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Supporting Information

Development of Photoactivated Fluorescent *N*-Hydroxyoxindoles and Their Application for Cell-Selective Imaging

Jinping Lai, An Yu, Letao Yang, Yixiao Zhang, Birju P. Shah, and Ki Bum Lee*^[a]

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MATERIALS

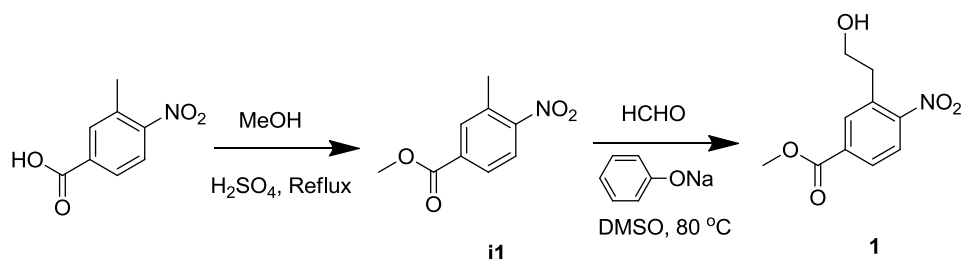
3-methyl-4-nitrobenzoic acid, 4-methyl-3-nitrobenzoic acid, methyl 4-amino-2-methoxybenzoate, 4-bromo-2-methylaniline, *N*-bromosuccinimide, paraformaldehyde, 3-hydroxy-3-methyl-2-butanone, malononitrile, 4-methyl-2-nitroanisole, 4-methyl-3-nitrophenol, 2-*tert*-butylaniline, tetrabutylammonium tribromide, iodomethane, sodium phenoxide, sodium perborate tetrahydrate, copper(I) cyanide, [1,1'-Bis(diphenylphosphino)ferrocene] dichloropalladium (Pd(dppf)Cl₂), dimethylzinc solution (2.0 M in toluene), diisobutylaluminum hydride (DIBAL, 1.0 M in THF), manganese(IV) oxide, PBS (pH7.4, 10 mM), these chemicals were purchased from Sigma-Aldrich. All chemicals were used as received, without any further purification. Solvents for NMR analysis (Cambridge Isotope Laboratories) were used as received.

METHODS

UV-vis absorption spectra were recorded on a Varian Cary 50 spectrophotometer. Fluorescence spectra were recorded on a Varian Cary Eclipse fluorescence spectrophotometer. ¹H NMR and ¹³C NMR was acquired on Varian 300, 400 or 500MHz NMR spectrometer. ESI-MS was collected on Finnigan LCQTM DUO LC/MS spectrometer. HPLC analysis was performed on Agilent 1100 series HPLC system. Luminescence digital photographs were taken with a Nikon D3000 camera. Cell fluorescence images were acquired on Nikon eclipse Ti inverted epifluorescence microscope with a Nikon Lens Light C-HGFI 130 W lamp. Confocal imaging was done using a Zeiss LSM 710-META confocal microscope. The photolysis of various ONPEs was carried out by exposure of ONPEs solution (methanol/PBS, 3:1 v/v) to a UV-lamp with 254 nm irradiation (3 mW·cm⁻²).

SYNTHESIS

I. Synthesis of ONPE 1



Methyl 3-methyl-4-nitrobenzoate (i1). To a solution of 3-methyl-4-nitrobenzoic acid (10 g, 55 mmol) in 100 mL methanol, concentrated H₂SO₄ (2 mL) was added dropwise and the solution was refluxed for 4 h. After cooling to room temperature, the solution was concentrated to 10 mL under vacuum, diluted with water (100 mL) and extracted with EtOAc (3×100 mL). The organic layer was washed with saturated NaHCO₃ and brine (100 mL each), dried over MgSO₄, filtered and concentrated to obtain **i1** as a pale yellow solid (96%).

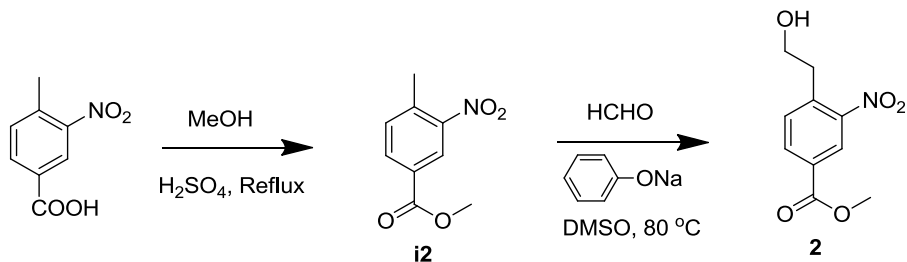
¹H-NMR (300 MHz, CDCl₃): δ8.03 (m, 1H), 7.97 (m, 2H), 3.96 (s, 3H), 2.62 (s, 3H) ppm.

Methyl 3-(2-hydroxyethyl)-4-nitrobenzoate (1).^{S1} To a solution of **i1** (2.92 g, 15 mmol) in DMSO (8 mL) was added paraformaldehyde (0.31 g, 10 mmol) and sodium phenoxide (25 mg). After stirring at 80°C for 3 h in dark, the reaction was diluted with water (100 mL) and extracted with EtOAc (3×100 mL). The organic layer was dried over MgSO₄, filtered, and concentrated. The crude product was purified by flash chromatography on silica gel (EtOAc: DCM= 1: 3, v/v) to get **1** as a light yellow solid (28%).

¹H NMR (300 MHz, CDCl₃): δ8.12 (d, *J* = 1.8 Hz, 1H), 8.03 (dd, *J* = 2.1, 8.4 Hz, 1H), 7.51 (d, *J* = 8.1 Hz, 1H), 3.97 (m, 5H), 3.18 (t, *J* = 6 Hz, 2H) ppm.

¹³C NMR (125 MHz, CDCl₃): δ165.44, 152.68, 134.16, 134.07, 133.93, 128.91, 124.91, 62.69, 52.99, 35.90 ppm.

II. Synthesis of ONPE 2



Methyl 4-methyl-3-nitrobenzoate (i2). To a solution of 4-methyl-3-nitrobenzoic acid (10 g, 55 mmol) in 100 mL methanol, concentrated H₂SO₄ (2 mL) was added dropwise and was refluxed for 4 h. After cooling to room temperature, the solution was concentrated to 10 mL under vacuum, and was diluted with water (100 mL) and extracted with EtOAc (3×100 mL). The organic layer was washed with saturated

NaHCO₃ and brine (100 mL each), dried over MgSO₄, filtered and concentrated to get **i2** as a pale yellow solid (95%).

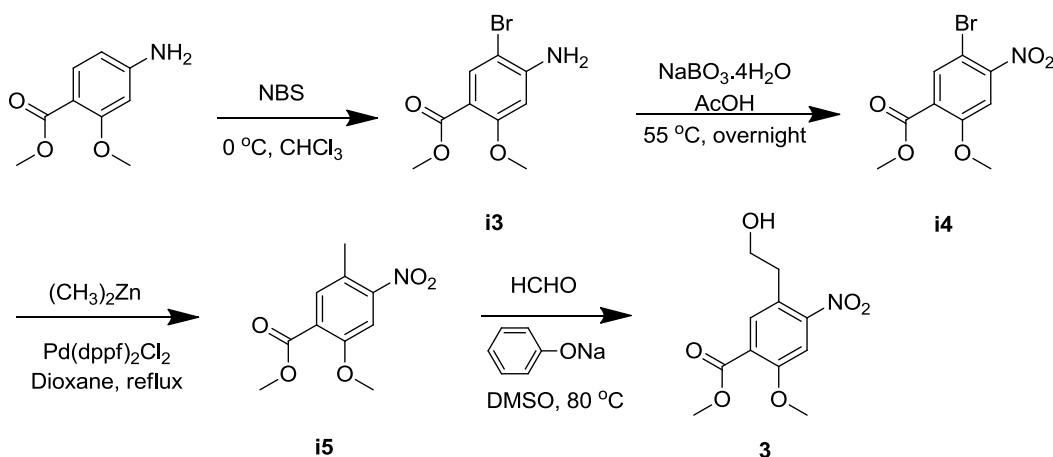
¹H NMR (300 MHz, CDCl₃): δ 8.60 (d, *J* = 1.8 Hz, 1H), 8.14 (dd, *J* = 1.8, 8.1 Hz, 1H), 7.44 (dd, *J* = 0.6, 8.1 Hz, 1H), 3.96 (s, 3H), 2.66 (s, 3H) ppm.

Methyl 4-(2-hydroxyethyl)-3-nitrobenzoate (2).^{S1} To a solution of **i2** (3 g, 15 mmol) in DMSO (8 mL) was added paraformaldehyde (0.31 g, 10 mmol) and sodium phenoxide (25 mg). After stirring at 80 °C for 3 h in dark, the reaction was diluted with water (100 mL) and extracted with EtOAc (3×100 mL). The organic layer was dried over MgSO₄, filtered, and concentrated. The crude product was purified by flash chromatography on silica gel (EtOAc : DCM = 1 : 3, v/v) to get **i1** as a light yellow solid. (24%)

¹H NMR (300 MHz, CDCl₃): δ 8.52 (d, *J* = 1.8 Hz, 1H), 8.16 (dd, *J* = 1.8, 8.1 Hz, 1H), 7.54 (d, *J* = 8.1 Hz), 3.96 (m, 5H), 3.20 (t, *J* = 6.3, 2H) ppm. (26%)

¹³C NMR (75 MHz, CDCl₃): δ 165.03, 149.74, 138.75, 133.26, 133.15, 129.83, 125.84, 62.23, 52.71, 36.09 ppm.

III. Synthesis of ONPE 3



Methyl 4-amino-5-bromo-2-methoxybenzoate (i3). To a solution of methyl 4-amino-2-methoxybenzoate (1.81 g, 10 mmol) in 100 mL CHCl₃ at 0 °C was slowly added NBS (1.78g, 10 mmol) under stirring in 1 h. The reaction was stirred at 0 °C for another 30 min. Thereafter, water (100 mL) was added to quench the reaction. The organic layer was collected and washed with water (100 mL), dried over MgSO₄, filtered and concentrated to obtain the crude compound, which was further purified *via* a flash column chromatography on silica gel using the indicated eluent (EtOAc : DCM = 1 : 4, v/v) to get the **i3** (73%).

¹H NMR (400 MHz, CDCl₃): δ 7.98 (s, 1H), 6.29 (s, 1H), 4.49 (s, 2H), 3.85 (s, 3H), 3.83 (s, 3H) ppm.

Methyl 5-bromo-2-methoxy-4-nitrobenzoate (i4). To a solution of **i3** (1.04 g, 4 mmol) in acetic acid (50 mL) was added NaBO₃·4H₂O 3.07 g, 20 mmol) and was stirred at 55 °C for overnight. After cooling to room temperature, the solution was neutralized with saturated NaHCO₃. The resulting solution was extracted with EtOAc (3 × 50 mL) and the organic layer was dried over MgSO₄, filtered and concentrated. A flash column chromatography on silica gel using DCM as eluent afforded the purified product **i4** (52%).

^1H NMR (300 MHz, CDCl_3): δ 8.10 (s, 1H), 7.42 (s, 1H), 3.96 (s, 3H), 3.93 (s, 3H)ppm.

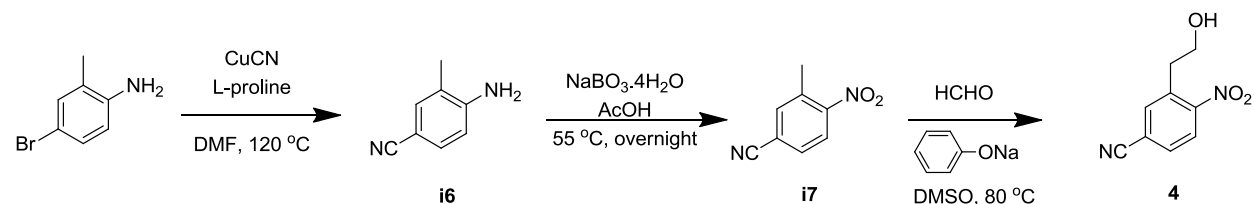
Methyl 2-methoxy-5-methyl-4-nitrobenzoate (i5).^{S2} [ENREF 2](#) To a solution of **i4** (67 mg, 0.3 mmol) and $\text{Pd}(\text{dppf})\text{Cl}_2$ (3 mg) in dry dioxane (1 mL) was added 350 μL of dimethylzinc solution (2.0 M in toluene) under argon. The mixture was refluxed in dark for 3 h and quenched by adding methanol (1 mL). The solution was diluted with 10 mL DCM, washed with HCl (1 mM), water and dried over MgSO_4 . The product was purified by flash chromatography on silica gel (DCM) to obtain **i5** (90%).

^1H NMR (300 MHz, CDCl_3): δ 7.72 (s, 1H), 7.56 (s, 1H), 3.95 (s, 3H), 3.93 (s, 3H), 2.54 (s, 3H)ppm.

Methyl 5-(2-hydroxyethyl)-2-methoxy-4-nitrobenzoate (3).^{S1} To a solution of **i5** (60 mg, 15 mmol) in DMSO (8 mL) was added paraformaldehyde (0.31 g, 10 mmol) and sodium phenoxide (25 mg). After stirring at 80 °C for 3 h in dark, the reaction was diluted with water (100 mL) and extracted with EtOAc (3 \times 100 mL). The organic layer was dried over MgSO_4 , filtered, and concentrated. The product was purified by flash chromatography on silica gel (EtOAc : DCM= 1: 3, v/v) to get **i1** (23%).

