Synthesis and Anaerobic Activity of Novel 1-Carba-1-dethiacephalosporins

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The synthesis and biological evaluation of novel 1-carba-1-dethiacephalosporins exhibiting activity against anaerobic pathogens are described. The nitrothiazole substituent was determined to be crucial to maintaining this activity. The pharmacokinetic parameters and initial toxicological profile of the lead compound are discussed.

The 1-carba-1-dethiacephalosporins represent a new class of antimicrobial agents having great potential for therapeutic use.[1] Loracarbef (Lorabid), the 1-carba analogue of cefaclor, represents the first marketed antibacterial of this class. Our efforts in this area have been directed toward the preparation and evaluation of new 1-carbacephalosporin derivatives taking advantage of their well-documented stability relative to their 1-sulfur counterparts.[2] One of our synthetic forays led us to the preparation of a series of 1-carba-3-thia derivatives which have demonstrated interesting activity against a variety of pathogenic organisms. In this report, we document the discovery, structure–activity relationship, and pharmacokinetics of 1-carba-3-thiathiazole cephalosporins that demonstrate potent activity against a wide array of clinically important anaerobic organisms.

The compounds were prepared via direct displacement of the triflate attached to C3 of a functionalized 1-carbacephalosporin.[3] Thus, treatment of **1** with a heterocyclic thiol in the presence of base provided the fully blocked product **2** (Scheme I). Although the desired Δ^3 isomer (**2**) was the major product in all cases, isomerization of the double bond to the C2–C3 position (the Δ^2 isomer) was observed on occasion. This undesired isomer was readily separated by chromatographic means at this stage or following the final synthetic transformation.[4] Removal of the blocking groups and purification by reverse-phase chromatography provided compounds **3**, suitable for biological evaluation (Table I). Although direct stability determinations on the final compounds were not made, all the 1-carbacephalosporins reported herein appeared stable to standard aqueous manipulations.

The first compound prepared in this series, LY206970 (**3a**), exhibited good activity against Gram-positive and Gram-negative aerobic organisms as shown in Table II. We were quite intrigued to discover that the compound also demonstrated good activity against a range of clinically-significant anaerobic organisms (Table III). Changing the side chain from the fluoroethyl oxime to the methyl oxime (**3b**, LY219641) had no significant effect on the activity against aerobes or anaerobes (Tables II and III).

At this stage in the development of the lead compound, we were concerned about potential toxicity resulting from the nitro aromatic substituent. Due to this concern, compound **3a** was prepared in gram quantities and evaluated to determine its pharmacokinetic parameters. The close analogue **3b** was prepared in quantity to determine its toxicological profile. While these studies were ongoing, we prepared compounds **3c–e** which were similar to the nitrothiazoles due to the presence of a strongly electron-withdrawing group attached to the thiazole ring. Unfortunately, none of these more innocuous-looking analogues exhibited desirable activity against anaerobes (Table III) although their activity against aerobes was similar to that of **3a**. In particular, we were most disappointed with these latter derivatives' lack of activity against the *Bacteroides* strains, in particular, *Bacteroides fragillis*, which represent a significant group of clinically relevant pathogens.

Scheme I

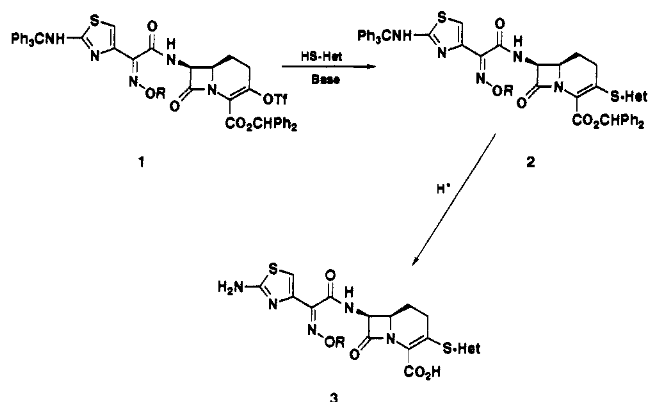

Table I

Compound	-Het	R
LY206970 (3a)	thiazole (S, N), NO_2	$-CH_2CH_2F$
LY219641 (3b)	thiazole (S, N), NO_2	$-CH_3$
LY235535 (3c)	thiazole (S, N), SO_2CH_3	$-CH_3$
LY235879 (3d)	thiazole (S, N), CN	$-CH_3$
LY225789 (3e)	thiazole (S, N), CF_3	$-CH_3$

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Table II. Antibacterial Activity of 1-Carbacephalosporins against Selected Aerobic Strains

test organism	MIC, μg/mL						
	3a	**3b**	**3c**	**3d**	**3e**	cefoxitin	cefotetan
Staphylococcus aureus X1.1	0.125	0.125	0.25	0.25	0.25	4	8
Staphylococcus epidermidis 222	1	0.5	1	1	1	4	16
Streptococcus pneumoniae PARK	0.008	0.008	0.008	0.008	0.008	0.5	4
Haemophilus influenzae RES 76	0.008	0.06	0.015	0.125	0.06	2	1
Escherichia coli EC14	0.5	0.5	0.06	0.5	0.25	2	0.125
Klebsiella pneumoniae X26	0.008	0.125	0.03	0.25	0.03	1	0.125
Enterobacter aerogenes C32	1	2	0.25	0.5	0.5	>128	8
Pseudomonas aeruginosa X239	>128	>128	>128	128	128	>128	>128

