Electronic Supplementary Material (ESI) for Chemical Communications.

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

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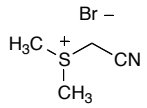
Supporting Information

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. D_2O 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. ^{1}H NMR, ^{13}C NMR, and ^{19}F 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.

(1) C. T. Lollar, K. M. Krenek, K. J. Bruemmer and A. R. Lippert, *Org. Biomol. Chem.* 2014, **12**, 406.



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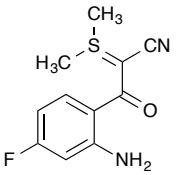
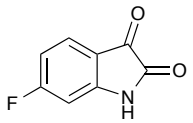
Sulfonium Salt (1). Bromoacetonitrile (7.5 mL, 100 mmol, 1.0 equiv.) was added to dimethyl sulfide (7.2 mL, 100 mmol, 1.0 equiv.) and stirred in a round bottom flask overnight. The solids were carefully broken apart with a spatula and with minimal exposure to the atmosphere. Residual reagent was removed under vacuum to yield the sulfonium salt **1** as a white solid (17 g, 93%). ^{1}H NMR (500 MHz, CD_3OD) δ 4.85 (s, 2H), δ 3.09 (s, 6H); ^{13}C NMR (125 MHz, CD_3OD) δ 112.04, δ 29.48, δ 25.89.



3-(2-amino-4-fluorophenyl)-2-(dimethyl-l^4-sulfanylidene)-3-oxopropanenitrile. 4-fluoroanthranlic acid (77.4 mg, 0.499 mmol, 1.0 equiv) was dissolved in 5 mL CH_2Cl_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_2Cl_2 and 10 mL 1M K_2CO_3, extracted four times with 10 mL CH_2Cl_2, dried over sodium sulfate, filtered, washed with extra CH_2Cl_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 $C_{11}H_{11}N_2OFS$ [M+H]+ 239.0649, found 239.0652.



6-fluoro-1*H*-indole-2,3-dione (6-fluoroisatin). 3-(2-amino-4-fluorophenyl)-2-(dimethyl-l^4-sulfanylidene)-3-oxopropanenitrile was dissolved in 2:1 THF:H_2O (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 CH_2Cl_2 and 20 mL H_2O, extracted three times with 20 mL CH_2Cl_2, washed with 20 mL brine, dried over sodium sulfate, filtered, washed with extra CH_2Cl_2, and concentrated. Purification of the crude product was accomplished by silica gel column chromatography (3:1 Hexane:EtOAc) to yield 6-fluoro-1*H*-indole-2,3-dione (61.8 mg, 80%). mp 197–199 ºC (lit.[2] 197 ºC); ^{1}H NMR (500 MHz, $CDCl_3$) 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); ^{13}C NMR (125 MHz, $CDCl_3$) 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 $C_8H_4NO_2F$ [M-H]- 164.0153, found 164.0159.

Peroxynitrite ($ONOO^-$). $ONOO^-$ was synthesized from H_2O_2 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, H_2O_2 (30% wt. in H_2O, 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, MnO_2 (10 mg, 0.115 mmol) was added to decompose the unreacted H_2O_2. 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 CH_2Cl_2. 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 ^{1}H NMR and GC/MS (Figure S1). ^{19}F NMR and HPLC were performed on the reaction mixture of 5-

(2) P. W. Sadler, *J. Org. Chem.* 1956, **21**, 169.
(3) R. M. Uppu, *Anal. Biochem.* 2006, **354**, 165.

fluoroisatin and $ONOO^-$ without work-up. ^1H NMR (500 MHz, D_2O) δ 7.52 (dd, 1H, *J* = 9.2, 2.9 Hz), δ 7.29 (dd, 1H, *J* = 9.2, 5.0 Hz), δ 7.20 (td, 1H, *J* = 8.5, 2.9 Hz); ^{19}F NMR (470 MHz, D_2O) δ –59.9; LRMS (EI) calcd for $C_7H_6NO_2F$ [M]+ 155.04, found 155.1.

