THE SYNTHESIS AND BIOLOGICAL CHARACTERISTICS OF NEW ORALLY ACTIVE CEPHEMS

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The chemotherapeutic significance of oral antibacterial agents is evidenced by their ability to generate annual sales of several billion dollars. Although many useful agents are currently available, the search for new, more potent compounds with broader spectra of activity continues. Our own efforts in this area have been influenced by the activity and pharmacokinetic properties of FK-482 (**1**).[1,2]

FK-482 is characterized by a (*Z*)-2-(2-aminothiazol-4-yl)-2-hydroxyiminoacetamido group at C-7 of a 3-vinyl cephalosporin nucleus. The 3-vinyl nucleus has previously been shown to support oral bioavailability with other side chains attached to the C-7 nitrogen.[3~5] Thus, we chose to append the (*Z*)-2-(2-aminothiazol-4-yl)-2-hydroxyiminoacetyl group to the 3-chlorocephalosporin and 3-chloro-1-carba-1-dethiacephalosporin nuclei since they serve as the foundation for the well known oral agent Ceclor (cefaclor) and the recently introduced Lorabid (loracarbef).

Experimental

All reactions described herein were performed under an inert atmosphere of dry nitrogen in flame-dried glassware unless otherwise noted. All reagents were used as supplied unless stated otherwise. Melting points were recorded on a Thomas-Hoover apparatus and are uncorrected. ^{1}H NMR spectra were recorded at 300 MHz with a General Electric QE-300 instrument, at 270 MHz with a Brucker W-M instrument and at 90 MHz with a JEOL FX-90 instrument. Chemical shifts are recorded in parts per million (δ) relative to tetramethylsilane. IR spectra were recorded on a Nicolet MX-1 FT-IR, optical rotations were measured on a Perkin-Elmer 241 spectrometer, and UV spectra were obtained on a Cary 219. The mass spectral data were obtained on either a CEC-21-140 or a Varian MAT-731 spectrometer. All MPLC separations were conducted on Merck Lobar columns (LiChroprep RP-18) with the help of a Fluid Metering Inc. pump. Analytical HPLC separations were performed on a Varian chromatographic system utilizing a MicroPak MCH-5 N-cap 15 cm × 4 mm column and a variable wavelength UV detector set to record at 254 nm.

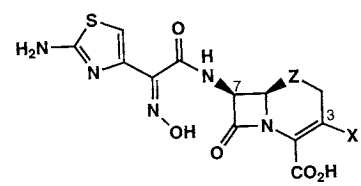
Preparation of *p*-Nitrobenzyl (7*S*,6*R*)-7-[[2-[Tritylamino)-4-thiazolyl]trityloximinoacetyl]amino]-3-chloro-3-cephem-4-carboxylate (**4a**)

To a solution of **2** (1.65 g, 2.46 mmol) in CH_2Cl_2 (25 ml) was added *N*-methylmorpholine (0.5 ml, 4.93 mmol) and $POCl_3$ (0.23 ml, 2.46 mmol) at 0°C and stirred for 15 minutes. To the resulting solution *N*-methylmorpholine (0.5 ml, 4.93 mmol) and **3a** (1.0 g, 2.46 mmol) was added and the reaction stirred for 1 hour at room temperature (RT). The reaction was diluted with EtOAc, washed with 1 N HCl, saturated $NaHCO_3$ solution and brine, dried over $MgSO_4$, filtered and concentrated. The crude product was purified by flash chromatography on silica gel (2% EtOAc-CH_2Cl_2) to give 1.47 g (58%) of **4a** as a light yellow foam: mp >105°C (dec); IR (KBr) cm^{-1} 3379, 3051, 1791, 1738, 1688, 1523, 1492, 1448, 1347, 1217; ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.95 (d, J=12 Hz, 1H), 8.75 (s, 1H), 8.14 (d, J=9 Hz, 2H), 7.71 (d, J=9 Hz, 2H), 7.29~7.08 (m, 30H), 6.56 (s, 1H), 5.96~5.87 (m, 1H), 5.42~5.24 (m, 3H), 3.89 (ABq, J=18 Hz, 2H): UV (EtOH) 248 nm (ε=29,600).

