Synthesis and evaluation of piperazine and homopiperazine analogues of JS-K, an anti-cancer lead compound

Rahul S. Nandurdikar[a], Anna E. Maciag[b], Michael L. Citro[b], Paul J. Shami[c], Larry K. Keefer[a], Joseph E. Saavedra[b,*], Harinath Chakrapani[a,*]

[a]Chemistry Section, Laboratory of Comparative Carcinogenesis, National Cancer Institute at Frederick, Frederick, Maryland 21702, USA. [b]Basic Sciences Program, SAIC-Frederick, National Cancer Institute at Frederick, Frederick, Maryland 21702, USA. [c]Division of Oncology, Department of Internal Medicine, University of Utah, Salt Lake City, Utah 84112, USA.

Table of Contents

General. Nitric oxide gas was purchased from Matheson Gas Products (Montgomeryville, PA). Starting materials were purchased from Aldrich Chemical Co. (Milwaukee, WI) unless otherwise indicated. NMR spectra were recorded on a Varian UNITY INOVA spectrometer; chemical shifts (δ) are reported in parts per million (ppm) downfield from tetramethylsilane. The NMR spectra of compounds **1b**, **11**-**15** were recorded at 70 °C in DMSO-d_6. Ultraviolet (UV) spectra were recorded on an Agilent Model 8453 or a Hewlett-Packard model 8451A diode array spectrophotometer. Elemental analyses were performed by Midwest Microlab (Indianapolis, IN). Nitric oxide measurements were performed using a Sievers Nitric Oxide Analyzer (NOA), model 280i (Instruments Business Group, Boulder, CO). Chromatography was performed on a Biotage SP1 Flash Purification System. Prepacked silica gel flash chromatography columns were purchased from Silicycle (Quebec City, Canada). Compounds **1a**[1], **2a**[1] and **16**[2] were prepared by using reported methods.

***O*^{2}-(2,4-Dinitrophenyl) 1-[(4-*t*-Butoxycarbonyl)homopiperazin-1-yl]diazen-1-ium-1,2-diolate (1b) (RN-1-12).** A solution of 1-(butyloxycarbonyl)homopiperazine (10 g, 0.05 mol) in methanol (2 mL) was treated with 25% methanolic sodium methoxide (9.3 mL) and ether (10 mL). The resulting solution was charged with 50 psi of NO and stirred overnight. A solid precipitate that resulted was collected by filtration, washed with ether and dried under vacuum to afford sodium 1-[(4-*t*-butoxycarbonyl)homopiperazin-1-yl]diazen-1-ium-1,2-diolate (7.67 g, 54%). UV (0.01 M NaOH) λ_{max} (ε) 250 nm (7.2 $mM^{-1}cm^{-1}$); ^{1}H NMR (D_2O with 10% NaOD) δ 1.96 (s, 9H), 3.18-4.79 (m, 8H).

Sodium 1-[(4-*t*-butoxycarbonyl)homopiperazin-1-yl]diazen-1-ium-1,2-diolate (4.8 g, 27.1 mmol) was dissolved in ice cold 5% aqueous sodium bicarbonate solution (60 mL), and this mixture was treated with a slurry of 1-fluoro-2,4-dinitrobenzene (1.1 g, 27.1 mmol) in *t*-BuOH (30 mL) and THF (5 mL). A yellow precipitate resulted, which was purified by column chromatography ($CHCl_3$:ethyl acetate 9:1) to afford **1b** (3.8 g, 53%): mp 117-120 °C; UV (DMSO) λ_{max} (ε) 317 nm (10.1 $mM^{-1}cm^{-1}$); ^{1}H NMR (DMSO-d_6, 70 °C) δ 1.35 (s, 9H), 1.90 (quintet, *J* = 6.0 Hz, 2H), 3.38 (t, *J* = 6.0 Hz, 2H), 3.63 (t, *J* = 6.0 Hz, 2H), 3.92 (t, *J* = 6.0 Hz, 2H), 3.99 (t, *J* = 6.0 Hz, 2H), 7.81 (d, *J* = 9.4 Hz, 1H), 8.51 (dd, *J* = 9.4, 2.8 Hz, 1H), 8.82 (d, *J* = 2.8 Hz, 1H); ^{13}C NMR (DMSO-d_6, 70 °C) δ 24.5, 27.6, 43.4, 45.0, 49.7, 50.0, 78.7, 117.4, 121.2, 129.2, 136.6, 141.5, 152.9, 153.8. Anal. Calcd for $C_{16}H_{22}N_6O_8$: C, 45.07; H, 5.20; N, 19.71, Found: C, 45.16; H, 5.19; N, 19.63.

***O*^{2}-(2,4-Dinitrophenyl) 1-[4-Homopiperazin-1-yl]diazen-1-ium-1,2-diolate Hydrochloride (2b) (RN-1-15).** To an ice-cold solution of **1b** (0.47 g, 1.1 mmol) in ethyl acetate (30 mL), concentrated HCl (2 mL) was added dropwise. The reaction mixture was allowed to attain room temperature and stirred at room temperature for 12 h. The yellow solid was filtered and dried under vacuum. The compound **2b** obtained (0.3 g, 75%) was used in next step without further purification: mp 146-149 °C (decomposition); UV (DMSO) λ_{max} (ε)

313 nm (12.6 $mM^{-1}cm^{-1}$); ^1H NMR (CD_3OD, 25 °C) δ 2.18 (quintet, *J* = 6.0 Hz, 2H), 3.24 (t, *J* = 4.8 Hz, 2H), 3.36-3.39 (m, 2H), 4.03 (t, *J* = 6.0 Hz, 2H), 4.24 (t, *J* = 6.0 Hz, 2H), 7.91 (d, *J* = 9.2 Hz, 1H), 8.54 (dd, *J* = 9.2, 2.8 Hz, 1H), 8.87 (d, *J* = 2.8 Hz, 1H), 9.57 (*broad*, 2H); ^{13}C NMR (CD_3OD, 25 °C) δ 32.5, 53.3, 54.2, 55.3, 58.6, 127.2, 131.2, 139.2, 146.1, 151.2, 162.7. A sample of this compound failed to provide satisfactory results in the elemental analysis test. Further evidence for identity and purity of this compound was inferred from the NMR spectra (Figure S1 and S2) and by conversion to derivatives **11**-**15** that provided satisfactory analytical data.

