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Research paper

Discovery of substituted oxadiazoles as a novel scaffold for DNA gyrase inhibitors

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ABSTRACT

DNA gyrase and topoisomerase IV are type IIa topoisomerases that are essential bacterial enzymes required to oversee the topological state of DNA during transcription and replication processes. Their ATPase domains, GyrB and ParE, respectively, are recognized as viable targets for small molecule inhibitors, however, no synthetic or natural product GyrB/ParE inhibitors have so far reached the clinic for use as novel antibacterial agents, except for novobiocin which was withdrawn from the market. In the present study, a series of substituted oxadiazoles have been designed and synthesized as potential DNA gyrase inhibitors. Structure-based optimization resulted in the identification of compound **35**, displaying an IC_{50} of 1.2 μM for *Escherichia coli* DNA gyrase, while also exhibiting a balanced low micromolar inhibition of *E. coli* topoisomerase IV and of the respective *Staphylococcus aureus* homologues. The most promising inhibitors identified from each series were ultimately evaluated against selected Gram-positive and Gram-negative bacterial strains, of which compound **35** inhibited *Enterococcus faecalis* with a MIC_{90} of 75 μM. Our study thus provides further insight into the structural requirements of substituted oxadiazoles for dual inhibition of DNA gyrase and topoisomerase IV.

1. Introduction

The incidence of infections resistant to all currently used antibacterial drugs has increased dramatically in the past decade [1]. The emerging resistance extends to both Gram-positive and Gram-negative organisms and constitutes a serious threat to successful antibacterial therapy [2]. One approach to tackling this issue successfully is to develop novel agents that inhibit known and novel bacterial targets via unique binding sites or via novel modes of action [3].

DNA gyrase and topoisomerase IV are bacterial type IIa topoisomerases and pivotal enzymes required in overseeing the topological state of DNA during transcription and replication processes [4]. While DNA gyrase is essential for the initiation of DNA replication, elongation of nascent DNA and the negative supercoiling of DNA during replication, topoisomerase IV is involved primarily in DNA decatenation at the end of replication [5]. The inhibition of one or both of these enzymes results in the disruption of DNA synthesis, in turn leading to cell death. DNA gyrase and topoisomerase IV are heterotetramers, formed by association of subunits GyrA and GyrB or ParC and ParE, respectively [6–8]. DNA gyrase and topoisomerase IV are clinically validated targets, their druggability being well established in two classes of antibiotics - the fluoroquinolones and aminocoumarins. The fluoroquinolones interact with the GyrA and ParC domain, while aminocoumarins target the GyrB and ParE domains [9]. Of note, DNA gyrase and topoisomerase IV enzymes act in an ATP-dependent manner, relying on their ATPase domains, GyrB and ParE, respectively, for providing the energy required for the extensive conformational changes [6]. Their ATP binding pockets exhibit high homology among bacteria, but at the same time low homology with eukaryotes [4]. They have been recognized early on as potential targets for small molecule inhibitors [7,10,11], however, to date, no synthetic or natural product GyrB/ParE inhibitors since novobiocin which was withdrawn from the market have reached the clinic due to different issues such as resistance, toxicity and permeability [12]. The GyrB/ParE ATP-binding subunits, therefore,

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are still insufficiently exploited targets for the development of novel antibacterial agents [13].

Novobiocin and clorobiocin belong to a class of structurally closely related naturally occurring antibiotics, the aminocoumarins (see Fig. 1). Clorobiocin features a pyrrole moiety replacing the carbamate group of novobiocin and a chlorine atom instead of a methyl group. The most essential interaction between these aminocoumarins and the ATP-binding site is achieved in *E. coli* via Asp73 residue and the associated water molecule [7]. Cyclothialidines (see Fig. 1) constitute the second class of naturally occurring DNA gyrase ATPase inhibitors [14–16]. These provide proof of tolerance of the ATP binding site of DNA gyrase to significant chemical diversity [7]. The X-ray crystal structures of coumarins and cyclothialidines bound to the ATP-binding pocket of DNA gyrase further have reinforced that conclusion, revealing that the carbamate of novobiocin, the 2-carboxypyrrole of clorobiocin, and the phenol group of cyclothialidine, are all able to interact with the Asp73 residue and its hydrogen-bonded conserved water molecule [6].

The crystal structure of DNA gyrase B was first solved by Wigley in 1991, laying the groundwork for further research in this field [17]. The X-ray structure information on the binding modes of cyclothialidine and novobiocin to the ATP binding site of GyrB [18,19] as well as the ligand-based inhibitor design [15,16] had established a broad knowledge of the structure-activity relationships (SAR), providing a strong basis for the rational design of novel inhibitors and at the same time enabling further optimization of lead structures.

