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Facile synthesis and biological evaluation of 3,3-diphenylpropanoyl piperazines as T-type calcium channel blockers

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ABSTRACT

We have developed a facile synthesis of 3,3-diphenylpropanamides using Meldrum's acid derivatives as amide coupling components. The in vitro biological evaluation of the title compounds led to the identification of compound **1h**, which has good inhibitory activity against T-type calcium channel (IC_{50} = 0.83 μM).

Voltage gated calcium channels regulate the calcium influx into cells in response to membrane depolarization.[1] On the basis of encoding of the pore-forming α-subunit, they are divided into three main families: Ca_V 1.x (L-type), Ca_V 2.x (N-, P/Q-, R-type), and Ca_V 3.x (T-type). Among them, T-type calcium channels, also known as low voltage activated calcium channels, play a crucial role in the excitability of both central and peripheral neurons.[1] In particular, they are mainly expressed in the CNS regions, such as the dorsal root ganglion (DRG), the dorsal horn, and the thalamus, and take responsibility for modulating neuronal excitability. Three different genes coding for T-type calcium channels, Ca_V 3.1 (α_{1G}), Ca_V 3.2 (α_{1H}) and Ca_V 3.3 (α_{1I}) have been identified and the physiological and pathological role of each subtype in nervous system has not been clearly understood due to lack of selective blocker for them.[2] Recent genetic studies indicated that they involve the expression of spontaneous absence epilepsy, the enhancement of sleep, and the attenuation of neuropathic pain.[3] Therefore, selective T-type calcium channel blockers may have high potentials for treatment of CNS related disorders such as epilepsy, insomnia, and pain.

Several T-type calcium channel blockers have been discovered as described in Figure 1. Flunarizine, a neuroleptic, exhibited potent inhibitory activities against α_{1G} and α_{1I} subtypes (K_d = 0.53 and 0.84 μM, respectively).[4] Another antipsychotic agent, pimozide, also showed T-type calcium channel blocking activity with high binding affinity (K_d = ~40 nM).[5] While these compounds are not selective calcium channel blockers, mibefradil (Posicor®, Roche), approved by the FDA for the treatment of hypertension, is described as the first selective T-type calcium channel blocker.[6] Although it was withdrawn from the market due to unfavorable drug–drug interaction,[7] it is still used widely as a biological probe to investigate the function of T-type calcium channels in neuronal tissues. Recently, it was reported that the intraperitoneal administration of mibefradil yields efficacy in animal models of neuropathic pain.[8]

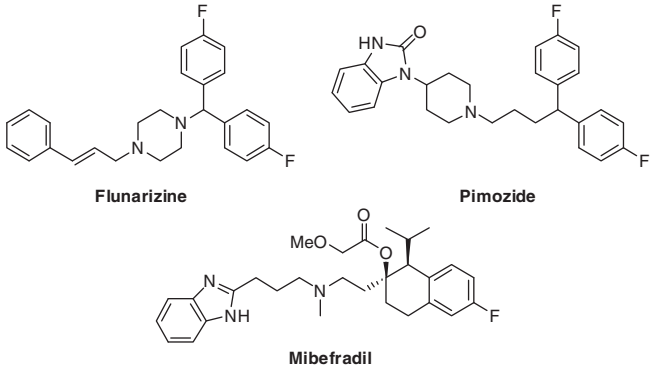

Figure 1. Structures of flunarizine, pimozide, and mibefradil.

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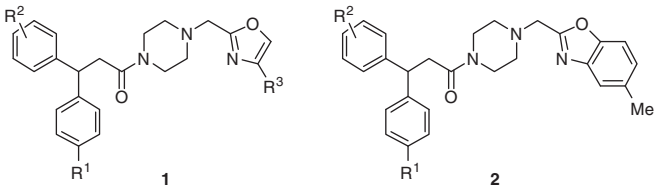
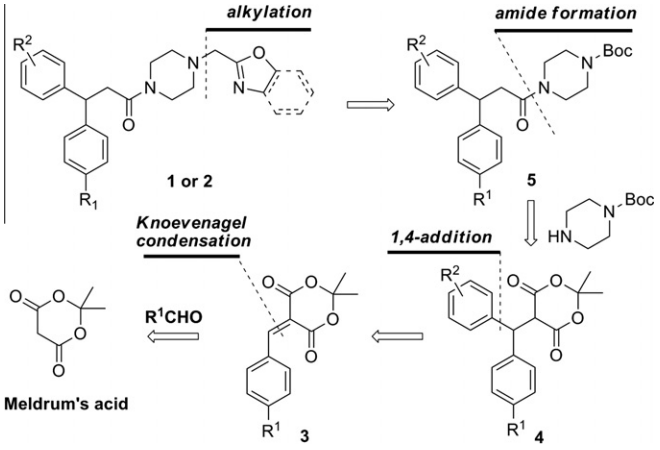


Figure 2. Structures of our target molecules **1** and **2**.

