Supplementary Information

Novel fluorescent lapachone-based BODIPY: Synthesis, computational and electrochemical aspects, and subcellular localisation of a potent antitumour hybrid quinone

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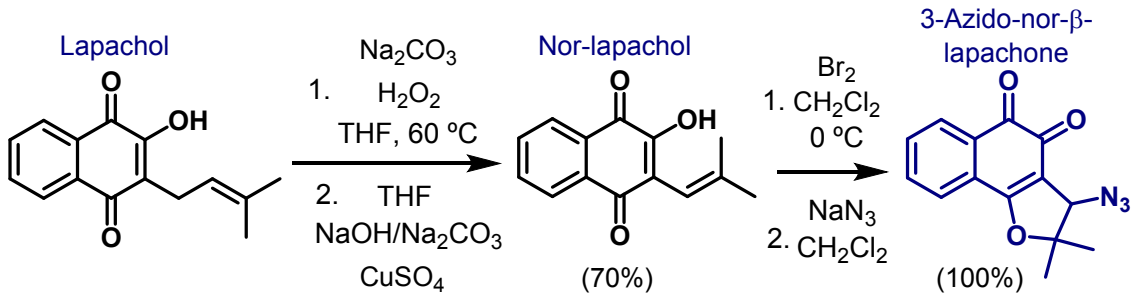
Contents

Chemistry

Materials and methods

Melting points were obtained on Thomas Hoover and are uncorrected. Analytical grade solvents were used. Column chromatography was performed on silica gel (SilicaFlash G60 UltraPure 60-200 µm, 60 Å). ^{1}H and ^{13}C NMR spectra were recorded at 300 K using a Bruker AVANCE DRX400 spectrometer. All samples for NMR were prepared in $CDCl_3$ containing TMS as internal reference. Chemical shifts (δ) are given in ppm and coupling constants (J) in Hertz. High resolution mass spectra (electrospray ionization) were obtained using a MicroTOF Ic – Bruker Daltonics instrument.

Synthesis of the Quinone and BODIPY derivatives



Scheme S1. Synthesis of the 3-azido-nor-β-lapachone (**4**).

Lapachol was initially extracted from the heartwood of *Tabebuia* sp. (*Tecoma*) and purified by a series of recrystallizations. Initially, nor-lapachol was synthesized by Hooker oxidation methodology[1] and data are consistent with those reported in the literature.[2] Nor-lapachol was obtained as an orange solid (160 mg, 0.7 mmol, 70% yield); m.p. 121-122 °C.[33] ^{1}H NMR (400 MHz, $CDCl_3$, 303 K) δ: 8.13 (ddd, J = 7.5, 1.5 and 0.5 Hz, 1H), 8.10 (ddd, J = 7.5, 1.5 and 0.5 Hz, 1H), 7.76 (td, J = 7.5, 7.5 and 1.5 Hz, 1H), 7.69 (td, J = 7.5, 7.5 and 1.5 Hz, 1H), 6.03-5.99 (m, 1H), 2.0 (d, J = 1.5 Hz, 3H), 1.68 (d, J = 1.2 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$, 303 K) δ: 184.7, 181.5, 151.1, 143.6, 134.9, 133.0, 132.9, 129.5, 126.9, 126.0, 120.9, 113.6, 26.5, 21.7.

Synthesis of 3-azido-2,2-dimethyl-2,3-dihydronaphtho[1,2-*b*]furan-4,5-dione, 3-azido-nor-β-lapachone (4): To a solution of nor-lapachol (228 mg, 1.0 mmol) in 25 mL of chloroform, 2 mL of bromine was added. The bromo intermediate precipitated immediately as an orange solid. After removal of bromine, by adding

dichloromethane and then removing the organic solvent with dissolved bromine by rotary evaporator, an excess of sodium azide (2 mmol) was added in CH_2Cl_2 and the mixture was stirred overnight. The crude reaction mixture was poured into 50 mL of water. The organic phase was extracted with organic solvent, dried over anhydrous Na_2SO_4, filtered, and evaporated under reduced pressure. The azido product **4** was obtained after recrystallization as an orange solid (263 mg, 0.98 mmol, 98% yield); m.p. 200-202 °C. ^{1}H NMR (400 MHz, $CDCl_3$, 303 K) δ: 8.14 (ddd, J = 6.9, 2.1 and 0.9 Hz, 1H), 7.72-7.65 (3H, m), 4.77 (1H, s), 1.67 (3H, s), 1.55 (3H, s). ^{13}C NMR (100 MHz, $CDCl_3$, 303 K) δ: 180.3, 175.2, 170.2, 134.5, 132.7, 131.1, 113.5, 129.5, 125.1, 126.7, 95.5, 67.3, 27.1, 21.9. Data are consistent with those reported in the literature.[3]

BODIPYs **1**, **2** and **3** were synthesized according to the literature.[4,5,6] For the synthesis of BODIPY **2**, we have followed the procedure previously described by Jiao and coworkers.[5] A mixture of BODIPY **1** (230 mg, 0.684 mmol) and $CuCl_2{\cdot}2H_2O$ (176 mg, 1.03 mmol) in CH_3CN (15 mL) was stirred at reflux for 2 h. After cooling down to room temperature, the reaction mixture was poured in diethyl ether (50 mL), washed with water (3 × 50 mL), dried over anhydrous Na_2SO_4, filtered and evaporated under vacuum. The residue was purified through column chromatography on silica, using C_6H_{14}/DCM (4:1 – 1:1) as eluent, from which the desired product **2** was obtained in 56% yield (142 mg). Data are consistent with those reported in the literature.[5] ^{1}H NMR (400 MHz, $CDCl_3$, 303 K) δ: 7.97 (s, 1H), 7.52-7.42 (m, 3H), 6.70 (d, J = 4.3 Hz, 1H), 6.66 (d, J = 4.3 Hz, 1H), 6.55 (d, J = 4.4 Hz, 1H), 6.42 (d, J = 4.3 Hz, 1H). ^{13}C NMR (75 MHz, $CDCl_3$, 303 K) δ: 145.9, 145.7, 135.4, 133.8, 131.7, 131.6, 130.7, 130.3, 130.2, 130.0, 128.4, 119.7, 119.2.

For the synthesis of BODIPY **3**: To a solution of compound **2** (90 mg, 0.242 mmol) in acetonitrile (10 mL) under stirring at room temperature, propargylamine (39 μL, 33 mg, 0.608 mmol, 2.5 eq) was added. After 1 hour under stirring at room temperature TLC control showed full conversion of the starting material. Solvent was evaporated under reduced pressure and, after purification *via* silica column chromatography (C_6H_{14}/DCM, 3:2 – 1:3), the desired product **3** (61 mg, 0,156 mmol, 65% yield) was obtained. ^{1}H NMR (400 MHz, $CDCl_3$, 303 K) δ: 7.46-7.42 (m, 3H), 7.38-7.32 (m, 1H), 6.73 (d, J = 4.9 Hz, 1H), 6.55 (br, 1H), 6.31 (dd, J = 3.6, 2.3 Hz, 1H), 6.28 (d, J = 4.9 Hz, 1H), 6.20 (d, J = 3.6 Hz, 1H), 4.22-4.17 (m, 2H), 2.40 (s, 1H). ^{13}C NMR (101 MHz, $CDCl_3$, 303 K) δ: 160.6, 135.0, 133.6, 132.6, 131.5, 131.0, 130.4, 129.5, 127.2,

127.1, 118.4, 113.0, 109.8, 76.6, 72.9, 33.0. ESI/HRMS (m/z) $[M+Na]^+$: 412.0310 Cald. for $[C_{32}H_{23}BCl_2F_2N_6O_3Na]^+$: 412.0367

Synthesis of the lapachone-based BODIPY 5

In a round bottom flask, 3-azido-nor-β-lapachone (**4**) (52 mg, 0.19 mmol), alkyne **3** (50 mg, 0.128 mmol), and 10 mL of CH_3CN were added. The reaction mixture was stirred until complete solubilization of the reagents after which, CuI (10% per mole) was added. The system was kept under inert atmosphere (Ar) until complete consumption of BODIPY (**3**) and monitored by TLC until its completion (20 hours). The solvent from the crude was evaporated under reduced pressure and it was purified by column chromatography on silica-gel, using eluents with an increasing polarity gradient mixture of hexane and ethyl acetate. Lapachone-based BODIPY (**5**) was obtained as a red solid (67 mg, 0.101 mmol, 79% yield); m.p. 179-183 ºC. ^{1}H NMR (400 MHz, $CDCl_3$, 303 K) δ: 1.15 (s, 3H), 1.75 (s, 3H), 4.68-4.78 (m, 2H), 5.98 (sl, 1H), 6.14 (d, J = 4.0 Hz, 1H), 6.27-6.27 (m, 1H), 6.31 (d, J = 4.0 Hz, 1H), 6.65 (d, J = 4.0 Hz, 1H), 6.93 (sl, 1H), 7.31 (sl, 1H), 7.31-7.33 (m, 1H), 7.41-7.43 (m, 2H), 7.66-7.74 (m, 3H), 7.78-7.80 (m, 1H), 8.09 (d, J = 8 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$, 303 K) δ: 21.1, 27.7, 40.2, 67.2, 95.9, 111.2, 112.0, 113.7, 118.5, 125.7, 126.7, 127.0, 128.1, 129.9, 130.5, 131.3, 131.5, 131.6, 132.0, 133.3, 133.9, 134.7, 134.8, 136.0, 136.1, 162.3, 171.5, 174.8, 180.2. EI/HRMS (m/z) $[M+Na]^+$: 681.1141 Cald. for $[C_{32}H_{23}BCl_2F_2N_6O_3Na]^+$: 681.1168.

Synthesis of nor-β-lapachone and β-lapachone

General procedure for the synthesis of nor-β-lapachone and β-lapachone: Sulfuric acid was slowly added to lapachol (1 mmol, 242 mg) or nor-lapachol (1 mmol, 228 mg) until complete dissolution of the quinone. Then, the solution was poured into ice and the precipitate formed was filtered and washed with water. Nor-β-lapachone and β-lapachone were recrystallized in an appropriate solvent, as for instance, ethanol.

Nor-β-lapachone was obtained as an orange solid (216 mg, 95% yield); m.p. 169-171 ºC. ^{1}H NMR (400 MHz, $CDCl_3$, 303 K) δ: 8.05-803 (m, 1H), 7.66-7.52 (m, 3H), 2.93 (s, 2H), 1.60 (s, 6H). ^{13}C NMR (100 MHz, $CDCl_3$, 303 K) δ: 181.3, 175.6, 168.7, 134.4, 131.8, 130.9, 129.2, 127.9, 124.5, 115.0, 93.7, 39.3, 28.4. Data are consistent with those reported in the literature.[7]

β-Lapachone was obtained as an orange solid (240 mg, 99% yield); m.p. 153-155 ºC. ^{1}H NMR (400 MHz, $CDCl_3$, 303 K) δ: 8.06 (dd, 1H, J = 7.6 and 1.4 Hz), 7.81 (dd, 1H, J = 7.8 and 1.1 Hz), 7.65 (ddd, 1H, J = 7.8, 7.6 and 1.4 Hz), 7.51 (td, 1H, J = 7.6, 7.6 and 1.1 Hz), 2.57 (t, 2H, J = 6.7 Hz), 1.86 (t, 2H, J = 6.7 Hz), 1.47 (s, 6H). ^{13}C NMR (100 MHz, $CDCl_3$, 303 K) δ: 179.8, 178.5, 162.0, 134.7, 132.6, 130.6, 130.1, 128.5, 124.0, 112.7, 79.3, 31.6, 26.8, 16.2. Data are consistent with those reported in the literature.[8]

NMR spectra of the nor-lapachol and 3-azido-nor-β-lapachone (4)

Nor-lapachol

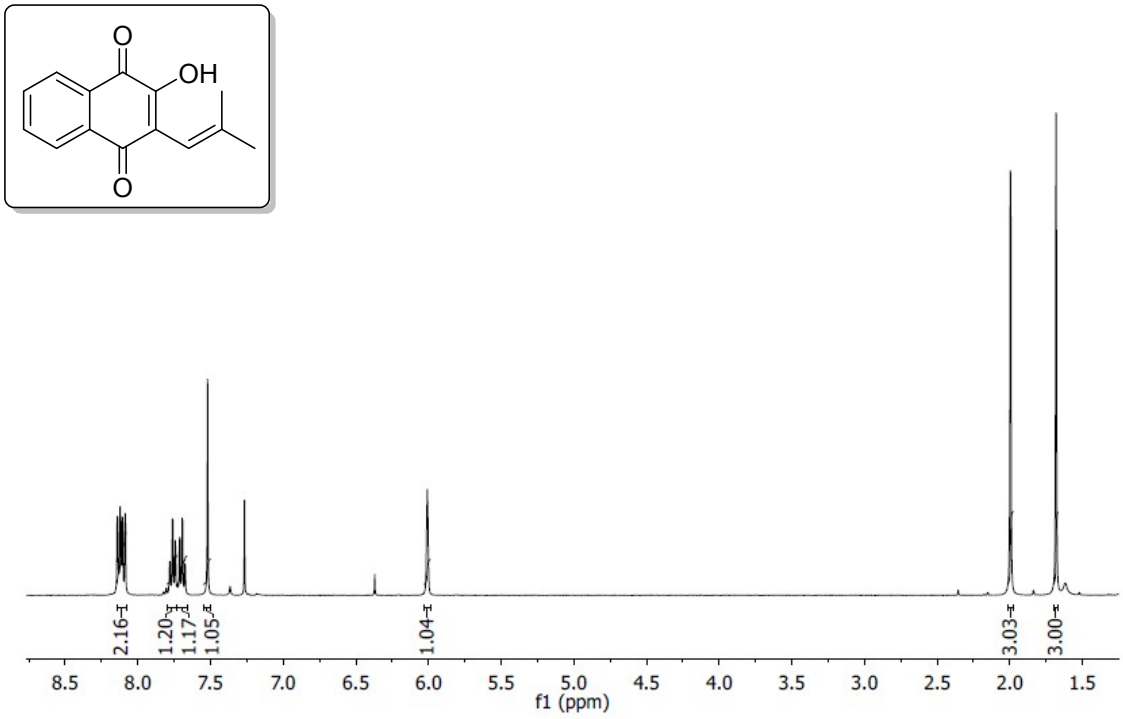


Figure S1. ^{1}H NMR spectrum of nor-lapachol at 400 MHz in $CDCl_3$ (300 K).

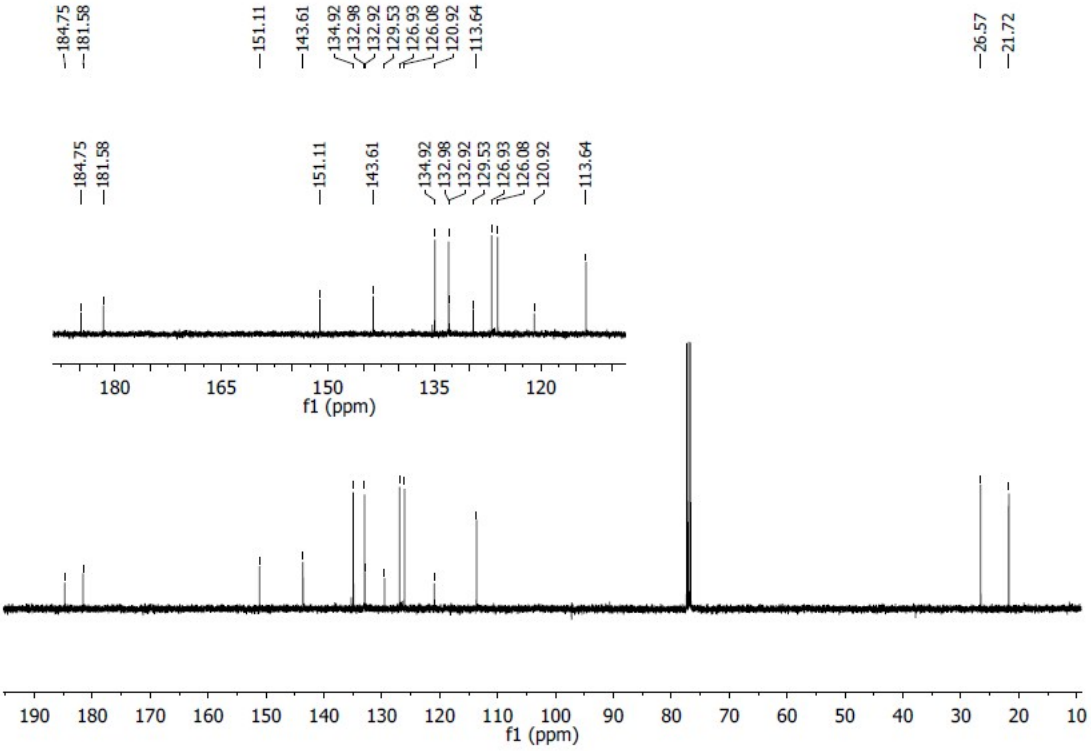


Figure S2. ^{13}C NMR spectrum of nor-lapachol at 100 MHz in $CDCl_3$ (300 K).

