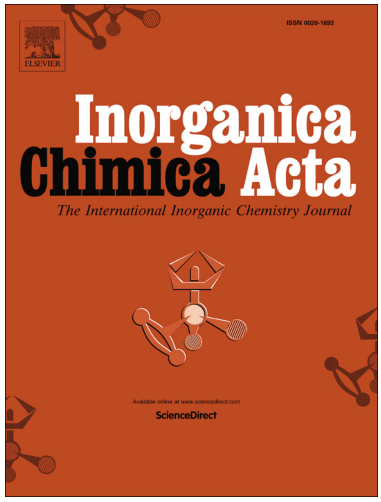


New Photo-CORMs: Deeply-coloured biocompatible Rhenium Complexes for the Controlled Release of Carbon Monoxide

Elham Kianfar, Christine Schäfer, Mohammad Reza Lornejad-Schäfer, Engelbert Portenkirchner, Günther Knör

PII: S0020-1693(15)00320-5
DOI: http://dx.doi.org/10.1016/j.ica.2015.05.035
Reference: ICA 16588

To appear in: *Inorganica Chimica Acta*

Received Date: 2 March 2015
Revised Date: 26 May 2015
Accepted Date: 28 May 2015

Please cite this article as: E. Kianfar, C. Schäfer, M.R. Lornejad-Schäfer, E. Portenkirchner, G. Knör, New Photo-CORMs: Deeply-coloured biocompatible Rhenium Complexes for the Controlled Release of Carbon Monoxide, *Inorganica Chimica Acta* (2015), doi: http://dx.doi.org/10.1016/j.ica.2015.05.035

New Photo-CORMs: Deeply-coloured biocompatible Rhenium Complexes for the Controlled Release of Carbon Monoxide

Elham Kianfar,[a] Christine Schäfer,[b] Mohammad Reza Lornejad-Schäfer,[b] Engelbert Portenkirchner [c] and Günther Knör *[a]

[a] Institute of Inorganic Chemistry, Johannes Kepler University Linz (JKU), 4040 Linz, Austria
[b] BioMed-zet Life Science GmbH, 4020 Linz, Austria
[c] Institute of Physical Chemistry, University of Innsbruck, 6020 Innsbruck, Austria
[*] Corresponding Author, Tel: +43 732 2468 5100, E-mail: guenther.knoer@jku.at

ARTICLE INFO

Article History
Received
Accepted
Available online

Keywords
Photochemistry • Carbon Monoxide • Heme Oxygenase Models • Rhenium Complexes • Cellular Uptake • Biocompatibility

ABSTRACT

Air- and water-stable rhenium carbonyl complexes are investigated as CO-carriers for studying the light-dependent release of small amounts of carbon monoxide under physiological conditions. The reported very low quantum yields are in a suitable range for studying potential applications as photochemical CO-releasing molecules (Photo-CORMs) without rapidly reaching cytotoxic levels of carbon monoxide. Preliminary results on bio-compatibility and selective uptake of the compounds into human colon carcinoma cells (Caco-2 cell line) are described.

1. Introduction

Carbon monoxide usually is considered to be highly toxic to humans, due to its fatal function as a respiratory inhibitor. Nevertheless, small quantities of this gaseous molecule are essential for a healthy life. As a result of enzymatic heme oxidation, a significant amount of gaseous CO is generated naturally in the human body where it acts as a small molecular messenger and plays an important role in regulating different cell functions. CO gas is known to dilate blood vessels and to induce beneficial anti-inflammatory, neuroprotective and anti-apoptotic effects.[1] Synthetic CO-releasing molecules (CORMs) are therefore widely used to explore the therapeutic potential of future carbon monoxide based drugs including applications in wound healing, organ transplantation and cardiovascular protection.

An interesting approach to control the release of small amounts of carbon monoxide under physiological conditions is to develop biocompatible precursor compounds that can be photochemically activated with visible light as an external signal (Photo-CORMs).[2,3] Here we describe the investigation of air- and water-stable rhenium carbonyl complexes that can be applied as non-cytotoxic CO-carriers for triggering the release of carbon monoxide under physiological conditions. Quantum yields for photochemical CO-release and preliminary studies on the incorporation of the metal complexes into cells of the human colon adeno-carcinoma cell line Caco-2 are reported.

