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Design and Synthesis of Dual Inhibitors Targeting Gyrase B and Par E

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Abstract: The structurally related bacterial topoisomerases DNA gyrase (GyrB) and topoisomerase IV (ParE) have long been recognized as prime candidates for the development of broad spectrum antibacterial agents. However, GyrB/ParE targeting antibacterials with spectrum that encompasses robust Gram-negative pathogens have not yet been reported. Using structure-based inhibitor design, we optimized novel inhibitor series with potent, dual targeting activity against GyrB and ParE.
Keywords : Topoisomerases, antibacterial agents.

Introduction

Bacterial resistance to antibiotics has become an important public health problem and there is a continuing need to develop newer and more potent antibiotics. Development of new antibacterial agents with novel mechanisms of action is required to overcome the problem of bacterial resistance that affects all currently used classes of antibiotics. Bacterial DNA gyrase(ParE) and topoisomerase IV (ParE) are type II topoisomerases involved in modifying the DNA topology during the replication process by unlinking DNA and facilitating chromosome segregation [1]. Topoisomerase IV forms a C_2E_2 tetramer responsible for segregation of the chromosome at cell division [1,2,3]. The ParC subunit castalytic domain is the site of topoisomerizationfor catalyzing the double-stranded DNA break [4,5]whereasParE subunit catalyzes the hydrolysis of ATP and provide free energy necessary for these reactions [6,7,8]. The GyrB and GyrAsubunits of the DNA gyrase A_2B_2 tetramer which catalyzes negative DNA supercoiling during the initiation and elongation processes of DNA replication [9,10], have extensive sequence homology with, respectively, ParE and ParC subunits.Bacterial DNA gyrase(GyrB) and topoisomerase IV (ParE) are well established and attractive targets for the development of antibacterial agents. In recent years there has been considerable interest in discovering and developing novel inhibitors containing that target both GyrB and ParE enzymes. In both Gram-negative andGram-positive bacterial resistance is commonly associated with mutations in topoisomerase GyrB or ParE or mediated by efflux [12,13]or is associated with both mechanisms [11,13,14] that a single residue (GyrB I78 and ParE M74) is responsible for the differences in novobiocin potency between these type II topoisomerases.

GyrB and ParE havestructural similarities in their conserved active sites, subunit organization, and antibiotic sensitivity to coumarins and quinolones[15,16] and are also independently essential for bacterial growth. The structural similarity of GyrBto ParE places both enzymes into the GHKL phosphotransferase superfamily [17].

The structural similarity between 43-kDa *E. coli*ParEand 43-kDa *E. coli*GyrBis remarkable[14]. The majordifference between these two structures are in the long α-helices that are present at the C terminus of each monomer. In case of GyrB, the long α-helices from each monomer stack themselves in close proximity, whereas these long α-helices display greater openness in ParE dimer compared to the GyrB structure.The distance between the Cα carbons betweenresidue A383 and its equivalent (A383′) in the ParE dimer was found to be 22.8 Å, whereas thedistance between the Cα carbons two equivalent structural residues (R386-R386′) in GyrB is 9.8 Å. In ParEenzyme structural differences was discovered to start at the D217 residue and continued tillto the end of the protein.The equivalent residue (G220) in GyrBis glycine and this explain the shift in the α-helices. The relevance for α-helical openness in TopoIV function is not known, which could be an artifact of crystal formation or in full-length ParEthe extent of openness may be different.

The prokaryotic type II topoisomerases differ sufficiently from eukaryotic type II topoisomerases with highly conserved catalytic domains within many clinically relevant bacteriaand are considered attractive antibacterial targets. Based on these properties design of specific bacterial inhibitors with broad spectrum of action is possible and designed dual-target inhibitor with equal potency will have the potential the potential to reduce the development resistance because two independent resistant mutations from each target would be required for the development of resistance[18].

