5th International Electronic Conference on Medicinal Chemistry

1-30 November 2019

chaired by Dr. Jean Jacques Vanden Eynde

sponsored by pharmaceuticals

Tackling bacterial resistance using antibiotics as ionic liquids and organic salts

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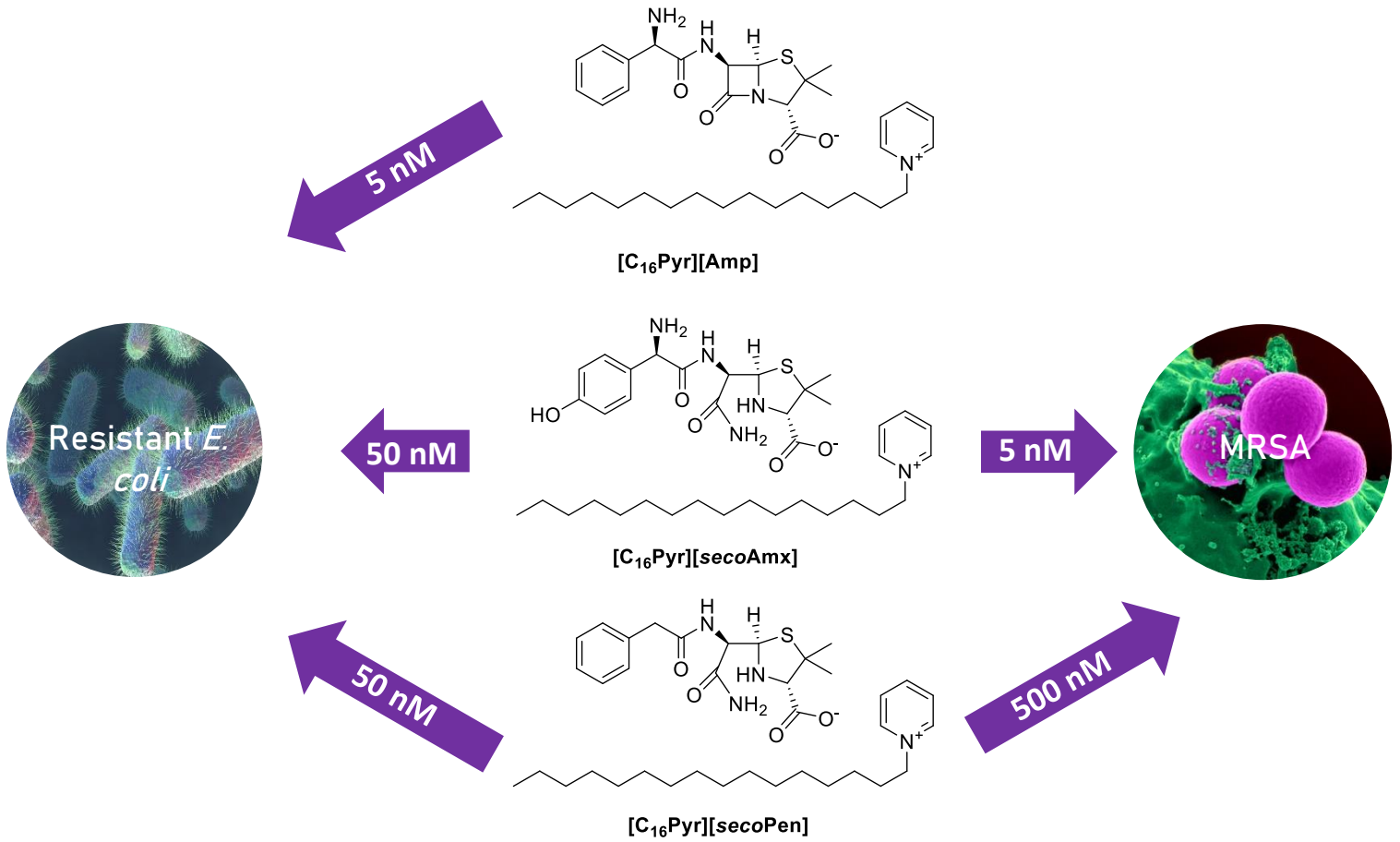
Tackling bacterial resistance using antibiotics as ionic liquids and organic salts

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.

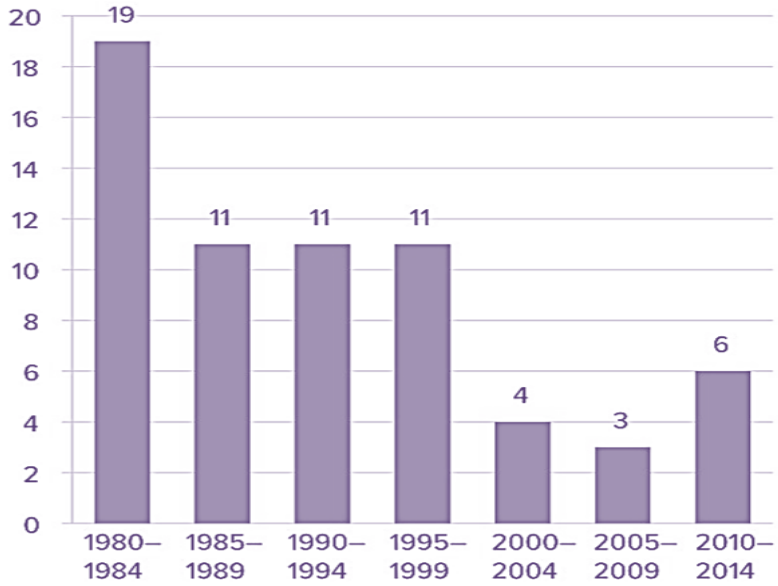
Ionic Liquids and Organic Salts from Active Pharmaceutical Ingredients (API-OSILs) have risen in academia for over 10 years as an efficient formulation for drugs with low bioavailability and permeability, as well as reduction or elimination of polymorphism, thereby potentially enhancing their pharmaceutical 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; β-lactam antibiotics; Ionic Liquids; MRSA

