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Original article

Synthesis and structure–activity relationship study of substituted caffeate esters as antinociceptive agents modulating the TREK-1 channel

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ABSTRACT

The TWIK-related K^+ channel, TREK-1, has recently emerged as an attractive therapeutic target for the development of a novel class of analgesic drugs. It has been reported that TREK-1 −/− mice were more sensitive than wild-type mice to painful stimuli, suggesting that activation of TREK-1 could result in pain inhibition. Here we report the synthesis of a series of substituted caffeate esters (**12a**–**u**) based on the hit compound CDC **2** (cinnamyl 3,4-dihydroxyl-α-cyanocinnamate). These analogs were evaluated for their ability to modulate TREK-1 channel by electrophysiology and for their *in vivo* antinociceptive activity (acetic acid induced-writhing assay) leading to the identification a series of novel molecules able to activate TREK-1 and displaying potent analgesic activity *in vivo*.

1. Introduction

Ion channels are major drug targets whose study can contribute to the understanding of the physiopathology of pain and the evolution of therapeutic analgesics [1–3]. The TREK-1 (TWIK-related K^+ channels 1) channel belongs to the two pore-domain potassium (K2P) channel family and was first identified in 1998 [4]. Human TREK-1 is highly expressed in the peripheral sensory system, particularly in small dorsal root ganglion (DRG) neurons that are associated with nociception [5,6]. The functionality of the TREK-1 channels results from their sensitivity to a variety of stimuli. They are gated by membrane stretching (mechano-activation), pH (intracellular acidosis), temperature (heat), polyunsaturated fatty acids (PUFAs) such as arachidonic acid, and some general volatile anesthetics (chloroform, diethyl ether, and nitrous oxide). They can also be down-modulated by PKA/PKC phosphorylation pathways and tonically inhibited by the actin cytoskeleton [7]. Structurally, the TREK-1 channels are transmembrane (TM) proteins with intracellular N- and C-termini [8]. The pore for K^+ conduction results from the assembly of two dimers, each containing four helical transmembrane segments (TM1–TM4), arranged in tandem with two pore domains (Fig. 1).

Recent studies have reported that mice with a disrupted TREK-1 gene (TREK-1 −/−) were more sensitive than wild-type mice to

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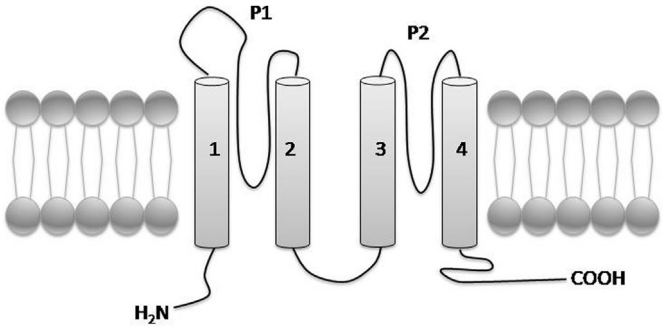

Fig. 1. Topology of TREK-1 channel.

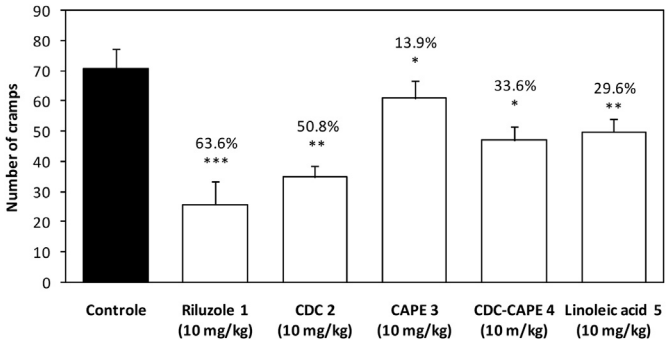

Fig. 3. Antinociceptive effect of compounds **1**–**5** (10 mg/kg, s.c.) against acetic acid-induced abdominal writhes in mice ($n = 8$). Each bar represents the mean ± SEM of eight experimental values. The percentage (%) of writhes inhibition (as compared to vehicle) is given above each bar and in Table 1. *t*-Test: *≤0.05, **≤0.01, ***≤0.001.

heat and mechanical stimulations [9,10], suggesting that TREK-1 channels are important in pain perception. TREK-1 channel could represent a novel molecular target of interest for the discovery of a new class of analgesics [11].

