MOE coupled with AutoDock Vina molecular docking and virtual screening empowered discovery of tetrapeptides inhibitors of Y-49 β-lactamase

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ABSTRACT

Tuberculosis (TB), caused by *Mycobacterium tuberculosis*, continues to be a worldwide health concern. The failure to control TB is due to the emergence of *M. tuberculosis* strains that are multiply drug resistant towards the front line antimycobacterial drugs such as isoniazid and rifampicin. One of the most effective resistance mechanisms to β-lactam antibiotics involves the production of β-lactamases which can cleave the amide bond in the target β-lactam ring. The intrinsic resistance to β-lactam antibiotics was demonstrated to be mainly due to the presence of a chromosomally-encoded gene (*blaC*) in *M. tuberculosis* for a Class A, Ambler β-lactamase (BlaC). The BlaC enzyme has been already validated as one of the lead therapeutic targets of tuberculosis therapy. In the last decade, many research studies proved that the main approach to overcome the resistance to β-lactam antibiotics the high throughput screening of new non β-lactam scaffolds, displaying inhibitory activity against β-lactamases. The spectrum of anti-TB drugs consisting of non β-lactam scaffolds has been expanded by the development of arginine and lysine-rich peptides which proved to be effective in neutralizing bacterial resistance, when administered in combination with antibiotics. Conceivably, the cationic-based peptides can be selectively enriched in natural or unnatural amino acids which can be further developed as β-lactamase inhibitors and additives of β-lactams, to help to prevent or reduce cleavage of the β-lactam ring. Following this logic strategy, we employed an original platform for molecular docking and structure-based drug design (SBDD) aimed to rational design novel tetrapeptides displaying inhibitory activity against the Y-49 enzyme, a class A β-lactamase, from *Mycobacterium tuberculosis.* The tetrapeptide pharmacophore was derived from the original sequence RRGHYY which was found to inhibit class A *Bacillus anthracis* Bla1, (K_i = 42 μM) and class A TEM-1 β-lactamase, (K_i = 136 μM) (*Protein Eng Des Sel* 16:853-860). Herein, we present the *in-silico* docking screening using a combined Autodock Vina and MOE software which lead to the discovery of 2HN-R-X-H-Y-CONH2 as potential competitive inhibitors of beta-lactamase having low micromolar-hundreds nanomolar inhibitory constant. X was varied with all 20 natural L- aminoacids and a structure-activity relationship (SAR) emerged highlighting the structural features required to ensure complementarity between the ligand peptide and the Y-49 enzyme. In addition, the drug-like properties of the tetrapeptides were assessed by the StarDrop-ADMET module. The combined structural complementarity and the drug-like properties criteria lead to the discovery of potential tetrapeptides inhibitors which were further tested using β-lactamase enzyme inhibition assays. The SBDD and SAR presented herein will enable further discovery of novel pharmacophores of linear and cyclic tetrapeptides with D- and unnatural amino acids with improved selectivity and anti-Y 49 β lactamase activity.

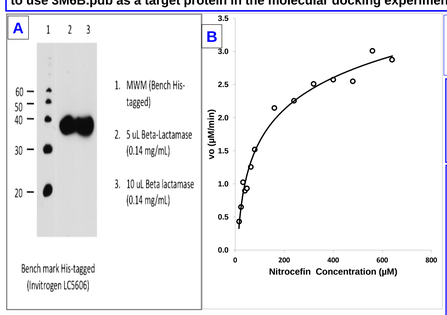
INTRODUCTION

➢Beta-lactamase enzymes. The simplest classification for these enzymes is by protein sequence, whereby the beta-lactamases are classified into four molecular classes: A,B,C and D, based on conserved and distinguishing amino acid motifs (1-3). Classes A, C, and D include enzymes that hydrolyze their substrates by forming an acyl enzyme through an active site serine, whereas class B β-lactamases are metalloenzymes that utilize at least one active-site zinc ion to facilitate β-lactam hydrolysis. The production of β-lactamases in both Gram-negative and Gram-positive bacteria is one of the most efficient and prevalent mechanisms of resistance to β-lactam antibiotics hydrolyzing the drugs before they can reach their target and exert the desired effect. All of these resistance mechanisms are important and each bacterium can create a combination of defenses depending on the selective pressures placed on it (1-7).

