Short Communication

Synthesis and SAR Study of T-Type Calcium Channel Blockers. Part II

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3,4-Dihydroquinazoline derivatives have been known to be the novel and potent T-type calcium channel blockers. From a systematic variation of 3,4-dihydroquinazoline derivative **5c** (**KYS05043**), plausible SAR results were established. It was revealed that a 5-(dimethylamino)pentylamino group at R^1, a biphenyl group at R^2, and a benzyl amido group at R^3 in the 3,4-dihydroquinazoline backbone are closely related with the channel selectivity (T/N-type) as well as the potency based on the discovery of **6k** (**KYS05090**).

Keywords: Blockers / 3,4-Dihydroquinazoline / SAR study / T-type calcium channel

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Introduction

Calcium channels are the primary route for translating electrical signals into the biochemical events underlying key processes such as neurotransmitter release, cell excitability, and gene expression [1]. Among calcium channels, T-type or low voltage activated (LVA) calcium channels are thought to contribute to neuronal excitability and also play crucial roles in the control of blood pressure [2], and they promise to provide the important therapeutic targets for the treatment of epilepsy, neuropathic pain, and cardiovascular diseases such as hypertension and angina pectoris [3]. Therefore, many researchers have been awaiting a specific T-type calcium channel blocker for the exact understanding of the pathophysiological role of T-type channel since the withdrawal of mibefradil in 1996 (Fig. 1). For this reason, we have also tried to identify new compounds with higher potency and selectivity for T-type channel and reported

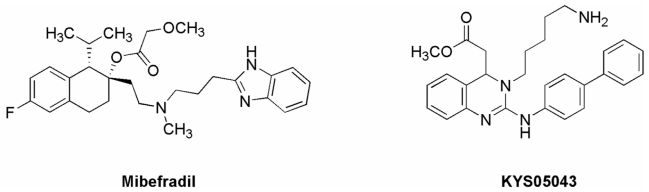

Figure 1. Mibefradil and KYS05043.

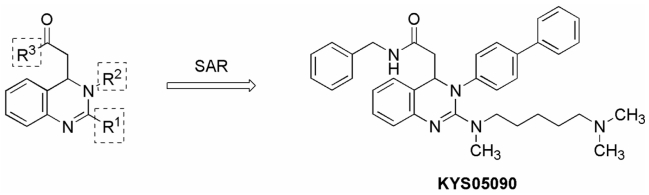

Figure 2. SAR study *via* the modifications of R^1, R^2, and R^3 substituents.

the synthesis of novel T-type channel blockers based on a 3,4-dihydroquinazoline backbone [4–7]. Through an intensive SAR (structure-activity relationship) study, we had recently discovered the lead-like compound **KYS05090** starting form **KYS05043** shown in Fig. 2 [5, 7]. Herein, we will discuss the detailed structure-activity relationship of 3,4-dihydroquinazoline based on the discovery of **KYS05090** (Fig. 2).

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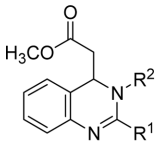
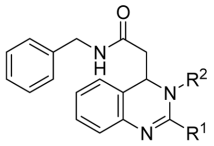
Abbreviations: low voltage activated (LVA)

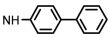
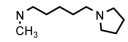
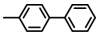
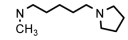
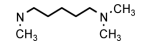
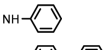
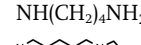
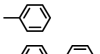
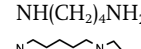
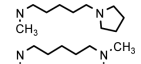
Table 1. *In vitro* calcium channel blocking effects of 3,4-dihydroquinazoline derivatives.



