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Cholinium-Based Ionic Liquids with Pharmaceutically Active Anions†

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Novel ionic liquids (ILs) containing cholinium as a benign cation combined with anions based on five active pharmaceutical ingredients (APIs), namely, nalidixic acid, niflumic acid, 4-amino-salicylic acid, pyrazinoic acid and picolinic acid, were prepared *via* a simple and sustainable two-step anion exchange reaction. The solubility of the prepared pharmaceutically active ILs (API-ILs) in both water and simulated biological fluids at 25 °C and 37 °C, as well as the solubility of the parent APIs, were measured. Further, *in vitro* cytotoxicity levels for both cholinium-based API-ILs and parent APIs were established using two different human cells lines, namely Caco-2 colon carcinoma cells and HepG2 hepatocellular carcinoma cells. Herein, the dual nature of ILs is exploited by combining the cheap, available and essential nutrient cholinium cation with pharmaceutically active anions, upgrading the chemical, physical and biopharmaceutical properties, particularly melting point, aqueous solubility and the potential to penetrate cell membranes of the parent APIs, without impair their cytotoxicity response which prompt opportunities for creating further advances in pharmaceutical challenges.

Introduction

According to the commonly accepted definition, ILs are salts with a melting point below the conventional temperature of 100 °C[1]. Due to their unique chemical and physical properties addressable by their dual-functional nature and their design flexibility which allow target synthesis of "tuned" materials, ILs display considerable potential in a variety of biomedical topics, including the formulation of biologically active compounds for delivery into the human body[2–7], as well as the solubilisation of biochemical compounds of paramount importance such as DNA[8,9] and nucleic acid bases[10–12].

An emerging research field of interest is the use of ILs in pharmaceutical applications. The potential of ILs as pharmaceutical solvents to dissolve poorly soluble APIs has been reported[13]. Additionally, their use as drug delivery vehicles has been investigated[14–16]. Moreover, some works have taken a further step in developing ILs that are themselves the APIs[2–7].

The first attempt to specifically prepare API-ILs was accomplished by Rogers and co-workers[2], who reported on the potential of this poorly exploited drug phase. Several studies have been recently published on API-ILs by an increasing number of research groups. Our group reported novel ILs containing antibiotics, ampicillin as an API anion[5,7] and tetracycline as an API cation[6], while Bica *et al.*[17] reported ILs based on analgesic, anti-pyretic and anti-inflammatory compounds (acetylsalicylic and salicylic acids). Hough *et al.*[2] and MacFarlane and co-workers[3] addressed API-ILs containing various active cations and anions. Hough-Troutman *et al.*[18] exploited the dual nature of ILs by combining anti-bacterial quaternary ammonium cations with artificial sweetener anions. Also, Cybulski and co-workers[19] prepared ILs based on antiseptic/disinfectant cations and amino acids anions, which were found to be very effective against bacteria and fungi.

The pharmaceutical industry has attributed more than 40% of the failures in new drug development to poor biopharmaceutical properties, particularly poor water solubility[20]. Water insolubility can shelve or invalidate new drug development, and thus significantly affect the much needed reformulation of currently marketed products[21–23]. In recent years, approximately 70% of new drug candidates have shown poor aqueous solubility[24]. Nowadays, close to 40% of the marketed immediate-release oral drugs are categorized as practically insoluble (<0.1 mg/ml)[25]. The poor aqueous sol-

† Electronic Supplementary Information (ESI) available: Experimental procedure, parent APIs attributes, ^{1}H and ^{13}C NMR, ESI-MS, elemental analysis, solubility of the cholinium-based API-ILs and parent APIs in the buffer solutions at 37 °C (PAS, Fig. S2; PYR, Fig. S3; PIC, Fig. S4) and cytotoxicity assay details. See DOI: 10.1039/b000000x/

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ubility of drug candidates plays one of the major roles in drug research and development. The low aqueous solubility of orally administered drugs leads to limited dissolution rate and low bioavailability. Compounds with aqueous solubility lower than 0.1 mg/ml mostly present dissolution-limited absorption[26]. In such cases, dose escalation is required until the blood concentration attains the therapeutic drug concentration range. However, this procedure might originate topical toxicity in the gastrointestinal tract upon oral administration, and impels to a reduction in patient compliance. Additionally, the manufacturing costs increase since a large amount of API is needed in the drug development and production.

