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Discovery of potent T-type calcium channel blocker

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Abstract—The intensive SAR study of 3,4-dihydroquinazoline series led to the most potent compound **10** (**KYS05090**: $IC_{50} = 41 \pm 1$ nM) against T-type calcium channel and its potency is nearly comparable to that of Kurtoxin. As a small organic molecule, this compound showed the highest blocking activity reported to date.

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] Therefore, T-type calcium channels are important therapeutic targets for the treatment of epilepsy, neuropathic pain, and cardiovascular diseases such as hypertension and angina pectoris.[3] For example, a T-type calcium channel blocker, mibefradil (Posicor™), was used in treatment of hypertension and stable angina.[4] Shortly following its introduction, mibefradil was withdrawn from the US market in May 1998 because of potential harmful interactions with other drugs.[5] Since the withdrawal of mibefradil, efforts aimed at discovery of new T-type calcium channel blockers have intensified.[6] We have recently reported that 3,4-dihydroquinazoline (**KYS05001**), as a new scaffold, showed T-type calcium channel blocking effect comparable to mibefradil.[7] In a development of this class, we have also reported that 2-aminopentyl-substituted 3,4-dihydroquinazoline (**KYS05044**) is more potent than **KYS05001** (Fig. 1).[7c] As a result of further investigation of structure–activity relationships in **KYS05044** series, we found that **KYS05090** is the most potent T-type calcium channel blocker reported to date. Herein, we report the synthesis and biological evaluation of **KYS05090** compound.

Compound **KYS05090** was synthesized by the same procedure as described previously by our group as shown in Schemes 1 and 2. Amine nucleophile **4**, *N*,*N*-dibenzyl-*N*′-methylpentane-1,5-diamine, was prepared from 1,5-diaminopentane **1**. Monoprotection of diamine **1** with di-*tert*-butyl dicarbonate afforded compound **2** and the other amine group was treated with 2 equivalents of benzyl bromide to provide dibenzyl compound **3**. The reduction of Boc group with lithium aluminum hydride gave a secondary amine **4** (Scheme 1).[8] Azaphosphorane compound **6** was prepared from 2-nitrocinnamic acid **5** via 3 steps. The coupling of **6** with biphenylyl isocyanate gave the carbodiimide **7**, which was treated with amine **4** to provide 3,4-dihydroquinazoline derivative **8** via tandem nucleophilic addition and intramolecular conjugate addition. Hydrolysis of compound **8** with LiOH afforded the free carboxylic acid **9**. The coupling reaction of **9** with benzylamine, EDC, and HOBt followed by hydrogenation with 10% Pd/C in the presence of formaldehyde afforded the compound **10** (**KYS05090**). For comparison of biological activity, ester compound **8** was also treated with 10% Pd(C) under the same condition to provide **11** (**KYS05089**) as shown in Scheme 2. Both of *N*,*N*-dimethylated compounds (**10** and **11**) are formed *via* the tandem debenzylation-reductive amination: that is, debenzylated free amine group was further reacted with formaldehyde to convert into imine group,

