Synthesis and Antibacterial Activities of New (α-Hydrazinobenzyl)cephalosporins

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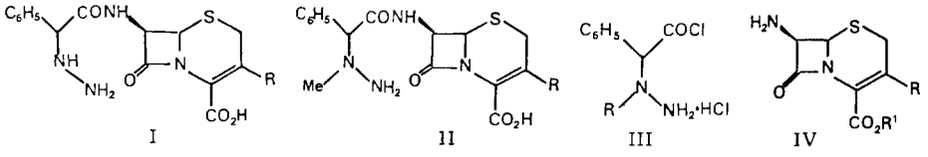
Received December 30, 1982

Some (α-hydrazinobenzyl)cephalosporins, I (R = Me, CH_2OAc, Cl) and II (R = Me, CH_2OAc), structurally related

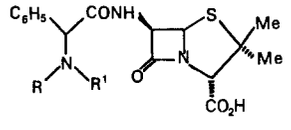


I II III IV

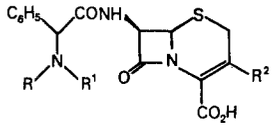
to cephalexin, cephaloglycin, and cefaclor have been prepared and evaluated in vitro for their antimicrobial activity. The synthesis involves the condensation of the chloride hydrochloride III (R = H or Me) with the 7-aminocephem derivatives IV. The hydrazino compound I (R = Cl), an analogue of cefaclor, resulted in being the most active compound of the series.

A great effort has been made to relate the chemical features of semisynthetic cephalosporins and penicillins to their potency, broadness of spectrum of activity, stability to acids, and resistance to enzymic inactivation, in order to design new drugs with superior antimicrobial and pharmacological properties.[1] Nevertheless, it is very hard to foresee at present the biological activity of newly designed β-lactam antibiotics.

A large number of α-substituted derivatives of phenylacetyl-APA, -ACA, and -ADCA have been synthesized. The introduction in the α position of a hydrophilic substituent, in particular an amino group, leads to a widening of the broadness of the spectrum of the activity, in particular against Gram-negative bacteria.[1e] Furthermore, (α-amino-α-phenylacetamido)penicillin [ampicillin (1)][1b,e] and -cephalosporins [e.g., cephalexin (3) and cephaloglycin (4)][1b-e] are stable to gastric acids and have effective intestinal absorption.



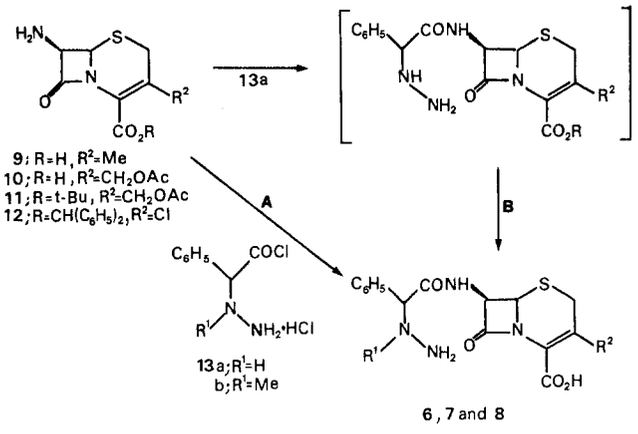
1 ; R = R¹ = H
2a; R = H, R¹ = NH_2
b; R = Alkyl, R¹ = NH_2



3 ; R = R¹ = H, R² = Me
4 ; R = R¹ = H, R² = CH_2OAc
5 ; R = R¹ = H, R² = Cl
6 a; R = H, R¹ = NH_2, R² = Me
b; R = Me, R¹ = NH_2, R² = Me
7 a; R = H, R¹ = NH_2, R² = CH_2OAc
b; R = Me, R¹ = NH_2, R² = CH_2OAc
8 ; R = H, R¹ = NH_2, R² = Cl