General Procedure for Synthesis of Compounds 3-15. A solution of **2a** or **2b** in CH_2Cl_2 was reacted with a solution of 1-1.5 equivalents of the electrophile and 2-2.2 equivalents of triethylamine. The reaction mixture was diluted with CH_2Cl_2, and then washed with dilute HCl and aqueous sodium bicarbonate. The organic layer was separated, dried ($MgSO_4$), and filtered, and the solvent was removed under reduced pressure to form a solid. This was then triturated with ether, filtered and dried under vacuum.

***O*^{2}-(2,4-Dinitrophenyl) 1-[4-(2-Fluoroethoxycarbonyl)piperazin-1-yl]diazen-1-ium-1,2-diolate (3) (JS-50-123).** Starting from **2a** (102 mg, 0.29 mmol), triethylamine (88 μL, 0.6 mmol), and 2-fluoroethyl chloroformate (27 μL, 0.29 mmol), **3** was isolated as a yellow solid (107 mg, yield 87%). mp 119-120 °C; UV (ethanol) λ_{max} (ε) 299 nm (17.9 $mM^{-1}cm^{-1}$); ^1H NMR ($CDCl_3$) δ 3.64-3.66 (m, 4H) 3.75-3.78 (m, 4H), 4.34-4.36 (m, 1H), 4.41-4.43 (m, 1H), 4.56-4.58 (m, 1H), 4.68-4.70 (m, 1H), 7.66 (d, *J* = 9.2 Hz, 1H), 8.47 (dd, *J* = 2.7, 9.2 Hz, 1H), 8.89 (d, *J* = 2.7 Hz, 1H); ^{13}C NMR δ 42.4, 50.5, 65.0 (*J* = 19.9 Hz), 81.56 (*J* = 169.4 Hz), 117.7, 122.2, 129.1, 142.5, 153.6, 154.4. Anal. Calcd for $C_{13}H_{15}N_6O_8F$: C, 38.81; H, 3.76; N, 20.89. Found: C, 38.90; H, 3.80; N, 20.76.

***O*^{2}-(2,4-Dinitrophenyl) 1-[4-(Vinyloxycarbonyl)piperazin-1-yl]diazen-1-ium-1,2-diolate (4) (JS-50-124).** Starting from **2a** (133 mg, 0.38 mmol), triethylamine (117 μL, 0.8 mmol), and vinyl chloroformate (36 μL, 0.38 mmol), **4** was isolated as a yellow solid (105 mg, yield 72%). mp 140-142 °C; UV (ethanol) λ_{max} (ε) 299 nm (17.6 $mM^{-1}cm^{-1}$); ^1H NMR ($CDCl_3$) δ 3.66-3.68 (m, 4H), 3.78 (b, 4H), 4.55 (dd, *J* = 6.2, 1.8 Hz, 1H) 4.84 (dd, *J* = 14.0, 1.8 Hz, 1H), 7.15-7.23 (dd, *J* = 14.0, 6.2 Hz, 1H), 7.66 (d, *J* = 9.3 Hz , 1H), 8.45-8.48 (dd, *J* = 2.3, 9.2 Hz,1H), 8.89 (d, *J* = 2.7 Hz, 1H); ^{13}C NMR ($CDCl_3$) δ 42.2, 50.5, 96.5, 117.8, 122.2, 129.1, 137.5, 142.1, 142.6, 152.0, 153.6. Anal. Calcd for $C_{13}H_{14}N_6O_8$: C, 40.84; H, 3.69; N, 21.98. Found: C, 40.93; H, 3.78; N, 21.71.

***O*^{2}-(2,4-Dinitrophenyl) 1-[4-(Allyloxycarbonyl)piperazin-1-yl]diazen-1-ium-1,2-diolate (5) (JS-51-94).** Starting from **2a** (210 mg, 0.60 mmol), triethylamine (184 µL, 1.3 mmol), and allyl chloroformate (64 µL, 0.60 mmol), **5** was isolated as a yellow solid (213 mg, yield 89%). mp 119-120 °C; UV (ethanol) λ_{max} (ε) 300 nm (16.3 $mM^{-1}cm^{-1}$); ^{1}H NMR ($CDCl_3$) δ 3.63-3.65 (m, 4H), 3.74-3.77 (m, 4H), 4.62-4.64 (m, 2H), 5.24-5.35 (m, 2H), 5.90-6.00 (m, 1H) 7.66 (d, *J* = 9.4 Hz, 1H), 8.47 (dd, *J* = 2.7, 9.4 Hz, 1H), 8.89 (d, *J* = 2.7 Hz, 1H); ^{13}C NMR ($CDCl_3$) δ 42.3, 50.5, 66.7, 117.7, 118.2, 122.2, 129.1, 132.4, 137.4, 142.5, 153.7, 154.6. Anal. Calcd for $C_{14}H_{16}N_6O_8$: C, 42.42; H, 4.07; N, 21.21. Found: C, 42.50; H, 3.99; N, 21.09.

