Synthesis, photophysics and biomolecules interactive studies of new hybrid benzo-2,1,3-thiadiazoles

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General information

The reactions were monitored by TLC carried out on Merck silica gel (60 F254) by using UV light as visualizing agent and 5% vanillin in 10% H_2SO_4 and heat as developing agents. Baker silica gel (particle size 0.040–0.063mm) was used for flash chromatography. 4,7-diiodobenzo[c][1,2,5]thiadiazole was synthesized according previous literature.[1]

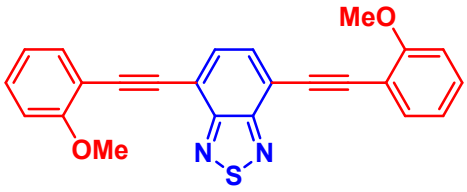
Hydrogen nuclear magnetic resonance spectra (^{1}H NMR) were obtained at 400 MHz on Bruker Avance III HD spectrometer. Spectra were recorded in $CDCl_3$ or DMSO-d_6 solutions. Chemical shifts are reported in ppm, referenced to tetramethylsilane (TMS) as the external reference. Coupling constants (*J*) are reported in Hertz. Abbreviations to denote the multiplicity of the signals are s (singlet), d (doublet), dd (double doublet), t (triplet) and m (multiplet). Carbon-13 nuclear magnetic resonance spectra (^{13}C NMR) were obtained at 100 MHz on Bruker Avance HD III spectrometer. Chemical shifts are reported in ppm, referenced to the solvent peak of $CDCl_3$ or DMSO-d_6. Low-resolution mass spectra were obtained with a Shimadzu GCMS-QP 2010 Plus mass spectrometer. High-resolution mass spectra (HRMS) were recorded on a Bruker Micro TOF-QII spectrometer 10416. UV-vis electronic absorption spectra were recorded using Shimadzu UV2600 spectrophotometer (data interval, 2.0 nm) using dichloromethane (DCM) or dimethyl sulfoxide (DMSO) as solvent at 250-800 nm range. Steady-state emission fluorescence spectra of samples in DCM or DMSO solutions were measured with a Varian Cary 50 fluorescence spectrophotometer (slit 2.0 mm/2.0 mm; emission/excitation). Fluorescence quantum yield values (Φ_f) of the derivatives in solutions were determined by comparing the corrected fluorescence spectra with that of 9,10-diphenylanthracene (DPA) in chloroform solution (Φ_f = 0.65, λ_{exc} = 366 nm) as the standard as the fluorescence yield.[2]

General procedure for the synthesis of 4,7-bis((2-methoxyphenyl)ethynyl)benzo[*c*][1,2,5]thiadiazole (1):[3]

To a 10 mL Schlenk tube containing the 4,7-diiodobenzo[*c*][1,2,5]chalcogenodiazole (0.5 mmol), were added 2-ethynylanisole (1.2 mmol), $PdCl_2(PPh_3)_2$ (10 mol%), CuI (10 mol%) and Et_3N (2.5 mL). Then, the reaction mixture was stirred at 70 °C for 12 h under N_2 atmosphere. After this time, the solution was cooled to room temperature, diluted with dichloromethane (20 mL), and

[1] M. Shimada, M. Tsuchiya, R. Sakamoto, Y. Yamanoi, E. Nishibori, K. Sugimoto and H. Nishihara, *Angew. Chem., Int. Ed.*, 2016, **55**, 3022. G.
[2] Heinrich, S. Schoof and H. Gusten, *J. Photochem.*, 1974/1975, **3**, 315.
[3] B. A. D. Neto, A. S. Lopes, G. Ebeling; R. S. Gonçalves, E. V. U. Costa, F. H. Quina and J. Dupont, *Tetrahedron*, 2005, **61**, 10975.

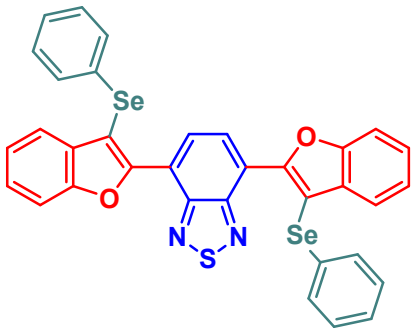
washed with water (3x 20 mL). The organic phase was separated, dried over $MgSO_4$ and concentrated under vacuum. The obtained product **1** was purified by column chromatography on neutral alumina using a mixture of ethyl acetate/hexane (10:90) as the eluent.



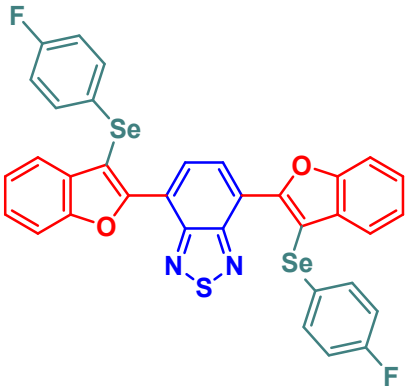
4,7-bis((2-methoxyphenyl)ethynyl)benzo[*c*][1,2,5]thiadiazole (1): Yield: 0.143 g (72%); yellow solid; mp: 137-139 °C. ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 7.90 (s, 2H), 7.58 (dd, J = 7.6 and 1.7 Hz, 2H), 7.50 – 7.43 (m, 2H), 7.15 (d, J = 8.1 Hz, 2H), 7.04 (m, 2H), 3.90 (s, 6H). ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 159.90, 153.71, 133.34, 132.68, 131.24, 120.66, 116.38, 111.58, 110.71, 93.79, 89.22, 55.81. MS (relative intensity) m/z: 396 (100); 361 (12); 277 (23); 190 (4); 131(10). HRMS calcd. for $C_{24}H_{16}N_2O_2S$: $[M]^+$ 396.0932. Found: 396.0909.

General procedure for the synthesis of 4,7-bis(3-(arylselanyl)benzofuran-2-yl)benzo[c][1,2,5]thiadiazoles (3a-d):

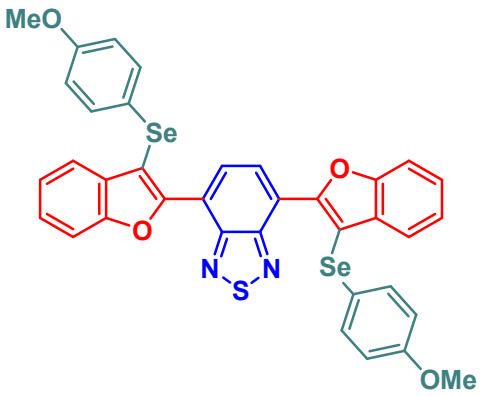
To a 10 mL round bottom flask containing an appropriated diaryl diselenide **2a-d** (0.50 mmol) and ethanol (3 mL), was added TClCA (trichloroisocyanuric acid) (0.75 mmol). Then, the reaction mixture was stirred at room temperature for 15 minutes under air. After this, we added the bis-alkynyl BTD **1** (0.25 mmol) and the homogenous reaction mixture was stirred at 60 °C for additional 3 hours. After this time, the solution was cooled to room temperature, diluted with dichloromethane (20 mL) and washed with water (3x 20 mL). The organic phase was separated, dried over $MgSO_4$ and concentrated under vacuum. The obtained products **3a-d** were purified by chromatography on neutral alumina using a mixture of ethyl acetate/hexane (10:90) as the eluent.



4,7-bis(3-(phenylselanyl)benzofuran-2-yl)benzo-2,1,3-thiadiazole (3a): Yield: 0.530 g (78%); yellow solid; mp: 245-247 °C. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 8.15 (s, 2H), 7.65 (d, J = 8.2 Hz, 2H), 7.64 – 7.37 (m, 4H), 7.34 – 7.24 (m, 6H), 6.86 (t, J = 8.6 Hz, 6H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 155.19, 154.23, 153.06, 130.96, 130.74, 130.06 (2C), 130.02, 129.22 (2C), 126.55, 125.87, 124.07, 123.60, 121.75, 111.68, 105.49. MS (relative intensity) m/z: 680 (70), 520 (76), 443 (38), 366 (100), 44(84). HRMS calcd. for $C_{34}H_{20}N_2O_2SSe_2$: $[M]^+$ 679.9576. Found: 679.9553.

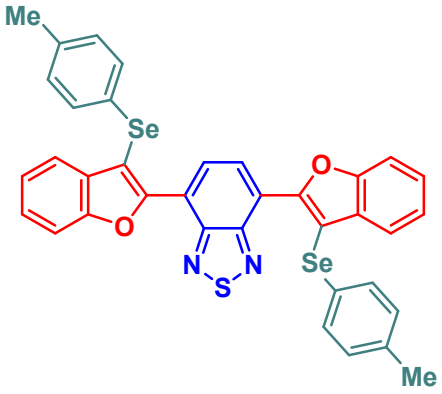


4,7-bis(3-(4-fluorophenylselanyl)benzofuran-2-yl)benzo-2,1,3-thiadiazole (3b): Yield: 0.372 g (52%); orange solid; mp: 233-235 °C. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 8.15 (s, 2H), 7.65 (d, J = 8.2 Hz, 2H), 7.45 – 7.36 (m, 4H), 7.34 – 7.29 (m, 4H), 7.27 – 7.21 (m, 2H), 6.86 (t, J = 8.5 Hz, 4H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 161.9 (d, J = 246.8 Hz) 155.2, 153.8, 153.0, 132.4 (d, J = 7.8 Hz, 2C), 130.5, 129.8, 125.9, 125.2 (d, J = 3.2 Hz, 2C), 124.1, 123.6, 121.6, 116.4 (d, J = 21,8 Hz), 111.7, 106.0. MS (relative intensity) m/z: 716 (68), 556 (81), 461 (31), 366 (100), 278 (27). HRMS calcd. for $C_{34}H_{18}F_2N_2O_2SSe_2$: $[M]^+$ 715.9387. Found: 715.9355.



4,7-bis(3-(4-methoxyphenylselanyl)benzofuran-2-yl)benzo-2,1,3-thiadiazole (3c): Yield: 0.481 g (65%); orange solid; mp: 204-206 °C. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 8.16 (s, 2H), 7.64 (d, J = 8.3 Hz, 2H), 7.45 (d, J = 7.6 Hz, 2H), 7.37 (t, J = 7.7 Hz, 2H), 7.31 (d, J = 8.8 Hz, 4H), 7.28 – 7.19 (m,

2H), 6.71 (d, J = 8.8 Hz, 4H), 3.71 (s, 6H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 158.94, 155.10, 153.30, 153.05, 132.89 (2C), 130.79, 129.92, 125.68, 124.09, 123.43, 121.72, 120.49, 114.90 (2C), 111.58, 106.75, 55.19. MS (relative intensity) m/z: 740 (45), 556 (81), 461 (32), 366 (100), 278 (27). HRMS calcd. for $C_{36}H_{24}N_2O_4SSe_2$: $[M]^+$ 739.9787. Found: 739.9765.



