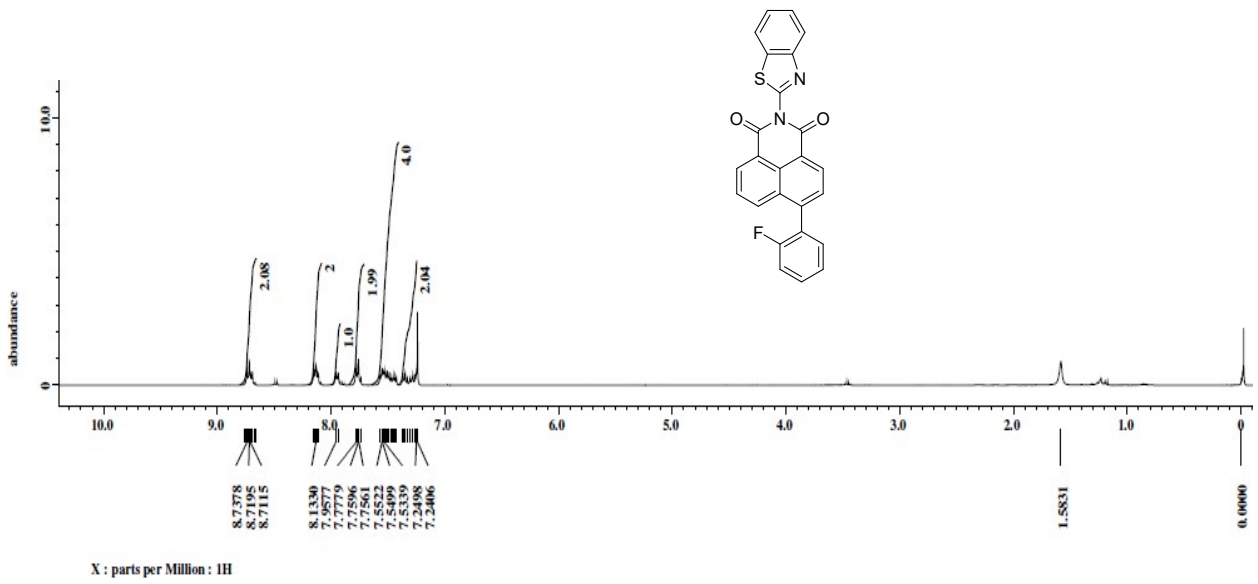


Figure S17: [1]H NMR spectrum of 2-(benzo[*d*]thiazol-2-yl)-6-(2-flourophenyl)-1*H*-benzo[*de*] isoquinoline-1,3(2*H*)-dione (**12**)

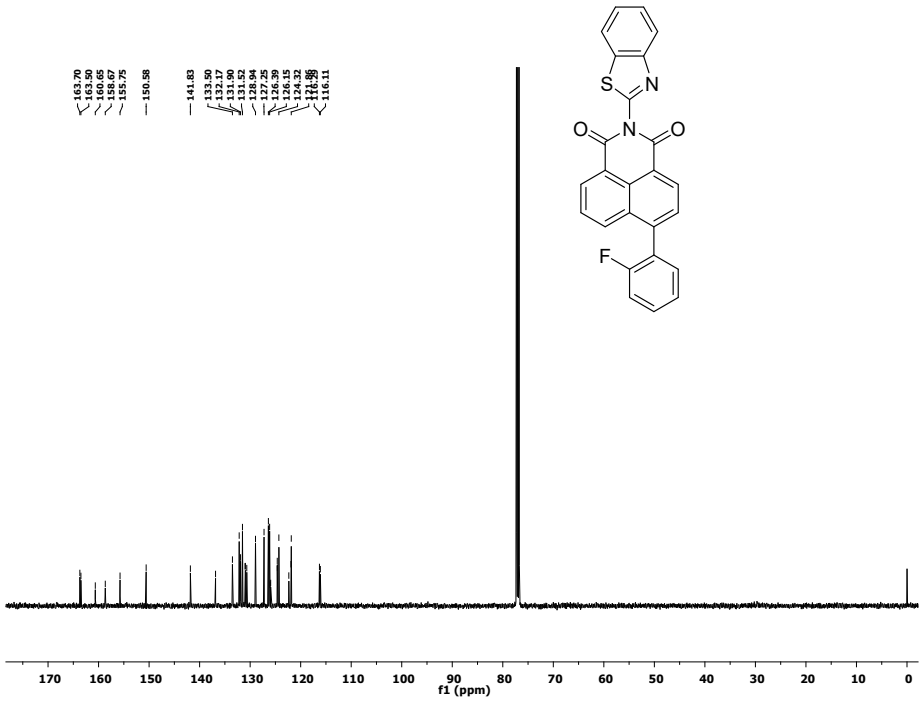


Figure S18: ^{13}C NMR spectrum of 2-(benzo[*d*]thiazol-2-yl)-6-(2-flourophenyl)-1*H*-benzo[*de*] isoquinoline-1,3(2*H*)-dione (**12**)

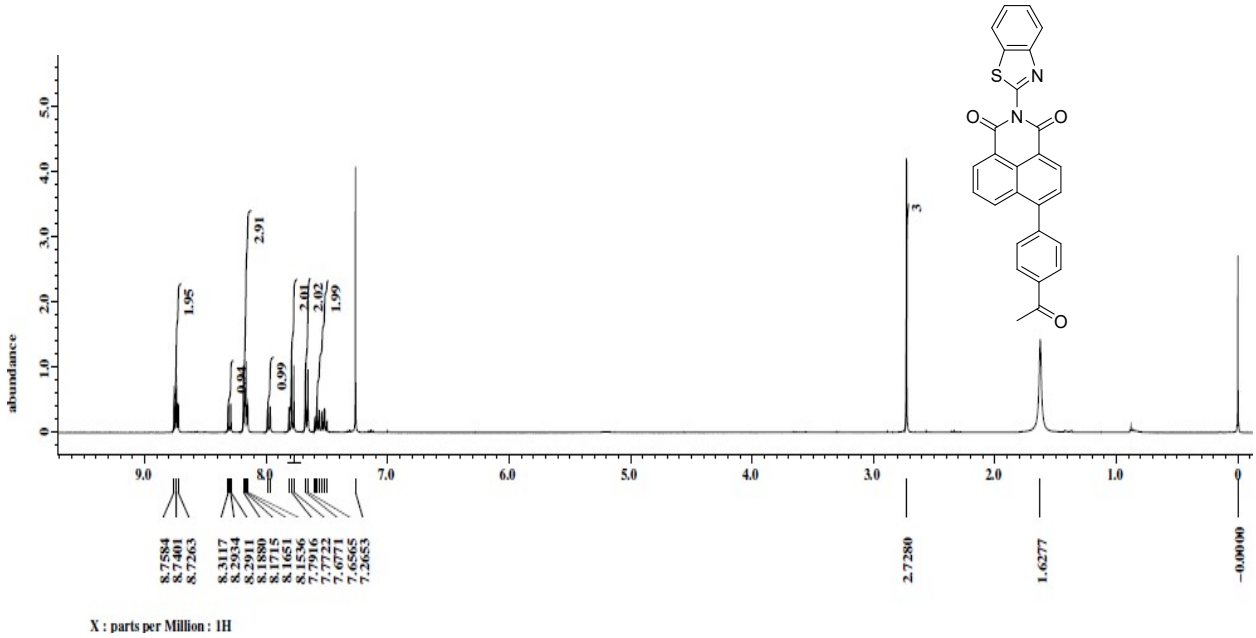


Figure S19: ^{1}H NMR spectrum of 6-(4-acetylphenyl)-2-(benzo[*d*]thiazol-2-yl)-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione (**13**).

