Bioorganic & Medicinal Chemistry Letters 20 (2010) 958–962

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

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

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ARTICLE INFO

Article history:
Received 3 November 2009
Revised 11 December 2009
Accepted 14 December 2009
Available online 22 December 2009

Keywords:
DNA gyrase enzyme
Virtual screening
Molecular docking
Antibacterial agents
Drug design

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]

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doi:10.1016/j.bmcl.2009.12.060

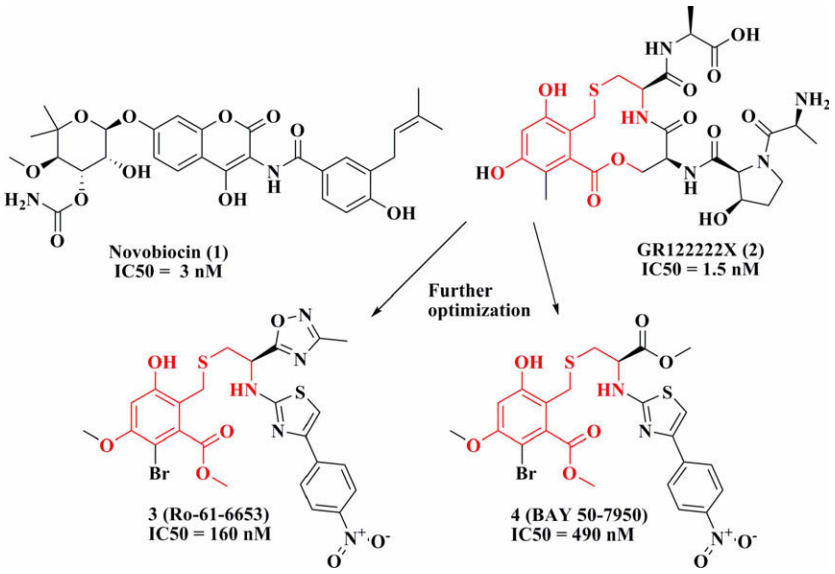


Figure 1. Structures of DNA gyrase B inhibitors: novobiocin (**1**), cyclothialidine GR122222X (**2**) and two synthetic analogues (**3** and **4**). The shared structural features of compounds (**2**–**4**) are colored in red.

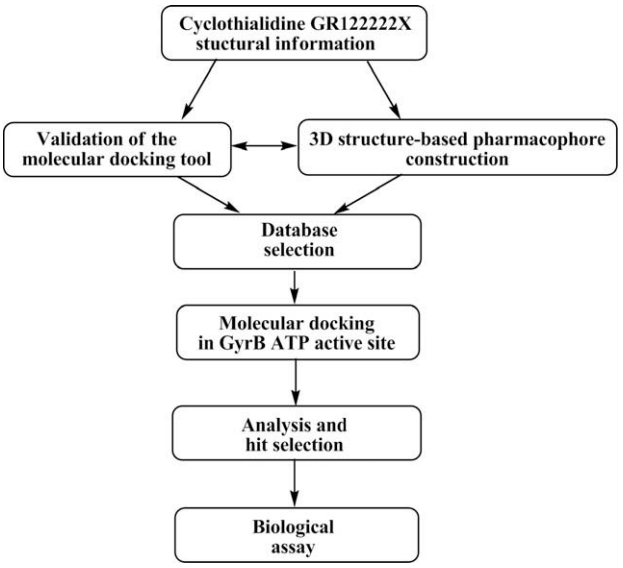


Figure 2. Outline of the virtual screening (VS) workflow used in the identification of novel DNA gyrase B inhibitors.

