Supplementary Information for

Investigation of the Superoxide Anion-Triggered Chemiluminescence of Coelenterazine Analogs

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1. General Synthetic Procedure

Reagents and solvents were purchased from Merck and used without further purification. All reactions involving oxygen or moisture-sensitive compounds were carried out under dry nitrogen atmosphere. Ice-water and silicon baths were used for reactions at low and high temperatures, respectively, with all reaction temperatures referring to the external bath. Organic extracts were dried over anhydrous Na_2SO_4, filtered and concentrated using a rotary evaporator (Büchi® Rotavapor® R-210, Büchi® B-491 Heating Bath 120V, KNF Neuberger D-79112 Vacuum Pump N 035.1.2 AN.18).

Reactions were monitored by thin-layer chromatography (TLC) using aluminum-backed Merck 60 F_{254} silica gel plates and *n*-hexanes-ethyl acetate solvent systems. After visualization under ultraviolet light at 254 nm and 365 nm, the plates were developed by immersion in a solution containing a mixture of *p*-anisaldehyde (2.5%), acetic acid (1%), and sulfuric acid (3.4%) in 95% ethanol followed by heating. Compounds were systematically named following IUPAC recommendations with ChemDraw v20.0.0.41 (Perkin-Elmer, Waltham, MA, USA).

NMR spectra were recorded in Acetone-d_6 or MeOH-d_4 solutions on a Bruker NMR spectrometer (Bruker Advance III 400 MHz Ascend, 9.4 Tesla), and chemical shifts are reported on the δ scale (ppm) using the residual solvent signals [δ = 2.050 ppm (^{1}H, qu, Acetone-d_6), 2.840 (^{1}H, s, Acetone-d_6)] or [δ = 3.31 ppm (^{1}H, qu, MeOH-d_4), 4.78 ppm (^{1}H, s, MeOH-d_4)] as internal standards. Coupling constants (*J*) are reported in Hz. FT-MS analysis were done on a LTQ OrbitrapTM XL hybrid mass spectrometer (Thermo Fischer Scientific, Bremen, Germany) controlled by LTQ Tune Plus and Xcalibur 2.1.0.

ESI = Electrospray ionization; EtOAc = Ethyl acetate; EtOH = Ethanol; NMR = Nuclear magnetic resonance; FT-MS = Fourier transform mass spectrometry; qu = quintet; *r.t.* = Room temperature; s = singlet.

1.1. 6-(4-Fluorophenyl)-2-methylimidazo[1,2-a]pyrazin-3(7H)-one (CLA-2)

A solution of 3-bromo-5-(4-fluorophenyl)pyrazin-2-amine (**F-Clm**) (0.931 mmol, 1 equiv) and methylglyoxal (1.396 mmol, 1.5 equiv) in EtOH (9 mL) was deoxygenated with N_2. Then the resulting mixture was cooled to 0 ºC, HCl (37%, 3.35 mmol, 3.6 equiv) was added, and the solution was stirred up to room temperature, and stirred first at 70 ºC for 2.5 h and then at r.t. overnight. The resulting solution was concentrated under reduced pressure to give a brown oil, which was redissolved in the minimum amount of EtOAc, precipitated with diethyl ether, and vacuum-dried to afford 6-(4-fluorophenyl)-2-methylimidazo[1,2-*a*]pyrazin-3(7*H*)-one (**CLA-2**) as an ochre solid [0.212 g, 94 %].

^{1}H NMR (400 MHz, Acetone) δ = 9.31 – 9.30 (d, *J* = 1.3 Hz, 1H), 9.26 – 9.24 (d, *J* = 1.4 Hz, 1H), 8.24 – 8.19 (m, 2H), 7.33 – 7.28 (m, 2H), 2.65 – 2.53 (s, 3H). **^{1}H NMR** (400 MHz, MeOD) δ = 9.05 – 9.04 (d, *J* = 1.4 Hz, 1H), 8.61 – 8.58 (d, *J* = 1.4 Hz, 1H), 7.96 – 7.92 (m, 2H), 7.18 – 7.11 (m, 2H), 2.94 – 2.30 (s, 3H). **FTMS-ESI (+)**: m/z: calcd for $[C_{13}H_{11}FN_3O]^+$: 244.0886 $[M+H]^+$; found 244.0886 $[C_{13}H_{11}FN_3O]^+$. **FTMS-ESI (-)**: m/z: calcd for $[C_{13}H_9FN_3O]^-$: 242.0730 $[M+H]^+$; found 242.0765 $[C_{13}H_9FN_3O]^-$.

