

Photophysical properties of novel cinnoline-fused naphthalimide derivative and its prospects for theranostics

Marina A. Pavlova, Daria E. Tyupakova, Pavel A. Panchenko and Olga A. Fedorova

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General

Melting points were measured on Melt-temp melting point electrothermal apparatus and were uncorrected. LC-ESI-MS analyses were performed using acetonitrile solutions on a Shimadzu LCMS-2020 liquid chromatography mass spectrometer. ^1H and ^{13}C NMR spectra were recorded on an Avance 300 (Bruker) and Inova 400 (Agilent) spectrometers. The measurements were performed in DMSO- d_6 solutions. The chemical shifts (given as δ) were determined with an accuracy of 0.01 ppm relative to the signals corresponding to the residual solvents and recalculated to the internal standard (TMS); the spin-spin coupling constants (J) were measured with an accuracy of 0.1 Hz. The numbering of carbon atoms in the compounds used by us for the description of ^1H and ^{13}C NMR spectra below is shown in Figure S1.

The absorption spectra were taken on a Cary 300 spectrophotometer (Agilent Technologies) and an AvaSpec-2048-USB2 fiber-optic spectrophotometer. The light intensity was measured by a Nova P/N 7Z01500 power meter equipped with 3A-FS P/N 7Z02628 thermal power/energy measurement sensor. The fluorescence measurements were performed using a Fluorolog3-221 spectrofluorimeter (Horiba Jobin Yvon). For measuring the photophysical properties compound **1** was dissolved in DMSO at 1.9 mM (stock solution). The stock solution were further diluted with acetone, the final DMSO content in the cuvette did not exceed 1% vol. Solvents used in spectroscopic studies was of HPLC grade. Spectral measurements were carried out in air-saturated solutions at ambient temperature. All measured fluorescence spectra were corrected for the nonuniformity of detector spectral sensitivity. Coumarin 481

in acetonitrile ($\varphi^{\text{fl}} = 0.08$)^{S1} was used as reference for the fluorescence quantum yield measurements. The fluorescence quantum yields were calculated by the Eq. (1)^{S2},

$$\varphi^{\text{fl}} = \varphi_{\text{R}}^{\text{fl}} \frac{S}{S_{\text{R}}} \cdot \frac{(1 - 10^{-A_{\text{R}}})n^2}{(1 - 10^{-A})n_{\text{R}}^2} \quad (1)$$

wherein φ^{fl} and $\varphi_{\text{R}}^{\text{fl}}$ are the fluorescence quantum yields of the studied solution and the standard compound respectively; A and A_{R} are the absorptions of the studied solution and the standard respectively; S and S_{R} are the areas underneath the curves of the fluorescence spectra of the studied solution and the standard respectively; and n and n_{R} are the refraction indices of the solvents for the substance under study and the standard compound.

The quantum yields of singlet oxygen (Φ_{Δ}) were estimated in acetone by using 1,3-diphenylisobenzofuran (DPBF) as a singlet oxygen chemical trap and tetraphenylporphyrin (TPP) as a reference compound ($\Phi_{\Delta} = 0.7$).^{S3,S4} Photosensitizers (**1** and TPP) with DPBF (40 μM) were dissolved in 2.5 mL of acetone in spectrofluorimetric cells equipped with a magnetic stirrer. The solutions were irradiated with monochromatic light (500 nm) using the excitation unit (a xenon lamp and excitation monochromator) of a FluoroLog-3 spectrofluorometer for different time intervals, monitoring the absorption intensity of the DPBF at 414 nm. The $^1\text{O}_2$ quantum yields were calculated by Eq. (2):

$$\Phi_{\Delta} = \Phi_{\Delta}^{\text{R}} \frac{V}{V_{\text{R}}} \cdot \frac{1 - 10^{-A_{\text{R}}}}{1 - 10^{-A}} \quad (2)$$

wherein V and V_{R} are the rate constants of DPBF bleaching in solutions containing **1** (V) and reference compound TPP (V_{R}), which were found as the tangents of the slope of the linear absorption curves at 414 nm as a function of the irradiation time; A and A_{R} are absorption values at excitation wavelength (500 nm) of the solutions containing the studied PS and reference compound (TPP) respectively. The accuracy of Φ_{Δ} estimation was about 10%. The graphs of the dependence of optical density at 414 nm on irradiation time used for Φ_{Δ} calculations are presented in Fig. S 12-13.

Quantum chemical calculations were carried out by the MOPAC 2016 program package using the PM6 semiempirical method.^{S5} CI calculations were performed at optimized geometries, which reached gradient variations less than 0.01 kcal/mol. The solvent effect was included in geometry optimizations following the “COnductorlike Screening MOdel” (COSMO) implemented in MOPAC 2016. A dielectric constant of $\varepsilon = 20$ and a refraction index of solvent (n) such that $n^2 = 2$ were used. The CI included eight occupied and eight unoccupied MOs.

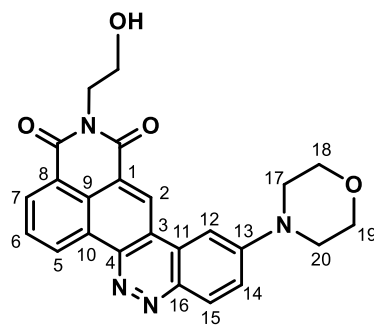


Figure S1. A numbering of carbon atoms in compound **1** (for compounds **3-6** the same numbering of the atoms of the naphthalimide ring was used).

Synthesis

All synthetic reagents were purchased from commercial sources. (3-Morpholino-phenyl)boronic acid was purchased from ABCR GmbH (Germany). The reaction course and purity of the final products was followed by TLC on aluminium oxide (TLC Aluminium oxide 60 F₂₅₄, Merck). Column chromatography was conducted over aluminium oxide (Aluminium oxide, particle size 0.04-0.20 mm, ChromLab). Flash chromatography was performed at IsoleraPrime (Biotage). Microwave synthesis system NOVA 2S (PreeKem, China) was used for synthesis of compound **6**.

