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Supporting Information

Human Cytotoxicity, Hemolytic Activity, Anti-Inflammatory Activity and Aqueous Solubility of Ibuprofen-based Ionic Liquids

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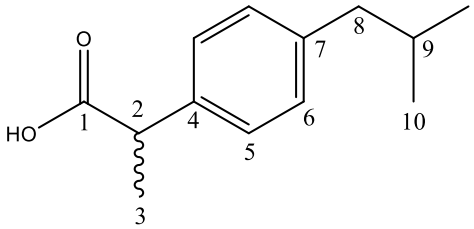
Characterization of the Ibuprofen-based Ionic Liquids

The prepared ibuprofen-based ionic liquids, ibuprofen and ibuprofen sodium salt were completely characterized by ^{1}H and ^{13}C NMR in order to check their expected structures. Additionally, the quantitative integration of their characteristic ^{1}H NMR resonance peaks unfold the expected cation/anion correlations. Also, there were no peaks assigned to impurities in the ^{1}H NMR spectra.

Ibuprofen

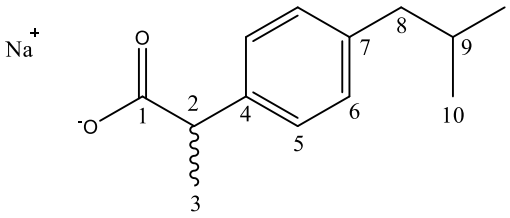


^{1}H NMR (400 MHz, D_2O): δ/ppm = 7.20 (d, *J* = 8.0 Hz, 2H, 6), 7.15 (d, *J* = 8.3 Hz, 2H, 5), 3.58 (m, 1H, 2), 2.40 (d, *J* = 7.1 Hz, 2H, 8), 1.75 (dd, *J* = 13.5, 6.6 Hz, 1H, 9), 1.33 (d, *J* = 7.2 Hz, 3H, 3), 0.79 (d, *J* = 6.6 Hz, 6H, 10).

^{1}H NMR (400 MHz, DMSO): δ/ppm = δ 12.25 (s, 1H, OH), 7.19 (d, *J* = 7.9 Hz, 2H, 6), 7.11 (d, *J* = 7.9 Hz, 2H, 5), 3.63 (q, *J* = 7.1 Hz, 1H, 2), 2.42 (d, *J* = 7.1 Hz, 2H, 8), 1.81 (dp, *J* = 13.5, 6.9 Hz, 1H, 9), 1.35 (d, *J* = 7.1 Hz, 3H, 3), 0.86 (d, *J* = 6.6 Hz, 6H, 10). ^{13}C NMR (101 MHz, DMSO): δ/ppm = 175.95 (s, 1), 140.02 (s, 7), 138.94 (s, 4), 129.43 (s, 6), 127.57 (s, 5), 44.74 (s, 8), 44.68 (s, 2), 30.03 (d, *J* = 8.9 Hz, 9), 22.42 (d, *J* = 43.1 Hz, 10), 18.91 (d, *J* = 16.1 Hz, 3).

^{1}H NMR (400 MHz, $CDCl_3$): δ/ppm = δ 7.25 (d, *J* = 7.8 Hz, 2H, 6), 7.13 (d, *J* = 7.8 Hz, 2H, 5), 3.74 (q, *J* = 7.1 Hz, 1H, 2), 2.48 (d, *J* = 7.1 Hz, 2H, 8), 1.87 (tt, *J* = 13.4, 6.7 Hz, 1H, 9), 1.52 (s, 3H, 3), 0.93 (d, *J* = 6.6 Hz, 6H, 10). ^{13}C NMR (101 MHz, $CDCl_3$): δ/ppm = 180.61 (s, 1), 140.87 (s, 7), 137.00 (s, 4), 129.40 (s, 6), 127.29 (s, 5), 45.05 (s, 8), 44.94 (s, 2), 30.17 (s, 9), 22.40 (s, 10), 18.11 (s, 3).

