6th International Electronic Conference on Medicinal Chemistry
1-30 November 2020
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Antitumor and osteogenic activity of bisphosphonate-based Organic Salts and Ionic Liquids

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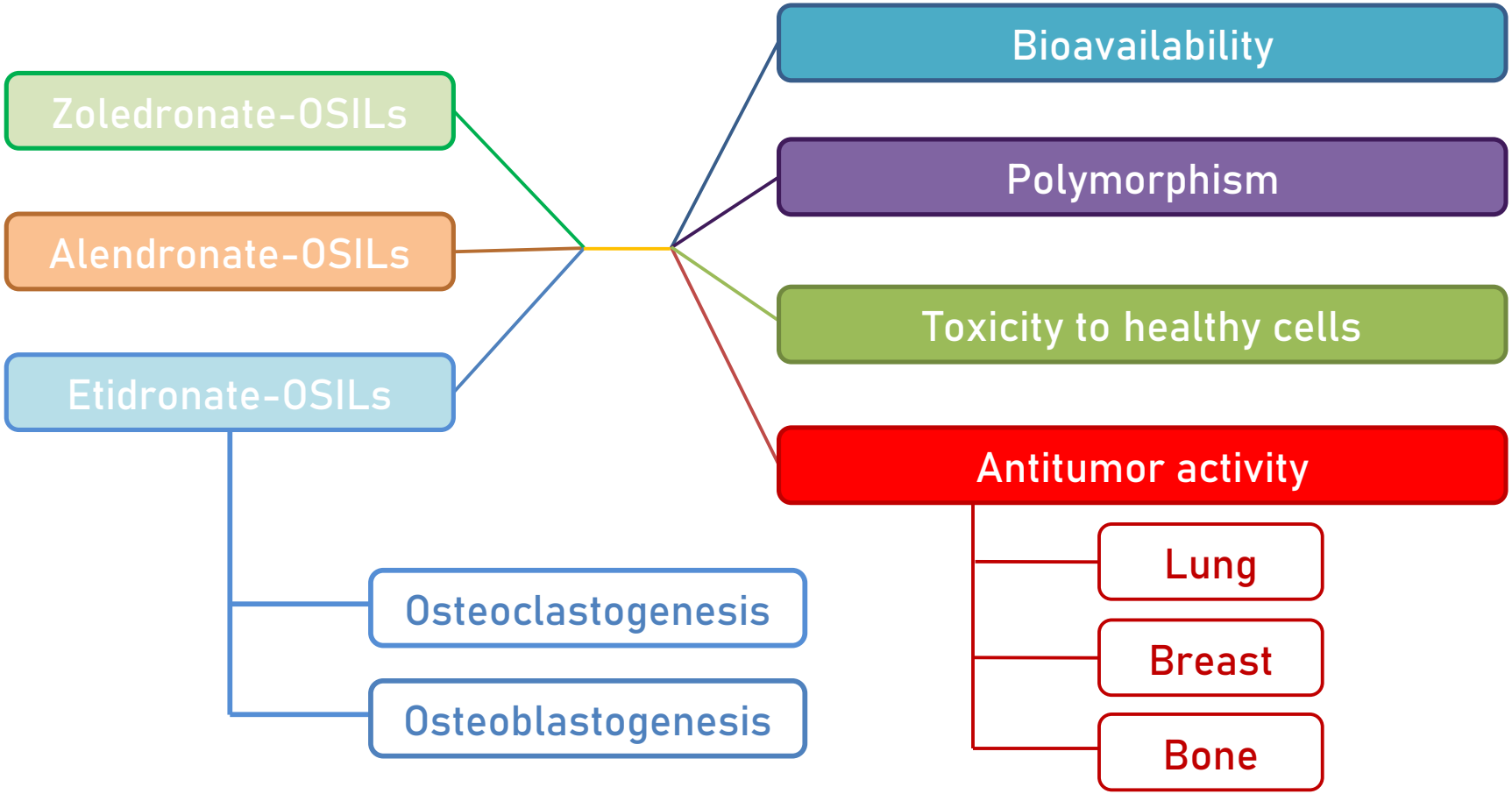
Antitumor and osteogenic activity of bisphosphonate-based Organic Salts and Ionic Liquids