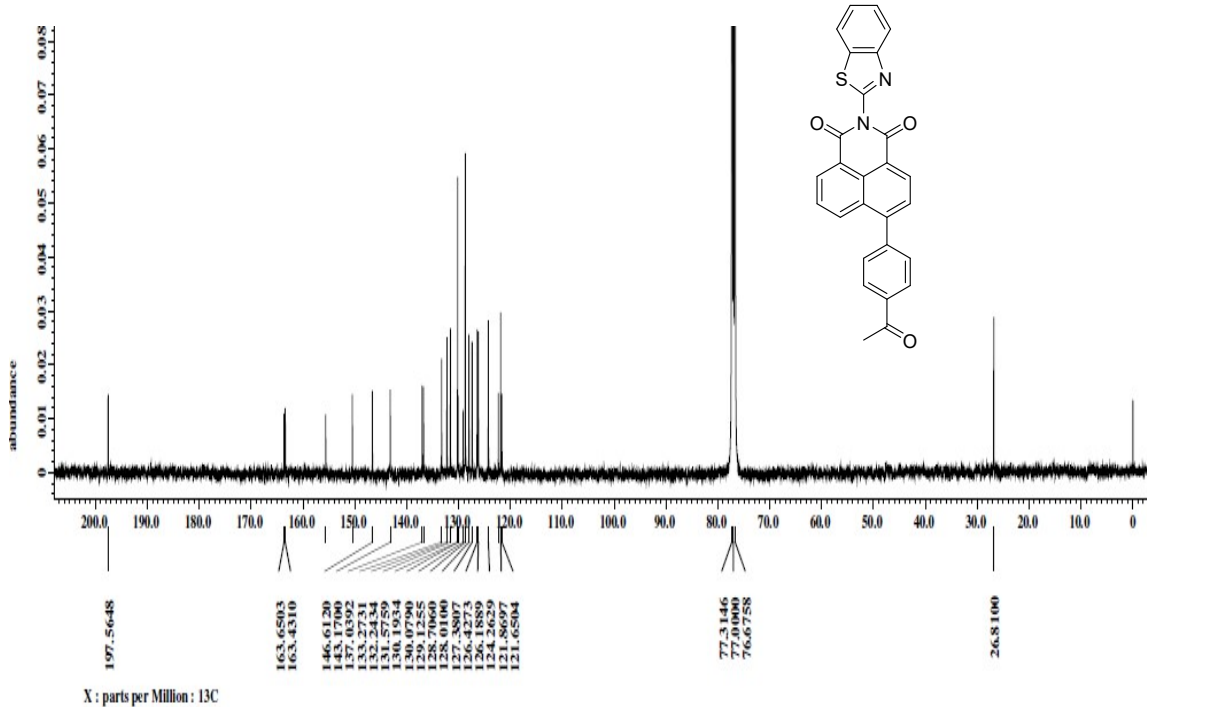


Figure S20: ^{13}C NMR spectrum of 6-(4-acetylphenyl)-2-(benzo[*d*]thiazol-2-yl)-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione (**13**)

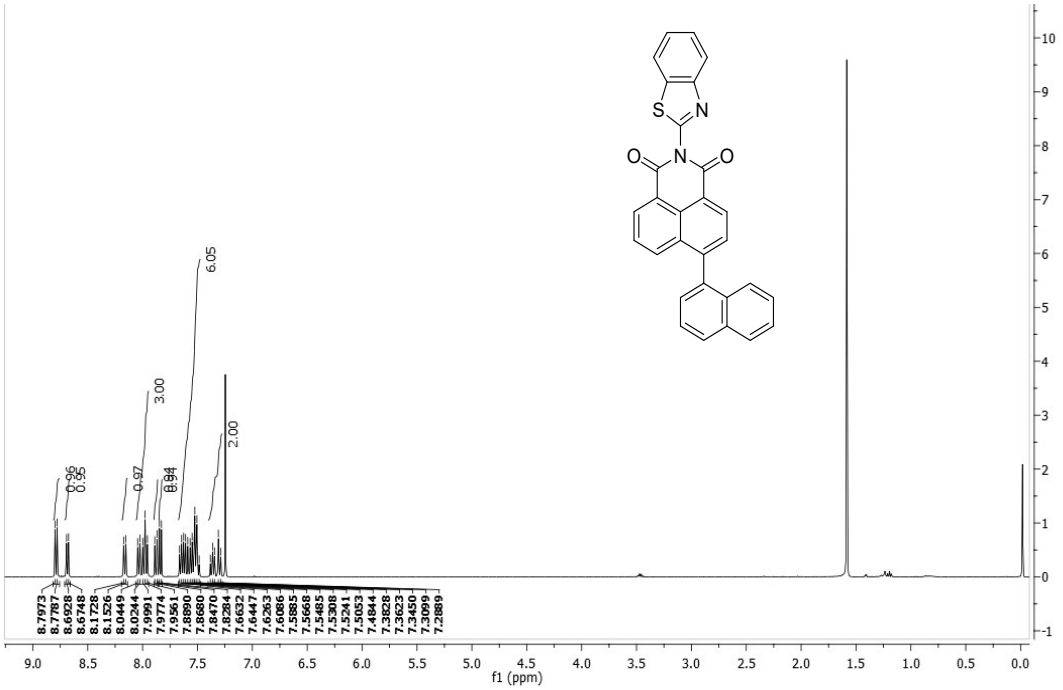


Figure S21: ^{1}H NMR spectrum of 2-(benzoic[*d*]thiazol-2-yl)-6-(naphthalen-1-yl)-1*H*-benzo [*de*] isoquinoline-1, 3(2*H*)-done (**14**)

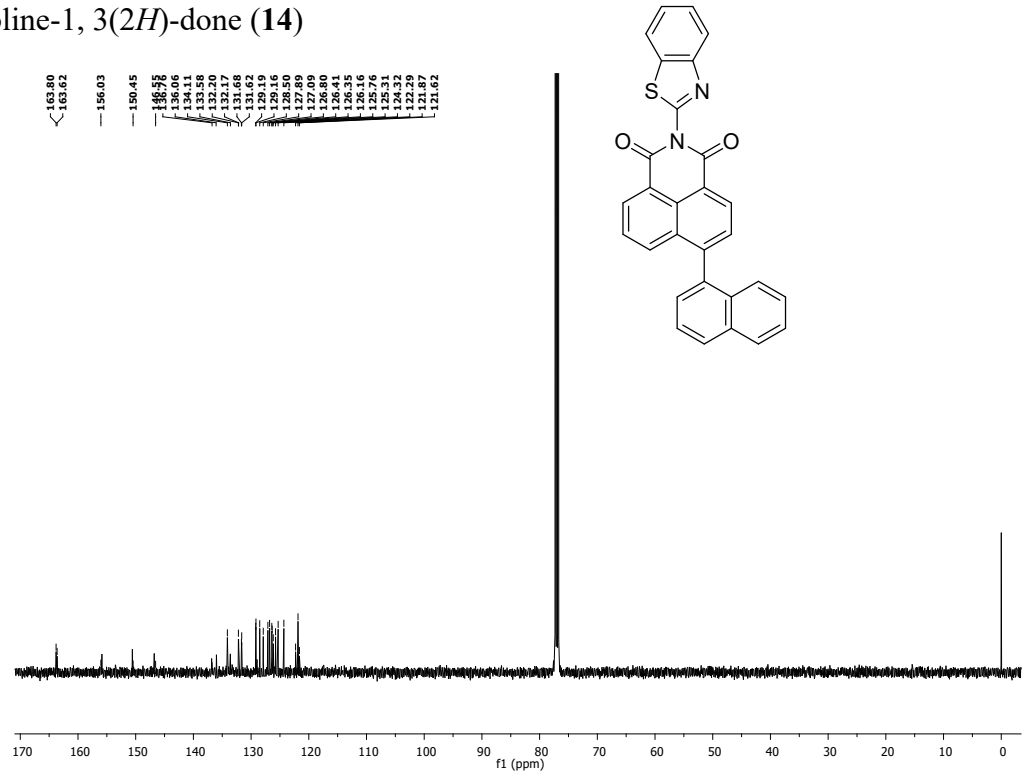


Figure S22: ^{13}C NMR spectrum of 2-(benzo[*d*]thiazol-2-yl)-6-(naphthalen-1-yl)-1*H*-benzo[*de*] isoquinoline-1,3(2*H*)-dione (**14**)

