IMIDAZOLIDINONES STRUCTURALLY-RELATED TO PENICILLINS: SYNTHESIS, MOLECULAR MODELING AND BIOLOGICAL EVALUATION

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SUMMARY

Several bicyclic imidazolidinones structurally-related to penicillins have been prepared from penam precursors. The compounds were compared to benzylpenicillin as active reference, using the methods of theoretical chemistry. Structures 7 and 8, in which the acyl group of the side-chain was anchored, via a "one-atom spacer", at position N-7 of the imidazolidinone ring, appeared as good penicillin mimics. However, they were found devoid of significant antibacterial activity.

KEY WORDS

bicyclic imidazolidinone, penicillin mimic, beta-lactam antibiotic, molecular modeling

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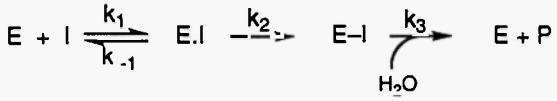
INTRODUCTION

The remarkable antibacterial effect of penicillins **1** results from their capacity to disrupt the biosynthesis of the bacterial cell wall by inhibiting D,D-transpeptidases involved in the peptidoglycan cross-linking /1/. Penicillin binding proteins (PBPs) are generally membrane-bound serine peptidases that recognise D-alanyl-D-alanine peptide termini. The affinity of penicillins **1** for these enzymes arises from their structural and conformational similarity to the peptide substrate. Their interaction with PBPs leads to the cleavage of the beta-lactam ring and the formation of a covalent acyl-enzyme intermediate **2** (Scheme 1) in which the carbonyl group of the antibiotic is connected to the oxygen atom of the active serine residue of the target protein. Good inhibitors are compounds forming relatively stable acyl-enzyme intermediates, weakly sensitive towards the hydrolysis which regenerates the active enzyme. This implies that in the kinetic equation (Scheme 1), the k_2 step is much more rapid than the k_3 step.

It has been widely assumed that the biological activity of penicillins **1** is due to the inherent strain of the beta-lactam ring or to the reduced amide resonance which should increase the electrophilic character of the carbonyl function ("acylating power") and, therefore, the susceptibility towards serine attack /2/. However, according to the recent kinetic experiments of Page /3/, strained lactams possessing a pyramidal geometry at the nitrogen atom are not absolutely necessary for either high chemical reactivity or antibiotic activity. Thus, less strained topological analogs of the beta-lactam drugs could be considered as potential new synthetic antibiotics /4/.

We became interested in the design and synthesis of original acylating molecules with the potential of blocking the PBPs irreversibly (k_3=0). We examined the bicyclic imidazolidinones **5** to **9** (Scheme 2) as penicillin mimics. Nucleophilic attack of the active serine residue could, in principle, lead to two different acyl-enzyme intermediates **3** and **4**, but in both cases a carbamate linkage would be formed, connecting the potential inhibitor and the target enzyme. The carbamate function is expected to be much more resistant towards hydrolysis than a simple ester.

The first problem arising from such a structural modification of the beta-lactam ring is the expected lower sensitivity of the carbonyl group to nucleophilic attack. The second problem is the efficiency of the molecular recognition ("goodness-of-fit") between the penicillin



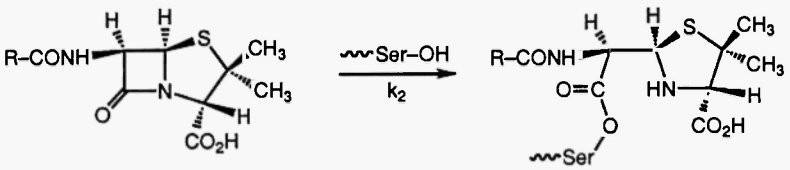
E : enzyme (D,D-peptidase)

I : inhibitor (penicillin 1)

E.I : Michaelis complex

E–I : acyl-enzyme intermediate

P : product (penicilloïc acid)



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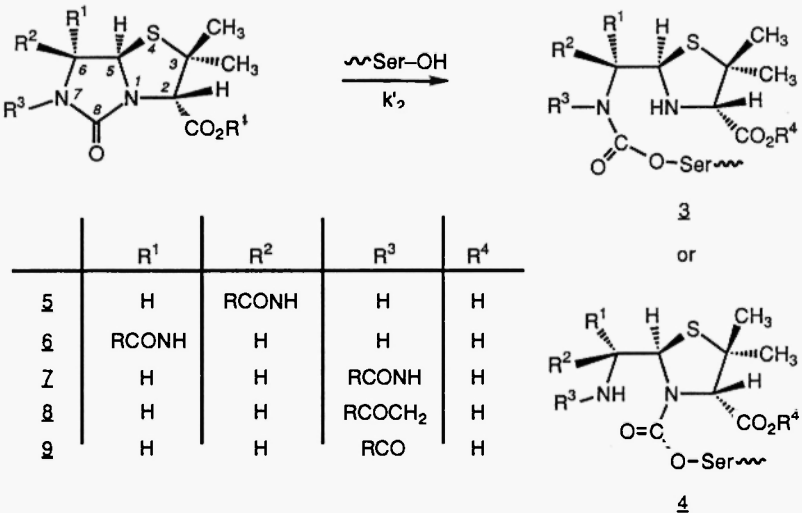
Scheme 1: Reaction of penicillins with PBPs.



	R^1	R^2	R^3	R^4
5	H	RCONH	H	H
6	RCONH	H	H	H
7	H	H	RCONH	H
8	H	H	$RCOCH_2$	H
9	H	H	RCO	H

Scheme 2: Bicyclic imidazolidinones as penicillin mimics.

mimic and the host-protein, which is essential for biological activity. Therefore, we considered various possibilities for anchoring the side-chain on the imidazolidinone ring (Scheme 2). The structures **5** and **9** were previously synthesized and found to be devoid of significant antibiotic activity /5,6/. We report now on the evaluation of compound **6**, bearing the acylamino side-chain at position C-6 with a non-natural configuration, and compounds **7** and **8**, functionalized at position N-7. In the last two families, the carbonyl group of the side-chain is further out from the imidazolidinone ring compared to structure **9** (NH and CH_2 spacers).

The imidazolidinones **5** to **9** have been compared to the benzylpenicillin **1** (R=$PhCH_2$) as active reference, using the methods of theoretical chemistry. The spatial disposition of the functional groups (side-chain and carboxyl function) on both sides of the potentially scissile bond N-1/C-8 has been explored.

The new synthesized products **6** to **8** were tested *in vitro* against representative bacterial strains.

MATERIALS AND METHODS

Chemicals

Reagents (analytical grade) and solvents were supplied by Merck and Aldrich. Dichloromethane and ethyl acetate were dried over phosphorous pentoxide at reflux, then distilled. Methanol was refluxed over calcium oxide and distilled. Diethyl ether and tetrahydrofurane were dried over lithium aluminium hydride and distilled under argon atmosphere. Triethylamine was stored over potassium hydroxide pellets. The rotations (±0.3°C) were determined on a Perkin-Elmer 241MC polarimeter, in chloroform (spectroscopic grade) as solvent, at 20°C. The IR spectra were taken with a Perkin-Elmer 297 instrument, in dichloromethane as solvent (unless otherwise mentioned) and calibrated with polystyrene. The ^1H NMR spectra were recorded on Varian XL-200 or Varian Gemini 200 spectrometers, in chloroform-d as solvent (unless otherwise mentioned), with tetramethylsilane as internal standard. The mass spectra were obtained on a Varian MAT 44 instrument in the electronic impact mode (EI, 70 eV) or on a Finnigan MAT TSQ 70 instrument in fast atom bombardment mode (FAB). The microanalyses were performed at the University of Vienna

(Austria) and at Continental Pharma (Belgium). The results indicated by the symbols of the elements were within ±0.3% of the theoretical values. The synthesized products were purified by column chromatography with Merck silica gel 60 (70-230 mesh ASTM). The R_F values were determined on Merck silica gel 60 TLC plates (F 254, 0.2 mm).

