European Journal of Pharmaceutics and Biopharmaceutics 137 (2019) 227–232

Contents lists available at ScienceDirect

European Journal of Pharmaceutics and Biopharmaceutics

journal homepage: www.elsevier.com/locate/ejpb

Research paper

Enhancement of water solubility of poorly water-soluble drugs by new biocompatible *N*-acetyl amino acid *N*-alkyl cholinium-based ionic liquids

Ana R. Jesus[a], Mário R.C. Soromenho[a], Luís R. Raposo[b], José M.S.S. Esperança[a], Pedro V. Baptista[b], Alexandra R. Fernandes[b], Patrícia M. Reis[a,*]

[a] LAQV, Requimte, Departamento de Química, Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa, Caparica 2829-516, Portugal
[b] UCIBIO, Life Sciences Department, Faculdade Ciências e Tecnologia, Universidade Nova de Lisboa, Caparica 2829-516, Portugal

ARTICLE INFO

Keywords:
Amino acids
Cytotoxicity
Ionic Liquids
Drug Delivery
Bioavailability

ABSTRACT

The major challenge of the pharmaceutical industry is to find potential solvents for poorly water-soluble drug molecules. Ionic liquids (ILs) have attracted this industry as (co-) solvents due to their unique physicochemical and biological properties. Herein, a straightforward approach for the enhancement of the water solubility of paracetamol and sodium diclofenac is presented, using new biocompatible *N*-acetyl amino acid *N*-alkyl cholinium-based ionic liquids as co-solvents (0.2–1 mol%). These new ionic liquids were able to increase the water solubility of these drugs up to four times that in pure water or in an inorganic salt solution. In the presence of these ILs, the drugs lipophilicity (log P was not significantly changed for paracetamol, but for sodium diclofenac it was possible to decrease significantly its lipophilicity. Concerning cytotoxicity in human dermal fibroblasts it was observed that ILs did not show a significant toxicity, and were able to improve cell viability compared with the respective precursors.

1. Introduction

In pharmaceutical industry, the introduction of new drugs in the market is a very challenging goal, mainly due to the fact that most drugs are poorly water-soluble and therefore present low gastro-intestinal absorption [1]. The methods reported in literature [2,3] to enhance the solubility of such drugs include (a) pH modification and salt formation, [4] (b) co-solvency and surfactant solubilization, [2,5] (c) amorphous forms, solid dispersions and cocrystals formation, [6] (d) polymeric micelles formation, [7] (e) inclusion complexation, [8] (f) size reduction and nanonization, [9,10] (g) solid lipid nanoparticles formation, [11] (h) encapsulation in liposomes and proliposomes, [12] and (i) microemulsions and self-emulsifying drug delivery systems. [13,14]

The solubility enhancement process of hydrophobic drugs plays a key role in the formulation development to achieve the bioavailability and therapeutic action of the drug at the target site. Poorly water-soluble drugs lead to poor drug absorption and poor bioavailability via oral drug administration, due to their high hydrophobicity. The common polar organic solvents excipients that are used to enhance the poor solubility of drugs are pyridine, dimethylformamide (DMF) and dimethyl sulfoxide (DMSO). [14,15] However, they are volatile, flammable, and toxic solvents, hence not ideal as pharmaceutical ingredients.

Ionic liquids (ILs), i.e. ionic compounds with melting point below 100 °C, have shown interesting properties that can be controlled by the tunability of both cation and/or anion, with a multidisciplinary application in chemical, biological, engineering, environmental and material sciences. [16]

The application of these new materials in pharmaceutical industry and medicine are emerging, as well as their biological application as active pharmaceutical ingredients. [17,18] The ability of ionic liquids to dissolve poorly water-soluble drugs has gained more attention from researchers as a new tool for drug delivery. [1,19,20] ILs can be tailored to promote the dissolution of a specific drug or a class of drugs (e.g. poorly soluble oral drugs), potentially increasing their concentration for delivery and enhancing drug permeation to boost therapeutic efficacy.

Ionic liquids have showed potential to be used as co-solvents to solve similar problems. [19,21–23] They possess dual capability of dissolving both polar and nonpolar species and the most useful feature is that they do not evaporate even at high temperatures. The use of ILs can markedly improve the pharmacokinetic and pharmacodynamics properties of drugs. [24] Many drug molecules show poor permeability, thus an excipient, such as lipid-based formulations, may be added

* Corresponding author.
E-mail address: preis@fct.unl.pt (P.M. Reis).

https://doi.org/10.1016/j.ejpb.2019.03.004
Received 28 November 2018; Received in revised form 4 February 2019; Accepted 1 March 2019
Available online 02 March 2019
0939-6411/

externally to enhance permeation transiently by facilitating drug permeability through the intestinal epithelium or by stimulating chylomicron secretin, which results in enhanced drug uptake through lymphatic system, bypassing the initial metabolism in the liver. [25]

The major challenge is the development of non-toxic and more biodegradable ILs with high dissolution capability. [1] Cholinium-based ILs are some of the most biodegradable, less toxic, easily accessible and cost-effective ILs, when compared with other ILs such as pyridinium and imidazolium-based ILs. [26,27] Cholinium amino acid based ionic liquids have sparked recent interest throughout the scientific community due to their allegedly low cost and natural origin. They are prepared from non-toxic renewable feedstocks, endowed with solubilizing power, sometimes selective and always really eco-friendly. [28]

Amino acids have been reported as biocompatible anions for ionic liquids with multiple applications. [29–33] The thermal stability of acetyl amino acids is greater than the corresponding amino acids. [34] For this reason our work was focused in the synthesis of new and non-toxic *N*-acetyl amino acid *N*-alkyl cholinium-based ionic liquids, and in the improvement of water solubility of oral drugs, as paracetamol and sodium diclofenac using these ILs as co-solvents.

