Structure-activity Relationship of 1β-Methylcarbapenem to Its Antibacterial Activity: Effect of the C-2 Side Chain and the 1β-Methyl Group

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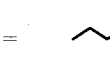
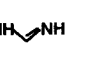
Previously, we described the synthesis and biological properties of carbapenem compounds having a 5'-aminocarbonyl pyrrolidin-3'-ylthio group as C-2 side chain and demonstrated that meropenem (MEPM) having a 1β-methyl group and the 5'-dimethylaminocarbonyl-2-pyrrolidinylthio group exhibits an extended antibacterial spectrum including anti-pseudomonal activity and high stability to renal dehydropeptidase-I (DHP-I)[1]. So far, only two other carbapenems, imipenem (IPM) and panipenem (PAPM), have been approved for clinical use. The structure of MEPM differs from that of IPM and PAPM by the presence of a 1β-methyl group and the basicity of the amino group in the C-2 side chain. The basicity of MEPM is much lower than that of IPM and PAPM[2~4], therefore, its physicochemical properties differ from those of the others. We have shown that the basicity of the C-2 side chain, but not the presence of a 1β-methyl group, correlates with their degree of neurotoxicity[5] and nephrotoxicity[6]. On the other hand, we reported that in the case of MEPM[1,7], the introduction of a 1β-methyl group improves not only the stability to dehydropeptidase-I (DHP-I) but also the activity against Gram-negative bacteria including *Pseudomonas aeruginosa*. However, there are no reports describing the structure-activity relationships between the antimicrobial activity and the basicity of the C-2 side chain.

We studied structure-activity relationships involved in intrinsic antimicrobial activity, focusing on the basicity of the C-2 side chain and the introduction of a methyl group at the C-1 position. In terms of activity against *P. aeruginosa*, not only the affinities of antimicrobial agents to the targets, penicillin binding proteins (PBPs), but also the permeability through the outer membrane (OM) influence their effectiveness[8]. Therefore, we determined the affinity of carbapenem compounds for the PBPs of *P. aeruginosa*, expecting this would lead to additional information concerning permeability through the OM of the tested compound.

The carbapenem compounds presented here were synthesized in our laboratories according to the reported procedures[1,9~13]. The MICs were measured by two-fold agar dilution method[1]. *P. aeruginosa* cell membranes were prepared and the affinities of the carbapenems for PBPs were determined by means of a competition assay using [^{14}C]benzylpenicillin as described[14]. Since the PBPs 1a/1b, 2, and 3 of *P. aeruginosa* are reported as the targets of the β-lactam antibiotics[15], affinities for

Table 1. Antimicrobial activity of 1β-methyl carbapenem compounds and their affinities for the PBPs of *P. aeruginosa*.

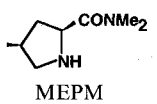
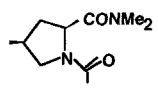
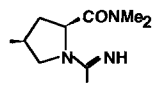
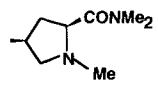
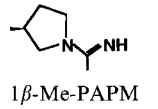
	R= 					
	1β-Me-THM		1β-Me-IPM			
	1a	**1b**	**1c**	**2a**	**2b**	**2c**
			MIC (μg/ml)			
S.a. FDA209P	0.025	0.20	0.05	0.05	0.20	0.05
S.e. IAM1296	0.78	0.78	0.78	0.39	0.39	0.39
S.py. Cook	≤0.013	0.05	≤0.013	≤0.013	0.025	≤0.013
S.pn. Type III	≤0.013	≤0.013	0.025	≤0.013	≤0.013	0.025
E.c. NIHJ JC-2	0.10	0.025	0.10	0.05	0.39	0.10
K.p. ATCC10031	0.05	0.025	0.10	0.05	0.025	0.05
P.m. GN2425	0.20	0.10	0.20	0.20	0.20	0.20
S.m. X100	0.20	0.10	0.20	0.10	3.13	0.10
P.a. T	3.13	100	6.25	6.25	200	25
P.a. IFO3451	1.56	50	3.13	1.56	50	3.13
H.i. IID983	0.78	0.10	0.78	0.10	0.05	0.20
Affinities for pseudomonal PBPs			IC_{50} (μg/ml)[a]			
1a	0.35	>10	1.7	0.68	7.7	n.t.
1b	0.60	3.0	3.2	0.96	1.3	
2	0.37	0.03	0.49	0.71	0.36	
3	0.33	0.27	0.94	0.57	0.40	

[a] Values indicate concentrations of compounds required to reduce [^{14}C]benzylpenicillin binding by 50%.

