VOL. 53 NO. 10, OCT. 2000 THE JOURNAL OF ANTIBIOTICS pp. 1086 — 1095

Synthesis and Biological Activity of the Penem Antibiotic MEN 10700 and Its Orally Absorbed Ester MEN 11505

FEDERICO MARIA ARCAMONE[a], MARIA ALTAMURA[b], ENZO PERROTTA[b], ATTILIO CREA[c], STEFANO MANZINI[c], DAVIDE POMA[d], ALDO SALIMBENI[d], ANTONIO TRIOLO[b] and CARLO ALBERTO MAGGI[b]

[a] CNR-ICOCEA,
Via Piero Gobetti 101, I-40129 Bologna, Italy
[b] Menarini Ricerche S.p.A.,
Via dei Sette Santi 3, I-50131 Florence, Italy
[c] Menarini Ricerche S.p.A.,
Via Tito Speri 10, I-00040 Pomezia, Italy
[d] Lusochimica S.p.A.,
Via Giotto 1, I-23871 Lomagna, Italy

(Received for publication July 25, 2000)

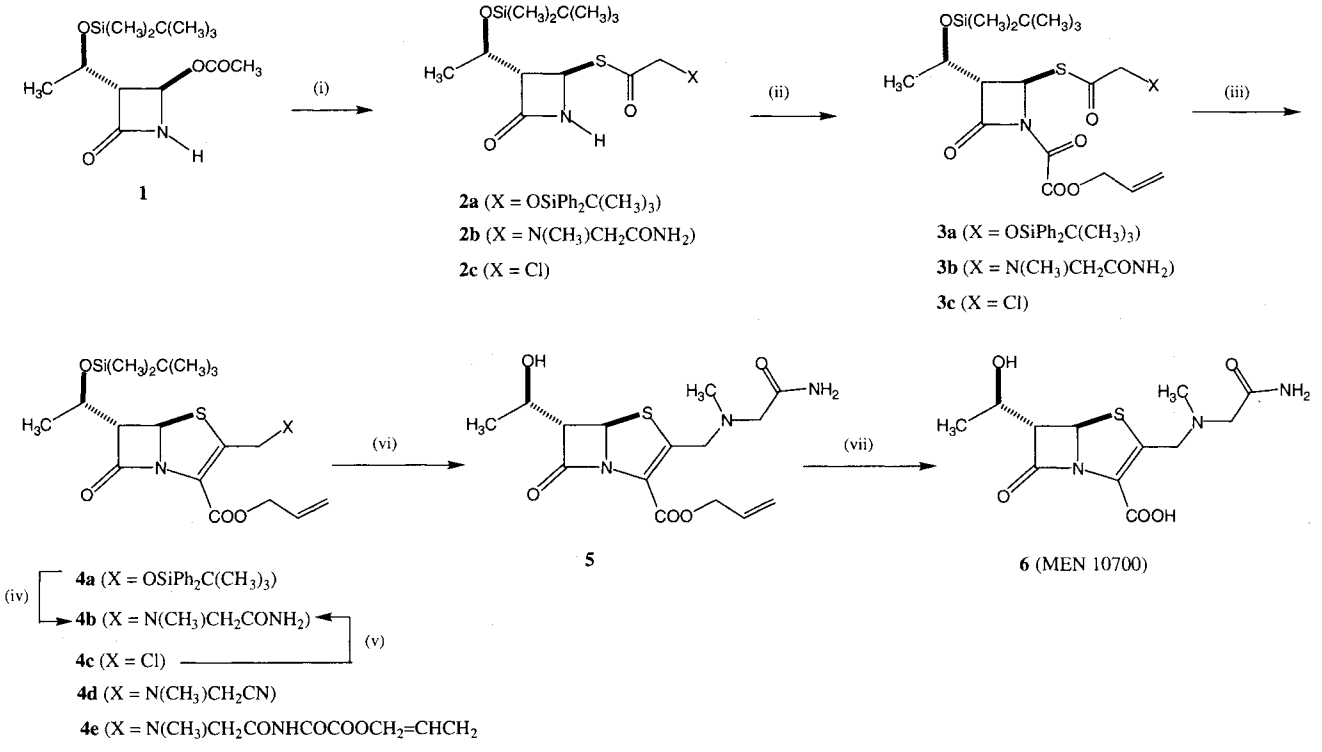
The synthesis and biological properties of the new penem antibiotic MEN 10700 (**6**) and of its selected oral prodrug MEN 11505 (**8f**) are described. MEN 10700 showed a broad spectrum of activity, with high potency both on Gram-positive and Gram-negative strains. It also exhibited good antibacterial activity toward anaerobes and on strains selected for their resistance to other antibacterial agents (cefotaxime- or ceftazidime-resistant Gram-negative strains, ciprofloxacin-resistant *E. coli*, extended spectrum β-lactamase producing and cephalosporinase inducible enterobacteria). MEN 10700 showed a very high stability to enzymatic degradation by renal dehydropeptidase DHP-I. After oral administration in rats of the pivaloyloxymethyl ester prodrug MEN 11505, the relative bioavailability of MEN 10700 was calculated as F=43%.

The development of bacterial resistance to existing antibiotics is a phenomenon of concern to the clinician, being a major cause of failure in the treatment of infectious diseases. Over the last years, increased resistance to established antibiotics has provided a continuous drive to the development of new antimicrobial agents. In addition, the emergence of opportunistic infections in immunocompromised individuals has created a need for effective therapy for unusual pathogens.

Due to their wide spectrum of activity, high potency and low toxicity, β-lactam antibiotics still retain a prominent role among the various classes of antibacterials. Several β-lactam antibiotics with improved antibacterial and pharmacokinetic profile have been introduced in clinical therapy over the past 20 years.[1] Most of them have been obtained through peripheral modifications of the nuclei of naturally occurring penicillins and cephalosporins, a number of compounds belong to the novel classes[2] of carbapenems, trinems and penems. Among them, penems retain the unique feature of being totally synthetic molecules, lacking a natural counterpart and designed[3] as a hybrid between penicillins and cephalosporins. Their antibacterial spectrum includes anaerobic bacteria as well as Gram-positive and most Gram-negative pathogens: their small size and compact shape both contribute[4] to the ability of penem compounds to rapidly cross the outer membrane of Gram-negative bacteria. In addition, stability to β-lactamases and to the renal dehydropeptidase DHP-I, and, most notably, the possibility of oral administration, provide considerable advantages of the penem series. Among the penems only one compound (faropenem)[5,6] is currently marketed for the oral therapy of respiratory tract, skin and soft tissue infections; other penems, *e.g.* ritipenem acoxil[7,8] and sulopenem,[9] are in the advanced phases of

Dedication: to the Memory of Sir EDWARD ABRAHAM.

Scheme 1.



