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Discovery of potent transient receptor potential vanilloid 1 antagonists: Design and synthesis of phenoxyacetamide derivatives

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ABSTRACT

We aimed to discover a novel type of transient receptor potential vanilloid 1 (TRPV1) antagonist because such antagonists are possible drug candidates for treating various disorders. We modified the structure of hit compound **7** (human TRPV1 IC_{50} = 411 nM) and converted its pyrrolidino group to a (hydroxyethyl)methylamino group, which substantially improved inhibitory activity (**15d**; human TRPV1 IC_{50} = 33 nM). In addition, **15d** ameliorated bladder overactivity in rats in vivo.

Transient receptor potential vanilloid 1 (TRPV1) is a Ca^{2+}-permeable non-selective cation channel composed of six transmembrane domains. The channel is activated by stimuli including heat, acids, and endogenous substances such as anandamide and lipoxygenase products, as well as by chemical compounds such as capsaicin (**1**) and resiniferatoxin (**2**) (Fig. 1).[1] TRPV1 antagonists are expected to have therapeutic potential for various disorders such as hyperalgesia, neuropathic pain, migraine, irritable bowel syndrome, osteoarthritis, and overactive bladder.[2] We therefore initiated a research program to discover a novel type of TRPV1 antagonist.

Capsazepine (**3**) was the first competitive TRPV1 antagonist to be discovered and exhibits antihyperalgesic activity in rats.[3] Subsequently, various structural classes of TRPV1 antagonists have been reported, including SB-704598 (**4**), ABT-102 (**5**), and AMG-517 (**6**) (Fig. 2).[4]

Here we report the design, synthesis, and biological evaluation of novel TRPV1 antagonists that, compared with SB-705498 (**4**), are more potent TRPV1 inhibitors.

First, we screened a chemical library in order to find antagonists to human TRPV1 recombinantly expressed in Chinese hamster ovary K1 cells.[5] We found a phenoxyacetamide derivative (**7**; Fig. 3) with moderate inhibitory activity against human TRPV1 (hTRPV1 IC_{50} = 411 nM). Aiming to improve TRPV1 inhibition, we explored structural modifications of **7**.

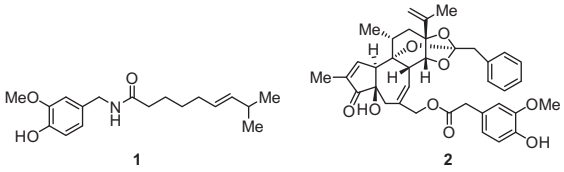

Figure 1. Representative TRPV1 agonists.

Phenoxyacetamide derivatives were efficiently synthesized as shown in Scheme 1. Coupling between amine **8** and nitropyridine **9**, followed by reduction of the nitro group provided aminopyridine **11**. Phenoxyacetic acid **14** was prepared by O-alkylation of phenol **12**, followed by hydrolysis of *tert*-butyl phenoxyacetate **13**. Phenoxyacetamide derivatives **15a**–**n** were obtained by using a coupling reagent for the condensation reaction between aminopyridine **11** and phenoxyacetic acid **14**. The obtained compounds were converted into corresponding salts for pharmacological tests as appropriate and were evaluated for antagonistic activity toward hTRPV1.

We evaluated the impact of changes of the left-part pyrrolidine moiety at the 2-position of the pyridine ring (Table 1). Introduction of a hydroxyl group at the 3-position of pyrrolidine did not markedly affect antagonistic activity toward hTRPV1 (**15a**). Conversion

Abbreviations: CYP, cyclophosphamide; HBSS, Hank's balanced salt solution; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; hTRPV1, human transient receptor potential vanilloid 1; SEM, standard error of mean; TRPV1, transient receptor potential vanilloid 1.

