Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 5, No. 6, pp. 555-558, 1995

0960-894X/95 $9.50+0.00

0960-894X(95)00072-0

SYNTHESIS AND ANTIBACTERIAL ACTIVITY OF MEN 10700, A NEW PENEM ANTIBIOTIC

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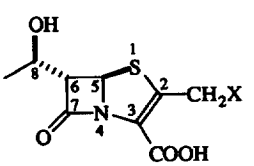
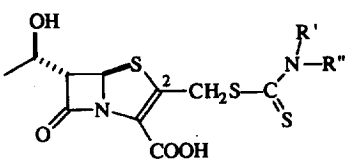
Abstract. The new penem antibiotic **Men 10700** (**3**), bearing an amino acid derived amide as C-2 side chain, was synthesized. **Men 10700** exhibited high potency and a broad spectrum of activity against Gram positive and Gram negative microorganisms.

The penems are a synthetic class of highly potent, broad spectrum ß-lactam antibiotics structurally related to the naturally occurring penicillins, cephalosporins and carbapenems [1]. The penem skeleton, born as a hybrid between penicillins and cephalosporins, has been the object of extensive synthetic work since its first synthesis in 1978 [2] and a number of penem derivatives, such as ritipenem [3a], furopenem [3b] and sulopenem [3c], are now in clinical trials. While the presence of the 1(R)-hydroxyethyl moiety at C-6, as in **1**, is now considered to be a fundamental requisite to confer chemical and ß-lactamase stability, a large differentiation is possible in the nature of C-2 substituent. In the course of our research program on new penems, we focused our attention on 2-CH_2X substituted penems **1** bearing a heteroatom linked to the bicyclic skeleton through a methylene spacer. As a part of these studies, we reported [4] on the synthesis and antibacterial activity of penem dithiocarbamates **2**: these compounds exhibited potent *in vitro* antibacterial activity against Gram positive bacteria, including heterogeneous methicillin resistant *Staphylococcus aureus* strains. However, their activity against Gram negative strains was considerably lower.



1



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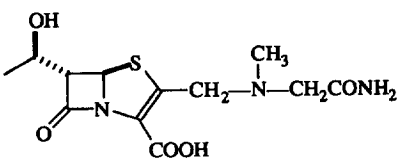
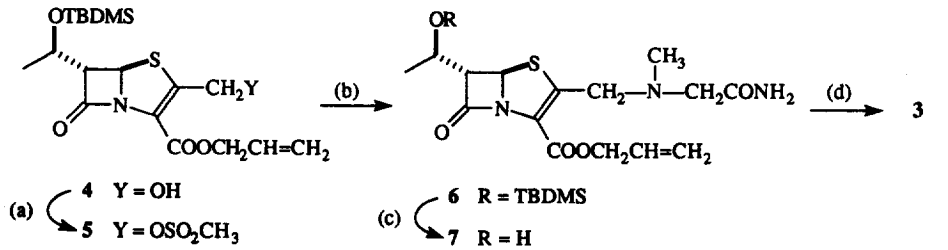


3 (Men 10700)

In the search for 2-substituted penems endowed with a better balanced antibacterial spectrum, we turned then our attention to nitrogen substituted methyl penems bearing amino acid derived side chains. We reasoned that insertion of amino acid derived moieties as small, polar groups, should improve the penetration through the outer membrane of Gram negative bacteria: in facts, in the previous series, our best results were obtained when

a N-methyl glycinamide was inserted (**2**, R' = CH_3, R" = CH_2CONH_2) as the terminal group on the dithiocarbamate. In addition, natural and unnatural amino acids should provide us a large pool of side chains with tunable biological and chemical features: incorporation of amino acid derived side-chains in other series of ß-lactam compounds has been used to improve spectrum [5] or pharmacokinetic properties, as oral absorption. [6]

We have synthesized [7] a number of new amino acid derived, amido substituted penems (**1**, X = $-NR^1-(CHR^2)_n-CONR^3R^4$): among them, the N-methyl glycinamido derivative **3** (**Men 10700**) emerged as a potent, broad spectrum, antibacterial agent.



Scheme 1. TBDMS = tert-butyldimethylsilyl. Reagents: (a): CH_3SO_2Cl, TEA, THF, 0-5°C, 1h. (b): $CH_3NH-CH_2CONH_2$, TEA, DMSO, 16 h, r. t. (c): TBAF, AcOH, THF, 24 h, r. t. (d): $Pd(PPh_3)_4$, triphenylphosphine, THF, 30 min, 35°C.

