Electronic Supplementary Material (ESI) for Journal of Materials Chemistry B.

Supporting Information for:

Fluorescent nitric oxide donor for the detection and killing of *Pseudomonas aeruginosa*

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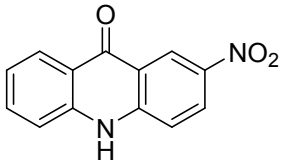
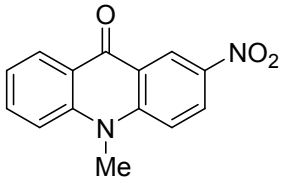
I. General Information

The following abbreviations are used throughout: rt= room temperature, NaH = sodium hydride, DMF = *N,N*-dimethylformamide, MeI = methyl iodide, $SnCl_2 \cdot 2H_2O$ = tin (II) chloride dihydrate, HCl = hydrochloric acid, NaOH = sodium hydroxide, THF = tetrahydofuran, DCM = dichloromethane, Boc = *tert*-Butyloxycarbonyl, EtOAc = ethyl acetate, $CDCl_3$ = deuterated chloroform, d_6-DMSO = deuterated dimethylsulfoxide, NaOD = sodium deuteroxide, and D_2O = deuterium oxide.

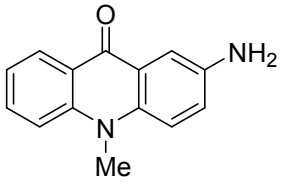
II. Experimental Procedures



2-nitroacridin-9(10H)-one (2). 9(10H)-Acridone (0.976 g, 5 mmol) was stirred in 36% acetic acid (65 mL, 0.077 M) for 5 minutes. A mixture of concentrated nitric acid (68%, 4.7 mL, 1 M) and glacial acetic acid (10.43 mL, 0.5 M) was slowly added, and the reaction was stirred for 5 hours at 55 °C. The hot reaction mixture was poured over 50 g of ice, and the precipitate was filtered. The precipitate was heated to boiling in absolute ethanol and filtered while hot to remove acridone. The solid was heated in boiling glacial acetic acid, filtered while hot, and rinsed with water to remove the 4-isomer. A pure yellow powder was obtained (821 mg, 67%). ^{1}H NMR (400 MHz, d_6-DMSO) δ 12.34 (s, 1H), 8.97 (d, *J* = 2.7 Hz, 1H), 8.45 (dd, *J* = 9.2, 2.7 Hz, 1H), 8.23 (d, *J* = 7.9 Hz, 1H), 7.81 (t, *J* = 7.0 Hz, 1H), 7.66 (d, *J* = 9.2 Hz, 1H), 7.58 (d, *J* = 8.3 Hz, 1H), 7.36 (t, *J* = 7.5 Hz, 1H). Spectra matched the previously reported values.[1]

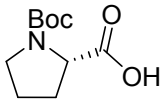


10-methyl-2-nitroacridin-9(10H)-one (3). A previously reported synthetic procedure was modified as follows.[2] To a solution of **2** (200 mg, 0.833 mmol) in DMF (6.7 mL, 0.125 M) at 0 °C was added NaH (83.2 mg, 2.08 mmol), and the reaction was stirred 30 minutes at 0 °C. Iodomethane (129 µL, 2.08 mmol) was added and the reaction was heated at 60 °C for 18 hours. The reaction was cooled to rt, quenched with 15 mL of deionized water, and then the precipitate was filtered. The crude precipitate was recrystallized from 25:1 DMF:H_2O and rinsed with diethyl ether, affording the title compound as yellow crystals (145.2 mg, 69%). ^{1}H NMR (400 MHz, d_6-DMSO) δ 9.03 (d, *J* = 2.9 Hz, 1H), 8.51 (dd, *J* = 9.6, 2.8 Hz, 1H), 8.34 (d, *J* = 8.2 Hz, 1H), 8.04 (d, *J* = 9.6 Hz, 1H), 7.98-7.87 (m, 2H), 7.45 (t, *J* = 7.3 Hz, 1H), 4.00 (s, 3H). Spectra matched the previously reported values.[2]



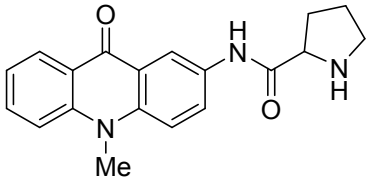
2-amino-10-methylacridin-9(10H)-one (4). A previously reported synthetic procedure was modified as follows.[3] To a solution of **3** (254.3 mg, 1 mmol) in 8 N HCl (19.2 mL, 0.052 M), a mixture of $SnCl_2 \cdot H_2O$ (67.7 mg, 3 mmol) in 8 N HCl (28.6 mL, 0.035 M) was added. The solution

was heated under reflux at 120 °C for 1 hour. After cooling to rt, 4 M NaOH was added until the mixture reached pH 11, then the precipitate was filtered and rinsed with water. The crude product was purified by flash column chromatography (SiO_2, 70% ethyl acetate/hexanes to 100% ethyl acetate gradient), affording the title compound as a bright orange solid (122.8 mg, 55%). ^{1}H NMR (400 MHz, d_6-DMSO) δ 8.27 (d, *J* = 8.1 Hz, 1H), 7.76-7.67 (m, 2H), 7.61 (d, *J* = 9.2 Hz, 1H), 7.46 (d, *J* = 2.8 Hz, 1H), 7.25 – 7.12 (m, 2H), 5.23 (s, 2H), 3.85 (s, 3H). Spectra matched the previously reported values.[3]



(tert-butoxycarbonyl)-L-proline (5). A previously reported synthetic procedure was followed.[4] To a solution of L-proline (1.151 g, 10 mmol) in aqueous NaOH (20 mL, 0.5 M) and THF (5 mL, 2 M), di-tert-butyl dicarbonate (2.575 g, 11.8 mmol) was added portion-wise over 20 min The title compound was obtained as a white crystalline solid (1.73 g, 80%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.50 (s, 1H), 4.39 – 4.18 (m, 1H), 3.61 – 3.27 (m, 2H), 2.29 (m, 1H), 2.15-1.80 (m, 3H), 1.44 (s, 9H). Spectra matched the previously reported values.[4]

