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Research paper

Study on chemical modification and analgesic activity of *N*-(4-tert-butylphenyl)-4-(3-chloropyridin-2-yl) piperazine-1-carboxamide

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ABSTRACT

N-(4-Tert-butylphenyl)-4-(3-chloropyridin-2-yl) piperazine-1-carboxamide (BCTC) is a potent and extensively studied urea-based TRPV1 antagonist. Although BCTC was effective in alleviating chronic pain in rats, it showed obvious hyperthermia side-effect and unsatisfactory pharmacokinetic profile, therefore, it was not developed further. In order to enrich the structural types of urea-based TRPV1 antagonists, two series of novel analogs, in which the pyridine ring of BCTC was replaced with a mildly basic pyrimidine ring or 1,2,3,4-tetrahydro-β-carboline scaffold, were designed and synthesized. Advancing the structure-activity relationship of these two series led to the discovery of *N*-(4-methoxyphenyl)-1,3,4,9-tetrahydro-2*H*-pyrido[3,4-*b*]indole-2-carboxamide (**7o**), with an improved pharmacological and tolerability profile compared with the lead compound BCTC.

1. Introduction

Pain, especially chronic pain, is a significant problem in public health management affecting one-fifth of the global population [1]. Among different targets involved in pain transmission [2,3], the transient receptor potential vanilloid 1 (TRPV1) receptor has been the focus of pain-related research since its discovery [4]. TRPV1, a calcium permeable nonselective cation channel expressed on sensory neurons, is selectively activated by multiple factors such as protons (pH < 6.8), heat (>43 °C), endogenous ligands, and natural vanilloids including capsaicin and resiniferatoxin [5]. The expression of TRPV1 is upregulated under certain nerve injury and inflammatory disease conditions. Furthermore, TRPV1 antagonists relieved pain of inflammation, osteoarthritis, and, even, cancer in rodent models [4]. Therefore, the TRPV1 antagonists are considered as an attractive analgesic [4,6]. Numerous potent TRPV1 antagonists have been developed so far, but owing to their hyperthermic effect in preclinical trials, none have advanced beyond the clinical trial stage [7,8]. Novel TRPV1 antagonists with excellent analgesic effect and no hyperthermic adverse-effect are yet to be developed.

Piperazine ureas represent one of the major classes of TRPV1 antagonists. Among them, *N*-(4-(tert-butyl) phenyl)-4-(3-chloropyridin-2-yl) piperazine-1-carboxamide (BCTC) is the most representative antagonist of this family. It showed potent analgesic activity in the animal models of inflammatory and neuropathic pain [9]. However, BCTC inhibited TRPV1 activation by capsaicin (CAP), protons, and, especially, protons, which led to obvious hyperthermia. Further optimization efforts undertaken during the past decade led to the development of several series of BCTC analogs. As far as we know, the structure of BCTC can be divided into A-, B- and C-regions (Fig. 1). These structural modifications mainly included the following: (i) To improve the pharmacokinetic profile of BCTC, the central piperazine core was bioisosterically replaced with 2-methylpiperazine [10] or tetrahydropyridine ring [11,12]. (ii) Several amide [13] or quinazoline [14] and 6,6-heterocycle [15] analogs have been prepared by substituting the piperazine urea in

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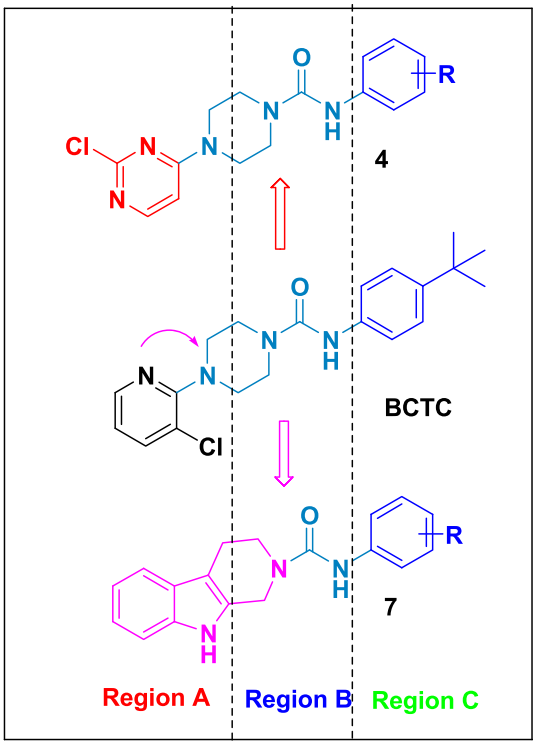
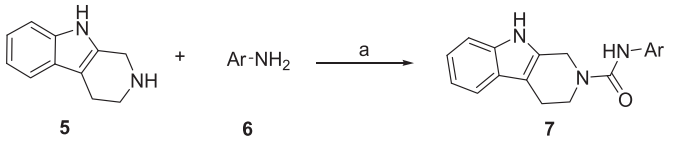


Fig. 1. Design of TRPV1 antagonists by chemical modification of the pyridine unit of BCTC.

