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BIOACTIVE PROPERTIES OF IRON-CONTAINING CARBON MONOXIDE-RELEASING MOLECULES (CO-RMS)

Philip Sawle[1], Jehad Hammad[1], Ian J. S. Fairlamb[2], Benjamin Moulton[2], Ciara T. O'Brien[2], Jason M. Lynam[2], Anne K. Duhme-Klair[2], Roberta Foresti[1] and Roberto Motterlini[1]

Vascular Biology Unit, Department of Surgical Research, Northwick Park Institute for Medical Research, Harrow, Middlesex, United Kingdom (PS, JH, RF, RM)

Department of Chemistry, University of York, Heslington, York, United Kingdom (IJSF, BM, CTOB, JML, AKDK)

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Corresponding author: Roberto Motterlini, Vascular Biology Unit, Department of Surgical Research, Northwick Park Institute for Medical Research, Harrow, Middlesex, HA1 3UJ, United Kingdom. Phone: +44-20-8693181; FAX: +44-20-8693270; e-mail: r.motterlini@imperial.ac.uk.

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ABSTRACT

Carbon monoxide-releasing molecules (CO-RMs) are compounds capable of delivering controlled amounts of CO within a cellular environment. Ruthenium-based carbonyls (CORM-2 and CORM-3) and boronacorbonates (CORM-A1) have been shown to promote vasodilatory, cardioprotective and anti-inflammatory activities in a variety of experimental models. Here we extend our previous studies by showing that CORM-F3, an irontricarbonyl complex which contains a 2-pyrone motif, liberates CO *in vitro* and exerts pharmacological actions that are typical of CO gas. Specifically, CORM-F3 caused vasorelaxation in isolated aortic rings and inhibited the inflammatory response (e.g. nitrite production) of RAW264.7 macrophages stimulated with endotoxin in a dose-dependent fashion. By analyzing the rate of CO release, we found that when the bromide at the 4-position of the 2-pyrone in CORM-F3 is substituted with a chloride group (CORM-F8), the rate of CO release is significantly decreased (4.5 fold) and a further decrease is observed when the 4- and 6-positions are substituted with a methyl group (CORM-F11) or a hydrogen (CORM-F7), respectively. Interestingly, the compounds containing halogens at the 4-position and the methyl at the 6-position of the 2-pyrone ring (CORM-F3 and CORM-F8) were found to be less cytotoxic compared to other CO-RMs when tested in RAW246.7 macrophages. Thus, iron-based carbonyls mediate pharmacological responses that are achieved through liberation of CO and the nature of the substituents in the organic ligand have a profound effect on both the rate of CO release and cytotoxicity.

INTRODUCTION

The biological function of carbon monoxide (CO) and its versatile role as a cellular messenger in mammals is presently attracting the interest of several investigators. CO regulates vessel tone and blood pressure (Wang, 1998; Motterlini et al., 1998), exerts anti-inflammatory actions (Otterbein et al., 2000), inhibits platelet aggregation (Brune and Ullrich, 1988) and acts as a signaling mediator in both apoptotic and anti-apoptotic processes (Song et al., 2004; Zhang et al., 2004). The importance of CO in biology and medicine can be better appreciated by considering the multifunctional activities of heme oxygenase-1 (HO-1), a redox-sensitive inducible enzyme that generates CO upon degradation of heme during conditions of intense oxidative or nitrosative stress (Motterlini et al., 2002c). Indeed, the induction of HO-1 appears to be central in the adaptation of tissues to a multitude of threatening conditions (Otterbein et al., 2003b). The signaling effects elicited by increased CO production in various experimental models are currently under scrutiny as they may reveal important clues about the cytoprotective nature of the HO-1 pathway. In line with this concept, recent studies have reported that low concentrations of CO gas can be utilized for therapeutic purposes in the resolution of various pathological states (Otterbein et al., 2003c; Lavitrano et al., 2004; Otterbein et al., 2003a; Nakao et al., 2003). Notably, the identification and characterization of compounds that liberate CO in biological environments could offer a unique opportunity to implement the development of novel strategic approaches based on CO therapy (Motterlini et al., 2003).

Until recently, the use of CO gas administered to cellular and animal experimental models was the only available approach to mimic the physiological role of the HO-1/CO pathway. The discovery that transition metal carbonyls can act effectively as CO-releasing molecules (CO-RMs) in a number of *in vitro*, *ex vivo* and *in vivo* models demonstrated that

the delivery of CO is attainable by storing this gas in a stable chemical form (carbonyl transition metal complex), which could carry and supply CO to tissues in a more controllable fashion than achieved with CO gas (Motterlini et al., 2002b; Motterlini et al., 2003). The metal complexes, dimanganese decacarbonyl (CORM-1) and tricarbonyldichloro ruthenium(II) dimer (CORM-2), were the first to show promising CO-dependent pharmacological activities such as relaxation of blood vessels, inhibition of coronary vasoconstriction and mitigation of acute hypertension (Motterlini et al., 2002a). The findings on these two metal carbonyls, which are sparingly soluble in water, prompted the design of additional compounds with similar chemical structures and improved bioactive properties. Tricarbonylchloro(glycinato)ruthenium(II) (CORM-3), the first prototypic water-soluble CO-RM, was subsequently synthesized and our experiments confirmed that CO, rapidly liberated from this compound, exerts important biological effects including vasodilatation and hypotension (Foresti et al., 2004), cardioprotection against ischemia-reperfusion injury and myocardial infarction (Clark et al., 2003; Guo et al., 2004), prevention of organ graft rejection following heart transplantation (Clark et al., 2003), as well as inhibition of the inflammatory response in macrophages (Sawle et al., 2005). More recently, we have reported on the vasorelaxant and hypotensive properties of CORM-A1, a boron-based carbonylating agent that liberates CO in a pH-dependent manner, and that under physiological conditions, releases CO at a slower rate compared to CORM-3 (Motterlini et al., 2005b; Sandouka et al., 2006). Thus, based on the chemistry of metal carbonyls and decarbonylation reactions in aqueous solutions we have identified compounds that meet the criteria of biologically active CO carriers and proposed that CO-RMs could be developed as suitable pharmaceutical agents (Motterlini et al., 2003; Motterlini et al., 2005a). In addition, CO-RMs may be used as an expedient to identify and elucidate new mechanisms mediated by CO (Chatterjee, 2004; Taille et al., 2005; Sandouka et al., 2005) and an increasing number of reports substantiates