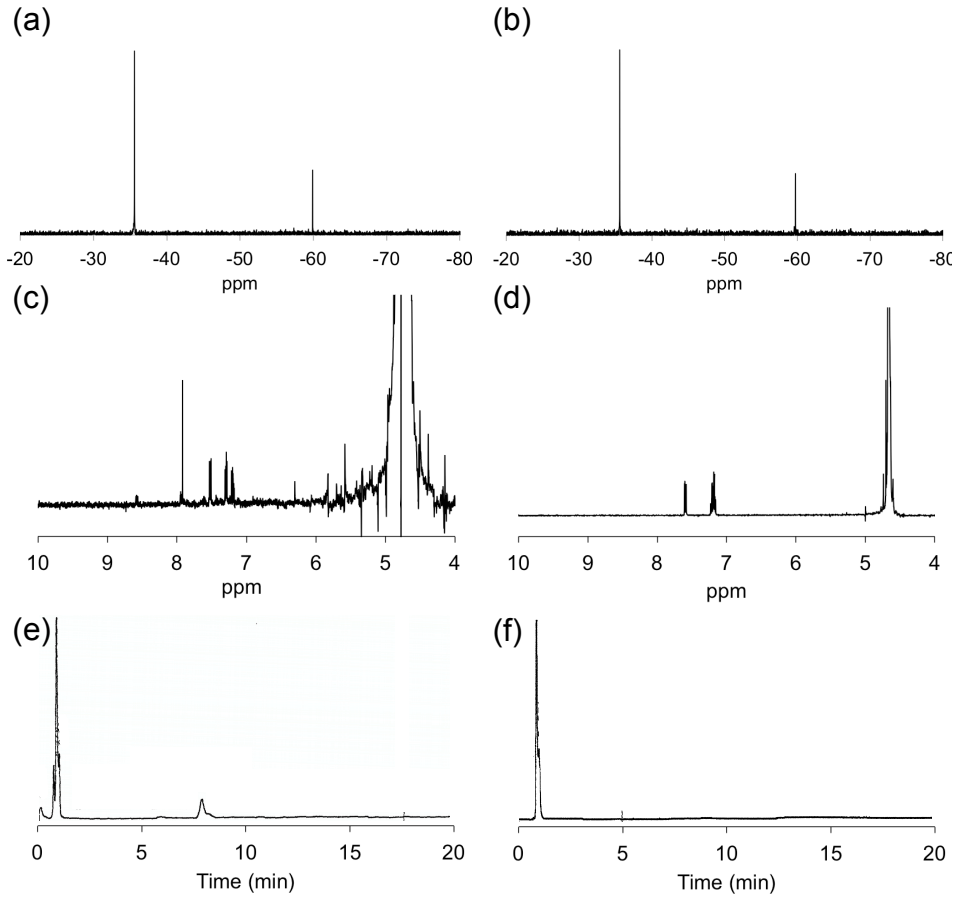


Figure S1. Confirmation of formation of 5-fluoroanthranilic acid from the reaction of 5-fluoroisatin with $ONOO^-$. (a) ^{19}F NMR spectra of 1 mM 5-fluoroisatin and 1.5 mM $ONOO^-$ in HEPES at pH 7.4. Spectra were acquired with 128 scans at 11.7 T using an internal reference peak of trifluoroacetic 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) ^1H NMR spectra of the crude reaction extract of 10 mM 5-fluoroisatin and 15 mM $ONOO^-$ in D_2O. (d) ^1H NMR spectra of 10 mM 5-fluoroanthranilic acid in D_2O. (e) HPLC trace of 500 μM 5-fluoroisatin and 750 μM $ONOO^-$ in HEPES at pH 7.4. (f) HPLC trace of 375 μM 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_2O lock in a reference tube at 25 °C. A 0.1% or 0.001% solution of

trifluoroacetic acid in D_2O 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-fluoroisaitn and 6-fluoroisatin, respectively.

