Identification of two natural coumarin enantiomers for selective inhibition of TRPV2 channels

Qiqi Zhou1 | Yuntao Shi2 | Hang Qi1 | Huijie Liu1 | Ningning Wei1 | Yong Jiang2 | KeWei Wang1

1Department of Pharmacology, Qingdao University School of Pharmacy, Qingdao, China  
2State Key Laboratory of Natural and Biomimetic Drugs, School of Pharmaceutical Sciences, Peking University, Beijing, China

Correspondence  
Ningning Wei and KeWei Wang, Department of Pharmacology, Qingdao University School of Pharmacy, Qingdao 266021, China.  
Email: weiningning@qdu.edu.cn (N. W.) and wangkw@qdu.edu.cn (K. W. W.)

Yong Jiang, State Key Laboratory of Natural and Biomimetic Drugs, School of Pharmaceutical Sciences, Peking University, Beijing 100191, China.  
Email: yongjiang@bjmu.edu.cn

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Abstract  
Thermosensitive transient receptor potential vanilloid 2 (thermoTRPV2) is a non-selective Ca2+-permeable cation channel broadly expressed, and is implicated in the pathology of diseases such as diabetes and pancreatitis. However, the physiological and pharmacological functions of TRPV2 channels have not been extensively investigated because of the absence of specific modulators. In this study, we report a pair of natural coumarin derivative enantiomers (−)-murraxocin (B304-1) and (+)-murraxocin (B304-2) from *Murraya exotica* for their selective inhibition of TRPV2 channels expressed in HEK293 cells and native TRPV2 currents in differentiated brown adipocytes. Whole-cell patch clamp recordings confirmed the enantiomers B304-1 and B304-2 could selectively inhibit the agonist mediated activation of TRPV2 current with IC50 values of 22.2 ± 7.8 μM and 3.7 ± 0.7 μM, respectively. Molecular docking and site-directed mutagenesis revealed a key residue I600 of TRPV2 critical for the binding of the enantiomers. Furthermore, B304-1 and B304-2 significantly reversed TRPV2 agonist-induced inhibition of mouse brown adipocyte differentiation. Taken together, our identification of two natural coumarin enantiomers provides valuable tools and chemical leads for further elucidation of TRPV2 channel function, and pharmacological modulation of thermoTRPV2 in brown adipocytes may represent a new therapeutic strategy for treatment of energy imbalance or metabolic disorders.

Keywords  
brown adipocytes, differentiation, murraxocin, TRPV2 channel

Abbreviations: 2-APB, 2-aminoethoxydiphenyl borate; BAT, brown adipose tissue; HFD, high fat diet; HVA, high-voltage-activated; LPC, lysophosphatidylcholine; LVA, low-voltage-activated; *Pparγ*, peroxisome proliferator-activated receptor γ; TRPM8, transient receptor potential melastatin 8; TRPV1, transient receptor potential vanilloid 1; TRPV3, transient receptor potential vanilloid 3; TRPV4, transient receptor potential vanilloid 4; *Ucp1*, uncoupling protein 1.

Qiqi Zhou and Yuntao Shi contributed equally to this study.

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1 INTRODUCTION

Transient receptor potential vanilloid 2 (TRPV2) is a heat-activated and nonselective Ca$^{2+}$-permeable cation channel of temperature-sensitive TRPV subfamily.\(^1,2\) ThermoTRPV2 is activated by extremely noxious heat (>53°C),\(^3\) and comprises a homotetramer with an ion-permeation pathway at the center in fourfold symmetry.\(^4,5\) Each subunit is composed of six trans-membrane spanning domains with a pore-forming loop between 5th and 6th transmembrane domains. TRPV2 channel shares high sequence identity (>50%) with TRPV1 and displays close structural homology.\(^6,8\) Similar to TRPV1 structure, TRPV2 pore region contains two constrictions, one at the selectivity filter (upper gate), and the other at bundle-crossing of S6 (lower gate), but the agonist-free full-length TRPV2 has wider upper and lower gates in comparison with closed or agonist-activated TRPV1, indicating that TRPV2 channel can accommodate partially hydrated Ca$^{2+}$, Na$^+$, and K$^+$ ions, as well as large organic cations in the apo state.\(^4,5\)

TRPV2 is widely expressed in tissues including adipose tissue, and involved in a variety of physiological processes and functions.\(^9-13\) Brown adipose tissue (BAT) is metabolically active in adult humans,\(^14,15\) and primarily functions as a source of energy for thermoregulation.\(^16\) Brown adipocytes are first differentiated from mesenchymal stem cells to brown pre-adipocytes before full development into brown adipocytes.\(^16,17\) The differentiated brown adipocyte contains numerous mitochondria and distinct lipid droplets.\(^18\) The presence of an uncoupling protein 1 (Ucp1) in mitochondria uncouples the electron transport chain, and causes the generation of heat allowing for nonshivering thermogenesis.\(^15,19\) Brown adipose tissue activation also improves glucose homeostasis and insulin sensitivity in humans, suggesting a therapeutic potential of BAT in protection against type 2 diabetes and obesity.\(^16,20,21\)

As a polymodal channel, TRPV2 can also be activated by membrane stretch, cell swelling\(^22-24\) as well as a number of chemical ligands, such as 2-aminoethoxydiphenyl borate (2-APB) and lysophosphatidylcholine (LPC).\(^25,26\) Molecules like ruthenium red, SKF96365 and tranilast can act as TRPV2 antagonists. However, most of these ligands are not specific for TRPV2.\(^1\) Ruthenium red inhibits a broad range of ion channels including TRPV1, TRPV3, and TRPA1,\(^27\) and SKF96365 also blocks other channels such as potassium channels, low-voltage-activated T-type calcium channels and some TRPCs channels.\(^28,29\) Tranilast is an antiallergic drug used for curing bronchial asthma, allergic rhinitis, and other allergic diseases, and it stabilizes the cell membrane of mast cells and basophils, prevents degranulation, and inhibits the release of allergic mediators, such as histamine and serotonin.\(^30\) Because there is a lack of specific inhibitors for TRPV2, the physiological and pharmacological functions of TRPV2 in tissues such as brown adipose tissues remain largely unknown. Therefore, it is necessary to identify TRPV2-specific inhibitors that can be used either as tools for study of TRPV2 function or lead compounds for potential therapy in energy imbalance or metabolic diseases such as obesity.\(^31,32\)

In this study, we identified two novel natural coumarin enantiomers, (−)-murraxocin and (+)-murraxocin, also named B304-1 and B304-2, respectively, from the roots of Murraya exotica plant. Both B304-1 and B304-2 can specifically inhibit TRPV2 channels expressed in HEK-293 cells and native TRPV2 currents in differentiated mouse brown adipocytes. B304-1 or B304-2 also can reverse TRPV2 agonist-induced inhibition of mouse brown adipocyte differentiation. Our identification of natural coumarin enantiomers provides essential pharmacological tools or chemical leads for further understanding of TRPV2 channel physiology and pharmacology in BAT and other tissues expressing TRPV2.

