β-LACTAM ANTIBIOTICS. I

COMPARATIVE STRUCTURE—ACTIVITY RELATIONSHIPS OF 6-ACYLAMINOPENICILLANIC ACID DERIVATIVES AND THEIR 6-(D-α-ACYLAMINOPHENYLACETAMIDO) PENICILLANIC ACID ANALOGUES

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Structure-activity relationships were compared for two series of penicillins containing a common acyl group. In series I, which included most of the penicillins in clinical use, the acyl moiety was linked directly to the amino group of the penicillin nucleus. For series II the acyl moiety was linked to the amino group of D-α-aminobenzylpenicillin (ampicillin). In the majority of cases a striking similarity between the two series was observed. The correlation provided both a valuable means of classifying and predicting the antibacterial properties of one series from a knowledge of the other.

Although several types of derivatives of the 6-amino group of the penicillin nucleus have been examined[1~15] all the semisynthetic penicillins in clinical use contain an amide bond as the link between the nucleus and the side-chain (**1a**). The influences that many of these acyl side-chains exerted on both the degree and spectrum of antibacterial activity has been discussed in several excellent reviews[16~22]. Some workers have commented[16,21,24] that, whatever the molecular interaction might be between receptor and substrate, much of the antibacterial properties of these penicillins is related to the nature of the substitution in the vicinity of the amide bond attached to the nucleus.

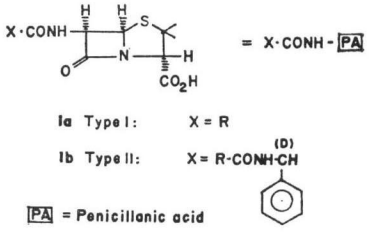
This paper reports the structure-activity relationships of a less familiar series of penicillins in which the same range of acyl side-chains are situated at a position more remote from the nucleus, namely D-α-acylamino benzylpenicillins (**1b**). For the purpose of this discussion the former class will be referred to as series (or type) I penicillins and the higher homologues as series (or type) II penicillins. It is unlikely that any of the type II penicillins in this paper would have any significant advantages over their type I analogues in terms of *in vitro* activity, although some members would appear to provide at least a useful alternative.

Materials and Methods

A variety of synthetic procedures[16,18,23] well known in peptide and penicillin chemistry were used to couple the acyl side-chains to the amino group of either 6-aminopenicillanic acid (6-APA) or ampicillin, to produce type I and II penicillins respectively.

The methods included prior activation of the carboxy group of the side-chains by acid chloride, mixed anhydride

Formula 1.



(*e.g.* isobutoxyformic) or 'activated' ester (*e.g.* N-succinimido) derivatives, without any protection of the thiazolidine carboxy group. Alternatively a carbodiimide coupling procedure was used in a few cases which necessitated protection of the thiazolidine carboxy group as a water labile trimethylsilyl ester or a benzyl ester which was finally removed by catalytic hydrogenolysis.

All the penicillins used in the tests for antibacterial activity were substantially pure by various criteria. Purity was assessed by N.M.R. and I.R. spectroscopic analysis, biochromatographic evidence and other physical data such as hydroxylamine[26] and/or iodometric[27] assay. Elemental analytical data was not available for all the penicillins.

Minimum inhibitory concentrations (MIC) were determined by two-fold serial dilution of the compounds in nutrient agar (pH 7.4) in Petri dishes which were inoculated with a replicating device delivering an inoculum of approximately 0.001 ml ($\sim 5 \times 10^5$ cells per drop). In all cases the inoculum was an undiluted 24-hour nutrient broth culture of the test organism. Inhibition of growth was recorded after incubation over-night at 37°C.

