Bioorganic & Medicinal Chemistry 26 (2018) 2632–2639

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Design, synthesis and biological evaluation of novel hydrogen sulfide releasing capsaicin derivatives

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ARTICLE INFO

Article history:
Received 9 March 2018
Revised 13 April 2018
Accepted 13 April 2018
Available online 17 April 2018

Keywords:
Capsaicin
Hydrogen sulfide
TRPV1 agonist
Analgesic
Anticancer
Gastric mucosa irritation

ABSTRACT

Capsaicin (CAP), the prototypical TRPV1 agonist, is the major active component in chili peppers with health-promoting benefits. However, its use is limited by the low bioavailability and irritating quality. In this study, for improving the activity of CAP and alleviating its irritating effects, a series of H_2S-releasing CAPs were designed and synthesized by combining capsaicin and dihydro capsaicin with various hydrogen sulfide donors. The resulting compounds were evaluated their TRPV1 agonist activity, analgesic activity, anticancer activities, H_2S-releasing ability, and gastric mucosa irritation. Biological evaluation indicated that the most active compound **B_9**, containing 5-(4-hydroxyphenyl)-3*H*-1,2-dithiole-3-thione moiety as H_2S donor, had better analgesic activity and displayed more potent cytotoxic effects on the test cell lines than the lead compound CAP. Furthermore, the preferred compound, **B_9** reduced rat gastric mucosa irritation caused by CAP. Notably, the improved properties of this derivative are associated with its H_2S-releasing capability.

1. Introduction

Capsaicin (*trans*-methyl-*N*-vainillyl-6-nonenamide, CAP) is a well-known natural product that is consumed worldwide and has been used as a spice, food additive, and drug.[1] Research on CAP's biological effects has demonstrated its analgesic,[2,3] anticancer,[4–9] anti-inflammation,[10,11] cardioprotective,[12] antioxidation,[13–15] and antiobesity activities,[16,17] which function through activating the transient receptor potential vanilloid 1 (TRPV1).[2,13,18] TRPV1 is a non-selective, ligand-operated cationic channel located primarily in the small fibers of nociceptive sensory neurons.[19] Furthermore, this receptor is the first membrane receptor that exhibits a tumor-suppressing effect associated with the down regulation of the EGFR.[20,21] However, CAP is classified as an irritant, causing localized burning sensation, erythema, or stinging, and aerosolized CAP can induce coughing or sneezing,[22] and as a lipophilic substance, CAP's low aqueous solubility greatly hinders its oral bioavailability.[23] Although efforts have been made to modify the original structure of CAP, no derivatives with improved pharmaceutical and pharmacological profile and reduced its irritation have been reported to date. Thus, there is an urgent need to develop a new strategy to modify the structure of CAP.

Hydrogen sulfide (H_2S) as a signaling and/or effector molecule plays a pivotal role in diverse physiological and pathophysiological processes including inflammation, cancer, pain perception, cardio protection, atherosclerosis, neuro modulation, hypertension, hemorrhagic shock, and gastric mucosal integrity.[24] A number of hybrid compounds which deals with the covalent incorporation of a H_2S donating group into the structure of a biologically active derivative, are currently under intensive investigation possessing a broad spectrum of activities.[25] In particular, because of its anti-inflammatory properties and cytoprotective effects, adjunctive H_2S has been considered aplausible means for improving the anti-inflammatory activity and safety of drugs. Structurally diverse H_2S-releasing nonsteroid anti-inflammatory drugs (NSAIDs) have been developed.[26–29] Several H_2S-donor NSAIDs exhibit greater anti-inflammatory and anti-cancer properties than their parent drugs but with less gastrointestinal and cardiorenal toxicity; and these H_2S releasing NSAIDs may even promote ulcer healing. Therefore, these results prove that the linkage of an H_2S releasing moiety to a biologically active agent may reduce the toxicity and improve the activity of the original compound.

