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Structure-activity relationships of cephem analogs

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Abstract - Hydrolysis rates, log k^{NMR}_{obsd} of 3'-Z-7α-methoxy-1-oxacephems were found to correlate linearly with ^{13}C NMR chemical shift differences between C_3 and C_4, $\Delta\delta(4\text{-}3)$, IR β-lactam bands $\nu_{C=O}$, or Hammet's σ_I values of group Z. Compounds were classified based on the product structures of the alkaline hydrolysis and its kinetics, both of which vary depending on the leavability of Z. A linear correlation of antibacterial potency against sensitive gram-negative bacteria (log $1/C_N$) with σ_I was found among compounds of low Z leavability. With compounds of high Z leavability, exceedingly high antibacterial potency was observed. With substitution of alkyl groups for the p-hydroxylphenyl group in latamoxef 10, the high antibacterial potency and broad antibacterial spectrum were maintained when the volume of the alkyl group was optimal. Further substitution of a cyano group for the carboxyl group narrowed the antibacterial spectrum.

INTRODUCTION

Penicillin inhibits the growth of bacteria by interfering with the enzymes for biosynthesis of the bacterial cell wall peptidoglycan which constitutes a network structure composed of linear glycan chains and peptide chains connecting two glycan chains. One of the effects of penicillin is to inhibit a D-Ala-D-Ala peptidase that catalyzes the peptide bridging by transfer of the second terminal D-alanyl group in a peptide branch of a glycan chain to the amino group in a peptide branch of another glycan chain. Penicillin possesses a moiety structurally similar to that of the C-terminal D-Ala-D-Ala of the peptide chain and irreversibly inhibits the serine hydroxy group of the active center in the transpeptidase as penicilloyl enzyme (ref. 1). The inhibition of bacterial growth subsequently causes loss of control of murein hydrolase activity, resulting in lysis of the bacterial cell wall (ref. 2).

Several factors influencing the in vitro antibacterial activity of cephems can be considered: i) formation of the Michaelis complex with the target enzymes, ii) formation of acyl enzymes, iii) stability of acyl enzymes, iv) stability to the hydrolysis by β-lactamases, and v) permeability through bacterial outer membranes. Many investigations on the correlations of the chemical reactivity of the β-lactam ring with the physicochemical properties of the β-lactam antibiotics have been reported (ref. 3). Also, studies have been conducted on the action mechanism of the β-lactam antibiotics by examining the hydrolysis of their β-lactam ring in alkaline aqueous solution and that catalyzed by β-lactamases (ref. 4).

This report tries to clarify the relationships between the structure of cephem analogs and their in vitro antibacterial activity against gram-negative bacteria in terms of changes in physicochemical and chemical properties induced by chemical modifications at the 1-, 3'-, 7α-, and 7β-positions of the cephem compounds. In vitro antibacterial activity can be defined by two factors, namely, the antibacterial potency, which is indicated by MICs against sensitive gram-negative strains, and the antibacterial spectrum, which gives the effective range of resistant gram-negative strains.

EFFECTS OF CHEMICAL MODIFICATION AT 1- AND 3′-POSITIONS AND 7β-AMIDO CHAIN ON *IN VITRO* ANTIBACTERIAL ACTIVITY

Table 1 shows MICs against representative gram-negative strains obtained by chemical modification of X, Y, Z, and R^1-groups. The left four strains, Escherichia. coli H, E. coli NIHJ JC-2. E. coli EC-14, and Klebsiela pneumoniae SRL-1, are sensitive gram-negative bacteria while the other three, penicillinase-producing E. coli 73(R) and cephalosporinase-generating E. coli 377(R) and Enterobacter cloacae 233, are resistant. Substitution of 1-methyl-1H-tetrazol-5-ylthio (STet) group for hydrogen atom at the 3'-position of 2 and 4 also increased the antibacterial potency. Substitution of a carboxyl group for the hydrogen atom at the benzylic position of the 7β-amido group of 1 and 5 lowered the MICs against the resistant strains, especially cephalosporinase-generating E. coli 377(R) and Enterobacter cloacae 233. Although substitution of the 7α-methoxy group for hydrogen in 1 increased the MICs in general, that in 3 significantly lowered the MIC against penicillinase-producing resistant E. coli 73(R).

