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SYNTHESIS AND MECHANISTIC STUDIES OF A 'TETRAZOLE-TETHERED' CEPHALOSPORIN-QUINOLONE HYBRID

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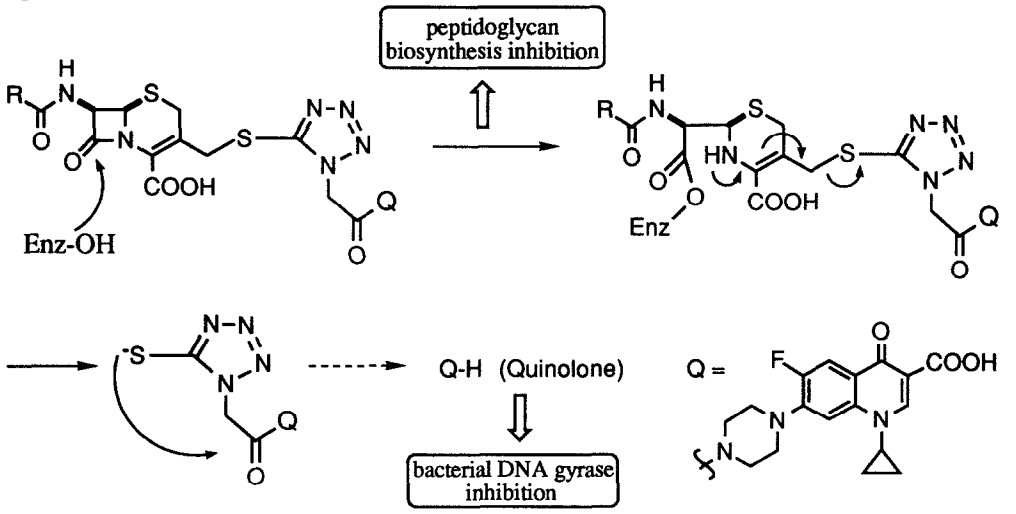
Abstract. The synthesis and antibacterial activity of a novel 'tetrazole-tethered' cephalosporin-quinolone hybrid is described. The *in vitro* spectrum of **7** mirrored that of a third-generation cephalosporin. Quinolone-like activity was not observed. β-Lactamase-accelerated hydrolysis of **7** produced tetrazolylquinolone **12** which (*via* independent synthesis) proved to be a weak antibacterial and unable to form free quinolone.

Background and Introduction

Dual-action β-lactam antibacterials[1] are molecular hybrids of β-lactam antibiotics and other antibacterials constructed in a way that allows both components to exert their bactericidal properties. We have previously described dual-action cephalosporins (DACs) which contain quinolones joined *via* ester, thioester, carbamate, ammonium or amine linkages[2-5] and we recently synthesized carbapenem and penem analogs.[6] The mechanistic picture which has emerged from this work indicates that the intact dual-action agent is acted upon by bacterial enzymes[7] which can lead to inhibition of peptidoglycan biosynthesis[8] resulting in the loss of bacterial cell wall production. The 3'-substituent of these cephalosporins is a quinolone which is released as a consequence of β-lactam cleavage and which exhibits its characteristic mode of bacterial killing *via* inhibition of bacterial DNA gyrase.[9]

New ways to release quinolone antibacterials by enzymatic processes that act upon the β-lactam nucleus of a dual-action antibacterial agent are being vigorously explored by a number of research groups. We decided to construct a cephalosporin-quinolone hybrid which contains a C-3'-thiotetrazoleacetic acid moiety[10] to which a quinolone could be attached *via* an amide bond. This hybrid should possess improved hydrolytic stability compared to DACs containing labile quinolone linking groups, and should exhibit good cephalosporin activity since cephems bearing C-3' thiotetrazole substituents are rather potent broad-spectrum antibacterial agents.[10] A consequence of β-lactam cleavage would be the release of thiotetrazole[11] tethered to a quinolone. By its design, an intramolecular neighboring group "assisted" amide cleavage process[12] liberating free quinolone may be anticipated, resulting in a dual mode of antibacterial activity (see Figure 1).

