19F Magnetic Resonance Probes for Live-Cell Detection of Peroxynitrite Using an Oxidative Decarbonylation Reaction

Kevin J. Bruemmer, a Sara Merrikhiaghi, a Christina T. Lollar, a Siti Nur Sarah Morris, b Johannes H. Bauer, b,c and Alexander R. Lippert*a,a,c

Departments of aChemistry and bBiological Sciences and cCenter for Drug Discovery, Design, and Delivery (CD4), Southern Methodist University, Dallas, TX 75275-0314, USA

Supporting Information

General Methods. 5-fluoroisatin was purchased from TCI (Portland, OR) and used without further purification. Diisopropylethylamine (DIPEA) was purchased from Alfa Aesar (Ward Hill, MA) and distilled from KOH before use. D2O was purchased from Cambridge Isotopes (Cambridge, MA). All other reagents were purchased from Sigma-Aldrich (St. Louis, MO). 6-fluoroisatin was synthesized using a previously published procedure.1 UV/Vis spectra were acquired on a Beckman Coulter DU 800 Spectrometer in the Department of Chemistry at Southern Methodist University. Fluorescence spectra were acquired on a Hitachi F7000 in the Department of Chemistry at Southern Methodist University. 1H NMR, 13C NMR, and 19F NMR spectra were taken on a JEOL ECX series 11.7 T NMR spectrometer in the Department of Chemistry at Southern Methodist University. Chemical shifts are reported in the standard notation of parts per million. High performance liquid chromatography was performed on an Agilent 1100 Series HPLC in the Department of Chemistry at Southern Methodist University. Gas chromatography–mass spectrometry was performed on an Agilent Technologies 6850 Series II GC-MS (EI source) in the Department of Chemistry at Southern Methodist University. High-resolution mass spectroscopy was performed on a Shimadzu IT-TOF (ESI source) at the Shimadzu Center for Advanced Analytical Chemistry at the University of Texas, Arlington.

**Sulfonium Salt (1).** Bromoacetonitrile (7.5 mL, 100 mmol, 1.0 equiv.) was added to dimethyl sulfide (7.2 mL, 100 mmol, 1.0 equiv.) and stirred in a round bottom flask overnight. The solids were carefully broken apart with a spatula and with minimal exposure to the atmosphere. Residual reagent was removed under vacuum to yield the sulfonium salt 1 as a white solid (17 g, 93%). \(^1\)H NMR (500 MHz, CD$_3$OD) δ 4.85 (s, 2H), δ 3.09 (s, 6H); \(^13\)C NMR (125 MHz, CD$_3$OD) δ 112.04, δ 29.48, δ 25.89.

3-(2-amino-4-fluorophenyl)-2-(dimethyl-4'-sulfanylidene)-3-oxopropanenitrile. 4-fluoroantranlic acid (77.4 mg, 0.499 mmol, 1.0 equiv) was dissolved in 5 mL CH$_2$Cl$_2$. HBTU (226.7 mg, 0.5977 mmol, 1.2 equiv), sulfonium salt 1 (272.1 mg, 1.494 mmol, 3.0 equiv), and DIPEA (0.26 mL, 1.50 mmol, 3.0 equiv) were added, in that respective order. The solution was stirred vigorously for 75 minutes. The reaction mixture was poured into a separatory funnel containing 10 mL CH$_2$Cl$_2$ and 10 mL 1M K$_2$CO$_3$, extracted four times with 10 mL CH$_2$Cl$_2$, dried over sodium sulfate, filtered, washed with extra CH$_2$Cl$_2$, and concentrated. The crude sulfur ylide was used in the next step without further purification. \(^1\)H NMR (500 MHz, CDCl$_3$) δ 7.50 (br t, 1H, J = 6.9 Hz), δ 6.20–6.17 (dd, 1H, J = 2.3, 11.5 Hz), δ 6.11 (td, 1H, J = 2.3, 11.5 Hz), δ 5.50 (br s, 2H), δ 2.60 (s, 6H); HRMS calcd for C$_{11}$H$_{11}$N$_2$OFS [M+H]$^+$ 239.0649, found 239.0652.