BCTC. (iii) By employing the conformational restriction of imidazole [16] or pyrrolidinyl [17] as a bioisosteric alternate for the amide bond of BCTC, two series of novel TRPV1 antagonists were developed. The structural analysis of BCTC and its analogs revealed that the chemical modification of the pyridine unit of BCTC is rare, and most of these studies focused on the structure-activity relationship (SAR) of regions B and C. Therefore, to enhance the structural novelty of analogs of BCTC, we tried to replace the pyridine group with suitable moieties that allow a better interaction with the TRPV1 receptor. It is known that the pyrimidine moiety is widely used in the pharmaceutical industry. Compared with pyridine structure, pyrimidine structure provides more versatility in the synthesis because of lower π-electron density [18]. Thus, we initially chose bioisosteric pyrimidine to replace the pyridine of BCTC in order to increase the interaction with the TRPV1 receptor.



Scheme 2. Synthesis of the target compounds **7**. Reagents and conditions: (a) triphosgene/DMAP, CH_2Cl_2, one pot.

Evodiamine and rutaecarpine possess vanilloid activity and can be considered as conformationally restricted tryptamine derivatives, which are similar to *N*-arachidonoylserotonin (an endogenous modulator of TRPV1, AA-5-HT, Fig. 2) [19] to some extent. More recently, we investigated the rigidified derivatives, designed using conformationally constrained analogs of tryptamine with a triazole ring, which behaved as relatively potent TRPV1 antagonists [20]. Here, with the aim to develop novel TRPV1 antagonists with improved pharmacological profile, we designed novel analogs based on the hypothesis that the conformationally restricted analog of tryptamine can be used to replace the pyridine moiety of BCTC. Based on the modification of the pyridine unit of BCTC, two series of novel TRPV1 antagonists were synthesized and evaluated in this study (Fig. 1).

2. Results and discussion

2.1. Chemistry

The target compounds, **4** and **7**, were prepared as shown in Schemes 1 and 2, respectively. In order to obtain compounds **4a-4z**, the synthesis of intermediate **3** was critical. Using a nucleophilic aromatic substitution reaction, piperazine (**2**) was mostly introduced at position 4 of 2,4-dichloropyrimidine (**1**). This reaction yielded two regioisomers, and 2-chloro-4-(piperazin-1-yl) pyrimidine (**3**) was yielded as the major product. The regiochemistry of this reaction was verified by X-ray crystallographic analysis of compound **4g** (Fig. 3), which also confirmed the structure of **3**. In the presence of triphosgene, intermediate **3** was coupled with the aromatic amines to obtain the desired compounds **4a-4ae** with acceptable yield. In addition, another series of derivatives, **7a-7ab**, were also successfully synthesized by treating commercially available tetrahydro-β-carboline (**5**) with aromatic amines and triphosgene in the presence of *N*,*N*-dimethylaminopyridine (DMAP). The details of chemical synthesis are presented in the supporting information.

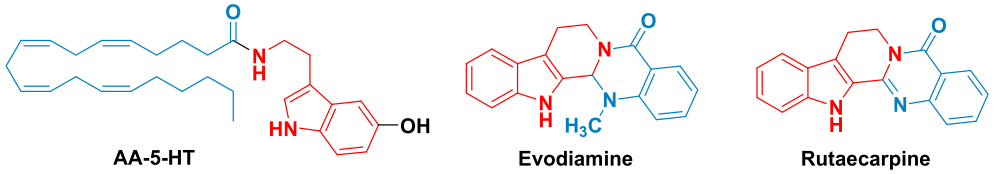
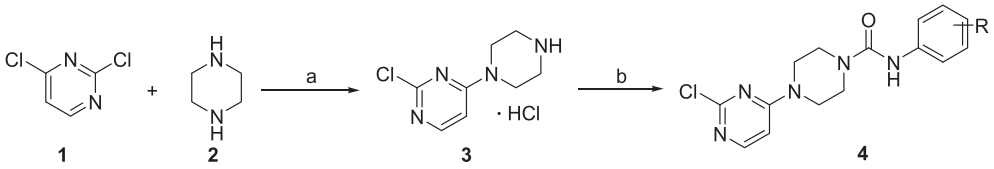


Fig. 2. Structure of AA-5-HT, evodiamine, and rutaecarpine.



Scheme 1. Synthesis of the target compounds **4**. Reagents and conditions: (a) CH_2Cl_2, 0 °C-rt; (b) aromatic amines, triphosgene/DMAP, CH_2Cl_2, one pot.

