Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry.

Supporting Information for

Development of an ATP-Independent Bioluminescent Probe for Detection of Extracellular Hydrogen Peroxide

Justin J. O'Sullivan, Marie C. Heffern

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Supplementary Figures

Supplementary Table 1. Photoluminescent properties of bor-DTZ/Nluc.

$\lambda_{max\ (nm)}$[a]	500
Molar luminescence at lower limit of detection (10 µM) (integrated photon flux/µM)	13.7 x 10^4
Molar luminescence at saturation (250 µM) (integrated photon flux/µM)	14.0 x 10^5

See supplementary methods for details. **a.** determined in the presence of 100 µM H_2O_2 and Nluc (0.4 µg/mL).

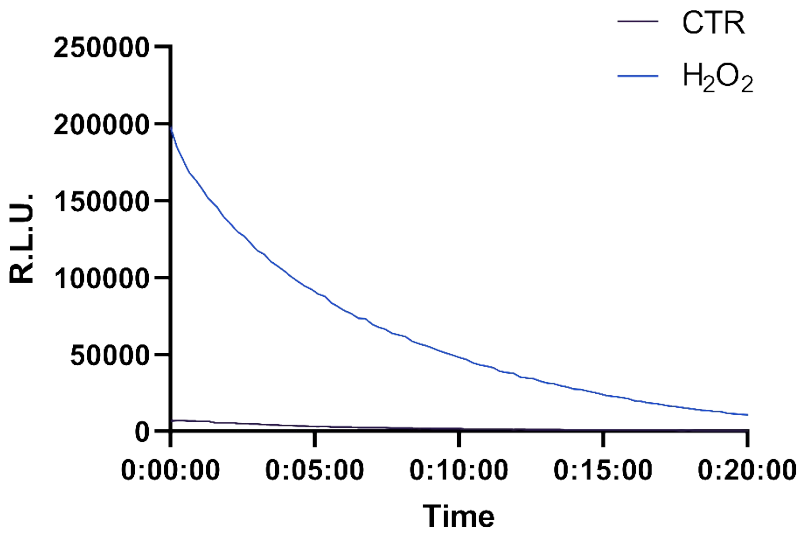


Figure S1. Representative kinetic curve of luminescence of bor-DTZ (1 µM) with rNluc (0.4 µg/mL) measured over twenty minutes in the presence and absence of H_2O_2 (100 µM). All solutions in DPBS, pH 7.4, 37°C, emission range: 300-850 nm.

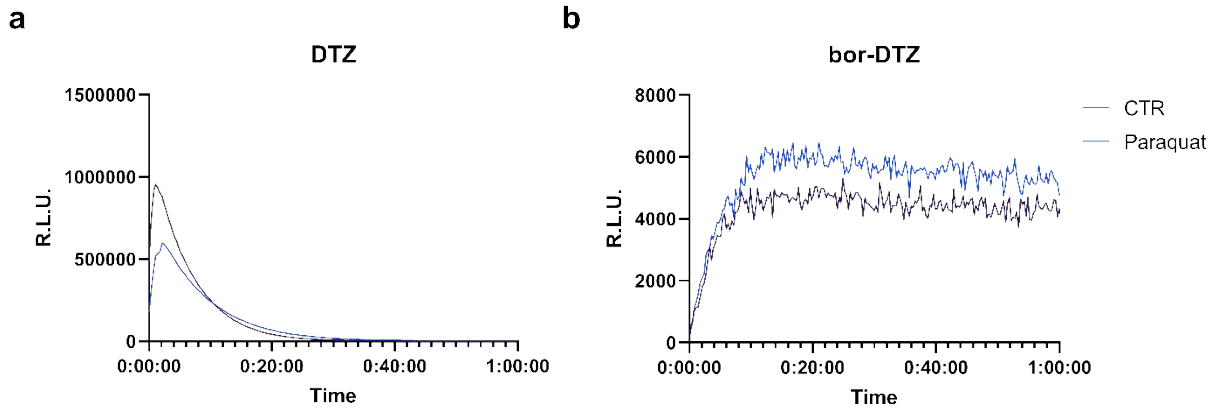


Figure S2. Representative kinetic curves of intracellular luminescence of (a) DTZ (10 μM) or (b) Bor-DTZ (10 μM) in Nluc MDA-MB-231 cells that have either been untreated (CTR) or treated with paraquat (500 μM) for 24 hours. All solutions in DPBS , pH 7.4, 37°C, emission range: 300-850 nm.

