Bioorganic & Medicinal Chemistry Letters 19 (2009) 5355–5358

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Spiro-naphthyridinone piperidines as inhibitors of *S. aureus* and *E. coli* enoyl-ACP reductase (FabI)

Peter B. Sampson [†,*], Christine Picard, Sean Handerson, Teresa E. McGrath, Megan Domagala, Andrew Leeson, Vladimir Romanov, Donald E. Awrey [†], Dhushy Thambipillai, Elias Bardouniotis, Nachum Kaplan, Judd M. Berman, Henry W. Pauls [†]

Affinium Pharmaceuticals Inc. 1243 Islington Ave., Suite 600, Toronto, Ontario, Canada M8X 1Y9

ARTICLE INFO

Article history:
Received 11 June 2009
Revised 27 July 2009
Accepted 28 July 2009
Available online 6 August 2009

Keywords:
Enoyl-ACP reductase
Naphthyridinone

ABSTRACT

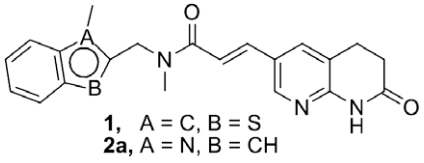
Spiropiperidine naphthyridinone inhibitors of *Staphylococcus aureus* and *Escherichia coli* FabI have been prepared. Compounds **14a** and **14c** were identified as having sub-nanomolar *E. coli* FabI activity and are among the most potent FabI inhibitors yet described. The structural model of **14a** bound to *E. coli* FabI is shown.

Hospital acquired bacterial infections due to methicillin-resistant *Staphylococcus aureus* (MRSA) have developed into a serious healthcare concern which necessitates the development of new antibacterial drugs.[1] The identification of new therapeutics which functions by a novel mechanism of action and circumvent resistance has been an ongoing challenge for anti-infective drug discovery.[2] Recently, there has been interest in targeting bacterial fatty acid biosynthesis as a viable approach to new antimicrobial agents.[3–7]

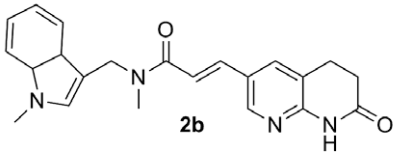
The enzyme FabI is one of the enoyl-ACP reductases of the FASII system. The enzyme requires the cofactor NAD(P)H as hydride source to carry out the reduction of an enoyl-ACP to the corresponding acyl-ACP (Scheme 1). FabI has been shown to be the target of the antimicrobial agent triclosan, as well as a number of small molecule inhibitors which possess excellent antimicrobial activity.[6]

Since no analogous protein is used in mammals for similar transformations, inhibitors of FabI do not interfere with mammalian fatty acid biosynthesis. FabI has been advanced as an attractive target for the potential treatment of systemic bacterial infections.[4–8]

Recent work by Brinster et al.[9] argues against the validity of targeting FASII for antimicrobial therapy. Exogenous fatty acids present in human serum were shown to reduce sensitivity to drug-induced FASII inhibition when treated with relatively weak inhibitors cerulenin and triclosan. The highly potent FabI inhibitors presented herein, may aid in addressing the drugability of the FASII pathway in pathogenic bacteria.

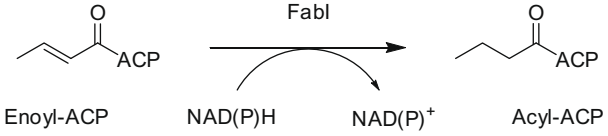


1, A = C, B = S
2a, A = N, B = CH



2b

Compounds of the ene-amide series which contain the naphthyridinone ring system (**1**, **2a**, and **2b**)[6], exhibit excellent potency against FabI. In this series, the inhibition of FabI correlates well with antibacterial activity as these compounds exhibit potent activity against a number of drug-resistant strains of *S. aureus*. Moreover, naphthyridinone analogues have been reported to be effective in vivo in a *S. aureus* thigh model infection in rats.[6]



Scheme 1. FabI biosynthetic reaction.

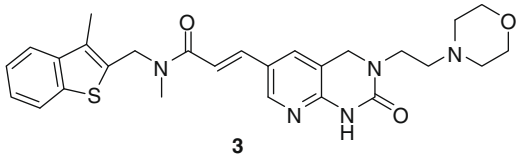
* Corresponding author. Tel.: +1 416 581 8550x5020.
E-mail address: psampson@uhnresearch.ca (P.B. Sampson).
† Present address: Campbell Family Institute for Breast Cancer Research, 101 College Street, Toronto, ON, Canada M5G 1L7.

