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Study on the potential anti-cancer activity of phosphonium and ammonium-based ionic liquids

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ABSTRACT

The anti-cancer activity and cytotoxicity of phosphonium and ammonium-based ionic liquids (ILs) have been determined for the first time via NCI's in vitro 60 human tumor cell lines. The preliminary SAR showed that the chain length of alkyl substitution on the cations plays crucial role towards anti-tumor activity and cytotoxicity of these ionic liquids. In general, phosphonium-based ILs were found to be more active and less cytotoxic as compared to ammonium ILs. Cell line data and hollow fiber study has demonstrated the potential of ILs to be developed as therapeutic agent.

Ionic liquids/salts have emerged as new class of compounds with unique properties. Most attractive feature is the potential to tailor their physiochemical properties by changing cation/anion combination, which is not possible for molecular compounds. This feature has been explored dexterously in finding their applications in diverse fields such as synthesis, materials, specialty chemicals etc. making ionic liquid as new and broad area of scientific research.[1] There has been a phenomenal growth in past two decades, resulting in few thousand publications on ionic liquids. Still there are only limited studies with mixed results regarding the toxicity and safety of these compounds.[2] This has caused skeptism for their use for biomedical applications, specifically as therapeutic agents and drugs. On the other hand, 'The poison is in the dose' as quoted by Paracelsus, famous physician and occultist of 14th century, suggests that toxicity could be a desirable property, which has been seen with many toxins originally used as poisons but found to be medically important.[3] This is because, toxins by their nature are biodynamic substances, as they affect the functioning of the victim's body, suggesting that they could potentially become important source of medicines.[4] Understanding and managing the toxicity of small molecule toxins is a major challenge in the discovery and development of new drugs.[5] Therefore, development of new anti-cancer agents that reduce the toxicity associated with existing chemotherapies and those targeted at circumventing tumor resistance mechanism is a major focus of drug discovery efforts.[5]

Once a compound is found to have desirable drug like property, its bioavailability also could become a challenge. Therefore, as literature shows one attractive and commonly used approach to overcome the problems of solubility and stability of Active Pharmaceutical Ingredients (APIs) is the salt formation of APIs. Numerous acids and bases with varying physio-chemical properties are utilized for this purpose with an estimated 50% of all APIs administered as salts.[6] There are well known examples where pharmaceutically active cations and anions combine together and the resulted salt exhibits therapeutic effects of both of its components.[7] Many of these ionic salts exhibit similar characteristics as seen now in the ionic liquids.[8] Therefore, it is logical to think that ionic liquids with their tunable properties and toxicities could potentially be designed as anti-cancer, anti-viral and other therapeutic agents. These 'Therapeutic Ionic Liquids' expectantly offer distinctly different properties. If therapeutic response is seen, then the major advantage of ionic liquids would be in managing/tuning their toxicity while tailoring the physio-chemical and pharmacological properties necessary for desired therapeutic application. This possibility motivated us to explore the anti-cancer activity of representative phosphonium and ammonium-based ionic liquids shown in Table 1. To the best of our knowledge, this is the first report where the anti-tumor activity of this class of compound has been explored.

The ionic liquids **1**–**7** used in these studies were purchased from Merck KgaA (EMD Chemicals), Darmstadt, Germany. Details of the methodology for NCI 60 cell line screening are described at http://dtp.nci.nih.gov/branches/btb/ivclsp.html.[9] Briefly, the panel is organized into nine subpanels representing diverse histologies: leukemia, melanoma, and cancers of lung, colon, kidney, ovary,

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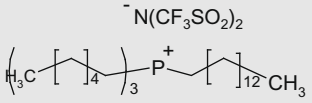
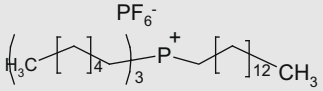
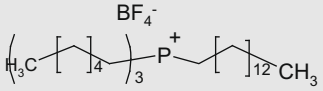
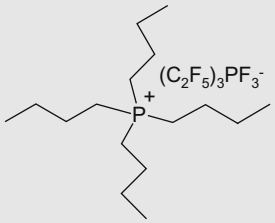
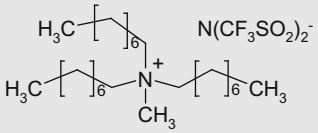
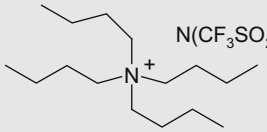
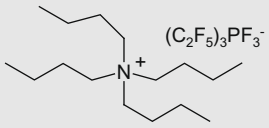
Table 1
List of compounds screened for NCI 60 cell lines

