Computational Analysis of Bacterial Protein-Inhibitory Interactions

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Overview

- 19 Honokiol-inspired analogs shown to have inhibitory bioactivity[1] were initially docked with five potential protein targets using AutoDock Vina, a computational docking program.
- The binding energy of Honokiol-inspired analogs with cell division protein FtsA were found to have a relatively strong positive correlation between Ln(MIC). Six structural variations of FtsA were further explored. Strong positive correlations emerged between the binding energies of the analogs and the FtsA proteins found in *Staphylococcus aureus*.
- Analysis of residue-ligand interactions suggested that the Honokiol-inspired analogs act as competitive inhibitors of ATP, disrupting cell division and bacterial growth.
- Insights into these antimicrobial agents mode of action informed the design of three additional analogs.

Methods and Materials

Minimum Inhibitory Concentration: Minimum inhibitory concentration assays of the analogs against three representative Gram-positive bacteria (*S. Epidermidis, E. Faecalis* and *Methicillin-Resistant S. aureus* (MRSA)) was undertaken in a previous study. This data was averaged and and the natural log was calculated.

AutoDock Vina[2]: A program in the AutoDock Suite that predicts a set of optimal bound conformations and binding energy of small molecule ligands with a target protein. The program also analyzes protein-ligand binding residues.

- Simplifications: assumes a rigid receptor and flexible ligand with flexibility specified by the user. Users must also specify search space around the receptor that the docking algorithm will explore.
- Accuracy: Binding energy is given in kcal per mol with errors of roughly 2-3 kcal/mol[3].
- AutoDock Vina scores the conformations and free energies based on steric interactions:
  - Steric interactions
  - Hydrogen-bonding
  - Hydrophobicity/hydrophilicity

Computed Atlas of Surface Topography of proteins (CASTp 3.0)[4] : A web server that locates, delineates and measures the topological properties of protein structures using the alpha shape method. The server was used to identify pockets on the protein's surface as potential binding sites. Pockets with volumes greater than 50 Angstroms were considered.

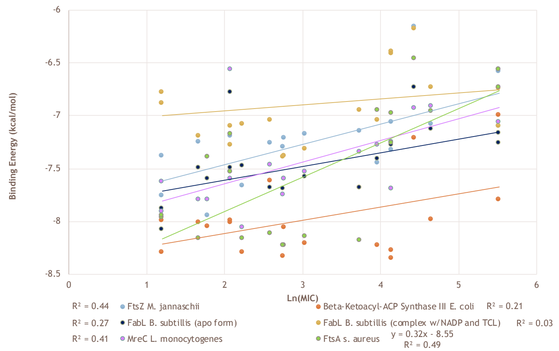
PyMOL[5]: An open-source molecular visualization system that was used to edit protein files and analyze AutoDock Vina results.

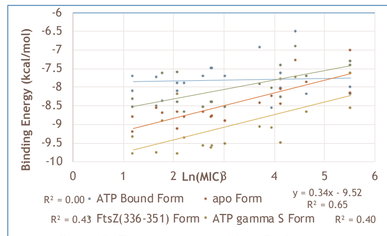
Analysis: The natural log of minimum inhibitory content (Ln(MIC)) and binding energy was graphed using a simple linear regression. If the protein is the target of the ligands, then the degree of binding of the ligand to the protein should be linearly correlated to the concentration needed to inhibit bacterial growth. Small ln(MIC), strong inhibition, is correlated to more negative binding energy (i.e. stronger binding).

Results

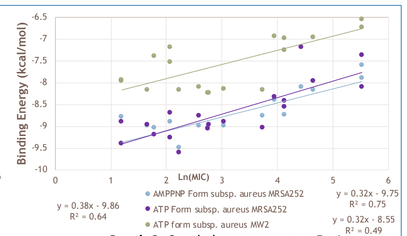
I. Minimum Inhibitory Concentration(MIC) vs Binding Energies

Graph 1. Initial Proteins Graphed by Ln(MIC) v. Binding Energy (kcal/mol)





Graph 2. *Thermotoga maritima* FtsA



Graph 3. *Staphylococcus aureus* FtsA

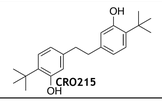
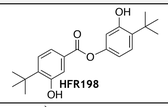
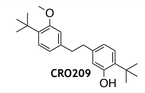
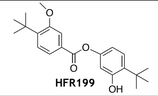
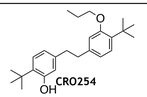
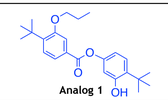
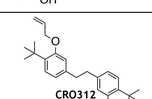
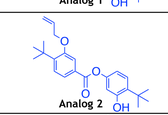
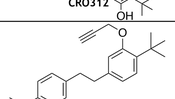
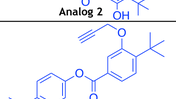
II. Identifying Best Analogs