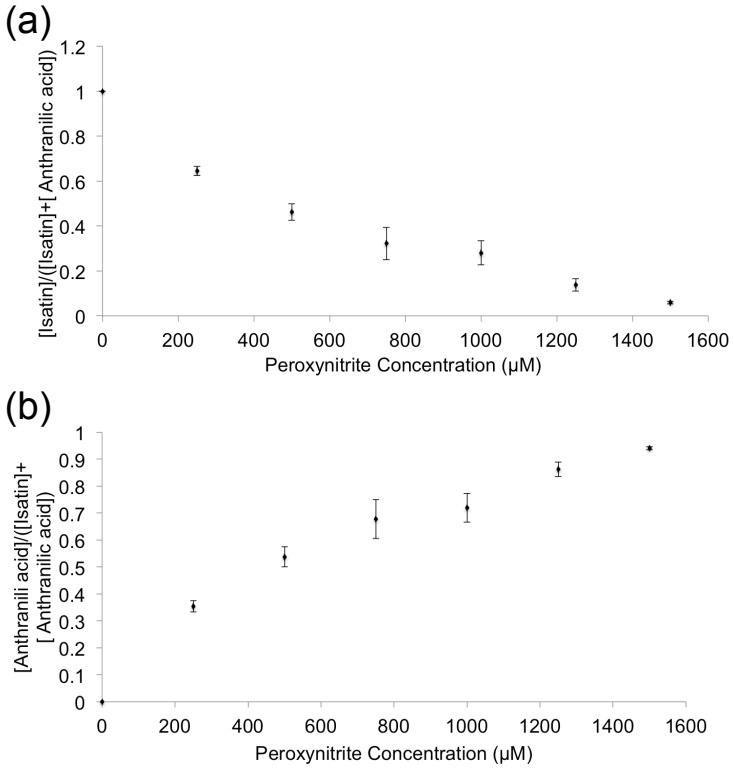


Figure S2. Integrated peak intensities for the reaction of 5-fluoroisatin with $ONOO^-$ represented as **(a)** [Isatin]/([Isatin]+[Anthranilic acid]) and **(b)** [Anthranilic acid] /([Isatin]+[Anthranilic acid]). Data points are an average between two repeats of the response testing, and error bars are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

two experiments used to generate the average.

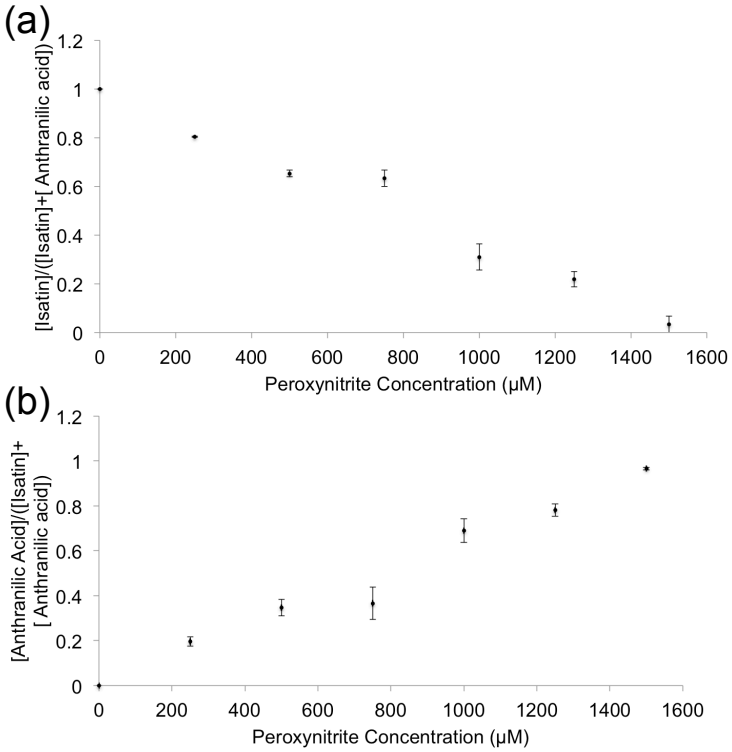


Figure S3. Integrated peak intensities for the reaction of 6-fluoroisatin with $ONOO^-$ represented as **(a)** [Isatin]/([Isatin]+[Anthranilic acid]) and **(b)** [Anthranilic acid] /([Isatin]+[Anthranilic acid]). Data points are an average between two repeats of the response testing, and error bars are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

^{19}F NMR Selectivity Tests. Selectivity tests were conducted using ^{19}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}F NMR scans were acquired and processed according to the general methods for NMR spectroscopy section.

