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Design, synthesis, and biological evaluation of 1,3-dioxoisoindoline-5-carboxamide derivatives as T-type calcium channel blockers

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Abstract—A small molecule library of 1,3-dioxoisoindoline-5-carboxamides **4** was designed based on the pharmacophore model, synthesized and biologically evaluated as potential T-type calcium channel blockers. The most active compounds **4d** and **4n** show T-type calcium channel blocking activity with IC_{50} values of 0.93 and 0.96 μM, respectively.

Influx of calcium ions into cells is involved in numerous intracellular events. Calcium ions are required for contraction of skeletal muscle and heart, release of neurotransmitters and hormones, induction of cell death, activation of various protein kinases and signaling cascades, and so on.[1] Precise control of calcium influx is therefore critical. Calcium influx is regulated by various calcium channels, mainly voltage-dependent calcium channels and ligand-dependent calcium channels.[2] Voltage-dependent calcium channel is a principal route through which calcium ions enter the cytosol. Voltage-dependent calcium channels are divided into high-voltage activated (N-, L-, P/Q, and R-type) and low voltage activated (T-type) channels based on the amount of cellular depolarization required for activation.[2]

Agents which inhibit or activate calcium channels have been shown to be useful as therapies for treating a wide variety of diseases and disorders.[3] Calcium channel blockers are the most widely used class of drugs in the treatment of cardiovascular diseases such as hypertension and angina pectoris.[4] These calcium channel blockers mainly block L-type calcium channel and show side effects such as bradycardia and atrioventricular block.[4] On the other hand, a T-type calcium channel blocker, mibefradil, was used in treatment of hypertension and stable angina pectoris with much less side effects than L-type calcium channel blockers (Fig. 1).[5] However, mibefradil was briefly marketed under the trade name Posicor™ and withdrawn because of unfavorable drug–drug interactions.[6]

In addition to the cardiovascular effects, T-type calcium channels play crucial roles in the control of neuropathic pains which are caused mainly by hyperexitable neurons.[7] T-Type calcium channel blockers such as ethosuximide and methsuximide are efficacious for treatment of epilepsy and absence seizure derived from hyperexitable neurons (Fig. 1).[8,9] Evidences have been accumulated that T-type calcium channel blockers are efficacious in the treatment of neuropathic pain.[8,10] T-type calcium channels have also been reported to be involved in various intracellular events such as neuronal differentiation,[11] cisplatin-induced apoptosis,[12] and $p21^{ras}$ signaling pathway.[13]

Since the withdrawal of mibefradil and the increased interest in the T-type calcium channels as a therapeutic target, there have been significant efforts to develop selective T-type calcium channel blockers.[14] Herein we report design, synthesis, and biological evaluation of novel T-type calcium channel blockers.

To design novel T-type calcium channel blockers, (1) a pharmacophore model was generated from active compounds,[15] (2) core features were defined to find a

Keywords: T-type calcium channel blockers; 1,3-Dioxoisoindoline-5-carboxamides; Pharmacophore model.
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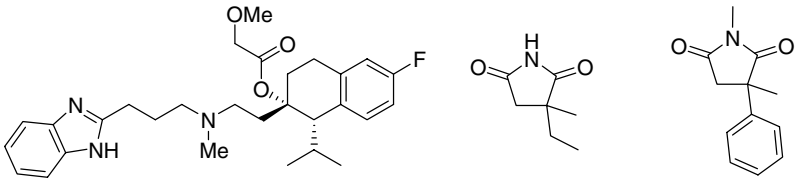


mibefradil **ethosuximide** **methsuximide**

Figure 1. T-type calcium channel blockers.