The binding modes of the reported inhibitors revealed an overlapping site of interaction, namely a set of hydrogen bonds provided by Asp73 and an associated conserved water molecule. This interaction is essential, and almost indispensable. Nevertheless, other unique structural and electronic characteristics of the binding-site pocket have also been taken into account in the design process. The common structural features of synthesized inhibitors also include functionalities making use of interactions with (*i*) Arg136; (*ii*) an additional hydrophobic pocket composed of Val71, Val43, Val167, and Ala147; (*iii*) a hydrophobic floor consisting of Pro79, Ile78 and Ile94 and (*iv*) and a π-stacking ceiling formed by Arg76 in a Glu50-Arg76 salt bridge. The ligand-based and fragment-based approaches as well as the targeted screening programs have identified numerous GyrB inhibitors with diverse scaffolds, such as novobiocin and cyclothialidine analogs [15,16,20], arylaminotriazines [21], arylaminopyrimidines [2], indazoles [22], pyrazolopyridones [23], pyrrolopyrimidines [11,13], pyridylureas [24], pyrrolamides [25] and tricyclic pyrimidinoindoles [26], thus providing several additional starting points for medicinal chemists (structures are shown in Fig. 2).

Currently, there is a pressing need for new antibacterial compounds [27]. Compound **XIV** (Fig. 3), a low nanomolar GyrB inhibitor based on a thiazole-piperidine central core has recently been disclosed as part of a series of promising pyrrolamide GyrB inhibitors [9]. Its development, however, was discontinued mostly due to unfavorable pharmacokinetics. Therefore, we explored possible replacements of the thiazole-piperidine core of **XIV** to find another scaffold that could provide different SAR and optimization opportunities. Oxadiazoles are considered important constituents of biologically active compounds, exhibiting a wide range of effects, which makes them attractive building blocks for a variety of purposes [28–30]. The main objective of the present study was to identify small molecule inhibitors of DNA gyrase based on a novel phenyl-substituted oxadiazole central scaffold by utilising their structural similarity to the known pyrrolamide **XIV**.

2. Results and discussion

2.1. Design

Our design was based on the structures of the recently disclosed series of pyrrolamide GyrB inhibitors carrying a thiazole-piperidine central core and represented by compound **XIV** [9]. Making use of both ligand-based as well as structure-based approaches, we chose this inhibitor as lead and designed a small library of its analogs based on a substituted 5-phenyl-1,2,4-oxadiazole central scaffold (Fig. 3). The thiazole-carboxylic acid moiety was bioisosterically replaced by an oxadiazole-carboxylic acid. The aromatic nature of this heterocycle is key for optimizing π-stacking with Arg76 and hydrogen bond interactions with Arg136, while the pendant carboxylic acid function provides an additional interaction with Arg136. The Glu50-Arg76 salt bridge has also been taken into account, as it is prone to a π-cation interaction with the oxadiazole moiety of the inhibitors.

The pyrrole NH group of **XIV** has been shown to interact with the Asp73-water H-bond motif in the GyrB ATP-binding pocket, indicating a certain degree of similarity of this series to clorobiocin and kibdelomycin [31]. Of note, some of the reported ATP-competitive DNA gyrase inhibitors (e.g. clorobiocin) feature substituted pyrrole moieties in their structures. It has been demonstrated that, by attaching small lipophilic groups to the pyrrole ring, stronger interactions with the left-hand side lipophilic pocket of GyrB can be achieved. Similarly, several DNA gyrase inhibitors possessing pyrrole, dibromopyrrole and bromopyrrole moieties in their structures have been identified [32–36]. Thus, the 5-methyl-3,4-dichloropyrrole moiety of the parent compound was replaced by various heterocyclic moieties, such as indole, pyrrole, 4-bromopyrrole, 4,5-dibromopyrrole and 5-fluoroindole moieties to probe the chemical space of the left-hand side lipophilic pocket. The selection of compounds for the synthesis was guided and corroborated by molecular docking of candidate molecules into the DNA gyrase ATP-binding site (PDB code: 4DUH). Analysis of the results of molecular docking of compound **19** in the ATP-binding site of *E. coli* DNA gyrase revealed possible hydrophobic interactions of the indole moiety in the hydrophobic pocket formed by Val43, Val71, Val120 and Val167.

Moreover, **19** was also predicted to form two hydrogen bonds – one with Asp73 side chain and the other with the conserved water

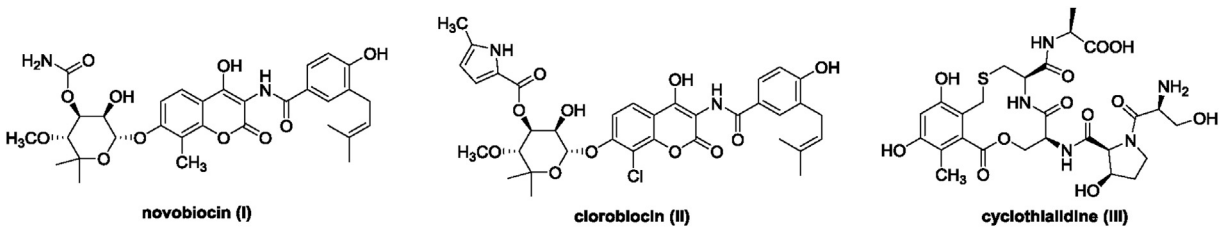

Fig. 1. Naturally occurring DNA gyrase inhibitors.