Compound#	NSC#	Structure	Activity*
1	747251		Active
2	747252		Active
3	747253		Active
4	747273		Active
5	747259		Active
6	747120		Inactive
7	747121		Inactive

* Compounds showing 60% tumor growth inhibition in 8 or more cell lines were considered to be active.

breast, prostate, and central nervous system. The cells are grown in supplemented RPM1 1640 medium for 24 h. The test compounds **1–7** were dissolved in DMSO and incubated with cells at five concentrations with 10-fold dilutions, the highest being 10^{-4} M and the others being 10^{-5}, 10^{-6}, 10^{-7}, and 10^{-8} M. The assay is terminated by addition of cold trichloroacetic acid, and the cells are fixed and stained with sulforhodamine B. Bound stain is solubilized, and the absorbance is read on an automated plate reader. The cytostatic parameter that is, 50% growth inhibition (GI_{50}) was calculated from time zero, control growth, and the five concentration level absorbance. The cytotoxic parameter that is, inhibitory concentrations (LC_{50}) represent the average of two independent experiments.[9] The in vitro screening is a two-stage process started with the evaluation of all the ionic liquids against the 60 human tumor cell lines with a single dose of 10.0 μM, which is done by following same protocol as for five dose screening. The output from the single dose screen was reported as a mean graph (given in the Supplementary data with general interpretation). Only the compounds which showed more then 60% of growth inhibition in at least 8 tumor cell lines were selected for further testing and the others were assumed as inactive. This primary one dose screening showed that compounds (**ILs**) **1**–**5** were active, while **6** and **7** were declared inactive (Table 1). A secondary screening was performed on active compounds **1**–**7**, in order to determine their cytostatic and cytotoxic activities. The compounds were evaluated at five concentration levels (100, 10, 1.0, 0.1 and 0.01 μM). On the bases of growth inhibition parameters, a structure activity relationship was obtained.

The log mean values for GI_{50} and LC_{50} in 60 cell lines for all the compounds except **3** are provided in Table 2 along with the log delta value (the maximum sensitivity in excess of the mean) and the log range (the maximum difference between the least sensitive and the most sensitive cell lines). These parameters provided insights into selectivity and potency of anti-tumor agents. Large values of the delta and range indicate high selectivity for some histological cancers over others. The drug response curves of **3** (given in the Supplementary data) for all the cell lines show further growth of the cells after their complete death and hence the results were not conclusive. This may be due to the precipitation of the compound at higher concentration because of its low solubility in the media and hence low cell membrane permeability. The lower median log GI_{50} of **1** and **2** show that these two compounds are most active followed by **5** and **4**. Also, the lower delta and range values indicated that all these compounds **1**, **2**, **4** and **5** are highly sensitive towards all 60 cell lines showing no selectivity for any particular case (Table 2). The high median log LC_{50} value of **4**, along with the low delta and range values, indicates the complete absence of cytotoxicity against all cell lines. Compound **1** is comparatively more cytotoxic with large delta and range values due to the high specificity for some of the Leukemia, melanoma and breast cancer cell lines. In contrast, compounds **2** and **5** were found to be most cytotoxic as indicated by lower median log LC_{50} values (Table 2).