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

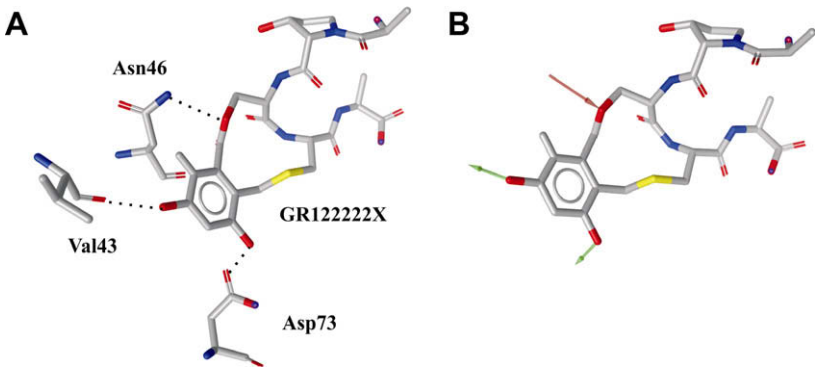


Figure 3. (A) Bound conformation of cyclothialidine GR122222X (**2**) with important GyrB interacting residues in the ATP-binding site. (B) The selected identified 3D-pharmacophore features of **2** (red arrow—hydrogen bond acceptor, green arrow—hydrogen bond donor).

Table 1
Selected hits from the virtual screening campaign with inhibitory activity against the DNA gyrase

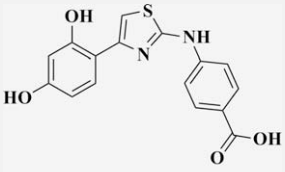
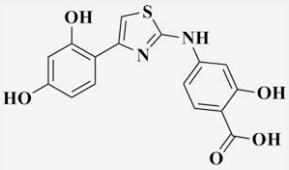
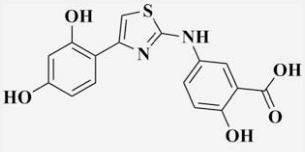
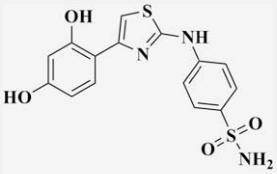
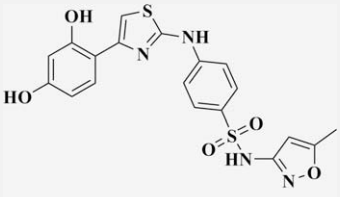
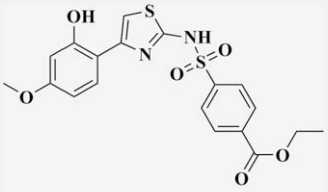
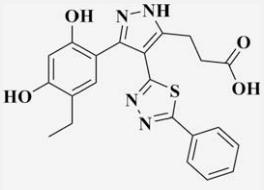
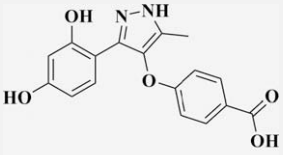
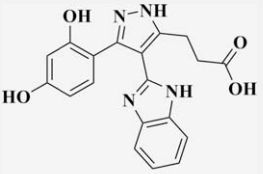
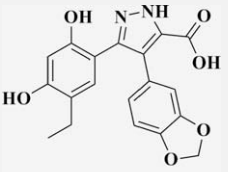
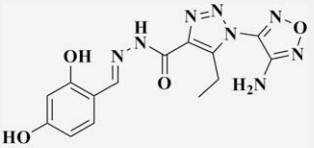
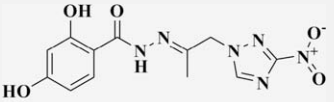
Chemical structure	IC_{50} (μM)
	56
5 	39
6 	36
7 	25
8 	46
9 	>500
10 	>500
11  12	>500

Table 1 (*continued*)

Chemical structure	IC_{50} (μM)
	>500
13 	>500
14 	>500
15 	>500
16	

* IC_{50} of 0.015 μM was measured for novobiocin; as reference standard (compared to 0.003 μM of Ref. 23).

