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Structural Features of Piperazinyl-Linked Ciprofloxacin Dimers Required for Activity Against Drug-Resistant Strains of *Staphylococcus aureus*

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Abstract—We previously demonstrated that piperazinyl-linked fluoroquinolone dimers possess potent antibacterial activity against drug-resistant strains of *Staphylococcus aureus*. In this study, we report the preparation and evaluation of a series of incomplete dimers toward ascertaining structural features of piperazinyl-linked ciprofloxacin dimers that render these agents refractory to fluoroquinolone-resistance mechanisms in *Staphylococcus aureus*.

Bacterial resistance to the fluoroquinolone antibiotics is seriously challenging the clinical application of this class of antimicrobial agents.[1] We recently reported the discovery of novel piperazinyl-linked fluoroquinolone dimers that posses potent antibacterial activity against drug resistant strains of *Staphylococcus aureus* (*S. aureus*), including fluoroquinolone resistant strains possessing NorA efflux-mediated and topoisomerase IV substitution-mediated resistance mechanisms (Fig. 1).[2] Susceptibility testing demonstrated that both the fluoroquinolone monomers and linker comprising the dimer impact antibacterial potency. For example, the linker affording the most active symmetric dimer of one fluoroquinolone was not necessarily the optimal linker for a symmetric dimer of a different fluoroquinolone. Furthermore, antibacterial activities of the dimers do not correlate with the inherent activity of the parent fluoroquinolone monomers used to form the dimers.

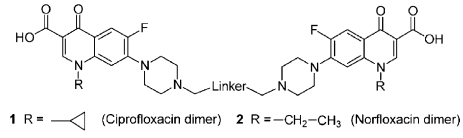

Figure 1. Piperazinyl-linked fluoroquinolone dimers possessing potent activity against drug-resistant strains of *S. aureus*.

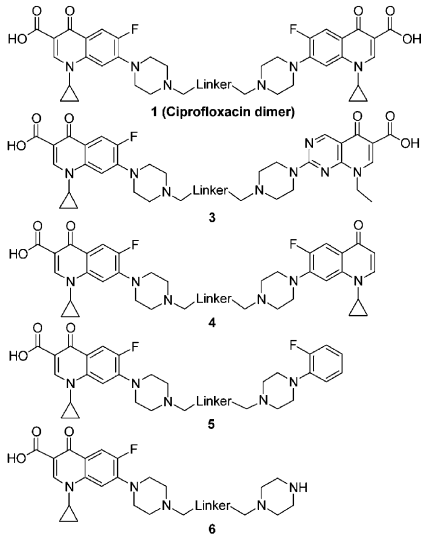

Figure 2. Comparison of a piperazinyl-linked ciprofloxacin dimer and incomplete dimers having varied structural features comprising one half of the dimer.

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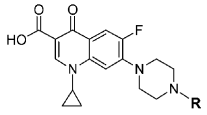
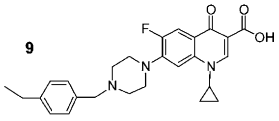
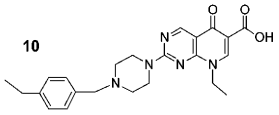
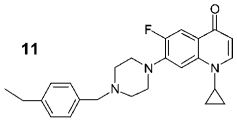
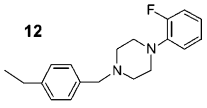
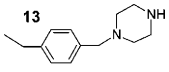
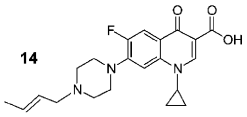
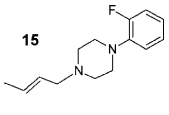
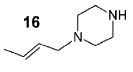
To begin determining the core structural features of the fluoroquinolone dimers responsible for potent activity against drug-resistant strains of *S. aureus*, we envisioned preparing a series of partial or 'incomplete' dimers where one half of the dimer consists of various components of the fluoroquinolone structure (Fig. 2). To this end, synthesis of the desired piperazinyl-linked ciprofloxacin derivatives was achieved using a two-step strategy (Scheme 1). First, reactive intermediates **7** and **8** were prepared by alkylation of the terminal piperazine nitrogen of ciprofloxacin with 1,4-bis-chloromethylbenzene and *trans*-1,4-dichloro-2-butene, respectively (Scheme 1).[3] Intermediates **7** and **8** were then coupled to piperazine and a series of mono-*N*-substituted piperazine derivatives to afford the desired *p*-xylenyl-linked and 1,4-*trans*-2-butenyl-linked ciprofloxacin analogues (**11**–**13**, **15**, **16**, Table 1).[4] Minimum inhibitory concentrations (MICs) of these derivatives were then determined against a panel of drug-resistant strains of *S. aureus* (Table 1).[5]

