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Successful reduction of off-target hERG toxicity by structural modification of a T-type calcium channel blocker

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ABSTRACT

To obtain an optimized T-type calcium channel blocker with reduced off-target hERG toxicity, we modified the structure of the original compound by introducing a zwitterion and reducing the basicity of the nitrogen. Among the structurally modified compounds we designed, compounds **5** and **6**, which incorporate amides in place of the original compound's amines, most appreciably alleviated hERG toxicity while maintaining T-type calcium channel blocking activity. Notably, the benzimidazole amide **5** selectively blocked T-type calcium channels without inhibiting hERG (hERG/T-type $\geqslant$ 220) and L-type channels (L-type/T-type = 96), and exhibited an excellent pharmacokinetic profile in rats.

Voltage-gated calcium channels are important regulators of calcium ion influx upon membrane potential depolarization in a number of cell types. This property translates electrical signals on the membrane surface into an intracellular chemical signal; the rise in cytosolic calcium concentration triggers cellular processes such as muscle contraction, hormone secretion, and gene transcription.[1–3] Voltage-dependent calcium channels are divided into high voltage-activated (HVA) and low voltage-activated (LVA) calcium channels by activation voltage range.[4] HVA calcium channels are classified into L-type and non-L-type (N-, P-, Q-, R-), and LVA calcium channels are also called T-type calcium channels. T-type calcium channels are expressed throughout the body, including in nervous tissue, heart, kidney, smooth muscle, and many endocrine organs.[5] Moreover, T-type calcium channels' properties differ from those of L-type channels: T-type channels have a more negative range of activation and inactivation and faster gating kinetics, and are resistant to standard L-type calcium channel blockers such as dihydropyridines, diltiazem and verapamil. Thus, T-type calcium channels are now anticipated to be novel targets for the treatment of various cardiovascular disorders such as heart failure, arrhythmia, hypertension, and neurological disorders such as epilepsy and pain.[6]

In a previous paper,[7] we identified α,α′-disubstituted phenylacetate derivatives **1** and **2** as T-type calcium channel blockers (Fig. 1). Compound **1**, which has a benzimidazole moiety in its side chain, seems to be one of the most potent and selective small molecule T-type calcium channel blockers ever found. However, the absolute hERG IC_{50} value of compound **1** was too low for development as a drug, though the ratio of T-type to hERG IC_{50} was moderately high (hERG IC_{50}/T-type IC_{50} = 21.8). In recent years, blockade of the hERG channel has arisen as a significant hurdle for drug discovery, as it can lead to a heart rhythm disorder known as long QT interval syndrome.[8] Even some commercial drugs such as terfenadine, sertindole, and grepafloxacine have been withdrawn from market because of hERG inhibition.

To identify compounds with decreased off-target effects against hERG channels, we structurally modified the original compounds **1** and **2**. A number of simple structural modifications to mitigate hERG channel activity have been reported including controlling lipophilicity,[9] reducing pKa,[10] and introducing zwitterions.[11] In this paper we reduce undesirable hERG toxicity in T-type calcium channel blockers by structurally modifying **1** and **2** to allow formation of zwitterions and reduce basicity of the nitrogen.

First, we attempted to make our inhibitors zwitterionic to reduce hERG inhibition, a widely accepted approach. Zwitterions appear to have lower hERG-blocking activity because of poor cell permeability and/or conformational changes relative to parent compounds.[12] Since compounds **1** and **2** possess amine and ester groups, which are acid derivatives, we can easily obtain zwitterions **3** and **4** by simple ester hydrolysis of compounds **1** and **2**, respectively (Scheme 1).

