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Novel organic salts based on fluoroquinolone drugs: Synthesis, bioavailability and toxicological profiles

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ABSTRACT

In order to overcome the problems associated with low water solubility, and consequently low bioavailability of active pharmaceutical ingredients (APIs), novel organic salts containing fluoroquinolones (e.g. ciprofloxacin and norfloxacin) were prepared, using an optimized synthetic procedure based on direct protonation, with different biocompatible counter ions such as mesylate, gluconate and glycolate. All the prepared organic salts were characterized by spectroscopic techniques, mass spectrometry and thermal analysis.

Solubility studies in water and simulated biological fluids at 25 °C and 37 °C were also performed. Additionally, octanol-water and phospholipid-water partition coefficients were measured at 25 °C. The cytotoxicity and anti-inflammatory efficacy using an human cell model of intestinal epithelia (Caco-2 cells) were also evaluated and compared to those of the parent APIs. The adequate selection of the biocompatible anions allows the tuning of important physical, thermal and toxicological properties.

1. Introduction

Fluoroquinolones are broad-spectrum antibiotics that play an important role in treatment of serious bacterial infections for human and veterinary use (King et al., 2000). Ciprofloxacin and norfloxacin are examples of second generation fluoroquinolone drugs and are primarily effective against gram negative bacteria. To increase the activity against gram positive and some anaerobic bacteria, a third-generation of fluoroquinolones was developed. Ciprofloxacin and norfloxacin were the first fluoroquinolones approved for clinical medicine use (Pallo-Zimmerman et al., 2010). Ciprofloxacin, patented in 1983 by Bayer A.G. and approved for use in the USA four years later, was the most successful compound in its generation (Sharma et al., 2010). This drug is very effective in the treatment of a wide range of infections such as urinary tract infection, osteomyelitis (bone infection), ENT infections (ears, nose and throat), and some DST (gonococcal and chronic bacterial prostatis) (Emmerson and Jones, 2003). Norfloxacin was patented in 1978 and was the first fluoroquinolone to receive US Food and Drug Administration approval. It is very effective against both gram positive and gram negative bacteria and it is used in infections of the urinary, biliary and respiratory tracts (Holmes et al., 1985).

Fluoroquinolones contain more than one functional groups that may become charged by protonation or deprotonation at physiological pH. In aqueous solutions a dynamic equilibrium of several protolytic forms (anionic, neutral, zwiterionic and cationic) can be found and the crystal structures of neutral and zwiterionic forms were described for norfloxacin and ciprofloxacin (Nikaido and Thanassi, 1993). The presence of several functional groups in crystal structures, which are able to be protonated, can strongly influence the properties of the fluoroquinolones namely their bioavailability (Nikaido and Thanassi, 1993). On the other hand, ciprofloxacin and norfloxacin are administrated in solid form and show low water solubility (0.08 mg/mL (Gale et al., 1978; Hurley and Wier, 1951) and 0.45 mg/mL (Swanson et al., 1983) at 283 K, respectively). Ciprofloxacin is also available in hydrochloride form

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and despite having higher solubility than the neutral form (17.8 mg/mL at 283 K) this API is still considered a poor soluble drug (Hough et al., 2007; Variankaval et al., 2008).

Spontaneous polymorphic conversion of some active pharmaceutical ingredients (APIs) crystalline forms and the consequent change of their pharmaceutical properties is among the most preeminent challenges that pharmaceutical industry is facing today. The conversion of APIs in different forms might lead to a dramatic change in drug solubility, greatly affecting their final bioavailability (Hough et al., 2007). In many APIs development processes, the phase II trials fails due to the low efficacy of the drugs related to the low bioavailability as a consequence of the low solubility. In order to overcome this problem many strategic routes have been followed such as, for example, co-crystals, amorphous forms and new delivery process systems. Since the mid-1990s ionic liquids (ILs) appeared as a new class of compounds, being currently applied in different research areas due to its unusual characteristics (Ferraz et al., 2012; Ferraz et al., 2011; Zhang et al., 2009). Ionic liquids are generally defined as organic salts, composed entirely by ions, whose melting point is below 100 °C being many of them liquid at room temperature (RTILs) (MacFarlane et al., 2006; Rogers and Voth, 2007; Torimoto et al., 2010; Zhang et al., 2009). The most attractive feature of this class of compounds is that the adequate cation–anion combination enables the tuning of relevant physical, chemical, thermal and biological properties to a desired application. Consequently, the formulation of active pharmaceutical ingredients as ionic liquids (APIs-ILs) has proved to be a good alternative to solve the polymorphism problem as well as to improve APIs bioavailability (Variankaval et al., 2008).