The importance of the carboxyl group for potent activity of the dimers against drug-resistant *S. aureus* is exemplified by comparison of the symmetric *p*-xylenyl-linked ciprofloxacin dimer **9** and analogues **10** and **11**, which contain the virtually inactive pipemidic acid and

Table 1. Antibacterial activity of fluoroquinolone dimers and incomplete dimers against drug-resistant strains of *S. aureus*[5]

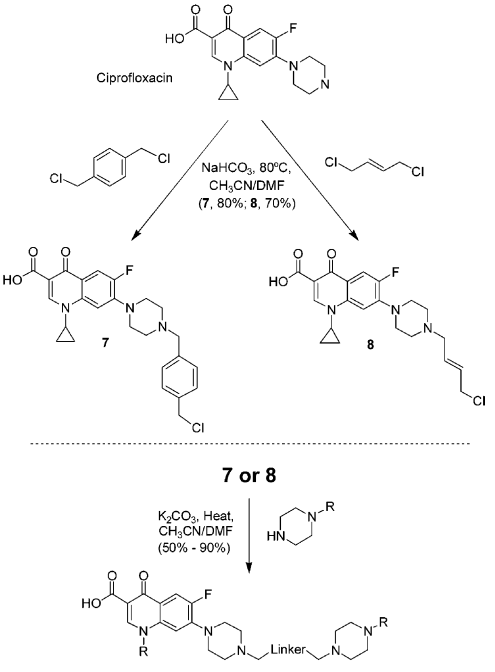


Compd[4]	Strains of *S. aureus*[a]				
	SA 1199	SA 1199-3	SA 1199B	MRSA 494	GISA 992
Ciprofloxacin monomer	0.125	1	8	0.5	32
Pipemidic acid monomer	>16	>16	>16	>16	>16
Descarboxy ciprofloxacin monomer	>16	>16	>16	>16	>16
R =					
9 	0.125	0.125	0.125	0.125	4
10 	<0.03	<0.03	<0.03	0.125	2
11 	0.25	0.5	0.5	1	>16
12 	0.5	0.5	0.5	0.5	16
13 	0.5	0.5	1	0.25	>16
14 	0.03	0.06	0.03	0.125	>16
15 	0.5	0.5	0.5	0.5	>16
16 	1	2	2	2	>16

[a]SA-1199, wild-type isolate; SA 1199-3, laboratory-derived mutant of SA 1199 that inducibly overexpresses *norA*, no gyrase or topoisomerase IV mutations; SA 1199B, constitutively overexpresses *norA* and harbors a topoisomerase IV A subunit substitution (A116E); MRSA 494, methicillin-resistant SA isolate; GISA 992, vancomycin-insensitive SA.

descarboxy ciprofloxacin monomers, respectively (Table 1). Compound **10**, which contains pipemidic acid in place of one ciprofloxacin in the dimer, is more potent or equipotent than ciprofloxacin dimer **9** against all strains of drug-resistant *S. aureus* tested. In contrast, incomplete dimer **11**, which contains descarboxy ciprofloxacin in place of one ciprofloxacin,[6] is less active than **9** against all strains. To our surprise, although the descarboxy ciprofloxacin dimer **11** is generally less active than parent dimer **9**, **11** does not have increased MICs against SA 1199-3 or SA 1199B.

These strains possess varied levels of FQ resistance where SA 1199-3 is a laboratory-derived mutant of SA 1199 that inducibly overexpresses the *norA* encoded efflux pump and has no DNA gyrase or topoisomerase IV mutations, and SA 1199B is a derivate of SA 1199 that constitutively overexpresses *norA* and harbors a topoisomerase IV A subunit substitution (A116E) known to correlate with raised FQ MICs.[7] Therefore, while the increased potency of dimers **9**, **10** and **14** over ciprofloxacin against SA 1199-3 and SA 1199B requires both carboxyl groups, this is not a requirement to simply maintain activity against the fluoroquinolone resistant strains. This observation is more remarkable with incomplete dimer analogues **12**–**13** and **15**–**16**, where only piperazine or fluorophenyl piperazine are linked to ciprofloxacin. In general, the inherent antibacterial activity of these analogues is lower than that of the full dimers (e.g., **9** and **14**) and even ciprofloxacin itself, however, this inherent level of activity against SA 1199 is maintained against SA 1199-3 and SA 1199B, indicating these simplified analogues are still refractory to the efflux-mediated and mutation-mediated resistance mechanisms (Table 1).[8,9]