Sodium Ibuprofen



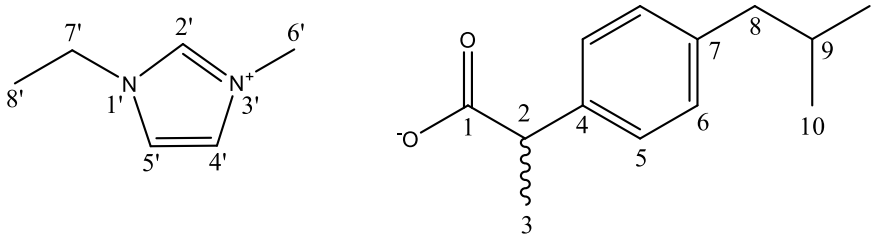
^{1}H NMR (500 MHz, D_2O): δ/ppm = 7.19 (d, *J* = 8.0 Hz, 2H, 6), 7.13 (d, *J* = 7.9 Hz, 2H, 5), 3.53 (q, *J* = 7.1 Hz, 1H, 2), 2.40 (d, *J* = 7.1 Hz, 2H, 8), 1.82– 1.69 (m, 1H, 9), 1.31 (d, *J* = 7.2 Hz, 3H, 3), 0.79 (d, *J* = 6.6 Hz, 6H, 10). ^{13}C NMR (126 MHz, D_2O): δ/ppm = 183.98 (d, *J* = 3.0 Hz, 1), 140.67 (s, 7), 140.44 (s, 4), 129.41 (s, 6), 127.10 (s, 5), 48.12 (d, *J* = 3.9 Hz, 8), 44.15 (d, *J* = 3.9 Hz, 2), 29.68 (s, 9), 21.56 (s, 10), 18.41 (s, 3).

^{1}H NMR (500 MHz, DMSO): δ/ppm = 7.17 (d, *J* = 8.0 Hz, 2H, 6), 6.96 (d, *J* = 8.0 Hz, 2H, 5), 3.21 (q, *J* = 7.1 Hz, 1H, 2), 2.37 (d, *J* = 7.1 Hz, 2H, 8), 1.87 – 1.70 (m, 1H, 9), 1.22 (t, *J* = 8.2 Hz, 3H, 3), 0.86 (d, *J* = 6.6 Hz, 6H, 10). ^{13}C NMR (101 MHz, DMSO) : δ/ppm = 177.55 (s, 1), 143.79 (s, 7), 137.88 (s, 4), 128.48 (s, 6), 127.68 (s, 5), 49.04 (s, 8), 44.84 (s, 2), 30.19 (s, 9), 22.69 (s, 10), 20.74 (s, 3).

^{1}H NMR (400 MHz, $CDCl_3$): δ/ppm = 7.24 (d, *J* = 8.0 Hz, 2H, 6), 7.12 (d, *J* = 8.0 Hz, 2H, 5), 3.74 (q, *J* = 7.0 Hz, 1H, 2), 2.47 (d, *J* = 7.2 Hz, 2H, 8), 1.94 – 1.75 (m, 1H, 9), 1.52 (d, *J* = 7.2 Hz, 3H, 3), 0.92 (d, *J* = 6.6 Hz, 6H, 10). ^{13}C NMR (101 MHz, $CDCl_3$): δ/ppm = 179.40 – 176.46

(m, 1), 142.58 – 138.96 (m, 7), 137.83 – 135.65 (m, 4), 129.42 (s, 6), 127.25 (s, 5), 45.04 (s, 8), 44.74 – 44.31 (m, 2), 30.16 (s, 9), 22.38 (s, 10), 18.20 (s, 3).

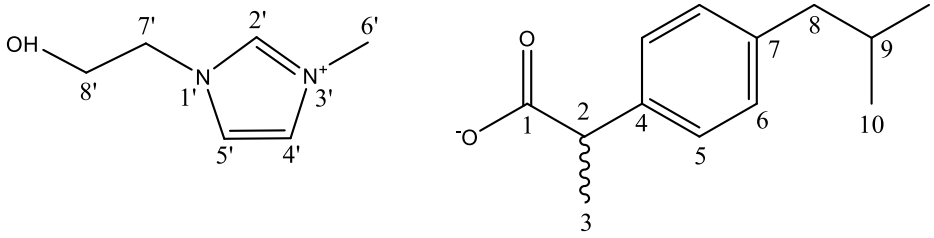
1-Ethyl-3-methylimidazolium ibuprofenate ([C_2C_1Im][Ibu])



