Bioorganic & Medicinal Chemistry Letters 18 (2008) 3565–3569

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Design and synthesis of 2-pyridones as novel inhibitors of the *Bacillus anthracis* enoyl-ACP reductase

Suresh K. Tipparaju [a], Sipak Joyasawal [a], Sara Forrester [b], Debbie C. Mulhearn [b], Scott Pegan [b], Michael E. Johnson [b,*], Andrew D. Mesecar [a,b,*], Alan P. Kozikowski [a,*]

[a] Drug Discovery Program, Department of Medicinal Chemistry and Pharmacognosy, University of Illinois at Chicago, 833 S. Wood Street, Chicago, IL 60612, USA
[b] Center for Pharmaceutical Biotechnology, University of Illinois at Chicago, 900 S. Ashland Avenue, Chicago, IL 60607, USA

ARTICLE INFO

Article history:
Received 20 March 2008
Revised 28 April 2008
Accepted 1 May 2008
Available online 4 May 2008

Keyword:
Anthrax

ABSTRACT

Enoyl-ACP reductase (ENR), the product of the FabI gene, from *Bacillus anthracis* (*Ba*ENR) is responsible for catalyzing the final step of bacterial fatty acid biosynthesis. A number of novel 2-pyridone derivatives were synthesized and shown to be potent inhibitors of *Ba*ENR.

Fatty acids are an essential source of energy for organisms from all taxa. Fatty acid synthesis in mammals is substantially different from that in bacteria. In mammals, the fatty acid synthesis involves a single multifunctional enzyme–acyl carrier protein (ACP) complex. In bacteria, the synthesis utilizes several small monofunctional enzymes that operate in conjunction with ACP-associated substrates.[1] Thus, it is possible to selectively target key enzymes in the bacterial fatty acid biosynthesis.[2]

The final and rate-determining step of chain elongation in the bacterial fatty acid biosynthesis is the reduction of enoyl-ACP to an acyl-ACP, which is catalyzed by the enzyme–enoyl–acyl carrier protein reductase. Considerable research over the past few years has shown that ENR is the target of a number of known antibacterial agents, including isoniazid,[3] diazaboranes,[4] and triclosan.[5] Targeting ENR is an attractive approach for the development of novel antibacterials, which has been validated by the recent discovery of several other small molecule inhibitors,[6] including those that exhibit potent in vitro activity against clinical isolates of methicillin-resistant *Staphylococcus epidermidis* and *Staphylococcus aureus*.[7]

As part of an on-going program to develop novel therapeutics for anthrax, we recently reported that the diphenyl ether-triclosan and a number of its aryl ether derivatives inhibit *Ba*ENR.[8] Although triclosan is a potent inhibitor of *Ba*ENR (IC_{50} = 0.5 μM, MIC = 3.1 μg/mL),[9] a major caveat in developing triclosan or its derivatives as drugs is the metabolic liability of the phenolic hydroxyl group.[10] In addition, triclosan-like diphenyl ethers are fairly lipophilic molecules and pose serious solubility problems. In order to overcome these structural drawbacks, it is important to design compounds in which the phenolic group of ring A of triclosan is replaced by a more metabolically stable functionality. We report herein our efforts toward addressing these issues by developing novel scaffolds that replace the phenolic ring of triclosan with other heterocyclic rings that retain the essential structural features of triclosan required for interaction with ENR, such as π-stacking of the aromatic ring with NAD^+, hydrogen bonding of the phenolic OH group with the ribose, as well as optimal flexibility of the two rings in order to allow complementary interactions with the active site.[9]