In this study, we have prepared 20 new biocompatible ionic liquids based on the *N*-alkyl cholinium cation (Fig. 1A) and *N*-acetyl amino acids anions (Fig. 1B), aiming the improvement on the water solubility of two drugs, paracetamol (Fig. 2A) and sodium diclofenac (Fig. 2B).

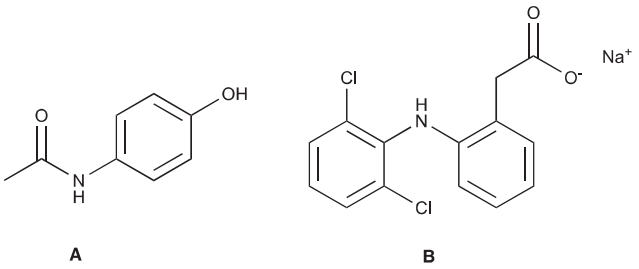


Fig. 2. Chemical structure **(A)** Paracetamol, **(B)** Sodium Diclofenac.

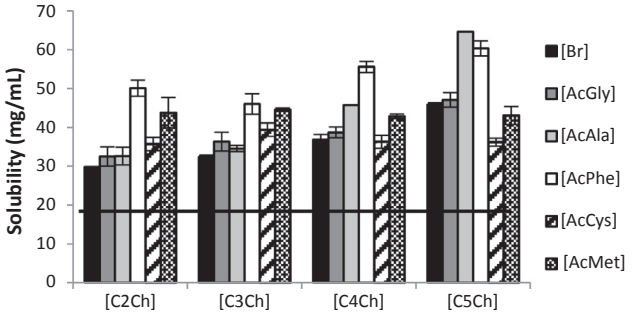


Fig. 3. Solubility of paracetamol (horizontal line corresponds to the water solubility).

2. Results and discussion

2.1. Solubility assays

Several studies to improve solubility of poorly water-soluble drugs in ionic liquids and/or deep eutectic solvents have already been reported. [21,35] However, these studies focus only on the determination of the maximum solubility of drugs in pure ionic liquids/deep eutectic solvents. Herein, we propose the improvement of water solubility of two commercial drugs using a small percentage (0.1–1 mol%) of *N*-acetyl amino acid *N*-alkyl cholinium-based ionic liquids as co-solvents.

In this study, we have determined the solubility of paracetamol and sodium diclofenac in pure water at 25 °C. Our results were 15.0 mg/mL and 20.4 mg/mL, which are very similar to those reported in literature, 14.9 mg/mL [36] and 19.4 mg/mL, [37] respectively.

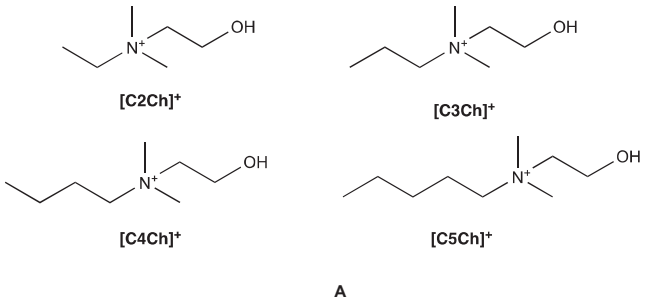
To mimic the body temperature, we have measured the solubility of these drugs in water at 37 °C. At this temperature the maximum solubility of paracetamol and sodium diclofenac were 18.0 mg/mL and 34.5 mg/mL, respectively. Then, the same approach for the determination of maximum solubility of both drugs in the presence of 0.2–1 mol% of *N*-acetyl amino acid *N*-alkyl cholinium-based ionic liquids was used and the results are presented accordingly.

Regarding the results obtained for paracetamol (Fig. 3), all the ionic liquids promoted a higher solubility of the drug (29–65 mg/mL) when compared to pure water or using an aqueous solution of an inorganic salt (e.g. KBr). For instance, with only 1 mol% of ionic liquid, such as [C5Ch][AcAla], [C4Ch][AcPhe] or [C5Ch][AcPhe], it was possible to achieve total paracetamol solubilization of about 55–65 mg/mL, which represents a 3-fold improvement on the drug solubility in comparison to pure water.

Analysing the effect of the alkyl chain in the cation moiety in the solubility of paracetamol (Fig. 3) it was possible to observe that ILs containing an acetyl phenylalanine-based anion contributed in general to the highest values of solubility, independently of the cation moiety. Likewise, ILs containing a C5-cholinium cation, for any type of anion also contributed to a significant improvement in solubility of paracetamol.

To understand the influence of IL concentration in the solubility of paracetamol, the ionic liquids, which have shown better results, namely [C5Ch][AcAla], [C5Ch][AcPhe], and [C4Ch][AcPhe], were also used at a smaller IL concentration (0.5 mol%). Fig. 4 shows that, even with half amount of IL, it is possible to achieve more than double the water solubility of paracetamol in pure water (18.0 mg/mL) or in the presence of the same % of an inorganic salt (18.5 mg/mL).