^1H NMR (300 MHz, CDCl_3): δ 7.81 (s, 1H), 7.50 (s, 1H), 3.95 (m, 8H), 3.11 (t, J = 5.4 Hz, 2H)ppm.

IV. Synthesis of ONPE 4



4-amino-3-methylbenzonitrile (i6).^{S3} [ENREF 1](#) To a solution of 4-bromo-2-methylaniline (500 mg, 2.7 mmol) in DMF (3 mL) was added CuCN (480 mg, 5.4 mmol) and L-proline (115 mg, 1 mmol). The reaction mixture was stirred under argon at 120 °C for 24 h. After cooling to room temperature, the reaction mixture was diluted with EtOAc (30 mL) and washed with water (3 \times 10 mL). The organic layer was dried over MgSO_4 , filtered and concentrated. A flash column chromatography on silica gel using the indicated eluent (EtOAc : hexane = 1 : 2, v/v) afforded the purified product **i6** as a purple solid. (33%)

^1H NMR (500 MHz, CDCl_3): δ 7.31 (m, 1H), 6.63 (d, J = 8.5 Hz, 1H), 4.08 (s, 2H), 2.15 (s, 3H) ppm.

^{13}C NMR (125 MHz, CDCl_3): δ 151.71, 136.71, 134.86, 130.92, 125.47, 117.10, 116.85, 20.13 ppm.

3-methyl-4-nitrobenzonitrile (i7). To a solution of **i6** (30 mg, 0.227 mmol) in acetic acid (3 mL) was added $\text{NaBO}_3 \cdot 4\text{H}_2\text{O}$ (0.21 g, 1.36 mmol) and was stirred at 55 °C overnight. After cooling to room temperature, the reaction was quenched with water (10 mL) and the solution was neutralized with saturated NaHCO_3 . The resulting solution was extracted with EtOAc (3 \times 100 mL) and the organic layer was dried over MgSO_4 , filtered, concentrated, and purified *via* flash column chromatography on silica gel using the indicated eluent (EtOAc : hexane = 1 : 2, v/v) afforded **i7** as an orange solid (67%).

^1H NMR (500 MHz, CDCl_3): δ 8.00 (d, J = 8.5 Hz, 1H), 7.65-7.68 (m, 2H), 2.62 (s, 6H) ppm.

^{13}C NMR (125 MHz, CDCl_3): δ 148.92, 134.42, 131.82, 122.26, 120.46, 114.44, 100.53, 17.23 ppm.

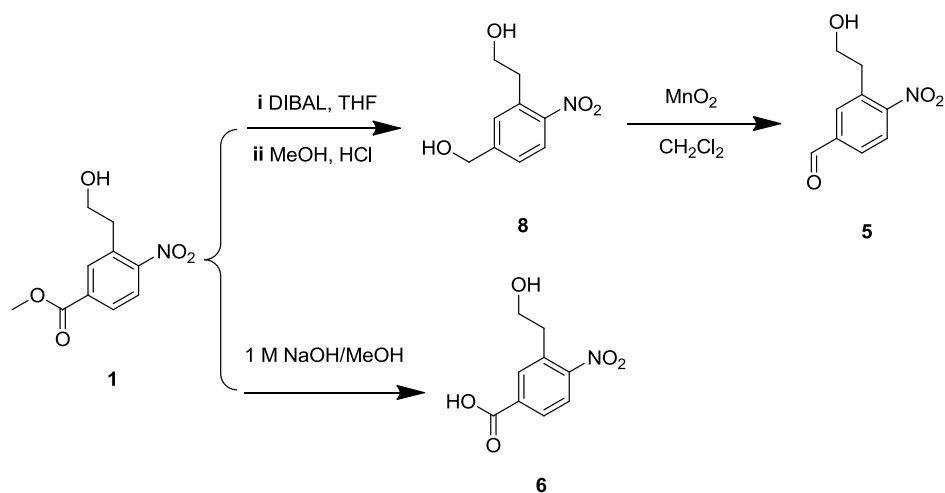
3-(2-hydroxyethyl)-4-nitrobenzonitrile (4).^{S1} To a solution of **i7** (31 mg, 0.19 mmol) in DMSO (1 mL) was added paraformaldehyde (3.6 mg, 0.12 mmol) and sodium phenoxide (1 mg). After stirring at 80 °C

for 3 h in dark, the reaction was diluted with water (15 mL) and extracted with EtOAc (3 × 20 mL). Combined organic layer was dried over MgSO₄ and concentrated. A flash column chromatography on silica gel (EtOAc : hexane = 1:2, v/v) was used to purify the product to obtain **4** as a yellow solid (30%).

¹H NMR (500 MHz, CDCl₃): δ 7.96 (d, *J* = 8.5 Hz, 1H), 7.79 (d, *J* = 1.5 Hz, 1H), 7.70 (dd, *J* = 1.5, 8.5 Hz, 1H), 3.97 (t, *J* = 6.0 Hz, 3H), 3.17 (t, *J* = 6.0 Hz, 3H) ppm.

¹³C NMR (125 MHz, CDCl₃): δ 152.34, 136.90, 135.48, 131.37, 125.46, 117.09, 116.72, 62.11, 35.43 ppm.

V. Synthesis of ONPEs **5**, **6** and **8**



2-(5-(hydroxymethyl)-2-nitrophenyl)ethan-1-ol (8**)**.^{S4} A solution of **1** (0.53 g, 0.37 mmol) in anhydrous THF (2 mL) at 0 °C was added 5 mL of DIBAL (1.0 M in THF) dropwise under argon. After stirring at 0 °C for 30 min, the reaction was warm to room temperature and stirred for another 3 h in dark. Thereafter, the reaction was quenched with methanol (10 mL), diluted with HCl (0.1M, 50 mL), and extracted with EtOAc (3×100 mL). Combined organic layer was dried over Na₂SO₄, filtered and concentrated. Purification by flash column chromatography on silica gel (EtOAc : DCM = 3:1, v/v) gave **8** as an orange solid (66%).

¹H NMR (300 MHz, CDCl₃): δ 7.97 (d, *J* = 8.1 Hz, 1H), 7.44 (s, 1H), 7.39 (d, *J* = 8.4 Hz, 1H), 4.80 (d, *J* = 5.1 Hz, 2H), 3.98 (dd, *J* = 3.3, 11.4 Hz, 2H), 3.21 (t, *J* = 6.6 Hz, 2H) ppm.

3-(2-hydroxyethyl)-4-nitrobenzaldehyde (5**)**. To a solution of **8** (100 mg, 0.51 mmol) in anhydrous DCM (3 mL) was added MnO₂ (227 mg, 2.61 mmol). The reaction mixture was stirred at room temperature overnight in dark. Thereafter, the reaction was diluted with EtOAc (20 mL), and filtered through celite to remove the MnO₂. The solution was concentrated and the crude compound was purified *via* a flash column chromatography on silica gel (EtOAc : DCM = 1 : 3, v/v) to give **5** (78%).

¹H NMR (500 MHz, CDCl₃): δ 10.1 (s, 1H), 8.00 (d, *J* = 8.5 Hz, 1H), 7.96 (s, 1H), 7.89 (d, *J* = 8.5 Hz, 1H), 3.99 (t, *J* = 5.0 Hz, 2H), 3.21 (t, *J* = 5.0 Hz, 2H) ppm.

¹³C NMR (125 MHz, CDCl₃): δ 190.63, 153.41, 138.66, 134.20, 128.48, 125.52, 62.47, 35.72 ppm.

3-(2-hydroxyethyl)-4-nitrobenzoic acid (6**)**. To a solution of **1** (0.45 g, 2 mmol) in methanol (10 mL) was added 0.5 mL of NaOH aqueous solution (1 M). The reaction mixture was stirred at room

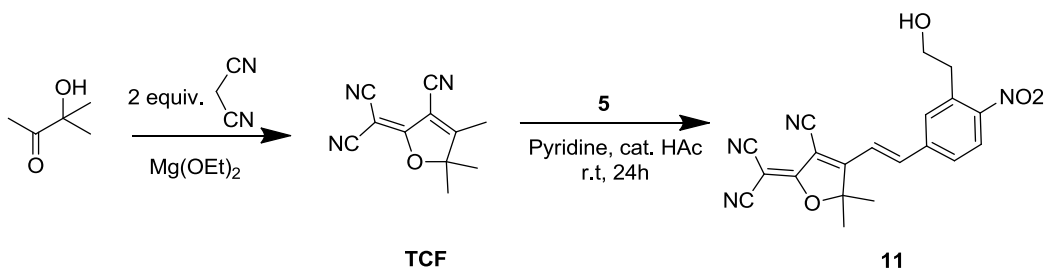
^1H NMR (300 MHz, CDCl_3): δ 7.40 (d, $J = 2.7$ Hz, 1H), 7.16 (d, $J = 8.7$ Hz, 1H), 7.00 (dd, $J = 3, 8.4$ Hz, 1H), 3.80 (s, 3H), 2.45 (s, 3H)ppm.

2-(4-methoxy-2-nitrophenyl)ethanol (10).^{S1} To a solution of **i8** (2.50 g, 15 mmol) in DMSO (8 mL) was added paraformaldehyde (0.31 g, 10 mmol) and sodium phenoxide (25 mg). After stirring at 80°C for 3 h in dark, the reaction was diluted with water (100 mL) and extracted with EtOAc (3×100 mL). The organic layer was dried over MgSO_4 , filtered, and concentrated. The product was purified by flash chromatography on silica gel (EtOAc : DCM= 1: 1, v/v) to obtain **10** (22%).

^1H NMR (300 MHz, CDCl_3): δ 7.41 (d, $J = 2.7$ Hz, 1H), 7.30 (d, $J = 8.7$ Hz, 1H), 7.08 (dd, $J = 2.8, 8.7$ Hz, 1H), 3.87-3.84 (br, 5H), 3.06 (t, $J = 6.6$ Hz, 2H), 2.52 (s, 1H) ppm.

^{13}C NMR (75 MHz, CDCl_3): δ 158.48, 149.97, 133.61, 125.64, 119.70, 109.40, 62.66, 55.80, 53.46 ppm.

VIII. Synthesis of TCF-ONPE derivative 11



2-(3-cyano-4,5,5-trimethylfuran-2(5H)-ylidene)malononitrile (TCF). The TCF was synthesized *via* a reported approach.^{S5} Briefly, to a solution of 3-hydroxy-3-methyl-2-butanone (1.0 g, 10 mmol) in absolute ethanol was added malononitrile (1.33 g, 20 mmol) and lithium (1 mg). The solution was stirred at 75 °C overnight in dark. After cooling to room temperature, the solution was concentrated. The residue was collected and washed by water, and was recrystallized from ethanol to obtain **TCF** (87%).

^1H NMR (300 MHz, CDCl_3): δ 2.38 (s, 3H), 1.65 (s, 6H) ppm.

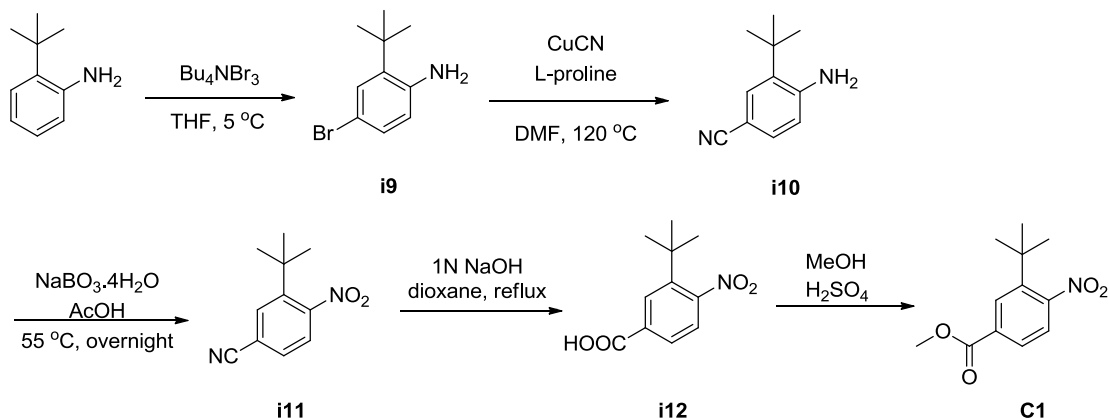
(E)-2-(3-cyano-4-(3-(2-hydroxyethyl)-4-nitrostyryl)-5,5-dimethylfuran-2(5H)-ylidene)malononitrile (11). A solution of **5** (25.4 mg, 0.130 mmol) and **TCF** (38.6 mg, 0.194 mmol) in pyridine (2 mL) was added drops of acetic acid. The mixture was stirred at 40 °C in dark overnight. Thereafter, the reaction mixture was concentrated and was diluted with EtOAc (50 mL). The organic layer was washed with diluted HCl (0.1M, 100 mL) and water (100 mL), dried over MgSO_4 and concentrated. Flash column chromatography on silica gel (EtOAc : hexane = 1: 1, v/v) was used to purify the product. Compound **11** was obtained as an orange solid (15%).

^1H NMR (500 MHz, DMSO): δ 8.00-8.04 (m, 3H), 7.91 (d, $J = 16.5$ Hz, 1H), 7.37 (d, $J = 17$ Hz, 1H), 3.66 (t, $J = 6.0$ Hz, 2H), 3.04 (t, $J = 6.0$ Hz, 2H), 1.813 (s, 3H), 1.810 (s, 3H) ppm.

