Supporting Information

A Chalcone-based Fluorescent Responsive Probe for Selective Detection of Nitroreductase Activity in Bacteria

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Materials and Methods

All reagents were purchased from commercial sources (Sigma Aldrich, TCI, Carlo Erba, Acros, and Merck) and used without further purification. The reactions were monitored by thin-layer chromatography carried out on silica gel plates (60F254, MERCK, Germany) and visualized by UV light (254 nm). Column chromatography was performed over silica gel 60 (70–230 mesh, MERCK, Germany). NMR spectra were recorded on a Bruker-500 MHz spectrometer (^{1}H at 500 MHz and ^{13}C at 125 MHz) at room temperature unless other mentioned. Chemical shifts of ^{1}H NMR are reported in ppm from the solvent resonance ($CDCl_3$ 7.26 ppm, CD_3OD 3.30 ppm). Data are reported as the followings: chemical shift, multiplicity (s = singlet, br = broad, d = doublet, t = triplet, q = quartet, m = multiplet), coupling constants, and number of protons. Proton decoupled ^{13}C NMR spectra were also recorded in ppm ($CDCl_3$ 77.0, CD_3OD 49.1 ppm). Analytical thin layer chromatography (TLC) was performed on EM Reagents 0.25 mm silica-gel 60-F plates and visualized with UV light. Flash chromatography was performed using silica gel 60 (230–400 mesh). Mass spectrometry was measured under ESI conditions.

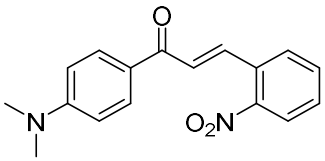
General procedure for the preparation of chalcones (3a-c)

4-Dimethylamino acetophenone **1** (0.67 mmol) and different substituted (*o*-, *m*-, or *p*-) nitrobenzaldehydes **2** (0.67 mmol) in MeOH (2 mL) were added in 2 mL of MeOH containing KOH (3.35 mmol). The mixture was stirred for 18 h at room temperature (25 ºC). After that, the yellow precipitate was filtered off followed by washing with ice cold MeOH (20 mL), and finally dried under vacuum. The products were obtained as the corresponding chalcones (**3a-c**).

General procedure for the reduction of chalcones (4a-c)

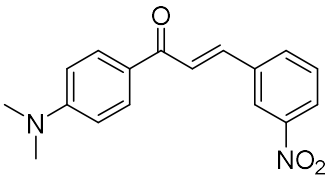
A mixture of chalcone (**3a**, **3b**, or **3c**) (50 mg, 0.168 mmol) and $SnCl_2.2H_2O$ (190 mg, 0.84 mmol) in THF (1 mL) was sonicated at 50 ºC for 20 min. The mixture was diluted with ethyl acetate (10 mL) and washed with brine three times. The organic layer was collected and dried with $MgSO_4$. After removal of the solvent, the product was purified on a silica-gel column using ethyl acetate/hexanes (1:1 v/v) as the eluent to give the corresponding product (**4a-c**) as a yellow solid.

1. ^{1}H NMR, ^{13}C NMR, and MS results of compound 3a-c



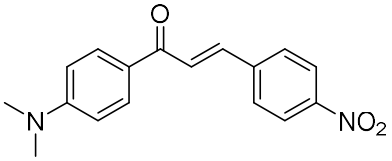
(*E*)-1-(4-(dimethylamino)phenyl)-3-(2-nitrophenyl)prop-2-en-1-one (3a)

95.30 mg, 48% yield; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.15 – 8.07 (m, 4H), 7.81 (d, J = 8.5 Hz, 1H), 7.75 (d, J = 8.0 Hz, 1H), 7.66 (ddt, J = 8.5, 6.5, 2.0 Hz, 1H), 7.43 (td, J = 6.5, 1.5 Hz, 1H), 6.82 (d, J = 9.0 Hz, 2H), 3.03 (s, 6H), ^{13}C NMR (125 MHz, $CDCl_3$) δ 157.4, 151.6, 136.6, 129.6, 129.3, 128.7, 127.5, 126.8, 125.6, 118.5, 112.4, 40.5, HRMS (ESI) calcd for $C_{17}H_{16}N_2Na_1O_3$ $[M+Na]^+$ = 319.1053, found 319.1061.



(*E*)-1-(4-(dimethylamino)phenyl)-3-(3-nitrophenyl)prop-2-en-1-one (3b)

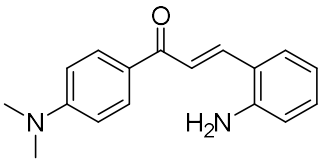
129.05 mg, 65% yield; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.49 (t, J = 2.0 Hz, 1H), 8.20 (ddd, J = 8.2, 2.3, 1.1 Hz, 1H), 8.01 (d, J = 9.0 Hz, 2H), 7.88 (d, J = 7.5 Hz, 1H), 7.77 (d, J = 16.0 Hz, 1H), 7.67 (d, J = 16.0 Hz, 1H), 7.57 (t, J = 8.0 Hz, 1H), 6.74 (d, J = 9.0 Hz, 2H), 3.09 (s, 6H), ^{13}C NMR (125 MHz, $CDCl_3$) δ 187.0, 153.7, 148.9, 139.7, 137.5, 134.5, 131.2, 130.1, 126.0, 125.1, 124.3, 122.2, 111.4, 40.5, HRMS (ESI) calcd for $C_{17}H_{16}N_2Na_1O_3$ $[M+Na]^+$ = 319.1053, found 319.1063.



(*E*)-1-(4-(dimethylamino)phenyl)-3-(4-nitrophenyl)prop-2-en-1-one (3c)

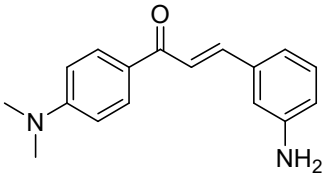
154.86 mg, 78% yield; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.24 (d, J = 9.0 Hz, 2H), 7.99 (d, J = 9.0 Hz, 2H), 7.77 (d, J = 15.5 Hz, 1H), 7.74 (d, J = 8.5 Hz, 2H), 7.66 (d, J = 15.5 Hz, 1H), 6.74 (d, J = 9.0 Hz, 2H), 3.09 (s, 6H), ^{13}C NMR (125 MHz, $CDCl_3$) δ 186.9, 153.7, 148.4, 142.0, 139.7, 131.3, 128.9, 126.3, 126.1, 124.4, 111.5, 40.5, HRMS (ESI) calcd for $C_{17}H_{16}N_2Na_1O_3$ $[M+Na]^+$ = 319.1053, found 319.1065.

2. ^{1}H NMR, ^{13}C NMR, and MS results of compound 4a-c



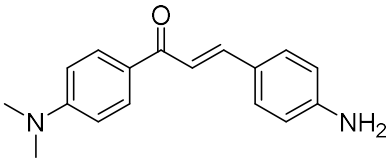
(*E*)-3-(2-aminophenyl)-1-(4-(dimethylamino)phenyl)prop-2-en-1-one (4a)

13.42 mg, 30% yield; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.07 (d, J = 9.0 Hz, 2H), 7.77 (d, J = 15.5 Hz, 1H), 7.57 (d, J = 15.5 Hz, 1H), 7.17 (t, J = 7.5 Hz, 1H), 7.07 (d, J = 8.0 Hz, 1H), 7.02 (t, J = 2.5 Hz, 1H), 6.84 (d, J = 9.0 Hz, 2H), 6.76 – 6.66 (m, 1H), 5.25 (s, 2H), 3.12 (s, 6H), ^{13}C NMR (125 MHz, DMSO) δ 186.6, 153.8, 149.5, 143.2, 136.0, 131.0, 129.8, 125.7, 121.7, 116.8, 116.5, 113.9, 111.3, 40.1, HRMS (ESI) calcd for $C_{17}H_{19}N_2O_1$ $[M+H]^+$ = 267.1492, found 267.1482.



