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Research paper

Synthesis and T-type calcium channel-blocking effects of aryl(1,5-disubstituted-pyrazol-3-yl)methyl sulfonamides for neuropathic pain treatment

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ABSTRACT

A novel series of aryl(1,5-disubstituted pyrazol-3-yl)methyl sulfonamide derivatives was designed, synthesized, and evaluated for T-type calcium channel (α_{1G} and α_{1H}) inhibitory activity. We identified several compounds, **9a**, **9b**, **9g**, and **9h** that displayed good T-type channel inhibitory potency with low hERG channel and CYP450 inhibition. Among them, **9a** and **9b** exhibited neuropathic pain alleviation effects in mechanical and cold allodynia induced in the SNL rat model. Compounds **9a** and **9b** displayed better efficacy than mibefradil and gabapentin against cold allodynia. In particular, compound **9a** seemed to be valuable as shown fast acting performance in mechanical neuropathic pain model.

1. Introduction

Calcium homeostasis plays an important role in the pathogenesis of neurodegenerative disease, stroke, pain, and epilepsies [1]. Since calcium entry into cells via voltage-activated calcium channels (VACCs) was discovered through experiments on action potential (AP) recordings in crayfish muscle fibers, biophysical and pharmacological studies have identified five different subtypes of high-voltage-activated (HVA) calcium channels – L, N, P, Q, and R – and one low-voltage-activated channel (LVA), the T type [2–4]. The discovery of specific blockers for different calcium channels types is a focus in new drug development for neuronal calcium channel-related diseases because each calcium channel subtypes contributes to the differing physiologies of neuronal cells.

T-type channels have been studied for pain treatment since their discovery and characterization in dorsal root ganglion (DRG) neurons [5], which implicates the T-type channel in sensitization to pain responses (e.g., by enhancing the excitability of nociceptors, as seen in animal models of type 1 and type 2 peripheral diabetic neuropathies (DPN) [6]. T-type knock-out mice were shown to be hyperalgesic to visceral pain, which is likely related to a T-type calcium channel-dependent anti-nociceptive mechanism operating in the thalamus [7]. T-type calcium channels have three different subtypes, $Ca_v3.1$ (α_{1G}), $Ca_v3.2$ (α_{1H}), and $Ca_v3.3$ (α_{1I}), which have different biophysical functions; thus, inhibitors of T-type calcium channels are an emerging therapeutic area for the treatment of pain, especially neuropathic pain [8,9].

Over 26 million patients worldwide suffer from neuropathic pain syndrome. Neuropathic pain is characterized by spontaneous hypersensitive pain responses that can persist long after the original nerve injury has healed. Although current pharmacological treatments for neuropathic pain include combinations of agents such as opioids, tricyclic antidepressants, anti-convulsant agents, and non-steroidal anti-inflammatory (NSAID) analgesics, treatment failure is high with these approaches, only providing ~30–50% alleviation of pain in ~50% of patients [10]. Thus, the effective treatment of neuropathic pain remains a great medical challenge.

Gabapentin (1-(aminomethyl)cyclohexene acetic acid) is considered by physicians to be the gold standard treatment for neuropathic pain, although its effectiveness may be limited in some cases and varies among individuals [11]. Mibefradil, the first selective T-type calcium channel blocker, was developed by

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researchers at Roche and exhibited antihyperalgesic effects in a neuropathic pain spinal-nerve-ligation (SNL) rat model, although it was marketed briefly as an antihypertensive agent, it was withdrawn from market due to a drug-drug interaction concerns [12,13]. Targeting the T-type calcium channel for the treatment of neuropathic pain is attractive pathophysiologically due to the strong connection, both mechanically and pharmacologically, between epilepsy and neuropathic pain, but no selective T-type calcium channel blocker has been developed to date. Clinical agents with T-type channel-blocking activity, such as the antiepileptic drugs zonisamide, ethosuximide, and phenytoin, may provide important clues for the development of potent neuropathic pain drugs. The synthetic peptide ziconotide, an N-type calcium channel blocker, has been used clinically in chronic and severe pain; however, its use is limited due to inconvenient treatment options – such as intrathecal administration – and serious side effects [14]. Thus, the development of orally active T-type calcium channel inhibitors would have advantages for overcoming the difficulties associated with current chronic and neuropathic pain treatments. Zonisamide is a widely used broad-spectrum antiepileptic, which is known to inhibit both Na channels and T-type calcium channels [15]. The percentage of inhibition of the T-type channel current in cultured neurons from rat cerebral cortex is ~60% at 500 μM zonisamide, without inhibition of L-type calcium currents. Zonisamide also inhibits T-type currents in cultured neuroblastoma cells (~40%, 50 μM) [16]. Open-label case studies in the clinic have reported that zonisamide can be an effective treatment for neuropathic pain [17].

Here, we describe the synthesis of a new series of pyrazolyl-connected bezenesulfonamides (**1**) based on structures relevant to known antiepileptics, such as zonisamide, ethosuximide, and phenytoin, and the results of a biological evaluation of T-type calcium channel-blocking activities *in vitro* and neuropathic pain alleviating efficacy *in vivo*.

Zonisamide is composed of a benzoxazolidine core connected to methyl sulfonamide, ethoximide consists of a cyclic diketoamide with methyl and ethyl groups, and phenytoin was constructed with a cyclic urea unit having two phenyl groups. Inspired in these known chemical structures, we designed aryl sulfonamides with a pyrazole ring bearing aromatic phenyl and aliphatic alkyl substituents by combining fragment of these antiepileptics (Fig. 1).

2. Chemistry

Synthesis of the final compounds was carried out according to Scheme 1. A Claisen condensation reaction of commercially

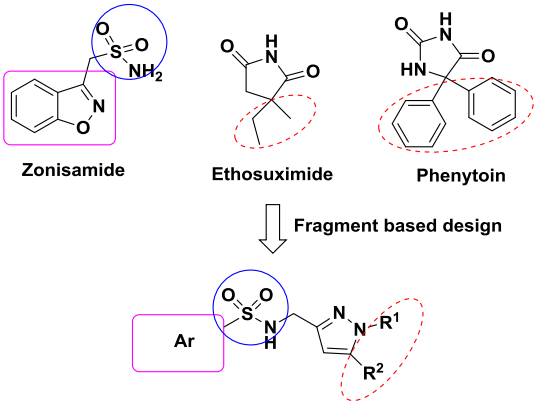


Fig. 1. Structure of antiepileptics with T-type channel blocking activity, and designed molecules.

