Antibacterial Activity Against Sensitive and Resistant bacteria of Organic salts and Ionic Liquids based on β-lactam Antibiotics and Their Hydrolysates

Ricardo Ferraz [1,2,*] , Dário Silva [3] , Ana Rita Dias [1,2], Vitorino Dias [1], Miguel M. Santos [3] , Luís Pinheiro [3], Cristina Prudêncio [1,4], João Paulo Noronha [3],
Željko Petrovski [3],* and Luís C. Branco [3],*

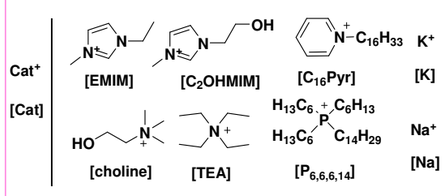
[1] Ciências Químicas e das Biomoléculas (CQB) e Centro de Investigação em Saúde e Ambiente (CISA), Escola Superior de Saúde do Instituto Politécnico do Porto, 4400-330 Porto, Portugal; [2] LAQV-REQUIMTE, Departamento de Química e Bioquímica, Faculdade de Ciências, Universidade do Porto, Rua do Campo Alegre 687, 4169-007 Porto, Portugal; [3] LAQV-REQUIMTE, Departamento de Química, Faculdade de Ciências e Tecnologia da Universidade Nova de Lisboa, 2829-516 Caparica, Portugal; [4] i3S, Instituto de Inovação e Investigação em Saúde, Universidade do Porto, 4099-002 Porto, Portugal,
E-mail: z.petrovski@fct.unl.pt

Introduction

- Organic salts and ionic Liquids (OSILs) containing active pharmaceutical ingredients (OSIL-APIs) have been presented as good drug delivery tool for APIs and drug modulations [1-3]. Our group described the suitable combination of pharmaceutical drugs and counter-ions as an innovative approach to improve the original bioavailability, reduce polymorphism as well as enhanced the therapeutic effect [1,2]. Herein, the synthesis and activity of some antimicrobial OSIL-APIs containing amoxicillin and penicillin in the hydrolysate form of β-lactam antibiotics as well as ciprofloxacin and norfloxacin as fluoroquinolone antibiotics [4,5].

β-Lactam hydrolysates derived OSIL-APIs

Ionic liquids and organic salts based on the ammonia hydrolysate anion of penicillin G and amoxicillin (α-amide of benzyl penicilloic acid and of amoxicillin penicilloic acid, respectively), abbreviated here as [*seco*-Pen] and [*seco*-Amx], respectively, were prepared by an ammonia buffered reaction procedure that was recently developed by our group [6].



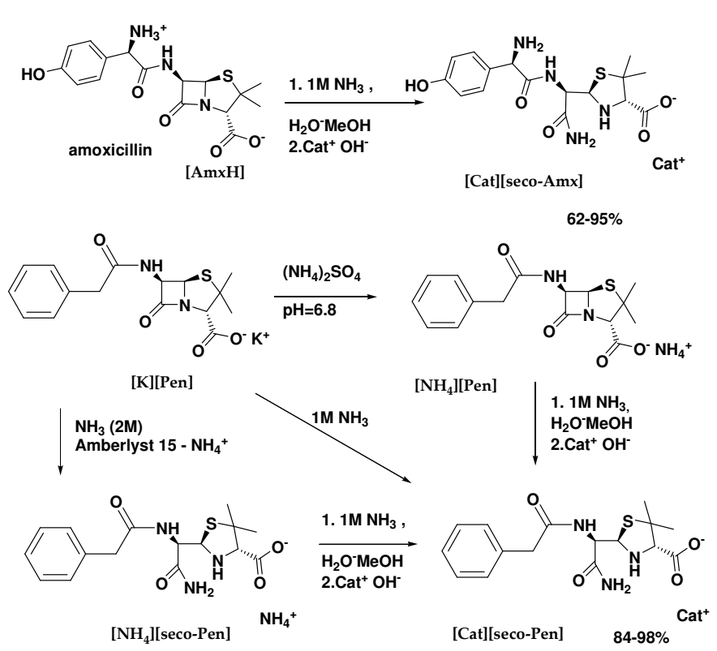


Figure 1 *– Synthesis of OSIL-APIs based on ammonia hydrolysate of amoxicillin and penicillin*