^{1}H NMR (400 MHz, D_2O): δ/ppm = 8.58 (s, 1H, 2′), 7.37 (s, 1H, 4′), 7.30 (s, 1H, 5′), 7.18 (d, J = 7.9 Hz, 2H, 6), 7.12 (d, J = 7.9 Hz, 2H, 5), 4.11 (q, J = 7.4 Hz, 2H, 7′), 3.77 (s, 3H, 6′), 3.53 (m, 1H, 2), 2.38 (d, J = 7.1 Hz, 2H, 8), 1.74 (dp, J = 13.5, 6.7 Hz, 1H, 9), 1.39 (t, J = 7.4 Hz, 3H, 8′), 1.30 (d, J = 7.2 Hz, 3H, 3), 0.78 (d, J = 6.6 Hz, 6H, 10). ^{13}C NMR (101 MHz, DMSO): δ/ppm =183.92 (s, 1), 140.71 (s, 7), 140.42 (s, 4), 129.40 (s, 6), 127.12 (s, 5), 123.36 (s, 4′), 121.78 (s, 5′), 48.18 (s, 7′), 44.70 (s, 8), 44.17 (s, 2), 35.49 (s, 6′), 29.69 (s, 9), 21.58 (s, 10), 18.43 (s, 3), 14.40 (s, 8′).

^{1}H NMR (400 MHz, DMSO): δ/ppm = 9.48 (s, 1H, 2′), 7.79 (s, 1H, 4′), 7.70 (s, 1H, 5′), 7.14 (d, *J* = 7.8 Hz, 2H, 5), 6.94 (d, *J* = 7.8 Hz, 2H, 6), 4.18 (q, *J* = 7.3 Hz, 2H, 7′), 3.84 (s, 3H, 6′), 3.15 (q, *J* = 7.1 Hz, 1H, 2), 2.37 (d, *J* = 7.1 Hz, 2H, 8), 1.78 (dp, *J* = 13.3, 6.8 Hz, 1H, 9), 1.40 (t, *J* = 7.3 Hz, 3H, 8′), 1.19 (d, *J* = 7.1 Hz, 3H, 3), 0.86 (d, *J* = 6.6 Hz, 6H, 10). ^{13}C NMR (101 MHz, DMSO): δ/ppm = 175.76 (s, 1), 144.45 (s, 2′), 137.55 (s, 7), 137.27 (s, 4), 128.35 (s, 6), 127.64 (s, 5), 123.97 (s, 4′), 122.38 (s, 5′), 49.65 (s, 7′), 44.86 (s, 8), 44.50 (s, 2), 36.06 (s, 6′), 30.19 (s, 9), 22.69 (s, 10), 20.86 (s, 3), 15.60 (s, 8′).

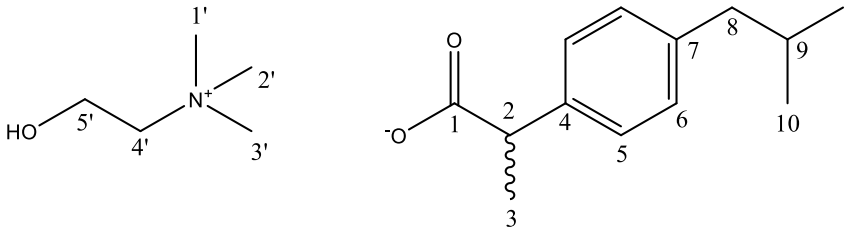
1-(2-Hydroxyethyl)-3-methylimidazolium ibuprofenate ([$C_{2(OH)}C_1$Im][Ibu])



^{1}H NMR (500 MHz, D_2O): δ/ppm = 7.41 (d, *J* = 1.9 Hz, 1H, 4′), 7.36 (d, *J* = 1.8 Hz, 1H, 5′), 7.18 (dd, *J* = 4.7, 3.2 Hz, 2H, 6), 7.12 (dd, *J* = 4.9, 3.0 Hz, 2H, 5), 4.26 – 4.19 (m, 2H, 7′), 3.88 – 3.78 (m, 5H, 6′, 8′), 3.51 (m, 1H, 2), 2.39 (dd, *J* = 6.9, 3.5 Hz, 2H, 8), 1.75 (ddd, *J* = 20.1, 10.1, 4.9 Hz, 1H, 9), 1.30 (dd, *J* = 7.1, 3.6 Hz, 3H, 3), 0.91 – 0.68 (m, 6H, 10). ^{13}C NMR (126 MHz, D_2O) : δ/ppm = 183.97 (s, 2′), 140.68 (s, 7), 140.44 (s, 4), 129.39 (s, 6), 127.09 (s, 5), 123.52 (s, 4′), 122.35 (s, 5′), 59.70 (s, 7′), 51.45 (s, 8), 48.15 (s, 2), 44.15 (s, 8′), 35.61 (s, 6′), 29.67 (s, 9), 21.55 (s, 10), 18.40 (s, 3).