2 MATERIALS AND METHODS

2.1 Materials

The roots of Murraya exotica were collected from Guangdong Province, China, in 2014 and identified by Prof. Pengfei Tu at Peking University. A voucher specimen (no. ME201402) has been deposited in the Herbarium of the Peking University Modern Research Center for Traditional Chinese Medicine. Most of the chemicals were obtained from Sigma (St. Louis, USA). TRPV2 antibodies (ACC-039) were purchased from Alomone (Jerusalem, Israel). All animal experiments were approved by the Institutional Animal Care and Use Committee of Qingdao University Health Science Center and were carried out in compliance with national and institutional guidelines for the care and use of laboratory animals.

2.2 Extraction and isolation of coumarin derivatives

The powdered roots of Murraya exotica (26 kg) were extracted using 95% of aqueous ethanol (ethanol: H$_2$O = 19:1, 400 L in total) for thrice to give rise to crude extracts (1.6 kg). The extract was suspended in H$_2$O and fractionated sequentially with petroleum ether and CH$_2$Cl$_2$. The CH$_2$Cl$_2$-soluble portion (600 g) was subjected to silica gel column chromatography and eluted with a stepwise gradient of petroleum ether-EtOAc (from 10:1 to 3:5, v/v) to obtain 14 fractions. Fraction 10 was recrystallized with methanol to afford racemic compound B304 (10.5 g). Chiral separation of racemic mixture was performed on a Supercritical Fluid Chromatography (Waters SFC350, Massachusetts, USA), using a Lux i-Cellulose-5 (3.0 × 1000 mm, 3 μm) column, and methanol (with 0.1% of
performed at room temperature of 22 ± 2.0°C. CellSens Dimension at 4-s intervals. All experiments were signals were collected with a CCD camera and recorded by

7.2) for 40 minutes at room temperature before wash twice

Esters of B304-1 and B304-2

A portion of compounds B304-1 and B304-2 (10 mg, 0.033 mM) was separately dissolved in 1 mL of CH2Cl2. (+)-α-Methyl-α-(trifluoromethyl) phenylacetyl (MTPA) chloride (50 mg, 0.2 mM) or (R)-MTPA chloride (50 mg, 0.2 mM), DMAP (4 mg, 0.033 mM), and triethylamine (50 μL, 0.33 M) were added to the solution under N2 gas protection and stirred overnight at room temperature. The products were separated by preparative TLC, with EtOAc-Hexane (2:3) as elution solvent to yield (R)- and (S)-MTPA esters 1R (4.3 mg) /1S (6 mg), and 2R (5 mg)/2S (6.3 mg). The 1H NMR data of the (R)- and (S)-MTPA esters (1R/1S, and 2R/2S) were recorded and analyzed.

Preparations of the (R)- and (S)-MTPA Esters of B304-1 and B304-2

2.3 | Preparations of the (R)- and (S)-MTPA Esters of B304-1 and B304-2

2.4 | Cell culture and transient transfection

Human embryonic kidney (HEK) 293 cells were cultured in Dulbecco's modified Eagle's medium supplemented with 10% of fetal bovine serum at 37°C with 5% of CO2. The HEK-293 cells were passaged at 2-day to 3-day intervals. Cells were passaged 6-24 hours before transfection, and plated on glass coverslips. Transient transfection was performed by adding 2-3 μL of Lipofectamine 2000 (Invitrogen, California, USA) and 2.5 μg of mouse TRPV2 cDNAs (accession number NM_011706.2). The mixture was added into a 35 mm cell culture dish. All cDNA clones were verified by sequencing. Patch-clamp recordings and calcium imaging were performed between 18 and 36 hours after transfection.

2.5 | Calcium imaging

HEK-293 cells overexpressing TRPV2 channels were loaded with 2 mM Fluo-8 (Abcam, Cambridge, MA, USA) in Ringer's solution [140 mM NaCl, 5 mM KCl, 2 mM MgCl2, 10 mM d-glucose, 10 mM HEPES, and 2 mM CaCl2, (pH 7.2)] for 40 minutes at room temperature before wash twice with the same solution. Ringer’s solution was used during imaging. The cells were challenged sequentially by agonist, antagonist with 2-APB, 2-APB, and ionomycin. Fluorescent signals were collected with a CCD camera and recorded by CellSens Dimension at 4-s intervals. All experiments were performed at room temperature of 22 ± 2.0°C.

2.6 | Measurement of intracellular calcium level in FlexStation 3 multi-mode microplate reader assay

Changes in intracellular calcium level ([Ca2+]i) in HEK293 cells were measured by fluorescent calcium-sensitive dyes using the Cal-520 PBX Calcium Assay Kit (AAT Bioquest, CA, USA). The fluorescent signals were read by FlexStation 3 Multi-Mode Microplate Reader (Molecular Devices, San Francisco, USA). TRPV2-transfected HEK-293 cells or differentiated brown adipocytes were seeded at a density of 30 000 cells/well in 96-well black-walled plates and grown for overnight at 37°C with 5% of CO2. Cells were loaded with the dye for 1.5 hours at 37°C. Loading and imaging were performed in Hanks’ balanced salt solution (137 mM NaCl, 5.4 mM KCl, 0.4 mM KH2PO4, 0.1 mM Na2HPO4, 1.3 mM CaCl2, 0.8 mM MgSO4, 5.5 mM glucose, 4 mM NaHCO3, and 20 mM HEPES, pH 7.4). The compounds were added onto cells at 17 seconds, and then, corresponding TRP agonist was added at the time point of 100 seconds, and the relative fluorescence unit values were continuously measured for 80 seconds. The wavelength of excitation light was set as 485 nm, and the wavelength of emission was set at 525 nm, and fluorescence intensity was measured at an interval of 1.6 seconds.33,34

