

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

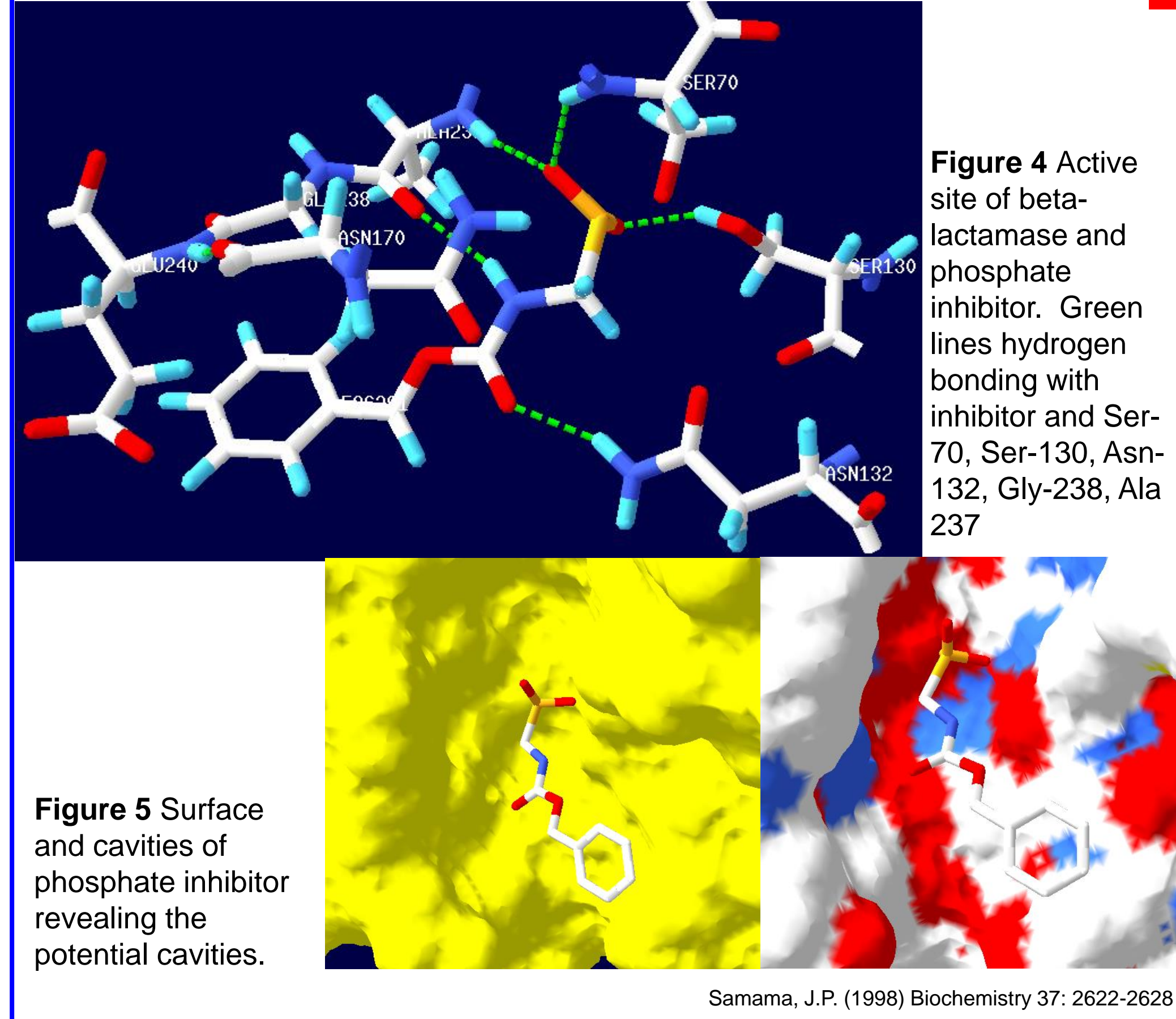


Todd J. Sullivan, Hussein W. Kafel, Kayla R. Bedel-Franklin, Charles A. Burt, Eric J. Bayer, Leticia De Souza, Tessa Peredy, Morgan A. Voulo, Zach Galasinski, Douglas Fleischmann, Kerin Ingegneri, Benjamin J. Alper, Joseph Audie, Chemistry Department, Sacred Heart University, Fairfield, Connecticut, United States 06825, USA