The complete in vitro anti-cancer data recorded on 60 subpanel cell lines for the five most active compounds is reported in Table 3. The ionic liquids **6** and **7** having ammonium cation with short alkyl chain (C-6) substitution were found to be inactive. Increase in alkyl chain length contributed towards anti-cancer activity as can be seen in case of **5** (having C-8 alkyl substitution) which was highly sensitive towards all 60 tumor cell lines showing overall potency with GI_{50} values ranging from 0.046 to 1.900 μM. However, with increase in activity, cytotoxicity was also found to be increased which is evident by the LC_{50} values <10 μM in more than 90% cell lines. Similar trend was observed with phosphonium based ionic liquids, however, these were found to be more active as compared to their ammonium counter part. Compound **4** with C-6 alkyl substitution was the least active phosphonium IL showing overall potency towards all cell line with GI_{50} values in range of 1.150–10.70 μM. While it was completely non-toxic to all the cell lines with LC_{50} >100 μM in all cases, except for COLO 205 (a colon cancer cell line) having LC_{50} 9.10 μM. Interestingly, **4** and **7** both have similar anion but **4** is significantly active and completely non-toxic for all 60 cell lines, while **5** was completely inactive. Although **4** and **7** both have similar alkyl substitution on the cations but

Table 2
Cytostatic (GT_{50}) and cytotoxic (LC_{50}) parameters for **1**, **2**, **4**, and **5**

Compound	GI_{50}			LC_{50}		
	Median	Delta	Range	Median	Delta	Range
1	−7.01	0.79	1.66	−4.34	2.69	3.01
2	−7.04	0.56	1.48	−5.28	1.10	2.38
4	−5.46	0.48	1.48	−4.02	1.02	1.04
5	−6.42	0.91	1.61	−5.22	0.95	2.17

Table 3
Antitumor activity ($GI_{50}/\mu M$)[a] and toxicity ($LC_{50}/\mu M$)[b] data of compounds selected for 5 dose studies for the NCI60-cell lines screen