crystal structure of the complex is not available in the PDB protein database. Therefore, protein structure of GyrB in complex with clorobiocin, a novobiocin analogue (**1**) from the same bacterial species (*Escherichia coli*) was retrieved from the PDB (entry 1KZN)[24] and all crystal waters were removed. GyrB active site was defined 8 Å around reference ligand clorobiocin. In addition, a pharmacophoric constrain was added, determining Asp73 as a hydrogen bond acceptor, an important interaction observed in the complex with GR122222X.[23] Previously, it has been demonstrated that the free phenol group is essential for enzyme inhibition as it is able to form a vital hydrogen bond network with the Asp73.[10] Furthermore, this residue is highly conserved among prokaryotes and hydrogen bonding network was observed between the protein, crystal water molecule, and several inhibitors.[23,24] The phenol moiety was also one of the fragments considered in previous fragment-based drug design efforts which resulted in potent inhibitors.[10,13] During the docking calculation all calculated poses were kept and were visually examined afterwards.

The FlexX calculated binding modes of **2** were quite consistent. The representative bound conformation is depicted in Figure 3A. It strongly resembles the experimentally determined structure of **2**.[23] As the RMSD comparison of the docked conformation with the experimental one was not possible, the evaluation was based primarily on the visual comparison of the FlexX-generated conformations with the available reference data.[23] It was gratifying to observe that the docked positions of the resorcinol and lactone moieties strongly resembled the reference conformation of **2** in the GyrB ATP-binding site. The hydroxyls of the resorcinol moiety form hydrogen bonds with the protein (Asp73 and Val43) and lactone interacts with Asn46. All these data are in line with the avail-

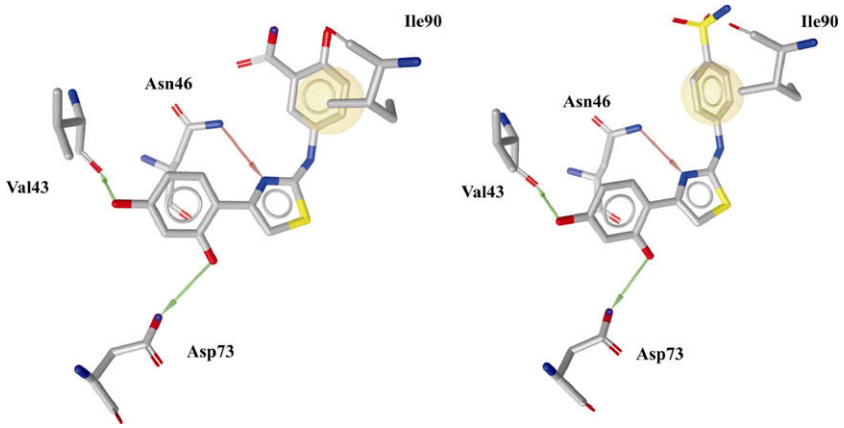


Figure 4. FlexX-calculated binding geometries of VS hit thiazole compounds **7** (left) and **8** (right) docked into the GyrB ATP-binding site and selected GyrB interacting residues Identified LigandScout 3D structure-based pharmacophore features are also displayed (the yellow sphere depicts the hydrophobic interaction).

able structural data. The docking procedure was further validated by generating the bound conformation of clorobiocin and comparing it with the experimental one (Fig. 1S of the Supplementary data). In addition, FlexX screening tool was validated using known inhibitors of the GyrB from cyclothialidine class from the literature (see Supplementary data for further details). The validation results showed that the similar binding modes can be obtained for the whole compound class giving more confidence in the screening tool used during the subsequent large scale docking calculations.

Subsequently, a 3D structure-based pharmacophore model was constructed using LigandScout software—a powerful structure-based pharmacophore generator.[28] The constructed pharmacophore model highlighted, in particular, three pharmacophoric features (Fig. 3B) for VS procedure. These features comprised two hydrogen bonds between hydroxylic groups of the resorcinol ring and Asp73 and Val43 of the GyrB subunit (acting as hydrogen bond donors) and a hydrogen bond between Asn46 and the lactone ring oxygen of **2** (acting as a hydrogen bond acceptor).