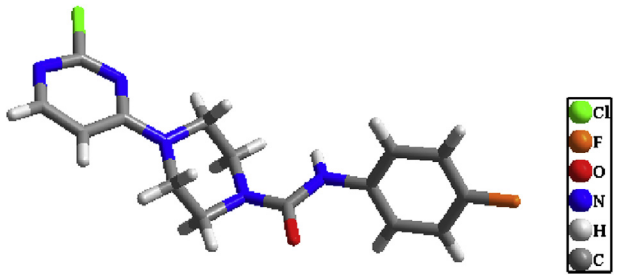


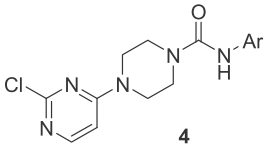
Fig. 3. X-ray crystal structure of **4g**.

2.2. In vitro evaluation

We initiated the exploration of the SAR of pyrimidine analogs **4a-4ae**. Compounds **4a-4ae** were compared with the prototype antagonist BCTC for their blockade ability of CAP or protons-induced activation of human TRPV1 channels. Unexpectedly, when the pyridine ring of BCTC was replaced with 2-chloropyrimidin (**4a**), the activity of the antagonist decreased significantly. This result suggests that the type of region A is important for the activity of such antagonists. Modifications of aryl moiety of BCTC were performed. As shown in Table 1, a variety of substituents such as alkyl, halogen group, bulky group (*tert*-butyl and *i*-propyl), and, even, the multi-substituted benzene groups were investigated. We found that the introduction of electron-withdrawing groups (for example fluoro or trifluoromethyl) on the phenyl ring resulted in higher potency. In addition, 2-CF_3 analog **4h** was the most effective compound in this series, but its activity was still significantly lower than that of the lead compound BCTC. Furthermore, the substitution of the phenyl group at region C with other types of aryl rings, that is, pyridine (**4ac** and **4ad**) and isoquinoline (**4ae**), resulted in a dramatic loss of activity. This prompted us to further investigate novel scaffolds.

For comparison with the 2-chloropyrimidin analogs, the tetrahydro-β-carboline scaffold that has been proved to have TRPV1 activity [19,20], was coupled with the aryl fragment to prepare ureas **7** (Table 2). As shown in Table 2, it is interesting to note that replacing the 2-chloropyrimidin with tetrahydro-β-carboline resulted in a significant increase in the antagonist activity. For instance, the first compound of this class, **7a**, which contains the *p*-^{t}Bu-phenyl fragment, displayed more potent antagonism than **4a**

Table 1
In vitro ability of compounds **4a-4ae** to inhibit the activation of hTRPV1 receptors.



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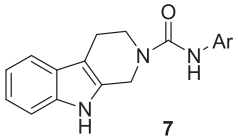
Compounds	Ar	hTRPV1(CAP)[a] IC_{50} (μM) or % inhib @ 10 μM	hTRPV1(pH)[b] % inhib @ 10 μM
4a	*p*-^{t}Bu-phenyl	29 ± 6 %	14 ± 3
4b	*o*-Br-phenyl	1.072 ± 0.615	55 ± 4
4c	*m*-Br-phenyl	1.451 ± 0.647	46 ± 7
4d	*p*-Br-phenyl	ND	ND
4e	*o*-F-phenyl	0.787 ± 0.034	66 ± 7
4f	*m*-F-phenyl	0.952 ± 0.147	57 ± 9
4g	*p*-F-phenyl	1.141 ± 0.318	45 ± 11
4h	*o*-CF_3-phenyl	0.136 ± 0.042	94 ± 5
4i	*m*-CF_3-phenyl	0.543 ± 0.178	61 ± 9
4j	*p*-CF_3-phenyl	0.874 ± 0.229	53 ± 7
4k	*p*-Cl-phenyl	1.248 ± 0.341	31 ± 11
4l	*o*-^{i}Pr-phenyl	7.531 ± 0.875	29 ± 4
4m	*m*-^{i}Pr-phenyl	ND	ND
4n	*o*-CH_3O-phenyl	9.417 ± 1.014	23 ± 6
4°	*p*-CH_3O-phenyl	41 ± 7 %	ND
4p	*o*-NO_2-phenyl	0.854 ± 0.269	62 ± 8
4q	3,4-diCH_3O-phenyl	37 ± 6 %	ND
4r	2,5-diCH_3-phenyl	ND	ND
4s	2,4-diCH_3-phenyl	ND	ND
4t	2,4,6-triCH_3-phenyl	35 ± 12 %	23 ± 5
4u	2-CH_3-5-C_2H_5-phenyl	ND	ND
4v	3-Cl-4-CH_3-phenyl	45 ± 10 %	17 ± 3
4w	2,5-diCl-phenyl	ND	ND
4x	3,4-diCl-phenyl	54 ± 9 %	27 ± 7
4y	4-Cl-2-NO_2-phenyl	43 ± 5 %	24 ± 9
4z	4-CH_3-2-NO_2-phenyl	ND	15 ± 4
4aa	2-CH_3O-4-NO_2-phenyl	42 ± 11 %	17 ± 6
4 ab	2,4,6-triCl-phenyl	68 ± 12 %	37 ± 7
4ac	4,6-dimethylpyridin-2-	ND	ND
4ad	2-chloropyridin-3-	ND	ND
4ae	isoquinolin-5-	22 ± 9 %	ND
BCTC		0.031 ± 0.034	97 ± 2

Unless otherwise stated, all values are the mean (SEM of at least three separate experiments).
ND, not determined.
[a] Human TRPV1 receptor activated by capsaicin.
[b] Human TRPV1 receptor activated by low pH (5.0).