Following the high intrinsic value of APIs and the relevance of structure and composition in the context of both intellectual property and bioavailability, a systematic approach to the development of a new broad class of APIs has been put forward in recent years, API-ILs[2]. API-ILs are attractive to the pharmaceutically industry because they offer multiple opportunities to modify the chemical and physical properties of an API (e.g. melting point, chemical stability, physical stability, solubility, bioavailability, dissolution rate) with a relatively simple approach[5]. Additionally, it allows a synergetic action upon the appropriate selection of the counter-ion. API-ILs counter-ions that are suitable for pharmaceutical use remain to be fully enumerated, but over 370 substances are listed as "Generally Regarded As Safe (GRAS)" by the FDA (U.S. Food and Drug Administration), including vitamins, food additives and other well-accepted substances. Additionally, exceedingly safe drugs, such as paracetamol and aspirin, are also valid counter-ions.

The access of ILs into the biosciences has been delayed mainly due to the toxicity of the counter-ions[5]. An alternative approach capable of overcoming this drawback is the development of ILs from components with well characterized biodegradable and toxicological properties. Natural metabolites are superior candidates to design low toxicity ILs. A striking example is the cholinium cation (cf. Figure 1), formerly classified as Vitamin B4[27]. The combination of this cation as well as related ones (e.g. betainium cations) with appropriate anions is a suitable approach to form "drinkable" ILs[5,28,29]. Albeit, the inclusion of a systematic evaluation of the toxicological properties is considered mandatory for IL product development[30].

In the present study, the development of novel API-ILs through the combination of the chemical and biological properties of the cholinium cation with those of pharmaceutically active anions, exploiting the dual nature of ILs, has been accomplished. Cholinium is an essential nutrient[31], which is necessary for the normal growth of cells and its dietary deficiency in adults animals and humans causes fatty liver as well as other abnormalities. Additionally, ILs with the benign cholinium cation were found to be highly biocompatible[32], e.g. the cholinium alkanoates are less toxic than their corresponding sodium salts. Also, the cytotoxicity of a family of cholinium phosphate ILs has been measured for J774 murine macrophage cell line, revealing that the toxicity essentially depends on the employed anion[33]. The structures of the APIs brought into play in this work are depicted in Figure 1, and their most relevant attributes are briefly described in the ESI.†

Herein, we report a straightforward synthetic procedure, characterization and thermal properties of five novel ILs based on the cholinium cation combined with anions based on the following APIs–nalidixic acid (NAL), niflumic acid (NIF), 4-amino-salicylic acid (PAS), pyrazinoic acid (PYR) and picolinic acid (PIC). Further, the solubility in water at 25 °C and buffer solutions suitable for dissolution testing at 37 °C (body temperature) were evaluated. In the latter case, simulated gastric fluid without enzymes–interchangeable with 0.1 N HCl (pH 1.0), simulated intestinal fluid without enzymes–interchangeable with phosphate standard buffer pH 6.8, and a 0.15 M NaCl–isotonic ionic strength solution were used. The solubility of the parent APIs were also estimated. Additionally, to ascertain whether our *a priori* assumption of a high biocompatibility of the cholinium-based API-ILs is valid, we have performed *in vitro* cytotoxicity assays with two different human cells lines, namely Caco-2 colon carcinoma cells and HepG2 hepatocellular carcinoma cells. We report experimental data on the cytotoxicity of the cholinium-based API-ILs and parents APIs in order to identify the toxicity levels for continued development in pharmaceutical formulations.

Results and Discussion

Cholinium serves many physiological functions due to its incorporation in membrane components, signaling molecules, and neurotransmitters[34]. Because of the prevalence of cholinium in the human body, cholinium-based API-ILs have been prepared and investigated within the frame of this work for their potential use in pharmaceutical applications. Plus, cholinium salts such as the chloride ($[N_{1112OH}]Cl$ –the cation "supplier", cf. Figure 1) are nowadays cost-efficient chemicals, produced on a scale of millions per year. Therefore, cholinium-based API-ILs should not only be biocompatible, but also offer economic advantages. The structures of the cholinium-based API-ILs prepared in this study are shown in Figure 1.