Syntheses of 6-epi-penicillins

6-Epi-penicillins were prepared according to the procedure of Vanderhaeghe /7/ and esterified under standard conditions.

Pivaloyloxymethyl 6-epi-benzylpenicillinate 10a: IR 3420, 1780, 1765, 1685, 1505, 1116, 990 cm^{-1}; ^1H NMR (D_2O exchange) δ 1.20 (s, 9H, tBu), 1.42 (s, 3H, Me), 1.55 (s, 3H, Me), 3.57 (s, 2H, $PhCH_2$), 4.43 (s, 1H, H-3), 5.00 (br s, 1H, J<1 Hz, H-6), 5.08 (br s, 1H, J<1 Hz, H-5), 5.75 (sharp AB q, 2H, $COO\text{-}CH_2$), 7.27 (s, 5H, Ph).

p-Nitrobenzyl 6-epi-benzylpenicillinate 11a: IR 3420, 1779, 1750, 1684, 1609, 1525, 1510, 1351 cm^{-1}; ^1H NMR (D_2O exchange) δ 1.38 (s, 3H, Me), 1.56 (s, 3H, Me), 3.58 (s, 2H, $PhCH_2$), 4.5 (s, 1H, H-3), 4.91 (br s, 1H, J<1 Hz, H-6), 5.15 (br s, 1H, J<1 Hz, H-5), 5.20 (s, 2H, $COOCH_2$), 7.25 (s, 5H, Ph), 7.47 and 8.16 (two d, 4H, J=8 Hz, $PhNO_2$).

Pivaloyloxymethyl 6-epi-phenoxymethylpenicillinate 10b: IR 3410, 1781, 1765, 1692, 1600, 1590, 1520, 1493, 1232, 1112, 987 cm^{-1}; ^1H NMR (D_2O exchange) δ 1.20 (s, 9H, tBu), 1.48 (s, 3H, Me), 1.60 (s, 3H, Me), 4.50 (br s, 3H, $PhOCH_2$ + H-3), 5.21 (br s, 2H, H-5 + H-6), 5.78 (sharp AB q, 2H, $COOCH_2O$), 6.76-7.50 (m, 5H, PhO).

Trichloroethyl 6-epi-phenoxymethylpenicillinate 12b: IR 3410, 1785, 1765, 1592, 1600, 1590, 1518, 1492, 1231, 1205, 1180, 1160 cm^{-1}; ^1H NMR (D_2O exchange) δ 1.55 (s, 3H, Me), 1.63 (s, 3H, Me), 4.50 (s, 2H, $PhOCH_2$), 4.60 (s, 1H, H-3), 4.73 (s, 2H, $COOCH_2$), 5.20 (br s, 1H, J<1 Hz, H-6), 5.27 (br s, 1H, J<1 Hz, H-5), 6.76-7.35 (m, 5H, PhO).

Syntheses of 6-epi-acylamino-imidazolidinones

The imidazolidinones were prepared in two steps, according to the procedure previously described /5,6/, from **10**-**12**.

PIV ester of 6-epi-phenylacetamido-imidazolidinone 13a (44%): IR 3450 (NH), 3420 (NH), 1745 (CO esters + CO urea), 1680 (CO

amide), 1495, 1410, 1240, 1110, 990 cm^{-1}; ^1H NMR δ 1.21 (s, 9H, tBu), 1.47 (s, 3H, Me), 1.54 (s, 3H, Me), 3.57 (s, 2H, $PhCH_2$), 4.65 (s, 1H, H-2), 5.22 (br s, 1H, J~0.2 Hz, H-5), 5.62 (br d, 1H, J=7.6 Hz, H-6), 5.72 and 5.87 (two AB d, 2H, J=5.6 Hz, $COOCH_2O$), 5.84 (br s, 1H, NH-7), 6.62 (br d, 1H, J=7.6 Hz, NH amide), 7.20-7.40 (m, 5H, Ph); Anal. $C_{22}H_{29}O_6N_3S$ (C, H, N).

PNB ester of 6-epi-phenylacetamido-imidazolidinone 14a (54%): IR 3450, 3420, 1740 (br), 1680, 1610, 1528, 1495, 1400, 1350, 1240, 1215, 1181, 1110, 910, 853 cm^{-1}; ^1H NMR δ 1.39 (s, 3H, Me), 1.53 (s, 3H, Me), 3.53 (s, 2H, $PhCH_2$), 4.71 (s, 1H, H-2), 5.23 (br s, 1H, J~ 0.2 Hz, H-5), 5.24 (sharp AB q, 2H, J=9 Hz, $COOCH_2$), 5.61 (br d, 1H, J=8 Hz, H-6), 6.23 (s, 1H, NH-7), 6.93 (br d, 1H, J=8 Hz, NH amide), 7.20-7.40 (m, 5H, Ph), 7.51 and 8.21 (two d, 4H, J=8.8 Hz, $PhNO_2$); Anal. $C_{23}H_{24}O_6N_4S$ (C, H, N).

6-Epi-phenylacetamido-imidazolidinone 6a (60%): the PNB ester of **14a** was cleaved by catalytic hydrogenation over Pd-C as catalyst (ethylacetate, 20°C, 40 psi). IR(KBr) 3600-2800 (br), 1725 (br), 1760, 1535, 1420, 1243 cm^{-1}; ^1H NMR (acetone-d6) δ 1.53 (s, 6H, Me X 2), 3.56 (s, 2H, $PhCH_2$), 4.58 (s, 1H, H-2), 5.31 (s, 1H, H-5), 5.54 (br d, 1H, J=7.8 Hz, H-6), 6.64 (m, 1H, NH-7), 7.20-7.40 (m, 5H, Ph), 8.26 (m, NH amide); Mass (FAB) m/e 349 (M, 20%), 348 (M-1, 100%), 316 (7%), 305 (M-CO_2, 21%), 304 (29%), 270 (6%), 261 (21%), 227 (16%), 214 (M-$PhCH_2CONH_2$, 23%), 213 (82%), 207 (37%), 183 (24%), 169 (M-$PhCH_2CONH_2$-CO_2, 51%), 136 (18%), 113 (37%).

PIV ester of 6-epi-phenoxyacetamido-imidazolidinone 13b (55%): IR 3445, 3410, 1745 (br), 1690, 1600, 1593, 1513, 1495, 1400, 1238, 1110, 990 cm^{-1}; ^1H NMR δ 1.22 (s, 9H, tBu), 1.50 (s, 3H, Me), 1.57 (s, 3H, Me), 4.51 (s, 2H, $PhOCH_2$), 4.71 (s, 1H, H-2), 5.38 (br s, 1H, J<0.2 Hz, H-5), 5.76 (AB d, 1H, J=5.6 Hz, COOCHO), 5.78 (br d, 1H, J=8.3 Hz, H-6), 5.86 (br s, 1H, H-7), 5.90 (AB d, 1H, J=5.6 Hz, COOCHO), 6.92-7.10 (m, 3H, PhO), 7.30-7.40 (m, 2H, PhO), 7.42 (br d, 1H, J=8.3 Hz, NH amide); Anal. $C_{22}H_{29}O_7N_3S$ (C, H, N).

TCE ester of 6-epi-phenoxyacetamido-imidazolidinone 15b (48%): IR 3450, 3415, 1760, 1740, 1690, 1600, 1593, 1513, 1496, 1440, 1400, 1240, 1178, 1158 cm^{-1}; ^1H NMR δ 1.57 (s, 3H, Me), 1.63 (s, 3H, Me), 4.51 (s, 2H, $PhOCH_2$), 4.71 and 4.85 (two AB d, 2H, J=11.7 Hz, $COOCH_2$), 4.83 (s, 1H, H-2), 5.40 (br s, 1H, J<0.2 Hz, H-5), 5.79 (br d, 1H, J=8.4 Hz, H-6), 6.07 (br s, 1H, NH-7), 6.90-7.10 and 7.30-

7.40 (two m, 3H + 2H, PhO), 7.42 (br d, 1H, J=8.4 Hz, NH amide); Anal. $C_{18}H_{20}O_5N_3SCl_3$ (C, H, N).