Table III. Antibacterial Activity of 1-Carbacephalosporins against Selected Anaerobic Strains

test organism	MIC, μg/mL						
	3a	**3b**	**3c**	**3d**	**3e**	cefoxitin	cefotetan
Clostridium difficile 2994	4	2	0.125	0.06	0.06	32	16
Clostridium perfringens 81	1	2	0.25	0.25	0.06	2	1
Clostridium septicum 1128	1	≤0.5	16	1	0.5	4	4
Eubacterium aerofaciens 1235	4	8	32	16	16	8	4
Peptococcus asaccharolyticus 1302	≤0.5	≤0.5	0.25	0.25	0.125	≤0.5	1
Peptococcus prevoti 1281	≤0.5	≤0.5	0.25	0.5	0.5	1	8
Peptostreptococcus anaerobius 1451	≤0.5	≤0.5	0.5	0.5	0.5	2	128
Peptostreptococcus intermedius 1264	≤0.5	≤0.5	0.03	0.03	0.015	2	8
Propionibacterium acnes 79	≤0.5	≤0.5	0.03	0.03	0.008	2	1
Bacteroides fragillis 111	4	8	>128	>128	128	8	8
Bacteroides fragillis 1877	1	2	32	16	4	4	8
Bacteroides fragillis 1936B	4	4	>128	>128	128	8	8
Bacteroides thetaiotaomicron 1438	≤0.5	≤0.5	2	4	4	2	1
Bacteroides melaninogenicus 1856/28	2	4	0.125	0.25	0.125	4	128
Bacteroides melaninogenicus 2736	1	4	32	32	32	8	128
Bacteroides vulgatis 1211	1	2	16	16	8	8	4
Bacteroides corrodens 1874	8	8	>128	>128	128	4	8
Fusobacterium symbosium 1470	1	1	8	2	2	8	128
Fusobacterium necrophorum 6054A	≤0.5	≤0.5	0.008	0.25	0.25	≤0.5	>128

The pharmacokinetic profile of compound **3a** was evaluated in male Sprague–Dawley rats. Following intravenous administration the C_{max} was 54.2 μg/mL and the area under the plasma time curve (AUC) was 11.1 μg·h/mL. The limited sensitivity of the bioassay, the result of high (98%) plasma protein binding, precluded determination of the plasma half-life. The urinary recovery was very low at 5.2% of the administered dose. This could result from either biliary elimination, metabolism, or renal elimination followed by chemical breakdown of **3a** in the urine; however, at the present time we can not discriminate among these possibilities.

Although the pharmacokinetic parameters of this compound were not extremely desirable, further investigations were suspended when it was determined that the nitrothiazole analogue **3b** was mutagenic in L5178Y mouse lymphoma cells.[5] Unfortunately, we have not found any other 1-carbacephalosporin derivatives to date which exhibit the significant activity against anaerobic organisms demonstrated by **3a** and **3b**. Clearly, this unique activity is a function of the nitro substituent, although the exact nature of the effect is not known at the present time.

Experimental Section

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-5N-cap 15 cm × 4 mm column and a variable-wavelength UV detector set to record at 254 nm.

Benzhydryl [7*S*,6*R*)-7-[[[(2-Tritylamino)-4-thiazolyl][(2-fluoroethyl)oximino]acetyl]amino]-3-[(4- or 5-substituted-thiazol-2-yl)thio]-1-carba-1-dethia-3-cephem-4-carboxylate (2). A solution of the appropriate 2-mercaptothiazole (1.2 equiv)[6-9] and 1 (1 equiv) in CH_3CN (to provide a concentration of ca. 1.5 M in 1) was treated with diisopropylethylamine (1.2 equiv) and the resulting solution stirred overnight at room temperature. The reaction was concentrated and the crude product was purified by flash chromatography on silica gel (10% EtOAc/CH_2Cl_2) to provide the desired products **2a–e** in yields of 11–98%. The spectral data for **2a–e** are available as supplementary material.

(7*S*,6*R*)-7-[[(2-Amino-4-thiazolyl)[(2-fluoroethyl)oximino]acetyl]amino]-3-(4- or 5-substituted-thiazol-2-yl)thio]-1-carba-1-dethia-3-cephem-4-carboxylic Acid (3a–e). To a solution of 2 (1 equiv) and triethylsilane (4 equiv) in CH_2Cl_2 (8 mL/g of 2) at 0 °C was added a volume of trifluoroacetic acid equal to that of the CH_2Cl_2, and the resulting reaction was stirred for 15 min. The cooling bath was removed and the reaction stirred for an additional 15 min at room temperature. The reaction was diluted with toluene and CH_3CN and concentrated. The crude product was purified by reverse-phase MPLC eluting with 10–25% CH_3CN/H_2O to provide the desired product 3 in yields of 25–52%. Spectral data for **3a–e** are available as supplementary material.