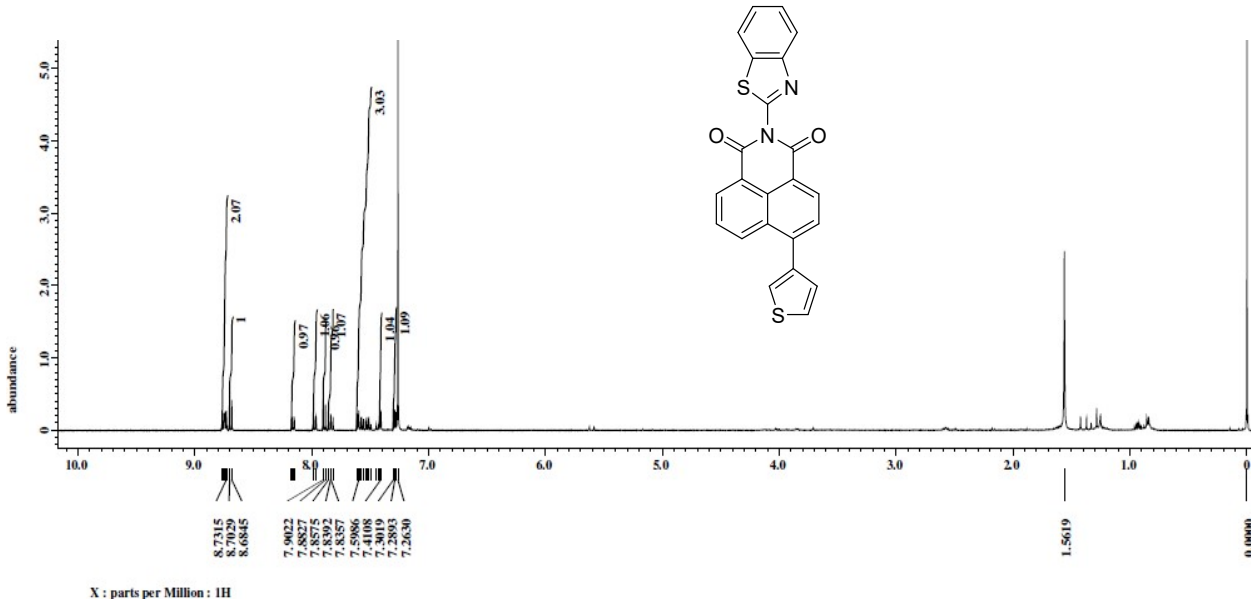


Figure S23: ^{1}H NMR spectrum of 2-(benzo[*d*]thiazol-2-yl)-6-(thiophen-3-yl)-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione (**15**)

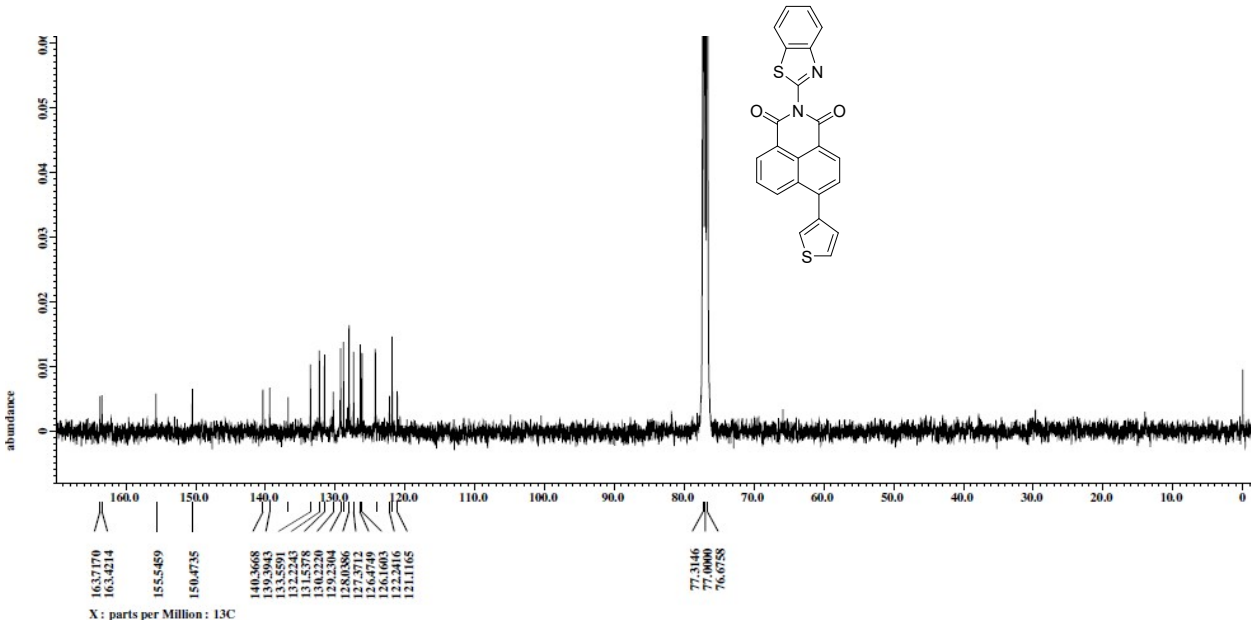


Figure S24: ^{13}C NMR spectrum of 2-(benzo[*d*]thiazol-2-yl)-6-(thiophen-3-yl)-1*H*-benzo[*de*] isoquinoline-1,3(2*H*)-dione (**15**)

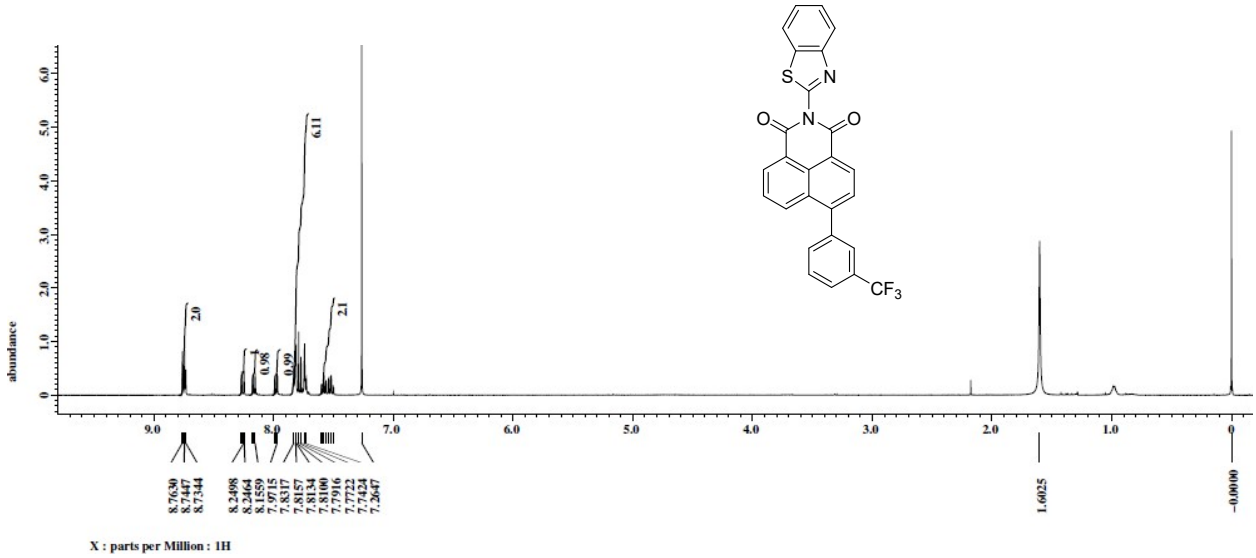


Figure S25 : ^{1}H NMR spectrum of 2-(benzo[*d*]thiazol-2-yl)-6-(3-(triflouromethyl)phenyl)-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-Dione (**16**)