Introduction

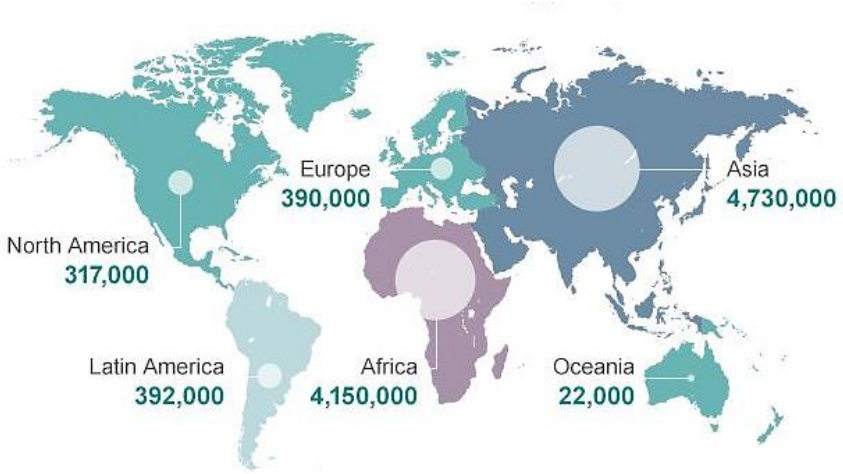
Approved # of antibiotics since 1980



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



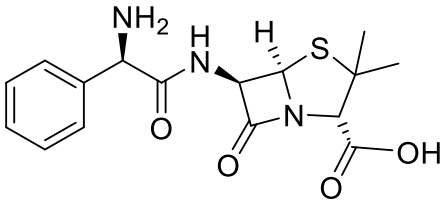
Reproduced from *Review on Antimicrobrial Resistance 2014*

10 million deaths by 2050

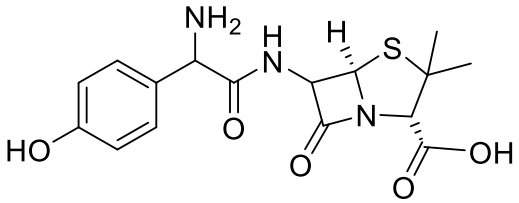
75b€ associated costs

Growing need for more effective antibiotics

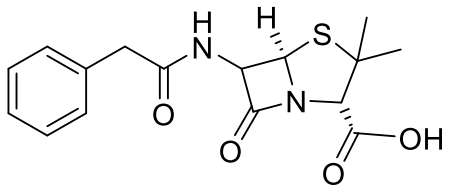
Introduction



Ampicillin



Amoxicillin



Penicillin

Bacteria resistance to β-lactam antibiotics

β-lactamases enzymatic degradation

Mutations in porine channels (*Gram*-negative)

Efflux pumps (*Gram*-negative)