Determination of Minimum Inhibitory Concentration (MIC). The minimum inhibitory concentrations for aerobic bacteria were determined according to the agar dilution method given in the National Committee for Clinical Laboratory Standards document M7-A2, Vol. 10, no. 8, "Methods for Dilution Antimicrobial Susceptibility Test for Bacteria that Grow Aer-

obically", 2nd ed. The minimum inhibitory concentrations for anaerobic bacteria were determined according to the agar dilution method given in the NCCLS document M11-A2, Vol. 10, no. 15, "Methods for Antimicrobial Testing of Anaerobic Bacteria", 2nd ed.

Determination of Pharmacokinetic Profile. Male Sprague–Dawley rats were dosed intravenously with **3a** at 20 mg/kg in saline. Dosing and blood sampling were carried out through an indwelling jugular vein cannula, thus permitting serial sampling from individual rats. Plasma levels and cumulative urinary recoveries were determined from samples collected over a 6-h time course. Antibiotic concentrations were determined with an agar well diffusion assay (bioassay) employing *Micrococcus luteus* (ATCC9241) as the bacterial test strain. The detection limit of the assay was 3.2 μg/mL of plasma. AUC was calculated using Simpson's rule. Urinary recovery was calculated as the percent of the administered dose recovered in the urine.

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Supplementary Material Available: Experimental data (melting point, IR, NMR, and MS) for 2a–e and 3a–e (2 pages). Ordering information is given on any current masthead page.

References

(1) Copper, R. D. G. Novel β-lactam structures—The carbacephems. In *The Chemistry of Beta-Lactams*; Page, M. E. Ed.; Chapman and Hall: London, 1992; pp 272–305.

(2) Blaszczak, L. C.; Brown, R. F.; Cook, G. K.; Hornback, W. J.; Hoying, R. C.; Indelicato, J. M.; Jordan, C. L.; Katner, A. S.; Kinnick, M. D.; McDonald, J. H., III; Morin, J. M., Jr.; Munroe, J. E.; Pasini, C. E. Comparative Reactivity of 1-Carba-1-dethiacephalosporins with Cephalosporins. *J. Med. Chem.* **1990**, *33*, 1656–1662.

(3) Cook, G. K.; McDonald, J. H., III; Alborn, W., Jr.; Boyd, D. B.; Eudaly, J. A.; Indelicato, J. M.; Johnson, R.; Kasher, J. S.; Pasini, C. E.; Preston, D. A. 3-Quaternary Ammonium 1-Carba-1-dethiacephems. Wu, C. Y. E. *J. Med. Chem.* **1989**, *32*, 2442–2450.

(4) During large-scale synthesis of LY219641 (**5**) it was discovered that formation of the Δ^2 isomer could be avoided by using benzhydryl (7*S*,6*R*)-7-(phenoxyacetamido)-3-[[(trifluoromethyl)sulfonyl]oxy]-1-carba-1-dethia-3-cephem-4-carboxylate as starting material instead of benzhydryl (7*S*,6*R*)-7-[[[(2-tritylamino)-4-thiazolyl]-methoximinoacetyl]amino]-3-[[(trifluoromethyl)sulfonyl]oxy]-1-carba-1-dethia-3-cephem-4-carboxylate and reacting this with the sodium salt of the 2-mercapto-5-nitrothiazole (formed by reaction of the free SH thiazole with NaH). This resulted in the formation of an orange, crystalline solid that contained only the Δ^3 isomer.

(5) Richardson, K. K., Toxicology division, Lilly Research Laboratories, unpublished observations.

(6) Scott, W.; Watt, G. W. Reactions in the Thiazole Series. I. Reactions of 2-Chlorobenzothiazoles with Thioureas. *J. Org. Chem.* **1937**, *2*, 148–156.

(7) Yamamoto, Y. On the Synthesis and Antibacterial Activity of 2-Amino-4-methyl-5-thiazolyl Alkyl Sulfides and Sulfones. *J. Pharm. Soc. Jpn.* **1952**, *72*, 1124–1128. Kanno, S.; Ohno, M. Reactions of p-Nitrophenylsulfenylchloride on Aromatic Heterocyclic Compounds. I. Synthesis of Thiazolylsulfones. *J. Pharm. Soc. Jpn.* **1949**, *69*, 503–507.

(8) Doyle, M. P.; Siegfried, B.; Dellaria, J. F., Jr. Alkyl Nitrite–Metal Halide Deamination Reactions. 2. Substitutive Deamination of Arylamines by Alkyl Nitrites and Copper(II) Halides. A Direct and Remarkably Efficient Conversion of Arylamines to Aryl Halides. *J. Org. Chem.* **1977**, *42*, 2426–2431.

(9) Buchman, E. R.; Reims, A. O.; Sargent, H. Mercaptothiazoles: Oxidation and Alkylation Studies. *J. Org. Chem.* **1941**, *6*, 764–773.