Inhibition of New Delhi metallo-beta-lactamase byhydroxy(thiol)pyrone and hydroxy(thiol)pyridinone compounds.

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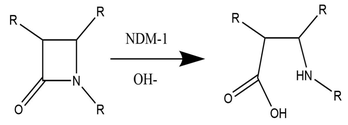
Abstract

Bacteria has developed naturally to resist antibiotics and this is a major threat for human health. New Delhi metallo-beta-lactamase 1 (NDM-1) is an enzyme produced by several genera of bacteria that render the bacteria resistant to many antibiotics. Currently there is no inhibitor of NDM-1 available in clinical therapy, thus making an essential need for research and development of a NDM-1 inhibitor. In this research we collected data using *AutoDock* programs to estimate the binding affinity of four potential molecule inhibitors. The molecules were maltol, thiomaltol, DMHP, and DMHTP which act as ligands to inhibit the NDM-1 enzyme by binding to the active site and blocking the catalytic enzyme activity and killing the bacteria.

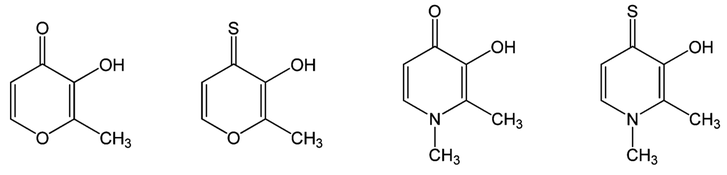
Introduction

Antibiotics play an important role in the treatment of bacterial infections, reducing mortality and preserving human life. However, β-lactamases are major defensive mechanisms in gram negative bacteria against antibiotics. The prevalent mechanisms of bacterial resistance include reduced cell wall permeability, antibiotic efflux and enzyme-mediated drug degradation [1]. Accompanied by the worldwide use of β-lactams, β-lactamase-mediated antimicrobial resistance has drawn ever more concern as the enzyme-encoding genes are often located on transferable units and are easily spread between pathogens [1]. Based on their molecular properties and/or amino acid sequences, β-lactamases can be divided into classes A, B, C and D, with classes A, C and D exhibiting hydrolytic capability depending on a serine in the active site. Class B requires either one or two zinc ions as the nucleophile and thus are known as metallo-β-lactamases (MBLs) [1]. There are three subclasses of MBLs (B1, B2 and B3), of which the class B1 New Delhi metallo-β-lactamase 1 (NDM-1). First reported in 2008, NDM-1 was initially identified in *Klebsiella pneumoniae* and *Escherichia coli*. NDM-1 is an enzyme produced by particular strains of bacteria to resist beta-lactam antibiotics and cause infections. Bacteria with the NDM-1 gene are part of a larger group of suberbug bacteria that are extremely hard to treat and can spread easily in hospitals NDM-1 hydrolyzes and inactivates beta-lactam antibiotics through breaking the beta-lactam ring as shown below.



Scheme 1 NDM-1 enzyme hydrolyzation

Maltol, thiomaltol, DMHP (Dimethylheptylpyran), and DMHTP are the four molecules selected as potential inhibitors for NDM-1 enzyme. Zn(II) in NDM-1 is more strongly bound by the softer O,S-donor ligands. In addition, they were selected based on Dr. Kim's experience in this field. Every molecule has a different binding affinity based on virtual screening *AutoDock Vina* and *AutoDock 4.2*.



Maltol **Thiomaltol** **DMHP** **DMHTP**

Scheme 2 Structural Formulae of the ligands

Results

Molecular Docking by AutoDock Vina

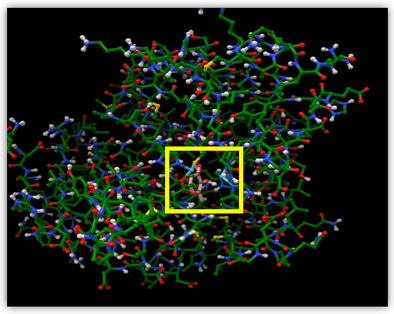
Table 1. Computed affinities by *Autodock Vina* for hydroxy(thiol)pyrone and hydroxy(thiol)pyridinone compound.

Configuration	Maltol Affinity (kcal/mol)	Thiomaltol Affinity (kcal/mol)	DMHP Affinity (kcal/mol)	DMHTP Affinity (kcal/mol)
1	-5.3	-4.6	-5.4	-5.0
2	-4.7	-4.4	-5.2	-5.0
3	-4.5	-4.4	-4.9	-4.8
4	-4.4	-4.1	-4.9	-4.7
5	-4.3	-4.1	-4.8	-4.0
6	-4.3	-3.9	-4.5	-4.0
7	-4.2	-3.9	-4.5	-4.0
8	-4.1	-3.9	-4.4	-3.8
9	-4.0	-3.7	-4.4	-3.8

In Table 1 there are 9 configurations were provided as shown in the table. However, not all of these configurations show the molecule near to Zn^{2+} ions site. binding affinity between a ligand and its target molecule may be affected by the presence of other molecules.