Abstract: Osteoclast-mediated bone loss disorders are chronically treated with bisphosphonates (BPs). In addition, they have recently shown potential antitumor activity. However, BPs suffer from several drawbacks such as polymorphism and low bioavailability which are related with the common side effects (e.g. muscle, joint and bone pain, numbness) associated with these drugs. Thus, there is a need to develop new ways to increase BPs' bioavailability while reducing toxicity. Active Principle Ingredients as Organic Salts and Ionic Liquids (API-OSILs) has been one of the focus of our group over the last years. The combination of drugs as anions or cations with biocompatible organic counter ions has proven to be an innovative approach to tackle drug polymorphism as well as to improve water solubility, permeability and corresponding bioavailability and biological activity. In this communication, we report the preparation of anionic etidronate, alendronate and zoledronate-based BP-OSILs in quantitative yields. The polymorphic profile of the prepared BP-OSILs and their solubility in water and biological fluids, as well as toxicity towards human healthy and lung, breast and bone cancer cell lines will be presented. Finally, the effect of etidronate-OSILs on osteoblast- and osteoclastogenesis will also be disclosed.

Keywords: API-OSILs; Antitumor; Bioavailability; Bisphosphonates; Osteogenesis.

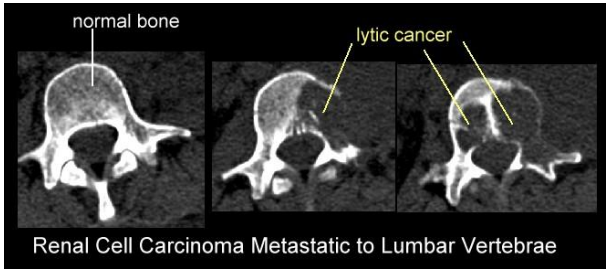
OSTEOCLASTS
H^+ erode hydroxyapatite
Cathepsin K
Proteases

OSTEOBLASTS
Collagen type 1
Hydroxyapatite

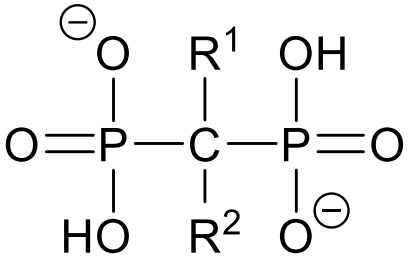
Osteoporosis

Paget's disease

Bone osteolytic metastases

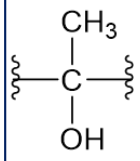
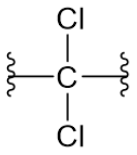
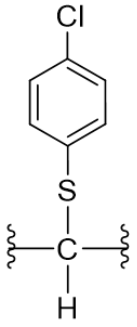
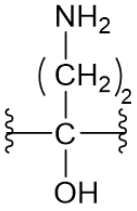
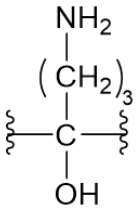
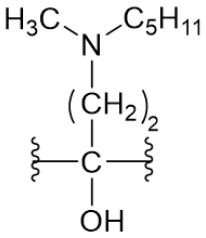
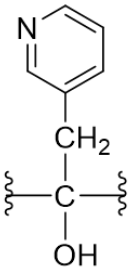
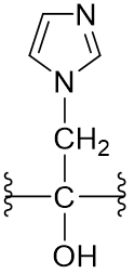


Anti-bone resorption drugs



bisphosphonates (BPs)

- Resistant to hydrolysis
- Ability to functionalize
- Enhanced affinity for calcium from hydroxyapatite
- Inhibit bone resorption

Relative potency							
							
etidronate	clodronate	tiludronate	pamidronate	alendronate	ibandronate	risedronate	zoledronate
1	10	10	100	500	1000	2000	10000

Pharmacokinetics

Bisphosphonate	Oral Bioav.	Food Effect	Metab	Vd	PPB	Urine	Plasma Clr	Terminal $T_{1/2}$
Alendr	0.7%	Decr	None	28L	78%	50%		10 years
Etidron	1-6%	Decr	None	1.4L/kg		30-50%	6 hrs	>90days
Pamidr	NA?	NA	None			51%		>300 days
Risedr	0.7%	Decr	None	6.3L/kg	24%	50%		
Tiludr	6%	Decr	Very Little					