➢Finding the suitable protein target for molecular docking experiments. This research focused on discovery of novel tetrapeptides scaffolds, potential inhibitors of Y-49, a class A β-lactamase. The host for the recombinant protein expression was *E. Coli* Top10 (Invitrogen), containing the pTrcHis B plasmid (Invitrogen), expressing Y-49 from *Mycobacterium tuberculosis,* a gift from Douglas S. Kernodle, M. D., Vanderbilt University School of Medicine, Division of Infectious Diseases, Nashville, TN (5). The docking experiments used as target protein the X-Ray structure of a β-lactamase in complex with an inhibitor ligand (ertapenem): 3M6B.pdb (6 and Figure 4). However, we wanted to confirm that the protein used for kinetics and enzyme inhibition assays has the same amino acid sequence with the protein used for molecular docking experiments (3M6B.pdb). The Y-49 recombinant β-lactamase was identified previously as being the protein produced by a chromosomal gene coming from *M. tuberculosis* strain H37Rv sequenced by the Sanger Center Cambridge, UK (5). The complete genome of H37Rv has 3999 coding genes. The list of expressed proteins in this genome was retrieved and a β-lactamase-like protein plus a class A β-lactamase was found (accession number NP_216584.1 and locus tag Rv2068c). We used a combination of bioinformatics tools and performed sequence alignments using the DNA and amino acid sequences of Y-49 and other beta lactamases proteins and found that the recombinant Y-49 β-lactamase we used for the kinetics and inhibition assays had the same amino acid sequence with the X-Ray crystallized 3M6B.pdb (Figure 1).

➢Discovery of peptides inhibitors of beta-lactamase. We decided to start the search for potential inhibitors using tetrapeptides derived from a pharmacophore represented by a 6-mer sequence discovered by W. Huang *et al.* during a phage display screening assay. The 6-mer linear peptide RRGHYY inhibited class A *Bacillus anthracis* Bla1, (Ki = 42 μM) and class A TEM-1 β-lactamase, (Ki = 136 μM).

Figure 1: Y-49 recombinant β-lactamase protein produced by a chromosomal gene coming from *M. tuberculosis* strain H37Rv (5, A). The list of expressed proteins in this genome was retrieved and a β-lactamase-like protein, a class A β-lactamase was found having the following identifiers: accession number NP_216584.1 and locus tag Rv2068c; the protein BLAST search against the annotated NCBI protein database retrieved the Y-49 as a class A β-lactamase (A); further alignment of the Y-49 amino acid sequence with the 3M6B.pdb revealed a 100% identity of the two proteins, except for a 42 amino acid sequence located at N-terminus of Y-49 (highlighted in yellow). The 100% identity between the active site of the two proteins justified us to use 3M6B.pdb as a target protein in the molecular docking experiments.



Kinetic Parameters	Literature Values	Experimental Values
k_{cat} (s^{-1})	31	15.34 ± 0.5
K_m (μM)	81	95.71 ±10.1
k_{cat} / K_m (μM^{-1} s^{-1})	0.21	0.16 ±0.05

Alignment of the Y-49 amino acid sequence with the 3M6B.pdb revealed a 100% identity of the two proteins, except for a 42 amino acid sequence located at N-terminus of Y-49 (highlighted in yellow).

Amino Acid Sequence for Locus Tag Rv2068c H37Rv Strain

MRNRGFGRRELLVAMAMLVSVTGCARHASGARPASTTLPAGADLADRFAELERRYDA RLGVVVPATGTTAAIEYRADERFAFCSTFKAPLVAAVLHQNPLTHLDKLITYTSDDIRSIS PVAQQHVQTGMTIGQLCDAAIRYSDGTAANLLLADLGGPGGGTAAFTGYLRSLGDTVS RLDAEEPELNRDPPGDERDTTTPHAIALVLQQLVLGNALPPDKRALLTDWMARNTTGA KRIRAGFPADWKVIDKTGTGDYGRANDIAVVWSPTGVPYVVAVMSDRAGGGYDAEPR EALLAEAATCVAGVLA

Score	Expect	Method	Identities	Positives	Gaps	Frame
536 bits(1382)	0.0()	Compositional matrix adjust.	265/265(100%)	265/265(100%)	0/265(0%)	

Features:

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            GGGYDAEPREALLAEAATCVAGVLA
Sbjct  283  GGGYDAEPREALLAEAATCVAGVLA  307
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METHODS AND RESULTS

1. Selection of the pdb target of interest: Beta-lactamase (BlaC) in complex with *ERTAPENEM (3M6B.pdb)*

Extract the x,y,z coordinates (x=-6.619; y=-6.952; z=2.546) of the original 1RG ligand from the complex 3M6B.pdb and perform directed rigid docking using Vina Autodock &MOE

Discovery of *novel tetrapeptides* inhibitors of Y-49 (Class A, β-lactamase) using SBDD approaches