The novel β-Lactam hydrolysates derived OSIL-APIs were tested against sensitive and resistant bacteria **(Table** 1 and **2).** The activity of prepared compounds was compared with standard antibiotics and their hydrolysates [Na][*seco*-Amx], [K][Pen] and [K][*seco*-Pen].

Table 1 – *Antimicrobial activity of ammonia hydrolysed penicillin and amoxicillin based OSIL-APIs expressed as Minimum inhibitory concentrations (MICs in mM) and Relative Decrease of Inhibitory Concentration (RDIC)*

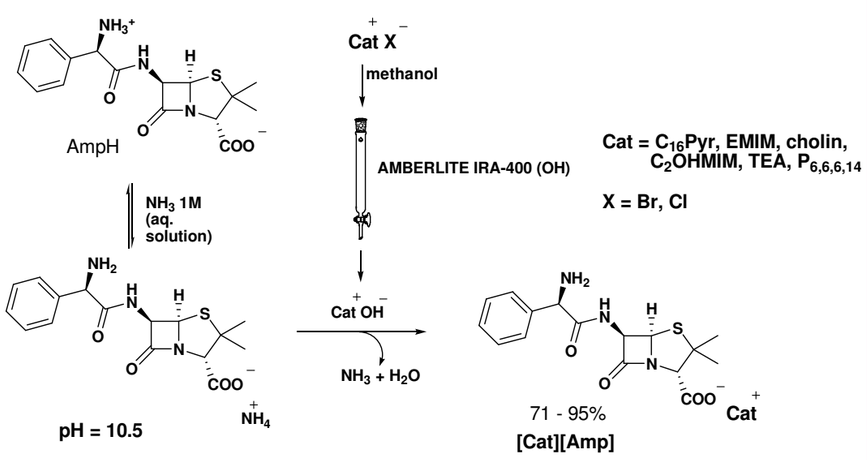
Compound	S. aureus ATCC25923	RDIC	E. coli ATCC25922	RDIC
Amx	0.050	1	0.005	1
Na[seco-Amx]	>5.0	<0.01	>5.0	<0.001
[EMIM][seco-Amx]	5.0	0.01	2.5	0.002
[C_2OHMIM][seco-Amx]	0.050	1	5.0	0.001
[Choline][seco-Amx]	>5.0	<0.01	>5.0	<0.001
[$P_{6,6,6,14}$][seco-Amx]	>5.0	<0.01	0.5	0.01
[C_{16}Pyr][seco-Amx]	>5.0	<0.01	0.050	0.1
K[Pen]	0.500	1	0.500	1
K[seco-Pen]	>5.0	<0.1	>5.0	<0.1
[EMIM][seco-Pen]	>5.0	<0.1	>5.0	<0.1
[C_2OHMIM][seco-Pen]	0.005	**100**	>5.0	<0.1
[Choline][seco-Pen]	>5.0	<0.1	5.0	0.1
[TEA][seco-Pen]	>5.0	<0.1	0.050	**10**
[$P_{6,6,6,14}$][seco-Pen]	>5.0	<0.1	>5.0	<0.1
[C_{16}Pyr][seco-Pen]	>5.0	<0.1	>5.0	0.1

Table 2 – *Minimum inhibitory concentrations (mM) and relative decrease of inhibitory concentration (RDIC) of the new compounds derivate of penicillin and amoxicillin that were produced on resistant strains.*