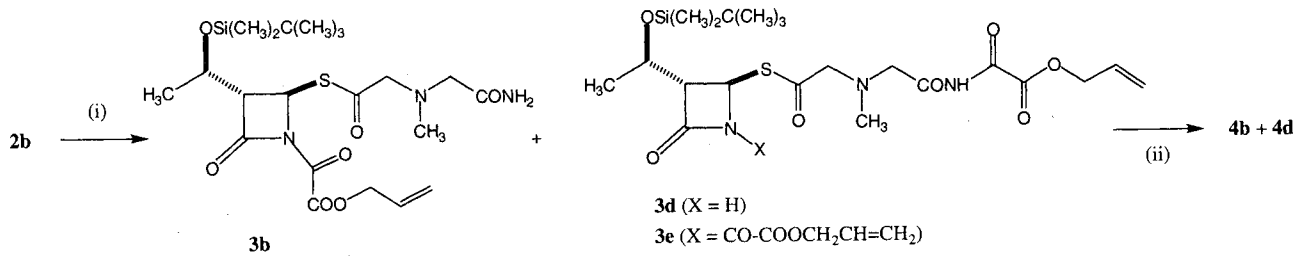
Reagents: (i): $HSCOCH_2X$ (see Exp. Section); (ii): allyl oxalyl chloride, NEt_3, toluene, 30', 5°C; (iii): $P(OEt)_3$, toluene, 110°C, 3-4 h; (iv): $NBu_4^+F^-$, AcOH, THF, 2h, then: CH_3SO_2Cl, NEt_3, THF, 0°C, 1h, then: $CH_3NHCH_2CONH_2$, DMSO, rt, 20 h; (v): NaI, DMF, then: $CH_3NHCH_2CONH_2$, rt, 2 h; (vi): $NBu_4^+F^-$, AcOH, THF, 20h; (vii): $Pd(PPh_3)_4$, PPh_3, AcOH, THF, 30', 40°C.

clinical evaluation. They all share a number of well established structural features, such as the presence of a free carboxy group at C-3 and 1-hydroxyethyl at C-6 and the (8*R*, 6*S*, 5*R*) configuration. A large differentiation is allowed in the nature of C-2 group. With the aim of obtaining potent, broad spectrum antibacterial agents by improving the penetration through the outer membrane of Gram-negative bacteria, we carried out the synthesis of a series of penems bearing amino acid related side chains in position 2 as small, polar groups.[10] Among them, the 2-sarcosinamidomethyl derivative **6** (MEN 10700) was selected for further development. In this paper we will describe the synthesis and biological properties of MEN 10700 and its selected oral prodrug MEN 11505 (**8f**).

Chemistry

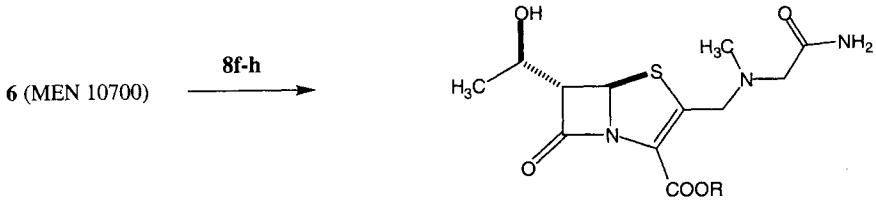
The initial quantity of **6**, necessary for screening tests, was prepared through the classical method[11] for penems (Scheme 1), starting from the reaction of silyl protected 4-acetoxyazetidinone **1** with (2-*tert*-butyldiphenylsilyloxy)-thioacetic acid to give **2a**; following nitrogen acylation to obtain **3a** and ring closure led to the bis-silyl protected penem **4a**. Selective silyl deprotection, followed in sequence by mesylate formation on the primary alcoholic group and nucleophilic exchange with sarcosinamide,[10] finally gave the fully protected 2-(sarcosinamido)methyl penem **4b**. Usual deprotections steps allowed to obtain the desired compound **6** (MEN 10700). This method suffers however of a number of drawbacks that render it unsuitable for the preparation of multigram quantities of penem **6**. The most critical step is the synthesis of **4b** from **4a**. Insufficient selectivity is achieved both during silyl deprotection on the primary alcohol in position 2 and nucleophilic exchange on mesylate with sarcosinamide: this last reaction step suffers competition from β-lactam ring opening by the same

Scheme 2.



Reagents: (i): allyl oxalyl chloride, NEt_3, toluene, 30', 5°C; (ii): $P(OEt)_3$, toluene, 110°C, 6 h

Scheme 3.



reagent.[10] In addition, the low stability of penem mesylate, together with the length of the whole synthetic pathway and the high cost of some reagents prompted us to look for shorter and more practicable methods.

A more straighforward reaction pathway can be outlined, that removes the need for protecting groups in the side-chain by reacting directly the 4-acetoxy-azetidinone **1** with *N*-thiocarboxymethyl-sarcosinamide to give **2b**. Nitrogen acylation to **3b** and penem ring closure should allow to obtain the protected penem **4b**. Again, when we tested this synthetic route experimentally, selectivity appeared as the crucial problem, as acylation of **2b** with allyl oxalyl chloride also occurred at the primary amide in the side chain (Scheme 2), giving a mixture of mono- and di-acylated azetidinones **3b**, **3d**, **3e** (see Experimental Section for details). Consequently, when the mixture was heated in toluene in the presence of triethylphosphite in order to close the thiazoline ring, the cyanomethyl substituted penem **4d** was obtained[12] together with the desired carboxyamido substituted penem **4b**, probably through elimination[13] from **3e** or from the corresponding penem **4e**. Any attempt to use the nitrile group in the C-2 side-chain as a temporary masked form of the primary amide failed, due to the harsh conditions requested to recover the amide from the corresponding nitrile.

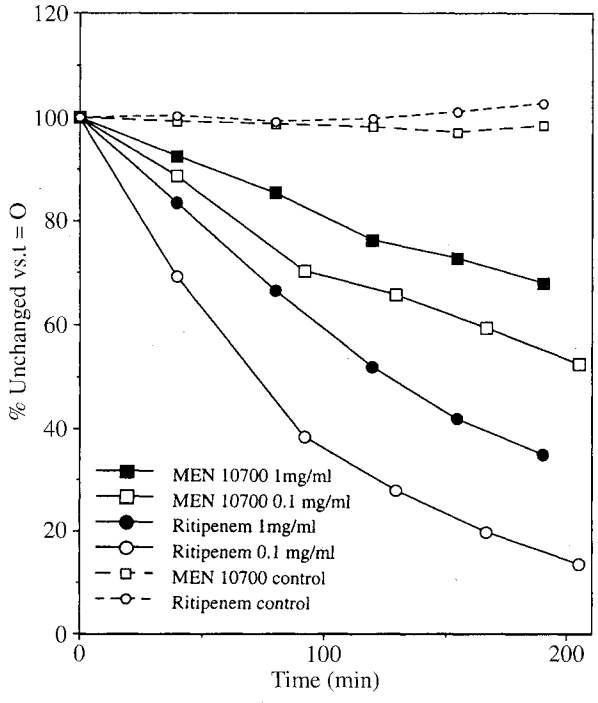
Although more selective conditions could be found for acylation of **2b**, the method remained, in our hands, unsuitable for large scale synthesis. On the other hand, good results were obtained building up the sarcosinamide side-chain in two steps, that is reacting **1** with the simple and easily available 2-chlorothioacetic acid[14] to give **2c**, acylating the β-lactam nitrogen to **3c**, closing the penem ring to obtain **4c** and finally exchanging chlorine with sarcosinamide to obtain **4b** through the intermediate *in situ* formation of the corresponding 2-iodomethyl penem. This method allowed us the synthesis of multigram quantities of **6** and is presently scaled up.

