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Ferracyclic carbonyl complexes as anti-inflammatory agents

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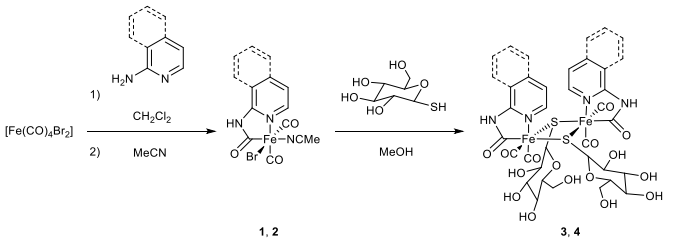
Reaction of $Fe(CO)_4Br_2$ with 2-aminopyridine and 2-aminonapthalene yields ferracyclic iron(II) complexes bearing two CO ligands. Irradiation with *visible* light releases these two CO molecules. Substitution of halide in parent complexes by thioglucose provides significantly and raises the quantum yield for CO release is raised by around five times. The complexes show anti- inflammatory activity in a TNF assay in the *dark*.

Carbon monoxide (CO) acts a bioregulator and exerts a wide range of protective effects in tissues at physiological concentrations. Over the past decade, the therapeutic potential of CO in various conditions including neurodegenerative and cardiovascular diseases has been recognized[1,2] and is likely to be partly due to their anti-inflammatory properties. Preclinical trials have shown that exogenous CO can act as a therapeutic agent,[3] bringing about a range of beneficial effects including inflammatory suppression[4,5] and vasodilation.[6,7] A critical challenge to overcome is the inherent toxicity of CO and to optimize its therapeutic delivery in a safe and tightly-controlled manner. One leading strategy is the application of metal carbonyl complexes bearing labile CO ligands. These carbon monoxide releasing molecules (CORMs) allow for molecular storage of one or more CO equivalents, which will only liberate the gas when subjected to a particular trigger mechanism.

A particularly attractive approach to controlled release of CO is to use an external stimulus to provide both special and temporal control. The leading method to achieve this at present is to use (visible) light to degrade dark-stable molecules: so-called photoCORMS.[8–13] Whilst Mn(I) complexes have received particular interest in the development of visible-light photoCORMs,[14–16] the potential of iron-based systems, has received more limited attention.[17–20] Appropriately chelating this transition metal offers the prospect of creating benign photoCORM systems complementary to the more extensive family of manganese-based systems.



Scheme 1. Synthesis of complexes.

We have previously reported a structural mimic of the photosensitive [Fe]-hydrogenase active site (**1**, Scheme 1),[21,22] featuring a low-spin Fe(II) centre and bearing two discrete CO ligands. We reasoned that the combination of a pyridyl chromophore with the CO-rich metal centre could provide a platform on which to build novel iron-based photoCORMs activated by visible light. Gratifyingly, the photoactivity of **1** was readily confirmed by monitoring the infrared (IR) region, with rapid loss of the starting materials *cis*-CO signature over a two hour time frame on irradiation with *visible* light.

Extending the aromatic system of **1** by introduction of a naphthyl ring was facile, and proceeded to yield the light-sensitive **2** by the same route (Fig. 1, top).

A central requirement for any practical photoCORM is that it is deliverable in aqueous media.[23] Introduction of a saccharide group offers the possibility to both confer water solubility and to influence cellular targeting. Ruthenium- and molybdenum-based photoCORMs bearing peptide or protected sugar functions have been described but these are only active in the ultraviolet region (UV).[24,25] By exploiting dinuclear cores related to **1** and bearing thioglucose ligands, we have now developed photoCORMs which release CO in the visible region and are soluble in water.

