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In silico discovery of 2-amino-4-(2,4-dihydroxyphenyl)thiazoles as novel inhibitors of DNA gyrase B

Matjaž Brvar [a,†], Andrej Perdih [a,†], Marko Oblak [b], Lucija Peterlin Mašič [c], Tom Solmajer [a,*]

[a] National Institute of Chemistry, Hajdrihova 19, 1001 Ljubljana, Slovenia
[b] Lek Pharmaceuticals, d.d., Verovškova 57, 1526 Ljubljana, Slovenia
[c] Faculty of Pharmacy, University of Ljubljana, Aškerčeva 7, 1001 Ljubljana, Slovenia

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ABSTRACT

Cyclothialidines are a class of bacterial DNA gyrase B (GyrB) subunit inhibitors, targeting its ATP-binding site. Starting from the available structural information on cyclothialidine GR122222X (**2**), an in silico virtual screening campaign was designed combining molecular docking calculations with three-dimensional structure-based pharmacophore information. A novel class of 2-amino-4-(2,4-dihydroxyphenyl)thiazole based inhibitors (**5**–**9**) with low micromolar antigyrase activity was discovered.

The emergence of bacterial resistance to most of the clinically used antibiotics is driving an urgent need for the development of novel and effective antibacterial agents.[1] The main challenge remains the discovery of highly potent antibacterials with broad spectrum of efficacy and improved safety profile.[2,3]

One of the well established targets of the antibacterial agents is the DNA gyrase, a unique bacterial type II topoisomerase originating from the gyrase, HSP90, histidine kinase, MutL (GHKL) enzyme superfamily that catalyzes the introduction of negative supercoils into the DNA using the concurrent ATP hydrolysis.[4–6] DNA gyrase consists of two subunits, gyrase A (GyrA) and gyrase B (GyrB), that together form a functional heterodimer structure A2B2. While the function of the GyrA subunit is primarily the breakage and reunion of the bacterial DNA, the GyrB subunit possesses an ATP-ase activity. In the absence of the ATP, DNA gyrase catalyzes only the relaxation of supercoiled DNA but not the introduction of negative supercoils.[5,6]

Quinolones are the only class of the DNA gyrase inhibitors currently used in clinical practice. They act by inhibiting the GyrA subunit, thus interfering with the DNA cleavage and religation reactions.[7] The coumarins (e.g., novobiocin, **1**) and cyclothialidines (e.g., GR122222X, **2**), natural antibiotics from the *Streptomyces* organisms, are the most studied inhibitors of the GyrB subunit. Both classes act as competitive inhibitors of the ATP-binding site on the GyrB subunit, thus inhibiting the ATP-dependent step in the enzyme catalytic cycle.[8] The inhibitory mechanism has also been characterized with radiolabelled benzoylcyclothialidine and dihydronovobiocin and by structural analysis.[9] More recently, GyrB inhibitors from various chemical classes have been reported including indazole,[10] pyrazole,[11] benzimidazole,[12] phenol[13] and indolinone.[14]

First coumarin GyrB inhibitors were discovered in 1950s and novobiocin (**1**) (IC_{50} = 3nM) was approved for clinical use in 1960s but was withdrawn from the market due to its hazardous side effects and toxicity.[9] The cyclothialidine GR122222X (**2**) with IC_{50} value of 1.5 nM was found to be a potent and selective inhibitor of the GyrB as well[15–17] (Fig. 1). The core structure of **2** comprises a 12-membered lactone ring with an integrated pentapeptide chain (Ala-Cys-Ser-Hyp-Ser) attached to the resorcinol moiety. Despite its excellent in vitro activity cyclothialidine **2** possesses no antibacterial activity, due to its insufficient penetration of the bacterial cell wall. Attempts to optimize the structure of **2** resulted in compounds **3** and **4** with promising antibacterial activities[18–20] (Fig. 1). Alongside the cyclothialidine class, flavonoids (e.g., quercetin) incorporating resorcinol moiety were also shown to be potent GyrB inhibitors.[21] The binding mode of quercetin to the ATP-binding site of gyrase B has been fully characterized by a combination of different physico-chemical approaches.[22]

* Corresponding author. Tel.: +386 (0)1 4760 277; fax: +386 (0)1 4760 300.
E-mail address: tom.solmajer@ki.si (T. Solmajer).
† These two authors contributed equally to this work.

