Electronic Supplementary Material (ESI) for Chemical Science.
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Ultraspecific live imaging of the dynamics of zebrafish neutrophil granules by a histopermeable fluorogenic benzochalcone probe

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ELECTRONIC SUPPORTING INFORMATION

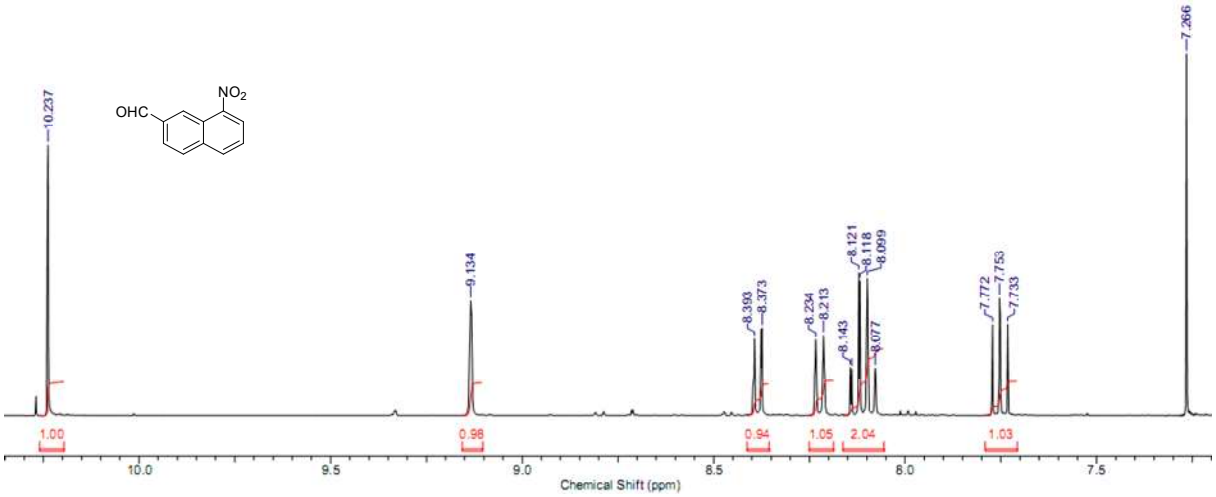
Chemistry

Synthesis and structural characterization.

Reagents and solvents were the best grades commercially available and were used as received. Pure nitric acid was prepared by slowly adding one volume of ice-cold sulphuric acid (95-98 %) to one volume of ice-cold nitric acid (65 %) under stirring, followed by distillation under vacuum at a temperature inferior to 55 °C. 8-Nitro-2-naphthaldehyde **2** and 6-nitro-2-methylnaphthalene **10** were prepared according to [A] and [B] respectively, but the procedures described herein correspond to preparative scale synthesis of the two compounds. Column chromatography was performed with silica gel 60 (9385 Merck). TLC were performed on aluminium plates coated with silica gel $60F_{254}$ (554 Merck) and visualized with UV light (254 and 366 nm). NMR spectra (^{1}H and ^{13}C) were recorded on a Varian Oxford AS-400 (400 MHz), and calibrated using tetramethylsilane for ^{1}H spectra (δ 0.00 ppm), and solvent signal for ^{13}C spectra ($CDCl_3$: δ 77.23 ppm, d_6-DMSO: δ 39.51 ppm). High-Resolution mass spectra (HRMS) were recorded on a Micromass LCT Premier spectrometer (Waters).

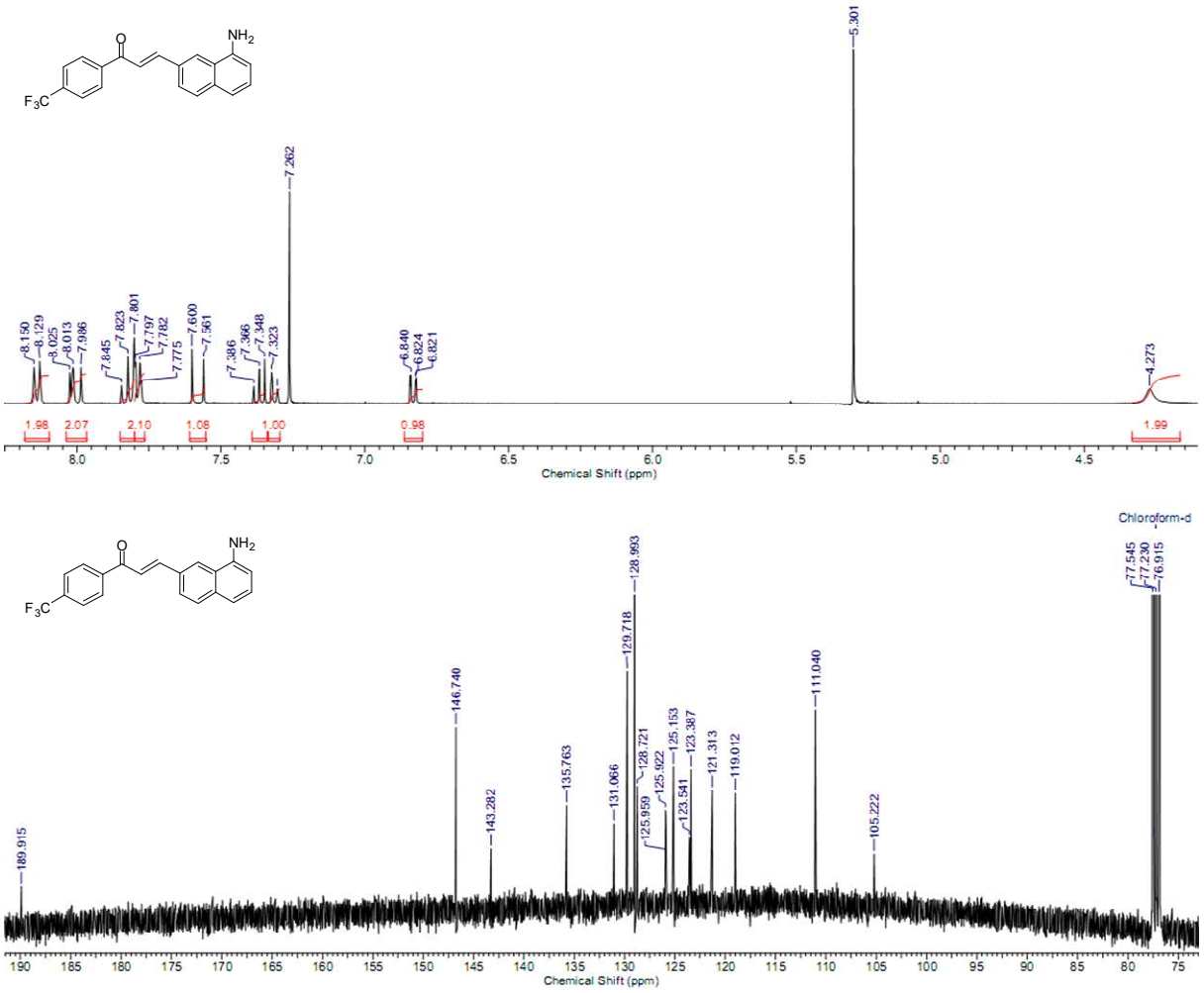
8-Nitro-2-naphthaldehyde (2) [*Described in ref. A*]

To glacial acetic acid (90 mL) is slowly added concentrated sulphuric acid (2.3 mL, 42.2 mmol) at room temperature with stirring. The solution is cooled in a water bath then primed with nitrogen. Solid β-naphthaldehyde **1** (4.5 g, 28.85 mmol) is added, followed by pure nitric acid (48 mL, 1.15 mol), dropwise and under vigorous stirring. At the end of the addition, the mixture is primed with nitrogen and allowed to react for 17 hrs. The reaction mixture is poured in ice-water mixture (700 mL) under stirring, and the formed yellow precipitate filtered over a Büchner. The solid is washed abundantly with water until neutral filtrate is obtained, dried by succion, then dessicated ($CaCl_2$) under vacuum. The crude product is dissolved in CH_2Cl_2 (150 mL) then treated with silica (60 g), and the solvent evaporated under reduced pressure. The complex mixture of nitro-2-naphthaldehyde regioisomers is repeatedly chromatographed (SiO_2, toluene / EtOAc / *n*-hexane 10:15:75 v/v/v), yielding 8-nitro-2-naphthaldehyde **2** (754 mg, 13 %) as a pale-yellow crystalline solid (R_f = 0.41 in toluene / EtOAc / *n*-hexane 10:15:75 v/v/v, silica TLC). ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.24 (*d*, *J* = 0.8 Hz, 1H, CH), 9.13 (*d*, *J* = 0.8 Hz, 1H, CH), 8.38 (*dd*, *J* = 8.0 Hz, *J* = 1.2 Hz, 1H, CH), 8.22 (*d*, *J* = 8.4 Hz, 1H, CH), 8.13 (*dd*, *J* = 8.6 Hz, *J* = 1.4 Hz, 1H, CH), 8.09 (*d*, *J* = 8.8 Hz, 1H, CH), 7.75 (*dd*, *J* = 7.6 Hz, *J* = 8.0 Hz, 1H, CH); HRMS (ESI+) m/*z* calculated for $C_{11}H_8NO_3$ $[M+H]^+$, 202.0504, found 202.0508.



(E)-3-(8-aminonaphthalen-2-yl)-1-(4-(trifluoromethyl)phenyl)prop-2-en-1-one (3)

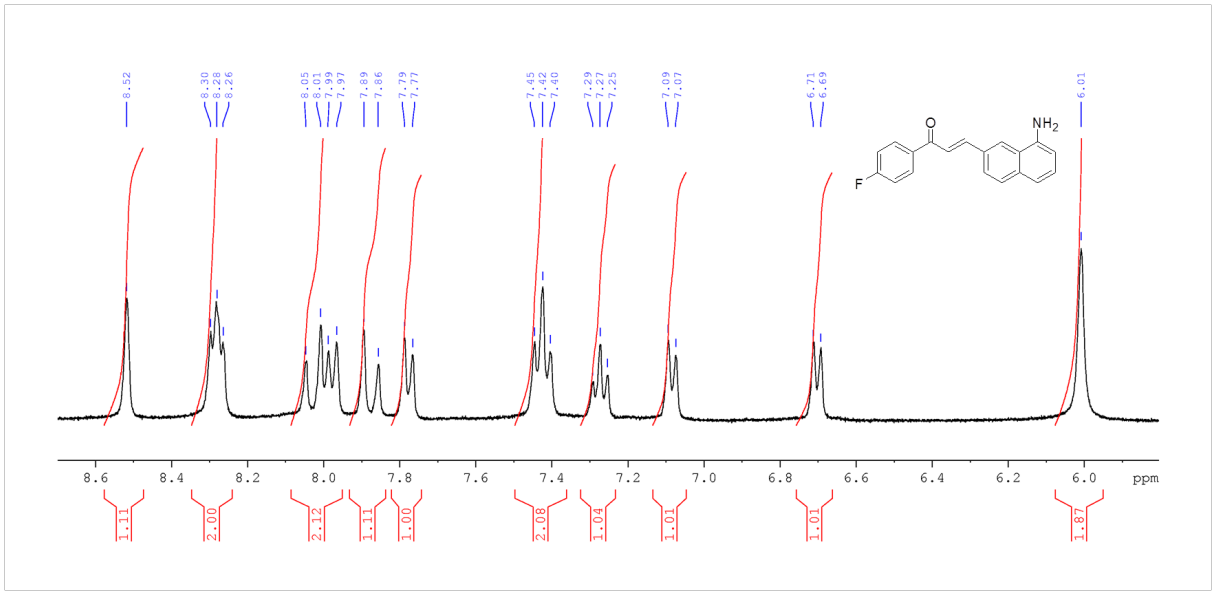
To a solution of 8-nitro-2-naphthaldehyde **2** (20 mg, 99.4 μmol) in absolute EtOH (800 μL) is added 4-trifluoromethylacetophenone (19 mg, 101 μmol) followed by NaOH (3 mg, 75 μmol). The mixture is stirred at room temperature for 24 hrs then treated with $SnCl_2$ (94 mg, 0.495 mmol). The mixture is further stirred for 4 hrs then treated with aqueous NaOH (1 M, 10 mL) and extracted with CH_2Cl_2 (10 mL). The organic phase is dried (Na_2SO_4) and evaporated under reduced pressure. The crude product is purified by column chromatography (SiO_2, EtOAc / *n*-hexane 25:75 v/v), furnishing 4'-trifluoromethyl-3-aminobenzochalcone **3** (13 mg, 38 % for two steps) as a red crystalline solid (R_f = 0.4 in EtOAc / *n*-hexane 25:75 v/v, silica TLC). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.14 (*d*, *J* = 8.0 Hz, 2H, CH), 8.01 (*s*, 1H, CH), 8.0 (*d*, *J* = 15.6 Hz, 1H, CH), 7.83 (*d*, *J* = 8.8 Hz, 1H, CH), 7.79 (*d*, *J* = 8.0 Hz, 2H, CH), 7.79 (*d*, *J* = 8.8 Hz, 1H, CH), 7.58 (*d*, *J* = 15.6 Hz, 1H, CH), 7.37 (*dd*, *J* = 8.0 Hz, *J* = 8.0 Hz, 1H, CH), 7.31 (*d*, *J* = 7.6 Hz, 1H, CH), 6.83 (*dd*, *J* = 7.6 Hz, *J* = 1.2 Hz, 1H, CH), 4.27 (*bs*, 2H, NH_2); ^{13}C NMR (100 MHz, $CDCl_3$) δ 189.9, 146.7, 143.3, 135.8, 131.1, 129.7, 129.0, 128.7, 126.0, 125.9 (two peaks), 125.8, 125.1, 123.5, 123.4, 121.3, 119.01, 111.0, 105.22; HRMS (ESI+) m/*z* calculated for $C_{20}H_{15}NOF_3$ $[M+H]^+$, 342.1106, found 342.1104.

