Bioorganic & Medicinal Chemistry Letters xxx (2013) xxx–xxx

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Synthesis and biological evaluation of 1-(2-hydroxy-3-phenyloxypropyl)piperazine derivatives as T-type calcium channel blockers

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ARTICLE INFO

Article history:
Received 24 September 2012
Revised 10 December 2012
Accepted 21 December 2012
Available online xxxx

ABSTRACT

To obtain selective and potent inhibitor for T-type calcium channel by ligand based drug design, 2-hydroxy-3-phenoxypropyl piperazine derivatives were synthesized and evaluated for in vitro activities. Compound **6m** and **6q** showed high selectivity over *h*ERG channel (IC_{50} ratio of *h*ERG/α_{1G} **6m** = 8.5, **6q** = 18.38) and they were subjected to measure pharmacokinetics profiles. Among them compound **6m** showed an excellent pharmacokinetic profile in rats.

Voltage-dependent calcium channels are the main pathway for calcium ion influx to the cellular membrane. The mechanism used is the difference in membrane potential, and this plays an important physiological role in the excitability cells.[1] Based on electrophysiological and pharmacological characteristics, voltage-dependent calcium channels are classified as either high-voltage-activated (L, N, P, Q/R type) channel or low-voltage-activated (T-type) channels. Calcium channels consist of four subunits: α_1, $\alpha_2\delta$, β, and γ. The main structural component of voltage-dependent calcium channels is the α_1 subunit, which can be divided by molecular cloning into 10 subtypes; Ca_v 1.1–1.4 (L-type), Ca_v 2.1–2.3 (N, P, Q/R-type), Ca_v 3.1–3.3 (T-type).[2]

T-type calcium channels are expressed throughout the body, including nervous tissue, heart, kidney, smooth muscle, and many endocrine organs. Therefore, selective T-type calcium channel blockers may have high potentials for the treatment of some types of cancer, hypertension, cardiac arrhythmia, and CNS-related disorders[3] such as epilepsy, neurophatic pain[3] and insomnia.[4]

Mibefradil, the first marketed selective T-type calcium channel blocker, was withdrawn from the market due to its drug–drug interaction.[5] Unfortunately, there are no potent and selective T-type calcium channel blockers which are available for clinical use until now.

To discover a new structural motif of T-type calcium channel blockers, a three-dimensional common feature pharmacophore model was derived from eight structurally diverse T-type calcium channel blockers including mibefradil.[6] HipHop algorithm implemented in the CATALYST software was employed for hypothesis generation and the derived query was used for screening of commercial library such as ion channel and Maybridge 2001. The virtual screening identified hit compound (**1**) which overlaid well on our five feature phamacophore including three hydrophobic groups, one hydrogen bond acceptor and one positive ionizable feature (Fig. 1). Two hydrophobic groups matched with halogen substituents on benzyl moiety and another hydrophobic group mapped well onto phenyl ring of phenoxy moiety. Positive ionizable group mapped onto piperazine moiety and hydrogen bond acceptor matched well with hydroxyl group. From the above feature, we designed and synthesized a series of 1-(2-hydroxy-3-phenoxypropyl)piperazine derivatives (**4a–q**, **5a–r**, **6a–r**).

Synthesis of 1-(2-hydroxy-3-phenoxypropyl)piperazine derivatives is elucidated in Scheme 1. The phenols (**2**) were reacted with chiral epichlorohydrin to give oxirane (**3a–f**),[7] which was subjected to S_N2 displacement with derivatives of piperazine to give the target compounds **4a–q**, **5a–r** and **6a–r** (Scheme 1).[8]

As shown in Table 1, the first in vitro activities of the synthesized compounds **4a–q**, **5a–r** and **6a–r** were assayed using the FDSS6000 HTS (high-throughput screening) system.[9] Our initial synthetic plan was to fix the chloride on phenyl group (X = Cl) similar to compound **1**, and to change various piperazines. Also we'd like to figure out the relation between biological activities and compound chirality (**4a–q**). Most compounds in series of **4a–q** exhibited greater than 40% inhibitory activity against the α_{1G} calcium channel at 10 μM concentration. However, %inhibition values of compounds **4a–q** did not reach the hit compound (**1**, %inhibition 54.41) and mibefradil (%inhibition 81.00). Thus, the substituent on the phenyl group was changed to fluoride **5a–r** or to trifluromethyl group **6a–r**. The %inhibition values increased with both fluoride

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http://dx.doi.org/10.1016/j.bmcl.2012.12.072

