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Chain Branching Approach in Structure Modification of TRPV1 Receptor Antagonist MK056 and its Analogs

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A series of chain branched 1,3-dibenzylthiourea derivatives were designed, synthesized, and evaluated for their antagonist activity against TRPV1. The synthesized chain branched 1,3-dibenzylthioureas **9a**-**g** were tested for their antagonist activities against TRPV1 by $^{45}Ca^{2+}$-influx assay using neonatal rat cultured spinal sensory neurons. Fluorinated ethyl-branched analog **9g** showed the most potent antagonist activity with an IC_{50} value of 0.41 μM, but all of the chain branched analogs were less potent than the parent compounds MK-056 and SC-0030, indicating that chain branching on the benzylic position of B-ring is detrimental to potency. Optimized receptor binding seems to be interfered by chain branching, and resulted in decrease in potency.

Key words: Chain branching, TRPV1, 1,3-Dibenzylthioureas, Antagonist, $^{45}Ca^{2+}$-influx assay

INTRODUCTION

Chain branching is one of the molecular modification method used in drug design and lead development (Silverman, 2004). However, as a result of larger molar volumes and shapes of branched compounds, chain branching usually will lower the potency of a compound. When lipophilicity is among the important parameters for potency of a drug or lead, a branched alkyl chain is less lipophilic than the corresponding straight alkyl chain. Optimized receptor binding interaction might also be interfered by chain branching. For these reasons, chain branching was not frequently used as expected, compared to other molecular modification methods. Nonetheless, newly created stereogenic centers by chain branching can alter both the binding mode and the selectivity to a receptor or an enzyme, ultimately resulting in a major pharmacological change. As ever increasing importance of receptor as drug target, it is often required a molecular modification method that could invoke receptor antagonism from the known agonist structure of a receptor. In this sense, chain branching could be a promising method for this important pharmacological change (Yoon et al., 2003). In addition, chain branching can lead to marked alterations in the physicochemical and biological properties of chain branched analogues. Thus, the introduction of a branched chain into biologically prevalidated drug scaffolds represents an important way of drug design.

On the other hand, TRPV1 is a ligand-gated non-selective cation channel vanilloid receptor 1 (VR1) with high Ca^{2+} permeability (Szallasi et al., 2007), emerging as a novel target for the treatment of pain with entirely different mechanism from the previously known (Westaway, 2007). Based on the premise that direct blockage of TRPV1 by its antagonists could be a promising way to shut down the pathway involved in nociception (Rami and Gunthorpe, 2004), extensive works have been done to develop new TRPV1 antagonists during the past decade (Gunthorpe and Chizh, 2009; Khairatkar-Joshi and Szallasi, 2009; Kym et al., 2009; Lambert, 2009). We have also reported the dibenzylthiourea analogs including MK-056 (**1**) (Park et al., 2004), and SC-0030 (**2**) (Suh et al., 2003), which exhibit highly potent competitive TRPV1 antagonist effects. Based on chain branching approach mentioned

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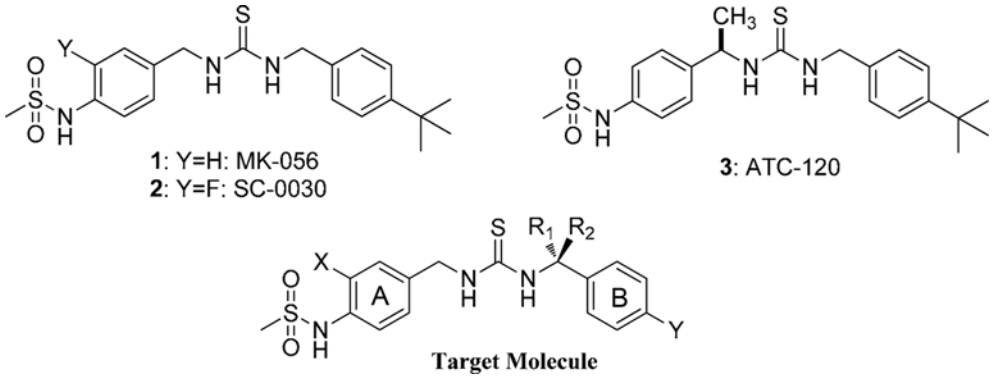