available diethyloxalate **1** with 4-methyl-2-pentanone or acetophenone yielded a 1,3-diketoester compound bearing isopropyl **2** or phenyl **3**, and ring cyclization with the corresponding hydrazine to give 5-isopropyl-1-phenyl-pyrazolyl-3-ethyl ester **4** or 1-*t*-butyl-5-phenyl-pyrazoyl-3-ethyl ester **5**. The ester functionality of **4** was transformed to aldehyde **6** by reduction with DIBAL-H, followed by reaction with hydroxyl amine to give oxime derivative **7**. The key intermediate 5-isopropyl-1-phenyl-pyrazolyl-3-methylamine **8** was prepared by reduction of oxime derivative 7 with LAH. Sulfonylation of the amine with arylsulfonyl chlorides having various aromatic rings, such as phenyl, mono-substituted phenyl (3-fluoro, 3-chloro, 4-iodo, 4-methyl, 4-*t*-butyl), di-substituted phenyl (2-fluoro-5-methyl, 2-methyl-3-chloro, 2,6-dichloro, 2,6-difluoro), benzyl, phenethyl, naphthyl, and quinoyl rings, gave aryl substituted 3-isopropyl-2-phenyl-pyrazol-5-yl)methyl sulfonamide derivatives **9a**–**9p**.

In an attempt to understand the biological effects of the hydrophobic group according to the substituted position, we synthesized pyrazolyl sulfonamides having a *t*-butyl group instead of a phenyl group on the 2-*N* position, and phenyl or 4-fluoro phenyl moieties on the 3-position, which were converted to the position of the hydrophobic phenyl group. The (1-*t*-butyl-5-phenyl-pyrazol-3-yl)methylamine **10** was prepared from the 1-*t*-butyl-5-phenyl-pyrazoyl-3-ethyl ester **5** through a functional group transformation with similar methods to compound **4**. Next, sulfonylation of amines derivative **10** with phenylsulfonyl chloride yielded 2*N*-*t*-butyl-3-phenylsubatituted pyrazolyl methylsulfonamides **11**.

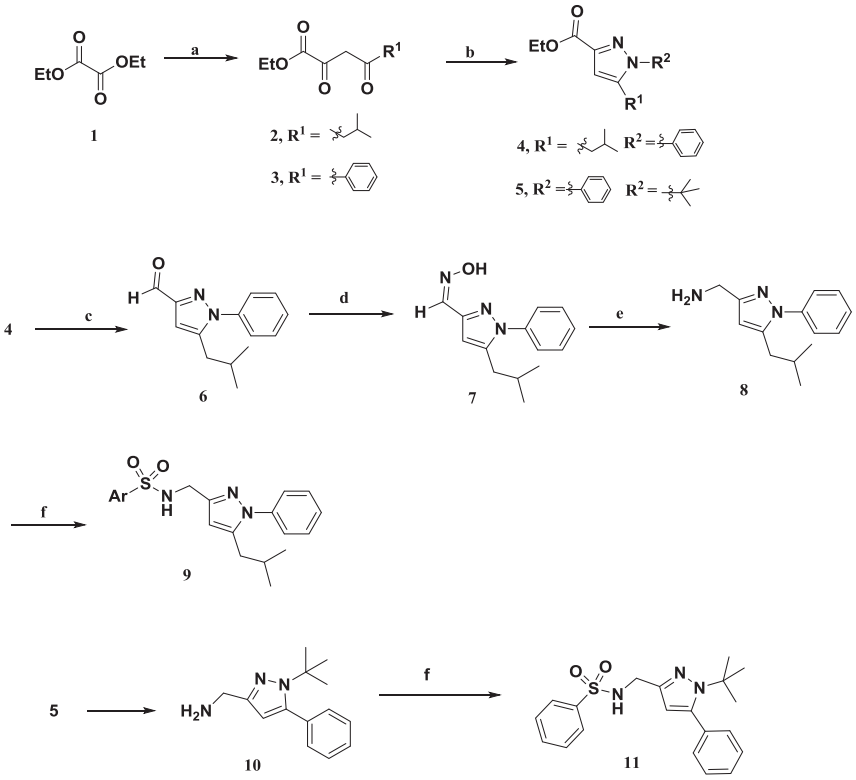
3. Results and discussion

In vitro inhibitory activities of the compounds synthesized were evaluated against T-type calcium channels including α_{1G} or α_{1H} using a fluorescence-based high-throughput screening (HTS) system FDSS6000 assay and whole-cell patch-clamp methods. The results for the (2-*N*-phenyl-3-isopropyl)pyrazolyl-5-methyl substituted arylsulfonamide derivatives **9a**–**9p** and **11** are summarized in Table 1. Most of tested compounds showed similar inhibitory potency against both T-calcium channel subtypes, α_{1G} and α_{1H}. The results indicated that compounds **9g** (R_1 = Bn) and **9h** ($R_1 = (CH_2)_2Ph$) showed the most activity against α_{1G} (70.53%) and α_{1H} (70.53%), respectively.

Variation of the aryl substituent strongly affected the inhibitory activity. The activity increased with methylene unit addition to the phenyl group in R_1. The inhibitory activity order was **9a** (R_1 = Ph, 61.42%) < **9g** ($R_1 = CH_2Ph$, 65.69%) < **9h** ($R_1 = CH_2CH_2Ph$, 70.53%). Except for **9b** (R_1 = 3-FPh, 64.10%), introducing any substituent on the phenyl (**9c**, **9d**, **9e**, **9f**, **9i**, **9j**, **9k**, and **9l**) showed lower activity than the unsubstituted phenyl group, with the aryl groups showing 36–57% inhibition. Moreover, larger aromatic substituents, such as naphthalene (**9m**, **9n**) and quinoline (**9o**), showed lower potency in the range of 19–29%, while the 1-methylnaphthyl-substituted **9p** almost lost activity (7%). Inhibitory activities of compounds **9i**, **9k,** and **9o** were higher against α_{1H} than α_{1G}. Compounds **9a**–**9p,** composed of aromatic R^1 (Ph) and aliphatic R^2 (iso-propyl)-bearing pyrazole groups, and compound **11a** – with a dispositioned aliphatic R^1 (*t*-butyl) and aromatic R^2 (Ph) group – showed lower inhibitory activity than **9a**.

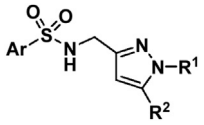
In an attempt to determine the selectivity of other calcium channels and the hERG channel, selected compounds showing >60% in FDSS were evaluated for IC_{50} against two T-type channel subtypes, the N-type channel and the hERG channel (Table 2).

