Electronic Supplementary Information (ESI)

Ditopic boronic acid and imine-based naphthalimide fluorescence sensor for Copper(II)

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1. General Methods

All chemical reagents and solvents were analytical grade and purchased from commercial suppliers. Compound **BNP**, **1** and **2** were prepared by the established literature procedure.[S1] ^{1}H NMR and ^{13}C NMR spectra were recorded on the Bruker AV-300 spectrometer with chemical shifts reported in ppm (in $CDCl_3$, TMS as internal standard) at room temperature. Electrospray mass spectra were recorded using a Bruker micro TOF spectrometer using reserpine as calibrant.

UV-vis absorption spectra were recorded on a Perkin Elmer spectrophotometer. Fluorescence measurements were performed on a Perkin Elmer Luminescence spectrophotometer LS 50B, utilising sterna silica (quartz) cuvettes with 10 mm path length and four sides polished. Spectral-grade solvents were used for measurements of UV-vis absorption and fluorescence.

(E)-6-(propylamino)-2-((2-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzylidene)amino)-1H-benzo[de]isoquinoline-1,3(2H)-dione (BNP)

2-amino-6-(propylamino)-1H-benzo[de]isoquinoline-1,3(2H)-dione (0.27 g, 1.0 mmol) was dissolved in absolute ethanol (20 mL). An excess of 2-aldehyde boronic acid ester (0.50 g, 2.0 mmol) was added and the mixture was refluxed for 3 h. After the mixture was cooled to room temperature, the precipitate produced was filtered and washed with hexane (3×10 mL) to give a yellow solid (434.60 mg, 90% yield).^{1}H NMR (300 MHz, DMSO-d_6, ppm): δ_H 9.14(s, 1H), 8.72(d, J = 10.0 Hz, 1H), 8.47(d, J = 8.7 Hz, 1H), 8.29 (d, J = 10.1 Hz, 1H), 8.22 (d, J = 8.5 Hz, 1H), 7.86-7.81(m, 2H), 7.72-7.60 (m, 3H), 6.78 (d, J = 10.3 Hz, 1H), 3.61-3.50(m, 2H), 1.78-1.69 (m, 2H), 1.25(s, 1H), 0.99(t, 3H, J = 8.6 Hz) ppm. ^{13}C NMR (75 MHz, DMSO-d_6): δ_C 170.8, 160.8, 160.3, 151.4, 137.7, 135.8, 135.1, 131.6, 131.5, 131.4, 129.2, 127.2, 124.6, 122.2, 120.5, 107.4, 104.4, 84.4, 44.9, 24.8, 21.5, 11.9 ppm. HRMS (ESI μTOF) m/z calcd for $C_{28}H_{30}N_3O_4B$ $[M + H]^+$ 484.2408, found 484.2406.

(E)-2-(benzylideneamino)-6-(propylamino)-1H-benzo[de]isoquinoline-1,3(2H)-dione (1)

2-amino-6-(propylamino)-1H-benzo[de]isoquinoline-1,3(2H)-dione (60 mg, 0.22 mmol) was dissolved in absolute ethanol (20 mL). An excess of benzaldehyde (36 mg, 0.33 mmol) was added and the mixture was refluxed for 3 h. After the mixture was cooled to room temperature, the precipitate produced was filtered and washed with hexane(3×10 mL). Yield: 90%. ^{1}HNMR (300 MHz, DMSO-d_6, ppm): δ=8.79(d, J= 9.9 Hz, 1H), 8.71(s, 1H), 8.49(d, J= 8.7 Hz, 1H), 8.31(d, J= 10.2 Hz, 1H), 8.00-7.89(m, 2H), 7.34(t, J= 9.3 Hz, 1H), 7.67-7.55(m, 2H), 6.84(d, J= 10.5 Hz, 1H), 3.40(t, J= 8.1 Hz, 2H), 1.84-1.66 (m, 2H), 1.02 (t, 3H, J= 8.7 Hz). ^{13}C NMR (75 MHz, DMSO-d_6): δ=171.5, 160.8, 160.3, 151.4, 135.1, 133.0, 132.8, 131.3, 129.4, 129.3, 129.0, 124.7, 122.4, 120.6, 107.6, 104.4, 45.0, 21.5, 11.9. HRMS (ESI) calcd for $C_{22}H_{19}N_3O_2$ [M^++H] 358.1556, found 358.1564.

(3-(1,3-dioxo-6-(propylamino)-1H-benzo[de]isoquinolin-2(3H)-yl)phenyl)boronic acid (2)

