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Discovery of a Novel Allosteric Binding Region within DNA Gyrase and its Significance to Antibacterial Drug Discovery

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By 2050 it is believed that *ca.* 10 million deaths will occur globally every year due to antimicrobial resistance.[1] This will cost the world economy *ca.* $100 trillion. There is no doubt that the continual evolution of antibiotic resistance is an existential threat to human beings. The discovery of penicillin in 1928 by Alexander Fleming yielded the start of the antibiotic era, revolutionising the treatment of bacterial infections and preventing an inconceivable number of fatalities, notably during World War II. Antibiotics have since become a staple in modern medical procedures.[2] However, following what is known as the "golden period" of antimicrobial drug discovery in the mid-to-late 20th century, a decline in new antibiotic approval by the FDA, alongside a rise in antimicrobial resistance (AMR), has led to a staggering increase in untreatable bacterial infections.[3] This can also be partially attributed to the unfortunate withdrawal of investment from "Big Pharma" companies in antibiotic drug discovery programmes. The total cost of development for an anti-infective drug is estimated at $500-800 million.[4] The lack of said significant breakthroughs illustrate why companies are hesitant to financially commit to such projects, where there are often more lucrative and viable options.[3, 5]

Bacterial type II topoisomerase enzymes, such as DNA gyrase and topoisomerase IV, are essential proteins that modulate the topology of DNA in bacteria during DNA transcription, replication, and other DNA-associated processes.[6, 7] The primary function of DNA gyrase is to introduce "negative supercoils" into bacterial DNA through an ATP-dependent mechanism. Topoisomerase IV serves a different function, primarily eliminating entanglements that occur naturally in DNA during DNA replication.

Both enzymes are comprised of two proteins, coded for by the *gyrA* and *gyrB* genes for DNA gyrase, and the *parC* and *parE* genes for topoisomerase IV.[8, 9] These two proteins are composed of four subunits, forming heterotetrameric protein complexes: A_2B_2 for DNA gyrase, and C_2E_2 for

topoisomerase IV. They are well-validated targets for antibiotics; the fluoroquinolone antibiotics (FQs) being one of the most important, clinically-used classes of antibiotics available. This is because they are renowned for possessing a "dual-targeting" mechanism, where they can of inhibit both DNA gyrase and topoisomerase IV simultaneously.

The prospect of achieving dual-targeting is an immensely attractive one for the discovery of novel antimicrobial agents, as the inhibition of two important enzymes simultaneously presents bacteria with a more difficult task of evolving resistance.[10, 11] Prominently, DNA gyrase and topoisomerase IV possess a high degree of structural and sequence similarity, but possess limited sequence similarity to human topoisomerase II, allowing for the design of selective inhibitors for bacterial topoisomerase enzymes over the human enzyme.[12]

The well-established regions on bacterial type II topoisomerases are associated with their DNA- and ATP-binding sites.[8, 13] FQs bind to the gyrase-DNA complex and "trap" the bound DNA within gyrase by forming key interactions within the DNA-binding site through a water-metal ion bridge.[14] Despite the success of "dual-targeting" inhibitors and the relatively slow rate at which bacterial resistance has evolved to these drugs, resistance is growing within the clinic, primarily through the development of point mutations.[15] For example, amino acid variation of Ser-83 to Phe and Tyr residues, and Asp-87 to Asn have been introduced within the GyrA domain of DNA gyrase, and Glu-84 to Lys modification within the ParC domain of topoisomerase IV.[16]

A promising approach to combating FQ resistance may lie in targeting allosteric binding sites present in DNA gyrase and topoisomerase IV. Allosteric sites are regions that are remote to the active site within a protein – the site that is responsible for carrying out its primary function, *i.e.* the DNA-binding site in the case of the topoisomerases.

Chan *et al.* report one such example within a *Staphylococcus aureus* DNA gyrase structure containing a thiophene-carboxamide-based inhibitor [17, 18] Upon examination of the co-crystal structure, it was discovered that the inhibitor was bound within a pocket between the GyrA and GyrB subunits. This region is remote from the FQ binding site, and as such is an allosteric inhibition site. The inhibitor was observed to be very potent towards *E. coli* DNA gyrase (IC_{50}: 0.30 μM) and importantly is not cross-resistant with the FQs. However it lacked any form of significant potency against topoisomerase IV (IC_{50}: >540 μM). Whilst potent against DNA gyrase, the dual-targeting mechanism associated with the FQs was clearly not observed for this compound. Unfortunately, the development of this inhibitor was later terminated due to *in vivo* toxicity.

A later publication by the same team describes examples of fused 5-6-heterocyclic inhibitors that incorporated or entirely replaced the thiophene moeity.[18] Their most potent inhibitor was determined against *E. coli* DNA gyrase (IC_{50}: 0.16 μM); 4-fold less active than the parent thiophene inhibitor, but notably also displayed some mild *E. coli* topoisomerase IV inhibition (IC_{50}: ~90 μM - decatenation of kinetoplast DNA inhibition assay). It was also observed to be weakly active against human topoisomerase IIα (IC_{50}: ~210 μM), and was found to possess cytotoxicity and cardiac ion channel inhibition (hERG and $Na_V1.5$) which resulted in the termination of these compounds. These data however offer significant promise for the future development of dual allosteric DNA gyrase/topoisomerase IV inhibitors. The design was developed further by Thalji *et al.*, resulting in a novel molecular design equipotent against DNA gyrase and more potent than the parent compound against topoisomerase IV. It remained weakly inactive against human topoisomerase II, showing the continual opportunity for selective targeting.

There is therefore potential for future development with this allosteric site, as different molecular designs appear to validate the nature of the residues involved in intermolecular bonding. Notably, with both the Chan *et al.* and Thalji *et al*. inhibitors, it was observed within two X-ray crystal structures (PDB: 5NPP and 6QX1 respectively) the predicted interactions between the different inhibitors and both Arg-630 and Glu-634. Targeting these two amino acid residues appears to be key to the inhibition of DNA gyrase, though minimal activity against topoisomerase IV to-date has likely resulted in crystal structure procurement challenging, if not impossible. It therefore remains to be seen if sufficient activity can be achieved against topoisomerase IV following a similar molecular design. This would potentially validate the existence of a similar allosteric site present within the topoisomerase IV crystal structure, and aid the rationalisation of a potentially novel dual-targeting approach that is not cross-resistant with the FQs. In this pursuit, the authors are using advanced computational modelling techniques to design and subsequently synthesise dual-targeting inhibitors tailored to this allosteric site.

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Disclosures

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