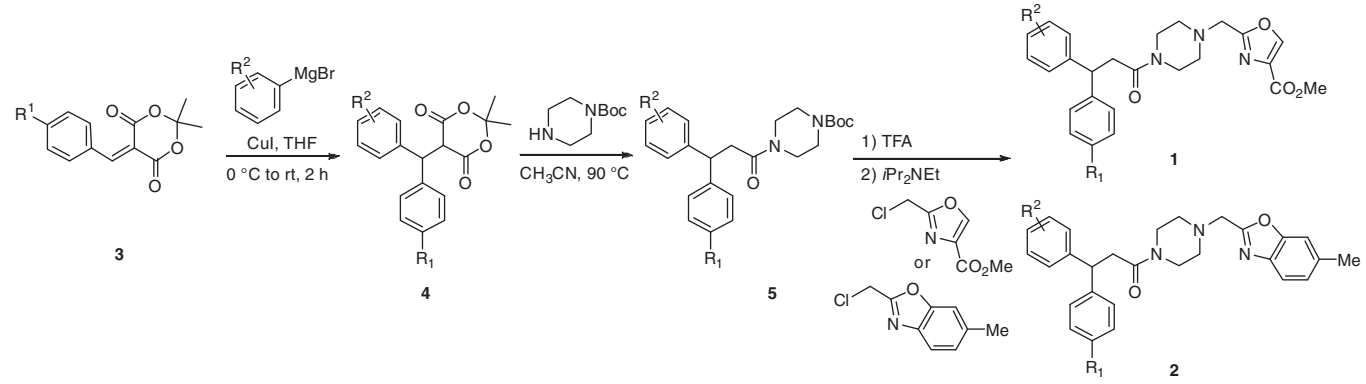


Scheme 1. Synthetic plan toward 3,3-diphenylpropanoylamides.

Inspired by such compounds, together with our compounds reported in the recent literatures,[9–11] we have designed new molecular scaffolds **1** and **2** having a 3,3-diphenylpropanamide and an oxazole or benzoxazole to search for new T-type calcium channel blockers (Fig. 2). Based upon analysis of the common features of those compounds, we hypothesized that the hydrophobic diphenyl-propanoyl group and the piperazine ring probably contribute to T-type calcium channel blocking activity. It is expected that the incorporation of various substituents into the diphenyl moiety of this target molecule might affect its affinity for T-type calcium channel. In addition, we could examine the effect of the functionalized heteroaromatic rings such as oxazole or benzoxazole on the T-type calcium channel blockade.

To achieve the synthesis of a library of target molecules, we need more convenient methods for substituted benzhydryl formation and amide coupling reactions.[12] To this end, we conceived a synthetic plan using Meldrum's acid as the key functional material (Scheme 1).[13] Indeed, alkylidene Meldrum's acids **3** are strong electrophiles, which can readily react with various phenyl cuprates to afford substituted benzhydryl Meldrum's acids **4**. Nucleophilic cleavage of this intermediate by piperazine would give the desired 3,3-diphenylpropanamide **5**. It should be noted that there would be no harmful by-products generated in this amide formation process. Accordingly, the use of Meldrum's acid would provide the final amide compounds in a practical and expedient manner. In this Letter, we report a facile synthesis of the 3,3-diphenylpropanoyl piperazine derivatives **1** and **2** using Meldrum's acid as the coupling component and in vitro biological activities of these compounds against α_{1G} subtype of T-type calcium channels.