The results indicated that most of the compounds tested showing some inhibitory activity against the N-type calcium and hERG channels. The inhibition of later is known to induce cytotoxicity. Among them, compound **9b** was most active compound for



Scheme 1. Reagents and conditions: (a) R^1COCH_2Cl, NaOEt, EtOH, rt, yield: (b) R^2hydrazine, EtOH, 0 °C to rt, yield 85–87%, (c) DIBAL-H, DCM, −78 °C, yield 86%, (d) hydroxylamine HCl, TEA, DCM, rt, yield 97%; (e) LAH, DCM, 0 °C to rt, 99%, and (f) $ArSO_2Cl$, TEA, DCM, yield 67–91%.

Table 1
Percent inhibition of aryl-*N*-((1-R^1-5-R^2-pyrazol-3-yl)methyl) sulfonamides against the T-type (α_{1G} and α_{1H}) calcium channels.



Compd.	Ar	R^1	R^3	FDSS % inhibition (10 μM)[a]	
				α_{1G}	α_{1H}
9a	Ph	Ph	*iso*-butyl	61.42	63.86
9b	3-FPh	Ph	*iso*-butyl	64.10	62.72
9c	3-ClPh	Ph	*iso*-butyl	57.57	55.74
9d	4-IPh	Ph	*iso*-butyl	46.37	44.92
9e	4-CH_3Ph	Ph	*iso*-butyl	57.26	62.21
9f	4-C$(CH_3)_3$Ph	Ph	*iso*-butyl	46.88	33.42
9g	CH_2Ph	Ph	*iso*-butyl	65.69	70.53
9h	CH_2CH_2Ph	Ph	*iso*-butyl	70.53	68.61
9i	2-F, 5-CH_3Ph	Ph	*iso*-butyl	54.86	70.04
9j	2-CH_3, 4-IPh	Ph	*iso*-butyl	22.65	26.61
9k	2,6-F_2Ph	Ph	*iso*-butyl	45.09	69.31
9l	2,6-Cl_2Ph	Ph	*iso*-butyl	36.93	56.04
9m	1-Naphthyl	Ph	*iso*-butyl	24.32	25.25
9n	2-Naphthyl	Ph	*iso*-butyl	19.77	24.86
9o	8-Quinoyl	Ph	*iso*-butyl	29.78	61.36
9p	CH_2-1-Naphthyl	Ph	*iso*-butyl	7.09	6.66
11a	Ph	*tert*-butyl	Ph	55.47	10.44
11b	Ph	*tert*-butyl	3-FPh	51.55	53.47

[a] % inhibition value was obtained using FDSS6000 fluorescence-based high-throughput screening assay on HEX293 cells.

the T-type calcium channel, showing 3.20 ± 0.62 and 2.08 ± 0.05 half inhibition concentrations against α_{1G} and α_{1H}, respectively, and also showing low inhibition potency against N-type (IC_{50} = 14.5 μM) and hERG (IC_{50} = 21 μM).

Inhibitory activities of selected compounds against human cytochrome P450 were evaluated to assess drug-drug interactions, and microsomal stability was evaluated to compound stability (Table 3). Recently, researchers have reported that CYP2D6 metabolizes 25% of clinically used drugs with significant polymorphism. In particular, several central nervous system and cardiovascular system-related drugs are substantially metabolized by CYP2D6 [18]. CYP3A4 is also involved in the metabolism of over 50% of clinical drugs. Thus, these two subtypes (CYP2D6 and CYP3A4) play more important roles in drug-drug interactions than CYP1A2 and CYP2C9 [19]. The results presented show that most of the tested compound showed no inhibition of CYP2D6, except **9h**, and compounds **9a**, **9b**, and **9g** showed low inhibition of CYP3A4. Thus, **9a**, **9b**, and **9g** would not be expected to cause drug-drug interactions. Unfortunately, the selected compounds have unsatisfactory human hepatic microsomal stability except for compound **9h**.

To identify neuropathic pain relief efficacy of our new T-type calcium channel blocking compounds, **9a**, **9b**, **9g**, and **9h**, we evaluated mechanical and cold allodynia in a rat SNL model (Chung's model) [20].

Behavioral testing for neuropathic pain was performed with rats at 14 days after surgical manipulation [21–23]. We orally administered 100 mg of the selected compound (**9a**, **9b**, **9g**, and **9h**) to each SNL rat and evaluated them in comparison with gabapentin- and mibefradil-administered animals. Gabapentin is used to treat neuropathic pain clinically, and mibefradil was the first T-type calcium channel blocker; here, these agents were used as references.

Table 2
In vitro activities of synthesized compounds **9** and **11** against T-type (α_{1G} and α_{1H}), N-type calcium, and hERG channels.

Compd.	T-type Ca^{2+} channel (IC_{50})[a]		N-type Ca^{2+} channel		hERG channel
	α_{1G} (μM)	α_{1H} (μM)	% inhibition[b]	IC_{50} (μM)[a]	IC_{50} (μM)
9a	5.65 ± 0.50	5.80 ± 0.61	38.21	ND[c]	21.00 ± 2.92
9b	3.20 ± 0.62	2.08 ± 0.05	55.60	14.50 ± 3.87	14.50 ± 3.86
9g	12.71 ± 2.31	9.01 ± 1.46	22.31	ND[c]	6.55 ± 0.55
9h	12.36 ± 0.36	14.60 ± 2.11	42.18	ND[c]	8.73 ± 1.70

[a] IC_{50} values (±SD) were obtained from a dose-response curve using a patch clamp.
[b] % inhibition was obtained using a patch clamp at 10 μM.
[c] Not determined.

Table 3
Cytochrome P450 inhibition and microsomal stability of selected compounds.

Compd.	% Control of CYP-450 (10 μM)[a]				HLM[f] stability
	1A2[b]	2D6[c]	2C9[d]	3A4[e]	Remaining %
9a	36.64	207.58	38.4	69.59	13.88
9b	45.58	204.06	30.4	80.10	1.72
9g	41.9	115.86	34.39	137.81	1.27
9h	NT	45.23	45.23	38.69	88.15

[a] Values represent the remaining % activities and the mean ± SD from triplicate experiments.
[b] α-naphthoflavon.
[c] Quinidine.
[d] Sulfaphenazol.
[e] Ketoconazole.
[f] HLM, human liver metabolic.

