



# 5th International Electronic Conference on Medicinal Chemistry

1-30 November 2019

chaired by Dr. Jean Jacques Vanden Eynde

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## Tackling bacterial resistance using antibiotics as ionic liquids and organic salts

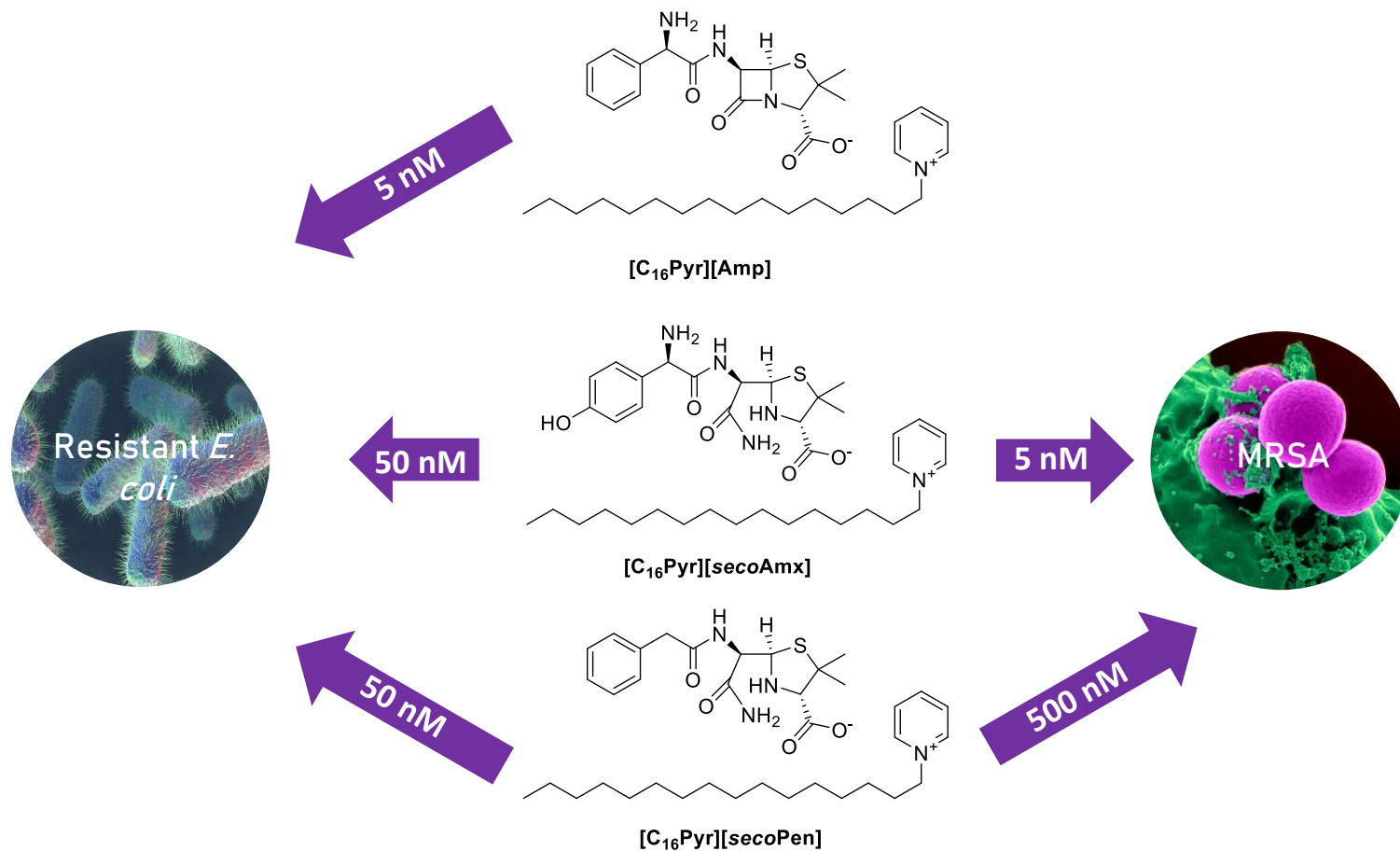
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# Tackling bacterial resistance using antibiotics as ionic liquids and organic salts

## Graphical Abstract



**Abstract:** Bacterial resistance to current antibiotics has a major impact on worldwide human health, leading to 700K deaths every year. The development of novel antibiotics did not present significant progress, namely regarding clinical trials, over the last years due to low returns. Thus, innovative alternatives must be devised to tackle the continuous rise of antimicrobial resistance.

Ionic Liquids and Organic Salts from Active Pharmaceutical Ingredients (API-OSILs) have risen in academia for over 10 years as an efficient formulation for drugs with low bioavailability and permeability, as well as reduction or elimination of polymorphism, thereby potentially enhancing their pharmaceutical efficiency. To the best of our knowledge, our group is the first to perform research on the development of API-OSILs from antibiotics as a way to improve their efficiency. More specifically, we have successfully combined ampicillin, penicillin and amoxicillin as anions with biocompatible organic cations such as choline, alkylpyridiniums and alkylimidazoliums.