3-azido-nor-β-lapachone (4)

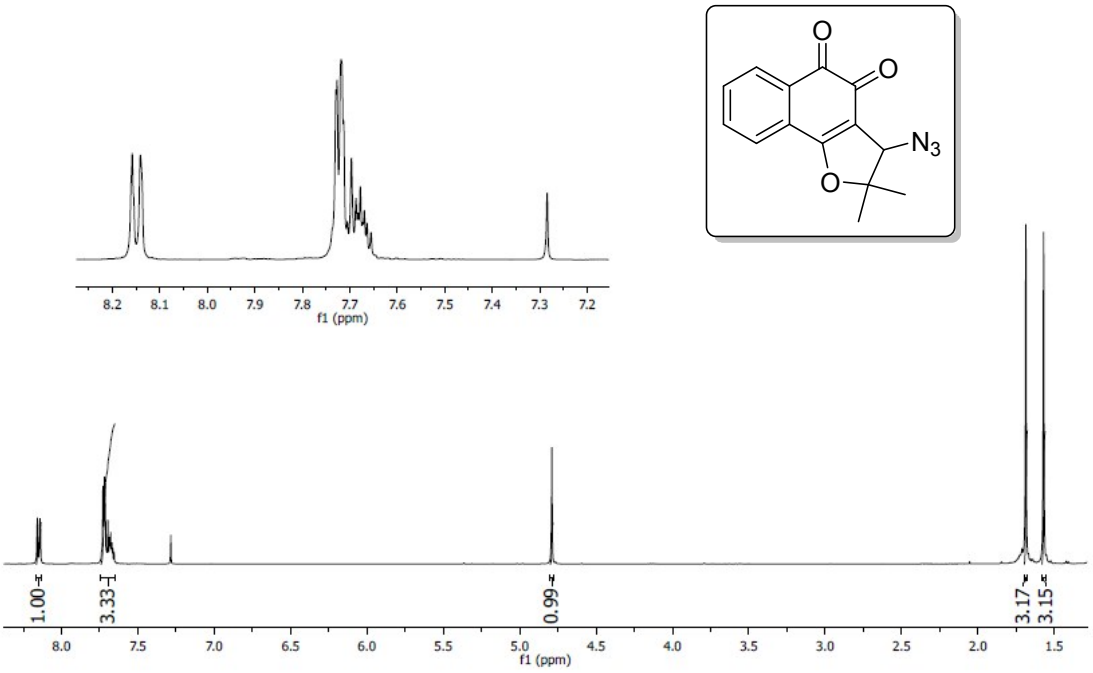


Figure S3. ^{1}H NMR spectrum of 3-azido-nor-β-lapachone **(4)** at 400 MHz in $CDCl_3$ (300 K).

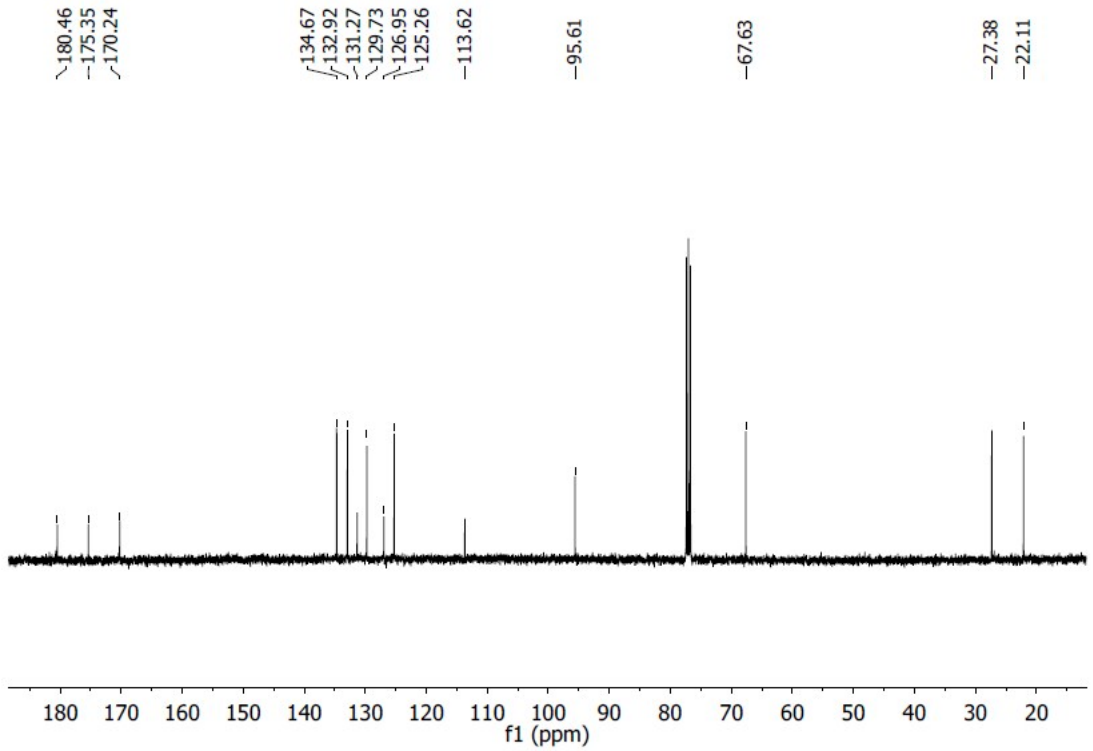
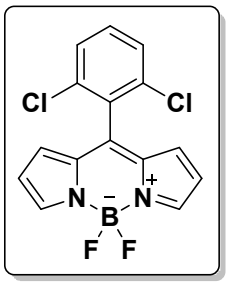


Figure S4. ^{13}C NMR spectrum of 3-azido-nor-β-lapachone (**4**) at 100 MHz in $CDCl_3$ (300 K).

NMR spectra of the BODIPY derivatives 1, 2 and 3

Compound 1



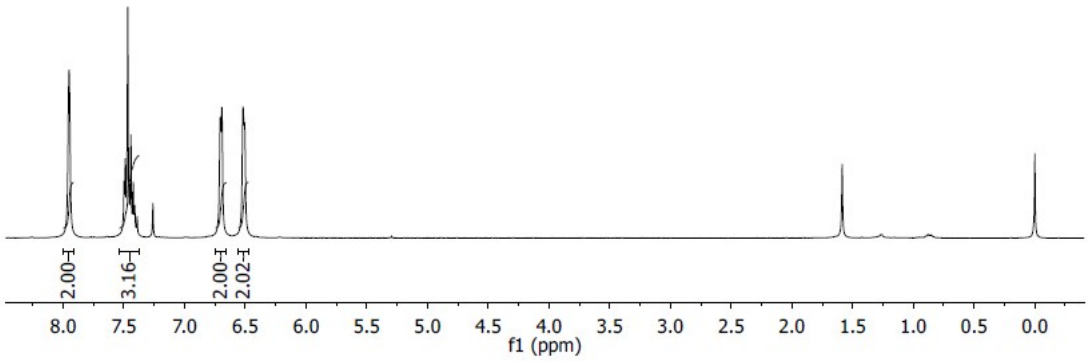


Figure S5. ^{1}H NMR spectrum of **1** at 400 MHz in $CDCl_3$ (300 K).

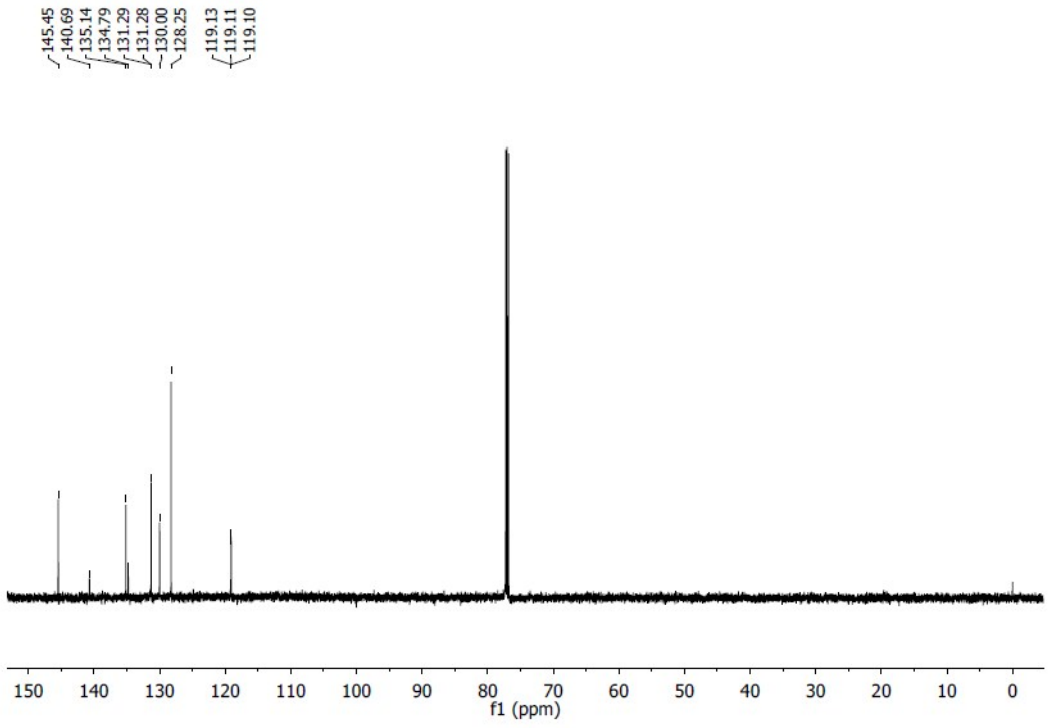
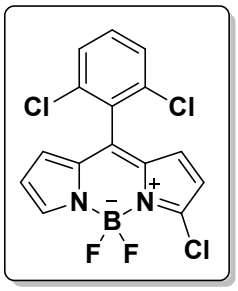


Figure S6. ^{13}C NMR spectrum of **1** at 100 MHz in $CDCl_3$ (300 K).

Compound 2



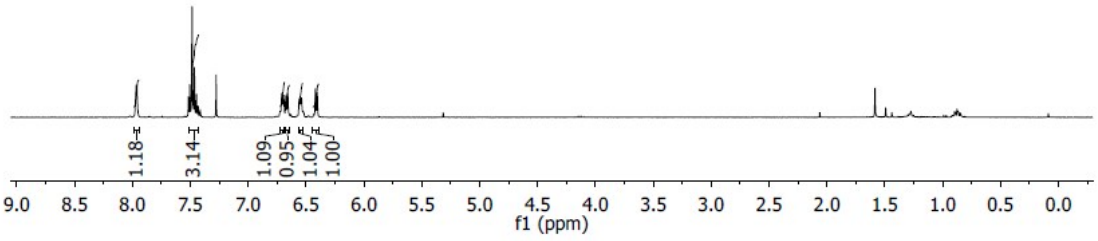


Figure S7. ^{1}H NMR spectrum of **2** at 400 MHz in $CDCl_3$ (300 K).

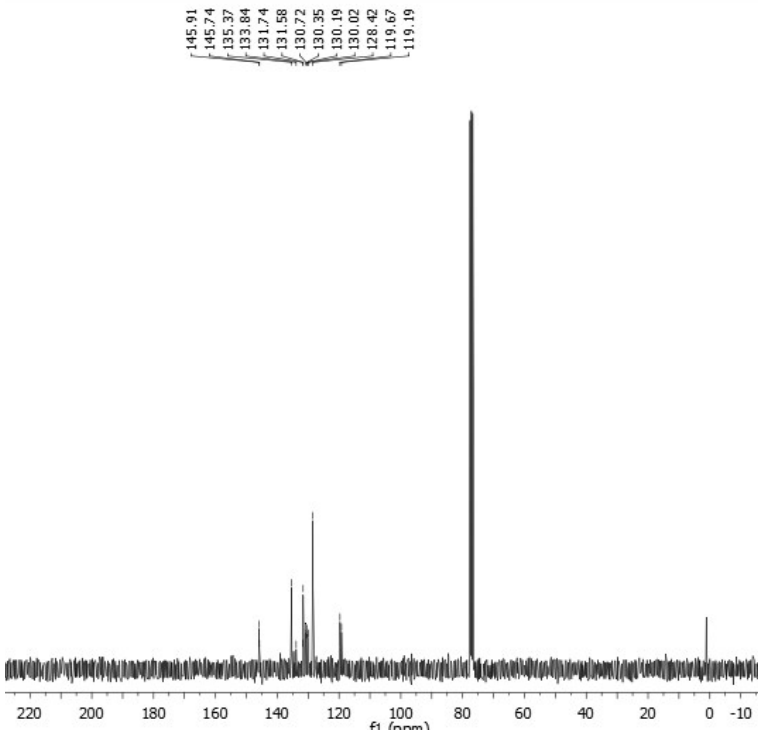
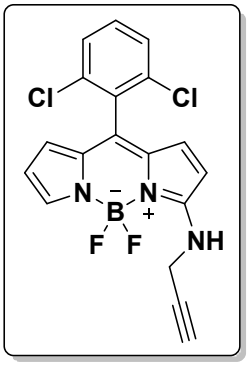


Figure S8. ^{13}C NMR spectrum of **2** at 100 MHz in $CDCl_3$ (300 K).

Compound 3



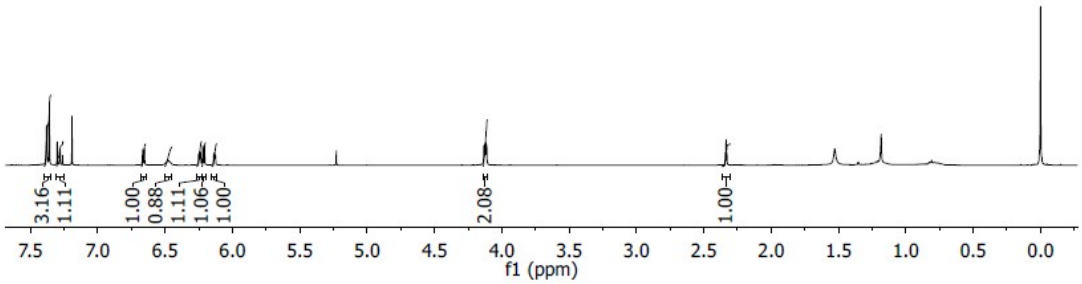


Figure S9. ^{1}H NMR spectrum of **3** at 400 MHz in $CDCl_3$ (300 K).

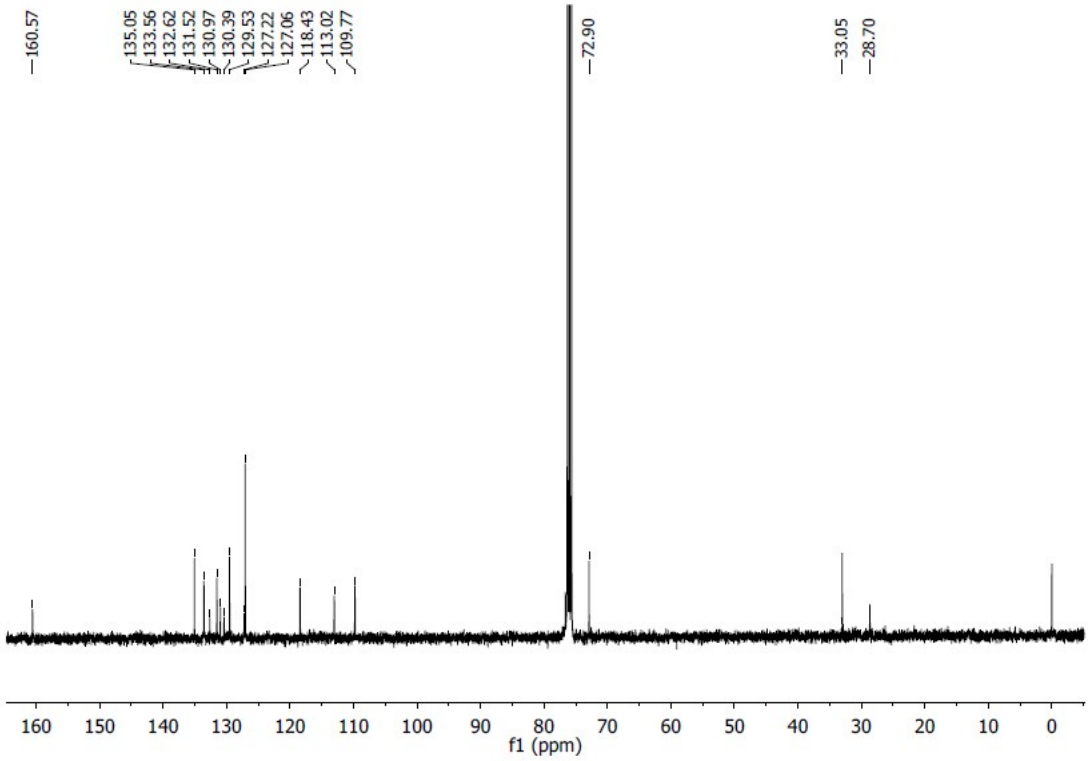


Figure S10. ^{13}C NMR spectrum of **3** at 100 MHz in $CDCl_3$ (300 K).

