

New Pyridazinium-based Ionic Liquids: An Eco-friendly Ultrasound-assisted Synthesis, Characterization and Biological Activity

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ABSTRACT

An eco-friendly ultrasound-assisted procedure was developed for the preparation of a series of novel pyridazinium ionic liquids (ILs) 1–8. The structures of the novel ILs were established based on their FT-IR, ¹H, ¹³C NMR and mass spectra. Furthermore, the antimicrobial and anticancer activities of ILs 1–8 have been also investigated. The results of these screening experiments revealed that the ILs exhibited good to moderate antibacterial activity, such as IL 7, compared with standard drugs. Some of the newly synthesized ILs were also tested *in vitro* against human hepatocellular carcinoma (HEPG2), human breast adenocarcinoma (MCF7) and human colon carcinoma (HCT116) cell lines. Among the synthesized ILs, IL 8 was found to exhibit promising antiproliferative effects and produced the lowest IC₅₀ values among the tested ILs.

KEYWORDS

Eco-friendly procedure, ionic liquid, ultrasound irradiation, antibacterial, antifungal and anticancer activity.

1. Introduction

The level of research interest directed towards ionic liquids (ILs) has grown considerably during the past two decades because of their many outstanding properties, including their negligible vapour pressure, great thermal stability, nonflammability, good electrical conductivity, moderate viscosity and excellent solubility towards organic and inorganic compounds.^{1–2} Based on their attractive properties, ILs were extensively employed as an alternative green solvent to the common organic solvents.³ Furthermore, ILs have been used in a broad range of applications in a variety of different fields, including the electrodeposition of metals⁴ and the development of corrosion inhibitors,^{5–7} as well as numerous applications in polymer science and catalysis.^{8–9}

On the basis of these findings, a series of novel functionalized pyridazinium-based ILs 1–7 is communicated using an efficient green ultrasound-assisted reaction. Our group recently reported the synthesis of another interesting IL known as 1-(2-(4-nitrophenyl)-2-oxoethyl)pyridazinium bromide, which will be referred to hereafter as IL 8.⁷ The antimicrobial and antitumor activities of ILs 1–8 were evaluated in the current study and the results of these experiments have been discussed in detail.

2. Results and Discussion

2.1. Chemistry

Numerous reports have recently been published pertaining to the use of ultrasonic irradiation to promote a variety of organic transformations.^{10–11} With this in mind, ultrasonic irradiation was used in the current study as an eco-friendly approach for

the successful construction of new pyridazinium-based ILs 1–8. Briefly, a solution of pyridazine was treated with several different alkyl halides under ultrasonic irradiation in a closed system (Scheme 1).

Conventional *N*-alkylation conditions generally require long reaction times and provide moderate to low yields of the desired products. However, the use of ultrasound technology can lead to a significant decrease in the reaction time (5 h) required for an *N*-alkylation reaction compared with conventional conditions.¹² The yields obtained in the current study for the synthesis of ILs 1–8 under ultrasonic irradiation are shown in Table 1.

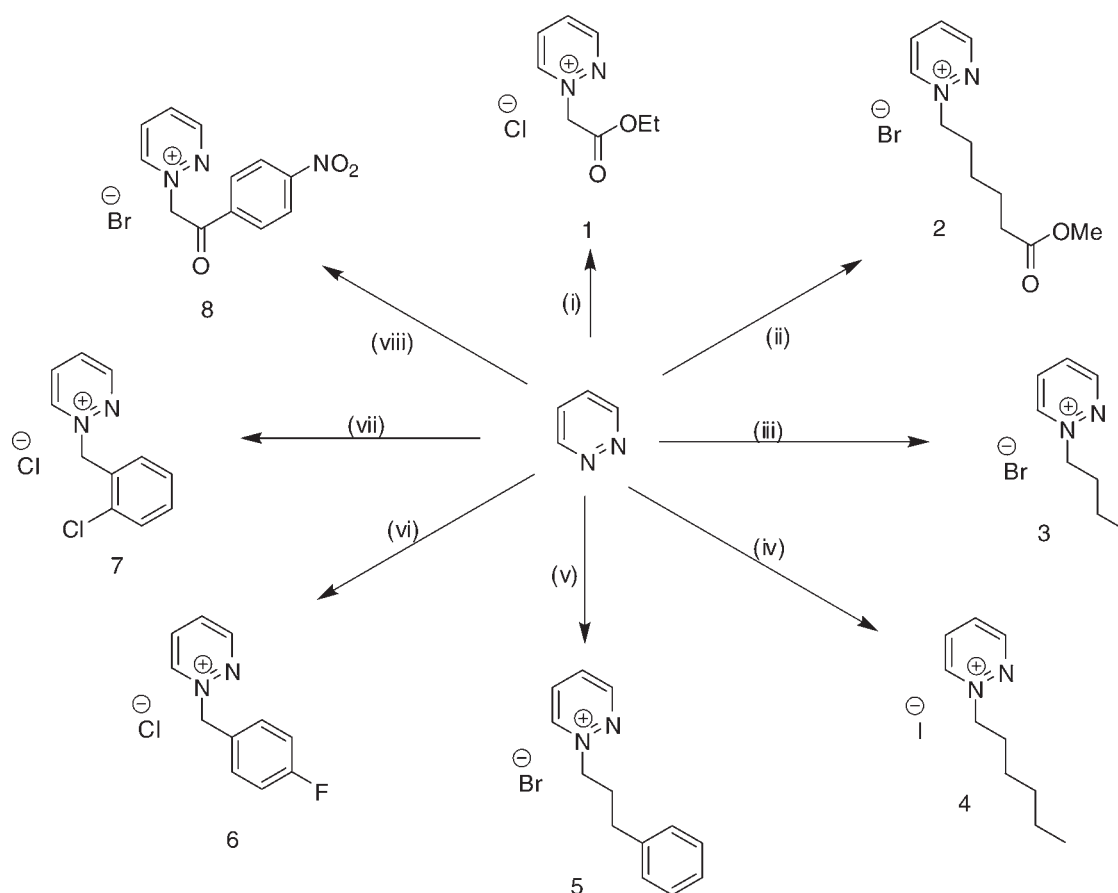
The newly synthesized ILs 1–8 were characterized by ¹H NMR, ¹³C NMR, FT-IR and LCMS analyses. The characterization of IL 2 was discussed in detail as the most representative compound of this new family of ILs.

The *N*-alkylation of pyridazine with methyl 6-bromohexanoate afforded 2, which result in the appearance of a singlet at 3.59 ppm due to the methyl protons of OCH₃ group. The five methylene protons of the IL2 appeared respectively as a triplet at 4.88 ppm for NCH₂CH₂, a triplet at 2.32 ppm for COCH₂CH₂, a quintet at 2.03 ppm for COCH₂CH₂, a sextet at 1.58 ppm for CH₂CH₂CH₂ and as a quintet at 2.03 for NCH₂CH₂. The ¹H NMR spectrum for 2 displays also two type of signals related to the pyridazinium protons. Two doublets at 9.70 and 10.16 ppm represent CHN=NCH and two doublets of doublet at 8.69 and 8.80 originate from NCHCHCHCHN.

The ¹³C NMR spectra of ILs 2 contained signal is consistent with the presence of a carbonyl group (C=O) at 173.2ppm. The signals of the five CH₂ groups and a CH₃ group appeared respectively at 64.2 ppm for (NCH₂), 51.2 ppm for (OCH₃), 32.9 ppm for (COCH₂), and 23.7, 24.7, 29.0 ppm for CH₂CH₂CH₂. The ¹³C NMR

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Scheme 1

N-alkylation of pyridazine under ultrasonic irradiation (US) conditions. (i) RX / toluene, 70 °C, 5 h. (i) R = $-\text{CH}_2\text{CO}_2\text{Et}$ for 1; (ii) $-(\text{CH}_2)_5\text{CO}_2\text{Me}$ for 2; (iii) $-(\text{CH}_2)_4\text{CH}_3$ for 3; (iv) $-(\text{CH}_2)_5\text{CH}_3$ for 4; (v) $-(\text{CH}_2)_3\text{Ph}$ for 5; (vi) $4\text{-FC}_6\text{H}_4\text{CH}_2-$ for 6; (vii) $2\text{-ClC}_6\text{H}_4\text{CH}_2-$ for 7; (viii) $4\text{-NO}_2\text{C}_6\text{H}_4\text{CH}_2-$ for 8; X = Cl, Br, I.

Table 1 Synthesis of pyridazinium-based ILs 1–8 under ultrasonic irradiation conditions.

Ionic liquid	RX	Yield /% <i>N</i> -Alkylation ^a
1	$\text{EtO}_2\text{C}(\text{CH}_2)\text{Cl}$	84
2	$\text{MeO}_2\text{C}(\text{CH}_2)_5\text{Br}$	86
3	$\text{CH}_3(\text{CH}_2)_4\text{Br}$	89
4	$\text{CH}_3(\text{CH}_2)_5\text{I}$	87
5	$\text{Ph}(\text{CH}_2)_3\text{Br}$	87
6	$4\text{-FC}_6\text{H}_4\text{CH}_2\text{Cl}$	88
7	$2\text{-ClC}_6\text{H}_4\text{CH}_2\text{Cl}$	89
8	$4\text{-NO}_2\text{C}_6\text{H}_4\text{CH}_2\text{Br}$	80

^a US: time 5 h, temperature 70 °C.

spectra also displayed the signals of the four tertiary carbons which appeared, respectively, at 150.0 and 154.4 ppm for the $\text{CHN}=\text{NCH}$, 136.0 and 136.5 ppm for the NCHCHCHCHN groups.

The structure of IL 2 was also confirmed by the FT-IR spectra in which an absorption band at 1731 cm^{-1} indicated clearly the presence of a carbonyl group (C=O) belonging to an ester. And finally, LCMS spectra of ILs 2 contained a peak at 209.3 ppm related the desired mass ions $[\text{M}-\text{Br}]^+$.

Generally, the ^1H NMR spectra of ILs 1 and 3–8 all contained signals in the range of 8.4–10.0 ppm, which were attributed to the pyridazinium protons. The phenyl protons of the pendent aromatic rings in ILs 5–8 were observed at 7.3–8.1 ppm. The characteristic ethyl ester protons of IL 1 appeared as a triplet and quartet at 1.25 and 4.25 ppm, respectively.

The ^{13}C NMR spectra of ILs 1 and 8 contained signals consistent with the presence of a carbonyl group at δ_{C} 165.7 and 189.7 ppm, respectively. The ^{13}C NMR spectra of ILs 1 and 3–8 all contained signals corresponding to aromatic carbons, including those of the pyridazinium ring, in the range of δ_{C} 116–162 ppm. Furthermore, the ^{13}C NMR spectra of ILs 1 and 3–8 contained carbon signals corresponding to CH_2 and CH_3 groups with the usual chemical shifts.

The success of this new strategy for the *N*-alkylation of pyridazine was also confirmed by the FT-IR spectra of ILs 1 and 3–8. The FT-IR spectra of IL 1 contained an absorption band at 1730 cm^{-1} , which indicated the presence of a carbonyl group (C=O) belonging to an ester. Furthermore, the FT-IR spectrum of IL 8 contained a peak at 1637 cm^{-1} , which was consistent with the presence of a carbonyl group (C=O) belonging to a ketone. Finally, the LCMS spectra of ILs 1 and 3–8 contained peaks consistent with the desired mass ions $[\text{M}-\text{X}]^+$.

2.2 Antimicrobial Properties

Several recent reports showed that ILs possess promising biological activities.^{13–15} Encouraged by these results, we proceeded to evaluate the antimicrobial activities of ILs 1–8 against four strains of bacteria, including two Gram-positive and two Gram-negative strains, and four strains of fungi.

2.2.1 Antifungal Properties

In recent years, extensive research appeared in literature regarding the design of new families of antifungal agents presumably due to the increasing resistance of fungi towards

existing antifungal agents. The antifungal activities of ILs 1–8 were screened *in vitro* against *Aspergillus fumigatus*, *Penicillium italicum*, *Candida albicans* and *Geotrichum candidum*. The antifungal activities were measured using the agar diffusion method with Sabouraud's agar medium. The results of these experiments are summarized in Table 2 and represented graphically in Fig. 1.

As shown in Table 2, the zones of inhibition fluctuated from 21.3 mm for IL 7 to 13.1 mm for IL 3, and were therefore comparable to those observed of the antibiotic Amphotericin B, which was used as a positive control.

2.2.2. Antibacterial Properties

The antibacterial activities of ILs 1–8 were tested *in vitro* against two Gram-positive (*Staphylococcus aureus* and *Bacillus subtilis*) and to Gram-negative (*Pseudomonas aeruginosa* and *Escherichia coli*) bacteria. The antibacterial activities of ILs 1–8 were determined using the agar diffusion method with Mueller-Hinton agar medium.

The results of these experiments are displayed in Table 3 and represented graphically in Fig. 2, and show that ILs 1–8 exhibited similar levels of antibacterial activity towards *S. aureus*, *B. subtilis*, *P. aeruginosa* and *E. coli* to both Ampicillin and Gentamicin.

Furthermore, IL 7 exhibited the highest activity towards all of the microorganisms tested in the current study at a concentration of 30 $\mu\text{g mL}^{-1}$. The higher activity of IL 7 was attributed to its 2-chlorobenzyl group.

2.2.3. Minimum Inhibitory Concentration (MIC)

Since ILs 2–3 and 5–8 showed good Inhibition zone, they were selected in order to test the *in vitro* Minimum Inhibitory Concentration (MIC) and the obtained results are summarized in Table 4.

The MIC of the tested ILs showed good to moderate activity against eight types of human pathogens comparing with drugs standard and the range of MIC levels varied from 0.99 μM to more than 260 μM .

2.2.4. Cytotoxic Activity of Compounds 1, 2 and 8 against Tumor Cell Lines

ILs 1, 2 and 8 were selected for further evaluation based on their *in vitro* activities, and their cytotoxic activities were evaluated towards a series of human cancer cell lines, including HEPG2 (human hepatocellular carcinoma), MCF-7 (human breast adenocarcinoma) and HCT116 (colon carcinoma) cells. Cell growth and cell viability measurements were recorded

Table 2 Antifungal activity of ILs 1–8 against four fungi. (The concentration used in the antimicrobial test was 1 mg mL⁻¹.)

Compound	Inhibition zone ^a /mm			
	<i>A. fumigatus</i>	<i>P. italicum</i>	<i>C. albicans</i>	<i>G. candidum</i>
1	—	—	—	—
2	19.3 ± 0.63	17.8 ± 0.44	—	21.4 ± 0.43
3	18.3 ± 0.73	16.4 ± 0.52	—	20.1 ± 0.76
4	13.1 ± 0.66	15.0 ± 0.25	—	15.9 ± 0.36
5	15.8 ± 0.25	16.3 ± 0.17	—	18.3 ± 0.35
6	19.3 ± 0.43	17.4 ± 0.66	—	19.2 ± 0.52
7	20.1 ± 0.29	18.1 ± 0.24	—	21.3 ± 0.38
8	18.1 ± 0.33	16.3 ± 0.54	—	19.3 ± 0.66
Amphotericin B	23.7 ± 0.10	21.9 ± 0.12	19.8 ± 0.20	28.7 ± 0.22

^a Results are the mean of three repetitions.