Scheme 1. Synthetic approach employed for the synthesis of piperazinyl-linked ciprofloxacin analogues described in Table 1.[3,4]

In summary, we have shown that potent activity of piperazinyl-linked ciprofloxacin dimers against drug-resistant strains of *S. aureus* requires the presence of a carboxyl group on both halves of the dimer structure. These results indicate that each half of the dimer might participate in equivalent-type interactions at the homodimeric topoisomerase interface; however, unique or non-equivalent interactions with the topoisomerase, the DNA, or the putative topoisomerase–DNA complex are possible. The observation that all piperazinyl-linked dimers and incomplete dimers maintain their level of activity against SA 1199 across strains SA 1199-3 and SA 1199B indicates that piperazine substitution renders these agents refractory to the NorA efflux pump and the topoisomerase IV point mutation present in SA 1199B.[9]

References and Notes

1. (a) Hooper, D. C. *Biochem. Biophys. Acta* **1998**, *1400*, 45. (b) Gootz, T.; Brighty, K. *Med. Res. Rev.* **1996**, *16*, 433. (c) Hooper, D. C. *Emerg. Infect. Dis.* **2001**, *7*, 337. (d) Acar, J. *Clin. Inf. Dis.* **1997**, *24*, S67.
2. Kerns, R. J.; Rybak, M. J.; Kaatz, G. W.; Vaka, F.; Cha, R.; Grucz, R. G.; Diwadkar, V. U.; Ward, T. D. *Bioorg. Med. Chem. Lett* **2003**, *13*, 1745.
3. Synthesis of **7** and **8** was achieved by heating ciprofloxacin with 5 equivalents of bis-alkyl chloride in DMF/acetonitrile at 80 °C for 12–24 h in the presence of powdered $NaHCO_3$. The product was precipitated from the cooled reaction mixture with excess diethyl ether, centrifuged, decanted and the white solid separated by flash silica gel chromatography (20:1; CH_2Cl_2:CH_3OH). Products were characterized by ^1H NMR and mass spectroscopic analysis.
4. Incomplete dimers **11**–**13**, **15**, **16** were prepared by heating **7** or **8** with the corresponding piperazine derivatives in acetonitrile at 60–85 °C for 12 to 48 h. When complete by TLC, the reaction mixture was cooled, filtered, precipitated with excess diethyl ether, centrifuged and the recovered white solid dried. Purification was achieved using semi-preparative reversed-phase (C-18) HPLC employing a linear gradient of acetonitrile in water (0.1% trifluoroacetic acid), affording separation of the desired products as a single peak. Acetonitrile was evaporated and the remaining aqueous samples lyophilized providing products in good to excellent isolated yield (50–90%). Products were characterized by ^1H NMR, mass spectroscopic analysis, and analytical HPLC, showing a single peak at $\lambda = 220$ nm and $\lambda = 254$ nm. Dimers **9**, **10** and **14** were prepared as previously reported.[2]
5. MICs were determined by broth microdilution following NCCLS guidelines using 2-fold dilutions of test compound. Error for MICs is therefore ± one dilution.
6. Decarboxylated ciprofloxacin was prepared as previously reported and the structure confirmed by ^1H NMR and mass spectroscopic analysis; Reuman, M.; Eissenstat, M. A.; Weaver, J. D., III *Tetrahedron Lett*. **1994**, *35*, 8303.
7. Ng, E.; Trucksis, M.; Hooper, D. *Antimicrob. Agents Chemother*. **1996**, *40*, 1881.
8. We have previously shown that symmetric fluoroquinolone dimers do not interfere with NorA-mediated efflux of ethidium bromide in SA 1199-B at concentrations up to five times the MIC and are not competitive substrates for this pump. Therefore it is likely that the incomplete dimers here similarly are not substrates for the NorA pump, accounting for their ability to evade this efflux mechanism.
9. (a) The primary target of ciprofloxacin in *S. aureus* is topoisomerase IV. One explanation for the observed activity

against SA 1199B is that the primary target of the dimers has switched from topoisomerase IV to DNA gyrase. Investigations are underway to determine target selectivity of the piperazinyl-linked fluoroquinolone dimers. (b) Previous reports of other fluoroquinolone analogues having varied substitutents at the C-7 position have shown that hydrophobic or bulky substituents at this position often result in analogues that are not substrates for the NorA efflux pump system. Piddock, L. J. V.; Zhu, M. *Antimicrob. Agents Chemother.* **1991**, *35*, 2423. Takenouchi, T.; Tabata, F.; Iwata, Y.; Hanzawa, H.; Sugawara, M.; Ohya, S. *Antimicrob. Agents Chemother.* **1996**, *40*, 1835.