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4-Quinolones as Noncovalent Inhibitors of High Molecular Mass Penicillin-Binding Proteins

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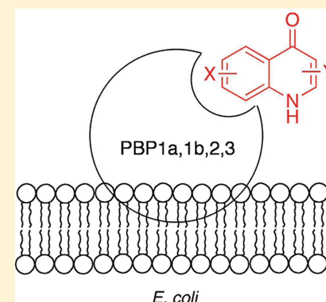
Supporting Information

ABSTRACT: Penicillin-binding proteins (PBPs) are important bacterial enzymes that carry out the final steps of bacterial cell wall assembly. Their DD-transpeptidase activity accomplishes the essential peptide cross-linking step of the cell wall. To date, all attempts to discover effective inhibitors of PBPs, apart from β-lactams, have not led to new antibiotics. Therefore, the need for new classes of efficient inhibitors of these enzymes remains. Guided by a computational fragment-based docking procedure, carried out on *Escherichia coli* PBP5, we have designed and synthesized a series of 4-quinolones as potential inhibitors of PBPs. We describe their binding to the PBPs of *E. coli* and *Bacillus subtilis*. Notably, these compounds bind quite tightly to the essential high molecular mass PBPs.



KEYWORDS: *Penicillin-binding protein, DD-peptidase, noncovalent inhibitor, 4-quinolone*

Bacterial resistance to β-lactam antibiotics continues to rise.[1−4] The targets of these antibiotics are the penicillin-binding proteins (PBPs) or DD-peptidases, which catalyze the final peptide cross-linking steps in peptidoglycan synthesis.[5,6] There are a number of variants of these enzymes, which appear to possess somewhat different activities, including transpeptidase, carboxypeptidase, and endopeptidase.[7,8]

Beyond β-lactams, few effective DD-peptidase inhibitors have been discovered, and, of these, none have been developed into new antibiotics. Direct substrate (and their derived transition states) analogues have not proved successful.[8−10] Thus we, and others,[11−14] following a variety of approaches, have sought new leads. We describe here some results of a computational, fragment-based approach. We were particularly interested in exploring small molecules that interacted with the conserved, polar residues of the DD-peptidase active site.[5,6] A platform that strongly interacted with those residues could then be expanded for particular applications. We initially focused especially on neutral ligands since the thermodynamics of ion binding are complicated.

An initial lead that interested us was the 4-quinolone **1**, composed from fragments and docked[15,16] into the active site of *Escherichia coli* PBP5 [this enzyme was chosen because a crystal structure is available[17] and substrates are available for direct assay in vitro[8]], as shown in Figure 1. We were impressed by the spanning of the active site by a 4-quinolone and envisioned the prospect of further interactions with oxyalkyl substituents. We therefore obtained the 4-quinolones **2−10** and assessed their potential as ligands of *E. coli* and *Bacillus subtilis* PBPs. Strikingly, we found that they bind quite tightly to the essential high molecular mass PBPs such as *E. coli* PBP **1a**/**1b**, **2**, and **3**.

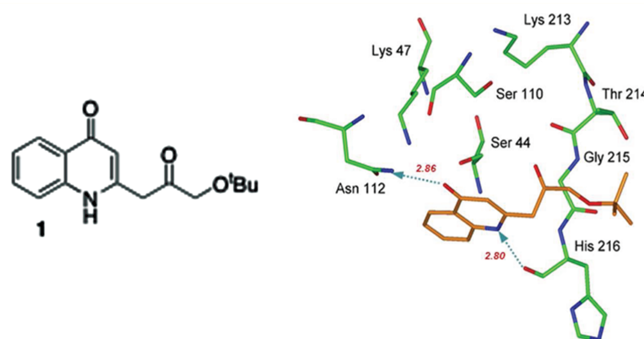


Figure 1. Computational docking of **1** into the *E. coli* PBP5 active site.

The 4-quinolones were either purchased (**2** and **3**) or synthesized (Scheme 1). The classical Conrad−Limpach cyclization of an aniline and β-ketoester is a standard synthetic route for the preparation of 4-quinolones[18] and seemed appropriate for our purpose. To synthesize 4-quinolones **4−8** with side chain functionality at C-2 and C-3, we developed shorter and more efficient syntheses than those previously reported (synthetic details may be found in the Supporting Information).