Results and Discussion

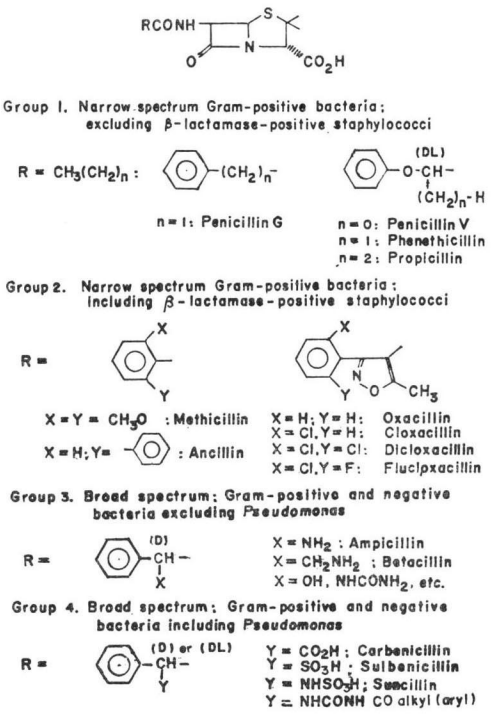
Semi-synthetic penicillins have often been classified for convenience into various groups according to their antibacterial spectra and the nature of their acyl side-chains. One such classification[19], summarised in Fig. 1, places *inter alia* the better known penicillins, such as those already well established in clinical practice or at present undergoing clinical trials, into one of four groups.

The first group consists mainly of penicillins with hydrophobic alkyl, aryl and aralkyl acid side-chains which confer activity against streptococci and non-β-lactamase-producing staphylococci with negligible, or only modest, activity against Gram-negative bacteria. The second group is represented by the sterically hindered side-chains[16] which produced penicillins with good stability to staphylococcal β-lactamase. The third and fourth groups are the broad-spectrum penicillins obtaided usually by the introduction of functional groups in the α-position of phenylacetic acid. Basic amino and guanidino functional groups produced penicillins with good activity against several Gram-negative organisms but little or no activity, against *Pseudomonas aeruginosa* or the indole-positive *Proteus* species.

Activity against these more penicillin-resistant Gram-negative organisms is achieved by the introduction of strongly acidic functional groups. The more neutral functional groups in the α-position can give rise to either type of broad-spectrum, or even narrow-spectrum, activity and will be discussed later.

The MIC activity data shown in Tables 2~5 have been represented schematically in three groups, *viz.*, good, moderate or poor, for each organism, or group of organisms, in

Fig. 1. 6-Acylaminopenicillins acids (Type 1)



order to make the comparison between the two series easier. The ranges of activities in each category for a particular organism(s) are given in Table 1. It is difficult to draw a sharp dividing line between what is universally accepted as good, moderate or poor activity for even a well-established class of antibiotics such as penicillins. However, the categories listed in this paper have been suggested with the benefit of the experience gained in these laboratories from testing many hundreds of penicillins over several years and relating these data to the activities of the clinically effective penicillins[19].

For example, carbenicillin (No. 29, Table 3) has a rather high average MIC of 50 μg/ml against several strains of *Ps. aeruginosa* but, nevertheless, has proved itself useful clinically against pseudomonas infections. Only relatively few penicillins, and no reported cephalosporins, exhibit even this degree of activity against this organism. Therefore an MIC of 50 μg/ml against *Ps. aeruginosa* may be regarded as good for β-lactam antibiotics whereas such a level of activity against *Salmonella typhi* or *Escherichia coli* may be regarded as poor since many broad-spectrum penicillins have average MIC's of 1.25~5.0 μg/ml against these organisms. Many of the type I/II pairs in this study which are placed in the same activity categories had in fact the same MIC values or differed only by a factor of two. No activities ranked in the same category differed by a factor greater that four (*i.e.* two dilutions).

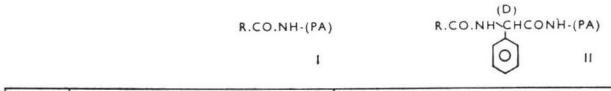
In Table 2 a comparison is made of those side-chains which gave rise to narrow-spectrum penicillins of groups I (Nos. 1, 3 and 5) and 2 (Nos. 7 and 9), in Fig. I, and their II analogues. The similarity is good for those penicillins (Nos. 1~6) inactive against β-lactamase-producing *Staphylococcus aureus*. However, where the type I penicillin (Nos. 7 and 9) was stable to the β-lactamase and had activity against this organism its type II analogue showed no such activity.