4,7-bis(3-(*p*-tolylphenylselanyl)benzofuran-2-yl)benzo-2,1,3-thiadiazole (3d): Yield: 0.375 g (53%); yellow solid; mp: 239-241 °C. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 8.15 (s, 2H), 7.66 (d, J = 8.2 Hz, 2H), 7.47 (d, J = 7.7 Hz, 2H), 7.38 (t, J = 8.4 Hz, 2H), 7.31 – 7.16 (m, 6H), 6.96 (d, J = 7.9 Hz, 4H), 2.24 (s, 6H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 155.14, 153.92, 153.07, 136.58, 130.82, 130.48 (2C), 130.01 (3C), 126.98, 125.76, 124.06, 123.51, 121.77, 111.63, 105.90, 20.98. MS (relative intensity) m/z: 708 (72), 548 (100), 455 (43), 366 (83), 266 (24). HRMS calcd. for $C_{36}H_{24}N_2O_2SSe_2$: $[M]^+$ 707.9889. Found: 707.9869.

FIGURES

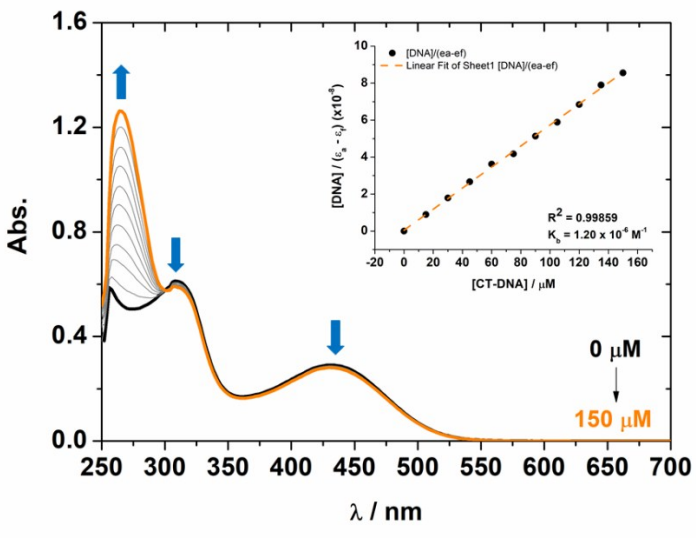


Figure S1. UV–vis titration absorption spectra of **3b** derivative, in a DMSO/Tris-HCl buffer (pH 7.4) mixture. The concentration of CT-DNA ranged from 0 to 150 μM. *Insert graph* shows the plot of [DNA]/($\varepsilon_a - \varepsilon_f$) *versus* [DNA].

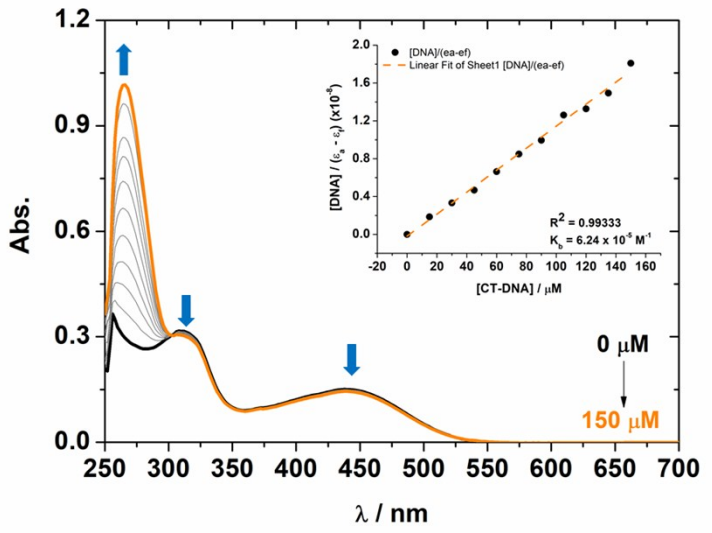


Figure S2. UV–vis titration absorption spectra of **3c** derivative, in a DMSO/Tris-HCl buffer (pH 7.4) mixture. The concentration of CT-DNA ranged from 0 to 150 μM. *Insert graph* shows the plot of [DNA]/($\varepsilon_a - \varepsilon_f$) *versus* [DNA].

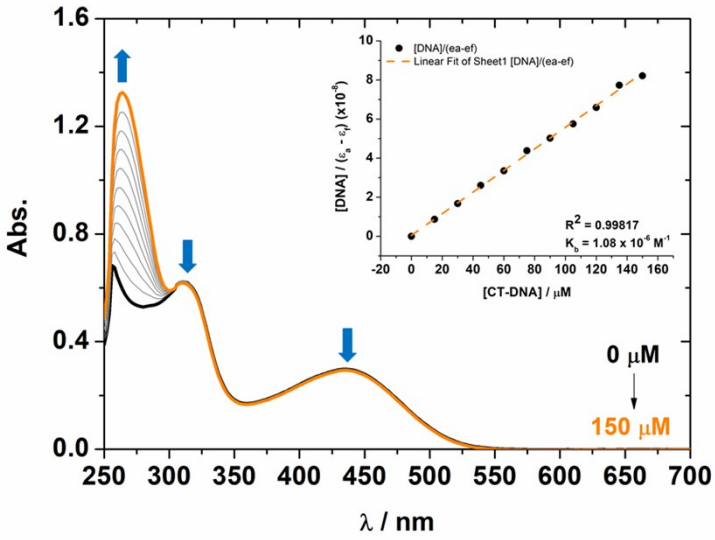


Figure S3. UV–vis titration absorption spectra of **3d** derivative, in a DMSO/Tris-HCl buffer (pH 7.4) mixture. The concentration of CT-DNA ranged from 0 to 150 μM. *Insert graph* shows the plot of [DNA]/($\varepsilon_a - \varepsilon_f$) *versus* [DNA].

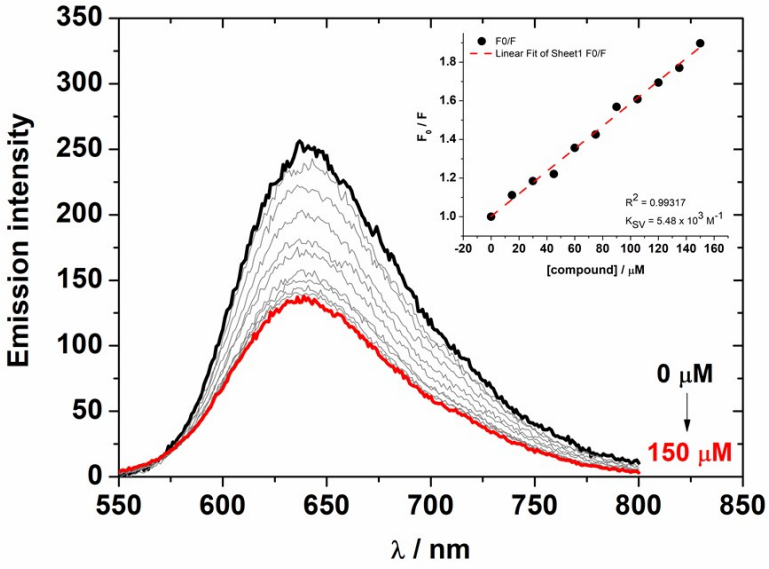


Figure S4. Steady-state emission fluorescence spectra of EB-DNA adduct in the presence of **3b**, in a DMSO/Tris-HCl pH 7.4 buffer mixture at λ_{exc} = 510 nm. The arrow indicates the changes in fluorescence intensities at increasing concentrations of samples. Insert graph shows the plot of F_0/F *versus* [compound].

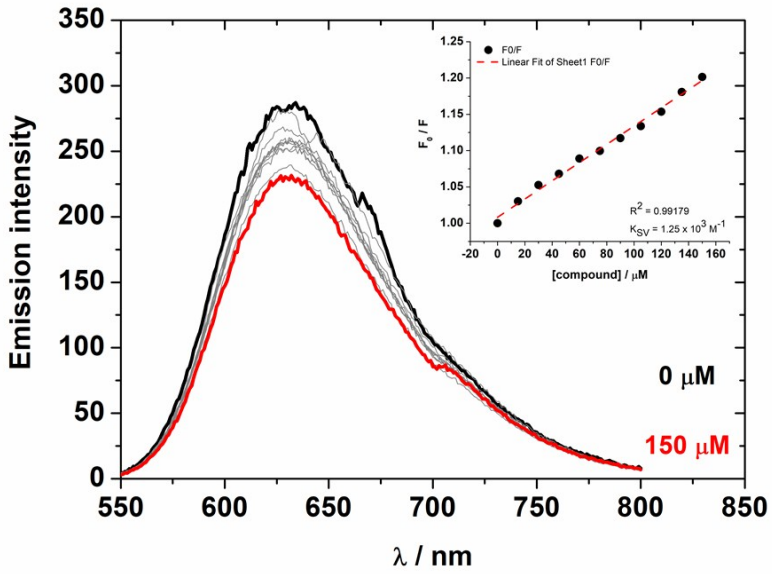


Figure S5. Steady-state emission fluorescence spectra of EB-DNA adduct in the presence of **3c**, in a DMSO/Tris-HCl pH 7.4 buffer mixture at λ_{exc} = 510 nm. The arrow indicates the changes in fluorescence intensities at increasing concentrations of samples. Insert graph shows the plot of F_0/F *versus* [compound].

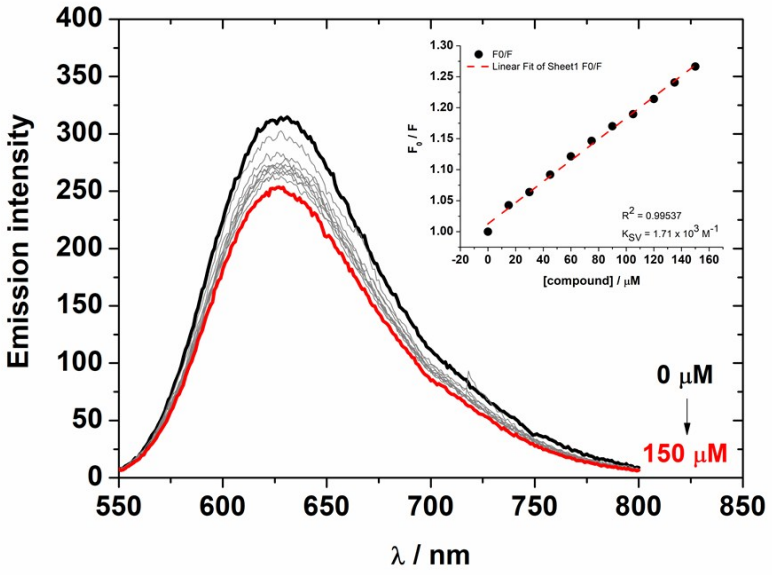


Figure S6. Steady-state emission fluorescence spectra of EB-DNA adduct in the presence of **3d**, in a DMSO/Tris-HCl pH 7.4 buffer mixture at λ_{exc} = 510 nm. The arrow indicates the changes in fluorescence intensities at increasing concentrations of samples. Insert graph shows the plot of F_0/F *versus* [compound].

