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2,3,4,5-Tetrahydro-1H-pyrido[2,3-*b* and *e*][1,4]diazepines as inhibitors of the bacterial enoyl ACP reductase, FabI

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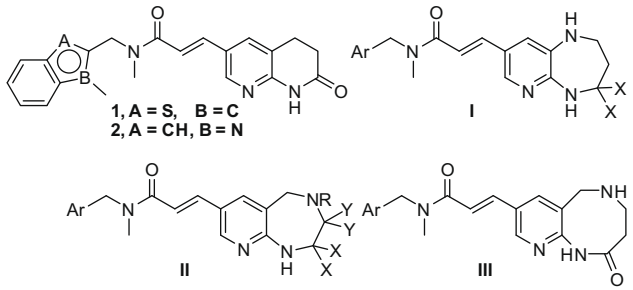
ABSTRACT

In the search for new antibacterial agents, the enzyme FabI has been identified as an attractive target. Employing a structure guided approach, the previously reported ene-amide series of FabI inhibitors were expanded to include 2,3,4,5-tetrahydro-1H-pyrido[2,3-*b* and *e*][1,4]diazepines. These novel series incorporate additional H-bonding functions and can be more water soluble than their naphthyridinone progenitors; diazepine **16c** is shown to be efficacious in a mouse infection model.

There is an urgent demand for new antibiotics due to an increase in drug resistant pathogenic bacteria.[1] One approach to combat antibiotic resistance is to target novel mechanisms of action,[2–4] and the enzymes of the bacterial fatty acid biosynthesis cycle (FASII) cycle are among the attractive targets.[5–7]

In bacteria, fatty acid biosynthesis is carried out by a series of sequential enzymatic steps.[7] In certain pathogenic bacteria (e.g., *Escherichia coli* and the genus *Staphylococcus*), an enoyl acyl carrier protein reductase, designated FabI, is responsible for the terminal step in the synthesis, and its corresponding gene is essential.[8] In contrast, similar transformations in humans are carried out by an unrelated multifunctional enzyme designated FASI.[9] This has led to the pursuit of specific FabI inhibitors as novel antibacterial agents[10] which would not be expected to interfere with comparable human biochemical processes.

A recent publication has challenged the validity of targeting FASII in general, and FabI in particular, for antibiotic therapy.[11] This work showed that inhibition of this pathway by the relatively weak FabI inhibitors triclosan and cerulenin can be bypassed by the addition of exogenous fatty acids. The availability of additional tools, as represented by potent FabI inhibitors, would be helpful in understanding the role of the FASII pathway in pathogenic bacteria.



Novel structures, namely the naphthyridinyl-ene-amides, were identified as potent FabI inhibitors by GSK.[10] Representatives of this class, benzothiophene **1** and indole **2** were shown to possess activity against *Staphylococci* and selected *E. coli* strains. For the purposes of our anti-infectives program, we required compounds suitable for both oral and intravenous dosing. However, inhibitor **1**, the more potent congener, could not be readily formulated for iv dosing. Indole **2**, although more aqueous soluble, achieved only modest blood levels upon oral dosing in rats.

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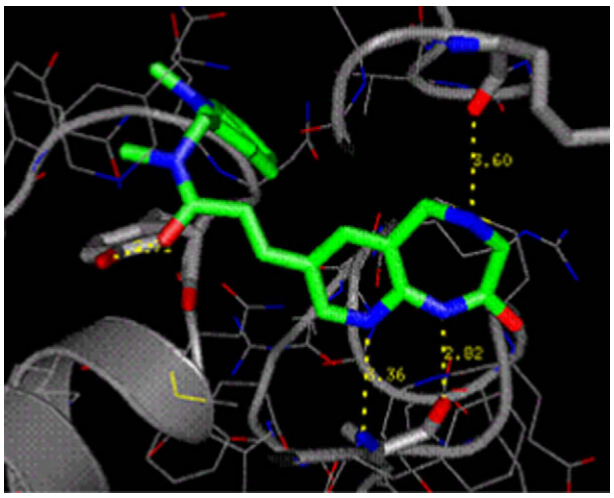


Figure 1. Model of compound **16c** docked in the *S. aureus* FabI active site.[13] H-bonds shown as yellow dashes.

