# Triazine-Benzimidazole Conjugates: Synthesis, Spectroscopic and Molecular Modelling Studies for Interaction with calf thymus DNA

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## Experimental Section

### Chemistry

All materials were obtained from commercial suppliers and used without further purification unless otherwise noted. Melting points were determined in open capillaries and were uncorrected. Reactions were monitored by thin-layer chromatography (TLC) carried out on silica gel plates (GF 254) using UV light as visualizing agents. Column chromatography was performed with silica gel 60-120. $^{1}$H NMR and $^{13}$C NMR spectra were recorded on Jeol-400 ($^{1}$H, 400 MHz; $^{13}$C, 100 MHz) spectrometer at ambient temperature, using $CDCl_3$, DMSO-$d_6$ and trifluoroacetic acid (TFA) as solvents. Chemical shifts are reported in parts per million (ppm) with TMS as an internal reference and *J* values are given in Hz. Data are reported as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, bs = broad singlet, m = multiplet), coupling constants and number of protons. Mass spectrometric data were recorded at Waters Micromass Q-Tof Micro. Elemental analysis was done with Thermo Scientific (Flash 2000) analyzer. Hexane:ethyl acetate and chloroform:methanol were the adopted solvent systems.

**Synthesis of (4,6-dichloro-[1,3,5]triazin-2-yl)-(4-fluoro-phenyl)-amine (1)[1] :** To a stirred solution of cyanuric chloride (10 g, 0.054 mol) in anhydrous THF (150 mL), 4-fluoroaniline (7.21 g, 0.065 mol) was added at 0-5 $^{0}$C. To this mixture, 10% $NaHCO_3$ was added and stirred at the same temperature for 6 h. The resulted reaction mixture was then poured into crushed ice, filtered, dried and column chromatographed on silica gel in ethylacetate:hexane (1:4) to afford the desired product (4,6-dichloro-[1,3,5]triazin-2-yl)-(4-fluoro-phenyl)-amine **(1)** as white solid; (11.93 g, 85%); mp: 222-224 $^{o}$C.

**Synthesis of 4-(1*H*-benzimidazol-2-yl)-phenylamine (2)[2] :** A mixture of 4-aminobenzoic acid (5 g, 5.78 mmol) and *o*-phenylenediamine (3.9 g, 3.68 mmol) were stirred in polyphosphoric acid (12.5 gm) at 200 $^{0}$C for 5 h. The reaction was monitored with TLC. The reaction mixture was cooled and poured into crushed ice. The precipitate was then stirred in cold water. Ammonium hydroxide solution was added until pH 7 was achieved. The resulting solid was filtered and washed several times with methanol and column chromatographed on silica gel in ethylacetate:hexane (4:1) to afford the desired product 4-(1*H*-benzimidazol-2-yl)-phenylamine (**2**) as white solid; yield: 82%; mp: 207-209 $^{o}$C; $^{1}$H NMR (400 MHz, DMSO-$d_6$): δ = 7.96 (d, 2H, *J* = 8.72 Hz, ArH), 7.55-7.51 (m, 2H, ArH), 7.16-7.14 (m, 2H, ArH), 6.75 (d, 2H, *J* = 8.24 Hz, ArH), 4.45 (bs, 2H, $NH_2$); $^{13}$C NMR (100 MHz, $CDCl_3$ + DMSO-$d_6$): δ = 152.2, 148.4, 127.5, 120.9, 118.6, 113.8 (ArC); MS (ESI), m/z: 209.2 ($M^{+}$+1).

**Synthesis of *N*-[4-(1*H*-benzimidazol-2-yl)-phenyl]-6-chloro-*N*'-(4-fluoro-phenyl)-[1,3,5]triazine-2,4-diamine (3):** To a stirred solution of (4,6-dichloro-[1,3,5]triazin-2-yl)-(4-fluoro-phenyl)-amine **(1)** (10 g, 0.026 mol) in anhydrous THF (150 mL), 4-(1*H*-benzimidazol-2-

yl)-phenylamine (**2**) (8.06 g, 0.038 mol) was added at room temperature. To this mixture, 10% $K_2CO_3$ was added and stirred for 24 h. The resulted reaction mixture was then poured into crushed ice, filtered, dried and column chromatographed on silica gel using ethylacetate:hexane (4:1) to afford the product *N*-[4-(1*H*-benzimidazol-2-yl)-phenyl]-6-chloro-*N*'-(4-fluoro-phenyl)-[1,3,5]triazine-2,4-diamine **(3)** as white solid; yield: 82%; mp: 249-251$^oC$; $^1H$ NMR (400 MHz, $CDCl_3$ + DMSO-$d_6$): δ = 10.12 (bs, 1H, NH), 9.97 (bs, 1H, NH), 8.19 (d, 1H, *J* = 8.72 Hz, ArH), 8.14 (d, 1H, *J* = 7.76 Hz, ArH), 8.00 (d, 1H, *J* = 3.20 Hz, ArH), 7.86 (d, 2H, *J* = 7.80 Hz, ArH), 7.71-7.67 (m, 2H, ArH), 7.60 (s, 1H, ArH), 7.22-7.19 (m, 2H, ArH), 7.10-7.06 (m, 2H, ArH); $^{13}C$ NMR (100 MHz, $CDCl_3$): δ = 169.4, 141.2, 134.9, 133.9, 133.6, 131.4, 129.1, 128.4, 126.3, 122.3, 119.8, 118.0, 110.3, 108.3 (ArC); MS (ESI), m/z: 431.8 ($M^+$+1); Anal. Calcd for $C_{22}H_{15}ClFN_7$: C, 61.19; H, 3.50; N, 22.70, Found: C, 61.18; H, 3.47; N, 22.71.

**General procedure for the synthesis of compounds 4-15:** To a stirred solution of *N*-[4-(1*H*-benzimidazol-2-yl)-phenyl]-6-chloro-*N*'-(4-fluoro-phenyl)-[1,3,5]triazine-2,4-diamine **(3)** (0.200 g, 0.463 mmol) in 1,4-dioxane (20 mL), amines (0.556 mmol) and potassium carbonate (0.095g, 0.695 mmol) were added and heated at 110 $^oC$ for 6-8 hrs. Extracted the reaction mixture with chloroform, dried over $Na_2SO_4$, filtered and concentrated to get crude product which was then purified through column chromatography using chloroform:methanol (50:1) as eluents to give compounds **4-15**.

***N*-[4-(1*H*-Benzimidazol-2-yl)-phenyl]-*N*'-(4-fluoro-phenyl)-6-morpholin-4-yl-[1,3,5]triazine-2,4-diamine (4):** White solid; yield: 75%; mp: 248-250 $^oC$; $^1H$ NMR (400 MHz, $CDCl_3$): δ = 8.00 (d, 2H, *J* = 4.72 Hz, ArH), 7.83 (s, 1H, NH), 7.72 (d, 2H, *J* = 8.24 Hz, ArH), 7.52-7.48 (m, 3H, ArH), 7.07 (t, 3H, *J* = 8.68 Hz, ArH), 6.96 (s, 1H, ArH), 6.80 (s, 1H, ArH), 3.82-3.76 (m, 4H, mor-$CH_2$), 3.70-3.69 (m, 4H, mor-$CH_2$); $^{13}C$ NMR (100 MHz, $CDCl_3$ + DMSO-$d_6$): δ = 163.7, 159.1, 148.6, 144.5, 134.8, 131.5, 128.2, 125.2, 122.1, 119.6, 114.9, 114.6, 114.4, 113.3 (ArC), 65.9 (O-$CH_2$), 43.4 (N-$CH_2$); MS (ESI), m/z: 482.5 ($M^+$+1); Anal. Calcd for $C_{26}H_{23}FN_8O$: C, 64.72; H, 4.80; N, 23.22, Found: C, 64.65; H, 4.77; N, 23.18.

***N*-[4-(1*H*-Benzimidazol-2-yl)-phenyl]-*N*'-(4-fluoro-phenyl)-6-piperidin-1-yl-[1,3,5]triazine-2,4-diamine (5):** White solid; yield: 78%; mp: 252-254 $^oC$; $^1H$ NMR (400 MHz, $CDCl_3$): δ = 8.00 (d, 2H, *J* = 8.28 Hz, ArH), 7.73 (bs, 1H, NH), 7.72 (d, 2H, *J* = 7.72 Hz, ArH), 7.53-7.49 (m, 3H, ArH), 7.05-7.00 (m, 4H, ArH), 6.85 (s, 1H, ArH), 3.79-3.78 (d, 4H, *J* = 5.04 Hz, N-$CH_2$), 1.55-1.51 (m, 6H, $CH_2$); $^{13}C$ NMR (100 MHz, $CDCl_3$ + DMSO-$d_6$): δ = 147.6, 131.1, 128.0, 125.1, 123.3, 120.8, 116.0, 114.7, 114.5, 113.1 (ArC), 43.5 (N-$CH_2$), 24.8 ($CH_2$), 21.6 ($CH_2$); MS (ESI), m/z: 480.5 ($M^+$+1); Anal. Calcd for $C_{27}H_{25}FN_8$: C, 67.48; H, 5.24; N, 23.32, Found: C, 67.50; H, 5.20; N, 23.39.

***N*-[4-(1*H*-Benzimidazol-2-yl)-phenyl]-*N*'-(4-fluoro-phenyl)-6-pyrrolidin-1-yl-[1,3,5]triazine-2,4-diamine (6):** White solid; yield: 72%; mp: 242-244 $^oC$; $^1H$ NMR (400 MHz, $CDCl_3$): δ = 7.96 (d, 1H, *J* = 8.68 Hz, ArH), 7.70 (d, 2H, *J* = 8.72 Hz, ArH), 7.59-7.52 (m, 4H, ArH), 7.25-7.22 (m, 1H, ArH), 7.10 (bs, 1H, NH), 7.02-6.97 (m, 4H, ArH), 3.59-3.57 (m, 4H, N-$CH_2$), 1.96-

1.95 (m, 4H, $CH_2$); $^{13}C$ NMR (100 MHz, $CDCl_3$): δ = 163.9, 163.6, 152.4, 143.0, 135.9, 135.5, 134.2, 129.1, 128.0, 126.2, 121.3, 115.4, 115.2, 109.1 (ArC), 47.2 (N-$CH_2$), 25.3 ($CH_2$); MS (ESI), m/z: 466.5 ($M^+$+1); Anal. Calcd for $C_{26}H_{23}FN_8$: C, 66.94; H, 4.97; N, 24.02, Found: C, 66.85; H, 4.93; N, 24.15.

***N*-[4-(1*H*-Benzimidazol-2-yl)-phenyl]-*N*'-(4-fluoro-phenyl)-6-(4-methyl-piperazin-1-yl)-[1,3,5]triazine-2,4-diamine (7):** White solid; yield: 77%; mp: 268-270 $^oC$; $^1H$ NMR (400 MHz, $CDCl_3$): δ = 7.97 (d, 2H, *J* = 8.72 Hz, ArH), 7.71 (bs, 1H, NH), 7.63 (d, 2H, *J* = 7.76 Hz, ArH), 7.48-7.45 (m, 2H, ArH), 7.25-7.23 (m, 3H, ArH), 7.13 (s, 1H, ArH), 7.02 (t, 2H, *J* = 8.24 Hz, ArH), 3.82-3.80 (m, 4H, N-$CH_2$), 2.43-2.41 (m, 4H, N-$CH_2$), 2.32 (s, 3H, $CH_3$); $^{13}C$ NMR (100 MHz, $CDCl_3$): δ = 164.9, 164.3, 151.8, 138.6, 138.5, 135.7, 135.0, 134.2, 129.1, 127.9, 126.2, 126.0, 122.1, 118.9, 116.4, 116.0, 115.5, 115.2 (ArC), 54.7 (N-$CH_2$), 43.1 (N-$CH_2$) 14.0 (N-$CH_3$); MS (ESI), m/z: 495.5 ($M^+$+1); Anal. Calcd for $C_{27}H_{26}FN_9$: C, 65.44; H, 5.29; N, 25.44, Found: C, 65.35; H, 5.25; N, 25.50.

***N*-(2-Amino-ethyl)-*N*'-[4-(1*H*-benzimidazol-2-yl)-phenyl]-*N*''-(4-fluoro-phenyl)-[1,3,5]triazine-2,4,6-triamine (8):** White solid; yield: 72%; mp: 273-275 $^oC$; $^1H$ NMR (400 MHz, $CDCl_3$): δ = 8.02 (bs, 1H, NH), 7.50-7.48 (m, 3H, ArH), 7.31 (d, 3H, *J* = 7.32 Hz, ArH), 7.11 (d, 2H, *J* = 8.24 Hz, ArH), 7.06 (d, 2H, *J* = 7.32 Hz, ArH), 6.95 (d, 2H, *J* = 7.36 Hz, ArH), 3.50-3.48 (m, 2H, N-$CH_2$), 2.93 (t, 2H, *J* = 5.48 Hz, N-$CH_2$); $^{13}C$ NMR (100 MHz, $CDCl_3$): δ = 166.4, 152.5, 142.9, 135.9, 135.8, 129.1, 128.0, 126.3, 115.4, 115.2, 109.1 (ArC), 43.5 (N-$CH_2$), 41.4 (N-$CH_2$); MS (ESI), m/z: 455.4 ($M^+$+1); Anal. Calcd for $C_{24}H_{22}FN_9$: C, 63.28; H, 4.87; N, 27.68, Found: C, 63.35; H, 4.80; N, 27.60.

**2-[4-[4-(1*H*-Benzimidazol-2-yl)-phenylamino]-6-(4-fluoro-phenylamino)-[1,3,5]triazin-2-ylamino]-ethanol (9):** White solid; yield: 69%; mp: 261-263 $^oC$; $^1H$ NMR (400 MHz, $CDCl_3$): δ = 8.02 (bs, 1H, NH), 7.44-7.42 (m, 2H, ArH), 7.29-7.26 (m, 5H, ArH), 7.03-6.98 (m, 3H, ArH), 6.90-6.84 (m, 2H, ArH), 3.81-3.79 (m, 2H, O-$CH_2$), 3.57-3.53 (m, 2H, N-$CH_2$), 1.84 (bs, 1H, OH); $^{13}C$ NMR (100 MHz, $CDCl_3$): δ = 166.5, 164.1, 152.5, 142.6, 135.7, 129.1, 128.0, 126.3, 122.0, 115.4, 115.1, 109.1 (ArC), 47.2 (O-$CH_2$), 43.7 (N-$CH_2$); MS (ESI), m/z: 456.4 ($M^+$+1); Anal. Calcd for $C_{24}H_{21}FN_8O$: C, 63.15; H, 4.64; N, 24.55, Found: C, 63.05; H, 4.60; N, 24.65.

***N*-[4-(1*H*-Benzimidazol-2-yl)-phenyl]-*N*'-(4-fluoro-phenyl)-*N*''-(2-morpholin-4-yl-ethyl)-[1,3,5]triazine-2,4,6-triamine (10):** White solid; yield: 74%; mp: 278-280 $^oC$; $^1H$ NMR (400 MHz, $CDCl_3$): δ = 7.94-7.90 (m, 2H, ArH), 7.61 (d, 1H, *J* = 9.16 Hz, ArH), 7.55-7.51 (m, 3H, ArH, NH), 7.50-7.43 (m, 3H, ArH), 7.23-7.21 (m, 1H, ArH), 7.02 (d, 1H, *J* = 6.40 Hz, ArH), 6.99 (d, 1H, *J* = 2.28 Hz, ArH), 6.97 (d, 1H, *J* = 1.84 Hz, ArH), 3.71-3.65 (m, 4H, O-$CH_2$), 3.53-3.46 (m, 2H, N-$CH_2$), 2.56-2.49 (m, 2H, N-$CH_2$), 2.46-2.42 (m, 4H, N-$CH_2$); $^{13}C$ NMR (100 MHz, $CDCl_3$): δ = 166.0, 164.6, 152.5, 142.9, 135.8, 129.1, 128.0, 126.3, 122.1, 121.7, 115.5, 115.2, 109.1 (ArC), 66.9 (O-$CH_2$), 53.4 (N-$CH_2$), 47.2 (N-$CH_2$), 37.1 (N-$CH_2$); MS (ESI), m/z: 525.5 ($M^+$+1); Anal. Calcd for $C_{28}H_{28}FN_9O$: C, 63.99; H, 5.37; N, 23.98, Found: C, 64.10; H, 5.33; N, 24.05.

**2-{4-[4-[4-(1*H*-Benzimidazol-2-yl)-phenylamino]-6-(4-fluoro-phenylamino)-[1,3,5]triazin-2-yl]-piperazin-1-yl}-ethanol (11):** White solid; yield: 73%; mp: 274-276 $^{o}$C; $^{1}$H NMR (400 MHz, $CDCl_3$): δ = 7.97 (d, 1H, *J* = 8.28 Hz, ArH), 7.61 (d, 2H, *J* = 8.28 Hz, ArH), 7.50-7.44 (m, 3H, ArH), 7.26-7.24 (m, 3H, ArH), 7.03-6.97 (m, 3H, ArH), 6.81 (bs, 1H, NH), 3.92 (t, 2H, *J* = 0.44 Hz, O-$CH_2$), 3.83-3.79 (m, 4H, N-$CH_2$), 3.67 (t, 2H, *J* = 4.12 Hz, N-$CH_2$), 2.55-2.53 (m, 4H, N-$CH_2$), 1.83 (bs, 1H, OH); $^{13}$C NMR (100 MHz, $CDCl_3$): δ = 152.4, 143.0, 135.9, 134.3, 131.6, 129.0, 128.0, 126.2, 116.2, 110.7, 109.1, 109.0 (ArC), 66.9 (O-$CH_2$), 47.1 (N-$CH_2$), 43.7 (N-$CH_2$), 43.6 (N-$CH_2$); MS (ESI), m/z: 525.5 ($M^+$+1); Anal. Calcd for $C_{28}H_{28}FN_9O$: C, 63.99; H, 5.37; N, 23.98, Found: C, 64.11; H, 5.29; N, 23.91.

***N*-[4-(1*H*-Benzimidazol-2-yl)-phenyl]-*N*'-benzyl-*N*''-(4-fluoro-phenyl)-[1,3,5]triazine-2,4,6-triamine (12):** White solid; yield: 78%; mp: 247-249 $^{o}$C; $^{1}$H NMR (400 MHz, $CDCl_3$): δ = 9.09 (d, 1H, *J* = 6.88 Hz, ArH), 8.81 (d, 2H, *J* = 7.80 Hz, ArH), 8.52 (s, 1H, ArH), 8.45 (bs, 1H, NH), 8.06-8.04 (m, 3H, ArH), 7.99 (d, 1H, *J* = 7.76 Hz, ArH), 7.91 (d, 3H, *J* = 8.24 Hz, ArH), 7.86 (d, 1H, *J* = 7.76 Hz, ArH), 7.74 (d, 1H, *J* = 6.40 Hz, ArH), 7.35-7.28 (m, 3H, ArH), 7.16 (t, 1H, *J* = 7.36 Hz, ArH), 5.98 (s, 2H, N-$CH_2$); $^{13}$C NMR (100 MHz, $CDCl_3$): δ = 168.7, 152.6, 142.1, 135.6, 133.4, 133.1, 132.7, 132.5, 129.1, 128.2, 128.1, 126.3, 116.9, 110.8, 109.5 (ArC), 47.3 (N-$CH_2$); MS (ESI), m/z: 502.5 ($M^+$+1); Anal. Calcd for $C_{29}H_{23}FN_8$: C, 69.31; H, 4.61; N, 22.30, Found: C, 69.22; H, 4.58; N, 22.39.

***N*-Allyl-*N*'-[4-(1*H*-benzimidazol-2-yl)-phenyl]-*N*''-(4-fluoro-phenyl)-[1,3,5]triazine-2,4,6-triamine (13):** White solid; yield: 70%; mp: 288-290 $^{o}$C; $^{1}$H NMR (400 MHz, $CDCl_3$): δ = 9.04 (d, 1H, *J* = 7.36 Hz, ArH), 8.45 (m, 1H, ArH), 7.97 (d, 2H, *J* = 7.80 Hz, ArH), 7.83 (d, 1H, *J* = 7.76 Hz, ArH), 7.73 (d, 2H, *J* = 5.96 Hz, ArH), 7.43-7.39 (m, 2H, ArH), 7.29-7.25 (m, 2H, ArH, NH), 7.15-7.11 (m, 2H, ArH), 6.20-6.10 (m, 1H, allylic-CH), 5.40-5.29 (m, 4H, allylic-$CH_2$); $^{13}$C NMR (100 MHz, $CDCl_3$): δ = 148.5, 144.5, 143.8, 142.7, 133.9, 130.8, 129.6, 129.5, 128.4, 122.7, 122.0, 120.6, 120.1, 110.0, 109.9, 108.8 (ArC), 54.6 (N-$CH_2$); MS (ESI), m/z: 452.4 ($M^+$+1); Anal. Calcd for $C_{25}H_{21}FN_8$: C, 66.36; H, 4.68; N, 24.76, Found: C, 66.48; H, 4.64; N, 24.88.

***N*-[4-(1*H*-Benzimidazol-2-yl)-phenyl]-*N*'-cyclohexyl-*N*''-(4-fluoro-phenyl)-[1,3,5]triazine-2,4,6-triamine (14):** White solid; yield: 72%; mp: 281-283 $^{o}$C; $^{1}$H NMR (400 MHz, $CDCl_3$): δ = 7.98 (d, 2H, *J* = 7.60 Hz, ArH), 7.73-7.63 (m, 3H, ArH), 7.52-7.47 (m, 2H, ArH), 7.24-7.16 (m, 3H, ArH), 7.13 (bs, 1H, NH), 7.01 (t, 2H, *J* = 8.04 Hz, ArH), 5.05 (bs, 1H, NH), 3.81 (s, 1H, N-CH), 2.04-2.02 (m, 2H, $CH_2$), 1.76-1.63 (m, 4H, $CH_2$), 1.38-1.21 (m, 4H, $CH_2$); $^{13}$C NMR (100 MHz, $CDCl_3$ + TFA): δ = 152.5, 147.6, 141.5, 130.8, 128.3, 126.7, 123.5, 123.4, 121.3, 116.7, 116.6, 115.8, 115.5, 113.6 (ArC), 51.7 (CH), 31.9 ($CH_2$), 25.0 ($CH_2$), 24.7 ($CH_2$); MS (ESI), m/z: 494.5 ($M^+$+1); Anal. Calcd for $C_{28}H_{27}FN_8$: C, 68.00; H, 5.50; N, 22.66, Found: C, 68.19; H, 5.46; N, 22.75.

