Available online at www.sciencedirect.com
ScienceDirect
Bioorganic & Medicinal Chemistry Letters 17 (2007) 2807–2810
Bioorganic & Medicinal Chemistry Letters

Small molecule inhibitors of *E. coli* primase, a novel bacterial target

Atul Agarwal,* Shirley Louise-May, Jane A. Thanassi, Steven D. Podos, Jijun Cheng, Christy Thoma, Cuixian Liu, Jason A. Wiles, David M. Nelson, Avinash S. Phadke, Barton J. Bradbury, Milind S. Deshpande and Michael J. Pucci*

Achillion Pharmaceuticals, 300 George Street, New Haven, CT 06511, USA

Received 24 January 2007; revised 20 February 2007; accepted 22 February 2007
Available online 25 February 2007

Abstract—Bacterial primase is essential for DNA replication in Gram-positive and Gram-negative bacteria. It is also structurally distinct from eukaryotic primases, and therefore an attractive, but under-explored, target for therapeutic intervention. We applied virtual screening to discover primase inhibitors, and subsequently several commercially available analogs of these initial hits showed potent primase inhibition and in vitro antibacterial activity. This work provides a 3D pharmacophore for primase ligands, SAR trends, and leads that can be further optimized.

Recent efforts in antibacterial research involve identification of new targets in order to address the challenge of antibiotic resistance. Bacterial primase is one such novel and under-explored bacterial target that is essential for DNA replication in Gram-positive and Gram-negative bacteria.

DNA polymerases are incapable of de novo DNA synthesis and require primers. These short RNA primers are synthesized by RNA polymerases called 'primases' on single-stranded DNA (ssDNA) that are then extended by the replicative DNA polymerase(s). Polymerases synthesize nucleic acids in a 5′–3′ direction that requires asymmetrical synthesis of antiparallel DNA strands. The 'leading' strand of DNA is made continuously and needs to be primed only once. However, the 'lagging' strand is formed discontinuously and this synthesis needs to be initiated multiple times during the course of replication. During lagging-strand synthesis of the *Escherichia coli* genome, primase (DnaG) proteins need to transcribe ~2000–3000 RNA primers at a rate of about one primer per second.[1] Sequence analysis indicates that bacterial primases are structurally distinct from known DnaG homologs identified in prokaryotes, eukaryotes, and bacteriophage.

Keywords: DNA primase; Antibacterial; Gram-negative; Gram-positive; DnaG; In silico screening; Virtual screening; Pharmacophore; MIC.
* Corresponding authors. Tel.: +1 203 624 7000; fax: +1 203 752 5454; e-mail addresses: aagarwal@achillion.com; mpucci@achillion.com

The primase enzyme is comprised of three structural domains: a 12-kDa N-terminal Zn^{2+} binding domain, a 36-kDa catalytic domain, and a 15-kDa C-terminal domain. A 3D structure of the entire enzyme from pathogenic bacterium is currently unavailable. Therefore, we analyzed the 3D structure[2,3] of the catalytic domain to predict 'druggable' sites and then virtually screened commercially available drug-like compounds in order to identify ligands for these sites. Analogs of validated leads were identified using data-mining techniques, acquired, and evaluated in vitro to build a SAR dataset.

We applied Tripos[4] sln filters to the databases of 20 vendors and derived a library of drug-like compounds that is comprised of molecules with non-reactive functionalities, at least one ring and up to eight rotatable bonds with a calculated log P of $\leqslant 5$ and MW, 275–500 amu. Three-dimensional coordinates for the resultant 500K compounds were generated using CONCORD, and finally, Schrodinger utilities were used to add hydrogens, representing ionization states at the physiological pH, and to energy minimize compounds.[5]

Commercial software, GRID,[6] was used to calculate interaction energies between the 3D structure of *E. coli* DNA primase, 1DDE (pdb code),[2] and a set of probes: chlorine, sp^3 Carbon, sp^2 Oxygen, and sp^3 Nitrogen. Since most drug-like compounds include Carbon, Nitrogen, Oxygen and halogens, the proximity of preferred interaction sites of these atoms in an accessible region of the protein would indicate the presence of a putative

0960-894X/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2007.02.056

'druggable' binding site. We identified three such putative binding sites, A, B, and C, in 1DDE (Fig. 1). Since currently available computational methods cannot distinguish productive from non-productive small molecule binding sites,[7] we carried out in silico screening in all the three predicted small molecule binding sites.