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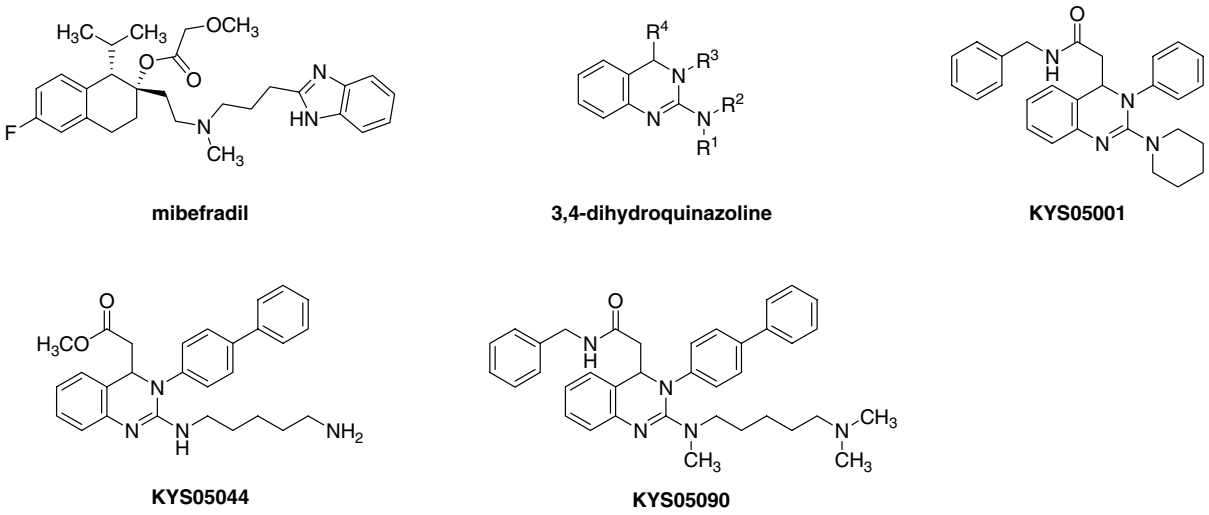
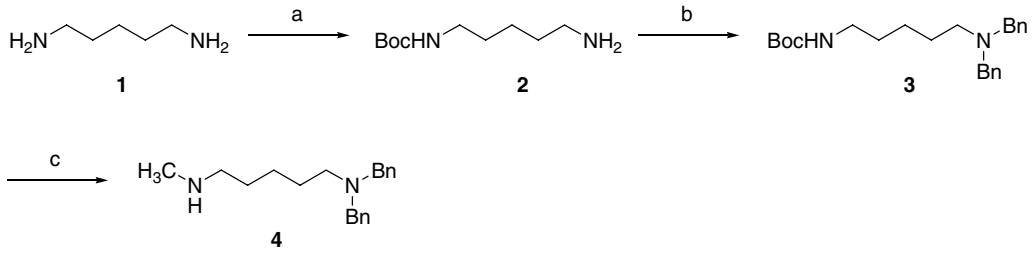


Figure 1. Mibefradil and 3,4-dihydroquinazoline series.



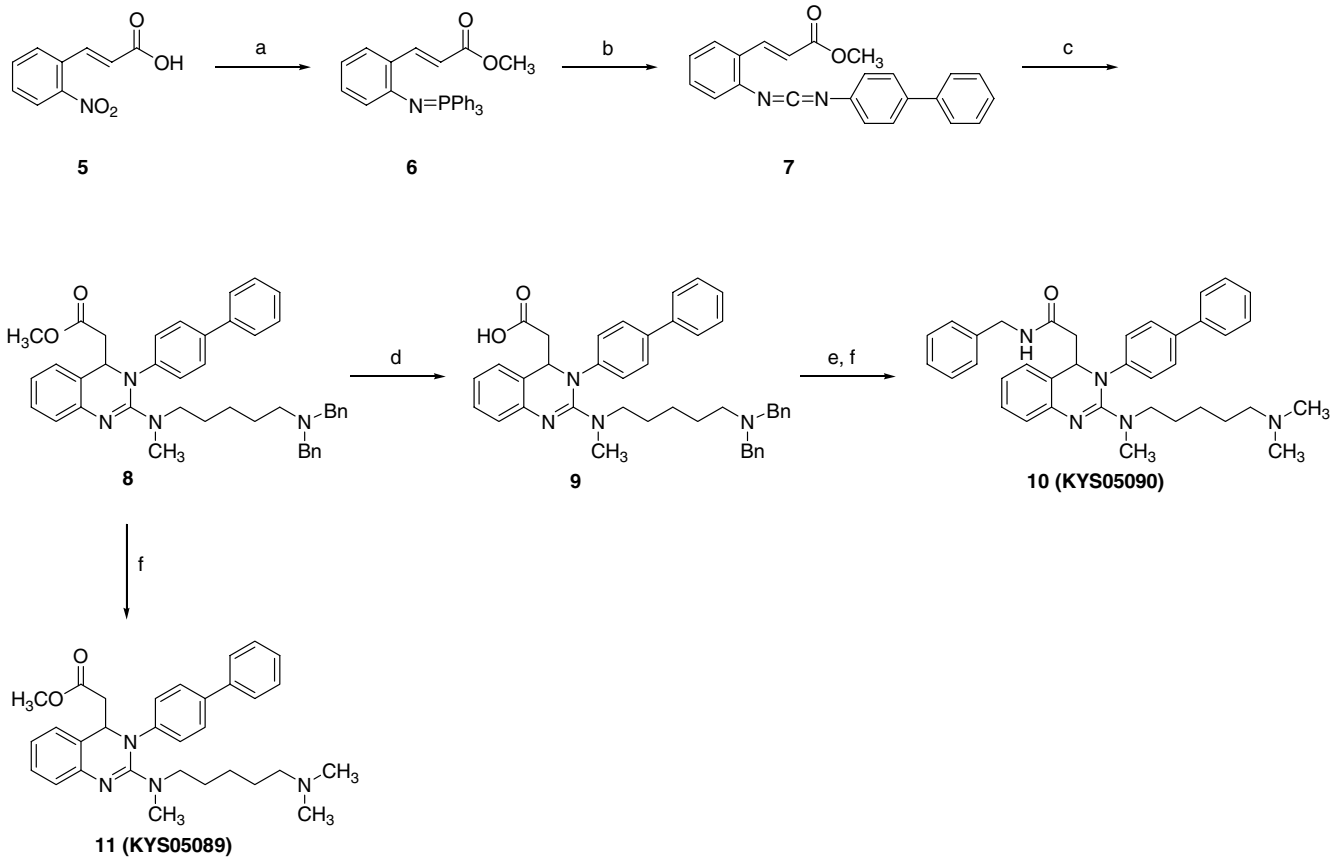
Scheme 1. Reagents and conditions: (a) (^{t}Boc)$_2$O, CH_2Cl_2, rt, 51%; (b) 2 equiv of benzyl bromide, Na_2CO_3, CH_2Cl_2, reflux, 49%; (c) $LiAlH_4$, THF, 70 °C, 60%.