Mutations in PBPs

Degradation of the β-lactam ring

Altered binding site

Low affinity for such antibiotics

Protein is no longer inhibited

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

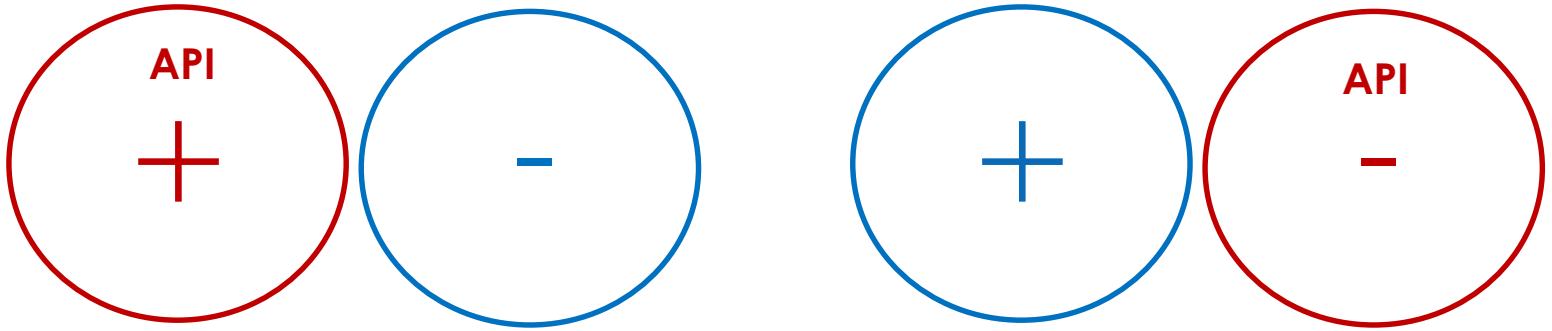
IL

Tuneable solubility of the IL in aqueous or organic solvents

Negligible vapour pressure

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

3RD GENERATION IONIC LIQUIDS



New physical, chemical and biochemical properties

Modulate biopharmaceutical drug classification

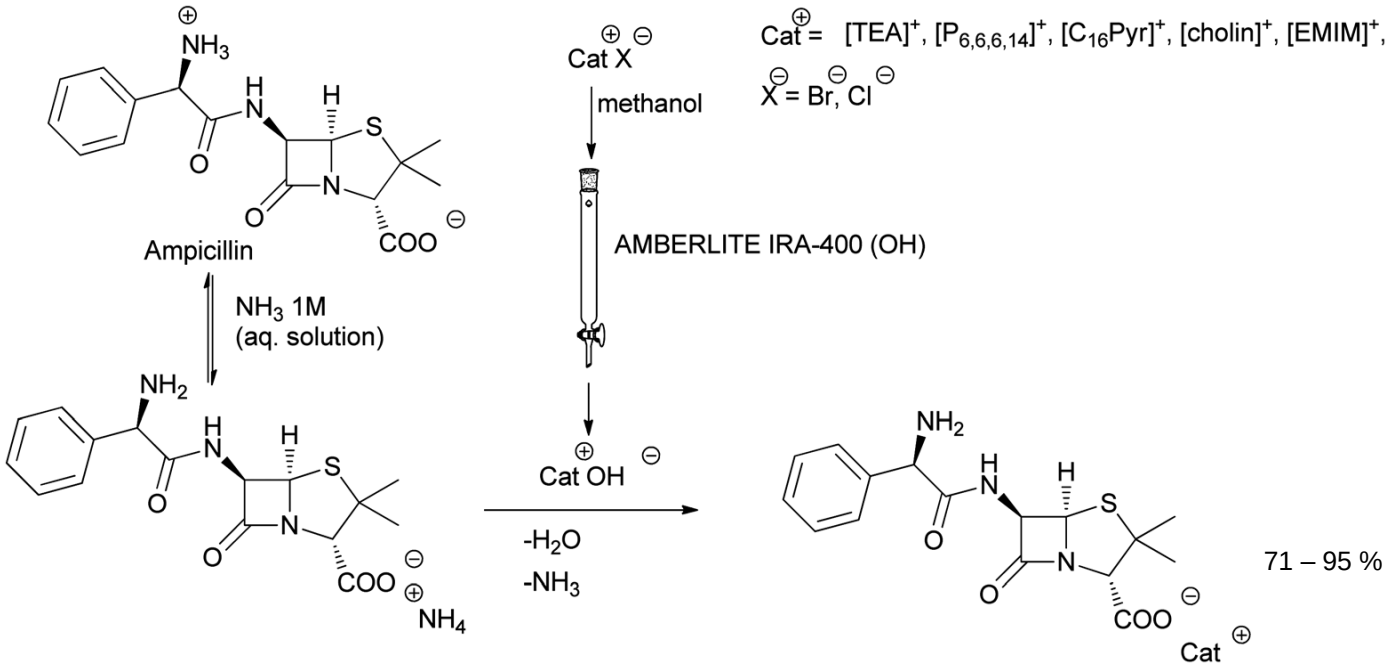
- Water solubility
- Permeability
- Drug formulation
- Toxicity and metabolism

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

Results and discussion

Ampicillin

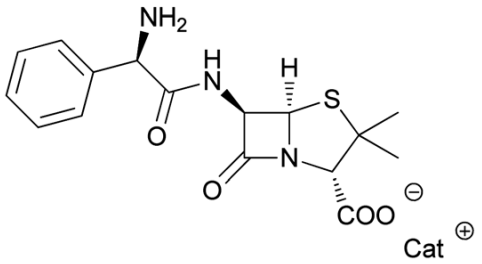
Neutralization method

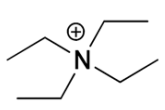


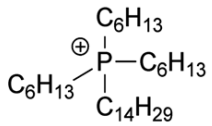
The hydroxide cation is prepared by passing a methanolic solution of halide salt through an ion-exchange column and subsequently added to ampicillin in 1M ammonium buffer solution.