6-Epi-phenoxyacetamido-imidazolidinone 6b (73%): the TCE ester of **15b** was cleaved by treatment with zinc in aqueous formic acid at 0°C. IR (KBr) 3600-2800, 3430, 1740, 1715, 1640, 1600, 1595, 1530, 1496, 1441, 1420, 1200, 1190 cm^{-1}; ^1H NMR (acetone - d6) δ 1.53 (br s, 6H, Me X 2), 4.55 (br s, 3H, $PhOCH_2$ + H-2), 5.37 (br s, 1H, J<0.2 Hz, H-5), 5.66 (br d, 1H, J=8.2 Hz, H-6), 6.92-7.06 and 7.24-7.38 (two m, 3H + 2H, PhO), 7.52 (br s, 1H, NH-7), 8.68 (br d, 1H, J=8.2 Hz, NH amide); Mass (EI) m/e 365 (M, 9%), 364 (M-1, 65%), 332 (7%), 320 (M-CO_2, 16%), 286 (4%), 277 (13%), 243 (11%), 223 (17%), 214 (M-$PhOCH_2CONH_2$, 10%), 213 (53%), 183 (100%), 169 (M-$PhOCH_2CONH_2$-CO_2, 44%), 150 (29%), 127 (23%).

Syntheses of 7-nitroso-imidazolidinones

Imidazolidinone **16-17** in ether solution and $NaNO_2$ (10 equiv.) in water solution were mixed and vigorously stirred at 0°C. An aqueous solution of HNO_3 (10 equiv.) was added dropwise for 30 min at 0°C. After 10 h at 20°C, the organic layer was separated, washed with 5% $NaHCO_3$, dried, concentrated and chromatographed.

PIV ester of 7-nitroso-imidazolidinone 18 (86%): IR 1769 (br) 1483, 1462, 1370, 1185, 1160, 1110, 992, 838 cm^{-1}; ^1H NMR δ 1.24 (s, 9H, tBu), 1.57 (s, 3H, Me), 1.66 (s, 3H, Me), 3.95 (AB d X d, 1H, J=12.5 and 2.8 Hz, H-6 trans), 4.13 (AB d X d, 1H, J=12.5 and 7.5 Hz, H-6 cis), 4.84 (s, 1H, H-2), 5.65 (d X d, 1H, J=7.5 and 2.8 Hz, H-5), 5.82 and 5.95 (two AB d, 2H, J=5.5 Hz, $COOCH_2O$); Anal. $C_{14}H_{21}O_6N_3S$ (C, H, N).

PMB ester of 7-nitroso-imidazolidinone 19 (90%): IR 1770, 1748, 1615, 1518, 1461, 1376, 1355, 1180 (br), 1038, 835 cm^{-1}; ^1H NMR δ 1.44 (s, 3H, Me), 1.61 (s, 3H, Me), 3.83 (s, 3H, OMe), 3.94 (AB d X d, 1H, J=13.6 and 2.9 Hz, H-6 trans), 4.10 (AB d X d, 1H, J=13.6 and 7.5 Hz, H-6 cis), 4.83 (s, 1H, H-2), 5.19 (sharp AB q, 2H, J=11 Hz, $COOCH_2$), 5.66 (d X d, 1H, J=7.5 and 2.9 Hz, H-5), 6.92 and 7.34 (two d, 4H, J=8.7 Hz, aryl); Anal. $C_{16}H_{19}O_5N_3S$ (C, H, N).

Syntheses of 7-acylamino-imidazolidinones

Zinc dust (3 equiv.) was added at 20°C, under vigorous stirring, to a solution of **18-19** in HOAc-H_2O (2:1, v/v). After 10 min, the reaction

mixture was diluted with EtOAc and H_2O, filtered and neutralized by addition of solid $NaHCO_3$ in small portions (pH~8). Extractions with EtOAc, drying on Na_2SO_4 and concentration gave crude amine **20-21**. This material was dissolved in CH_2Cl_2 and treated at 0°C successively with Et_3N (1 equiv.) and acid chloride (1 equiv.). After 2 h at 20°C, the solution was washed (0.01 N HCl, then 5% $NaNCO_3$), dried, evaporated and chromatographed.

PIV ester of 7-phenylacetamido-imidazolidinone 22a (26%): IR 3400, 1746 (br), 1700, 1479, 1405, 1373, 1329, 1160, 1112, 984 cm^{-1}; ^1H NMR δ 1.23 (s, 9H, tBu), 1.50 (s, 3H, Me), 1.59 (s, 3H, Me), 3.65 (s, 2H, $PhCH_2$), 3.66 (AB d X d, 1H, J=9 and 2.4 Hz, H-6 trans), 4.08 (AB d X d, 1H, J=9 and 7.5 Hz, H-6 cis), 4.70 (s, 1H, H-2), 5.66 (d X d, 1H, J=7.5 and 2.4 Hz, H-5), 5.79 and 5.89 (two AB d, 2H, J=5.5 Hz, $COOCH_2O$), 7.35 (sharp m, 5H, Ph), 7.46 (s, 1H, NH); Anal. $C_{22}H_{29}O_6N_3S$ (C, H, N).

PMB ester of 7-phenylacetamido-imidazolidinone 23a (30%): IR 3400, 1740 (br), 1700, 1612, 1515, 1471, 1407, 1323, 1240, 1212, 1173, 1030, 960, 823 cm^{-1}; ^1H NMR δ 1.36 (s, 3H, Me), 1.50 (s, 3H, Me), 3.59 (AB d X d, 1H, J=9.1 and 2.4 Hz, H-6 trans), 3.59 (s, 2H, $PhCH_2$), 3.79 (s, 3H, OMe), 4.02 (AB d X d, 1H, J=9.1 and 7.5 Hz, H-6 cis), 4.64 (s, 1H, H-2), 5.09 (s, 2H, $COOCH_2$), 5.63 (d X d, 1H, J=7.5 and 2.4 Hz, H-5), 6.87 (d, 2H, J=8.6 Hz, aryl), 7.22-7.36 (sharp m + d, 7H, aryl); Anal. $C_{24}H_{27}O_5N_3S$ (C, H, N).

7-Phenylacetamido-imidazolidinone 7a (95%): the PMB ester of **23a** was cleaved by heating for 1 h at 70°C in HCOOH-H_2O (9:1, v/v). IR 3400, 3300-2800, 1737 (br), 1690, 1610, 1500 (br), 1470, 1410, 1350, 1240, 1179, 1116, 1030 cm^{-1}; ^1H NMR δ 1.52 (s, 3H, Me), 1.56 (s, 3H, Me), 3.58 (s, 2H, $PhCH_2$), 3.62 (AB d X d, 1H, J=8 and 1.5 Hz, H-6 trans), 4.03 (AB d X d, 1H, J=8 and 6.5 Hz, H-6 cis), 4.57 (s, 1H, H-2), 5.56 (d X d, 1H, J=6.5 and 1.5 Hz, H-5), 7.29 (s, 5H, Ph), 8.28 (br s, 1H, NH), 8.30 (m, 1H, COOH); Mass (EI) m/e 349 (M, 2%), 275 (5%), 272 (2%), 257 (2%), 231 (M-$PhCH_2CO$, 10%), 215 (M-$PhCH_2CONH$, 5%) 154 (15%), 139 (20%), 121 (15%), 91 ($PhCH_2$, 100%), 85 (37%), 71 (75%).

PIV ester of 7-o,o'-(dimethoxy)benzamido-imidazolidinone 22c (27%): IR 3405, 1750 (br), 1698, 1599, 1474, 1460, 1240, 1115, 980 cm^{-1}; ^1H NMR δ 1.20 (s, 9H, tBu), 1.48 (s, 3H, Me), 1.60 (s, 3H, Me), 3.79 (AB d X d, 1H, H-6 trans), 3.80 (s, 6H, OMe X 2), 4.25 (AB d X d, 1H, J=9 and 7.5 Hz, H-6 cis), 4.72 (s, 1H, H-2), 5.67 (d X

d, 1H J=7.5 and 2.2 Hz, H-5), 5.75 and 5.84 (two AB d, 2H, J=5.5 Hz, $COOCH_2O$), 6.52 (d, 2H, J=8.4 Hz, aryl), 7.28 (t, 1H, aryl), 7.63 (s, 1H, NH); Anal. $C_{23}H_{31}O_8N_3S$ (C, H, N).