the feasibility of this new approach (Stanford et al., 2003; Jozkowicz et al., 2003; Xi et al., 2004; Arregui et al., 2004; Tongers et al., 2004; Rattan et al., 2004; Allanson and Reeve, 2005; Desmard et al., 2005).

Thus, the identification of new chemicals that have the propensity to liberate CO may provide important information for optimizing therapies based on the use of CO in its naked form (as a gas). Here we extend our knowledge on the bioactivity of CO-RMs in tissue and cellular systems by analyzing the profile of CO release, cytotoxicity, vasorelaxation and anti-inflammatory properties of a class of iron-containing metal carbonyls. A rationale on how the chemical structure of these new compounds can be modified to retain CO-dependent effects and reduce cellular toxicity is presented.

MATERIALS AND METHODS

Synthesis of CORM-F compounds. The chemical structure of the CO-RMs used in this study is reported in Figure 1. As shown, the common denominator of all compounds is a 2-pyrone ring which is coordinated to an irontricarbonyl complex ($Fe(CO)_3$) and has different ring substituents, such as halogens (Cl or Br) and methyl groups (Me). These irontricarbonyl complexes were synthesized according to a general procedure described previously (Fairlamb et al., 2003).

Preparation of solutions and reagents. Stock solutions of CO-RMs (in DMSO) were prepared freshly on the day of the experiments and used within 1 min from the preparation. Lipopolysaccharide (LPS - *E. Coli* serotype 026:B6) was obtained from Sigma (Poole, Dorset, UK). All other chemicals were reagent grade and obtained from Sigma unless otherwise stated.

Cell culture. Murine RAW264.7 monocyte macrophages were purchased from the European Collection of Cell Cultures (Salisbury, Wiltshire, UK) and cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 units/ml penicillin and 0.1 mg/ml streptomycin. Cultures were maintained at 37°C in a 5% CO_2 humidified atmosphere and experiments were conducted on cells at approximately 80-90% confluence as previously described (Motterlini et al., 2002a; Sawle et al., 2005). RAW264.7 macrophages were treated with increasing concentrations of CO-RMs (10-200 μM) for 24 h using 24 well plates. At the end of the incubation period, the culture medium was collected for the lactate dehydrogenase (LDH) release assay and the nitrite assay, while cells were utilized for assessing cell metabolism using the Alamar blue assay.

Determination of CO release using the myoglobin assay. The release of CO from iron-containing CO-RMs was assessed spectrophotometrically by measuring the conversion of deoxymyoglobin (deoxy-Mb) to carbonmonoxy myoglobin (MbCO) as previously reported by our group (Motterlini et al., 2002a; Clark et al., 2003; Motterlini et al., 2003). A small aliquot of concentrated CO-RM solutions (final concentration of CO-RM: 10, 20 and 40 μM) was added to 1 ml deoxy-Mb solution (final concentration of Mb: 53 μM) in phosphate buffer and changes in the Mb spectra were recorded over time. The concentration of MbCO formed was quantified by measuring the absorbance at 540 nm (extinction coefficient = 15.4 M^{-1} cm^{-1}) and plotted over time. The amount of MbCO formed is expressed in the graph as μM and the initial rate was calculated from the fitted curves. Because we used 1 ml of solution containing myoglobin, the rate of MbCO formed (and by inference the amount of CO liberated over time) is reported in the text as nmoles/min [moles = volume (liters) * M].

Cell viability and cell injury assays. Cell viability was determined in macrophages using an Alamar Blue assay kit and carried out according to the manufacturer's instructions (Serotec, Oxford, UK) as previously reported by us (Clark et al., 2000). The assay is based on the detection of metabolic activity of living cells using a redox indicator which changes from an oxidised (blue) form to a reduced (red) form. The intensity of the red colour is proportional to the metabolism of the cells, which is calculated as the difference in absorbance between 570 nm and 600 nm and expressed as a percentage of control. Lactate dehydrogenase (LDH) released in the medium was also measured and used as an index of cellular damage.

Nitrite assay. Macrophages were exposed for 24 hr to LPS (1 μg/ml) in the presence or absence of CO-RMs (10, 50 and 100 μM) and nitrite levels were determined at the end of the

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incubation. After incubation, nitrite levels were determined using the Griess method as previously described by our group (Sawle et al., 2005). This measurement is an indication of NO production following stimulation of inducible nitric oxide synthase (iNOS). Briefly, the medium from treated cells cultured in 24 well plates was removed and placed into a 96 well plate (50 µl per well). The Griess reagent was added to each well to begin the reaction, the plate was shaken for 10 min and the absorbance read at 550 nm on a Molecular Devices VERSAmax plate reader. The nitrite level in each sample was calculated from a standard curve generated with sodium nitrite (0 µM to 300 µM in cell culture medium).