In a previous paper,[2] the α-amino group of ampicillin (1) was replaced with the bulkier and more polar hydrazino group (unsubstituted or *N*-alkyl substituted) in order to get improved Gram-negative antibacterial activity and penicillinase resistance without loss of stability to acids. It was found that these replacements lead to compounds **2** with a broad spectrum of antibacterial activity both in vitro and in vivo. In addition, the (α-hydrazinobenzyl)-penicillins **2** are acid resistant and exhibit moderate penicillinase resistance. The most active penicillins in this series appeared the ones with the unsubstituted hydrazino group (**2a**) and the *N*-methyl substituted compound (**2b**, R = Me).[3] On the basis of these results we planned the synthesis and microbiological evaluation of the (α-hydrazino-α-phenylacetamido)cephalosporin derivatives **6** and **7**, in which **6a** and **7a** correspond to cephalexin (**3**) and cephaloglycin (**4**), respectively. Furthermore, we extended our research in preparing the α-hydrazino-α-phenylacetamido derivative (**8**) of 7β-amino-3-chloro-3-cephem-4-carboxylic acid, taking as a model cefaclor (**5**), which is an orally active cephalosporin with better Gram-negative antibacterial activity than cephalexin itself.[1d]

Scheme I



9; R = H, R² = Me
10; R = H, R² = CH_2OAc
11; R = t-Bu, R² = CH_2OAc
12; R = $CH(C_6H_5)_2$, R² = Cl

13a; R¹ = H
b; R¹ = Me

6, 7 and 8

The amino group in the side chain of the (α-aminobenzyl)penicillin (1) and -cephalosporins (**3–5**) created a new chiral center, and the *R* epimers of these compounds exhibit much higher activity, especially against Gram-negative bacteria, than the *S* isomers.[1b,c] The same trend was observed in the case of (α-hydrazinobenzyl)penicillins. Therefore, the cephalosporins under examination (**6–8**) were prepared with the α-hydrazino-α-phenylacetamido side chain having the *R* configuration while the α-(1-methylhydrazino)-α-phenylacetamido derivative **6b** was obtained as an epimeric mixture.

Chemistry. The (α-hydrazinobenzyl)cephalosporins **6–8** have been prepared as shown in Scheme I, according to method A or B.

Method A involves the reaction, in the presence of 1,2-propylene oxide,[4] of suitable 3-substituted 7β-amino-3-

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(2) Libassi, G.; Monguzzi, R.; Broggi, R.; Broccali, G.; Carpi, C.; Pifferi, G. *J. Antibiot.* **1977**, *30*, 376.
(3) Pifferi, G.; Nasi, F.; Monguzzi, R.; Pinza, M.; Broccali, G., *Proc. Int. Congr. Chemother. 10th* **1978**, *1*, p 609.
(4) Looker, B. E.; Attenburrow, J. I.; Wilson, E. M. German Offen. 2 063 268, 1971.

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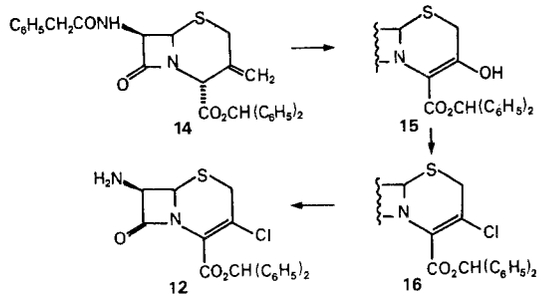
Scheme II



cephem-4-carboxylic acids (**9** and **10**), protected as trimethylsilyl esters, with the acid chloride of the α-hydrazino-α-phenylacetic acid hydrochlorides (**13a** or **13b**).[2] The *R* form of these acids has been used in all the cases, except for the preparation of **6b**, in which the *RS* form has been used. This reaction was followed by mild hydrolysis to the free acids **6** and **7b**. Method B consists of the reaction of the respective *tert*-butyl and benzhydryl esters of 3-substituted 7β-amino-3-cephem-4-carboxylic acid (**11** and **12**) with the acid chloride hydrochloride **13a**, in the presence of 1,2-propylene oxide. The intermediate esters were directly deprotected by treatment with trifluoroacetic acid and anisole, and the free acids (**7a** and **8**) were isolated in pure form.