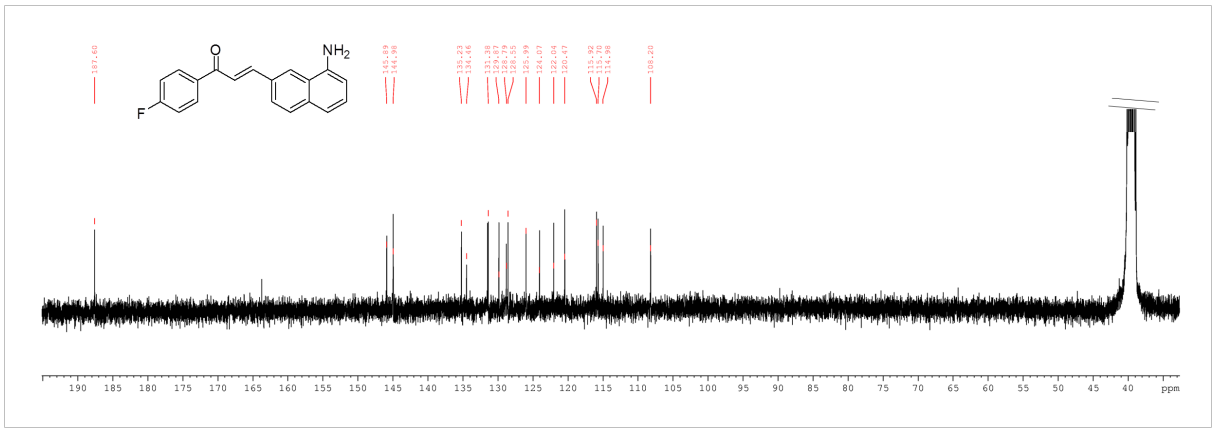


(E)-3-(8-aminonaphthalen-2-yl)-1-(4-fluorophenyl)prop-2-en-1-one (4)

To a solution of 8-nitro-2-naphthaldehyde **2** (20 mg, 99.4 µmol) in absolute EtOH (800 µL) is added 4-trifluoroacetophenone (14 mg, 101 µmol) followed by NaOH (3 mg, 75 µmol). The mixture is stirred at room temperature for 24 hrs then treated with AcOH (800 µL). Following priming with nitrogen and addition of Pd-C (10 %, 3 mg), the mixture is hydrogenated (1 atm) at room temperature for 1.5 hrs. After priming with nitrogen and cooling in an ice bath, the reaction is treated with aqueous NaOH (1 M, 14 mL) and extracted with CH_2Cl_2 (3 x 5 mL). The organic phase is dried (Na_2SO_4) and evaporated under reduced pressure. The crude product is purified by column chromatography (SiO_2, EtOAc / *n*-hexane 25:75 v/v), furnishing 4'-fluoro-3-aminobenzochalcone **4** (9 mg, 31 % for two steps) as a dark red solid (R_f = 0.45 in EtOAc / *n*-hexane 30:70 v/v, silica TLC). ^{1}H NMR (400 MHz, d$_6$-DMSO) δ 8.52 (*s*, 1H, CH), 8.28 (*dd*, *J* = 8.0 Hz, *J* = 8.0 Hz, 2H), 8.03 (*d*, *J* = 15.0 Hz, 1H, CH), 7.98 (*d*, *J* = 8.0 Hz, 1H, CH), 7.87 (*d*, *J* = 15.0 Hz, 1H, CH), 7.78 (*d*, *J* = 8.0 Hz, 1H, CH), 7.42 (*dd*, *J* = 8.0 Hz, *J* = 8.0 Hz, 2H, CH), 7.27 (*dd*, *J* = 8.0 Hz, *J* = 8.0 Hz, 1H, CH), 7.08 (*d*, *J* = 8.0 Hz, 1H, CH), 6.70 (*d*, *J* = 8.0Hz, 1H, CH), 6.01 (*bs*, 2H, NH_2); ^{13}C NMR (100 MHz, d$_6$-DMSO)

δ 187.6, 145.9, 145.0, 135.2, 134.5, 131.4, 129.9, 128.8, 128.5, 126.0, 124.1, 122.0, 120.5, 115.9, 115.7, 115.0, 108.2; HRMS (ESI+) m/*z* calculated for $C_{19}H_{15}NOF$ $[M+H]^+$, 292.1138, found 292.1137.

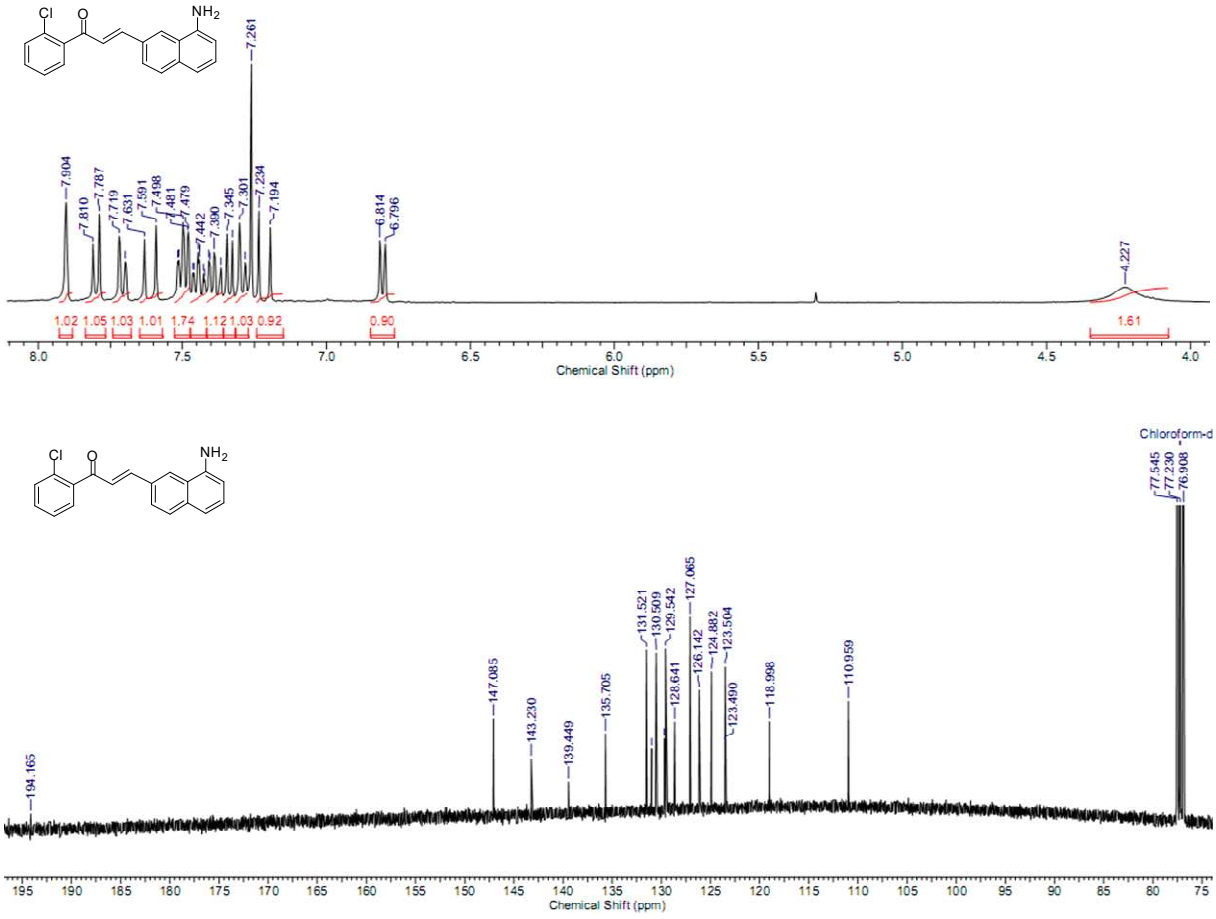




(E)-3-(8-aminonaphthalen-2-yl)-1-(2-chlorophenyl)prop-2-en-1-one (5)

To a solution of 8-nitro-2-naphthaldehyde **2** (20 mg, 99.4 µmol) in absolute EtOH (800 µL) is added 2-chloroacetophenone (15.5 mg, 100 µmol) followed by NaOH (4 mg, 100 µmol). The mixture is stirred at room temperature for 24 hrs then treated with $SnCl_2$ (94 mg, 0.495 mmol). The mixture is further stirred for 4 hrs then treated with aqueous NaOH (1 M, 10 mL) and extracted with CH_2Cl_2 (10 mL). The organic phase is dried (Na_2SO_4) and evaporated under reduced pressure. The crude product is purified by column chromatography (SiO_2, EtOAc / *n*-hexane 20:80 v/v), furnishing 2'-chloro-3-aminobenzochalcone **5** (8 mg, 26 % for two steps) as a red crystalline solid (R_f = 0.27 in EtOAc / *n*-hexane 20:80 v/v, silica TLC). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.90 (*s*, 1H, CH), 7.80 (*d*, *J* = 9.2 Hz, 1H, CH), 7.71 (*d*, *J* = 8.8 Hz, 1H, CH),