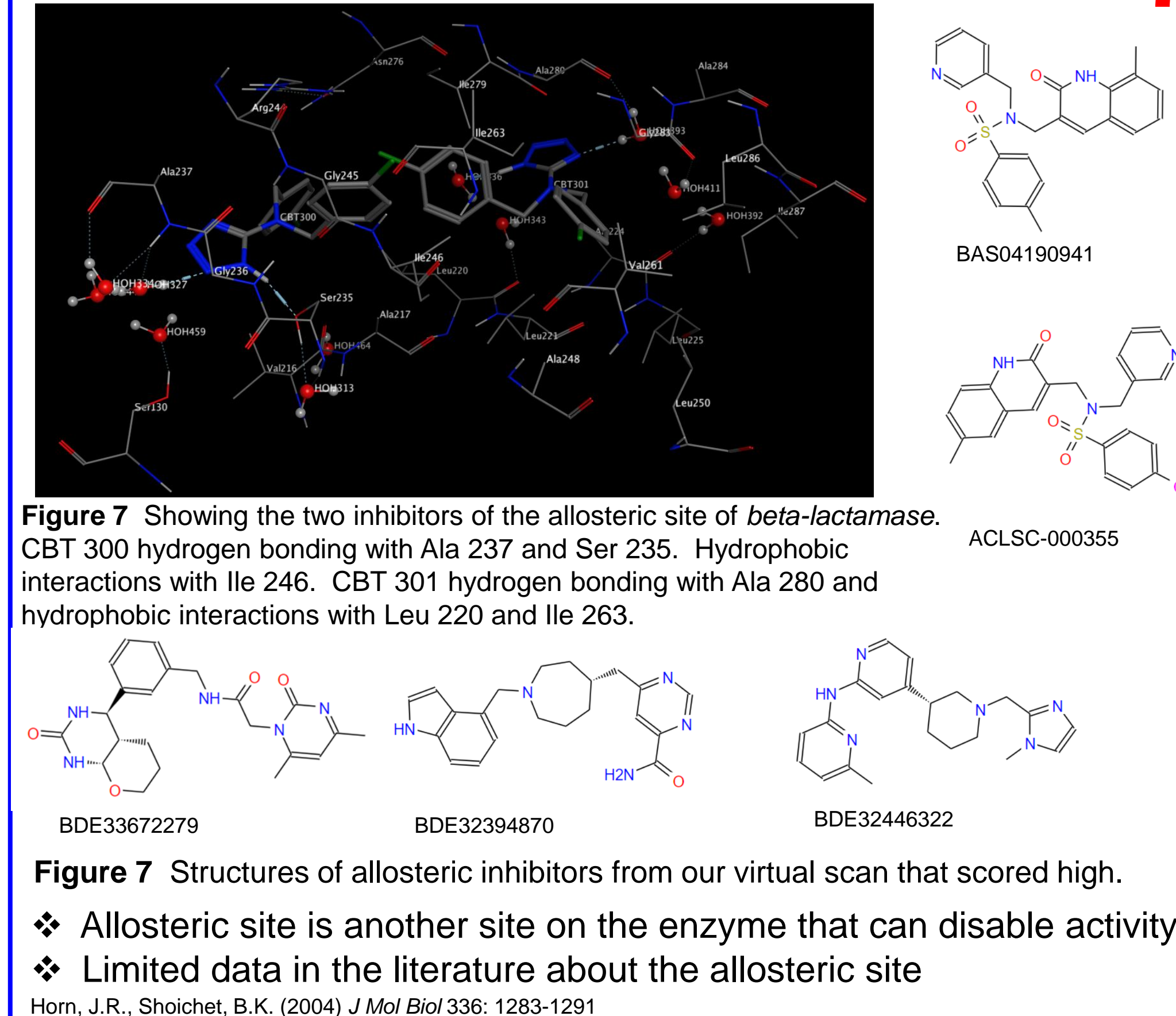
## Abstract

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 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  $\mu\text{M}$  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-lactamase drug candidate will be identified and that our computational work-flow will prove useful on other targets.