which was hydrogenated to afford *N*,*N*-dimethylated compound.[9]

The biological activity of new synthesized compounds (**10** and **11**) 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.[10] The % inhibition of Ca^{2+} current was measured at 10 μM concentration of the synthesized compounds. For this purpose, two assay methods were employed: high-throughput screening (FDSS6000 system)[11] and patch–clamp assay using a single cell.[10] High-throughput screening is applied for the preliminary screening. On the other hand, patch–clamp assay is more accurate and sensitive but measures Ca^{2+} current one by one. Compounds were primary-screened by high-throughput screening and secondary-screened by patch–clamp assay to obtain IC_{50} values. Their in vitro blocking data are summarized in Table 1 and the data of **KYS05001** and **KYS05044** were also inserted for comparison.

With respect to % inhibition at 10 μM concentration, both the new synthetic compounds (**10** and **11**) exhibited lower % inhibitory activity than mibefradil under high-throughput screening (FDSS6000 system). However, accurate patch–clamp assay showed that both compounds were more or less active than mibefradil but superior to **KYS05044**. Compound **10** exhibited the most potency among them. With respect to IC_{50} value, both compounds (**10** and **11**) were found to be more potent than the other compounds. In accordance with % inhibition data, compound **10** exhibited the most potency (IC_{50} = 41 ± 1 nM). This potency is nearly comparable to that of Kurtoxin (IC_{50} = 15 nM), a peptide compound from the venom of the south African scorpion.[12] To the best of our knowledge, **10** (**KYS05090**) shows the most powerful activity compared to small organic molecules reported to date. With respect to channel selectivity (T/N-type calcium channel), both compounds (**10** and **11**) showed less selectivity than parent compound **KYS05044** based on % inhibition at 10 μM concentration. To determine the exact channel selectivity of **10** (**KYS05090**), however, we obtained its IC_{50} value (4.9 μM) against N-type channel (α_{1B}) by patch–clamp assay. As a result, compound **10** (**KYS05090**) showed higher selectivity for T-type over N-type calcium channel than based on % inhibition [Selectivity index: 119.5 = 4.9 μM (N-type)/0.041 μM (T-type)].

In summary, new compounds **10** and **11** (**KYS05090** and **KYS05089**) were synthesized as a result of further structure–activity relationship study in **KYS05044** series.[13] Compound **10** (**KYS05090**) showed the comparable potency to Kurtoxin as well as high selectivity for T-type calcium channel. The detailed result of SAR study in **KYS05044** series will be reported in due course.



Scheme 2. Reagents and conditions: (a) (i) H_2SO_4, MeOH, 70 °C; (ii) $SnCl_2{\cdot}2H_2O$, EtOAc, 70 °C; (iii) PPh_3, C_2Cl_6, Et_3N, toluene, reflux, 60% (3 steps); (b) 4-biphenylyl isocyanate, toluene, rt, 80%; (c) compound **4**, toluene, rt, >99%; (d) LiOH, THF-H_2O (1:1), 70 °C, >99%; (e) $PhCH_2NH_2$, HOBt, EDC, THF-CH_2Cl_2 (1:1), 0 °C to rt, >79%; (f) HCHO, H_2, 10% Pd/C, CH_3OH, 4 days, rt, 62% (for **10**) and 60% (for **11**).

