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Lipidic peptides. XI. Quantitative structure-activity relationships of a series of lipidic amino acid conjugates of β-lactam antibiotics

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Summary

A series of lipidic amino acid conjugates of β-lactam antibiotics were synthesized and the in vitro and in vivo activity was determined against a variety of Gram-positive and -negative bacteria. The chemical structures of the compounds were characterized by indicator variables showing the presence or absence of a substituent at a certain position, and by their lipophilicity as the calculated logarithmic value of octanol/water partition coefficient (clog *P*). Stepwise linear regression analysis was applied for revealing quantitative structure-activity relationships (QSAR). The in vitro activities of the compounds did not correlate with in vivo activities indicating the influence of absorption processes. The in vivo oral and subcutaneous activities were influenced by the lipophilicity of the compounds. The optimum clog *P* values for high in vivo oral and subcutaneous activity were around 6 and 8, respectively. The presence of the long lipidic side chain at position 1 decreased the activity against *E. coli* and *Pseudomonas aeruginosa*. Loss of activity against the non-β-lactamase-producing *S. aureus* was observed for compounds with a lipidic side chain at position 2.

Introduction

Due to their bifunctional nature, the lipidic amino acids and peptides have the capacity to be chemically conjugated to drugs with a wide variety of functional groups. The resulting lipidic amino acid conjugates are expected to possess a degree of membrane-like character, sufficient to facilitate the passage of poorly absorbed drugs across biological membranes to reach their site of action (Gibbons et al., 1990). Therefore, a series of lipidic amino acid conjugates of β-lactam antibiotics were synthesized and their in vitro and in vivo antibacterial activities were determined (Hughes et al., 1991; Toth et al., 1991a,b). Quantitative structure-activity relationship (QSAR) studies first introduced by Hansch and Fujita (1964) provide a valuable tool for revealing the structural requirements for the activity and the role of lipophilicity in the absorption of the com-

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pounds. The application of indicator variables in QSAR assists drug design by providing information about the substituent type and position for maximum activity.

In this study, QSAR investigations are presented using the combined methods of Hansch and Fujita (1964) and Free and Wilson (1964), as suggested by Kubinyi (1976), applying both indicator variables and physico-chemical parameters (octanol/water partition coefficients, log P). The objective was a better understanding of the role of lipidic amino acid substituents at various positions of β-lactam antibiotics in modifying their in vivo and in vitro antibacterial activity.

Materials and Methods

The synthesis and the biological activity of the compounds **1–26** (Table 1 were described elsewhere (Hughes et al., 1991; Toth et al., 1991a,b). The logarithmic value of their octanol/water partition coefficients (clog P) was calculated from the chemical structure by the ProLogP™ expert

