Communications to the editor

SYNTHESIS AND *IN VITRO* ANTIBACTERIAL ACTIVITY OF 7-TRIFLUOROMETHYLTHIOACETAMIDO CEPHAMYCINS RELATED TO SK&F 59962 (CEFAZAFLUR)

Sir:

Various 7α-methoxycephalosporins (cephamycins) have been reported to show enhanced antibacterial activity against certain gram-negative organisms relative to their unmethoxylated analogs.[1,2] Consequently, several synthetic methods have been developed for producing cephamycin analogs by introducing a 7α-methoxy group into the cephalosporin structure.[3~7] This enables one to determine whether the introduction of a 7α-methoxy group into an already promising cephalosporin molecule would improve its antimicrobial profile. Previously we reported the broad-spectrum antibacterial activities of 7-trifluoromethylthioacetamido-3-(1-methyl-1H-tetrazol-5-ylthiomethyl)-3-cephem-4-carboxylic acid (cefazaflur, compound **9**) and some closely related analogs.[8] We have extended this work to several of the corresponding 7α-methoxy analogs. The synthesis and *in vitro* antibacterial activities of these derivatives are reported here.

Cephalosporins **7**, **8** and **9** were prepared by acylation of the appropriate 7-amino-3-cephem-4-carboxylic acids with the *N*-hydroxysuccinimide ester of trifluoromethylthioacetic acid as described previously.[8] Attempts to introduce the 7α-methoxy group directly into **9** by treatment of its *tert*-butyl ester with lithium methoxide and *tert*-butyl hypochlorite[5] were unsuccessful. Since it appeared unlikely that this procedure would be more successful when applied to analogs having at the 3 position substituents other than methyltetrazolethiomethyl, the 7α-methoxycephalosporins **4** and **5**, as well as **6**, were synthesized by coupling the appropriate *tert*-butyl 7-amino-7α-methoxy-3-cephem-4-carboxylates (**1**, **2** and **3**) to trifluoromethylthioacetic acid with DCC. Intermediate **1** was prepared from 7-ADCA *tert*-butyl ester by thiomethylation of its benzaldehyde SCHIFF base followed by mercury-catalyzed methoxy exchange.[4] Compound **2** was obtained by direct methoxylation of the *p*-nitrobenzylcarbamate of 7-ACA *tert*-butyl ester with lithium methoxide and *tert*-butyl hypochlorite.[6] The previously unreported **3** was synthesized from *tert*-butyl-7-amino-3-(1-methyl-1H-tetrazol-5-ylthiomethyl)-3-cephem-4-carboxylate[9] by a procedure analogous to that used to obtain **1**. The *tert*-butyl esters were cleaved by treatment with trifluoroacetic acid and the cephamycins, **4**, **5** and **6**, were converted to their sodium salts with 30% sodium 2-ethylhexanoate in isopropyl alcohol. The ir, nmr and elemental analyses of all final products were consistent with their structure.

The *in vitro* antibacterial activities of the three cephamycins (**4**, **5** and **6**) as well as the corresponding cephalosporins (**7**, **8** and **9**) are presented in Table 1. Inspection of these data reveals several distinct differences in the spectrum of activities of the methoxylated versus unmethoxylated analogs. Insertion of the methoxyl group into the 7-ADCA derivative **7** results in a compound (**4**) which is devoid of antibacterial activity. This agrees with previously reported results for the 7-thienylacetamido-7α-methoxydesacetoxycephalosporanic acid.[4] For the analogs which

Scheme 1. Synthesis of 7-trifluoromethylthioacetamido cephamycins

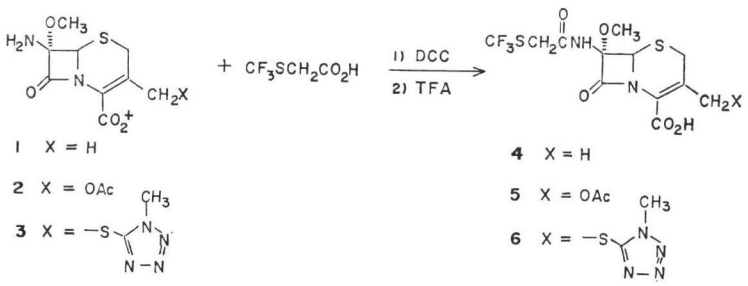
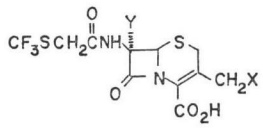
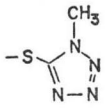
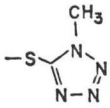


Table 1. *In vitro* activities of 7-trifluoromethylthioacetamido cephalosporins and cephamycins



Compound	Y	X	Minimum inhibitory concentration (μg/ml)*									
			1 *S.a.*(R)	2 *S.a.*(S)	3 *E.c.*	4 *K.p.*	5 *Sal.p.*	6 *Sh.p.*	7 *Ent.a.*	8 *Ent.c.*	9 *Ser.m.*	10 *Pr.m.* (+)
4	OCH_3	H	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200
5	OCH_3	OAc	1.6	3.1	6.3	6.3	3.1	1.6	12.5	6.3	12.5	12.5
6	OCH_3		0.8	1.6	3.1	1.6	1.6	1.6	6.3	1.6	6.3	3.1
7	H	H	6.3	6.3	25	25	25	50	200	50	>200	NT
8	H	OAc	0.4	0.4	1.6	1.6	0.4	3.1	3.1	3.1	>200	200
9**	H		0.4	0.2	0.4	0.4	0.8	0.8	1.6	1.6	200	50
	Cephalothin		0.4	0.2	3.1	1.6	0.8	1.6	12.5	6.3	>200	>200
	Cefoxitin		1.6	3.1	6.3	12.5	6.3	3.1	200	25	25	25