## Active Site



## Allosteric Site



## Results-Molecular Modeling on Hit

R <sub>1</sub>	R <sub>2</sub>	R <sub>3</sub>	R <sub>4</sub>	R <sub>5</sub>	R <sub>6</sub>	R <sub>7</sub>	R <sub>8</sub>	R <sub>9</sub>	R <sub>10</sub>	R <sub>11</sub>	R <sub>12</sub>	R <sub>13</sub>	R <sub>14</sub>	R <sub>15</sub>	PLANTS	Score
H	H	OH	H	H	CH <sub>3</sub>	H	H	OH	H	H	C=O	-84.124				
H	H	H	C <sub>2</sub> H <sub>5</sub> (OH) <sub>2</sub>	H	CH <sub>3</sub>	H	H	OH	H	C=O	-103.200					
H	H	H	B(OH) <sub>2</sub>	H	CH <sub>3</sub>	H	H	OH	H	C=O	-102.000					
H	H	H	H	H	C <sub>2</sub> H <sub>5</sub>	H	H	OH	H	C=O	-101.800					
H	H	C <sub>2</sub> H <sub>5</sub> (OH) <sub>2</sub>	H	H	CH <sub>3</sub>	H	H	OH	H	C=O	-101.400					
H	H	H	H	H	C <sub>2</sub> H <sub>5</sub>	H	H	OH	H	C=O	-101.300					
H	H	H	H	H	C <sub>2</sub> H <sub>5</sub>	H	H	OH	H	C=O	-99.630					
H	H	OH	H	H	C <sub>2</sub> H <sub>5</sub>	H	H	OH	H	C=O	-99.399					
H	H	OH	H	H	C <sub>2</sub> H <sub>5</sub>	H	H	OH	H	C=O	-99.299					
H	H	C <sub>2</sub> H <sub>5</sub> (OH) <sub>2</sub>	H	H	CH <sub>3</sub>	H	H	OH	H	C=O	-99.270					
H	H	OH	H	H	C <sub>2</sub> H <sub>5</sub>	H	H	OH	H	C=O	-99.203					
H	H	H	H	H	C <sub>2</sub> H <sub>5</sub>	H	H	OH	H	C=O	-98.850					
H	H	H	CH	H	CH <sub>3</sub>	H	H	OH	H	C=O	-97.970					
H	H	H	H	H	C <sub>2</sub> H <sub>5</sub>	H	H	OH	H	C=O	-97.090					
H	H	H	H	H	C <sub>2</sub> H <sub>5</sub>	H	H	OH	H	C=O	-97.070					
H	H	H	H	H	C <sub>2</sub> H <sub>5</sub>	H	H	OH	H	C=O	-96.120					
H	H	H	H	H	C <sub>2</sub> H <sub>5</sub>	H	H	OH	H	C=O	-93.947					
H	H	OH	H	H	CH <sub>3</sub>	H	H	CH <sub>3</sub> CO <sub>2</sub> H	H	C=O	-93.544					
H	H	OH	H	H	CH <sub>3</sub>	H	H	C <sub>2</sub> H <sub>5</sub> SO <sub>3</sub> F	H	C=O	-93.393					
H	H	OH	H	H	CH <sub>3</sub>	H	H	CH <sub>3</sub> PO(OH) <sub>2</sub>	H	C=O	-92.547					
H	H	H	H	H	C <sub>2</sub> H <sub>5</sub>	H	H	OH	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.

