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Novel T-type calcium channel blockers: Dioxoquinazoline carboxamide derivatives

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Abstract—T-type calcium channel is one of therapeutic targets for the treatment of cardiovascular diseases and neuropathic pains. Since the withdrawal of mibefradil, a T-type calcium channel blocker, there have been a lot of efforts to develop T-type calcium channel blockers. A small molecule library of dioxoquinazoline carboxamide derivatives containing 155 compounds was designed, synthesized, and biologically evaluated for T-type calcium channel blocking activity. Among those compounds synthesized, the compound **1n** shows the most potent T-type calcium current blocking activity with an IC_{50} value of 1.52 μM, which is comparable to that of mibefradil.

1. Introduction

T-type calcium channel blockers have drawn more attention since mibefradil was approved for the treatment of angina pectoris and hypertension.[1] It reduced blood pressure and heart rate without suppressing myocardial contraction. Mibefradil also had beneficial effects in various animal models of heart failure and improved survival rate in a rat chronic myocardial infarction model. This was possible by accompanied antiarrhythmic effects through T-type calcium channel blockade.[2] Unfortunately, the drug had to be withdrawn from the market due to drug interaction at the cytochrome P-450 3A4 enzyme which was unrelated to T-type calcium channel blockade.[3] However, there were some stark findings reported that a genetic loss-of-function model, a mouse lacking the $Ca_V3.2$ T-type calcium channel, surprisingly demonstrated that calcium influx through VSM (vascular smooth muscle) T-type calcium channels is an essential mediator of normal relaxation, not contraction, of coronary arteries.[4] There is another report that the effect of mibefradil on blood pressure and small vessel myogenic tone is mediated by the $Ca_V1.2$ L-type calcium channel.[5] Therefore, it becomes very controversial that the cardiovascular effect of mibefradil results from the blockade of T-type calcium channel.

On the other hand, T-type calcium channels play crucial roles in the control of neuropathic pains which are caused by hyperexitable neurons.[6] The role of T-type calcium channels in pain has also been addressed using specific genetic modulation of T-type calcium channel isoforms. $Ca_V3.1$ knockout mice were shown to have an increased sensitivity to visceral pain, but no difference in acute pain thresholds or responses to a cutaneous pain simulus compared to wild-type mice.[7] $Ca_V3.2$ antisense knockdown reduced T-type currents in small and medium-sized DRG (dorsal root ganglion) cells.[8] The antisense treatment resulted in major anti-nociceptive and anti-hyperalgesic, suggesting that $Ca_V3.2$ plays a major pronociceptive role in acute and chronic pain states. Together, the results of these two studies suggest that the different T-type calcium channel isoforms can be anti-nociceptive or pronociceptive. Perhaps the major problem with elucidating the exact role of T-type calcium channels in neuropathic pain is the current lack of highly specific pharmacological agents.

Keywords: T-type calcium channel blockers; Dioxoquinazoline carboxamide derivatives.
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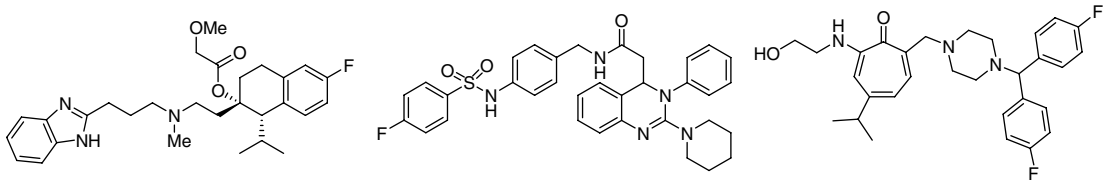


Figure 1. T-type calcium channel blockers.

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 (Fig. 1).[9] Herein we report design, synthesis, and biological evaluation of novel T-type calcium channel blockers.

2. Results and discussion

2.1. Design of small molecule library

Previously, a pharmacophore model was generated by using in-house hit compounds and mibefradil.[10] The pharmacophore model was used for the virtual screening of the small molecule library from ChemDiv and Maybridge2001 in order to obtain new scaffolds of T-type calcium channel blockers.[11] Among the virtual hit compounds, selected compounds were bought and biologically screened, resulting in novel scaffolds of several T-type calcium channel blockers. A thioxoquinazolinone carboxamide derivative is one of the interesting scaffolds and its derivatives show potent inhibitory activity against T-type calcium channels (Fig. 2). From the pharmacophore analysis, the sulfur in the thioxoquinazolinone carboxamide derivative was found to be acting as a hydrogen bonding acceptor. Because oxygen atom is a stronger hydrogen bonding acceptor than sulfur atom,[12] a small molecule library was designed by using dioxoquinazoline carboxamide derivatives (Scheme 1).

Two building blocks, R^1 and R^2 groups, were added to the novel key scaffold (the dioxoquinazoline) to fulfill the common features of the pharmacophore model: one building block R^1 is mainly composed of substituted phenyl and benzyl groups and alkyl groups, and the other building block R^2 is composed of cyclic tertiary aminoalkyl groups. By combination of various R^1 and R^2 groups, a small molecule library of T-type calcium channel blockers was constructed (Scheme 1).