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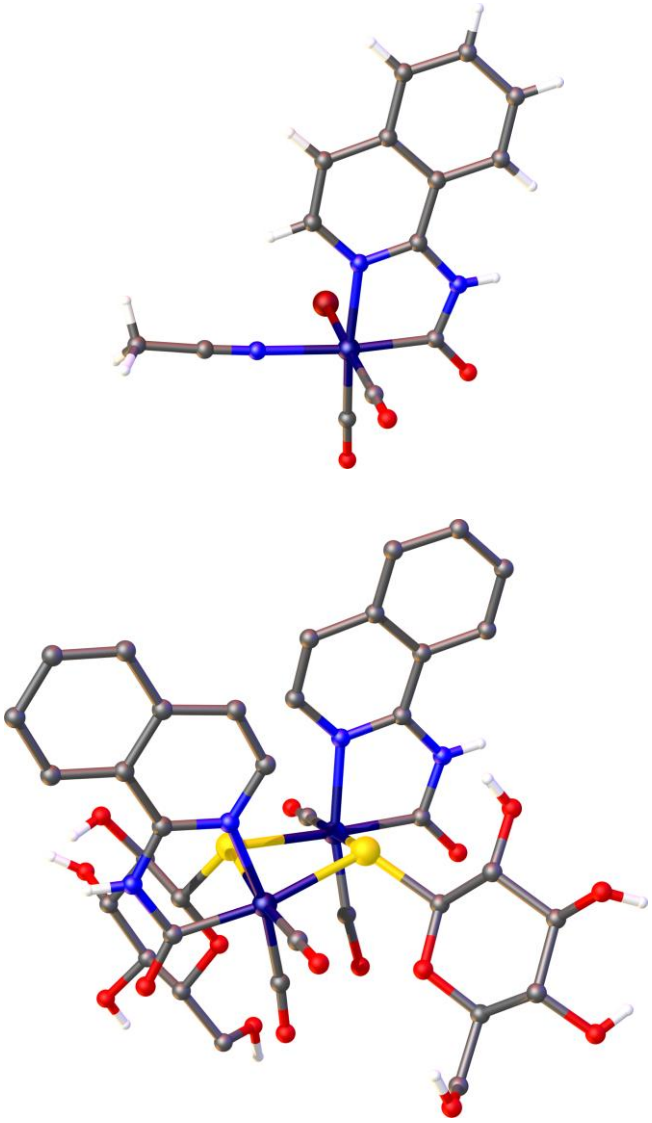

Figure 1. Ball-and-stick representations of the structure of **1**·MeCN (top) and **4**·3(MeOH)·1.5(H_2O) (bottom); solvent molecules and hydrogen atoms bound to carbon (bottom) are omitted for clarity. For ORTEP representations see ESI.‡,§

Replacement of the halide in **1** has been established using simple aromatic thiols,[21,22] and was successful in generating **3** and **4** in MeOH solution. The latter was readily crystallised as the methanol/water solvate (Fig. 1), confirming the presence of the desired Fe–S linkage along with dimer formation.[21] Whilst the parent complexes are insoluble in pure water, **3** can be dissolved in water at concentrations of at least 35 mM. The ultraviolet spectra of **1**–**4** in DMSO reveal that whilst the most significant bands are in the ultraviolet region, the compounds exhibit absorption into the visible (Fig. 2, λ > 400 nm). On a per-metal basis, the monomeric and dimeric systems show similar UV-visible behaviours in the ultraviolet and visible ranges.

Quantitative assessment of photoCORM potential was carried out using the myoglobin assay[26] with irradiation from a cold visible light source at two power levels (0.216 W and 0.840 W). Carbon monoxide release was monitored in buffer solution, with the metal complexes added dissolved in water

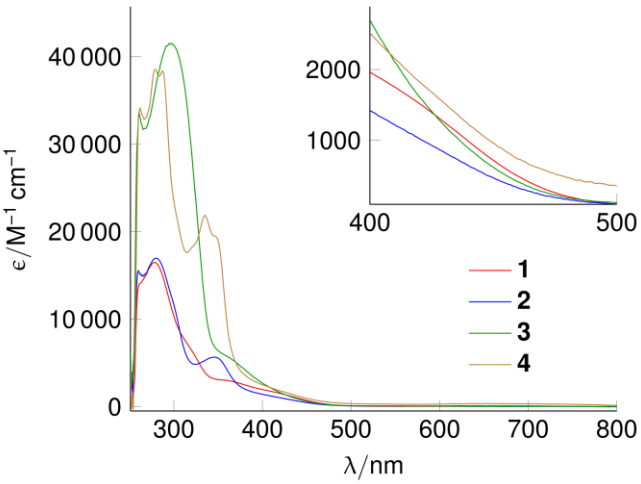

Figure 2. UV-vis spectra for photoCORMs in DMSO.

(**3**), water–DMSO 49 : 1 (**4**) or DMSO (**1** and **2**). The assays were performed at concentrations of 10 µM, 20 µM and 30 µM. The apparent rates of CO photorelease (k_{CO}) and quantum yields (Φ) are given in Table 1. Both complexes **3** and **4** release two molecules of CO per mole (*i.e.* one per metal centre) during the assay. With the proviso that varying amounts of DMSO were required to solubilise the complexes, it is possible to compare the release behaviours of the systems: the rate of CO release in the dimeric complexes is significantly raised compared with the monomeric parents (Fig. 3).

