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In-silico study of potential carboxylic acid derivatives as D-glutamate ligase inhibitors in *Salmonella typhi*

Muhammad I. Qadir*, Hina Mushtaq, Tahira Mobeen

Institute of Molecular Biology & Biotechnology, Bahauddin Zakariya University, Multan, Pakistan

**Corresponding author: mrimranqadir@hotmail.com*

Abstract

Salmonella typhi is food-borne as well as water-borne pathogen, which is the main cause of typhoid fever. This disease is affecting people in both developing and underdeveloped countries. The emergence of multidrug resistance in S. typhi has encouraged researchers towards targeting novel pathways. UDP-N-acetylmuramoyl-L-alanine: D-glutamate ligase enzyme is involved in cell wall synthesis of the bacterium. Studies have shown that two carboxylic acids; acetic acid and lactic acid, have anti-bacterial activity. Present in-silico study investigated the potential of acetic acid derivative (2,4-Dihydroxybenzyliminodiacetic acid) and lactic acid derivative (ammonium lactate) to inhibit the peptidoglycan synthesis. These derivatives showed good potential as inhibitors of target protein. These compounds can have a pharmaceutical application in drug development against the disease.

Key words: Anti-Salmonella; carboxylic acids; D-glutamate ligase; molecular docking; typhoid.

1. Introduction

Drug discovery has become smooth and robust through bioinformatics (Firat *et al*., 2016). Remarkable progress in protein expression, genome sequencing, high throughput crystallography, NMR and protein three-dimensional structures and many other techniques have contributed to scientific spur of drug discoveries. The prominent role of bioinformatics and structural biology assisting in optimization and drug target identification is well established. The bioinformatics data base and software now contribute to drug discovery by structure determination and high-throughput techniques of screening of fragment binding (Blundell *et al*., 2006). Computational biology can establish possible platform for antibiotic discovery. Novel antibiotics can be sought by exploring the available natural products, making alterations in the cell for compound penetration, developing species-specific drug. Moreover, the dormant bacterial persisters can possibly be eradicated by identifying prodrugs (Lewis, 2013).

Typhoid fever is a bacterial disease. It is one of the leading causes of desolation and fatality (Nagshetty *et al*., 2009). It is transmitted through the ingestion of contaminated food (Black *et al*., 1985) and water (Luby *et al*., 1998); contaminated by the faeces or urine of infected people. Close contact with typhoid carriers (Tran *et al*., 2005) and flooding (Vollaard *et al*., 2004) are other causes of typhoid. Infected people develop fever and symptoms including abdominal pain, headache, weakness, loss of appetite, constipation etc. Intestinal hemorrhage, vomiting, and diarrhea are late onset of the disease (Lin *et al*., 2000). Some people get "rose spot" on the chest.

Typhoid commonly occurs in population of low income countries, facing poor sanitary conditions. This disease affects more than 21.5 million people each year. The epidemic of typhoid fever is common, the most susceptible are children and adults between 5 and 19 years old (Sinha *et al*., 1999). An estimate of the global burden of typhoid fever, with a total 16 million including 600,000 deaths annually, was presented at a meeting of the Pan American Health Organization in 1984 and subsequently published in 1986 (Edelman and Levine, 1986). The global illness incidence in 2000 was an estimated 21,650,974 cases with 216,510 deaths in Bangladesh (Crump *et al*., 2004).

The causative agent of typhoid fever is *Salmonella typhi*. It is a rod-shaped gram negative bacterium. *Salmonella typhi* is a food born pathogen that is progressively more difficult to control. *Salmonella typhi* are now resistant to multiple drugs like ciprofloxacin, ampicillin, chloramphenicol, co-trimoxazole, streptomycin (Rowe *et al*., 1997). These resistant bacterial strains are now an alarming issue, especially in the Indian subcontinent and Southeast Asia (Effa *et al*., 2011).

Owing to the increased resistance, there is a demand for alternative compounds with antibacterial properties. Lactobacillus found in dairy products is reported to release bacteriocins; bio-preservative properties of Lactobacillus is mainly due to production of organic acids like lactic acid and acetic acid and they can be used as potential drugs for different gastrointestinal and uro-genital infections (Šušković *et al*., 2010). A previous study reported effect of lactic acid on the outer membrane permeability of

Pseudomonas aeruginosa, Escherichia coli O157:H7 and Salmonella enterica serovar Typhimurium. In addition to the antimicrobial property of lactic acid, it was found to function as a permeabilizer of the outer membrane of gram-negative bacteria (Alakomi *et al*., 2000). Moreover, bactericidal effect of acetic acid was reported against *S. enterica* serovar Typhimurium (Rhee *et al*., 2003).

