SYNTHESIS AND BIOLOGICAL ACTIVITY OF A NEW SEMISYNTHETIC CEPHALOSPORIN, CN-92,982

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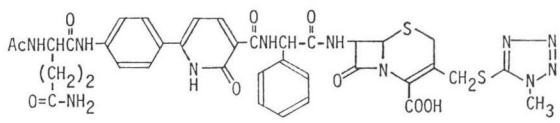
A new broad spectrum semisynthetic cephalosporin (CN-92,982) was prepared from the condensation of an acetylaminoacylaminophenyl pyridone with *trans*-7-[(D-2-phenylglycyl) amino]-3-[[(1-methyl-1H-tetrazol-5-yl)thio]methyl]-Δ^3-cephem-4-carboxylic acid. The new cephalosporin displayed an *in vitro* antibacterial spectrum similar to other cephaloglycine types such as cefoperazone and SM-1652. The compound produced a high and prolonged blood level following a single intramuscular dose in a dog.

Cephalosporins which possess a broad spectrum of activity against both Gram-positive and Gram-negative types of bacteria are of considerable clinical interest especially for the treatment of hospital acquired infections. This is especially true if such agents have activity profiles which are similar to the aminoglycosides and retain their activities against the aminoglycoside resistant and β-lactamase producing strains. Such an agent, designated as CN-92,982, has been prepared in these laboratories and its chemical synthesis and preliminary biological properties are reported.

The structure of CN-92,982 is shown in Fig. 1. The N-acetylamino acid moiety in the side chain has the *S* or natural glutamine configuration and the total side chain is attached to a cephaloglycin-type substrate.

Fig. 1.



Synthesis

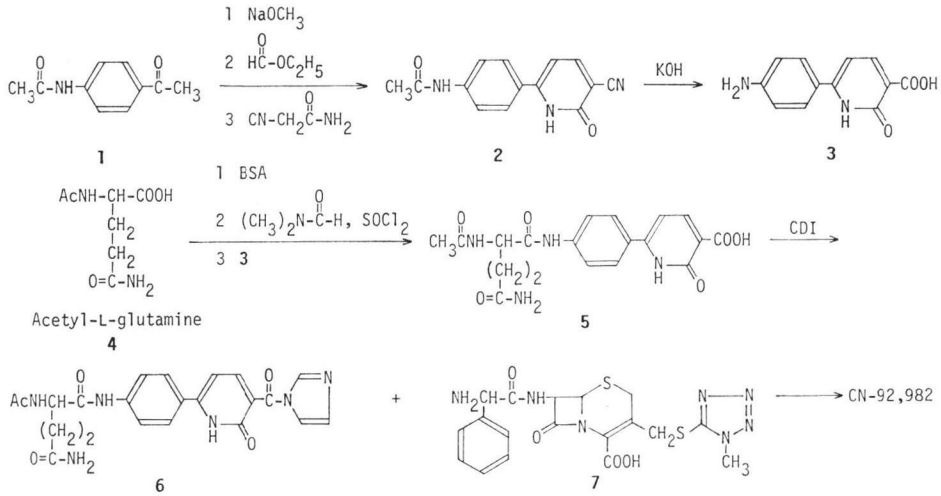
Its chemical synthesis is shown in Fig. 2. The synthesis features the utilization of an optically active N-acetylamino acid chloride in a condensation with an arylamine under conditions which cause no detectable racemization of the chiral center. Activation of the side chain carboxyl group is accomplished *via* the imidazolide **6**[3] for coupling with the cephalosporin substrate.

Antimicrobial Activity

The *in vitro* antibacterial spectrum is shown in Table 1 and points out the relative potencies of CN-92,982 compared with the other broad spectrum cephaloglycin-type analogs cefoperazone[1] and SM-1652.[2] It can be seen that CN-92,982's antibacterial spectrum quantitatively resembles that of SM-1652 more closely than cefoperazone particularly against *Serratia marcescens* and *Proteus vulgaris*.

The relatively high molecular weight (882) of CN-92,982 compared with other β-lactams might

Fig. 2.



suggest a prolongation of drug level in blood and tissue with resultant longer half-life values and high persistent blood levels. This was found to be the case in at least two animal species studied. Blood level studies in mice and dogs showed that CN-92,982 produced higher serum concentrations with longer duration of effective levels than similar doses of cefoperazone. This data is presented in Tables 2 and 3.

Although the half-life values of each drug in mice do not differ significantly, the blood concentrations at comparable dosing are higher with CN-92,982 and persist for much longer intervals. For example at single SC doses of 30 mg/kg measurable blood levels of CN-92,982 are maintained up to 4 hours *vs* 1 hour for cefoperazone. At doses of 300 mg/kg the measurable blood levels of CN-92,982 persist for 6 hours *vs* 2 hours for cefoperazone.