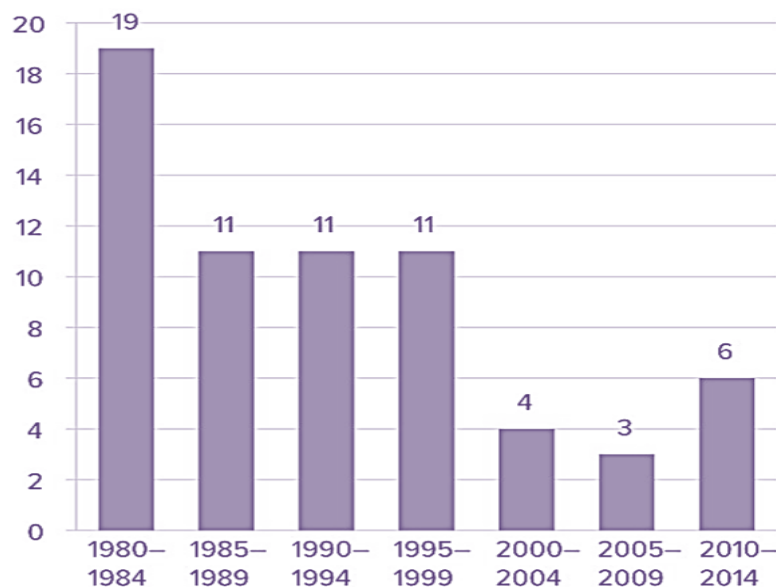
In this communication, we present our latest developments in the synthesis and physicochemical (DSC) characterization of OSILs from these antibiotics, in addition to *in vitro* antimicrobial activity data, in particular towards MRSA and multi-resistant *E. coli*, as well as sensitive strains of gram-positive and gram-negative bacteria.

**Keywords:** API-OSILs; bacterial resistance;  $\beta$ -lactam antibiotics; Ionic Liquids; MRSA



# Introduction

Approved # of antibiotics since 1980

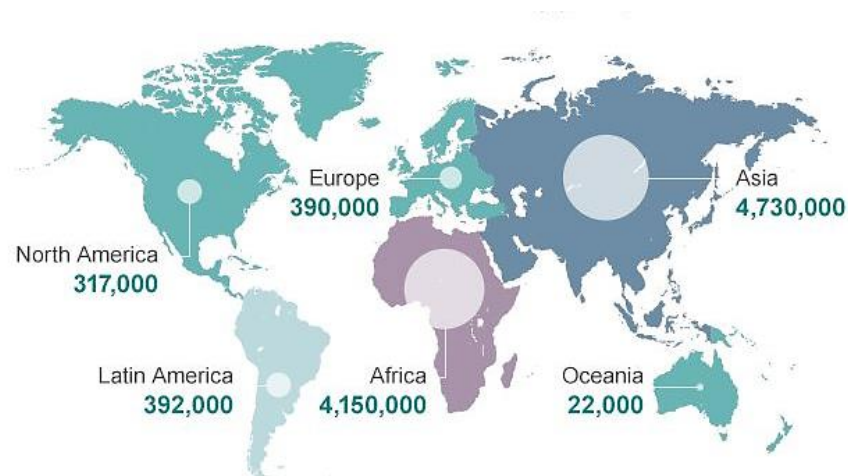


Reproduced from C. Lee Ventola, MS. (2015). *The Antibiotic Resistance Crisis*. Pharmacy & Therapeutics, Vol.40, N. 4



Low returns from clinical trials

Estimated deaths by resistant bacteria in 2050



Reproduced from *Review on Antimicrobial Resistance 2014*

**10 million deaths by 2050**

**75b€ associated costs**

Growing need for more effective antibiotics



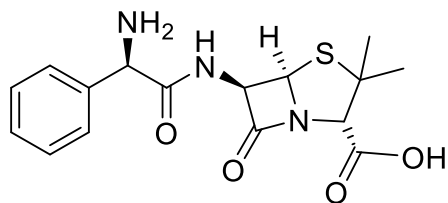
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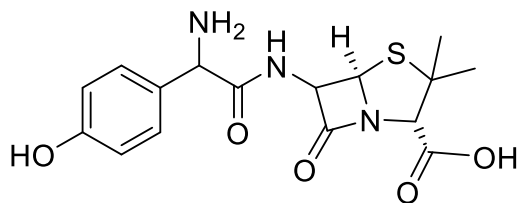


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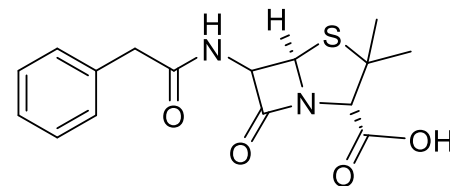
# Introduction



Ampicillin

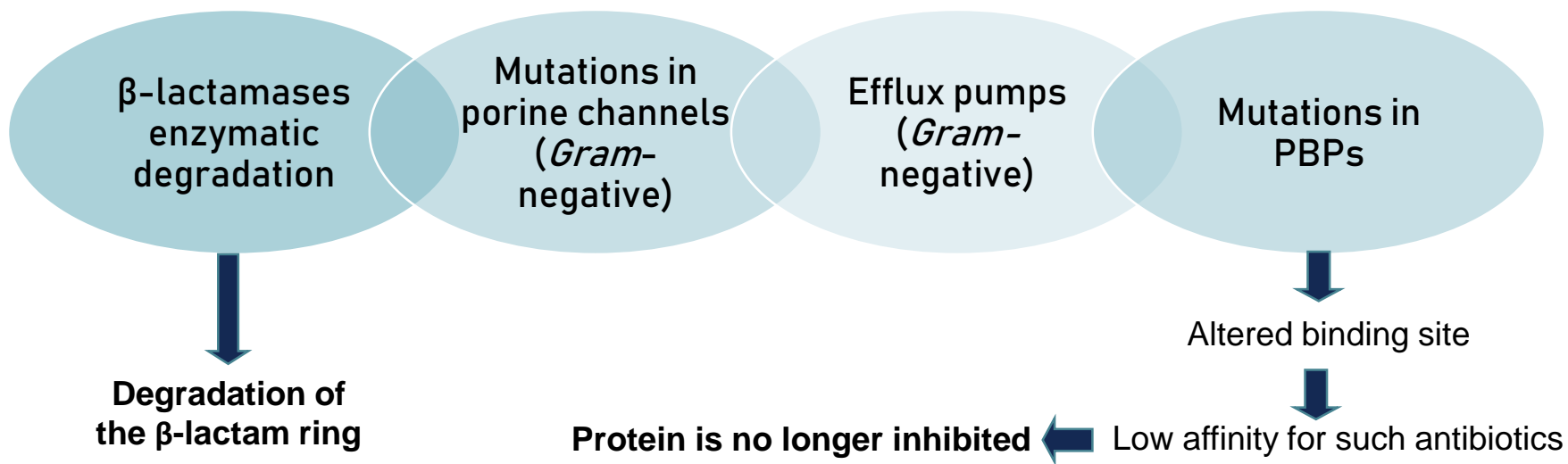


Amoxicillin



Penicillin

## Bacteria resistance to $\beta$ -lactam antibiotics



# PROBLEMS TO BE ADDRESSED

**Bioavailability**  
Low solubility of APIs in water and biological fluids  
Poor permeability across biological membranes