Our structure optimization studies were based on predicted binding interactions of 2-pyridones with the enzyme active site. Triclosan and synthesized pyridones were docked into the *Ba*ENR crystal structure[9] using the GOLD-docking program.[11] It was observed that the 2-pyridones dock to *Ba*ENR in the same binding pocket as that of triclosan, and maintain similar H-bonding interactions with the residues in the active site. Figure 1 shows the similarities in the binding geometry of triclosan and a representative 2-pyridone, **2**. Figure 2 shows the crystal structure of triclosan bound to *Ba*ENR and the GOLD docking conformations of compounds **2** and **35** in the active site. The X-ray structure of triclosan bound to *Ba*ENR shows that the phenolic hydroxyl group on ring A is involved in two hydrogen bonds, one with Tyr157 (OH) and the other to the 2′-hydroxyl group of nicotinamide ribose (shown in Fig. 2A).[9] These interactions appear to be preserved in the GOLD-docking conformation of the pyridones as well, and show that the oxygen on the carbonyl group of ring A is involved in a hydrogen bonding interaction with Tyr157 (OH) and the NAD^+ (shown in Fig. 2B and C). As seen in Figure 1, the ring A pocket of the

* Corresponding authors. Tel.: +1 312 996 9114; fax: +1 312 413 9303 (M.E.J.); tel.: +1 312 996 1877; fax: +1 312 413 9303 (A.D.M.); tel.: +1 312 996 7577; fax: +1 312 413 0577 (A.P.K.).
E-mail addresses: mjohnson@uic.edu (M.E. Johnson), mesecar@uic.edu (A.D. Mesecar), kozikowa@uic.edu (A.P. Kozikowski).

0960-894X/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2008.05.004

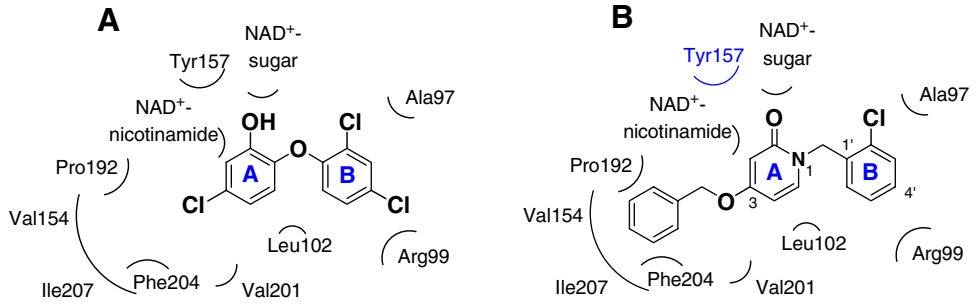


Figure 1. Schematic representation of residues in the binding pockets of rings A and B of (A) triclosan and (B) compound **2**.

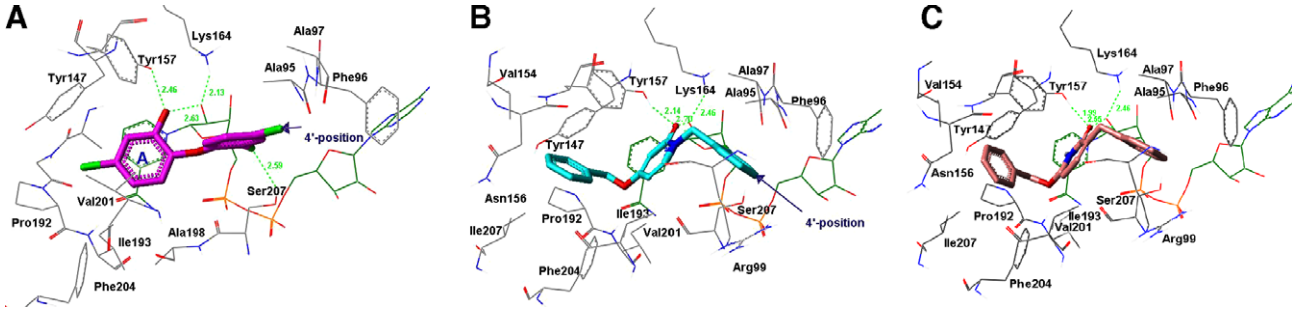
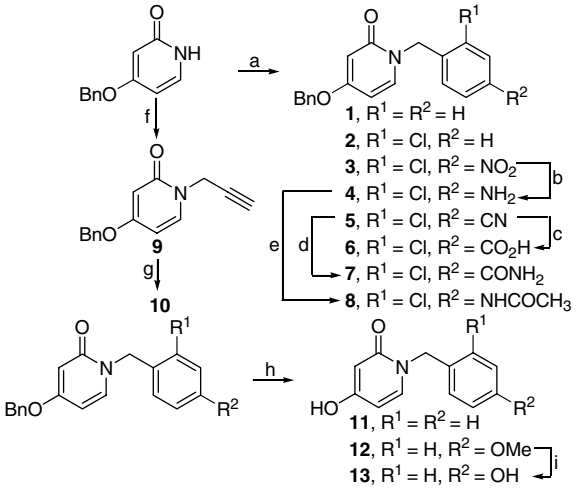