minimum scaffold, (3) building blocks were attached to the scaffold to match other common features from the pharmacophore model, and (4) a small molecule library was generated. A pharmacophore model was generated by using the in-house hits obtained from commercial libraries[15d] as well as active compounds from literatures.[14,15c] Analysis of the pharmacophore model gave six common features such as two hydrogen bond acceptors, one positively ionizable point, and three hydrophobic centers (Fig. 2). The two hydrogen bond acceptors and the middle hydrophobic center were assigned as core features, and 1,3-dioxoisoindoline-5-carboxamide, which could be easily constructed starting from the commercially available sources, was selected as the minimum scaffold (Scheme 1). The 1,3-dioxoisoindoline-5-carboxamide has two hydrogen bond acceptors properly positioned and one hydrophobic center in the middle. Two building blocks, R^1 and R^2, were attached to the 1,3-dioxoisoindoline-5-carboxamide core. One building block R^1 with substituted phenyl or benzyl groups constitutes a hydrophobic center, while the other building block R^2 has saturated heterocyclic tertiary amines corresponding to the positively ionizable point and hydrophobic center in the far right side of the pharmacophore model (Fig. 2). By combination of various R^1 and R^2, a small molecule library of T-type calcium channel blockers was constructed (Scheme 1). One of the designed compounds was mapped to the pharmacophore model, which was well-positioned with satisfying the six common features of the pharmacophore model (Fig. 3).

The designed T-type calcium channel blockers of the small molecule library were synthesized from commercially available 1,2,4-benzenetricarboxylic acid anhydride **2** (Scheme 2). 1,2,4-Benzenetricarboxylic acid anhydride **2** was treated with various anilines and benzylamines (R^1NH_2, Scheme 2) in acetic acid under refluxing conditions to give 2-substituted 1,3-dioxoisoindoline-5-carboxylic acids **3** in 58–96% yields. The carboxylic acids **3** were converted to the corresponding acyl halides by using oxalyl chloride and a catalytic amount of DMF in anhydrous CH_2Cl_2. Treatment of the crude acyl halides with *N*-aminoalkyl-piperidine, pyrrolidine or morpholine (R^2NH_2, Scheme 2) in CH_2Cl_2 provided 1,3-dioxoisoindoline-5-carboxamide derivatives **1**, which were treated with 1 N HCl in ethereal solution to give the corresponding HCl salts **4** in 17–98% overall yields starting from the carboxylic acids **2** (Scheme 2).