Spectra of the Lapachone-Based BODIPY (5)

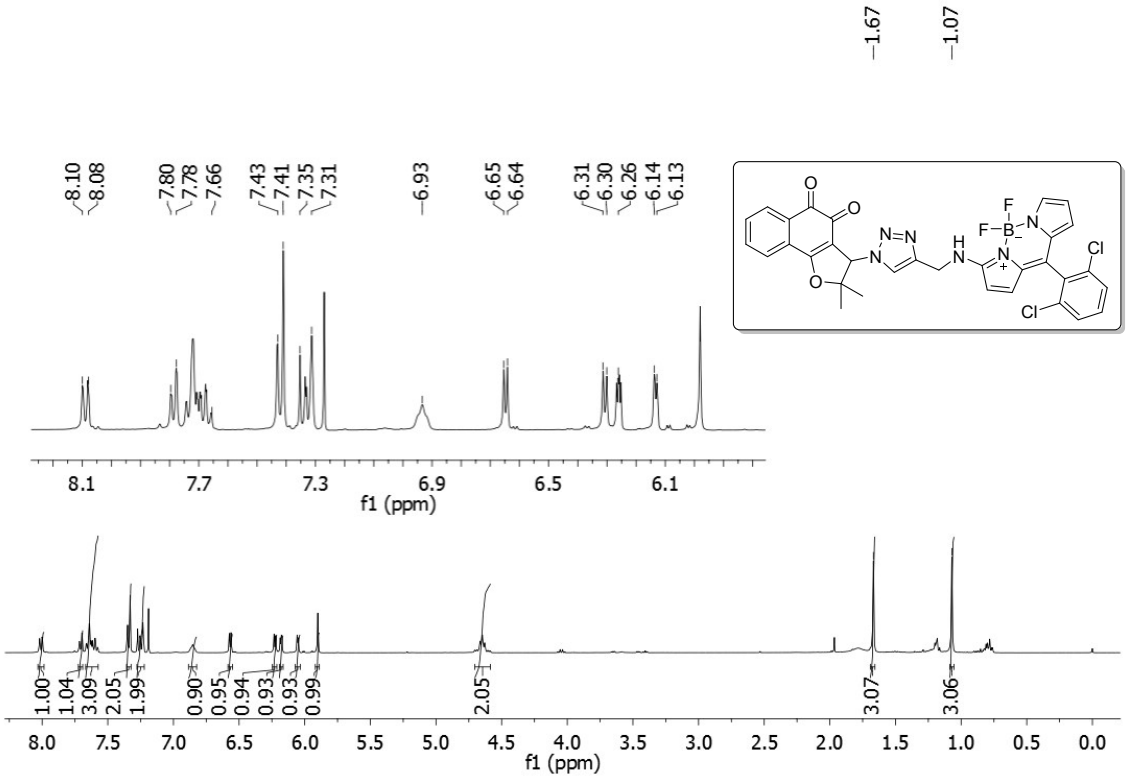


Figure S11. ^{1}H NMR spectrum of **5** at 400 MHz in $CDCl_3$ (300 K).

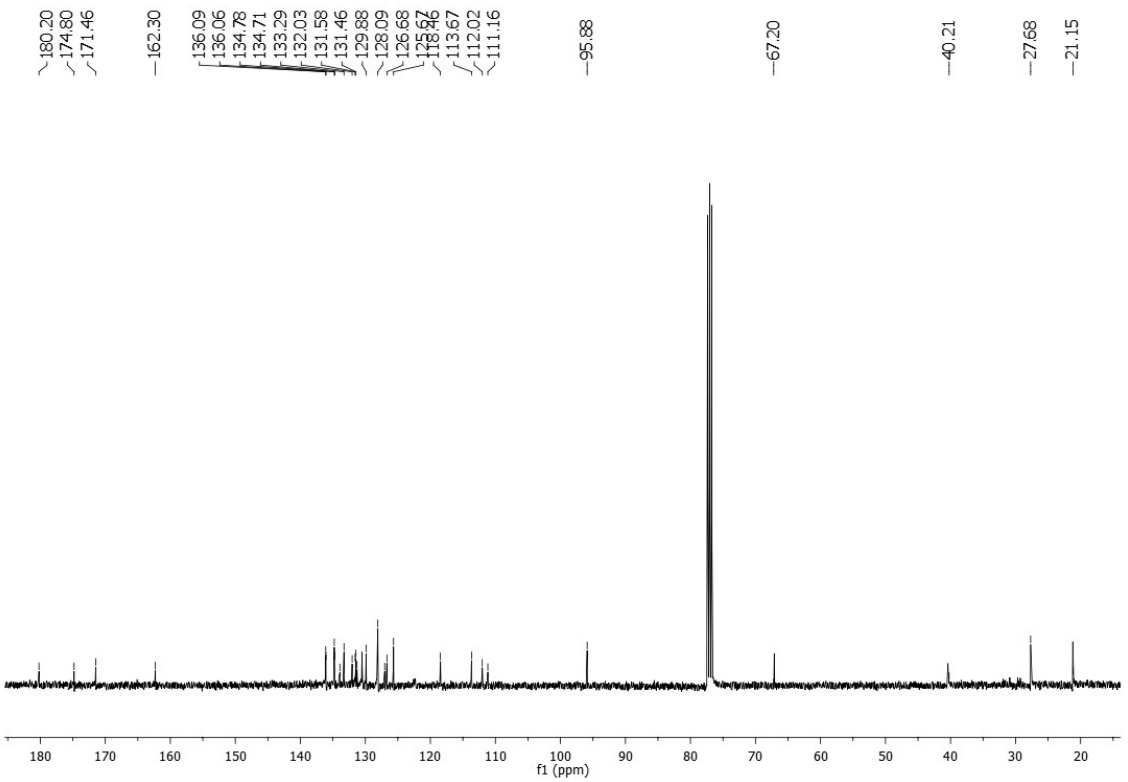


Figure S12. ^{13}C NMR spectrum of **5** at 100 MHz in $CDCl_3$ (300 K).

β-lapachone

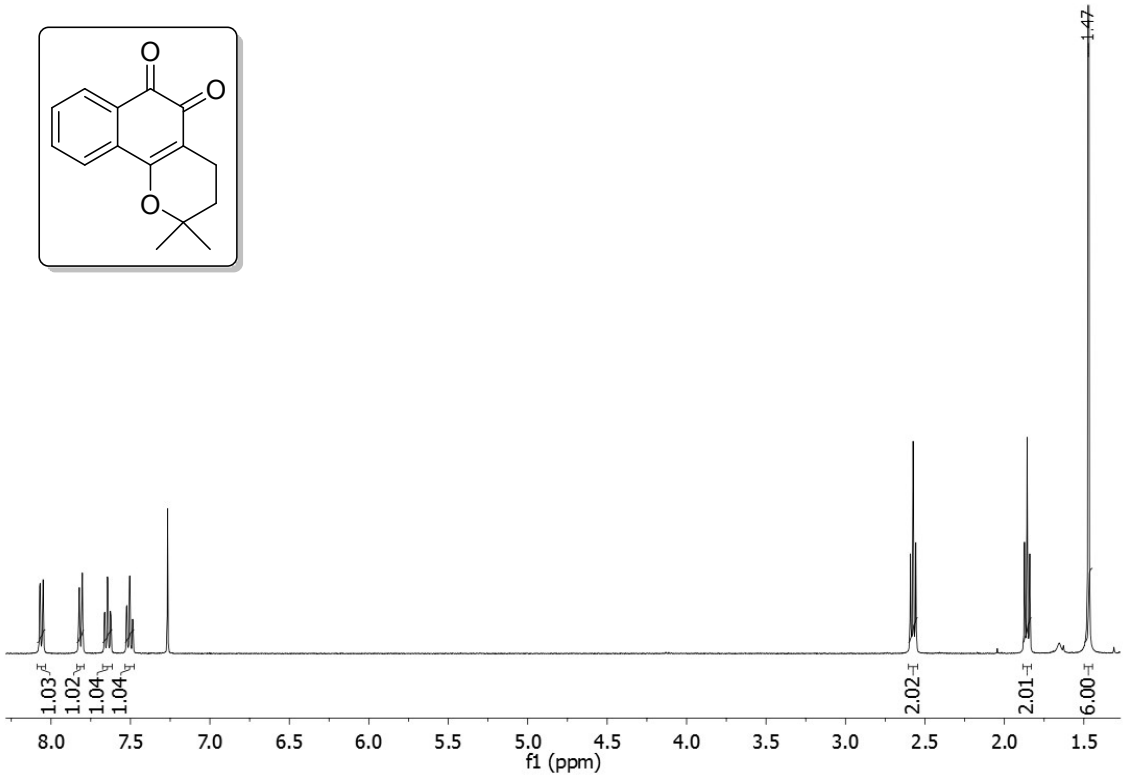


Figure S13. ^{1}H NMR spectrum of β-lapachone at 400 MHz in $CDCl_3$ (300 K).

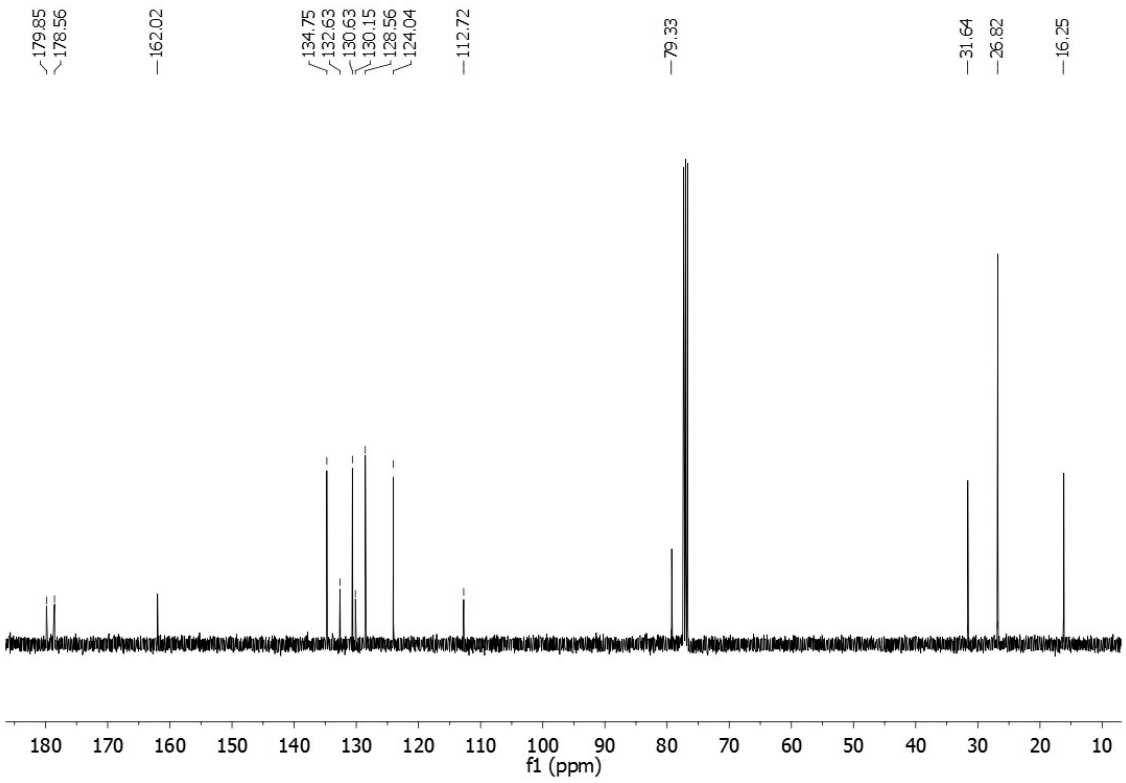


Figure S14. ^{13}C NMR spectrum of β-lapachone at 100 MHz in $CDCl_3$ (300 K).

Nor-β-lapachone

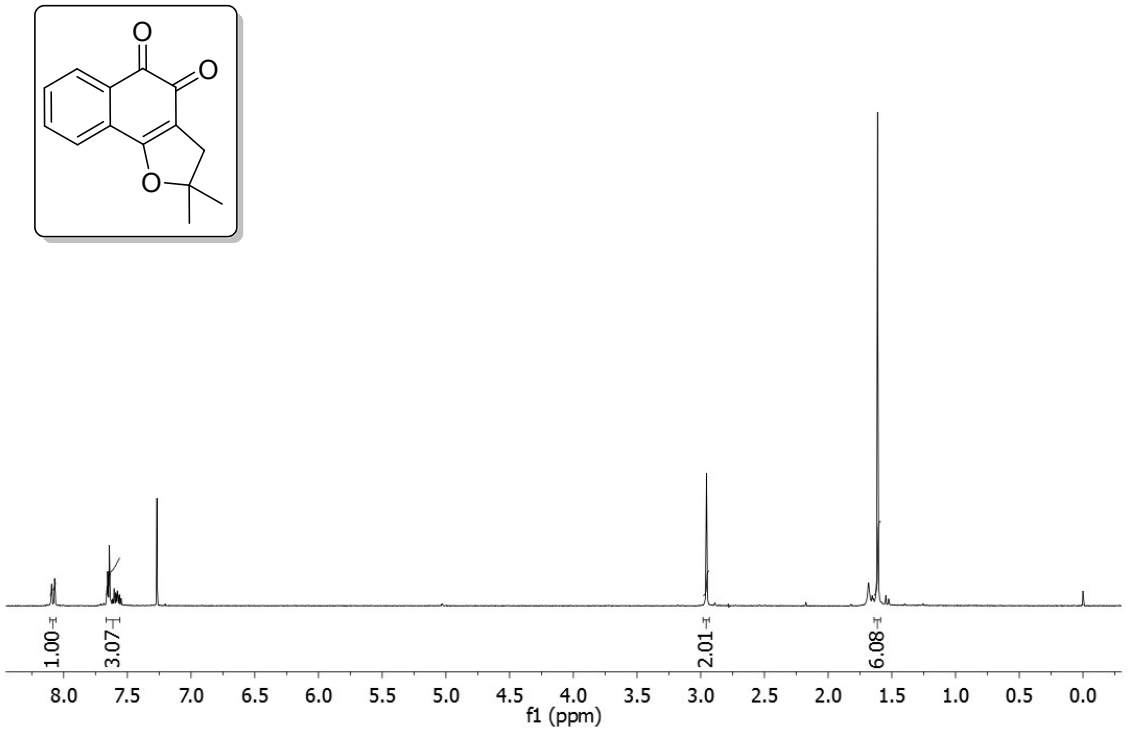


Figure S15. ^{1}H NMR spectrum of nor-β-lapachone at 400 MHz in $CDCl_3$ (300 K).

