Semisynthetic Cephalosporins. Synthesis and Structure–Activity Relationships of 7-(1-Pyrryl)- and 7-(1-Indolyl)acetamidocephalosporin Derivatives

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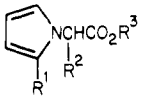
A series of 1-pyrrole- and 1-indoleacetamido derivatives of 3-heteroaryl-substituted cephalosporins was prepared. The most active compound in the series was 7-[[2-(1-pyrryl)acetyl]amino]-3-[[(1-methyltetrazol-5-yl)thio]-methyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid (**6**), which showed comparable potency in vitro and in vivo to that of cefazolin, and, in addition, was more potent than cefazolin against *Enterobacter* sp. and *Providencia stuartii*.

This paper describes the synthesis and structure–activity relationships of a family of 1-pyrryl- and 1-indolylcephalosporin derivatives substituted at the 3 position with heteroarylthiomethyl groups. In view of the fact that many 3-heteroarylthiomethyl-substituted cephalosporins show greater in vitro potency, especially against gram-negative microorganisms, than the corresponding 3-acetoxymethyl compounds,[1] we undertook the investigation of a number of 3-heteroarylthiomethyl cephalosporin analogues of compounds which had only been described in the literature as substituted by a 3-acetoxymethyl function.[2]

Chemistry. The compounds studied (Table I) were prepared by coupling the respective acetic acid derivatives[3] with the corresponding 3-substituted 7-aminocephalosporanic acids,[4] using the general mixed anhydride method of Spencer et al.[5]

1(1*H*)-Pyrroleacetic acid (**1a**) was best prepared as



1a, $R^1 = R^2 = R^3 = H$
b, $R^1 = C(=O)H$; $R^2 = R^3 = H$
c, $R^1 = R^3 = H$; $R^2 = CO_2Et$
d, $R^1 = H$; $R^2 = CO_2Et$; $R^3 = Et$
e, $R^1 = R^3 = H$; $R^2 = CO_2H$

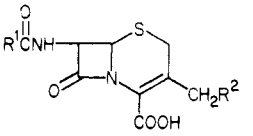
described by Gloede et al.[6] from 2,5-diethoxytetrahydrofuran and glycine. The considerably greater acidity of 2-formylpyrrole, as compared to pyrrole, enabled its direct N-alkylation when treated with bromoacetic acid in the presence of sodium hydroxide to give **1b**. The monoethyl ester of pyrrolemalonic acid, **1c**, was prepared by partial hydrolysis[7] of the diester **1d** which was prepared by Gloede's[6] procedure from diethyl aminomalonate hydrochloride.[8] Total hydrolysis of **1d** gave the dicarboxylic acid **1e**.

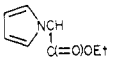
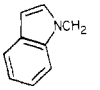
The trichloroethoxycarbonyl-protected amino acid **2** was prepared according to Scheme I.

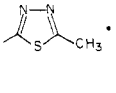
Acid **2** was coupled to the *tert*-butyl ester of 7-aminodeacetoxycephalosporanic acid using *N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ) as the coupling agent to give the cephalosporin derivative **3**. Free amino acid **4** was liberated by first treating **3** with zinc in acetic acid and then with trifluoroacetic acid.

Biological Results and Discussion. The minimal inhibitory concentrations of the cephalosporins shown in Table I against a variety of gram-positive and gram-negative bacteria are presented in Table II. It is apparent that compounds substituted at the 3 position by heteroarylthiomethyl groups were more potent than analogues having a methyl or acetoxymethyl group at that position. As a class, the unsubstituted pyrrole derivatives were more potent than the analogous indole derivatives. In addition, the formyl-substituted pyrrole derivatives were generally less potent than the unsubstituted pyrrole analogues,