6-fluoro-1H-indole-2,3-dione (6-fluoroisatin). 3-(2-amino-4-fluorophenyl)-2-(dimethyl-4'-sulfanylidene)-3-oxopropanenitrile was dissolved in 2:1 THF:H$_2$O (6.4 mL: 3.2 mL) before Oxone (463.3 mg, 0.7531 mmol, 1.5 equiv) was added. The stirring reaction was
allowed to proceed for 10 minutes. The reaction mixture was poured into a separatory funnel containing 20 mL CH₂Cl₂ and 20 mL H₂O, extracted three times with 20 mL CH₂Cl₂, washed with 20 mL brine, dried over sodium sulfate, filtered, washed with extra CH₂Cl₂, and concentrated. Purification of the crude product was accomplished by silica gel column chromatography (3:1 Hexane:EtOAc) to yield 6-fluoro-1H-indole-2,3-dione (61.8 mg, 80%). mp 197–199 ºC (lit.² 197 ºC); ¹H NMR (500 MHz, CDCl₃) δ 7.82 (br s, 1H), δ 7.66 (q, 1H), δ 6.81 (t, 1H, J = 1.7 Hz), δ 6.64 (d, 1H, J = 8.6 Hz); ¹³C NMR (125 MHz, CDCl₃) δ 180.54, δ 170.16, δ 168.20 (d, J = 39.4 Hz), δ 159.05 (d, J = 13.1 Hz), δ 151.31 (d, J = 11.9 Hz), δ 128.70 (d, J = 11.9 Hz), δ 111.36 (d, J = 23.8 Hz), 100.83 (d, J = 27.4 Hz); HRMS calcd for C₈H₄NO₂F [M-H] - 164.0153, found 164.0159.

Peroxynitrite (ONOO⁻). ONOO⁻ was synthesized from H₂O₂ and isopentyl nitrite in a mixed solvent system, using a procedure slightly modified from a previous description.³ The solvent system, 4.5 mL of 0.55 M NaOH and 5 mL isopropyl alcohol, was added to an open round bottom flask at room temperature. Then, H₂O₂ (30% wt. in H₂O, 0.24 mL, 2.4 mmol, 1.2 equiv) was added to the solvent system, followed by isopentyl nitrite (0.27 mL, 2.0 mmol, 1 equiv). The reaction was stirred for 15 min at rt, and then quenched with 5 mL of 5 M NaOH. Immediately to the quenched reaction mixture, MnO₂ (10 mg, 0.115 mmol) was added to decompose the unreacted H₂O₂. The quenched reaction mixture was stirred for 5 additional min and then filtered. The filtrate was poured into a 250 mL separatory funnel and washed four times with 40 mL CH₂Cl₂. The concentration of ONOO⁻ in the remaining aqueous layer was measured by UV/Vis spectrometry using an extinction coefficient at 302 nm of 1670 M⁻¹ cm⁻¹. This method typically provides 95–120 mM ONOO⁻ in an alkaline solution.

Formation of 5-fluoroanthranilic acid. The production of 5-fluoroanthranilic acid was confirmed by reacting 10 mM 5-fluoroisatin with 15 mM ONOO⁻ for five minutes at room temperature. The reaction mixture was poured into 20 mL 2.5 M HCl, extracted with 30 mL ethyl acetate, and concentrated. The crude product was analyzed by ¹H NMR and GC/MS (Figure S1). ¹⁹F NMR and HPLC were performed on the reaction mixture of 5-

fluoroisatin and ONOO\(^-\) without work-up. \(^1\)H NMR (500 MHz, D\(_2\)O) \(\delta\) 7.52 (dd, 1H, \(J = 9.2, 2.9\) Hz), \(\delta\) 7.29 (dd, 1H, \(J = 9.2, 5.0\) Hz), \(\delta\) 7.20 (td, 1H, \(J = 8.5, 2.9\) Hz); \(^{19}\)F NMR (470 MHz, D\(_2\)O) \(\delta\) –59.9; LRMS (EI) calcd for C\(_7\)H\(_6\)NO\(_2\)F [M]+ 155.04, found 155.1.