We reasoned that ring expanded analogs incorporating basic nitrogen atoms, as represented by compounds **I**, **II**, and **III** would have modified physicochemical properties vis a vis the naphthyridinones. The calculated aqueous solubility is significantly enhanced by such modifications, especially if the carbonyl of the saturated ring is absent, that is, X,X or Y,Y = H,H.[12]

Examination of the putative binding modes of these analogs (Fig. 1), as deduced from X-ray structures of representative naphthyridinones,[13] suggests an additional benefit. Compound **16c** was subjected to a molecular dynamics simulation using AMBER.[14] The simulation revealed favorable free energies of binding compared to that for compound **1**. Compound **16c** had a low desolvation penalty and an additional H-bond between the amine NH and the Lys199 carbonyl of FabI. Similar interactions are predicted for compounds **I**, **II**, and **III**. Herein we present results on a series of seven- and eight-membered heterocyclic FabI inhibitors.[15]

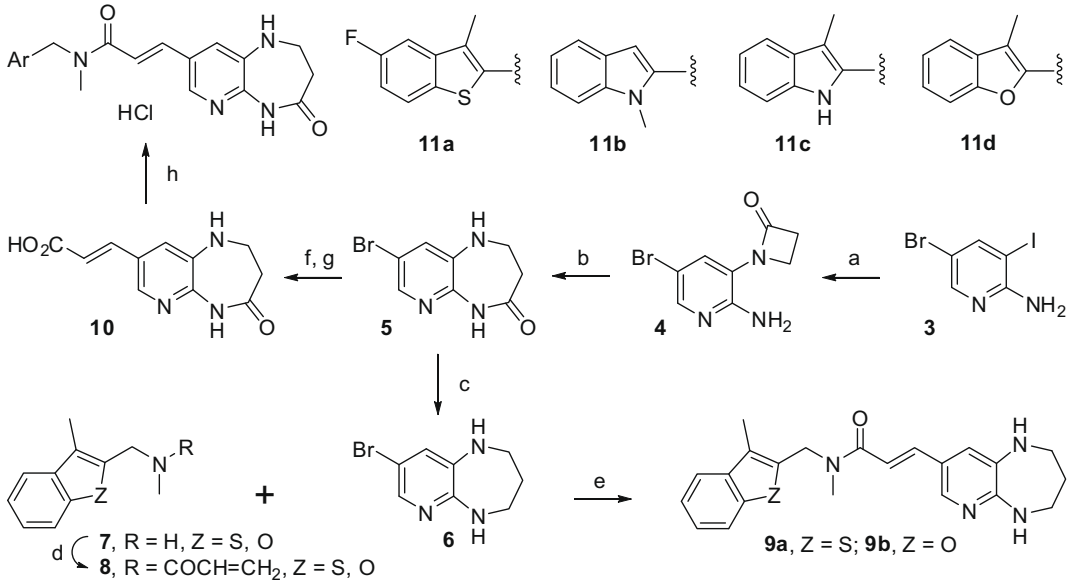
The left-hand side heterocyclic arylmethyl amines used in Schemes 1–3 were generally prepared from aldehydes, by reductive amination, as previously described.[10]

Scheme 1 details the synthesis of 1,5-diazepin-2-ones **11a–d** and 1,5-diazepines **9a**,**b**. The key step for the latter is a Heck coupling of acrylamide **8** with bromo-diazepine **6**. Diazepinone **5** was reduced to yield **6**; the acrylamide coupling partner **8** was prepared by acylation of amine **7**. Iodobromoaminopyridine **3** was selectively N-arylated with azetidinone under palladium-catalyzed conditions to furnish **4** which was subsequently converted to **5** by a ring expansion.[16] Heck coupling of **5** with *tert*-butyl acrylate, followed by cleavage gave acid **10**. The diazepinones **11a-d** were obtained by coupling **10** with N-methylated benzyl amines.