2.7 | Electrophysiology

Macroscopic currents of TRPV2 channels were recorded in whole-cell patch clamp configuration using a HEKA EPC10 amplifier driven by PatchMaster software. Patch pipettes were prepared from borosilicate glass and fire-polished to resistance about ~4.0 MΩ. For whole-cell recordings of TRPV2 and TRPM8 currents expressing in HEK 293 cells, both bath and pipette solutions contained 130 mM NaCl, 0.2 mM EDTA, and 3 mM HEPES, pH 7.2-7.4, adjusted with NaOH. Membrane potential was held at 0 mV, and currents were elicited by a protocol consisting of a 400-ms step to +80 mV followed by a 400-ms step to −80 mV at 1-s intervals. For ramp recording of TRPV1, TRPV3, and TRPV4 currents, cell membrane potential of HEK 293 cells was held at 0 mV with 500-ms voltage ramp from −100 to +100 mV at 1-s intervals. For whole-cell recordings of mouse brown adipocytes, the bath solution contained 140 mM NaCl, 5 mM KCl, 2 mM MgCl2, 2 mM CaCl2, 10 mM HEPES, and 10 mM glucose, pH 7.4, adjusted with NaOH. The recording pipette was filled with a solution containing: 140 mM KCl, 5 mM EGTA, and 10 mM HEPES, pH 7.4, adjusted with KOH. Membrane potential was held at −60 mV, and voltage-ramp pulses from −100 to 100 mV for 300 ms were applied in a 1-s intervals. All currents were analyzed at ±100 mV and all patch clamp recordings were performed at room temperature and data were analyzed with Igor Pro (Wave-metrics) and Origin 8.6 (OriginLab).
For whole-cell recordings of Nav1.4 and Nav1.5 channel, the bath solution contained 140 mM NaCl, 3 mM KCl, 1 mM MgCl$_2$, 1 mM CaCl$_2$, 10 mM HEPES, and 10 mM glucose (pH 7.3, adjusted with NaOH). The recording pipette was filled with a solution contained 140 mM CsF, 10 mM NaCl, 1 mM EGTA, and 10 mM HEPES (pH 7.3, adjusted with CsOH). Membrane potential was held at −120 mV, and voltage pulses was −10 mV for 100 ms at 1s intervals. For whole-cell recordings of Kv11.1 channel, pipette solutions contained with 130 mM KCl, 1 mM MgCl$_2$, 12.5 mM EGTA, and 10 mM HEPES (pH 7.3, adjusted with KOH). Extracellular solutions contained with 137 mM NaCl, 4 mM KCl, 1.8 mM CaCl$_2$, 1.2 mM MgCl$_2$, and 10 mM HEPES and 10 mM glucose (pH 7.4, adjusted with NaOH). Holding potential was −80 mV, and step currents were elicited with 100 ms pulses from −60 to +60 mV in 10 mV increments before return to −50 mV. Tail currents were measured at −50 mV. For two-electrode voltage clamp recordings of acetylcholine receptors α3β2 and α7 channels, oocytes were harvested and injected with cRNA encoding nAChR subunits before gravity-perfused with ND96 (96 mM NaCl, 2 mM KCl, 1.8 mM CaCl$_2$, 1 mM MgCl$_2$, 5 mM HEPES, pH 7.1-7.5) containing 1 μM of atropine (ND96A) with or without compound at a rate of 5 mL/min. Glass pipettes electrodes were filled with 3 M KCl with resistance of 0.5-1.0 M and. All recordings were made at room temperature (22 ± 2°C).

2.8 Primary culture and oil red O staining of mouse brown adipocytes

Pre-adipocytes from BAT were isolated from ten C57BL6/J male mice (1-2 day old) and cultured in Dulbecco’s modified Eagle’s medium supplemented with 20% of fetal bovine serum and 0.5% of penicillin/streptomycin at 37°C with 5% of CO$_2$. When pre-adipocytes were grown to 100% confluency, the medium was replaced with induction medium that contained 500 μM of 3-isobutyl-1-methylxanthine and 1 μM of dexamethasone (day 0). After 48 hours (day 2), change of induction medium with the differentiation medium containing 50 μM of triiodothyronine (T3) and 5 mg/L insulin was made for 6 more days (day 8) and the medium was changed every 2 days. For the differentiation experiments, compounds were added when medium was replaced with the differentiation medium for 6 days to induce differentiation.

Oil red O staining was performed using oil red O dye. Oil red O staining was done according to previously reported methods. Briefly, adipocytes were fixed with 4% of formalin and incubated for at least 1 hour at room temperature (RT). After fixation, the cells were washed twice with purified water, and then, washed with 60% of isopropanol for 5 minutes at RT. The cells were dried completely at RT, and oil red O solution was added, and then, incubated for 10 min at RT. Oil red O solution was removed by adding pure water, and the cells were washed four times with purified water. Images were obtained under a microscope (Nikon Ti-E, Tokyo Met, Japan) for analysis.

2.9 RNA extractions and quantitative real-time PCR

Total RNAs were extracted from cells using TRIzol reagent according to the manufacturer’s protocol and reversely transcribed into cDNA using FastKing RT Kit (TIANGEN, Beijing, China). Mouse gene copy numbers were determined by quantitative real-time PCR using SYBR Green (TIANGEN, Beijing, China) according to the manufacturer’s instruction using primers previously reported. The results were normalized by comparing the level of 36B4 mRNA in each sample.

2.10 Western blot

Cell lysates were prepared using RIPA lysis buffer containing cocktail phosphatase inhibitor and protease inhibitor (Thermo Fisher Scientific). The protein concentration in the supernatant was determined with the BCA protein assay kit. Protein samples were denatured and separated to 10% of SDS-PAGE, and then, transferred to polyvinylidene fluoride (PVDF) membranes (MilliporeSigma). After blocking with 5% of nonfat milk in TBST (25 mM Tris-HCl, pH 7.4, 137 mM NaCl, 2.7 mM KCl, and 0.05% of Tween 20) for 1 hour at RT and the membranes were incubated with primary antibodies against TRPV2 (1:200; Alomone Lab, Jerusalem, Israel) and β-actin (1:10,000; Abcam, Cambridge, MA, USA) overnight at 4°C. After extensive washing in TBST buffer for 15 minutes, the membranes were incubated with secondary antibody for 1 hour at RT before washed again with TBST buffer. Signals were detected using an ECL Western blotting detection system.

