Bioorganic & Medicinal Chemistry Letters 25 (2015) 3468–3475

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Synthesis of ciprofloxacin dimers for evaluation of bacterial permeability in atypical chemical space

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ARTICLE INFO

Article history:
Received 18 May 2015
Revised 30 June 2015
Accepted 2 July 2015
Available online 9 July 2015

Keywords:
Chemical space
Middle space
Bacterial permeability
Fluoroquinolone
DNA gyrase
Antibacterial
Antibiotic
Dimer

ABSTRACT

We describe the synthesis and evaluation of a library of variably-linked ciprofloxacin dimers. These structures unify and expand on the use of fluoroquinolones as probes throughout the antibiotic literature. A dimeric analog (**19**) showed enhanced inhibition of its intracellular target (DNA gyrase), and translation to antibacterial activity in whole cells was demonstrated. Overall, cell permeation was governed by physicochemical properties and bacterial type. A principal component analysis demonstrated that the dimers occupy a unique and privileged region of chemical space most similar to the macrolide class of antibiotics.

The golden age of antibiotics ushered in such a sense of security that the field was considered mature by the 1980s.[1] More than two hundred antibacterial drugs have been developed since the advent of penicillin. However, the majority are later-generation variants of only twelve chemotypes, with few novel classes to counter the evolutionary selection for bacterial resistance.[2] Unsuccessful discovery research, compounded by emerging resistance, is together referred to as the antibiotic crisis.[3–6] After a period of deprioritization of antibiotic discovery research, the reentry of some drug makers has been accompanied by renewed research approaches.[7] For example, there is growing awareness that cellular permeation of bacteria can be neither well-predicted nor neglected.[8–11]

Our interest in this area emanated from a desire to explore permeation of synthetic compounds in the 'middle space' between drug-like small molecules, and biologics.[12] While this chemical space is known to have physicochemical challenges,[13] the impact of such properties on bacterial cell permeability is poorly understood.[14]

To design probes for this purpose, we planned a library of variably-linked dimers of ciprofloxacin (Fig. 1). This prototypical fluoroquinolone has previously been used as a building block in homodimeric and heterodimeric antibiotic probes, but with limited biological data and antibiotic rationale.[15,16] Ciprofloxacin was first chemically modified resulting in three derivatized monomers to which was appended various linkers. Linkers and their placement at the basic piperazine were guided by reports of monomeric structure-activity relationships.[17] Supporting the design strategy was a crystal structure-based computational model for how dimers might interact with the target DNA gyrase complex,[18] as well as biochemical tracking of target inhibition to inform cellular assays. As proposed a decade ago, the possibility of bisintercalation into two fluoroquinolone binding sites was also considered.[19]

Library components included three ciprofloxacin-derivatized monomers and four modular linkers (e.g., aryl, amine, peptide, and PEG), which were combined to give a dimer library with which to study the impact of dimerization, linker functionality, and overall physicochemical properties in biochemical and cellular assays.

Building blocks **1**–**6** were prepared as shown in Scheme 1. Peptide coupling, followed by benzyl esterification and amine deprotection afforded amine-functionalized monomers **2** and **3**. Alternatively, acid-based monomers were prepared via halogen displacement, affording **5** and **6**. Thus, the synthesis of a diverse library of dimers linked directly at the basic piperazine amine (e.g., **4**), or at an added linker group (**2**, **3**, **5**, **6**) was enabled.

