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Mechanistic insights into the *in vitro* metal-promoted oxidation of (di)azine hydroxamic acids: evidence of HNO release and N,O-di(di)azinoyl hydroxylamine intermediate

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Support information

Experimental section

General Protocol for the ^{1}H NMR following of the $K_3[Fe^{III}(CN)_6]$ mediated oxidation of (di)azine hydroxamic acids 1, 2 and 3

The isonicotinohydroxamic acid (1 eq) was added in phosphate buffer solution (0.1 M, pH 7.4) (3 mL). After solubilization of the substrate, $K_3[Fe(CN)_6]$ (2.5 eq) was added to the hydroxamic acid solution. The mixture was stirred at room temperature and analyzed by ^{1}H NMR spectroscopy for 322 h.

General protocol for the synthesis of *N,O*-diaroylhydroxylamines 4, 5 and 6

The hydroxamic acid (1 eq.) was added in phosphate buffer (0.1 M, pH 7.4) (3 mL). After solubilization of the substrate, $K_3[Fe(CN)_6]$ (2.5 eq) was added to hydroxamic acid solution. The mixture was stirred at room temperature for 24 h. The mixture was extracted with EtOAc. The organic phase was concentrated under reduced pressure, and the solid residue was dried under vacuum before characterization.

***N,O*-diisonicotinoylhydroxylamine (4)**

From isonicotinohydroxamic acid (0.100 g, 0.734 mmol) and $K_3[Fe(CN)_6]$ (0.604 g, 1.835 mmol).

^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 8.91(d, *J* = 4.4 Hz, 2H), 8.81 (d, *J* = 4.4 Hz, 2H), 7.98 (d, *J* = 4.4 Hz, 2H) and 7.79 (d, *J* = 4.4 Hz, 2H) (400 MHz, D_2O) δ (ppm): 8.80 (tt, *J* = 4.6, 1.6 Hz, 4H), 8.22 (dt, *J* = 5.2, 1.6 Hz, 2H), 8.13 (dt, *J* = 4.6, 1.6 Hz, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ (ppm): 167.7 (C=O), 166.0 (C=O), 149.7 (CH x 2), 147.8 (CH x 2), 146.0 (C), 137.5 (C), 123.4 (CH x 2), 122.7 (CH x 2). IR Symmetric stretching (ν_s), Antisymmetric stretching (ν_{as}), Symmetric bending (δ_s) and Twisting (τ) (cm^{-1}): 3465-3297 (ν_s N-H), (ν_s C-H), 1719 (ν_s C=O/ bonding to O), 1592 (ν_s C=O/ bonding to N) 1560 - 1499 (ν_sC=N, ν_sC=C), 1420 (δ_s N-H), 1269 (δ_s C-H), 1059 - 1015 (ν_{as} C=N), 854 (τ C-H). HRMS (ESI): *m/z* calcd. for $C_{12}H_9N_3O_3$: 244.0722 found: 244.0726.

***N,O*-dinicotinoylhydroxylamine (5)**

From nicotinohydroxamic acid (0.100 g, 0.734 mmol) and $K_3[Fe(CN)_6]$ (0.604 g, 1.84 mmol). ^1H NMR (400 MHz, DMSO-d_6) δ (ppm): 9.17 (dd, *J* = 2.2, 0.9 Hz, 1H), 9.00 (dd, *J* = 2.3, 0.9 Hz, 1H), 8.86 (dd, *J* = 4.8, 1.7 Hz, 1H), 8.68 (dd, *J* = 4.8, 1.7 Hz, 1H), 8.39 (dt, *J* = 8.0, 2.0 Hz, 1H), 8.18 (dt, *J* = 7.9, 2.0 Hz, 1H), 7.62 (ddd, *J* = 8.0, 4.9, 0.9 Hz, 1H), 7.50 – 7.45 (m, 1H). HRMS (ESI^+): *m/z* calcd. for $C_{12}H_9N_3O_3$: 244.0722, found: 244.0726.

***N,O*-dipyrazinoylhydroxylamine (6)**

From pyrazinohydroxamic acid (0.100 g, 0.719 mmol) and $K_3[Fe(CN)_6]$ (0.592 g, 1.797 mmol). MS (DCI/CH_4): *m/z* = 230 [M**9** + $C_2H_5^+$], 217 [M**9** + CH_3^+], 202 [M**9** + H^+] and *m/z* = 124 [M**14** + H^+].