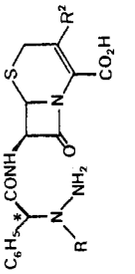
The benzhydryl ester of 3-chloro-7β-amino-3-cephem-4-carboxylic acid (**12**), previously reported in the patent literature,[5] has been prepared (Scheme II) from the known 3-methylenecepham derivative **14**[6a] in the following manner. Ozonolysis of **14** according to previously reported methods[6] gave the 3-hydroxy-3-cephem derivative **15**, which was treated with $SOCl_2$ in DMF to afford the corresponding 3-chloro-3-cephem derivative **16**. Deacylation of **16** with PCl_5 in dry pyridine, followed by the addition of isobutyl alcohol, led to the desired intermediate **12**.

All the new (α-hydrazinobenzyl)cephalosporins show their zwitterionic nature by displaying ammonium and carboxylate stretching bands at 2600-2100 and ~1600 cm^{-1}, respectively. The ^1H NMR spectra exhibit a 0.2–0.3-ppm upfield shift of the benzylic proton with respect to the corresponding (α-aminobenzyl)cephalosporins. This may be due to the increased distance of the benzylic proton from the center of salification in the hydrazino compounds.

Results

The minimum inhibitory concentrations of the newly synthesized cephalosporins are reported in Table I, together with those of cephalexin, cephaloglycin, and cefaclor taken as reference standards. Contrary to what could be expected on the basis of previous results in the penicillin series,[2,3] replacement of the amino group of the model with a hydrazino group led to cephalosporins less active both on Gram-positive and Gram-negative strains. The comparison between the activities of the couples of compounds **6a**, **7a** and **6b**, **7b** shows that the 1-methyl substitution on the hydrazine group improves the activity on Gram-positive and Gram-negative sensitive bacteria, as well as on Gram-positive resistant strains. Also, in the case of the hydrazino compounds, the 3-chloro substitution gives the most interesting derivative, **8**. The activity of **8** against

Table I. Physical Properties and Antibacterial Activities of Cephalosporin Compounds



compd	R	R^2	config at C*	mp, °C	method	yield, %	$[\alpha]^{20}D$, deg	formula[a]	MIC,[b] μg/mL: Gram positive	Gram-positive resistant	Gram negative	Gram-negative resistant
6a	H	CH_3	*R*	158–160	A	51	+83.4[c]	$C_{16}H_{18}N_4O_4S$	12.50	62.9	>100	>100
6b	CH_3	CH_3	*RS*	185–190	A	47	+116.2[c]	$C_{17}H_{20}N_4O_4S$	6.74	50.0	100	>100
7a	H	CH_2OAc	*R*	dec	B	39	+57.0[d]	$C_{18}H_{20}N_4O_6S$	1.68	31.5	73.4	>100
7b	CH_3	CH_2OAc	*R*	183–186	A	36	+61.2[e]	$C_{19}H_{22}N_4O_6S$	1.33	24.9	54.5	>100
8	H	Cl	*R*	202	B	34	+22.6[d]	$C_{15}H_{15}ClN_4O_4S$	1.33	31.4	21.4	50
cephalexin (3)									0.58	24.9	5.7	7.8
cephaloglycin (4)									1.33	19.8	8.5	79.3
cefaclor (5)									0.16	15.7	1.0	7.8

[a] All compounds were analyzed for C, H, Cl, N, and S. [b] The in vitro antibacterial activities were evaluated by the twofold serial agar dilution method with a multiinocular device (see ref 7). Results are reported as geometrical averages of the MIC's. [c] *c* 0.05 in pH 4.4 buffer. [d] *c* 0.2 in THF/1 N HCl (1:1). [e] *c* 1 in 1 N HCl.

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(6) (a) Scartazzini, R.; Bickel, H. *Helv. Chim. Acta* **1974**, *57*, 1919. (b) Chauvette, R. R.; Pennington, P. A. *J. Am. Chem. Soc.* **1974**, *96*, 4986. (c) Chauvette, R. R.; Pennington, P. A. *J. Med. Chem.* **1975**, *18*, 403.
(7) Steers, E.; Foltz, E. L.; Graves, B. S. *Antibiot. Chemother.* **1959**, *9*, 307.

Gram-positive bacteria is comparable to that of cephaloglycin, whereas the activity toward Gram-negative bacteria is moderately lower. However, the lower activity of 8 vs. cefaclor discouraged further in vitro and in vivo studies.