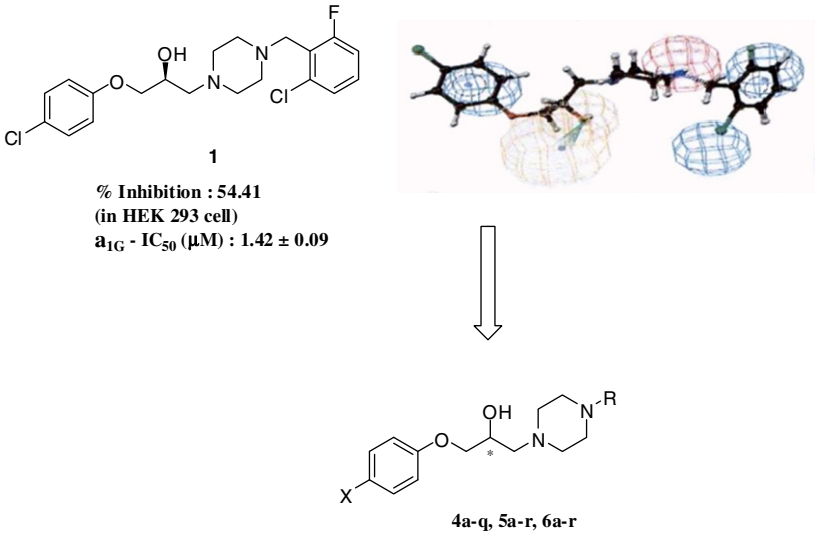
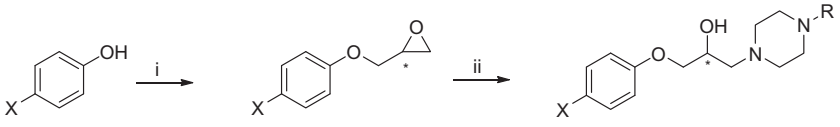
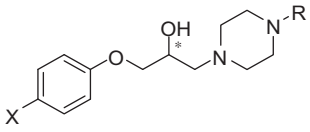


Figure 1. Designed structures of T-type calcium channel blocker and the structural mapping with **2** of **1**. Red sphere: positive ionizable, yellow sphere: hydrogen bond acceptor; Blue sphere: hydrophobic group.



Scheme 1. Reagents and conditions: (i) (R/S)-epichlorohydrin, K_2CO_3, 2-butanone, reflux; (ii) piperazine derivatives, MeOH, rt.

Table 1
In vitro activities for T-type calcium channel blockers of 2-hydroxy-3-phenoxypropyl piperazine derivatives using FDSS6000 HTS system



Entry	Compound (R/S)	X	R	%inhibition[a] (10 μM)	
				R-configuration	*S*-configuration
1	**4a**/**4j**	Cl	2-Fluorophenyl	46.93	35.90
2	**4b**/**4k**	Cl	4-Fluorophenyl	39.69	28.32
3	**4c**/**4l**	Cl	2-Methoxyphenyl	47.38	48.81
4	**4d**/**4m**	Cl	3-Methoxyphenyl	44.60	45.99
5	**4e**/**4n**	Cl	3-Chlorobenzyl	45.95	52.18
6	**4f**/**4o**	Cl	4-Chlorobenzyl	42.12	56.46
7	**4g**/**4p**	Cl	3,4-Dichlorobenzyl	48.61	41.17
8	**4h**/**1**[b]	Cl	2-Chloro-6-fluorobenzyl	40.86	54.41
9	**4i**/**4q**	Cl	2-Fluorobenzyl	35.76	41.46
10	**5a**/**5j**	F	2-Fluorophenyl	51.15	42.00
11	**5b**/**5k**	F	4-Fluorophenyl	47.48	49.99
12	**5c**/**5l**	F	2-Methoxyphenyl	50.67	41.02
13	**5d**/**5m**	F	3-Methoxyphenyl	61.30	44.46
14	**5e**/**5n**	F	3-Chlorobenzyl	61.10	65.79
15	**5f**/**5o**	F	4-Chlorobenzyl	50.14	65.76
16	**5g**/**5p**	F	3,4-Dichlorobenzyl	69.45	64.18
17	**5h**/**5q**	F	2-Chloro-6-fluorobenzyl	56.45	62.07
18	**5i**/**5r**	F	2-Fluorobenzyl	23.77	20.34
19	**6a**/**6j**	CF_3	2-Fluorophenyl	50.21	48.88
20	**6b**/**6k**	CF_3	4-Fluorophenyl	53.74	59.22
21	**6c**/**6l**	CF_3	2-Methoxyphenyl	56.24	50.84
22	**6d**/**6m**	CF_3	3-Methoxyphenyl	43.94	68.55
23	**6e**/**6n**	CF_3	3-Chlorobenzyl	43.13	43.42

Please cite this article in press as: Park, J.-e.; et al. *Bioorg. Med. Chem. Lett.* (2013), http://dx.doi.org/10.1016/j.bmcl.2012.12.072

Table 1 (*continued*)

Entry	Compound (R/S)	X	R	%inhibition[a] (10 μM)	
				R-configuration	*S*-configuration
24	**6f**/**6o**	CF_3	4-Chlorobenzyl	61.54	56.57
25	**6g**/**6p**	CF_3	3,4-Dichlorobenzyl	38.81	45.62
26	**6h**/**6q**	CF_3	2-Chloro-6-fluorobenzyl	44.57	55.09
27	**6i**/**6r**	CF_3	2-Fluorobenzyl	45.46	48.58
Mibefradil				81.00	

[a] %inhibition value was obtained at 10 μM.
[b] Hit compound from pharmacophore modeling.