Experimental

Fragment-based drug discovery (FBDD) is an alternative approach to high-throughput screening for the generation of fragment leads for targets in both industry and academia. Small-molecule drug discovery has always been challenge for the researchers. Fragment-based lead discovery is a potential solution and becoming increasingly popular [19] for lead identification and optimization. The goal FBDD is to build drug leads in pieces by small molecular fragments. These small fragment leads are then either linked together or expanded. The fragments are small molecules weighing less than 200–300 Da, with fewer than 15–20 heavy atoms. The concept of fragment-based lead discovery was first proposed 25 years ago [20,21], but after advent of molecular modeling and drug design tools, FBDD became very popular, with the discovery of few drug candidates and there have been considerable recent advances in the area of fragment merging and linking. The advantage of FBDD discovery is that the designed molecules are likely to have a higher ‘ligand efficiency’ to the molecules discovered through conventional methods.

Various approaches for fragment linking have proven successful with carbonic anhydrase and click chemistry was successfully used to discover bovine CA II inhibitors [22,23]. In another approach of FBDD, a surface histidine residue was exploited to bind to a metal-chelating fragment [24].In recent years fragment based drug design has been successfully applied for the discovery of dipeptidyl peptidase-IV (DPP-IV)[25], thrombin, ribonuclease A[26], p38a MAP kinase[27], matrix metalloproteinase (MMP-13)[28], hepatitis C virus NS3 protease–NS4A cofactor complex (ns4a–ns3p)[29]and PDE4[30]inhibitors.

The main objective of our present study was to identify dual of the DNA GyrB and ParE enzymes and for this we employed the concept of fragment-based drug design[31,32] and designed 10 compounds with high binding affinity for both DNA GyrB and ParE enzymes. The X-ray crystal structures of DNA GyrB (PDB code 4DUH) and IV ParE (PDB code 1S14) subunit were prepared using the Protein Preparation Wizard available in the Schrodinger Suite 2014. Fragment librarywas built from known inhibitors and fragments were prepared using Schrodinger’s LigPrep software. Ligands were desalted and tautomers generated. The OPLS2005 force field was used for geometric optimization. Additional settings for both protein and ligand preparation utilized default parameters.

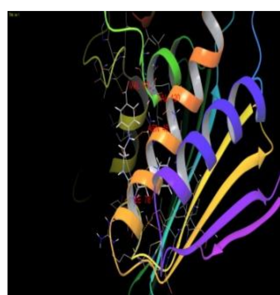

Figure 1.Docking of compound most active ligand in the active site of 1KZN

Figure 1. Docking of compound most active ligand in the active site of 1S14

Table1. Docking results of designed molecules in the active site of ParE (PDBID:1S14)

Ligand	GScore	DockScore	LipophilicEvdW	HBond	Electro	LowMW	ExposPenal	RotPenal
1	-7.33	-7.33	-4.52	-2.19	-0.44	-0.2	0	0.33
2	-6.67	-6.67	-4.38	-2.59	-0.28	-0.27	0.36	0.48
3	-6.13	-6.13	-4.9	-0.99	-0.44	-0.32	0	0.52
4	-5.83	-5.83	-4.97	-0.97	-0.15	-0.15	0	0.41
5	-5.78	-5.78	-4.62	-0.75	-0.58	-0.21	0.02	0.44
6	-5.77	-5.77	-4.68	-1.04	-0.31	-0.17	0	0.42
7	-5.76	-5.76	-4.26	-1.27	-0.14	-0.17	0	0.32
8	-5.58	-5.57	-5.18	-0.53	-0.14	-0.12	0	0.39
9	-5.56	-5.56	-4.66	-0.7	-0.39	-0.41	0	0.6
10	-5.79	-5.52	-4.43	-0.89	-0.17	-0.18	0	0.43

Table1. Docking results of designed molecules in the active site of GyrB (PDBID:1KZN)