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

Compounds	HEK293 cell (T-type: α_{1G})		HEK293 cell (N-type: α_{1B})	Selectivity (T/N-type) at 10 μM
	% Inhibition (10 μM)[a]	IC_{50} (μM)[b]	% Inhibition (10 μM)[a]	
KYS05001	90.1 ± 2.3	1.16 ± 0.04	28.1 ± 1.7	3.2
KYS05044	82.5 ± 0.7	0.56 ± 0.10	No blocking[c]	>100
KYS05089 (11)	90.1 ± 2.3 (57.9)[d]	0.23 ± 0.03	24.4 ± 2.8	3.7
KYS05090 (10)	98.0 ± 1.6 (42.9)[d]	0.041 ± 0.001	70.6 ± 3.1	1.4 (119.5)[e]
Mibefradil	95.9 ± 1.7 (77.6)[d]	1.34 ± 0.49	67.6 ± 1.2	1.4

[a] % Inhibition value (±SE) was obtained by repeated procedures ($n \geqslant 4$) under patch–clamp assay.
[b] IC_{50} value was determined from the dose–response curve.
[c] 'No blocking' means the inhibition was less than 1%.
[d] Data by HTS (FDSS6000 system).
[e] Selectivity index based on IC_{50} values.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2007.08.070.

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10. (a) Kim, T.; Choi, J.; Kim, S.; Kwon, O.; Nah, S. Y.; Han, Y. S.; Rhim, H. *Biochem. Biophys. Res. Commun.* **2004**, *324*, 401; (b) For the recordings 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 MΩ 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 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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13. Spectral data of the compounds **10** and **11**: for compound **10** (**KYS05090**), ^{1}H NMR (400 MHz, $CDCl_3$)δ 7.72–6.98 (18H, m, Ph), 5.32 (1H, dd, J = 9.1 and 4.6 Hz, $COCH_2CH$), 4.50 (2 H, d, J = 5.8 Hz, $PhCH_2$–), 3.50–3.20 (2 H, m, CH_3N–CH_2), 2.71–2.33 (4 H, CH_3–N and COC*H*), 2.44 (1H, dd, J = 14.5 and 5.2 Hz, COC*H*), 2.30–2.29 (2 H, m, –NCH_2), 2.21 (6 H, s, 2 × N–CH_3), 1.65–1.35 (4 H, m, 2 × –CH_2), 1.25–1.10 (2 H, m, CH_2); ^{13}C NMR (100 MHz, $CDCl_3$)δ 170.1, 153.9, 145.3, 143.8, 140.4, 138.4, 136.7, 128.8, 128.6, 128.1, 128.0, 127.8, 127.4, 127.1, 127.0, 126.2, 125.1, 122.8, 122.4, 122.2, 61.2, 59.7, 49.5, 45.2, 43.8, 41.8, 35.3, 27.1, 27.0, 24.7; MS (FAB+), *m/z* (relative intensity, %) 596.7([M+Na]$^+$, 100), 574.7 ([M+H]$^+$, 30); MS (FAB-), *m/z* (relative intensity, %) 572.7([M-H]$^+$, 100); HRMS (FAB+) calcd for $C_{37}H_{44}N_5O$: [M+H]$^+$ = 574.3546, found = 574.3516; for compound **11** (**KYS05089**), ^{1}H NMR (400 MHz, $CDCl_3$)δ 7.52–6.88 (13 H, m, Ph), 5.11 (1H, dd, J = 10.4 and 4.5 Hz, $COCH_2CH$), 3.75 (3H, s, OCH_3), 3.50 (1H, m, CH_3N–*CH*), 3.15 (1H, m, CH_3N–*CH*), 2.88–2.82 (4H, m, CH_3–N and COC*H*), 2.51 (1H, dd, J = 15.5 and 4.6 Hz, COC*H*), 2.25–2.23 (2H, m, –NCH_2), 2.20 (6H, s, 2 × N–CH_3), 1.52–1.45 (4H, m, 2 × –CH_2), 1.24–1.23 (2H, m, CH_2); ^{13}C NMR (100 MHz, $CDCl_3$)δ 171.9, 153.4, 145.4, 144.2, 140.3, 136.8, 128.8, 128.4, 127.9, 127.1, 126.8, 125.4, 124.8, 122.9, 122.6, 122.1, 61.2, 59.7, 49.5, 45.2, 43.8, 41.8, 35.3, 27.1, 27.0, 24.7; HRMS (FAB+) calcd for $C_{31}H_{39}N_4O_2$: [M+H]$^+$ = 499.3073, found = 499.3060.