SEMISYNTHETIC PENICILLINS. A STRUCTURE - ACTIVITY STUDY OF A NEW SERIES OF ACYL AMINO ACID - PYRIDONE AND PYRIMIDONE AMOXICILLIN ANALOGS

T. H. HASKELL*, P. W. K. WOO, E. D. NICOLAIDES, M. P. HUTT, G. G. HUANG, J. P. SANCHEZ, D. DEJOHN, C. L. HEIFETZ, U. KROLLS, E. LUNNEY and T. F. MICH

Warner-Lambert/Parke-Davis, Pharmaceutical Research Division
Ann Arbor, Michigan 48105, U.S.A.

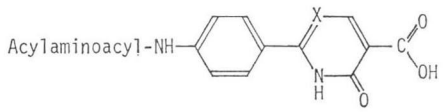
(Received for publication April 24, 1981)

The synthesis and biological activities of a series of 12 new semisynthetic penicillins is described. These compounds consisted of acylated amino acid analogs of 6-substituted-1,2-dihydro-2-oxonicotinic acid and 2-substituted-3,4-dihydro-4-oxo-5-pyrimidinecarboxylic acid attached to amoxicillin. The effect of the amino acid substituent, chirality of amino acid and acyl function on biological properties is discussed.

As part of a program designed to discover classical β-lactam derivatives possessing outstanding biological activity against troublesome Gram-negative bacteria,[1] we wish to report a series of amino acid analogs of 6-(4-aminophenyl)-1,2-dihydro-2-oxonicotinic acid condensed with amoxicillin. The antibacterial spectra of the twelve compounds reported herein resemble that of piperacillin and azlocillin in breadth and potency. These compounds are of interest because of their favorable potencies against strains of *Pseudomonas aeruginosa, Klebsiella pneumoniae, Enterobacter cloacae,* indole positive *Proteus* and *Escherichia coli.* Furthermore, these compounds show very potent *in vivo* activity in experimental mouse infections when compared directly with the marketed penicillin entities ticarcillin, azlocillin, and piperacillin.

The side chains described herein are acylated amino acid derivatives which are attached *via* a peptide linkage to 6-(4-aminophenyl)-1,2-dihydro-2-oxonicotinic acid[2] as shown in Fig. 1, **a**. A brief structure-activity study is presented whereby the effect of the a) type of amino acid, b) chirality of amino acid, and c) type of acyl function on biological activity has been determined. Furthermore, the effect of exchanging the 6-substituted-2-oxopyridine moiety with a 2-substituted-4-oxo-5-pyrimidinecarboxylate group (Fig. 1, **b**) on microbiological potency is presented.

Fig. 1.



a X = CH b X = N

The substituted 2-oxonicotinic acid side chains were prepared, in most instances, by the reaction of a mixed anhydride on the silylated 6-(4-aminophenyl)-1,2-dihydro-2-oxonicotinic acid. The mixed anhydrides were prepared from the acylated amino acids using either methyl or isobutyl chloroformate at low temperatures. Little to no racemization occurred. In two instances, however, (**10**[2] and **9**) the VILSMIER acid chloride method was used.

The 2-(4-aminophenyl)-3,4-dihydro-4-oxo-5-pyrimidinecarboxylic acid was prepared as shown in Fig. 2. Condensation with the acyl amino acid was accomplished by the mixed anhydride procedure. The side chains prepared are listed in Table 1.

Fig. 2.

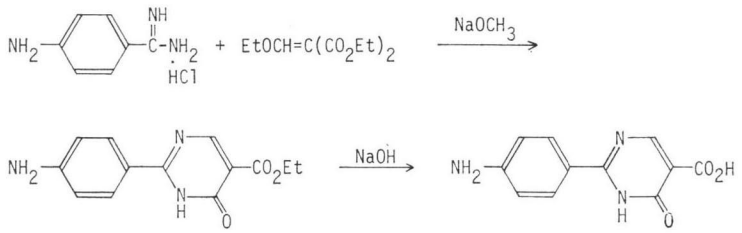
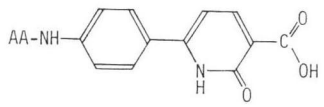


Table 1. Summary of side chains.