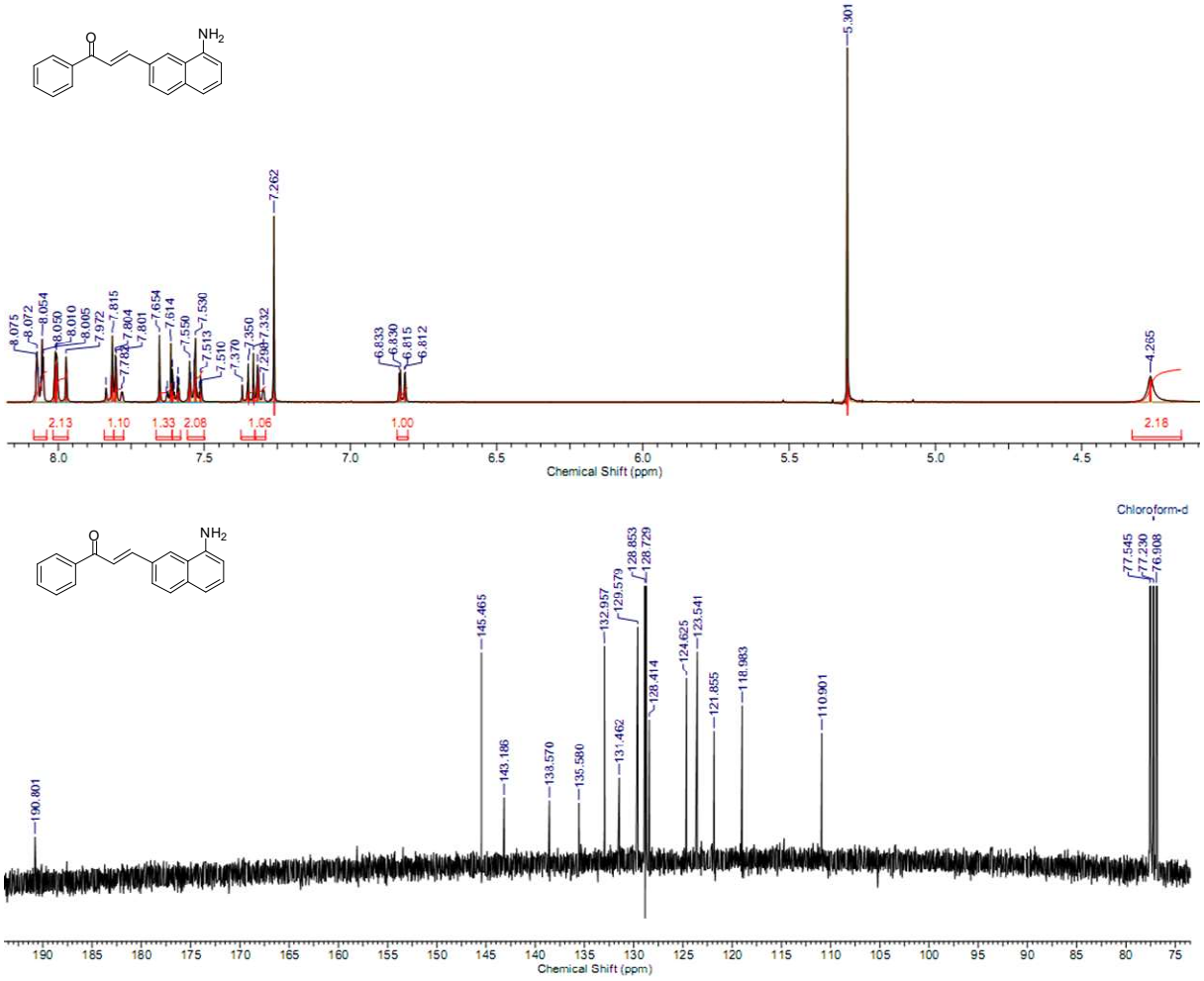
7.61 (*d*, *J* = 16.0 Hz, 1H, CH), 7.50 (*m*, 2H, CH), 7.44 (*ddd*, *J* = 9.0 Hz, *J* = 7.8 Hz, *J* = 1.6 Hz, 1H, CH), 7.39 (*ddd*, *J* = 9.2 Hz, *J* = 7.2 Hz, *J* = 1.6 Hz, H, CH), 7.33 (*d*, *J* = 7.6 Hz, 1H, CH), 7.29 (*d*, *J* = 8.0 Hz, 1H, CH), 7.21 (*d*, *J* = 16.0 Hz, 1H, CH), 6.80 (*d*, *J* = 7.2 Hz, 1H, CH), 4.23 (*bs*, 2H, NH_2); ^{13}C NMR (100 MHz, $CDCl_3$) δ 194.2, 147.1, 143.2, 139.4, 135.7, 131.5, 131.0, 130.5, 129.7, 129.5, 128.6, 127.1, 126.1, 124.9, 123.5 (two peaks), 119.0, 110.9; HRMS (ESI+) m/*z* calculated for $C_{19}H_{15}NOCl$ $[M+H]^+$, 308.0842, found 308.0846.



(E)-3-(8-aminonaphthalen-2-yl)-1-phenylprop-2-en-1-one (6)

To a solution of 8-nitro-2-naphthaldehyde **2** (20 mg, 99.4 µmol) in absolute EtOH (800 µL) is added acetophenone (12 mg, 101 µmol) followed by NaOH (3 mg, 75 µmol). The mixture is stirred at room temperature for 72 hrs, evaporated, and treated with AcOH (800 µL). Following priming with nitrogen and addition of Pd-C (10 %, 3 mg), the mixture is hydrogenated (1 atm) at room temperature for 1.5 hrs. After priming with nitrogen and cooling in an ice bath, the reaction is treated with aqueous NaOH (1 M, 14 mL) and extracted with a CH_2Cl_2 / EtOAc 50:50 v/v mixture (3 x 5 mL). The organic phase is dried (Na_2SO_4) and evaporated under reduced pressure. The crude product is purified by column chromatography (SiO_2, EtOAc / *n*-

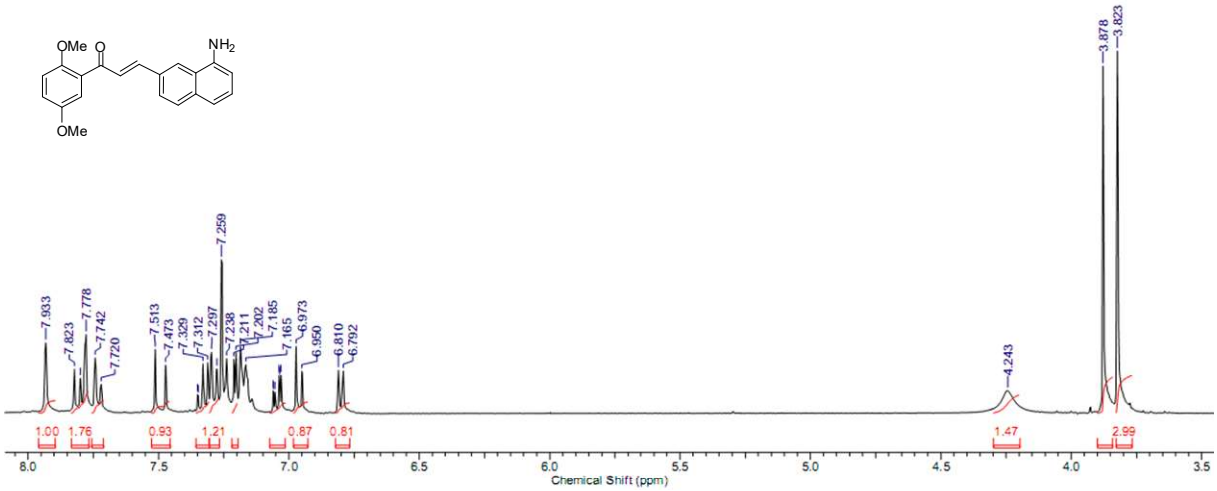
hexane 22:78 v/v), furnishing 3-aminobenzochalcone **6** (10 mg, 37 % for two steps) as a dark red crystalline solid (R_f = 0.45 in EtOAc / *n*-hexane 30:70 v/v, silica TLC). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.06 (*ddd*, *J* = 8.6 Hz, *J* = 6.9 Hz, *J* = 1.4 Hz, 2H, CH), 8.0 (*s*, 1H, CH), 7.99 (*d*, *J* = 15.6 Hz, 1H, CH), 7.82 (*d*, *J* = 8.4 Hz, 1H, CH), 7.79 (*dd*, *J* = 8.6 Hz, *J* = 1.0 Hz, 1H, CH), 7.63 (*d*, *J* = 15.6 Hz, 1H, CH), 7.61 (*dddd*, *J* = 8.3 Hz, *J* = 8.3 Hz, *J* = 1.2 Hz, *J* = 1.2 Hz, 1H, CH), 7.53 (*ddd*, *J* = 8.3 Hz, *J* = 6.6 Hz, *J* = 1.4 Hz, 2H, CH), 7.35 (*dd*, *J* = 8.0 Hz, *J* = 7.2 Hz, 1H, CH), 7.31 (*d*, *J* = 8.0 Hz, 1H, CH), 6.82 (*dd*, *J* = 7.2 Hz, *J* = 1.2 Hz, 1H, CH), 4.26 (*bs*, 2H, NH_2); ^{13}C NMR (100 MHz, $CDCl_3$) δ 190.8, 145.5, 143.2, 138.6, 135.6, 133.0, 131.5, 129.6, 128.8, 128.7, 128.4, 124.6, 123.6, 123.5, 121.8, 119.0, 110.9; HRMS (ESI+) m/*z* calculated for $C_{19}H_{16}NO$ $[M+H]^+$, 274.1232, found 274.1232.

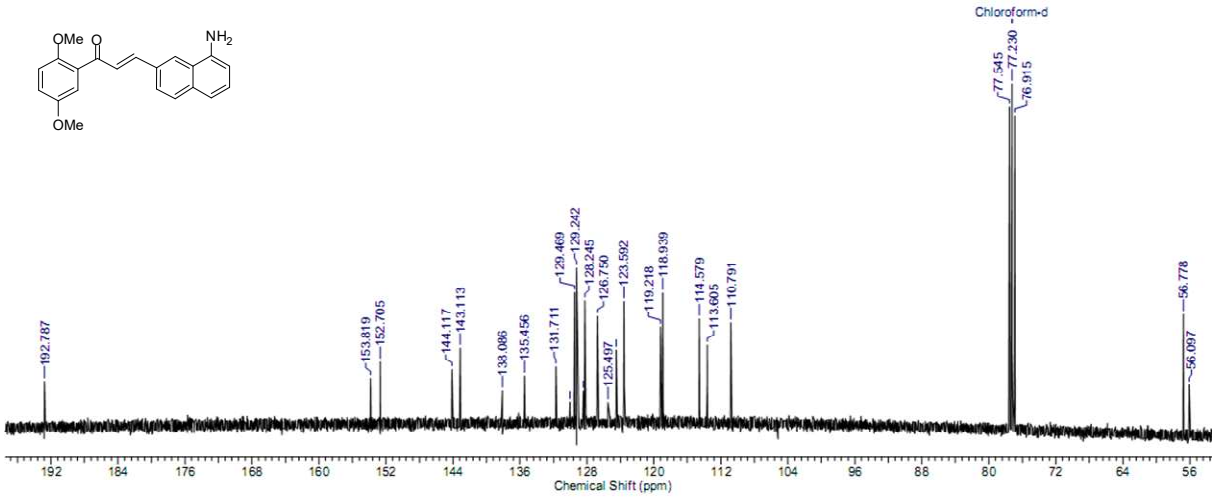


(E)-3-(8-aminonaphthalen-2-yl)-1-(2,5-dimethoxyphenyl)prop-2-en-1-one (7)

To a solution of 8-nitro-2-naphthaldehyde **2** (20 mg, 99.4 µmol) in absolute EtOH (800 µL) is added 2,5-dimethoxyacetophenone (18 mg, 100 µmol) followed by NaOH (3 mg, 75 µmol). The mixture is stirred at room temperature for 72 hrs, evaporated to dryness, and the residue retaken in AcOH (800 µL). Following priming with nitrogen and addition of Pd-C (10 %, 3

