NMR studies on NO Synthase Isoforms Inhibition by $Re(CO)_3$-complexes

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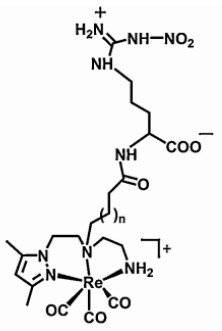
Nitric oxide (NO) is a key mammalian modulator that results from the catalytic oxidation of L-arginine to L-citruline by the heme-containing enzyme nitric oxide synthase (NOS), which has three distinct isoforms: endothelial (eNOS), neuronal (nNOS) and inducible (iNOS). Besides mediating several physiological functions, NO overproduction by nNOS and iNOS has been associated to several clinical disorders including stroke, Alzheimer's and Parkinson's disease, cancer, among others.[1]

Monitoring NOS expression would allow earlier intervention on clinical patients, namely related to disease diagnosis and treatment. Radionuclide–based single-photon emission Computed Tomography (SPECT), one of the most sensitive imaging techniques, represents an interesting approach to evaluate protein expression. The design of innovative radioactive probes for *in vivo* targeting of NOS is a promising goal. A set of $^{99m}Tc(CO)_3$-complexes containing NOS-recognizing units (*e.g.* L-Arginine derivatives, guanidine or S-methylisothiourea moieties) were established[2] and $Re(CO)_3$-complex analogs were studied as "non radioactive" surrogates.

Enzymatic activity of iNOS in the presence of Re1 and Re2 complexes (Fig. 1) revealed inhibitory action characterized by K_I's of 84 µM and 6 µM, respectively. Additionally, both Re complexes permeate through macrophage cell membranes and interact with the cytosolic target enzyme, inhibiting NO biosynthesis in LPS-induced macrophages.[3]

NMR saturation-transfer difference (STD) experiments were performed in iNOS / Re1 and Re2 samples. Results allow to identify binding moieties for each compound and to shed light on structural elements characterizing their selectivity and affinity properties. Nearest interacting protons present in binding moieties were recognized, complementing our docking studies,[4] elucidating how Re1 and Re2 interact and how they can be used to probe NOS isoforms. Moreover, interaction evidences revealing Re complexes selectivity toward NOS isoforms will help in the design of new specific inhibitors.

Structure-activity relationship will be assessed complementarily with X-ray crystallography and Isothermal Titration Calorimetry studies to understand the full spectra of NOS isoforms and interesting complexes.



Re1 (n = 1) and Re2 (n = 4)

Fig. 1 - Organometallic $Re(CO)_3$ complexes containing a pendant Nω-NO2-L-arginine moiety.

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