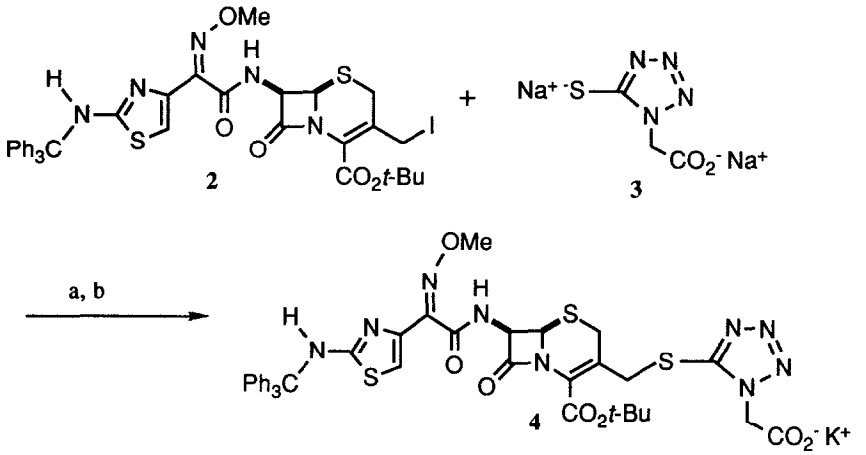
Figure 1



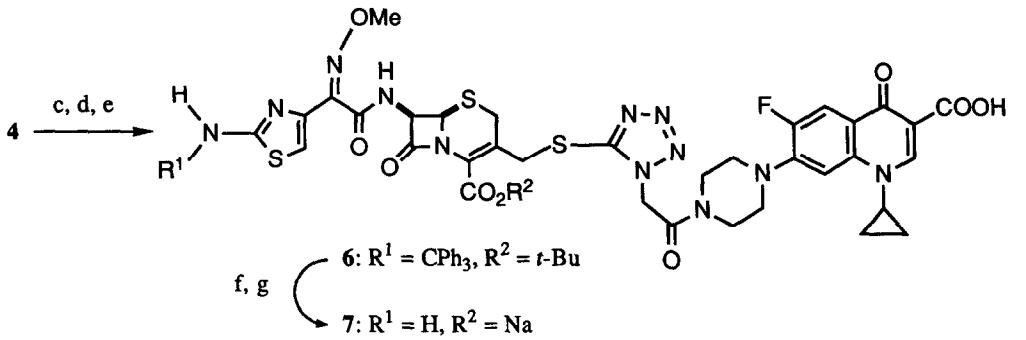
Chemistry

The synthesis of a "tetrazole-tethered" cephalosporin-quinolone hybrid required three distinct operations (Scheme 1). 3-Iodomethylcephem **2**[13] was reacted with tetrazolethiolate **3**[14] to afford product **4**. This adduct was activated *via in situ* generation of the corresponding acyl chloride which condensed with silylated quinolone **5** to yield the amide product **6**. Subsequent treatment with trifluoroacetic acid cleaved both the *N*-trityl and the *t*-butyl ester protecting groups to give a crude product which upon reverse-phase chromatography afforded pure product **7**.

Scheme 1



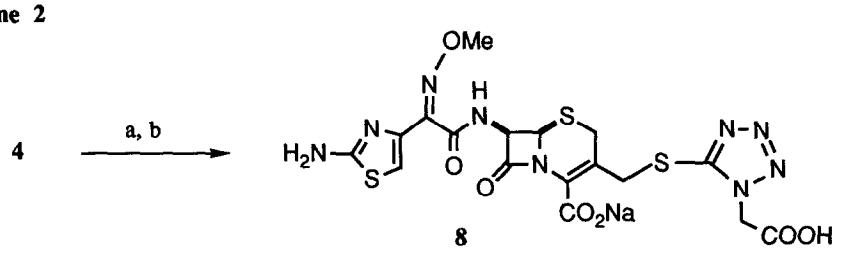
Scheme 1 (continued):



a) DMF, R.T.; b) Aq. potassium biphthalate buffer, 76%; c) ClCOCOCl, *i*-Pr_2NEt / CH_2Cl_2; d) bis(TMS)ciprofloxacin **5** (pre-mixed MSTFA, ciprofloxacin **1**); e) Aqueous work-up, 52%; f) TFA, anisole / CH_2Cl_2; g) $NaHCO_3$ / H_2O-CH_3CN reverse-phase chromatography, 60%.