The resistance of the strain of *S. aureus* used in this study, like the majority of penicillin-resistant staphylococci, can be attributed primarily to the destruction of the penicillin by the β-lactamase enzyme[28]. Therefore a comparison of the activities of a penicillin against the sensitive and resistant strains of *S. aureus* is an indication of the stability of the penicillin to staphylococcal β-lactamase. All the penicillins in this group were very active against non-β-lactamase-producing *S. aureus*. Consequently the difference in the activities of the two series containing sterically hindered side-chains against β-lactamase-producing *S. aureus* is mainly due

Table 1. Classification of antibacterial activities. (Values in μg/ml)

Organisms		Good	Moderate	Poor
1. Gram-positive organisms	*Staphylococcus aureus* (β-lactamase-negative) *Streptococcus pyogenes*	0.02~0.5	1.25~5.0	>5.0
	Staphylococcus aureus (β-lactamase-positive) *Streptococcus faecalis*	0.12~2.5	5.0~25	>25
2. Gram-negative organisms	*Escherichia coli* *Salmonella typhi* *Proteus mirabilis* *Proteus morganii* (indole-positive)	1.25~5.0	12.5~50	>50
	Pseudomonas aeruginosa	12.5~50	125~250	>250

Table 2. Narrow spectrum (Gram-positive) penicillins.

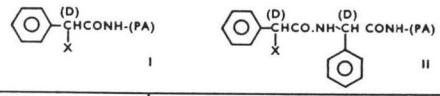


No.	PENICILLIN R	STRUCTURAL TYPE	ANTIBACTERIAL ACTIVITY (IN VITRO) GRAM-POSITIVE ORGANISMS: Staphylococcus aureus	Staphylococcus aureus *	Streptococcus faecalis	Streptococcus pyogenes	GRAM-NEGATIVE ORGANISMS: Escherichia coli	Salmonella typhi	Pseudomonas aeruginosa	Proteus mirabilis	Proteus morganii *
1[a]	$PhCH_2$-	I									
2		II									
3[b]	$PhOCH_2$-	I									
4		II									
5[c]	(DL) PhO-CH(CH$_3$)-	I									
6		II									
7[d]	2,6-dimethoxyphenyl	I									
8		II									
9[e]	2-chloro-5-methylisoxazol-4-yl	I									
10		II									

a, Penicillin G; b, Penicillin V; c, Phenethicillin d, Methicillin e, Cloxacillin

* β-lactamase producing strains ▨ GOOD ▩ MODERATE □ POOR

Table 3. Broad spectrum penicillins (excluding *Pseudomonas*).



NUMBER	PENICILLIN X	STRUCTURAL TYPE	ANTIBACTERIAL ACTIVITY (IN VITRO) GRAM-POSITIVE ORGANISMS: Staphylococcus aureus	Staphylococcus aureus *	Streptococcus faecalis	Streptococcus pyogenes	GRAM-NEGATIVE ORGANISMS: Escherichia coli	Salmonella typhi	Pseudomonas aeruginosa	Proteus mirabilis	Proteus morganii *
11[a]	.NH_2	I									
12		II									
13[b]	.N_3	I									
14		II									
15	.$NHCONH_2$	I									
16		II									
17	.SO_2NH_2	I									
18		II									
19	.$ONHCONH_2$	I									
20		II									
21	.$OCOCH_3$	I									
22		II									
23	.OH	I									
24		II									
25	.$NH.C(NH)NH_2$	I									
26		II									
27	.Cl	I									
28		II									

* β-lactamase producing strains a, Ampicillin b, Azidocillin ▨ GOOD ▩ MODERATE □ POOR

to a difference in their relative stabilities to staphylococcal β-lactamase and not due to other factors such as penetration to the active site(s) in the bacterium.

The reason for the resistance of some Gram-negative bacteria, including *Pseudomonas*, is often more complex than the presence of the β-lactamase(s) they produce[20]. Hence the absence of anti-pseudomonas activity of cloxacillin (No. 9) compared with the moderate activity of its type II analogue (No. 10) need not necessarily simply be due to the greater stability of the latter compound to pseudomonas β-lactamase; especially since cloxacillin already shows very good stability to this enzyme. Furthermore, in contrast to the situation with *S. aureus* and

P. morganii, the amount of β-lactamase produced by the strain of *Ps. aeruginosa* used in this study is probably too small to be a significant factor for the resistance of the organism.

