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Research Article

In Vitro Anticancer and Antivirus Activities of Cyano- and Bis(Trifluoromethylsulfonyl)imide-Based Ionic Liquids

Małgorzata Musiał,* Agnieszka B. Olejniczak,* Marta Denel-Bobrowska, Edward Zorębski, and Marzena Dzida*

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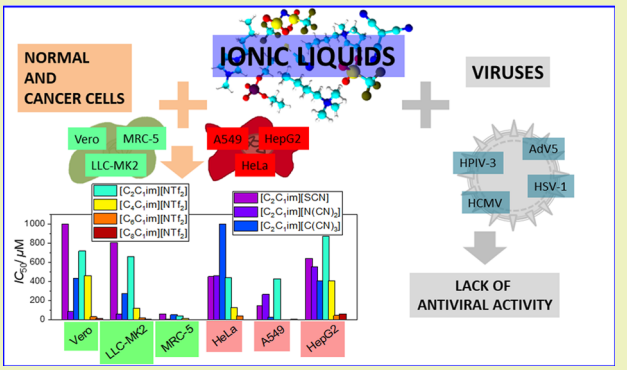
ABSTRACT: In the current work, we present a broad analysis of cytotoxicity toward normal (kidney and lung) and cancer (lung, liver, and cervix) cells of ionic liquids (ILs) with imidazolium cations and cyano-based or bis(trifluoromethylsulfonyl)imide anions. Additionally, we verify if ILs might be candidates for new potential virucidal agents. We observed the highest biological activity for 1-methyl-3-octylimidazolium bis(trifluoromethylsulfonyl)imide, whereas the least toxic was 1-ethyl-3-methylimidazolium thiocyanate. We found that all investigated ILs revealed a lack of antiviral activity against viruses: human parainfluenza virus type 3 (HPIV-3), human adenovirus 5 (AdV5), human herpesvirus 1 (HSV-1), and human herpesvirus 5 (HCMV) in nontoxic concentrations.



KEYWORDS: *ionic liquids, cytotoxicity, nontumor cells, cancer cells, anticancer activity, antiviral activity*

INTRODUCTION

Ionic liquids (ILs) quickly became popular because of their unusual properties.[1] Their features may be designed using different combinations of cations and anions.[2,3] They are chemically and thermally stable, possess low volatility and high ionic/electrical conductivity, and many more.[1] For these reasons, ILs quickly found applications in many areas, for example, in analytics, synthesis, catalysis, separation processes, electrochemistry.[1] Although ILs, being nonflammable and nonvolatile compounds, were initially considered as "green solvents", it is now clear that they can pose a risk to humans and the environment (for example, through solubility in water), and their toxicity is often high as well as their biodegradability is questionable. Therefore, the successful application progress of ILs has been somewhat inhibited by their potential toxicity to humans and the environment. Nevertheless, this opened new applications based on their biological activity, for example, in the pharmaceutical industry.[4−6] Recently, Cho et al.[7] reviewed the toxic effects of ILs, and one can note from this study that systematic investigation was performed, for example, toward bacteria, yeast, and fungi; however, less data we may find for mammalian cells, in particular, human cells. Up to date, among others, studies were conducted for human colorectal adenocarcinoma (CaCo-2) cells, cervical cancer (HeLa) cells, human immortal keratinocyte cells, human pancreatic cancerous cells, human hepatocellular carcinoma cells, human breast cancerous cells, human renal cancer tumor cells, and human skin cells.[7] According to a minireview of Dias et al.,[5] several types of ILs have been tested for their anticancer activity so far, including combinations of pyridine, pyrrolidine, and piperidine-based cations with organic and inorganic anions (*e.g.*, bromide, Br^-; chloride, Cl^-; and tetrafluoroborate, $[BF_4]^-$).[4] Additionally, recently, imidazolium-based ILs modified with fluorinated phenylacetylamides have been investigated for their anticancer activity by Rezki and co-workers.[8] In turn, Al-Blewi et al.[9] synthesized ILs with pyridinium hydrazones as cations and tested them for biological activity. Moreover, Perez et al.,[10] apart from monocationic pyridinium-based ILs, also investigated their dicationic counterparts. The authors observed that dicationic analogs possess lower cytotoxicity. Notably, based on the concept that ILs are tunable compounds, modeling of ILs with appropriate biological activity (low or high, depending on the potential application) was also discussed. Overall, considering the generalized structure−activity relationship reported by Cho et al.,[7] the side-chain length is currently the most significant indicator of biological activity. In addition to the cationic (or anionic) side-chain length, the head group has been investigated, but this does not distinctly influence IL

