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cis-trans-[Re(II)(CO)2Br2L2]n complexes

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CO Releasing Properties and Cytoprotective Effect of \textit{cis-trans}- [Re^{II}(CO)_{2}Br_{2}L_{2}]\textsuperscript{n} Complexes

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\textit{Re}^{II}\textsuperscript{-}based CO-RMs

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Abstract

The carbon monoxide (CO) releasing properties of a series of Rhenium(II)-based complexes of general formula \(\text{cis-trans-}[\text{Re}^\text{II}(\text{CO})_2\text{Br}_2\text{L}_2]_n\) and \(\text{cis-trans-}[\text{Re}^\text{II}(\text{CO})_2\text{Br}_2\text{N}\cap\text{N}]\) (where \(\text{L} = \text{monodentate}\) and \(\text{N}\cap\text{N} = \text{bidentate ligand}\)) are reported. Complexes evaluated in this study were obtained from direct ligand substitution reactions of the \(\text{cis-}[\text{Re}^\text{II}(\text{CO})_2\text{Br}_4]^{2-}\) synthon (2) recently described.\(^1\) All molecules have been fully characterized. The solid state structures of the \(\text{cis-trans-}[\text{Re}^\text{II}(\text{CO})_2\text{Br}_2\text{L}_2]_n\) (with \(\text{L} = \text{N-methylimidazole (3), benzimidazole (4) and 4-picoline (5)}\)) and the \(\text{cis-trans-}[\text{Re}^\text{II}(\text{CO})_2\text{Br}_2\text{N}\cap\text{N}]\) (with \(\text{N}\cap\text{N} = 4,4'\text{-dimedthyl-2,2'-bipyridine (8) and 2,2' \text{-dipyridylamine (11)}\}) adducts are presented. The release of CO from the \(\text{cis-trans-}[\text{Re}^\text{II}(\text{CO})_2\text{Br}_2\text{L}_2]_n\) complexes was assessed spectrophotometrically by measuring the conversion of deoxymyoglobin (Mb) to carbonmonoxy myoglobin (MbCO). Only compounds bearing monodentate ligands were found to liberate CO. The rate of CO release was found to be pH-dependent with half lives (\(t_{1/2}\)) under physiological conditions (25 °C, 0.1 M phosphate buffer, pH 7.4) varying from ca. 6 to 43 min. At lower pH values the time required to fully saturate Mb with CO liberated from the metal complexes gradually decreased. Complex 2 and the \(\text{cis-trans-}[\text{Re}^\text{II}(\text{CO})_2\text{Br}_2\text{Im}_2]\) adduct (with \(\text{Im} = \text{imidazole (6)}\)) show a protective action against “ischemia-reperfusion” stress of neonatal rat ventricular cardiomyocytes (NRCs) in culture.
Introduction.

In recent years carbon monoxide (CO) has been acknowledged as a fundamental (small-molecule) gaseous messenger in humans. The endogenous production of CO is associated with the heme metabolic pathway and the action of a family of enzymes known as heme oxygenases (HO’s). HO’s catalyze the oxidation of heme to biliverdin resulting in the production of free iron and CO (scheme 1). The distribution of HO’s is strictly tissue specific and HO-derived CO carry on a number of local physiological functions. Carbon monoxide acts as a signaling molecule in cytoprotective signaling cascade activated by various stress stimuli. Similar to nitric monoxide, it plays a fundamental role in the circulatory system by improving vasorelaxation and cardiac blood supply. CO also appears to influence neurotransmission in the hypothalamic-pituitary-adrenal axis like NO and there is evidence that CO might influence our circadian rhythm (the 24-hour cycle in the biochemical, physiological or behavioural processes) by interacting with NPAS2 (the human “clock” protein). Furthermore, carbon monoxide was reported to attenuate arteriosclerotic lesions associated with chronic graft rejection.

Scheme 1. Heme-catalyzed endogenous production of CO.
As the importance of CO is increasingly recognized, there is a growing interest in the pharmacological and medicinal applications of carbon monoxide.\textsuperscript{16-22} Direct inhalation of CO has been viewed as a novel therapeutic approach but reports on tolerance to CO exposure are contradictory.\textsuperscript{4, 23, 24} There is a very delicate balance between CO induced tissue hypoxia and therapeutic effects. Furthermore, the direct use of the gaseous molecule poses problems associated with its safe handling and delivery to specific target sites in a controlled and measurable fashion. An alternative approach to the administration of carbon monoxide is the use of CO-releasing molecules (CO-RMs). An obvious choice for CO-RMs are transition metal carbonyl complexes with one or more CO ligands.

Several complexes have been evaluated to date,\textsuperscript{19, 25-31} but the pioneering work of Motterlini and Mann has resulted in the discovery of the fac-[RuCl(glycinato)(CO)$_3$] complex (CORM-3) as the most promising compound for the CO release \textit{in vivo}.\textsuperscript{5, 32-34} The chemistry and therapeutic effects of CORM-3 are well documented. The Ru complex releases 1 mol of CO within 10 min after dissolving in water and it has been shown that CORM-3 significantly reduces blood pressure \textit{in vivo} and relaxes precontracted aortic rings \textit{in vitro}.\textsuperscript{5, 32-34} The cardioprotective functions of CORM-3 have also been described.\textsuperscript{5}

We have recently reported that the one electron reduction of \textit{cis}-[Re$^{\text{III}}$(CO)$_2$Br$_4$] (1) results in the stable Re$^{\text{II}}$ synthon \textit{cis}-[Re$^{\text{II}}$(CO)$_2$Br$_4$]$^{2-}$ (2).\textsuperscript{1} While the chemistry of 1 is largely underdeveloped, we have demonstrated that 2 reacts well with pyridine-type ligands.\textsuperscript{1} The chemistry of rhenium complexes in the oxidation state +II ($d^5$ configuration) is rare if compared to the other oxidation states of this metal ion. However, the related paramagnetism together with the open shell electronic configuration makes such complexes interesting for various applications in medicine, magnetochemistry or catalysis. Here we show that 2 and several other [Re$^{\text{II}}$(CO)$_2$Br$_4$L$_2$] complexes (where L = monodentate ligand) act as CO-releasing molecules and that under physiologically relevant conditions the rate of CO release is comparable to that of CORM-3. The complexes presented in this study represent a first example of metal-based CO-RMs in which the central metal ion is not found in a $d^6$, $d^8$ or $d^{10}$ configuration. In our view, the open shell $d^5$ configuration of the Re system herein described represents an advantage over the more robust $d^6$, $d^8$ or $d^{10}$ systems for which physical stimuli (e.g. UV radiation) are often needed in order
to elicit dissociation of carbon monoxide from the metal core.\textsuperscript{19, 25, 26, 30, 31, 35, 36} We further show that selected \textit{cis-trans-}[Re\textsuperscript{n}(CO)\textsubscript{2}Br\textsubscript{2}L\textsubscript{2}]\textsuperscript{n} complexes induce a protective action against “ischemia-reperfusion” stress of neonatal rat ventricular cardiomyocytes (NRCs) in culture.