TABLE 1

Chemical structure of the investigated compounds

Type No.	Type	Type A: R^1	Type B: R^2	Type B: R^3
1	A	Boc–NH–CH(C_8H_{17})–CO–	COOH	–
2	A	Boc–NH–CH($C_{12}H_{25}$)–CO–	COOH	–
3	A	Ph–CH_2(NH_2)–CO–	COOH	–
4	A	Ph–CH[(NHCOCH(C_8H_{17})–NH–Boc]–CO–	COOH	–
5	A	Ph–CH[NHCOCH(C_5H_{11})–NH–Boc]–CO–	COOH	–
6	B	Boc–NH–CH(C_8H_{17})–CO–	COOH	CH_2OCOCH_3
7	B	Boc–NH–CH($C_{12}H_{25}$)–CO–	COOH	CH_2OCOCH_3
8	B	Boc–[NH–CH(C_8H_{17}–CO)]$_2$–CO–	COOH	CH_2OCOCH_3
9	B	Ph–CH(NH_2)–CO–	COOH	CH_2OCOCH_3
10	B	Ph–CH[NHCOCH(C_8H_{17})–Boc]–CO–	COOH	CH_3
11	B	Ph–CH[NHCOCH($C_{12}H_{25}$)–Boc]–CO–	COOH	CH_3
12	A	Ph–CH_2–CO–	COOH	–
13	B	(C_4H_4O)–C(=NOCH$_3$)–CO–	COOH	CH_2OCONH_2
14	A	Ph–CH_2–CO–	COOCH(C_6H_{13})COOH	–
15	A	Ph–CH_2–CO–	COOCH($C_{12}H_{25}$)COOH	–
16	A	Ph–CH_2–CO–	COOCH($C_{14}H_{29}$)COOH	–
17	A	Ph–CH(NH_2)–CO–	COOCH(C_6H_{13})COOCH$_3$	–
18	B	(C_4H_4O)–C(=NOCH$_3$)–CO–	COOCH(C_6H_{13})COOCH$_3$	$OCONH_2$
19	B	(C_4H_4O)–C(=NOCH$_3$)–CO–	COOCH($C_{14}H_{29}$)COOCH$_3$	$OCONH_2$
20	A	Ph–CH_2–CO–	$COOCH_2OCOCH(C_8H_{14})NHBoc$	–
21	A	Ph–CH_2–CO–	$COOCH_2CH_2OCOCH(C_8H_{14})NHBoc$	–
22	A	Ph–CH_2–CO–	$COO(CH_2)_3OCOCH(C_8H_{14})NHBoc$	–
23	A	Ph–CH_2–CO–	$COOCH_2CH_2OH$	–
24	B	(C_4H_4O)–C(=NOCH$_3$)–CO–	$COOCH_2OCOCH(C_8H_{17})NHBoc$	$OCONH_2$
25	B	(C_4H_4O)–C(=NOCH$_3$)–CO–	$COOC_2H_4OCOCH(C_8H_{17})NHBoc$	$OCONH_2$
26	B	(C_4H_4O)–C(=NOCH$_3$)–CO–	$COOC_3H_6OCOCH(C_8H_{17})NHBoc$	$OCONH_2$

Boc, $C(CH_3)_3$–O–CO–; Ph, phenyl ring.

TABLE 2

Antibiotic activity and log P values of the investigated compounds

Compound	A	B	C	D	E	F	G	log *P*
1	8.23	0.51	32.9	257	16.45	103	103	5.84
2	29.5	3.69	230	230	29.5	46.1	46.1	7.91
3	0.15	0.15	0.32	0.15	0.15	3.22	3.22	1.15
4	6.46	0.40	1.61	0.40	3.23	10.0	80.8	5.84
5 [a]	91.8	1.48	5.93	0.74	11.85	5.33	ndr	4.87
6	14.7	0.46	231	231	57.2	46.1	46.1	4.41
7	104	3.34	209	209	209	41.8	41.8	6.49
8	87.2	11.2	176	176	176	35.1	35.1	7.50
9 [a]	ndr	ndr	ndr	ndr	ndr	23.9	17.9	−0.91
10	100	6.48	50.2	100	50.2	40.5	40.5	5.78
11	186	11.9	186	186	186	37.1	37.1	7.34
12	5.37	0.16	21.5	0.35	21.5	1.34	9.67	1.89
13	72.9	0.30	0.31	1.17	4.70	8.47	58.8	−1.45
14	255	4.08	255	16.3	32.6	2.67	11.20	5.10
15 [a]	ndr	ndr	ndr	ndr	ndr	9.23	9.23	8.21
16 [a]	ndr	ndr	ndr	ndr	ndr	i.a.	27.40	9.25
17 [a]	ndr	ndr	ndr	ndr	ndr	ndr	2.52	5.46
18 [a]	ndr	ndr	ndr	ndr	ndr	4.13	ndr	2.12
19 [a]	ndr	ndr	ndr	ndr	ndr	i.a.	i.a.	6.28
20	197	0.2	197	0.7	197	2.0	2.0	7.95
21	192	0.38	192	3.1	24	2.0	38	8.18
22	188	188	188	188	93	2.0	37	8.12
23	164	10	330	42	164	66	66	1.68
24 [a]	172	172	22	172	22	ndr	ndr	4.61
25 [a]	170	170	170	170	42	ndr	ndr	4.84
26	239	239	118	239	59	4.5	15	0.45

[a] Compounds were not included in the calculations because of the missing activity data.