Aortic ring preparation. Transverse ring sections were prepared from thoracic aortas of male adult Sprague Dawley rats (350 g) as previously described (Sammut et al., 1998; Motterlini et al., 2002a; Foresti et al., 2004). After removal of superficial fat and connective tissue, the aorta was cut into rings and mounted in organ baths containing normal Krebs Henseleit solution with the following composition (mM): (NaCl 118, KCl 4.7, KH_2PO_4 1.2, $MgSO_4.7H_2O$ 1.2, $NaHCO_3$ 22, glucose 11, K^+ EDTA 0.03, $CaCl_2$ 2.5). The bath solution was maintained at 37 °C and bubbled continuously with a mixture of 95% O2 and 5% CO2. Aortic rings were equilibrated for 60 min at a resting tension of 2 g and pre-contracted with KCl (110 mM) before specific experimental protocols were initiated. Responses were recorded isometrically via a force-transducer connected to a Power Lab recording system (AD Instruments, UK). The extent of vasorelaxation over time elicited by a single addition of CO-RMs (100 µM) was assessed in aortic rings pre-contracted with phenylephrine (1 µM) and compared with the effect produced by the vehicle (DMSO). Vasorelaxation was expressed as a reduction (in percentage) of the initial contraction produced by phenylephrine.

Statistical Analysis. Statistical analysis was performed using ANOVA combined with Bonferroni test. Differences were considered to be significant at $P < 0.05$.

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RESULTS

Release of CO from iron-containing CO-RMs

The extent and rate of CO released from the iron-containing CO-RMs was assessed by measuring the conversion of deoxymyoglobin (deoxy-Mb) to carbon monoxide myoglobin (MbCO) using a method previously described by us (Motterlini et al., 2002a; Clark et al., 2003). The absorption spectra and the amount of MbCO formed over time after reacting deoxy-Mb with CORM-F3, an iron(0)tricarbonyl-2-pyrone complex, which contains a bromide at 4-position and a methyl group at 6-positionof the 2-pyrone ring, are represented in Figures 2A and 2B, respectively. As shown, CORM-F3 gradually liberates CO over time and the calculated rate of CO release is 0.19 nmoles/min. Notably, when the bromide at the 4-position of the 2-pyrone is substituted with a chloride group as in CORM-F8, the rate of CO release is significantly decreased to 0.041 nmoles/min (Figures 3A and 3B). A further substantial decrease in the rate of CO release is observed when the methyl group at the 6-position is substituted with a hydrogen as in CORM-F7 (0.007 nmoles/min, see Figures 4A and 4B) or the halogen at the 4-position is substituted with another methyl group as in CORM-F11 (no CO release detected, see Figures 5A and 5B). These data clearly indicate the importance of the halogen group at the 4-position of the 2-pyrone ring present in both CORM-F3 and CORM-F8 in modulating the liberation of CO from these iron(0)tricarbonyl complexes.

Cytotoxicity profile of iron-containing CO-RMs

The two compounds showing CO release (CORM-F3 and CORM-F8) were also found to be less cytotoxic compared to other CO-RMs when tested in RAW246.7 macrophages. The percentage of lactate dehydrogenase (LDH) release was measured as an index of cell injury

whereas the Alamar Blue assay was used to determine the degree of cell viability after incubation of CO-RMs for 24 h. As shown in Figures 2C and 2D, cells incubated with increasing concentrations of CORM-F3 showed that this compound did not cause any detectable cytotoxicity when used at concentrations between 10 and 100 μM, whereas 200 μM CORM-F3 caused a 44 ± 3.1 % in LDH release and 21 ± 1.5 % loss in cell viability. CORM-F8, started to cause some degree of toxic effects already at 100 μM (LDH release = 6.0 ± 1.1 %), and at a higher concentration (200 μM) this compound promoted a 27 ± 1.9 % in LDH release and 44 ± 3.6 % decrease in cell viability (Figures 3C and 3D). Both CORM-F7 and CORM-F11 were more damaging to the cells since at 100 μM they already caused a significant LDH release (Figures 4C and 5C, respectively) and at 200 μM they promoted a marked decrease in cell viability (Figures 4D and 5D, respectively).

Anti-inflammatory and vasodilatory properties of CORM-F3

Since CORM-F3 showed the most promising profile in terms of CO release and cell injury, this compound was investigated further to assess its potential anti-inflammatory and vasodilatory properties. Addition of CORM-F3 (10-100 μM) to RAW264.7 macrophages, stimulated with lipopolysaccharide (LPS, 1 μg/ml), resulted in a significant and concentration-dependent decrease in nitrite production (Figure 6A). Moreover, addition of 100 μM CORM-F3 to isolated aortic rings pre-contracted with phenylephrine elicited a marked and significant vasodilatory effect (30.9 ± 1.3 %) compared to control groups treated with vehicle, DMSO (2.2 ± 1.2 %, $p<0.05$). These data suggest that CO released from CORM-F3 exerts bioactive functions that are typical of CO gas.

DISCUSSION

The development of CO-releasing molecules (CO-RMs), compounds that carry and release CO into biological systems, was initiated from the need for a tool that would mimic the biological functions of heme oxygenase-derived CO in mammals. This led to the creation of a new strategic approach for the therapeutic delivery of this gas in a controlled and safe manner (Motterlini et al., 2003). The results obtained with transition metal carbonyls such as the tricarbonyldichlororuthenium(II) dimer (CORM-2) (Motterlini et al., 2002a) and tricarbonylchloro(glycinato)ruthenium(II) (CORM-3) (Clark et al., 2003), as well as the carbonylating agent sodium boranocarbonate (CORM-A1) (Motterlini et al., 2005b), substantiated the concept that a "solid form" of CO could be utilized to simulate the pharmacological activities typical of CO gas and HO-1 induction (Motterlini et al., 2005a). From a chemical and pharmacological perspective, the discovery that the rate of CO release from CO-RMs can be finely modulated to achieve a "fast" or "slow" release and consequently allows the modification of the ensuing biological effect (Motterlini et al., 2005b; Motterlini et al., 2005a) offers a unique opportunity to better understand the mechanism of CO liberation from these compounds. This is particularly true for transition metal carbonyl complexes where the bond of the CO groups can be rendered more or less labile depending on the type of ligand coordinated to the metal center (Motterlini et al., 2003; Motterlini et al., 2005a); in addition, the different substituents can also affect the degree of toxicity and, therefore, the overall biological activity of the compound.

