Supporting Information to Accompany: Indirect and Direct Detection of the 4-(Benzothiazol-2-yl)phenylnitrenium Ion from a Putative Metabolite of a Model Anti-Tumor Drug

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Kinetics and Azide Trapping Experiments:

Kinetic experiments were carried out at 30 °C in aqueous 0.02 M phosphate buffer, pH 7.1, (5 vol% CH_3CN-H_2O, μ=0.5 ($NaClO_4$)). Solutions were incubated in cuvettes in the thermostatted cell holder of a UV-vis spectrophotometer for ca. 20 min before kinetic runs were initiated by injecting 15 μL of a 5.0 mM solution of **3** in CH_3CN into 3 mL of the buffer to obtain an initial concentration of **3** of 2.5×10^{-5} M. Data were collected at 319 nm (λ_{max} of **3**), 272 nm, 250 nm, and 221 nm (λ_{max} of **6**). Absorbance vs. time data were fit to a consecutive first-order rate equation at all wavelengths. HPLC kinetics were collected with identical reaction solutions at 10 °C. Reactions were monitored by periodic 20 μL injections onto an HPLC (C-8 reverse phase column, 70/30 MeOH/H_2O eluent, 1 mL/min, monitored by

UV absorbance at 212 nm and 330 nm). Peak area vs. time data were fit to a first-order rate equation (**3**), or a consecutive first-order rate equation (**6**).

A series of NaN_3 solutions in the concentration range 0.2 mM to 2 mM were prepared in 0.02 M phosphate buffer, pH 7.1, (5 vol% CH_3CN-H_2O, μ=0.5 ($NaClO_4$)). Solutions were incubated at 30 °C for ca. 20 min before 15 μL of a 5.0 mM solution of **3** in CH_3CN was added to 3 mL of the buffer. Reactions run for HPLC analysis were carried out in Teflon capped round bottom glass vials immersed in a water bath. A control experiment in phosphate buffer in the absence of NaN_3 was also included. Reactions run for kinetics were treated as described above except that kinetics were monitored at 350, 339, and 320 nm. Reaction mixtures were analyzed in duplicate after completion of the reaction by 20 μL injections of the reaction mixture from each vial onto an HPLC using the conditions described above. Product peak areas were plotted against [N_3^-].

Laser Flash Photolysis Experiments:

Laser flash photolysis was carried out using a Nd:YAG laser (308 nm, ca. 20 ns pulse) with a 1 cm light path. All reactions were performed at ambient temperature (22 °C). Solutions of **3** were made in O_2 saturated pH 7.1, 0.02 M phosphate buffer (5 vol% CH_3CN-H_2O, μ=0.5($NaClO_4$)) by injecting 15 μL of a 9.3 mM stock solution of **3** into 3 mL of buffer, so that the initial concentration of **3** was 4.7×10^{-5} M. These solutions had an absorbance of ca. 1.0 at 308 nm with a 1 cm light path. Since **3** undergoes hydrolysis to **6** under these conditions, all solutions were used promptly after mixing, and experiments were completed within 5 min of mixing. Concentrations of N_3^- were in the range from 0-1 mM.

Transient absorbance spectra were monitored in the range 430-690 nm. Spectra were obtained with a delay of 20 ns after the laser pulse. Transient spectra were collected with a 20 ns time window. Kinetics measurements were made at 570 nm for time spans that ranged from 1.25 μs to 5.00 μs depending on quenching rates. The kinetics measurements at 570 nm were averaged four times for each determination. Between each measurement, the sample was re-mixed to avoid depletion of reactants within the volume of the cuvette exposed to the flash. Kinetics traces were fit to a standard first-order rate equation. Data taken less than 10 ns after the flash were excluded from the fitting procedure.