5a-k



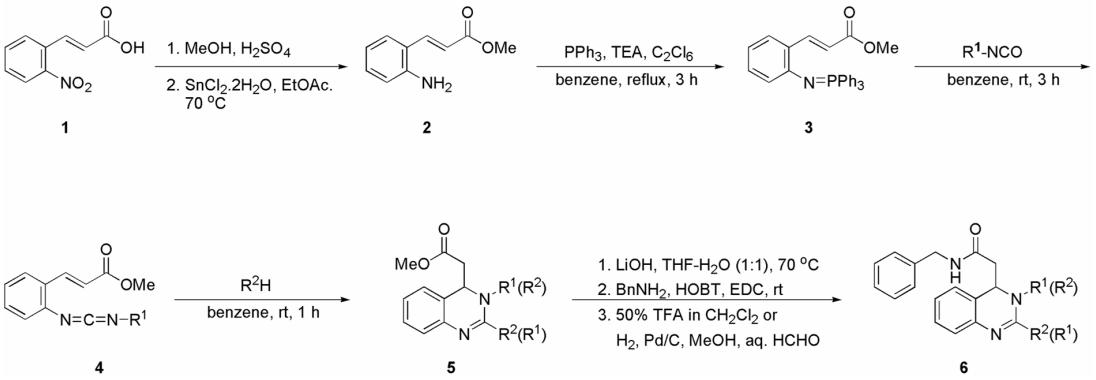
6a-k

Entry	R_1	R_2	HEK293 cells T-Type (α_{1G})		HEK293 cell N-Type (α_{1B})	Selectivity (T/N-Type)[c]
			% Inhibition[a] (10 μM)	IC_{50} (μM)[b]	% Inhibition (10 μM)	
5a		$(CH_2)_5NH_2$	51.9 ± 1.1	9.16 ± 0.32	3.0 ± 2.6	17.3
5b		$(CH_2)_5NHBoc$	90.0 ± 0.7	2.27 ± 0.49	54.2 ± 2.1	1.6
5c (KYS05043)		$(CH_2)_5NH_2$	84.1 ± 1.6	0.30 ± 0.09	7.5 ± 0.7	11.2
5d		$(CH_2)_5NHBoc$	88.5 ± 0.4	0.37 ± 0.08	94.9 ± 1.7	0.9
5e	$NH(CH_2)_4NH_2$		84.1 ± 0.6	1.84 ± 0.15	4.3 ± 2.6	19.5
5f	$NH(CH_2)_4NHBoc$		87.7 ± 1.2	2.02 ± 0.15	53.1 ± 2.5	1.7
5g			59.5 ± 1.1	5.84 ± 0.44	10.7 ± 2.3	5.6
5h	$NH(CH_2)_4NH_2$		82.5 ± 0.7	0.56 ± 0.10	No Blocking[d]	⩾100
5i	$NH(CH_2)_4NHBoc$		86.5 ± 0.5	0.68 ± 0.18	98.6 ± 1.3	0.9
5j			94.9 ± 1.2	0.34 ± 0.04	36.5 ± 0.2	2.6
5k			90.1 ± 2.3	0.23 ± 0.03	24.4 ± 2.8	3.7
6a		$(CH_2)_5NHBoc$	92.9 ± 1.7	1.20 ± 0.12	35.0 ± 1.8	2.7
6b		$(CH_2)_5NH_2$	67.4 ± 1.1	4.54 ± 0.62	No Blocking[d]	⩾ 100
6c		$(CH_2)_5NHBoc$	88.5 ± 0.6	0.17 ± 0.03	30.1 ± 1.1	2.9
6d		$(CH_2)_5NH_2$	91.8 ± 1.9	0.14 ± 0.01	15.2 ± 2.4	6.0
6e	$NH(CH_2)_4NHBoc$		97.5 ± 1.2	0.57 ± 0.05	75.1 ± 0.1	1.3
6f	$NH(CH_2)_4NH_2$		72.4 ± 0.3	3.13 ± 0.27	1.7 ± 1.1	42.5
6g			68.1 ± 0.5	4.20 ± 0.20	46.3 ± 0.3	1.5
6h	$NH(CH_2)_4NHBoc$		88.3 ± 1.5	0.16 ± 0.02	16.6 ± 0.7	5.3
6i	$NH(CH_2)_4NH_2$		83.8 ± 1.4	0.13 ± 0.01	8.3 ± 1.8	10.1
6j			88.1 ± 1.7	0.26 ± 0.01	11.7 ± 5.8	7.5
6k (KYS05090)			98.0 ± 1.6	0.04 ± 0.00	70.6 ± 3.1 (4.9 μM)[e]	1.4 (119.5)[f]
Mibefradil			95.9 ± 1.7	1.34 ± 0.49	67.6 ± 1.2	1.4

[a] Percent inhibition value (± SE) was obtained by repeated procedures (n ≥ 4).
[b] IC_{50} value was determined from the dose-response curve.
[c] % inhibition ratio at 10 μM.
[d] "No blocking" means that the inhibition was less than 1%.
[e] IC_{50} value against N-type channel.
[f] Selectivity value based on IC_{50} value ratio.