Table 2
In vitro ability of compounds **7a-7 ab** to inhibit the activation of hTRPV1 receptors.



7

Compounds	Ar	hTRPV1(CAP)[a] IC_{50} (μM) or % inhib @ 10 μM	hTRPV1(pH)[b] % inhib @ 10 μM
7a	*p*-^{t}Bu-phenyl	0.125 ± 0.084	57 ± 7
7b	*o*-Br-phenyl	0.387 ± 0.056	62 ± 16
7c	*m*-Br-phenyl	0.424 ± 0.115	59 ± 10
7d	*p*-Br-phenyl	0.212 ± 0.065	74 ± 5
7e	*o*-F-phenyl	0.365 ± 0.115	65 ± 4
7f	*m*-F-phenyl	0.463 ± 0.081	52 ± 11
7g	*p*-F-phenyl	0.317 ± 0.128	67 ± 6
7h	*o*-CF_3-phenyl	0.336 ± 0.079	37 ± 7
7i	*m*-CF_3-phenyl	0.569 ± 0.212	33 ± 6
7j	*p*-CF_3-phenyl	0.302 ± 0.124	45 ± 9
7k	*p*-Cl-phenyl	0.284 ± 0.082	61 ± 11
7l	*o*-^{i}Pr-phenyl	0.138 ± 0.079	69 ± 14
7m	*m*-^{i}Pr-phenyl	0.179 ± 0.087	23 ± 6
7n	*o*-CH_3O-phenyl	0.217 ± 0.097	33 ± 8
7o	*p*-CH_3O-phenyl	0.091 ± 0.057	43 ± 7
7p	2-CH_3-5-C_2H_5-phenyl	0.347 ± 0.146	35 ± 6
7q	4-CH_3-2-NO_2-phenyl	ND	15 ± 5
7r	2,5-diCH_3-phenyl	0.684 ± 0.122	34 ± 6
7s	2,4-diCH_3-phenyl	0.389 ± 0.175	47 ± 9
7t	2-CH_3O-4-NO_2-phenyl	0.594 ± 0.115	28 ± 6
7u	2,5-diCl-phenyl	0.765 ± 0.148	19 ± 8
7v	3,4-diCl-phenyl	0.568 ± 0.126	28 ± 5
7w	2,4,6-triCl-phenyl	0.098 ± 0.075	93 ± 7
7x	4-Cl-2-NO_2-phenyl	ND	51 ± 12
7y	3-Cl-4-CH_3-phenyl	0.297 ± 0.126	55 ± 13
7z	3,4-diCH_3O-phenyl	0.481 ± 0.134	36 ± 11
7aa	2-chloropyridin-3-	ND	21 ± 8
7 ab	isoquinolin-5-	ND	15 ± 7
BCTC		0.024 ± 0.046	98 ± 2

Unless otherwise stated, all values are the mean (SEM of at least three separate experiments).
ND, not determined.
[a] Human TRPV1 receptor activated by capsaicin.
[b] Human TRPV1 receptor activated by low pH (5.0).

against CAP and proton (pH 5.0) activation. An interesting trend was observed during the analysis of SAR for substituents of benzene moiety, performed to gain a deeper insight. In the CAP test, the antagonism activity of *para*-substituted analogs was slightly stronger than that of *ortho*- or *meta*-substituted analogs. Typical examples include phenyls substituted with bromine (**7d** vs. **7b**, **7c**), fluorine (**7g** vs. **7e**, **7f**), and trifluoromethyl (**7j** vs. **7h**, **7i**) (Table 2). Furthermore, the electron-donating group favored the antagonism activity of the second structural class. For instance, *p*-^{t}Bu-phenyl (**7a**) and *p*-CH_3O-phenyl (**7o**) analogs exhibited good antagonism. Thereafter, we introduced multiple substitutions on the benzene ring and replaced the benzene ring with heteroaryl groups (such as pyridine and isoquinoline). As illustrated in Table 2, during the introduction of multiple substitutions on the phenyl ring, a modest decrease in antagonism activity was observed. Unexpectedly, a complete loss in antagonism activity was observed when the phenyl ring was replaced with a 2-chloropyridin ring (**7aa**) and isoquinoline ring (**7ab**). In the pH assay, almost all analogs exhibited a partial blockade (<74%) of protons-inducted activation of TRPV1 at 10 μM dose, except for 2,4,6-triCl-phenyl (**7w**, 93%) derivative and BCTC (98%), which manifested a full blockade of acid activation. It is noteworthy that several temperature-neutral analogs have strong antagonistic effects on capsaicin-induced activation of TRPV1, but only partially blocked acid-activated TRPV1 [4,21,22].