**Figure S1.** Confirmation of formation of 5-fluoroanthranilic acid from the reaction of 5-fluoroisatin with ONOO\(^-\). (a) \(^{19}\)F NMR spectra of 1 mM 5-fluoroisatin and 1.5 mM ONOO\(^-\) in HEPES at pH 7.4. Spectra were acquired with 128 scans at 11.7 T using an internal reference peak of trifluoroacetic acid at –35.6 ppm. (b) \(^{19}\)F NMR spectra of 1 mM 5-fluoroanthranilic acid in HEPES at pH 7.4. Spectra were acquired with 128 scans at 11.7 T using an internal reference peak of trifluoroacetic acid at –35.6 ppm. (c) \(^1\)H NMR spectra of the crude reaction extract of 10 mM 5-fluoroisatin and 15 mM ONOO\(^-\) in D\(_2\)O. (d) \(^1\)H NMR spectra of 10 mM 5-fluoroanthranilic acid in D\(_2\)O. (e) HPLC trace of 500 \(\mu\)M 5-fluoroisatin and 750 \(\mu\)M ONOO\(^-\) in HEPES at pH 7.4. (f) HPLC trace of 375 \(\mu\)M 5-fluoroanthranilic acid in HEPES at pH 7.4. 5-fluoroanthranilic acid has a retention time of 0.8 minutes and 5-fluoroisatin has a retention time of 8.2 minutes.

**ONOO\(^-\) Response Testing using \(^{19}\)F NMR Spectroscopy.** \(^{19}\)F NMR spectra were acquired using a D\(_2\)O lock in a reference tube at 25 °C. A 0.1% or 0.001% solution of
trifluoroacetic acid in D$_2$O in the insert tube was used as an internal reference. The number of scans was changed depending on the concentration of the probe in solution (128 scans for response and selectivity testing, 7300 scans for cell testing). Overnight NMR experiments were conducted for cell experiments. NMR spectra were processed using Delta NMR software and graphed using Microsoft Excel. For response and selectivity studies, the free induction decay spectra were processed using DC balance, hamming, Fourier transform, machine phase, baseline correction, and absolute value. For cell testing, the free induction decay spectra were processed using a Fourier transform, baseline correction, and absolute value. ONOO$^-$ was synthesized at 96 mM, and then added to a 500 µL solution of 1 mM isatin in 100 mM HEPES buffered at pH 7.39. Spectra were taken for various concentrations of ONOO$^-$ for a total of seven spectra containing 0, 0.25, 0.5, 0.75, 1.0, 1.25, or 1.5 mM ONOO$^-$. Peak integrations are shown in Figure S2 and S3 for the 5-fluoroisatin and 6-fluoroisatin, respectively.

**Figure S2.** Integrated peak intensities for the reaction of 5-fluoroisatin with ONOO$^-$ represented as (a) [Isatin]/([Isatin]+[Anthranilic acid]) and (b) [Anthranilic acid]/([Isatin]+[Anthranilic acid]). Data points are an average between two repeats of the response testing, and error bars are the distance from the average to the values of the
two experiments used to generate the average.

Figure S3. Integrated peak intensities for the reaction of 6-fluoroisatin with ONOO\(^-\) represented as (a) [Isatin]/([Isatin]+[Anthranilic acid]) and (b) [Anthranilic acid]/([Isatin]+[Anthranilic acid]). Data points are an average between two repeats of the response testing, and error bars are the distance from the average to the values of the two experiments used to generate the average.

\(^{19}\text{F NMR Selectivity Tests.}\) Selectivity tests were conducted using \(^{19}\text{F NMR}\) spectroscopy. Various reactive sulfur, oxygen, and nitrogen (RSON) species were tested. Each test was performed at 1.5 equivalents of the RSON species to the probe, except for GSH, which was tested at 10 equivalents. After preparation, each reaction mixture was added to an NMR tube, and then 128 \(^{19}\text{F NMR}\) scans were acquired and processed according to the general methods for NMR spectroscopy section.

\textbf{ONOO}^\(-\): 3.9 µL of 96 mM ONOO\(^-\) (final concentration 750 µM) was added to 492 µL of 500 µM isatin in 100 mM HEPES buffered at pH 7.39.
OH•: 1 mg Fe(ClO₄)₂ (final concentration 750 µM) was added to an 8 mL solution of 500 µM isatin and H₂O₂ (final concentration 750 µM) in sodium phosphate buffer (100 mM, pH 7.4) at room temperature as described previously.¹

**Glutathione (GSH):** GSH testing was conducted at a 10:1 equivalence. 1.5 mg GSH (final concentration 5 mM) was added to a solution of 500 µL of 500 µM isatin in 100 mM HEPES buffered at pH 7.39.