Fig. 1. Chemical structure of 1,3-dibenzylthioureas

previously, we also designed and synthesized methyl branched ATC-120 (**3**) (Ryu et al., 2004) as TRPV1 antagonist, showing 2-fold more potent antagonist activity compared with parent compound MK-056. This chain branching has become a key element to designing novel TRPV1 antagonists having dibenzylthiourea scaffold. Encouraged with the result shown by ATC-120, we try to introduce a stereogenic center at another benzylic position in MK-056 and SC-0030, as shown in Fig. 1.

MATERIALS AND METHODS

The melting points were obtained using Büchi 535 melting point apparatus and were uncorrected. Optical rotations were measured on a JASCO DIP 1000 digital polarimeter. ^{1}H-NMR and ^{13}C-NMR spectra were obtained on a Varian Inova 400 spectrometer and the chemical shifts are reported as values in parts per million (δ) relative to tetramethylsilane (TMS) as an internal standard. The infrared spectra (IR) were recorded on a JASCO FT/IR-430 spectrophotometer. Thin layer chromatography (TLC) was carried out on 0.25 mm E. Merck precoated silica gel glass plates ($60F_{254}$). Column chromatography was performed using the forced flow of indicated solvent on Merck Kieselgel 60 (230-400 mesh). Chiral HPLC was performed using a Shimadzu LC-10AS pumping system and Shimadzu SPD-10A UV detector with a chiral column (Chiralcel OD, 0.46 cm (Φ) × 25 cm, Daicel Chemical Ind., Ltd). Unless otherwise noted, the materials were obtained from commercially available sources and were used without further purification. THF was freshly distilled from sodium benzophenone ketyl under an argon atmosphere. Benzene, DCM, DMF, triethylamine (TEA) and toluene were freshly distilled under a nitrogen atmosphere with calcium hydride.

(*R*)-*N*-(1-(4-(tert-butyl)phenyl)ethyl)formamide (6a)

$AlCl_3$ (815 mg, 6.11 mmol) was added portionwisely to a stirred mixture of (*R*)-*N*-(1-phenylethyl)formamide (**5a**, 456 mg, 3.05 mmol) and *t*-butyl chloride (664 μL, 6.11 mmol) in anhydrous dichloroethane (10 mL) at −40°C. The mixture was stirred for 90 min at −40°C, then quenched by addition of finely crushed ice and 10% hydrochloric acid. The layers were separated, and the aqueous layer was extracted twice with dichloromethane (20 mL). The combined organic layers were neutralized with aq. $NaHCO_3$ solution, washed with water and brine, dried over anhydrous Na_2SO_4, and concentrated *in vacuo*. The residue was purified by column chromatography (silica gel; 33% ethyl acetate in *n*-hexane) to afford the title compound **6a** as a solid (194.5 mg, 31%). m.p. 81-83°C; $[\alpha]_D^{24}$ +135 (*c* 0.5, $CHCl_3$); ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.09 (s, 1H), 7.30 (d, 2H, *J* = 8.2 Hz), 7.19 (d, 2H, *J* = 8.2 Hz), 5.75 (bs, 1H), 5.14 (quint, *J* = 7.2 Hz), 1.45 (d, 3H, *J* = 6.8 Hz), 1.24 (s, 3H); IR (KBr) cm^{-1} 3326, 2959, 1663, 1460, 1382.

(*S*)-*N*-(1-(4-(tert-butyl)phenyl)ethyl)formamide (6b)

By the same procedure as described for the preparation of compound **6a**, title compound **6b** was obtained from (*S*)-*N*-(1-phenylethyl)formamide (**5b**) as solid in 60% yield. Analytical data are identical with those of **6a** except optical rotation value. $[\alpha]_D^{25}$ −132 (*c* 0.9, $CHCl_3$).