^{13}C NMR (125 MHz, DMSO): δ 176.8, 174.0, 150.7, 144.1, 138.1, 133.3, 127.7, 124.8, 118.4, 112.4, 111.5, 110.4, 101.5, 99.5, 60.7, 55.2, 35.0, 28.9, 24.9 ppm.

MS (ESI): Calculated 376.1, found 375.1 [M-H]⁻.

VII. Synthesis of C1



4-bromo-2-(tert-butyl)aniline (i9). To a solution of 2-tert-butylaniline (1.49 g, 10 mmol) in THF (20 mL) at 5 °C was added a solution of tetrabutylammonium tribromide (4.38 g, 9.1 mmol) in THF (3 mL) dropwise. After stirring at 4 °C for 30 min, the reaction was quenched with water (10 mL). The mixture was diluted with diethyl ether (200 mL) and washed with saturated NaHCO_3 solution (200 mL) and brine (200 mL). Resulting solution was dried over Na_2SO_4 , filtered and concentrated to give **i9** as brown oil (98%).

$^1\text{H NMR}$ (300 MHz, CDCl_3): δ 7.32 (d, $J = 2.4$ Hz, 1H), 7.12 (dd, $J = 2.4, 8.4$ Hz, 1H), .56 (d, $J = 8.4$ Hz, 1H), 4.11 (s, 2H), 1.40 (s, 9H) ppm.

4-amino-3-(tert-butyl)benzonitrile (i10).^{S3} A solution of CuCN (179 mg, 2 mmol), L-proline (115 mg, 1 mmol) and **9** (228 mg, 1 mmol) in DMF (3 mL) was stirred at 120 °C under argon for 2 days. After cooling to room temperature, the reaction mixture was diluted with EtOAc (15 mL) and washed with water (3×10 mL). The organic layer was dried over Na_2SO_4 filtered and concentrated. A flash column chromatography on silica gel using the indicated eluent (EtOAc : hexane = 1 : 2, v/v) afforded the **i10** (47%).

$^1\text{H NMR}$ (300 MHz, CDCl_3): δ 7.47 (d, $J = 1.8$ Hz, 1H), 7.29 (dd, $J = 1.8, 8.4$ Hz, 1H), 6.61 (d, $J = 8.4$ Hz, 1H), 4.11 (s, 2H), 1.40 (s, 9H) ppm.

3-(tert-butyl)-4-nitrobenzonitrile (i11). The solution of **i10** (81.3 mg, 0.47 mmol) and $\text{NaBO}_3 \cdot 4\text{H}_2\text{O}$ (431 mg, 2.8 mmol) in acetic acid (5 mL) was stirred at 55 °C overnight. After cooling to room temperature, the reaction was quenched with water (10 mL), and neutralized with saturated NaHCO_3 solution, and extracted with EtOAc (3×100 mL). Combined organic layer was dried over MgSO_4 , filtered and concentrated. A flash column chromatography on silica gel using the indicated eluent (EtOAc : hexane = 1 : 2, v/v) afforded the purified product **i11** as an orange solid (66%).

$^1\text{H NMR}$ (500 MHz, CDCl_3): δ 7.87 (d, $J = 2.0$ Hz, 1H), 7.62 (dd, $J = 2.0, 8.5$ Hz, 1H), 7.41 (d, $J = 8.5$ Hz, 1H), 1.42 (s, 9H) ppm.

3-(tert-butyl)-4-nitrobenzoic acid (i12). A mixture of **i11** (63 mg, 0.31 mmol) in 1,4-dioxane (2 mL) and 1 mL of NaOH aqueous solution (1 M) was refluxed overnight. After cooling to room temperature, the mixture was diluted with water (10 mL) and the pH of the solution was adjusted to 1 using HCl solution

(1M). The resulting solution was extracted with EtOAc (3×100 mL). Combined organic layer was washed with brine (100 mL), dried over NaSO₄, filtered and concentrated to get product **i12** (82%).

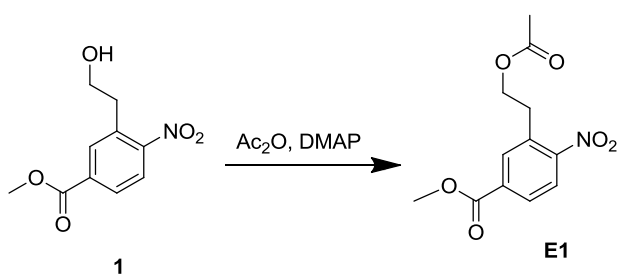
¹H NMR (500 MHz, CDCl₃): δ 8.33 (d, *J* = 1.8 Hz, 1H), 8.00 (dd, *J* = 1.8, 8.1 Hz, 1H), 7.40 (d, *J* = 8.1 Hz, 1H), 1.45 (s, 9H) ppm.

Methyl 3-(tert-butyl)-4-nitrobenzoate (C1). A solution of **i12** (57 mg, 0.26 mmol) in methanol (5 mL) was added few drops of concentrated H₂SO₄ and was refluxed for 5 h. After cooling to room temperature, the reaction mixture was diluted with water (50 mL) and extracted with EtOAc (2 × 50 mL). The organic layer was washed with saturated NaHCO₃ and dried over NaSO₄, filtered and concentrated to get **C1** as a pale yellow solid. (98%)

¹H NMR (500 MHz, CDCl₃): δ 8.24 (d, *J* = 2.0 Hz, 1H), 7.93 (dd, *J* = 2.0, 8.0 Hz, 1H), 7.34 (d, *J* = 8.0 Hz, 1H), 3.94 (s, 3H), 1.41 (s, 9H) ppm.

¹³C NMR (125 MHz, CDCl₃): δ 165.6, 153.5, 141.8, 132.2, 130.6, 128.4, 124.2, 52.8, 36.1, 30.8 ppm.

VIII. Synthesis of E1



To a solution of **1** (0.45 g, 2 mmol) and 10 mg of DMAP in CHCl₃ (10 mL) at 0 °C was added 1 mL of acetic anhydride. The reaction mixture was stirred 0 °C for 30 min and recovered to room temperature for 3 h. Thereafter, the reaction was diluted with water (20 mL), extracted with EtOAc (3 × 100 mL) and washed with brine. The organic layer was dried over Na₂SO₄ and concentrated in vacuum to afford **E1**, no further purification required (93%).

¹H NMR (300 MHz, CDCl₃): δ 7.99 (d, *J* = 1.6 Hz, 2H), 7.90 (d, *J* = 8.1 Hz, 1H), 4.33 (t, *J* = 6.3 Hz, 1H), 3.94 (s, 3H), 3.24 (t, *J* = 6.3 Hz, 1H), 1.98 (s, 3H) ppm.

¹³C NMR (75 MHz, CDCl₃): δ 170.69, 165.02, 152.32, 133.76, 133.01, 128.94, 124.75, 63.47, 52.74, 31.95, 20.72 ppm.

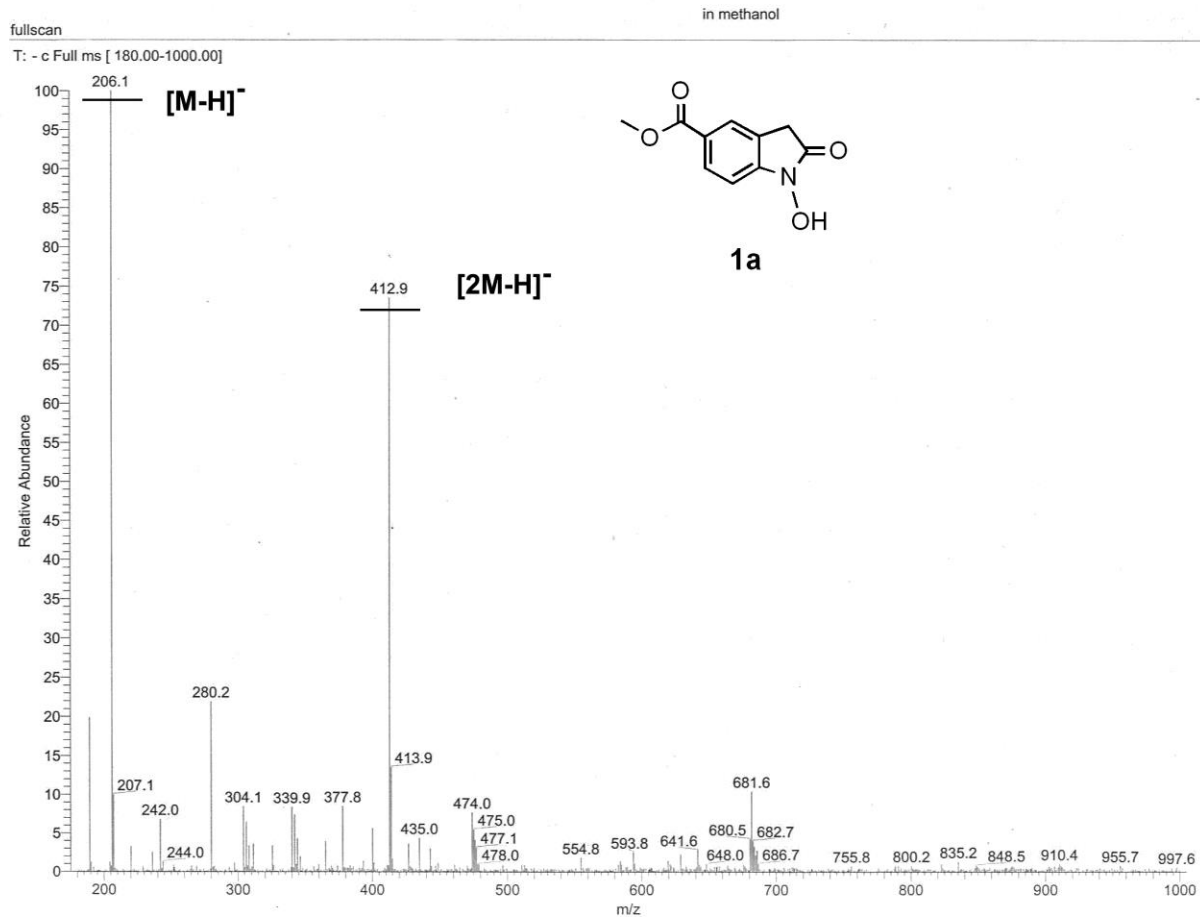


Figure S1. Mass spectrum analysis of the photolysis product of **1**.

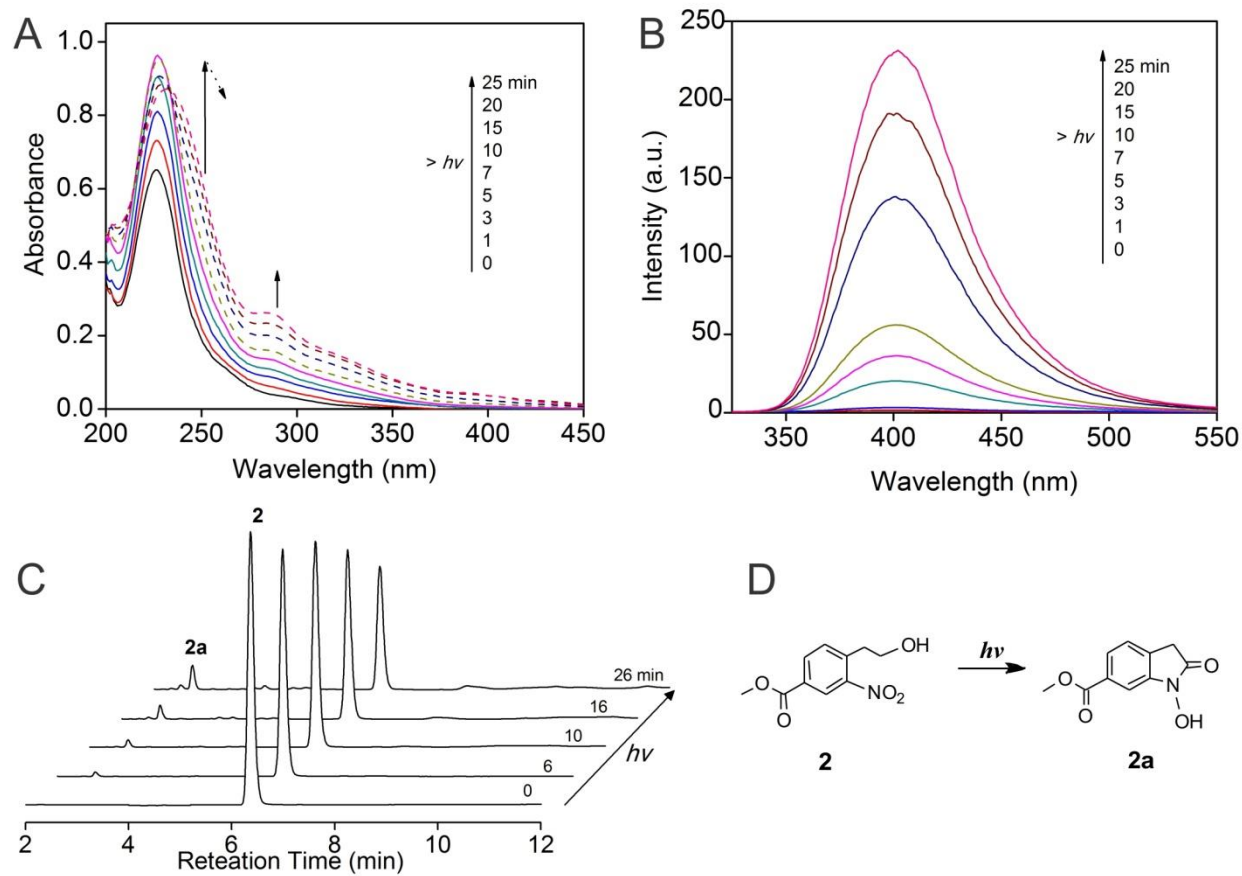


Figure S2. (A) UV-vis absorption spectrum, (B) Fluorescence emission spectrum and (C) HPLC analysis of the photolysis reaction of **2** to **2a** (D). MeOH/PBS (3:1 v/v, pH7.4, 10 mM PBS), [**2**] = 5 μ M.