Following the Conrad−Limpach reaction, equimolar quantities of aniline and β-ketoesters **11b**,**c** were reacted in a desiccator over concentrated sulfuric acid to yield corresponding Schiff bases **12b**,**c**. Thermal cyclization of **12b**,**c** in DowTherm A resulted in the construction of quinolone-2-

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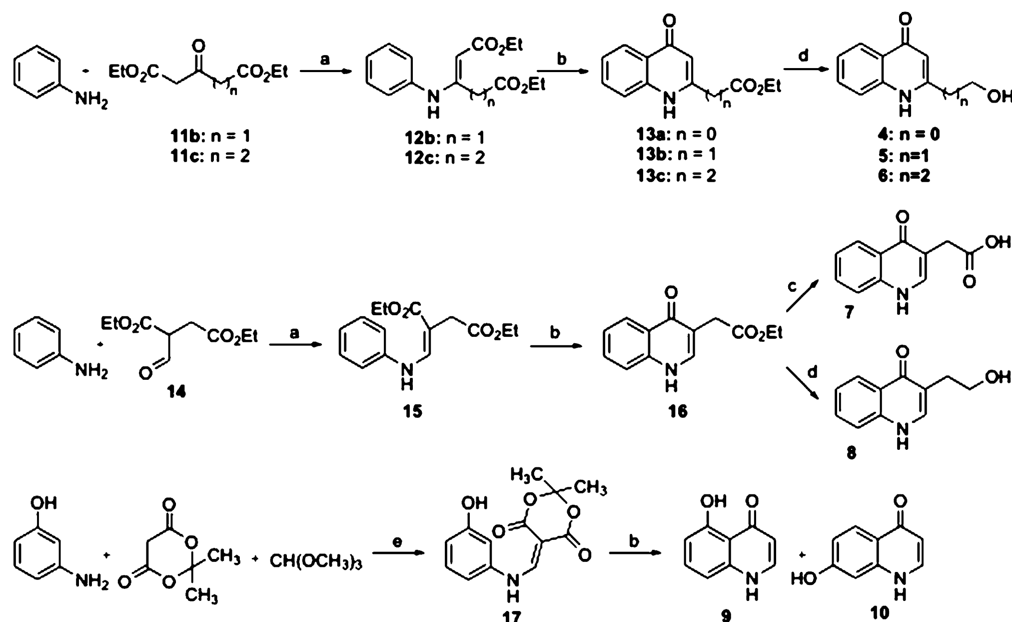
Scheme 1. Synthesis of 4-Quinolones[a]



[a]Reagents and conditions: (a) desiccator over H_2SO_4 (conc), rt, 2 days; (b) DowTherm A, 250 °C, 20 min; (c) NaOH (2 N), rt, 15 h; (d) $BH_3{\cdot}SMe_2$, THF, rt, 15 h; (e) MeCN, reflux, 2 h.

carboxylic acid ethyl esters **13b,c**. Reduction of esters **13a–c** by the borane-dimethyl sulfide ($BH_3{\cdot}SMe_2$) complex provided the corresponding alcohols **4–6** with varied length side chains.

By a similar procedure to that employed to obtain **4–6**, treatment of aniline with ethyl-α-formyl succinate (**14**) led to the synthesis of quinolone-3-carboxylic acid ethyl ester **16**. This was followed by hydrolysis of **16** to acid **7** or reduction with the borane–dimethyl sulfide complex to alcohol **8**.

To access the hydroxy-4-quinolones **9** and **10**, Meldrum's acid and 3-hydroxyaniline were subjected to a condensation reaction in the presence of trimethyl orthoformate to form the corresponding arylaminomethylene derivative **17**. Thermal ring closure of the Meldrum's acid derivative afforded a mixture of 5- and 7-hydroxy-4-quinolone analogues in low yield. The challenging separation of the isomers was performed by repetitive RP-HPLC on a C-18 analytical column. All products were characterized as described in the Supporting Information and were at least 95% pure by NMR estimation.

The dissociation constants (K_i) of these molecules from their PBP complexes were measured by their competition with the irreversible reaction of the fluorescent penicillin Bocillin FL with membrane-bound PBPs of *E. coli* and *B. subtilis,* as described previously.[10] Some typical experimental data is shown in Figure 2. The resulting K_i values from the *E. coli* measurements are presented as Table 1.

The results show that the parent molecule, 4-quinolone (**2**), at concentrations up to 1 mM, does not bind measurably to any *E. coli* PBP. The derivatives **3–10**, however, do bind to the various PBPs to varying degrees. All compounds bound most tightly to PBP4, which is a nonessential low molecular mass class C PBP with a distinctive substrate-binding site.[8,19] The weakest binding was to the low molecular mass class A PBPs **5** and **6**. Notably, after PBP4, the tightest binding is to the high molecular mass PBPs **1a**/**1b**, **2**, and **3**. The latter enzymes in bacteria are generally essential to bacterial survival and are the β-lactam antibiotic targets. Of the various 4-quinolones tested, **4** was generally the tightest binding, with K_i values of around 30

