Brief Article

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Discovery of Non-pungent Transient Receptor Potential Vanilloid 1 (TRPV1) Agonist as Strong Topical Analgesic

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ABSTRACT: Paradoxically, some TRPV1 agonists are, at the organismal level, both non-pungent and clinically useful as topical analgesics. Here, we describe the scaled-up synthesis and characterization in mouse models of a novel, non-pungent vanilloid. Potent analgesic activity was observed in models of neuropathic pain, and the compound blocked capsaicin induced allodynia, showing dermal accumulation with little transdermal absorption. Finally, it displayed much weaker systemic toxicity compared to capsaicin and was negative in assays of genotoxicity.

■ INTRODUCTION

The Transient Receptor Potential Vanilloid 1 (TRPV1) is a ligand-gated and nonselective cation channel that is expressed in the spinal cord and brain and is localized on neurons in sensory ganglia, with peripheral projection to the skin, muscles, joints and gut and with central terminals to the spinal dorsal horn.[1] TRPV1 can be activated by multiple factors such as protons, heat, endogenous ligands and natural vanilloids including capsaicin and resiniferatoxin. Activation of TRPV1 triggers an influx of calcium and sodium, which initiates a cascade of events associated with pain transmission, including membrane depolarization, neuronal firing, and release of pain transmitters.[2]

Capsaicin (CAP, **1**) is the prototype of TRPV1 agonists[3] and is clinically used as an analgesic in topical formulations.[4,5] The high concentration of capsaicin (8% capsaicin) as a transdermal patch (Qutenza) is specifically indicated for the management of neuropathic pain associated with postherpetic neuralgia.[6,7] Topical application of capsaicin causes an initial activation of TRPV1, causing pungency. However, the persistent activation of the channel results in high intracellular levels of calcium, ultimately leading to desensitization/defunctionalization to a variety of noxious stimuli through functional and morphological alterations to the peripheral ends of nerve fibers and consequent pain relief. Topical application of capsaicin thus combines two actions, a long-term therapeutic effect following an initial adverse effect of pungency at the application site as manifested by erythema, pain, pruritus and papules.[6]

Zucapsaicin (**2**) is the *cis*-isomer of capsaicin and was approved as a topical analgesic in 2010 for use in conjunction with oral COX-2 inhibitors or NSAIDs to relieve severe pain in adults with osteoarthritis of the knee.[8] The advantage of zucapsaicin is the lesser degree of local irritation such as stinging, burning and erythema in patients.

Resiniferatoxin (RTX, **3**) is an extremely potent irritant tricyclic diterpene. RTX has proven to function pharmacologically as an ultrapotent agonist for TRPV1, displaying 10^3- to 10^4-fold greater potency than capsaicin.[9] The actions of RTX are mediated by binding, with picomolar affinity, directly to the capsaicin-binding site on the TRPV1 receptor.[10] Whereas capsaicin under normal conditions produces only short term desensitization of TRPV1 mediated responses, the apparent desensitization to RTX can be of very long duration, lasting for weeks and can thus be applicable for chronic pain relief.[11-13]

For drug development targeting TRPV1, a recognized and very important concept is that the physiological response to agonist engagement is a complex integral of ligand-target interaction, feedback from cellular signaling mechanisms, and pharmacokinetics. For example, resiniferatoxin caused partial separation of acute toxicity versus desensitization/ defunctionalization in rats relative to capsaicin[14] and olvanil has long been known as a non-pungent TRPV1 agonist.[15] Thus, whereas initial evaluation of ligands for receptor engagement is a valuable first step, *in vivo* evaluation is essential to further define the pattern of response.

In our program to develop a non-pungent, highly efficacious TRPV1 agonist to avoid the adverse effects associated with the use of capsaicin, we investigated a number of diverse series of TRPV1 ligands and recently discovered MDR-652 (**4**) as a clinical candidate for use in a topical gel. Structurally, MDR-652 contains the three principal pharmacophores previously

designated for capsaicin[16] with the 3-fluoro-4-(hydroxymethyl)phenyl (A-region), urea (B-region) and 2-(*t*-butyl)-4-(3-chlorophenyl)thiazole (C-region) groups corresponding to the 4-hydroxy-3-methoxyphenyl (A-region), amide (B-region) and 8-methylnonenyl (C-region) groups, respectively, in capsaicin.

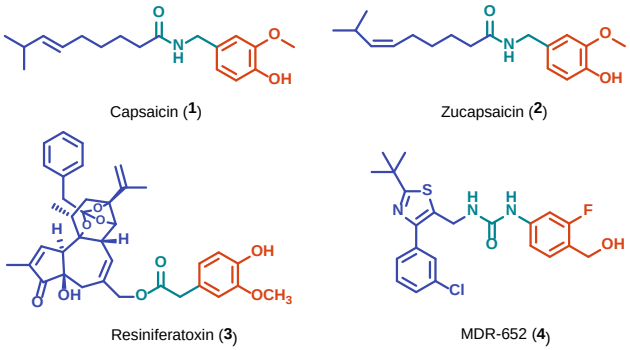


Figure 1. Representative TRPV1 agonists

In this paper, we described the optimized scale-up synthesis of **4**, the agonistic activity of **4** *in vitro* and *in vivo*, and its analgesic activities in acute and neuropathic pain models by topical and intraperitoneal (i.p.) administration, respectively. In addition we conducted the pharmacokinetic and toxicological studies of **4** for its development as a topical agent.