(*R*)-*N*-(1-(4-(tert-butyl)phenyl)propyl)formamide (6c)

By the same procedure as described for the preparation of compound **6a**, title compound **6c** was obtained from (*S*)-*N*-(1-phenylpropyl)formamide (**5c**) as liquid in 59% yield. $[\alpha]_D^{24}$ +116 (*c* 0.5, $CHCl_3$); ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.13 (d, 1H, *J* = 6.4 Hz), 7.32-6.97 (m, 4H), 5.76 (bs, 1H), 4.90 (quint, 1H, *J* = 7.6 Hz), 1.83-

1.75 (m, 2H), 1.24 (s, 9H), 0.85 (t, 3H, J = 7.2 Hz); IR (NaCl, neat) cm^{-1} 3277, 2964, 1658, 1460, 1382.

(*R*)-1-(4-(tert-butyl)phenyl)ethanamine (7a)

To a solution of (*R*)-*N*-(1-(4-(tert-butyl)phenyl)ethyl) formamide (**6a**, 50 mg, 0.244 mmol) in ethanol (5 mL) was added 1N aqueous KOH solution (1 mL) at room temperature. The reaction mixture was concentrated *in vacuo*, and then diluted with dichloromethane (30 mL). The organic layer was washed with water and brine, dried over anhydrous Na_2SO_4, and concentrated *in vacuo* to afford the title compound **7a** as liquid (37 mg, 86%). $[\alpha]_D^{25}$ +10.7 (*c* 0.8, $CHCl_3$); ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.29 (d, 2H, J = 8.2 Hz), 7.20 (d, 2H, J = 8.2 Hz), 4.08 (br s, 2H), 1.24 (s, 9H); IR (NaCl, neat) cm^{-1} 3356, 3285, 1578, 1460, 1363.

(*S*)-1-(4-(tert-butyl)phenyl)ethanamine (7b)

By the same procedure as described for the preparation of compound **7a**, title compound **7b** was obtained from (*S*)-*N*-(1-(4-(tert-butyl)phenyl)ethyl)formamide (**6b**) as liquid in 90% yield. Analytical data are identical with those of **7a** except optical rotation value. $[\alpha]_D^{25}$ −11.0 (*c* 1.0, $CHCl_3$).

(*R*)-1-(4-(tert-butyl)phenyl)propan-1-amine (7c)

By the same procedure as described for the preparation of compound **7a**, title compound **7c** was obtained from (R)-N-(1-(4-(tert-butyl)phenyl)propyl)formamide (**6c**) as liquid in 28% yield. $[\alpha]_D^{25}$ +6.94 (*c* 0.5, $CHCl_3$); ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.29-7.17 (m, 4H), 4.07-3.83 (m, 1H), 1.73 (s, 2H), 1.25 (s, 9H), 0.79 (s, 3H); IR (NaCl, neat) cm^{-1} 3315, 2963, 1556, 1460, 1363.

(*R*)-*N*-(4-((3-(1-phenylethyl)thioureido)methyl) phenyl)methanesulfonamide (9a)