ILs are conventionally prepared by anion exchange of halide salts with metal salts or acids[35]. One of the major concerns within the IL community, is the need to prepare halide-free ILs. These inorganic contaminations are a drawback for the alkaline salt metathesis synthetic route, that can be avoided by the use of acids instead of salts[36].

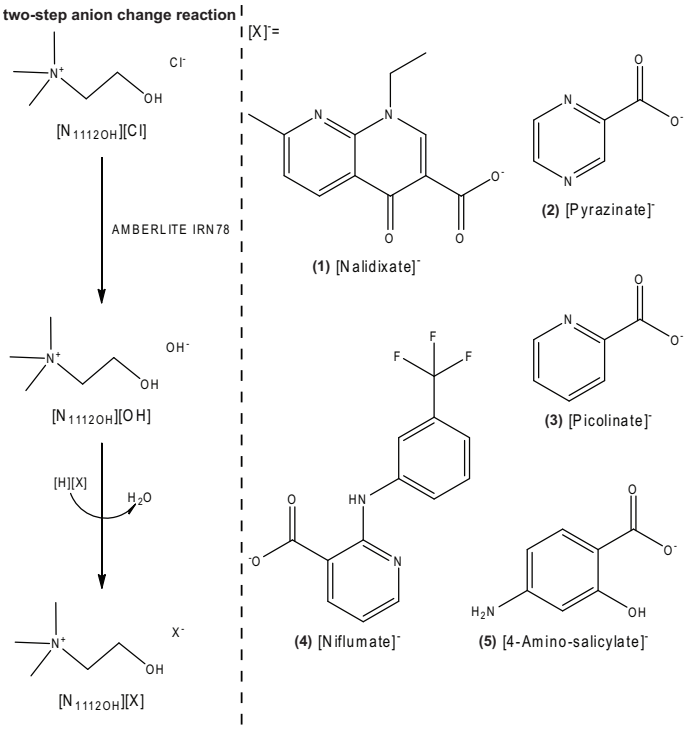

Fig. 1 Two-step anion exchange reaction and chemical structure of the ions used for preparation of the cholinium-based API-ILs. The abbreviations of the prepared cholinium-based API-ILs are as follow, (1) [N_{1112OH}][NAL], (2) [N_{1112OH}][PYR], (3) [N_{1112OH}][PIC], (4) [N_{1112OH}][NIF] and (5) [N_{1112OH}][PAS].

However, due to the fact that an anion exchange by weaker acids than hydrohalic acids cannot be efficiently performed[28], this method is unsuitable for the present study. Instead, we implemented ion exchange resin methods, as develop by Ohno *et al.*, which are being successfully used as alternative anion exchange processes[37]. Therefore, the cholinium-based API-ILs were synyhesised *via* the two-step anion exchange reaction illustrated in Figure 1. In this synthetic procedure, an anion exchange resin in the OH form (SUPELCO AMBERLITE IRN78) was used to prepare an aqueous solution of cholinium hydroxide ([N_{1112OH}][OH]) from cholinium chloride ([N_{1112OH}]Cl) aqueous solution. The [N_{1112OH}][OH] was then neutralized by the dropwise addition of adequate aqueous API solution. Neat cholinium-based API-ILs were obtained after eliminating the excess water and API by evaporation and washing, respectively. All isolated products were completely characterized by ^{1}H and ^{13}C NMR, ESI mass spectra and elemental analysis in order to check their expected structures and final purities. Additionally, the quantitative integration of their characteristic ^{1}H NMR resonance peaks unfold the expected cation/anion correlations. The water content, determined by Karl Fischer titration, was less than 0.05 wt%. The chloride content (halide impurities), quantified by a Chloride Ion Selective Electrode, was found to be less than 0.04 wt%. The prepared cholinium-based API-ILs were further characterized by thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC).

Despite the fact that several studies[38–40] assumed that the inclusion of carboxylic acid groups in ILs would readily produce strong hydrogen bonds, and consequently high melting points (T_m) or glass transition temperatures (T_g), some works prepared low-melting ILs incorporating carboxylate groups[28,32]. In this work, four out of the five prepared cholinium-based API-ILs have T_m and T_g below 100 °C, and two of them ([N_{1112OH}][NIF] and [N_{1112OH}][PIC]) are liquid at room temperature. [N_{1112OH}][PIC] shows no melting or freezing behavior in the DSC measurements, even when slowly heated and cooled at a scan rate of 1 °C/min. Only [N_{1112OH}][PAS] was obtained as a molten salt (T_m = 117.40 °C). Particularly relevant is the decrease of the initial melting points of the parent APIs (listed in Table 1) by their conjugation with the cholinium cation. The thermal properties (melting points and glass transition temperatures) of the synthesized cholinium-based API-ILs and parent APIs are summarized in Table 1.