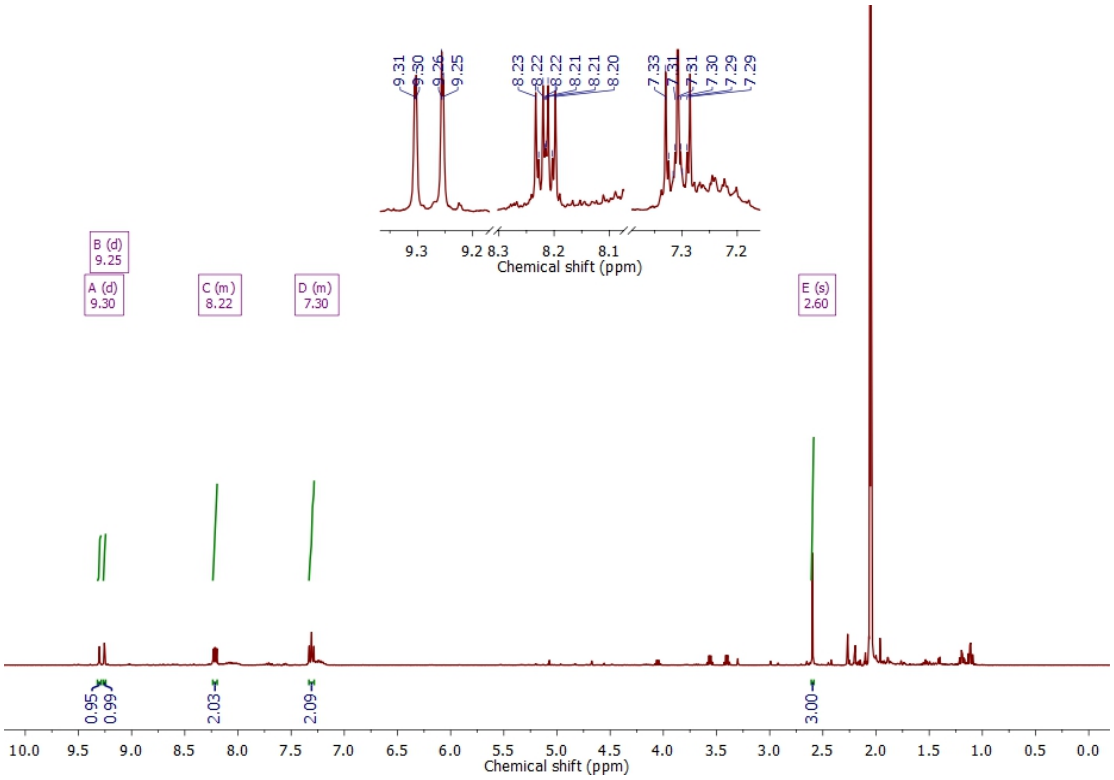

Figure S1. ^{1}H NMR spectrum (400 MHz, Acetone) for 6-(4-fluorophenyl)-2-methylimidazo[1,2-*a*]pyrazin-3(7*H*)-one (**CLA-2**). δ = 9.31 – 9.30 (d, *J* = 1.3 Hz, 1H), 9.26 – 9.24 (d, *J* = 1.4 Hz, 1H), 8.24 – 8.19 (m, 2H), 7.33 – 7.28 (m, 2H), 2.65 – 2.53 (s, 3H).

