Allosteric Inhibition of Bacterial Enzymes: A Biochemical Approach to Combat Antimicrobial Resistance

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Abstract*: *The emergence of widespread antimicrobial resistance (AMR) has become one of the most pressing health crises in modern medicine, leading to prolonged illness, high morbidity and mortality, and escalating healthcare costs. The development of antibacterial drugs has traditionally focused on molecules that inhibit the active site, but these inhibitors are frequently rendered ineffective by bacterial enzyme mutations. Allosteric inhibition, or binding to areas of the enzyme other than the active site, is an alternative approach for downregulating bacterial metabolism and thus either killing pathogen or limiting its growth with reduced likelihood of resistance.

Here we review the literature and present distinct secondary data analysis on allosteric inhibition in these drivers, such as β-lactamases (BLs), DNA gyrase, and dihydrofolate reductase. These kinetic data obtained in quantitative assays from literature show that some inhibitors with as low IC 50 values as 100 nM will give >80% catalytic efficiency reduction, which consequently lead to the restoration of antibiotic susceptibility in resistant bacterial strains. The allosteric site is known to induce some conformational changes as shown by crystallographic data, but these changes are not sufficient to explain the ability of ligand binding at the allosteric site to restrict substrate access to the active site. Recommendations for integrating allosteric inhibitor discovery in antimicrobial development pipelines is mentioned in the conclusion of the paper

Index Terms*— *Allosteric inhibition, antimicrobial resistance, bacterial enzymes, enzyme kinetics, structural biology, drug design

I. INTRODUCTION

Antimicrobial resistance (AMR) is spreading on a global scale, eroding the effectiveness of many front-line antibiotics, and putting decades of medical advances in danger. The World Health Organization (WHO) has also said that if immediate action is not taken, minor infections could again become fatal. The key driver of this process is the ability certain bacteria have to create inexpensive enzymes that destroy antibiotics or modify their intended target. For example, β-lactamases break down the β-lactam ring in penicillin's and cephalosporins, which makes them inactive. Fluoroquinolone resistance results from mutations in DNA gyrase, dihydrofolate reductase mutations cause trimethoprim resistance. Allosteric inhibition comes with an important characteristic: since the inhibitors bind to sites of a given enzyme distant from those found in the active site, control over conformation supports control without having to compete directly for binding with substrates. This potentially lowers the rate of resistance, as mutations in the active site may not disrupt inhibitor binding.

II. LITERATURE REVIEW AND THEORETICAL BACKGROUND

The notion of allosteric modulation has been well defined in enzymology with early studies regarding metabolic enzymes (e.g. Phosphofructokinase). Recent structural biology advances in bacterial systems have revealed additional pockets just outside the active site that can be drugged. For example, Ehmann et al. [1] reported a distal binding pocket located in the class A β-lactamases, approximately 18 Å away from the catalytic serine. Binding to this site results in subtle changes to the Ω-loop and limits access for substrates. Similarly, Collin et al. [2] GyrB subunit of DNA gyrase as an allosteric pocket near the ATPase domain with potential to hand-on mechanism has been demonstrated.

III. METHODOLOGY

The secondary data used were extracted from peer-reviewed biochemical journals, structural databases

(Protein Data Bank; PDB) and enzyme kinetics repositories. Inclusion criteria included:

- Inhibitory assay(enzyme – II) statement for Km, kcat and Ki values of various bacterial-allosteric Inhibitors.
- Characterisation of Binding Partners (X-ray crystallography or cryo-EM)
- Verification of rescued antibiotic activity by in vitro or in vivo model.
- The data were normalised to pH 7.4, 37 °C for comparison between studies

IV. RESEARCH AND DISCUSSIONS

This review summarizes representative allosteric inhibitors against clinically relevant bacterial enzymes (Table I). One of these was active against the TEM-1 β-lactamase, with a Ki of 120 nM and an 85% reduction in kcat/Km [3]. Likewise, a DNA gyrase inhibitor which bound to the GyrB allosteric site demonstrated a Ki of 90 nM and resulted in four-fold reduction in supercoiling activity with concomitant restoration of ciprofloxacin-susceptibility against resistant E. coli [4]. Allosteric dihydrofolate reductase inhibitors that act at a site proximal to the folate-binding pocket displayed micromolar potency and functioned in combination with trimethoprim.

As shown in Fig. 1 Binding of the allosteric inhibitor (red) in a distal TEM-1 β-lactamase merely 18 Å away from active serine. This causes a conformational change in the Ω-loop that interferes with binding of β-lactam antibiotics to the active site. These structural changes provide a mechanism that underpins the reduced catalytic efficiency observed.

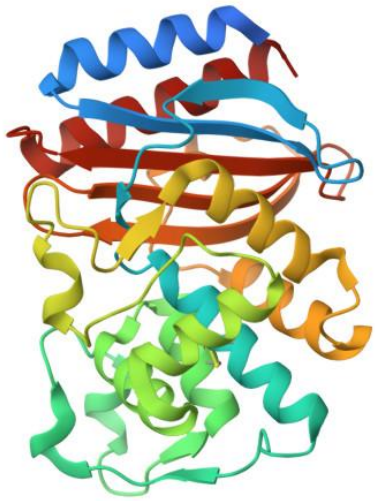

Fig. 1- Structural representation of TEM-1 β-lactamase showing allosteric inhibitor (red) bound at distal site (PDB ID: 1ZG4).

V. CONCLUSIONS AND FUTURE DIRECTIONS

This makes allosteric inhibition a particularly exciting new frontier in the fight against antimicrobial resistance. This method avoids many common active-site target discovery problems by focusing on the less conserved binding sites, distal from conventional inhibition regions of bacterial enzymes. The data from secondary assays show that inhibitors having nanomolar potency can decrease substantially enzyme activity and restore the drug sensitivity. Efforts to computationally map allosteric sites, high-throughput screening campaigns of small molecule candidates and the rational design of dual-site inhibitors that bind both active and allosteric pockets are some possibilities for future work. Combination strategies like these could be the basis of next-generation antibiotics that can halt or even reverse AMR trends.

REFERENCE

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