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Review article

Dual targeting DNA gyrase B (GyrB) and topoisomerse IV (ParE) inhibitors: A review

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ABSTRACT

GyrB and ParE are type IIA topoisomerases and found in most bacteria. Its function is vital for DNA replication, repair and decatenation. The highly conserved ATP-binding subunits of DNA GyrB and ParE are structurally related and have been recognized as prime candidates for the development of dual-targeting antibacterial agents with broad-spectrum potential. However, no natural product or small molecule inhibitors targeting ATPase catalytic domain of both GyrB and ParE enzymes have succeeded in the clinic. Moreover, no inhibitors of these enzymes with broad-spectrum antibacterial activity against Gram-negative pathogens have been reported. Availability of high resolution crystal structures of GyrB and ParE made it possible for the design of many different classes of inhibitors with dual mechanism of action. Among them benzimidazoles, benzothiazoles, thiazolopyridines, imidiazopyridazoles, pyridines, indazoles, pyrazoles, imidazopyridines, triazolopyridines, pyrrolopyrimidines, pyrimidoindoles as well as related structures are disclosed in literatures. Unfortunately most of these inhibitors are found to be active against Gram-positive pathogens. In the present review we discuss about studies on novel dual targeting ATPase inhibitors.

Contents

1. Introduction

As bacterial resistance to antibiotics has become an important public health problem, there is a continuing need to develop newer and more potent antibiotics. The bacterial type II DNA topoisomerases, Gyrase B (GyrB) and topoisomerase IV (ParE) are essential and highly conserved enzymes that regulate changes in DNA topology and integrity during replication, recombination, and transcription in an ATP-dependent reaction. Both enzymes are also independently essential for bacterial growth. Although there are specific structural and functional differences between GyrB and

Abbreviations: ADP, adenosine diphosphate; Arg, arginine; Asp, aspartic acid; ATP, adenosine triphosphate; CC_{50}, concentration which results in the death of 50 percent of the host cells; C_{max}, maximum serum concentration; DNA, deoxyribonucleic acid; FQR, fluoroquinoline resistant; Glu, glutamine; HEPES, N-(2-hydroxye thyl)piperazine-N'-2-ethanesulfonic acid; IC_{50}, half maximal inhibitory concentration; Ile, isoleucine; K_i, inhibitory constant; MDR, multi drug resistant; Met, methionine; MIC, minimum inhibitory concentration; MRSA, methicillin resistant *Staphylococcus aureus*; MSSA, methicillin susceptible *Staphylococcus aureus*; nM, nano mole; pK_a, acid dissociation constant; TC_{50}, the compound concentration that causes 50% cell death; TopIV, topoisomersae IV; Tyr, tyrosine; $T_{1/2}$, half life; VISA, vancomycin intermediate *Staphylococcus aureus*; VRE, vancomycin-resistant *Enterococci*; VRSA, vancomycin-resistant *Staphylococcus aureus*.

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ParE, both enzymes catalyse DNA double stranded breaks and subsequent ligation and they both bind and hydrolyse ATP to provide the energy required to perform these tasks. The effectiveness of quinolones and fluoroquinolones is limited due to prevalence of target-based resistance [1,2] and this prompted the search for new types of compounds with new mechanisms of action against the type II topoisomerases. Being attractive and well established target for the development of antibacterial agents, there has been considerable interest in discovering and developing novel inhibitors containing a single pharmacophore that target both GyrB and ParE enzymes. There are fewer known inhibitors that bind to GyrB. Examples include the coumarins, novobiocin and coumermycin A1, cyclothialidine, cinodine, and clerocidin. These chemical classes have provided significant structural and mechanistic interest. But despite being potent inhibitors of gyrase supercoiling, the coumarins have not been widely used as antibiotics. They are generally not suitable due to their low permeability in bacteria, eukaryotic toxicity, and poor water solubility [3]. Novobiocin was shown to inhibit DNA replication by competitively inhibiting the ATPase of GyrB [4]. Although toxicity has limited the use of novobiocin in the clinic but it showed excellent antimicrobial activity for both methicillin-susceptible and -resistant *Staphyloccocus aureus* in combination with rifampicin [5]. In addition, this drug combination prevented the emergence of resistance *in vitro* to each drug in the combination and was found to be useful as mutations to novobiocin resistance in GyrB arised [6,7].

Further study revealed that resistance to novobiocin in *S. aureus* is predominantly due to the successive point mutations first occurred specifically in the GyrB gene, followed by a point mutation in the ParE gene and then an additional point mutation in the GyrB gene [8]. These findings revealed that DNA gyrase is the primary target and that topoisomerase IV is the secondary target for novobiocin. Results also suggested that the accumulation of point mutations in both the GyrB and the ParE genes is associated with high-level resistance to novobiocin in *S. aureus* and interactions of novobiocin and coumermycin A1 with GyrB differ at the molecular level [9].