General protocol for the synthesis of *N*-methylisonicotinamide, *N*-methylnicotinamide, and *N*-methylpyrazinamide 16,17 and 18

The hydroxamic acid (1 eq) and NH_2CH_3 40% in water (10 eq) was added to a phosphate buffer solution (0.1 M, pH 7.4) (3 mL). After solubilization of the substrate, $K_3[Fe(CN)_6]$ (2.5 eq) was added to hydroxamic acid solution. The mixture was stirred at room temperature for 2 h. The solvent was removed by evaporation under reduced pressure. The crude material was purified by silica gel flash column chromatography using a mixture of MeOH/DCM (0-100 / 10-90) as eluent.

***N*-methylisonicotinamide (16)**

From isonicotinohydroxamic acid (0.10 g, 0.734 mmol), NH_2CH_3 40% in water (0.228 g, 7.35 mmol) and $K_3[Fe(CN)_6]$ (0.604 g, 1.84 mmol). Yield = 79% (0.078 g), white solid. ^1H NMR (400 MHz, DMSO-d_6) δ (ppm): 8.78 – 8.65 (m, *J* = 4.44, 3H (In this case, the N-H part, there is in the same zone of the C-H of the ring), 7.73 (d, *J* = 4.44, 2H), 2.80 (d, *J* = 4.6 Hz, 3H). ^{13}C NMR (101 MHz, D_2O) δ (ppm): 164.99 (C=O), 150.22 (CH x 2), 141.36 (C), 121.10 (CH x 2), 26.25 (CH_3). IR Symmetric stretching (ν_s), antisymmetric stretching (ν_{as}), symmetric bending (δ_s) and twisting

(τ) (cm^{-1}): 3348-3304 (ν_s N-H), 3000-2852 (ν_s C-H), 1644 (ν_s C=O), 1543 - 1494 (ν_sC=N, ν_sC=C), 1407 (δ_s N-H), 1311 (δ_s C-H), 1065 (ν_{as} C=N), 838 (τ C-H). UV-Vis (H_2O) λ_{max}/nm ($\varepsilon/M^{-1}cm^{-1}$) = 266 (2706), 232 (4032), 221 (4535), 213 (5750). HRMS (DCI/CH_4): *m/z* calcd. for [($C_7H_8N_2O$) + H^+] 137.0715, found: 137.0712. Elemental Anal. calcd. for $C_7H_8N_2O{\cdot}0.15H_2O$: C, 60.55; H, 6.03; N, 20.17. Found: C, 60.95; H, 6.13; N, 19.73. Retention Factor (MeOH/DCM 10%) = 0.41. M.p. = 113 °C.

***N*-methylnicotinamide (17)**

From nicotinohydroxamic acid (0.10 g, 0.734 mmol), NH_2CH_3 40% in water (0.228 g, 7.35 mmol) and $K_3[Fe(CN)_6]$ (0.604 g, 1.84 mmol). Yield = 68 % (0.067 g), white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 8.98 (d, *J* = 2.3 Hz, 1H), 8.69 (dd, *J* = 4.9, 1.6 Hz, 1H), 8.62 (s, 1H), 8.16 (dt, *J* = 8.0, 2.0 Hz, 1H), 7.49 (dd, *J* = 8.0, 4.8 Hz, 1H), 2.80 (d, *J* = 4.6 Hz, 3H). ^{13}C NMR (101 MHz, D_2O) δ (ppm): 165.18 (C=O), 151.73 (CH), 148.25 (CH), 134.80 (CH), 129.96 (C), 123.47 (CH), 26.20 (CH_3). IR Symmetric stretching (ν_s), antisymmetric stretching (ν_{as}), symmetric bending (δ_s) and twisting (τ) (cm^{-1}): 3331 (ν_s N-H), 3091-2854 (ν_s C-H), 1644 (ν_s C=O), 1549 - 1484 (ν_sC=N, ν_sC=C), 1412 (δ_s N-H), 1315 (δ_s C-H), 1029 (ν_{as} C=N), 829 (τ C-H). UV-Vis (H_2O) λ_{max}/nm ($\varepsilon/M^{-1}cm^{-1}$) = = 263 (3286), 212 (6723). HRMS (DCI/CH_4): *m/z* calcd. for [($C_7H_8N_2O$) + H^+]: 137.0670, found: 137.0706. Elemental Anal. calcd. for $C_7H_8N_2O{\cdot}0.2H_2O$: C, 60.16; H, 6.06; N, 20.04. Found: C, 60.07; H, 5.87; N, 19.67. TLC Retention Factor (MeOH/DCM 10%) = 0.47. M.p = 108 °C.