***O*^{2}-(2,4-Dinitrophenyl) 1-[4-(Propargyloxycarbonyl)piperazin-1-yl]diazen-1-ium-1,2-diolate (6) (RN-1-37).** Starting from **2a** (150 mg, 0.43 mmol), triethylamine (120 µL, 0.86 mmol), and propargyl chloroformate (60 µL, 0.65 mmol), **6** was isolated as a yellow solid (145 mg, yield 86%). mp 92-96 °C; UV (DMSO) λ_{max} (ε) 304 nm (14.2 $mM^{-1}cm^{-1}$); ^{1}H NMR ($CDCl_3$) δ 2.51 (t, *J* = 2.4 Hz, 1H), 3.65 (t, *J* = 5.0 Hz, 4H), 3.77 (t, *J* = 5.0 Hz, 4H), 4.74 (d, *J* = 2.4 Hz, 2H), 7.67 (d, *J* = 9.2 Hz, 1H), 8.65 (dd, *J* = 9.2, 2.4 Hz, 1H), 8.88 (d, *J* = 2.4 Hz, 1H); ^{13}C NMR ($CDCl_3$) δ 42.4, 50.5, 53.5, 75.0, 77.8, 117.7, 122.2, 129.1, 137.4, 142.5, 153.6, 153.9. Anal. Calcd for $C_{14}H_{14}N_6O_8$. 0.2EtOAc: C, 43.15; H, 3.82; N, 20.40. Found: C, 43.29; H, 3.75; N, 20.55.

***O*^{2}-(2,4-Dinitrophenyl) 1-[4-(2,2,2-Trichloroethoxycarbonyl)piperazin-1-yl]diazen-1-ium-1,2-diolate (7) (JS-43-63).** Starting from **2a** (446 mg, 1.28 mmol), triethylamine (356 µL, 0.6 mmol), and 2,2,2-(trichloroethyl) chloroformate (172 µL, 1.28 mmol), **7** was isolated as a yellow solid (485 mg, yield 78%). mp 115-116 °C; UV (ethanol) λ_{max} (ε) 298 nm (14.1 $mM^{-1}cm^{-1}$); ^{1}H NMR ($CDCl_3$) δ 3.67-3.70 (m, 4H), 3.82-3.84 (b, 4H), 4.79 (s, 2H), 7.67 (d, *J* = 9.2 Hz, 1H), 8.47 (dd, *J* = 9.2, 2.8 Hz, 1H), 8.88 (d, *J* = 2.8 Hz, 1H); ^{13}C NMR ($CDCl_3$) δ 42.6, 50.5, 75.3, 95.3, 117.8, 122.2, 129.1, 137.5, 142.6, 153.0, 153.6. Anal. Calcd for $C_{13}H_{13}Cl_3N_6O_8$: C, 32.02; H, 2.69; Cl, 21.81; N, 17.23. Found: C, 32.08; H, 2.79; Cl, 21.81; N, 16.94

***O*^{2}-(2,4-Dinitrophenyl) 1-[4-(Ethylmercaptocarbonyl)piperazin-1-yl]diazen-1-ium-1,2-diolate (8) (JS-50-125).** Starting from **2a** (105 mg, 0.30 mmol), triethylamine (86 µL, 0.62 mmol), and *S*-ethyl chloroformate (31 µL, 0.30 mmol), **8** was isolated as a yellow solid (112 mg, yield 93%). mp 147-149 °C; UV (ethanol) λ_{max} (ε) 299 nm (17.9 $mM^{-1}cm^{-1}$); ^{1}H NMR ($CDCl_3$) δ 1.31 (t, *J* =7.1 Hz, 3H) 2.96 (q, *J* = 7.3 Hz, 2H), 3.65-3.68 (m, 4H), 3.80 (b, 4H), 7.66 (d, *J* = 9.3 Hz, 1H) 8.45 (dd, *J* = 9.3, 2.7 Hz, 1H), 8.89 (d, *J* = 2.7 Hz, 1H); ^{13}C NMR ($CDCl_3$) δ 15.3, 25.0, 50.4, 117.7, 122.2, 129.1, 137.5, 142.6, 153.6, 167.8. Anal. Calcd for $C_{13}H_{16}N_6O_7S$: C, 39.00; H, 4.03; N, 20.99, S, 8.01. Found: C, 38.98; H, 4.06; N, 20.83; S. 7.95.

***O*^{2}-(2,4-Dinitrophenyl) 1-[4-(Ethylaminocarbonyl)piperazin-1-yl]diazen-1-ium-1,2-diolate (9) (JS-50-188).** Starting from **2a** (156 mg, 0.45 mmol), triethylamine (124 μL, 0.90 mmol), and ethyl isocyanate (40 μL, 0.50 mmol), **9** was isolated as a yellow solid (118 mg, yield 69%). mp 144-145 ^{o}C; UV (ethanol) λ_{max} (ε) 299 nm (9.3 $mM^{-1}cm^{-1}$); ^{1}H NMR ($CDCl_3$) δ 1.17 (t, *J* = 7.3 Hz, 3H), 3.27-3.33 (m, 2H), 3.62-3.67 (m, 8H), 4.82 (*broad*, 1H), 7.67 (d, *J* = 9.3 Hz, 1H), 8.45 (dd, *J* = 9.3, 2.9 Hz, 1H), 8.89 (d, *J* = 2.9 Hz, 1H); ^{13}C NMR ($CDCl_3$) δ 15.5, 35.9, 42.5, 50.4, 117.5, 117.9, 122.0, 129.2, 153.7, 157.0. Anal. Calcd for $C_{13}H_{17}N_7O_7$: C, 40.73; H, 4.47; N, 25.58. Found: C, 40.80; H, 4.50; N, 25.51

