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Anti-inflammatory choline based ionic liquids: Insights into their lipophilicity, solubility and toxicity parameters

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ABSTRACT

The impact on *in vivo* efficacy and safety of two novel ionic liquids based on the association of choline with non-steroidal anti-inflammatory drugs, ketoprofen and naproxen forming IL-APIs, was evaluated. Their lipophilicity, solubility and toxicity were assessed aiming the illustration of the pharmaceutical profile and potential toxic impact.

Partition coefficient was determined using micelles of hexadecylphosphocholine and UV–Vis derivative spectroscopy. Additionally, solubility in phosphate buffer pH 7.4 was measured using a modified shake flask method and UV–Vis spectroscopy as detection technique. Ultimately, toxicity was considered resorting to a fully automated cytochrome *c* oxidase assay based on microfluidics. The obtained results demonstrated that the IL-APIs' drug format has the ability to interact with biological membranes and also improves solubility up to 58 times. Moreover, it was evidenced that, although being a nutrient, choline influences the IL-APIs' toxicity. The studied anti-inflammatory IL-APIs exhibited promising properties regarding their incorporation in pharmaceutical formulations.

1. Introduction

The pharmaceutical industry faces nowadays unprecedented challenges and amendments as a consequence of strong demands related with economic viability and environmental sustainability. The investment on research and development is forecast to decline since the risks associated with drug discovery seem to not be in accordance with the current economic demands. It is known that 90% of the experimental drugs cannot reach the market mainly due to lack of efficacy, toxicity and inadequate pharmacokinetics [1,2]. Indeed, approximately 40% of the novel drug candidates fail to obtain approval because of poor pharmaceutical properties such as reduced solubility or decreased permeation across the blood-brain barrier [3]. Moreover, the demands regarding the environmental impact of drug discovery are on the frontline of the principles of Green Chemistry [4].

Typically, the pharmaceutical industry relies on crystalline forms of active pharmaceutical ingredients (APIs) with claimed advantages in terms of purity, thermal stability, manufacturing and handling. However, solid APIs often suffer from low solubility and polymorphism conversion, which can have a negative impact on drug bioavailability and ultimately on its efficacy [2,5]. The issue of efficacy is quite important considering that this is the most frequent reason for failure in phase II clinical trials [6]. Salt formation is the most common and cost-effective strategy to overcome the problems faced by solid forms of APIs, being estimated that around 50% of all drugs utilized in the pharmaceutical industry are salts with improved properties regarding the corresponding ionizable drugs [5,7].

Ionic liquids (ILs) emerged as a new class of compounds with peculiar properties that make them suitable for distinct pharmaceutical applications [8]. In the past few years, ILs have been explored as reaction media for the synthesis of APIs [9], as pharmaceutical solvents [10] and as part of drug delivery systems [11,12]. Additionally, several authors have reported ILs that are themselves the APIs (IL-APIs) [13–17]. From the pharmaceutical point of view, an IL approach in the design of novel APIs seems to be appropriate as it enables a large number of possible cation-anion combinations while providing singular properties unreachable in solid salts, namely improved solubility and absence of

Abbreviations: APIs, active pharmaceutical ingredients; chol [KTP], choline ketoprofenate; chol [NAP], choline naproxenate; CytCox, cytochrome *c* oxidase; FeC, ferrocytochrome *c*; HDPC, hexadecylphosphocholine; ILs, ionic liquids; IL-APIs, pharmaceutically active ionic liquids; KTP, ketoprofen; NAP, naproxen; SIA, sequential injection analysis.

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polymorphic forms [5,18,19]. Moreover, the appropriate combination of cations and anions empowers the chemical manipulation of compounds with specific purposes related with the manufacturing process, the stability of formulations and their bioavailability [18,20]. The research in this field has been focused mostly on the synthesis of new IL-APIs, their physico-chemical characterization and *in vitro* assessment of the expected pharmacological activity [14,17,21]. As far as we know there are relatively few studies exploring the pharmaceutical properties of IL-APIs for prediction of their *in vivo* behavior and there is still little information regarding the toxicity of these compounds [16,22–26].

Thus, in this work, we studied for the first time the lipophilicity, solubility and toxicity of two anti-inflammatory IL-APIs based on choline (Fig. 1). The selected IL-APIs include distinct anions with well-known analgesic, anti-inflammatory and anti-pyretic activity, namely ketoprofen (KTP) and naproxen (NAP) [27], combined with the choline cation, an essential nutrient that is necessary for many critical functions in the human body [28].

The lipophilicity of the anti-inflammatory IL-APIs was assessed through the calculation of partition coefficient (expressed as log *D*) by derivative spectroscopy. The determination was based on the study of the interaction of the selected IL-APIs with a membrane model aiming to evaluate the distribution of drugs between aqueous and lipid phases. It is nowadays accepted and demonstrated that membrane models are more adequate for this purpose than biphasic solvent systems like octanol-water since they mimic the lipid bilayer of biological membranes [29,30]. Indeed, biomimetic membrane models are able to consider the hydrophobic, hydrogen bond, dipole-dipole and electrostatic interactions between drug and membrane, whereas octanol-water systems can only model nonpolar interactions [31,32]. In the present work, micelles of hexadecylphosphocholine (HDPC) were used as membrane model since they are easy and fast to prepare, have high stability and few spectral interferences, and circumvent the use of toxic organic solvents [33,34].