The effects of introduction of the position 1-atoms, 7α-methoxy, and benzylic carboxyl groups were investigated more precisely, keeping the 3'-substituent unchanged. The effects on MICs are shown in Table 2 (ref. 5). Introduction of the C_1-atom increased the antibacterial potency in the order of oxygen, sulfur, and carbon atoms. Substitution effects of the carboxyl group and the 7α-methoxy group are shown by the rather higher MICs indicated by the squares in Table 2 where the compounds lack the relevant functional groups.

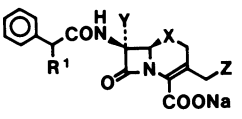
The second factor considered to influence in vitro antibacterial activity was the formation of acylenzymes. This was examined by observing the change in antibacterial potency based on both the pseudo-first order rates of the hydrolysis of β-lactam ring (log k^{UV}_{obsd}) and infrared absorption bands for C=0 stretching of the β-lactam carbonyl ($\nu_{C=O}$)(Table 3)(ref. 5). The k^{UV}_{obsd} values were measured by the disappearance of the UV absorption at about 265 nm in a buffer solution of pH 9.2 and at 35°C. The $\nu_{C=O}$ values were measured for the sodium salts in dry DMSO. As a parameter defining the antibacterial potency, logarithms of the reprocicals of geometrical means of MICs (mol/L) for the four sensitive gram-negative strains (log $1/C_N$) were calculated. The mean increase in the antibacterial potency ($1/C_N$) and chemical reactivity (k^{UV}_{obsd}) for each conversion are also shown in Table 3. Significant increases in both the potency and chemical reactivity for the S-O conversion are noteworthy, as compared with those obtained for the introduction of the 7α-methoxy or the benzylic carboxyl group. For carbacephem 10c, although the chemical reactivity increased moderately, a significant decrease in antibacterial potency was observed.

EFFECTS OF SUBSTITUTION OF Z AT THE 3′-POSITION

Substitution effects of various Z of oxacephems 4, 5, and 11-17 on the antibacterial potency were investigated. The significantly fluctuant MIC values against the four sensitive gram-negative strains and the log $1/C_N$ calculated from them are shown in Table 4 (ref. 6). Their physicochemical and chemical properties are shown in Table 5. Although the invariance of δC_8 may imply similar electronic circumstances of the lactam carbonyl, the variable $\Delta\delta$(4-3), i.e., the difference between chemical shifts C_4 and C_3, indicate the large dependency of polarization of the C_3-C_4 double bond on Z. Dependencies on Z of the infrared carbonyl frequency measured in DMSO and the pseudo-first order rates of the hydrolysis, measured by ^{1}H NMR method in a buffer solution of heavy water of pD 10.4 and at 35°C, are also obvious.

As shown in Table 6, fairly good correlations of $\Delta\delta$(4-3), $\nu_{C=O}$ of lactam carbonyl, and log k^{NMR}_{obsd} with σ_I were obtained (r = 0.927, 0.973, and 0.939, respectively). The conclusion reached is that the inductive effect of Z influences the C_3-C_4 double bond polarization and, accordingly, destabilizes the vibrationally excited state of the stretching vibration of the carbonyl and stabilizes the transition state of the alkaline hydrolysis, resulting in the observed acceleration of the reaction. There is only a poor correlation of either σ_I or log k^{NMR}_{obsd} with log $1/C_N$ values, indicating that only the pseudo-first order hydrolysis rate can not be used to define the antibacterial potency of even limited strains of the sensitive gram-negative bacteria.