^{1}H NMR (400 MHz, DMSO): δ/ppm = 9.31 (s, 1H, 2′), 7.73 (s, 1H, 4′), 7.67 (s, 1H, 5′), 7.15 (d, *J* = 7.8 Hz, 2H, 6), 6.96 (d, *J* = 7.8 Hz, 2H, 5), 4.21 (t, *J* = 4.9 Hz, 2H, 7′), 3.83 (s, 3H, 6′), 3.69 (dd, J = 13.8, 8.9 Hz, 2H, 8′), 3.18 (q, J = 7.1 Hz, 1H, 2), 2.36 (t, J = 8.1 Hz, 2H, 8), 1.87 – 1.68 (m, 1H, 9), 1.19 (d, J = 7.1 Hz, 3H, 3), 0.85 (d, J = 6.6 Hz, 6H, 10). ^{13}C NMR (101 MHz, DMSO) : δ/ppm = 176.29 (s, 1), 144.09 (s, 2′), 137.75 (s, 7), 137.62 (s, 4), 128.45 (s, 6), 127.64 (s, 5), 123.62 (s, 4′), 123.14 (s, 5′), 59.66 (s, 7′), 52.02 (s, 8), 49.39 (s, 2), 44.84 (s, 8′), 36.05 (s, 6′), 30.20 (s, 9), 22.70 (s, 10), 20.77 (s, 3).

Cholinium ibuprofenate ([$N_{1112(OH)}$][Ibu])



^{1}H NMR (500 MHz, D_2O): δ/ppm = 7.19 (d, *J* = 8.1 Hz, 2H, 6), 7.13 (d, *J* = 8.0 Hz, 2H, 5), 4.01 – 3.91 (m, 2H, 5′), 3.60 – 3.46 (m, 1H, 2), 3.46 – 3.36 (m, 2H, 4′), 3.10 (s, 9H, 1′,2′,3′), 2.40 (d, *J* = 7.1 Hz, 2H, 8), 1.82 – 1.67 (m, 1H, 9), 1.30 (d, *J* = 7.2 Hz, 3H, 3), 0.79 (d, *J* = 6.6 Hz, 6H, 10). ^{13}C NMR (126 MHz, D_2O) : δ/ppm = 183.96 (s, 1), 140.69 (s, 7), 140.43 (s, 4), 129.41 (s, 6), 127.10 (s, 5), 68.57 – 65.25 (m, 5′), 55.55 (s, 4′), 54.66 – 53.28 (m, 1′, 2′, 3′), 48.15 (s, 8), 44.15 (s, 2), 29.68 (s, 9), 21.56 (s, 10), 18.41 (s, 3).

^{1}H NMR (500 MHz, DMSO): δ/ppm = 7.15 (d, *J* = 8.0 Hz, 2H, 5), 6.96 (d, *J* = 8.0 Hz, 2H, 6), 3.90 – 3.73 (m, 2H, 5′), 3.18 (q, *J* = 7.1 Hz, 1H, 2), 3.09 (s, 9H, 1′, 2′, 3′), 2.37 (d, *J* = 7.1 Hz, 2H, 8), 1.77 (dq, *J* = 20.1, 6.7 Hz, 1H, 9), 1.20 (d, *J* = 7.1 Hz, 3H, 3), 0.86 (d, *J* = 6.6 Hz, 6H, 10). ^{13}C NMR (126 MHz, DMSO) : δ/ppm = 176.34 (s, 1), 144.07 (s, 7), 137.77 (s, 4), 128.46 (s, 6), 127.64 (s, 5), 67.68 (s, 5′), 55.43 (s, 4′), 54.01 – 53.24 (m, 1′, 2′, 3′), 49.35 (s, 8), 44.84 (s, 2), 30.19 (s, 9), 22.70 (s, 10), 20.79 (s, 3).

Figure S1. DSC profile and analysis with TA Instruments Universal Analysis V4.5A software at 1ºC/min of ibuprofen.