**Polymorphism**  
Distinct crystalline forms of a solid drug

**Drug resistance**  
Efficiency reduction of drugs

**Drug delivery**  
Lack of systemic site-targeting of the drug



**Organic Salts and Ionic Liquids can be the alternative approach to address such API problems**



# IONIC LIQUIDS

Organic salts with melting points lower than 100 °C composed by an organic cation and an inorganic or organic anion

**The physical and structural properties of the ILs are dependent on the cation-anion combinations**

*High thermal and chemical stability*

*High ionic conductivity*

*Tuneable solubility of the IL in aqueous or organic solvents*

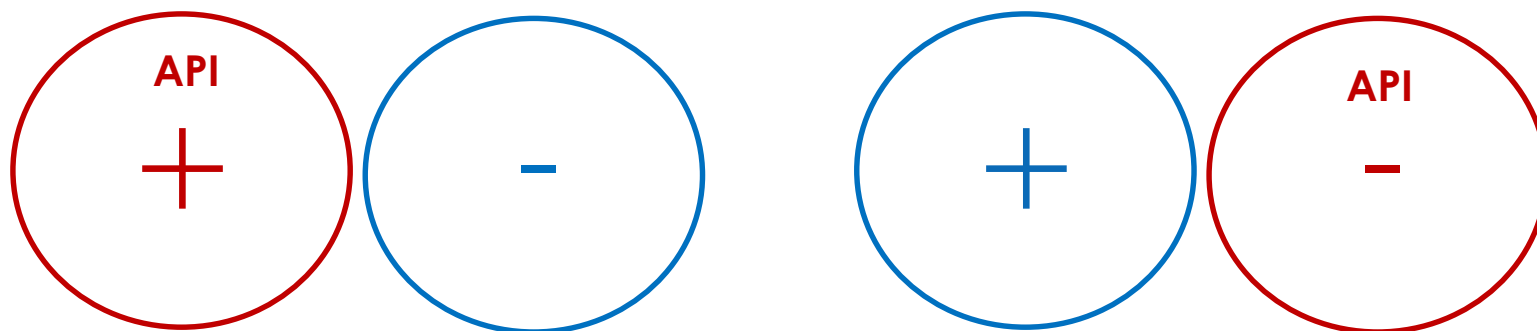
IL

*Negligible vapour pressure*

*Adjustable solubility of  $scCO_2$ , organic compounds and transition metal complexes in the Ionic Liquid*



# 3<sup>RD</sup> GENERATION IONIC LIQUIDS



New physical, chemical and biochemical properties



Modulate biopharmaceutical drug classification

Water solubility

Permeability

Drug formulation

Toxicity and metabolism

W. L. Hough, *et al*, *New J. Chem.* **2007**, 31, 1429; *ChemMedChem* **2011**, 6, 975; *Annual Rev. Chem. Biom. Eng.* **2014**, 5, 527



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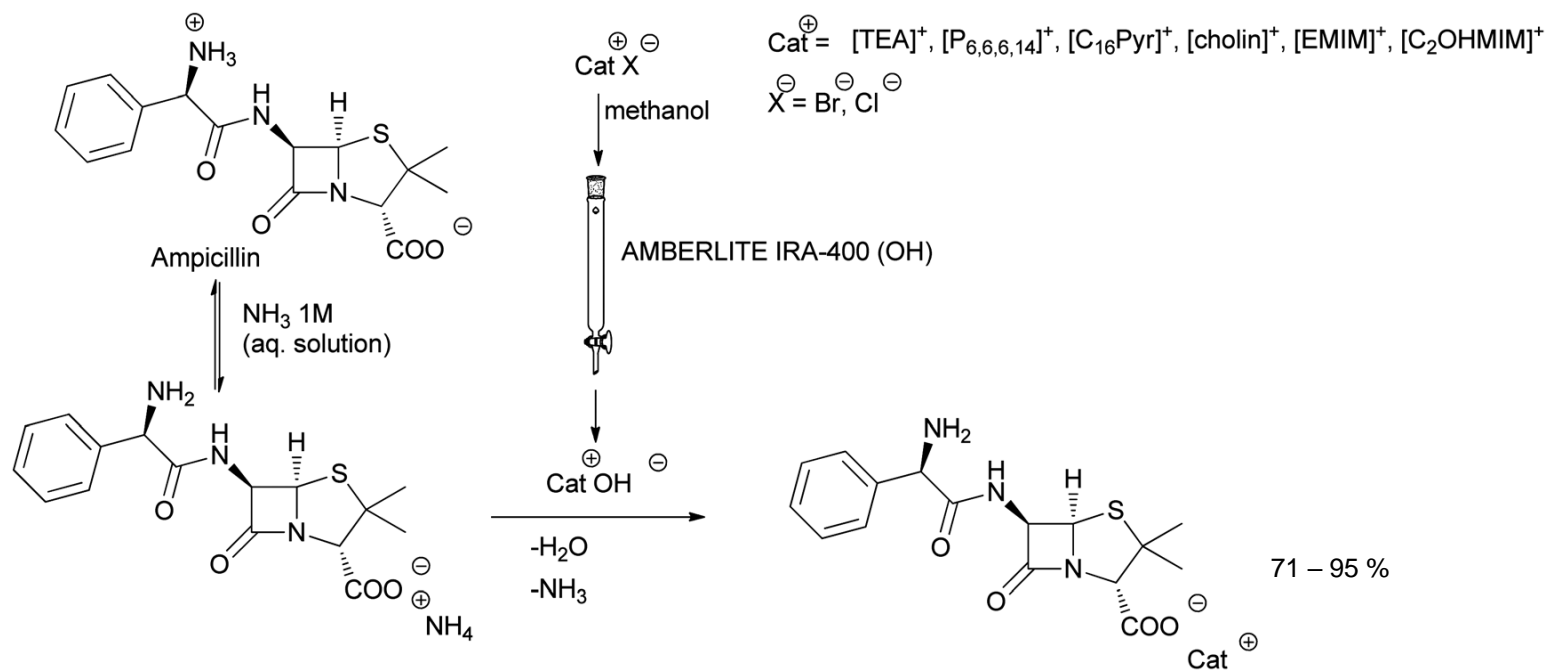
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### Neutralization method

