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Syntheses and studies of quinolone-cephalosporins as potential anti-tuberculosis agents

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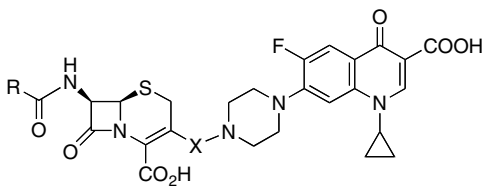
Abstract—The syntheses and anti-tuberculosis activity of quinolone-cephalosporin conjugates (**1** and **2**) are described. Both showed broad-spectrum antibacterial activity and significant anti-TB activity. The carbamate-linked quinolone-cephem **2** showed better antimycobacterial activity, including anti-TB activity, than the direct amine-linked quinolone-cephem **1**, while quinolone-cephem **1** was slightly more effective against some Gram-negative bacterial strains.

Tuberculosis (TB) is one of the leading causes of death and suffering worldwide. The increasing microbial resistance, toxicity, and side effects of currently used anti-tuberculosis drugs also emphasize the urgent need for new, safer, and more effective anti-tuberculosis agents.[1–5] Quinolonyl-β-lactams are a class of multifunctional β-lactams and some examples showed high potency and broad antibacterial activity against a range of clinically significant bacterial pathogens, especially against newly emerging strains of quinolone- and β-lactam-resistant bacteria, for example, methicillin-resistant ciprofloxacin-resistant (MRCR) *Staphylococcus aureus*, penicillin-resistant *Streptococcus pneumoniae*, and vancomycin-resistant *Enterococcus faecium*.[6]

However, no anti-TB activity data of quinolonyl-β-lactams were reported. Based on our previous work on the synthesis of a carbacephalosporin-quinolone conjugate,[7] we describe here the syntheses and anti-TB activity of direct amine-linked quinolonyl-cephem **1** and carbamate-linked quinolonyl-cephem **2** (see Fig. 1).

The synthesis of the direct amine-linked quinolone-cephalosporin **1** is shown in Scheme 1. 3-Chloromethyl-7-phenylacetylamino cephalosporanic acid *p*-methoxybenzyl ester (GCLE) was converted to 3-iodomethyl cephalosporin **3** in 92% yield according to the literature procedure.[8] The key intermediate **4** was obtained in 30% yield by coupling compound **3** with ciprofloxacin that had been pretreated with *N*-methyl-*N*-(trimethylsilyl) trifluoro acetamide (MSTFA).[9] Subsequent deprotection with TFA in the presence of anisole provided the desired product **1** in 70% yield.

The synthesis of carbamate-linked quinolone-cephalosporin compound **2** is shown in Scheme 2. This synthesis began with a controlled hydrolysis to remove the acetyl group of commercially available 7-amino cephalosporanic acid (7-ACA). Reaction with phenylacetyl chloride



1. X = CH_2 **2**. X = CH_2OCO

Figure 1. QLAs: quinolone-cephalosporin conjugates.

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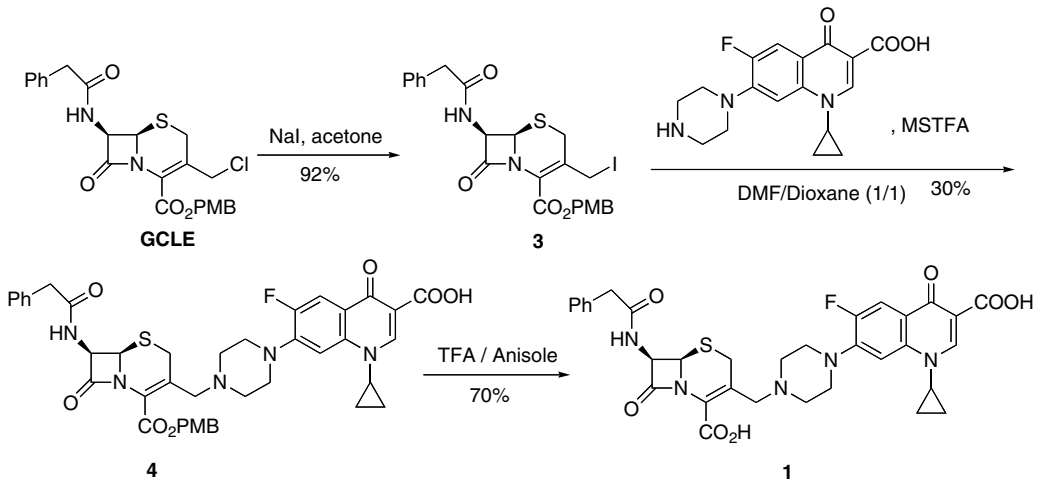
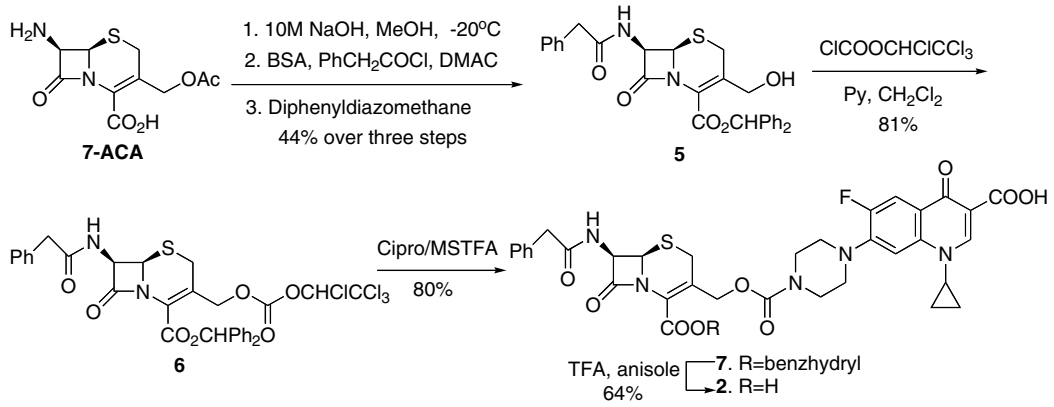