* The *in vitro* antibacterial activities are reported as minimum inhibitory concentrations (MIC) in μg/ml. The MICs were determined by the twofold agar dilution method on Trypticase soy agar buffered to pH 6.0. Organisms selected for inclusion in this table are: *S.a.* (R), *Staphylococcus aureus* HH 127 (penicillin G resistant); *S.a.* (S), *Staphylococcus aureus* 23390 (Smith); *E.c.*, *Escherichia coli* 12140; *K.p.*, *Klebsiella pneumoniae* 4200; *Sal.p.*, *Salmonella paratyphi* ATCC 12176; *Sh.p.*, *Shigella paradysenteriae* HH 117; *Ent.a.*, *Enterobacter aerogenes* ATCC 13048; *Ent. c.*, *Enterobacter cloacae* HH 31254; *Ser.m.*, *Serratia marcescens* ATCC 13880; *Pr.m.* (+), *Proteus morganii* 179 (indole-positive). NT=not tested.

** Cefazaflur, SK&F 59962.

have acetoxymethyl or methyltetrazolethiomethyl at the 3 position, the gram-positive activities (organisms 1 and 2) of the cephamycins are lower than those of the corresponding cephalosporins. This is also true for the activity against those gram-negative bacteria (organisms 3~8) which are usually sensitive to cephalosporins. On the other hand, these cephamycins are more active against gram-negative organisms 9 and 10 which are relatively insensitive to cephalosporins. *In vitro* activities of cephamycins **5** and **6** compare favorably with those of cefoxitin[10,11] throughout the entire spectrum studied. Based on these data, compound **9** (cefazaflur) still appears to provide the most advantageous combination of gram-positive and gram-negative activities. However, the favorable broad-spectrum antibacterial activity of compound **6** as compared to that of cefoxitin calls for further biological studies of this cephamycin analog.

Acknowledgement

We wish to thank Dr. G. L. DUNN and Dr. J. R. E. HOOVER for their advice and assistance during the course of this work and in the preparation of the final manuscript.

R. M. DEMARINIS
J. V. URI
J. A. WEISBACH

Smith Kline & French Laboratories
1500 Spring Garden Street, Philadelphia,
Pennsylvania 19101, U.S.A.

(Received July 12, 1976)

References

1) NAGARAJAN, R.; L. D. BOECK, M. GORMAN, R. L. HAMILL, C. E. HIGGENS, M. M. HOEHN, W. M. STARK & J. G. WHITNEY: β-Lactam antibiotics from *Streptomyces*. J. Am. Chem. Soc. 93: 2308~2310, 1971
2) STAPLEY, E. O.; M. JACKSON, S. HERNANDEZ, S. B. ZIMMERMAN, S. A. CURRIE, S. MOCHALES, J. M. MATA, H. B. WOODRUFF & D. HENDLIN: Cephamycins, a new family of β-lactam antibiotics. I. Production by Actinomycetes, including *Streptomyces lactamdurans* nov. sp. Antimicr. Agents & Chemoth. 2: 122~131, 1972
3) CAMA, L. D.; W. J. LEANZA, T. R. BEATTIE & B. G. CHRISTENSEN: Substituted penicillin and cephalosporin derivatives. I. Stereospecific introduction of the C-6(7) methoxy group. J. Am. Chem. Soc. 94: 1408~1410, 1972
4) JEN, T.; J. FRAZEE & J. R. E. HOOVER: A stereospecific synthesis of $C_{6(7)}$ methoxypenicillin and -cephalosporin derivatives. J. Org. Chem. 38: 2857~2859, 1973
5) KOPPEL, G. A. & R. E. KOEHLER: Functionalization of $C_{6(7)}$ of penicillins and cephalosporins. A one-step stereoselective synthesis of 7α-methoxycephalosporin C. J. Am. Chem. Soc. 95: 2403~2404, 1973
6) LUNN, W. H. W. & E. V. MASON: The synthesis of 7α-methoxy-7β-amidocephalosporanic acids by methoxylation of 7β-(*p*-nitrobenzyloxycarboxamido) cephalosporanic acid. Tetrahedron Lett. 1974: 1311~1313, 1974
7) YANAGISAWA, H.; M. FUKUSHIMA, A. ANDO & H. NAKAO: A novel general method synthesizing for 7α-methoxycephalosporins. Tetrahedron Lett. 1975: 2705~2708, 1975, and references therein.
8) DEMARINIS, R. M.; J. R. E. HOOVER, G. L. DUNN, P. ACTOR, J. V. URIL & J. A. WEISBACH: A new parenteral cephalosporin, SK&F 59962: 7-Trifluoromethylthioacetamido-3-(1-methyl-1H-tetrazol-5ylthiomethyl)1-3-cephem-4-carboxylic acid. Chemistry and structure-activity relationships. J. Antibiotics 28: 463~470, 1975
9) DEMARINIS, R. M.; J. R. E. HOOVER, L. L. LAM, J. V. URI, J. R. GUARINI, L. PHILLIPS, P. ACTOR & J. A. WEISBACH: Semisynthetic cephalosporins. Synthesis and structure-activity relationships of 7-sulfonylacetamido-3-cephem-4-carboxylic acids. J. Med. Chem. 19: 754-759, 1976
10) WALLICK, H. & D. HENDLIN: Cefoxitin, a semisynthetic cephamycin antibiotic: susceptibility studies. Antimicr. Agents & Chemoth. 5: 25~32, 1974
11) HAMILTON-MILLER, J. M. T. & W. BRUMFITT: Cephamycins: A review, prospects and some original observations. Infection 3: 183~188, 1975