Ligand	GScore	DockScore	LipophilicEvdW	HBond	Electro	LowMW	ExposPenal	RotPenal
1	-5.81	-5.8	-4.3	-1.71	-0.22	-0.27	0.18	0.48
2	-5.73	-5.73	-4.27	-1.01	-0.41	-0.12	0.08	0.39
3	-5.73	-5.73	-4.56	-0.96	-0.5	-0.41	0.1	0.6
4	-5.47	-5.47	-4.14	-1.05	-0.45	-0.27	0.35	0.48
5	-5.44	-5.43	-4.69	-0.35	-0.23	-0.21	0	0.44
6	-5.18	-5.17	-4.3	-1	-0.34	-0.17	0.21	0.42
7	-5.08	-5.08	-4.34	-0.65	-0.14	-0.32	0.25	0.52
8	-5.05	-5.04	-4.58	-0.7	-0.11	-0.17	0.09	0.42
9	-5.04	-5.03	-3.39	-1.23	-0.32	-0.15	0	0.41
10	-5.03	-5.02	-4.48	-0.7	-0.16	-0.21	0.08	0.44

Resultsand Discussion

In case of 1KZN (GyB)most of the compounds formed multiple hydrogen bonding with Asn46, Ala 96, Ser121,Asp73,Arg76, residues. Compound **1** which showed highest docking 5.81 Kcal /molexhibited hydrogen bonding with Asn46 and Ala 96. Some of the compounds also showed Pi-pi-stacking with Ala96. In case of 1S14 (ParE) most of the compounds exhibited hydrogen bonding with Asn1042, Arg1132, Val1118 and Asp1069 residues. Compound which showed highest docking score 7.3 Kcal/mol exhibited hydrogen bonding with Asn42. Some of the compound also exhibited Pi-cation interaction with Arg1072.However, in comparison to the 4-{[4'-methyl-2'-(propanoylamino)-4,5'-bi-1,3-thiazol-2-yl]amino}benzoic acid, no hydrogen bonding was observed with Arg136 in most of the compounds 3a-l. In compound 3a H-atom of the hydroxyl group of the side chain at second position of the thiazolidinone ring formed H-bond (2.06 Ao) with OH function of the carboxylic group of Asp 73 where as the O-atom of same group formed two H-bonds with the –NH function of the amide linkage between Gly 77 and Arg 76 and with -OH function of the carboxylic group of Thr 165 (1.83 Ao and 2.25 Ao respectively) (Figure 1 and 2). In compound 3c O-atom of hydroxyl group of the side chain at second position of the thiazolidinone ring formed two H-bonds with NH function of amide linkage between Gly 102 and Lys 103 and NH function of amide linkage between Lys 103 and Phe 104 and (2.13 and 2.39Ao respectively). In compound 3c another H-bond (1.76 Ao) was observed between H-atom of hydroxyl group of the side chain and >C=O function of the amide linkage between His 99 and Ala 100 residues (Figure 3 and 4).

1S14 (ParE)

Hydrogen bonding interaction was observed in all the designed compounds (Table 3). However, in comparison to the standard drug novobiocin, no hydrogen bonding was observed in the designed compounds with Arg1132 and Asp1077 residues. In all designed compounds hydrogen bonding was observed between H-

atom of hydroxyl group of the side chain at the second position of the thiazolidinone ring with Asp 1069, Glu 1046, Asn 1042, Arg 1072, Gly 1073 or Thr 1163 residues. In compound 3a and 3c H-atom of hydroxyl group of the side chain at second position of the thiazolidinone ring formed H-bonds (2.20 and 1.88 Ao respectively) with >C=O function of the amide linkage between Asp 1045 and Glu 1046 residues (Figure 5-8).