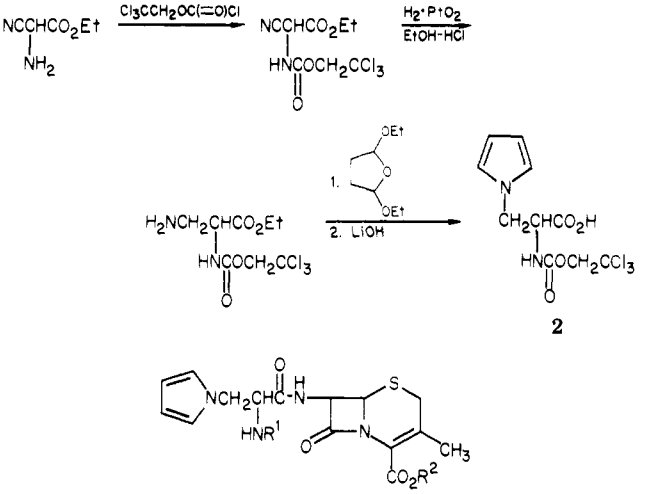
Table I. 7(1*H*)-Pyrrole- and 7(1*H*)-Indoleacetamidocephalosporins



compd	R^1	R^2 [c]	yield, %[a]
5[b]		a	53
6		c	20
7		d	46
8		a	18
9		c	35
10		d	22
11		a	19
12		b	58
13		c	25
14		d	21

[a] Compounds 6–14 gave satisfactory elemental analyses and had the expected NMR spectra. [b] See ref 2. [c] a = acetoxy; b = hydrogen; c =  ; d =  .

Scheme I



3, $R^1 = C(=O)OCH_2CCl_3$; $R^2 = C(CH_3)_3$
4, $R^1 = R^2 = H$

Table II. Activity in Vitro of 7(1*H*)-Pyrrole- and 7(1*H*)-Indoleacetamidocephalosporins

	min inhibitory concn, μg/mL[a]											
organism	5	6	7	8	9	10	11	12	13	14	cephalothin	cephazolin
gram-positive												
S. aureus[b]	1.6	0.8	0.8	1.6	1.6	0.8	6.2	3.1	0.2	0.2	0.4-0.8	0.4-1.6
S. aureus	0.8	0.4	0.2	0.8	0.8	0.4	1.6	1.6	0.1	0.1	0.1-0.2	0.2-0.4
S. epidermidis	3.1	1.6	0.8	3.1	3.1	1.6	3.1	6.2	0.4	0.4	0.4-0.8	0.8-1.6
St. faecalis	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	100	>100
St. pneumoniae	0.4	0.1	0.1			0.2	0.4	1.6		⩽0.025	0.1	0.1
St. pyogenes	0.4	0.1	0.1	0.4	0.2	0.2	0.4	0.8	0.05	0.05	0.1-0.2	0.1-0.2
gram-negative												
Ent. aerogenes	>100	25	100	>100	100	>100	25	>100	50	100	50->100	50->100
Ent. cloacae	>100	6.2	100	>100	>100	>100	25	>100	50	100	>100	50-100
E. coli	6.2	0.8	12.5	100	12.5	50	25	>100	12.5	50	6.2-12.5	0.8-1.6
K. pneumoniae	6.2	1.6	6.2	50	12.5	50	25	>100	12.5	50	3.1-12.5	1.6-3.1
Pr. mirabilis	12.5	6.2	25	100	50	100	50	>100	50	50	6.2-12.5	6.2-12.5
Pr. vulgaris	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
Prov. stuartii	50	6.2	25	>100	50	>100	12.5	>100	25	25	100	50->100
Ps. aeruginosa	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
S. schottmuelleri	3.1	0.4	6.2	50	6.2	12.5	25	>100	6.2	25	0.8-1.6	1.6
S. marcescens	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100

[a] Determined by serial twofold dilution of the compound in Mueller-Hinton agar and inoculation of the agar surface with an appropriately diluted 18-24-h broth culture. Agar plates were incubated at 37 °C for 17 h and the lowest concentration causing complete or virtually complete inhibition of visible growth was considered to be the minimal inhibitory concentration. [b] Benzylpenicillin-resistant strain.

Table III. Activity in Vivo of Compound 6 and Cefazolin

	ED_{50}, mg/kg per dose[a]	
organism	6	cefazolin
S. aureus[b]	72	22
St. pyogenes	2.3	(1.8)[c]
E. coli	2.5	0.6
K. pneumoniae	67	42
Pr. mirabilis	100	79
S. schottmuelleri	2.5	2.2

[a] Male albino CD-1 mice weighing 20 (±1) g were infected by intraperitoneal injection of a bacterial suspension to produce uniformly lethal infections. Groups of ten mice each were treated subcutaneously with appropriate concentrations of antibiotic at 1 and 4 h after infection. The number of mice in each group surviving the challenge for 4 days was recorded, and the ED_{50} (the dose in mg/kg required to protect 50% of the infected mice) was determined by the method of Reed and Muench [*Am. J. Hyg.*, 27, 493 (1938)]. [b] Benzylpenicillin-resistant strain. [c] ED_{50} for cephalexin administered orally.

especially with regard to activity against gram-negative bacteria. Compound **4**, the higher homologue pyrrole derivative with an α-amino substituent, was essentially inactive (data not shown).