Organic Salts and Ionic Liquids from Bisphosphonates

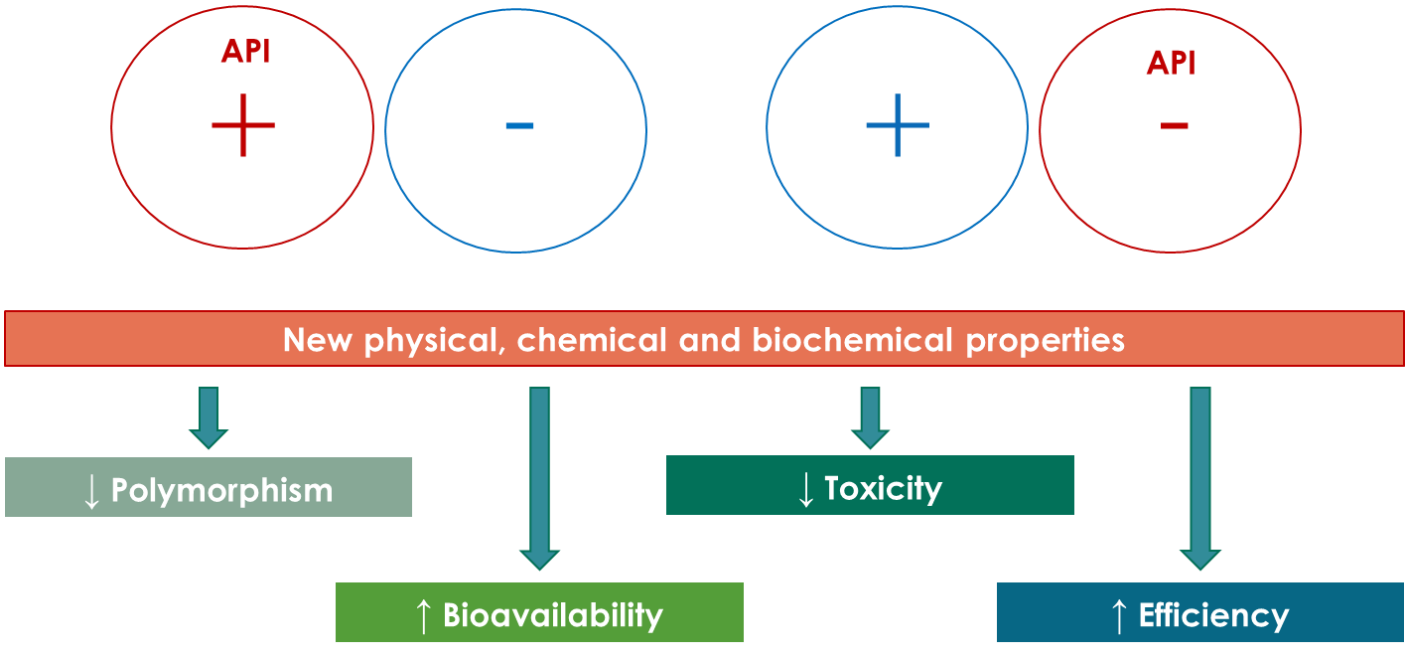
Common side effects

- muscle, joint and bone pain
- muscle spasms
- numbness

Polymorphic profile

Different crystalline forms with distinct pharmacological effects

Third generation of Ionic Liquids



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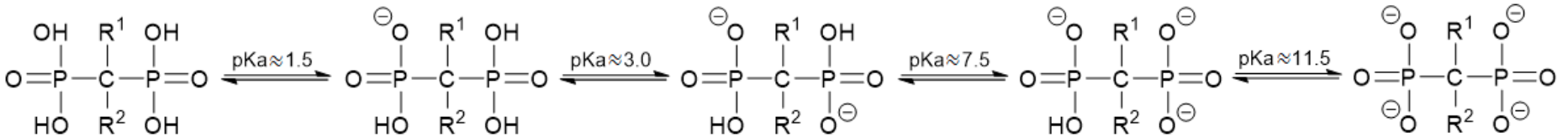
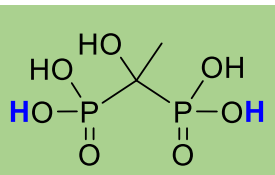
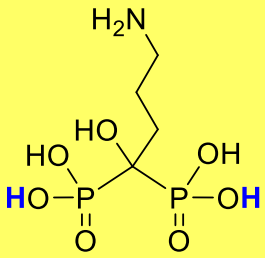


METHODOLOGY



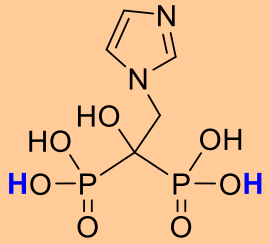
etidronic acid

Chem. Sci., submitted



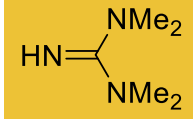
alendronic acid

Pharmaceutics **2020**, *12*, 293

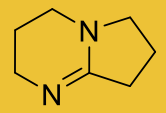


zoledronic acid

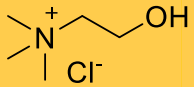
ChemMedChem **2019**, *14*, 1767



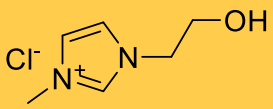
[TMG]