***N*-methylpyrazinamide (18)**

From pyrazinohydroxamic acid (0.10 g, 0.729 mmol) NH_2CH_3 40% in water (0.226 g, 7.29 mmol) and $K_3[Fe(CN)_6]$ (0.600 g, 1.82 mmol). Yield = 69% (0.068 g), white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 9.17 (d, *J* = 1.4 Hz, 1H), 8.89 (s, 1H), 8.85 (d, *J* = 2.5 Hz, 1H), 8.71 (dd, *J* = 2.4, 1.6 Hz, 1H), 2.83 (d, *J* = 4.8 Hz, 3H). ^{13}C NMR (101 MHz, D_2O) δ (ppm): 163.29 (C=O), 147.37 (CH), 144.87 (C), 143.35 (CH), 143.32 (CH), 25.98 (CH_3). IR Symmetric stretching (ν_s),

antisymmetric stretching (ν_{as}), symmetric bending (δ_s) and twisting (δ) (cm^{-1}): 3358 (ν_s N-H), 2929 (ν_s C-H), 1669 (ν_s C=O), 1583 - 1542 (ν_sC=N, ν_sC=C), 1404 (δ_s N-H), 1294 (δ_s C-H), 1054 - 1024 (ν_{as} C=N), 868 (τ C-H). UV-Vis (H_2O) λ_{max}/nm ($\varepsilon/M^{-1}cm^{-1}$) = 310 (657), 270 (7045), 209 (8544). HRMS (DCI/CH_4): *m/z* calcd. for [($C_6H_7N_3O$) + H^+]: 138.0667, found: 137.0662. TLC Retention Factor (MeOH/DCM 10%) = 0.58. M.p = 110 °C.

General protocol for the synthesis of sodium isonicotinoate, nicotinoate and pyrazinoate molecules

To a mixture of the suitable carboxylic acid (1 eq) in water (8 mL) was added a solution of NaOH (0.85 eq) in water (2 mL). The solution was stirred at room temperature for 30 min. The solvent was removed by evaporation under reduced pressure at 50 °C. The solid obtained was characterized.

Sodium Isonicotinoate (7b)

From isonicotinic acid (0.12 g, 0.975 mmol) and NaOH (0.033 g, 0.817 mmol). Yield = 99.0% (0.140 g), white solid. ^{1}H NMR (400 MHz, D_2O) δ (ppm): 8.64 (d, *J* = 2H), 7.78 (d, *J* = 2H). ^{13}C NMR (101 MHz, D_2O) δ (ppm): 173.28 (C=O), 148.42 (CH x 2), 147.47 (C), 124.19 (CH x 2), 49.50 (reference: CH_3OD). IR Symmetric stretching (ν_s), Antisymmetric stretching (ν_{as}), Symmetric bending (δ_s) and Twisting (τ) (cm^{-1}): 1576 (ν_s C=O), 1528 - 1408 (ν_s C=N, C=C), 1300 (δ_s C-H), 1012 (ν_{as} C=N), 760 (τ C-H). UV-Vis (H_2O) λ_{max}/nm ($\varepsilon/M^{-1}cm^{-1}$) = 268 nm (2343 $M^{-1}cm^{-1}$), 211 nm (6575 $M^{-1}cm^{-1}$). Elemental Anal. calcd. for $NaC_6H_4O_2 \cdot 0.1H_2O$: C, 49.06; H, 2.88; N, 9.54. Found: C, 49.10; H, 2.52; N, 9.45. Retention Factor (MeOH/DCM 50%) = 0.6.

Sodium Nicotinoate (8b)

From nicotinic acid (0.12 g, 0.975 mmol) and NaOH (0.033 g, 0.817 mmol). Yield = 99.5 % (0.14 g), white solid. ^{1}H NMR (300 MHz, D_2O) δ (ppm): 8.95 (dd, *J* = 2.2, 0.9, 1H), 8.62 (dd, *J* = 5.1, 1.7, 1H), 8.34 (ddd, *J* = 8.0, 2.2, 1.7, 1H), 7.59 (ddd, *J* = 8.0, 5.1, 0.9, 1H). ^{13}C NMR (101 MHz,