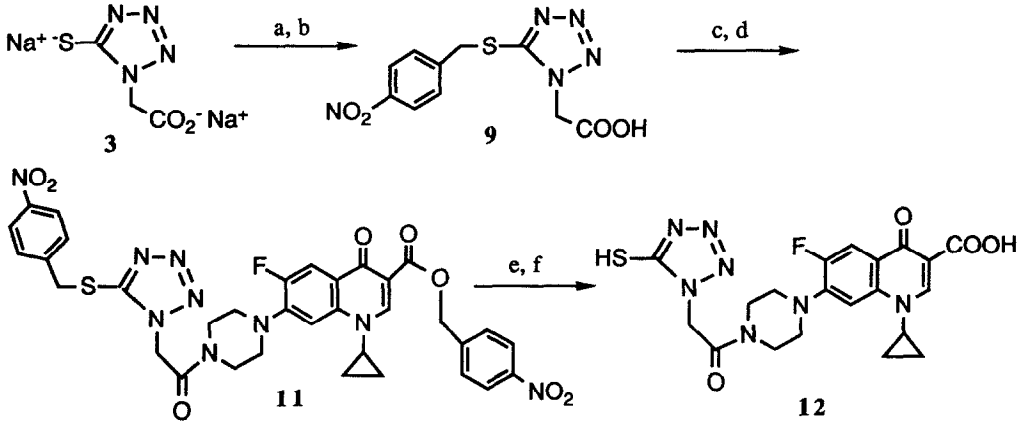
In order to determine the inherent antibacterial activity of **7**, *in vitro* evaluation of the individual components of this hybrid, quinolone **1** (ciprofloxacin) and tetrazolethiolate **3**, along with the putative β-lactam hydrolysis product tetrazolylquinolone **12**, and cephem-tetrazole **8**, was necessary. Cephem-tetrazole **8** was readily available *via* deprotection of **4** (Scheme 2). Tetrazolylquinolone **12** was prepared by coupling the acyl chloride of thiotetrazoleacetic acid *para*-nitrobenzyl (pNB) thioether **9** with ciprofloxacin *para*-nitrobenzyl ester **10** (Figure 2) followed by removal of both pNB protecting groups (Scheme 3). Cephem alcohol **13**[1] (Figure 2) was also chosen to be evaluated in these screens.

Scheme 2



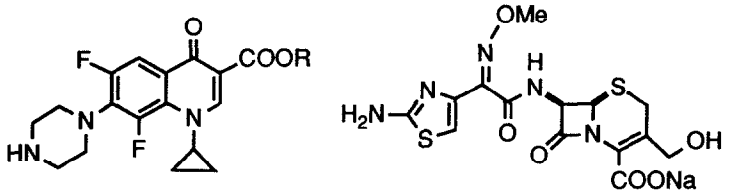
a) TFA, anisole / CH_2Cl_2; b) $NaHCO_3$ / H_2O-CH_3CN, 50%.

Scheme 3



a) *para*-nitrobenzyl bromide / DMF; b) H^+ / H_2O, 35%; c) ClCOCOCl, *i*-Pr_2NEt / CH_2Cl_2; d) ciprofloxacin *para*-nitrobenzyl ester **10**, 69%; e) H_2, Pd-C / DMF; f) H^+ / H_2O, 30%.

