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The design and synthesis of transient receptor potential vanilloid 3 inhibitors with novel skeleton

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ABSTRACT

Transient receptor potential vanilloid 3 (TRPV3) channel as a member of thermo TRPV subfamily is primarily expressed in the keratinocytes of the skin and sensory neurons, and plays critical roles in various physiological and pathological processes such as inflammation, pain sensation and skin disorders. However, TRPV3 studies have been challenging, in part due to a lack of research tools such as selective antagonists. Recently, we synthesized a series of cinnamate ester derivatives and evaluated their inhibitory activities on human TRPV3 channels expressed in HEK293 cells using whole-cell patch clamp recordings. And, we identified two potent TRPV3 antagonists **7c** and **8c** which IC_{50} values were 1.05 μM and 86 nM, respectively. It also showed good selectivity to other subfamily members of TRPV, such as TRPV1 and TRPV4.

1. Introduction

TRPV3, a member of the TRPV subfamily, is a non-selective cation channel widely distributed over the peripheral and central nervous system with high calcium permeability and ligand-gated property [1,2]. As a temperature sensor, TRPV3, in addition to responding to temperature stimulation (>33 °C), can also be activated by some chemical agonists and is also regulated by a variety of physiological factors of the body [3–6]. Although its physiological functions have yet to be fully elucidated, unique attributes of TRPV3 have distinguished it from other vanilloid family members. Unlike other vanilloid TRPs, TRPV3 has sensitization characteristics, that is, the number of stimulation channels increases in a certain range, and the channel current also gradually increases [7].

Although many natural products can activate TRPV3, they have little selectivity, such as carvacrol, camphor, menthol, thymol, etc [8]. The most commonly used agonist in current research is the synthetic compound 2-aminoethoxybiphenyl borate (2-APB) [9,10]. Studies have shown that chemical activation of TRPV3 increases the release of many proinflammatory and inflammatory factors, such as ATP, nitric oxide, prostaglandin E2, nerve growth factor, interleukin (IL)-1α, tumor necrosis factor (TNF)-α and IL-6 [11–16]. After TRPV3, which is expressed in skin keratinocytes, is activated, the released factors can cause skin itching and dermatitis. Recently, several studies have reported that patients with Olmsted syndrome (OS) [17–19] caused by TRPV3 gene mutation also have obvious skin pruritus, and studies have shown that the expression of TRPV3 in patients with allergic dermatitis is significantly increased. These indicated that TRPV3 is a key molecule of the skin signaling pathway, and inhibiting TRPV3 overexpression may exert a beneficial influence on skin health.

As so far, only a few compounds that can inhibit TRPV3 are reported, such as osthole, forsythoside B and ruthenium red [20–22]. Moreover, Example #64, Example #82 and FTP-THQ developed by Hydra Bioscience (Fig. 1), GRC15133, GRC17173 and GRC15300 (unpublished structures) of Glenmark pharmaceutical company [23] and 74a reported by AbbVie company [24] showed a good inhibitory activity on TRPV3. In terms of endogenous inhibitors, isoprene pyrophosphate [25] and 17 (R)-resolvin D1 have been studied [26]. However, there is still a lack of specific antagonists.

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2. Results and discussion

Previous work showed that forsythoside B has a certain inhibitory activity against TRPV3 (IC_{50} = 6.7 μM) [21]. We proposed that cinnamate ester was the crucial fragment, and a series compounds were synthesized. In the design of the target compound, the basic structure skeleton was divided into three parts (Part A, B, C) (Fig. 2). H, hydroxyl, acetyl, trifluoromethoxy substituents were introduced into the benzene ring of Part A, and various substituents are introduced into the benzene ring of Part B. Part C was consisted of α,β-unsaturated ester and carbonyl. Through a series of modification, a total of 23 target compounds were synthesized.

2.1. Chemistry

The synthetic route of 17 target compounds is shown in Scheme 1. By esterification reaction of acids and alcohol/phenol with EDCI, DMAP, triethylamine in CH_2Cl_2 as solvent overnight gave compounds **1**–**5, 6a-6f** in yields of 80–87%. Then, the target compounds **7a-7f** were obtained by dissolving **6a-6f** in the solution of methanol and THF added with potassium carbonate for 2 h, and the yields were about 60–76%.

In addition, the α,β-unsaturated carbonyl compounds were obtained through the aldol condensation reaction. Under the condition of 10% sodium hydroxide, compound **8a-8f** was synthesized overnight in anhydrous ethanol, and the yields were about 82–89%. Then, the protecting group MOM removed with 3 N HCl condition in methanol solution for 3 h to obtain target compounds **9a** and **9b** in 72–76% yields (Scheme 2).

2.2. Biological assays

2.2.1. Preliminary screening of TRPV3 inhibitory activity

The activities of 23 compounds were determined by patch clamp technique in 50 μM concentration, and forsythoside B was used as positive control. The results of inhibiting TRPV3 activity are shown in the following table. In Table 1**, 17** ester compounds were modified and obtained. Compounds **1**–**5** showed lower TRPV3 inhibitory activity than the positive control. When the trifluoromethoxy group was substituted

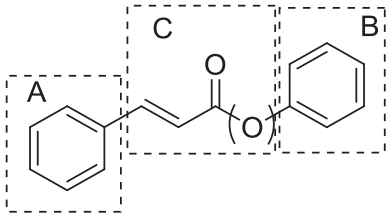


Fig. 2. Modification of cinnamate ester.

in the *para*- position of Part A, the activities of compounds **4** and **5** were higher than those of compounds **1** and **2**. The inhibitory activities of compounds **1** and **4** were similar, but the inhibitory activities of benzyl ester **5** were much higher than that of benzyl ester **2**.