***O*^{2}-(2,4-Dinitrophenyl) 1-[4-(Diethoxyphosphoryl)piperazin-1-yl]diazen-1-ium-1,2-diolate (10) (JS-51-58).** Starting from **2a** (181 mg, 0.52 mmol), triethylamine (181 μL, 1.3 mmol), and diethyl chlorophosphate (75 μL, 0.52 mmol), **10** was isolated as a yellow solid (205 mg, yield 88%). mp 80-81 ^{o}C; UV (ethanol) λ_{max} (ε) 299 nm (14.0 $mM^{-1}cm^{-1}$); ^{1}H NMR ($CDCl_3$) δ 1.35 (t, *J* = 7.1 Hz, 6H), 3.39-3.42 (m, 4H), 3.61-3.64 (m, 4H), 4.06-4.11 (q, *J* = 7.1 Hz, 4H), 7.67 (d, *J* = 9.3 Hz, 1H), 8.52 (dd, *J* = 9.3, 2.7 Hz, 1H), 8.88 (d, *J* = 2.7 Hz, 1H); ^{13}C NMR ($CDCl_3$) δ 16.2, 43.3, 50.8, 51.0, 62.8, 117.5, 122.3, 129.2, 142.4, 153.7. Anal. Calcd for $C_{14}H_{21}N_6O_9P$: C, 37.51; H, 4.72, N, 18.75. Found: C, 37.23; H, 4.73; N, 16.91. A copy of the ^{1}H and ^{13} C NMR spectra is available in Figures S3 and S4 in support of purity of this compound.

***O*^{2}-(2,4-Dinitrophenyl) 1-[4-(2-Fluoroethoxycarbonyl)homopiperazin-1-yl]diazen-1-ium-1,2-diolate (11) (RN-1-18).** Starting from **2b** (120 mg, 0.33 mmol), triethylamine (90 μL, 0.66 mmol), and 2-fluoroethyl chloroformate (50 μL, 0.50 mmol), **11** was isolated as a yellow solid (122 mg, yield 89%). mp 82-86 ^{o}C; UV (DMSO) λ_{max} (ε) 314 nm (14.0 $mM^{-1}cm^{-1}$); ^{1}H NMR (DMSO-d_6, 70 ^{o}C) δ 1.93 (quintet, *J* = 6.0 Hz, 2H), 3.47 (t, *J* = 6.0 Hz, 2H), 3.69-3.73 (m, 2H), 3.95 (t, *J* = 6.0 Hz, 2H), 4.02 (t, *J* = 6.0 Hz, 2H), 4.23 (dt, *J* = 29.6, 4.0 Hz, 2H), 4.55 (dt, *J* = 47.6, 4.0 Hz, 2H), 7.79 (d, *J* = 9.2 Hz, 1H), 8.50 (dd, *J* = 9.2, 2.8 Hz, 1H), 8.82 (d, *J* = 2.8 Hz, 1H); ^{13}C NMR (DMSO-d_6, 70 ^{o}C) δ 24.7, 43.8, 45.1, 49.4, 50.1, 64.0 (*J* = 19.5 Hz), 81.6 (*J* = 165.3 Hz), 117.4, 121.2, 129.2, 136.6, 141.6, 153.0, 154.4. Anal. Calcd for $C_{14}H_{17}FN_6O_8$: C, 40.39; H, 4.12; N, 20.19. Found: C, 40.62; H, 4.15; N, 19.88.

***O*^{2}-(2,4-Dinitrophenyl) 1-[4-(Vinyloxycarbonyl)homopiperazin-1-yl]diazen-1-ium-1,2-diolate (12) (RN-1-17).** Starting from **2b** (145 mg, 0.40 mmol), triethylamine (117 μL, 0.8 mmol), and vinyl chloroformate (60 μL, 0.6 mmol), **12** was isolated as a yellow solid (105 mg, yield 66%). mp 112-115 ^{o}C; UV (DMSO) λ_{max} (ε) 315 nm (13.7 $mM^{-1}cm^{-1}$); ^{1}H NMR (DMSO-d_6, 70 ^{o}C) δ 1.95 (quintet, *J* = 5.2 Hz, 2H), 3.51 (*broad*, 2H), 3.75 (*broad*, 2H), 3.97 (t, *J* = 6.0 Hz, 2H), 4.04 (t, *J* = 6.0 Hz, 2H), 4.40-4.50 (*broad*, 1H), 4.69-4.81 (*broad*, 1H), 7.04-7.07 (*broad*, 1H), 7.79 (d, *J* = 9.2 Hz, 1H), 8.50 (dd, *J* = 9.2, 2.8 Hz, 1H), 8.82 (d, *J* = 2.8 Hz, 1H);

^{13}C NMR (DMSO-d_6, 70 ^{o}C) δ 25.1, 43.9, 45.2, 49.4, 50.0, 95.6, 117.5, 121.2, 129.2, 136.6, 141.6, 142.1, 152.0, 152.9. Anal. Calcd for $C_{14}H_{16}N_6O_8$: C, 42.43; H, 4.07; N, 21.21. Found: C, 42.50; H, 3.99; N, 21.11.