2.2. Synthesis

The designed T-type calcium channel blockers were synthesized from dimethyl 2-aminoterephthalate **2**. Dimethyl 2-aminoterephthalate **2** was treated with isocyanates **3** (R^1-NCO) in 1,4-dioxane solution under basic conditions to give cyclized dioxoquinazolines **4** in 41–92% yields (Scheme 2).[13] The compounds **4** were hydrolyzed to the corresponding carboxylic acids **5** in 88–99% yields.[14] In the case of commercially unavailable alkyl isocyanates such as methyl, propyl, and cyclohexyl isocyanates, the corresponding amines such as methyl, propyl, and cyclohexyl amines were used for the synthesis of dioxoquinazolines (Scheme 2). Therefore, dimethyl 2-aminoterephthalate **2** was converted to the corresponding isocyanates **6** by treatment with triphosgene in toluene under reflux conditions. The isocyanates **6** were treated with alkyl amines under basic conditions to give urea analogs **7** in 22–50% yields from compounds **2**. The urea analogs **7** underwent cyclization and hydrolysis simultaneously in a mixture of 10% NaOH and methanol to give dioxoquinazoline carboxylic acids **5** in 54–98% yields.

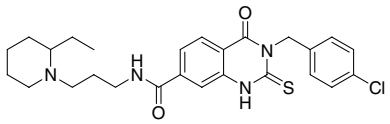


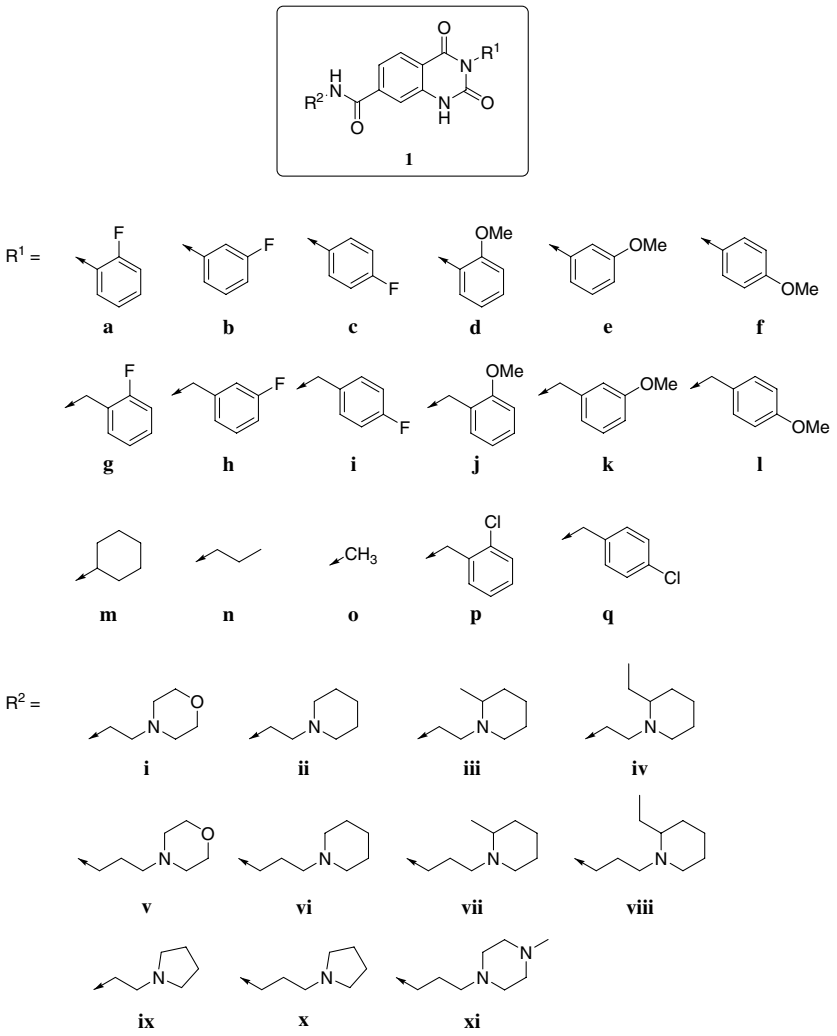
Figure 2. A thioxoquinazolinone derivative.