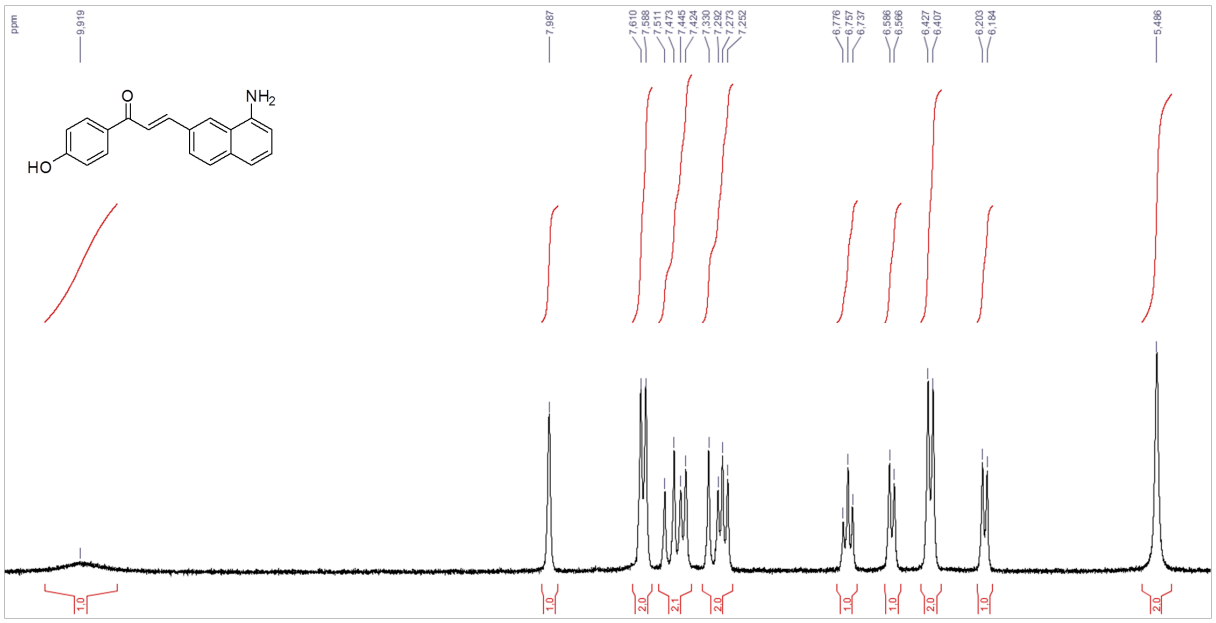
mg), the mixture is hydrogenated (1 atm) at room temperature for exactly 40 min as to minimize reduction of the carbon-carbon double bound. After priming with nitrogen and cooling in an ice bath, the reaction is treated with aqueous NaOH (1 M, 14 mL) and extracted with a CH_2Cl_2 / EtOAc 50:50 v/v mixture (3 x 5 mL). The organic phase is dried (Na_2SO_4) and evaporated under reduced pressure. The crude product is purified by column chromatography (SiO_2, EtOAc / *n*-hexane 27:73 v/v), furnishing 2',5'-dimethoxy-3-aminobenzochalcone **7** (6 mg, 18 % for two steps) as a red crystalline solid (R_f = 0.33 in EtOAc / *n*-hexane 27:73 v/v, silica TLC). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.93 (*s*, 1H, CH), 7.80 (*d*, *J* = 16.0 Hz, 1H, CH), 7.79 (*d*, *J* = 8.4 Hz, 1H, CH), 7.73 (*d*, *J* = 8.8 Hz, 1H, CH), 7.49 (*d*, *J* = 16.0 Hz, 1H, CH), 7.33 (*ddd*, *J* = 8.0 Hz, *J* = 7.4 Hz, *J* = 0.8 Hz, 1H, CH), 7.29 (*d*, *J* = 8.4 Hz, 1H, CH), 7.20 (*d*, *J* = 3.6 Hz, 1H, CH), 7.04 (*dd*, *J* = 9.2 Hz, *J* = 3.2 Hz, 1H, CH), 6.96 (*d*, *J* = 9.2 Hz, 1H, CH), 6.80 (*d*, *J* = 7.2 Hz, 1H, CH), 4.24 (*bs*, 2H, NH_2), 3.88 (*s*, 3H, CH_3), 3.82 (*s*, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 192.8, 153.8, 152.7, 144.1, 143.1, 135.4, 131.7, 130.0, 129.5, 128.2, 126.7, 124.5, 123.6 (two peaks), 119.2, 118.9, 114.6, 113.6, 110.8, 56.8, 56.1; HRMS (ESI+) m/*z* calculated for $C_{21}H_{20}NO_3$ $[M+H]^+$, 334.1443, found 334.1443.

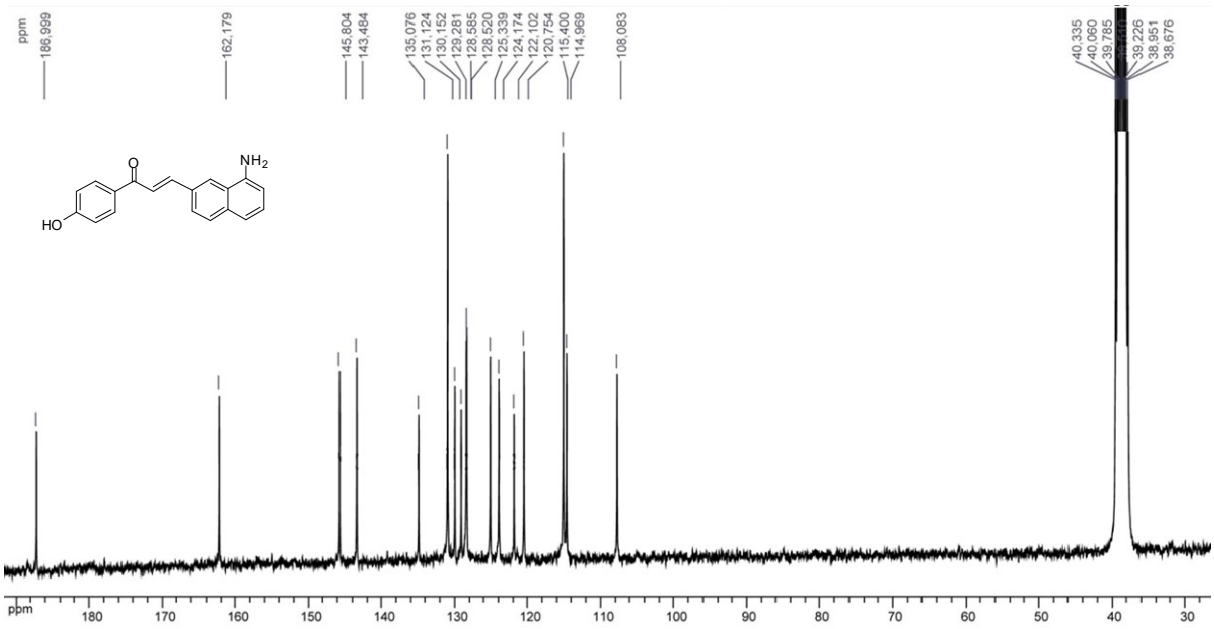




(E)-3-(8-aminonaphthalen-2-yl)-1-(4-hydroxyphenyl)prop-2-en-1-one (HAB) 8

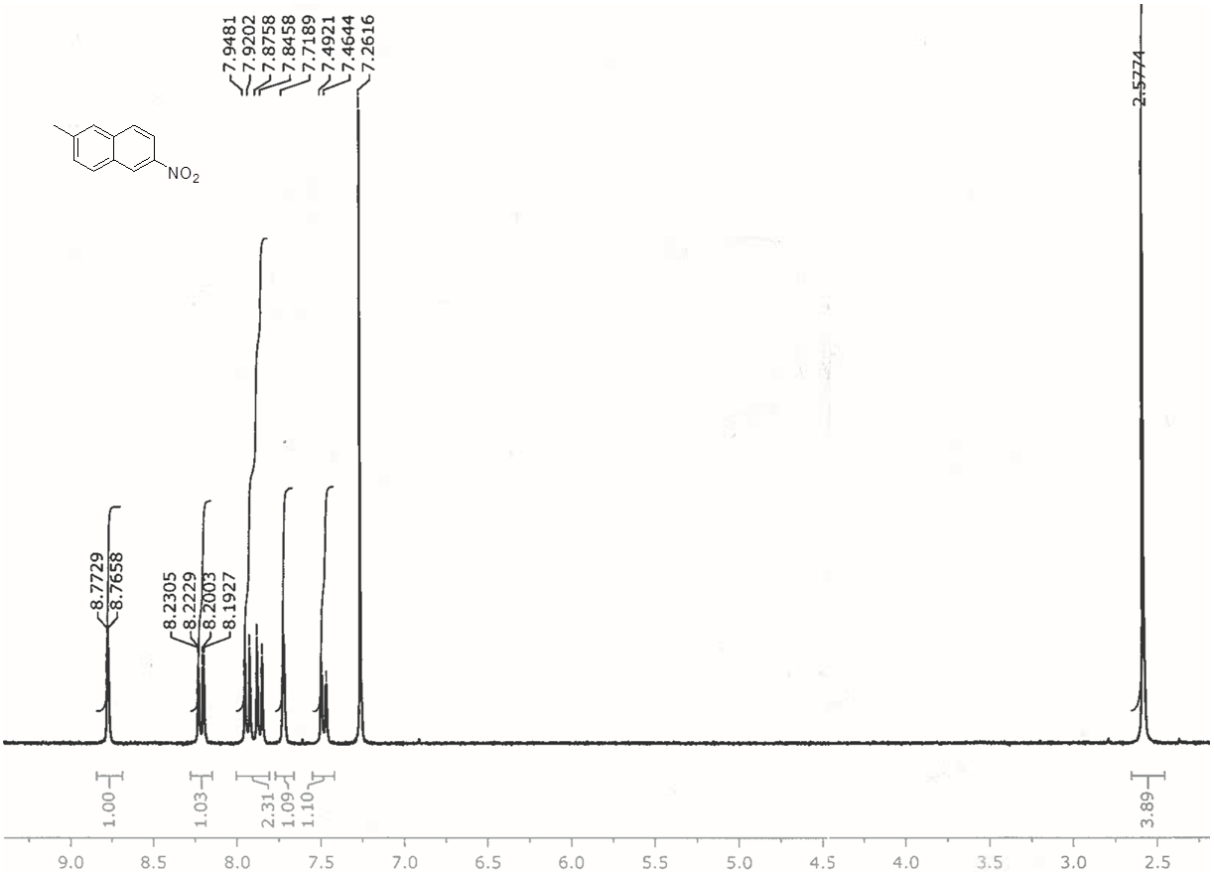
To a solution of 8-nitro-2-naphthaldehyde **2** (20 mg, 99.4 µmol) in absolute EtOH (800 µL) is added 4-hydroxyacetophenone (14 mg, 103 µmol) followed by NaOH (8 mg, 200 µmol). The mixture is stirred at room temperature for 72 hrs then treated with $SnCl_2$ (188 mg, 0.99 mmol). The mixture is further stirred for 4 hrs then sequentially treated with water (1 mL), aqueous NaOH (1 M, 2 mL), and aqueous saturated $NaHCO_3$ (2 mL). The mixture is extracted with a CH_2Cl_2 / EtOAc 50:50 v/v mixture (5 x 5 mL) (10 mL). The organic phase is dried (Na_2SO_4) and evaporated under reduced pressure. The crude product is purified by column chromatography (SiO_2, EtOAc / *n*-hexane 40:60 v/v), furnishing 4'-hydroxy-3-aminobenzochalcone **8** (20 mg, 69 % for two steps) as an orange solid (R_f = 0.35 in EtOAc / cyclohexane 40:60 v/v, silica TLC). ^{1}H NMR (400 MHz, d$_6$-DMSO) δ 9.92 (*bs*, 1H, OH), 7.99 (*s*, 1H, CH), 7.60 (*d*, *J* = 8.4 Hz, 2H, CH), 7.49 (*d*, *J* = 15.2 Hz, 1H, CH), 7.43 (*d*, *J* = 8.4 Hz, 1H, CH), 7.31 (*d*, *J* = 15.2 Hz, 1H, CH), 7.26 (*d*, *J* = 8.4 Hz, 1H, CH), 6.76 (*dd*, *J* = 7.6 Hz, *J* = 8.0 Hz, 1H, CH), 6.58 (*d*, *J* = 8.0 Hz, 1H, CH), 6.42 (*d*, *J* = 8.4 Hz, 2H, CH), 6.19 (*d*, *J* = 7.6 Hz, 1H, CH), 5.49 (*bs*, 2H, NH_2); ^{13}C NMR (100 MHz, d$_6$-DMSO) δ 187.0, 162.2, 145.8, 143.5, 135.1, 131.1, 130.1, 129.3, 128.6, 128.5, 125.3, 124.2, 122.1, 120.7, 115.4, 115.0, 108.1; HRMS (ESI+) m/*z* calculated for $C_{19}H_{16}NO_2$ $[M+H]^+$, 290.1181, found 290.1177.