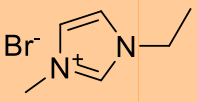
[DBN]



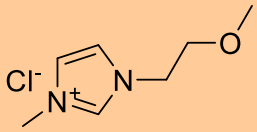
[Ch]Cl



[C_2OHMIM]Cl



[EMIM]Br



[C_3OMIM]Cl

direct deprotonation

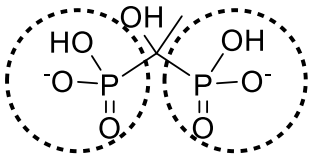
Cation$^+$X$^-$

A-26(OH)

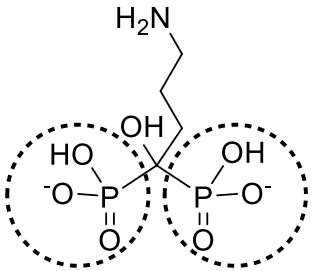
Cation$^+$OH$^-$

quantitative yields

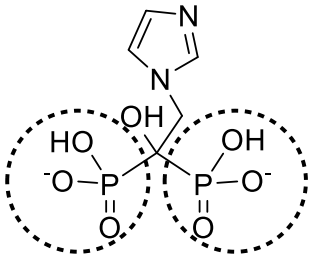
- H_2O



[ETI]



[ALN]