**Experimental Section**

Chemicals and solvents were purchased from standard sources. Anhydrous dimethoxyethane (DME) was purchased from Aldrich and degassed prior to use. All other solvents were distilled and degassed prior to use. (NEt\textsubscript{4})\textsubscript{2}[ReBr\textsubscript{3}(CO)\textsubscript{3}], compounds 1, 2, 6, 7, 12 and 13 were synthesized as previously described.\textsuperscript{1, 37, 38} All other complexes were synthesized under nitrogen with standard techniques. Elemental analyses (EA) were performed on a Leco CHNS-932 elemental analyser. IR spectra were recorded in a PerkinElmer Spectrum BX FT-IR spectrometer. UV-visible spectra were recorded on a Varian Carry 50 Scan spectrometer. Cyclic voltammetry experiments were performed and data analyzed with a Metrohm 757 VA Computertrace package version 2.0. For the determination of rhenium uptake by the cells, cultured NRCs (10\textsuperscript{6} cells/petri-dish, 5 days after isolation) were incubated for 5, 10, 30, 60 and 120 min in the presence of 30 µM of the rhenium complexes. The medium was collected for analysis and the cells were triple-washed free from the extracellular ReII with phosphate buffer and wet-burned in 200 µl concentrated metal free (TraceSelect) HNO\textsubscript{3} (Fluka). Cell culture medium samples were acidified with 0.2 M HNO\textsubscript{3}. The amount of rhenium in the cells and the medium samples were analysed with flame atomic absorption spectroscopy (F-AAS) using an AA240FS spectrometer (Varian AG, Zug, Switzerland). Crystallographic data were collected at 183(2) K with Mo K\textsubscript{a} radiation (\(\lambda = 0.7107 \text{ Å}\)) that was monochromated with help of a graphite on an Oxford Diffraction Xcalibur system with a Ruby detector. Suitable crystals were covered with oil (Infineum V8512), mounted on top of a glass fiber and immediately transferred to the diffractometer. The program suite CrysAlis\textsuperscript{pro} was used for data collection, semi-empirical absorption correction and data reduction.\textsuperscript{39} Structures were solved with direct methods using SIR97\textsuperscript{40} and were refined by full-matrix least-squares methods on F\textsuperscript{2} with SHELXL-97.\textsuperscript{41} The structures were checked for higher symmetry with help of the program Platon.\textsuperscript{42}
Detection of CO release using the myoglobin assay.

The release of CO from the \( \text{cis-trans-}[\text{Re}^{II}(\text{CO})_2\text{Br}_2\text{L}_2]^n \) complexes was assessed spectrophotometrically by measuring the conversion of deoxymyoglobin (Mb) to carbonmonoxymyoglobin (MbCO) as previously reported.\(^5, 19, 21\) A small aliquot of a freshly prepared concentrated solution of the selected Re\(^{II}\) complex (in methanol 2, in DMSO all other complexes) was added to 1 ml of the Mb solution in phosphate buffer prepared at different pHs (7.4, 6.3 and 5.8; final concentrations: 30 \( \mu \)M for Re\(^{II}\) complex and Mb). Changes in the Mb spectra were recorded over time at 25 °C. The methanol or DMSO content of the solution never exceeded 0.5%. The amount of MbCO formed was determined by measuring the absorbance at 540 nm (extinction coefficient = 15.4 M\(^{-1}\) cm\(^{-1}\)). The MbCO concentration was plotted over time and directly related to the equivalents of CO released from the compounds. The half-life of CO release from the Re\(^{II}\) complexes at different pH’s was then estimated from the graphs. Control experiments were run under identical conditions but without addition of the metal complexes. All manipulations were performed under a pure N\(_2\) atmosphere in a wet box.

In Vitro Model of “Ischemia-Reperfusion” Stress of Cardiomyocyte.

Animal keeping, breeding and experimentation were reviewed, approved, and carried out in accordance with the Swiss animal protection laws and institutional guidelines. Neonatal rat ventricular cardiomyocytes (NRCs) were isolated from Wistar rat pups (postnatal day 3-4) as described elsewhere.\(^43\) The cells were kept in culture for 4 days and then used for experiments. The culture medium was exchanges for a glucose-free medium titrated to pH 6.0 and the cells were placed into a hypoxic incubator and exposed to 1% O\(_2\), 5% CO\(_2\) and 94% N\(_2\) for 16 h to simulate ischemic conditions. Thereafter, reperfusion was simulated by replacing the medium for one containing 5 mM glucose titrated to pH 7.4 and oxygen concentration in the incubator was increased to 20%. This protocol will be referred to as “ischemia-reperfusion” stress. The tested compounds were freshly dissolved in DMSO (30
mM stock solutions) before the experiment and added to obtain a final concentration of 30 µM immediately after the incubation medium was been changed (at the “onset of reperfusion”). A corresponding amount of DMSO was added to the control non-treated cells. Following 12 h of “reperfusion” the cells were stained with a mixture of propidium iodide (PI, 0.5 µg/ml) and Hoechst 33342 reagent (0.1 µg/ml) in phosphate buffer for 10 min and the number of Hoechst 33342- and PI-positive nuclei was assessed using fluorescent microscope equipped with DAPI (409 nm excitation /450 nm emission) and Cy3 (550 nm excitation /570 nm emission) filters and CCD chamber. The images obtained for the PI and Hoechst 33342 fluorescent signals were then analysed using MCID image analysis software pack. The total number of cells (Hoechst 33342-positive) and the total number of dead cells (PI-positive) was obtained. NRCs were plated (~ 1 million cells per dish) and grown in petri dishes of 4.5 cm² area. After 4 days most cells were confluent and contracted rhythmically in concert. Counted were 5 areas in each dish at pre-fixed positions amounting to a total of 400 cells counted per dish. The statistical analysis was performed using normality test with the following non-paired Student’s test using GraphPad Prism 5 (GraphPad Software).

