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SYNTHESIS OF NEW PENEM DITHIOCARBAMATES

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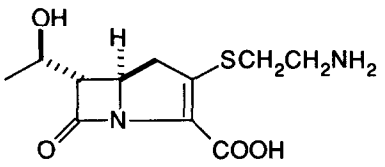
Abstract: Penem dithiocarbamates **10** were synthesized from the alcohol **6**, in high yields and favourable ratio between the penem and the undesired penam isomers. *In vitro* antibacterial activity showed a dependence on polarity and steric hindrance of the side chain at C-2. Best results were obtained with the piperazino derivatives **10 l-n**, while catechol **10 p** did not show the expected improvements in anti-pseudomonal activity.

The intense interest in the study of β-lactam antibiotics has led, in the last fifteen years, to the continuous introduction of new classes of these compounds [1]: their wide spectrum of activity associated with very low toxicity levels ensures them an outstanding role in antibacterial chemotherapy.



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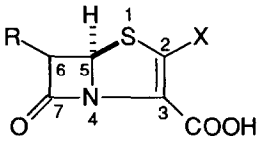

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Among the wide range of compounds described since the discovery of the natural carbapenem thienamycin **1** [2] in 1976, the highly potent, broad spectrum penem antibiotics **2** [3] still retain the unique feature of being totally synthetic ones, lacking a natural counterpart. The penem skeleton, born as a hybrid between penicillins and cephalosporins, has been extensively modified in each position since its first syntheses in 1978.[4] Early work allowed to establish some general point: the presence of a free carboxy group in position 3, as for penicillins or cephalosporins; the 5*R*,6*S* (trans) configuration as for carbapenems. Furthermore, the thienamycin-like 1(*R*)-hydroxyethyl side chain at C-6 is now considered to be a fundamental requisite to confer chemical and β-lactamase stability.

On the other hand, a large differentiation is still possible in the nature of the X substituent in position 2 : several series of 2-heteroatom [3b] or 2-methyl substituted (**2**, X = CH_2Y) [3c] penems have been synthesized in recent years.

In the course of our studies on new 2-methyl substituted penems [5], we focused our attention on the synthesis of (2-thiocarbamoylthio)methyl penems. The presence of a heteroatom in the side chain in position 2 of the penem nucleus had been related [3b] to an enhanced chemical reactivity of the β-lactam linkage and

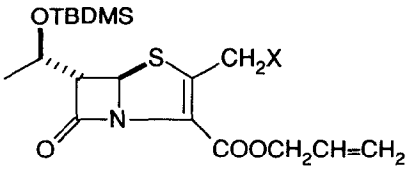
therefore to a higher biological activity. Several series of penems bearing a nitrogen, oxygen or sulfur atom at C-2 have been reported and, more particularly, a number of 2-alkylthiomethyl derivatives (**3**, R = alkyl) have been synthesized in the past [6a], but their preparation is not simple owing to the inclination of these derivatives to undergo double bond shift (Scheme 1) toward the usually more stable 2-alkylthiomethylene penam (the "exo" form **4**).

Scheme 1

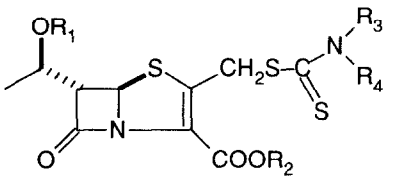
3 **4**

The electron-withdrawing character of the substituent at the exocyclic sulfur seems to play a role [6b] in the penem/penam equilibrium, stabilizing the penem form. We reasoned that the previously unknown penem dithiocarbamates should give a particularly favourable ratio between the penem and penam isomers.

We synthesized penem dithiocarbamates **10** starting from the well known 2-hydroxymethyl penem **5** [7]: the alcohol **5**, activated as its mesylate **6** (MsCl, TEA, THF, 30 min, 0°C) [6a] or the chloride **7** ($CaCl_2$, DMSO on **6**, 20°C, 3 h),[5] was allowed to react with alkaline or ammonium dithiocarbamates [8] derived from aliphatic or heterocyclic amines (DMSO, 20°C, 1 h).[9]



