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Bioorganic & Medicinal Chemistry Letters 13 (2003) 1635–1638

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Antibacterial Activity of Quinolone–Macrocycle Conjugates

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Received 3 December 2002; revised 12 March 2003; accepted 19 March 2003

Abstract—Novel quinolone–macrocycle conjugates displayed submicromolar antibacterial activity against *Escherichia coli* and *Staphylococcus aureus* bacterial strains. An analogous open-chain structure was not active at 100 μM against the same pathogenic strains.

We previously reported the low micromolar in vitro bacterial translation inhibition of a series of 14-membered macrocycles.[1,2] In this paper, we describe the antibacterial activity of another class of 14-membered macrocycles derived from a mixture-based combinatorial library. From high-throughput screening we identified three mixtures displaying weak antibacterial activity in our whole cell *Escherichia coli* assay (Fig. 1).

A common structural feature of the macrocycles is the quinolone functionality appended to the endocyclic aryl group. Identical macrocycles missing the quinolone functionality (aryl amine analogues) lacked antibacterial activity against *E. coli* and *Staphylococcus aureus* (>100 μM).[2] The quinolone was derived from the incorporation of nalidixic acid in the last step of the solid-phase synthesis (Scheme 1). Since 2% of the total macrocycles with the nalidixoyl functionality were active, the antibacterial activity is highly dependent upon the side chain substitution pattern. The active macrocycles possess one positively charged side chain (TFA salts) and at least one aryl side chain functionality. Intrigued by the antibacterial activity of these quinolone–macrocycle conjugates, we initiated a follow-up study.

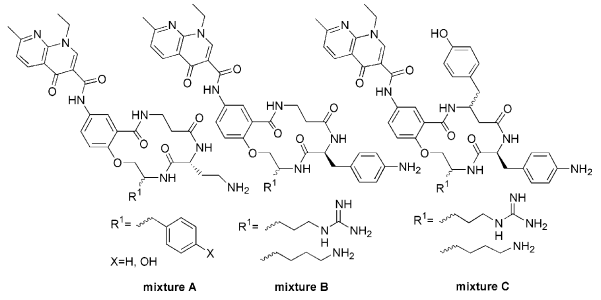
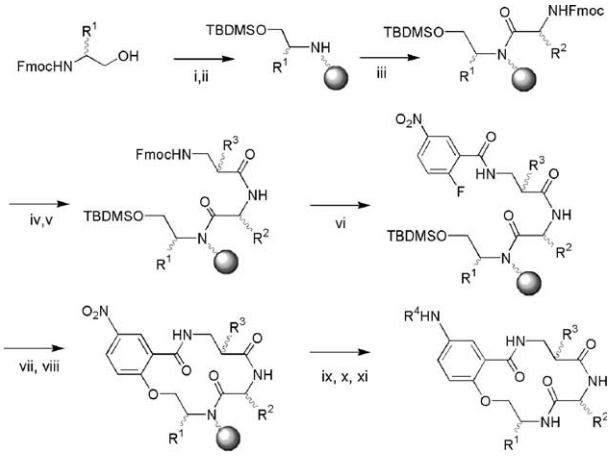


Figure 1. Macrocyclic mixtures A, B and C with weak antibacterial activity against *E. coli* (MIC = 50–100 μM).



Scheme 1. Reagents and conditions: (i) 1.3 equiv Fmoc-NHCH(R^1)CH_2OH, 2.0 equiv NaOH, MeOH, 4 h; 2.2 equiv AcOH, 30 min; 1.0 equiv ArgoGel™-MB-CHO, $CH(OMe)_3$, 24 h; 2 equiv BH_3pyridine, 2 equiv AcOH, 24 h; (ii) 3.0 equiv TBDMS-Cl, 3.0 equiv TEA, 0.1 equiv DMAP, DCM, 24 h; (iii) 0.2 M Fmoc-α-amino acid, 0.2 M DIC, DMF, 24 h; (iv) 20% piperidine, DMF; (v) 0.12 M Fmoc-$NHCH_2CHR^3CO_2H$, 0.12 M HATU, 0.25 M collidine, DMF, 24 h; (vi) 0.12 M 2-fluoro-5-nitrobenzoic acid, 0.12 M HATU, 0.25 M collidine, DMF/DCM (1/1; v/v), 24 h; (vii) 0.2 M TREAT-HF, THF, 24 h; (viii) 0.2 M DBU, DMF; (ix) 1.5 M $SnCl_2$, DMF/EtOH (10:1); (x) 0.12 M carboxylic acid (R^4CO_2H), 0.12 M HATU, 0.25 M collidine, DMF, 24 h; (xi) TFA/TIS (95:5).