1DDE was prepared for docking computations by deleting the water molecules and then energy minimizing the protein 3D structure to a gradient of 0.3 Å using the impref utility of the Schrodinger suite of software. Subsequently, grids were calculated around amino acids, Tyr267 (site A), Arg201 (site B), and Arg155 (site C), using Glide[5] for the three druggable sites predicted. A 'bounding box' of 16 Å and 'enclosing box' that would fit ligands up to the length of 20 Å defined the space to explore at each site for ligand docking. Next, the formatted library of compounds was flexibly docked into the predicted 'druggable' sites. The docked compounds were rank ordered by the 'glide score', and the top 2500 ligands of each binding site were visually inspected to select structurally diverse, putative ligands that complemented the polar and non-polar residues of the binding sites in the appropriate ionization states.

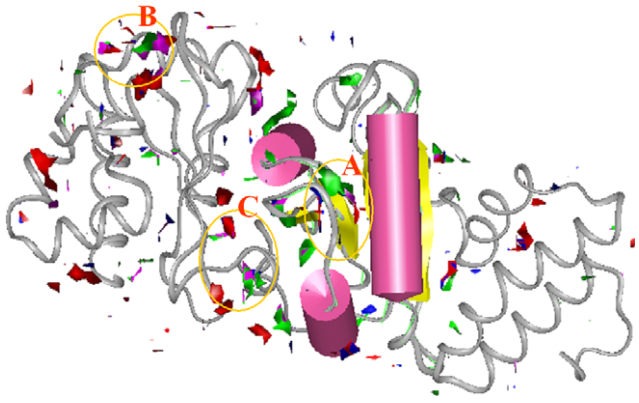


Figure 1. Ribbon representation of 1DDE. α-Helices of the 'toprim' domain are shown as pink cylinders and β-strands as yellow arrows. Preferred binding sites for chlorine, sp^3 Carbon, sp^2 Oxygen, and sp^3 Nitrogen are indicated with magenta, green, red, and blue contours, respectively. A, B, and C are the three putative 'druggable' binding sites.

Compounds selected from in silico screening were purchased and their activities were determined in an *E. coli* primase scintillation proximity assay (SPA).[8] Using a single-stranded DNA template, the double-stranded product of the primase reaction is preferentially captured on coated SPA beads and quantified by β-scintillography. The in vitro antibacterial activity was determined by MIC as described by the CLSI[9] for efflux pump-deficient *E. coli* (ACH 0002).

The 'glide score' ranked ligands were visually inspected to select 79 compounds for purchase; 33 for site A, 7 for site B, and 39 for site C. The 68 of 79 compounds available were evaluated in the enzyme inhibition assay to discover four primase inhibitors (Fig. 2). All four primase inhibitors discovered belonged to the compound set selected from in silico screening of site C, and therefore, indicate that site C is a potentially 'druggable' site. Despite reasonable potency, the initial four primase inhibitors and their analogs purchased did not inhibit the bacterial growth in vitro.

The initial four hits were modeled and overlaid to deduce a 3D pharmacophore (Fig. 3) which was then used to further explore additional compounds for primase and bacterial growth inhibition.

A set of 2846 putative primase ligands was identified by data-mining the in-house database of commercially available compounds with our 3D pharmacophore. Clustering techniques and manual filtering for desirable analogs led to purchase of 34 structurally diverse compounds. Another eight primase inhibitors (IC_{50} < 100 μM) were discovered from the purchased set, three of which inhibited the bacterial growth in vitro as well (Fig. 4).

Analogs of compounds **5**–**7** were purchased to build a SAR dataset. The available analogs of **5** had a piperazine moiety instead of an acyclic amine and had significantly reduced primase inhibition activity. This decrease of activity could be due to sub-optimal location of the basic amine or absence of the ring attached oxygen (Table 1). The decrease in activity of **8** by substituting R^1: phenyl as in compound **9** or removing R^1: phenyl as in compound **12** suggests a well-defined binding region around R^1.

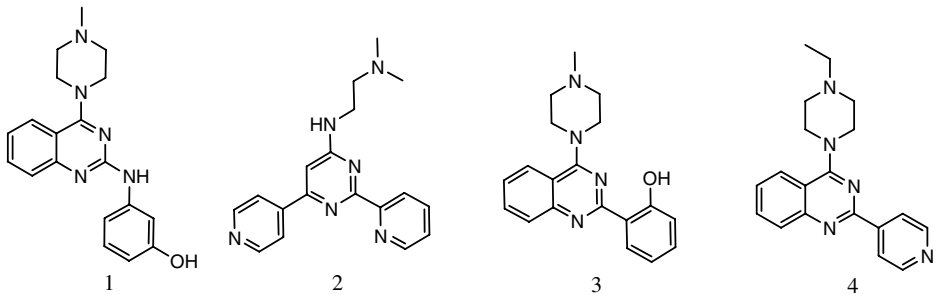


Figure 2. Inhibitors of *E. coli* primase enzyme.