$ONOO^-$: 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 $Fe(ClO_4)_2$ (final concentration 750 μM) was added to an 8 mL solution of 500 μM isatin and H_2O_2 (final concentration 750 μM) in sodium phosphate buffer (100 mM, pH 7.4) at room temperature as described previously.[4]

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.

$Na_2N_2O_3$: 1.2 mg Angeli's salt ($Na_2N_2O_3$) (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.

O_2^-: A 750 μM solution of superoxide was made by adding 1.5 mg KO_2 to 28 mL of 500 μM isatin in HEPES buffered at pH 7.39.

^{t}BuOOH: 0.5 μL of 0.728 M ^{t}BuOOH (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.

NO_2^-: 0.5 mg of $NaNO_2$ 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.

$KHSO_5$: 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.

H_2O_2: 3.75 μL of 100 mM H_2O_2 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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(4) T. Doura, Q. An, F. Sugihara, T. Matsuda and S. Sando, *Chem. Lett.* 2011, **40**, 1357.

Higher Concentration Selectivity Testing: Selectivity testing at equivalences greater than 1.5:1 $ONOO^-$ to isatin was conducted by reacting 40–120 mM H_2O_2 (Figure S4) and 1–10 mM OH• (Figure S5) with 1 mM 5-fluoroisatin and 6-fluoroisaitn.

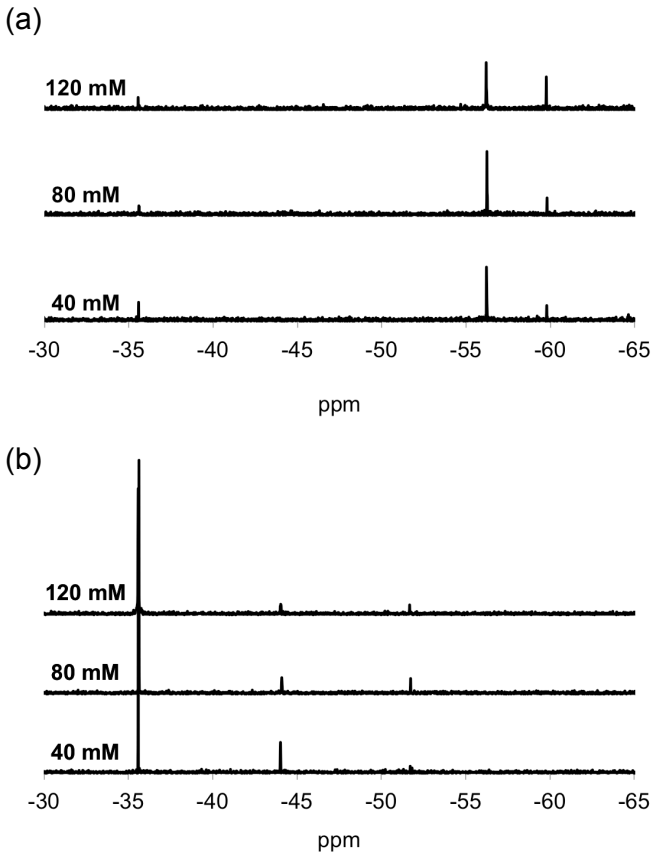

Figure S4. **(a)** Response of 5-fluoroisatin to high concentrations of H_2O_2. Reaction of 1 mM 5-fluoroisatin with 40, 80, or 120 mM H_2O_2. All reactions were performed in 100 mM HEPES at pH 7.4. Spectra were acquired with 128 scans at 11.7 T using an internal reference peak of 0.001% trifluoroacetic acid at –35.6 ppm. **(b)** Response of 6-fluoroisatin to high concentrations of H_2O_2. Reaction of 1 mM 6-fluoroisatin with 40, 80, or 120 mM H_2O_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.