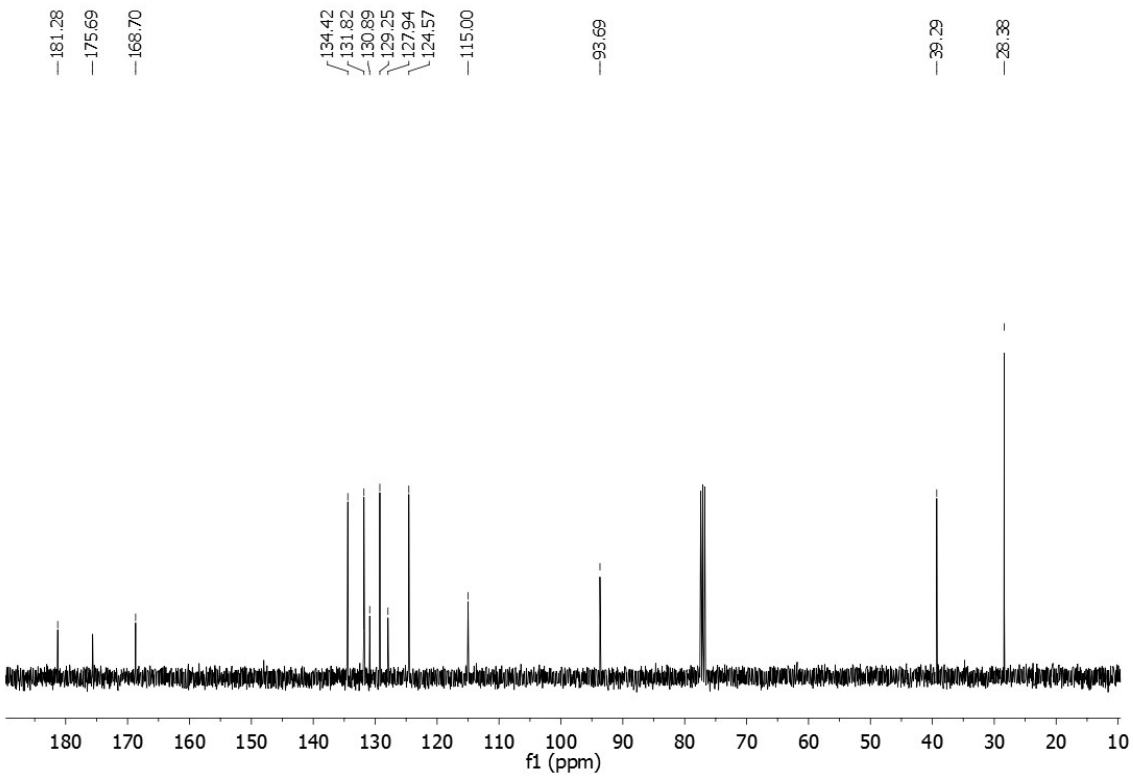


Figure S16. ^{13}C NMR spectrum of nor-β-lapachone at 100 MHz in $CDCl_3$ (300 K).

HRMS spectrum of compound 5

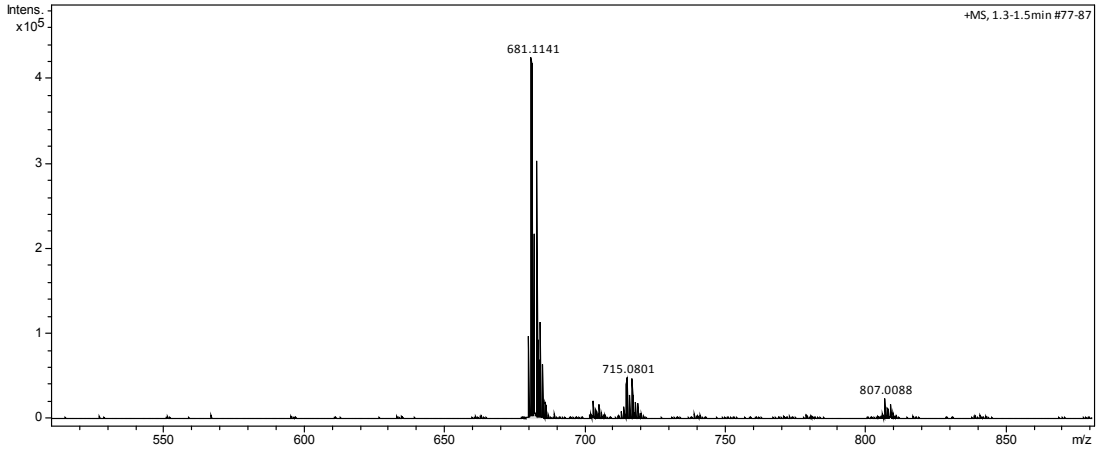


Figure S17. HRMS of compound **5**.

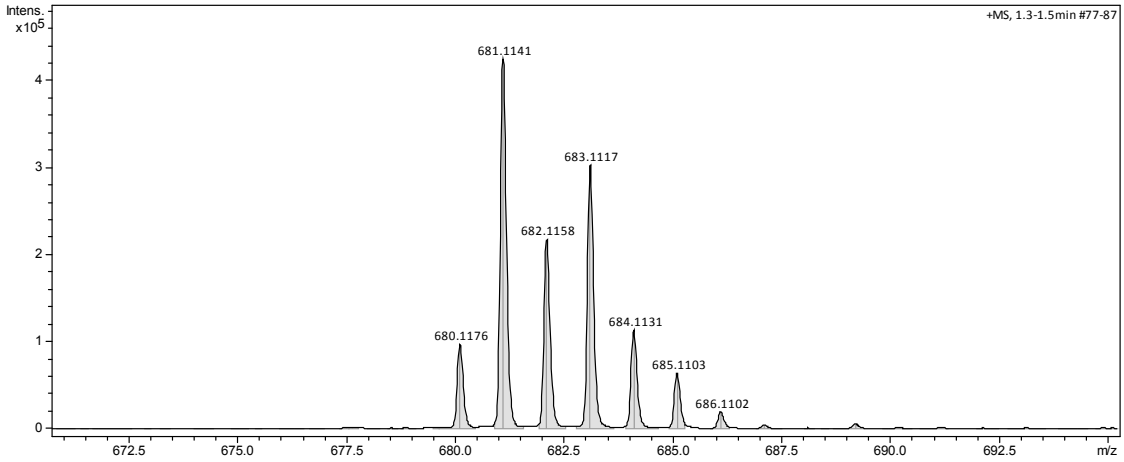


Figure S18. Expanded HRMS of compound **5**.

Photophysical Parameters

Absorption spectra were obtained on a Varian Cary 100 spectrophotometer at room temperature in the solvents described below. Steady state fluorescence spectra were obtained on a Varian Cary Eclipse spectrofluorimeter with a xenon arc lamp as the light source while using an excitation wavelength (λ_{exc}) corresponding to a higher absorption band. In all experiments, a quartz cuvette was employed with a 1 cm optical path length. Photophysical properties of **5** were studied in 6 different solvents, which varied in polarity and were either protic or aprotic: ethyl acetate, acetonitrile, DMSO, dichloromethane, methanol and toluene. The studied compound, regardless of the solvent, showed strong absorptions in the ultraviolet as well as strong fluorescent emissions (Figure S19).

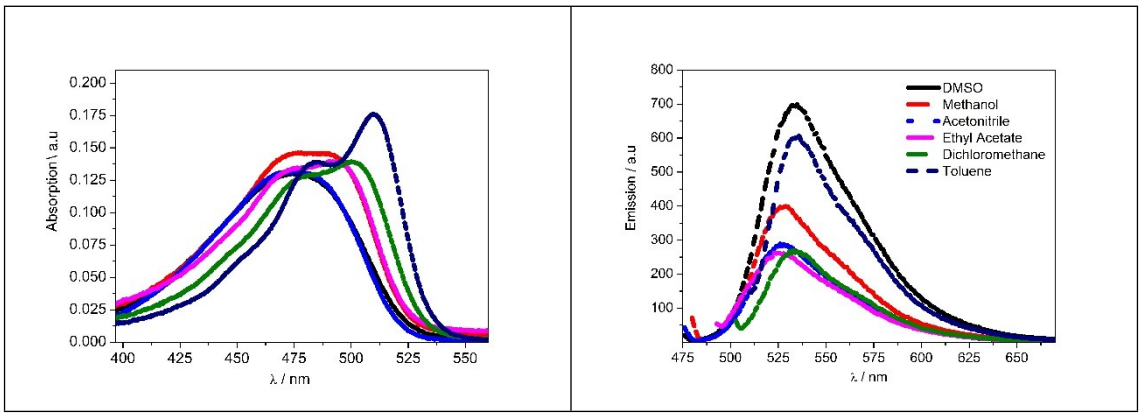


Figure S19. Absorption spectrum (left) and emission spectrum (right) of **5** in different solvents.

For solvatochromism studies, a dichloromethane solution of the initial concentration of 5.00 x 10^{-6} mol L^{-1} was prepared and an aliquot of 100 x 10^{-6} L was moved into a 10.0 mL volumetric flask. After the dichloromethane had completely evaporated, the volume of the recipient was completed with one of the solvents used in this investigation. For lapachone-based BODIPY (**5**) in different solvents, the absorption and emission spectra were recorded.

Molar absorption coefficients (ε) were obtained for the six solvents shown in Table S1. The molar absorption coefficients were calculated taking into account the absorption spectra (maximum absorption).

Table S1. UV-vis and fluorescence emission data (in different solvents) for lapachone-based BODIPY **5**. Concentration = 5 x 10^{-6} mol L^{-1}

Solvent	**λ_{max} (abs) (nm)**	**Log ε (ε)**	**λ_{max} (em) (nm)**	**Stokes Shift (nm)**
Ethyl acetate	493	4.44 (27800)	525	32
Acetonitrile	476	4.42 (26400)	527	51
DMSO	476	4.42 (26000)	533	57
Dichloromethane	501	4.44 (27800)	533	32
Methanol	489	4.46 (29000)	538	49
Toluene	509	4.55 (35200)	534	25

Quantum yields were obtained by a comparative method[9] using 500 x 10^{-6} mol L^{-1} fluorescein in 0.1 mol L^{-1} and NaOH(aq) as standard[10] (φ = 0.79[11]). The emission spectra from compound **5** were obtained. The results were plotted with the integrated fluorescence intensity vs. absorbance, to obtain the slope of the curve. A curve was obtained for **5** as well as for the standard. The quantum yield of the tested compound (Φ_x) was calculated, using the following formula, where Φ_{St} is the quantum yield of the standard, m_x and m_{St} are the slopes for **5** and standard compound, respectively, and n_x and n_{st} are the refractive indexes of the solvents. The value of the quantum yield observed for compound **5** was Φ_7 = 0.07.

$$\Phi_X = \Phi_{St}\left[\frac{m_x}{m_{St}}\right]\left[\frac{n_x}{n_{St}}\right]^2 \qquad (1)$$

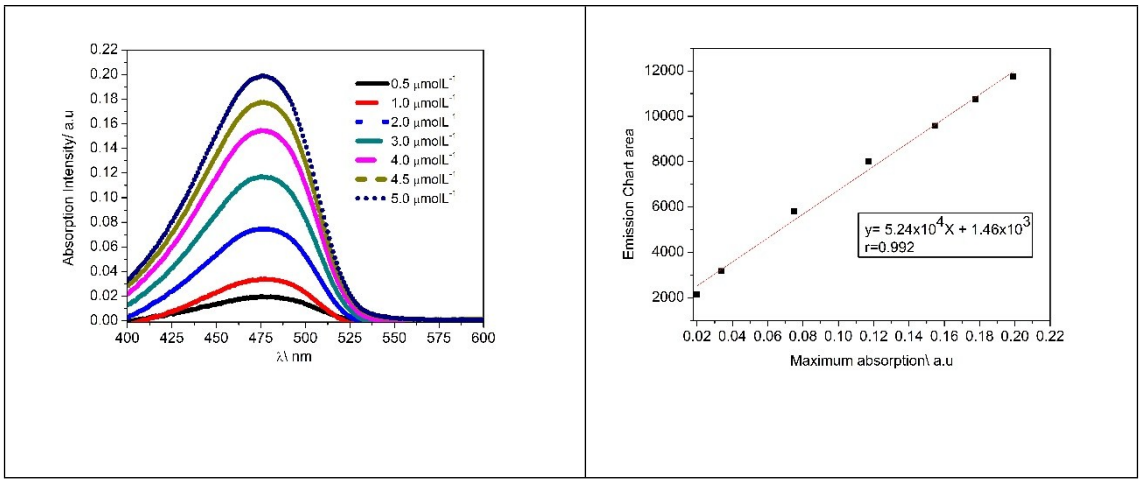


Figure S20. Absorption spectrum in DMSO (left) and calibration curve (right) of **5**.

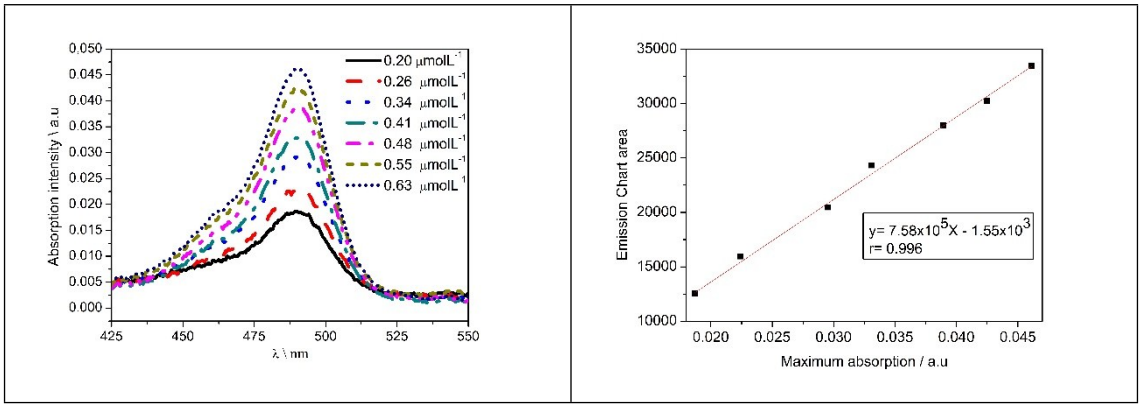


Figure S21. Absorption spectrum (left) and calibration curve (right) of fluorescein as standard.

Cell Staining Procedure

Cell culture

MCF-7, MDA-MB231, T47-D (human breast cancer cells' lineages) and PANC-1 (human pancreatic carcinoma, epithelial-like cell line) were maintained in appropriated culture medium as recommended to ATCC (American Type Culture Collection), supplemented with 10% of fetal bovine serum plus 100 IU/mL penicillin and 100 μg/mL streptomycin at 37 ºC, in 5% CO_2 atmosphere.

Fluorescence assay

Cells were seeded on 13 mm round glass coverslips on the bottom of a 24-well plate, allowed to adhere overnight and washed three times with serum-free medium for removal of non-adherent cells. After reaching confluence, the cells were separated in two samples (live samples and fixed samples). The live samples were incubated for 30 min with lapachone-based BODIPY (**5**) (**1 μM**), nor-β-lapachone, β-lapachone and 3-azido-nor-β-lapachone (**4**) (**100 μM**) at 37 ºC. These samples were washed three times with PBS 1X (pH 7.4) at room temperature and fixed in formaldehyde 3.7% for 30 min. The samples were washed again three times in PBS 1X (pH 7.4) at room temperature and the coverslips were mounted over glass slides using ProLong Gold Antifade (Invitrogen, OR, USA) according to the manufacturer's recommendations. The fixed samples were first washed three times in PBS 1X (pH 7.4) and then fixed in formaldehyde 3.7 % for 30 min. After fixative procedure, the samples were washed three times in PBS 1X (pH 7.4) at room temperature and incubated for 30 min with lapachone-based BODIPY (**5**) (**1 μM**), nor-β-lapachone, β-lapachone and 3-azido-nor-β-lapachone (**4**) (**100 μM**) at room temperature. The samples were washed three times in PBS 1X (pH 7.4) at room temperature and the coverslips were mounted over glass slides using ProLong Gold Antifade (Invitrogen, OR, USA) according to the manufacturer's recommendations. The negative control was performed by incubation of the samples in 0.01% of DMSO (Dimethyl sulfoxide), which was the diluent used. The samples were analyzed using a Leica Confocal Microscopy TCS SP5 and excited using 488 nm wavelength laser emission. All assays were performed in triplicate and three repetitions were done for each cell sample and experimental condition.

Co-staining compound 5 and Mitotracker

In order to confirm the morphological evidence that **5** accumulated in mitochondria, it was performed a co-staining assay with **5** and the commercial mitochondria marker Mitotracker™ (ThermoFisher Scientific, NY, USA), according to manufacturer's recommendations. Briefly, 3 x 10^5 MCF-7 cells (human breast adenocarcinoma cell), were seeded on 13 mm round glass coverslips on the bottom of a 24-well plate, allowed to adhere overnight and washed three times with serum-free medium for removal of non-adherent cells. After reaching confluence, the samples were incubated in 1 μM of **5** or 100 nM of Mitotracker™, for 30 minutes at 37 °C. The samples were washed three times in PBS (Phosphate Buffer Saline), pH 7.4 at 37 °C and the cells were fixed in 3.7 % formaldehyde solution for 30 minutes at room temperature. The samples were washed three times in PBS and the coverslips were mounted over glass slides using ProLong Gold Antifade (Invitrogen, OR, USA) according to the manufacturer's recommendations. The negative control was performed by incubation of the samples in 0.01% of DMSO, the diluent of **5**. The samples were analyzed using a Leica Confocal Microscopy TCS SP5. All assays were performed in triplicate and it was done in three independent repetitions.