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0960-894X/03/$ - see front matter
doi:10.1016/S0960-894X(03)00285-3

There is much interest in exploring new classes of quinolone antibacterial drugs (Fig. 2) because of the alarming growth of pathogenic bacterial drug resistance.[3–5] Quinolones target DNA gyrase in gram negative organisms, such as *E. coli* and target topoisomerase IV in gram positive organisms, such as *S. aureus*.[4] Both of these enzymes are essential for bacterial DNA replication.

Structure–activity relationship (SAR) studies on quinolones show the 3-carboxylic acid group to be crucial for antibacterial activity, making C-3 modifications generally not tolerated.[6] However, there are some exceptions to this rule. Obvious exceptions are the ester prodrug analogues which are converted in vivo back to the acids.[7] Interestingly, ciprofloxacin derivative **4** (Fig. 3) was reported to have antibacterial activity and the authors suggested the planarity between the 4-oxo group and the 3-carboxylic group must be important for DNA gyrase binding.[8] However, there was no activity in another C-3 derivatized planar quinoline system (**5**), suggesting that the structure–activity relationship is more complex.[9]

The macrocycles described in this paper represent novel C-3 modified quinolone conjugates. A preliminary SAR study was carried out, based upon the macrocycles from one MIC-active library well.

The general solid-phase synthesis of the 14-membered macrocycles was described previously.[1,2] The macrocycle can be varied in a combinatorial fashion using α-amino alcohols, α-amino acids, β-amino acids and carboxylic acids (Scheme 1). For Fmoc-protected amino alcohols, a one-pot Fmoc-deprotection/reductive amination protocol was followed using ArgoGel™–MB-CHO resin. The resin-bound amino alcohol was then *t*-butyldimethylsilyl (TBDMS)-protected and reacted with a Fmoc-protected α-amino acid. Following deprotection, an Fmoc-protected β-amino acid was coupled to the N-terminus. After another deprotection step, the cyclization linker 2-fluoro-5-nitrobenzoic acid, was incorporated into the backbone. The TBDMS protection was removed and the structure was cyclized by a base-induced S_NAr reaction. The aryl nitro group was reduced with tin(II) chloride and the resulting aryl amino group derivatized with a carboxylic acid. The macrocycles were cleaved from solid support with TFA.

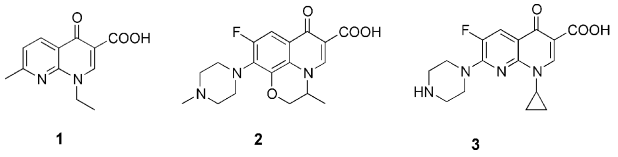


Figure 2. Representative quinolone antibacterials including nalidixic acid (**1**), ofloxacin (**2**) and ciprofloxacin (**3**).

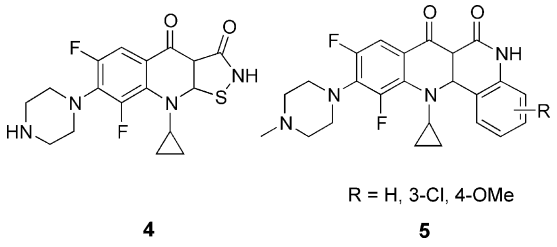


Figure 3. C-3 modified quinolones.

A library of 1350 macrocycles, incorporating nalidixic acid in the last synthesis step, were prepared using the IRORI technology for directed-sorting split-and-mix synthesis.[10] The macrocycle side chain diversity is displayed in Figure 4. Each IRORI MicroKan™ was loaded with an equimolar mixture of the TBDMS-protected amino alcohol resins. A total of four different resin mixtures derived from eight different amino alcohols were used in the library set. In the first combinatorial step, ten Fmoc-amino acids were reacted as single *d* or *l* isomers (step iii, Scheme 1). After deprotection, four β-amino acid racemic mixtures and Fmoc-β-alanine were coupled (step v, Scheme 1).

Each IRORI MicroKan's™ resin was cleaved into individual wells to give three to eight product macrocycles (0.1 mmol in total). After removing the cleavage solvent, the samples were dried and dissolved in DMSO to give mixture macrocycle concentrations of 20 mM. All wells were analyzed by LC/MS and the sum of the integrated peaks of the expected molecular ions was generally >85%.