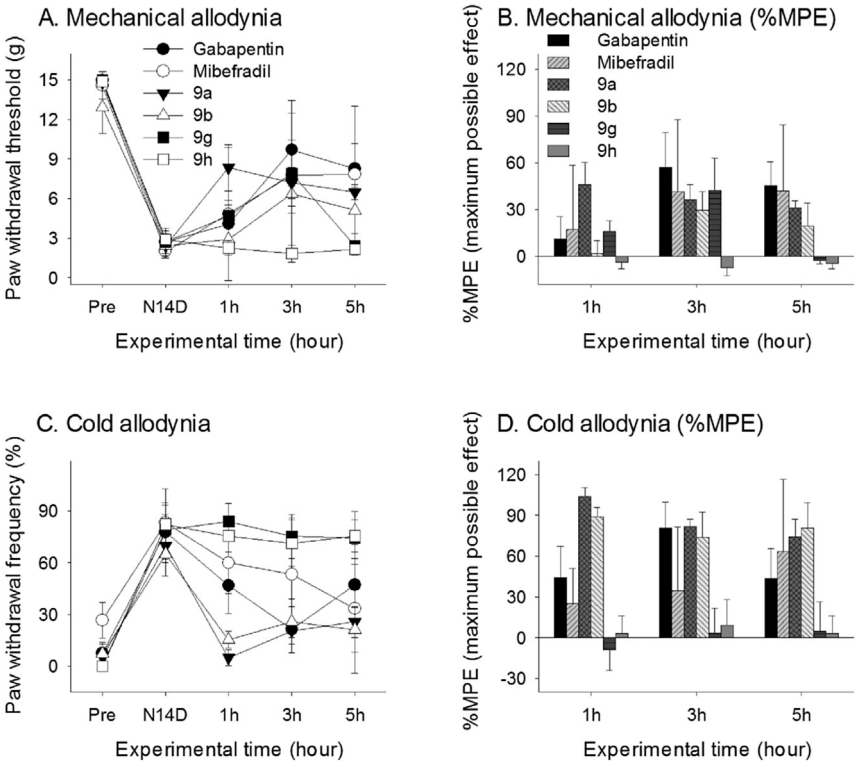


Fig. 2. Effects on mechanical allodynia (A and B) and cold allodynia (C and D) after oral administration of mibefradil (100 mg/kg, $n = 4$), gabapentin (100 mg/kg, $n = 4$), **9a** (100 mg/kg, $n = 4$), **9b** (100 mg/kg, $n = 4$), **9g** (100 mg/kg, $n = 4$), and **9h** (100 mg/kg, $n = 4$) in neuropathic pain-induced rats. Experimental time expressed as days after neuropathic pain injury (N) and hours after gabapentin, mibefradil, **9a**, **9b**, **9g**, or **9h** administration.

The *in vivo* pain relief results, including mechanical and cold allodynia, of our new compounds are summarized in Fig. 2. The result of each (see supplementary material) was represented to one figure.

In mechanical allodynia, the compound **9a** showed significant analgesic effect over the 3-folds higher than those of both gabapentin and mibefradil at 1 h after administration. The efficacy of **9b**, exhibiting the highest *in vitro* T-type calcium inhibitory activity, was increased gradually but the pain relieving efficacy showed lower than reference compounds. The caveats of compound **9b**

Table 4
Pharmacokinetic parameters following intravenous (n = 5) and oral (n = 5) administration (10 mg/kg) of **9b** to male rats.

	9b (mean ± SD[a])	
	iv	Oral
$AUC_{0-\infty}$ (μg min/ml)	120.98 ± 14.48	10.09 ± 3.96
AUC_{last} (μg min/ml)	120.68 ± 24.45	9.82 ± 3.62
Terminal half-life (min)	55.66 ± 4.96	66.80 ± 21.90
C_{max} (μg/ml)	–	0.28 ± 0.20
T_{max} (min)		26 (15–30)[b]
CL (ml/min/kg)	83.77 ± 11.69	–
MRT (min)	57.98 ± 7,19	–
Brain-to-plasma ratio (B/P) at 2 h	1.40	0.19
F (%)	8.3	

Abbreviations: $AUC_{0-\infty}$, total area under the plasma concentration–time curve from time zero to infinity; AUC_{last}, total area under the plasma concentration–time curve from time zero to the previous time; C_{max}, peak plasma concentration; T_{max}, time to reach C_{max}; CL, time-averaged total body clearance; MRT, mean residence time; *F*, bioavailability.
[a] SD: Standard deviation.
[b] Median (range) for T_{max}.

were that it had poor bioavailability ($F = 8.3$) and brain-to-plasma ratios (B/P = 0.19) when administered orally (Table 4). The pain alleviating effect of **9g** was potent at 3 h but lost efficacy rapidly within 5 h. The least active compound **9h** *in vitro* did not showed alleviating effect of mechanical allodynia (Fig. 2. **A** and **B**).

In cold allodynia, compound **9a** and **9b** having high *in vitro* potency, showed higher pain relief efficacy than gabapentin, and compound **9g** and **9h** having low *in vitro* potency exhibited almost no pain reducing effect (Fig. 2. **C** and **D**). Based on these results, we identified that the T-type calcium channel inhibitory effect *in vitro* of aryl(1,5-disubstituted-pyrazol-3-yl)methyl sulfonamides was well correlated on neuropathic pain alleviating efficacy *in vivo*, particularly in cold allodynia. The most potent T-type calcium channel blocking compound **9b** needs to be further optimized to develop a new drug candidate for the treatment of neuropathic pain.

4. Conclusions

A novel series of arylsulfonamide derivatives bearing 1,5-disubstituted pyrazol-3-yl methane were designed, inspired by the chemical motifs of known antiepileptics possessing T-type calcium inhibitory activity. The synthesis and structure activity relationships (SAR) of this series of compounds *in vitro* and in neuropathic pain-alleviating efficacy *in vivo* are reported in the current study. A small unsubstituted phenyl having a longer methylene unit on the Ar substituent displayed a positive effect on *in vitro* inhibitory activity against both α_{1G} and α_{1H} calcium channels. Among the tested compounds, we found that compounds **9a**, **9b**, **9g**, and **9h** exhibited selective inhibition against T-type calcium channels versus N-type, and showed low inhibition of hERG channels. Although three compounds exhibited good CYP450 liability, especially against 2D6 and 3A4, their microsomal stabilities were very low. Nevertheless, among selected compounds, **9a** and **9b** exhibited neuropathic pain alleviation effects in mechanical and cold allodynia induced in the SNL rat model. Compounds **9a** and **9b** displayed better efficacy than mibefradil and gabapentin against cold allodynia. In particular, compound **9a** seemed to be valuable as shown fast acting performance in mechanical pain. Thus, the aryl(1-phenyl-5-isopropyl-pyrazol-3-yl)methyl sulfonamide derivatives appear to be good T-type calcium blockers and could offer safe and effective oral neuropathic pain treatments. Based on these results, we will conduct further optimization studies with this class of compounds to achieve development of new neuropathic pain drug candidates.