Steady State Photolysis Experiments:

Steady state photolysis was performed in a Rayonet photochemical reactor in a jacketed quartz reactor kept at 10 °C. Luzchem LZC-UVB lamps that have emission in the range of 281-315 nm were used. A bank of 4 lamps arranged at 0 °, 90 °, 180 °, and 270 ° within the reactor were turned on several min before initiating the reaction by injection of 500 μL of a 4.8 mM stock solution of **3** in CH_3CN into a rapidly stirred 100 mL solution of O_2 saturated pH 7.1, 0.02 M phosphate buffer (μ=0.5($NaClO_4$)). This generated a solution with initial an concentration of **3** of 2.4×10^{-5} M. Photolysis lamps were turned off either 15 s or 30 s after initiation of the reaction. In the control experiment identical procedures were followed, except the lamps were not turned on. The photolysis process and subsequent decomposition of **3** and formation of **6** were analyzed as a function of time by 20 μL injections onto an HPLC (C-8 reverse phase column, 75/25 MeOH/H_2O eluent, 1 mL/min, monitored by UV absorbance at 212 nm and 330 nm).

Table S1. Rate constants obtained from UV absorbance measurements at 30 °C[a]

Conditions	$10^3 k_o$ (s^{-1})[b]	$10^4 k_1$ (s^{-1})[b]
pH 7.1 phosphate buffer	2.26 ± 0.05	5.33 ± 0.04
pH 7.1 phosphate buffer, 2 mM N_3^-	2.20 ± 0.34	

[a]Conditions: 5 vol% CH_3CN-H_2O, 30 °C, 0.02 M phosphate, μ = 0.5 ($NaClO_4$), pH 7.1, saturated with O_2. [b]Each rate constant is an average, with standard deviation, of two to three measurements taken at different wavelengths.

Table S2. Rate constants for quenching of 570 nm absorbance at 22 °C.[a]

$[N_3^-]$ (mM)	$10^{-6} k_{obs}$ (s^{-1})[b]
0	1.86 ± 0.07
0.25	3.03 ± 0.20
0.50	4.53 ± 0.18
0.75	5.54 ± 0.21
1.00	6.64 ± 0.54

[a]Conditions: 5 vol% CH_3CN-H_2O, 22 °C, 0.02 M phosphate, μ = 0.5 ($NaClO_4$), pH 7.1, saturated with O_2. [b]Each rate constant is the average of four runs with its standard deviation.