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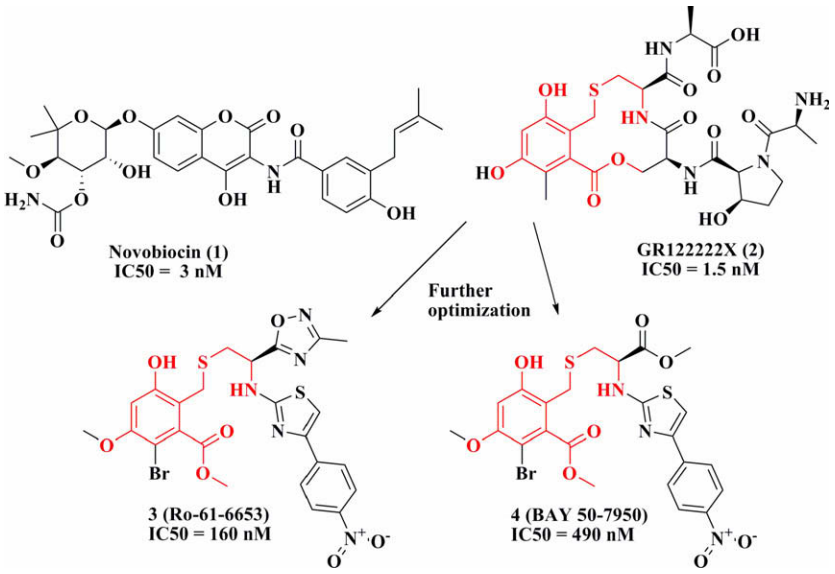

Figure 1. Structures of DNA gyrase B inhibitors: novobiocin (**1**), cyclothialidine GR122222X (**2**) and two synthetic analogues (**3** and **4**). The shared structural features of compounds (**2**–**4**) are colored in red.

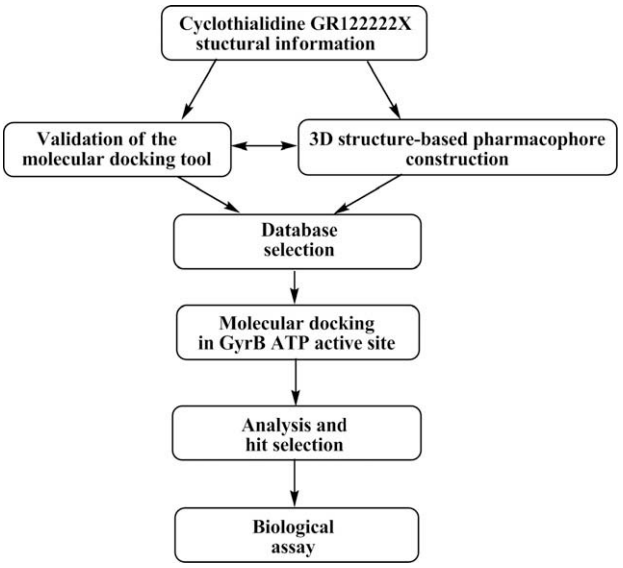

Figure 2. Outline of the virtual screening (VS) workflow used in the identification of novel DNA gyrase B inhibitors.

The crystal structures of compounds **1** and **2**[23] with 24 kDa N-terminal fragment of the DNA gyrase B protein offer valuable information for lead optimization process. It has been demonstrated that the noviose sugar of novobiocin (**1**) and resorcinol moiety of cyclothialidine GR122222X (**2**) are involved in a similar H-bond network interaction pattern as well as similar hydrophobic interactions in the ATP-binding site of GyrB.[23–26]

In our ongoing efforts to discover novel promising GyrB inhibitors as potential antibacterial agents[9] an in silico virtual screening campaign was designed, taking into account the available structural information on binding mode of cyclothialidine **2** into the ATP-binding site of GyrB.[9,23] The main objective of the VS campaign was to screen the available compound databases to identify novel low-molecular inhibitors mimicking the cyclothialidine (**2**) molecular recognition pattern. Additionally, we searched for potential conformational rigid replacements of the 12-membered lactone ring in cyclothialidine **2** that would retain the antigyrase B affinity and concurrently improve the drug-like properties.

The virtual screening protocol is schematically presented in Figure 2. In the first stage the available structural information on cyclothialidine GR122222X binding mode was used for the validation of the VS molecular docking tool FlexX.[27] Bound conformation of **2** is well described in the literature, however and regrettably the

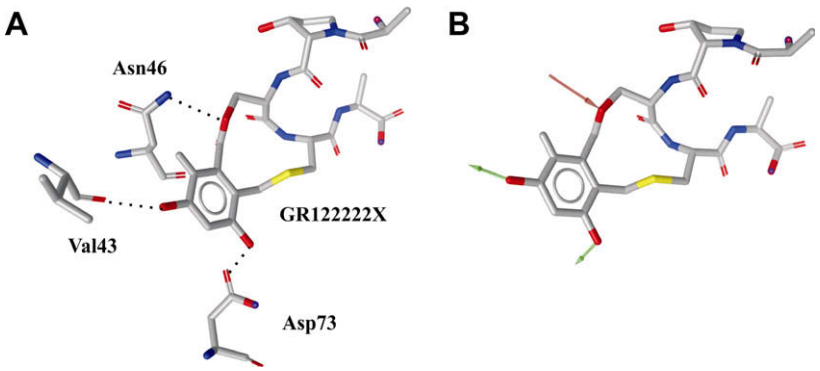

Figure 3. (A) Bound conformation of cyclothialidine GR122222X (**2**) with important GyrB interacting residues in the ATP-binding site. (B) The selected identified 3D-pharmacophore features of **2** (red arrow—hydrogen bond acceptor, green arrow—hydrogen bond donor).