Sodium diclofenac is more soluble in pure water, at 37 °C, than



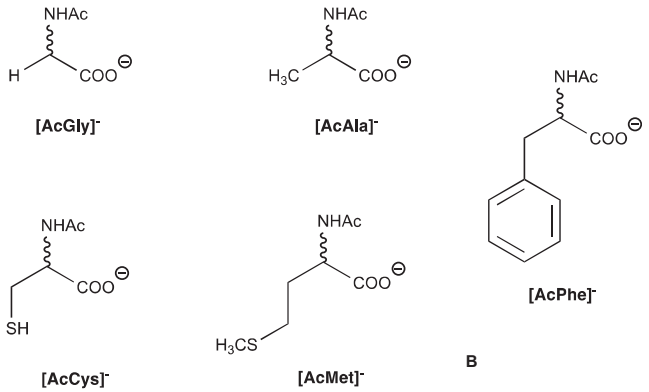


Fig. 1. (A) N-Alkyl cholinium-based cations, **(B)** Racemic N-Acetyl amino acid-based anions.

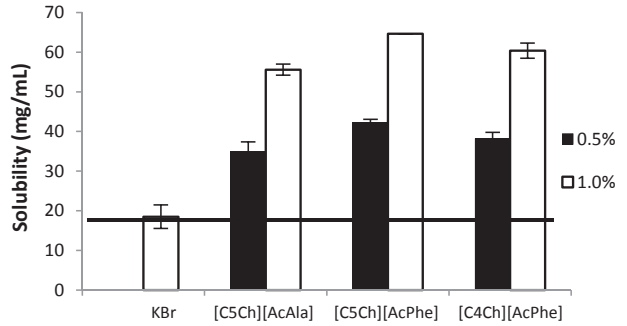


Fig. 4. Solubility of paracetamol at 0.5 mol% vs 1 mol% IL (horizontal line corresponds to the water solubility).

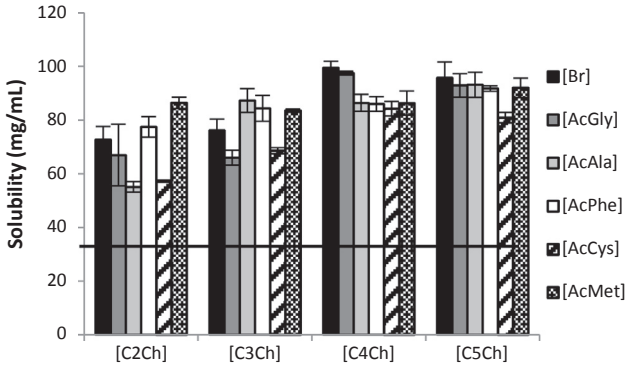


Fig. 5. Solubility of sodium diclofenac (horizontal line corresponds to the water solubility).

paracetamol (34.5 mg/mL vs 18.0 mg/mL) and it was decided to start at a lower IL concentration (0.2 mol%).

The results obtained for this drug are presented in Fig. 5. The ILs affording the best solubility values (92–100 mg/mL) at only 0.2 mol%, were [C4Ch]Br, [C4Ch][AcGly], [C5Ch]Br, [C3Ch][AcPhe], [C5Ch][AcGly], [C5Ch][AcMet], and [C5Ch][AcPhe], representing a 3-fold improvement on sodium diclofenac solubility in comparison to pure water.

Analysing the effect of the alkyl chain in the cation moiety in the solubility of sodium diclofenac, (Fig. 5) it was possible to conclude that, C5Ch and C4Ch-based ILs were the best co-solvents for this drug. Moreover, in general, ILs with an [AcMet] anion promoted higher solubilization of sodium diclofenac with any type of *N*-alkyl cholinium cation.

The relevance of IL amount in the solubility of the sodium diclofenac was also evaluated for the best candidates, and therefore the IL amount was reduced to half, 0.1%. The selected ionic liquids were [C4Ch]Br, [C5Ch]Br and [C4Ch][AcGly]. Again, Fig. 6 shows that

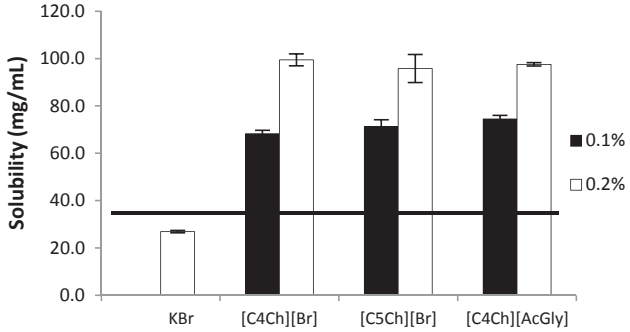


Fig. 6. Solubility of sodium diclofenac at 0.2% vs 0.1% IL (horizontal line corresponds to the water solubility).

reducing the amount of IL to half resulted in the decrease of solubility values, yet still up 2.5 times higher (68 – 75 mg/mL) that with pure water (34.5 mg/mL) or in the presence of the same amount of an inorganic salt (26.9 mg/mL).