On the basis of the above-mentioned studies, we hypothesized that hybridization of such H_2S-releasing moieties with CAP may release H_2S to exert synergistic biological activities with CAP. In addition, recent studies showed that the absence of a free 4-hydroxyl group of CAP could reduce its pungency, and they will be more suitable for eating and may improve lipid metabolism in humans

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https://doi.org/10.1016/j.bmc.2018.04.031
0968-0896/

through blocking the phenolic OH of capsaicin.[30] To test this hypothesis, we designed and synthesized a series of novel H_2S-donating derivatives, which were derived through the conjugation of capsaicin or dihydrocapsaicin at the C-4 positions to H_2S-donor substructures and were endowed with various H_2S-releasing capabilities. Their TRPV1 agonist activity, analgesic activity *in vivo*, *in vitro* anticancer activities, H_2S-releasing ability, and gastric mucosa irritation were biologically evaluated.

2. Results and discussion

2.1. Chemistry

We designed and synthesized a series of hydrogen sulfide releasing derivatives of capsaicin, including 14 compounds which were the direct combination of capsaicin and dihydrocapsaicin with various H_2S donors at the C-4 positions. Compared to the inorganic salts such as sodium sulfide (Na_2S) and sodium hydrogen sulfide (NaHS) salts, organic H_2S donors are able to release H_2S slowly and continuously at low concentrations similar to endogenous levels. Thus, a number of organic H_2S donors have been developed to date, including anethole dithiolethione (ADT), and its metabolite 5-(4-hydroxyphenyl)-3*H*-1,2-dithiole-3-thione (ADT-OH), 4-hydroxythiobenzamide (4-OH-TBZ), arylthiobenzamides, phosphordithioates (such as GYY4137), etc, which based on diverse mechanisms of H_2S release.[31,32] In this study we used one of the following moieties, ADT-OH, or 4-OH-TBZ, or 4-carbamothioylbenzoic acid for H_2S release and attached it to the capsaicin and dihydrocapsaicin at the C-4 positions. The target compounds were shown in the Table 1. The chemical synthesis details were presented in the experiment section.

2.2. Structure-activity relationship (SAR) analysis

The new compounds were tested for TRPV1 agonist activity, analgesic and anticancer activities, hydrogen sulfide release activity, and gastric mucosa irritation. Firstly, all of the new compounds were tested for TRPV1 agonist activity. In the TRPV1 agonist high throughput screening model, all the compounds enhance Ca^{2+} inflow (Table 2). Especially, compound **B_9** exerted potency better than the positive control capsaicin and dihydrocapsaicin.

Next, the antinociceptive activity *in vivo* of each compounds were evaluated by capsaicin test, abdominal constriction test, and tail-flick test (Fig. 1). The total time spent licking the paw was reduced by all test compounds in the capsaicin test and compounds **B_1**, **B_9** exhibited better potency than the lead compound capsaicin and dihydrocapsaicin. In the abdominal constriction test, basically all compounds reduced the number of writhes in proto-induced pain model, especially **B_9** was superior to positive control BCTC and capsaicin. In the tail-flick test, compounds **B_1**, **B_9** showed similar %MPE as BCTC. In this assay, all the test compounds had antinociceptive activity to a certain extend. Nevertheless, the ADT-OH derivatives of capsaicin **B_1** and **B_9** exhibited better antinociceptive activity than other compounds.