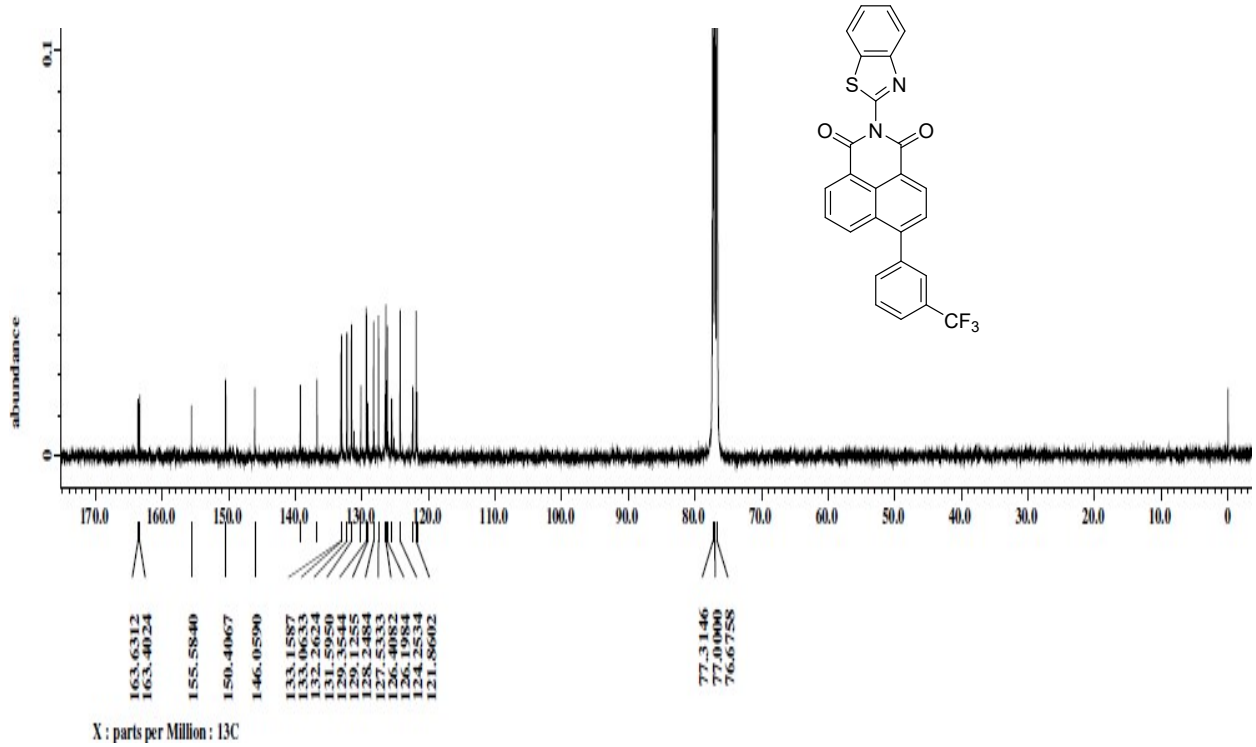


Figure S26 : ^{13}C NMR spectrum of 2-(benzo[*d*]thiazol-2-yl)-6-(3-(triflouromethyl)phenyl)-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione (**16**)

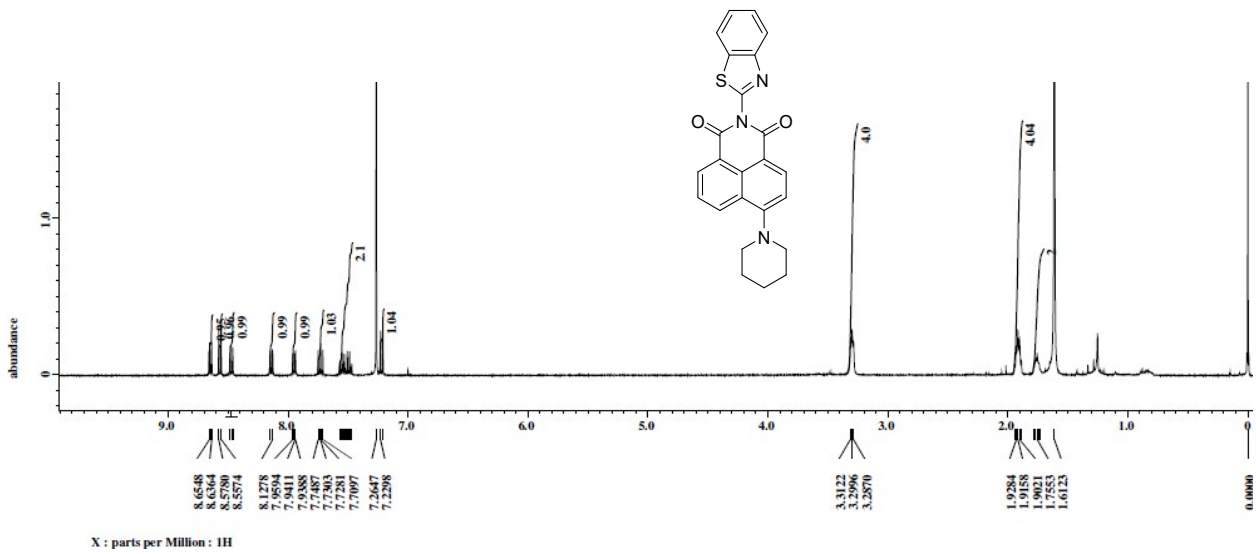


Figure S27: ^{1}H NMR spectrum of 2-(benzo[*d*]thiazol-2-yl)-6-piperdin-1-yl-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione (**17**)**.**

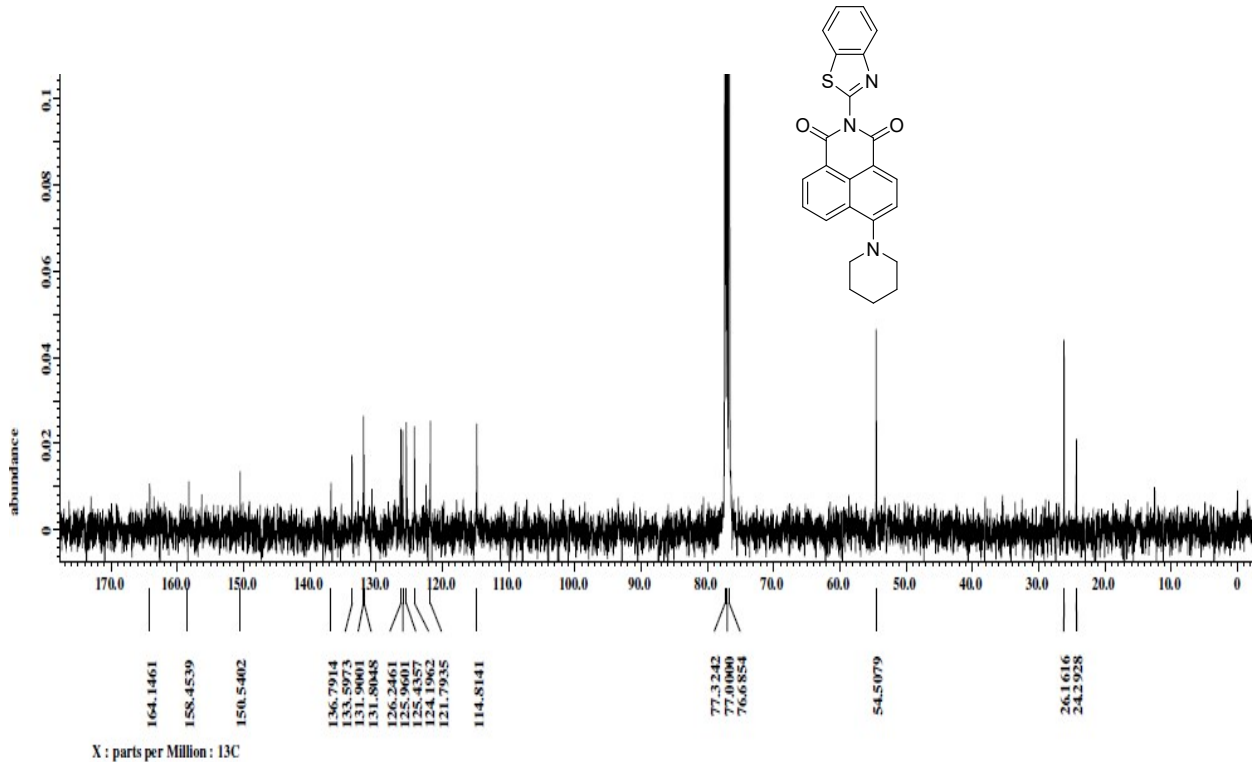


Figure S28 : ^{13}CNMR spectrum of 2-(benzo[*d*]thiazol-2-yl)-6-piperdin-1-yl-1*H*-benzo[*de*] isoquinoline-1,3(2*H*)-dione (**17**)**.**