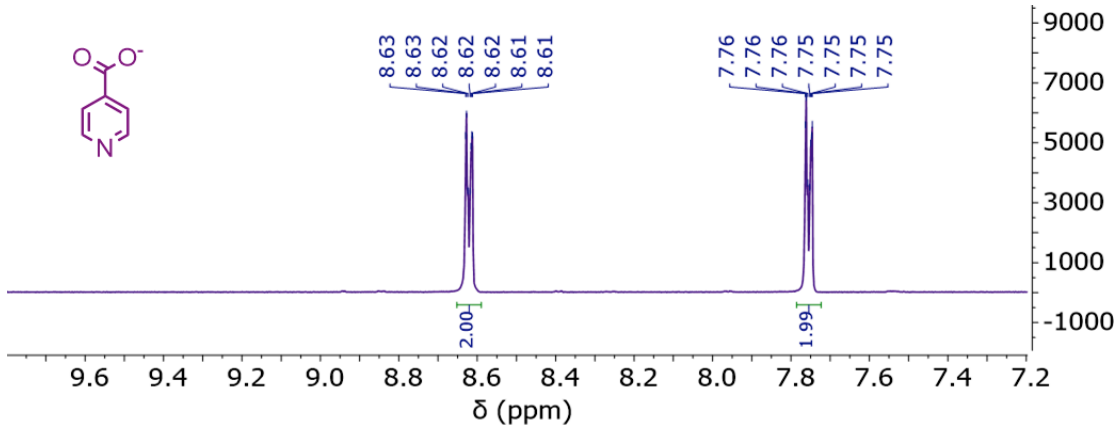
D_2O) δ (ppm): 172.98 (C=O), 149.74 (CH), 148.51 (CH), 139.65 (CH), 133.54 (C), 125.04 (CH), 49.50 (reference: CH_3OD). IR Symmetric stretching (ν_s), Antisymmetric stretching (ν_{as}), Symmetric bending (δ_s) and Twisting (τ) (cm^{-1}): 1610 (ν_s C=O), 1562 - 1403 (ν_s C=N, C=C), 1322 (δ_s C-H), 1029 (ν_{as} C=N), 752 (τ C-H). UV-Vis (H_2O) λ_{max}/nm ($\varepsilon/M^{-1}cm^{-1}$) = 272 nm (2137 $M^{-1}cm^{-1}$), 265 nm (2819 $M^{-1}cm^{-1}$), 260 nm (2549 $M^{-1}cm^{-1}$), 212 nm (7350 $M^{-1}cm^{-1}$). Elemental Anal. calcd. for $NaC_6H_4O_2 \cdot 0.4H_2O$: C, 47.32; H, 3.18; N, 9.20. Found: C, 47.35; H, 2.96; N, 9.48. Retention Factor (MeOH/DCM 50%) = 0.6.

Sodium pyrazinoate (9b)

From pyrazine-2-carboxylic acid (0.12 g, 0.970 mmol) and NaOH (0.033 g, 0.817 mmol). Yield = 87.5 % (0.124 g), white solid. ^{1}H NMR (400 MHz, D_2O) δ (ppm): 9.09 (d, *J* = 1.1 Hz, 1H), 8.70 (m, 2H). ^{13}C NMR (101 MHz, D_2O) δ (ppm): 170.87 (C=O), 148.54 (C), 146.46 (CH), 145.13 (CH), 144.79 (CH), 49.50 (reference: CH_3OD). IR Symmetric stretching (ν_s), Antisymmetric stretching (ν_{as}), Symmetric bending (δ_s) and Twisting (τ) (cm^{-1}) (cm^{-1}): 1620 (ν_s C=O), 1572 - 1428 (ν_s C=N, C=C), 1384 (δ_s C-H), 1012 (ν_{as} C=N), 844 (τ C-H). UV-Vis (H_2O). λ_{max}/nm ($\varepsilon/M^{-1}cm^{-1}$) = 313 nm (639.4 $M^{-1}cm^{-1}$), 271 nm (7074 $M^{-1}cm^{-1}$), 204 nm (7345 $M^{-1}cm^{-1}$). Elemental Anal. calcd. for $NaC_6H_4O_2$: C, 41.11; H, 2.07; N, 19.18. Found: C, 41.33; H, 1.65; N, 19.06. Retention Factor (MeOH/DCM 50%) = 0.5.

Figure S1

a)



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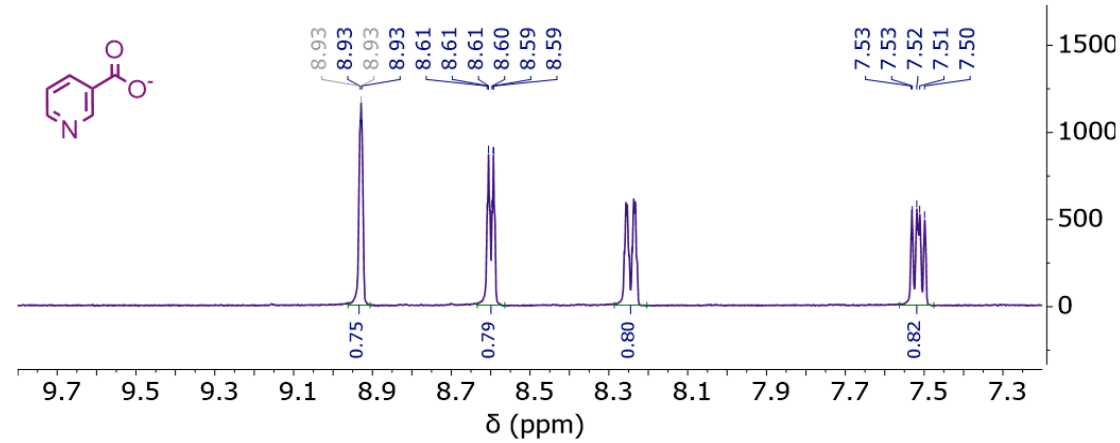