An increasing number of publications have showed that potency of hERG blockade is associated with basic nitrogens capable of protonation at physiological pH.[13] Therefore, lowering the basicity of the nitrogen should reduce unwanted hERG activity. With

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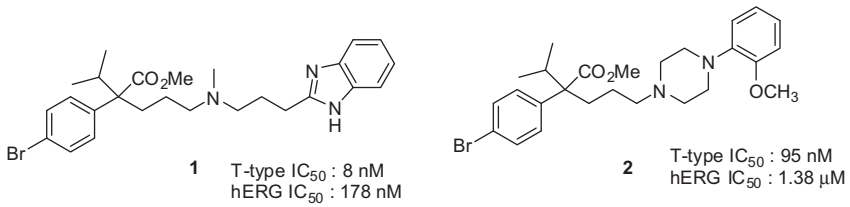
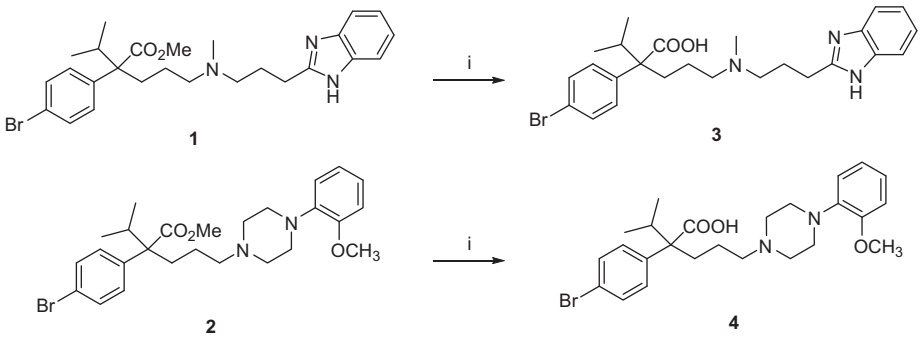


Figure 1. Profiles of α,α′-disubstituted phenylacetate derivatives **1** and **2**.



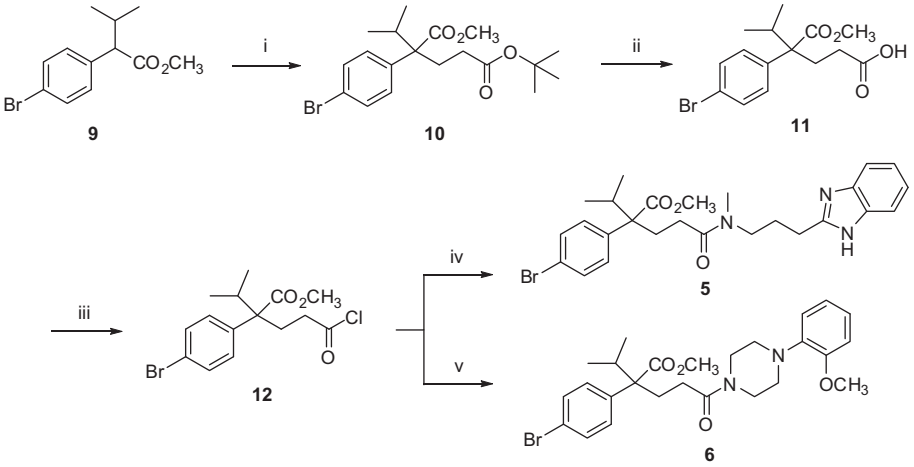
Scheme 1. Reagents and conditions: (i) (a) 1 N NaOH, MeOH/H_2O (1:1), 60 °C, 2 h; (b) 1 N HCl, 88% (**3**), 82% (**4**).

this concept in mind, our next efforts focused on diminishing nitrogen basicity by amide formation and nitrogen atom deletion.