Further, acetoxy group were introduced to the *para*- substitution of Part A in **6a-6f**. However, the activity of compound **6a** decreased significantly when *p*-methylbenzyl ester was retained. At the same time, compounds **6b**, **6c** and **6f** with higher inhibition rate than positive control were determined against TRPV3. The inhibitory activity of **6b** was better than that of **6c**, which indicated that fluorine substituted sites had a certain effect on the inhibitory activity. It was also showed that substitution of fluorine (compound **6b**) was better than that of trifluoromethyl (compound **6e**) and two halogen (compound **6d**). The difluoro substitution of ring B (compound **6f**) made the compound exhibiting good inhibitory activity of TRPV3, and its inhibitory rate was much higher than that of other compounds.

Still in Table 1, compounds **7a-7f** were showed to compare the activity with A-ring acetoxy substituents. It can be seen from the table that compound **7a** showed lower inhibition rate than positive control, which similar to compound **6a**. The difluoro substituted compound **6f** showed the best inhibition in these acetoxy substituted compounds, while the **7f** was not. Compound **7c** was identified as the best inhibitor in this series with inhibition rate value of 85.45%.

According to Table 2, these compounds were featured with α,β-unsaturated carbonyl as linker in Part C. When compound **9a** retained the hydroxyl of ring A and the fluorine of ring B, it displayed high inhibition. But when the substituent of ring B was changed by *p*-methyl (compound **9b**), it resulted in almost disappearance of the

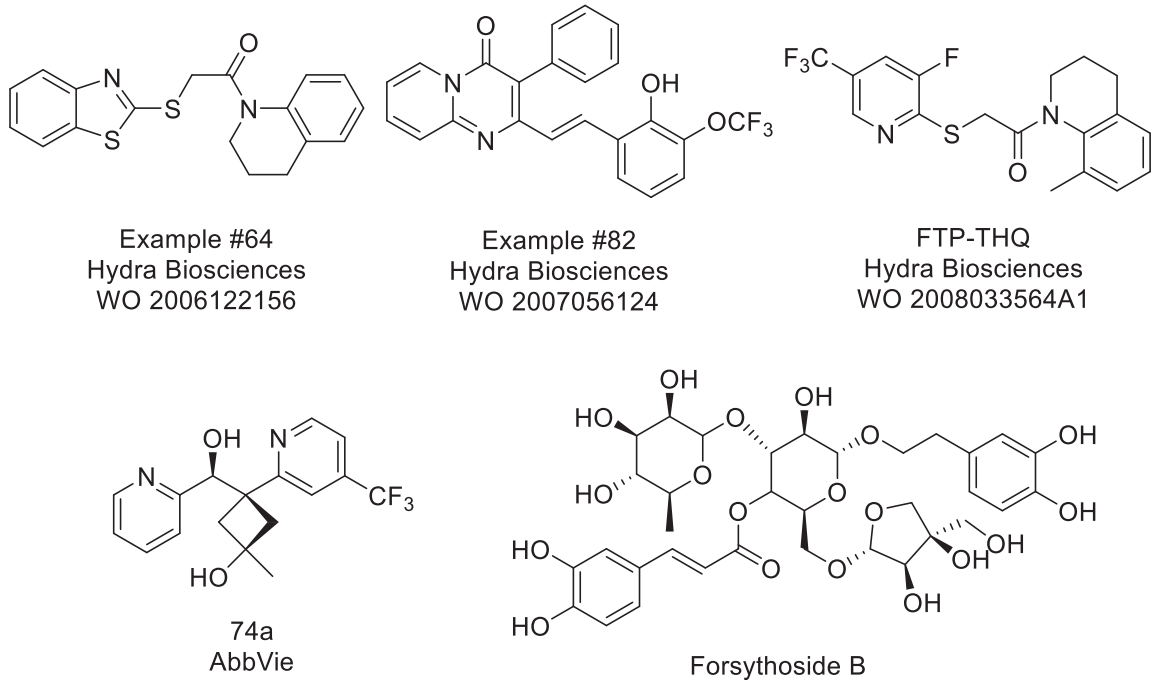
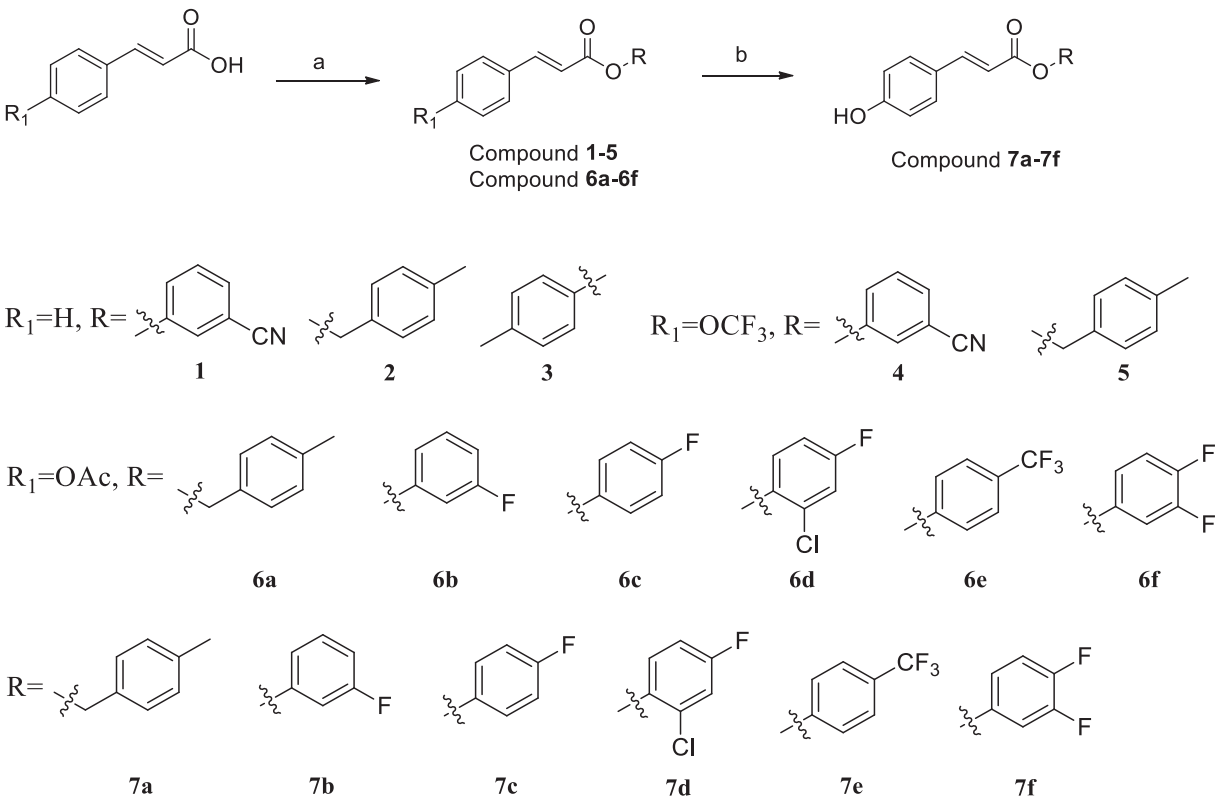
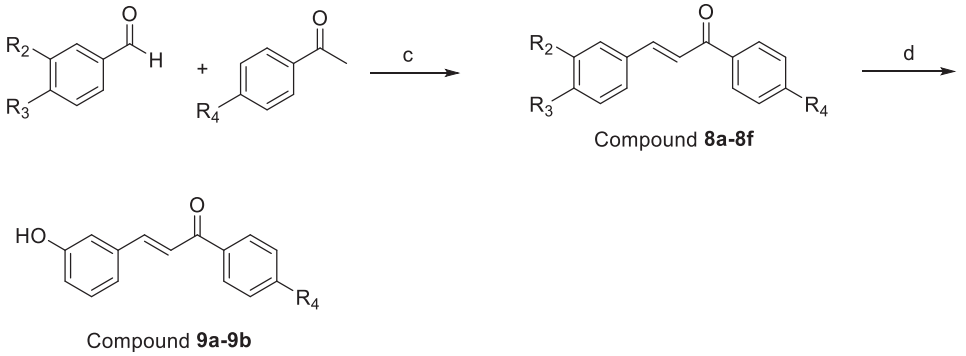