Photobleaching assay

Compound **5** (1 μM) was placed in a 96-well plate. The fluorescence measurement were recorded every 5 min interval for a total period of twelve h (Ex/Em = 480/520-560 nm) under a tungsten halogen light source. The values were represented as means (n = 3) and fitted to a non-linear regression one-phase exponential decay using GraphPad Prism 5.0 for Windows, GraphPad Software, (San Diego CA, USA).

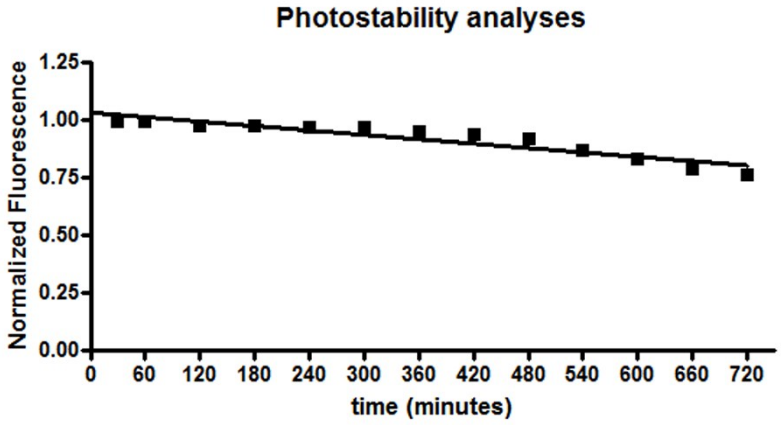


Figure S22. Photobleaching assay of compound **5**. No significant photobleaching was detected during the analyzed period.

Discussion

In order, to confirm the efficiency of our strategy in the preparation of lapachone-based BODIPY (**5**) as an important biomarker. We have evaluated lapachones without the BODIPY fluorescent moiety. β-Lapachone, nor-β-lapachone and 3-azido-nor-β-lapachone (**100 µM**) have produced a mild fluorescence emission even by it's use at 100 times more concentrated than lapachone-based BODIPY (**5**), previously applied in stain cells assay (**1 µM**), as observed in the Figures S26 (β-lap.), S27 (nor-β-lap.) and S28 (3-azido-nor-β-lap.), images A, B, D and E. The staining pattern observed in all cell samples was associated with peripheral cellular region and plasmatic membrane, none specific cellular section was staining.

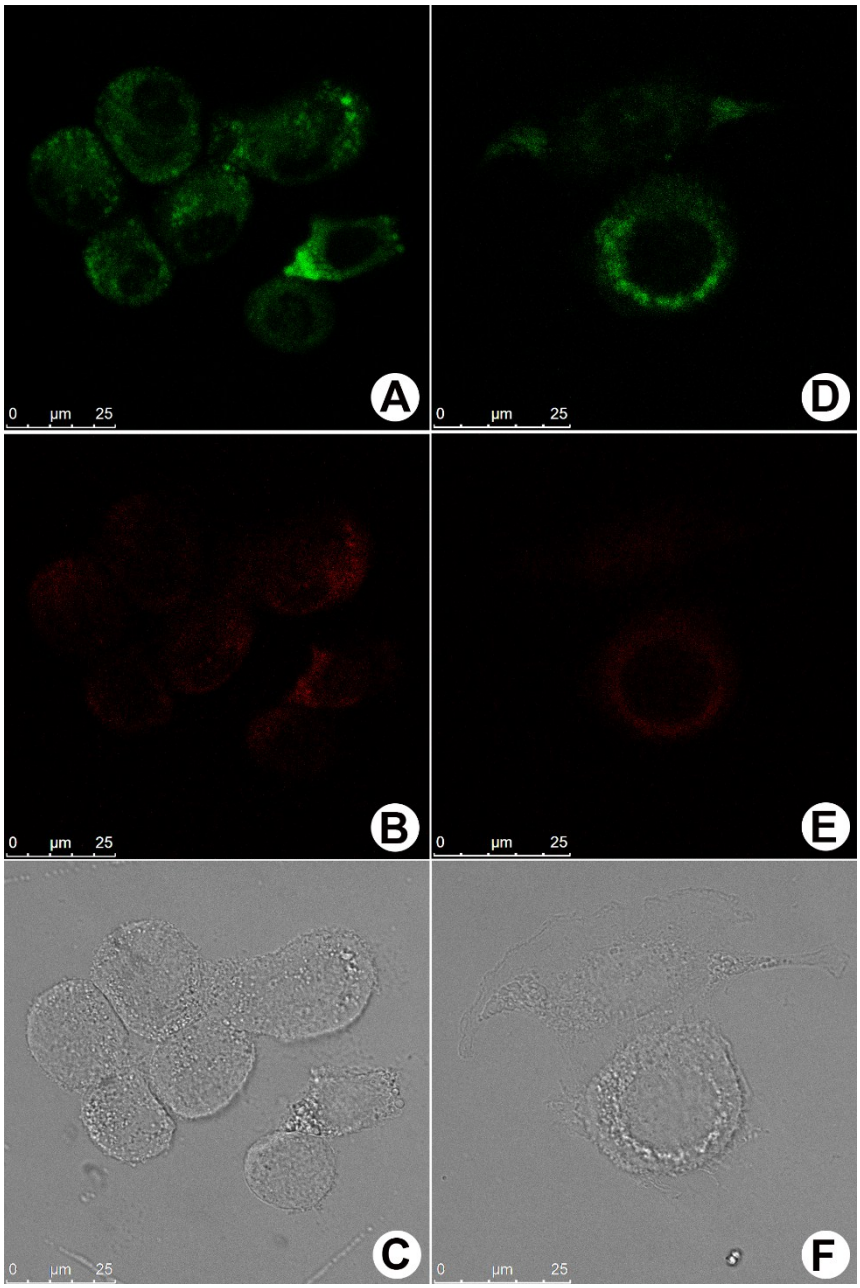


Figure S23. Fluorescent profile of MDA-MB231 cells incubated with lapachone-based BODIPY (**5**) (**1 µM**). Images A, B, D and E show live and fixed cell samples, respectively. These images also show the dual fluorescent signal emission (red and green). The images C and F show the normal morphological aspects of the samples by phase contrast microscopy. The fluorescent staining pattern is slight distributed to cell cytoplasm with a high accumulation near to the cell nuclei. No fluorescent signal could be detected inside of cellular nuclei, shown as black voids in the images. Reference scale bar 25 µm.

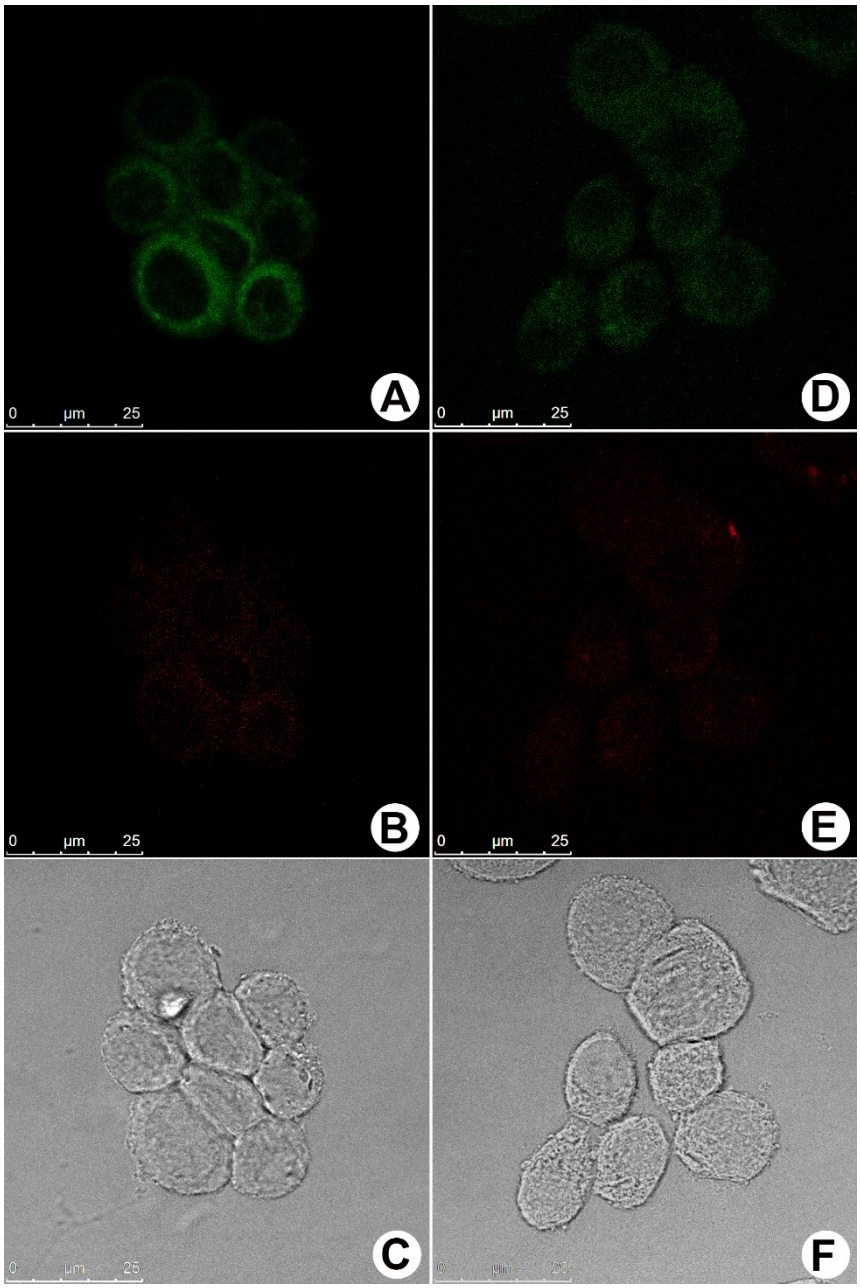


Figure S24. Fluorescent profile of T47-D cells incubated with lapachone-based BODIPY (**5**) (**1 µM**). Images A, B, D and E show live and fixed cell samples, respectively. These images also show the dual fluorescent signal emission (red and green). The images C and F show the normal morphological aspects of the samples, by phase contrast microscopy. The fluorescent staining pattern is slight distributed in cell cytoplasm with a high accumulation near to the cell nuclei. No fluorescent signal could be detected inside of cellular nuclei shown as black voids in the images. Reference scale bar 25 µm.

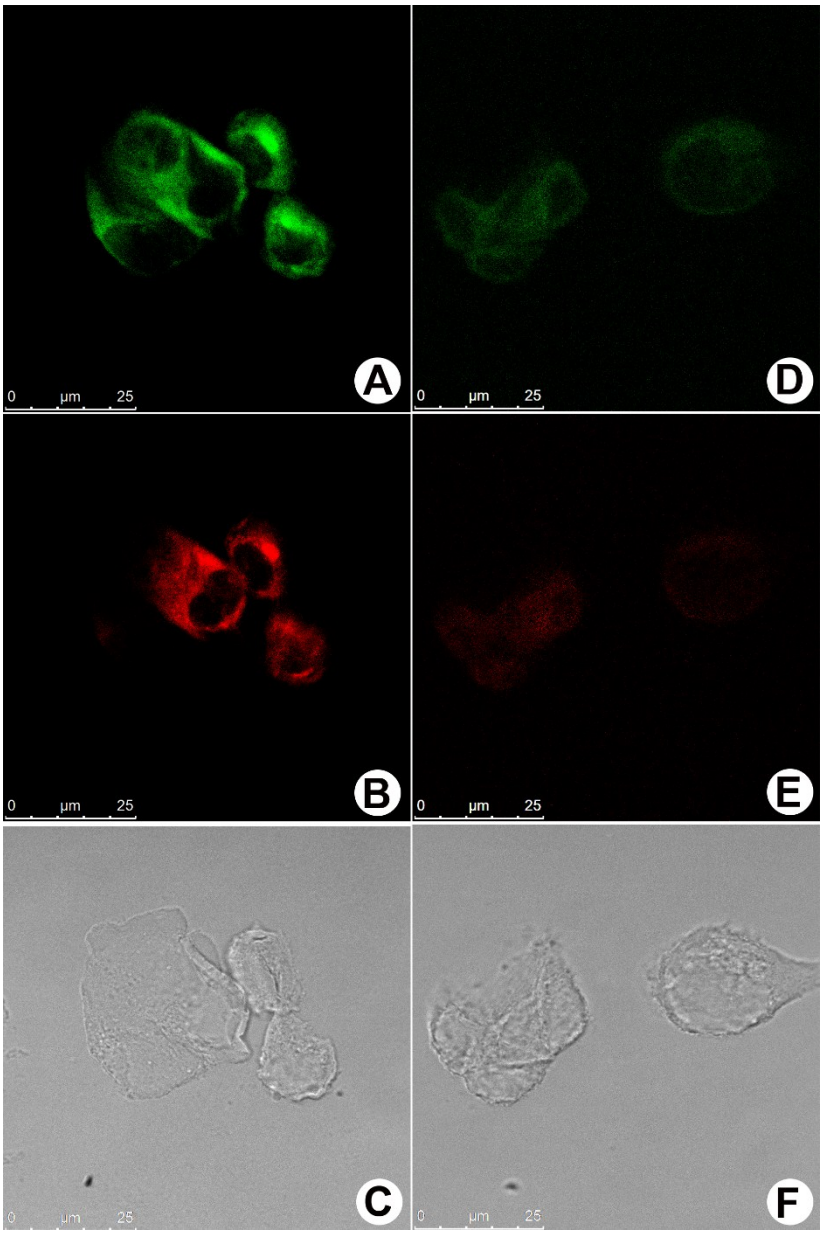
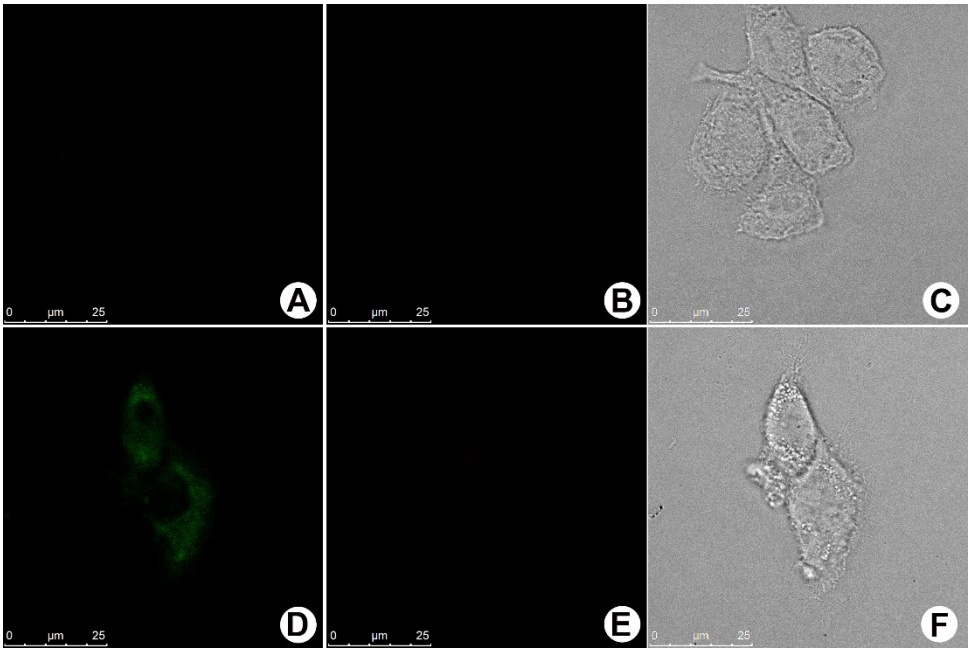


Figure S25. Fluorescent profile of PANC-1 cells incubated with lapachone-based BODIPY (**5**) (**1 µM**). Images A, B, D and E show live and fixed cell samples, respectively. These images also show the dual fluorescent signal emission (red and green). The images C and F show the normal morphological aspects of the samples, by phase contrast microscopy. The fluorescent staining pattern is slight distributed in cell cytoplasm with a high accumulation near to the cell nuclei. No fluorescent signal could be detected inside of cellular nuclei shown as black voids in the images. Reference scale bar 25 µm.

