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Synthesis and evaluation of α,α′-disubstituted phenylacetate derivatives for T-type calcium channel blockers

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ABSTRACT

We have synthesized and evaluated α,α′-disubstituted phenylacetate derivatives that were designed as T-type calcium channel blockers. Among them, compound **10e** (IC_{50} = 8.17 ± 0.48 nM) showed the most potent T-type calcium current blocking activity and higher potency than Mibefradil (IC_{50} = 1.34 ± 0.49 μM). The PK profile and subtype selectivity over L-type calcium channel were satisfied for further animal assay using disease model.

Voltage-dependent calcium channels have crucial roles in translating electrical signals into biochemical events such as enzyme activity, neurotransmitter release, neuronal excitability, neurite outgrowth, and gene transcription.[1] They are subdivided into two major classes, high-voltage activated (HVA or L-type) and low-voltage activated (LVA or T-type) calcium channels based on their biophysical and pharmacological properties. Mibefradil (Posicor®, Hoffman-La Roche) which was launched in 1998 for the treatment of hypertension and angina pectoris,[2–4] blocks T-type calcium channels at concentration lower than that needed to block L-type calcium channels.[5,6] Unfortunately, the drug was withdrawn from the market due to drug–drug interaction with antihistamine such as astemizole, but this side effect is not related to T-type calcium channel blockade.[7,8] T-type calcium channels are involved in cardiac pacemaking,[9] regulation of vascular tone, and secretion of various types of hormones.[10–13] Thus, T-type calcium channel is now considered to be a novel therapeutic target for the treatment of cardiovascular, neuronal, and endocrine systems.[14] Currently several compounds including Mibefradil analogues[15] have been reported to inhibit T-type calcium channels; however, none of them have shown high potency and selectivity to this channels. In this study, we designed and synthesized compounds with potency and selectivity to T-type calcium channel using the 3D ligand based pharmacophore model, which was generated by hypothesis approach (HipHop) implemented in CATALYST program[16,17] (Fig. 1). As a result of in vitro inhibition assay, most of compounds showed higher inhibition activities than Mibefradil. Especially, compound **10e** showed the most potent T-type calcium channel blocking activity and good selectivity over L-type calcium channel.

10a–g and **11a–j** were synthesized via a routine procedure shown in Schemes 1 and 2. Aminobenzimidazole intermediates **5a–c** were prepared from 4-methylaminobutyric acid **1**.[18] Protection of the secondary amine with benzyloxycarbonyl (Cbz) afforded compound **2**, which was then treated with isobutyl chloroformate and *o*-phenylenediamines to provide compounds **3a–c** in 59–94% yields. They were treated with *p*-toluenesulfonic acid in toluene under reflux condition to give cyclized benzimidazoles **4a–c** in 33–77% yields. Deprotection of Cbz group from the secondary amines with 10% Pd/C gave compounds **5a–c** in 69–95% yields.

The methods for the preparation of the title compounds were shown in Scheme 2. Starting from phenylacetic acids **6a–c**, the carboxylic acids were esterified to make esters **7a–c** in 90–95% yields, which were alkylated with *t*-BuOK and isopropyl bromide to give compounds **8a–c**. Compounds **9a–c** were obtained via treating **8a–c** with LDA and dibromopropane in THF under anhydrous condition. The coupling reaction of **9a–c** with previously prepared benzimidazole derivatives **5a–c** and commercially available piperazine derivatives under basic condition (K_2CO_3, EtOH) produced compounds **10a–g** in 20–46% yields and compounds **11a–j** in 47–69% yields, respectively.

In vitro calcium channel blocking activities of **10a–g** and **11a–j** were tested with T-type calcium channels expressed in HEK293 cells (α_{1G}). All the compounds exhibited promising activities on α_{1G} calcium channels expressed in HEK293 cells at 10 μM concentration by the whole-cell patch-clamp method in a preliminary assay.[19,20] Then the molar concentrations needed to produce 50%