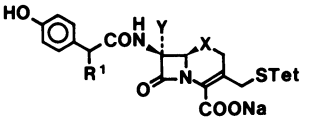
TABLE 1. MIC[a] of cephem analogs 1-6 and 1s-3s

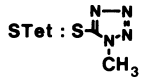


No.	X	Compound Y	Z	R^1	MIC (μg/ml) E.coli H	E.coli NIHJ JC-2	E.coli EC-14	Kleb. pneum. SRL-1	E.coli 73(R)	E.coli 377(R)	Enterobac. cloacae 233
1	O	H	H	H	3.13	12.5	6.25	3.13	50	>100	>100
1s	S	H	H	H	25	100	>100	50	>100	100	>100
2	O	H	H	COONa	6.25	6.25	6.25	6.25	12.5	6.25	6.25
2s	S	H	H	COONa	100	100	100	100	200	100	100
3	O	H	STet	COOH	0.05	0.1	0.1	0.1	25	n.d.	0.1
3s	S	H	STet	COOH	1.56	3.13	3.13	1.56	25	3.13	3.13
4	O	OCH_3	H	H	100	200	100	100	400	>400	>400
5	O	OCH_3	STet	H	0.1	0.78	0.39	0.39	1.56	0.78	50
6	O	OCH_3	STet	COONa	0.0125	0.05	0.05	0.05	0.2	0.05	0.05

a Minimal inhibitory concentrations.

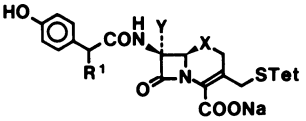
TABLE 2. MICs of cephem analogs 7-10, 7s-10s, and 10c





No.	X	Compound Y	R^1	MIC (μg/ml) E.coli H	E.coli NIHJ JC-2	E.coli EC-14	Kleb. pneum. SRL-1	E.coli 73(R)	E.coli 377(R)	Enterobac. cloacae 233
7	O	H	H	0.39	0.78	0.78	0.78	>100	12.5	>100
7s	S	H	H	0.78	3.13	3.13	1.56	25	50	>100
8	O	H	COONa	0.2	0.2	0.2	0.39	>100	0.2	0.39
8s	S	H	COONa	3.13	3.13	6.25	3.13	50	3.13	6.25
9	O	OCH_3	H	0.2	0.1	0.39	0.2	0.78	0.78	100
9s	S	OCH_3	H	0.78	12.5	3.13	1.56	12.5	6.25	>100
10	O	OCH_3	COONa	0.05	0.1	0.1	0.1	0.2	0.1	0.2
10s	S	OCH_3	COONa	0.2	0.78	0.78	0.39	3.13	0.39	0.78
10c	CH_2	OCH_3	COONa	3.13	6.25	6.25	3.13	25	3.13	3.13

TABLE 3. Substituent effects on physicochemical and chemical parameters of cephem analogs 7-10, 7s-10s, and 10c

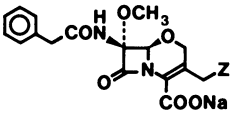


Comp. No.	log $1/C_N$[a] (mol/L)	IR $\nu_{C=O}$ cm^{-1}	log k^{UV}_{obsd} (h^{-1}) pH 9.2, 35°C
7	5.85	1778.2	-0.86
7s	5.42	1773.1	-1.64
8	6.35	1772.9	-0.64
8s	5.13	1768.1	-1.45
9	6.40	1779.2	-0.89
9s	5.29	1770.0	-1.62
10	6.83	1771.8	-0.75
10s	6.09	1767.8	-1.62
10c	5.07	1757.0	-1.16

a Geometrical mean of sensitive gram-negative strains E.coli H, E.coli NIHJ, JC-2, E.coli EC-14, and Kleb. pneum, SRL-1.

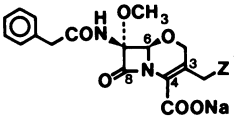
	Conversion	Mean increase $1/C_N$	K^{UV}_{obsd}
X	S → O	7.5	6.3
Y	H → OCH_3	2.9	1.2
R^1	H → COONa	2.3	0.7