■ RESULTS AND DISCUSSION

Chemistry. The scale-up synthesis of **4** is described in **Scheme 1**. For the synthesis of the A-region, commercially available 2-fluoro-4-nitrobenzoic acid **5** was esterified and then reduced to the corresponding alcohol **7**. The nitro group of **7** was reduced and then reacted with phenylchloroformate to provide the carbamate intermediate of the A-region **9** employed for the final coupling reaction. For the synthesis of the C-region, commercially available 3-chloroacetophenone **10** was alkylated with dimethylcarbonate to afford the β-ketoester **11**, which was chlorinated with sulfuryl chloride to afford the α-chloro-β-ketoester **12**. The intermediate was condensed with pivalthioamide **13**, prepared from pivalamide by a modified method[17], to provide the thiazole **14**. The ester of **14** was reduced to the corresponding alcohol **15** and then converted to the amine of the C-region **16**. Finally, the condensation reaction between carbamate **9** and amine **16** under basic conditions provided the final product **4**. Overall, **4** was obtained in kilogram scale through a convergent ten-stage synthesis from readily available **5** and **10**.

In vitro Activity. The binding affinity and agonistic potency of **4** were assessed *in vitro* by a binding competition assay with [^{3}H]resiniferatoxin (RTX) and by a functional $^{45}Ca^{2+}$ uptake assay using human/rat TRPV1 heterologously expressed in Chinese hamster ovary (CHO) cells, as previously described.[18]

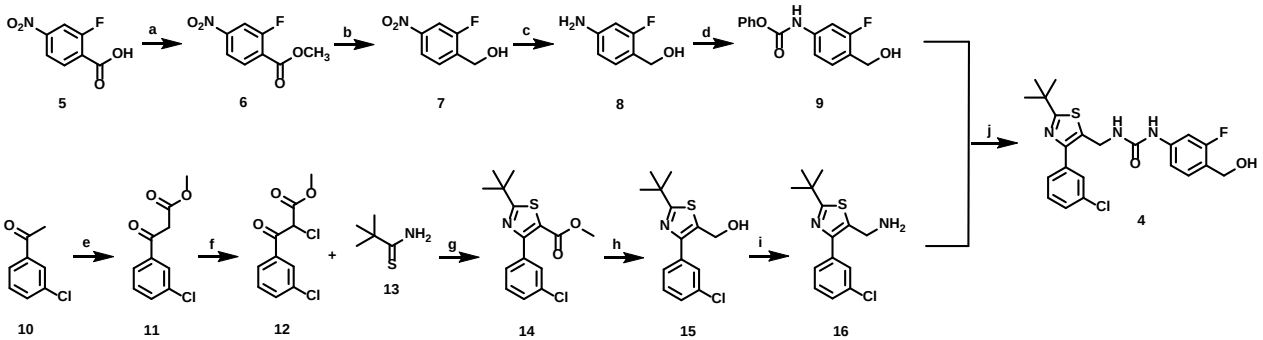
As shown in **Table 1**, **4** showed high affinity for human and rat TRPV1 with K_i = 11.4 and 23.8 nM that was ca. 80-fold more potent than that of capsaicin in both species. Also, it displayed highly potent agonism in *h*TRPV1 with an EC_{50} = 5.05 nM, which was ca. 5-fold more potent than that of capsaicin and zucapsaicin. Importantly, in both systems maximal calcium uptake stimulated by **4** was 50-60% that of capsaicin, indicating that it functions as a partial agonist (**Figure S1**). We have noted elsewhere that there is not full correspondence between measured TRPV1 ligand affinity and agonism assayed in cellular systems.[19] While both measures are valuable, we believe that the ligand affinity is a more robust measure, since the cellular agonism assays will further reflect the level of exogenous TRPV1 expressed in the cells, rate of compound uptake, etc. In any case, the most critical measure is actual *in vivo* activity. Were TRPV1 receptor affinity by an agonist to be the only factor determining response, then non-pungent agonists causing desensitization would be an unachievable goal.