***N*-[4-(1*H*-Benzimidazol-2-yl)-phenyl]-*N*'-(4-fluoro-phenyl)-6-hydrazino-[1,3,5]triazine-2,4-diamine (15):** White solid; yield: 66%; mp: 289-291 $^{o}$C; $^{1}$H NMR (400 MHz, $CDCl_3$ + DMSO-$d_6$): δ = 8.30 (s, 1H, NH), 8.25-8.12 (m, 3H, ArH), 8.00-7.85 (m, 3H, ArH), 7.76-7.59 (m, 3H, ArH), 7.35-7.19 (m, 3H, ArH); $^{13}$C NMR (100 MHz, $CDCl_3$ + DMSO-$d_6$): δ = 151.5, 138.4, 134.9, 132.9, 128.3, 127.2, 126.5, 125.9, 122.4, 117.6, 116.1, 114.7, 114.5 (ArC); MS (ESI), m/z: 427.4 ($M^+$+1); Anal. Calcd for $C_{22}H_{18}FN_9$: C, 61.82; H, 4.24; N, 29.49, Found: C, 61.80; H, 4.21; N, 29.40.

**General procedure for the synthesis of compounds 16-21:** To the stirred solution of *N*-[4-(1*H*-benzimidazol-2-yl)-phenyl]-6-chloro-*N*'-(4-fluoro-phenyl)-[1,3,5]triazine-2,4-diamine **(3)** (0.200 g, 0.463 mmol) in 1,4-dioxane (20 mL), arylboronic acids (0.556 mmol), $Pd(PPh_3)_4$ (10 mol%) and potassium carbonate (0.095g, 0.695 mmol) were added and refluxed for 8-10 hrs in an inert atmosphere. After the completion of reaction, extracted the reaction mixture with chloroform, dried over $Na_2SO_4$, filtered and concentrated to get crude product which was purified through column chromatography using chloroform:methanol (50:1) as eluents to give compounds **16-21**.

***N*-[4-(1*H*-Benzimidazol-2-yl)-phenyl]-*N*'-(4-fluoro-phenyl)-6-phenyl-[1,3,5]triazine-2,4-diamine (16):** White solid; yield: 69%; mp: 246-248 $^{o}$C; $^{1}$H NMR (400 MHz, $CDCl_3$): δ = 7.92 (d, 2H, *J* = 6.84 Hz, ArH), 7.69-7.60 (m, 3H, ArH, NH), 7.52 (d, 3H, *J* = 5.96 Hz, ArH), 7.45 (d, 4H, *J* = 4.60 Hz, ArH), 7.21-7.20 (m, 3H, ArH), 6.96 (t, 3H, *J* = 8.24 Hz, ArH); $^{13}$C NMR (100 MHz, $CDCl_3$): δ = 164.5, 160.3, 157.8, 152.2, 139.2, 135.8, 135.7, 134.4, 129.0, 127.9, 126.3, 122.9, 122.8, 119.0, 116.7, 115.5, 115.3 (ArC); EIMS, m/z: 473.5 ($M^+$+1); Anal. Calcd for $C_{28}H_{20}FN_7$: C, 71.02; H, 4.26; N, 20.71, Found: C, 71.23; H, 4.22; N, 20.79.

***N*-[4-(1*H*-Benzimidazol-2-yl)-phenyl]-6-(4-chloro-phenyl)-*N*'-(4-fluoro-phenyl)-[1,3,5]triazine-2,4-diamine (17):** White solid; yield: 68%; mp: 262-264 $^{o}$C; $^{1}$H NMR (400 MHz, $CDCl_3$ + DMSO-$d_6$): δ = 8.69 (d, 2H, *J* = 7.76 Hz, ArH), 8.10-8.05 (m, 4H, ArH), 7.84-7.77 (m, 2H, ArH), 7.69-7.63 (m, 4H, ArH), 7.56 (d, 1H, *J* = 7.36 Hz, ArH), 7.50 (d, 1H, *J* = 6.88 Hz, ArH), 7.28 (t, 2H, *J* = 6.88 Hz, ArH); $^{13}$C NMR (100 MHz, $CDCl_3$): δ = 170.0, 165.1, 164.4, 152.0, 138.8, 137.7, 135.8, 135.4, 134.0, 132.2, 132.1, 132.0, 129.7, 129.2, 128.6, 128.5, 128.0, 125.8, 119.2, 115.7, 101.5 (ArC); EIMS, m/z: 507.9 ($M^+$+1); Anal. Calcd for $C_{28}H_{19}ClFN_7$: C, 66.21; H, 3.77; N, 19.30, Found: C, 66.11; H, 3.74; N, 19.38.

***N*-[4-(1*H*-Benzimidazol-2-yl)-phenyl]-*N*'-(4-fluoro-phenyl)-6-thiophen-2-yl-[1,3,5]triazine-2,4-diamine (18):** White solid; yield: 67%; mp: 274-276 $^{o}$C; $^{1}$H NMR (400 MHz, $CDCl_3$): δ = 7.98 (d, 3H, *J* = 8.72 Hz, ArH), 7.73-7.62 (m, 4H, ArH), 7.54-7.50 (m, 3H, ArH), 7.35 (d, 2H, *J* = 3.6 Hz, ArH), 7.06 (t, 3H, *J* = 9.16 Hz, ArH); $^{13}$C NMR (100 MHz, $CDCl_3$): δ = 170.9, 165.1, 164.5, 152.0, 138.7, 136.8, 135.8, 134.1, 131.6, 129.2, 128.3, 128.0, 125.9, 119.1, 115.6, 101.4 (ArC); MS (ESI), m/z: 479.5 ($M^+$+1); Anal. Calcd for $C_{26}H_{18}FN_7S$: C, 65.12; H, 3.78; N, 20.45; S, 6.69, Found: C, 65.29; H, 3.75; N, 20.42; S, 6.75.

***N*-[4-(1*H*-Benzimidazol-2-yl)-phenyl]-*N*'-(4-fluoro-phenyl)-6-furan-2-yl-[1,3,5]triazine-2,4-diamine (19):** White solid; yield: 71%; mp: 248-250 $^{o}$C; $^{1}$H NMR (400 MHz, $CDCl_3$): δ = 7.95 (d, 2H, *J* = 8.24 Hz, ArH), 7.58-7.52 (m, 4H, ArH), 7.48-7.45 (m, 3H, ArH), 7.24-7.22 (m, 4H, ArH), 7.02-6.98 (m, 2H, ArH); $^{13}$C NMR (100 MHz, $CDCl_3$): δ = 170.6, 164.7, 152.2, 139.5, 135.9, 134.3, 132.5, 130.6, 129.1, 126.2, 122.8, 119.2, 115.7, 115.5, 102.2 (ArC); MS (ESI), m/z: 463.4 ($M^{+}$+1); Anal. Calcd for $C_{26}H_{18}FN_7O$: C, 67.38; H, 3.91; N, 21.16, Found: C, 67.46; H, 3.88; N, 21.29.

***N*-[4-(1*H*-Benzimidazol-2-yl)-phenyl]-*N*'-(4-fluoro-phenyl)-6-(2-methoxy-phenyl)-[1,3,5]triazine-2,4-diamine (20):** White solid; yield: 65%; mp: 288-290 $^{o}$C; $^{1}$H NMR (400 MHz, $CDCl_3$): δ 7.98 (d, 3H, *J* = 8.72 Hz, ArH), 7.81 (s, 1H, ArH), 7.67 (d, 3H, *J* = 8.00 Hz, ArH), 7.52-7.50 (m, 5H, ArH, NH), 7.27 (d, 2H, *J* = 2.76 Hz, ArH), 7.06 (t, 3H, *J* = 8.24 Hz, ArH), 3.96 (s, 3H, $OCH_3$); $^{13}$C NMR (100 MHz, $CDCl_3$): δ = 152.7, 144.8, 139.2, 135.5, 132.6, 132.0, 129.2, 129.0, 127.9, 127.4, 126.7, 126.2, 122.5, 121.5, 117.0, 113.9, 111.9, 109.4 (ArC), 55.3 ($OCH_3$); MS (ESI), m/z: 503.5 ($M^{+}$+1); Anal. Calcd for $C_{29}H_{22}FN_7O$: C, 69.17; H, 4.40; N, 19.47, Found: C, 69.03; H, 4.33; N, 19.41.

***N*-[4-(1*H*-Benzimidazol-2-yl)-phenyl]-*N*'-(4-fluoro-phenyl)-6-(4-methoxy-phenyl)-[1,3,5]triazine-2,4-diamine (21):** White solid; yield: 70%; mp: 266-268 $^{o}$C; $^{1}$H NMR (400 MHz, $CDCl_3$): δ = 7.97 (d, 3H, *J* = 8.72 Hz, ArH), 7.66 (d, 3H, *J* = 8.24 Hz, ArH), 7.52-7.50 (m, 3H, ArH), 7.32 (bs, 1H, NH), 7.28-7.27 (m, 3H, ArH), 7.25 (s. 1H, ArH), 7.05 (t, 3H, *J* = 8.72 Hz, ArH), 3.96 (s, 3H, $OCH_3$); $^{13}$C NMR (100 MHz, $CDCl_3$): δ = 163.2, 159.7, 157.0, 147.2, 136.4, 132.2, 130.2, 128.5, 127.6, 126.8, 122.4, 116.6, 114.0, 113.6, (ArC), 55.4 ($OCH_3$); MS (ESI), m/z: 503.5 ($M^{+}$+1); Anal. Calcd for $C_{29}H_{22}FN_7O$: C, 69.17; H, 4.40; N, 19.47, Found: C, 69.05; H, 4.37; N, 19.40.

## Materials and methods

The stock solution of ct-DNA (Sigma Chemical Co., USA) was prepared by dissolving an appropriate amount of ct-DNA in Millipore water. The purity of ct-DNA was verified by monitoring the ratio of the absorbance at 260 and 280 nm, and the ratio of $A_{260}/A_{280}$ was 1.82, indicating that ct-DNA was sufficiently free of protein contamination. The concentration of ct-DNA in terms of the nucleotide phosphate (i.e. nucleotide) was determined to be $2.56 \times 10^{-3}$ $molL^{-1}$ by UV-Vis absorption at 260 nm using a molar absorption coefficient of $\varepsilon_{260}$ = 6600 L $mol^{-1}cm^{-1}$(expressed as the molarity of phosphate groups). All stock solutions were diluted to the required concentrations with phosphate buffer (pH 7.4). All other reagents were of analytical reagent grade, and ultrapure water was used throughout the experiment. All stock solutions were stored at 0–4 $^{0}$C.

## UV-Vis absorption spectra

All the spectra were recorded at ambient temperature (300 K). UV-Vis spectra were recorded on Shimadzu-2400 PC spectrometer with 1 cm cuvette. Any background buffer signal was electronically subtracted. Absorption titrations were performed with constant ligand concentration of 20 μM and increasing ct-DNA concentration (up to 15 μM). The control experiment was done by adding equal volumes of buffer solution instead of DNA solution to the same concentration of compound. The control experiment shows no significant change in the absorption spectra of the compound.

**Fluorescence spectra**

Emission spectra were recorded with Varian Cary Eclipse fluorescence spectrometer. Fluorescence titration spectra were obtained with a constant ligand concentration (20 μM) and increasing amount of ct-DNA (up to 17 μM).

**Binding constants**

Binding constant K for the ligand–DNA complex was estimated from absorption and fluorescence titration data using the Benesi–Hildebrand equation-1 that suggested strong interactions with ct-DNA.

$$1/(A_f - A_{obs}) = 1/(A_f - A_{fc}) + \{1/[K(A_f - A_{fc})]\}[\text{ligand}] \quad \text{------ 1}$$

where K is the binding constant, $A_f$ is the absorbance of the free host, $A_{obs}$ is the absorbance observed, and $A_{fc}$ is the absorbance at saturation.

K was determined from the ratio of intercept to slope obtained from the linear fit of the plot of $1/(A_f - A_{obs})$ versus 1/[DNA], respectively. The control experiment was done by adding equal volumes of buffer solution instead of DNA solution to the same concentration of the compound. No significant change in the emission spectra was observed in control experiment.

**DNA melting studies**

Thermal melting curves were measured with Shimadzu-2400 PC spectrometer equipped with Peltier temperature controller system (±0.1 °C). ct-DNA was treated with compounds (ratio of DNA to compound = 1:1) and kept at 25 °C for 5 minutes. All the solutions were degassed before the experiment. The absorbance of ct-DNA (50 μM) in buffer (pH 7.4) was measured at 260 nm. The samples were heated from 20 °C to 100 °C and the absorbance was measured after attaining thermal equilibrium at a particular temperature with an interval of 1 °C. The absorbance of the compound was subtracted at each point. $T_m$ was determined from the 1$^{st}$ derivative of the absorbance vs. temperature curve.

**Competitive binding study of compounds 7, 9-11, 16 and 21 with ct-DNA in the presence of ethidium bromide and Hoechst 33258**

Competitive binding of ligands with DNA in the presence of intercalator ethidium bromide and classical minor groove binder Hoechst 33258 were also studied. Ethidium bromide and Hoechst 33258 (Sigma) were dissolved in Milli-Q water to prepare a stock solution with 1 mM strength and diluted immediately before use. Fluorescence spectra were taken in the presence of ethidium bromide and Hoechst 33258 (10 μM) while increasing the amount of ct-DNA (0 to 20 μM) and using an excitation wavelength of 480 nm and 353 nm. Compounds were added with an increasing concentration of 2 μM (up to 30 μM), mixed thoroughly after each addition and the emission spectrum was recorded with $\lambda_{ex}$ = 480 nm and $\lambda_{ex}$ = 353 nm.

**Viscosity measurements**

Viscosity measurements were conducted on a viscometer, immersed in a thermostatic water-bath maintained at a constant temperature of 25 ± 0.1 $^0$C. The experiments were carried out by adding different concentration of ligand into the viscometer while keeping the ct-DNA concentration constant. The flow time (t) of the solution through the capillary was measured with a digital stopwatch, and the average flow time of five parallel measurements was used to evaluate the relative viscosity of the samples. The data were presented as the relative viscosity of ct-DNA $(\eta/\eta_0)^{1/3}$ versus LDR (ligand-DNA ratio), where $\eta$ and $\eta_0$ represent the viscosity of ct-DNA in the presence and absence of ligand, respectively.

**Ionic strength and Iodide quenching effect**

Effects of ionic strength and iodide on the interaction between compounds and ct-DNA were studied by varying the concentration of NaCl and KI between 0 and 10 μM in a total volume of 3 ml containing compounds **7**, **9-11**, **16** and **21** (20 μM). In another set, a similar experiment was done in the presence of 15 μM of ct-DNA. It was performed in the presence and absence of ct-DNA.

**Docking studies**

Compounds were built using the builder tool kit of the software package Argus Lab 4.0.1.23 and energy minimized with semi-empirical quantum mechanical method PM3. Crystal coordinates of DNA (PDB ID 1BNA) was downloaded from protein data bank and in the molecule tree view of the software, the monomeric structures of the crystal co-ordinate was selected and the active site was defined as 15 Å around the ligand. Validation of the docking programme was checked by docking the known inhibitors of the respective enzymes in their binding sites. The molecule to be docked in the active site of the enzyme was pasted in the work space carrying the structure of the

enzyme. The docking programme implements an efficient grid based docking algorithm which approximates an exhaustive search within the free volume of the S19 binding site cavity. The conformational space was explored by the geometry optimization of the flexible ligand (rings were treated as rigid) in combination with the incremental construction of the ligand torsions. Thus, docking occurs between the flexible ligand parts of the compound and enzyme. The docking was repeated several times (approx. 10,000 iterations) until no change in the position of the ligand and a constant value of the binding energy was observed. The ligand orientation was determined by a shape scoring function based on Ascore and the final positions were ranked by lowest interaction energy values. H-bond interactions between the respective compound and enzyme were explored. During computational docking, a pose is typically generated, scored and compared to the previous pose(s). The current pose is then accepted or rejected on the basis of the score for that pose.