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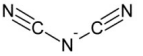
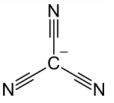
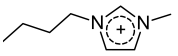
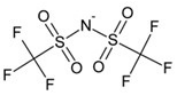
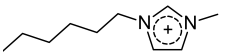
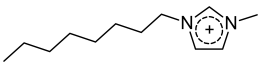
Table 1. Full Names, Acronyms, and Cation/Anion Structures of the Investigated ILs

Full name, acronym	Cation	Anion
1-ethyl-3-methylimidazolium thiocyanate, $[C_2C_1im][SCN]$		
1-ethyl-3-methylimidazolium dicyanamide, $[C_2C_1im][N(CN)_2]$		
1-ethyl-3-methylimidazolium tricyanomethanide, $[C_2C_1im][C(CN)_3]$		
1-ethyl-3-methylimidazolium bis(trifluoromethylsulfonyl)imide, $[C_2C_1im][NTf_2]$		
1-butyl-3-methylimidazolium bis(trifluoromethylsulfonyl)imide, $[C_4C_1im][NTf_2]$		
1-hexyl-3-methylimidazolium bis(trifluoromethylsulfonyl)imide, $[C_6C_1im][NTf_2]$		
1-methyl-3-octylimidazolium bis(trifluoromethylsulfonyl)imide, $[C_8C_1im][NTf_2]$		

toxicity. In our previous study,[11] we found that the type of anion and its lipophilic nature are also important.

On the other hand, information on the antiviral activity of ILs is very scarce. In addition to systematic research by Byrne et al.,[12] only three other studies by the Rossmanith group are focused on this topic.[13−15] Byrne et al.[12] investigated the stabilization of the tobacco mosaic virus, an essential genetic vector in plant biotechnology, employing protic ILs (PILs). The results indicated that ethylammonium and diethylammonium mesylate stabilized the tested virus. In contrast, keeping the virus particles in tripropylammonium and triethylammonium mesylate changed their secondary structure. Thus, the virus destabilized in PILs containing three alkyl chains (with at least two -CH_2- groups) substituted to the cation. In turn, in the first work of the Rossmanith group, the authors introduced a method that uses ILs to destabilize viral capsids in order to extract viral RNA and DNA.[13] In a second study, scientists analyzed the effect of various elements of IL structures on two basic unenveloped viruses:*Escherichia coli* phage MS2 and Listeria monocytogenes phage P100. At the end of that work, the authors stated that with respect to IL toxicity, outcomes from prokaryotes or eukaryotes are not assignable to viruses. In the last research, the Rossmanith group tested whether ILs could be employed for the liquid−liquid extraction of whole virus particles from aqueous systems, and as a result, the authors identified some ILs with good viral extraction properties.

Herein, we first investigate cytotoxicity toward two types of human cells, namely, "normal" (health) cell lines and cancer cell lines. Overall, six different cell lines: *Cercopithecus aethiops* normal kidney cells (Vero), *Macaca mulatta* normal kidney cells (LLC-MK2), human lung normal fibroblasts (MRC-5), human lung carcinoma cells (A549), human hepatocellular carcinoma (HepG2), and human cervix adenocarcinoma cells (HeLa) were studied. Notably, up to date, we could find systematic data only for HeLa and HepG2 cell lines. The rest of the data are mainly new. In the next step, we checked how the toxicity results translate into antiviral activity using human herpesvirus 1 (HSV-1, VR-539); human parainfluenza virus type 3 (HPIV-3, VR-93); human adenovirus 5 (AdV5, VR-5), and human herpesvirus 5 (HCMV, VR-1590).