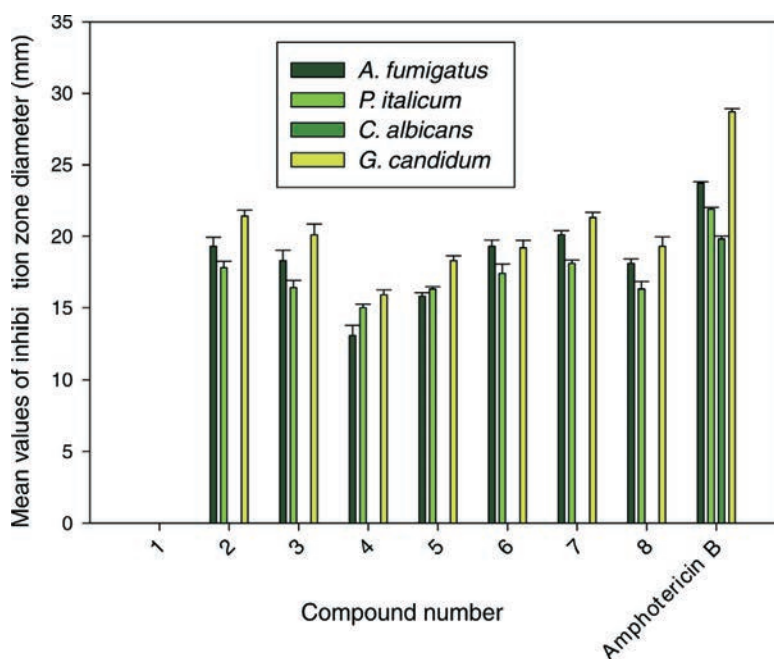
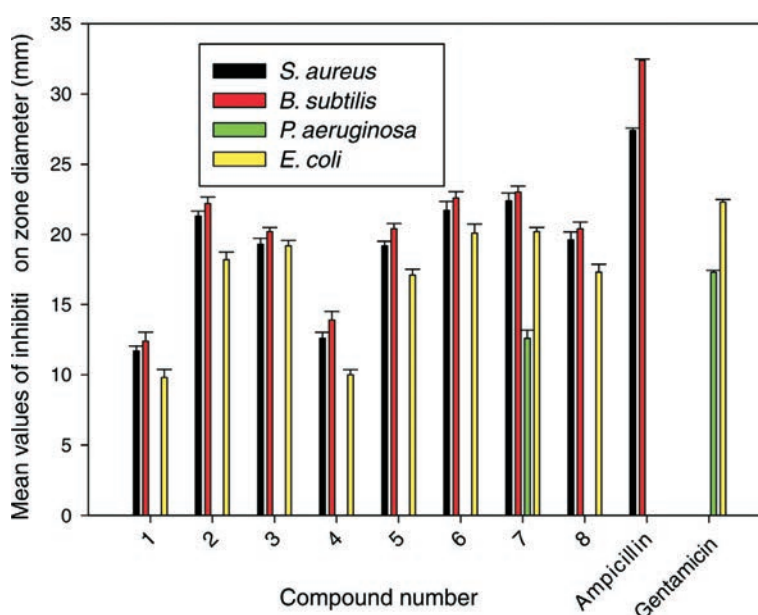


Figure 1 Antifungal activity of ILs 1–8 against *Aspergillus fumigatus*, *Penicillium italicum*, *Candida albicans* and *Geotrichum candidum*. (The concentration used in the antimicrobial test was 1 mg mL⁻¹.)

Table 3 Antibacterial activity of ILs 1–8 against four bacteria. (The concentration used in the antimicrobial test was 1 mg mL⁻¹.)

Compound	Inhibition zone ^a /mm			
	<i>S. aureus</i>	<i>S. aureus</i>	<i>S. aureus</i>	<i>S. aureus</i>
1	11.7 ± 0.36	12.4 ± 0.64	—	9.8 ± 0.58
2	21.3 ± 0.36	22.2 ± 0.46	—	18.2 ± 0.54
3	19.3 ± 0.42	20.2 ± 0.28	—	19.2 ± 0.36
4	12.6 ± 0.43	13.9 ± 0.62	—	10.0 ± 0.36
5	19.2 ± 0.31	20.4 ± 0.37	—	17.1 ± 0.43
6	21.7 ± 0.64	22.6 ± 0.44	—	20.1 ± 0.64
7	22.4 ± 0.55	23.0 ± 0.43	12.6 ± 0.58	20.2 ± 0.29
8	19.6 ± 0.58	20.4 ± 0.48	—	17.3 ± 0.58
Ampicillin	27.4 ± 0.18	32.4 ± 0.10	—	—
Gentamicin	—	—	17.3 ± 0.15	22.3 ± 0.18

^a Results are the mean of three repetitions.

**Figure 2** Antibacterial activity of ILs 1–8 against Gram-negative and Gram-positive bacteria. (The concentration used in the antimicrobial test was 1 mg mL⁻¹.)

according to a previously reported procedure.¹⁶ Cytotoxicity was evaluated using various viability assays (HCT cytotoxicity Assay and MTT Metabolic impairment Assay), which were performed by the Regional Center for Mycology & Biotechnology at Al-Azhar University, Egypt. The results of these assays are displayed in Figs. 3–5. The used concentrations are the same for all tested ILs and are expressed in mg mL⁻¹ and the results are the mean of three repetitions.

The results of the cytotoxicity assays showed that ILs 1, 2 and 8 exhibited dose-dependent activities towards all three cell lines, with increases in the dose leading to a reduction in survival. However, the dose responses were less pronounced for concentrations in the range of 25–50 mg mL⁻¹, but IL 8 was found to exhibit the higher antiproliferative activity in the range of 5 to 25 mg mL⁻¹.

The IC₅₀ values of ILs 1, 2 and 8 were found to be in the range of

Table 4 Antimicrobial activity expressed as MIC/mM^a.

Compound	Antifungal activity				Antibacterial activity			
	<i>A. fumigatus</i>	<i>P. italicum</i>	<i>C. albicans</i>	<i>G. candidum</i>	<i>S. aureus</i>	<i>B. subtilis</i>	<i>P. aeruginosa</i>	<i>E. coli</i>
2	13.5	27	—	3.38	3.38	1.69	—	27
3	33.8	13.5	—	8.43	16.8	8.43	—	16.8
5	111	111	—	27.9	13.9	6.98	—	55.9
6	17222.3	6.95	—	17.3	2.18	1.07	—	8.67
7	8.08	64.8	—	4.06	2.03	0.99	259.1	8.1
8	48.2	96.4	—	12.03	6.01	6.01	—	48.2
Amphotericin B	0.13	0.53	2.11	0.032	—	—	—	—
Ampicillin	—	—	—	—	0.17	0.04	—	—
Gentamicin	—	—	—	—	—	—	32.7	1.02

^a Results are the mean of three repetitions.

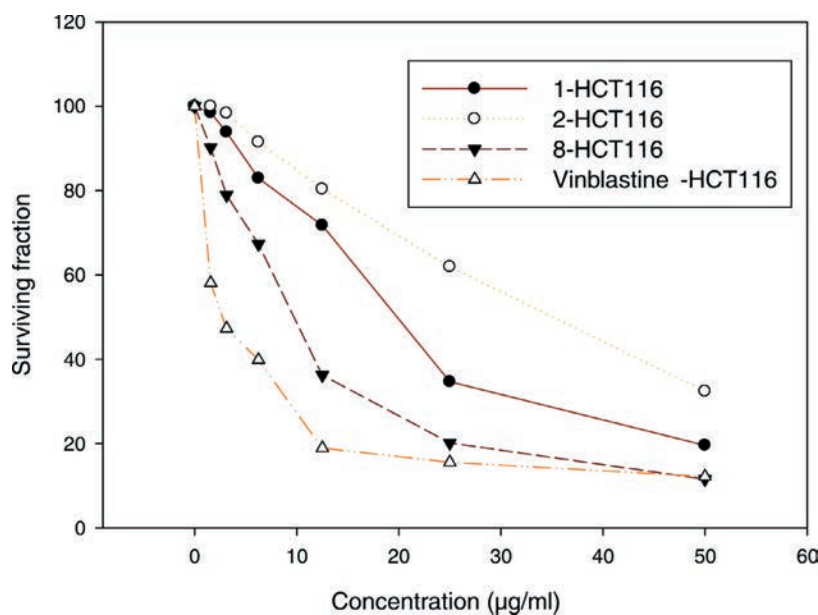


Figure 3 Cytotoxic activities of ILs 1, 2 and 8 against HCT116 cells.

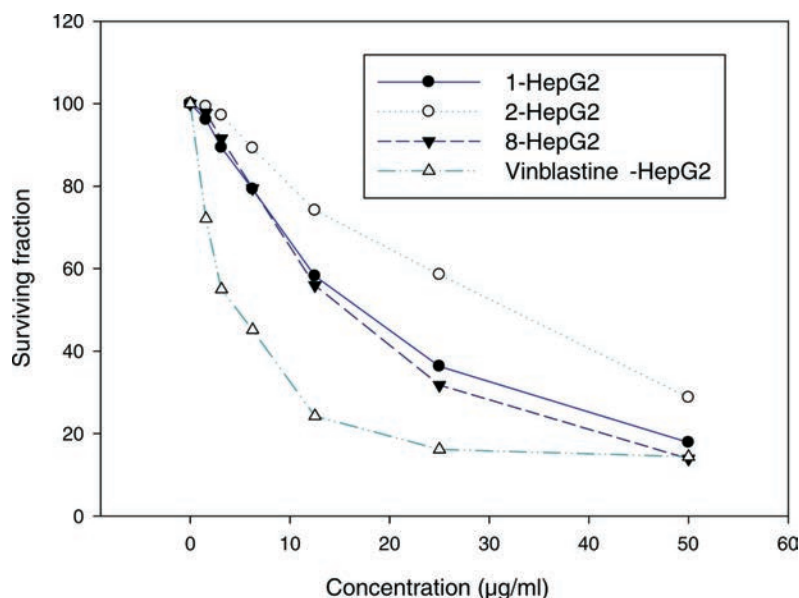


Figure 4 Cytotoxic activities of ILs 1, 2 and 8 against HepG2 cells.

30 to 140 μM , with IL 2 showing the weakest activity of the three different compounds (Table 5). Notably, IL 8 showed the highest activities of the three ILs tested towards all three cell lines, with IC_{50} values in the range of 30 to 51 μM , although the activities of this compound was less than those of Vinblastine, which was used as a positive control.

3. Experimental

All new compounds were characterized by ^1H NMR, ^{13}C NMR and IR spectroscopy and LCMS. ^1H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra were measured in DMSO and D_2O at room temperature. Chemical shifts (δ) were reported in ppm, with tetramethylsilane (TMS) as an internal standard. The Liquid chromatography–mass spectrometry LCMS (spectra were measured with a Micromass LCT (Liquid Chromatography Tandem) mass spectrometer (Model: Micromass LCT Classic, Agilent Technologies, Germany). IR spectra were recorded on a KBr disc with a Shimadzu 8201 PC FT-IR spectrophotometer (ν_{max} in cm^{-1}). Ultrasound-assisted reactions were performed with a

high-intensity ultrasonic processor SUB Aqua 5 Plus-Grant with a temperature controller (750 W), microprocessor controlled-2004. The ultrasonic frequency of the cleaning bath used is 25 KHz.

3.1. Synthesis

3.1.1. General Procedure for the Synthesis of *N*-alkylpyridazinium Halides 1–8 with Ultrasonic Irradiation

Pyridazine (1.0 eq.) and an alkyl halide (1.1 eq.) were added to toluene (15 mL) and placed in a closed vessel and exposed to irradiation for 5 h at 70 $^{\circ}\text{C}$ in a sonicator until a precipitate was formed. The obtained solid was filtered and washed with ethylacetate to afford the desired pyridazinium IL

3.2. Characterization

3.2.1. 1-(2-Ethoxy-2-oxoethyl)pyridazinium Chloride 1

This compound was obtained as brown solid; Mp 120–122 $^{\circ}\text{C}$;

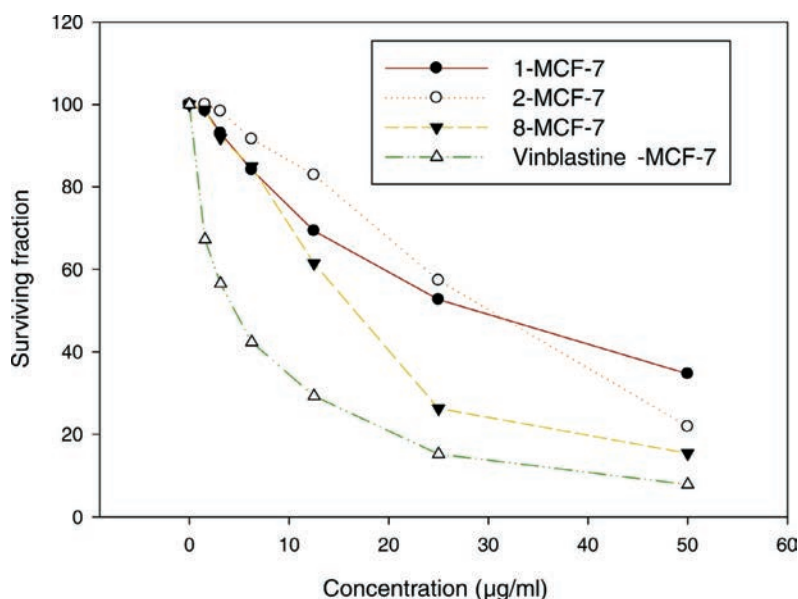


Figure 5 Cytotoxic activities of ILs 1, 2 and 8 against MCF7 cells.

Table 5 IC₅₀ (mg mL⁻¹ (mM)) values of compounds 1, 2 and 8 towards HCT116, HEPG2 and MCF7 cells.

Cell line	1	2	8	Vinblastine
HCT116	19.8 (97)	35.1 (120)	9.73 (30)	2.38 (2.93)
HEPG2	17.2 (85)	32.2 (110)	15.6 (48)	4.6 (5.67)
MCF7	28.7 (140)	30.2 (100)	16.6 (51)	4.6 (5.67)

yield 84 %; δ_{H} (DMSO-*d*₆, 400 MHz): 1.25 (t, *J* = 7.2 Hz, 3H), 4.26 (q, *J* = 7.2 Hz, 2H), 6.17 (s, 2H), 8.82–8.98 (2Ar-H), 9.82 (d, 1Ar-H), 10.42 (1Ar-H); δ_{C} (DMSO-*d*₆, 100 MHz): 13.8 (CH₃), 62.5 (CH₂), 64.2 (CH₂), 136.0 (CH), 137.8 (CH), 151.9 (CH), 154.7 (CH), 165.7 (CO); IR (ν_{max} cm⁻¹) 3000 (C-H Ar), 1748 (C=O), 1572–1446 (C=C), 1240 (C-N), 1027 (C-O) cm⁻¹; LCMS (M-Cl) 167 found for C₈H₁₁N₂O₂⁺. (Found: C, 47.35; H, 5.50; N, 13.76 %. Calc. for C₈H₁₁ClN₂O₂ (202.64); C, 47.42; H, 5.47; N, 13.82 %).

3.2.2. 1-(6-Methoxy-6-oxohexyl)pyridazinium Bromide 2

This compound was obtained as dark semi-solid; yield 86 %; δ_{H} (DMSO-*d*₆, 400 MHz): 1.34 (quint, *J* = 7.2 Hz, 2H), 1.58 (sextet, *J* = 7.2 Hz, 2H), 2.03 (quint, *J* = 7.2 Hz, 2H), 2.32 (t, *J* = 7.2 Hz, 2H), 3.59 (s, 3H), 4.88 (t, *J* = 7.2 Hz, 2H), 8.69 (1Ar-H), 8.80 (1Ar-H), 9.70 (1Ar-H), 10.16 (1Ar-H); δ_{C} (DMSO, 100 MHz): 23.7 (CH₂), 24.7 (CH₂), 29.0 (CH₂), 32.9 (CH₂), 51.2 (CH₃), 64.2 (CH₂), 136.0 (CH), 136.5 (CH), 150.0 (CH), 154.5 (CH), 173.2 (CO); IR (ν_{max} cm⁻¹) 2997 (C-H Ar), 1747 (C=O), 1595–1445 (C=C), 1195 (C-N), 998 (C-O) cm⁻¹; LCMS (M-Br) 209 found for C₁₁H₁₇N₂O₂⁺. (Found: C, 45.60; H, 6.02; N, 9.74 %. Calc. for C₁₁H₁₇BrN₂O₂ (289.17); C, 45.69; H, 5.93; N, 9.69 %).

3.2.3. 1-Pentylpyridazinium Bromide 3

This compound was obtained as brown solid; Mp 118–120 °C; yield 89 %; δ_{H} (400MHz, DMSO): 0.87 (t, 3H), 1.13 (m, 4H), 2.03 (quint, 2H), 4.89 (t, 2H), 8.68 (dd, 1Ar-H), 8.83 (dd, 1Ar-H), 9.71 (d, 1Ar-H), 10.20 (d, 1Ar-H); δ_{C} (100MHz, DMSO): 13.6 (CH₃), 21.5 (CH₂), 27.4 (CH₂), 29.1 (CH₂), 64.4 (CH₂), 136.0 (CH), 136.5 (CH), 149.8 (CH), 154.5 (CH). IR (ν_{max} cm⁻¹) 3032 (C-H, sp²), 1570–1471 (C=C), 1165 (C-N); LCMS (M-Br) 151 found for C₉H₁₅N₂⁺. (Found: C, 46.70; H, 6.48; N, 12.16 %. Calc. for C₉H₁₅BrN₂ (231.13); C, 46.77; H, 6.54; N, 12.12 %).

