Supplementary Information: Ferracyclic carbonyl complexes as anti-inflammatory agents

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Experimental

General

Reactions were carried out in dried Schlenk glassware under an atmosphere of pure nitrogen or argon. When necessary, the reactions were protected from light by wrapping the glassware in aluminium foil. Starting materials were purchased from Aldrich or Alfa Aesar and did not require further purification. The appropriate drying agents were used for solvent drying: dichloromethane (P_4O_{10}, Na_2CO_3), tetrahydrofuran sodium/benzophenone and acetonitrile (CaH_2). NMR spectra were recorded on Bruker Avance 300 (^{1}H at 300 MHz). IR spectra were recorded using a PerkinElmer SpectrumBX instrument. Elemental analyses were carried out at London Metropolitan University. Mass spectrometry was carried out at the National Mass Spectrometry Facility at Swansea University. Irradiation light source for myoglobin assays and quantum yield measurements were carried out using broadband visible light from a Krüss Optronic lamp (230 V, 150 W) at two different light intensities: 0.216 W and 0.840 W. 1-Thio-β-glucose[S1] was prepared following the literature procedures.

$FeBr(C_6H_5N_2O)(CO)_2(MeCN)$ (1)

This was synthesised following a method adapted from reported literature procedure.[S2] To a solution of $Fe(CO)_4Br_2$ (0.49 g, 1.49 mmol) in dichloromethane (60 cm^3) was added 2-aminopyridine (2 eq, 0.28 g, 3.02 mmol). The solution was left to react under stirring for 2 hours. Gas was evolved and a yellow precipitate formed over time. Once precipitation was complete, the supernatant was remove by decantation. The yellow product was recrystallized from acetonitrile affording brown crystals (0.23 g, 43 %). M.p. 108 °C. Found C 36.37, H 2.67, N 14.16 %; $C_9H_8BrFeN_3O_3{\cdot}C_2H_3N$ requires C 36.49, H 2.81, N 14.14 %. λ_{max}/nm (ε/M^{-1} cm^{-1}) (DMSO) 277 (16 500). ν_{max}/ cm^{-1} (MeCN) 205`, 1997, 1668, 1621. *m/z* (Orbitrap) 375.9 $[M + Na]^+$.

$FeBr(C_{10}H_7N_2O)(CO)_2(MeCN)$ (2)

$Fe(CO)_4Br_2$ (0.29 g, 0.77 mmol) was dissolved in dichloromethane (30 cm^3) and cooled to –40 °C.1-Aminoisoquinoline (0.19 g, 1.82 mmol) in dichloromethane (20 cm^3) was added drop wise (protected from light), and the resulting solution allowed to warm to –10 °C. The solution was allowed to react at room temperature for a further hour. The solvent was decanted to leave a yellow precipitate (intermediate salt $[C_5H_7N_2][FeBr_2(C_{10}H_7N_2O)(CO)_2]$).Recrystallization from acetonitrile (25 cm^3) yielded **2** (0.37 g, 48 %). M.p. 155 °C (dec.). Found C 41.40, H 2.34,N 10.16 %; $C_{14}H_{10}BrFeN_3O_3$ requires C 41.62, H 2.49,N 10.40 %. λ_{max}/nm (ε/M^{-1} cm^{-1}) (DMSO) 346 (5660), 280 (17 000). ν_{max}/ cm^{-1} (MeCN) 2053, 1990, 1662, 1613.

$\{Fe(C_6H_5N_2O)(CO)_2(\mu^2\text{-}SC_6H_{12}O_5)\}_2$ (3)

A solution of **1** (37 mg, 0.11 mmol) was dissolved in methanol (10 cm^3) and one equivalent of β-D-thioglucose was added from a stock solution of known concentration. The reaction was allowed to stir for 1 h. The solvent was removed under vacuum yielding a yellow solid (48 mg, 51 %). M.p. 95 °C. Found C 38.87, H 3.68, N 6.37 %; $C_{28}H_{34}Fe_2N_4O_{16}S_2$ requires C 39.18, H 3.99,N 6.53 %. λ_{max}/nm (ε/M^{-1} cm^{-1}) (DMSO) 295 (41 500). ν_{max}/ cm^{-1} (MeOH) 2046, 2025, 1977, 1674, 1622. *m/z* (Orbitrap) 857.0 [M^+].

$\{Fe(C_{10}H_7N_2O)(CO)_2(\mu^2\text{-}SC_6H_{12}O_5)\}_2$ (4)

A solution of **2** (5 mg, 12 µmol) was dissolved in methanol (10 cm^3) and one equivalent of β-D-thioglucose was added from a stock solution of known concentration. The reaction was allowed to stir for 1 h. The product was recrystallized from methanol affording yellow prisms (3.7 mg, 40 %). M.p 136 °C. λ_{max}/nm (ε/M^{-1} cm^{-1}) (DMSO) 335 (1960), 288 (3240) 278 (3250). ν_{max}/ cm^{-1} (MeOH) 2048, 2025, 1980, 1675, 1622. *m/z* (Orbitrap) 979.0 [M^+].

