Bioorganic & Medicinal Chemistry 26 (2018) 4567–4573

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

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

Novel benzodiazepines derivatives as analgesic modulating for Transient receptor potential vanilloid 1

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ARTICLE INFO

Keywords:
Benzodiazepine
Transient receptor potential vanilloid 1
Analgesic
Sedation

ABSTRACT

A new series of derivatives of 3-(7-chloro-5-(2-fluorophenyl)-2-oxo-2,3-dihydro-1*H*-benzo[e][1,4]diazepin-3-yl) propanoic acid were designed and synthesized as analgesic modulating for Transient receptor potential vanilloid 1. They were investigated for TRPV1 antagonistic activity in vitro, analgesic activity and sedative activity in vivo and aqueous solubility. Preliminary studies identified 3-(7-chloro-5-(2-fluorophenyl)-2-oxo-2,3-dihydro-1*H*-benzo[e][1,4]diazepin-3-yl)-N,N-dimethylpropanamide(Compound **11**), as a potent analgesic modulating for TRPV1 with potent activity and good aqueous solubility.

1. Introduction

Pain is an unpleasant feeling and also a global health challenge, which has strong clinical demand. In the past two decades, advancements have come in the form of quality improvement initiatives, the introduction of novel analgesics, the advent of innovative techniques and improved understanding of pain signaling.[1] Unfortunately, most analgesic drugs on the market today is saddled by some side-effects.[2] Transient receptor potential vanilloid type 1 (TRPV1) is a non-selective cation channel and found on unmyelinated C fibers in the periphery, for which many have been developed and entered clinical trials for the treatment of pain.[3,4] TRPV1 antagonists are usually made up of three parts, including: aryl interaction head, H-bond interaction linker and lipophilic sidechain tail.[5] For example, the TRPV1 antagonist BCTC (**1**), containing the three parts described above, possesses the potent analgesic activity.

Moreover, benzodiazepines drugs, which act on γ-aminobutyric acid type A ($GABA_A$), have a strong sedative anesthesia effect and are widely used in postoperative analgesia[6] (Fig.1), Diazepam (**2**) is usually used to be as sedative-hypnotic medicine. And Midazolam (**3**) was optimized from Diazepam, which possesses potent sedative and anesthetic activity.[7] Furthermore, optimization of adding the side chain of methyl propionate make Remimazolam (**4**) become safer and more effective, for its metabolite having very little activity, so that Remimazolam exerts and loses efficacy quickly.[8] However, a variety of side effects including dependence, unwanted sedation and amnesia, complicating their long-term use.[9–12] Thus, there is a great need for novel, potent analgesic drugs with improved safety and tolerability (Fig. 2 and 3).

In this context, in order to keep analgesia potency and reduce the side effects, we regard aromatic area of benzodiazepine derivatives as aryl interaction head, and γ-aminobutyric as linker, and connect them to different lipophilic sidechain by using amide bond, resulting in novel benzodiazepine derivatives as analgesic modulated for Transient receptor potential vanilloid 1.

2. Result and discussion

2.1. Chemistry

The synthetic route employed to prepare benzodiazepine derivatives **11–23** is shown in Scheme 1. Commercially available 2-Amino-5-chloro-2′-fluorobenzophenone reacted with Fmoc-L-Glu(OMe) under the catalysis of dicyclohexylcarbodiimide (DCC) in dichloromethane at room temperature for 4 h to produce intermediate **7**. The Fmoc protecting group of intermediate **7** then was removed by dissolving the intermediate in the mixed solvents of trimethylamine-dichloromethane, and the seven-membered ring was synthesized by dissolving in the mixed solvents of acetic acid-ethanol, producing methyl 4-amino-5-((4-chloro-2-(2-fluorobenzoyl)phenyl)amino)-5-oxopentanoate. Hydrolyzing methyl ester in THF-MeOH-H_2O with LiOH produced

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https://doi.org/10.1016/j.bmc.2018.08.002
Received 3 July 2018; Received in revised form 28 July 2018; Accepted 1 August 2018
Available online 01 August 2018
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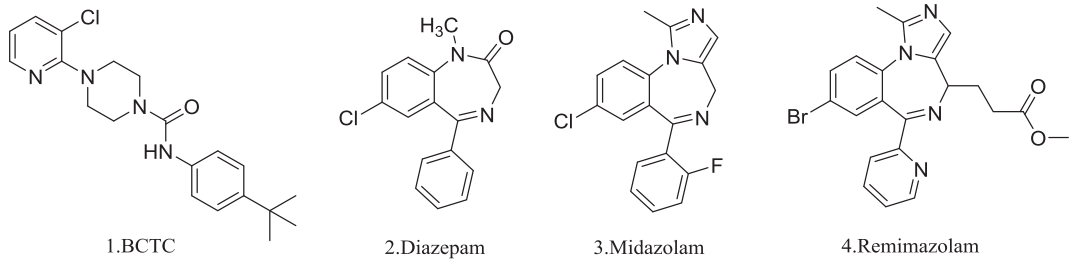

Fig. 1. Structure of BCTC and benzodiazepine sedative drugs.