5 X = OH
6 X = OSO_2CH_3
7 X = Cl

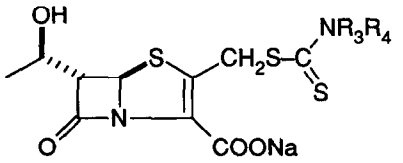


8 R_1 = TBDMS R_2 = $CH_2CH{=}CH_2$
9 R_1 = H R_2 = $CH_2CH{=}CH_2$
10 R_1 = H R_2 = Na, K

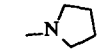
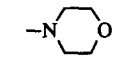
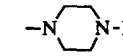
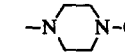
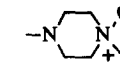
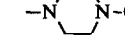
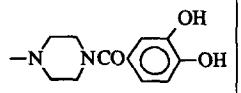
The reaction gave, as the unique products, the fully protected penem dithiocarbamates **8**. No penam isomer could be detected from the ^{1}H and ^{13}C NMR spectra. Deprotection of the secondary hydroxy group at C-8 (TBAF·3 H_2O, AcOH, THF, 20°C, 24 h, 90%) gave, however, a 3:1 mixture of the penem **9** and penam (**4**, R = CS-NR_3R_4, R' = H, R "= $CH_2CH{=}CH_2$) [10]. After deallylation at the carboxy group at C-3 ($Pd(PPh_3)_4$, PPh_3, sodium 2-ethyl hexanoate, AcOEt, 20°C, 30 min), [11] the isomeric mixture was separated [12] and the penems **10** tested for antibacterial activity. Principal results are summarized in Table 1.

Penem dithiocarbamates **10 a-p** exhibited potent *in vitro* antibacterial activity against gram positive bacteria and heterogeneous methicillin resistant *Staphylococcus aureus* strains. However, their activity against gram negative strains was considerably lower. As for the major part of the penems, compounds **10** were inactive against *Pseudomonas aeruginosa*.

Table 1. In vitro antibacterial activity of penem dithiocarbamates



10 a-p

Compound -NR_3R_4		MIC (μg/mL) [a]					
		S.a.	*S.a. MR*	*E.c.*	*E.c. DC2*	*Ps. aer.*	*Ps. aer. ß-*
a	$-N(CH_3)_2$	0,03	0,06	32	0,12	>32	1
b	$-N(CH_3)CH_2CONH_2$	0,12	0,03	8	0,5	>32	4
c	$-N(CH_2CH_2OH)_2$	0,25	0,5	32	4	>32	2
d	$-N(CH_2CH_2OAc)_2$	0,12	0,5	>32	2	>32	>32
e	$-N(CH_3)Ph$	0,03	0,25	>32	1	>32	>32
f	$-N(CH_3)CH_2Ph$	0,06	0,5	>32	1	>32	32
g	$-N(CH_3)OCH_2Ph$	0,03	0,12	>32	2	>32	32
h	$-N(CH_3)Py$ [b]	0,06	0,5	>32	1	>32	>32
i	$-N(CH_3)CH_2CH_2Py$ [b]	0,06	0,12	>32	2	>32	>32
j		0,03	0,12	>32	0,5	>32	>32
k		0,03	0,06	32	0,5	>32	0,5
l		0,12	0,25	8	4	>32	4
m		0,03	0,06	4	0,5	>32	4
n		0,03	0,03	4	2	>32	4
o		0,12	0,12	16	4	>32	16
p		0,25	1	>32	0,5	>32	-
Ampicillin/Sulbactam		0,25	0,12	8	1	32	0,25

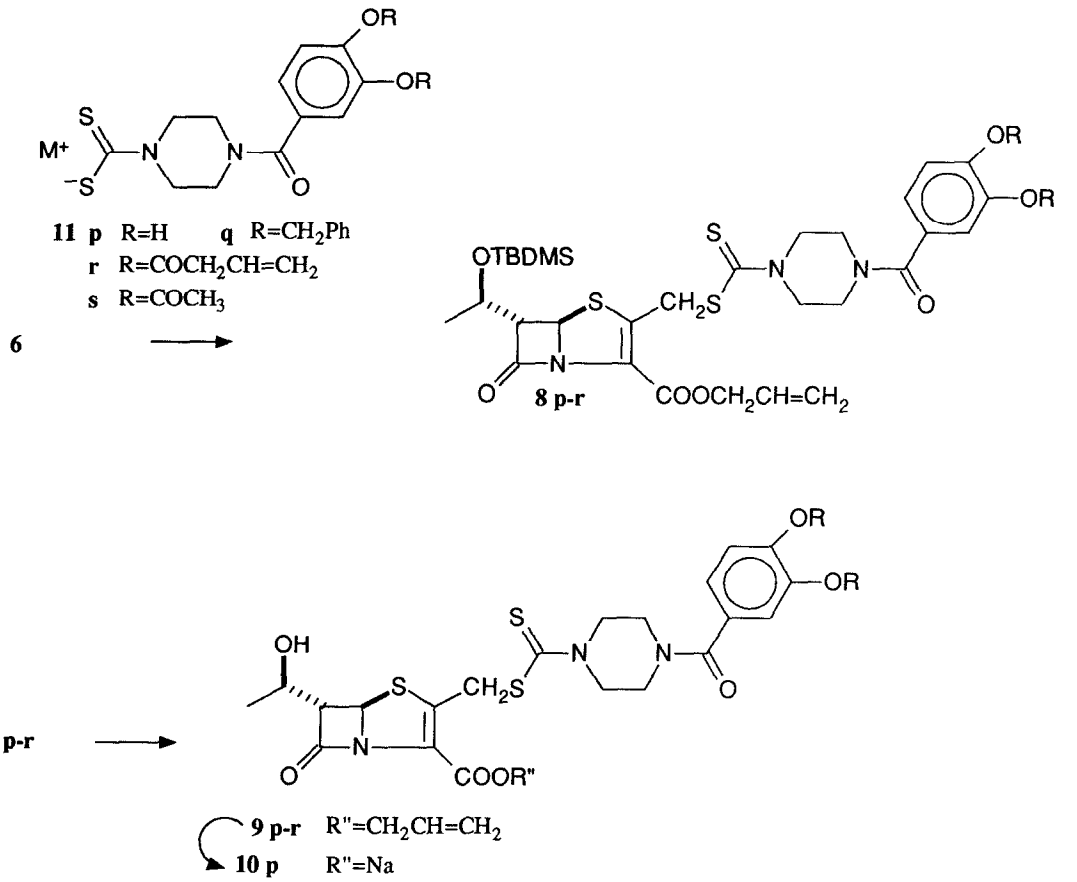
a. Minimum Inhibitory Concentrations (MIC's) were determined by a standard agar dilution method. Abbreviations: *S. a.*: *Staphylococcus aureus* ATCC 25923; *S. a.* MR: heterogeneous methicillin resistant *Staphylococcus aureus* MR 013; *E.c.*: *Escherichia coli* ATCC 25922; *E. c.*DC2 *Escherichia coli*, hyperpermeabile strain; *Ps. aer.*: *Pseudomonas aeruginosa* ATCC 27853; *Ps. aer.* ß-: *Pseudomonas aeruginosa* VR5 (ß-lactamase lacking hyperpermeabile strain).
b. Py = 2-pyridyl-

