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Supplementary Materials: Bioactivity of Ionic Liquids based on Valproate in SH-SY5Y Human Neuroblastoma Cell Line

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1. Detailed Synthetic Procedures and Analytical/Spectral Data for the Prepared Compounds

General remarks: Commercially available reagents were purchased from Aldrich (Saint Louis, MO, USA), BDH – laboratory reagents (Poole, UK) and Solchemar and were used as received. The solvents were from Valente & Ribeiro and distilled before used. The basic anion-exchange resin Amberlite IRA-400-OH (ion-exchange capacity 1.4 Eq/mL) was purchased from Supelco (Bellefonte, PA, USA). ^{1}H and ^{13}C NMR spectra in $(CD_3)_2SO$ or CD_3OD (from Euriso-Top, Saint Aubin, France) were recorded on a Bruker AMX400 spectrometer (Bruker, Karlsruhe, Germany) at room temperature unless specified otherwise. Chemical shifts are reported downfield in parts per million (ppm). IR spectra were measured on a Perkin Elmer 683 (Waltham, MA, USA).

General Procedure for the Synthesis of the Compounds

Preparation of (2-Hydroxyethyl)trimethylammonium 2-propylpentanoate [Ch][VPA]

Choline chloride (0.2778 g; 1.99 mmol) was dissolved in methanol and passed through ion-exchange column Amberlite IRA-400-OH (5 eq., flow rate 0.133 mL/mL/min = 8 BV/h). Then the choline hydroxide solution was slowly added to valproic acid (0.3181 g; 1.99 mmol; 1.10 eq.) dissolved in methanol. The mixture was stirred at room temperature for 1 h. The solvent and the valproic acid excess were then evaporated and then dried in vacuum for 24 h to provide desired product as liquid (0.4834 g; 98.2%). ^{1}H NMR (400.13 MHz, $CDCl_3$) δ = 0.84 (t, J = 7.0 Hz 6H), 1.24-1.53 (m, 8H), 2.12-2.17 (m, 1H), 3.33 (s, 9H), 3.67 (t, J = 4.6 Hz, 2H), 4.00 (m, 2H) ppm; ^{13}C NMR (100.62 MHz, $CDCl_3$) δ = 14.42, 21.14, 35.54, 47.78, 54.81, 56.25, 68.60, 182.18 ppm; IR (KBr): ν = 3410, 2958, 2933, 2873, 2078, 1638, 1552, 1488, 1476, 1468, 1411, 1381, 1356, 1311, 1280, 1222, 1170, 1133, 1110, 1084, 1052, 1007, 956, 925, 864, 751, 668, 618, 558, cm^{-1}. Anal. calcd for $C_{13}H_{29}NO_3$ 2 H_2O C 56.90, H 11.75, N 5.10, found: C 56.98, H 11.62, N 5.52.

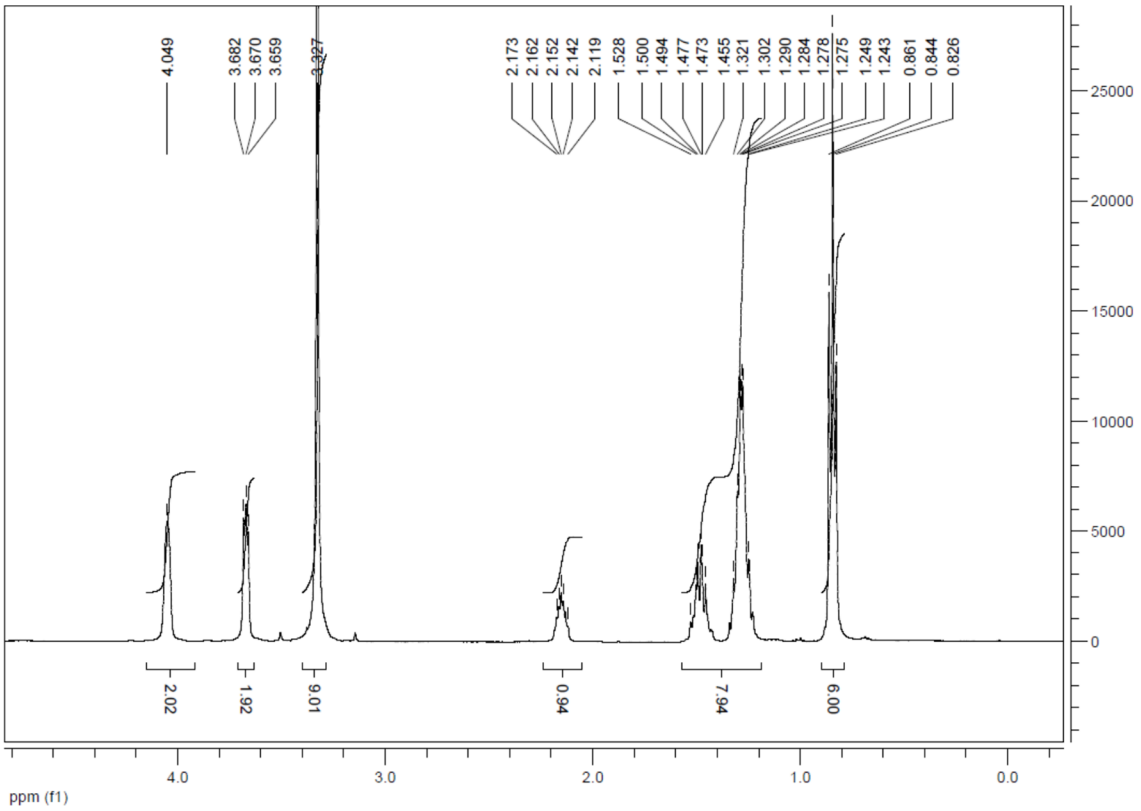

Figure S1. [Ch][VPA] ^{1}H NMR spectrum in $CDCl_3$.

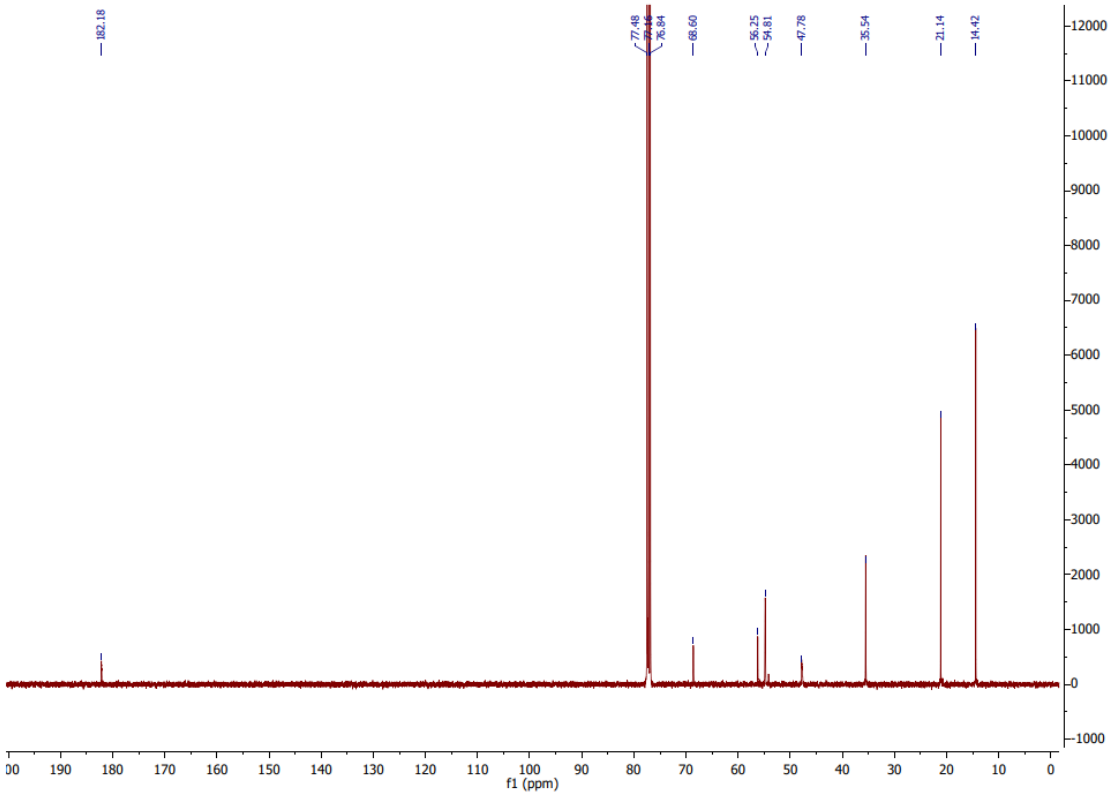

Figure S2. [Ch][VPA] ^{13}C NMR spectrum in $CDCl_3$.

