Integrated Screening for Beta-Lactamases Inhibitors Identification of Pharmaceutical Hits and Lead Optimization

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Abstract 1

Beta-lactamases are enzymes produced by bacteria that provide multi-resistance to beta-lactam antibiotics such as penicillins, cephalosporins, cephamycins, and carbapenems (ertapenem). Beta-lactamase provides antibiotic resistance by breaking the antibiotics' structure. Bacteria that produce carbapenemases are often referred to in the news media as "superbugs" because infections caused by them are difficult to treat. Recently, with the use of virtual screening and docking to identify and characterize novel beta-lactamase inhibitors as potential therapeutics to treat to treat a broad spectrum of Gram-positive and Gram-negative bacteria has been reported in the literature. Hence, there is interest in the development of new and improved beta-lactamase inhibitors. Here, we describe the use of an iterative in silico and in vitro work-flow for identifying novel beta-lactamase inhibitors. The first in silico arm of the work-flow involves the use of library design, virtual screening, docking, and consensus scoring to identify predicted hit compounds. The in vitro arm involves rapid assaying of predicted hits in an optimized beta-lactamase assay. Confirmed hits are passed into the second (optimization) in silico arm which involves ligand-based screening, docking, and consensus scoring. We have added more criteria to our in silico docking model such as libraries of sub-structures of known substrates and inhibitors. Currently we have identified ractopamine as a μM inhibitor, Pharmaceutical Hit and are performing rational based drug design/Structure Activity Relationships. Recently we have identified a 50 micro-molar inhibitor. Preliminary results appear encouraging, providing hope that a novel beta-lactamases drug candidate will be identified and that our computational work-flow will prove useful on other targets.

Introduction 2

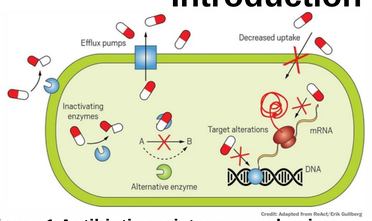

Figure 1 Antibiotic resistance mechanisms

- Pathogens have evolved several mechanisms to neutralize antibiotics
- They can use inactivating enzymes such as β-lactamases to destroy antibiotics containing β-lactam rings.
- They can increase the production of efflux pumps to spit antibiotics back out of the cell.
- They can alter the composition of their cell wall to decrease antibiotic uptake.
- They can alter the genetic targets of some antibiotics, and they can replace enzymes targeted by antibiotics with alternative enzymes that carry out the same function.

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Introduction 3

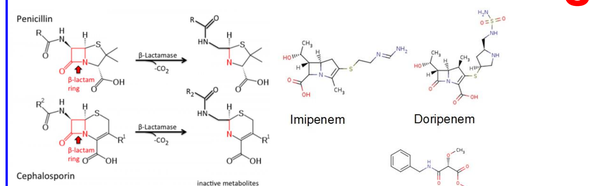


Figure 2. Core structures for penicillins & cephalosporin antibiotics & hydrolysis by beta-lactamase.

Figure 3 Natural Substrates of beta-lactamase

- Penicillins & cephalosporins share a common four-atom beta-lactam ring.
- Beta-lactamases are a family of enzymes produced by some gram-negative bacteria that provide a resistance to beta-lactam drugs by breaking the ring open by hydrolysis, which eliminates the molecule's antibacterial actions.
- Beta-lactam antibiotics are typically used to treat a broad spectrum of Gram-positive and Gram-negative bacteria

Taylor, S.C.; *Chirality Ind.* 2, 187-188 (1997)
Kurosaki, H.; *Biol. Pharm. Bull.* 38, 96-101 (2015)
http://tmedweb.tulane.edu/pharmwiki/doku.php/betalactam_pharm