3.2.4. 1-Hexylpyridazinium Iodide 4

This compound was obtained as greenish brown solid; Mp 62–64 °C; yield 87 %; δ_{H} (400MHz, DMSO-*d*₆): 0.85 (t, 3H), 1.30 (m, 6H), 2.02 (quint, 2H), 4.84 (t, 2H), 8.64 (1Ar-H), 8.77 (1Ar-H), 9.68 (1Ar-H), 10.04 (1Ar-H); δ_{C} (100MHz, DMSO-*d*₆): 13.8 (CH₃), 21.8 (CH₂), 25.0 (CH₂), 29.3 (CH₂), 30.5 (CH₂), 64.5 (CH₂), 136.0 (CH), 136.5 (CH), 149.8 (CH), 154.5 (CH). IR (ν_{max} cm⁻¹) 3028 (C-H, sp²), 1567–1441 (C=C), 1143 (C-N); LCMS (M-I) 165 found for C₁₀H₁₇N₂⁺. (Found: C, 41.05; H, 5.92; N, 9.53 %. Calc. for C₁₀H₁₇IN₂ (292.16); C, 41.11; H, 5.86; N, 9.59 %).

3.2.5. 1-(3-Phenylpropyl)pyridazinium Bromide 5

This compound was obtained as brown solid; Mp 100–102 °C; yield 87 %; δ_{H} (400MHz, DMSO-*d*₆): 2.35 (quint, 2H), 2.72 (t, 3H), 4.91 (t, 2H), 7.19–7.31 (m, 5H), 8.67 (1Ar-H), 8.77 (1Ar-H), 9.68 (1Ar-H), 10.04 (1Ar-H); δ_{C} (100MHz, DMSO-*d*₆): 30.9 (CH₂), 31.5 (CH₂), 64.2 (CH₂), 126.1 (CH), 128.3 (CH), 128.4 (CH), 136.0 (CH), 136.5 (CH), 140.3 (C), 150.0 (CH), 154.4 (CH); IR (ν_{max} cm⁻¹) 2997 (C-H, sp²), 1587–1473 (C=C), 1162 (C-N); LCMS (M-Br) 199 found for C₁₃H₁₅N₂⁺. (Found: C, 55.82; H, 5.49; N, 9.99 %. Calc. for C₁₃H₁₅BrN₂ (279.18); C, 55.93; H, 5.42; N, 10.08 %).

3.2.6. 1-(4-Fluorobenzyl)pyridazinium Chloride 6

This compound was obtained as beige solid; Mp 200–202 °C; yield 88 %; δ_{H} (400MHz, D₂O): 5.96 (s, 2H), 7.75 (Ar-H), 8.13 (2Ar-H), 8.82 (1Ar-H), 8.94 (1Ar-H), 9.78 (1Ar-H), 10.05 (1Ar-H); δ_{C} (100MHz, D₂O): 67.9 (CH₂), 116.1 (CH), 116.3 (CH), 132.0 (CH), 132.1 (CH), 136.1 (CH), 137.0 (CH), 149.2 (CH), 154.7 (CH), 162.0 (C); IR (ν_{max} cm⁻¹) 3038 (C-H, sp²), 1588–1470 (C=C), 1188 (C-N); LCMS (M-Cl) 189 found for C₁₁H₁₀FN₂⁺. (Found: C, 58.72; H, 4.55; N, 12.40 %. Calc. for C₁₁H₁₀ClF₂N₂ (224.66); C, 58.81; H, 4.49; N, 12.47 %).

3.2.7. 1-(2-Chlorobenzyl)pyridazinium Chloride 7

This compound was obtained as beige solid; Mp 176–178 °C; yield 89 %; δ_{H} (400MHz, D₂O): 6.09 (s, 2H), 7.35–7.45 (m, 3Ar-H), 7.58 (1Ar-H), 8.47 (1Ar-H), 8.53 (1Ar-H), 9.37 (1Ar-H), 9.71 (1Ar-H); δ_{C} (100MHz, D₂O): 66.3 (CH₂), 128.0 (CH), 129.1 (C), 130.2 (CH), 132.1 (CH), 134.7 (C), 136.0 (CH), 137.1 (CH), 133.2 (C), 149.6 (CH), 154.7 (CH); IR (ν_{max} cm⁻¹) 3043 (C-H, sp²), 1572–1474 (C=C), 1167 (C-N); LCMS (M-Cl) 205 found for

$C_{11}H_{10}ClN_2^+$; (Found: C, 54.71; H, 4.26; N, 11.59 %. Calc. for $C_{11}H_{10}Cl_2N_2O_2$ (241.12); C, 54.79; H, 4.18; N, 11.62 %).

3.2.8. 1-(2-(4-Nitrophenyl)-2-oxoethyl)pyridazinium Bromide **8**

This compound was obtained as beige solid; Mp 225–227 °C; yield 80 %; δ_H (400MHz, DMSO): 6.87 (s, 2H), 7.75 (2Ar-H), 8.13 (dd, 2Ar-H), 8.82 (1Ar-H), 8.94 (1Ar-H), 9.78 (1Ar-H), 10.05 (1Ar-H); δ_C (100MHz, DMSO): 70.5 (CH₂), 124.1 (CH), 129.9 (CH), 136.0 (CH), 137.6 (CH), 137.9 (C), 151.8 (CH), 154.7 (CH), 150.7 (C), 189.7 (CO); IR (ν_{max} cm⁻¹) 3112 (C-H, sp²), 1721 (C=O), 1591–1448 (C=C), 1112(C-N); LCMS (M-Br) 244 found for $C_{12}H_{10}N_3O_3^+$; (Found: C, 44.39; H, 3.19; N, 12.93%. Calc. for $C_{12}H_{10}BrN_3O_3$ (324.13); C, 44.47; H, 3.11; N, 12.96%).

3.3. Determination of IZ and MIC

The antimicrobial potential was estimated in terms of inhibition zone (IZ) measurements by agar disc diffusion method. The test was performed by transferring 24 h fresh bacterial cultures onto the surface of Muller–Hinton agar plates to yield confluent growth. The antibacterial potential was assayed using filter paper discs (6 mm i.d.) loaded with 10 μ g of tested ILs. Loaded discs were transferred onto the centre of inoculated Petri-plates which were then maintained for 2 h in a refrigerator at 4 °C to allow for the diffusion of the bioactive compound. The diameter of the inhibition zone was measured (in mm) after 24 h incubation at 37 °C. Sterile distilled water was used as a control. Clinical samples of *Escherichia coli*, *Enterobacter* sp., *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Bacillus cereus* and *Acinetobacter baumannii* were used as test strains.

The minimum inhibitory concentration (MIC) of tested ionic liquids were determined by CLSI microdilution-based method.¹⁷ Test compound was dissolved in sterile, distilled water and diluted to a final concentration of 256 μ g mL⁻¹ in Mueller-Hinton broth (Becton Dickinson, USA).¹⁸ Two-fold serial dilutions were prepared in a 96-well microtitre plate. Bacterial suspension containing approximately 1×10^8 CFU mL⁻¹ were prepared from 24 h agar plates with Mueller Hinton broth. Aliquots of 100 μ L of each bacterial suspension was mixed with 100 μ L serially diluted tested compound in microtitre plate.¹⁹ Uninoculated wells were prepared as control samples. Plates were incubated at 37 °C for 24 h. The MIC was defined as the lowest concentration of test compound producing no visible growth. All experiments were performed in triplicate.

4. Conclusion

A novel series of functionalized pyridazinium-based ILs has been successfully synthesized under ultrasonic irradiation conditions. The structures of these compounds were fully characterized by FT-IR, ¹H-NMR, ¹³C-NMR and mass spectrometry. Compared with standard antibiotics, the results of several antimicrobial screening experiments showed that our newly synthesized ILs exhibited good to moderate antimicrobial and antifungal activities. Furthermore, the cytotoxic activities of three of these new ILs were tested *in vitro* against three different human carcinoma cell lines. The results revealed that IL **8** could be considered as a promising antiproliferative agent with low IC₅₀ values against all three cell lines.

Supplementary Data

Supplementary data (¹H NMR, ¹³C NMR, Dept NMR, IR and

mass spectra) associated with this article are available in the online supplement.

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Supporting Information

New pyridazinium based ionic liquids: An eco-friendly ultrasound-assisted synthesis, characterization and biological activity

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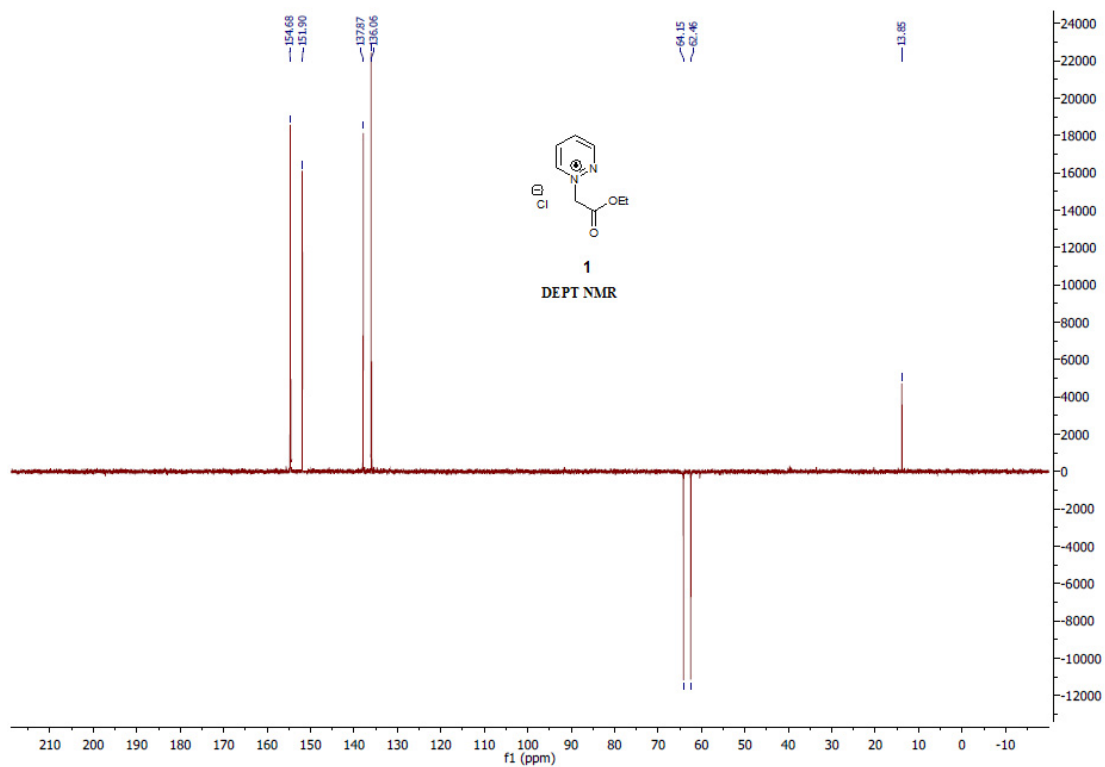
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^d*Department of Chemistry, Mutah University, Mutah 61710, Karak-Jordan.*

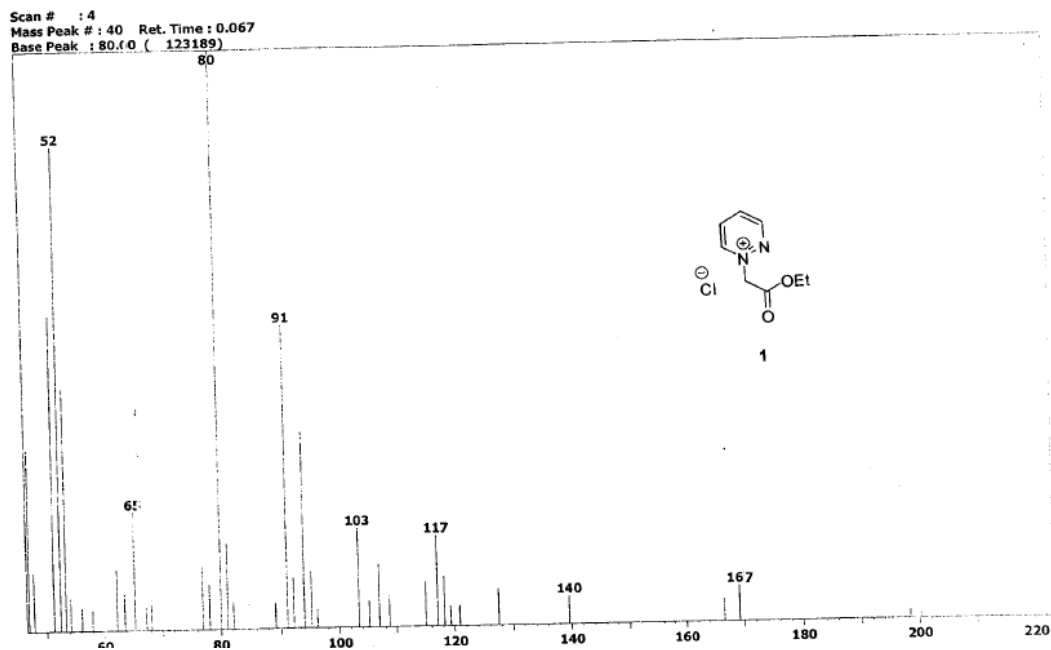
*E-mail: mousslim@mail.be

The following spectra are included:

- S1: ¹H NMR, ¹³C NMR, Dept135 NMR, mass and IR spectra of IL **1**
- S2: ¹H NMR, ¹³C NMR, Dept135 NMR, mass and IR spectra of IL **2**
- S3: ¹H NMR, ¹³C NMR, Dept135 NMR, mass and IR spectra of IL **3**
- S4: ¹H NMR, ¹³C NMR, Dept135 NMR, mass and IR spectra of IL **4**
- S5: ¹H NMR, ¹³C NMR, Dept135 NMR, mass and IR spectra of IL **5**
- S6: ¹H NMR, ¹³C NMR, Dept135 NMR, mass and IR spectra of IL **6**
- S7: ¹H NMR, ¹³C NMR, Dept135 NMR, mass and IR spectra of IL **7**
- S8: ¹H NMR, ¹³C NMR, Dept135 NMR, mass and IR spectra of IL **8**

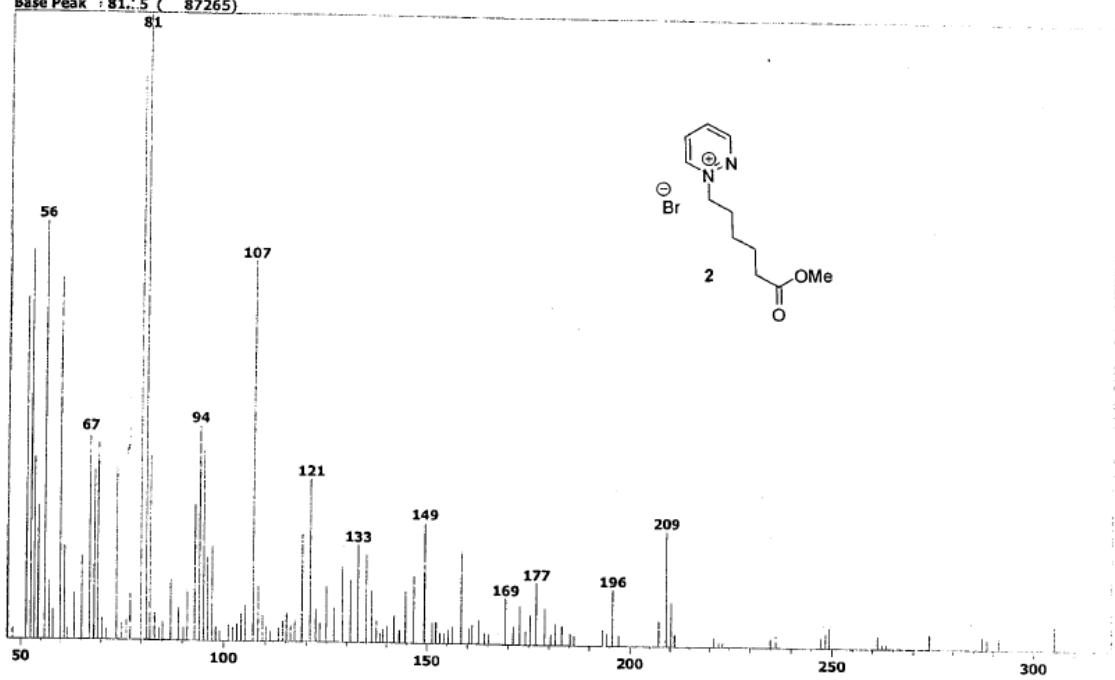


S1: DEPT- 135 NMR spectrum of IL 1 in DMSO (100 MHz)

