Letter

Syntheses and Antibacterial Activity of N-acylated Ciprofloxacin Derivatives Based on the Trimethyl Lock

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Syntheses and Antibacterial Activity of *N*-acylated Ciprofloxacin Derivatives Based on the Trimethyl Lock

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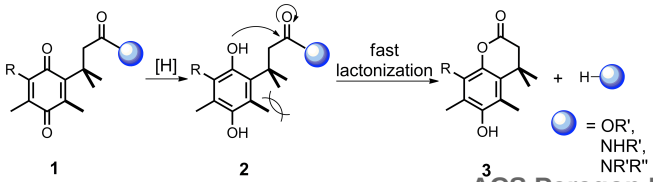
ABSTRACT: Several *N*-acyl ciprofloxacin quinone derivatives based on a trimethyl lock structure were synthesized and their *in vitro* antibacterial activity against a panel of clinically relevant bacteria was evaluated. A few new analogs displayed enhanced activity against Gram-positive species compared to the parent drug. Additionally, studies of **8-Cip**, which was the most potent compound tested, indicate that it may act through a dual-action mechanism.

Since their discovery in 1970s, fluoroquinolone antibiotics have played an important role in treatment of bacterial infections and have saved countless millions of lives. Targeting bacterial DNA gyrase and topoisomerase, fluoroquinolones exhibit their significant bactericidal activity by forming ternary complexes of enzyme-DNA-drug, and consequently blocking bacterial replication.[1,2] Their notable antimicrobial activity, excellent pharmacokinetic properties and few side effects have led to the widespread use of fluoroquinolones and, unfortunately, thus also the development of bacterial resistance.[3] Therefore, although many advances in the search for new fluoroquinolones have been already made, there is a sense of urgency for new and more effective derivatives.

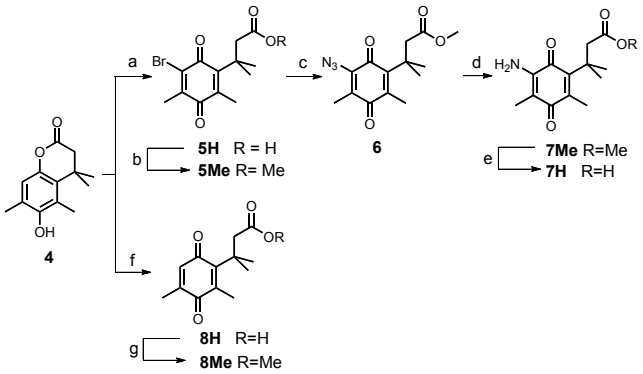
Recently, in our development of linkers to incorporate a drug release function in iron transport-mediated drug delivery, a series of carboxylic acids with a quinone "trimethyl lock" structure and their methyl esters were obtained as synthetic intermediates.[4,5] The chemical nature of the trimethyl lock is an *o*-hydroxylcinnamic acid derivative.[6] Reduction of the quinone trimethyl lock system generalized by structure **1** would lead to the corresponding hydroquinones **2** in which severe steric repulsion between three methyl groups promotes rapid lactonization with concomitant release of an alcohol or amine (Scheme 1).[7,8] Several quinone trimethyl lock compounds have been investigated for reductase-activated prodrugs such as those based on mustard[9] and oxindoles[10] for anticancer therapy. In ciprofloxacin, one of the best known fluoroquinolones, it has been demonstrated that chemical modification of the secondary amine in the piperazinyl ring by acylation does not always alter the effect of these molecules towards their bacterial target.[11] With the aim of probing the effects of the quinone trimethyl lock on antibacterial activity of fluoroquinonlones, we herein describe the syntheses of several ciprofloxacin derivatives with quinone trimethyl lock precursors as *N*-acyl substituents and studies of their *in vitro* antibacterial activity.

Scheme 1. Reaction of a quinone trimethyl lock to form lactone via reduction



The syntheses of trimethyl locks with different substituents on the quinone are shown in Scheme 2. Both forms **5H** and **8H** were derived from lactone **4** by oxidative ring opening of the lactone using bromine[8] and *N*-bromosuccinimide,[12] respectively. The acids were conveniently converted to their methyl esters **5Me** and **8Me** via thionyl chloride-mediated or acid catalyzed esterification in methanol. Nucleophilic substitution of **5Me** with NaN_3 in aqueous methanol provided azide **6** in excellent yield. A triphenylphosphine-mediated reduction of the azide afforded vinylogous amide **7Me** in 66% yield, which was subsequently hydrolyzed to furnish acid **7H**. Water-soluble carbodiimide-mediated coupling of ciprofloxacin to quinone trimethyl locks **5H**, **7H** and **8H** provided *N*-acyl ciprofloxacin derivatives **5-Cip**, **7-Cip** and **8-Cip** with different substituents (Br-, NH_2-, H-) on the quinone moieties in 44-71% yield (Scheme 3).