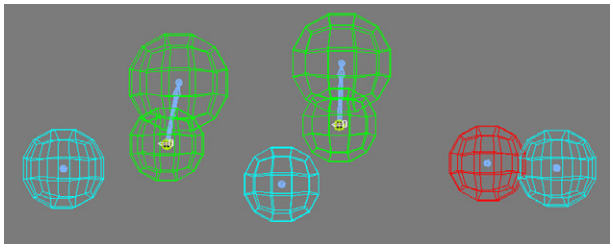
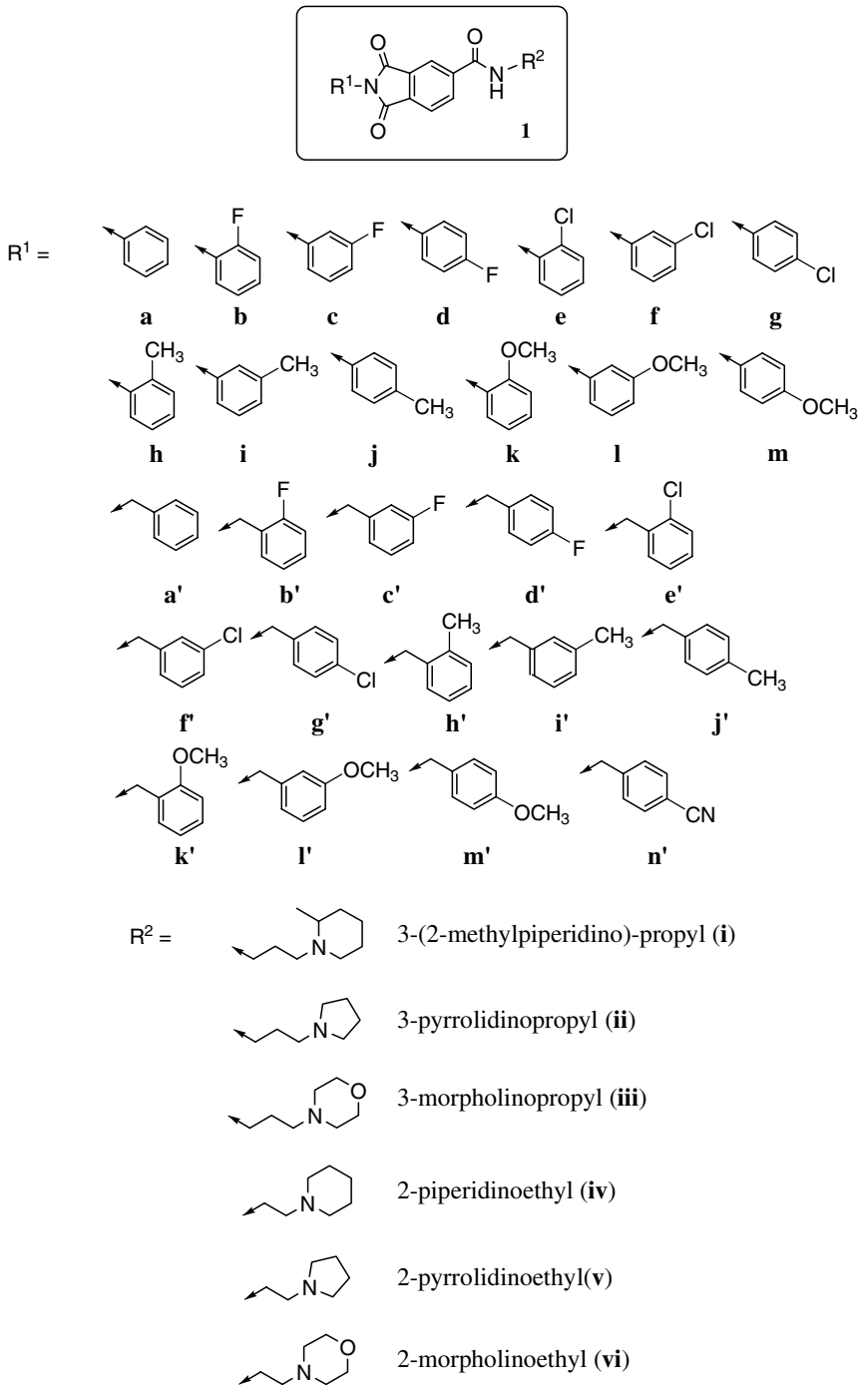


Figure 2. A pharmacophore model generated from hits and known active compounds. The features are shown in green (hydrogen acceptors), red (a positively ionizable point), and cyan (hydrophobic centers), respectively.

Total 132 compounds of **4** were synthesized with combination of two building blocks, R^1 and R^2. The biological activity of the synthesized compounds was evaluated against HEK293 cells which stably express both T-type calcium channel $Ca_v3.1$ with α_{1G} subunit and potassium channel Kir2.1.[16] The % inhibition of Ca^{2+} current was measured at certain molar concentration of the synthesized compounds. For this purpose, two assay methods were employed: FDSS6000 assay[17] and patch–clamp assay using a single cell[16]. FDSS6000 assay is developed for high-throughput screening and applied to the whole small molecule library. On the other hand, patch–clamp assay is more accurate and sensitive but measures Ca^{2+} current one by one. The synthesized 132 compounds were primary-screened by FDSS6000 assay and selected compounds with potent activity were secondary-screened by patch–clamp assay to obtain IC_{50} values (Table 1).

The preliminary calcium channel blocking activity of the synthesized compounds was obtained by FDSS6000 assay. The FDSS6000 assay results are summarized as follows: first, in general, 1,3-dioxoisoindoline-5-carboxamide derivatives **4** with benzyl group as R^1 are more potent than the corresponding phenyl-substituted analogs. Compounds **4** with fluorobenzyl groups as R^1 show T-type calcium channel blocking activity in 30–86% inhibition range at 100 μM, while compounds **4** with fluorophenyl groups are active only with 15–46% inhibition range at 100 μM (detailed data not shown). Second, biological activity also depends on R^2 groups, in which compounds **4** with piperidine-based group are more active than those with pyrrolidine group or relatively polar morpholino group. Third, the alkyl tether of R^2, either propylenes or ethylenes within 3-(2-methylpiperidino)-propyl and 2-piperidinoethyl groups, gives little effect on the inhibition of T-type calcium current.