III. ^{1}H and ^{13}C, ESI-TOF MS, and UV-Vis Spectra



6

$CDCl_3$, 400 MHz

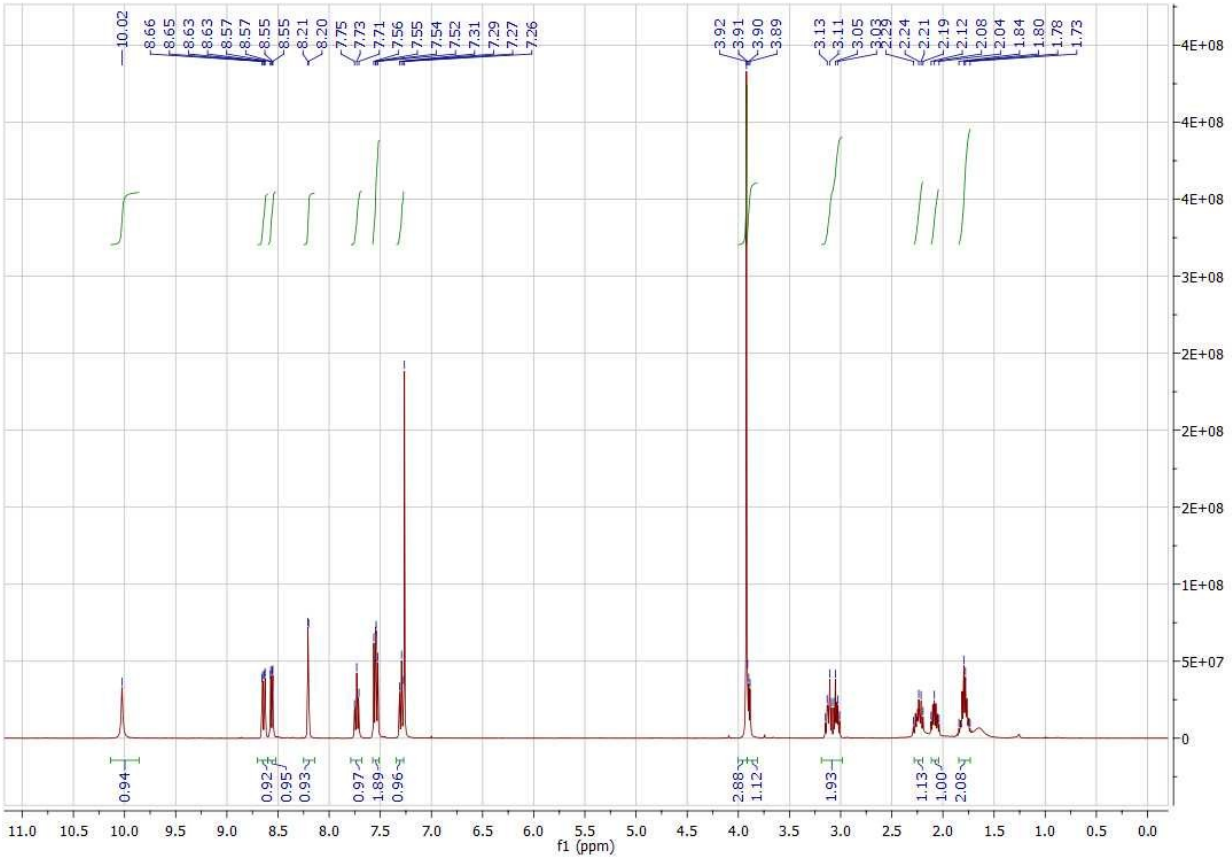
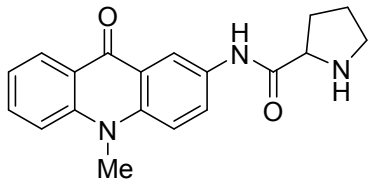


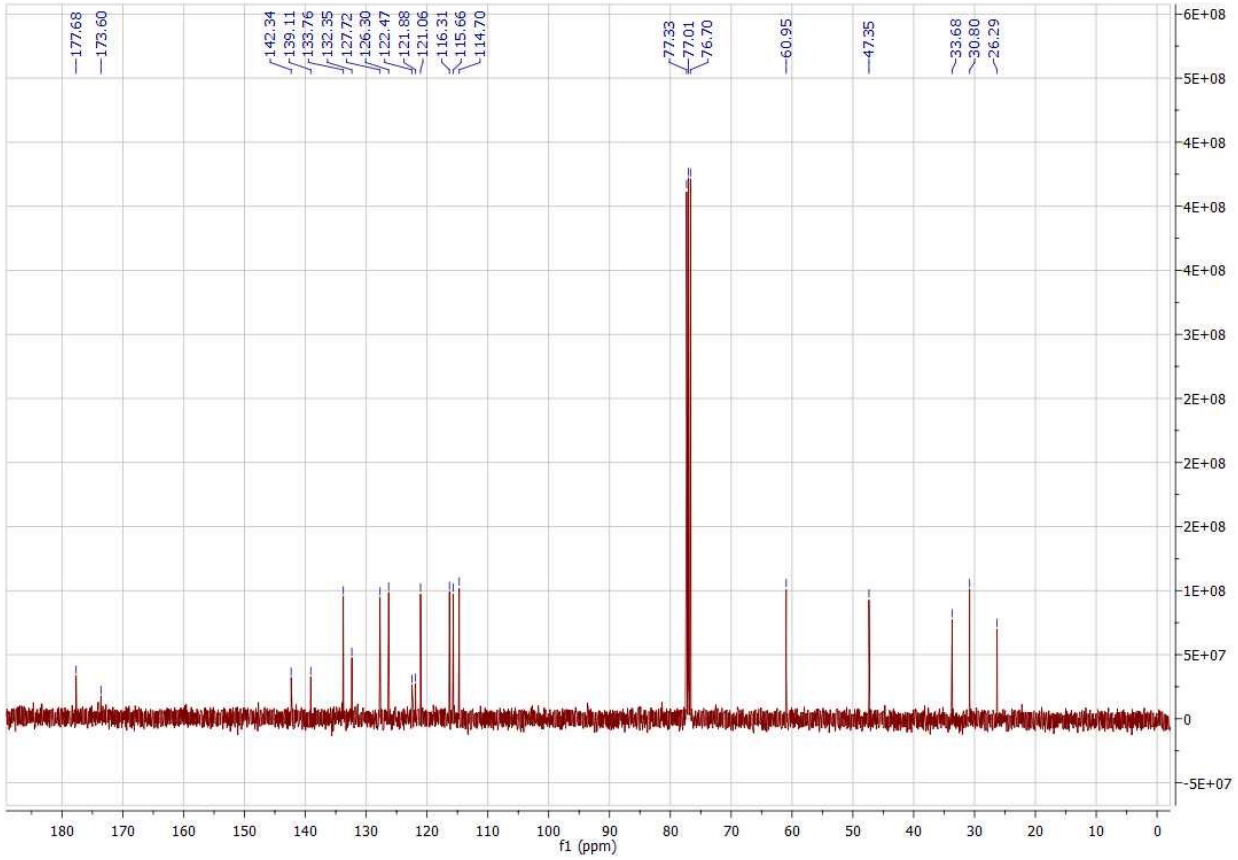
Figure S1. ^{1}H NMR of **6** in $CDCl_3$

