Highly Efficient and Low Toxic Skin Penetrants Composed of Amino Acid Ionic Liquids

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Materials and methods

1. Materials

Ibuprofen, proline, triethanolamine and an aqueous solution of choline hydroxide (48–50 wt%) were purchased from Tokyo Chemical Industry Co., Ltd (Tokyo, Japan). Pro-TBP (>98%) was purchased from Katayama Seiyakusyo Co., Ltd. Other chemicals used in this study were the highest purity commercially available.

2. Typical procedure for amino acid ester synthesis

All reagents were commercially available materials and were used without further purification. The typical procedure to synthesise the proline ethylester is as follows. First, 5 g of proline was dispersed into 50 mL of ethanol at room temperature. Then, two times the molar amount of $SOCl_2$ was added into the slurry and the resultant solution was stirred thoroughly for a day. Second, the excess $SOCl_2$ and ethanol were removed by evaporation after the reaction. As a result, the proline ethylester hydrochloride (ProOEt HCl) salt was derived. Third, the derived ProOEt HCl was added to 10 mL of distilled water and neutralised by the addition of two times the molar amount of ammonium solution. Fifty millilitres of ethyl acetate was also added and stirred for 2 h at room temperature. Subsequently, the organic layer were extracted and concentrated in vacuum to yield the ProOEt.

3. Chirality evaluation

Proline ethylester was hydrolysed to proline in 1 M NaOH solution for a day. The amount of L- and D-proline was measured by high performance liquid chromatography (HPLC) analysis with an optical resolution column (SUMICHIRAL OA-5000, 4.6 x 150mm). As a result, D-proline was not detected (i.e., under detection limit in the present experimental conditions). It was thus confirmed that the optical purity of the proline ethylester synthesized was sufficiently high.

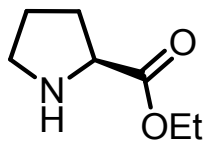
4. Synthesis of IL-API

The derived ProOEt was mixed with an equimolar amount of ibuprofen and stirred thoroughly at 40 °C for 2 h. Evaluation of purity of the derived material was carried out by ^{1}H-NMR analysis. Quantification of ibuprofen was conducted by employing a 4.6 × 150 mm ODS L-column (Chemical Evaluation and Research Institute, Japan), and a mobile phase composed of equivalent amount of 20 mM H_3PO_4 and methanol. The eluted ibuprofen was evaluated by measuring the absorbance at 230 nm and 40 °C.

Characterisation of IL constituents

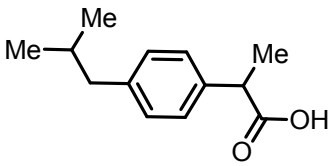
NMR : ^{1}H NMR spectra were obtained using a Bruker Ascend 400 MHz NMR spectrometer (Karlsruhe, Germany). Studies in deuterochloroform were conducted at 25 °C.

ProOEt



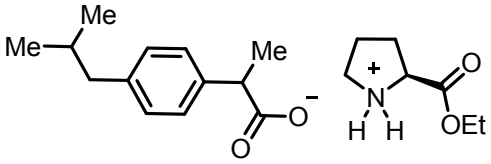
^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.28 (3H, t, 7.2 Hz), 1.72-1.88 (3H, m), 2.11-2.17 (1H, m), 2.52 (1H, brs), 2.90-2.96 (1H, m), 3.07-3.13 (1H, m), 3.77 (1H, dd, 6.0 Hz, 8.8 Hz), 4.19 (2H, q, 7.2 Hz).

Ibuprofen



^{1}H NMR ($CDCl_3$, 400 MHz): δ 0.89 (6H, d, 6.4 Hz), 1.50 (d, 3H, 6.4 Hz), 1.84 (1H, m), 2.44 (2H, d, 7.2 Hz), 3.71 (1H, q, 7.2 Hz), 7.10 (2H, d, 8.4 Hz), 7.22 (2H, d, 8.4 Hz).

Ibuprofen-ProOEt (**Fig. S1**)



^{1}H NMR ($CDCl_3$, 400 MHz): 0.89 (6H, d, 6.8 Hz) 1.25 (3H, t, 7.2 Hz), 1.44 (3H, d, 7.2 Hz), 1.68-1.72 (1H, m), 1.73-1.93 (3H, m), 2.14-2.23 (1H, m), 2.43 (2H, d, 7.2 Hz), 3.00-3.06 (2H, m), 3.61 (1H, q, 7.2 Hz), 3.99 (1H, dd, 8.8 Hz, 5.6 Hz), 4.18 (2H, q, 7.2 Hz), 7.06 (2H, d, 7.6 Hz), 7.23 (2H, d, 7.6 Hz), 8.98 (2H, s)

5. Skin permeation study

The skin penetration test was carried out with the ionic liquefied ibpurofen presented above. The control sample is a saturated solution of ibuprofen in equivolume of a mixture of phosphate buffer solution and ethanol. The phosphate buffer solution was prepared as follows. First, NaCl, KCl, Na_2HPO_4 and KH_2PO_4 were dissolved into distilled water to concentrations of 8 g/L, 0.2 g/L, 2.9 g/L and 0.2 g/L, respectively. The pH of the solution was then adjusted at 7.4 with HCl or NaOH.

Yucatan micro pig skin (female, 5 months of age) used for the skin permeation test was purchased from Charles River Laboratories International, Inc. First, the fat layer of skin was carefully removed, and placed into a Franz diffusion cell to fit the inner side of the skin with the receptor solution. As a receptor solution, 12 mL of equivolume of the mixture of phosphate buffered saline and ethanol was adopted with stirring at 37 °C. Then, 0.3 mL of ionic liquefied ibuprofen or control sample was drawn on the outer side of skin. Subsequently, an aliquot of sample was withdrawn from the receptor solution at fixed intervals for 48 or 96 h, and the concentration of ibuprofen in the solution was quantified by high performance liquid chromatography (HPLC) calibrated with the standard.