***O*^{2}-(2,4-Dinitrophenyl) 1-[4-(Allyloxycarbonyl)homopiperazin-1-yl]diazen-1-ium-1,2-diolate (13) (RN-1-25).** Starting from **2b** (200 mg, 0.55 mmol), triethylamine (155 μL, 1.1 mmol), and allyl chloroformate (90 μL, 0.83 mmol), **13** was isolated as a yellow solid (188 mg, yield 83%). mp 109-112 ^{o}C; UV (DMSO) λ_{max} (ε) 316 nm (13.3 $mM^{-1}cm^{-1}$); ^{1}H NMR (DMSO-d_6, 70 ^{o}C) δ 1.92 (quintet, *J* = 6.0 Hz, 2H), 3.47 (t, *J* = 6.0 Hz, 2H), 3.71 (t, *J* = 6.0 Hz, 2H), 3.95 (t, *J* = 6.0 Hz, 2H), 4.02 (t, *J* = 6.0 Hz, 2H), 4.50 (d, *J* = 4.8 Hz, 2H), 5.12 (d, *J* = 10.4 Hz, 1H), 5.22 (d, *J* = 17.2 Hz, 1H), 5.83-5.92 (m, 1H), 7.79 (d, *J* = 9.2 Hz, 1H), 8.50 (dd, *J* = 9.2, 2.8 Hz, 1H), 8.82 (d, *J* = 2.8 Hz, 1H); ^{13}C NMR (DMSO-d_6, 70 ^{o}C) δ 34.3, 53.1, 54.6, 59.0, 59.6, 74.5, 126.0, 126.9, 130.7, 138.7, 142.5, 146.1, 151.1, 162.5, 163.8. Anal. Calcd for $C_{15}H_{18}N_6O_8$: C, 43.91; H, 4.42; N, 20.48. Found: C, 43.85; H, 4.33; N, 20.33.

***O*^{2}-(2,4-Dinitrophenyl) 1-[4-(Propargyloxycarbonyl)homopiperazin-1-yl]diazen-1-ium-1,2-diolate (14) (RN-1-26).** Starting from **2b** (200 mg, 0.55 mmol), triethylamine (155 μL, 1.1 mmol), and propargyl chloroformate (90 μL, 0.83 mmol), **14** was isolated as a yellow solid (200 mg, yield 89%). mp 122-125 ^{o}C; UV (DMSO) λ_{max} (ε) 315 nm (14.9 $mM^{-1}cm^{-1}$); ^{1}H NMR (DMSO-d_6, 70 ^{o}C) δ 1.93 (quintet, *J* = 6.0 Hz, 2H), 3.30 (s, 1H), 3.47 (t, *J* = 6.0 Hz, 2H), 3.70 (t, *J* = 6.0 Hz, 2H), 3.95 (t, *J* = 6.0 Hz, 2H), 4.01 (t, *J* = 6.0 Hz, 2H), 4.65 (s, 2H), 7.79 (d, *J* = 9.2 Hz, 1H), 8.51 (dd, *J* = 9.2, 3.0 Hz, 1H), 8.82 (d, *J* = 3.0 Hz, 1H); ^{13}C NMR (DMSO-d_6, 70 ^{o}C) δ 34.4, 53.4, 54.7, 58.9, 59.6, 61.9, 86.2, 88.1, 126.9, 130.7, 138.7, 146.1, 151.1, 162.5, 163.4. Anal. Calcd for $C_{15}H_{16}N_6O_8$: C, 44.12; H, 3.95; N, 20.58. Found: C, 43.95; H, 3.87; N, 20.46.

***O*^{2}-(2,4-Dinitrophenyl) 1-[4-(Ethylmercaptocarbonyl)homopiperazin-1-yl]diazen-1-ium-1,2-diolate (15) (RN-1-19).** Starting from **2b** (120 mg, 0.33 mmol), triethylamine (90 μL, 0.66 mmol), and *S*-ethyl chloroformate (50 μL, 0.45 mmol), **15** was isolated as a yellow solid (115 mg, yield 84%). mp 58-62 ^{o}C; UV (DMSO) λ_{max} (ε) 314 nm (13.6 $mM^{-1}cm^{-1}$); ^{1}H NMR (DMSO-d_6, 70 ^{o}C) δ 1.15 (t, *J* = 7.2 Hz, 3H), 1.96 (quintet, *J* = 5.2 Hz, 2H), 2.80 (q, *J* = 7.2 Hz, 2H), 3.57 (t, *J* = 6.0 Hz, 2H), 3.79 (t, *J* = 5.2 Hz, 2H), 3.94 (t, *J* = 6.0 Hz, 2H), 4.05 (t, *J* = 5.2 Hz, 2H), 7.81 (d, *J* = 9.2 Hz, 1H), 8.50 (dd, *J* = 9.2, 2.8 Hz, 1H), 8.82 (d, *J* = 2.8 Hz, 1H); ^{13}C NMR (DMSO-d_6, 70 ^{o}C) δ 14.8, 23.7, 24.6, 44.1, 45.6, 49.5, 49.7, 117.5, 121.2, 129.2, 136.6, 141.6, 152.9, 166.4. Anal. Calcd for $C_{14}H_{18}N_6O_7S$: C, 40.58; H, 4.38; N, 20.28; S, 7.74. Found: C, 40.62; H, 4.40; N, 20.21; S, 7.65.

***O*^{2}-(2,4-Dinitrophenyl) 1-[4-Methylpiperazin-1-yl]diazen-1-ium-1,2-diolate Methiodide (JS-52-25) (17).** To a solution of **1b** (255 mg, 0.78 mmol) in CH_2Cl_2 (10 mL) was added iodomethane (62 μL, 1 mmol). After standing for overnight at room temperature, the crystallized product was collected by filtration and washed with CH_2Cl_2. The product **17** (158 mg, 43%) was dried under vacuum. mp 194-198 ^oC; UV (ethanol) λ_{max} (ε) 297 nm (11.7 $mM^{-1}cm^{-1}$); ^1H NMR (DMSO-d_6) δ 3.22 (s, 6H), 3.66-3.69 (m, 4H), 4.09-4.11 (m, 4H), 8.00 (d, J = 9.4 Hz, 1H), 8.60 (dd, J = 9.4, 2.8 Hz, 1H), 8.91 (d, J = 2.8 Hz, 1H); ^{13}C NMR (DMSO-d_6) δ 44.1, 50.9, 59.0, 118.5, 122.3, 130.2, 137.4, 142.8, 153.0. Anal. Calcd for $C_{12}H_{17}IN_6O_6$: C, 30.78; H, 3.66; N, 17.95; I, 27.10. Found: C, 30.83; H, 3.55; N, 17.78, I, 27.24.