Myoglobin assay

Horse heart myoglobin stock solution (approx.. 130 mg in 4 mL) was dissolved in phosphate buffered saline (PBS, 0.01 M, pH = 7.4). Sodium thionite (200-fold excess) stock solution in PSB was made. To obtain an UV-vis reference spectrum of deoxy-myoglobin, a disposable cuvette (1 cm pathlength) was filled with myoglobin solution (500 µL), sodium thionite (100 µL) and PBS(400 µL). To this, gaseous CO was passed through and a UV-vis spectrum run for carbonmonoxymyoglobin. For the CORM run: The metal carbonyl complex was dissolved in DMSO and added to a cuvette (10 µL). To this cuvette was added myoglobin (500 µL), sodium thionite (100 µL) and PBS (390 µL). A UV-vis spectrum was taken, followed by irradiation of visible light. The cuvette is removed from the light source and a spectrum run every 10 min.

Figures S1 and shows representative results from the myoglobin assay.

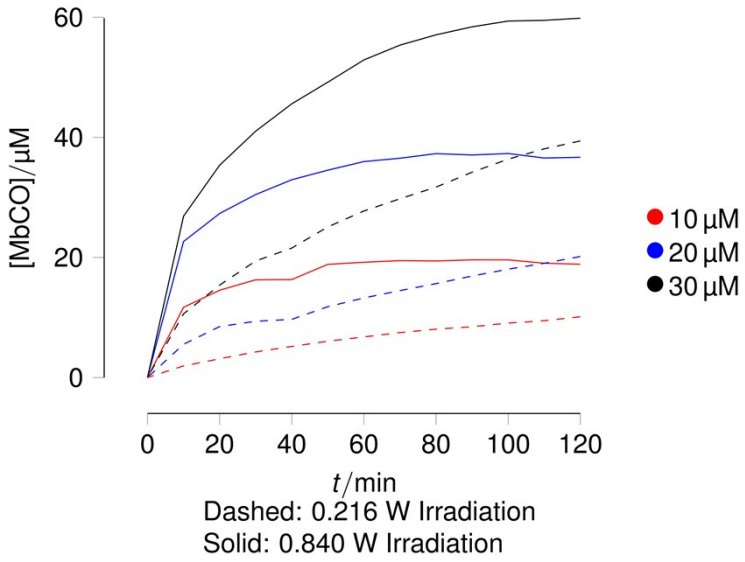

Figure S1: Plot of the amount of MbCO formed via CO liberated from complex **1**

For complexes **1** and **2**, the myoglobin assay curves were fitted using a single term exponential ($y = y_0 - Ae^{-kt}$), and the rate taken as an average across concentrations. For complexes **3** and **4**, a

two-term exponential ($y = y_0 - A_1 e^{-k_1 t} - A_2 e^{-k_2 t}$) was used, and the rate reported is the average for the slower k_2 term.

Control experiments established that no response was observed in the assay in the absence of complex, in the absence of light or in the absence of myoglobin.

Infra-red studies

In parallel experiments, CO release was tracked from the metal complexes using IR measurements. These confirmed the same qualitative pattern of CO release as the myoglobin assays revealed, with smooth loss of CO from the start of irradiation. Figure S3 shows a representative trace for complex **1**.