5. Experimental section

5.1. Chemistry

5.1.1. General

All reagents and solvents were commercially available and were used without further purification or drying. The reaction progress was monitored on thin-layer chromatography (Merck, silica gel 60F254) and viewed with a UV lamp at 254 and 365 nm. Compounds were purified by flash column chromatography (Merck, 230–400 mesh). ^{1}H and ^{13}C NMR FT-NMR spectra were recorded on a Bruker Advance 300 or 400 MHz spectrometer. Chemical shifts (δ) of protons and carbons are relative to trimethyl silyl (TMS) as an internal standard at 0 ppm, and coupling constants (*J*) are reported in Hz and presented as s (singlet), d (doublet), t (triplet) and (m, multiplet). Mass spectra (MS) were measured with a Q-TOF SYNAPT G2 (Waters MS Technologies, Manchester, UK).

5.1.2. (Z)-Ethyl 3-ethyl-2-hydroxy-6-methyl-4-oxohept-2-enoate (**2**)

2-Methyl-4-heptanone (2.05 mL, 13.0 mmol) was added at −78 °C to a solution of lithium bis(trimethylsilyl)amide (1 M in THF, 13.1 mL, 13.1 mmol) in diethyl ether (52 mL). The solution was stirred for 45 min, and mixed with diethyl oxalate (1.85 mL, 13.7 mmol) for 1 h. Thereafter, the reaction mixture was warmed to room temperature and stirred for an additional 18 h. It was acidified with 1 N HCl, added with water, and extracted with diethyl ether. The organic layer thus obtained was dried over magnesium sulfate, and then concentrated in a water bath (65 °C) only using a rotary evaporator to afford the title compound; ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.35 (s, 1H), 4.46 (q, $J = 7.12$ Hz, 2H), 2.35 (d, $J = 7.16$ Hz, 2H), 2.18 (m, 1H), 1.38 (t, $J = 7.12$Hz, 3H), 0.97 (d, $J = 6.61$ Hz, 6H).

5.1.3. Ethyl(4-ethyl-5-isobutyl-1-phenyl-1H-pyrazole)-3-carboxylate (**4**)

Phenyl hydrazine (1.28 mL, 13.0 mmol) was added to a solution of (Z)-ethyl(3-ethyl-2-hydroxy-6-methyl-4-oxohept-2-eno)ate (2.97 g, 13.0 mmol) in ethanol (40 mL). One hour later, 1 N HCl was added to the solution that was then stirred at 80 °C for 3 h. After removal of the solvent, the reaction mixture was added with water and extracted with dichloromethane. The organic layer was dried over magnesium sulfate, filtered in vacuo, and purified by column chromatography (hexane:EtOAc = 15:1 → 5:1) to afford 607 mg of the title compound. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.46–7.39 (m, 5H), 6.76 (s, 1H), 4.42 (q, $J = 7.12$ Hz, 2H), 2.51 (d, $J = 7.16$ Hz, 2H), 1.87–1.79 (m, 1H), 1.41 (t, $J = 7.12$ Hz, 3H), 0.86 (d, $J = 6.64$ Hz, 6H).

5.1.4. Ethyl 1-(tert-butyl)-5-phenyl-1H-pyrazole-3-carboxylate (**5**)

Diethyloxalate (0.71 ml, 5.25 mmol) was added slowly at 0 °C to a solution of acetophenone (0.59 mL, 5.00 mmol) and NaOEt (2.80 mL, 7.50 mmol) in ethanol (12 mL), and stirred for overnight. It was acidified with 1 N HCl, added with water, and extracted with dichloromethane. The organic layer was dried over magnesium sulfate, and then concentrated in a water bath (65 °C) only using a rotary evaporator to afford ethyl 2,4-dioxo-4-phenylbutanoate (**3**, 552 mg). Without further purification, (Z)-ethyl 2-hydroxy-4-oxo-4-phenylbut-2-enoate (86.3 mg, 0.392 mmol) was slowly added to a solution of *tert*-butyl hydrazine hydrogen chloride (50.1 mg, 0.392 mmol) in ethanol (2.5 mL). The solution was stirred overnight at room temperature. When the reaction was completed as measured by TLC (hexane:EtOAc = 6: 1), the reaction mixture was concentrated in vacuo. After the extraction of the concentrate with dichloromethane and water, the organic layer thus formed was dried over anhydrous sodium sulfate, filtered, concentrated, and purified by column chromatography (hexane:EtOAc = 15: 1 → 6: 1)

to afford the title compound (59.8 mg, 86%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.47–7.36 (m, 5H), 6.71 (s, 1H), 4.43 (q, J = 7.1 Hz, 2H), 1.54 (s, 9H), 1.43 (t, J = 7.1 Hz, 3H).

5.1.5. 4-Ethyl-5-isobutyl-1-phenyl-1H-pyrazole-3-carbaldehyde (**6**)

Dried ethyl 4-ethyl-5-isobutyl-1-phenyl-1*H*-pyrazole-3-carboxylate (454 mg, 1.51 mmol) was dissolved in dichloromethane (2.0 mL), and diisobutylaluminum hydride (1 M in hexane, 4.53 mL, 4.53 mmol) was added at −78 °C to the solution. It was stirred for 1.5 h, and mixed with methanol and 1 N HCl. After the temperature of the reaction mixture was elevated to room temperature, it was added with water and extracted with dichloromethane. The organic layer thus formed was dried over magnesium sulfate, filtered in vacuo, and purified by column chromatography (hexane:EtOAc = 10:1) to afford the title compound (332 mg, 85.8%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.1 (s, 1H), 7.52–7.41 (m, 5H), 6.76 (s, 1H), 2.55 (d, J = 7.17 Hz, 2H), 1.90–1.82 (m, 1H), 0.74 (d, J = 6.64 Hz, 6H).

5.1.6. 4-Ethyl-3-(hydroxyamino)methyl-5-isobutyl-1-phenylpyrazole (**7**)

Triethylamine (165 μL, 1.18 mmol) was added to a solution of hydroxylamine hydrogen chloride (82.5 mg, 1.18 mmol) in dichloromethane (2.5 mL). After stirring for 5 min, the pH of the solution was measured. A solution of dichloromethane in 4-ethyl-5-isobutyl-1-phenyl-1*H*-pyrazole-3-carbaldehyde (276 mg, 1.08 mmol) was added. The resulting reaction mixture was stirred for 5 h, added with water, and extracted with dichloromethane. The organic thus obtained was dried over magnesium sulfate, filtered, concentrated in vacuo, and purified to afford the title compound (284 mg, 97.0%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.27 (s, 1H), 7.49–7.36 (m, 5H), 7.21 (s, 1H), 6.53 (s, 1H), 2.55 (d, J = 7.16 Hz, 2H), 1.88–1.80 (m, 1H), 0.88 (d, J = 6.60 Hz, 6H).