Intramuscular administration in dogs at 30 mg/kg showed therapeutic concentrations for *Pseudomonas* of CN-92,982 after 8 hours *vs* 2 hours for cefoperazone. Here the half-life values of each drug, T 1/2 2.4 hours for CN-92,982 *vs* 0.9 hour for cefoperazone, were significantly different.

Table 1. Comparative *in vitro* activity.

Test organisms	MIC (μg/ml)*		
	CN-92,982	Cefoperazone	SM-1652
Pseudomonas aeruginosa # 28	3.1	3.1	1.6
Pseudomonas aeruginosa BRK 12-4-4	6.3	3.1	3.1
Pseudomonas aeruginosa UI-18	6.3	3.1	3.1
Escherichia coli	0.4	0.1	3.1
Proteus vulgaris	6.3	0.4	6.3
Enterobacter cloacae	1.6	0.2	6.3
Serratia marcescens	12.5	0.4	12.5
Klebsiella pneumoniae	0.8	0.1	3.1
Streptococcus faecalis	25	25	6.3
Staphylococcus aureus UC-76	0.4	1.6	1.6
Staphylococcus aureus S-18713**	3.1	3.1	3.1

* Microtitration broth dilution in TSB. Final inoculum approximately 10^4 CFU/ml for Gram-negative bacteria and 10^6 CFU/ml for *S. faecalis* and *Staphylococci*.

** β-lactamase producing, multi-resistant isolate.

This pharmacokinetic attribute of CN-92,982 was evident in *in vivo* chemotherapy studies in mice. The comparative protective potencies of the three cephalosporins against *Pseudomonas aeruginosa* (2 strains), *Enterobacter cloacae, Klebsiella pneumoniae* and a β-lactamase producing strain of *Staphy-*

Table 2. Comparative mouse blood levels (μg/ml)* after single SC doses.

Hours after dosing	CN-92,982		Cefoperazone	
	30 mg/kg	300 mg/kg	30 mg/kg	300 mg/kg
1/4		94	5	50
1/2	24	162	5	62
1	21	132	3	43
2	12	136	<3	15
4	4	56	<3	1
6	1	9	—	<1
t 1/2 (hour)	1.1	1.0	0.9	0.7

* Disk-agar diffusion microbiological assay; *Branhamella catarrhalis* 03596 in TSA.

Table 3. Comparative dog plasma levels (μg/ml) following single IM dose of 30 mg/kg*.

Hours post dose	CN-92,982	Cefoperazone
1/4	33	26
1/2	54	47
1	78	53
2	54	32
4	32	5
8	21	<0.5
24	2	—
t 1/2 (hour)	2.4	0.9

* Disk-agar diffusion microbiological assay; *Branhamella catarrhalis* 03596 in TSA.

Table 4. Comparative *in vivo* data.

Compound	PD_{50} Values in mg/kg in mice*				
	Pseudomonas aeruginosa		*Entero. cloacae* (IMM-11)	*Klebs. pneumoniae* (MGH-2)	*Staph.*** *aureus* (H-228)
	(UI-18)	(BRK)			
CN-92,982	42	50	10	8	2.8
Cefoperazone	94	54	0.3	3	7
SM-1652	70	24	5	12	4.8

* Total of two sc-doses, first concurrent with lethal IP challenge. All challenges mucinized except for *K. pneumoniae*.

** Penicillinase producing strain.

lococcus aureus are shown in Table 4.

Although the biological data presented is preliminary, the favorable *in vivo* properties of CN-92,982 in addition to its broad *in vitro* spectrum of activity and low mouse toxicity (LD_{50}>2,000 mg/kg) makes this agent a likely candidate for clinical study.

Experimental Section

N-[4-(5-Cyano-1,6-dihydro-6-oxo-2-pyridinyl)phenyl]acetamide (**2**)

A suspension of 330 g (6.1 moles) of sodium methoxide, 3 liters of tetrahydrofuran, and 2.5 liters of ether was stirred at room temperature and a suspension of 490 g (2.77 moles) of N-(4-acetylphenyl) acetamide, 416 g (5.54 moles) of ethyl formate, and 3 liters of tetrahydrofuran was added over a period of 1 hour. The suspension was stirred at room temperature overnight under nitrogen. The brown solid was collected and washed with tetrahydrofuran.

A solution of this salt in 9 liters of water was adjusted to pH 9.0 with HOAc and 388 g (4.6 moles) of 2-cyanoacetamide was added. The solution was heated at 90°C for 18 hours. The pH of the cooled suspension was adjusted to 5.8 with HOAc and the solid filtered and washed with H_2O, MeOH and ethyl acetate. Drying afforded 422 g of **2**. m.p. >350°C.