## $^{1}$H and $^{13}$C NMR spectra of compounds

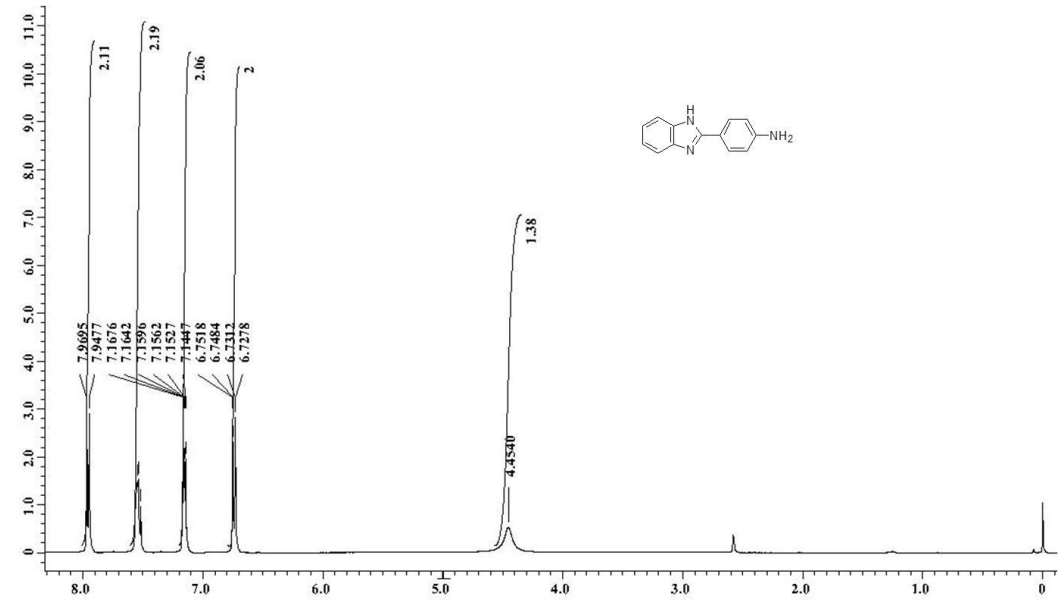


**Figure S1**. $^{1}$H NMR spectrum of compound **2** (DMSO-$d_6$).

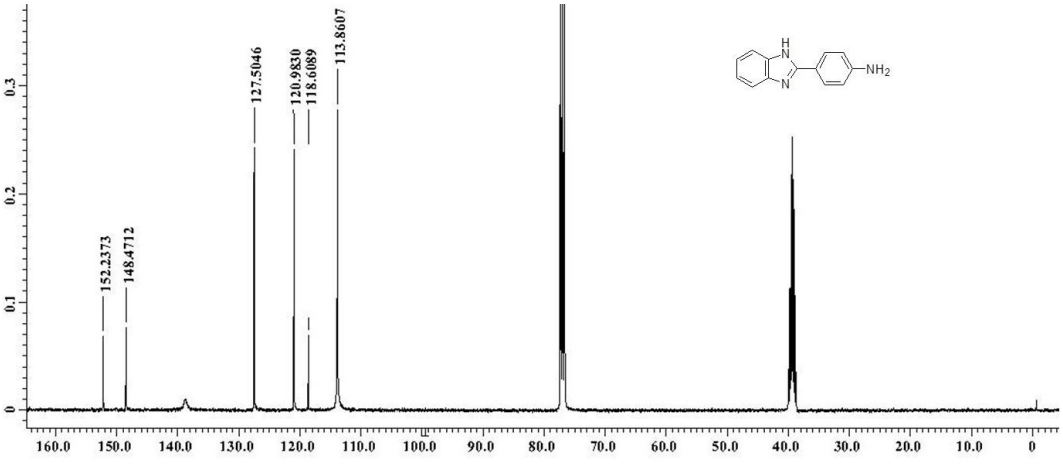


**Figure S2**. $^{13}$C NMR spectrum of compound **2** ($CDCl_3$ + DMSO-$d_6$).

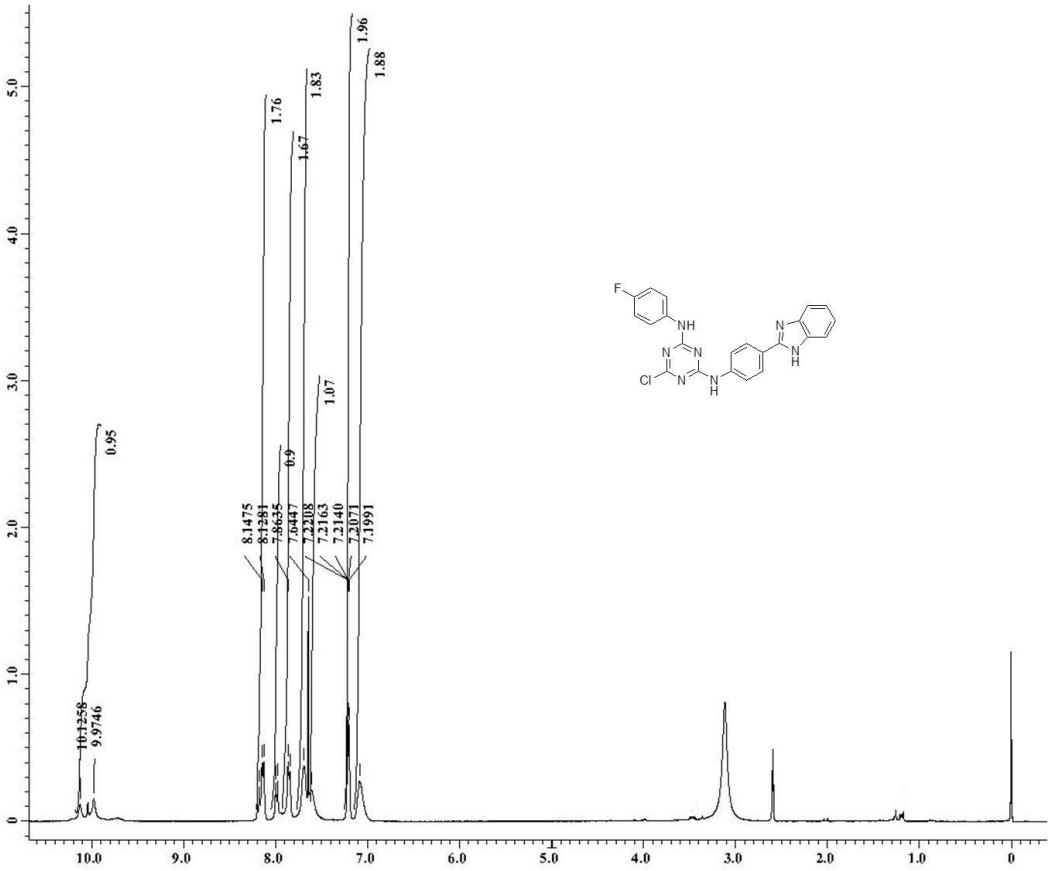


**Figure S3**. $^{1}$H NMR spectrum of compound **3** ($CDCl_3$ + DMSO-$d_6$).

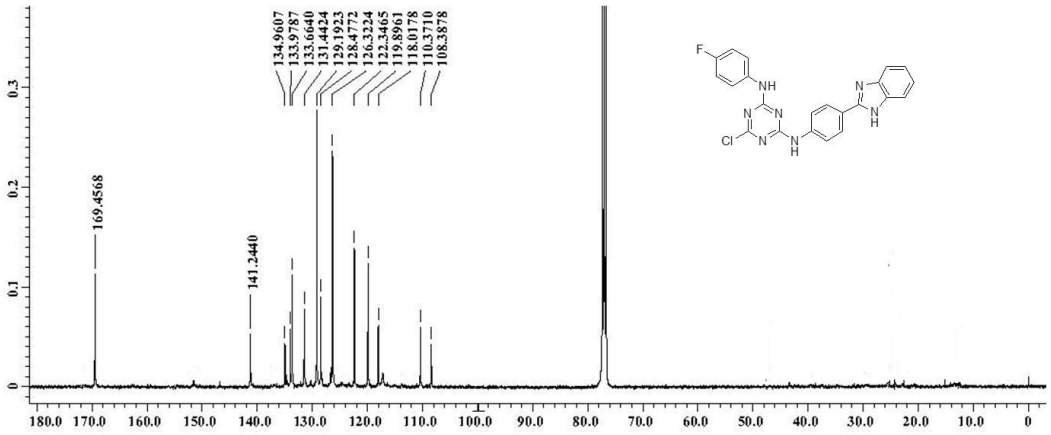


**Figure S4**. $^{13}$C NMR spectrum of compound **3** ($CDCl_3$).

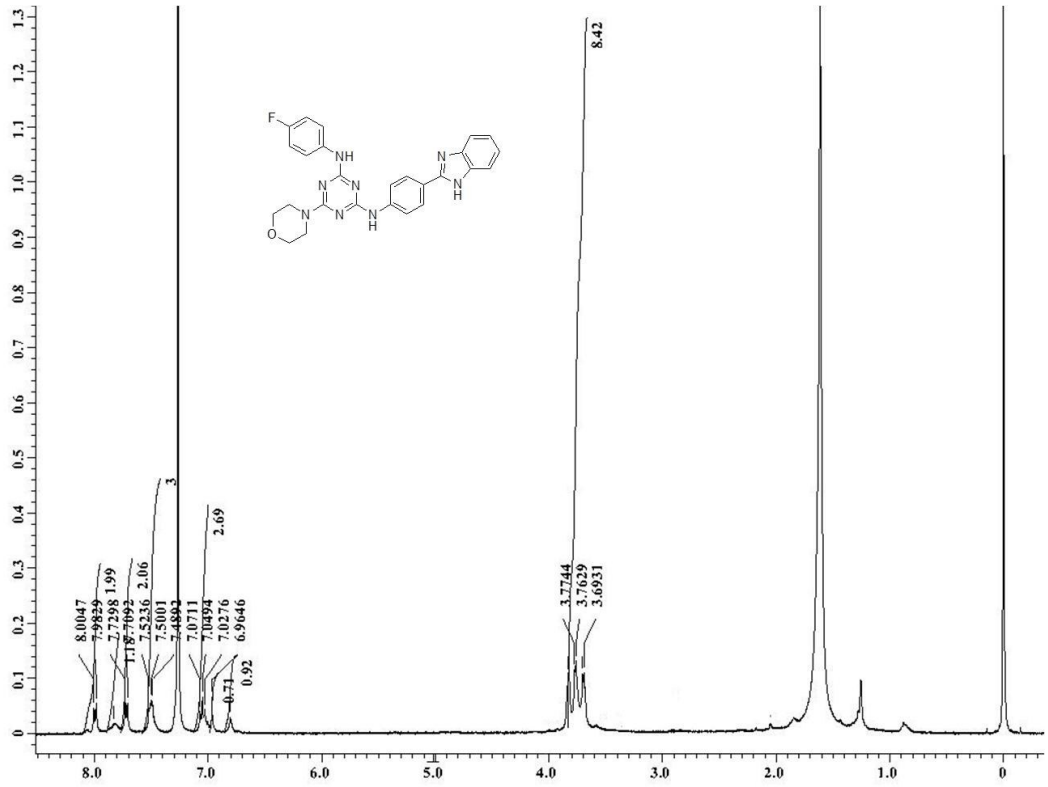


**Figure S5**. $^{1}$H NMR spectrum of compound **4** ($CDCl_3$).

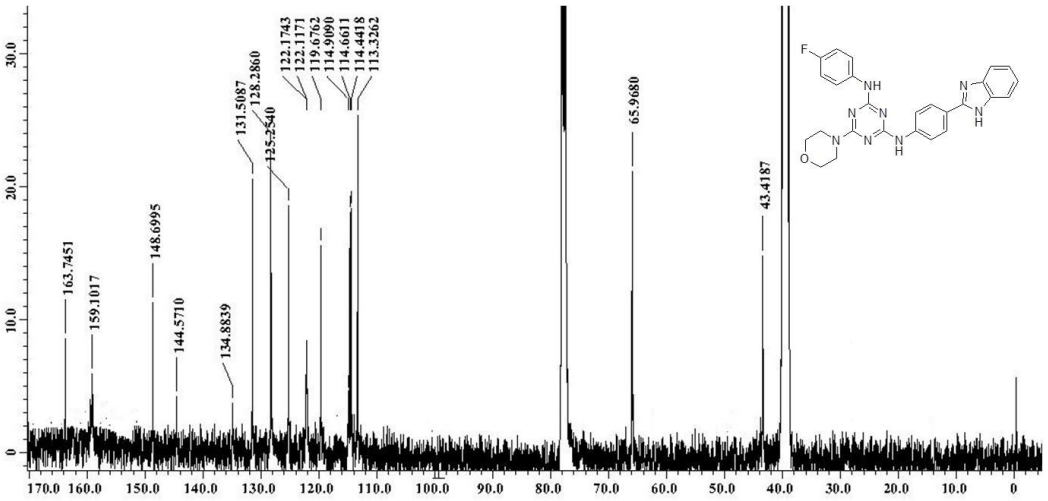


**Figure S6**. $^{13}$C NMR spectrum of compound **4** ($CDCl_3$ + DMSO-$d_6$).

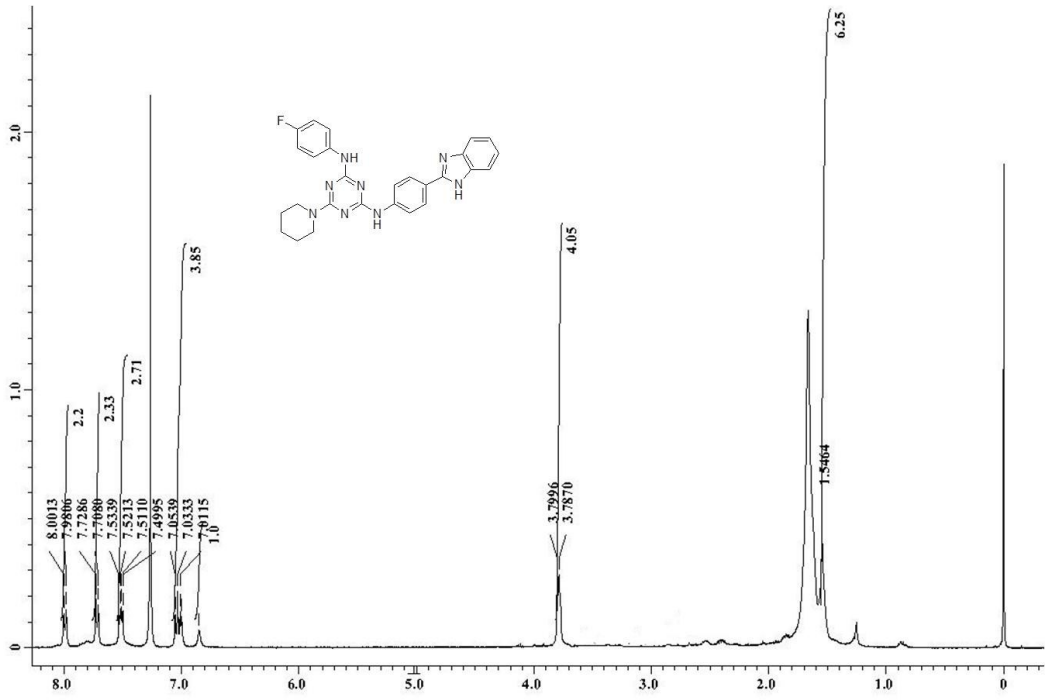


**Figure S7**. $^{1}$H NMR spectrum of compound **5** ($CDCl_3$).

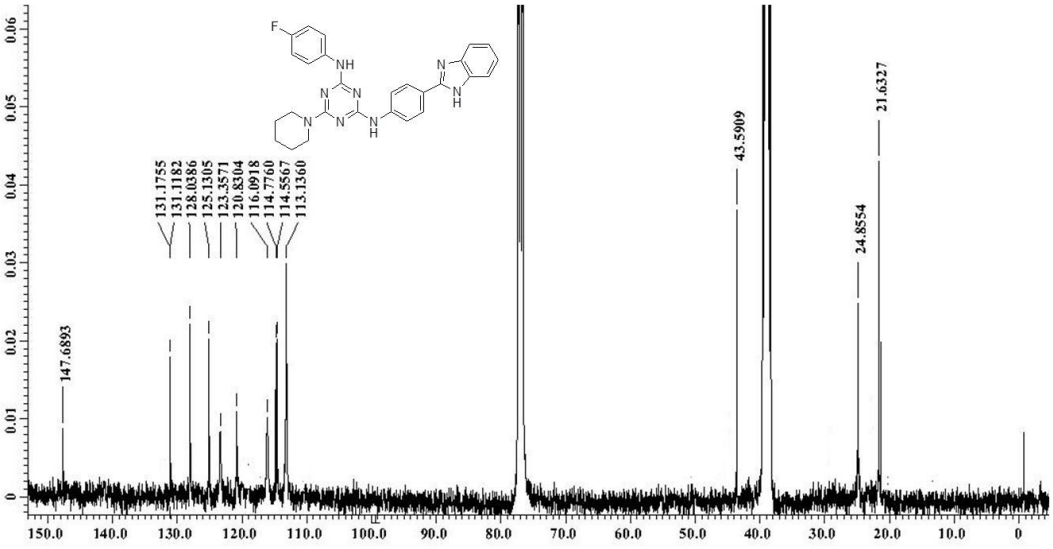


**Figure S8**. $^{13}$C NMR spectrum of compound **5** ($CDCl_3$ + DMSO-$d_6$).

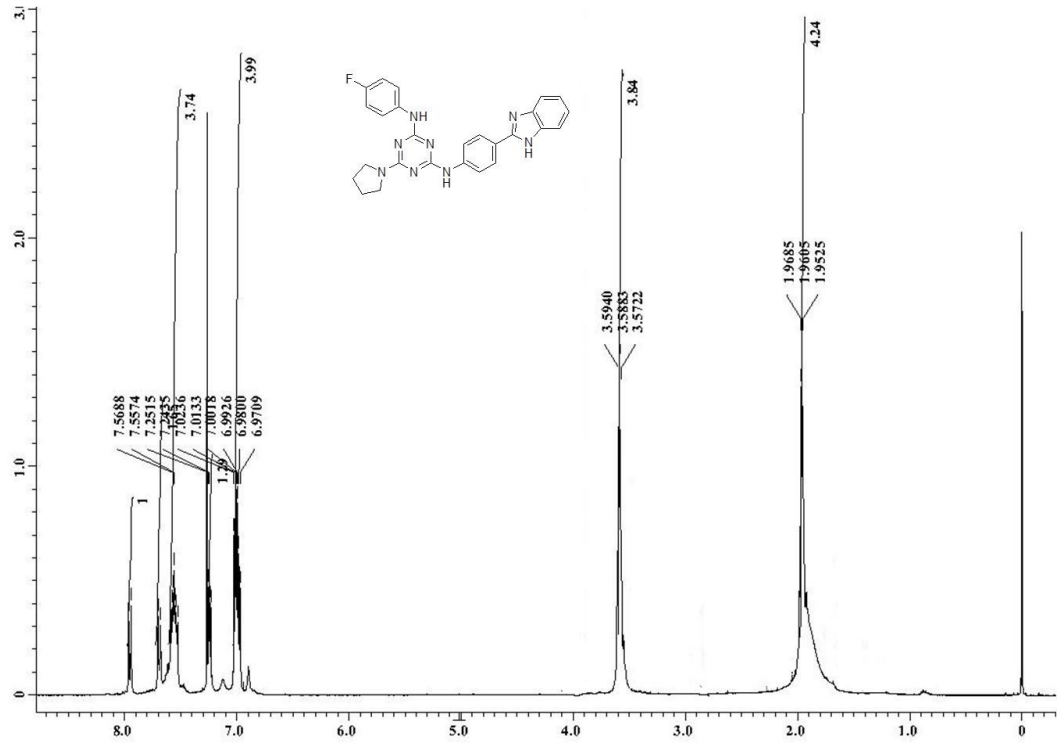


**Figure S9**. $^{1}$H NMR spectrum of compound **6** ($CDCl_3$).

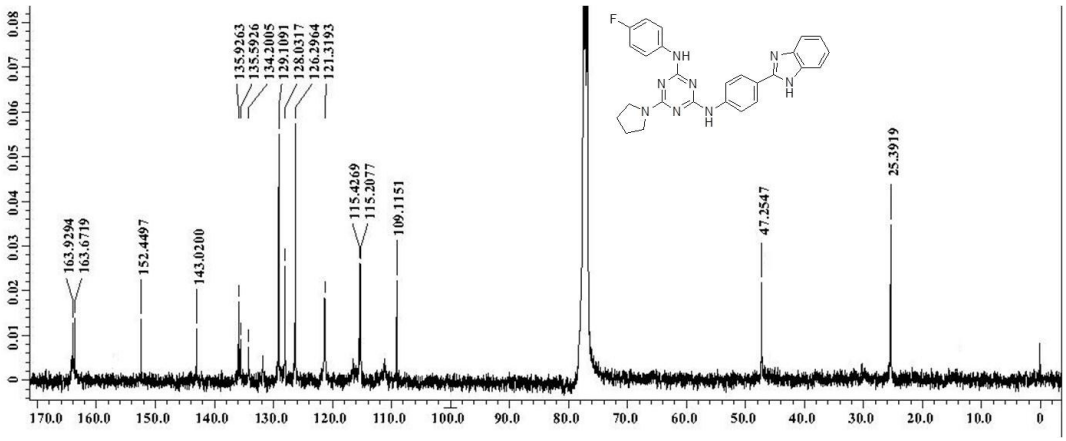


**Figure S10**. $^{13}$C NMR spectrum of compound **6** ($CDCl_3$).

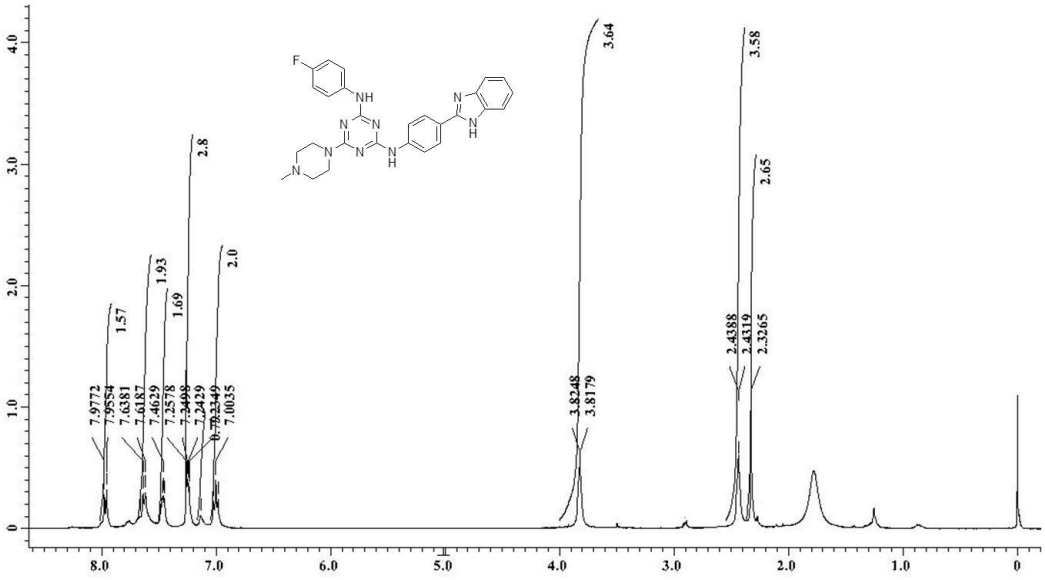


**Figure S11**. $^{1}H$ NMR spectrum of compound **7** ($CDCl_3$).

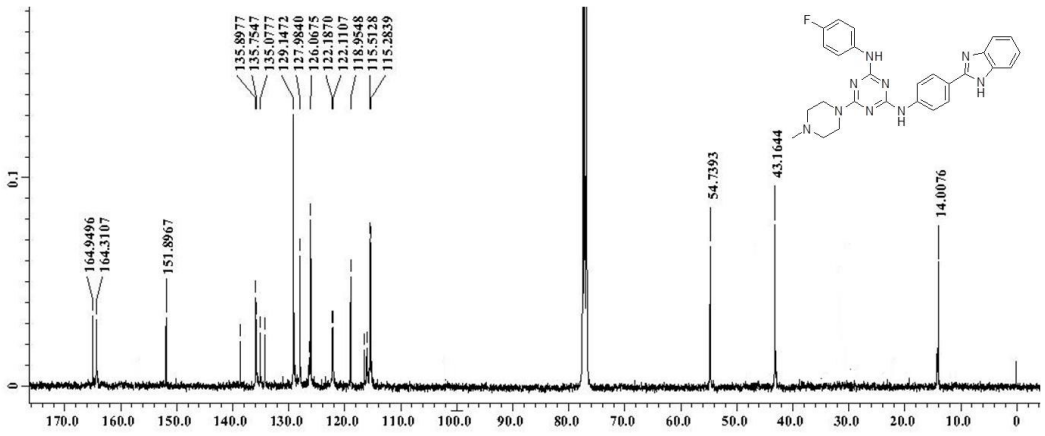


**Figure S12**. $^{13}C$ NMR spectrum of compound **7** ($CDCl_3$).

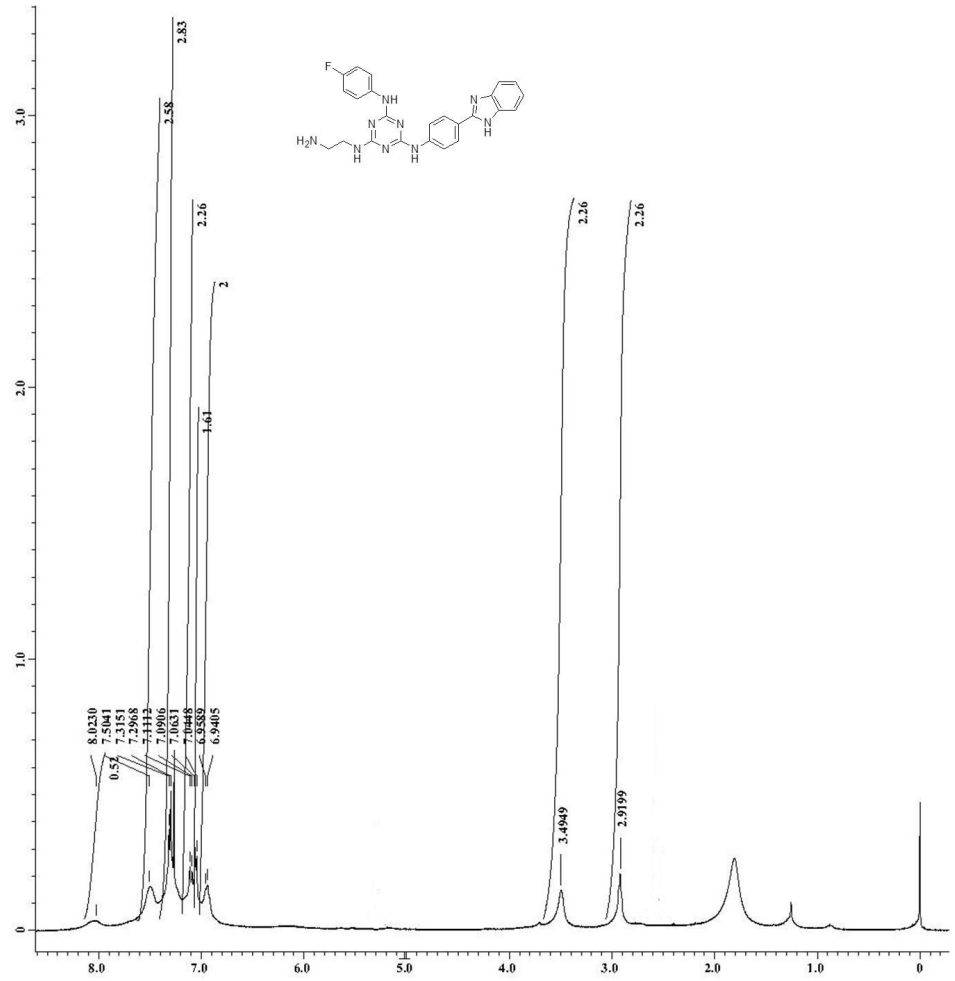