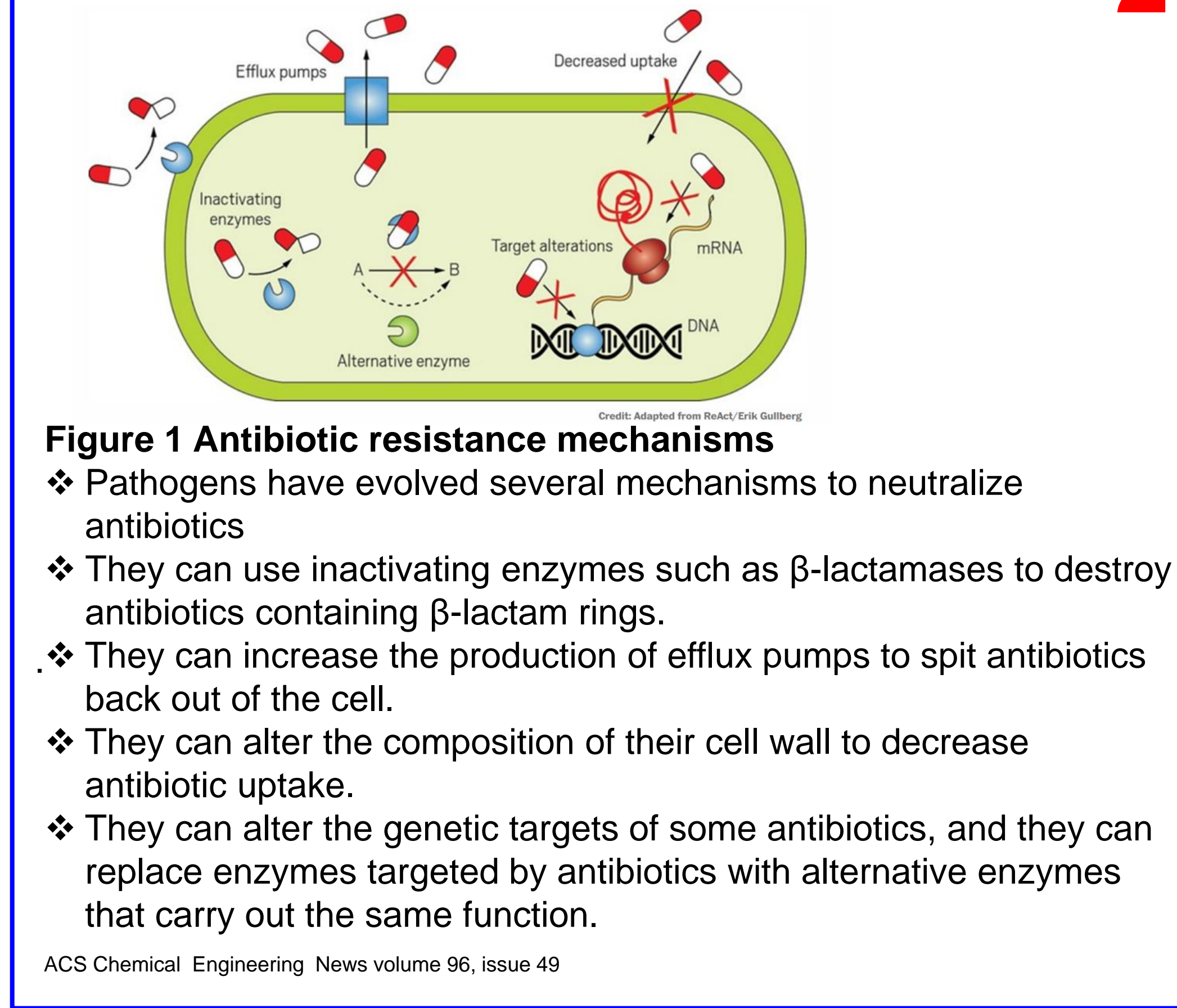
**Table (2) Synthesis Table of Ractopamine like compounds:** Using easily obtainable compounds and combining Ring A and B to produce ractopamine like derivatives.

R <sub>1</sub>	R <sub>2</sub>	R <sub>3</sub>	R <sub>4</sub>	R <sub>5</sub>	R <sub>6</sub>	R <sub>7</sub>	R <sub>8</sub>	R <sub>9</sub>	R <sub>10</sub>	R <sub>11</sub>	Plant Score
H	H	OMe	H	H	CH <sub>3</sub>	H	H	OMe	H	H	-66.627
H	H	OH	H	H	CH <sub>3</sub>	H	H	OH	H	H	-79.655
H	H	H	H	H	C <sub>6</sub> H <sub>5</sub>	H	H	OMe	H	H	-86.145
H	H	H	H	H	C <sub>6</sub> H <sub>5</sub>	H	H	OH	H	H	-86.288
H	H	H	H	H	C <sub>6</sub> H <sub>5</sub>	H	H	COOH	H	H	-88.942
H	H	H	H	H	C <sub>6</sub> H <sub>5</sub>	H	H	COOH	H	H	-84.840