Figure 2. (A) Crystal structure of triclosan bound to *Ba*ENR. ENR atoms are colored by atom type, NAD^+ is green, and triclosan is magenta. (B) GOLD-docking conformation of **2** against the crystal structure of *Ba*ENR. ENR atoms are colored by atom type, NAD^+ is green, and **2** is cyan. Distances between atoms that are close enough to be within hydrogen bonding range are shown in green. (C) GOLD-docking conformation of **35** against the crystal structure of *Ba*ENR. ENR atoms are colored by atom type, NAD^+ is green, and **35** is coral.

pyridones is surrounded by Val 154, Val 201, Ile 207, and Phe 204, and thus appears to be dominated by a high amount of hydrophobicity.

The synthesis of compounds **1**–**3**, **5**, and **9** involved selective N-alkylation of the commercially available 4-benzyloxy-2(1*H*)-pyridone with the corresponding benzyl halides according to the procedure described by Conreaux et al. (Scheme 1).[12] Reduction of **3** gave the amino compound **4**, which was further converted to the acetamide **8**. While alkaline hydrolysis of the benzonitrile **5** resulted in the carboxylic acid **6**, hydrolysis in the presence of hydrogen peroxide gave the carboxamide **7**. Isoxazole **10** was prepared by treating **9** with ethyl chlorooximidoacetate in the presence of a base.[13] Rapid and selective O-debenzylation of 4-benzyloxy-2(1*H*)-pyridone derivatives occurred when treated with Pd/C under atmospheric pressure of hydrogen to give compounds **11** and **12**. Compound **13** was obtained by treatment of **12** with BBr_3.

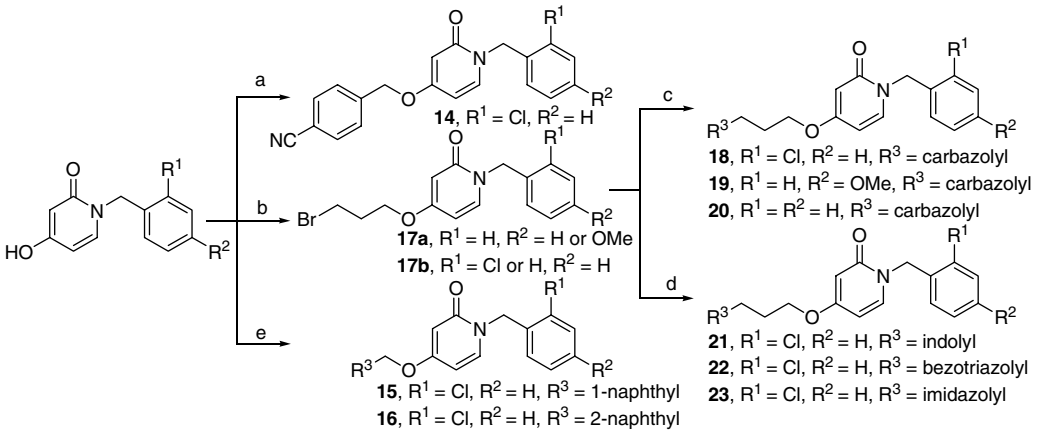


Scheme 1. Reagents and conditions: (a) KO*t*Bu, TBAI, THF, $ArCH_2X$, 0–25 °C, 16 h; (b) $NaBH_4$, $Cu(OAc)_2$, THF, 0 °C, 40 min; (c) 25% NaOH, EtOH, reflux, 20 h; (d) 35% H_2O_2, 3 N NaOH, EtOH, 30 °C, 20 h; (e) Ac_2O, DMAP, Et_3N, CH_2Cl_2, 0–25 °C, 3 h; (f) KO*t*Bu, TBAI, THF, propargyl bromide, 0–25 °C, 16 h; (g) ethyl chlorooximidoacetate, Et_3N, rt, 5 h; (h) Pd/C, H_2, MeOH, rt, 10–15 min; (i) BBr_3, CH_2Cl_2, −78 °C to rt, 12 h.