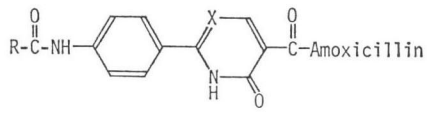


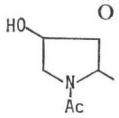
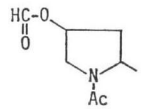
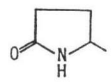
Amino acid	Chirality	Method	$E^{1\%}_{1cm}$ [a] λ 266,	330 nm	$[\alpha]_D^{25}$ [b]	Formula	Calcd. C	H	N	Found C	H	N
N-Ac alanyl	L	A	310,	577	−72°	$C_{17}H_{17}N_3O_5 \cdot 2H_2O$	53.82	5.58	11.08	53.59	5.76	10.93
N-Ac alanyl	D, L	A	327,	630	0	$C_{17}H_{17}N_3O_5 \cdot 0.4H_2O$	58.25	5.12	11.99	58.11	4.85	11.64
N-Ac alanyl	D	B	352,	660	+78°	$C_{17}H_{17}N_3O_5$	59.47	4.99	12.24	59.19	5.10	12.11
N-Formyl alanyl	L	B	332,	620	−50°*	$C_{16}H_{15}N_3O_5 \cdot 0.4H_2O$	57.11	4.73	12.48	57.51	4.54	12.06
N-Ac prolyl	L	A	307,	560	−97.5°	$C_{19}H_{19}N_3O_5$	61.78	5.18	11.38	61.66	5.41	11.34
N-Ac hydroxyprolyl	L	A	267,	498	−56°	$C_{19}H_{19}N_3O_6 \cdot 1.5H_2O$	55.52	5.40	10.22	55.75	5.55	10.44
N-Ac *O*-formyloxy prolyl	L	A	313,	558	+19.2°**	$C_{20}H_{19}N_3O_7 \cdot 0.5H_2O$	56.87	4.77	9.95	56.76	4.77	10.20
N-Ac γ-aminobutyryl		A	305,	598	0	$C_{18}H_{19}N_3O_5 \cdot 0.75H_2O$	58.28	5.57	11.33	58.21	5.51	11.12
Pyroglutamyl	L	C	322,	596	+15°**	$C_{17}H_{15}N_3O_5 \cdot 0.5H_2O$	58.29	4.60	11.99	58.61	4.35	12.02
N-Ac glutamyl[2]	L	C	294,	544	+19°**	$C_{19}H_{20}N_4O_6 \cdot H_2O$	54.54	5.30	13.39	54.67	5.30	13.36
N-Carbamoyl alanyl	L	B	331,	567	+12.5°*	$C_{16}H_{16}N_4O_5 \cdot H_2O$	53.03	5.00	15.46	53.00	4.59	16.34
N-Ac alanyl (pyrimidine)	L	A	614 at 308 nm		−72°	$C_{16}H_{16}N_4O_5 \cdot H_2O$	53.03	5.00	15.46	53.36	5.10	15.41

[a] In pH 7 buffer.
[b] In pH 7 buffer.
* In 75% DMF-pyridine.
** In DMSO.

The condensation of side chain to the amoxicillin nucleus was accomplished by activating the carboxyl function by the imidazolide procedure[3] in all but one case. The exception was with the *N*-acetyl-L-hydroxyproline analog (**6**) where the *N*-hydroxy succinimide ester was utilized.[4] The final products were isolated as their water soluble hydrated sodium salts by freeze drying from water. The structures and properties of the semisynthetic analogs prepared are given in Table 2.

Table 2.