Figure 2



1: R = H
10: R = *para*-nitrobenzyl

13

Results and Discussion

In this study, a distinguishing feature of great importance is manifested in the Minimum Inhibitory Concentrations (MICs) against *Pseudomonas aeruginosa* and methicillin-resistant quinolone-sensitive *Staphylococci.* Quinolones exhibit excellent potency while cephalosporins are often ineffective against these pathogens. Dual-action cephalosporins, by way of their targeted enzymatic release of free quinolone, approach *in vitro* potency of the respective quinolone component. In contrast to quinolone **1** (ciprofloxacin), both cephem **13** and cephem-tetrazole **8** failed to display significant activity against these bacterial strains but were potent against cephalosporin-susceptible Gram-positive pathogens (*Staphylococcus aureus* Smith, *Streptococcus pneumoniae* and *S. pyogenes*) as well as Gram-negative *Klebsiella pneumoniae* A, and *Proteus vulgaris.* This spectrum of antibacterial activity, representative of a third-generation cephalosporin, is mirrored in the MICs of "tetrazole-tethered" cephalosporin-quinolone hybrid **7**, indicating that a dual mode of antibacterial killing was not operative (Table 1). Tetrazolylquinolone **12** proved to be a weak antibacterial agent against both Gram-negative and Gram-

positive pathogens. To our knowledge, **12** is a novel quinolone and reflects a generally detrimental modification to the quinolone nucleus, namely piperazine nitrogen acylation. As judiciously selected, tetrazolethiolate **3** was inactive (MICs >128 μg/mL) in these assays and thus serves only as a tethering moiety.

Table 1: *In Vitro* Activity of Cephem-Quinolone **7** and Reference Compounds: MIC (μg/mL)

Organism	**7**	**12**	**8**	**13**	**1**
Escherichia coli 257	4	2	0.125	0.25	0.0078
E. coli TEM-1[a]	2	2	0.125	0.25	0.0156
Citrobacter freundii BS-16[a]	32	4	128	>128	0.0313
Klebsiella pneumoniae A	2	32	0.0625	0.0625	0.25
Enterobacter cloacae P99[a]	16	2	>128	>128	0.0078
Proteus vulgaris ATCC 6380[a]	4	4	≤0.0156	0.0625	0.0078
Pseudomonas aeruginosa 5712	>128	128	16	>128	1
Ps. aeruginosa 18SH[a]	128	32	>128	>128	0.25
Staphylococcus aureus Smith	4	32	8	16	0.125
S. aureus 67[b]	64	64	>128	>128	0.5
S. aureus 753[b]	128	64	>128	>128	0.25
Streptococcus pneumoniae 6301	≤0.0156	64	0.0313	0.0625	1
S. pyogenes 4	≤0.0156	64	0.0313	≤0.0157	0.5

(a = constitutive ß-lactamase producer; b = methicillin-resistant)

'Tetrazole-tethered' cephalosporin-quinolone **7** was subjected to hydrolysis at a physiological pH (7.4) and temperature (37°C) to determine the rate of release of tetrazolylquinolone **12** and free quinolone (ciprofloxacin) **1**. In several determinations, **7** was *exceptionally stable* towards hydrolysis and although half-life values varied considerably ($T_{1/2} \geq$ 11-16 days), the lone quinolone product formed in all cases was tetrazolylquinolone **12**, and ciprofloxacin was absent. In an attempt to accelerate β-lactam hydrolysis by interaction with a β-lactamase active against third-generation cephalosporins, a purified *Proteus vulgaris* lactamase[15] was reacted with the cephalosporin-quinolone hybrid **7**. While β-lactam hydrolysis *was* greatly accelerated (**7** was nearly completely consumed within one hour), *no* free ciprofloxacin **1** was detected and the formation of tetrazolylquinolone **12** was observed. Synthetic tetrazolylquinolone **12** was found to be resistant to hydrolysis at neutral pH; strongly basic conditions (pH 11-13) were needed to produce ciprofloxacin to any appreciable extent ($T_{1/2}$ = 2-3 h at pH ~12). (All studies were easily monitored by HPLC - see references 2-6).

Conclusions

The design and synthesis of a 'tetrazole-tethered' cephalosporin-quinolone **7** is described. This novel hybrid proved to be exceptionally stable to hydrolysis at physiological pH (7.4) and displayed cephalosporin-like antibacterial activity *in vitro* with no apparent quinolone activity. Hydrolysis studies of **7** with and without a

(*Proteus vulgaris*) β-lactamase demonstrated that upon β-lactam cleavage, tetrazolylquinolone **12** was released. This compound was a weak antibacterial and unable to release free ciprofloxacin **1**. These findings were confirmed via independent synthesis and stability studies.

Acknowledgments

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