6. Cytotoxicity evaluation

Cytotoxicity of ProOEt was investigated using mouse fibroblast L929 cells. The cells were seeded in a 96-well cell culture dish at 5,000 cells/well and then cultured in Dulbecco's modified Eagle medium (DMEM) containing 10 vol% fetal bovine serum (FBS) for 15 h at 37 °C in a CO_2 incubator. ProOEt was dissolved in Krebs Ringer Hepes buffered solution (KRH, pH 7.4) at 200 mM. After adjusting the pH of the solution to 7.4, the solution was mixed with 3-fold volume of DMEM containing 10 vol% FBS. The resultant solution containing ProOEt was then diluted with a mixture of 25% KRH/75% medium to a final concentration of 0.01–50 mM ProOEt. Aliquots of the solutions, 200 μL, were replaced with the medium in each well containing L929 cells. After 20 h of incubation, the solution was removed from each well and the wells were rinsed with KRH twice. Then, 150 μL medium containing 1/20 vol. of the reagent in a Cell-counting Kit-8 (Dojindo Molecular Technologies, Inc., Kumamoto, Japan) was added to each well to measure the mitochondrial activity of the cells. After 2 h of incubation at 37 °C in a CO_2 incubator, the absorbance of the supernatant at 450 nm ($A_{treated}$) was measured using a spectrophotometer. As a control, the value obtained for cells soaked in the mixture of 25% KRH/75% medium not containing ProOEt ($A_{control}$) was used. The cytotoxicity was expressed as the relative viability using the following formula:

$$\text{Relative viability (\%)} = (A_{treated}/A_{control}) \times 100. \quad (1)$$

Experiments were performed five times for each ProOEt concentration and data are presented as the mean ± standard deviation. The same protocol was applied to all the other additives, proline, Pro-TBP, triethanolamine and choline hydroxide. The aqueous pH of the stock solution of each additive was checked before adding the culture broth.

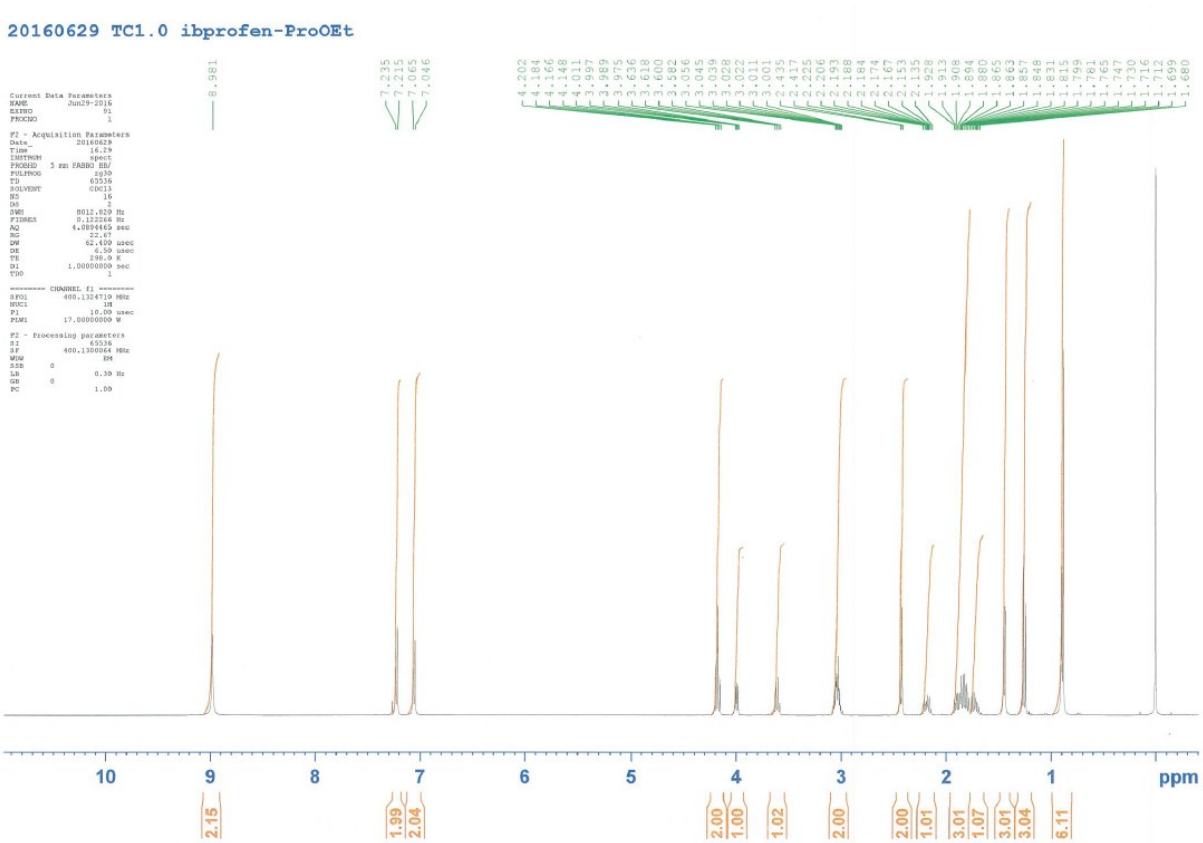


Figure S1. ^{1}H-NMR spectrum of ProOEt-ibuprofenate.