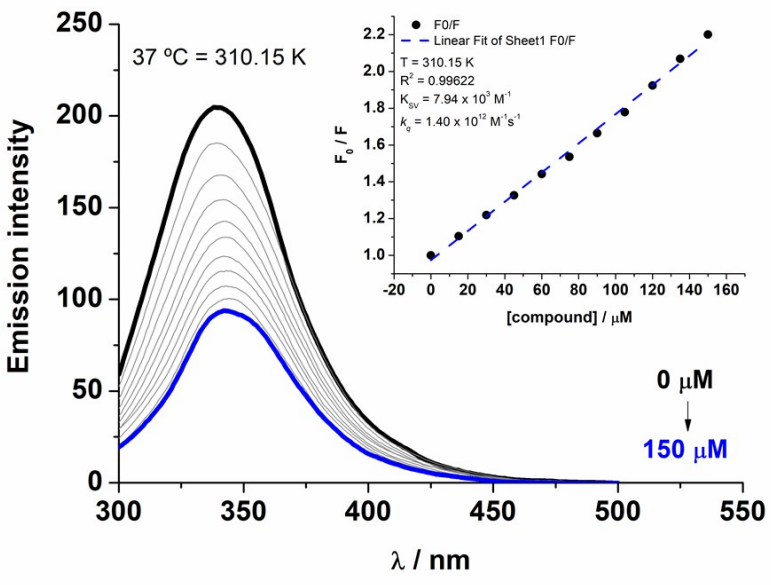


Figure S7. Steady-state HSA-emission fluorescence spectra of **3a**, in a DMSO/Tris-HCl buffer (pH 7.4) mixture at 310.15 K. The concentration of compounds ranged from 0 to 150 μM. *Insert graph* shows the plot of F_0/F *versus* [compound].

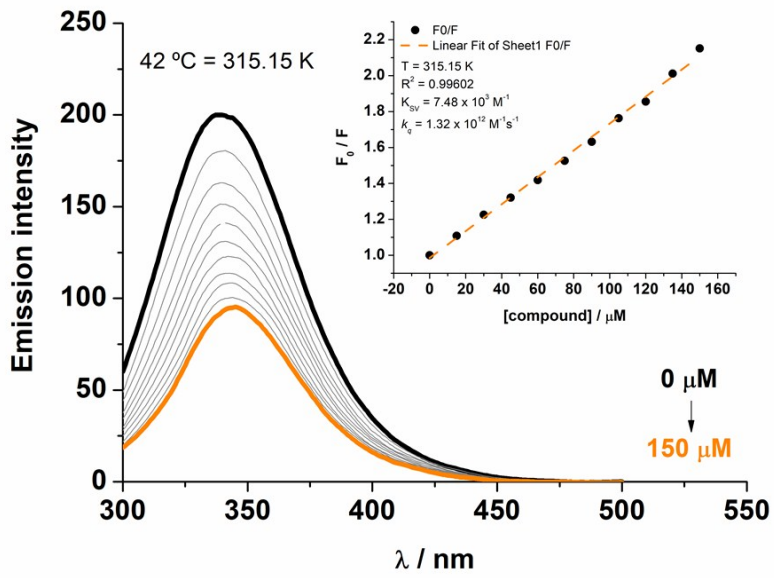


Figure S8. Steady-state HSA-emission fluorescence spectra of **3a**, in a DMSO/Tris-HCl buffer (pH 7.4) mixture at 315.15 K. The concentration of compounds ranged from 0 to 150 μM. *Insert graph* shows the plot of F_0/F *versus* [compound].

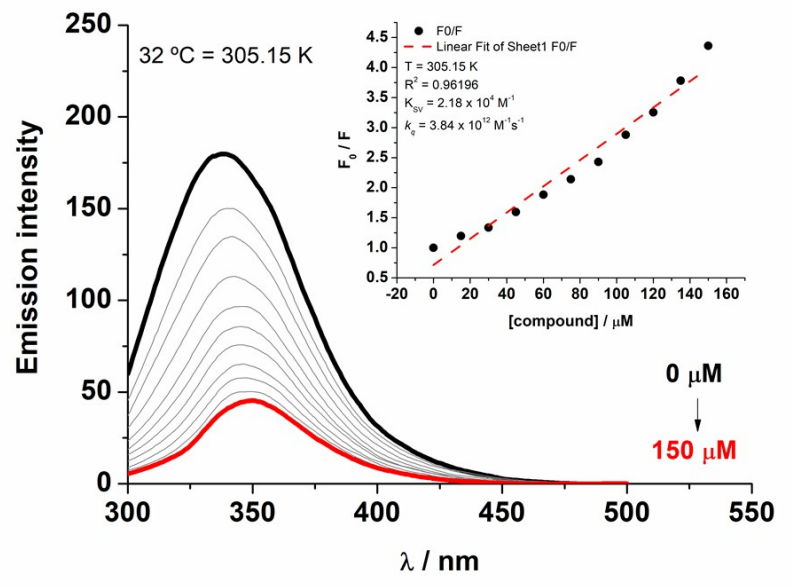


Figure S9. Steady-state HSA-emission fluorescence spectra of **3b**, in a DMSO/Tris-HCl buffer (pH 7.4) mixture at 305.15 K. The concentration of compounds ranged from 0 to 150 μM. *Insert graph* shows the plot of F_0/F *versus* [compound].

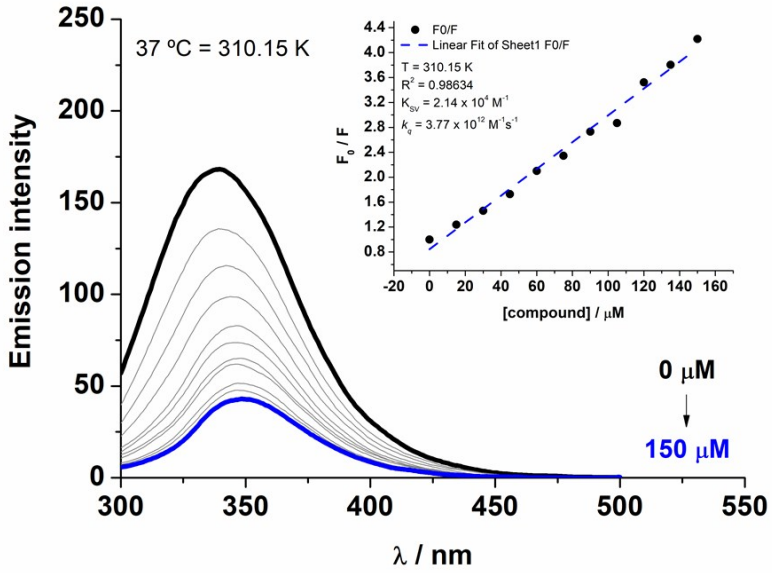


Figure S10. Steady-state HSA-emission fluorescence spectra of **3b**, in a DMSO/Tris-HCl buffer (pH 7.4) mixture at 310.15 K. The concentration of compounds ranged from 0 to 150 μM. *Insert graph* shows the plot of F_0/F *versus* [compound].

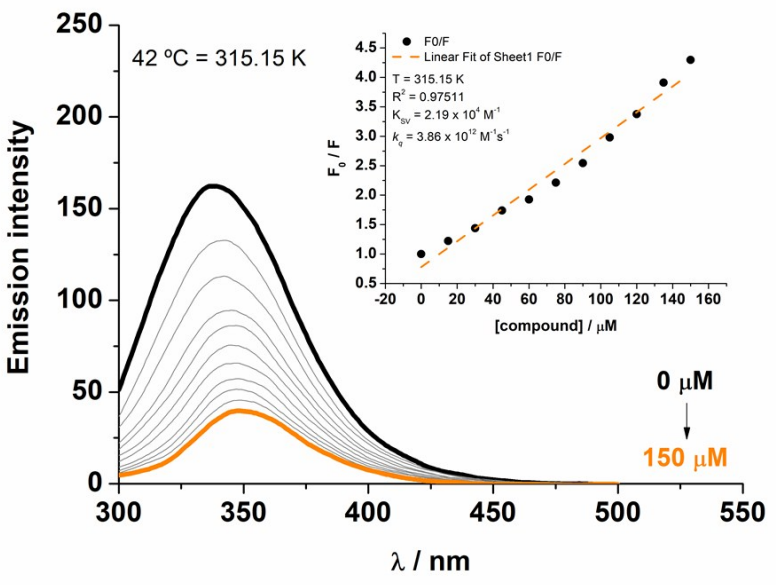


Figure S11. Steady-state HSA-emission fluorescence spectra of **3b**, in a DMSO/Tris-HCl buffer (pH 7.4) mixture at 315.15 K. The concentration of compounds ranged from 0 to 150 μM. *Insert graph* shows the plot of F_0/F *versus* [compound].

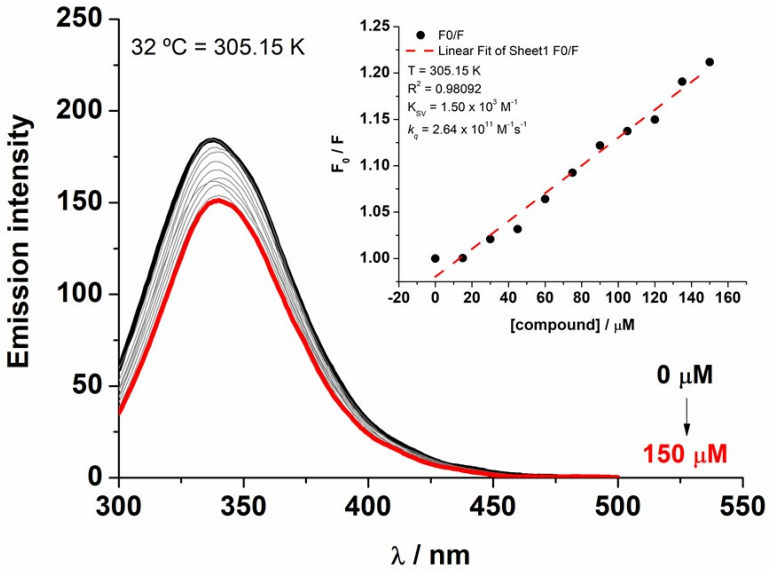


Figure S12. Steady-state HSA-emission fluorescence spectra of **3c**, in a DMSO/Tris-HCl buffer (pH 7.4) mixture at 305.15 K. The concentration of compounds ranged from 0 to 150 μM. *Insert graph* shows the plot of F_0/F *versus* [compound].