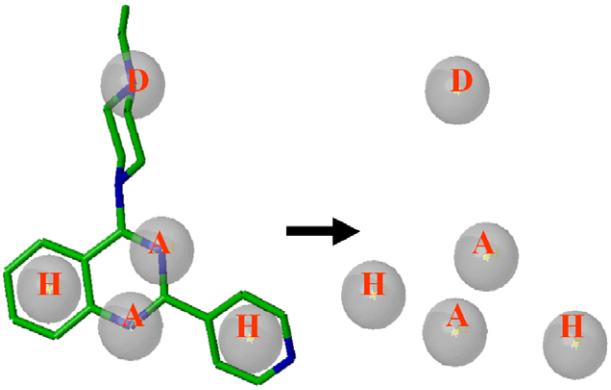
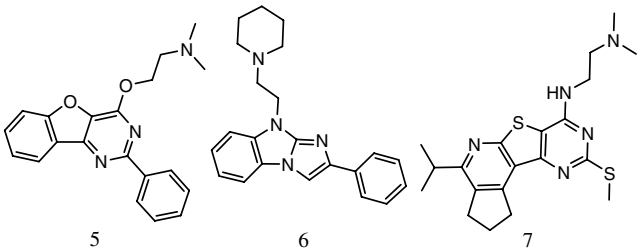


Figure 3. 3D query built using **4** as template. Carbon and nitrogen atoms of **4** are colored green and blue, respectively. The query consists of five features, two hydrophobes (H), two hydrogen bond acceptors (A), and a donor (D). All the features include a spatial tolerance defined by a sphere of 1 Å.



5
IC_{50}: 8.9 μM,
MIC Ec: 4 μg/mL

6
IC_{50}: 18.4 μM,
MIC Ec: 64 μg/mL

7
IC_{50}: 28.6 μM,
MIC Ec: 8 μg/mL

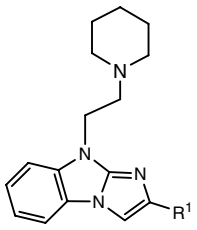
Figure 4. Primase inhibitors identified using 3D pharmacophore shown in Fig. 3.

The available imidazole analogs included aromatic ring variations of R^1. An overlay of the furan and imidazole skeletons superimposes R^1 substituents of the two series. As seen with furans, the para-substitution on R^1: phenyl decreases the activity of the imidazole analogs and supports the idea of limited amount of space available for R^1 (Table 2).

Analogs of **7** have a thiomethyl group instead of an aromatic ring at the equivalent position of imidazole, furan, and quinazoline analogs. The following two variations in compound **7**, a C8–C9 fused cyclohexyl ring and removal of the thiomethyl group (compound **17**, Table 3), resulted in a 2× drop in potency. Activity of compound **7** also drops with larger substitutions on the aliphatic amines, **18** and **19**. Compound **20** with a morpholine at C7 is about 3× less potent as compared to **21** that has a methyl at C7. The primase inhibition activity of **22** is increased about 3× when a smaller ring is fused through C8–C9, as seen in compound **23**. A comparison of **23** and **24** demonstrates that the activity decreases with a larger, un-branched, aliphatic group at C7. This limited SAR suggests interdependence of the substitutions at C7, C8, C9, and the aliphatic amine. As observed in the analogs of **5**, fixing the position of basic amine in a piperazine detracts from primase inhibition activity of thiophenes as well (**25**). The low MIC of compound **24** seems to be anomalous given the weak primase activity.

A primase inhibition IC_{50} of about 100 μM translated into weak but measurable in vitro antibacterial activity in all the three chemical series. Similar in vitro antibacterial activity trend was seen for *Staphylococcus aureus* as well (data not shown). Our data indicated the following SAR trends, increase in primase inhibition with a

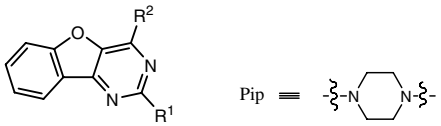
Table 2. Chemical structures of benzo[*d*]imidazo[2,1-*b*]imidazoles as primase inhibitors



Compound	R^1	IC_{50}[a] (μM)	MIC (Ec)[b] (μg/mL)
6	–Ph	26 (8.7)	64
14	–Ph-4-Me	99.5 (32.1)	64
15	–Ph-4-OMe	162.1 (49.9)	>64
16	–2-Thiophene	32.3 (9.0)	64

[a] Values are means of multiple experiments, standard deviation is given in parentheses.
[b] Values are means of two independent experiments.