2.3. In vivo evaluation

On the basis of the *in vitro* studies, the selected compounds (**4h**, **7a**, **7l**, **7o**, and **7w**) were screened for further studies *in vivo*. It is worth noting that the efficacy of the same TRPV1 receptor antagonist is different in different models of hyperalgesia. To evaluate the analgesic activity of each compound, we performed nociception tests in three different pain models, with BCTC as the control (Fig. 4). The rats were administered a single oral dose of 30 mg/kg test compounds, because oral administration of BCTC at 30 mg/kg dose has been reported to exhibit potent analgesic activity in various *in vivo* nociceptive trials in rats [23]. In the capsaicin model, all test compounds significantly reduced the total paw licking time compared with the vehicle. Particularly, compound **7o** exhibited higher potency than the positive control BCTC (Fig. 4A). In the proton-induced abdominal constriction model, BCTC, **4h**, and **7w** considerably reduced the number of writhes, whereas compounds **7a**, **7l**, and **7o** showed a certain inhibitory effect (Fig. 4B). This is consistent with results of studies *in vitro*, that is, **7a**, **7l**, and **7o** incompletely blocked proton (pH 5.0) activation, with a modest inhibition activity of 57%, 69%, and 43%, respectively. In the case of the tail-flick model, all compounds could increase %MPE in comparison with the vehicle. Interestingly, the highest %MPE produced by **7o** was significantly higher than that generated by the other

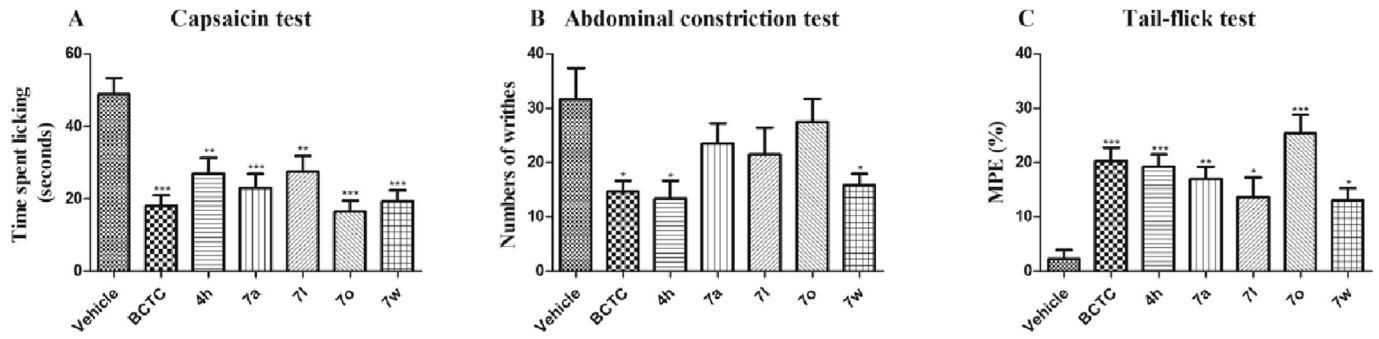


Fig. 4. Analgesic activities of synthesized compounds at 30 mg/kg dose after oral administration. (A) The antinociceptive effect in the capsaicin test; (B) suppression of acetic acid-induced writhing response; (C) inhibition of thermal nociception by synthesized compounds. Each bar represents the mean ± 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, and, ***p < 0.001 compared with the vehicle group.

drugs, for unknown reasons (Fig. 4C). It was observed that all the test compounds had a certain degree of antinociceptive activity. As shown in Fig. 4, **7o** showed good antinociceptive potency that was more potent than BCTC, in the models of capsaicin- and heat-induced pain. Importantly, **7o** showed a weak effect on low pH, indicating that it was probably free of hyperthermia.

In order to confirm our hypothesis, we carried out the body temperature investigation of test compounds using a rectal thermometer, with BCTC as the positive control (Fig. 5). Rats were administered a single oral dose of 30 mg/kg, and the rectal body temperatures were recorded prior to dosing and at 30, 60, 90, 120, 150, and 180 min after dosing. As shown in Fig. 5, a transient increase in body temperature was observed in rats following BCTC dosing. The mean temperatures of the BCTC group significantly differed from that of the vehicle-treated group (ANOVA/Dunnett's) at 30 and 60 min post dosing. Similar to the results of BCTC, compounds **4h**, **7a**, **7l**, and **7w** also caused transient hyperthermia in the test rats, with the mean temperatures at some evaluated time points being significantly higher than those in the control group. As expected, there was no significant difference between the compound **7o**-treated and vehicle-treated groups from before dosing to 180 min post dosing. In addition, the dose-dependency of body temperature was evaluated. As shown in Fig. 6, at all time points and doses of **7o**, the patterns of drug-related effects on body temperature were not obvious (no other significant differences were observed when compared with the control groups).