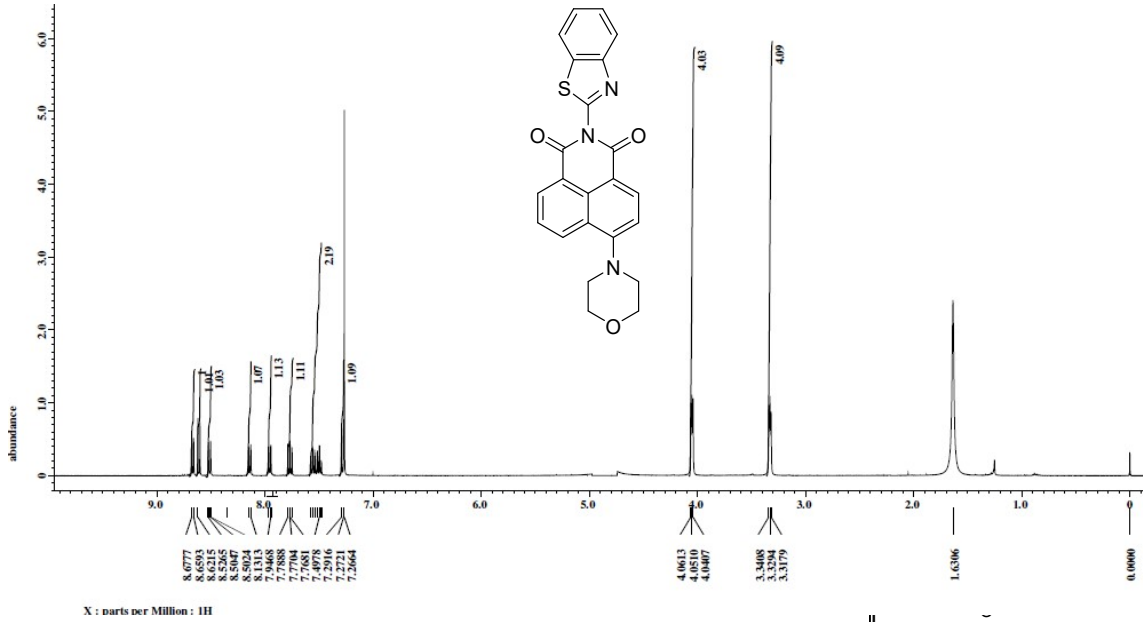


Figure S29 : ^{1}H NMR spectrum of 2-(benzo[*d*]thiazol-2-yl)-6-morpholino-1*H*-benzo[*de*] isoquinoline-1,3(2*H*)-dione (**18**)

Figure S30 : ^{1}H NMR spectrum of 2-(benzo[*d*]thiazol-2-yl)-6-morpholino-1*H*-benzo[*de*] isoquinoline-1,3(2*H*)-dione (**18**)

3. Supporting figures

3.1 Absorption and emission spectra

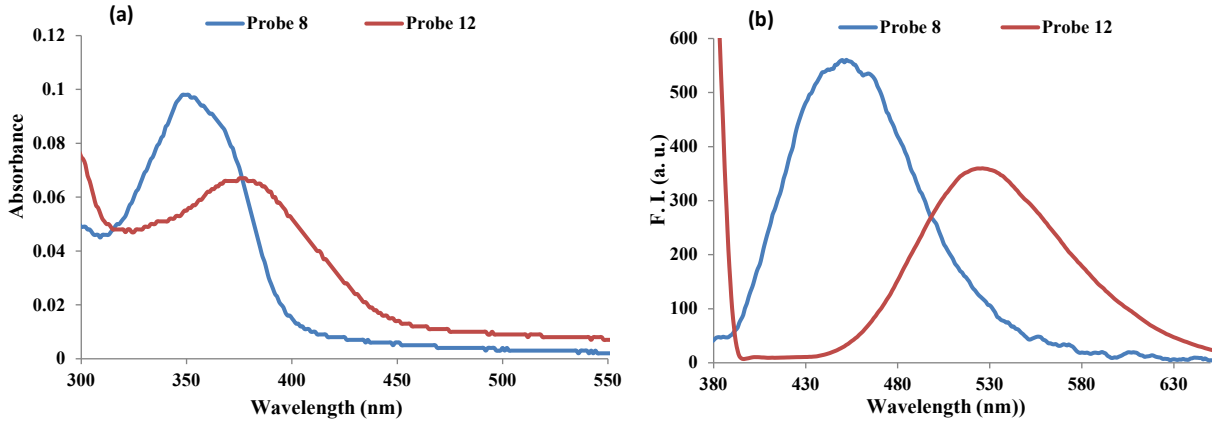
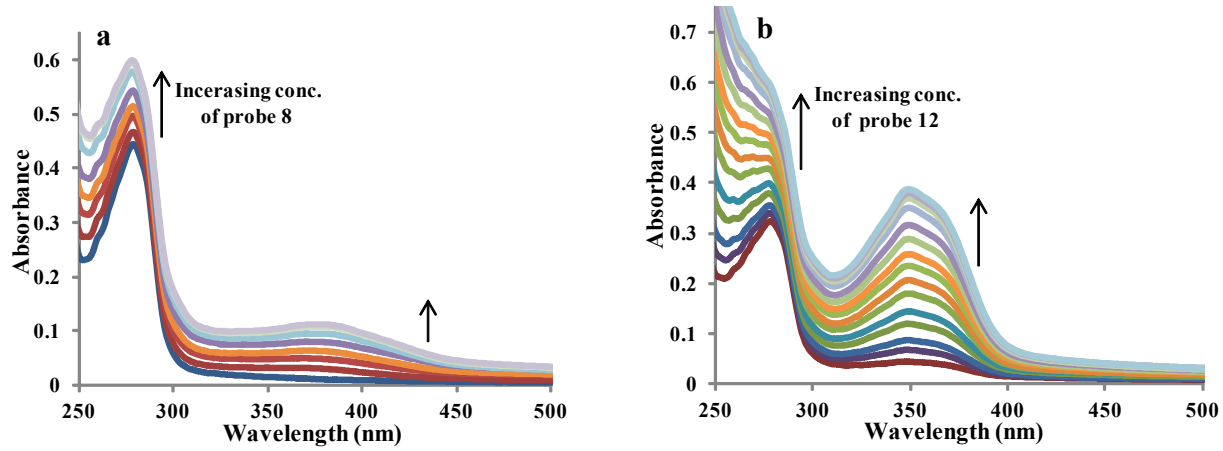


Figure S31: (a) absorption and (b) emission spectra of probes **8** (7 μM) and **12** (7 μM) in phosphate buffer at *p*H 7.4.



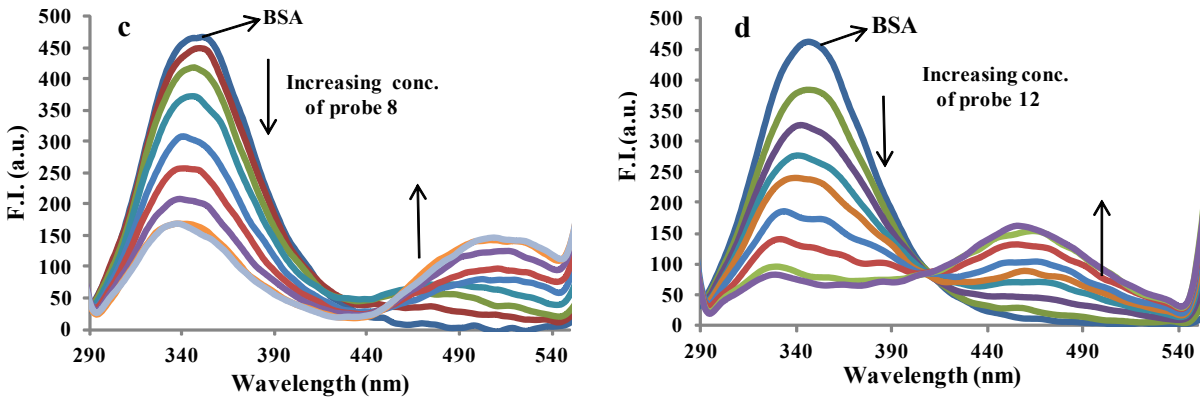


Figure S32: Effect of incremental addition of compounds (a) **8** and (b) **12** on the absorption spectra of BSA; and (c) **8** and (d) **12** on emission spectra of BSA in phosphate buffer at *p*H 7.4.