	1		**2**		**3**		**4**		**5**	
	GI_{50}	LC_{50}	GI_{50}	LC_{50}	GI_{50}	LC_{50}	GI_{50}	LC_{50}	GI_{50}	LC_{50}
Leukemia										
CCRF-CEM	0.026	8.458	0.038	2.140	0.034	nd	4.410	>100	0.190	2.440
HL-60(TB)	0.046	0.711	0.025	0.642	0.039	nd	2.420	>100	0.173	1.230
K-562	0.041	>100	0.087	>100	0.038	>100	1.680	>100	0.186	4.859
MOLT-4	0.069	>100	0.046	1.750	0.090	>100	10.70	>100	0.324	4.890
RPMI-8226	0.016	0.098	0.042	49.10	0.016	nd	1.640	>100	0.046	0.951
SR	0.072	8.085	0.047	3.580	0.107	>100	34.60	>100	0.162	5.134
Non-small cell lung cancer										
A549/ATCC	0.292	>100	0.377	9.840	0.391	>100	3.340	>100	1.900	8.580
EKVX	0.123	>100	0.069	4.600	0.206	nd	2.470	>100	0.215	4.310
HOP-62	0.435	>100	0.245	5.010	0.399	>100	5.170	>100	0.787	7.770
HOP-92	0.030	>100	0.025	3.350	0.038	nd	4.790	>100	0.206	4.470
NCI-H226	0.105	>100	0.088	4.230	0.189	nd	3.910	>100	0.478	4.410
NCI-H23	0.192	>100	0.143	9.260	0.236	>100	1.950	>100	0.308	6.330
NCI-H322M	0.321	>100	0.359	4.230	0.401	nd	6.120	>100	1.810	6.120
NCI-H460	0.315	>100	0.285	3.560	0.346	>100	2.840	>100	0.362	4.100
NCI-H522	0.105	>100	0.069	4.260	0.158	>100	3.420	>100	0.287	5.400
Colon cancer										
COLO 205	0.182	>100	1.730	>100	0.243	>100	1.700	9.160	0.292	>100
HCC-2998	0.240	>100	1.230	7.440	0.297	>100	2.410	>100	0.425	6.960
HCT-116	0.061	>100	0.050	6.960	0.084	>100	2.630	>100	0.319	9.900
HCT-15	0.291	>100	0.246	3.710	0.339	nd	2.880	>100	0.993	5.230
HT29	0.053	>100	0.057	6.490	0.061	>100	3.800	>100	0.305	5.170
KM12	0.049	>100	0.045	0.080	0.059	>100	2.890	>100	0.310	5.310
SW-620	0.049	>100	0.047	6.580	0.072	>100	2.870	>100	0.378	10.00
CNS cancer										
SF-268	0.072	>100	0.066	6.270	0.088	>100	7.440	>100	0.493	5.500
SF-295	0.205	>100	0.147	6.690	0.251	>100	4.070	>100	0.333	4.570
SF-539	0.174	>100	0.100	3.450	0.234	nd	5.290	>100	0.514	4.190
SNB-19	0.069	>100	0.068	7.830	0.097	>100	3.180	>100	0.587	8.250
SNB-75	0.076	>100	0.255	5.780	0.223	>100	4.510	>100	0.336	3.770
U251	0.041	5.620	0.037	3.610	0.037	nd	2.630	>100	0.334	4.320
Melenoma										
LOX IMVI	0.058	0.592	0.037	0.418	0.050		4.240	>100	0.192	1.080
MALME-3M	0.029	5.380	0.041	4.110	0.034	>100	2.000	>100	0.290	4.110
M14	0.052	>100	0.044	5.490	0.087	>100	3.050	>100	0.453	9.190
SK-MEL-2	0.027	>100	nd	nd	0.081	>100	8.250	>100	0.356	>100
SK-MEL-28	0.207	6.590	0.178	6.060	0.376	>100	1.800	>100	0.710	7.180
SK-MEL-5	0.079	0.829	0.052	0.663	0.074	nd	2.470	>100	0.177	0.677
UACC-257	0.103	>100	0.112	4.810	0.272	nd	2.380	>100	0.411	6.160
UACC-62	0.045	>100	0.043	4.180	0.061	nd	3.440	>100	0.335	4.100
Ovarian cancer										
IGROV1	0.071	>100	nd	nd	0.316	>100	7.460	>100	0.388	>100
OVCAR-3	0.051	6.760	0.036	3.100	0.118	>100	2.620	>100	0.315	4.180
OVCAR-4	0.073	>100	0.096	8.690	0.125	>100	4.340	>100	0.309	5.800
OVCAR-5	0.318	>100	0.279	7.590	0.337	nd	3.300	>100	1.240	8.170
OVCAR-8	0.086	>100	0.079	6.460	0.229	>100	5.120	>100	0.468	5.880
SK-OV-3	2.610	>100	0.254	5.000	0.331	>100	2.70	>100	0.565	5.120
Renal cancer										
786-0	0.253	>100	0.086	5.890	0.211	>100	5.290	>100	0.415	5.020
A498	0.310	>100	0.451	6.080	0.472	nd	2.360	>100	1.820	6.360
ACHN	0.260	>100	0.212	3.390	0.244	nd	2.920	>100	0.630	4.520
CAK-1	0.236	>100	0.207	4.480	0.350	>100	3.130	>100	0.084	3.280
RXF 393	0.141	>100	0.179	>100	0.352	>100	7.660	>100	0.464	5.750
SN12C	0.042	4.440	0.043	3.560	0.062	nd	3.310	>100	0.367	4.060
TK-10	0.341	>100	0.145	4.490	0.234	nd	2.570	>100	0.503	4.860
UO-31	0.350	>100	0.298	5.410	0.398	>100	4.330	>100	0.686	5.950
Prostate cancer										
PC-3	0.045	5.900	0.035	0.779	0.044	>100	3.780	>100	0.282	3.990
DU-145	0.156	>100	0.117	5.290	0.357	>100	6.020	>100	0.407	4.440
Breast cancer										
MCF7	0.144	>100	0.082	3.910	0.253	>100	3.050	>100	0.317	4.020
NCI/ADR-RES	0.730	>100	0.765	8.400	0.947	nd	3.080	>100	1.680	8.130
MDA-MB-231ATCC	0.056	>100	0.047	3.220	0.107	nd	6.980	>100	0.345	3.630
HS 578T	0.053	>100	0.077	9.390	0.084	>100	1.760	>100	0.417	>100
MDA-MB-435	0.045	19.80	0.038	8.460	0.050	>100	3.560	>100	0.335	5.700
BT-549	0.066	>100	0.061	4.370	0.094	>100	3.100	>100	0.421	4.420
T-47D	0.073	>100	0.068	20.40	0.089	>100	1.150	>100	0.428	>100
MDA-MB-468	0.036	2.560	0.044	4.240	0.053	>100	2.220	>100	0.119	2.110

nd: Not determined.
[a] GI_{50}: 50% Growth inhibition, concentration of drug resulting in a 50% reduction in net protein increase compared with control cells.
[b] LC_{50}: Lethal concentration, concentration of drug lethal to 50% of cells.