TABLE 4. MICs of oxacephems 4, 5, and 11-17



No.	Compound R[1]	MIC (μg/ml) E.coli H	E.coli NIHJ JC-2	E.coli EC-14	Kleb. pneum. SRL-1	log 1/C_N (mol/L)
4	H	100	200	100	100	3.49
11	CN	12.5	50	25	25	4.19
12	N⊕	12.5	12.5	12.5	12.5	4.53
13	OH	25	100	50	50	3.88
14	OCH_3	25	200	100	50	3.74
15	$OCOCH_3$	0.78	3.13	3.13	1.56	5.36
16	$OCONH_2$	0.78	3.13	0.78	3.13	5.43
17	SCH_3	50	>400	200	100	3.39
5	STet	0.10	0.78	0.39	0.39	6.16

TABLE 5. Physicochemical and chemical parametes of oxacephems 4, 5, and 11-17



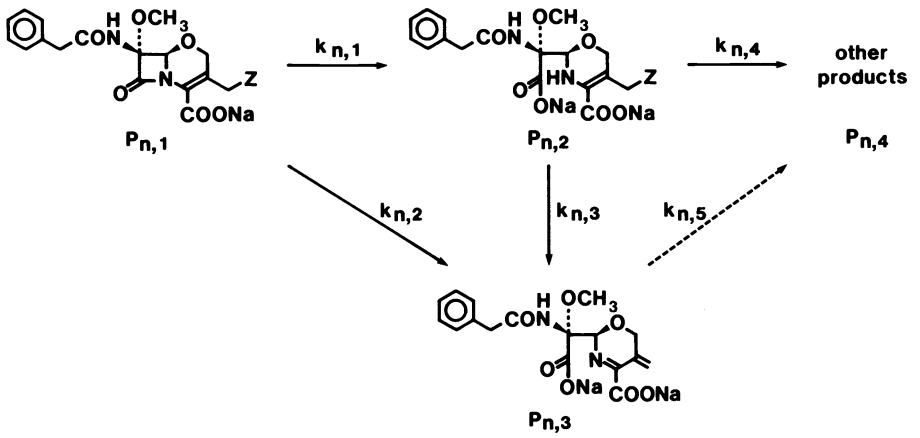
Comp. No.	^{13}C NMR (ppm) δC_8	$\Delta\delta$(4-3) in D_2O	X-ray analysis (Å) d[a,b]	N-C_8[b]	IR νc=O cm^{-1} in DMSO	σ_I	log k^{NMR}_{obsd} (h^{-1}) pD 10.4, 35°C
4	163.03	-2.61	0.264	1.375	1766.8	0.00	-1.315
17	162.99	2.58			1769.3	0.23	-0.996
13	163.36	-0.05			1770.5	0.25	-0.870
14	163.15	5.99	0.233	1.379	1771.9	0.27	-0.839
15	163.28	7.59			1774.3	0.39	-0.544
16	163.30	6.24			1772.8	0.46	-0.638
5	163.09	5.10	0.220	1.393	1772.2	0.53	-0.717
11	163.25	10.57			1774.6	0.56	-0.631
12	163.44	15.42			1776.9	1.09	0.143

a Distance between the lactam nitrogen atom and C_4,C_6,C_8 plane.
b Data obtained for the corresponding benzhydryl esters.

TABLE 6. Correlation of physicochemical and chemical parameters of oxacephems 4, 5, and 11-17

Correl. formula	Correl. coef. (r)	$\Delta\delta$(4-3) (ppm)	$\nu_{c=0}$ (cm^{-1})	σ_I	log k^{NMR}_{obsd} (h^{-1})	log 1/C_N (mol/L)
$\Delta\delta$(4-3)		1			0.927	
$\nu_{c=0}$			1		0.973	
σ_I				1	0.939	
log k^{NMR}_{obsd}		-1.04 + 0.0665 x $\Delta\delta$(4-3)	-174.38 + 0.0980 x $\nu_{C=0}$	-1.16 + 1.17 x σ_I	1	0.479
log 1/C_N					6.39 + 2.19 x log k^{NMR}_{obsd}	1

Scheme 1



Kinetics of alkaline hydrolysis of oxacephems 4, 5, 11-17

Interestingly, the structure of the alkaline hydrolysis products of the oxacephems and the reaction kinetics of their formation have been found to vary depending on the leavability as well as the σ_I of group Z. The structures of the products and the kinetics of the hydrolysis have been investigated by ^1H NMR and ^{13}C NMR spectroscopies, which were believed to afford information on the structures of the acyl enzymes and the kinetics of enzyme acylation. The structures of the hydrolysis products shown in Scheme 1 vary depending on the nature of Z and the data of the kinetics of the alkaline hydrolysis were calculated by assuming pseudo-first order kinetics of the hydrolysis (ref. 7).