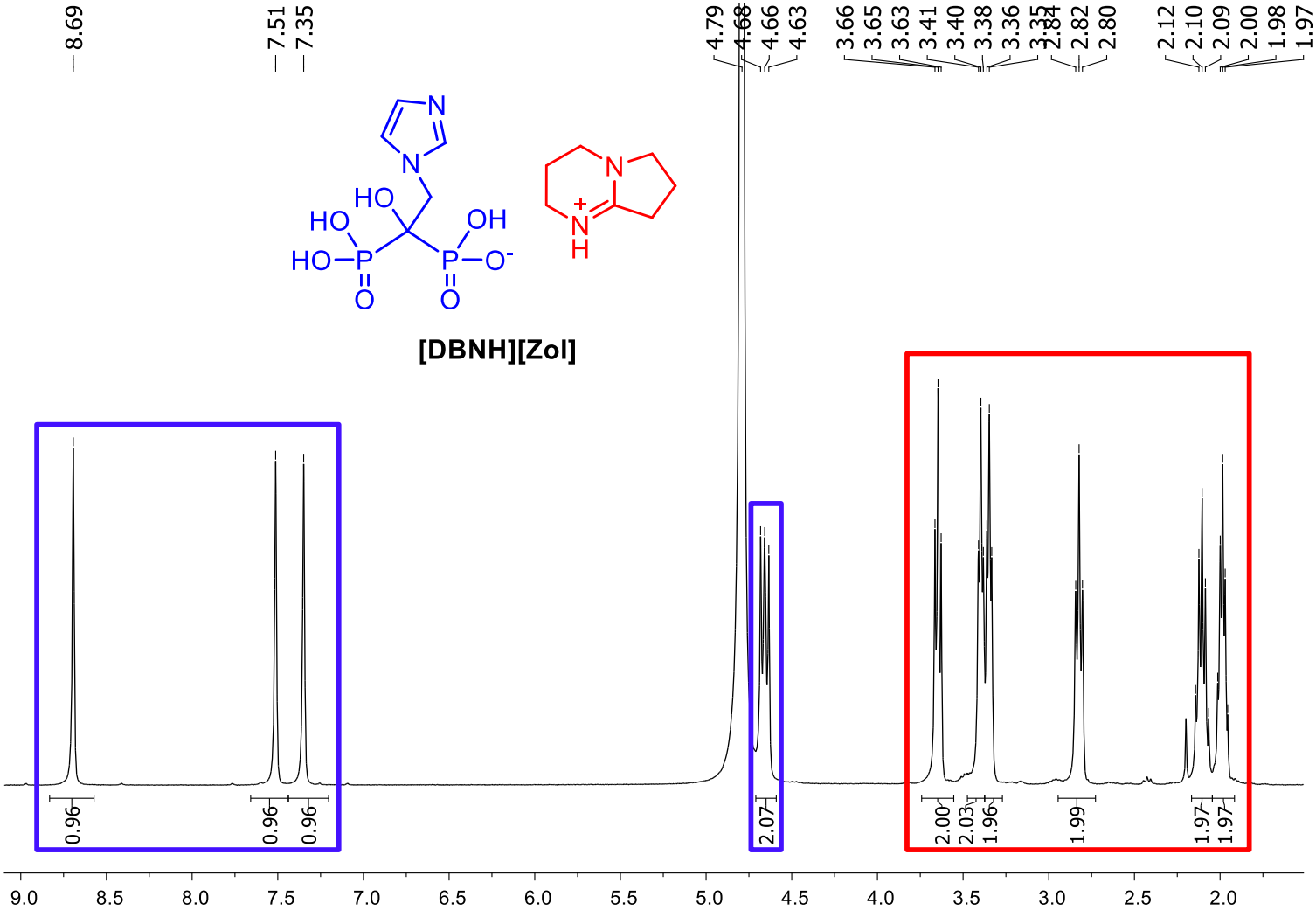


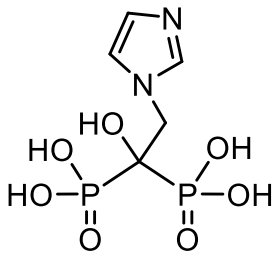
[ZOL]