(*E*)-3-(3-aminophenyl)-1-(4-(dimethylamino)phenyl)prop-2-en-1-one (4b)

18.77 mg, 42% yield; ^{1}H NMR (500 MHz, DMSO-*d6*) δ 8.07 (d, J = 9.0 Hz, 2H), 7.77 (d, J = 15.5 Hz, 1H), 7.56 (d, J = 15.5 Hz, 1H), 7.17 (t, J = 7.5 Hz, 1H), 7.07 (d, J = 7.5 Hz, 1H), 7.02 (t, J = 2.0 Hz, 1H), 6.84 (q, J = 2.0 Hz, 2H), 6.77 – 6.68 (m, 1H), 5.25 (s, 2H), 3.12 (s, 6H), ^{13}C NMR (125 MHz, DMSO) δ 186.6, 153.8, 149.5, 143.2, 136.0, 131.0, 129.8, 125.7, 121.7, 116.8, 116.5, 113.9, 111.3, 40.1, HRMS (ESI) calcd for $C_{17}H_{19}N_2O_1$ $[M+H]^+$ = 267.1490, found 267.1492.

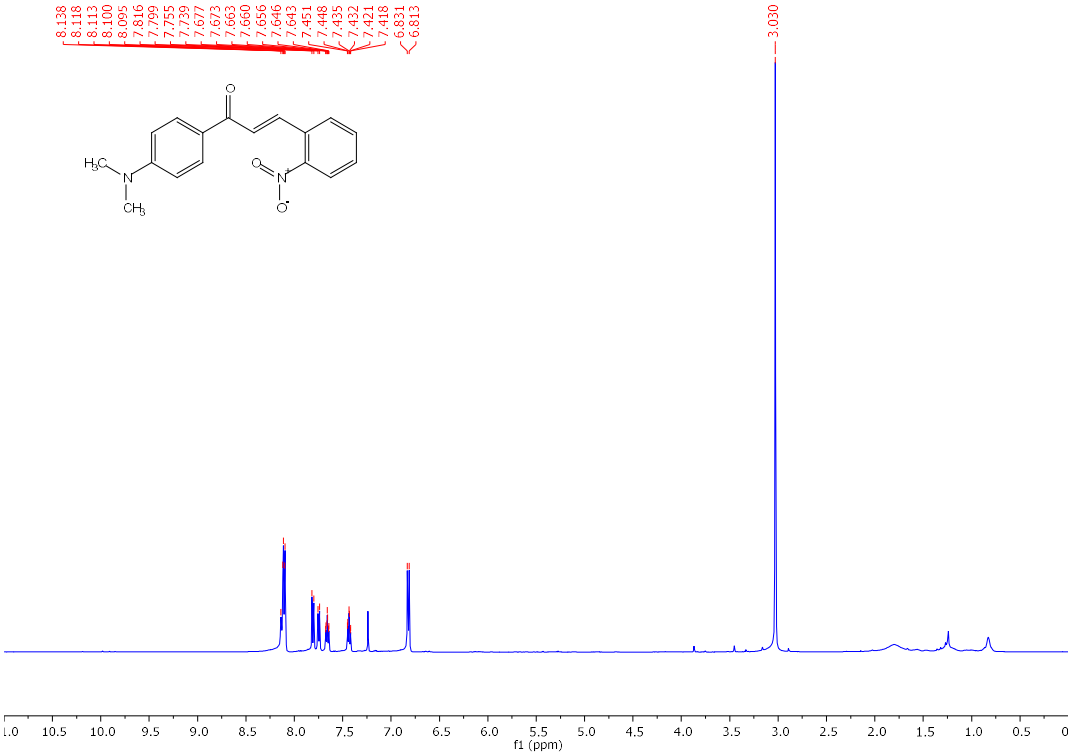


(*E*)-3-(4-aminophenyl)-1-(4-(dimethylamino)phenyl)prop-2-en-1-one (4c)

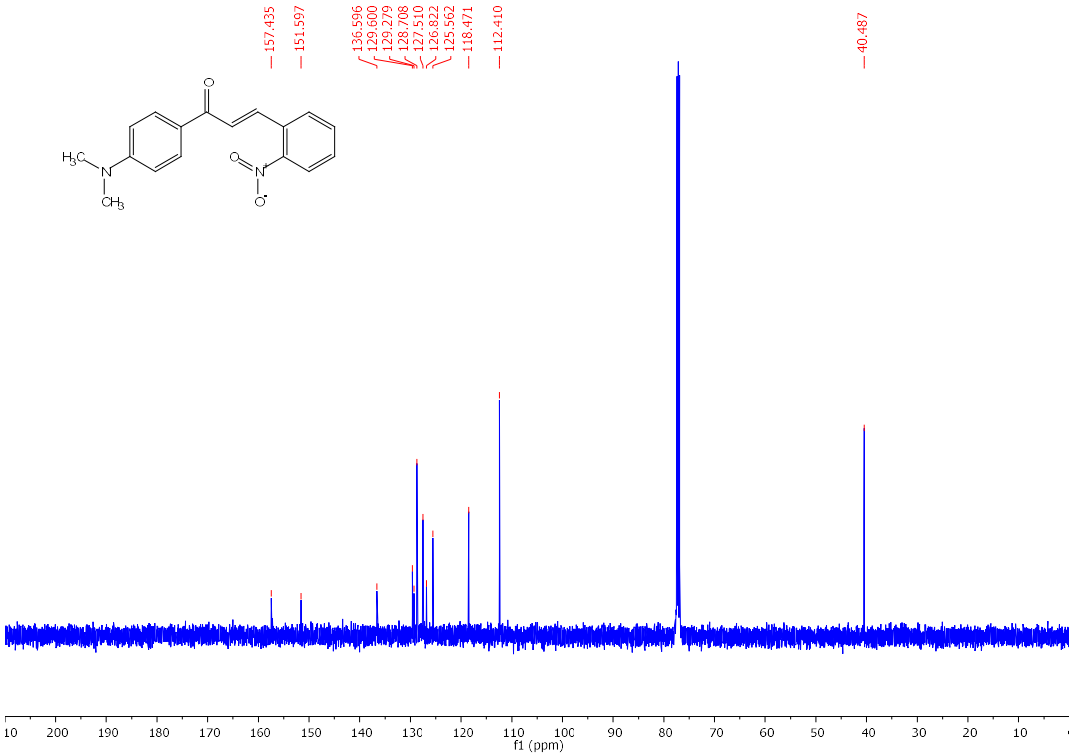
25.92 mg, 58% yield; ^{1}H NMR (500 MHz, DMSO-*d6*) δ 8.06 (d, J = 8.5 Hz, 2H), 7.62 – 7.57 (m, 4H), 6.82 (d, J = 9.0 Hz, 2H), 6.67 (d, J = 8.5 Hz, 2H), 5.83 (s, 2H), 3.11 (s, 6H), ^{13}C NMR (125 MHz, DMSO) δ 186.6, 153.5, 151.9, 143.6, 131.0, 130.7, 126.3, 122.9, 116.2, 114.1, 111.3, 40.1, HRMS (ESI) calcd for $C_{17}H_{19}N_2O_1$ $[M+H]^+$ = 267.1497, found 267.1492.