Due to the excellent *in vivo* and *in vitro* activities of compound **7o**, the pharmacokinetic properties of this compound was assessed in Sprague-Dawley rats (Table 3 and Fig. 7; the pharmacokinetic parameters of compound **4h** was also included for comparison). Compounds **4h** and **7o** were administered at 10 mg/kg dose by oral gavage to three rats. As shown in Table 3, compound **4h** exhibited

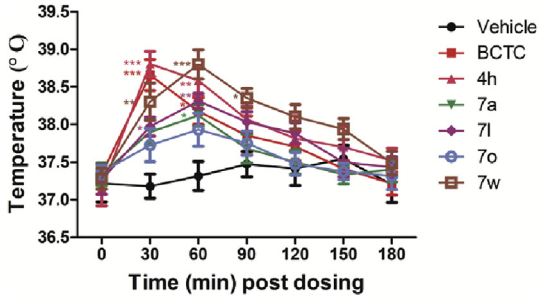


Fig. 5. Effects of compounds at 30 mg/kg dose after oral administration on body temperature in rats. Data are expressed as mean ± SEM (n = 6). *p < 0.05, **p < 0.01, and, ***p < 0.001 by Dunnett's multiple comparison test compared with the vehicle-treated group.

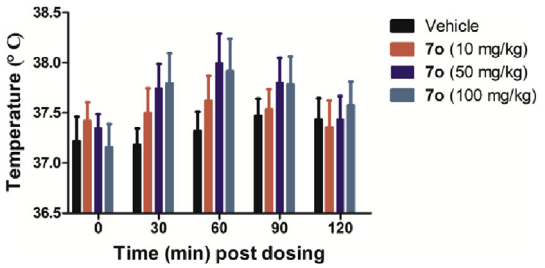


Fig. 6. Effects of compound **7o** at different doses on body temperature in rats. Data are expressed as mean ± SEM (n = 6). *p < 0.05, **p < 0.01, and, ***p < 0.001 by Dunnett's multiple comparison test compared with the vehicle-treated group.

rapid absorption (T_{max} = 0.106 ± 0.020 h), high C_{max} (278.163 ± 86.843 mg/mL), high AUC (622.907 ± 60.132 mg/mL × h), and modest half-life ($t_{1/2}$ = 1.581 ± 0.653 h). As shown in Table 3 and Fig. 7, the pharmacokinetic property of compound **7o** was obviously better than **4h** when administered orally. Upon oral administration, compound **7o** exhibited slower absorption (T_{max} = 3.933 ± 0.389 h), lower clearance (CL 0.136 ± 0.025 mL/h/kg), and a longer half-life ($t_{1/2}$ = 3.085 ± 0.484 h), which resulted in reasonable residence time (7.95 ± 0.86 h after a 10 mg/kg dose).

2.4. Molecular modeling

Furthermore, the docking study was carried out with a rat TRPV1 (PDB ID: 5IS0) model [24]. The binding interaction between antagonist **7o** and the receptor was analyzed, and its binding mode was compared with that of BCTC.

Table 3
Pharmacokinetic parameters of **4h** and **7o** following oral administration[a] to rats[b].

Parameters	Unit	Compound	
		4h	**7o**
$t_{1/2}$, k_a	h	0.016 ± 0.002	2.426 ± 0.109
$t_{1/2}$, k_{10}	h	1.581 ± 0.653	3.085 ± 0.484
k_a	1/h	44.072 ± 5.783	0.286 ± 0.013
k_{10}	1/h	0.479 ± 0.198	0.227 ± 0.036
V	mL/kg	0.036 ± 0.011	0.613 ± 0.204
CL	mL/h/kg	0.016 ± 0.002	0.136 ± 0.025
T_{max}	h	0.106 ± 0.020	3.933 ± 0.389
C_{max}	mg/mL	278.163 ± 86.843	7.050 ± 2.001
AUC $_{0\text{-inf}}$	mg/mL*h	622.907 ± 60.132	74.848 ± 13.564
MRT	h	2.304 ± 0.945	7.951 ± 0.855

[a] Compound was prepared in 0.5% sodium carboxymethyl cellulose and administered at 10 mg/kg dose.
[b] n = 3.