Table 1 Thermal properties (T_g, T_m and T_{onset}) of the cholinium-based API-ILs. The melting points of the parent APIs are also depicted to highlight the successful reduction of the initial melting points

Compound	T_g[a] / °C	T_m[b] / °C	T_{onset}[c] / °C
[N_{1112OH}][NIF]	-19.45	-4.70 (lit.,[41] 205.24)	188.47
[N_{1112OH}][PIC]	-52.45	— (lit.,[42] 137-138)	176.28
[N_{1112OH}][NAL]	-16.81	76.07 (lit.,[43] 227-229)	179.41
[N_{1112OH}][PYR]	-47.15	81.31 (lit.,[44] 225)	179.41
[N_{1112OH}][PAS]	-19.93	117.40 (lit.,[45] 151-153)	171.04

[a]Glass transition temperatures (T_g) and [b]melting temperatures (T_m) were determined by DSC at a heating rate of 1 °C/min ([N_{1112OH}][NIF]), 5 °C/min ([N_{1112OH}][PIC]), 1 °C/min ([N_{1112OH}][NAL]), 5 °C/min ([N_{1112OH}][PYR]), 1 °C/min ([N_{1112OH}][PAS]), after cooling to -90 °C under nitrogen. The melting temperatures of the parent APIs are also depicted enclosed in parentheses. [c]Decomposition temperatures (T_{onset}) were measured by TGA with a heating rate of 1 °C/min under nitrogen.

Figure 2 shows the DSC trace for [N_{1112OH}][PYR] (solid API-IL with the highest T_m) at 5 and 10 °C/min rates. [N_{1112OH}][PYR] remains liquid upon cooling (subcooled liquid state) until it reaches a glass transition at low temperature. The glass transition with enthalpic relaxation is reached at -47.15 °C in the heating part of the cycle at a scan rate of 5 °C/min (-45.99 °C at 10 °C/min). Upon heating above the glass transition, [N_{1112OH}][PYR] exhibits an exothermic crystallization peak at 21.04 °C (42.14 °C at 10 °C/min),

cold crystallization temperature, followed by an endothermic melting peak at 81.31 °C (82.63 °C at 10 °C/min). This API-IL shows thermal hysteresis.

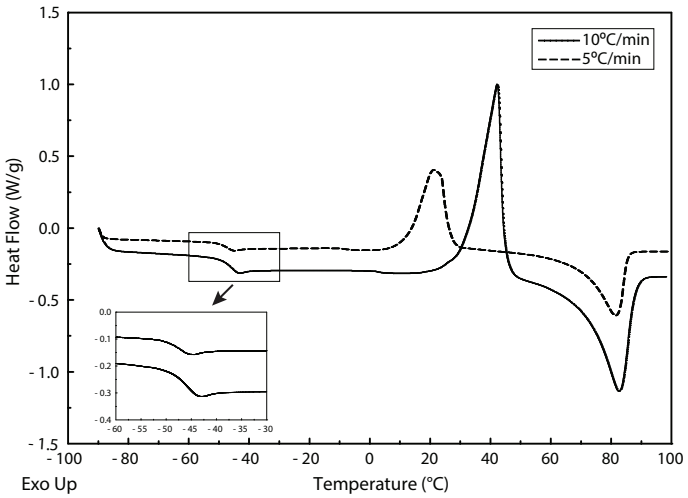

Fig. 2 DSC curves of [N_{1112OH}][PYR] at two different scan rates, 10 °C/min and 5 °C/min. This cholinium-based API-IL shows thermal hysteresis. [N_{1112OH}][PYR] remains liquid upon cooling (subcooled liquid state) until it reaches a glass transition at low temperature in the heating part of the cycle. Upon heating above the glass transition, [N_{1112OH}][PYR] exhibits an exothermic crystalization peak (cold crystalization temperature) followed by an endothermic melting peak.