A problem of permeability through the gram negative outer membrane could be involved in the generally low activity of penem dithiocarbamates against *E. coli* (see for comparison data relative to *E. coli* hyperpermeabile strain), while both permeability barriers and the β-lactamase action could be responsible of the inactivity against *Pseudomonas aeruginosa* (see data for standard and β-lactamase lacking hyperpermeabile *Pseudomonas* strains).

Modification of the side chain in aliphatic compounds **10 a-d** gave a slight improvement only in the case of the N-methyl-N-(2-glycinamido) derivative **10 b**, whereas introduction of a free (entry **10 c**) or masked (entry **10 d**) hydroxy group in the dithiocarbamate side chain did not improve the gram negative activity while slightly lowering the gram positive one. Also N-alkyl-N-aryl compounds **10 e-i** suffered from the lack of activity against some important gram negative strains. Best results were obtained in the heterocyclic series and especially with the polar piperazino derivatives **10 l-n**, bearing a second nitrogen atom on the heterocyclic ring.

To potentially improve the gram negative activity of our derivatives, we synthesized the catechol compound **10 p**. Many monobactams, penicillins and cephalosporins and a series of carbapenems [13] bearing a catechol moiety have been described recently, considering the potential ability of catechol-linked molecules to get access to bacteria using the *ton*B dependent iron uptake system [14]. To our knowledge, however, no synthesis of catechol penem has been reported yet.

Scheme 2



The preparation of **10 p** (Scheme 2) is not trivial, due to the difficulty to find suitable protecting groups for the catechol subunit. In fact, deprotection of catechol dibenzyl ether at the various stages (**8**, **9** or **10 q**) of the synthetic sequence did not give any result, although different catalysts and experimental conditions were exploited. Moreover, contemporary unmasking in **9 r** of the bis allyl carbonate at the catechol moiety and the allyl ester at C-3 ($Pd(PPh_3)_4$, PPh_3, AcOH, dry CH_2Cl_2 or THF) gave a complex mixture in which **10 p** was undetectable. On the other hand, the use of acetate as protecting group for the phenolic hydroxyl was discouraging due to the lability of catechol diacetate in the course of the synthetic sequence.

Finally, the synthesis of **10 p** was achieved in the simplest mode, by reacting the unprotected dithiocarbamate **11 p** with the mesylate **6** and following the usual sequence without the use of any catechol protecting group. Unfortunately, the sodium salt **10 p** did not give the expected improvements in terms of anti-pseudomonal activity.

In conclusion the synthesis of penem dithiocarbamates **10** could be achieved in high yields and with a favourable ratio between the penem and penam isomers. Although the antibacterial activity is still unsatisfactory, further research on modifications of the piperazino side chain of **10 l-n** might lead to an improvement of the activity against gram negative strains and also to a better understanding of the influence of the C-2 side chain on the biological activity of 2-substituted penem derivatives.

