Light Activated Release of a Metal Binding Pharmacophore from a Ru(II) Polypyridine Complex

Johannes Karges, Ryjul W. Stokes and Seth M. Cohen

Department of Chemistry and Biochemistry, University of California, San Diego, La Jolla, California 92093, United States.

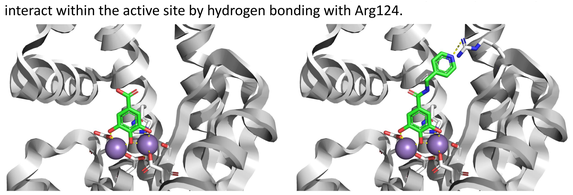
Metal Binding Pharmacophores

About 40-50% of all identified enzymes require a metal ion to perform their biological function. Despite comprising such a large space for drug development, less than 5% of recently approved therapies by the Food and Drug administration (FDA) target metalloenzymes, making them attractive and underrepresented pharmacological targets. While the metal ion can act as a structural domain, metal ions are also found in the active site to catalyze various transformations. The vast majority of metalloprotein inhibitors typically bind to the catalytic active site metal ion, hindering the activity of the enzyme.[1] To enable a tight coordination, compounds with charged or highly polar groups are necessary, which generates compounds that are generally hydrophilic. While improved water solubility can be desirable for a pharmacological modulator, these properties can also hamper their biological activity through various side effects or poor cellular permeability.[2] Among different strategies to retain these polar functional groups of a promising inhibitor, the hydrophilic groups can be protected using a prodrug strategy. To date, various protecting groups have been introduced and are currently utilized in clinically approved drugs. Despite their success, the regulation of the pharmacokinetic properties of the drug release represents a challenging research topic. As a complementary strategy, the release by an external trigger is considered favorable to regulate the dosing and location of a therapeutic, presenting the possibility to enable a selective treatment only at the target site.[3]

Design

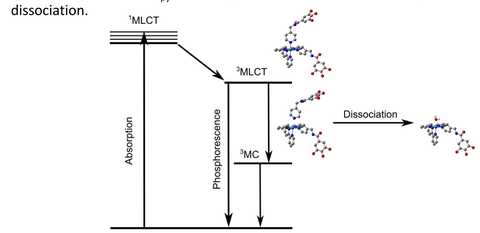
As a model metalloenzyme system, herein, the RNA-dependent RNA polymerase of the influenza virus was chosen. The viral N-terminal domain (PA_N) of the PA polymerase subunit is a validated target with no known human homolog, and is highly conserved over all circulating influenza strains. Inhibitors have been described that target the PA_N by binding to its dinuclear Mn^{2+} or Mg^{2+} active site.[4]

The basis of the compound design is gallic acid that has a known IC_{50} value (half maximal inhibitory concentration) of 4.2±1.0 µM against PA_N.[5] To enable elaboration of this compound for conjugation with a photoactive metal center, the binding pose of this compound was predicted in the active site. The three-dimensional geometry of the fragment was optimized using DFT calculations and the binding pose within the active site was anticipated using docking studies. While the hydroxyl group in the 4-position coordinates to both metal centers, the binding is further supported by the axial coordination of the hydroxyl groups in positions 3 and 5. To enable the coordination of a metal complex which can release the inhibitor upon a light trigger, a pyridine functionality was chosen as a conjugating group, which could be linked by a peptide bond. Docking studies of this molecule revealed that the additional pyridyl moiety could potentially also interact within the active site by hydrogen bonding with Arg124.

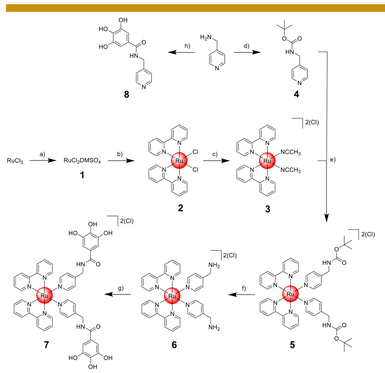


Photoejection Mechanism

As the basis for the photoreleasing moiety, Ru(II) polypyridine complexes were investigated due to their attractive chemical and photophysical properties. A derivative of $[Ru(2,2´\text{-bipyridine})_3]^{2+}$ was generated by exchanging one 2,2´-bipyridine ligand with two of the gallic acid-derived inhibitors. The mechanism of the photo-induced dissociation of the pyridine ligand from the scaffold was investigated by time-dependent DFT calculations. Upon irradiation, the Ru(II) polypyridine complex is excited to a singlet metal-to-ligand charge transfer (^{1}MLCT) state from which it can undergo an intersystem crossing process to a longer lived triplet ^{3}MLCT state. This excited state can decay back to the ground state upon emitting of a phosphorescence signal, making the complex also potentially suitable as an imaging probe. Alternatively, the excited triplet metal-centered-(^{3}MC) state can be populated, which can promote the photoinduced ligand loss of the monodentate pyridine derivative. The theoretical calculations indicate that the pyridine ligand has a distorted geometry and the Ru-N_{py} bond length is increased about 0.63 Å, which could explain the dissociation.



