Supplemental Figures to:

Identification and validation of larixyl acetate as a potent TRPC6 inhibitor

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Molecular Pharmacology
Supplemental Figure 1: Structure and chemical characterisation of Larixol

R<sub>f</sub> = 0.02 (Hexan: EE 9:1 (v:v))

T<sub>m</sub> = 100°C

<sup>1</sup>H-NMR (300 MHz, CDCl<sub>3</sub>) δ [ppm] = 0.68 (s, 3H, 20-CH<sub>3</sub>), 0.99 (s, 3H, 18/19-CH<sub>3</sub>), 1.01-1.60 (m, 12H), 1.16 (s, 3H, 18/19-CH<sub>3</sub>), 1.13 (d, 1H, 5-CH), 1.26 (s, 3H, 16-CH<sub>3</sub>), 2.04 (t, 1H, 7-CH), 2.66 (dd, 1H, 7-CH), 3.82 (dt, 1H, 6-CH), 4.65 (dd, 2H, 17-CH<sub>2</sub>), 5.04 (dd, 1H, 15-CH, J<sub>cis</sub> = 10.37 Hz, J<sub>geminal</sub> = 1.26 Hz), 5.18 (dd, 1H, 15-CH, J<sub>trans</sub> = 17.37 Hz, J<sub>geminal</sub> = 1.26 Hz), 5.89 (dd, 1H, 14-CH, J<sub>trans</sub> = 17.37 Hz, J<sub>cis</sub> = 10.37 Hz)

<sup>13</sup>C-NMR (75 MHz, CDCl<sub>3</sub>) δ [ppm] = 16.3 (20-CH<sub>3</sub>), 18.3 (11-CH<sub>2</sub>), 19.4 (2-CH<sub>2</sub>), 22.6 (18/19-CH<sub>3</sub>), 28.0 (16-CH<sub>3</sub>), 34.1 (4-C), 36.9 (18/19-CH<sub>3</sub>), 39.6 (1-CH<sub>2</sub>), 39.8 (10-CH<sub>2</sub>), 41.6 (12-CH<sub>2</sub>), 44.0 (3-CH<sub>2</sub>), 49.4 (7-CH<sub>2</sub>), 56.7 (9-CH), 60.8 (5-CH), 71.9 (6-CH), 73.8 (13-C), 108.6 (17-CH<sub>2</sub>), 111.9 (15-CH<sub>2</sub>), 145.4 (14-CH), 145.8 (8-C)

HRMS [M+Na<sup>+</sup>] calculated 329.24565 Da, found 329.24510 m/z.
Supplemental Figure 2: Structure and chemical characterisation of larix-6-yl monoacetate

\( R_f = 0.30 \) (Hexan: EE 4:1(v:v))

\(^1\)H-NMR (300 MHz, CDCl\(_3\)) \( \delta [\text{ppm}] = 0.73 \) (s, 3H, 20-CH\(_3\)), 0.83 (s, 3H, 18/19-CH\(_3\)), 1.00 (s, 3H, 18/19-CH\(_3\)), 1.03-1.80 (m, 12H), 1.26 (s, 3H, 16-CH\(_3\)), 1.40 (d, 1H, 5-CH), 2.02 (s, 3H, Ac-CH\(_3\)), 2.66 (dd, 1H, 7-CH), 4.63 (m, 1H, 17-CH\(_2\)), 4.91 (m, 1H, 17-CH\(_2\)), 4.97-5.06 (m, 2H), 5.19 (dd, 1H, 15-CH), 5.89 (dd, 1H, 14-CH)

\(^{13}\)C-NMR (75 MHz, CDCl\(_3\)) \( \delta [\text{ppm}] = 16.2 \) (20-CH\(_3\)), 18.2 (11-CH\(_2\)), 19.2 (2-CH\(_2\)), 22.2 (Ac-CH\(_3\)), 22.7 (18/19-CH\(_3\)), 28.0 (16-CH\(_3\)), 33.7 (4-C), 36.4 (18/19-CH\(_3\)), 39.3 (1-CH\(_2\)), 40.1 (10-CH\(_2\)), 41.5 (12-CH\(_2\)), 43.7 (3-CH\(_2\)), 44.4 (7-CH\(_2\)), 56.6 (9-CH), 57.8 (5-CH), 73.5 (6-CH), 73.7 (13-C), 109.7 (17-CH\(_2\)), 111.9 (15-CH\(_2\)), 144.5 (14-CH), 145.4 (8-C), 170.3 (Ac-CO)

HRMS [M+Na\(^+\)] calculated 371.25621 Da, found 371.25567 m/z
Supplemental Figure 3: Potency of larixol and larixyl acetate to inhibit Ca\(^{2+}\) entry through TRPC6 in the receptor-induced mode of activation.

To assess whether the TRPC6 inhibitors are effective when TRPC6 is activated in a receptor-mediated fashion, fluo-4-loaded HEK\(_{\text{TRPC6}}\) cells were preincubated for 5 min with 2 µM thapsigargin in the presence of various concentrations of larixol (A) or larixyl acetate (B), and then stimulated with ATP (300 µM), carbachol (1 mM), and thrombin (0.5 U/ml) to induce activation of endogenous phospholipase C. Concentration-response curves were fitted to a four parameter Hill equation, and resulting \(IC_{50}\) and Hill coefficients are indicated. For comparison, the concentration response curve of TRPC6 inhibition in the OAG-activated mode (Fig. 3D,E of the main manuscript) is superimposed as dashed lines.
Supplemental Figure 4: Effect of larixyl acetate on voltage-gated Ca^{2+} channels (Ca_{V1.2}).

HEK293 cells were transiently transfected with expression plasmids encoding the α1c77, the β_{2a}, and the α_{2δ} subunits of Ca_{V1.2} along with a yellow fluorescent protein as transfection marker. (A) Ba^{2+} currents through recombinant Ca_{V1.2} channels were measured in voltage step protocols, after stepping from a holding potential of -70 mV to the indicated voltages. Shown are means and S.E. of peak current amplitudes determined in 20 patched cells. (B-E) Superposition of depolarisation-induced Ba^{2+} currents in the presence of the indicated modulators, and 160 s after their wash-out (black line). (F,G) Statistical analysis of modulator effects on peak current densities (F) and on inactivation time constants $\tau_{\text{inact}}$ (monoeXponential fits; G) calculated from 6-9 experiments performed as shown in (B-E). Filled bars: values in the presence of the indicated modulators; open bars: values after wash-out of the respective modulator.