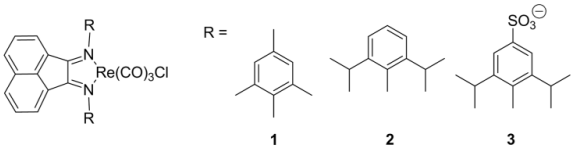


Fig. 1. Structures of the investigated rhenium(I)-complexes.

2. Results and discussion

The intensely red-brown coloured organometallic compounds **1**-**3** (Fig. 1) of the type *fac*-Re(1,2-diimine)$(CO)_3X$ with X = Cl selected for the present study have recently been synthesized and structurally characterized in our group.[4] In the solid state and in solution under ambient conditions, these chloro-tricarbonyl rhenium complexes display an excellent stability with no indication of decomposition or loss of CO. When exposed to light, however, the compounds are gradually transformed in a photochemical reaction sequence leading to permanent spectral changes shown here for the neutral complex **2** (Fig. 2). Irradiated solutions of **1** and **3** display a very similar behaviour (ESI†). Closer analysis of the light-induced spectral variations indicates a loss of the isosbestic points in the visible spectral region, which is consistent with the occurrence of a stepwise conversion.

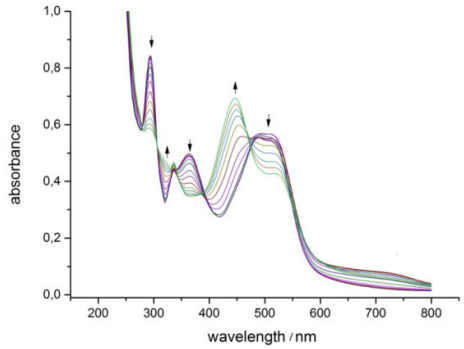


Fig. 2. Absorption spectral changes during irradiation of the rhenium carbonyl complex **2** in acetonitrile solution (298 K, 1 cm cell, c = 8.7×10^{-5} M, t = 0-2 h exposure to a 150 W Xe-lamp equipped with a water filter).

The quantum yields for this UV-light dependent photolysis of the rhenium complexes are rather low (< 1%). Values of φ(280 nm) = 2.9×10^{-3}, 4.8×10^{-3} and 3.8×10^{-3} were measured for compounds **1** and **2** in CH_3CN solution and for the water-soluble derivative **3** in aqueous solution, respectively. A quite similar reactivity with an irreversible wavelength-dependent photolysis in CH_3CN and a quantum yield maximum of $\varphi = 3 \times 10^{-3}$ in the UV-spectral region has been reported before for the corresponding rhenium complex with R = phenyl (Fig. 1).[5] The quantum yield for this decomposition reaction rapidly drops to zero with monochromatic light in the visible spectral range. In agreement with our earlier study and also considering the UV-photoreactivity reported for the closely related family of *fac*-Mn(1,2-diimine)$(CO)_3$X systems,[3,6] the spectral changes observed here (Fig. 2) are tentatively interpreted as a UV-light induced *fac* → *mer* isomerization of the complexes **1**-**3** and a photosubstitution of the coordinated chloro ligand against a solvent molecule. Such a reactivity pattern was also supported by positive ESI-mass spectrometry of the photolyzed samples. Comparing the trend of the quantum yield data confirms our assumption that the presence of more bulky aryl substituents on the nitrogen atoms of the 1,2-diimine ligand can favour the release of the rhenium bound chloride ligand, which is also of considerable interest for catalytic applications of these compounds.[7] Note that the UV-light induced reactivity described above, which is most probably involving the direct population of a high-lying repulsive metal-centered excited state of ligand field (^{1}MC) origin, does not lead to a permanent loss of CO. This is also consistent with the IR-spectroscopic data obtained after photolysis.[5]

Upon polychromatic irradiation in solution, however, the completely dark-stable complexes **1**-**3** were found to release free carbon monoxide in significant amounts. This is indicating a branching of the excited state deactivation pathways at lower energies, which can open additional photoreactivity patterns involving the chromophoric charge transfer (CT) excited state manifold[5,6] which is dominating the absorption properties of the compounds in the visible spectral region. It is interesting to note, that such a an additional photochemical reactivity arising from lower-lying charge transfer excited states of otherwise emissive metal-to-ligand (MLCT) or ligand-to-ligand (LLCT) origin could also explain the unusual non-luminescent behaviour reported earlier for this kind of rhenium complexes.[5]

Quantitative detection of photoinduced CO-loss by the complexes **1**-**3** was carried out with a direct FTIR-spectroscopic method described elsewhere in detail[7] (Fig. 3).