3.2 Linear binding plots for binding constant

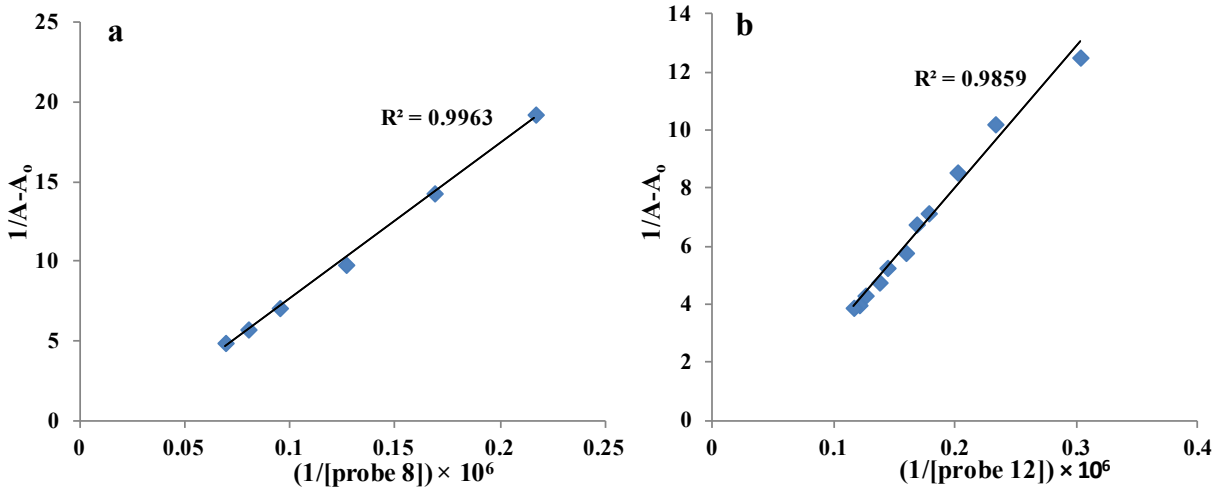


Figure S33: Plots of $1/(A - A_0)$ *vs.* 1/[Drug] for absorption spectra of compounds (a) **8** and (b) **12** in the presence of HSA in phosphate buffer.

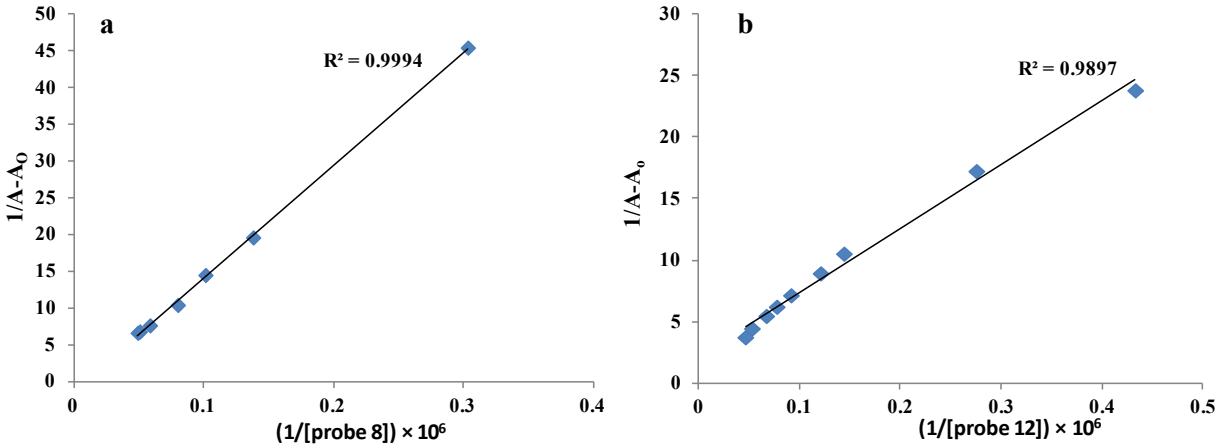


Figure S34 : Plots of $1/(A - A_0)$ *vs.* 1/[Drug] for absorption spectra of compounds (a) **8** and (b) **12** in the presence of BSA in phosphate buffer.

3.3 Stern-Volmer plots for determination of quenching constant

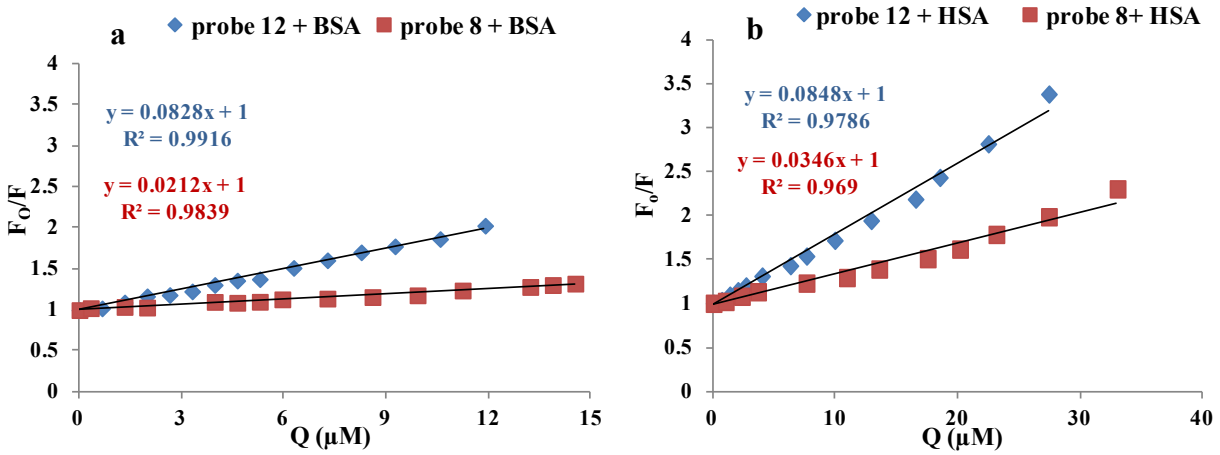


Figure S35: Stern-Volmer plots F_0/F versus [Q] for determination of quenching constant by fluorescence spectrometer titration of (a) BSA and (b) HSA with **8** and **12** in PBS buffer.

3.4 Modified Stern-Volmer plots for determination of binding site

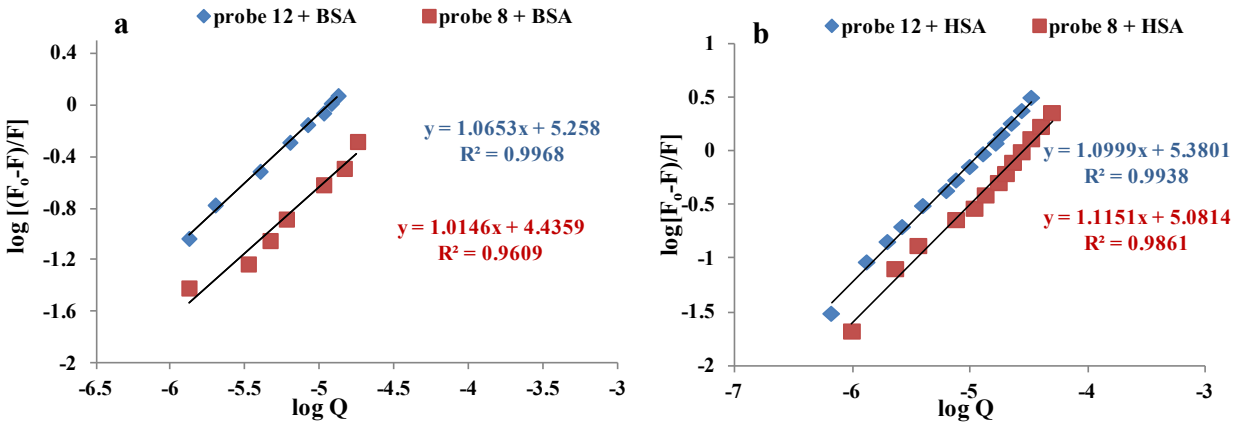


Figure S36: Modified Stern-Volmer plots of log [(F_o − F)/F] versus log [Q] for determination of number of binding sites by fluorescence spectrometer titration of (a) BSA and (b) HSA with **8** and **12** in PBS buffer.