Anal. Calcd. for $C_{14}H_{11}N_3O_2 \cdot 0.2\ H_2O$: C, 65.47, H, 4.47, N, 16.36
Found: C, 65.68, H, 4.70, N, 16.36

6-(4-Aminophenyl)-1,2-dihydro-2-oxo-3-pyridinecarboxylic acid (**3**)

A suspension of 422 g (1.67 moles) of **2** in 3.65 liters of H_2O containing 932 g of KOH was heated

at 105°C for 40 hours in a stainless steel autoclave. The cooled solution was acidified to pH 4.0 with 1.36 liters of conc. HCl and 400 g of KOH was added with stirring. After filtration, the pH was adjusted to 4.5 with conc. HCl and the solid was filtered, washed with H_2O, MeOH and ethyl acetate. Drying afforded 328 g of **3**. m.p. 314~316°C (dec.).

Anal. Calcd. for $C_{12}H_{10}N_2O_8$: C, 62.60, H, 4.38, N, 12.17
Found: C, 62.29, H, 4.32, N, 12.16

(*S*)-6-[4-[[2-(Acetylamino)-5-amino-5-oxopentyl]amino]phenyl]-1,2-dihydro-2-oxo-3-pyridinecarboxylic acid (**5**)

A mixture of 309 g (1.64 moles) of N-acetyl-L-glutamine, 227 g (1.12 mole) of N,N-bis(trimethylsilyl)acetamide (BSA) and 5.2 liters of THF was stirred at room temperature for 4 days. Insoluble material was filtered off and 59.4 g (0.81 mole) of dry DMF was added. The temperature was lowered to −35°C and a solution of 96.7 g (0.82 mole) of $SOCl_2$ in 522 ml of CH_2Cl_2 was added.

A mixture of 145.5 g (0.63 mole) of **3**, 217 g (1.07 mole) of BSA, 654 ml of THF and 1.67 liters of CH_2Cl_2 was stirred at room temperature for 3 days, filtered and washed with 900 ml of CH_2Cl_2 containing 0.01% of BSA. The cooled filtrate (−50°C) was added to the acid chloride solution over 1.5 hours keeping the temperature at −35°C. After stirring for 15 hours at this temperature the mixture was concentrated *in vacuo* at 37°C to a yellow slurry and 3.1 liters of cold 95% EtOH was added. The suspension was centrifuged and washed two times with 95% EtOH. The solid was suspended in 3.5 liters of H_2O and centrifuged. The process was repeated twice. The solid was washed with 1 liter of EtOH and finally with ether. Filtration and drying afforded 439 g of **5** as a light yellow solid. m.p. 255~256°C (dec.), $[\alpha]_D^{23}$ +18.1° (*c* 1, DMSO).

Anal. Calcd. for $C_{19}H_{20}N_4O_6 \cdot H_2O$: C, 54.54, H, 5.30, N, 13.39
Found: C, 54.67, H, 5.30, N, 13.36

(*S*)-2-(Acetylamino)-N^1-[4-[1,6-dihydro-5-(1*H*-imidazol-1-ylcarbonyl)-6-oxo-2-pyridine]phenyl] pentanediamide (**6**)

A mixture of 28 g (0.07 mole) of **5**, 17 g (0.15 mole) of carbonyl diimidazole and 200 ml of DMF was heated at 55°C for 1 hour. After standing overnight at room temperature, 500 ml of CH_3CN was added. The cooled mixture was filtered, washed with CH_3CN, ether and dried to give 27 g of imidazolide (**6**). $[\alpha]_D^{23}$ +18.3° (*c* 1, DMSO).

7-[[*N*-[[6-[4-[(*N*-Acetyl-L-glutaminyl)amino]phenyl]-1,2-dihydro-2-oxo-3-pyridinyl]carbonyl]-D-2-phenylglycyl]amino]-3-[[(1-methyl-1*H*-tetrazol-5-yl)thio]methyl]-Δ^3-cephem-4-carboxylic acid

A solution of 82.6 g (0.11 mole) of *trans*-7-[(D-2-phenylglycyl)amino]-3-[[(1-methyl-1*H*-tetrazol-5-yl) thio]methyl]-Δ^3-cephem-4-carboxylic acid (salt with 1.5 eq of *p*-toluene sulfonic acid)[4] (**7**), 45 g (0.1 mole) of **6** and 500 ml of DMA was stirred at 5°C for 30 minutes and at room temperature for 2 hours. The reaction mixture was treated with 64 ml (0.204 mole) of 3.2 M sodium 2-ethylhexanoate in DMA and the resulting solution poured into 2 liters of stirred ethyl acetate. The solid was filtered, washed with EtOAc, ether and dried. The powder was dissolved in 1.25 liters of H_2O and the pH adjusted to 2.0 with HCl. The solid was filtered, washed with H_2O and resuspended in 2.5 liters of H_2O. Addition of NaOH solution to pH 6.7 dissolved the solid. The solution was clarified by filtration and lyophilized to give 82.8 g of the sodium salt. $[\alpha]_D^{23}$ −212° (*c* 1, pH 7). HPLC assay 96%.

Anal. Calcd. for $C_{37}H_{37}N_{11}O_9S_2Na \cdot 3H_2O$: C, 47.38, H, 4.72, N, 16.42
Found: C, 47.22, H, 4.32, N, 17.17

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