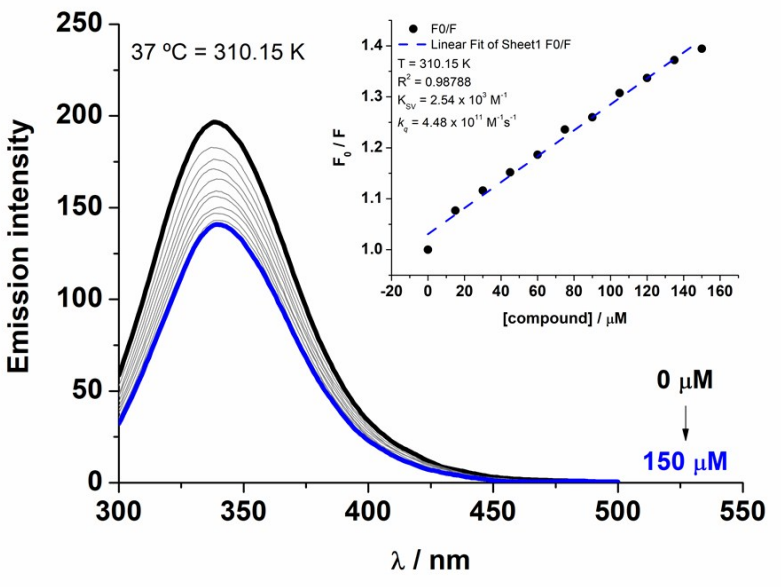


Figure S13. Steady-state HSA-emission fluorescence spectra of **3c**, in a DMSO/Tris-HCl buffer (pH 7.4) mixture at 310.15 K. The concentration of compounds ranged from 0 to 150 μM. *Insert graph* shows the plot of F_0/F *versus* [compound].

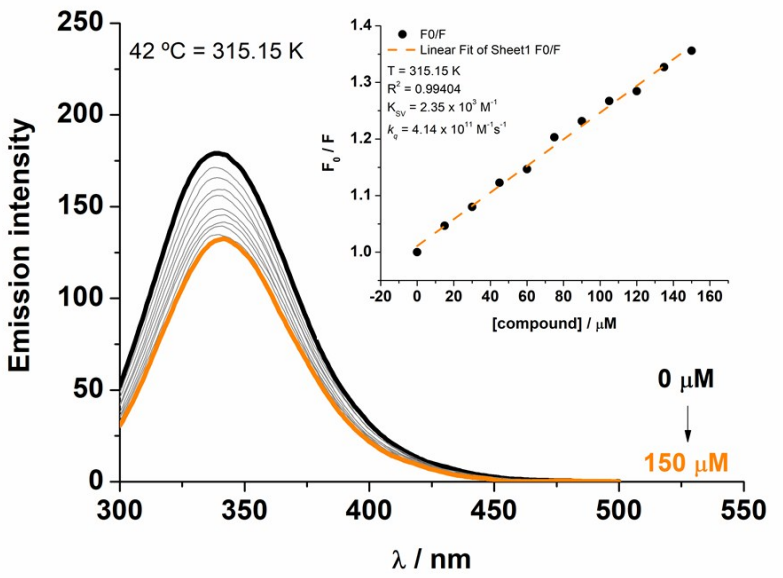


Figure S14. Steady-state HSA-emission fluorescence spectra of **3c**, in a DMSO/Tris-HCl buffer (pH 7.4) mixture at 315.15 K. The concentration of compounds ranged from 0 to 150 μM. *Insert graph* shows the plot of F_0/F *versus* [compound].

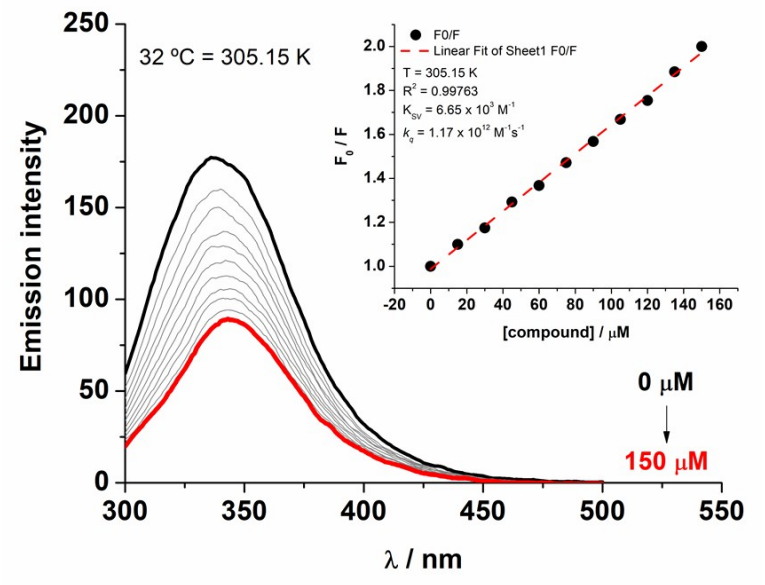


Figure S15. Steady-state HSA-emission fluorescence spectra of **3d**, in a DMSO/Tris-HCl buffer (pH 7.4) mixture at 305.15 K. The concentration of compounds ranged from 0 to 150 μM. *Insert graph* shows the plot of F_0/F *versus* [compound].

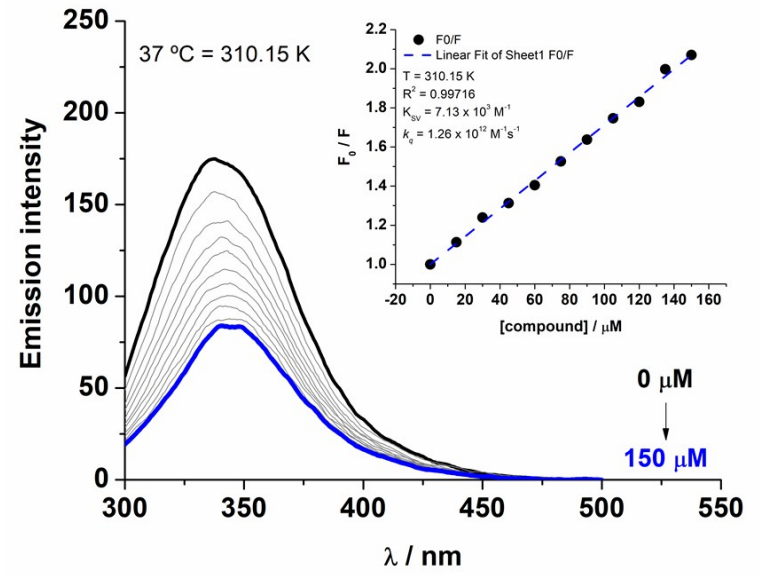


Figure S16. Steady-state HSA-emission fluorescence spectra of **3d**, in a DMSO/Tris-HCl buffer (pH 7.4) mixture at 310.15 K. The concentration of compounds ranged from 0 to 150 μM. *Insert graph* shows the plot of F_0/F *versus* [compound].

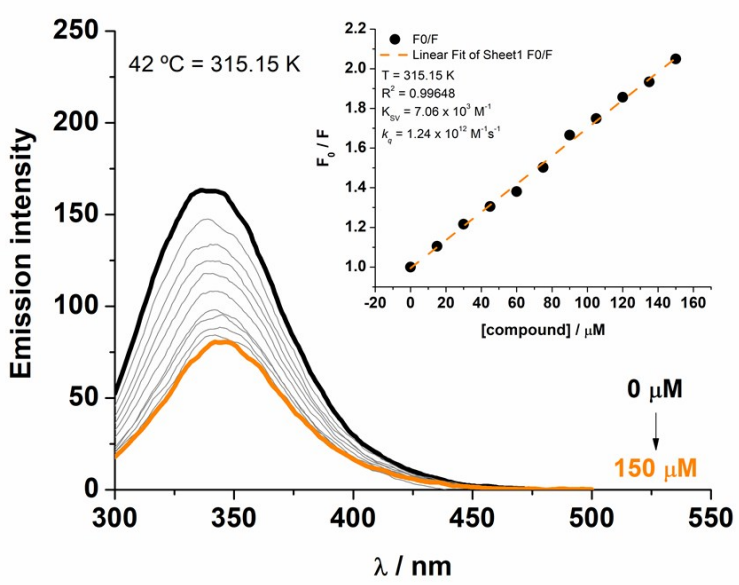


Figure S17. Steady-state HSA-emission fluorescence spectra of **3d**, in a DMSO/Tris-HCl buffer (pH 7.4) mixture at 315.15 K. The concentration of compounds ranged from 0 to 150 μM. *Insert graph* shows the plot of F_0/F *versus* [compound].

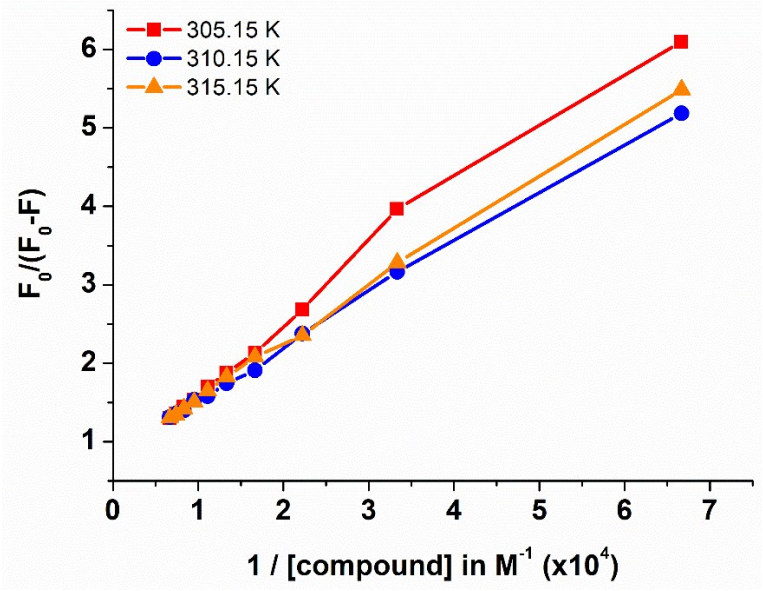


Figure S18. Modified Stern-Volmer plots for HSA:**3b** at three different temperatures.