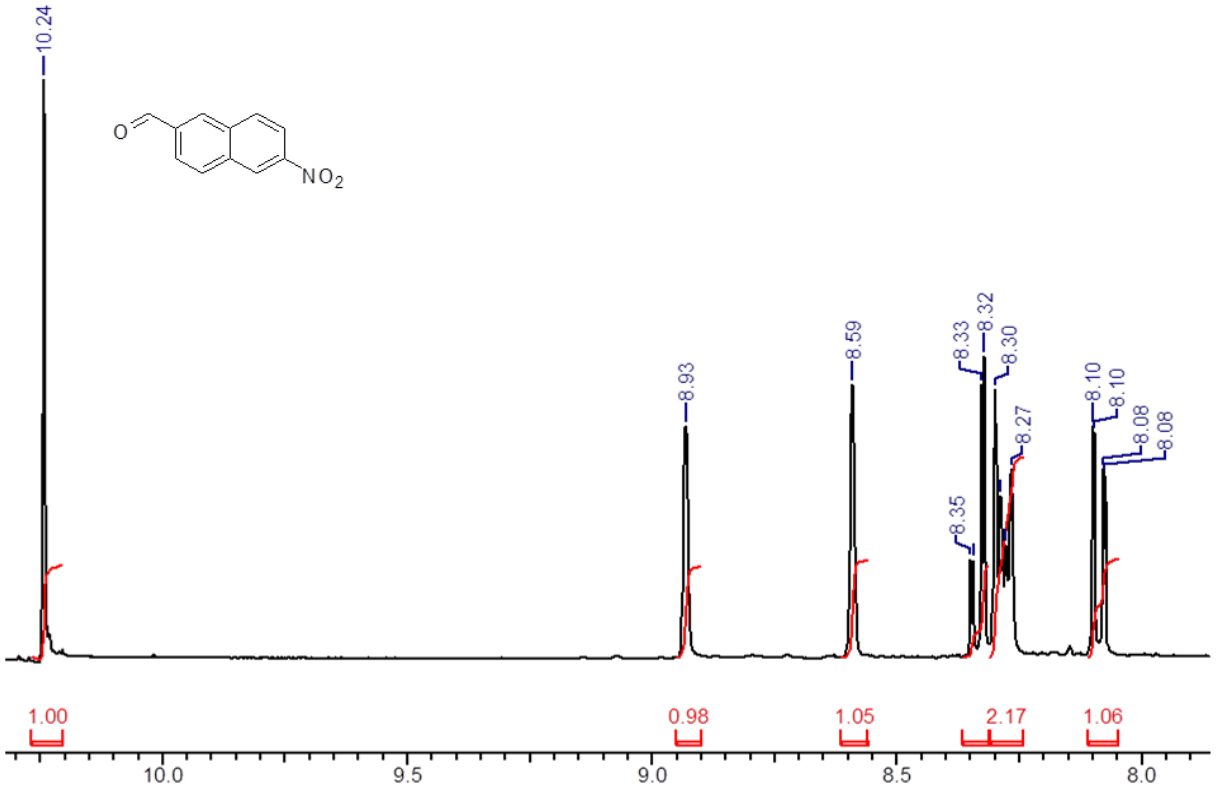
6-Nitro-2-methylnaphthalene (10) [*Described in ref. B*]

To a solution of β-methylnaphthalene **9** (5.0 g, 37.1 mmol) in acetic anhydride (90 mL), immersed in a water bath at room temperature, is added a solution of pure nitric acid (1.825 mL, 44.0 mmol) in acetic anhydride (10 mL) under stirring over the course of 15 minutes. The mixture is stirred 30 min at room temperature then slowly poured in a 50:50 mixture of ice and water (250 mL) under vigorous stirring. The yellow gum obtained is decanted then triturated with aqueous KOH 1N (50 mL), yielding a yellow precipitate which is abundantly washed with water then air-dried. The complex mixture of nitro-2-methylnaphthalene regioisomers is repeatedly chromatographed (SiO_2, toluene / *n*-hexane 10:90 v/v), yielding 6-nitro-2-methylnaphthalene **10** (765 mg, 11 %) as a pale-yellow crystalline solid (R_f = 0.21 in toluene / *n*-hexane 10:90 v/v, silica TLC). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.77 (*d*, *J* = 2.1 Hz, 1H, CH), 8.21 (*dd*, *J* = 2.3 Hz, *J* = 9.0 Hz, 1H, CH), 7.93 (*d*, *J* = 8.4 Hz, 1H, CH), 7.86 (*d*, *J* = 9.0 Hz, 1H, CH), 7.72 (*s*, 1H, CH), 7.48 (*d*, *J* = 8.4 Hz, 1H, CH), 2.58 (*s*, 3H, CH_3); HRMS (ESI+) m/*z* calculated for $C_{11}H_9NO_2Na$ $[M+Na]^+$, 210.0531, found 210.0539.



6-Nitro-2-naphthaldehyde (11)

A mixture of 6-nitro-2-methylnaphthalene **10** (535 mg, 2.86 mmol) and selenium oxide (634 mg, 5.72 mmol) is homogeneized in a mortar into a fine powder. The resulting mixture is packed in a thick-wall glass tube equipped with a gas outlet then heated at 150 °C for 15 hrs. The cooled mixture is extracted with $CHCl_3$ (5 x 8 mL), filtered over a pad of celite-545®, and evaporated under reduced pressure. The crude product is chromatographed (SiO_2, EtOAc / *n*-hexane 10:90 v/v), yielding by order of elution recovered 6-nitro-2-methylnaphthalene **10** (91 mg, 17 %) and 6-nitro-2-naphthaldehyde **11** (161 mg, 28 %, 45 % based on conversion) as an orange crystalline solid (R_f = 0.31 in EtOAc / *n*-hexane 10:90 v/v, silica TLC). ^{1}H NMR (400 MHz, $CDCl_3$ / d_6-DMSO 75:25 v/v, calibrated on TMS) δ 10.24 (*s*, 1H, CH), 8.93 (*s*, 1H, CH), 8.59 (*s*, 1H, CH), 8.33 (*dd*, *J* = 4.0 Hz, *J* = 8.0 Hz, 1H, CH), 8.29 (*d*, *J* = 8.0 Hz, 1H, CH), 8.28 (*d*, *J* = 8.0 Hz, 1H, CH), 8.09 (*dd*, *J* = 1.5 Hz, *J* = 8.0 Hz, 1H, CH); ^{13}C NMR (100 MHz, $CDCl_3$ / d_6-DMSO 75:25 v/v, calibrated on $CDCl_3$) δ 190.8, 146.0, 135.4, 134.1, 133.9, 132.5, 130.4, 130.1, 123.5, 123.3, 119.1; HRMS (ESI+) m/*z* calculated for $C_{11}H_8NO_3$ $[M+H]^+$, 202.0504, found 202.0508.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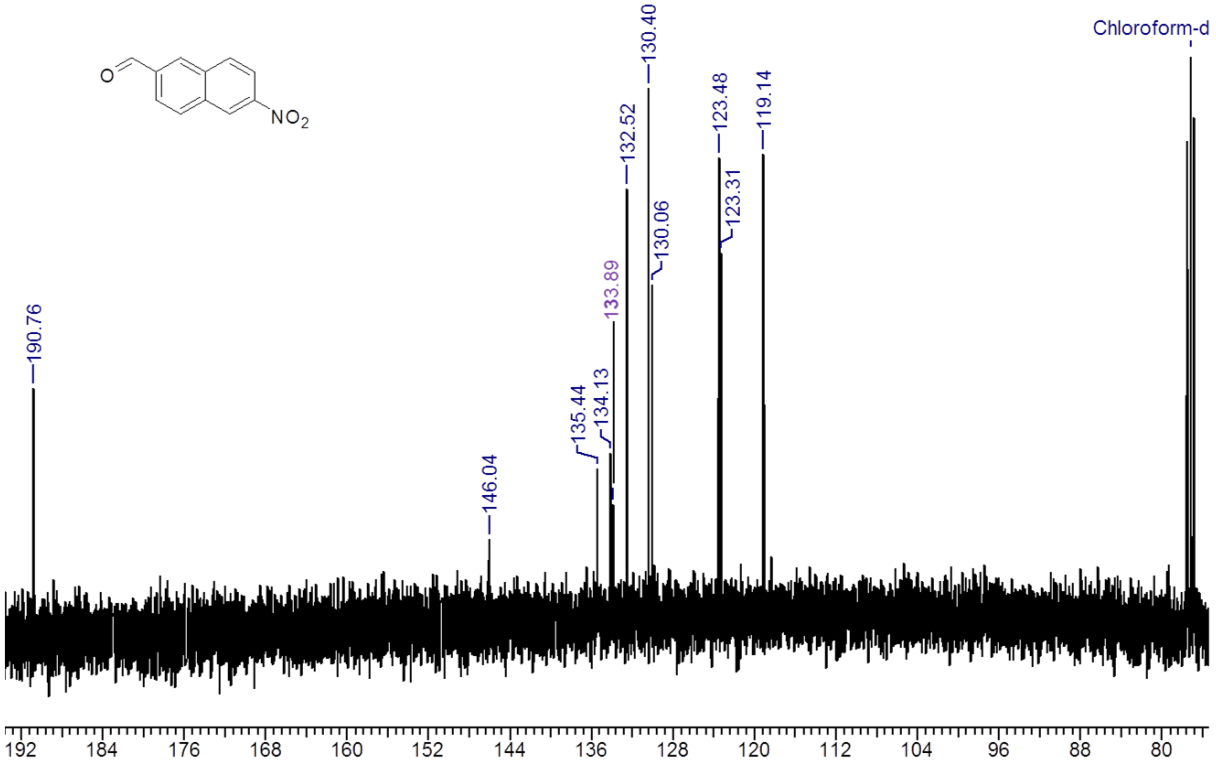
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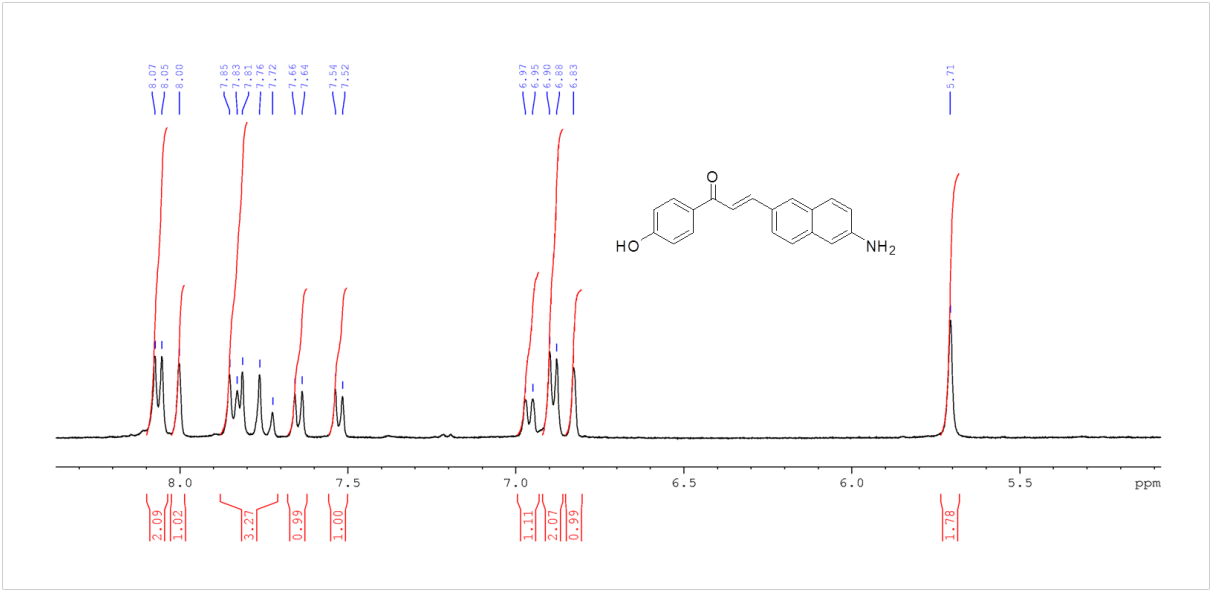
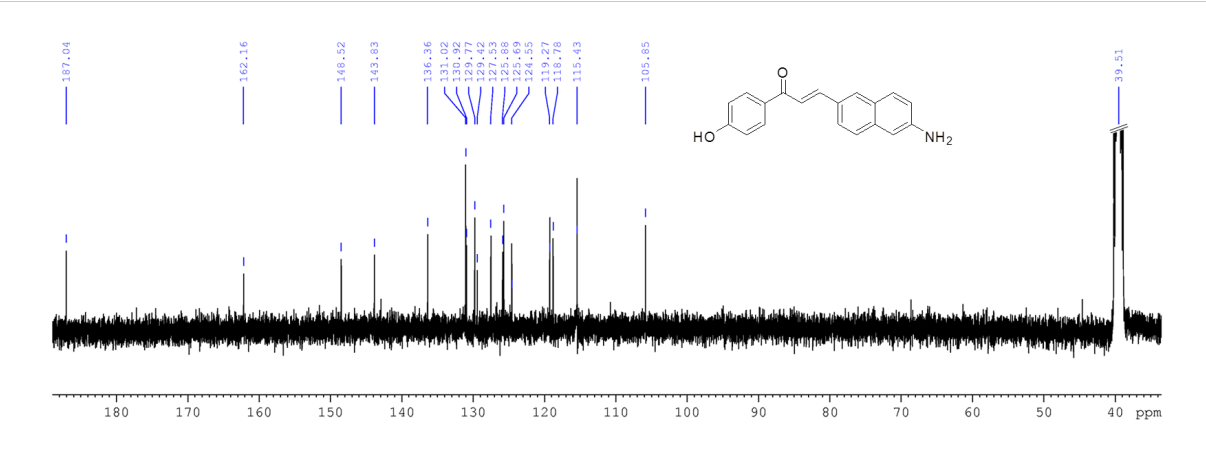
192
184
176
168
160
152
144
136
128
120
112
104
96
88
80