Scheme 2. Synthesis of quinone trimethyl locks 5H, 7H, 8H and their methyl esters[a]



[a]Reagents and conditions: (a) Br_2, AcOH, rt, 80% (b) $SOCl_2$, MeOH, reflux, 77%; (c) NaN_3, MeOH-H_2O, rt, 91%; (d) 1. PPh_3, CH_2Cl_2, rt; 2. AcOH, THF-H_2O, reflux, 66% for 2 steps; (e) LiOH, MeOH-H_2O, rt, 91%; (f) NBS, CH_3CN-H_2O-acetone, rt, 79%; (g) H_2SO_4 (cat.), MeOH, reflux, 95%.

Table 1. Diameter of growth inhibition zones (mm) in the agar diffusion antibacterial susceptibility assay

		5-Cip	5Me	7-Cip	7Me	8-Cip	8Me	Cip
1	*B.subtilis* ATCC 6633	35	0	36	0	39	0	20/24P[b]
2	*S. aureus* SG511	25	0	31	0	33	0	21[a]
3	*M. luteus* ATCC10240	19	0	17V	0	28	0	0[a]
4	*M. vaccae* IMET10670	37	0	24/30P	0	32/42P	0	23[a]
5	*A. baumannii* ATCC17961	18/23P	0	24/30P	0	26/32P	0	18/21P[a]
6	*P. aeruginosa* K799/wt	29/35P	0	22/32P	0	41	0	15[b]
7	*P. aeruginosa* K799/61	29/38P	0	31/35P	0	35/42P	0	19/24P[b]
8	*E. coli* X580	33/40P	0	31/37P	0	32/40P	0	23[c]
9	*E. coli* DC0	17/21P	0	16/23P	0	21/29P	0	18[a]
10	*E. coli* DC2	24	0	23	0	27	0	22[a]

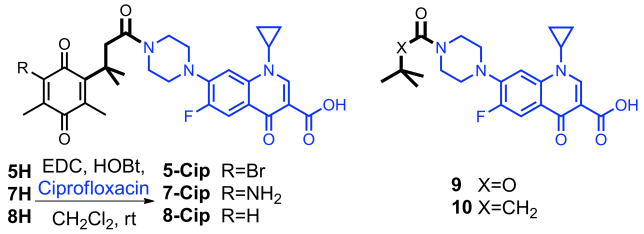
P, unclear inhibition zone/many colonies in the inhibition zone; V, very unclear inhibition zone. Exactly 50 μL of a 0.1 mM solution of each compound dissolved in 1:9 DMSO/MeOH was added to 9 mm wells in agar media (Standard I Nutrient Agar, Serva or Mueller Hinton II Agar, Becton, Dickinson and Company). Inhibition zones were read after incubation at 37 ºC for 24 h. *M. vaccae* was incubated 37ºC for 44 h; [a,b,c]Ciprofloxacin was tested at (a) 5 μg/mL, (b) 1.66 μg/mL, (c) 0.33 μg/mL in H_2O.

Table 2. MIC values of the *N*-acyl ciprofloxacin derivatives against selected bacterial strains

	5-Cip	7-Cip	8-Cip	9	10	8Me	Cip
B.subtilis ATCC 6633	<0.05	<0.05	<0.05	<0.05	<0.05	50	0.03
S. aureus SG511	0.1	<0.05	<0.05	0.4	0.4	>100	0.47
M. luteus ATCC10240	1.56	12.5	0.2	>100	12.5	50	8[c]
M. vaccae IMET10670	2	2	0.5	2	2	80	0.47
A. baumannii ATCC17961	0.78	0.6	0.39	>100	25	>100	0.24
P. aeruginosa K799/wt	5	2.5	1.25	>100	>100	>100	0.47
P. aeruginosa K799/61	2.5	5	2.5	>100	>100	>100	0.24
E. coli DC0	12.5	25	12.5	>100	>100	>100	0.47
E. coli DC2	8.33	12.5	6.25	>100	100	>100	0.24

[a]MIC values (μM) were determined using the broth microdilution method in Mueller-Hinton broth No.2 (MHII) with visual end point analysis according to the CLSI guidelines.[14]; [b]Each compound was tested in triplicate; [c]Data from ref 13.