5.1.7. (4-Ethyl-5-isobutyl-1-phenyl-1H-pyrazol-3-yl)methaneamine (**8**)

Dried 4-ethyl-3-(hydroxyamino)methyl-5-isobutyl-1-phenylpyrazole (282 mg, 1.04 mmol) was dissolved in a mixed solvent of diethyl ether (1.4 mL) and tetrahydrofuran (2.7 mL), and lithium aluminium hydride (1 M in diethyl ether, 2.29 mL, 2.29 mmol) was added at -0 °C to the solution. It was stirred for 30 min before for 30 min at room temperature. The temperature was reduced again to 0 °C to carefully add sodium sulfate hydrate to the solution. The reaction mixture was filtered through celite and sodium sulfate, concentrated in vacuo, and dried to afford the title compound (266 mg, 99.3%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.48–7.36 (m, 5H), 6.14 (s, 1H), 3.91 (s, 2H), 2.50 (d, J = 7.12 Hz, 2H), 2.48 (q, J = 7.57 Hz, 2H), 1.88–1.80 (m, 1H), 0.87 (d, J = 6.64 Hz, 6H).

5.1.8. N-[(5-Isobutyl-1-phenyl-1H-pyrazol-3-yl)methyl]benzenesulfonamide (**9a**)

Triethylamine (194 μL, 1.39 mmol) was added at 0 °C to a solution of (5-isobutyl-3-aminomethyl-1-phenyl)pyrazole (290 mg, 1.26 mmol) in dichloromethane (3.0 mL). The solution was stirred for 5 min, and mixed with benzenesulfonyl chloride (170 μL, 1.33 mmol). Subsequently, the solution was warmed to room temperature, and stirred for 1 h. Then, water and a saturated sodium hydrogen carbonate solution were added to the solution before extraction with dichloromethane. The organic layer thus obtained was dried over magnesium sulfate, concentrated in vacuo, and purified by column chromatography (hexane:EtOAc = 2:1) to afford the title compound (419 mg, 85.6%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.86 (d, J = 7.97 Hz, 2H), 7.56–7.37 (m, 6H), 7.25 (d, J = 6.64 Hz, 2H), 6.01 (s, 1H), 5.59 (br, 1H), 4.18 (d, J = 5.96 Hz, 2H), 2.43 (d, J = 7.17 Hz, 2H), 1.77–1.68 (m, 1H), 0.81 (d, J = 6.62 Hz, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 147.9, 144.3, 140.0, 139.5, 132.5, 129.1, 129.0, 128.1127.2, 125.7, 104.9, 41.1, 35.0, 28.3, 22.3. HRMS [ESI$^+$] m/z calcd for $C_{20}H_{23}N_3O_2S$ [M+H]$^+$: 370.1511, found: 370.1600.

5.1.9. N-[(5-Isobutyl-1-phenyl-1H-pyrazol-3-yl)methyl]-3-fluorobenzenesulfonamide (**9b**)

Triethylamine (121 μL, 0.871 mmol) was added at 0 °C to a solution of (5-isobutyl-3-aminomethyl-1-phenyl)pyrazole (182 mg, 0.792 mmol) in dichloromethane (3.0 mL). The solution was stirred for 5 min, and mixed with 3-fluorobenzenesulfonyl chloride (111 μL, 0.832 mmol). Subsequently, the solution was warmed to room temperature, and stirred for 1 h. Then, water and a saturated sodium hydrogen carbonate solution were added to the solution before extraction with dichloromethane. The organic layer thus formed was dried over magnesium sulfate, concentrated in vacuo, and purified by column chromatography (hexane:EtOAc = 3:1 → 2:1) to afford the title compound (262 mg, 85.5%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.64–7.62 (m, 1H), 7.53–7.51 (m, 1H), 7.45–7.35 (m, 4H), 7.28–7.19 (m, 3H), 6.01 (s, 1H), 5.89 (t, J = 5.81 Hz, 1H), 4.18 (d, J = 5.87 Hz, 2H), 2.41 (d, J = 7.19 Hz, 2H), 1.76–1.69 (m, 1H), 0.80 (d, J = 6.61 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 163.5, 161.0, 147.7, 144.4, 142.2, 142.1, 139.5, 130.7, 130.7, 129.1, 128.2, 125.6, 123.0, 123.0, 119.7, 119.5, 114.6, 114.4, 104.9, 41.0, 35.0, 28.3, 22.3. HRMS [ESI$^+$] m/z calcd for $C_{20}H_{22}FN_3O_2S$ [M+H]$^+$: 388.1417, found: 388.1498.

5.1.10. N-[(5-Isobutyl-1-phenyl-1H-pyrazol-3-yl)methyl]-3-chlorobenzenesulfonamide (**9c**)

Triethylamine (122 μL, 0.872 mmol) was added at 0 °C to a solution of (5-isobutyl-3-aminomethyl-1-phenyl)pyrazole (182 mg, 0.792 mmol) in dichloromethane (3.0 mL). The solution was stirred for 5 min, and mixed with 3-chlorobenzenesulfonyl chloride (117 μL, 0.832 mmol). Subsequently, the solution was warmed to room temperature, and stirred for 1 h. Then, water and a saturated sodium hydrogen carbonate solution were added to the solution before extraction with dichloromethane. The organic layer thus formed was dried over magnesium sulfate, concentrated in vacuo, and purified by column chromatography (hexane:EtOAc = 3:1 → 2:1) to afford the title compound (286 mg, 89.3%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.83–7.82 (m, 1H), 7.74–7.71 (m, 1H), 7.50–7.35 (m, 5H), 7.27–7.24 (m, 2H), 6.01 (s, 1H), 5.70 (br, 1H), 4.20 (d, J = 5.86 Hz, 2H), 2.42 (d, J = 7.18 Hz, 2H), 1.77–1.70 (m, 1H), 0.81 (d, J = 6.63 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 147.6, 144.4, 141.8, 139.5, 135.0, 132.5, 130.2, 129.1, 128.2, 127.3, 125.6, 125.3, 104.9, 41.1, 35.0, 28.3, 22.4.