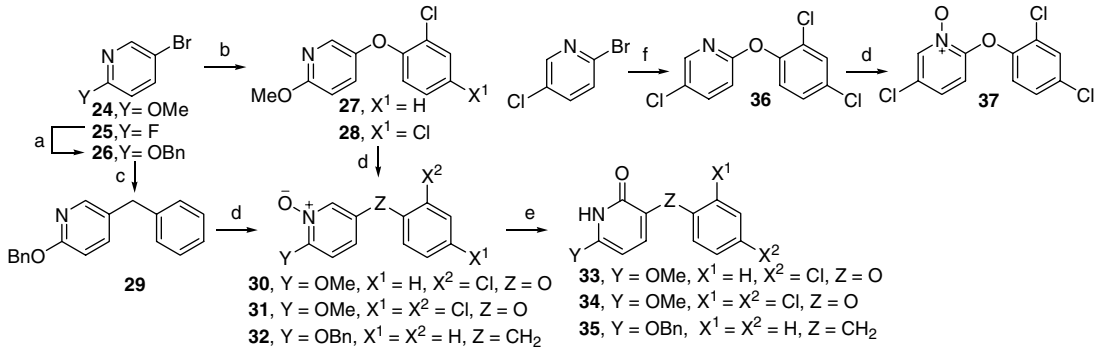
The above 4-hydroxy-pyridones were elaborated into a number of derivatives via a series of alkylation reactions (Scheme 2). Compound **14** was prepared by benzylation of 4-hydroxy-pyridone with 4-cyanobenzyl bromide. Naphthyl derivatives **15** and **16** were obtained by using a similar procedure. The common synthetic intermediates **17** were prepared by alkylating the 4-hydroxy-pyridones with 1,3-dibromopropane. Compounds **18**–**20** were prepared in turn by *N*-alkylation of carbazole with these intermediates. Compounds **21**–**23** were synthesized using a similar protocol.

The synthesis of the 3-substituted 2-pyridones and N-oxide derivatives is shown in Scheme 3. Pyridones **33**–**35** were synthesized by acetic anhydride mediated rearrangement of the corresponding N-oxides, followed by acidic hydrolysis.[14] The N-oxides were synthesized from the corresponding pyridines by *m*-CPBA oxidation. While the ethers **27** and **28** were prepared by standard coupling reactions, intermediate **29** was obtained by treating 2-benzoyloxy-5-bromopyridine with benzyltrimethyltin under Stille reaction conditions.

The compounds synthesized were evaluated for their *Ba*ENR inhibitory and anitibacterial activities.[15] Inhibitor **2** appears to be the best compound in this series and was considered as a lead. As expected from the interactions of hydrophobic residues located around the 3-position of the pyridone ring in the active site, replacing the benzyloxy group at this position with a more polar hydroxy group led to a substantial decrease in the ENR inhibitory activity (Table 1. compounds **11**–**13**). A four–fold improvement in the en-



Scheme 2. Reagents and conditions: (a) NaH, DMF/THF, 4-CN-PhCH$_2$Br, 0–25 °C, 12 h; (b) NaH, DMF, 1,3-dibromopropane, 0–25 °C, 6 h; (c) NaH, DMF, carbazole, 0–25 °C, 12 h; (d) For **21**: NaH, DMF, indole, rt, 16 h; for **22**: benzotriazole, DMF, K_2CO_3, rt, 12 h; for **23**: imidazole, KOH, THF, 85 °C, 16 h; (e) NaH, DMF/THF, 1- or 2-bromomethyl naphthalene, 0–25 °C, 12 h.