3.5 Modified Stern-Volmer plots for determination of the binding constant of drug displacement studies

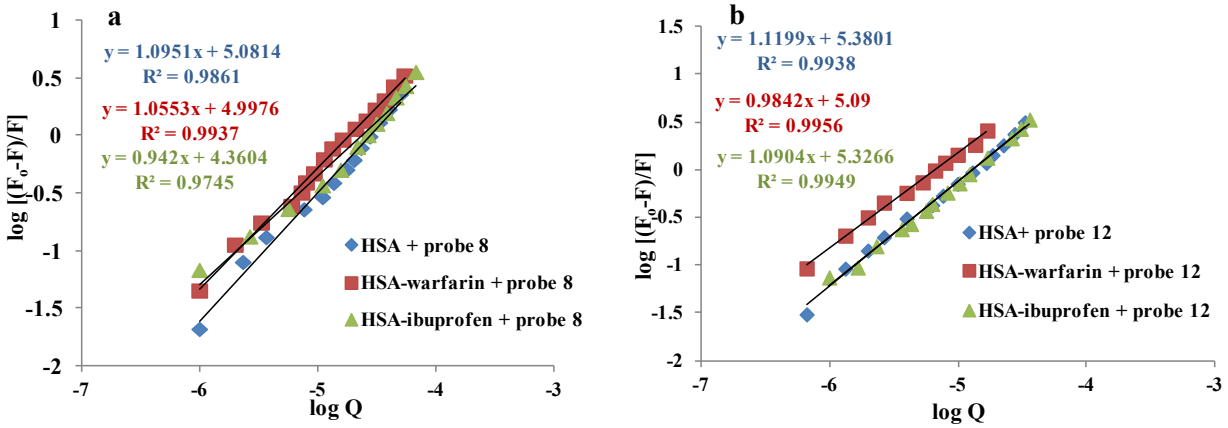
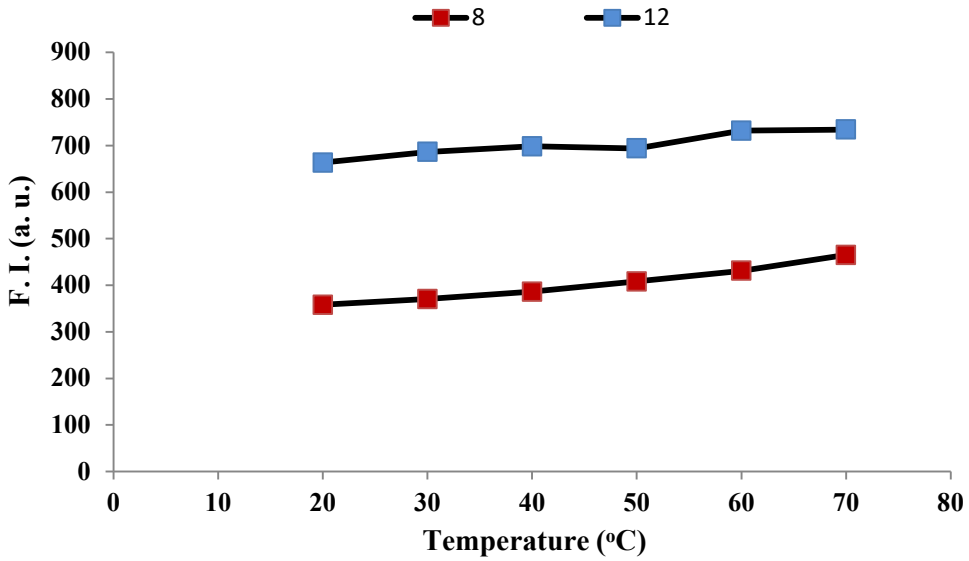


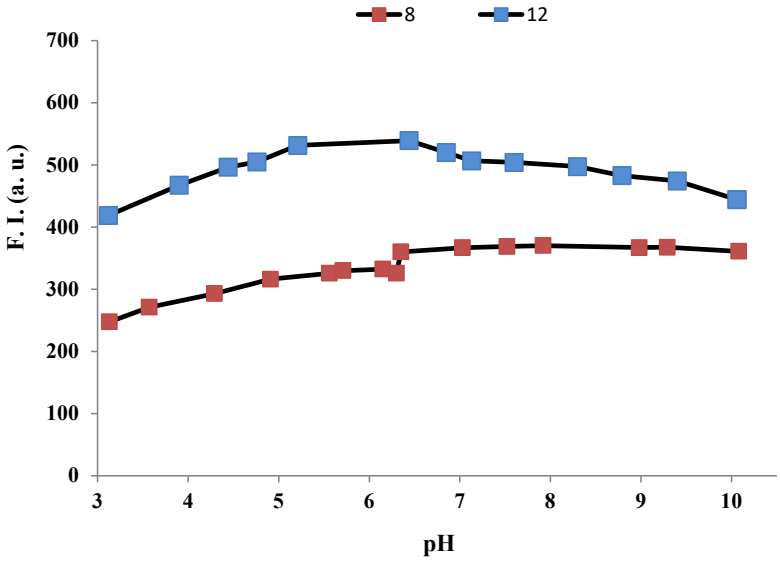
Figure S37: Modified Stern-Volmer plots of log [$(F_0 - F)/F$] versus log [Q] for determination of binding constant by fluorescence spectrometer titration of HSA with (a) **8** and (b) **12** in PBS buffer.

3.6 Temperature-dependent studies



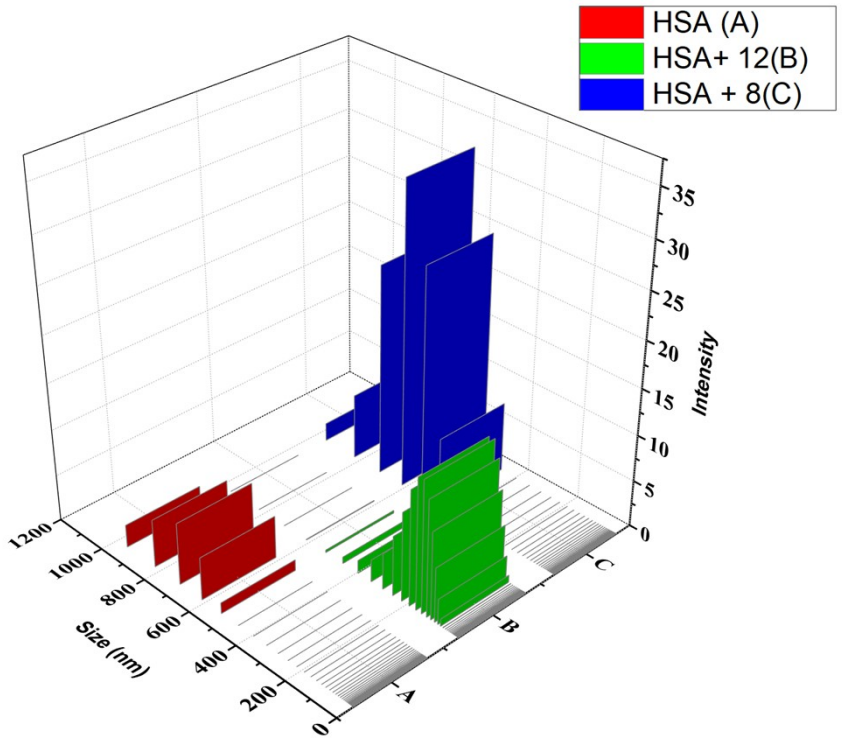
FigureS38: Fluorescence emission intensity of compounds **8** and **12** at emission wavelengths 508 and 448 nm respectively within temperature range 20-70 °C

3.7 pH studies



FigureS39: Fluorescence emission intensity of compounds **8** and **12** at emission wavelengths 508 and 448 nm respectively within pH range 3-10

3.8 DLS experiment



FigureS40: The changes observed in the intensity of average hydrodynamic size of human serum albumin (7μM) upon addition of 30 μM of compounds **8** and **12**

3.9 Cytotoxicity of compounds

3.9.1 Anticancer activity of compounds

Table S1: Anticancer activity of compounds at one dose concentration (10 μM) with selected cell lines.

Panel	Cell line	5	6	7	8	10	11	12	17	18	5-FU
Leukemia	CCRF-CEM	5.18	-	15.3	4.89	-	3.52	**40.5**	19.9	-	**57.1**
	HL-60(TB)	-	-	-	0.90	-	-	**38.5**	19.3	6.36	**47.9**
	K-562	12.3	1.54	15.3	8.51	-	3.84	**54.3**	**32.3**	-	**42.3**