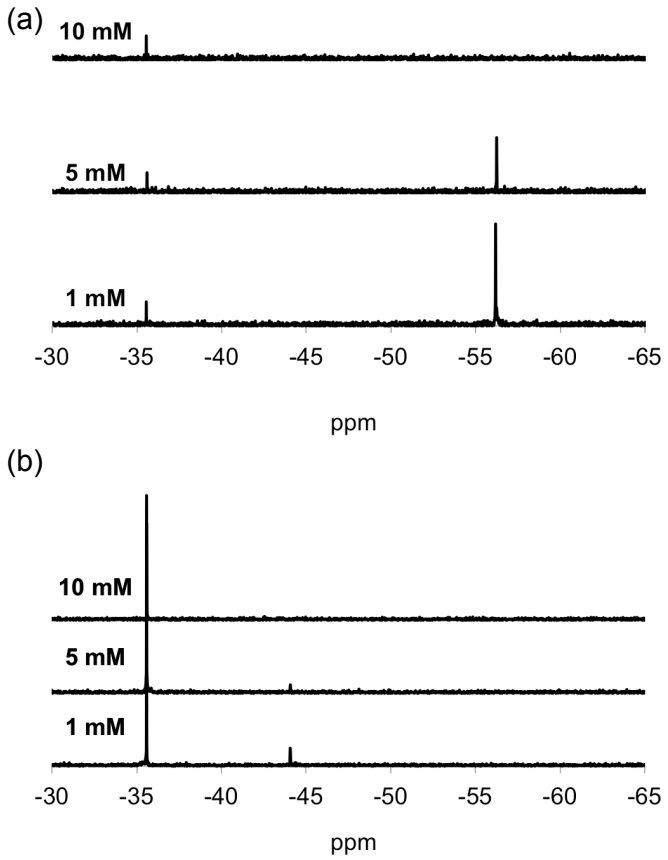

Figure S5. **(a)** Response of 5-fluoroisatin to high concentrations of •OH. Reaction of 1 mM 5-fluoroisatin with 1 mM, 5 mM, or 10 mM $Fe(ClO_4)_2$ and H_2O_2. All reactions were performed in 100 mM HEPES at pH 7.4. Spectra were acquired with 128 scans at 11.7 T using an internal reference peak of 0.001% 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_2O_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 λ_{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 $M^{-1}s^{-1}$ for the reaction between GSH and $ONOO^-$ at 21 ºC,[5] the room temperature measured by thermostat and thermometer. The value of k_2 = 1400 $M^{-1}s^{-1}$ for the reaction of GSH and $ONOO^-$ is the value measured at 37 ºC.[5]

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

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

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(5) k_2 = 495 $M^{-1}s^{-1}$ at 21 ºC was extrapolated from the Arrhenius plot in: W. H. Koppenol, J. J. Moreno, W. A. Pryor, H. Ischiropoulos and J. S. Beckman, *Chem. Res. Toxicol.* 1992, **5**, 834.

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_2O_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_2O_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.