Table 1. *In vitro* receptor activity[a]

	4	Capsaicin	Zucapsaicin
hTRPV1 (K_i, nM)	11.4 (±3.9)	1070 (±126)	1080 (±210)
hTRPV1 (EC_{50}, nM)[b]	5.05 (±0.88)	25.9 (±4.8)	28.2 (±2.3)
rTRPV1 (K_i, nM)	23.8 (±0.87)	1810 (±270)	
rTRPV1 (EC_{50}, nM)[c]	93 (±17)	44.8 (±3.8)	

[a] The values are the mean of at least three experiments. The saturating concentration of capsaicin (3 μM[b] or 1 μM[c]) was used to define maximal response. The EC_{50} of **4** is expressed relative to the maximal response that it induces.

Scheme 1. Scale-up synthesis of MDR-652 (**4**)[a]



[a] Reagents and conditions: (a) H_2SO_4, MeOH, reflux, overnight, 95%; (b) $NaBH_4$, MeOH, r.t., 3 h, 78%; (c) Zn, NH_4Cl, MeOH-H_2O, 50 °C, 4 h, 72%; (d) phenylchloroformate, pyridine, acetone, r.t., 4 h, 62%; (e) dimethylcarbonate, NaH, THF, 50 °C, overnight, 65%; (f) sulfuryl chloride, $CHCl_3$, reflux, 20 h, 72%; (g) MeOH, reflux, overnight, 71%; (h) $LiAlH_4$, THF, 0 °C, 85%; (i) i) $SOCl_2$, CH_2Cl_2, r.t., ii) NH_4OH, CH_3CN, 40 °C, overnight, 45%; (j) NEt_3, CH_3CN, 15-20 °C, 5 h, 81%.

In order to check the selectivity of **4** over other TRP family members, the intracellular calcium influxes mediated by four other nociceptive TRP channels were examined using a fluorescence imaging plate reader (FLIPR) assay with individual TRP-transfected cells. Upon application of **4**, statistically significant calcium influxes occurred only in TRPV1-transfected cells, but not in cells transfected with hTRPA1, mTRPA1, mTRPV3 and mTRPM8, whereas these cells all showed the expected calcium increases in response to the application of their appropriate TRP family member agonist (**Figure S2**), indicating that **4** specifically activates TRPV1.

Body Temperature Study. In order to confirm the agonistic activity of **4** *in vivo*, we performed the body temperature study of **4** using a rectal temperature probe in ICR mouse and with capsaicin as our control.[20,21] The administration of 3 mg/kg capsaicin by i.p. injection decreased the body temperature, showing the lowest temperature at 30 min. The coadministration of 3 mg/kg capsaicin with 0.5 mg/kg **4** caused a further decrease in body temperature compared with that of 3 mg/kg capsaicin alone (**Figure 2A**). In addition, **4** displayed a dose-dependent decrease of body temperature (**Figure 2B**), supporting that **4** displayed TRPV1 agonism in the intact animal.

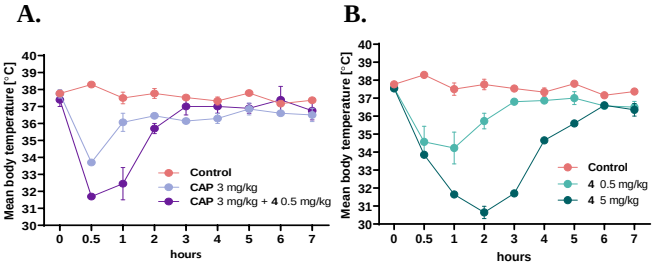


Figure 2. Body temperature study of **4** and capsaicin. Time profile of the hypothermic response of capsaicin and **4** in ICR mice. Capsaicin or/and **4** was administered intraperitoneally at time point zero, and the body temperature of mice was measured over 7 h. The data represents the mean±SEM (n=3).

Analgesic Activity. In order to evaluate analgesic activity of **4**, we conducted nociception assays in standard pain models. The formalin model is a standard model of pain in which 2% formalin is injected into the dorsal surface of a hindpaw and the time the animal spends licking the paw is recorded.[22,23] Analgesic evaluations both for 1st phase (5 min) and 2nd phase (20-30 min) were performed (**Figure 3**). The result revealed an excellent and dose-dependent analgesic profile after i.p. administration of **4** with analgesic activity (ED_{50} = 0.79 mg/kg for 1st phase and 0.16 mg/kg for 2nd phase) superior to gabapentin (ED_{50} = > 40 mg/kg). Of particular note, 1 mg/kg of **4** fully inhibited the 2nd phase pain response, showing ~100% of the maximum possible effect (MPE).

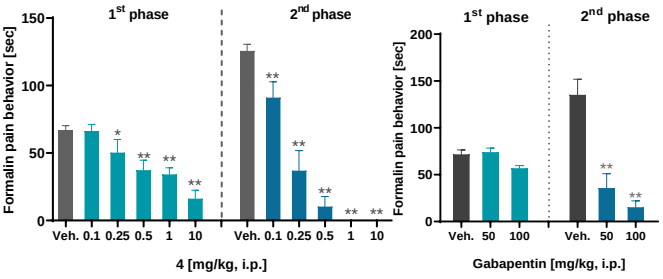


Figure 3. Analgesic activity of **4** in formalin model. Effect of **4** on the antinociceptive activity in the formalin test. Animals pretreated with a single injection of **4** showed significantly less licking compared with vehicle-treated animals. The data represents the mean±SEM (n=8). *P<0.05, **P<0.01 compared to vehicle.