(3-(6-bromo-1,3-dioxo-1H-benzo[de]isoquinolin-2(3H)-yl)phenyl)boronic acid (0.80 g, 2 mmol) and 3 mL propylamine were added into 30 mL of 2-methoxyl ethanol and refluxed for 3 h. After the mixture cooled to room temperature, the solvent was removed under vacuum and the residue was purified with column chromatography (silica gel, DCM–MeOH, 20:1, v/v) to give a yellow solid (0.69 g, 92% yield). ^{1}H NMR (300 MHz, DMSO-d_6, ppm): δ_H 8.77-8.72 (m, 1H), 8.43-8.39 (m, 1H), 8.26-8.22 (m, 1H), 8.13 (s, 1H), 7.82 (d, J = 7.2 Hz, 1H), 7.72 - 7.62 (m, 1H), 7.62 - 7.43 (m, 1H), 7.33 - 7.22 (m, 1H), 6.79 (dd, J_1 = 8.6 Hz, J_2 = 3.3 Hz, 1H), 6.69 - 6.65(m, 1H), 3.40 - 3.35 (m, 2H), 1.78 - 1.66 (m, 2H), 0.98 (t, 3H, J = 7.3Hz) ppm. ^{13}C NMR (75 MHz, DMSO-d_6, ppm): δ_c 164.4, 163.6 158.1, 151.2, 137.8, 136.2, 131.1, 129.7, 128.3, 124.6, 122.6, 120.6, 120.1, 116.6, 115.2, 107.9, 104.2, 44.9, 44.9, 21.5, 11.9 ppm. HRMS (ESI) calcd for $C_{21}H_{19}N_2O_4B$ $[M + H]^+$ 375.1516, found 375.154

Reference

S1. X. Qian, Y. Xiao, Y. Xu, X. Guo, J. Qian and W. Zhu, *Chem. Commun.*, 2010, **46**, 6418-6436

2. Absorption spectra of BNP

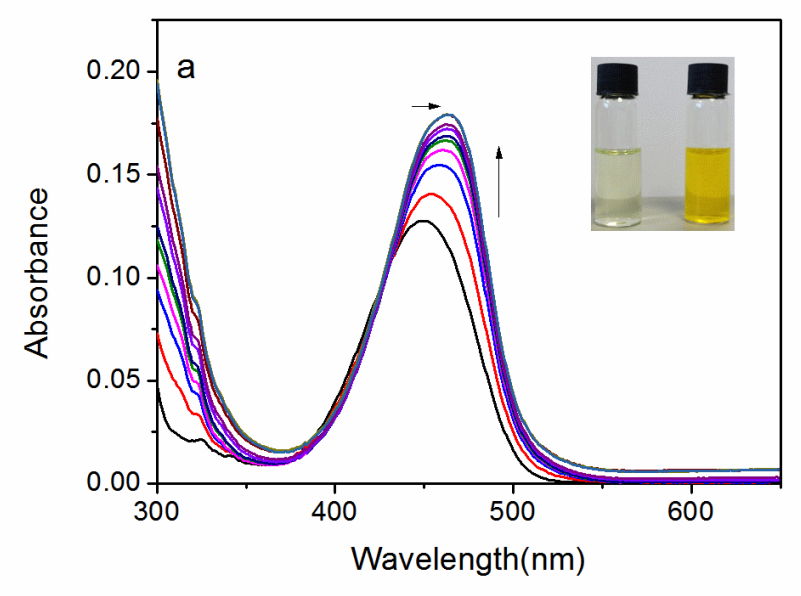


Figure S1. Absorption spectra of BN (1×10^{-5} M) in methanol in the presence of different amounts of Cu^{2+} (0 –30 equiv).

3. Association constant of BNP for Cu^{2+}

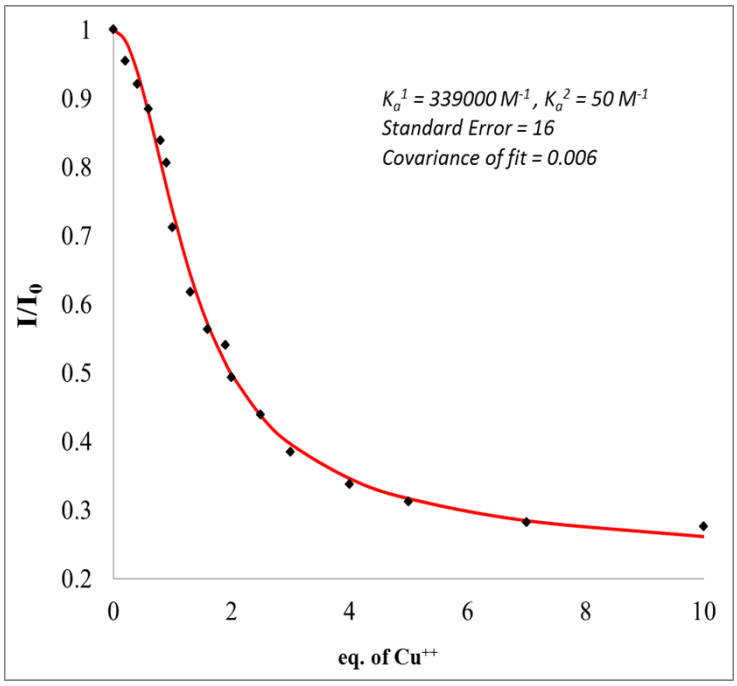


Figure S2. Fluorescence quenching response of BNP to the increments of Cu^{2+} equivalent in solution. Association constants, K_a^1 and K_a^2, were calculated by using MATLAB® m-files and fitted for 2:1 BNP: Cu^{2+} binding isotherm (*Thodarson, P., Chem. Soc. Rev.* **2011**, *40*, 1305–1323). Experimental data points, (black markers) and fitting curve (red line) for such equilibrium are reported in figures here together with standard error and covariance of fit.