2-(2-Hydroxyethyl)-6-nitro-1H-benzo[de]isoquinoline-1,3(2H)-dione (3). To a suspension of 4-nitro-1,8-naphthalic anhydride **2** (1.0 g, 4.10 mmol) in ethanol (42 ml) was added ethanolamine (0.25 ml, 4.10 mmol). After stirring for 2 h at 78 °C the reaction mixture was cooled to r.t. Then the product **3** was filtered out, washed with ethanol on a filter to give 0.896 g of product **3** as a white powder (77% yield). M.p. = 156 – 157 °C (*cf.* ref.^{S6}: m.p. = 157 – 160 °C.). ¹H NMR (300.13 MHz, DMSO-*d*₆, 21.5 °C): δ = 3.59 – 3.69 (m, 2H, CH₂CH₂OH), 4.16 (t, *J* = 6.4, 2H, CH₂CH₂OH), 4.83 (t, *J* = 6.1, 1H, OH), 8.04 – 8.14 (m, 1H, H(6)), 8.51 – 8.75 (m, 4H, H(2,3,5,7)).

6-Amino-2-(2-hydroxyethyl)-1H-benzo[de]isoquinoline-1,3(2H)-dione (4). A suspension of compound **3** (0.562 g, 1.96 mmol) in ethanol (10 ml) was heated to 50 °C. A solution of SnCl₂·2H₂O (3.163 g, 14.0 mmol) in 2.4 ml of concentrated hydrochloric acid (ρ = 1.18 g/ml) was added dropwise to the stirred suspension. After stirring for 1.5 h at 78 °C the reaction mixture was poured into 25 ml of water. The precipitate was filtered out, washed on a filter with water, 5% aqueous solution of NaOH, then again with water, ethanol to give 0.382 g of product **4** as an orange powder (76% yield). M.p. = 260 – 261 °C. (*cf.* ref.^{S6}: m.p. = 260 – 262 °C). ¹H NMR (300.13 MHz, DMSO-*d*₆, 22.9 °C): δ = 3.52 – 3.63 (m, 2H, CH₂CH₂OH), 4.12 (t, *J* = 6.6, 2H, CH₂CH₂OH), 4.78 (t, *J* = 5.9, 1H OH), 6.84 (d, *J* = 8.5, 1H, H(3)), 7.43 (s, 2H, NH₂), 7.61 – 7.69 (m, 1H, H(6)), 8.19 (d, *J* = 8.4, 1H, H(2)), 8.43 (d, *J* = 7.3, 1H, H(7)), 8.61 (d, *J* = 8.5, 1H, H(5)).

6-Amino-5-bromo-2-(2-hydroxyethyl)-1H-benzo[de]isoquinoline-1,3(2H)-dione (5). To a suspension of compound **4** (0.180 g, 0.70 mmol) in glacial acetic acid (4.4 ml) was added NBS (0.166 g, 0.93 mmol). The reaction mixture was heated at 78 °C for 2 h. The mixture was cooled to r.t. and poured into water. The precipitate was filtered out, washed on a filter with water to give 0.162 g of compound **5** as a light brown powder (yield 69 %). M.p. = 257 – 260 °C. ¹H NMR (300.13 MHz, DMSO-*d*₆, 20.7 °C): δ = 3.52 – 3.63 (m, 2H, CH₂CH₂OH), 4.08 (t, *J* = 6.9, 2H, CH₂CH₂OH), 4.79 (t, *J* = 5.9, 1H, OH), 7.46 (s, 2H, NH₂), 7.68 – 7.77 (m, 1H, H(6)), 8.33 (s, 1H, H(2)), 8.44 (d, *J* = 7.2, 1H, H(7)), 8.76 (d, *J* = 8.6, 1H, H(5)). ¹³C NMR (100.61 MHz, DMSO-*d*₆, 19 °C): δ = 41.56 (NCH₂CH₂), 57.86 (NCH₂CH₂), 101.48 (C(3)), 109.47 (C(1)), 119.93 (C(10)), 122.11 (C(8)), 125.31 (C(6)), 128.23 (C(9)), 129.34 (C(5)), 130.89 (C(7)), 135.68 (C(2)), 148.51 (C(4)), 162.19 (C=O), 163.50 (C=O). ESI-mass *m/z*: calculated: 334.00 for [C₁₄H₁₀BrN₂O₃]⁻, found: 332.90 ([C₁₄H₁₀⁷⁹BrN₂O₃]⁻), 334.90 ([C₁₄H₁₀⁸¹BrN₂O₃]⁻).

6-Amino-2-(2-hydroxyethyl)-5-(3-morpholinophenyl)-1H-benzo[de]isoquinoline-1,3(2H)-dione (6). Compound **5** (0.40 g, 1.19 mmol), (3-morpholinophenyl)boronic acid (0.27 g, 1.31 mmol) and Na₂CO₃ (0.378 g, 3.58 mmol) were dissolved in DMF, H₂O (6 ml, 1:1, v/v). The resulting mixture was purged with argon before addition of Pd[dppf]Cl₂ (0.088 g, 0.12 mmol). The reaction mixture was heated at 100 °C for 3 h with stirring in a microwave reactor (250 W). The mixture was cooled to r.t. and poured into water. The precipitate was filtered out, washed on a filter with water and recrystallized from methanol (3 ml) to give 390 g of product **6** as a brownish green powder (yield 78 %). M.p. > 251 °C (*decomp.*). ¹H NMR (400.02 MHz, DMSO-*d*₆, 18.0 °C): δ = 3.15 – 3.24 (m, 4H, CH₂(18,19)), 3.53 – 3.62 (m, 2H, NCH₂CH₂), 3.71 – 3.79 (m, 4H, CH₂(17,20)), 4.12 (t, *J* = 6.6, 2H, NCH₂CH₂), 4.81 (t, *J* = 5.9, 1H, OH), 6.87 – 6.97 (m, 3H, NH₂, H(14)), 6.98 – 7.07 (m, 2H, H(12,16)), 7.40 (t, *J* = 7.8, 1H, H(15)), 7.66 – 7.73 (m, 1H, H(6)), 8.06 (s, 1H, H(2)), 8.44 (d, *J* = 7.4, 1H, H(7)), 8.78 (d, *J* = 8.3, 1H, H(5)). ¹³C NMR (100.61 MHz, DMSO-*d*₆, 18 °C): δ = 41.40 (NCH₂CH₂), 48.23 (C(18,19)), 57.94 (NCH₂CH₂), 66.12 (C(17,20)), 114.45 (C(1)), 115.54 (C(12,16)), 119.75 (C(4)), 119.97 (C(10)), 121.06 (C(3)), 121.86 (C(8)), 124.56 (C(6)), 128.81 (C(9)), 129.74 (C(15)), 129.82 (C(5)), 130.73 (C(7)), 134.61 (C(2)), 138.89 (C(11)), 148.51 (C(4)), 151.66 (C(13)), 163.12 (C=O), 163.87 (C=O). ESI-mass *m/z*: calculated: 418.18 for [C₂₄H₂₄N₃O₄]⁺, found: 418.11 ([C₂₄H₂₄N₃O₄]⁺), 440.94 ([C₂₄H₂₄N₃O₄]⁻ Na⁺).