Table 1
Synthesis of the 3,3-diarylpropanoylpiperazine derivatives **1** and **2** from alkylidene Meldrum's acid



Entry	Code	R^1	R^2	Yield[a] (%)			
				4	**5**	**1**	**2**
1	**a**	F	H	77	35	74	91
2	**b**	F	4-F	64	63	74	71
3	**c**	F	3-F	59	69	64	73
4	**d**	Cl	H	42	54	70	84
5	**e**	Cl	4-F	91	64	89	87
6	**f**	Cl	3-F	51	54	74	68
7	**g**	Br	H	68	97	84	90
8	**h**	Br	4-F	59	76	71	61
9	**i**	Br	3-F	49	77	62	65
10	**j**	CF_3	H	50	64	69	72
11	**k**	CF_3	4-F	43	54	86	77
12	**l**	CF_3	3-F	27	34	78	65
13	**m**	MeO	H	39	47	81	91
14	**n**	MeO	4-F	71	53	65	55
15	**o**	MeO	3-F	75	54	85	32

[a] Isolated yields.

The synthesis of substituted diphenylmethyl Meldrum's acid is described in Table 1. Following the procedure reported by Fillion,[14] we carried out a Knoevenagel condensation of Meldrum's acid with benzaldehydes under pyrrolidinium acetate catalysis to obtain a series of alkylidene Meldrum's acids **3**. The conjugate addition of substituted phenyl Grignard reagents to **3** in the presence of copper(I) iodide successfully afforded substituted benzhydryl Meldrum's acids **4** in good to excellent yield.[15] In this case, a stoichiometric amount of the arylcuprate reagents is not necessary because the alkylidene Meldrum's acids are electrophilic enough to accept the phenylmagnesium bromide with a catalytic amount of a copper species.

Next, we explored the coupling reactions of compounds **4** with *N*-Boc-piperazine (Table 1). Several examples describe the formation of amides from the reaction between Meldrum's acids and nitrogen-containing nucleophiles.[16,17] Indeed, it is crucial to control the amount of amine to avoid formation of the piperazine salt due to the high acidity of the Meldrum's acid derivatives. Therefore, we attempted several reaction conditions, including thermal aminolysis using excess piperazine (either Boc-protected or unprotected), TFA promoted reaction, and microwave irradiation. Fortunately, we found that treatment of the Meldrum's acid derivatives **4** with one equivalent of *N*-Boc-piperazine in acetonitrile at 90 °C afforded the corresponding amides **5** in 34–97% yields, generating CO_2 and acetone as by-products.[18] It is noted that the reaction conditions are so mild and practical that we could easily isolate the products by simple purification procedures such as solvent evaporation and column chromatography. Having established the optimized conditions for amide formation, we then removed the Boc protecting group. Subsequent alkylation of the corresponding secondary amine with chloromethyl-oxazole[19a] or chloromethyl benzoxazole[19b] produced the final target molecules **1** and **2** in good yield.[20]

With a series of the compounds **1** in hand, we also prepared additional analogues replacing the ester functional group of **1** with alcohol, acid, and amide groups (Scheme 2). Reduction of **1** with $LiBH_4$ generated the corresponding alcohols **6** in low to moderate yields. Saponification of **1** under basic condition gave high yields of the acids **7**, which was subsequently coupled with morpholine to afford the amide derivatives **8**. In the case of $R^1 = CF_3$, $R^2 = 3$-F, the amide **8l** was alternatively obtained from the direct coupling reaction of the ester **1l** with morpholine in the presence of TBD (1,5,7-triazabicyclo[4.4.0]dec-5-ene) at 75 °C.