PIV ester of 7-(2-(2-tritylamino-4-thiazolyl)-2-(methoxy)-imino)-acetamido)-imidazolidinone 22d (27%): 2-(2-tritylamino-4-thiazolyl)-2-(methoxy)-iminoacetyl chloride was prepared from the corresponding acid /8/ treated with tetramethyl-α-chloroenamine /9/ in THF at 10°C. IR 3395, 3260, 1765-1730, 1690, 1530, 1487, 1445, 1408, 1371, 1260, 1215, 1155, 1105, 1035, 978 cm^{-1}; ^1H NMR δ 1.21 (s, 9H, tBu), 1.48 (s, 3H, Me), 1.57 (s, 3H, Me), 3.81 (AB d X d, 1H, J=8.9 and 2.9 Hz, H-6 trans), 4.04 (s, 3H, OMe), 4.15 (AB d X d, 1H, J=8.9 and 7.5 Hz, H-6 cis), 4.60 (s, 1H, H-2), 5.65 (d X d, 1H, J=7.5 and 2.9 Hz, H-5), 5.77 and 5.87 (two AB d, 2H, J=5.5 Hz, $COOCH_2O$), 6.96 (s, 1H, thiazol), 7.09 (s, 1H, NH-Tr), 7.28 (br s, Ph_3C), 8.25 (s, 1H, NH amide).

7-(2-(2-Amino-4-thiazolyl)-2-(methoxy)-imino)acetamido-imidazolidinone 22e (92%): the trityl group of **22d** was cleaved by heating at 70°C for 45 min in $HCOOH-H_2O$ (9:1, v/v). IR 3480, 3390, 3305, 3210, 1765-1720, 1690, 1620, 1605, 1530, 1480, 1371, 1107, 1030, 977 cm^{-1}; ^1H NMR δ 1.22 (s, 9H, tBu), 1.51 (s, 3H, Me), 1.62 (s, 3H, Me), 3.86 (AB d X d, 1H, J=9.1 and 2.3 Hz, H-6 trans), 4.0 (s, 3H, OMe), 4.29 (AB d X d, 1H, J=9.1 and 7.4 Hz, H-6 cis), 4.66 (s, 1H, H-2), 5.71 (d X d, 1H, J=7.4 and 2.3 Hz, H-5), 5.79 and 5.90 (two AB d, 2H, J=5.5 Hz, $COOCH_2O$), 5.85 (m, 2H, NH_2), 6.97 (s, 1H, thiazol), 7.29 (s, 1H, NH amide); Mass (DCI $CH_4/N_2O(+)$) m/e 529 (M+1, 22%), 513 (M-NH_3, 5%), 485 (M-CO_2, 4%), 394 (14%), 374 (7%), 344 (21%), 329 (M-side chain, 12%), 301 (34%), 285 (63%), 269 (13%), 258 (65%), 257 (100%), 256 (52%), 243 (24%), 229 (85%), 225 (45%), 214 (17%), 197 (22%), 183 (28%), 126 (39%), 103 (71%), 100 (33%).

Syntheses of 7-(benzoyl)-methyl-imidazolidinones

Powered K_2CO_3 (4 equiv.) was added to a mixture of **16** (**24**) and phenacyl bromide (4 equiv.) in acetone solution. The mixture was stirred for 96 h at 20°C, then worked up as usual.

PIV ester of 7-(benzoyl)-methyl-imidazolidinone 25 (39%): IR 1755, 1720, 1699, 1600, 1480, 1440, 1228, 1110, 990 cm^{-1}; ^1H NMR δ 1.22 (s, 9H, tBu), 1.52 (s, 3H, Me), 1.59 (s, 3H, Me), 3.64 (AB d X

d, 1H, J=10 and 1.6 Hz, H-6 trans), 4.01 (AB d X d, 1H, J=10 and 7.4 Hz, H-6 cis), 4.69 and 4.78 (two AB d, 2H, J=18 Hz, $PhCOCH_2$), 4.77 (s, 1H, H-2), 5.68 (d X d, 1H, J=7.4 and 1.6 Hz, H-5), 5.79 and 5.89 (two AB d, 2H, J=5.6 Hz, $COOCH_2O$), 7.42-7.66 and 7.90-8.04 (m, 3H + 2H, Ph); Anal. $C_{22}H_{28}O_6N_2S$ (C, H, N).

TCE ester of 7-(benzoyl)-methyl-imidazolidinone 26 (61%): IR 1760, 1720, 1698, 1600, 1480, 1442, 1228, 1179, 1154 cm^{-1}; ^1H NMR δ 1.59 (s, 3H, Me), 1.65 (s, 3H, Me), 3.67 (AB d X d, 1H, J=9.8 and 1.7 Hz, H-6 trans), 4.03 (AB d X d, 1H, J=9.8 and 7.4 Hz, H-6 cis), 4.70 and 4.80 (two AB d, 2H, J=17.6 Hz, Ph CH_2CO), 4.76 and 4.84 (two AB d, 2H, J=12 Hz, $COO\text{-}CH_2CCl_3$), 4.89 (s, 1H, H-2), 5.71 (d X d, 1H, J=7.4 and 1.7 Hz, H-5), 7.40-7.66 and 7.90-8.02 (m, 3H +2H, Ph); Anal. $C_{18}H_{19}O_4N_2SCl_3$ (C, H, N).

7-(Benzoyl)-methyl-imidazolidinone 8 (88%): the TCE ester of **26** was cleaved by treatment with zinc at 0°C in $HCOOH\text{-}H_2O$ (9:1, v/v). IR 3300-2800, 1745, 1717, 1695, 1600, 1480, 1446, 1224 cm^{-1}; ^1H NMR δ 1.58 (s, 3H, Me), 1.62 (s, 3H, Me), 3.64 (AB d X d, 1H, J=9.7 and 1 Hz, H-6 trans), 4.0 (AB d X d, 1H, J=9.7 and 7.5 Hz, H-6 cis), 4.66 (s, 1H, H-2), 4.69 and 4.79 (two AB d, 2H, J=18.2 Hz, $PhCOCH_2$), 5.60 (d X d, 1H, J=7.5 and 1 Hz, H-5), 7.40-7.65 and 7.87-8.03 (m, 3H + 2H, Ph); Mass (EI) m/e 334 (M, 5%), 289 (M-CO_2, 2%), 260 (18%), 257 (M-Ph, 7%), 229 (M-PhCO, 42%), 203 (3%), 149 (8%), 128 (7%), 105 (PhCO, 100%), 88 (68%), 77 (Ph, 85%).

Computational methods

The geometry of the imidazolidinones was fully optimized within the MNDO framework /10/ using the link 402 of the GAUSSIAN 86 program /11/. Conformational analyses were performed using the molecular mechanics MM2 program /12/. Geometrical adjustment and dynamic fit were included in the graphic ULYSSE package from the laboratory of Liège. It was run on a DATA GENERAL MV 7800 computer with an attached graphic processor GDC 2400.