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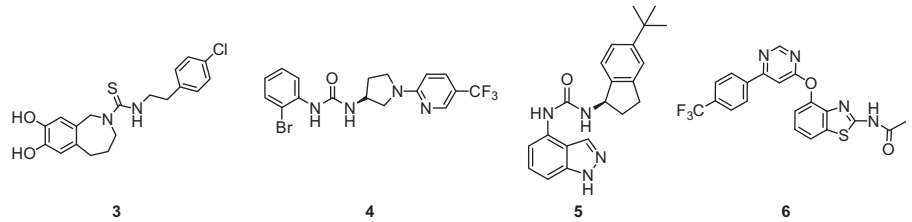


Figure 2. Representative TRPV1 antagonists.

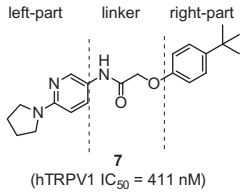
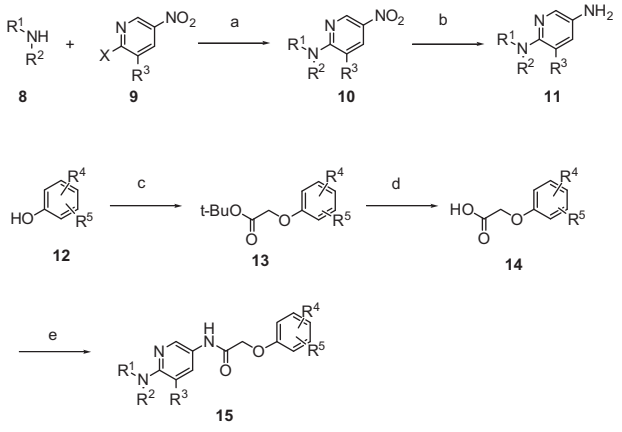


Figure 3. Compound discovered by a screening campaign.



(R^1, R^2) = (Me, Me), (Me, Et), (Me, CH_2CH_2OH), ($CH_2CH_2CH_2CH_2$), ($CH_2CH_2CH_2OHCH_2$),
R^3 = H, Me, Cl
(R^4, R^5) = (H, 4-*tert*-butyl), (H, H),(H, 4-*i*-Pr), (H, 4-CF_3), (H, 4-adamantyl),
(H, 2-*tert*-Butyl), (H, 3-*tert*-butyl), (2,4-di-*tert*-butyl),
(2-Br, 4-di-*tert*-butyl), (2-piperidinyl, 4-di-*tert*-butyl)
X = Cl, Br

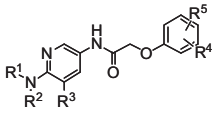
Scheme 1. Reagents and conditions: (a) K_2CO_3, dimethylformamide, rt, 72–99%; (b) Pd/C, H_2, MeOH, rt or Fe, NH_4Cl, tetrahydrofuran, EtOH, H_2O, 70 °C, 77–99%; (c) *tert*-butyl bromoacetate, K_2CO_3, acetone, 50 °C, 58–99%; (d) trifluoroacetic acid, CH_2Cl_2, 40 °C, 58–99%, (e) 11, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide, *N*,*N*-diisopropylethylamine, CH_2Cl_2, rt, 10–99%.

of a pyrrolidino group to dimethyamino group considerably improved activity (**15b**), while the activity of ethylmethylamino compound **15c** was comparable with that of **7**. To our surprise, (hydroxyethyl)methylamino compound **15d** showed the most potent activity with an IC_{50} = 33 nM. The effects of the hydroxyethyl group are not yet fully understood. The chlorine substituent at the 3-position of the pyridine ring of **15d** had a similar effect, but a methyl group was not favorable (**15e** and **15f**).

We next turned our attention to modifying the right part (Table 1; **15g**–**15o**). To clarify the substituent effects, we took **15d** as a starting point and modified the substituents of the right-part phenyl ring. Moving the *tert*-butyl group from the 4-position to the 2- or 3-position of the phenyl ring decreased antagonistic activity (**15g** and **15h**). Furthermore, we introduced hydrogen or substituents such as trifluoromethyl, isopropyl, and adamantly groups at the 4-position. However, these substituents were not as effective as the *tert*-butyl group. Adding a substituent at the 2-position of right-part phenyl ring in **15d** caused a decrease in antagonistic activity (**15m**, **15n**, and **15o**).

