European Journal of Medicinal Chemistry 66 (2013) 193–203

Contents lists available at SciVerse ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Original article

Design and synthesis of conformationally restricted capsaicin analogues based in the 1, 3, 4-thiadiazole heterocycle reveal a novel family of transient receptor potential vanilloid 1 (TRPV1) antagonists

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ARTICLE INFO

Article history:
Received 28 December 2012
Received in revised form
12 April 2013
Accepted 1 May 2013
Available online 21 May 2013

Keywords:
TRPV1 receptor
Restriction conformational
Capsaicin analogues
1, 3, 4-thiadiazole
Antagonist activity

ABSTRACT

4-hydroxy-3-methoxybenzaldehyde was used as starting material to obtain a number of 1, 3, 4-thiadiazole alkylamide derivatives. The pharmacological properties of these conformationally restricted capsaicin analogues were evaluated on HEK-293T cells transiently expressing TRPV1 receptor. By means of a highthroughput calcium imaging assay we find that 1, 3, 4-thiadiazoles (compounds **8**–**15**) act as potent antagonists of the capsaicin receptor, inhibiting both, the capsaicin- and temperature-dependent activation. Docking studies suggested a different binding orientation on the vanilloid binding site when compared with capsaicin analogues, such as 5-iodononivamide. Overall, our studies suggest that 1, 3, 4-thiadiazoles interact with capsaicin's binding region of the receptor, although using a different set of interactions within the vanilloid binding pocket.

1. Introduction

Transient receptor potential vanilloid 1 (TRPV1) proteins are non-selective, ligand-gated cation channels with permeability to calcium ions. They are well described as polymodal nociceptors allowing for the integration of multiple stimulus during the activation process. These include capsaicin, pH, PIP2, T° >43, and voltage [1–3]. TRPV1 receptors have a direct role in the physiology and physiopathology of the pain responses showing a strong correlation with their tissue expression, which includes peripheral terminals as well as specific brain areas related with the nociceptive process [4]. Thereby, TRPV1 receptors represent an important pharmacological target, which already have provided a broad diversity of drugs engineered for the treatment of inflammatory chronic and neuropathic pain [5,6].

Capsaicin was the first TRPV1 agonist described, even though its effects, such as burning pain, increment of the intracellular calcium and depolarization of DRG sensory neurons, and potential analgesic effects, were known long before to the identification of the receptor [7,8]. Structurally, capsaicin has been an important lead for drug development targeting TRPV1 receptors, leading to the synthesis and identification of important analogues with well described modifications [9–15]. This has allowed the identification of the minimal structural requirements essential to modulate TRPV1 activity. According to this, both capsaicin and capsaicin-like compounds can be divided into three pharmacophoric structural features (Fig. 1). These correspond to a region A with capacity of forming hydrogen bonds [9]; a polar region B [10], and a lipophilic region C [11]. Modifications performed on these regions have allowed the identification of a great diversity of compounds with antagonist activity [9–22]. Within the modifications described so far, stand out the antagonist effect induced by iodine atom in capsaicin analogues, such as 5-iodononivamide or 6-iodononivamide [25]. Likewise, modifications in the region B such as the substitution of the amide group by the urea or thiourea group notably increased the agonist or antagonist activity in both capsaicin analogues as in non-capsaicin-like compounds [10,16].

In order to increase the antagonist potency, several optimization strategies have been incorporated in TRPV1 compounds, in which the conformational restriction of linker groups has shown to be a useful way for this purpose. The conformational restriction has been

Abbreviations: DRG, dorsal root ganglion; rTRPV1, rat TRPV1; cTRPV1, chicken TRPV1; TLC, thin layer chromatography; Rf, retention factor.

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0223-5234/$ – see front matter © 2013 Published by Elsevier Masson SAS.
http://dx.doi.org/10.1016/j.ejmech.2013.05.001

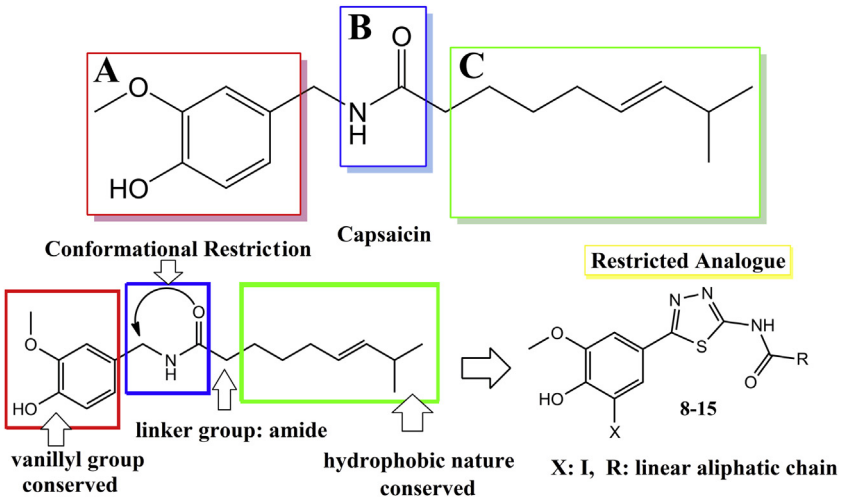

Fig. 1. Pharmacophoric features for capsaicin and design of conformationally restricted capsaicin analogues.