The database of commercially available compounds used in VS campaign was extracted from the ZINC database.[29] It comprised structures containing the resorcinol moiety as well as similar mono- or poly-substituted phenol analogues. Approximately 20,000 structures were used in our virtual screening campaign.

Obtained compounds were docked into the GyrB ATP-binding site, prepared as described above using the FlexX molecular docking software with the previously discussed pharmacophoric constraint. Each molecule was docked 10 times into the binding site.

After molecular docking procedure the analysis of the performed VS campaign was done and the obtained binding geometries were visually inspected. In particular, resorcinol derivatives displayed favorable binding behavior in the GyrB ATP active site.

Overall the group of selected hits can be described as analogues of initial cyclothialidine (**2**), principally from two series based on the five-membered ring attached to the resorcinol moiety. The first group (compounds **5**–**9**) has a thiazole moiety attached; the other group (compounds **11**–**14**) contains the heterocycle pyrazole. It has been previously demonstrated that monomethylated resorcinols retain their antigyrase activity thus also compound **10** was included in the hit selection.[13,19] Finally, compounds **15** and **16**, where the resorcinol is linked via the acylhydrazone moiety to the heterocycle showed favorable binding geometries in the initial analysis.

Selected compounds **5**–**16** were purchased and assayed for their antigyrase activity utilizing a standard high-throughput assay and novobiocin as a reference standard[30] (see Supplementary data for the compounds origin details and vendors quality control procedures). To prevent nonspecific (promiscuous) inhibition 0.002% TWEEN-20® was added in the reaction mixture.[31] The results are presented in Table 1.

Compounds **5**–**9** represent resorcinol derivatives linked directly to the 2-aminothiazole moiety. The amino group is further substituted with various *meta* and/or *para* substituted phenyl moieties. These compounds were all found to possess inhibitory activity in the low micromolar range with the IC_{50} values ranging between 25 and 56 μM. Interestingly, the thiazole ring is also present in *seco*-cyclothialidine inhibitors albeit it is not directly linked to the resorcinol moiety.[19] The FlexX predicted binding modes of the thiazole-containing compounds resemble the experimental bound conformation of the GR122222X (**2**). In Figure 4 the selected conformations of the most active compounds **7** and **8** are displayed. Hydroxylic groups of **7** (IC_{50} = 36 μM) and **8** (IC_{50} = 25 μM) both form hydrogen bond network with Asp73 and Val43 and act as hydrogen bond donors, which is in accordance with the previous published X-ray data.[23] Next important interaction is the H-bond between the nitrogen of the thiazole moiety (**7** and **8**) and Asn46 where nitrogen acts as a hydrogen bond acceptor. This interaction is complementary to the observed interaction between Asn46 and the lactone ring oxygen of GR122222X (**2**).[23] Thus, the thiazole moiety can, similarly to **2**, act not only as a proper linker but also supplying this important interaction for molecular recognition. Similar interaction pattern was observed for all docked compounds **5**–**9** of the thiazole series. The third substructure of the active compounds comprises differently substituted phenyl moieties. In the GyrB ATP-binding site this residues generally form hydrophobic interaction with the Ile90 residue which improves their binding free energy. The LigandScout software identified a hydrophobic feature on the phenyl ring (Fig. 4), which corroborates nicely this observation. No such pharmacophore feature could have been anticipated as favorable before the VS of small molecules was performed due to different chemical nature of the GR122222X moiety located in the vicinity of Ile90. Interestingly, the compound **10** was found to be inactive (IC_{50} >500 μM) suggesting in our limited structure–activity relationship that the distant phenyl moiety needs to be directly attached to the amino group. The FlexX determined conformations placed the ester group of compound **10** in the bulk of the GyrB, thus apparently decreasing its binding free energy.[32]