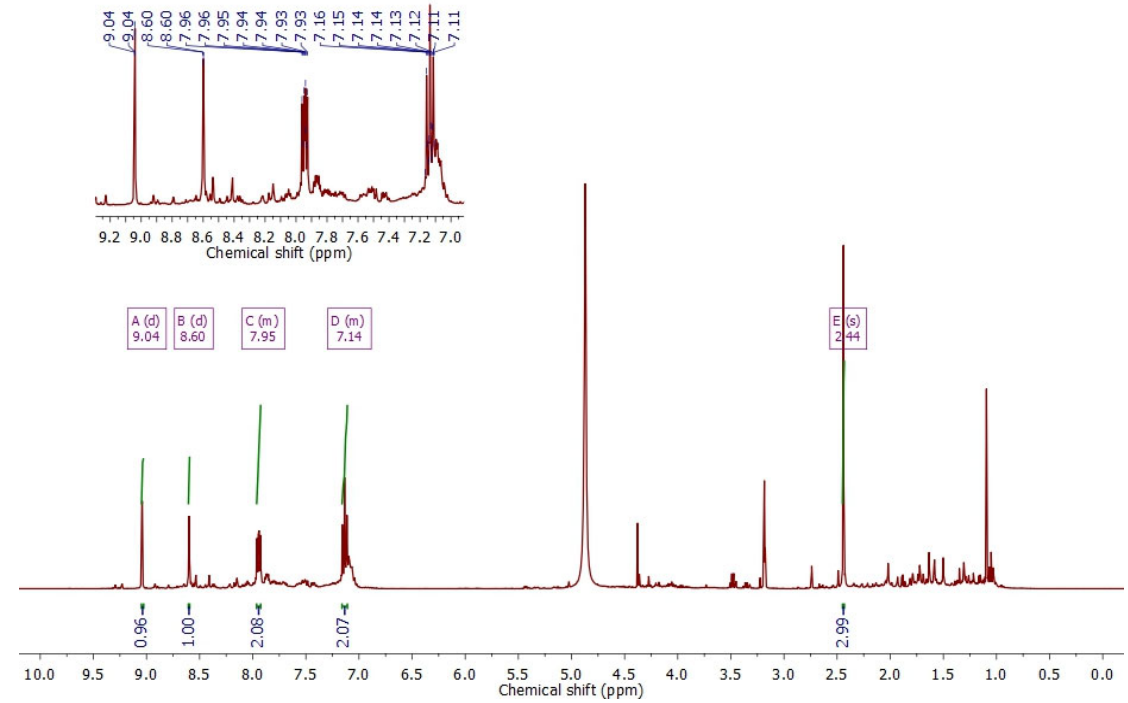

Figure S2. ^{1}H NMR spectrum (400 MHz, MeOD) for 6-(4-fluorophenyl)-2-methylimidazo[1,2-*a*]pyrazin-3(7*H*)-one (**CLA-2**). δ = 9.05 – 9.04 (d, *J* = 1.4 Hz, 1H),

8.61 – 8.58 (d, *J* = 1.4 Hz, 1H), 7.96 – 7.92 (m, 2H), 7.18 – 7.11 (m, 2H), 2.94 – 2.30 (s, 3H).