Finally, prodrugs **7f~h** were obtained (Scheme 3) through acylation of the acid **6** with suitable halogenomethyl carboxylates **8f~h**.

Biological Properties

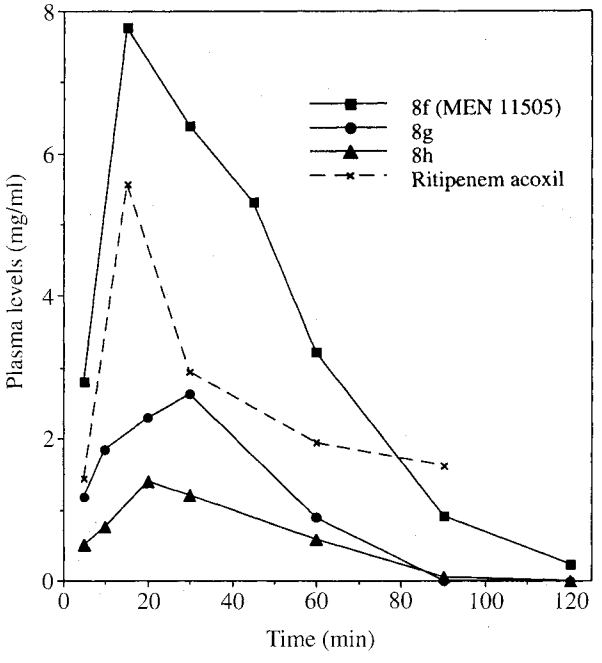
The *in vitro* antibacterial activity of MEN 10700 has been the subject of several studies, carried on with the aim of evaluating the potency and spectrum of activity of MEN 10700 in comparison to known antibiotics. In addition, studies have been carried out on resistant or difficult-to-treat strains to verify the potential value of the new compound for future therapy. In these studies, MEN 10700 showed a broad spectrum of activity, with high potency both on Gram-positive and Gram-negative strains. As for Gram-positive strains,[4,10,15] MEN 10700 exhibited potent activity against methicillin sensitive staphylococci (*S. aureus* and *S. epidermidis*). On methicillin-resistant staphylococci, MIC_{90} values were usually rather high (>32 μg/ml). However, a very large range of MICs was observed against methicillin-resistant *S. aureus* strains (MRSA)[15] with a number of strains falling within a reasonable therapeutic window. The activity of MEN 10700 against streptococci was usually very high (MIC_{90} around 0.06 μg/ml for penicillin-sensitive *S. pneumoniae*, *S. pyogenes*, *S. agalactiae*). A study[16] devoted to the evaluation of activity on penicillin susceptible, intermediate and resistant *S. pneumoniae* strains confirmed the sensitivity to MEN 10700 of highly resistant strains (MIC_{90}=2 μg/ml). MEN 10700 showed moderate activity on *E. faecalis* (MIC_{90}=8 μg/ml) and good activity on *Listeriae* (MIC_{90}=0.5 μg/ml). Concerning the Gram-negative strains, MEN 10700 showed good to excellent activity against most members of Enterobacteriaceae,[4,15,17] good activity on *Haemophilus influenzae* (MIC_{90}= 1 μg/ml)[18] and on Neisseriae (MIC_{90}=0.03 μg/ml for *N. meningitidis* and 0.25 μg/ml for *N. gonorrhoeae*).[19] MEN 10700 was inactive on *Pseudomonas aeruginosa* and *Xanthomonas maltophilia*, as for the other compounds of the penem class (ritipenem, faropenem) and most of the newer, orally available carbapenems and trinems (CS-834, sanfetrinem). MEN 10700 exhibited good antibacterial activity toward Gram-positive and Gram-negative anaerobes.[20] In addition, MEN 10700 repeatedly showed good activity on strains selected for their resistance to other agents, such as cefotaxime- or ceftazidime-resistant Gram-negative strains,[15,17] ciprofloxacin-resistant *E. coli*, extended spectrum β-lactamase producing and cephalosporinase inducible enterobacteria.[21] In comparison to the antibacterial agents used as reference compounds, MEN 10700 showed similar spectrum to imipenem (excluding *Pseudomonas*) and compared favourably to third generation cephalosporins, *e.g.* cefotaxime. The spectrum was similar to that of other penems, such as ritipenem and faropenem, with higher activity for MEN 10700 on *Enterobacter* and *Citrobacter*. Against anaerobes, MEN 10700 showed activity similar to that of imipenem, and was more potent than metronidazole, a "classical" anti-anaerobe agent, against all species except *C. difficile* and *P. anaerobius*. In general MEN 10700 was a much better anti-anaerobe agent than the cephalosporins and ciprofloxacin. MEN 10700 showed a very high stability to enzymatic degradation by renal dehydropeptidase DHP-I. The stability of MEN 10700 to DHP-I was evaluated in comparison to the penem ritipenem, incubating both compounds in the same solution to minimise differences. Results are outlined in Fig. 1: after 190 minutes incubation at 37% and at a substrate concentration of 1 mg/ml, 68% of the initial amount of MEN 10700 remained unchanged *vs.* 35% of ritipenem; at a substrate concentration of 0.1 mg/ml, residual amounts were 52% for MEN 10700 and 13% for ritipenem after 205 minutes. Literature data[22] for imipenem in comparable conditions gave only 5% of compound remaining after 3 hours (initial concentration: 1 mg/ml).

Fig. 1. Stability of MEN 10700 to renal dehydropeptidase DHP-I at 37°C compared with ritipenem (substrate concentration: 1 and 0.1 mg/ml).



After single i.v. administration in rats at a dose of 30 mg/kg, MEN 10700 showed a half-life of about 8 minutes, a volume of distribution of 573 ml/kg and a AUC

Fig. 2. Plasma levels in rats of MEN 10700 and ritipenem after oral administration of the prodrugs **8f**~**8g** and ritipenem acoxil (dose= 40 mg/kg).



of 655 mg/liter×minute; these values were compared with those for the penem ritipenem, showing a similar behaviour for both compounds. As in preliminary tests MEN 10700 did not show any valuable absorption after oral administration in rats, a number of its derivatives were synthesized and tested to allow oral administration of the compound. Derivatives were chosen among these esters or carbonates at C-3 carboxy group in which the ester (or carbonate) moiety could be easily cleaved *in vivo* by the hydrolytic action of esterases, therefore acting as MEN 10700 prodrugs. Best results were obtained with the pivaloyloxymethyl ester **8f** (MEN 11505), the (α-methyl)acetoxymethyl ester **8g** and the 1-ethylisopropyl carbonate **8h**. Plasma concentrations of MEN 10700 after oral administration in rats of the prodrugs **8f**~**h**, at a dose corresponding to 40 mg/kg of MEN 10700, are outlined in Fig. 2. An experimental comparison with ritipenem acoxil,[23] the oral prodrug of ritipenem, is also shown in Fig. 2. **8f** (MEN 11505) showed superior AUC values (283.2 mg/liter×minute) when compared with **8g** (AUC=105.5 mg/liter×minute) and **8h** (AUC=55.9 mg/liter×minute) and also with ritipenem acoxil (AUC=229 mg/liter×minute). The relative bioavailability of MEN 10700 after oral administration of MEN 11505 was calculated as F=43% *vs.* F=23% (lit.[23] data: F=41%) for ritipenem acoxil.