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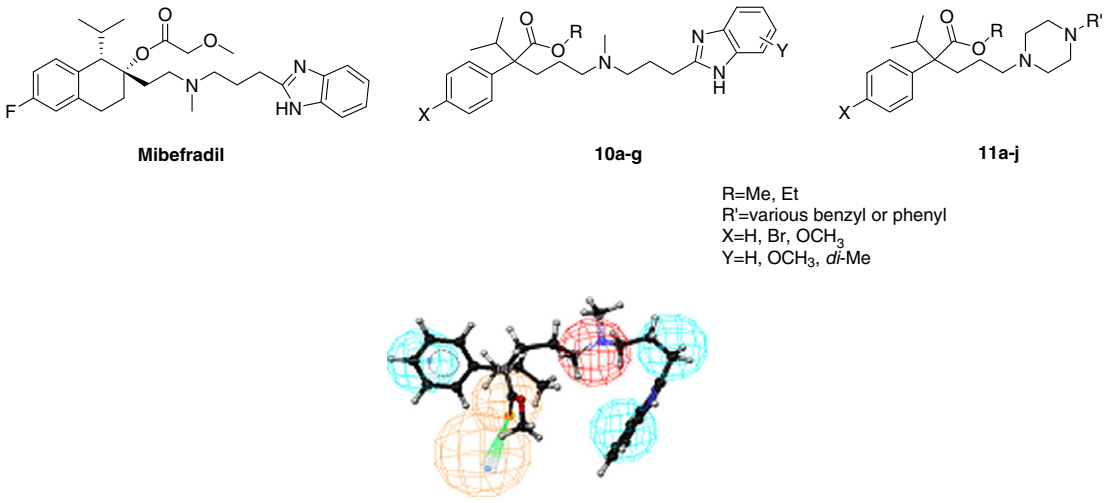
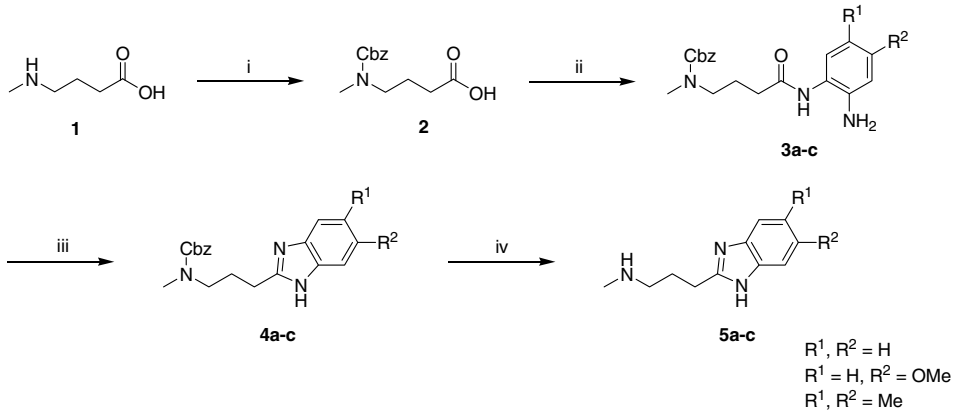
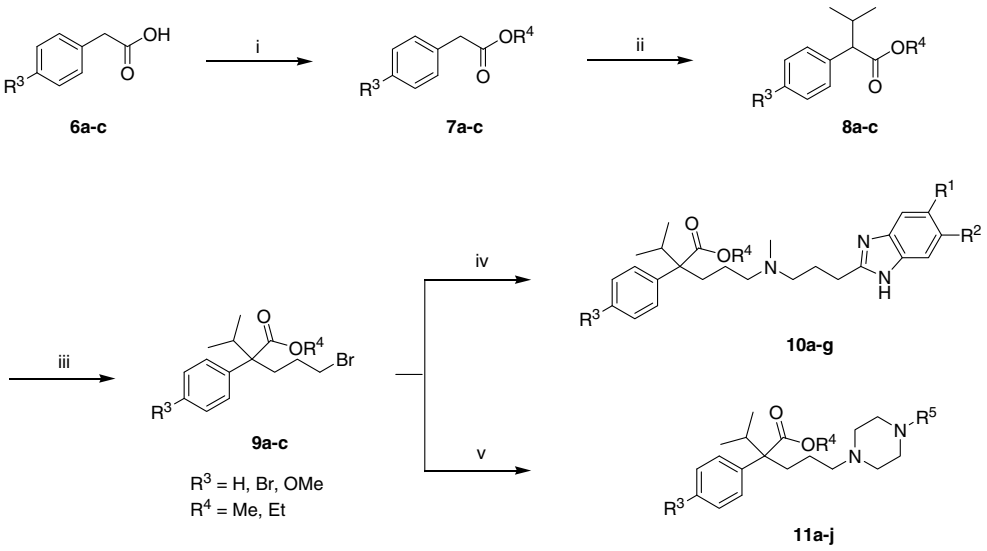


Figure 1. Designed structures for T-type calcium channel blocker and mapping result of suggested pharmacophore with **10a**.