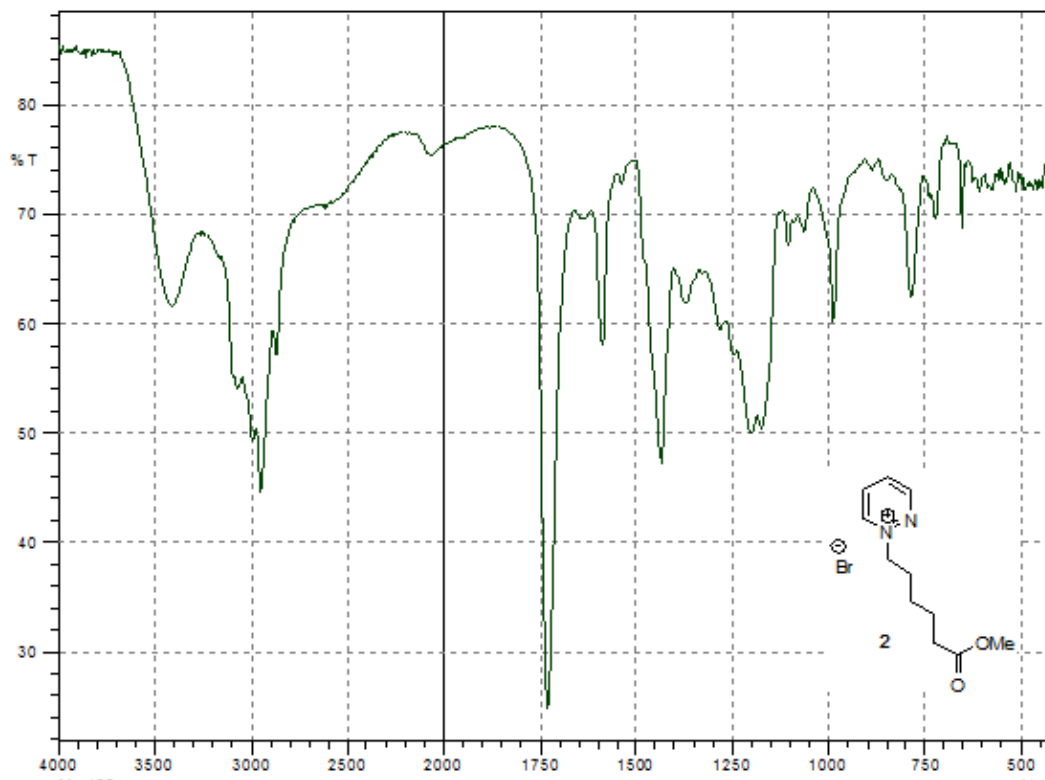


S1: (LCMS) Mass spectrum of IL 1

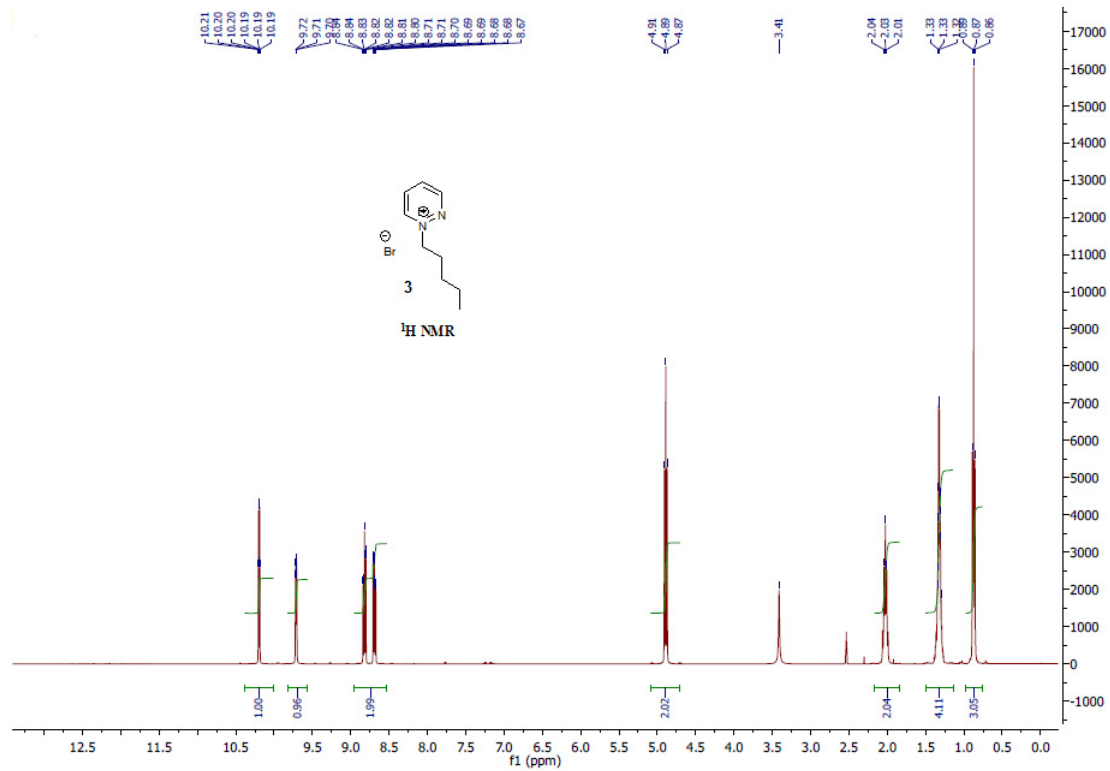
Scan # : 208
Mass Peak # : 123 Ret. Time : 3.467
Base Peak : 81.5 (87265)



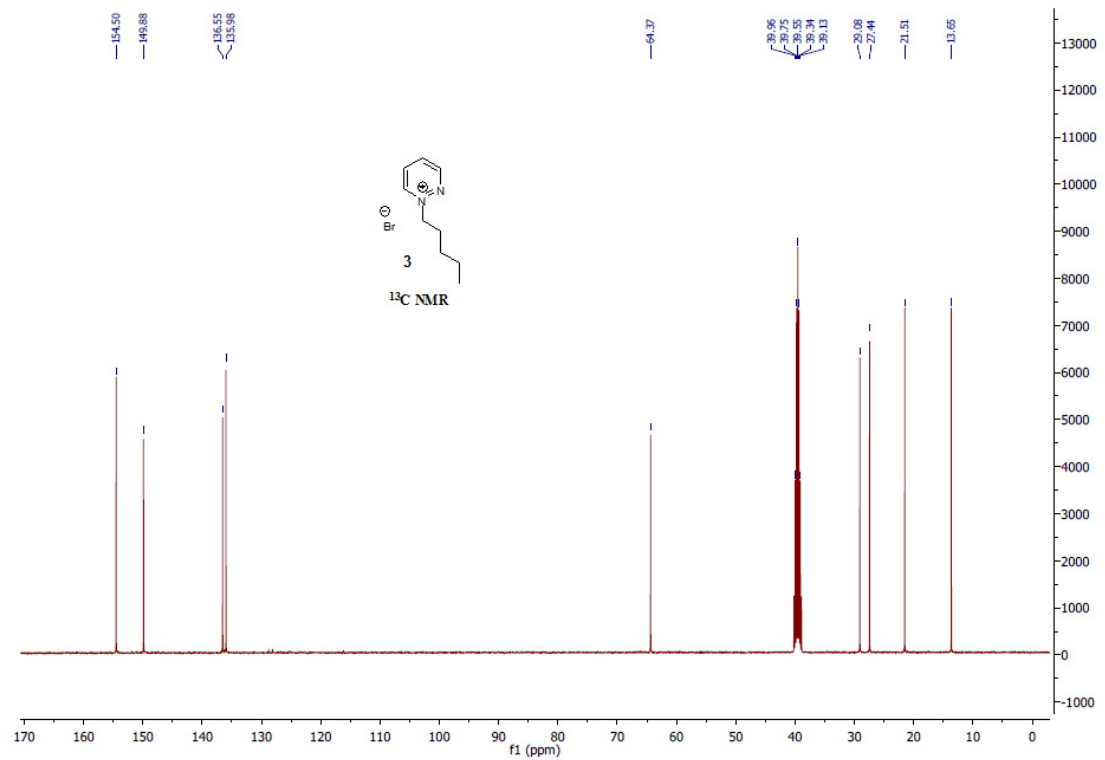
S2: (LCMS) Mass spectrum of IL 2



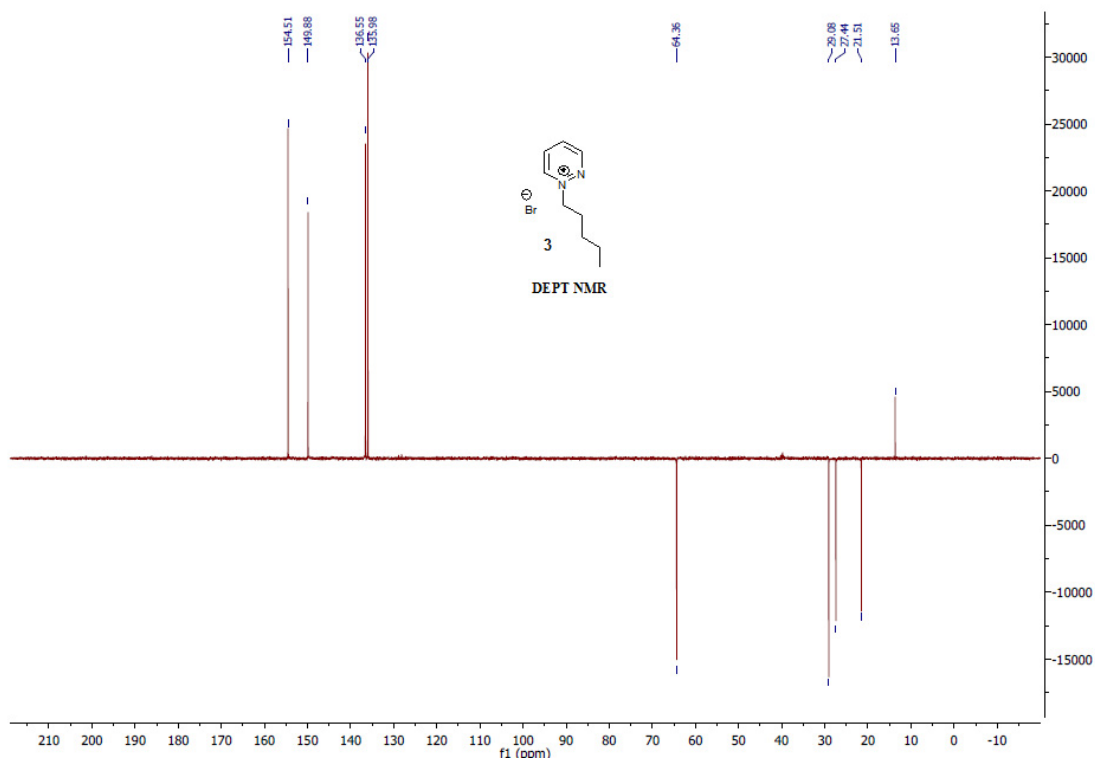
S2: IR spectrum of IL 2



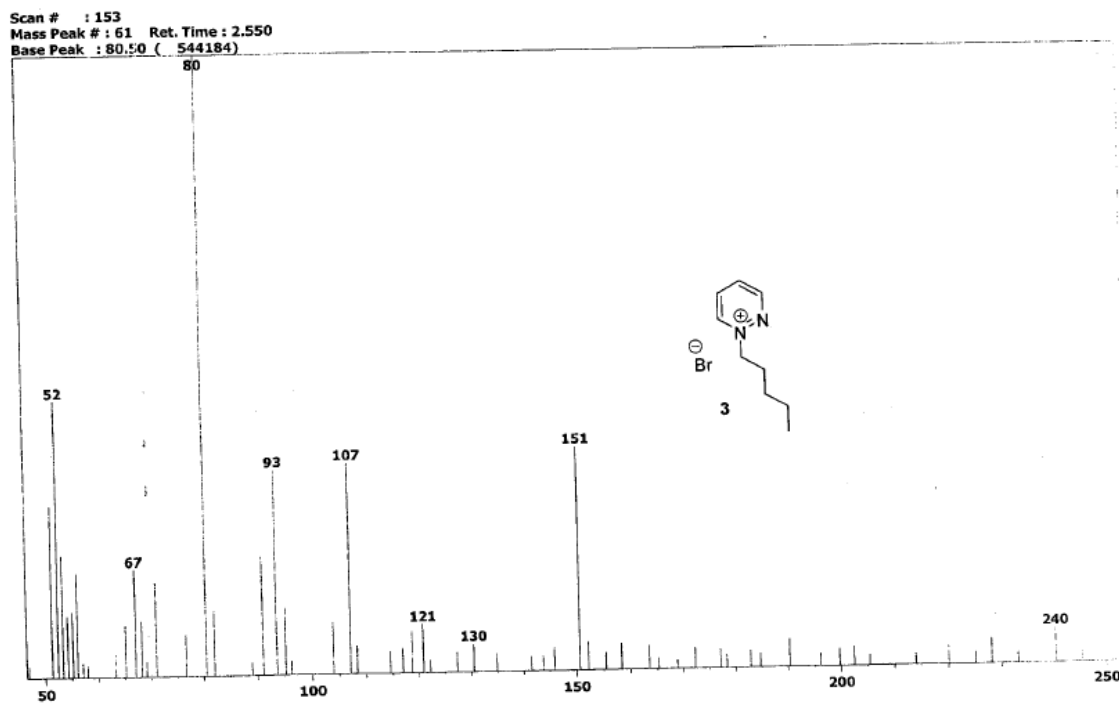
S3: ¹H NMR spectrum of IL 3 in DMSO (400 MHz)



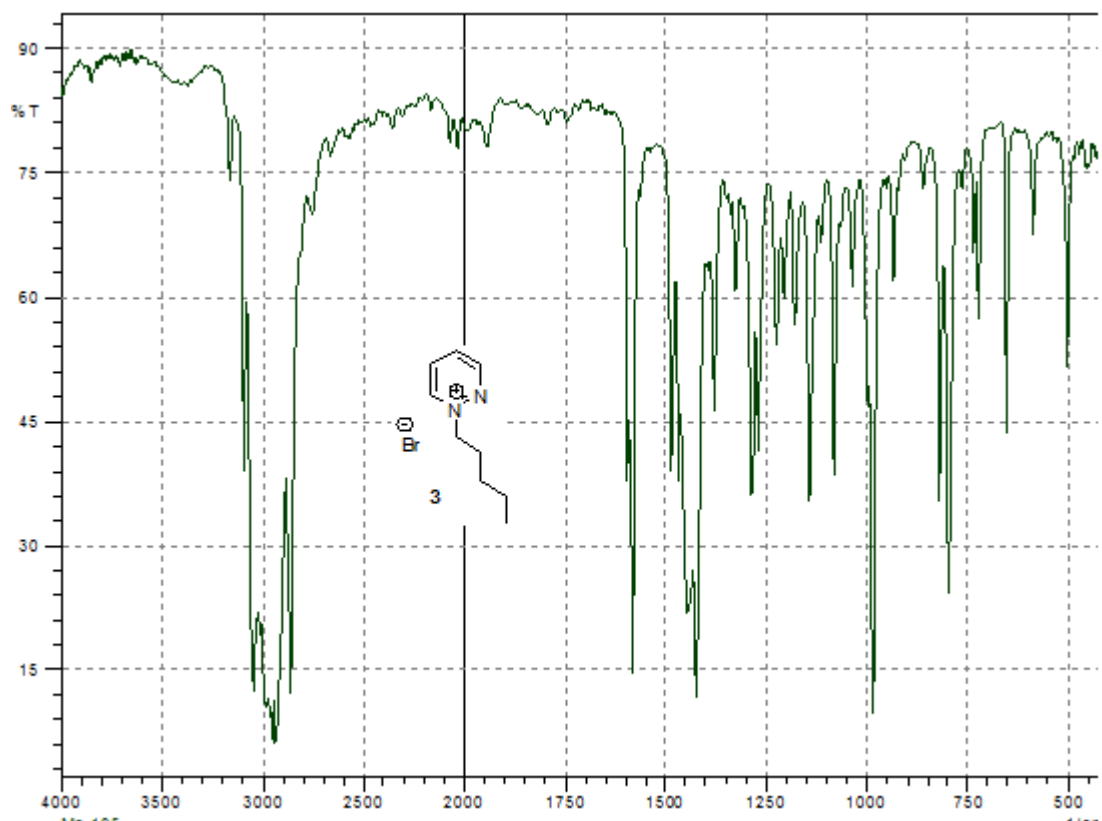
S3: ¹³C NMR spectrum of IL 3 in DMSO (100 MHz)