Subsequently, we evaluated the antitumor activity of the target compounds on antiproliferative activity by MTT assay using the human erythroleukemia K562 cell, human cervicai cancer Hela cell and the human breast MCF-7 cancer cell lines. We chose doxorubicin (DOXO) as the positive control, which is one of the most effective antineoplastic agents in clinical practice.[33] The results were summarized in Table 3. Some of the compounds exhibited moderate cytotoxic activity in the evaluation. And it is notable that basically all of H_2S-CAP derivatives displayed more potent than the lead compound CAP in its antiproliferative activity against K562, Hela and MCF-7 cells, suggesting that the presence of a H_2S-releasing group may be important to improve CAP's efficacy in tested cell lines. Furthermore, the types of the H_2S donor moiety, the type and length of the linkers which connected H_2S donor moiety to CAP, were important for compounds' activities. Analysis of structure activity relationships (SAR) among these compounds revealed that ADT-OH was crucial for the antiproliferative activity of H_2S-CAP hybrids because the ADT-OH -substituted H_2S-CAP with a linker bearing a acyl chain (**B_9**, **B_{10}**) showed strong cytotoxicity against the tested cell lines *in vitro*.

Furthermore, we tested whether such compounds also produced H_2S-associated biological effects *in vitro*. Current techniques for H_2S detection described in the literature include colorimetric[34,35] and electrochemical assays,[36] gas chromatography,[37] metal-induced sulfide precipitation,[38] and HPLC method.[39,40] However, these methods had a variety of disadvantages and difficult to implement for *in situ* detection, fluorescence spectroscopy might become the most attractive technique for *in vivo* detection of biorelated species by virtue of its high-sensitivity, real-time spatial imaging, and non-damaging detection in living cells or tissues.[41–43] The newly synthesized compounds **B_1**, **B_9**, **B_{11}** and **B_{13}** were tested using a fluorometric assay based upon the reaction of H_2S with dansyl azide (DNS-Az) to give the fluorescent related amide, which was detected with a HPLC system equipped with a fluorimetric detector.[44] The results of H_2S release in DMEM are reported in Fig. 2. After 1 h, lower amounts of H_2S were detected in this system, near to the limit of detection of the assay. Longer incubation times higher amount of H_2S were detected in most compounds, showing a steady release profile suitable for an H_2S therapeutic. Nevertheless, the 4-OH-ADT derivatives **B_1** and **B_9** produced high amounts of H_2S, whereas the 4-OH-TBZ derivative **B_{11}** as well as the 4-COOH-TBZ derivative **B_{13}**, released lower levels of H_2S. The most potent H_2S donor was **B_9**, a derivative of CAP bearing the 4-OH-ADT moiety through an ester linkage.

At last, mice were used to study whether the gastric mucosal irritation response caused by CAP could be relieved through blocking the phenolic OH of capsaicin. Previous research indicated that the oral LD_{50} value of capsaicin for male rats is 161.2 mg/kg.[45] Furthermore, long-term capsaicin treatments of >90 days and high doses (90–250 mg of capsaicin per day) have been shown as toxic and potentially carcinogenic, whereas small doses of capsaicin (0–29.9 mg of capsaicin per day) led to no or few deleterious effects in rodents.[46,47] On the basis of reports, a dose of 30 mg/kg of body weight was adopted in this paper. Oral administration of CAP suspension for 6 days induced marked ulcerative lesions on the gastric mucosal surface and exhibited high ulcer score, whereas other test compounds exhibited low ulcer scores (Fig. 3). The above results suggested that the H_2S-donating capsaicins possessed a reducing effect against irritation in rat stomach tissues induced by CAP.

3. Conclusions

In conclusion, we have synthesized a new series of H_2S-releasing derivatives of capsaicin as well as dihydrocapsaicin to improve its pharmaceutical profile, in view of their ability to release H_2S. In this study, we evaluated these new hybrids for their TRPV1 agonist activity, analgesic activity, anticancer activities, H_2S-releasing ability, and ulcerogenic action. The combined H_2S-releasing moiety strongly influenced the products' biological behavior. All H_2S-CAPs were able to enhance Ca^{2+} inflow, and most of them had antinociceptive activity to a certain extend. In addition, when tested on K562, Hela and MCF-7 cell lines, basically all of H_2S-CAP derivatives displayed more potent cytotoxic effects than those of the lead. Overall, compound **B_9** emerged as the most interesting member of the series, and animal studies showed that the gastric mucosa irritation caused by CAP was alleviated effectively. The