Experimental

General Methods

IR spectra were recorded on a Perkin Elmer FTIR 1710 spectrometer. NMR spectra were obtained using a Varian Gemini 200 spectrometer at 200 MHz and 50 MHz for ^{1}H and ^{13}C NMR spectra respectively. Mass spectra were acquired either by positive-ion Thermospray ionization (TS) with a Hewlett-Packard HP 5988A mass spectrometer, introducing the sample by flow-injection, or by positive-ion Electrospray ionization (ES) with a VG Quattro mass spectrometer, introducing the sample *via* direct infusion. Melting points were determined using a Buchi melting point apparatus and were not corrected. Optical rotations were obtained with a Perkin Elmer 241 polarimeter. TLC analyses were performed on Merck silica gel F_{254} plates. Analytical HPLC and UV analyses were performed with a Perkin Elmer Analyst liquid chromatograph equipped with a Perkin Elmer 235 diode array detector and work station. Column chromatography was performed using E. Merck Kieselgel 60 (70~230 mesh). Ritipenem was synthesised in our laboratories according to known procedures. DHP-I, freshly prepared from rat kidney, was supplied by the Institute of Biochemistry, University of Milan, and stored at −80°C.

N-Thiocarboxymethyl-sarcosinamide

Triethylamine (2.3 ml, 18.8 mmol) and *N,N*-dimethylaminopyridine (0.21 g, 1.71 mmol) were added in sequence to a mixture of 2.5 g (17.1 mmol) of *N*-carboxymethyl-sarcosinamide and 40 ml of *N,N*-dimethylformamide. The mixture was stirred for 15 minutes to obtain a clear solution and cooled to 0°C. 3.8 g (18.8 mmol) of 4-nitrophenyl chloroformate were added. After stirring for 2 hours at 0~5°C, the mixture was poured into iced water, filtered under vacuum, and the obtained yellowish solid washed on the filter with diethyl ether. The crude 4-nitrophenyl derivative was dissolved in 150 ml of dichloromethane. Triethylamine (1.1 ml, 7.86 mmol) was added and the solution cooled to 0°C. Gaseous hydrogen sulphide was bubbled trough the solution for 30 minutes. The solution was allowed to warm to room temperature. After 2 hours the excess hydrogen sulphide was removed with the aid of a nitrogen flow. The solution was concentrated under vacuum to a final volume of 20~30 ml. Diethyl ether (150 ml) was added and the mixture allowed

to stand overnight at 0~5°C. *N*-Thiocarboxymethyl-sarcosinamide (1.25 g, 45%) was obtained as a yellowish powder and used without further purification for the synthesis of **2b**. ^{1}H NMR (200 MHz, DMSO-d_6): ∂ 2.78 (3H, s); 3.77 (2H, s); 3.84 (2H, s); 7.62 (1H, br s); 7.86 (1H, br s); 8.96 (1H, br s). MS (TS): *m/z* 163 $(M+H)^+$.

(3*S*,4*R*)-3-[(*R*)-1(*tert*-Butyldimethylsilyloxy)ethyl]-4-(2-(*N*-carbamoylmethyl-*N*-methyl-amino)acetylthio)-2-azetidinone (**2b**)

1.24 ml (8.88 mmol) of triethylamine were added at 5°C to a mixture of 1.2 g (7.4 mmol) of *N*-thiocarboxymethyl-sarcosinamide in 30 ml of acetone. The mixture was allowed to warm to room temperature. 1.48 g (5.14 mmol) of (3*S*,4*S*)-4-acetoxy-3-(*R*)-1(*tert*-butyldimethylsilyloxy)-ethyl-2-azetidinone **1** and 4.7 ml of water were added in sequence, obtaining a clear solution. The pH of the solution was pH=9.1: a few drops of triethylamine were added in order to reach pH=9.5. The solution was stirred at room temperature for 3 hours. Acetone was removed by evaporation under vacuum and the residue taken up with ethyl acetate. The solution was washed with brine, dried over anhydrous sodium sulfate and evaporated. Column chromatography on silica gel (ethyl acetate/cyclohexane 9 : 1 v/v) gave (3*S*,4*R*)-3-(*R*)-1(*tert*-butyldimethylsilyloxy)-ethyl-4-(2-(*N*-carbamoylmethyl-*N*-methyl-amino)-acetylthio)-2-azetidinone as a white foam. Yield: 1.05 g (52%). ^{1}H NMR (200 MHz, $CDCl_3$): ∂ 1.22 (3H, d, *J*=6.3 Hz), 2.45 (3H, s), 3.19 (1H, dd, partially hidden by the previous peak), 3.43 (2H, s), 3.22 (2H, s), 4.26 (1H, m, CH_3–*CH*), 5.27 (1H, d, *J*=2.4 Hz, H-4), 5.71 (1H, br s, one of $CONH_2$), 6.39 (1H, s, β-lactam NH), 6.98 (1H, br s, one of $CONH_2$). MS (ES): *m/z* 390 $(M+H)^+$.

(3*S*,4*R*)-1-(Allyloxyoxalyl)-3-[(*R*)-1(*tert*-butyldimethylsilyloxy)ethyl]-4-(2-(*N*-carbamoylmethyl-*N*-methyl-amino)acetylthio)-2-azetidinone (**3b**)