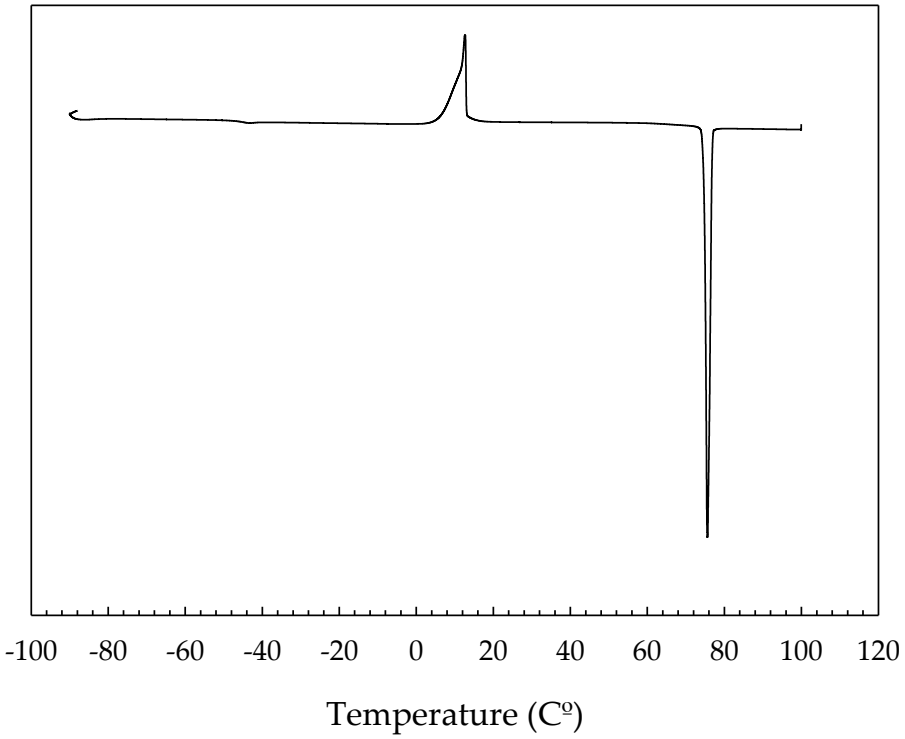


Figure S2. DSC profile and analysis with TA Instruments Universal Analysis V4.5A software at 1ºC/min of [C_2C_1Im][Ibu].

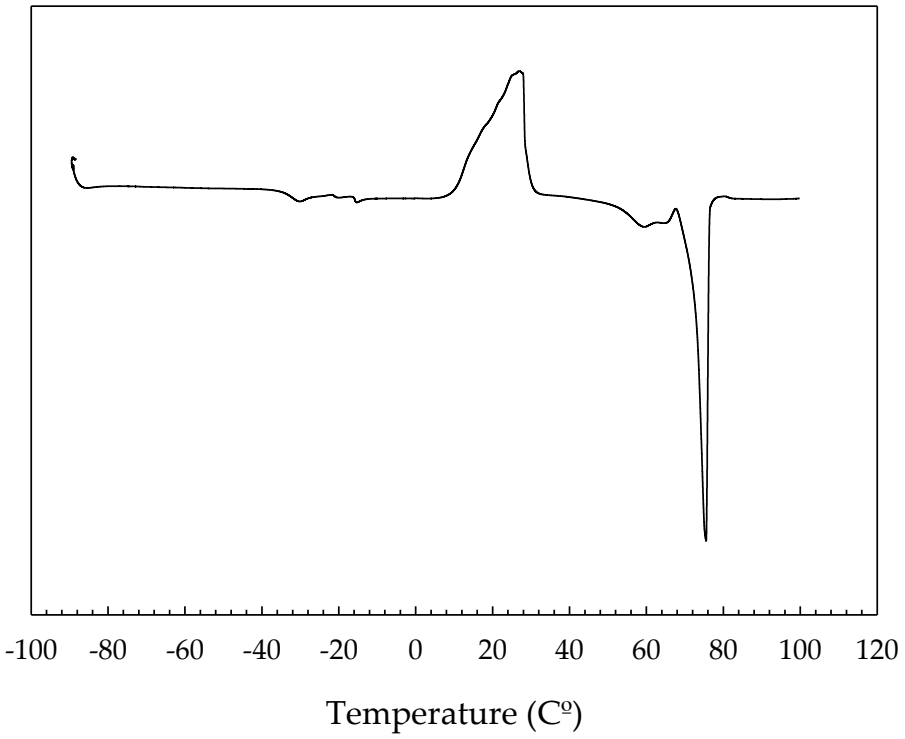


Figure S3. DSC profile and analysis with TA Instruments Universal Analysis V4.5A software at 1ºC/min of [$C_{2(OH)}C_1Im$][Ibu].