3. Spectrum NMR of chalcones (3a-c)

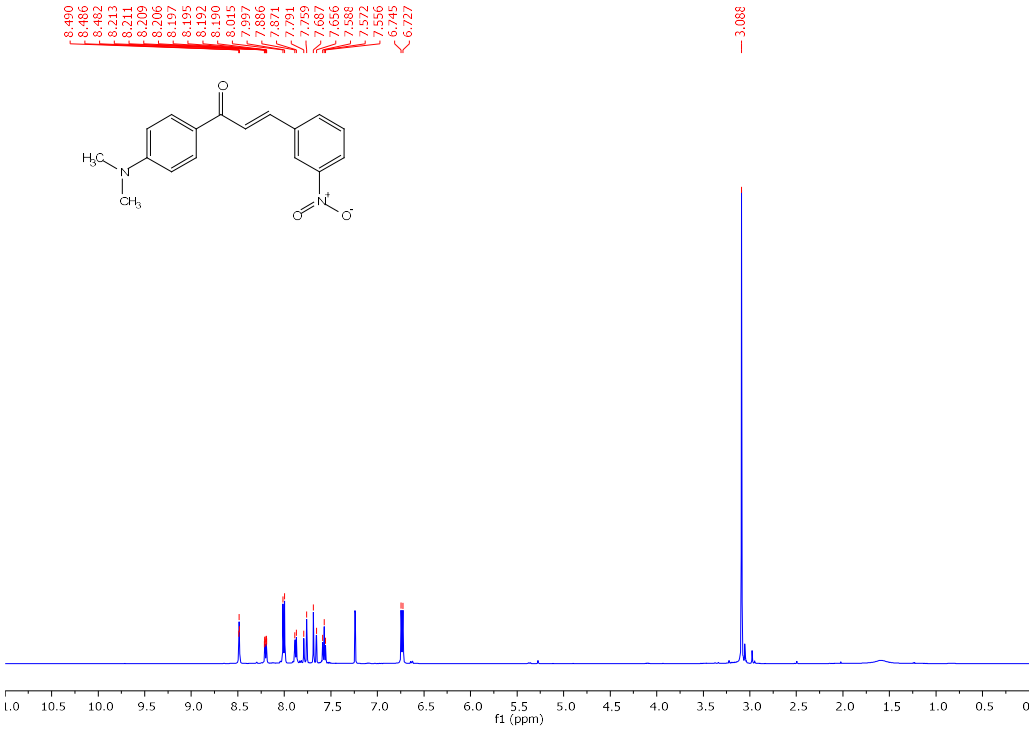
^{1}H NMR of compound **3a**



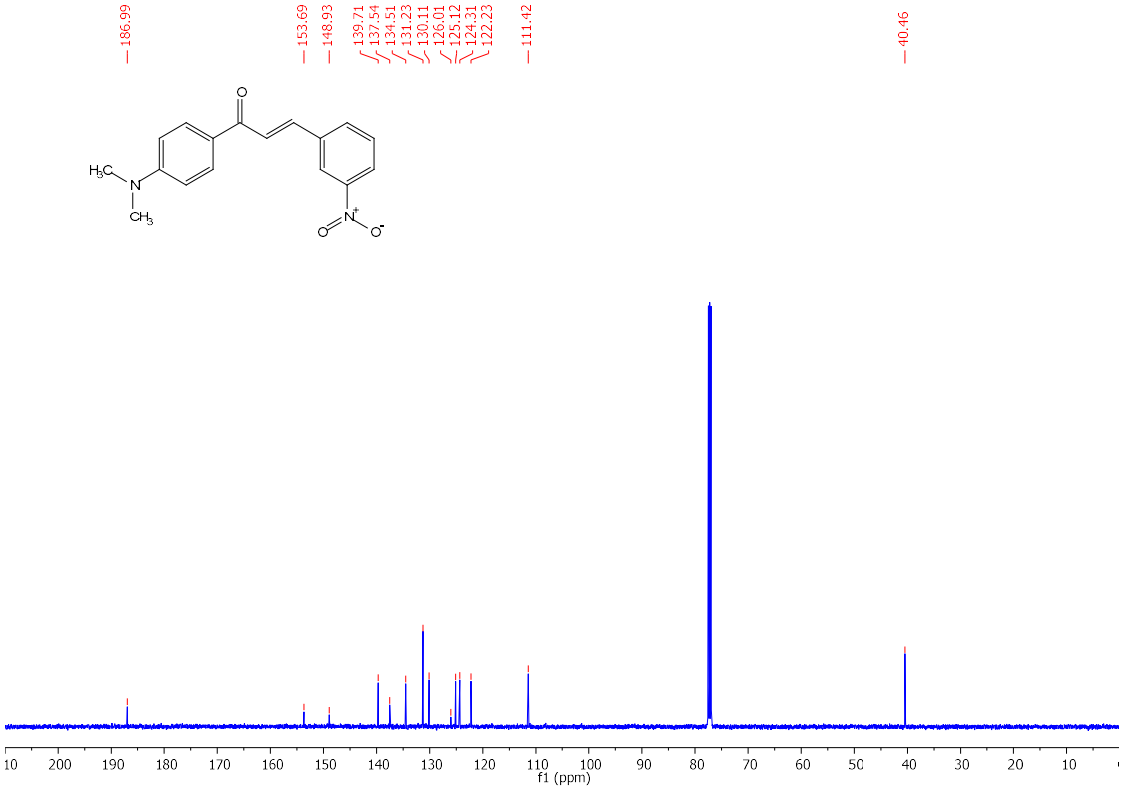
^{13}C NMR of compound **3c**



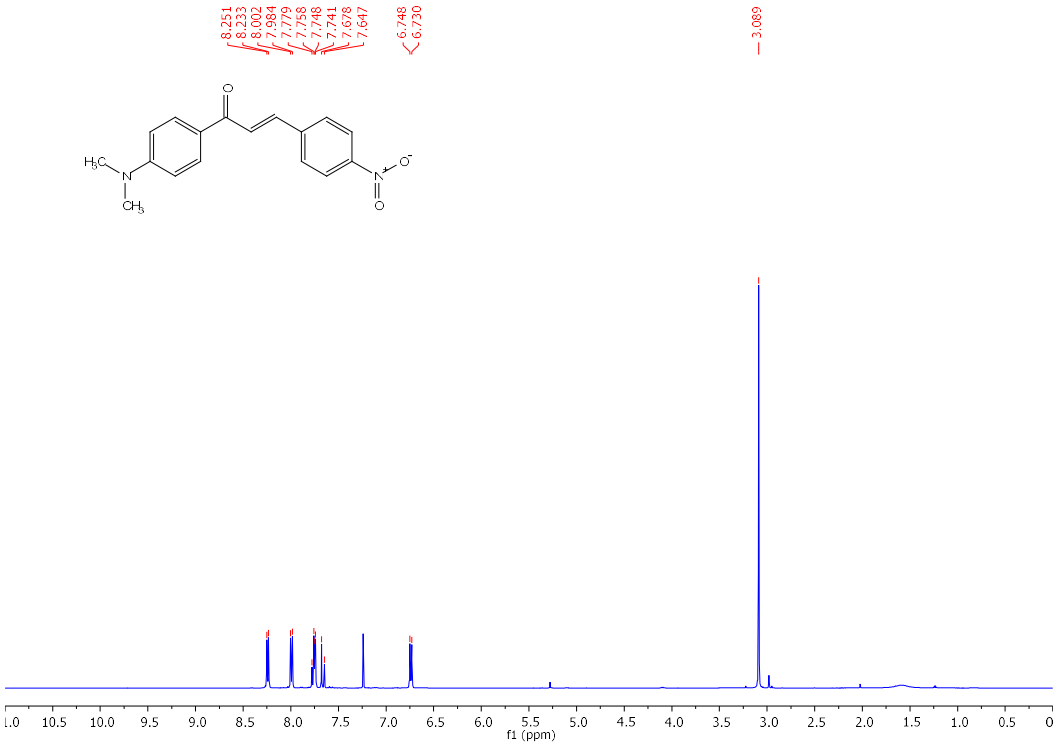
^{1}H NMR of compound **3b**



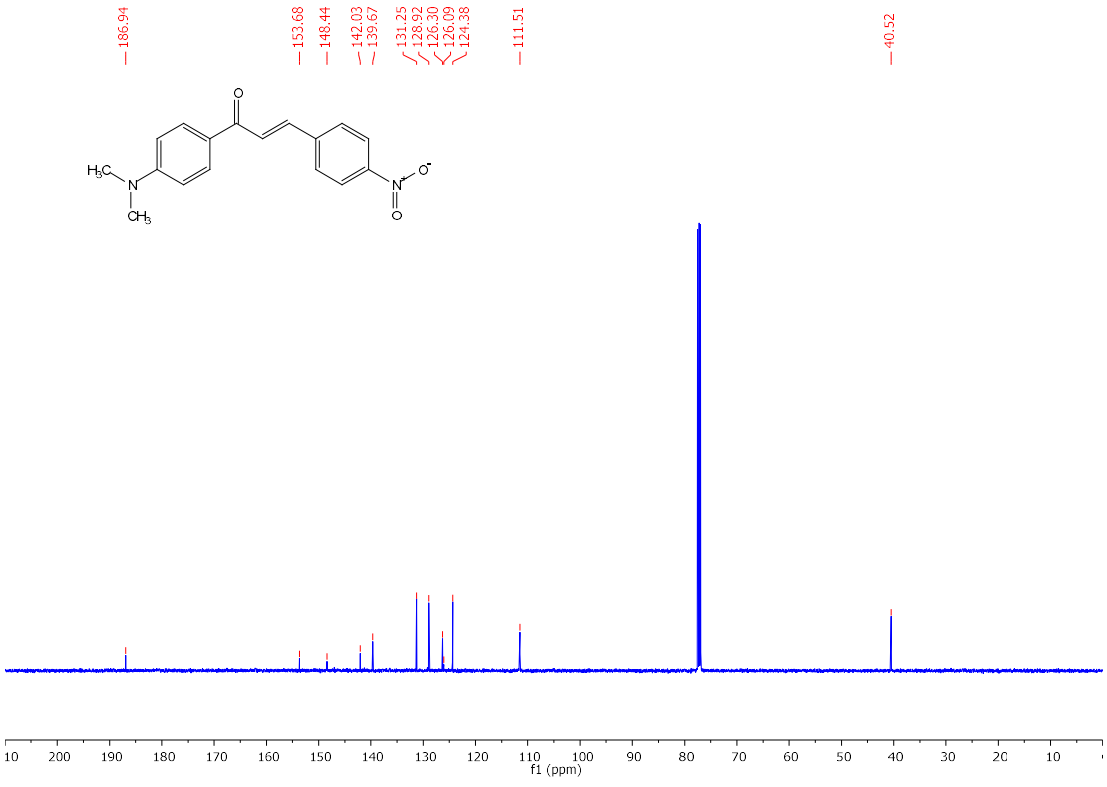