Here, we present data on a novel class of iron(0)tricarbonyl complexes containing a 2-pyrone motif and demonstrate that they function as CO-RMs in biological systems. There are precedents showing that 2-pyrone derivatives can be used in biological systems. Indeed, substituted 2-pyrones have been reported to inhibit proliferation of ovarian cancer cells *in vitro* and exhibited no major toxic effects when used at low micromolar concentrations (5-20 μM) in

normal cells (Fairlamb et al., 2004; Marrison et al., 2002). In the present study, complexation of the 2-pyrone with an iron(0)tricarbonyl unit serves to activate the ring system and the nature of the substitutents has a profound affect on both the rate and extent of CO release and the associated biological effects. Specifically, CORM-F3, which possesses a bromine substituent at position-4 and a methyl group at position-6 of the 2-pyrone, promotes the liberation of CO at a rate of 0.19 nmoles/min. Notably, the substitution of the bromine with a chlorine, as in CORM-F8, renders the compound less prone to release CO as the rate decreases by 4.6 fold. Additional substitutions at the 4- and 6-position with methyl groups (CORM-F11) further decrease the ability of the compound to liberate CO. Of significant interest is that the types of substituent(s) on the 2-pyrone ring not only influence the kinetics of CO release but also the extent of cytotoxicity inflicted to cells in culture. In fact, among the CO-RMs tested, CORM-F3 was the iron(0)tricarbonyl complex that caused less cell injury and, at the concentration of 100 μM, did not affect cell viability in RAW246.7 macrophages. Conceptually, these data are in line with previously reported results on ruthenium-based CO-RMs where replacement of a $Ru(CO)_3Cl$ motif from CORM-2 with a glycine to obtain CORM-3 resulted in marked reduction of the cytotoxic effects caused to smooth muscle cells in culture (Motterlini et al., 2002a; Motterlini et al., 2005a). The demonstration that iron-containing carbonyls function as CO-RMs is of some consequence since iron is a naturally occurring metal present in abundance in both structural and functional proteins, and can be easily removed and transported in the blood (*e.g.* by transferrin), whereas only traces of ruthenium can be found in biological systems (Finney and O'Halloran, 2003). Nevertheless, it is also important to emphasize that the inaccurate perception that transition metals are inherently toxic to mammals is inaccurate and needs to be revisited (Clarke, 2002) because substitution or coordination to appropriate biological ligands, as shown here and in previous reports (Clark et al., 2003; Motterlini et al., 2005a), can dramatically change the bioactive properties of the metal centre and shield from or eliminate the intrinsic toxicological

features of these specific compounds. In fact, ruthenium-based compounds are currently being developed as anticancer agents and other transition metals such as vanadium and gold have been used as central parts of molecules that have therapeutic properties in a variety of diseases (Clarke, 2002). This is an important aspect for the development of safe pharmaceuticals and it is envisaged that more effort from both synthetic organometallic, organic and medicinal chemists will substantially improve the pharmacological profile of iron and other transition metal carbonyl complexes (Motterlini et al., 2005a).

Thus, the presence of a bromine group at 4-position of the 2-pyrone ring in CORM-F3 gives rise to a relevant release of CO and a better cytotoxic outcome compared to the other CORM-F compounds. Consequently, this compound was tested further and it was found that at micromolar concentrations (10-100 μM) to exert both vasodilatory and anti-inflammatory properties in *ex-vivo* and *in vitro* systems, respectively. These findings are in agreement with previously published results from Motterlini and colleagues showing that a ruthenium-containing carbonyl (CORM-3) elicits profound vasorelaxation in isolated aortic rings (Foresti et al., 2004) and significantly reduces endotoxin-mediated nitrite production in activated macrophages (Sawle et al., 2005). The identification of CORM-F3 as a bioactive iron-containing carbonyl complex broadens the portfolio of molecules that are capable of exerting a physiological effect through the action of CO, since a wide range of CO carriers containing manganese (CORM-1), ruthenium (CORM-2 and CORM-3) and boron (CORM-A1) have been already investigated for their pharmacological properties to alleviate pathological conditions characterized by oxidative stress, acute hypertension, ischemic events and inflammatory states (Motterlini et al., 2002a; Clark et al., 2003; Foresti et al., 2004; Sawle et al., 2005; Sandouka et al., 2006; Motterlini et al., 2005a). Future work in the development of novel transition metal carbonyl complexes containing appropriate organic ligand architecture will implement the design of selective CO-RMs that can be adapted for the specific treatment of a certain disease states.

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Figure Legends

Figure 1. Chemical structure of irontricarbonyl-2-pyrone complexes. The difference in these compounds is the position of the substituents in the 2-pyrone ring with 4-bromo and 6-methyl in CORM-F3, 4-chloro and 6-methyl in CORM-F8, 4-chloro and 6-hydrogen in CORM-F7 and 4,6-dimethyl in CORM-F11.