Based on the preliminary information of the activities from the FDSS assay, 20 compounds were selected and biologically evaluated with patch–clamp assay



Scheme 1. Designed T-type calcium channel blockers with 1,3-dioxoisoindoline-5-carboxamide scaffold.

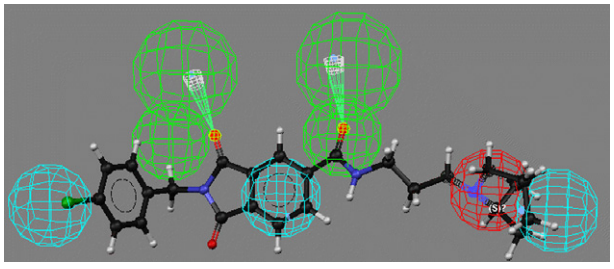
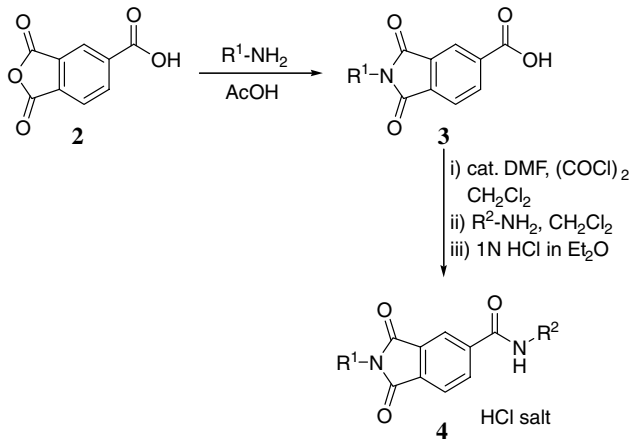


Figure 3. The mapping of one of designed compounds to the phamacophore model.

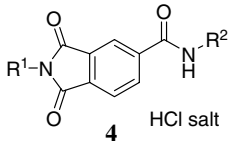
method to obtain IC_{50} values. The results are shown in Table 1. The selected compounds have substituted benzyl groups at R^1 and piperidine-based groups at R^2 except the compound **4t** which has a pyrrolidine substituent. According to the SAR study of the aromatic substituents of R^1, substituted benzyl groups show better blocking activity of T-type calcium current than a benzyl group without substitution (entry 8, **4h**). Among the aromatic substituents, chlorobenzyl groups (**4b–d** and **4l–n**) show better T-type blocking activity than the other substituted benzyl groups such as fluoro, methyl, and methoxy. Particularly, compounds **4d** and



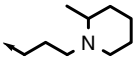
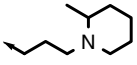
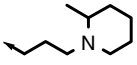
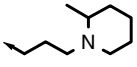
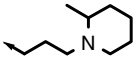
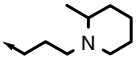
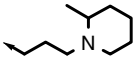
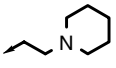
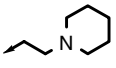
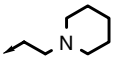
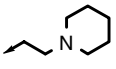
Scheme 2. Synthesis of the small molecule library.

4n[18] with a *p*-chlorobenzyl group as R^1 show the most potent blocking activity of T-type calcium current with an IC_{50} values of 0.93 and 0.96 μM, respectively, which are comparable to that of mebefradil, the positive control. Aromatic methyl substituents (**4e–g** and **4o–q**) also show moderate to potent activity. However, unlike **4d** and **4n**, compounds **4f** and **4p** with a methyl substituent at *meta* position instead of *para* show potent activity with an IC_{50} values of 1.84 and 1.93 μM, respectively. On the other hand, compounds **4** with a fluorobenzyl group (**4i–k**) or a methoxybenzyl group (**4r** and **4s**) show only moderate activity, except compound **4a** which has relatively potent activity with IC_{50} value of 2.03 μM. For the R^2 group, the 3-(2-methylpiperidino)-propyl-substituted analogs (**4a–g**) are generally more potent than the 2-piperidinoethyl-substituted ones (**4h–s**). Compound **4t** with 3-pyrrolidinopropyl group at R^2 shows