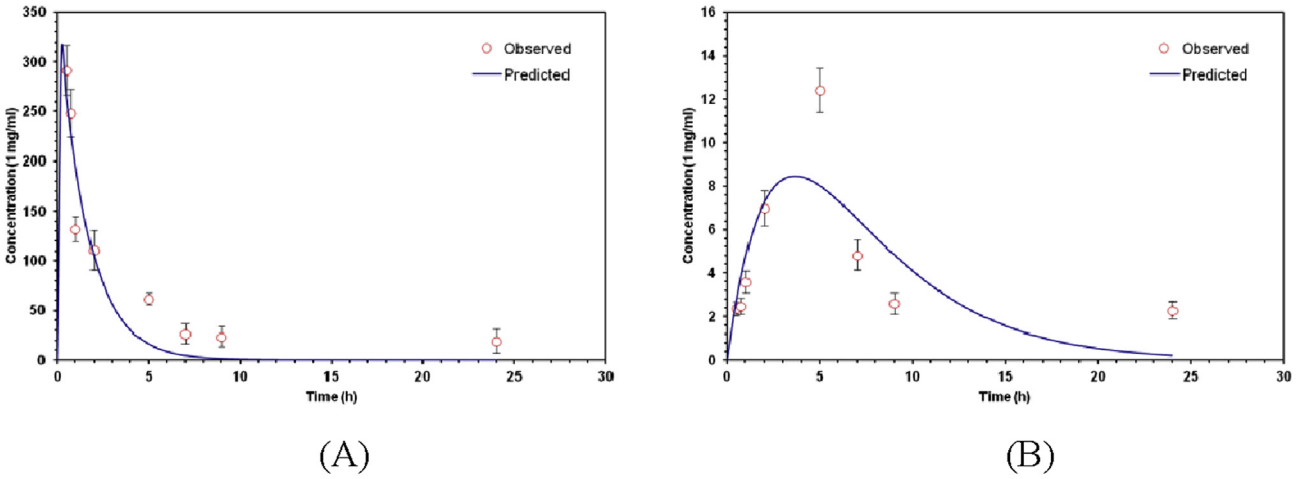


Fig. 7. (A) Plasma concentrations of compound **4h** at each time point after intragastric gavage (10 mg/kg) in rats. (B) Plasma concentrations of compound **7o** at each time point after intragastric gavage (10 mg/kg) in rats. Each point is the average concentration, and the bars are standard deviations of the mean (n = 3).