2.11 Molecular docking and site-directed mutagenesis

Molecular docking was performed using Schrödinger Glide (Maestro software suite 2015, Schrödinger, New York, USA). Molecules B304-1 and B304-2 were drawn using ChemBioDraw Ultra 14.0 (CambridgeSoft) and optimized for docking using a built-in program Ligprep in Maestro. The TRPV2 EM structure was obtained from Protein Data Bank (PDB code 5HI9) and prepared for the docking following the standard procedure of Maestro using the standard docking module SP. The binding pocket of ligand with
rTRPV2 was selected based on the reported inhibitor binding sites of TRPV2, TRPV1, and TRPA1 using Glide and ranked by the resulting score. The pore region of the rTRPV2 upper gate, which consists of M607, G606, and I605 (equivalent to M602, G601, and I600 in mTRPV2) was chosen to be the binding site for B304-1 and B304-2 based on its highest ranking. All mTRPV2 point mutations were made using QuikChange II XL site directed mutagenesis kit following the manufacturer’s instructions. All mutations were confirmed by sequencing.

3 | RESULTS

3.1 | Chiral separation and structure determination of two coumarin enantiomers B304-1 and B304-2

We obtained racemic B304 was as a white amorphous powder. Its molecular formula was deduced as C_{17}H_{20}O_{5} from the ^{13}C NMR and HRESIMS data (m/z 305.1388 [M+H]^+, calculated for C_{17}H_{21}O_{5}, 305.1389) (Figures S1 and S2).

The NMR data indicate that B304 is murraxocin, but its specific rotation approached zero and no Cotton effects observed (Figures S3 and S4), suggesting that B304 is a pair of enantiomers coexisted as a racemic mixture. Separation of B304 by chiral-phase HPLC gave rise to a pair of enantiomers B304-1 and B304-2 in an approximate ratio of 1:1 (Figure 1A-C). Their specific rotations were detected as [α]D^25 = -66.7 (c 0.01, MeOH) for B304-1 and [α]D^25 = +52.8 (c 0.01, MeOH) for B304-2 (Figure 1D). The absolute configurations of the 2′-OH of compounds B304-1 and B304-2 were determined by Mosher’s reactions with (S)-(+)−α-methoxy−α-trifluoromethyl phenylacetyl chloride and (R)-(−)−α-methoxy−α-trifluoromethyl phenylacetyl chloride ((S/R)-MTPA chloride), respectively, thus, generating their (S)-MTPA and (R)-MTPA esters of each compound (ΔδH = δS−δR) were calculated (Figure S5). Based on the results of ΔδH, the 2′-OH configurations of compounds B304-1 and B304-2 were deduced as S and R, respectively. The coupling constants between H-1′ and H-2′ in both B304-1 and B304-2 are 9.2-9.3 Hz (Table 1), indicating the threo-configurations.

**FIGURE 1** Chiral isolation and structure determination of natural coumarin derivative enantiomers (−)-murraxocin (B304-1) and (+)-murraxocin (B304-2). A,B, The chemical structures of compounds B304-1 (in red) and B304-2 (in blue). C, The chiral separation of compounds B304-1 and B304-2 by HPLC. D, The ECD spectra of compounds B304-1 (in red) and B304-2 (in blue)
Thus, the absolute configuration of B304-1 was identified as (1S, 2S) and B304-2 as (1R, 2R). Thus, the structures of B304-1 and B304-2 were defined as (-)-murraxocin and (+)-murraxocin, respectively. The (+)-murraxocin from natural sources is reported for the first time.

3.2 Identification of coumarin enantiomers B304-1 and B304-2 as novel TRPV2 antagonists in calcium imaging and fluorescence assays

We examined the effect of enantiomers B304-1 and B304-2 on Ca²⁺-permeable TRPV2 channels expressed in HEK-293 cells using calcium imaging assay. Application of B304-1 (100 μM) or B304-2 (100 μM) (blue bar) showed no elevation of intracellular calcium, while application of TRPV2 agonist 2-APB (1 mM) (red bar) or ionomycin (10 μM) (green bar) gave rise to a drastic elevation of intracellular calcium in HEK-293 cells transfected with mTRPV2 (Figure 2A,B, and also Figure S6), as compared with untransfected cells. Prior to the addition of second 2-APB challenge, there was a delayed calcium response for the combination of B304-1 and 2-APB (Figure 2A, far right panel) between the intervals. This apparent bump in calcium response is likely resulted from the relatively weak compound potency than the combination of B304-2 and 2-APB group (Figure 2B, far right panel) or tranilast and 2-APB group (Figure 2C, far right panel) that show the little rise of calcium rise. We further confirmed the inhibitory effect of B304-1 and B304-2 on the rise of intracellular calcium induced by 2-APB in TRPV2-expressing HEK-293 cells using calcium fluorescence assay in FlexStation3 format. As shown in Figure 2D, the addition of B304-1 at 100 or 500 μM resulted in a significant decrease of calcium fluorescent signals induced by 2-APB (1 mM). Similarly, B304-2 at 10 or 30 μM also caused dramatic decrease of intracellular calcium level (Figure 2E), as compared with the positive control of tranilast (20 μM). These results indicate that the enantiomers B304-1 and B304-2 are TRPV2 channel antagonists.

3.3 Dose-dependent and selective inhibition of TRPV2 current by enantiomers B304-1 and B304-2

To further evaluate the inhibitory effects of the enantiomers on TRPV2, we performed whole-cell patch clamp recordings of HEK-293 cells expressing TRPV2 channels. As shown in Figure 3A,B, B304-1 (30 μM) inhibited TRPV2 current activated by 2-APB (2 mM) about 40.0 ± 2.4% (n = 6), and B304-2 (30 μM) inhibited TRPV2 current about 84.3 ± 1.7% (n = 5), as compared with the positive control of tranilast (20 μM) that inhibited TRPV2 current about 86.1 ± 0.7% (n = 6). In addition, B304-1 and B304-2 also inhibited TRPV2 current activated by agonist probenecid (Figure S7).