Abbreviations: *S.a.*, *Staphylococcus aureus*; *S.e.*, *Staphylococcus epidermidis*; *S.py.*, *Streptococcus pyogenes*; *S.pn.*, *Streptococcus pneumoniae*; *E.c.*, *Escherichia coli*; *K.p.*, *Klebsiella pneumoniae*; *P.m.*, *Proteus mirabillis*; *S.m.*, *Serratia marcescens*; *P.a.*, *Pseudomonas aeruginosa*; *H.i.*, *Haemophilus influenzae*; THM, thienamycin.

n.t., not tested.

Table 2. Antimicrobial activity of 1β-methyl carbapenem compounds and their affinities for the PBPs of *P. aeruginosa*.

R =	 MEPM				 1β-Me-PAPM
	3a	**3b**	**3c**	**3d**	**4c**
			MIC (μg/ml)		
S.a. FDA209P	≤0.013	0.78	0.10	0.20	0.05
S.e. IAM1296	0.39	3.13	0.78	1.56	0.39
S.py. Cook	≤0.013	0.20	≤0.013	0.025	≤0.013
S.pn. Type III	≤0.013	0.10	0.025	≤0.013	≤0.013
E.c. NIHJ JC-2	≤0.013	0.78	0.10	0.025	0.10
K.p. ATCC10031	≤0.013	0.10	0.05	0.05	0.025
P.m. GN2425	≤0.013	0.10	0.20	0.10	0.20
S.m. X100	≤0.013	0.78	0.10	0.10	0.20
P.a. T	0.10	100	3.13	3.13	25
P.a. IFO3451	0.39	25	3.13	3.13	6.25
H.i. IID983	0.05	0.05	0.05	0.10	0.20
Affinities for pseudomonal PBPs			IC_{50} (μg/ml)[a]		
1a	0.96	>10	0.44	3.3	0.78
1b	0.84	2.3	3.0	0.81	2.5
2	0.13	0.19	0.39	0.83	0.59
3	0.06	0.41	0.20	0.17	0.53

[a] and abbreviations: See the footnote in Table 1.

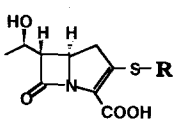
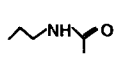
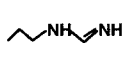
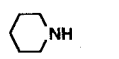
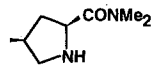
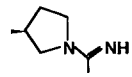
these PBPs are listed in the tables.

Table 1 shows the effect of altering the basicity of two 1β-methyl carbapenems, **1a** and **2a**, which have a highly basic side chain at the C-2 position upon the antimicrobial activity against Gram-positive and Gram-negative bacteria, and upon the affinities for the PBPs of *P. aeruginosa*. The calculated p*K*a values were 9.0 and 9.2, for **1a** and **2a**, respectively[16]. Blocking the basic amino group in the C-2 side chain by *N*-acetylation (**1b** and **2b**), slightly reduced the activity against *Staphylococcus aureus*, and increased that against some Gram-negatives such as *Haemophilus influenzae*. However, *N*-acetylation significantly reduced anti-pseudomonal activity. The affinity for pseudomonal PBP-1a and/or PBP-1b was significantly decreased, but those for PBP-2 and PBP-3 were unchanged or increased. A β-lactam exhibiting affinity for only PBP-3 can inhibit the growth of *P. aeruginosa*[15], so the significant reduction in pseudomonal activity indicated that the OM is less permeable to *N*-acetylated compounds. The results obtained with the des-methyl carbapenem compound were similar, as seen by comparing thienamycin (**1'a**) with *N*-acetyl thienamycin (**1'b**) (Table 3). *N*-acetylation causes the steric hindrance around the amino group, which may influence the antimicrobial activity. However, it was confirmed that such steric hindrance did not influence the antimicrobial activity by comparing the corresponding *N*-imidoyl derivatives. The *N*-amidino groups do not reduce the basicity and have been incorporated into IPM and PAPM. No significant change in the antimicrobial activity was observed with the formimidoyl (**1c**, 1β-methyl IPM) or the *N*-acetimidoyl derivative (**2c**), while the anti-pseudomonal activity was slightly reduced, in association with a decreased affinity for a few PBPs. Consequently, the basicity in the C-2 side chain plays an important role in the activity against *P. aeruginosa*, mainly by supporting passage through the OM.

Table 2 shows the effect of introducing selected substituents at the secondary amino group of MEPM, (**3a**, p*K*a=7.4)[2] on the antimicrobial activities. *N*-acetylation (**3b**) again led to a significant reduction in activity against *P. aeruginosa*, as seen with **1a**, **2a**, and **1'a**. In this case, the activity against all other organisms except for *H. influenzae* was also decreased. Introducing an *N*-acetimidoyl group (**3c**) reduced the activity against Gram-negatives except for *H. influenzae*, although the decrease in the anti-pseudomonal activity was less significant compared with that seen with *N*-acetylation. *N*-methylation of MEPM (**3d**) also reduced antimicrobial activity against various organisms, including *P. aeruginosa*. *N*-substitution of **3a** tended to reduce the affinity toward a few PBPs. Compound **3a** differs from compounds **1a**, **1'a**, or **2a** with respect to steric crowding around the amino group of the C-2 side chain, because of the 5'-substitution. Consequently, the bulkiness of additional *N*-substitution may affect its antimicrobial activity.