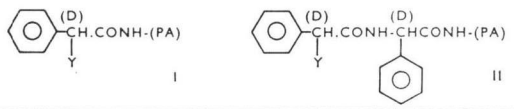
The weak binding of sterically hindered type I penicillins, such as methicillin (No. 7) and cloxacillin (No. 9), to staphylococcal β-lactamase, as indicated by their very low affinity constants, may be interpreted to imply that the 6-amide bond may be an important binding site to the active centre of the enzyme.[30] In any case the effect of steric hindrance near the side-chain amide bond in these penicillins somehow produces a substrate which induces a catalytically non-active conformation of the enzyme.[31] The corresponding type II analogues of methicillin and cloxacillin (Nos. 8 and 10 respectively) have a second amide bond in the side-chain further removed from nucleus. It would appear from the observed instability of these analogues to staphylococcal β-lactamase that steric hindrance around this amide bond does not induce a conformation of the enzyme which is unfavourable for hydrolysis. Therefore, either the second amide bond in these derivatives is not an important binding site to the active centre of staphylococcal β-lactamase or that these larger molecules are still sufficiently flexible to accommodate a favourable substrate—enzyme interaction.

Both types of penicillins in this group showed either poor, or only a modest, degree of activity against Gram-negative bacteria.

A comparison is made in Table 3 of α-substituted phenylacetyl side-chains (all D epimers) which conferred either good or modest broad-spectrum activity (excluding *Ps. aeruginosa* and indole-positive *Proteus*) in type I penicillins with their type II analogues.

In most cases the correlation was good. The guanidino penicillins (Nos. 25 and 26) showed a modest variation in their relative degrees of activity against two of the organisms, *viz.*, β-lactamase-positive *S. aureus* and *Ps. aeruginosa*. In neither case was the difference one of good *versus* poor activity. Compound 25 was distinguished as being the only penicillin in this

Table 4. Broad spectrum penicillins (including *Pseudomonas*).



No.	Y	STRUCTURAL TYPE	GRAM-POSITIVE ORGANISMS: Staphylococcus aureus	Staphylococcus aureus *	Streptococcus faecalis	Streptococcus pyogenes	GRAM-NEGATIVE ORGANISMS: Escherichia coli	Salmonella typhi	Pseudomonas aeruginosa	Proteus mirabilis	Proteus morganii *
29[a] 30	$.CO_2H$	I II									
31[b] 32	$.NHSO_3H$	I II									
▲33[c] 34	$.SO_3H$	I II				NO DATA AVAILABLE					NO DATA
35 36	.NHCON⟨ ⟩NH	I II									
37 38	.NHCONHCO–	I II									
39 40	$.NHCONHC(NH)NH_2$	I II									

▲results for this comparison were taken from S. Morimoto (J. Antibiotics, XXV(No.3), P.145 P.146. , 1973.

a, Carbenicillin; b, Suncillin; and c, Sulbenicillin

GOOD MODERATE POOR

*β-lactamase producing strains

category with good activity against β-lactamase-producing *S. aureus*. It has considerable stability to staphylococcal penicillinase which again may be partly due to steric crowding around the amide bond; in this instance perhaps due to a closely packed solvation shell around the highly polar, bulky guanidino function. Compound 24, which has an hydroxyl functional group, also had a moderate degree of anti-pseudomonas activity unlike its type I analogue (No. 23).

All the other pairs had poor activity against resistant *S. aureus*, *Ps. aeruginosa* and *Proteus morganii*. Compounds with an amino, hydroxyl (type I) or ureido group (Nos. 11, 12, 23, 15 and 16) had the greatest potency with the chloro, acetoxy and oxyureido penicillin (Nos. 27, 28, 21, 22, 19 and 20) possessing Gram-negative activity little better than that of penicillin G (No. 1, Table 2).

Introduction of strongly acidic functions in the α-position of phenylacetyl side-chains had again essentially the same influence in either type I or type II penicillins as shown in Table 4 (Nos. 29~34). In both series good anti-pseudomonas activity was achieved along with a level of broad-spectrum activity ranging from moderate to good against most of the other organisms represented in the Table. The strongly acidic α-(5-tetrazolyl)benzylpenicillin[21] and its type II analogue are not represented here, however, from the trend observed with the other acidic α-substituents again a good agreement between the two spectra would be expected. Examples 35 and 37 are important since they represent a fairly large class of anti-pseudomonas penicillins, namely, the α-(acylureido)benzylpenicillins. The similarity between them and their type II analogues (Nos. 36 and 38) is extremely poor, since the good anti-pseudomonas activity of the former is totally lacking in the latter. In fact compound 38 had little activity against any of the Gram-negative bacteria in this study. Consequently, the two types have have quite different anti-bacterial spectra and represent the second class for which the type I/II comparison differs markedly. A closer comparison of compounds 35 and 37 merits further consideration. MORIMOTO *et al.*[24]

Table 5. Miscellaneous penicillin types.