Fig. 1. The chemical structure of TRPV3 antagonists.



Scheme 1. The synthetic route of 17 target compounds. Reagents and conditions: (a) phenol or benzyl alcohol, EDCI, DMAP, Et_3N, CH_2Cl_2, 0 °C to rt, overnight, yields 80–87%; (b) K_2CO_3, CH_3OH, THF, rt, 2 h, yields 60–76%



8a R_2= , R_3=H, R_4=F

8b R_2= , R_3=H, R_4=CH_3

9a R_4=F

9b R_4=CH_3

8c R_2=H, R_3=OCF_3, R_4=F

8d R_2=H, R_3=F, R_4=F

8e R_2=H, R_3=Cl, R_4=F

8f R_2=H, R_3=Br, R_4=F

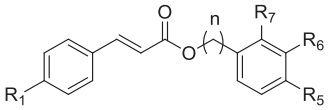
Scheme 2. The synthetic route of 6 target compounds. Reagents and conditions: (c) 10% NaOH, EtOH, rt, overnight; (d) 3 N HCl, CH_3OH, rt, 3 h, yields 72–76%

activity. Then, the fluorine substitution of ring B was retained, and ring A was modified to obtain **8c-8f** compounds. These compounds with halo substituted in ring B exhibited poor inhibitory activities. While the trifluoromethoxy substituted compound **8c** showed the excellent inhibition (77.17%).

2.2.2. IC_{50} values and TRPV selectivity

All compounds were tested on TRPV3 to evaluate their TRPV3 inhibitory activities in 50 μM concentration. Compound **7c** and **8c** performed well in this test, then the IC_{50} values were evaluated. In Fig. 3, The IC_{50} values were 1.05 μM and 86 nM, respectively. While the IC_{50}

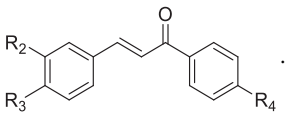
Table 1
The α,β-unsaturated ester target compounds of inhibitory activity *in vitro*



Compound	R_1	R_5	R_6	R_7	n	50 μM Inhibition rate (%)
1	H	H	CN	H	0	19.26
2	H	CH_3	H	H	1	32.6
3	H	CH_3	H	H	0	28.58
4	OCF_3	H	CN	H	0	29.12
5	OCF_3	CH_3	H	H	1	60.39
6a	OAc	CH_3	H	H	1	22.65
6b	OAc	H	F	H	0	75.59
6c	OAc	F	H	H	0	66.9
6d	OAc	F	H	Cl	0	56.54
6e	OAc	CF_3	H	H	0	58.8
6f	OAc	F	F	H	0	81.87
7a	OH	CH_3	H	H	1	47.24
7b	OH	H	F	H	0	63.99
7c	OH	F	H	H	0	85.45
7d	OH	F	H	Cl	0	79.56
7e	OH	CF_3	H	H	0	55.25
7f	OH	F	F	H	0	51.26
Forsythoside B*	–	–	–	–	–	61.07

* positive control.

Table 2
The α,β-unsaturated carbonyl target compounds of inhibitory activity *in vitro*



Compound	R_2	R_3	R_4	50 μM Inhibition rate (%)
9a	OH	H	F	73.58
9b	OH	H	CH_3	2.586
8c	H	OCF_3	F	77.17
8d	H	F	F	7.837
8e	H	Cl	F	21.28
8f	H	Br	F	41.03
Forsythoside B*	–	–	–	61.07

* positive control.

