Ecotoxicology and Environmental Safety 112 (2015) 22–28

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

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

Cytotoxicity towards CCO cells of imidazolium ionic liquids with functionalized side chains: Preliminary QSTR modeling using regression and classification based approaches

Marina Cvjetko Bubalo [a], Kristina Radošević [a], Višnja Gaurina Srček [a], Rudra Narayan Das [b], Paul Popelier [c], Kunal Roy [b,c,*]

[a] Laboratory for Cell Technology, Application and Biotransformations, Faculty of Food Technology and Biotechnology, University of Zagreb, Pierottijeva 6, HR-10000 Zagreb, Croatia
[b] Drug Theoretics and Cheminformatics Laboratory, Department of Pharmaceutical Technology, Jadavpur University, Kolkata 700032, India
[c] Manchester Institute of Biotechnology, University of Manchester, Manchester M1 7DN, United Kingdom

ARTICLE INFO

Article history:
Received 24 July 2014
Received in revised form
13 October 2014
Accepted 20 October 2014
Available online 4 November 2014

Keywords:
Imidazolium ionic liquids
CCO cell line
In vitro toxicity
Quantitative structure–toxicity relationship
Predictive toxicology

ABSTRACT

Within this work we evaluated the cytotoxicity towards the Channel Catfish Ovary (CCO) cell line of some imidazolium-based ionic liquids containing different functionalized and unsaturated side chains. The toxic effects were measured by the reduction of the WST-1 dye after 72 h exposure resulting in dose- and structure-dependent toxicities. The obtained data on cytotoxic effects of 14 different imidazolium ionic liquids in CCO cells, expressed as EC_{50} values, were used in a preliminary quantitative structure–toxicity relationship (QSTR) study employing regression- and classification-based approaches. The toxicity of ILs towards CCO was chiefly related to the shape and hydrophobicity parameters of cations. A significant influence of the quantum topological molecular similarity descriptor ellipticity (ε) of the imine bond was also observed.

1. Introduction

Over the past decades, ionic liquids (ILs) have been intensively studied as a green replacement for standard volatile and flammable organic solvents, which is reflected in the more than 30,000 scientific papers related to their preparation, characterization, application and impact on the environment. However, it was demonstrated that ILs were not intrinsically green (Cvjetko Bubalo et al., 2014a; Egorova and Ananikov, 2014) and could become potent water and soil contaminants if not handled properly. Consequently, their persistence in the environment, (bio)degradation, migration, bioaccumulation, and (eco)toxicity should be proactively assessed prior to their large-scale application (Cvjetko Bubalo et al., 2014a; Pham et al., 2010). So far, toxicity studies on ILs have been conducted by performing a series of tests on bacteria (Ranke et al., 2004; Matzke et al., 2007; Ventura et al., 2012), yeast (Zhu et al., 2013), algae (Cho et al., 2008; Latała et al., 2010), nematode (Swatloski et al., 2004), mammals (Yu et al., 2009), plants (Matzke et al., 2007; Zhang et al., 2013; Cvjetko Bubalo et al., 2014b; Wang et al., 2009) as well as in different mammalian (Ranke et al., 2004; Stepnowski et al., 2004, Stolte et al., 2007; Wang et al., 2007) and fish cell lines (Radošević et al., 2013), where depending on the used test-system and chemical structure, ILs showed moderate to high toxicity, in general. Nevertheless, ILs present a group of exceptional chemicals with vast possible variations in their structure, and thus it is crucial to understand the chemical and environmental factors controlling the behavior of ILs in the environment in order to design them as environmentally benign (Cvjetko Bubalo et al., 2014a).

