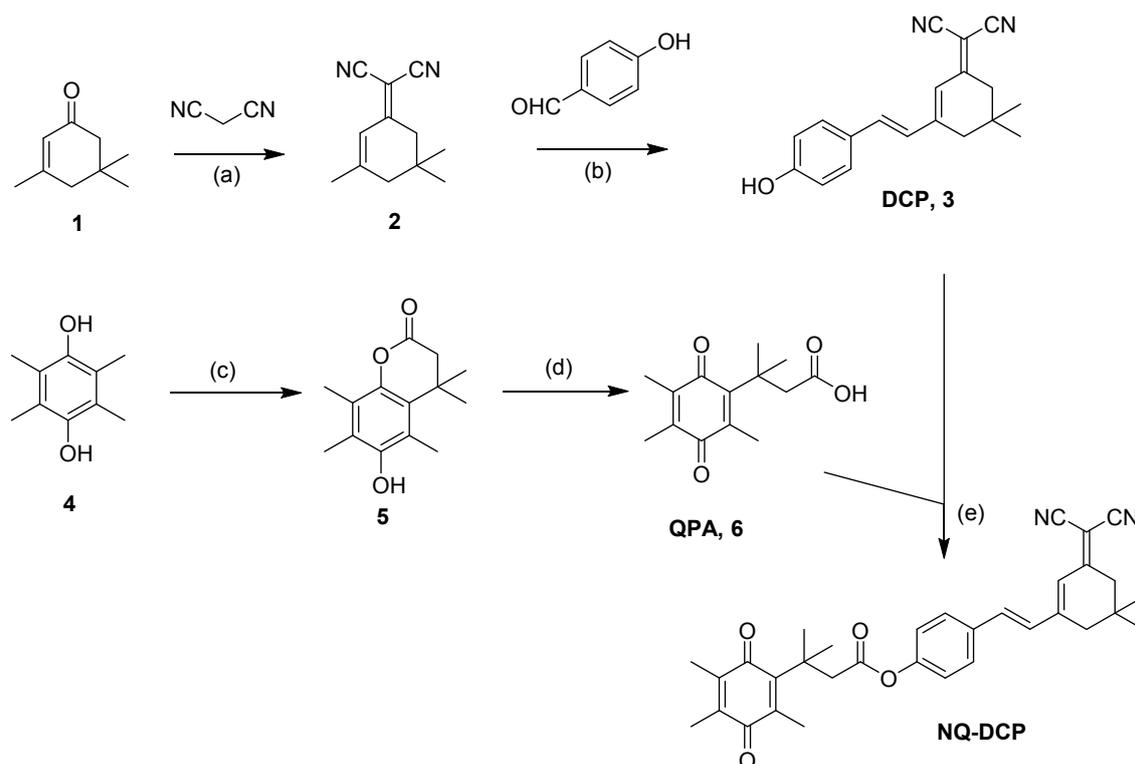


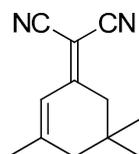
Supplementary Materials: Cancer-specific biomarker hNQO1 activatable fluorescent probe for imaging cancer cells in vitro and in vivo

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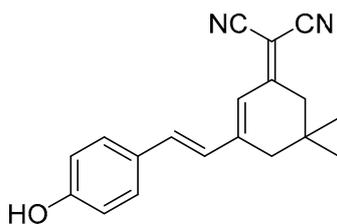


Scheme S1. Synthetic scheme of NQ-DCP. *Reagents & conditions:* (a) Ammonium acetate, acetic anhydride, acetic acid, toluene, reflux, 12 h; (b) piperidine, acetonitrile, 50 °C, 6 h; (c) methyl 3,3-dimethylacrylate, methanesulfonic acid, 70 °C, 2 h; (d) NBS, acetonitrile, water, rt, 1 h; (e) EDC, pyridine, rt, 10 h.