In vitro test (mmol/1×10^{-3}) of compounds and cefuroxime (10^3 cfu/ml inoculum). A, *S. aureus* β-lactamase-producing; B, *S. aureus* non-β-lactamase-producing; C, *E. coli*; D, *Ps. aeruginosa*; E, *C. perfringens*; F, in vivo ED_{50} s.c.; G, in vivo ED_{50} p.o., log *P*, logarithmic value of the octanol/water partition coefficient.

system developed by Compudrug Ltd, Budapest, Hungary (Van de Waterbeemd, 1986) based on the hydrophobic fragmental constants (Rekker, 1976).

The antibiotic activities and the clog *P* values of the compounds **1–26** are summarized in Table 2.

The chemical structures of the compounds for the QSAR study were characterized by indicator variables (Table 3). They were assigned on the basis of the following rules: R^1, R^2 and R^3 refer to substituent positions 1, 2 and 3, respectively, as shown in Table 1.

By means of the parameters listed in Table 3, each chemical structure could be uniquely described. The indicator variables extracted from

TABLE 3

Assignment of indicator variables used for characterizing the chemical structure

Sign	Represents	Position
R^1Boc, R^2Boc	$C(CH_3)_3$–O–CO–	1 and 2
R^1PhN	Ph–CH(NH–)–Co–	1
R^1Ph	Ph–CH_2–CO– (lacking NH_2)	1
R^1Fur	(C_4H_4O)–C(=$NOCH_3$)–CO–	1
R^1C, R^2C	number of –CH_2– groups in chain	1 and 2
R^2H	–COOH	2
R^2Me	–$COOCH_3$ at the end of side chain	2
R^2OH	–COOH at the end of side chain	2
R^3O	structure type A	
R^3NH_2	–CH_2OCONH_2	3
R^3Me	–CH_3	3
R^3OME	–CH_2OCOCH_3	3

TABLE 4

Indicator variables formed on the bases of the chemical structure of the compounds (for explanation of the symbols, see Table 3)

Compound No.	R^1					R^2					R^3			
	Boc	PhN	Ph	Fur	C	H	Me	Boc	OH	C	O	NH_2	Me	OMe
1	1	0	0	0	8	1	0	0	0	0	1	0	0	0
2	1	0	0	0	11	1	0	0	0	0	1	0	0	0
3	0	1	0	0	0	1	0	0	0	0	1	0	0	0
4	1	1	0	0	8	1	0	0	0	0	1	0	0	0
5	1	1	0	0	5	1	0	0	0	0	1	0	0	0
6	1	1	0	0	8	1	0	0	0	0	0	0	0	1
7	1	0	0	0	12	1	0	0	0	0	0	0	0	1
8	1	0	0	0	16	1	0	0	0	0	0	0	0	1
9	0	1	0	0	0	1	0	0	0	0	0	0	1	0
10	1	1	0	0	8	1	0	0	0	0	0	0	1	0
11	1	1	0	0	12	1	0	0	0	0	0	0	1	0
12	0	0	1	0	0	1	0	0	0	0	1	0	0	0
13	0	0	0	1	0	1	0	0	0	0	0	1	0	0
14	0	0	1	0	0	0	0	0	1	6	1	0	0	0
15	0	0	1	0	0	0	0	0	1	12	1	0	0	0
16	0	0	1	0	0	0	0	0	1	14	1	0	0	0
17	0	1	0	0	0	0	1	0	0	6	1	0	0	0
18	0	0	0	1	0	0	1	0	0	6	0	1	0	0
19	0	0	0	1	0	0	1	0	0	14	0	1	0	0
20	0	0	1	0	0	0	0	1	0	9	1	0	0	0
21	0	0	1	0	0	0	0	1	0	10	1	0	0	0
22	0	0	1	0	0	0	0	1	0	11	1	0	0	0
23	0	0	1	0	0	0	0	0	1	2	1	0	0	0
24	0	0	0	1	0	0	0	1	0	9	0	1	0	0
25	0	0	0	1	0	0	0	1	0	10	0	1	0	0
26	0	0	0	1	0	0	0	1	0	3	0	1	0	0

the chemical structure of the compounds are summarized in Table 4.