To a solution of 1.05 g (2.70 mmol) of (3*S*,4*R*)-3-(*R*)-1(*tert*-butyldimethylsilyloxy)ethyl-4-(2-(*N*-carbamoylmethyl-*N*-methyl-amino)acetylthio)-2-azetidinone in 10 ml toluene, at 5°C, under nitrogen atmosphere, 0.90 ml (6.48 mmol) of triethylamine and 0.44 g (2.97 mmol) of allyl oxalyl chloride ware added in sequence. The solution was stirred at the same temperature for 20 minutes and the reaction course monitored by TLC (ethyl acetate/cyclohexane 9 : 1 v/v) and HPLC (column: Bondclone 10 C18, 300×3.9 mm; mobile phase: acetonitrile/water 65 : 35 v/v; flow: 1 ml/minute; λ: 220 nm). At the end of the reaction, the mixture was diluted with toluene, washed with water, dried over anhydrous sodium sulfate and evaporated. The crude product was used for the following step. Separation of the crude product by column chromatography (ethyl acetate/cyclohexane, gradient from 25 : 75 to 90 : 10 v/v) allowed to obtain pure **3b**, **3d** and **3e**. The three acylation products could be identified by TLC (Rf, ethyl acetate/cyclohexane, 50 : 50 v/v) and HPLC (Rt in the conditions given) data and their structure confirmed by ^{1}H NMR analysis. **3b**: Rf: 0.18; Rt: 8.75; ^{1}H NMR (200 MHz, $CDCl_3$): ∂ 0.03 (3H, s), 0.08 (3H, s), 0.85 (9H, s), 1.22 (3H, d, *J*=6.3 Hz), 2.46 (3H, s), 3.24 (2H, s), 3.47 (1H, m, H-3), 3.50 (2H, s), 4.35 (1H, m, CH_3–*CH*), 4.8 (2H, m, $COOCH_2CH=CH_2$), 5.35 (2H, m, $COOCH_2CH=CH_2$), 5.92 (1H, m, $COOCH_2CH=CH_2$), 5.95 (1H, d, *J*=2 Hz, H-4), 6.95 and 6.18 (1H each, br s, $CONH_2$). **3d**: Rf: 0.66; Rt: 7.90; ^{1}H NMR (200 MHz, $CDCl_3$): ∂ 0.04 (3H, s), 0.05 (3H, s), 0.85 (9H, s), 1.17 (3H, d, *J*=6.1 Hz), 2.52 (3H, s), 3.15 (2H, m, H-3), 3.42 (2H, s), 3.64 (2H, s), 4.25 (1H, m, CH_3–*CH*), 4.75 (2H, m, $COOCH_2CH=CH_2$), 5.23 (1H, d, *J*=2 Hz, H-4), 5.32 (2H, m, $COOCH_2CH=CH_2$), 5.95 (1H, m, $COOCH_2CH=CH_2$), 6.72 (1H, s, β-lactam NH), 8.45 (1H, br s, CONHCOCOO–). **3e**: Rf: 0.86; Rt: 15.6; ^{1}H NMR (200 MHz, $CDCl_3$): ∂ 0.03 (3H, s), 0.08 (3H, s), 0.84 (9H, s), 1.22 (3H, d, *J*=6.3 Hz), 2.53 (3H, s), 3.42 (1H, m, H-3), 3.47 (2H, s), 3.63 (2H, s), 4.32 (1H, m, CH_3–*CH*), 4.77 (4H, m, $COOCH_2CH=CH_2$), 5.35 (4H, m, $COOCH_2CH=CH_2$), 5.9 (2H, m, $COOCH_2CH=CH_2$), 5.92 (1H, d, *J*=2.2 Hz, H-4). Several different experimental conditions were tested (Table 1): although the use of benzene as solvent gave the best selectivity (15 : 1 ratio of **3b** *vs.* **3d**+**3e**), the less toxic toluene was found preferable (85% total yield, 10 : 1 selectivity).

Allyl (5*R*,6*S*)-2-(*N*-(2-Acetamido)-*N*-methyl)-aminomethyl-6-[(*R*)-*tert*-butyldimethylsilyloxy)ethyl]-penem-3-carboxylate (**4b**) from **3b**

The crude **3b** (containing about 9% of **3d**+**3e**) coming from the previous step was dissolved in 14 ml of toluene. 1.02 ml (5.94 mmol) of triethylphosphite were added at 35~40°C, and the solution refluxed for 6 hours, allowed to cool to 35°C and evaporated under vacuum. Separation of the residue by column chromatography (ethyl acetate/cyclohexane 60 : 40 v/v) allowed to obtain allyl (5*R*,6*S*)-2-(*N*-(2-Acetamido)-*N*-methyl)aminomethyl-6-[(*R*)-*tert*-butyldimethylsilyloxy)ethyl]-penem-3-carboxylate **4b** (yield: 22%; white solid; ^{1}H NMR (200 MHz, $CDCl_3$): ∂ 0.07 (6H, s, $Si(CH_3)_2$); 0.87 (9H, s, $Si(CH_3)_3$); 1.24 (3H, d, *J*=6.2 Hz, CH_3–CH); 2.36 (3H, s, N–CH_3); 3.09 (2H, s, N–CH_2); 3.68 (1H, dd, *J*=1.6, 4.5 Hz, H-6); 3.76 and 3.88 (2H, ABq, *J*=16 Hz, N–CH_2); 4.24 (1H, m, H-8); 4.72 (2H,

Table 1. Acylation of the azetidinone **2b** with allyl oxalyl chloride.

Solvent	Base	Temp.	Time	Results (3b/(3d + 3e)
Toluene	TEA, 2.4 eq.	5°C	20 min	10:1
Benzene	TEA, 2.4 eq.	5°C	20 min	15:1
Toluene	DIPEA, 2 eq.	5°C	20 min	2:1
CH_2Cl_2	TEA, 2 eq.	5°C	20 min	2:1
CH_2Cl_2	TEA, 2 eq.	-60°C	3 h	6:1
CH_2Cl_2	Py, 2 eq.	5°C	20 min	< 1: 50
CH_2Cl_2	$CaCO_3$, 3 eq.	5°C	20 min	< 1: 50
CH_2Cl_2	Cs_2CO_3, 2 eq.	5°C	1 h	< 1: 50
Toluene	None	5°C	20 min	< 1: 50

TEA= triethylamine; DIPEA = diisopropylethylamine; Py = pyridine; eq. = equivalents (mol/mol **2b**)

m, $COOCH_2$); 5.33 (2H, m, CH=CH_2); 5.55 (1H, d, J=1.6 Hz, H-5); 5.70 (1H, br s, CONH); 5.82 (1H, m, CH=CH_2); 6.90 (1H, br s, CONH); ^{13}C NMR (50 MHz, $CDCl_3$): ∂ −5.1, −4.3, 18.0, 22.4, 25.7, 43.7, 55.1, 61.1, 61.9, 65.1, 65.7, 71.8, 118.6, 121.1, 131.5, 155.7, 159.5, 172.8, 172.9; MS (TS): m/z 470 $(M+H)^+$) and the corresponding nitrile **4d** (allyl (5R,6S)-2-(*N*-(cyanomethyl)-*N*-methyl)-aminomethyl-6-[(*R*)-*tert*-butyldimethylsilyloxy)ethyl]-penem-3-carboxylate: yield: 42%; ^{1}H NMR (200 MHz, $CDCl_3$): ∂ 0.08 (6H, s, $Si(CH_3)_2$); 0.88 (9H, s, $Si(CH_3)_3$); 1.24 (3H, d, J=6.2 Hz, CH_3–CH); 2.46 (3H, s, N–CH_3); 3.55 (2H, s, N–CH_2); 3.66 (1H, dd, J=1.6, 4.5 Hz, H-6); 3.76 and 3.88 (2H, ABq, J=16 Hz, N–CH_2); 4.24 (1H, m, H-8); 4.70 (2H, m, $COOCH_2$); 5.30 (2H, m, CH=CH_2); 5.54 (1H, d, J=1.6 Hz, H-5); 5.92 (1H, m, CH=CH_2); MS (ES), m/z 470 $(M+H)^+$.