4. Job's plot of BNP

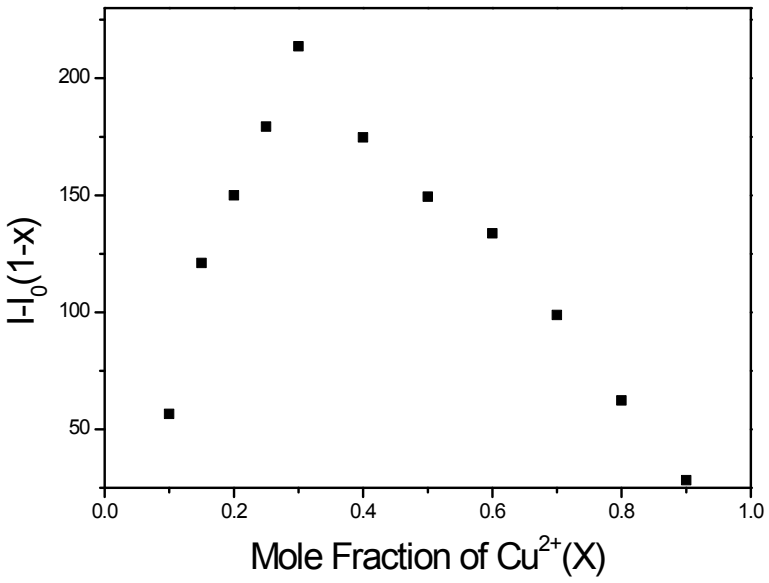


Figure S3. The Job's plot of sensor BNP in methanol. The total concentration of sensor BNP and Cu^{2+} is 20.0 μM

5. Selectivity of BNP

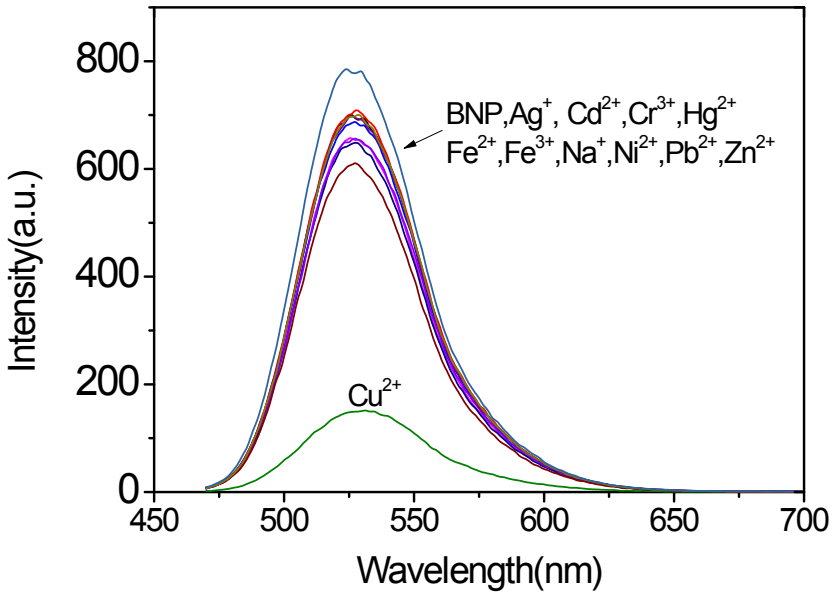


Figure S4. Fluorescent changes of BNP (10 μM) with various metal ions (100 μM) in methanol (λ_{ex} = 450 nm).

6. Selectivity of control compounds 1 and 2.

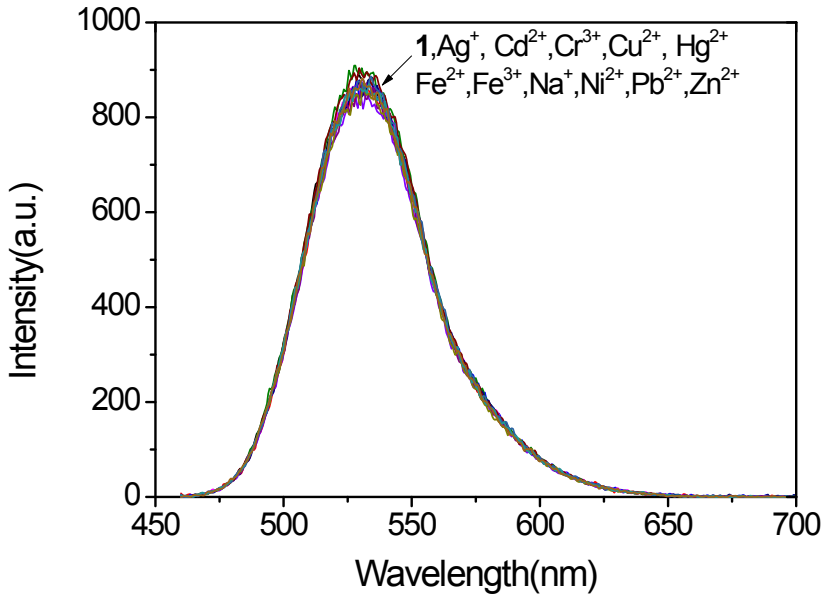


Figure S5. Emission spectra of control compound **1** upon addition 10 equiv. various metal ion with excitation wavelength at 450 nm in methanol. The concentration of **1** was 10 μM.