^{13}C NMR of compound **3b**



^{1}H NMR of compound **3c**

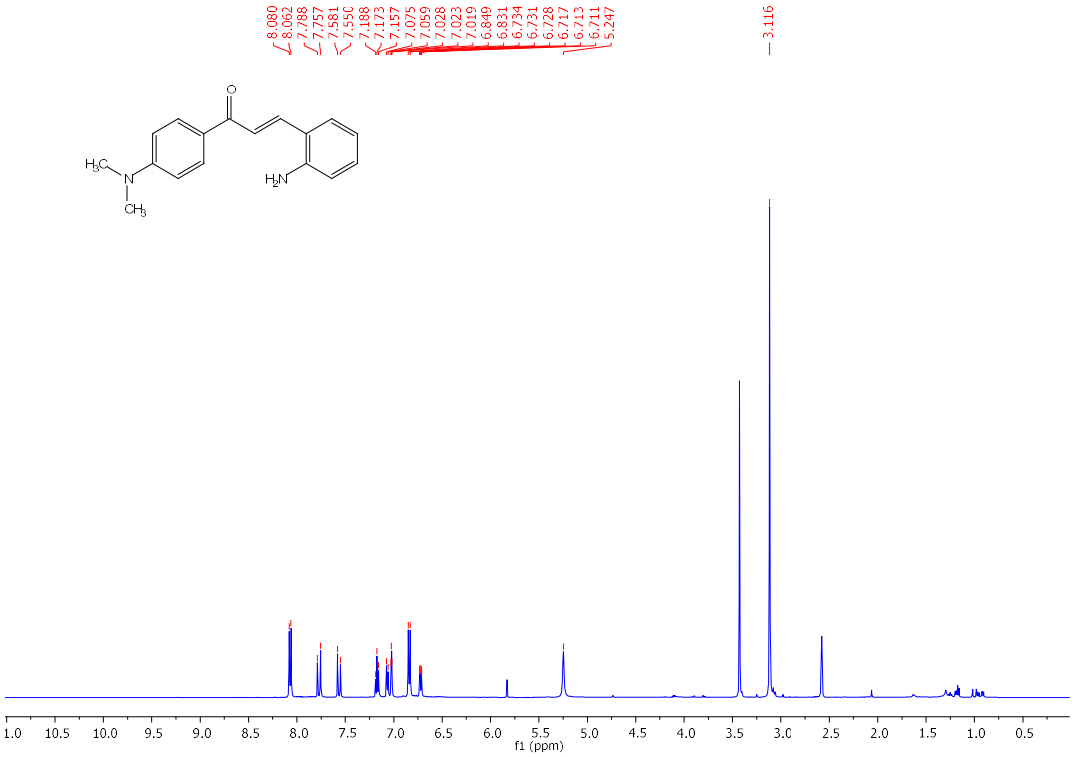


^{13}C NMR of compound **3c**

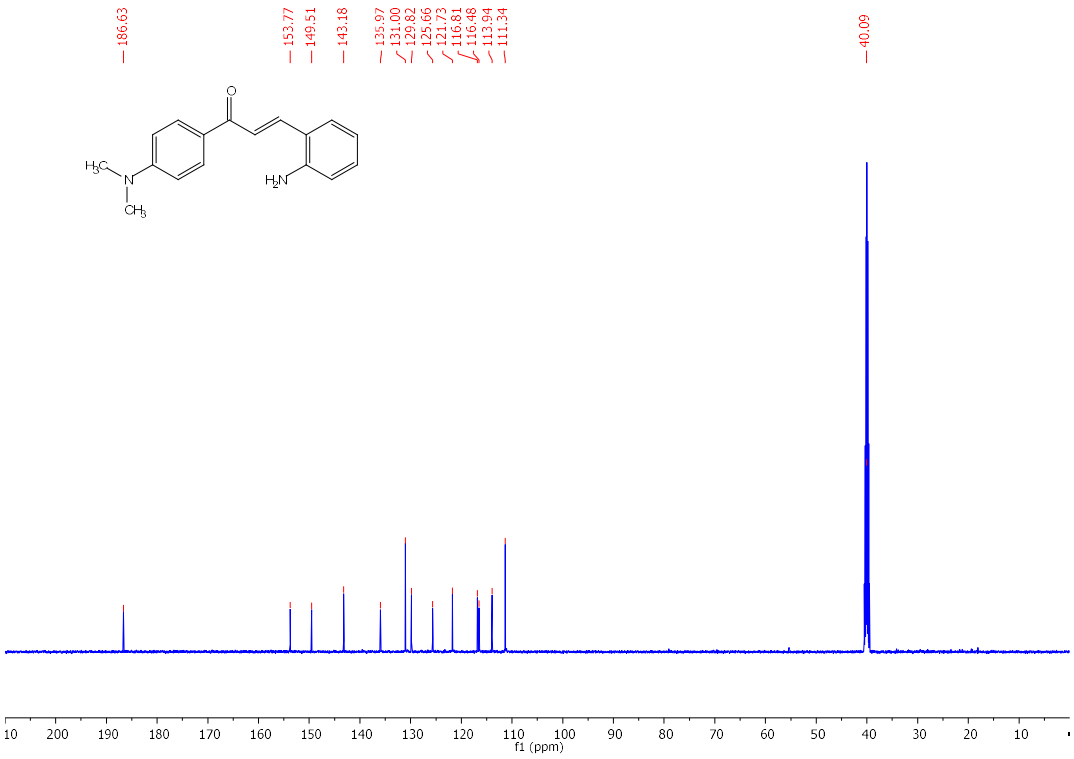


4. Spectrum NMR of chalcones (4a-c)

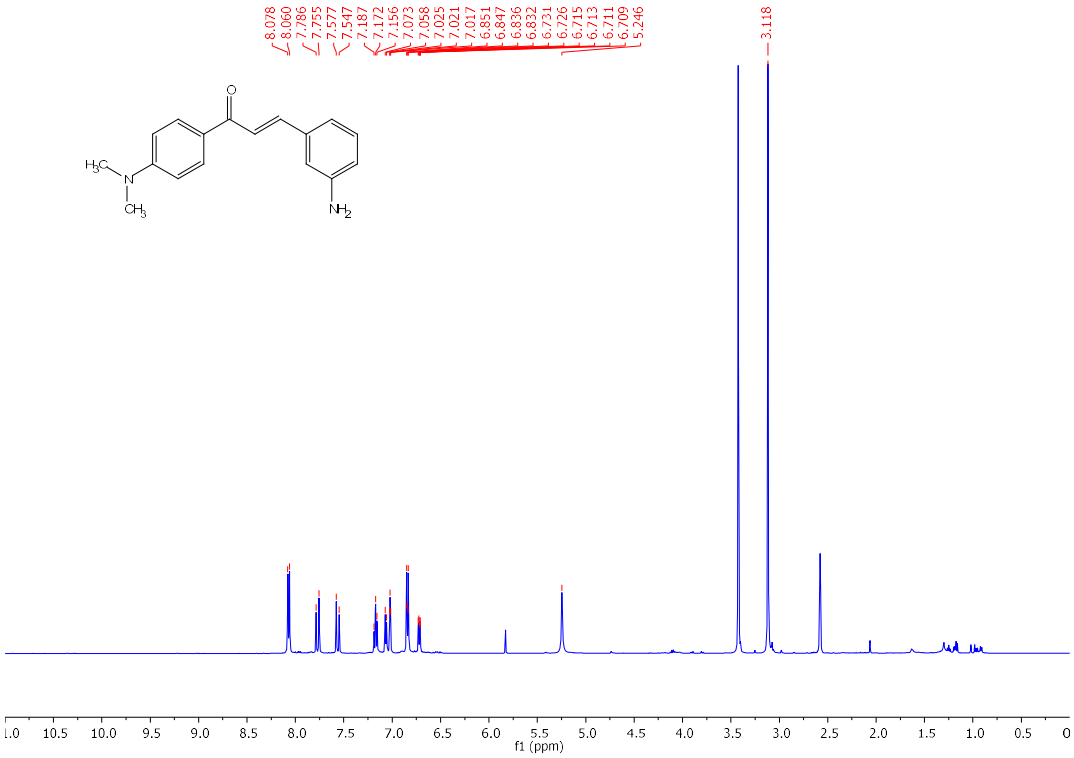
^{1}H NMR of compound **4a**



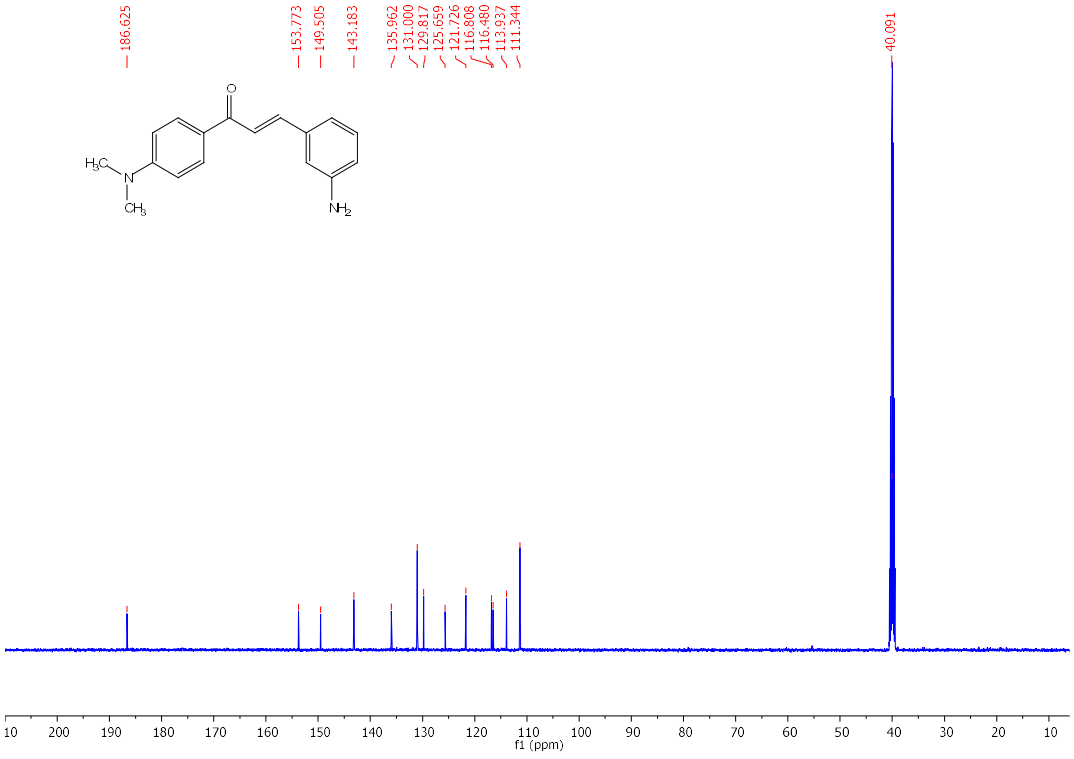