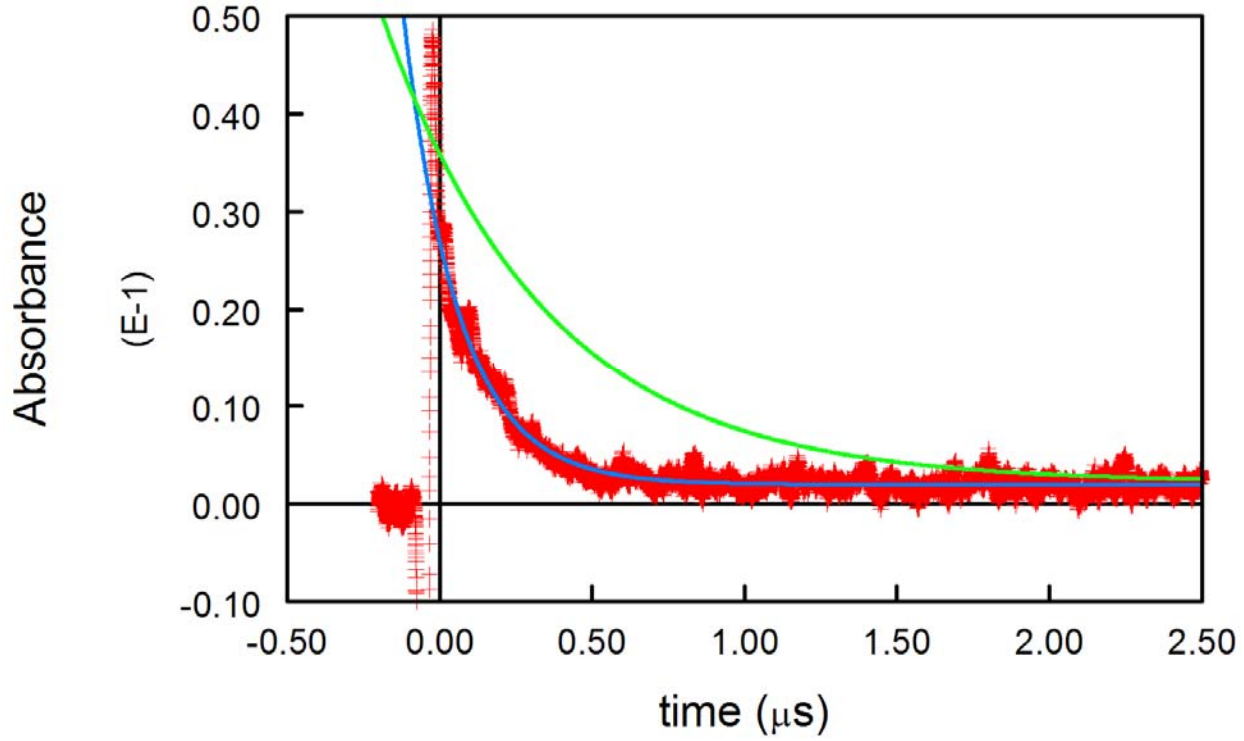


Figure S1. Decay of the absorbance at 570 nm in O_2-saturated pH 7.1 phosphate buffer, 0.75 mM N_3^-. Data taken 10 ns after the flash until the end of the run were fit to a standard first-order rate equation (blue curve). The green curve shows the least-squares fit of the time course of decay of the 570 nm absorbance in the absence of N_3^-. Absorbance data and the calculated curves are taken from the average of four runs.

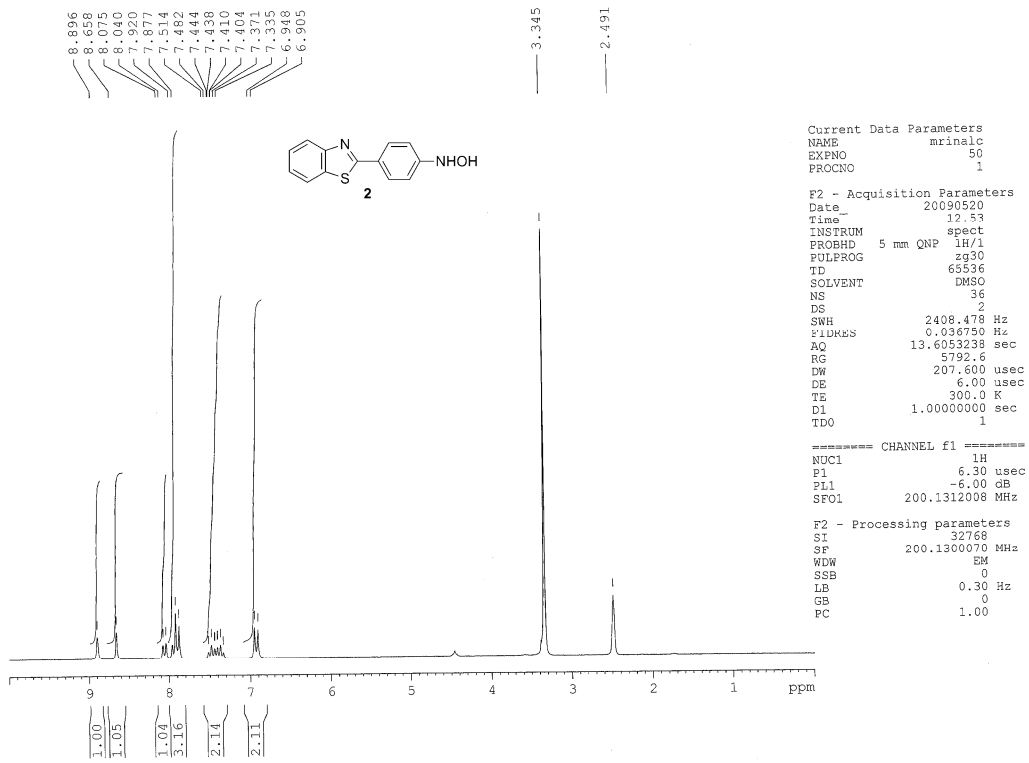
Synthesis:

***N*-(4-(Benzothiazol-2-yl)phenyl)hydroxylamine (2)**: 2-(4-Nitrophenyl)benzothiazole (0.001 mole, 0.256 g) was added to a three-neck 100 mL round bottom flask. Dry, freshly distilled THF (50 mL) was added and the mixture was stirred under nitrogen while 0.314 gm of 5% Pd/C was added. The flask was then put into an ethylene glycol-dry ice bath at -30 °C. After the flask had cooled for several minutes, 50 μL of hydrazine monohydrate was added via syringe and the mixture was stirred for 15 min. Then the flask was removed from the bath and allowed to reach room temperature. A 100μL aliquot was removed via syringe, diluted with 5 mL of 70/30 MeOH/H_2O and centrifuged for 3 minutes. A 20 μL sample was analyzed by HPLC (C-8 reverse phase column, 75/25 MeOH/H_2O eluent, 1 mL/min, monitored by UV absorbance at 320 nm). The reaction mixture contained both unreacted nitro

compound and the hydroxylamine product. The procedure was repeated with an additional 50 μL of hydrazine monohydrate. (Occasionally, additional small amounts of hydrazine hydrate (in 10 uL aliquots) were added to completely remove the nitro compound before quenching the reaction mixture.) When the peak corresponding to starting material had disappeared the reaction mixture was filtered through Celite and the filtrate was rotary evaporated quickly to obtain a light yellow solid that was purified by flash chromatography on silica gel using 60:40 EtOAc: pet ether eluent to yield 160 mg (66%) of the product: mp 182-188 °C with decomposition; IR 3318, 3135, 2834, 1606, 1471, 1433, 1232, 1178 cm^{-1}; ^{1}H NMR (200 MHz, DMSO-d_6) δ 6.92 (2H, d, J = 8.6 Hz), 7.33-7.51 (2H, m), 7.90 (2H, d, J = 8.6 Hz), 7.93 (1H, d, J = 7.8 Hz), 8.06 (1H, d J = 7.0 Hz), 8.66 (1H, s), 8.90 (1H, s).

***O*-Acetoxy-*N*-(4-(benzothiazol-2-yl)phenyl)hydroxylamine (3)**: A solution of 50 mg of **2** (0.206 mmol) and 29 μL of N-ethylmorpholine (0.229 mmol) in 30 mL of dry, freshly distilled benzene was stirred at room temperature for 15 minutes as **2** incompletely dissolved. Pyruvonitrile (16.5 μL, 0.206 mmol) was added via syringe in 3-4 μL portions over a period of 10 min. As the pyruvonitrile was added, **2** dissolved completely. After the last addition, the reaction mixture was stirred for another 15 min. The reaction mixture was transferred to a small separatory funnel and washed consecutively with 2 × 7 mL of ice cold 0.5 M NaOH, 2 × 7 mL of ice cold 5% aq $NaHCO_3$ and 2 × 7 mL of ice cold distilled water. The solution was dried over Na_2SO_4 and rotary evaporated to obtain 33 mg (60%) of a yellow solid: mp 71-81 °C with decomposition; IR 3258, 1756, 1606, 1476, 1434, 1203, 1176 cm^{-1}; ^{1}H NMR (500 MHz, CD_3CN) δ 2.20 (3H, s), 7.09 (2H, d, J = 8.8 Hz), 7.40 (1H, td, J = 7.6, 1.2 Hz), 7.50 (1H, td, J = 7.7, 1.3 Hz) 7.98 (2H, m), 8.01 (2H d, J = 8.8 Hz) 8.98 (1H, s); ^{13}C NMR (125.8 MHz, CD_3CN) δ 19.06 (2.20), 115.58 (7.09), 122.81 (7.98), 123.50 (7.98), 126.04 (7.40), 127.33 (7.40), 128.65, 129.27 (8.01), 135.64, 151.19, 155.05, 168.39, 171.09.