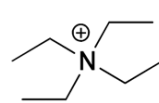
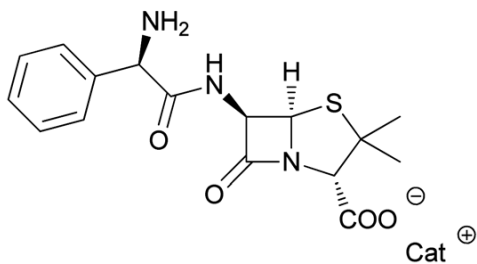


The hydroxide cation is prepared by passing a methanolic solution of halide salt through an ion-exchange column and subsequently added to ampicillin in 1M ammonium buffer solution.

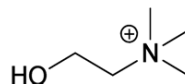
*Med. Chem. Comm.* **2012**, 3, 494



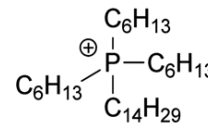
# Thermal Properties of Ampicillin-OSILs



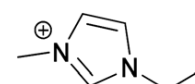
[TEA]<sup>+</sup>



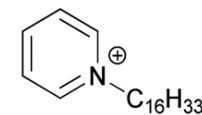
[cholin]<sup>+</sup>



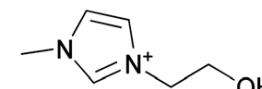
[P<sub>6,6,6,14</sub>]<sup>+</sup>



[EMIM]<sup>+</sup>



[C<sub>16</sub>Pyr]<sup>+</sup>



[C<sub>2</sub>OHMIM]<sup>+</sup>

✓ <sup>1</sup>H NMR

✓ <sup>13</sup>C NMR

✓ FTIR

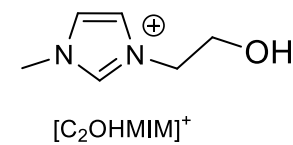
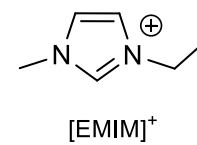
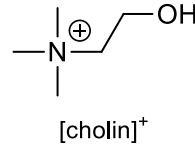
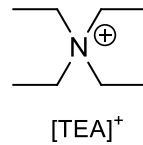
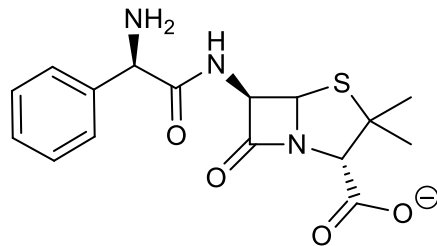
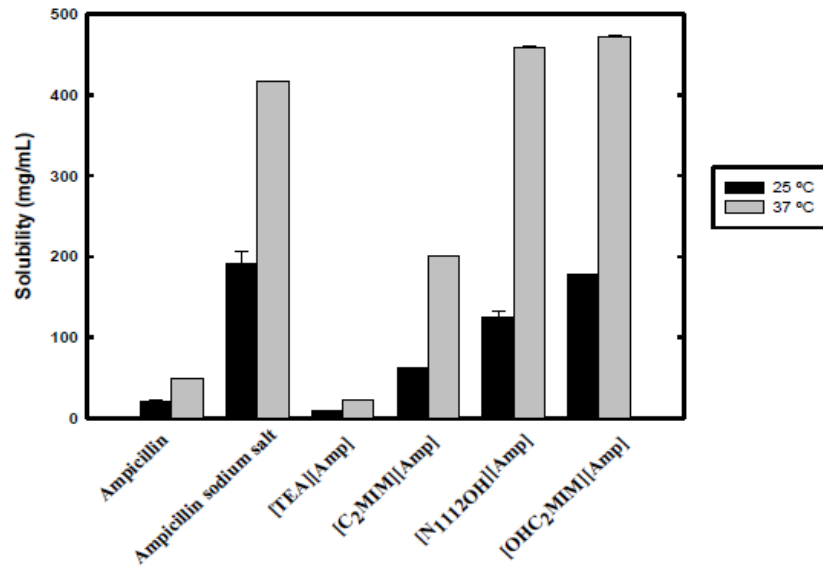
✓ Elemental analysis

✓ DSC

Compound	Physical State	T <sub>m</sub> <sup>a</sup> [°C]	T <sub>g</sub> <sup>b</sup> [°C]	T <sub>dec</sub> <sup>c</sup> [°C]
[TEA][Amp]	Pale yellow solid	79.0	-18.64	214.75
[P <sub>6,6,6,14</sub> ][Amp]	Yellow viscous liquid	-	-	297.65
[C <sub>16</sub> Pyr][Amp]	Pale yellow solid	86.0	-19.64	269.39
[cholin][Amp]	Pale yellow solid	58.0	-20.12	221.29
[EMIM][Amp]	Pale yellow solid	72.0	-17.86	239.64
[C <sub>2</sub> OHMIM][Amp]	Pale yellow solid	117.0	-20.84	246.40