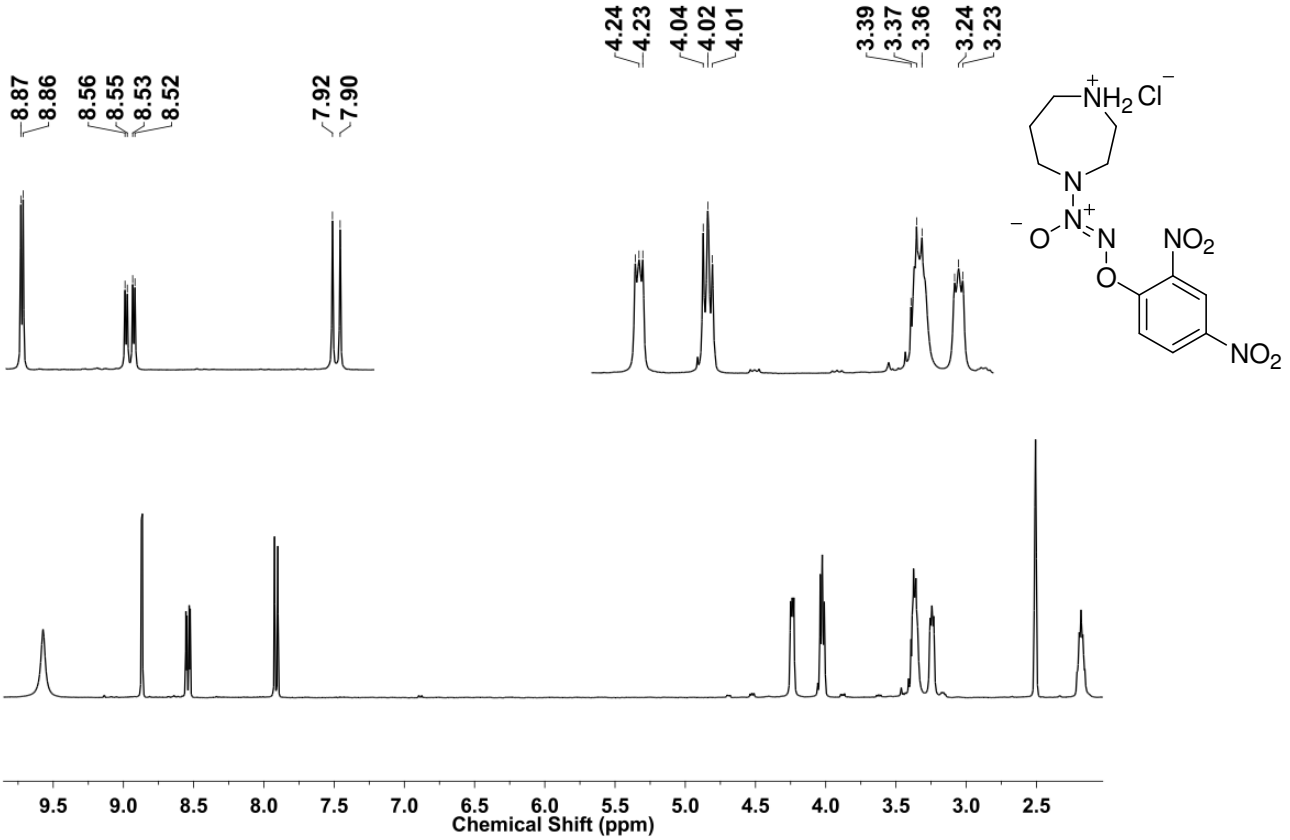

Figure S1. ^{1}H NMR Spectra of **2b** recorded at 25 °C in DMSO-d_6.