Table 2
Inhibitory activities of selected compounds against T-type calcium channel and *h*ERG

Compound	α_{1G}-IC_{50} (μM)	*h*ERG-IC_{50} (μM)	*h*ERG-IC_{50}/α_{1G}-IC_{50} (μM)
4o	1.08 ± 0.02	0.32 ± 0.09	0.30
5d	12	—	
5e	2.1 ± 0.78	NA	
5g	0.1 ± 0.05	0.87 ± 0.13	8.70
5h	12.33 ± 1.2	—	
5n	2.58 ± 1.23	—	
5o	1.56 ± 0.89	1.35 ± 0.05	0.87
5p	0.23 ± 0.01	0.29 ± 0.02	1.26
5q	11.38 ± 3.03	—	
6c	1.79 ± 1.1	3.95 ± 0.15	2.21
6g	1.27 ± 0.37	9.2 ± 0.5	7.24
6k	15.5 ± 6.3	—	
6m	0.48 ± 0.27	4.08 ± 1.28	8.50
6o	4.23 ± 0.42	—	
6q	0.76 ± 0.62	13.97 ± 8.3	18.38
1	1.42 ± 0.09	2.07 ± 5.04	1.46
Mibefradil	0.83 ± 0.19	1.40 ± 0.29	1.69

5a–**r** and trifluoromethyl group **6a**–**r**, indicating that the presence of fluoride on the phenyl ring is important for the inhibition. In addition, there was no significant effect between the stereochemistry of the compounds and respect to %inhibitory activities.

Compounds that showed above 55% inhibitory activity against T-type calcium channels were evaluated with the whole-cell patch-clamp method to measure the IC_{50} values for α_{1G} in HEK 293 cell and IC_{50} values for *h*ERG channel.[10] With this data obtained we could choose with the selective lead compound over *h*ERG/α_{1G} channel. Blocking of the *h*ERG channel can lead to a heart rhythm disorder, such as long QT syndrome which is characterized by prolonged action potential of ventricular muscle.[11] Thus, the meaning of the IC_{50} of *h*ERG/α_{1G} shows selective inhibition of the T-type calcium channel. Most compounds showed good activities against T-type calcium channel (Table 2). When R group was 4-chlorobenzyl, it had good potency in IC_{50} values of α_{1G}, but didn't showed selectivity between α_{1G} and *h*ERG channel (**4o**, **5o**). Whereas some compounds (**5g**, **5p**, **6m**, **6q**) showed strong potency than hit compound (**1**) and mibefradil. 3,4-Dichlorobenzyl substituent on R group showed that the compounds including (*R*)-configuration (**5g**, **6g**) showed better potency and selectivity than (*S*)-configuration. Biological activities of compound **6m** (R = 3-methoxyphenyl) and **6q** (2-chloro-6-fluorobenzyl) were affected by X group on phenyl, not R group on piperazine. Moreover compound **6m** (IC_{50} of α_{1G} = 0.83 ± 0.19, IC_{50} of *h*ERG = 4.08 ± 1.28) and **6q** (IC_{50} of α_{1G} = 0.76 ± 0.62, IC_{50} of *h*ERG = 13.97 ± 8.3) IC_{50} ratio of *h*ERG/α_{1G} channel than mibefradil (IC_{50} of α_{1G} = 0.48 ± 0.27, IC_{50} of *h*ERG = 1.40 ± 0.29) and they were chosen to perform further biological assay.

In order to determine the potential of compounds **6m** and **6q** as a lead compound for therapeutic targets, these were subjected to measure pharmacokinetic profiles. The pharmacokinetic data for **6m** and **6q** after oral administration in rats are presented Figure 2 and Table 3. Both compounds **6m** and **6q** were observed to be rapidly absorbed in plasma (T_{max}). Concentration–time profiles showed that compound **6m** lasted longer than **6q** in plasma and brain (Fig. 2). Moreover, compound **6m** possessed good oral bioavailability (*F*, 67.25%) and showed a good AUC_{brain}/AUC_{plasma} ratio of 346.5% (Table 3).