Although the API-IL [N_{1112OH}][PYR] is not liquid at room temperature, or even at body temperature–a more relevant temperature for pharmaceutical applications–(the T_m cut-off of API-ILs for use within the body should be around 37 °C), the two DSC traces at scan rates of 5 and 10 °C/min (see Figure 2) indicate the potential of the cholinium-based API-ILs approach to effectively reduce the melting point of the parent API (pyrazinoic acid; lit.[44], T_m = 225 °C). Additionally, [N_{1112OH}][PYR] exhibits cold crystallization temperature and thus depending on the cooling–heating cycles this API-IL can be easily handled as liquid at room temperature, which is advantageous for the development of new drug delivery systems, e.g. adsorption of API-ILs onto mesoporous silica-based materials, that are emerging as good candidates for controlled delivery of APIs[46].

Thermal stability studies were performed by TGA analysis for all the synthesized cholinium-based API-ILs. The onset points of decomposition (T_{onset}) are listed in Table 1, and they point out the influence of the organic cation on the thermal stability of these compounds. It can be observed that the thermal stability of all API-ILs prepared in this work are very similar.

Solubility data were determined for the prepared cholinium-based API-ILs ([N_{1112OH}][NAL], [N_{1112OH}][NIF], [N_{1112OH}][PAS], [N_{1112OH}][PYR], and [N_{1112OH}][PIC]), as well as for the parent APIs (nalidixic acid, niflumic acid, 4-amino-salicylic acid, pyrazinoic acid, and picolinic acid), in both water at 25 °C and buffer solutions suitable for dissolution tests at 37 °C (body temperature). In the latter case, simulated gastric fluid without enzymes–interchangeable with 0.1 N HCl (pH 1.0), simulated intestinal fluid without enzymes–interchangeable with phosphate standard buffer pH 6.8, and 0.15M NaCl–isotonic ionic strength were used. The solubility of the API-ILs and parent APIs in water at 25 °C is summarized in Table 2 (solubility data of the parent APIs reported in bibliography are also depicted when available). As mentioned above, poor water solubility is a limiting factor in the efficacy and bioavailability of an API. Figure 3 illustrates the water solubility at 25 °C of the cholinium-based API-ILs vs parent APIs, and clearly demonstrates that the cholinium-based API-ILs strategy is appropriate to overcome the solubility problems of the parent APIs, and consequently enhance their bioavailability and efficacy. The most relevant results were attained for [N_{1112OH}][NAL] and [N_{1112OH}][NIF], whose parent APIs (nalidixic acid and niflumic acid, respectively) are classified as practically insoluble in water (solubility $<$ 0.1 mg/ml), thus presenting dissolution-limited adsorption[26].

Table 2 Solubility of cholinium-based API-ILs and parent APIs in water at 25 °C (±0.1 °C), where the solubility is the overall mean of three independent experiments ± standard deviation

API	Solubility (mg/ml)	
	cholinium-based API-ILs	parent APIs[a]
NAL	95.12 ± 4.44	0.0282 ± 0.0008 (lit.,[47] 0.031)
NIF	1124 ± 37	0.0212 ± 0.0008 (lit.,[48–50] 0.019, 0.040, 0.085)
PAS	101.3 ± 2.8	4.554 ± 0.221 (lit.,[51–53] 1.996[b], 1.685[c], 3.216[d].)
PYR	254.9 ± 7.3	6.763 ± 0.061
PIC	984.3 ± 5.4	475.7 ± 8.1

[a]Solubility data of the parent APIs reported in bibliography are also depicted when available enclosed in parentheses. [b]@20 °C. [c]@23 °C. [d]@30 °C

Additionally, the determination of the solubility of an API under physiological pH conditions is recommended. The pH–solubility profile of the API should be determined at 37 °C in aqueous media with a pH in the range of 1–7.5. The solu-