We have selected combinations of imidazolium-based cations with cyano-based (thiocyanate $[SCN]^-$, dicyanoamide $[N(CN)_2]^-$, and tricyanomethanide $[C(CN)_3]^-$), and bis-(trifluoromethylsulfonyl)imide $[NTf_2]^-$ anions. Without a doubt, $[NTf_2]^-$-based ILs are the most popular and most frequently studied group among ILs because of their thermal and chemical stability. On the other hand, cyano-based ILs are an interesting class mostly because of the relatively weak cation-anion interactions, leading to greater ion mobility.[16] Accordingly, ILs with [CN]-based anions exhibit remarkably low viscosity and both high electrical and thermal conductivity, and these features are regarded as crucial properties for some electrochemical applications.[17,18] Both cyano-based and $[NTf_2]^-$-based ILs are considered as effective heat-transfer fluids and good components of ionanofluids.[11,19,20] In addition to the advantages of cyano-containing ILs mentioned above, we chose this group of ILs for another reason. More specifically, Mester et al.[21] found that anion chaotropy is the major factor influencing the antimicrobial activity of ILs with cation side-chain lengths lower than 6. ILs bearing chaotropic anions may thus be promising agents for virus inactivation. Virus-protection capsids contain protein units; hence, inactivation through denaturation could be possible. Con-

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sequently, to test this hypothesis, we used ILs with a nonviral cation (taking into account Mester et al.'s conclusions), namely, $[C_2C_1im]^+$ and the strongest chaotropic anion from Hofmeister series, that is, the thiocyanate anion. Subsequently, we also reviewed ILs containing more -CN groups.

We observed the highest biological activity against human health and cancer cells for the IL with the longest alkyl chain attached to the cation, namely, for 1-methyl-3-octylimidazolium bis(trifluoromethylsulfonyl)imide, $[C_8C_1im][NTf_2]$. Among ILs with the same amount of $-CH_2-$ groups substituted to cations, $[C_2C_1im][N(CN)_2]$ and $[C_2C_1im][SCN]$ have the highest and the lowest toxicity, respectively, toward normal human cells. Against cancer cell lines, no correlation with the type of anion used was found. Moreover, we found that all investigated ILs are inactive against viruses: HPIV-3, AdV5, HSV-1, and HCMV in nontoxic concentrations.

MATERIALS AND METHODS

Ionic Liquids. All investigated ILs were provided by Iolitec (Germany). The full names, acronyms, and structures of the tested samples are presented in Table 1. Cyano-based ILs, before measurements, were dried and degassed under argon at 2 mbar (a Heidolph rotary evaporator combined with the SC 920 G vacuum pump system) at 105 °C for 6 h, and then, the water content was determined via the Karl Fischer method by TitroLine 7500 (SI Analytics, Germany). The water contents were 200, 320, and 156 ppm in $[C_2C_1im][SCN]$, $[C_2C_1im][N(CN)_2]$ and $[C_2C_1im][C(CN)_3]$, respectively. The $[NTf_2]^-$-based ILs were used without purification. The water content was <40 ppm.

In Vitro Cytotoxicity and Antiviral Activity Studies. *Cytotoxicity Assay.* The cytotoxic properties of ILs were evaluated using cell lines: Vero (CCL-81, *Cercopithecus aethiops* normal kidney cells); LLC-MK2 (CCL-7, *Macaca mulatta* normal kidney cells), MRC-5 (CCL-171, human lung normal fibroblasts), A549 (CCL-185, human lung carcinoma cells), HepG2 (HB-8065, human hepatocellular carcinoma), and HeLa (CCL-2, human cervix adenocarcinoma cells). Cell lines were obtained from the American Type Culture Collection (ATCC; Manassas, Virginia, USA).

Stock solutions of each tested IL were prepared in the growth medium supplemented with 2% fetal bovine serum (FBS) at 100 mM.

The investigated cells were propagated in the minimum essential medium (MEM; Sigma-Aldrich Darmstadt, Germany) supplemented with 10% heat-inactivated FBS (Sigma-Aldrich Darmstadt, Germany) and 100 units/mL penicillin G with 100 mg/mL streptomycin (Sigma-Aldrich Darmstadt, Germany). Upon reaching 80−90% confluency, cells were harvested with 0.25% trypsin in 1 mM ethylenediaminetetraacetic acid (EDTA) (Life Technologies, Warsaw, Poland) and seeded into 96-well microplates at 2×10^5 cells/mL. After overnight incubation at 37 °C in a humidified atmosphere containing 5% CO_2, the culture medium was removed and replaced with 100 μL of freshly prepared solution of the tested ILs diluted with the growth medium supplemented with 2% FBS to ensure compound dissolution for obtaining concentrations ranging from 0.1 to 1000 μM. Cytotoxicity was evaluated by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. All experiments were carried out in triplicate. IL-treated and -untreated cells (control group) were incubated at 37 °C for 48 h in a humidified atmosphere containing 5% CO_2.