Live cells



Fixed cells

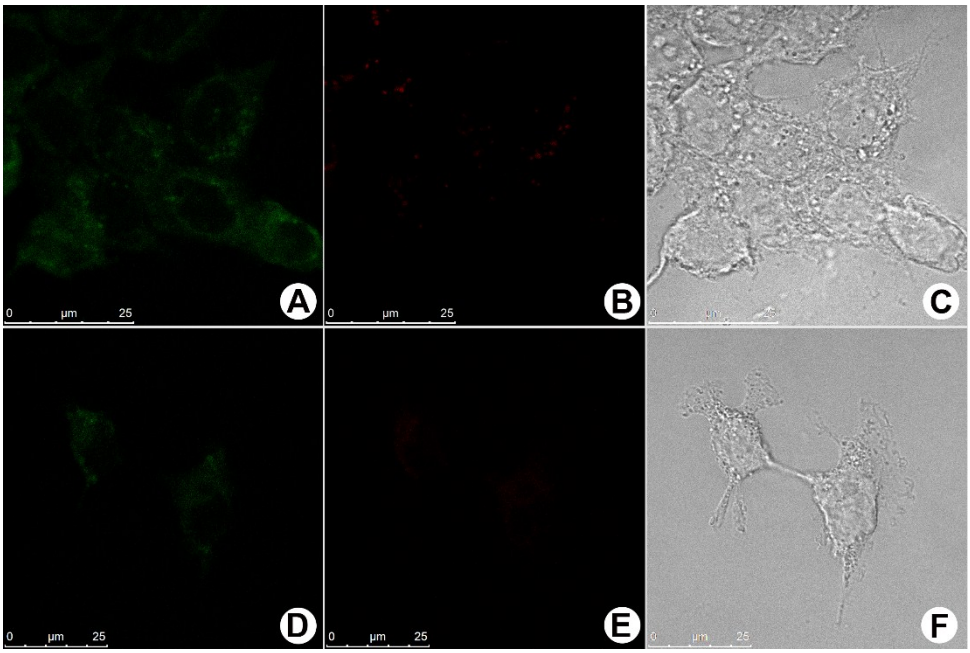
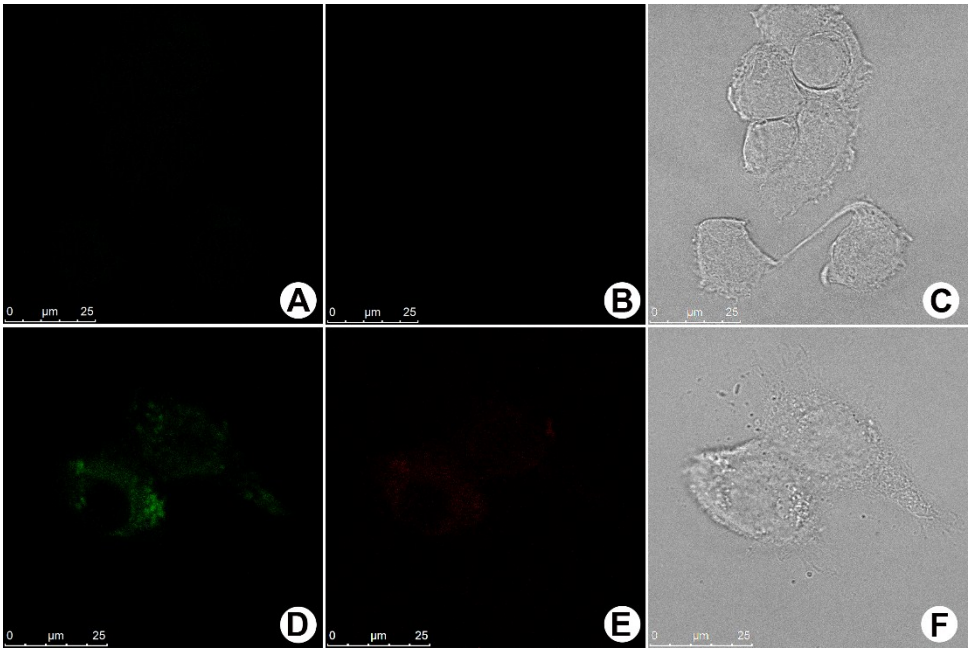


Figure S26. A mild fluorescent signal was detected in live and fixed cell samples incubated with β-lapachone (**100 µM**). Image A, B, D and E show faint fluorescent signal on channels green and red respectively. The images C, F show the cells normal morphological aspects by contrast phase microscopy. Images A, B and C show MCF-7 cells, images D, E and F show MDA-MB-231. Reference scale bar 25 µm.

Live cells



Fixed cells

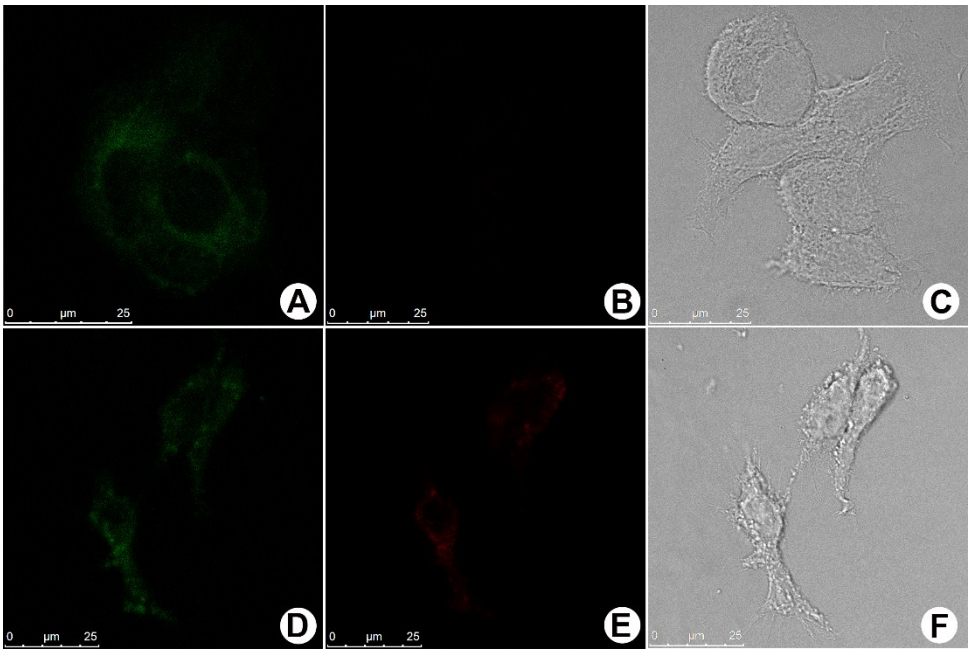
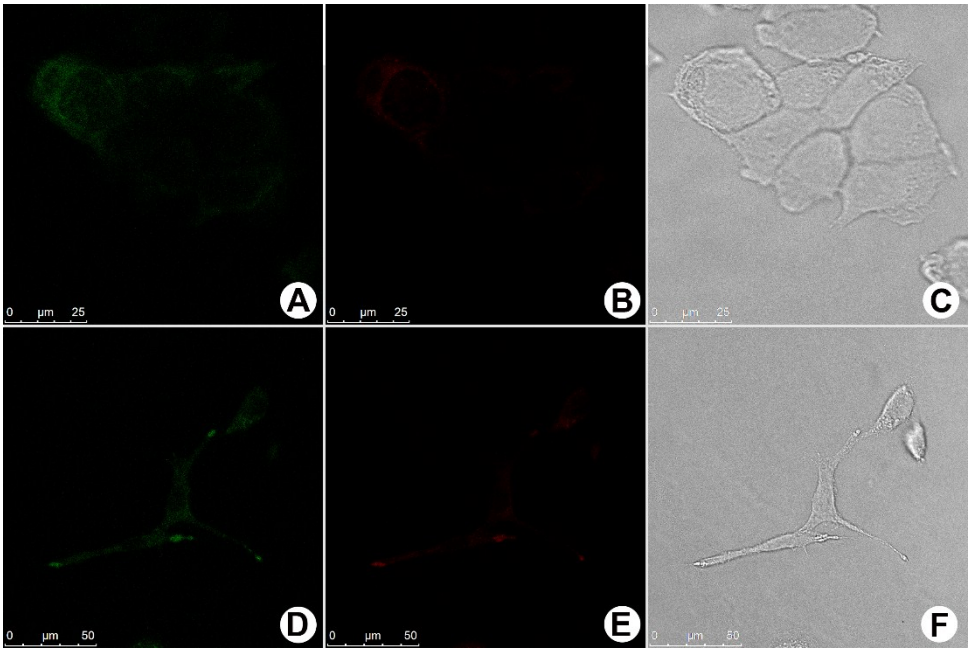


Figure S27. A mild fluorescent signal was detected in live and fixed cell samples incubated with nor-β-lapachone (**100 µM**). Image A, B, D and E show faint fluorescent signal on channels green and red respectively. The images C, F show the cells normal morphological aspects by contrast phase microscopy. Images A, B and C show MCF-7 cells, images D, E and F show MDA-MB-231. Reference scale bar 25 µm.

Live cells



Fixed cells

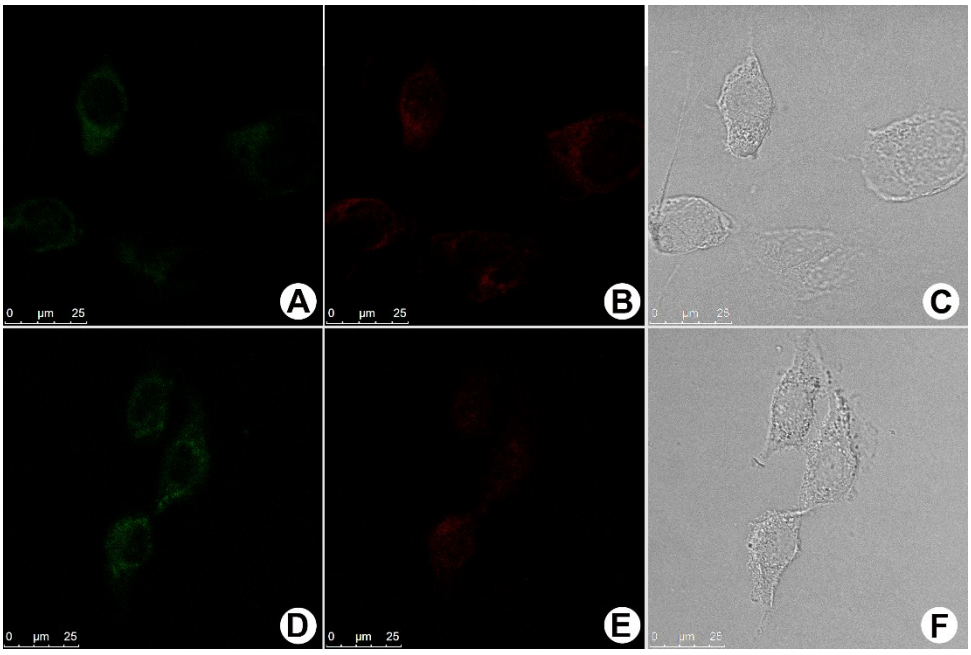


Figure S28. A mild fluorescent signal was detected in live and fixed cell samples incubated with 3-azido-nor-β-lapachone (**4**) (**100 µM**). Image A, B, D and E show faint fluorescent signal on channels green and red respectively. The images C, F show the cells normal morphological aspects by contrast phase microscopy. Images A, B and C show MCF-7 cells, images D, E and F show MDA-MB-231. Reference scale bar 25 µm.

Cytotoxicity against cancer cell lines – MTT assay

The cytotoxic activity of compound **5**, nor-β-lapachone, β-lapachone and 3-azido-nor-β-lapachone (**4**) were evaluated by MTT assay,[12] against cancer cell lines obtained from the National Cancer Institute (Bethesda, MD, USA). The cell was maintained in an RPMI 1640 medium supplemented with 10% fetal bovine serum, 2 mM glutamine, 100 U/mL penicillin and 100 μg/mL streptomycin, at 37 °C with 5% of CO_2. Cancer cell growth was quantified by the ability of living cells to reduce the yellow dye 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT) to a purple formazan product. Briefly, cells were plated in 96-well plates (0.1×10^6 cells/mL) and compound **5**, nor-β-lapachone, β-lapachone and 3-azido-nor-β-lapachone (**4**) dissolved in DMSO, were added to each plate well in a final concentration of 200 μM. Control group received the same amount of vehicle. After 60 minutes of incubation, the supernatant was replaced by fresh medium containing MTT (0.5 mg/mL). Three hours later, the MTT formazan product was dissolved in 150 μL DMSO and absorbance was measured at 595 nm (DTX-880, Beckman Coulter®). The final concentration of DMSO in the culture medium was kept constant, below 0.1% (v/v). All cell treatments were carried out with three replicates.

Table S2. Cytotoxic activity expressed by IC_{50} μM (95% CI) of compound **5**, nor-β-lapachone, β-lapachone and 3-azido-nor-β-lapachone (**4**) in cancer and normal cell lines after 72 h exposure, obtained by nonlinear regression for all cell lines from three independent experiments. *Data obtained for the positive control doxorubicin.

Compounds	**Prostate**		**Leukemia**			
	PC3	DU-145	HL-60	K562	MOLT-4	Jurkat
Quinone-Based BODIPY (**5**)	0.74 (0.63-0.72)	0.77 (0.72-0.80)	0.39 (0.36-0.42)	0.50 (0.44-0.54)	0.34 (0.28-0.37)	0.37 (0.33-0.44)
Nor-β-lapachone	1.30 (1.21-1.38)	1.74 (1.70-1.79)	1.43 (1.40-1.46)	1.55 (1.48-1.61)	0.88 (0.83-0.92)	1.26 (1.22-1.31)
β-Lapachone	1.52 (1.47-1.59)	1.87 (1.81-1.94)	1.38 (1.25-1.46)	1.51 (1.40-1.62)	0.79 (0.76-0.84)	1.22 (1.17-1.24)
3-Azido-nor-β-lapachone	1.81 (1.97-1.83)	1.76 (1.70-1.84)	3.16 (3.02-3.24	3.79 (3.61-3.87)	2.94 (2.88-3.05)	3.63 (3.47-3.80)
Doxorubicin	*0.05 (0.01-0.06)	*0.09 (0.04-0.12)	*0.03 (0.01-0.07)	*0.06 (0.02-0.11)	*0.04 (0.03-0.05)	*0.02 (0.01-0.03)

Compounds	**Colon**			**Breast**		
	HCT-116	HCT-8	SW620	MX-1	HS578t	MDA-MB231
Quinone-Based BODIPY (**5**)	1.04 (0.89-1.24)	0.98 (0.91-1.04)	0.95 (0.89-1.01)	1.71 (1.62-1.79)	1.79 (1.67-1.89)	2.02 (1.88-2.14)
Nor-β-lapachone	0.81 (0.76-0.90)	1.27 (1.22-1.31)	1.15 (1.09-1.25)	0.41 (0.33-0.47)	0.50 (0.45-0.57)	0.56 (0.52-0.61)
β-Lapachone	0.93 (0.85-1.03)	0.80 (0.75-0.83)	0.62 (0.53-0.71)	0.48 (0.42-0.51)	0.57 (0.53-0.62)	0.48 (0.37-0.56)
3-Azido-nor-β-lapachone	1.15 (1.02-1.27)	0.95 (0.89-1.06)	0.90 (0.83-0.99)	3.18 (3.06-3.29)	3.64 (3.55-3.79)	3.13 (2.95-3.28)
Doxorubicin	*0.21 (0.16-0.25)	*0.37 (0.31-0.42)	*0.39 (0.33-0.47)	*0.42 (0.39-0.45	*0.48 (0.41-0.56)	*0.44 (0.40-0.49)

Compounds	**Glioblastome**		**Melanoma**		**Lung**	**Normal**
	SF295	SF268	MDA-MB435	UACC62	NCI-H460	PBMC
Quinone-Based BODIPY (**5**)	2.17 (1.92-2.55)	2.11 (1.97-2.15)	1.79 (1.70-1.91)	1.97 (1.89-2.06)	1.89 (1.77-1.99)	3.26 (3.00-3.50)
Nor-β-lapachone	1.40 (1.29-1.51)	1.25 (1.18-1.30)	0.37 (0.31-0.40)	0.28 (0.23-0.31)	1.05 (0.93-1.10)	>21.9
β-Lapachone	0.88 (0.84-0.93)	0.94 (0.86-1.05)	0.22 (0.16-0.25)	0.25 (0.24-0.27)	0.83 (0.75-0.96)	>20.6
3-Azido-nor-β-lapachone	3.10 (3.03-3.17)	2.84 (2.75-2.98)	1.24 (1.19-1.31)	1.85 (1.72-2.04)	2.07 (2.01-2.13)	5.39 (5.16-5.58)
Doxorubicin	*0.47 (0.44-0.50)	*0.51 (0.49-0.53)	*0.85 (0.79-0.92)	*0.62 (0.57-0.66)	*0.74 (0.71-0.78)	*0.55 0.41-0.58

Analysis of reduced glutathione content

The spectrophotometer determination of 5-thio-2-nitrobenzoate (TNB) procedure, which was produced from 5,5'-dithionitrobenzoic acid (DTNB) was used for measurement of reduced glutathione (GSH) after Akerboom and Sies[13] with minor modifications. Briefly, tested compounds-treated cells with 7 and 15 μM were washed with 0.1 M, phosphate buffer saline (pH 7.4), and 3% TCA was added to precipitate the protein. The supernatant was neutralized with 2 M KOH, and the insoluble residue was removed by centrifugation (8000 x g for 15 min at 4 ºC). For the spectrophotometric determination, 910 μL of supernatant or of the standard glutathione solution, in the same phosphate-EDTA buffer, were mixed with 50 μL of 4 mg/mL NADPH in 0.5% (w/v) $NaHCO_3$, 20 μL of 6 U/mL glutathione reductase in phosphate-EDTA buffer, and 20 μL of 1.5 mg/mL DTNB in 0.5% $NaHCO_3$, and incubated for an hour. The increase in absorbance was measured at 412 nm. The results were normalized by protein content.[14] The concentration of GSH were expressed as μg GSH/mg protein.