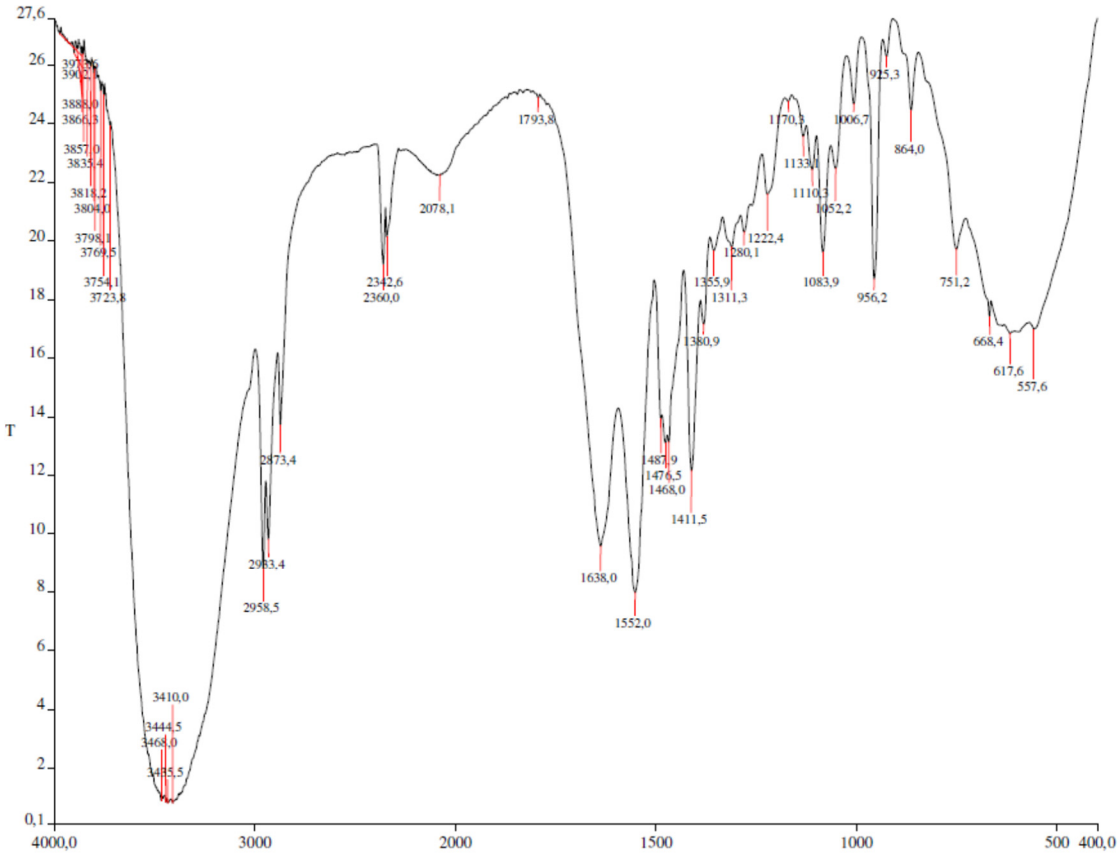

Figure S3. [Ch][VPA] IR spectrum in KBr.

Preparation of 1-hexadecylpyridinium 2-propylpentanoate [C_{16}Pyr][VPA]

Cetylpyridinium chloride (0.717 g; 2.003 mmol), was dissolved in methanol and passed through ion-exchange column Amberlite IRA-400-OH (5 eq., flux rate 0.133 mL/mL/min = 8 BV). Then the cetylpyridinium hydroxide solution was slowly added to valproic acid (0.3039 g; 2.107 mmol; 1.05 eq.) dissolved in methanol. The mixture was stirred at room temperature for 1 h. The solvent and the valproic acid excess were then evaporated and then dried in vacuum for 24 h to provide desired product as liquid (0.8835 g; 98.5%). ^{1}H NMR (400.13 MHz, CD_3OD) δ = 0.87-0.93 (m, 9H), 1.29-1.39 (m, 30H), 1.48-1.64 (m, 2H), 2.04 (q, J=7.3Hz), 2.29-2.21 (m, 1H), 4.67-4.63 (m, 2H), 8.13 (t, J=7.0Hz, 2H), 8.61 (tt, J=7.8Hz, 1.4Hz, 1H), 9.04-9.02 (m, 2H).; ^{13}C NMR (100.62 MHz, CD_3OD) δ = 14.50, 14.55, 21.98, 23.79, 27.24, 30.16, 30.52, 30.54, 30.66, 30.76, 30.81, 32.55, 33.13, 36.50, 63.17, 118.04, 129.56, 145.91, 145.96, 146.91, 182.56 ppm; IR (KBr): ν = 3460, 3413, 2957, 2924, 2854, 2730, 2680, 1702, 1640, 1578, 1502, 1488, 1466, 1406, 1378, 1356, 1306, 1276, 1249, 1209, 1176, 1109, 1064, 1040, 981, 816, 774, 746, 722, 685, 612, 550, 476 cm^{-1}. Anal. calcd for $C_{29}H_{53}NO_2$ .1.5 H_2O C 73.37, H 11.89, N 2.95, found: C 73.19, H 11.54, N 2.99.

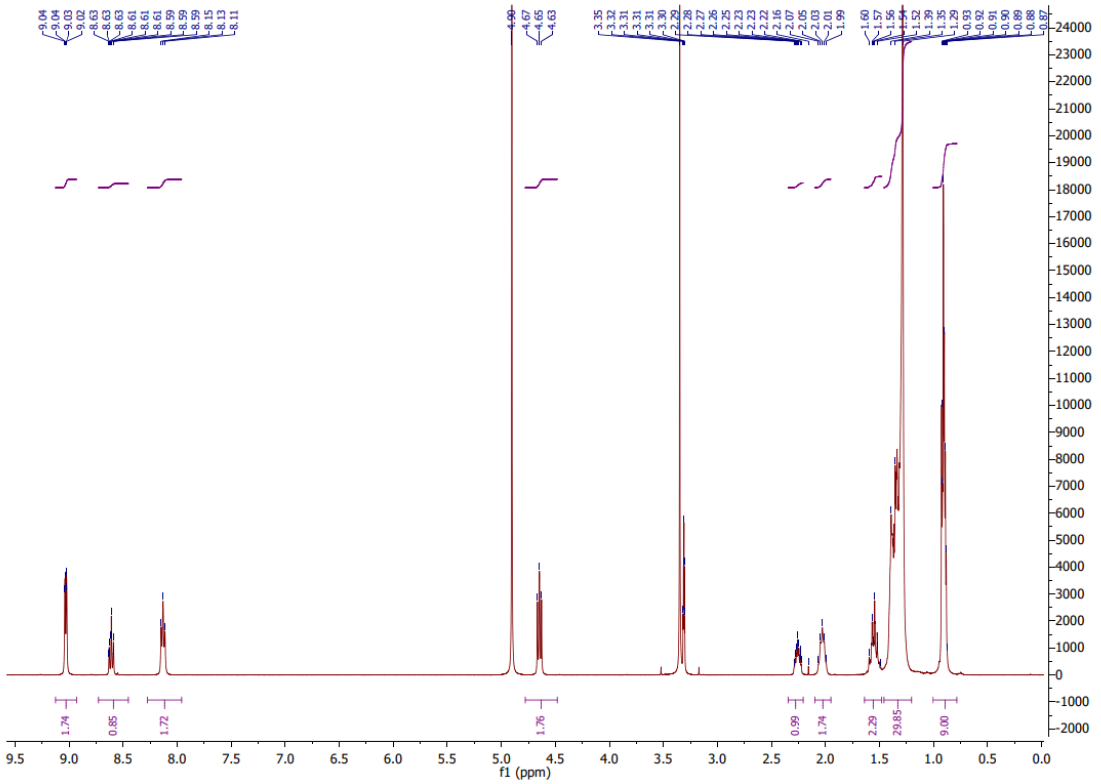

Figure S4. [C_{16}Pyr][VPA] ^{1}H NMR spectrum in CD_3OD.