The library was screened in MIC assays against *E. coli* and *S. aureus* bacterial strains at 100 μM.[11] Three wells were identified with weak antibacterial activity against *E. coli* (MIC = 50–100 μM). All other wells were considered inactive as they did not inhibit the assay >75% at 100 μM (total macrocycle concentration). These MIC-active wells did not inhibit an *E. coli* transcription/translation inhibition assay at 100 μM, suggesting a non-ribosomal RNA mechanism.[12]

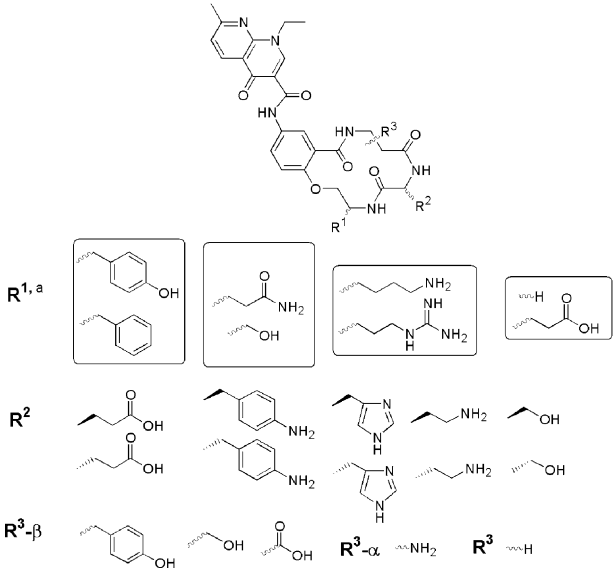
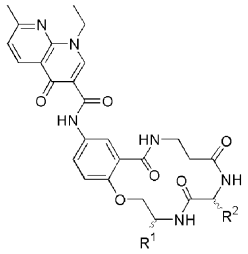
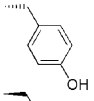
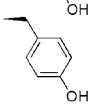


Figure 4. Mixture-based combinatorial library (15 R^1×10 R^2×9 R^3) [a]R^1 encapsulated product functionalities came from amino alcohol-derivatized resins that were pre-mixed in a single canister.

Table 1. Antibacterial activity of macrocycles based on mixture A



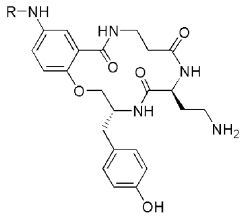
Compd	R^1	R^2	MIC[a] (μM), *E. coli*[b]	MIC (μM), *S. aureus*[b]
6			25–50	>100
7			25–50	>100
8			25–50	>100
9			25–50	>100
10			25–50	>100
11			25–50	>100
12			25–50	>100
13			25–50	>100

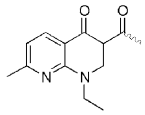
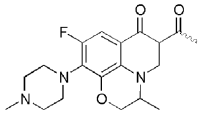
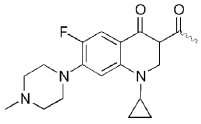
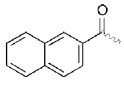
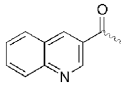
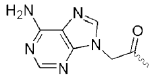
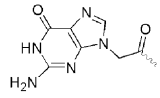
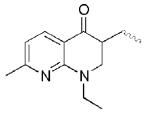
[a]Minimum inhibitory concentration that inhibited visible growth.
[b]Bacterial strains: *E. coli* ATCC 25922, *S. aureus* ATCC 13709.

We chose to deconvolute one well mixture to follow-up the antibacterial activity. All possible stereoisomers of mixture A were prepared as single compounds and the individual MIC values determined (Table 1). Macrocycles **6**–**13** gave MIC values = 25–50 μM against *E. coli* and were inactive against *S. aureus*. Using our assay systems, the macrocycles were found to be less potent than nalidixic acid (MIC values for nalidixic acid are 1.5–3.0 μM and 50–75 μM against *E. coli* and *S. aureus*, respectively).

In order to determine the importance of the aryl endocyclic substitution on MIC activity, other quinolones (ofloxacin and ciprofloxacin) and aryl functionalities were explored (Table 2). The quinolone-macrocycle conjugates **14** and **15** gave good activity against both gram negative (*E. coli*) and gram positive (*S. aureus*) bacterial strains. As a comparison, ofloxacin (**2**) gave MIC values of 0.39–0.78 and 12–25 against *E. coli* and *S. aureus* while ciprofloxacin gave MIC values of 0.04–0.08 and 0.3–0.6 against *E. coli* and *S. aureus*, in our

Table 2. Antibacterial activity of aryl-modified macrocycles



Compd	R	MIC[a] (μM), *E. coli*	MIC (μM), *S. aureus*
6		25–50[b]	>100[b]
1		1.5–3.0	50–75
14		1–3[b]	6–13[b]
2		0.39–0.78	12–25
15		0.1–0.2[b]	0.8–1.6[b]
3		0.04–0.08	0.3–0.6
16		>100	>100
17		>100	>100
18		>100	>100
19		>100	>100
20		>100	>100
21		>100	>100