To a solution of the *N*-(4-isothiocyanatomethylphenyl) methanesulfonamide (**8a**, 40.0 mg, 0.17 mmol) in dichloromethane (5 mL) was added (*R*)-(–)-α-4-methylbenzylamine (**4a**, 23.0 mg, 0.20 mmol) at room temperature. After being stirred for 12 h at room temperature, the reaction mixture was quenched with H_2O (1 mL) and diluted with dichloromethane (30 mL). The organic layer was washed with brine, dried over anhydrous Na_2SO_4, and concentrated *in vacuo*. The residue was purified by column chromatography (silica gel; 33% ethyl acetate in *n*-hexane) to afford the title compound **9a** as solid (33.0 mg, 46%). m.p. 69-71°C; $[\alpha]_D^{20}$ −33.14 (*c* 0.53, $CHCl_3$); ^{1}H-NMR (400 MHz, CD_3OD) δ 7.61 (s, 1H), 7.30-7.24 (m, 5H), 7.03 (d, 2H, J = 8.0 Hz), 6.92 (d, 2H, J = 8.0 Hz), 6.74 (s, 1H), 5.96 (s, 1H), 4.83 (s, 2H), 4.64 (d, 1H, J = 15.2 Hz), 4.49 (d, 1H, J = 15.2 Hz), 2.92 (s, 3H), 1.47 (d, 3H, J = 6.8 Hz); IR (KBr) cm^{-1} 3350, 3259, 3063, 3027, 2975, 2928, 1545, 1511; HRMS (FAB^+) calcd for $C_{17}H_{22}N_3O_2S_2$ (M+H) 364.1153, found 364.1157.

(*S*)-*N*-(4-((3-(1-phenylethyl)thioureido)methyl) phenyl)methanesulfonamide (9b)

By the same procedure described above, the product was obtained from **8a** and (*S*)-(-)-α-4-methylbenzylamine (**4b**) as solid in 52% yield. Analytical data are identical with those of **9a** except optical rotation value. $[\alpha]_D^{20}$ +35.1 (*c* 0.7, $CHCl_3$).

(*R*)-*N*-(4-((3-(1-(4-(tert-butyl)phenyl)ethyl)thioureido)methyl)phenyl)methanesulfonamide (9c)

By the same procedure described above, the product was obtained from **8a** and **7a** as solid in 62% yield. m.p. 88-90°C; $[\alpha]_D^{20}$ −17.0 (*c* 0.5, $CHCl_3$); ^{1}H-NMR (400 MHz, $CDCl_3$): 7.35 (d, 2H, J = 8.0 Hz), 7.23 (d, 2H, J = 8 Hz), 7.07 (d, 2H, J = 8.4 Hz), 7.00 (s, 1H), 6.96 (d, 2H, J = 8.4 Hz), 6.52 (s, 1H), 5.75 (s, 1H), 4.7 (dd, 2H, J = 14.8 Hz, 4.8 Hz), 4.58 (dd, 1H, J = 14.8 Hz, 4.8 Hz), 2.98 (s, 3H), 1.52 (d, 3H, J = 6.8 Hz), 1.3 (s, 3H); IR (KBr) cm^{-1} 3350, 3260, 3025, 2964, 1614, 1326, 1152; HRMS (FAB^+) calcd for $C_{21}H_{30}N_3O_2S_2$ (M+H) 420.1779, found 420.1775.

(*S*)-*N*-(4-((3-(1-(4-(tert-butyl)phenyl)ethyl)thioureido)methyl)phenyl)methanesulfonamide (9d)

By the same procedure described above, the product was obtained from **8a** and **7b** as solid in 62% yield. Analytical data are identical with those of **9c** except optical rotation value. $[\alpha]_D^{20}$ +16.5 (*c* 1.0, $CHCl_3$).

(*R*)-*N*-(4-((3-(1-(4-(tert-butyl)phenyl)propyl)thioureido)methyl)phenyl)methanesulfonamide (9e)

By the same procedure described above, the product was obtained from **8a** and **7c** as syrup in 34% yield. $[\alpha]_D^{20}$ −24.34 (*c* 0.71, $CHCl_3$); ^{1}H-NMR (400 MHz, $CDCl_3$): 7.29-6.98 (m, 8H), 6.83 (s, 1H), 6.63 (s, 1H), 5.71 (s, 1H), 4.67 (dt, 1H, J = 14.2 Hz, 6.0 Hz), 4.45 (dt, 2H, J = 12.6 Hz, 4.4 Hz), 2.90 (s, 3H), 1.86-1.66 (m, 2H), 1.24 (s, 9H), 0.83 (dt, 3H, J = 7.2 Hz, 2.0 Hz); IR (NaCl, neat) cm^{-1} 3259, 1547, 1512, 1328, 1152; HRMS (FAB^+) calcd for $C_{22}H_{32}N_3O_2S_2$ (M+H) 434.1936, found 434.1936.