No	R	STRUCTURAL TYPE	Staphylococcus aureus	Staphylococcus aureus *	Streptococcus faecalis	Streptococcus pyogenes	Escherichia coli	Salmonella typhi	Pseudomonas aeruginosa	Proteus mirabilis	Proteus morganii *
			GRAM-POSITIVE ORGANISMS				GRAM-NEGATIVE ORGANISMS				
41	$CH_3CH_2CH_2$·	I									
42		II									
43	CH_3COCH_2·	I									
44		II									
45	NH_2CH_2·	I									
46		II									
47	$HO_2C.CH_2$·	I									
48		II									
49	PhCH=N-N(Ph)·	I									
50		II									

*β-lactamase producing strains

GOOD MODERATE POOR

put forward the hypothesis that acylureido penicillins, such as example 37, had anti-pseudomonas activity due to the presence of a relatively acidic imido group (·NHCONHCO-). This conformed with the structural requirement of other anti-pseudomonas penicillins of having a unit; aryl/alkyl-CH(X^-)CONH- (X^-=negatively charged group) in the side-chain. This explanation is unsatisfactory however to explain the excellent anti-pseudomonas activity of a more recent series of acylureido benzylpenicillins[32], typified by compound 35, in which the acidic imido proton has now been replaced by an alkyl group (·NHCONCO-, alkyl), resulting in a fairly neutral class of side-chains.

Compound 44 (Table 5) is also at variance with this hypothesis. This compound bears an α-substituent, a β-ketoamide ($NHCOCH_2COCH_3$), closely related to the earlier class of acylureido penicillins since it also possesses an acidic proton. Nevertheless, despite the similarity, the good anti-pseudomonas activity of the acylureido penicillins is totally lacking in the β-ketoamido-penicillins.

In fact the β-ketoamide substituent of compound 44 had essentially the same spectrum as the corresponding α-acylamino penicillin (·$NHCOCH_2CH_2CH_3$; compound 42). However, for both substituents a good agreement was again found for the type I/II analogues. Compound 45 and 47 (Table 5) were included to illustrate the effect of replacing the phenyl ring by a proton in ampicillin and carbenicillin respectively. In the former case (compound 45) the same spectrum was obtained but with a lesser degree of Gram-negative activity than ampicillin. Its type II analogue (compound 46) possessed a similiar degree of activity to that of ampicillin.

Compound 47 differed considerably from carbenicillin (No. 29) in having no anti-pseudomonas activity. It is interesting that its type II analogue (compound 48) also lacked this activity and indeed a very similar result was obtained in general for the two types. Examples 49 and 50 illustrated that even for an unusual side-chain, such as N-phenyl benzylidene hydrazinoyl, an excellent correspondence between the two types was observed.

In the structural moiety of type I penicillins, R, X>CHCONH-, if R=aryl, heterocyclic or alkyl then the functional group, X, will largely determine the spectrum of activity, especially when X is strongly anionic or cationic. The R groups will have a modest influence on the degree of activity against a particular organism. In both type I and II penicillins R=H can have a marked effect in some cases (*e.g.*, X=CO_2H) but not in other (*e.g.*, X=NH_2) as discussed. The effects of a larger range of X and R substituents in type II penicillins will be the subject of a future publication in this series.

In conclusion, although it was known previously that the acyl moiety in 6-acylaminopenicillanic acids largely determined the spectrum of activity of the penicillin, the present study has shown that even when the acyl group is at a more remote position from the nucleus, as in the N-acylated ampicillins, it can also greatly influence the spectra of antibacterial activities observed. Furthermore, the nature of the acyl group is also important in determining the degree of correlation between the two series of penicillins.

There was no obvious reason *à priori* to expect the good correspondence observed in the

majority of cases for the two series, nor is it possible at this stage to offer any satisfactory explanation for the phenomenon. No such similarities would be expected between non-acyl derivatives of 6-APA and ampicillin, due to the generally very weak antibacterial activity of the former types.[17] The general patterns of structure-activity which have emerged from this study may be of some value as a means of predicting the properties of other N-acylated ampicillins.

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