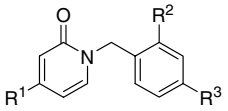
Scheme 3. Reagents and conditions: (a) benzyl alcohol, NaH, THF, rt, 12 h; (b) for **27**: KOH-$CuCO_3$, 2-chlorophenol, 180 °C, 15 h; For **28**: 2,4-dichlorophenol, KO*t*Bu, DMF, rt, 3 h, then at 45 °C under vacuum, Cu(OTf)$_2$·PhCH$_3$, DMF, reflux, 16 h; (c) trimethylbenzylstannane, Pd(PPh$_3$)$_4$, DMF, 80 °C, 12 h; (d) *m*-chloroperbenzoic acid, CHCl$_3$, rt, 12 h; (e) Ac_2O, reflux, 5 h, 1 N HCl, 100 °C, 12 h; (f) KOH–$CuCO_3$, 2,4-dichlorophenol, 180 °C, 15 h.

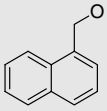
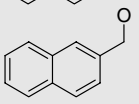
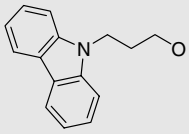
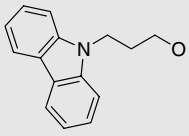
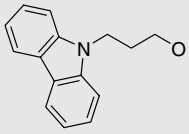
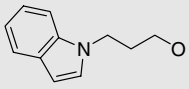
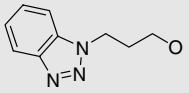
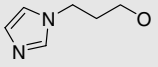
zyme inhibitory activity was observed when a chlorine atom is present at the 2′-position on the aromatic ring B (cf. **1** vs **2**). A similar improvement in the binding affinity of triclosan derivatives to the *Ba*ENR active site was recently observed by us.[8] Attachment of an electron withdrawing cyano group to the aromatic ring of the benzyloxy moiety resulted in **14**, which showed an ENR inhibitory activity lower than the lead compound. Thus, we explored the activities of compounds with other hydrophobic substitutents at the 3-position of the pyridone ring. Introduction of 1- and 2-naphthyl groups resulted in compounds **15** and **16** whose enzymatic activity was comparable to the lead compound. Introduction of a bulkier carbazole unit, tethered with a three-carbon chain on the other hand, decreased the *Ba*ENR inhibitory activity (Table 1, compounds **18**–**20**). Again, the importance of having a chlorine atom at the 2′-position on ring B to improve the inhibitory activity of these compounds is evident by comparing the activities of **18** and **20**. Replacing the carbazole unit with a smaller indole moiety resulted in twofold improvement in the ENR inhibitory activity (compound **21**), while a benzotriazolyl substitution resulted in the compound (**22**) with an ENR inhibitory activity comparable to that of the lead compound. Introduction of an imidazolyl unit did not improve the activity.

We explored the SAR of the 2-pyridones by functionalizing ring B. From the docking conformations of the lead compound into the *Ba*ENR X-ray crystal structure (Fig. 2B), we anticipated that hydrogen bond donors/acceptors at the 4′-position would be ideally positioned to interact with either Ala 97 or Arg 99. Hence, we synthesized compounds **3**–**8** with various functional groups at the 4′-position of the ring B. Compound **4**, bearing an amino group at the 4′-position turned out to be the best compound with an IC_{50} of 0.8 μM. Conversion of the amino functionality into an acetamide (compound **8**) reduced the *Ba*ENR inhibitory activity by half. Thus, it appears that the presence of an electron-donating group at the 4′-position is able to enhance the interaction of ligands with the enzyme active site. Attempts to replace the aromatic ring B of these 2-pyridones with an acetylene (compound **9**) or an isoxazole (compound **10**) were not successful in improving the activity (Table 2).