In a recent study, two derivatives of carboxylic acid (1H-pyrazole-3-carboxylic acid and pyridazinone) were synthesized and investigated for their antimicrobial activity (against *Staphylococcus aureus , Bacillus cereus, Escherichia coli* and *Pseudomonas putida*). The investigated compounds showed inhibitory effects on growth of these bacteria (Akbas *et al*., 2005).

Carboxylic acids can be used to target peptidoglycan-structural component of bacterial cell wall, as it plays a vital role in the survival of bacterial cell. UDP-N-acetylmuramoyl-L-alanine: D-glutamate ligase is one of the crucial enzyme that is vital for the intracellular biosynthesis of peptidoglycan (Tomašić *et al*., 2011). In biosynthetic pathway of bacterial peptidoglycan, the monomer unit of peptide is assembled by stepwise accumulation of L-alanine, D-glutamic acid, meso-diamino-pimelic acid or lysine, and D-alanyl-D-alanine to UDP-N-acetylmuramic acid (UDP-MurNAc)$_2$. Every step of reaction of adding peptide monomers is catalyzed by a highly customized ADP-forming ligase. The UDP-MurNAc-L-alanine: D-glutamate ligase, or D-glutamate-adding enzyme, catalyzes the addition of D-glutamate to UDP-MurNAc-L-Ala (UMA) (Auger *et al*., 1998). By inhibiting D-glutamate ligase enzyme the synthetic pathway of peptidoglycan formation can be shutdown. In a recent research, different classes of furan-based benzene-1,3-dicarboxylic acid derivatives were investigated and have been found to show a multiple MurC–MurF ligase inhibition (Perdih *et al*., 2015). Thus, the class of ligase enzymes constitutes as a target for the novel antibacterial drug to combat the emerging multi-drug resistant strains.

Molecular docking is a key tool in computer-assisted drug design and structural molecular biology. The aim of ligand-protein/target docking is to predict the principle binding mode(s) of a ligand with a protein/target molecule of known three-dimensional structure. In Successful docking methods high-dimensional spaces are effectively analyzed and a scoring function that appropriately ranks candidate docking is setup. It can be used to perform virtual screening on entire database of compounds and by ranking the result, inhibition capacity of the target molecule with ligand can be assessed (Morris and Lim-Wilby, 2008).

A recent study by Navesika & co-workers (2016), reported the antibiotic properties of a novel compound extracted from Nostoc, 9-Ethyliminomethy l-12 (morpholin-4-ylmethoxy) -5, 8,13,16-tetraaza-hexacene-2, 3 dicarboxylic acid (EMTAHDCA). Its antimicrobial potential was assessed with the help of comparative Molecular docking (Niveshika *et al*., 2016).

The aim of this study was to investigate two carboxylic acid derivatives, acetic acid derivative (2,4-Dihydrox ybenzyliminodiacetic acid) and lactic acid derivative (ammonium lactate) as potential inhibitor of UDP-Mur-N-Ac-L-alanine: D-glutamate ligase, through molecular docking approach.

2. Materials and methods

2.1 Accession of ligand:

The chemical structure of 2,4-dihydroxybenzylimin odiacetic acid and ammonium lactate were accessed from Pubchem (https://pubchem.ncbi.nlm.nih.gov/), and Zinc Data Base (www.zincdatabase org). We have found ADMET properties of ligand molecules by using (http://medchem-designer.software.informer.com/).