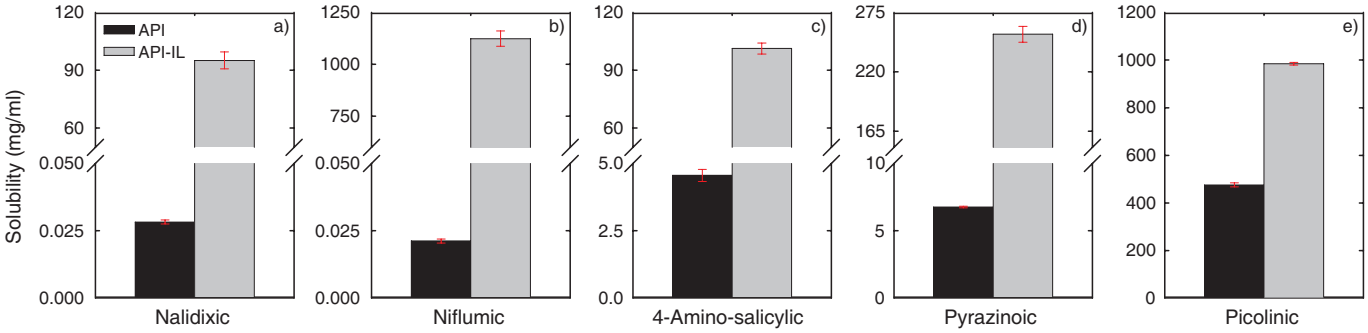

Fig. 3 Solubility of the prepared cholinium-based API-ILs and parent APIs in water at 25 °C.

bility results of the cholinium-based API-ILs and parents APIs in the above-mentioned buffer solutions at 37 °C are summarized in Table 3. The comparative analysis (cholinium-based API-ILs vs. parent APIs) of these solubilities, for the above-mentioned practically insoluble in water nalidixic and niflumic acids, is depicted in Figure 4. The solubilities in the buffer solutions at 37 °C of the remaining cholinium-based API-ILs ([N_{1112OH}][PAS], [N_{1112OH}][PYR], and [N_{1112OH}][PIC]), and respective parent APIs (4-amino-salicylic acid, pyrazinoic acid, and picolinic acid), are shown in Figures S2, S3, and S4 of the ESI.†

Table 3 Solubility of cholinium-based API-ILs and parent APIs in buffer solutions suitable for dissolution testing, simulated gastric fluid (pH 1.0), simulated intestinal fluid (pH 6.8) and isotonic ionic strength aqueous solution (0.15M NaCl), at 37 °C (±0.1 °C). The solubility is the overall mean of three independent experiments ± standard deviation

Compound	Solubility (mg/ml)		
	pH=1.0	pH=6.8	0.15M NaCl
[N_{1112OH}][NAL]	14.12±0.23	38.92±0.43	95.00±1.40
NAL	0.0515±0.0020	0.330±0.014	0.0570±0.0005
[N_{1112OH}][NIF]	77.56±3.76	313.5±10.8	952.8±46.1
NIF	0.688±0.027	1.600±0.024	0.0861±0.0014
[N_{1112OH}][PAS]	19.57±0.38	152.3±0.8	777.0±0.7
PAS	7.258±0.217	9.966±0.112	13.35±0.09
[N_{1112OH}][PYR]	67.17±2.20	318.5±8.1	220.7±10.5
PYR	8.547±0.137	13.28±0.50	9.161±0.313
[N_{1112OH}][PIC]	916.6±4.8	985.2±9.0	876.3±24.6
PIC	449.4±10.1	481.5±3.9	479.9±11.5

APIs display their specific potentials when they can penetrate cells through the cell membranes. The study of Kawai et al.[54] demonstrated that cholinium-like ILs clearly show the possibility to penetrate cell membranes. For that, the proposed cholinium-based API-ILs are considered to have a higher potential to penetrate cell membranes than the parent APIs. Ions cannot usually be transported through cell membranes by a simple diffusion process. However, it is well known that cholinium is transported through cell membranes for use as a material for the biosynthesis of phosphatidylcholine and acetylcholine[55,56]. The prepared cholinium-based API-ILs may be transported through cell membranes by a similar process. Additionally, in our work on ampicillin-based ILs[7], the cholinium-based ampicillin IL showed higher water solubility and octanol–water partition coefficient than ampicillin. The cholinium-based ampicillin IL also showed higher octanol–water partition coefficient than ampicillin sodium salt, though the water solubility values are similar.