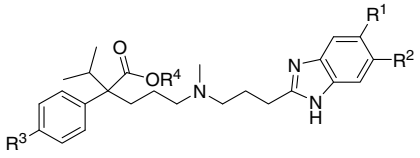
Scheme 1. Reagents and condition: (i) benzyl chloroformate, NaOH, EtOH/H_2O (1:1), 0 °C to rt, 63%; (ii) isobutyl chloroformate, *o*-phenylenediamines, Et_3N, THF, −15 °C to rt; (iii) *p*-TsOH, toluene, reflux; (iv) H_2, 10% Pd/C, MeOH, rt.



Scheme 2. Reagents and condition: (i) H_2SO_4, MeOH, reflux; (ii) isopropyl bromide, *t*-BuOK, DMF, 0 °C to rt; (iii) LDA, 1,3-dibromopropane, THF, −78 °C to rt; (iv) compound **5**, K_2CO_3, EtOH, reflux; (v) 1-substituted piperazine derivatives, Et_3N, NaI, CH_3CN, reflux.

inhibition of peak currents (IC_{50}) were measured and summarized in Tables 1 and 2 with Mibefradil as a positive control for comparison. According to the assay results, most of derivatives exhibited significant inhibitory activities on HEK293 cells and numbers of

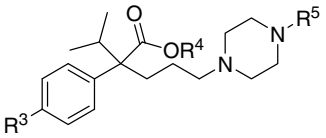
Table 1
In vitro calcium channel blocking effects of benzimidazole derivatives



10a-g

Compound	R^1	R^2	R^3	R^4	Patch-clamp HEK293 cell (T-type α_{1G}, $n = 3$)[21]	
					% Inhibition (10 μM)	IC_{50} (μM)
10a	H	H	H	Et	98.3 ± 0.9	0.25 ± 0.005
10b	Me	Me	H	Et	91.4 ± 0.4	0.10 ± 0.01
10c	H	H	OMe	Me	93.5 ± 3.2	0.19 ± 0.001
10d	OMe	H	OMe	Me	91.7 ± 1.9	0.88 ± 0.07
10e	H	H	Br	Me	96.9 ± 0.5	8.17 ± 0.48 (nM)
10f	OMe	H	Br	Me	94.5 ± 1.2	0.25 ± 0.01
10g	Me	Me	Br	Me	94.2 ± 0.4 (1 μM)	53.02 ± 4.87 (nM)
Mibefradil					95.9 ± 1.7	1.34 ± 0.49

Table 2
In vitro calcium channel blocking effects of piperazine derivatives



11a-j

Compound	R^3	R^4	R^5	Patch-clamp HEK293 cell (T-type α_{1G}, $n = 3$)[21]	
				% Inhibition (10 μM)	IC_{50} (μM)
11a	H	Et	4-Methoxybenzyl	96.3 ± 1.7	0.34 ± 0.02
11b	H	Et	4-Fluorobenzyl	94.0 ± 2.5	0.26 ± 0.03
11c	Br	Me	2-Fluorobenzyl	90.4 ± 3.2	0.74 ± 0.02
11d	Br	Me	3-Fluorobenzyl	89.3 ± 1.6	0.98 ± 0.11
11e	Br	Me	4-Fluorobenzyl	96.5 ± 1.7	1.11 ± 0.05
11f	Br	Me	3-Trifluorobenzyl	93.0 ± 1.5	0.28 ± 0.02
11g	Br	Me	2-Methoxyphenyl	94.1 ± 1.5	95.04 ± 14.78 (nM)
11h	Br	Me	4-Methoxybenzyl	96.0 ± 1.7	0.48 ± 0.08
11i	Br	Me	2,3,4-Trimethoxybenzyl	95.1 ± 1.9	0.32 ± 0.01
11j	Br	Me	3-Methylbenzyl	94.9 ± 1.5	0.39 ± 0.03
Mibefradil				95.9 ± 1.7	1.34 ± 0.49

compounds showed excellent blocking efficacy. Especially, compounds **10e**, **10g**, and **11g** had 14- to 160-fold higher potency than Mibefradil.