2.3 Chemical structure:

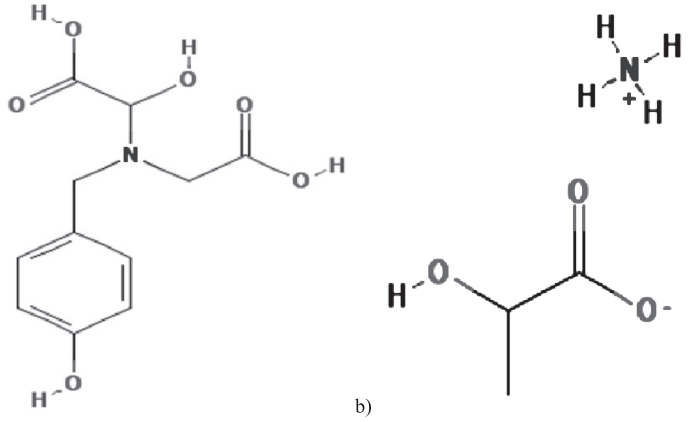

Fig. 1 (a) 2,4-dihydroxybenzyliminodiacetic acid; **(b)** Ammonium lactate

Accession of target:

The three-dimensional structure of target UDP-N-acetylmuramoyl-L-alanine: D-glutamate ligase enzyme was retrieved from protein data bank (PDB), by using the PDB ID: 1E0D (http://www.rcsb.org/pdb). The 3D structure of protein is shown in (Figure 2).

We have used Chimera software for finding different residues, for structure clarification and different molecular properties.

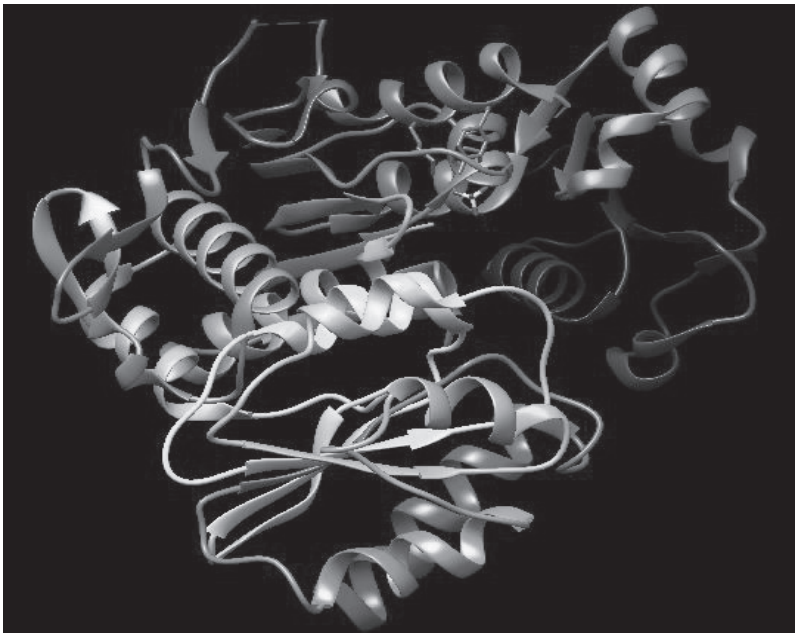

Fig. 2. 3D structure of target protein, UDP-N-acetylmuramoyl-L-alanine: D-glutamate ligase.

2.4 Drug scoring

We have used DSX online, (http://pc1664.pharmazie.uni-marburg.de/drugscore) for drug scoring. From this software, we obtained the information about tors_score, sas_score, atom-atom pairs and coordinated covalent bonds (Table 2).

2.5 Molecular docking

SwissDock, was used for molecular docking of the target and the ligand. The target protein was uploaded in PDB format, downloaded from protein data bank. Only one ligand can be uploaded for docking at one time, so the MOL2 file of one ligand was uploaded for docking in one time. The docked compound was analyzed using UCSF Chimera software.

3. Results

The study is conducted to find out the potential of selected derivatives against D-glutamate ligase. The three dimensional structure of target protein was assessed from protein data bank. Target has PDB ID: 1E0D. The binding pockets of target (UDP-N-acetylmuramoyl-L-alanine: D-glutamate ligase) was assessed by using Dogsite Scorer (dogsite.zbh.uni.hamburg.de) software. The results are given in Table 1. Interaction of ligand-target is shown in Figure 3 and 4 by using Chimera software as it shows best conformation of target with ligand molecules. The Information about the derivatives is given in Table 2, which include Pubchem ID. Zinc ID, molecular weight and their hydrogen bond. Docking results of ligand molecules, which include values of simple fitness, full fitness, intra full, inter full, surface full and ΔG (Kcal/mol) are given in Table 3. Drug scan is done for pharmacokinetics analysis in human body .This tool gives us information about tors_score, which is the sum of scores of each bond. A single bond (B—C) can have more than one torsion curves (A1--B--C--D1, A2--B--C--D1 ...). The score for a single bond is the mean of its possible torsions and sas_score. The ‹sas_score› is the solvent accessible surface score for solvation/desolvation contributions. The 'PCS'(per_contact_score) is the score divided by the number of atom-atom-interactions having any contribution to the total score. All information about drug scoring is given in Table 4.