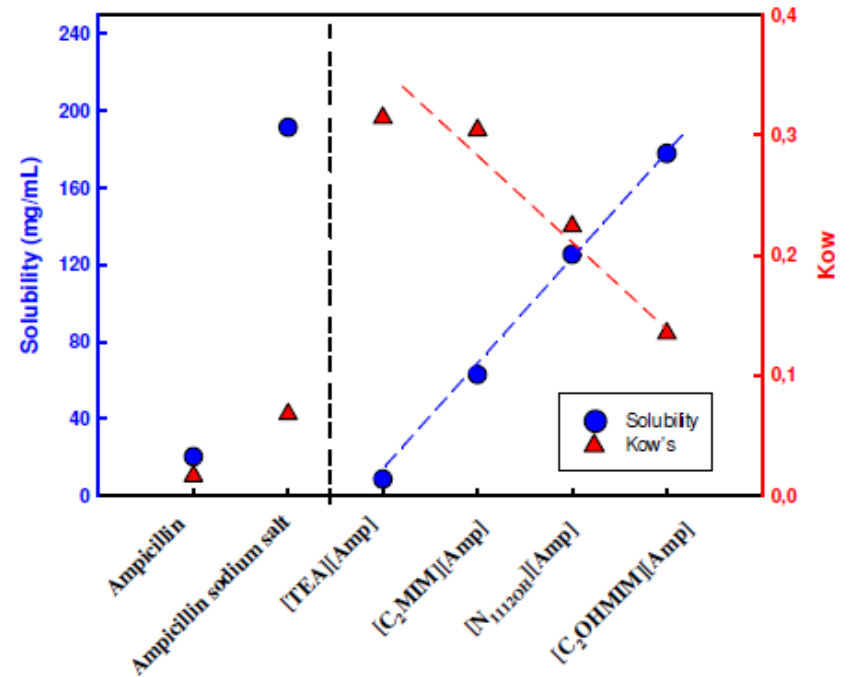


## Water solubility



Tuning of water solubility and octanol-water partition: **tunable bioavailability**

## Water solubility & partition coefficient



**OSIL approach is much more versatile than the traditional salt (Na<sup>+</sup>) approach**

*Int. J. Pharm.* **2013**, 456, 553

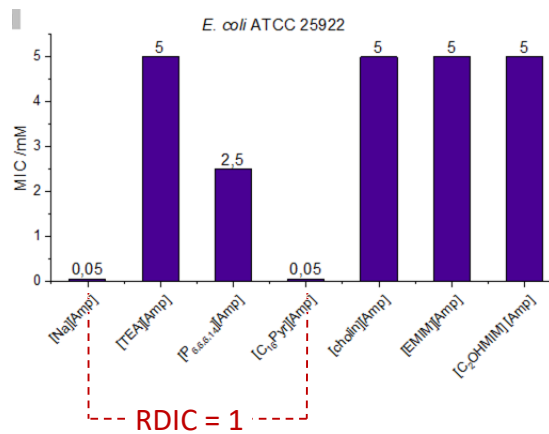
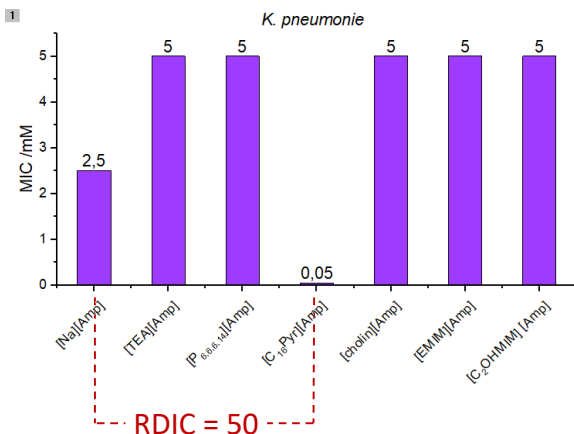


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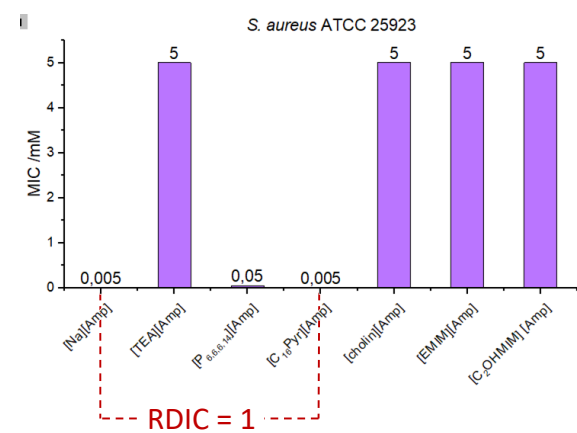
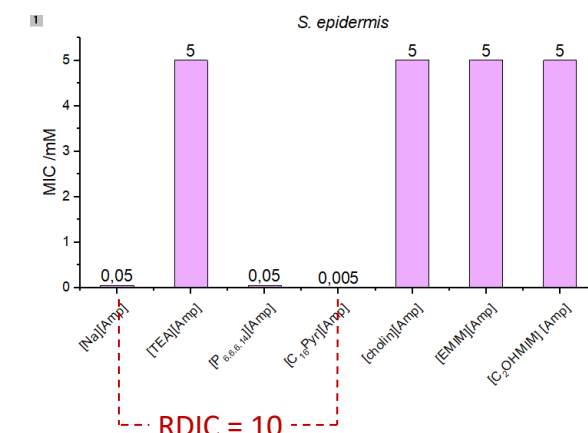
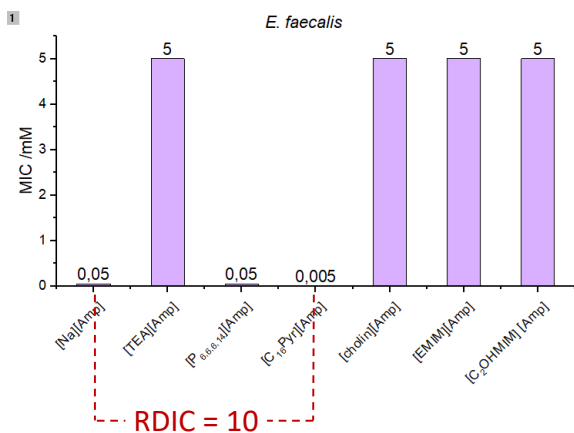
# MICs (mM) of API-OSILs against gram-negative sensitive strains