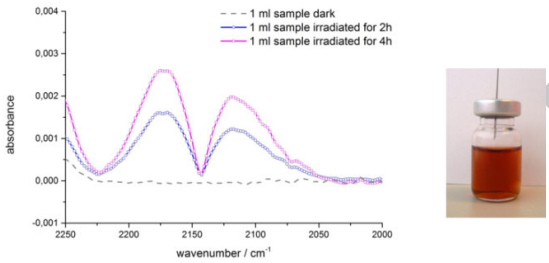


Fig. 3. Headspace gas FTIR difference absorption spectra of dark control (dashed line) and light-exposed samples of the rhenium carbonyl complex **2** in acetonitrile solution (298 K, GC vial, c = 4.1×10^{-4} M, t = 0, 2 and 4 h exposure to a 150 W Xe-lamp equipped with a water filter).

In a typical experiment, 2 mg (2.5 µmol) of **2** were dissolved in 6 ml of dry CH_3CN and the sample vial was sealed with a gas-tight septum inside a glove box. From dark control and irradiated samples, headspace gas was drawn with a syringe and injected into the FTIR measurement cell to detect the amount of free carbon monoxide. While no CO was lost in the dark, a quantum yield of φ(CO) = 0.04 was determined for CO-photorelease of the rhenium complex **2**. Comparable values of φ(CO) = 0.03 and φ(CO) = 0.01 were obtained with this method for complex **1** in CH_3CN and complex **3** in aqueous solution, respectively (ESI†).

Light-induced formation of free carbon monoxide with Photo-CORMs such as **2** is therefore in the activity range of 160 nmol CO/h/mg under the conditions reported here. It can be estimated that in 4h of photolysis about 50% conversion of the Photo-CORM takes place, assuming that only one carbon monoxide molecule per rhenium center can be ejected. Such a slow process may be partially due to a competing back-reaction of the photolyzed complex in the presence of dissolved CO. In aqueous solution the water soluble complex **3** is permanently releasing carbon monoxide with a rate of 40-50 nmol CO/h/mg when activated by light under the conditions reported here. Compared to many other compounds suggested as CORMs for potential biomedical applications, these low values at the first glance seem to be not very promising. It should be kept in mind, however, that the balance between any beneficial therapeutic action of carbon monoxide and negative effects of CO on oxygen transport and cellular respiration has to be very carefully controlled.[1a] In fact, the activity of natural heme oxygenase (HO) enzymes responsible for the controlled release of carbon monoxide in cells and tissues is typically in the range of around 1 nmol CO/h/mg,[8,9] which is still 50 times lower than the value of Photo-CORM **3** reported here. It should also be mentioned in this context that a convenient up- or down-regulation of this inducible heme oxygenase-like activity can be achieved at a given intrinsic quantum yield by controlling the applied light-intensity, which is a well-known general advantage of photochemical enzyme model systems.[10]

In a preliminary study for potential biomedical applications of the new Photo-CORMs as photoactivable metal-based drugs,[11] we have started to investigate the properties of the novel water-soluble derivative **3** under physiological conditions. Different concentrations of the

rhenium carbonyl complex up to 1000 µg/ml were applied in a cell culture medium (Fig. 4). It turned out that complex **3** displays excellent solubility in the test medium (DMEM without fetal bovine serum and phenol red).

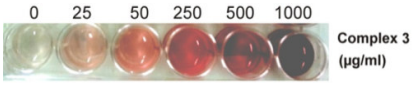


Fig. 4. Solubility of the rhenium carbonyl complex **3** at increasing concentrations in DMEM cell culture medium with 4.5 g/liter glucose, 4 mM glutamine, 100 units/ml penicillin, 100 mg/ml streptomycin, 1% nonessential amino acids.