^{1}H NMR of **2**:



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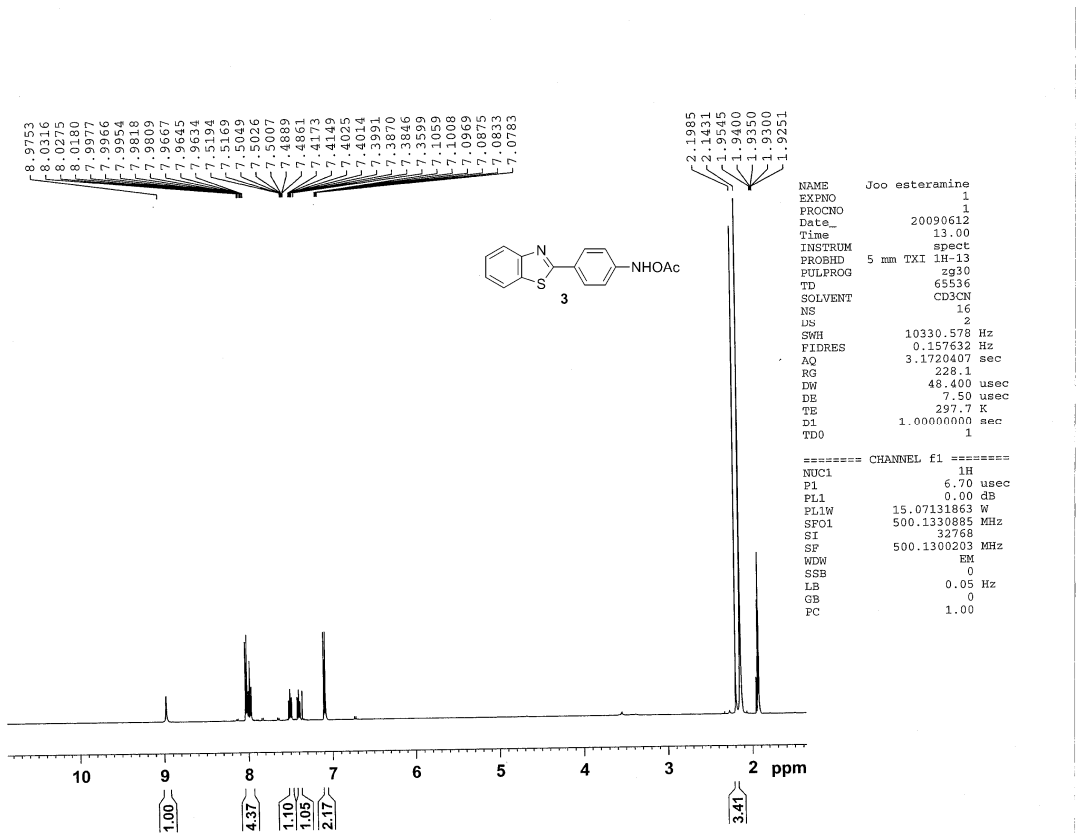
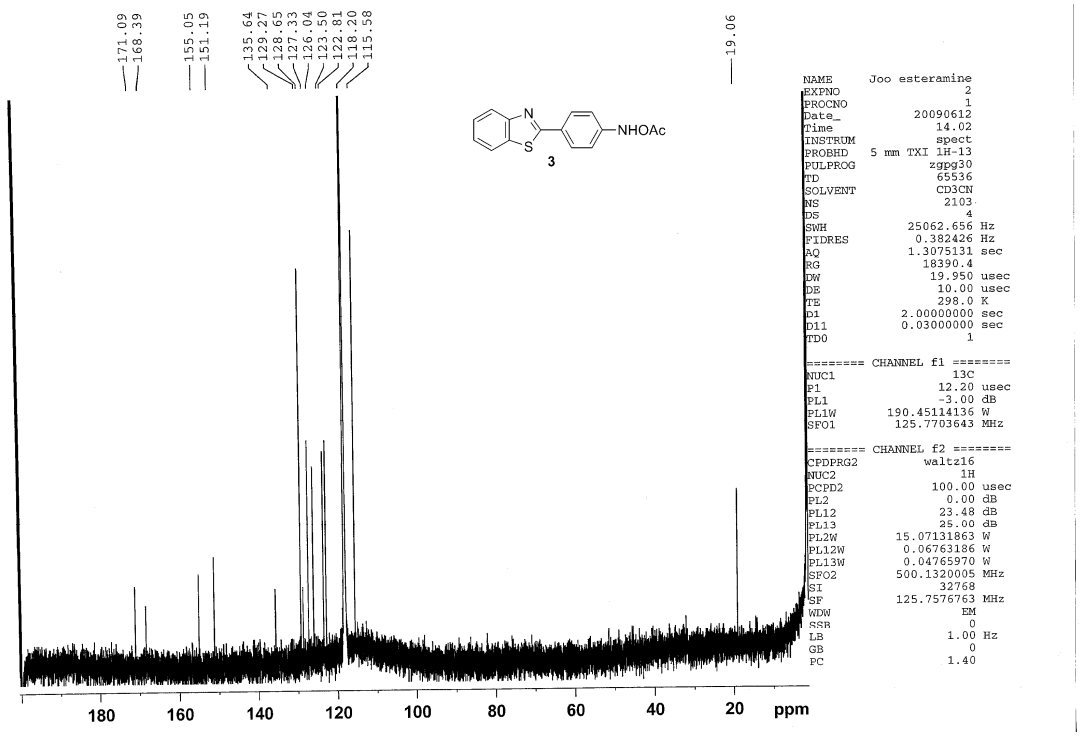