Among them, **10e**[22] exhibited the most potent T-type calcium current blocking activity (8.17 ± 0.48 nM). Thus, further evaluations of **10e** such as hERG inhibition, pharmacokinetic profile assay, and selectivity test over L-type calcium channel were carried out. According to the hERG inhibition assay, IC_{50} of **10e** was 178 nM (Table 3).[23] Although the absolute IC_{50} value of hERG inhibition was low, the relative ratio over two channels was moderately high (hERG IC_{50}/T-type α_{1G} IC_{50} = 21.8). Moreover, % inhibition value against L-type calcium channel at 1 μM of **10e** was 0%. It means that **10e** could distinguish T-type and L-type calcium channel effectively and block T-type calcium channel selectively (T-type/L-type > 1000). In order to examine in vivo dynamics, **10e** was subjected to pharmacokinetics analysis in mouse. **10e** was administered as single oral dose (50 mg/kg) and evaluated in the plasma and brain. Based on the pharmacokinetic data, compound **10e** exhibited competitive pharmacokinetic profiles in plasma. It is inferred that **10e** is metabolically stable in liver enzymes ($T_{1/2}$ = 1 h) and has comparable absorption in gastrointes-

Table 3
Selectivity data of compound **10e** against hERG and L-type calcium channel

Compound	T-type IC_{50} (nM)	hERG IC_{50} (μM)	% Inhibition of L-type at 1 μM
10e	8.17	0.178	0

Table 4
Plasma and brain pharmacokinetic parameters in mouse obtained via a single oral dose

	10e		
	Brain	Plasma	B/P[a]
Dose[b] (mg/kg)	50	50	
T_{max} (h)	1.0	1.0	
C_{max} (ng/ml)	274	1615	0.17
AUC_{0-t}[c] (h ng/ml)	973	3902	0.25

[a] $B/P = AUC_{brain}/AUC_{plasma}$.
[b] The vehicle of PK experiment: DMA/Cremophor EL/PBS (10/10/80 v/v %).
[c] Area under curve (AUC) values were calculated by linear trapezoidal rule.

tinal system. Also, **10e** could penetrate BBB and reach to brain in 25% ratio compared to plasma (Table 4).

In summary, **10a–g** and **11a–j** were designed and synthesized based on pharmacophore mapping study and most of compounds showed higher T-type calcium channel inhibition activities than Mibefradil. Among them, compound **10e** exhibited the most potent T-type calcium current blocking activity and good pharmacokinetic profiles. Furthermore, **10e** showed excellent selectivity over L-type calcium channel. Therefore, the series of title compounds were suggested to elucidate the potential of new therapeutics for T-type calcium channel associated disease such as hypertension, neuropathic pain, and migraine based on the fundamental etiology.

Acknowledgments

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21. Experimental procedure for patch-clamp (electrophysiological recording). For the recordings of α_{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 MΩ 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). α_{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).
22. Compound **10e**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.45 (br s, 2H), 7.39 (d, J = 8.6 Hz, 2H), 7.18 (dd, J = 6.0, 3.1 Hz, 2H), 7.10 (d, J = 8.6 Hz, 2H), 3.69 (s, 3H), 3.04 (t, J = 6.4 Hz, 2H), 2.48 (t, J = 5.8 Hz, 2H), 2.35–2.40 (m, 3H), 2.21 (s, 3H), 2.04–2.10 (m, 1H), 1.89–1.98 (m, 3H), 1.23–1.43 (m, 2H), 0.85 (d, J = 6.8 Hz, 3H), 0.74 (d, J = 6.8 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 175.0, 155.4, 138.6, 130.7, 130.1, 121.7, 120.5, 59.0, 58.4, 58.2, 51.7, 41.5, 34.6, 33.9, 28.9, 24.2, 22.7, 18.9, 18.0.; Anal. ($C_{26}H_{34}BrN_3O_2$2HCl) Calcd: C, 54.46; H, 6.33; N, 7.33. Found: C, 54.16; H, 6.23; N, 7.61.
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