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Ionic Liquids Based on Valproate as Antitumor Agents Against Human Neuroblastoma

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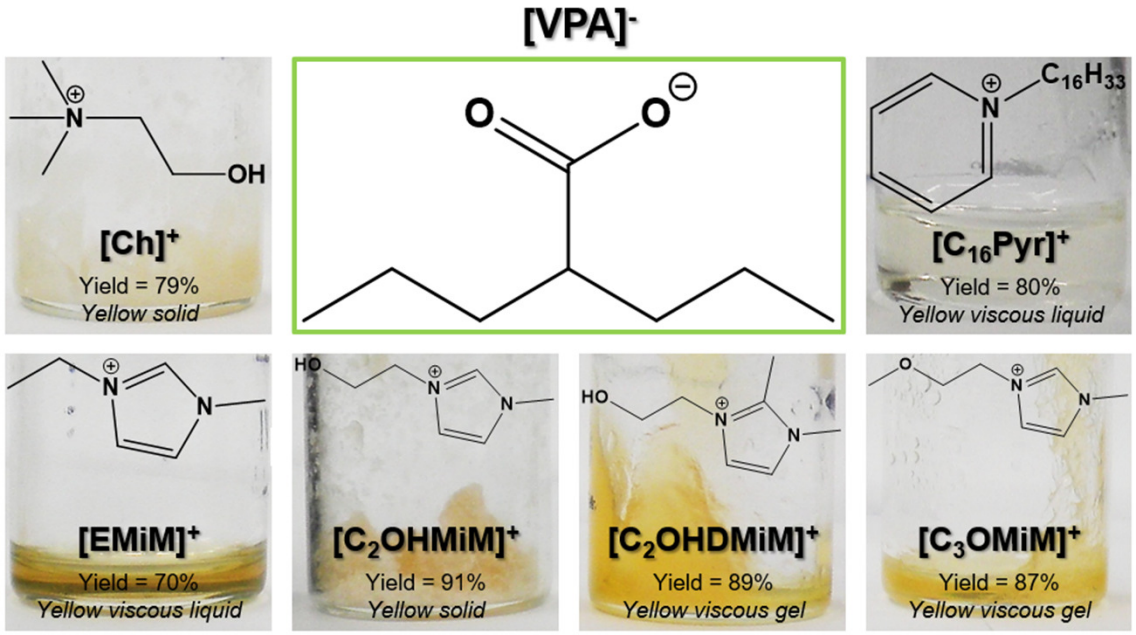
Ionic Liquids Based on Valproate as Antitumor Agents Against Human Neuroblastoma

Graphical Abstract



Ionic Liquids Based on Valproate Against Human Neuroblastoma

Abstract:

Ionic liquids (ILs) containing active pharmaceutical ingredients (APIs) have been reported as a successful approach to improve drug delivery and to overcome other drawbacks of pharmaceutical industry. Moreover, ILs-API derived from antibiotics revealed antimicrobial activity against sensitive bacteria and, particularly, increased for resistant species. The higher antimicrobial activity can be attributed to the improved drug delivery and solubility, but also to some specific interactions. On the other hand, the search for alternative and effective therapies to fight cancer pointed out ionic liquids as potential therapeutic agents with antitumor properties. In this context, several ILs with valproate (VPA) as API were synthesized and studied in terms of their bioactivity against neuroblastoma. The toxicity of the prepared ionic liquids was evaluated by MTT cell metabolic assay in human neuroblastoma SH-SY5Y and human primary Gingival Fibroblast (GF) cell lines, in which they showed inhibitory effects. Low cytotoxicity against GF cell lines was also observed, suggesting that these compounds are not toxic to human cell lines. 1-(2-hydroxyethyl)-2,3-dimethylimidazolium 2-propylpentanoate, [C2OHDMiM][VPA], demonstrated an outstanding antitumor activity against SH-SY5Y and lower activity against the non-neoplastic GF line. The herein assessed compounds played an important role in the modulation of the signalling pathways involved in the cellular behavior. This work also highlights the potential of these ILs-API as possible antitumor agents.

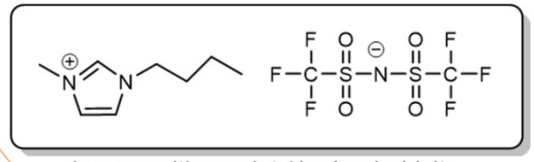
Keywords: Ionic liquids; valproic acid; antitumor agents; neuroblastoma; toxicity; signaling pathways

Ionic Liquids and Drugs

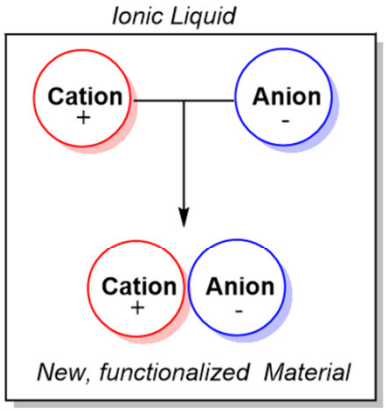
Ionic liquids (ILs): organic ionic compounds with melting point temperature below 100 ℃.