Preparation of (7*S*,6*R*)-7-[[2-[(Tritylamino)-4-thiazolyl]trityloximinoacetyl]amino]-3-chloro-3-cephem-4-carboxylic Acid (**5a**)

To a solution of **4a** (1.45 g, 1.42 mmol) in CH_3CN

Fig. 1.



1 (FK-482)	X=−CH=CH$_2$	Z=S
6a (LY215891)	X=−Cl	Z=S
6b (LY215890)	X=−Cl	Z=CH$_2$

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(140 ml) and H_2O (45 ml) at 40°C was added $NaHCO_3$ (14.28 g, 170 mmol). The suspension was stirred for 1 minute, then $Na_2S_2O_4$ (9.86 g, 56.7 mmol) was added as a solid over 2 minutes with gas evolution. After 6 minutes stirring, the reaction was poured into H_2O - CH_2Cl_2 and the pH was lowered to 3 with concentrated HCl. The aqueous layer was extracted with CH_2Cl_2, the organic layer was washed with brine, dried over Na_2SO_4, filtered and concentrated. The crude product was purified by flash chromatography on silica gel (2.5% AcOH - EtOAc) to yield 740 mg of **5a** (53%) as a yellow solid: ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.56 (d, J=9 Hz, 1 H), 7.78 (s, 1H), 7.38~7.05 (m, 30H), 6.57 (s, 1H), 5.92~5.84 (m, 1H), 5.28 (d, J=6 Hz, 1H), 3.80 (ABq, J=15 Hz, 2H).

Preparation of (7*S*,6*R*)-7-[[(2-Amino-4-thiazolyl)-hydroximinoacetyl]amino]-3-chloro-3-cephem-4-carboxylic Acid (**6a**)

To a solution of **5a** (740 mg, 0.75 mmol) in THF (6 ml) was added 75% formic acid (6 ml) and the reaction was stirred at 45°C for 2.5 hours. The reaction was diluted with CH_3CN and concentrated. The crude product was purified by C_{18} reverse phase medium pressure liquid chromatography (3% CH_3CN - H_2O) to yield 85 mg (27%) of **6a** as an off-white solid: mp >205°C; IR (KBr) cm^{-1} 1764, 1615, 1532, 1357; ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.44 (d, J=8.7 Hz, 1H), 7.11 (s, 2H), 6.61 (s, 1H), 5.62~5.56 (m, 1H), 5.07 (d, J=6.7 Hz, 1H), 3.54 (ABq, J=13.5 Hz, 2H); FAB-MS m/z 426 (M^+), 428 (M^{+2}).

Preparation of *p*-Nitrobenzyl (7*S*,6*R*)-7-[[2-[(Tritylamino)-4-thiazolyl]trityloximinoacetyl]-amino]-3-chloro-1-carba-1-dethia-3-cephem-4-carboxylate (**4b**)

To a solution of **2** (672 mg, 1.0 mmol) in CH_2Cl_2 (20 ml) was added *N*-methylmorpholine (0.22 ml, 2.0 mmol) and $POCl_3$ (0.09 ml, 1.0 mmol) at 0°C and stirred for 10 minutes. To the resulting solution *N*-methylmorpholine (0.22 ml, 2.0 mmol) and **3b** (388 mg, 1 mmol) was added and the reaction stirred for 4 hours at RT. The reaction was diluted with EtOAc, washed with 1 N HCl and brine, dried over $MgSO_4$, filtered and concentrated. The crude product was purified by flash chromatography on silica gel (2×; 40% EtOAc - hexanes followed by 4% EtOAc - CH_2Cl_2) to give 384 mg (38%) of **4b**: mp >140°C (dec); IR (KBr) cm^{-1} 3486, 1780, 1737, 1682, 1523, 1448, 1347, 1206; ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.43 (d, J=9 Hz, 1H), 8.73 (s, 1H), 8.20 (d, J=9 Hz, 2H), 7.68 (d, J=9 Hz, 2H), 7.27~7.07 (m, 30H), 6.57 (s, 1H), 5.58~5.54 (m, 1H), 5.40 (ABq, J=13.5 Hz, 2H), 3.96~3.88 (m, 1H), 2.58~2.40 (m, 2H), 1.78~1.64 (m, 1H), 1.30~1.19 (m, 1H); FD-MS m/z 1,004 (M^+), 1,006 (M^{+2}); UV (EtOH) 250 nm (ε=31,000).