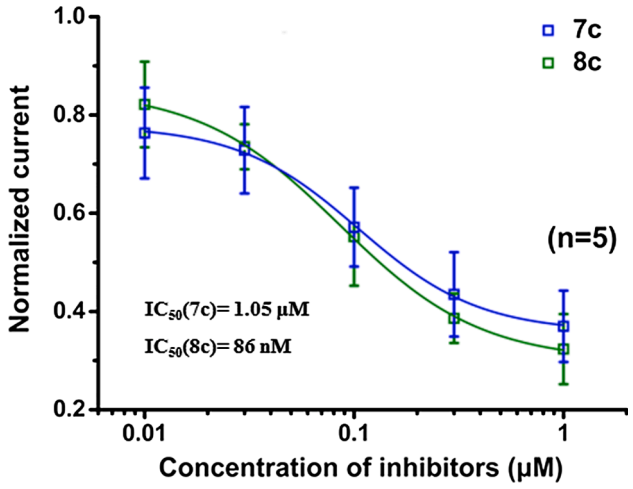


Fig. 3. IC_{50} values of Compound **7c** and **8c**.

value of Forsythoside B was tested as 6.71 μM. As an important branch of TRPV family, TRPV3 has been paid more and more attentions due to the lack of specific inhibitors. Accordingly, with the aim of characterizing the isoform selectivity profile, **7c** and **8c** were selected as the candidate to test its inhibitory activities against TRPV1 and TRPV4. We can see from the Fig. 4. The results of selectivity experiments showed that **7c** and **8c** hardly inhibited TRPV1 and TRPV4.

3. Conclusion

In summary, we reported that a novel series of cinnamate ester derivatives were designed, synthesized and evaluated against TRPV3. The target compounds were synthesized by the corresponding reaction based on bioisosterism. The results showed that the target compound **7c** and **8c** have high TRPV3 inhibitory activity, with IC_{50} values of 1.05 μM and 86 nM, respectively. Furthermore, **7c** and **8c** were tested have a good selectivity to TRPV1 and TRPV4. At the same time, the results of SAR research and analysis indicated that these target compounds have the potential of further structural optimization.

4. Experimental section

4.1. Chemistry

4.1.1. A general method for synthesis of compounds 1–5, 6a-6f and 7a-7f

To a stirred solution of cinnamic acid (1.5 mmol) in 8 mL dichloromethane solution was added alcohol or phenol (1 mmol), EDCI (2 mmol) and DMAP (0.05 mmol) at 0 °C. The triethylamine (2 mmol) was added to the previous liquor and the reaction was warmed to room temperature and stirred mechanically overnight. After that, the reaction mixture was quenched with saturated aqueous $NaHCO_3$ solution (20 mL) and extracted with CH_2Cl_2 (3 × 20 mL). The combined organic phases were washed with saturated aqueous NaCl solution (2 × 20 mL) and dried over anhydrous Na_2SO_4, then filtrated and evaporated the solvent under vacuum. The residue was subjected to silica gel column chromatography for purification using EtOAc/petroleum ether (1:6) as eluent to give compound **1**–**5** and **6a-6f** as white foam (80–87% yield). To a stirred **6a-6f** (0.4 mmol) and K_2CO_3 (0.12 mmol) were put into 2.5 mL methanol and 2.5 mL THF for 2 h. The reaction was acidified with 2 N HCl to pH 1. The resultant mixture was extracted with EtOAc (3 × 20 mL). The combined organic phases were washed with saturated aqueous NaCl solution (2 × 20 mL) and dried over anhydrous Na_2SO_4, then filtrated and evaporated the solvent under vacuum. The residue was subjected to silica gel column chromatography for purification using EtOAc/petroleum ether (1:3) as eluent to give compound **7a-7f** as white foam (60–76% yield).

4.1.2. 3-cyanophenyl cinnamate (1)

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.90 (d, J = 16.0 Hz, 1H), 7.60 (dd, J = 7.1, 2.3 Hz, 2H), 7.55 – 7.50 (m, 3H), 7.46 – 7.44 (m, 4H), 6.62 (d, J = 16.0 Hz, 1H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 164.69, 150.92, 147.78, 133.82, 131.12, 130.39, 129.49, 129.09, 128.45, 126.70, 125.47, 117.93, 116.25, 113.45. HRMS (ESI): *m/z* 250.0863 $[M+H]^+$; calcd for $C_{16}H_{12}NO_2$, 250.0868.

4.1.3. 4-methylbenzyl cinnamate (2)

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.74 (d, J = 16.0 Hz, 1H), 7.56 – 7.50 (m, 2H), 7.39 (dd, J = 3.8, 2.5 Hz, 3H), 7.33 (d, J = 8.0 Hz, 2H), 7.21 (d, J = 7.8 Hz, 2H), 6.49 (d, J = 16.0 Hz, 1H), 5.23 (s, 2H), 2.38 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 166.85, 145.06, 138.14, 134.39, 133.04, 130.32, 129.29, 128.89, 128.49, 128.10, 126.80, 118.00, 66.35, 21.25. HRMS (ESI): *m/z* 275.1043 $[M+Na]^+$; calcd for $C_{17}H_{16}NaO_2$, 275.1048.