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

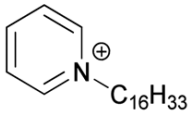
Thermal Properties of Ampicillin-OSILs



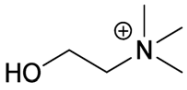

[TEA]+



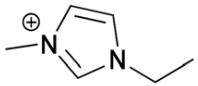
[P6,6,6,14]+



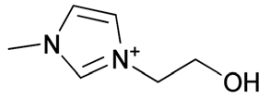
[C16Pyr]+



[cholin]+



[EMIM]+

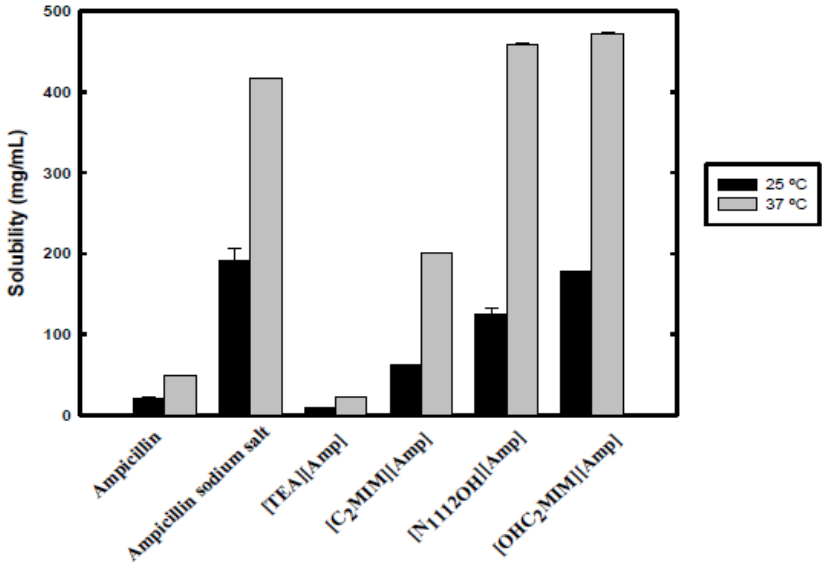


[C2OHMIM]+

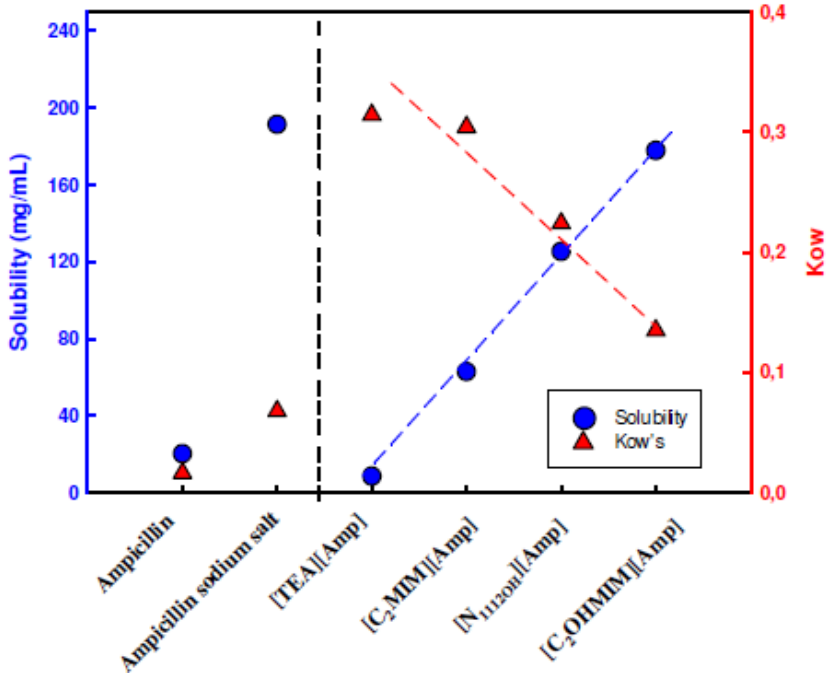
✓ ^{1}H NMR ✓ ^{13}C NMR ✓ FTIR ✓ Elemental analysis ✓ DSC

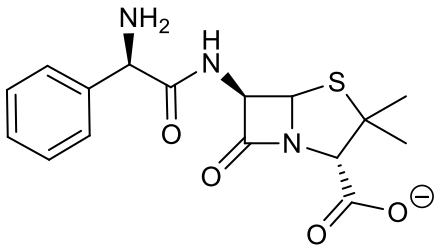
Compound	Physical State	T_m[a] [°C]	T_g[b] [°C]	T_{dec}[c] [°C]
[TEA][Amp]	Pale yellow solid	79.0	-18.64	214.75
[$P_{6,6,6,14}$][Amp]	Yellow viscous liquid	-	-	297.65
[C_{16}Pyr][Amp]	Pale yellow solid	86.0	-19.64	269.39
[cholin][Amp]	Pale yellow solid	58.0	-20.12	221.29
[EMIM][Amp]	Pale yellow solid	72.0	-17.86	239.64
[C_2OHMIM] [Amp]	Pale yellow solid	117.0	-20.84	246.40

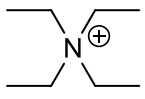
Water solubility



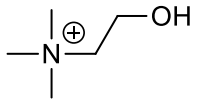
Water solubility & partition coefficient