(E)-3-(6-aminonaphthalen-2-yl)-1-(4-hydroxyphenyl)prop-2-en-1-one (12)

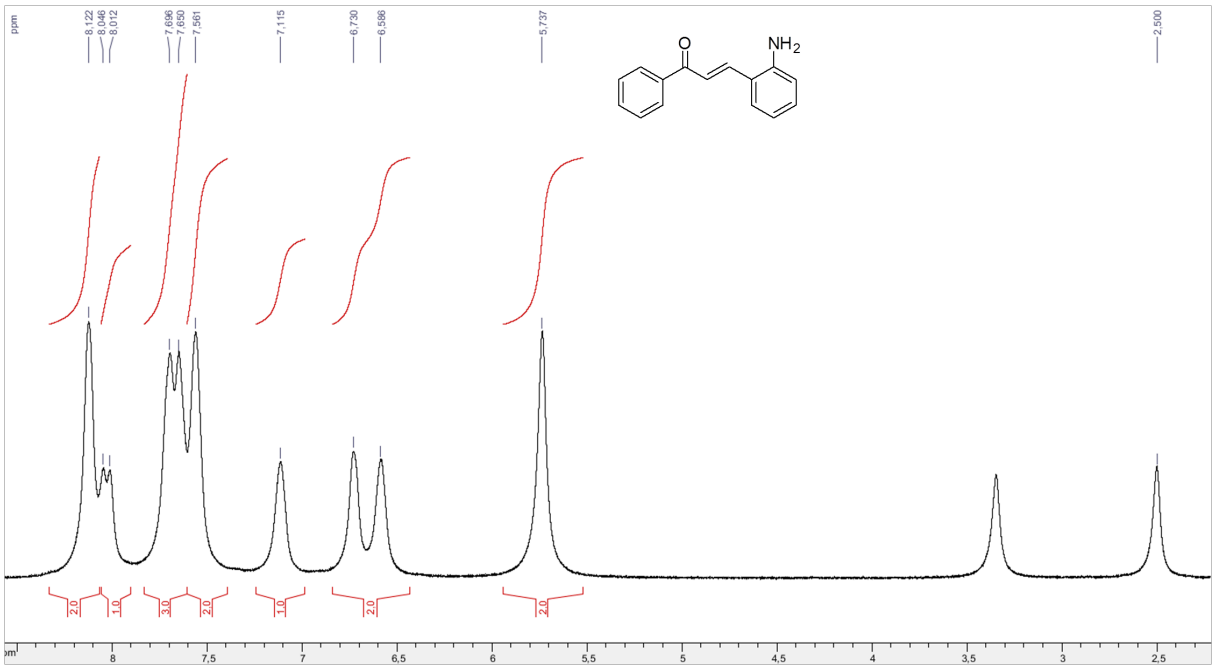
To a solution of 6-nitro-2-naphthaldehyde **11** (20 mg, 99.4 µmol) in absolute EtOH (800 µL) is added 4-hydroxyacetophenone (14 mg, 103 µmol) followed by NaOH (8 mg, 200 µmol). The mixture is stirred at room temperature for 72 hrs then diluted with AcOH (800 µL) and placed under an atmosphere of argon. Powdered iron (56 mg, 0.99 mmol) is added, and the mixture is refluxed under vigorous stirring for 15 hrs. The cooled reaction mixture is evaporated to dryness under reduced pressure, treated with aqueous saturated $NaHCO_3$ (5 mL) and extracted with EtOAc (5 x 5 mL). The organic phase is dried (Na_2SO_4) and evaporated under reduced pressure, yielding and orange solid. The crude product is purified by column chromatography (SiO_2, EtOAc / *n*-hexane 40:60 v/v), furnishing 4'-hydroxy-5-aminobenzochalcone **12** (16 mg, 56 % for two steps) as an orange solid (R_f = 0.30 in EtOAc / cyclohexane 45:55 v/v, silica TLC). ^{1}H NMR (400 MHz, d_6-DMSO) δ 8.06 (d, *J* = 8.0 Hz, 2H, CH), 8.0 (*s*, 1H, CH), 7.84 (*d*, *J* = 8.0 Hz, 1H, CH), 7.83 (*d*, *J* = 16.0 Hz, 1H, CH), 7.74 (*d*, *J* = 16.0 Hz, 1H, CH), 7.65 (*d*, *J* = 8.0 Hz, 1H, CH), 7.53 (*d*, *J* = 8.0 Hz, 1H, CH), 6.96 (*d*, *J* = 8.0 Hz, 1H, CH), 6.89 (*d*, *J* = 8.0 Hz, 2H, CH), 6.83 (*s*, 1H, CH), 5.71 (*bs*, 2H, NH2)); ^{13}C NMR (100 MHz, d_6-DMSO) δ 187.0, 162.2, 148.5, 143.8, 136.4, 131.0, 130.9, 129.8, 129.4, 127.5, 125.9, 125.7, 124.5, 119.3, 118.8, 115.4, 105.8; HRMS (ESI+) m/*z* calculated for $C_{19}H_{16}NO_2$ $[M+H]^+$, 290.1181, found 290.1180.

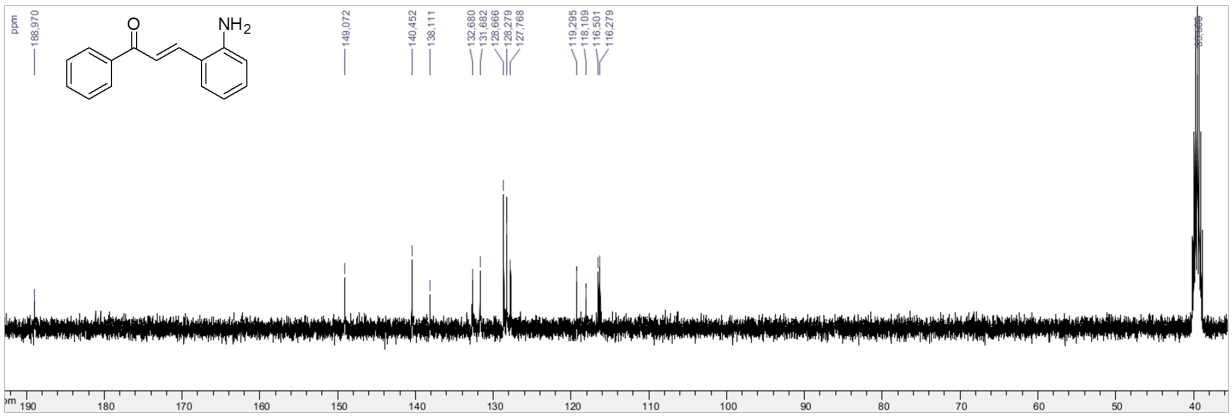




(E)-3-(2-aminophenyl)-1-phenylprop-2-en-1-one (14)

To a solution of 2-nitrobenzaldehyde **13** (100 mg, 0.66 mmol) in absolute EtOH (1.5 mL) is added acetophenone (80 mg, 0.66 mmol) followed by NaOH (26.5 mg, 0.66 mmol). The mixture is stirred at room temperature for 72 hrs then evaporated to dryness, and the residue is retaken in AcOH (5 mL). Following priming with nitrogen and addition of Pd-C (10 %, 25 mg), the mixture is hydrogenated (1 atm) at room temperature for exactly 25 min to minimize degradation of the unstable product formed. After priming with nitrogen, the mixture is quickly filtered over a pad of celite-545® that is further rinsed with a MeCN / H_2O 6:4 v/v mixture (*ca.* 5 mL) until a final volume of 10 mL is obtained. The solution of crude product is purified by preparative RP-C_{18} column chromatography (MeCN / H_2O 6:4 v/v) at a flow rate of 20 mL / min. The selected fractions are deprived of MeCN by evaporation under reduced pressure resulting in the precipitation of pure 2-aminochalcone **14** (18 mg, 12 % for two steps) as a curry-yellow solid (R_f = 0.26 in EtOAc / cyclohexane 25:75 v/v, silica TLC). ^{1}H NMR (400 MHz, d_6-DMSO, c = 5-30 mg/mL) δ 8.12 (*bs*, 2H, CH), 8.03 (*d*, *J* = 16.0 Hz, 1H, CH), 7.67 (*d*, *J* = 16.0 Hz, 1H, CH), 7.67 (*bs*, 2H, CH), 7.56 (*bs*, 1H, CH), 6.73 (*bs*, 1H, CH), 6.59 (*bs*, 1H, CH), 5.74 (*bs*, 2H, NH_2); ^{13}C NMR (100 MHz, $CDCl_3$) δ 189.0, 149.1, 140.4, 138.1, 132.7, 131.7, 128.7, 128.3, 127.8, 119.3, 118.1, 116.5, 116.3; HRMS (ESI+) m/*z* calculated for $C_{15}H_{14}NO$ $[M+H]^+$, 224.1075, found 224.1073.





Photophysical study.

Fluorescence emission spectra were recorded on a Hitachi F4500 spectrofluorimeter equipped with a thermostated cell holder set at 20.0 ± 0.1 °C, and the samples were placed in a quartz cell of 10 mm path length. Both slit width settings of excitation and emission monochromators were adjusted to 5.0 nm. Stock solutions of chalcones (10 mM) were made in $CHCl_3$ (when using *n*-hexane, *n*-heptane or *n*-octane for fluorescence measurement) or in DMSO (when using all other solvents described in the study). Final concentration of compound (50 μM) was obtained by diluting 10 μL of the stock solutions in 1990 μL of the desired solvent. Fluorescence quantum yields were measured using coumarin 153 in EtOH (Φ = 0.38) or ICG in DMSO as the standards (Φ = 0.12) according to [C] and [D], respectively. Regarding fluorescence experiments of **HAB** in biological media, we used TRIS (10 mM, pH 7.0)

containing NaCl (100 mM) and $MgCl_2$ (5 mM). The protein used was bovine serum albumin fraction V (66 kDa, Sigma).

Table S1: Photophysical properties of selected 3-amino- and 5-aminobenzochalcones and reference fluorophores including 2-aminochalcone 14

Compound	B ring	λ_{ex} [nm] [a]	λ_{em} [nm] [a]	Δ[b]	Φ[a,c]
3 (4'-CF_3, 3-NH_2)	Naph.	430	567	137	38.2 ± 1.9
6 (4'-H, 3-NH_2)	Naph.	417	552	135	46.5 ± 2.2
8 (4'-OH, 3-NH_2)	Naph.	411	548	137	54.0 ± 0.3
12 (4'-OH, 5-NH_2)	Naph.	402	512	110	2.4 ± 0.2
14 (4'-H, 2-NH_2)	Ph.	405	497	92	5.5 ± 1.0
DPP[d]	-	321	483	162	21.0 ± 0.8
ICG[e]	-	795	824	29	8.3 ± 0.5[f]

[a] Measured in toluene. [b] Stokes shift. [c] Relative fluorescence quantum yields (in %) measured using coumarin 153 in EtOH as the standard (Φ = 0.38)[C]. [d] *N*-Dansyl-*N'*-phenylpiperazine. [e] Indocyanine green®. [f] Fluorescence quantum yield (in %) measured using ICG in DMSO as the standard (Φ = 12.0) [D].