Scheme 3. Synthesis of *N*-acyl ciprofloxacin derivatives



N-Acyl ciprofloxacin quinone derivatives **5-Cip**, **7-Cip** and **8-Cip** were initially screened for antibiotic activity against representative Gram-positive and Gram-negative bacteria as well as *Mycobacterium vaccae* using agar diffusion assays (Table 1). All three ciprofloxacin derivatives displayed strong antibacterial activities when tested at 0.1 mM, as indicated by the large zones of inhibition they induced. The trimethyl lock cores, as represented by methyl esters **5Me**, **7Me** and **8Me**, were generally inactive. Moreover, all three ciprofloxacin derivatives showed moderate to good activity against *Mycobacterium vaccae*, a non-pathogenic model organism for *Mycobacterium tuberculosis* that causes tuberculosis, suggesting their potential in anti-tuberculosis agent development.

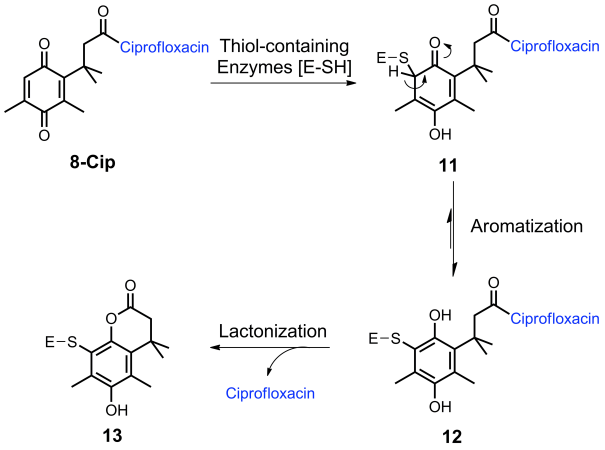
All ciprofloxacin derivatives were subjected to further assays to determine their minimum inhibitory concentrations (MIC) (Table 2). Two additional *N*-acyl derivatives **9** and **10** that have similar trimethyl substituted side chain structures but without the quinone fragment were also synthesized and included in the assay. In general, the trimethyl lock ciprofloxacin quinone derivatives possessed equal or enhanced *in vitro* activity against Gram-positive species compared to the parent drug. Among the three derivatives, compound **8-Cip** was the most active with MIC values less than 0.05 μM and 0.2 μM against *S. aureus* and *M. luteus*, respectively, against which ciprofloxacin is less active (MIC in 0.47 μM and 8 μM, respectively). On the other hand, **9** and **10** were less active than the trimethyl lock ciprofloxacin derivatives, indicating that the quinone structure plays a key role for the activity enhancement observed. In fact, trimethyl lock compound **8Me** has very weak antibacterial activity (MIC=50-80 μM) against the Gram-positive species tested except *S. aureus*, suggesting that the trimethyl lock ciprofloxacin derivatives may be dual-action antibiotics.

The trimethyl lock ciprofloxacin derivatives displayed decreased activity (2-50 fold) against mycobacteria and Gram-negative bacteria compared to the parent drug, presumably because of the loss of zwitterionic structure of ciprofloxacin,

which favors membrane penetration.[15] However, **9** and **10** were virtually inactive (MIC>100 μM) against Gram-negative bacteria, suggesting that the quinone fragment partially compensates for the loss of activity caused by the non-zwitterionic structure.

Based on results from antibacterial assays and the chemical nature of the trimethyl lock system, we have proposed modes of action of the trimethyl lock ciprofloxacin derivatives. While all three derivatives could serve as prodrugs which are activated by potential reductases as depicted in Scheme 1, compound **8-Cip** may have an additional dual-action mechanism by serving as a prodrug and as a covalent enzyme inhibitor, which is consistent with its superior antibacterial activity. Conjugate addition of sulfur nucleophiles to quinones is well known and has been exploited in design of certain enzyme inhibitors.[16] Similarly, the activation of compound **8-Cip** could be initiated by the reductive alkylation with certain thiol-containing enzymes (Scheme 4). The resulting thiol-quinone adduct **11** would be expected to aromatize to the corresponding hydroquinone **12**. The rapid lactonization of **12** induced by the trimethyl lock could release ciprofloxacin and meanwhile result in a covalent inactivated enzyme **13**. In contrast, such a pathway with an aromatization step could not be established for compound **5-Cip** and **7-Cip**.