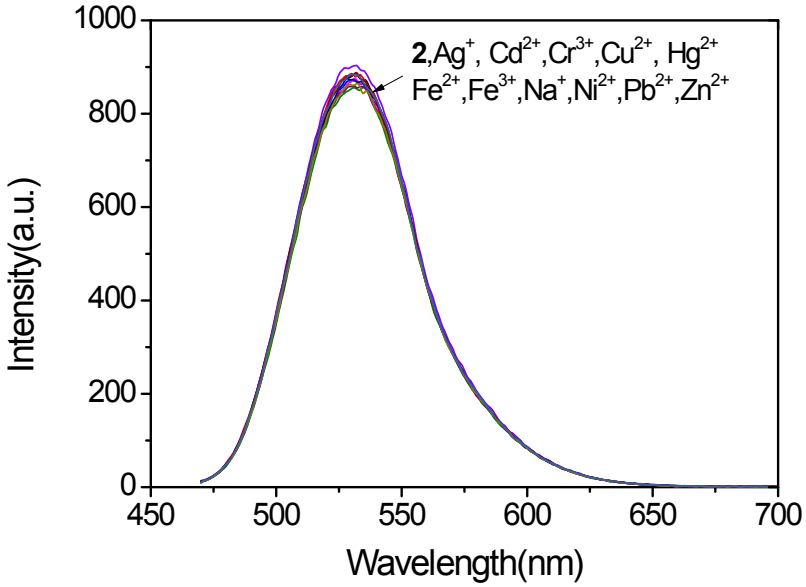


Figure S6. Emission spectra of control compound **2** upon addition 10 equiv. various metal ion with excitation wavelength at 450 nm in methanol. The concentration of **2** was 10 μM.

7. Emission spectra of BNP upon addition of 100mM different saccharides.

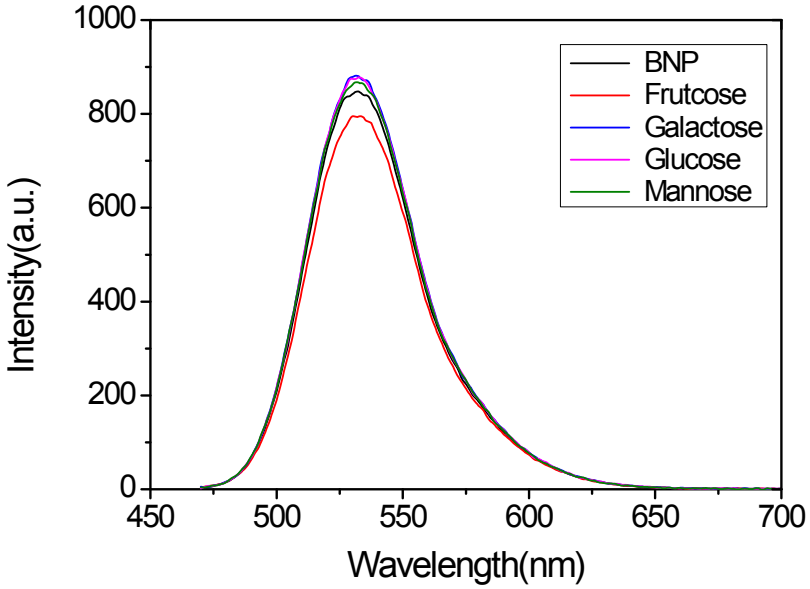


Figure S7. Emission spectra of BNP upon addition of 100mM each relevant analyst with excitation wavelength at 450 nm in methanol. The concentration of BNP was 10 μM.

8. Cell Culture

(HeLa, PC-3) cells were grown as monolayers in T75 tissue culture flasks, and cultured in Eagle's Minimum Essential Medium (EMEM) for HeLa, Roswell Park Memorial Institute medium (RPMI) for PC-3, supplemented with 10% foetal bovine serum, 1% L-glutamine (200 mM), 0.5% penicillin/streptomycin (10 000 IU mL^{-1}/10 000 mg mL^{-1}). Cells were maintained at 37 ^{o}C in a 5% CO_2 humidified atmosphere and grown to approximately 85% confluence before being split using a 2.5% trypsin solution. For microscopy, cells were seeded into glass bottomed Petri dishes and incubated for 12 h for HeLa and 48 h for PC-3 to ensure adhesion. Cells were plated in 35 mm uncoated 1.5 mm thick glass-bottomed dishes as 3×10^5 cells per dish and incubated for at least 24 h prior to imaging experiment. **BNP** complexes were prepared as 5 mM solutions in DMSO, and diluted to 50 μM with serum free EMEM. Cells were washed 5 times with 1 mL Hank's Balanced Salt Solution (HBSS) and incubated with the 100 μM (1% DMSO) of **BNP** compounds at 37 ^{o}C for 15 min. Background autofluorescence was measured by imaging the cells in 1 mL of serum free EMEM only. Immediately prior to imaging cells were washed 3 times with 1 mL HBSS and returned to serum free EMEM.