^{13}C NMR of compound **4a**



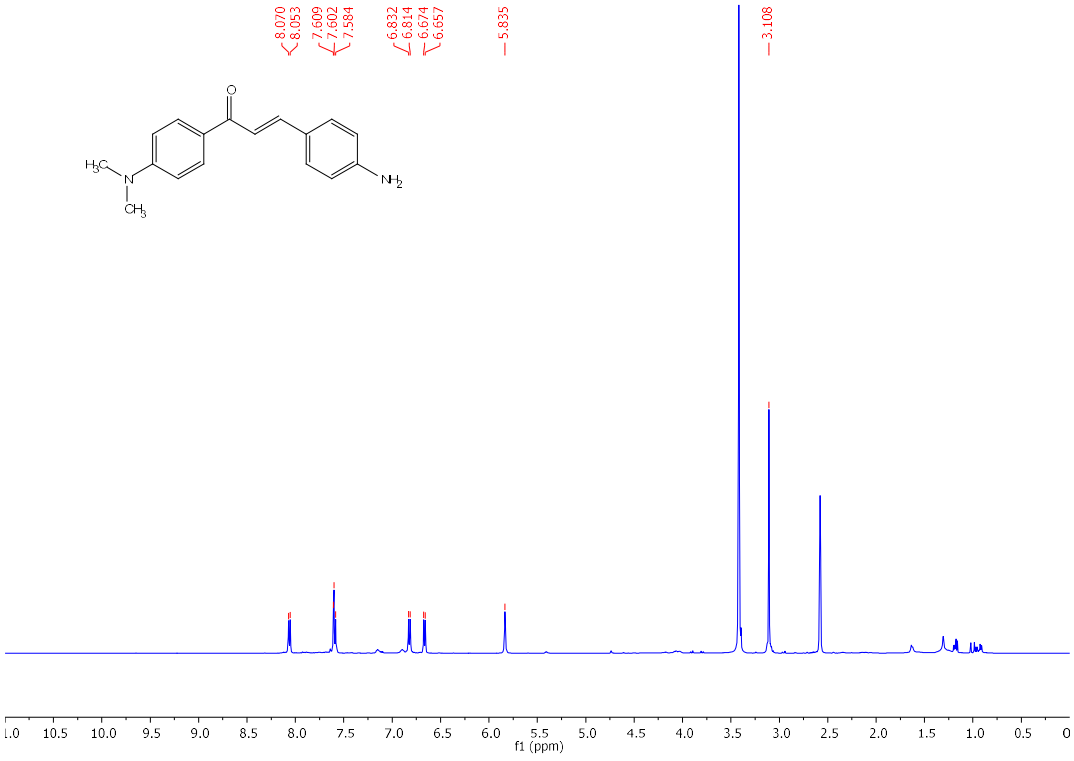
^{1}H NMR of compound **4b**



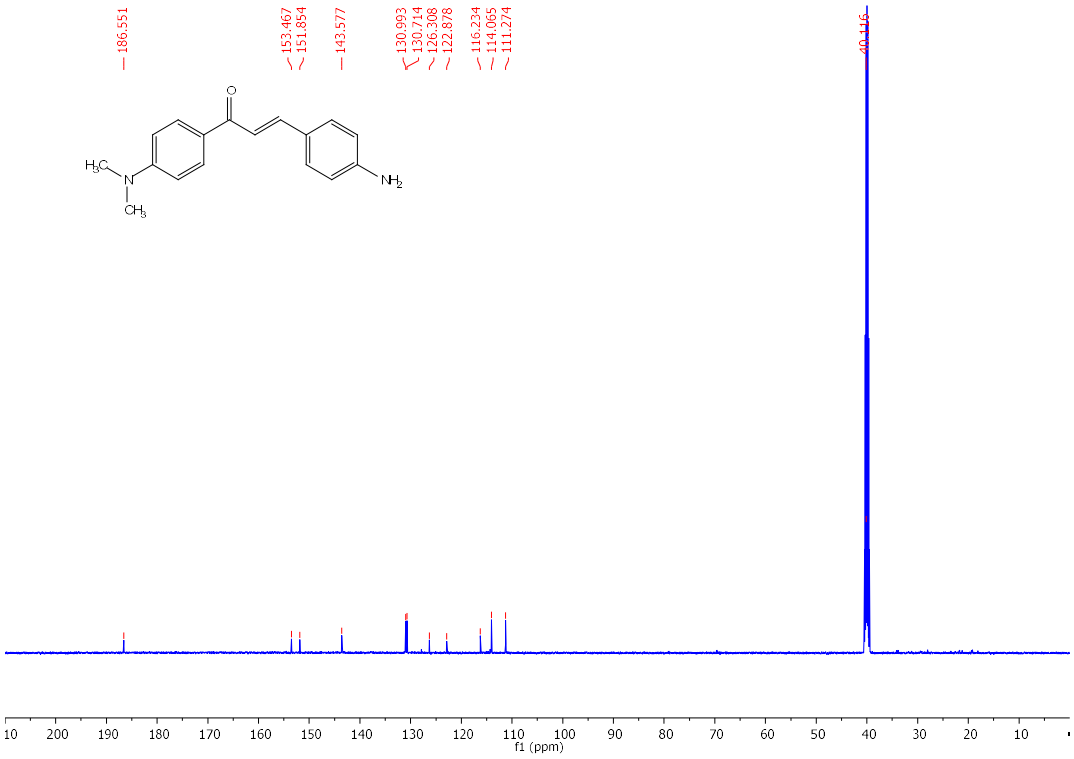
^{13}C NMR of compound **4b**



^{1}H NMR of compound **4c**



^{13}C NMR of compound **4c**



5. Absorption and fluorescence spectroscopic analyses