Preparation of (7*S*,6*R*)-7-[[2-[(Tritylamino)-4-thiazolyl]trityloximinoacetyl]amino]-3-chloro-1-carba-1-dethia-3-cephem-4-carboxylic Acid (**5b**)

To a solution of **4b** (310 mg, 0.31 mmol) in CH_3CN (25 ml) and H_2O (10 ml) at 45°C was added $NaHCO_3$ (3.12 g, 37.0 mmol). The suspension was stirred for 1 minute, then $Na_2S_2O_4$ (2.15 g, 12.3 mmol) was added as a solid over 2 minutes with gas evolution. After 5 minutes stirring, the reaction was poured into H_2O - CH_2Cl_2 and the pH was lowered to 4 with concentrated HCl. The aqueous layer was extracted with CH_2Cl_2 (2×) and the combined organics were washed with brine, dried over Na_2SO_4, filtered and concentrated. The crude product was purified by flash chromatography on silica gel (2.5% AcOH - EtOAc) to yield 194 mg of **5b** (62%) as a white solid: ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.45 (d, J=12 Hz, 1H), 8.77 (s, 1H), 7.36~7.03 (m, 30H), 6.59 (s, 1H), 5.54~5.46 (m, 1H), 3.90~3.82 (m, 1H), 2.57~2.23 (m, 2H), 1.76~1.62 (m, 1H), 1.24~1.15 (m, 1H).

Preparation of (7*S*,6*R*)-7-[[(2-Amino-4-thiazolyl)-hydroximinoacetyl]amino]-3-chloro-1-carba-1-dethia-3-cephem-4-carboxylic Acid (**6b**)

To a solution of **5b** (410 mg, 0.47 mmol) in THF (3 ml) was added 50% formic acid (3 ml) and the reaction was stirred at 45°C for 4 hours. The reaction was diluted with CH_3CN and concentrated. The crude product was purified by C_{18} reverse phase medium pressure liquid chromatography (4% CH_3CN - H_2O) to yield 73 mg (38%) of **6b** as an off-white solid: mp >200°C; IR (KBr) cm^{-1} 1751, 1617, 1532, 1357; ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.12 (d, J=9.2 Hz, 1H), 7.10 (s, 2H), 6.65 (s, 1H), 5.29~5.22 (m, 1H), 3.73~3.64 (m, 1H), 2.49~2.21 (m, 2H), 1.86~1.67 (m, 2H); FAB-MS m/z 408 (M^+), 410 (M^{+2}).

Pharmacology

Male Sprague-Dawley rats were dosed intravenously with test compounds at 20 mg/kg in 0.9% saline. Dosing and blood sampling were carried out through an indwelling jugular vein cannula, thus permitting serial sampling from individual rats. Plasma levels were determined from samples col-

lected over a 6-hour time course. Male CD-1 mice were dosed both orally and intravenously with test compound at 20 mg/kg in 0.9% saline. The 0~4 hours cumulative urinary recovery was collected in 0.1 M sodium citrate buffer, pH 6.5, from animals placed in metabolism cages. Plasma and urine samples were stored at −70°C prior to analysis.

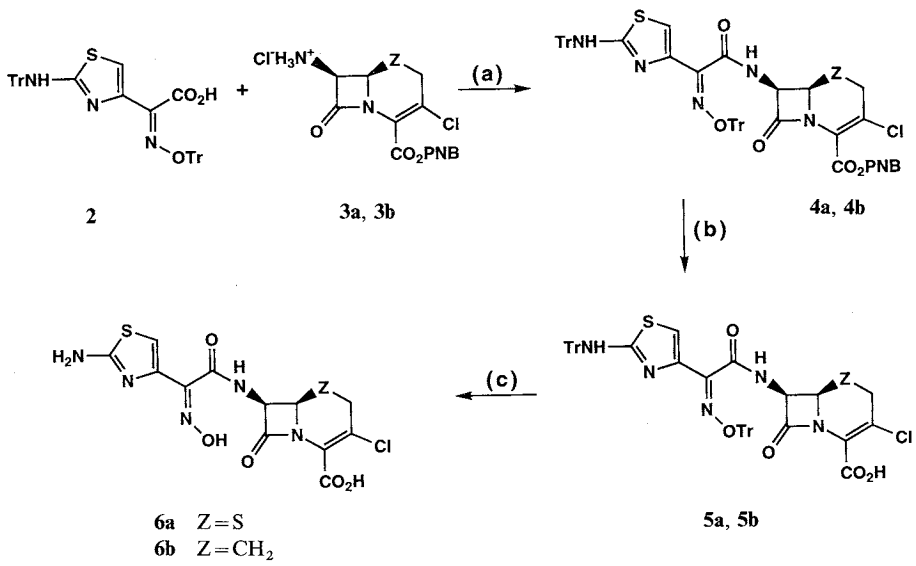
Oral bioavailability was calculated as the oral/intravenous ratio of antibacterial activity recovered in the urine following a 20 mg/kg dose.