Active Site 4

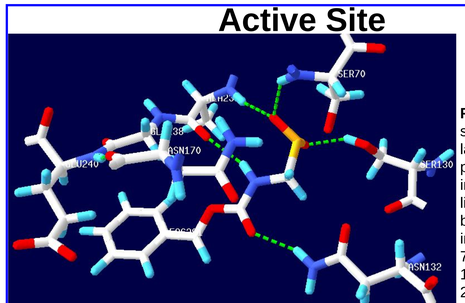


Figure 4 Active site of beta-lactamase and phosphate inhibitor. Green lines hydrogen bonding with inhibitor and Ser-70, Ser-130, Asn-132, Gly-238, Ala 237

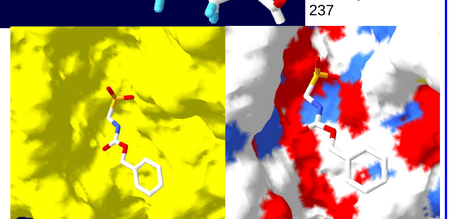
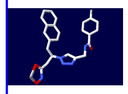


Figure 5 Surface and cavities of phosphate inhibitor revealing the potential cavities.

Samama, J.P. (1998) Biochemistry 37: 2622-2628

Molecular Docking 5

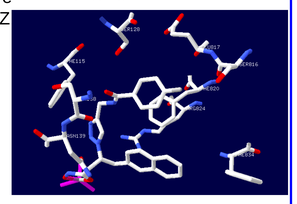


Virtual Libraries based on physical properties drug like

Docking Software Vega-ZZ MOE

Predicted ligand poses and binding affinities rescoring using four other criteria

Protein-Receptor



- Generates poses and ΔGs
- Each virtual compound has ten modeled poses
- Sort out duplicates with Pivot table the six different binding criteria
- Two different soft ware systems, Novel way to evaluate data
- Filter virtual compounds via similarity structures
- Look for hydrogen bonding interactions
- Analyze fit in binding pockets look for cavities in enzyme
- Look for acceptable pose buy compound test on *in vitro* assay
- Generate pharmacological hit

Known Inhibitors 6

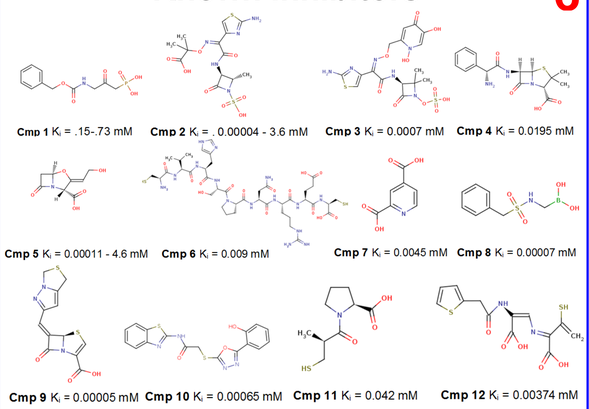


Figure 6 Structures of known beta-lactamase inhibitors.

Crowder, *ChemMedChem* 9, 2445-2448 (2014)
Prati, F.; *J. Med. Chem.* 53, 7852-7863 (2010)
Levesque, R.C.; *J. Antimicrob. Chemother.* 55, 252-255 (2005)
Bonomo, R.A.; *Antimicrob. Agents Chemother.* 54, 2867-2877 (2010)
Arakawa, Y.; *Antimicrob. Agents Chemother.* 53, 69-74 (2009)
Page, M.I.; *Biochemistry* 44, 8578-8589 (2005)
Galleni ; *Antimicrob. Agents Chemother.* 51, 2136-2142 (2007)
Pratt, R.F.; *J. Org. Chem.* 71, 4778-4785 (2006)
Bonomo, R.A.; *J. Antimicrob. Chemother.* 69, 682-690 (2014)
Pratt, R.; *Bioorg. Med. Chem.* 18, 282-291 (2010)
Vakulenko, S.B.; *Biochemistry* 54, 588-597 (2015)
Appl Microbiol. **17** (6): 783–6.