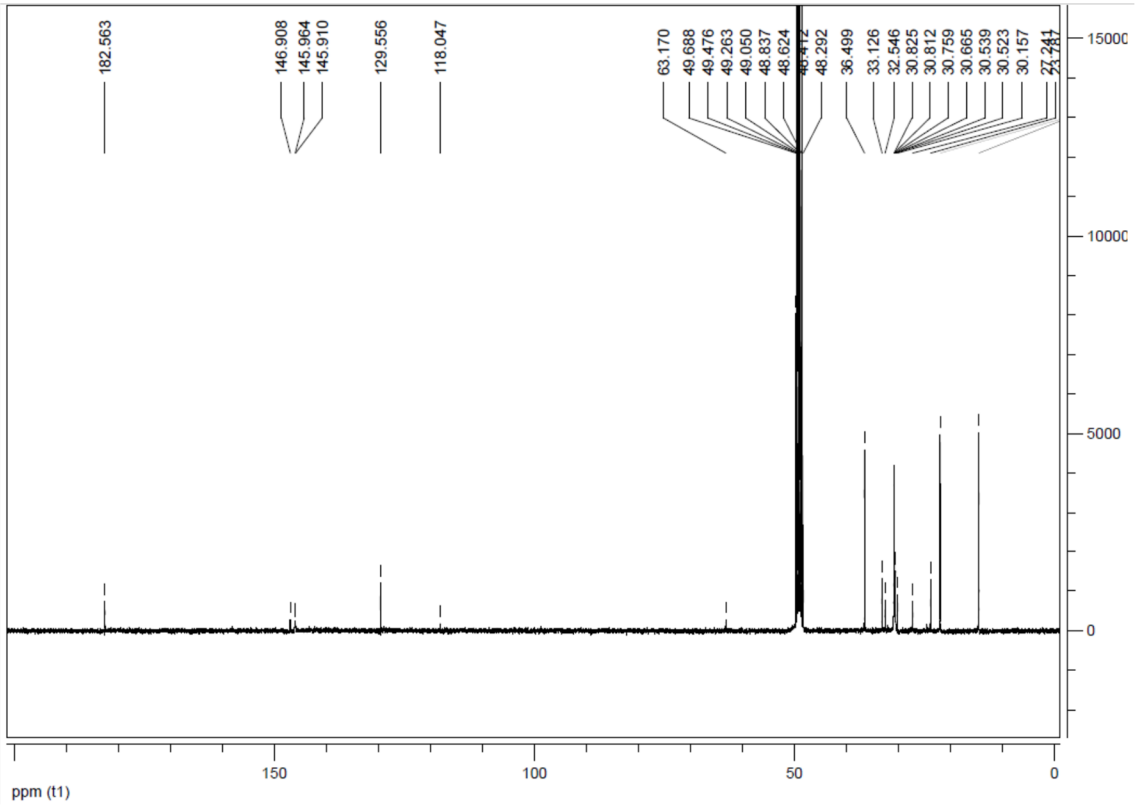

Figure S5. [C_{16}Pyr][VPA] ^{13}C NMR spectrum in CD_3OD.

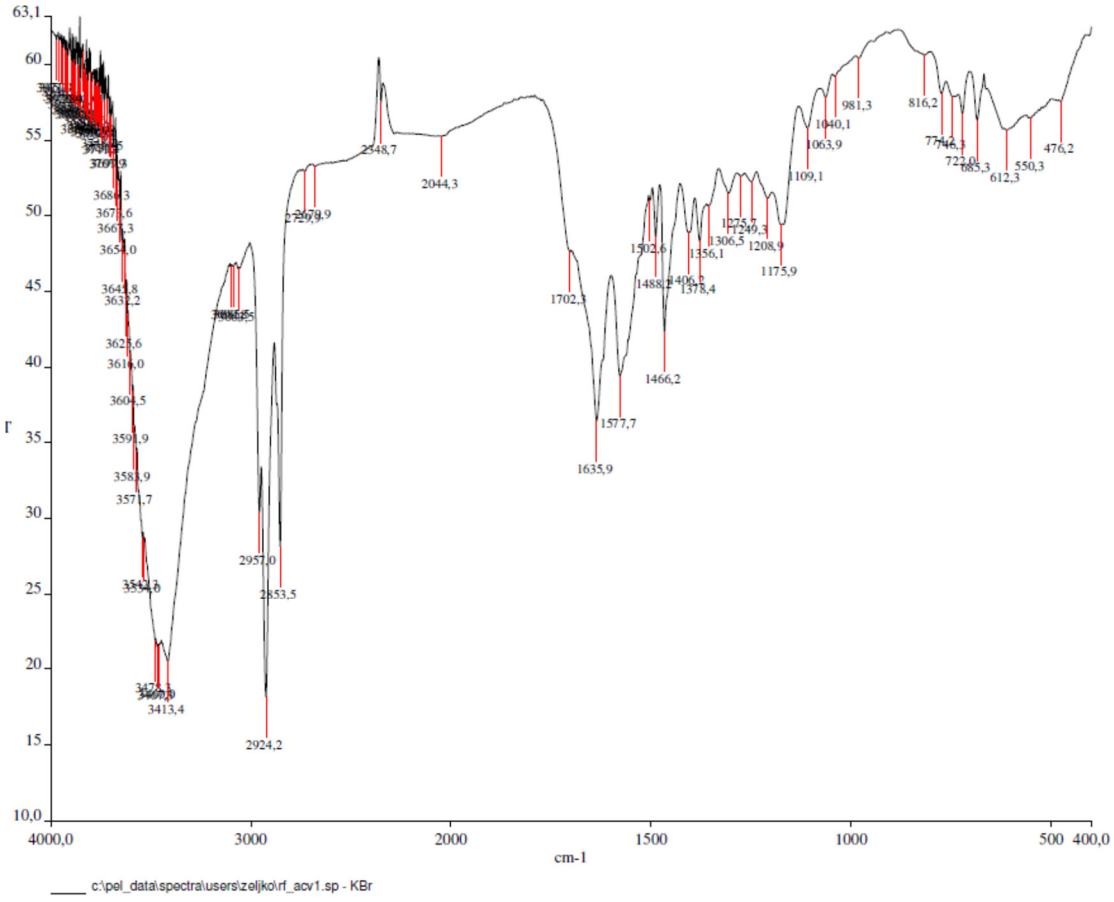

Figure S6. [C_{16}Pyr][VPA] IR spectrum in KBr.

Preparation of 1-ethyl-3-methylimidazolium 2-propylpentanoate [EMiM][VPA]

1-ethyl-3-methylimidazolium bromide (0.3839 g; 2.00 mmol) was dissolved in methanol and passed through ion-exchange column Amberlite IRA-400-OH (5 eq., flow rate 0.133 mL/mL/min = 8 BV/h). Then the 1-ethyl-3-methylimidazolium hydroxide solution was slowly added to valproic acid (0.3032 g; 2.10 mmol; 1.05 eq.) dissolved in methanol. The mixture was stirred at room temperature for 1 h. The solvent and the valproic acid excess were then evaporated and dried in vacuum for 24 h to provide desired product as liquid (0.5032 g; 98.7%). ^{1}H NMR (400.13 MHz, $CDCl_3$) δ = 0.85 (t, *J* = 6.9 Hz, 6H), 1.29-1.62 (m, 11H), 2.22-2.26 (m, 1H), 4.04 (s, 3H), 4.36 (q, *J* = 7.4 Hz, 2H), 5.44 (bs, 1H), 7.18 (s, 1H), 11.26 (s, 1H) ppm; ^{13}C NMR (100.62MHz $CDCl_3$) δ = 14.42, 15.58, 21.12, 35.61, 36.47, 45.11, 48.05, 120.85, 122.85, 139.91, 182.29; IR (KBr): ν = 3429, 3158, 3115, 2958, 2933, 2873, 2124, 1638, 1556, 1466, 1406, 1380, 1356, 1310, 1328, 1296, 1250, 1171, 1110, 1032, 960, 851, 754, 701, 669, 649, 623, 558 cm^{-1}. Anal. calcd for $C_{14}H_{26}N_2O_2$ C 66.11, H 10.30, N 11.01, found: C 65.97, H 10.53, N 10.55.