Antibiotic concentrations were determined with an agar well diffusion assay (bioassay) employing *Escherichia coli* (ATCC4157) as the bacterial test strain. Standard curves were generated from rat plasma spiked with the compound under study. Urine samples were analyzed by comparison to a standard curve prepared in 0.1 M sodium citrate buffer, pH 6.5. Urine samples were diluted with citrate buffer so that the drug concentration would fall into the range of the standard curve.

Results and Discussion

Preparation of the two compounds was accomplished as shown in Scheme 1. Thus, acylation of the appropriate nucleus followed by removal of the protecting groups provided the desired products. Both **6a** and **6b** exhibited potent Gram-positive and Gram-negative antibacterial activity as determined by an agar dilution method[6] and summarized in Table 1. Unfortunately, the compounds were inactive against *Pseudomonas*.

Scheme 1.



Tr = Triphenylmethyl

Reagents: (a) $POCl_3$, *N*-methylmorpholine, CH_2Cl_2, (b) $NaHCO_3$, $Na_2S_2O_4$, H_2O/CH_3CN, (c) 75% formic acid/THF.

Table 1. Antibacterial activity against selected organisms.

Test organisms	MIC μg/ml		
	1 (FK-482)	**6a** (LY215891)	**6b** (LY215890)
Staphylococcus aureus X1.1	0.5	0.25	1
S. epidermidis 222	0.5	2	4
Streptococcus pneumoniae PARK	0.06	0.125	0.25
Haemophilus influenzae RES 76	0.25	0.125	0.25
Escherichia coli EC14	0.125	0.06	0.06
Klebsiella pneumoniae X26	0.06	0.015	0.06
Enterobacter aerogenes C32	0.05	0.25	0.25
Pseudomonas aeruginosa X239	>128	>128	>128

Table 2. Urinary recovery of antibacterial activity in CD-1 mice.

Compound	Urinary recovery (% dose)		Oral bioavailability[a] (%)
	iv	po	
1 (FK-482)	58.9	15.0	25.5
6a (LY215891)	47.2	≤7.1	≤14.8
6b (LY215890)	49.1	16.2	32.9

[a] Oral bioavailability = urinary recovery (po)/urinary recovery (iv)

The pharmacokinetic profiles of the 3-chloro derivatives **6a** and **6b** were evaluated in male Sprague-Dawley rats following intravenous administration. The drug concentration at 30 minutes was 11.5 μg/ml for **6a** and 14.7 μg/ml for **6b**. The limited sensitivity of the plasma assays precluded half-life determination, however, the results suggest that these compounds were rapidly cleared.

The oral absorption of compounds **1**, **6a** and **6b** was tested experimentally in CD-1 mice. Oral bioavailability was calculated as the po/iv ratio of antibacterial activity recovered in the urine following a 20 mg/kg dose. The results, shown in Table 2, demonstrate that the oral bioavailability of the 1-carbacephalosporin analogue **6b**, while relatively low, is greater than that of **6a** and **1**.

This observation combined with the well documented stability of the 1-carbacephalosporins relative to their 1-sulfur counterparts[7] has prompted us to select **6b** for more detailed studies. In addition, esters of these types of compounds have been demonstrated to exhibit enhanced oral bioavailability.[5,8~11] Thus, our intention is to prepare such ester prodrugs of **6b** in the hope of obtaining well-absorbed antimicrobial agents for clinical studies. The results of these continuing studies will be reported in due course.

Acknowledgments

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