**PROLI NONO-ate:** A solution of 237.8 mM PROLI NONO-ate was made in 0.01 M NaOH and stored at 0 °C during the day of the experiment. 1.6 µL of this solution was added to a solution of 497 µL (final concentration, 750 µM) of 500 µM isatin in 100 mM HEPES buffered at pH 7.39.

**Na₂N₂O₃:** 1.2 mg Angeli’s salt (Na₂N₂O₃) (final concentration 100 mM) was added to 100 µL of 0.01 M NaOH. On the same day, 3.75 µL (final concentration 750 µM) of the resulting 100 mM solution of Angeli’s salt was added to 497 µL of 500 µM isatin in 100 mM HEPES.

**O₂⁻:** A 750 µM solution of superoxide was made by adding 1.5 mg KO₂ to 28 mL of 500 µM isatin in HEPES buffered at pH 7.39.

**¹BuOOH:** 0.5 µL of 0.728 M ¹BuOOH (final concentration 750 µM) was added to 499.5 µL of 500 µM isatin in 100 mM HEPES buffered at pH 7.39.

**GSNO:** 1.3 mg S-nitrosoglutathione (final concentration 750 µM) was added to a solution of 5 mL 500 µM isatin in 100 mM HEPES buffered at pH 7.39.

**NO₂⁻:** 0.5 mg of NaNO₂ was added to a solution of 10 mL of 500 µM isatin in 100 mM HEPES buffered at pH 7.39.

**OCl⁻:** 2.5 µL of 0.15 M NaOCl (final concentration 750 µM) was added to a solution of 500 µL of 500 µM isatin in 100 mM HEPES buffered at pH 7.39.

**KHSO₅:** 1.2 mg of Oxone (final concentration 750 µM) was added to a solution of 10 mL of 500 µM isatin in 100 mM HEPES buffered at pH 7.39.

**H₂O₂:** 3.75 µL of 100 mM H₂O₂ solution (final concentration 750 µM) was added to a solution of 500 µL of 500 µM isatin in 100 mM HEPES buffered at pH 7.39.

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Higher Concentration Selectivity Testing: Selectivity testing at equivalences greater than 1.5:1 ONOO− to isatin was conducted by reacting 40–120 mM H₂O₂ (Figure S4) and 1–10 mM OH• (Figure S5) with 1 mM 5-fluoroisatin and 6-fluoroisain.

Figure S4. (a) Response of 5-fluoroisatin to high concentrations of H₂O₂. Reaction of 1 mM 5-fluoroisatin with 40, 80, or 120 mM H₂O₂. All reactions were performed in 100 mM HEPES at pH 7.4. Spectra were acquired with 128 scans at 11.7 T using an internal reference peak of 0.001% trifluoroacetic acid at −35.6 ppm. (b) Response of 6-fluoroisatin to high concentrations of H₂O₂. Reaction of 1 mM 6-fluoroisatin with 40, 80, or 120 mM H₂O₂. All reactions were performed in 100 mM HEPES at pH 7.4. Spectra were acquired with 128 scans at 11.7 T using an internal reference peak of 0.1% trifluoroacetic acid at −35.6 ppm.
Figure S5. (a) Response of 5-fluoroisatin to high concentrations of •OH. Reaction of 1 mM 5-fluoroisatin with 1 mM, 5 mM, or 10 mM Fe(ClO$_4$)$_2$ and H$_2$O$_2$. All reactions were performed in 100 mM HEPES at pH 7.4. Spectra were acquired with 128 scans at 11.7 T using an internal reference peak of 0.001% trifluoroacetic acid at −35.6 ppm. (b) Response of 6-fluoroisatin to high concentrations of •OH. Reaction of 1 mM 6-fluoroisatin with 1 mM, 5 mM, or 10 mM Fe(ClO$_4$)$_2$ and H$_2$O$_2$. All reactions were performed in 100 mM HEPES at pH 7.4. Spectra were acquired with 128 scans at 11.7 T using an internal reference peak of 0.1% trifluoroacetic acid at −35.6 ppm.