NDM-1 Protein

This first picture shows 3D structure of NDM-1 and where Zinc (II) is located. There are two Zn^{2+} ions in one NDM-1 molecule as shown in yellow box. This is where the ligands must bind in order to inactivate the enzyme.

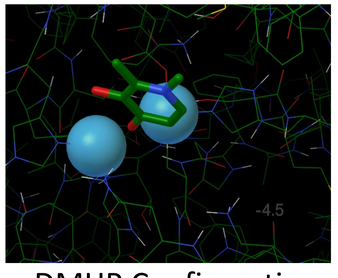


Molecular Docking by AutoDock 4.2

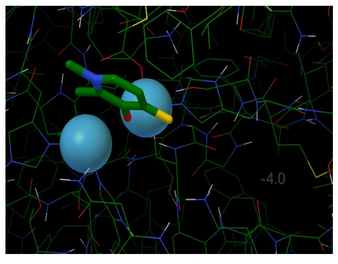
There are Zn^{2+} ions at the protein active site drawn as blue spheres. The green molecule in the top is DMHP and the one in the bottom is DMHTP.

DMHP in that configuration has -4.5 kcal/mol binding affinity while DMHTP has -4.0 kcal/mol. Both ligands are located close to the Zn^{2+} ions where they more likely to bind.

Although thiomaltol has nine configurations with variety affinities, it did not bind close to Zn^{2+} ions, therefore, it was excluded from this section.

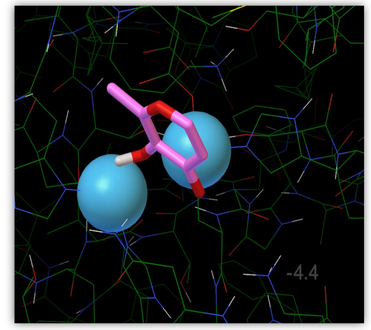


DMHP Configuration



DMHTP Configuration

In the right picture, maltol is shown in pink and zinc ions are blue spheres. maltol molecule has -4.4 kcal/mol binding affinity which is very close to DMHP's affinity. In addition, maltol was the nearest molecule to Zn^{2+} ions among the other molecules according to the 3D structure on *AutoDock 4.2*. Therefore, it was selected to be tested as an inhibitor for NDM-1 enzyme. However, DMHP might be tested as well in future investigation. Moreover, based on other researches, maltol worked efficiently as metalloprotein inhibitor.



Maltol Configuration

IC_{50} (50% Inhibition Concentration)

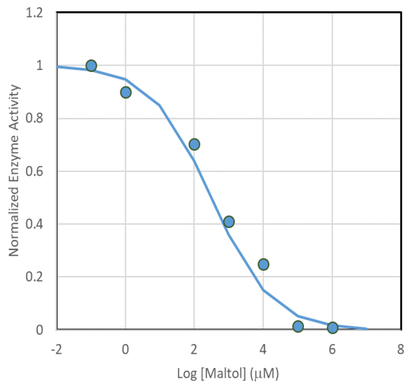


Figure 1 illustrates 0.13 µg/mL NDM-1 activity with different concentrations of maltol for 10 min, followed by assaying enzyme activity with 36 µM nitrocefin. The activity of the enzyme decreases when higher concentrations of maltol is used. The enzyme became inactive when approximately 6.0 µM of maltol is added. The IC_{50} was calculated to be 350 ± 25 µM which is considered too high to be a potential drug candidate.

Figure 1. Enzyme activity of 0.13 µg/mL NDM-1 with various concentrations of maltol

Conclusion

AutoDock 4.2 illustrates the visual binding between the molecule and the protein, but it cannot estimate the lowest and most efficient concentration of the molecule.

In addition, *AutoDock vina* provides various of binding affinities for each potential molecule. However, The binding affinities amounts are fluctuating from one molecule to another with different locations around the protein.

Determining the best configuration was based in the distance from Zn^{2+} ions and secondly, on the lowest affinity value. This test showed similar results from DMHP molecule and maltol, which leads to the selection of maltol as the first option.

According to the results, maltol cannot be considered as potential drug candidate to kill the bacteria with NDM-1 gene because it has high inhibition concentration. Therefore, there is a further investigation to find a potent molecule for NDM-1 inhibition.

References

1. Chaves, Sílvia, et al. "Complexes of Hydroxy(Thio)Pyrone and Hydroxy(Thio)Pyridinone with Zn(Ii) and Mo(Vi). Thermodynamic Stability and Insulin-Mimetic Activity." *Metallomics*, vol. 2, no. 3, 2010, pp. 220–227., doi:10.1039/b914169c.
2. Shi, Cheng & Chen, Jiaxing & Xiao, Bin & Kang, Xinyue & Lao, Xingzhen & Zheng, Heng. (2019). Discovery of NDM-1 inhibitors from natural products. Journal of Global Antimicrobial Resistance. 18. 10.1016/j.jgar.2019.02.003.