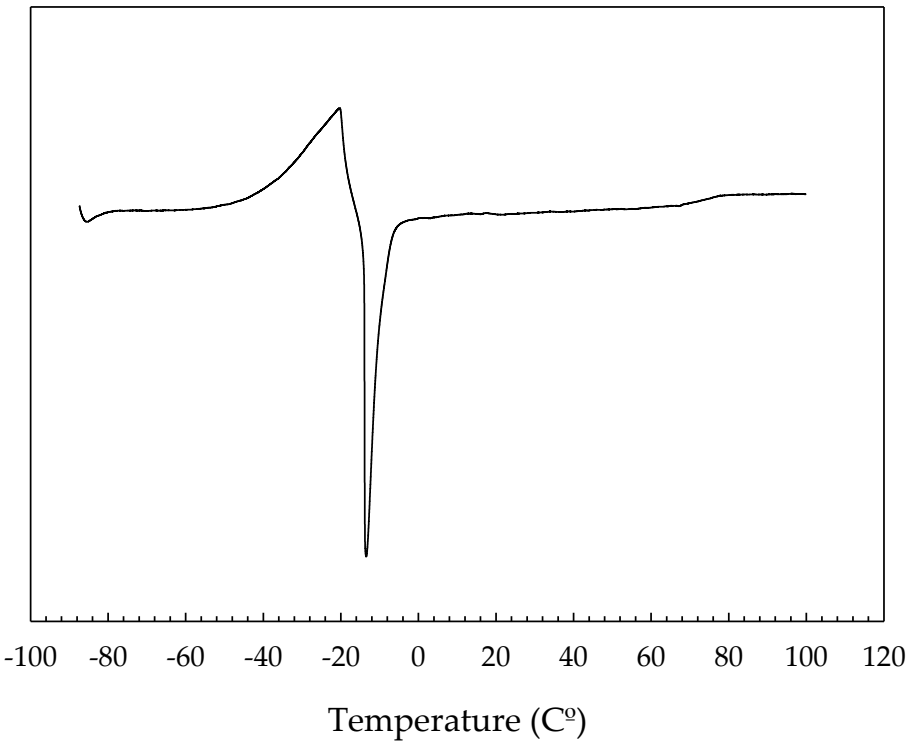


Figure S4. DSC profile and analysis with TA Instruments Universal Analysis V4.5A software at 1ºC/min of [$N_{1112(OH)}$][Ibu].