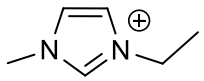




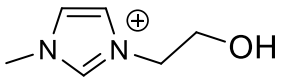
[TEA]$^+$



[cholin]$^+$



[EMIM]$^+$



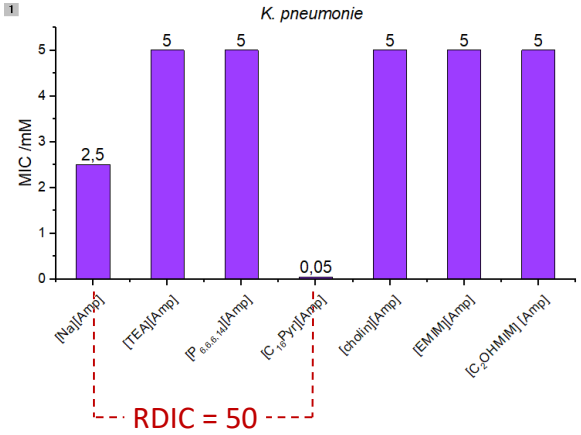
[C_2OHMIM]$^+$

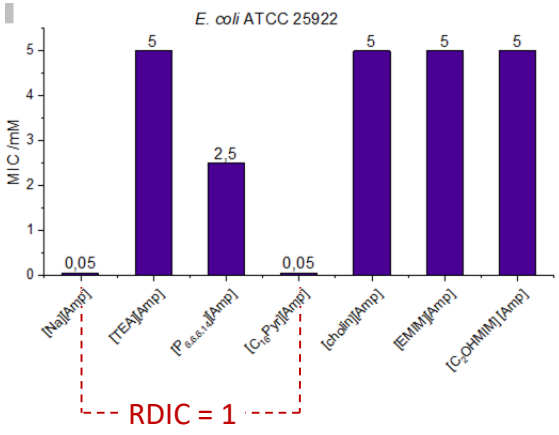
Tuning of water solubility and octanol-water partition: ***tunable bioavailability***

OSIL approach is much more versatile than the traditional salt (Na^+) approach

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

MICs (mM) of API-OSILs against *gram*-negative <u>sensitive</u> strains



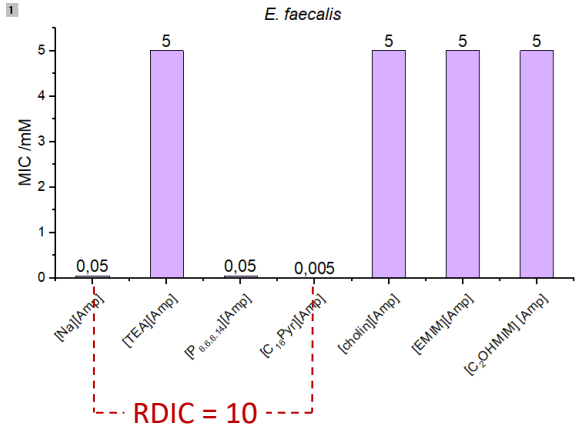


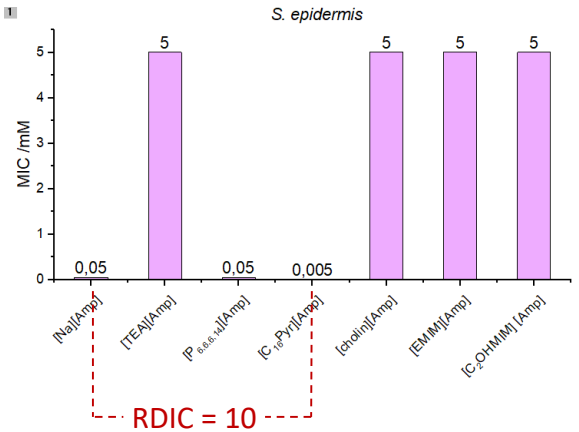
10–50x increased activity was found for $[C_{16}Pyr][Amp]$

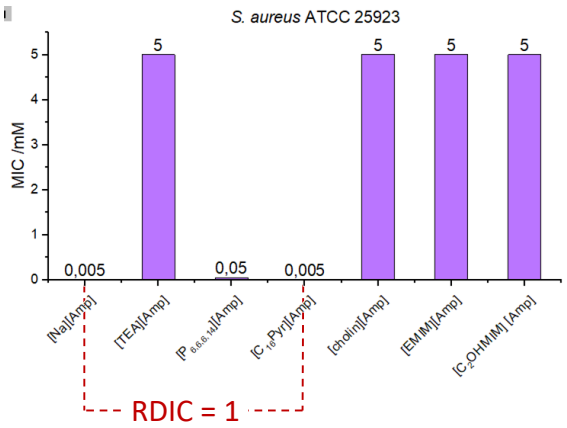
(Threshold: 5 mM)