TBARS assay

The lipid peroxidation was determined by the reaction of thiobarbituric acid (TBA) with malondialdehyde (MDA). The assays were performed according to Salgo and Pryor with modifications.[15] Cells were incubated with compound **5**, nor-β-lapachone, β-lapachone and 3-azido-nor-β-lapachone (**4**) (7 and 15 μM) for 24 h and then lysed with 15 mM Tris-HCl for 1 h. Two mL of trichloroacetic acid (0.4 mg/mL) and HCl (0.25 M) was added to the lysate, which was then incubated with 7 mg/mL TBA for 15 min at 100 ºC. The mixture was centrifuged at 900 g for 15 min. As TBA reacts with other products of lipid peroxidation in addition to MDA, results are expressed in terms of thiobarbituric reactive species (TBARS), which are determined by absorbance at 532 nm. Hydrolyzed 1,1,3,3-tetramethoxypropane was used as the standard. The results were normalized by protein content (Lowry, 1951).

Table S3. Effect of compound **5**, nor-β-lapachone, β-lapachone and 3-azido-nor-β-lapachone (**4**) on reduced glutathione (GSH) content and lipid peroxidation after 24 h exposure.

Treatments		**GSH (µg/mg protein)**	**MDA equivalents (nmol/mg protein)**
	Concentrations	Mean ± S.E.M.	Mean ± S.E.M.
NC[a]	-	6.80 ± 0.51	3.72 ± 1.15
PC[b]	50 µM	2.04 ± 0.30*	26.07 ± 3.15*
Compound **5**	5 µg/mL (~7.58 µM)	4.52 ± 0.33*	9.25 ± 2.07*
	10 µg/mL (~15.16 µM)	3.10 ± 0.56*	15.83 ± 2.51*
Nor-β-lapachone	7 µM	3.58 ± 0.10*	13.75 ± 3.10*
	15 µM	2.37 ± 0.22*	19.35 ± 1.54*
β-Lapachone	7 µM	3.13 ± 0.25*	12.75 ± 1.17*
	15 µM	2.19 ± 0.10*	19.51 ± 2.15*
3-Azido nor-β-lapachone (**4**)	7 µM	5.07 ± 0.22*	6.28 ± 1.05*
	15 µM	3.74 ± 0.10*	11.62 ± 0.75*

[a]Negative control (0.1% DMSO); [b]Positive control (H_2O_2); *p< 0.05 as compared to negative control, by ANOVA followed by Tukey's test. Data are presented as means ± standard error of the mean (S.E.M) for three independent experiments in triplicate.

Electrochemical studies

Cyclic voltammetry (CV) experiments were performed with a conventional three electrode cell in an Autolab PGSTAT-30 potentiostat (Echo Chemie, Utrecht, the Netherlands) coupled to a PC microcomputer, using GPES 4.9 software. The working electrode was a glassy carbon (GC) BAS (d = 3 mm), the counter electrode was a Pt wire and the reference electrode, an Ag|AgCl, Cl^- (saturated), all contained in a one-compartment electrochemical cell with a volumetric capacity of 5 mL. The GC electrode was cleaned up by polishing with alumina on a polishing felt (BAS polishing kit). The solvent used in aprotic media studies was *N*,*N*-Dimethylformamide extra dry 99.8% acquired from Acrós Organics. In CV experiments, the scan rate varied from 10 to 1000 mV s^{-1}. Electrochemical reduction and oxidation were performed in aprotic media (DMF + $TBAPF_6$ 0.1 mol L^{-1}) at room temperature (25 ± 2 °C).

Lapachone-based BODIPY (**5**) (1 x 10^{-3} mol L^{-1}) was added to the supporting electrolyte and the solution was deoxygenated with argon, before the measurements by cyclic voltammetry, in different potential intervals. The same procedure was performed with BODIPY **3**. To prove the origin of wave IIIa, a potential conditioning at -1.8 V was applied for different times (from 15 s up to 120 s) and CV and DPV were run, to observe the effects on wave IIIa.

Table S4. Major electrochemical parameters for BODIPY (**3**), quinone-based BODIPY (**5**), nor-β-lapachone, β-lapachone and 3-azido-nor-β-lapachone (**4**) (c = 1 x 10^{-3} mol L^{-1}), in DMF + $TBAPF_6$, 0.1 mol L^{-1}, ν = 100 mV s^{-1}.

Compounds	*E*pIc (V)	*E*pIIc (V)	*E*pIIIc (V)	*E*pIa (V)	*E*pIIa (V)	*E*pIIIa (V)
BODIPY (3)	-1.065	-1.891	_	-0.969	-0.001	-
Lapachone-based BODIPY (5)	-0.548	-1.053	-1.921	-0.459	-0.920	-0.023
Nor-β-Lap.	-0.641	-1.127	-	-0.548	-1.073	-
β-Lap.	-0.670	-1.136	-	-0.579	-1.034	-
3-Azido-nor-β-lapachone (4)	-0.575	-1.239	-	-0.510	-1.045	-

β-Lapachone, nor-β-lapachone and 3-azido-nor-β-lapachone (**4**) were also investigated by cyclic voltammetry in DMF + $TBAPF_6$, to allow comparison with the new hybrid derivative **5** and its fluorescent BODIPY precursor (**3**). Data are listed in Table S4.

The first two successive one-electron redox systems (Ic/Ia, IIc/IIa), for β-lapachone, nor-β-lapachone and compound **5**, resemble the typical reduction behaviour of the quinone moiety, in aprotic medium, in absence of proton donor. The first step, Ic/Ia couple, is reversible and has a diffusional nature and is related to the reduction of the quinone (represented as Q) to generate an anion radical or semiquinone ($Q^{\bullet -}$, peak Ic); this latter intermediate is in turn reoxidized into the neutral quinone Q (peak Ia). The second electron transfer (IIc/IIa), is quasi-reversible and corresponds to the reduction of the semiquinone to the diamagnetic dianion species (Q^{2-}). As shown in Table S4, the facility of reduction in terms of the quinones, represented by *E*pIc is the following: lapachone-based BODIPY > 3-azido-nor-β-lapachone > nor-β-lapachone > β-lapachone. For compound **5**, *E*pIIc is a combination of *E*pIc of compound **3** + *E*pIIc of nor-β-lapachone derivative, despite being less intense (in terms of current) than expected. Its *E*pIIIc correlates to *E*pIIc of BODIPY **3**, as well as peaks that appeared in *E*pIIIa (compound **5**) and *E*pIIa (compound **3**).

A mixed CV composed of the particular CV of each compound, including BODIPY **3** (Figures S30) and the combined CV, obtained after addition of compound **5** in the solution containing nor-β-lapachone is shown in Figure S30.

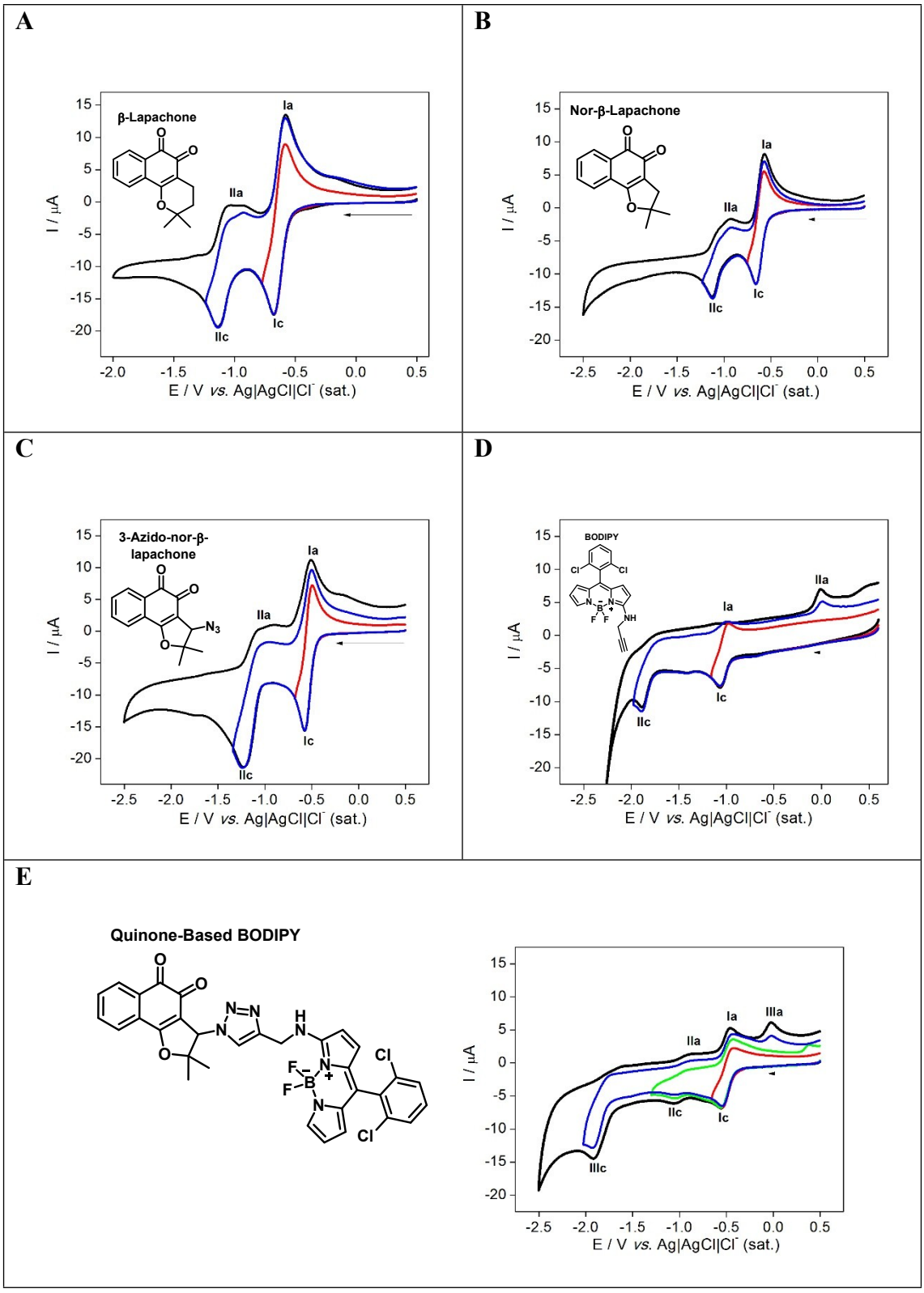


Figure S29. Cyclic voltammetry (CV) in DMF + $TBAPF_6$ (0.1 M), glassy carbon electrode, potential range: 0.5 V up to -2.9 V, with adequate potential invertion, $\nu = 0.1$ V s^{-1}. A: β-lapachone (1 mM), B: nor-β-lapachone (1 mM), C: 3-azido-nor-β-lapachone (**4**) (1 mM), D: BODIPY **3** (1 mM), E: lapachone-based BODIPY (**5**) (1 mM).

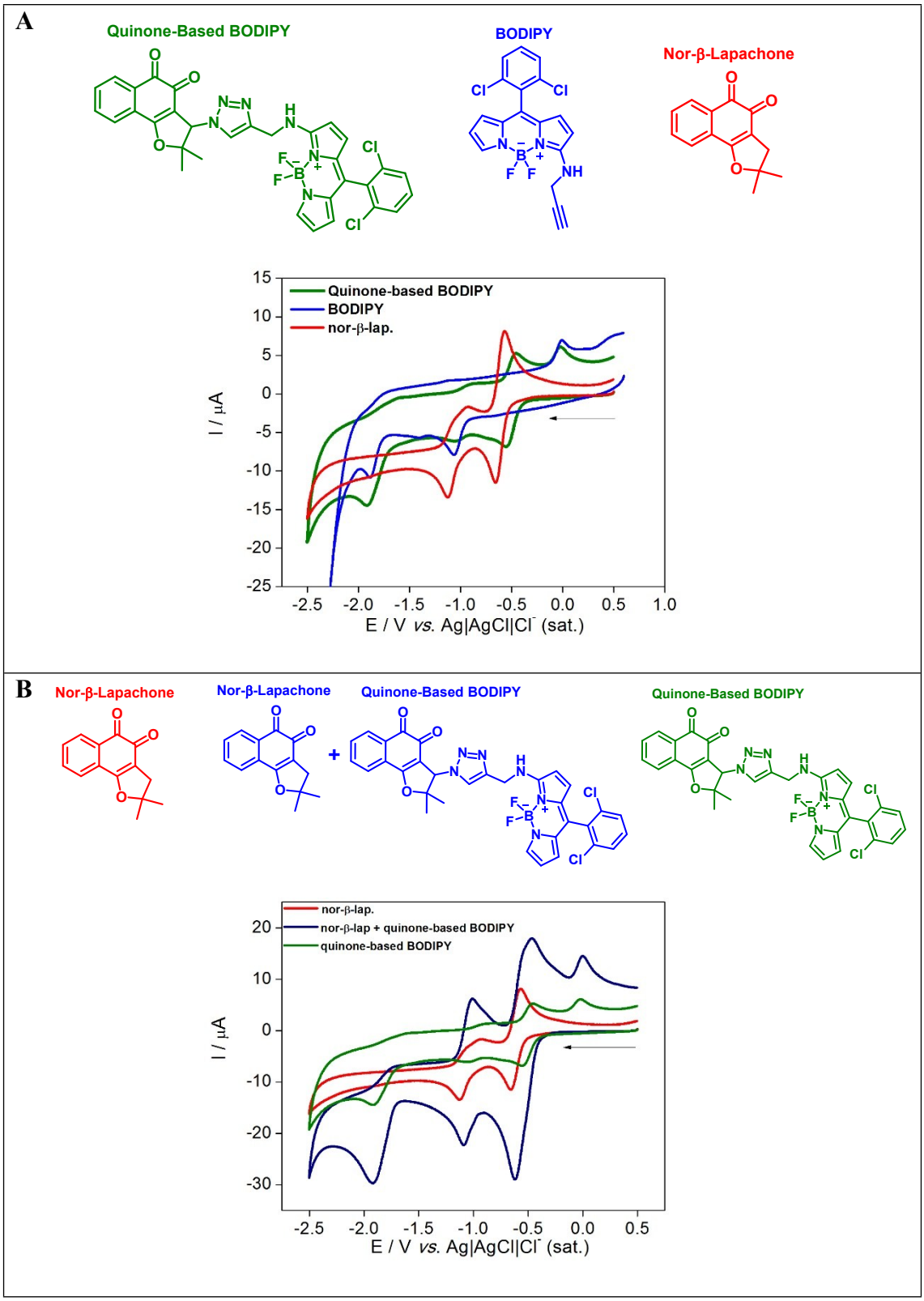


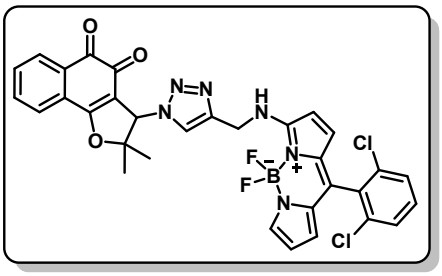
Figure S30. Cyclic voltammetry (CV) in DMF + $TBAPF_6$ (0.1 M), glassy carbon electrode, potential range: 0.5 V up to -2.9 V, with adequate potential inversion, $\nu = 0.1$ V s^{-1}. A: Combined CVs of nor-β-lapachone and compounds **3** and **5**. B: CVs of the solution containing both nor-β-lapachone and compound **5**.