Dioxoquinazoline carboxylic acids **5** were converted to the corresponding amides **1** by treatment with cyclic tertiary aminoalkylamines (R^2–NH_2, Scheme 1). Some of cyclic tertiary aminoalkylamines are commercially available. Commercially unavailable amines (R^2–NH_2) were synthesized in two steps (Scheme 3). Piperidine derivatives **8** underwent S_N2 reaction with *N*-bromoethylphthalimide or *N*-bromopropylphthalimide to give compounds **9** in 27–77% yields.[15] Compounds **9** were treated with hydrazine hydrate in ethanol to afford the desired piperidinoalkylamines **10** in 67–87% yields.[16] To synthesize the desired dioxoquinazoline carboxamide derivatives **1**, dioxoquinazoline carboxylic acids **5** were converted to the corresponding acyl halides by treating with $SOCl_2$ under reflux conditions or treating with oxalyl chloride and a catalytic amount of DMF in CH_2Cl_2 at room temperature (Scheme 2). Then, treatment of the intermediate acyl halides with cyclic tertiary aminoalkylamines (R^2–NH_2) provided dioxoquinazoline carboxamide derivatives **1** in 5–82% overall yields starting from the carboxylic acids **5**.[17]

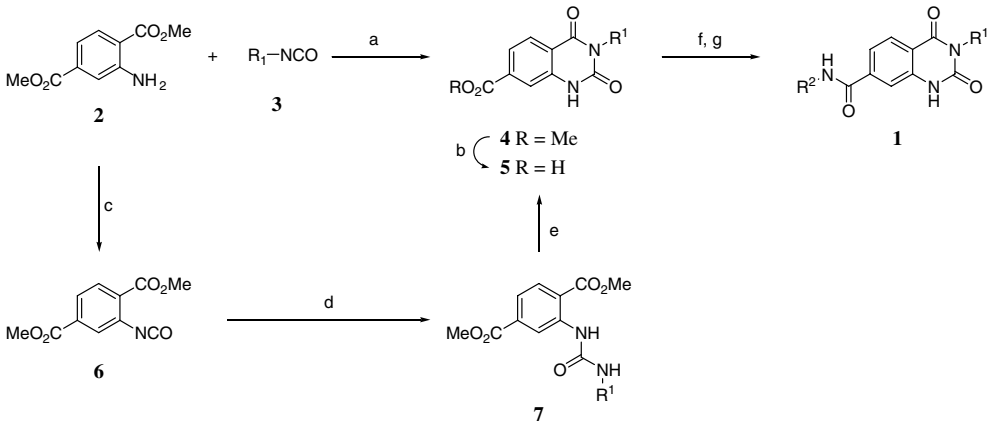
Total 155 compounds of dioxoquinazoline carboxamide derivatives **1** were synthesized with combination of two building blocks, R^1 and R^2 groups.

2.3. Biological activity

The synthesized compounds **1** of the small molecule library were biologically evaluated against HEK293 cells which stably express both T-type calcium channel $Ca_V3.1$ with α_{1G} subunit and potassium channel

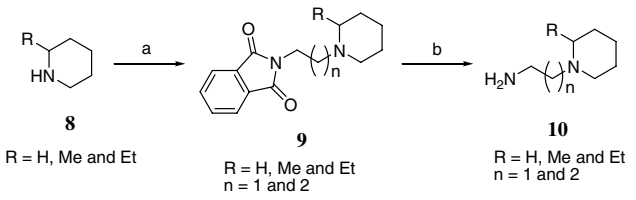


Scheme 1. A small molecule library of dioxoquinazoline carboxamide derivatives **1**.



Scheme 2. Reagents and conditions: (a) TEA, 1,4-dioxane, reflux, 41–92%, (b) 10% NaOH/1,4-dioxane (1:4), rt, 88–99%, (c) triphosgene, toluene, reflux, (d) R^1–NH_2, TEA, 1,4-dioxane, reflux, 22–50% (two-step yields), (e) 10% NaOH/MeOH (1:3), reflux, 54–98%, (f) $SOCl_2$, reflux or $(COCl)_2$, cat. DMF, CH_2Cl_2, rt, (g) R_2–NH_2, CH_2Cl_2, rt, 5–82% (two-step yields).

Kir2.1.[18] Two assay methods were employed: FDSS6000 assay and patch clamp assay. FDSS6000 assay was developed for high throughput screening and applied to the whole small molecule library of dioxoquinazoline carboxamide derivatives **1**.[19] Patch clamp assay using a single cell is more sensitive and more accurate. However, the patch clamp assay records the currents of the single cell one by one so that the work is laborious. The whole



Scheme 3. Reagents and conditions: (a) *N*-bromoethylphathalimide or *N*-bromopropylphthalimide, K_2CO_3, CH_3CN, reflux, 27–77%; (b) hydrazine hydrate, EtOH, reflux, 67–87%.

synthesized 155 compounds were primarily screened by FDSS6000 assay to obtain % inhibition at a certain molar concentration (Table 1). From the FDSS6000 results, 15 compounds with the most T-type current blocking activity were selected and screened by patch clamp assay to obtain IC_{50} values (Table 2).

