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In silico fragment-based discovery of indolin-2-one analogues as potent DNA gyrase inhibitors

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Abstract—We describe here the fragment-based design of potent DNA gyrase inhibitors. Using the tools of virtual screening and NMR spectroscopy we identified the binding of two low-molecular weight fragments (2-aminobenzimidazole and indolin-2-one) to the 24 kDa N-terminal fragment of DNA gyrase B. Further in silico optimization of indolin-2-one led to the discovery of potent DNA gyrase inhibitors.

DNA gyrase is a bacterial enzyme that catalyzes the introduction of negative supercoils in a closed-circular DNA using the energy of the ATP hydrolysis. Since it is found only in prokaryotes and is vital for their survival, it has become an attractive target for antibacterial agents.[1] DNA gyrase consists of two subunits, GyrA and GyrB, and forms an A_2B_2 complex.[2] GyrA is a catalytic site for DNA breakage and reunion, while the ATP-binding site is found in the subunit GyrB. Coumarins (novobiocin, Fig. 1) are natural antibiotics that inhibit the ATPase activity by blocking the binding of ATP to subunit GyrB. Low solubility and permeability, high toxicity, and insufficient in vivo activity of coumarins currently prevent their therapeutic use. Newly developed triazine (**1**) and indazole (**2**) inhibitors (Fig. 1) have also been unable to overcome all of these coumarin deficiencies.[3,4] Therefore, design of novel potent DNA gyrase B inhibitors is at present still of great importance.[5]

Recently, we have characterized the binding site of quercetin, a natural flavonoid that inhibits the DNA gyrase activity by binding to the subunit GyrB.[6] Based on the results of NMR spectroscopy experiments we have constructed a structural model of quercetin in the ATP-binding site with residue Asp73 forming one of the crucial hydrogen bonds with the hydroxyl group of quercetin.

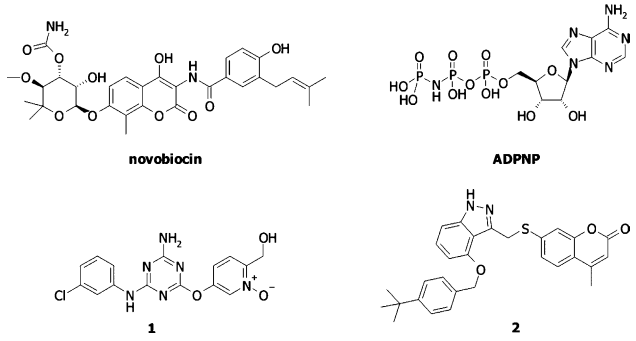

Figure 1. Inhibitors of DNA gyrase B.

In this work, we employed the concept of fragment-based drug design, which already showed promising results in case of indazole inhibitors, to search for novel inhibitors of DNA gyrase B.[3] This approach utilizes identification of weak-binding fragments (molecules with M_r = 120–250) toward active site pockets and their

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subsequent linking or optimization into lead compounds.[7] Biophysical methods, including NMR spectroscopy, X-ray crystallography, and mass spectrometry, as well as various computational tools were utilized to assist this process.[8]

The crystal structure of 43 kDa N-terminal fragment of DNA gyrase B in complex with ADPNP (PDB code 1EI1)[9] was taken as a starting-point. Using LUDI module as implemented in INSIGHT2000[10] we have identified several interaction sites (HB-donors, HB-acceptors, and hydrophobic areas) that complement the central part of the ATP-binding pocket near residue Asp73 (Fig. 2A). This highly conserved residue among prokaryotes (eukaryotes have Asn residue at this position) is vital for binding as it takes part in hydrogen bonding network observed between protein, crystal water molecule, and several inhibitors.[11]