Next, we examined the analgesic activity of **4** in the spinal nerve ligation (SNL) model. The SNL model is a standard model of neuropathic pain in which the L5 spinal nerve is tightly ligated with a surgical suture to cause neuropathic pain. The ligation was performed 14 days prior to compound testing and mechanical stimulation was induced with von Frey filaments (up & down method).[24] The i.p. administration of **4** exhibited an excellent and dose dependent analgesic profile and its activity (ED_{50} = 0.5-2 mg/kg) was again superior to gabapentin (ED_{50} = > 30 mg/kg). The 5-10 mg/kg dose of **4** blocked the neuropathic pain completely, indicating 100% maximum possible effect (MPE) (**Figure 4A**). In addition, the subcutaneous injection (s.c.) of **4** also displayed an excellent analgesic outcome with maximum effect at 30 min after administration, indicating that the route of administration did not change the strong analgesic profile (**Figure 4B**).

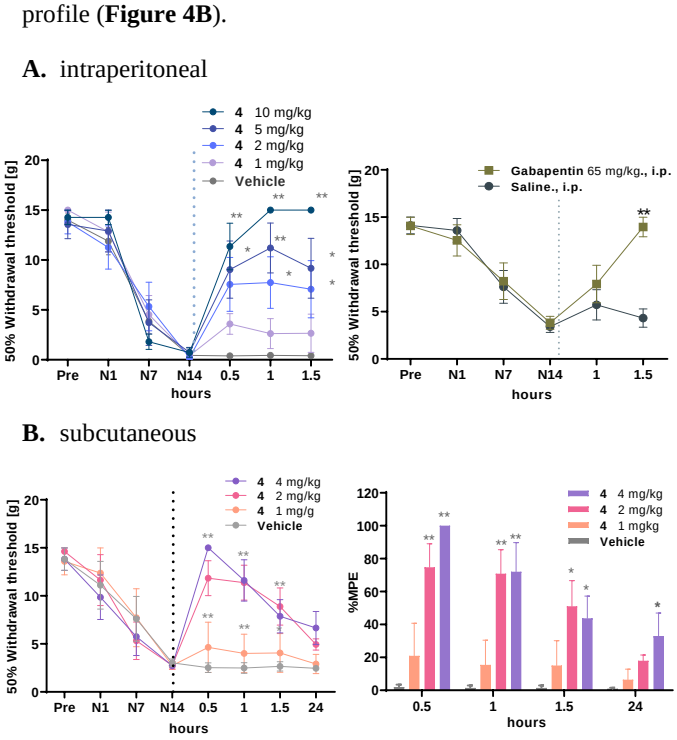


Figure 4. Analgesic activity of **4** in neuropathic pain model. Rats treated with **4** had significantly higher thresholds to pain caused by von Frey filament post dosing. The data represents the mean±SEM (n=7-8). *p<0.05, **P<0.01 compared to vehicle.

For the target engagement study of **4**, we examined the analgesic profile of **4** in the capsaicin-induced allodynia model.[25,26] Capsaicin, a full agonist of TRPV1, evokes pain after injection into the rodent paw. After topical gel application of **4**, the foot withdrawal frequency to mechanical stimulus was monitored over the 5 min period following capsaicin injection. As shown in **Figure 5**, **4** showed an excellent analgesic profile in a dose-dependent manner against capsaicin-induced allodynia, indicating that the analgesic activity of **4** was mediated by the desensitization of TRPV1.

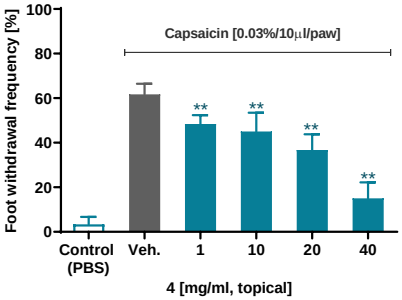


Figure 5. Target engagement study of **4**. **4** inhibited capsaicin induced mechanical hyperalgeisa in mice. The data represents the mean±SEM (n=7-8).

Pharmacokinetic Study. To examine the topical permeability of **4**, we conducted an *in vitro* transdermal drug delivery test using diffusion cells, comparing **4** to capsaicin cream (**Figure 6**).[27,28] Using the Franz cell diffusion system with porcine skin, **4** in carbopol gel, prepared as a 1% formulation, was applied to examine dermal accumulation and systemic absorption. The result indicated that almost all of the **4** applied remained in the skin, comprised of epidermis and dermis, and showed much higher dermal accumulation compared to that of capsaicin. As a result, a negligible amount of **4** was detected in the acceptor cell, in contrast to the case with capsaicin, suggesting that there would be little systemic absorption of **4**. The result supported the concept that **4** has promising potential as a safer dermal agent with minimal potential systemic absorption.