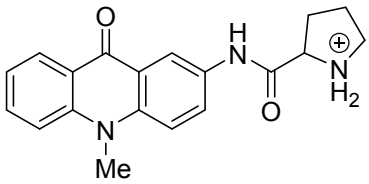


6

$CDCl_3$, 101 MHz

Figure S2. ^{13}C NMR of **6** in $CDCl_3$





7

d_6-DMSO, 400 MHz

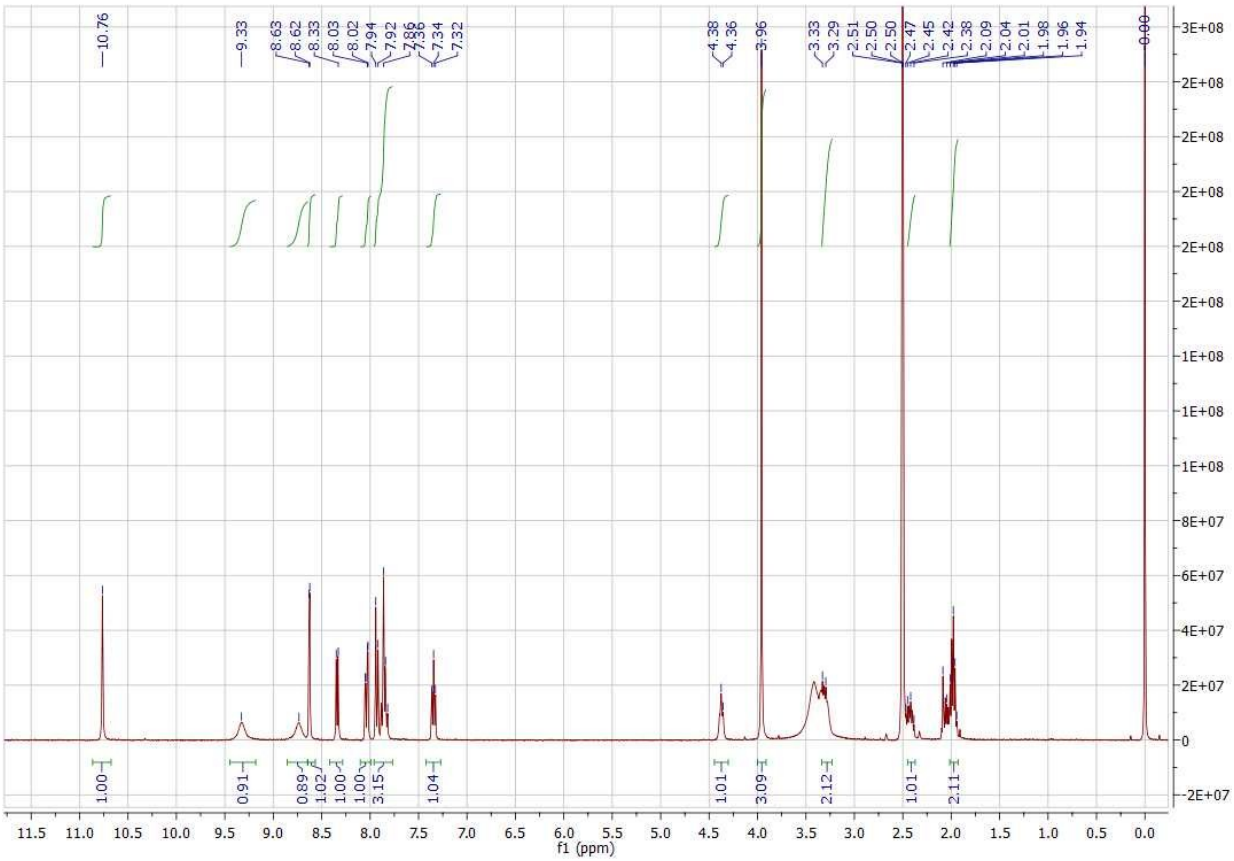
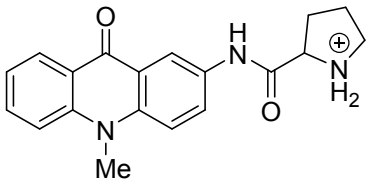


