^{99m}Tc/Re-tricarbonyl complexes containing pendant acetamidine moieties for iNOS targeting

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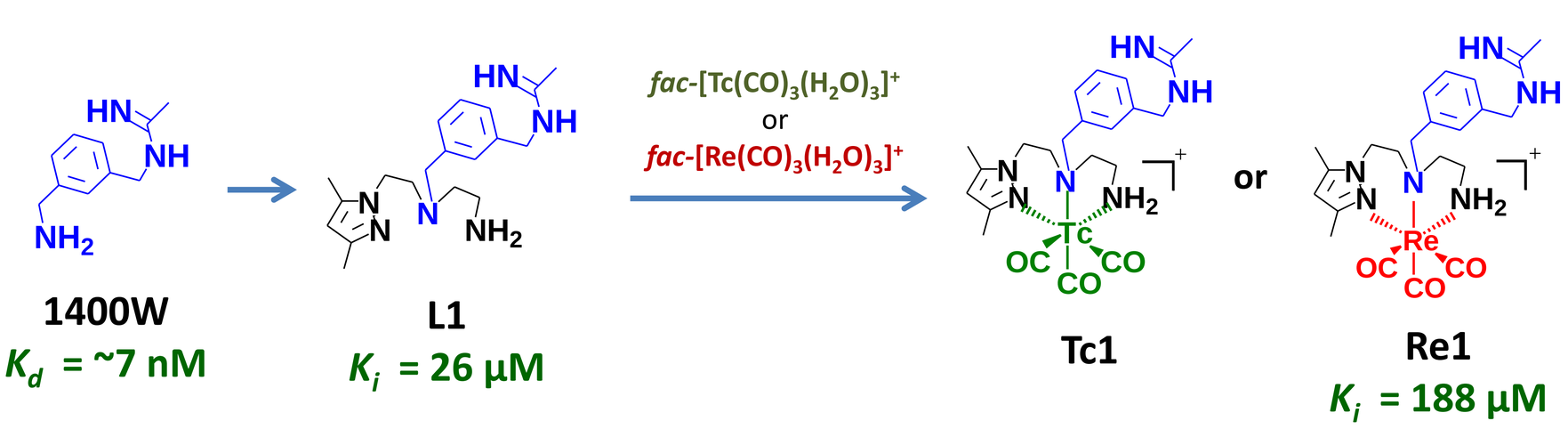
INTRODUCTION

Nitric Oxide Synthase (NOS) is the enzyme that catalyzes the biosynthesis of Nitric Oxide (NO) and L-citrulline from L-Arg in the presence of molecular oxygen. The *in vivo* imaging of NOS by nuclear techniques (SPECT/PET) could provide insights into NO/NOS-related diseases.[1 – 3] Herein we report the preparation of novel ^{99m}Tc/Re(CO)$_3$-complexes containing derivatives of the iNOS selective inhibitor **1400W**. The inhibitory potency of **L1** and corresponding Re(I) complex **Re1** towards iNOS was assessed *in vitro*. Docking and Molecular Dynamics simulations studies were performed to shed light on the specific molecular interactions responsible for the different affinities of the compounds to the enzyme.

RESULTS AND DISCUSSION

• ENZYMATIC RESULTS

^{99m}Tc/Re(CO)$_3$-complexes containing a 1400W pendant moiety for iNOS recognition