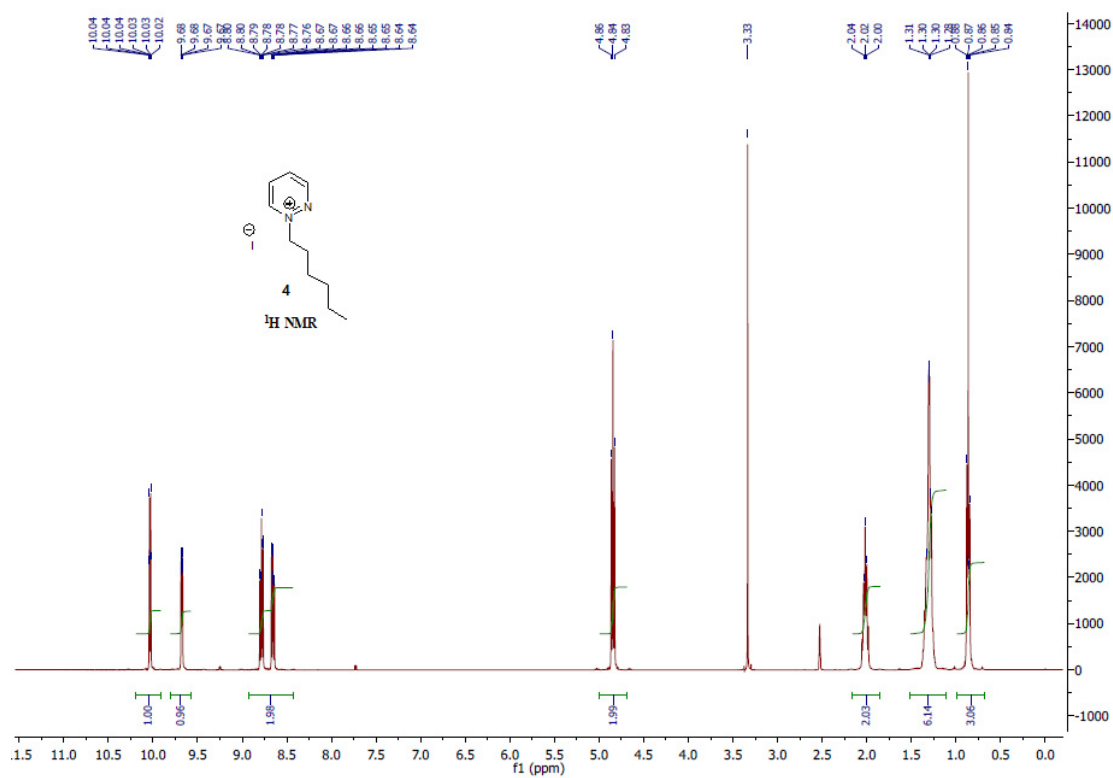
S3: DEPT- 135 NMR spectrum of IL 3 in DMSO (100 MHz)



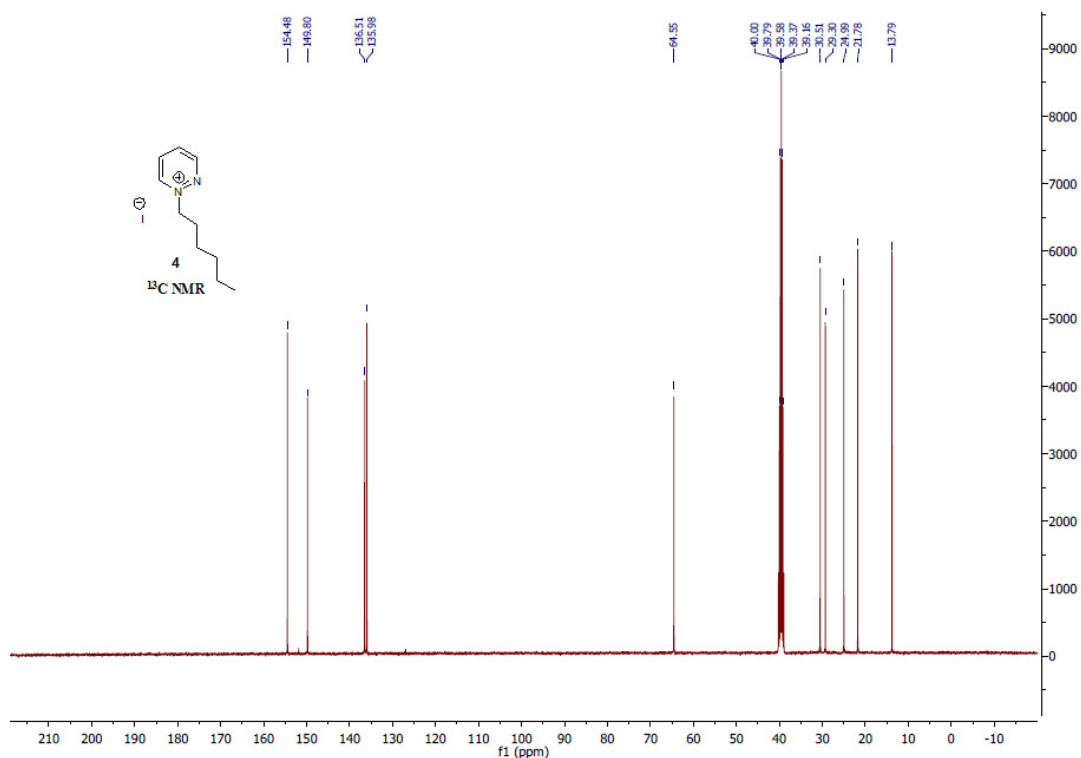
S3: (LCMS) Mass spectrum of IL 3



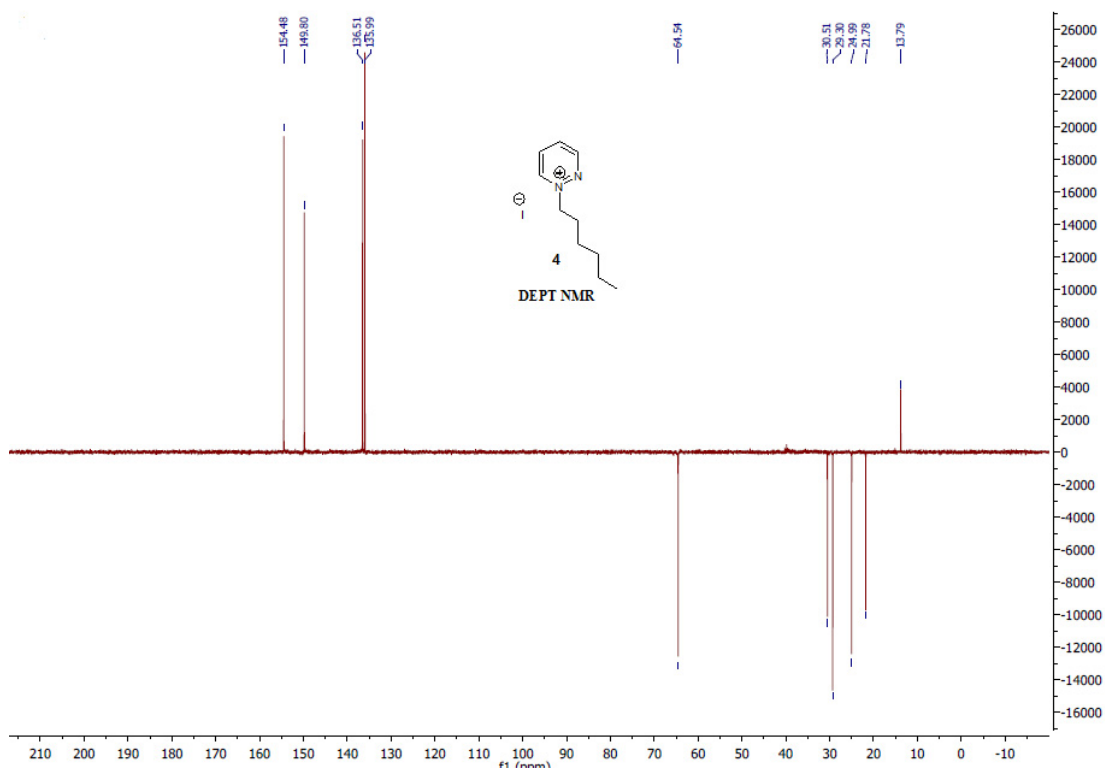
S3: IR spectrum of IL 3



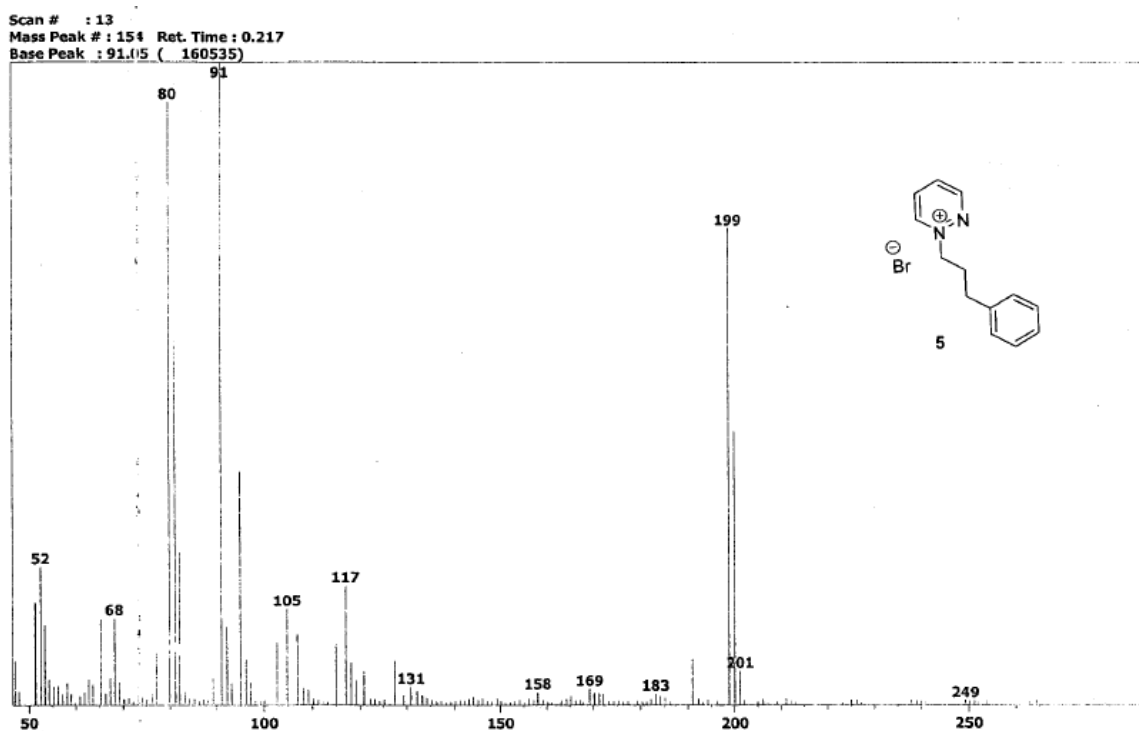
S4: ¹H NMR spectrum of IL 4 in DMSO (400 MHz)



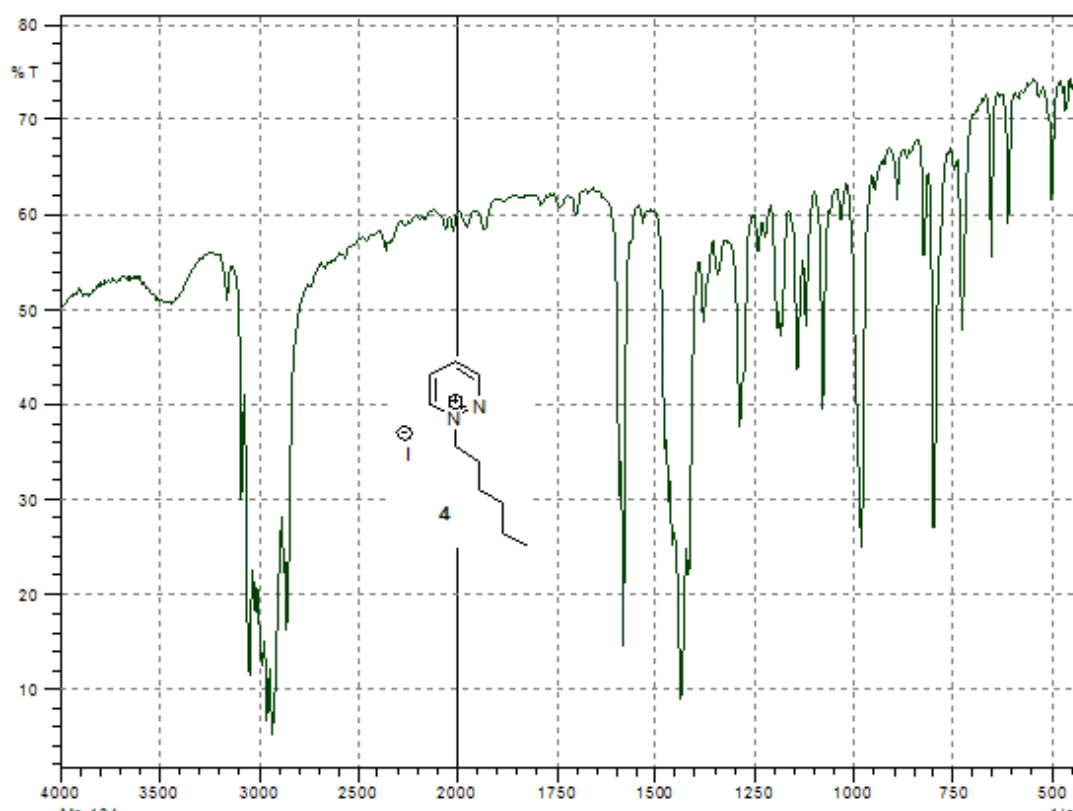
S4: ¹³C NMR spectrum of IL 4 in DMSO (100 MHz)



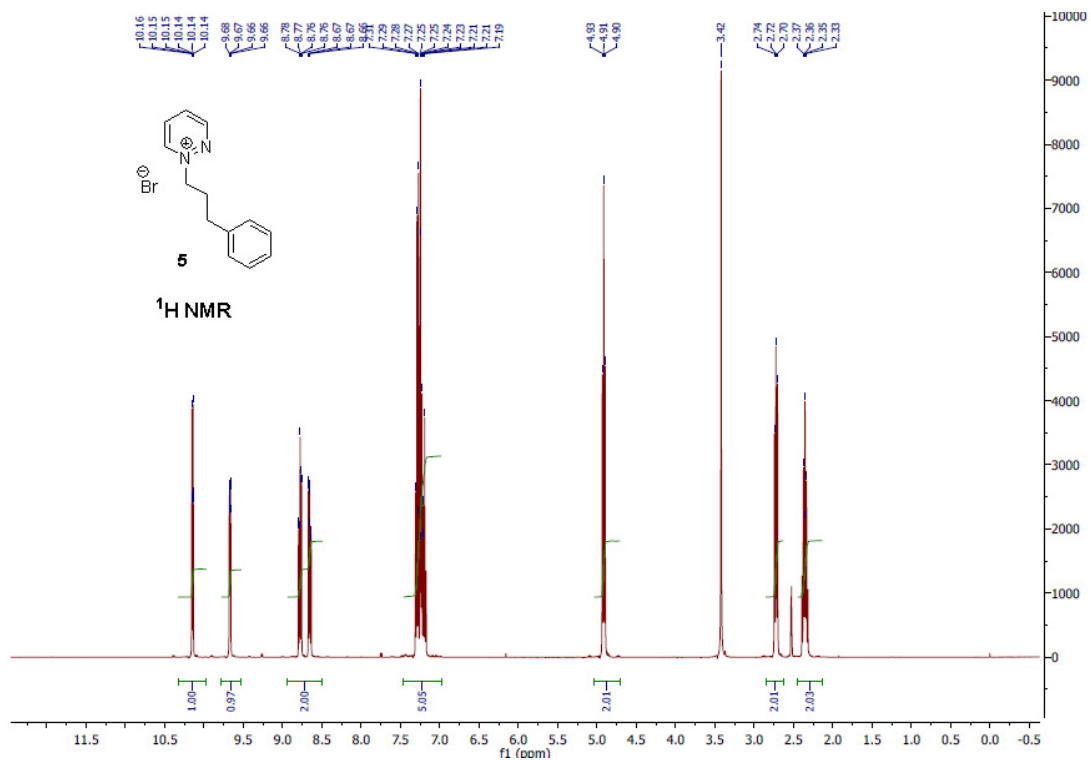
S4: DEPT- 135 NMR spectrum of IL 4 in DMSO (100 MHz)