Both paracetamol and sodium diclofenac are taken at 6–8 h intervals, hence we have studied their solubility in selected ionic liquids aqueous solutions after 6 h. After the 6 h period it was observed that the solubility was practically the same than after 24 h. (see graphics in ESI)

2.2. Octanol-water partition coefficient and log P

In the context of pharmacokinetics the partition coefficient (log P) can be useful to estimate the distribution profile of a drug within the body, which has a great influence in its ADME profile. It also helps scientist to determine the most likely way of administration. In the case of an orally absorbed drug it has to pass first through lipid bilayers in the intestinal epithelium. Therefore, it is necessary a certain lipophilicity to partition into that bilayer but also require some hydrophilicity so that will not partition out again. [38]

According to literature, paracetamol has a log P value ranging from 0.30 to 0.56, [39] which means that it is primarily found in the blood serum; on the other hand, sodium diclofenac has a log P higher than 4, meaning that is distributed to hydrophobic areas such as lipidic bilayers of cells [40,41].

In this study, we have showed that *N*-acetyl amino acids *N*-alkyl cholinium-based ionic liquids were able to improve water solubility of paracetamol and sodium diclofenac. However, a question remained, whether is it possible to improve pharmacokinetic parameters of these drugs to meliorate their ADME profile. To answer this question, we have determined the log P value of these drugs using ionic liquids as co-solvents.

We have selected ionic liquids that promoted the maximum solubility for each drug and the corresponding halogenated precursor, [C5Ch][AcAla], [C5Ch][AcPhe] and [C5Ch]Br.

Initially, it was necessary to prepare a calibration curve for each drug in octanol-saturated water, by UV–Vis spectrophotometry. The absorbance of a range of known concentrations of each drug was measure, maintaining absorbance values below 1.

Then, an amount within the solubility range of each drug was mixed with equal amounts of mutually saturated water and octanol. After mixing and separation of layers, the absorbance of water-rich phase was measured, and concentration of drug was calculated based on the calibration curve.

Log P values for each drug in aqueous solution, and in the presence of the corresponding percentage of ionic liquid was determined using Eqs. (1) and (2), and results are presented in Table 1. However, log P of sodium diclofenac was not determined due to the fact that the separation of octanol-water phases was not possible. [42]

The results obtained for paracetamol indicate that its log P decreases slightly in the presence of a 1 mol% ionic liquid solution. This indicates that in ILs aqueous solutions paracetamol becomes even more hydrophilic, and therefore with a higher affinity for the aqueous phase. As mentioned previously there must be a hydrophilicity/lipophilicity

Table 1
Log P values for paracetamol and sodium diclofenac.

System/Media	Log P
Paracetamol/water	0.423 ± 0.015
Sodium diclofenac/water	–
Paracetamol/water + 1 mol% [C5Ch]Br	0.034 ± 0.006
Paracetamol/water + 1 mol% [C5Ch][AcAla]	0.093 ± 0.017
Paracetamol/water + 1 mol% [C5Ch][AcPhe]	− 0.051 ± 0.120
Sodium diclofenac/water + 0.2 mol% [C5Ch]Br	1.81 ± 0.16
Sodium diclofenac/water + 0.2 mol% [C5Ch][AcAla]	1.93 ± 0.20
Sodium diclofenac/water + 0.2 mol% [C5Ch][AcPhe]	1.93 ± 0.01

balance to achieve an ideal oral drug. This methodology, however did not afford the desired results for paracetamol as it only meliorated its solubility (although paracetamol is already considered a soluble drug) but did not improved its distribution coefficient.

However, the most outstanding results were obtained with sodium diclofenac with log P values below 2. It is important to highlight that in ionic liquid solutions we did not encounter the same problems described for the determination of log P of sodium diclofenac in water.

In literature there is not much information regarding the log P of sodium diclofenac but the log P of the active compound diclofenac (acidic form) is higher than 4. [43] In this study, ionic liquids not only significantly improve the solubility of this drug but also meliorate its log P value down to 1.91–1.93. According to literature the optimum log P values are between 0 and 3. [44]

2.3. Cytotoxicity evaluation

We have chosen only few ionic liquids to study their cytotoxicity vs the cytotoxicity of their halogen precursors. Therefore, [C3Ch][AcGly], [C4Ch][AcPhe], [C5Ch][AcAla], and [C5Ch][AcPhe], which afforded the best results in terms of solubility, were selected as well as their precursors [C3Ch]Br, [C4Ch]Br, and [C5Ch]Br.

The results presented in Fig. 7 B), D), F) and G) and in table 2 show that the ILs displayed IC_{50} values ranging from 19.3 to 122.7 mM. Also, these ILs showed less toxicity compared to the respective precursors (IC_{50} ranging from 17.5 mM to 75.1 mM) as observed in Fig. 7(A), (C) and (E) and Table 2.

As the *N*-alkyl chain of the cation moiety increases there is a decrease in the IC_{50} value, indicating a higher lipophilicity, and therefore a higher ability to permeate the cell membrane. Moreover, for the same cation (C5Ch) a phenylalanine anion also induces a decrease in the IC_{50} value for being a more hydrophobic anion than alanine itself.

Interestingly, these IC_{50} values for the tested ILs are similar or higher than those previously described in the literature for paracetamol [45] reinforcing their low cytotoxicity in normal human dermal fibroblasts.

3. Conclusions

This work proved that a small percentage of *N*-acetyl amino acid *N*-alkyl cholinium-based ionic liquids can improve solubility of paracetamol and sodium diclofenac by 2- to 4-fold as compared to their solubility in water or in the presence of the same percentage of an inorganic salt. More specifically, sodium diclofenac which is an oral drug that belongs to BCS class II (poor aqueous solubility), is converted in a class I drug by the addition of small quantities of these new ILs, that lower the lipophilicity of the drug, mimicking a high permeability and high solubility compound.