Stepwise linear regression analysis was carried out by the Drugidea™ program system developed for drug design (Chemicro Ltd, Budapest, Hungary). The dependent variables were always the antibiotic activity data, and the independent variables were selected according to their significance level (set to 95%) in explaining the variance of the dependent variable.

Results and Discussion

The correlation coefficients among the various antibiotic activity data and the clog *P* values are summarized in a correlation matrix shown in Table 5. The activity data did not show a close correlation with each other because they were obtained from different strains.

In vitro activities

The variance of the MIC of the compounds on *S. aureus*[a] could be described by Eqn 1:

$$\begin{aligned} MIC_{S.aureus^a} = &-184.4(\pm 17.7)R^2H \\ &- 54.3(\pm 18.4)R^3O \\ &+ 76.32(\pm 27.2)R^3Me + 251.1 \end{aligned} \quad (1)$$

$$n = 17; \quad r = 0.973; \quad s = 32.2 \quad F = 36.9$$

where n is the number of compounds, r denotes the multiple correlation coefficient, s is the stan-

TABLE 5

Correlation coefficients of the variables used in the equations presented in a correlation matrix

	S. aur[a]	*S. aur*[b]	*E. coli*	*Ps. aerug.*	*C. perfr.*	R^1Boc	R^1PhN	R^1Fur	R^1C	R^2H	R^2C	R^3O	R^3NH_2	R^3Me	log *P*	ED_{50sc}	ED_{50po}
S. aur[a]	1.00																
S. aur[b]	0.51	1.00															
E. coli	0.48	−0.01	1.00														
Ps. aerug.	0.02	0.44	0.26	1.00													
C. perfr.	0.35	−0.07	0.61	0.24	1.00												
R^1Boc	−0.49	−0.43	−0.05	0.39	0.16	1.00											
R^1PhN	−0.38	−0.32	−0.33	−0.16	−0.17	0.50	1.00										
R^1Fur	0.30	0.68	−0.26	0.17	−0.27	−0.45	−0.33	1.00									
R^1C	−0.38	−0.39	0.11	0.48	0.37	0.92	0.30	−0.42	1.00								
R^2H	−0.82	−0.60	−0.43	0.06	−0.11	0.74	0.53	−0.36	0.68	1.00							
R^2C	0.68	0.56	0.28	−0.05	0.03	−0.65	−0.47	0.30	−0.60	−0.88	1.00						
R^3O	−0.14	−0.32	0.01	−0.49	−0.25	−0.19	−0.07	−0.55	−0.31	−0.12	0.12	1.00					
R^3NH_2	0.41	0.84	−0.11	0.35	−0.17	−0.38	−0.28	0.84	−0.35	−0.51	0.43	−0.46	1.00				
R^3Me	0.12	−0.20	−0.04	0.11	0.23	0.37	0.51	−0.17	0.35	0.27	−0.24	−0.37	0.14	1.00			
log *P*	0.16	−0.12	0.37	0.27	0.36	0.43	−0.01	−0.51	0.48	0.34	0.31	0.16	−0.25	0.20	1.00		
$ED_{50s.c.}$	−0.33	−0.28	0.17	0.57	0.18	0.60	0.06	−0.23	0.52	0.35	−0.45	0.17	−0.18	0.16	0.06	1.00	
$ED_{50p.o.}$	−0.38	−0.20	−0.12	0.28	−0.15	0.55	0.04	0.01	0.41	0.30	−0.36	−0.01	−0.19	−0.07	0.06	0.70	1.00

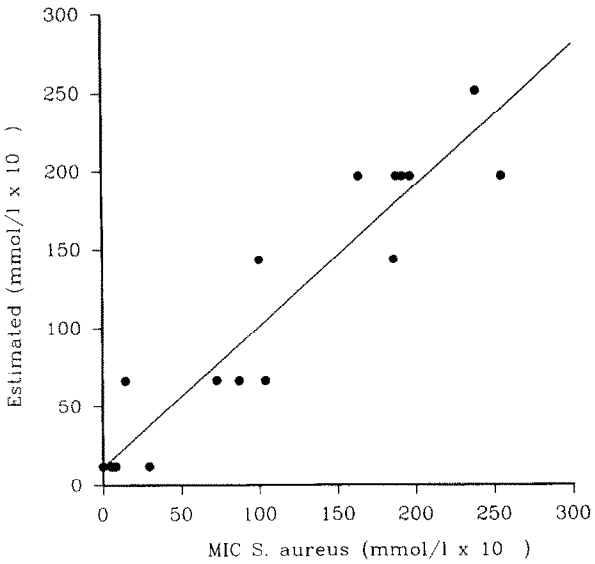

Fig. 1. Plot of the measured and calculated (using Eqn 1) minimum inhibitory concentration (MIC) values of the compounds (mmol/l$\times 10^{-3}$) obtained in vitro against β-lactamase-producing *S. aureus*.

