Using a Molecular Modeling and Native State Mass Spectrometry Quick Screening Approach to Understand the Inhibition of IMP-1 Variants

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Overview

In this study we used a molecular modeling and native state mass spectrometry quick screening approach to determine if a variety of inhibitors would be capable of inhibiting IMP-1 and the variant IMP-78. The inhibitors chosen showed promising inhibition towards IMP-1. Native state mass spectrometry was used to confirm the mechanism of inhibition of each inhibitor. Some differences were observed between the inhibition of IMP-1 and IMP-78. Future studies are needed to elucidate the differences in inhibition. The results of this study will help the future design of MBL inhibitors and highlight the importance of including MBL variants in inhibitor design.

Introduction

Bacterial infections are most commonly treated by the use of β -lactam antibiotics[1]. β -lactam antibiotics inhibit the transpeptidases, penicillin-binding proteins (PBPs), involved in cell well biosynthesis[2]. Resistance to β-lactam antibiotics has become increasingly prevalent since the introduction of antibiotics. Each year in the United States approximately two million people acquire bacterial infections that are resistant to one or more antibiotics and about 23,000 people die due to this resistance[3]. A common mechanism for β-lactam resistance is the production of β-lactamases that hydrolyze the β-lactam ring, thus rendering the drugs inactive[1]. Today there are more than 2000 β-lactamases, but this study will focus on the B1 subclass known as metallo-β-lactamases (MBLs). MBLs are capable of inactivating all β-lactam antibiotics, except monobactams, and do not have any known clinical inhibitors. Thus, the development of MBL inhibitors is crucial[4]. Most studies focus on the three most clinically relevant MBLs which include: Imipenemase MBL (IMP-1); Verona integrin-encoded MBL (VIM-2); and New Delhi MBL (NDM-1). Over time each of these MBLs have evolved and now have many variants whose inhibition is poorly studied. This study will focus on IMP-1 and the variant IMP-78 (S262G/V67F), whose two mutations are near the active site. The inhibition of IMP-1 is widely studied, however, it is not known whether an inhibitor of IMP-1 is also capable of inhibition IMP-78. Native state electrospray ionization mass spectrometry (ESI-MS) was used to quickly determine the mechanism of inhibition. Molecular modeling and molecular dynamic (MD) simulations were used to further probe the binding of inhibitors. The binding affinity of each inhibitor for IMP-1 and IMP-78 will be determined using ITC. Lastly, microbiological studies (MICs) will be performed to determine *in vivo* activity against meropenem.

Results

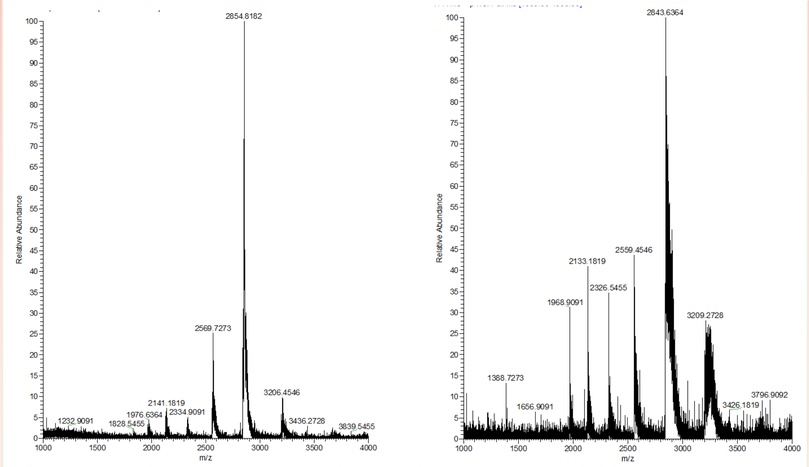

Figure 1. Native mass spectra of IMP-1 (left) and IMP-78 (right)

Native state electrospray ionization mass spectrometry was used to analyze IMP-1 and IMP-78. Figure 1 (left) shows the mass spectrum of IMP-1. The +9 peak (2,854 m/z) corresponds to the mass of IMP-1 with 2 eq Zn(II) bound (25,568 Da). Figure 1 (right) shows the mass spectrum of IMP-78. The +9 peak (2,843 m/z) corresponds to the mass of IMP-78 with 2 eq Zn(II) bound (25,615 Da).