11-(2-Hydroxyethyl)-2-morpholino-10H-benzo[c]isoquinolino[4,5-*gh*]cinnoline-10,12(11H)-dione (1). To a solution of compound **6** (0.315 g, 0.75 mmol) in acetonitrile, nitrosyl tetrafluoroborate (0.097 g, 0.83 mmol) was added at 0 °C. The reaction mixture was stirred for 2 hours at 0 °C. Then it was neutralized with saturated aqueous NaHCO₃ solution and left for a night. The precipitate was filtered out, washed on a filter with water. The residue was purified by column chromatography on Al₂O₃ in the dichloromethane – methanol system using gradient elution (v/v from 1:0 to 2:1) to give 98 mg of product **1** (30% yield). M.p. > 242 °C (*decomp.*). ¹H NMR (400.02 MHz, DMSO-*d*₆, 20.6 °C): δ = 3.63

– 3.78 (m, 6H, CH₂(18,19), NCH₂CH₂), 3.80 – 3.90 (m, 4H, CH₂(17,20)), 4.23 (t, *J* = 6.5, 2H, NCH₂CH₂), 4.88 (t, *J* = 5.8, 1H, OH), 7.89 (d, *J* = 8.3, 1H, H(14)), 8.06 (d, *J* = 1.4 1H, H(12)), 8.13–8.21 (m, 1H, H(6)), 8.53 (d, *J* = 9.3, 1H, H(15)), 8.65 (d, *J* = 7.3, 1H, H(7)), 9.68 (s, 1H, H(2)), 9.85 (d, *J* = 8.2, 1H, H(5)). ¹³C NMR (100.61 MHz, DMSO-*d*₆, 18 °C): δ = 42.12 (NCH₂CH₂), 46.95 (C(18,19)), 57.82 (NCH₂CH₂), 65.96 (C(17,20)), 99.79 (C(3)) 100.47 (C(12)), 118.16 (C(10)), 120.22 (C(14)), 122.60 (C(8)), 123.60 (C(9)), 126.84 (C(2)), 127.14 (C(11)), 128.98 (C(6)), 129.72 (C(5)), 130.67 (C(7)), 132.11 (C(15)), 138.41 (C(1)), 141.23 (C(4)), 143.20 (C(16)), 153.04 (C(13)), 163.58 (2×C=O). ESI-mass *m/z*: calculated: 429.15 for [C₂₄H₂₁N₄O₄]⁺, found: 429.25.

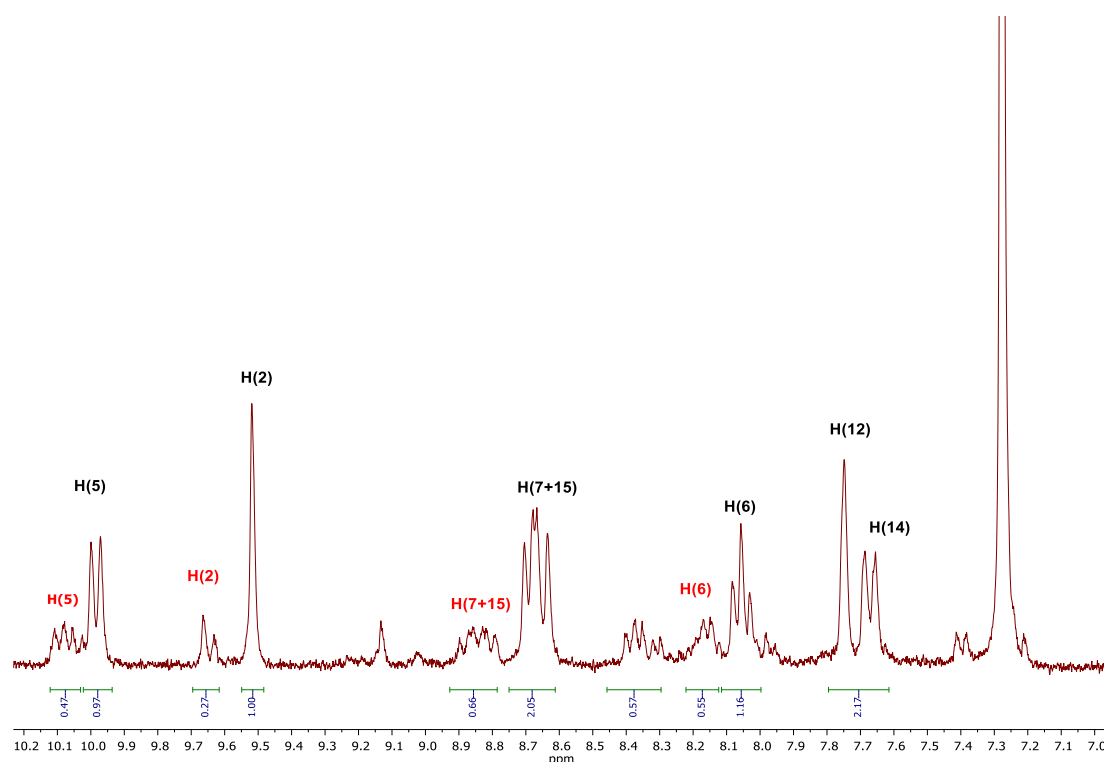


Figure S2. ¹H NMR spectrum (CDCl₃) spectrum of the precipitate obtained after the diazotization reaction with NOBF₄ followed the described methodology.^{S7} The signals of the *ortho*-isomer **1'** are marked in red.