monoanion *or* ***dianion***

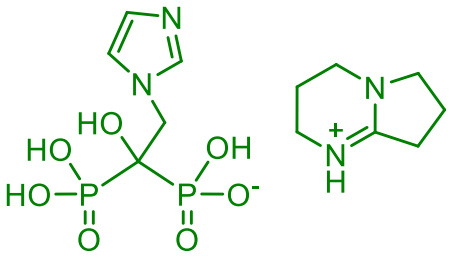
- ✓ NMR
- ✓ FTIR
- ✓ EA
- ✓ DSC

^{1}H NMR SPECTROSCOPY

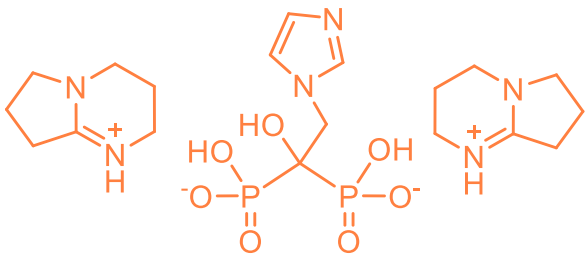




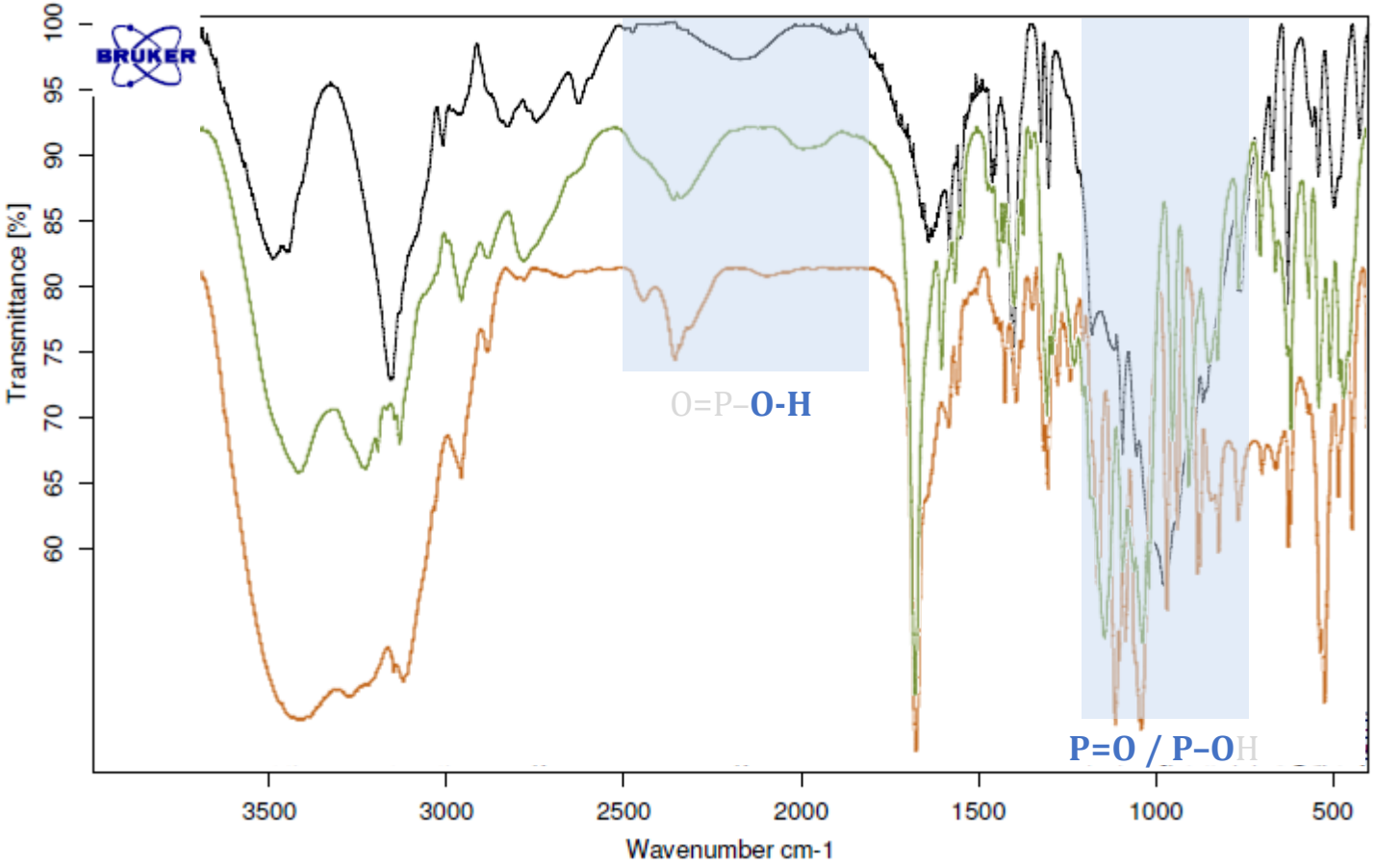
zoledronic acid



[DBNH][Zol]

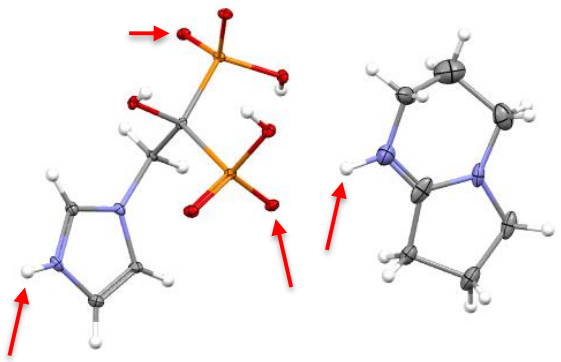


$[DBNH]_2[Zol]$



Salt	Physical state	T_m /°C	T_c /°C	T_g /°C
ETI	White solid	199.0	-	-
[TMGH][ETI]	White solid	166.6;189.9	-	38.5
[DBNH][ETI]	White solid	195.2	-	27.7
Na[ALN]	White solid	259.3	-	-
[TMGH][ALN]	White solid	48.1;162.7	107.1*	-
[DBNH][ALN]	White solid	130.3;133.2	-	-
[C_2OHMIM][ALN]	Colorless paste	-	-	64.5
[Ch][ALN]	White solid	141.2	-	74.9
ZOL	White solid	214.0; 230.0	-	-
[TMGH][ZOL]	White solid	225.3	-	-
[DBNH][ZOL]	White solid	208.7	-	45.7
[Ch][ZOL]	White solid	220.4	-	78.4
[EMIM][ZOL]	White solid	198.0	-	29.5
[C_2OHMIM][ZOL]	White solid	143.8;195.9	170.1*	57.3
[C_3OMIM][ZOL]	White solid	125.9;185.0	139.8*	45.7