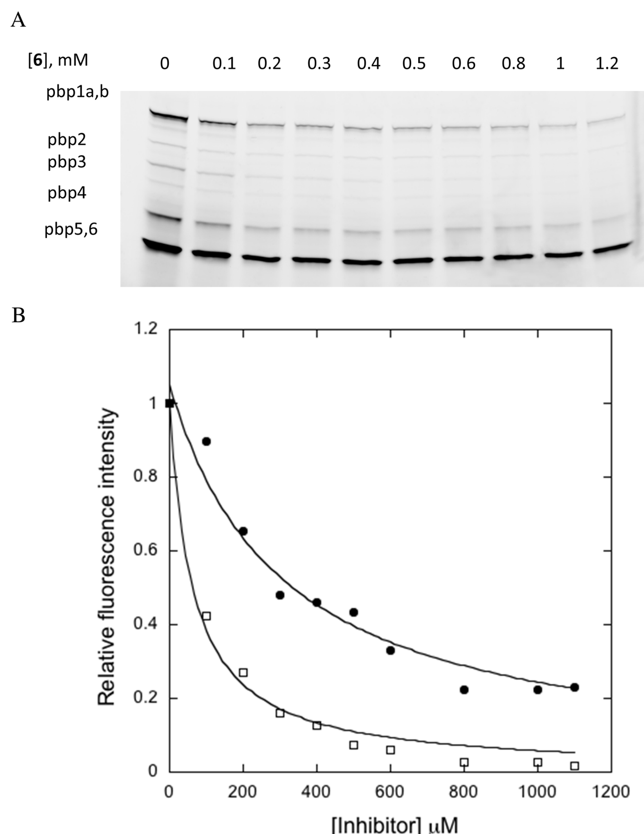


Figure 2. (A) Extent of fluorescence labeling of *E. coli* PBPs by Bocillin FL (20 μM) in the presence of various concentrations of the 4-quinolone **6**. (B) Extent of Bocillin FL (20 μM) labeling of membrane-bound *E. coli* PBP2 as a function of the concentrations of **6** (□) and **7** (●). In each case, the ordinate is normalized to the observed initial intensity. Other details of these experiments are found in the Supporting Information.

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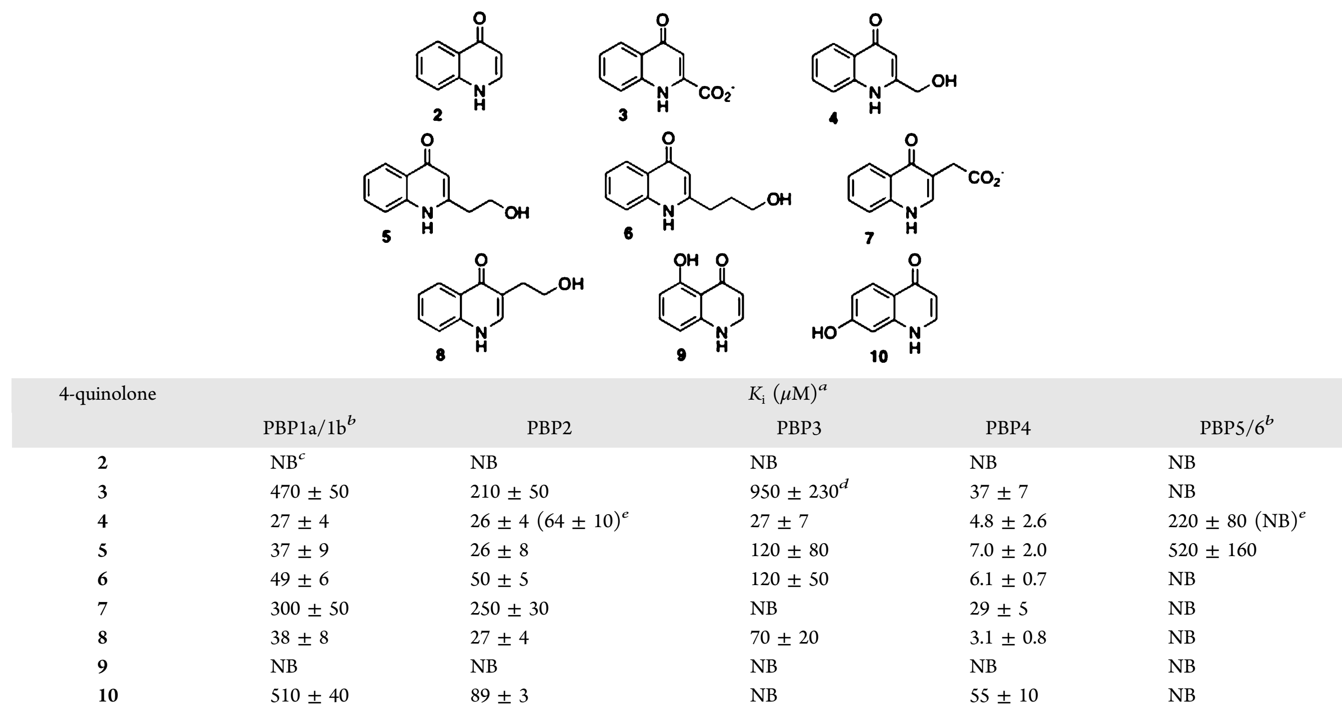
Table 1. Dissociation Constants of 4-Quinolones from *E. coli* PBPs in Membrane Preparations