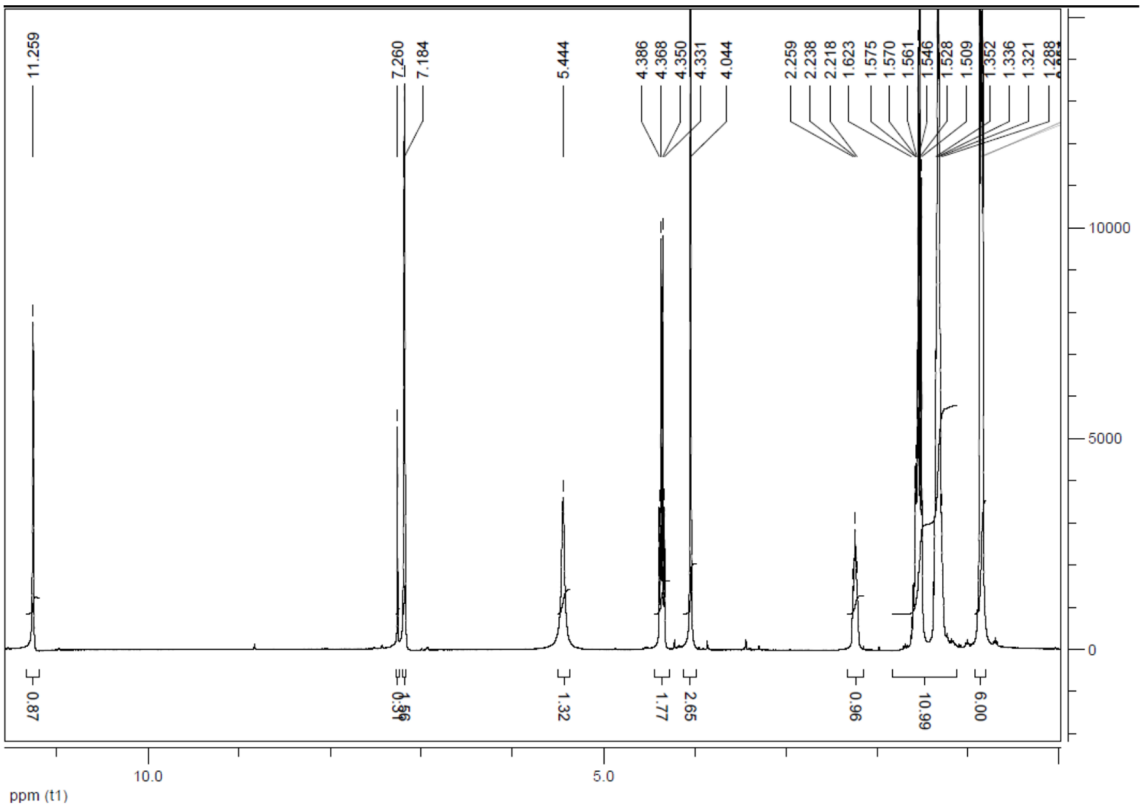

Figure S7. [EMiM][VPA] ^{1}H NMR spectrum in $CDCl_3$.

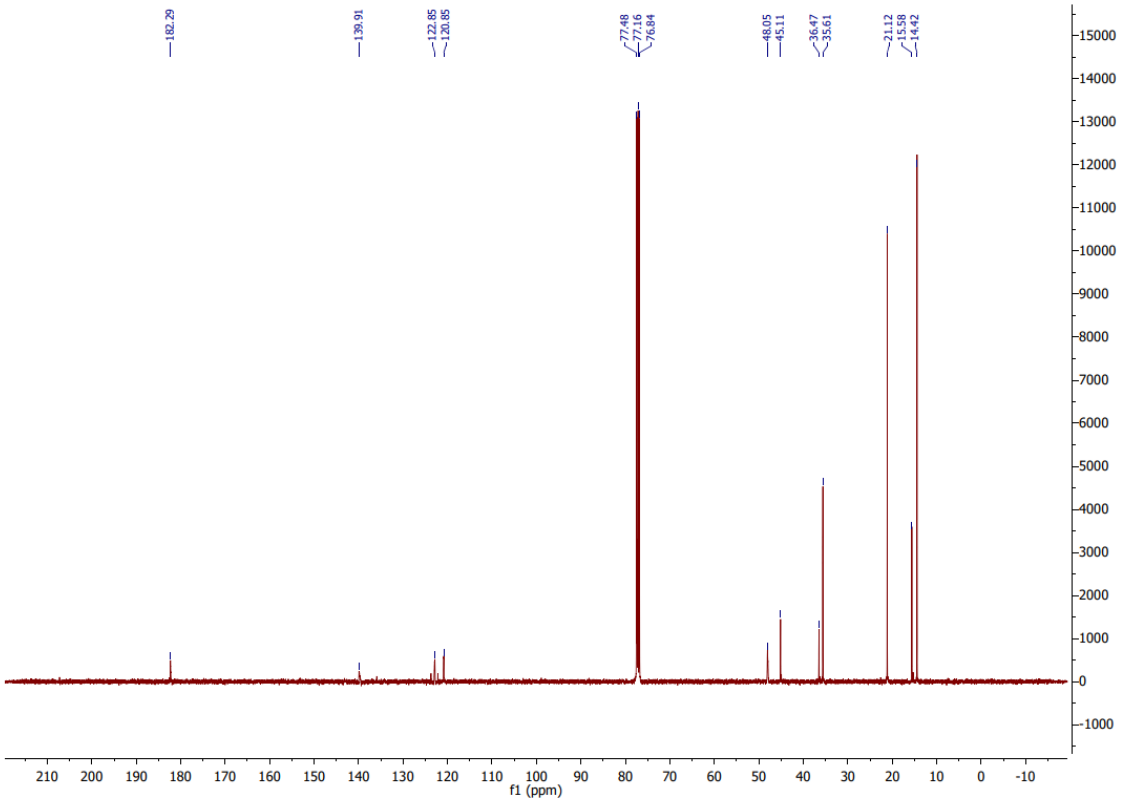

Figure S8. [EMiM][VPA] ^{13}C NMR spectrum in $CDCl_3$.

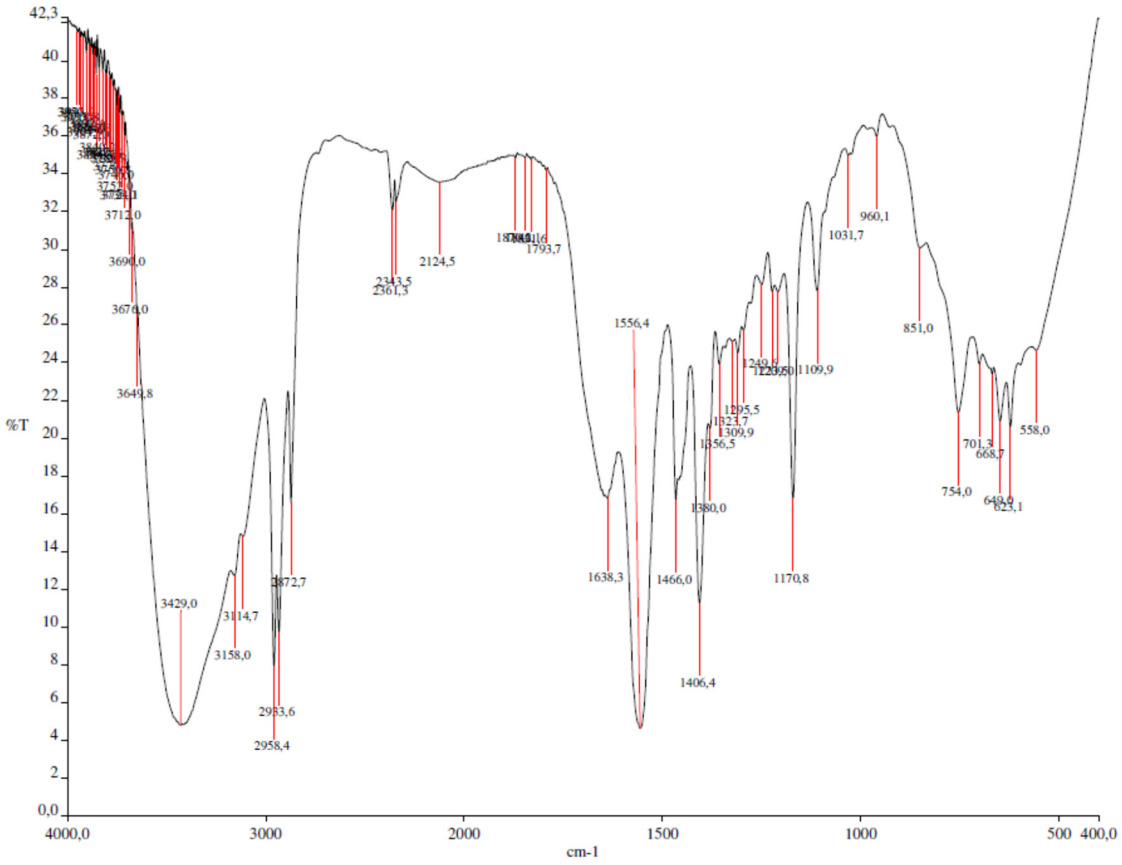

Figure S9. [EMiM][VPA] IR spectrum in KBr.