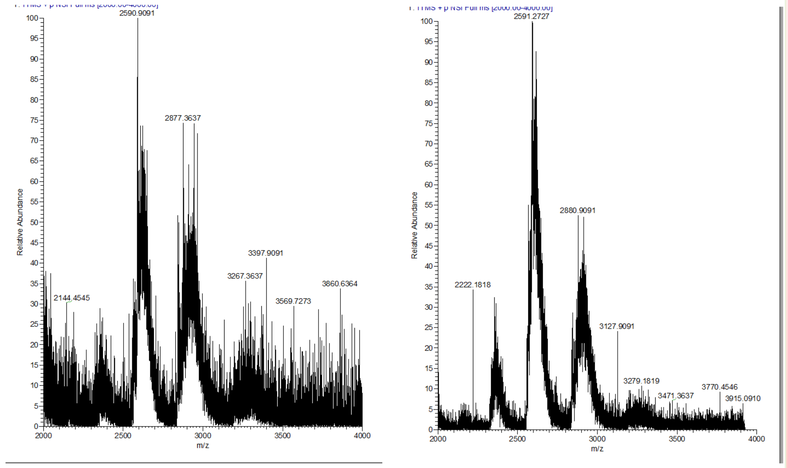

Figure 2. Native mass spectra of IMP-1 (left) with 1 eq compound QPX 7546 bound and IMP-78 (right) with 1 eq compound QPX 7546.

Native mass spectrometry revealed the formation of a ternary complex between IMP-1 and QPX 7546 and the variant IMP-78 and QPX 7546. Of the 6 different inhibitors analyzed, only 3 formed a ternary complex with both IMP-1 and IMP-78. The +9 peak (2,591 m/z) in Figure 2 (left) corresponds to the mass of 1 eq compound QPX 7546 bound to 2 Zn(II) bound IMP-1 (25,904 Da). The +9 peak (2,591 m/z) in Figure 2 (right) corresponds to the mass of 2 Zn(II) bound IMP-78 with 1 eq of compound QPX 7546 bound (25,951 Da).

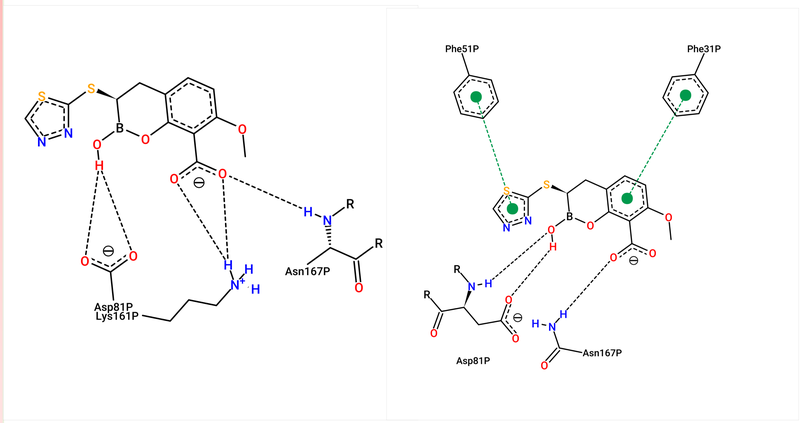

Figure 3. Molecular model of IMP-1 (left) and IMP-78 (right) with compound QPX 7546.

The molecular modeling of IMP-1 and IMP-78 showed binding of the compound QPX 7546, in agreement with the native MS results. QPX 7546 interacted with the following residues in both IMP-1 and IMP-78: Asp81 and Asn167. In the model of IMP-1 and QPX 7546, there is also interaction with Lys161. While in the model of IMP-78 and QPX 7546 there is a hydrophobic interaction and pi-pi stacking between QPX 7546 and the Phe31 residue.

Conclusions and Future Work

In conclusion, the results of native MS showed differences in the inhibition of IMP-1 and IMP-78 by the six inhibitors studied. Three of the inhibitors formed a ternary complex with IMP-1, but did not form a ternary complex with IMP-78. As this study is currently ongoing, there is future work to be completed. To investigate whether one or both amino acid mutations in IMP-78 prevent inhibitor binding, IMP-6 (S262G) and IMP-10 (V67F) will be analyzed using native state ESI-MS. The remainder of the molecular modeling and molecular dynamic (MD) simulations will be completed on each inhibitor with IMP-1 and IMP-78. Molecular modeling and MD simulations will be performed for the 3 inhibitors that bind IMP-1 but not IMP-78 using IMP-6 and IMP-10. The binding affinity of each inhibitor for IMP-1 and IMP-78 will be determined using ITC. Lastly, microbiological studies (MIC's) will be conducted.

References

(1)Aitha, M., et al. *J. Inorg. Biochem.* **2014**, *136, 40-46.*
(2)Brem, J., et al., *Nat. Chem.* **2014**, *6, 1084-1090.*
(3)Frieden, T. *Broch. – US Cent. Dis. Control Prev.* **2013**
(4)Ju, L. C., et al. *Trends in Pharmacological Sciences.* **2018**, *39 (7), 635-647.*