Biological methods

The MIC of the imidazolidinones (PIV esters and free acids) were measured by microdilution tests in 96-microwell plates (Nunc, Rotkilde, Denmark) as described by Thrupp /13/, using a final

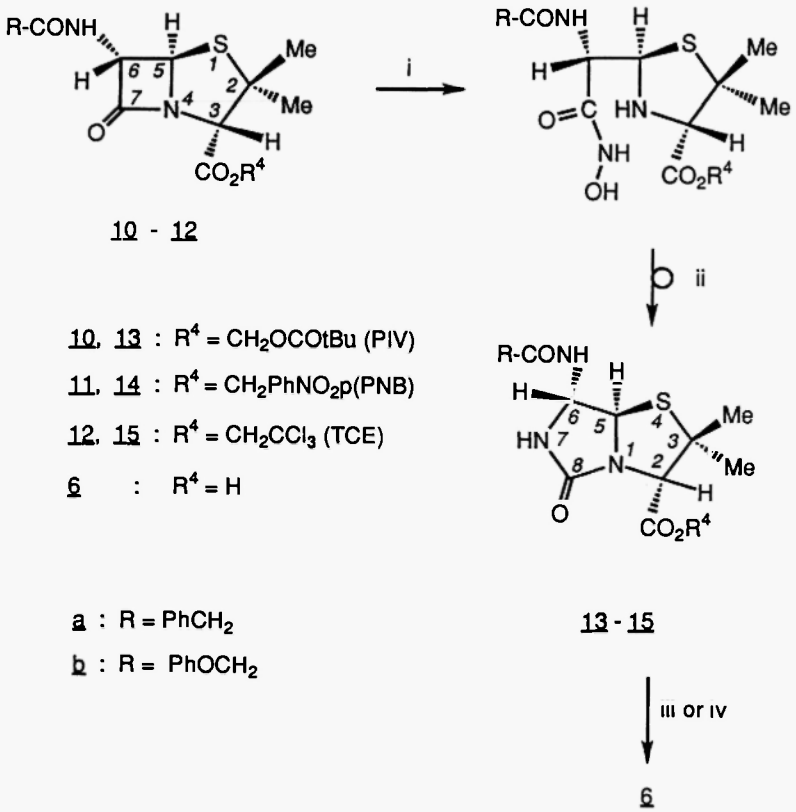
inoculum of 10^5 CFU/ml and Mueller-Hinton broth (BBL, Microbiological Systems, Cockeyville, PA, U.S.A.). To allow the growth of *Streptococcus*, 5% horse blood (Gibco, Ghent, Belgium) was added to the culture medium. The range of concentrations obtained by 2-fold dilutions were from 200 to 0.006 μM. For water insoluble products, dimethylsulfoxide was used as solvent, but its concentration never exceeded 2%. Ampicillin and benzylpenicillin PIV esters (when PIV ester imidazolidinones were tested) were included in each experiment as active reference antibiotics. The bacterial strains were obtained from Institut Pasteur (Paris, France): *Escherichia coli* ATCC 25422, *Klebsiella pneumoniae* ATCC 10.031, *Pseudomonas aeruginosa* ATCC 27853, *Proteus mirabilis* NTC 8309, *Serratia marcescens* ATCC 4003, *Salmonella typhimurium* LT2 60.62, *Staphylococcus aureus* ATCC 25923 and *Streptococcus pyogenes* ATCC 8668. *Proteus vulgaris*, a sensitive laboratory strain, was provided by Prof. J. M. Ghuysen, (University of Liège, Belgium). Hydrolysis of the PIV esters (20 mM) was performed by incubation for 120 min at 37°C in the presence of human plasma. Then the solutions were diluted adequately and tested as above /13/.

RESULTS AND DISCUSSION

Synthetic chemistry

A general strategy for the preparation of bicyclic imidazolidinones **5** from penicillin precursors has been previously reported /5/; it involved the opening of the beta-lactam ring by hydroxylamine, the Lossen rearrangement of the resulting hydroxamic acid and the intramolecular trapping of the isocyanate intermediate. All the sequence was performed without racemization /5/. The 6-epi-imidazolidinones **6** were similarly prepared (Scheme 3) from 6-epi-penicillinate precursors, obtained by base-catalysed epimerisation of N-trimethylsilyl benzyl- or phenoxy-methylpenicillins /7/.

Thus, beta-lactams **10a**, **11a**, **10b** and **12b** were smoothly cleaved by addition of hydroxylamine in methanol solution to yield the corresponding hydroxamic acids. Their rapid rearrangement under treatment with N,N-diethylaminopropyne at low temperature and subsequent intramolecular cyclization gave respectively the imidazolidinones **13a**, **14a**, **13b** and **15b** in moderate overall yields



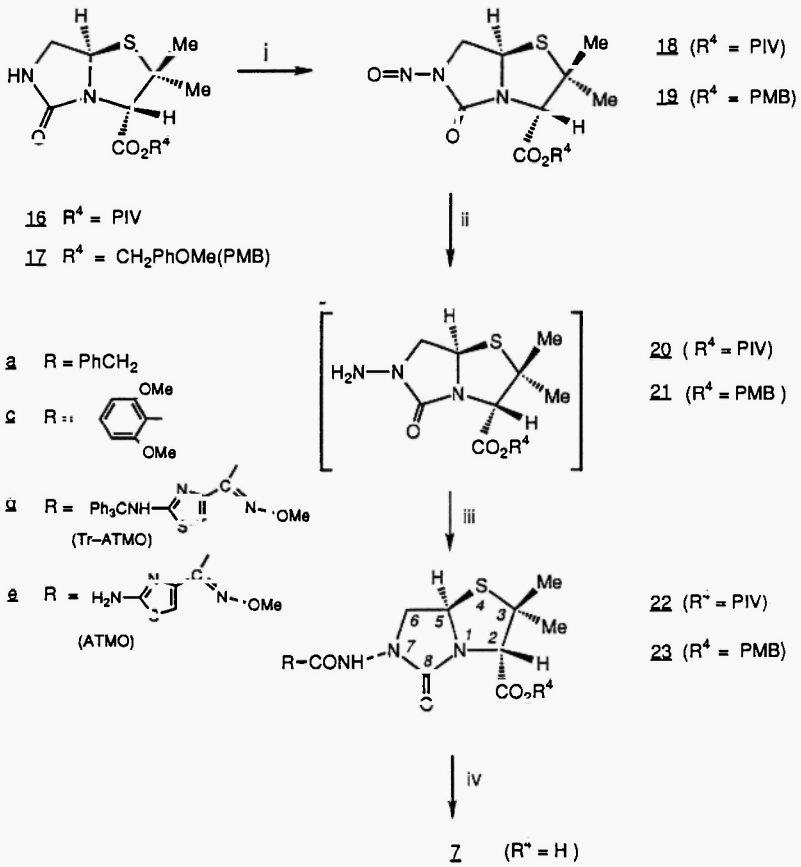
(i) H_2N–OH, CH_3OH, - 40° to 20°C ; (ii) Me-C≡C-NEt_2 , CH_2Cl_2, -60° to 20°C ; column-chromatography on SiO_2 ; (iii) R^2 = PNB : H_2, Pd-C, EtOAc, 20°C ; (iv) R^2 = TCE : HCO_2H : H_2O 9 : 1, Zn, 0°C, 2 h.

Scheme 3: Synthesis of 6-epi-acylamino-imidazolidinones.

(44% to 55%). The *trans* relationship between the protons H-5 and H-6 was confirmed by their very small coupling constant value (J=0-0.2 Hz) in the ^1H NMR spectra. Conventional deprotections of the p-nitrobenzyl (PNB) and trichloroethyl (TCE) esters afforded the free acids **6a** and **6b**.

The same strategy allowed the preparation of the 7-unsubstituted imidazolidinones **16**-**17** from the corresponding penicillanic esters /6/.

They were readily converted into the N-nitroso derivatives **18-19** (Scheme 4) by reaction with sodium nitrite in a two-phase system (ether - aqueous HNO_3). The subsequent reduction to give the 7-amino intermediates **20-21** was somewhat troublesome. Over-reduction into starting imidazolidinones, resulting from N-N cleavage, occurred very rapidly. However, using small quantities of zinc and very



(1) NaNO2 (10eq), ether, HNO_3–H_2O, 0° to 20°C ; (ii) Zn, HOAc : H_2O 2 : 1, 35°C, 5 - 10 min then $NaHCO_3$, pH ~ 8 ; (iii) R^1COCl, Et_3N, CH_2Cl_2, 0° to 20 °C and chromatography ; (iv) HCO_2H : H_2O 9 : 1, 75 °C, 1 h.