TABLE 1 The NMR data of compound B304 (in CDCl₃, δ in ppm)

| Position | δH (J in Hz) | δC |
|----------|--------------|----|
| 2        | 161.4        |    |
| 3        | 6.28, d (9.5)| 114.2 |
| 4        | 7.64, d (9.5)| 143.4 |
| 5        | 7.41, d (8.6)| 128.9 |
| 6        | 6.88, d (8.6)| 108.0 |
| 7        | 160.5        |    |
| 8        | 114.2        |    |
| 9        | 153.8        |    |
| 10       | 113.3        |    |
| 1’       | 5.16, d (8.8)| 75.8 |
| 2’       | 4.92, d (8.8)| 76.4 |
| 3’       | 143.7        |    |
| 4’       | 4.66, 4.71, m| 112.8 |
| 5’       | 1.72, s      | 17.3 |
| 1”       | 3.44-3.47, 3.51-3.54, m| 65.2 |
| 2”       | 1.21, t (7.0)| 15.3 |
| 7-OCH₃   | 3.95, s      | 56.2 |

To determine the dose-dependent inhibition of TRPV2 by the enantiomers, we perfused different concentrations of B304-1 or B304-2. As a control, we first determined the dose-dependent activation of TRPV2 by 2-APB that gave rise to an EC₅₀ of 1.2 ± 0.03 mM, n = 8 (Figure 3C). Adding different concentrations of B304-1 caused a dose-dependent inhibition of TRPV2 current evoked by 2-APB (2 mM) with an IC₅₀ value of 22.2 ± 7.8 μM (n = 5-7), with a Hill coefficient of 0.88 (Figure 3D). More significantly, application of different concentrations (10 nM to 300 μM) of B304-2 resulted in a dose-dependent inhibition of TRPV2 current with an IC₅₀ value of 3.7 ± 0.7 μM (n = 5-7) with a Hill coefficient of 1.04 (Figure 3D). These results indicate that both B304-1 and B304-2 inhibit TRPV2 current in dose-dependent manner, whereas B304-2 is about sixfold more potent than B304-1.

To further confirm the selectivity of B304-1 and B304-2 on other members of TRPV channels, we also tested their effects on TRPV1, TRPV3, and TRPV4 channels individually expressed in HEK-293 cells. TRPV2 current was elicited by voltage ramps from −100 to +100 mV in the presence of channel agonist 2-APB. As shown in Figure 3E, B304-1 or B304-2 at 200 μM had no inhibitory effect on TRPV1 current activated by the specific agonist capsaicin (1 μM). Similarly, B304-1 or B304-2 had no inhibitory effect on either TRPV3 current induced by 2-APB (200 μM) or TRPV4 current evoked by 100 nM GSK1016790A (GSK) (Figure 3F,G). These results demonstrate that B304-1 and B304-2 are selective for inhibition of TRPV2 among tested TRPV channels.
3.4 Inhibition of endogenous TRPV2 currents by the enantiomers in differentiated mouse brown adipocytes

TRPV2 has been shown to be important for the maintenance of thermogenic function in brown adipose tissue (BAT) and also for the regulation of brown adipocyte differentiation.\(^{37,40}\)

To recapitulate the expressions of TRPV2 and other TRPV channels in BAT, we examined their expressions in primary cultured mouse pre-adipocytes and differentiated mouse brown adipocytes. The RT-PCR and western blot analysis may reveal that TRPV2 mRNA expression level was the highest among the tested TRPV1, TRPV3, and TRPV4 channels in the differentiated brown adipocytes (Figure 4A,B), assuming the similar efficiency of primer pairs and antibodies used in the assay. We also examined the expressions of
A series of murraxocin derivatives were synthesized and modified by esterizing the 2'-OH group of murraxocin to investigate the murraxocin-induced TRPV2 inhibition (Figures 6 and S10). Derivative 3, 4, 5, 10, and 7 were expected to change the -OH electronegativity to neutral or positively charged. Derivative 16 was negatively charged but had a carboxylic acid distal and elongated chain at the 2'-OH position (Figure 6A). However, no inhibition was observed with any of the 2'-OH group derivatives in our electrophysiology assay (Figure 6B,C). This observation suggest that the 2'-OH group is required for B304-induced inhibition of mouse brown adipocyte differentiation.
Sites of the enantiomers bound to TRPV2 revealed by molecular docking and site-directed mutagenesis

To understand the molecular mechanism underlying the inhibitory effect of the enantiomers on TRPV2, we performed molecular docking of compounds B304-1 and B304-2 into a recent EM structure of rat TRPV2 (PDB: 5HI9) using the Glide model of Schrödinger. The docking results show that both B304-1 and B304-2 are confined in the sites near upper gate of the ion conductance pathway, consisting of three residues, M607, G606, and I605 of rTRPV2 (equivalent to M602, G601, and I600 in mouse TRPV2), forming noncovalent interactions with these residues and blocking the channel pore (Figure 7A,B). The binding scores of B304-1 and B304-2 with rTRPV2 are −4.1 and −5.7, respectively. Upon binding,
B304-2 was recognized by rTRPV2 residues I605, G606, M607, and Y634 (equivalent to I600, G601, M602, and Y629 in mTRPV2). The backbone carbonyl of residue G606 in the selectivity filter of pore loop forms the hydrogen bond with the 2′-OH group of B304-2, the N-end of the same G606 also forms the hydrogen bond with 2-carbonyl of the coumarin ring of B304-2. Several van der Waals interactions were also detected including the two side chains of residue M607 and 5′-methyl and 4′-alkene groups of B304-2, and residue I605 and 7-methoxy group of B304-2, as well as residue Y634 and H-3 of B304-2. In comparison, B304-1 bound to rTRPV2 in a different orientation (Figure 7A,B), and was recognized by T604, I605 and Y634 (equivalent to T599, I600, and Y629 in mTRPV2). The 2′-OH group forms two hydrogen bonds with I605 and Y634, and several van der Waals forces were also observed for B304-1 bound to rTRPV2.