Cyclic voltammetry of the solutions containing all the compounds of interest (Figure S30 B) have shown that the electrochemical behaviour of lapachone-based BODIPY (**5**) is a combination of the ones from the precursors, BODIPY (**3**) and non-substituted nor-β-lapachone, showing the increase of the currents of the first reduction peak Ia, Ia, IIa, and the appearance of the peaks IIIc and IIIa.

Computational details

DFT calculations indicate that both HOMO-2 and HOMO-1 are dispersed throughout nor-β-lapachone-based BODIPY (Figure S31). The HOMO orbital is mainly in the BODIPY portion and the LUMO is mainly in the quinone moiety. For β-lapachone, nor-β-lapachone and 3-azido-nor-β-lapachone (**4**), the HOMO is mainly in the aromatic ring (Figures S32-S34). For 3-azido-nor-β-lapachone (**4**), some contribution from the azide group to the HOMO is observed, but not for the LUMO (Figure S34). The HOMO-LUMO gap was calculated to be 3.51, 3.38 and 3.46 eV respectively. The coupling between the quinone with the BODIPY moiety reduces this gap to 2.12 eV in nor-β-lapachone-based BODIPY.

Cartesian coordinated of the optimized structure of nor-β-lapachone-based BODIPY (**5**).



C	-2.147075	3.587930	0.462468
C	-2.437308	2.228118	0.513361
C	-0.867147	4.017699	0.128116
C	0.129038	3.093275	-0.162548
C	-0.171432	1.717623	-0.116200
C	-1.451721	1.289830	0.225316

C	1.503561	3.577730	-0.516671
C	2.588849	2.513419	-0.881172
C	2.155420	1.146648	-0.788255
C	0.897614	0.788085	-0.437330
O	1.787385	4.750296	-0.524164
O	3.704892	2.860045	-1.211688
O	0.681159	-0.528763	-0.406333
C	1.889327	-1.206721	-0.938738
C	2.988114	-0.084479	-0.954032
C	1.544868	-1.615772	-2.364699
C	2.169956	-2.391344	-0.038732
N	4.002426	-0.196034	0.095252
N	3.690527	0.046918	1.380017
N	4.754390	-0.179522	2.089356
C	5.291848	-0.584700	-0.017965
C	5.768920	-0.573123	1.271597
C	7.104753	-0.953113	1.826366
N	8.205491	-0.134415	1.340304
C	9.385206	-0.627038	0.926102
N	10.483993	0.140668	0.837509
C	9.698738	-1.957183	0.480515
C	11.017410	-1.946881	0.107729
C	11.524784	-0.637567	0.328061
B	10.607920	1.635062	1.282140
N	12.087755	2.025360	1.064925
F	10.228229	1.742064	2.625371
F	9.768642	2.429426	0.501113
C	12.787096	-0.120651	0.161970
C	13.069504	1.210330	0.523594
C	14.258279	1.953789	0.437530
C	13.980280	3.228939	0.927635
C	12.634029	3.233173	1.303526
C	13.872776	-0.967395	-0.393247
C	14.649249	-1.794159	0.426674

C	15.686442	-2.566042	-0.083129
C	15.962471	-2.521787	-1.442982
C	15.210008	-1.718046	-2.288874
C	14.176459	-0.954634	-1.759859
Cl	14.316637	-1.863619	2.138449
Cl	13.235052	0.045068	-2.835829
H	-2.918256	4.313765	0.686249
H	-3.432211	1.895200	0.780363
H	-0.620633	5.070474	0.087176
H	-1.666874	0.230357	0.263989
H	3.537564	-0.104805	-1.894575
H	2.389017	-2.138689	-2.817116
H	1.308650	-0.740005	-2.971044
H	0.682452	-2.281805	-2.366422
H	3.076557	-2.902796	-0.366199
H	1.339956	-3.095987	-0.091061
H	2.299772	-2.071871	0.993278
H	5.752772	-0.803211	-0.965045
H	7.038673	-0.878730	2.915738
H	7.325843	-1.992005	1.585003
H	8.202840	0.836899	1.622780
H	15.418794	-1.674704	-3.348577
H	9.016511	-2.788319	0.436382
H	11.592806	-2.772489	-0.277548
H	15.194791	1.585005	0.052346
H	14.660867	4.059933	1.010300
H	12.040523	4.029192	1.723147
H	16.265906	-3.188024	0.584181
H	16.769465	-3.118793	-1.847193

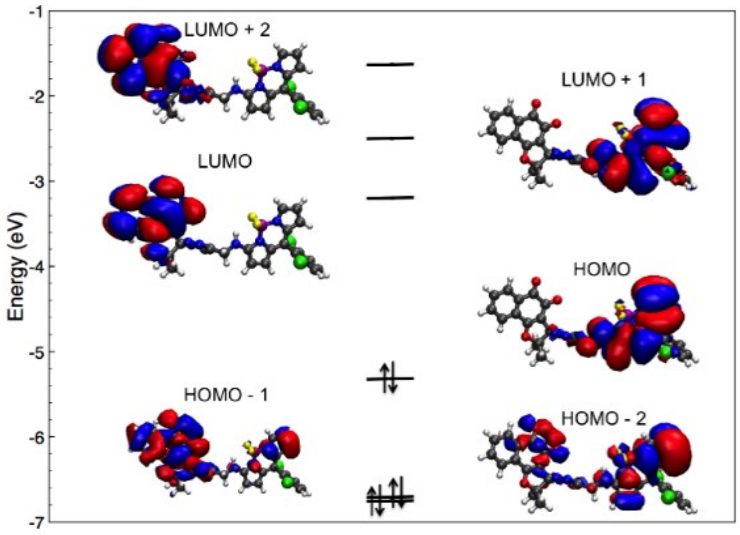
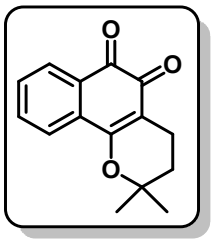


Figure S31. Frontier orbitals in probe **5**. The orbitals were calculated at the B3LYP/TZVP level and plotted with an isovalue of 0.008. C (Gray), H (white), O (red), N (blue), Cl (green), F (yellow), and B (purple).

Cartesian coordinated of the optimized structure of β-lapachone



C	-6.402213	2.282560	0.329186
C	-6.358004	0.900526	0.484351
C	-5.226027	2.984684	0.099982
C	-4.009502	2.312502	0.018973
C	-3.959838	0.917689	0.185522
C	-5.146929	0.220075	0.416584
C	-2.765606	3.076129	-0.262562
C	-1.447621	2.267836	-0.422209
C	-1.498428	0.825785	-0.245354
C	-2.669070	0.213336	0.078683
O	-2.750654	4.279290	-0.375064

O	-0.414928	2.864470	-0.663417
O	-2.771588	-1.099973	0.329567
C	-0.224011	0.046093	-0.398124
C	-0.325826	-1.316107	0.281576
C	-1.665656	-2.007237	0.017718
C	-1.872044	-3.178775	0.965754
C	-1.826837	-2.425535	-1.442686
H	-7.347141	2.808098	0.383524
H	-7.271616	0.345989	0.659645
H	-5.227995	4.058753	-0.032556
H	-5.118617	-0.852558	0.540953
H	0.600222	0.625413	0.019772
H	0.009326	-0.064244	-1.462530
H	0.473205	-1.977734	-0.059573
H	-0.208300	-1.193462	1.361434
H	-2.854586	-3.628128	0.814481
H	-1.798439	-2.839808	2.000030
H	-1.111499	-3.941935	0.795084
H	-1.688406	-1.579098	-2.115285
H	-1.091078	-3.190722	-1.695728
H	-2.823378	-2.837442	-1.607420

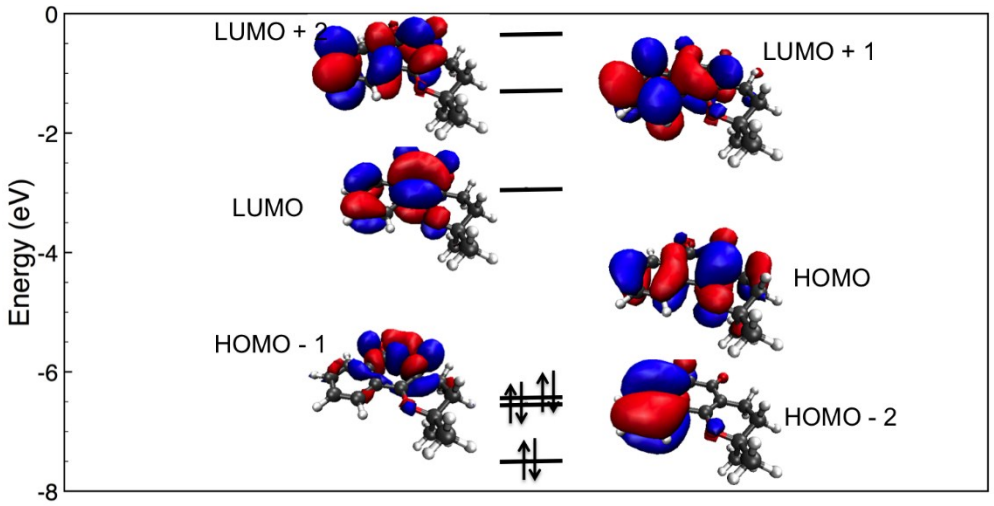
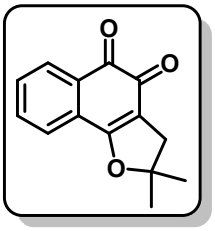


Figure S32. Frontier orbitals in β-lapachone. The orbitals were calculated at the B3LYP/TZVP level and plotted with an isovalue of 0.02. C (Gray), H (white), O (red). Cartesian coordinated of the optimized structure of nor-β-lapachone.



C	-6.357094	2.639672	0.281325
C	-6.506456	1.257032	0.329286
C	-5.089461	3.200604	0.156514
C	-3.966112	2.385214	0.074499
C	-4.124127	0.986978	0.119612
C	-5.392207	0.427988	0.251469
C	-2.603882	2.998191	-0.063937
C	-1.367603	2.044938	-0.182298
C	-1.663931	0.641350	-0.128773
C	-2.926044	0.170985	0.014855
O	-2.442130	4.194446	-0.084311
O	-0.250227	2.510069	-0.308806
O	-3.025545	-1.160586	0.052471
C	-0.710526	-0.513044	-0.193975

H	-2.486235	-3.261818	-1.453069
C	-1.664212	-1.739883	-0.157861
C	-1.408904	-2.662105	1.018251
C	-1.733853	-2.472847	-1.487205
H	-7.227137	3.280716	0.341515
H	-7.492246	0.820434	0.428156
H	-4.951619	4.273382	0.119540
H	-5.496515	-0.648043	0.288570
H	-0.015342	-0.509427	0.649331
H	-0.106100	-0.496681	-1.103269
H	-1.994372	-1.777786	-2.287171
H	-0.766393	-2.922304	-1.718890
H	-2.146255	-3.465171	1.049710
H	-1.460394	-2.101441	1.952845
H	-0.414577	-3.105063	0.935513

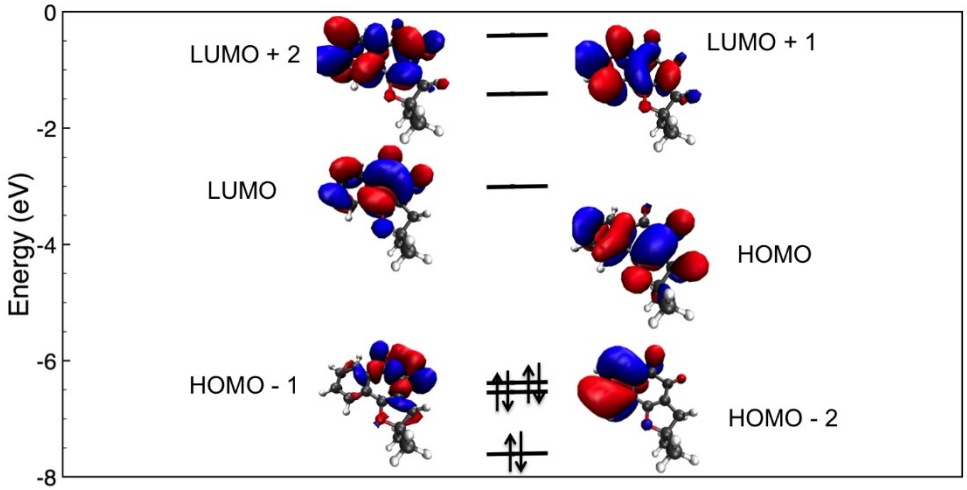
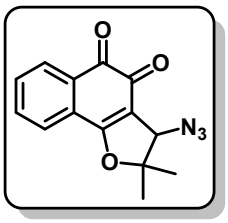


Figure S33. Frontier orbitals in nor-β-lapachone. The orbitals were calculated at the B3LYP/TZVP level and plotted with an isovalue of 0.02. C (Gray), H (white), O (red).

Cartesian coordinated of the optimized structure of 3-azido-nor-β-lapachone (**4**).



C	-6.299430	2.623984	0.420723
C	-6.402494	1.257795	0.662833
C	-5.077360	3.177843	0.052873
C	-3.952210	2.371291	-0.075825
C	-4.062149	0.989253	0.172075
C	-5.286946	0.437032	0.536677
C	-2.647129	2.981617	-0.492947
C	-1.386954	2.055569	-0.559753
C	-1.645776	0.661281	-0.340423
C	-2.865404	0.182108	0.006122
O	-2.552962	4.149106	-0.783048
O	-0.286659	2.522582	-0.785363
O	-2.909987	-1.138680	0.186267
C	-0.658076	-0.467561	-0.333505
H	-2.641534	-3.018573	-1.630287
C	-1.608431	-1.703316	-0.268723
C	-1.215212	-2.780833	0.717104
C	-1.867798	-2.251648	-1.666773
H	-7.170911	3.258397	0.519517
H	-7.353382	0.827783	0.950865
H	-4.978441	4.236839	-0.146673
H	-5.356875	-0.626627	0.720825
H	-0.256122	-3.212746	0.429975
H	-1.116844	-2.372642	1.720505
H	-2.197229	-1.458066	-2.339156
H	-0.955873	-2.695935	-2.068355
H	-1.968028	-3.569675	0.717295

N	0.234781	-0.414817	0.853011
N	1.275595	0.223777	0.707435
N	2.260215	0.771883	0.686527
H	-0.051002	-0.502712	-1.241009

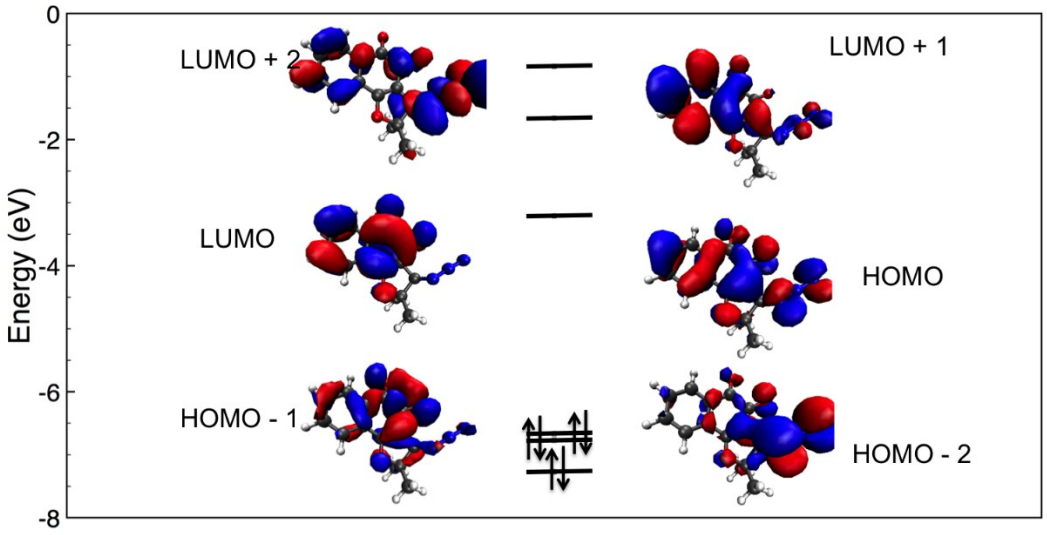


Figure S34. Frontier orbitals in 3-azido-nor-β-lapachone (**4**). The orbitals were calculated at the B3LYP/TZVP level and plotted with an isovalue of 0.02. C (Gray), H (white), O (red), N (blue).

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