**Figure S13**. $^{1}H$ NMR spectrum of compound **8** ($CDCl_3$).

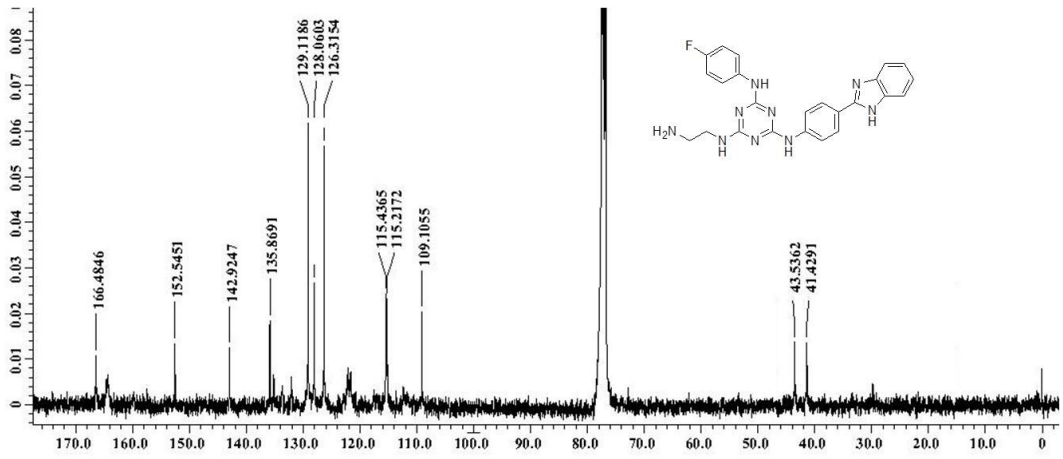


**Figure S14**. $^{13}C$ NMR spectrum of compound **8** ($CDCl_3$).

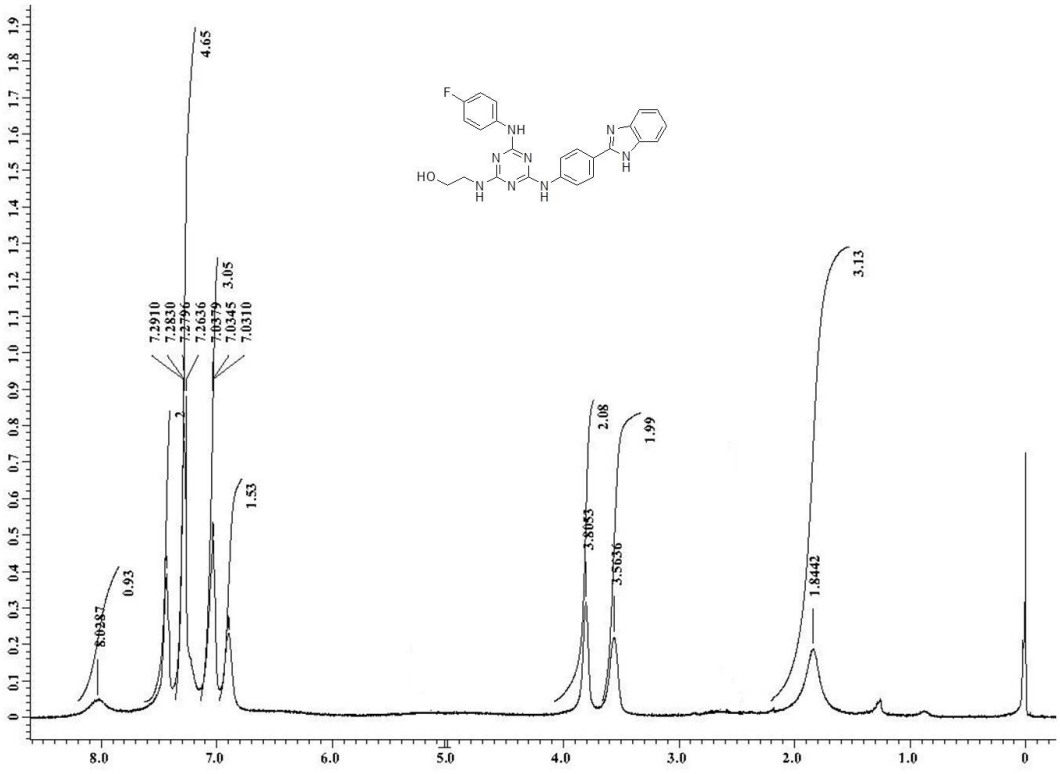


**Figure S15**. $^{1}H$ NMR spectrum of compound **9** ($CDCl_3$).

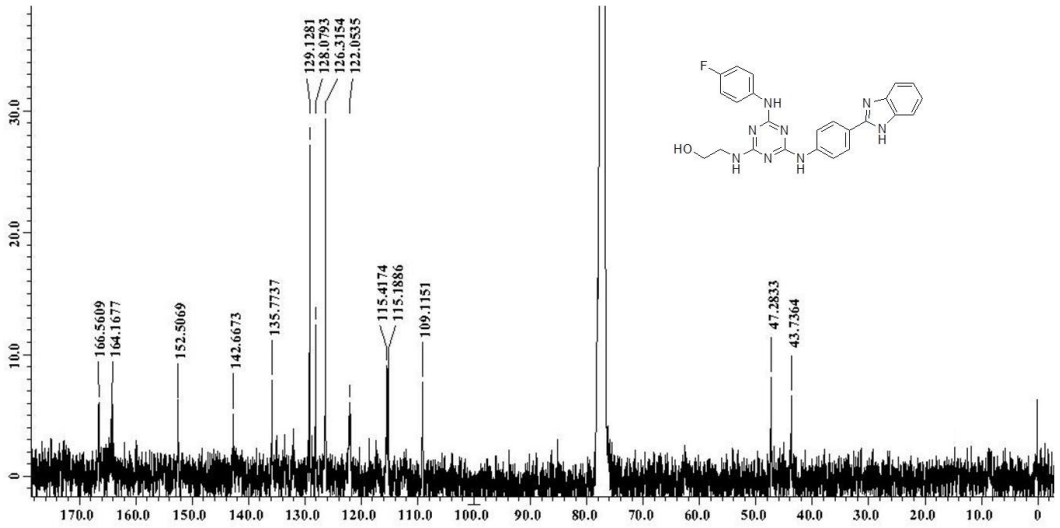


**Figure S16**. $^{13}C$ NMR spectrum of compound **9** ($CDCl_3$).

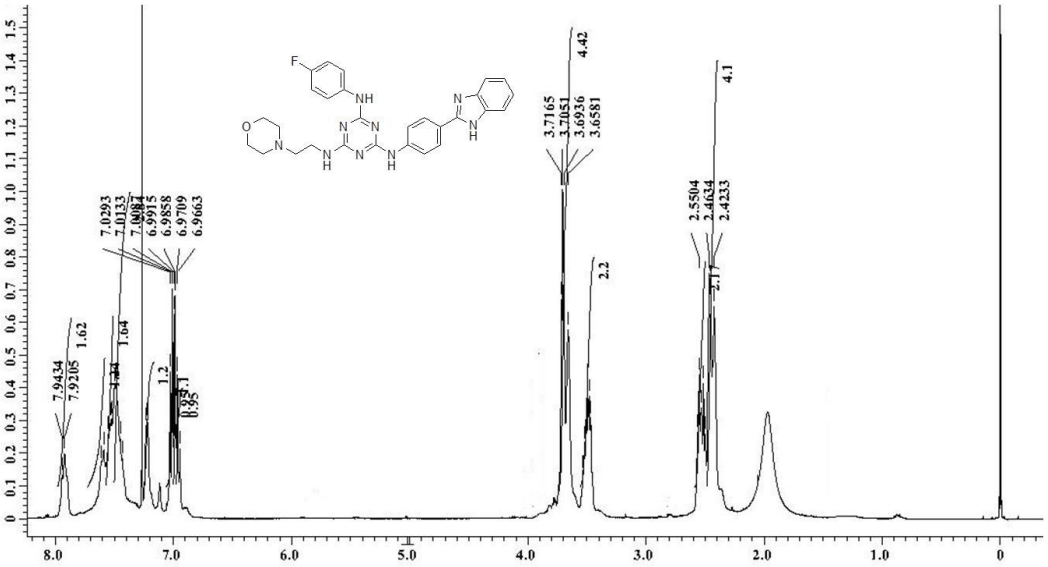


**Figure S17**. $^{1}H$ NMR spectrum of compound **10** ($CDCl_3$).

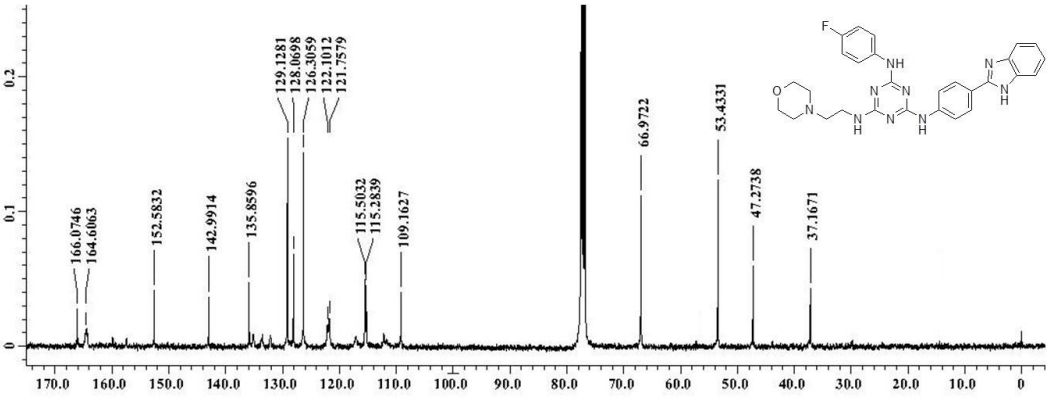


**Figure S18**. $^{13}C$ NMR spectrum of compound **10** ($CDCl_3$).

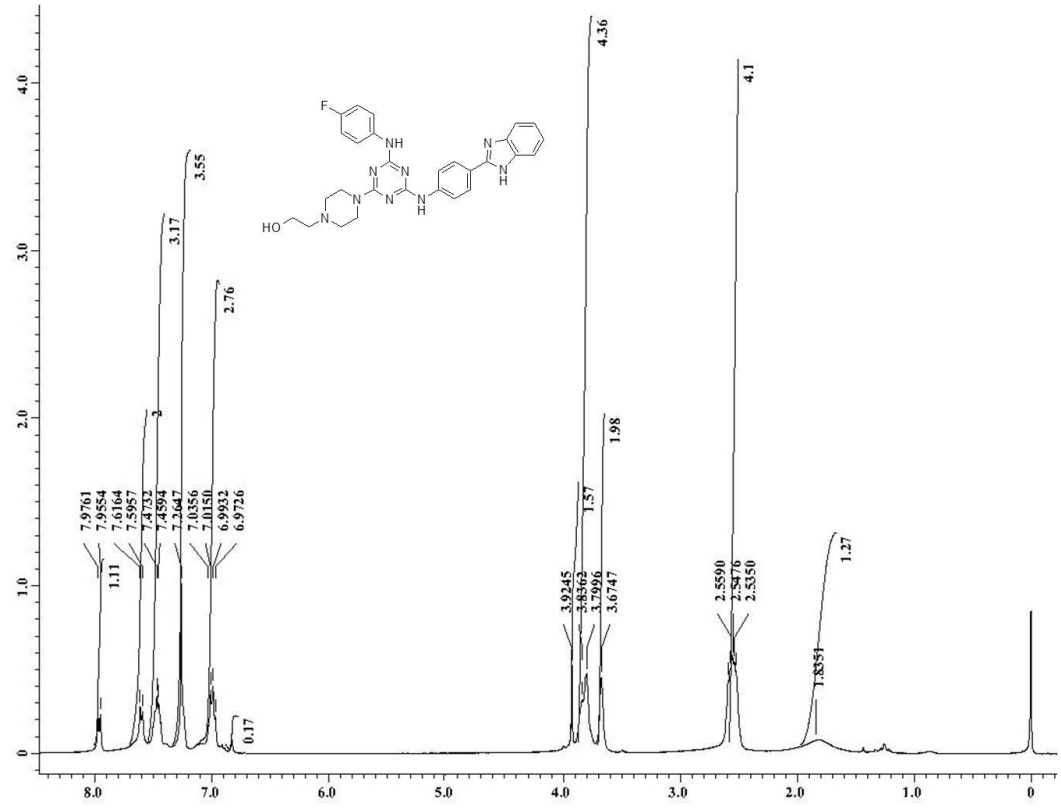


**Figure S19**. $^{1}H$ NMR spectrum of compound **11** ($CDCl_3$).

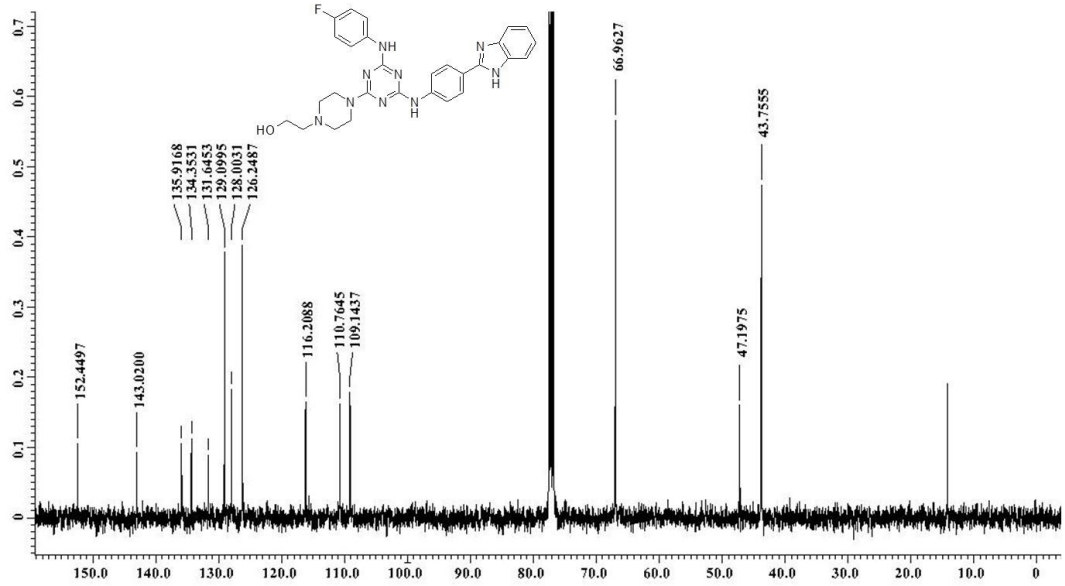


**Figure S20**. $^{13}C$ NMR spectrum of compound **11** ($CDCl_3$).

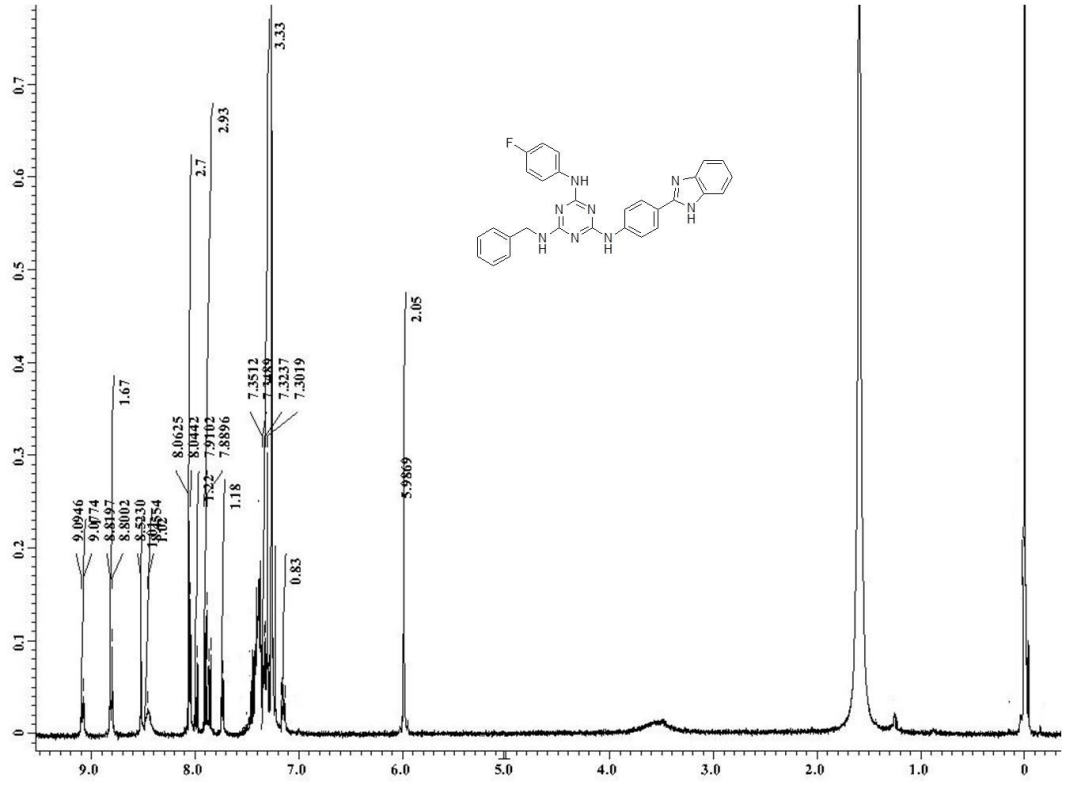


**Figure S21**. $^{1}$H NMR spectrum of compound **12** ($CDCl_3$).

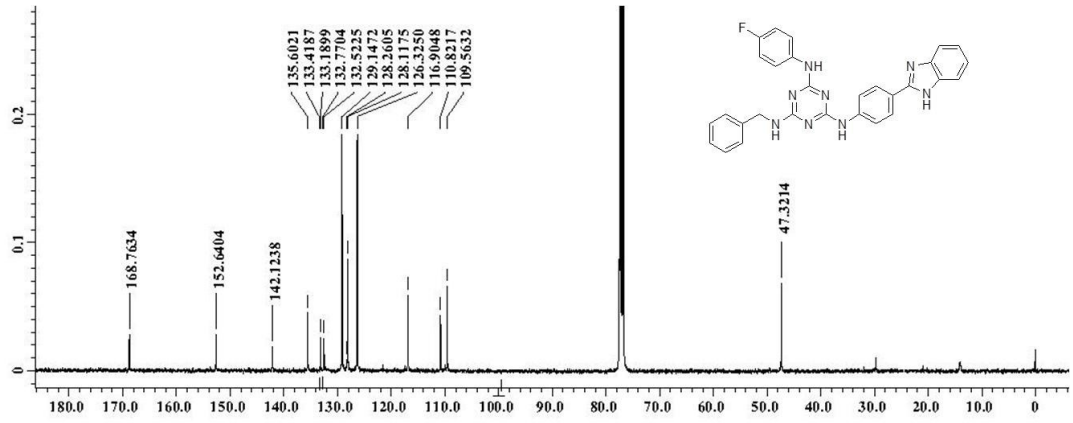


**Figure S22**. $^{13}$C NMR spectrum of compound **12** ($CDCl_3$).

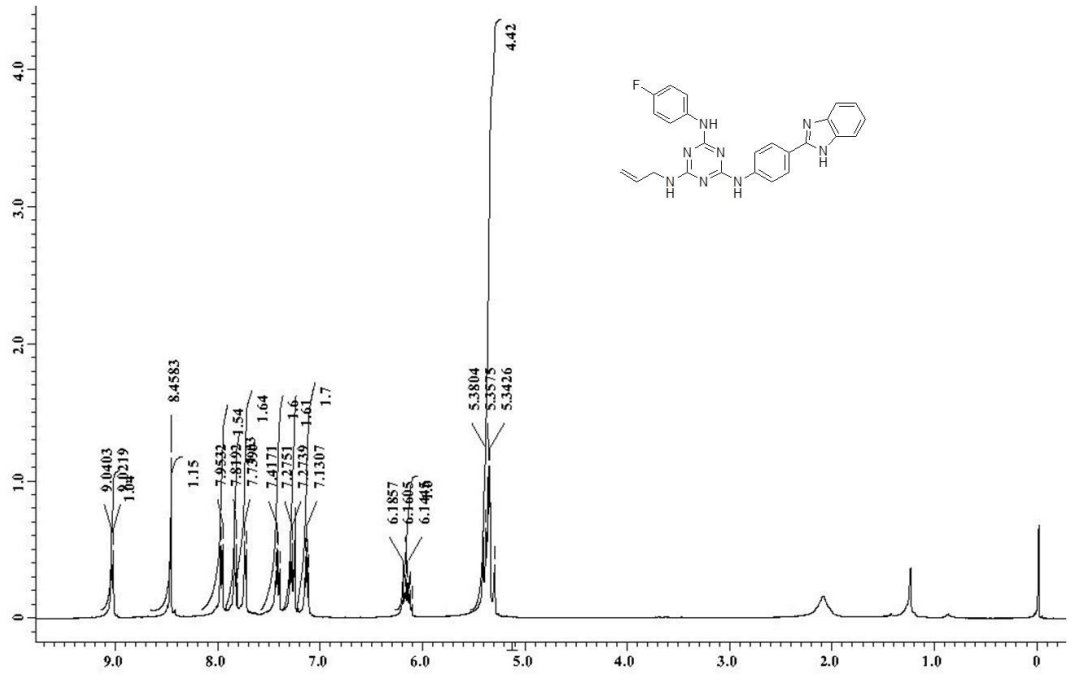


**Figure S23**. $^{1}$H NMR spectrum of compound 1**3** ($CDCl_3$).

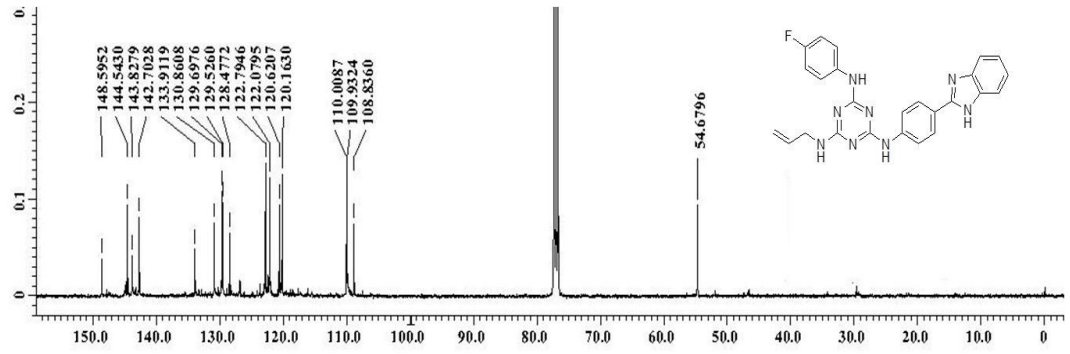


**Figure S24**. $^{13}$C NMR spectrum of compound 1**3** ($CDCl_3$).

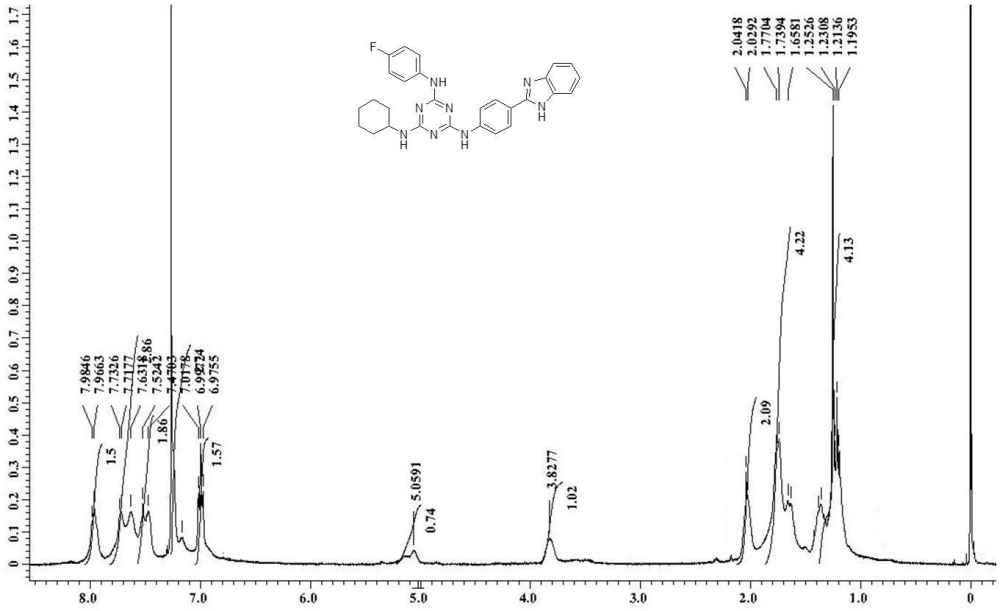


**Figure S25**. $^{1}H$ NMR spectrum of compound **14** ($CDCl_3$).

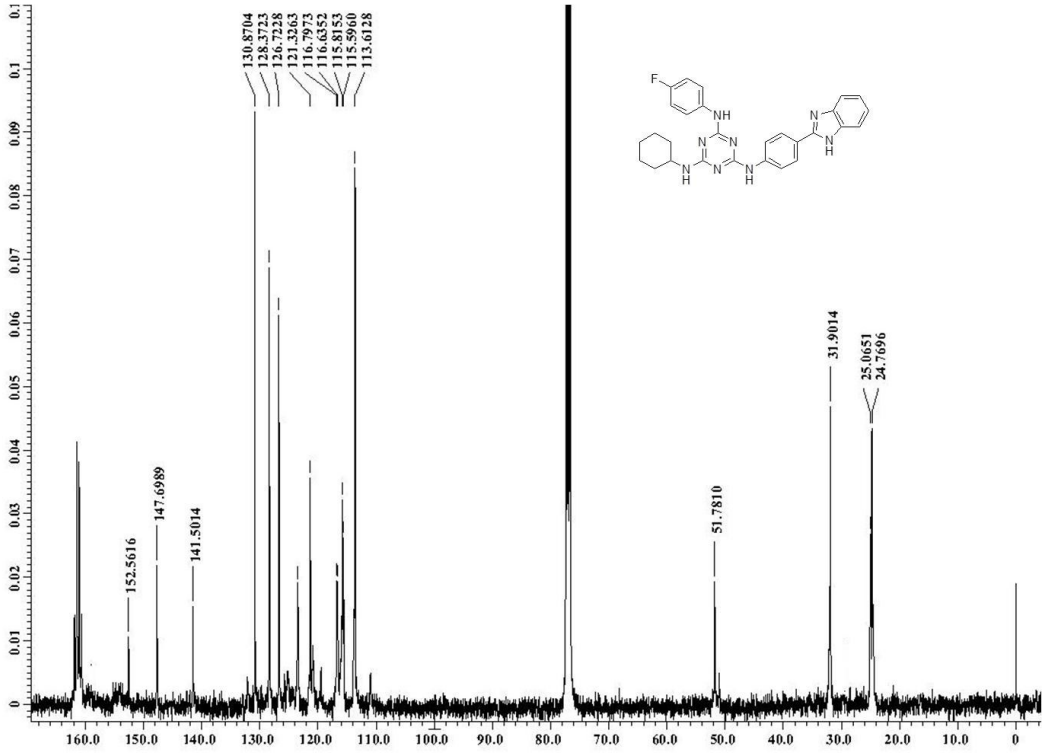


**Figure S26**. $^{13}C$ NMR spectrum of compound **14** ($CDCl_3$ + TFA).

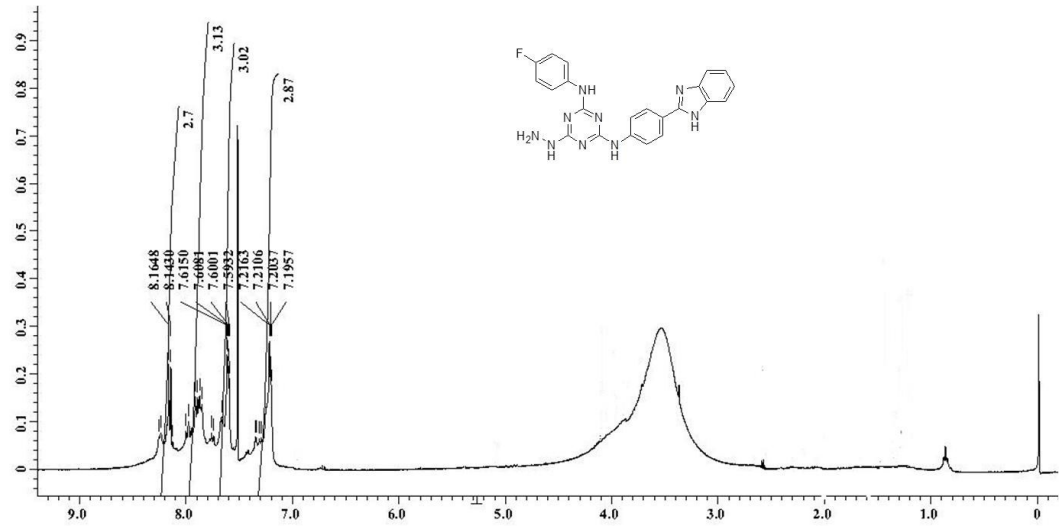