9.Imaging experiments

The fluorescent uptake of the **BNP** complex was imaged by laser-scanning confocal microscopy. Initial experiments for cell viability and uptake were recorded using a Zeiss LSM 510 META microscope irradiating at 488 nm with emission filtered between 505 and 535 nm. 5 eq. of $CuCl_2$ was consequently added into the serum free medium; cells were incubated 15 minutes and washed three times with HBSS prior to imaging. The same experiment was repeated at the OCTOPUS facility at the Research Complex at Harwell, using single photon and 2-photon laser irradiation with the specifications below.

Two photon (690-1000 nm) wavelength laser light was obtained from the mode-locked titanium- sapphire laser Mira (Coherent Laser Co., Ltd.) produced by 180 femtosecond pulse frequency of 75 MHz. This laser-pumped solid-state continuous wave 532 nm laser (Verdi V18, Coherent Laser Co., Ltd.). This can also be used for the fundamental output of the oscillator 915 ± 2 nm. The laser beam was focused to a diffraction-limited spot by the water immersion UV calibration target (Nikon VC × 60, NA1.2) and the specimen on a microscope stage of the modified Nikon TE2000-U, with UV illumination optical emission. The focused laser beam raster scanning used an X - Y galvanometer (GSI Lumonics Corporation). Fluorescence emission was collected without de-scanning, bypassing the scanning system and passed through a coloured glass (BG39) filter. In normal operation mode and line scan frame and pixel clock signal was generated with an external fast microchannel plate photomultiplier tube as detector (R3809 - U, Hamamatsu, Japan) synchronisation. These were linked to a Time Correlated Single Photon Counting (TCSPC) PC module SPC830 for the lifetime measurements with 915 nm excitation and emission in the range between 360 and 580 nm.

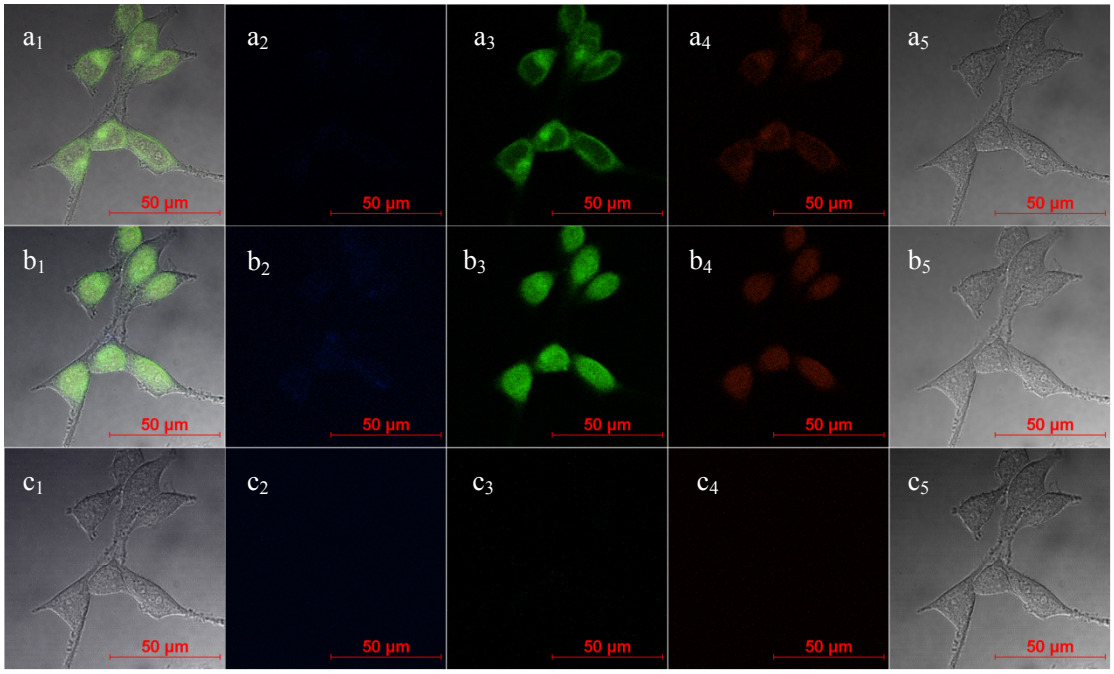


Figure S8: Confocal fluorescence imaging of cervical cancer (HeLa) cells 37 °C, 15 minutes incubation with the addition of 100 µM of **BNP**, 1% DMSO, λ_{ex}= 405 nm (a), 488 nm (b) and 543 nm (c), band-pass filtered at 420-480 nm (a_2, b_2, c_2), long-pass filtered at 515 nm (a_3, b_3, c_3) and long-pass filtered at 605 nm (a_4, b_4, c_4), respectively. (a_1), (b_1) and (c_1) are overlaid images of (a_2)-(a_5), (b_2)-(b_5) and (c_2)-(c_5) micrographs respectively.