incorporated mainly in the linker group located between regions B and C, through of the substitution by polar heterocyclic groups, such as pyrimidine [17], oxazol [18], thiazol [19] among other groups, achieving potent compounds known as conformationally restricted TRPV1 antagonist. However, the linker group between regions A and B corresponding to methylene or amino groups, has been conserved in these kind of antagonists because the absence or the substitution by non-flexible groups, such as carbonyl group has shown to cause lost or decreasing of the activity on TRPV1 receptors [17–22]. Although it is true, that many compounds with restriction conformational have been reported as potent antagonist TRPV1 and several modifications have been studied for expand the three pharmacophoric areas mentioned before, the changes on the linker group between regions A and B through conformational restriction with heterocyclic groups is little frequent.

Thereby, in this work have been proposed the design of conformationally restricted capsaicin analogues by replacing the amide group of capsaicin with the 1, 3, 4-thiadiazole heterocycle, followed by the incorporation of an amide group as linker group between regions B and C (Fig. 1). This isosteric replacement has allowed evaluating both the isosteric efficiency of this heterocycle and the effect of conformational restriction between regions A and B for these capsaicin analogues, which have not been evaluated before through of the substitution by a heterocyclic group. Thus, from 4-hydroxy-3-methoxybenzaldehyde as starting material, were carried out several steps of synthesis, which allowed obtaining 1, 3, 4-thiadiazole alkylamides (**8**–**15**) as novel conformationally restricted capsaicin analogues. These analogues included both iodinated derivatives on the fifth position of vanillyl group and non-iodinated derivatives, in order to evaluate the effect induced by iodine atom on this kind of derivatives, due to the differences of agonist effect and antagonist effect, observed between compounds non-iodinated and iodinated, respectively on TRPV1 receptors [14,17]. So, as a consequence of the high polarity and the conformational restriction induced by 1, 3, 4-thiadiazole heterocycle, together with the effect of the amide group as linker group were obtained a novel family of potent TRPV1 antagonists.

2. Results and discussion

2.1. Chemistry

The 1, 3, 4-thiadiazole alkylamide derivatives (**8**–**15**) were synthetized from 4-hydroxy-3-methoxybenzaldehyde in three steps. Previously, 4-hydroxy-3-methoxybenzaldehyde was iodinated (Scheme 1-I) through *in situ* oxidation of potassium iodide with commercial hypochlorite (4.7%). This iodination method allowed obtaining the monoiodinated product (**1**), with a yield of 72%.

According to Scheme 1-II the first step consisted in the synthesis of heterocycle 1, 3, 4-thiadiazole through oxidative cyclization of the thiosemicarbazones **4** and **5** with an aqueous solution of ferric chloride. Thus, 2-amino-1, 3, 4-thiadiazole **6** and **7** were obtained with good yields that corresponded to 60% and 45% respectively. The next step, involved the formation of amides between 2-amino-1, 3, 4-thiadiazole **6** or **7** and acyl derivatives coming from linear aliphatic carboxylic acids with chains of different longitude. According to Scheme 2, the carboxylic acids were converted to acyl chlorides with thionyl chloride. Later, *in situ* and at room temperature the 2-amino-1, 3, 4-thiadiazole **6** or **7** was added into the reaction medium followed by the addition of pyridine. The esterified amide derivatives were obtained by this method, therefore the last step of synthesis consisted in an alkaline hydrolysis reaction with NaOH 0.1 M under reflux at 60 °C approximately for achieve the formation of a medium of reaction homogenous. This method led to obtaining the 1, 3, 4-thiadiazole alkylamide derivatives **8**–**15**, which were purified by chromatography and crystallization, achieving satisfactory yields of synthesis.

2.2. Biological evaluation

All the 1, 3, 4-thiadiazole derivatives obtained were tested in transiently transfected HEK-293T cells expressing rat TRPV1 (rTRPV1). The biological activity of the derivatives was evaluated on the activation of the rTRPV1 receptor by temperature and capsaicin. TRPV1 activation was measured by means of a highthroughput calcium influx assay; following the fluorescence of Fluo-4AM loaded cells in a real-time PCR thermocycler [23]. This method allowed us to follow multiple conditions simultaneously during a temperature ramp as well as to maintain controlled temperatures.

First, the response to temperature changes for control conditions was evaluated from 22 °C to 56 °C. These results are shown in Fig. 2a in which the temperature-induced calcium influx was normalized with respect to the fluorescence obtained at 50 °C for rTRPV1. The temperature activation curve for rTRPV1 receptors rose around 40 °C and it saturated at 54 °C. The calcium influx induced by increasing temperature was modest or not observed in the cultures previously incubated with reported TRPV1 blockers, such