In addition, it was possible to observe that the cytotoxicity of the ILs in human dermal fibroblasts decreased in comparison with the corresponding precursors.

Thus, these new biocompatible ionic liquids are promising vehicles for aqueous administration of poorly water-soluble drugs.

4. Experimental section

Detailed analysis data of all the synthesized *N*-acetyl amino acids and ionic liquids are given in the ESI.

4.1. Materials and methods

Amino acid phenyl-L-alanine (99%), *N*-acetyl-L-glycine (99%), *N*-acetyl-DL-methionine (99%), *N*-acetyl-L-cysteine (≥99.5%), *N,N*-Dimethyl ethanolamine (≥99.5%), and acetic anhydride (≥98%) were purchased from Aldrich and used as received. Glacial acetic acid (100%) was purchased from Merck. Amino acid DL-alanine (99%), bromoethane (98%), 1-bromopropane (99%), 1-bromobutane (≥98%) and 1-bromopentane (99%) were purchased from Alfa Aesar and used as received.

4.1.1. General procedure for the synthesis of *N*-acetyl amino acids

Amino acid (10 g) was mixed with glacial acetic acid (50 mL). Acetic anhydride (1.1 equiv.) was added to the reaction vessel and the mixture was kept at 70 °C for 10–30 min, until total dissolution of the initial product. Acetic acid was then removed under vacuum and the *N*-acetyl amino acid was recrystallized from ethyl acetate. The final compound was obtained in 95–99% yield and dried in high vacuum overnight and high purity was confirmed by NMR experiments.

4.1.2. General procedure for the synthesis of $[C_nCh]Br$

The synthesis was carried out as reported elsewhere. [46] In general, *N,N*-Dimethyl ethanolamine (0.2 mmol) was mixed with the alkylating agent, $[C_nH_{2n+2}]Br$ (2.0 equiv.), and *n*-hexane (50 mL) in a 100 mL pressure reaction vessel. The mixture was kept at 80–90 °C for 24 h for shorter alkyl chains (n = 2,3) and 48 h for longer alkyl chains (n = 4,5), and then allowing it to cool down to room temperature. The precipitated bromide salts were thoroughly washed with EtOAc and/or acetone to remove unreacted amine and/or alkylating agent. Ionic liquids were then dried in the vacuum for 1–2 days, and their purity confirmed by NMR. The bromide salts were obtained in 95–99% yield.

4.1.3. General procedure for the metathesis reaction

The synthesis was carried out as reported elsewhere. [47] An aqueous solution of *N*-alkyl choline bromide (10 mmol) was passed slowly through an anion exchange column Amberlite™ IRN-78. Then, the corresponding hydroxide solution was slowly added to an aqueous solution of *N*-acetyl amino acid (1.0 equiv.). The reaction mixture was stirred at room temperature for 2–3 h prior to water removal by evaporation. Ionic liquids were obtained in quantitative yield and dried in high vacuum for 3–4 days at 50–60 °C prior to the coulometric assays to guarantee minimum water content. Coulometric Karl-Fischer titrations yielded final water contents below 1000–1500 ppm depending on the IL. Moreover, $AgNO_3$ test was used to confirm the absence of halogen presence in the final IL.

4.1.4. Solubility assays

A calibration curve was prepared for each drug, in water, at their maximum absorbance wavelength, 243 nm for paracetamol and 277 nm for sodium diclofenac, at 25 °C. Absorbance values were kept below 1.

4.1.5. General procedure for the determination of solubility limit of paracetamol

Aqueous solutions (5 mL) of ionic liquids (1 mol%) were prepared and paracetamol (325 mg) was added to each sample (at this concentration we are above the solubility limit for all the samples). The solution was stirred for 24 h at 37 °C until equilibrium was reached. Keeping the solutions in a 37 °C bath, the non-dissolved drugs were filtered off through a 0.25 μm syringe filter, and 10 μL of each filtered solution was diluted to 50 mL of MilliQ® water. Triplicates were prepared for each sample and maximum absorbance was measured at 243 nm. Solubility of paracetamol in the presence of each ionic liquid was calculated based on the calibration curve.

4.1.6. General procedure for the determination of solubility limit of sodium diclofenac

Aqueous solutions (5 mL) of ionic liquids (0.2 mol%) were prepared and sodium diclofenac (500 mg) was added to each sample (at this concentration we are above the solubility limit for all the samples). The solution was stirred for 24 h at 37 °C until equilibrium was reached. Keeping the solutions in a 37 °C, the non-dissolved drugs were filtered off through a 0.25 μm syringe filter, and 10 μL of each filtered solution was diluted to 50 mL of MilliQ® water. Triplicates were prepared for