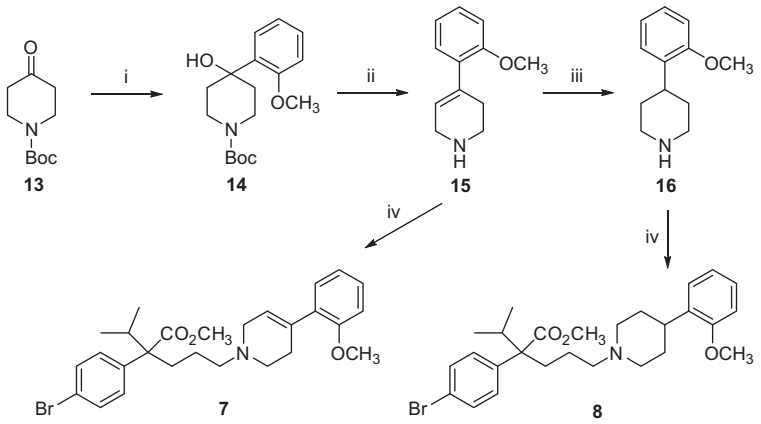
Structural modification of amines **1** and **2** to amides **5** and **6** is described in Scheme 2. To introduce acid functionalities, which are easily transformed to amides, into **1** and **2**, a Michael reaction was used to obtain diester **10**; specifically, **9**[7] was treated with *t*-butyl acrylate, *t*-BuOK, and 18-crown-6 in THF. Chemoselective hydrolysis of *t*-butyl ester **10** with TFA gave mono-acid **11**, which was converted to acid chloride **12** by reaction with SO_2Cl_2. Coupling of **12** with the amine functions of **1** and **2** afforded the corresponding amides **5** and **6** in good yield, respectively.

As another approach to reduce nitrogen basicity, we synthesized compounds **7** and **8**, which lack the nitrogen in the piperazine ring of **2** (Scheme 3). To obtain 4-methoxyphenylpiperidine, which lacks the nitrogen at the N-4 position of 4-methoxyphenylpiperazine, we started from commercially available Boc-protected 4-piperidone **13**. A Grignard reaction of **13** with 2-methoxyphenylmagnesium bromide gave tertiary alcohol **14**. When compound **14** was treated with trifluoroacetic acid, concomitant alcohol dehydration and Boc deprotection reactions took place to afford **15**. Hydrogenation of **15** with Pd/C under hydrogen atmosphere in EtOH provided saturated compound **16**. Alkylation of **15** and **16** with bromopropylphenylacetate afforded nitrogen-deleted compounds **7** and **8**, respectively.

We evaluated the abilities of synthetic compounds **3**–**8**, structurally modified according to our strategy described above (Table 1), to block T-type and hERG channels.[7] As shown in Table 1, zwitterionic compounds **3** and **4** displayed significantly lower off-target hERG activity than the original compounds, but also had considerably reduced T-type calcium channel blocking activity. These compounds also had limited solubility at physiological pH. Therefore, replacement of the ester to acid to confer zwitterionic properties was not a satisfactory strategy for our purposes. On the other hand, notable success was achieved in the case of amide modification. Amide compounds **5** and **6** blocked hERG channels only at high concentrations, with IC_{50} values of over 10 μM, which



Scheme 2. Reagents and conditions: (i) *t*-butyl acrylate, *t*-BuO^-K^+, 18-crown-6, THF, 0 °C–rt, 24 h, 68% (recycle); (ii) CF_3CO_2H/CH_2Cl_2 (1:1), rt, 1 h, 94%; (iii) SO_2Cl_2, 60 °C, 2 h, 100%; (iv) [3-(1H-benzimidazol-2-yl)propyl]methylamine, Et_3N, CH_2Cl_2, 0 °C, 2 h, 75%; (v) 4-(2-methoxyphenyl)piperazine, Et_3N, CH_2Cl_2, 0 °C, 2 h, 88%.



Scheme 3. Reagents and conditions: (i) 2-methoxyphenylmagnesium bromide, THF, 0 °C, 2 h, 52%; (ii) CF_3CO_2H/CH_2Cl_2 (1:1), rt to reflux, 3 h, 72%; (iii) Pd/C (10%), H_2, EtOH, 5 h, rt, 100%; (iv) methyl isopropyl-(3-bromopropyl)-4-bromophenylacetate, Et_3N, NaI, CH_3CN, reflux, 62% (**7**), 55% (**8**).