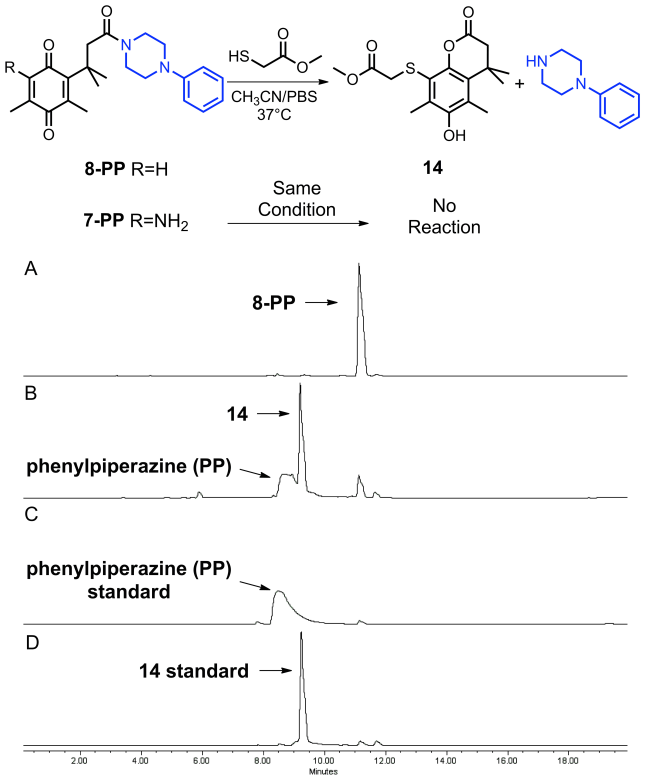
Scheme 4. Proposed modes of action of compound 8-Cip



To determine whether **8-Cip** could be activated in the presence of thiol-containing enzymes as proposed, model reactions were examined for the reaction of thiols with *N*-acyl phenylpiperazine **8-PP**, a more soluble alternative to **8-Cip**, and the reaction was monitored by HPLC (Scheme 5). The thiol methyl mercaptoacetate was chosen for the model studies principally because its pKa (7.9) was similar to that of the thiol in cysteine (pKa = 8), which is ubiquitous in biological systems. We were pleased to find that in the presence of the thiol, **8-PP** (retention time = 11.1 min) was largely converted to lactone **14** (retention time = 9.2 min) with concomitant release of the phenylpiperazine (retention time = 8.5 min) in buffered aqueous acetonitrile after 3 h. In sharp contrast, no reaction was observed for compound **7-PP**, a representative system lacking the aromatization pathway, under the same conditions that led to rapid lactonization for **8-PP**. These data provide evidence for **8-Cip** being a dual-action prodrug that might utilize a unique thiol-activated pathway.

In summary, we have described the syntheses of a series of ciprofloxacin derivatives with a quinone trimethyl lock as the *N*-acyl substituent and their antibacterial activity. Among the derivatives, compound **8-Cip** displayed enhanced activity against several Gram-positive species compared to the parent drug. The superior activity of **8-Cip** suggests that it may act through a dual-action mechanism by serving as a prodrug and as a covalent thiol-containing enzyme inhibitor. This dual-action mechanism also has great potential for use in enzyme-activated prodrug strategies for site-selective drug delivery. Future work will focus on syntheses of other drug derivatives based on **8H** and examine the release of drug in appropriate enzyme assays.

Scheme 5



Top: model reaction for enzyme activation of trimethyl lock derivatives; Bottom: HPLC studies of the activation of **8-PP**: panel A, **8-PP** standard; panel B, **8-PP** after treatment with methyl mercaptoacetate in CH_3CN/PBS solution at 37 °C for 3 h; panel C, phenylpiperazine (PP) standard; panel D, **14** standard.

ASSOCIATED CONTENT

Experimental procedures, copies of spectral data, and characterization data. 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.

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ABBREVIATIONS

DNA, deoxyribonucleic acid; Cip, ciprofloxacin; MIC, minimum inhibitory concentration required to inhibit the growth of 90% of organisms; HPLC, high-performance liquid chromatography; PP, phenylpiperazine.

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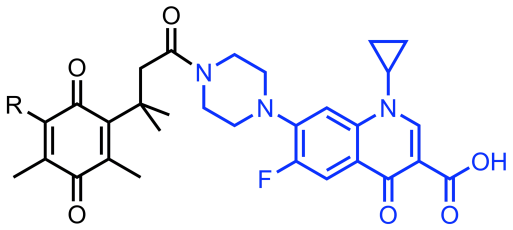

R=Br, NH_2, H

8-Cip (R=H)
Dual Action
Fluoroquinolone
Derivative