^{13}C NMR of **3**:

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TD0                        1

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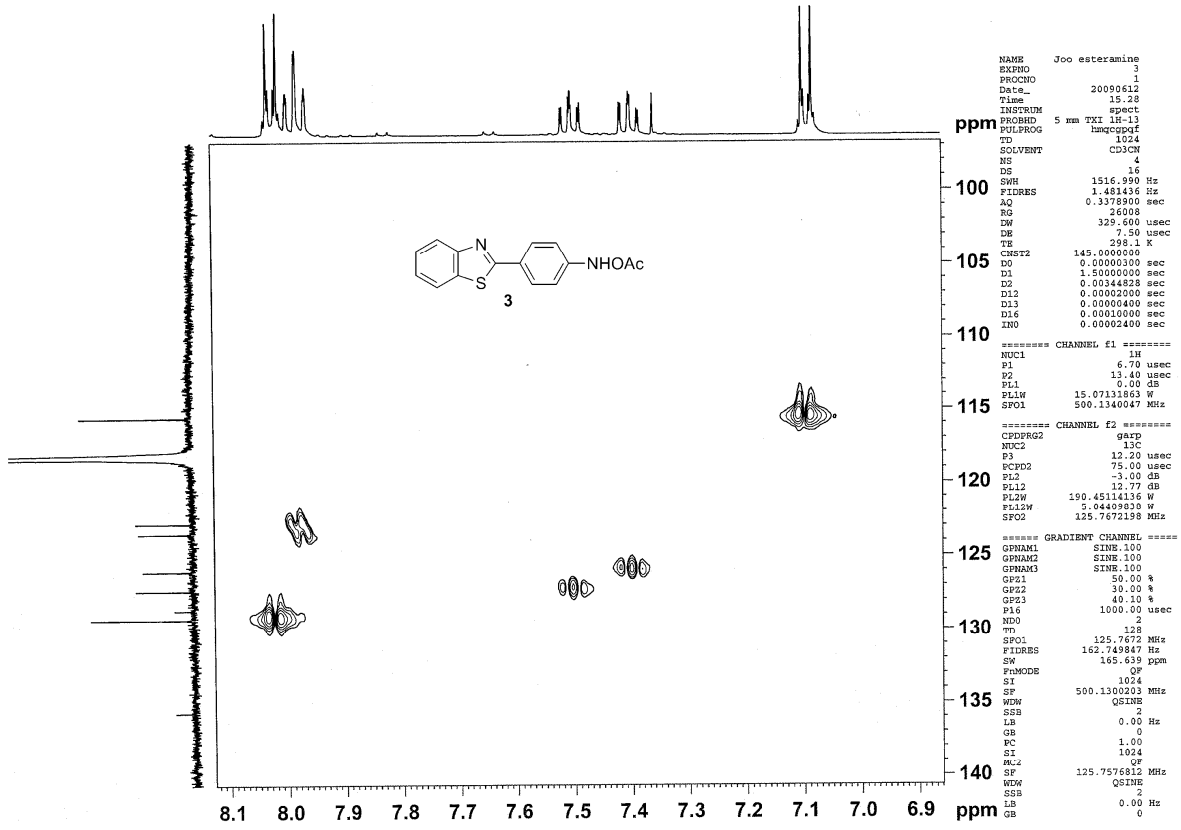
HMQC of **3**:

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