As shown in Table 7, the measured oxacephems were classified into classes 1, 2 and 3 depending on the ratio of the relative yield of $P_{n,2}$ to $P_{n,3}$ when the starting oxacephems $P_{n,1}$ disappeared. Class 1 oxacephems 12, 15, 16, and 5 form only $P_{n,3}$; the formation of $P_{n,2}$ has not been observed. Contrary to this, class 3 oxacephems 4 and 11 form only $P_{n,2}$. Class 2 oxacephems 13, 14, and 17 are intermediates between classes 1 and 3; formation of $P_{n,2}$ and their gradual degradation to $P_{n,3}$ were observed. The occurrence of parallel reactions, deacetylation of 15 to 13 and ammonolysis of 16 with the ammonia generated from the eliminated carbaminic acid made analysis of the hydrolysis more difficult and these parallel reactions had to be corrected in order to obtain the individual pseudo-first order rates.

TABLE 7. Classification of oxacephems 4, 5, and 11-17

Class	Comp. No.	Ratio of formation of product(%)			Pseudo first order rate (h^{-1}) (X 10^2)				
		$P_{n,2}$	$P_{n,3}$	$P_{n,4}$	k^{NMR}_{obsd}	$k_{n,1}$	$k_{n,2}$	$k_{n,3}$	Other ks
1	12	0	100	0	125.9		(125.9)		
	15	-	76	-	25.5		30.8		a)
	16	0	100	0	45.0		22.8		b)
	5	0	100	0	20.43				
2	13	9	32	59	16.41	15.3	0.0	4.6	c)
	14	30	27	43	20.38	19.9	0.1	3.2	d)
	17	37	26	37	11.04				
3	4	100	0	0	4.84	(4.84)			
	11	100	0	0	28.86	(28.86)			

a) $k_{15\text{-}13}$: 12.2. b) $k_{16\cdot NH_3}$: 6840 $L^{-1}\cdot h^{-1}$. c) $k_{n,4}$: 8.4. d) $k_{n,4}$: 5.0.

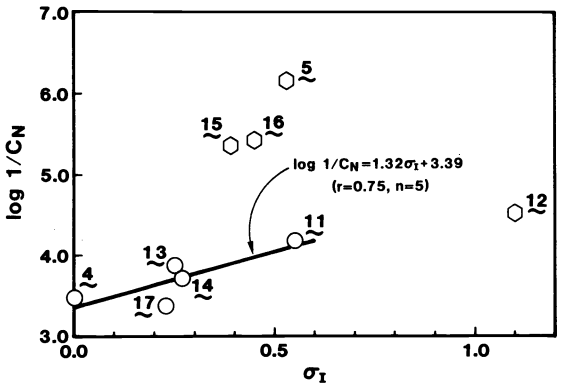


Fig. 1. σ_I-log $1/C_N$ Correlation of oxacephems 4, 5, and 11-17

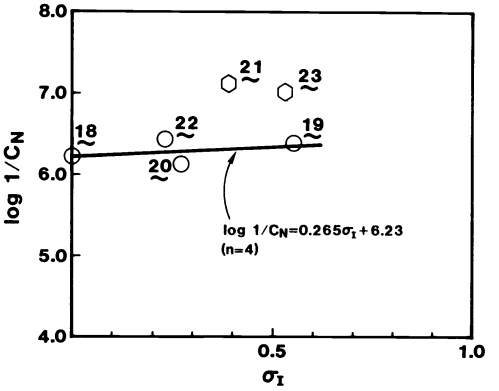


Fig. 2. σ_I-log $1/C_N$ Correlation of cephems 18-23

Based on these classifications, the oxacephems can be divided into two groups, one which generates $P_{n,2}$ (indicated by circles in Fig. 1) and the other which does not form $P_{n,2}$ (indicated by hexagons). Among the circles, a fairly good correlation of log $1/C_N$ with σ_I was obtained.