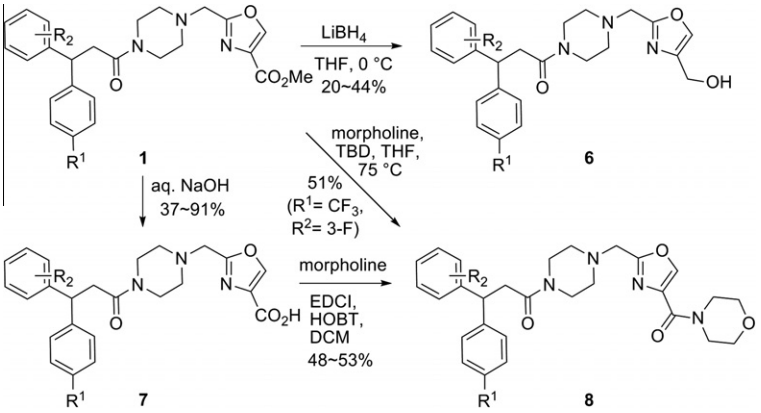
Using the FDSS6000 HTS (high-throughput screening) system,[21] we evaluated the calcium channel inhibitory activity of the 3,3-diphenylpropamide derivatives **1**, **2**, **6**, **7**, and **8** against the T-type calcium channel α_{1G} subtype in HEK293 cells. The in vitro efficacies of the synthesized compounds are summarized in Table 2. In general, the % inhibition values of oxazole esters **1** are relatively higher than those of benzoxazole **2**. However, it seems that the electronic character and position of the R^1 substituents in the diphenyl units have a crucial effect on structure–activity relationship. When $R^1 = F$, the potencies of compound **1a–c** are almost similar to those of **2a–c** (entries 1–3 and 16–18). The oxazole esters **1** are more potent than the corresponding benzoxazoles **2** when the R^1 substituents are other electron-withdrawing groups such as Cl, Br, and CF_3. In the case of electron-donating methoxy group (R^1 = OMe), however, the benzoxazoles **2m–o** exhibit significantly higher %-inhibition compared with the oxazole esters **1m–o** (entries 13–15 and 28–30).

On the other hand, the functional group of oxazole component also plays an important role for the inhibition. In fact, when the substituent on the oxazole moiety was changed into the alcohol or the acid (compounds **6** or **7**), lower inhibitory activity in FDSS assay was observed. This result indicated that the binding site of oxazole substituents in **6** and **7** may occupy a hydrophobic region of T-type calcium channel pores. In addition, morpholine amides **8** exhibited better channel blocking activities in comparison to **6** and **7** even if they were similar to those of the esters **1**.

Table 2
In vitro T-type calcium channel blocking activity of **1**, **2**, and **6–8**

Entry	Compd	% Inhibition[a]	Entry	Compd	% Inhibition[a]
1	**1a**	27.69	24	**2i**	18.02
2	**1b**	33.53	25	**2j**	22.39
3	**1c**	44.54	26	**2k**	9.84
4	**1d**	35.01	27	**2l**	26.42
5	**1e**	45.06	28	**2m**	63.53
6	**1f**	24.53	29	**2n**	23.26
7	**1g**	42.83	30	**2o**	52.02
8	**1h**	42.12	31	**6c**	13.66
9	**1i**	36.60	32	**6f**	19.20
10	**1j**	60.40	33	**6i**	21.32
11	**1k**	51.77	34	**6l**	19.24
12	**1l**	57.65	35	**6o**	4.90
13	**1m**	22.82	36	**7b**	3.84
14	**1n**	24.54	37	**7e**	3.62
15	**1o**	25.47	38	**7h**	5.42
16	**2a**	42.67	39	**7k**	3.62
17	**2b**	30.95	40	**7n**	7.68
18	**2c**	44.93	41	**8c**	36.87
19	**2d**	25.94	42	**8f**	48.63
20	**2e**	15.50	43	**8i**	44.64
21	**2f**	32.24	44	**8l**	53.41
22	**2g**	13.61	45	**8o**	19.69
23	**2h**	11.15	46	Mibefradil	78.92

[a] % Inhibition value was obtained at 10 μM.



Scheme 2. Transformation of **1** to alcohols **6**, acids **7**, and amides **8**.

Table 3
Inhibitory activities of selected compounds against T-type calcium and hERG channels

Entry	Compd	IC_{50}[a] (μM)	hERG[a] (μM)
1	**1e**	8.80 ± 2.20	—[b]
2	**1g**	21.15 ± 2.31	—[b]
3	**1h**	0.86 ± 0.23	2.23 ± 0.90
4	**1j**	17.05 ± 0.93	—[b]
5	**1k**	1.73 ± 0.24	3.83 ± 1.13
6	**2a**	1.29 ± 0.12	2.27 ± 0.63
7	**2m**	2.48 ± 0.13	1.16 ± 0.21
8	Mibefradil	1.34 ± 0.49	1.40 ± 0.29

[a] IC_{50} value (±SD) was obtained from a dose–response curve.
[b] Not available.