(3*S*,4*R*)-3-[(*R*)-*tert*-Butyldimethylsilyloxy)ethyl]-4-(2-chloroacetylthio)-2-azetidinone (**2c**)

To a solution of 50 g (0.174 mol) of (3*S*,4*S*)-4-acetoxy-3-(*R*)-1(*tert*-butyldimethylsilyloxy)ethyl-2-azetidinone **1** in anhydrous dioxane (400 ml), at 20°C, under nitrogen atmosphere, were added 83.3 g (0.261 mol) of zinc iodide and, after 15 minutes stirring, 38.3 g (0.346 mol) of 2-chlorotioacetic acid. The mixture was cooled to 12°C and a solution of 26.5 ml (0.190 mol) of triethylamine in 50 ml of dioxane was added dropwise over 1 hour. After stirring for 2 hours at the same temperature, 5.0 ml (0.036 mol) of triethylamine were added dropwise. The mixture was stirred for 30 minutes, poured in a cold 3% $NaHSO_3$ solution in water and extracted with ethyl acetate. The organic phase was washed with 3% $NaHSO_3$, 5% $NaHCO_3$, water, 10% NaCl and dried over anhydrous Na_2SO_4. Evaporation of the solvent under vacuum gave an orange-yellow solid. Addition of pentane and filtration through a buchner funnel gave a pale yellow solid that was washed with pentane on the filter giving a white solid. Yield: 57 g (97%). mp: 81.4°C; ^{1}H NMR (200 MHz, $CDCl_3$): ∂ 0.07 (3H, s, Si–CH_3); 0.08 (3H, s, Si–CH_3); 0.88 (9H, s, $Si(CH_3)_3$); 1.21 (3H, d, J=6.3 Hz, CH_3–CH); 3.23 (1H, dd, J=2.3, 4.0 Hz, H-3); 4.22 (2H, s, S–CO–CH_2Cl); 4.27 (1H, qd, J=3.7, 6.3 Hz, CH_3–CH); 5.32 (1H, d, J=2.3 Hz, H-4); 6.4 (1H, br s, N–H); ^{13}C NMR (50 MHz, $CDCl_3$): ∂ −5.1, −4.3, 17.9, 22.3, 25.7, 48.0, 52.4, 64.6, 65.4, 166.1, 194.8; MS (TS): m/z 338 $(M+H)^+$, 355 $(M+NH_4)^+$.

(3*S*,4*R*)-1-(Allyloxyoxalyl)-3-[(*R*)-*tert*-butyldimethylsilyloxy)ethyl]-4-(2-chloroacetylthio)-2-azetidinone (**3c**)

To a solution of 19 g (0.056 mol) of **2c** in anhydrous toluene (150 ml), at 0~3°C, under nitrogen atmosphere, 14.1 ml (0.113 mol) of allyl oxalyl chloride were added. The solution was stirred for 5 minutes at the same temperature and a solution of 11.7 ml (0.084 mol) of triethylamine in toluene (10 ml) added dropwise. The mixture was stirred for 90 minutes at the same temperature. 3.9 ml (0.028 mol) of triethylamine were added and the mixture stirred for other 90 minutes, filtered, and the filtrate washed several times at 5°C with 5% aqueous $NaHCO_3$, with water and with 10% NaCl. After drying over anhydrous Na_2SO_4 the mixture was filtered and the toluene solution used for the following step. Alternatively, **3c** could be isolated in 98% yield as a brown viscous oil. ^{1}H NMR (200 MHz, $CDCl_3$): ∂ −0.09 (3H, s, Si–CH_3); −0.04 (3H, s, Si–CH_3); 0.85 (9H, s, $Si(CH_3)_3$); 1.24 (3H, d, J=6.3 Hz, CH_3–CH); 3.52 (1H, t, J=3.0 Hz, H-3); 4.26 (2H, s, S–CO–CH_2Cl); 4.38 (1H, qd, J=3.0, 6.3 Hz, CH_3-CH); 4.70~4.82 (2H, m, $COOCH_2$); 5.22~5.46 (2H, m, CH=CH_2); 5.80~6.06 (1H, m, CH=CH_2); 5.97 (1H, d, J=3.0 Hz, H-4); ^{13}C NMR (200 MHz, $CDCl_3$): ∂ −5.2, −4.3, 17.8, 21.7, 25.6, 47.9, 53.8, 64.7, 66.3, 67.4, 120.1, 130.5, 154.5, 159.0, 162.9, 190.7; MS (TS): m/z 468 $(M+NH_4)^+$.

Allyl (5*R*,6*S*)-2-Chloromethyl-6-[(*R*)-*tert*-butyldimethylsilyloxy)ethyl]-penem-3-carboxylate (**4c**)

To the toluene solution (250 ml) containing crude **3c** (0.055 mol) was added triethylphosphite (20.7 ml, 0.121 mol) and the mixture refluxed for 3 hours. The solution is allowed to cool to room temperature, most of the solvent evaporated under vacuum and the residue, dissolved in 320 ml of *N*,*N*-dimethylformamide, was used for the following step without any purification. Alternatively, **4c** could be isolated in 82% yield as a yellow oil.[24]

Allyl (5*R*,6*S*)-2-(*N*-(2-Acetamido)-*N*-methyl)-aminomethyl-6-[(*R*)-*tert*-butyldimethylsilyloxy)ethyl]-penem-3-carboxylate (**4b**) from **4c**

To the solution of **4c** in 320 ml of *N*,*N*-dimethylformamide, obtained as described for the previous step, at room temperature, under nitrogen atmosphere, were added 16.9 g (0.114 mol) of sodium iodide. After 30 minutes, 9.78 g (0.111 mol) of sarcosinamide were added and the mixture was stirred for about 2 hours. At the end of the reaction, the mixture was poured into iced 3% aq. $NaHSO_3$ (200 ml) and extracted three times with ethyl acetate. The organic phase was washed with 5% aq. $NaHCO_3$ (3×70 ml), with water and with brine. After drying over anhydrous Na_2SO_4, the solvent was evaporated under vacuum, giving an orange-red oil, that was crystallised by the aid of cold cyclohexane/*n*-hexane 1 : 5 v/v, filtered and washed with hexane. Yield: 10.8 g (42% overall yield from **2b**). White solid.

(5*R*,6*S*)-2-(*N*-(2-Acetamido)-*N*-methyl)aminomethyl-6-[(1*R*)-hydroxyethyl]-penem-3-carboxylic Acid **6** (MEN 10700)

The synthesis of **6** from **4b** through **5** has already been described.[10] Compound **6** was obtained after freeze-drying as a white powder; mp: 133.5~170.6°C (dec.); optical rotation: $[\alpha]_{20}^{D}=+103°$ (c=0.01 g/ml, water); ^{1}H NMR (200 MHz, DMSO-d_6): ∂ 1.15 (3H, d, J=6.35 Hz, CH_3–CH), 2.29 (3H, s, N–CH_3), 3.03 (2H, ABq, C–CH_2–N), 3.70 (1H, dd, J=6.35 Hz, J=1.50 Hz, H-6), 3.79 (2H, s, N–CH_2), 3.94 (1H, m, H-8), 5.17 (1H, d, OH), 5.53 (1H, d, J=1.50 Hz, H-5), 7.06 (1H, br s, CONH), 7.18 (1H, br s, CONH), 13.45 (1H, br s, COOH); ^{13}C NMR (50 MHz, D_2O): ∂ 24.9 (CH_3–CH), 46.1 (N–CH_3), 57.4 (CH_2-2′), 60.5 (N–CH_2), 68.1 (C-5), 69.5 (C-8), 75.1 (C-6), 135.7 (C-2), 141.1 (C-3), 169.7 (COOH), 173.7 ($CONH_2$), 180.2 (β-lactam C=O); IR (KBr, cm^{-1}): 3600~3200 (OH), 3343 (NH_2), 3182 (NH_2), 2972 (CH_3 in N–CH_3) 2700~2500 (COOH), 1777 (β-lactam C=O), 1692 (C=O in $CONH_2$ (amide band I) and COOH), 1611 (C=C, five membered ring), 1575 ($CONH_2$, amide band II), 1454 (CH_2), 1374 (C–N stretch, amide band III), 1289 (C–O(H) stretch); UV (λ_{max}, H_2O): 194, 250, 316 nm; MS (ES^+): m/z 316 (MH^+), 338 (MNa^+).