2. Results and discussion

2.1. Identification of hit molecules

A literature survey allowed us to identify several small organic molecules able to module the TREK-1 channels. Riluzole **1** (Fig. 2), a neuroprotective agent currently used in the treatment of amyotrophic lateral sclerosis [12], was reported as an activator of TREK-1 [13,14]. This activation was reported to be transient followed by an inhibition, process attributable to an increase in the intracellular cAMP concentration by riluzole that produces a PKA-dependant inhibition of TREK-1. Cinnamyl 3,4-dihydroxyl-α-cyanocinnamate (CDC **2**, Fig. 2) and caffeic acid phenylethyl ester (CAPE **3**, Fig. 2) were also reported as TREK-1 activators [15]. Danthi et al. carried out a structure–activity relationship (SAR) study but failed to improve the gating activity of the caffeate esters. Because of the reported activities of **2** and **3**, we also decided to consider the CAPE–CDC hybrid molecule **4** [16,17]. Finally, Danthi et al. also reported that polyunsaturated fatty acids, such as linoleic acid **5** (Fig. 2), were able to activate TREK-1 channels [18].

The antinociceptive activity of compounds **1**–**5** (10 mg/kg, administered subcutaneously) was evaluated by using the acetic acid-induced writhing test in mice. Compared to the controlled mice (vehicle), all the compounds were able to inhibit the induced abdominal writhes (Fig. 3, Table 1). The most active compounds were found to be riluzole **1** (63.6% inhibition) while linoleic acid **5** showed a moderate analgesic activity (29.6% inhibition). CDC **2** showed potent analgesic activity (50.8% inhibition) while CAPE **3** and CDC–CAPE-hybrid **4**, which are structurally similar to **2**, showed low to moderate antinociceptive effect with respectively 13.9% and 33.6% inhibition. This result correlated with the ability of these agents to activate TREK-1, since 10 μM of CDC is required to increase TREK-1 current by 7-fold compared to 40 μM of CAPE [15]. These results suggest that the distance between the two aromatic rings and/or the substitution at the α-position of the ester could be an important feature for optimal pharmacological activity.

Although it has been found to possess high analgesic activity, riluzole **1** is non-specific for TREK-1 activation. The drug has been widely studied and many pharmacological activities have been associated with this molecule [19–22]. However no bioactivity data have been reported on CDC **2**, which also displays a good drug-like profile (Fig. 4A and B) [23]. We further evaluated the analgesic activity of CDC **2** in the acetic acid-induced writhing assay and determined an ID_{50} of 8.0 mg/kg (Fig. 4C). No noticeable sedative or adverse effect was observed at the doses tested.

2.2. Synthesis of substituted caffeate esters

Since no information was available about the binding of CDC **2** to the TREK-1 channel, and the 3D structure of TREK-1 was not available at the time, [8] we decided to perform hit optimization by conventional structure–activity relationship (SAR) studies around the general structure **12**, studying the influence of various substituents (R^1–R^3, X) on the pharmacological activity of the analogs. We developed two routes to obtain α-substituted cinnamate esters **12** (Scheme 1). Condensation of aldehyde **9** with carbonyl compound **8**, which could be obtained from the carboxylic acid **7** and synthon **6** (route A), would lead to analogs **12** with variations in R^1, R^2 and X. Alternatively esterification of cinnamic acid derivatives **10** with synthon **6**, the α,β-unsaturated acid emanating from a condensation between aldehyde **9** and carboxylic acid **7** (route B) would lead to analogs **12** bearing different R^3 substituents.

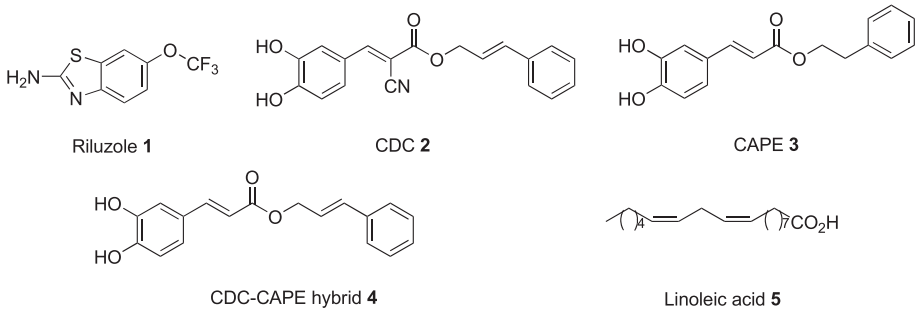

Fig. 2. The chemical structures of compounds **1**–**5**.