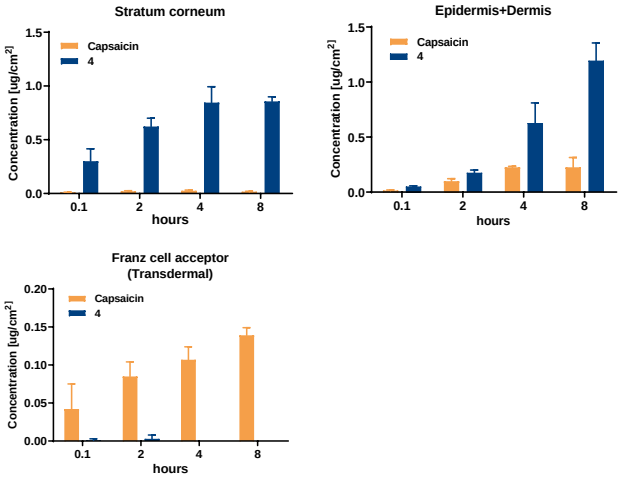


Figure 6. *In vitro* transdermal delivery test. Retention and permeation profile of **4** through pig abdominal skin using Franz diffusion cell. The data represents the mean±SD (n=3)

Toxicology Study. In order to investigate the safety of agonist **4** as a topical agent, we performed skin irritation and edema tests comparing it to capsaicin to examine the toxicity associated with TRPV1 agonism. To evaluate skin irritation, we conducted the ear scratching test in which a solution of **4** or capsaicin was applied to the ears of mice and then the number of scratching movements was recorded during the following 30 min period (**Figure 7A**).[29] For evaluating edema, we performed the ear swelling test in which a solution of **4** or capsaicin was applied to the ears of mice and then ear punch biopsies were collected after 30 min and weighed (**Figure 7B**).[29] Against the general idea that all TRPV1 agonists evoke skin irritation and edema similarly, **4** showed minimal signs of skin irritation and edema in the animal models whereas capsaicin displayed high irritation and edema in a concentration-dependent manner. The result indicated that **4** showed non-pungency and little edema associated with TRPV1 agonism, in contrast to the behavior of capsaicin in animal models.

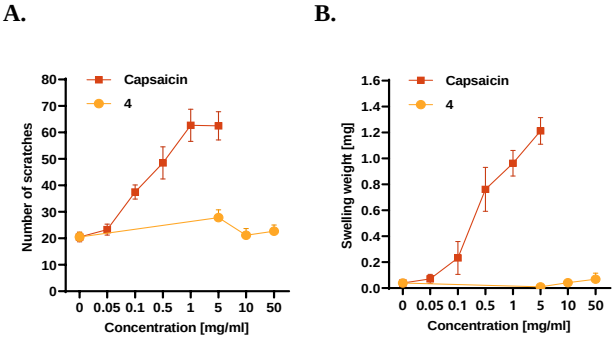


Figure 7. Skin irritation and edema test. (a) ear scratching behavior and (b) ear swelling elicited by **4** and capsaicin. The data represents the mean±SEM (n=7-8).

Finally, we conducted standard toxicity studies of **4** compared to capsaicin (**Table 2**). In a single-dose toxicity study, the LD_{50} of **4** was found to be higher than 200 and 2000 mg/kg in i.p. and p.o. administration, respectively, whereas the LD_{50} of capsaicin had low values[30,31], indicating that **4** was much safer than capsaicin. In addition, in a genotoxicity study, **4** proved to be negative in all assays, including the Ames assay[32] or bacterial reverse mutation study, the *in vitro* chromosome aberration assay[33] and the *in vivo* micronucleus assay[34] in the rat. Furthermore, no pathological problems were observed after daily topical application for 4 weeks in the rat.

Table 2. Toxicity study of 4

	4 (mg/kg)	**CAP** (mg/kg)
Single-dose tox, LD_{50} (i.p., mice)	>200	7.5[a]
Single-dose tox, LD_{50} (p.o, mice)	>2000	118.8[b]
AMES assay	negative	
In vitro chromosome aberration assay	negative	
In vivo micronucleus assay, rat (i.p.)	negative	

[a] ref 30, [b] ref 31

■ CONCLUSION

In this work, we developed a non-pungent and highly efficacious TRPV1 agonist as a potential topical analgesic agent for neuropathic pain. MDR-652 (**4**) demonstrated high affinity and potent agonism in human and rat TRPV1 *in vitro*. The functional agonism of **4** was confirmed *in vivo* by showing hypothermia in body temperature studies in the mouse both in protocols of coadministration with capsaicin and of single administration. **4** exhibited an excellent analgesic profile in the standard pain models - formalin and spinal nerve ligation - and was superior to capsaicin and gabapentin. In addition, **4** displayed a dose-dependent analgesic profile toward capsaicin-induced allodynia, indicating it engaged in TRPV1 for its analgesic activity. An *in vitro* transdermal delivery test indicated that most of the **4** remained in the skin and would have

little systematic absorption whereas the majority of the capsaicin was detected in the acceptor cell, indicating that **4** has promising PK properties as a topical agent. In toxicology studies, **4** showed minimal signs of skin irritation and edema in animal models and a much higher LD_{50} (*i.e*. lower toxicity) compared to that of capsaicin and **4** was negative in all genotoxicity studies.