	MOLT-4	-	-	-	5.64	-	-	22.9	18.2	-	**43.1**
	RPMI-8226	7.44	-	15.9	6.57	5.20	6.63	**45.5**	22.5	-	**41.4**
	SR	10.7	3.22	29.1	15.5	1.13	0.14	**70.1**	23.4	4.08	24.8
Lung	HOP-62	7.25	-	26.3	14.5	18.6	12.1	-	5.96	1.84	**52.2**
	HOP-92	22.2	19.5	17.1	25.1	-	7.20	**51.0**	24.6	-	**50.6**
	NCI-H226	3.09	5.39	-	7.24	4.76	9.00	24.3	21.2	8.18	**69.5**
	NCI-H322M	12.4	7.41	-	1.26	11.6	7.16	18.9	27.5	17.5	**40.5**
	NCI-H460	9.67	5.96	-	4.20	7.22	-	27.3	3.78	-	NT
	NCI-H522	10.2	0.37	19.5	13.8	5.33	2.73	19.5	**30.7**	16.2	**58.0**
Colon	COLO 205	-	0.66	4.68	-	-	-	27.7	5.89	-	NT
	HCT-116	7.71	0.72	13.2	7.02	-	-	**38.8**	23.4	8.50	17.8
	HCT-15	1.29	-	-	-	-	-	**54.8**	**40.6**	7.48	26.5
CNS	SNB-75	-	5.69	-	11.3	7.79	-	-	27.0	10.7	**65.9**
Melanoma	LOX IMVI	1.64	5.77	6.07	5.28	6.42	12.6	21.2	11.1	4.55	**30.4**
	MALME-3M	2.06	1.21	24.0	**33.9**	23.4	27.3	-	23.7	17.5	**58.2**
	SK-MEL-5	2.13	7.18	5.50	1.76	3.73	4.08	**32.6**	5.18	5.98	**66.3**
	UAAC-62	17.0	5.53	18.2	12.8	9.33	17.5	**57.5**	25.6	23.9	**94.4**
Ovarian	IGROV1	25.2	12.5	13.1	19.2	28.5	17.2	28.1	22.9	6.13	**51.2**
	OVCAR-4	-	-	13.1	-	-	-	16.4	17.4	2.62	**59.4**
Renal	CAKI-1	11.1	14.5	26.0	4.53	20.6	19.2	25.0	19.2	14.6	**60.6**
	TK-10	-	-	-	-	-	17.7	29.5	-	-	**67.1**
	UO-31	**36.9**	26.3	**40.7**	23.6	**36.5**	29.6	**55.4**	**51.4**	**33.7**	**41.3**
Prostate	PC-3	12.4	1.25	25.1	-	15.0	12.2	**44.2**	**41.0**	9.41	**58.2**
Breast	MDA-MB-231	17.5	0.64	27.6	10.0	1.60	17.3	**43.2**	26.49	20.8	**78.1**
	T-47D	0.88	11.3	12.6	-	-	-	21.36	21.78	-	**56.7**

NT – Not Tested. - indicated inactive, **Orange bold** –30-40% GI, **black bold** – 40-50 % GI, **pink bold** – 50-70 % GI, **blue bold** – 70-100 % GI, 5-FU = 5-fluorouracil

3.9.2 Cytotoxicity of probe 12 towards normal cell line

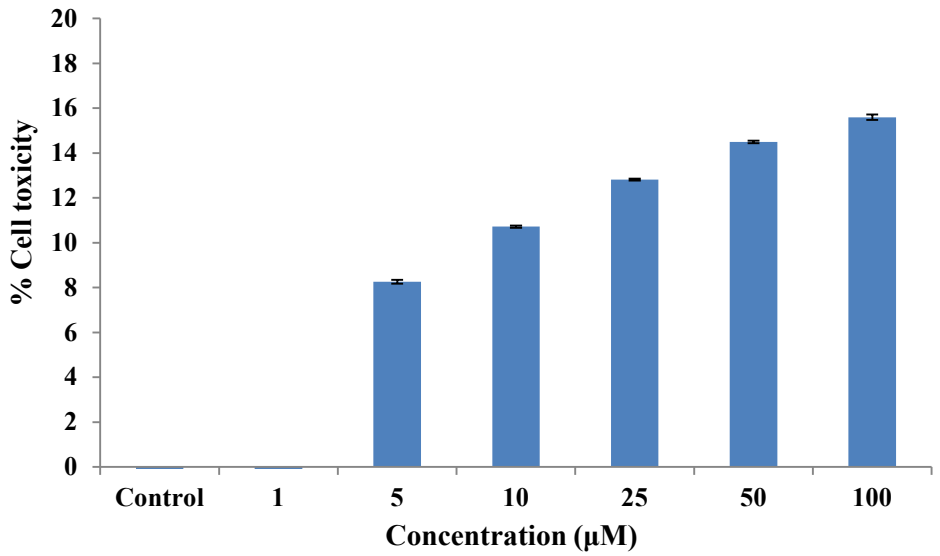


Figure S41 : Cytotoxic effect of compound towards human normal cell line (Hek293).

3.10. Molecular docking with BSA

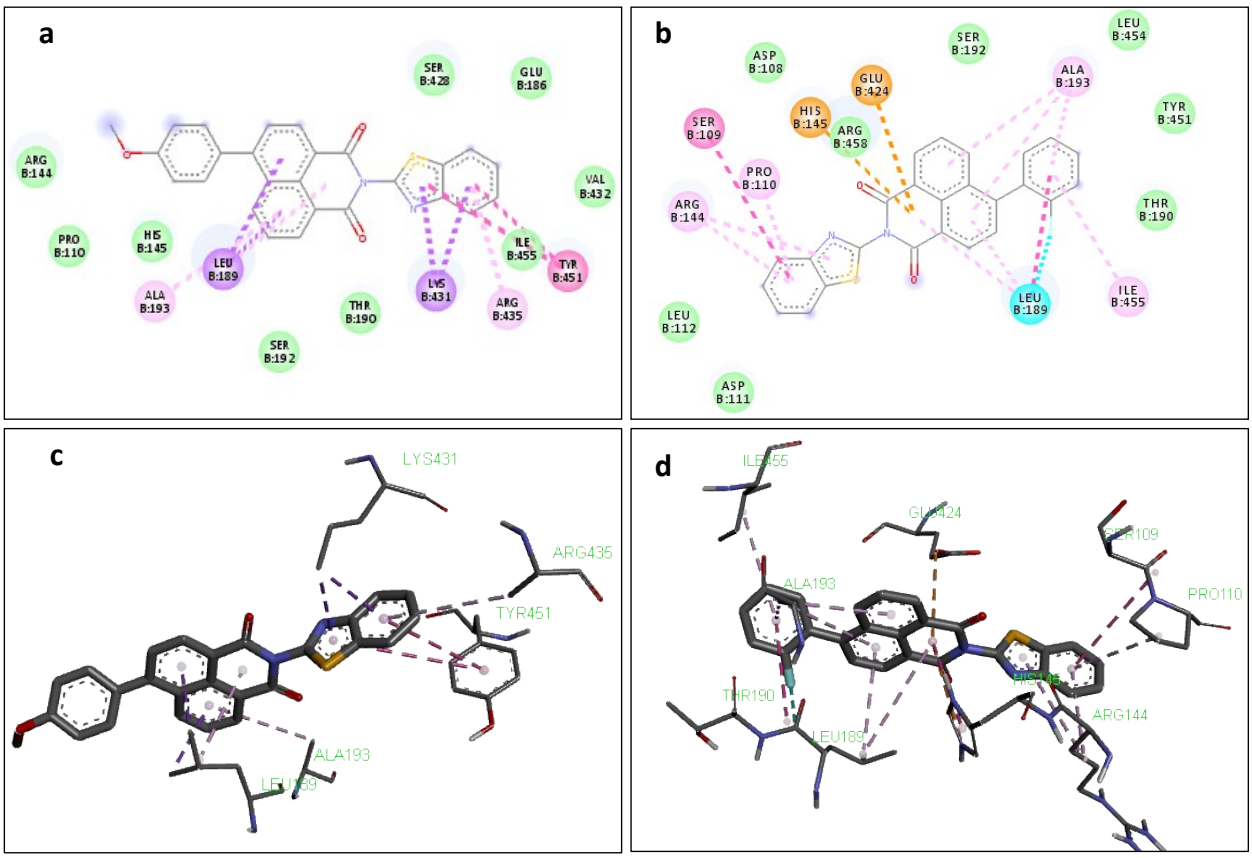


Figure S42: 2-D view of amino acid residues surrounding (a) **8** and (b) **12;** 3-D view conformations of (c) **8** and (d) **12** with BSA (pdb: 3V03).

3.11 Photophysical properties

Table S2: Photophysical properties of compounds 5-18

Compound Entry	Absorption Maxima (nm)	Emission Maxima (nm)	Extinction Coefficient (M^{-1} cm^{-1})	Quantum Yield
5	333	450	4140	0.595
6	362	471	14571	0.523
7	366	463	15142	0.675
8	378	508	9000	0.609
9	349	478	2000	0.562
10	334	449	7428	0.631
11	361	465	6857	0.654
12	348	448	45000	0.582
13	352	485	13714	0.524
14	350	460	13142	0.686
15	385	505	9571	0.541
16	380	490	8527	0.565
17	430	520	15857	0.431
18	410	481	18285	0.473