No.	R	Chirality	X	$[\alpha]_D^{23}$ pH 7	$\lambda_{max}^{pH\,7}$ nm ($E_{1cm}^{1\%}$)	Purity* (%)
1	CH_3-CH(NHAc)-	L	C	−492°	269 (160) 355 (424)	88
2	CH_3-CH(NHAc)-	D	C	−440°	269 (152) 355 (381)	86
3	CH_3-CH(NHAc)-	DL	C	−475°	268 (150) 358 (405)	93
4	CH_3-CH(NH-C(=O)-H)-	L	C	−314°	268 (160) 358 (415)	95
5		L	C	−340°	268 (157) 358 (336)	79***
6		L	C	−468°	269 (157) 358 (410)	91
7	$AcNH(CH_2)_3$-		C	−245°	268 (143) 359 (388)	94
8		L	C	−366°	268 (147) 358 (363)	94
9		L	C	−210°	268 (158) 358 (393)	95
10	$NH_2C(=O)$-$(CH_2)_2$-CH(NHAc)-	L	C	−414°	268 (144) 357 (371)	92
11	CH_3-CH(NH-C(=O)-NH_2)-	L	C	+181°**	267 (120) 358 (370)	97
12	CH_3-CH(NHAc)-	L	N	+83°	317 (350)	95

* Determined by iodometric titration.[6]

** In 75% DMF - 25% pyridine.

*** An improved procedure for synthesis of this compound has been developed. P.W.K. Woo manuscript in preparation.

Biological Results

The *in vitro* antibacterial spectra of the semisynthetic penicillins, against eight strains of clinically important bacteria, are shown in Table 3. The table also includes the MIC values of piperacillin,

Table 3. Comparative *in vitro* activity (MIC, μg/ml) of the penicillins in Table 2.

Compound	1	2	3	4	5	6	7	8	9	10	11	12	Piperacillin	Ticarcillin	Mezlocillin
P. aeruginosa #28	0.8	1.6	1.6	1.6	3.1	1.6	1.6	0.8	0.8	3.1	1.6	1.6	1.6	12.5	12.5
P. aeruginosa BRK 12-4-4	1.6	3.1	3.1	1.6	3.1	1.6	3.1	0.8	1.6	3.1	1.6	1.6	3.1	25	25
P. aeruginosa UI-18	0.8	1.6	1.6	1.6	1.6	1.6	1.6	0.8	3.1	1.6	1.6	0.8	1.6	12.5	12.5
E. coli Brig.	6.3	6.3	3.1	3.1	3.1	3.1	3.1	3.1	1.6	3.1	3.1	6.3	1.6	3.1	3.1
E. coli Vogel	0.8	1.6	0.8	0.8	0.4	0.8	0.8	0.4	0.8	0.8	1.6	0.4	0.8	1.6	0.8
P. vulgaris	6.3	3.1	1.6	3.1	1.6	3.1	3.1	1.6	1.6	3.1	3.1	0.8	0.4	0.8	0.8
E. cloacae	1.6	6.3	3.1	3.1	3.1	6.3	6.3	1.6	1.6	3.1	6.3	6.3	0.8	3.1	6.3
S. marcescens	3.1	6.3	3.1	3.1	12.5	6.3	3.1	6.3	3.1	3.1	3.1	12.5	0.8	3.1	1.6
K. pneumoniae	3.1	12.5	3.1	6.3	12.5	12.5	3.1	6.3	6.3	12.5	6.3	50	3.1	>50	6.3
S. faecalis	0.8	1.6	0.4	0.8	1.6	0.8	0.8	0.8	1.6	1.6	0.8	1.6	1.6	25	0.8
S. aureus UC-76	0.4	0.4	0.2	0.8	3.1	0.8	0.1	0.4	0.4	0.8	0.8	0.4	0.8	0.4	0.8

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 *S. aureus*.

Table 4. Comparative *in vivo* activity PD_{50} values in mice.*

Compound	*P. aeruginosa*		*E. cloacae* IMM-11	*K. pneumoniae* MGH-2	LD_{50}** (mg/kg)
	UI-18	BRK 12-4-4			
1	37	32	22	78	>6,000
2	24	14	36	76	770
3	18	22	32	47	3,100
4	18	24	34	62	>2,000
5	20	16	16	64	>2,000
6	26	38	18	66	2,200
7	38	34	22	92	>2,000
8	24	28	15	36	1,860
9	34	30	17	32	>2,000
10	20	28	9	84	>2,000
11	22	34	28	68	1,600
12	23	22	38	186	>2,000
Piperacillin	150	100	20	38	>2,000
Ticarcillin	240	186	12	3,200	4,200
Mezlocillin	560	1,360	50	64	>2,000

* Total of two doses administered s.c.; 0 and 2 hours post infection.
** Single dose given i.v.

ticarcillin, and mezlocillin. The MIC values of the analogs correspond more closely to those of piperacillin than the other two clinical compounds for most of the bacteria listed.