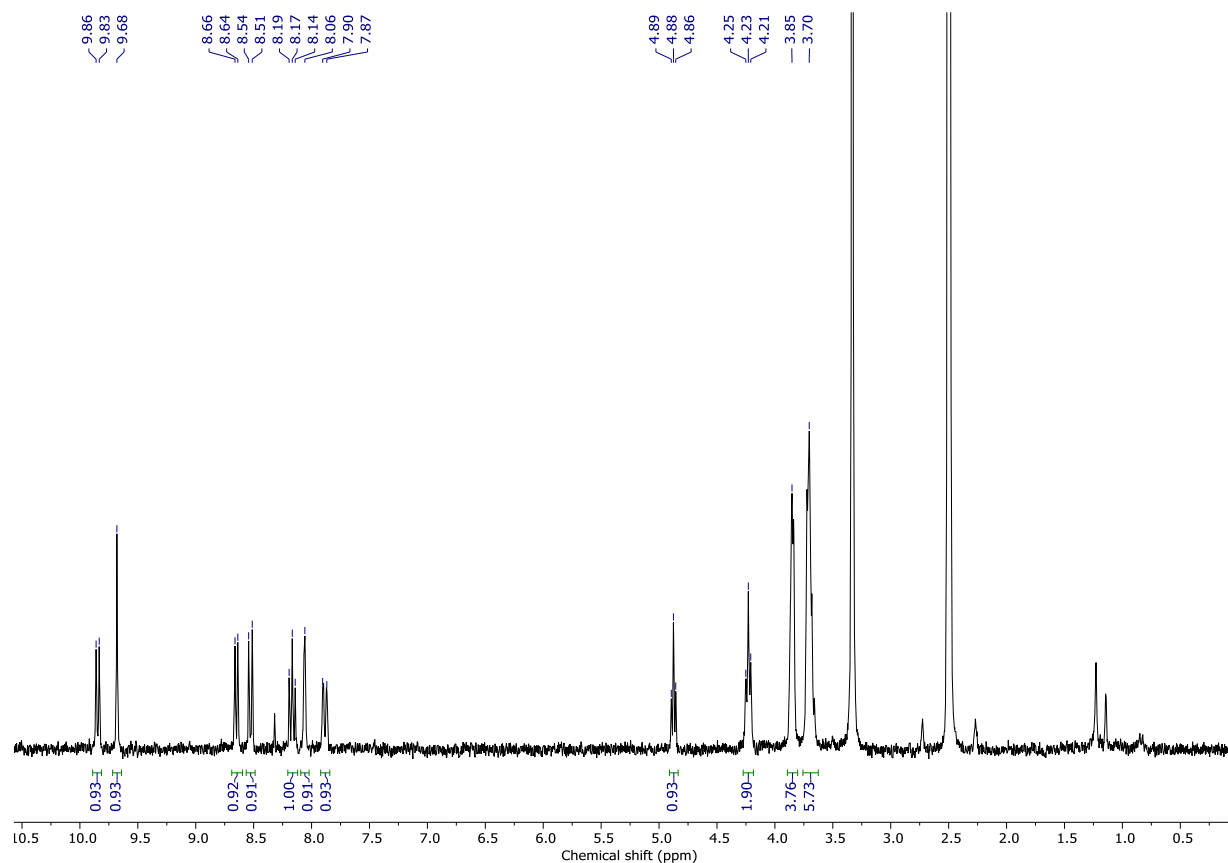


Figure S3. ¹H NMR spectrum of **1** in DMSO-*d*₆.