Pyrazole-containing compounds **11**–**14** were found to be inactive (IC_{50} >500 μM). Following a careful inspection of the FlexX calculated binding modes the lack of interaction with Asn46 and the absence of interactions in the distal heterocyclic moiety was observed in all cases. These compounds were found to possess the hydrophobic interaction with Ile90 along with formed hydrogen bond network with Asp73 and Val43 residues (see Fig 3S of Supplementary data for the binding mode of the compound **11**). In the VS

selection procedure it was assumed that this interaction pattern might still enable a sufficient molecular recognition and thus provide an alternative scaffold to the already selected thiazole compounds. Unfortunately, the measured activity did not corroborate this hypothesis. Thus, these molecules follow the cyclothialidine (**2**) pharmacophore interaction pattern to a much smaller extent as thiazole derivatives and exhibit much lower binding affinity. Alternatively, the intramolecular hydrogen bond interaction between the resorcinol hydroxyl and pyrazole nitrogen (**11**–**14**) or acylhydrazone (**15**) could decrease the favorable hydrogen bond interaction with Asp73. Compounds **15** and **16** with acylhydrazone linker were also inactive (IC_{50} >500 μM).

Interestingly, compounds of this class were recently found to be inhibitors of the functionally different but structurally homologous protein HSP90[33] and their inactivity against DNA gyrase points to the possibility of achieving selectivity in targeting ATP-binding site within the same protein superfamily.

In summary, by applying structure-based drug design techniques, combining molecular docking calculations coupled with three-dimensional structure-based pharmacophore information a novel class of DNA gyrase B inhibitors **5**–**9** containing the 2-amino-4-(2,4-dihydroxyphenyl) thiazole moiety was discovered. The inhibitory activity of these compounds was found to be in a low micromolar range. These compounds show prospective for further hit to lead development as their low-molecular weight (Mw = 250–350 D) and favorable agreement with the Lipinski rules renders them highly drug-like compounds.[34] The disclosed structures provide novel promising leads that could by further optimization result in compounds used as effective antibacterials.

Acknowledgments

This work was supported by the Ministry of Higher Education, Science and Technology of the Republic of Slovenia. The authors are grateful to the National Institute of Biology, Department of Genetic Toxicology and Cancer Biology (Drs. M. Filipič and B. Zegura) for hospitality and assistance with the biological testing.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2009.12.060.

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30. Maxwell, A.; Burton, N. P.; O'Hagan, N. *Nucl. Acids Res.* **2006**, *34*, e104: The assay was performed on black streptavidin-coated 96-well microplates which were first rehydrated using wash buffer (20 mM Tris–HCl (pH 7.6), 137 mM NaCl, 0.01% (w/v) BSA, 0.05% (v/v) Tween 20). Then biotinylated oligonucleotide was immobilized onto the wells and the excess oligo was washed off with wash buffer. Enzyme assay was carried out in reaction volume of 30 μL using 1 μg relaxed pNO1 as the substrate and 1.5 U gyrase from *E. coli*. Tested compounds were added as 100× stock in DMSO and final concentration of DMSO was 1% respectively. Reactions were incubated at 37 °C for 30 min, and then TF buffer (50 mM NaOAc (pH 5.0), 50 mM NaCl, 50 mM $MgCl_2$) was added to the wells and incubated at room temperature for another 30 min to allow triplex formation. Any unbound plasmid was washed off with TF buffer and SYBR Gold (Invitrogen) in T10 buffer (10 mM Tris–HCl (pH 8) and 1 mM EDTA) was added and allowed to stain for 20 min. After mixing, fluorescence was read using Tecan fluorimeter (Ex: 485 nm and Em: 535 nm) and Magellan software. IC_{50} values were determined by measuring residual activity at eight (500, 250, 125, 62.5, 31.3, 15.6, 7.8 and 3.9 μM, respectively) different concentrations and represent the concentration of inhibitor where residual activity is 50%.
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