Experimental Section

Melting points were determined with a Mettler FP 52 apparatus equipped with a Reichert Neovar microscope and are uncorrected. IR spectra were taken on paraffin oil mulls on Perkin-Elmer 157 and 197 spectrometers. ^{1}H NMR spectra were detected with a Perkin-Elmer R 12B 60-MHz spectrometer with Me_4Si or sodium 3-(trimethylsilyl)-1-propanesulfonate (DSS) as internal standards. Evaporations were made in vacuo (rotating evaporator). Magnesium sulfate was always used as the drying agent.

Benzhydryl 3-Chloro-7β-(α-phenylacetamido)-3-cephem-4-carboxylate (16). A solution of benzhydryl 3-hydroxy-7β-(α-phenylacetamido)-3-cephem-4-carboxylate (15; 2.0 g, 4.0 mmol) [prepared as previously described[6a] by ozonolysis of benzhydryl 3-methylene-7β-(α-phenylacetamido)cepham-4α-carboxylate (14)] in anhydrous DMF (10 mL) was treated with thionyl chloride (0.86 mL, 12 mmol) and stirred at room temperature for 5 h. The mixture was diluted with ethyl acetate and washed with aqueous $NaHCO_3$. The organic phase was dried and treated with charcoal. The solvent was evaporated, and the residue was triturated with Et_2O to give 16 (1.56 g, 75%): mp 162–164 °C dec; IR ν_{max} 1775 (β-lactam), 1725 (ester), 1655 (amide) cm^{-1}; ^{1}H NMR (Me_2SO-d_6) δ 3.47 (s, 2, PhCH_2), 3.75 and 3.99 (AB q, 2, J = 18.6 Hz, SCH_2), 5.26 (d, 1, J = 4.6 Hz, CHS), 5.81 (dd, 1, J = 8.2 and 4.6 Hz, NHCH), 7.03 (s, 1, CHPh$_2$). Anal. ($C_{28}H_{23}ClN_2O_4S$) C, H, N, Cl.

Benzhydryl 7β-Amino-3-chloro-3-cephem-4-carboxylate (12). A stirred solution of 16 (1.45 g, 2.8 mmol) in CH_2Cl_2 (5 mL) was cooled to -20 °C and treated with anhydrous pyridine (5 mL and PCl_5 (0.87 g, 4.2 mmol). The mixture was stireed at -15 °C for 2 h and then treated with isobutyl alcohol (10 mL); after 2 h, it was treated with H_2O and adjusted to pH 7.5 with aqueous NH_3. The organic phase was dried, treated with charcoal, and evaporated to dryness. The residue was purified by column chromatography on silica gel, eluting with a 1:1 mixture of ethyl acetate–hexane, to yield 12 (0.3 g, 27%): mp 158–162 °C dec; IR ν_{max} 1770 (β-lactam), 1735 (ester) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 3.43 and 3.75 (AB q, 2, J = 18.6 Hz, CH_2S), 4.68 (d, 1, J = 4.6 Hz, CHS), 4.93 (d, 1, J = 4.6 Hz, NH_2CH), 7.04 (s, 1, CHPh$_2$). Anal. ($C_{20}H_{17}N_2O_3S$) Cl, N, S.

General Procedure for the Preparation of (α-Hydrazinobenzyl)cephalosporins 6a,b and 7b. Method A. This method is illustrated by the synthesis of 7β-[(*R*)-α-hydrazino-α-phenylacetamido]-3-methyl-3-cephem-4-carboxylic acid (6a). A mixture of 9 (1.07 g, 5 mmol) and hexamethyldisilazane (1.25 mL, 6 mmol) in anhydrous MeCN 10 mL) and CH_2Cl_2 (10 mL) was refluxed for 1 h, and the solvent was evaporated at reduced pressure. To a residue, dissolved in anhydrous MeCN (15 mL), was added propylene oxide (10 mL). The resulting solution was cooled to −20 °C and treated under stirring with 13a[2] (1.3 g, 5.87 mmol). The solution was stirred at room temperature for 2 h, and the precipitate was collected by filtration and dissolved in MeOH (30 mL). The solution was stirred at 0 °C with silica gel (1 g) and filtered. The solvent was evaporated, and the residues was triturated with ethyl acetate to yield 6a (0.63 g, 51%): mp 158–160 °C dec; IR ν_{max} 1775 (β-lactam), 1680 (amide), 1550 (carboxylate) cm^{-1}; ^{1}H NMR (D_2O–CF_3CO_2H) δ 2.07 (s, 3, CH_3), 3.14 and 3.38 (AB q, 2, J = 18.6 Hz, CH_2S), 4.84 (d, 1, J = 4.6 Hz, CHS), 4.97 (s, 1, PhCH), 5.56 (d, 1, J = 4.6 Hz, NHCHCH).