Allosteric Site 7

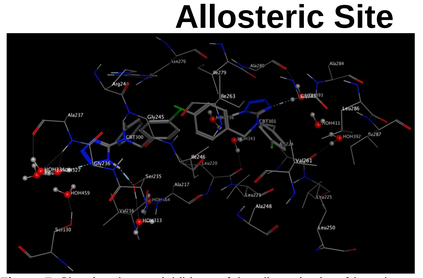
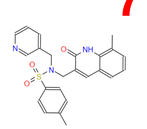
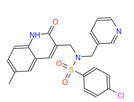


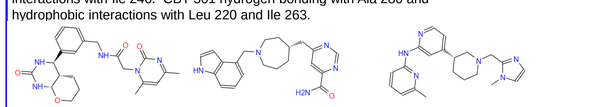
Figure 7 Showing the two inhibitors of the allosteric site of *beta-lactamase*. CBT 300 hydrogen bonding with Ala 237 and Ser 235. Hydrophobic interactions with Ile 246. CBT 301 hydrogen bonding with Ala 280 and hydrophobic interactions with Leu 220 and Ile 263.



BAS04190941



ACLSC-000355



BDE33672279 BDE32394870 BDE32446322

Figure 7 Structures of allosteric inhibitors from our virtual scan that scored high.

- Allosteric site is another site on the enzyme that can disable activity
- Limited data in the literature about the allosteric site

Horn, J.R., Shoichet, B.K. (2004) *J Mol Biol* 336: 1283-1291

Results-Molecular Modeling 8

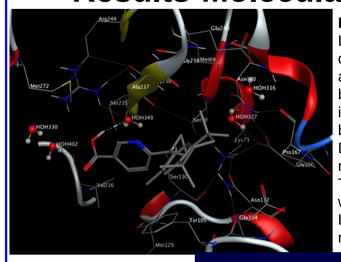


Figure 8 Repurposed drug LG100268 is a cancer and anti-viral drug that shows low micro-molar activity (50 μM) *in Vitro*. Active site of beta-lactamase and LG100268 inhibitor. Light blue lines hydrogen bonding with inhibitor and Arg-244. Data generated via molecular modeling. Π stacking interactions with Tyr-105. Hydrophobic interactions with Ser-130, Ser-70, Val-216, and Lys-73. Data generated via molecular modeling.

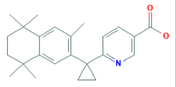


Figure 9 Repurposed drug LG100268 is a cancer and anti-viral drug that shows low micro-molar activity (50 μM) *in Vitro*.

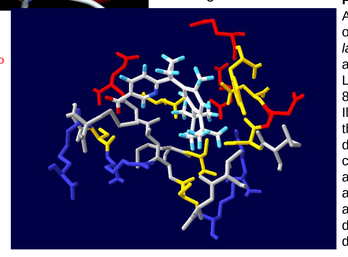


Figure 10 Active site of *beta-lactamase* and LG100268 inhibitor. Illustrating the different classes of amino acids to aid in drug design.

Results 9

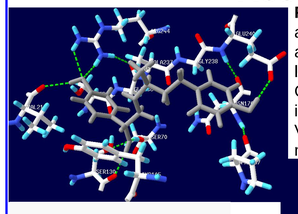


Figure 11 Ractopamine is a beta agonist drug shows high micro-molar activity *in Vitro*. Active site of beta-lactamase and ractopamine inhibitor. Green lines hydrogen bonding with inhibitor and Ser-70, Ser-130, Arg-244, Val-216, Glu 240. Data generated via molecular modeling.

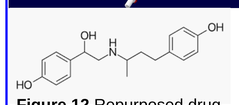


Figure 12 Repurposed drug **Ractopamine** is a feed additive, banned in many countries, to promote leanness in animals raised for their meat. Pharmacologically, it is a phenol-based TAAR1 agonist and β adrenoreceptor agonist that stimulates β_1 and β_2 adrenergic receptors. It is a low micro-molar inhibitor of *beta-lactamase*.