0960-894X/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2009.07.129

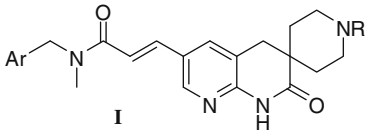
One drawback to this series is the high lipophilicity and inherent low aqueous solubility (compounds such as **1** exhibit aqueous solubility <1 μg/mL). The purpose of this study was to improve the solubility of the FabI inhibitors while retaining excellent in vitro potency.

Utilizing the available co-structure of naphthyridinone **2b** with *Escherichia coli* FabI[10], a structure guided approach was implemented to determine specific regions of the molecule which may tolerate the addition of a solubilizing functionality. Given the high homology between the active sites of *S. aureus* and *E. coli* FabI, this approach should apply the inhibition of the latter as well. The fatty acid binding pocket of the FabI active site, where the benzothiophene and indole moieties of **1**, **2a**, and **2b** bind, is hydrophobic in nature. It was clear from the crystal structure (Fig. 1) and SAR, that charged and highly polar functions are not tolerated in this pocket. The region in which the naphthyridinone resides is more hydrophilic in nature. The binding energy of the naphthyridinone is derived from a strong hydrogen bonding network between the backbone carbonyl and amide hydrogen of Ala97 and the pyridyl nitrogen and amide hydrogen of the inhibitor. Therefore, it was desirable to keep this interaction intact. The region 'above' the naphthyridinone ring is exposed to solvent water and we focused attention in this region for the introduction of basic functionalities.

Pyridopyrimidinones, carrying a basic tether (e.g., **3**), were previously evaluated as equivalent right hand side analogues.[11] We were encouraged to find that potency was not reduced upon introduction of basic functionalities in the right hand side region of the molecule. The spiropiperidine modification was designed using the crystal structure of the morpholine analogue **3**, bound to *S. aureus* FabI.[12]; this structure revealed that the basic tether was projected away from the active site into bulk water. It was envisioned that the piperidine ring of prototype **I** would occupy the same space as the morpholine tether of **3** and confer enhanced solubility by nature of its basic nitrogen.



3



I

The spiropiperidine modification of the previously disclosed naphthyridinone series was prepared as outlined in Scheme 2. The dibromide **4** was obtained in 3 steps from 2-aminonicotinic acid.[6] The enolate of the Boc-protected isonipecotate **5** was prepared and added slowly to **4** which produced the expected benzylic alkylation, followed by cyclization to give the desired spirocycle **6**. A Heck coupling of **6** with benzyl acrylate, followed by sodium hydroxide saponification provided the acrylic acid **8**.

The *N*-methyl acrylic acid **12** was prepared as shown in Scheme 3. Ethyl *N*-methyl-4-piperidine carboxylate **9** was prepared directly from ethyl isonipecotate.[13] The lithium enolate of **9** was prepared and added directly to dibromide **4** to give the spirocycle **10**. A Heck reaction of **10** with *tert*-butyl acrylate and subsequent TFA mediated deprotection gave the acrylic acid **12**.

The target compounds were prepared as outlined in Scheme 4. The left-hand side amines **13a–e** were prepared by reductive amination of the corresponding aldehydes.[6,10] The acrylic acids **8** and **12** were coupled in the presence of EDC with *N*-methyl amines **13a–e**, followed by treatment with HCl in ether to give the amides

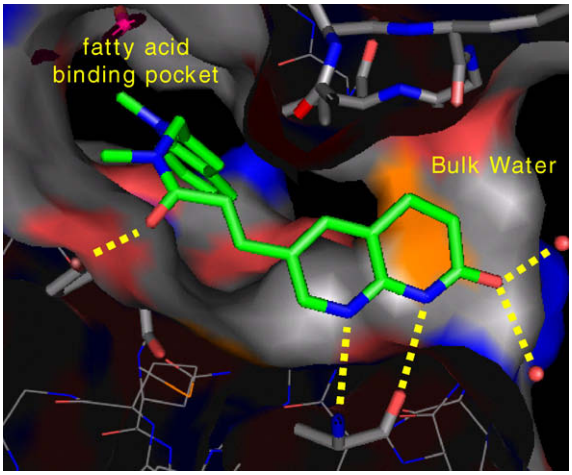


Figure 1. Crystal structure of compound **2b** in the *E. coli* FabI active site, hydrogen bonds are shown as yellow dashes.

14 and **15** as the hydrochloride salts. Instances where **8** was used as the acid coupling partner, the Boc-protected material was obtained from the coupling reaction. Addition of excess HCl in ether resulted in removal of the *tert*-butyl carbamate and formation of the hydrochloride salt.