Pivaloyloxymethyl (5*R*,6*S*)-2-(*N*-(2-Acetamido)-*N*-methyl)-aminomethyl-6-((*R*)-1-hydroxyethyl)-penem-3-carboxylate (**8f**, MEN 11505)

1 g (3.17 mmol) of **6** was dissolved, under nitrogen atmosphere, in 20 ml of anhydrous *N*,*N*-dimethylformamide. 525 mg (3.8 mmol) of potassium carbonate, 1.02 g (3.17 mmol) of tetrabutylammonium bromide and 0.92 ml (6.34 mmol) of chloromethyl pivalate were added in sequence. After stirring at room temperature for 6 hours, the reaction mixture was poured into iced brine (25 ml) and extracted with ethyl acetate (5×80 ml). The organic phase was dried over anhydrous Na_2SO_4 and evaporated under vacuum, giving a crude oil that was washed several times with hexane and purified by column chromatography (ethyl acetate/methanol 95 : 5 v/v), allowing to obtain **8f** as a white solid. Yield: 710 mg (52%); mp: 94~95°C (dec.); optical rotation: $[\alpha]_{D}^{25}=+111.6°$ (c=0.01 g/ml, methanol); ^{1}H NMR (200 MHz, DMSO-d_6): ∂ 1.14 (3H, d, CH_3–CH, J=6.2 Hz); 1.15 (9H, s, $(CH_3)_3C$); 2.26 (3H, s, N–CH_3); 2.98 (2H, AB system, C–CH_2–N); 3.77 (1H, dd, H-6); 3.79 (2H, s, N–CH_2); 3.95 (1H, m, H-8); 5.20 (1H, d, OH); 5.57 (1H, d, H-5); 5.75 and 5.84 (2×1H, 2×d, AB system, $COOCH_2OCO$); 6.99 (1H, br s, CONH); 7.17 (1H, br s, CONH); IR (KBr, cm^{-1}): 3600~3100 (OH), 3364 (NH_2), 3197 (NH_2), 2979 and 2874 (CH_3 in N–CH_3), 2801 (C–N stretch in tertiary amine, N–CH_3 and –N–CH_2), 1796 (β-lactam C=O), 1752 (C=O stretch in C=C=C=O), 1719 (C=O stretch in $CH_2OCOtBu$), 1651 (C=O in $CONH_2$ (amide band I); UV (λ_{max}, H_2O): 208, 247, 326 nm; MS (ES^+): m/z 430 (MH^+), 452 (MNa^+). A similar procedure allowed to obtain **8g** (mp: 147~148°C (dec.); ^{1}H NMR (200 MHz, $CDCl_3$): ∂ 1.33 (3H, d, CH_3–CH, J=6.2 Hz); 1.54 (3H, d, CH_3–CH, J=5.4 Hz); 2.1 (3H, s, CH_3CO); 2.37 (3H, s, N–CH_3); 3.05 (2H, s, N–CH_2); 3.60~3.85 (total 3H, m); 4.23 (1H, m, H-8); 5.59 (1H, d, H-5); 5.95 (1H, br s, CONH); 6.88 (1H, br s, CONH), 6.92 (1H, m)) and **8h** (mp: 130~131°C (dec.); ^{1}H NMR (200 MHz, $CDCl_3$): ∂ 1.31 (3H, d, CH_3–CH); 1.35 (2×3H, 2d, $(CH_3)_2CH$); 1.58 (3H, d, CH_3–CH–O); 2.37 (3H, s, N–CH_3); 3.09 (2H, s, N–CH_2); 3.71 (1H, dd, H-6); 3.82 (1H, Abq, NCH_2); 4.23 (1H, m, H-8); 4.91 (1H, m, $(CH_3)_2CH$); 5.56 (1H, d, H-5); 6.85 (1H, m).

Stability of **6** and Ritipenem to Dehydropeptidase-I (DHP-I)

Aliquots of 0.5 ml of a solution containing 2.00 mg/ml of **6** and 2.00 mg/ml of ritipenem in 0.025 M Tris-HCl buffer (pH=7) were added with 0.5 ml of rat DHP-I and the samples incubated at 37°C for different times. Control: aliquots of 0.5 ml of the same solution were added with 0.5 ml of water and stored at 37°C. For the parallel study at a substrate concentration of 0.1 mg/ml, 1 ml of the solution containing **6** and ritipenem was diluted to 10 ml with 0.025 M Tris-HCl buffer (pH=7). The stability was followed for about 3 hours, analysing the residual **6** and ritipenem by HPLC (column: Lichrosorb RP-18 5 μ, 250×4.6 mm, Merck; mobile phase: 0.02 M potassium dihydrogen phosphate buffer pH=3/acetonitrile 93 : 7 v/v; flow rate: 1 ml/minute; λ: 320 nm; Rt (minute): 9.3 for **6** and 3.3 for ritipenem).

Plasma Levels of **6** (MEN 10700) after Oral Administration in Rats of **8f~h**

Sprague Dawley male rats, with a weigh range of 350~400 g, were dosed orally by gavage with a quantity of **8f~h** corresponding to 40 mg/kg of **6** and dissolved in sterile saline at a concentration of (2 mg of **6**)/ml. The volume administered was 10 ml/kg. Blood samples of approximately 0.15~0.25 ml each were collected from each rat after 5, 10, 15, 20, 30, 45, 60, 90, 120 minutes in heparinized tubes by means of a cannula inserted in the jugular vein. The blood was centrifuged for 10 minutes at 4°C, the blood cells discarded and the plasma obtained was stored at −20°C pending analysis. Plasma concentrations of **6** were determined by HPLC (column: Supelcosil LC-1, 5 μm, 250×4.6 mm; mobile phase: 0.066 M phosphate buffer pH=7.0/acetonitrile 95 : 5; flow: 1 ml/minute; λ: 306 nm).

Acknowledgements

We thank B. T. Biotecnica s.r.l., Saronno, Italy, for evaluation of DHP stability. Many thanks are due to Prof. J. M. T. HAMILTON-MILLER for encouraging discussions and to Mr. V. D'ARANNO for the technical support.