**Synthesis of [ReBr₂(CO)₂(MeIm)] (3).** 100 mg of 2 (0.122 mmol) were suspended in 15 ml of DME and 32 mg of N-methylimidazole (MeIm, 3 eq.) were added. The mixture was heated to 60 °C for 3.5 h and stopped when the red suspension had become a yellow solution and a yellow precipitate had formed. The mixture was filtered while still hot. A bright yellow solid of 3 was collected, dried in vacuo and recrystallized from a CH₂Cl₂/hexane mixture giving dark red crystals. Yield: 49 mg, 71%. Anal. Calc. for C₁₀H₁₂Br₂N₄O₂Re (566.2): C 21.21%, H 2.14%, N 9.89%. Found: C 21.89%, H 2.32%, N 9.64%. I.r. (solid state, KBr, cm⁻¹): ν CO 1982, 1825. Single crystals suitable for x-ray diffraction were grown by slow diffusion of hexane into a CH₂Cl₂ solution of the compound.

**Synthesis of [ReBr₂(CO)₂(BzIm)] (4).** 100 mg of 2 (0.122 mmol) were suspended in 15 ml of DME and 46 mg of benzimidazole (BzIm, 3 eq.) were added. The mixture was heated to 60 °C for 3.5 h and stopped when the red suspension had become a yellow solution and a yellow precipitate had formed. The mixture was filtered while still hot. A bright yellow solid of 4 was collected and dried in vacuo. The
crude product was purified by loading a CH$_3$OH solution of 4 onto a chromatofix C18 filter. This was washed with a 15% CH$_3$OH solution in water and then extracted with CH$_3$OH. Yield: 39 mg, 50%. Anal. Calc. for C$_{16}$H$_{12}$Br$_2$N$_4$O$_2$Re (638.3): C 30.11%, H 1.89%, N 8.78%. Found: C 29.99%, H 1.82%, N 8.47%. I.r. (solid state, KBr, cm$^{-1}$): $\nu_{CO}$ 1992, 1833.

Single crystals suitable for x-ray diffraction were grown by slow diffusion of hexane into a CH$_2$Cl$_2$ solution of the compound giving dark red crystals.

**Synthesis of [ReBr$_2$(CO)$_2$(4-pic)$_2$] (5).** 100 mg of 2 (0.122 mmol) were suspended in 10 ml of DME and ca. 10 eq. 4-methylpyridine (4-pic, 100 µl) were added. After stirring overnight, a brownish precipitate was filtered off and dried in vacuo. The crude product was recrystallized from a CH$_2$Cl$_2$/hexane mixture giving dark red crystals of 5 which were found suitable for x-ray diffraction. Yield: 50 mg, 70%. Anal. Calc. for C$_{14}$H$_{12}$Br$_2$N$_2$O$_2$Re (588.3): C 28.58%, H 2.40%, N 4.76%. Found: C 28.39%, H 2.69%, N 4.88%. I.r. (solid state, KBr, cm$^{-1}$): $\nu_{CO}$ 1992, 1830.

**Synthesis of [ReBr$_2$(CO)$_2$(4,4-Mebipy)] (8).** 100 mg of 2 (0.122 mmol) were suspended in 15 ml of DME and 28 mg of 4,4'-dimethyl-2,2'-bipyridine (4,4'-Mebipy, 1.25 eq.) were added. The suspension was heated to 60 °C for 3.5 h and stopped when the red suspension had become a deep red solution. The mixture was allowed to cool to room temperature (RT) and then filtered. The dark red solution was dried in vacuo leaving 8 as a dark red solid. Yield: 70 mg, quantitative. Anal. Calc. for C$_{14}$H$_{12}$Br$_2$N$_2$O$_2$Re (586.3): C 28.68%, H 2.04%, N 4.78%. Found: C 29.04%, H 2.52%, N 4.23%. I.r. (solid state, KBr, cm$^{-1}$): $\nu_{CO}$ 1996, 1813. Single crystals suitable for x-ray diffraction were grown by slow diffusion of hexane into a CH$_2$Cl$_2$ solution of the compound giving dark red crystals.

**Synthesis of [ReBr$_2$(CO)$_2$(phd)] (9).** 100 mg of 2 (0.122 mmol) were suspended in 15 ml DME and 93 mg of 1,10-phenanthroline-5,6-dione (phd, 1.25 eq.) were added. The suspension was heated to 60 °C for 3.5 h and stopped when the red suspension had become a deep red solution. The mixture was allowed to cool to RT and then filtered. The dark red solution was dried in vacuo leaving 9 as a dark red solid. The crude product was recrystallized from a CH$_2$Cl$_2$/hexane mixture. Yield: 60 mg, 81%. Anal.
Calc. for $\text{C}_{16}\text{H}_8\text{Br}_2\text{N}_2\text{O}_4\text{Re}$ (612.2): C 27.47%, H 0.99%, N 4.58%. Found: C 28.11%, H 1.13%, N 4.38%. I.r. (solid state, KBr, cm$^{-1}$): $\nu_{C=O}$ 1996, 1859.

**Synthesis of $[\text{ReBr}_2(\text{CO})_2(4,7\text{-Mephen})]$ (10).** 100 mg of 2 (0.122 mmol) were suspended in 15 ml DME and 26 mg (1.25 eq.) of 4,7-dimethyl-1,10-phenanthroline (4,7-Mephen) were added. The suspension was heated to 60 °C for 3.5 h and stopped when the red suspension had become a deep red solution. The mixture was allowed to cool to RT and then filtered. The dark red solution was dried in vacuo leaving 10 as a dark red solid. The crude product was recrystallized from a $\text{CH}_2\text{Cl}_2$/hexane mixture. Yield: 70 mg, quantitative. Anal. Calc. for $\text{C}_{16}\text{H}_{12}\text{Br}_2\text{N}_2\text{O}_2\text{Re}$ (610.3): C 31.49%, H 1.98%, N 4.59%. Found: C 31.67%, H 1.53%, N 4.76%. I.r. (solid state, KBr, cm$^{-1}$): $\nu_{C=O}$ 1997, 1844.

**Synthesis of $[\text{ReBr}_2(\text{CO})_2(2,2\text{-dipy-NH})]$ (11).** 100 mg of 2 (0.122 mmol) were suspended in 15 ml DME and 21 mg (1.25 eq.) of 2,2-dipyridylamine (2,2-dipy-NH) were added. The suspension was stirred at RT for 12 h and then filtered. The light orange residue was identified as a mixture of unreacted starting materials and $\text{Et}_4\text{NBr}$. The remaining solution was dried in vacuo leaving a dark yellow/green oil. This was dissolved in $\text{CHCl}_3$ (1 ml) and hexane was slowly added. Compound 11 was then collected by filtration as a dark red solid. Yield: 42 mg, 60%. Anal. Calc. for $\text{C}_{12}\text{H}_9\text{Br}_2\text{N}_3\text{O}_2\text{Re}$ (573.2): C 25.14%, H 1.58%, N 7.33%. Found: C 24.89%, H 1.58%, N 7.25%. I.r. (solid state, KBr, cm$^{-1}$): $\nu_{C=O}$ 1992, 1837. Single crystals suitable for x-ray diffraction were grown by slow diffusion of ether into an acetone solution of the compound giving dark red crystals.
Results and Discussion.