Taking into account the low solubility of the anions incorporated in the selected compounds it was evaluated if this property was improved in the IL format [35,36]. This was performed through the determination of thermodynamic solubility using the shake flask method. The solubility issue is of great importance since it is known that solubility affects both *in vitro* assay results and *in vivo* oral bioavailability. Indeed, poor aqueous solubility is one of the main causes for low systemic exposure and, consequently, lack of *in vivo* efficacy [3].

Considering the pharmaceutical potential of IL-APIs, their toxicity was evaluated resorting to a fully automated cytochrome *c* oxidase (CytCox) assay aiming to predict IL-APIs' safety. Enzymatic inhibition assays have already proved to be useful in clarifying the impact of ILs' structural elements on toxicity and the way they should be changed to decrease hazardous potential. The methodology presented the additional advantage of automation of the assay based on sequential injection analysis (SIA) as it guarantees a precise control of the reaction conditions and reduces the consumption of reagents as well as the production of hazardous effluents, being in good agreement with the present concerns of Green Chemistry [37,38].

With this work, it is then expected to contribute to the understanding of the potentialities of chol [KTP] and chol [NAP] to replace their APIs counterparts. For that, these compounds were submitted to new assays to evaluate more pharmaceutical properties and toxicity in order to complement the information gathered during their synthesis and physico-chemical characterization as well as their binding affinity data [26]. The comparison of the IL-APIs' profile with the respective starting materials aims to highlight some of the features of APIs in the IL format. Moreover, by contributing to the revealing of the drug-like nature of these compounds it is also expected to give early warning of potential difficulties in formulation, process development and safety that would otherwise increase development time/cost and delay their possible clinical introduction.

2. Experimental

2.1. Chemicals

All solutions were prepared using chemicals of analytical reagent grade and high purity water (Milli-Q water) with a specific conductance < 0.1 μS cm^{-1}.

KTP (2-(3-Benzoylphenyl)propionic acid), Na [NAP] ((*S*)-6-Methoxy-α-methyl-2-naphthaleneacetic acid sodium salt), Tris (2-Amino-2-(hydroxymethyl)-1,3-propanediol), CytCox from bovine heart (EC 1.9.3.1), cytochrome *c* and DL-dithiothreitol (*threo*-1,4-Dimercapto-2,3-butanediol) were all purchased from Sigma-Aldrich and used as supplied. HDPC was obtained from Cayman Chemical and used without further purification. chol [KTP] and chol [NAP] (99%) were synthesized and characterized as reported in our previous work [26]. The tested IL-APIs were stored at room temperature in a carefully controlled anhydrous environment.

The partition coefficient and solubility assays were performed in phosphate buffer 0.1 mol L^{-1}, pH 7.4 (I = 0.15 mol L^{-1}). A stock suspension of HDPC 600 μmol L^{-1} was prepared daily in the described phosphate buffer solution and a suspension of HDPC 150 μmol L^{-1} was prepared by suitable dilution of the stock suspension in the same buffer. In the toxicological assays, a Tris-HCl buffer 0.01 mol L^{-1} (pH 7.0), containing KCl 0.12 mol L^{-1} was used as carrier in the flow system. CytCox 0.2 U mL^{-1} was reconstituted in Tris-HCl enzyme dilution buffer 0.01 mol L^{-1} (pH 7.0), with sucrose 0.25 mol L^{-1}, and divided in 12 working solutions, which were stored at −20 °C. Each aliquot of working solution was reconstituted with enzyme dilution buffer at a final concentration of 0.01 U mL^{-1}. Ferrocytochrome *c* (FeC) solution was prepared daily by the combination of 25 μL of DL-dithiothreitol 0.1 mol L^{-1} solution, previously prepared, 13.5 mg of cytochrome *c* and water to a final volume of 5 mL.

In the partition coefficient assays solutions of chol [KTP] and KTP 250 μmol L^{-1}, chol [NAP] and Na [NAP] 800 μmol L^{-1} were prepared

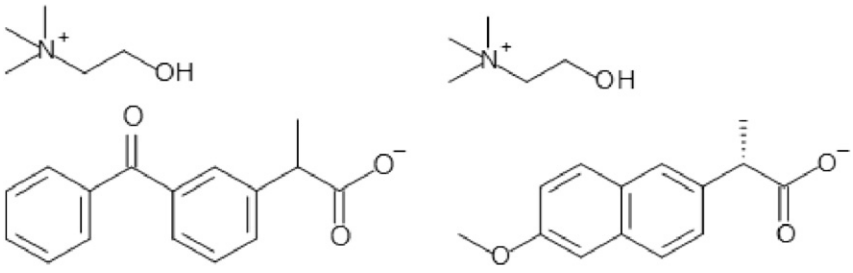

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Fig. 1. Chemical structures of the studied IL-APIs: (1) choline ketoprofenate (chol [KTP]) and (2) choline naproxenate (chol [NAP]).