We also investigated cell morphology effects and cellular uptake of the rhenium carbonyl complex **3** with the human cell line Caco-2 in culture (ESI†). Caco-2 cells are derived from human colorectal adenocarcinoma and form monolayers (like human intestinal epithelium) under conventional culture conditions.[12] They have been widely used as a potent in-vitro model to predict drug absorption in humans.[13,14] Cell morphology did not change markedly after 24 h treatment with the rhenium carbonyl complex **3** which was dissolved in the test medium (Fig. 5a, 5b). Therefore, we suppose no significant cytotoxicity up to 24 h. However, regions of intense cellular uptake of the red-brown coloured complex were located within the cell monolayer (Fig. 5c), and therefore also the dose-dependent uptake of **3** into differentiated Caco-2 cells was spectrophotometrically analyzed after cell lysis (ESI†). These observations are currently investigated more closely by us.

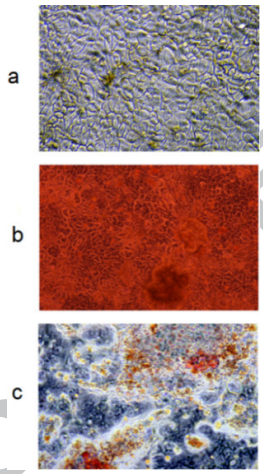


Fig. 5. Cell morphology monitored with a light microscope after differentiated Caco-2 cells were treated with compound **3** for 24 hours. a) control (0 µg/ml); b) 1000 µg/ml carbonyl complex **3**; c) 1000 µg/ml carbonyl complex **3** after removal of the culture medium and washing step.

A selective absorption and/or accumulation of the rhenium carbonyl complex **3** in a distinct subpopulation of intestinal cells, for instance colon cancer stem cells, would be of great interest for new biomedical applications against colorectal cancer. Colorectal cancer is one of the leading causes of cancer deaths and metastatic colorectal cancer is badly curable with currently available systemic therapeutic options.[15] It should be kept in mind that the Caco-2 cell line consists of several subpopulations. For instance, this cell line has previously been demonstrated to sustain a subpopulation of cancer stem cells with tumor initiating capacity characterized by the expression of the CD133 and CD44 surface markers.[16]

A reliable quantitative measure for potential dark cytotoxicity and cytolysis mediated by the Photo-CORM **3** in vitro using the lactate dehydrogenase (LDH) assay[17] could not be obtained, because the deeply-coloured rhenium carbonyl complex (Fig. 1) interfered with LDH-detection usually carried out at a wavelength of 490 nm in commercially available enzymatic assays (data not shown). Therefore, in order to determine the potential toxic effect of the carbonyl complex **3** in Caco-2 cells, we tested poly ADP-ribose polymerase (PARP) cleavage[18] as an apoptosis marker (ESI†). It turned out that neither the cell morphology nor PARP cleavage was affected in the dark control or illuminated cells in the presence and absence of Photo-CORM **3**, and also the protein expression of the reference marker GAPDH was relatively unchanged (Fig. 6). Therefore, we assume that apoptosis effects by Photo-CORM **3**, as well as light-induced apoptosis (light control), and apoptosis potentially induced by the experimental setting used (dark control) did not a play role. Photo-CORM **3** will therefore be very useful for further *in vitro* studies to explore new biomedical applications because it is not leading to cell death under these conditions.

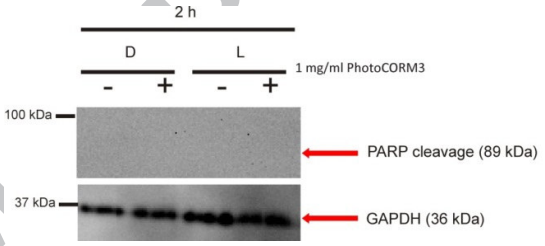


Fig. 6. Effect of carbonyl complex **3** on the apoptosis marker "PARP cleavage" using western blot analysis. D: dark control, L: light exposed samples in the presence (+) or absence (-) of **3**.