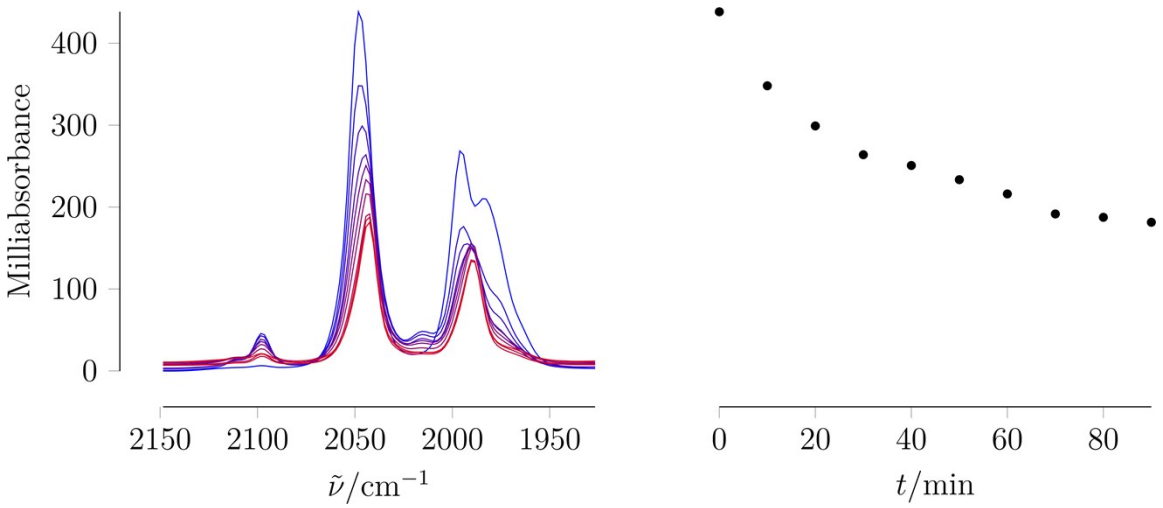

Figure S3: Left: Infrared Spectra of complex **1** Feduring visible light ('high power') triggered CO release in THF. Data ranges from 0 min(blue, prior to illumination) to90 min(red), measured at intervals of 10 min. Right: Carbonyl intensity at 2047 cm^{-1}decrease over time of illumination

Quantum yield measurements

All quantum yields were carried out using a liquid phase potassium ferrioxalate actinometer. The actinometer was prepared in complete darkness by mixing $FeCl_3$ (24.4 g, 150 mmol) in water (100 cm^3) with $K_2C_2O_4$ (76.0 g, 457 mmol) in water (300 cm3) and allowing to stir for 90 min, after which time a green precipitate formed. The supernatant was removed and the precipitate recrystallised from hot water three times. The actinometer stock solution was prepared by dissolving the $K_3Fe(C_2O_4)_3\}$ precipitate (8.94 g, 18.2 mmol) in distilled water (800 cm^3) followed by addition of sulphuric acid (1.0 M, 100 cm^3) and making up to 1000 cm^3 in distilled water. The irradiation was carried out in a darkroom using a red photographic safelight. The light intensity in photochemical reaction was determined by irradiating the ferrioxalate solution and monitoring the subsequent changed in absorbance at 510 nm. For each actinometeric measurement a cuvette was charged with 1 cm^3 (V_1) of ferrioxalate solution. The cuvette was placed at a specified distance away from the light source and irradiated for a set period of time. After the allocated irradiation time (t), the solution was well mixed and an aliquot volume (0.5 cm^3, V_2) was added to a volumetric flask (10 cm^3, V_3). A buffer solution (half of V_2, 0.25 cm^3) and phenanthroline solution (0.3 % by weight, 2 cm^3) was added. The solution was made up to the mark with distilled water (7.25 cm^3). The solution was mixed and allowed to develop for 1 h. The absorbance was measured at 510 nm using distilled water as a reference.

X-Ray crystallography

For each sample, crystals were suspended in oil, and one was mounted on a glass fibre and fixed in the cold nitrogen stream of the diffractometer. Data were collected using Mo-Kα (λ = 0.71073 Å) radiation using a Rigaku Saturn724+ diffractometer equipped with confocal mirrors (**2**) or Oxford Diffraction Xcalibur-3 CCD diffractometer equipped with a graphite monochromator (**4**), and were processed using CrystalClear-SM Expert (**2**)[S3] or CrysAlisPro (**4**)[S4]. Structures were determined using a dual-space approach in SHELXT[S5] and refined by full-matrix least-squares methods on F^2 in SHELXL.[S6] Non-hydrogen atoms were refined with anisotropic thermal parameters. The nitrogen-bound hydrogen in **2** was located in the Fourier difference map and freely refined; all other hydrogen atoms were included in idealized positions and their U_{iso} values were set to ride on the U_{eq} values of the parent atom. In **2**, the data were sufficiently strong to assign hydrogen atoms to each OH/water group but not to place every one based on the difference map. Some close contacts in the structure resulted. A small amount of residual electron density is also likely due to the presence of a partial-occupancy MeOH which could not be modelled satisfactorily,

CCDC 1432518 and 1432519 contain the supplementary crystallographic data for this paper.