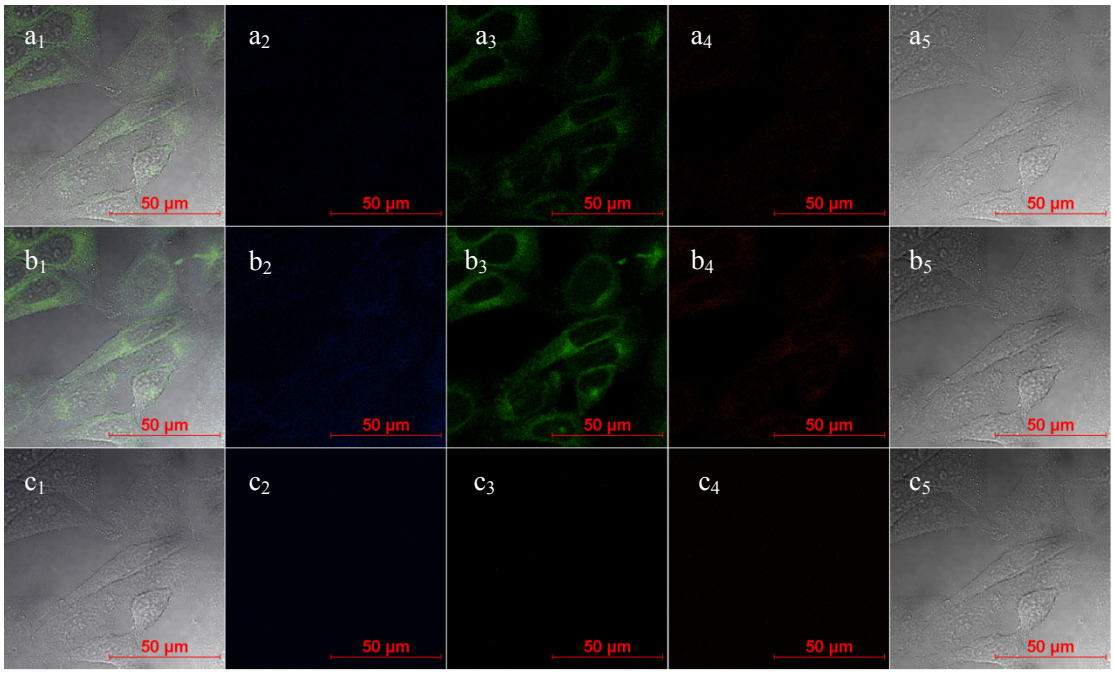


Figure S9: Confocal fluorescence imaging of cervical cancer (HeLa) cells 37 °C, 15 minutes incubation with the addition of 100 µM of **BNP**, 5 eq $CuCl_2$, 1% DMSO, λ_{ex}= 405 nm (a), 488 nm (b) and 543 nm (c), band-pass filtered at 420-480 nm (a_2, b_2, c_2), long-pass filtered at 515 nm (a_3, b_3, c_3) and long-pass filtered at 605 nm (a_4, b_4, c_4), respectively. (a_1), (b_1) and (c_1) are overlaid images of (a_2)-(a_5), (b_2)-(b_5)

and (c_2)-(c_5) micrographs respectively.