Absorption, distribution, metabolism and excretion properties (ADME) were assessed by using MedChem Designer. log+P - two models: artificial neural network ensemble and constructed by our automatic model builder

ADMET Modeler. log+D estimation of octanol-water distribution coefficient at user-defined pH. The results collected from this software are shown in Table 5. After docking, the hydrophobicity surface of target with ligand molecules is shown in Figure 5. The interactions between the ligand and the enzyme were competitive.

Table 1. The active pockets of target 1E0D

Name	Volume [A^{3}]	Surface [A^{2}]	Lipo Surface [A]	Depth [A]	Drug Score
	1210.37	1397.02	764.65	23.29	0.61
P1	442.88	737.20	556.87	17.83	0.39
P3	272.38	492.78	347.41	11.47	0.17
P4	265.47	446.84	311.63	15.63	0.09
P2	277.57	406.83	239.50	14.39	0.04
P5	220.42	302.49	165.45	11.49	0.04
P7	162.75	289.14	155.49	14.24	0.00
P10	118.27	267.56	147.48	8.89	0.00
P8	157.38	374.60	137.35	10.98	0.00
P11	110.59	218.34	124.42	6.85	0.00
P6	203.84	235.12	107.09	10.23	0.02
P9	128.77	218.02	95.42	9.22	0.00

Table 2. Pubchem ID, Zinc entry and other properties of ligand molecules

S.r #	Pubchem ID	Zinc Entry	Molecular Formula	Molecular Weight g/mol	H bond Donor count	H bond acceptor count	Rotatable bond count	x log P
1	53444430	968695	C11H13NO6	255.22402	3	7	6	-2.16
2	62358	4658560	C3H9NO3	107.10846	1	3	1	-0.71

Table 3. Docking results and other drug related properties of ligand molecules

S.r #	Pubchem ID	Name	Simple fitness	Full fitness	Inter full	Intra full	Surface full	Energy	Delta G	Clusters
1	53444430	2,4-dihydroxyben-zyliminodiacetic acid	1.92617	-2239.8308	-53.4749	93.3201	288.434	1.92617	-7.351239	0
2	62358	Ammonium lactate	-10.9642	-2260.9175	-75.8931	52.3807	289.335	-10.9642	-7.757989	0

Table 4. Drug scoring results based on several properties of ligand molecules

S.r #	Pubchem ID	Name	PCS	Number/ Rank	tors_score	sas_score	RMSD	Drug score
1	53444430	2,4-dihydroxybenzyl-iminodiacetic acid	3.658	0/1	0.00	3.658	None	3
2	62358	Ammonium lactate	-1.377	0/1	0.00	-1.377	None	-1

Table 5. ADMET Properties of finalized molecules

Name	MlogP	S+logP	S+log D	M_NO	T_PSA	HBD_H
Zinc00968695	-4.754	-1.771	-2.634	7.000	118.300	4.000
Zinc04658560	-1.109	-0.624	-3.128	3.000	57.530	2.000