Next, we selected several compounds with over 40% inhibition of Ca^{2+} current in FDSS assay and examined the IC_{50} values of them using the whole-cell patch-clamp method.[22] While **2m** exhibited the highest % of inhibition values in the preliminary FDSS assay, **1h** proved to be the best analogue with an IC_{50} value of 0.86 μM. In spite of difference between fluorescence-based assay and electrophysiological assay, our filtering process using FDSS system is necessary because compounds active in both methods could provide more reliable in vitro data to be eventually applied to in vivo experiment. Besides, the preliminary evaluation procedure to filter compounds allowed us to reduce excessive workload for laborious patch-clamp assays.

Additionally, we investigated the IC_{50} values of the selected compounds against hERG channel, which plays an important role as a potential target for cardiac side effects (Table 3). In fact, we found that compound **1h** displayed twofold higher activity for T-type over hERG channel (IC_{50} = 2.23 μM). Compared with IC_{50} values of mibefradil against T-type and hERG channels, **1h** is not only more potent than mibefradil, but also less effective to hERG inhibition. Therefore, compound **1h** will be further investigated as a viable T-type calcium channel blocker.

In summary, we have developed an efficient synthesis of the 3,3-diphenylpropanamides **1** and **2** which exhibit potential T-type calcium channel blocking activity. In particular, the use of Meldrum's acid as a coupling agent provided a practical method that allows the preparation of propanoyl piperazines in good to excellent yield. The in vitro biological evaluation of the title compounds has led to the discovery of compound **1h**, which is comparable to mibefradil in terms of both potency and hERG channel inhibition. These results suggest that the 3,3-diphenylpropanoyl piperazine analogue **1h** will be a potential lead compound to discover an effective T-type calcium channel blocker.