3. Conclusion

In summary, we have reported here the characterization of three deeply coloured rhenium(I) carbonyl complexes suitable for the photochemically controlled release of small amounts of carbon monoxide in solution. Among these compounds, a sulfonated *fac*-Re(1,2-diimine)$(CO)_3Cl$ derivative (**3**) is described, which displays several highly desirable properties necessary for potential biomedical applications as a Photo-CORM including excellent aqueous solubility, air- and water-stability and a reasonable compatibility with physiological conditions. First very promising observations on apparently negligible dark-cytotoxicity and selective cellular uptake are reported. Moreover, the observed quantum yields for light-triggered CO release are in an advantageous range, which should allow to photochemically mimick some of the beneficial protective effects of an up-regulated inducible heme oxygenase (HO-1) function on demand without risking to reach otherwise toxic levels of carbon monoxide under physiological conditions, which might be a serious problem with many other CORMs reported in the literature.[1]

Acknowledgments

Financial support of this work by the Austrian Science Fund (FWF project P25038: "Functional Light-responsive

Metal Carbonyl Systems“) and the State of Upper Austria is gratefully acknowledged.

Notes and references

† Electronic Supplementary Information (ESI) available: Experimental details and further data. See DOI:

1 R. Motterlini, R. Foresti, *Antioxid. Redox Signal.* 2014, **20**, 1810; J. Chen, *Rev. Neurosci.* 2014, **25**, 269; R. Motterlini, L. E. Otterbein, *Nat. Rev. Drug Discovery* 2010, **9**, 728; B. E. Mann, *Top. Organomet. Chem.* 2010, **32**, 247; R. Alberto, R. Motterlini, *Dalton Trans.* 2007, **17**, 1651; R. Motterlini, J. E. Clark, R. Foresti, P. Sarathchandra, B. E. Mann, C. J. Green, *Circulation Res.* 2002, **90**, e17; Maines, M. D., Heme Oxygenase: Clinical Applications and Functions, CRC, Boca Raton, FL, 1992.

2 S. García-Gallego, G. J. L. Bernardes, *Angew. Chem. Int. Ed.* 2014, **53**, 9712; R. D. Rimmer, A. E. Pierri, P. C. Ford, *Coord. Chem. Rev.* 2012, **256**, 1509; G. Knör, U. Monkowius, *Adv. Inorg. Chem.* 2011, **63**, 235; U. Schatzschneider, *Inorg. Chim. Acta*, 2011, **374**, 19.

3 V. Yempally, S. J. Kyran, R. K. Raju, W. Y. Fan, E. N. Brothers, D. J. Darensbourg, A. A. Bengali, *Inorg. Chem.* 2014, **53**, 4081; S. H. Heinemann, T. Hoshi, M. Westerhausen, A. Schiller, *Chem. Commun.* 2014, **50**, 3644; S. J. Carrington, I. Chakraborty, P. K. Mascharak, *Chem Commun.* 2013, **49**, 11254; P. Peng, C. Wang, Z. Shi, V. K. Johns, L. Ma, J. Oyer, A Copik, R. Igarashia, Y. Liao, Org. *Biomol. Chem.* 2013, **11**, 6671; J. S. Ward, J. M. Lynam, J. W. B. Moir, D. E. Sanin, A. P. Mountford, A. P., I. J. S. Fairlamb, *Dalton Trans.* 2012, **41**, 10514.

4 E. Kianfar, U. Monkowius, E. Portenkirchner, G. Knör, Z. *Naturforsch.* 2014, **69b**, 691.

5 G. Knör, M. Leirer, A. Vogler, *J. Inf. Recording* 1998, **24**, 69; G. Knör, M. Leirer, T. Keyes, J. Vos, A. Vogler, *Eur. J. Inorg. Chem.* 2000, 749.

6 I. R. Farrell, A. Vlček Jr., *Coord. Chem. Rev.* 2000, **208**, 87; F. Hartl, P. Rosa, L. Ricard, P. Le Floch, S. Záliš, *Coord. Chem. Rev.* 2007, **251**, 557.