To confirm the residues critical for the compound binding obtained from the docking data, we mutated five key residues of TRPV2 to alanine (mTRPV2 T599A, I600A, G601A, M602A, and Y629A). The mTRPV2 T599A and I600A mutants were functional as determined for the channel activation with agonist 2 mM 2-APB, indicating that TRPV2 pharmacology was undisrupted. Administration of 100 μM of B304-1 or B304-2 resulted in an inhibition of mTRPV2 T599A mutant activated by 2-APB (2 mM) comparable to 50 μM of tranilast that had the same inhibitory effect on mTRPV2 T599A mutant (Figure 7D), suggesting that T599 residue is unlikely a binding site for B304-1. However, when the I600A mutant was tested, neither B304-1 nor B304-2 (100 μM) nor tranilast (50 μM) showed inhibition on TRPV2 current activated by 2 mM 2-APB (Figure 7E), indicating that the residue I600 forming van der Waals force with B304-2 or hydrogen bond with B304-1 is critical for their inhibitory effects. Conversely, there is a detectable increase of inward current in the I600A mutant by the two compounds, thus, likely attributing to the feature of the compounds. We were unable to observe any inhibitory effect of B304-1 or B304-2 on the G601A, M602A, and Y629A mutants, because G601A and M602A were insensitive to 2 mM 2-APB (Figure S9A,B), and Y629A was desensitized to 2 mM 2-APB (Figure S9C). These results are consistent with the previous observations that residues nearby the selectivity filter are often important for maintaining channel activation, and mutating these residues can disrupt the channel function.5

4 | DISCUSSION

The goal of this study was to identify selective TRPV2 inhibitors that can be used as either pharmacological tools or chemical leads for further understanding of the channel function because there is a lack of specific TRPV2 inhibitors. Using a combination of calcium imaging, calcium fluorescence assay, patch-clamp recordings, molecular modeling, and site-directed mutagenesis, we screened and identified two natural coumarin derivative enantiomers (−)-muraxocin (named B304-1) and (+)-muraxocin (named B304-2) from murraya exotica plant that selectively inhibit TRPV2 channel.

The plant murraya species are widely used as herbal medicines in China for pain relief, promotion of blood circulation and dissipation of blood stasis syndrome.49 Muraxocin has been found in murraya paniculata (L.) Jack,45 murraya exotica,50 and Boenninghausenia albiflora51 to exhibit insecticidal activity against forest insect pests such as Plectoptera reflexa, Clostera cupreata, and Crypsiptya coecalis.52 Interestingly, the chiral configuration of 2′-OH and 1′-ethoxy groups in (−)-muraxocin and (+)-muraxocin leads to a significant difference in their inhibitory effects on TRPV2, indicating further chemical modifications of muraxocins may...
lead to discovery of more potent and selective inhibitors of TRPV2.

TRPV2 can be activated by heat (>53°C), membrane stretch, and cell swelling, and the linker domain (also known as the membrane-proximal domain, Pro320-Leu373) is important for thermosensation. However, mice lacking TRPV2 display an intact heat sensitivity, and human TRPV2 is even heat-insensitive. In recent report, oxidation of methionine residues activates the high-threshold heat-sensitive ion channel TRPV2. TRPVs, including TRPV2, have ankyrin repeats in their N-terminal regions. Because the ankyrin repeats interact with certain cytoskeletal proteins, this region of TRPV2 might be also important for acceptance of applied mechanical signals. The absence of specific TRPV2 modulators has significantly impeded the understanding of the channel function and pharmacology. The fact that the
FIGURE 4 TRPV2 expressions in brown pre-adipocytes and adipocytes, and inhibition of TRPV2-mediated current by B304-1 and B304-2 in differentiated brown adipocytes. A, Real-time PCR analyses of TRPV1, TRPV2, TRPV3, and TRPV4 expressions in differentiated brown adipocytes after 35 thermal cycles. Control (C) lanes indicate the bands from template plasmid cDNAs. Lanes with (+) indicate reverse transcriptions of indicated genes, and lanes labeled with (−) indicate reactions without reverse transcription. B, Western blot analysis of TRPV2, TRPV1, and TRPV3 expression in 6-day-differentiated brown adipocytes. C, Real-time PCR analysis of TRPV2 expression in pre-adipocytes (red) and 6-day-differentiated brown adipocytes (blue). mRNA levels are expressed as the means ± SEM. from six independent tests, **P < .01 vs pre-adipocytes. One-way ANOVA followed by two-tailed t test with Bonferroni correction. D, E, Western blot analysis (D) and densitometry quantification (E) of TRPV2 expression in pre-adipocytes, and 6-day-differentiated brown adipocytes. F, Left panels, whole-cell currents of TRPV2 activated by agonist 2 mM 2-APB in pre-adipocytes (top) or 6-day-differentiated brown adipocytes (bottom). Right panels, current-voltage curves from left panels for pre-adipocytes (top) and 6-day differentiated adipocytes (bottom) in response to 2-APB (2 mM) or tranilast (50 µM) at time points indicated by 0, 1, 2, and 3. G,H, Left panels, TRPV2-like currents activated by 2 mM 2-APB and inhibition of the current by co-application of 100 µM B304-1 (G) or co-addition 100 µM B304-2 (H). Right panels, current-voltage curves from left panels in response to voltage ramps from −100 to +100 mV. (G) under control condition (0) after addition of 2 mM 2-APB (1), co-addition of 100 µM B304-1 and 2 mM 2-APB (2), and washout (3). (H) under control condition (0) after addition of 2 mM 2-APB (1), co-addition of 100 µM B304-2 and 2 mM 2-APB (2), and back to baseline (3).

channel is activated by high heat with the activation threshold above 53°C also presents a challenge in understanding the channel physiology. Although TRPV2 activators such as 2-aminoethoxydiphenyl borate (2-APB),3 probenicid,5 cannabinoids,6 and lysophospholipids26 have been developed sporadically, none of them, however, are specific for TRPV2 channels. This discomfiture also occurs in current TRPV2 antagonists that are poorly selective over other TRP channels. For instance, ruthenium red inhibits a broad range of ion channels including TRPV1, TRPV3, and