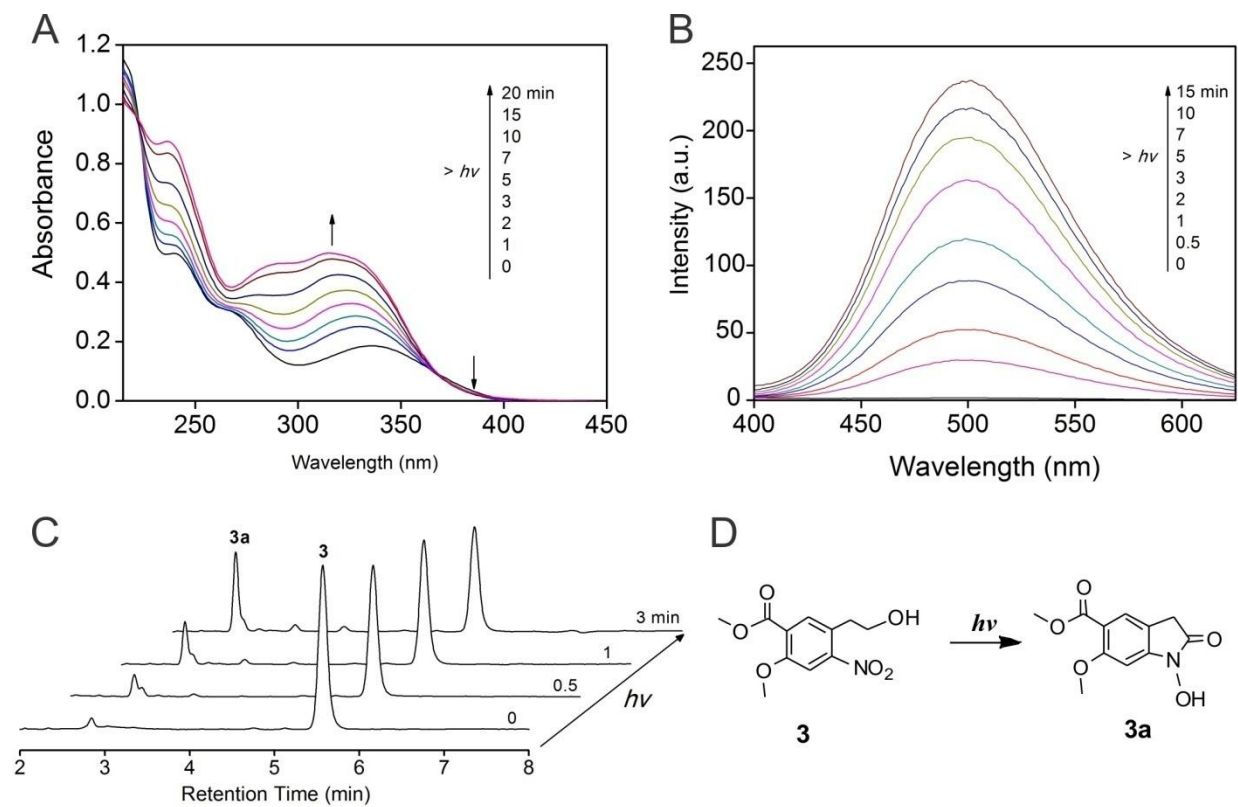


Figure S3. (A) UV-vis absorption spectrum, (B) Fluorescence emission spectrum and (C) HPLC analysis of the photolysis reaction of **3** to **3a** (D). MeOH/PBS (3:1 v/v, pH 7.4, 10 mM PBS), $[3] = 5 \mu\text{M}$.

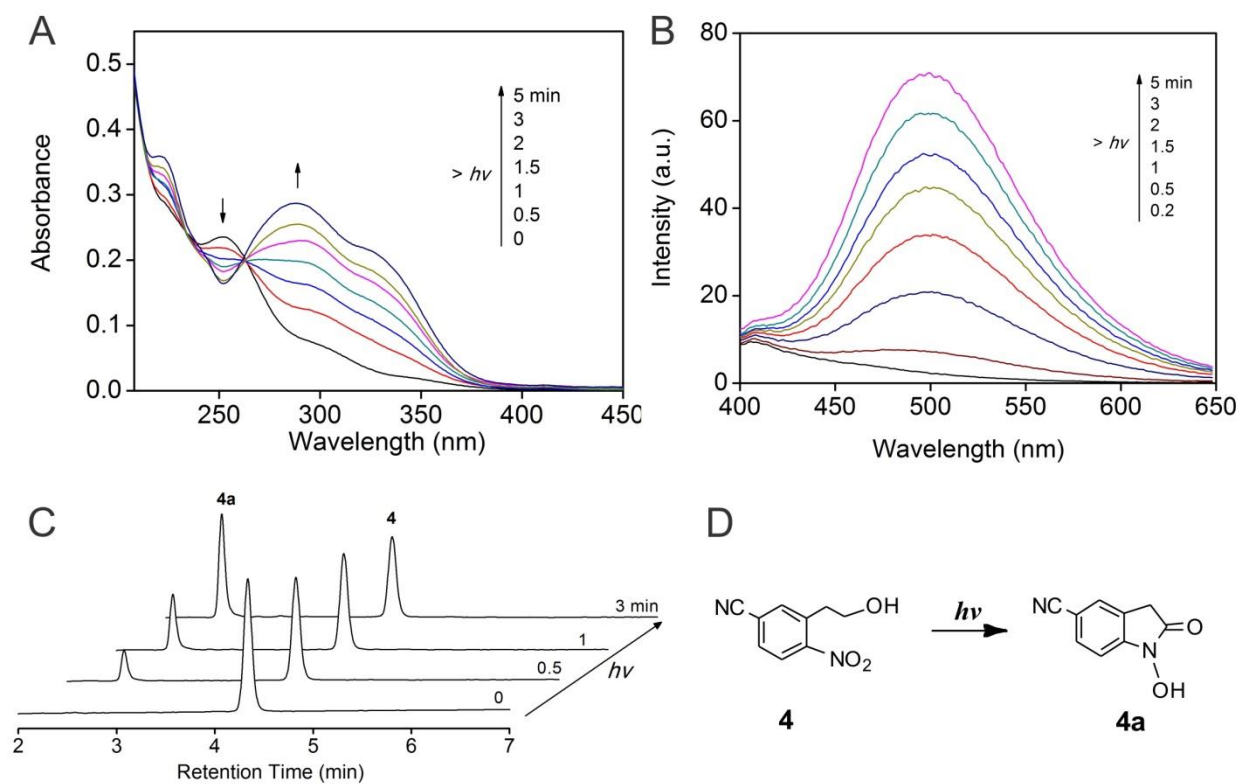


Figure S4. (A) UV-vis absorption spectrum, (B) Fluorescence emission spectrum and (C) HPLC analysis of the photolysis reaction of **4** to **4a**(D). MeOH/PBS (3:1 v/v, pH7.4, 10 mM PBS), [**4**] = 5 μ M.

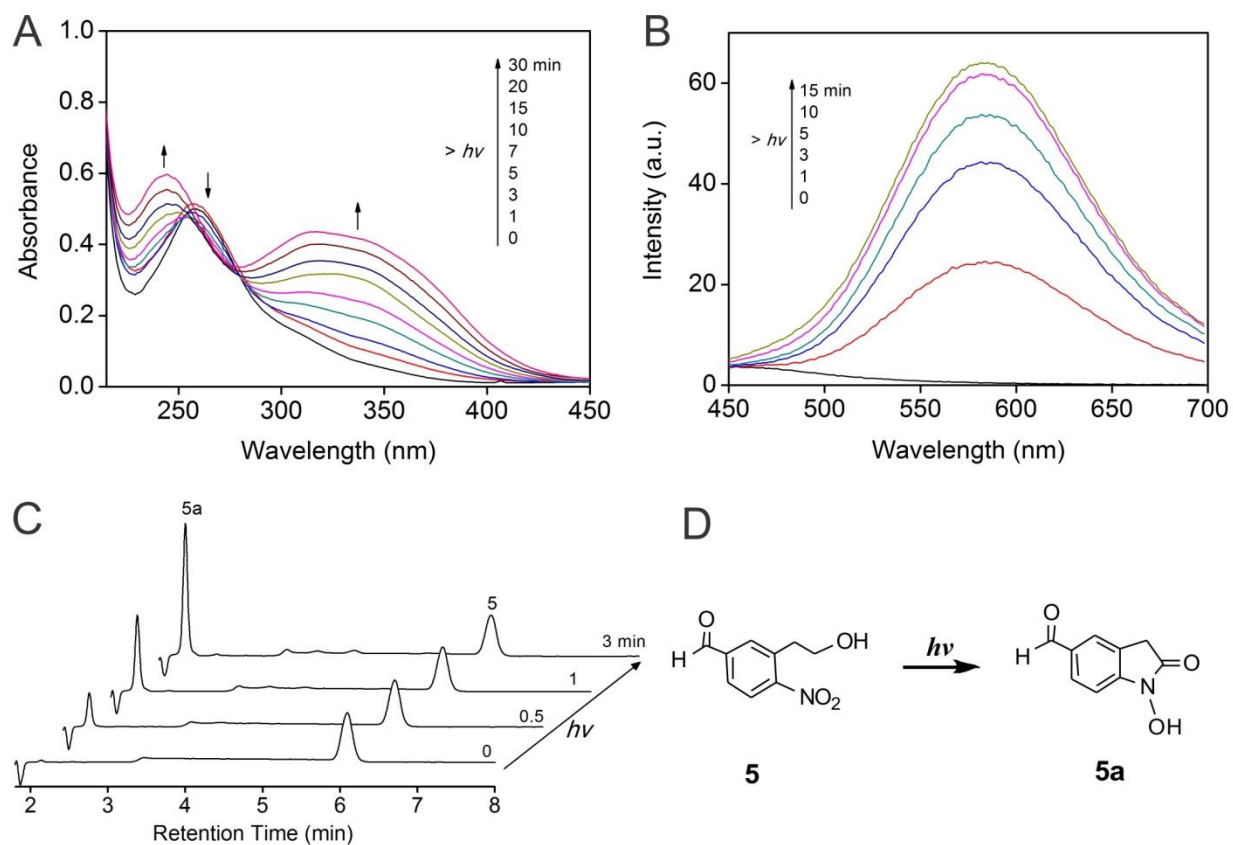


Figure S5. (A) UV-vis absorption spectrum, (B) Fluorescence emission spectrum and (C) HPLC analysis of the photolysis reaction of **5** to **5a**(D). MeOH/PBS (3:1 v/v, pH7.4, 10 mM PBS), [**5**] = 5 μ M.

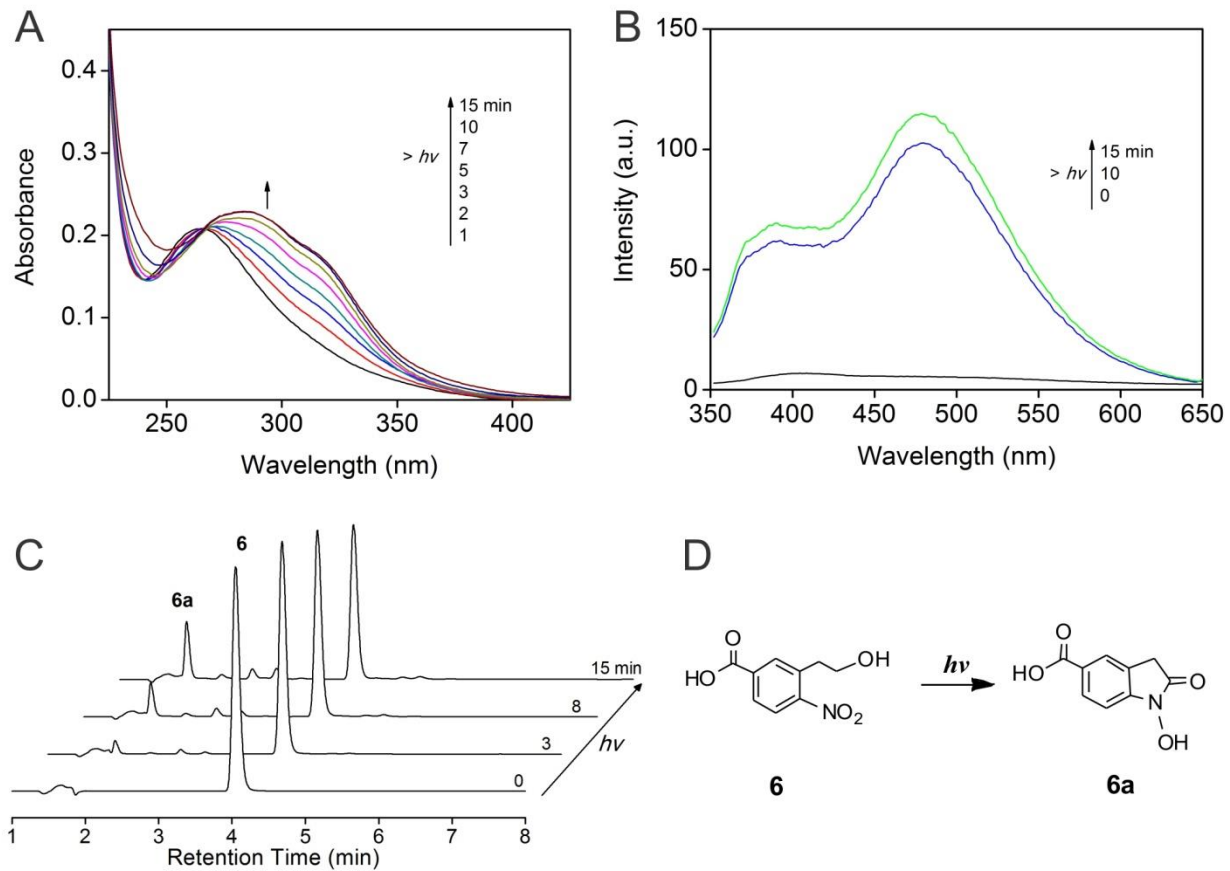


Figure S6. (A) UV-vis absorption spectrum, (B) fluorescence emission spectrum and (C) HPLC analysis of the photolysis reaction of **6** to **6a**(D). MeOH/PBS (3:1 v/v, pH7.4, 10 mM PBS), [**6**] = 5 μ M.