Procedure for the Preparation of (α-Hydrazinobenzyl)-cephalosporins 7a and 8. Method B. This method is illustrated by the synthesis of 3-chloro-7β-[(*R*)-α-hydrazino-α-phenylacetamido]-3-cephem-4-carboxylic acid (8). A stirred solution of 12 (2.75 g, 6.9 mmol) in CH_2Cl_2 (30 mL) and propylene oxide (7 mL) was cooled to −30 °C and treated with 13a[2] (1.68 g, 7.5 mmol). Stirring was continued at −20 °C for 1 h and at room temperature for 30 min, and the solvent was evaporated. The residue was crystallized from 2-propanol and dissolved at 0 °C in a 1:1 mixture of anisole and trifluoroacetic acid (7 mL). The solution was stirred for 40 min at 0 °C and treated with Et_2O (45 mL). The resulting precipitate was collected and taken up with a 2-propanol–H_2O mixture. The pH was adjusted to 4.8 with aqueous $NaHCO_3$, and the suspension was filtered. The aqueous phase was concentrated to a small volume at reduced pressure, and 2-propanol was added. The precipitate was collected to obtain 8 (0.9 g, 34%): mp 202 °C dec; IR ν_{max} 1760 (β-lactam), 1665 (amide), 1600 (carboxylate) cm^{-1}; ^{1}H NMR (CD_3COCD_3–CF_3CO_2H) δ 3.52 and 3.87 (AB q, 2, J = 17.3 Hz, CH_2S), 5.20 (d, 1, J = 4.6 Hz, CHS), 5.46 (s, 1, PhCH), 5.84 (d, 1, J = 4.6 Hz, NHCHCH).

Acknowledgment. The authors thank Dr. M. Visconti for spectroscopic assistance.

Registry No. 6a, 57820-56-1; 6b (isomer 1), 86688-18-8; 6b (isomer 2), 86688-19-9; 7a, 57820-58-3; 7b, 86632-57-7; 8, 86632-58-8; 9 trimethylsilyl ester, 41360-37-6; 10 trimethylsilyl ester, 55633-18-6; 11, 6187-87-7; 12, 53994-70-0; (*R*)-13a, 54193-10-1; (*R*)-13b, 63903-96-8; (*RS*)-13b, 54186-58-2; 14, 51762-03-9; 15, 54639-48-4; 16, 63821-56-7.

A Deuterium Isotope Effect on the Inhibition of Gastric Secretion by *N,N*-Dimethyl-*N'*-[2-(diisopropylamino)ethyl]-*N'*-(4,6-dimethyl-2-pyridyl)urea. Synthesis of Metabolites

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The use of isotopic substitution to retard the oxidative metabolism of the gastric antisecretory agent *N,N*-dimethyl-*N'*-[2-(diisopropylamino)ethyl]-*N'*-(4,6-dimethyl-2-pyridyl)urea (1) and improve its antisecretory potency was examined. The pyridine ring methyl hydrogens of 1 were replaced with either deuterium or fluorine. The hexadeuterated analogue (12) was found to be ~2.1 times more potent than the protio form (1) as an inhibitor of gastric acid secretion stimulated by gastrin tetrapeptide. The hexafluoro analogue (11) was 0.4 times as potent as 1. A useful pyridine ring synthesis was developed to prepare the metabolites of 1, 10a (4-hydroxymethyl) and 10b (6-hydroxymethyl), and the hexafluoro analogue 11. These syntheses involved the condensation of 1,3-diketones with an appropriately N-substituted amidinoacetate.

A recent report from these laboratories described a series of aminoalkyl-substituted pyridylureas as inhibitors of gastric acid secretion.[1] One member of this series, *N,N*-dimethyl-*N'*-[2-(diisopropylamino)ethyl]-*N'*-(4,6-di-

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