Generation 1

Solvents
Physical Properties

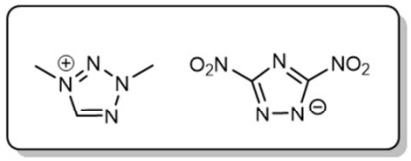


lower melting point / hydrophobicity



Generation 2

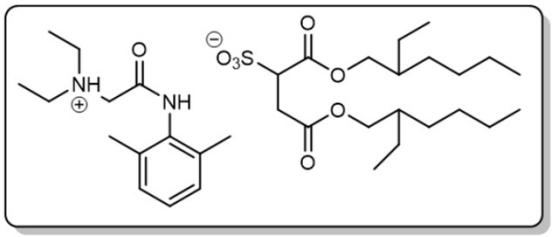
Advanced Materials
Chemical and Physical Properties



energy density / oxygen balance

Generation 3

Pharmaceuticals
Biological
Biological and Chemical or Physical Properties



local anesthetic / emollient

ILs can be used as greener alternative to toxic and volatile organic solvents (**first generation of ILs**).

ILs properties can be exploited in material chemistry, electrochemistry, catalysis (**second generation of ILs**).

ILs can be prepared with active pharmaceutical ingredients (**third generation of ILs or IL-APIs**).

Ionic liquid: some similarities and differences with other compounds/systems

Completely Ionic	Partially Ionic	Melting point >100 °C	Melting point <100 °C but >25 °C	Melting point <25 °C
Ionic liquid (IL) and Room temperature Ionic liquid (RTIL) Organic salt (OS) Group of Uniform Materials based on Organic Salt (GUMBOS)	Protic Ionic liquid (PIL) Deep eutectic system/solvent (DES) Natural DES (NADES)	OS GUMBOS	GUMBOS DES, NADES IL, PIL	DES, NADES RTIL, PIL

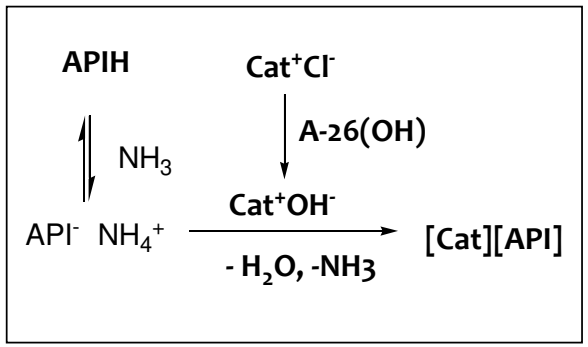
Most of our work with pharmaceutical compounds is about :

OSs and ILs containing active pharmaceutical ingredients (OS-APIs and IL-APIs) and frequently these two groups are considered together as novel OSIL-APIs

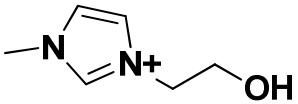
OSIL-API are an extended group unifying physicochemical properties of all OS and all groups of ILs (including RTILs and PILs too).

Differences in physicochemical properties of OSIL-APIs (e.g. mp, solubility, lipophilicity) help us to easier evaluate counterion contribution to their activities and spot some specific counterion contributions (enhancement vs potentiation).

Ampicillin-based Ionic Liquids by *Buffer Neutralization method*

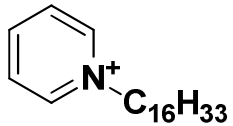


Compound	Yield %
[TEA][Amp]]	76.0
[$P_{6,6,6,14}$][Amp]	80.0
[P_{16} Pyr][Amp]	76.4
[cholin][Amp]	70.7
[EMIM][Amp]	94.6
[C_2OHMIM][Amp]	86.8



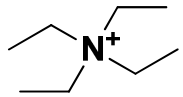
[C_2OHMIM]$^+$

Pale yellow solid
(m.p 117ºC)



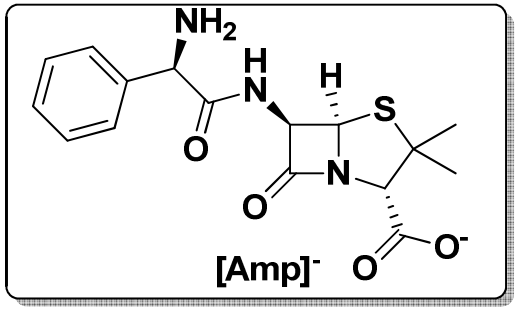
[C_{16}Pyr]+

Pale yellow solid (m.p 86 ºC)