Table 1
T-type and hERG channel blocking activities and clog *P* values of compounds **1–8**

Compd	T-type IC_{50} (α_{1G}, nM)	hERG IC_{50} (μM)	clog *P*
3	1360	2.34	3.16
4	3260	3.96	2.86
5	56	12.5	5.40
6	654	16.8	4.77
7	3240	1.26	6.76
8	2650	0.78	6.46
1	8.17	0.178	5.84
2	95	1.38	5.70

is stable enough to proceed with drug development. Although the T-type calcium channel blocking activities of **5** and **6** were somewhat inferior to those of the original compounds **1** and **2**, their activities were still potent enough for use as T-type calcium channel inhibitors. Particularly, amide **5**,[14] which includes a benzimidazole moiety, was highly selective between T-type and hERG channels (hERG IC_{50}/T-type $IC_{50} \geqslant 220$). Piperazine amide **6** displayed the most appreciably mitigated hERG off-target toxicity among the modified compounds **3–8**, and effectively inhibited T-type calcium channels. The lack of inhibition of hERG channels by amides **5** and **6** seems to result from conformational and lipophilic differences from parent compounds **1** and **2**. Nitrogen-deleted compounds **7** and **8** yielded disappointing results; both demonstrated seriously weakened blocking of T-type calcium channels and greater off-target hERG toxicity compared to reference compound **2**. From these results, we infer that the piperazine nitrogen is important for T-type channel blocking activity, and unwanted hERG blockade is related lipophilicity (clog *P* values in Table 1), as it is known that the increased lipophilicity enhances hERG binding.[15]

Table 2
Selectivity data of **5** and **6** between T-type and L-type calcium channel

Compd	T-type IC_{50} (α_{1G}, nM)	L-type IC_{50} (α_{1C}, μM)	Ratio (L/T)
5	56	5.42	96.8
6	654	5.90	9.0

Since compounds **5** and **6**, the modified versions containing amides in place of the original compounds' amines, showed improved T-type channel selectivity as intended, we further performed selectivity tests against other types of calcium channels and pharmacokinetic analysis in rats to understand their *in vivo* dynamics. As shown in Table 2, amides **5** and **6** were less potent in blocking L-type[16] than T-type calcium channels. Although absolute IC_{50} values of **5** and **6** against L-type channels were similar, compound **5** was much more selective in blocking T-type channels than compound **6**.

Pharmacokinetic parameters for amides **5** and **6** following intravenous and oral doses in rat are shown in Table 3. Compound **5** exhibited acceptable plasma concentration-time profiles following intravenous and oral administration, with excellent AUC values and 60% estimated bioavailability, despite a relatively high lipophilicity (clog *P* = 5.40). Further, its pharmacokinetic profile upon intravenous injection was remarkably improved compared to parent compound **1**. On the contrary, compound **6** showed reasonable pharmacokinetic properties upon intravenous injection, but its plasma concentration was low following oral administration. To understand the pharmacokinetic improvement of compound **5**, we measured the stability of compounds **1**, **5** and **6** in rat plasma and human microsomes. Each compound was stable in rat plasma (± 10% after 2 h), but the stability of compounds **5** and **6** in human microsomes was greater than that of compound **1** ($T_{1/2}$ of

Table 3
Mean (±SD[a]) pharmacokinetic parameters of **5** and **6** after intravenous and oral administration (10 mg/kg) to SD male rats ($n = 3$)

	1	**5**		**6**	
	Intravenous	Intravenous	Oral	Intravenous	Oral
[b]$AUC_{0-\infty}$ (μg min/ml)	54.36	577.3 ± 106.6	348.1 ± 52.07	304.6 ± 19.43	28.03 ± 7.498
half-life (min)	77.72	105.5 ± 35.35	47.72 ± 2.077	294.0 ± 45.91	132.4 ± 59.03
[c]C_{max} (μg/ml)	–	–	4.582 ± 0.7809	–	0.218 ± 0.0418
[d]T_{max} (min)	–	–	30	–	30
F (%)	–	60.29		9.204	

[a] SD: standard deviations.
[b] $AUC_{0-\infty}$: total area under the plasma concentration–time curve from time zero to time infinity.
[c] C_{max}: peak plasma concentration.
[d] T_{max}: time to reach C_{max}.