Figure 2 3M6B.pdb was used as a protein template in docking experiments involving the private collection of tetrapeptides derived from the sequence 2HN-RXHY-COOH. *VINA-Autodock* and MOE software were used to assess the ΔG (free energy) of interaction between the tetrapeptides and the beta-lactamase target, generating the structure-activity relationship (SAR) for the potential inhibitors of Y-49-β-lactamase (which has the same amino acid sequence as 3M6B.pdb)

Pharmacophore structure used in the structure-based design of *tetrapeptides* inhibitors of beta-lactamase (2)

RRGHYY
Pharmacophore B

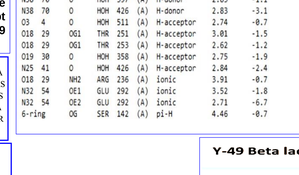


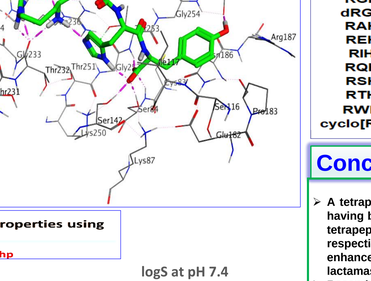
Figure 3

Criteria I: Generate a tetrapeptide library which will ensure better drug-like properties than the original hexamer sequence

Criteria II: Key physical-chemical structural features of the tetrapeptide 2HN-RXHY-COOH library .

Determine the free energy (ΔG) of interaction between the *tetrapeptide inhibitors* and the target beta-lactamase Y-49.

Original ligand: RG_308 3M6B.pdb (6) (ERTAPENEM)

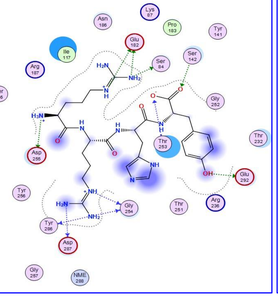


***Poseview* of the RRHY tetrapeptide in the active site of the3M6B highlighting the steric, hydrogen bonds and the electrostatic interactions established with selected residues in the active site of beta lactamase; (Autodock/Vina/MOE)**

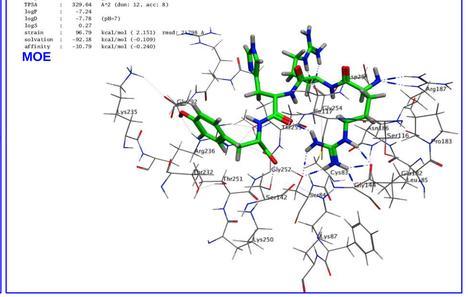
Predicted ΔG= -7.6 kcal/mol Vina Autodock

Ligand Interactions Report
RRHY

Ligand	Receptor		Interaction	Distance	E (kcal/mol)
O4 30	OE2	GLU 292 (A)	H-donor	2.62	-3.9
N6 50	OD1	ASP 255 (A)	H-donor	3.38	-6.0
N6 50	OD2	ASP 255 (A)	H-donor	3.09	-4.5
N7 63	OE1	GLU 182 (A)	H-donor	2.82	-4.8
N9 69	OG	SER 84 (A)	H-donor	2.75	-4.1
N9 69	OE1	GLU 182 (A)	H-donor	2.83	-7.2
N9 69	OE2	GLU 182 (A)	H-donor	2.73	-3.4
N10 81	O	GLY 254 (A)	H-donor	2.84	-3.5
N11 84	O	ASP 287 (A)	H-donor	2.75	-2.6
N12 87	O	GLY 254 (A)	H-donor	3.31	-1.2
N12 87	O	TYR 286 (A)	H-donor	2.87	-1.5
O2 15	N	THR 253 (A)	H-acceptor	2.84	-0.5
O2 15	OG1	THR 253 (A)	H-acceptor	2.55	-2.6
O3 16	OG	SER 142 (A)	H-acceptor	2.54	-0.5
N6 50	OD1	ASP 255 (A)	ionic	3.38	-2.4
N6 50	OD2	ASP 255 (A)	ionic	3.09	-3.9
N7 63	OE1	GLU 182 (A)	ionic	2.82	-5.8
N7 63	OE2	GLU 182 (A)	ionic	3.95	-0.6
N9 69	OE1	GLU 182 (A)	ionic	2.83	-5.8
N9 69	OE2	GLU 182 (A)	ionic	2.73	-6.6



MOE



***Poseview* of the RAHY tetrapeptide in the active site of the 3M6B; highlighting the steric, hydrogen bonds and the electrostatic interactions established with selected residues in the active site of beta lactamase; (Autodock/Vina/MOE)**