We briefly explored the activities of *C*-substituted 2-pyridones that are structurally similar to the *N*-substituted 2-pyridones discussed above (compounds **33**–**35**). These C-substituted pyridones are capable of existing in their enol form as hydroxypyridines, and thus closely mimic triclosan in structure. The activities of these compounds are shown in Table 2. It is gratifying to note that the novel *C*-substituted 2-pyridone, **35** showed a 10-fold improvement in ENR inhibitory activity over its N-substituted analog **1**. The GOLD-docking conformation of **35** in Figure 2C suggests a nearly identical orientation of the *C*-substituted 2-pyridones compared to the N-substituted pyridones. Although the origin of improved activity of compound **35** is not completely clear at this stage, the pyridone NH and the nicotinamide ring are about 3.6 Å apart and thus ligand binding stabilization from an N–H$\cdots\pi$ interaction cannot be ruled out.[16] Moderate ENR inhibition was observed by the 3-phenoxy-2-pyridones **33** and **34**. Among the pyridine N-oxides, compound **37** exhibited modest ENR inhibition, while compound **30** was inactive.

Table 1
BaENR inhibitory activities of compounds

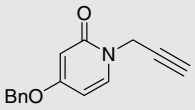
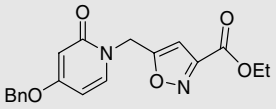
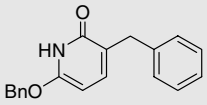
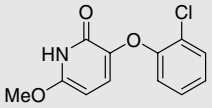
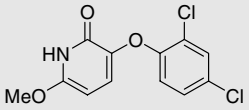
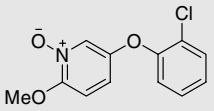
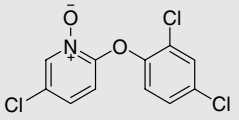


Compound	R^1	R^2	R^3	IC_{50} (μM)
11	OH	H	H	>100[a]
12	OH	H	OMe	>100[a]
13	OH	H	OH	>100[a]
1	BnO	H	H	6.8 ± 0.8
2[b]	BnO	Cl	H	1.5 ± 0.1
14	4-CN-PhCH$_2$O	Cl	H	2.7 ± 0.4
15		Cl	H	1.5 ± 0.8
16		Cl	H	1.1 ± 0.1
18		Cl	H	>6[c]
19		H	OMe	>3[d]
20		H	H	>100[a]
21		Cl	H	0.8 ± 0.2
22		Cl	H	1.6 ± 0.3
23		Cl	H	28.0 ± 6.2
5	BnO	Cl	CN	7.0 ± 1.1
7	BnO	Cl	$CONH_2$	3.6 ± 0.3
6[b]	BnO	Cl	COOH	23.8 ± 2.5
3	BnO	Cl	NO_2	3.7 ± 1.1
4	BnO	Cl	NH_2	0.8 ± 0.1
8	BnO	Cl	NHAc	1.8 ± 0.5

[a] *Ba*ENR inhibition was less than 30% at 100 μM.
[b] MIC values against ΔANR *B. anthracis*[15] for compound **2**: 16 μg/mL, for compound **6**: 74 μg/mL. MICs for all other compounds in the table are >80 μg/mL.
[c] The inhibitor precipitated at concentrations >6 μM.
[d] The inhibitor precipitated at concentrations >3 μM.

In conclusion, we have identified certain 2-pyridone derivatives as novel, small-molecule inhibitors of bacterial enoyl-ACP reductase (ENR) from *B. anthracis*. Compound **2** showed good ENR-inhibitory activity as well as reasonable antibacterial activity, thus providing a lead compound for further development. Compounds **4** and **21** show nearly a twofold improvement in ENR inhibitory activity over compound **2**. Compound **35**, a 'reversed' pyridone, is also an encouraging lead for the development of a new class of ENR inhibitors. Current efforts focus on further improvement of

Table 2
*Ba*ENR inhibitory activities of compounds

Compound[a]	Structure	IC_{50} (μM)
9		>100[b]
10		>100[b]
35		0.7 ± 0.4
33		18.8 ± 4.2
34		23.7 ± 11.7
30		>100[b]
37		21.4 ± 7.7

[a] MIC values against ΔANR *B. anthracis* were >100 μg/mL.
[b] *Ba*ENR inhibition was less than 30% at 100 μM.

*Ba*ENR inhibition and improving antibacterial activities of these compounds.

Acknowledgments

This work was funded by a grant from NIH/NIAID (U19 AI056575) and a contract from DOD (W81XWH-07-1-0445).