Synthesis of **3** (Scheme 1) started from well-known 2-hydroxymethyl penem intermediate **4** [8]: the primary hydroxy group in **4** was activated as the mesylate **5** and allowed to react with sarcosinamide hydrochloride to give **6** [9] (65% overall yield from **4**). Deprotection at C-8 with tetrabutylammonium fluoride and removal of the allyl ester moiety by palladium catalysis, [10] followed by reverse phase column chromatography, gave (5R,6S)-2-[N-methyl-N-(2-acetamido)]-aminomethyl-6-[(1R)-hydroxyethyl]-penem-3-carboxylic acid **3** (56% overall yield from **6**) as a white solid; mp 95-6°C. ^1H NMR (200 MHz, D_2O) δ 1.25 (3H, d, J = 6.2 Hz, CH_3-C), 2.90 (3H, s, N-CH_3), 3.98 (2H, s, N-CH_2), 3.98 (1H, dd, J = 1.4, 6.4 Hz, H-6), 4.13-4.28 (1H, m, H-8), 4.25 (2H, s, CH_2-2'), 5.69 (1H, d, J = 1.4 Hz, H-5). ^{13}C NMR (50 MHz, D_2O) δ 24.9 (CH_3-C), 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 (ß-lactam C=O). IR (KBr) cm^{-1} 1761 (ß-lactam C=O), 1682, 1605. FAB-MS (m/z) 316 $(M + H)^+$. $[\alpha]^{20}_D$: +96° (*c* 0.1, H_2O). UV: λ_{max} (H_2O) 254, 315 nm. Chemical half-lives (HPLC determination at 37°C): 4 h (pH 1.7), 50 h (pH 3.7), 120 h (pH 7.0), 30 h (pH 9.0).

Antibacterial activity of **3** was first tested against a number of standard and modified Gram positive and Gram negative strains (Table 1). Compound **3** showed outstanding activity against standard *S. aureus* strains, heterogeneous methicillin resistant *S. aureus*, and against *E. coli* standard strain. Against all these strains activity of **3** was very similar to ritipenem and imipenem, by far exceeding that of ampicillin-sulbactam.

It is interesting to note how the difference in minimum inhibitory concentrations between standard and hyperpermeabile *E. coli* strains, previously showed for **2**, and indicating in that case a problem in permeability through the outer Gram negative bacterial membrane, disappeared for **3**, supporting the hypothesis of enhanced permeability of the compound, when bearing the small, polar, sarcosinamide derived, side chain. Activity of **3** against *E. coli* did not show any significant change when *E. coli* strains producing known beta-lactamases were used and constantly remained at the imipenem level. Comparison with two close analogues of **3**, the N,N-dimethylamide **8** and the n-butylester **9** (X = $-N(CH_3)CH_2CON(CH_3)_2$ and $-N(CH_3)CH_2COOC_4H_9$, respectively, in general formula **1**) brought into evidence the importance of a primary amido moiety: in fact, the

ester compound did not show any significant activity against Gram negative strains, while dimethylamide **8** led to a neat decrease of potency all over the spectrum, confirming the subtle influence on biological properties of a change in the amino acid derivative. As most of penem antibiotics [11], **3** did not show activity against *Pseudomonas aeruginosa*.

Table 1. *In vitro* antibacterial activity * of compounds **2** and **3** (**Men 10700**) in comparison with ritipenem, imipenem and ampicillin-sulbactam, as determined by the agar dilution technique.

	MIC (μg/mL)													
Com pound	*S. a.* ATCC 29213	*S. a.* ATCC 25923	*S. a.* MR 13	*S. a.* MR 09	*E. f.* ATCC 29212	*E. coli* ATCC 25922	*E. coli* DC2	*E. coli* TEM1	*E. coli* TEM2	*E. coli* SHV	*E. coli* L	*Ps. aer.* ATCC 27853	*Ps. aer.* VR5	*Ps. aer.* G242
2	0.12	0.12	0.03	2	8	8	0.5	8	8	16	16	>32	4	8
3	0.06	<0.03	0.06	1	4	0.25	0.25	0.25	0.5	0.5	0.25	>32	0.5	4
8	0.25	0.12	0.25	4	8	0.5	1	1	1	1	0.5	>32	4	0.5
9	0.5	0.25	0.5	16	8	32	1	>32	>32	>32	>32	>32	>32	>32
RITI	0.12	0.06	0.12	2	2	0.5	1	1	1	1	1	>32	0.5	1
IMI	<0.03	<0.03	0.12	0.25	0.5	0.12	0.5	0.25	0.25	0.25	0.12	1	0.25	0.25
A-S	0.25	0.03	0.12	4	1	8	1	>32	8	>32	8	32	0.12	>32

* Minimum Inhibitory Concentrations (MIC) determined in Mueller Hinton 2 Medium, bioMerieux. Inoculum: 10^4 cells/ml. Incubation: 24 hours at 37°C. Abbreviations: *S. a.* : *Staphylococcus aureus*; *S. a.* MR 13: heterogeneous methicillin resistant *S. aureus*; *S. a.* MR 09: homogeneous methicillin resistant *S. aureus*; *E. f.*: *Enterococcus faecalis*; *E. coli* DC2: hyper-permeable *E. coli* strain; *E. coli* TEM1, TEM2, SHV: strains producing known beta-lactamases; *E. coli* L: ß-lactamase lacking strain; *Ps. aer.*: *Pseudomonas aeruginosa*; *Ps. aer.* VR5: ß-lactamase lacking hyper-permeable strain; *Ps. aer.* G242: hyper-permeable strain. RITI = ritipenem, IMI = imipenem, A - S = ampicillin - sulbactam.