Figure S3. ^{1}H NMR of **7** in d_6-DMSO



7

d_6-DMSO, 101 MHz

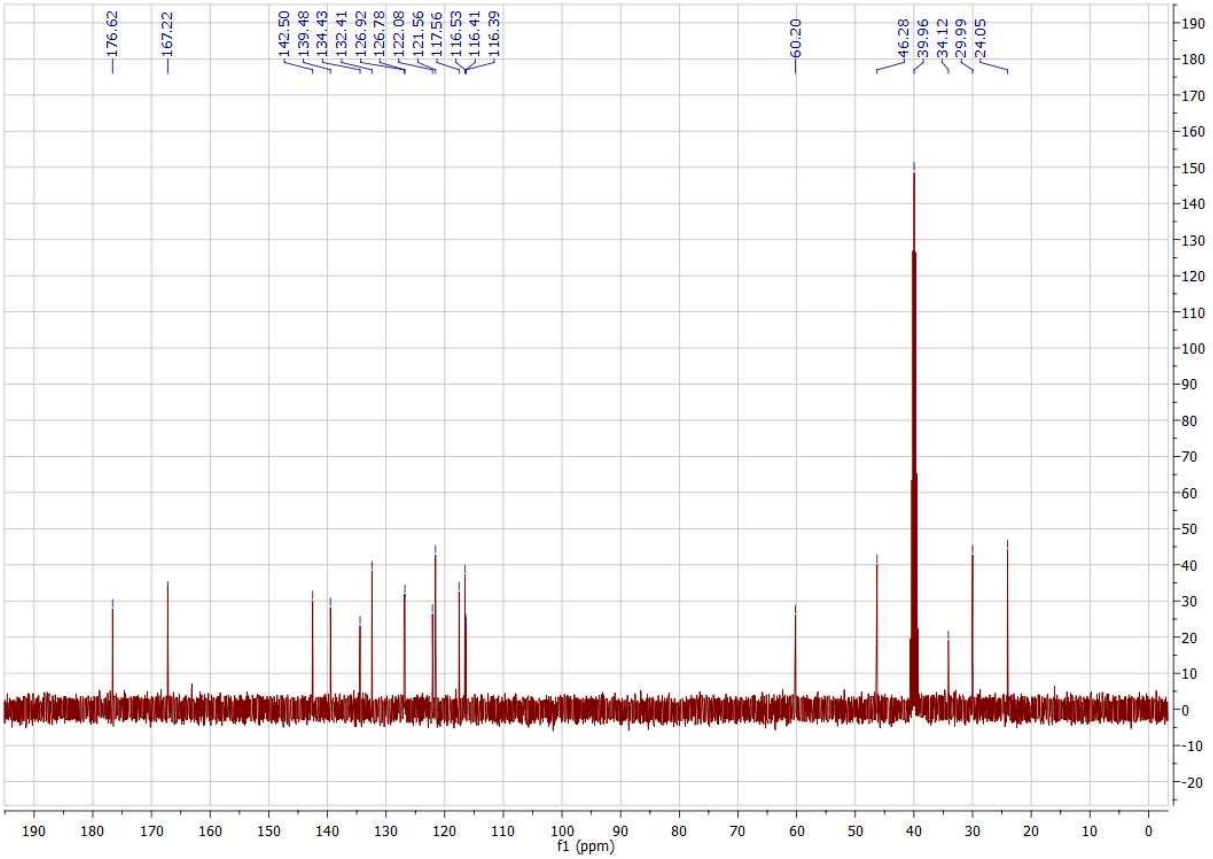
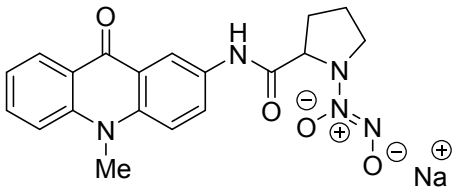


Figure S4. ^{13}C NMR of **7** in d_6-DMSO



8

0.5 mM NaOD in D_2O, 400 MHz

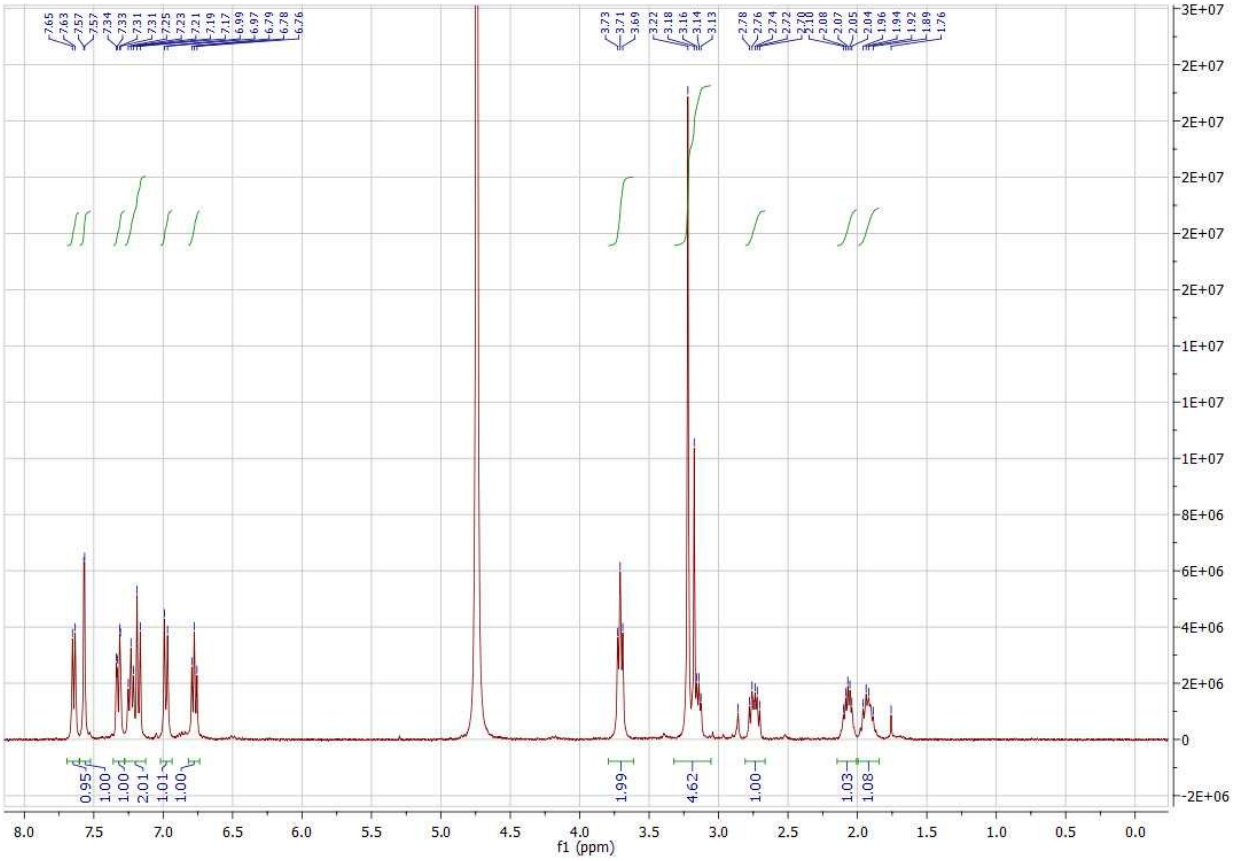
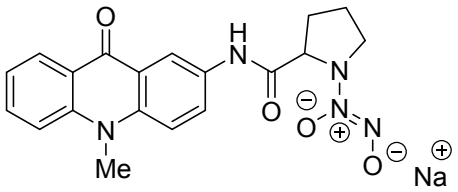