After incubation with drugs, the cells were treated with dye solution MTT (Sigma-Aldrich Darmstadt, Germany) (25 μL, 5 mg/mL) for 2 h and lysed with solvent solution (100 μL) containing dimethylformamide (DMF; Sigma-Aldrich Darmstadt, Germany) (45 mL), 13.5 g of sodium dodecyl sulfate (SDS; Sigma-Aldrich Darmstadt, Germany), and distilled water (55 mL). After overnight incubation at 37 °C, optical density at 550 nm with a reference wavelength of 670 nm was measured on a microplate spectrophotometer Varioskan Lux (Thermo Fisher Scientific, Waltham, Massachusetts, USA). The cytotoxic concentration was defined as the concentration required to reduce the cell number by 50% compared to the untreated controls and was calculated by the linear regression analysis of the dose-response curves obtained from the data.

Antiviral Assay. The antiviral properties of ILs were evaluated using viruses: HSV-1,VR-539; HPIV-3, VR-93; AdV5, VR-5; and HCMV, VR-1590. Viruses were obtained from ATCC (Manassas, Virginia, USA).

Stock solutions of each tested IL were prepared in the growth medium supplemented with 2% FBS at 100 mM.

The investigated cells were propagated in the MEM (Sigma-Aldrich Darmstadt, Germany) supplemented with 10% heat-inactivated FBS (Sigma-Aldrich Darmstadt, Germany) and 100 units/mL penicillin G with 100 mg/mL streptomycin (Sigma-Aldrich Darmstadt, Germany). Upon reaching 80−90% confluency, the cells were harvested with 0.25% trypsin in 1 mM EDTA (Life Technologies, Warsaw, Poland) and seeded into 96-well microplates at 2×10^5 cells/mL. After overnight incubation of cells at 37 °C in a humidified atmosphere containing 5% CO_2, the culture medium was removed, and the cells were inoculated with the respective virus solution in MEM supplemented with 2% FBS [HSV-1 MOI 0.005, 1000 virions/mL; HPIV-3 MOI 0.01, 2000 virions/mL; AdV5 MOI 0.005, 1000 virions/mL, HCMV 20 PFU (plaque-forming units) per well]. After 1 h (HSV-1, HPIV-3, and AdV5) or 2 h adsorption period (HCMV), residual virus was removed, and the infected cells were further incubated with 100 μL of MEM supplemented with 2% FBS containing the compound concentrations ranging from 0.1 to 1000 μM. All experiments were carried out in triplicate. The cell monolayers were treated with ILs at 37 °C in a humidified atmosphere containing 5% CO_2, until the typical cytopathic effect (CPE) was visible. Viral infection was evaluated by the MTT assay (as described previously) or plaque reduction assay (HCMV). The number of HCMV plaques was counted under a microscope, and the antiviral activity was expressed as the concentration required to reduce the number of viral plaques to 50% of control (virus infected but untreated).

RESULTS AND DISCUSSION

Our initial studies over the cytotoxicity of ILs on normal human dermal fibroblasts in the context of their applications show that different behaviors depend on the IL structure.[11] We reported that two main factors influence cytotoxicity toward skin cells (normal human dermal fibroblasts, NHDF): *i*) the type of anion and *ii*) the length of the alkyl chain substituent to ions. We found that ILs with $[NTf_2]^-$ anions have the highest cytotoxic activity toward NHDF, and thus, there is also a great possibility that they have activity toward cancer cells. This is highly probable as the $[NTf_2]^-$ anion is generally recognized as toxic and rather dangerous. Therefore, in the current work, we extended the toxicity analysis to cancer cells, among others. Additionally, we verify in this study if ILs can be pretenders as new potential virucidal agents and if so, whether structure−activity relationship principles can be applied to viruses or whether new/additional rules are needed.

ILs examined herein differed in several structural characteristics, such as anions and the length of the cationic alkyl side chains (see Table 1). It should be stressed that the obtained data for the investigated ILs are mainly new. Only Wang et al.[22] reported EC_{50} values (effective concentration of the test material that causes a reduction of processes by 50%, including growth or reproductive activity) for the same imidazolium homologous series with $[NTf_2]^-$ anions toward HeLa cells.