Biology

Reagents

3-Nitro-2'-hydroxy-4',6'-dimethoxychalcone **15**, flavokawain A **16** (4,4',6'-trimethoxy-2'-hydroxychalcone) and 4-methylchalcone **19** were prepared by Claisen-Schmidt condensation according to [E]. Cardamonin **17** (2',4'-dihydroxy-6-methoxychalcone), 4-hydroxy-3-methoxy-4'-chlorochalcone **18**, and Sudan Black were purchased from Sigma-Aldrich.

Microscopic imaging in live zebrafish embryo and larva.

Zebrafish care and maintenance. Wild-type AB fish were obtained from the Zebrafish International Resource Center (Eugene, OR). The Tg(*mpx*:GFP)^{i114}, *mfap-4*:mCherry-F, Tg(UAS-E1b:nfsB.mCherry)^{c264} transgenic zebrafish lines have been previously described [F]. The Tg(*mpx*:GAL4.VP16)^{i222} line was kindly provided by Prof. S. Renshaw (Sheffield University, Sheffield, UK). Eggs were obtained by marble-induced spawning, bleached according to protocols described in The Zebrafish Book [G], and then kept in Petri dishes containing Volvic source water and, from 24 hours post fertilization (hpf) onwards, with 0.003% 1-phenyl-2-thiourea (Sigma-Aldrich) to prevent melanin synthesis. Embryos were reared at 28 °C or 24 °C according to the desired speed of development. All timings in the

text refer to the developmental stage at the reference temperature of 28.5°C [H]. Embryos and larvae were anaesthetized with 200 µg/ml tricaine (Sigma-Aldrich) during the *in vivo* imaging.

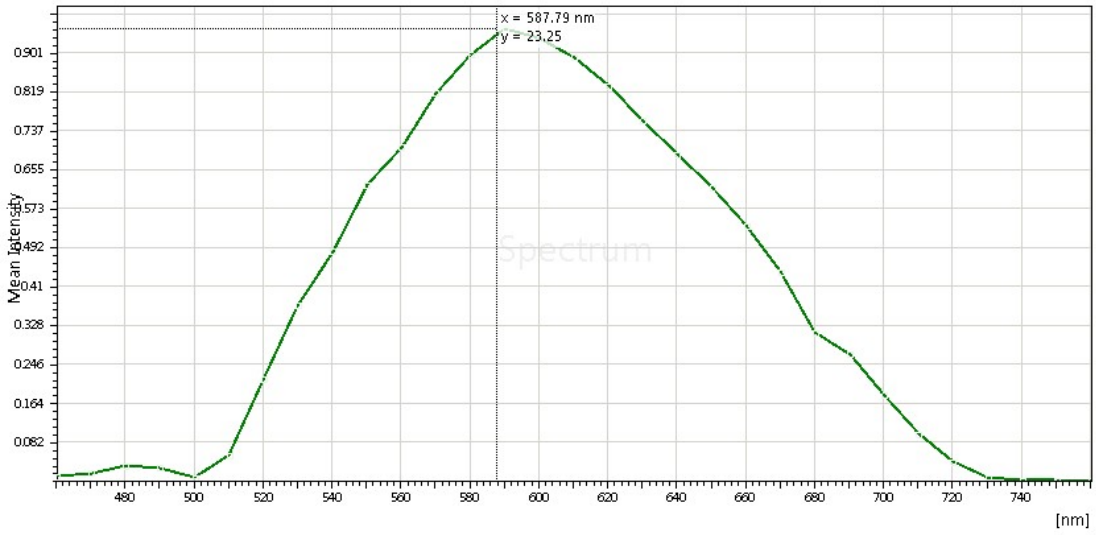


Figure S2. HAB fluoresces mainly in the yellow-orange region of the visible spectrum when excited at 448 or 488 nm *in vivo*. 72 hpf zebrafish larva was incubated with **HAB** (10 µM) for 1 hr at room temperature, anaesthetized, and imaged with a SP8 confocal microscope. Regions of interest (ROI) corresponding to magnified sections of neutrophiles granules population (one per cell) were selected for fluorescence analysis. The emission spectrum shown is representative of several ROIs.

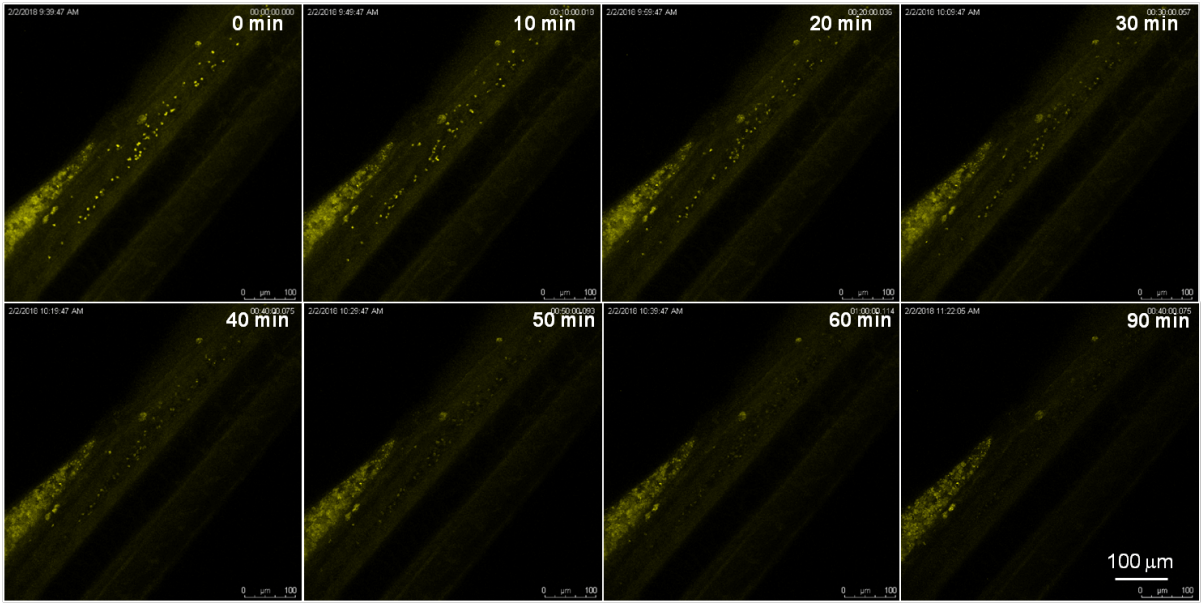


Figure S3. HAB freely diffuses outside live zebrafish neutrophil granules over time. A 72 hpf zebrafish larva was incubated with **HAB** (10 μM) for 1 hr at room temperature, washed, anaesthetized, and time-lapse imaged in the ventral tail to analyze **HAB** stained granule dynamics under conditions of minimal photobleaching by acquisition performed every 10 min. Frames extracted at various time points from the maximum intensity projection of the acquisition, following excitation at 488 nm and emission collected between 550-650 nm are shown, demonstrating a progressive loss of **HAB** signal over time.

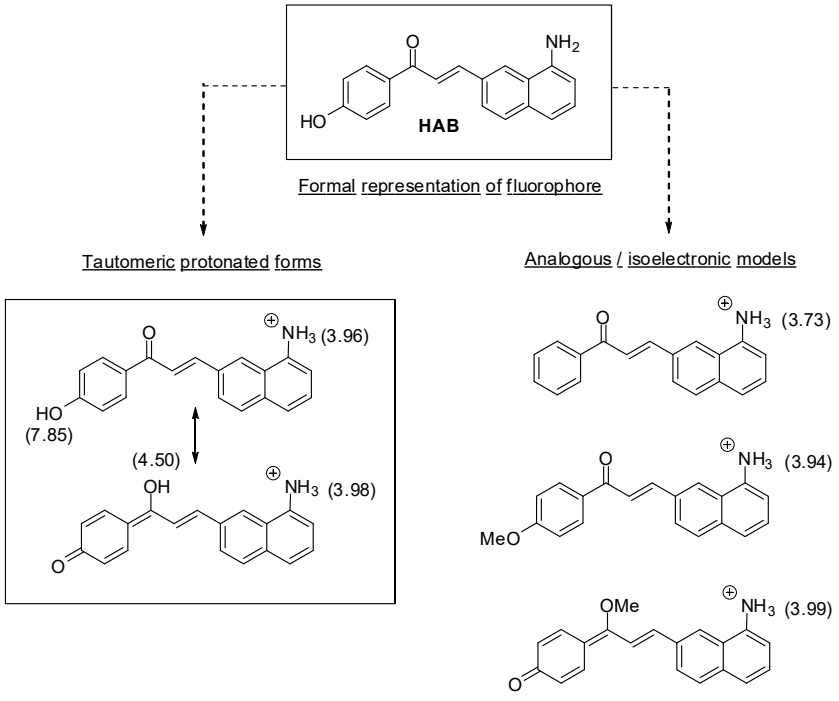
Video S4. HAB is highly photostable in live zebrafish neutrophil granules. A 72 hpf zebrafish larva was incubated with **HAB** (10 μM) for 1 hr at room temperature, anaesthetized in tricaine containing **HAB** (10 μM), mounted in agarose containing **HAB** (10 μM), and time-lapse imaged in the ventral tail to analyze **HAB** stained granule dynamics under conditions of maximal photobleaching by continuous illumination by the 488 nm laser (time-lapse interval of 20 sec and 27 serial Z-optical sections of 2 μm were imaged). The maximum intensity projection is shown.

Video S5 (related to Fig. 6): High-resolution confocal imaging of HAB in zebrafish larvae harboring mCherry neutrophils. A 72 hpf transgenic zebrafish larva harbouring mCherry + neutrophils was incubated with **HAB** (10 μM) for 1 hr at room temperature then anaesthetized and mounted in **HAB** containing medium as specified in Video S4, and imaged in the ventral tail region. The **HAB** labeled mCherry+ neutrophils were observed at high resolution using a confocal fluorescence microscope where the fluorescence (**HAB**: EX 448 nm, EX 550-650 nm; mCherry: EX 552 nm, EM 660-750 nm) was combined with DIC microscopy to visualize the neutrophil intracellular structures. A Z-stack movie of 43 optical sections (0.4 μm each) is shown, with the single fluorescence channels on the left (mCherry, HAB), followed by the DIC channel and the overlay of the fluorescence and DIC channels. Two neutrophils located at different Z planes are shown. Comparing the DIC and fluorescence images, it appears that **HAB** labels neutrophil granules that are, as expected, constantly moving into the cytoplasm and filling almost all the space therein. Scale bar: 5 μm

Video S6 (related to Fig. 6): **Dynamic co-localization of HAB with neutrophil granules *in vivo*.** A 72 hpf transgenic zebrafish larva harbouring mCherry-labeled neutrophils was incubated with **HAB** (10 μM) for 1 hr at room temperature then anaesthetized and mounted in

HAB containing medium as specified in Video S4, then time-lapse imaged in the ventral tail region to analyze **HAB** stained granule dynamics (time interval = 47 sec). A mCherry+ **HAB** labeled migrating neutrophil constantly changing its shape, with its highly dynamic moving granules is visible (top center) over time from the beginning until the end of the video. By 47" to 15':48" (time indicated top right on the video) two migrating neutrophils cross the field. By 21':20" to 33:12" four migrating neutrophils cross the field. Acquisition was performed for about 33 min. A single Z section of 1µm was selected for the video (on 34 Z section acquired). Combined single fluorescence (mCherry and **HAB**), DIC microscopy and merged images are shown (from left to right). Scale bar: 25 µm

Figure S7: pKa values of HAB were calculated using the ACD software (Advanced Chemistry Development Inc.), copyright® 1994-2002. Predictions (values in brackets) were done for the two amino tautomers of **HAB** along with isoelectronic and analogous compounds.