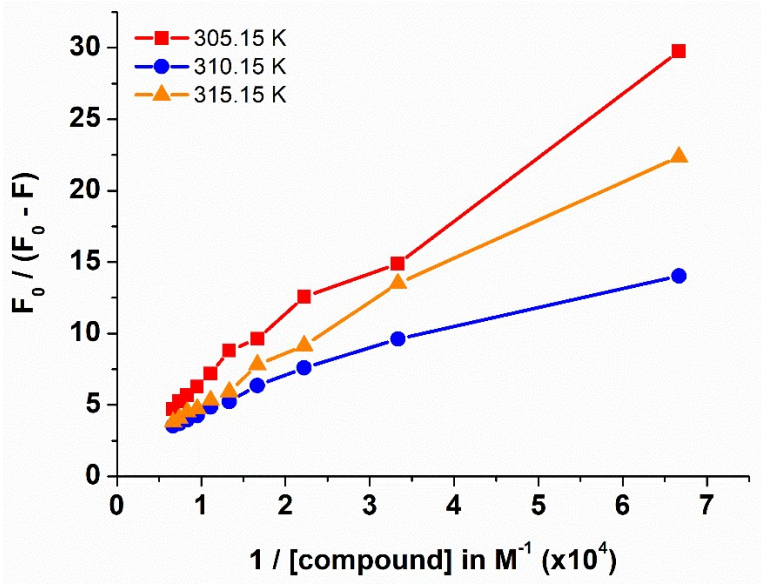


Figure S19. Modified Stern-Volmer plots for HSA:**3c** at three different temperatures.

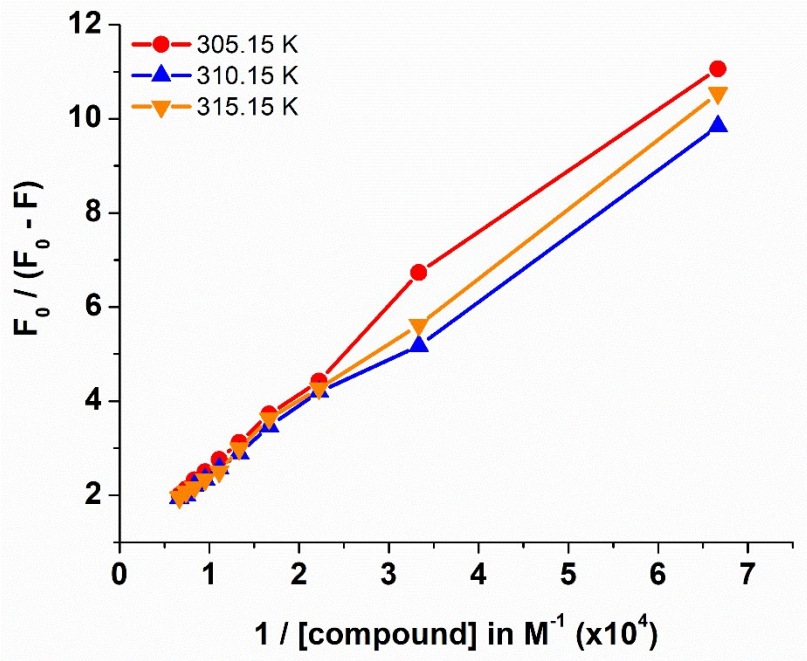


Figure S20. Modified Stern-Volmer plots for HSA:**3d** at three different temperatures.

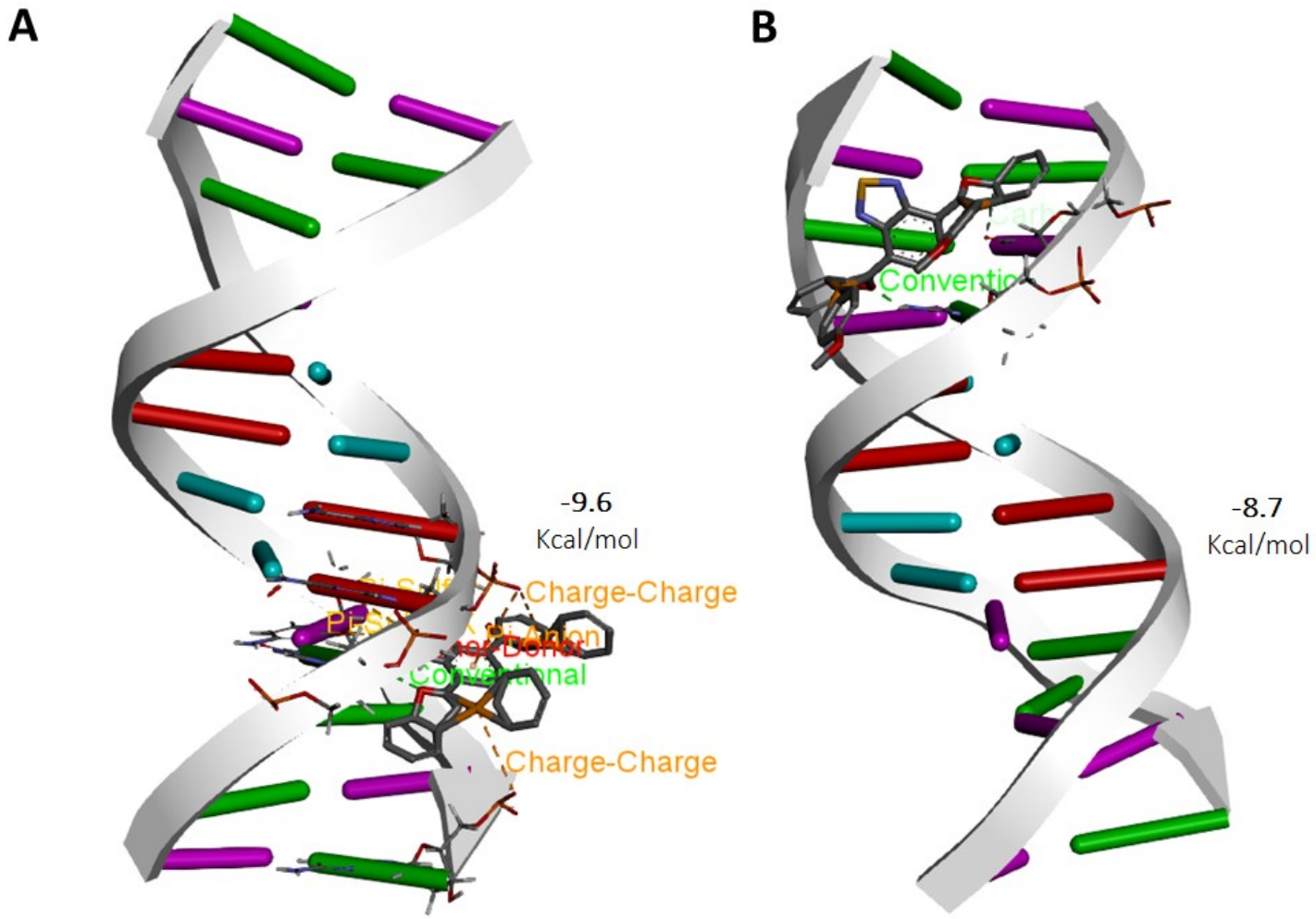


Figure S21. Molecular docking obtained binding modes and protein-ligand of molecules (A) **3a** and (B) **3c** in the minor groove of DNA structure.

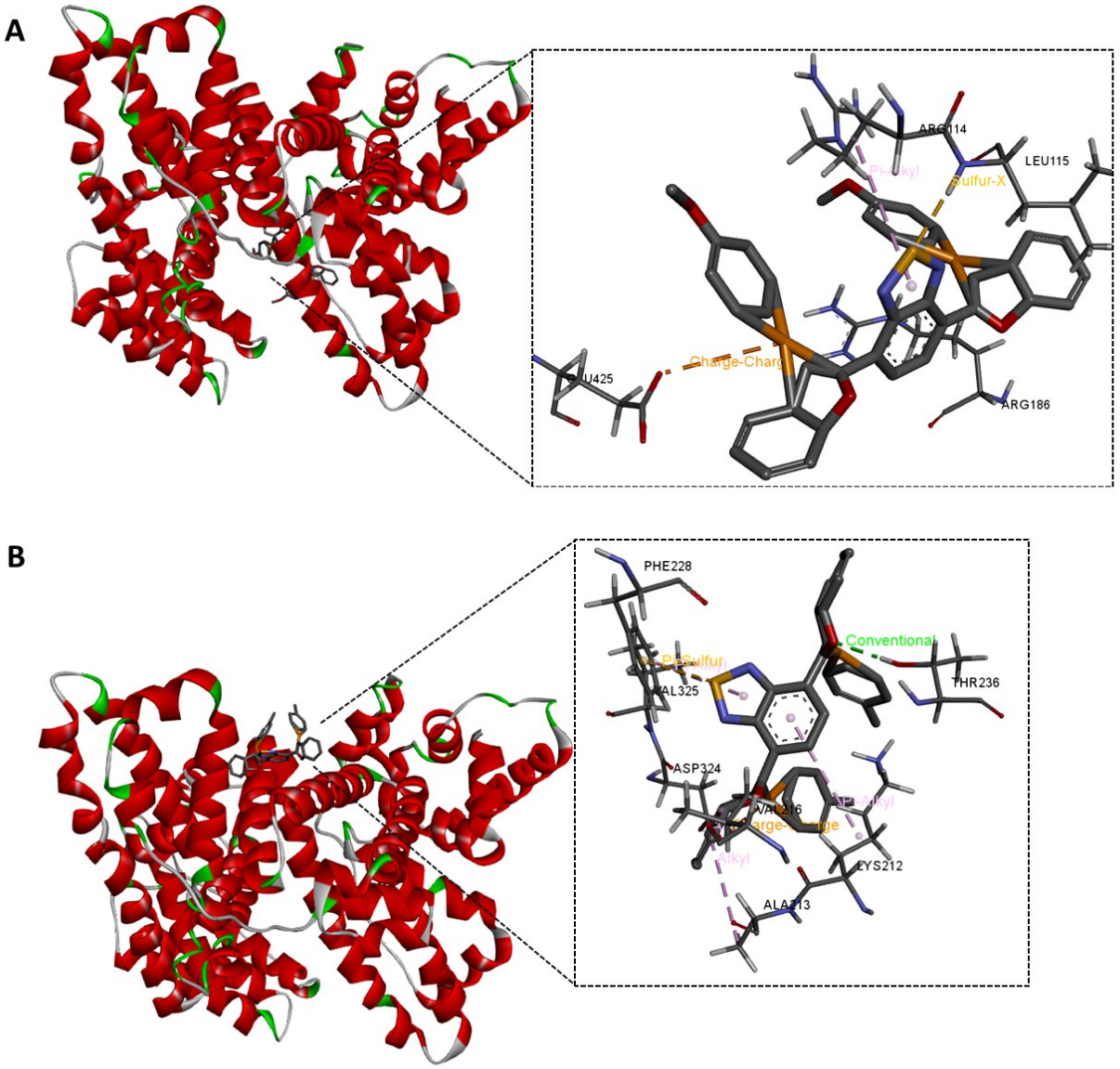


Figure S22. Molecular docking obtained binding modes and protein-ligand interactions of molecules (A) **3c** and (B) **3d** in the pocket atoms of HSA structure.