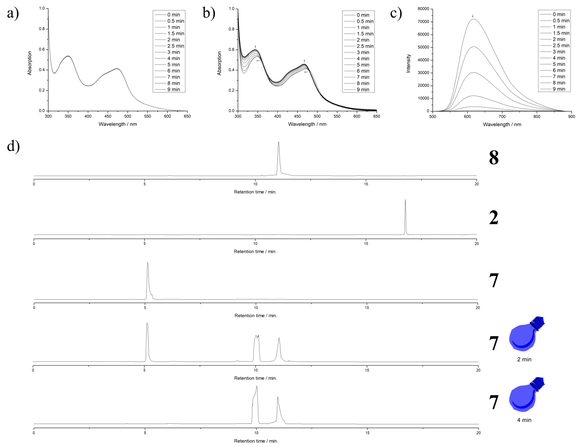
Synthesis



Synthetic strategy for the synthesis of the compounds. a) EtOH, 5h; DMSO, 150 °C, 3h; b) 2,2´bipyridine, LiCl, DMF, 4h, nitrogen atmosphere; c) AgOTf, CH_3CN, 3h; d) $ZrCl_4$, Boc_2O, CH_3CN, 1h; e) EtOH, 50 °C, 8h, nitrogen atmosphere; f) DCM/MeOH (4:1), HCl in Et_2O (2M), overnight; g) 1. gallic acid, $SOCl_2$, DCM, 2h, 2. DCM, 5h; h) gallic acid, $SOCl_2$, DCM, 2h.

Photophysical Activity

The ability to release the enzyme inhibitor from the Ru(II) coordination sphere of **7** was investigated using various experimental techniques. Compound **7** was incubated in H_2O and the temporal change of the absorption spectrum was monitored in the dark as well as upon irradiation at 450 nm. While no changes in the dark were observed (a), the absorption spectrum did show clear shifts after irradiation for 1 min with two isosbestic points (b), indicating changes in the molecular structure of the compound. Within 3 min, new absorption characteristics were asymptotically reached. The changes of **7** were also investigated by following the luminescence properties of the Ru(II) polypyridine complex. Immediately after exposure to the light, a drastic decrease of the luminescence signal of the metal complex was observed (c). After irradiation for 3 min, no emission could be detected, indicating that the generated photoproduct is photo-inactive. Following this assessment, the release of the enzyme inhibitor was studied by HPLC analysis (d). After a 2 min irradiation, the HPLC trace showed some remaining Ru(II) polypyridine-inhibitor conjugate (R_t = 5.1 min), the released enzyme inhibitor (R_t = 11.0 min), as well as the appearance of a new Ru complex-based product peak (R_t = 9.9-10.1 min) as identified by the characteristic retention time and absorption spectrum. Upon doubling of the irradiation time to 4 min, full conversion was reached with the complete release of the enzyme inhibitor. The release of inhibitor from the light irradiated sample was further confirmed by mass spectrometry.

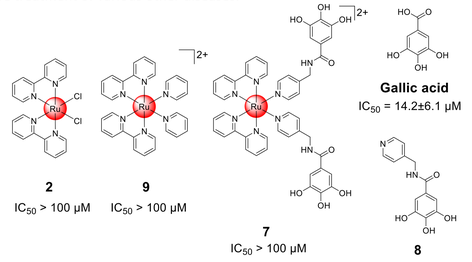


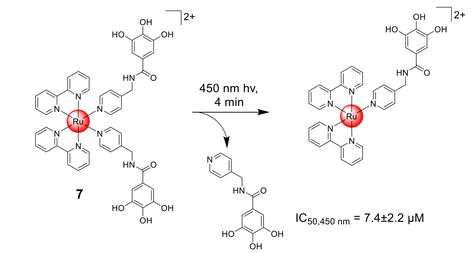
Biochemical Activity

To determine the biological activity of the Ru(II) polypyridine-PA_N inhibitor conjugate **7**, a Förster resonance energy transfer (FRET)-based enzymatic assay was utilized. All Ru-based compounds **2**, **7**, and **9** showed essential no significant inhibition (IC_{50} >100 µM) in the dark, indicating that the 'metallocaged' compounds are effectively sequestered. Importantly, compound **8**, the product of the ligand ejection, showed inhibitory activity in the micromolar range (IC_{50} = 9.3±3.7 µM) similar to gallic acid (IC_{50} = 14.2±6.1 µM).

Following this, the biological effect of the conjugate **7** upon irradiation was studied. The PA_N enzyme-compound mixture was irradiated at 450 nm (0.84 J/cm^2) for 4 min, as previous investigations have shown that this light dose is necessary for a complete photorelease of the enzyme inhibitor from the Ru(II) center. It is important to note, that exposing the enzyme to a 4 min irradiation did not alter its activity. While complex **7** showed no inhibition effect in the dark ($IC_{50,dark}$ >100 µM), upon light exposure enzyme inhibition was observed ($IC_{50,450\ nm}$ = 7.4±2.2 µM). Similar light irradiation of complex **9** showed negligible enzymatic inhibition, confirming that the Ru(II) polypyridine complex acts purely as a caging agents.

Overall, we believe that the approach of computationally-guided compound design, as well as the combination of a photoactive metal complex with an organic inhibitor holds great potential for selective photo-triggered inhibitor release and can open avenues for the selective treatment of various other diseases.





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