Scheme 4: Synthesis of N-7-acylamino-imidazolidinones.

short reaction times, it was possible to obtain crude mixtures containing the desired 7-amino-imidazolidinones **20**-**21** (30% yield), together with unreacted **18**-**19** (20%-30%) and NH-imidazolidinones **16**-**17** (50%-40%).

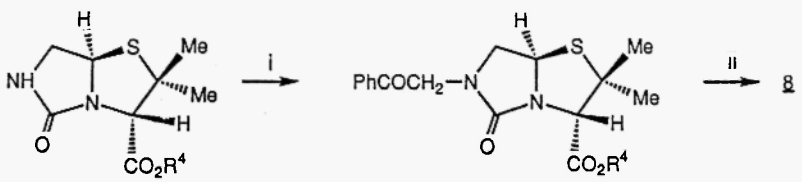
Direct acylation with acid chlorides bearing representative antibiotic side-chains yielded the imidazolidinones **22**-**23** in which the acylamino residue has been anchored at position N-7. The products **22a**, **22c**, **22d** and **23a** were isolated, after the column-chromatography on silica gel, in rather poor overall yields (25%-30%) from the nitroso precursors **18**-**19**. Cleavage of the *p*-methoxybenzyl ester **23a** with wet formic acid at 70°C gave the corresponding free acid **7a**. These experimental conditions also hydrolyzed the N-trityl protecting group of the cefotaxime side-chain /8/ in **22d**, giving the free amine **22e**.

N-Alkylation of imidazolidinones **16** and **24** with phenacyl bromide (Scheme 5) was realized in acetone solution and in the presence of powdered potassium carbonate. Large excesses of reagents and long reaction times were needed for obtaining reasonable yields (50%) of compounds **25**-**26**. Conventional deprotection of the trichloroethyl ester **26** gave the corresponding free acid **8** (R=Ph).

All the new compounds were fully characterized by their spectroscopic data (IR, NMR, Mass); physical parameters of the imidazolidinone esters ($R^4 \neq H$) are given in Table 1.

Computational chemistry

A recent conformational study /14/ of various classes of bacterial D,D-peptidase inhibitors pointed out common geometrical features in



16 (R^4 = PIV) **25** (R^4 = PIV)

24 (R^4 = TCE) **26** (R^4 = TCE)

(i) $PhCOCH_2Br$ (4eq.), K_2CO_3 (8 eq.), acetone, 20 °C, 96 h ;

(ii) HCO_2H–H_2O 9 : 1, Zn, 0°C, 4 h.

Scheme 5: Synthesis of N-7-phenacyl-imidazolidinones.

TABLE 1

Physical parameters of imidazolidinone esters

cpd	R^1	R^2	R^3	R^4	R_F[a]	$[\alpha]_D$[b]
13a	$PhCH_2CONH$	H	H	PIV	0.32 (60:40)	+196.7° (0.74%)
14a	$PhCH_2CONH$	H	H	PNB	0.34 (60:40)	+179.1° (0.77%)
13b	$PhOCH_2CONH$	H	H	PIV	0.44 (60:40)	+183.9° (0.985%)
15b	$PhOCH_2CONH$	H	H	TCE	0.49 (60:40)	+162.4° (0.615%)
18	H		NO	PIV	0.46 (97.5:2.5)	+383.9° (0.655%)
19	H		NO	PMB	0.54 (95:5)	+408.7° (1.755%)
22a	H	H	$PhCH_2CONH$	PIV	0.47 (80:20)	+158.0° (0.41%)
23a	H	H	$PhCH_2CONH$	PMB	0.44 (80:20)	+161.3° (0.315%)
22c	H	H	$(MeO)_2C_6H_3CONH$	PIV	0.48 (70:30)	+139.3° (0.465%)
22d	H	H	Tr-ATMO	PIV	0.47 (80:20)	+ 94.8° (0.70%)
22e	H	H	ATMO	PIV		+137.6° (0.675%)
25	H	H	$PhCOCH_2$	PIV	0.51 (90:10)	+161.7° (0.635%)
26	H	H	$PhCOCH_2$	TCE	0.49 (95:5)	+159.3° (0.70%)

[a] CH_2Cl_2 - EtOAc (v/v)
[b] $CHCl_3$ (conc.)

all the compounds examined, including benzylpenicillin, cephapyrin, 6-spiro-epoxypenicillins, thienamycins, lactivicin and penem-like gamma-lactams. This was also valid for the model substrate Ac_2-L-Lys-D-Ala-D-Ala. In all these ligands, the oxygen atom of the side-chain is orientated in the same way in relation to the carbonyl of the scissile amide bond and the carboxylic function.

A similar study has been performed on the imidazolidinones **5** to **9** (Scheme 2; R=$PhCH_2$ in **5**, **6**, **7** and **9**; R=Ph in **8**).

Conformational analysis

The atomic coordinates of the molecules were determined by MNDO optimization, leading to the geometries shown in Figure 1. It should be noted that in compound **7**, the extra nitrogen atom adopted a pseudo sp^3 character, breaking the conjugation of the amide bond.

Using the optimized geometries as a starting point, investigation of the energetically accessible conformational space of the side-chains was performed by molecular mechanics in the MM2 framework /12/. Assuming that the amide bond is planar, only one dihedral angle ϕ_1 (Fig. 1) could be responsible for the orientation of the carbonyl group of the side-chain in structures **5**, **6** and **7**. Plots of energy as a function of ϕ_1 showed for molecule **5** a wide domain of stability between 170° and 320° (Fig. 2A), similar to the one found in penicillins /14/. In contrast, stable conformers of **6** adopted a ϕ_1 value between -60° and 190° (Fig. 2B); these conformers are not close to the ones found in active compounds /14/. Permitted values of ϕ_1 were 100°-110° or 260° -290° in **7** (Fig. 2C). In structure **8**, the orientation of the side-chain carbonyl group depends on two dihedral angles, ϕ_1 and χ_1 (Fig. 1). The energy map calculated by steps of ten degrees around both torsional angles showed a large domain of stability for that molecule (Fig. 3).

Superposition with benzylpenicillin

Comparison of the structures was first by a common orientation of the lactam rings. Five atoms of the imidazolidinone ring were superimposed on the corresponding ones of the beta-lactam ring in benzylpenicillin by minimizing the sum of the squared distances between each pair of atoms (Fig. 4).