Cytotoxicity toward Normal and Cancer Cell Lines. We chose two types of human cell lines, namely, healthy (Vero, LLC-MK2, and MRC-5) and cancer (A549, HepG2, and HeLa) cell lines. Currently, cancer is a major health problem

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and a leading cause of death worldwide, responsible for nearly 10 million deaths in 2020.[23] Moreover, the COVID-19 pandemic has intensified the problem of cancer deaths because of the delay in diagnosis and therapy. There is a need to find new drugs that will be effective in the treatment of cancer at various stages of advancement.

Selected for our studies, cancer cell lines represent cancer types with a high global incidence and, especially, high mortality rate. The most frequent cause of cancer deaths in 2020 was lung cancer (1.80 million deaths). Also, a very large number of deaths from liver cancer were recorded in 2020 (830.000 deaths). Although cervical cancer is one of the most preventable and treatable malignant diseases, in 2018, it was the fourth most frequent cancer in women, representing 7.5% of all female cancer deaths.[24]

Among the analyzed cell lines, HeLa[10,22,25−28] and HepG2[29−32] were the most investigated, in relation to ILs. HeLa cells represent prototypical cells of the human epithelium. HeLa is the oldest and the most widely immortal human cell line used in research. The cell line was found to be extremely durable and fertile, and over the past few decades, it has contributed to many milestones in medicine such as the advancement of polio vaccine or research on cancer, acquired immunodeficiency syndrome (AIDS), or leukemia. On the other hand, HepG2 is an immortalized cell line consisting of human liver cancer cells and is widely used in drug metabolism and hepatotoxicity studies. For these two cell lines, a mechanism of cell death was proposed. More precisely, Wan et al.[30] observed that 1-hexadecyl-3-methylimidazolium chloride $[C_{16}C_1im]Cl$ induced genetic toxicity, oxidative stress, and apoptosis in HepG2 cells. In turn, Ma and Li[32] presented toxicity mechanisms (i.e., biochemical disturbance and oxidative stress) of 1-methyl-3-octadecylimidazolium bromide $[C_{18}C_1im]Br$ in HepG2 cells. The same type of cell death (apoptosis) was detected for HeLa cells by Perez et al.[10]

The representative dependence of cell viability (absorbance) after exposure to various concentrations of ILs measured by the MTT assay for MRC-5 cells is presented in Figure 1. All the determined IC_{50} values are given in Table 2 and depicted in Figure 2. Herein, both tested imidazolium IL series with cyano-based and $[NTf_2]^-$ anions exhibited cytotoxic activity against "normal" and cancer cells. The susceptibility and

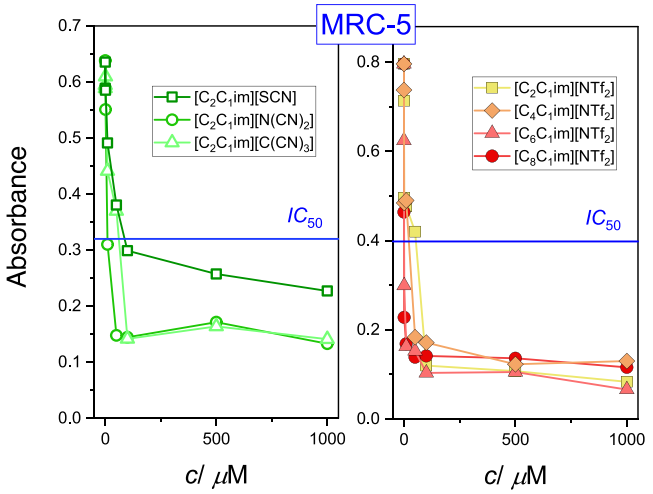

Figure 1. Concentration-dependent MRC-5 cell viability (absorbance) after exposition on ILs.