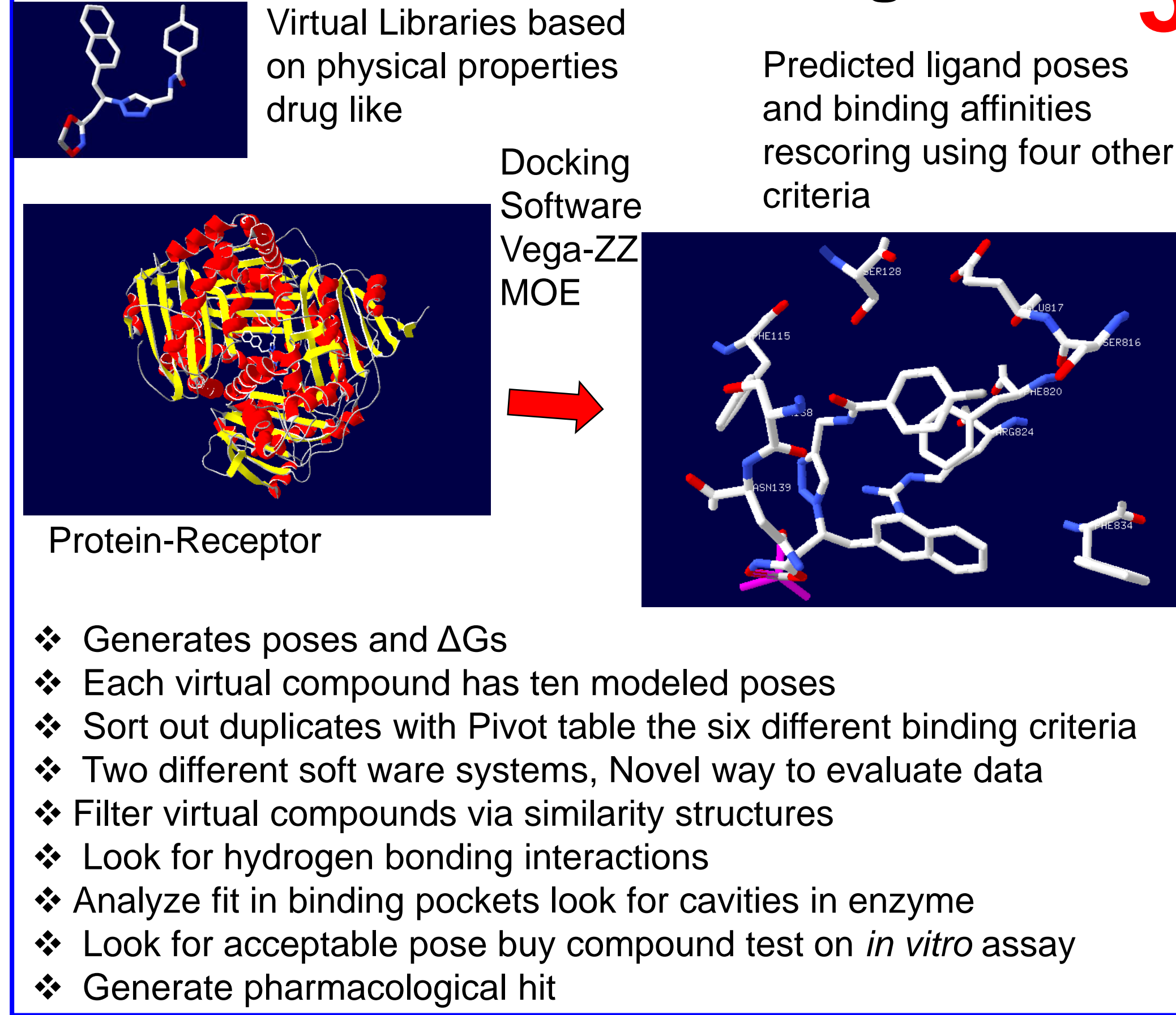
**Table (2) Synthesis Table of Ractopamine like compounds:** Using easily obtainable compounds and combining Ring A and B to produce ractopamine like derivatives.