Compound	E. Coli CTX M9	RDIC	E. coli CTX M2	RDIC	MRSA ATCC 43300	RDIC
Amx	> 5	1	> 5	1	> 5	1
Na[seco-Amx]	>5	-	>5	-	>5	-
[EMIM][seco-Amx]	>5	-	> 5	-	> 5	-
[C_2OHMIM][seco-Amx]	> 5	-	> 5	-	5	>1
[$P_{6,6,6,14}$][seco-Amx]	0.05	**>100**	1.0	>5	> 5	-
[C_{16}Pyr][seco-Amx]	0.05	**>100**	0.05	**>100**	0.005	**>1000**
[Choline][seco-Amx]	0.5	**>10**	0.05	**>100**	0.5	**10**
K[Pen]	>5	1	> 5	1	> 5	1
K[seco-Pen]	>5	-	>5	-	>5	-
[EMIM][seco-Pen]	>5	-	>5	-	> 5	-
[C_2OHMIM][seco-Pen]	>5	-	>5	-	> 5	-
[Choline][seco-Pen]	1.0	>5	>5	-	1.0	**>5**
[$P_{6,6,6,14}$][seco-Pen]	0.5	**>10**	0.5	**>10**	**> 5**	-
[C_{16}Pyr]seco-[Pen]	0.5	**>10**	0.5	**>10**	0.05	**>100**
[TEA][seco-Pen]	>5	-	> 5	-	> 5	-

Ampicillin derived OSIL-APIs

Ampiciline based organic salts and ionic liquids were prepared in good yield using buffer neutralization method [6] (see ***Figure 2***). Except two (choline and C_2OHMIM) they are all ionic liquids as their m.p. are bellow 100 ºC.



Ampicillin based IL-APIs were first tested against sensitive gram-positive and sensitive and resistant gram-negative bacteria **(Table 3** and **4).** The activity of prepared compounds was compared with standard antibiotic [Na][Amp].

Table 3 – *Antimicrobial activity of ampicillin based IL-APIs against gram sensitive bacteria expressed as RDIC*

Strains / Comp.	Gram-negative		Gram-positive		
	E. coli ATCC 25922	*K. pneumoniae*	*S. aureus ATCC* 25923	*E. fecalis*	*S. epidermis*
Na[Amp]	1	1	1	1	1
[$P_{6,6,6,14}$][Amp]	0.02	0.5	0.1	1	1
[C_{16}Pyr][Amp]	0.1	**50**	1	**10**	**10**
[C_2OHMIM][Amp]	0.01	-	-	0.01	0.02

RDIC(OSIL-API)= MIC(API)/MIC(OSIL-API)

Table 4 – *Minimum inhibitory concentrations (mM) and relative decrease of inhibitory concentration (RDIC) of the compounds derivate of ampicillin that were produced on resistant strains.*

Strains / Comp.	*E. coli* TEM CTX M9		*E. coli* CTX M2		*E. coli* AmpC Mox2	
	MIC	RDIC	MIC	RDIC	MIC	RDIC
Na[Amp]	>5	1	>5	1	>5	1
[$P_{6,6,6,14}$][Amp]	0.5	**>10**	0.5	**>10**	>5	-
[C_{16}Pyr][Amp]	0.005	**>1000**	0.05	**>100**	>5	-
[TEA][Amp]	>5	-	>5	-	>5	-
[cholin][Amp]	>5	-	>5	-	>5	-
[EMIM][Amp]	>5	-	>5	-	>5	-
[C_2OHMIM][Amp]	>5	-	>5	-	5	>1

Conclusions

The prepared OSIL-APIs derived from penicillin G, amoxicillin and ampicillin show enhanced activities against several bacteria strains, particularly against resistant ones. The RDIC prove to be a good tool to monitor effectiveness of OSIL-APIs in respect to parent antibiotics. In case of sensitive strains the activity of novel OSIL-APIs was usually not greatly increased but in case of resistant species the, MICs at the nanomolar scale were measured, representing RDIC values as high as >1000. The cationic species were mostly ineffective when in the form of halide salts (not shown here). These results highlight OSIL-APIs as a promising solution to fight against antibiotic resistance.

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