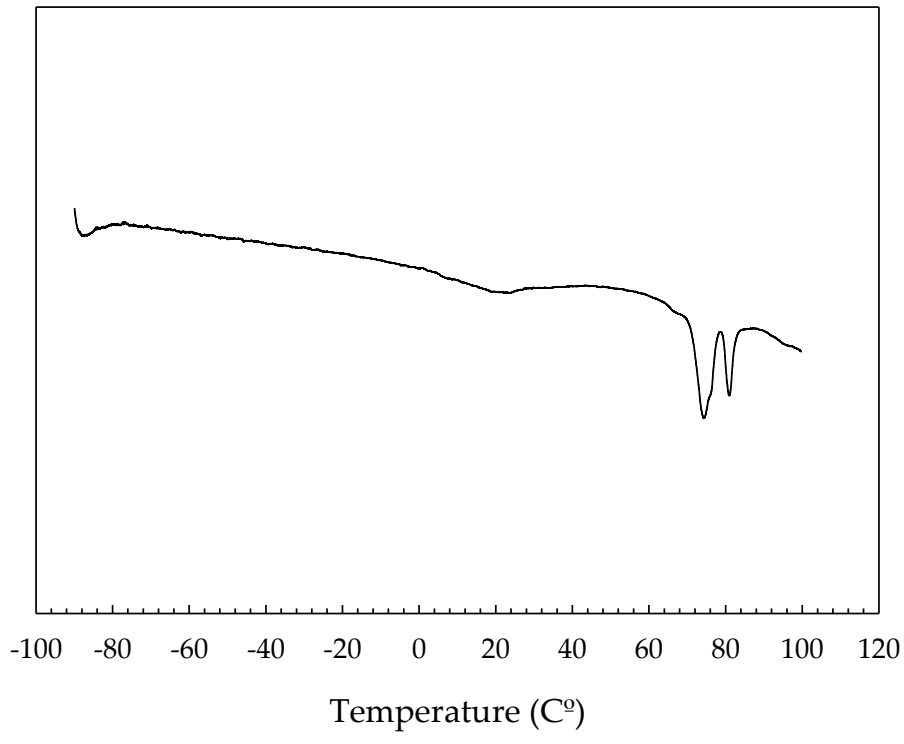


Table S1. Chemical shifts for the hydrogens in the position 2 and 3 (as depicted chemical structure of ibuprofen on the right) for ibuprofen, sodium ibuprofen and API-ILs in D_2O.

Compounds	H2 (δ/ppm)	H3 (δ/ppm)
Ibuprofen	3.58	1.33
Na[Ibu]	3.53	1.31
[C_2C_1Im][Ibu]	3.53	1.30
[$C_{2(OH)}$C1Im][Ibu]	3.51	1.30
[$N_{1112(OH)}$][Ibu]	3.53	1.30

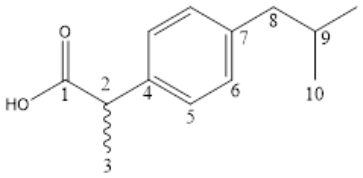


Figure S5. Non-linear regression fitting curves, calculated Log EC_{50} ± standard deviation and respective R-squared and p-value for ibuprofen **(A)**, [C_2C_1Im][Ibu] **(B)**, [$C_{2(OH)}C_1Im$][Ibu] **(C)**, [$N_{1112(OH)}$][Ibu] **(D)**, [C_2C_1Im]Cl **(E)**, [$C_{2(OH)}C_1Im$]Cl **(F)** and [$N_{1112(OH)}$]Cl **(G)** in Caco-2 cell line.