In vivo chemotherapy data in mice is shown in Table 4. The mouse protective effect is expressed as the PD_{50} in mg/kg and points out the superiority of this new class of compounds over the marketed penicillins with respect to the two *P. aeruginosa* strains studied. The acute intravenous toxicity data in mice is also reported as LD_{50} values in mg/kg of body weight.

Structure - Activity Studies

1. Amino Acid Substituent

Variations in the structure of the acylated amino acid substituted in the side chain moiety had very little effect on the *in vitro* potency and breadth of antibacterial spectra. This also held true for *in vivo* potency in mice as shown by the very similar PD_{50} values in Table 4. The main differences appear to be in acute intravenous toxicity which could be related to aqueous solubility. In general the more insoluble analogs were the most toxic.

2. Chirality of Amino Acid

The effect of amino acid chirality on bacterial potency and spectra was shown to be minimal as illustrated by comparative MIC and PD_{50} data on the acetyl alanine analogs **1**, **2**, and **3**. There was, however, a surprising difference in acute intravenous toxicity with the D, L, and DL compounds. The addition of an optically active center to a chiral molecule produces diastereoisomers which can alter the solubility and molecular conformation of the penicillin which could conceivably affect the toxic potential of the molecule.

3. Acyl Function on *N* and *O*

The nature of acyl function on nitrogen and oxygen has little effect on potency. The biological activities of the *N*-formylalanyl (**4**), *N*-acetylalanyl (**1**), *N*-carbamoyl-L-alanyl (**11**), and the cyclic amide, L-pyroglutamyl (**9**), were indistinguishable. The presence or absence of the free hydroxyl group in the hydroxyproline analogs **6** and **7** also had no effect on biological activity.

4. Pyridone *vs* Pyrimidone

Both the 2-oxonicotinic and 4-oxo-5-pyrimidinecarboxylate type confer excellent *in vitro* and *in vivo* activities against strains of *P. aeruginosa, E. coli, Proteus vulgaris,* and *Streptococcus faecalis.* However, the pyrimidinone penicillins as a class are inferior to their pyridinone counterparts with respect to *Klebsiella, Serratia,* and possibly *Enterobacter* strains. This correlation is valid for several other amino acid pyrimidinone types not included herein.

Experimental

A. 2-(4-Aminophenyl)-4-hydroxy-5-pyrimidinecarboxylic Acid

A solution of 14.8 g (0.642 mole) of sodium in 750 ml of dry ethanol was stirred at 0°C and 44.6 g (0.215 mole) of 4-aminobenzenecarboximidamide dihydrochloride[5] was added. The mixture was stirred five minutes under nitrogen and 46.2 g (0.214 mole) of diethyl (ethoxymethylene)propanedioate was added. After stirring for 30 minutes, the mixture was refluxed for 4 hours and allowed to stand overnight at room temperature. The salt was filtered and washed with 2-propanol. The salt was suspended in 214 ml of 2 N potassium hydroxide and stirred at 70°C for 4 hours. After treating with a small amount of charcoal, the filtrate was added to 325 ml of 2 N HCl with stirring. The acid was filtered, washed with water, ethanol, ether, and dried to give 50.8 g of the title compound, mp 312~314°C (dec). The product was recrystallized from DMA-water to give 44.5 g, mp 313~314°C (dec). λ_{max} (pH 7) 227 nm ($E^{1\%}_{1cm}$ 460), 331 nm (920).