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9. Altamura, M.; Arcamone, F.; Giannotti, D.; Pestellini, V.; Sbraci, P. *PCT Int. Appl. WO 91 17995* (*Chem. Abstr.* **1992**, *116*, 173882). A typical procedure, referred to the 1-pyrrolidine derivative **8 j**, is as follows: **5** (2 g, 5 mmol) was dissolved in anhydrous THF (50 mL). Triethylamine (0.84 mL, 6 mmol) and methanesulfonyl chloride (0.46 mL, 6 mmol) were added in sequence, at 0/+5°C, under nitrogen atmosphere and the resulting mixture stirred at the same temperature for 30 min. After filtration, the solution was evaporated under vacuum, and the oily residue taken up in ethyl ether and washed at 10°C with water and 5% $NaHCO_3$. The organic layer was dried over Na_2SO_4 and evaporated to give a yellow solid. The crude **6** was dissolved in DMSO (50 mL). Solid ammonium 1-pyrrolidine dithiocarbamate (0.98 g, 6 mmol) was added. After stirring at room temperature for 1 h, the solution was poured into iced water. **8 j** precipitated as a pale yellow solid and was filtered and washed on the filter with water. Yield: 2.33 g (88%). The crude product could be crystallized from cold petroleum ether. Yield: 2 g (76%). mp 119-120°C. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 0.05 (6H, s), 0.85 (9H, s), 1.18 (3H, d, J = 6.3 Hz), 1.90-2.18 (4H, m), 3.60-3.70 (2H, m), 3.67 (1H, dd, J = 1.7, 4.4 Hz), 3.85-3.99 (2H, m), 4.14-4.27 (1H, m), 4.61-4.79 (2H, m), 4.84 and 4.99 (2H, AB_q, J = 14.5 Hz), 5.18-5.48 (2H, m), 5.51(1H, d, J = 1.7 Hz), 5.82-6.03 (1H, m). UV (CH_3CN): 248, 262, 329 (nm).

10. **9 j** : ^{1}H NMR (200 MHz, $CDCl_3$) δ: 1.25$^{\#}$ (3H, d, J = 6.3 Hz), 1.30* (3H, d, J= 6.3 Hz) , 1.86 -2.15 (4H, m), 2.7-3.0 (1H, bs), 3.38* (1H, dd, J = 1.4, 7.1 Hz) , 3.56-3.70 (2H, m), 3.69$^{\#}$ (1H, dd, J = 1.5, 6.3 Hz), 3.81-3.96 (2H, m), 4.08-4.30 (1H, m), 4.59-4.82 (2H, m), 4.78$^{\#}$ and 4.91$^{\#}$ (2H, AB_q, J = 15 Hz), 5.18-5.28 (2H, m), 5.41* (1H, d, J=1.5 Hz) , 5.48* (1H, d, J = 1.4 Hz) , 5.51$^{\#}$ (1H, d, J = 1.5 Hz), 5.80-6.02 (1H, m), 7.19* (1H, d, J = 1.5 Hz) [# = penem, * = penam isomer]. Measures of Nuclear Overhauser Effect (NOE) between H-2', H-3 and H-5 could be used to determine the geometry of the exo double bond in position 2 and the configuration at C-3 in the penam isomer. Observed NOE (DMSO d_6, 200 MHz, delay time: 6 s) between H-3 and H-5 allowed to presume a Z double bond geometry. Very low NOE was observed between H-3 and H-5, while no NOE was detected between H-3 and H-6. The last result should suggest a α orientation for H-3.

11. Jeffrey, P.D.; McCombie, S.W. *J. Org. Chem.* **1982**, *47*, 587. At the given reaction time, no further isomerization was detected (HLPC and NMR data).

12. Separation was performed by reverse phase column chromatography (LichroPrep® RP-18, 40-63 μm, water/acetone 95:5). Penem isomer **10 j**: ^{1}H NMR (200 MHz, D_2O) δ: 1.24 (3H, d, J = 6.5 Hz), 1.82-2.14 (4H, m), 3.55-3.72 (2H, m), 3.72-3.88 (2H +1H, m), 4.08-4.28 (1H, m), 4,74 and 4,89 (2H, AB_q, J = 15 Hz), 5.51 (1H, d, J = 1.3 Hz); UV (H_2O): 257, 312 (nm). Penam isomer **4 j** (R = CS-N$(CH_2)_4$, R' = H, R" = Na): ^{1}H NMR (200 MHz, D_2O) δ: 1.26 (3H, d, J = 6.3 Hz), 1.82-2.12 (4H, m), 3.50 (1H, dd, J = 1.4, 6.3 Hz), 3.60-3.73 (2H, m), 3.73-3.87 (2H, m), 4.30-4.65 (1H, m), 5.34 (1H, d, J = 1.3 Hz), 5.38 (1H, d, J = 1.4 Hz), 6.81 (1H, d, J = 1.3 Hz); UV (H_2O): 275 (nm).

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