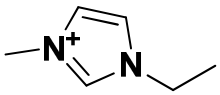


[TEA]+

Pale yellow solid
(m.p 79ºC)

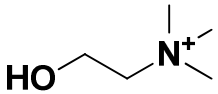


[Amp]$^-$



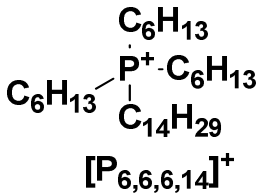
[EMIM]$^+$

Pale yellow solid
(m.p 72ºC)



[$N_{1,1,1,2OH}$]$^+$

Pale yellow solid (m.p 58ºC)

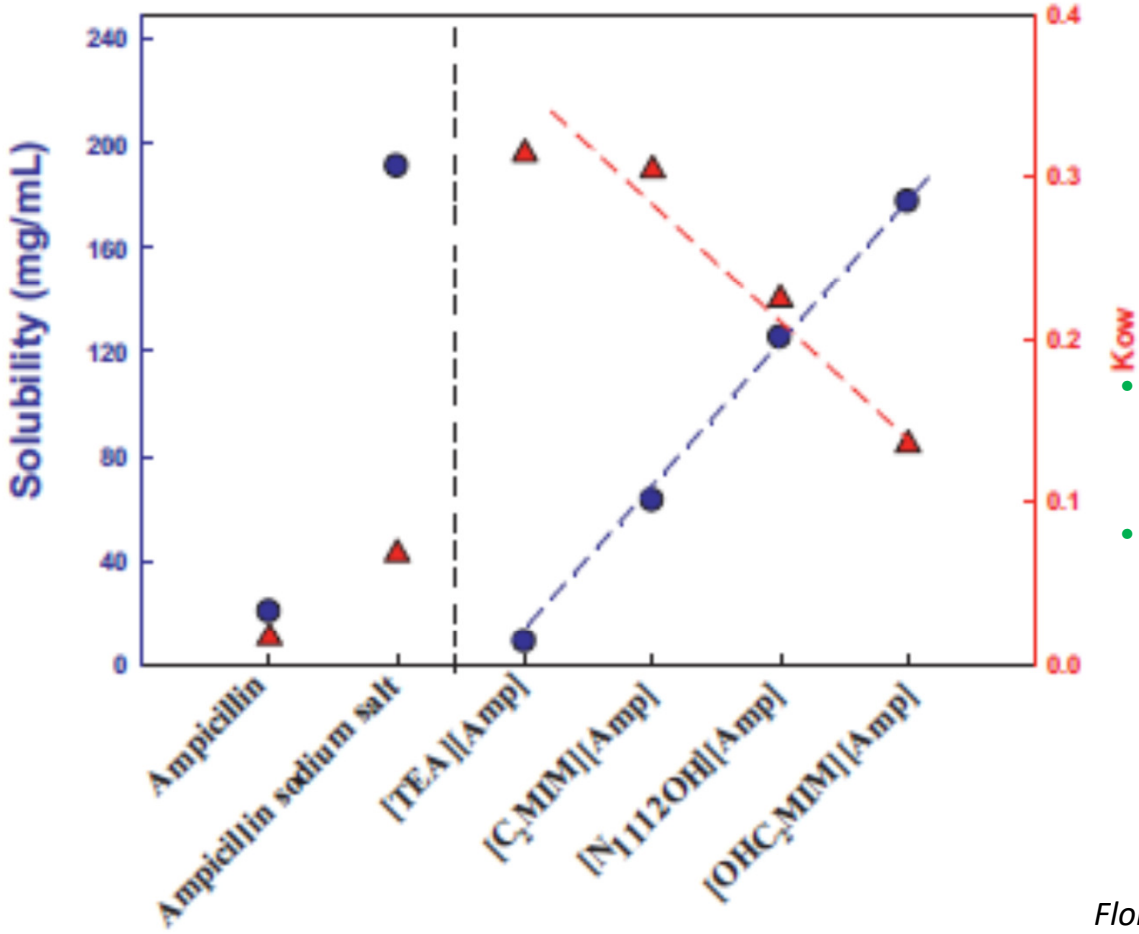


[$P_{6,6,6,14}$]$^+$

Yellow Viscous
Liquid

Ferraz R. et al. *Med.Chem.Commun* **2012**, *3*, 494-497

Comparative analysis between the aqueous solubility (●) and octanol-water partition coefficient (▲) of ampicillin, ampicillin sodium salt and ampicillin-based ILs at 25 °C.



- **Significant improvement of Bioavailability**
- **Elimination of polymorphism**

Florindo, C. et al. Inter. J. Pharmaceutics **2013**, *456*, 553-559.