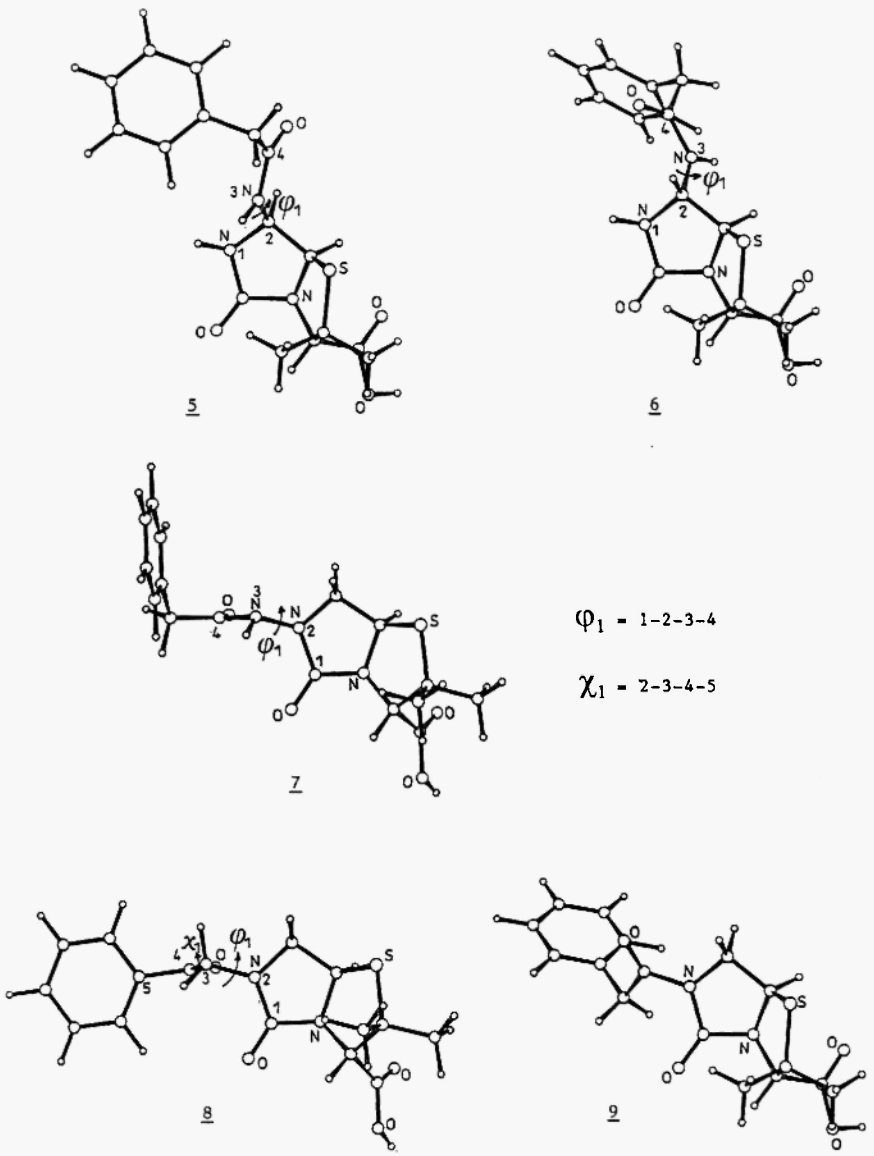


Fig. 1: Optimized geometries of molecules **5** to **9**.

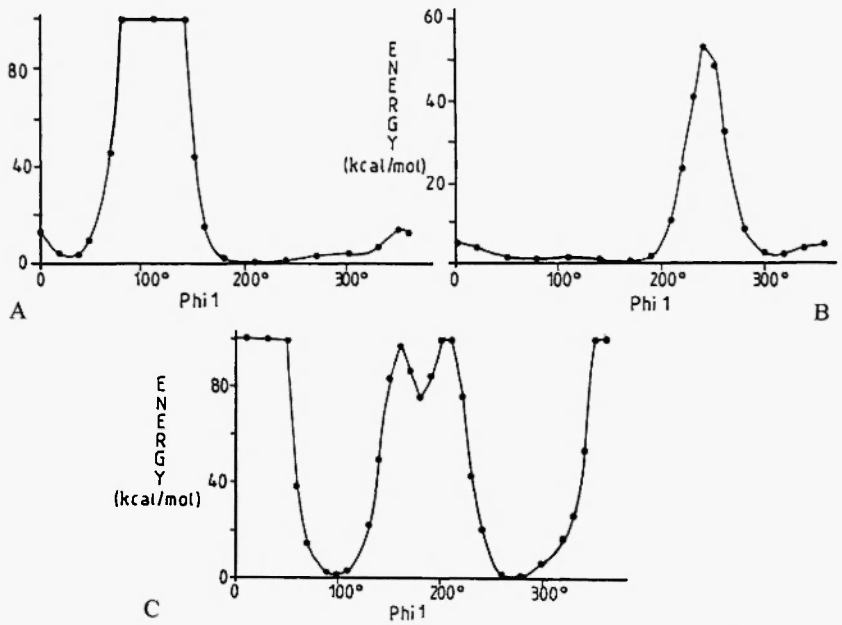


Fig. 2: Energetically accessible conformational space of the side-chains for molecules **5**, **6** and **7**.

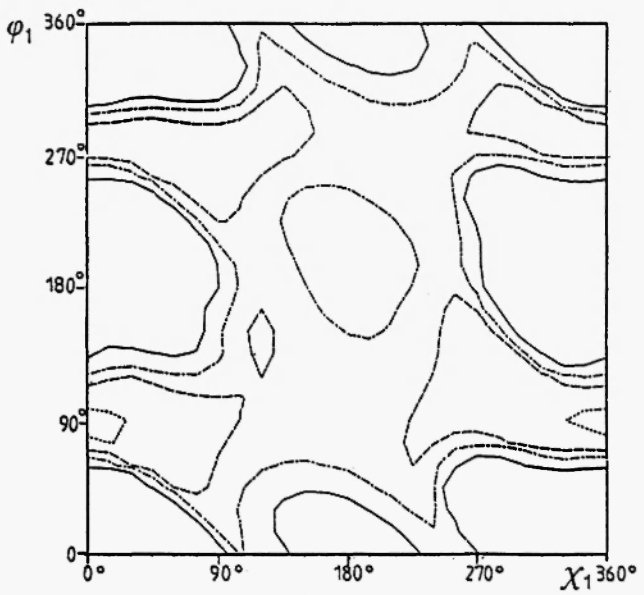


Fig. 3: Energetically accessible conformational space of the side-chain for molecule **8**.

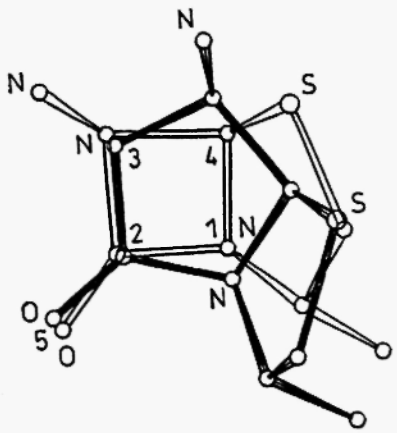


Fig. 4: Imidazolidinone and beta-lactam superposition.

After this fit, the lactam carbonyl bond of the superimposed structures almost coincided, whereas distances between fitted atoms were less than 0.5 Å. In this way, the carbon bearing the carboxylic function was at least 1 Å away from the acid substituant in penicillin, and the atoms of the acyl group of the side-chains lay far away from each other (Table 2). The oxygen atom present in all side-chains could now be compared to the corresponding one in benzylpenicillin, and eventually brought as close as possible by modification of the conformations. This dynamical fit was performed by the interactive graphic program ULYSSE, rotating the side-chains in search for the best agreement. In most cases, distances between carbonyl groups remained important (Table 3). In molecules **5** and **6**, the branching of the side-chain in the beta-position in regard to the scissile bond never allowed a common conformation with benzylpenicillin. The dynamical fit only orientated the carbonyls in the same direction (Fig. 5A,B). The NH groups of the side-chain of **7** and benzylpenicillin were orientated in the same way, and the oxygen atoms of the carbonyls were rather close to each other (Fig. 5C), but this adjustment required 6.84 kcal/mole (Table 3). In compound **9** minimization of the distance between the side-chain oxygen atoms at the level of 1.38 Å cost more than 9 kcal/mole (Fig. 5E). A good agreement was found with **8**, but in this case the benzene ring masked the carbonyl group (Fig. 5D).