From the high throughput screening by FDSS6000 assay, several structure–activity relationships were obtained (Table 1): (1) one of the building blocks, R^1 group, prefers to have substituted benzyl groups (**g**–**l**, **p**, and **q**) instead of substituted phenyl groups (**a**–**f**). Among the substituted benzyl groups, *para*-substituents (fluoro **i**, chloro **q**, and methoxy **l**) were preferred to *ortho*- or *meta*-substituents for activity. Among the alkyl groups, a cyclohexyl group **m** shows good T-type calcium current blocking activity and was selected for the patch clamp assay rather than a methyl or a propyl group (**n** or **o**) of relatively small size. (2) The other building block, R^2 group, prefers piperidine-based groups (**ii**–**iv** and **vi**–**viii**) to morpholine-based groups (**i** and **v**) or a piperazine-based group (**xi**). The compounds **1** with morpholine-based groups (**i** and **v**) or a piperazine-based group (**xi**) show little T-type calcium current blocking activity. (3) There was no significant difference in T-type calcium current blocking activity between piperidinoethyl groups (**ii**–**iv**) and piperidinopropyl groups (**vi**–**viii**).

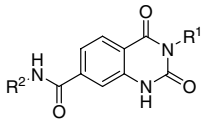
According to the FDSS6000 assay results, 15 compounds were selected and biologically evaluated against T-type calcium channel to obtain IC_{50} values with patch-clamp assay method. The results are shown in Table 2. The selected 15 compounds (**1a**–**1o**) show moderate to potent T-type calcium current blocking activity with IC_{50} values from 1.52 to 16.87 μM. Compound **1b** was the only example with a cyclohexyl group as R^1 group instead of a substituted benzyl group, which shows T-type current blocking activity with an IC_{50} value of 7.96 μM. The position of the substituents in a benzyl group prefers *para*-position instead of *ortho*- or *meta*-position. The compound **1d** with a *para*-fluorobenzyl group as R^1 group shows more potent blocking activity against T-type calcium channel than the compound **1c** with a *meta*-fluorobenzyl group. The compound **1n** with a *para*-chlorobenzyl group is more active than the compound **1o** with an *ortho*-chlorobenzyl group. The compound **1n** shows the most potent T-type calcium channel blocking activity among this series of compounds **1** with an IC_{50} value of 1.52 μM, which is comparable to the activity of the positive control, mibefradil (entry 16). Among the *para*-substituents such as chloro, fluoro, and methoxy, the chloro-substituent shows the most potent activity. The compound **1e** with a *para*-chlorobenzyl group is more active than the compound **1d** with a *para*-fluorobenzyl group. The compound **1g** is more active than the compounds **1f** and **1h**. Compounds **1i**, **1l**, and **1n** are more active than the compounds **1j**, **1k**, and **1m**, respectively. For the R^2 building block, most R^2 groups are piperidine-based groups except compounds **1a** and **1b**, which have a pyrrolidinoethyl group. The compound **1a** shows potent T-type calcium current blocking activity with an IC_{50} value of 2.46 μM. In most cases, the compounds **1i**–**1o** with piperidinopropyl groups (**vi**–**viii**, Scheme 1) show slightly better blocking activity than the compounds **1c**–**1h** with piperidinoethyl groups (**ii**–**iv**). The compounds **1i**, **1m**, and **1n** are more active than the compounds **1e**, **1f**, and **1g**, respectively. Among the substituents on piperidine-based R^2 groups such as hydrogen (**ii** and **vi**), methyl (**iii** and **vii**), and ethyl (**iv** and **viii**), ethylpiperidine-based R^2 groups (**iv** and **viii**) show better blocking activity. The compounds **1f**, **1m**, and **1n** with ethylpiperidine-based R^2 groups (**iv** and **viii**) are more active than the compounds **1d**, **1k**, and **1l**, respectively. However, the compound **1g** with an ethylpiperidinoethyl group (**iv**) is slightly less active than the compound **1e** with a piperidinoethyl group (**ii**). It is consistent with the SAR results that the compound **1n**, which has best R^1 and R^2 building blocks, shows the most potent T-type calcium current blocking activity.

2.4. Discussion

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. Like other calcium channels, T-type calcium channels are composed of an α_1-subunit and auxiliary β- and α_2-δ-subunits. The α_1-subunits form the channel pore, sense the membrane voltage, and define the basic pharmacological properties of the channel. Calcium channel blockers including a selective T-type calcium channel blocker, mibefradil, are assumed to bind with high affinity to the channel pore, resulting in the blockade of calcium entry.[20]

In our efforts to identify novel T-type calcium channel blockers with a new scaffold, a small molecule library of dioxoquinazoline carboxamide derivatives **1** was designed based on the pharmacophore model. Total 155 compounds were synthesized and biologically evaluated by FDSS6000 assay and patch-clamp assay. The compound **1n** shows the most potent inhibitory activity against T-type calcium channel, which was identified as a novel T-type calcium channel blocker. Our designed compounds were derived from the pharmacophore model which has six common features with mibefradil and in-house hit compounds. Therefore, the synthesized compounds with inhibitory activity are also expected to have binding affinity to the channel pore composed of α_1-subunits to inhibit calcium entry. It was expected that the compound **1n** would be much more active than our original lead compound **VH04**, because oxygen

Table 1. %inhibition of T-type calcium current of synthesized compounds by FDSS6000 assay[a]