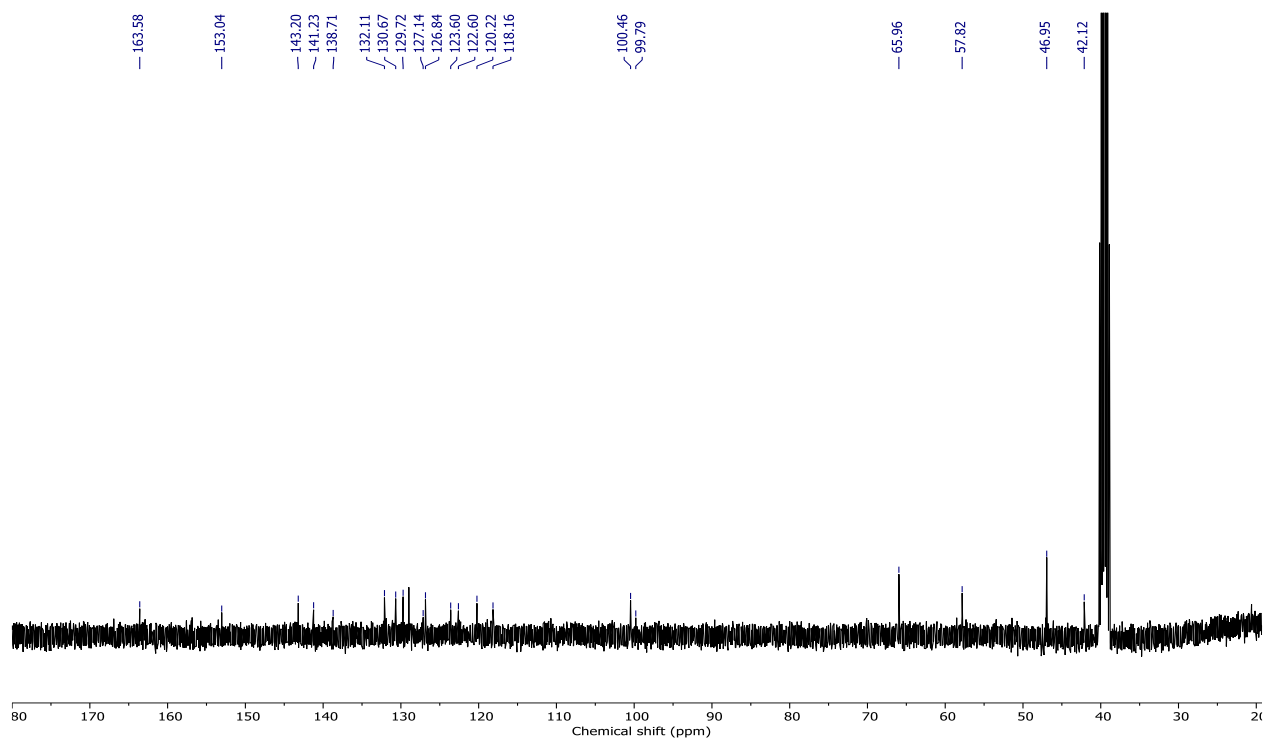


Figure S4. ¹³C NMR spectrum of **1** in DMSO-*d*₆.