c)

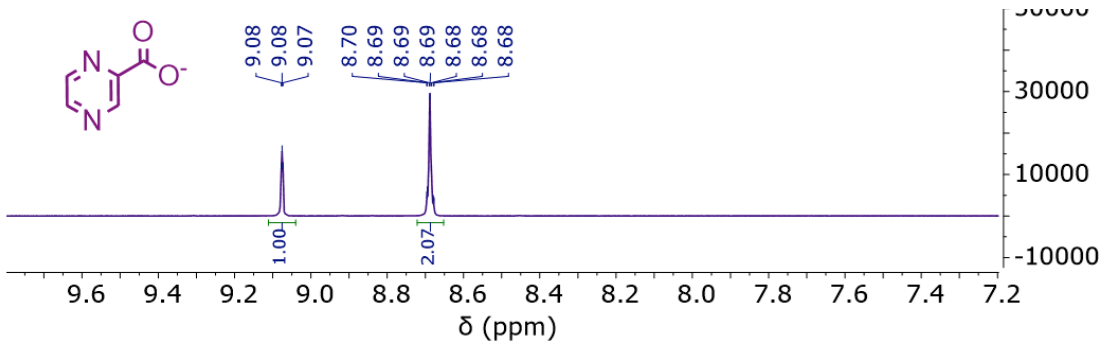
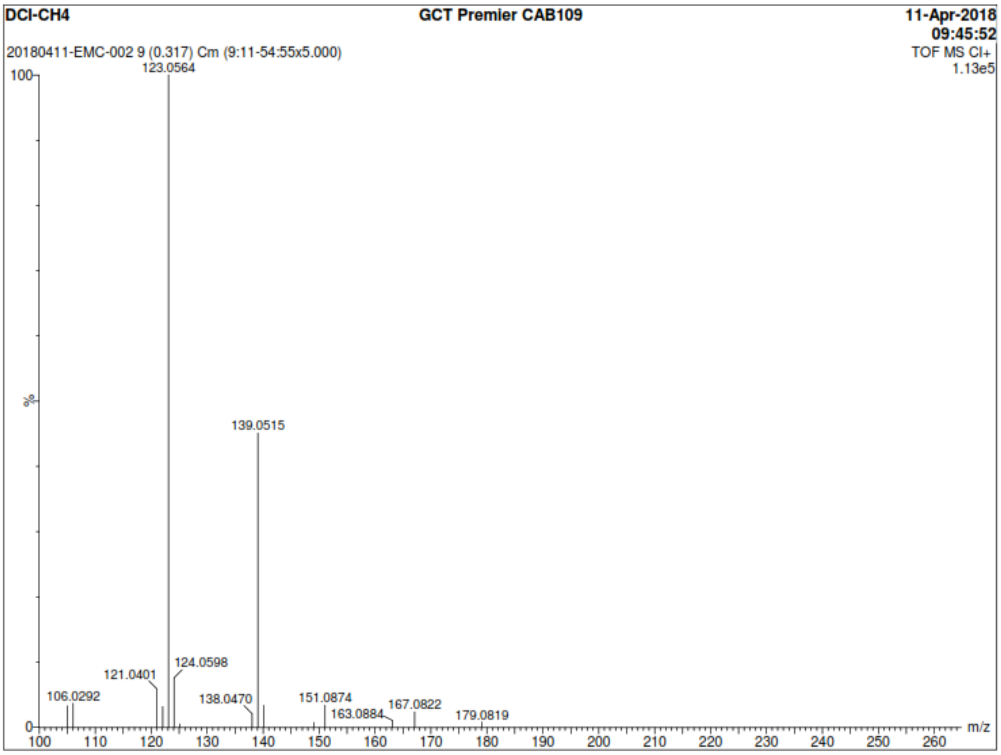


Figure S1. ^{1}H NMR spectra of a) sodium isonicotinoate, b) sodium nicotinoate acid, c) sodium pyrazinoate.