It is important to emphasize that the use of the cholinium cation can effectively modify the solubility in water and buffer solutions suitable for dissolution testing of the parent APIs and consequently enhances its bioavailability, efficacy, and potential membrane permeability. Solving bioavailability problems of active pharmaceutical ingredients is a major challenge for the pharmaceutical industry, since nearly half of the new active substances being identified in high-throughput screening are either insoluble or poorly soluble in water[57].

Before API-ILs can be used for pharmaceutical applications, broad data such as cytotoxicity effects is required to establish biocompatibility. In this work, cytotoxicity assays were performed using two different human cell lines, Caco-2 colon carcinoma cells and HepG2 hepatocellular carcinoma cells, that are suitable and useful *in vitro* models for the intestinal barrier and hepatic metabolism studies, respectively[58,59]. Further, cytotoxicity test in largely dedifferentiated cancer cells lines such as Caco-2 and HepG2 cells provide a convenient screening method for obtaining first rough estimates for the toxic potential of chemical substances[60].

In vitro cytotoxicity of representative cholinium-based API-ILs ([N_{1112OH}][NAL], [N_{1112OH}][NIF] and

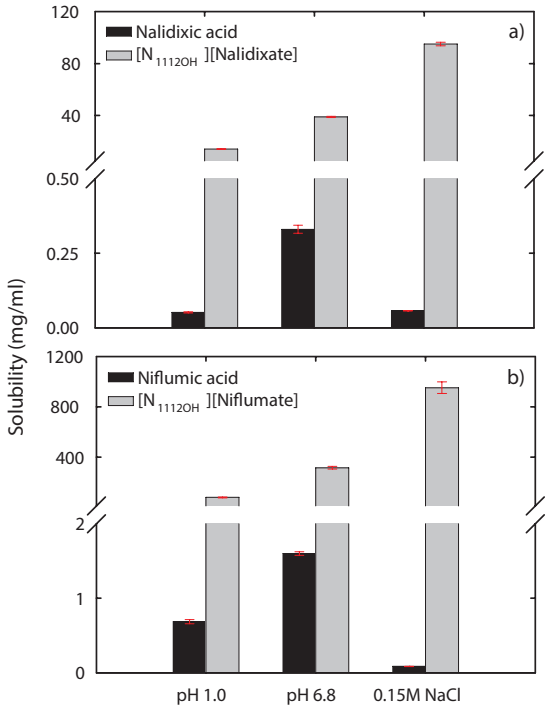

Fig. 4 Solubility of (a) [N_{1112OH}][NAL] and nalidixic acid (parent API), and (b) [N_{1112OH}][NIF] and niflumic acid (parent API) in buffer solutions suitable for dissolution testing, simulated gastric fluid (pH 1.0), simulated intestinal fluid (pH 6.8) and isotonic ionic strength aqueous solution (0.15M NaCl), at 37 °C.

[N_{1112OH}][PYR]) and parent APIs (nalidixic acid, niflumic acid and pyrazinoic acid, respectively) were measured using a proper MTS tetrazolium assay[61]. Due to the low solubility of the parent APIs in media-based solutions, all the stock solutions of the API-ILs and parent APIs were prepared first in Milli-Q water (around parent API solubility) and then the minimum possible dilution with culture medium, composed of 0.5% FBS, was performed to avoid precipitation (APIs and/or media nutrients). Cells were exposed up to six independent dilutions of each API-IL and parent API in 96-well plates for 24 h. Each plate contained a negative control (cells with culture medium), and a positive control (cells with 100 % DMSO). Concentrations ranged from 500 to 6000 μM for [N_{1112OH}][PYR] and pyrazinoic acid, from 20 to 58 μM for [N_{1112OH}][NAL] and nalidixic acid, and from 10 to 35 μM for [N_{1112OH}][NIF] and niflumic acid. Further details of the MTS assay and method are provided in the ESI.†

The dose-response cytotoxicity curves are depicted in Figure 5. No significant difference was observed in the metabolic activity of the cells within the incubation of the cholinium-based API-ILs and parent APIs with both cell lines in the concentration range tested. The course of the cytotoxicity curves did not allow a valid calculation of EC_{50} (i.e. effective concentration reducing cell viability to 50 %) due to the limited solubility of the parent APIs in the cell culture medium. Albeit, the studied concentrations lie above/in range of the pharmacokinetic parameter maximum plasma concentration of the parent APIs (170.03–202.26 μM for pyrazinoic acid[62]; 53.82–61.58 μM for nalidixic acid[63]; 20.20–25.16 μM for niflumic acid[64]), which is above possible intracellular concentrations.