We next evaluated the pharmacological effects of **15d** in vivo in an overactive bladder rat model, because **15d** was a potent TRPV1 inhibitor in vitro. Specifically, we evaluated the effects of **15d** on capsaicin-induced bladder contraction in rats (mechanistic model)[6] and on cyclophosphamide (CYP)-induced cystitis in rats (disease model)[7] (Figs. 4 and 5). At a dose of 3 mg/kg, iv, **15d** suppressed capsaicin (30 μg/kg, iv)-induced bladder contraction in rats. Moreover, at a dose of 3 mg/kg (iv), **15d** reduced the voiding frequency in rats with CYP-induced cystitis and pollakiura.

Table 1
Antagonist activity of phenoxyacetamide derivatives toward hTRPV1



Compound	R^1	R^2	R^3	R^4	R^5	htrpv1 IC_{50} (nM)[a]
4 (SB-120						704598)
7	$-(CH_2)_4-$		H	H	4-*tert*-Butyl	411
15a	$-(CH_2)_2-CH_2OHCH_2-$		H	H	4-*tert*-Butyl	516
15b	Me	Me	H	H	4-*tert*-Butyl	59
15c	Et	Me	H	H	4-*tert*-Butyl	583
15d	$(CH_2)_2OH$	Me	H	H	4-*tert*-Butyl	33
15e	$(CH_2)_2OH$	Me	Me	H	4-*tert*-Butyl	130
15f	$(CH_2)_2OH$	Me	Cl	H	4-*tert*-Butyl	44
15g	$(CH_2)_2OH$	Me	H	H	3-*tert*-Butyl	2620
15h	$(CH_2)_2OH$	Me	H	H	2-*tert*-Butyl	796
15i	$(CH_2)_2OH$	Me	H	H	H	>10 μM
15j	$(CH_2)_2OH$	Me	H	H	4-i-propyl	110
15k	$(CH_2)_2OH$	Me	H	H	4-CF_3	>10 μM
15l	$(CH_2)_2OH$	Me	H	H	4-Admanthyl	>10 μM
15m	$(CH_2)_2OH$	Me	H	2-*tert*-Butyl	4-*tert*-Butyl	107
15n	$(CH_2)_2OH$	Me	H	2-Br	4-*tert*-Butyl	86
15o	$(CH_2)_2OH$	Me	H	2-Piperidinyl	4-*tert*-Butyl	941

[a] Inhibition assays were carried out in hTRPV1-transfected Chinese hamster ovary K1 cells. IC_{50} were determined from data at various concentrations of derivatives in triplicate.

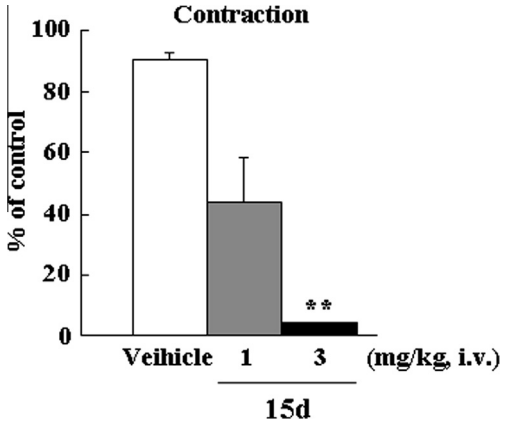


Figure 4. Effect of **15d** on capsaicin-induced bladder contraction in rats. Compound **15d** dose-dependently suppressed capsaicin-induced bladder contraction in rats. Data are shown as the mean ± SEM of 4 animals. $^{**}P$ <0.01 compared with the vehicle group.