The high potency and broad spectrum of activity exhibited by **Men 10700** as a new antibiotic become evident in light of the data of Table 2, which collects minimum inhibitory concentrations values (as MIC_{90}) against clinical isolates, compared with imipenem (as one of the most potent and broadest spectrum ß-lactam antibiotic now available), ampicillin-sulbactam and amoxicillin. Tested microorganisms included Gram positive, Gram negative, aerobic and anaerobic strains. **Men 10700** showed higher activity than standard compounds, comprising imipenem, against both methicillin-sensitive and methicillin-resistant *S. aureus* strains. Activity of **Men 10700** against *S. epidermidis* was ten-fold greater than imipenem and more than 100-fold greater than ampicillin-sulbactam and amoxicillin. Our compound exhibited equal potency when compared with standards against a range of different *Streptococcus* species, although it was less potent against *Clostridium perfringens*. As for Gram negative spectrum, **Men 10700** was constantly more potent than ampicillin-sulbactam and amoxicillin, reaching imipenem MIC_{90} values against *Haemophilus influenzae*, *Klebsiella pneumoniae*, *Citrobacter freundii*, *Escherichia coli* and *Proteus spp.* Especially noteworthy is the activity against *Enterobacter*, generally considered as a highly resistant nosocomial pathogen.

Further evaluations are now in progress in order to establish the *in vivo* efficacy of the new compound.

Acknowledgment. We wish to thank Dr. Laura Lorenzi and Mr. Daniele Caglio for synthetic work and Dr. Antonio Triolo for the registration of mass spectra. Many thanks are due to Prof. Roberta Fontana (University of Verona) for helpful discussions. This work was supported in part by MURST (IMI Grant No. 53529).

Table 2. *In vitro* antibacterial activity * (MIC_{90}) of **Men 10700 (3)** against clinical isolates

Micro-organism (no. of strains)	MIC_{90} (μg/mL)			
	Men 10700	Imipenem	Amp. - Sulb.	Amoxicillin
Staphylococcus aureus MS (6)	0.0075	0.015	1	1
Staphylococcus aureus MR (20)	4	>32	32	>32
Staphylococcus epidermidis (13)	0.015	0.25	4	4
Streptococcus pyogenes A (31) [a]	0.06	0.06	0.03	0.015
Streptococcus pneumoniae (23) [a]	0.06	0.06	0.03	0.015
Streptococcus agalactiae B (21)	0.06	0.015	0.06	0.06
Clostridium perfringens (27)	0.5	0.03	0.12	0.06
Enterococcus faecalis (47)	8	2	2	0.5
Enterococcus faecium (47)	>64	>64	>64	32
Listeria monocytogenes (20)	0.5	0.03	0.5	0.5
Branhamella catarrhalis (27)	0.25	0.03	0.5	2
Haemophilus influenzae (21) [b]	2	1	1	1
Bacteroides fragilis (15)	2	0.25	4	32
Aeromonas spp. (21)	1	0.06	>32	>32
Klebsiella pneumoniae (20)	0.5	0.25	16	>64
Acinetobacter anitratus (20)	16	0.5	32	n. t.
Pseudomonas aeruginosa (11)	>64	8	>64	n. t.
Xanthomonas maltophilia (9)	>64	>64	>64	n. t.
Enterobacter aerogenes (19)	2	4	>64	n. t.
Escherichia coli (21)	0.25	0.12	8	n. t.
Citrobacter freundii (20)	1	0.5	>64	n. t.
Yersinia spp. (20)	0.5	0.25	32	>64
Proteus mirabilis (16)	1	2	32	n. t.
Proteus vulgaris (10)	2	2	8	n. t.
Providencia rettgerii (10)	2	1	32	n. t.
Providencia stuartii (10)	2	2	64	n. t.
Morganella morganii (10)	2	2	16	n. t.

* MICs determined in Mueller Hinton 2 Medium, bioMerieux (aerobes), inoculum: 10^4 cells/ml, incubation: 24 hours at 37°C; in Wilkins-Chalgren Agar, Oxoid (anaerobes), inoculum: 10^5 cells/ml, incubation: 24-48 hours at 37°C. [a]) MICs determined in Tryptone Soya Agar, Oxoid + 1% Supplement B, Bacto; inoculum: 10^4 cells/ml, incubation: 24-48 hours at 37°C. [b]) MICs determined in Haemophilus Test Medium Base, Oxoid + Haemophilus Test Medium Supplement, Oxoid; inoculum: 10^4 cells/ml, incubation: 24-48 hours at 37°C.

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(*Received in Belgium* 28 *November* 1994; *accepted* 16 *January* 1995)