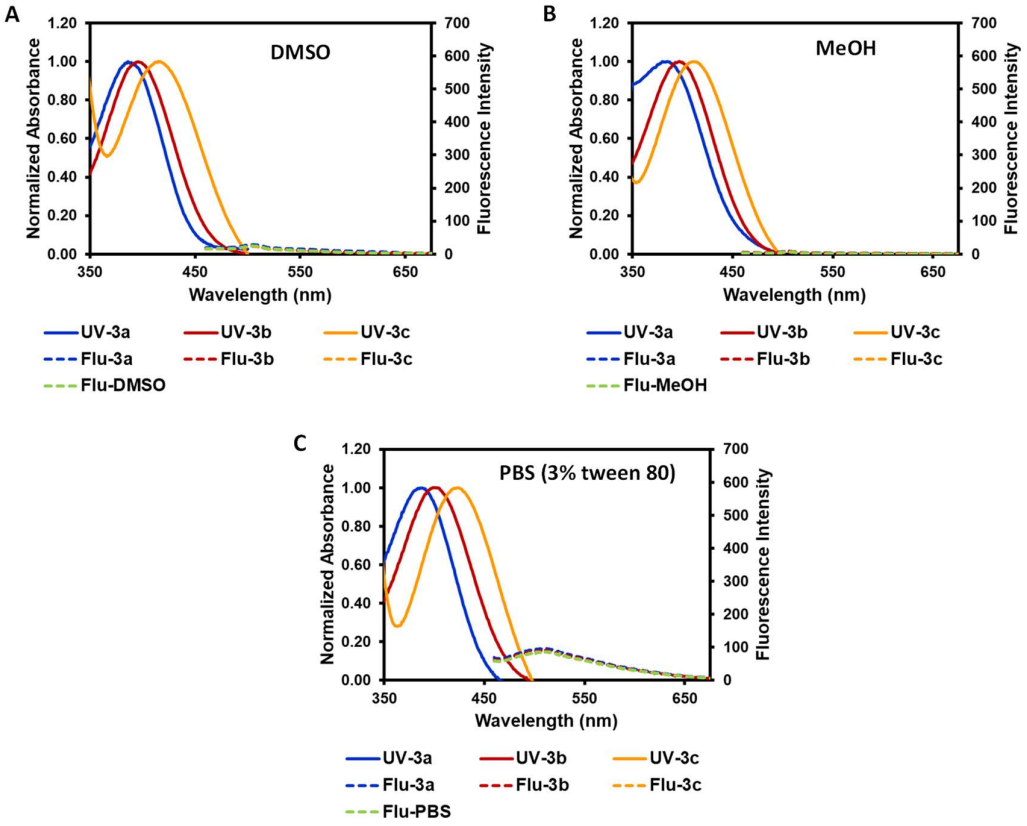


Fig S1. Absorption and fluorescence spectra of chalcones **3a-c** in DMSO (A), MeOH (B), and PBS (C), λ_{ex} = 405 nm.

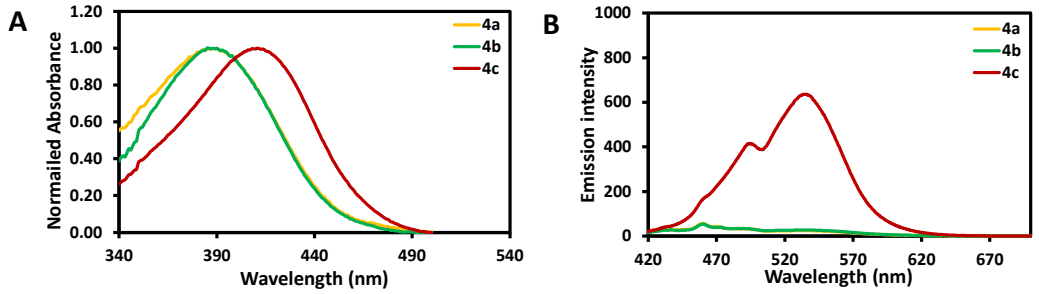


Fig S2. (A) Absorption and (B) fluorescence spectra of chalcones **4a-c** in MeOH, λ_{ex} = 405 nm.