(1) Miniyala, B.; Bhattacharya, 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.2003.12.004>  
(2) Roethli, P. A.; McFadden, R. M.; Yang, H.; Hvaslin, P.; Hui, H.; Graupe, M.; Gallagher, B.; Chao, J.; Hesselegger, J.; Dutschek, P.; Zheng, J.; Lu, B.; Tumas, D. B.; Perry, J.; Halcomb, R. L. Identification and Optimization of Pindolol-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/jm400487m>

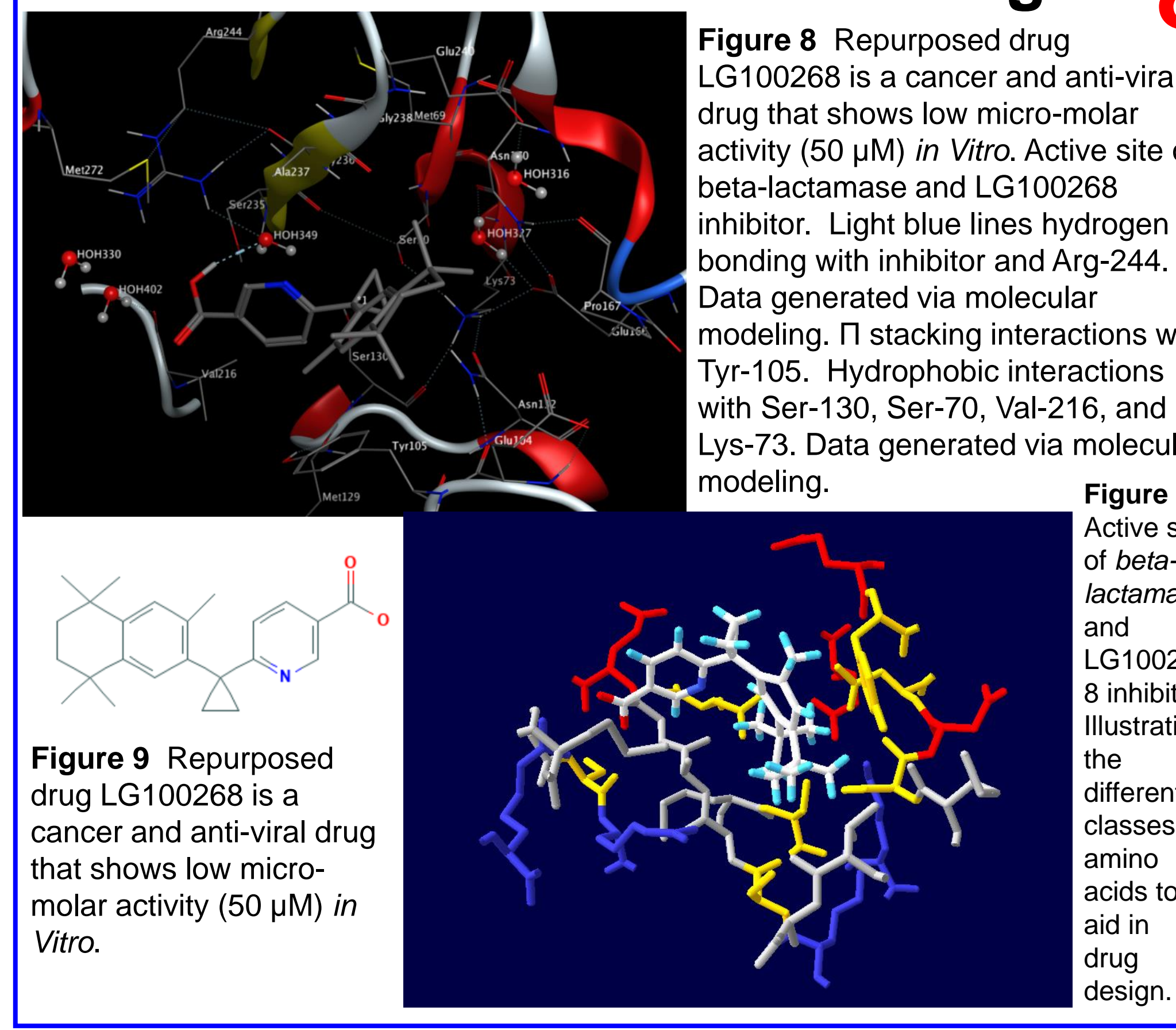