1400W K_d = ~7 nM

L1 K_i = 26 µM

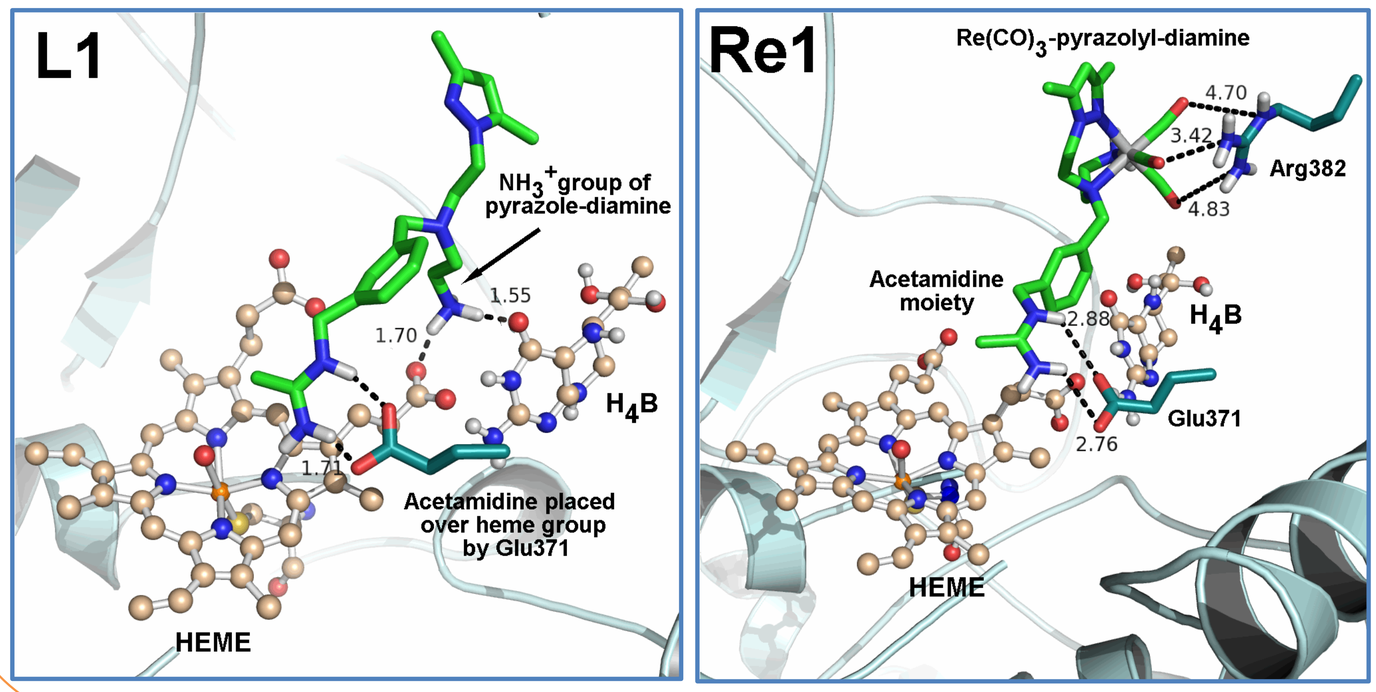
Tc1

Re1 K_i = 188 µM

- The kinetic parameters (K_i) of murine iNOS were determined using the oxyhemoglobin NO capture assay.
- **L1** (*Ki* = 26 µM) and **Re1** (*Ki* = 188 µM) showed lower affinities to the enzyme compared to the free inhibitor **1400W**.

• COMPUTATIONAL RESULTS

Docking studies (Autodock software) were combined with MD simulations to rationally predict the binding mode of **L1** and **Re1** inside iNOS active pocket and to understand their structural differences.



Structural micro-environment around L1 and Re1

L1 is anchored above the heme group by residue Glu371. The NH_3^+ group of the pyrazolyl-diamine chelator is hydrogen-bonded to the heme propionate A and to the O atom of the H_4B co-factor. Coordination of **L1** to the "Re(CO)$_3$" core through the pyrazolyl-diamine prevents this interaction leading a complex with lower affinity to the iNOS.

* MD simulations (8ns) were performed with NAMD Software and CHARMM27 FF. CHARMM FF parameters for **L1** were generated using the ParaChem Server. Parameters for Heme, H_4B and "Re (CO)$_3$" were kindly provided by Cho *et al*. and Reichert *et al.*. [4-5] RESP charges were obtained at the B3LYP/6-31G* level of theory for H, C, N, O and B3LYP/SDD for Re using the R.E.D. Server.

CONCLUSIONS

- New Re(I) and ^{99m}Tc(I) complexes comprising a pyrazolyl-diamine backbone for stabilization of the metal core and a 1400W moiety for iNOS recognition were introduced.
- Derivatization of the pyrazolyl-diamine chelator with 1400W to give **L1** and subsequent coordination to "Re(CO)$_3$" (**Re1**) gave compounds with lower affinity to iNOS.
- The absence of a free NH_3^+ group in **Re1** limits the interaction of the complex with iNOS.

References

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