Anal. Calcd. for $C_{11}H_9N_3O_3$: C, 57.14; H, 3.92; N, 18.17
Found: C, 56.94; H, 3.80; N, 17.86

B. Side Chain Synthesis

Method A

a) Silylation: To a suspension of 20.2 g (88 mmole) of 6-(4-aminophenyl)-1,2-dihydro-2-oxo-3-pyridinecarboxylic acid[2] in 500 ml of CH_2Cl_2 was added 37 ml (263 mmole) of Et_3N followed by 34 ml (262.5 mmole) of chlorotrimethylsilane. The mixture was stirred at room temperature for

45 minutes.

b) Mixed anhydride: To a suspension of 175 mmole of *N*-acetylated amino acid in 440 ml of acetonitrile was added 175 mmole of 4-methylmorpholine and the mixture cooled to −20°C. Isobutyl chloroformate 25 ml (192.5 mmole) was added dropwise with stirring over 15 minutes and the mixture was stirred for 40 minutes at −20°C.

To this mixture was added the silylated pyridinone acid prepared above keeping the temperature below −15°C. The mixture was stirred 4 hours at 5°C and overnight at room temperature. 2-Propanol (100 ml) was added and the product was filtered after 20 minutes and washed with CH_2Cl_2 followed by ether. Analytical samples could usually be prepared by crystallization from DMF-ethanol.

Method B

To a solution of 8.35 g (37.5 mmole) of *N*-[(phenylmethoxy)carbonyl]-L-alanine in 100 ml of acetonitrile was added 4.1 ml (37.5 mmole) of 4-methylmorpholine. After cooling to −20°C the mixture was treated with 2.9 ml (37.5 mmole) of methyl chloroformate and stirred for 30 minutes.

A silylated solution of the nicotinic acid, prepared from 5.76 g (25 mmole) of 6-(4-aminophenyl)-1,2-dihydro-2-oxo-3-pyridinecarboxylic acid described in Method A, was added to the above solution at −10°C and allowed to stir overnight at room temperature. The mixture was filtered and the filtrate evaporated to dryness *in vacuo*. Acetone (100 ml) and H_2O (250 ml) were added and the product filtered, washed with acetone - H_2O (1: 3), ether, and dried. The *N*-[(phenylmethoxy)carbonyl]-L-alanyl compound (7.7 g) was obtained.

$[\alpha]_D^{23}$ −56.3° (*c* 1, MeOH (pH 7)). λ_{max} (pH 7) 264 nm ($E_{1cm}^{1\%}$ 257), 330 nm (481).

A suspension of 6.5 g (15 mmole) of the *N*-[(phenylmethoxy)carbonyl]-L-alanyl pyridone acid in 60 ml of 30% HBr in acetic acid was stirred at room temperature for 40 minutes. The solution was filtered and the product isolated by adding 275 ml of ethyl acetate and 100 ml of ether, filtering, and washing with ethyl acetate and ether. The dried solid was suspended in water and the pH raised to 10.2 with conc. NH_4OH. The filtered solution was evaporated *in vacuo* and the product isolated by filtration, washing with water, ethanol, and ether. The dried product weight was 4.0 g, $[\alpha]_D^{23}$ +6.7° (*c* 1, MeOH - H_2O (pH 10)).

N-Acylation of the alanyl compound was accomplished by dissolving 3.3 mmole of compound in 6 ml DMF containing 1.5 ml of Et_3N and adding the anhydride at 5°C. After stirring for 2 hours at room temperature the mixture was poured into water, acidified to pH 3 with HCl and isolated by filtration, washing, and drying.

N-Carbamoylation of the alanyl compound was accomplished by dissolving 2.53 g of the amino acid in 100 ml of water containing two equivalents of triethylamine and adding 3.4 g (five equivalents) of potassium cyanate. After standing over night the mixture was evaporated to dryness *in vacuo*. The process was repeated by adding an additional 10 equivalents of cyanate. Evaporation to a small volume followed by acidification to pH 2.5 precipitated the product and afforded 2.6 g of the carbamoyl derivative.

Method C

The silylated derivative of 6-(4-aminophenyl)-1,2-dihydro-2-oxo-3-pyridinecarboxylic acid was prepared from 23 g of pyridone acid as described in Method A.

A solution of 22.1 g (0.1 mole) of L-pyroglutamic acid chloride[7] in 100 ml of CH_2Cl_2 was added slowly to the above silylated mixture at 5°C. The mixture was stirred at 5°C for 30 minutes and at room temperature for 3 days. The solvent was evaporated *in vacuo* and 100 ml of MeOH was added. The solid was filtered, washed with MeOH, and dried affording 27 g of 6-[4-(L-pyroglutamylamino) phenyl]-1,2-dihydro-2-oxo-3-pyridinecarboxylic acid.