Synthesis of Complexes.

A summary of the reactions of 2 with different mono-, bi- and tridentate ligands is given in scheme 2. As we have previously shown, complex 2 reacts well with monodentate nitrogen containing aromatic ligands. Thus the reaction with N-methylimidazole (MeIm), 4-picoline (4-pic) or benzimidazole (BzIm) gave the corresponding cis-trans-[Re\textsuperscript{II}(CO)\textsubscript{2}Br\textsubscript{2}L\textsubscript{2}] complex (3 with L = MeIm, 4 with L = BzIm, 5 with L = 4-pic). The direct substitution reaction of 2 with this type of ligands allowed to isolate 3, 4 and 5 in a short time and in good isolated yields (>50%). With the exception of 3, which was found to be hygroscopic and slowly decomposed over time, 4 and 5 appear indefinitely stable as solids under aerobic conditions.

Scheme 2. Synthesis of Re\textsuperscript{II} complexes evaluated in this study. All compounds were obtained via substitution reactions from the common cis-[Re\textsuperscript{II}(CO)\textsubscript{2}Br\textsubscript{4}]\textsuperscript{2+} (2) precursor.
Complexes 3-5 are soluble in common organic solvents like methanol, CH\textsubscript{2}Cl\textsubscript{2}, acetonitrile or DMSO, and, even under aerobic conditions, were stable for days. X-ray quality crystals of 3, 4 and 5 could be grown from CH\textsubscript{2}Cl\textsubscript{2} and hexane and the x-ray structures of these are given in figure 1. In all cases the ligands L substituted the two bromides *trans* to the CO’s giving [Re\textsuperscript{II}(CO)\textsubscript{2}Br\textsubscript{2}L\textsubscript{2}] complexes with a *cis-trans-cis* arrangement of the ligands. Since the *cis* arrangement of the two COs is the only structural compulsion, other isomers are expected as well but were not observed. We propose that the *trans*-labilizing effect of the CO ligands may account for this selectivity.

A common feature of the complexes 3-5 is the bending of the two *trans* bromides away from the CO’s with an average Br-Re-Br angle of 171.5°. Bond lengths and angles of 3 and 5 were found similar to the related imidazole and pyridine complexes *cis-trans*-\([\text{Re}^{\text{II}}(\text{CO})_{2}\text{Br}_{2}(\text{Im})_{2}]\) (6) and *cis-trans*-\([\text{Re}^{\text{II}}(\text{CO})_{2}\text{Br}_{2}(\text{py})_{2}]\) (7) previously described.\textsuperscript{1} When reacted with other heterocyclic ligands like tetrahydrofuran or thiophene, 2 was always recovered unreacted indicating that, under similar experimental conditions, these ligands are not strong enough to replace the bound bromides even if present in large excess.

While the MeIm and 4-pic ligands were mainly selected in order to provide data for comparison to the Im and py adducts 6 and 7, BzIm was also selected as a simple model for the interaction of 2 with purine bases. In 4 the two BzIm ligands are found in a head-to-tail (HT) conformation. Our previous results of the interaction of DNA bases with the fac-\([\text{Re}^{\text{I}}(\text{CO})_{3}(\text{H}_{2}\text{O})_{3}]^{+}\) complex have shown that neither hydrogen bonding interactions nor steric factors are important in determining the orientation of the bases around the Re\textsuperscript{I} core.\textsuperscript{44-48} Theoretical calculations indicated that the energy difference between rotamers is only ca. 5 Kcal/mol and we have shown that the different head-to-head (HH) or HT conformers observed in the solid state structures of the adducts are a result of packing effects.\textsuperscript{45} Although any comments on a possible interaction of 2 with DNA is premature, we expect also the BzIm and other purine-type ligands to be able to rotate around *cis*-\([\text{Re}(\text{CO})_{2}]^{2+}\) core. Thus, we suggest that HT conformer observed in the solid state structure of 4 is unlikely to be the predominant form in solution.
Figure 1. ORTEP presentation of complexes 3 (A), 4 (B) and 5 (C). Selected bond lengths (Å) and angles (°) for 3 are: Re(1)-Br(1) 2.5301(5); Re(1)-Br(2) 2.5261(5); Re(1)-N(1) 2.183(3); Re(1)-N(3) 2.172(3); Re(1)-C(1) 1.931(5); Re(1)-C(1) 1.930(4); Br(1)-Re(1)-Br(2) 171.802(16); for 4: Re(1)-Br(1) 2.5194(4); Re(1)-N(1) 2.186(3); Re(1)-C(1) 1.920(4); Br(1)-Re(1)-Br(1) 168.67(2); and for 5: Re(1)-Br(1) 2.5146(3); Re(1)-N(1) 2.207(2); Re(1)-C(1) 1.957(4); Br(1)-Re(1)-Br(1) 173.867(17). Ellipsoids are drawn at 50% probability. Hydrogen atoms are omitted for clarity. Atoms labeled with underscore ( _ ) are symmetry generated.
Reaction of 2 with bidentate bipyridine or phenantroline type ligands, proceeded smoothly with no reduction of the Re$^{II}$ center. Thus 4,4'-dimethyl-2,2'-bipyridine (4,4'-Mebipy), 1,10-phenantroline-5,6-dione (phd), 4,7-dimethyl-1,10-phenantroline (4,7-Mephen) and 2,2'-dipyridylamine (2,2'-dipy-NH) gave the corresponding cis-trans-[Re$^{II}$(CO)$_2$Br$_2$N∩N] complexes (8 with N∩N = 4,4'-Mebipy, 9 with N∩N = phd, 10 with N∩N = 4,7-Mephen and 11 with N∩N = 2,2'-dipy-NH; see scheme 2).

Table 1. Spectroscopic and electrochemical properties of complexes 2-13.