Overall, compound **6** was the most potent and had the broadest spectrum. Its in vitro and in vivo potency was generally comparable to that of cefazolin against organisms sensitive to the latter (Tables II and III). In addition, as shown in Table II, compound **6** was more potent than cefazolin against *Enterobacter* sp. and *Providencia stuartii*. In a subsequent study in vitro against 11 strains of *Enterobacter* sp., compound **6** inhibited nine at a concentration of 25 μg/mL while cefazolin inhibited only two at the concentration (Table IV). In other in vitro studies (data not shown) neither compound **6** nor cefazolin displayed noteworthy activity against strains of *Neisseria gonorrhoeae*, *Haemophilus influenzae*, *Bacteroides fragilis*, *Fusobacterium varium*, or *Clostridium perfringens*.

In summary, compound **6** is considered to be an interesting cephalosporin worthy of further investigation.

Experimental Section

Satisfactory elemental analyses were obtained for all new compounds.

Table IV. Minimal Inhibitory Concentrations of 6 and Cefazolin against 11 Strains of *Enterobacter* sp.[a]

	no. inhibited by concn (μg/mL)						
compd	3.1	6.2	12.5	25	50	100	>100
6	1	1	2	5			2
cefazolin			1	1	1	2	6

[a] See footnote *a*, Table II.

(2-Formyl-1-pyrryl)acetic Acid (1b). To an ice-cold solution (about 0 °C) of sodium hydroxide (8.8 g in 30 mL of water) was added 9.5 g (0.1 mol) of pyrrole-2-carboxaldehyde followed by the addition of bromoacetic acid (14 g, 0.11 mol) in small portions. The solution was stirred at room temperature for 18 h. Only a small trace of the pyrrole-2-carboxaldehyde could be detected by thin-layer chromatography. The aqueous solution was first washed with chloroform and then acidified to pH 3 with 6 N hydrochloric acid. The acidified solution was extracted with ethyl acetate which was dried over magnesium sulfate, filtered, and flash evaporated. An oil was obtained which was crystallized from ethyl acetate–benzene to give 9.7 g of product (63% yield): mp 141–143 °C; NMR (Me_2SO-d_6 + D_2O) δ 5.10 (s, 2), 6.3 (q, 1), 7.1 (q, 1), 7.25 (m, 1), and 9.58 (s, 1).

Diethyl (1*H*-Pyrrol-1-yl)malonate (1d). To a boiling solution of 32.6 g (0.4 mol) of sodium acetate in 750 mL of glacial acetic acid was added diethyl aminomalonate hydrochloride. The clear solution was refluxed for 5 min; then 25.1 g (about 0.2 mol) of 2,5-diethoxytetrahydrofuran (90–95% pure) was added in one portion and the mixture boiled an additional 2 min. The acetic acid was flash evaporated and the residue was distilled. Diethyl (1*H*-pyrrol-1-yl)malonate was obtained in 64% yield: bp 143–146 °C (0.1 mmHg). The NMR spectrum indicated a small amount of impurity. The distilled ester (14.3 g) was chromatographed on 220 g of silica gel, eluted with 3:2 ether–hexane, to give 12.9 g of the diester shown to be a single spot on the thin-layer chromatography plate: NMR ($CDCl_3$) δ 1.22 (t, 6), 4.24 (q, 4), 5.42 (s, 1), 6.18 (t, 2), 6.80 (t, 2).

Ethyl (1-Pyrryl)malonate (1c). To a solution of **1d** (4.5 g, 0.02 mol) in 25 mL of absolute ethanol was added 25 mL (0.02 mol) of 0.8 N ethanolic potassium hydroxide. This solution was flash evaporated, and the residue was dissolved in 25 mL of water and washed twice with 50 mL of ether. The aqueous phase was separated, acidified to pH of 2.5, saturated with sodium chloride, and extracted twice with 80 mL of ether. The ether extracts were combined, dried, and evaporated to give 2.3 g (58 % yield) of the monoethyl ester as an oil: NMR ($CDCl_3$) δ 1.22 (t, 3), 4.3 (q, 2), 5.45 (8.1), 6.22 (t, 2), 6.8 (t, 2).