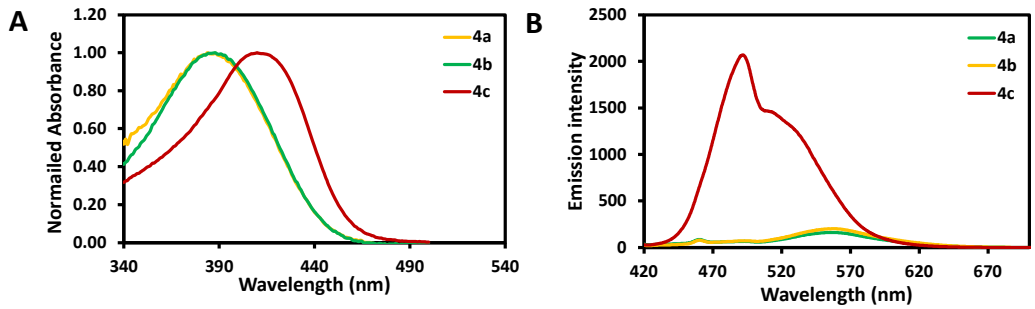


Fig S3. (A) Absorption and (B) fluorescence spectra of chalcones **4a-c** in DMSO, λ_{ex} = 405 nm.

6. Optimizations in the Gas phase

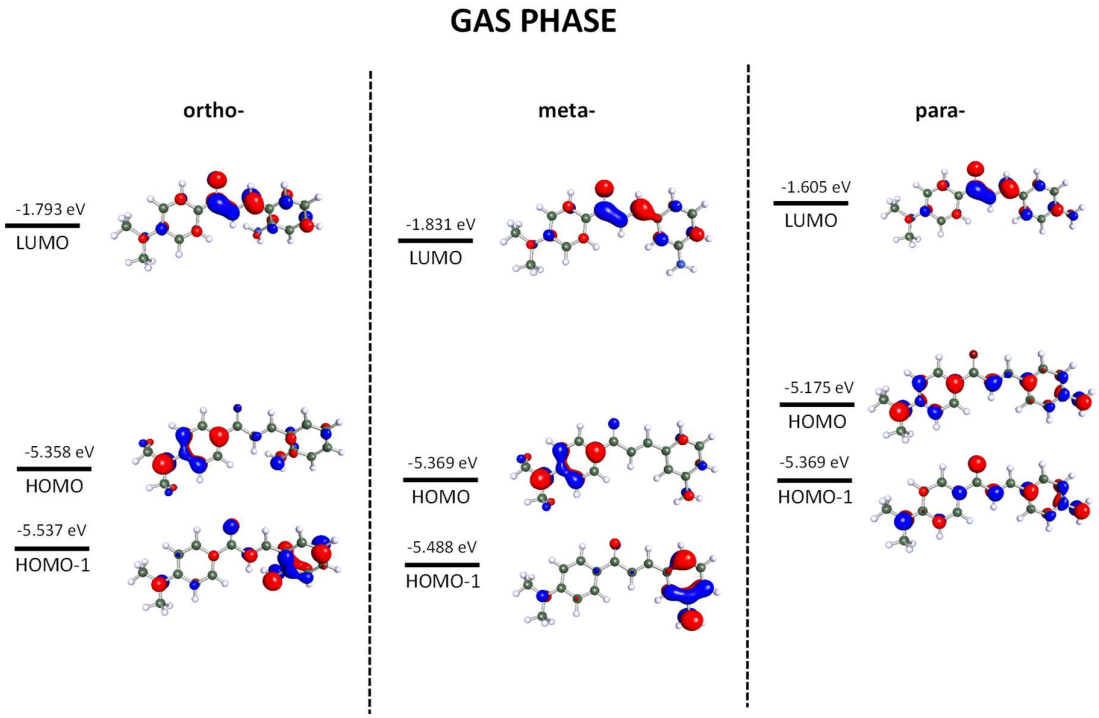


Fig S4. TD-DFT calculation (HOMO-LUMO) of **3a-c** in gas phase.

7. Fluorescence response of 3c with various concentrations of NTR

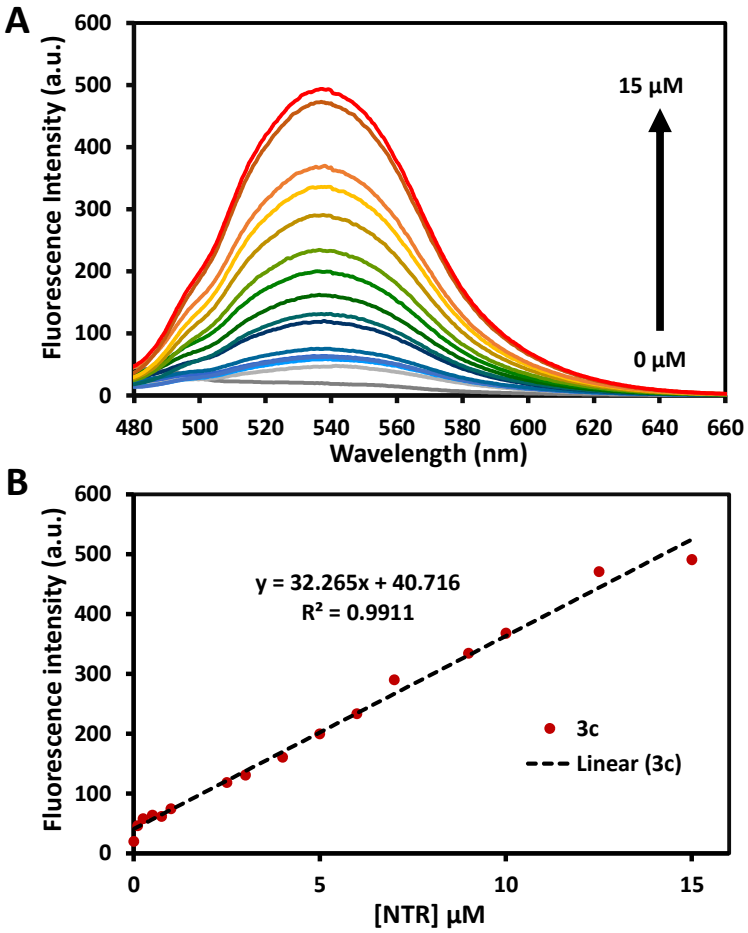


Fig S5. Fluorescence responses of **3c** (10 μM) to different concentrations of nitroreductase (NTR) in PBS buffer (0.01 M, pH 7.4) with 1% DMSO and NADH (50 μM) at 37°C for 5 min. Excitation wavelength = 405 nm.

8. Detection of bacterial NTR using 3c (varies incubated time 2, 6, 12 and 24 h)

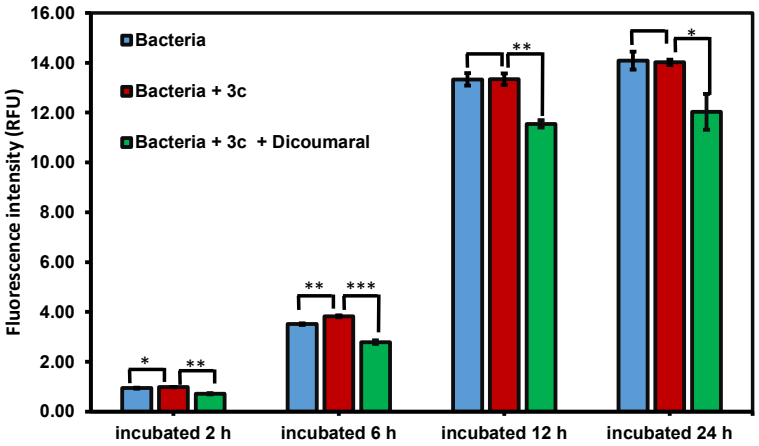


Fig S6. The detection of *E. coli* using NTR probe (**3c**). The fluorescence was measured at the wavelength 535 nm (λ_{ex} = 405 nm) after the bacterial cells were treated with 10 μM NTR probe (**3c**) with and without dicoumarol (0.2 mM) under different incubation time (2, 6, 12 and 24 h) at 37 °C. Statistical analysis is based on one-way ANOVA (*P < 0.05, **P < 0.01, ***P < 0.001)