Figure S5. ^{1}H NMR of **8** 0.5 mM NaOD in D_2O



8

0.5 mM NaOD in D_2O, 101 MHz

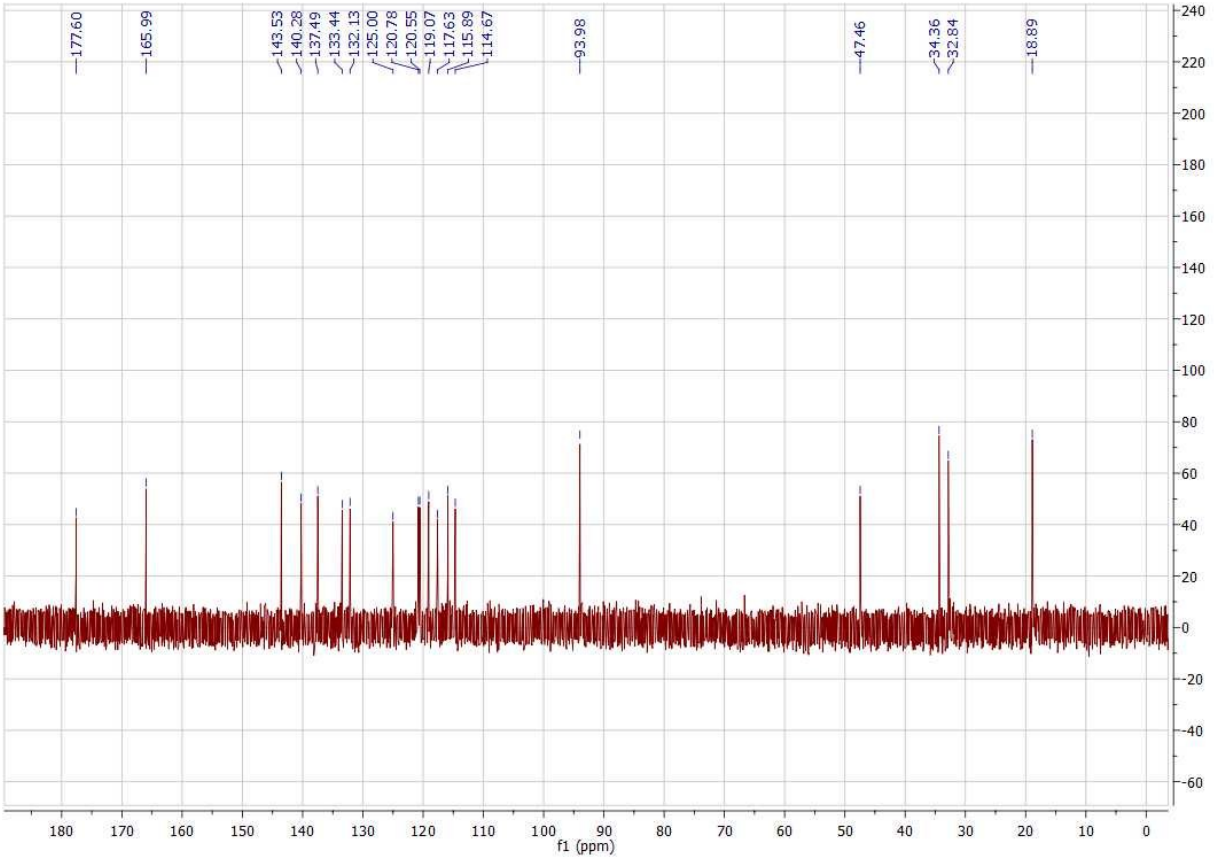


Figure S6. ^{13}C NMR of **8** 0.5 mM NaOD in D_2O

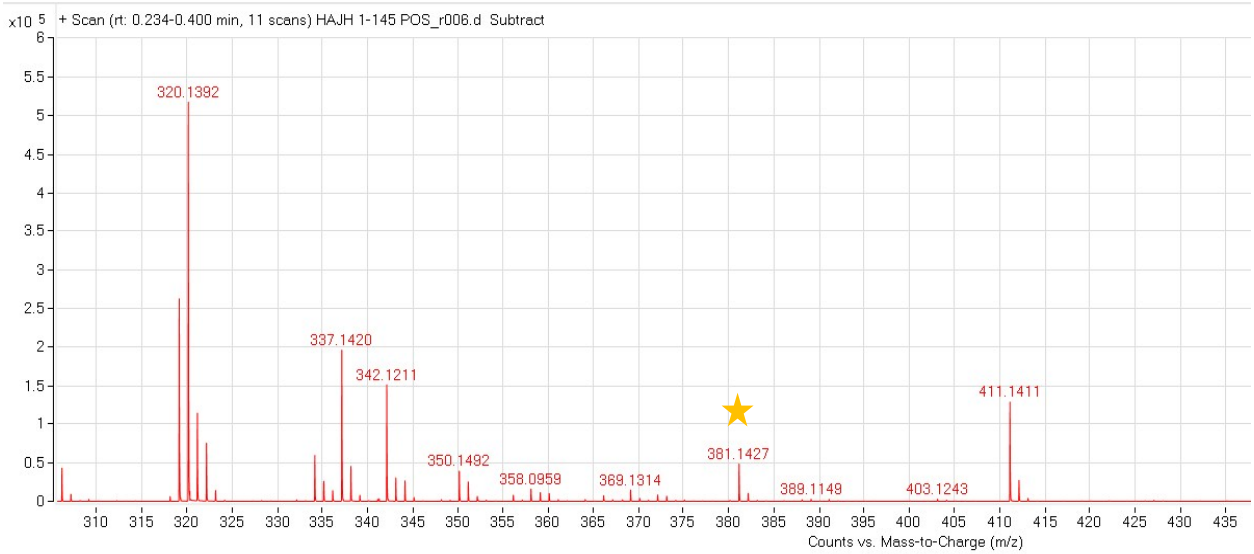


Figure S7. Electrospray ionization time-of-flight mass spectrometry (ESI-TOF MS) spectrum showing correct mass of **8**. The experiment was run in a solvent system of 80 v/v% methanol, 19.5 v/v% water, and 0.5 v/v% ammonia, with a pH ≈ 12 to stabilize the diazeniumdiolate