Minimum Inhibitory Concentrations (MICs in mM) on the Sensitive Bacterial Strains Tested

Comp. \ Strains	Gram-negative				Gram-positive		
	E. coli ATCC 25922	*E. coli CTX M9*	*E. coli TEM1*	*K. pneumoniae*	*S. aureus ATCC 25923*	*E. fecalis*	*S. epidermis*
Na[Amp][a]	0.05	0.05	0.05	2.5	0.005	0.05	0.05
[TEA][Amp]	>5	>5	>5	>5	>5	>5	>5
[$P_{6,6,6,14}$][Amp]	2.5	0.05	>5	5	0.05	0.05	0.05
[C_{16}Pyr][Amp]	0.5	0.05	0.05	**0.05**	0.005	**0.005**	**0.005**
[C_{16}Pyr][Cl]	0.5	0.5	2.5	2.5	0.5	0.5	2.5
[$N_{1,1,1,2OH}$][Amp]	>5	>5	>5	>5	>5	>5	>5
[EMIM][Amp]	>5	>5	>5	>5	>5	>5	>5
[C_2OHMIM][Amp]	5	>5	>5	>5	>5	5	2.5

[a] The [Na][Amp] was used as control.

Ferraz R. et al. *RSC Advances* **2014**, *4*, 4301-4307.

Relative Decrease of Inhibitory Concentration(RDIC) of ampicillinate anion in [Cat][Amp] (for ampicillin sensitive bacteria)

In order to obtain results easier to compare ratio of MIC values of [Na][Amp] and [Cat][Amp] is combined in table of so-called RDIC values(relative decrease of inhibitory concentration).

$$\text{RDIC ([Cat][Amp])} = \frac{MIC(Na[Amp])}{MIC\ ([Cat][Amp])}$$

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 Values on the Ampicillin Resistant Bacterial Strains Tested

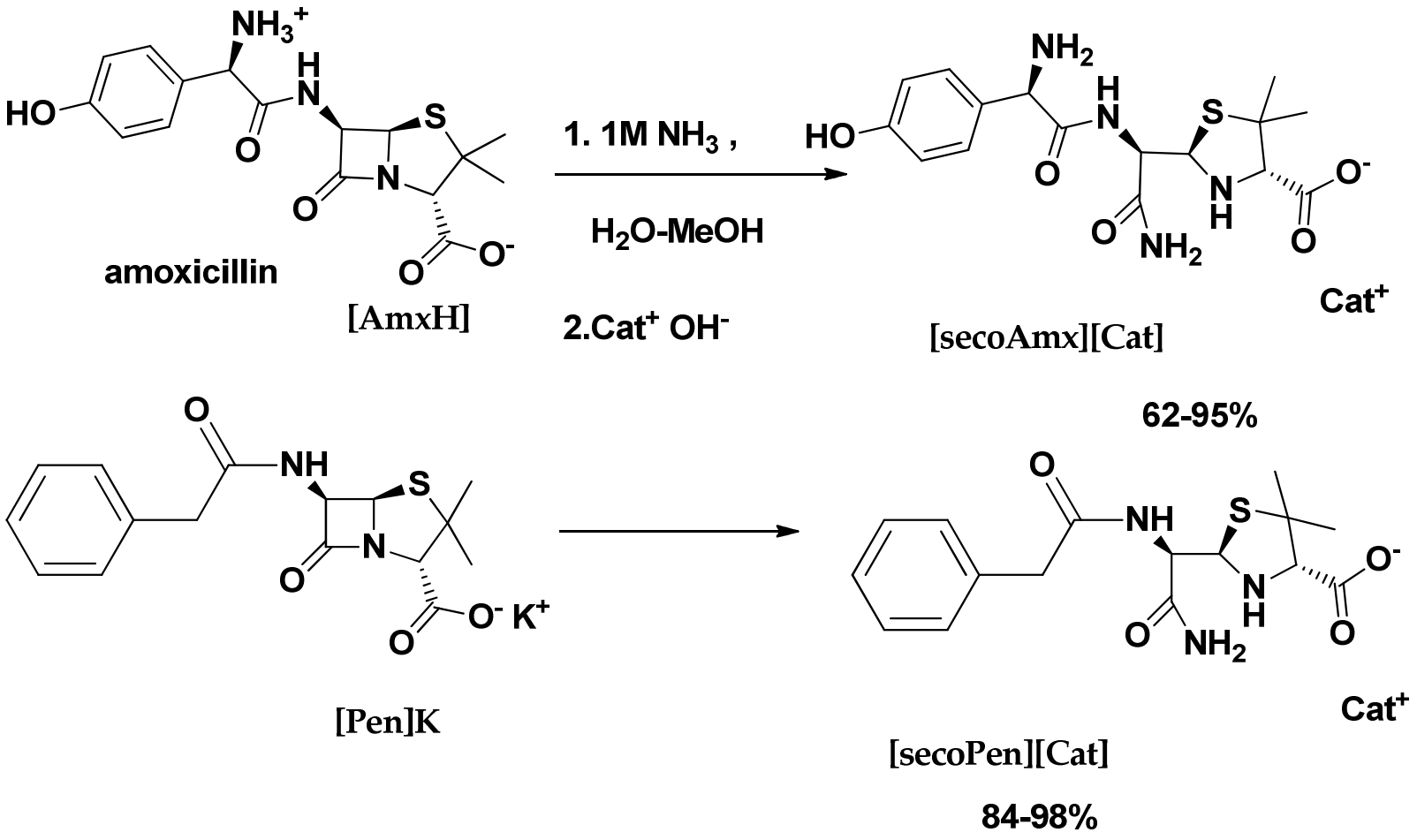
Comp. \ Strains	*E. coli* TEM CTX M9	*E. coli* CTX M2	*E. coli* AmpC Mox2
Na[Amp][a)]	1	1	1
[$P_{6,6,6,14}$][Amp]	**>10**	**>10**	-
[C_{16}Pyr][Amp]	**>1000**	**>100**	-

