Introduction

The influenza virus infects about 3 billion people worldwide and kills hundreds of thousands of people each year. The virus frequently mutates, making it a difficult target for vaccine. A mutation in a membrane protein, the M2 channel, causes the virus to be more resistant to antivirals and influenza. Neuraminidase inhibitors are also vulnerable to viral resistance.

We are working to design compounds that inhibit influenza M2 in a unique, possibly irreversible way. i.e. metal complexes of Cu(II) with IDA (1,10-phenanthroline) have been shown to kill the highly conserved activity centering H3N2 strains. These compounds have also been shown to inhibit M2 proton conductance in liposomes and full-length M2 protein expressed in oocytes, and viral infection of MDCK cells. Absorption spectroscopy was found to be a useful method of identifying metal-complex stability in different buffer solutions.

Methods & Materials

Compound Synthesis

Liposome Assay

Oocyte Assay

Viral Mini-Plaque Assay

Figure 1: Molecular Dynamics Simulations of AMT in M2. Structure of M2 22-62 WT (a) before and (b) after binding AMT. Seitenko et al., 2001. AMT is shown as a ball and stick model in red. The S31N mutation site is marked in yellow. The His-37 site is marked in blue. The N-terminal domain and the transmembrane domain are marked in green and yellow, respectively.

Figure 2: Membrane current traces for Xenopus laevis oocytes transfected with Udorn/72 M2 WT, Udorn/72 M2 S31N, and Udorn/72 M2 WT and S31N. The traces were recorded with a two-electrode voltage-clamp apparatus at Vm=-20 mV. Oocyte current is shown in red. The background current is shown in black. The background current is due to the leakage of the oocyte membrane.

Figure 3: Intradiscal Complexes Transformed from Cu(IDAA), Cu(IDA), Cu(IDA), Cu(AMT-IDA), Cu(CO-IDAA), Cu(AMT-IDAA)

Figure 4: LCQ-Dynasty Purify Analysis of compound and metal were used to determine compound identity.

Results

Figure 5: Stability Assay for Cu(IDAA).

Figure 6: Potentiometric pH measurements of Cu(IDAA) in the presence and absence of Udorn/72 M2 WT, Udorn/72 M2 S31N, and Udorn/72 M2 WT and S31N. The pH change due to titrationsreaction through the M2 liposomes accumulate in the cytoplasmatic region of the M2 channel. The pH change due to titrationsreaction through the M2 liposomes accumulate in the cytoplasmatic region of the M2 channel.

Figure 7: Stability Assay for Cu(IDAA).

Figure 8: Membrane current traces for Xenopus laevis oocytes transfected with Udorn/72 M2 WT and S31N. The traces were recorded with a two-electrode voltage-clamp apparatus at Vm=-20 mV. Oocyte current is shown in red. The background current is shown in black. The background current is due to the leakage of the oocyte membrane.

Figure 9: Intradiscal Complexes Transformed from Cu(IDAA), Cu(IDA), Cu(AMT-IDA), Cu(CO-IDAA), Cu(IDA), Cu(AMT-IDAA)

Figure 10: Transient Oocyte TEVC Protein Current Inhibition. Parental X. laevis m (or 68-14, X. laevis S31N) and Udorn/72 m (or 68-14, Udorn/72 S31N) were subjected to 10 min exposure to 10 µM metal complexes. In the absence of metal complexes, the net inward current shown in red is due to background current. A small decrease in current is observed in the presence of metal complexes.

Figure 11: Intradiscal Complexes Transformed from Cu(IDAA), Cu(IDA), Cu(AMT-IDA), Cu(CO-IDAA), Cu(IDA), Cu(AMT-IDAA)

Figure 12: EC50 of Drug Candidates Against Influenza A Virus in Mini-Plaques. The drug candidates were evaluated in a virus infection assay using MDCK cells infected with the virus. The EC50 values were determined using a least-squared fit of a binding function in KaleidaGraph.

Figure 13: EC50 of Drug Candidates. The drug candidates were evaluated in a virus infection assay using MDCK cells infected with the virus. The EC50 values were determined using a least-squared fit of a binding function in KaleidaGraph.

Acknowledgements

The authors thank the NIH for providing grants for influenza research, and several colleagues for help with project design and execution. The authors also thank the following for their support: Dr. DeGrado, W. F.; Lamb, R. A.; Pinto, L. H.; Cu(II) Inhibition of the M2 proton channel. J. Am. Chem. Soc. 2001, 123, 274.

References