<table>
<thead>
<tr>
<th>Complex</th>
<th>ν$_{CO}$ (cm$^{-1}$)$^a$</th>
<th>E$_{1/2}$ (mV)$^b$</th>
<th>λ$_{max}$ (nm)$^c$</th>
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<tr>
<td>[Re(CO)$_2$Br$_4$]$^2$ (2)$^1$</td>
<td>1972, 1796</td>
<td>-120</td>
<td>412</td>
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<tr>
<td>[Re(CO)$_2$Br$_2$(MeIm)$_2$] (3)</td>
<td>1982, 1825</td>
<td>dec.</td>
<td>418</td>
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<tr>
<td>[Re(CO)$_2$Br$_2$(BzIm)$_2$] (4)</td>
<td>1992, 1833</td>
<td>-195</td>
<td>418</td>
</tr>
<tr>
<td>[Re(CO)$_2$Br$_2$(4-pic)$_2$] (5)</td>
<td>1992, 1830</td>
<td>-90</td>
<td>423</td>
</tr>
<tr>
<td>[Re(CO)$_2$Br$_2$(Im)$_2$] (6)$^1$</td>
<td>1988, 1826</td>
<td>dec.</td>
<td>418</td>
</tr>
<tr>
<td>[Re(CO)$_2$Br$_2$(py)$_2$] (7)$^1$</td>
<td>1990, 1825</td>
<td>-76</td>
<td>425</td>
</tr>
<tr>
<td>[Re(CO)$_2$Br$_2$(4,4'-Mebipy)] (8)</td>
<td>1996, 1813</td>
<td>-104</td>
<td>421</td>
</tr>
<tr>
<td>[Re(CO)$_2$Br$_2$(phd)] (9)</td>
<td>1996, 1859</td>
<td></td>
<td>428</td>
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<tr>
<td>[Re(CO)$_2$Br$_2$(4,7-Mephen)] (10)</td>
<td>1997, 1844</td>
<td>-92</td>
<td>423</td>
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<tr>
<td>[Re(CO)$_2$Br$_2$(2,2'-dipy-NH)] (11)</td>
<td>1992, 1837</td>
<td>-86</td>
<td>427</td>
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<tr>
<td>[Re(CO)$_2$Br$_2$(bipy)] (12)$^1$</td>
<td>1989, 1837</td>
<td>-80</td>
<td>424</td>
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<td>[Re(CO)$_2$Br$_2$(phen)] (13)$^1$</td>
<td>1993, 1862</td>
<td>-74</td>
<td>426</td>
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$^a$ KBr. $^b$ Potentials are reported vs. Ag/AgCl reference electrode in CH$_3$OH with 0.1 M TBAPF$_6$ as an electrolyte. All processes are one electron and refer to the Re$^{II}$ → Re$I$ reduction. $^c$ in CH$_3$OH 2, in CH$_2$Cl$_2$ all others.

Complexes 8-11, isolated in good yield (>60%), are soluble in common organic solvents like methanol, CH$_2$Cl$_2$, acetonitrile or DMSO. They were found to be stable for days both as solids and in solution even
under aerobic conditions. Complexes 8-11 show similar spectroscopic and electrochemical properties to the related cis-trans-[Re\textsuperscript{II}(CO)\textsubscript{2}Br\textsubscript{2}(bipy)] (12) and cis-trans-[Re\textsuperscript{II}(CO)\textsubscript{2}Br\textsubscript{2}(phen)] (13) previously published.\textsuperscript{1} These properties are listed in table 1.

**Figure 2.** ORTEP presentation of 8 (top) and 11. Selected bond lengths (Å) and angles (°) are for 8:  
Re(1)-Br(1) 2.5207(4); Re(1)-N(1) 2.169(3); Re(1)-C(1) 1.948(4); Br(1)-Re(1)-Br(1\_2) 175.491(18);  
for 11: Re(1)-Br(1) 2.5074(18); Re(1)-Br(2) 2.5366(17); Re(1)-N(1) 2.200(8); Re(1)-C(1) 1.935(12);  
Br(1)-Re(1)-Br(2) 174.61(7); Ellipsoids are drawn at 50% probability. Hydrogen atoms are omitted for clarity.

X-ray quality crystals of 8 and 11 could be grown from a CH\textsubscript{2}Cl\textsubscript{2}/hexane and an acetone/ether mixture respectively. The x-ray structures of 8 and 11 are given in figure 2. As expected, in the complexes the N\textsuperscript{-}N ligands were found trans to the CO’s and the two bromides bent away from the carbonyl groups forming an average angle of 175.1°. In 8 the 4,4\textsuperscript{-}Mebipy ligand is not flat but the two pyridines are twisted so as to form an angle of ca. 15°. While such feature is not uncommon for bipyridine type
ligands, a search of the CCDC has revealed that the only other monomeric rhenium complex showing such a severe twist of the bound pyridines is the cis-trans-[Re\(^{1}(CO)\(_2\)(PPh\(_3\))(POEt\(_3\))(4,4'-Mebipy)]\(^+\) recently reported by Ishitani.\(^{49}\) In 11 binding of 2,2'-dipy-NH occurs via the pyridine units. This feature makes complex 11 particularly interesting because derivatization of the bridging amine (e.g. via alkylation, or by appending a peptide vector) could offer new labeling strategies with Re\(^{II}\) based complexes or help for a targeted delivery of CO-RMs. Crystallographic details are given in table 2.

**Table 2.** Crystallographic data for compounds 3-5, 8 and 11.

<table>
<thead>
<tr>
<th>Compound</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>8</th>
<th>11</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Formula</strong></td>
<td>C(<em>{10})H(</em>{12})Br(_2)N(_4)O(_2)Re</td>
<td>C(<em>{16})H(</em>{12})Br(_2)N(_4)O(_2)Re</td>
<td>C(<em>{14})H(</em>{14})Br(_2)N(_2)O(_2)Re</td>
<td>C(<em>{14})H(</em>{12})Br(_2)N(_2)O(_2)Re</td>
<td>C(<em>{12})H(</em>{8})Br(_2)N(_3)O(_2)Re</td>
</tr>
<tr>
<td><strong>FW</strong></td>
<td>566.26</td>
<td>638.32</td>
<td>588.29</td>
<td>586.28</td>
<td>573.24</td>
</tr>
<tr>
<td><strong>T, K</strong></td>
<td>183(2)</td>
<td>183(2)</td>
<td>183(2)</td>
<td>183(2)</td>
<td>183(2)</td>
</tr>
<tr>
<td><strong>space group</strong></td>
<td>C2/c</td>
<td>C2/c</td>
<td>Pcca</td>
<td>C2/c</td>
<td>Pnma</td>
</tr>
<tr>
<td><strong>crystal system</strong></td>
<td>monoclinic</td>
<td>monoclinic</td>
<td>orthorhombic</td>
<td>monoclinic</td>
<td>orthorhombic</td>
</tr>
<tr>
<td><strong>Z</strong></td>
<td>8</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td><strong>a, Å</strong></td>
<td>30.1515(16)</td>
<td>10.6383(4)</td>
<td>13.33059(15)</td>
<td>6.50367(15)</td>
<td>7.8516(3)</td>
</tr>
<tr>
<td><strong>b, Å</strong></td>
<td>7.62680(19)</td>
<td>12.4396(3)</td>
<td>8.38345(9)</td>
<td>18.6076(4)</td>
<td>14.3410(5)</td>
</tr>
<tr>
<td><strong>c, Å</strong></td>
<td>15.1080(7)</td>
<td>13.7647(4)</td>
<td>15.52929(19)</td>
<td>13.4408(3)</td>
<td>12.8531(6)</td>
</tr>
<tr>
<td><strong>β, deg</strong></td>
<td>119.758(7)</td>
<td>99.358(3)</td>
<td>90</td>
<td>101.791(2)</td>
<td>90</td>
</tr>
<tr>
<td><strong>V, Å(^3)</strong></td>
<td>3016.1(2)</td>
<td>1797.34(9)</td>
<td>1735.50(3)</td>
<td>1592.26(6)</td>
<td>1447.27(10)</td>
</tr>
<tr>
<td><strong>d(_{calc}), g/cm(^3)</strong></td>
<td>2.494</td>
<td>2.359</td>
<td>2.252</td>
<td>2.446</td>
<td>2.631</td>
</tr>
<tr>
<td><strong>R1(wR2)(^d)</strong></td>
<td>0.0319 (0.0569)</td>
<td>0.0253 (0.0670)</td>
<td>0.0200 (0.0640)</td>
<td>0.0302 (0.0840)</td>
<td>0.0384, (0.0654)</td>
</tr>
<tr>
<td><strong>largest diff. peak / hole (e Å(^{-3}))</strong></td>
<td>1.639 and -0.894</td>
<td>1.170 and -0.842</td>
<td>0.848 and -1.208</td>
<td>1.122 and -1.403</td>
<td>2.852 and -1.043</td>
</tr>
</tbody>
</table>