References

1) (a) SUTCLIFFE, J. & N. H. GEORGOPAPADAKOU, *Eds.*: Emerging Targets in Antibacterial Chemotherapy. Chapman and Hall. New York, 1992. (b) PAGE, M. I., *Ed.*: The Chemistry of β-Lactams. Blackie Academic and Professional. Glasgow, 1992. (c) NICCOLAI, D.; L. TARSI, R. J. THOMAS: The renewed challenge of antibacterial chemotherapy. Chem. Commun. 1997: 2333~1342, 1997

2) (a) WISE, R.: The carbapenem and penem antibiotics—a brief review. Antimicrob. Newsl. 7: 73~80, 1990. (b) MITSUHASHI, S. & G. FRANCESCHI, *Eds.*: Penem Antibiotics. Japan Scientific Society Press—Springer Verlag. Tokyo—Berlin, 1991. (c) BENTLEY, P. H. & P. J. O'HANLON, *Eds.*: Anti-Infectives. Recent Advances in Chemistry and Structure-Activity Relationships. Royal Society of Chemistry, Spec. Publ. No. 198, 1997. (d) BRYSKIER, A.: Penems: new oral β-lactam drugs. Exp. Opin. Invest. Drugs 4: 705~724, 1995

3) ERNEST, I.; J. GOSTELI, C. W. GREENGRASS, W. HOLICK, D. E. JACKMAN, H. R. PFAENDLER & R. B. WOODWARD: The penems, a new class of β-lactam antibiotics: 6-acylaminopenem-3-carboxylic acids. J. Am. Chem. Soc. 100: 8214~8222, 1978

4) FONTANA, R.; M. ALTAMURA, F. ARCAMONE, A. MAZZARIOL, G. MORANDOTTI, R. SPERNING & G. CORNAGLIA: MEN 10700, a new penem antibiotic: *in vitro* activity and its correlation with β-lactamase stability, PBP affinity and diffusion through the bacterial cell wall. J. Antimicrob. Chemother. 41: 513~525, 1998

5) INOUE, E. & S. MITSUHASHI: *In vitro* antibacterial activity and β-lactamase stability of SY5555, a new oral penem antibiotic. Antimicrob. Agents Chemother. 38: 1974~1979, 1994

6) CORMICAN, M. G. & R. N. JONES: Evaluation of the *in-vitro* activity of furopenem (SY5555 or SUN5555) against respiratory tract pathogens and beta-lactamase producing bacteria. J. Antimicrob. Chemother. 35: 535~539, 1995

7) FRANCESCHI, G.; M. FOGLIO, M. ALPEGIANI, C. BATTISTINI, A. BEDESCHI, E. PERRONE, F. ZARINI & F. ARCAMONE: Synthesis and biological properties of sodium (5*R*,6*S*,8*R*)-6α-hydroxyethyl-2-carbamoyloxymethyl-2-penem-3-carboxylate (FCE 22101) and its orally absorbed esters FCE 22553 and FCE 22891. J. Antibiotics 36: 938~941, 1983

8) WISE, R. & R. C. BALLARD: Review of the *in-vitro* evaluation of FCE 22101. J. Antimicrob. Chemother. 23: (Suppl. C): 7~16, 1989

9) MINAMIMURA, M.; Y. TANIYAMA, E. INOUE & S. MITSUHASHI: *In vitro* antibacterial activity and β-lactamase stability of CP-70,429, a new penem antibiotic. Antimicrob. Agents Chemother. 37: 1547~1551, 1993

10) ALTAMURA, M.; E. PERROTTA, P. SBRACI, V. PESTELLINI & F. ARCAMONE: 2-Substituted penems with amino acid-related side chains: synthesis and antibacterial activity of a new series of β-lactam antibiotics. J. Med. Chem. 38: 4244~4256, 1995

11) (a) ALPEGIANI, M.; A. BEDESCHI, E. PERRONE, F. ZARINI & G. FRANCESCHI: 2-(Heteroatom-Substituted)methyl penems. III. Nitrogen derivatives. Heterocycles 27: 1329~1340, 1988. (b) CORRAZ, A. J.; S. L. DAX, N. K. DUNLAP, N. H. GEORGOPAPADAKOU, D. D. KEITH, D. L. PRUESS, P. L. ROSSMAN, R. THEN, J. UNOWSKY & C. WEI: Dual-action penems and carbapenems. J. Med. Chem. 35: 1828~1839, 1992

12) CONNOLLY, S.; K. W. MOORE, M. D. COOKE, J. C. WALMSLEY & P. H. BENTLEY: The synthesis and biological activity of a novel series of 2-aryl penems. J. Antibiotics 44: 1169~1171, 1991

13) LIBEREK, B. & R. KASPRZYKOWSKA: Side reactions of monoaminodicarboxylic acids in peptide synthesis. Int. J. Pept. Protein Res. 31: 58~62, 1988
14) SATSUMABAYASHI, S.; H. TAKAHASHI, T. TANAKA & S. MOTOKI: The synthesis of 1,3-dithiolanone derivatives. J. Org. Chem. 38: 3953~3954, 1973
15) HAMILTON-MILLER, J. M. T. & S. SHAH: *In vitro* microbiological assessment of a new penem, MEN 10700. J. Antimicrob. Chemother. 39: 575~584, 1997
16) PANKUCH, G. A.; D. B. HOELLMAN, M. R. JACOBS & P. C. APPELBAUM: Antipneumococcal activity of MEN 10700, a new penem, compared to other compounds, by MIC and time-kill. J. Antimicrob. Chemother. 44: 381~384, 1999
17) VAROTTO, F.; E. PARRAVIDINI, E. M. DE LUCA, A. CASINI & F. FRASCHINI: *In-vitro* antibacterial activity of MEN 10700, a new penem antibiotic. Chemotherapy 45: 405~410, 1999
18) MOREILLON, P.; M. GIDDEY, CH. DURUSSEL, M. P. GLAUSER & J. BILLE: *In vitro* activity of the new penem MEN 10700 against 114 Swiss clinical isolates of *Haemophilus influenzae* susceptible or resistant to amoxicillin. Abstracts of papers of 39th Intersci. Conf. on Antimicrob. Agents Chemother., No. F-23, San Francisco, 1999
19) HAMILTON-MILLER, J. M. T. & S. SHAH: *In vitro* activity of a novel penem, MEN 10700, against meningococci and gonococci, and the effect of a cysteine containing supplement. J. Antimicrob. Chemother. 42: 553~555, 1998
20) HAMILTON-MILLER, J. M. T. & S. SHAH: Comparative anti-anaerobic activity of MEN 10700, a penem antibiotic. Int. J. Antimicrob. Agents 10: 321~324, 1998
21) MOREILLON, P.; M. GIDDEY, CH. DURUSSEL, J. ETIENNE, M. P. GLAUSER & J. BILLE: *In vitro* activity of the new penem MEN 10700 against clinical isolates of enterobacteria producing extended-spectrum beta-lactamases (ESBL) or inducible cephalosporinases (CEPHASE). Abstracts of papers of 39th Intersci. Conf. on Antimicrob. Agents Chemother., No. F-24, San Francisco, 1999
22) MITSUHASHI, S. & G. FRANCESCHI, *Eds.*: Penem Antibiotics. Japan Scientific Society Press—Springer Verlag. Tokyo—Berlin, p. 58, 1991
23) DELLA BRUNA, C.; D. JABES, G. SEBBEN & A. SANFILIPPO: *In vitro* and *in vivo* evaluation of the penem FCE 22101 and its orally absorbed ester FCE 22891. G. Ital. Chemioter. 30: 125~130, 1983
24) ALTAMURA, M. & E. PERROTTA: An efficient synthesis of 2-(halogenomethyl)penems J. Org. Chem. 58: 272~274, 1993