5.1.11. N-[(5-Isobutyl-1-phenyl-1H-pyrazol-3-yl)methyl]-4-iodobenzenesulfonamide (**9d**)

Triethylamine (32.6 μL, 0.234 mmol) was added at 0 °C to a solution of (5-isobutyl-3-aminomethyl-1-phenyl)pyrazole (48.7 mg, 0.212 mmol) in dichloromethane (1.0 mL). The solution was stirred for 5 min, and mixed with a solution of 4-iodobenzenesulfonyl chloride (68.2 mg, 0.223 mmol) in dichloromethane. Subsequently, the solution was warmed to room temperature, and stirred for 1 h. Then, water and a saturated sodium hydrogen carbonate solution were added to the solution before extraction with dichloromethane. The organic layer thus formed was dried over magnesium sulfate, concentrated in vacuo, and purified by column chromatography (hexane:EtOAc = 3:1 → 2:1) to afford the title compound (86.3 mg, 82.0%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.76 (d, J = 8.16 Hz, 2H), 7.53 (d, J = 8.24 Hz, 2H), 7.46–7.38 (m, 3H), 7.23 (d, J = 7.60 Hz, 2H), 5.95 (s, 1H), 5.72 (br, 1H), 4.18 (d, J = 5.80 Hz, 2H), 2.40 (d, J = 7.16 Hz, 2H), 1.75–1.68 (m, 1H), 0.81 (d, J = 6.60 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 147.6, 144.4, 139.9, 139.5, 138.1, 129.2, 128.7, 128.2, 125.7, 104.8, 99.7, 41.0, 35.0, 28.3, 22.4.

*5.1.12. N-[(5-Isobutyl-1-phenyl-1H-pyrazol-3-yl)methyl]-4-methylbenzenesulfonamide (**9e**)*

Triethylamine (38.4 μL, 0.276 mmol) was added at 0 °C to a solution of (5-isobutyl-3-aminomethyl-1-phenyl)pyrazole (57.5 mg, 0.251 mmol) in dichloromethane (1.0 mL). The solution was stirred for 5 min, and mixed with a solution of *p*-toluenesulfonyl chloride (50.5 mg, 0.263 mmol) in dichloromethane. Subsequently, the solution was warmed to room temperature, and stirred for 1 h. Then, water and a saturated sodium hydrogen carbonate solution were added to the solution before extraction with dichloromethane. The organic layer thus formed was dried over magnesium sulfate, concentrated in vacuo, and purified by column chromatography (hexane:EtOAc = 3:1 → 2:1) to afford the title compound (87.7 mg, 91.2%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.74 (d, J = 8.24 Hz, 2H), 7.44–7.37 (m, 3H), 7.27–7.24 (m, 4H), 6.00 (s, 1H), 5.43 (br, 1H), 4.16 (d, J = 5.92 Hz, 2H), 2.42 (d, J = 7.20 Hz, 2H), 2.39 (s, 3H), 1.76–1.69 (m, 1H), 0.81 (d, J = 6.64 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 148.0, 144.3, 143.2, 139.6, 137.0, 129.6, 129.1, 128.1, 127.3, 125.6, 104.9, 41.1, 35.0, 28.3, 22.3, 21.5.

*5.1.13. N-[(5-Isobutyl-1-phenyl-1H-pyrazol-3-yl)methyl]-4-tert-butylbenzenesulfonamide (**9f**)*

Triethylamine (35.2 μL, 0.252 mmol) at 0 °C was added to a solution of (5-isobutyl-3-aminomethyl-1-phenyl)pyrazole (52.6 mg, 0.229 mmol)indichloromethane (1.0 mL). The solution was stirred for 5 min, and mixed with a solution of 4-*tert*-butylbenzenesulfonyl chloride (56.5 mg, 0.241 mmol) in dichloromethane. Subsequently, the solution was warmed to room temperature, and stirred for 1 h. Then, water and a saturated sodium hydrogen carbonate solution were added to the solution before extraction with dichloromethane. The organic layer thus formed was dried over magnesium sulfate, concentrated in vacuo, and purified by column chromatography (hexane:EtOAc = 3:1 → 2:1) to afford the title compound (65.7 mg, 67.3%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.80 (d, J = 8.52 Hz, 2H), 7.49 (d, J = 8.52 Hz, 2H), 7.45–7.37 (m, 3H), 7.28–7.25 (m, 2H), 6.04 (s, 1H), 5.26 (br, 1H), 4.18 (d, J = 5.88 Hz, 2H), 2.43 (d, J = 7.16 Hz, 2H), 1.78–1.70 (m, 1H), 1.33 (s, 9H), 0.82 (d, J = 6.64 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 156.3, 148.0, 144.3, 139.6, 136.8, 129.1, 128.1, 127.1, 126.0, 125.6, 104.9, 41.2, 35.1, 35.1, 31.1, 28.3, 22.4.

*5.1.14. N-[(5-Isobutyl-1-phenyl-1H-pyrazol-3-yl)methyl] benzylsulfonamide (**9g**)*

Triethylamine (32.6 μL, 0.234 mmol) was added at 0 °C to a solution of (5-isobutyl-3-aminomethyl-1-phenyl)pyrazole (48.7 mg, 0.212 mmol) in dichloromethane (1.5 mL). The solution was stirred for 5 min, and mixed with a solution of phenylmethanesulfonyl chloride (43.0 mg, 0.223 mmol) in dichloromethane. Subsequently, the solution was warmed to room temperature, and stirred for 1 h. Then, water and a saturated sodium hydrogen carbonate solution were added to the solution before extraction with dichloromethane. The organic layer thus formed was dried over magnesium sulfate, concentrated in vacuo, and purified by column chromatography (hexane:EtOAc = 3:1 → 2:1) to afford the title compound (64.2 mg, 78.9%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.49–7.45 (m, 2H), 7.42–7.38 (m, 1H), 7.36–7.32 (m, 2H), 7.26 (br, 5H), 6.17 (s, 1H), 4.97 (br, 1H), 4.25 (s, 2H), 4.19 (d, J = 5.88 Hz, 2H), 2.49 (d, J = 7.16 Hz, 2H), 1.84–1.77 (m, 1H), 0.85 (d, J = 6.60 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 148.7, 144.6, 139.7, 130.8, 129.4, 129.2, 128.7, 128.6, 128.2, 125.7, 105.1, 59.1, 41.3, 35.1, 28.4, 22.4. HRMS [ESI$^+$] m/z calcd for $C_{22}H_{25}N_3O_2S$ [M+H]$^+$: 384.1667, found: 384.1742.