behavior of cell lines to the tested ILs vary considerably, depending on the type of anions and the amount of $-CH_2-$ groups attached to the cation. As expected, cytotoxic activity increases with alkyl chain elongation, which is already widely described in the literature.[7] As a consequence, the highest biological activity was received for $[C_8C_1im][NTf_2]$. The aforementioned compound was the most cytotoxic to the tested tumor cell lines at a concentration as low as 0.77 μM, particularly being effective for HeLa cells. The same compound was cytotoxic to MRC-5 and LLC-MK2 cell lines. However, compound $[C_6C_1im][NTf_2]$ was less active and showed cytotoxic effects on A549 and MRC-5 cell lines (IC_{50} = 3.17 and 0.42 μM). The same trend with the length of the alkyl chain n was revealed by Wang et al.[22] for EC_{50} of the analogous $[NTf_2]$-based IL series and also for another imidazolium, ammonium, pyridinium, and phosphonium-based IL series with $[BF_4]^-$ and Br^- anions toward HeLa cells (see Table S1 in the Supporting Information). In a study by Perez et al.,[10] against the same cell line, an analogous trend was marked (see Table S1). Additionally, one can note based on Wang et al.[22] results that the structure of the cation (ammonium, pyridinium, or imidazolium) with a similar number of -CH_2- groups attached to nitrogen does not influence toxicity toward HeLa cells significantly, which is consistent with our previous observation for NHDF cell lines even if aliphatic quaternary ammonium ILs have been reported to be less hazardous in many other cytotoxicity studies.[33] However, importantly, not only does the elongation of the alkyl chain length exert influence but also cation/anion functionalization may play a role, as clearly seen in the results presented by Jovanovic-Santa et al.[34] or Ferraz et al.[35] (see Table S1). Ferraz et al.[35] showed that ILs with hydroxyl-functionalized cations ($[C_2OHC_1im]^+$) are much more cytotoxic against HepG2 cells than ILs with nonfunctionalized cations ($[C_2C_1im]^+$). These results stay in contrast with the conclusion presented in a very recently published review that polar substituents reduce toxicity.[33] On the other hand, cytotoxicity data toward HeLa and A549 cell lines (see again Table S1) reported by Jovanovic-Santa et al.[34] are consistent with this rule. The results are different for each cell line. Therefore, new investigations are still needed. We can also notice from Wang et al.[22] and Chen et al.[36] results toward HeLa and A549 cell lines, respectively, that anions have a large impact on the obtained EC_{50} values, especially its hydrophobic character, for example, ILs with $[BF_4]^-$ anion are less toxic than ILs with $[NTf_2]^-$ anion (see Table S1). Herein, comparing cyano- and $[NTf_2]^-$-based ILs with the same amount of $-CH_2-$ groups attached to cations, the cytotoxic activity of $[C_2C_1im][N(CN)_2]$ is the highest, and $[C_2C_1im]$-$[SCN]$ showed noncytotoxic activity against Vero and LLC-MK2 cell lines. According to our results, another interesting finding is connected with $[C_2C_1im][N(CN)_2]$, which is the most toxic toward nontumor cell lines among $[C_2C_1im]$-based ILs; however, cytotoxic activity against the A549 cell line is lower than that obtained for $[C_2C_1im][SCN]$ and $[C_2C_1im]$-$[C(CN)_3]$ but higher than that obtained for $[C_2C_1im][NTf_2]$. Certainly, $[C_2C_1im][N(CN)_2]$, being much more active to nontumor than tumor cells (see Figure 2), cannot be considered as a new potential anticancer agent. One can notice from Figure 2 that $[C_2C_1im][SCN]$ is slightly more active against the A549 cell line.

From Figure 2, (see also Table 2) it is evident that the IL cytotoxic effect is also influenced by the chosen cell line. The diversity of cell viability of different cell lines after exposure to

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Table 2. Cytotoxicity Assay Results in Concentrations Ranging from 0.1 to 1000 μM on Vero, LLC-MK2, MRC-5, HeLa, A549, and HepG2 Cells

	IC_{50}[a] value/μM					
		normal cells			cancer cells	
IL	Vero	LLC-MK2	MRC-5	HeLa	A549	HepG2
$[C_2C_1im][SCN]$	>1000	806.7 ± 11.6	60.17 ± 2.84	450.0 ± 98.5	143.3 ± 27.5	641.7 ± 62.9
$[C_2C_1im][N(CN)_2]$	86.00 ± 1.73	62.67 ± 2.08	7.33 ± 0.31	460.0 ± 72.1	265.0 ± 13.2	550.0 ± 78.1
$[C_2C_1im][C(CN)_3]$	430.0 ± 26.5	270.0 ± 10.0	53.17 ± 1.61	>1000	28.33 ± 2.89	408 ± 142
$[C_2C_1im][NTf_2]$	716.7 ± 23.6	656.7 ± 15.3	39.00 ± 1.00	436.7 ± 20.2	428.3 ± 11.6	868.7 ± 18.6
$[C_4C_1im][NTf_2]$	460.0 ± 15.0	122.3 ± 19.7	14.83 ± 2.36	125.0 ± 21.8	3.10 ± 0.66	408.3 ± 52.0
$[C_6C_1im][NTf_2]$	31.67 ± 2.08	19.33 ± 1.15	0.42 ± 0.03	39.67 ± 6.25	3.17 ± 0.76	45.67 ± 8.16
$[C_8C_1im][NTf_2]$	15.83 ± 1.44	3.50 ± 0.50	0.13 ± 0.01	0.77 ± 0.23	4.83 ± 1.61	63.00 ± 2.65
Cisplatin[b]				24.11 ± 1.20	31.95 ± 1.59	23.05 ± 1.16