(1*H*-Pyrrol-1-yl)malonic Acid (1e). A solution of 4.5 g (0.02

mol) of 1d in 25 mL of absolute ethanol was mixed with 50 mL of 0.8 N alcoholic potassium hydroxide (0.04 mol) and stirred overnight at room temperature. The solution was evaporated to dryness. The NMR [(D_2O) δ 5.3 (s, 1), 6.48 (t, 2), 7.0 (t, 2)] of the residue indicated that the desired dipotassium salt was obtained. The residue was dissolved in a small amount of water and acidified to pH 2 with 6 N hydrochloric acid. The aqueous phase was extracted with ether. The ether extracts were combined, dried, and evaporated to give 1.6 g (47%) of 1e as an oil: NMR (Me_2SO-d_6) δ 5.41 (s, 1), 6.02 (t, 2), 6.72 (t, 2).

α-[[(2,2,2-Trichloroethoxy)carbonyl]amino]-1*H*-pyrrole-1-propanoic Acid (2). Ethyl aminocyanoacetate *p*-toluenesulfonate[9] (20.4 g, 68 mmol) and 29 g (137 mmol) of 2,2,2-trichloroethyl chloroformate were refluxed in 500 mL of ethyl acetate for 14 h. A stream of nitrogen was passed through the reaction mixture to remove the formed HCl. The reaction mixture was then washed with 100 cm^3 of dilute aqueous HCl, 100 mL of dilute aqueous $NaHCO_3$, and 100 cm^3 of water. The organic layer was dried over $MgSO_4$, filtered, and evaporated to dryness. The oily residue was taken up in 50 mL of CH_2Cl_2 and treated with hexane to crystallize out 17.8 g (86% yield) of ethyl α-[[(2,2,2-trichloroethoxy)carbonyl]amino]-α-cyanoacetate.

Five grams of the above compound was dissolved in 150 mL of 14% ethanolic HCl and treated with 1 g of platinum oxide under hydrogen in a Parr shaker at 40 psi for 2 h. The catalyst was removed by filtration through Celite and the filtrate was evaporated to dryness in vacuo to leave a clear oil. This was dissolved in 150 cm^3 of chloroform and washed with two portions of 50 cm^3 of dilute aqueous HCl. After washing the combined acidic layers with chloroform they were basified with 2 N sodium hydroxide and extracted with three portions of 100 mL each of chloroform. The organic extracts were combined, dried over $MgSO_4$, filtered, and evaporated to dryness to give 4.1 g of a clear oil which was ethyl α-[[(2,2,2-trichloroethoxy)carbonyl]-amino]-1-propanoate.

The above compound (3.53 g, 11.46 mmol) was refluxed in 30 mL of acetic acid for 1 h with 2.5 g (13.02 mmol) of 2,5-diethoxytetrahydrofuran. The dark reaction mixture was evaporated to dryness. After charcoaling, the light brown residue was chromatographed on silica gel using 20% hexane in chloroform as the eluent. The desired fractions were combined and crystallized from dichloromethane–hexane to give 1.73 g (42% yield) of ethyl α-[[(2,2,2-trichloroethoxy)carbonyl]amino]-1*H*-pyrrole-1-propanoate.

The above ester (1.37 g, 3.83 mmol) was dissolved in 10 mL of THF and 3 cm^3 of water was added. This mixture was treated with 91.8 mg (3.83 mmol) of lithium hydroxide at room temperature overnight. The reaction mixture was then poured into 100 cm^3 of dilute aqueous $NaHCO_3$ and washed three times with 50 cm^3 of chloroform. The aqueous layer was acidified with dilute aqueous HCl and again extracted three times with 100 cm^3 of chloroform. The organic extracts were dried over $MgSO_4$, filtered, and evaporated to dryness to give 1.17 g of solid. This was recrystallized from chloroform–hexane to give 1.02 g (64% yield) of **2**: NMR ($CDCl_3$) δ 4.45 (br d, 2 H), 4.57 (br m, 1 H), 4.78 (s, 2 H), 5.65 (d, 1 H), 6.23 (t, 2 H), 6.68 (t, 2 H), 10.3 (s, 1 H).