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

MICs (mM) of API-OSILs against *gram*-positive <u>sensitive</u> strains

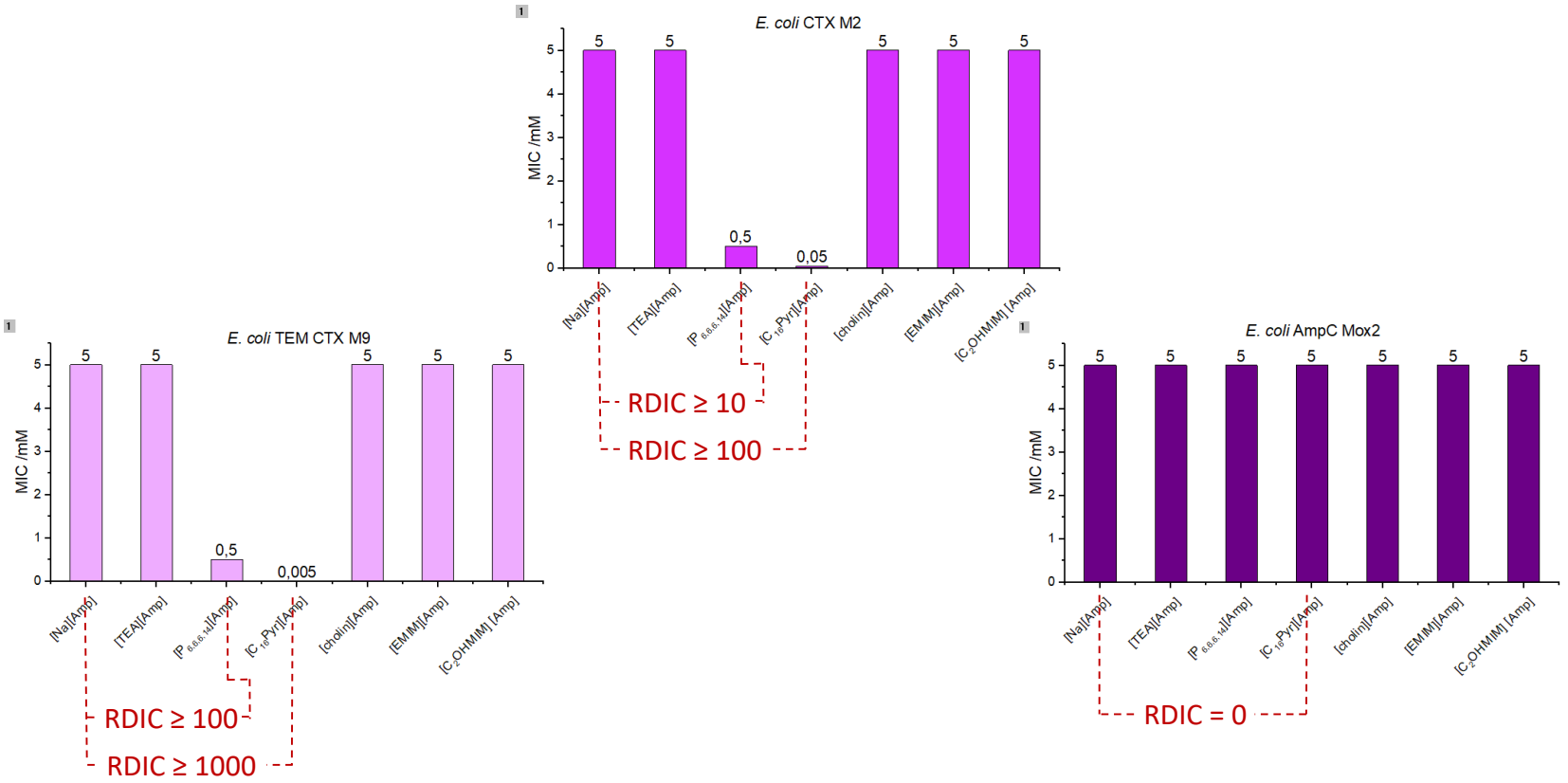






RDIC: Relative Decrease in Inhibitory Concentration

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



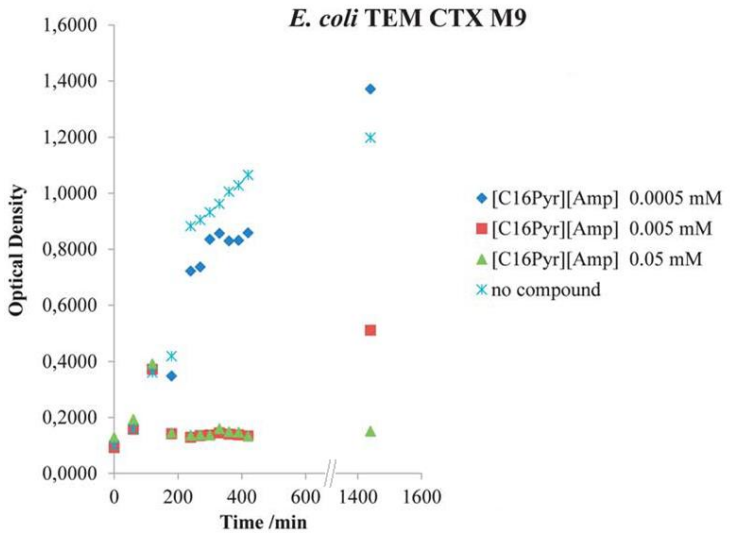
[C_{16}Pyr][Amp] was at least 100 to 1000 times more efficient against two of the Ampicillin-*resistant* E. coli strains tested in *in vitro* studies

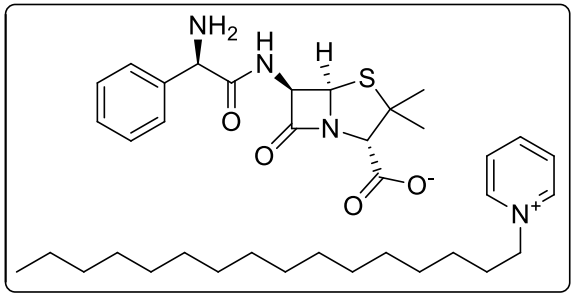
(Threshold: 5 mM)