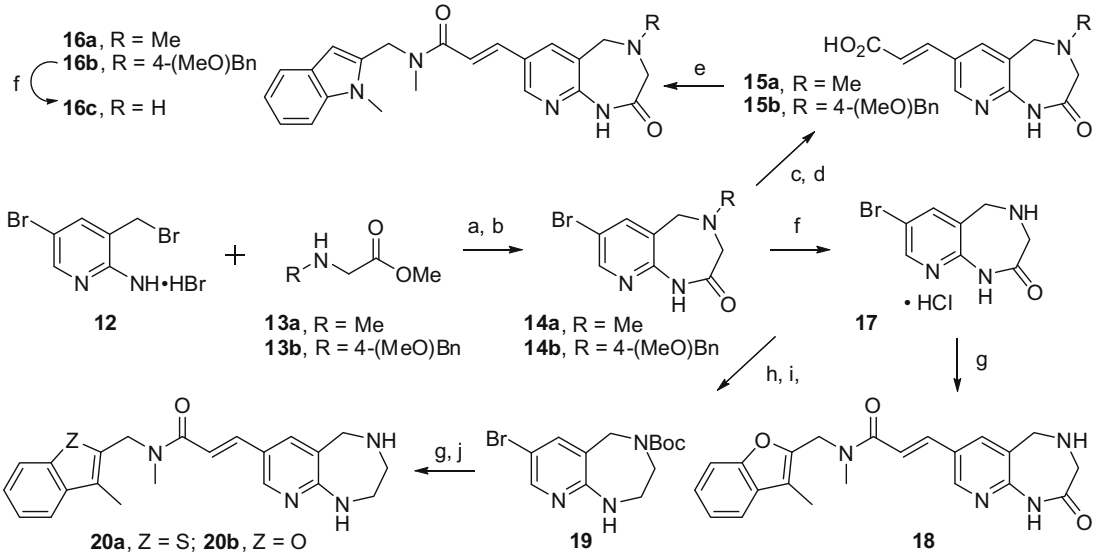
The syntheses of 1,4-diazepin-2-ones **16a**,**c**, **18** and 1,4-diazepine **20a**,**b** are shown in Scheme 2. Compound **12**[10] was reacted with substituted glycine methyl esters **13a**,**b** to give the corresponding amino esters which were cyclized with NaH in DMSO to provide the diazepinones **14a**,**b**. The ene-amides were constructed by Heck coupling. For example, **14a**,**b** was coupled with *tert*-butyl acrylate followed by deprotection and then amidation to provide **16a**,**b**. Compound **16c** was prepared by subsequent removal of the methoxybenzyl group. The preparation of **18** was similar. In this instance, the methoxybenzyl group was removed prior to the Heck reaction; bromide **17** was coupled with acrylamide **29** to give **18**. Reduction of **17** followed by Boc protection gave bromide **19** which was subjected to Heck coupling as usual to obtain the diazepine **20**.

The diazepinone **27** and diazocinone **28** were obtained according to Scheme 3. Reaction of 2-chloro-3-cyanopyridine with ethyl glycine ester gave compound **21** while palladium-catalyzed N-arylation with azetidinone gave **22**. These nitriles were reduced by hydrogenation with palladium on activated carbon to give the cyclized products in one pot. Filtration followed by bromination yielded compounds **23** and **24**. Heck coupling with *tert*-butyl acrylate, followed by *tert*-butyl cleavage with trifluoroacetic acid gave the acids **25** and **26**. The final amides were prepared by standard amide couplings. Reduction of **24** with LAH, followed by protection of the secondary amine with a Boc group gave **30**. Heck coupling with **29** and deprotection of the Boc group furnished the desired diazocine **31**.

The data for compounds prepared in this study are presented in Table 1. The 1,5 diazepinones **11a-d** were effective FabI inhibitors, however, all things being equal (**11b** vs **2**), no in vitro benefit over



Scheme 1. Reagents and conditions: (a) azetidin-2-one, $Pd_2(dba)_3$, xantphos, Cs_2CO_3, toluene, 90 °C; (b) $Ti(OiPr)_4$, toluene, 110 °C; (c) $LiAlH_4$, THF; (d) acryloyl chloride, Et_3N, THF; (e) *tert*-butyl acrylate, $Pd_2(dba)_3$, P(*t*-Bu)$_3$, (*i*-Pr)$_2$EtN, DMF, 100 °C; (f) *tert*-butyl acrylate, $Pd(OAc)_2$, P(*o*-tolyl)$_3$, (*i*-Pr)$_2$EtN, EtCN, DMF, 100 °C; (g) (i) TFA, CH_2Cl_2; (ii) 4 M HCl/dioxane; (h) $ArCH_2NHCH_3$, EDC, HOBt, DMF.