(*R*)-*N*-(4-((3-(1-(4-(tert-butyl)phenyl)ethyl)thioureido)methyl)-2-fluorophenyl)methanesulfonamide) (9f)

By the same procedure described above, the product was obtained from **8b** and **7a** as solid in 19% yield. m.p. 70-72°C; $[\alpha]_D^{22}$ −40.56 (*c* 0.1, $CHCl_3$); ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.30 (s, 1H), 7.26 (d, 2H, J = 8.4 Hz), 7.15 (d, 2H, J = 8.4 Hz), 6.88 (s, 2H), 5.24 (s, 1H), 4.74

(d, 1H, *J* = 12.4 Hz), 4.51 (d, 1H, *J* = 12.4 Hz), 2.83 (s, 3H), 1.38 (d, 3H, *J* = 6.8 Hz), 1.21 (s, 9H); IR (KBr) cm^{-1} 3354, 3259, 3069, 2963, 2926, 1590, 1547, 1510, 1332, 1157; HRMS (FAB^+) calcd for $C_{21}H_{29}N_3O_2S_2$ (M+H) 438.1685, found 438.1687.

(*R*)-*N*-(4-((3-(1-(4-(tert-butyl)phenyl)propyl) thioureido)methyl)-2-fluorophenyl)methane sulfonamide) (9g)

By the same procedure described above, the product was obtained from **8b** and **7c** as solid in 39% yield. $[\alpha]_D^{25}$ −37.7 (*c* 0.6, $CHCl_3$); ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.30 (s, 1H), 7.26 (d, 2H, *J* = 8.4 Hz), 7.15 (d, 2H, *J* = 8.4 Hz), 6.88 (s, 2H), 5.24 (s, 1H), 4.74 (d, 1H, *J* = 12.4 Hz), 4.51 (d, 1H, *J* = 12.4 Hz), 2.83 (s, 3H), 1.38 (d, 3H, *J* = 6.8 Hz), 1.21 (s, 9H); 7.39-6.66 (m, 7H), 6.61 (s, 1H), 5.74 (s, 1H), 4.89- 4.80 (m, 1H), 4.49 (dt, 2H, *J* = 14.0 Hz, 4.8 Hz), 2.99 (s, 3H), 1.93-1.73 (m, 2H), 1.30 (s, 9H), 0.92 (dt, 3H, *J* = 7.0 Hz, 1.2 Hz); IR (KBr) cm^{-1} 3257, 2963, 1547, 1512, 1332, 1157; HRMS (FAB^+) calcd for $C_{22}H_{31}FN_3O_2S_2$ (M+H) 452.1842, found 452.1840.

Culture of DRG neurons

DRG neurons were prepared from neonatal Sprague-Dawley rats. DRGs of all spinal levels were dissected aseptically and collected. Ganglia were incubated sequentially for 30 min at 37°C in 200 U/mL collagenase and 2.5 mg/mL trypsin. The digestion was halted by an addition of an equal volume of DME/F12 medium supplemented with 10% horse serum. The ganglia were then triturated through a fire-polished Pasteur pipet, filtered through nylon membrane, and spun down. Dissociated cells were plated onto Terasaki plates previously coated with 10 μg/mL poly-D-ornithine at a density of 1500-1700 neurons/well. The cells were then cultured for 3 days in DME/F12 medium containing 1.2 g/L sodium bicarbonate, 15 mM HEPES, 50 mg/L gentamycin, and 10% horse serum, diluted 1:1 with identical medium conditioned by C6 glioma cells (2 days on a confluent monolayer) in a humidified atmosphere at 37°C containing 5% CO_2. Medium was supplemented with 200 ng/mL nerve growth factor. Cytosine arabinoside (100 μM) was added for the first 2 days to kill dividing nonneuronal cells.