Side Chain Imidazolides

A suspension of 1.0 g of side chain acid in 10 ml of DMF containing 1.2 g of carbonyl diimidazole was heated with stirring to 50~60°C for 1 hour. After standing overnight at room temperature, an equal volume of CH_2Cl_2 and 20 ml of ether were added. The solid was filtered, washed with CH_2Cl_2, ether and dried *in vacuo*. The imidazolides could be recrystallized from DMF - CH_2Cl_2 mixtures and are stable when stored at room temperatures in the absence of moisture. They were usually used for

condensing with amoxicillin without further purification.

Hydroxysuccinimide Ester of 6-[4-(*N*-Acetyl-4-hydroxy-L-prolylamino)-phenyl]-1,2-dihydro-2-oxo-nicotinic Acid

A mixture of 5.0 g (13 mmole) of the *N*-acetyl hydroxyprolyl nicotinic acid, 1.5 g (13 mmole) of *N*-hydroxysuccinimide and 2.7 g (13 mmole) of *N,N'*-dicyclohexylcarbodiimide in 75 ml of DMA was stirred at room temperature for 16 hours. The urea was removed by filtration and the filtrate was evaporated to a syrup *in vacuo*. The solid, which was isolated by adding 100 ml of 2-propanol followed by 100 ml of ether and filtering was used directly in the condensation step with amoxicillin.

General Method for Condensing Side Chain Imidazolides with Amoxicillin

A mixture of 27 mmole of the imidazolide, 30 mmole of amoxicillin trihydrate and 150~190 ml of DMA was stirred at 0~5°C for 4~6 hours. The reaction mixture was added to 1~2 liters of cold water and the pH was adjusted to 2.5 with 6 N HCl with stirring. The solid was filtered and washed with cold water. The product was suspended in 300~500 ml of water and the pH was adjusted to 6~7 with 1 N sodium hydroxide. The solution was clarified by filtration and lyophilized to afford the penicillin sodium salt.

The *N*-hydroxysuccinimide ester of the hydroxyproline analog side chain can be isolated and utilized in the same manner as described for the imidazolides.

References

1) KALTENBRONN, J. S.; T. H. HASKELL, L. DOUB, J. KNOBLE, D. DEJOHN, U. KROLLS, N. JENESEL, G. HUANG, C. L. HEIFETZ & M. W. FISHER: CI-867, a new broad spectrum semisynthetic penicillin. J. Antibiotics 32: 621~625, 1979
2) MICH, T. F.; G. HUANG, P. W. K. WOO, J. P. SANCHEZ, C. L. HEIFETZ, D. SCHWEISS, U. KROLLS, M. P. HUTT, T. CULBERTSON & T. H. HASKELL: Synthesis and biological activity of a new semisynthetic cephalosporin, CN-92,982. J. Antibiotics 33: 1352~1356, 1980
3) STAAB, H. A.; M. LÜKING & F. DÜRR: Darstellung von Imidazoliden. Synthese von Amiden, Hydraziden, and Hydroxamsäuren nach der Imidazolidmethode. Chem. Ber. 95: 1275~1283, 1962
4) ANDERSON, G. W.; J. ZIMMERMAN & F. M. CALLAHAN: The use of esters of N-hydroxysuccinimide in peptide synthesis. J. Am. Chem. Soc. 86: 1839~1842, 1964
5) SHAW, E. & D. W. WOOLEY: Indole carboxamidines and aminomethylindoles as antimetabolites of serotinin. J. Am. Chem. Soc. 79: 3561~3564, 1957
6) GOODEY, V. R.; K. N. REED & J. STEPHENS: Chemical and microbiological assay of penicillin. J. Pharm. & Pharmacol. 7: 692~701, 1976
7) EBERHARDT, H.: 5-Oxo-2-pyrrolidinecarboxylic acid phenylalkanol esters. Ger. Offen 2,456,634, 1976