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http://dx.doi.org/10.1016/j.bmcl.2015.07.010
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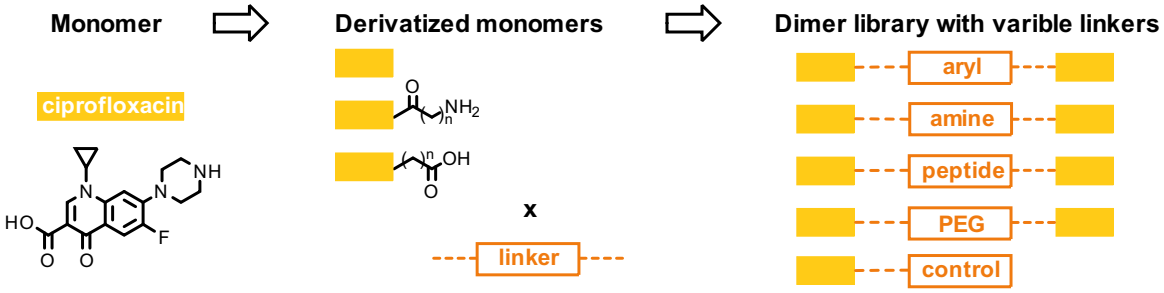
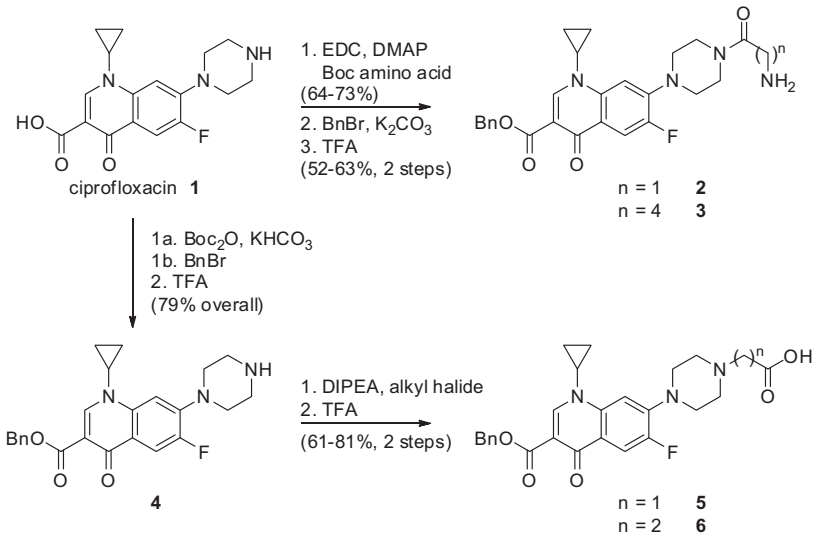
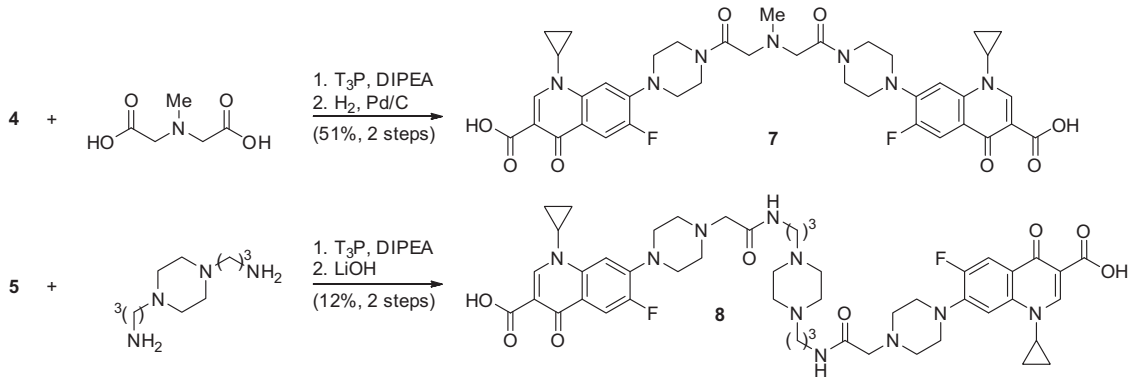


Figure 1. Design of fluoroquinolone dimer library.



Scheme 1. Synthesis of building blocks (**2**–**6**).



Scheme 2. Synthesis of amine-linked dimers (**7** and **8**).

Dimers containing basic amines were next prepared (**7**–**8**, Scheme 2). We rationalized that these basic moieties may productively associate with DNA in or near the target complex and influence physicochemical properties (e.g., aq solubility).

Peptide-linked dimers were prepared from amide coupling reactions (**9**–**11**, Scheme 3). Polyethylene glycol (PEG)-based linker components afforded dimers with acceptable chemical stability and solubility.

Direct PEG-based linkers were also explored. Although not anticipated to improve binding near anionic DNA, a report by Pinter and co-workers suggested that fluoroquinolone analogs bearing longer PEG chains (n_{ave} = 24–30) were able to enter Gram-negative bacteria cells without concomitant membrane disruption.[20] We thus prepared one PEG dimer with a carbamate hinge (**14**, Scheme 4), and a series with alkyl hinges made from displacement reactions (**12**, **13**, and **15**–**16**, Scheme 4).

Building upon related work from Kerns and colleagues,[19,21–24] we prepared their most potent dimer as a control (**17**, Scheme 5). Analysis of ciprofloxacin analogs indicated that while benzylation is moderately tolerated,[25] aryl acylation[26,27] is well-tolerated.