*5.1.15. N-[(5-isobutyl-1-phenyl-1H-pyrazol-3-yl)methyl]-2-phenylethanesulfonamide (**9h**)*

Triethylamine (80.9 μL, 0.580 mmol) was added at 0 °C to a solution of (5-isobutyl-3-aminomethyl-1-phenyl)pyrazole (121 mg, 0.528 mmol) in dichloromethane (1.5 mL). The solution was stirred for 5 min, and mixed with a solution of 2-phenylethanesulfonyl chloride (113 mg, 0.553 mmol) in dichloromethane. Subsequently, the solution was warmed to room temperature, and stirred for 1 h. Then, water and a saturated sodium hydrogen carbonate solution were added to the solution before extraction with dichloromethane. The organic layer thus formed was dried over magnesium sulfate, concentrated in vacuo, and purified by column chromatography (hexane:EtOAc = 2:1) to afford the title compound 187 mg (89.3%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.50–7.42 (m, 3H), 7.34–7.24 (m, 5H), 7.13–7.10 (m, 2H), 6.22 (s, 1H), 5.40 (t, J = 5.94 Hz, 1H), 4.38 (d, J = 5.97 Hz, 2H), 3.33–3.27 (m, 2H), 3.13–3.07 (m, 2H), 2.51 (d, J = 7.20 Hz, 2H), 1.89–1.75 (m, 1H), 0.87 (d, J = 6.63 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 148.7, 144.6, 139.6, 138.1, 129.2, 128.7, 128.3, 128.2, 126.8, 125.8, 105.0, 54.2, 40.9, 35.1, 29.8, 28.4, 22.4. HRMS [ESI$^+$] m/z calcd for $C_{22}H_{27}N_3O_2S$ [M+H]$^+$: 398.1824, found: 398.1903.

5.2. Biological evaluation

5.2.1. In vitro evaluation of inhibitory activity against T-type calcium channel

5.2.1.1. High throughput T-type calcium channel inhibitory assay. T-type calcium channels (α1G, α1H) were stably expressed in HEK293 cells [24] and were grown in modified Eagle's medium supplemented with puromycin (1 μg/mL), streptomycin (100 mg/mL), penicillin (100 U/mL), geneticin (500 mg/mL), and 10% (v/v) fetal bovine serum at 37 °C in a humid atmosphere of 95% air and 5% CO_2. Cells were seeded in 96-well black-wall clear-bottom plates at a density of 4 × 10^4 cells/well and were used the next day in a HTS FDSS6000 assay [25]. Cells were incubated for 60 min at room temperature with 5 mM 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, 13.8 glucose (pH 7.4). 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 the FDSS6000 and related software (Hamamatsu, Tokyo, Japan).

5.2.1.2. Electrophysiological recordings (whole-cell patch clamp assay). For recordings of T-type Ca^{2+} currents (α1G, α1H), a standard whole-cell patch-clamp method was used as described previously [26]. T-type Ca^{2+} currents were evoked every 15 s by a 50-ms depolarizing voltage step from −100 mV to −30 mV. The external solution contained (in mM) 140 NaCl, 2 $CaCl_2$, 10 HEPES, and 10 glucose (pH 7.4). The molar concentrations of test compounds required to produce 50% inhibition of peak currents (IC_{50}) were determined by fitting raw data into dose-response curves. The current recordings were obtained using an EPC-9 amplifier and the Pulse/Pulsefit software (HEKA, Elektronik, Lambrecht/Pfalz, Germany).

5.2.2. Evaluation of inhibitory activity of hERG channels

The inhibitory activity of hERG channels by the synthesized compounds was determined using CHO-K1 Tet-On hERG cells purchased from IonGate Biosciences GmbH (Frankfurt, Germany). After cell culture and preparation, an automated patch-clamp device, NPC-16 Patchliner (Nanion Technologies, Munich, Germany) was used for whole-cell recordings. The hERG channel assay was performed as described for the patch-clamp method [26]. Whole-cell recordings were analyzed using the Patchmaster/Fitmaster

(HEKA Elektronik), IGOR Pro (WaveMetrics Inc., Portland, OR, USA), and GraphPad Prism 4 (GraphPad Software, Inc., La Jolla, CA, USA) software.

5.2.3. Measurement of CYP450 inhibition

Inhibition of human CYP450 enzyme activities, including CYP1A2, 2D6, 2C9, and 3A4, was measured using the Vivid CYP450 kit (Invitrogen, Madison, WI, USA) according to the manufacturer's instructions. Briefly, the test compounds were diluted with water in a 96-well plate and the Master Pre-Mix (450 BACULOSOMES Regent and Regeneration system) were added. After incubating of the mixture for 15 min at 37 °C, the reaction was initiated by adding the Vivid CYP450 substrates and NADPH buffer. A fluorescence plate reader was used to measure the remaining enzyme activity. Positive controls were prepared by diluting solutions to 10 mM.

5.2.4. Metabolic stability in human liver microsomes

A metabolic stability assay of selected compound was performed by incubating human liver microsomes (HLM, UltraPool HLM 250 Mixed Gender Pooled Donor, Corning Gentest Co., Woburn, MA, USA) in the presence of NADPH (#44332000, Oriental Yeast Co., Tokyo, Japan) as the cofactor at 37 °C, as described previously. Briefly, human liver microsomes were incubated with a 1 μM solution of the test compounds in potassium phosphate buffer (PPB) in the presence of the cofactor NADPH (1.2 mM) at 37 °C. The reaction was terminated by addition of cold acetonitrile containing 0.1 μg of internal standard after a 30-min incubation. After removing precipitated protein by centrifugation, the supernatants were analyzed by LC-MS/MS.

5.2.5. In vivo efficacy test of neuropathic pain in SNL model

For the *in vivo* efficacy test for neuropathic pain in a spinal nerve injury rat model, two behavioral tests (mechanical allodynia and cold allodynia) were performed by previously described methods [21]. Briefly, 2 weeks after the nerve surgery, the rats underwent postoperative behavioral tests and were treated orally with 100 mg/kg of compounds **9a**, **9b**, **9g**, **9h**, mibefradil, or gabapentin. Rats were adapted to the transparent plastic box, and given freedom of movement with a wire mesh floor to permit access to the plantar surface of the hind paw. After administration, the tests were re-evaluated at 1 h, 3 h, and 5 h. For mechanical allodynia tests [22], a von Frey filament (Stoelting, Wood Dale, IL, USA) was touched five times during every 3–4 s on the hind paw. For cold allodynia, the rat was placed under a transparent plastic box on a metal mesh floor and acetone was applied to the plantar surface of the hind paw. To quantify cold allodynia sensitivity of the paw, brisk withdrawal according to five applications of acetone at 2-min intervals was measured. The frequency of paw withdrawal was expressed as a percentage [(no. of trials accompanied by brisk foot withdrawal/total no. of trials] × 100].

The results of behavioral tests, including both mechanical and cold allodynia, are expressed as % MPE. 100% MPE values near 100 indicate normal thresholds, whereas values near 0 indicate allodynia.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2016.07.032.

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