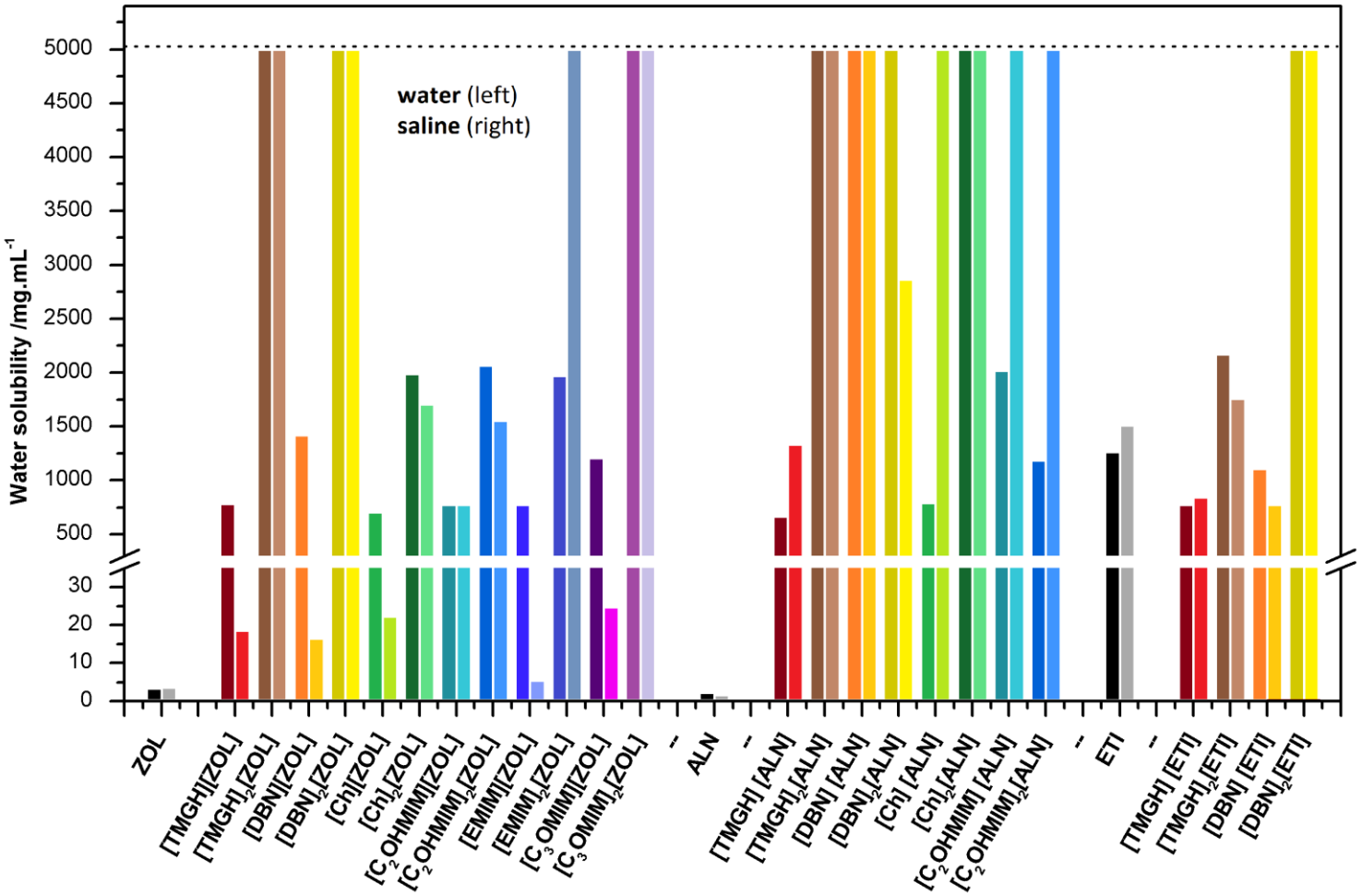
* Cold crystallization



[DBNH][Zol]
one cationic DBN unit and one zwitterionic Zol entity bearing two anionic phosphonate groups and one cationic imidazolium moiety.

- 11 monoanionic solid salts and 1 RTIL
- Lower T_m than parent drugs
- 7 non-polymorphic
- 11 dianionic RTILs and 1 salt