**10-50x increased activity was found for [C<sub>16</sub>Pyr][Amp]**  
 (Threshold: 5 mM)

RSC Advances 2014, 4, 4301

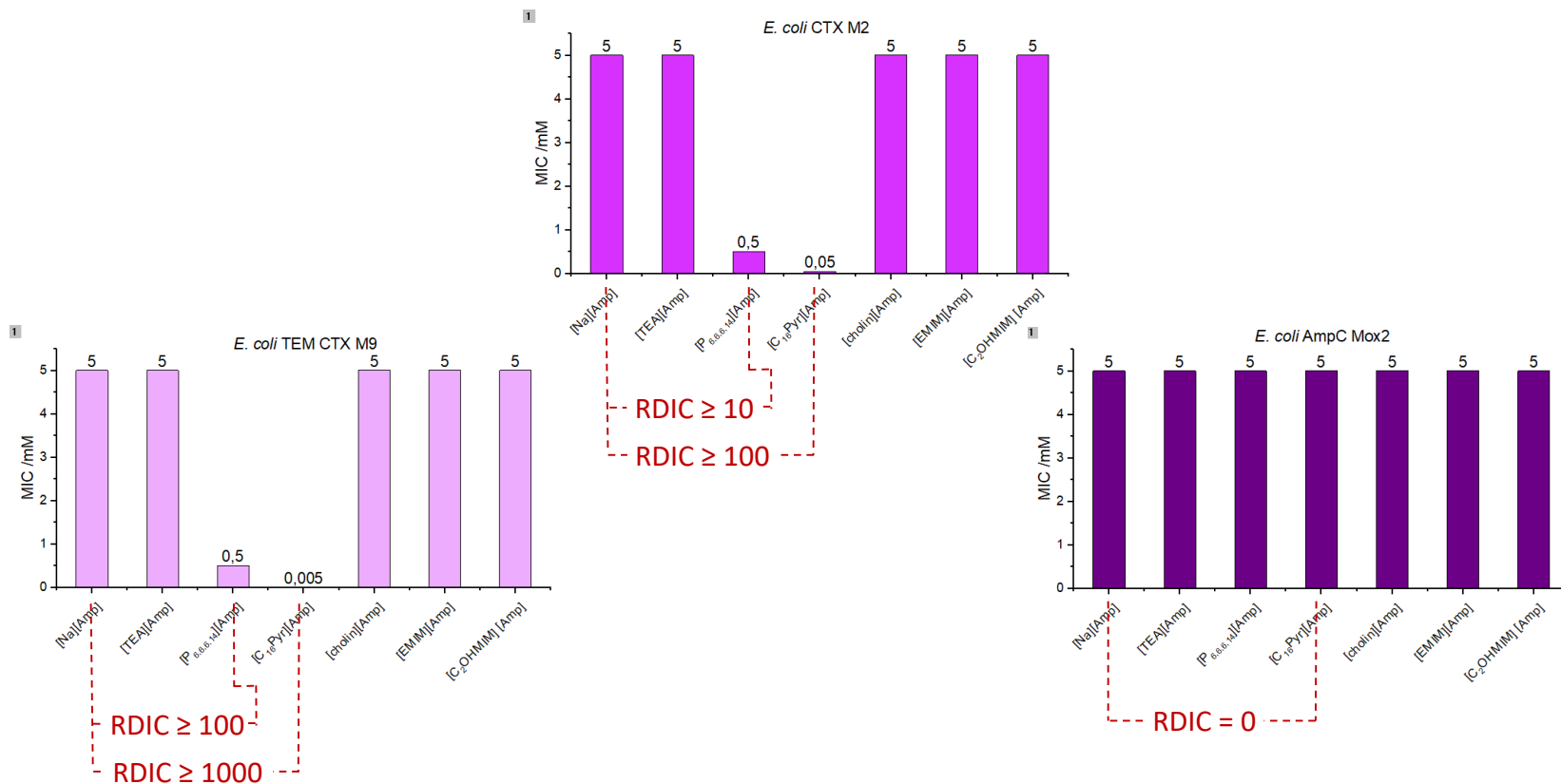
# MICs (mM) of API-OSILs against gram-positive sensitive strains



RDIC: Relative Decrease in Inhibitory Concentration



# MICs (mM) of Amp-OSILs against *E. coli* resistant strains



**[C<sub>16</sub>Pyr][Amp] was at least 100 to 1000 times more efficient against two of the Ampicillin-resistant *E. coli* strains tested in *in vitro* studies**

(Threshold: 5 mM)