In the first step, we initiated virtual screening of MDL-ACD and MDL-SCD databases[12] to obtain the central fragment (molecules with $M_r < 180$) that would satisfy the interaction sites defined by LUDI as shown above. In addition, virtual screening was performed also on the basis of topological similarity to adenine (ADPNP), triazine (**1**), and indazole (**2**) rings, respectively. Tanimoto coefficient was set to a relatively low value >0.5 to allow higher structural diversity of the resulting hits. According to the 'rule of three,'[13] water solubility and commercial availability this selection process resulted in two fragments (2-aminobenzimidazole (**3**) and indolin-2-one (**4**); Fig. 2B). Subsequently, we characterized their binding to 24 kDa N-terminal fragment of DNA gyrase B (GyrB24), which comprises the ATP-binding site, by using NMR spectroscopy.[6b] The ^{15}N HSQC experiments of ^{15}N-isotopically labeled GyrB24 protein from *Escherichia coli* were performed in the presence of 2-aminobenzimidazole and indolin-2-one, respectively. By mapping the chemical shift differences of the apo and bound protein we were able to identify the residues, which are directly involved in the interaction with each fragment, using the assignment of nearly all backbone N–H chemical shifts in the ^{15}N HSQC spectra. Residues with most pronounced shift differences are clustered into four regions around residues Ala47, Arg76, Val120, and Val167 (Fig. 3A) that delineate the ATP-binding site. Similar pattern of shift differences was observed for both fragments indicating their homologous binding mode in the ATP-binding pocket. These results confirmed our initial hypothesis about structural requirements for the binding of central fragment.

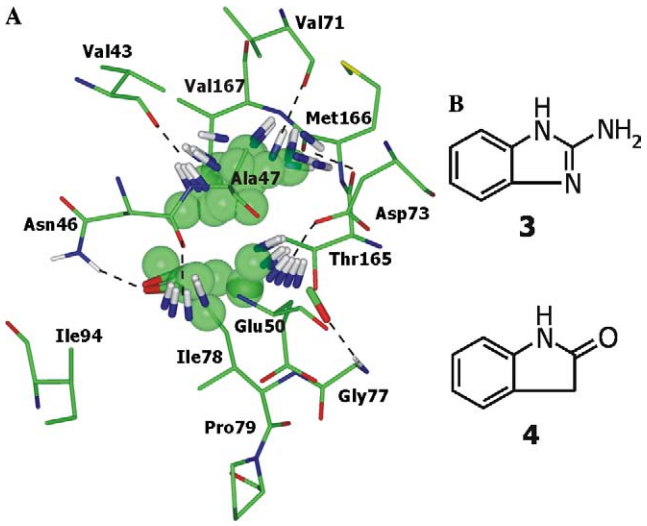

Figure 2. (A) Interaction sites in the central part of the ATP-binding pocket. Hydrogen bond acceptors and donors are presented as green-red and blue-gray capsules, respectively. Two hydrophobic regions are displayed with green spheres. (B) Fragments of 2-aminobenzimidazole (**3**) and indolin-2-one (**4**) obtained by virtual screening of the database.

We also performed ^{15}N HSQC titration experiments with increasing fragment concentrations and determined the dissociation constant K_D for both fragments upon binding to the GyrB24 protein (Fig. 3B). In agreement with docking studies, lower dissociation constant of fragment **3** (K_D = 2 mM) compared to that of fragment **4** (K_D = 10 mM) can be explained with the higher number of hydrogen bonds formed between 2-aminobenzimidazole and residues Asp73 and Gly77, respectively.

Due to a shortage of commercially available substituted 2-aminobenzimidazoles with free primary amino group (~20) in the MDL database compared to substituted indolin-2-ones (~11.000), we decided on in silico optimization of indolin-2-one, despite its 5-fold lower affinity.