**Figure S27**. $^{1}$H NMR spectrum of compound **15** ($CDCl_3$ + DMSO-$d_6$).

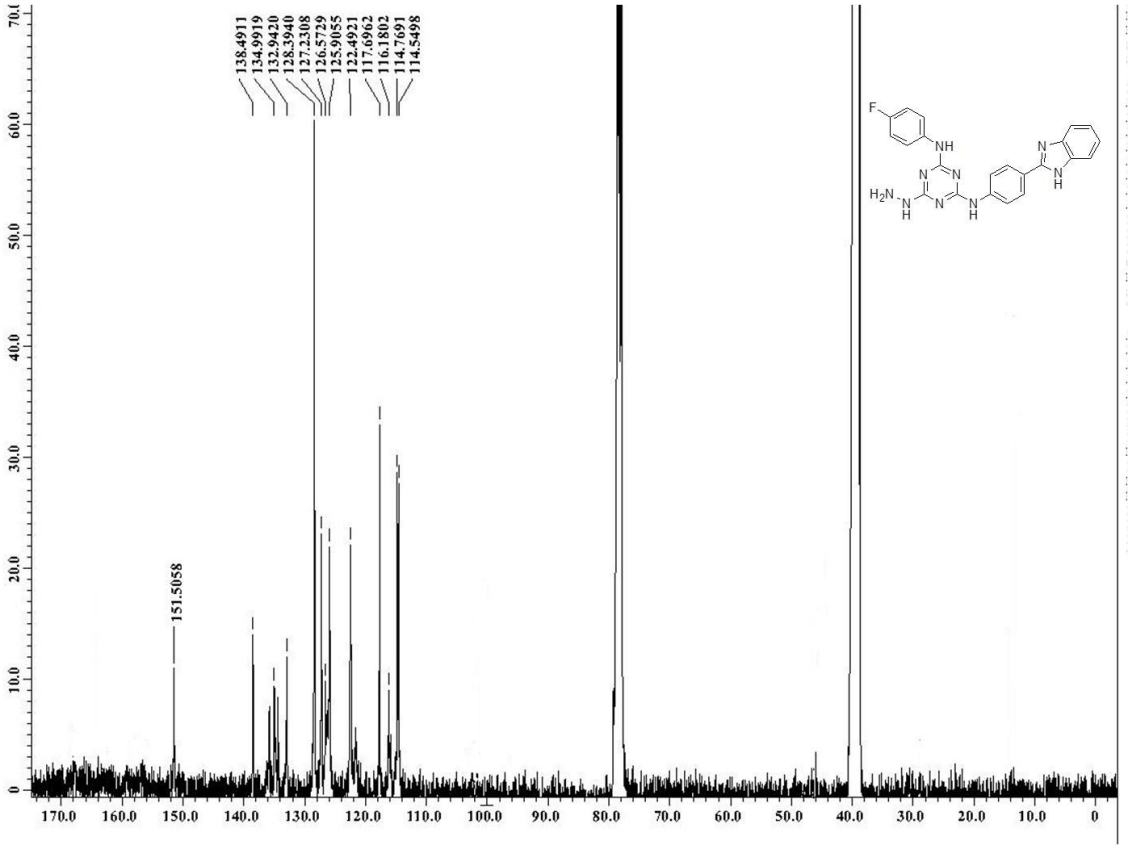


**Figure S28**. $^{13}$C NMR spectrum of compound **15** ($CDCl_3$ + DMSO-$d_6$).

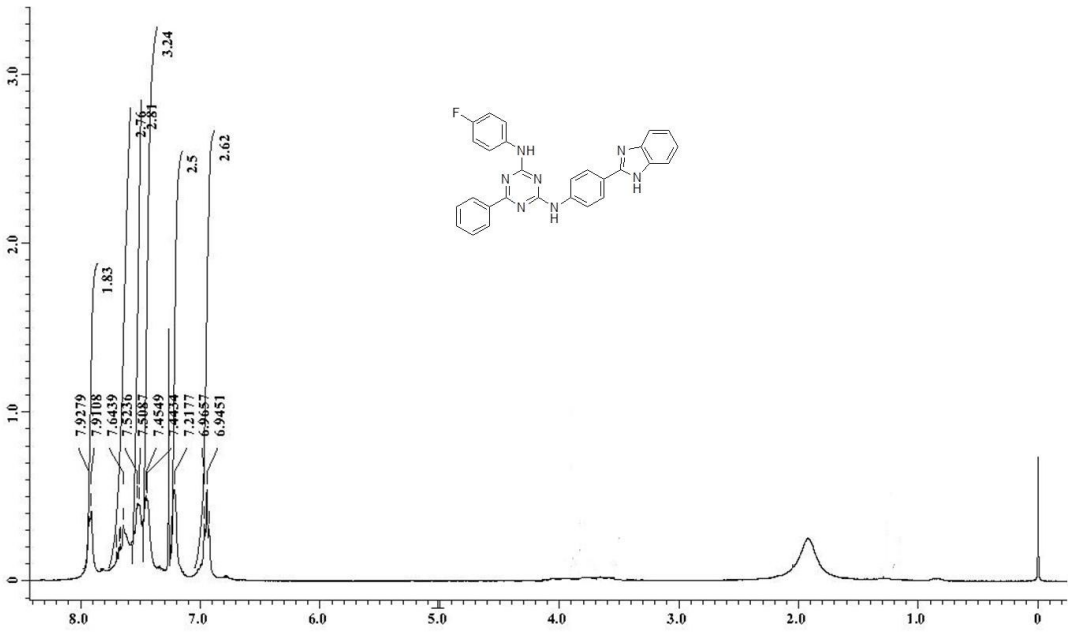


**Figure S29**. $^{1}H$ NMR spectrum of compound **16** ($CDCl_3$).

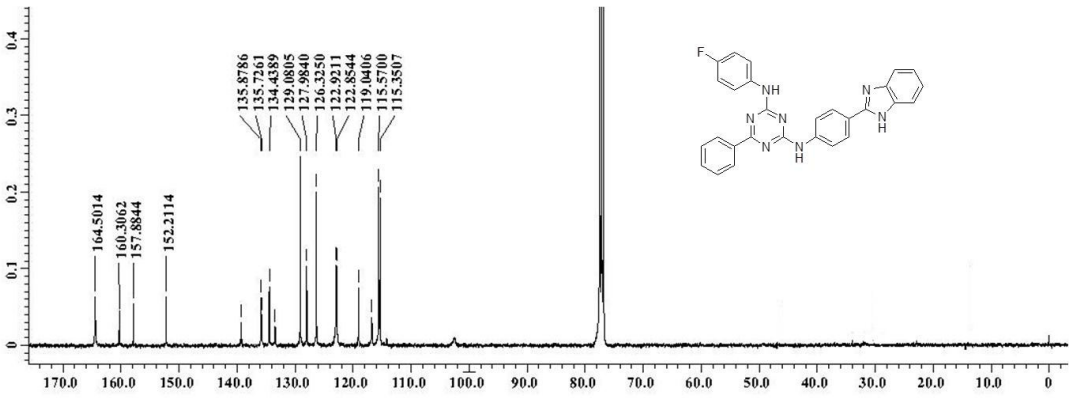


**Figure S30**. $^{13}C$ NMR spectrum of compound **16** ($CDCl_3$).

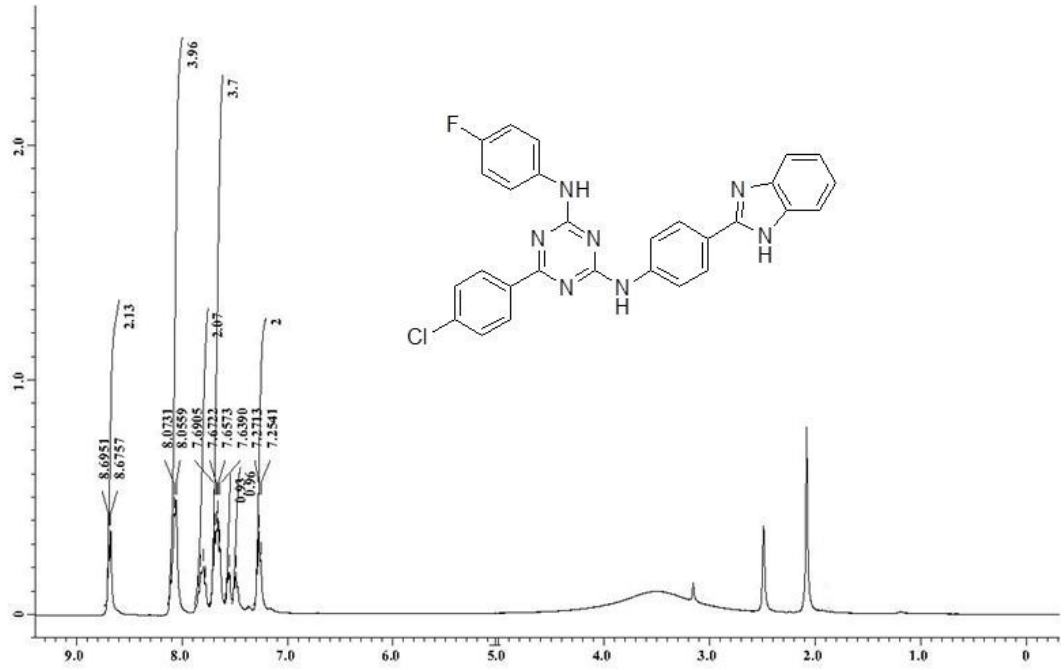


**Figure S31**. $^{1}$H NMR spectrum of compound **17** ($CDCl_3$ + DMSO-$d_6$).

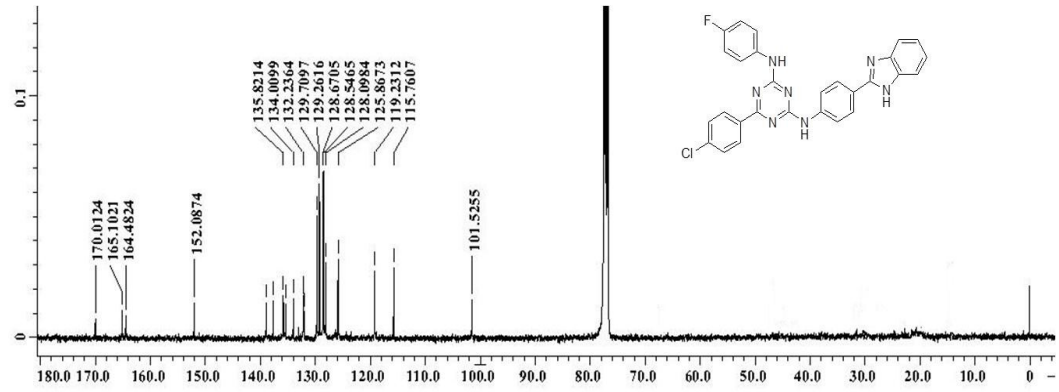


**Figure S32**. $^{13}$C NMR spectrum of compound **17** ($CDCl_3$).

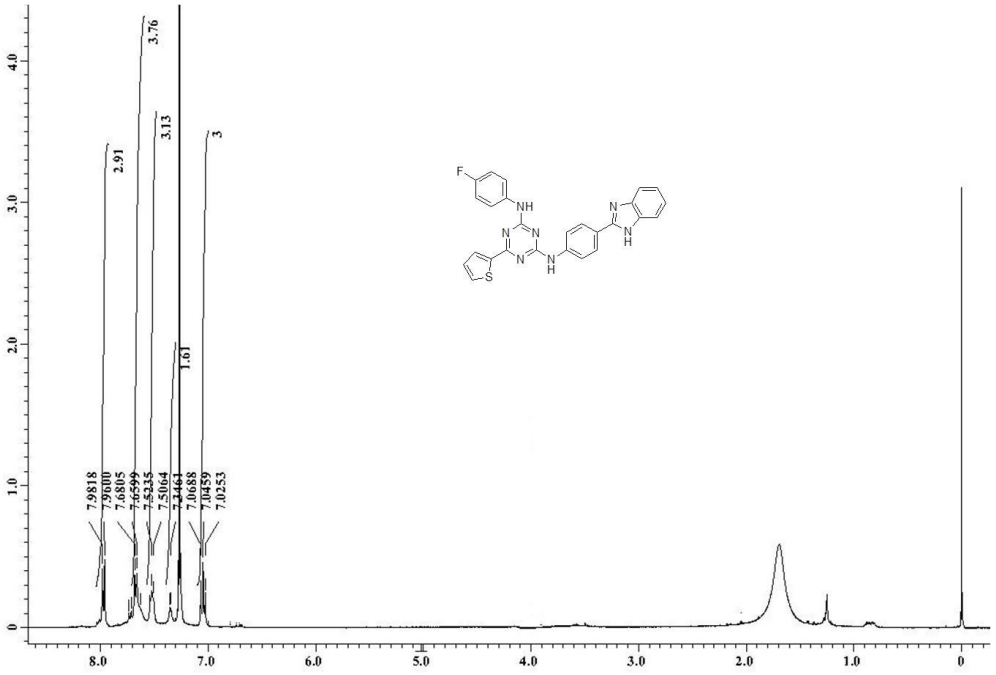


**Figure S33**. $^{1}$H NMR spectrum of compound **18** ($CDCl_3$).

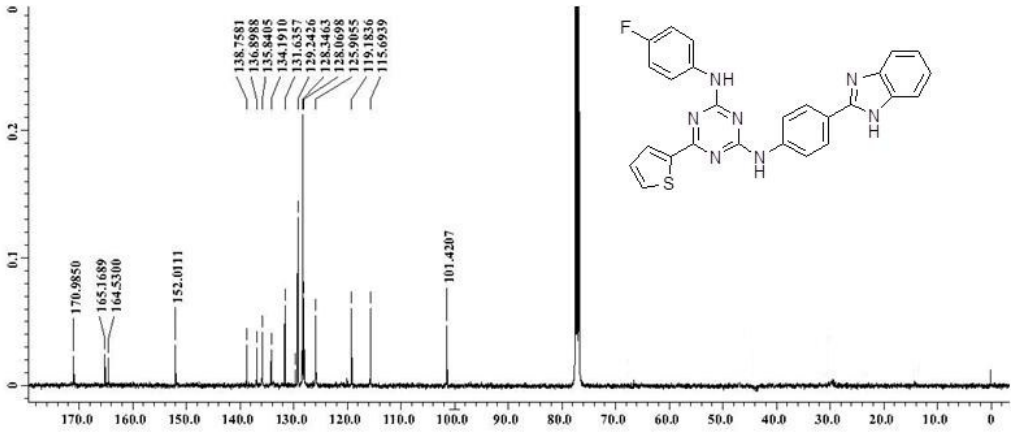


**Figure S34**. $^{13}$C NMR spectrum of compound **18** ($CDCl_3$).

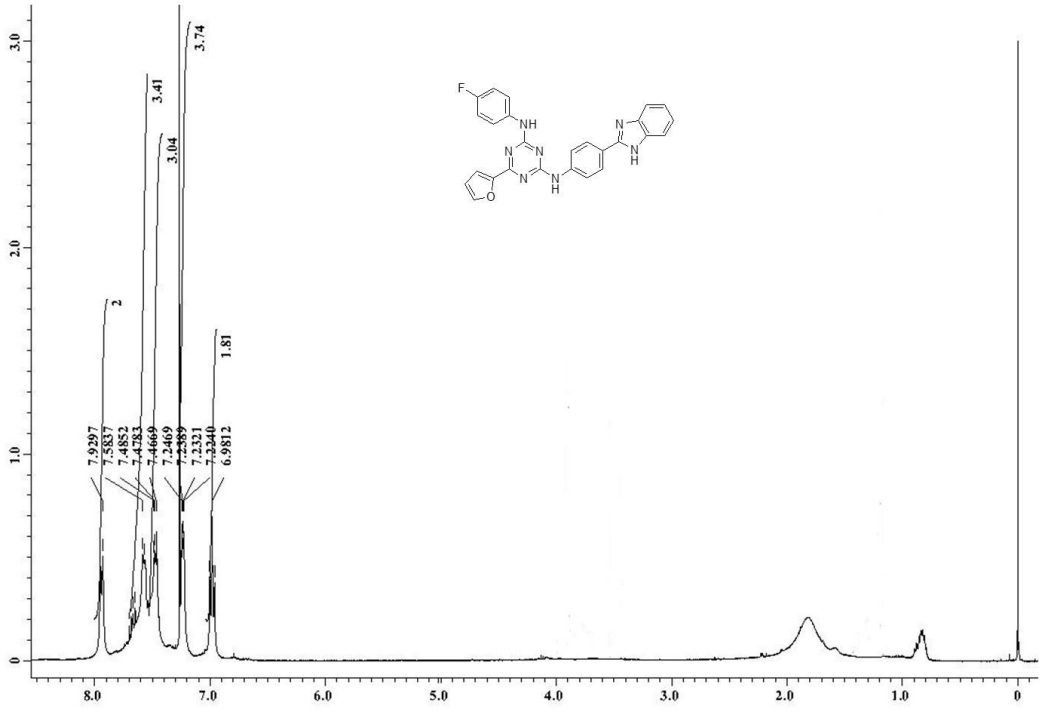


**Figure S35**. $^{1}H$ NMR spectrum of compound **19** ($CDCl_3$).

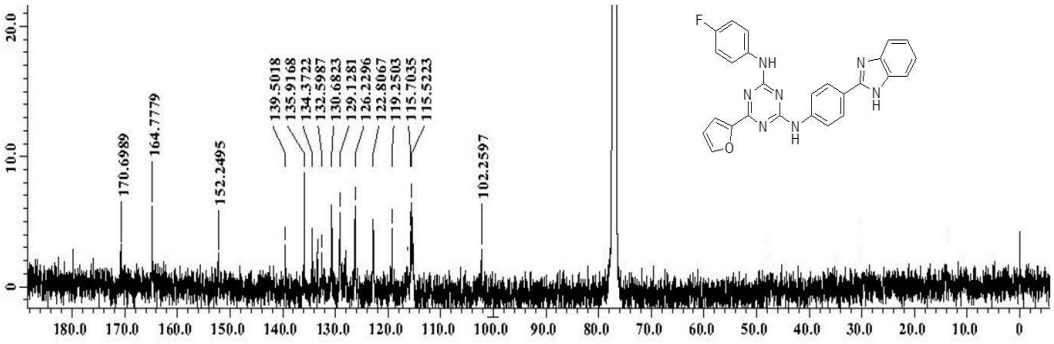


**Figure S36**. $^{13}C$ NMR spectrum of compound **19** ($CDCl_3$).

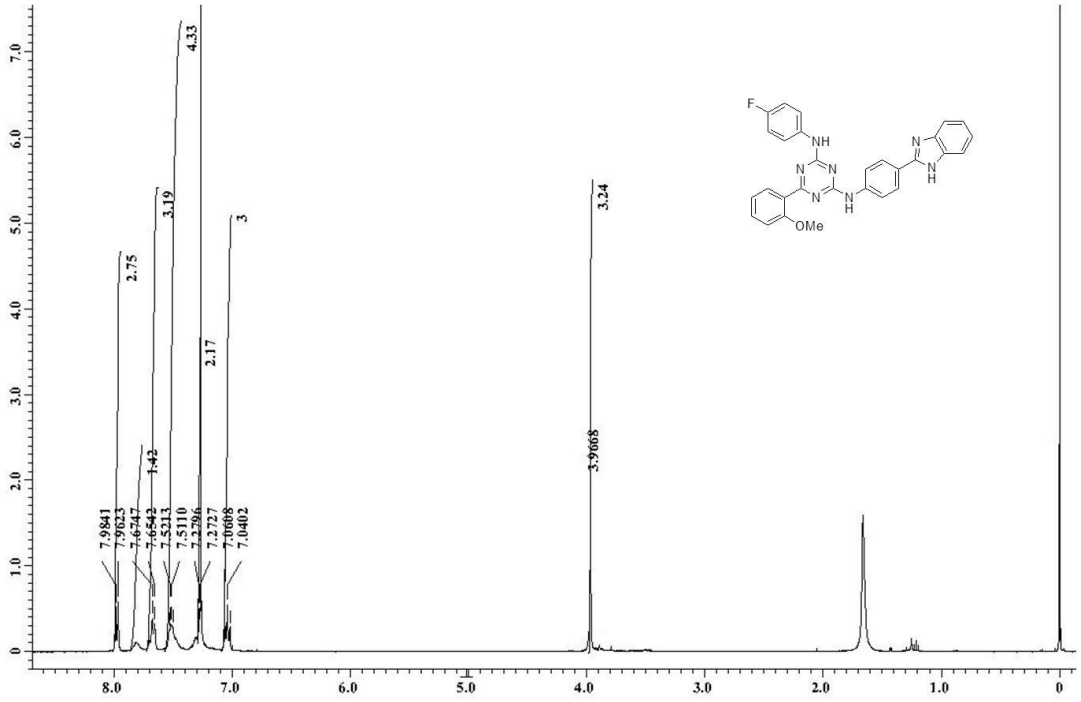


**Figure S37**. $^{1}H$ NMR spectrum of compound **20** ($CDCl_3$).

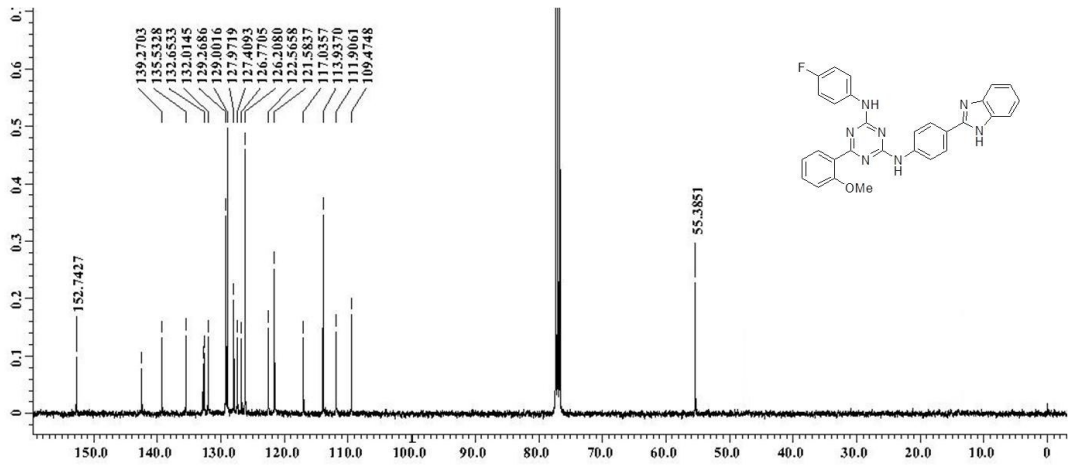


**Figure S38**. $^{13}C$ NMR spectrum of compound **20** ($CDCl_3$).

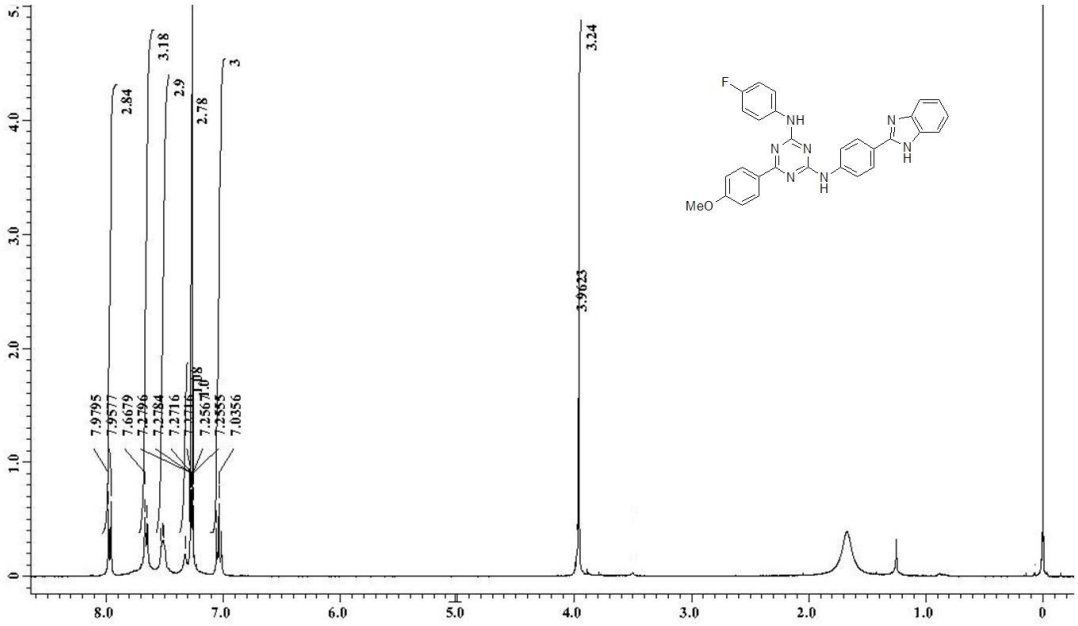