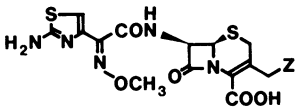
These findings led to the conclusions that the pseudo-first order alkaline hydrolysis rate is solely correlated with σ_I of group Z and, accordingly, the inductive effect of group Z affects the chemical reactivity of the β-lactam ring and ii) that the antibacterial potency may be determined primarily by the acylating ability of the β-lactam ring and subsequently by the leavability of group Z at the level of the acylated target enzymes.

To find whether these conclusions could be applicable to another series of cephem analogs, the cephems shown in Table 8 were investigated (ref. 8). The variances in the MIC values for this series were very small. The correlation coefficient of log $1/C_N$ with σ_I shown in Fig. 2 was obviously smaller than that for 7α-methoxy-oxacephems with the phenylacetamido side chain shown in Fig. 1.

EFFECTS OF STRUCTURES OF THE 7β-AMIDO SIDE CHAIN ON *IN VITRO* ANTIBACTERIAL ACTIVITY

The broad-spectrum antibiotic latamoxef disodium 10 possesses very high stability to β-lactamases. Two independent effects of the 7α-methoxy and benzylic carboxyl groups on attaining the stability to penicillinases and cephalosporinases, respectively, have demonstrated by measuring the enzymatic hydrolysis rates of 7-10 and 7s-10s shown in Table 9 (ref. 9).

TABLE 8. MICs of cephems 18-23

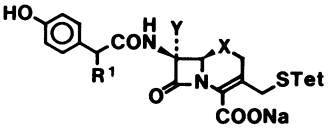


Compound No.	Z	MIC (μg/ml) E.coli H	E.coli NIHJ JC-2	E.coli EC-14	Kleb. pneum. SRL-1	log $1/C_N$ (mol/L)
18	H	0.1	0.39	0.39	0.2	6.23
19	CN	0.1	0.39	0.2	0.1	6.40
20	OCH_3	0.2	1.56	0.39	0.2	6.14
21	$OCOCH_3$	0.025	0.05	0.05	0.025	7.11
22	SCH_3	0.1	0.2	0.39	0.2	6.45
23	STet	0.125	0.2	0.1	0.025	7.04

In order to clarify the roles of the carboxyl group as well as the p-hydroxyphenyl group in 10, MICs of alkylmalonamido- and alkylcyanoacetamido -7α-methoxy-oxacephems 24-30 and 31-35 shown in Tables 10 and 11, respectively, were examined (ref. 10). With all the alkyl groups studied in the alkylmalonamido series, relatively low MICs were obtained. The obvious effect of the length on the antibacterial potency was revealed by good parabolic dependency of log $1/C_N$ on V_W, substituent van der Waals volume (ref. 11) (Fig. 3.), or π, substituent contributions to log P, where P is partition coefficient of compounds between water and butanol.

Although the cyano group in cyanoacetamido series did not lower the MICs of Enterobacter cloacae 233, a similar dependency of log $1/C_N$ on the length of the alkyl group to that described above was seen (Fig. 3). The fact that almost the same alkyl length in these two series exhibited the highest

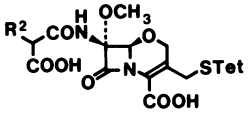
TABLE 9. Hydrolysis rates of cephem analogs 7-10, 7s-10s by β-lactamases



X = O, S

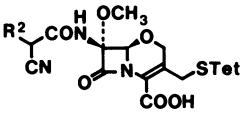
Compd. No.	Y	R^1	Hydrolysis rate by Penicillinases	Hydrolysis rate by cephalosporinases
7, 7s	H	H	high	high
8, 8s	H	COONa	high	low
9, 9s	OCH_3	H	low	high
10, 10s	OCH_3	COONa	low	low