RSC Advances 2014, 4, 4301



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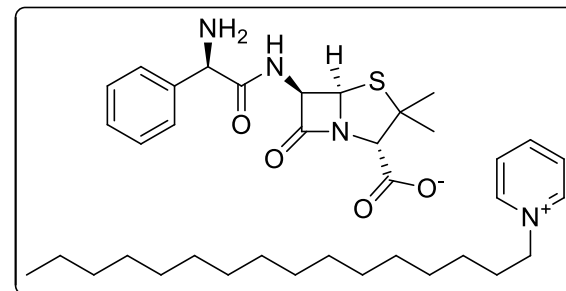
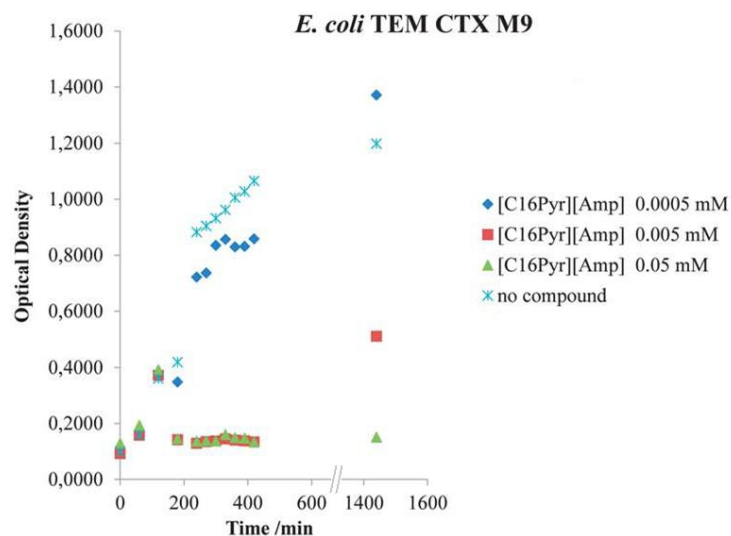
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# Growth inhibition of resistant *E. coli* bacteria strains

The growth of *E. coli* TEM CTX M9 and CTX M2 was efficiently inhibited by [C<sub>16</sub>Pyr][Amp]

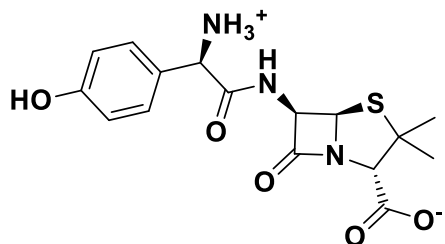


*RSC Advances* **2014**, 4, 4301



# Results and discussion

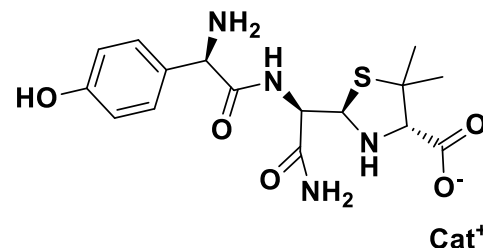
## Penicillin and Amoxicillin



Amoxicillin

1. 1M NH<sub>4</sub>OH, H<sub>2</sub>O-MeOH

2. [Cat<sup>+</sup>]OH<sup>-</sup>



[*seco*-Amx][Cat]

62-95%

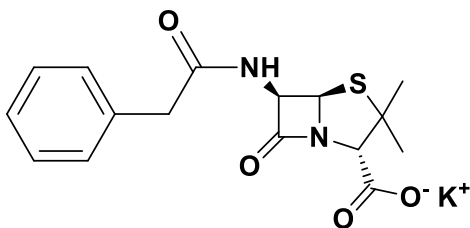
✓ <sup>1</sup>H NMR

✓ <sup>13</sup>C NMR

✓ FTIR

✓ Elemental analysis

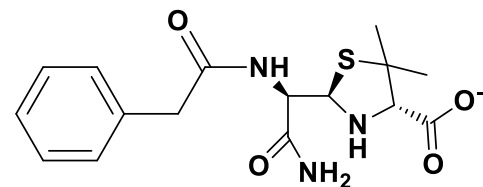
✓ DSC



Potassium Penicillin G

1. (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, H<sub>2</sub>O-MeOH

2. [Cat<sup>+</sup>]OH<sup>-</sup>



[*seco*-Pen][Cat]

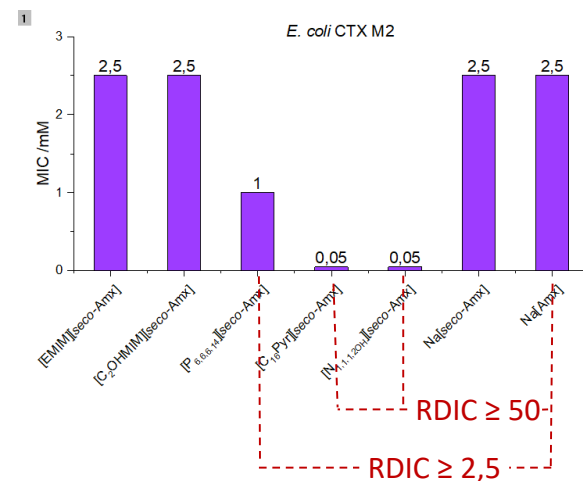
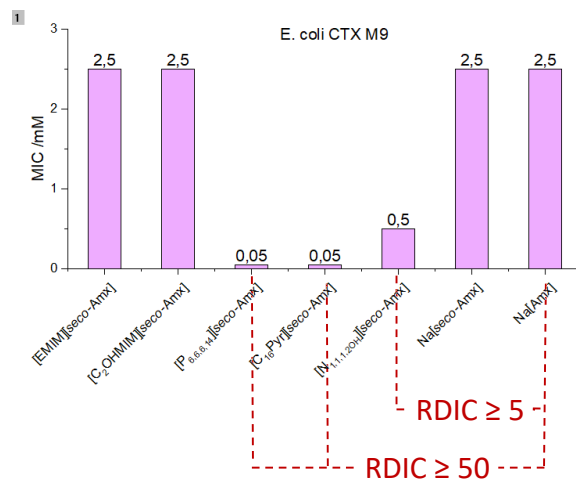
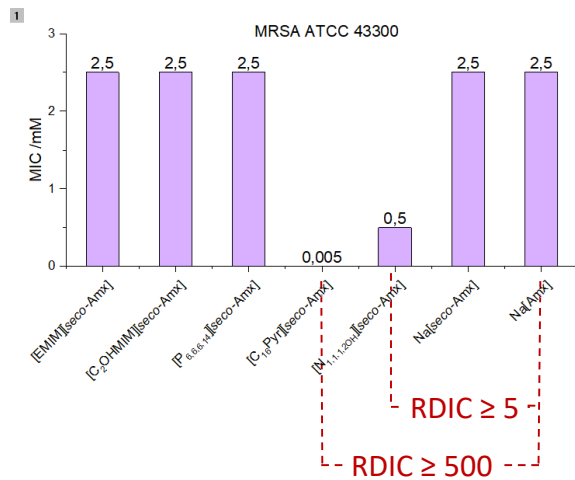
84-98%