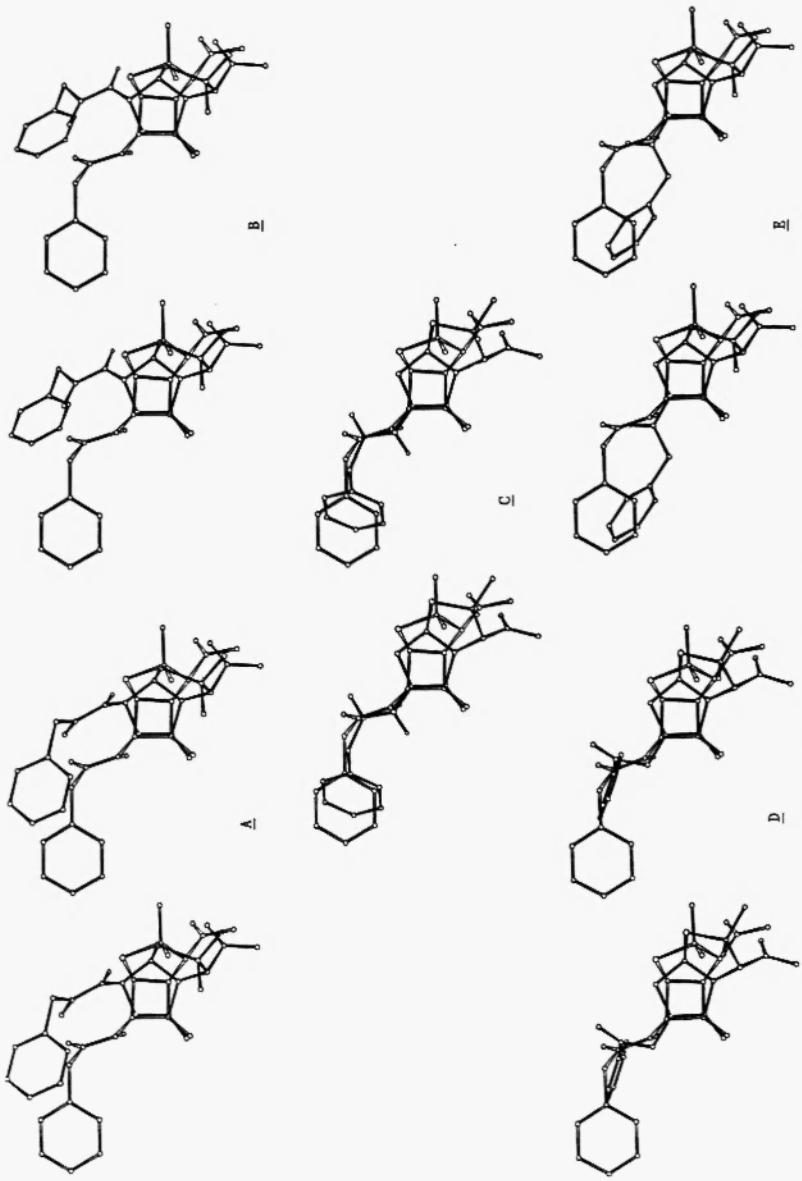


Fig. 5: Dynamical fit of imidazolidinones **5** to **9** with benzylpenicillin.

TABLE 2

Distances (Å) between atoms of the acyl side-chains after common orientation of benzylpenicillin and imidazolidinones

side-chain atoms	5	6	7	8	9
N	2.28	3.28	0.58		
H	2.36	4.72	0.82		
C	2.46	3.38	2.04	2.99	1.95
O	2.06	2.05	2.66	3.39	2.85

TABLE 3

Distances (Å) between corresponding atoms of the acyl side-chain of imidazolidinones after dynamical fit to benzylpenicillin

side-chain atoms	5	6	7	8	9
N	2.28	3.28	0.58		
H	2.65	4.76	1.82		
C	2.06	3.24	1.12	1.87	1.95
O	1.35	1.42	1.06	0.90	1.38
relative energy (kcal/mole)	1.96	1.62	6.84	5.12	9.62

The above results were derived after a relative orientation of the skeletons based on the best coincidence of the lactam ring atoms only. Another fit could superimpose, by the same method, five pairs of atoms in the lactam ring *and* the carbon atom of the fused ring bearing the carboxylic function. Comparison between cephalosporins and penicillins /14/ had shown that the relative position of the carboxylic group towards the scissile bond of antibiotics was not critical /15,16/. Nevertheless, it compelled a 10° rotation of the imidazolidinone ring, giving rise to a worse agreement of the side-chains.

Biological evaluation

Imidazolinones belonging to the new families **6**, **7** and **8** were evaluated for antibacterial activity *in vitro*. The free acids **6a**, **6b**, **7a**, **7c**, **7e** and **8** were found inactive against representative gram-positive and gram-negative microorganisms at concentrations of up to 100 μM. The biodegradable /17/ pivaloyloxymethyl esters **13a**, **13b**, **22a**, **22c**, **22e** and **25** were tested as such and after their hydrolysis catalyzed

with human serum; the compounds were also devoid of activity at concentrations of up to 200 μM.

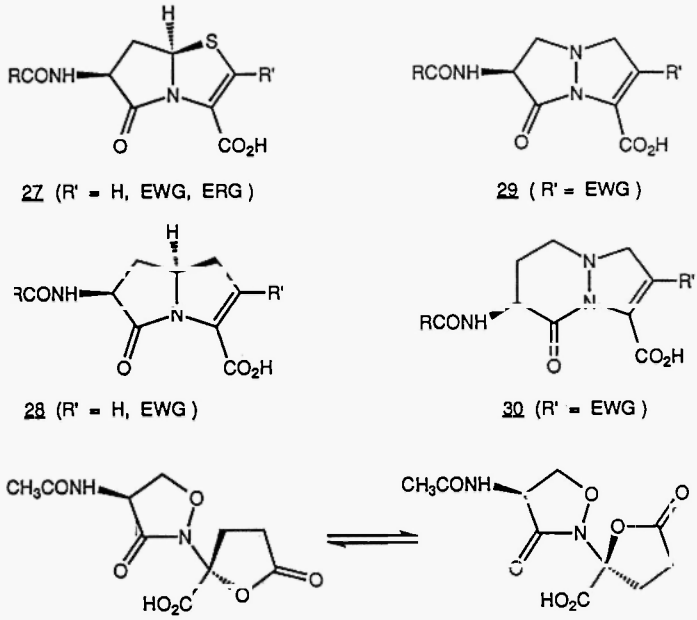
The tested compounds in families **6** and **7** were equipped with the acyl side-chains typical of the classical beta-lactam antibiotics, e.g. side-chains of penicillin G (R = benzyl), penicillin V (R = phenoxymethyl), methicillin (R = o,o'-dimethoxyphenyl) and cefotaxime (RCO = 2-(2-amino-4-thiazolyl)-2-(methoxy)-imino)-acetyl; ATMO). For synthetic reasons, compounds in family **8** were equipped with a benzoyl residue (R=phenyl).

Thus, independently of the nature of the side-chain residues R and the position for side-chain anchoring (C-6 up, C-6 down or N-7), the imidazolidinones **5** to **9** (Scheme 2) structurally related to the penicillins appeared devoid of antibacterial properties /5,6/.

CONCLUSION

During the last few years several non beta-lactam analogs of antibiotics have been derived (Scheme 6). The gamma-lactams **27** /18,19/ and **28** /18/, structurally related to penems and carbapenems, exhibited only weak activities. On the other hand, the pyrazolidinones **29** /20,21/, structurally related to carbapenems, showed excellent antibacterial properties. But their homologs, the tetrahydro-pyridazinones **30**, were inactive /22/. Concomitantly with the synthetic efforts in the design of penicillin mimics, a natural antibiotic possessing the isoxazolidinone structure was isolated from bacterial culture filtrates; the lactivicin **31** /23/ was shown to interact with the PBPs in the same way as the beta-lactams. This interesting discovery reinforced the idea that non beta-lactamic structures could interact with the penicillin binding proteins (PBPs).

Our results suggest that the imidazolidinones **5**, **6** and **9** cannot be accomodated in the protein active sites where penicillin is recognized. The goodness-of-fit appears somewhat better for the imidazolidinones **7** and **8** in which the acyl group of the side-chain is anchored at position N-7 via a short arm (one-atom spacer). Yet, the biological inactivity of the compounds **7** and **8** could result from the poor electrophilic character of their imidazolidinone carbonyl function. Therefore, more reactive imidazolidinones related to the penem or carbapenem families (like **29**) would probably represent good candidates for antibacterial activity. Such compounds, bearing EWG or



Scheme 6: Non beta-lactam analogs of antibiotics.

ERG substituents (see Scheme 6), are currently being evaluated using a theoretical model /24/ based on the reaction of the scissile amide bond with the duplex molecule "methanol-water", via a six-membered transition-state in a concerted mechanism /19/.

A current challenge in antibiotherapy is the discovery of new structures which will overcome the bacterial resistance mainly due to the production of beta-lactamases /25/. We hope that non beta-lactams may provide a solution for this problem.

ACKNOWLEDGEMENTS

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