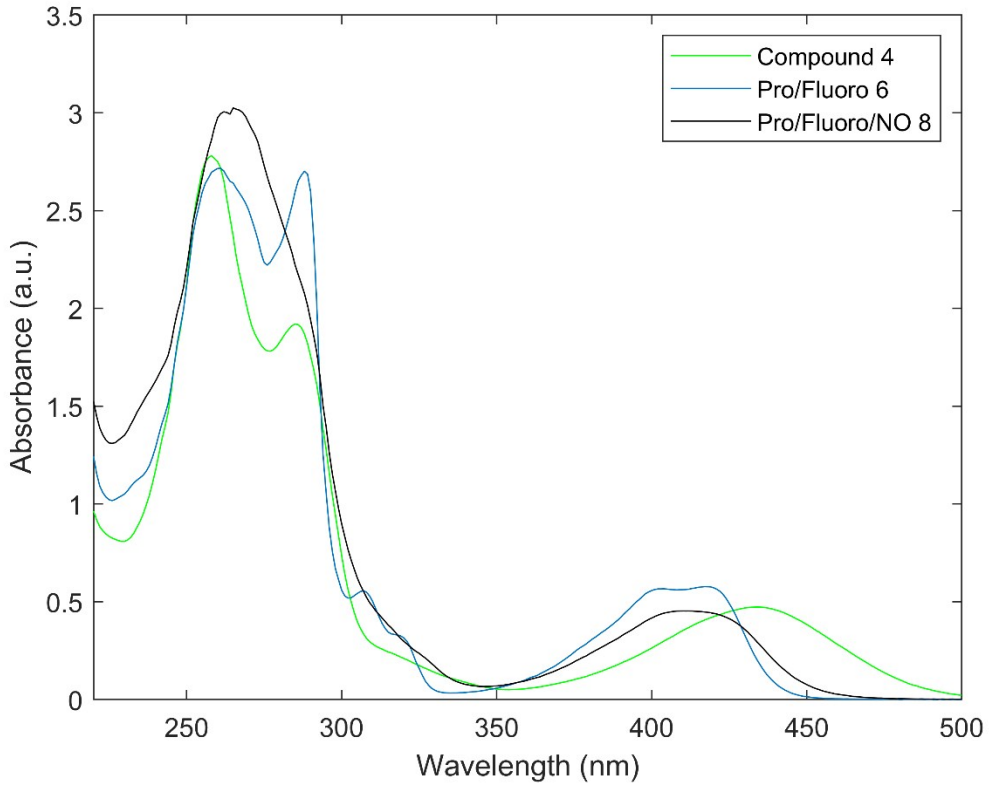


Figure S8. UV-Vis spectra of compound **4** (1 mM in EtOH), Pro/Fluoro **6** (1 mM in EtOH) Pro/Fluoro/NO **8** (1 mM in 0.01 M NaOH)

IV. Bacterial Detection Study Figures

Photographs were obtained using an iPhone 7 with the flash disabled. Photographs are for illustrative purposes only as colors shown may not be as seen by eye.