**Figure S39**. $^{1}$H NMR spectrum of compound **21** ($CDCl_3$).

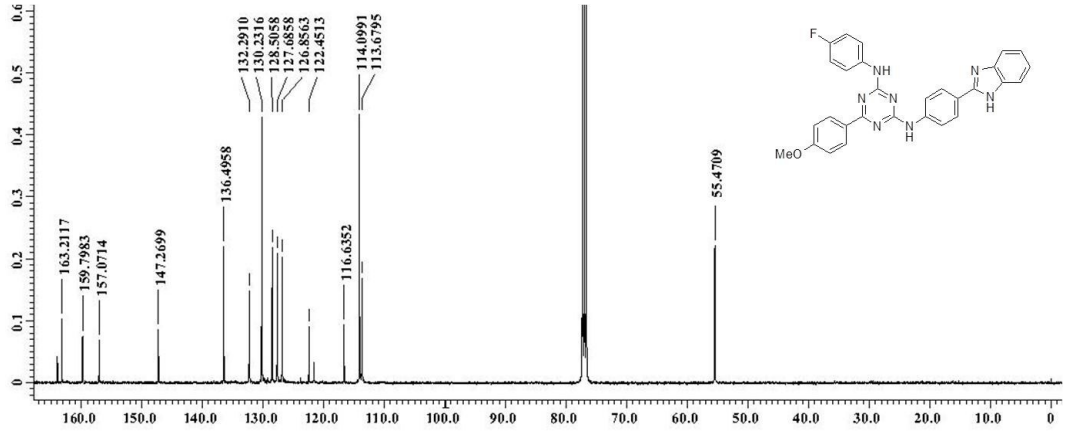


**Figure S40**. $^{13}$C NMR spectrum of compound **21** ($CDCl_3$).

**Absorption and emission spectra of compounds 4-6, 8, 12, 14-15, 18** and **20**.

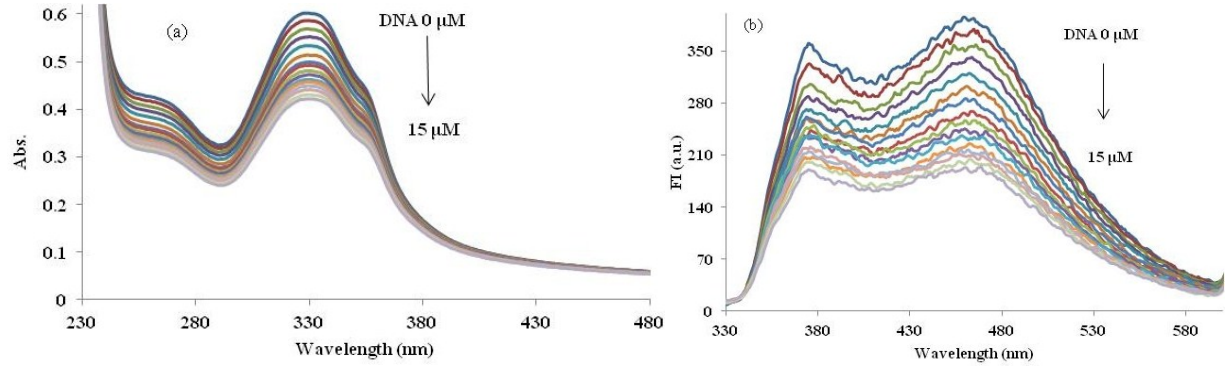


**Figure S41.** (a) Absorption (b) emission spectra of compound **4** (20 µM) with increasing concentration of ct-DNA in phosphate buffer.

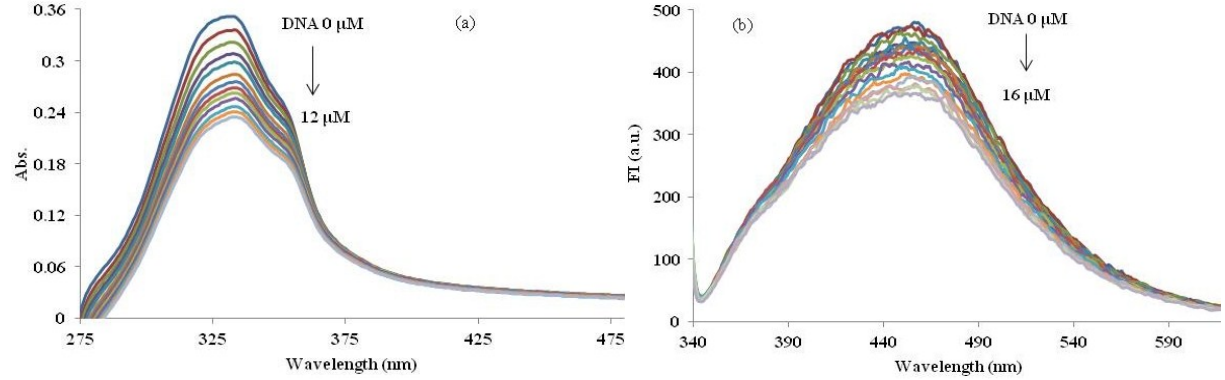


**Figure S42.** (a) Absorption (b) emission spectra of compound **5** (20 µM) with increasing concentration of ct-DNA in phosphate buffer.

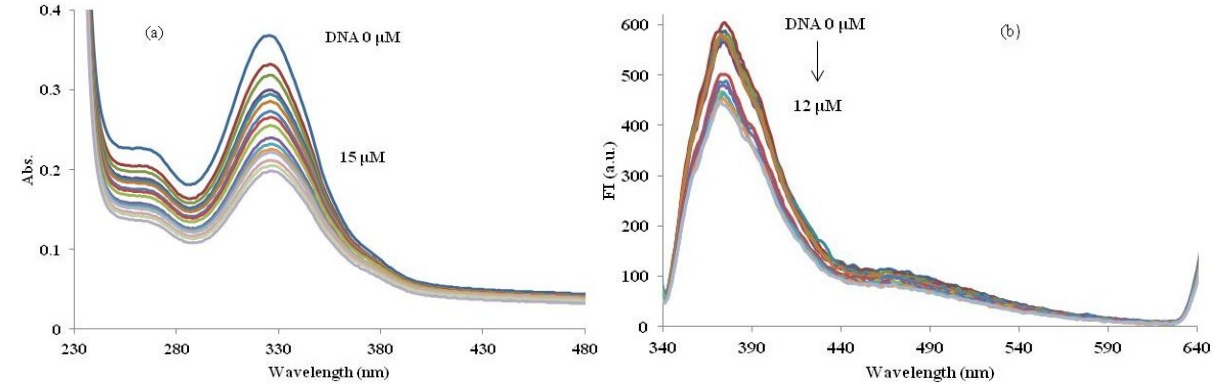


**Figure S43.** (a) Absorption (b) emission spectra of compound **6** (20 µM) with increasing concentration of ct-DNA in phosphate buffer.

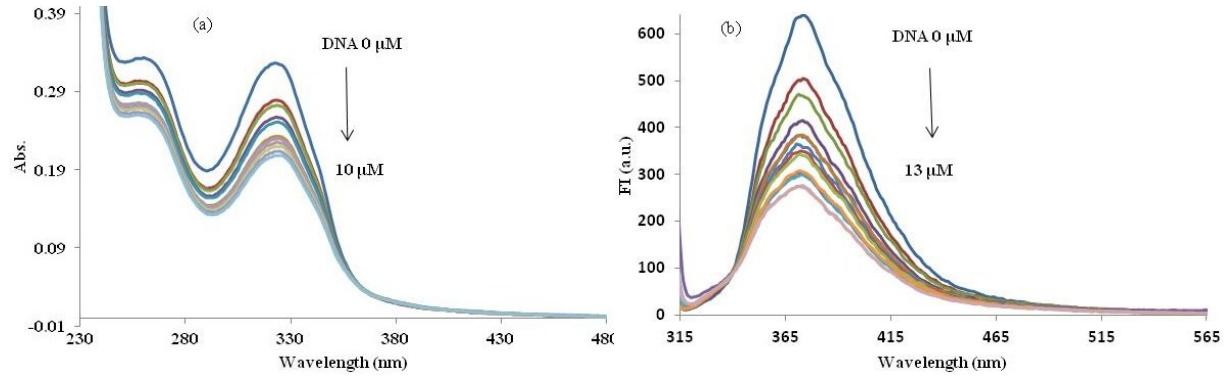


**Figure S44.** (a) Absorption (b) emission spectra of compound **8** (20 µM) with increasing concentration of ct-DNA in phosphate buffer.

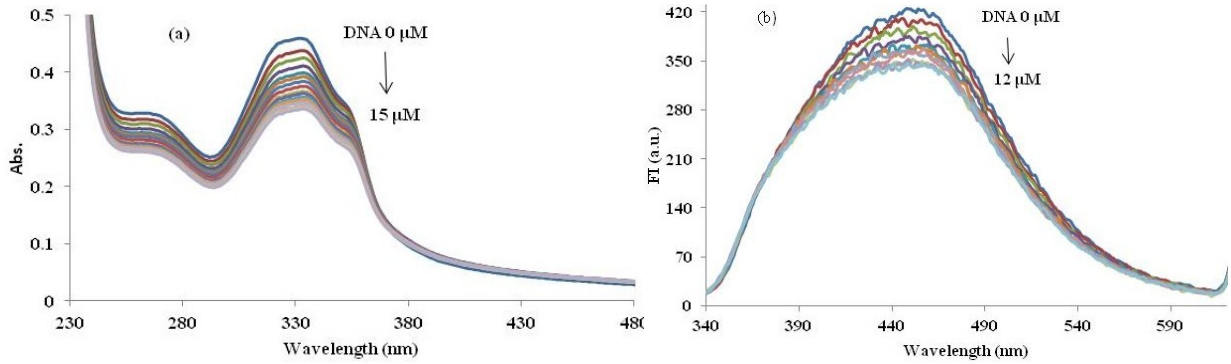


**Figure S45.** (a) Absorption (b) emission spectra of compound **12** (20 µM) with increasing concentration of ct-DNA in phosphate buffer.

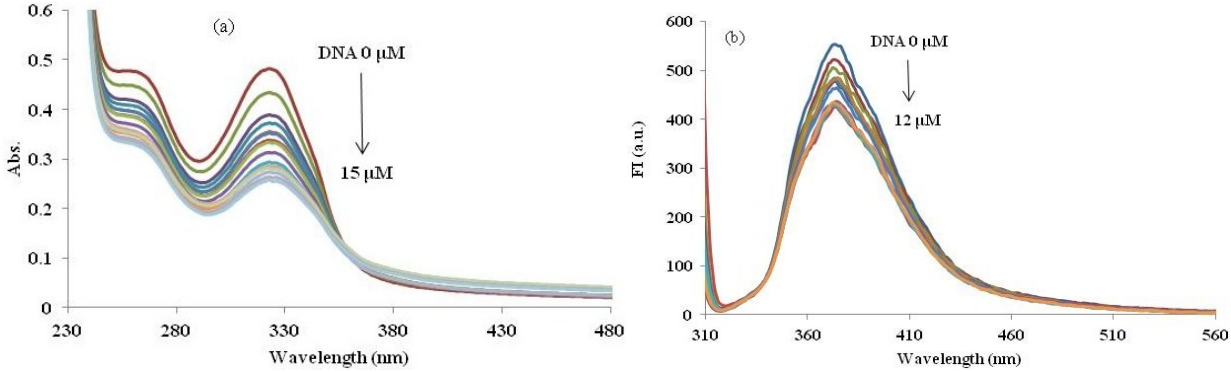


**Figure S46.** (a) Absorption (b) emission spectra of compound **14** (20 µM) with increasing concentration of ct-DNA in phosphate buffer.

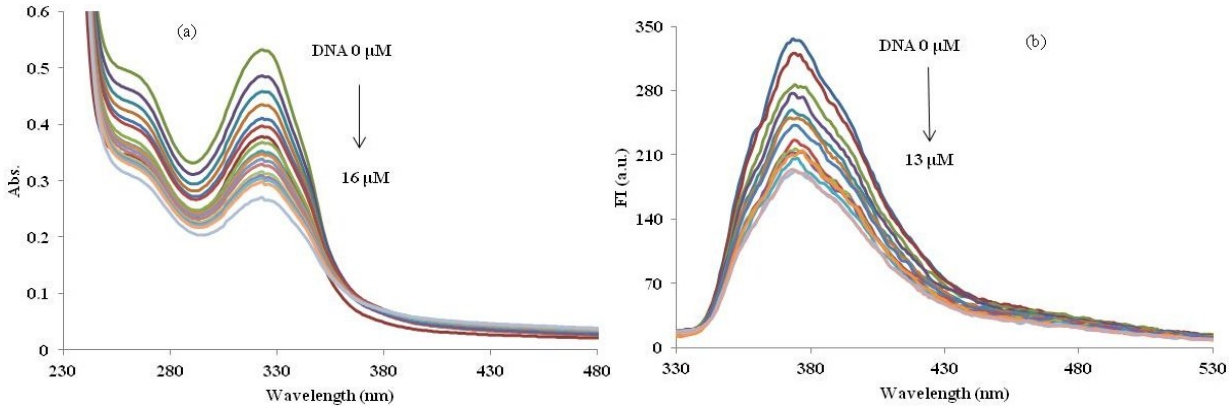


**Figure S47.** (a) Absorption (b) emission spectra of compound **15** (20 µM) with increasing concentration of ct-DNA in phosphate buffer.