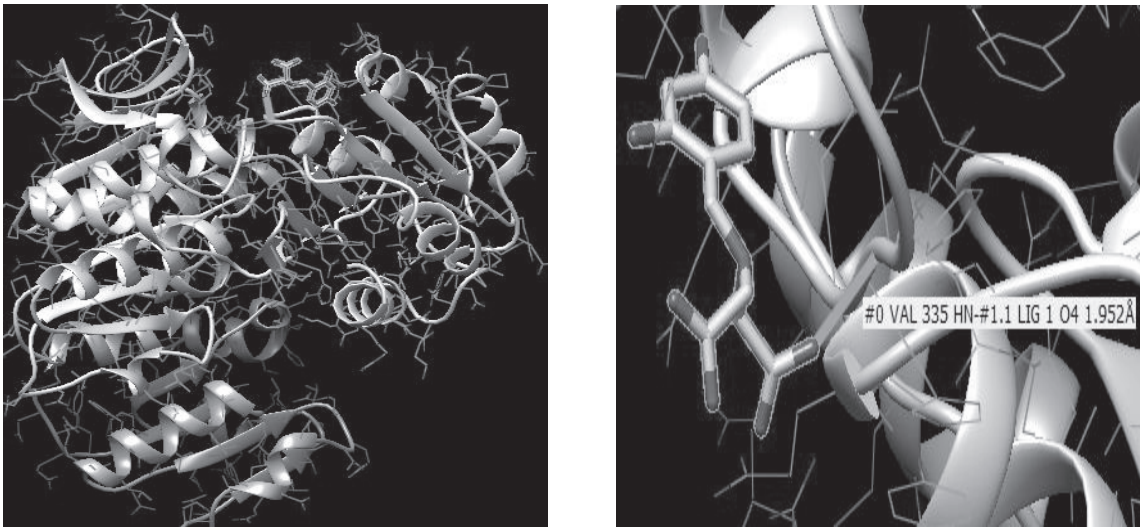

Fig. 3. Complete docked (a) and zoomed structure (b) of 2,4-Dihydroxybenzy-imino-di-acetic acid. Hydrogen interaction was seen with bond distance of 1.952 °A.