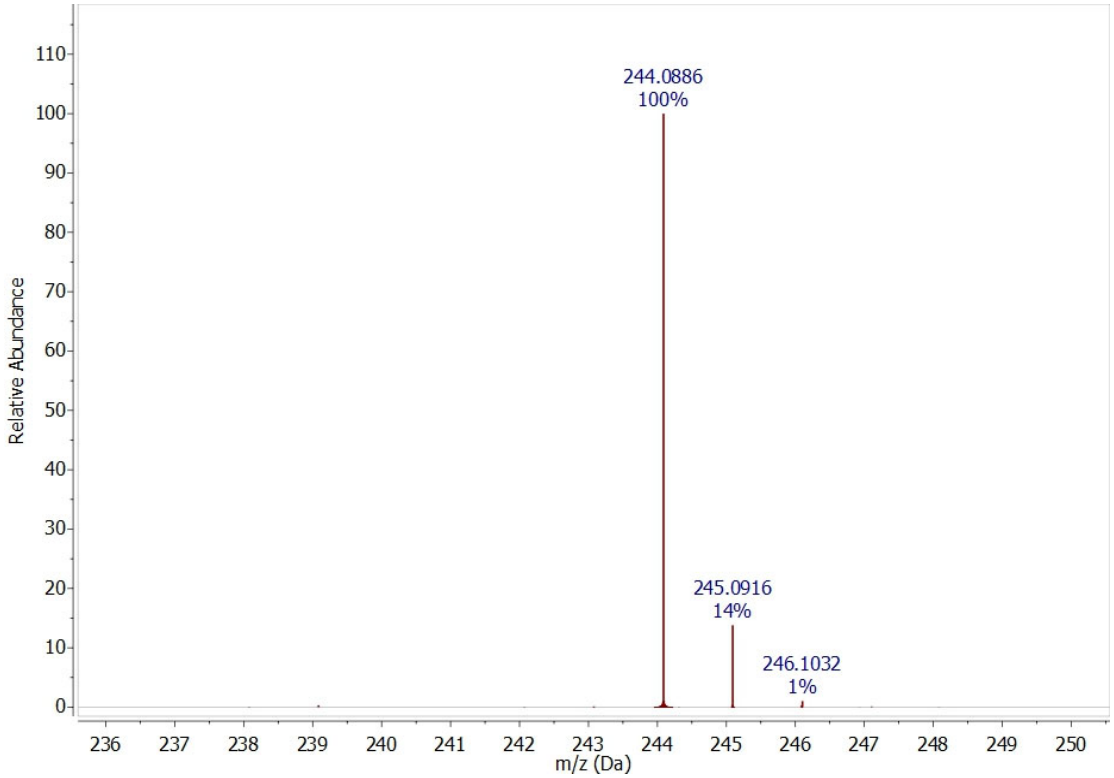

Figure S3. FTMS-ESI (+) spectrum for 6-(4-fluorophenyl)-2-methylimidazo[1,2-*a*]pyrazin-3(7*H*)-one (**CLA-2**), m/z: calcd for $[C_{13}H_{11}FN_3O]^+$: 244.0886 $[M+H]^+$; found 244.0886 $[C_{13}H_{11}FN_3O]^+$.

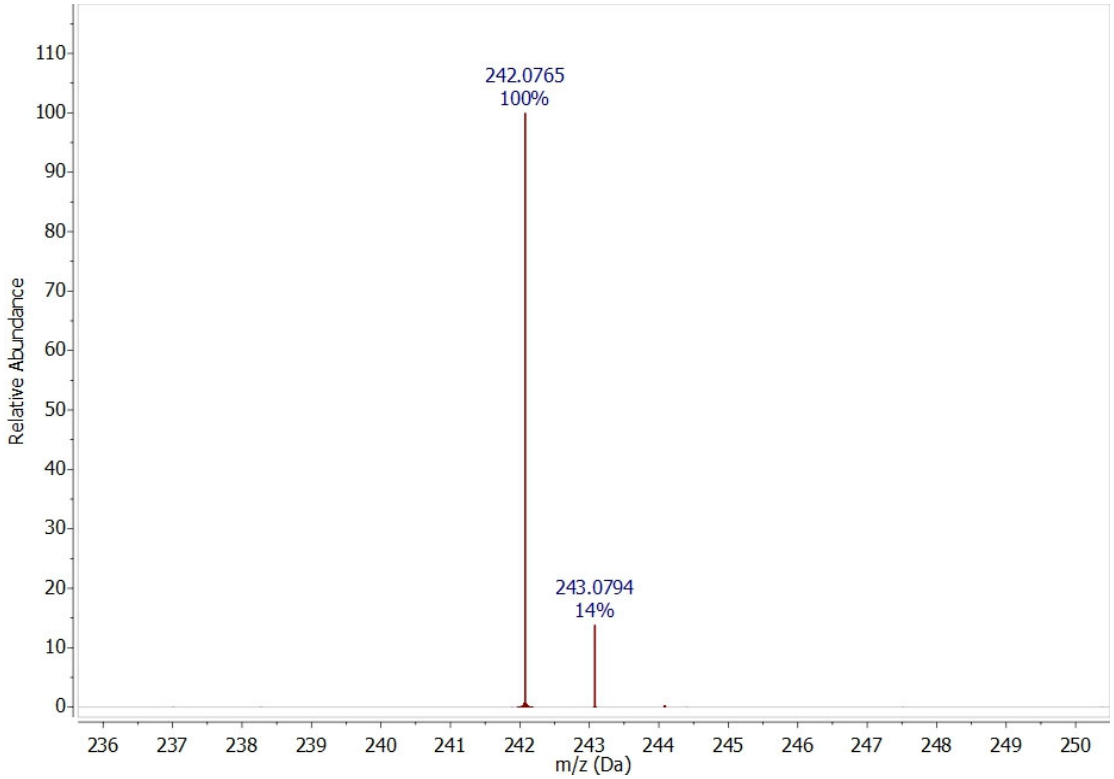

Figure S4. FTMS-ESI (-) spectrum for 6-(4-fluorophenyl)-2-methylimidazo[1,2-*a*]pyrazin-3(7*H*)-one (**CLA-2**), m/z: calcd for $[C_{13}H_9FN_3O]^-$: 242.0730 $[M+H]^+$; found 242.0765 $[C_{13}H_9FN_3O]^-$.