Taken together, these results suggest that **4** is a highly potent and efficacious TRPV1 ligand with agonist activity having a promising topical PK profile and no significant toxicity, and it is currently under clinical development as a topical agent for neuropathic pain.

■ EXPERIMENTAL SECTION

General. All chemical reagents were commercially available. Melting points were determined on a melting point Buchi B-540 apparatus and are uncorrected. Silica gel column chromatography was performed on silica gel 60, 230–400 mesh, Merck. ^{1}H and ^{13}C NMR spectra were recorded on a JEOL JNM-LA 300 at 300 MHz, Bruker Analytik DE/AVANCE Digital 400 at 400 MHz. Chemical shifts are reported in ppm units with Me_4Si as a reference standard. Mass spectra were recorded on a 6460 Triple Quad LC−MS instrument. The purity was determined by high-performance liquid chromatography (HPLC) and was confirmed to be ≥95%. HPLC was performed on a Water Alliance 2695 separation Module instrument using a Hydrosphere C18 column (4.6 mm × 250 mm, 5 μm) with a 1.0 mL/min flow rate.

1-((2-(tert-Butyl)-4-(3-chlorophenyl)thiazol-5-yl)methyl)-3-(3-fluoro-4-(hydroxymethyl) phenyl)urea (4). To a solution of phenylcarbamate **9** (1.1 kg, 4.21 mol) in acetonitrile was added amine **16** (963 g, 3.43 mol) followed by triethylamine (567 mL, 4.06 mol). After stirring at 15-20 °C for 5 h, the solvent was removed *in vacuo* and the residue was dissolved in EtOAc. The organic layer was washed with 1 N HCl, water, $NaHCO_3$ solution, brine, dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by silica gel column chromatography with EtOAc/hexanes (1:2) as eluent to provide **4** (1.25 kg, 81%). For analytical purity, the solid was recrystallized five times using EtOAc/hexanes (1:10) to yield the >99% pure form of **4** (1.01 kg) as a white solid. m.p = 132 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.57 (s, 1H, Ar-H), 7.45-7.42 (m, 1H, Ar-H), 7.35 - 7.31 (m, 2H, Ar-H), 7.26 - 7.24 (m, 2H, Ar-H), 6.91 - 6.87 (dd, J_1 = 2.01 Hz, J_2 = 2.22 Hz, 1H, Ar-H), 6.64 (s, 1H, -CH_2NH), 5.24 (t, *J* = 5.85 Hz, 1H, CH_2OH), 4.63 (bd, 4H, α-CH_2, -CH_2OH), 1.42 (s, 9H, -$C(CH_3)_3$); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 177.77, 158.75, 154.79, 147.24, 104.75, 136.65, 133.91, 133.22, 130.38, 129.50, 127.99, 127.60, 126.99, 121.49, 113.18, 104.37, 56.48, 37.26, 36.09, 30.478 (3C); HR-MS (FAB) calcd for $C_{22}H_{23}ClFN_3O_2S$ $[M + H]^+$ 448.1183, found 448.1262. Purity 99.99% (eluent: 0.1 N phosphoric acid : acetonitrile (45:55, v/v), retention time: 18.64 min)

Methyl 2-fluoro-4-nitrobenzoate (6). To a solution of 2-fluoro-4-nitrobenzoic acid (**5**, 3 kg, 15.06 mol) in MeOH (40 L) was added H_2SO_4 (180 mL) and heated to reflux for 15 h. After completion of the reaction by TLC, MeOH was removed under reduced pressure and the residue was partitioned between EtOAc and water. The aqueous layer was extracted with EtOAc (15 L x 3) and the combined organic layers were washed with brine, dried over $MgSO_4$ and filtered. The filtrate was concentrated *in vacuo* to provide **6** (2.98 kg, 95%) as a brown oil, which was used for the next reaction without further purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.11 (t, *J* = 6.14 Hz, 1H), 8.05 (d, *J* = 6.94 Hz, 1H), 8.00 (d, *J* = 6.36 Hz, 1H), 3.97 (s, 3H); Mass (FAB) *m/z* 200 $[M + H]^+$.