Figure 2. Profile of CO release from CORM-F3 and effects on cell viability in murine macrophages. (**A**) Absorption spectra of carbon monoxy myoglobin (MbCO) formed over time after reaction of CORM-F3 (40 μM) with deoxy-myoglobin (deoxy-Mb) in phosphate buffer (pH=7.4) (see Materials and Methods). As a positive control, the spectrum of MbCO formed by bubbling deoxyMb with CO gas for 5 minutes is shown (saturated MbCO). (**B**) Quantification of MbCO formed over time after reaction of deoxy-Mb with CORM-F3 (40 μM). (**C**) Percentage of LDH released from RAW246.7 macrophages after 24 h incubation with increasing concentrations (10-200 μM) of CORM-F3. Data are expressed as a percentage of the total LDH released after treatment of cells with triton (1%). (**D**) Cell viability was assessed 24 hr after exposure of macrophages to CORM-F3 (10-200 μM) using the Alamar Blue assay (see Materials and Methods). Results represent the mean ± S.E.M. of 6 independent experiments. * indicates $P < 0.05$ vs. control (0 μM).

Figure 3. Profile of CO release from CORM-F8 and effects on cell viability in murine macrophages. (**A**) Absorption spectra of carbon monoxy myoglobin (MbCO) formed over time after reaction of CORM-F8 (40 μM) with deoxy-myoglobin (deoxy-Mb) in phosphate buffer (pH=7.4) (see Materials and Methods). (**B**) Quantification of MbCO formed over time after reaction of deoxy-Mb with CORM-F8 (40 μM). (**C**) Percentage of LDH released from RAW246.7 macrophages after 24 h incubation with increasing concentrations (10-200 μM) of

CORM-F8. Data are expressed as a percentage of the total LDH released after treatment of cells with triton (1%). (**D**) Cell viability was assessed 24 hr after exposure of macrophages to CORM-F8 (10-200 μM) using the Alamar Blue assay (see Materials and Methods). Results represent the mean ± S.E.M. of 6 independent experiments. * indicates $P < 0.05$ vs. control (0 μM).

Figure 4. Profile of CO release from CORM-F7 and effects on cell viability in murine macrophages. (**A**) Absorption spectra of carbon monoxy myoglobin (MbCO) formed over time after reaction of CORM-F7 (40 μM) with deoxy-myoglobin (deoxy-Mb) in phosphate buffer (pH=7.4) (see Materials and Methods). (**B**) Quantification of MbCO formed over time after reaction of deoxy-Mb with CORM-F7 (40 μM). (**C**) Percentage of LDH released from RAW246.7 macrophages after 24 h incubation with increasing concentrations (10-200 μM) of CORM-F7. Data are expressed as a percentage of the total LDH released after treatment of cells with triton (1%). (**D**) Cell viability was assessed 24 hr after exposure of macrophages to CORM-F7 (10-200 μM) using the Alamar Blue assay (see Materials and Methods). Results represent the mean ± S.E.M. of 6 independent experiments. * indicates $P < 0.05$ vs. control (0 μM).

Figure 5. Profile of CO release from CORM-F11 and effects on cell viability in murine macrophages. (**A**) Absorption spectra of carbon monoxy myoglobin (MbCO) formed over time after reaction of CORM-F11 (40 μM) with deoxy-myoglobin (deoxy-Mb) in phosphate buffer (pH=7.4) (see Materials and Methods). (**B**) Quantification of MbCO formed over time after reaction of deoxy-Mb with CORM-F11 (40 μM). (**C**) Percentage of LDH released from RAW246.7 macrophages after 24 h incubation with increasing concentrations (10-200 μM) of CORM-F11. Data are expressed as a percentage of the total LDH released after treatment of

cells with triton (1%). (**D**) Cell viability was assessed 24 hr after exposure of macrophages to CORM-F11 (10-200 μM) using the Alamar Blue assay (see Materials and Methods). Results represent the mean ± S.E.M. of 6 independent experiments. * indicates $P < 0.05$ vs. control (0 μM).

Figure 6. Anti-inflammatory and vasodilatory activities of CORM-F3. (**A**) RAW246.7 macrophages were incubated for 24 h with 1 μg/ml lipopolysaccharide (LPS) alone or in the presence of increasing concentrations of CORM-F3 (10-100 μM) and nitrite levels, an index of NO production, were measured as described in Materials and Methods. Data represent the mean ± S.E.M. of 6 independent experiments. * indicates $P < 0.05$ vs. control (white bar); † indicates $P < 0.05$ vs. LPS alone (grey bar). (**B**) Isolated aortic rings were pre-contracted with phenylephrine (1 μM) and CORM-F3 (100 μM) was then added as reported in Materials and Methods. The percentage of relaxation mediated by CORM-F3 was compared to the ones elicited by the vehicle (DMSO), which was used as a control group. Data represent the mean ± S.E.M. of 5 independent experiments. * indicates $P < 0.05$ vs. control (CON).