4.1.4. p-tolyl cinnamate (3)

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.86 (d, J = 16.0 Hz, 1H), 7.59 (dd, J = 6.2, 3.2 Hz, 2H), 7.46 – 7.40 (m, 3H), 7.20 (d, J = 8.2 Hz, 2H), 7.05 (d, J = 8.4 Hz, 2H), 6.63 (d, J = 16.0 Hz, 1H), 2.36 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 165.63, 148.54, 146.40, 135.43, 134.22, 130.65, 129.97, 128.99, 128.29, 121.31, 117.41, 20.92. HRMS (ESI): *m/z* 239.1067

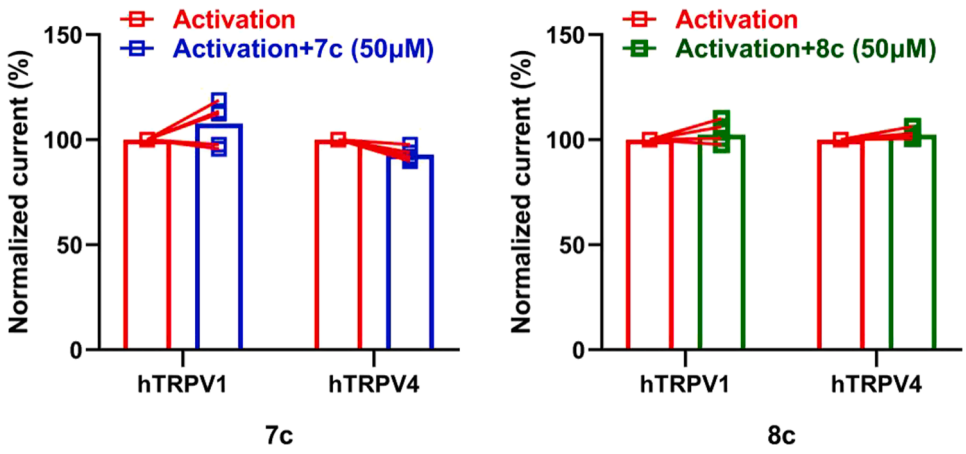


Fig. 4. Selectivity of compound **7c** and **8c** to other subfamily members of TRPV.

$[M+H]^+$; calcd for $C_{16}H_{15}O_2$, 239.1072.

*4.1.5. (E)-3-cyanophenyl 3-(4-(trifluoromethoxy)phenyl)acrylate **(4)***

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.87 (d, J = 16.0 Hz, 1H), 7.64 (d, J = 8.7 Hz, 2H), 7.58 – 7.50 (m, 3H), 7.47 – 7.43 (m, 1H), 7.28 (d, J = 8.4 Hz, 2H), 6.59 (d, J = 16.0 Hz, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 164.38, 150.99, 150.97, 150.84, 145.92, 132.39, 130.44, 129.94, 129.60, 126.63, 125.42, 121.65, 121.27, 119.08, 117.88, 117.25, 113.53. ^{19}F NMR (376 MHz, $CDCl_3$) δ −57.70. HRMS (ESI): *m/z* 356.0505 $[M+Na]^+$; calcd for $C_{17}H_{10}F_3NNaO_3$, 356.0510.

*4.1.6. (E)-4-methylbenzyl 3-(4-(trifluoromethoxy)phenyl)acrylate **(5)***

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.68 (d, J = 16.0 Hz, 1H), 7.53 (t, J = 5.6 Hz, 2H), 7.31 (d, J = 8.0 Hz, 2H), 7.21 (t, J = 8.7 Hz, 4H), 6.44 (d, J = 16.0 Hz, 1H), 5.21 (s, 2H), 2.37 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.52, 150.44, 143.27, 138.26, 133.01, 132.91, 129.52, 129.33, 128.54, 121.67, 121.16, 121.16, 119.10, 118.99, 77.38, 77.06, 76.74, 66.52, 21.23. ^{19}F NMR (376 MHz, $CDCl_3$) δ −57.74. HRMS (ESI): *m/z* 359.0866 $[M+Na]^+$; calcd for $C_{18}H_{15}F_3NaO_3$, 359.0871.

*4.1.7. (E)-4-methylbenzyl 3-(4-acetoxyphenyl)acrylate **(6a)***

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.69 (d, J = 16.0 Hz, 1H), 7.53 (d, J = 8.6 Hz, 2H), 7.32 (d, J = 8.0 Hz, 2H), 7.20 (d, J = 7.8 Hz, 2H), 7.12 (d, J = 8.6 Hz, 2H), 6.43 (d, J = 16.0 Hz, 1H), 5.21 (s, 2H), 2.37 (s, 3H), 2.31 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 169.16, 166.76, 152.13, 143.94, 138.18, 132.99, 132.14, 129.97, 129.31, 129.24, 128.51, 128.44, 122.15, 118.18, 115.91, 66.42, 21.24, 21.14. HRMS (ESI): *m/z* 333.1097 $[M+Na]^+$; calcd for $C_{19}H_{18}NaO_4$, 333.1103.

*4.1.8. (E)-3-fluorophenyl 3-(4-acetoxyphenyl)acrylate **(6b)***

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.85 (d, J = 16.0 Hz, 1H), 7.61 (d, J = 8.1 Hz, 2H), 7.36 (dd, J = 15.2, 7.6 Hz, 1H), 7.17 (d, J = 8.0 Hz, 2H), 6.97 (dd, J = 15.8, 8.9 Hz, 3H), 6.57 (d, J = 16.0 Hz, 1H), 2.33 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 169.11, 164.83, 164.16, 161.70, 152.56, 151.69, 151.58, 145.94, 131.74, 130.25, 130.16, 129.55, 122.32, 117.47, 117.43, 117.00, 112.97, 112.76, 109.90, 109.66, 21.15. ^{19}F NMR (376 MHz, $CDCl_3$) δ −111.06. HRMS (ESI): *m/z* 323.0690 $[M+Na]^+$; calcd for $C_{17}H_{13}FNaO_4$, 323.0696.