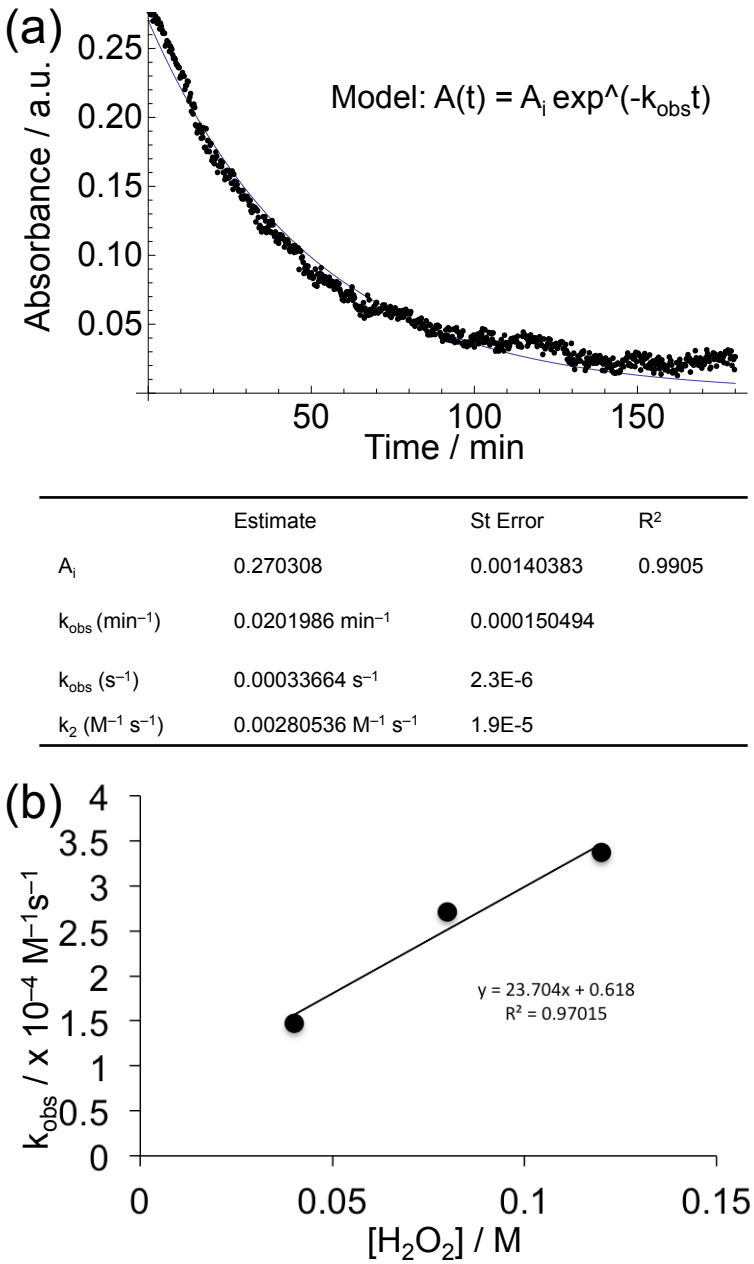
$$A(t) = A_i \exp\text{^}(-k_{obs}t) \quad (2)$$

A representative fit is given in Figure S6a. A linear correlation between k_{obs} and $[H_2O_2]$ indicates the reaction behaves as a 2nd order reaction within this concentration range (Figure S6b). Each H_2O_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 ºC.

$$d[Isatin]/dt = -k_{obs}[Isatin] \quad (3)$$

$$d[Isatin]/dt = -k_2[ONOO^-][Isatin] \quad (4)$$

$$k_{obs} = k_2[ONOO^-] \quad (5)$$



	Estimate	St Error	R^2
A_i	0.270308	0.00140383	0.9905
k_{obs} (min^{-1})	0.0201986 min^{-1}	0.000150494	
k_{obs} (s^{-1})	0.00033664 s^{-1}	2.3E-6	
k_2 (M^{-1} s^{-1})	0.00280536 M^{-1} s^{-1}	1.9E-5	

Figure S6. Kinetics of the reaction between isatin and H_2O_2. **(a)** Representative time course of the reaction of 500 μM isatin and 120 mM H_2O_2 in PBS buffered to pH 7.4. Absorbance values were measured at 410 nm. **(b)** Plot of k_{obs} versus [H_2O_2].

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 H_2O 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).

