Natural and synthetic flavonoid modulation of TRPC5 channels

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Introduction

TRPC proteins form tetrameric cation channels permeable to Na^+ and Ca^{2+} (Figure 1). TRPC channels can consist of homomers or heteromers of subunits (Figure 1B,C), each with their own biological functions. Several TRPC multimers have been implicated in human disease. For example, pro-inflammatory roles of heteromeric TRPC1/C5 channels in cardiovascular/metabolic disease and rheumatoid arthritis have been identified in Leeds.

Because of their key roles in signal transduction/integration and the role of specific TRPC channels in modulating pathological cellular phenotypes, TRPC proteins receive increasing attention as potential drug targets. However, small molecule pharmacology of TRPC channels is relatively under-developed. Although several natural and synthetic modulator series exist, they often lack potency, selectivity and/or a well-defined mode of action. Here we sought new TRPC5 channel modulators by testing natural products isolated from Traditional Chinese Medicines (TCMs).

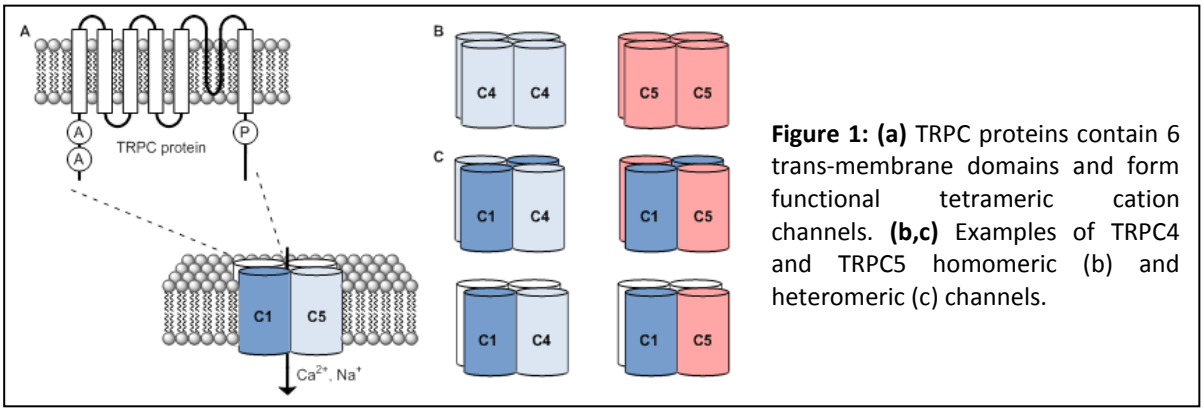

Figure 1: (a) TRPC proteins contain 6 trans-membrane domains and form functional tetrameric cation channels. **(b,c)** Examples of TRPC4 and TRPC5 homomeric (b) and heteromeric (c) channels.

Results

Through a screen of a small number of natural products from TCMs, we identified the flavonol galangin as an inhibitor of TRPC5 channels over-expressed in HEK 293 cells (IC_{50} 0.45 µM against TRPC5-mediated Ca^{2+} entry). Galangin also inhibited Ca^{2+} entry through endogenous TRPC5-containing channels, although with 5–15 times less potency. Galangin is a flavonol isolated from *A. officinarum* and other members of the ginger family. The related natural flavonols kaempferol and quercetin were also TRPC5 inhibitors, but with less potency than galangin. Myricetin and luteolin lacked effect, and the flavone apigenin had the reverse effect, stimulating TRPC5.

Structure-activity relationships of natural flavonols (Figure 2A) and a set of synthetic, mono-substituted flavonols (Figure 2B) led to the design of a new compound, AM12, which combines the hydroxylation pattern of galangin with an ortho-Br substituent on the phenyl ring (Figure 2C). AM12 inhibited TRPC5-mediated Ca^{2+} entry with an IC_{50} of 0.28 µM (Figure2D,E). In subsequent electrophysiology experiments, AM12 was shown to rapidly inhibit both TRPC5 and TRPC4 channels, and – to a lesser extent – heteromeric TRPC1/C5 channels. AM12 was highly selective with respect to TRPC3, TRPV4 and TRPM2 channels, and endogenous Ca^{2+} release signal evoked by thapsigargin or ATP.