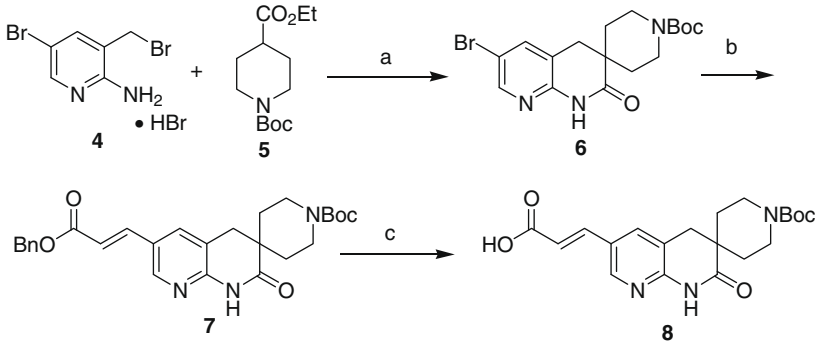
Table 1 clearly shows that one of the objectives of this work was met in that the spiropiperidines demonstrate enhanced solubility relative to the naphthyridinones. This is illustrated by comparing solubilities for compounds **14b** and **14c** with compounds **2** and **1** respectively. In both instances, solubility is enhanced several orders of magnitude by grafting on the spiropiperidine ring. The solubilities of the spiropiperidine analogs are generally in the 0.1–1 mg/mL range.

As shown in Table 2, the spiropiperidine modification resulted in compounds that were equipotent to the parent naphthyridinones in terms of *S. aureus* FabI IC_{50}. Moreover, a correlation can be observed between the IC_{50} results and the whole cell activity against *S. aureus* (Table 2). In the case of **14b** and **14e**, a noticeable increase in MIC against the MRSA strain was observed compared to **1**. The *N*-methyl derivatives **15a**, **15c**, and **15e**, appear to improve potency and whole cell activity compared to the analogues which carry the free amine. It is possible that increased whole cell activity is related to the enhanced permeability of the methylated species.

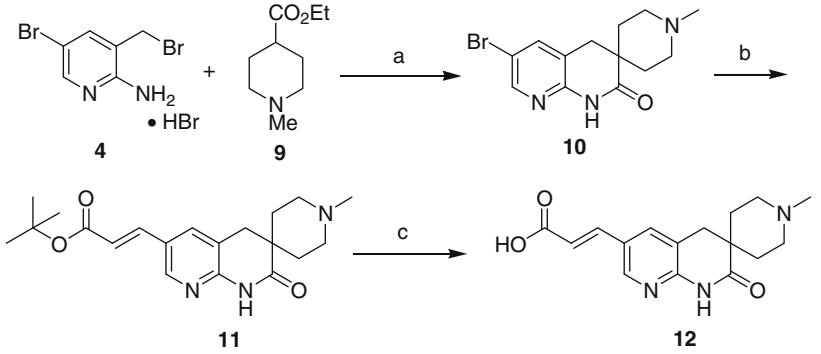
Excellent potency was observed with respect to *E. coli* FabI, with **14a** and **14c** showing sub-nanomolar potency. In contrast with *S. aureus*, the free amine analogues appear to exhibit improved MIC values relative to the *N*-methyl series. The MIC's of this series also offer a dramatic improvement over the naphthyridinone compounds, as is evident in the high MIC values for **1**.

The model structure of **14a** bound to *E. coli* FabI is depicted in Figure 2. The inhibitor is shown to exhibit binding characteristics which are consistent with the results obtained for previous co-structures, such as that obtained for the morpholine analogue **3**.[12]

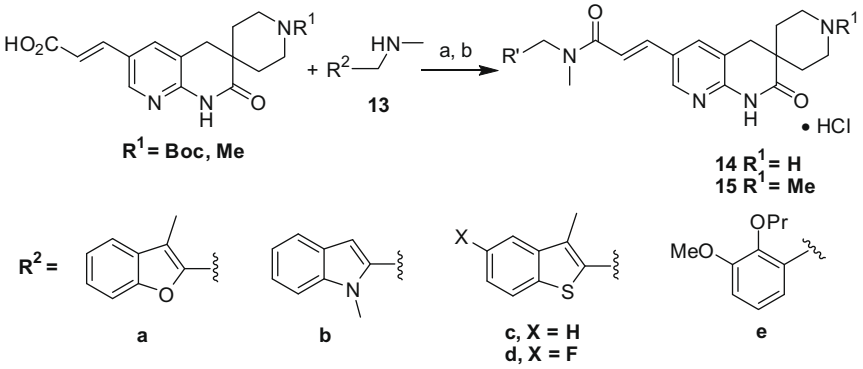
The benzofuran is nestled in a hydrophobic pocket consisting of residues Tyr146, Met159, Phe203, and Tyr156. The linker amide carbonyl engages in the canonical H-bond network to the phenolic hydrogen of Tyr156 and the 2′-hydroxyl of the co-factor. The pyridyl nitrogen and amide hydrogen of the naphthyridinone ring participate in a hydrogen bonding network with Ala95. The piperidine ring, which is directed towards solvent, also appears to be engaged in a hydrogen-bonding interaction with the carbonyl of Leu195. The piperidine nitrogen resides in a similar position to the morpholine nitrogen of **3**, further underscoring the tolerance of charged species in this region.