\(^d\) [I>2sigma(I)]
CO Releasing Properties of cis-trans-[Re^{II}(CO)_2Br_2L_2]^n Complexes.

The carbon monoxide releasing properties of cis-trans-[Re^{II}(CO)_2Br_2L_2]^n complexes were evaluated by the myoglobin assay (figure 3). This assay was shown to be a reliable method for assessing the amount and kinetic of CO liberation from CO-releasing molecules.\textsuperscript{5,19,21} In these experiments, an aliquot of a freshly prepared concentrated solution of the [Re^{II}(CO)_2Br_2L_2]^n complex was added to a buffered solution of horse skeletal myoglobin (Mb), freshly reduced with excess sodium dithionite under N\textsubscript{2}. With the exception of 2 all complexes are insoluble in water. However, stock solutions of 2 in water were found to generate an inactive form of the complex due to the rapid CO release from this compound. Thus solutions of 2 were prepared in methanol while stock solutions of 3-13 were prepared in DMSO. The final methanol or DMSO content of the buffered aqueous solution never exceeded 0.5%. The conversion of Mb to carbon monoxide myoglobin (MbCO) was followed over time by measuring the changes in the absorption spectra of the Q band region of this protein at pH 7.4, 6.3 and 5.8 after addition of the Re^{II} complex. The maximal absorption peak of Mb at 560 nm was rapidly converted over time to spectrum of MbCO, with two maximal absorption peaks at 540 and 578, respectively. A typical spectrum is shown in figure 3.

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{spectrum.png}
\caption{A typical spectrum of conversion of deoxy-myoglobin (Mb) to carbon monoxide myoglobin (MbCO) by a cis-trans-[Re^{II}(CO)_2Br_2L_2]^n complex. Here reproduced is the spectrum change of a 30 \textmu M}
\end{figure}
Mb solution after the addition of species 4 (30 μM, 25°C, 0.1 M phosphate buffer pH 7.4, spectra intervals = 5min).

Only compounds bearing monodentate ligands (i.e. compounds 2-7) elicited the spectral changes associated with CO release. All other complexes bearing a Ν∩Ν type of ligand did not liberate CO. The amount of MbCO formed over time after addition of the ReII complex to the Mb solution was calculated according to the known extinction coefficients. The MbCO concentration was directly related to the equivalents of CO released from the compounds and these were plotted as a function of time. Figure 4 shows the pH-dependent rate of CO release of different cis-trans-[ReII(CO)2Br2L2]n complexes. The half-lives (t_{1/2}) of CO release from the ReII complexes at different pH’s were estimated from these graphs and are listed in table 3.

**Table 3.** Half−lives (t_{1/2}, min, 25 °C)^a for the release of 1 equivalent of CO by cis-[Re(CO)2Br2L2]n complexes at different pH’s.\(^b\)

<table>
<thead>
<tr>
<th>Complex (^c)</th>
<th>t_{1/2} at pH 5.8</th>
<th>t_{1/2} at pH 6.3</th>
<th>t_{1/2} at pH 7.4</th>
</tr>
</thead>
<tbody>
<tr>
<td>[Re(CO)2Br4]2 (2)</td>
<td>1.0</td>
<td>2.5</td>
<td>5.7</td>
</tr>
<tr>
<td>[Re(CO)2Br5(MeIm)]2 (3)</td>
<td>19.9</td>
<td>27.0</td>
<td>40.7</td>
</tr>
<tr>
<td>[Re(CO)2Br5(BzIm)]2 (4)</td>
<td>8.4</td>
<td>12.3</td>
<td>14.0</td>
</tr>
<tr>
<td>[Re(CO)2Br5(4-pic)]2 (5)</td>
<td>15.2</td>
<td>20.3</td>
<td>23.6</td>
</tr>
<tr>
<td>[Re(CO)2Br5(Im)]2 (6)</td>
<td>29.8</td>
<td>41.3</td>
<td>42.3</td>
</tr>
<tr>
<td>[Re(CO)2Br5(py)]2 (7)</td>
<td>9.7</td>
<td>10.2</td>
<td>17.2</td>
</tr>
</tbody>
</table>

\(^a\) Half−lives (t_{1/2}) were estimated from the graphs shown in figure 4. \(^b\) 0.1 M phosphate buffer. \(^c\) Complexes 8-13 do not release CO.
Figure 4. The pH-dependent rate of CO release of different cis-trans-[Re^{II}(CO)_2Br_2L_2]^n complexes. Conditions: 30 μM Mb and corresponding Re^{II} species, 25°C, 0.1 M phosphate buffer pH 7.4( ), 6.3 ( ), 5.8 ( ).

When the experiments were performed under conditions of a limiting amount of the metal complexes, taking into account the molar extinction coefficient of MbCO\(^{10}\), it was found that approximately 1 mol of CO was released per mole of the corresponding [Re^{II}(CO)_2Br_2L_2]^n species. Thus only one CO ligand is liberated from the metal complexes 2-7. The CO detection via this assay proceeded as expected with three isosbestic points at 552, 567 and 587 nm clearly visible in the spectrum (see figure 3). As described above, changes in the spectrum are indicative of the conversion of Mb to MbCO.