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

Growth inhibition of resistant *E. coli* bacteria strains

The growth of *E. coli* TEM CTX M9 and CTX M2 was efficiently inhibited by [C_{16}Pyr][Amp]

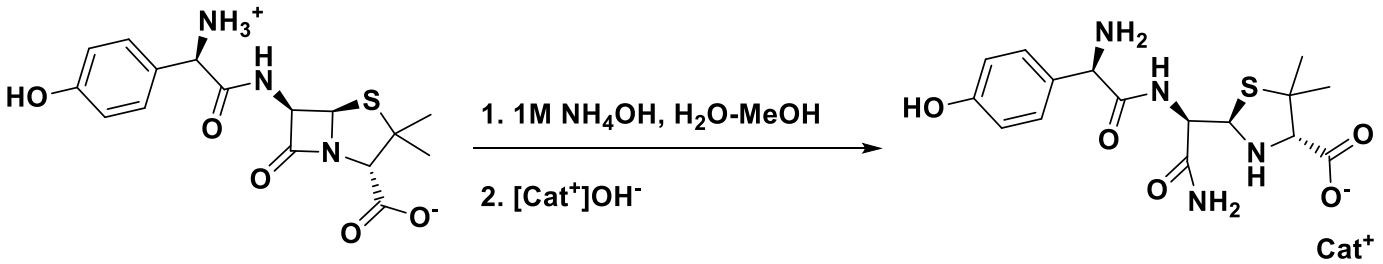




RSC Advances **2014**, *4*, 4301

Results and discussion

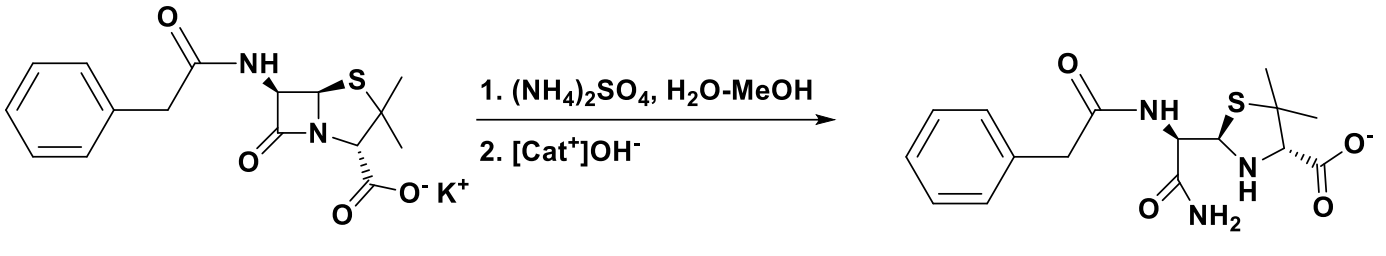
Penicillin and Amoxicillin



Amoxicillin

[*seco*-Amx][Cat]

62-95%



Potassium Penicilin G

[*seco*-Pen][Cat]

84-98%

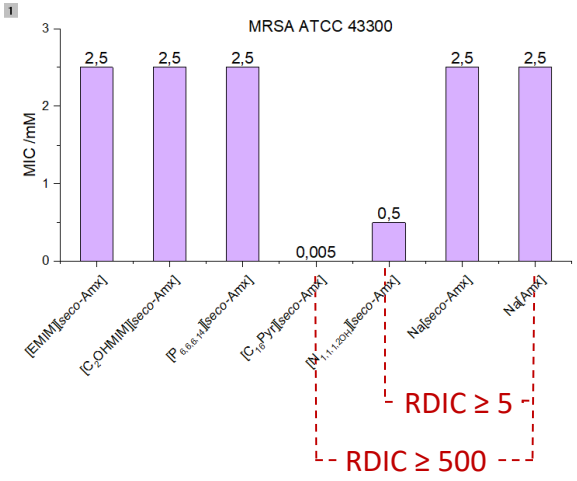
- ✓ ^{1}H NMR
- ✓ ^{13}C NMR
- ✓ FTIR
- ✓ Elemental analysis
- ✓ DSC

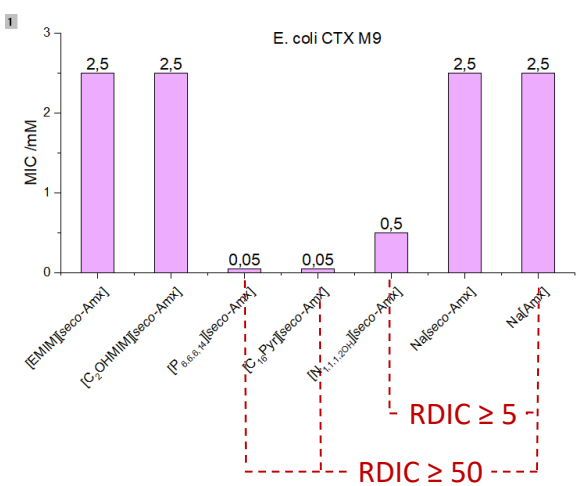
Cat^+ = EMIM, C_2OHMIM, $N_{1,1,1,2OH}$, TEA, $P_{6,6,6,14}$, $C_{16}Pyr$, Na, K.