changing from ammonium to phosphonium made huge difference in activity. Based on the single dose data compounds **1**, **2** and **3** having same cation were found to be active even though compound has different anion. These three compounds were found to be highly sensitive against all 60 tumor cell lines showing overall potency with GI_{50} values ranging from 0.016 to 2.610 μM for **1**, 0.025 to 1.730 μM for **2** and 0.016 to 0.947 μM for **3**. Despite having same cation the cytotoxicity profiles of **1**–**3** were quite different. Compound **3** with tetrafluoroborate (BF_4^-) anion did not afford conclusive results due to its less solubility and hence less cell membrane permeability, as discussed earlier. Compound **2** having hexafluorophosphate (PF_6^-) anion was found to be most toxic of all the phosphonium bases ionic liquids toxic with LC_{50} <10 μM for 95% of cell lines. In contrast, compound **1** with bis(trifluoromethanesulfonyl)imide ($(CF_3SO_2)_2N^-$) anion was non-toxic having LC_{50} >100 μM for more than 75% of the cell lines. These results indicate that anions have significant effect on the cytotoxicity of ionic liquids and the cations seem to play a major role towards cytostatic activity. Of course, it would require more studies and extensive screening to establish this point.

The high sensitivity and non-toxicity of compound **1** resulted in selection for screening in hollow fiber assay. These experiments were carried out at the National Cancer Institute. The detailed protocol can be obtained from http://dtp.nci.nih.gov/branches/btb/hfa.html.[10] In summary, a panel consisting of breast (MDA-MB-231), non-small cell lung (NCI-H23 and NCI-H522), colon (SW-620 and COLO 205), melanoma (UACC-62, MDA-MB-435 and LOXIMVI), ovarian (OVCAR-5 and OVCAR-3) and CNS (U251 and SF-295) cell lines was used to screen in vivo activity. The cell lines were cultivated in RPMI-1640, containing 10% FBS and 2 mM glutamine and the suspension is flushed into 1 mm (internal diameter) polyvinylidene fluoride hollow fiber. A total of 3 different tumor lines were prepared for each experiment so that each mouse would receive 3 intraperitoneal (IP) implants (1 of each tumor line) and 3 subcutaneous (SC) implants (1 of each tumor line). The compounds were solubilized in 10% DMSO in saline/Tween 80R and mice are treated with either a high or a low dose (at 37.5 and 15 mg/kg/dose) using a QD X 4 schedule (four daily treatments) administered intraperitoneally. The day after the last dose, the fibers were collected and assessed for viable cell mass using an MTT dye conversion assay. Altogether, 12 cell lines are studied resulting in 48 possible test combinations (12 cell lines × 2 sites × 2 doses). A score of 2 is assigned for each compound dose, which results in a 50% or greater reduction in viable cell mass. Compounds with a combined IP+SC score 20, a SC score 8 or a net cell kill of one or more cell lines are referred for further studies.[10] Compound **1** showed the IP score of 12 (out of 24) and SC score of 4 (out of 24), with a total of 16 (out of 48) with no net cell kill. Although these results were not sufficient for NCI criteria of further xenograft testing, the overall study has clearly established the potential of these new class of compounds i.e. ionic liquids to be developed as anti-cancer drugs.

In summary, for the first time anti-tumor activity of phosphonium and ammonium-based ionic liquids has been determined using NCI 60 human tumor cell lines. With increase in alkyl chain length significant improvement in anti-tumor activity can be achieved. In general, phosphonium-based ILs were found to be more active then ammonium ILs. Results clearly show that the 'tunability' of ionic liquids (changing the cation/anion combination and modifying the cation with different substituents) can control their biological activity and cytotoxicity. This property of ILs could play a major role in their therapeutic applications, such as in cancer therapy. Further investigation into the mechanism of action with more extensive screening of these compounds may lead to their potential utility as drugs.

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Supplementary data

The one dose data of all the compounds **1**–**7** and drug response curves of active compounds **1**–**5**. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2009.06.086.

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