Figure S2

a)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139.0515
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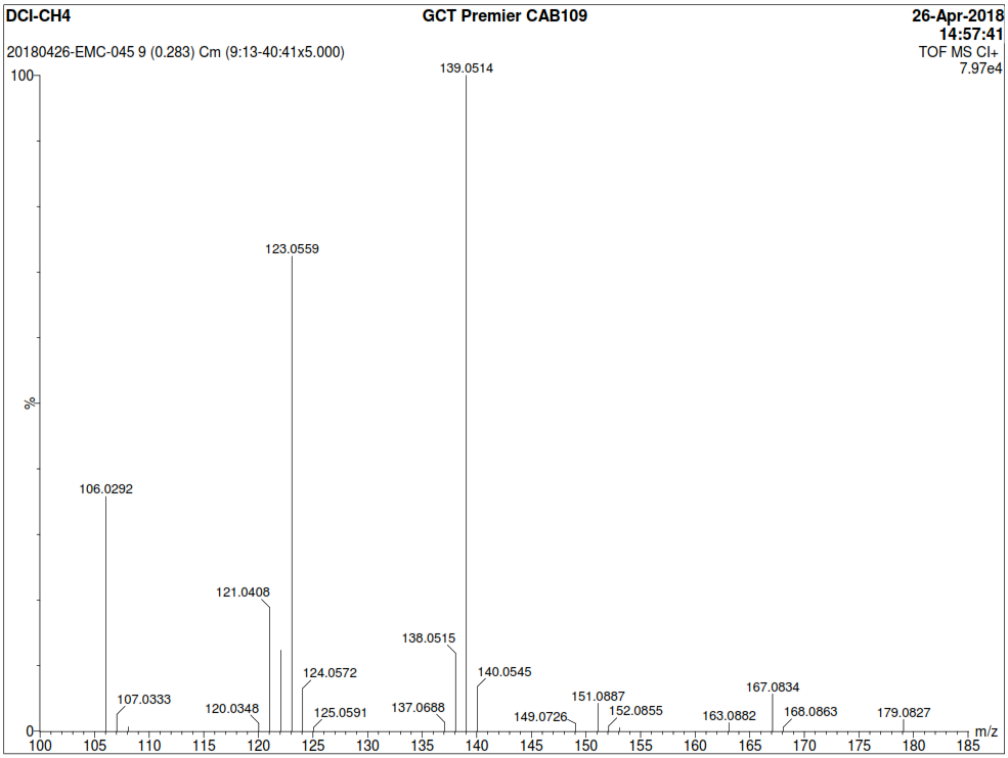
%
m/z

b)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125.0591
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%
m/z

c)