Compound **3a** exhibited the highest anti-pseudomonal activity among this series of carbapenem compounds despite lower basicity of the C-2 side chain. The introduction of a methylene spacer between the amino carbonyl group and pyrrolidine ring at the 5'-position of MEPM increased the basicity of C-2 side chain[6]. We

Table 3. Antimicrobial activity of 1-des-methyl carbapenem compounds and affinities for the PBPs of *P. aeruginosa*.

 R =						
	THM		IPM		desMe-MEPM	PAPM
	1'a	**1'b**	**1'c**	**2'a**	**3'a**	**4'c**
			MIC (μg/ml)			
S.a. FDA209P	≤0.013	0.05	≤0.013	≤0.013	≤0.013	≤0.013
S.e. IAM1296	0.78	0.39	0.20	0.20	0.78	0.20
S.py. Cook	≤0.013	≤0.013	≤0.013	≤0.013	≤0.013	≤0.013
S.pn. Type III	0.025	0.025	≤0.013	≤0.013	≤0.013	≤0.013
E.c. NIHJ JC-2	0.20	0.10	0.20	0.10	≤0.013	0.10
K.p. ATCC10031	0.10	0.05	0.10	0.05	≤0.013	0.10
P.m. GN2425	0.78	0.20	0.78	0.39	0.10	0.39
S.m. X100	0.39	0.20	0.39	0.20	0.05	0.10
P.a. T	6.25	>100	1.56	12.5	0.39	12.5
P.a. IFO3451	3.13	50	1.56	3.13	0.78	3.13
H.i. IID983	3.13	1.56	1.56	0.39	0.39	0.39
Affinities for pseudomonal PBPs			IC_{50} (μg/ml)[a]			
1a	0.15	>10	0.40	0.11	0.49	0.06
1b	0.17	2.5	0.81	0.38	2.6	5.3
2	0.08	2.7	0.23	0.03	0.87	0.35
3	0.75	0.87	0.53	0.09	0.38	0.31

[a] and abbreviations: See the footnote in Table 1.

reported that these compounds have lower activity against *P. aeruginosa* than MEPM[17]. We concluded that the basicity in the *P. aeruginosa*. The presence of a basic C-2 side chain is important for anti-pseudomonal activity, but the degree of the basicity does not directly correlate with this activity. These results show that the antimicrobial activity of carbapenem antibiotics can be optimized without increasing their neurotoxicity and nephrotoxicity.

Table 3 lists the results for several des-methyl carbapenems, to show the effect of the β-methyl group at the C-1 position. As for compounds having a highly basic side chain (compare **1'a**, **1'c**, **2'a**, and **4'c** with the corresponding 1β-methyl substituted compounds), the introduction of a 1β-methyl group generally increased the activity against Gram-negative bacteria (such as *H. influenzae*), except for *P. aeruginosa*, but tended to decrease the activity against *S. aureus*. Introducing a 1β-methyl group influenced activity against *P. aeruginosa*, depending on the C-2 side chain. Although the affinities of all compounds toward the pseudomonal PBPs tended to be lowered by introducing a 1β-methyl group, the anti-pseudomonal activity tended to increase for **1'a** and **2'a**, but tended to decrease for compounds having an amidino group (**1'c** and **4'c**). On the other hand, introducing a 1β-methyl group to compounds having a less basic side chain or that having a neutral side chain (compare **3'a** with **3a** or **1'b** with **1b**), affected the anti-pseudomonal activity in a different way. The affinities of **3a** and **1b** for PBP-2 and 3 were increased by introducing a 1β-methyl group, and the anti-pseudomonal activity of **3a** was clearly enhanced. The activity against other Gram-negative bacteria was improved by a 1β-methyl group in analogy to compounds having a highly basic side chain. Thus, it was found that the 1β-methyl group of carbapenem compounds can not only improve their stability against DHP-I, but also affect their antimicrobial activity.

In summary, we found that the basicity of the C-2 side chain is important to increase antimicrobial activity against *P. aeruginosa* by improving permeability through the OM. However, the strength of the basicity did not directly correlate with the anti-pseudomonal activity. The basicity of the C-2 side chain was not important for activity against other microorganisms, and masking the basicity rather increased the activity against *H. influenzae*. Further studies regarding this microorganism are on going. We also showed that the introduction of a 1β-methyl group generally improved the activity against Gram-negative bacteria, but variably affected the anti-pseudomonal activity depending on the C-2 side chain. This information may be helpful for designing new β-lactam antibiotics.

Acknowledgments

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