Preparation of 1-(2-hydroxyethyl)-3-methylimidazolium 2-propylpentanoate [C_2OHMiM][VPA]

1-(2-hydroxyethyl)-3-methylimidazolium chloride (0.3224 g; 1.98 mmol) was dissolved in methanol and passed through ion-exchange column Amberlite IRA-400-OH (5 eq., flow rate 0.133 mL/mL/min = 8 BV/h). Then the 1-(2-hydroxyethyl)-3-methylimidazolium hydroxide solution was slowly added to valproic acid (0.3028 g; 2.10 mmol; 1.06 eq.) dissolved in methanol. The mixture was stirred at room temperature for 1 h. The solvent and the valproic acid excess were then evaporated and then dried in vacuum for 24 h to provide desired product as liquid (0.5032 g; 98.7%). ^{1}H NMR (400.13 MHz, ($CDCl_3$) δ = 0.82 (t, J = 7.0Hz, 6H), 1.15-1.39 (m, 6H), 1.40-1.62 (m, 2H), 2.14 (dq, J_1=8.3 and J_2=4.2Hz, 1H), 3.85-3.87 (m, 2H), 3.90 (s, 3H), 4.11 (bs, 1H), 4.32-4.34 (m,2H), 7.10 (t, 1.8Hz, 1H), 7.21 (d, J=2.3Hz, 1H), 10.50 (s, 1H).; ^{13}C NMR (100.62 MHz, $CDCl_3$) δ = 14.32, 21.04, 35.48, 36.30, 47.67, 52.40, 59.56, 122.24, 139.83, 182.54 ppm; IR (KBr): ν = 3435, 3410, 3401, 2958, 2933, 2873, 2126, 1734, 1638, 1552, 1491, 1466, 1452, 1409, 1380, 1341, 1311, 1295, 1225, 1167, 1110, 1068, 944, 837, 753, 701, 668, 651, 622 cm^{-1}. Anal. calcd for $C_{14}H_{26}N_2O_3.H_2O$ C 58.31, H 9.71, N 9.71, found: C 58.31, H 9.61, N 10.15.

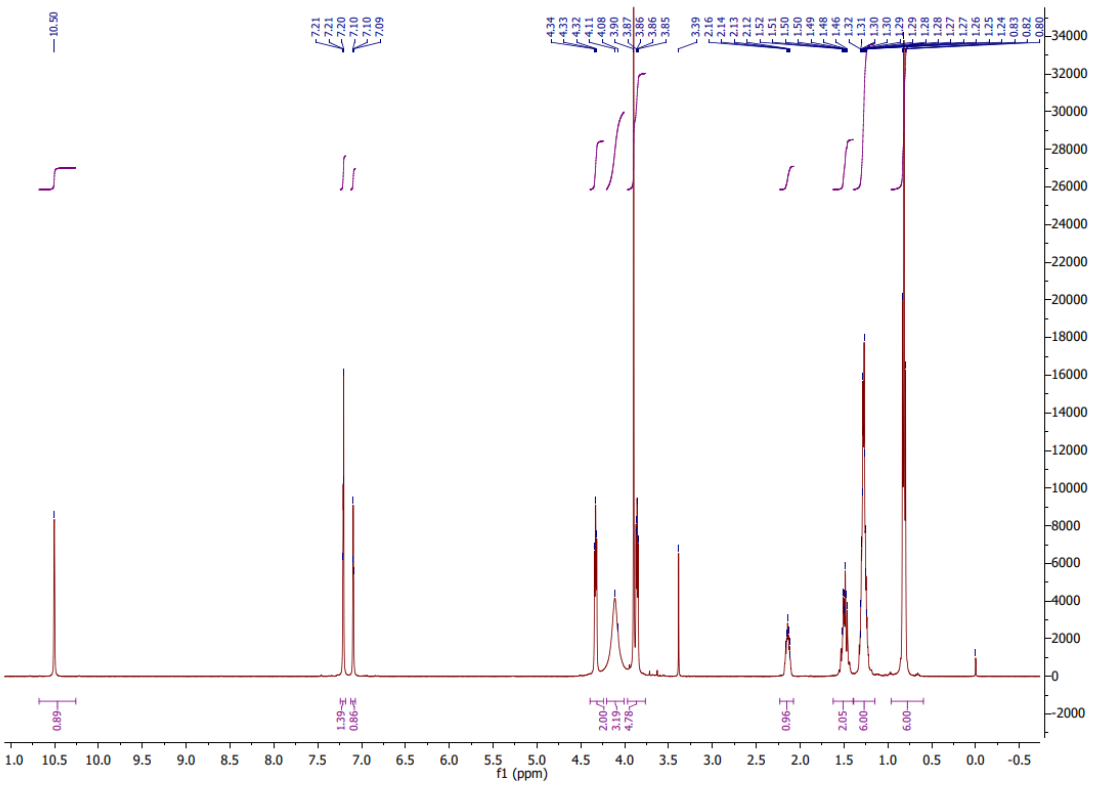

Figure S10. [C_2OHMiM][VPA] ^{1}H NMR spectrum in $CDCl_3$.

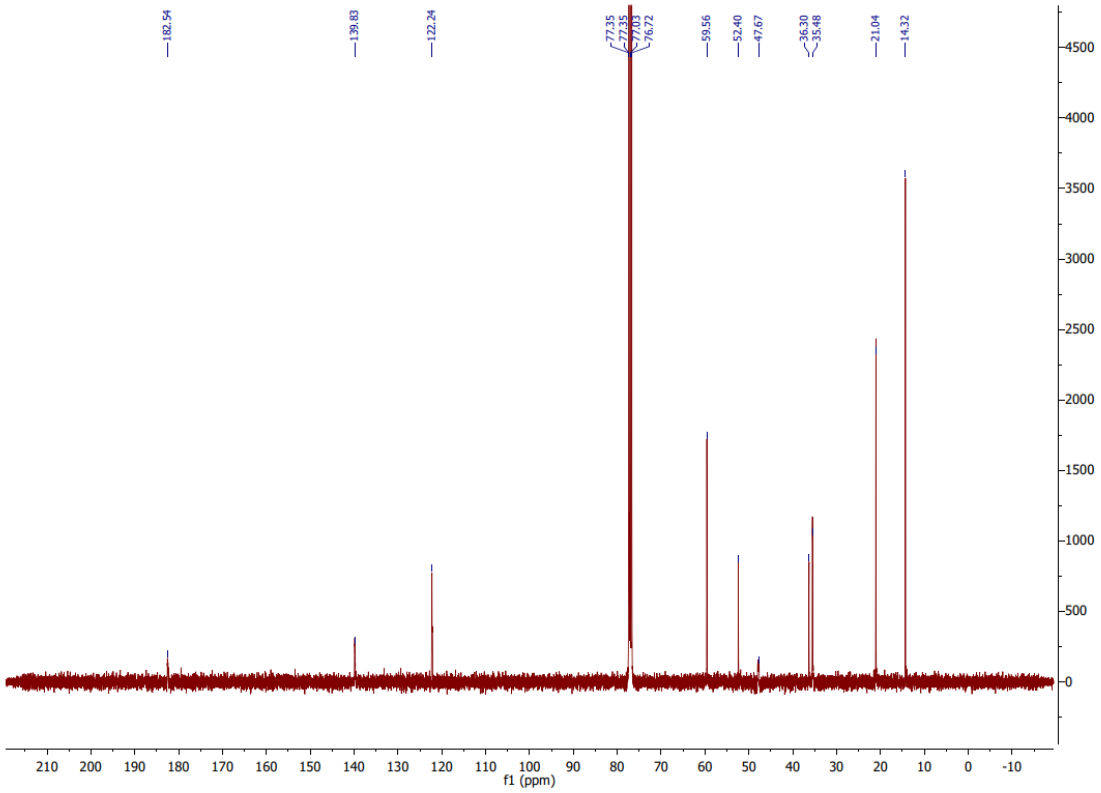

Figure S11. [C_2OHMiM][VPA] ^{13}C NMR spectrum in $CDCl_3$.

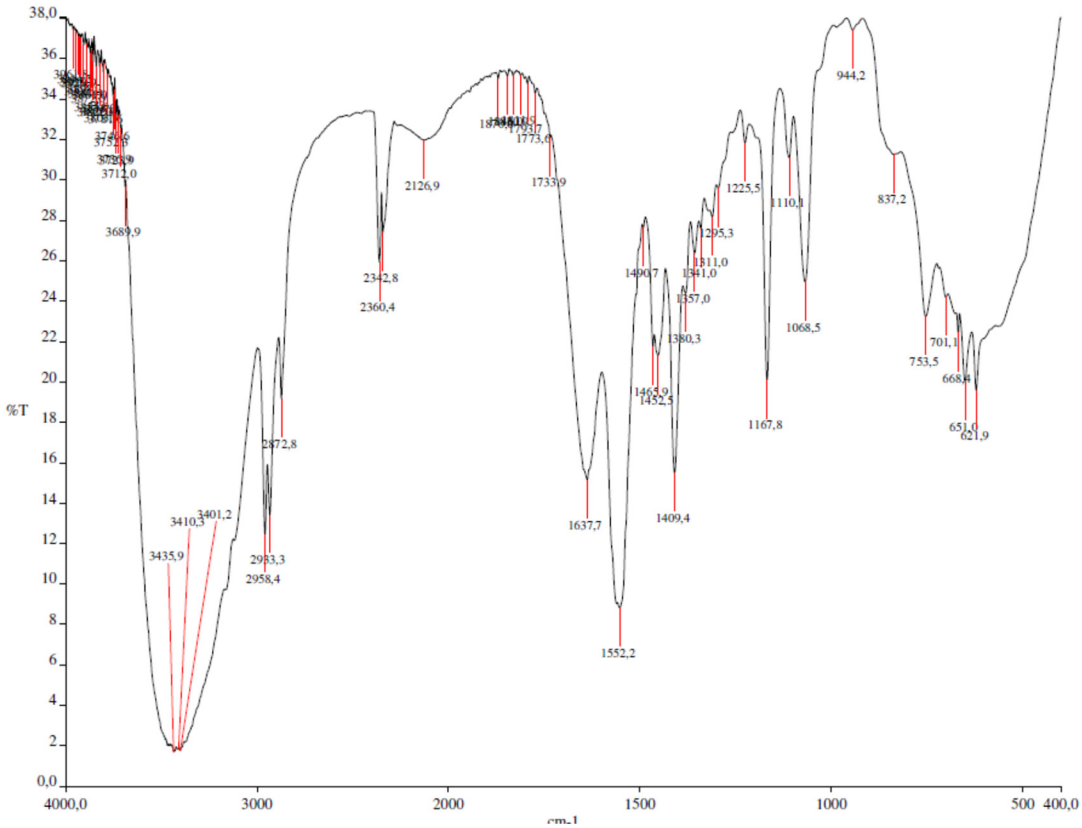

Figure S12. [C_2OHMiM][VPA] IR spectrum in KBr.