References

1. Zechiedrich, E. L., and Cozzarelli,N. R., Replications of topoisomerases IV and DNA gyrase in DNA unlinking during replication in Escherichia coli. Genes Dev.1995, 9, 2859-2869.
2. Adams, D.E.,Shekhtman,E.M.,Zechiedrich, E.L., Schmid,M.B.,Cozzarelli,N. R.,The role of topoisomerase IV in partitioning bacterial replicons and the structure of catenated intermediates in DNA replication. Cell,1992, 71, 277-288.
3. Kato, J., Nishimura,Y., Imamura,R.,Niki,H., Higara,S.,and Suzuki,H.,New topoisomerase essential for chromosome segregation in E. coli. Cell, 1990, 63, 393-404.
4. Kato, J.,Nishimura, Y.,Imamura,R.,Niki,H., Higara,S.,and Suzuki, H., New topoisomerase essential for chromosome segregation in E. coli. Cell, 1990, 63, 393-404.
5. Wang,J. C., DNA topoisomerases. Annu. Rev. Biochem.,1996, 65, 635-692.
6. Ali, J.A., Jackson, A.P.,Howells, A.J.,and Maxwell, A., The 43-kilodalton N-terminal fragment of the DNA gyrase B protein hydrolyzes ATP and binds coumarin drugs. Biochemistry, 1993, 32, 2717-2724.
7. Berger, J.M., Gamblin,S. J.,Harrison,S.C., and Wang,J. C., Structure and mechanism of DNA topoisomerase II. Nature, 1996, 379,225-232.
8. Drlica, K., and Zhao, X., DNA gyrase, topoisomerase IV, and the 4-quinolones. Microbiol. Mol. Biol. Rev., 1997, 61, 377-392.
9. Gellert, M.K., Mizuuchi, M. H., O'Dea, and Nash,H. A.,DNA gyrase: an enzyme that introduces superhelical turns into DNA. Proc. Natl. Acad. Sci., 1976, 73, 3872-3876.
10. Wang, J. C., DNA topoisomerases. Annu. Rev. Biochem.1996, 65, 635-692.
11. Laskowski, R. A., MacArthur, M. W., Moss, D. S., and Thornton, J.M.,Procheck: a program to check the stereo chemical quality of protein structure. J. Appl. Crystallogr. 1993, 26, 283-291.
12. Contreras, A.,and Maxwell, A.,Gyr, B., mutations which confer coumarin resistance also affect DNA supercoiling and ATP hydrolysis by Escherichia coli DNA gyrase. Mol. Microbiol., 1992, 6, 1617-1624.
13. Lewis, R. J., Singh, O. M. P., Smith, C. V., Skarzynski, T., Maxwell,A., Wonacott,A. J.,Wigley.D. B.,The nature of inhibition of DNA gyrase by the coumarins and cyclothialidine revealed by X-ray crystallography. EMBO J., 1996, 15, 1412-1420.
14. Steven Bellon., Jonathan,D., Parsons., Yunyi Wei., Koto Hayakawa., Lora L. Swenson., Paul S. Charifson., Judith A. Lippke., Robert Aldape., and Christian H. Gross., Crystal Structures of Escherichia coli Topoisomerase IV ParE Subunit (24 and 43 Kilodaltons): a Single Residue Dictates Differences in Novobiocin Potency against Topoisomerase IV and DNA Gyrase. Antimicrob. Agents Chemother., 2004, 48 (5), 1856-1864. doi: 10.1128/AAC.48.5.1856-1864.
15. Khodursky, A. B.,Zechiedric,E. L.,and. Cozzarelli,N. R., Topoisomerase IV is a target of quinolones in Escherichia coli. Proc. Natl. Acad. Sci. USA 1995, 92, 11801-11805.
16. Peng, H., and Marians,K. J., 1993. Escherichia coli topoisomerase IV purification, characterization, subunit structure and subunit interactions. J. Biol. Chem. 268:24481-24490.
17. Dutta, R., and Inouye,M., 2000. GHKL, an emergent ATPase/kinase superfamily. Trends Biochem. Sci. 25:24-28.
18. Drlica, K., 2001. Antibiotic resistance: can we beat the bugs? Drug Discov. Today 6:714-715.
19. Various approaches for fragment linking have proven successful with carbonic anhydrase and click chemistry was successfully used to discover bovine CA II inhibitors [70]. In another approach of FBDD, a surface histidine residue was exploited to bind to a metal-chelating fragment [71].
20. Jahnke, W., Erlanson, D.A., (Eds): Fragment-Based Approaches in Drug Discovery in series Methods and Principles in Medicinal Chemistry (Series Eds: R Mannhold, H Kubinyi, GFolkers). Weinheim, Germany: Wiley-VCH; 2006, vol 34.
21. Jencks, W.P.,On the attribution and additivity of binding energies. ProcNatlAcadSci USA 1981, 78, 4046-4050.
22. Shuker, S.B., Hajduk, P.J., Meadows, R.P., Fesik, S.W., Discovering high-affinity ligands for proteins: SAR by NMR. Science 1996, 274:1531-1534.