*4.1.9. (E)-4-fluorophenyl 3-(4-acetoxyphenyl)acrylate **(6c)***

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.84 (d, J = 16.0 Hz, 1H), 7.64 – 7.56 (m, 2H), 7.19 – 7.06 (m, 6H), 6.57 (d, J = 16.0 Hz, 1H), 2.33 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 169.12, 165.31, 161.46, 159.04, 152.51, 146.61, 146.58, 145.71, 131.80, 129.51, 123.07, 122.98, 122.31, 117.14, 116.22, 115.99, 21.15. ^{19}F NMR (376 MHz, $CDCl_3$) δ −117.04. HRMS (ESI): *m/z* 323.0690 $[M+Na]^+$; calcd for $C_{17}H_{13}FNaO_4$, 323.0696.

*4.1.10. (E)-2-chloro-4-fluorophenyl 3-(4-acetoxyphenyl)acrylate **(6d)***

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.89 (d, J = 16.0 Hz, 1H), 7.62 (d, J = 8.6 Hz, 2H), 7.24 – 7.16 (m, 4H), 7.08 – 6.98 (m, 1H), 6.61 (d, J = 16.0 Hz, 1H), 2.33 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 169.07, 164.32, 161.21, 158.75, 152.64, 146.47, 143.36, 131.67, 129.62, 127.91, 127.80, 124.60, 124.51, 122.32, 117.67, 117.41, 116.27, 114.85, 114.62, 21.16. ^{19}F NMR (376 MHz, $CDCl_3$) δ −114.39.

*4.1.11. (E)-4-(trifluoromethyl)phenyl 3-(4-acetoxyphenyl)acrylate **(6e)***

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.87 (d, J = 16.0 Hz, 1H), 7.68 (d, J = 8.5 Hz, 2H), 7.62 (d, J = 8.6 Hz, 2H), 7.31 (d, J = 8.6 Hz, 2H), 7.18 (d, J = 8.5 Hz, 2H), 6.59 (d, J = 16.0 Hz, 1H), 2.33 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 169.09, 164.71, 153.30, 152.65, 146.26, 131.66, 129.59, 128.22, 127.90, 126.88, 126.84, 126.80, 126.76, 125.27, 122.57, 122.36, 122.15, 116.78, 21.14. HRMS (ESI): *m/z* 373.0658 $[M+Na]^+$; calcd for $C_{18}H_{13}F_3NaO_4$, 373.0664. ^{19}F NMR (376 MHz, $CDCl_3$) δ −62.20.

*4.1.12. (E)-3,4-difluorophenyl 3-(4-acetoxyphenyl)acrylate **(6f)***

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.84 (d, J = 16.0 Hz, 1H), 7.61 (d, J = 8.5 Hz, 2H), 7.19 (dd, J = 18.6, 8.8 Hz, 3H), 7.10 – 7.02 (m, 1H), 6.95 – 6.89 (m, 1H), 6.55 (d, J = 16.0 Hz, 1H), 2.33 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 169.09, 164.85, 152.63, 151.43, 151.29, 149.56, 149.44, 148.95, 148.81, 147.12, 146.99, 146.42, 146.39, 146.34, 146.30, 146.20, 131.64, 129.57, 122.35, 117.69, 117.65, 117.62, 117.59, 117.39, 117.20, 116.67, 111.81, 111.61, 21.14. ^{19}F NMR (376 MHz, $CDCl_3$) δ −134.65 (d, J = 21.4 Hz), −140.99 (d, J = 21.4 Hz). HRMS (ESI): *m/z* 341.0596 $[M+Na]^+$; calcd for $C_{17}H_{12}F_2NaO_4$, 341.0601.

*4.1.13. (E)-4-methylbenzyl 3-(4-hydroxyphenyl)acrylate **(7a)***

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.66 (d, J = 16.0 Hz, 1H), 7.40 (d, J = 8.5 Hz, 2H), 7.31 (d, J = 8.0 Hz, 2H), 7.19 (d, J = 7.9 Hz, 2H), 6.84 (d, J = 8.6 Hz, 2H), 6.33 (d, J = 16.0 Hz, 1H), 5.21 (s, 2H), 2.36 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 167.74, 158.28, 158.13, 145.32, 145.17, 138.18, 132.98, 130.07, 129.31, 128.45, 126.96, 126.86, 115.98, 115.96, 115.10, 114.98, 66.48, 66.43, 21.24. HRMS (ESI): *m/z* 291.0992 $[M+Na]^+$; calcd for $C_{17}H_{16}NaO_3$, 291.0997.

*4.1.14. (E)-3-fluorophenyl 3-(4-hydroxyphenyl)acrylate **(7b)***

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.81 (d, J = 15.9 Hz, 1H), 7.50 (d, J = 8.3 Hz, 2H), 7.36 (dd, J = 14.8, 7.9 Hz, 1H), 7.00 – 6.91 (m, 3H), 6.87 (d, J = 8.4 Hz, 2H), 6.47 (d, J = 15.9 Hz, 1H). ^{13}C NMR (101 MHz, DMSO) δ 165.44, 163.91, 161.48, 160.97, 152.15, 152.04, 147.65, 131.29, 131.21, 131.12, 125.30, 118.70, 118.67, 116.40, 113.19, 113.13, 112.98, 110.50, 110.26. ^{19}F NMR (376 MHz, CD_3OD) δ −113.69. HRMS (ESI): *m/z* 257.0619 $[M - H]^+$; calcd for $C_{15}H_{10}FO_3$, 257.0614.

*4.1.15. (E)-4-fluorophenyl 3-(4-hydroxyphenyl)acrylate **(7c)***

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.80 (t, J = 13.7 Hz, 1H), 7.48 (d, J = 8.6 Hz, 2H), 7.16 – 7.03 (m, 4H), 6.85 (d, J = 8.6 Hz, 2H), 6.47 (d, J = 15.9 Hz, 1H), 5.57 (s, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.03, 161.44, 159.01, 158.19, 146.68, 133.06, 130.34, 126.92, 123.11, 123.03, 116.20, 116.03, 115.97, 115.10, 114.26. ^{19}F NMR (376 MHz, $CDCl_3$) δ −117.13. HRMS (ESI): *m/z* 257.0619 $[M - H]^+$; calcd for $C_{15}H_{10}FO_3$, 257.0614.