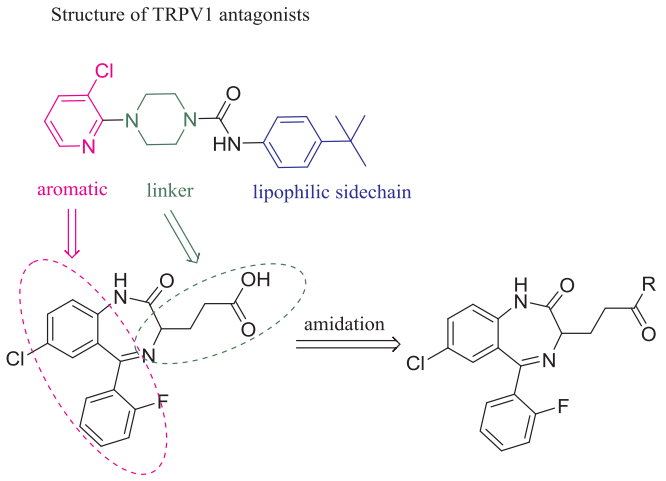

Fig. 2. Modified benzodiazepines structure by amidation according to the common structure of TRPV1 antagonists.

intermediate **10**. Compound **10** reacted with differently substituted primary amines or amino heteroaromatic compounds to produce corresponding targets **11–23** in moderate yields. Total yield range is 40 to 65%.

2.2. Biological studies

2.2.1. Transient receptor potential vanilloid type 1 antagonistic activity assays in vitro

In order to test the antagonistic activity of the compounds, we used TRPV1 over-expressed cells HEK-293 for vitro activity test (Table 1). Compound **11**, **19**, **20** and **23** has relatively good antagonistic activity against capsaicin-activated TRPV1. But the inhibition rate is lower than that of positive control BCTC, and the remaining compounds have weaker antagonistic activity. The more carbon atoms of the aliphatic chain connected at the terminal nitrogen atom, the weaker its antagonistic activity is; compound with six-membered nitrogen-containing heterocycle is more potent than one with five-membered nitrogen-containing heterocycle, and the inhibition rate significantly increases when the benzyl group is connected to the terminal nitrogen. However, as the steric hindrance between the aromatic ring and the three-carbon linker increases, the inhibition rate decreases.

2.2.2. Analgesic activity in vivo

The TRPV1 antagonist BCTC with better analgesic activity was used as a positive control. In the capsaicin test, results showed that all the compounds can reduce the time of the mice licking paw to varying degrees, so all the compound are able to reduce the pain induced by capsaicin. Among them, compound **12**, **13**, **14**, **15** and **22** have best analgesic activities. And their analgesic activity was better than that of positive control BCTC; In abdominal constriction test, compound **11**, **12**, **17**, **19** and **23** can significantly reduce the number of abdominal constriction. Among them **11**, **12**, **17** and **23** have better analgesic activity than positive control BCTC; In the tail-flick test, compared with the blank group, except **12**, **13**, **17** and **21**, the remaining compounds can increase the mice MPE%, Compounds **11**, **15**, **16**, **18**, **19** and **20** have a significant effect of reducing the pain induced by the hot. **16**, 18 and **20** have better analgesic activity than BCTC (Table 2).

2.2.3. Sedative activities screened in vivo

2.2.4. Aqueous solubility test

Data in the Table 3 displays that expect for compound **16**, **19** and **22**, aqueous solubility of other compounds has improved to varying degrees compared to BCTC.

2.3. Docking results

Docking experiments have been conducted to study structure-activity relationships of target compounds (Fig. 4). Results show that both BCTC and Compound **11** occupied the cavity of the TRPV1 ion channel. BCTC molecular has aromatic H-bond interactions with the Key residue ALA 566 and THR 550. Compound **11** with designed novel structures

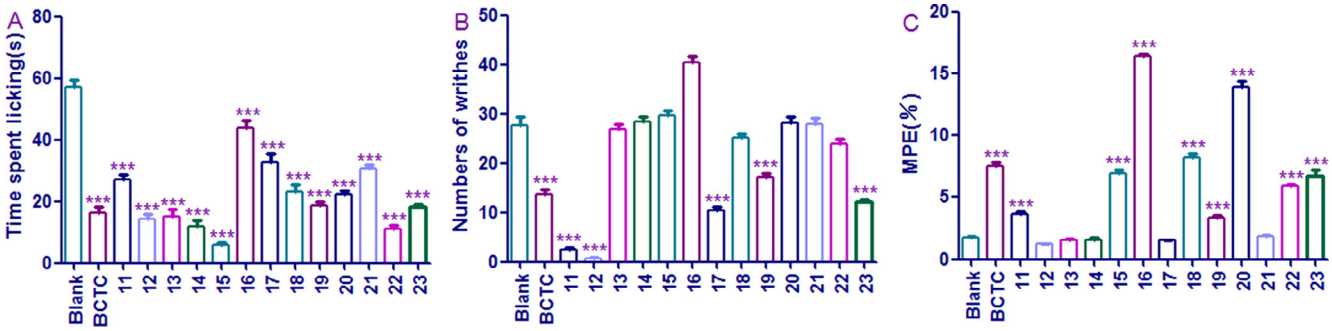

Fig. 3. Antinociceptive activities of compound **11–23**. (A) The results of capsaicin test; (B) The results of abdominal constriction test; (C) The results of tail-flick test. Each bar represents the mean ± SEM (n = 6). Statistical analysis was evaluated using a one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison test. $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$ compared with the vehicle group.