FIGURE 5 Reversal of TRPV2 agonist-induced inhibition of mouse brown adipocyte differentiation by B304-1 and B304-2. A, Oil red O staining and the number of 6-day-differentiated mouse brown adipocytes after pharmacological treatment by 2-APB alone, 2-APB+tranilast, 2-APB+B304-1, and 2-APB+B304-2. The control group indicates differentiation medium with solvent (0.2% of DMSO). Scale bar indicates 100 µm. B,C, Ucp1 (B), Ppary (C) mRNA levels after various treatment in 6-day-differentiated mouse brown adipocytes. D, Total cDNAs per well after indicated pharmacological treatments in 6-day-differentiated brown adipocytes. Data are the means ± SEM. n = 3. *P < .05, **P < .001, and ****P < .0001 vs control group; #P < .05, ##P < .001, and ###P < .0001 vs 2-APB group. One-way ANOVA followed by two-tailed t test with Bonferroni correction.
TRPA1. SKF96365 originally identified as a blocker of receptor-mediated calcium entry is widely used as a blocker of TRPCs channels and high-voltage-activated (HVA) and low-voltage-activated (LVA) T-type Ca2+ channels. Tranilast is an antiallergic drug that is considered as a relatively selective inhibitor of TRPV2, but it is also involved in many biological pathways that limit its use for elucidation of specific TRPV2 functions. In the present study, both B304-1 and B304-2 selectively inhibit TRPV2 over other tested channels such as TRPV1, TRPV4, TRPM8, voltage-gated K+ channels Kv7.2/3 and Kv11.1, voltage-gated Nav1.4 and Nav1.5, and also ligand-gated acetylcholine receptors α3β2 and α7 (Figures 3 and S8). More interestingly, B304-2 (IC50 = 3.7 µM) exhibits a better potency over tranilast (IC50, 10-100 µM) for inhibition of TRPV2. B304-2, therefore, can serve as a better tool for studying TRPV2 function both in vitro and in vivo, although its ideal structure and activity relation (SAR) for inhibition of TRPV2 requires further investigations.

A recent report has shown that TRPV2 mRNA expression levels were significantly increased in iBAT and sWAT from HFD-induced obese mice. Similar increases were also observed in iBAT and sWAT from db/db mice. And some observations indicate that brown adipose tissue in adult human plays a role in energy expenditure through adaptive thermogenesis for antiobesity strategy. Modulation of BAT function appears be an intriguing way to treat human obesity and related metabolic disorders. The important role of TRPV2 in brown adipocytes is suggested by its higher expression level in differentiated brown adipocytes than pre-adipocytes. It has been shown that activation of TRPV2 by agonist 2-APB inhibits the differentiation of brown adipocytes, and TRPV2 antagonist SKF96365 reverses 2-APB-induced inhibition of mouse brown adipocyte differentiation in its pre-adipocyte stage. In differentiated brown adipocytes TRPV2 promotes thermogenesis, whereas TRPV2 knockout mice exhibit difficulty in maintaining body temperature in cold environment, as well as a tendency of developing obesity under high fat diet (HFD) treatment. These observations for TRPV2-dependent inhibition of brown adipocyte differentiation apparently contradicts the involvement of TRPV2 in the thermogenesis, because thermogenesis occurs only in the differentiated brown adipocytes and the functional expression of polymodal TRPV2 can vary during differentiation, which might explain the different functions of TRPV2 in mouse brown adipocytes.

Our molecular docking suggests that the two enantiomers B304-1 and B304-2 inhibit TRPV2 by binding at its upper gate for block of the channel pore. In this binding model, B304-2 interacts with the residue I605, G606, M607, and Y634, whereas B304-1 interacts with the residue T604, I605, and Y634, which is consistent with I605A point mutation.
that is insensitive to block by either B304-1 or B304-2. Our SAR analysis of the 2′-OH group of murraxocin shows that modification of the murraxocin 2′-OH group to any tested neutral groups, positive or negative charged groups abolishes the inhibition effect of murraxocin on 2-APB-activated TRPV2 (Figures 6 and S10). These results indicate that these hydrogen bonds formed between B304-2 and G606, B304-1, and I605 or Y634 increase their binding affinity.

The docking results also explain the sixfold difference in activity between B304-1 and B304-2, in which the residue G606 that forms hydrogen bonds with B304-2, but not B304-1, is a crucial part of the binding. It is also worth

**FIGURE 7**  Putative binding sites for B304-1 and B304-2 in rat TRPV2 channel. A, Side view (up) and top-down (down) view B304-1 with rat TRPV2 subunits. B, A side view (up) and top-down view (down) for B304-2 with rat TRPV2 subunits. C, Residue sequence alignment and comparison of the S5-P-S6 regions between rat TRPV2 and mouse TRPV2 channels. D,E, Current traces of mouse TRPV2 T599A (D) and I600A (E) mutants expressed in HEK-293 cells in responses to 2-APB alone and 2-APB with B304-1 or B304-2 or tranilast
mentioning that although residues T604, I605, G606, M607, and Y634 near the pore are quite conserved in the members of TRP family, their subtle structural differences in the pore region might explain the selective inhibition of TRPV2 by B304-1 and B304-2 for over other TRPs (Figure 7). For instance, the upper gate of TRPV2 channel is wider than that of TRPV1, likely accommodating B304-1 and B304-2 better in TRPV2 pore than TRPV1. TRPV2 shows the variable pharmacological regulation by different stimuli between species. In this study, we have only show the pharmacological inhibition of mouse TRPV2 by B304-1 or B304-2, however, it is possible that the two enantiomers-mediated pharmacological effects are different depending on channel species such as human TRPV2 that has not been tested in this study.

In summary, we find two coumarin enantiomers B304-1 and B304-2 from the roots of *Murraya exotica* that selectively inhibit TRPV2 channels. The identification of both B304-1 and B304-2 provides essential pharmacological tools or leads for further understanding of the channel physiology and pharmacology in BAT and other tissues expressing TRPV2.

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CONFLICT OF INTEREST
The authors declare that they have no conflicts of interest with the contents of this article.

AUTHOR CONTRIBUTIONS
Q.Q. Zhou, N.N. Wei, Y.T. Shi, Y. Jiang, and K.W. Wang designed research; Q.Q. Zhou, N.N. Wei, Y.T. Shi, H. Qi, and H.J. Liu performed research; Q.Q. Zhou, Y.T. Shi, and H.J. Liu analyzed data; Q.Q. Zhou, N.N. Wei, Y.T. Shi, Y. Jiang, and K.W. Wang wrote the paper, and all authors have read, commented on, and approved the content of the manuscript.