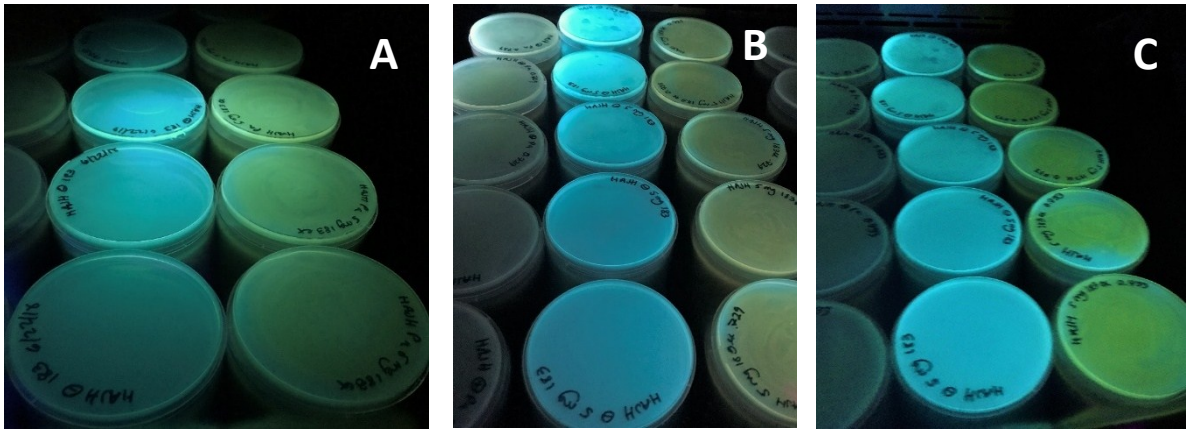


Figure S9. Colonies of *Pseudomonas aeruginosa* after incubation in the presence of **7**. Postive control (left)- inoculated with *P. aeruginosa*, no Pro/Fluoro/H^+ **7**; Negative control (middle)- not inoculated with *P. aeruginosa*, Pro/Fluoro/H^+ **7**; Experiment (right)- inoculated with *P. aeruginosa*, Pro/Fluoro/H^+ **7**. Trial 1 (A), Trial 2 (B), Trial 3 (C).

V. Nitric Oxide Release Data

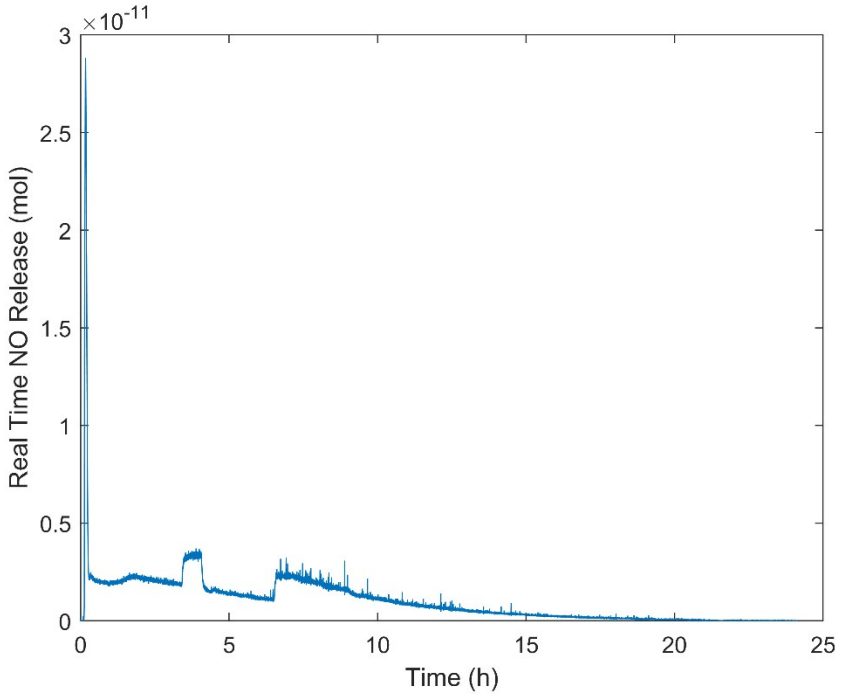


Figure S10. Representative plot of real-time NO release of Pro/Fluoro/NO at 0.1 mM in nutrient broth at 37 ˚C

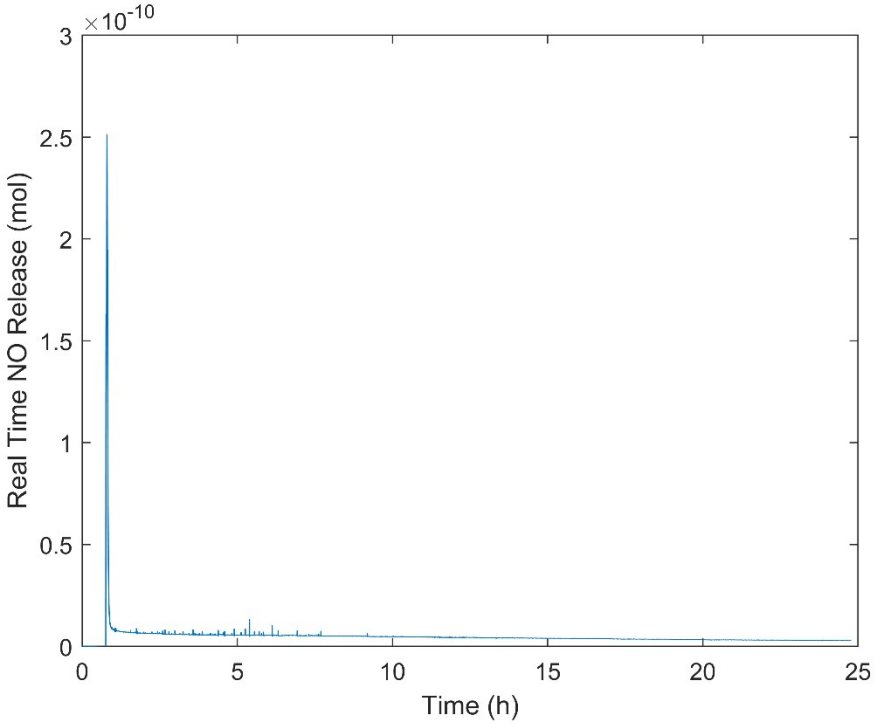


Figure S11. Representative plot of real-time NO release of Pro/Fluoro/NO at 1 mM in nutrient broth at 37 ˚C