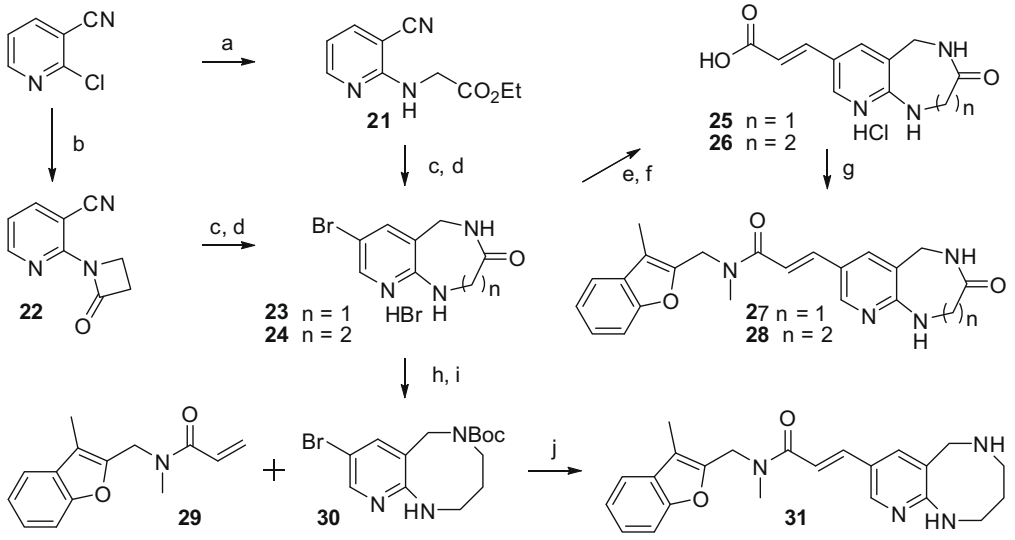
Scheme 2. Reagents and conditions: (a) Et_3N, DMF; (b) NaH, DMSO; (c) *tert*-butyl acrylate, $Pd(OAc)_2$, P(*o*-tol)$_3$, (*i*-Pr)$_2$EtN, EtCN, DMF 100 °C; (d) TFA, CH_2Cl_2; (e) $ArCH_2NHCH_3$, EDC, HOBt, DMF; (f) ACE-Cl, DCE; (g) **8** or **29**, $Pd(OAc)_2$, P(*o*-tol)$_3$, (*i*-Pr)$_2$EtN, EtCN, DMF; (h) $LiAlH_4$, THF; (i) $(Boc)_2O$, Et_3N, CH_2Cl_2; (j) (i) TFA, CH_2Cl_2; (ii) 4 M HCl/dioxane.

and above the naphthyridinones is apparent. The benzofuran analog **11d** possesses superior activity against *Staphylococcus aureus* and *E. coli* FabI. In addition, excellent antibacterial activities were obtained against wild type *S. aureus* and, more importantly, two resistant strains: MRSA and TRSA. Water solubility is somewhat enhanced but not pH sensitive.

At acidic pH the 1,4 diazepinones are more soluble than their 1,5 isomers. Qualitatively this is understood by the chemical modification involved, that is, compounds **16a**,**c** and **18** incorporate a basic amine. The enzyme assay indicates that the most potent 1,4 diazepinones (**16c**, **18**) have a free NH available for interaction with the carbonyl of Lys 199. Congener **18** showed excellent antibacterial activity. However, in head to head comparisons, the 1,5 diazepinones are more effective than the 1,4 isomers against FabI; models indicate a tighter H-bond to Lys199.

In compound **27** the carbonyl is migrated from the two to the three position of the diazepinone ring; a marginally less potent compound results. It is interesting that FabI active site can also tolerate an eight-membered ring analog. The diazocinone **28** is equipotent to its seven-membered ring analog **27**.

To further improve solubility, we prepared analogs in which the carbonyl groups were absent. As expected, solubilities of compounds **9b**, **20b**, and **31** are improved in relation to their comparators **11d**, **18**, and **28**, respectively, especially at acidic pH. Against *S. aureus* FabI, activity of **9b** and **31** has decreased significantly. This suggests that the carbonyl group might be involved in an important hydrogen bond or is affecting the hydrogen bonding capabilities of the proton on the adjacent nitrogen. It has been shown that the naphthyridine moiety of the right-hand side forms two H-bonds with a backbone alanine residue of *S. aureus* FabI and this donor–acceptor network is required for optimal potency.[13] Note, however, that against *E. coli* FabI a reduction in activity is not seen. In fact, entries **9b**, **20a**, and **31** are 5–10-fold better than that measured for *S. aureus* FabI**.** This indicates subtle differences in the binding of these inhibitors; further results to be reported separately.