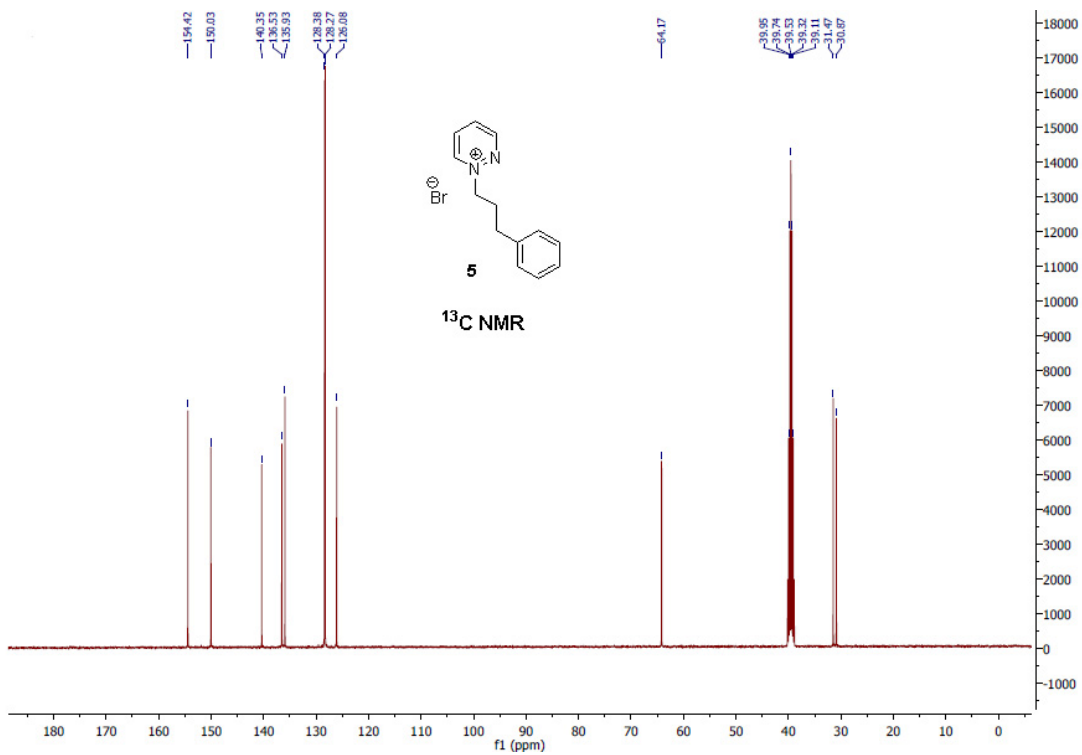
S4: (LCMS) Mass spectrum of IL 4



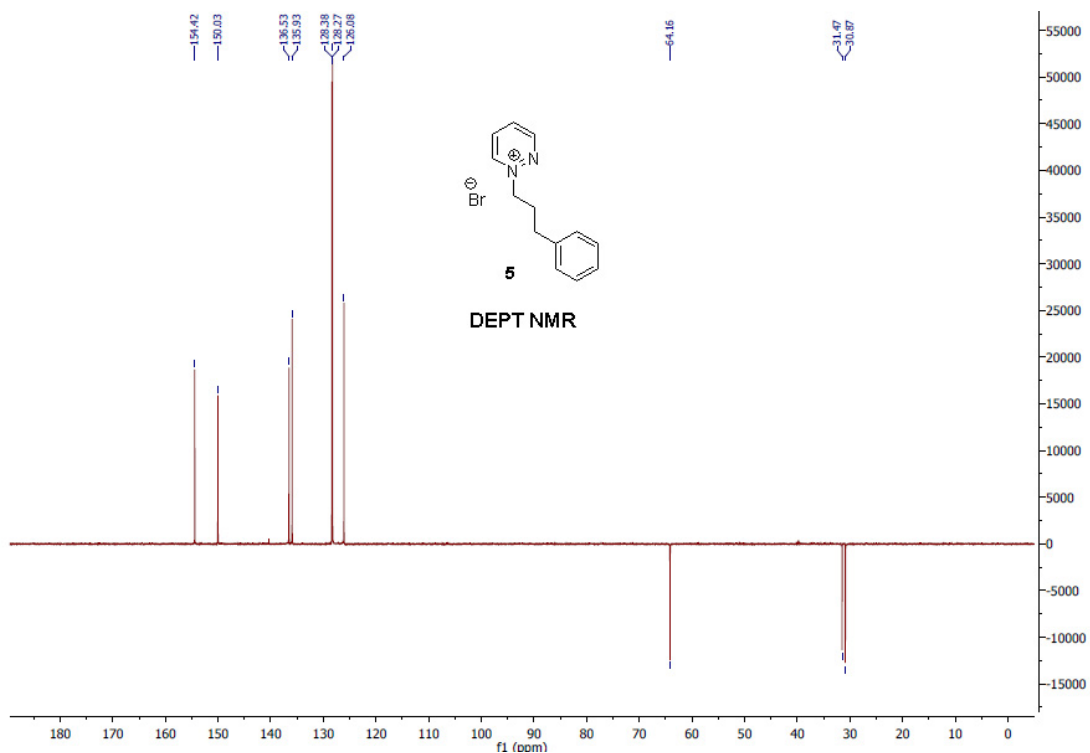
S4: IR spectrum of IL 4



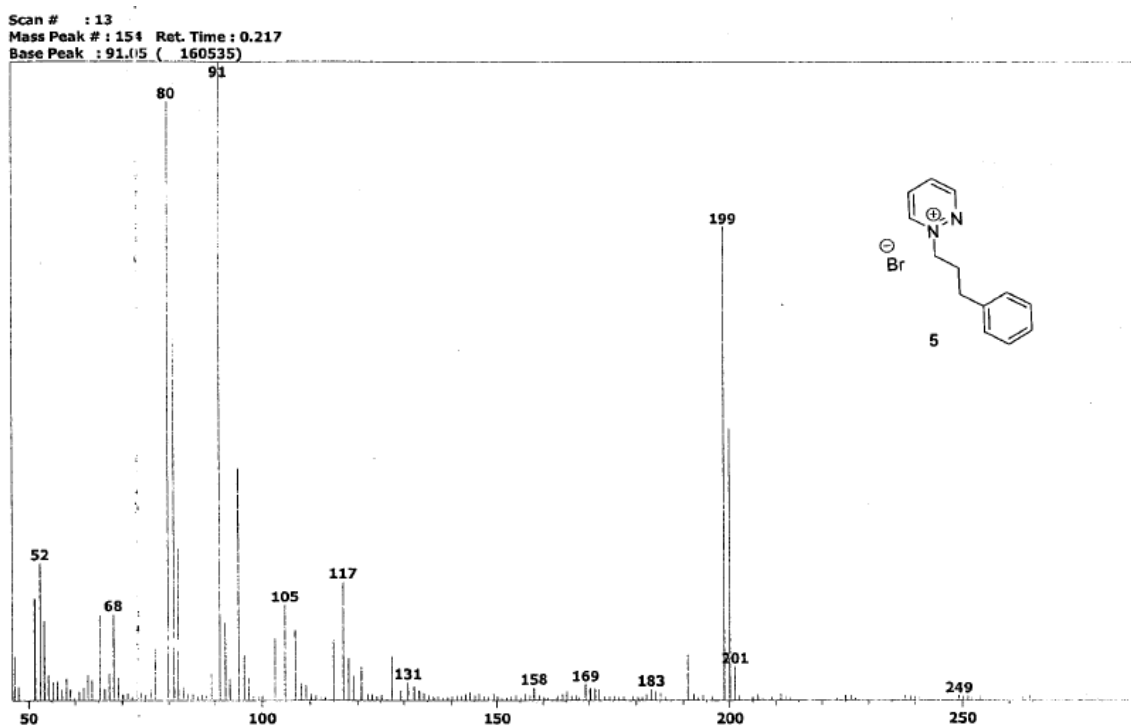
S5: ¹H NMR spectrum of IL 5 in DMSO (400 MHz)



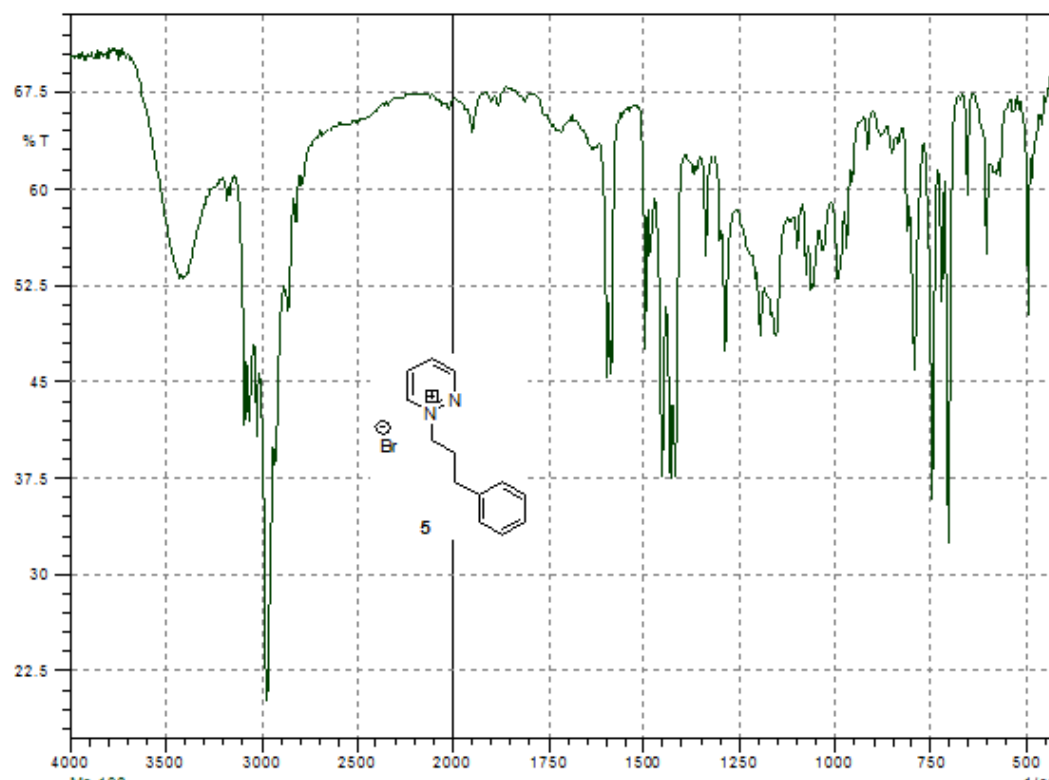
S5: ¹³C NMR spectrum of IL 5 in DMSO (100 MHz)



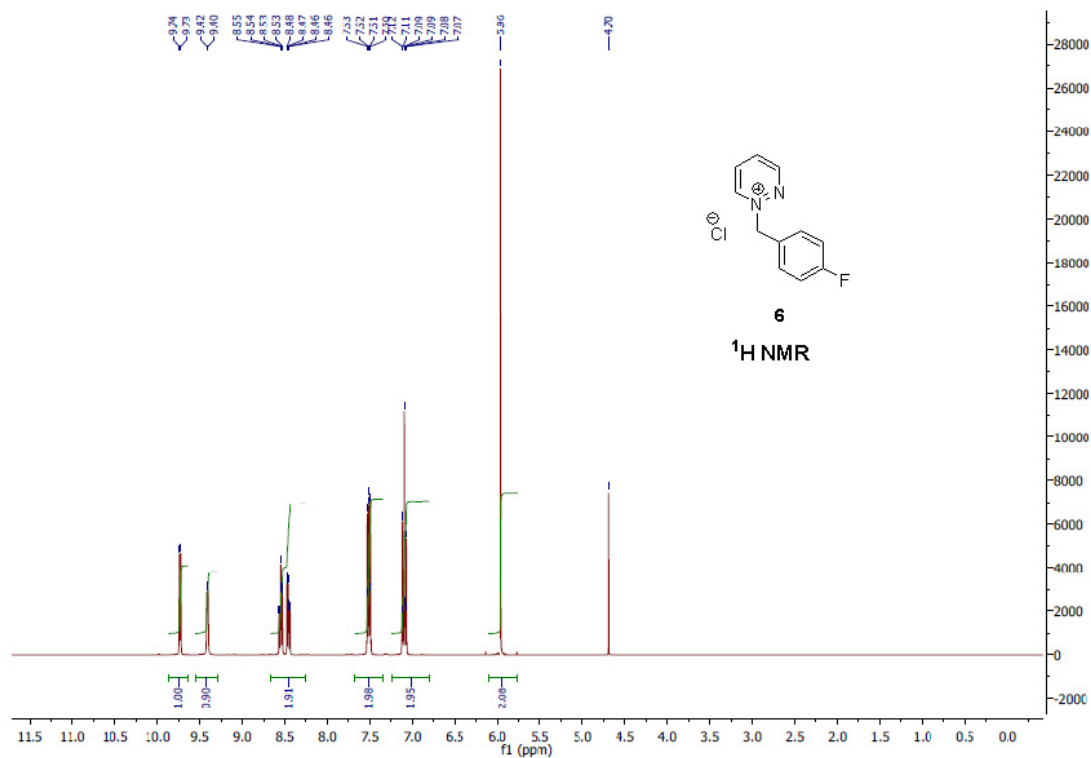
S5: DEPT- 135 NMR spectrum of IL 5 in DMSO (100 MHz)



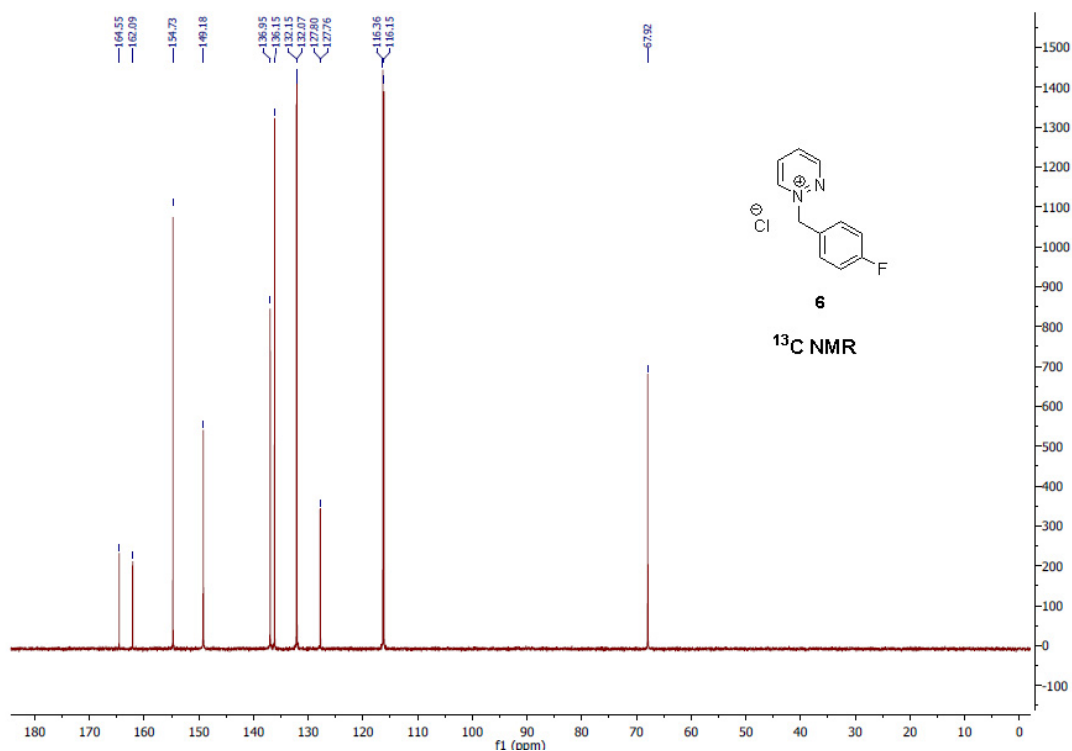
S5: (LCMS) Mass spectrum of IL 5



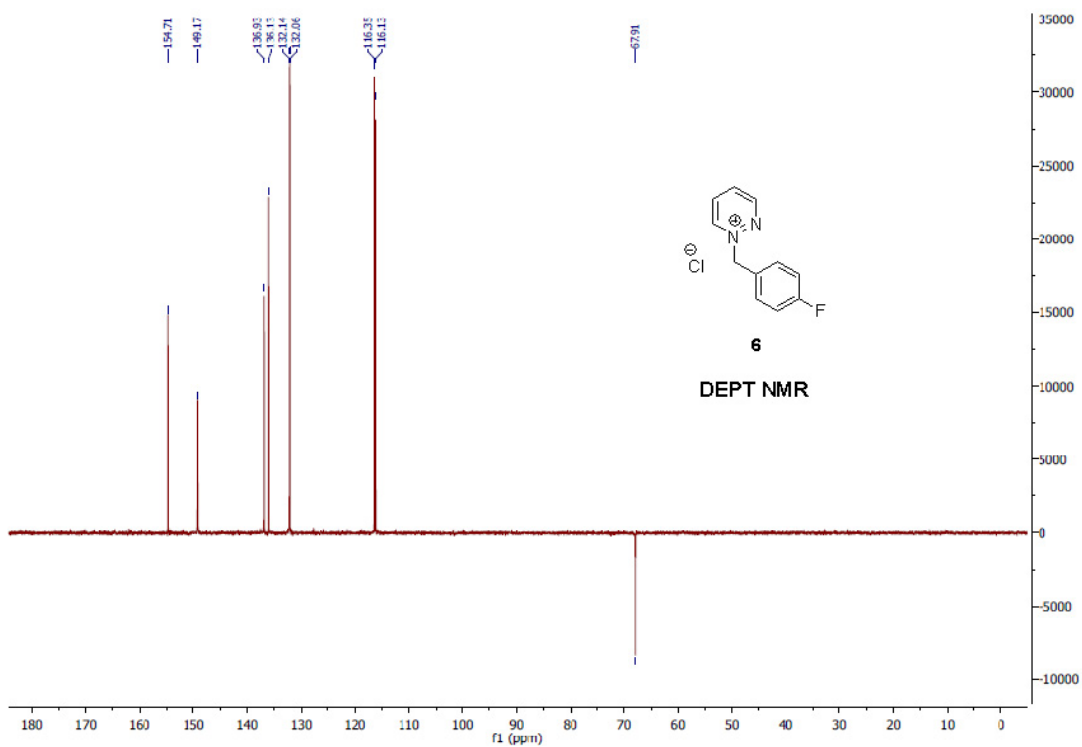
S5: IR spectrum of IL 5



S6: ¹H NMR spectrum of IL 6 in D₂O (400 MHz)