Figure 1

CORM-F3

CORM-F7

CORM-F8

CORM-F11

Figure 2

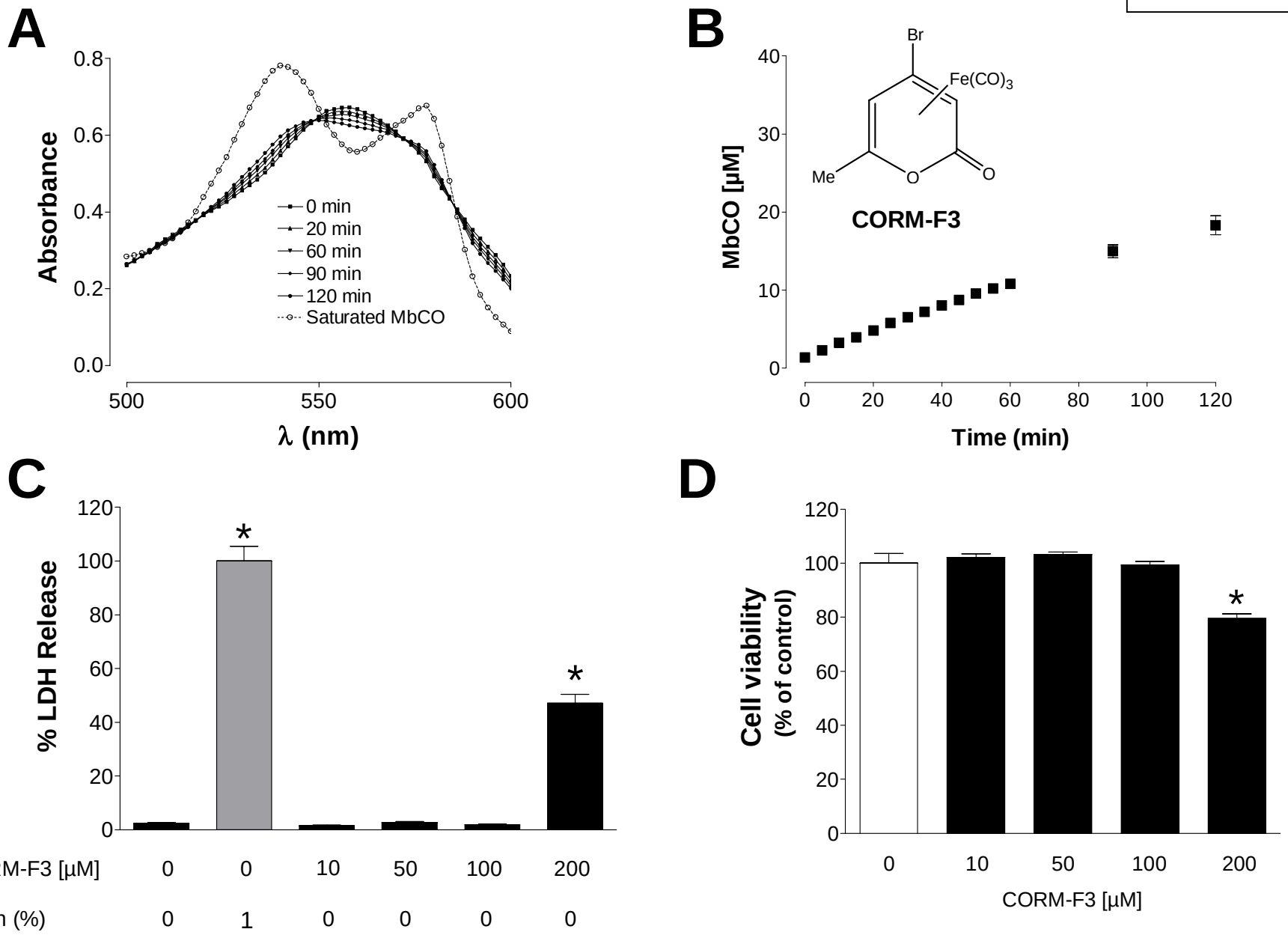


Figure 3