Scheme 3. Reagents and conditions: (a) glycine ethyl ester, DMF, K_2CO_3, 100 °C; (b) azetidin-2-one, Pd(dba), xantphos, Cs_2CO_3, toluene, 90 °C; (c) Pd/C, H_2, HOAc; (d) Br_2; (e) *tert*-butyl acrylate, $Pd(OAc)_2$, P(*o*-tol)$_3$, (*i*-Pr)$_2$EtN, DMF, 100 °C; (f) (i) TFA, CH_2Cl_2; (ii) 4 M HCl/dioxane; (g) 3-methylbenzofuran-2-CH_2NHCH_3, EDC, HOBt, DMF; (h) $LiAlH_4$, THF; (i) $(Boc)_2O$, Et_3N, CH_2Cl_2; (j) (i) $Pd(OAc)_2$, P(*o*-tol)$_3$, (*i*-Pr)$_2$EtN, EtCN, DMF; (ii) TFA, CH_2Cl_2.

Table 1
In vitro activity and physicochemical data for compounds used in this study

Compd	FabI IC_{50}[17] (nM)		MIC[18] (μg/mL)				p*Ka*[e]	Solubility[f] (μg/mL)	
	S. aureus	*E. coli*	*S. aureus*[a]	*MRSA*[b]	*TRSA*[c]	*E. coli*[d]		pH 4.0	pH 7.4
1	22	64	0.125	⩽0.063	4	4	3.6	0.2	0.3
2	100	150	⩽0.016	0.031	0.5	⩽0.063	3.6	6.2	5.6
11a	31	25	⩽0.016	⩽0.016	0.125	1	4.9	31	38
11b	130	136	0.25	0.125	1	1	4.9	25	33
11c	200	840	0.031	0.063	2	4	4.9	39	37
11d	7	25	⩽0.016	⩽0.016	⩽0.016	⩽0.063	4.9	1.5	1.7
9a	26	14	⩽0.016	⩽0.016	⩽0.016	⩽0.063	8.4	30	1.1
9b	67	12	⩽0.016	0.031	0.5	⩽0.063	8.4	>100	29
16a	510	NT	4	0.5	8	>32	4.6	73	9.7
16c	130	330	0.125	0.125	1	1	4.8	>100	82
18	43	29	⩽0.063	⩽0.063	0.125	⩽0.063	4.8	170	20
20a	57	9	⩽0.016	0.031	2	⩽0.063	7.7	100	33
20b	48	14	0.063	0.063	4	⩽0.063	7.7	>650	440
27	60	39	⩽0.063	⩽0.063	4	⩽0.063	4.2	NT	NT
28	61	30	⩽0.016	0.031	0.5	⩽0.063	4.8	46	17
31	170	16	0.25	0.5	8	8	8.3	1400	210

The following strains were from the Affinium bacterial collection or the American Type Culture Collection (Manassas, VA): [a]wild type *S. aureus* 29213. [b]Methicillin resistant *S. aureus* 43300. [c]Triclosan resistant *S. aureus* 934335. [d]*E. coli* efflux pump mutant. [e]Advanced Chemistry Development software calculated p*Ka*. [f]Solubility measured by Shake Flask or Millipore MultiScreen Solubility Filter Plate Method. NT not tested.

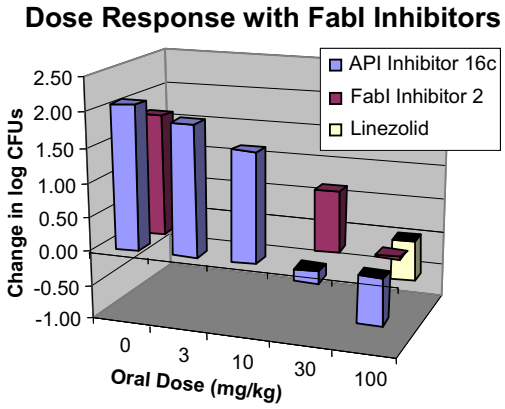


Figure 2. Neutropenic murine thigh infection model.[19]

Selected inhibitors were tested in a neutropenic mouse thigh infection model. Figure 2 shows that the diazepine **16c** was comparable to Linezolid at 100 mg/kg orally and superior to earlier members of this series namely indole **2**. This is despite the fact that indole **2** possesses superior in vitro numbers, which underscores the importance of ancillary properties in the optimization of in vivo efficacy.

In conclusion, we described a series of seven- and eight-membered heterocyclic inhibitors of *S. aureus* and *E. coli* FabI. These compounds are potent enzyme inhibitors with excellent antibacterial activity. These inhibitors were designed to exhibit modified physiochemical properties; improved efficacy results. These data, underscored by the in vivo efficacy, tend to support FabI as a target for antibacterial therapy.

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