Synthesis and Biological Evaluation of Isophthalamide Derivatives as T-type Calcium Channel Blockers

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Since mibefradil, a selective T-type calcium channel blocker, was withdrawn from the market in 1998 due to drug-drug interaction, there have been efforts to discover novel T-type calcium blockers.[1,2] Mibefradil had been approved for the treatment of angina pectoris and hypertension by the FDA in 1997. According to accumulation of new findings on T-type calcium channels, it has been reported that T-type calcium channels play crucial roles in the control of pain which are caused by hyperexitable neurons.[3] The role of T-type calcium channels in pain has been addressed using specific genetic modulation of T-type calcium channel isoforms. In the case of $Ca_V3.1$ knockout ($\alpha_{1G}^{-/-}$) mice, it was observed that, after L5 spinal nerve ligation, spontaneous pain responses were reduced and a threshold for paw withdrawal was increased in response to mechanical stimulation.[4] $Ca_V3.2$ antisense treatment resulted in major anti-nociceptive and anti-hyperalgesic effect, suggesting that $Ca_V3.2$ plays a major pronociceptive role in acute and chronic pain states.[5] Together, the results of these two studies suggest that blocking T-type calcium channels should reduce nociceptive pain and neuropathic pain.

Herein we report design, synthesis and biological evaluation of novel isophthalamide derivatives as T-type calcium channel blockers. Recently, we designed 1,3-dioxoisoindoline-5-carboxamide derivatives with assistance of a pharmacophore model generated and synthesized those compounds, of which the biological results were reported (Figure 1).[6] Based on the previous SAR (structure-activity relationship) study, new isophthalamide derivatives **2** were designed, synthesized and biologically evaluated (Figure 1).

The isophthalamide derivatives were synthesized in 3 steps starting from isophthalic acid monoester **3** (Scheme 1). Isophthalic acid monoester **3** underwent amide coupling with various benzyl amines by treatment with *N*-(3-dimethylaminopropyl)-*N'*-ethyl-carbodiimide hydrochloride (EDCI) and 1-hydroxybenzotriazole (HOBt) to give compounds **4** in

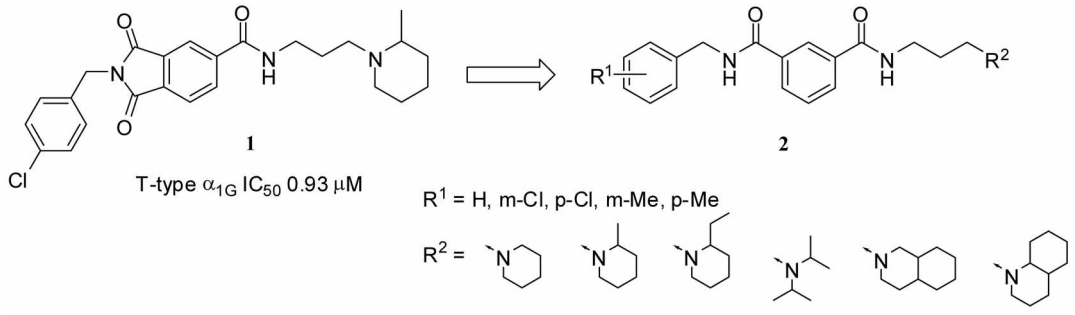
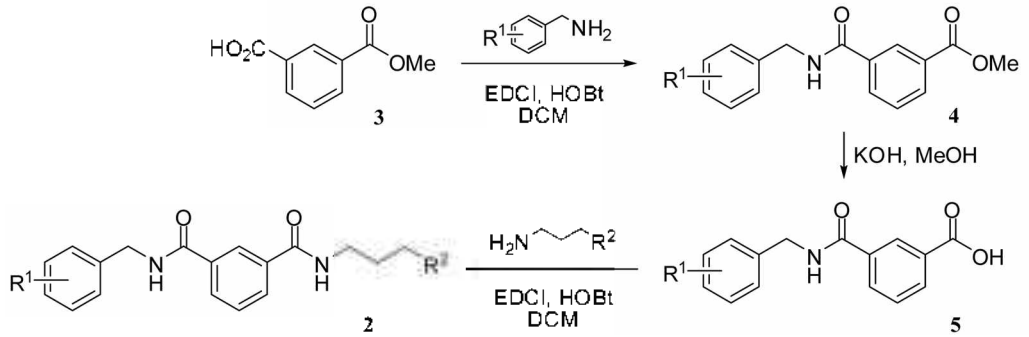


Figure 1. Designed isophthalamide derivatives.



Scheme 1. Synthesis of isophthalamide derivatives.

70-82% yields. The compounds **4** were hydrolyzed to give the corresponding benzoic acids **5**, which were transformed into the desired isophthalamide derivatives **2** in 22-68% yields by coupling with various 3-R^2-propylamines in the presence of EDCI and HOBt.