(2-Fluoro-4-nitrophenyl)methanol (7). To a solution of **6** (2.9 kg, 14.56 mol) in MeOH (50 L) at 0 °C was added $NaBH_4$ (2.75 kg, 72.8 mol) portionwise. After stirring for 5 h at room temperature, the reaction mixture was cooled to 0 °C, water was carefully added, and the solution concentrated moderately. The residue was extracted with EtOAc (10 L x 3) and the combined organic layers were washed with water (10 L) and brine (10 L), dried over $MgSO_4$ and filtered. The filtrate was concentrated *in vacuo* to provide **7** (2.0 kg, 78%) as a pale brown oil, which was used for the next reaction without further purification. ^{1}H-NMR (300 MHz, $CDCl_3$) δ 8.08 (dd, *J* = 2.01, 8.43 Hz, 1H), 7.92 (dd, *J* = 2.22, 9.54 Hz, 1H), 7.71 (t, *J* = 7.32 Hz, 1H), 4.88 (d, *J* = 4.88 Hz, 2H); Mass (FAB) *m/z* 172 $[M + H]^+$.

(4-Amino-2-fluorophenyl)methanol (8). To stirred solution of **7** (2 kg, 11.68 mol) in MeOH at 0 °C was added zinc dust (3 kg, 45.87 mol**,** 10 μm) followed by ammonium chloride (2.46 kg, 45.98 mol) in water (13 L). The ice bath was removed, and the resulting mixture was stirred at 50 °C for 4 h. After completion, the reaction mixture was filtered through a thin Celite pad with MeOH and the filtrate was concentrated *in vacuo*. The crude residue was crystallized with EtOAc/hexanes (1:3) as eluent to provide **8** (1.16 kg, 72%) as a pale yellow solid. ^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.13 (t, *J* = 8.04 Hz, 1H), 6.41 (m, 2H), 4.61 (d, *J* = 5.88 Hz, 2H), 3.77 (*b*s, 2H); Mass (FAB) *m/z* 142 $[M + H]^+$.

Phenyl (3-fluoro-4-(hydroxymethyl)phenyl)carbamate (9). To a stirred solution of **8** (1.15 kg, 8.15 mol) in acetone (30 L) was added pyridine (750 mL, 24.44 mol) followed by phenyl chloroformate (1.075 L, 8.15 mol) at 0°C. After stirring at room temperature for 5 h, the reaction mixture was concentrated *in vacuo*. The residue was extracted with EtOAc (15L x 3) and washed with water (10 L) and brine (10 L). The combined organic layers were dried over $MgSO_4$, filtered and concentrated *in vacuo*. The resulting residue was dissolved in EtOAc and recrystallized with EtOAc/hexanes (1:10) to provide **9** (1.32 kg, 62%) as a pale yellow solid. ^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.38-7.43 (m, 4H), 7.24 (m, 1H), 7.20 (d, *J* = 2.22 Hz, 2H), 7.10 (dd, *J* = 2.01, 8.07 Hz, 1H), 4.71 (d, *J* = 5.13 Hz, 2H); Mass (FAB) *m/z* 262 $[M + H]^+$.

Methyl 3-(3-chlorophenyl)-3-oxopropanoate (11). To a 100 L reactor charged with THF (50 L) was carefully added sodium hydride (3.83 kg, 159.64 mol) surrounded by an ice bath. 3-Chloroacetophenone (**10**, 5 kg, 32.34 mol) in THF (5 L) was added dropwise to the mixture over 1 h at 0 °C, followed by dropwise addition of dimethyl carbonate (5.45 L, 64.68 mol) in THF (6 L) at 0 °C. The reaction mixture was stirred at 0 °C for 30 min and then heated to 50 °C for 4 h. The mixture was cooled to 0 °C and quenched by the addition of aqueous NH_4Cl solution. The resulting mixture was extracted with EtOAc (20 L x 3), washed with water, dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by silica gel column chromatography with EtOAc/hexanes (1:10) as eluent to provide **11** (4.5 kg, 65%) as a brown oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.91 (d, *J* = 1.6 Hz, 1H), 7.79-7.81 (m, 1H), 7.75 (m, 0.3 H, enol form), 7.62-7.64 (m, 0.3 H, enol form), 7.56 (dd, *J* = 8.2, 3.2 Hz, 1H), 7.42 (t, *J* = 8.0 Hz, 1H), 7.34 (m, 0.3 H, enol form), 3.97 (s, 2H), 3.80 (s, 1H, enol form), 3.75 (s, 3H); Mass (FAB) *m/z* 213 $[M + H]^+$.