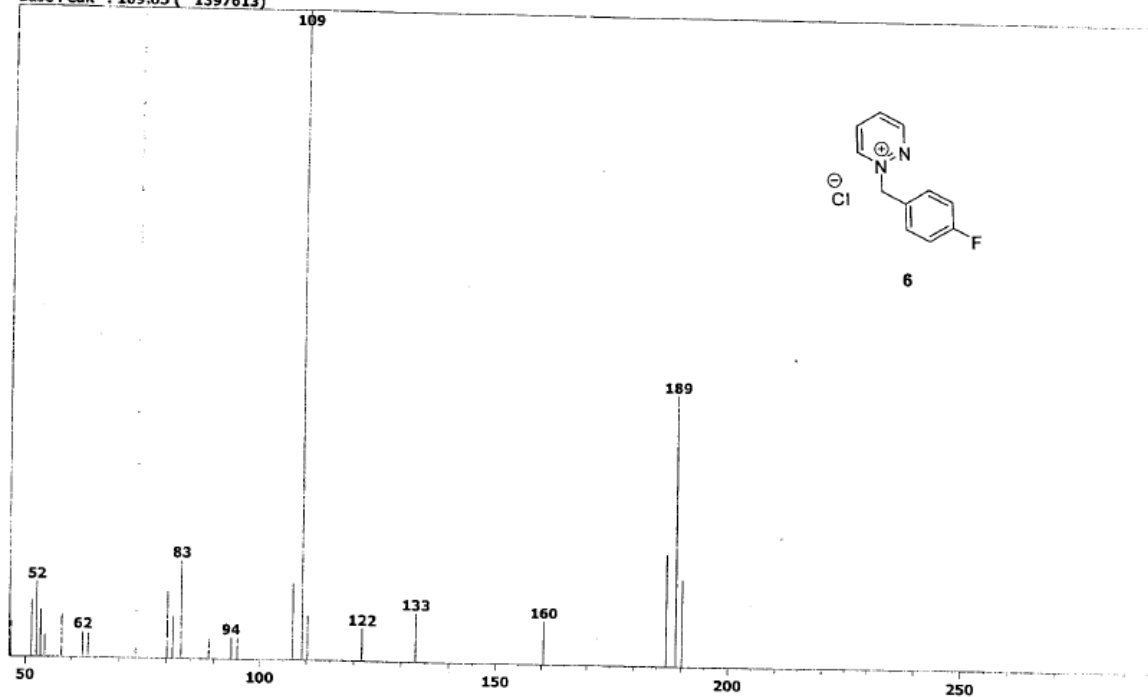


S6: ¹³C NMR spectrum of IL 6 in D₂O (100 MHz)

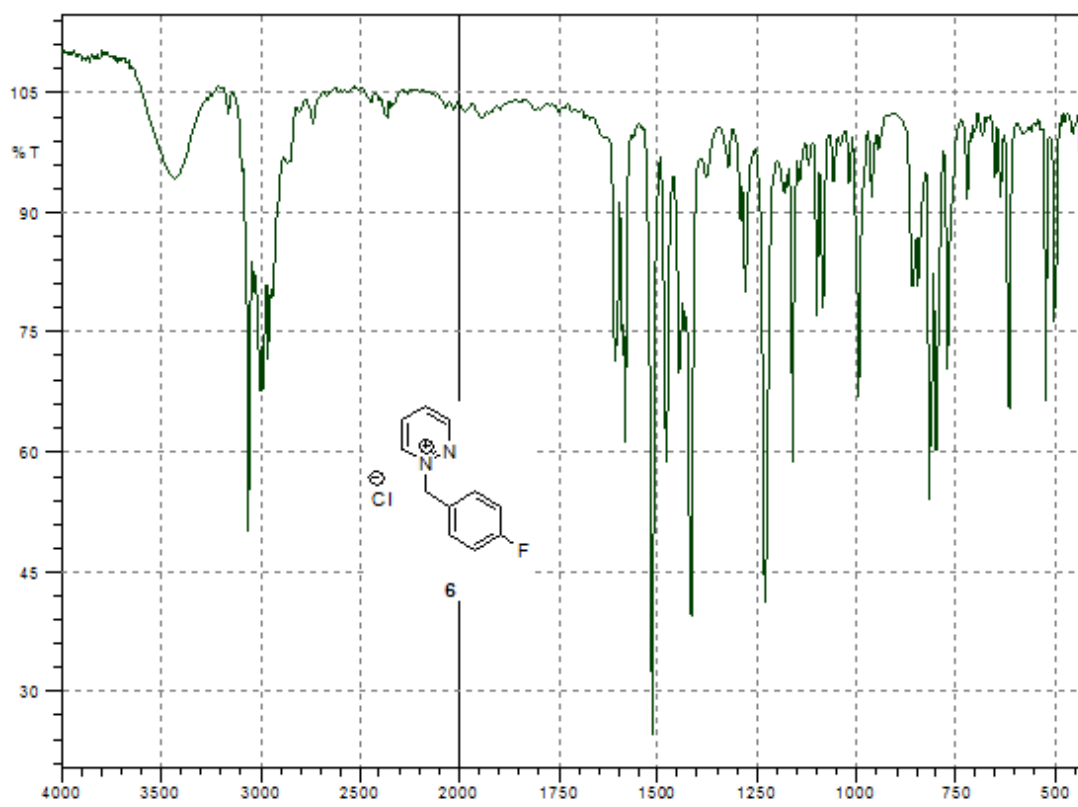


S6: DEPT- 135 NMR spectrum of IL 6 in D₂O (100 MHz)

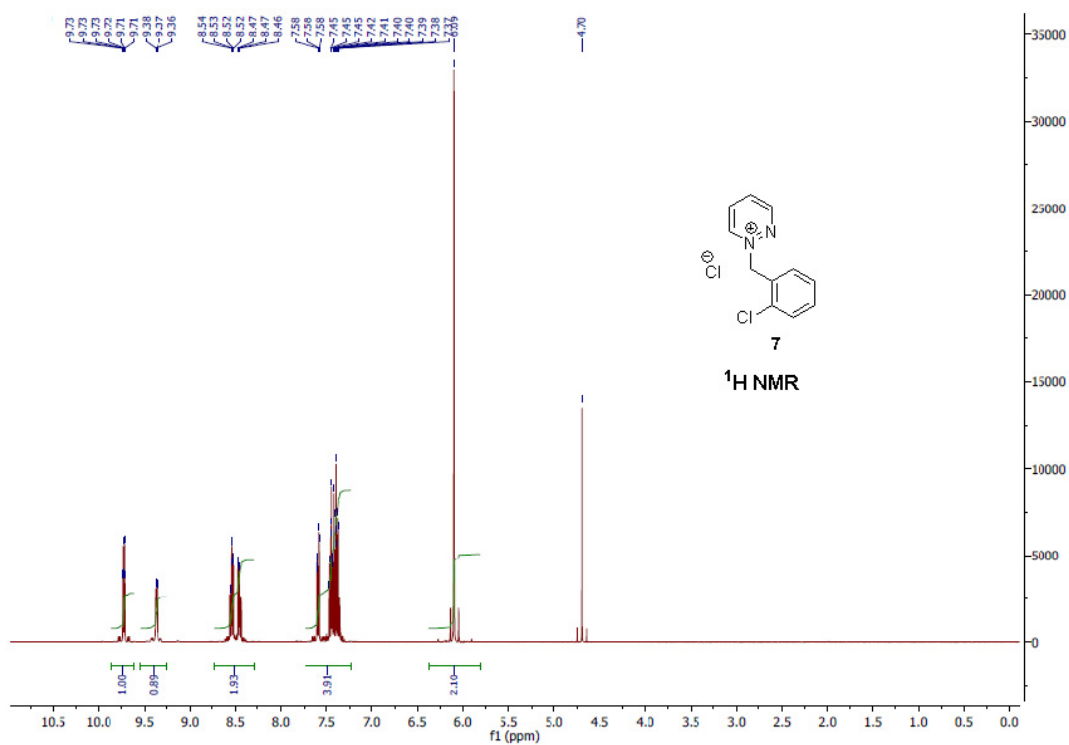
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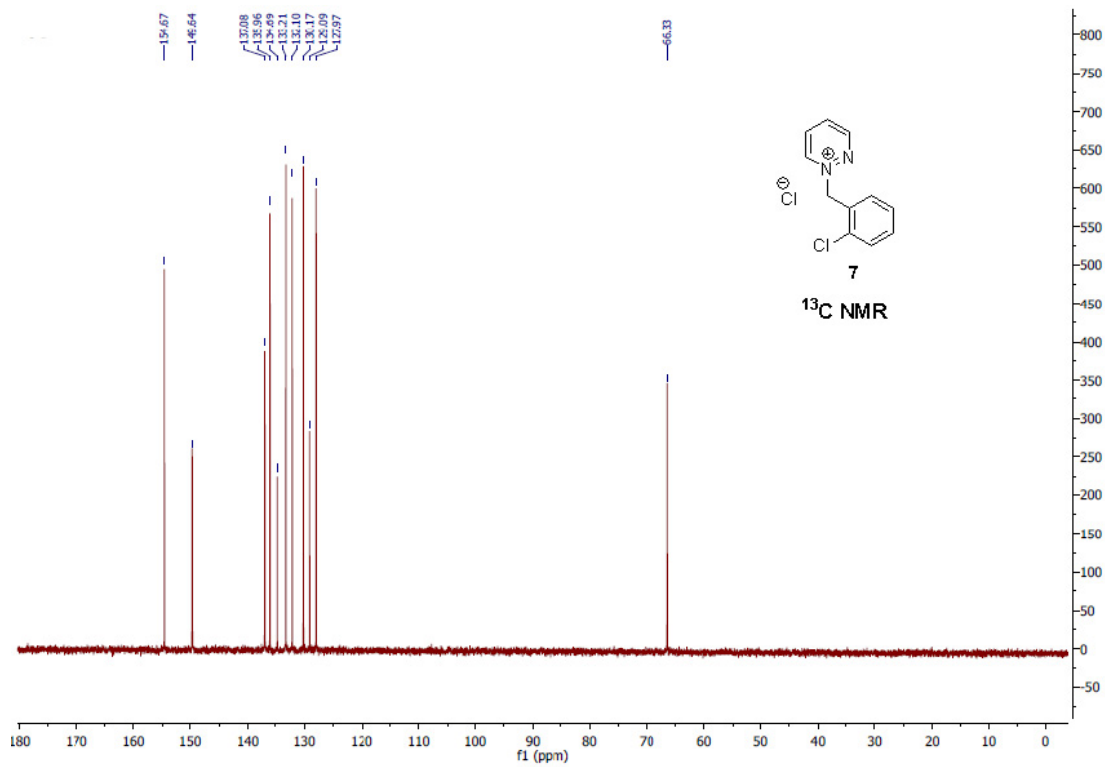
S6: (LCMS) Mass spectrum of IL 6



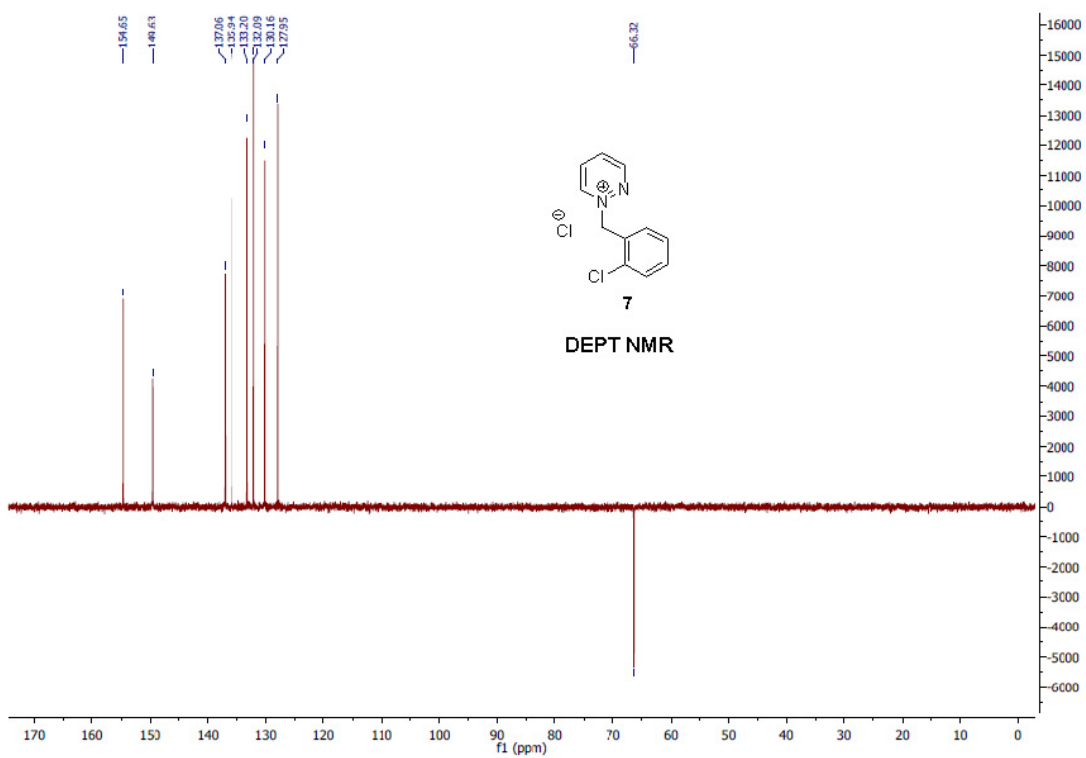
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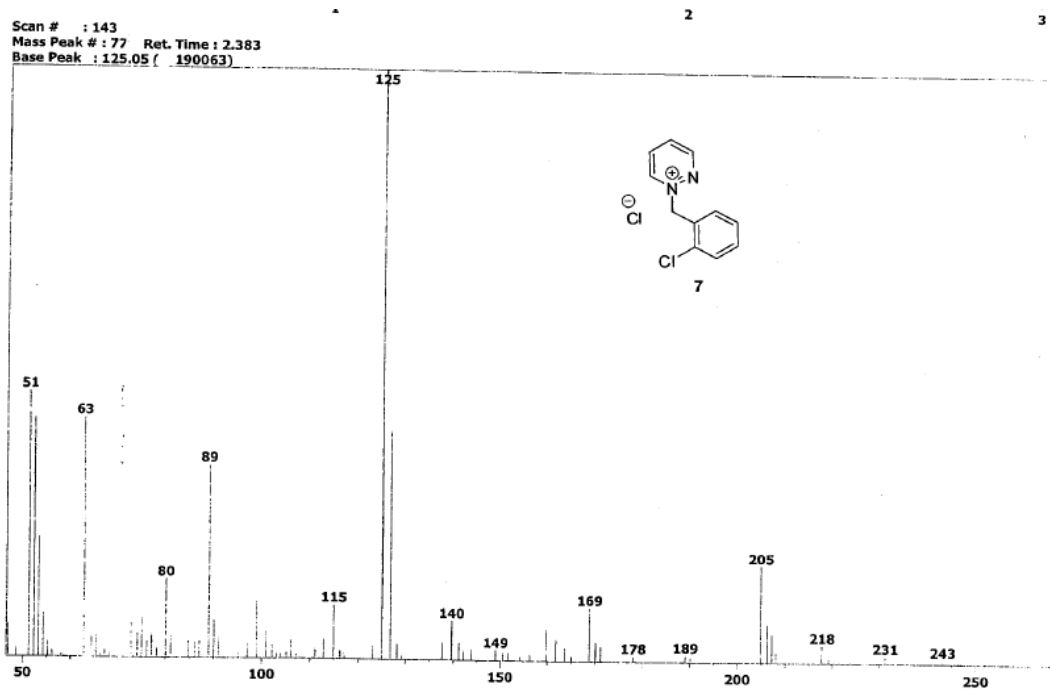
S7: ¹H NMR spectrum of IL 7 in D₂O (400 MHz)