Synthesis of 7-[[2-Amino-3-(1*H*-pyrrol-1-yl)propyl]-amido]deacetoxycephalosporanic Acid (4). **2** (270 mg, 0.82 mmol), 240 mg (0.82 mmol) of *tert*-butyl 7-aminodeacetoxycephalosporanate, and 220 mg (0.89 mg) of EEDQ were stirred in 50 mL hydrocarbon-stabilized chloroform for 16 h at room temperature and under argon. The reaction mixture was diluted with another 50 mL of chloroform and washed with 50 mL of dilute aqueous HCl, 50 mL of dilute aqueous $NaHCO_3$, and 50 mL of water. The organic layer was dried over $MgSO_4$, filtered, and evaporated to dryness to give 425 mg (73% yield) of the diprotected cephalosporin.

The latter material (1.625 g, 2.79 mmol) was dissolved in 30 mL of formic acid and treated with 1.83 g (2.79 mmol) of zinc dust for 1 h at 0 °C. The reaction mixture was filtered through Celite and poured into 100 cm^3 of water. This acidic aqueous layer was washed with 50 mL of ethyl acetate before being basified with dilute aqueous $NaHCO_3$ and extracted three times with 50 mL each of chloroform. The chloroform extracts were combined, dried over $MgSO_4$, filtered, and evaporated to dryness to give 525 mg of a clear oil. This was dissolved in 10 cm^3 of trifluoroacetic acid (TFA) and allowed to react at room temperature for 10 min. At this point the excess TFA was removed by evaporation in vacuo to give 455 mg (47% yield from diprotected cephalosporin) of 4 as the diastereoisomeric mixture: NMR (CD_3CN–TFA) δ 2.17 (s, 3 H), 3.46 (q, 2 H), 4.48 (s, 2 H), 5.08 (d, 1 H), 5.68 (q, 1 H), 6.12 (t, 2 H), 6.78 (t, 2 H).

7-[2-Carboethoxy-2-(1-pyrrylacetyl)]aminocephalosporanic Acid (11). A solution of 1c (1 g, 5.07 mmol) and 0.87 g (5.2 mmol) of *N,N'*-carbonyldiimidazole in 10 mL of dimethylformamide was stirred at room temperature for 1 h. In a second flask, 7-aminocephalosporanic acid (1.37 g, 5.07 mmol) was dissolved in 35 mL of chloroform upon the addition of 2.05 g (20.3 mmol) of triethylamine and was stirred under nitrogen for 30 min in the presence of Linde 4A molecular sieves. The two solutions were combined and the mixture was stirred for 4 h after the addition of 0.5 g of charcoal. The reaction mixture was filtered and concentrated by flash evaporation, and the residue was dissolved in 25 mL of water and washed several times with ether. The aqueous phase was covered with 50 mL of ethyl acetate and was then acidified to pH 2.5 with 6 N hydrochloric acid. A small amount of solid was removed by filtration and the organic phase was separated, dried, and concentrated to 5 mL. The title compound was obtained as a powder (425 mg, 19% yield) upon addition of the ethyl acetate solution to hexane: NMR (Me_2SO-d_6–D_2O) δ 1.30 (t, 3), 2.08 (s, 3), 3.5 (br s, 2), 4.31 (q, 2), 5.0 (m, 3), 5.55 (s, 1), 5.8 (m, 1), 6.22 (m, 2), 6.9 (m, 2).

References and Notes

(1) For a general view of structure–activity relationships, see M. Gorman and C. W. Ryan, "Cephalosporins and Penicillins, Chemistry and Biology", E. H. Flynn, Ed., Academic Press, New York, N.Y., 1972, Chapter 12.
(2) See ref 1, pp 572–573.
(3) 1*H*-Indoleacetic acid is commercially available from Aldrich Chemical Co., Inc.
(4) G. L. Dunn, J. R. E. Hoover, D. A. Berges, J. J. Taggart, L. D. Davis, E. M. Dietz, D. R. Jakas, N. Yim, P. Actor, J. V. Uri, and J. A. Weisbach, *J. Antibiot.*, **29**, 65 (1976).
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