Scheme 2. Preparation of the spiro-naphthyridinone piperidines: (a) LDA, THF, −78 °C, 55%; (b) benzyl acrylate, $Pd(OAc)_2$, $(oTol)_3P$, DMF, propionitrile, 100 °C, 50%; (c) 1 N NaOH, MeOH, 76%.



Scheme 3. Preparation of the *N*-methyl spiro-naphthyridinone piperidines: (a) LDA, THF, −78 °C, 59%; (b) *tert*-butyl acrylate, $Pd(OAc)_2$, $(oTol)_3P$, DMF, propionitrile, 100 °C, 28%; (c) TFA, CH_2Cl_2, 100%.



Scheme 4. Preparation of the FabI Inhibitors: (a) EDC, HOBt, DIPEA, DMF, (b) HCl, ether.

Table 1
Enzyme inhibitory activity and aqueous solubility of FabI inhibitors

Compound	FabI IC_{50}[a] (nM)		Measured solubility[b] (μg/mL)	
	S. aureus	*E. coli*	pH 4.0	pH 7.4
1	30 (±)	n/a	0.2	0.3
2	50 (±1)	139 (±8)	6.2	5.8
3	26 (±1)	7 (±2)	>67	6.1
14a	49 (±3)	0.7 (±0.06)	1500	100
14b	132 (±5)	17(±1)	>100	>100
14c	48 (±4)	0.4 (±0.05)	22	4.8
14e	71 (±6)	n/a	700	520
15a	20 (±2)	1.8 (±0.2)	83	43
15c	14 (±3)	2.4 (± 0.3)	360	110
15d	28 (±)	24 (±2)	790	110
15e	26 (±2)	55 (±4)	1500	1000

[a] *Note*: the biochemical assay was performed as described[14]; n/a; not assayed.
[b] Solubility measured by modified shake flask method or Millipore MultiScreen Solubility Filter Plate Method.

Table 2
Antibacterial profile for FabI inhibitors[15]

Compound	*S. aureus* 29213 MIC (μg/mL)	*S. aureus* 43300 (MRSA strain) MIC (μg/mL)	*E. coli* AG100A acrAB⁻ MIC (μg/mL)
1	0.125	0.063	4
2	⩽0.016	0.016	⩽0.063
3	⩽0.063	⩽0.063	⩽0.063
14a	0.031	0.031	⩽0.063
14b	0.5	2	0.5
14c	⩽0.016	0.063	⩽0.063
14e	0.125	0.5	2
15a	⩽0.016	⩽0.016	0.25
15c	⩽0.016	⩽0.016	⩽0.063
15d	⩽0.016	⩽0.016	⩽0.063
15e	0.031	0.125	4

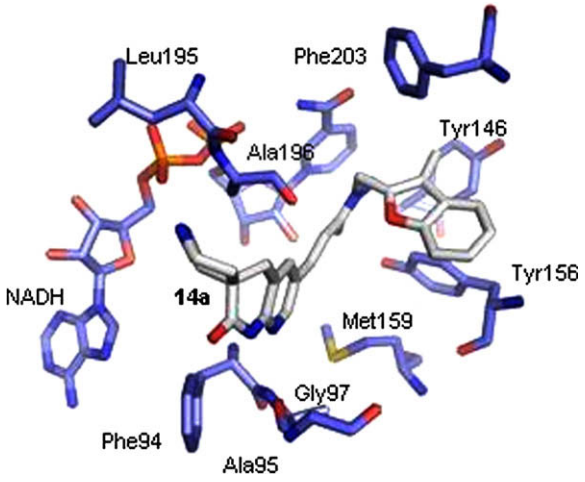


Figure 2. Model of **14a** and NADH bound in the active site of *E. coli* FabI. For clarity, only the side chains of the residues which define the hydrophobic pocket and those surrounding the piperidine ring are shown. The amide carbonyl of the inhibitor is shown to make a hydrogen bond interaction with the 2′-hydroxyl of the nicotinamide ribose of NADH and the hydroxyl of Tyr156. The nitrogen of the piperidine ring makes a hydrogen bond interaction with the carbonyl of Leu195.