The assay data is also indicative of a change of mechanism: whilst release from the monomeric systems may be fitted using a single exponential function, a two-term exponential is required to model the behaviour of the dimeric systems. A two-step mechanism involving distinct loss mechanisms for the first and second CO equivalents from **3** and **4**, possibly driven by strong hydrogen bonding, may explain this behaviour. To date we have been unable to identify signals corresponding to such an intermediate, and are pursuing this both synthetically and by ultrafast pulse-probe methods.

Typically, the term 'photoCORM' is applied to any molecule which releases CO on exposure to (ultraviolet or visible) light,[9] and so a positive response in the myoglobin assay is normally regarded as sufficient to describe materials as photoCORMs. This is an effective screening method given the increasing number of putative photoCORMs reported in recent years, but

Table 1. Apparent rate of CO release (k_{CO}) and associated quantum yields (Φ) for **1**–**4** at two different irradiation powers.

Complex [b]	Ave. rate (k_{CO})[a]/(10^{-3} min^{-1})		Quantum yield (Φ)[b]/10^{-5}	
	0.216 W	0.840 W	0.216 W	0.840 W
1[c]	7.7(10)	15.9(6)	15.4	1.39
2[c]	7.9(6)	23(4)	15.8	1.49
3[d]	17.1(15)	107(6)	17.0	9.31
4[e]	17.5(4)	111(4)	17.4	9.67

a] As determined as the average of myglobin assays at 10 µM, 20 µM and 30 µM solutions. [b] Using a broadband visible light source. [c] Dissolved in DMSO. [d] Dissolved in water. [e] Dissolved in water–DMSO 49 : 1.

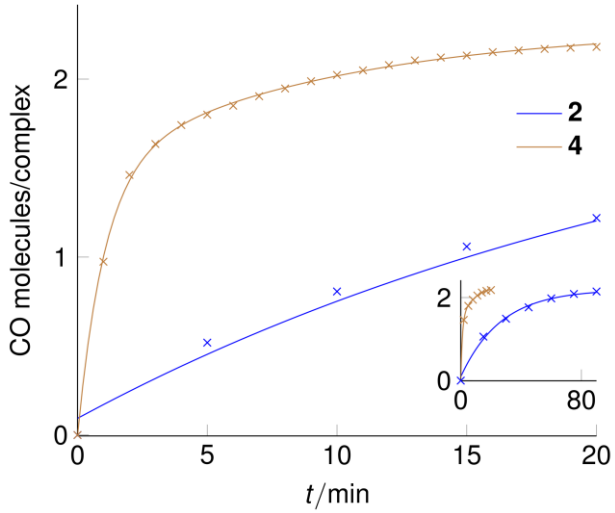

Figure 3. CO release from **2** and **4** as assessed by a myoglobin assay at a light power of 0.840 W, $[complex]_0$ = 30 μM. Points: experimental data; lines: exponential fits. Inset shows full time course for release from **2**.

can only be a precursor to more detailed in vitro testing for activity in a biological context.

There are several different approaches to in vitro testing of putative photoCORMs. Cell viability screening,[27–31] is vital to establish that photoCORMs are not themselves harmful, but does not directly provide information on active therapeutic potential. Studies on anti-cancer,[32–35] anti-microbial[36,37] and ion channel activity[38] have been reported in recent years, but cover only a small number of the total reports of putative photoCORMs. Anti-inflammatory activity is well-established for CO and is therefore an area which is a key target for (photo)CORM application. However, to date we are unaware of any photoCORM systems shown to exhibit anti-inflammatory activity *in vitro*.[39]

Anti-inflammatory activity for complexes **1**–**4** was assessed by studying the inhibitory effects on lipopolysaccharide-induced tumour necrosis factor-alpha (TNF) secretion in human THP_1 monocytes[40] We have previously validated this in vitro model of inflammation.[40,41] We investigated the anti-inflammatory activity of complexes **1**–**4**, both in the light and in the dark (Fig. 4); control experiments established that the compounds did not affect cell viability at the concentrations used (IC_{50} >1 mM for all compounds, data not shown). Surprisingly, *in the light* there was no evidence for any activity for compounds **1** or **3**, with limited efficacy for complex **4**. On irradiation, complexes **2** and **4** showed statistically-significant activity, though in the latter case the degree of suppression was small (13.9%). In contrast, assay experiments carried out in the dark revealed 51% suppression of inflammatory response for compound **1** and 72 % suppression for compound **2**. The more elaborate structures **3** and **4** again were disappointingly ineffective, with no significant suppression of TNF release.