1

Entry	R^1	R^2	% inhibition at 100 μM	%inhibition at 10 μM
1	**a**	**i**		—[b]
2	**b**	**i**	3.8	
3	**c**	**i**	—[b]	
4	**d**	**i**		—[b]
5	**e**	**i**		—[b]
6	**f**	**i**		5.8
7	**g**	**i**		—[b]
8	**h**	**i**		7.9
9	**i**	**i**		12.8
10	**j**	**i**		—[b]
11	**k**	**i**		2.8
12	**l**	**i**	—[b]	
13	**m**	**i**		—[b]
14	**n**	**i**	—[b]	
15	**o**	**i**		—[b]
16	**p**	**i**		—[b]
17	**q**	**i**		29.6
18	**a**	**ii**	7.2	
19	**b**	**ii**	3.4	
20	**c**	**ii**	12.5	
21	**d**	**ii**		—[b]
22	**e**	**ii**	—[b]	
23	**f**	**ii**		—[b]
24	**g**	**ii**		6.3
25	**h**	**ii**	64.6	
26	**i**	**ii**	65.0	
27	**j**	**ii**		3.8
28	**k**	**ii**	62.9	
29	**l**	**ii**		23.1
30	**m**	**ii**	52.2	
31	**n**	**ii**	18.0	
32	**o**	**ii**	17.6	
33	**p**	**ii**		30.0
34	**q**	**ii**		44.2
35	**g**	**iii**		9.8
36	**h**	**iii**		15.4
37	**i**	**iii**		14.7
38	**j**	**iii**		—[b]
39	**k**	**iii**		11.0
40	**l**	**iii**		22.9
41	**m**	**iii**		21.7
42	**p**	**iii**		7.9
43	**q**	**iii**		31.8
44	**g**	**iv**		21.8
45	**h**	**iv**		19.4
46	**i**	**iv**		33.6
47	**j**	**iv**		1.6
48	**k**	**iv**		31.0
49	**l**	**iv**		33.7
50	**m**	**iv**		32.3
51	**p**	**iv**		13.1
52	**q**	**iv**		53.9
53	**a**	**v**	2.8	
54	**b**	**v**	15.1	
55	**c**	**v**	9.2	
56	**d**	**v**		—[b]
57	**e**	**v**		—[b]
58	**f**	**v**		5.2
59	**g**	**v**		—[b]
60	**h**	**v**		—[b]
61	**i**	**v**	—[b]	
62	**j**	**v**		3.7
63	**k**	**v**	28.9	
64	**l**	**v**	36.0	
65	**m**	**v**	36.3	
66	**n**	**v**	9.2	
67	**o**	**v**		2.2
68	**p**	**v**		7.6
69	**q**	**v**		9.7
70	**g**	**vi**		8.7
71	**h**	**vi**		—[b]
72	**i**	**vi**		14.9
73	**j**	**vi**		—[b]
74	**k**	**vi**		—[b]
75	**l**	**vi**		35.2
76	**m**	**vi**		10.6
77	**p**	**vi**		—[b]
78	**q**	**vi**		34.8
79	**a**	**vii**		—[b]
80	**b**	**vii**	—[b]	
81	**c**	**vii**		—[b]
82	**d**	**vii**		—[b]
83	**e**	**vii**	12.3	
84	**f**	**vii**	1.5	
85	**g**	**vii**	13.9	
86	**h**	**vii**	47.8	
87	**i**	**vii**	56.6	
88	**j**	**vii**	12.7	
89	**k**	**vii**	36.9	
90	**l**	**vii**	42.3	
91	**m**	**vii**	48.3	
92	**n**	**vii**	12.5	
93	**o**	**vii**	8.5	
94	**p**	**vii**		1.8
95	**q**	**vii**		40.8
96	**g**	**viii**		13.4
97	**h**	**viii**		21.8
98	**i**	**viii**	68.3	
99	**j**	**viii**		7.6
100	**k**	**viii**		15.7
101	**l**	**viii**		29.3
102	**m**	**viii**		26.1
103	**p**	**viii**		34.4
104	**q**	**viii**		44.0
105	**a**	**ix**	6.5	
106	**b**	**ix**	4.3	
107	**c**	**ix**		—[b]
108	**d**	**ix**		—[b]
109	**e**	**ix**	13.1	
110	**f**	**ix**	10.5	
111	**g**	**ix**	24.9	
112	**h**	**ix**		6.9
113	**i**	**ix**	47.2	
114	**j**	**ix**		—[b]
115	**k**	**ix**	27.8	
116	**l**	**ix**	39.4	
117	**m**	**ix**	57.7	
118	**n**	**ix**	6.7	
119	**o**	**ix**	—[b]	
120	**p**	**ix**		15.5

(*continued on next page*)

Table 1 (*continued*)