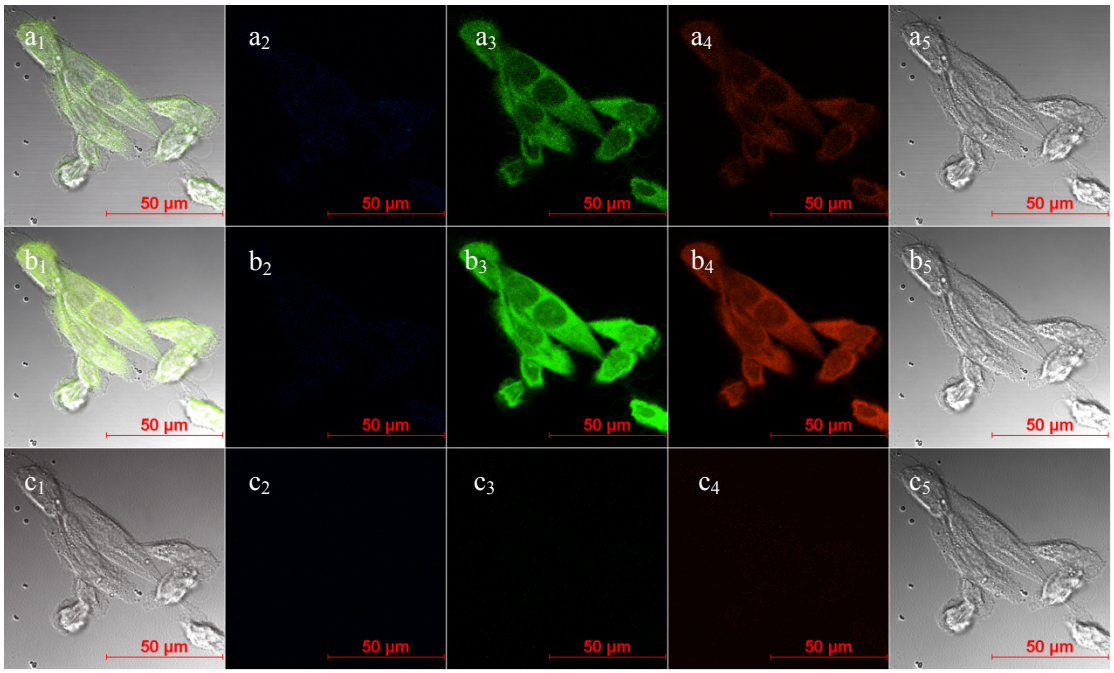
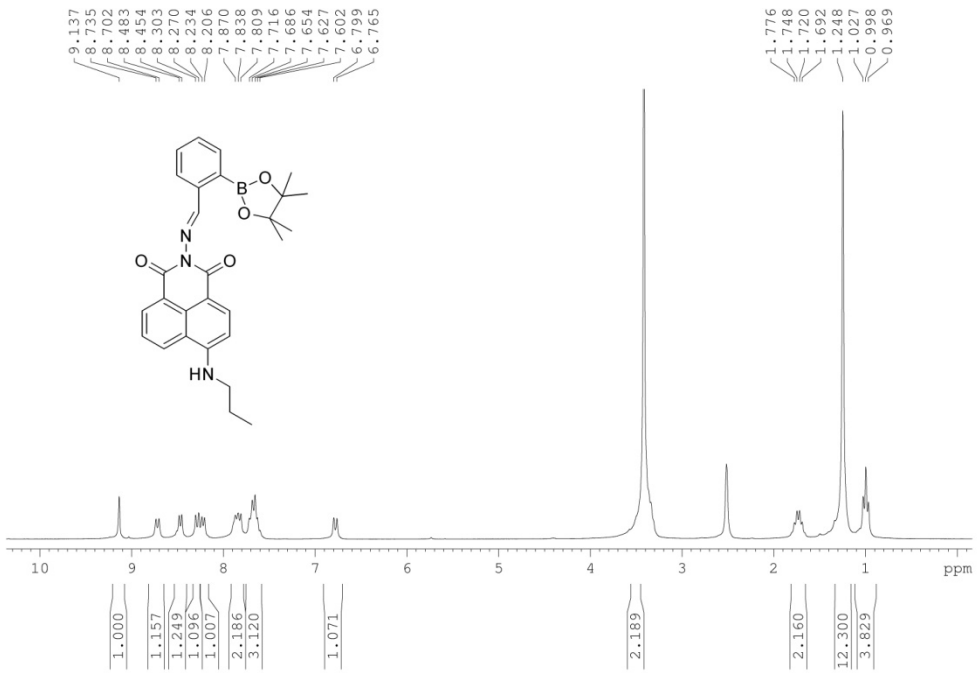
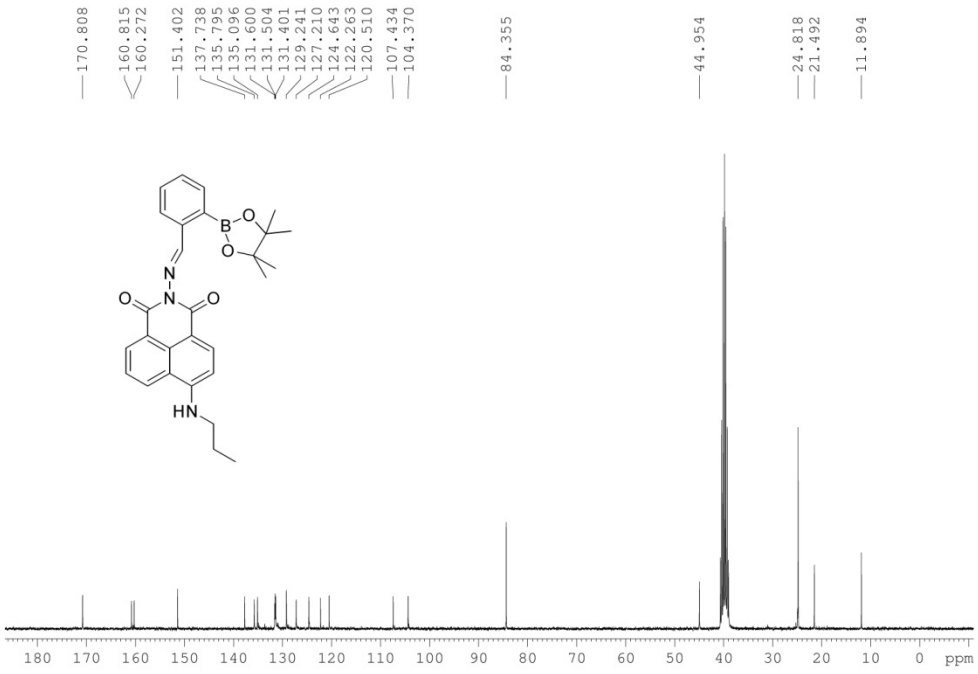
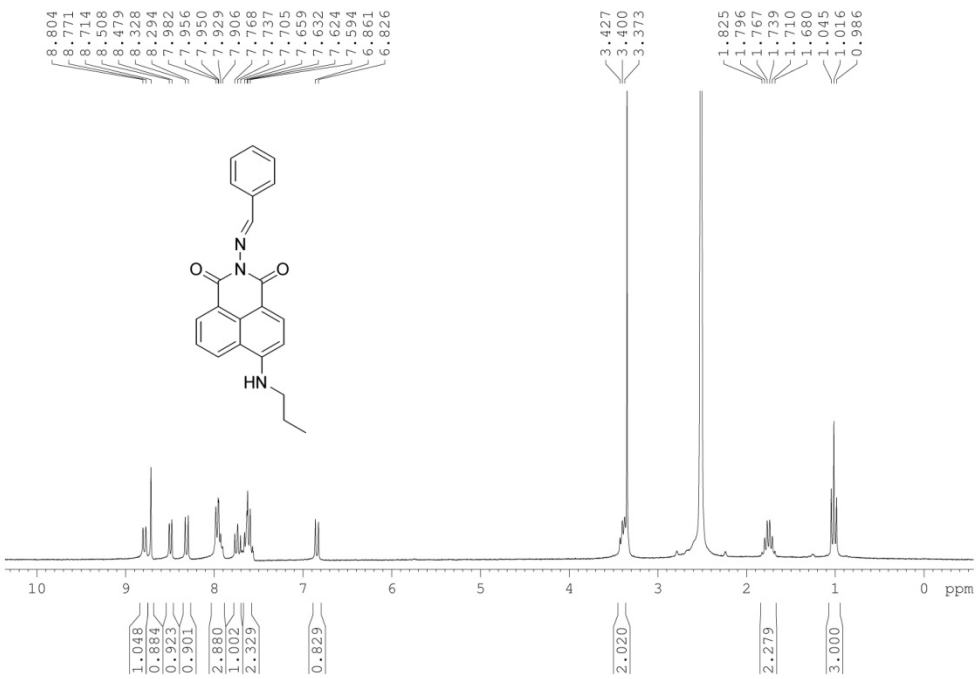