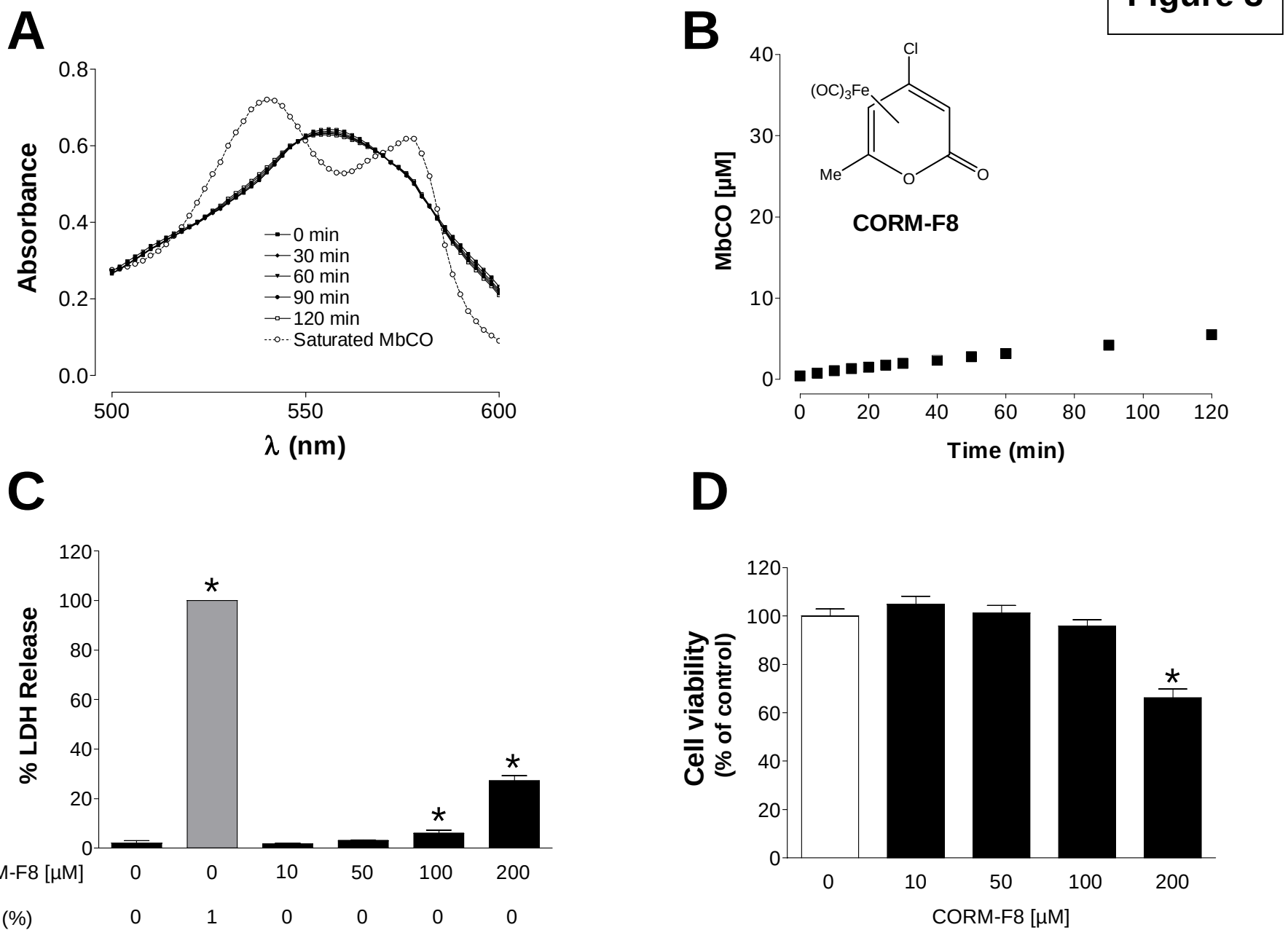


Figure 4

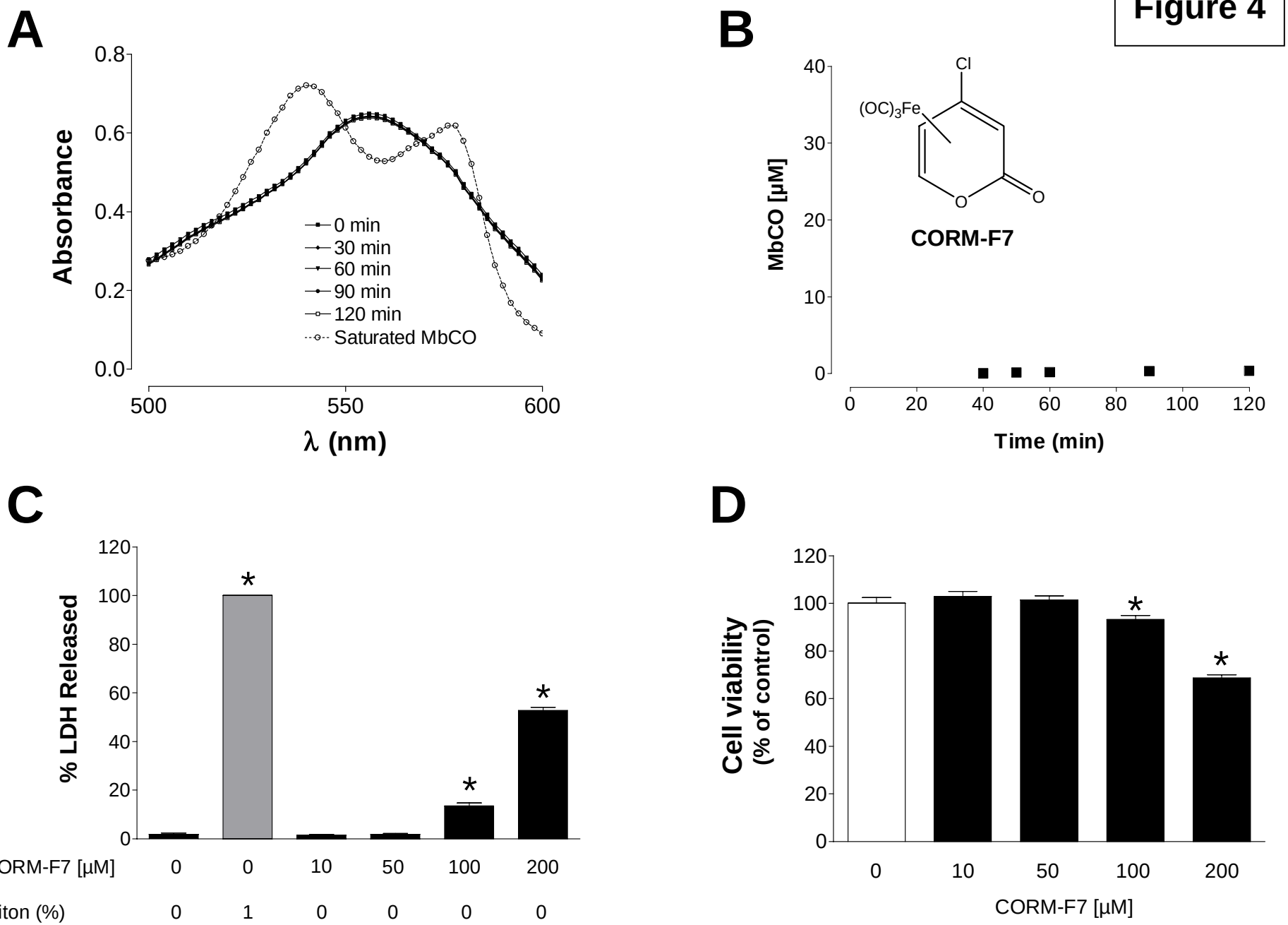


Figure 5

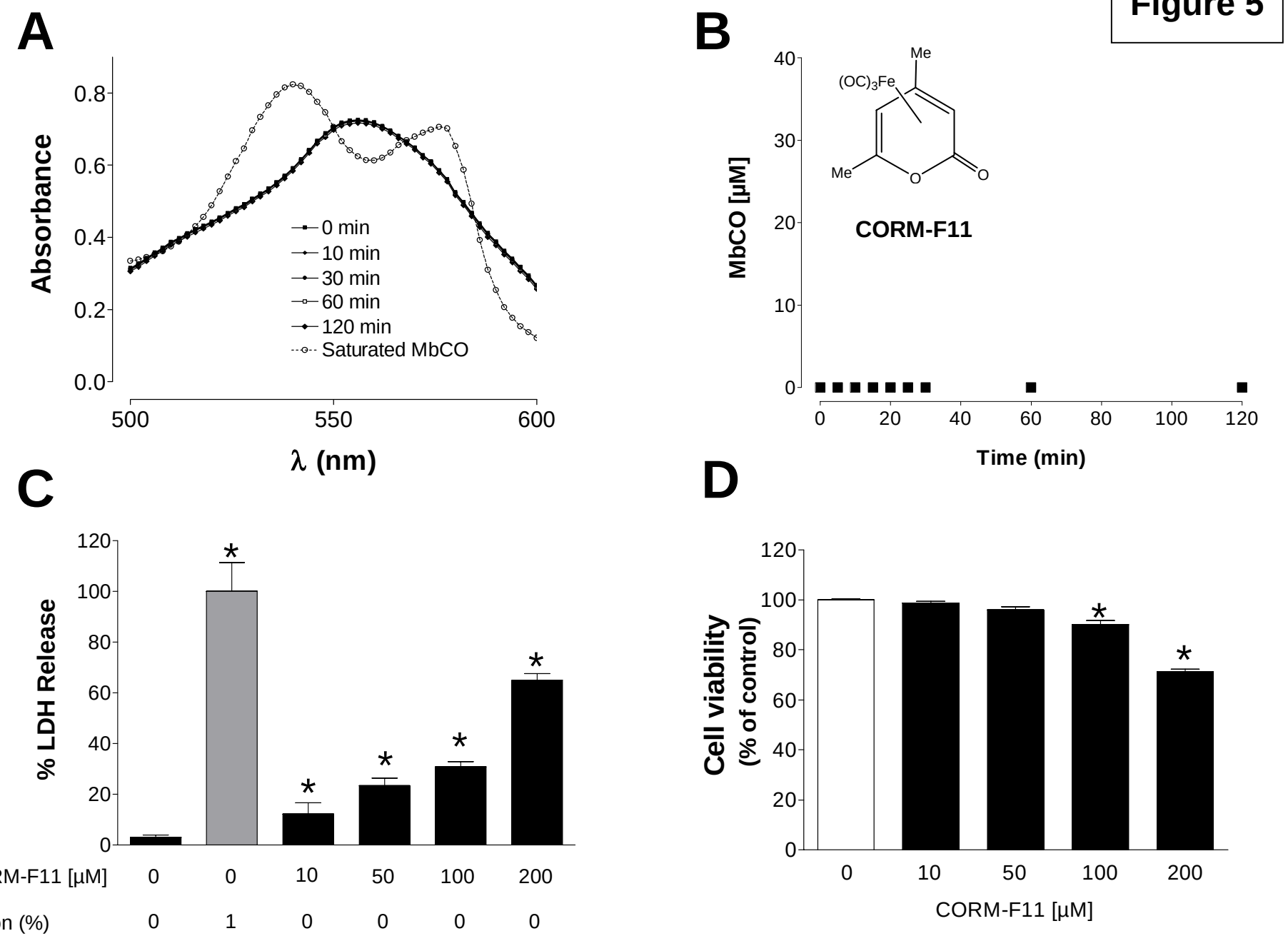