Entry	R^1	R^2	% inhibition at 100 μM	%inhibition at 10 μM
121	**q**	**ix**		31.8
122	**a**	**x**		—[b]
123	**b**	**x**	—[b]	
124	**c**	**x**		5.8
125	**d**	**x**	1.5	
126	**e**	**x**	—[b]	
127	**f**	**x**	—[b]	
128	**g**	**x**		5.8
129	**h**	**x**	9.8	
130	**i**	**x**	34.2	
131	**j**	**x**		—[b]
132	**k**	**x**	13.2	
133	**l**	**x**	26.8	
134	**m**	**x**		—[b]
135	**n**	**x**		—[b]
136	**o**	**x**		—[b]
137	**p**	**x**		6.2
138	**q**	**x**		23.1
139	**a**	**xi**	2.4	
140	**b**	**xi**		—[b]
141	**c**	**xi**		—[b]
142	**d**	**xi**		—[b]
143	**e**	**xi**		—[b]
144	**f**	**xi**	—[b]	
145	**g**	**xi**	7.3	
146	**h**	**xi**	8.3	
147	**i**	**xi**	25.5	
148	**j**	**xi**		—[b]
149	**k**	**xi**	20.1	
150	**l**	**xi**		20.6
151	**m**	**xi**		—[b]
152	**n**	**xi**	—[b]	
153	**o**	**xi**	3.4	
154	**p**	**xi**		5.7
155	**q**	**xi**		17.4

[a] % inhibition was obtained at 10 and 100 μM, alternatively.
[b] Not active.

atom is a better hydrogen bonding acceptor than sulfur atom. However, the compound **1n** is found to be less active than the compound **VH04** (Fig. 2), which implies that there might be more important features than hydrogen bonding to be addressed.

Several structure–activity relationships were identified from this study. The R^1 group prefers a substituted benzyl group to a substituted phenyl group (Table 1). Among the benzyl groups, *para*-substituted benzyl groups show more inhibitory activity than *ortho*- or a *meta*-substituted benzyl groups and the best substituent on a benzyl group in this study is a chloro-substituent (Table 2). The R^2 group prefers cyclic tertiary amines according to the pharamacophore model. Among the cyclic tertiary amines, an ethylpiperidinopropyl group shows the best inhibitory activity against T-type calcium current (Table 2). The tether between the core dioxoquinazoline carboxamide and the tertiary amine prefers a propyl group. A bulkier group in the tertiary amine is preferred such as an ethylpiperidino-group instead of a simple piperidino-group. Further modification of the compound **1n** is under study, based on the information of the SAR study.

3. Conclusion

A small molecule library of dioxoquinazoline carboxamide derivatives **1** was designed, synthesized, and biologically evaluated to develop novel T-type calcium channel blockers. Among total 155 compounds, the compound **1n** shows the most potent T-type calcium current blocking activity. Further evaluations of the compound **1n** such as selectivity for other calcium channels, pharmacokinetics, and neuronal analgesic effect are in progress.

4. Materials and methods

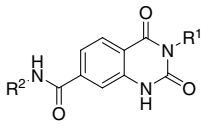
4.1. Experimental

All the commercially available reagents were obtained from Aldrich and Fluka, and generally used without further purification. ^{1}H NMR and ^{13}C NMR spectra were obtained on Bruker Advance 400 (or 300) spectrometer. Nuclear magnetic resonance spectra were acquired at 400 (or 300) MHz for ^{1}H and 100 MHz for ^{13}C NMR. Infrared spectra were obtained on a Perkin-Elmer 16FPC FT-IR spectrometer using KBr pellet, $CHCl_3$ or neat. GC/MSD was obtained on a Hewlett Packard 5890. HRMS spectra were obtained on a JMS-700 mass spectrometer (Jeol). Analytical thin layer chromatographies (TLC) were carried out on precoated silica gel plates (Merck Kieselgel 60F254, layer thickness 0.25 mm). Flash column chromatographies were conducted with silica gel grade 230–400 mesh (Merck Kiesegel 60 Art 9385).

4.1.1. Methyl 3-(4-chlorobenzyl)-2,4-dioxoquinazoline-7-carboxylate (4). A solution of dimethyl aminoterephthalate (3.54 g, 16.9 mmol) in 1,4-dioxane (60 mL) was treated with triethylamine (5.8 mL, 42 mmol) and 1-chloro-4-isocyanatomethyl-benzene (2.9 mL, 22 mmol) at room temperature. The resulting mixture was heated up to 90 °C and stirred for 3 days. The reaction mixture was cooled down to room temperature and the precipitate was filtered, washed with diethylether, and dried to afford the desired product **4** (2.38 g, 6.90 mmol) in 41% yield: ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.73 (s, 1H), 8.06 (d, J = 8.2 Hz, 1H), 7.79 (s, 1H), 7.71 (d, J = 8.0 Hz, 1H), 7.36 (br s, 4H), 5.06 (s, 2H), 3.89 (s, 3H).