Figure S10: Confocal fluorescence imaging of prostate cancer (PC-3) cells 37 ^{o}C, 15 minutes incubation with the addition of 100 µM of **BNP**, 1% DMSO, λ_{ex}= 405 nm (a), 488 nm (b) and 543 nm (c), band-pass filtered at 420 -480 nm (a_2, b_2, c_2), long-pass filtered at 515 nm (a_3, b_3, c_3) and long-pass filtered at 605 nm (a_4, b_4, c_4), respectively. (a_1), (b_1) and (c_1) are overlaid images of (a_2)-(a_5), (b_2)-(b_5) and (c_2)-(c_5) micrographs respectively.

10. ^{1}H NMR and ^{13}C NMR spectra



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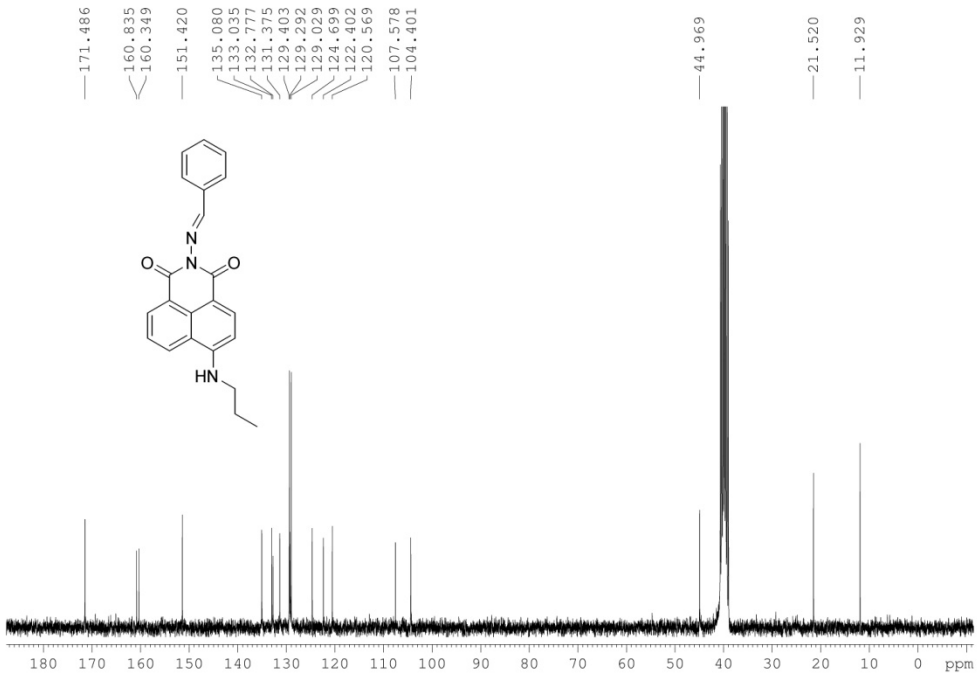
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150
140
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120
110
100
90
80
70
60
50
40
30
20
10
0
ppm

Figure S11. ^{1}H NMR and ^{13}C NMR spectra of BNP and control compounds 2, 3.