dard error of the estimate and F represents the Fisher-test value. The plot of the measured and calculated MIC values according to Eqn 1 is shown in Fig. 1. Compounds type A were active against *S. aureus*[a] when a carboxyl-substituted tetrahydrothiazole ring was present. The activity is significantly decreased when $R^3 = CH_3$. The hydrophobicity of the compounds or the length of the alkyl chain did not play an important role in this case.

The variance of the MIC of the compounds against the non-β-lactamase producing *S. aureus*[b] strain could be described by Eqn 2:

$$MIC_{S.aurcus^b} = 6.1(\pm 2.5)R^2C + 220.1(\pm 41.5)R^3NH_2 + 0.5 \quad (2)$$

$n = 17$; $r = 0.919$; $s = 40.2$; $F = 17.5$

Eqn 2 shows that the activity of the compounds against *S. aureus*[b] is strongly disfavoured by the presence of the CH_2OCONH_2 group at the 3-position. Increasing the number of carbon atoms in the substituent at position 2 also decreased the activity of the compounds. Positions 2 and 3 seem to be important sites for determining activity against *S. aureus*.

The structural requirements for the in vitro activity of the compounds against *E. coli* could be described quantitatively by Eqn 3:

$$MIC_{E.coli} = 13.6(\pm 3.7)R^1C - 173.1(\pm 47.0)R^2H + 118.8(\pm 55.8)R^2OH + 173.8 \quad (3)$$

$n = 17$; $s = 64.4$; $r = 0.912$; $F = 9.6$;

The activity could be increased by the presence of the carboxyl group at position 2. The activity decreases, however, when a lipophilic substituent at position 2 terminates with a carboxyl group or when there is a long hydrophobic side chain at position 1.

The structure-activity relationship of the compounds against *Pseudomonas aeruginosa* could be described by Eqn 4:

$$MIC_{Ps.aerug.} = 12.8(\pm 3.4)R^1C + 202.8(\pm 80.8)R^3NH_2 + 36.2 \quad (4)$$

$n = 17$; $s = 75.7$; $r = 0.861$; $F = 8.5$

This suggests that a long alkyl chain at position 1 and the $-CO-NH_2$ group at position 3 should be avoided. These two structural parameters decreased the activity of the compounds against *Ps. aeruginosa*.

The variance of the activity data against *Clostridium perfringens* could be described very poorly (but statistically significantly at 95% probability level) by Eqn 5:

$$MIC_{C.perfr.} = -157.1(\pm 61.1)R^2H - 16.7(\pm 7.6)R^2C + 90.1(\pm 38.2)R^3OMe + 1.8(\pm 0.7)(\log P)^2 + 142.3 \quad (5)$$

$n = 20$; $r = 0.729$; $s = 56.1$; $F = 4.3$

The long alkyl chain at position 2 is advantageous for the activity, but the optimum log P is around 3.6. A carboxyl group at position 2 is also beneficial (the β-weight being only slightly higher for the carboxyl group than that of the long alkyl chain). An acetoxymethyl group at position 3 decreased the activity of the compounds.

