Available online at www.sciencedirect.com
SCIENCE DIRECT®
Bioorganic & Medicinal Chemistry Letters
Bioorganic & Medicinal Chemistry Letters 16 (2006) 995–998

η^4-Pyrone iron(0)carbonyl complexes as effective CO-releasing molecules (CO-RMs)

Ian J. S. Fairlamb,[a,*] Anne-Kathrin Duhme-Klair,[a] Jason M. Lynam,[a] Benjamin E. Moulton,[a] Ciara T. O'Brien,[a] Philip Sawle,[b] Jehad Hammad[b] and Roberto Motterlini[b,*]

[a]*Department of Chemistry, University of York, Heslington, York YO10 5DD, UK*
[b]*Vascular Biology Unit, Department of Surgical Research, Northwick Park Institute for Medical Research, Harrow, Middlesex HA1 3UJ, UK*

Received 18 August 2005; revised 24 October 2005; accepted 26 October 2005
Available online 11 November 2005

Abstract—The CO-releasing properties of iron(0)tricarbonyl complexes bearing a 2-pyrone ligand have been evaluated. In this report, we demonstrate that the intrinsic stability of the (η^4-2-pyrone)$Fe(CO)_3$ complex influences the extent and rate of CO release, which is affected by the presence of a halogen substituent on the 2-pyrone ring. The cell viability index has been highlighted for the active carbon monoxide-releasing molecules (CO-RMs), demonstrating that these complexes and related derivatives are a promising new class of compounds with potential therapeutic applications.

The development of carbon monoxide-releasing molecules (CO-RMs) has become a recent target for therapeutic intervention.[1] It is well known that CO is generated in mammals during the degradation of heme by constitutive (HO-2) and inducible (HO-1) heme oxygenase enzymes. It is responsible for important physiological functions and is a fundamental signaling mediator.[2] CO possesses vasodilatory properties,[3] controls the proliferation of vascular smooth muscle cells,[4] suppresses the rejection of transplanted hearts,[5] mediates potent anti-inflammatory effect,[6] and promotes protection against ischemic tissue injury.[7] When low concentrations of CO gas are administered, beneficial therapeutic effects have been observed, although the associated toxicity and inherent poor selectivity of CO, as a gas, are clearly not ideal. The method of choice, potentially, for taking advantage of the biological role of CO, is to utilize a 'CO-carrier' such as a metal carbonyl complex. Motterlini et al. have previously shown that certain transition metal carbonyl complexes function as carbon monoxide-releasing molecules (CO-RMs) in biological systems.[8]

Dimanganese decacarbonyl (CORM-1) and tricarbonyldichloro ruthenium(II)-dimer (CORM-2) were the first identified complexes to demonstrate an inherent capacity to liberate CO in biological models and promote relaxation of blood vessels in vitro, attenuate coronary vasoconstriction in isolated hearts, and reduce acute hypertension in vivo.[9] The synthesis of tricarbonylchloro(glycinato)ruthenium(II) (CORM-3), the first prototypic water-soluble CO-RM, confirmed the pharmacological effects of metal carbonyls in mediating protection against ischemia and myocardial infarction,[10] vasodilatation, and hypotension[11] as well as prevention of organ graft rejection following heart transplantation.[10b] More recently, transition metal carbonyls and other classes of CO-RMs have been used to assess the role of CO gas as an important signaling factor in a variety of experimental models.[12] Thus, the identification of other metal carbonyls that have the ability to release CO, and are biologically compatible, will provide important information on the chemistry of these compounds in aqueous environments and implement the design of novel pharmaceuticals for therapeutic purposes.

Here we report on the chemical and biological features of a group of 2-pyrone iron(0)carbonyl complexes. They

Keywords: Vasorelaxation; Carbon monoxide; Transiton metal carbonyl complexes; Therapeutic agents.
* Corresponding authors. Tel.: +44 1904 434091; fax: +44 1904 432516; e-mail addresses: ijsf1@york.ac.uk; r.motterlini@imperial.ac.uk

0960-894X/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2005.10.085

represent the first reported CO-RMs containing iron and an η^4-coordinated diene ligand.

2-Pyrone (2*H*-pyran-2-one) **1**, and related compounds, are ubiquitous in nature (Fig. 1).[13] Natural and non-natural 2-pyrone compounds exhibit diverse bioactivity.[14] For example, substituted 2-pyrones **2** inhibit human ovarian carcinoma (A2780) and human chronic myelogenous leukemia (K562) cell lines at the sub-micromolar level (IC_{50}), exhibiting insignificant toxicity in normal cells.[15] In these cellular systems, the 2-pyrone appears to behave as a pro-drug, where carbonyl ring-opening leads to the bioactive form.[16] 6-Chloro-2-pyrones inhibit yeast cholesterol esterase from *Candida cylindracea* in a similar manner.[17]