Synthesis

Referring to the structures listed in Table 1, 3,4-dihydroquinazoline derivatives were easily prepared according to Scheme 1 using our set-up procedure [4 – 6]. The iminophosphorane derivative **3** was directly prepared by the Appel's method (PPh_3-C_2Cl_6-Et_3N reagent system) from methyl 2-aminocinnamate **2** [8], which was derived from commercially available 2-nitrocinnamic acid **1** using a procedure described earlier [6]. The intermolecular aza-



Reactants: R^1-NCO = Ph-NCO, 4-Ph-Ph-NCO; R^2H = $H_2N(CH_2)_5NH_2$, $H_2N(CH_2)_5NHBoc$, 1-pyrrolidinyl$(CH_2)_5NH(CH_3)$, $Bn_2N(CH_2)_5NH(CH_3)$.

Scheme 1. Synthesis of the 3,4-dihydroquinazoline derivative **6**.

Wittig reaction of iminophosphorane **3** with aryl isocyanate (R^1-NCO) in benzene provided carbodiimide **4**, which was treated with an amine nucleophile to afford the 3,4-dihydroquinazoline **5**. In the case of the primary amine (R^2H) attack on carbodiimide **4**, the resulting two regioisomers **5** could be separated by silica gel column chromatography and their structures could be completely elucidated by ^{1}H-NMR technique such as NOESY as reported earlier [5]. The hydrolysis of compound **5** with LiOH provided the free carboxylic compound, which was coupled with benzylamine in the presence of EDC and HOBT, afforded the benzyl amide **6** [9]. Finally, deprotection of *tert*-butoxy (50% TFA in CH_2Cl_2) or dibenzyl group (H_2, Pd/C, MeOH, aq. HCHO) was carried out to provide the corresponding 3,4-dihydroquinazoline derivative **6** as depicted in Scheme 1 [7].

Results and discussion

The *in-vitro* calcium channel blocking activities of 3,4-dihydroquinazoline derivatives were determined in T-type (α_{1G}) and N-type channels (α_{1B}), respectively, stably expressed in HEK293 cells, by whole-cells patch-clamp methods at 10 μM concentration [10]. For the exact potency of the compounds, their IC_{50} values required to produce 50% inhibition of α_{1G} T-type currents were again determined from fitting raw data into dose-response curves. *In-vitro* blocking data of the compounds are summarized in Table 1.

Compared to mibefradil (96%), most of compounds showed good inhibitory activity (>84%) against T-type calcium channel (α_{1G}) at a concentration of 10 μM except **5a**, **5g**, **6b**, **6f**, and **6g**. Among them, compounds **6e** and **6k** showed better activity (>97%) than mibefradil. With

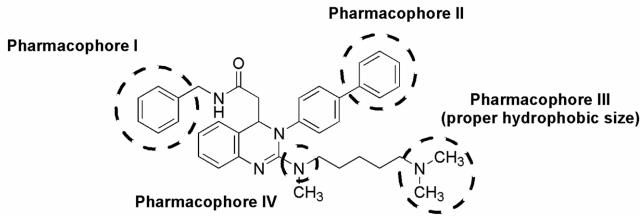


Figure 3. Pharmacophores of 3,4-dihydroquinazlone compound for T-type channel blocking.

respect to IC_{50} values, both of percent inhibition and IC_{50} values showed linear relationships except for **5b.** Moreover, fourteen synthetic compounds are more potent than mibefradil. In particular, compound **6k** (**KYS05090**) is most potent and approximately 33-fold more potent (IC_{50} = 40 nM) than the reference. Based on this structure-activity relationship, more hydrophobic moieties are required at both R^1 and R^2 substituents irrespective of the position when comparing the phenyl group with the biphenyl group (for example, **5a**–**b** vs. **5c**–**d** & **6a**–**b** vs. **6c**–**d**).