4.1.2. 3-(4-Chlorobenzyl)-2,4-dioxoquinazoline-7-carboxylic acid (5, R^1 = 4-chlorobenzyl). Methyl 3-(4-chlorobenzyl)-2,4-dioxoquinazoline-7-carboxylate **4** (2.54 g, 7.37 mmol) was dissolved in a 1:4 mixture of 10% NaOH and 1,4-dioxane, and the resulting mixture was stirred at room temperature for 1 h. The reaction mixture was neutralized with concd HCl and the resulting precipitate was filtered, washed with water, and dried to afford the desired product **5** (2.22 g, 6.71 mmol) in 91% yield: ^{1}H NMR (300 MHz, DMSO-d_6) δ 13.52 (s, 1H), 11.73 (s, 1H), 8.02 (d, J = 8.2 Hz, 1H), 7.78 (s, 1H), 7.69 (d, J = 8.2 Hz, 1H), 7.36 (br s, 4H), 5.07 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 166.80, 162.05, 150.56, 139.92, 137.75, 136.65, 132.21, 131.46, 129.25, 128.77, 128.31, 123.14, 116.82, 43.22.

Table 2. IC_{50} values of T-type calcium channel blocking activity of selected compounds by patch clamp assay



1

Entry	Compound	R^1	R^2	IC_{50} (μM)
1	**1a**	**q**	**ix**	2.46 ± 0.22
2	**1b**	**m**	**ix**	7.96 ± 0.61
3	**1c**	**h**	**ii**	16.87 ± 2.38
4	**1d**	**i**	**ii**	9.23 ± 0.68
5	**1e**	**q**	**ii**	2.08 ± 0.36
6	**1f**	**i**	**iv**	7.98 ± 0.55
7	**1g**	**q**	**iv**	3.38 ± 0.36
8	**1h**	**l**	**iv**	5.97 ± 1.23
9	**1i**	**q**	**vi**	1.95 ± 0.25
10	**1j**	**l**	**vi**	3.11 ± 1.28
11	**1k**	**i**	**vii**	4.63 ± 0.53
12	**1l**	**q**	**vii**	3.72 ± 0.20
13	**1m**	**i**	**viii**	3.31 ± 0.19
14	**1n**	**q**	**viii**	1.52 ± 0.23
15	**1o**	**p**	**viii**	2.79 ± 0.36
16	mibefradil			1.43 ± 0.49

4.1.3. 3-Cyclohexyl-2,4-dioxoquinazoline-7-carboxylic acid (5, R^1 = cyclohexyl). A solution of dimethyl 2-aminoterephthalate **2** (3.0 g, 14 mmol) and triphosgene (5.1 g, 17 mmol) in toluene (60 mL) was refluxed for 6 h. The resulting mixture was concentrated to dryness to give compound **6**. The compound **6** was dissolved in 1,4-dioxane (60 mL) and treated with triethylamine (0.2 mL). The reaction mixture was warmed up to 90 °C and stirred for 60 h. The resulting mixture was cooled to room temperature and diethyl ether (100 mL) was added to the mixture. The precipitate was filtered and washed with diethyl ether to give the desired product, dimethyl 2-(3-cyclohexylureido)-terephthalate **7** (1.75 g, 3.22 mmol) in 22% yield: ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.66 (s, 1H), 9.00 (s, 1H), 7.97 (d, J = 8.2 Hz, 1H), 7.51–7.48 (m, 2H), 4.75 (br s, 1H), 3.88 (s, 3H), 3.86 (s, 3H), 2.38 (br s, 2H), 1.82–1.54 (m, 4H), 1.29–1.11 (m, 4H).

Dimethyl 2-(3-cyclohexylureido)-terephthalate **7** (1.51 g, 5.23 mmol) was dissolved in a 1:3 mixture of 10% NaOH and MeOH, and the reaction mixture was stirred at 70 °C for 3 h. The resulting mixture was cooled to room temperature and acidified with concd HCl to give a precipitate. The precipitate was filtered, washed with water, and dried to give the desired product **5** (0.80 g, 2.8 mmol) in 54% yield: ^{1}H NMR (300 MHz, DMSO-d_6) δ 13.47 (s, 1H), 11.50 (s, 1H), 7.99 (d, J = 8.2 Hz, 1H), 7.71 (s, 1H), 7.65 (dd, J = 8.2 Hz, J = 1.3 Hz, 1H), 4.76–4.68 (m, 1H), 2.42–2.34 (m, 2H), 1.81–1.77 (m, 2H), 1.65–1.58 (m, 2H), 1.32–1.16 (m, 4H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 166.68, 162.27, 150.61, 139.86, 136.70, 128.38, 122.80, 117.52, 116.42, 53.47, 28.74, 26.43, 25.55.