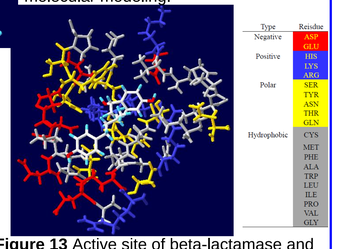


Figure 13 Active site of beta-lactamase and ractopamine inhibitor. Illustrating the different classes of amino acids to aid in drug design. For example the gray hydrophobic amino acids that could be exploited in future analogues.

Results-Molecular Modeling on Hit 10

R_1	R_2	R_3	R_4	R_5	R_6	R_7	R_8	R_9	R_{10}	R_{11}	R_{12}	PLANTS Scc
H	H	OH	H	H	CH_3	H	H	OH	H	H	C=O	-84.124
H	H	H	$C_2H_4B(OH)_2$	H	CH_3	H	H	OH	H	H	C=O	-103.200
H	H	H	$B(OH)_2$	H	CH_3	H	H	OH	H	H	C=O	-102.500
H	H	H	H	H	$C_{11}H_{15}$	H	H	OH	H	H	C=O	-101.800
H	H	$C_2H_4B(OH)_2$	H	H	CH_3	H	H	OH	H	H	C=O	-101.400
H	H	H	H	H	$C_{10}H_{13}$	H	H	OH	H	H	C=O	-101.300
H	H	H	H	H	C_9H_{11}	H	H	OH	H	H	C=O	-99.630
H	H	OH	H	H	$C_4H_7N_4S$	H	H	OH	H	H	C=O	-99.399
H	H	OH	H	H	$C_{10}H_{17}$	H	H	OH	H	H	C=O	-99.290
H	H	$C_2H_4B(OH)_2$	H	H	CH_3	H	H	OH	H	H	C=O	-99.270
H	H	OH	H	$C_9H_{12}O$	CH_3	H	H	OH	H	H	C=O	-99.203
H	H	H	H	H	$C_{10}H_8$	H	H	OH	H	H	C=O	-98.850
H	H	H	CH	H	CH_3	H	H	OH	H	H	C=O	-97.970
H	H	H	H	H	C_9H_{11}	H	H	OH	H	H	C=O	-97.090
H	H	H	H	H	C_6H_9	H	H	OH	H	H	C=O	-97.070
H	H	H	H	C_6H_5O	CH_3	H	H	OH	H	H	C=O	-96.120
H	H	H	H	H	C_2H_5	H	H	OH	H	H	C=O	-93.947
H	H	OH	H	H	CH_3	H	CH_2CO_2H	H	H	H	C=O	-93.544
H	H	OH	H	H	CH_3	H	$C_2H_4SO_2F$	H	H	H	C=O	-93.393
H	H	OH	H	H	CH_3	H	$CH_2PO(OH)_2$	H	H	H	C=O	-92.547
H	H	H	H	H	C_6H_5	H	H	OH	H	H	C=O	-92.270

Table (1): Virtual and Synthetic Ractopamine derivatives: Using molecular-docking software (Swiss-PdbViewer and VEGA ZZ), changes to Ring A and B were made based on knowledge of natural substrates, known inhibitors and knowledge of the three-dimensional structure of TEM-1 Beta-Lactamase. Binding energy, represented by PLANTS score, is based on algorithms called ant colony optimization which utilize protein flexibility to maximize hydrogen bonding between the ligand and active site. Ractopamine derivative PLANTS scores and Interacting Residues are shown in the data table above. Addition of hydrogen-bond rich functional groups at R3 and R4 and mainly aromatic compounds at R6 result in the greatest decrease in PLANTS score.