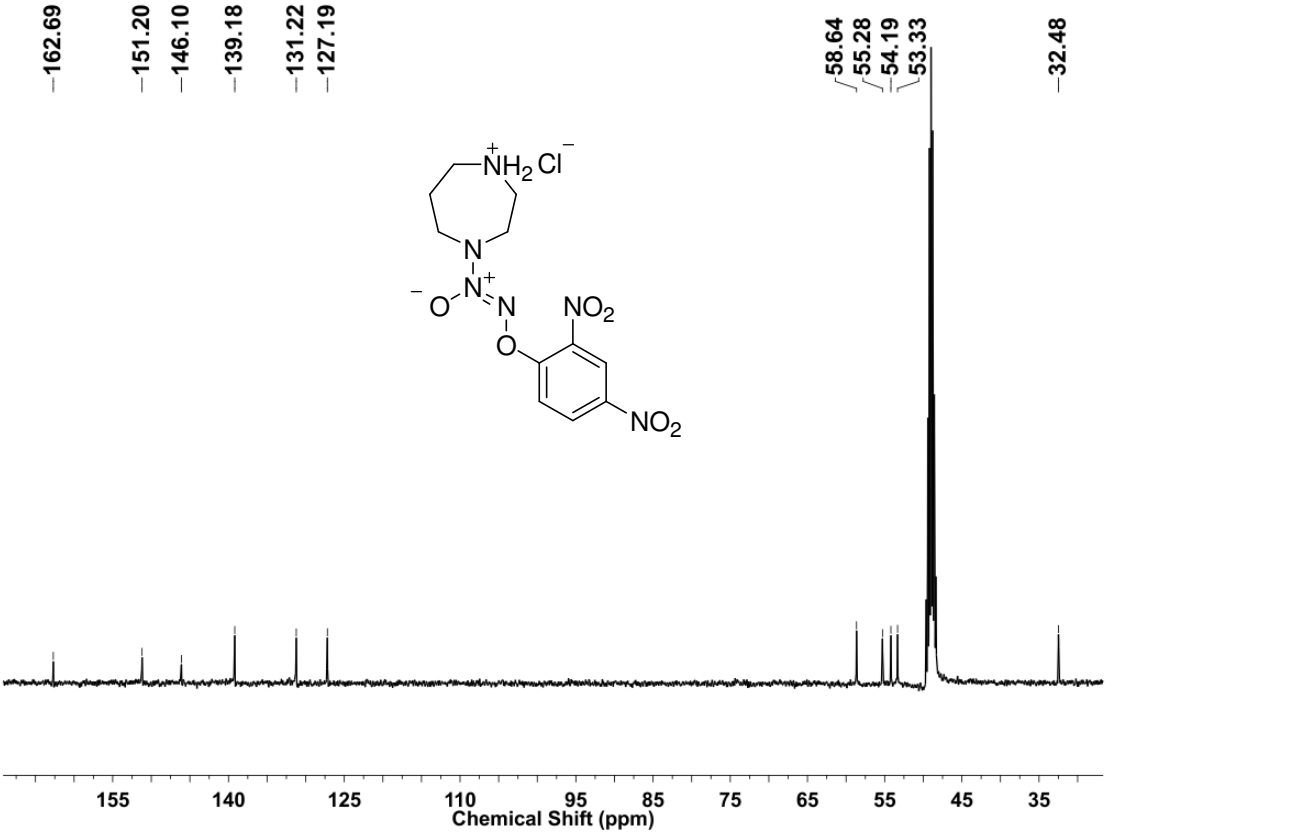

Figure S2. ^{13}C NMR spectrum of **2b** recorded at 25 °C in DMSO-d_6.

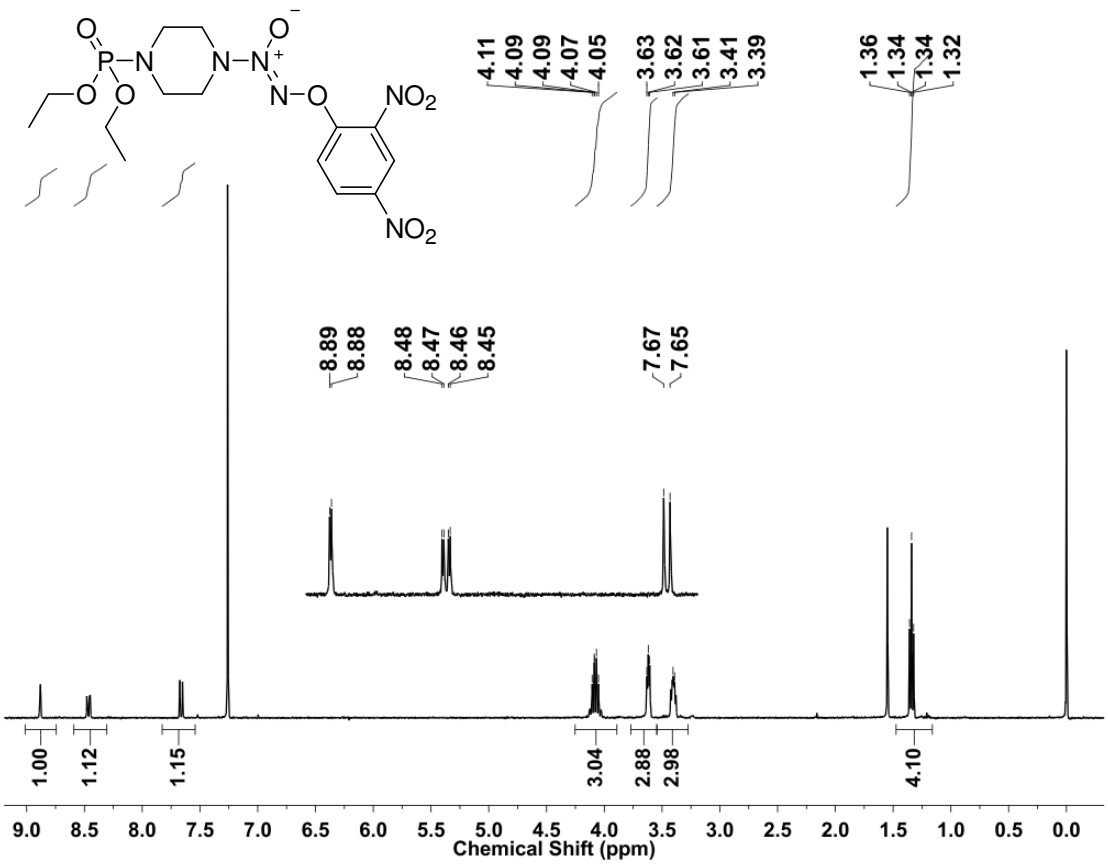

Figure S3. ^{1}H NMR spectrum of **10** recorded at 25 °C in $CDCl_3$.