TABLE 10. MICs of oxacephems 24-30

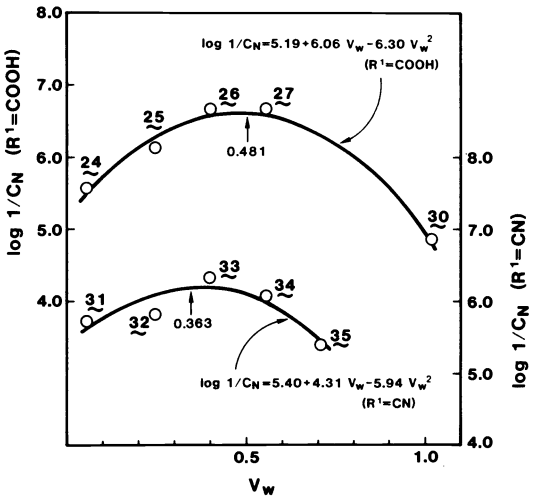


No.	Compound R^2	MIC (μg/ml) E.coli H	E.coli NIHJ JC-2	E.coli EC-14	Kleb. pneum. SRL-1	E.coli 73(R)	E.coli 377(R)	Enterobac. cloacae 233	log $1/C_N$ (mol/L)
24	H	3.13	1.56	3.56	3.13	1.56	1.56	25	5.58
25	CH_3	0.39	0.39	0.2	0.39	0.39	0.2	0.39	6.13
26	C_2H_5	0.1	0.1	0.1	0.1	0.2	0.1	0.1	6.66
27	$(CH_2)_2CH_3$	0.1	0.1	0.1	0.1	0.39	0.1	0.1	6.67
28	$CH(CH_3)_2$	0.39	0.78	0.39	0.39	1.56	0.39	0.78	6.01
29	$CH_3CH(CH_3)_2$	0.2	0.2	0.39	0.39	1.56	0.39	0.39	6.24
30	$(CH_2)_5CH_3$	1.56	25	12.6	6.25	50	25	12.5	4.84

TABLE 11. MICs of oxacephems 31-35



No.	Compound R^2	MIC (μg/ml) E.coli H	E.coli NIHJ JC-2	E.coli EC-14	Kleb. pneum. SRL-1	E.coli 73(R)	E.coli 377(R)	Enterobac. cloacae 233	log $1/C_N$ (mol/L)
31	H	0.78	0.78	0.78	0.78	100	100	100	5.72
32	CH_3	0.78	0.78	0.39	0.78	0.39	0.78	100	5.81
33	C_2H_5	0.2	0.2	0.2	0.2	0.39	0.39	12.5	6.34
34	$(CH_2)_2CH_3$	0.2	0.39	0.39	0.78	1.56	0.78	100	6.06
35	$(CH_2)_3CH_3$	0.39	3.13	3.13	3.13	6.25	3.13	100	5.40



	V_w
H	0.056
C_1	0.245
C_2	0.399
C_3	0.553
C_4	0.707
C_6	1.015

Fig. 3. Parabolic dependency of log $1/C_N$ on van der Waal volume of R^2

antibacterial potency led to the conclusion that not the π but the V_w affects the antibacterial potency, because the optimal log P values of each set of compounds in both series must be completely different from each other when the large difference between π values of carboxylate and cyano groups is taken into account.

CONCLUSION

Electron-withdrawing 3'-substituent Z in 7β-phenylacetamido-7α-methoxy-oxacephems polarizes the C_3-C_4 double bond and stabilizes the transition state in alkaline hydrolysis and, probably, also that in acylation of the active center of the target enzymes, thus enhancing the ability to acylate the target enzymes. This is likely to increase the antibacterial potency. In addition to these effects, when Z has high leavability, after acylation of the target enzymes, it may be eliminated to give $P_{n,3}$-type acyl enzymes, thus enhancing the antibacterial potency because of stabilization of the resulting acyl enzymes. Of the two types of broad-spectrum antibiotic studied, the p-hydroxyphenyl group in latamoxef 10 may be replaced by ethyl or n-propyl without loss of antibacterial potency. These three groups possess a similar optimal volume and may fit in a suitable cavity in the target enzymes to cause the maximal antibacterial potency.

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