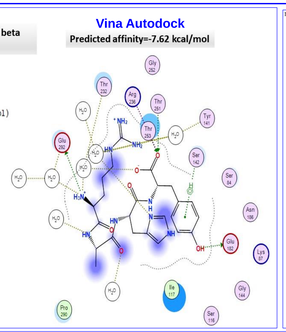
Ligand RAHY tetrapeptide docked pose in the active site of beta lactamase 3M6B.pdb (*Autodock* Vina and MOE)

Vina Autodock
Predicted affinity=-7.62 kcal/mol

Ligand Interactions Report

3M6B: HYDROLASE/ANTIBIOTIC / AUTOGEN_RAHY.out_1ligand_1

Ligand	Receptor		Interaction	Distance	E (kcal/mol)
O16 26	OE1	GLU 182 (A)	H-donor	2.57	-1.1
N28 48	O	HOH 381 (A)	H-donor	3.08	-1.7
N32 54	OE2	GLU 292 (A)	H-donor	2.71	-5.4
N32 54	O	HOH 341 (A)	H-donor	2.69	-3.3
N32 54	O	HOH 358 (A)	H-donor	2.77	-5.9
N36 67	O	HOH 426 (A)	H-donor	2.85	-3.5
N38 70	O	HOH 397 (A)	H-donor	2.85	-1.1
N38 70	O	HOH 426 (A)	H-donor	2.83	-3.1
O3 4	O	HOH 511 (A)	H-acceptor	2.74	-0.7
O18 29	OG1	THR 251 (A)	H-acceptor	3.01	-1.5
O18 29	OG1	THR 253 (A)	H-acceptor	2.62	-1.2
O19 30	O	HOH 358 (A)	H-acceptor	2.75	-1.9
N25 41	O	HOH 426 (A)	H-acceptor	2.84	-2.4
O18 29	NH2	ARG 236 (A)	ionic	3.91	-0.7
N32 54	OE1	GLU 292 (A)	ionic	3.52	-1.8
N32 54	OE2	GLU 292 (A)	ionic	2.71	-6.7
6-ring	OG	SER 142 (A)	pi-H	4.46	-0.7



MOE

Y-49 Beta lactamase tetrapeptides inhibitors: assessment of ADMET properties using OPTIBRIUM SeeSAR and StarDrop software
http://www.optibrium.com/stardrop/stardrop-adme-qsar-models.php

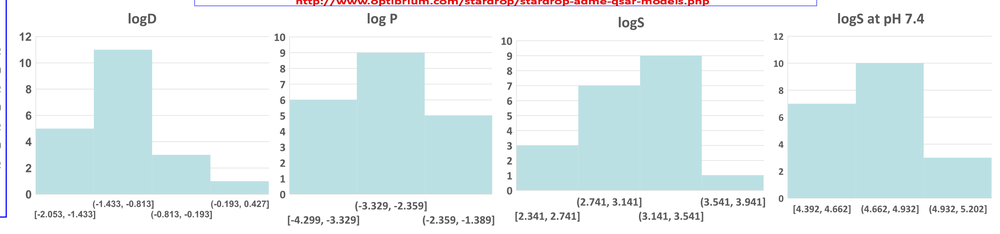


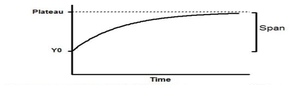
Figure 4

Structure-activity relationship (SAR) of selected lead tetrapeptides inhibitors of Y-49 beta-lactamase

One-phase association Best-fit values	Control (-I)	(+) RRHY	(+) dRGHY	(+) dRRHY	(+) RGHY
Y0	= 0	= 0	= 0	= 0	= 0
Plateau	0.2641	0.2661	0.576	0.276	0.262
K (observed rate)	0.007507	0.005303	0.001847	0.004799	0.002462
Tau	133.2	188.6	541.5	208.4	406.1
Half-time	92.33	130.7	375.4	144.4	281.5
Span	= 0.2641	= 0.2661	= 0.576	= 0.276	= 0.262

