Synthesis and activity of penicillin G hydroxamic acid derivatives against *S. aureus*, *S. pneumoniae*, and intraphagocytic *L. monocytogenes* .

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ABSTRACT

Background: Hydroxamic acid derivatives of beta-lactams (carboxyl group replaced by a CO-N(R1)-O-R2 moiety) are not ionizable at physiological pH, which could allow for improved penetration in phagocytic cells. We have synthesized new series of N-hydroxybenzylpenicillin amides with substituents of the N and O atoms to study activity against both extracellular and intracellular bacteria.

Methods: Syntheses were made with the mixed anhydride method with starting compounds prepared by modified Gabriel's and Doleschall's procedures for O-alkylated and N-monoalkylated derivatives, respectively. MICs were determined by agar dilution and activity on intracellular L. m. evaluated on infected murine J774 macrophages (Chanteux et al., JAC 52:610-5, 2003). The absence of conversion into Pen G in microbiological and cell culture media was checked by HPLC.

Results: Structures were confirmed by 1H-NMR, 13C-NMR, IR, and HR mass spectrometry. No release of free Pen G was detected. MICs (mg/L) are shown in Table.

Bacteria	PenG	Oxa	1 R1 = H R2 = CH_3	2 R1 = H R2 = Bz	3 R1 = CH_3 R2 = H	4 R1 = iPr R2 = H	5 R1 = R2 = CH_3
S.a.(n=4)	0.03-0.25	0.25	0.25-0.5	0.25-1	0.06-0.25	0.125-0.5	1-2
S.p.(n=6)	≤ 0.03	0.06-0.25	0.06-0.25	0.5-2	0.06-0.125	< 0.03	8-32
L.m.	0.06		4	2			

1-4 are weak acids (pK approx. 9); 5 is not an acid.

On intracell. L.m., Pen G and 2 were both static at an extracell. concentr. (Ce) of 6 X the MIC, but 1 was already static at Ce = 1 x the MIC.

Conclusion: Replacing the free carboxyl group of beta-lactams by a weak acid does not necessarily suppress antibacterial activity and may confer enhanced activity against intracell. bacteria at equipotent Ce.

INTRODUCTION

The activity of β-lactam antibiotics is critically dependent on the presence of a free carboxyl function or of an equivalent acidic group at a strategic distance from the β-lactam ring in order to mimic a D-Ala-D-Ala moiety (1,2). This acid function, however, is responsible for the lack of cellular penetration and tissue accumulation of β-lactams (3).

Hydroxamic acid derivatives of beta-lactams keep the acidic character of the classical carboxyl-substituted beta-lactams, while being not ionizable at physiological pH, which could allow their improved penetration in eucaryotic cells.

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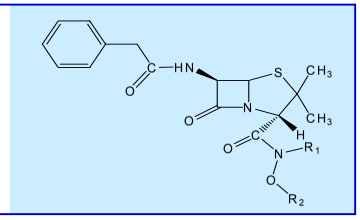
AIM OF THE STUDY

- to synthesize hydroxamic derivatives of penicillin G
- to evaluate if their activity against Gram-positive is maintained
- to test for their intracellular activity against *L. monocytogenes* .



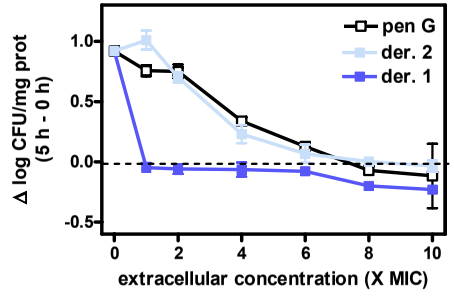
ANTIBACTERIAL ACTIVITY

bacteria	strain	Penicillin G	Oxacillin	1 R_1 = H R_2 = CH_3	2 R_1 = H R_2 = Bz	3 R_1 = CH_3 R_2 = H	4 R_1 = iPr R_2 = H	5 R_1 = CH_3 R_2 = CH_3
S. aureus	ATCC 25923	0.03	0.25	0.5	0.25	0.125	0.25	1
	N3012755	0.03	0.25	0.25	0.5	0.06	0.125	1
	N2043549	0.25	0.25	0.5	0.25	0.125	0.5	2
	n2043561	0.03	0.25	0.5	1	0.25	0.25	1
S. pneumoniae	N212417	< 0.03	0.25	0.25	1	0.06	< 0.03	16
	N3010230	<0.03	0.06	0.125	2	0.06	< 0.03	8
	N2121732a	0.03	0.5	0.06	0.5	0.06	< 0.03	16
	N3091907	< 0.03	0.125	0.06	1	0.125	< 0.03	32
	N3070964	0.03	0.25	0.25	1	0.06	< 0.03	8
	n2120865	< 0.03	0.25	0.25	1	0.125	< 0.03	16
L. monocytogenes		0.06		4	2			

METHODS

Synthesis of N-hydroxybenzylpenicillin derivatives: All compounds were prepared by the mixture anhydride method (4). They were characterized by infrared and nuclear magnetic resonance (^{1}H NMR and ^{13}C NMR) spectroscopy, melting points and high-resolution mass spectrometry.
Microbiological assays:. MIC were determined by standard microdilution techniques according to NCCLS guidelines using ATCC strains and clinical isolates of *S. aureus* and *S. pneumoniae*
Intracellular activity against *L. monocytogenes*: Intracellular activity was testing in a model of J774 mouse macrophages over a 5 h period, following the procedure described in our previous publications (see 5 for example).
Stability of the molecules was checked by HPLC in all condictions of biological assays.

INTRACELLULAR ACTIVITY



CONCLUSIONS

• derivatives 1,3, and 4 are as active, and derivative 2 is slightly less active as comparators. Derivative 5, which is not acidic, is only poorly active.
⇒ replacing the free carboxylic group of beta-lactam by a weak acid does not necessarily suppress antibacterial activity.

• derivative 1 shows bacteriostatic activity against intracellular *Listeria monocytogenes* at extracellular concentrations lower (in multiples of MIC) than derivative 2 or penicillin G
⇒ The non ionizable character of these derivatives may be one of the characteristics that improves intracellular activity, but other factors need to be evidenced to explain the difference in activity between derivatives 1 and 2.

• no conversion into penicillin G is detected in the microbiological or cell culture media
⇒The synthesized derivatives are not prodrugs of penicillin G and are thus active *per se*.

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