Cat<sup>+</sup> = EMIM, C<sub>2</sub>OHMIM, N<sub>1,1,1,2</sub>OH, TEA, P<sub>6,6,6,14</sub>, C<sub>16</sub>Pyr, Na, K.

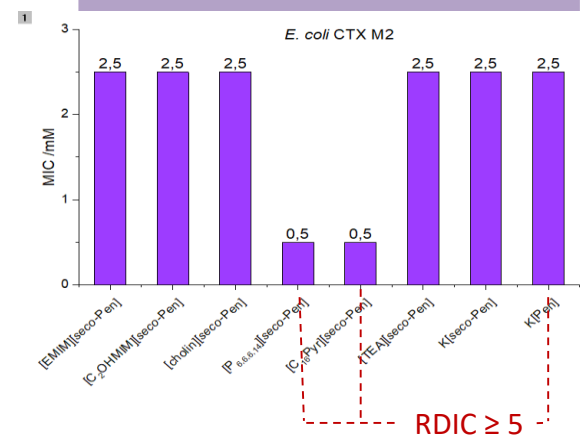
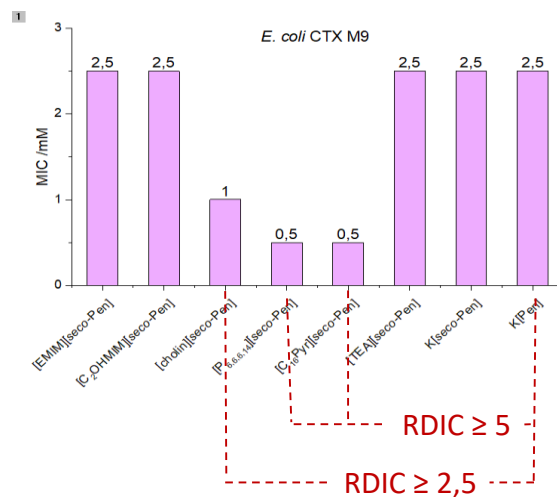
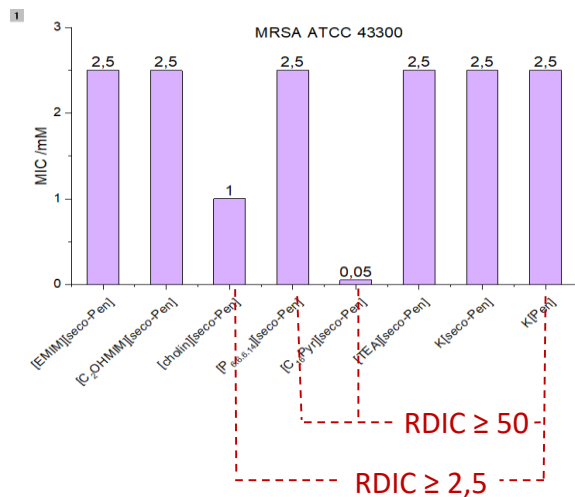
Using the same (for Amoxicillin) or a different (for Penicillin G) procedure, hydrolyzed (*secondary*) β-lactam antibiotic cations were obtained



## However, against resistant bacteria...



(Threshold: 2,5 mM)





# Conclusions

Using a simple and straightforward neutralization procedure, we were able to:

- Synthesize six Amp-OSILs, five *seco*Amx-OSILs and six *seco*Pen-OSILs;
- The  $\beta$ -lactam ring was conserved in Amp, while on the other two families it was disrupted;
- Amp polymorphism was eliminated, while water solubility and  $K_{ow}$  can be modulated according to the cation-anion combination;
- Against sensitive bacteria,  $[C_{16}Pyr][Amp]$  was found to be 10-50 times more efficient than  $Na[Amp]$ ;
- $[C_{16}Pyr][Amp]$  showed a relative decrease in inhibitory concentration (RDIC) between at least 100 to 1000 towards *E. coli* resistant strains;
- $[C_{16}Pyr][secoAmx]$  and  $[C_{16}Pyr][secoPen]$  were particularly effective against MRSA (RDIC  $\geq 500$  and  $\geq 50$ );
- The activity of *seco*Amx and *seco*Pen OSILs was surprising but it is not unprecedented - reversible inactivation of  $\beta$ -lactam antibiotic mediated by enzyme active site of PBPs in *Enterococcus faecium* was recently described (see Edoo, Z. *et al. Scientific Report* **2017**, 7: 9136);
- We are optimizing the structure of the cations in order to further enhance the antimicrobial activity of these antibiotics, and we are currently determining MICs for Amp-OSILs towards MRSA in addition to PBP2a – API-OSILs interaction studies for a deeper understanding of the action mechanism
- We have optimized the procedure for the preparation of Amx-OSILs and further studies are underway.



# Acknowledgments

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