Figure 1. Table of FEBs for ligands with *S. aureus* MRSA252

Analog	Binding Energy of AMPPNP Form of *S. aureus* (kcal/mol)	Binding Energy of ATP Form of *S. aureus* (kcal/mol)
CRO215	-8.8	-8.9
CRO254	-8.9	-9.0
CRO209	-9.0	-9.0
CRO312	-9.1	-9.0
CRO262	-9.3	-9.3
HFR198	-9.4	-9.4
HFR199	-9.6	-9.5
Analog 1	**-9.3**	**-9.3**
Analog 2	**-9.3**	**-9.3**
Analog 3	**-9.7**	**-9.7**
ATP	-10	-10.4

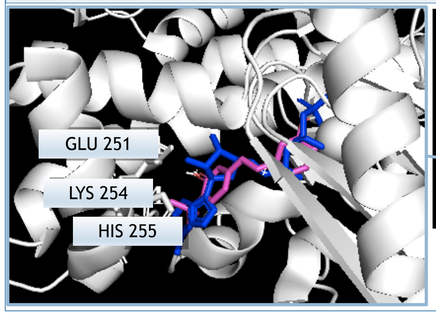
II. Structure of Best Analogs and Design of New Analogs

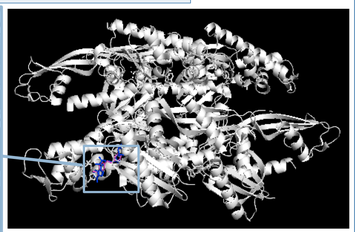
Figure 2.

Phenol Protection	Linker Group		
None		 CRO215	 HFR198
		 CRO209	 HFR199
		 CRO254	 Analog 1
		 CRO312	 Analog 2
		 CRO262	 Analog 3

IV. Residue-Ligand Interactions

Figure 3. Binding of CRO215 (pink) at the binding site of AMPPNP (blue) in *S. aureus MRSA252*[7].





Discussion

- A strong positive correlation was found between the analogs' ln(MIC) and binding energy resulting from the docking with cell-division protein FtsA expressed in *Staphylococcus aureus* subsp. aureus MW2. FtsA is necessary for cell division and inhibition of this protein leads to filamentation and eventual cell death. Binding of ATP to FtsA is required for its proper functioning[8].
- Six other versions of FtsA expressed in both *Staphylococcus aureus* and *Thermotoga maritima* were analyzed in the same way with strong positive correlations found between the analogs' average ln(MIC) and binding energy with FtsA as it is expressed in *Staphylococcus aureus*.
- *Staphylococcus aureus* subsp. MRSA252 is a gram-positive bacteria that grows in anaerobic and aerobic conditions. It is a methicillin-resistant strain of *Staphylococcus aureus* and is a major cause of community- and hospital- acquired infections. This form of FtsA most likely matches the bacteria screened in the minimum inhibitory concentration assays which included MRSA.
- Further analysis of ligand-residue interactions on AutoDock Vina and PyMol showed that the 19 Honokiol-inspired analogs were specifically binding in the ATP (or AMPPNP) binding spot of FtsA and may be acting as competitive inhibitors to ATP and inhibiting cell division. Although these analogs may be acting as competitive inhibitors to ATP, ATP consistently had a lower binding energy with FtsA which indicates that a higher concentration of the analogs compared to ATP is required for an inhibitory effect.
- The analogs with the lowest predicted binding energy were identified and the features of these analogs were used to design three new analogs. Initial docking of these three newly designed compounds with *Staphylococcus aureus* subsp. MRSA252 FtsA revealed lower binding energies compared to the parent compound CRO215, with Analog 3 having among the lowest predicted binding energies among all the compounds, suggesting that it may be an effective antibacterial agent.

Conclusions

- This research explores the cell-division protein FtsA as a potential target for antibacterial agents and potential FtsA inhibitors.
- Honokiol-inspired analogs which have shown to have inhibitory bioactivity may be acting at competitive inhibitors to ATP binding with the cell-division protein FtsA.
- Three new analogs, including 4-(*tert*-butyl)-3-hydroxyphenyl 4-(*tert*-butyl)-3-(prop-2-yn-1-loxy)benzoate, are proposed and preliminary predicted binding energy suggests that these analogs may work as antibacterial agents. Further biological experiments should be conducted to explore the effectiveness of these analogs as antibacterial agents.
- Further research should be conducted into the role of FtsA in cytokinesis as FtsA inhibitors may be appealing antibacterial agents.

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