1 = 120 min, $T_{1/2}$ of **5** = 240 min, $T_{1/2}$ of **6** = 220 min). From these results, we infer that the improvement of pharmacokinetic properties for **5** and **6** results from enhanced microsomal stability.

In summary, among the series of structurally modified compounds based on parent compounds **1** and **2** we designed, we found that modifying amines to amides was most effective in mitigating hERG toxicity and maintaining potent, selective inhibitory activity against T-type calcium channels. Of the two amide compounds **5** and **6**, benzimidazole amide **5** was more selective in blocking T-type calcium channels over hERG (hERG/T-type $\geqslant$ 220) and L-type channels (L-type/T-type = 96). Furthermore, compound **5**'s pharmacokinetic profiles in rats following intravenous and oral administration suggest suitability for further development, and its bioavailability (*F* value) was over 60%. Taken together, these results suggest that compound **5** holds promise as a potent, selective and orally available T-type calcium channel blocker for the treatment of disorders related to this channel such as hypertension, neuropathic pain and migraine.

Acknowledgements

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14. Compound **5**: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.7 (dd, *J* = 5.8, 2.9 Hz, 2H), 7.46 (d, *J* = 8.6 Hz, 2H), 7.43 (dd, *J* = 5.8, 2.9 Hz, 2H), 7.09 (d, *J* = 8.6 Hz, 2H), 6.45 (bs, 1H), 3.78 (s, 3H), 3.47–3.41 (m, 2H), 3.25 (bs, 2H), 2.97 (s, 3H), 2.52–2.45 (m, 1H), 2.42–2.36 (m, 2H), 2.26–2.17 (m, 1H), 2.10 (bs, 2H), 2.02–1.95 (m, 1H), 0.94 (d, *J* = 6.7 Hz, 3H), 0.84 (d, *J* = 6.7 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 174.90, 174.57, 152.96, 138.35, 131.78, 131.12, 129.98, 125.40, 120.81, 114.25, 58.82, 51.85, 46.39, 35.74, 35.43, 32.26, 29.81, 24.47, 22.92, 18.77, 18.47. HRMS: calcd for $C_{26}H_{32}BrN_3O_3 + H^+$, 513.1627; found (ESI, $[M+H]^+$), 513.1624.
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16. *Transient expression of L-type Ca^{2+} channels in HEK 293 cells:* Plasmid cDNAs for channel subunits were cloned into the following vectors: α1C (X58696) in pCR3, rat β2a (M80545) and rat α2δ (M86621) in pCDNA3 (Invitrogen, Carlsbad, CA), and green fluorescence protein (GFP) in pEGFP-N1 (Clontech, Cambridge, UK). HEK 293 cells in 90% confluency were split with trypsin-EDTA and replated on 35-mm dishes (Corning) at a density of 2×10^5/dish. One day after plating, these cells were transfected with 3, 1, 1, and 0.7 μg of α1C, β2a, α2δ, and GFP cDNAs, respectively, using a calcium phosphate transfection kit (Invitrogen), and incubated for at least 24 h in a CO_2 incubator at 37 °C. Successfully transfected cells were identified by green fluorescence, and electrophysiological recordings were taken between 2 and 4 days after transfection.
Solutions and drugs: To isolate Ca^{2+} currents, patch pipettes were filled with an internal solution containing: 120 mM *N*-methyl-D-glucamine (NMG)-methanesulfonate (MS), 20 mM tetraethylammonium (TEA)-MS, 20 mM HCl, 11 mM EGTA, 1 mM $CaCl_2$ H_2O, 10 mM HEPES, 4 mM Mg-ATP, 0.3 mM Na_2-GTP, and 14 mM creatine phosphate (pH 7.2, 290 mOsm/kg). The external recording solution contained: 145 mM TEA-MS, 10 mM HEPES, 10 mM $CaCl_2$, 15 mM glucose, and 0.0003 mM tetrodotoxin (TTX) (pH 7.4, 325 mOsm/kg).
Electrophysiological recordings: Ca^{2+} currents were recorded using the whole-cell ruptured configuration of the patch clamp technique.