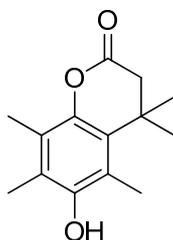
Synthesis of NQ-DCP



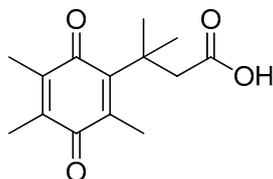
2-(3,5,5-trimethylcyclohex-2-en-1-ylidene) malononitrile (2): To a stirred solution of 2.25 mL (15.0 mmol) isophorone (1) and 1.49 g (22.5 mmol) of malononitrile in 50 mL toluene were added 0.29 g (3.75 mmol) of ammonium acetate, 0.5 mL of glacial acetic acid, and 1.0 mL of acetic anhydride. The reaction mixture was heated at 120 °C for 12 h under air atmosphere in the dark. Reaction mixture was allowed to cool to room temperature, solvent was removed under diminished pressure, neutralized with saturated sodium carbonate solution and extracted with three 30 mL portion of ethyl acetate. The combined organic layer was dried over anhydrous Na_2CO_3 , and concentrated. The crude product was purified by chromatography on a silica gel column. Elution with 9:1 hexanes-ethyl acetate afforded compound 2 as pale yellow solid: yield 1.98 g (71%); silica gel TLC R_f 0.37 (4:1 hexanes/ethyl acetate); ^1H NMR (400 MHz, CDCl_3) δ 1.01 (s, 6H), 2.03 (s, 3H), 2.18 (s, 2H), 2.51 (s, 2H) and 6.62 (s, 1H); ^{13}C NMR (100 MHz, CDCl_3) δ 25.3, 27.8, 32.4, 42.6, 45.6, 78.2, 112.4, 113.2, 120.5, 159.9 and 170.4.



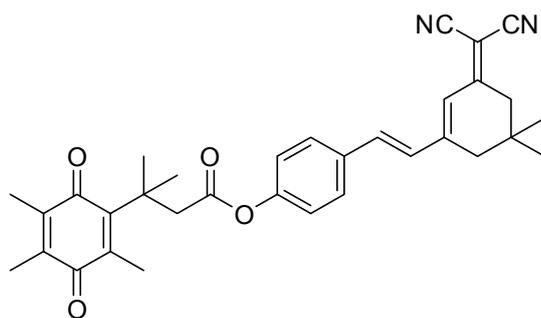
(E)-2-(3-(4-hydroxystyryl)-5,5-dimethylcyclohex-2-en-1-ylidene)malononitrile (DCP, 3): To a stirred solution of 1.92 g (10.3 mmol) of compound 2, 0.15 mL of piperidine in 40 mL of acetonitrile was added 1.89 g (15.5 mmol) of 4-hydroxybenzaldehyde. The reaction mixture was stirred at 50 °C for 6 h. After the reaction mixture was allowed to cool to room temperature, the formed precipitate was filtered, washed with acetonitrile, and recrystallized with ethanol to obtain compound 3 as yellow crystal: yield 2.36 g (79%); ^1H NMR (400 MHz, CDCl_3) δ 1.08 (s, 6H), 2.46 (s, 2H), 2.59 (s, 2H), 6.81 (d, 1H, $J = 14.8$ Hz), 6.86 (d, 2H, $J = 7.8$ Hz), 7.02 (d, 1H, $J = 16.0$ Hz) and 7.42 (d, 2H, $J = 8.0$ Hz); ^{13}C NMR (100 MHz, CDCl_3) δ 28.0, 32.0, 39.2, 43.0, 77.7, 112.9, 113.7, 116.1, 122.8, 127.0, 128.6, 129.4, 136.8, 154.4, 157.2 and 169.5.



Synthesis of 6-hydroxy-4,4,5,7,8-pentamethylchroman-2-one (5): To a stirred solution of 2.25 g (13.5 mmol) of 2,3,5,6-tetramethylbenzene-1,4-diol (4) in 15 mL of methanesulfonic acid was added 1.98 mL (1.85 g, 16.2 mmol) of methyl 3,3-dimethylacrylate at room temperature. The reaction mixture was stirred at 70 °C for 2 h. After cooling to room temperature, the reaction mixture was diluted with 150 mL of water and extracted three 70 mL portions of dichloromethane. The combined organic extracts were washed with 100 mL of saturated NaHCO_3 solution, 100 mL of NaCl solution, dried over anhydrous Na_2SO_4 and concentrated under diminished pressure. The crude product was recrystallization from 30% CHCl_3 in hexanes gave compound 5 as white solid: yield 2.56 g (81%); ^1H NMR (400 MHz, CDCl_3) δ 1.45 (s, 6H), 2.19 (s, 3H), 2.22 (s, 3H), 2.36 (s, 3H), 2.55 (s, 2H) and 4.75 (s, 1H); ^{13}C NMR (100 MHz, CDCl_3) δ 12.3, 12.6, 14.5, 27.7, 35.5, 46.1, 118.9, 121.8, 123.4, 128.2, 143.5, 148.8 and 168.9.