**Competition Kinetics Studies.** Kinetics of the reaction of isatin with ONOO$^-$ were measured on a Hitachi F7000 Spectrophotometer with $\lambda_{ex} = 312$ nm and measuring the emission intensity at 405 nm. Glutathione (GSH) was used as a reaction competitor. Stock solutions were prepared of 20 mM isatin in dimethylformamide (DMF), 6.615 mM ONOO$^-$ in 0.01 M NaOH, and 200 mM GSH in 100 mM pH 7.4 HEPES. Each measurement was repeated a total of four times. The apparent second order rate constant $k_1$ for the reaction between isatin and ONOO$^-$ was determined by using equation (1), where [GSH] is the concentration of GSH that gives a 50% reaction
inhibition and $k_2 = 495 \, \text{M}^{-1}\text{s}^{-1}$ for the reaction between GSH and ONOO$^-$ at 21 °C$^5$, the room temperature measured by thermostat and thermometer. The value of $k_2 = 1400 \, \text{M}^{-1}\text{s}^{-1}$ for the reaction of GSH and ONOO$^-$ is the value measured at 37 °C.$^5$

$$k_1[\text{isatin}][\text{ONOO}^-] = k_2[\text{GSH}][\text{ONOO}^-] \quad (1)$$

Tests were performed with the following conditions:

**200 μM isatin in 100 mM pH 7.4 HEPES**

40 μL isatin (final concentration 200 μM) was added to 3960 μL of 100 mM pH 7.4 HEPES and vortexed for 5 seconds. 1 mL of the solution was added to a cuvette and a fluorescence spectrum was acquired.

**200 μM isatin and 20 μM ONOO$^-$ in 100 mM pH 7.4 HEPES**

40 μL isatin (final concentration 200 μM) was added to 3952 μL of 100 mM pH 7.4 HEPES and vortexed for 5 seconds. 998 μL of the solution was transferred to an Eppendorf tube and 2.09 μL ONOO$^-$ (final concentration 20 μM) was added. The tube was vortexed for 5 seconds, transferred to a cuvette, and a fluorescence spectrum was acquired.

**200 μM isatin, 20 μM ONOO$^-$, and 5 mM GSH in 100 mM pH 7.4 HEPES**

40 μL isatin (final concentration 200 μM) and 100 μL GSH (final concentration 5 mM) was added to 3862 μL pH 7.4 HEPES and vortexed for 5 seconds. 998 μL of the solution was transferred to an Eppendorf tube and 2.09 μL ONOO$^-$ (final concentration 20 μM) was added. The tube was vortexed for 5 seconds, transferred to a cuvette, and a fluorescence spectrum was acquired.

**200 μM isatin, 20 μM ONOO$^-$, and 10 mM GSH in 100 mM pH 7.4 HEPES**

40 μL isatin (final concentration 200 μM) and 200 μL GSH (final concentration 10 mM) was added to 3752 μL pH 7.4 HEPES and vortexed for 5 seconds. 998 μL of the solution was transferred to an Eppendorf tube and 2.09 μL ONOO$^-$ (final concentration 20 μM) was added. The tube was vortexed for 5 seconds, transferred to a cuvette, and a fluorescence spectrum was acquired.

**200 μM isatin, 20 μM ONOO$^-$, and 15 mM GSH in 100 mM pH 7.4 HEPES**

40 µL isatin (final concentration 200 µM) and 300 µL GSH (final concentration 15 mM) was added to 3652 µL pH 7.4 HEPES and vortexed for 5 seconds. 998 µL of the solution was transferred to an Eppendorf tube and 2.09 µL ONOO\(^-\) (final concentration 20 µM) was added. The tube was vortexed for 5 seconds, transferred to a cuvette, and a fluorescence spectrum was acquired.

**200 µM isatin, 20 µM ONOO\(^-\), and 20 mM GSH in 100 mM pH 7.4 HEPES**

40 µL isatin (final concentration 200 µM) and 400 µL GSH (final concentration 20 mM) was added to 3552 µL pH 7.4 HEPES and vortexed for 5 seconds. 998 µL of the solution was transferred to an Eppendorf tube and 2.09 µL ONOO\(^-\) (final concentration 20 µM) was added. The tube was vortexed for 5 seconds, transferred to a cuvette, and a fluorescence spectrum was acquired.