However, after full CO saturation of Mb the molar absorptivity in the Q band region of MbCO increased over time beyond the expected calculated value. All three isosbestic points were lost. There was no further spectral change in terms of the position of the maximal absorption peaks at 540 and 578, but only an apparent increase in their molar absorptivity. This was true for all [Re^{II}(CO)_2Br_2L_2]^n species tested and for all pH’s. This type of spectral changes have been previously attributed to an increase in the turbidity of the solution as a result CO-RM precipitation.\(^{50}\) It is also possible that the apparent increase in the molar absorptivity in the Q band region of MbCO may be due to an interaction of the resulting Re complexes with the protein. This interaction might explain the increase in absorption intensity due to further lowering of MbCO symmetry.\(^{27}\)

The rate of CO loss from 2-7 was found to be pH-dependent with half lives (t\(_{1/2}\)) under physiological conditions (pH 7.4) varying from ca. 6 (for 2) to 43 min (for 6, table 3). At lower pH values the time required to fully saturate Mb with CO liberated from the metal complexes gradually decreased. This was generally true for all compounds except for [Re(CO)_2Br_2(Im)]\(_2\) (6) and [Re(CO)_2Br_2(py)]\(_2\) (7) where only small difference were detected between pH 7.4 and 6.3 and between pH 6.3 and 5.8 respectively (table 3). The pH-dependent CO-cleavage of the cis-trans-[Re^{II}(CO)_2Br_2L_2]^n species is not yet fully
understood. It may be due to a cis-labilizing effect of potential π-donors such as H₂O which could substitute the bound bromide ions. Also, as previously suggested, this type of pH-dependence may point to an associatively activated substitution mechanism. This is reasonable in light of the fact that all Re species investigated are 17e- complexes.

Complex 2 was found to be the most rapid CO-releasing molecule (CO-RM) in all cases while the imidazole adducts the slowest. The overall order for the rate of CO release is: 2 > 4 ≈ 7 > 5 > 3 ≈ 6. At pH 7.4 and at 25 °C saturation of Mb with CO liberated from 2 was reached within 30 min (t₁/₂ = 5.7 min). This value is comparable to that of the fac-[RuCl(glycinato)(CO)₃] complex (CORM-3) whose t₁/₂ at pH 7.4 and at 37 °C has been reported to be ca. 1 min.⁵,²¹

**Protective Action of Selected cis-trans-[Reᴵᴵ(CO)₂Br₂L₂]ⁿ Complexes Against “Ischemia-Reperfusion” Stress of Cardiomyocyte.**

The fac-[RuCl(glycinato)(CO)₃] complex (CORM-3) has been shown to protect cardiac cells against hypoxia-reoxygenation stress and ischemia-reperfusion injury.⁵ Ischemia refers to an absolute or relative shortage of the blood supply to an organ, resulting in a shortage of oxygen, glucose and other nutrients transported via the blood. In order to test if cis-trans-[Reᴵᴵ(CO)₂Br₂L₂]ⁿ complexes also showed a protective action against “ischemia-reperfusion” stress of cardiac cells, neonatal rat ventricular cardiomyocytes (NRCs) kept under simulated ischemic conditions (i.e. glucose-free medium, pH 6.0, 1% O₂, 5% CO₂ and 94% N₂ for 16 h) were treated with compounds 2, 5, 6 and 7 at the “onset of reperfusion” (i.e. at the onset of normoxic conditions, 5 mM glucose, pH 7.4, 20% O₂). The Reᴵᴵ complexes were selected so as to encompass the widest range of t₁/₂ of CO release (see table 3) in order to understand if the rate of CO loss may represent a determining factors in our results.

Under normoxic conditions (i.e. normal 20% O₂ level) almost 100% of NRCs stayed alive either in the absence or in the presence of 30 μM of complexes 2, 6 and 7. However, complex 5 proved to be very toxic by killing over 90% of cells within 12 h of treatment. In a second protocol the toxicity of low pH and glucose omission was tested and the cells were incubated for 16 h in glucose-free well-
oxygenated medium at pH of 6.0. Thereafter “reperfusion” step was simulated by replacing the medium by that containing 5 mM glucose at pH of 7.4. As in the “ischemia-reperfusion” protocol Re" complexes were added immediately after the medium replacement and the incubation duration fixed to 12 h. Similar to that under normoxic conditions, cell viability was not affected by these manipulations, except for complex 5, which again lead to over 90% cell mortality (data not shown). Finally the cells were exposed to a simulated ischemia-reperfusion. Ischemic condition was simulated by exposing the cells to glucose-free medium of pH 6.0 in hypoxic incubator (1% O₂, 5% CO₂ and 94% N₂). After 16 h of “ischemia” the selected Re" complexes 2, 5, 6 and 7 at final concentration of 30 µM were administered into the cell culture medium as it was changed to high glucose well-oxygenated medium of pH 7.4, namely at the “onset of reperfusion”. Their cytoprotective efficacy was tested 12 h after the administration. Exposure of cells to the "ischemia-reperfusion" procedure reduced cell viability to ~75% (i.e. 25% dead cells) in the absence of Re" complexes (control in figure 5). Addition of complex 2 during “reperfusion” increased cell survival by 67% (~9% dead cells) and complex 6 by 83% (~4% dead cells), while complexes 5 and 7 displayed no significant protective effect (see figure 5).

Figure 5. Cytoprotective effects of selected cis-trans-[Re"(CO)₂Br₂L₂]ₙ complexes (30 µM) against “ischemia-reperfusion” stress of neonatal rat ventricular cardiomyocytes (NRCs) as described in the experimental section. Numbers on the abscissa refer to Re" complexes whose CO release half-lives are
given in table 3. Controls were subjected to the “ischemia-reperfusion” procedure without addition of Re\textsuperscript{II} complexes. Bars indicate the % of PI-positive (dead) cells. Asterisks indicate statistical significance: *) p < 0.035 and **) p < 0.005.

Figure 6 shows representative histochemical staining of cardiac cell cultures under different conditions. Propidium iodide (PI) only enters cells whose plasma membrane is damaged (dead cells) and stains the nucleic DNA (false red color). Hoechst 33342 permeates all cells and stains DNA in nuclei and mitochondria in living and dead cells (false green color). Superimposed nuclear staining in the merged pictures appear in yellow (selected nuclei are indicated by white arrows). The first vertical row shows cells under normoxia (no dead cells visible). The second vertical row contains cells subjected to the "ischemia-reperfusion" protocol without Re\textsuperscript{II} complex displaying ca. 25% dead cells (control in figure 5). In the third row the number of dead cells was reduced by complex 6 added during the reperfusion phase. None of the compounds tested was able to reduce cell mortality when present in the culture during the hypoxic phase alone. Taken together, these findings indicated that the CO released from the Re\textsuperscript{II} complexes attenuated the oxidative toxicity during reoxygenation but was unable to support cell survival under conditions of oxygen deprivation as CO further reduced O\textsubscript{2} availability.