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2008.05.004.

References and notes

1. (a) Rock, C. O.; Jackowski, S. *Biochem. Biophys. Res. Commun.* **2002**, *292*, 1155; (b) Wakil, S. J. *Biochemistry* **1989**, *28*, 4523.
2. (a) Campbell, J. W.; Cronan, J. E., Jr. *Annu. Rev. Microbol.* **2001**, *55*, 305; (b) Heath, R. J.; White, S. W.; Rock, C. O. *Prog. Lipid Res.* **2001**, *40*, 467.
3. Rozwarski, D. A.; Grant, G. A.; Barton, D. H.; Jacobs, W. R., Jr.; Sacchettini, J. C. *Science (New York, NY)* **1998**, *279*, 98.
4. Levy, C. W.; Baldock, C.; Wallace, A. J.; Sedelnikova, S.; Viner, R. C.; Clough, J. M.; Stuitje, A. R.; Slabas, A. R.; Rice, D. W.; Rafferty, J. B. *J. Mol. Biol.* **2001**, *309*, 171. and references cited therein.
5. (a) McLeod, R.; Muench, S. P.; Rafferty, J. B.; Kyle, D. E.; Mui, E. J.; Kirisits, M. J.; Mack, D. G.; Roberts, C. W.; Samuel, B. U.; Lyons, R. E.; Dorris, M.; Milhous, W. K.; Rice, D. W. *Int. J. Parasitol.* **2001**, *31*, 109; (b) Ref. 8 and references cited therein.
6. (a) Kitagawa, H.; Kumura, K.; Takahata, S.; Iida, M.; Atsumi, K. *Bioorg. Med. Chem. Lett.* **2007**, *15*, 1106; (b) Kitagawa, H.; Ozawa, T.; Takahata, S.; Iida, M.; Saito, J.; Yamada, M. *J. Med. Chem.* **2007**, *50*, 4710. and references cited therein; (c) Moir, D. T. *Curr. Drug Targets Infect. Disord.* **2005**, *5*, 297. and references cited therein.

7. Karlowsky, J. A.; Laing, N. M.; Baudry, T.; Kaplan, N.; Vaughan, D.; Hoban, D. J.; Zhanel, G. G. *Antimicrob. Agents Chemother.* **2007**, *51*, 1580.
8. Tipparaju, S. K.; Mulhearn, D. C.; Klien, G. M.; Chen, Y.; Tapadar, S.; Bishop, M. H.; Yang, S.; Chen, J.; Ghassemi, M.; Santarsiero, B. D.; Cook, J. L.; Johlfs, M.; Mesecar, A. D.; Johnson, M. E.; Kozikowski, A. P. *ChemMedChem*, in press.
9. Klein, G. M.; Bernard, D. S.; Tipparaju, S. K.; Pegan, S.; Bishop, M. H.; Kozikowski, A. P.; Mesecar, A. D. *Biochemistry* **2007**, accepted for publication.
10. Wang, L. Q.; Falany, C. N.; James, M. O. *Drug Metab. Dispos.* **2004**, *32*, 1162.
11. The synthesized pyridones were docked into the *B. anthracis* ENR active site from the crystal structure with PDB code 2QIT. GOLD docking was performed using the default parameters and the ChemScore scoring function. GOLD reference: Verdonk, M. L.; Cole, J. C.; Hartshorn, M. J.; Murray, C. W.; Taylor, R. D. *Proteins* **2003**, *52*, 609.
12. Conreaux, D.; Bossharth, E.; Monteiro, N.; Desbordesb, P.; Balmea, G. V. *Tetrahedron Lett.* **2005**, *46*, 7917.
13. Kozikowski, A. P.; Adamczyk, M. *J. Org. Chem.* **1983**, *48*, 366.
14. Shone, R. L.; Coker, V. M.; Moormann, A. E. *J. Heterocycl. Chem.* **1975**, *12*, 389.
15. Detailed description of methods for biological assay is provided in Supplementary information.
16. Meyer, E. A.; Castellano, R. K.; Diederich, F. *Angew. Chem., Int. Ed.* **2003**, *42*, 1210.