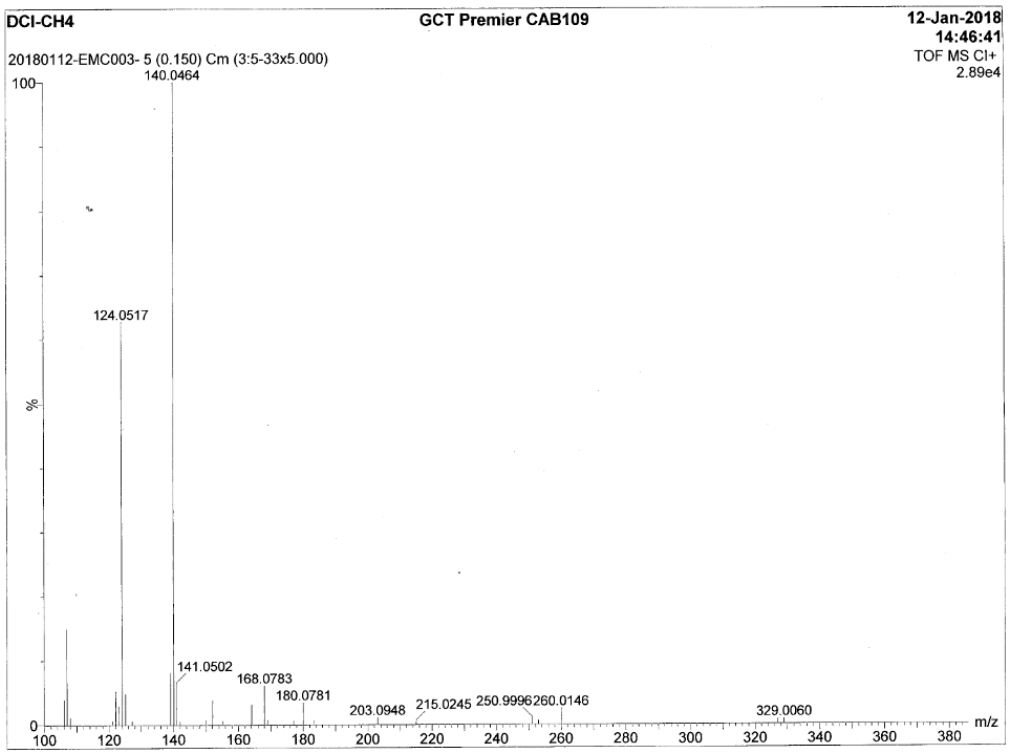

Figure S2. Mass spectra of a) isonicotionohydroxamic acid, b) nicotinohydroxamic acid and c) pyrazinohydroxamic acid.

Figure S3

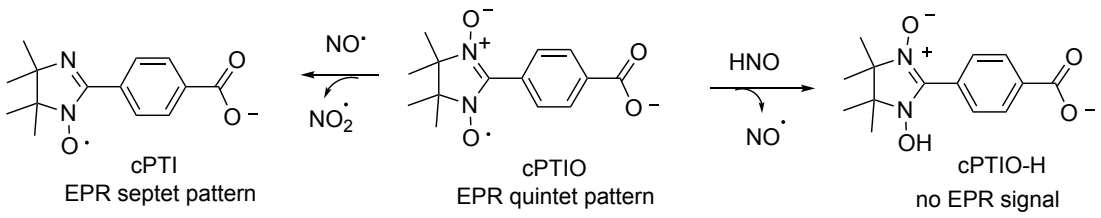

Figure S3. Reactions of cPTIO with NO. or HNO, and EPR patterns of the products.