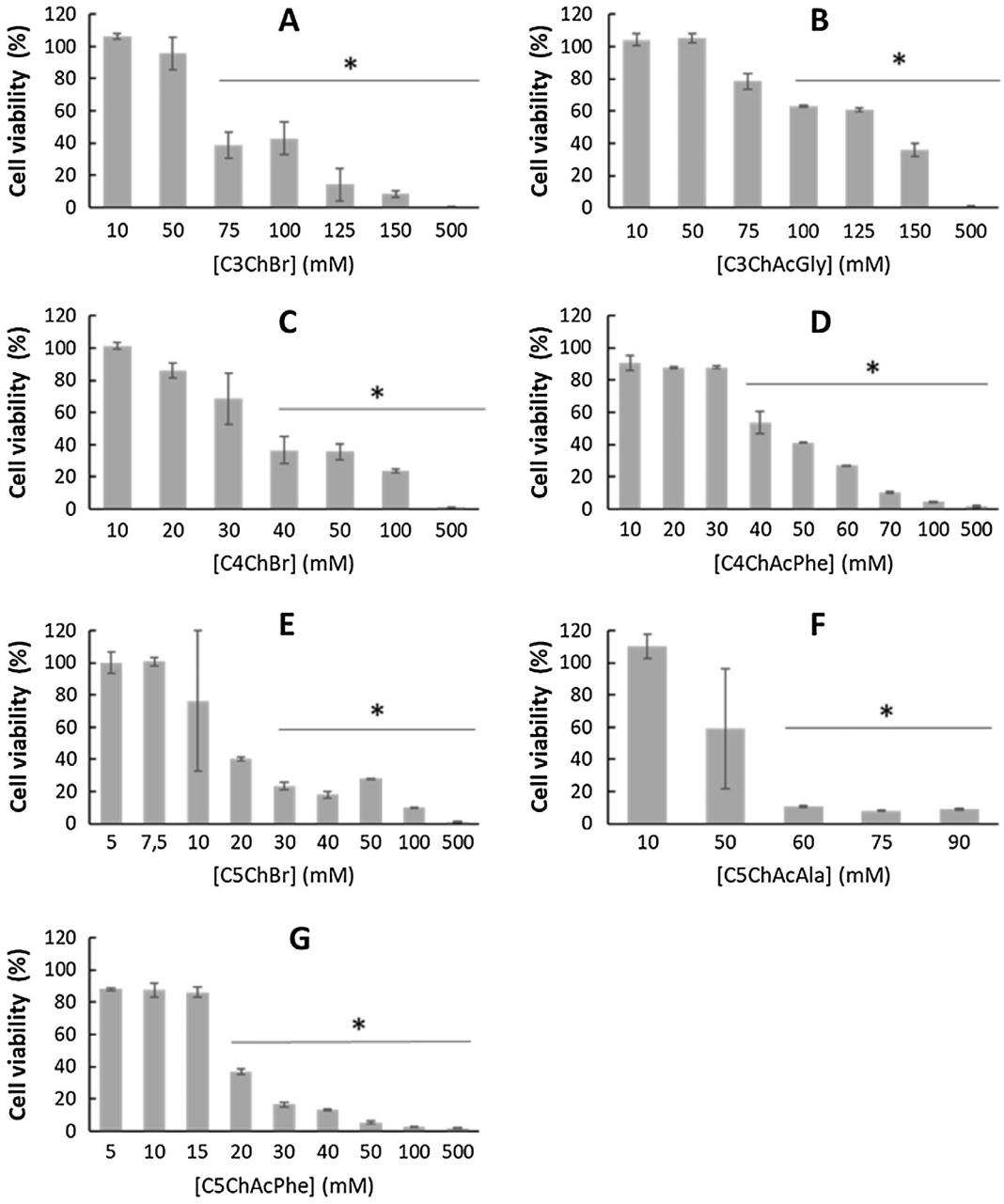


Fig. 7. Cytotoxicity of the precursors and respective derived ionic liquids (A) [C3Ch]Br, (B) [C3Ch][AcGly], (C) [C4Ch]Br, (D) [C4Ch][AcPhe], (E) [C5Ch]Br, (F) [C5Ch][AcAla], and (G) [C5Ch][AcPhe] in normal human dermal fibroblasts after 48 h exposure to different concentrations of the respective ILs. The results are expressed as the average ± SD from three independent biological assays. The symbol * indicates that the viabilities are statistically significant with $p < 0.01$ when compared to the control.

Table 2
Relative IC_{50} of the precursors and respective derived ionic liquids [C3Ch]Br, [C3Ch][AcGly], [C4Ch]Br, [C4Ch][AcPhe], [C5Ch]Br, [C5Ch][AcAla], [C5Ch][AcPhe] after 48 h exposure in normal human dermal fibroblasts.

Compound	IC_{50} (mM)
[C3Ch]Br	75.1
[C3Ch][AcGly]	122.7
[C4Ch]Br	33.3
[C4Ch][AcPhe]	46.4
[C5Ch]Br	17.5
[C5Ch][AcAla]	28.4
[C5Ch][AcPhe]	19.3

each sample and maximum absorbance was measured at 277 nm. The solubility of sodium diclofenac in the presence of each ionic liquid was calculated based on the calibration curve.

4.1.7. General procedure for the determination of partition coefficient and log P

The K_{ow} and log P values of paracetamol and sodium diclofenac in the presence of ionic liquids were determined using the shake-flask method. Water and octanol were both saturated prior to the experiments. Saturation was achieved by mixing equals amounts of water and octanol and stirring for 3 days at room temperature. Then, the mixture was transferred to a separation funnel, and layers were allowed to completely separate for 2 days. The octanol-saturated aqueous layer was used to prepare the ionic liquid solutions at 0.2 or 1 mol%, prior to the assays. The experimental setup consisted of 15 mL glass vials

equipped with a magnetic stirrer, in which random but known amounts of drug were dissolved in 1 g of IL solution and mixed with 1 g of water-saturated octanol. The solutions stirred vigorously for 18–24 h and then sat for some hours until full separation of organic and aqueous layers. Three independent experiments were performed. Octanol and water layers were separated and centrifuged for 30 min at 2000 rpm. The drug concentration in the water-rich phase was analysed in a UV–Vis VWR® spectrophotometer, model UV-6300PC, using a previously prepared calibration curve. Samples were diluted until the absorbance was smaller than 1.