4-quinolone	K_i (μM)[a]				
	PBP1a/1b[b]	PBP2	PBP3	PBP4	PBP5/6[b]
2	NB[c]	NB	NB	NB	NB
3	470 ± 50	210 ± 50	950 ± 230[d]	37 ± 7	NB
4	27 ± 4	26 ± 4 (64 ± 10)[e]	27 ± 7	4.8 ± 2.6	220 ± 80 (NB)[e]
5	37 ± 9	26 ± 8	120 ± 80	7.0 ± 2.0	520 ± 160
6	49 ± 6	50 ± 5	120 ± 50	6.1 ± 0.7	NB
7	300 ± 50	250 ± 30	NB	29 ± 5	NB
8	38 ± 8	27 ± 4	70 ± 20	3.1 ± 0.8	NB
9	NB	NB	NB	NB	NB
10	510 ± 40	89 ± 3	NB	55 ± 10	NB

[a]The uncertainties quoted are from fitting the data to eq 1. Within the limits cited, these values could be reproduced in separate experiments. [b]These were not resolved on the gel. [c]NB: no binding observed at 1 mM ligand. [d]The large uncertainties of the K_i values of PBP3 reflect the faintness of this band on the gel (see Figure 1). [e]Experiments carried out with purified enzymes in solution.

μM for each of the high molecular mass enzymes. The position of the hydroxyl group in **4** seems moderately important, as judged by the generally lesser effectiveness of **5**, **6**, and **8**. All of the alkanols are, however, much more effective than the parent quinolone **2**. The carboxylates, **3** and **7**, are distinctly less potent, presumably reflecting the cost of dehydration and/or inserting a charge into a more hydrophobic medium than bulk water. Aromatic ring hydroxylation (**9** and **10**) gave only modest improvement over the case of **2**.

The association of **4** with two purified enzymes, *E. coli* PBP2 and PBP5, in solution was also determined. As reported in Table 1, binding by the membrane-associated enzymes appears somewhat stronger. This could arise from differences in the structure/dynamics of the membrane-associated holoenzymes from those of the truncated solubilized enzymes in solution, or perhaps from sequestration of the neutral **4** to the membrane.

That the 4-quinolones **3**−**10** should bind to PBPs other than the PBP5 template is not unexpected because the active site components to which the quinolones were predicted to bind (Figure 1) are conserved in all of the PBPs and, for catalysis, are arranged in a very similar fashion. It seems likely that the additional polar functionality of **3**−**10**, not present in **2**, interacts favorably with some elements of the diverse set of active site functional groups shown in Figure 1.

A further notable result was that **3**−**10** did not have antibiotic activity against *E. coli* (MIC > 1 mM). The direct conclusion is that they are unable to reach their target enzymes in vivo. This conclusion was confirmed by experiments to directly measure the binding of **3**−**10** to the target PBPs in vivo; none was observed. This may suggest that the outer membrane of *E. coli* blocked their access. This explanation is made less likely, however, by the additional observation that, although **3**−**10** also bind to *B. subtilis* high molecular mass PBPs in membranes (Supporting Information, Figure S1), they do not have any antibacterial activity against the Gram-positive *B. subtilis*, which lacks an outer membrane.

This research has shown that small suitably functionalized 4-quinolones are quite effective inhibitors of the high molecular mass PBPs of *E. coli* and *B. subtilis*. Although the present molecules appear to have no antimicrobial activity themselves, it is possible that more elaborated derivatives may have such activity as well as stronger affinity for the PBPs. The search for such compounds is in progress. Structural studies would also be informative.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Detailed experimental procedures—organic syntheses, characterization of products, bacteria and membrane preparation, and determination of binding constants—and a figure demonstrating the reaction of 4-quinolones with *B. subtilis* PBPs. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

ABBREVIATIONS

MIC, minimum inhibitory concentration; PBP, penicillin-binding protein; RP-HPLC, reverse phase high pressure liquid chromatography; THF, tetrahydrofuran

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