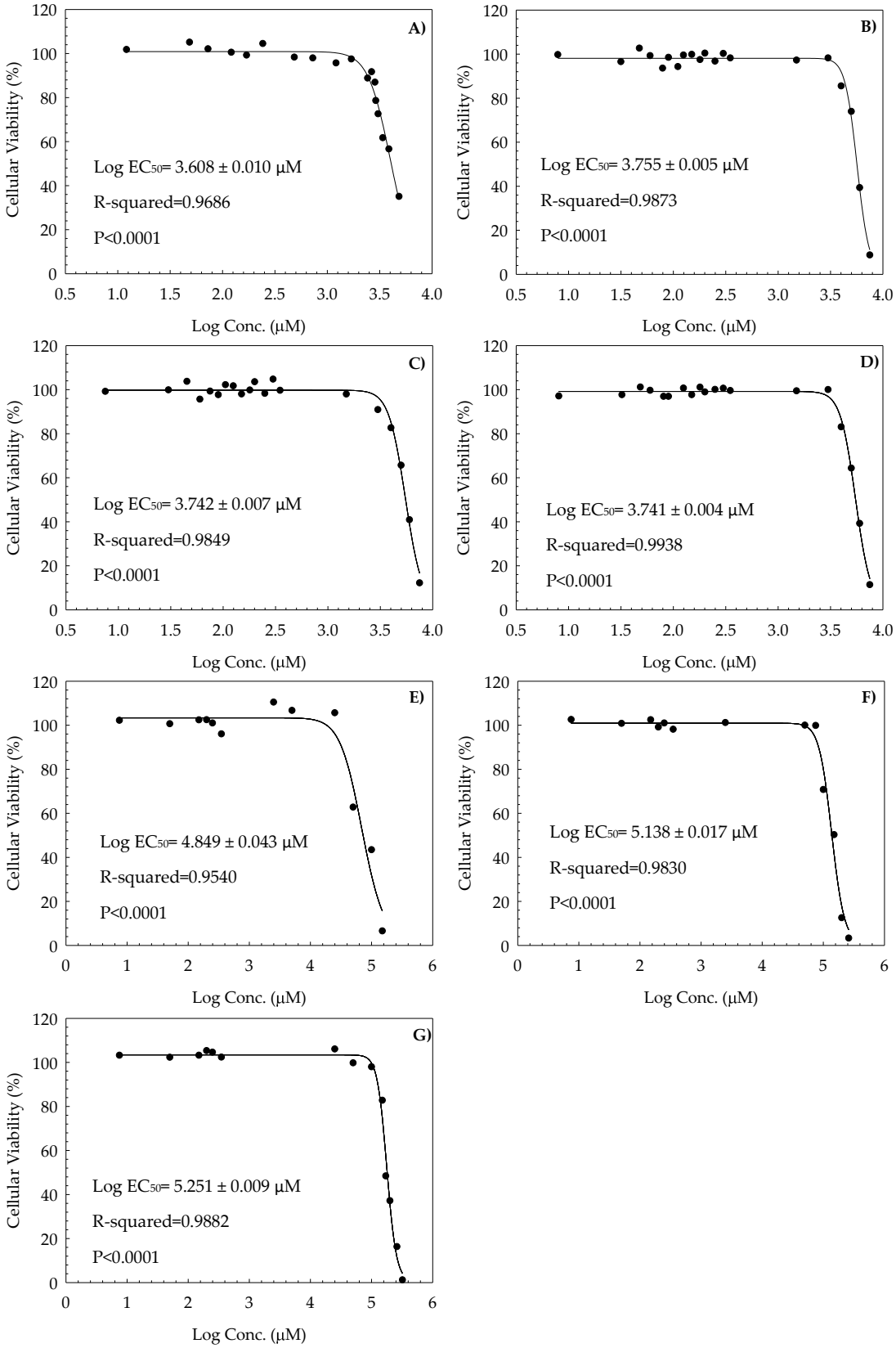


Figure S6. EC_{50} values of ibuprofen, ibuprofen-based ILs and IL/salt cation "suppliers" for the API-ILs ([C_2C_1Im]Cl, [$C_{2(OH)}C_1Im$]Cl, [$N_{1112(OH)}$]Cl) in Caco-2 cell line exposed to the compounds for 24h. The R-squared was greater than 0.9540 an $P < 0.0001$ for all fitted curves.

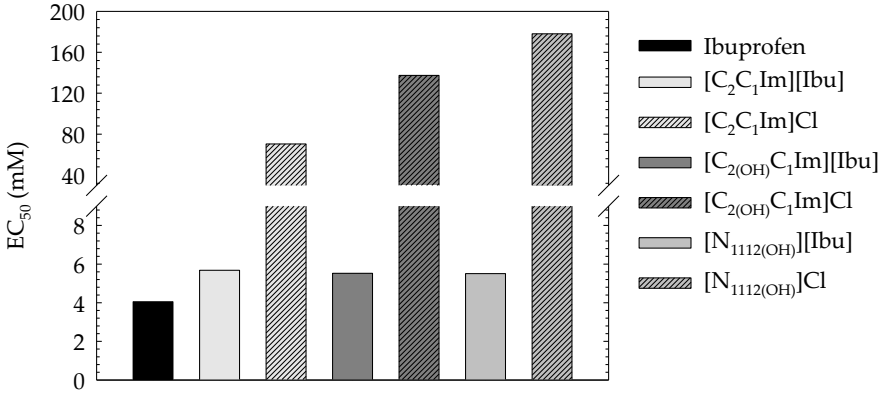


Table S2. Inhibition of BSA denaturation in PBS pH 7.4 at different concentrations for the neutral and salt form of ibuprofen and API-ILs. The presented value is the mean of at least two independent measures ± standard deviation.

Concentration (mM)	Inhibition of BSA denaturation (%)	Concentration (mM)	Inhibition of BSA denaturation (%)
Ibuprofen		**Sodium Ibuprofen**	
0.727	19.93 ± 2.00	0.657	20.15 ± 1.10
1.697	42.85 ± 1.97	1.533	36.97 ± 1.61
2.424	67.66 ± 2.49	2.190	61.24 ± 4.24
3.636	76.40 ± 1.32	3.286	69.54 ± 4.81
4.848	74.34 ± 1.32		
$[C_2C_1Im][Ibu]$		**$[C_{2(OH)}C_1Im][Ibu]$**	
0.474	23.30 ± 1.15	0.451	43.67 ± 3.21
1.106	48.77 ± 2.52	1.053	70.99 ± 3.81
1.580	55.56 ± 2.73	1.504	82.25 ± 0.44
2.370	63.27 ± 2.08	2.256	86.27 ± 0.95
$[N_{1112(OH)}][Ibu]$			
0.485	31.79 ± 1.57		
1.131	39.66 ± 1.33		
1.616	50.93 ± 2.00		
2.424	59.41 ± 0.79		