Through a structure guided approach, we have shown that potent inhibition of *S. aureus* and *E. coli* FabI can be retained upon addition of functionalities which improve naphthyridinone aqueous solubility. Antibacterial activity is maintained or improved, offering additional opportunities to target FabI for Staphylococcal infections.

References and notes

1. Chu, D. T. W.; Plattner, J. J.; Katz, L. *J. Med. Chem.* **1996**, *39*, 3853.
2. Projan, S. J. *Curr. Opin. Pharmacol.* **2002**, *2*, 513.
3. White, S. W.; Zheng, J.; Zhang, Y.; Rock, C. O. *Annu. Rev. Biochem.* **2005**, *74*, 791.
4. Heerding, D. A.; Chan, G.; DeWolf, W. E.; Fosberry, A. P.; Janson, C. A.; Jaworski, D. D.; McManus, E.; Miller, W. H.; Moore, T. D.; Payne, D. J.; Qiu, X.; Rittenhouse, S. F.; Slater-Radosti, C.; Smith, W.; Takata, D. T.; Vaidya, K. S.; Yuan, C. C. K.; Huffman, W. F. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 2061.
5. Miller, W. H.; Seefeld, M. A.; Newlander, K. A.; Uzinskas, I. N.; Burgess, W. J.; Heerding, D. A.; Yuan, C. C. K.; Head, M. S.; Payne, D. J.; Rittenhouse, S. F.; Moore, T. D.; Pearson, S. C.; Berry, V.; DeWolf, W. E.; Polizzi, B. J.; Qiu, X.; Janson, C. A. *J. Med. Chem.* **2002**, *45*, 3246.
6. Seefeld, M. A.; Miller, W. H.; Newlander, K. A.; Burgess, W. J.; DeWolf, W. E.; Elkins, P. A.; Head, M. S.; Jakas, D. R.; Janson, C. A.; Keller, P. M.; Manley, P. J.; Heerding, D. A.; Chan, G.; Fosberry, A. P.; Jaworski, D. D.; Moore, T. D.; Payne, D. J.; Pearson, S.; Polizzi, B. J.; Qiu, X.; Rittenhouse, S. F.; Uzinskas, I. N.; Wallis, N. G.; Huffman, W. F. *J. Med. Chem.* **2003**, *46*, 1627.
7. Lu, H.; Tonge, P. J. *Acc. Chem. Res.* **2008**, *41*, 11.
8. Moir, D. T. *Curr. Drug Targets Infect. Disord.* **2005**, *5*, 297.
9. Brinster, S.; Lamberet, G.; Stael, B.; Trieu-Cuot, P.; Gruss, A.; Poyart, C. *Nature* **2009**, *458*, 83.
10. The coordinates were obtained from the PBD under the accession code 1MFP.
11. Berman, J; Sampson, P.; Pauls, H.W.; Ramnauth, J.; Manning, D.; Surman, M.D.; Xie, D.; Decornez, H.Y.; Pct. Intl. Appl. WO 2004052890, 2004.
12. Clarke, T.; Buzadzja, K.; Dorsey, M.; Abstract of papers, 44th Interscience Conference on Antimicrobial Agents and Chemotherapy, Washington, DC, October, 2004; Abstract F-1527.
13. Cignarella, G.; Villa, S.; Barlocco, D. *Eur. J. Med. Chem.* **1994**, *29*, 115.
14. *S. aureus* FabI inhibition assays were carried out as described in Ref. 5, using a FabI enzyme concentration of 3 nM. *E. coli* FabI inhibition assays were carried out in half area 96 well plates containing 150 μL of 100 mM MOPS pH 7.2, 50 mM ammonium acetate, 3% glycerol, 25 μM crotonyl-ACP, 50 μM NADH, and 0.5 nM enzyme. Consumption of NAD(P)H was monitored at 340 nm and a titration of standard compound was included on each dose response plate as a positive control.
15. MIC testing was performed on bacterial strains from the Affinium bacterial collection or the American Type Culture Collection (Manassas, VA) according to CLSI guidelines using the microdilution method in 96 wellplates: National Committee for Clinical Laboratory Standards 2003. Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically, Fifth Ed., Approved Standard M7-A6. NCCLS, Wayne, PA, USA.