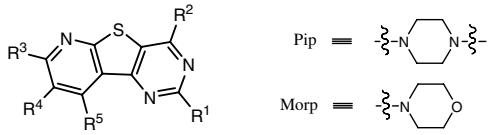
Table 1. Chemical structures of benzo[*d*]pyrimido[5,4-*b*]furans as primase inhibitors



Compound	R^1	R^2	IC_{50}[a] (μM)	MIC (Ec)[b] (μg/mL)
5	–Ph	$-O(CH_2)_2NMe_2$	3.4 (1.6)	4
8	–Ph	–Pip-4-Me	42.5 (0.8)	>64
9	–4-Cl-Ph	–Pip-4-Me	>200	>64
10	–Et	–Pip-4-Et	170.5 (14.8)	>64
11	–H	$-Pip-4-(CH_2)_2OH$	138 (4.2)	64
12	–H	–Pip-4-Me	85.1 (28.8)	64
13	–H	–Pip-4-cPentyl	122.4 (20.6)	>64

[a] Values are means of multiple experiments, standard deviation is given in parentheses.
[b] Values are means of two independent experiments.

Table 3. Chemical structures of pyrido[3′,2′:4,5]thieno[3,2-*d*]pyrimidines as primase inhibitors



Compound	R^1	R^2	R^3	R^4–R^5	IC_{50}[a] (μM)	MIC (Ec)[b] (μg/mL)
7	SMe	$-NH(CH_2)_2NMe_2$	-*i*Pr	$-(CH_2)_3-$	26.6 (2.8)	8
17	H	$-NH(CH_2)_2NMe_2$	-*i*Pr	$-(CH_2)_4-$	61.0 (5.7)	16
18	SMe	$-NH(CH_2)_2NEt_2$	-*i*Pr	$-(CH_2)_3-$	36.0 (19.2)	>64
19	SMe	$-NH(CH_2)_2$-1-Morp	-*i*Pr	$-(CH_2)_3-$	>200	>64
20	SMe	$-NH(CH_2)_2NEt_2$	-1-Morp	$-CH_2OC(Me)_2CH_2-$	45.0 (13.8)	64
21	SMe	$-NH(CH_2)_2NEt_2$	Me	$-CH_2OC(Me)_2CH_2-$	16.9 (3.5)	32
22	SMe	$-NH(CH_2)_2NMe_2$	-*i*Bu	$-CH_2OC(Me)_2CH_2-$	79.2 (5.6)	>64
23	SMe	$-NH(CH_2)_2NMe_2$	-*i*Bu	$-(CH_2)_3-$	22.0 (6.9)	4
24	SMe	$-NH(CH_2)_2NMe_2$	-*n*Bu	$-(CH_2)_3-$	103.2 (31.5)	8
25	SMe	–Pip-4-Et	-*i*Pr	$-(CH_2)_3-$	>200	>64

[a] Values are means of multiple experiments, standard deviation is given in parentheses.
[b] Values are means of two independent experiments (ND, not determined).

non-polar R^1 and decrease with a piperazine ring in furan (**5**) and thiophene (**7**) analogs.

We have successfully applied virtual screening methods to discover bacterial primase inhibitors that exhibit both in vitro enzyme inhibition and antibacterial activity. Further optimization of these compounds is underway to ascertain that the observed antimicrobial activity is due to the inhibition of primase. This work provides lead primase inhibitors and SAR guidelines for further drug discovery effort and a 3D pharmacophore for the primase inhibition activity.

References and notes

1. (a) Marians, K. J. *Annu. Rev. Biochem.* **1992**, *61*, 673; (b) Aravind, L.; Leipe, D. D.; Koonin, E. V. *Nucleic Acids Res.* **1998**, *26*, 4205.
2. Keck, J. L.; Roche, D. D.; Lynch, A. S.; Berger, J. M. *Science* **2000**, *287*, 2482.
3. Podobnik, M.; McInerney, P.; O'Donnell, M.; Kuriyan, J. J. *J. Mol. Biol.* **2000**, *300*, 353.
4. Tripos, Inc. 1699 South Hanley Road, St. Louis, MO 63144-2913, US.
5. Schrodinger, Inc. 120 West 45th Street, 32nd floor, New York, NY 10036-4041, US.
6. Molecular Discovery, Via Stoppani, 38, 06087-Ponte San Giovanni-PG, Italy.
7. Hajduk, P. J.; Huth, J. R.; Fesik, S. W. *J. Med. Chem.* **2005**, *48*, 2518.
8. Zhang, Y.; Yang, F.; Kao, Y.-C.; Kurilla, M. G.; Pompliano, D. L.; Dicker, I. B. *Anal. Biochem.* **2002**, *304*, 174.
9. Clinical and Laboratory Standards Institute. 2001. Performance standards for antimicrobial susceptibility testing: 11th informational supplement. Vol. 21, no. 1, M100-S11. Clinical and Laboratory Standards Institute, Wayne, PA.