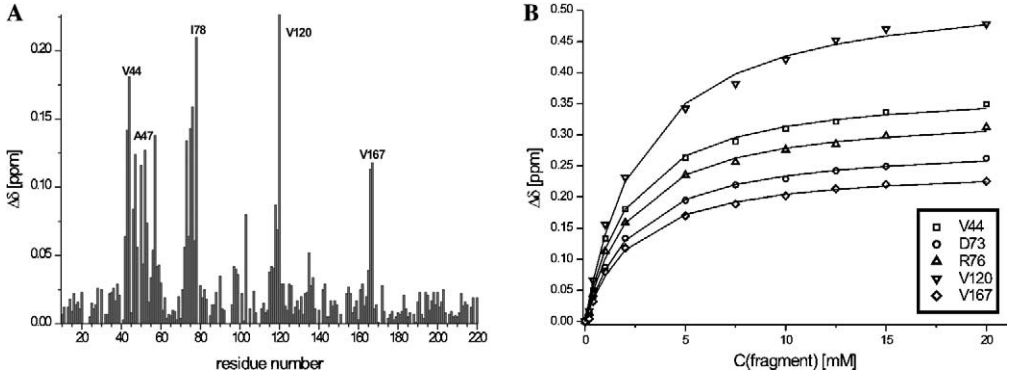

Figure 3. (A) Chemical shift changes of GyrB24 backbone amide resonances observed by ^{15}N HSQC experiments upon addition of fragment **3**. (B) Chemical shift differences of selected GyrB24 backbone resonances as a function of fragment **3** concentration. Dissociation constant K_D = 2 mM for 2-aminobenzimidazole was determined as an average value for residues with most pronounced shift differences.

In this step, LUDI was used again to define the interaction sites in the rest of the ATP-binding pocket and to search for fragments that could bind in close vicinity of indolin-2-one-binding site as determined by NMR spectroscopy. Typical fragments that met structural requirements defined by LUDI were five- to seven-membered monocyclic and seven- to ten-membered bicyclic heterocyclic rings with up to four heteroatoms (N, O, S). Coumarin ring was also recognized among heterocycles mentioned above. This structural element of novobiocin and compound **2** (Fig. 1), which stacks on the Glu50-Arg76 salt bridge and forms two strong hydrogen bonds with positively charged side chain of Arg136, is essential for their high in vitro antigyrase activity.[14]

Ten indolin-2-one derivatives containing the above-mentioned heterocyclic system (such as benzene, naphthalene, imidazole, and pyridine rings) that follow the Lipinski rule of five[15] were selected from the MDL database and their inhibitory activity was evaluated in the DNA gyrase supercoiling assay at compound concentration 100 μM (Fig. 4).[16] Only compound **6** with substituted imidazole ring and ethylidene linker to indolin-2-one inhibited the gyrase activity at this concentration. Maximum non-effective concentration (MNEC)[17] for compound **6** was determined at 50 μM.

Next, seven close structural analogues of compound **6** were purchased to investigate the importance of particular group for activity (Fig. 5). Since tautomerization of imidazole ring permits the proton positioning on either of the nitrogen atoms, indolin-2-ones with indole and pyrazole rings (**19**–**21**; Fig. 5) were also selected. The limited SAR of compounds **6** and **15**–**21** indicates that imidazole ring appears to be essential for antigyrase activity. Imidazole ring is likely to be located in hydrophobic part of the active site, most probably in the vicinity of residues Ile78, Pro79, and Ile94. The activity of compound **17** (without the ethyl group at position 2 in imidazole ring) decreased considerably compared to compound **6**, while the additional methyl group at position 4 (compound **18**) increased the activity by 2-fold. Phenyl ring at position 2 (compound **16**) is probably too bulky to be accommodated in the active site. Substantial reduction in activity for compounds with substituted indole and pyrazole ring can be explained by their different spatial conformation. Namely, intramolecular hydrogen bond between carbonyl oxygen of indolin-2-one moiety and amino group of imidazole ring that could stabilize compounds **6** and **15**–**18** in *Z*-conformation cannot be formed in case of indole or pyrazole ring (**19**–**21**). Additional reason for inactivity of compounds **19**–**21** could be that a congested active site cannot accommodate the larger side chains of these compounds, as hypothesized for compound **16**.

Thus, compound HTS05063 (**18**) displays the highest in vitro activity of this series of indolin-2-ones in DNA gyrase supercoiling assay (MNEC = 25 μM). This is still 500-fold lower activity compared to novobiocin;