The first attempt to specifically prepare API-ILs was accomplished by Rogers and co-workers (Hough et al., 2007), who explored the potential scope of this poorly exploited drug phase. Since then, API-ILs have been studied by a number of groups. Bica and co-workers studied ILs based on analgesic, anti-pyretic and anti-inflammatory compounds (acetylsalicylic and salicylic acids) (Bica et al., 2010). Hough et al. (Hough-Troutman et al., 2009) and MacFarlane et al. (Dean et al., 2008) published API-ILs containing various active cations/anions, while Cybulski and co-workers (Cybulski et al., 2011) prepared ILs based on antiseptic/disinfectant cations and enzyme/amino acids anions, which were found to be very effective against bacteria and fungi. These API-ILs compounds offer improved properties, such as increased stability, solubility, permeability and drug delivery, as compared to the corresponding solid pharmaceutical forms (Ferraz et al., 2012). Also, the use of an active drug in the liquid form (at room temperature) can avoid some of the issues of polymorphism associated with crystalline solids and, thus, dramatically influence the drug's solubility and dosages (Bica et al., 2010; Hough et al., 2007; Stoimenovski et al., 2010). The use of this modular ionic liquids-based strategy to produce tuneable active pharmaceutical ingredients is very attractive since it allows infinite new possibilities, challenges and opportunities. Besides the elimination of polymorphism and the adjustable solubility, the question of how ionic liquids conjugated with pharmaceutical ingredients could enhance the transport across the membrane is of interest within the APIs context (Dean et al., 2008; Stoimenovski et al., 2010; Stoimenovski and MacFarlane, 2011; Variankaval et al., 2008).

Herein, we proposed a sustainable synthetic strategy to prepare two fluoroquinolone cations (ciprofloxacin and norfloxacin) combined with mesylate, gluconate and glycolate as biocompatible anions. Solubility studies were also performed using water and simulated biological fluids at 25 °C and 37 °C. The distribution of a drug between the aqueous (gastrointestinal fluids, plasma, extracellular medium) and the lipid (cellular membrane) media is the key step in the drug's absorption and distribution processes (Florindo et al., 2013; Hansch and Dunn, 1972; Leo et al., 1971b). For that purpose, octanol-water partition coefficients (K_{ow}), using the shake flask method, were also determined, in order to investigate and compare the potential of membrane permeation of the ciprofloxacin and norfloxacin based ILs against the parent APIs. However, the isotropic nature of octanol makes it a poor mimetic system for biomembranes. More structured membrane models have been emerging, such as lipid vesicles (Helmut et al., 2008), or micelles, amongst others (Serebryany et al., 2012). Hexadecylphosphocholine (HDPC) is a micelle forming zwitterionic phospholipid molecule that possesses a positive choline and a negative phosphoryl group close to a hydrophobic core, closely resembling phosphatidylcholine, the major constituent of the cell membrane. Therefore, HDPC micelles can be used as a biomimetic model, and are especially useful for spectrophotometric determinations since their spectral interference is negligible. In this work, HDPC-water partition coefficients (K_p) were also measured and the results compared to the octanol-water partition coefficients. Finally, cytotoxicity and anti-inflammatory activity of the synthesized fluoroquinolones salts and original APIs were investigated with an in vitro model of human intestinal epithelia (Caco-2 cells).

2. Materials and methods

2.1. Materials

Ciprofloxacin, ciprofloxacin.HCl and norfloxacin were generously provided by Bayer HealthCare AG with high purity (>99.8%) and were used as supplied. Commercially available reagents were purchased from Aldrich, BDH – laboratory reagents and Solchemar and were used as received. The solvents were from Laborspirit and distilled before used. Octanol spectrophotometric grade, hexadecylphosphocholine and Hepes were purchased from Sigma–Aldrich and used as supplied. ^{1}H and ^{13}C NMR spectra in D_2O, $(CD_3)_2SO$ or CD_3OD (from Euriso-Top) were recorded on a Bruker AMX400 spectrometer. Chemical shifts are reported downfield in parts per million (ppm). FTIR spectra were measured on a PerkinElmer 683.

All experiments and solutions were prepared using high purity water (Milli-Q water) with a specific conductance <0.1 μS/cm. For the solubility assays, in order to simulate the physiological conditions, a phosphate standard buffer solution pH 6.8 and an aqueous solution of 0.15 M NaCl – isotonic ionic strength from Sigma–Aldrich were used. DMEM medium, fetal bovine serum (FBS), penicillin–streptomycin solution, transferrin, trypsin–EDTA solution and TNF-α (tumor-necrosis factor-α) were from Invitrogen (Paisley, UK). IL-1β and LPS (Lipopolysaccharide from *Escherichia coli*) were purchased from Sigma (St. Quentin Fallavier, France). IL-8 Elisa Kit from Peprotech (Rocky Hill, NJ, USA), human colon carcinoma Caco-2 (Bbe1 clone) cells from American type culture collection (ATCC-CRL 2102, Braunschweig, Germany) were used in this work.

The organic salts synthesized and used in this work were ciprofloxacin mesylate ([Cip][Mes]), ciprofloxacin gluconate ([Cip][Glu]), ciprofloxacin glycolate ([Cip][Gly]), norfloxacin mesylate ([Nor][Mes]), norfloxacin gluconate ([Nor][Glu]) and norfloxacin glycolate ([Nor][Gly]). Their chemical structure and respective acronym is shown in Scheme 1.

2.2. Synthesis of organic salts based on ciprofloxacin and norfloxacin

2.2.1. Ciprofloxacin mesylate, [Cip][Mes]

Ciprofloxacin (1.00 g; 3.02 mmol) was added to a flask containing 50 mL of methanol. A solution of mesylic acid 1 M (3.02 mL; 3.02 mmol) was slowly added to the ciprofloxacin solution. The reaction mixture was stirred at room temperature for 24 h. Then, the solvent was removed and the final product was dried in vacuum for