Preparation of 1-(2-hydroxyethyl)-2,3-dimethylimidazolium 2-propylpentanoate [C_2OHDMiM][VPA]

1-(2-hydroxyethyl)-2,3-dimethylimidazolium chloride (0.6536 g; 3.70 mmol) was dissolved in methanol and passed through ion-exchange column Amberlite IRA-400-OH (5 eq., flow rate 0.133 mL/mL/min = 8 BV/h). Then the 1-(2-hydroxyethyl)-2,3-dimethylimidazolium hydroxide solution was slowly added to valproic acid (0.609 g; 4.22 mmol; 1.14 eq.) dissolved in methanol. The mixture was stirred at room temperature for 1 h. The solvent and the valproic acid excess were then evaporated and dried in vacuum for 24nh to provide desired product as liquid (1.3054 g; 88.6%). ^{1}H NMR (400.13 MHz, CD_3OD) δ = 0.90 (t, *J*=7.1Hz, 6H), 1.18-1.63 (m, 8H), 2.20 (dd, J_1=9.2 and J_2=4.7 Hz, 1H), 2.61-2.63 (m, 3H*), 3.80 (s, 3H), 3.85-3.88 (m, 2H), 4.25-4.27 (, 2H), 7.51 (dd, J_1=15.5 and J_2=2.1 Hz) ppm; ^{13}C NMR (100.62 MHz, CD_3OD) δ = 14.74, 22.27, 35.42, 37.18, 50.50, 51.86, 61.42, 122.60, 123.61, 185.39 ppm; IR (KBr): ν = 3468, 3434, 2959, 2933, 2873, 2121, 1749, 1639, 1590, 1546, 1453, 1410, 1383, 1358, 1359, 1311, 1281, 1250, 1167, 1117, 1070, 944, 869,848, 754, 713, 667 cm^{-1}. Anal. calcd for $C_{15}H_{28}N_2O_3$ C, 63.35; H, 9.92; N, 9.85, found: C 63.59, H 10.05, N 10.25.

* CH_3 group lacking intensity mostly due to H-D exchange, see for example [1].

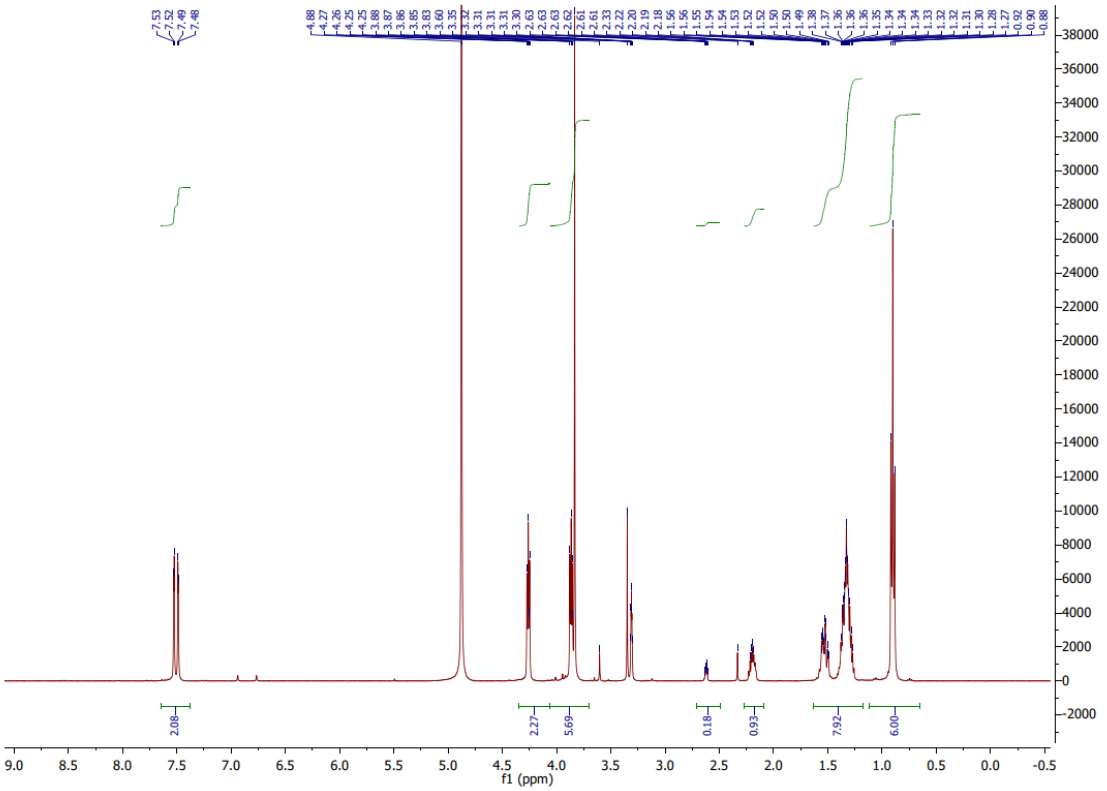

Figure S13. [C_2OHDMiM][VPA] ^{1}H NMR spectrum in CD_3OD.

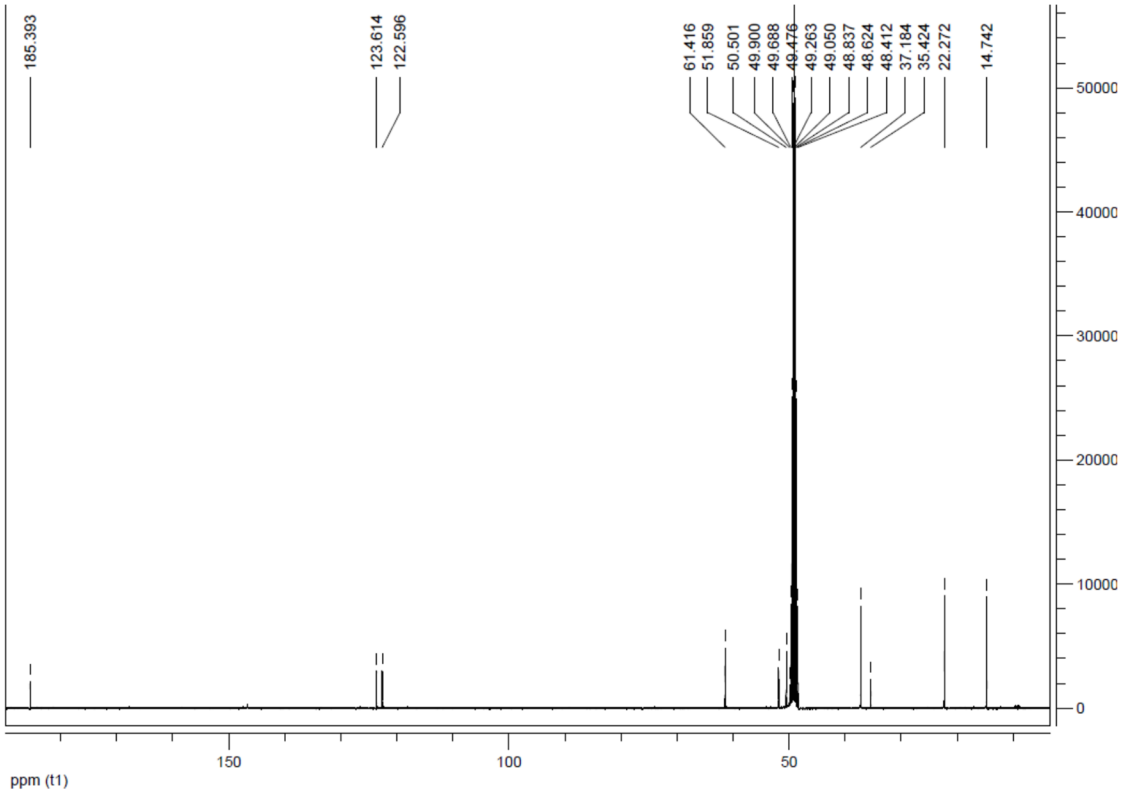

Figure S14. [C_2OHDMiM][VPA] ^{13}C NMR spectrum in CD_3OD.