Scheme 1. Procedure for synthesis of amine-linked quinolone-cephalosporin conjugate.



Scheme 2. Procedure for synthesis of carbamate-linked quinolone-cephalosporin conjugate.

followed by treatment with diphenyldiazomethane provided **5** in 44% yield for three steps.[10] Subsequent coupling with tetrachloroethyl chloroformate gave intermediate carbonate **6** in 81% yield. Direct reaction with ciprofloxacin under basic aqueous conditions followed by deprotection gave the desired product, **2**, in 51% yield for two steps.[11]

Quinolone-cephalosporin compounds **1**, **2**, **4**, **7**, as well as GCLE, ciprofloxacin, mixtures, and precursors **3** and **5**, were tested for their antibacterial activities against various strains of Gram-positive and Gram-negative bacteria, several strains of mycobacteria, as well as against *Mycobacterium tuberculosis* (Table 1).[12–17] Compound **2** was found to be comparable to or by 1 to 2 dilution steps more active than compound **1** for all fast growing mycobacteria, and also for *M. tuberculosis* H37Rv. Interestingly, the very hydrophobic benzhydryl ester, compound **7**, was the most active compound against enterobacteria (*Escherichia coli* and *Enterobacter cloacae*), against staphylococci and enterococci as well as fast growing mycobacteria. On the other hand, **7** and ciprofloxacin were less effective against *Micrococcus luteus*, while conjugates **1**, **2**, and **4** were highly active toward *M. luteus*. For the Gram-negative bacteria and for the enterococcus strain used here, ciprofloxacin alone was still more active than the corresponding β-lactam conjugates **1**, **4**, and **7**. As noted, the hydrophobic protecting group of **7** increased activity against staphylococci and enterococci relative to compounds **1**, **2**, and **4** that are more hydrophobic. Generally, compounds **1** and **2** with hydrophilic properties (or without hydrophobic protecting groups) were found to be more active than conjugate **4** with a hydrophobic (PMB) protecting group. Activity of all compounds against *Pseudomonas aeruginosa* did not depend on outer membrane permeability since essentially the same MIC values were obtained for the wild-type strain and for the mutant strain with an impaired outer membrane barrier. Activity of all compounds (except for the inactive precursor β-lactams **3** and **5**) against *E. coli* was increased for the permeability mutant DC2 in contrast to the wild-type strain DC0.

Compounds **1**, **2**, and **7** also showed good anti-TB activity, but starting GCLE, **4**, and conjugate precursors **3** and **5** displayed weak to no activity. Carbamate-based conjugate **2** was active against *M. tuberculosis* H37Rv

Table 1. Antibacterial activity of quinolone-cephalosporin compounds

Species/strains	MIC (μM)							
	1	**4**	**2**	**7**	Cipro[a]/GCLE tested separately	**3**	**5**	GCLE + Cipro
Escherichia coli DC0 wild type	3.12	25	3.12	1.56	0.4/200	200	>200	3.12
Escherichia coli DC2 permeability mutant	0.8	6.25	0.8	0.2	<0.1/NT[e]	200	200	NT
Enterobacter cloacae P99	0.1	0.4	0.2	<0.1	<0.1/NT	100	200	NT
Micrococcus luteus ATCC 10240	0.4	3.12	0.2	12.5	25/50	100	25	50
Pseudomonas aeruginosa KW 799/WT wild type	1.56	12.5	3.12	1.56	0.8/100	100	100	3.12
Pseudomonas aeruginosa KW 799/61 permeability mutant	1.56	12.5	3.12	1.56	0.4/50	100	100	1.56
Mycobacterium vaccae IMET 10670	3.12	3.12	1.56	1.56	0.8/100	100	200	6.25
Mycobacterium smegmatis SG987	3.12	12.5	3.12	1.56	0.8/NT	>200	200	NT
Mycobacterium aurum SB66	0.4	0.8	0.2	0.2	0.1/NT	100	50	NT
Mycobacterium aurum DSM 43436	0.4	1.56	0.2	0.2	0.1/NT	50	25	NT
Mycobacterium fortuitum B	3.12	12.5	0.8	0.8	0.2/100	200	200	1.56
Staphylococcus aureus SG 511	1.56	1.56	0.4	0.2	0.8/1.56	6.25	3.12	1.56
Staphylococcus aureus 134/93 MRSA	200	>200	200	100	50/NT	>200	>200	NT
Enterococcus faecalis 1528 VRE	12.5	50	6.25	3.12	1.56/NT	200	200	NT
Mycobacterium tuberculosis H37Rv MABA[b]/GAS[c]	3.8	62.3	1.6	8.0	1.8/31.5	>128	>128	1.8
Vero cells IC_{50} (μM)[d]	>128	13.1	>128	>128	NT/NT	NT	NT	NT