**Kinetics of Isatin and H\(_2\)O\(_2\).** The rate constant was determined by performing the reaction under pseudo first-order conditions using 500 µM isatin and 40, 80, and 120 mM H\(_2\)O\(_2\) in 200 mM PBS at pH 7.4 to provide the apparent 1st order rate constant, \(k_{obs}\). Data was fitted to equation (2) using Mathematica (Wolfram, Champaign, IL), where \(A(t)\) is the absorbance at 410 nm, \(k_{obs}\) is the observed 1st order rate constant, and \(t\) is time in minutes.

\[
A(t) = A_i \exp(-k_{obs}t) \tag{2}
\]

A representative fit is given in Figure S6a. A linear correlation between \(k_{obs}\) and [H\(_2\)O\(_2\)] indicates the reaction behaves as a 2nd order reaction within this concentration range (Figure S6b). Each H\(_2\)O\(_2\) concentration was repeated 2–3 times and the apparent 2nd order rate constant \(k_2\) was determined from equation 3–5. The \(k_2\) values determined from 7 independent experiments were averaged to give \(k_2 = 3.3 \pm 0.4 \times 10^{-3}\) M\(^{-1}\)s\(^{-1}\) at 21 °C.

\[
d[\text{Isatin}]/dt = -k_{obs}[\text{Isatin}] \tag{3}
\]

\[
d[\text{Isatin}]/dt = -k_2[\text{ONOO}^-][\text{Isatin}] \tag{4}
\]

\[
k_{obs} = k_2[\text{ONOO}^-] \tag{5}
\]
Figure S6. Kinetics of the reaction between isatin and H₂O₂. (a) Representative time course of the reaction of 500 µM isatin and 120 mM H₂O₂ in PBS buffered to pH 7.4. Absorbance values were measured at 410 nm. (b) Plot of $k_{obs}$ versus [H₂O₂].

HPLC Response and Selectivity Studies. Stock solutions of 10 mM 5-fluoroisatin and 5-fluoroanthranilic acid were made in acetonitrile (MeCN). 25 µL of the MeCN stock solution was added to 475 µL 20 mM HEPES (final concentration 500 µM) and then injected into the HPLC, and detected with a UV/Visible detector at 245 nm. The mobile phase consisted of MeCN and H₂O at a flow rate of 1.000 mL/min. A gradient of the
mobile phase was implemented over the course of a 20 minute collection, with an isocratic solution of 0% MeCN for 3 minutes, a gradient from 0% MeCN to 40% MeCN for seven minutes, a gradient from 40% MeCN to 100% MeCN for five minutes, and then an isocratic solution of 100% MeCN for five minutes. 5-fluoroanthranilic acid has a retention time of 0.8 minutes and 5-fluoroisatin has a retention time of 8.2 minutes under these conditions. The peak integrations were calibrated with respect to 5-fluoroisatin and 5-fluoroanthranilic acid concentrations (Figure S7). Selectivity studies were performed as described above and HPLC traces were acquired after 15 minutes (Figure S8a). Blank HPLC traces of the RSON species without any 5-fluoroisatin were also acquired (Figure S8b). The peaks for both 5-fluoroanthranilic acid and 5-fluoroisatin were quantified using the standard calibration curves and corrected using the values in the blank HPLC traces (Figure S9).