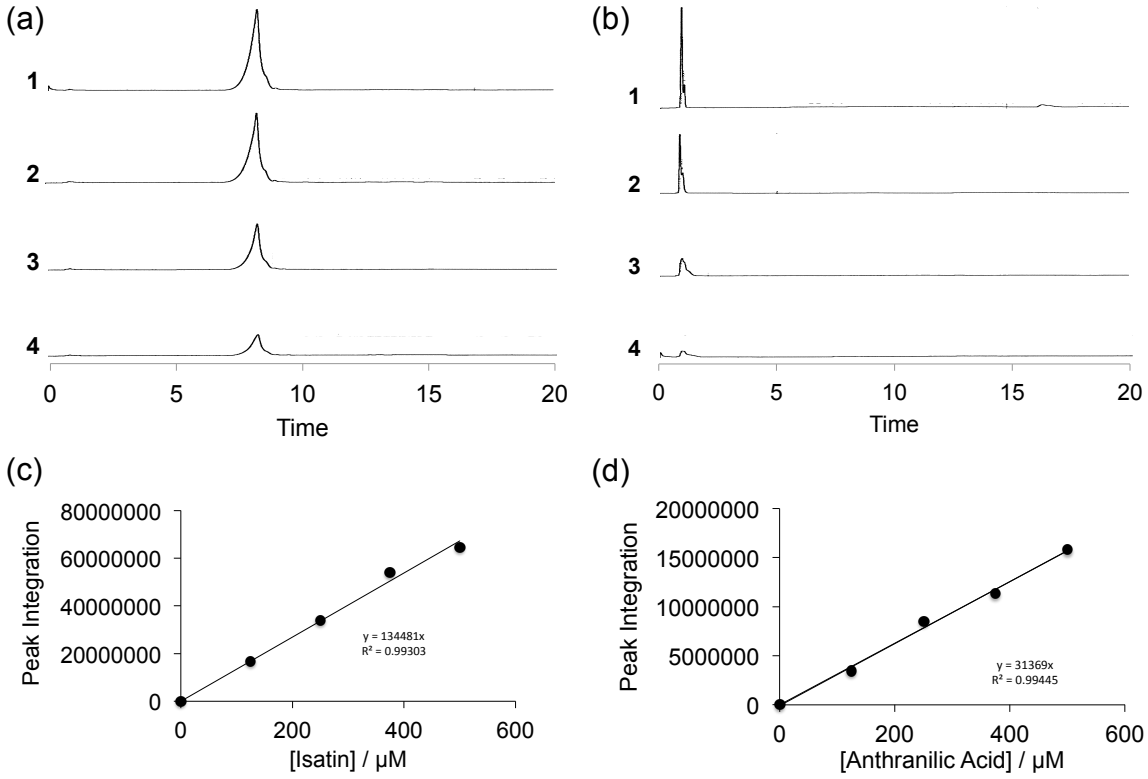


Figure S7. **(a)** Calibration of 5-fluoroisatin. HPLC traces of **1.** 500 µM **2.** 375 µM **3.** 250 µM and **4.** 125 µM 5-fluoroisatin. **(b)** Calibration of 5-fluoroanthranilic acid. HPLC traces of **1.** 500 µM **2.** 375 µM **3.** 250 µM and **4.** 125 µM 5-fluoroanthranilic acid. **(c)** Peak integrations of 5-fluoroisatin traces versus concentration. **(d)** Peak integrations of 5-fluoroanthranilic acid traces versus concentration.