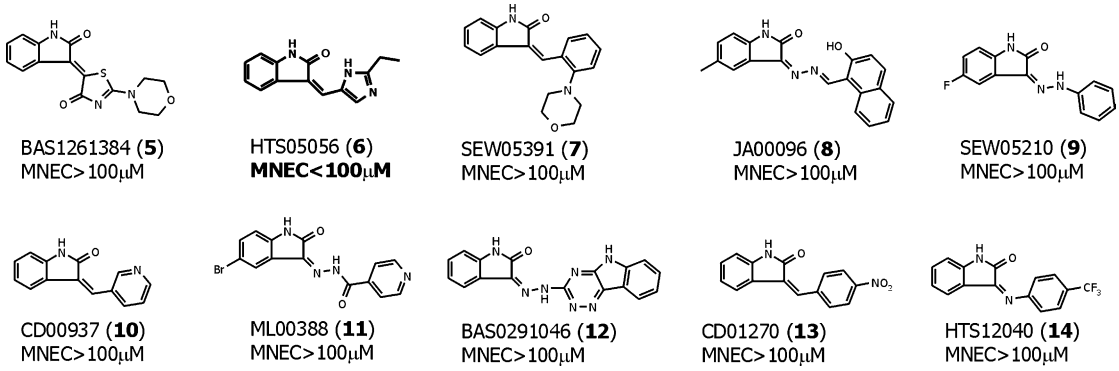

Figure 4. In vitro activity of substituted indolin-2-ones against *Escherichia coli* DNA gyrase supercoiling activity. Inhibition was measured as maximum non-effective concentration of inhibitor (MNEC), which showed no inhibition of supercoiling activity (as a reference, MNEC for novobiocin was determined at 0.05 μM). For details see Refs. 16 and 17.

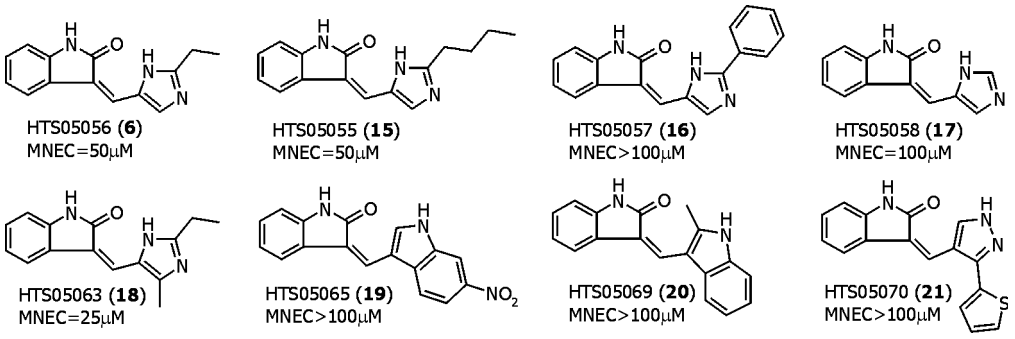

Figure 5. In vitro activity of structural analogues of compound **6** against *Escherichia coli* DNA gyrase supercoiling activity. For details see Figure 4 and Refs. 16 and 17.

however, its relatively low molecular weight (M_r = 253.3) and lipophilicity as estimated by the logarithm of the octanol/water partition coefficient ($\log P = 1.82$)[18] provide a sound basis for further optimization. Successful applications of the fragment based discovery methodology[7,8] point to the necessity of adding another substituent onto the scaffold of promising lead compound in order to improve on desired inhibitory activity from micromolar to nanomolar concentration range.

DNA gyrase belongs to the GHKL ATPase superfamily[19] and is therefore closely structurally related to the large family of protein kinases. The conservation of structural features within the ATP binding cleft led to the belief that specificity would be difficult to achieve. However, the clarified structural basis for selectivity and potency of protein kinase inhibitors has yielded several small-molecule ATP-competitive inhibitors that are in various stages of development.[20] Not surprisingly, indolin-2-ones are potent inhibitors of VEGF and PDGF receptor tyrosine kinases,[21] JNK protein kinases,[22] casein kinase-1,[23] etc.

In conclusion, we report here a class of compounds with indolin-2-one structural scaffold as potent DNA gyrase inhibitors. Based on the available 3D structure of DNA gyrase B and structural similarity of known gyrase inhibitors, we completed a successful search for fragments that would bind to the ATP-binding site. Binding of two fragments, indolin-2-one and 2-aminobenzimidazole, was demonstrated using the ^{15}N HSQC NMR spectroscopy of isotopically labeled protein. Further in silico optimization of indolin-2-one led us to discovery of a compound HTS05063 that inhibits the DNA gyrase supercoiling activity in the low micromolar range.