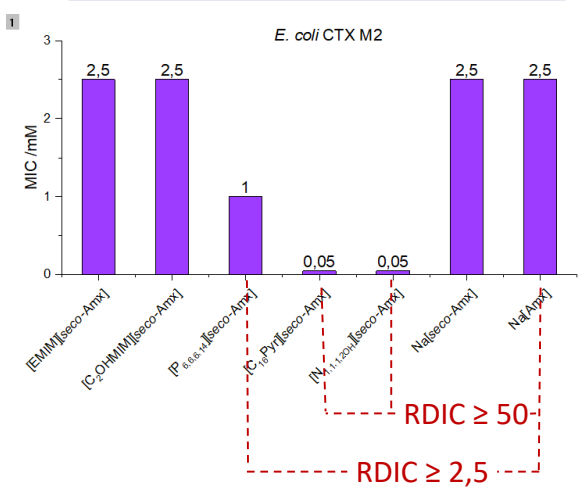
Using the same (for Amoxicillin) or a different (for Penicillin G) procedure, hydrolized (*secondary*) β-lactam antibiotic cations were obtained

However, against resistant bacteria…

Amoxicillin-OSILs

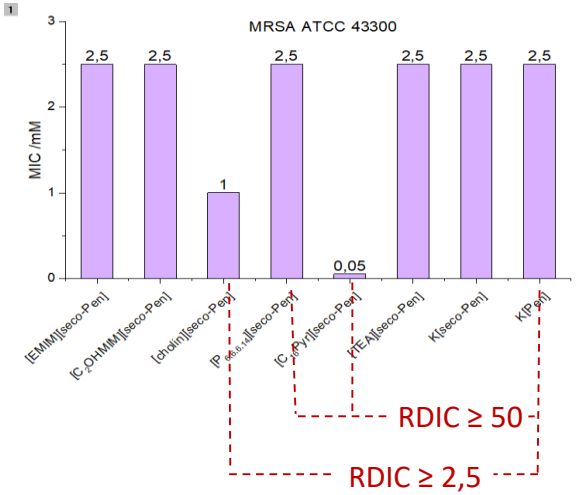


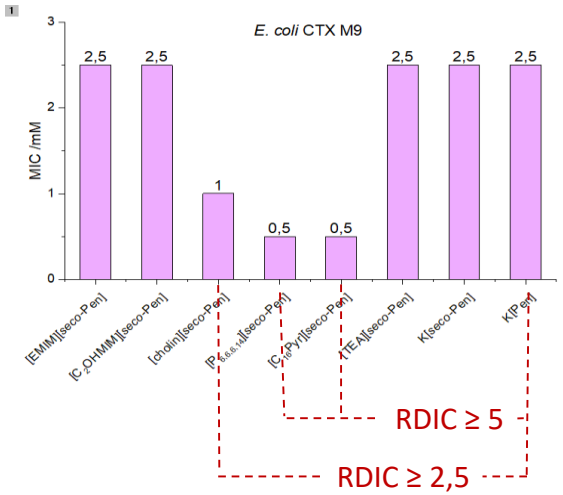


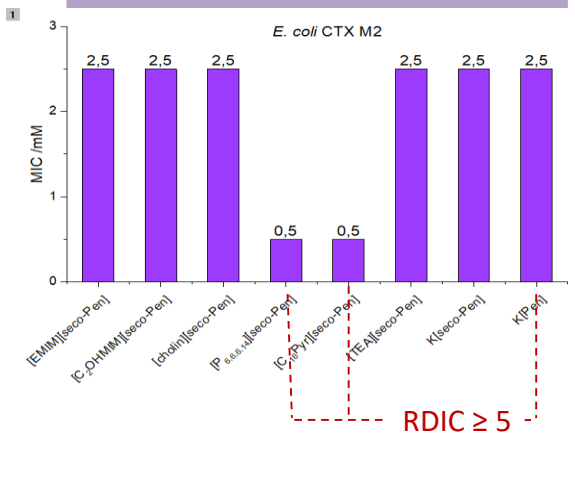


(Threshold: 2,5 mM)

Penicillin-OSILs







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 β-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][*seco*Amx] and [C_{16}Pyr][*seco*Pen] were particularly effective against MRSA (RDIC ≥ 500 and ≥ 50)
- The activity of *seco*Amx and *seco*Pen OSILs was surprising but it is not unprecendent - reversible inactivation of β-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

Work supported by FCT-MCTES (PTDC/QUI-QOR/32406/2017, PTDC/BIA-MIC/31645/2017, PEst-C/LA0006/2013, IF/0041/2013/CP1161/CT00), by the Associate Laboratory for Green Chemistry LAQV and by the Unidade de Ciências Biomoleculares Aplicadas-UCIBIO which are financed by national funds from FCT/MCTES (UID/QUI/50006/2013 and UID/Multi/04378/2013, respectively) and co-financed by the ERDF under the PT2020 Partnership Agreement (POCI-01-0145-FEDER-007265 and POCI-01-0145-FEDER-007728, respectively). Authors also thank Solchemar.