Secondly, the benzylamido group exhibited a higher potency than the methyl ester group at the R^3 substituent (**5** vs. **6**), which means that a hydrophobic moiety is also required at R^3 position. In the case of the R^1 position, the terminal part of the chain is required to have a proper hydrophobic moiety, when comparing the activity of **6k** (IC_{50} = 40 nM) with that of **6j** (IC_{50} = 0.26 μM). With regard to the N-type channel (α_{1B}), in the meanwhile, two compounds (**5d** and **5i**) showed higher inhibitory activity against the N-type channel and, thus, poor channel selectivity. However, two compounds (**5h** and **6b**) did not block the N-type channel (less than 1%) and thus showed the highest selectivity for the T-type channel. However,

these data did not provide the general relationship between N-type channel selectivity and N-type channel blocking effect. In case of the most potent compound **6k** (**KYS05090**), we obtained its IC_{50} value (4.9 μM) against N-Type channel (α_{1B}) by patch – clamp assay. As a result, this compound showed higher selectivity (ca. 120 = 4.9/0.04) for T-type over N-type calcium channel compared to a value (ca. 1.4) based on the percent inhibition ratio.

Based on the discovery of lead-like **6k** (**KYS05090**), in summary, we have obtained the following structure – activity relationship (SAR) together with the previous result (pharmacophore IV) as illustrated in Fig. 3. Further studies to acquire more information about structure – activity relationships are in progress in our laboratory.

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The authors have declared no conflict of interest.

References

[1] J. W. Barclay, A. Morgan, R. D. Burgoyne, *Cell Calcium* **2005**, *38*, 343 – 353; X. Zheng, J. A. Bobich, *Brain Res. Bull*. **1998**, *47*, 117 – 128; B. Himpens, L. Missiaen, R. Casteels, *J. Vasc. Res.* **1995**, *32*, 207 – 219; A. J. Levi, P. Brooksby, J. C. Hancox, *Cardiovasc. Res*. **1993**, *27*, 1743 – 1757.

[2] L. H. Opie, W. H. Frishman, U. Thadani in *Calcium Channel Antagonists (calcium entry blockers) in Drugs for the Heart* (Ed.: L. H. Opie), 4th Ed., W. B Saunders, *Philadelphia,* **1994**, p. 50; D. R. Abernethy, J. B. Schwartz, *N. Engl. J. Med*. **1999**, *341*, 1447 – 1457; U. Thadani, *Curr. Opin. Cardiol.* **1999**, *14*, 349 – 358.

[3] M. T. Nelson, S. M. Todorovic, *Curr. Pharm. Des.* **2006**, *12*, 2189 – 2197; E. Perez – Reyes, *Physiol. Rev*. **2003**, *83*, 117 – 161; G. Vassort, J. Alvarez, *J. Cardiovasc. Electrophysiol*. **1994**, *5*, 376 – 393.

[4] J. Y. Choi, H. N. Seo, M. J. Lee, S. J. Park, *et al*., *Bioorg. Med. Chem. Lett.* **2007**, *17*, 471 – 475; S. J. Park, S. J. Park, M. J. Lee, H. Rhim, *et al*., *Bioorg. Med. Chem.* **2006**, *14*, 3502 – 3511.

[5] H. Rhim, Y. S. Lee, S. J. Park, B. Y. Chung, J. Y. Lee, *Bioorg. Med. Chem. Lett*. **2005**, *15*, 283 – 286.

[6] Y. S. Lee, B. H. Lee, S. J. Park, S. B. Kang, *et al*., *Bioorg. Med. Chem. Lett*. **2004**, *14*, 3379 – 3384.

[7] H. N. Seo, J. Y. Choi, Y. J. Choe, Y. Kim, *et al*., *Bioorg. Med. Chem. Lett*. **2007**, *17*, 5740 – 5743.

[8] R. Appel, M. Halstenberg in *Organophosphorus Reagents in Organic Synthesis* (Ed.: J. I. G. Cadogan) Academic Press, London, **1979**, p. 378ff; T. Okawa, N. Osakada, S. Eguchi, A. Kakehi, *Tetrahedron* **1997**, *53*, 16061 – 16082.

[9] A. Gaucher, Y. Zuliani, D. Cabaret, M. Wakselman, J.-P. Mazaleyrat, *Tetrahedron Asymmetry* **2001**, *12*, 2571 – 2580; M. K. Dhaon, R. K. Olesen, K. Ramasamy, *J. Org. Chem*. **1982**, *47*, 1962 – 1965.

[10] A. Monteil, J. Chemin, E. Bourinet, G. Mennessier, *et al*., *J. Biol. Chem*. **2000**, *275*, 6090 – 6100; T. Kim, J. Choi, S. Kim, O. Kwon, *et al*., *Biochem. Biophys. Res. Commun.* **2004**, *324*, 401 – 408.