7 E. Portenkirchner, K. Oppelt, C. Ulbricht, D. A. M. Egbe, H. Neugebauer, G. Knör, N. S. Sariciftci, *J. Organomet. Chem.* 2012, **716**, 19; E. Portenkirchner, E. Kianfar, N. S. Sariciftci, G. Knör, *ChemSusChem* 2014, **7**, 1347; M. Klein, U. Neugebauer, A. Gheisari, A. Malassa, T. M. A. Jazzazi, F. Froehlich, M. Westerhausen, M. Schmitt, J. Popp, *J. Phys. Chem. A*, 2014, **118**, 5381.

8 H. J. Vreman, D. K. Stevenson, *Analyt. Biochem.* 1988, **168,** 31.

9 E. F. Chang, R. J. Wong, H. J. Vreman, T. Igarashi, E. Galo, F. R. Sharp, D. K. Stevenson, L. J. Noble-Haeusslein, *J. Neurosci.* 2003, **23**, 3689.

10 G. Knör, *Chem. Eur. J.* 2009, **15**, 568.

11 N. A. Smith, P. J. Sadler, *J. Phil. Trans. R. Soc. A* 2013, **371**, 20120519.

12 M. Pinto, S. Robine-Leon, M.-D. Appay, M. Kedinger, N. Triadou, E. Dussaulx, B. Lacroix, P. Simon-Assmann, K. Haffen, J. Fogh, A. Zweibaum, *Biol. Cell* 1983, **47**, 323.

13 P. Artursson, *J. Pharm. Sci.* 1990, **79,** 476.

14 C. Schäfer, K. R. Schröder, O. Höglinger, S. Tollabimazraehno, M. R. Lornejad-Schäfer, *Cell Physiol. Biochem.* 2013, **32**, 431.

15 J. Ferlay, E. Steliarova-Foucher, J. Lortet-Tieulent, S. Rosso, J. W. W. Coebergh, H. Comber, D. Forman, F. Bray, *Eur. J. Cancer* 2013, **49**, 1374.

16 N. Haraguchi, M. Okhuma, H. Sakashita, S. S. Matsuzaki, F. Tanaka, K. Mimori, Y. Kamohara, H. Inoue, M. Mori, *Ann. Surg. Oncol.* 2008, **15**, 2927.

17 C. Korzeniewski, D. M. Callewaert, *J. Immunol. Meth.* 1983, **64**, 313.

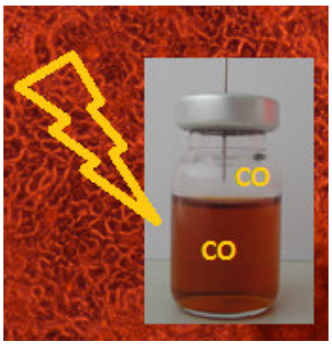
18 S. Elmore, *Toxicol Pathol.* 2007, **35**, 495.

Synopsis for the Graphical Abstract

Bioorganometallic Photochemistry

Elham Kianfar, Christine Schäfer, Mohammad Reza Lornejad-Schäfer, Engelbert Portenkirchner, Günther Knör *

New Photo-CORMs: Deeply-coloured biocompatible Rhenium Complexes for the Controlled Release of Carbon Monoxide



Air- and water-stable rhenium carbonyl complexes are investigated as CO-carriers for studying the light-dependent release of small amounts of carbon monoxide under physiological conditions. The reported very low quantum yields are in a suitable range for studying potential applications as photochemical CO-releasing molecules (Photo-CORMs) without rapidly reaching cytotoxic levels of carbon monoxide. Preliminary results on bio-compatibility and selective uptake of the compounds into human colon carcinoma cells (Caco-2 cell line) are described.

New Photo-CORMs: Deeply-coloured Biocompatible Rhenium Complexes for the Controlled Release of Carbon Monoxide

Elham Kianfar,[a] Christine Schäfer,[b] Mohammad Reza Lornejad-Schäfer,[b] Engelbert Portenkirchner [c] and Günther Knör *[a]

Highlights :

- **Non-toxic dark-stable and water-soluble carbonyl carrier**
- **Excellent biocompatibility and efficient cellular uptake**
- **Light-triggered carbon monoxide release characterized**
- **Photochemical modelling of heme oxygenase function**

Keywords:

- **Photochemistry**
- **Carbon Monoxide**
- **Heme Oxygenase Models**
- **Rhenium Complexes**
- **Cellular Uptake**
- **Biocompatibility**