The contrast between myoglobin and TNF assay results here is striking. The myoglobin data establish that complexes **1**–**4** release CO in response to irradiation (and not by hydrolysis), but it is clear from the TNF data that this is not

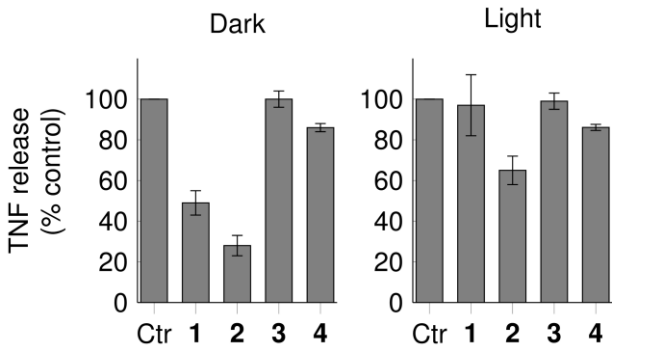

Figure 4. Anti-inflammatory effects of photoCORMs. THP-1 monocytes were pre-treated with photoCORMs (50 μM) for 30 min prior to addition of lipopolysaccharide (for 3 h) to stimulate TNF secretion. TNF in cell supernatants was measured by ELISA (BD Biosciences). Values are relative to control LPS-activated DMSO-treated cells (means, ± SD, three biological replicates).

under stress is not sufficient for activity. One may speculate responsible for suppressing the inflammatory response; as noted in Table 1, release of CO in the myoglobin assay is dependent on illumination of the substrates. Thus releasing CO close to cells that this points to a requirement for cellular uptake as part of the overall mode of action of these compounds. The structural variations present in the complexes may offer some insight. Complexes **3** and **4** are dimeric and may therefore be too large to enter viable cells, despite their favourable solubility characteristics. Efforts to elucidate the background to this contrasting behaviour are ongoing.

In summary, a family of photoCORM molecules have been developed featuring a ferracyclic core. All release CO in response to irradiation with *visible light* as detected by a myoglobin assay. However, anti-inflammatory activity is only seen in the dark, suggesting an alternative mode of action is in play. The results here emphasise the need to look beyond the myoglobin assay to *in vitro* testing in the development of practical leads for therapeutic photoCORM systems.

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Conflicts of interest

There are no conflicts to declare.

Notes and references

‡ Crystal data for **2**·MeCN: Empirical formula $C_{16}H_{13}BrFeN_4O_3$; Formula weight 445.06; Crystal size/mm^3 0.09 × 0.06 × 0.01; Crystal system triclinic; Space group *P*–1; *a*/Å 8.0450(6); *b*/Å 8.9535(6); *c*/Å 12.1950(9); *α*/° 96.322(6); *β*/° 91.386(5); *γ*/° 92.148(5); *V*/Å^3 872.11(11); *Z* 2; MoKα (*λ* = 0.71075); Reflections collected 15 491; Independent reflections 3981; R_{int} = 0.0492; Data/restraints/parameters 3981/1/232; R_1 [I >= 2σ(I)] 0396; wR_2 [all data] 0.1148.

§ Crystal data for **4**·3(MeOH)·1.5(H_2O). Empirical formula $C_{39}H_{51}Fe_2N_4O_{20.5}S_2$; Formula weight 1079.66; Crystal size/mm^3 0.38 × 0.35 × 0.25; Crystal system monoclinic; Space group *C*2; *a*/Å 23.8608(7); *b*/Å 13.3771(5); *c*/Å 15.4092(5); *β*/° 101.705(3); *V*/Å^3 4816.2(3); *Z* 4; MoKα (λ = 0.71073); Reflections collected 35 958; Independent reflections 10 847; R_{int} = 0.0634, ; Data/restraints/parameters 10847/23/644; R_1 [I >= 2σ(I)] 0.0619, wR_2 [all data] 0.1696; Flack parameter –0.02(3)**.**

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