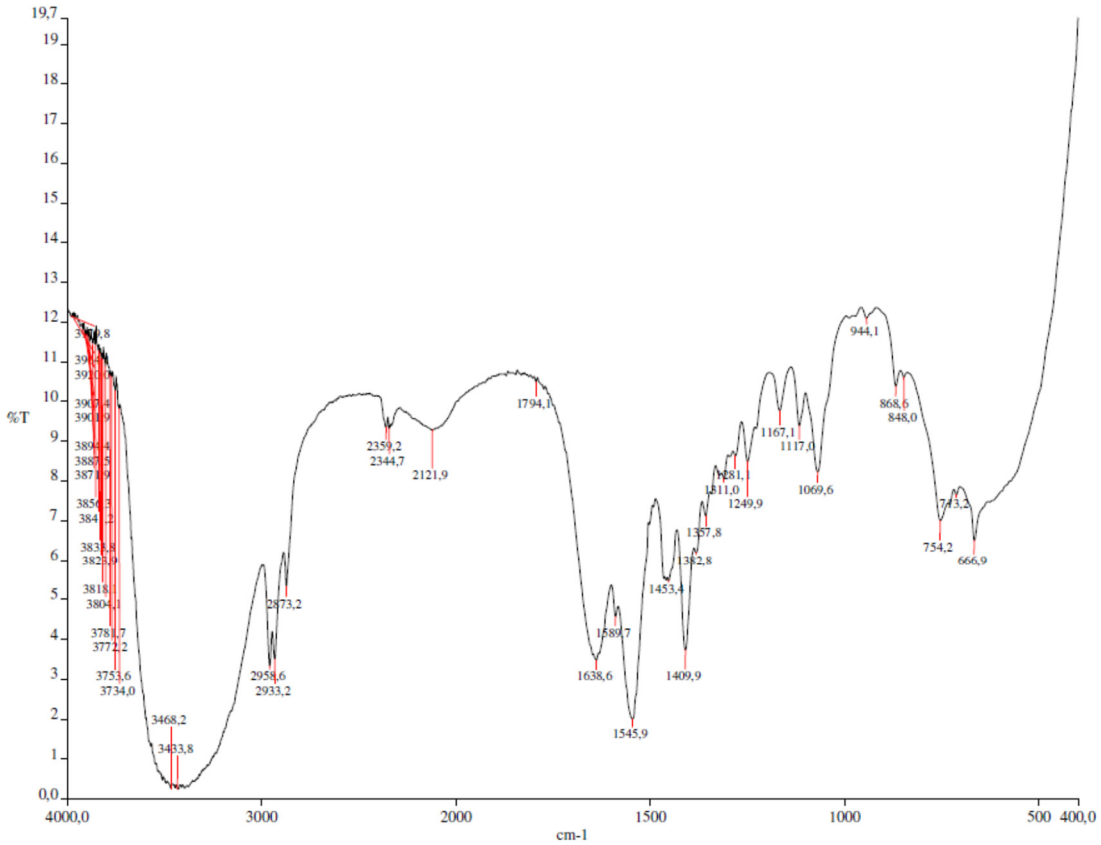

Figure S15. [C_2OHDMiM][VPA] IR spectrum in KBr.

Preparation of 1-(2-methoxyethyl)-3-methylimidazolium 2-propylpentanoate [C_3OMiM][VPA]

1-(2-methoxyethyl)-3-methylimidazolium chloride (0.7098 g; 4.02 mmol) was dissolved in methanol and passed through ion-exchange column Amberlite IRA-400-OH (5 eq., flow rate 0.133 mL/mL/min = 8 BV/h). Then the 1-(2-methoxyethyl)-3-methylimidazolium hydroxide solution was slowly added to valproic acid (0.6064 g; 4.20 mmol; 1.05 eq.) dissolved in methanol. The mixture was stirred at room temperature for 1 h. The solvent and the valproic acid excess were then evaporated and dried in vacuum for 24h to provide desired product as liquid (1.3427 g; 94.1%). ^{1}H NMR (400.13 MHz, $CDCl_3$) δ = 0.90 (t, *J* = 7.2 Hz 6H), 1.26-1.56 (m, 8H), 2.17-2.24 (m, 1H), 3.36 (s, 1H), 3.73 (t, *J* = 4.8 Hz, 2H), 3.94 (s, 3H), 4.39 (t, *J* = 4.8, 2H), 7.57 (d, *J* = 1.8 Hz, 1H), 7.62 (d, *J* = 1.8 Hz, 1H) ppm; ^{13}C NMR (100.62 MHz, $CDCl_3$) δ = 14.74, 22.27, 36.47, 37.18, 50.47, 50.78, 59.17, 71.18, 124.13, 124.70, 185.33 ppm; IR (KBr): ν = 3434, 3411, 2958, 2933, 2873, 2090, 1639, 1563, 1552, 1454, 1409, 1384, 1357, 1341, 1311, 1295, 1225, 1170, 1118, 1079, 1036, 1012, 968, 940, 832, 753, 702, 652, 622 cm^{-1}. Anal. calcd for $C_{15}H_{28}N_2O_3$: C, 63.35; H, 9.92; N, 9.85, found: C 63.31, H 9.52, N 10.14.

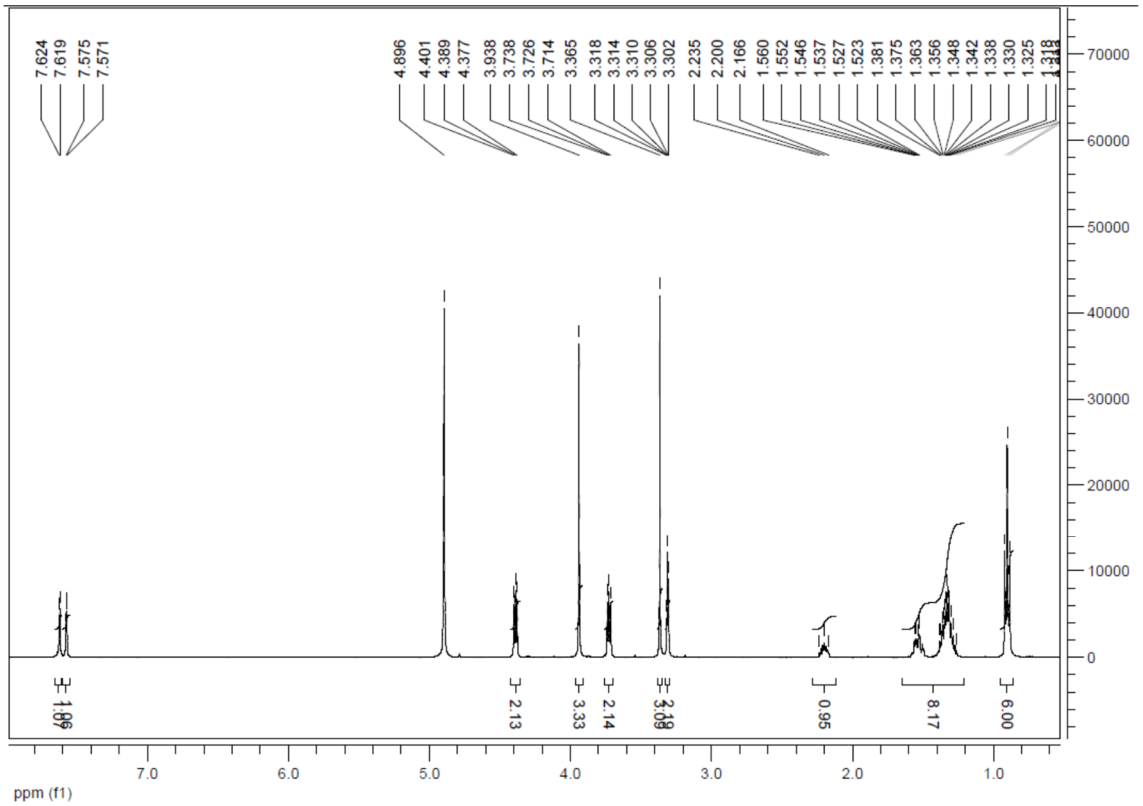

Figure S16. [C3OMiM][VPA] ^{1}H NMR spectrum in CD_3OD.