GEOMETRICAL PARAMETERS

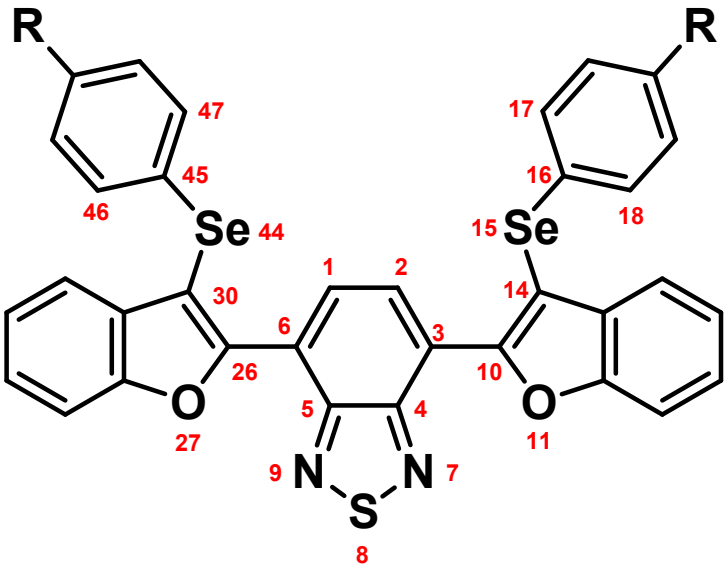


Figure S23. Atom indexes description for compounds **3a–3d**.

Table S1. Selected geometrical parameters for compounds **3a–3d**. All distances are in Angstroms and angles in degrees.

Parameter		**Compound**			
Name	**Index**	**3a**	**3b**	**3c**	**3d**
b1	3-10	1.45	1.45	1.45	1.45
a1	7-8-9	100.7	100.7	100.7	100.7
a2	30-44-45	100.2	100.1	96.6	99.0
a3	14-15-16	99.5	99.0	98.4	99.2
d1	5-6-26-30	39.2	40.1	40.5	37.6
d1*	5-6-26-27	33.2	34.5	35.7	32.4
d2	2-3-10-14	134.0	135.0	127.0	134.0
d2*	4-3-10-11	140.0	143.2	134.0	142.0
d3	14-15-16-17	136.0	135.0	118.0	132.1
d4	30-44-45-46	26.8	29.1	45.15	30.1

SELECTED SPECTRA

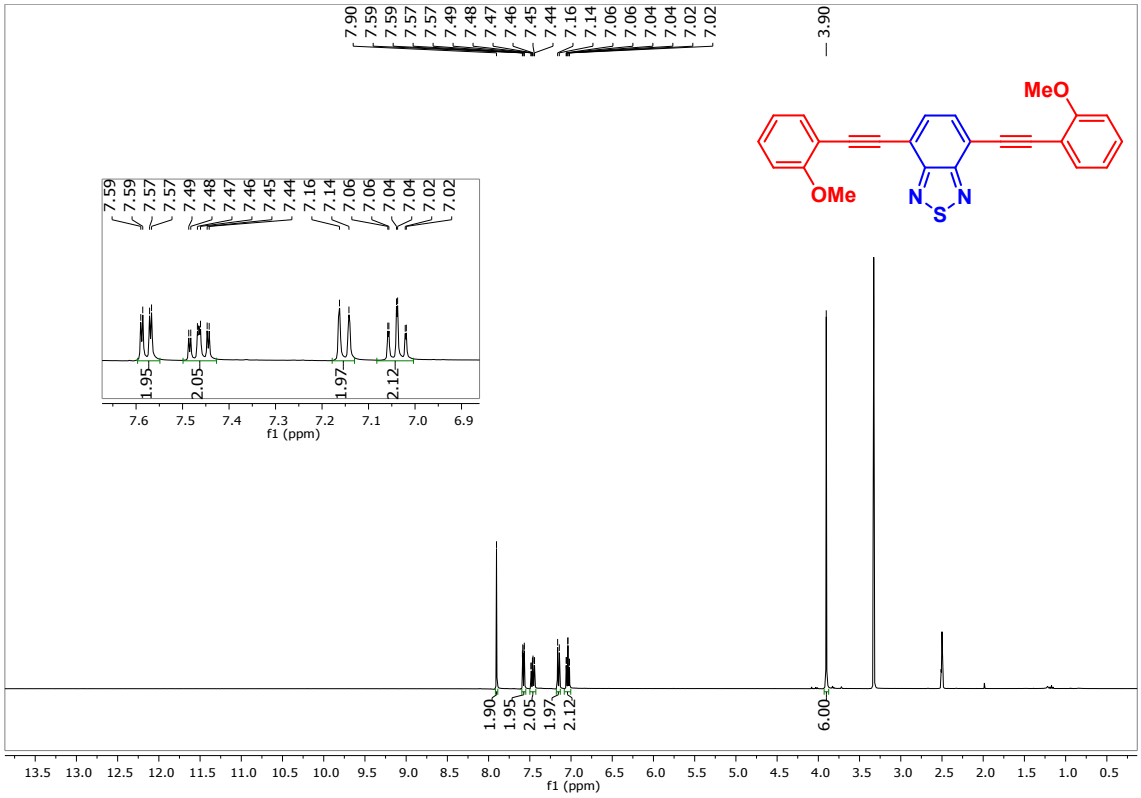


Figure S24. ^{1}H NMR (400 MHz) spectrum for compound **1** in DMSO-d_6.

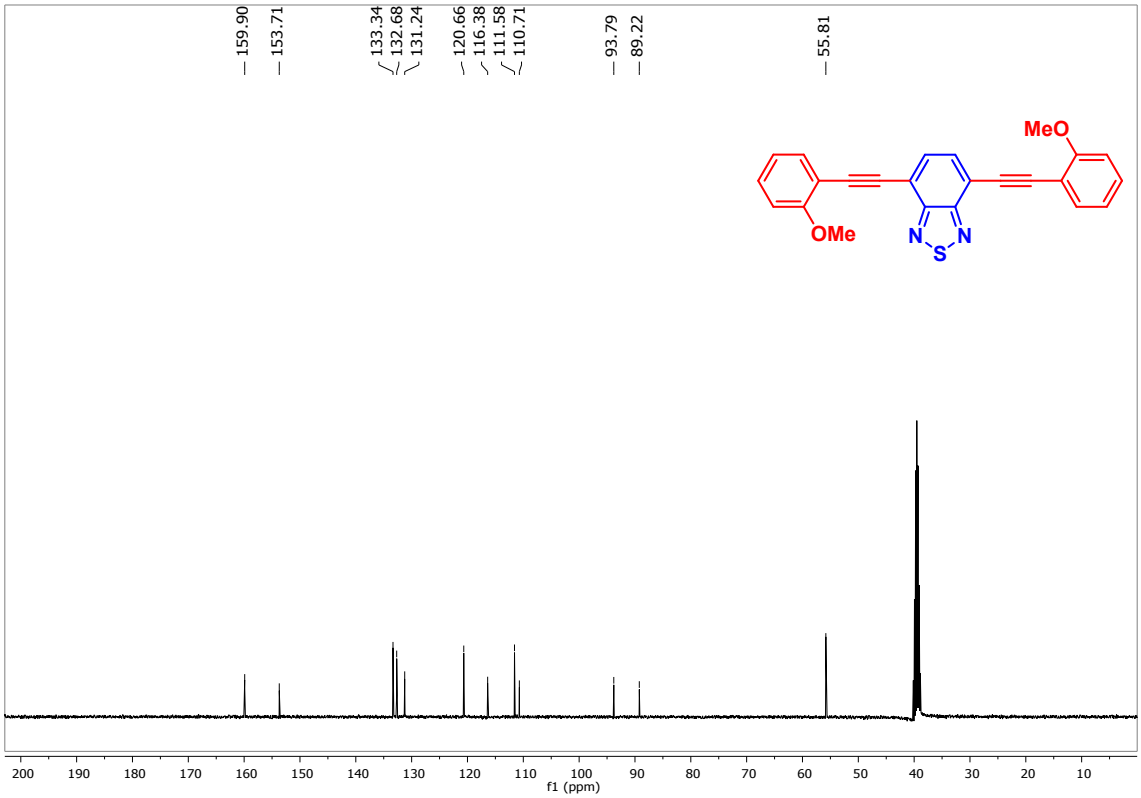


Figure S25. ^{13}C NMR (100 MHz) spectrum for compound **1** in DMSO-d_6.

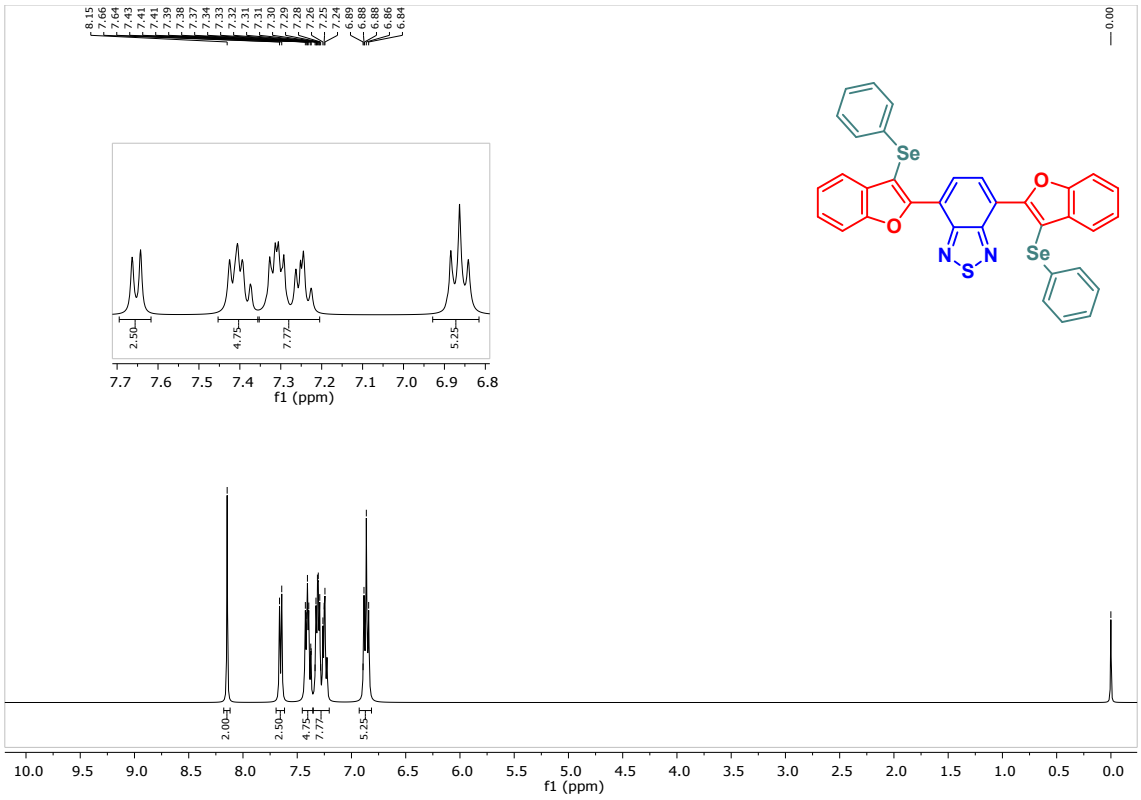


Figure S26. ^{1}H NMR (400 MHz) spectrum for compound **3a** in $CDCl_3$.