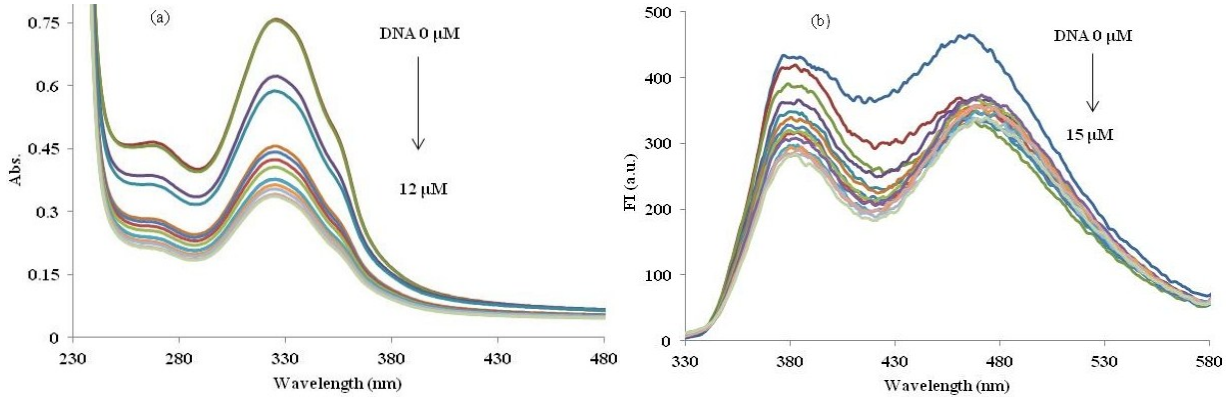


**Figure S48.** (a) Absorption (b) emission spectra of compound **18** (20 µM) with increasing concentration of ct-DNA in phosphate buffer.

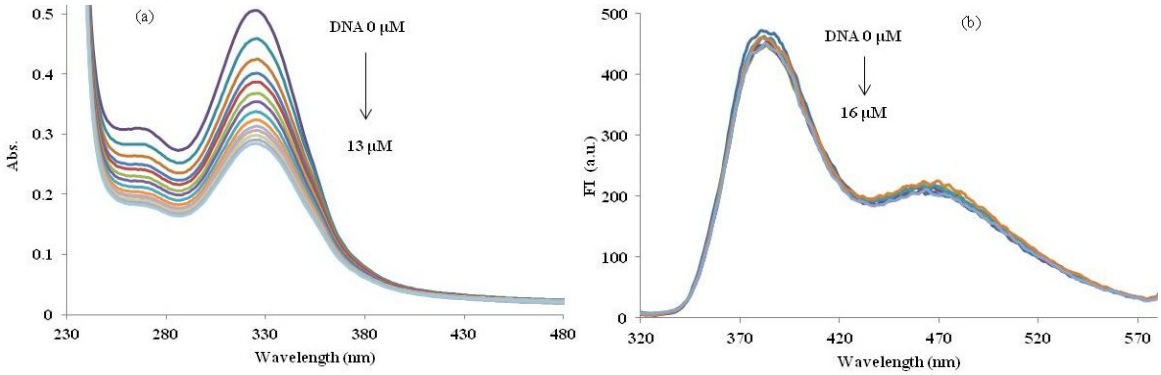


**Figure S49.** (a) Absorption (b) emission spectra of compound **20** (20 µM) with increasing concentration of ct-DNA in phosphate buffer.

## Inner Filter Effect[3]

The inner-filter effect refers to the absorbance (or optical dispersion) of light at the excitation or emission wavelength by the compounds present in the solution. Usually, the optics of commercial fluorimeter focuses the exciting light and collects the emission from the centre of the cuvette. Therefore, when absorption at the excitation wavelength is significant, less light reaches the centre of the sample and thus the fluorescence of the fluorophore is reduced, while absorption at the emission wavelength reduces the emitted light that reaches the detector. This is a problem whenever the ligand used in a titration absorbs at the excitation and/or emission wavelengths. Also any dilution of the fluorophore upon ligand titration needs to be corrected. Due to the non-linear nature of the inner-filter effect this may require special attention. If the geometry of the instrument is such that the collected intensity comes exactly from the centre of the cuvette, the inner filter effect can be estimated from:

$$F_{corr} = F_{obs} \times 10^{\left[\frac{A_{em}+A_{ex}}{2}\right]}$$

Where $F_{corr}$ and $F_{obs}$ are corrected and uncorrected fluorescence intensities, $A_{ex}$ is absorbance of solution at excitation wavelength and $A_{em}$ is absorbance of solution at emission wavelength.

**Table S1 Correction factors of compounds for inner filter effect**

| Compound No. | Correction factor | Compound No. | Correction factor |
|---|---|---|---|
| **4** | 1.4588 | **11** | 1.5541 |
| **5** | 1.3335 | **12** | 1.5153 |
| **6** | 1.3630 | **14** | 1.3677 |
| **7** | 1.1468 | **15** | 1.3899 |
| **8** | 1.2119 | **16** | 1.1494 |
| **9** | 1.4910 | **18** | 1.3598 |
| **10** | 1.4471 | **21** | 1.2373 |

**Linear Binding plots to calculate binding constant**

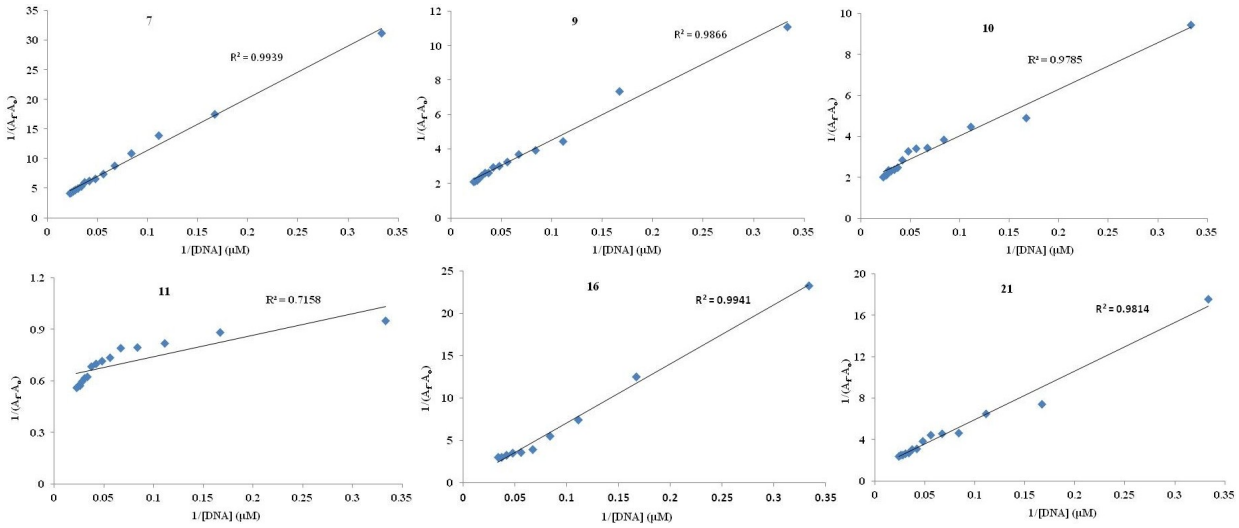


**Figure S50.** Plot of $1/(A_f - A_0)$ vs. 1/[DNA] for absorption spectra of compounds **7, 9-11, 16** and **21** in the presence of ct-DNA.

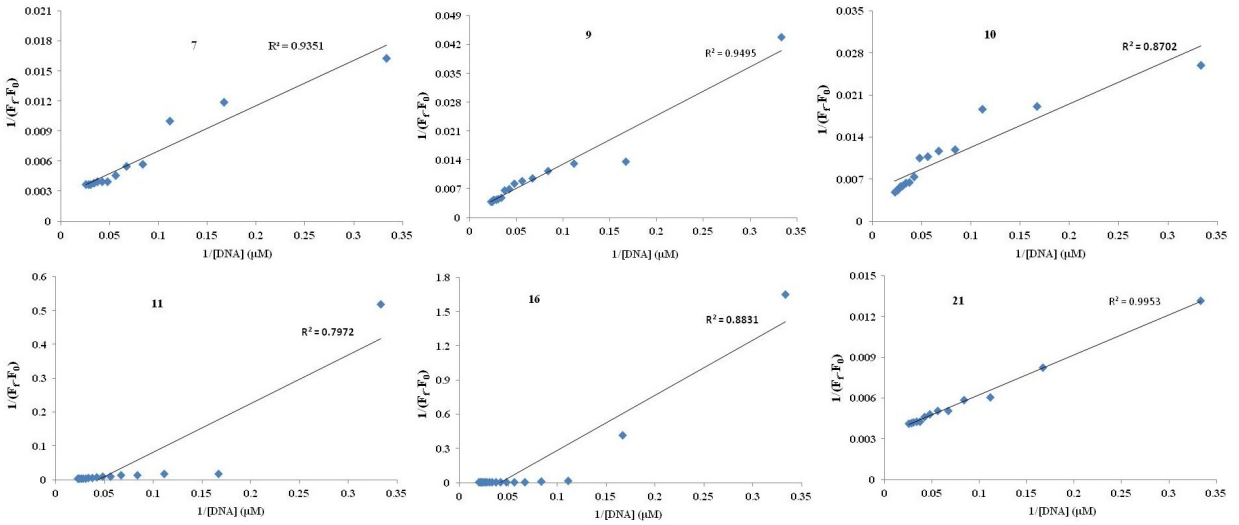


**Figure S51.** Plot of $1/(F_f - F_0)$ vs. 1/[DNA] for emission spectra of compounds **7, 9-11, 16** and **21** in the presence of ct-DNA.

## Competitive binding study

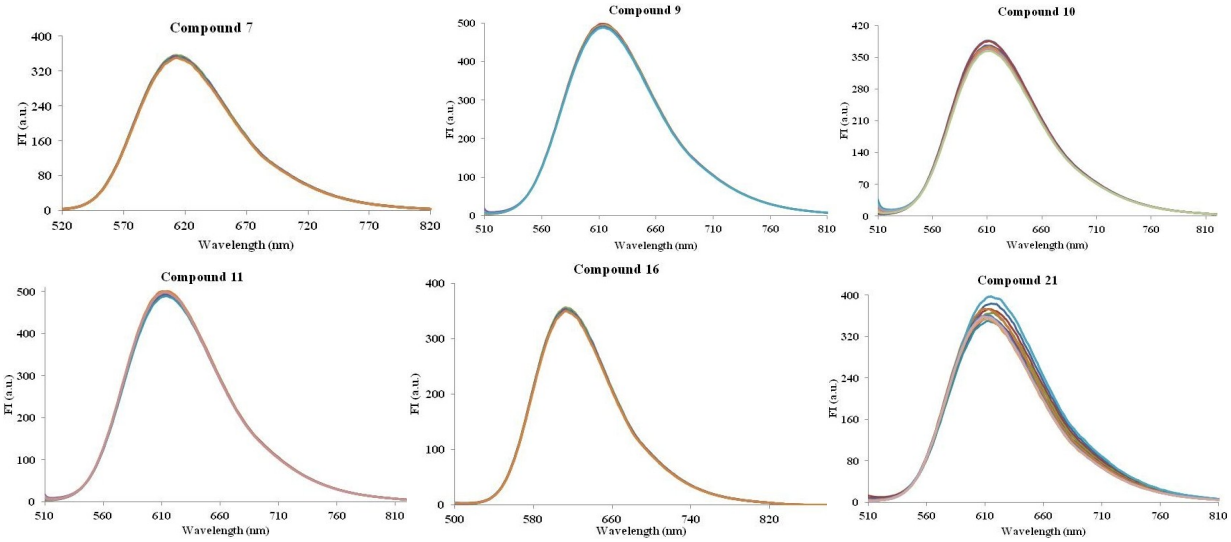


**Figure S52.** Gradual addition of compounds **7, 9-11, 16** and **21** shows no significant change in the fluorescence intensity of ethidium bromide bound ct-DNA.

**Effect of ionic strength on fluorescence quenching of compound.DNA complexes** (**7, 9-11, 16** and **21**).

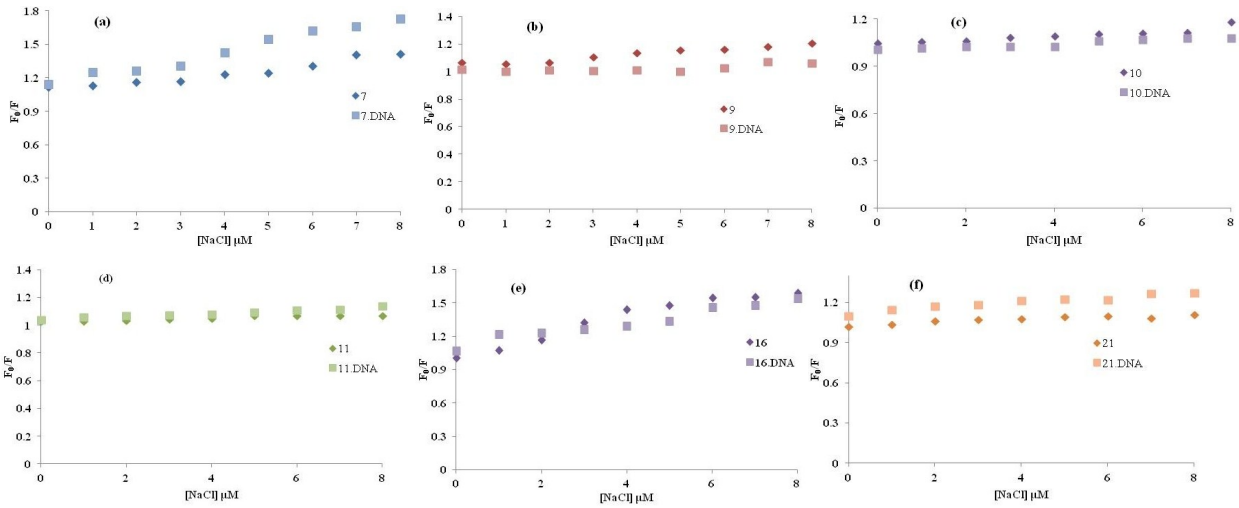


**Figure S53.** Fluorescence quenching plots of (a) compound **7;** (b) compound **9;** (c) compound **10;** (d) compound **11;** (e) compound **16;** (f) compound **21** and compound.DNA complexes by NaCl.

**Effect of iodide on fluorescence quenching of compound.DNA complexes (7, 9-11, 16 and 21).**

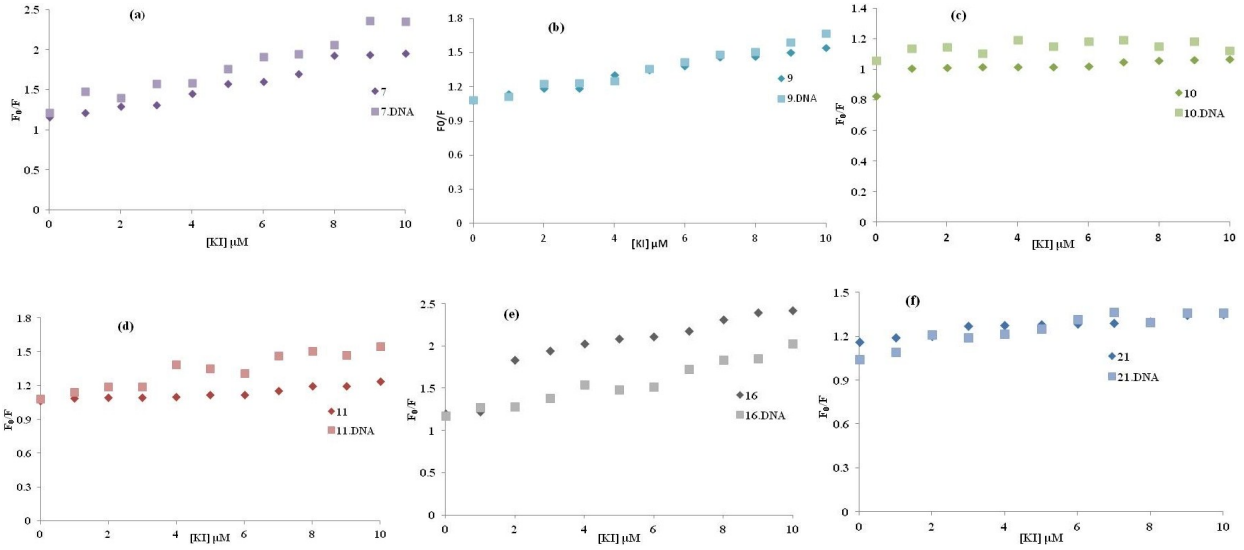


**Figure S54.** Fluorescence quenching plots of (a) compound **7;** (b) compound **9;** (c) compound **10;** (d) compound **11;** (e) compound **16;** (f) compound **21** and compound.DNA complexes by iodide.