*4.1.16. (E)-2-chloro-4-fluorophenyl 3-(4-hydroxyphenyl)acrylate **(7d)***

^{1}H NMR (400 MHz, CD_3OD) δ 7.82 (t, J = 17.0 Hz, 1H), 7.55 (d, J = 8.6 Hz, 2H), 7.34 (dd, J = 8.3, 2.9 Hz, 1H), 7.26 (dd, J = 9.0, 5.2 Hz, 1H), 7.17 – 7.08 (m, 1H), 6.84 (d, J = 8.6 Hz, 2H), 6.55 (d, J = 15.9 Hz, 1H). ^{13}C NMR (101 MHz, CD_3OD) δ 169.06, 165.18, 164.49, 162.73, 151.79, 147.67, 136.96, 134.24, 131.68, 131.57, 129.39, 128.85, 128.76, 120.86, 120.59, 119.53, 118.50, 118.27, 115.67. ^{19}F NMR (376 MHz, CD_3OD) δ −116.68. HRMS (ESI): *m/z* 315.0195 $[M+Na]^+$; calcd for $C_{15}H_{10}ClFNaO_3$, 315.0200.

*4.1.17. (E)-4-(trifluoromethyl)phenyl 3-(4-hydroxyphenyl)acrylate **(7e)***

^{1}H NMR (400 MHz, DMSO) δ 7.73 – 7.79 (m, 3H), 7.66 (d, J = 8.7 Hz, 2H), 7.45 (d, J = 8.4 Hz, 2H), 6.84 (d, J = 8.6 Hz, 2H), 6.65 (d, J = 15.9 Hz, 1H). ^{13}C NMR (101 MHz, DMSO) δ 165.35, 161.00, 154.07, 147.92, 131.34, 127.35, 127.31, 127.27, 127.24, 126.93, 126.61, 125.88, 125.30, 123.40, 123.17, 116.39, 112.97. ^{19}F NMR (376 MHz, CD_3OD) δ −63.48. HRMS (ESI): *m/z* 307.0588 $[M - H]^+$; calcd for $C_{16}H_{10}F_3O_3$, 307.0582.

*4.1.18. (E)-3,4-difluorophenyl 3-(4-hydroxyphenyl)acrylate **(7f)***

^{1}H NMR (400 MHz, DMSO) δ 10.16 (s, 1H), 7.78 (d, J = 15.9 Hz, 1H), 7.65 (d, J = 8.6 Hz, 2H), 7.56 – 7.40 (m, 2H), 7.13 – 7.05 (m, 1H), 6.83 (d, J = 8.6 Hz, 2H), 6.61 (d, J = 15.9 Hz, 1H). ^{13}C NMR (101 MHz, DMSO) δ 165.49, 160.90, 150.94, 150.80, 149.04, 148.92, 148.49, 148.35, 147.76, 147.13, 147.11, 147.04, 147.01, 146.63, 146.50, 131.32, 125.33, 119.30, 118.16, 117.97, 116.37, 112.98, 112.73, 112.54. ^{19}F NMR (376 MHz, CD_3OD) δ −138.05 (d, J = 20.8 Hz), −144.31 (d, J = 20.8 Hz). HRMS (ESI): *m/z* 275.0525 $[M - H]^+$; calcd for $C_{15}H_9F_2O_3$, 275.0520.

4.1.18.1. A general method for synthesis of compounds 9a, 9b and 8c-8f.

The acetophenone (1.3 mmol) and 10% NaOH (3 mmol) were dissolved in anhydrous ethanol (5 mL) under stirring for 10 min. Then the benzaldehyde (1.2 mmol) was added to the previous liquor and the reaction mixture was stirred overnight at room temperature. After that, the resultant mixture was extracted with EtOAc (3 × 20 mL). The combined organic phases were washed with saturated aqueous NaCl solution (2 × 20 mL) and dried over anhydrous Na_2SO_4, then filtrated and evaporated the solvent under vacuum. The residue was subjected to silica gel column chromatography for purification using EtOAc/petroleum ether (1:15) as eluent to give compounds **8a-8b** as oil and **8c-8f** as white foam. To a stirred solution of **8a-8b** (1 mmol) in methanol solution (5 mL) was added 3 N HCl (3 mmol). The reaction mixture was stirred at room temperature for 3 h and then quenched with saturated aq.$NaHCO_3$ (20 mL). The resultant mixture was extracted with EtOAc (3 × 20 mL). The combined organic phases were washed with saturated aqueous NaCl solution (2 × 20 mL) and dried over anhydrous Na_2SO_4, then filtrated and evaporated the solvent under vacuum. The residue was subjected to silica gel column chromatography for purification using EtOAc/petroleum ether (1:10) as eluent to give compound **9a**, **9b** as light yellow foam (72–76% yield).

*4.1.18.2. (E)-1-(4-fluorophenyl)-3-(3-hydroxyphenyl)prop-2-en-1-one **(9a)**.* ^{1}H NMR (400 MHz, DMSO) δ 9.64 (d, J = 2.5 Hz, 1H), 8.24 (dd, J = 8.4, 5.7 Hz, 2H), 7.84 (d, J = 15.6 Hz, 1H), 7.65 (d, J = 15.6 Hz, 1H), 7.42 – 7.35 (m, 2H), 7.31 (d, J = 7.6 Hz, 1H), 7.28 – 7.22 (m, 2H), 6.88 (dd, J = 7.9, 1.2 Hz, 1H). ^{13}C NMR (101 MHz, DMSO) δ 188.23, 166.76, 164.26, 158.20, 144.92, 136.37, 134.75, 134.72, 132.04, 131.94, 130.35, 122.15, 120.41, 118.36, 116.37, 116.16, 115.80. HRMS (ESI): *m/z* 243.0816 $[M+H]^+$; calcd for $C_{15}H_{12}FO_2$, 243.0821.