Methyl 2-chloro-3-(3-chlorophenyl)-3-oxopropanoate (12). To a solution of **11** (4.5 kg, 21.16 mol) in chloroform (40 L) was

added dropwise sulfuryl chloride (1.7 L, 21.16 mol) at 0 °C and the solution was then heated to reflux for 20 h. The reaction was allowed to cool to room temperature and diluted with water. The organic layer was washed with water (20 L x 3), dried over $MgSO_4$, filtered and concentrated *in vacuo*. The residue was purified by silica gel column chromatography with EtOAc/hexanes (1:5) as eluent to provide **12** (3.76 kg, 72%) as a pale brown oil. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.96 (t, *J* = 1.8 Hz, 1H), 7.86 (dt, *J* = 7.8, 1.4 Hz, 1H), 7.60 (dq, *J* = 7.9, 1.1 Hz, 1H), 7.45 (t, *J* = 8.0 Hz, 1H), 5.59 (s, 1H), 3.88 (s, 3H); Mass (FAB) *m/z* 246 $[M + H]^+$.

2,2-Dimethylpropanethioamide (13). To a solution of pivalamide (2.6 kg, 25.70 mol) in diethyl ether (40 L) and toluene (20 L) was added phosphorus pentasulfide (4 kg, 17.99 mol) and the mixture was stirred at 45 °C for 5 h to provide a tacky yellow solid and a supernatant layer. The supernatant was filtered and the yellow solid was extracted with diethyl ether (5 L x 3). The combined organic layers were evaporated *in vacuo* and the residue was purified by silica gel column chromatography with EtOAc/hexanes (1:1) as eluent to provide **13** (2.87 kg, 63 %) as a pale brown oil. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.62 (s, 1H), 6.96 (s, 1H), 1.36 (s, 9H) ; Mass (FAB) *m/z* 118 $[M + H]^+$.

Methyl 2-(tert-butyl)-4-(3-chlorophenyl)thiazole-5-carboxylate (14). To a solution of **12** (3.7 kg, 15.04 mol) in MeOH (30 L) was added thioamide **13** (1.85 kg, 15.78 mol) and the solution was then refluxed for 15 h. The reaction mixture was concentrated *in vacuo* and the residue was dissolved in EtOAc (20 L). The organic layer was washed with water, brine, dried over $MgSO_4$, and concentrated *in vacuo*. The residue was recrystallized with isopropyl alcohol/hexanes to provide **14** (3.28 kg, 71%) as a pale yellow solid. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.77 (d, *J* = 1.4 Hz, 1H), 7.67 (td, J = 4.4, 2.3 Hz, 1H), 7.40-7.31 (2H), 3.80 (s, 3H), 1.47 (s, 9H); Mass (FAB) *m/z* 310 $[M + H]^+$.

(2-(tert-Butyl)-4-(3-chlorophenyl)thiazol-5-yl)methanol (15). To a solution of **14** (3.2 kg, 10.35 mol) in THF (60 L) was added lithium aluminium hydride (420 g, 11.06 mol) at 0 °C and stirring was continued at room temperature for 4 h. The reaction mixture was cooled to 0 °C, water was added dropwise, and the mixture was then concentrated *in vacuo*. The residue was dissolved in EtOAc and the organic layer was washed with water, brine, dried over $MgSO_4$, and filtered. The filtrate was concentrated *in vacuo* to provide **15** (2.47 kg, 85%) as a brown oil, which was used for the next reaction without further purification. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.67 (d, *J* = 1.8 Hz, 1 H), 7.51 (td, *J* = 4.4, 2.3 Hz, 1 H), 7.29-7.35 (m, 2 H), 4.79 (s, 2 H), 2.44 (s, 1 H), 1.44 (s, 10 H); Mass (FAB) *m/z* 282 $[M + H]^+$.

(2-(tert-Butyl)-4-(3-chlorophenyl)thiazol-5-yl)methanamine (16). To a solution of **15** (2.45 kg, 8.71 mol) in CH_2Cl_2 (30 L) was added thionyl chloride (548 mL, 7.56 mol) at 0 °C. After stirring at room temperature for 2 h, the reaction mixture was concentrated *in vacuo*. The residue was dissolved in acetonitrile (30 L) and then tetrabutyl ammonium bromide (100 g) was added followed by dropwise addition of ammonium hydroxide solution (20 L) at 0 °C. After stirring at 40 °C for 15 h, the reaction mixture was concentrated *in vacuo*, and the residue was diluted with water. The aqueous layer was extracted with EtOAc (10L x 3) and the combined organic layers were washed with water, brine, dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by silica gel column chromatography with EtOAc/hexanes (1:2) as eluent to provide **16** (1.1 kg, 45%) as a yellow solid. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.63 (t, *J* = 1.8 Hz, 1 H), 7.48 (dt, *J* = 7.4, 1.6 Hz, 1 H), 7.28-7.36 (m, 2 H), 4.14 (s, 2 H), 1.44 (s, 10 H); Mass (FAB) *m/z* 281 $[M + H]^+$.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at http://pubs.acs.org.

Molecular formula strings (CSV); Agonism data of **4**; Selectivity study of **4**; Biological and pharmacokinetic methods.

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS

TRPV1, Transient Receptor Potential Vanilloid 1; CAP, capsaicin; RTX, resiniferaxin; CHO, chinese hamster ovary; SNL, spinal nerve ligation; MPE, maximum possible effect

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