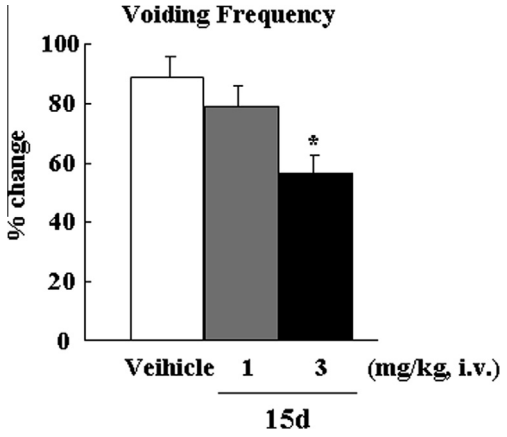


Figure 5. Effect of **15d** on pollakiuria in rats with CYP-induced cystitis. Compound **15d** dose-dependently reduced voiding time in rats with CYP-induced cystitis with pollakiuria. Data are shown as the mean ± SEM of 4 animals. $^{*}P$ <0.05 compared with the vehicle group.

In conclusion, we identified hit compound **7**, which has a phenoxyacetamide structure, from our chemical library. The pyrrolidino group of **7** was converted to other substituents. As a result, we found that **15d** bearing a (hydroxyethyl)methylamino group has potent antagonistic activity toward hTRPV1. Moreover, we demonstrated that **15d,** which showed the most potent antagonistic activity in vitro, ameliorated bladder overactivity in rats in vivo. These data suggest that **15d** has potential as a drug for treating overactive bladder. To broaden the therapeutic potential, we plan to evaluate the discovered TRPV1 antagonists in other disease models.

Acknowledgments

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5. *Calcium mobilization assays:* hTRPV1-expressing Chinese hamster ovary K1 cells were seeded into 96-well plates and grown to 90% confluency. The cells were then incubated at rt for 90 min in the Hank's balanced salt solution (HBSS)/4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) buffer (20 mM HEPES, HBSS; pH 7.4) supplemented with calcium-sensitive fluorescent dye (2 μM, Fluo4-AM, Dojindo). The wells were then washed three times with HBSS–HEPES buffer. Addition of 100 nM capsaicin to the wells resulted in concentration-dependent elevation of intracellular calcium levels and subsequent activation of Fluo4 fluorescence. Fluorescence imaging plate readers (FLIPR, Molecular Devices) were used to monitor changes in the fluorescence and to determine the maximum fluorescence signal during 120 s after the application of capsaicin by statistics (FLIPR Software). The derivatives at various concentrations were added to the wells together with capsaicin. The maximum signals by capsaicin in the presence or absence of the derivatives at various concentrations were averaged in triplicate and then, IC_{50} values (the concentration required for 50% inhibition of the control reaction) were determined in GraphPad Prism, version 4 (GraphPad Software).
6. *Capsaicin-induced bladder contraction in rats*: Female Sprague–Dawley rats were anesthetized with urethane (1.0 g/kg, ip), and then a catheter was inserted into the bladder through the external urethral orifice. Physiological saline at rt was infused into the bladder (3.0 mL/h, 5 min; 0.25 μL) to obtain the basal intravesical pressure. Capsaicin (30 μg/kg, iv) was injected at 10 min intervals to induce bladder contraction. Stable bladder contractions (control) were obtained, **15d** or the vehicle (20% cremophor EL) was administered (iv) which was followed by additional application of capsaicin. A percent change in the capsaicin-induced bladder contraction was calculated. Statistical significance was evaluated using Bartlett's test followed by Williams' tests compared to vehicle group. *P* values <0.05 were considered significant.
7. *Cystometry in pollakiuria rats with CYP-induced cystitis*: Female Sprague–Dawley rats were used. Cystitis was induced by administering cyclophosphamide (150 mg/kg, ip) to rats. At 4 h after application, an intravesical catheter was implanted and then physiological saline at rt was infused into the bladder (3.6 mL/h) to obtain continuous cystometrograms. After a stable voiding frequency was obtained, **15d** or the vehicle (20% cremophor EL) was administered (iv). Voiding times for 20 min before and after the administration was recorded; the percent change in voiding frequency was calculated for each group. Statistical significance was evaluated by Bartlett's test followed by Williams' test for comparison with the vehicle group. *P* values <0.05 were considered significant.