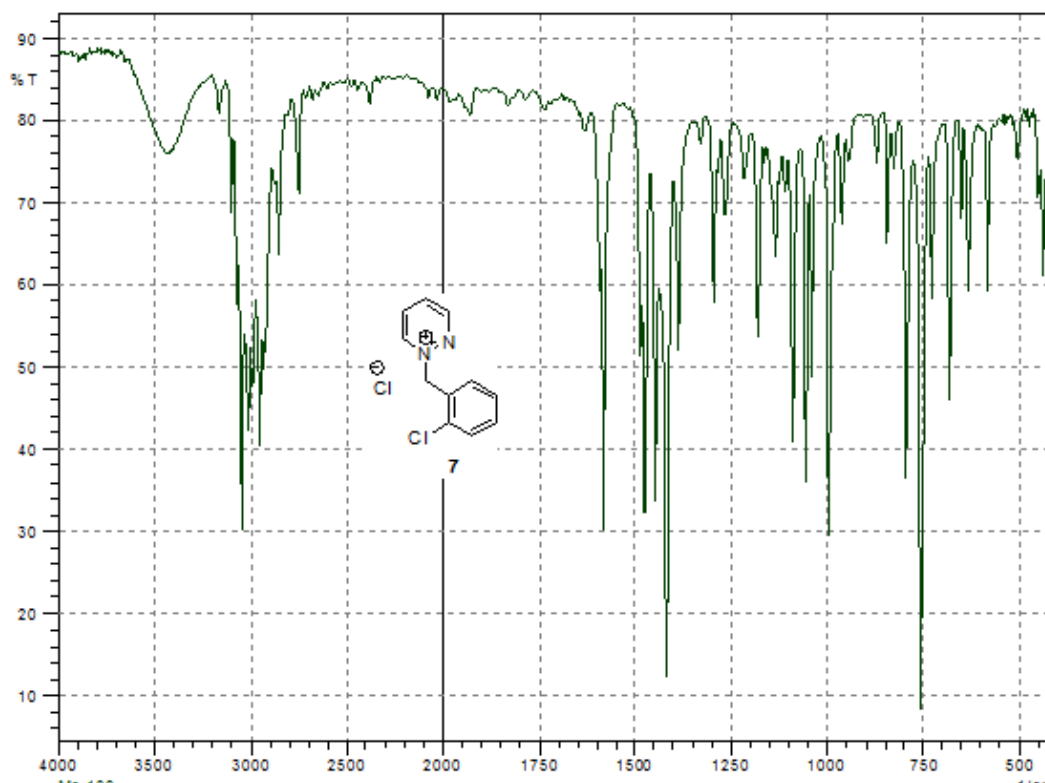
S7: ¹³C NMR spectrum of IL 7 in D₂O (100 MHz)



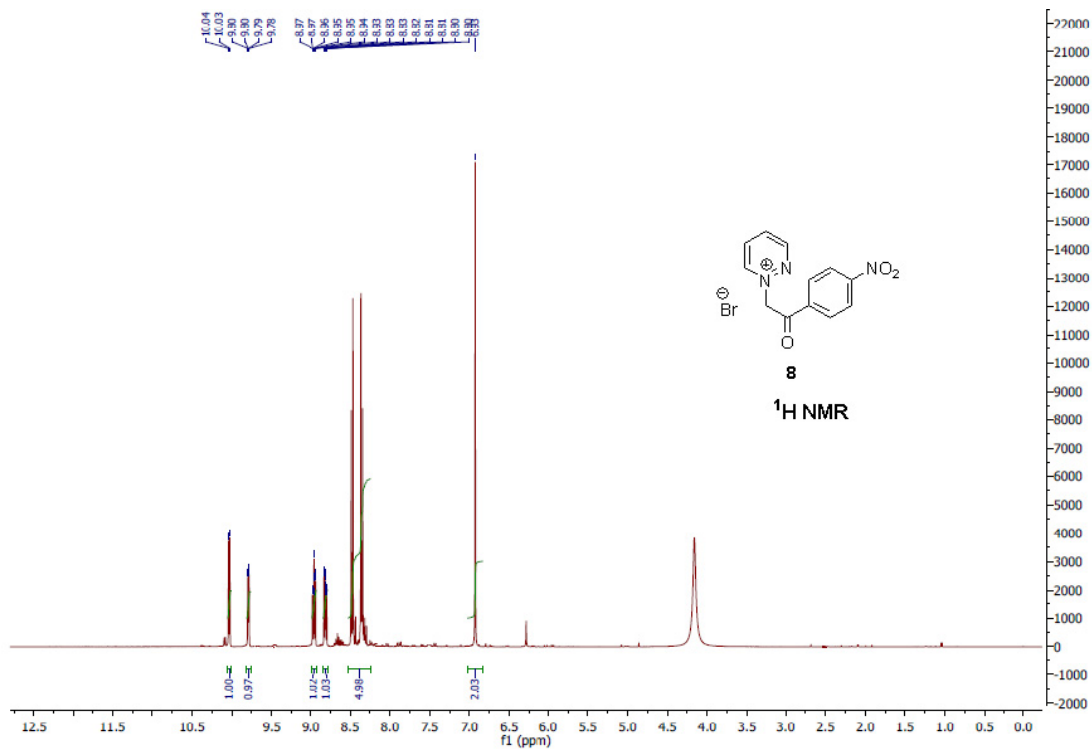
S7: DEPT- 135 NMR spectrum of IL 7 in D₂O (100 MHz)



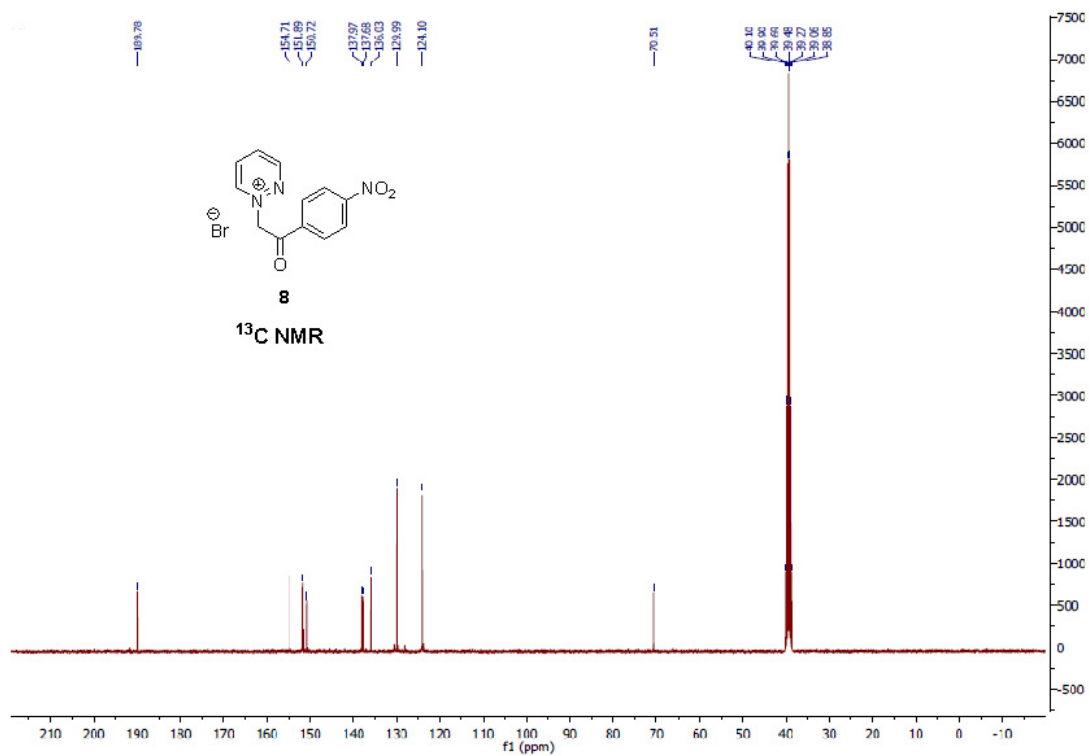
S7: (LCMS) Mass spectrum of IL 7



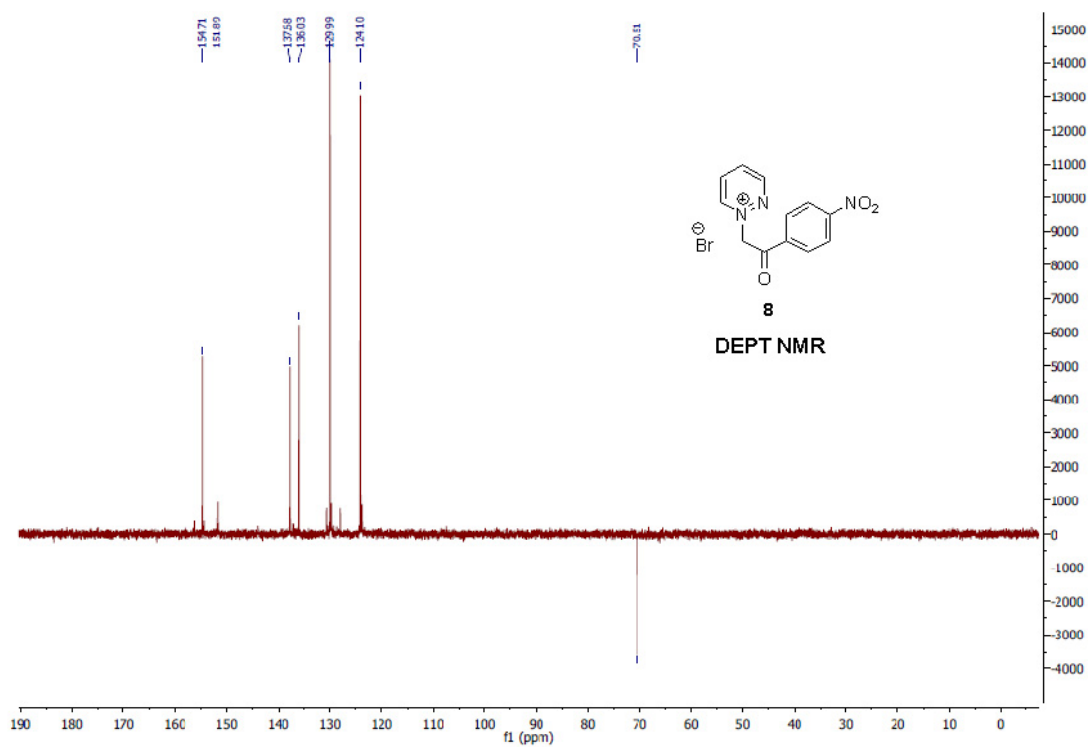
S7: IR spectrum of IL 7



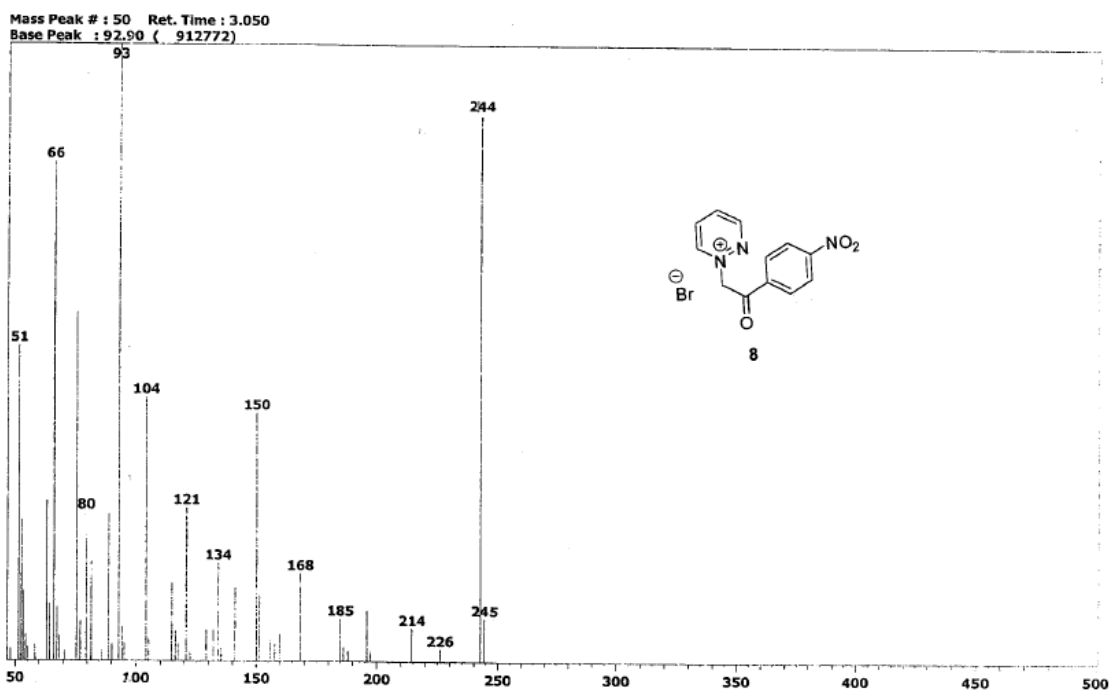
S8: ¹H NMR spectrum of IL 8 in DMSO (400 MHz)



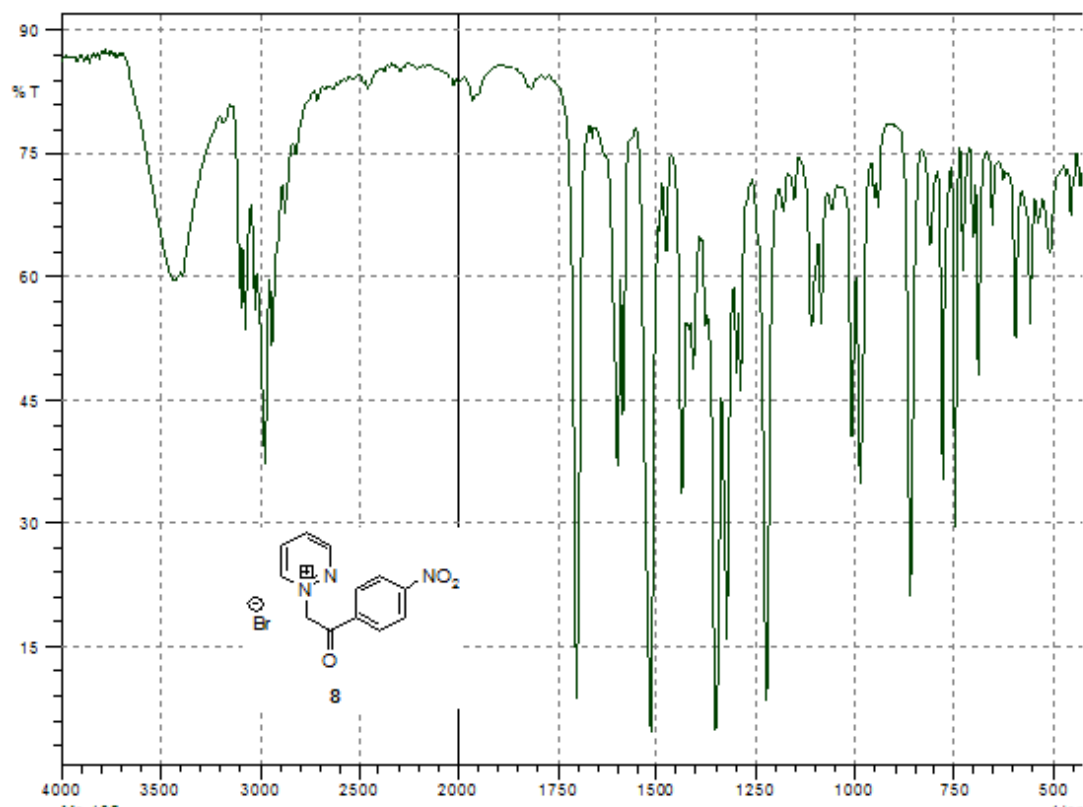
S8: ¹³C NMR spectrum of IL 8 in DMSO (100 MHz)



S8: DEPT- 135 NMR spectrum of IL 8 in DMSO (100 MHz)



S8: (LCMS) Mass spectrum of IL 8



S8: IR spectrum of IL 8