[a]Minimum inhibitory concentration that inhibited visible growth.
[b]MIC values determined from samples purified to >95% by preparative HPLC.

assays systems (Table 2). The antibacterial activity of quinolone-macrocycles **14** and **15,** being somewhat weaker, roughly parallels the activity of the quinolone acids. The other aryl functionalities (**16**–**20**) failed to provide active macrocycles (all MIC values >100 μM). As a modified analogue of **6**, macrocycle **21** was prepared missing the quinolone 3-carboxyl group.[13] The antibacterial activity was not maintained (*E. coli* MIC value >100 μM) showing that the 3-carboxyl moiety is

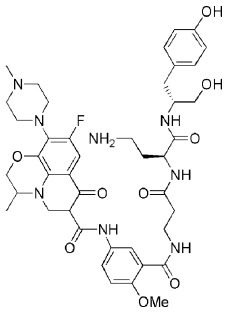
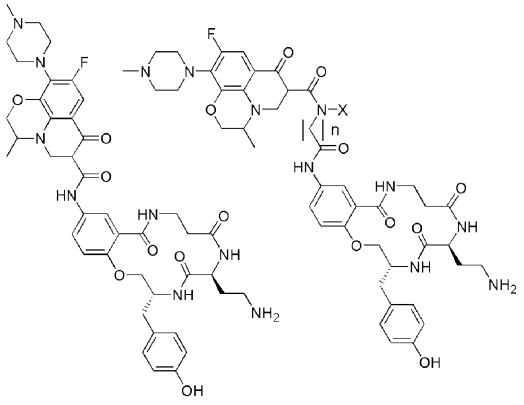


Figure 5. Open chain analogue **22**.

Table 3. Quinolone-macrocycle linker SAR



14 **23-28**

Compd[a]	Spacer	MIC[b] (μM), *E. coli*	MIC[b] (μM), *S. aureus*
14	None	1–3	6.3–13
23	$n=1$, X = H	1–3	25–50
24	$n=1$, X = Me	3–6	25–50
25	$n=2$, X = H	1–3	6.3–13
26	$n=3$, X = H	1–3	13–25
27	$n=4$, X = H	1–3	13–25
28	$n=5$, X = H	1–3	13–25

[a]All compounds were purified to >95% purity by preparative HPLC.
[b]Minimum inhibitory concentration of compound that inhibited visible growth.

required for activity. To explore the importance of the macrocyclic portion on activity, structure **22** (Fig. 5) was prepared as an open chain analogue of **14**. Analogue **22** did not show MIC activity against *E. coli* and *S. aureus* bacterial strains at 100 μM, highlighting the importance of the constrained macrocycle ring. Furthermore, if the antibacterial activity of **14** came from a mechanism of enzymatic amide bond hydrolysis, which would release free quinolone acid, structure **22** would be anticipated to be active.

In addition, we explored some quinolone–macrocycle linker SAR using amino acid-based linkers (Table 3).[14] The amino acids glycine (for macrocycle **23**) and sarcosine (for macrocycle **24**) were incorporated into the solid phase synthesis scheme, and the length of the linker increased further with longer glycine-type mimetics (macrocycles **25**–**28**). Macrocycle **23**, incorporating glycine as the linker, gave similar activity against *E coli* bacteria and weaker activity against *S. aureus* bacteria, compared to parent macrocyle **14**. Macrocycle **24**, incorporating the peptoid linker, was slightly less potent than the parent. Macrocycle **25**, incorporating the β-alanine linker, gave the same MIC activity as parent macrocycle **14** against both bacterial strains. Furthermore, the antibacterial activity did not vary with the longer spacers (macrocycles **26**–**28**). Surprisingly, the SAR profile for these linker–modified macrocycles is relatively flat.

We have identified novel quinolone-macrocycle conjugates with good activity against *E. coli* and *S. aureus* bacterial strains. The MIC activity roughly parallels the analogous quinolone acids. This study suggests that C-3 quinolone modifications of this type are tolerated and are anticipated to be useful for the development of new quinolone-based antibacterial agents.

Acknowledgements

This work was supported by the Department of Defense through DARPA Grant BAA 98-25-544 and NIST Advanced Technology Program Grant 97-01-0135.

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13. Macrocycle **21** was prepared by the reductive amination of the macrocyclic arylamine with the carbaldehyde analogue of ciprofloxicin (Lesher, G. Y.; Gruett, M. D. U.S. Patent 3873554, 1975).
14. The synthesis of similar macrocycles with amino acid–based linkages is described in ref 2.