A number of small molecule GyrB and ParE inhibitors have been identified but none has reached the market. It is desirable to have a new, effective inhibitor that targets the ATP binding sites in both the GyrB and ParE subunits and it would be useful for treating various bacterial infections. There are several reviews appeared in literature [10–19] describing the approaches to design the inhibitors of GyrB and ParE enzymes. High resolution structural information on GyrB and ParE has been instrumental in stimulating the research effort for new synthetic chemotypes that inhibits these enzymes but currently there are no compounds in the clinic. Discovery of GyrB and ParE dual inhibitors started with the solution of structures of *Escherichia coli* ParE (43kda N-terminal fragment) complexed with adenylyl-imidodiphosphate (ADPNP) [20,21] and *E. coli* ParE (24 kDa N-terminal) complexed with novobiocin (PDB-ID: 1S14) [22]. GyrB and ParE enzymes have structural similarities in their conserved active sites (Figs. 1 and 2), subunit organization, and antibiotic sensitivity to coumarins and quinolones [23–25]. These structural similarities can be utilized for the development of novel small-molecule inhibitors by dual targeting of ATPase binding sites in both GyrB and ParE subunits. Novobiocin was found to be approximately 5-fold less potent as a ParE inhibitor (*E. coli* IC_{50} 210 nM) than as GyrB inhibitor (*E. coli* IC_{50} 46 nM) and based on mutation studies this difference in potency was attributed to the single residue difference (GyrB Ile78 to ParE Met74). Exchange of these single-position equivalent amino acid residues in ParE (Met74) and in GyrB (Ile78) resulted in a 20-fold (to 12 nM) increase in the potency of novobiocin against topoisomerase IV. However, the corresponding exchange in GyrB (Ile78 Met) yielded a 20-fold decrease in the potency of novobiocin (to

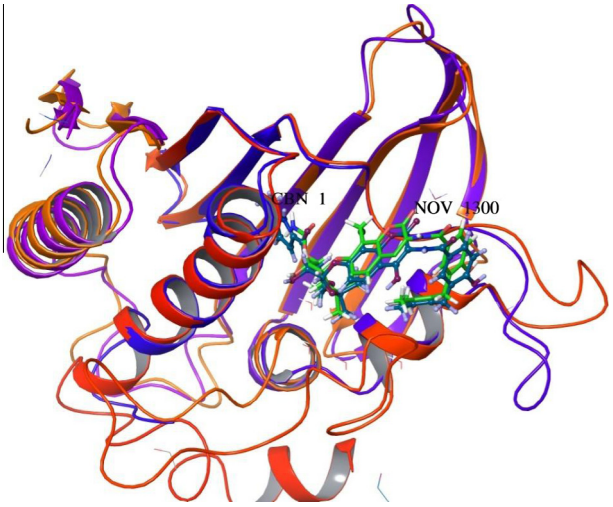

Fig. 1. The superimposition of the catalytic pockets of GyrB chain A (PBD ID 1KZN, Orange red color) and ParE chain A (PBD ID 1S14, blue color) structures is shown (Alignment Score: 0.081, RMSD: 1.424 Angstrom), (NOV: novobiocin in stick representation, CBN 1: in ball and stick representation).

1.0 μM) [22]. The sensitivity of novobiocin potency to single-amino-acid substitutions in GyrB and ParE suggested that they are independently vulnerable to resistance mutations and hence targeting both type II topoisomerases will result in more durable therapies. In GyrB mutagenesis work, when GyrB Ile78 residue was changed to alanine or leucine an increase in the novobiocin IC_{50} was observed by factors of 30 and 14, respectively [26]. In contrast, substitution of more conserved valine resulted in a twofold increase in IC_{50}, suggesting that a branching of the hydrophobic side chain at the β-carbon is important for novobiocin affinity. Additional structural information of *E. coli* ParE ATP binding site co-crystallized with 1-(4-acetyl-6-pyridin-3-yl-1H-benzimidazol-2-yl)-3-ethylurea (PDB ID: 3FV5) is given by Wei and co-workers [27].

2. GyrB and ParE dual inhibitors

Dual inhibitor of GyrB and ParE ATPase subunits is highly attractive in reducing the frequency of development of drug resistance, as the probability of two simultaneous mutations occurring in both essential targets is low [22]. Availability of crystal structures of GyrB and ParE has made possible the design of inhibitors with dual mechanism. But unfortunately all of these GyrB/ParE inhibitors seem to target Gram-positive pathogens, and none have so far progressed for the clinical use. In this review we discuss novel dual-targeting GyrB/ParE ATPase inhibitors disclosed in literatures and patents.

In an effort to discover novel antibacterial agents, pyrazole derivative (**1**) (Fig. 3) was identified as dual inhibitors of DNA gyrase and topoisomerase IV. It showed inhibitory activity against *S. aureus* DNA gyrase and topoisomerase IV (IC_{50} 128 μg/mL in both cases) with weak antibacterial activity against methicillin resistant (MRSA), penicillin resistant *Streptococcus pneumoniae* (PRSP), vancomycin-resistant *Enterococcus faecalis* (VRE) and multidrug efflux pump mutant *E. coli* K901 (MIC 64 μg/mL in all cases) [28]. Further structural modification of compound **1** yielded compounds **2** and **3** (Fig. 3) with potent antibacterial activity against MRSA (MIC 4 and 1 μg/mL, respectively), PRSP (8 and 2 μg/mL, respectively), VRE (8 and 2 μg/mL, respectively) and multidrug efflux pump mutant *E. coli* K901 (4 μg/mL in both cases). The inhibitory activity of compounds **1–3** was examined against DNA gyrase