Mathematical model used to fit the kinetics data

$Y = Y0 + (Plateau - Y0) * (1 - exp(-K * x))$

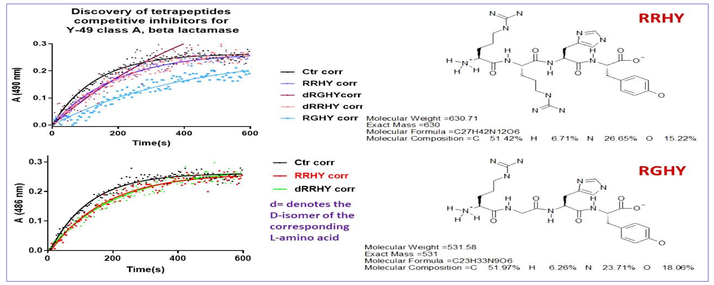


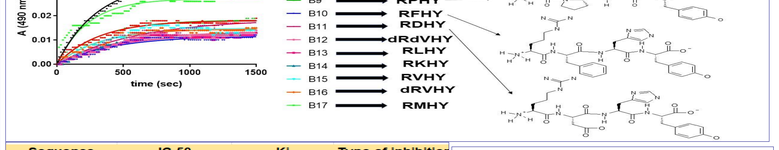
Experimental kinetics were run under *pseudo-1st order conditions* using a nitrocefin, substrate, concentration <Km

250 uL final reaction assay volume

If *competitive inhibition*, i.e same "Plateau (vmax) Then the Ki is calculated using the formula:
Ki= [I]/[(Kuninhibited/Kinhibited)-1]
[I]=the inhibitor final concentration in the assay

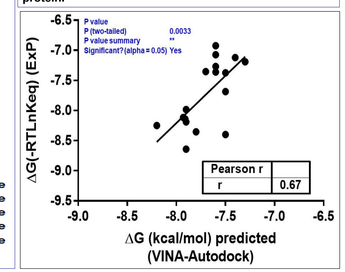
25 uM nitrocefin (0.26 Km for nitrocefin)
2.5nM enzyme
230 uL Hepes, pH 7.0
12.5 uL Inhibitor





Sequence	IC-50	Ki	Type of inhibition
RVHY	7.97	6.33	competitive
RMHY	1.94	1.53	competitive
RLHY	3.89	0.81	uncompetitive
RPHY	9.14	1.91	uncompetitive
RPHY	9.14	1.9	uncompetitive
RNHY	5.76	1.2	uncompetitive
RKHY	18.32	3.81	uncompetitive
RFHY	11.27	2.35	uncompetitive
RDHY	8.07	1.68	uncompetitive
dRVHY	3.72	0.77	uncompetitive
dRdVHY	18.52	3.85	uncompetitive
RRHY	15.14	13.12	competitive
dRRHY	4.12	3.26	competitive
RGHY	1.63	1.29	competitive
dRGHY	0.97	0.76	competitive
RAHY	7.10	6.54	competitive
REHY	8.76	1.82	uncompetitive
RIHY	10.30	10.3	noncompetitive
RQHY	6.47	6.47	noncompetitive
RSHY	8.50	8.5	noncompetitive
RTHY	9.52	9.52	noncompetitive
RWHY	7.50	7.5	noncompetitive
cyclo[RRHY]	2.52	1.9	competitive

VINA_Autodock provided a statistically significant correlation between the experimental and *in silico* predicted free energy of interaction between the tetrapeptides binders and the target beta lactamase protein.



Conclusions

- A tetrapeptide library (2HN-RXHY-COOH) with arginine in position 1, histidine in position 3, ending with tyrosine in position 4, and having both free NH3 and COOH ends, was constructed by performing SAR where X was replaced with all 20 L-amino acids. New lead tetrapeptides were discovered: RLHY has Ki of 0.81 uM while the tetrapeptides dRGHY and dRVHY have Ki of 760 nM and 770 nM, respectively. In all cases the replacement of L-isomer of Arg at the N-terminus with the D-isomer (dR) resulted in at least 2 fold enhanced inhibitory activity. Moreover, the cyclic analogue cyclo [RRHY] increased at least six fold the affinity for the Y-49 beta lactamase, from Ki of 13.12 uM characterizing the linear RRHY peptide to a Ki of 1.9 uM for the cyclo [RRHY].
- Recently, Caitlyn M. Rotondo et al. (2015, (7)) discovered novel nanomolar peptide inhibitors of metallo-beta-lactamases (MBL class). All the lead peptides inhibitors of MBL are poly-Arginine based sequences(derived from one of the lead sequence, Ac-Cys-Tyr-β-Ala-$(Arg)_6$-Val-Leu-Arg-OH). This recent report support our findings from SBDD approach where the arginine based tetrapeptides are low uM inhibitors of Y-49 beta-lactamase.
- Predicted ADMET properties of peptides from 2HN-RXHY-COOH series suggests that tetrapeptides have better drug-like properties than the original hexamer peptide RRGHYY pharmacophore from which were originally developed; thus the linear and cylized tetrapeptides could be used as new scaffolds for developing potent anti-Y49 beta lactamase inhibitors.

References:
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