Electronic Supplementary Material (ESI) for RSC Advances.

New transient receptor potential TRPV1, TRPM8 and TRPA1 channel antagonists from a single linear β,γ-diamino ester scaffold

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Electronic Supplementary Information

Animal behavioral assays

Male C57BL/6J mice (25-27 g) were obtained from in house-bred stock originally from Harlan Laboratories. All experiments were approved by the Institutional Animal and Ethical Committee of the Universidad Miguel Hernandez where experiments were conducted and they were in accordance with the guidelines of the Economic European Community and the Committee for Research and Ethical Issues of the International Association for the Study of Pain. All parts of the study concerning animal care were performed under the control of veterinarians. CFA emulsion (1:1 oil/saline, 0.5 mg/mL) was injected into the plantar surface (50 µL) of the left hind paw of mice [1]. Compound was administered at 2 mg/kg i.p. 24 h after CFA injection. Thermal hyperalgesia was monitored 24 h after CFA injection and up to 6 h after administering the compounds with an Ugo Basile Plantar Test (Hargreaves Apparatus). In brief, mice were habituated to an apparatus consisting of individual Perspex boxes on an elevated glass table. A mobile radiant heat source was located under the table and focused on the hind paw. Paw withdrawal latencies were defined as the time taken by the mouse to remove its hind paw from the heat source. A cutoff point of 25 s was set to prevent tissue damage. The mechanical allodynia was monitored 24 h after CFA injection and up to 6 h after administering the compounds. Paw withdrawal latency to mechanical stimulation was assessed with an automated testing device consisting of a blunt-end metal filament (0.5 mm in diameter) that is pushed against the plantar surface of the paw with increasing force until the paw is withdrawn (Dynamic Plantar Aesthesiometer; Ugo Basile). The maximum force was set at 50 g to prevent tissue damage and the ramp speed was 2.5 g/s. Mice were placed in test cages with a metal grid bottom. They were kept in the test cages for 30-40 min to allow accommodation. The paw withdrawal latency was obtained as the mean of 3 consecutive assessments at each time point (at least 10 s between repeated measurements of the same paw). Data are expressed as mean ± SEM. Analysis of the time course effects of compound **4b** in the CFA model was carried out with 2-way ANOVA with replicates followed by the Bonferroni post hoc test and significance level preset to $p<0.05$.

1. Ciardo, M.G., et al., *Whirlin increases TRPV1 channel expression and cellular stability*. Biochim Biophys Acta, 2015. **1863**(1): p. 115-127.

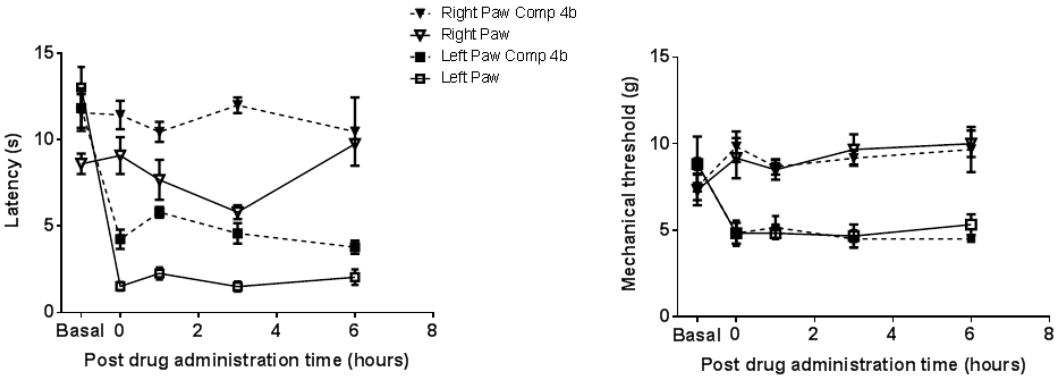

Figure S1. Effect of compound **4b** in the CFA-induced paw inflammation model.Time course of mechanical hyperalgesia in rats after injection of CFA 0.5 mg/mL (50µL) into the right hind paw with and without administration of compound (2 mg/kg, i.p.). The diagrams show the paw withdrawal latencies in response to a heat source (A) or mechanical stimulation (B) ($n \geq 6$ rats/group). Data are given as mean ± SEM n = 6.