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20. *Experimental procedure for compound* **4h**, **5h**, and **1h**. Compound **4h**: To a solution of the alkylidene Meldrum's acid **3h** (600 mg, 1.93 mmol) and copper iodide (37 mg, 0.193 mmol) in THF (19 ml) cooled to 0 °C was added 4-fluorophenylmagnesium bromide (2.0 M, 1.93 ml, 3.86 mmol). The reaction mixture was stirred for 2 h and then quenched with aq NH_4Cl (20 mL). The solution was neutralized with 1 N HCl (up to pH 7), extracted with CH_2Cl_2 (3 × 25 mL), washed with brine (30 mL) and dried over $MgSO_4$. The solvent was removed under reduced pressure to give a crude oil, which was purified by column chromatography on silica gel (hexane/EtOAc = 4:1) to give diphenylmethyl Meldrum's acid **4h** (462 mg, 59%) as a yellowish oil. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.46–7.41 (m, 2H), 7.27 (m, 2H), 7.17 (d, J = 8.5 Hz, 2H), 7.04–6.97 (m, 2H), 5.34 (d, J = 2.2 Hz, 1H), 4.24 (d, J = 2.6 Hz, 1H), 1.78 (s, 3H), 1.57 (s, 3H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 164.4, 164.3, 162.0 (^1J = 245.3 Hz), 139.0, 135.2 (^4J = 3.3 Hz), 131.6, 131.0 (^3J = 8.1 Hz), 130.8, 121.3, 115.5 (^2J = 21.2 Hz), 105.3, 51.1, 47.6, 28.3, 27.5. Compound **5h**: A solution of **4h** (438 mg, 1.08 mmol) and *tert*-butyl piperazine-1-carboxylate (200 mg, 1.08 mmol) in CH_3CN (11 mL) was stirred at 75 °C for 15 h. The solvent was removed under reduced pressure. The remaining crude oil was purified by column chromatography (hexane/EtOAc = 1:1) on silica gel to afford the 3,3-diphenylpropanoylamide **5h** (404 mg, 76%) as a clear pinkish oil. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.42–7.35 (m, 2H), 7.21–6.91 (m, 6H), 4.63 (t, J = 7.4 Hz, 1H), 3.51–3.25 (m, 8H), 2.99 (d, J = 7.4 Hz, 2H), 1.45 (s, 9H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 169.3, 161.5 (^1J = 244.0 Hz), 154.5, 142.7, 139.2 (^4J = 3.2 Hz), 131.7, 129.5, 129.2 (^3J = 8.0 Hz), 120.5, 115.5 (^2J = 21.1 Hz), 80.4, 45.8, 45.5, 41.6, 38.8, 28.4. HRMS (m/z): [M+H$^+$] calcd for $C_{24}H_{29}BrFN_2O_3$ 491.1346, Found 491.1349. Compound **1h**: To a solution of **5h** (306 mg, 0.623 mmol) in CH_2Cl_2 (6 mL) at 23 °C was added TFA (1.53 mL, 20.6 mmol). The reaction mixture was stirred for 2 h, then the solvent and the remaining TFA were removed under reduced pressure. After being completely dried in a high vacuum, the crude material was dissolved in DMF (3 mL) and treated with diisopropylethylamine (0.54 mL, 3.11 mmol) and methyl 2-(chloromethyl)oxazole-4-carboxylate (109 mg, 0.623 mmol). The mixture was stirred at 75 °C for 15 h. On completion of the reaction (monitored by TLC), the solution was cooled to 75 °C and CH_2Cl_2/water was added into the solution. The organic layer was separated and washed with water and brine, dried over $MgSO_4$, and concentrated. The crude oil was purified by column chromatography (hexane/EtOAc = 1:4) to afford the oxazole derivative **1h** (215 mg, 71%) as a oil. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.21 (s, 1H), 7.37–7.34 (m, 2H), 7.14–7.03 (m, 4H), 6.95–6.89 (m, 2H), 4.57 (t, J = 7.4 Hz, 1H), 3.89 (s, 3H), 3.70 (s, 2H), 3.56–3.53 (m, 2H), 3.38–3.35 (m, 2H), 2.94 (d, J = 7.5 Hz, 2H), 2.42–2.31 (m, 4H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 169.1, 161.55 (^1J = 243.4 Hz), 161.51, 161.46, 144.6, 143.0, 139.3 (^4J = 3.2 Hz), 133.3, 131.7, 129.5, 129.3 (^3J = 7.9 Hz), 120.4, 115.5 (^2J = 21.2 Hz), 54.1, 52.7, 52.4, 52.3, 45.9, 45.5, 41.6, 38.7. HRMS (m/z): [M+H$^+$] Calcd for $C_{25}H_{26}BrFN_3O_4$ 530.1091, Found 530.1095.
21. *Experimental procedure for FDSS6000 assay*. HEK293 cells which stably express both a_{1G} and Kir2.1 subunits were grown in Dulbecco's modified Eagle's medium supplemented with 10% (v/v) fetal bovine serum, penicillin (100 U/mL), streptomycin (100 μg/mL), geneticin (500 μg/mL), and puromycin (1 μg/mL) at 37 °C in a humid atmosphere of 5% CO_2 and 95% air. Cells were seeded into 96-well black wall clear bottom plates at a density of 4 × 10^4 cells/well and were used the next day for high-throughput screening (HTS) FDSS6000 assay. For FDSS6000 assay, cells were incubated for 60 min at room temperature with 5 μM fluo3/AM and 0.001% Pluronic F-127 in a Hepes-buffered solution composed of (in mM): 115 NaCl, 5.4 KCl, 0.8 $MgCl_2$, 1.8 $CaCl_2$, 20 Hepes, and 13.8 glucose (pH 7.4). During the fluorescence-based FDSS6000 assay, a_{1G} T-type Ca^{2+} channels were activated using high concentration of KCl (70 mM) in 10 mM $CaCl_2$ contained Hepes-buffered solution and the increase in $[Ca^{2+}]_i$ by KCl-induced depolarization was detected. During the whole procedure, cells were washed using the BIO-TEK 96-well washer. All data were collected and analyzed using FDSS6000 and related software (Hamamatsu, Japan).

22. *Experimental procedure for patch-clamp (electro-physiological recording).* For the recordings of a_{1G} T-type Ca^{2+} currents, the standard whole-cell patch-clamp method was utilized. Briefly, borosilicate glass electrodes with a resistance of 3–4 MX were pulled and filled with the internal solution contained (in mM): 130 KCl, 11 EGTA, 5 Mg-ATP, and 10 Hepes (pH 7.4). The external solution contained (in mM): 140 NaCl, 2 $CaCl_2$, 10 Hepes, and 10 glucose (pH 7.4). a_{1G} T-type Ca^{2+} currents were evoked every 15 s by a 50 ms depolarizing voltage step from −100 mV to −30 mV. The molar concentrations of test compounds required to produce 50% inhibition of peak currents (IC_{50}) were determined from fitting raw data into dose–response curves. The current recordings were obtained using an EPC-9 amplifier and PULSE/PULSEFIT software program (HEKA, Germany).