Synthesis of 3-methyl-3-(2,4,5-trimethyl-3,6-dioxocyclohexa-1,4-dien-1-yl)butanoic acid (QPA, 6): To a stirred solution of 2.00 g (8.54 mmol) of compound 5 in a solution of 70 mL of acetonitrile and 30 mL of water was added 1.67 g (9.39 mmol) of *N*-bromosuccinimide at room temperature. After 60 min, acetonitrile was removed from the reaction mixture, the residue was extracted three 30-mL portions of dichloromethane. The combined organic layer was dried over anhydrous Na_2SO_4 and concentrated under diminished pressure. The crude product was purified by chromatography on a silica gel column. Elution with 4:1 hexanes-ethyl acetate afforded compound 6 as yellow solid: yield 1.64 g (77%); silica gel TLC R_f 0.23 (7:3 hexanes/ethyl acetate); ^1H NMR (400 MHz, CDCl_3) δ 1.43 (s, 6H), 1.92 (s, 3H), 1.95 (s, 3H), 2.13 (s, 3H) and 3.01 (s, 2H); ^{13}C NMR (100 MHz, CDCl_3) δ 12.2, 12.6, 14.4, 29.0, 38.1, 47.4, 138.5, 139.2, 143.1, 152.1, 178.6, 187.6 and 191.0.



(E)-4-(2-(3-(dicyanomethylene)-5,5-dimethylcyclohex-1-en-1-yl)vinyl)phenyl 3-methyl-3-(2,4,5-trimethyl-3,6-dioxocyclohexa-1,4-dien-1-yl)butanoate (NQ-DCP, 7): To a stirred solution of 125 mg (0.50 mmol) of compound 6 in 1 mL of dry pyridine was added 193 mg (0.75 mmol) of EDC at room temperature. After 20 min a solution of 194 mg (0.50 mmol) of compound 3 in 0.2 mL of pyridine was added to the reaction mixture and stirred for 10 h at room temperature. The reaction mixture was diluted with 20 mL of 1N HCl and extracted with two 20 mL portions of ethyl acetate. Combined organic layer was dried over Na₂SO₄ and concentrated under diminished pressure. The obtained crude product was purified by chromatography on a silica gel column. Elution with 4:1 hexanes-ethyl acetate afforded compound 7 as yellow solid: yield 222 mg (85%); silica gel TLC *R_f* 0.22 (7:3 hexanes/ethyl acetate); ¹H NMR (400 MHz, CDCl₃) δ 1.08 (s, 6H), 1.53 (s, 6H), 1.93 (s, 3H), 1.94 (s, 3H), 2.18 (s, 3H), 2.46 (s, 2H), 2.60 (s, 2H), 3.26 (s, 2H), 6.83 (s, 1H), 6.92 (d, 1H, *J* = 16.1 Hz), 7.00 (s, 1H, *J* = 16.1 Hz), 7.03 (d, 2H, *J* = 7.8 Hz) and 7.49 (d, 2H, *J* = 8.6 Hz); ¹³C NMR (100 MHz, CDCl₃) δ 12.1, 12.6, 14.4, 28.0, 28.9, 32.0, 38.4, 39.1, 42.9, 47.6, 78.8, 112.7, 113.4, 122.2, 123.7, 128.6, 129.3, 133.4, 135.7, 138.6, 139.3, 142.8, 151.3, 151.7, 153.6, 169.2, 171.1, 187.3 and 190.8.

1.H and 13C NMR spectrums of intermediates and NQ-DCP