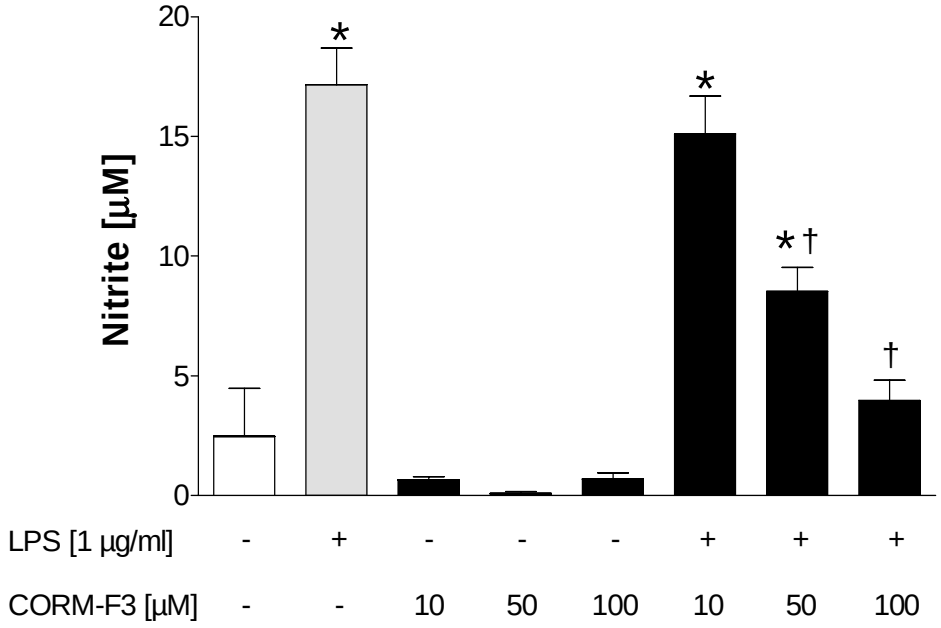


Figure 6

A



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