[a]The values are the mean ± SD of three independent experiments. [b]Reported in ref 39.

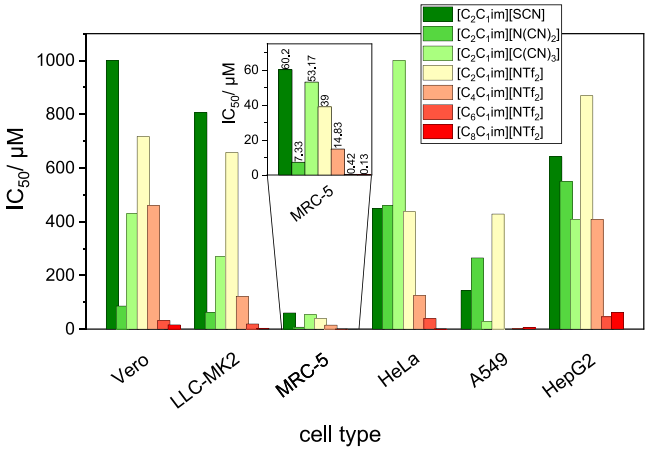

Figure 2. Cytotoxicity of the tested ILs toward Vero, LLC-MK2, MRC-5, HeLa, A549, and HepG2 cells.

the same IL may be due to the specific characteristics and/or properties of each cell type. Considering all investigated cells, MRC-5 showed the highest sensitivity to the analyzed compounds, undeniably higher than the rest cell types. MRC-5 is a well-characterized human diploid fibroblast cell line that retains the predominantly diploid normal karyotype of the original tissue cells.[37]

To see a complete picture of the obtained toxicity, we compared our results toward HeLa cells with the literature results on the toxicity of classical industrial solvents and KCl reported by Malich et al.[38] The IC_{50} values of dichloromethane, xylene, ethanol, phenol, and KCl after 3 h of incubation using the MTT assay are 71,430, 52,430, 1501.43 × 10^3, 42,680, and 688.57 × 10^3 μM, respectively. Thus, even for $[C_2C_1im][SCN]$ (generally the least toxic among studied ILs), almost 100 times lower concertation is enough to affect cells in comparison to the most harmful solvent compared here, namely, phenol. Notably, Stepnowski et al.[25] showed that in the case of HeLa cells, the incubation time does not significantly affect the inherent sensitivity of the test systems. Thus, these results can be compared even after different incubation times.

Antiviral Activity. Viruses are able to infect most organisms, including bacteria, algae, fungi, plants, insects, and vertebrates. Viruses infect a host cell in order to replicate. Viral particles contain a genome that may be DNA or RNA wrapped in a protein coat called a capsid or nucleocapsid.[40] For our studies, one RNA virus and three DNA viruses were selected, namely, DNA viruses: human herpesvirus 1, human herpesvirus 5 (both Herpesviridae family), human adenovirus 5 (Adenoviridae family), and RNA virus: human parainfluenza virus type 3 (Paramyxoviridae family).[41]

There are eight human herpesviruses (HSV-1 to HSV-8) that establish lifelong infection in the host and avoid the immune system by a latent infection. Investigated human herpesvirus 1 (known as herpes simplex virus) is associated primarily with orolabial infections,[42] whereas human herpesvirus 5 (also known as human cytomegalovirus, HCMV) is commonly associated with immunosuppression, and for this reason, hosts such as neonates, transplant recipients, and patients with AIDS are particularly susceptible to severe viral infection.[43] There are >70 types of naturally occurring human adenoviruses.[44] Investigated adenovirus 5 is associated with commonly occurring mild respiratory illnesses of children.[45] Currently, adenovirus-based vectors are commonly used in genetic therapeutic applications.[44] Human parainfluenza virus-3 is second in frequency to RSV (respiratory syncytial virus) as a cause of bronchiolitis, especially in infants under 6 months of age, and occurs also in school-aged children and occasionally in adolescents and adults.[46]