In vivo activities

The in vivo data did not show correlation with the in vitro data on the same strain (*S. aureus*[b]), indicating the importance of other parameters (e.g., absorption, distribution, metabolism, excretion) which may influence activity in vivo. The compounds were tested in vivo against the non-β-lactamase producing *S. aureus* strain after oral and subcutaneous administration. The following equation describes the structure-activity relationships:

$$\begin{aligned} ED_{50}(po) = {} & 136.8(\pm 19.8)R^1Boc \\ & - 50.7(\pm 13.0)R^2H \\ & + 61.0(\pm 10.8)R^3O \\ & - 32.9(\pm 6.2)\log P \\ & + 2.9(\pm 0.6)(\log P)^2 + 42.6 \qquad (6) \end{aligned}$$

$n = 17; \quad r = 0.92; \quad s = 13.5; \quad F = 10.8$

The plot of the measured ED_{50}(po) against the calculated value is presented in Fig. 2. The presence of a Boc group in the substituent at position 3 decreased the in vivo activity. Replacement of the five-membered ring with a six-membered one is not advantageous (Eqn 3). The free carboxyl group at position 2 increased the activity. A log P value of 5.9 was found to be optimal for absorption. As for the β-weights of the independent variables in Eqn 3, log P has the highest value (-3.87), followed by the R_1Boc variable (2.49). The difference between Eqns 2 and 3 provided information about the differences between the in vitro and in vivo activity. Considering the two equations together it can be concluded, that a lipidic side chain at position 1 and the tetrahydrothiazine ring increased the absorption of the compounds while the 'Boc' ending and/or an excessively long hydrocarbon side chain at position 2 decreased the activity.

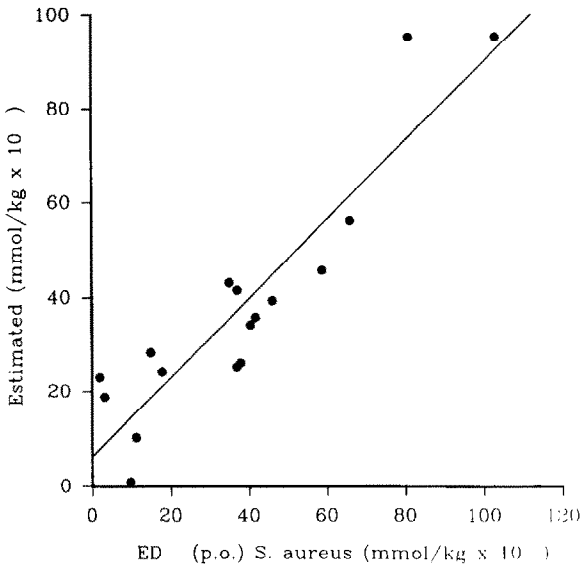

Fig. 2. Plot of the measured and calculated (using Eqn 6) ED_{50} concentration values (mmol/kg$\times 10^{-3}$) of the compounds obtained in vivo after oral administration to mice previously infected by a non-β-lactamase-producing *S. aureus*.

A different equation was obtained for the description of the variance of the in vivo activity after subcutaneous administration. These results may reflect the different absorption processes.

$$\begin{aligned} ED_{50s.c.} = {} & 129.0(\pm 26.4)R^1Boc \\ & - 35.9(\pm 11.9)R^1PheN \\ & - 105.6(\pm 32.8)R^1Fur \\ & - 57.8(\pm 20.6)R^2H \\ & - 24.7(\pm 13.6)R^3OMe \\ & - 33.4(\pm 12.6)\log P \\ & + 2.3(\pm 1.1)(\log P)^2 + 121.6 \qquad (7) \end{aligned}$$

$n = 17; \quad r = 0.904; \quad s = 16.4; \quad F = 5.7$

The hydrophobicity of the compounds played an important role besides several structural requirements. The optimum log P value for subcutaneous absorption is around 7.3 when calculated from the regression coefficients in Eqn 7. A Boc-containing substituent at position 1 decreased the activity, as did the presence of the (C_4H_4O)–$C(=NOCH_3)$–CO– group at the same position. The Ph–$CH_2(NH_2)$–CO– group is the most advantageous at position 1. The presence of carboxyl and acetoxymethyl groups at positions 2 and 3, respectively, also enhanced the subcutaneous activity of the compounds.

In conclusion, the in vitro and in vivo activity of lipidic amino acid conjugates of β-lactam antibiotics could be described by structural parameters of the compounds using indicator variables for the presence or absence of the substituents at certain positions of substitution. The hydrophobic character of the compounds was one of the most important factors concerning the in vivo activity against non-β-lactamase-producing *S. aureus* after oral administration.

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