[a] Ciprofloxacin from BioChemika.
[b] MABA, microplate alamar blue assay.[15]
[c] GAS, glycerol-alanine-salts.[16]
[d] Concentration resulting in 50% inhibition of the growth of VERO cells.[17]
[e] NT, not tested.

with an MIC of 1.6 μM. Interestingly, its benzhydryl ester **7** also was active with an MIC of 8.0 μM. The amine-linked quinolone-cephalosporin compound **1** was slightly less active than carbamate **2**. Compounds **1**, **2**, and **7** were found not to be cytotoxic against Vero cells, thus, making them worthy of consideration as potential anti-tuberculosis agents.

In summary, we have synthesized direct amine-linked quinolone-cephem **1** and carbamate-linked quinolone-cephem **2**, and evaluated them and their corresponding esters **4** and **7** for antibacterial activity. Of all conjugates, **2** had superior antimycobacterial/anti-TB activity.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2006.08.045.

References and notes

1. Bodiang, C. K. *Scott. Med. J.* **2000**, *45*, 25.
2. VanScoy, R. E.; Wilkowske, C. J. *Mayo Clin. Proc.* **1999**, *74*, 1038.
3. Long, R. *Can. Med. Assoc. J.* **2000**, *163*, 425.
4. Gazdic, A. *Med. Arch.* **1998**, *52*, 207.
5. Pozniak, A. *Int. J. Tuberc. Lung Dis.* **2000**, *4*, 993.
6. Hershberger, P. M.; Demuth, T. P., Jr.. In *Resolving the Antibiotic Paradox: Progress in Understanding Drug Resistance and Development of New Antibiotics*; Rosen, B. P., Mobashery, S., Eds.; Kluwer Academic/Plenum: New York, 1998; Vol. 13, p 239.
7. Stocksdale, M. G.; Ramurthy, S.; Miller, M. *J. Org. Chem.* **1998**, *63*, 1221.
8. Sugiyama, I.; Komatsu, Y.; Yamauchi, H. *J. Antibiot.* **1992**, *45*, 103.
9. Ma, X.; Davis, B. W.; O'hara, T. L.; Anthony, D. R.; Billings, D. M.; Maston, P. A.; White, R. E.; Krauss, H. J.; Berk, J. D.; Wood, N. A.; Loenigs, P. M.; Shah, S. K.; Shinabarger, D. L.; Twinem, T. L.; McOsker, C. C.; Kraft, W. G.; Rourke, F. J.; Demuth, Jr., T. P. In *34th Interscience Conference on Antimicrobial Agents Chemotherapy, Orlando, FL, October 4–7*, 1994.
10. Gonzalez, M.; Rodriguez, Z.; Tolon, B.; Rodriguez, J. C.; Velez, H.; Valdes, B.; Lopez, M. A.; Fini, A. *Il Farmaco* **2003**, *58*, 409.
11. Demuth, T. P., Jr.; White, R. E.; Tietjen, R. A.; Ebetino, F. H.; Kraft, W. G.; Andersen, J. A.; McOsker, C. C.; Walling, M. A.; Davis, B. W.; Rourke, F. J. In *200th National Meeting of the American Chemical Society, Washington, DC, August 20–24*; American Chemical Society: Washington, DC, 1990; MEDI-154.
12. National Committee for Clinical Laboratory Standards. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically, approved standard. NCCLS Document M7-A5, 5th ed.; National Commit-

tee for Clinical Laboratory Standards: Villanova, PA, 2000.
13. Heinisch, L.; Wittmann, S.; Stoiber, T.; Berg, A.; Ankel-Fuchs, D.; Möllmann, U. *J. Med. Chem.* **2002**, *45*, 3032.
14. Afonin, S.; Glaser, R. W.; Berditchevskaja, M.; Wadhwani, P.; Gührs, K-H.; Möllmann, U.; Perner, A.; Ulrich, A. S. *ChemBioChem* **2003**, *4*, 1151.
15. Collins, L.; Franzblau, S. G. *Antimicrob. Agents Chemother.* **1997**, *41*, 1004.
16. De Voss, J. J.; Rutter, K.; Schroeder, B. G.; Su, H.; Zhu, Y., ; Barry, C. E., III *Proc. Natl. Acad. Sci. U.S.A.* **2000**, *97*, 1252.
17. Falzari, K.; Zhu, Z.; Pan, D.; Liu, H.; Hongmanee, P.; Franzblau, S. G. *Antimicrob. Agents Chemother.* **2005**, *49*, 1447.