The development of predictive chemometric models constitutes an essential part of the toxicity assessment of different chemicals including ILs. It helps in predicting the toxicity of untested compounds and it aids in deriving a rational basis for correlating the toxicity of chemicals with their structural attributes while reducing the number of experiments involving animals. Structure-toxicity relationship studies for ILs have already prompted scientists to develop a variety of predictive models in order to estimate the toxicity of ILs, while avoiding the costs associated with the production and testing of new ILs (Luis et al., 2007, 2010; García-Lorenzo et al., 2008; Torrecilla et al., 2010; Fatemi and Izadiyan, 2011; Alvarez-Guerra and Irabien, 2011; Cho

* Corresponding author at: Jadavpur University, Department of Pharmaceutical Technology, Drug Theoretics and Cheminformatics Laboratory, Raja S C Mullick Road, Kolkata 700032, India. Fax: +91 33 28371078.
E-mail address: kroy@pharma.jdvu.ac.in (K. Roy).

http://dx.doi.org/10.1016/j.ecoenv.2014.10.029
0147-6513/

et al., 2013; Das and Roy, 2013, 2014). The cytotoxic effects of ionic liquids have largely been attributed to lipophilic interactions with cell membranes and cellular proteins, leading to disruption of membrane or protein function (Stolte et al., 2007). The influence of the cationic head group and alkyl side chain on cytotoxicity is also mainly driven by the lipophilicity of the compound. Functionalized side chains (polar ether, hydroxyl and nitrile functional groups within the side chains) lead to lower toxicity. The anions also play a significant role in the cytotoxicity of ILs (Stolte et al., 2006). Cruz-Monteagudo et al. identified several cytotoxicophores of ILs: cationic linear alkyl side chain of length >5; anions with highly fluorinated alkyl side chains (a fluorocarbonated side chain of length ≥ 2, or two or more trifluoromethyl groups); cationic aromatic N-heterocycles with linear alkyl side chain of length ≥ 4; six-membered aromatic rings with a methyl substituent, which can be either the cation head group or its substituent (Cruz-Monteagudo et al., 2013).

Recently, we reported the toxicity towards a CCO fish cell line of imidazolium ILs containing different anions and alkyl chain lengths as the substituent at the cation ring (Radošević et al., 2013). Because some authors (Samori et al., 2007) reported that the presence of functionalized groups affects the toxicity of ILs, we tested the toxicity toward a CCO cell line of *N*-alkoxyl- and amino-substituted imidazolium-based ILs, as well as alkenyl-, alkynyl-, and benzyl-substituted imidazolium-based ILs. These data, together with previously obtained data on toxicity of imidazolium ILs, were used to build preliminary models based on the QSTR approach to elucidate chemical and structural factors controlling the toxicity of ILs. Note that this is the first attempt to build QSTR models of ILs for the endpoint of CCO cell line toxicity.

2. Materials and methods

2.1. Ionic liquids

Seven ILs evaluated for the cytotoxicity towards the CCO cells in this study (1,2-dimethyl,3-pentylimidazolium bis(trifluoromethanesulfonyl)imide [C_5mmim][Tf_2N], 1-methyl-3-pentoxyimidazolium bis(trifluoromethanesulfonyl)imide [C_5omim][Tf_2N], 1-heptyl-2,3-dimethylimidazolium bis(trifluoromethanesulfonyl)imide [C_7mmim][Tf_2N], 1-allyl-3-methylimidazolium bis(trifluoromethanesulfonyl)imide [$C_{3\text{-}2}$mim][Tf_2N], 1-methyl-3-propargylimidazolium bis(trifluoromethanesulfonyl)imide [$C_{3\text{-}3}$mim][Tf_2N], 1-benzyl-3-methylimidazolium bis(trifluoromethanesulfonyl)imide [bzmim][Tf_2N] and 1-(3-*N*,*N*-dimethylaminopropyl)-3-methylimidazolium bis(trifluoromethanesulfonyl)imide [apmim][Tf_2N] were synthesized as reported previously (Cvjetko et al., 2012). The IUPAC names, abbreviations and molecular structures of the newly synthesized ILs are given in Table 1, together with seven other ILs whose cytotoxicity towards CCO cells was reported previously by Radošević et al. (2013). We have used all 14 ILs for our *in silico* modeling work in this study.