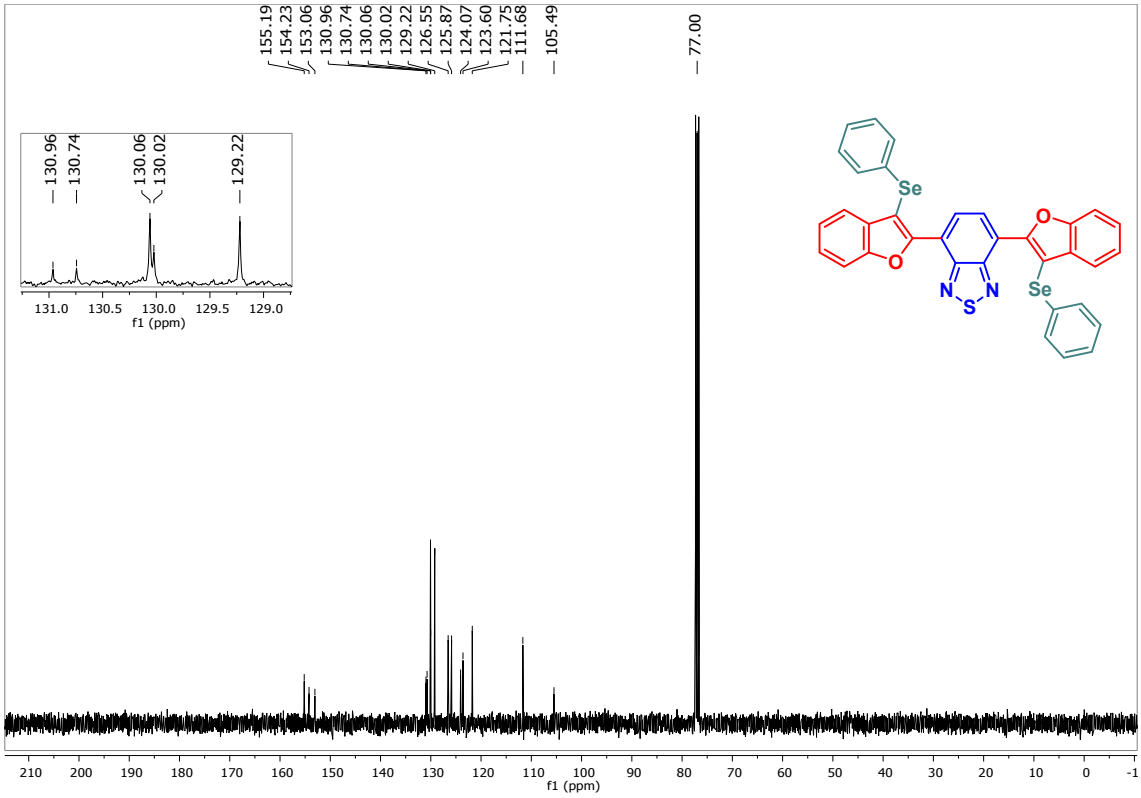


Figure S27. ^{13}C NMR (100 MHz) spectrum for compound **3a** in $CDCl_3$.

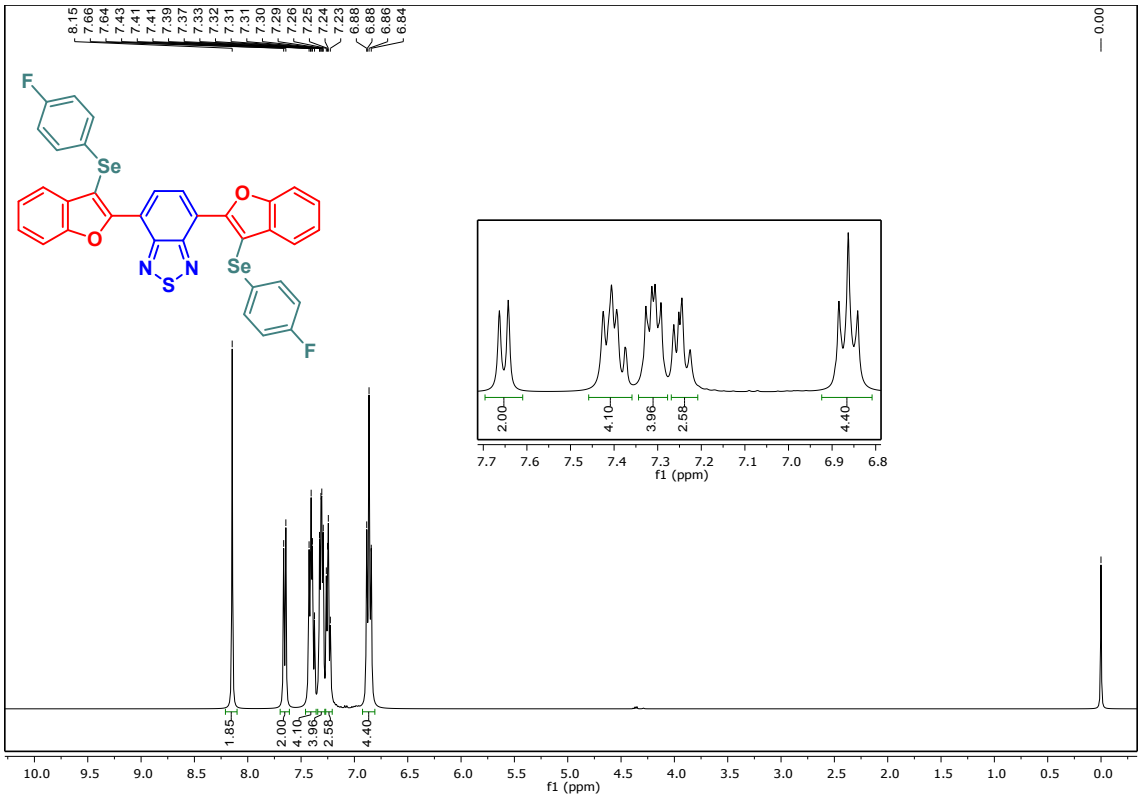


Figure S28. ^{1}H NMR (400 MHz) spectrum for compound **3b** in $CDCl_3$.

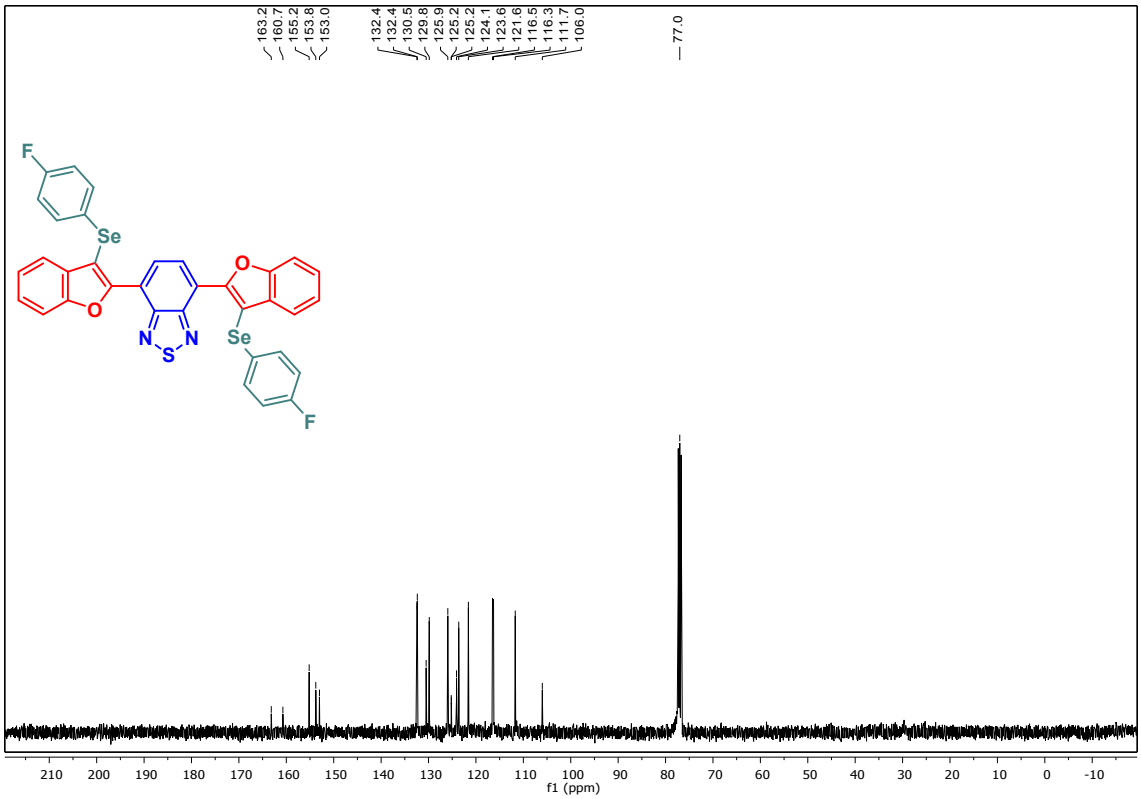


Figure S29. ^{13}C NMR (100 MHz) spectrum for compound **3b** in $CDCl_3$.