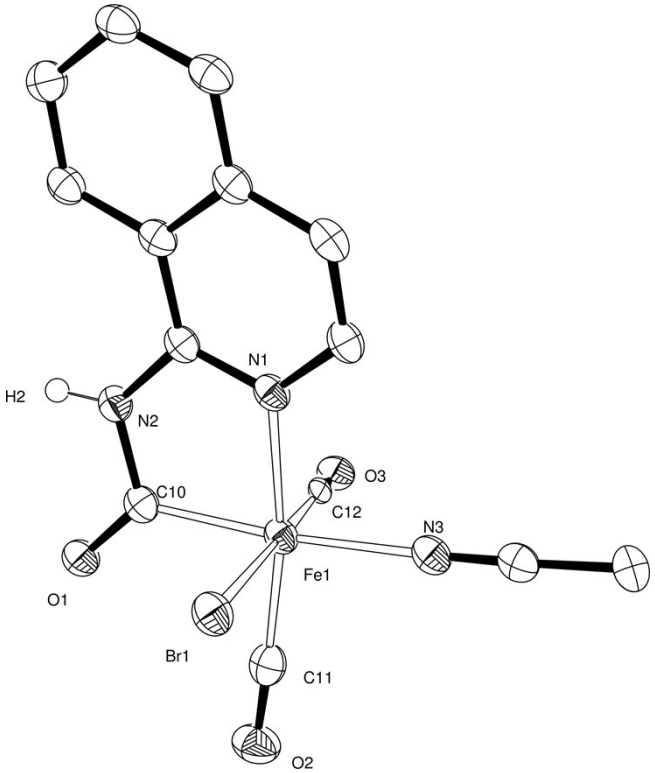

Figure S2: ORTEP representation of the structure of **2**.MeCN showing 50 % probability ellipsoids; one molecule of MeCN and hydrogen atoms attached to carbon have been omitted for clarity.

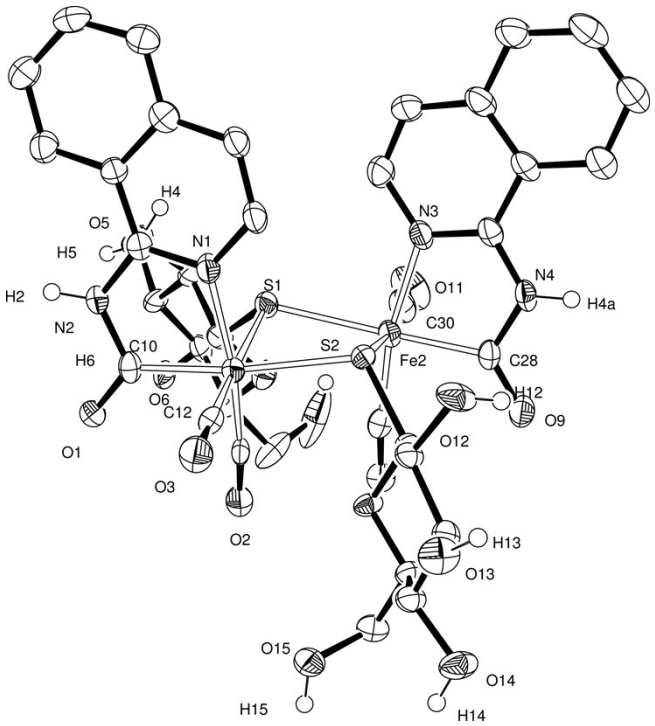

Figure S3: ORTEP representation of the structure of complex **4** showing 50 % probability ellipsoids; hydrogen atoms attached to carbon have been omitted for clarity.

Cell cultures

Human monocytic THP-1 cells (from the European Collection of Authenticated Cell Cultures, ECACC) were cultured in RPMI 1640 medium (Hyclone, GE Healthcare Sciences), supplemented with 10 % foetal bovine serum, 2 mM L-glutamine, 100 units/mL Penicillin and 100 µg/mL Streptomycin (Gibco). The cells were maintained at 37 °C in a humidified atmosphere with 5% CO_2 and passaged twice weekly.

Cell viability assay

Cell viability was measured as previously described.[S7] Briefly, 3×10^4 cells were seeded in 100 µl volume in 96-well tissue culture-treated plates and varying concentrations of photoCORM molecules (100 pM–1 mM) or DMSO vehicle control added for 72 h. Following this, cell proliferation was determined using a Cell Titer 96 Aqueous non-radioactive assay (Promega). MTS reagent was added for 4 h, followed by absorbance measured at 492nm using a BMG Optima plate reader (BMG Labtech).

TNFα ELISA

TNFα secretion was measured as previously described.[S7] Briefly, 5×10^5 THP-1 cells were seeded in 24-well plates and photoCORM molecules or DMSO vehicle control added. Cells were stimulated with LPS (1 µg/ml) for 3 h. Supernatants were collected following centrifugation and stored at –80 °C until further use. TNFα concentrations in supernatants were measured using a human TNF ELISA kit (BD Biosciences) according to the manufacturer's instructions. Absorbance of samples was measured at 450 nm with subtraction of absorption values at 570 nm.

References

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[S3] *CrystalClear-SM Expert*, Rigaku, The Woodlands, Texas, USA, **2014**.

[S4] *CrysAlisPro*, Agilent Technologies, Abingdon, UK, 2014.

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[S7] J. Reed, M. J. Stephenson, K. Miettinen, B. Brouwer, A. Leveau, P. Brett, R. J. M. Goss, A. Goossens, M. A. O'Connell, A. Osbourn, *Metab. Eng.* **2017**, *42*, 185−193.