2.2. Cell culture

The CCO fish ovary cell line was purchased from the American Type Culture Collection (ATCC: CRL-2772). Cells were cultured in 25 cm^2 T-flasks in Dulbecco's Modified Eagle's Medium (DMEM, Gibco, UK) supplemented with 10% (v/v) fetal bovine serum (FBS, Gibco, UK) and maintained at 30 °C in a humidified atmosphere of 5% CO_2.

2.3. Cytotoxicity assay

The cytotoxicity of ILs was determined using a WST-1 assay (Roche, Germany). Prior to the cytotoxicity assays, the water content of the ILs was determined by Karl Fischer titration (Mettler Toledo C20X compact Coulometric) and was typically found to vary between 1–3% (w/w). The water content value of each IL was considered when preparing stock solutions in order to obtain more accurate EC_{50} values. The CCO cells from the exponential growth phase were seeded at a density of 5×10^4 cells/well into 96-well plates in 100 μL of media. After overnight cell growth, the culture medium was replaced with a fresh one containing tested ILs within the range of 0.1–10 mmol L^{-1}, and cells were incubated for 72 h. After the incubation period, 10 μL of tetrazolium salt WST-1 {4-[3-(4-iodophenyl)-2-(4-nitrophenyl)-2H-5-tetrazolio]-1,3-benzene disulfonate) was added to each well and the cells were incubated for an additional 4 h. The absorbance was spectrophotometrically quantified at 450 nm on the microplate reader (Tecan, Switzerland). The experiments were performed three times for each IL concentration and the results were expressed as a cell viability, *i.e.* percentage of treated cells *versus* control cells (mean ± standard deviation). The EC_{50} values were obtained from the dose–response curves using equations of best-fitted trend-lines.

2.4. Morphological assessment by fluorescent microscopy

Briefly, CCO cells were seeded in six-well plates (Corning, USA) at a concentration of 1×10^5 cells/mL, allowed to attach for 24 h and exposed for 72 h to 1 mmol L^{-1} of selected ILs. Following exposure, the cells were washed with sterile PBS and stained by a mixture of acridine orange (AO) (100 μg/mL in PBS) and ethidium bromide (EB) (100 μg/mL in PBS) for 10 min. The cells were examined using a fluorescent microscope Olympus BX51 (Olympus, Japan) with integrated camera.

2.5. Quantitative structure–toxicity relationship (QSTR) modeling

We have attempted to develop classification- and regression-based QSTR models using the set of 14 imidazolium ILs (out of which seven compounds were previously synthesized and tested; Cvjetko et al., 2012) for their toxicity toward a CCO cell line. The regression-based model should be able to accurately predict the quantitative toxicity values while the classification-based model could initially be used for filtering the ILs out into toxic and non-toxic classes. Considering the limited size of the data set and limited chemical diversity, this attempt may be considered as preliminary one. The toxicity values were converted into the molar negative logarithmic unit (pEC_{50}, M) and a threshold value of $pEC_{50} = 3.0$ M, based on the average value of the quantitative toxicity entities, was chosen for the two-group classification analysis such that those bearing a pEC_{50} value less than 3 were treated as non-toxic while those with more than 3 as toxic. Various two-dimensional descriptors (Table S1 in Supplementary material) computed using PaDEL-Descriptor software (version 2.11) (Yap, 2011) and chemometric tools (*i.e.* linear discriminant analysis (LDA) (Fisher, 1936) and stepwise multiple linear regression (MLR) (Darlington, 1990) were employed to perform classification- and regression-based analyses, respectively. Although we tried to incorporate predictor variables defining chemical features of both the cations and anions, the best model was obtained employing cationic descriptors only. The LDA analysis was performed using a stepping algorithm, F to enter = 4.0 and F to remove = 3.9, with a tolerance value of 0.01 as a strategy for the selection of essential chemical features. An effort was also made in deriving a regressional QSTR model on the available quantitative toxicity values employing stepwise regression analysis (objective function F to enter = 4.0 and F to remove = 3.9) as a descriptor selection tool. During the regression analysis, we have used a pool of quantum topological molecular similarity (QTMS) descriptors (Popelier, 1999) for cations along with the 2D-descriptors as listed