Important note:

In resistant bacteria MIC(Na[Amp]) is not definite value. Here is represented by highest tested concentation >5mM.

In respect to parent antibiotic we managed to improve activity using ILs particularly against resistant bacteria (**RDIC values up to >1000 as for [C_{16}Pyr][Amp] against *E. coli* TEM CTX M9**).

NH_3-based Buffer Neutralization Method and Penicillin and Amoxicillin



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

Ferraz, R. *et al. Pharmaceutics* **2020**, *12,* 221.

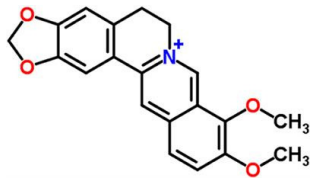
Minimum Inhibitory Concentrations (mM) of the New Compounds Produced on Resistant Strains and Relative Decrease of Inhibitory Concentration (RDIC)

Compound	E. coli CTX M9	***RDIC***	*E. coli* CTX M2	***RDIC***	MRSA ATCC 43300	***RDIC***
[EMIM][secoAmx]	>5	-	> 5	-	> 2.5	-
[C_2OHMIM][secoAmx]	> 5	-	> 5	-	5	>1
[$P_{6,6,6,14}$][secoAmx]	**0.05**	>100	**1.0**	>5	> 5	-
[C_{16}Pyr][secoAmx]	**0.05**	**>100**	**0.05**	**>100**	**0.005**	**>1000**
[$N_{1,1,1,2}$OH][secoAmx]	0.5	>10	0.05	>100	0.5	>10
Na[secoAmx]	>5	-	>5	-	>5	-
Amx	> 5	1	> 5	1	> 5	1
[EMIM][secoPen]	>5	-	>5	-	> 5	-
[C_2OHMIM][secoPen]	>5	-	>5	-	>5	-
[Choline][secoPen]	**1.0**	>5	>5	-	**1.0**	>5
[$P_{6,6,6,14}$][secoPen]	**0.5**	>10	**0.5**	>10	>5	-
[C_{16}Pyr][secoPen]	**0.5**	**>10**	**0.5**	**>10**	**0.05**	**>100**
[TEA][secoPen]	>5	-	> 5	-	>5	-
K[secoPen]	>5	-	>5	-	>5	-
K[Pen]	>2.5	1	> 25	1	> 2.5	1

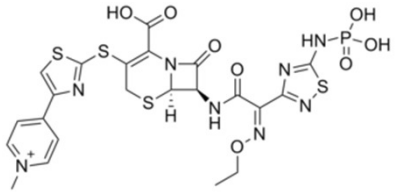
The activity of seco-Amx and seco-Pen OSILs was surprising but it is not unprecendent –recently is discribed reversible inactivation of β-lactam antibiotic mediated by enzyme active site of PBPs in *Enterococcus faecium* (see Edoo, Z. *et al. Scientific Report* **2017**, 7: 9136).

Conclusion: Antibiotic OSIL-APIs

- Great increase of MIC values of OSIL-APIs congaing [$C_{16}Pyr^+$] cation against resistant *E coli* species and MRSA ATCC 43300 is achieved (RDIC values up to >1000).
- Similar examples were also found in literature. So, berberine in the presence of ampicillin and oxacillin also greatly increased MIC against MRSA. Specific interaction (allosteric activation) of PBP2a with an alkylpyridinium compound, antibiotic ceftaroline is also discovered (Mobashery, S. *et al. Iubmb Life* **2014**, 66, 572–577).