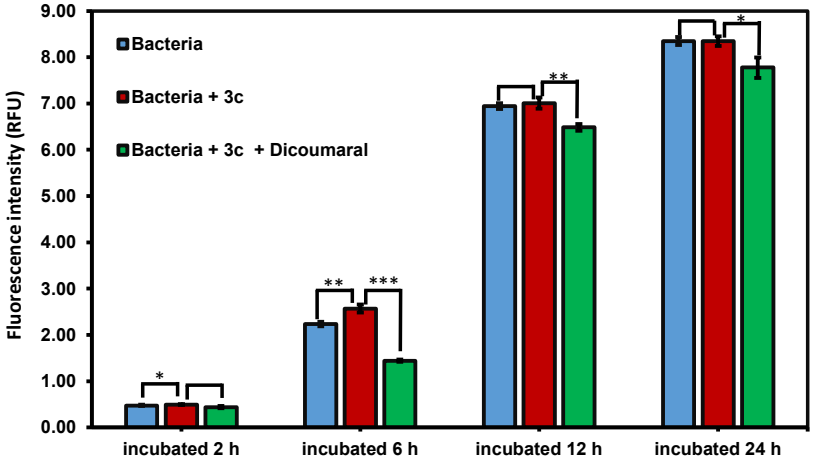


Fig S7. The detection of *S. aureus* using NTR probe (**3c**). The fluorescence was measured at the wavelength 535 nm (λ_{ex} = 405 nm) after the bacterial cells were treated with 10 μM NTR probe (**3c**) with and without dicoumarol (0.2 mM) under different incubation time (2, 6, 12 and 24 h) at 37 °C. Statistical analysis is based on one-way ANOVA (*P < 0.05, **P < 0.01, ***P < 0.001)

9. Detection of NTR in other bacteria strains

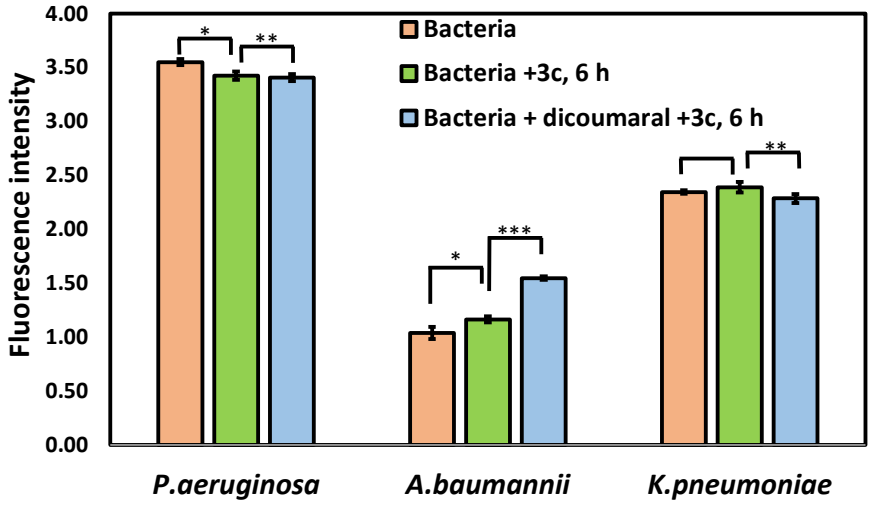


Fig S8. The detection of bacteria using NTR probe (**3c**). The fluorescence was measured at the wavelength 535 nm (λ_{ex} = 405 nm) after the bacterial cells were treated with 10 μM NTR probe (**3c**) with and without dicoumarol (0.2 mM) and incubated 6 h. Bacteria cells. PA: *P. aeruginosa*, AB: *A. baumannii*, and KP: *K. pneumonia.* Statistical analysis is based on one-way ANOVA (*P < 0.05, **P < 0.01, ***P < 0.001)

10. Overexpression and purification of NfsB

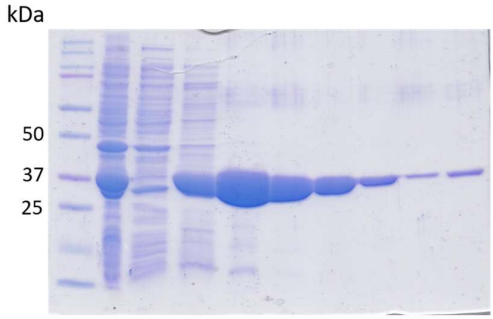


Fig S9. The SDS-PAGE analysis of NfsB purification. The pure fractions were collected.

11. HPLC analysis

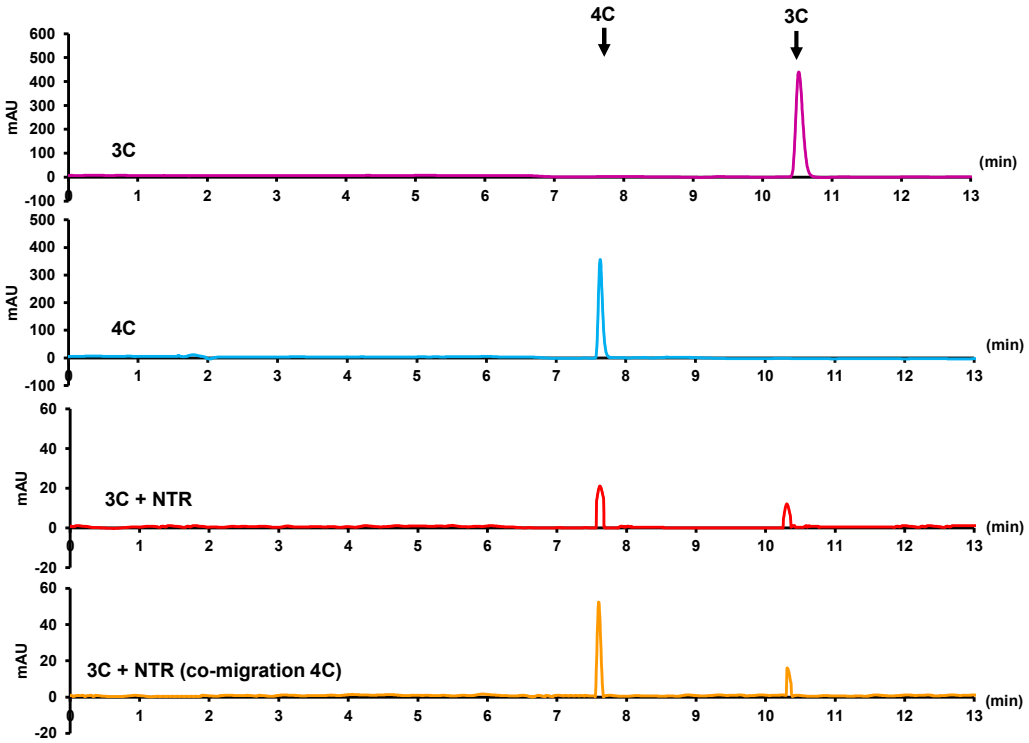


Figure S10. HPLC analysis of probe **3c** reduction catalyzed by NfsB.

12. Linear relationship between NTR and various concentration of 3c

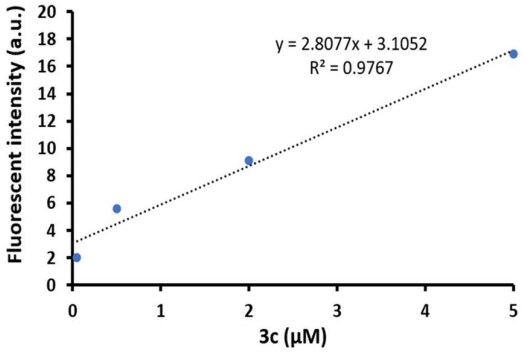


Figure S11. The calibration curve of **4c** determined by fluorescence spectroscopy (Excitation: 405 nm. Emission: 535 nm).