The concentration of drug in the octanol-rich phase was directly calculated by subtracting the amount in the water-rich phase to the initial amount dissolved.

K_{ow} and log P of the three samples were determined using Eqs. (1) and (2), and the mean value was taken.

$$K_{ow} = [drug]_{oct}/[drug]_{aq} \quad (1)$$

$$\log P = \log K_{ow} \quad (2)$$

4.2. Cytotoxicity evaluation

4.2.1. Cell culture

Normal primary dermal fibroblasts, ATCC® PCS-201–010, (ATCC, Manassas, VA, USA), were cultivated in Dulbecco's modified Eagle's medium (DMEM) (Thermofisher Scientific, Waltham, Ma, EUA) supplemented with 10% (v/v) Fetal Bovine Serum, FBS, (Thermofisher Scientific) 100 U/mL penicillin and 100 mg/mL streptomycin (Thermofisher Scientific) at 37 °C in an atmosphere with 5% (v/v) CO_2 and 99% (v/v) relative humidity.

4.2.2. Viability assays

The cellular viability was evaluated as previously described. [48] Fibroblast cells were incubated in 96-well plates (SPL Life Sciences, Co., Ltd. Naechon-myeon, South Korea) at a concentration of 7500 cells per well, for 48 h with different concentrations of ILs [C5Ch][AcAla], [C3Ch][AcGly], [C4Ch][AcPhe], [C5Ch][AcPhe], or the precursors [C5Ch]Br, [C4Ch]Br and [C3Ch]Br, ranging from 5 to 500 mM. Cell viability was evaluated using the CellTiter 96® AQueous One Solution Cell Proliferation Assay System (Promega, Fitchburg, Wi, USA) according to the manufacturer's instructions. Viable cells produce formazan by reduction of the 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)–2H-tetrazolium salt (MTS). The formazan product was quantified by measuring the absorbance at 490 nm in an Infinite M200 microplate reader (Tecan, Mannedorf, Switzerland).

GraphPad Prism v6.01 (GraphPad Software Inc, La Jolla, California) was used to perform statistical analysis. Data is expressed as average ± standard deviation from at least 3 independent experiments.

Acknowledgements

The authors thank Fundação para a Ciência e Tecnologia, FCT/MCTES (Portugal) for financial support through Investigator contracts (IF/00355/2012 – J.M.S.S. Esperança and IF/00621/2015 – P.M. Reis), and through project IF/00621/2015.

This work was also partially supported by the Associated Laboratory Research Unit for Green Chemistry, Technologies and Clean Processes, LAQV/Requimte (financed by national funds from FCT/MCTES, UID/QUI/50006/2019 and co-financed by the ERDF under the PT2020, POCI-01-0145-FEDER-007265) and by the Unidade de Ciências Biomoleculares Aplicadas - UCIBIO (financed by national funds from FCT/MCTES, UID/Multi/04378/2019 and co-financed by the ERDF under the PT2020 Partnership Agreement, POCI-01-0145-FEDER-007728).

The NMR spectrometers are part of The National NMR Facility, supported by FCT/MCTES (RECI/BBB-BQB/0230/2012).

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejpb.2019.03.004.