Figure S4

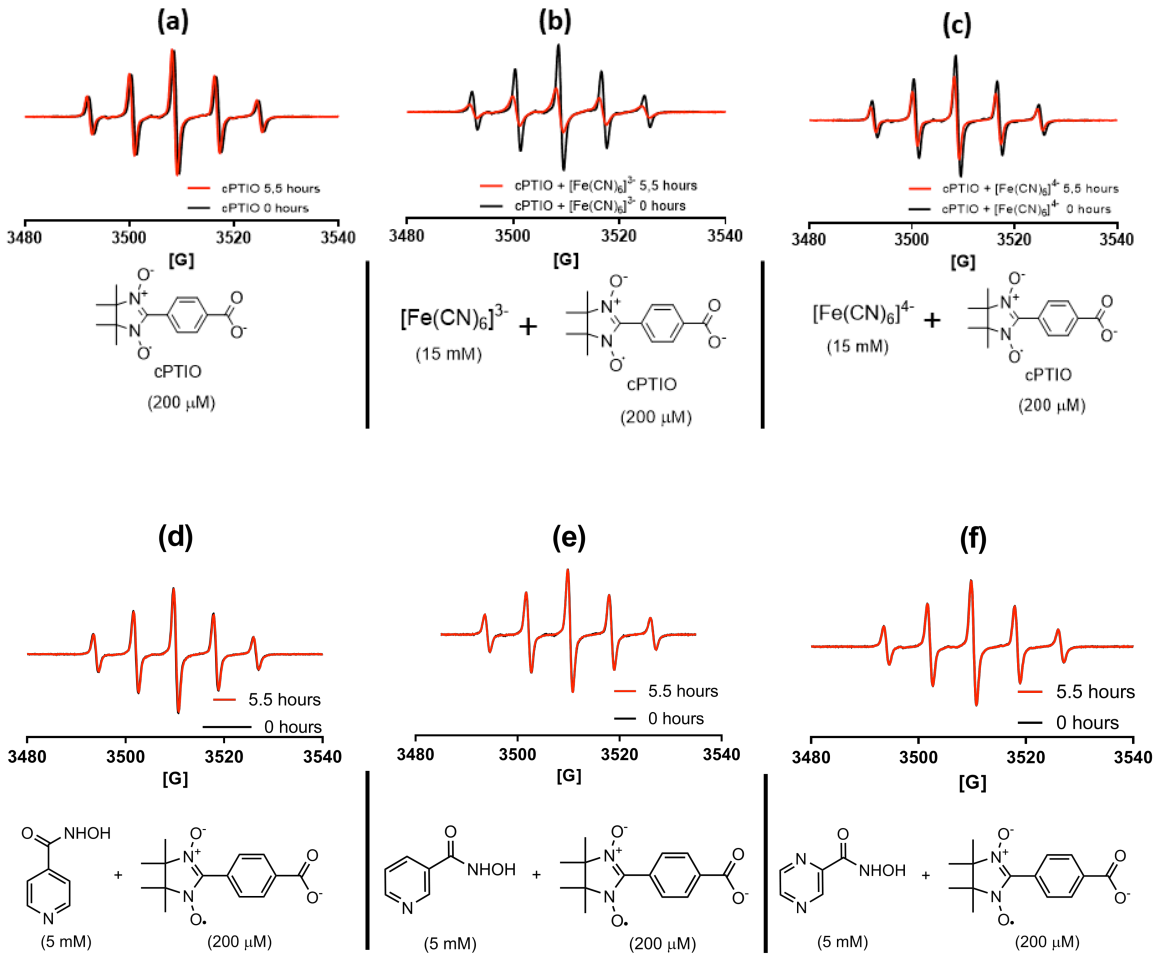

Figure S4a. EPR spectra of the controls: a) cPTIO (200 µM), b) cPTIO (200 µM) and $[Fe(CN)_6]^{3-}$ (15 mM), c) cPTIO (200 µM) and $[Fe(CN)_6]^{4-}$ (15mM), d) cPTIO (200 µM) and isonicotinohydroxamic acid (5 mM), e) cPTIO (200 µM) and nicotinohydroxamic acid (5 mM), and f) cPTIO (200 µM) and pyrazinohydroxamic acid (5 mM) 5.5 hours in phosphate buffer 40 mM, pH 7.4 at room temperature.

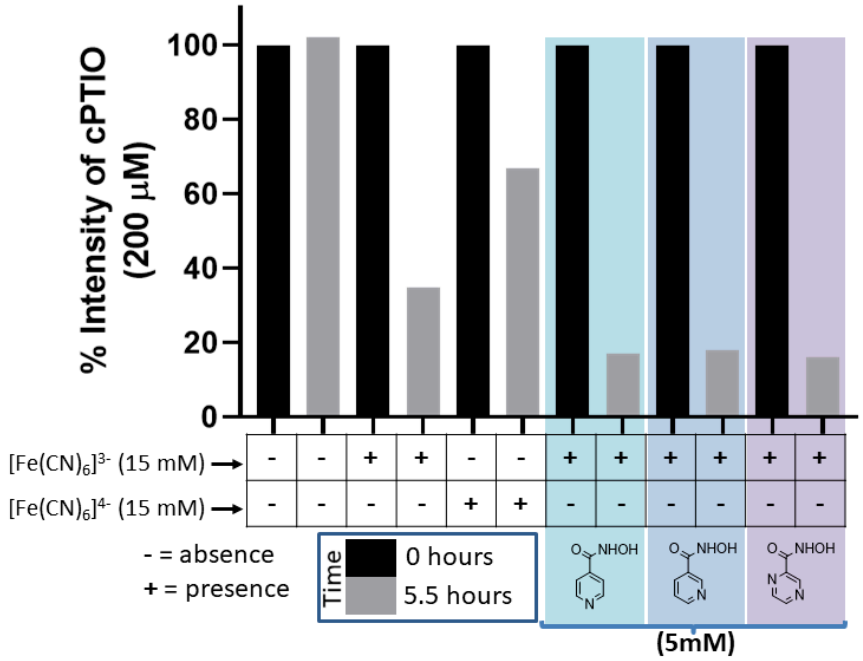

Figure S4b: EPR signal intensity of the reaction of isonicotinohydroxamic acid, nicotinohydroxamic acid and pyrazinohydroxamic acid (5 mM) with $[Fe(CN)_6]^{3-}$ (15mM) in the presence of cPTIO (200 µM) at 0 and 5.5 h in phosphate buffer 40 mM (pH 7.4) and at room temperature.

Figure S5

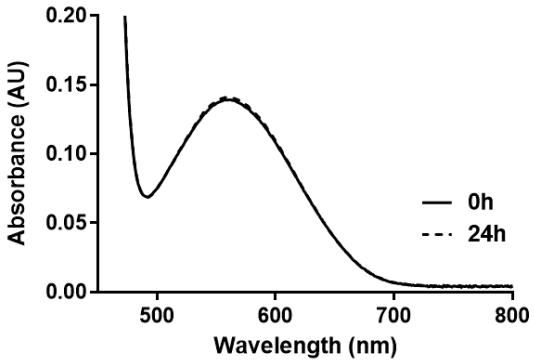

Figure S5. UV-vis absorption monitoring at 0-24 h of the evolution of a mixture of cPTIO (150 µM) and $K_3[Fe^{III}(CN)_6]$ (15 mM) in 40 mM phosphate buffer (pH 7.4) at room temperature.