Figure S7. (a) Calibration of 5-fluoroisatin. HPLC traces of 1. 500 \( \mu\)M 2. 375 \( \mu\)M 3. 250 \( \mu\)M and 4. 125 \( \mu\)M 5-fluoroisatin. (b) Calibration of 5-fluoroanthranilic acid. HPLC traces of 1. 500 \( \mu\)M 2. 375 \( \mu\)M 3. 250 \( \mu\)M and 4. 125 \( \mu\)M 5-fluoroanthranilic acid. (c) Peak integrations of 5-fluoroisatin traces versus concentration. (d) Peak integrations of 5-fluoroanthranilic acid traces versus concentration.
Figure S8. Selectivity of 5-fluoroisatin. (a) HPLC traces of the reaction of 500 µM 5-fluoroisatin with 750 µM various RSON species, except for GSH, which was reacted at 5 mM. 1. ONOO\(^-\) 2. •OH 3. GSH 4. •NO 5. Na\(_2\)N\(_2\)O\(_3\) 6. KO\(_2\) 7. \({}^7\)BuOOH 8. GSNO 9. NO\(_2^-\) 10. ClO\(^-\) 11. KHSO\(_5\) 12. H\(_2\)O\(_2\). (b) Blank HPLC traces for 750 µM of various RSON species donors and blanks, except for GSH, which was measured at 5 mM. 1. ONOO\(^-\) 2. •OH 3. GSH 4. •NO 5. Na\(_2\)N\(_2\)O\(_3\) 6. KO\(_2\) 7. \({}^7\)BuOOH 8. GSNO 9. NO\(_2^-\) 10. ClO\(^-\) 11. KHSO\(_5\) 12. H\(_2\)O\(_2\).

Figure S9. Quantification of HPLC traces in Figure S8. (a) Quantification of 5-fluoroanthranilic acid from the HPLC traces in Figure S8a. In samples with significant overlap, the integrations of the donor traces in Figure S8b were subtracted from the traces in Figure S8a. (b) Quantification of 5-fluoroisatin from the HPLC traces in Figure S8a.

Detection Limit. The detection limit was determined by integrating five blank regions in the \(^{19}\)F NMR spectra acquired using 7300 scans. The mean and standard deviation of these integrated values was determined and a detection limit for each spectrum was
evaluated as the blank value plus 3 x standard deviation. This value was averaged over six independent spectra and compared with a 5-fluoroanthranilic acid standard to give a detection limit of 1.04 µM 5-fluoroanthranilic acid.

**Cell Culture and IFN-γ Stimulation with $^{19}$F NMR Detection of ONOO$^-$.** Cell experiments were performed using human lung carcinoma epithelial cells (A549, ATCC) at 90% confluency. F-12 Kaighn’s modification (ATCC) containing 10% fetal calf serum (ATCC) was used as the media. Cells were seeded into a six-well plate (TPP) 24 hours before incubating with 50 µM 5-fluoroisatin and 0.1% BSA as a vehicle control ($n = 5$) or 50 ng/mL IFN-γ ($n = 5$) at 37 °C and 5% CO$_2$ for 6–18 hours. A sample of the cellular media was then transferred to an NMR tube and spectra were acquired with 7300 scans at 11.7 T. Quantification of the conversion of 5-fluoroisatin to the corresponding 5-fluoroanthranilic acid was performed on each of the acquired $^{19}$F NMR spectra. The concentration of 5-fluoroanthranilic acid was determined by comparison to the trifluoroacetic acid insert standard.

**Cell Culture and IFN-γ Stimulation Dihydrorhodamine 123 Imaging of ONOO$^-$.** Cell experiments were performed using human lung carcinoma epithelial cells (A549, ATCC) at 90% confluency. F-12 Kaighn’s modification (ATCC) containing 10% fetal calf serum (ATCC) was used as the media. Cells were seeded into a six-well plate (TPP) 24 hours before incubating with 10 µM DHR 123 for 30 min in PBS at 37 °C. Cells were then washed with PBS to remove any free probe before incubating with 0.1% BSA as a vehicle control ($n = 3$) or 50 ng/mL IFN-γ ($n = 3$) at 37 °C and 5% CO$_2$ for 6 hours. At 6 hours, cells were imaged with an EVOS-fl fluorescence microscope (Life Technologies) using the GFP filter set (Figure S10).
Figure S10. Fluorescence microscopy images of ONOO− detection in A549 cells using dihydrorhodamine 123. (a) A549 cells incubated with 10 µM dihydrorhodamine 123 for 30 min at 37 °C, washed and then incubated with a vehicle control for 6 hours. (b) A549 cells incubated with 10 µM dihydrorhodamine 123 for 30 min, washed, and then incubated with 50 ng/mL IFN-γ for 6 hours. (c) Quantification of the mean emission intensity of replicates (n = 3) of experiments described in (a) and (b). Statistical analysis was performed with a two-tailed Student’s t-test; error bars are S.D.