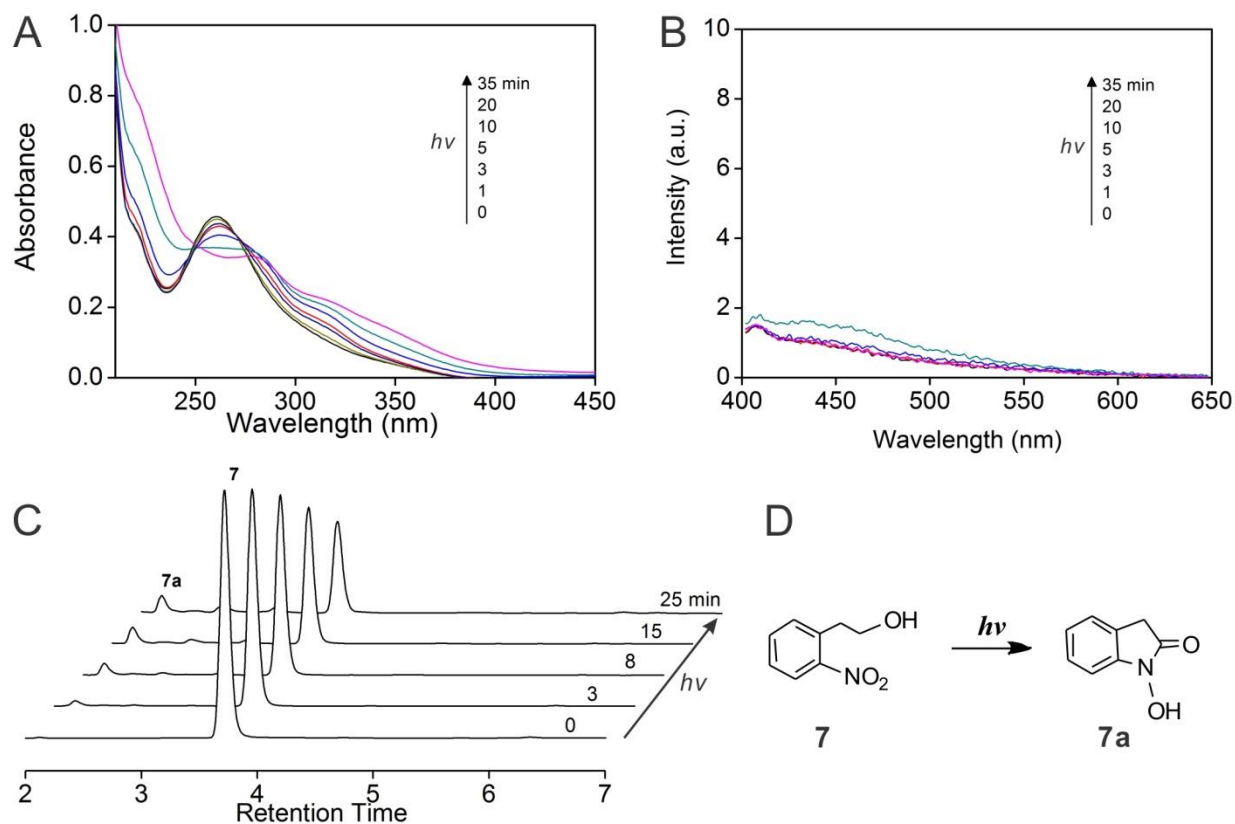


Figure S7. (A) UV-vis absorption spectrum, (B) fluorescence emission spectrum and (C) HPLC analysis of the photolysis reaction of **7** to **7a**(D). MeOH/PBS (3:1 v/v, pH7.4, 10 mM PBS), [**7**] = 5 μ M.

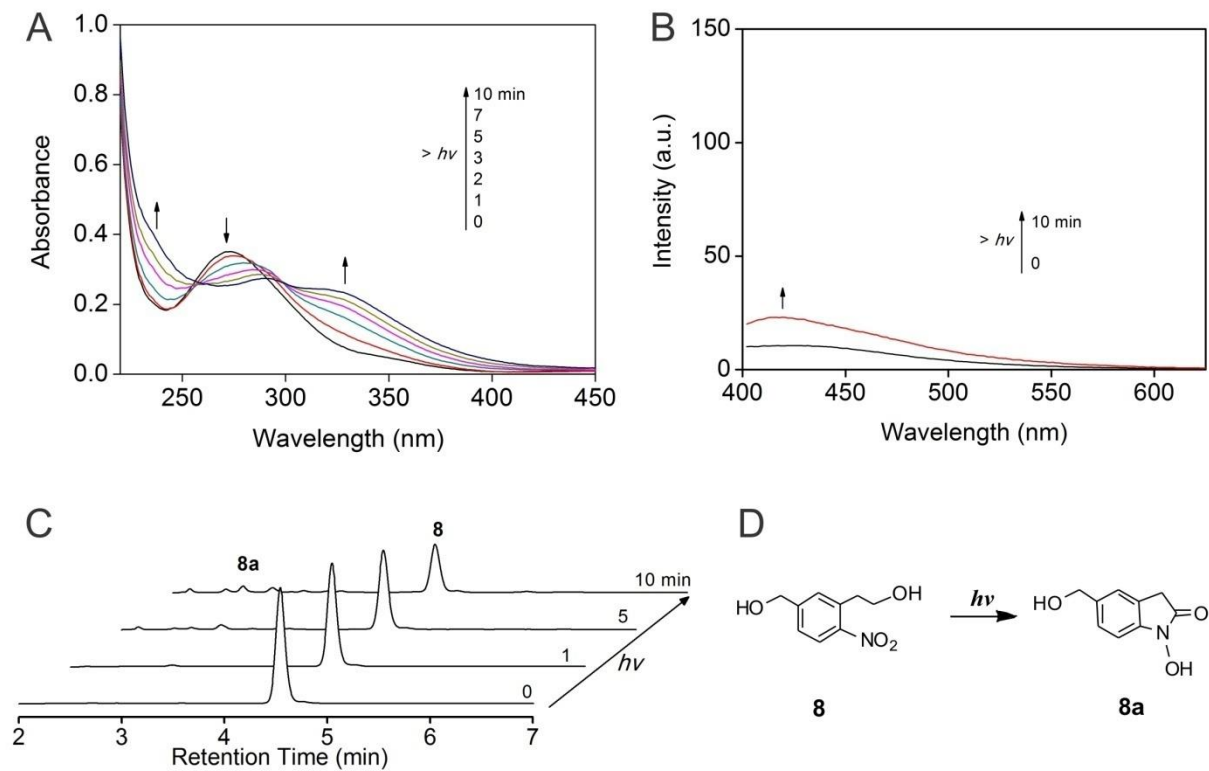


Figure S8. (A) UV-vis absorption spectrum, (B) fluorescence emission spectrum and (C) HPLC analysis of the photolysis reaction of **8** to **8a** (D). MeOH/PBS (3:1 v/v, pH 7.4, 10 mM PBS), [**8**] = 5 μ M.

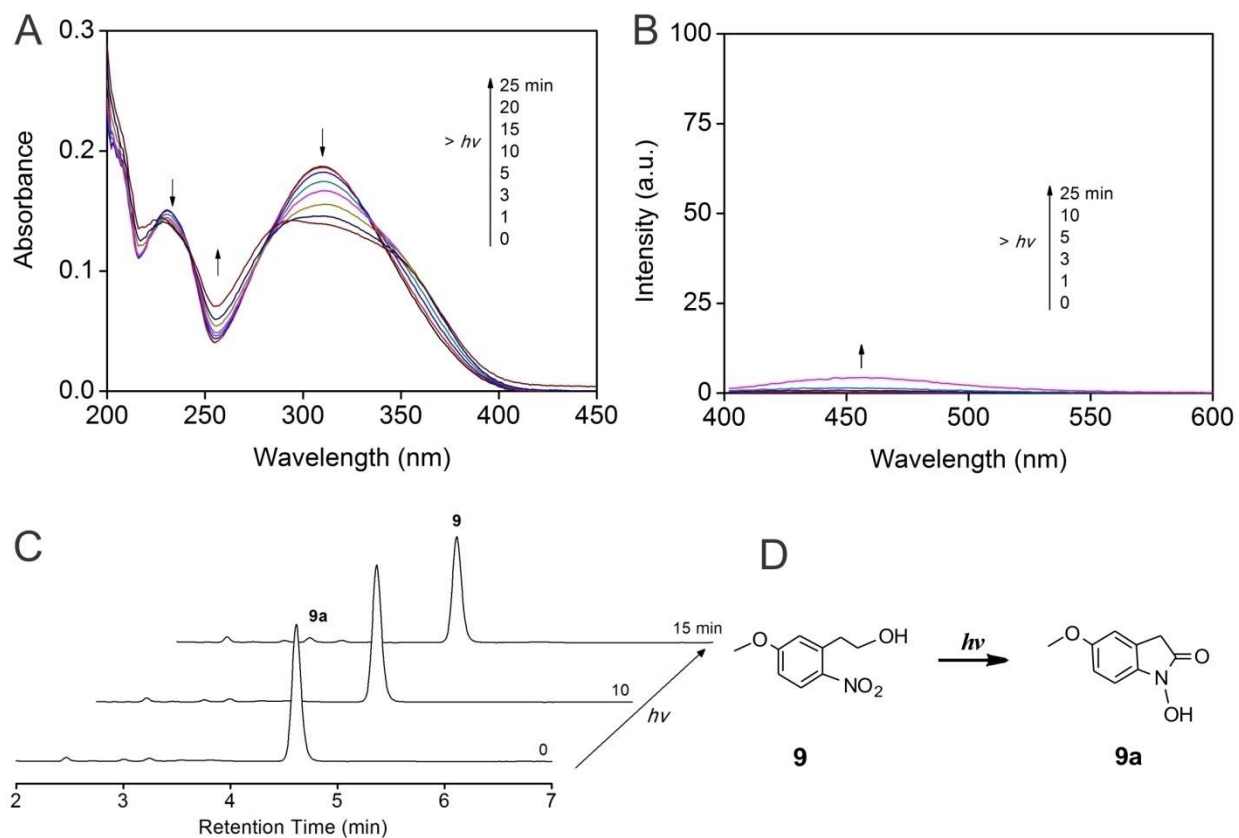


Figure S9. (A) UV-vis absorption spectrum, (B) fluorescence emission spectrum and (C) HPLC analysis of the photolysis reaction of **9** to **9a**(D). MeOH/PBS (3:1 v/v, pH7.4, 10 mM PBS), [**9**] = 5 μ M.