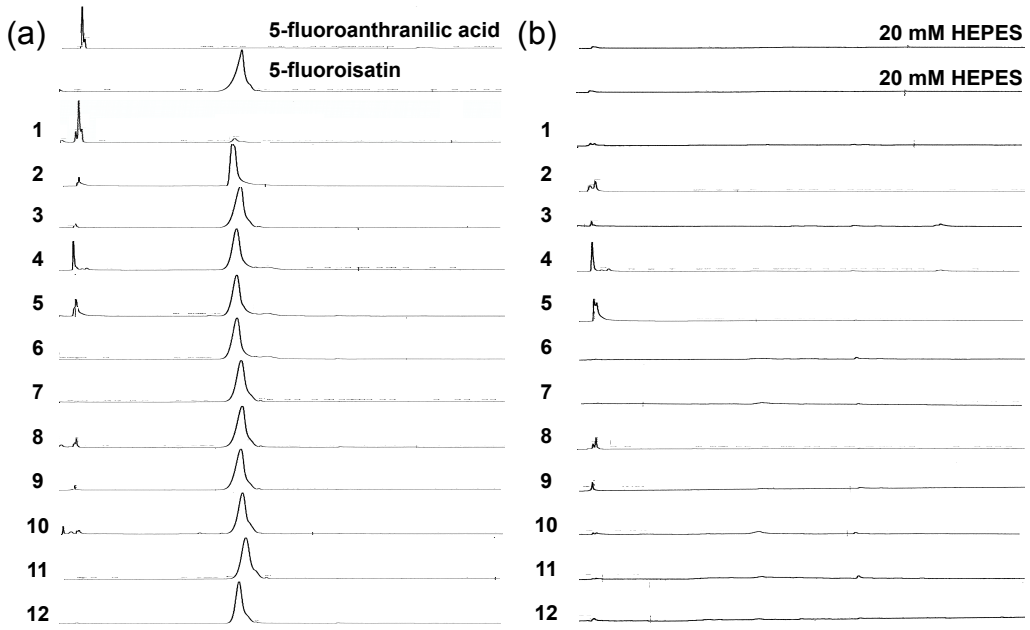


Figure S8. Selectivity of 5-fluoroisatin. **(a)** HPLC traces of the reaction of 500 μM 5-fluoroisatin with 750 μM various RSON species, except for GSH, which was reacted at 5 mM. **1.** $ONOO^-$ **2.** •OH **3.** GSH **4.** •NO **5.** $Na_2N_2O_3$ **6.** KO_2 **7.** ^{t}BuOOH **8.** GSNO **9.** NO_2^- **10.** ClO^- **11.** $KHSO_5$ **12.** H_2O_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.** $Na_2N_2O_3$ **6.** KO_2 **7.** ^{t}BuOOH **8.** GSNO **9.** NO_2^- **10.** ClO^- **11.** $KHSO_5$ **12.** H_2O_2.

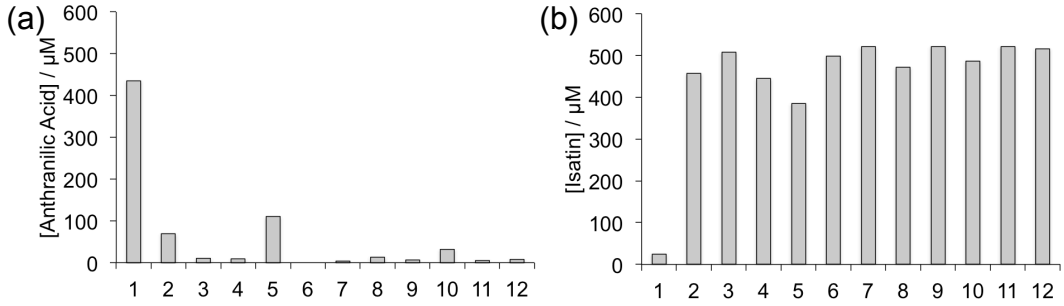


Figure S9. Quantification of HPLC traces in Figure S8. **(a)** Quantification of 5-fluoroanthranilic acid from the HPLC traces in Figure S8a. In samples with significant overlap, the integrations of the donor traces in Figure S8b were subtracted from the traces in Figure S8a. **(b)** Quantification of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

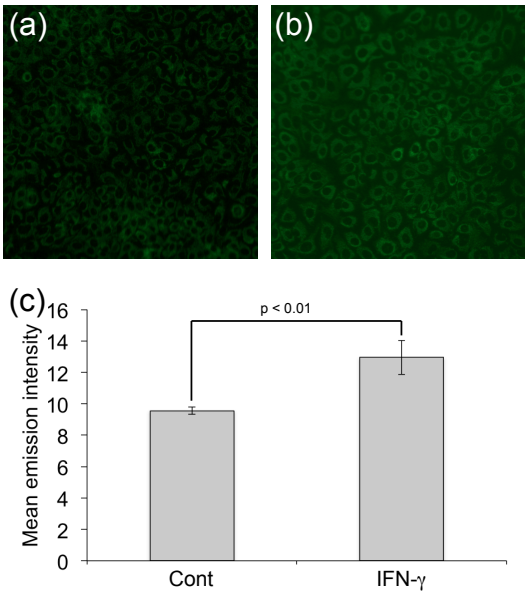


Figure S10. Fluorescence microscopy images of $ONOO^-$ detection in A549 cells using dihydrorhodamine 123. **(a)** A549 cells incubated with 10 μM dihydrorhodamine 123 for 30 min at 37 ºC, washed and then incubated with a vehicle control for 6 hours. **(b)** A549 cells incubated with 10 μM dihydrorhodamine 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.