References and notes

1. Maxwell, A. *Trends Microbiol.* **1997**, *5*, 102.
2. Reece, R. J.; Maxwell, A. *Crit. Rev. Biochem. Mol. Biol.* **1991**, *26*, 335.
3. Boehm, H. J.; Boehringer, M.; Bur, D.; Gmuender, H.; Huber, W.; Klaus, W.; Kostrewa, D.; Kuehne, H.; Luebbers, T.; Meunier-Keller, N.; Mueller, F. *J. Med. Chem.* **2000**, *43*, 2664.
4. Poyser, J. P.; Telford, B.; Timms, D.; Block, M. H.; Hales, N. J. WO Patent 99/01442, 1999.
5. Maxwell, A.; Lawson, D. M. *Curr. Top. Med. Chem.* **2003**, *3*, 283.
6. (a) Plaper, A.; Golob, M.; Hafner, I.; Oblak, M.; Solmajer, T.; Jerala, R. *Biochem. Biophys. Res. Commun.* **2003**, *306*, 530; (b) Grdadolnik, S. G.; Oblak, M.; Šolmajer, T.; Jerala, R. In *Drug Discovery and Design: Medical Aspects*; Matsoukas, J., Mavromoustakos, T., Eds.; IOS Press: Amsterdam, 2002; Vol. 55, pp 312–322.
7. Rees, D. C.; Congreve, M.; Murray, C. W.; Carr, R. *Nat. Rev. Drug Discovery* **2004**, *3*, 660.
8. Erlanson, D. A.; McDowell, R. S.; O'Brien, T. *J. Med. Chem.* **2004**, *47*, 3463.
9. Brino, L.; Urzhumtsev, A.; Mousli, M.; Bronner, C.; Mitschler, A.; Oudet, P.; Moras, D. *J. Biol. Chem.* **2000**, *275*, 9468.
10. (a) Boehm, H. J. *J. Comput. Aided Mol. Des.* **1992**, *6*, 61; (b) INSIGHT2000; Accelrys, San Diego, CA.
11. Lewis, R. J.; Singh, O. M.; Smith, C. V.; Skarzynski, T.; Maxwell, A.; Wonacott, A. J.; Wigley, D. B. *EMBO J.* **1996**, *15*, 1412.
12. http://www.discoverygate.com/.
13. Congreve, M.; Carr, R.; Murray, C.; Jhoti, H. *Drug Discovery Today* **2003**, *8*, 876.
14. Holdgate, G. A.; Tunnicliffe, A.; Ward, W. H.; Weston, S. A.; Rosenbrock, G.; Barth, P. T.; Taylor, I. W.; Pauptit, R. A.; Timms, D. *Biochemistry* **1997**, *36*, 9663.
15. Lipinski, C. A.; Lombardo, F.; Dominy, B. W.; Feeney, P. J. *Adv. Drug Delivery Rev.* **1997**, *23*, 3.
16. DNA gyrase supercoiling assay kits were obtained from John Innes Enterprises Ltd. (Norwich Research Park, United Kingdom), courtesy of Prof. A. Maxwell. Assays were performed according to the manufacturer's instructions.
17. Angehrn, P.; Buchmann, S.; Funk, C.; Goetschi, E.; Gmuender, H.; Hebeisen, P.; Kostrewa, D.; Link, H.; Luebbers, T.; Masciadri, R.; Nielsen, J.; Reindl, P.; Ricklin, F.; Schmitt-Hoffmann, A.; Theil, F. P. *J. Med. Chem.* **2004**, *47*, 1487.
18. The log*P* value for compound HTS05063 was calculated using ACD/Labs7.0 software.
19. Dutta, R.; Inouye, M. *Trends Biochem. Sci.* **2000**, *25*, 24.
20. (a) Toledo, L. M.; Lydon, N. B.; Elbaum, D. *Curr. Med. Chem.* **1999**, *6*, 775; (b) Cohen, P. *Nat. Rev. Drug Discov.* **2002**, *1*, 309.
21. Sun, L.; Tran, N.; Tang, F.; App, H.; Hirth, P.; McMahon, G.; Tang, C. *J. Med. Chem.* **1998**, *41*, 2588.
22. Luk, K. C.; Mahaney, P.; Mischke, S. G. WO Patent 00/35906, 2000.
23. Mashhoon, N.; DeMaggio, A. J.; Tereshko, V.; Bergmeier, S. C.; Egli, M.; Hoekstra, M. F.; Kuret, J. *J. Biol. Chem.* **2000**, *275*, 20052.