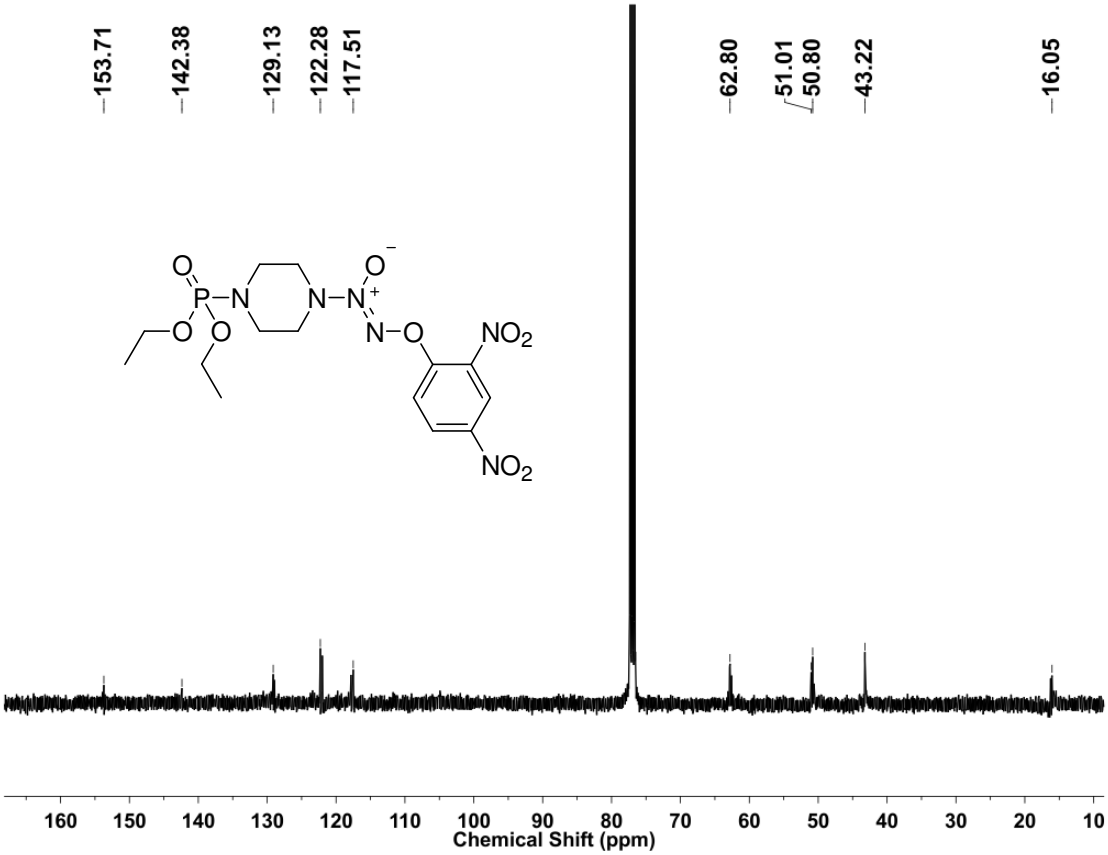

Figure S4. ^{13}C NMR Spectrum of **10** recorded at 25 °C in $CDCl_3$.

Cell Culture and Cytotoxicity Assays. HL-60 and U937 cell lines were obtained from American Type Culture Collection (ATCC, Manassas, VA). Cells were maintained in RPMI 1640 medium (Gibco, Invitrogen, Carlsbad, CA) supplemented with 10% fetal calf serum (Gemini Bio-Products, Sacramento, CA), 100 U/mL penicillin and 2 mM glutamine, at 37 °C and 5% CO_2. The CellTiter 96 non-radioactive cell proliferation assay (MTT assay, Promega, Madison, WI), performed according to the manufacturer's protocol, was used to measure cell growth. Cells were seeded in 96-well plates at the density of 10^4 per well and allowed to grow for 24 h before addition of the drugs. Diazeniumdiolate prodrugs were prepared as 10 mM stock solution in DMSO (Sigma, St. Louis, MO). Increasing drug concentrations in 10 μL of PBS were added to 100 μL of the culture medium for 72 h. Each compound concentration was represented in six repeats, and the screening was performed as at least two independent experiments.

Gluathione-activated NO Release. Calibration of the Sievers Nitric Oxide Analyzer (NOA), model 280i (Instruments Business Group, Boulder, CO) was performed by injecting of various volumes of known concentrations of NO in helium (50 ppm, 500 ppm and 5%) certified standards into the reaction chamber and recording the peaks. Samples and reaction chambers were incubated at 37 °C. The contents of the reaction chamber were sparged with argon and swept into the chemiluminescence detector. Data were recorded using Agilent Chemstation software and processed using Microsoft Excel. Approximately 3.5 mL of pH 7.4 buffer containing GSH (3.6-3.9 mM) and diethylenetriaminepentaacetic acid (DTPA, 50 μM) was placed into the reaction chamber of the NOA and then sparged for several minutes with argon. A DMSO solution (10 mM) of the prodrug (50-100 μL) was injected into the reaction chamber and nitric oxide release was recorded. Total amount of NO released was determined by measuring the area under the curve.

Intracellular NO Release. The intracellular level of nitric oxide after diazeniumdiolate prodrug treatment was quantified using the NO-sensitive fluorophore 4-amino-5-methylamino-2',7'-difluorofluorescein diacetate (DAF-FM diacetate; Invitrogen, Carlsbad, CA). HL-60 cells were loaded with 2.5 μM DAF-FM diacetate in Hanks' balanced salt solution (HBSS) at 37 °C and 5% CO_2. After 30 min of incubation the cells were collected by centrifugation, rinsed with HBSS to remove excess probe, and resuspended in fresh HBSS. The compounds were added to the cells at 5 μM final concentration. After 40 min incubation the fluorescence of the benzotriazole derivative formed on DAF-FM's reaction with aerobic NO was analyzed using a Perkin Elmer LS50B luminescence spectrometer with the excitation source at 495 nm and emission at 515 nm. The mean value of three independent experiments is reported.

References

1. Saavedra, J. E.; Booth, M. N.; Hrabie, J. A.; Davies, K. M.; Keefer, L. K. *J. Org. Chem.* **1999**, *64*, 5124.

2. Shami, P. J.; Saavedra, J. E.; Bonifant, C. L.; Chu, J.; Udupi, V.; Malaviya, S.; Carr, B. I.; Kar, S.; Wang, M.; Jia, L.; Ji, X.; Keefer, L. K. *J. Med. Chem.* **2006**, *49*, 4356.