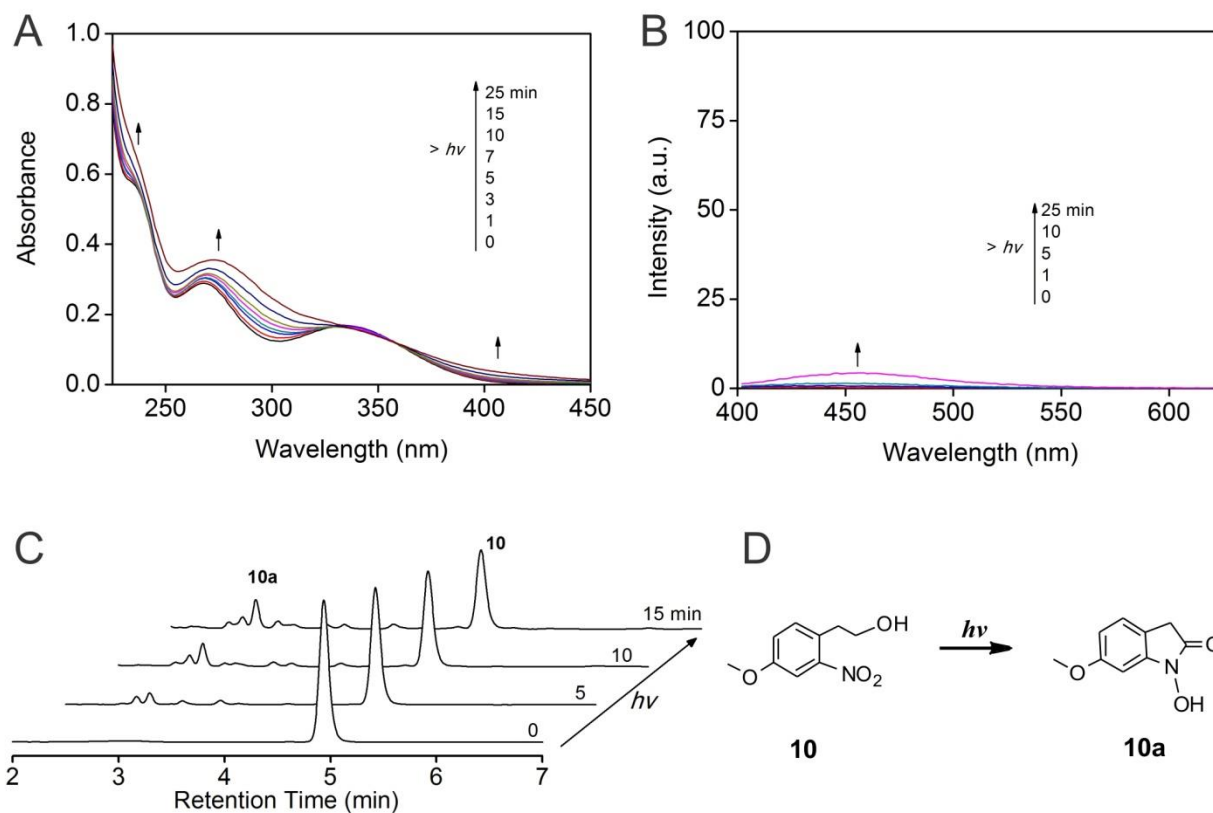


Figure S10. (A) UV-vis absorption spectrum, (B) fluorescence emission spectrum and (C) HPLC analysis of the photolysis reaction of **10** to **10a** (D). MeOH/PBS (3:1 v/v, pH7.4, 10 mM PBS), [**10**] = 5 μ M.

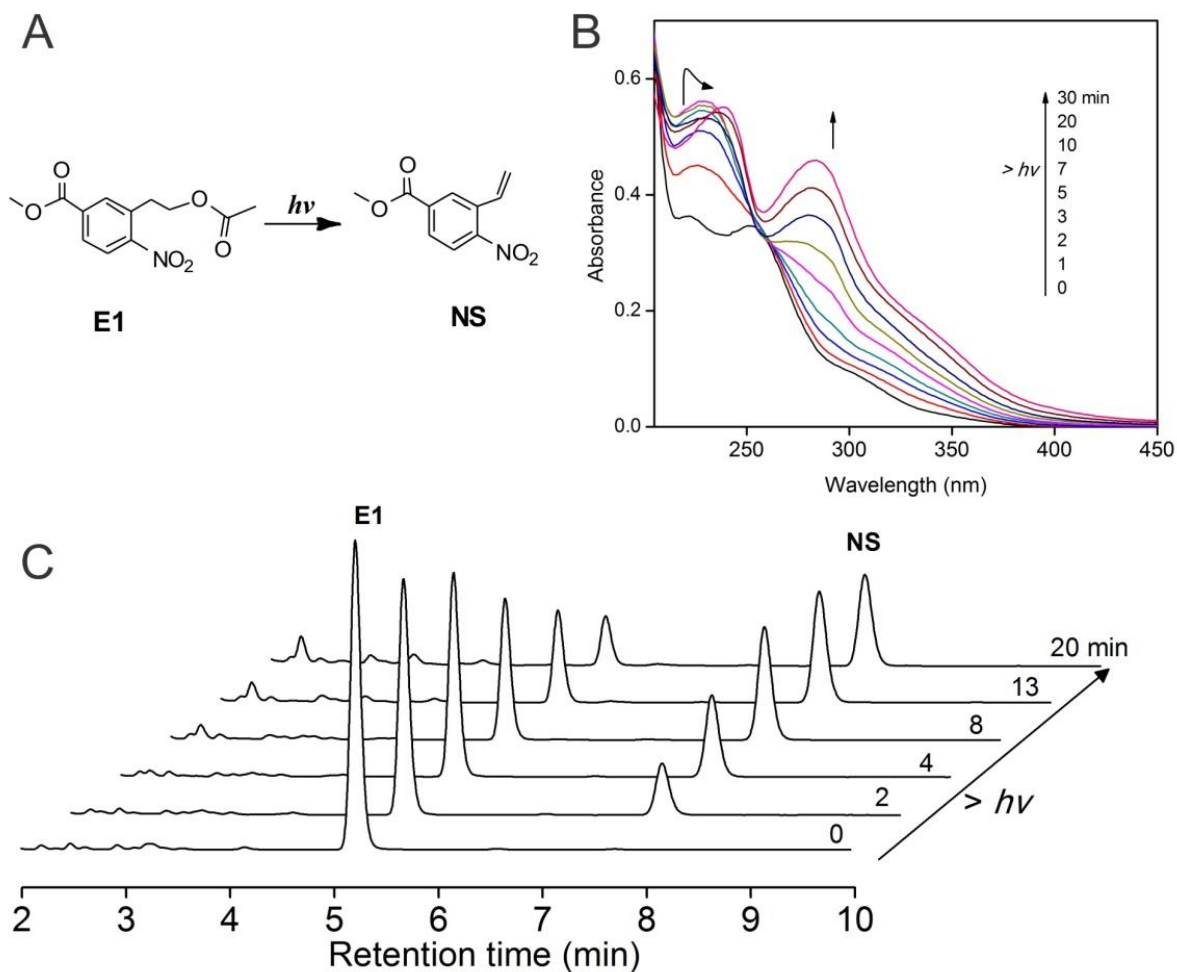


Figure S11. (A) Photolysis of the esterification product (**E1**) of **1** leads to the formation of nonfluorescent nitrostyrene (**NS**). (B) UV-vis absorption spectrum and HPLC analysis monitoring of the photolysis reaction of **E1** to **NS**. MeOH/PBS (3:1 v/v, pH7.4, 10 mM PBS), [**E1**] = 5 μ M.

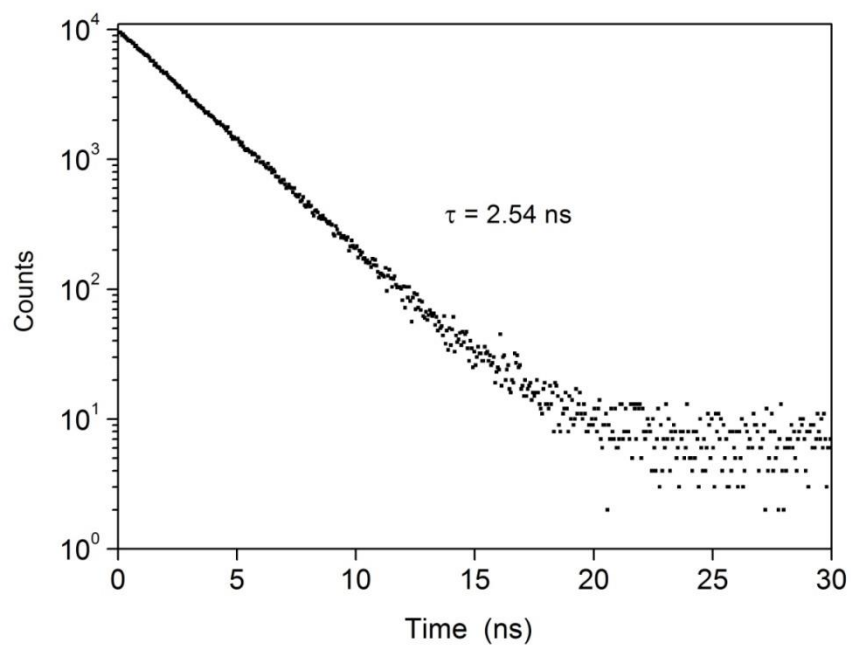


Figure S12. Fluorescence lifetime measurement of 1a. (MeOH/PBS, 3:1 v/v, pH7.4, 10 mM PBS)

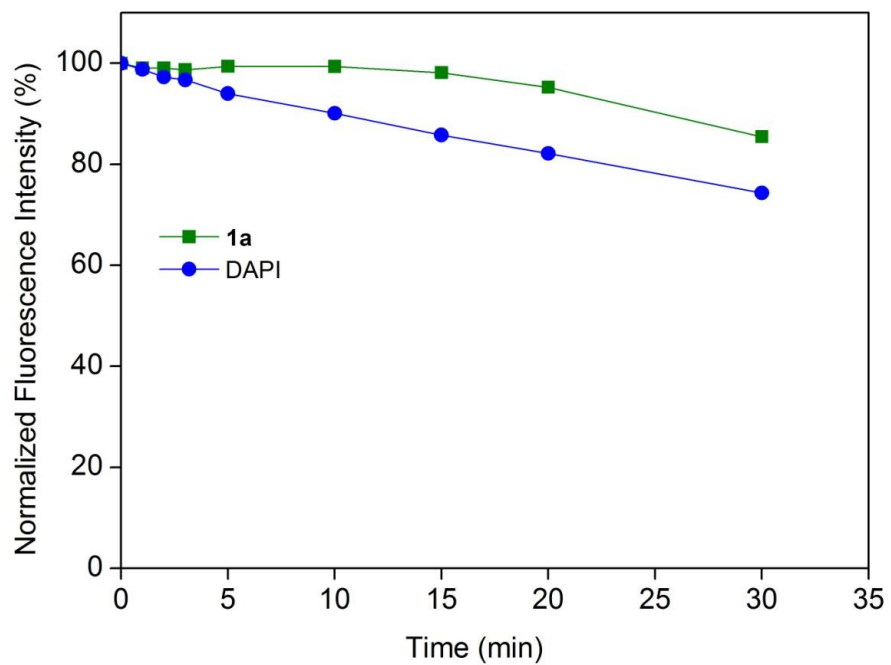


Figure S13. Study on the photostability of **1a** and **DAPI**. (5 μM of dyes in MeOH/PBS, 3:1 v/v, pH7.4, 10 mM PBS. Photolysis was performed at 365 nm. For **1a**, $\lambda_{\text{ex}} = 365 \text{ nm}$, $\lambda_{\text{em}} = 520 \text{ nm}$; for **DAPI**, $\lambda_{\text{ex}} = 365 \text{ nm}$, $\lambda_{\text{em}} = 468 \text{ nm}$. Photostability was calculated by using integrated fluorescence emission).

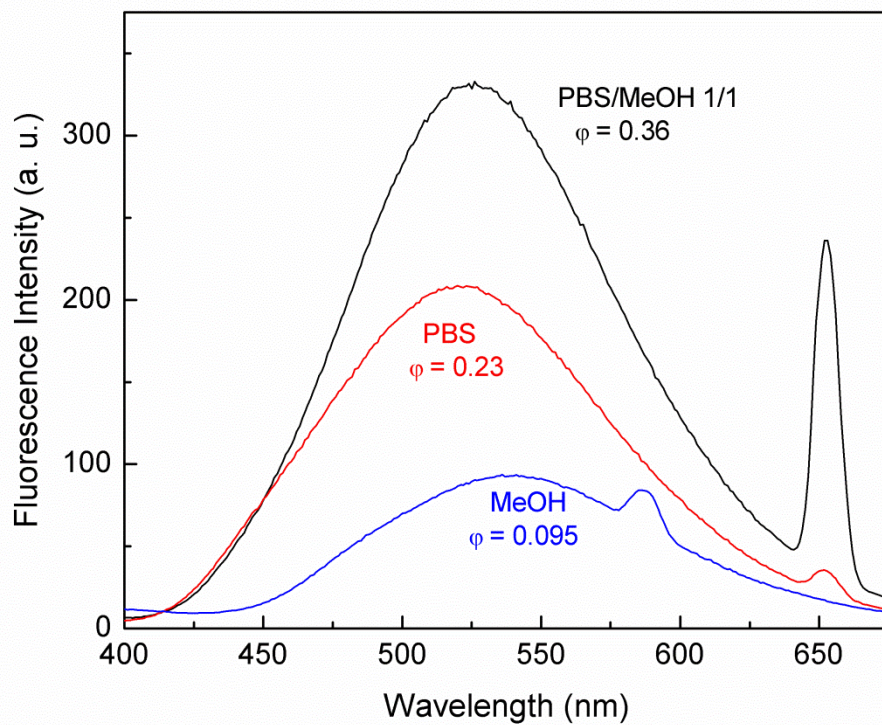


Figure S14. Solvent polarity-dependent fluorescence emission spectrum and fluorescence quantum yield of **1a**.