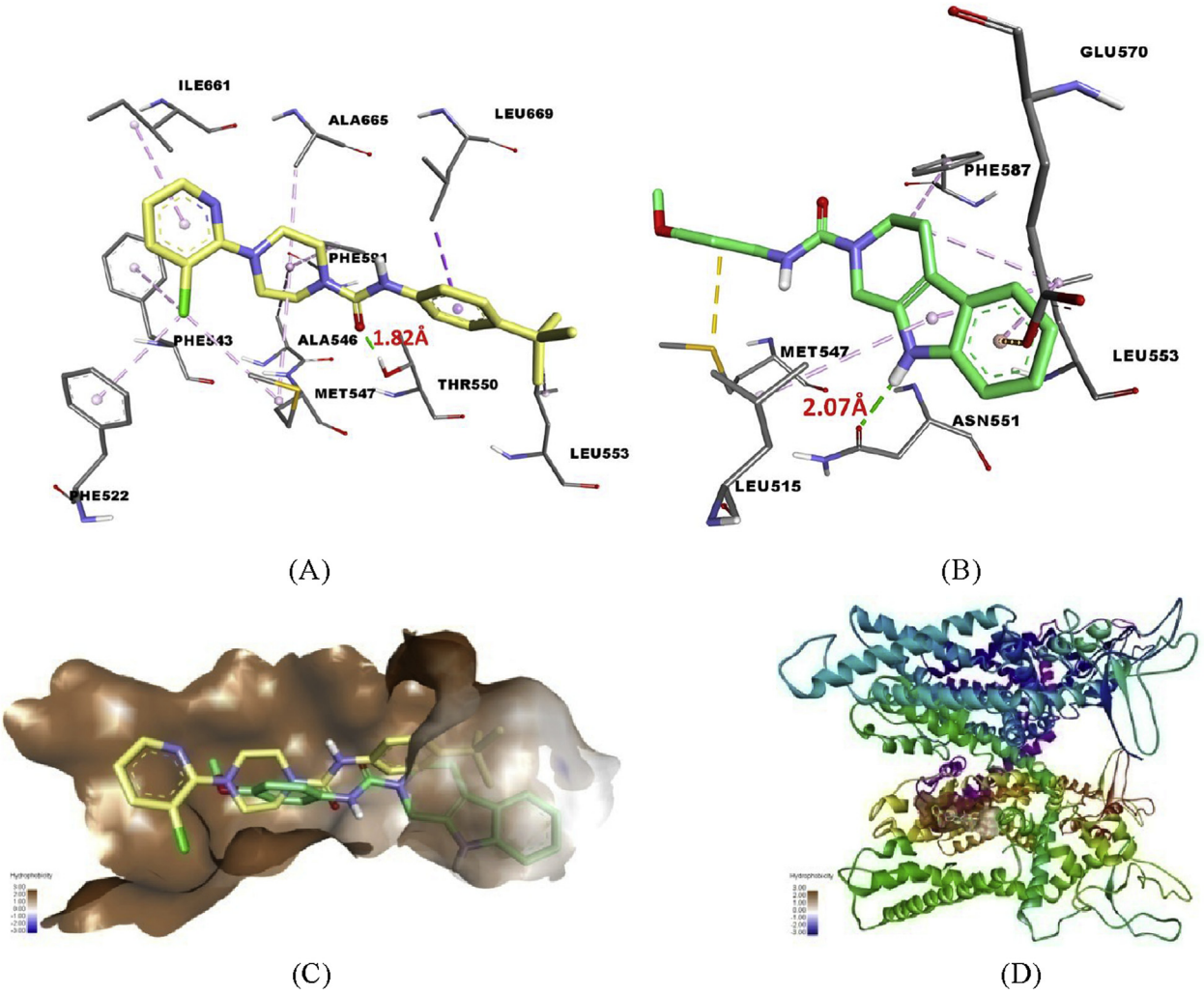


Fig. 8. Molecular modeling of compound **7o** and BCTC. (A) Three dimensional model of BCTC that interacts with the key amino acids of rTRPV1. Hydrogen bonds are shown as green dashed lines. (B) Three dimensional model of compound **7o** that interacts with the key amino acids of rTRPV1. Hydrogen bonds are shown as green dashed lines. (C) Three dimensional model of compounds in the hydrophobic pocket of rTRPV1. Compound **7o** is depicted by sticks colored by atom type (C green, O red, N blue, polar H white). BCTC is depicted by sticks colored by atom type (C yellow, O red, Cl green, N blue, polar H white). (D) Three dimensional model of compounds that interacts with the hydrophobic transmembrane region of rTRPV1. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

As illustrated in Fig. 8A, when binding to rTRPV1, BCTC achieved favorable contacts with diverse residues of amino acids. An oxygen atom of the urea formed a hydrogen bond with Thr550. The piperazine ring occupied the main hydrophobic receptor slot and interacted tightly with Ala665, Ala546, Phe591, and Met547 via hydrophobic interaction. Furthermore, we also observed hydrophobic interactions between the 4-*tert*-butylphenyl group and Leu553, Leu669; the 3-chloropyridine group and Met547, Phe543, Phe522, and Ile661. Correspondingly, **7o**, which has tetrahydro-β-carboline in the A-region, exhibited excellent coordination with the binding site. As shown in Fig. 8B, tetrahydro-β-carboline occupied the deep bottom hole and participated in the hydrophobic interactions with Phe587, Leu553, and Leu515, along with electrostatic interaction with Glu570. In addition, the NH in the A-region formed a hydrogen bond with Asn551. Moreover, the aromatic group in the C-region interacted with Met547. Finally, as shown in panels C and D of Fig. 8, compound **7o** was more suitable for the active hydrophobic pocket than BCTC. On the basis of these data, compound **7o** was characterized as a potent TRPV1 antagonist.

3. Conclusions

In the present study, we synthesized two series of novel TRPV1 antagonists and compared their pharmacology with the prototype compound BCTC, in various *in vitro* and *in vivo* assays. The analysis of SAR indicated that *N*-phenyl-3,4-dihydro-1*H*-pyrido[3,4-b]indole-2(9*H*)-carboxamides displayed better antagonism than the corresponding 4-(4-chloropyrimidin-2-yl)-*N*-phenylpiperazine-1-carboxamides. The potency of *N*-phenyl-3,4-dihydro-1*H*-pyrido[3,4-b]indole-2(9*H*)-carboxamides was enhanced as the electron-donating effect of the mono-substituent phenyl progressively increased. The most effective antagonist **7o** displayed selective antagonism, and it exhibited a strong antagonism towards capsaicin for hTRPV1, but showed a weak antagonism to low pH and avoided hyperthermia. Additionally, compared with **4h**, compound **7o** showed favorable pharmacokinetic characteristics in rats when administered orally. The docking study of compounds BCTC and **7o** in the rTRPV1 homology model indicated that **7o** exhibited a high binding affinity, compared with than that of BCTC. This study provides a novel scaffold for further investigation of related TRPV1 antagonists.

Crystallographic data (excluding structure factors) on the structure of compounds have been deposited in the Cambridge Crystallographic Data Centre as supplementary publication numbers CCDC-1965573 and 1965572. Copies of the data can be obtained, free of charge, from CCDC, 12 Union Road, Cambridge CB2 1EZ, UK [fax: +44(0) 1223 336033 or e-mail: deposit@ccdc.cam.ac.uk].

Declaration of competing interest

The authors declare that they have no known competing financial interest or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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