## Introduction



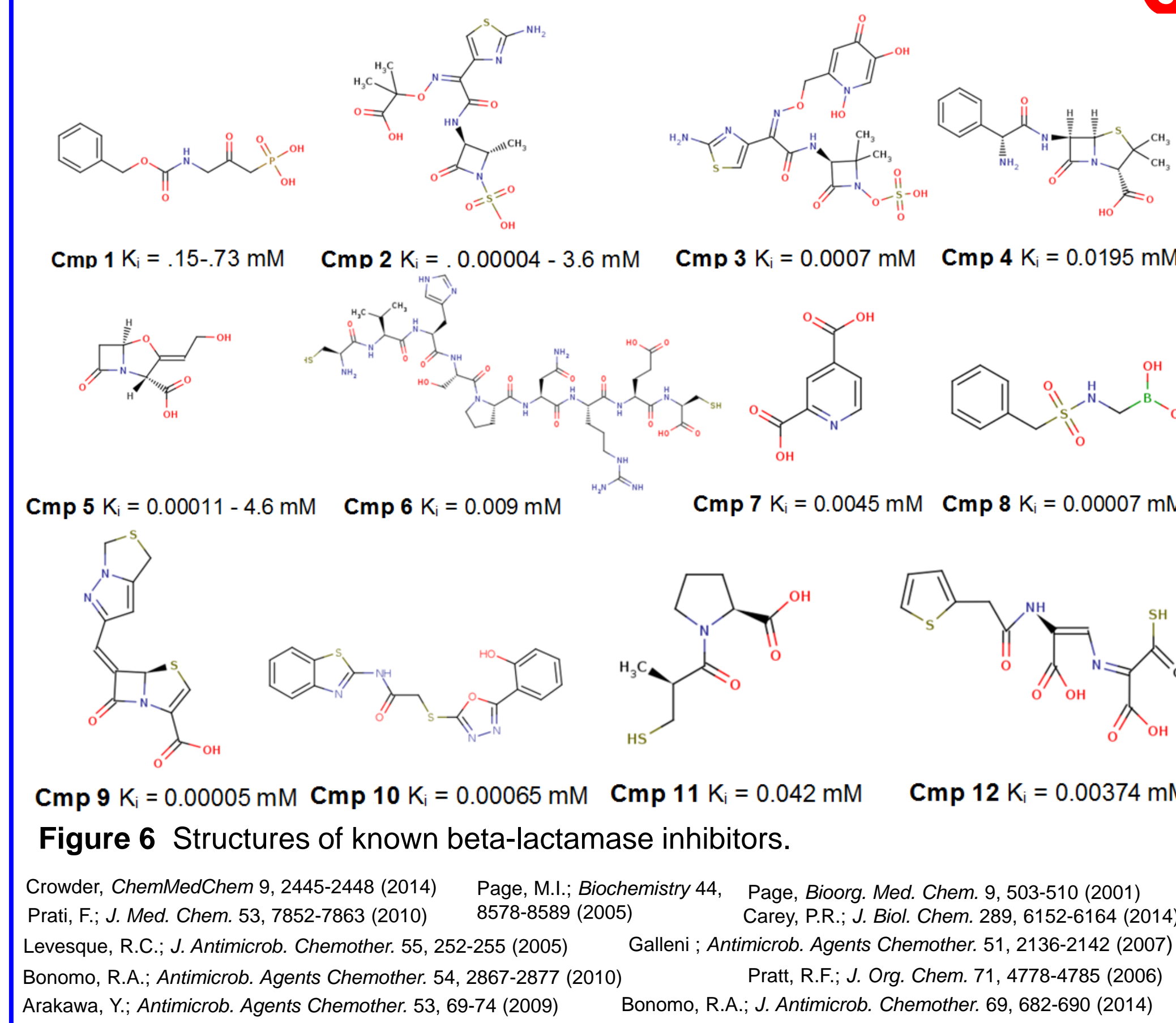
## Molecular Docking



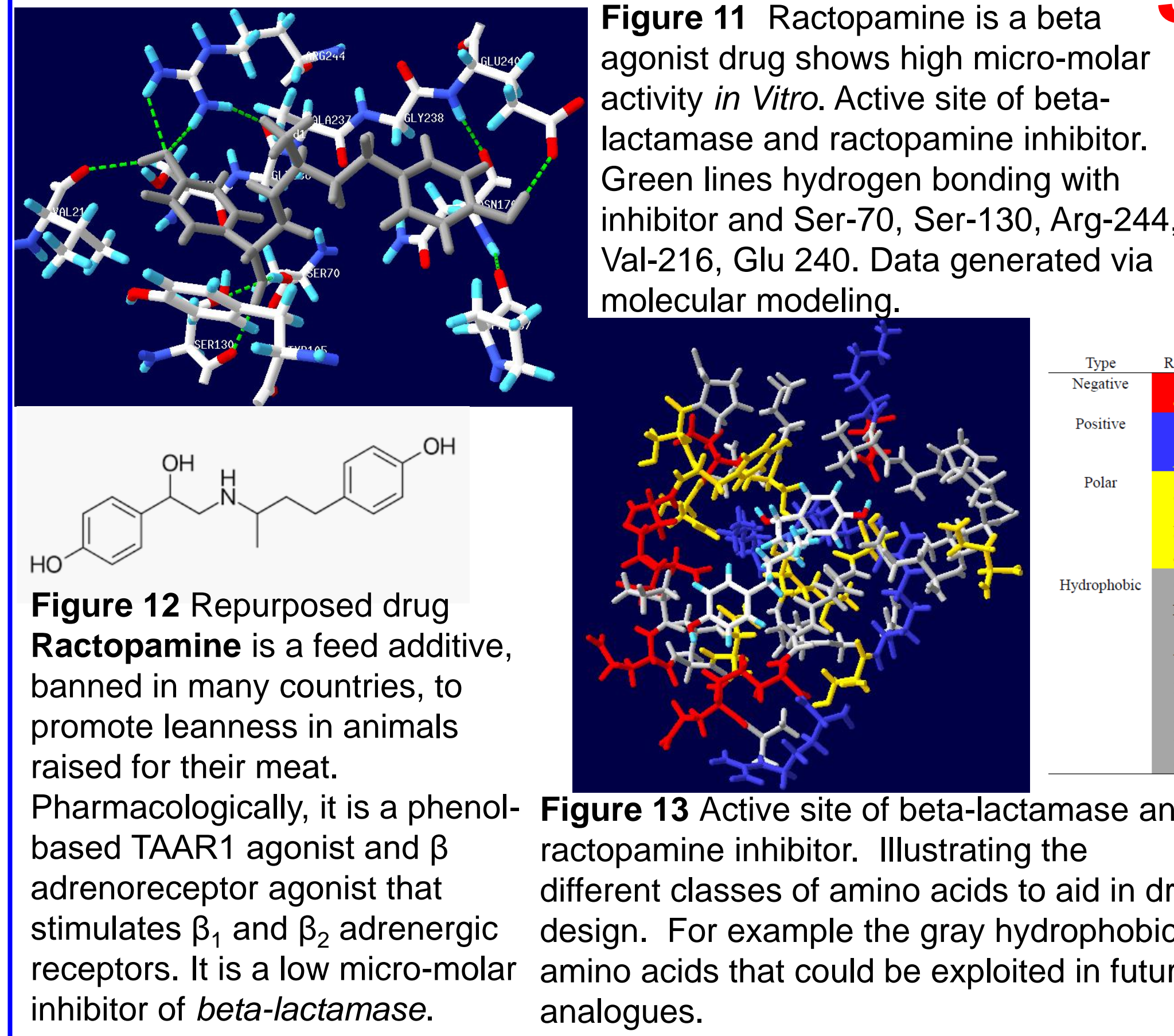
## Results-Molecular Modeling



## Known Inhibitors



## Results



## Conclusion Future Work