Total 26 isophthalamide derivatives **2a-z**, thus prepared, were biologically evaluated against α_{1G} ($Ca_V3.1$) T-type calcium channel in HEK293 cells which stably express both T-type calcium channel $Ca_V3.1$ and potassium channel Kir2.1.[7] All the synthesized compounds were screened by fluorescence-based HTS (high throughput screening) FDSS600 assay,[8] and the %-inhibitions of Ca^{2+} current measured at 10 μM concentration of the isophthalamide derivatives are summarized in Table 1. In general, compounds with *m*-Cl (**2g-2l**, Table 1) or *p*-Cl (**2m-2r**, Table 1) group as R^1 showed higher activity than the corresponding compounds with H (**2a-2f**, Table 1), *m*-Me (**2s-2v**, Table 1) and *p*-Me (**2w-2z**, Table 1) substituents. Among the compounds tested, compounds with high %-inhibition (**2i**, **2l**, **2m**, **2n** and **2o**, Table 1) were selected for the patch-clamp assay which is more accurate and more sensitive.[9] The patch-clamp assay is a very time-consuming process because it measures %-inhibition of Ca^{2+} current with a single cell at each concentration with one compound, and thus, only 5 compounds were selected for accurate screening. The selected compounds were found to be active with IC_{50} values between 2.66 μM to 13.53 μM, and among those, compound **2l** showed activity against α_{1G} T-type calcium channel with an IC_{50} value of 2.66 μM, which is comparable to that of mibefradil.

Table 1. Activity of isophthalamides **2** against α_{1G} ($Ca_V3.1$) T-type calcium channel

en.	compd.	R^1	R^2	HTS[a] %inh (10 μM)	patch-clamp[b] IC_{50} (μM)
1	**2a**	H	piperidin-1-yl	15.71	–[c]
2	**2b**	H	2-methylpiperidin-1-yl	12.76	–[c]
3	**2c**	H	2-ethylpiperidin-1-yl	29.61	–[c]
4	**2d**	H	diisopropylamino	0.37	–[c]
5	**2e**	H	octahydroisoquinolin-2(1H)-yl	37.80	–[c]
6	**2f**	H	octahydroquinolin-1(2H)-yl	29.35	–[c]
7	**2g**	*m*-Cl	piperidin-1-yl	39.87	–[c]
8	**2h**	*m*-Cl	2-methylpiperidin-1-yl	15.68	–[c]
9	**2i**	*m*-Cl	2-ethylpiperidin-1-yl	72.01	6.77 = 0.20
10	**2j**	*m*-Cl	diisopropylamino	44.45	–[c]
11	**2k**	*m*-Cl	octahydroisoquinolin-2(1H)-yl	44.39	–[c]
12	**2l**	*m*-Cl	octahydroquinolin-1(2H)-yl	49.07	2.66 = 0.12
13	**2m**	*p*-Cl	piperidin-1-yl	51.90	13.53 = 0.99
14	**2n**	*p*-Cl	2-methylpiperidin-1-yl	48.46	11.74 ± 1.22
15	**2o**	*p*-Cl	2-ethylpiperidin-1-yl	59.21	6.86 = 0.18
16	**2p**	*p*-Cl	diisopropylamino	39.58	–[c]
17	**2q**	*p*-Cl	octahydroisoquinolin-2(1H)-yl	42.35	–[c]
18	**2r**	*p*-Cl	octahydroquinolin-1(2H)-yl	46.09	–[c]
19	**2s**	*m*-Me	piperidin-1-yl	19.74	–[c]
20	**2t**	*m*-Me	2-methylpiperidin-1-yl	6.84	–[c]
21	**2u**	*m*-Me	2-ethylpiperidin-1-yl	21.10	–[c]
22	**2v**	*m*-Me	diisopropylamino	10.68	–[c]
23	**2w**	*p*-Me	piperidin-1-yl	0.95	–[c]
24	**2x**	*p*-Me	2-methylpiperidin-1-yl	3.76	–[c]
25	**2y**	*p*-Me	2-ethylpiperidin-1-yl	15.83	–[c]
26	**2z**	*p*-Me	diisopropylamino	15.31	–[c]
27			mibefradil	78.92	1.43 ± 0.49

[a]Fluorescence-based HTS (high throughput screening) assay. [b]For the recordings of α_{1G} T-type Ca^{2+} currents, the standard whole-cell patch-clamp method was utilized as previously described. [c]Not determined.

Based on the results of the SAR study described above, it is clear that the bulky R^2 substituent increases the biological activity of the corresponding compound. Thus, compound **2l** with octahydroquinolin-1-(2H)-yl group is more active than **2i** with 2-ehtylpiperidin-1-yl group. Also, the compound **2o** with 2-ehtylpiperidin-1-yl group is more active than the compound **2n** with 2-mehtylpiperidin-1-yl group, which, in turn, is more active than the compound **2m** with piperidin-1-yl group.

In summary, the SAR study of isophthalamides **2** with R^1 and R^2 substituents revealed that the bulky R^2 is favored for high biological activity, which provides valuable insights into the design and optimization of novel α_{1G} T-type calcium channel blockers.

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