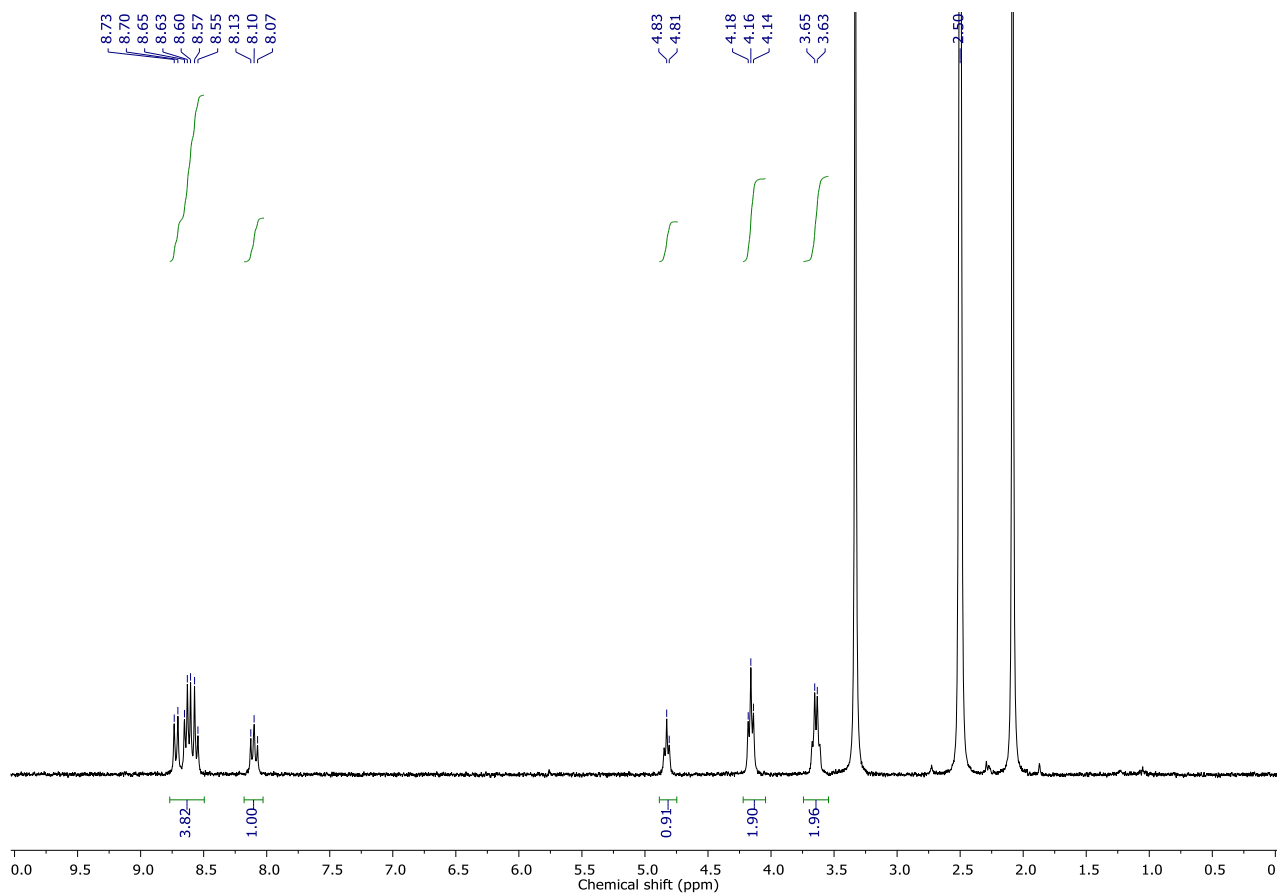


Figure S5. ¹H NMR spectrum of **3** in DMSO-*d*₆

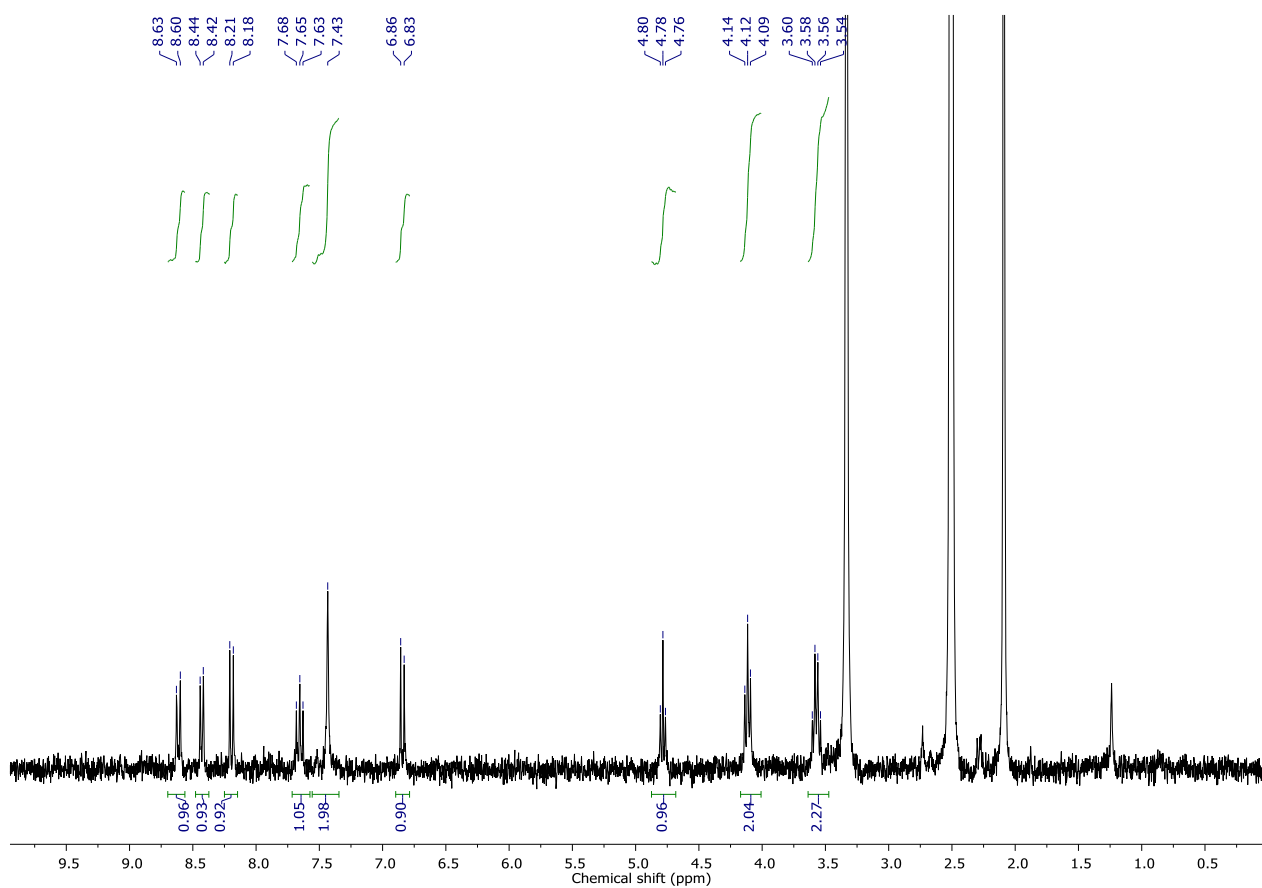


Figure S6. ¹H NMR spectrum of **4** in DMSO-*d*₆

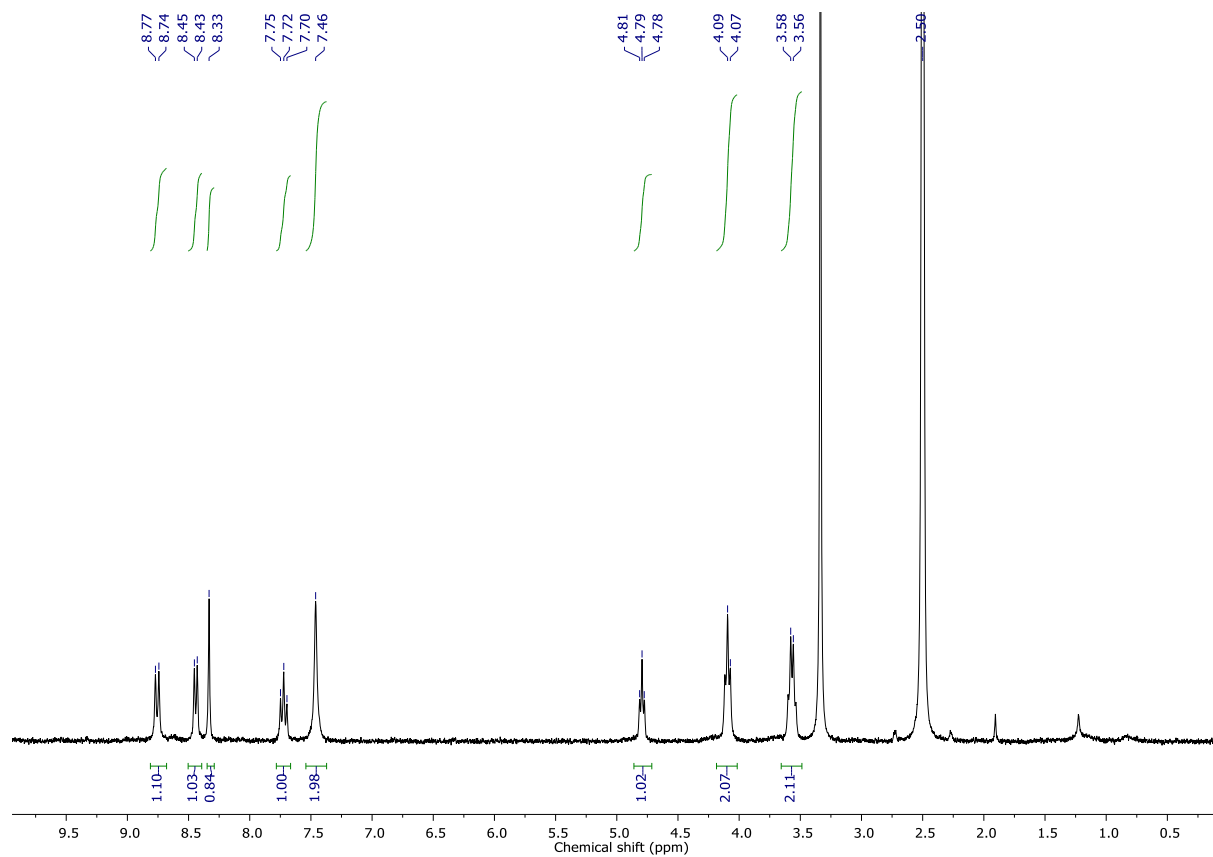


Figure S7. ¹H NMR spectrum of 5 in DMSO-*d*₆

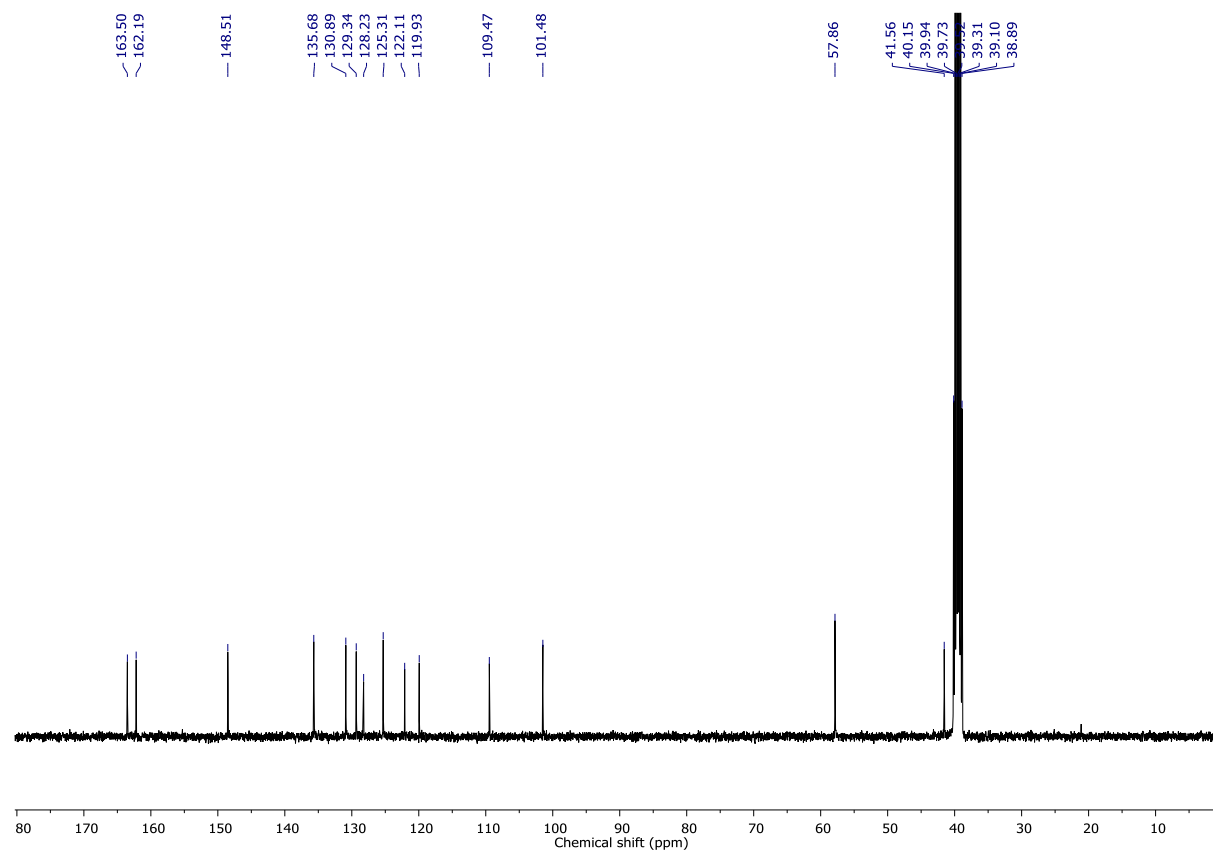


Figure S8. ¹³C NMR spectrum of 5 in DMSO-*d*₆

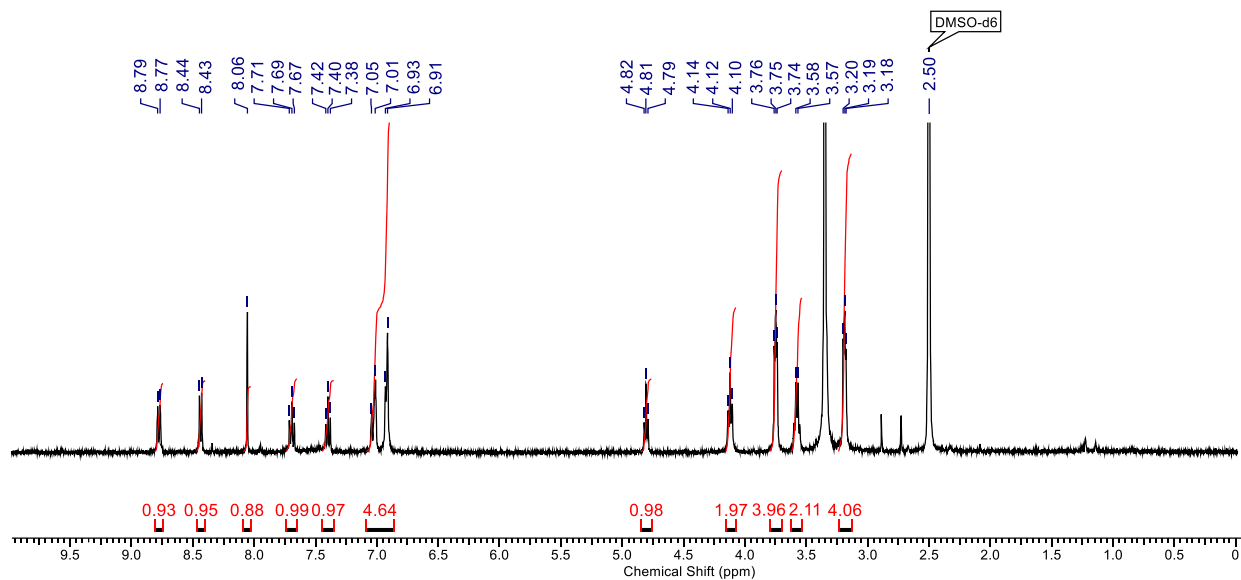


Figure S9. ^1H NMR spectrum of **6** in $\text{DMSO-}d_6$

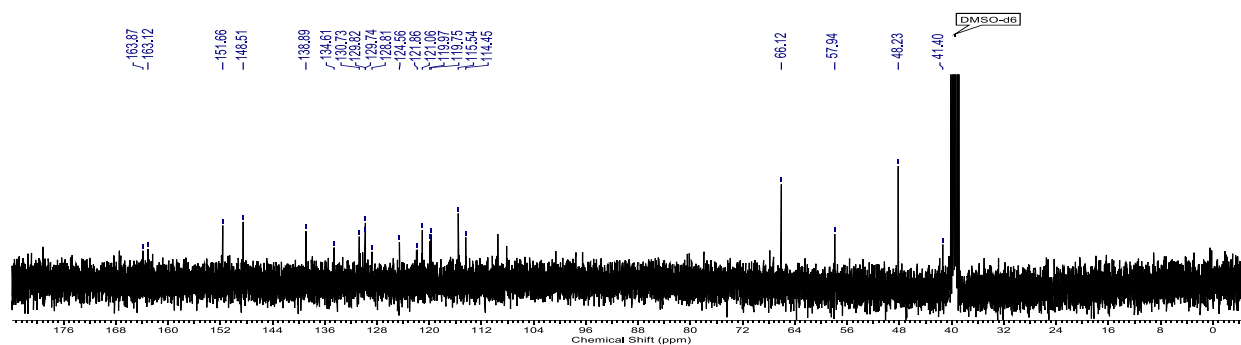


Figure S10. ^{13}C NMR spectrum of **6** in $\text{DMSO-}d_6$