References

[1] H.D. Williams, Y. Sahbaz, L. Ford, T.-H. Nguyen, P.J. Scammells, C.J.H. Porter, Chem. Commun. 50 (2014) 1688.
[2] S. Kalepu, V. Nekkanti, Acta Pharmaceutica Sinica B 5 (2015) 442.
[3] B. Dhillon, N.K. Goyal, R. Malviya, P.K. Sharma, Global J. Pharmacol. 8 (2014) 26.
[4] A.T.M. Serajuddin, Adv. Drug Deliv. Rev. 59 (2007) 603.
[5] K. Kawakami, N. Oda, K. Miyoshi, T. Funaki, Y. Ida, Eur. J. Pharm. Sci. 28 (2006) 7.
[6] L. Yu, Adv. Drug Deliv. Rev. 48 (2001) 27.
[7] C. Oerlemans, W. Bult, M. Bos, G. Storm, J.F.W. Nijsen, W.E. Hennink, Pharm. Res. 27 (2010) 2569.
[8] T. Loftsson, M.E. Brewster, J. Pharm. Sci. 85 (1996) 1017.
[9] R.H. Müller, C. Jacobs, O. Kayser, Adv. Drug Deliv. Rev. 47 (2001) 3.
[10] K. Kraft Walter, B. Steiger, D. Beussink, N. Quiring John, N. Fitzgerald, E. Greenberg Howard, A. Waldman Scott, J. Clin. Pharmacol. 44 (2013) 67.
[11] S. Xie, L. Zhu, Z. Dong, X. Wang, Y. Wang, X. Li, W. Zhou, Coll. Surf. B Biointerf. 83 (2011) 382.
[12] K.Y. Janga, R. Jukanti, A. Velpula, S. Sunkavalli, S. Bandari, P. Kandadi, P.R. Veerareddy, European J. Pharmaceut. Biopharmaceutics 80 (2012) 347.
[13] A. Müllertz, A. Ogbonna, S. Ren, T. Rades, J. Pharm. Pharmacol. 62 (2010) 1622.
[14] C.W. Pouton, Eur. J. Pharm. Sci. 29 (2006) 278.
[15] C. Agatemor, K.N. Ibsen, E.E.L. Tanner, S. Mitragotri, Bioeng. Transl. Med. 3 (2018) 7.
[16] Z. Lei, B. Chen, Y.-M. Koo, D.R. MacFarlane, Chem. Rev. 117 (2017) 6633.
[17] K.S. Egorova, E.G. Gordeev, V.P. Ananikov, Chem. Rev. 117 (2017) 7132.
[18] Y. Sahbaz, H.D. Williams, T.H. Nguyen, J. Saunders, L. Ford, S.A. Charman, P.J. Scammells, C.J. Porter, Mol. Pharm. 12 (2015) 1980.
[19] J.L. Shamshina, P.S. Barber, R.D. Rogers, Expert Opinion Drug Delivery 10 (2013) 1367.
[20] V. Jaitely, A. Karatas, A.T. Florence, Int. J. Pharm. 354 (2008) 168.
[21] K.B. Smith, R.H. Bridson, G.A. Leeke, J. Chem. Eng. Data 56 (2011) 2039.
[22] J. Resende de Azevedo, J.-J. Letourneau, F. Espitalier, M.I. Ré, J. Chem. Eng. Data 59 (2014) 1766.
[23] J.L. Shamshina, R.D. Rogers, Therapeutic Delivery 5 (2014) 489.
[24] N. Adawiyah, M. Moniruzzaman, S. Hawatulaila, M. Goto, MedChemComm 7 (2016) 188.
[25] S. Kalepu, M. Manthina, V. Padavala, Acta Pharmaceutica Sinica B 3 (2013) 361.
[26] M. Halayqa, A. Pobudkowska, U. Domańska, M. Zawadzki, Eur. J. Pharm. Sci. 111 (2018) 270.
[27] K.D. Weaver, H.J. Kim, J. Sun, D.R. MacFarlane, G.D. Elliott, Green Chem. 12 (2010) 507.
[28] L. Gontrani, Biophys. Rev. 10 (2018) 873.
[29] S. Kirchhecker, D. Esposito, Curr. Opin. Green Sustain. Chem. 2 (2016) 28.
[30] H. Ohno, K. Fukumoto, Acc. Chem. Res. 40 (2007) 1122.
[31] K. Fukumoto, M. Yoshizawa, H. Ohno, J. Am. Chem. Soc. 127 (2005) 2398.
[32] O.J. Curnow, R. Yunis, RSC Adv. 6 (2016) 70152.
[33] M.P. Pereira, R.d. Souza Martins, M.A.L. de Oliveira, F.I. Bombonato, RSC Adv. 8 (2018) 23903.
[34] S. Bhattacharyya, F.U. Shah, J. Mol. Liq. 266 (2018) 597.
[35] C. Lu, J. Cao, N. Wang, E. Su, MedChemComm 7 (2016) 955.
[36] R.A. Granberg, Å.C. Rasmuson, J. Chem. Eng. Data 44 (1999) 1391.
[37] L.F. Žilnik, A. Jazbinšek, A. Hvala, F. Vrečer, A. Klamt, Fluid Phase Equilib. 261 (2007) 140.
[38] Stoner, C. L.; Troutman, M. D.; Laverty, C. E. In Cancer Drug Design and Discovery; Neidle, S., Ed.; Academic Press: New York, 2008, p 131.
[39] M.F. Harris, J.L. Logan, J. Chem. Educ. 91 (2014) 915.
[40] A.A. Somani, K. Thelen, S. Zheng, M.N. Trame, K. Coboeken, M. Meyer, K. Schnizler, I. Ince, S. Willmann, S. Schmidt, Br. J. Clin. Pharmacol. 81 (2016) 137.
[41] B. Chuasuwan, V. Binjesoh, J.E. Polli, H. Zhang, G.L. Amidon, H.E. Junginger, K.K. Midha, V.P. Shah, S. Stavchansky, J.B. Dressman, D.M. Barends, J. Pharm. Sci. 98 (2009) 1206.
[42] A. Avdeef, K.J. Box, J.E. Comer, C. Hibbert, K.Y. Tam, Pharm. Res. 15 (1998) 209.
[43] T. Scheytt, P. Mersmann, R. Lindstädt, T. Heberer, Water Air, Soil Pollution 165 (2005) 3.
[44] H. Pajouhesh, G.R. Lenz, NeuroRx 2 (2005) 541.
[45] Y.C. Toh, T.C. Lim, D. Tai, G. Xiao, D. van Noort, H. Yu, Lab Chip 9 (2009) 2026.
[46] A.J. Costa, M.R. Soromenho, K. Shimizu, I.M. Marrucho, J.M. Esperanca, J.N. Lopes, L.P. Rebelo, ChemPhysChem 13 (2012) 1902.
[47] A. Costa, A. Forte, K. Zalewska, G. Tiago, Z. Petrovski, L.C. Branco, Green Chem. Lett. Rev. 8 (2015) 8.
[48] A. Maron, K. Czerwinska, B. Machura, L. Raposo, C. Roma-Rodrigues, A.R. Fernandes, J.G. Malecki, A. Szlapa-Kula, S. Kula, S. Krompiec, Dalton Trans. 47 (2018) 6444.