Table 1. T-type calcium channel blocking activity of selected 1,3-dioxoisoindoline-5-carboxamide derivatives **4** by patch–clamp assay

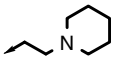
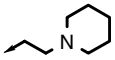
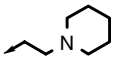
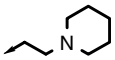
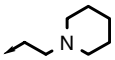
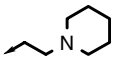
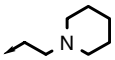
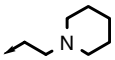
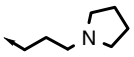


4

Entry	Compound	R^1	R^2	IC_{50}[a] (μM)
1	**4a**	*p*-F-benzyl		2.03 ± 0.12
2	**4b**	*o*-Cl-benzyl		1.52 ± 0.19
3	**4c**	*m*-Cl-benzyl		1.81 ± 0.46
4	**4d**	*p*-Cl-benzyl		0.93 ± 0.06
5	**4e**	*o*-Me-benzyl		2.07 ± 0.45
6	**4f**	*m*-Me-benzyl		1.84 ± 0.21
7	**4g**	*p*-Me-benzyl		3.53 ± 0.43
8	**4h**	Benzyl		22.78 ± 9.23
9	**4i**	*o*-F-benzyl		14.86 ± 1.12
10	**4j**	*m*-F-benzyl		8.52 ± 0.75
11	**4k**	*p*-F-benzyl		8.88 ± 1.89

(*continued on next page*)

Table 1 (*continued*)

Entry	Compounds	R^1	R^2	IC_{50}[a] (μM)
12	**4l**	*o*-Cl-benzyl		5.82 ± 0.82
13	**4m**	*m*-Cl-benzyl		4.09 ± 1.55
14	**4n**	*p*-Cl-benzyl		0.96 ± 0.07
15	**4o**	*o*-Me-benzyl		8.28 ± 2.95
16	**4p**	*m*-Me-benzyl		1.93 ± 0.23
17	**4q**	*p*-Me-benzyl		5.82 ± 0.35
18	**4r**	*m*-OMe-benzyl		14.12 ± 2.06
19	**4s**	*p*-OMe-benzyl		5.25 ± 0.80
20	**4t**	*p*-Cl-benzyl		1.62 ± 0.16
21		Mibefradil		1.34 ± 0.49

[a] IC_{50} value (±SD) was obtained from a dose–response curve.

Table 2. Selectivity of compounds **4d** and **4n** against N-type calcium channel

Entry	Compound	T-type IC_{50}[a] (μM)	N-type IC_{50}[a] (μM)	SI[b]	Cytotoxicity CC_{50}[c] (μM)
1	**4d**	0.93 ± 0.06	54.72 ± 6.81	59	>100
2	**4n**	0.96 ± 0.07	33.40 ± 4.16	35	>100

[a] IC_{50} value (±SD) was obtained from a dose–response curve.
[b] SI (selectivity index) = N-type IC_{50} value/T-type IC_{50} value.
[c] MTT assay with 0.1% DMSO as negative control and H_2O_2 as positive control.

potent T-type blocking activity with IC_{50} value of 1.62 μM, but it is less active than compounds **4d** and **4n**, which have piperidine-based R^2 group and the same R^1 group.