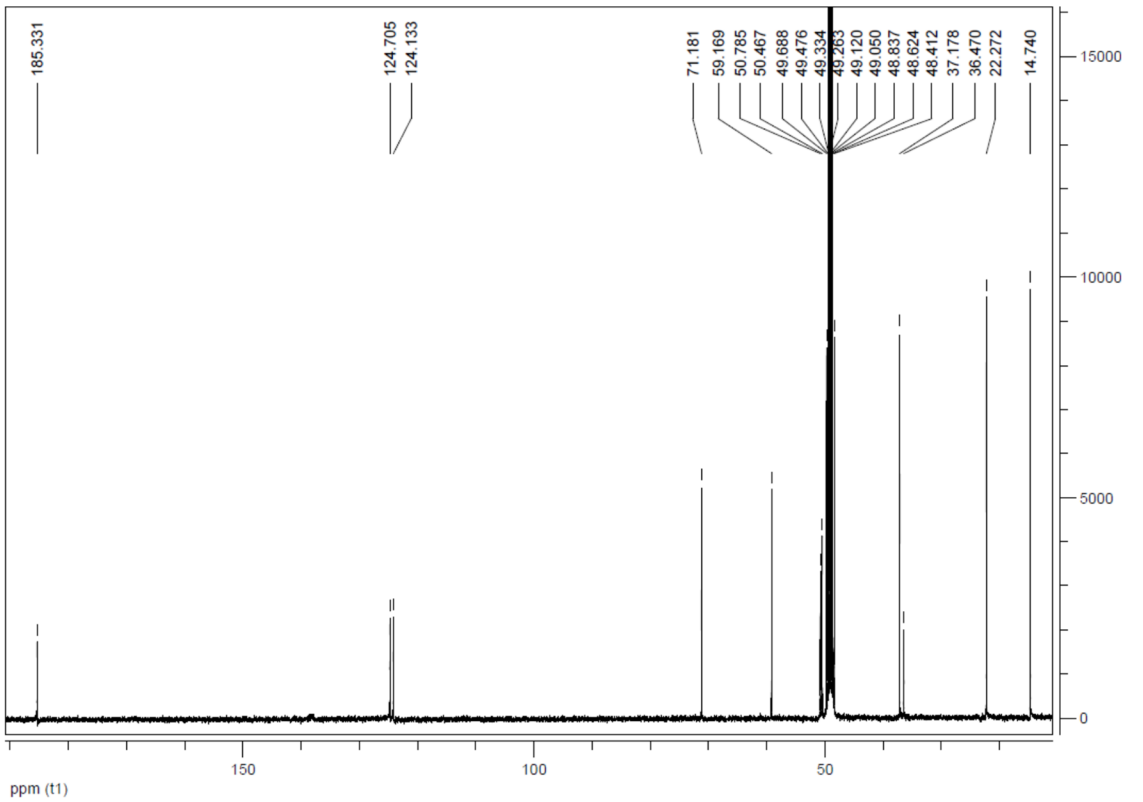

Figure S17. [C3OMiM][VPA] ^{13}C NMR spectrum in CD_3OD.

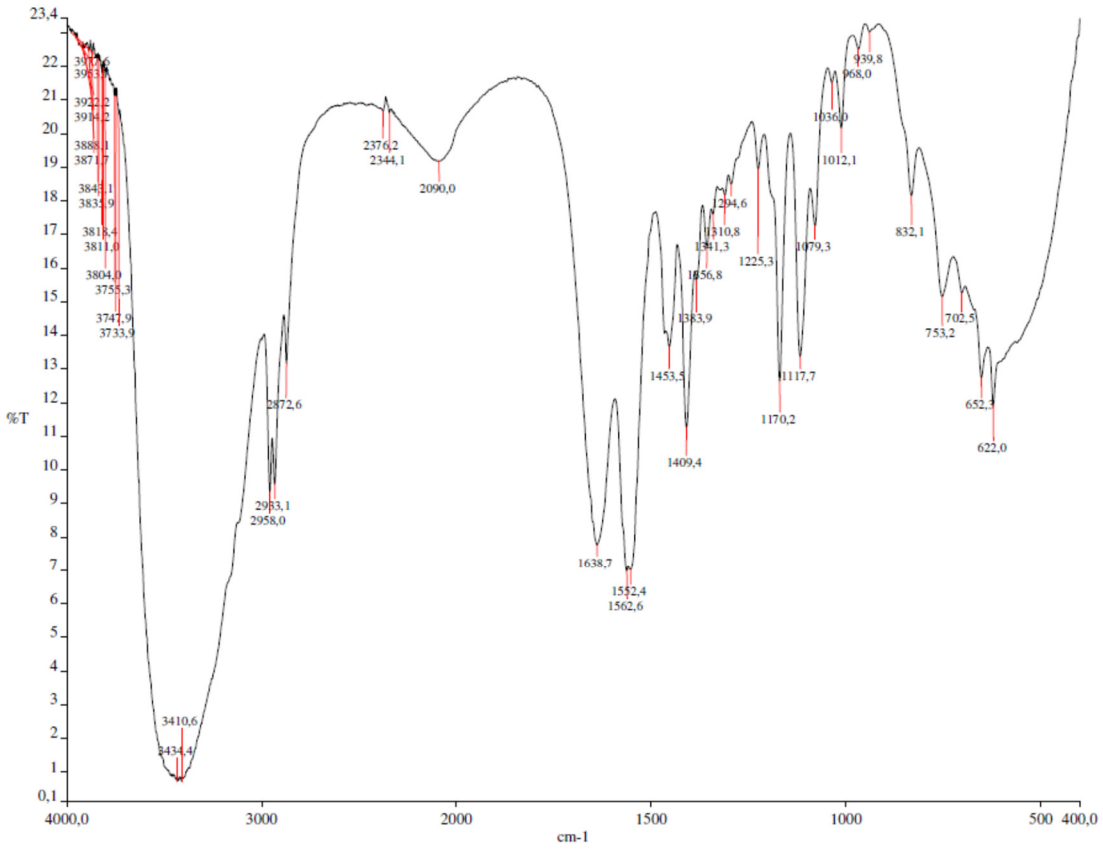

Figure S18. [C3OMiM][VPA] IR spectrum in KBr.

2. Cell Culture Studies

Procedure**:** Cells were maintained in a-minimal essential medium (a-MEM) containing 10% fetal bovine serum, 100 IU/mL penicillin, 2.5 mg/mL streptomycin, 2.5 mg/mL amphotericin B, and 50 mg/mL ascorbic acid. At ~70–80% confluence, cells were enzymatically detached with 0.05% trypsin and 0.5 mm EDTA and seeded at 10^4 cells/cm^2. After an attachment period of 24 h, the culture medium was renewed and supplemented with various concentrations (0.005–500 mM) of the ampicillin-based ILs. Cell cultures were maintained in a 5% CO_2 humidified atmosphere at 37 °C. Cellular viability/proliferation was assessed by MTT assay on days 1, 3, and 5 of the culture. This assay is based on the reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide to a purple formazan product by viable cells. Briefly, cultured cells were incubated at 37 °C with MTT (0.5 mg/mL) for 4 h. The culture medium was then removed; the stained product was dissolved in DMSO, and absorbance was measured at λ = 550 nm in an ELISA plate reader. Results were expressed as absorbance per square centimeter (A/cm^2) [2]. Half-maximal inhibitory concentration (IC_{50}) and median lethal dose (EC_{50}) values were obtained by nonlinear regression analysis of concentration–effect curves, using GraphPad Prism software [3]. The definition of IC_{50} is given by Sebaugh [4] as "the response corresponding to the 50% control (the mean of the 0% and 100% assay controls)" and is used to measure the efficacy of a compound in inhibiting any biochemical or biological function. EC_{50} is the concentration of a drug that gives half-maximal [4].

References

1. Zanatta, M.; dos Santos, F.P.; Biehl, C.; Marin, G.; Ebeling, G.; Netz, P.A.; Dupont, J. Organocatalytic Imidazolium Ionic Liquids H/D Exchange Catalysts. *J. Org. Chem.* **2017**, *82*, 2622–2629. https://doi.org/10.1021/acs.joc.6b03029.
2. Gomes, P.S.; Santos, J.D.; Fernandes, M.H. Cell-induced response by tetracyclines on human bone marrow colonized hydroxyapatite and Bonelike®. *Acta Biomater.* **2008**, *4*, 630–637. https://doi.org/10.1016/j.actbio.2007.12.006.
3. Barros, A.V.; Araujo, L.M.; de Oliveira, F.F.; da Conceicao, A.O.; Simoni, I.C.; Fernandes, M.J.B.; Arns, C.W. In Vitro Evaluation of the Antiviral Potential of *Guettarda angelica* Against Animal Herpesviruses. *Acta Sci. Vet.* **2012**, *40*, 1–7.
4 Sebaugh, J.L. Guidelines for accurate EC50/IC50 estimation. *Pharm. Stat.* **2011**, *10*, 128–134. https://doi.org/10.1002/pst.426.