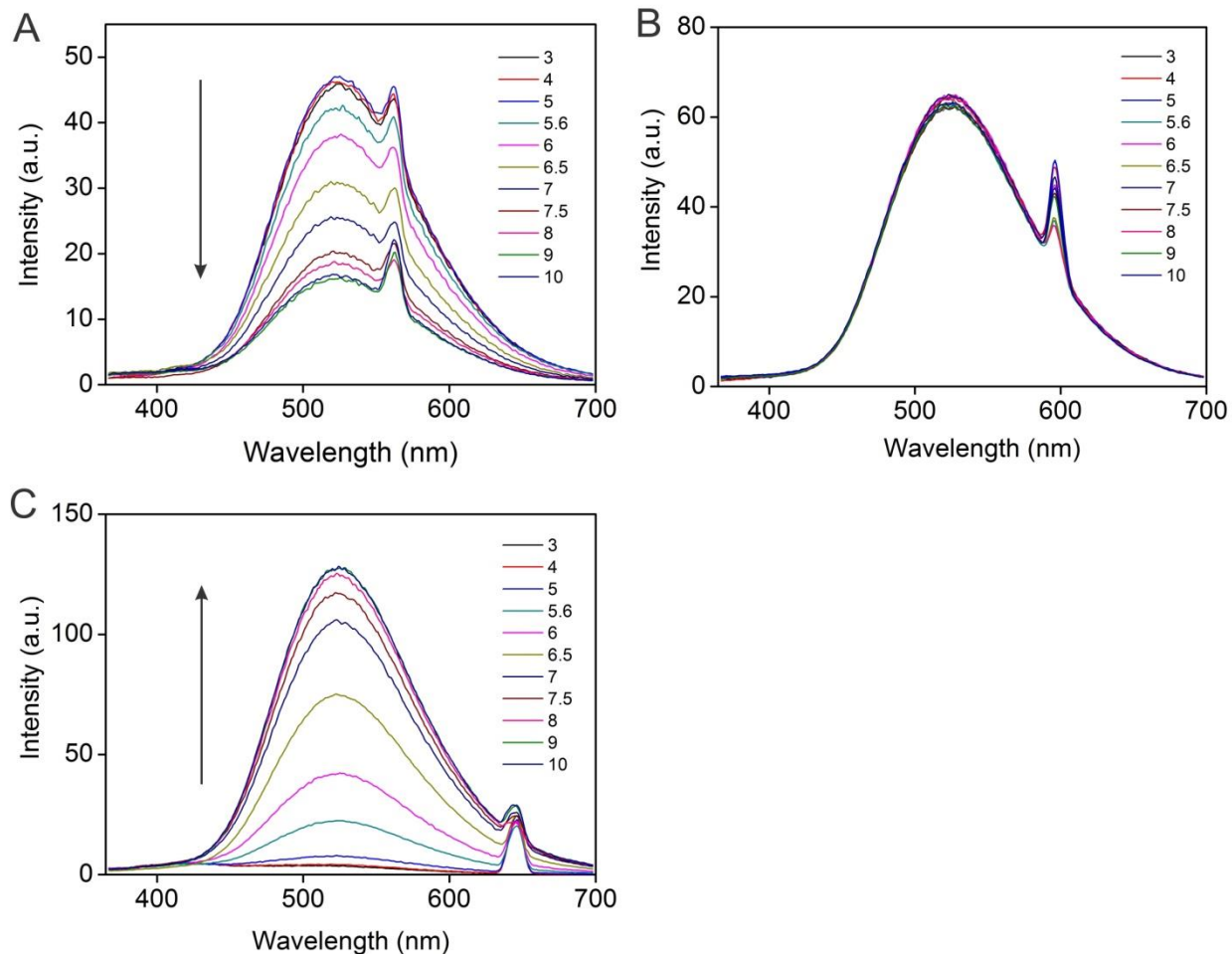


Figure S15. Fluorescence emission spectrum of **1a** under different pH conditions with different excitation wavelengths. (A) 281 nm, (B) 299 nm, and (C) 324 nm.

NOTE: The fluorescence intensity can be calculated using equation $F=2.303I_0\phi A$, where F is the fluorescence intensity, I_0 is the excitation density at the excitation wavelength, ϕ is the fluorescence quantum yield and A is the absorbance at the excitation wavelength. When the excitation wavelength was set at 299 nm (Figure S15B), which is the wavelength of isosbestic point (Figure 2B), the solution at different pH will have a same absorbance A , thus the fluorescence intensity is determined by the ϕ . It's shown in Figure S15B that the fluorescence intensity retains constants while pH changes, indicates that both the acidic and basic form of **1a** has a same ϕ , and the fluorescence intensity changes in Figure S15a and S15c are due to the changes in the absorbance. These results strong indicated that both in acid and base solution, the **1a** has a same fluorescent structure, the deprotonic **1a**, as showed in Figure 2D.

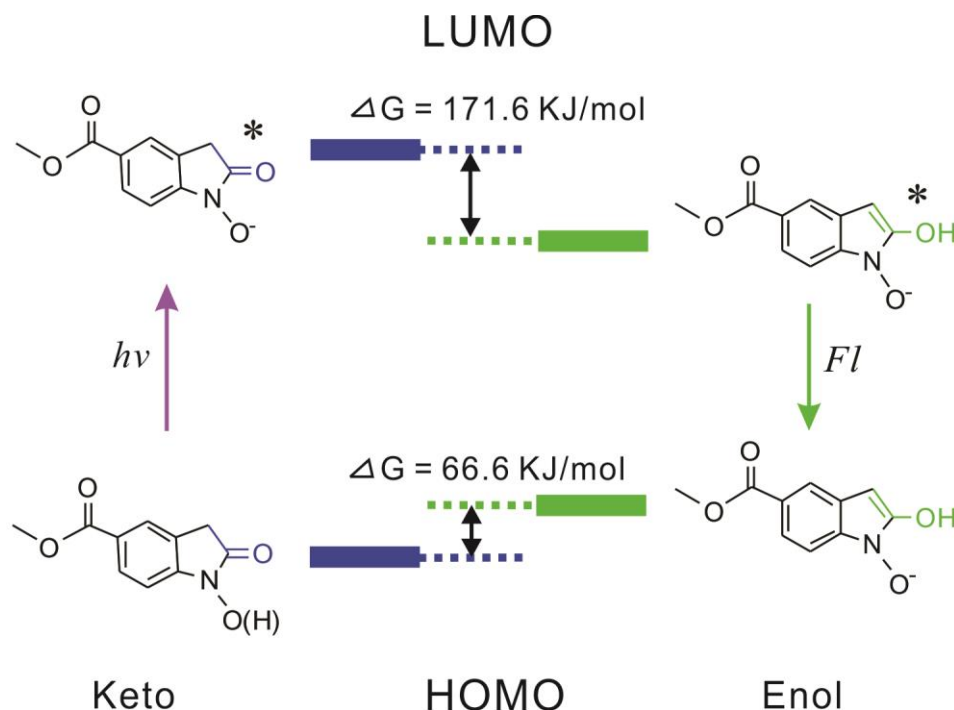


Figure S16. Schematic illustration of the proposed excited state keto-enol tautomerization of **1a**, where only the enol form is the fluorescent moiety. HOMO and LUMO calculations of the keto and enol show that enol form is more stable at excited state than the keto form.

NOTE: To further support this hypothesis and better understanding the mechanism behind the unique fluorescence of the small molecule, Gibbs' free energy change (ΔG) in the excitation involved in the keto-enol tautomerism were calculated. All calculations were performed in the Gaussian 09 suite software.⁸⁶ Using 6-311+G(d,p) basis set for DFT method (B3LYP), the ground states of the keto and enol form were optimized and the Gibbs free energies were obtained from the frequency study of optimized keto and enol form. Water was set as the default solvent in the calculation. The free Gibbs energy change for keto-enol tautomerism in the ground state was calculated to be 66.59 kJ/mol (0.02536 Ha). Based on the optimized ground state of keto and enol form, the first excited states of keto and enol were described and optimized using Cis method with 6-31G(d,p) basis. The Gibbs' free energies were obtained (-737.463183 Ha for enol form and -737.52856 Ha for keto form) and the change of Gibbs' free energy in the enol* to keto* tautomerism was calculated to be 171.6473 kJ/mol (0.065377 Ha). The Gibbs' free energy change in the tautomerization clearly indicates the enol to keto transformation is favored in ground state while keto to enol transformation is favored in the excited states. (**Detailed calculation results see Supporting Information page S30**).

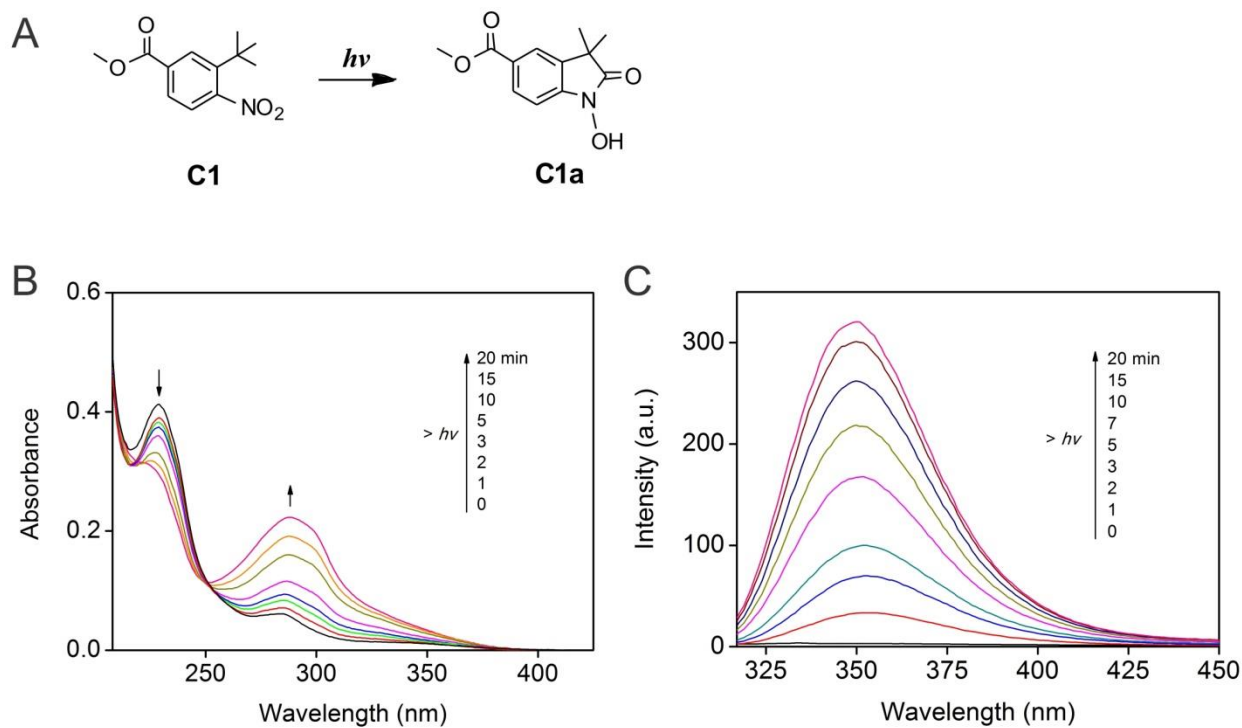


Figure S17. (A) The photolysis of controlled compound methyl 3-(*tert*-butyl)-4-nitrobenzoate compound (**C1**) produces a substituted oxindole without the α -proton (**C1a**), which will not precede keto-enol tautomerization. (B) UV-vis absorption spectrum and (C) Fluorescence emission spectrum analysis of the photolysis. MeOH/PBS, 3:1 v/v, pH7.4, 10 mM PBS; [**C1**] = 5 μ M.

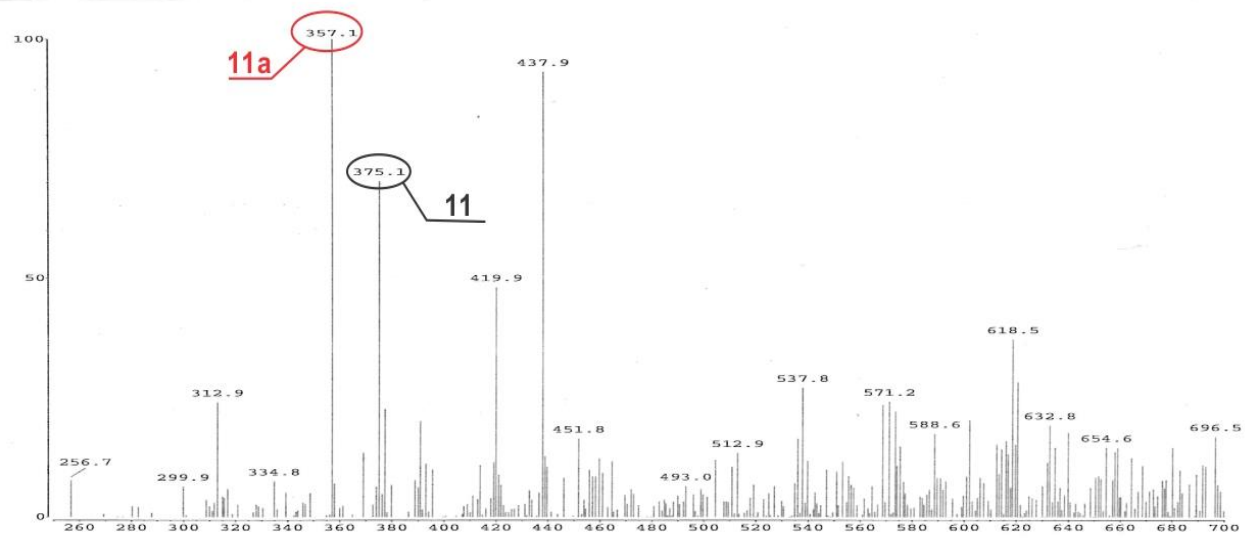
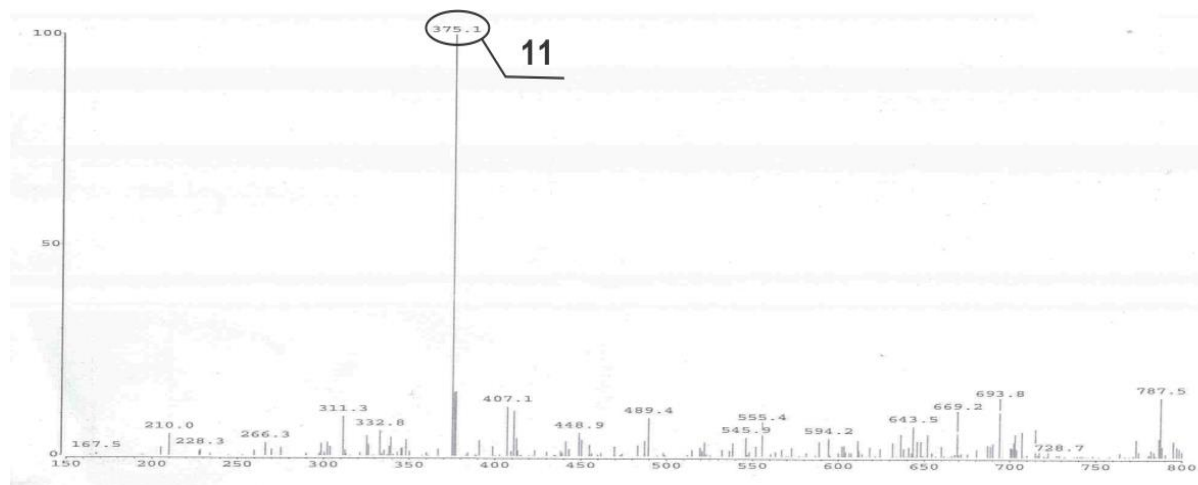
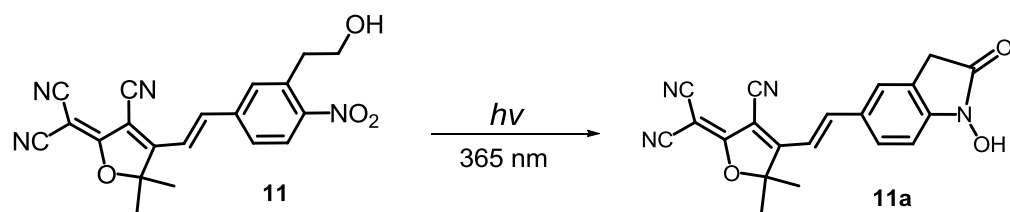