In summary, the series of 2-hydroxy-3-phenoxypropyl derivatives **4a**–**q**, **5a**–**r** and **6a**–**r** was designed, synthesized and evaluated as T-type calcium channel blockers. Using the FDSS assay system, we rapidly screened the title compounds and selected several hav-

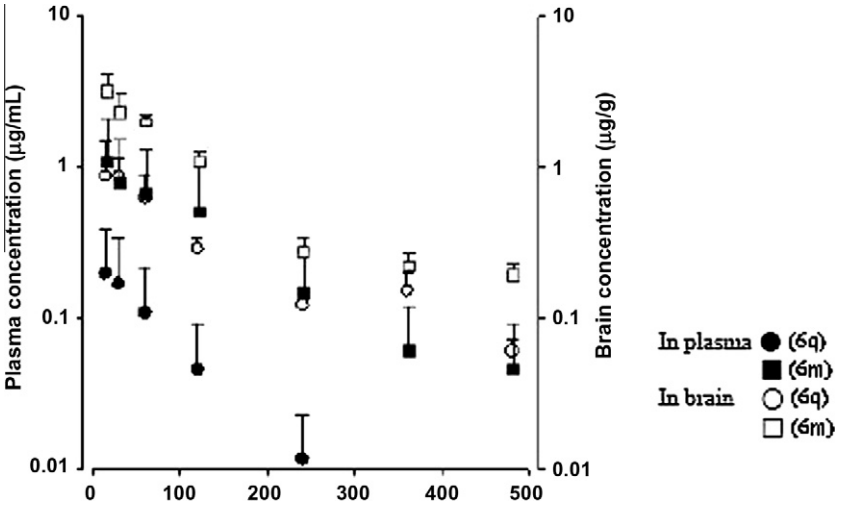


Figure 2. Mean plasma (•) or brain (°) concentration–time profiles after oral administration of **6m** and **6q** at a dose of 10 mg/kg to mice (n = 3 per time point). Bars represent standard deviation.

Table 3
Brain pharmacokinetic parameters after oral administration of **6m** (10 mg/kg) and **6q** (10 mg/kg) to ICR mice (n = 3 per time point)

	6m		6q	
	Plasma	Brain	Plasma	Brain
$AUC_{0-\infty}$ (μg min/ml or μg min/g)	198.1	686.6	16.55	—
AUC_{last} (μg min/ml or μg min/g)	188.5	497.4	15.61	119.1
Terminal half-life (min)	102.4	474.3	56.54	185.9
C_{max} (μg/mL)	1.566	4.662	0.1974	0.8702
T_{max} (min)	15	15	15	30
AUC_{brain}/AUC_{plasma} (%)	346.5		763.0	
F (%)	67.25		9.935	

Values are presented as mean. C_{max}, peak plasma and brain concentration; T_{max}, time to reach C_{max}; AUC (area under the plasma concentration versus time curve) = dose/clearance; F, bioavailability; AUC_{brain}/AUC_{plasma}, equal to B/P ratio.

ing high potency. Especially compound **6m** and **6q** showed high selectivity over *h*ERG channel and against T-type calcium channel (IC_{50} ratio of *h*ERG/α_{1G} **6m** = 8.5, **6f** = 18.38). Compound **6m** showed excellent pharmacokinetic profile in rats. Our results show promise because we gained proof of concept using a ligand-based drug design with a new target. Further assays with neuropathic pain models and structural optimization will be followed with this scaffold.

Acknowledgment

We thank Medifron DBT for pain animal test. This work was supported by the research fund of Korea Institute of Science and Technology (2E 22760).

Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2012.12.072.

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8. All data of compounds such as experimental procedure, and spectroscopy data are mentioned on Supplementary data.
9. *Experimental procedure for the FDSS6000 assay:* HEK 293cells which express both stable 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 40,000 cells/well and were used on the next day for high-throughput screening (HTS) FDSS 6000 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, 13.8 glucose (pH 7.4). During fluorescence-based FDSS6000 assay, α_{1G} T-type Ca^{2+} channels were activated using a high concentration of KCl (70 mM) in 10 mM $CaCl_2$ contained a HEPES-buffered solution, and the increase in $[Ca^{2+}]_i$ by KCl-induced depolarization was detected. Throughout the entire procedure, cells were washed in a BIO-TEK 96-well washer. All data were collected and analyzed using FDSS6000 and related software (Hamamatsu, Japan).
10. Experimental procedure for the patch-clamp test (electro-physiological recording): For the recording 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 MX were pulled and filled with the internal solution containing (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 to −30 mV. The molar concentration 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). For more details, see: (a) Rhim, H.; Lee, Y. S.; Park, S. J.; Chung, B. Y.; Lee, J. Y. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 283; (b) Choi, K. H.; Song, C.; Shin, D.; Park, S. *Biochim. Biophys. Acta, Biomembr.* **2011**, *1808*, 1560.
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