4.1.4. 2-(3-(2-Ethylpiperidin-1-yl)propyl)isoindoline-1,3-dione (9). A solution of 2-ethylpiperidine (2.63 mL, 19.7 mmol) in acetonitrile (50 mL) was treated with 2-(3-bromopropyl)-isoindol-1,3-dione (4.4 g, 16 mmol) and K_2CO_3 (3.4 g, 25 mmol). The resulting mixture was heated up to 100 °C and stirred for 6 h. After concentration, the residue was diluted with CH_2Cl_2 (100 mL) and washed with water and the organic layer was dried over $MgSO_4$, filtered, and concentrated. The residue was purified with a 1:1 mixture of hexane and EtOAc by column chromatography on silica gel to give the desired product **9** (3.8 g, 13 mmol) in 81% yield: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.78–7.74 (m, 2H), 7.67–7.63 (m, 2H), 3.67–3.60 (m, 2H), 2.79–7.64 (m, 2H), 2.43–2.34 (m, 1H), 2.11 (bs, 2H), 1.82–1.77 (m, 2H), 1.55–1.44 (m, 6H), 1.21–1.19 (m, 2H), 0.78 (t, J = 7.4 Hz, 3H).

4.1.5. 3-(2-Ethylpiperidin-1-yl)propan-1-amine (10). A solution of 2-(3-(2-ethylpiperidin-1-yl)propyl)isoindoline-1,3-dione **9** (3.8 g, 13 mmol) in ethanol (50 mL) was treated with hydrazine monohydrate (2.5 mL, 50 mmol) at room temperature and the resulting mixture was refluxed for 1 h. The reaction mixture was cooled down to room temperature. The precipitate was filtered off and the filtrate was concentrated. The residue was diluted with 30 mL EtOAc and the precipitate was filtered off. The filtrate was concentrated to dryness to give the desired product **10** (1.6 g, 9.4 mmol) in 73% yield: ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.66–2.61 (m, 2H), 2.35–2.25 (m, 1H), 2.10–2.06 (m, 2H), 1.57–1.36 (m, 10H), 1.24–1.21 (m, 2H), 0.79 (t, J = 7.4 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 61.66, 51.50, 51.43, 40.91, 29.35, 28.12, 25.30, 23.32, 23.21, 10.08.

4.1.6. 3-(4-Chlorobenzyl)-*N*-(3-(2-ethylpiperidin-1-yl)propyl)-2,4-dioxoquinazoline-7-carboxamide (1n). 3-(4-Chlorobenzyl)-2,4-dioxoquinazoline-7-carboxylic acid **5** (90 mg, 0.27 mmol) was dissolved in thionyl chloride (4 mL) and the resulting solution was refluxed for 2 h. After concentration to dryness, the resulting acyl chloride was dissolved in CH_2Cl_2 (4 mL) and treated with 3-(2-ethylpiperidin-1-yl)propan-1-amine **10** (101 mg, 0.598 mmol) at 0 °C. The reaction mixture was stirred at room temperature for 2 h. After concentration, the residue was purified with a 10:1 mixture of CH_2Cl_2 and methanol by column chromatography on silica gel to give the desired product **1n** (58 mg, 0.12 mmol) in 44% yield: ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.65 (s, 1H), 8.71 (bs, 1H), 7.99 (d, J = 8.3 Hz, 1H), 7.59–7.55 (m, 2H), 7.34 (br s, 4H), 5.06 (s, 2H), 3.26–3.25 (m, 2H), 2.75–2.66 (m, 2H), 2.22 (bs, 1H), 2.12 (bs, 2H), 1.65–1.60 (m, 2H), 1.49–1.37 (m, 6H), 1.24–1.22 (m, 2H), 0.78 (t, J = 7.2 Hz, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 165.46, 162.08, 150.66, 141.33, 139.94, 136.72, 132.21, 130.02, 128.78, 128.15, 120.99, 115.77, 115.12, 61.04, 51.53, 50.90, 43.19, 38.65, 29.55, 25.99, 25.62, 23.63, 23.54, 10.03; IR (KBr) 3278, 1720, 1658, 1649 cm^{-1}; HR-MS (FAB, M+H) calcd for $C_{28}H_{38}ClN_4O_3$ 483.2163, found 483.2166.

4.2. Biological assay

4.2.1. FDSS6000 assay. HEK293 cells which stably express both α_{1G} and Kir2.1 subunits were grown in Dulbecco's modified Eagle's medium supplemented with 10% (v/v) fetal bovine serum, penicillin (100 U/mL), streptomycin (100 μg/mL), geneticin (500 μg/mL), and puromycin (1 μg/mL) at 37 °C in a humid atmosphere of 5% CO_2 and 95% air. Cells were seeded into 96-well black wall clear bottom plates at a density of 4×10^4 cells/well and were used the next day for high-throughput screening (HTS) FDSS6000 assay. For FDSS6000 assay, cells were incubated for 60 min at room temperature with 5 μM 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, and 13.8 glucose (pH 7.4). During the fluorescence-based FDSS6000 assay, α_{1G} T-type Ca^{2+} channels were activated using high concentration of KCl (70 mM) in 10 mM $CaCl_2$ contained Hepes-buffered solution and 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 FDSS6000 and related software (Hamamatsu, Japan).

4.2.2. Patch clamp assay. For the recordings of α_{1G} T-type Ca^{2+} currents, the standard whole-cell patch-clamp method was utilized as previously described.[21] 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).

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