Atomic absorption spectroscopy (AAS) measurements indicated that complexes 2, 6 and 7 did not enter the cells through the cell surface membrane during a 3 h incubation period (data not shown). Compound 5, on the contrary, was actively taken into the cells with the uptake rate of ~ 40 \(\mu\)mol/(10\(^6\) cells*h). Rhenium concentration in the medium decreased to <7-8 \(\mu\)M after 2 h of exposure suggesting that the compound was accumulating in the cells against the gradient and/or rapidly metabolised. Of note, accumulation of complex 5 was not followed by an immediate 90% mortality suggesting that not the compound itself, but rather the product(s) of its metabolism (such as 4-methylpyridine), were the cause of its elevated toxicity. These findings indicate that the cytoprotective action exerted by 2 and 6 may be attributed to the extracellular release of CO while the high toxicity of complex 5 may be related to its membrane permeability and accumulation into the cells. Our results
further suggest that the rate of CO release is not a determining factor for the protection of NRCs. Both complex 2 (t$_{1/2}$ of CO loss = ca. 6 min) and 6 (t$_{1/2}$ = ca. 43 min) protect cardiomyocytes in our “ischemia-reperfusion” protocol. Compound 7 (t$_{1/2}$ = ca. 17 min) displayed no significant protection while 5 (t$_{1/2}$ = ca. 24 min) was toxic.

**Figure 6.** Representative histochemical staining of cardiac cell cultures under different conditions. Top horizontal row, staining of nuclear DNA (red) of dead cells with propidium iodide (PI). Middle row, staining of DNA (green) in nuclei and mitochondria (additional stain uptake in cell bodies) of dead and live cells with Hoechst 33342. Bottom row, merged pictures with yellow nuclei where the two stains overlay (selected cases highlighted by white arrows). Normoxia, in the absence of "ischemia-reperfusion" no dead cells were observed; "ischemia-reperfusion" protocol induced around 25% dead
cells; addition of ReII complex 6 significantly reduced the number of dead cells. Each picture measures 830 µm in width and 650 µm in height, yielding an area of 0.54 mm².

With regard to the cytoprotective effect, all synthesized ReII complexes contain two molecules of carbon monoxides (CO). The myoglobin assay indicated that all complexes tested released one CO per molecule within 2 hours albeit at different rates (see table 3). Given the fact that the tested complexes (with the exception of 5) remain outside the cells, it must be assumed that the released CO that is known to be lipid soluble, readily enters the cells to exert its biological function. Whether the second CO will be released later during the 12 h of reperfusion is not known at present. It could be that metabolic interactions in the medium leads to structural rearrangements entailing release of the second CO molecule with progressing time. Anyhow, the results clearly indicate that complex 6 with the slowest CO release rate ($t_{1/2} = \text{ca. 43 min}$) offers the most extensive protection as compared to the slower CO releasing complex 2. Complex 7 displays no protection and the most lipophilic complex 5, which enters the cells, seems to precipitate cell mortality (figure 5). It is possible that in these two cases toxic properties may compete and outweigh the beneficial effects of CO (pyridines are cytotoxic themselves).

Conclusion.

The CO releasing properties of a new class of CO-RMs of general formula $\text{cis-trans-}[\text{Re}^{II}(\text{CO})_2\text{Br}_2\text{L}_2]^n$ (where L = monodentate ligand) have been described. Only complexes bearing monodentate ligands were found to liberate CO. The rate of CO loss of the ReII compounds was found to be pH-dependent with half lives under physiological conditions varying from ca. 6 to 43 min. This feature makes the complexes potentially useful for medicinal applications. Because the rate of CO release can be controlled by the appropriate choice of L, fine tuning of the coordination sphere of the $\text{cis-trans-}$
[Re\(^{II}\)(CO)\(_2\)Br\(_2\)]^0\) core would in principle allow for the design of compounds with specific rates of CO loss. In turn this feature allows controlling the rate of CO release and the selection of specific complexes when specific concentrations of CO are required. Selected cis-trans-[Re\(^{II}\)(CO)\(_2\)Br\(_2\)L\(_2\)]\(^n\) complexes were found to induce a protective action against “ischemia-reperfusion” stress of neonatal rat ventricular cardiomyocytes in culture. Our biological results appear to indicate that the rate of CO release might not be a determining factor for the protection of cardiomyocytes. However, the possibility of derivatizing the cis-trans-[Re\(^{II}\)(CO)\(_2\)Br\(_2\)]^0 core with water soluble and biologically compatible ligands while retaining the fundamental CO-releasing properties, makes this class of CO-RMs unique in the field. Taken together, application of CO could open new therapeutic strategies for preventing pathologic developments in cardiovascular disease. However, due to its high affinity to the various heme proteins CO is also one of the most toxic substances, particularly when exogenously administered. Furthermore, potential treatment doses require careful evaluation and monitoring in order to avoid toxic side effects. The mechanism by which the cis-trans-[Re\(^{II}\)(CO)\(_2\)Br\(_2\)L\(_2\)]\(^n\) (where L = monodentate ligand) species release CO is currently being investigated and efforts are directed towards the development of biocompatible CO releasing compounds coupled to water soluble scaffold moieties.

**Acknowledgements**

The Swiss National Science Foundation (Ambizione PZ00P2_121989/1 and Grant# 310030_124970/1) is gratefully acknowledged for financial support. We thank Nikolay Bogdanov (Institute of Veterinary Physiology) for his assistance in the isolation of the neonatal rat cardiomyocytes.
References.

The carbon monoxide (CO) releasing properties of a series of Re\textsuperscript{II}-based complexes of general formula \textit{cis-trans-}[Re\textsuperscript{II}(CO)\textsubscript{2}Br\textsubscript{2}L\textsubscript{2}]\textsuperscript{n} (where L = monodentate ligand) are described. It is shown that the rate of CO loss from the \textit{cis-trans-}[Re\textsuperscript{II}(CO)\textsubscript{2}Br\textsubscript{2}]\textsuperscript{0} core can be easily controlled by varying the coordinated L. Selected \textit{cis-trans-}[Re\textsuperscript{II}(CO)\textsubscript{2}Br\textsubscript{2}L\textsubscript{2}]\textsuperscript{n} complexes induce a protective action against "ischemia-reperfusion" stress of neonatal rat ventricular cardiomyocytes (NRCs) in culture.