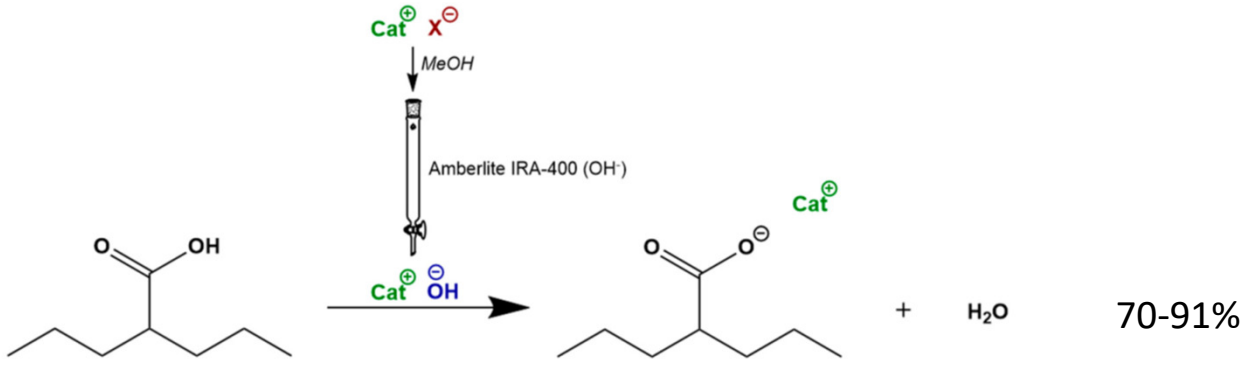
berberine



ceftaroline

- The greatly amplified activity beta-lactam antibiotics by with [$C_{16}Pyr^+$] (potentiation - up to 2 or 3 orders of magnitude) against resistant bacteria is thus probably due to a similar synergic effect of both ions and is in contrast with the unspecific increase due just to change in physicochemical properties (enhancement – several times).
- Combination therapy -antibiotic potentiation by combinations of antibiotics with non- antibiotic is suggested recently as a solution to fight antibiotic resistance. (See: Tiers, M. *& Wright, G. D. Nature Reviews Microbiology* **2019**, *17*, 141-155. So it acts as antibiotic potentiator (see Chalwa, M. *et al. Frontiers in Microbiology* **2022**, *13*, 887251 .

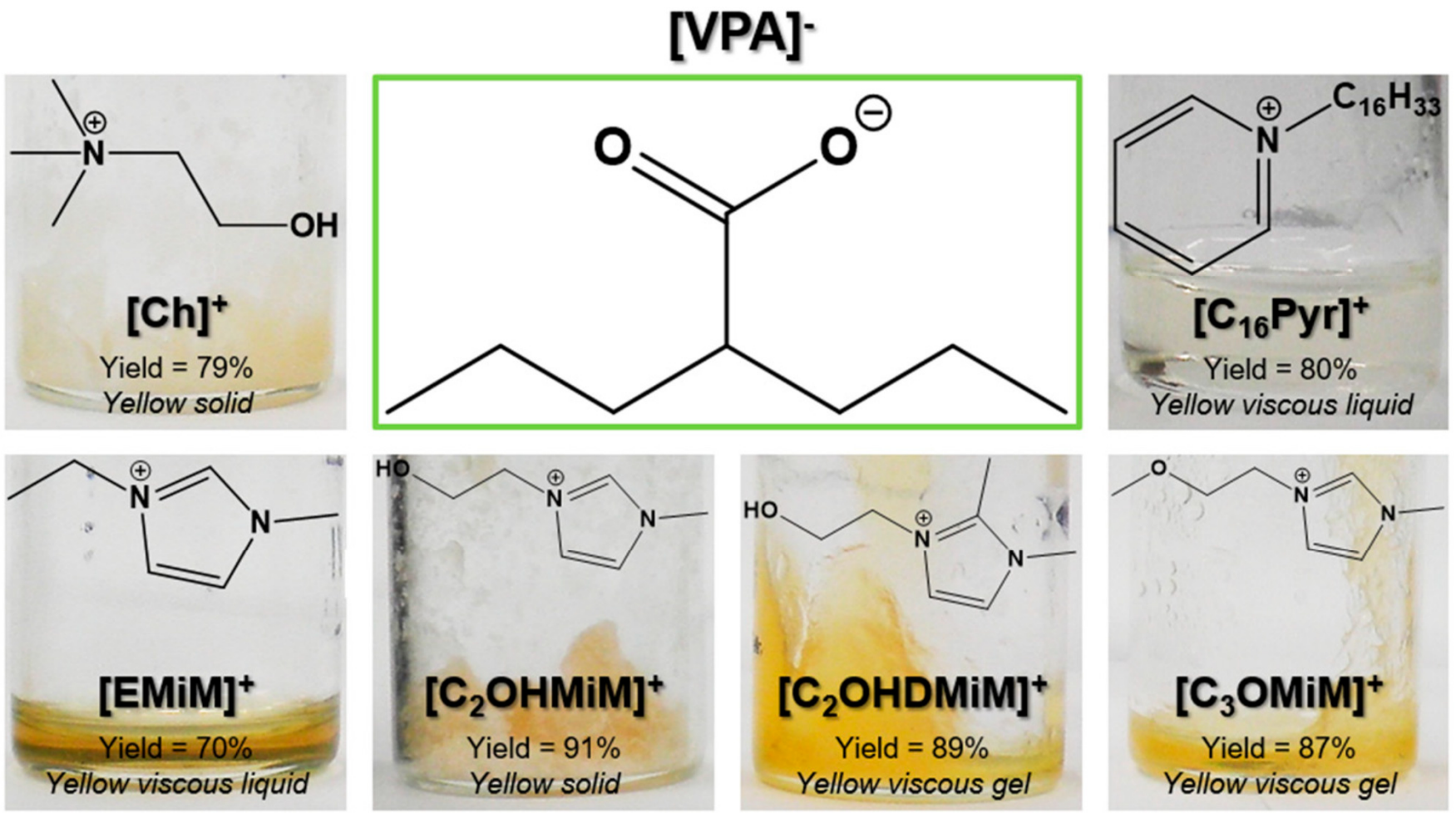
Bioactivity of Ionic Liquids Based on Valproate in SH-SY5Y Human Neuroblastoma Cell Line