In our efforts to identify novel T-type calcium channel blockers, a small molecule library of 1,3-dioxoisoindoline-5-carboxamide derivatives **4** were designed, synthesized, and biologically evaluated by FDSS6000 assay and patch–clamp assay. Several structure–activity relationships were identified from this study. The R^1 group prefers benzyl groups to phenyl groups. Among the *para* substituents in benzyl groups, the T-type blocking activity order is Cl > OMe ≈ Me > F > H within 2-piperidinoethyl groups as R^2, and the activity order is Cl > F > Me > H within 3-(2-methylpiperidino)-propyl groups as R^2. Among the *meta* substituents, the activity order is Me > Cl > F > OMe > H within a 2-piperidinoethyl group as R^2. There exists a little bit difference in the T-type blocking activity according to the length of alkyl tethers in R^2 groups and the position of substituents in benzyl groups. For the R^2 groups, piperidine-based groups show better activity than pyrrolidine-based groups and morpholine-based groups. The alkyl tethers of the R^2 groups prefer propylenes to ethylenes, based on the IC_{50} values by patch–clamp assay.

N-type calcium channel is one of high-voltage activated channels, while T-type calcium channel is a low-voltage activated channel. To determine the selectivity of the most active compounds **4d** and **4n** for calcium channels, the two compounds were further biologically evaluated against N-type calcium channel along with cytotoxicity (Table 2). The compounds **4d** and **4n** show much less N-type calcium channel blocking activity with IC_{50} values of 54.72 and 33.40 μM, respectively, than T-type calcium channel blocking activity. The compounds **4d** and **4n** are selective to T-type calcium channel without cytotoxicity up to 100 μM. N-type calcium channel blockers are being developed for the treatment of severe chronic

pain for cancer or AIDS patients.[19] On the other hand, T-type calcium channel blockers are usually for the treatment of migraine.[19] Therefore, the selectivity between two calcium channels may be very important.

In summary, a new series of 1,3-dioxoisoindoline-5-carboxamide derivatives **4** were designed, synthesized, and biologically evaluated to develop novel effective T-type calcium channel blockers. Among total 132 compounds, **4d** and **4n** show the most potent T-type calcium channel blocking activity. Further evaluations of compounds **4d** and **4n** such as pharmacokinetics and neuronal analgesic effect are in progress.

Acknowledgment

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18. Spectral data of the compounds **4d** and **4n**: for compound **4d** (HCl salt, two diastereomers), ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.36 (br s, 1H), 9.15–9.02 (m, 1H), 8.39–8.22 (m, 2H), 7.99 (d, 1H, J = 7.8 Hz), 7.43–7.22 (m, 4H), 4.77 (s, 2H), 3.63–3.50 (m, 0.3H), 3.49–3.30 (m, 2H), 3.30–2.96 (m, 4H), 2.96–2.80 (m, 0.7H), 2.07–1.77 (m, 2H), 1.88–1.55 (m, 5H), 1.55–1.38 (m, 1H), 1.28 (d, 2.1H, J = 6.0 Hz), 1.21 (d, 0.9H, J = 6.5 Hz); ^{13}C NMR (100 MHz, DMSO-d_6) δ 167.67, 167.58, 165.05, 140.10, 135.98, 134.20, 134.12, 132.56, 132.36, 129.86, 129.02, 123.92, 122.02, 58.68, 51.44, 50.19, 47.41, 37.39, 31.37, 22.98, 22.21, 17.64; HRMS (FAB, M+1) Calcd for $C_{25}H_{29}ClO_3N_3$: 454.1892. Found 454.1887: for compound **4n** (HCl salt), ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.26 (br s, 1H), 9.29 (t, 1H, J = 5.0 Hz), 8.39–8.28 (m, 2H), 7.99 (d, 1H, J = 8.1 Hz), 7.42–7.26 (m, 4H), 4.77 (s, 2H), 3.78–3.64 (m, 2H), 3.58–3.41 (m, 2H), 3.29–3.13 (m, 2H), 2.98–2.82 (m, 2H), 1.89–1.61 (m, 5H), 1.43–1.29 (m, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 167.61, 167.55, 165.27, 139.74, 135.96, 134.33, 134.25, 132.56, 132.31, 129.87, 129.01, 123.88, 122.28, 55.49, 52.62, 34.72, 22.75, 21.75; HRMS (FAB, M+1) Calcd for $C_{23}H_{25}ClO_3N_3$: 426.1576. Found 426.1582.
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