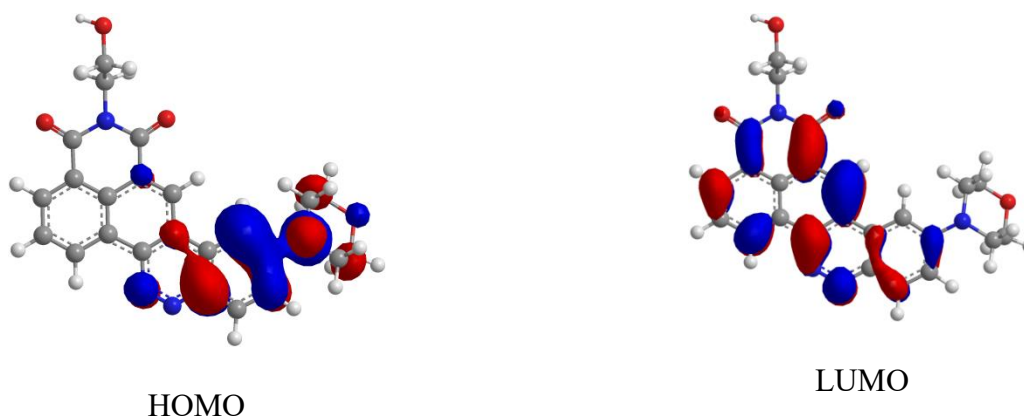


Figure S11. The distribution of electron density over the frontier molecular orbitals of compound **1** (MOPAC 2016, PM6).

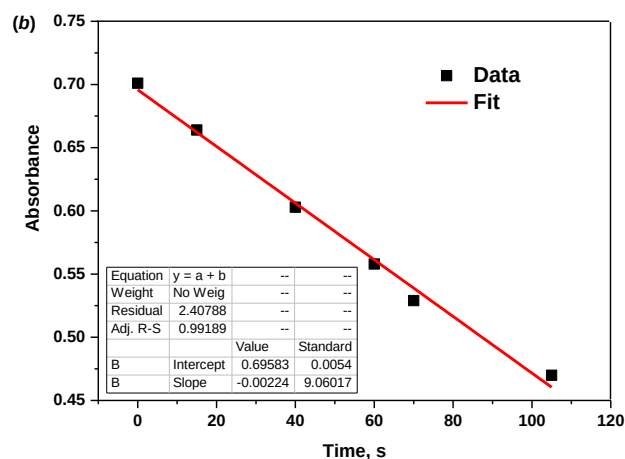


Figure S12. The dependence of the electron density of the mixture of **1** and DPBF at 414 nm on the irradiation time (black squares – experimental data, red line – linear fit, adjusted $R^2 = 0.99$), used in calculating the quantum yield of singlet oxygen generation.

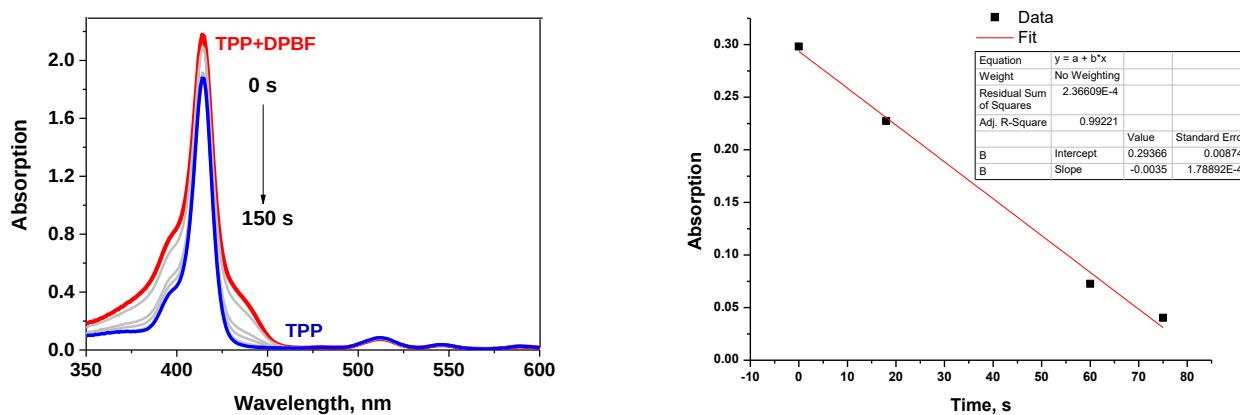


Figure S13. Changes in absorption of TPP at 414 nm upon exposure in presence of DPBF ($4.0 \cdot 10^{-5} \text{M}$) in acetone. Changes in the UV/Vis absorption spectrum of a mixed solution containing TPP and DPBF ($4.0 \cdot 10^{-5} \text{M}$) in acetone upon irradiation at 500 nm; b) the dependence of the electron density of the mixture of TPP and DPBF at 414 nm on the irradiation time (black squares), the linearization of this function (red line) and its parameters used in calculating the quantum yield of singlet oxygen generation.

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