23. Mocharla, V.P., Colasson, B., Lee, L.V., Roper, S., Sharpless, K.B., Wong, C.H., Kolb, H.C.,In situ click chemistry: enzyme-generated inhibitors of carbonic anhydrase II. AngewChemInt Ed Engl 2004, 44:116-120.
24. Jude, K.M., Banerjee, A.L., Haldar, M.K., Manokaran, S., Roy, B., Mallik, S., Srivastava, D.K., Christianson, D.W., Ultrahigh resolution crystal structures of human carbonic anhydrases I and II complexed with 'two-prong' inhibitors reveal the molecular basis of high affinity. J Am ChemSoc 2006, 128:3011-3018.
25. Rummey, C., Nordhoff, S., Thiemann, M., Metz, G.,Insilico fragment based discovery of DPP-IV S1 pocket binders. Bioorg MedChemLett 2006, 16:1405-1409.
26. Hartshorn, M.J., Murray, C.W.,Cleasby, A., Frederickson, M., Tickle, I.J., Jhoti, H., Fragment-based lead discovery using X-ray crystallography. J Med Chem 2005, 48:403-413.
27. Ciulli, A., Williams, G., Smith, A.G., Blundell, T.L., Abell, C., Probing hot spots at protein-ligand binding sites: a fragment-based approach using biophysical methods. J Med Chem 2006,49:4992-5000.
28. Wu, J., Rush, T.S., III, Hotchandani, R., Du, X., Geck, M., Collins, E., Xu, Z.B., Skotnicki, J., Levin, J.I., Lovering, F.E., Identification of potent and selective MMP-13 inhibitors. Bioorg Med ChemLett 2005, 15:4105-4109.
29. Wyss, D.F., Arasappan, A., Senior, M.M., Wang, Y.S., Beyer, B.M., Njoroge, F.G., McCoy, M.A., Non-peptidic small-molecule inhibitors of the single-chain hepatitis C virus NS3 protease/NS4A cofactor complex discovered by structure-based NMR screening. J Med Chem 2004, 47:2486-2498.
30. Krier, M., Araujo-Junior, J.X., Schmitt, M., Duranton, J., Justiano-Basaran, H., Lugnier, C., Bourguignon, J.J., Rognan, D.,Design of small-sized libraries by combinatorial assembly of linkers and functional groups to a given scaffold: application to the structure-based optimization of a phosphodiesterase 4 inhibitor. J Med Chem 2005, 48:3816-3822.
31. Oblak,M.,Grdadolnik,S.G.,Kotnik,M.,Jerala,R.,Filipic,M.,Solmajer.T.,Insilico.fragment-based discovery of indolin-2-one analogues as potent DNA gyrase inhibitors. Bioorg. Med. Chem. Lett. 15 (2005) 5207-5210.
32. Manchester,J. I.,Dussault,D. D., Rose,J. A.,Boriack-Sjodin,P.A.,Uria-Nickelsen, G.M., Ioannidis, S.,Bist, P., Fleming, K. G., Hull. Discovery of a novel azaindole class of antibacterial agents targeting the ATPase domains of DNA gyrase and Topoisomerase IV. Bioorg. Med. Chem. Lett. 22 (2012) 5150-5156.

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