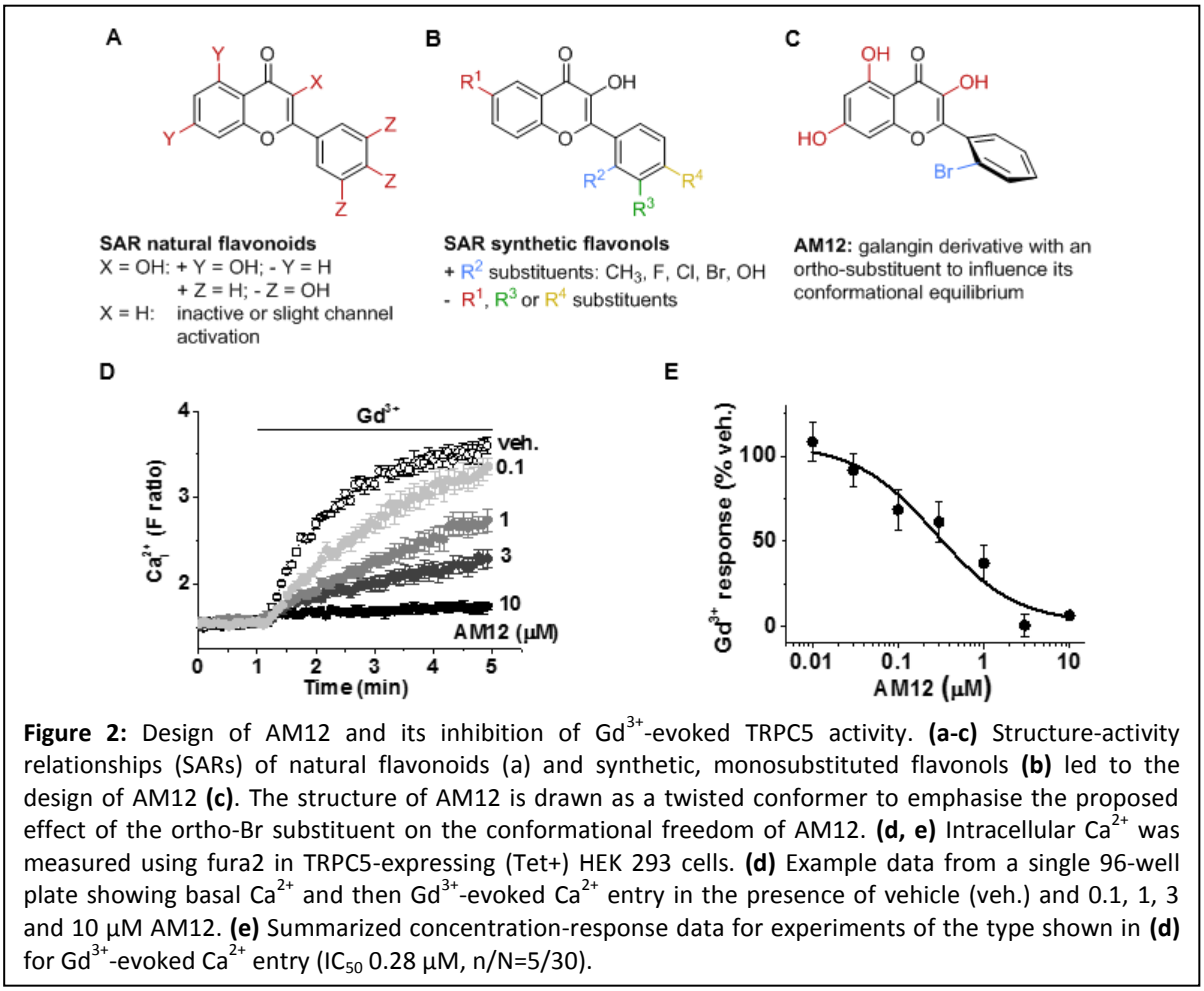

Figure 2: Design of AM12 and its inhibition of Gd^{3+}-evoked TRPC5 activity. **(a-c)** Structure-activity relationships (SARs) of natural flavonoids (a) and synthetic, monosubstituted flavonols **(b)** led to the design of AM12 **(c)**. The structure of AM12 is drawn as a twisted conformer to emphasise the proposed effect of the ortho-Br substituent on the conformational freedom of AM12. **(d, e)** Intracellular Ca^{2+} was measured using fura2 in TRPC5-expressing (Tet+) HEK 293 cells. **(d)** Example data from a single 96-well plate showing basal Ca^{2+} and then Gd^{3+}-evoked Ca^{2+} entry in the presence of vehicle (veh.) and 0.1, 1, 3 and 10 µM AM12. **(e)** Summarized concentration-response data for experiments of the type shown in **(d)** for Gd^{3+}-evoked Ca^{2+} entry (IC_{50} 0.28 µM, n/N=5/30).

TRPC5-containing endogenous channels are up-regulated in differentiated adipocytes and inhibition of channel function in vivo by a dominant-negative mutant TRPC5 raises circulating adiponectin levels, which is expected to have a cardioprotective effect. Therefore flavonoids may act as natural regulators of adipocyte biology at least in part via modulation of Ca^{2+} entry through TRPC5-containing channels, conferring a mechanism for integration with the environment via dietary intake. It should be noted, however, that flavonoids are not specific for TRPC5 channels. In addition, the effects of synthetic flavonols on TRPC5 activity show that potency and mode of action of flavonols on TRPC5 channels depends strongly on subtle changes of substituent patterns.

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