Valproic acid (valproate) is a drug used against epilepsy and bipolar disorder.

Recent studies conducted in vivo and in vitro have demonstrated the antitumor activity of valproate and modulation of numerous signaling pathways. Valproate is effective against several types of cancer, including neuroblastoma and shows potent antitumor effect with alteration of DNA methylation in neuroblastoma.
Gu, S. *et al. Anticancer Drugs* **2012**, *23*, 1054–1066.

Bioactivity of Ionic Liquids Based on Valproate in SH-SY5Y Human Neuroblastoma Cell Line



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Bioactivity of Ionic Liquids Based on Valproate in SH-SY5Y Human Neuroblastoma Cell Line

Bioactivity of valproate based IL-APIs was tested against Neuroblastoma Cell Line SH-SY5Y and Non-Neoplastic Gingival Fibroblasts (GF) . MTT metabolic assays were performed, allowing to determine the IC_{50} and EC_{50},i.e., the drug's concentration needed to reduce by half the cellular metabolic activity and cell viability, in Neuroblastoma cell line SH-SY5Y and in Gingival Fibroblasts (GF).

Compounds	IC_{50} (μM)			EC_{50} (μM)		
	GF	SH-SY5Y		GF	SH-SY5Y	
	Day 1	Day 1	Day 3	Day 1	Day 1	Day 3
VPA [1]	294.2	0.633	n.d.	299.3	60.72	n.d.
[Ch][VPA]	n.d.	0.049	n.d.	n.d.	0.158	3528
[C_{16}Pyr][VPA]	75.54	1.408	1.411	76.07	4.358	1.681
[EMiM][VPA]	1058	1.038	n.d.	>1058	0.086	126.0
[C_2OHMiM][VPA]	23.66	31.09	3560	27.42	21.03	2.824
[C_2OHDMiM][VPA]	n.d.	0.263	227.8	n.d.	6.000	44.58
[C_3OMiM][VPA]	1374	0.646	n.d.	>1374	27.30	14.71

Bioactivity of Ionic Liquids Based on Valproate in SH-SY5Y Human Neuroblastoma Cell Line