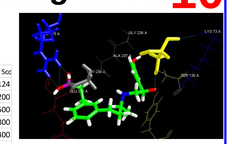


Figure 14 Docked Ractopamine derivative (R_4: $C_2H_4B(OH)_2$,R_3: H) ligand in TEM-1 active site. Critical and nearby residues labeled based on properties.

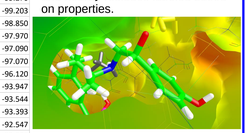
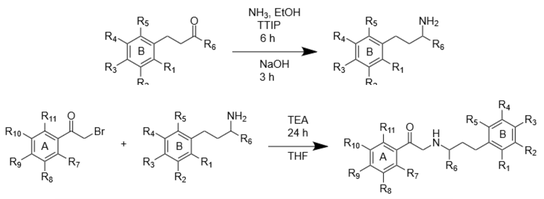


Figure 15 Docked Ractopamine derivative (R_4: $C_2H_4B(OH)_2$,R_3: H) ligand in TEM-1 active site showing topological polar surface area and active-site cavities.

Korb, O., Stützle, T. & Exner, T.E. An ant colony optimization approach to flexible protein–ligand docking. *Swarm Intell.* 2009, 49, 49–84. https://www.ncbi.nlm.nih.gov/pubmed/19125657
Opassi, G., Gesu, A. & Massarotti, A. The hitchhiker's guide to the chemical-biological galaxy. *Drug Discovery Today.* 2018, 23, 565—574. https://www.ncbi.nlm.nih.gov/pubmed/29330125

Synthetic Approach Initial SAR 11



R_1	R_2	R_3	R_4	R_5	R_6	R_7	R_8	R_9	R_{10}	R_{11}	Plant Score
H	H	OMe	H	H	CH_3	H	H	OMe	H	H	-66.627
H	H	OH	H	H	CH_3	H	H	OH	H	H	-79.655
H	H	H	H	H	C_6H_5	H	H	OMe	H	H	-86.145
H	H	H	H	H	C_6H_5	H	H	OH	H	H	-86.288
H	H	H	H	H	C_6H_5	H	H	H	COOH	H	-88.942
H	H	H	H	H	C_6H_5	H	H	COOH	H	H	-84.840

Table (2) Synthesis Table of Ractopamine like compounds: Using easily obtainable compounds and combining Ring A and B to produce ractopamine like derivatives.

(1) Miriyala, B.; Bhattacharyya, S.; Williamson, J. S. Chemoselective Reductive Alkylation of Ammonia with Carbonyl Compounds: Synthesis of Primary and Symmetrical Secondary Amines. *Tetrahedron* **2004**, *60* (7), 1463–1471. https://doi.org/10.1016/j.tet.2003.12.024.
(2) Roethle, P. A.; McFadden, R. M.; Yang, H.; Hrvatin, P.; Hui, H.; Graupe, M.; Gallagher, B.; Chao, J.; Hesselgesser, J.; Duatschek, P.; Zheng, J.; Lu, B.; Tumas, D. B.; Perry, J.; Halcomb, R. L. Identification and Optimization of Pteridinone Toll-like Receptor 7 (TLR7) Agonists for the Oral Treatment of Viral Hepatitis. *J. Med. Chem.* **2013**, *56* (18), 7324–7333. https://doi.org/10.1021/jm400815m.

Conclusion Future Work 12

- Using physical properties for the ligands, multiple parameters for docking studies allows smaller more manageable libraries/data
- SAR studies on ractopamine
- Biological assay/testing of the interesting compounds from docking studies to be evaluated *in vitro* on the target enzyme
- Initial identification of the pharmaceutical hit optimize via virtual compounds
- Identification of the pharmacophore
- Look for biological clues that will drive the synthetic chemistry
- Are there cavities in the enzyme that can be build into
- Find new hydrogen bonding interactions
- Fragment based drug design
- Generate synthetic schemes to generate congeners of the pharmaceutical hit
- Continue molecular modeling to support synthetic chemistry
- Evaluate pharmacokinetics ADME