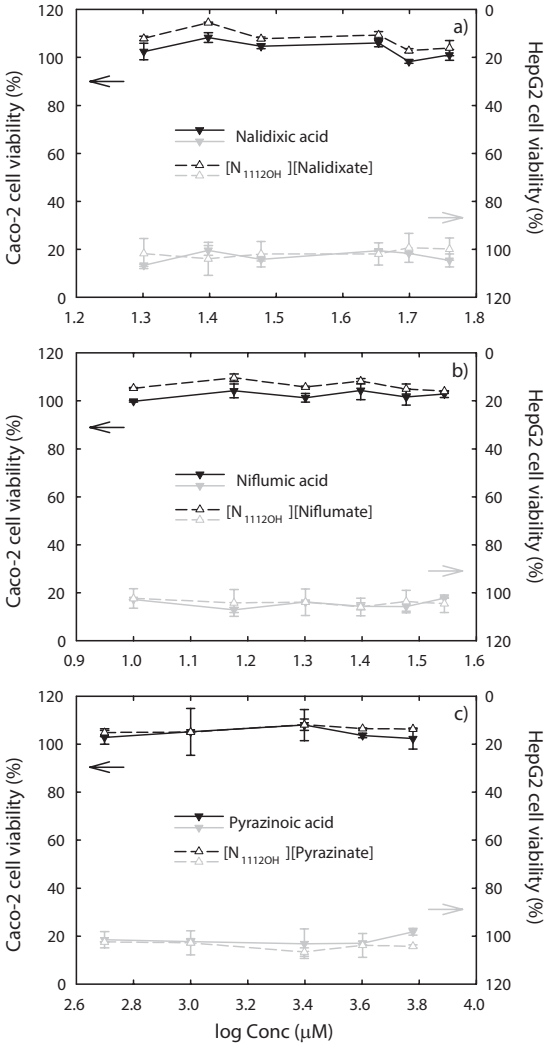

Fig. 5 Cytotoxicity profile of (a) [N_{1112OH}][NAL] and nalidixic acid, (b) [N_{1112OH}][NIF] and niflumic acid, and (c) [N_{1112OH}][PYR] and pyrazinoic acid in the Caco-2 and HepG2 cell lines. Both Caco-2 and HepG2 cells were exposed for 24 h to the respective cholinium-based API-IL and parent API at the concentrations shown and assessed for metabolic activity as described in the ESI†.

The cell viability of the API-ILs is comparable to those of the parent APIs (Figure 5), indicating that the use of a mod-

ular IL strategy based on the cholinium cation produced no significant effect on the API system cytotoxicity. The analysis of the *in vitro* cytotoxicity assays indicates the suitability and biocompatibility of the proposed cholinium-based API-ILs for pharmaceutical formulations.

Conclusions

In the present work, we report five new cholinium-based ILs with active pharmaceutical anions directly derived from the "APIs pool" (nalidixic acid, niflumic acid, 4-amino-salicylic acid, pyrazinoic acid, and picolinic acid). The cholinium cation, due to its particular properties–biocompatibility, low toxicity, high water solubility and numerous functions supported in organisms–, improves the aqueous solubility and the potential to penetrate cell membranes of the parent APIs. A modular IL strategy based on the benign, cheap and available cholinium cation can provide a platform for improved activity and new treatment options of pharmaceuticals. The feasibility to surpass problems such as reduced aqueous solubility and bioavailability, and polymorphism, might give discarded APIs a second life, or stimulate the acceptance of new APIs candidates. Cytotoxicity test on human cells lines have furthermore substantiated that cholinium-based API-ILs and parent APIs display similar cytotoxic response which spur opportunities for creating further advances in pharmaceutical challenges. The pharmacological action of the prepared API-ILs should be tested to verify if a potential synergetic effect between the ion pair exists, consequently leading to new therapeutic advantages. The development of API-ILs represents a paradigm that offers many opportunities related to drug development and delivery. This is a very appealing and fast moving field of research that may play a significant role in the future of healthcare, taking ILs from the benchtop to the bedside.

Acknowledgments

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