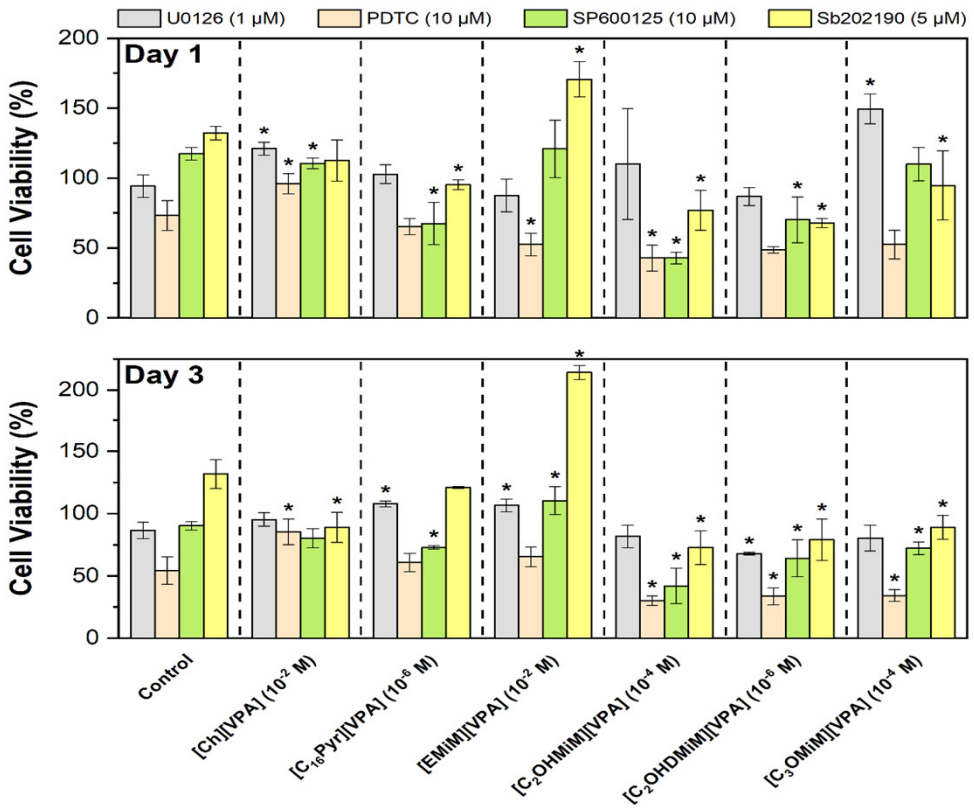
IC_{50} results for neuroblastoma SH-SY5Y cell line:

- On day 1, both [Ch][VPA] and [C_2OHDMiM][VPA] exhibited the lowest values, indicating the highest toxicity; highest toxicity of [Ch][VPA] was unexpected as choline is considered an essential nutri-ent for normal cell metabolism.
- remaining ionic liquids were classified according to their toxicity, in which the latter revealed the less promising result: [C_3OMiM][VPA] > [EMiM][VPA] > [C_{16}Pyr][VPA] > [C_2OHMiM][VPA].
- On day 3, [C_{16}Pyr][VPA] presented higher toxicity, followed by [C_2OHDMIM][VPA]. Furthermore, with the exception of [C_2OHMiM][VPA], the other ILs-API, as well as the control, did not display any IC_{50} in the tested concentration range (10^{-7} to 10^{-2} M).
- For EC_{50}, the results are in accordance with the IC_{50} assays on both days 1 and 3, being, in general, align to those already reported in literature.

Regarding GF:

- VPA is one of the most toxic compounds studied herein. High cytotoxicity values were exhibited for [C_{16}Pyr][VPA] and [C_2OHMiM][VPA].
- On the contrary, [Ch][VPA] and [C_2OHDMiM][VPA] proved to be the least toxic ones.

Cell-Signaling Pathways in Cellular Behavior of Human Tumor Cell Line SH-SY5Y



Cell viability in Neuroblastoma cultures of SH-SY5Y cells supplemented with different inhibitors of cell-signaling pathways: U0126, PDTC, SP600125 and Sb202190, which are the inhibitors of the MEK, NFkB, JNK and p38 pathways, respectively.

Cell-Signaling Pathways in Cellular Behavior of Human Tumor Cell Line SH-SY5Y

- Two inhibitors U0126 and SP600125 (inhibitors of MEK and JNK pathways, respectively)did not promote significant changes in the cell viability during the experiment period. However,
- PDTC (inhibitor of NFkB pathway) decreased the cell viability between days 1 and 3 of the culture in all the studied compounds, whereas Sb202190 (inhibitor of p38 pathway) revealed an increase during the same period.
- Sb202190 (inhibitor of p38 patway) exclusively modified the cell response in cultures supplemented with [EMIM][VPA], resulting in an increase on viability and contrary to what was observed for the negative control.

Conclusions

- OSIL-APIs can be used successfully to modulate drug (APIs) activity by increase of solubility and partition coefficient.
- Modulation of those factors may increase drug bioavailability but overall increase of activity is usually only several times in respect to parent drug (API) alone and not higher then oner order of magnitude, so OSIL-APIs can therefore act as drug enhancers.
- The most significant contribution of OSIL-APIs is, however, when counterion too, targets some specific site and act as drug potentitiator. In this case the increase in activity is of two or three orders of magnitude in respect to parent drug, so in case of antibiotics and resistant bacteria completer reversal of bacterial resistance is possible.
- In respect antitumor VPA based IL-APIs against Neuroblastoma SH-SY5Y cell lines similar tendency (of enhancement) seems plausible.
- In addition here IL-APIs managed to diminish toxicity of VPA against GF, suggesting that compounds are not toxic for human cells and can increase selectivity against neuroblastoma.
- Further research is needed to elucidate the mechanism of action of OSIL-APIs.

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