Figure S18. The MS spectra monitoring the photolysis of **11** to **11a**.

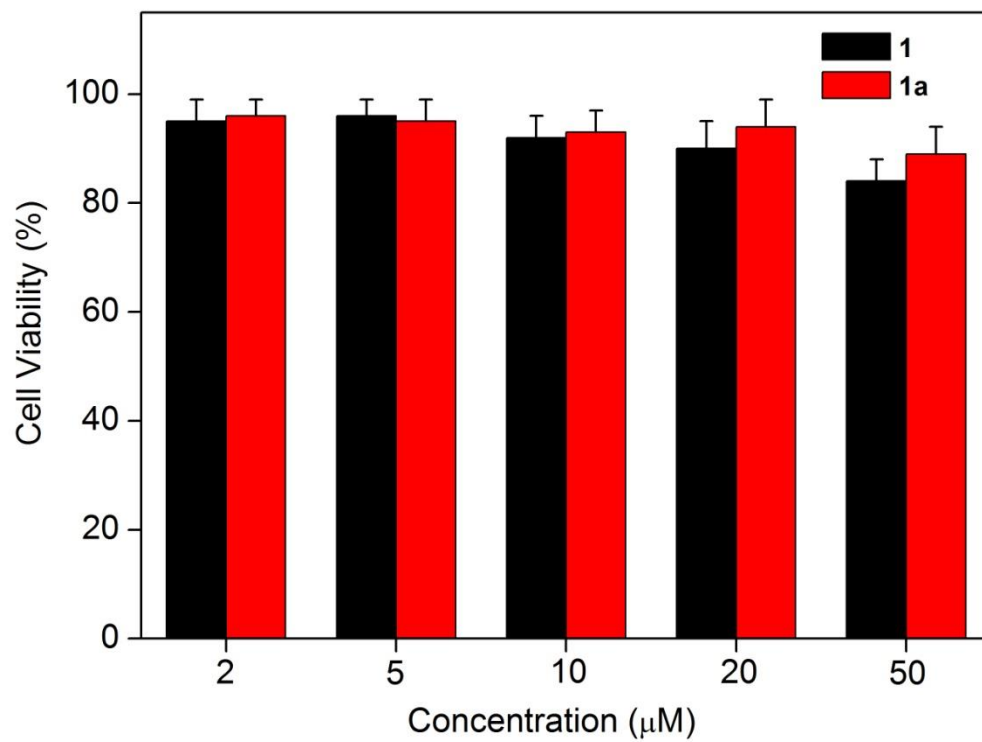


Figure S19. Analysis of viability and proliferation of HeLa cells in the presence of increasing concentrations of **1** and **1a**.

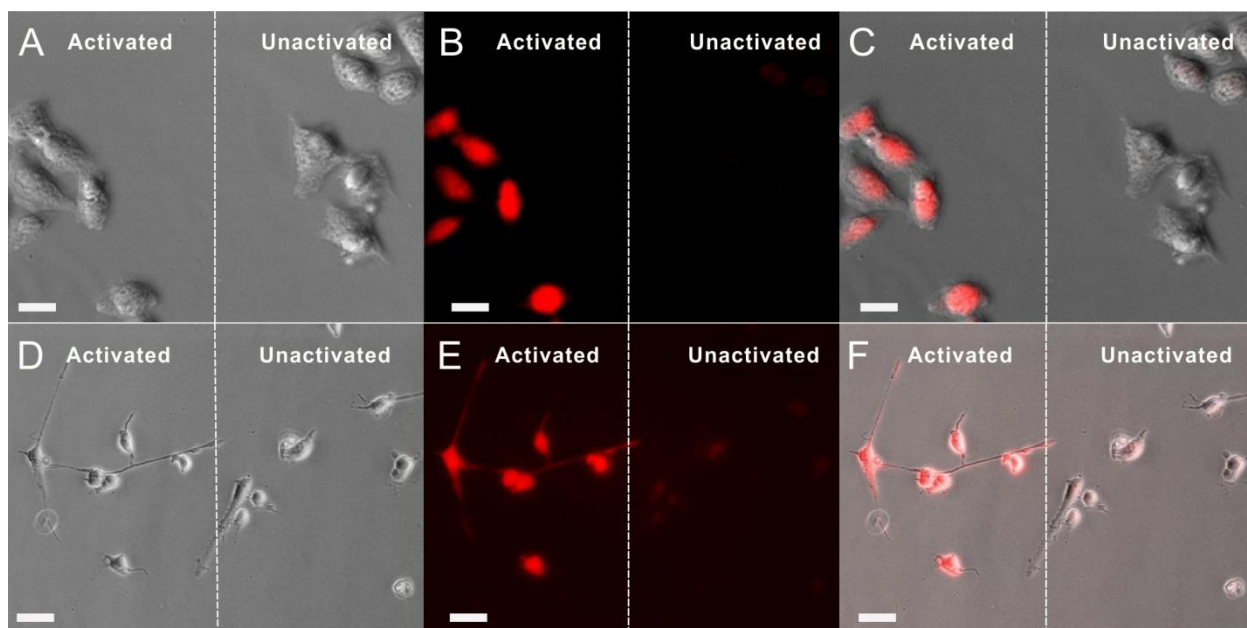


Figure S20. Photomask-assisted spatially-controlled cell-selective imaging of HeLa cells (A-C) and hNSCs (D-F). Bright field images (A and D), fluorescent images (B and E) and their merged images (C and F). UV-activation was carried out by using the UV excitation channel of the microscope (exposure time was 30 s). Fluorescent images were collected using Texas Red channel of the microscope (excitation 550-580 nm, emission 600-650 nm). Scale bar is 20 μm .

Absolute energies of ground state and excited state of keton and enol form at optimized geometries

Ground state of keton form:

Charge = 0 Multiplicity = 1

C	0.62395	-0.82219	0.01586
C	-0.75279	-0.70733	0.02231
C	-1.34029	0.5702	0.02128
C	-0.59755	1.74471	0.00916
C	0.79093	1.61273	0.00081
C	1.40757	0.35185	0.00501
H	1.10265	-1.79447	0.01516
H	-1.08033	2.7146	0.00419
H	1.42116	2.49493	-0.0093
C	2.8915	0.30707	-0.00505
O	3.6144	1.29118	-0.01555
O	3.37309	-0.95385	-0.00151
C	-1.85371	-1.74074	0.02698
H	-1.82902	-2.40998	-0.83935
H	-1.85316	-2.3706	0.92316
C	-3.1425	-0.91109	-0.00748
O	-4.29918	-1.28699	-0.05225
N	-2.72683	0.41272	0.0405
O	-3.59393	1.4724	-0.12634
H	-3.9083	1.6964	0.76708
C	4.80722	-1.07768	-0.0117
H	5.00866	-2.14787	-0.00807
H	5.22583	-0.61382	-0.90753
H	5.23923	-0.60526	0.87322

Sum of electronic and zero-point Energies=	-742.151691
Sum of electronic and thermal Energies=	-742.138381
Sum of electronic and thermal Enthalpies=	-742.137437
Sum of electronic and thermal Free Energies=	-742.192081

Ground state of enol form:

Charge = 0 Multiplicity = 1

C	0.95563	-0.99289	0.0086
C	-0.41967	-0.75487	0.01283
C	-0.87297	0.59473	0.04135
C	-0.01064	1.68842	0.02976
C	1.35042	1.42246	0.0087
C	1.83678	0.09607	0.00462
H	1.33835	-2.00498	-0.00103
H	-0.38884	2.70271	0.03704
H	2.06261	2.23705	-0.00172
C	3.30169	-0.10055	-0.01035
O	4.1293	0.79231	-0.0228
O	3.67317	-1.40379	-0.00903
N	-2.24873	0.53824	0.09436
O	-3.06989	1.61135	-0.18772
H	-3.40595	1.92102	0.6671
O	-3.99406	-0.99167	-0.01002
H	-4.17422	-1.9386	-0.0657
C	-2.66943	-0.78219	0.00769
C	-1.57868	-1.60969	-0.00813
H	-1.59983	-2.68798	-0.0099
C	5.10249	-1.44462	-0.02565
H	5.43023	-2.46319	-0.02551
H	5.4631	-0.95403	-0.90553
H	5.48363	-0.94719	0.84164

Sum of electronic and zero-point Energies=	-742.126299
Sum of electronic and thermal Energies=	-742.112763
Sum of electronic and thermal Enthalpies=	-742.111819
Sum of electronic and thermal Free Energies=	-742.166718

Excited state of keton form:

Charge = 0 Multiplicity = 1

C	0.59948	-0.85544	-0.0157
C	-0.73448	-0.73148	-0.01165
C	-1.34902	0.58515	-0.00537
C	-0.57685	1.78488	-0.0195
C	0.76263	1.66289	-0.02572
C	1.41644	0.34485	-0.01392
H	1.07471	-1.81617	-0.01899
H	-1.0639	2.74121	-0.02651
H	1.39282	2.52902	-0.03842
C	2.84763	0.29793	0.00116
O	3.57007	1.28147	0.00562
O	3.34899	-0.94623	0.00937
C	-1.84312	-1.75582	-0.00794
H	-1.84023	-2.39046	-0.88685
H	-1.83037	-2.3947	0.86771
C	-3.10319	-0.9087	-0.00233
O	-4.24743	-1.24879	-0.00304
N	-2.68354	0.41087	0.01654
O	-3.55416	1.43018	-0.06633
H	-3.87478	1.60538	0.81244
C	4.75658	-1.07565	0.02282
H	4.95784	-2.13625	0.02632
H	5.19442	-0.62064	-0.85552
H	5.17795	-0.61806	0.90789

Sum of electronic and zero-point Energies=	-737.422809
Sum of electronic and thermal Energies=	-737.409624
Sum of electronic and thermal Enthalpies=	-737.408680
Sum of electronic and thermal Free Energies=	-737.463183

Excited state of enol form:

Charge = 0 Multiplicity = 1

C	0.04823	0.00804	0.
C	-1.31628	0.24764	0.00266
C	-1.81979	1.62734	0.01534
C	-0.90278	2.70833	0.01831
C	0.45906	2.43013	0.01499
C	0.93101	1.12389	0.00767
H	0.44113	-0.98903	-0.00929
H	-1.26569	3.71911	0.02543
H	1.17009	3.23259	0.01885
C	2.41062	0.92797	0.00729
O	3.19524	1.83012	0.00626
O	2.7578	-0.33951	0.00906
C	-2.43563	-0.55564	-0.0147
H	-2.48343	-1.62757	-0.03373
C	-3.64577	0.3505	0.00778
N	-3.10931	1.69793	0.02129
O	-4.41725	0.12346	-1.11856
H	-5.14471	0.73161	-1.11484
C	4.14913	-0.64348	0.0101
H	4.21242	-1.71941	0.01317
H	4.62007	-0.2413	-0.87484
H	4.61977	-0.23631	0.89292
O	-4.42843	0.2156	1.14697
H	-4.88506	-0.61329	1.1103

Sum of electronic and zero-point Energies=	-737.488913
Sum of electronic and thermal Energies=	-737.476241
Sum of electronic and thermal Enthalpies=	-737.475297
Sum of electronic and thermal Free Energies=	-737.528560

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