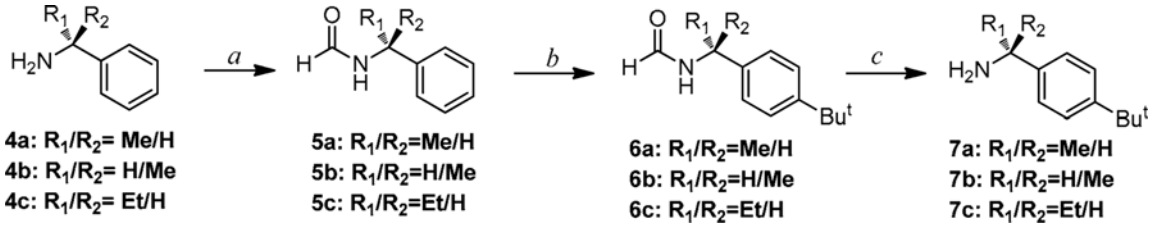
$^{45}Ca^{2+}$ Uptake assays

Terasaki plates containing DRG neurons grown for 3 days were equilibrated with four washes of HEPES (10 mM, pH 7.4)-buffered calcium- and magnesium-free Hank's balanced salt solution. The solution in each well was removed from the individual wells. For antagonistic studies, medium (10 μL) containing 10 μCi/mL $^{45}Ca^{2+}$ and 0.5 M capsaicin together with the test concentration of the compound was added to each well. The neurons were incubated at room temperature for 10 min, and then the Terasaki plates were washed six times in HEPES (10 mM, pH 7.4)-buffered calcium- and magnesium-free Hank's balanced salt solution and dried in an oven. Sodium dodecyl sulfate (0.3%, 10 μL) was then added to dissolve the cells and extract the $^{45}Ca^{2+}$. The contents of each well were transferred to scintillation vials and counted in 3 mL of aquasol-2 scintillant. Antagonistic activities of test compounds were given as IC_{50} (the concentration of the compound necessary to reduce the response to 0.5 μM capsaicin by 50%). The IC_{50} values were estimated at least three replicates at each concentrated. Each compound was tested at least in two independent experiments.

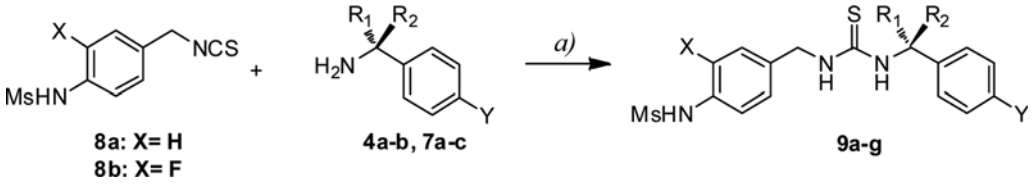
RESULTS AND DISCUSSION

MK-056 (**1**) and SC-0030 (**2**) were also chosen as reference compounds in order to clarify the chain branching effect. Because these compounds represent the most important scaffolds in 1,3-dibenzylthioureas with high antagonist activity. Thus, target compounds in this study were the methyl and ethyl branched derivatives of MK-056 (**1**) and SC-0030 (**2**). These targets were synthesized via the route outlined in Scheme 1-2. Optically active methyl or ethyl branched benzylamines **7a-c** were synthesized via the route outlined in Scheme 1. Commercially available, optically active α-methyl or α-ethylbenzylamines **4a-c** were formylated in the presence of HCOOH and Ac_2O according to the literature methods (Iwata and Kuzuhara, 1989). Friedel-Crafts alkylation of formamides **5a-c** using *tert*-butyl chloride and $AlCl_3$ under standard condition gave the corresponding *tert*-butylated product **6a-c** in moderate yields. Hydrolysis of formamides **6a-c** with KOH gave desired corresponding benzylamines **7a-c** in good yields. The requisite isothiocyanates **8a** and **8b** were prepared according to the previously reported method (Lee et al., 2003; Suh et al., 2005). Finally, **8a** and **8b** were treated with optically active α-branched benzylamine **4a-c** and **7a-c** respectively in dry dichloromethane to give the chain branched 1,3-dibenzylthioureas **9a-g** as target compounds.