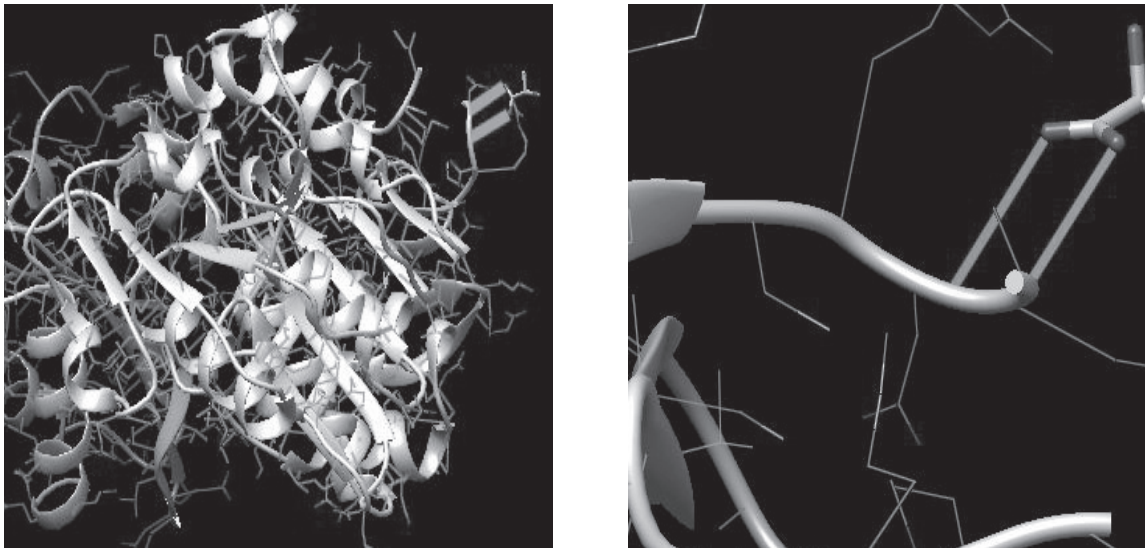

Fig. 4. Complete docked (a) and zoomed structure (b) of ammonium lactate

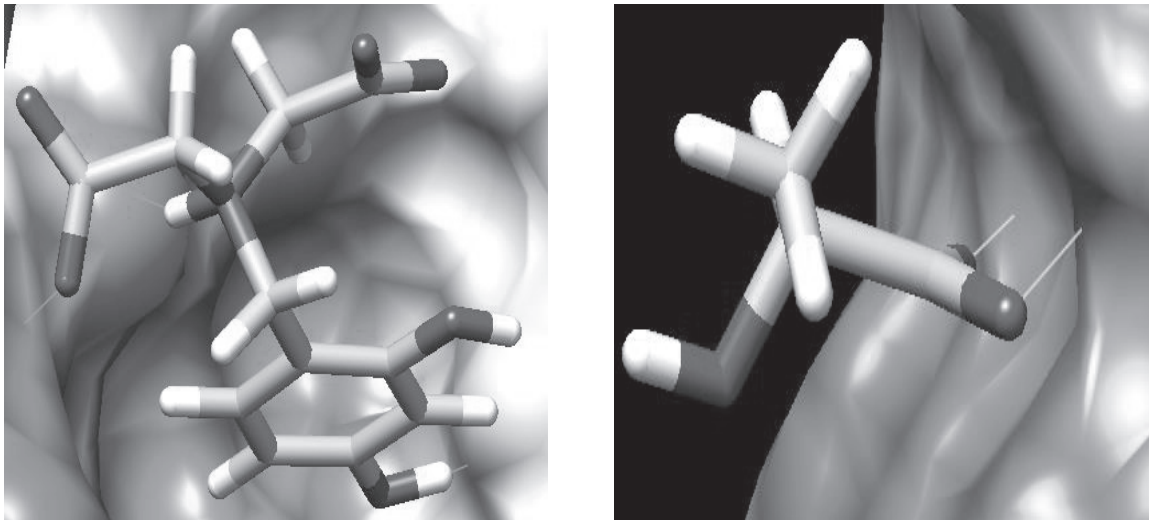

Fig. 5. (a) Hydrophobic surface of target with 2,4-Dihydroxybenzyliminodiacetic acid; and (b) with ammonium lactate

4. Discussion

For a suitable ligand, the count of hydrogen bonds donor must be less than five. Here, we have proposed two new ligand molecules, derivative of acetic acid, 2, 4-dihydroxybenzyliminodiacetic acid, and derivative of lactic acid, ammonium lactate, against D-glutamate enzyme involved in peptidoglycan synthesis. During protein-ligand interaction, 2, 4-Dihydroxybenzyliminodiacetic acid form one H-bond with selected chain (437aa) of target UDP-N-acetylmuramoyl-L-alanine: D-glutamate ligase while lactic acid derivative, ammonium lactate makes two H-bonds with selected chain (437aa) of target protein (Figure 3,4 respectively). These compounds can inhibit the cell wall synthesis by interfering the action of UDP-N-acetylmuramoyl-L-alanine:D: D-glutamate ligase enzyme that cross-links peptidoglycan chains to form rigid cell envelop (Van Heijenoort, 2001). Therefore, these compounds have a potential to be used in anti-bacterial drug development.

Binding of the ligand molecules on to the surface of target protein are analysed, confirming that these ligand molecules can enter the substrate-binding region of the protein active sites. Hydrogen bond can decide the structure of ligand molecules, the successful binding of the ligand and binding strength. Therefore, hydrogen bond between the target and the ligand molecules are important during interaction procedure shown in (Figure 3, 4 respectively).

The calculated final docked energy of 2, 4-dihydroxybenzyliminodiacetic acid and ammonium lactate compounds are -7.351239 and -7.757989 Kcal/mol respectively. Docking results clearly shows that these antibacterial compounds accurately interact with ligase protein and can get pharmaceutical application in future as potential anti-bacterial drug.

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دراسة عن استخدام مشتقات حامض كاربوكسيليك

محمد قادر*، هينا مشتاق

معهد البيولوجيا الجزيئية والتكنولوجيا الحيوية، جامعة بهاء الدين زكريا، مولتان، باكستان

*mrimranqadir@hotmail.com

خـلاصـة

السالمونيلا التيفية هي عدوى في الطعام أو الماء وهي المسبب الرئيسي لحمى التيفوئيد. يؤثر هذا المرض على الناس في الدول المتقدمة وغير المتقدمة. ظهور مقاومة لأدوية معالجة السالمونيلا التيفية أدى إلى تشجيع الباحثين لإيجاد حلول مبتكرة. UDP-N-acetylmuramoyl-L-alanine: D-glutamate انزيم ليجاس يشترك في خلايا جدار التوليف للبكتريا. أثبتت الدراسات أن الحامضين الكاربوكسيليك؛ حامض الاستيك وحامض اللكتيك لهم نشاط مضاد للبكتريا. في هذه الدراسة نبحث عن استخدام مشتقة لحامض الأستيك (Dihydroxybenzyliminodiacetic acid-2،4)، ومشتقة لـ (ammonium lactate لمنع توليف peptidoglycan. أوضحت هذه المشتقات أن لها إمكانات جيدة كموانع للبروتين المستهدف. يمكن أن يكون لهذه المركبات تطبيقات لتطوير أدوية ضد هذا المرض.