Solubility studies



BP-OSILs >>> BPs

dianions > monoanions

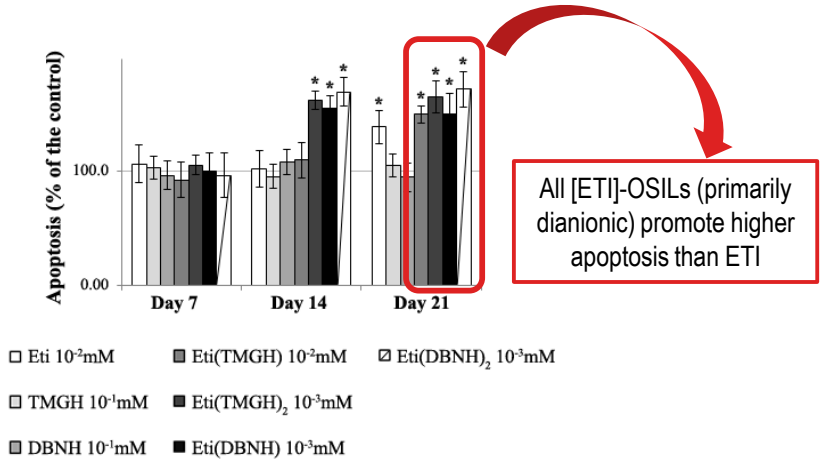
ALN-OSILs > ZOL-OSILs ≈ ETI-ILs

19 compounds are fully soluble

Compound	IC_{50} /mM		
	Fibroblasts	T47D	MG63
Pactitaxel	1.91×10^{-5}	6.46×10^{-6}	8.19×10^{-6}
ETI	15.6	48.9	61.1
[TMGH][ETI]	**n.d.**	**2.7×10^{-7}**	1.6
$[TMGH]_2[ETI]$	1.4×10^{-3}	9.1×10^{-4}	12.0
[DBNH][ETI]	**11.4**	**9.3×10^{-4}**	**2.0×10^{-3}**
$[DBNH]_2[ETI]$	**18.6**	n.d.	**2.0×10^{-3}**

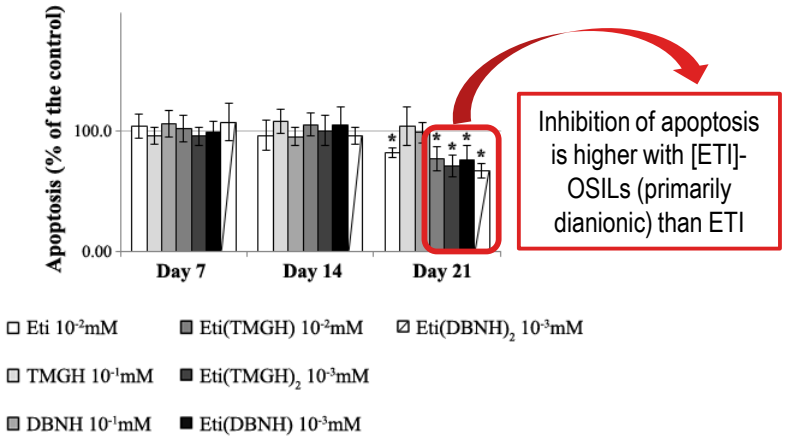
Peripheral Blood Mononuclear Cells (PBMC) as precursors of osteoclasts

❶ *Apoptosis quantification (caspase-3 activity)*



Human Mesenchymal Stem Cells (HMSC) as precursors of osteoblasts

❶ *Apoptosis quantification (caspase-3 activity)*



CONCLUSIONS

- 24 new Organic Salts and Ionic Liquids from etidronic, alendronic and zoledronic acids in quantitative yields
- Sustainable and green Amberlyst resin-based method
- Monoanionic are salts and dianionic are RTILs
- Characterization by NMR (^{1}H, ^{13}C), FTIR, DSC, elemental analysis and single crystal XRD (for [DBNH][ZOL])
- Tunability of water solubility and thermal properties according to the cation and degree of ionization
- Decrease of systemic toxicity and enhancement of antitumor activity as low as nanomolar scale
- [ETI]-based OSILs display higher anti-osteoclast and pro-osteoblast activity than ETI and protonated superbases
- Osteoclastogenesis is inhibited through the MEK ([TMGH]) and PKC ([DBNH]) pathways
- Osteoblastogenesis is enhanced through the NFkB ([TMGH]), PKC ([DBNH]) and JNK (both)
- New avenue for **modulation of bone metabolism associated with bone cancer cells, particularly when increased bone resorption is present.**

ACKNOWLEDGMENTS

Synthesis
Luís C. Branco (LAQV@REQUIMTE, FCT-NOVA)
CHARM group @ FCT-NOVA

Bone metabolism and cytotoxicity studies
Sónia Teixeira (FMD-UPorto)
M. H. Fernandes (FMD-UPorto)
João Costa-Rodrigues (ESS-IPPorto)

DSC facilities
Madalena Dionísio (LAQV@REQUIMTE, FCT-NOVA)

DESignBIOtechHealth (Norte-01-0145-FEDER-000024)
PTDC/QUI-QOR/32406/2017
RECI/BBBBQB/0230/2012
PEst-C/LA0006/2013
PTDC/CTM/103664/2008
IF/0041/2013/CP1161/CT005 (LCBranco)
Associate Laboratory for Green Chemistry LAQV (**UID/QUI/50006/2020**)