Antibiotic activity of new penams (6-APA) and cephems (7-ACA) with bulky T-shaped side-chains.

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ABSTRACT

Objectives: Discovered more than 50 years ago, beta-lactam antibiotics still remain of large interest owing to their potentially large spectrum and low intrinsic toxicity. Although a large number of semi-synthetic derivatives have been obtained but few have explored the possibility to use bulky side chains in an attempt to more fully block the catalytic crevice in PBPs. In this context, we have synthesized penam and cephem derivatives bearing two morpholine rings attached to their side-chains. We report here on two typical compounds (DEMO-Pen and DEMO-Cef) modelled after benzylpenicillin and the corresponding methoxy-acetyl-cephem.
Methods: DEMO-Pen and DEMO-Cef were formed via the mixed anhydride method. The whole side chain was first obtained by bis-alkylation of methyl 3,5-dihydroxybenzoate with N-chloroethylmorpholine, and made into a pentafluorophenyl ester. The latter was coupled with 6-APA and 7-ACA to give the corresponding penam and cephem derivatives. MIC's were determined by common agar dilution method in comparison with ampicillin and cefadroxil against both collection strains and clinical isolates.
Results: The structures and stabilities of DEMO-Pen and DEMO-Cef were confirmed by ^{1}H NMR, ^{13}C NMR, IR and the high-resolution mass . DEMO-Pen was active against Gram (+) bacteria, *H. influenzae*, and beta-lactamase producing *M. catarrhalis*, but not against other Gram (-) bacteria. DEMO-Cef was more active than cefadroxil against Gram (+) but inactive against Gram(-).
Conclusion: Contrary to most beliefs, bulky side-chains do not prevent penams and cephems to reach the active site of PBP's. This opens the way to renewed efforts in the synthesis of novel beta-lactam derivatives.

INTRODUCTION

Although a large number of semi-synthetic derivatives of beta-lactams have been obtained, only a few of them have been designed to completely fill the catalytic crevice in PBPs by addition of a bulky side chain.

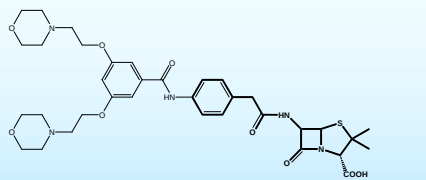
To investigate the influence of such steric effects on antibiotic activity, we have synthesized penam and cephem derivatives bearing two morpholine rings attached to their side-chains. Simple acyl derivatives of p-aminobenzylpenicillin are, indeed, known to be active antibiotics (1,2).

DESIGN OF NEW DERIVATIVES

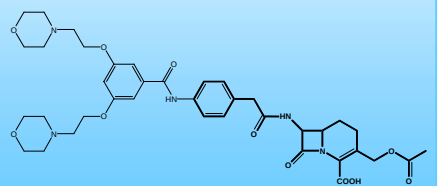
We report on two typical compounds (DEMO-Pen and DEMO-Cef) modeled after benzylpenicillin and the corresponding methoxy-acetyl-cephem. The bulky side chain has been attached to the p-amino function of p-aminobenzylpenicillin and N-(p-aminophenyl acetyl)-7-aminocephalosporanic acid, respectively. Morpholino rings were choosen to ensure a good water solubility and to favor an extended conformation of the side-arms by formation of hydrogen bonds with the solvent. The low hydrophobicity of these rings should avoid the shrinking of the molecule to a stacked conformation.

Acknowledgments:

L.E. was the recipient of a post-doctoral fellowship of the Université catholique de Louvain.

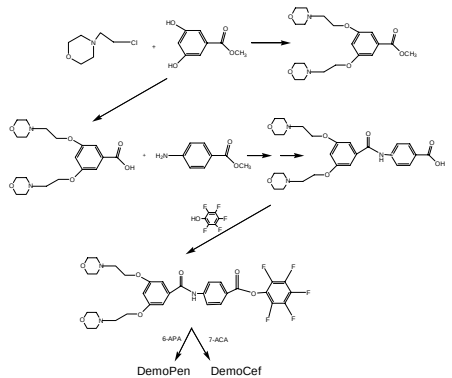


DEMO pen



DEMO cef

Strategy for the synthesis of DEMO pen and DEMO cef



Antibacterial activity

Bacteria	Mechanisms of resistance	MIC (µg/ml) [a]			
		Ampicillin	DEMOPen	Cefadroxil	DEMOcef
COLLECTION STRAINS					
Staphylococcus aureus ATCC 25923		< 0.125	0.25	2	2
Haemophilus influenzae ATCC 49247		4	16	256	> 256
CLINICAL ISOLATES					
Staphylococcus aureus ((N0100587)		0.25	1	4	4
Streptococcus pneumoniae (N0100368)		< 0.03	0.125	1	< 0.06
Streptococcus pneumoniae (N0100621)	MLSB	< 0.03	0.06	1	< 0.06
Streptococcus pyogenes (N101617)		< 0.03	< <0.06	< 0.06	< 0.06
Streptococcus pyogenes (N100582)		< 0.03	< 0.06	< 0.06	< 0.06
Listeria monocytogenes (Mont-Godinne collection 93)		0.125	2	16	4
Listeria monocytogenes (Mont-Godinne collection 108)		0.25	2	16	4
Moraxella catarrhalis (N0100483)	Beta-lactamase (BRO-1 ?)	0.25	< 0.125	8	64
Clostridium perfringens (Mont-Godinne collection 64)		0.25	2	32	8

[a] determined by geometric dilutions using standard procedures

METHODS

DEMOPen and DEMOCef were formed via the mixed anhydride method. The horizontal stem of the T-shaped side-chain was first obtained by bis-alkylation of methyl 3,5-dihydroxybenzoate with N-chloroethylmorpholine and saponification. The resulting acid was coupled with the p-amino function of methyl p-aminophenylacetate. The so-obtained peptidic bond, associated with its two adjacent benzene rings, formed the vertical stem of the T-shaped side-chain. The corresponding pentafluorophenyl ester was synthesized and coupled with 6-APA and 7-ACA to give the sterically crowded derivatives DEMOPen and DEMOCef (in good yield, 75-80% for the last step).
MIC's were determined by common agar dilution method against both collection strains and clinical isolates, in comparison with ampicillin and cefadroxil.

RESULTS

The structures and stabilities of DEMO-Pen and DEMO-Cef were confirmed by ^{1}H NMR, ^{13}C NMR, IR and high-resolution mass spectroscopy.
DEMO-Pen was active against Gram (+) bacteria, beta-lactamase producing *M. catarrhalis*, and to a low extent on *H. influenzae*, but not against other Gram (-) bacteria. DEMO-Cef was more active than cefadroxil against Gram (+) but inactive against Gram(-).

CONCLUSIONS

Bulky side-chains do not abolish the activity of beta-lactam antibiotics, meaning that they probably do not prevent penams and cephems to reach the active site of PBP's.

The present data may open interesting perspectives for the synthesis of novel bulky beta-lactam derivatives.

REFERENCES

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2. Tosoni, A. L.; Moloney, P. J.; Goldsmith, L.; Glass, D. G. U.S. Patent 2,978,447, 1961; Chem. Abstr. 1961, 55, 199521