Video S8 (related to Figure 7d). HAB-labeled neutrophil granules are recruited to the forming phagosome during phagocytosis of zymosan by neutrophil. A 72 hpf zebrafish larva was incubated with **HAB** (10 µM) for 1h at room temperature to stain neutrophil granules, immediately injected subcutaneously with Cy5-zymosan (*ca.*500 zymosan particles) to analyse **HAB** labelled granules dynamics during neutrophil phagocytosis, and live imaged

every min. from 20 min p. i. (t = 0 on the movie) to 1 hr p. i. (t = 40 on the movie) by confocal fluorescence microscopy. Four **HAB**-labeled neutrophils were tracked over time while they engulf red zymosan (white arrows; labelled with number 1 to 4) to show the recruitment of **HAB**-positive granules to the nascent phagosome containing the zymosan particle. The maximum intensity projection from 41 Z-sections every 1 µm is shown. Scale bar: 10µm.

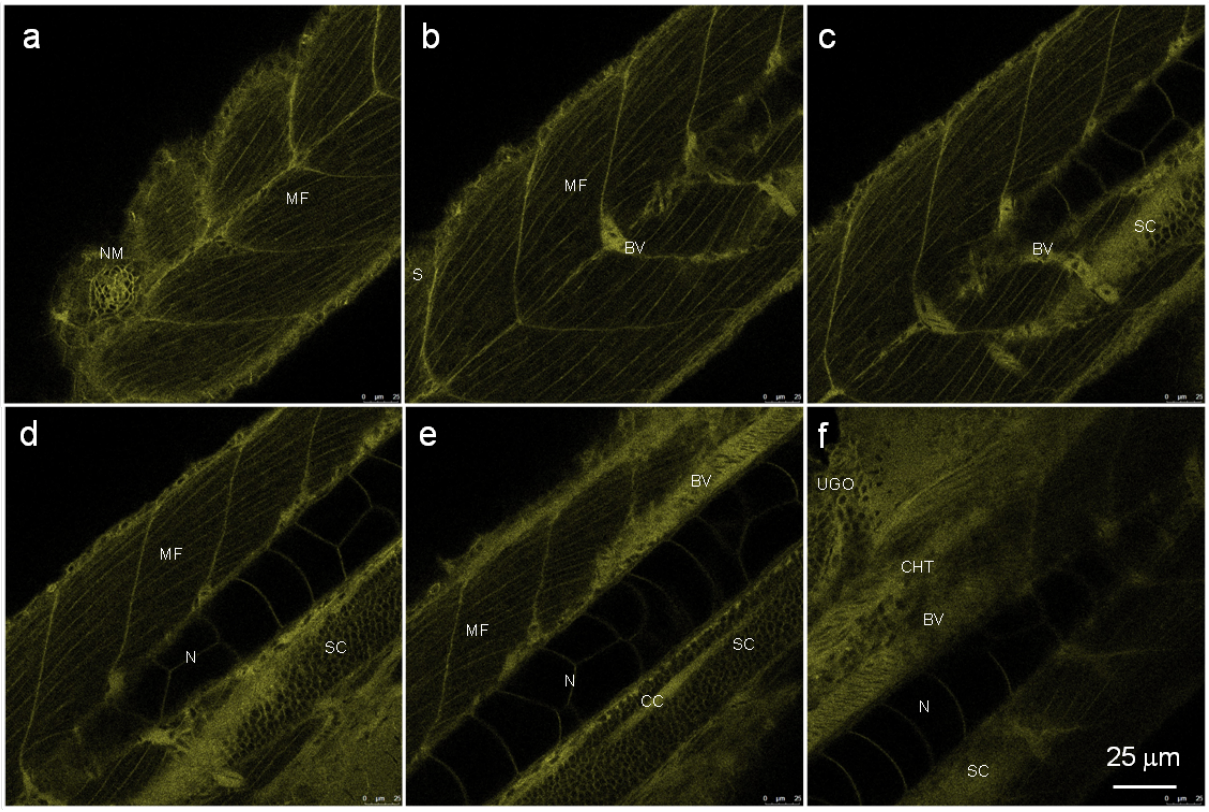


Figure S9. Chalcone 18 behaves as an interstitial fluorescent label of zebrafish histology *in vivo*. (**a-f**) Confocal fluorescence imaging of **18** (10 µM) in live transgenic 72 hpf zebrafish larvae following excitation at 488 nm and detection in the 550-650 nm range. Single 2 µm serial optical sections are shown. Abbreviations used: NM, trunk neuromast; MF, muscle fibers; S, somites; BV, blood vessels; N, notochord; SC, spinal cord; CC, central canal; CHT, caudal hematopoietic tissue; UGO, urogenital opening. Selected frames (2 µm serial optical sections) from a Z-stack are shown.

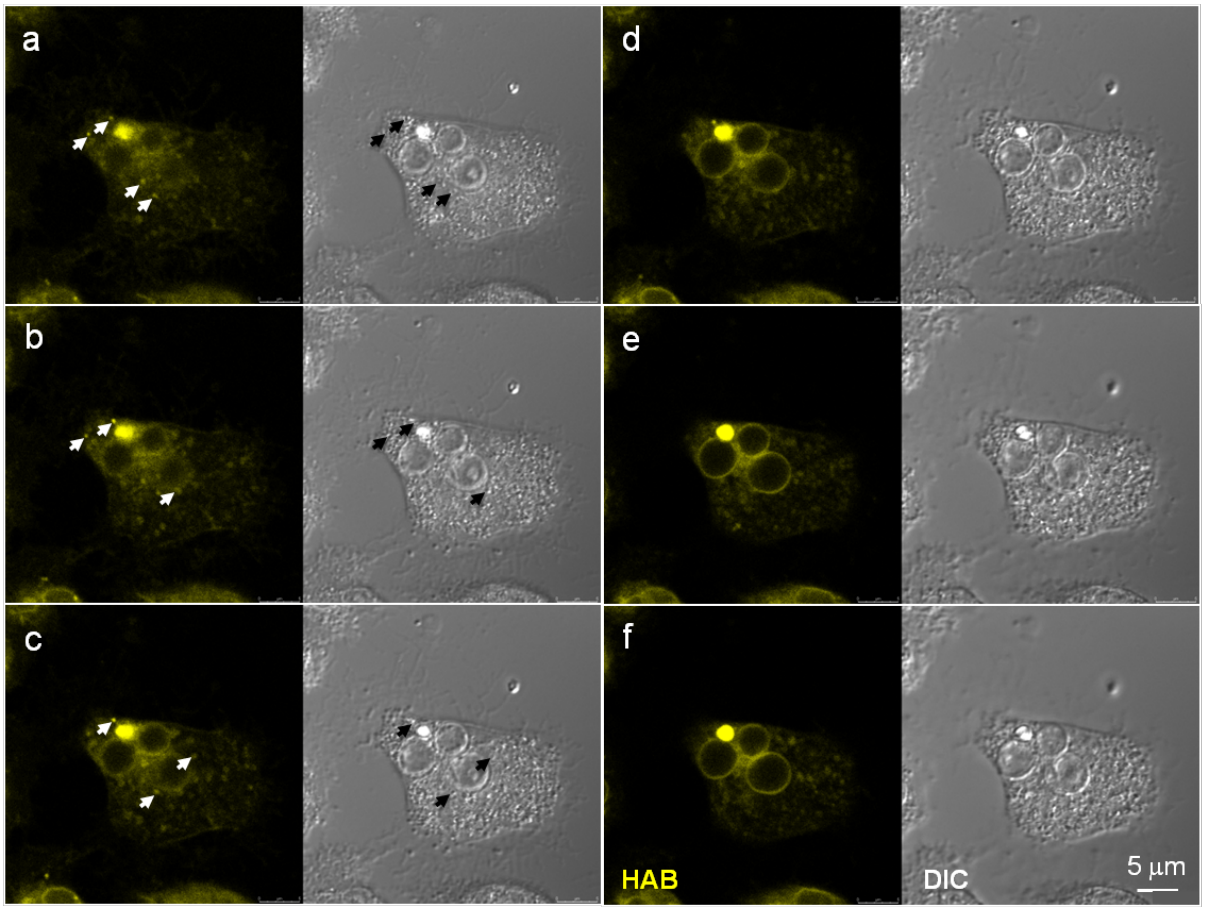


Figure S10 HAB labels granules in live human primary neutrophils. Confocal fluorescence (left) and DIC imaging (right) of **HAB** (10 µM) in live human neutrophils following excitation at 488 nm and detection in the 580-650 nm range. The yellow-orange color is indicative of the fluorescence seen with the naked eye. Single 0.4 µm serial optical sections are shown. Arrows point to the co-localisation of **HAB** signals with granular substructures seen by DIC imaging.

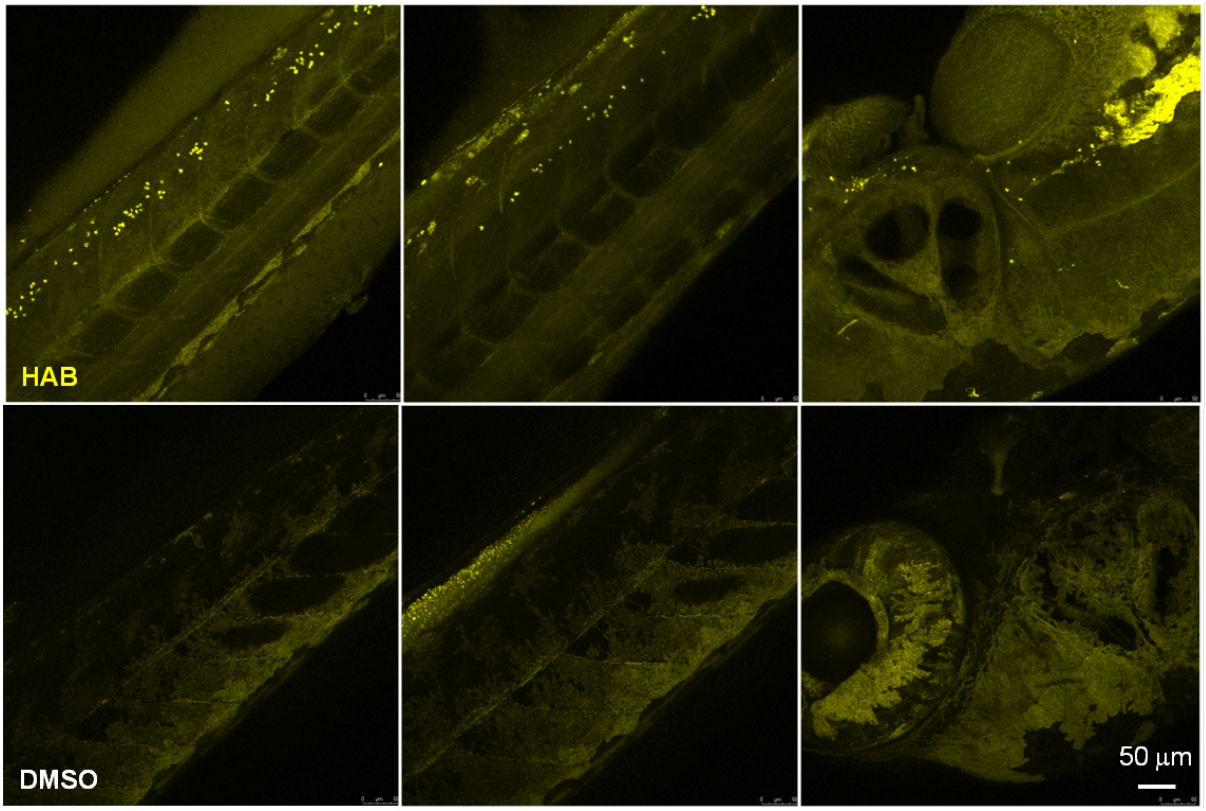


Figure S11. HAB labels 6 days-old zebrafish larva. A 6 days-old zebrafish larva was incubated with **HAB** (10 µM) for 1 hr at room temperature, anaesthetized, mounted in **HAB** containing medium then live imaged to analyze **HAB**. Top panel, **HAB** labeling of neutrophil granules in various regions of the larva, from the trunk to the head (left to right). Bottom panel, control DMSO-treated larva, to show the fluorescent background of zebrafish tissues at 6 dpf (from the trunk to the head, left to right). Maximum intensity projections are shown.

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