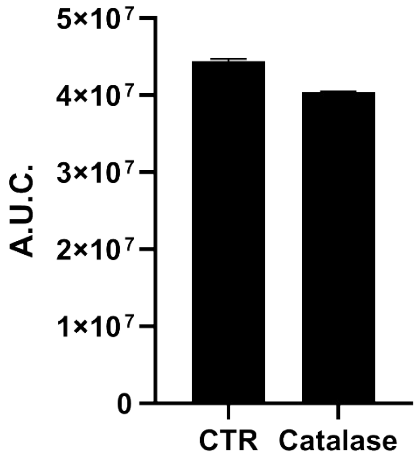


Figure S3. Calculated area under the curve of luminescence over 30 minutes of cell media from secNluc MDA-MB-231 cells in the presence or absence of catalase (1 x 10^4 U/L) with 1 μM DTZ. Error bars denotes SEM, n=3.

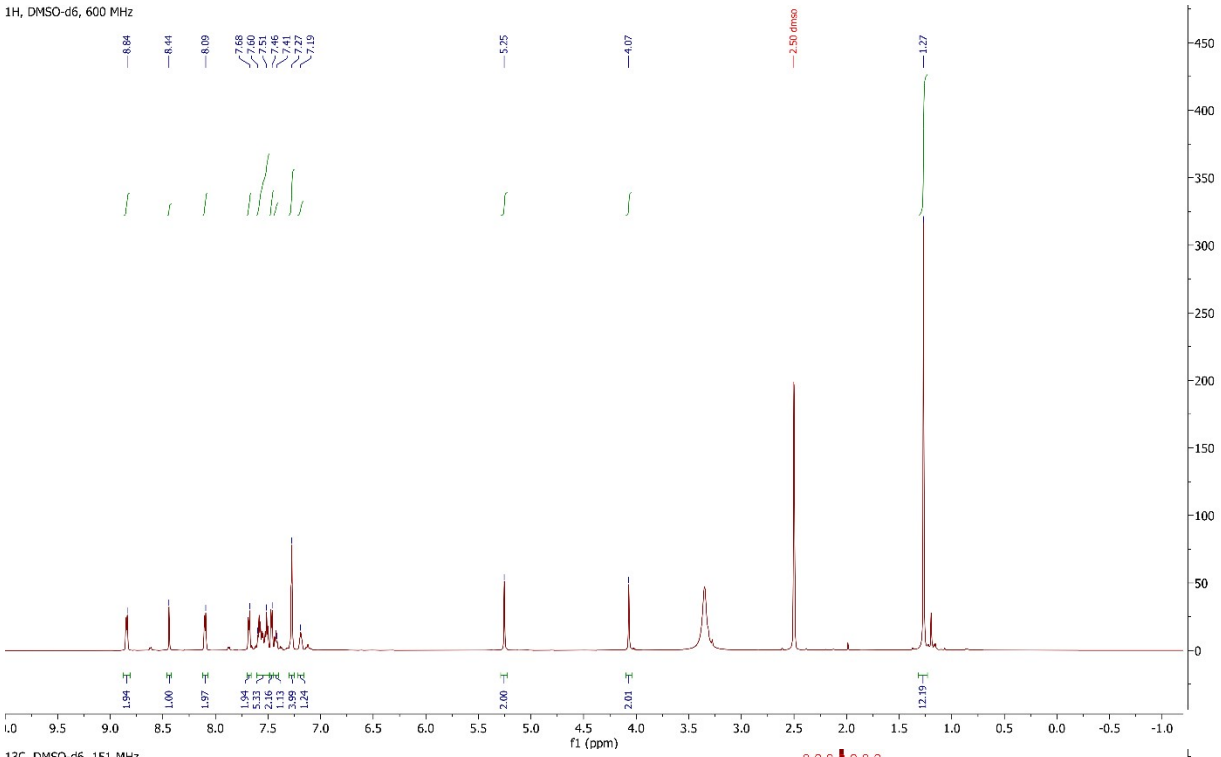
Supplementary Methods

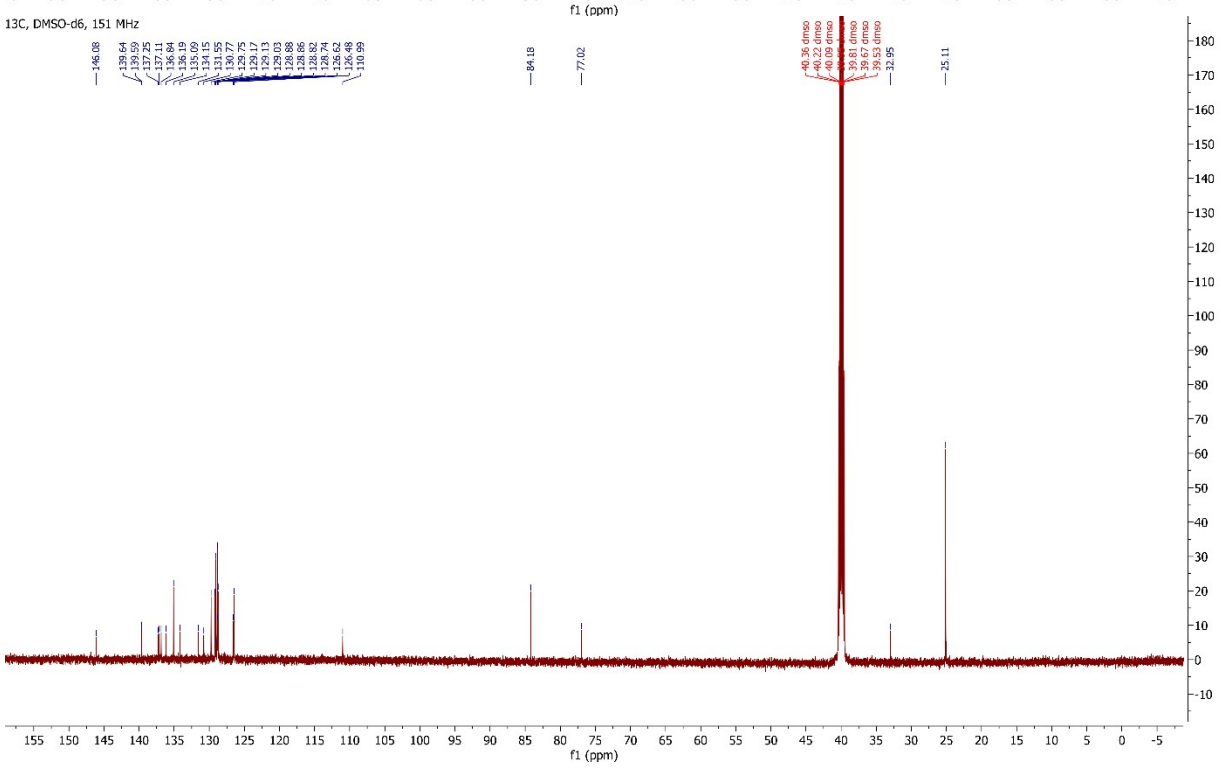
Determination of λ_{max} of emission for bor-DTZ/Nluc. Stock solutions of bor-DTZ, rNluc, and H_2O_2 were made as previously described in the main text. These solutions were combined in a well of a 96-well, white, opaque plate at final concentrations of 1 μM bor-DTZ, 0.4 μg/mL rNluc, and 100 μM H_2O_2. The luminescence spectrum was immediately recorded from 300 to 800 nm at a 5-nm step interval.

Determination of molar luminescence. For more details on experimental methods, see methods for *in vitro* luminescence assays in the main text. The measured luminescence of bor-DTZ (1 μM) in the presence of rNluc (0.4 μg/mL) at the lower and upper limits of detection were integrated over twenty minutes to obtain an integrated photon flux value and divided by the concentration of probe to obtain molar luminescence as photon flux per μM of probe.

Supplementary NMRs

Bor-DTZ


1H, DMSO-d6, 600 MHz
8.84
8.44
8.09
7.68
7.60
7.51
7.46
7.41
7.27
7.19
5.25
4.07
2.50 dmso
1.27
1.94
1.00
1.97
1.94
5.33
2.16
1.13
3.99
1.24
2.00
2.01
12.19
f1 (ppm)


13C, DMSO-d6, 151 MHz
146.08
139.64
139.59
137.25
137.11
136.84
136.19
135.09
134.15
131.55
130.77
129.75
129.17
129.13
129.03
128.88
128.86
128.82
128.74
126.62
126.48
110.99
84.18
77.02
40.36 dmso
40.22 dmso
40.09 dmso
39.81 dmso
39.67 dmso
39.53 dmso
32.95
25.11
f1 (ppm)