REFERENCES
1. Peralvarez-Marin A, Donate-Macian P, Gaudet R. What do we know about the transient receptor potential vanilloid 2 (TRPV2) ion channel? *FEBS J.* 2013;280:5471-5487.
2. Flockerzi V. An introduction on TRP channels. *Handb Exp Pharmacol.* 2007;222:1-19.
3. Caterina MJ, Rosen TA, Tominaga M, Brake AJ, Julius D. A capsaicin-receptor homologue with a high threshold for noxious heat. *Nature.* 1999;398:436-441.
4. Zubcevic L, Herzik MA Jr, Chung BC, Liu Z, Lander GC, Lee SY. Cryo-electron microscopy structure of the TRPV2 ion channel. *Nat Struct Mol Biol.* 2016;23:180-186.
5. Huyhn KW, Cohen MR, Jiang J, et al. Structure of the full-length TRPV2 channel by cryo-EM. *Nat Commun.* 2016;7:11130.
6. Cromer BA, McIntyre P. Painful toxins acting at TRPV1. *Toxicon.* 2008;51:163-173.
7. Kojima I, Nagasawa M. Trpv2. *Handb Exp Pharmacol.* 2014;222:247-272.
8. Zhang X, Hu M, Yang Y, Xu H. Organellar TRP channels. *Nat Struct Mol Biol.* 2018;25:1009-1018.
9. Iwata Y, Katanosaka Y, Arai Y, Komamura K, Miyatake K, Shigekawa M. A novel mechanism of myocyte degeneration involving the Ca2+-permeable growth factor-regulated channel. *J Cell Biol.* 2003;161:957-967.
10. Stokes AJ, Wakano C, Del Carmen KA, Koblan-Huberson M, Turner H. Formation of a physiological complex between TRPV2 and RGA protein promotes cell surface expression of TRPV2. *J Cell Biol.* 2005;94:669-683.
11. Stokes AJ, Shimoda LM, Koblan-Huberson M, Adra CN, Turner H. A TRPV2-PKA signaling module for transduction of physical stimuli in mast cells. *J Exp Med.* 2004;200:137-147.
12. Che H, Yue J, Tse HF, Li GR. Functional TRPV and TRPM channels in human preadipocytes. *Eur J Appl Physiol.* 2014;6:947-959.
13. Sugio S, Nagasawa M, Kojima I, Ishizaki Y, Shibusaki K. Transient receptor potential vanilloid 2 activation by focal mechanical stimulation requires interaction with the actin cytoskeleton and enhances growth cone motility. *FASEB J.* 2017;31:1368-1381.
14. van Marken Lichtenbelt WD, Vanhommmerig JW, Smulders NM, et al. Cold-activated brown adipose tissue in healthy men. *N Engl J Med.* 2009;360:1500-1508.
15. Cypress AM, Lehman S, Williams G, et al. Identification and importance of brown adipose tissue in adult humans. *N Engl J Med.* 2009;360:1509-1517.
16. Reddy NL, Tan BK, Barber TM, Randeva HS. Brown adipose tissue: endocrine determinants of function and therapeutic manipulation as a novel treatment strategy for obesity. *BMC Physiol.* 2014;1:13.
17. Rosenwald M, Wolfrum C. The origin and definition of brite versus white and classical brown adipocytes. *Adipocyte.* 2014;3:4-9.
18. Cannon B, Nedergaard J. Brown adipose tissue: function and physiological significance. *Physiol Rev.* 2004;84:277-359.
19. Bartelt A, Bruns OT, Reimer R, et al. Brown adipose tissue activity controls triglyceride clearance. *Nat Med.* 2011;17:200-205.
20. Tang Y, He Y, Li C, et al. RPS3A positively regulates the miR-148a-5p expression and mitochondrial function of human periaortic adipose tissue and is associated with coronary artery diseases. *Cell Discovery.* 2018;4:52.
21. Munakata Y, Yamada T, Imai J, et al. Olfactory receptors are expressed in pancreatic beta-cells and promote glucose-stimulated insulin secretion. *Sci Rep-UK.* 2018;8:1499.
22. Muraki K, Iwata Y, Katanosaka Y, et al. TRPV2 is a component of osmotically sensitive cation channels in murine aortic myocytes. *Circ Res.* 2003;93:829-838.
23. Iwata Y, Katanosaka Y, Arai Y, Shigekawa M, Wakabayashi S. Dominant-negative inhibition of Ca2+ influx via TRPV2...
ameliorates muscular dystrophy in animal models. *Hum Mol Genet*. 2009;18:824-834.

24. Nagasawa M, Kojima I. Translocation of TRPV2 channel induced by focal administration of mechanical stress. *Physiol Rep*. 2015;3:e12296.

25. Monet M, Gkika D, Lehen’kyi V, et al. Lysophospholipids stimulate prostate cancer cell migration via TRPV2 channel activation. *Biochim Biophys Acta - Mol Cell Res*. 2009;1793:528-539.

26. Ramsey IS, Delling M, Clapham DE. An introduction to TRP channels. *Ann Rev Physiol*. 2006;68:619-647.

27. Singh A, Hildebrand ME, Garcia E, Snutch TP. The transient receptor potential calcium-permeable channels. *Exp Pharmacol Therap*. 2015;91:15-28.

28. Sun W, Uchida K, Takahashi N, et al. Activation of TRPV2 negates the effects of exfoliation of beta-amyloid plaques in Alzheimer’s disease. *J Biol Chem*. 2007;282:15894-15902.

29. Tian YQ, Ding P, Yan XH, Hu WJ. Discussion on quality control of preparations with cortex moutan in volume I pharmacopoeia of People’s Republic of China (2005 edition). *Zhongguo Zhong Yao Za Zhi*. 2008;33:339-341.

30. Varga T, Czinnerer Z, Nagy L. PPARs are a unique set of fatty acid regulated transcription factors controlling both lipid metabolism and inflammation. *Biochim Biophys Acta - Mol Basis Dis*. 2011;1812:1007-1022.

31. ZHOU et al. How to cite this article: Zhou Q, Shi Y, Qi H, et al. Identification of two natural coumarin enantiomers for selective inhibition of TRPV2 channels. *The FASEB Journal*. 2020;34:12338-12353. https://doi.org/10.1096/fj.201901541R