**Docking Results**

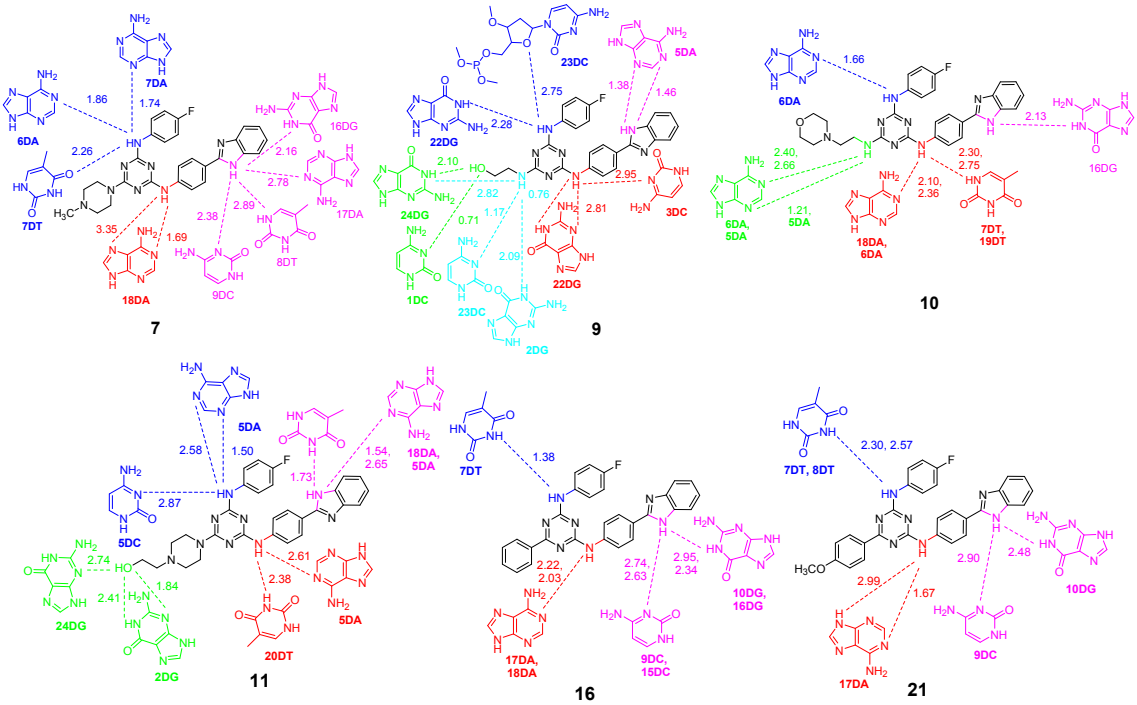


**Figure S55.** H-bonding pattern of compounds **7**, **9-11**, **16** and **21** with DNA base pairs.

**Table S2**. Charges (PM3, AM and HF) on different atoms of compounds **7** and **9**.

| | | Compound 7 | | | | Compound 9 | | |
|---|---|---|---|---|---|---|---|---|
| Sr. No. | Atoms | PM3* | AM** | HF** | Atoms | PM3* | AM** | HF** |
| 1 | N | 0.0481 | -0.2611 | -0.6939 | C | 0.1150 | 0.1687 | 0.8756 |
| 2 | C | -0.2198 | -0.0078 | -0.1134 | N | -0.3252 | -0.2897 | -0.7247 |
| 3 | C | -0.2118 | -0.0711 | -0.1073 | C | 0.1091 | 0.1655 | 0.8539 |
| 4 | N | -0.1021 | -0.2924 | -0.6384 | N | -0.3253 | -0.2806 | -0.7056 |
| 5 | C | -0.2720 | -0.0997 | -0.2497 | C | 0.1073 | 0.1653 | 0.8548 |
| 6 | C | -0.2117 | -0.0714 | -0.0982 | N | -0.3205 | -0.2791 | -0.7086 |
| 7 | C | -0.2209 | -0.0075 | -0.1295 | C | -0.1054 | -0.0188 | 0.2054 |
| 8 | C | 0.1407 | 0.2856 | 0.8762 | C | -0.1356 | -0.0514 | -0.1821 |
| 9 | N | -0.3356 | -0.3941 | -0.6572 | C | -0.2219 | -0.1528 | -0.2766 |
| 10 | C | 0.1103 | 0.2767 | 0.8234 | C | 0.0639 | 0.0870 | 0.3832 |
| 11 | N | -0.3218 | -0.3792 | -0.7927 | C | -0.2157 | -0.1712 | -0.2767 |
| 12 | C | 0.1048 | 0.2732 | 0.8830 | C | -0.1917 | -0.1296 | -0.2369 |
| 13 | N | -0.3171 | -0.3749 | -0.7995 | F | -0.0981 | -0.0999 | -0.3933 |
| 14 | C | -0.1006 | 0.0773 | 0.1712 | C | -0.0827 | -0.0241 | 0.2034 |
| 15 | C | -0.1441 | -0.1076 | -0.0742 | C | -0.2236 | -0.1534 | -0.2358 |
| 16 | C | -0.2202 | -0.1430 | -0.2561 | C | -0.1313 | -0.0801 | -0.1926 |
| 17 | C | 0.0610 | 0.0719 | 0.4106 | C | -0.0365 | -0.0189 | -0.1259 |
| 18 | C | -0.2129 | -0.1381 | -0.2291 | C | -0.1770 | -0.1041 | -0.2327 |
| 19 | C | -0.1964 | -0.1449 | -0.2446 | C | -0.1638 | -0.0737 | -0.1819 |
| 20 | F | -0.0981 | -0.1178 | -0.4478 | C | -0.0925 | 0.0110 | 0.6175 |
| 21 | C | -0.0810 | 0.1153 | 0.1359 | N | -0.1111 | -0.1148 | -0.6339 |
| 22 | C | -0.2274 | -0.1900 | -0.1822 | N | 0.1742 | -0.1788 | -0.7888 |
| 23 | C | -0.1304 | -0.0509 | -0.1379 | C | -0.1743 | -0.1059 | -0.2162 |
| 24 | C | -0.0418 | -0.0626 | -0.0115 | C | -0.1732 | -0.1165 | -0.2300 |
| 25 | C | -0.1747 | -0.0894 | -0.2367 | C | -0.2127 | -0.1440 | -0.2437 |
| 26 | C | -0.1691 | -0.1552 | -0.1705 | C | -0.1202 | -0.0657 | -0.2013 |
| 27 | C | -0.0914 | 0.0807 | 0.4756 | C | -0.0723 | -0.0655 | 0.1526 |
| 28 | N | -0.1094 | -0.1592 | -0.5662 | C | -0.2069 | -0.0700 | 0.2073 |
| 29 | N | 0.1710 | -0.2378 | -0.7958 | N | 0.1143 | -0.1392 | -0.7779 |
| 30 | C | -0.1743 | -0.1340 | -0.1120 | N | 0.1106 | -0.1435 | -0.7824 |
| 31 | C | -0.1733 | -0.1105 | -0.2023 | H | 0.2273 | 0.1560 | 0.2728 |
| 32 | C | -0.2129 | -0.1527 | -0.2153 | H | 0.2180 | 0.1502 | 0.2647 |
| 33 | C | -0.1202 | -0.0646 | -0.0966 | H | 0.2179 | 0.1498 | 0.2659 |
| 34 | C | -0.0731 | -0.0661 | 0.0851 | H | 0.2008 | 0.1394 | 0.2488 |
| 35 | C | -0.2061 | -0.0213 | 0.1498 | H | 0.2026 | 0.1390 | 0.2461 |
| 36 | N | 0.1132 | -0.2513 | -0.7566 | H | 0.2284 | 0.1581 | 0.2829 |
| 37 | N | 0.1137 | -0.2616 | -0.7675 | H | 0.1830 | 0.1395 | 0.2445 |
| 38 | H | 0.1314 | 0.0921 | 0.1735 | H | 0.2245 | 0.1576 | 0.2681 |
| 39 | H | 0.1672 | 0.1247 | 0.2203 | H | 0.1359 | 0.1977 | 0.3385 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 40 | H | 0.1325 | 0.0960 | 0.1773 | H | 0.1984 | 0.1404 | 0.2485 |
| 41 | H | 0.1135 | 0.0640 | 0.1565 | H | 0.1899 | 0.1393 | 0.2449 |
| 42 | H | 0.0884 | 0.0447 | 0.1339 | H | 0.1928 | 0.1398 | 0.2430 |
| 43 | H | 0.1147 | 0.0824 | 0.1688 | H | 0.2100 | 0.1492 | 0.2617 |
| 44 | H | 0.1148 | 0.0821 | 0.1689 | H | 0.1357 | 0.1967 | 0.3491 |
| 45 | H | 0.1321 | 0.0964 | 0.1786 | H | 0.1316 | 0.1979 | 0.3528 |
| 46 | H | 0.1147 | 0.0646 | 0.1565 | N | 0.0292 | -0.1872 | -0.7505 |
| 47 | H | 0.1681 | 0.1259 | 0.1916 | C | -0.2635 | -0.0797 | -0.2282 |
| 48 | H | 0.1298 | 0.0915 | 0.1812 | C | -0.0689 | -0.0121 | -0.0791 |
| 49 | H | 0.2309 | 0.1603 | 0.3398 | N | -0.3190 | -0.3368 | -0.6788 |
| 50 | H | 0.2170 | 0.1503 | 0.2093 | C | 0.1347 | 0.1846 | 0.3364 |
| 51 | H | 0.2175 | 0.1511 | 0.2183 | C | 0.1787 | 0.1352 | 0.2809 |
| 52 | H | 0.1999 | 0.1373 | 0.2047 | C | 0.1350 | 0.0761 | 0.2177 |
| 53 | H | 0.2022 | 0.1349 | 0.2054 | C | 0.0978 | 0.0771 | 0.2077 |
| 54 | H | 0.2251 | 0.1598 | 0.2483 | O | 0.0839 | 0.0561 | 0.1765 |
| 55 | H | 0.1887 | 0.1215 | 0.1999 | H | 0.2182 | 0.2101 | 0.3781 |
| 56 | H | 0.2274 | 0.1686 | 0.3387 | | | | |
| 57 | H | 0.1377 | 0.2504 | 0.3476 | | | | |
| 58 | H | 0.1983 | 0.1329 | 0.2116 | | | | |
| 59 | H | 0.1899 | 0.1296 | 0.1979 | | | | |
| 60 | H | 0.1928 | 0.1313 | 0.1965 | | | | |
| 61 | H | 0.2101 | 0.1447 | 0.2249 | | | | |
| 62 | H | 0.1331 | 0.2497 | 0.3620 | | | | |
| 63 | H | 0.1295 | 0.2505 | 0.3593 | | | | |

**Table S3**. Charges (PM3, AM and HF) on different atoms of compounds **10** and **11**.

| | **Compound 10** | | | | **Compound 11** | | |
|---|---|---|---|---|---|---|---|
| Sr. No. | Atoms | PM3* | AM** | HF** | Atoms | PM3* | AM** | HF** |
| 1 | C | 0.1105 | 0.1611 | 0.8777 | C | 0.1412 | 0.1643 | 0.7522 |
| 2 | N | -0.3357 | -0.2884 | -0.6610 | N | -0.3362 | -0.2990 | -0.6432 |
| 3 | C | 0.1117 | 0.1679 | 0.7292 | C | 0.1110 | 0.1639 | 0.7275 |
| 4 | N | -0.3282 | -0.2769 | -0.6026 | N | -0.3241 | -0.2839 | -0.6079 |
| 5 | C | 0.1067 | 0.1722 | 0.8172 | C | 0.1055 | 0.1617 | 0.7197 |
| 6 | N | -0.3177 | -0.2873 | -0.8038 | N | -0.3181 | -0.2758 | -0.6012 |
| 7 | C | -0.1029 | -0.0595 | 0.1168 | C | -0.1015 | -0.0454 | 0.1513 |
| 8 | C | -0.1948 | -0.1080 | -0.1509 | C | -0.1952 | -0.1229 | -0.1499 |
| 9 | C | -0.2147 | -0.1351 | -0.2164 | C | -0.2133 | -0.1329 | -0.2132 |
| 10 | C | 0.0617 | 0.0707 | 0.3972 | C | 0.0624 | 0.0839 | 0.4099 |
| 11 | C | -0.2196 | -0.1657 | -0.2324 | C | -0.2202 | -0.1691 | -0.2374 |
| 12 | C | -0.1387 | -0.1315 | -0.1706 | C | -0.1425 | -0.0473 | -0.1363 |
| 13 | F | -0.0985 | -0.1031 | -0.4494 | F | -0.0978 | -0.0998 | -0.4443 |
| 14 | C | -0.0815 | -0.0231 | 0.1671 | C | -0.0812 | -0.0205 | 0.1703 |
| 15 | C | -0.2227 | -0.1513 | -0.1809 | C | -0.2241 | -0.1516 | -0.1811 |
| 16 | C | -0.1327 | -0.0768 | -0.1320 | C | -0.1302 | -0.0774 | -0.1324 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 17 | C | -0.0374 | -0.0152 | -0.0030 | C | -0.0373 | -0.0162 | -0.0032 |
| 18 | C | -0.1781 | -0.1050 | -0.2431 | C | -0.1757 | -0.1042 | -0.2425 |
| 19 | C | -0.1611 | -0.0754 | -0.1487 | C | -0.1641 | -0.0761 | -0.1480 |
| 20 | C | -0.0911 | 0.0111 | 0.4737 | C | -0.0941 | 0.0119 | 0.4741 |
| 21 | N | -0.1103 | -0.1131 | -0.5605 | N | -0.1105 | -0.1142 | -0.5615 |
| 22 | N | 0.1710 | -0.1786 | -0.7943 | N | 0.1765 | -0.1794 | -0.7947 |
| 23 | C | -0.1742 | -0.1062 | -0.1120 | C | -0.1742 | -0.1059 | -0.1118 |
| 24 | C | -0.1732 | -0.1164 | -0.2011 | C | -0.1732 | -0.1167 | -0.2013 |
| 25 | C | -0.2127 | -0.1443 | -0.2149 | C | -0.2130 | -0.1444 | -0.2149 |
| 26 | C | -0.1203 | -0.0653 | -0.0957 | C | -0.1201 | -0.0653 | -0.0958 |
| 27 | C | -0.0727 | -0.0652 | 0.0840 | C | -0.0729 | -0.0651 | 0.0842 |
| 28 | C | -0.2057 | -0.0703 | 0.1513 | C | -0.2078 | -0.0701 | 0.1516 |
| 29 | N | 0.1078 | -0.1415 | -0.7462 | N | 0.1110 | -0.1425 | -0.7467 |
| 30 | N | 0.1126 | -0.1242 | -0.7565 | N | 0.1141 | -0.1406 | -0.7448 |
| 31 | H | 0.1992 | 0.1396 | 0.2115 | H | 0.1998 | 0.1390 | 0.2128 |
| 32 | H | 0.2169 | 0.1474 | 0.2173 | H | 0.2176 | 0.1473 | 0.2203 |
| 33 | H | 0.2180 | 0.1468 | 0.2095 | H | 0.2177 | 0.1512 | 0.2215 |
| 34 | H | 0.2299 | 0.2275 | 0.3607 | H | 0.2287 | 0.1547 | 0.2261 |
| 35 | H | 0.2014 | 0.1389 | 0.2074 | H | 0.2021 | 0.1377 | 0.2055 |
| 36 | H | 0.2269 | 0.1589 | 0.2511 | H | 0.2260 | 0.1580 | 0.2499 |
| 37 | H | 0.1870 | 0.1387 | 0.2087 | H | 0.1846 | 0.1382 | 0.2079 |
| 38 | H | 0.2209 | 0.1540 | 0.2222 | H | 0.2228 | 0.1538 | 0.2209 |
| 39 | H | 0.1371 | 0.1969 | 0.3482 | H | 0.1366 | 0.1973 | 0.3483 |
| 40 | H | 0.1984 | 0.1401 | 0.2131 | H | 0.1982 | 0.1400 | 0.2128 |
| 41 | H | 0.1899 | 0.1391 | 0.1997 | H | 0.1899 | 0.1390 | 0.1995 |
| 42 | H | 0.1928 | 0.1398 | 0.1983 | H | 0.1928 | 0.1396 | 0.1980 |
| 43 | H | 0.2100 | 0.1495 | 0.2263 | H | 0.2100 | 0.1492 | 0.2258 |
| 44 | H | 0.1329 | 0.1968 | 0.3589 | H | 0.1337 | 0.1953 | 0.3574 |
| 45 | H | 0.1305 | 0.1993 | 0.3612 | H | 0.1308 | 0.1946 | 0.3566 |
| 46 | N | 0.0357 | -0.1379 | -0.6960 | N | 0.0468 | 0.1772 | -0.7200 |
| 47 | C | -0.2301 | -0.0759 | -0.1118 | C | -0.2195 | 0.0762 | -0.1318 |
| 48 | C | -0.2219 | -0.0841 | -0.1027 | C | -0.2092 | -0.1046 | -0.1348 |
| 49 | O | -0.1080 | -0.2389 | -0.6636 | N | -0.0985 | -0.2287 | -0.6321 |
| 50 | H | -0.2521 | -0.0901 | -0.1152 | O | -0.2206 | -0.2603 | -0.6796 |
| 51 | H | -0.2550 | -0.0820 | -0.1229 | C | -0.2180 | -0.1305 | -0.1563 |
| 52 | H | -0.1032 | -0.1250 | -0.1199 | C | -0.2476 | -0.0608 | -0.0086 |
| 53 | H | -0.1066 | -0.0136 | 0.0319 | C | -0.0806 | -0.0631 | -0.0206 |
| 54 | H | -0.2450 | -0.3323 | -0.7499 | C | -0.3172 | -0.1172 | -0.1324 |
| 55 | H | 0.1546 | 0.1227 | 0.2206 | H | 0.1690 | 0.2039 | 0.3740 |
| 56 | H | 0.1577 | 0.0825 | 0.1711 | H | 0.1311 | 0.1241 | 0.2245 |
| 57 | H | 0.1270 | 0.0737 | 0.1605 | H | 0.1148 | 0.0638 | 0.1516 |
| 58 | H | 0.1402 | 0.1012 | 0.1760 | H | 0.1309 | 0.1139 | 0.1996 |
| 59 | H | 0.1142 | 0.1038 | 0.1819 | H | 0.1364 | 0.0717 | 0.1486 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 60 | H | 0.1366 | 0.0751 | 0.1633 | H | 0.1197 | 0.0960 | 0.1683 |
| 61 | H | 0.1485 | 0.1182 | 0.1994 | H | 0.1705 | 0.1121 | 0.1981 |
| 62 | H | 0.1005 | 0.0850 | 0.1790 | H | 0.1295 | 0.0706 | 0.1576 |
| 63 | H | 0.1364 | 0.0715 | 0.1609 | H | 0.1182 | 0.1113 | 0.1848 |
| 64 | H | 0.1009 | 0.0923 | 0.1588 | H | 0.1215 | 0.0709 | 0.1606 |
| 65 | H | 0.1367 | 0.1077 | 0.1816 | H | 0.1211 | 0.1123 | 0.1860 |
| 66 | H | 0.1369 | 0.0759 | 0.1681 | H | 0.1067 | 0.1030 | 0.1776 |
| 67 | H | 0.1464 | 0.2017 | 0.3960 | H | 0.2093 | 0.1106 | 0.1925 |

**Table S4.** Charges (PM3, AM and HF) on different atoms of compounds **16** and **21**.

| | **Compound 16** | | | | **Compound 21** | | | |
|---|---|---|---|---|---|---|---|---|
| Sr. No. | Atoms | PM3* | AM** | HF** | Atoms | PM3* | AM** | HF** |
| 1 | C | 0.1939 | 0.1826 | 0.6009 | C | 0.1966 | 0.1516 | 0.6190 |
| 2 | N | -0.2901 | -0.3105 | -0.6764 | N | -0.2903 | -0.2603 | -0.6465 |
| 3 | C | 0.0851 | 0.2348 | 0.8926 | C | 0.0816 | 0.1537 | 0.7413 |
| 4 | N | -0.3006 | -0.3136 | -0.6523 | N | -0.2962 | -0.2715 | -0.6126 |
| 5 | C | 0.0811 | 0.2278 | 0.9182 | C | 0.0811 | 0.1619 | 0.8116 |
| 6 | N | -0.2976 | -0.3217 | -0.7715 | N | -0.2758 | -0.2766 | -0.8015 |
| 7 | C | -0.1017 | 0.0498 | 0.2911 | C | -0.1039 | -0.0304 | 0.1722 |
| 8 | C | -0.2126 | -0.1328 | -0.1866 | C | -0.1432 | -0.1078 | -0.0899 |
| 9 | C | -0.2037 | -0.1488 | -0.2474 | C | -0.2187 | -0.1583 | -0.2554 |
| 10 | C | 0.0511 | 0.0738 | 0.3952 | C | 0.0625 | 0.0798 | 0.4096 |
| 11 | C | -0.2074 | -0.1478 | -0.2274 | C | -0.2131 | -0.1679 | -0.2297 |
| 12 | C | -0.2041 | -0.1461 | -0.2233 | C | -0.1951 | -0.1317 | -0.2446 |
| 13 | F | -0.0992 | -0.1072 | -0.4515 | F | -0.0976 | -0.1021 | -0.4483 |
| 14 | C | -0.0841 | 0.0799 | 0.3221 | C | -0.0854 | -0.0176 | 0.1701 |
| 15 | C | -0.2093 | -0.1522 | -0.2276 | C | -0.2000 | -0.1037 | -0.1578 |
| 16 | C | -0.1324 | -0.0698 | -0.1598 | C | -0.1366 | -0.0885 | -0.1450 |
| 17 | C | -0.0358 | -0.0349 | -0.0405 | C | -0.0343 | -0.0191 | -0.0016 |
| 18 | C | -0.1723 | -0.1034 | -0.1974 | C | -0.1746 | -0.0986 | -0.2409 |
| 19 | C | -0.1828 | -0.1331 | -0.1571 | C | -0.1820 | -0.1252 | -0.1439 |
| 20 | C | -0.0960 | 0.0659 | 0.5849 | C | -0.0939 | 0.0119 | 0.4723 |
| 21 | N | -0.1082 | -0.1528 | -0.6030 | N | -0.1075 | -0.1142 | -0.5598 |
| 22 | N | 0.1750 | -0.2485 | -1.0315 | N | 0.1705 | -0.1793 | -0.7943 |
| 23 | C | -0.1746 | -0.1306 | -0.1299 | C | -0.1744 | -0.1057 | -0.1119 |
| 24 | C | -0.1721 | -0.1126 | -0.2168 | C | -0.1724 | -0.1169 | -0.2013 |
| 25 | C | -0.2126 | -0.1530 | -0.2287 | C | -0.2125 | -0.1445 | -0.2151 |
| 26 | C | -0.1195 | -0.0648 | -0.1263 | C | -0.1198 | -0.0655 | -0.0957 |
| 27 | C | -0.0736 | -0.0649 | 0.0855 | C | -0.0737 | -0.0653 | 0.0838 |
| 28 | C | -0.2065 | -0.0210 | 0.3298 | C | -0.2049 | -0.0703 | 0.1509 |
| 29 | N | 0.1276 | -0.2598 | -1.0015 | N | 0.1143 | -0.1407 | -0.7805 |
| 30 | N | 0.1408 | -0.2360 | -0.9843 | N | 0.1154 | -0.1327 | -0.7653 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 31 | C | -0.0909 | -0.0526 | -0.1294 | C | -0.1286 | -0.0623 | -0.0805 |
| 32 | C | -0.1333 | -0.0743 | -0.1395 | C | -0.0873 | -0.0398 | -0.1764 |
| 33 | C | -0.2145 | -0.1482 | -0.2288 | C | -0.3023 | -0.2163 | -0.2659 |
| 34 | C | -0.1612 | -0.1011 | -0.1698 | C | 0.1218 | 0.0742 | 0.3912 |
| 35 | C | -0.2128 | -0.1478 | -0.2365 | C | -0.2610 | -0.1191 | -0.2031 |
| 36 | C | -0.1373 | -0.0791 | -0.1280 | C | -0.0986 | -0.0882 | -0.1227 |
| 37 | H | 0.2900 | 0.2021 | 0.3613 | O | -0.1932 | -0.2054 | -0.7295 |
| 38 | H | 0.2149 | 0.1508 | 0.2285 | C | -0.1491 | -0.0741 | -0.1538 |
| 39 | H | 0.2158 | 0.1514 | 0.2317 | H | 0.2303 | 0.2198 | 0.3524 |
| 40 | H | 0.1996 | 0.1377 | 0.2170 | H | 0.2183 | 0.1447 | 0.2063 |
| 41 | H | 0.2076 | 0.1467 | 0.2267 | H | 0.2179 | 0.1490 | 0.2169 |
| 42 | H | 0.2277 | 0.1630 | 0.2669 | H | 0.2008 | 0.1389 | 0.2048 |
| 43 | H | 0.1890 | 0.1350 | 0.2193 | H | 0.2085 | 0.1495 | 0.2194 |
| 44 | H | 0.2210 | 0.1559 | 0.2430 | H | 0.2277 | 0.1609 | 0.2492 |
| 45 | H | 0.1375 | 0.2493 | 0.3833 | H | 0.1895 | 0.1389 | 0.2098 |
| 46 | H | 0.1987 | 0.1360 | 0.2162 | H | 0.2164 | 0.1473 | 0.2202 |
| 47 | H | 0.1903 | 0.1321 | 0.1998 | H | 0.1375 | 0.1978 | 0.3488 |
| 48 | H | 0.1933 | 0.1328 | 0.1982 | H | 0.1989 | 0.1402 | 0.2132 |
| 49 | H | 0.2104 | 0.1497 | 0.2339 | H | 0.1903 | 0.1390 | 0.1995 |
| 50 | H | 0.1445 | 0.2547 | 0.3986 | H | 0.1932 | 0.1395 | 0.1979 |
| 51 | H | 0.1338 | 0.2432 | 0.3892 | H | 0.2103 | 0.1492 | 0.2261 |
| 52 | H | 0.2191 | 0.1544 | 0.2582 | H | 0.1411 | 0.1998 | 0.3660 |
| 53 | H | 0.1974 | 0.1364 | 0.2107 | H | 0.1315 | 0.1999 | 0.3636 |
| 54 | H | 0.1927 | 0.1341 | 0.2141 | H | 0.2173 | 0.1589 | 0.2588 |
| 55 | H | 0.1972 | 0.1361 | 0.2139 | H | 0.2095 | 0.1484 | 0.2163 |
| 56 | H | 0.2114 | 0.1532 | 0.2424 | H | 0.2187 | 0.1511 | 0.2291 |
| 57 | | | | | H | 0.2156 | 0.1536 | 0.2314 |
| 58 | | | | | H | 0.0969 | 0.0700 | 0.1734 |
| 59 | | | | | H | 0.1185 | 0.1046 | 0.1928 |
| 60 | | | | | H | 0.0937 | 0.0653 | 0.1555 |

*Obtained from Argus Lab, **Obtained from Gaussian – 09 Window

## References


**1.** A. Solankee and Y. Prajapati, *Rasayan J. Chem.* 2009, **2**, 9.

**2.** P.T. Selvam, P.P. Radhika, S. Janagaraj and A.S. Kumar, *Research in Biotechnology,* 2011*,* **2***,* 50.

**3**. J. R. Lakowicz, *Principles of Fluorescence Spectroscopy*, Kluwer Academic/Plenum, New York, 1999.