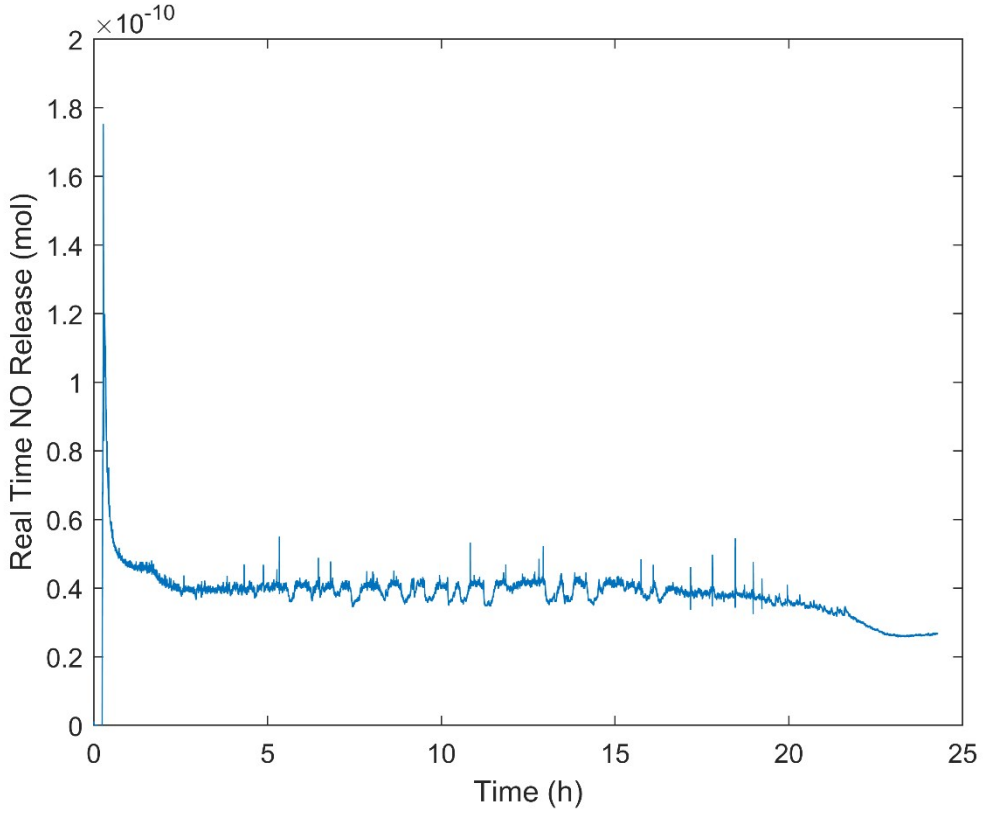


Figure S12. Representative plot of real-time NO release of Pro/Fluoro/NO at 10 mM in nutrient broth at 37 °C

VI. Cell Viability Assay Figures

Photographs were obtained using an iPhone 7 with the flash disabled. Photographs are for illustrative purposes only as colors shown may not be as seen by eye.

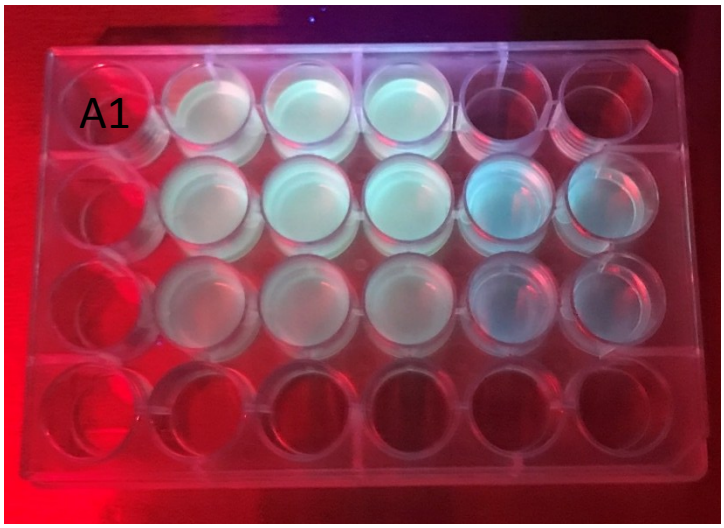


Figure S13. 24 well plate after 24 hour incubation. A2-A4: *P. aeruginosa*, positive control; B2-B4: 1 mM Pro/Fluoro/NO, *P. aeruginosa*; B5-B6: 1 mM Pro/Fluoro/NO; C2-C4: 100 µM Pro/Fluoro/NO, *P. aeruginosa*; C5-C6: 100 µM Pro/Fluoro/NO

VII. References

(1) Gao, C.; Liu, F.; Luan, X.; Tan, C.; Liu, H.; Xie, Y.; Jin, Y.; Jiang, Y. Novel Synthetic 2-Amino-10-(3,5-Dimethoxy)Benzyl-9(10H)-Acridinone Derivatives as Potent DNA-Binding Antiproliferative Agents. *Bioorg. Med. Chem.* **2010**, *18* (21), 7507–7514.

(2) Stopka, T.; Marzo, L.; Zurro, M.; Janich, S.; Würthwein, E.-U.; Daniliuc, C. G.; Alemán, J.; Mancheño, O. G. Oxidative C-H Bond Functionalization and Ring Expansion with TMSCHN2 : A Copper(I)-Catalyzed Approach to Dibenzoxepines and Dibenzoazepines. *Angew. Chem. Int. Ed.* **2015**, *54* (17), 5049–5053.

(3) Manfroni, G.; Paeshuyse, J.; Massari, S.; Zanoli, S.; Gatto, B.; Maga, G.; Tabarrini, O.; Cecchetti, V.; Fravolini, A.; Neyts, J. Inhibition of Subgenomic Hepatitis C Virus RNA Replication by Acridone Derivatives: Identification of an NS3 Helicase Inhibitor. *J. Med. Chem.* **2009**, *52* (10), 3354–3365.

(4) Stache, E. E.; Seizert, C. A.; Ferreira, E. M. Molecular Scaffolds with Remote Directing Groups for Selective Palladium-Catalyzed C–H Bond Functionalizations. *Chem. Sci.* **2012**, *3* (5), 1623.

(5) Boużyk, A.; Jóźwiak, L.; Błażejowski, J. 1 H and 13 C NMR Spectroscopy of 9-Acridinones. *J. Mol. Struct.* **2002**, *612* (1), 29–38.