The synthesized chain branched 1,3-dibenzylthioureas **9a-g** were tested for their antagonist activities against TRPV1 by $^{45}Ca^{2+}$-influx assay using neonatal rat cultured spinal sensory neurons (Wood et al., 1988). The results are summarized in Table I. MK-056 (**1**) and SC-0030 (**2**) were used as reference compounds. As shown in Table I, (*R*)-isomers are uniformly more



Scheme 1. Reagents and conditions: a) HCOOH, Ac_2O, 96-97%; b) t-BuCl, $AlCl_3$, $ClCH_2CH_2Cl$, 59%; c) KOH. EtOH, 86%



Scheme 2. Reagents and conditions: a) DCM, rt, 46-74%

potent than (*S*)-isomers. When removing *tert*-butyl group on the phenyl ring, antagonist activities were dropped drastically as shown by **9a** and **9b**. This result is consistent the with the previous results that 4-positioned *tert*-butyl group on the B ring is very reluctant to permit chemical modification (Li et al., 2009). Introduction of branched methyl group on MK-056 (**9c** and **9d**) dropped potency by a factor of 5 to 50-fold depending on their absolute configurations. Eutomer **9c** with (*R*)-configuration exhibited 0.55 μM of IC_{50} value as antagonist, and a eudismic ratio of 8.2. Ethyl-branched analog **9e** with (*R*)-configuration showed a slightly a less potent activity compared to **9c**. However, in case of the fluorinated analogs of SC-0030, ethyl-branched analog **9g** were more potent than methyl-branched analog **9f**. Among the tested compounds, fluorinated ethyl-branched analog **9g** showed the most potent antagonist activity with an IC_{50} value of 0.41 μM. All of chain branched analogs were less potent than the parent compounds MK-056 and SC-0030, indicating that chain branching on the benzylic position of B-ring is detrimental to the potency. It is expected that reduction in lipophilicity resulted by chain branching could be counterbalanced by the increased hydrocarbon moiety. Accordingly, a decrease in potency by chain branching is due to the poor binding of analogs to the receptor, and not to the changed lipohilicities of the analogs. An increase in the bulk on the benzylic position of B-ring seems to be weakening the receptor binding. A high eudismic ratio of enantiomers provides a piece of evidence that optimized receptor binding is interfered by chain branching, resulted in decrease in the potency of our analogs. While most of the analogs we explored failed to exhibit the improved their potency over the parent compounds MK-056 and SC-003, our report would serve as an important basis for the development of the highly potent and novel TRPV1 antagonist with suitable pharmacological properties. Further work on the therapeutically useful TRPV1 antagonist based on our current results is underway.

Table I. $^{45}Ca^{2+}$-Uptake inhibition by chain branched analogues

Compound	X	R_1	R_2	Y	$^{45}Ca^{2+}$ influx activity (μM)[a]	
					Agonist (EC_{50})	Antagonist (IC_{50})
9a	H	Me	H	H	>100	8.9
9b	H	H	Me	H	>100	>30
9c	H	Me	H	*t*-Bu	>100	0.55
9d	H	H	Me	*t*-Bu	>100	4.5
9e	H	Et	H	*t*-Bu	>100	0.74
9f	F	Me	H	*t*-Bu	>100	0.64
9g	F	Et	H	*t*-Bu	>100	0.41
MK-056	H	H	H	*t*-Bu	>100	0.11
SC-0030	F	H	H	*t*-Bu	>100	0.04

[a]EC_{50} (the concentration of derivatives necessary to produce 50% of the maximal response) and IC_{50} values (the concentration of derivatives necessary to reduce the response to 0.5 μM capsaicin by 50%) were estimated with at least three replicates at each concentration. Each compound was tested in two independent experiments. Antagonist data were fitted with a sigmoidal function.

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