*4.1.18.3. (E)-3-(3-hydroxyphenyl)-1-(p-tolyl)prop-2-en-1-one **(9b)**.* ^{1}H NMR (400 MHz, CD_3OD) δ 7.99 (dd, J = 8.3, 1.9 Hz, 2H), 7.75 – 7.63 (m, 2H), 7.42 – 7.33 (m, 2H), 7.28 – 7.21 (m, 2H), 7.15 (s, 1H), 6.92 – 6.85 (m, 1H), 2.45 (s, 3H). ^{13}C NMR (101 MHz, CD_3OD) δ 190.63, 157.74, 144.75, 144.02, 136.18, 135.37, 129.68, 129.09, 128.96, 128.42, 121.49, 119.85, 117.53, 114.40, 20.26. HRMS (ESI): *m/z* 237.0921 $[M - H]^+$; calcd for $C_{16}H_{13}O_2$, 237.0916.

*4.1.18.4. (E)-1-(4-fluorophenyl)-3-(4-(trifluoromethoxy)phenyl)prop-2-en-1-one **(8c)**.* ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.08 – 7.98 (m, 2H), 7.76 (d, J = 15.7 Hz, 1H), 7.65 (d, J = 8.7 Hz, 2H), 7.45 (d, J = 15.7 Hz, 1H), 7.24 (d, J = 7.8 Hz, 2H), 7.18 – 7.14 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 188.49, 167.00, 164.47, 150.64, 143.19, 134.33, 134.30, 133.37, 131.18, 131.09, 129.90, 122.35, 121.66, 121.23, 119.10, 115.96, 115.75. ^{19}F NMR (376 MHz, $CDCl_3$) δ −61.65, −109.09. HRMS (ESI): *m/z* 311.0690 $[M+H]^+$; calcd for $C_{16}H_{11}F_4O_2$, 311.0695.

*4.1.18.5. (E)-1,3-bis(4-fluorophenyl)prop-2-en-1-one **(8d)**.* ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.08 – 8.02 (m, 2H), 7.78 (d, J = 15.7 Hz, 1H), 7.67 – 7.61 (m, 2H), 7.43 (d, J = 15.6 Hz, 1H), 7.21 – 7.15 (m, 2H), 7.15 – 7.08 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 188.60, 166.92, 165.39, 164.39, 162.88, 143.73, 134.47, 134.44, 131.14, 131.04, 130.45, 130.36, 121.27, 121.25, 116.30, 116.08, 115.90, 115.69. ^{19}F NMR (376 MHz, $CDCl_3$) δ −105.42, −108.82. HRMS (ESI): *m/z* 267.0592 $[M+Na]^+$; calcd for $C_{15}H_{10}F_2NaO$, 267.0597.

*4.1.18.6. (E)-3-(4-chlorophenyl)-1-(4-fluorophenyl)prop-2-en-1-one **(8e)**.* ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.08 – 8.03 (m, 2H), 7.76 (d, J = 15.7 Hz, 1H), 7.60 – 7.56 (m, 2H), 7.48 (d, J = 15.7 Hz, 1H), 7.42 – 7.38 (m, 2H), 7.22 – 7.15 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 188.55, 166.97, 164.44, 143.55, 136.59, 134.39, 134.36, 133.26, 131.17, 131.08, 129.63, 129.31, 121.97, 115.95, 115.73. ^{19}F NMR (376 MHz, $CDCl_3$) δ −109.18. HRMS (ESI): *m/z* 283.0296 $[M+Na]^+$; calcd for $C_{15}H_{10}ClFNaO$, 283.0302.

*4.1.18.7. (E)-3-(4-bromophenyl)-1-(4-fluorophenyl)prop-2-en-1-one **(8f)**.* ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.09 – 8.01 (m, 2H), 7.75 (d, J = 15.7 Hz, 1H), 7.58 – 7.54 (m, 2H), 7.53 – 7.47 (m, 3H), 7.22 – 7.15 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 188.53, 166.98, 164.44, 143.61, 134.37, 134.34, 133.69, 132.27, 131.17, 131.08, 129.83, 124.96, 122.06, 115.95, 115.74. ^{19}F NMR (376 MHz, $CDCl_3$) δ −109.14. HRMS (ESI): *m/z* 326.9791 $[M+Na]^+$; calcd for $C_{15}H_{10}BrFNaO$, 326.9797.

4.2. Screening of TRPV3 inhibitory activity

HEK-293 cells were seeded into a small dish. On the next day, HEK-293 cells were transiently transfected with hTRPV3 plasmid. After 4 h, the medium was changed and incubated overnight in a 5% CO_2 incubator at 37 °C. On the third day, the whole cell current was recorded by patch clamp amplification system. The extracellular fluid containing 2-APB (50 μM) was perfused first, and then the extracellular fluid containing different concentrations of inhibitor and 2-APB (50 μM) was perfused. The maximum outward current mediated by mTRPV3 channel induced by 2-APB and the outward current mediated by hTRPV3 channel under the action of 2-APB were recorded. The half inhibitory concentration (IC_{50}) was calculated according to the inhibition rate of outward current mediated by hTRPV3 channel [21].

When we evaluated the channel selectivity of the compounds, we selected known potent agonists for each channel to control, 1 μM capsaicin for TRPV1 and 0.1 μM GSK101 for TRPV4 [21].

Declaration of Competing Interest

The authors declare that they have no known competing financial interests 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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