The antiviral activity of ILs was investigated only in four studies, which was described with some details in the Introduction. Herein, we chose other viruses for studies; thus, the obtained results are entirely new. As demonstrated by Mester et al.,[21] at the enzymatic level and with short-term exposure to bacteria, the overall increase in IL toxicity is related to the chaotropicity of the anions. The Rossmanith group suggested that the chaotropicity of the IL anion could be especially promising for the inactivation of enveloped viruses. Because of their lipid envelope, comparable to the bacterial membrane, inactivation through denaturation could be possible. Thus, for investigation, we chose, among others, $[C_2C_1im][SCN]$ with the strongest chaotropic anion in consideration of the Hofmeister series. Note that the cytotoxicity toward nontumor and cancer cell lines is in good accordance with other published studies showing a correlation between the number of side chains and IL toxicity.[7] This is important because the results obtained for normal cells and HeLa were used as a basis to test whether ILs could be applied as antiviral agents.

One can notice from Table 3 that all the compounds revealed a lack of antiviral activity against viruses: HPIV-3, AdV5, HSV-1, and HCMV in nontoxic concentrations (see Table 2 for comparison). Note that similar to the outcome of Fister et al.,[13] our present study indicates that a chaotropic

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Table 3. Results of the Antiviral Activity of the Investigated Compounds in Concentrations Ranging from 0.1 to 1000 μM against HSV-1, HPIV-3, AdV5, and HCMV

	IC_{50} value/μM			
IL	HSV-1 (Vero)	HPIV-3 (LLC-MK2)	ADV5 (HeLa)	HCMV (MRC-5)
$[C_2C_1im][SCN]$	>1000	>806.7	>450.0	>60.17
$[C_2C_1im][N(CN)_2]$	>86.00	>62.67	>460.0	>7.33
$[C_2C_1im][C(CN)_3]$	>430.0	>270.0	>1000	>53.17
$[C_2C_1im][NTf_2]$	>1000	>763.3	>68.33	>8.17
$[C_4C_1im][NTf_2]$	>716.7	>656.7	>436.7	>39.00
$[C_6C_1im][NTf_2]$	>460.0	>122.3	>125.0	>14.83
$[C_8C_1im][NTf_2]$	>31.67	>19.33	>39.67	>0.42

effect of the tested ILs on nonenveloped and enveloped viruses could also be excluded under the selected test conditions because even for ILs with $[SCN]^-$ anion, we did not observe antiviral activity in nontoxic concentrations.

CONCLUSIONS

In summary, it was found that all tested compounds were more cytotoxic against the MRC-5 cell line than against Vero and LLC-MK2 cell lines. The most toxic toward nontumor cell lines were $[C_8C_1im][NTf_2]$ and $[C_6C_1im][NTf_2]$. In turn, $[C_2C_1im][N(CN)_2]$ showed moderate activity toward Vero and LLC-MK2 cell lines, but $[C_2C_1im][SCN]$, $[C_2C_1im][C(CN)_3]$, $[C_2C_1im][NTf_2]$, and $[C_4C_1im][NTf_2]$ were inactive against both cell lines. It was also found that the tested ILs were more active against the A549 cell line. Their activity was lower against the HeLa cell line and the lowest toward the HepG2 cell line. Generally, in the series of $[NTf_2]^-$-based ILs, the obtained results indicate that the toxicity of ILs increased with the alkyl chain length, and this effect can be related to higher lipophilic properties for ILs with longer cationic side chains. Regarding cyano-based ILs toward tumor cell lines, they were rather inactive. However, assessing only normal cells, ILs with $[N(CN)_2]^-$ anion showed increased toxic activity.

Importantly, none of the tested ILs could be readily classified as antiviral agents against tested viruses at nontoxic concentrations. Anion chaotropicity does not exhibit virucidal activity.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acssuschemeng.1c06580.

Table containing the cytotoxicity assay results on HeLa, HepG2, A549 cells reported in the literature for different cation and anion structures (PDF)

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Notes

The authors declare no competing financial interest.

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