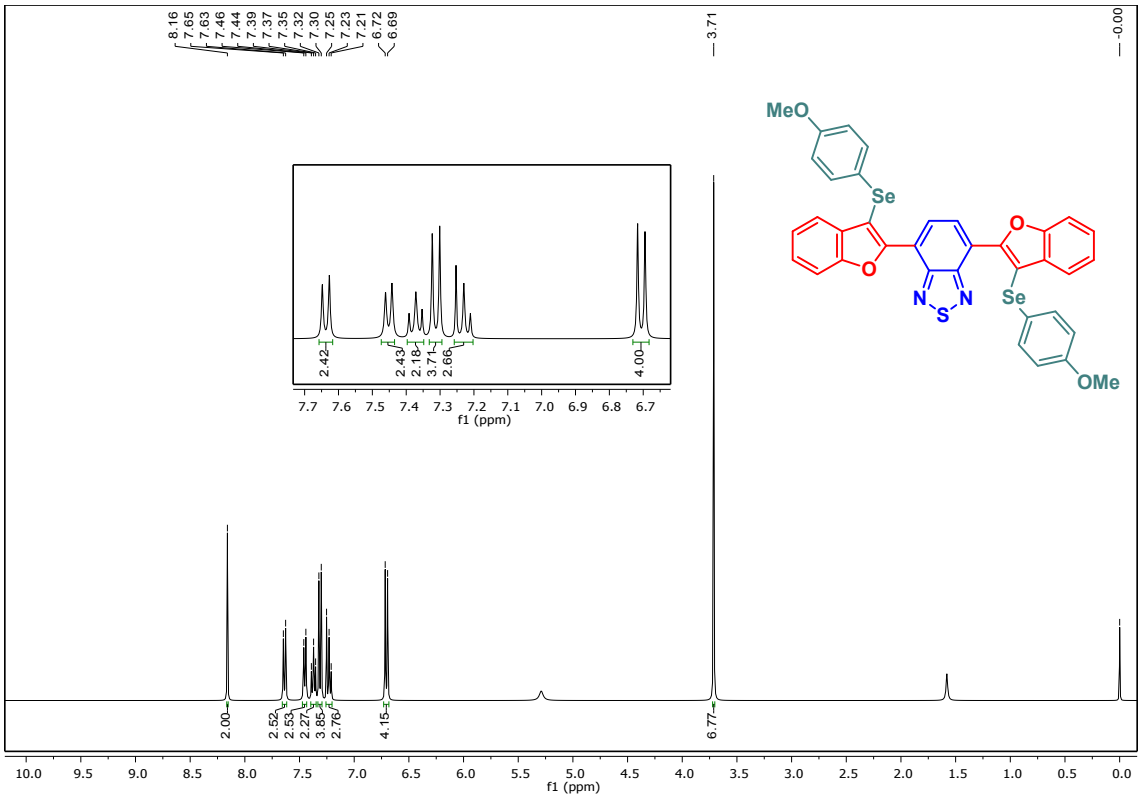


Figure S30. ^{1}H NMR (400 MHz) spectrum for compound **3c** in $CDCl_3$.

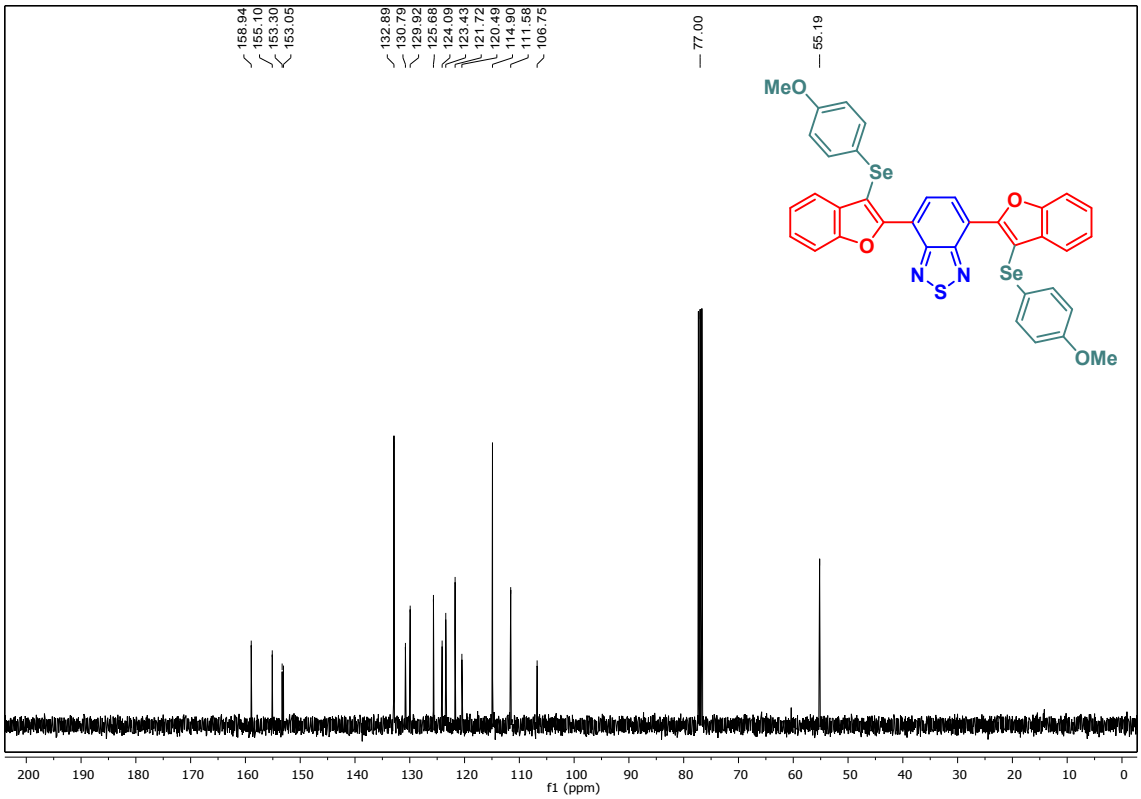


Figure S31. ^{13}C NMR (100 MHz) spectrum for compound **3c** in $CDCl_3$.

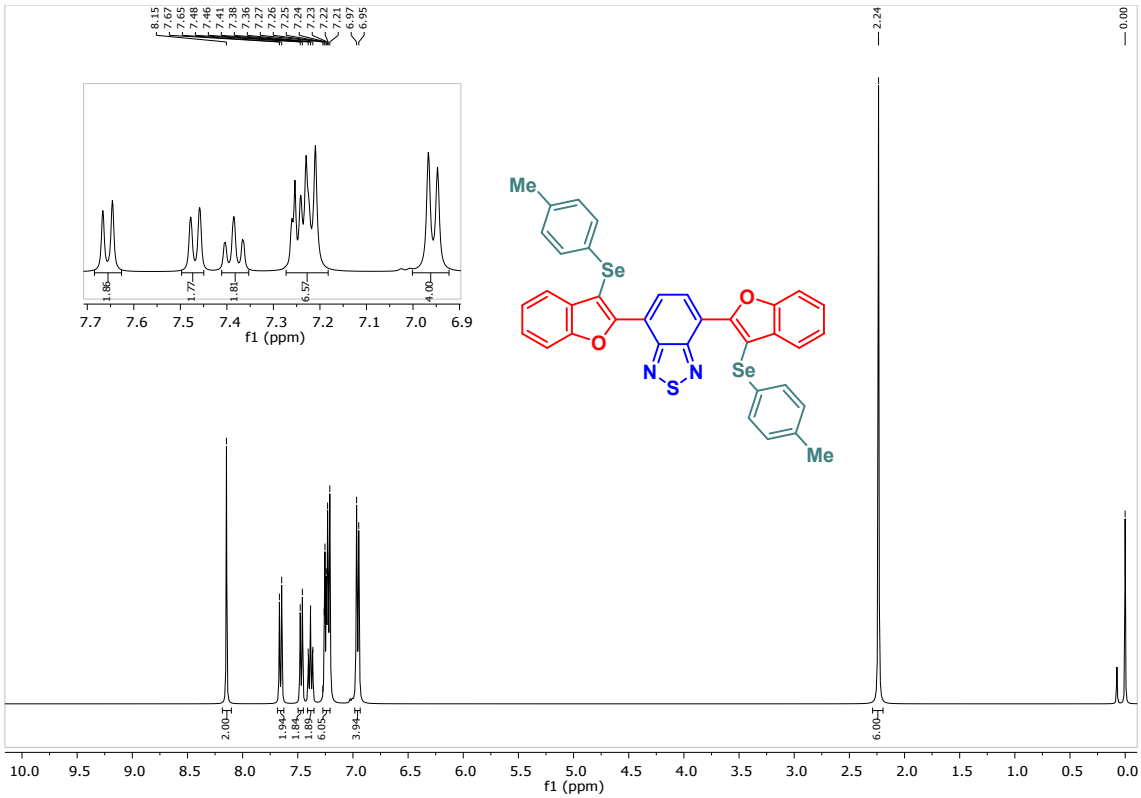


Figure S32. ^{1}H NMR (400 MHz) spectrum for compound **3d** in $CDCl_3$.

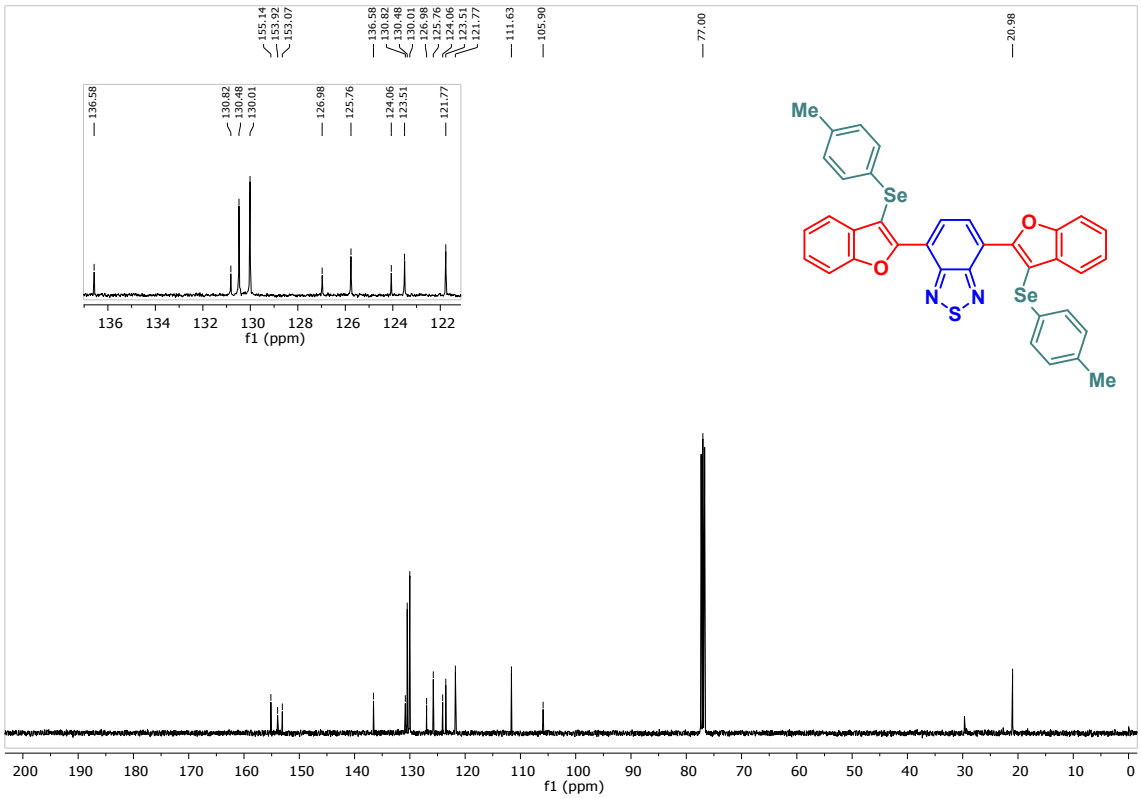


Figure S33. ^{13}C NMR (100 MHz) spectrum for compound **3d** in $CDCl_3$.