Complexation of the 2-pyrone in an η^4-diene-like fashion to an irontricarbonyl unit '$Fe(CO)_3$' serves to activate the 2-pyrone ring-system (Fig. 2). The synthesis of such compounds, although challenging, is made possible by the employment of $Fe_2(CO)_9$[18] or Grevels' reagent,[19] $[Fe(coe)_2(CO)_3]$ (coe = *cis*-cyclooctene).[20]

To rationalize the CO-releasing activity of complexes **3a**–**c**, several new (η^4-2-pyrone)$Fe(CO)_3$ complexes: **3d**,**e**, and **g** were synthesized according to Scheme 1.[21]

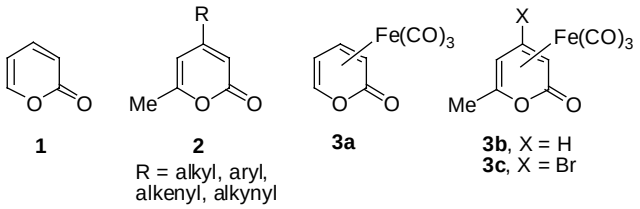


Figure 1. 2-Pyrones and related 2-pyrone metal carbonyl complexes.

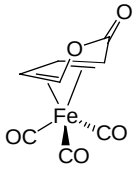
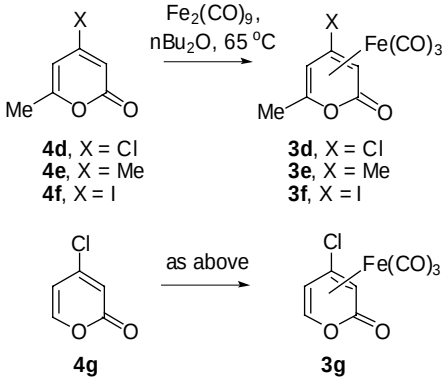


Figure 2. 'Bent-form' of parent 2-pyrone on η^4-coordination to Fe^0.



Scheme 1. Synthesis of novel (η^4-2-pyrone)$Fe(CO)_3$ complexes.

4-Chloro-**4d**, 4-iodo-**4f**, and 4,6-dimethyl-2-pyrone **4e** were reacted with Fe_2CO_9 in *n*-Bu_2O at 65 °C. Complex **3d** was isolated in 13% yield (based on recovered **4d**), and some hydrodechlorination was seen affording **3b** as a minor product (2%).[22] Complex **3e** was produced in 22% yield (based on recovered **4e**). We have previously established that Grevels' reagent may be used to access complexes such as **3c** in higher yield.[20] However, the difficulty in handling this reagent led us to revert to the more traditional preparative method for these preliminary studies.

The coordination mode in the solid state (X-ray),[23] as in the solution state, is very similar to complexes **3a**–**c**.[20] Hydrodeiodonation in **3f** leads to the isolation of **3b** in 7% yield, exclusively. Complexation of 4-chloro-2-pyrone **4g** provides **3g** in 32% yield (based on recovered **4g**) with no observable hydrodechlorination.

The amount of CO released from 2-pyrone carbonyls was assessed by measuring the conversion of deoxymyoglobin (deoxy-Mb) to carbon monoxide myoglobin (MbCO), as previously described.[9–12] The typical spectra of deoxy-Mb and MbCO are represented in Figure 3A. As shown, deoxy-Mb (50 μM) is rapidly converted into MbCO after bubbling CO gas (1%) for 2 min into the solution. A similar profile is observed when deoxy-Mb is reacted with 60 μM **3c** (see Fig. 3B) and the amount of MbCO formed over time is reported in Figure 3C. From the fitted curve it is calculated that the initial rate of CO release from **3c** is approximately 0.19 μM/min. For comparison, under thermal conditions $Fe(CO)_5$ does not result in any appreciable formation of MbCO over a similar period of time—light activation is required to initiate CO release in this case.[9]

The bioactive properties of **3c** were confirmed by examining the effect of this compound on vessel relaxation. Transverse ring sections were prepared from thoracic aortas of male adult Sprague–Dawley rats (350 g) and mounted in an organ bath containing oxygenated (95% O_2 and 5% CO_2) Krebs–Henseleit buffer.[11] The extent of vasorelaxation over time elicited by two consecutive additions of **3c** (100 μM) was assessed in aortic rings pre-contracted with phenylephrine (1 μM). As shown in Figure 3D, **3c** produced a 42% relaxation after the first addition and a further 35% decrease in tension was observed following the second addition. These results are in agreement with the vasodilatory role of CO liberated from primary transition metal carbonyl sources, for example, $Mn_2(CO)_{10}$.[8,9] The potential toxic effects of CO-RMs that were capable of releasing CO were also tested. RAW246.7 murine macrophages (involved in immune response) were incubated for 24 h and the cell viability was determined using an Alamar blue assay as previously described.[24]

As shown in Table 1, the IC_{10} (concentration at which 10% of cells are not viable) for **3c** is 132 μM. This indicates that the concentration of **3c** used for causing vasorelaxation (100 μM) is not toxic and confirms that the pharmacological effect of **3c** is due to the liberated CO. In addition, the small degree of toxicity caused by