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Synthesis and biological evaluation of novel T-type calcium channel blockers

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Abstract—3,4-Dihydroquinazoline analogues substituted by *N*-methyl-*N*-(5-pyrrolidinopentyl)amine at the 2-position were synthesized and their blocking effects were evaluated for T- and N-type calcium channels. Compound **11b** (**KYS05080**), compared to mibefradil ($IC_{50} = 1.34 \pm 0.49$ μM), was about 5-fold potent ($IC_{50} = 0.26 \pm 0.01$ μM) for T-type calcium channel (α_{1G}) blocking and its selectivity of T/N-type was also improved (7.5 versus 1.4 of mibefradil).

T-type (low-threshold) calcium channels are known to be implicated in pathogenesis of epilepsy and neuropathic pain.[1,2] In spite of this, the pharmacology of T-type channels is complex because many drugs have been found to block T-type currents.[3] Unfortunately, none of these compounds has high selectivity for these channels. Therefore it is difficult to isolate T-type current pharmacologically. Mibefradil has been marketed worldwide for the treatment of hypertension and angina for a short period before it was withdrawn due to drug–drug interactions (Fig. 1). Mibefradil binds to skeletal muscle L-type calcium channels and brain voltage-gated sodium channels.[4,5] It also can block potassium and chloride channels. Obviously, this makes it not an ideal tool for in vitro or in vivo studies on T-type channels. Studies of calcium channels expressed in oocytes have identified kurtoxin as a promising tool for functional and structural studies of low-threshold T-type calcium channels.[6] This peptide, isolated from the venomous scorpion *Parabuthus transvaalicus*, inhibits expressed low-threshold $Ca_v3.1$ (α_{1G}) and $Ca_v3.2$ (α_{1H}) calcium channels with relatively high potency and high selectivity. In thalamic neurons, however, kurtoxin blocks the composite high-threshold calcium channel to about 50% as well as T-type calcium channel almost completely in these cells.[7] Therefore, only limited progress has been made to date in the quest to identify both potent and selective compounds except kurtoxin and mibefradil for T-type channel blockade.

In previous studies, we have tried to identify new compounds with higher potency and selectivity for T-type channel and reported some lead-like compounds containing 3,4-dihydroquinazoline ring as novel scaffold.[8–10] Among them, **KYS05044** and **KYS05050** were found to be superior to mibefradil with respect to both potency and selectivity for T-type channel (Fig. 1).[9]

However, both compounds have an innate synthetic problem for the further development as clinically applicable drug due to the difficulty in separating two regioisomers (**iii** and **iv**) stemmed from a primary amine in the synthetic procedure (Fig. 2).[9] In order to obtain a desired compound (**iii**) among two regioisomers, therefore, an attacking nucleophile should be a secondary amine. On the other hand, a computational mapping of compound **KYS05050** (most active among this series compounds) into 6-feature 3D pharmacophore hypothesis, which was generated from our previous results,[11] hypothesized that its blocking activity would increase when one phenyl group is removed from the parent

Keywords: T-type calcium channel; Channel blockers; 3,4-Dihydroquinazoline; Regioisomers.
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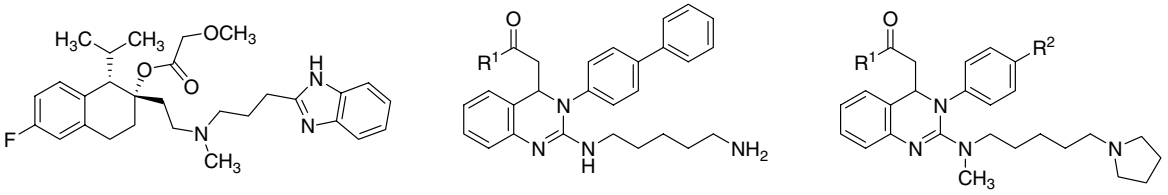


Figure 1. Mibefradil and 3,4-dihydroquinazoline derivatives.

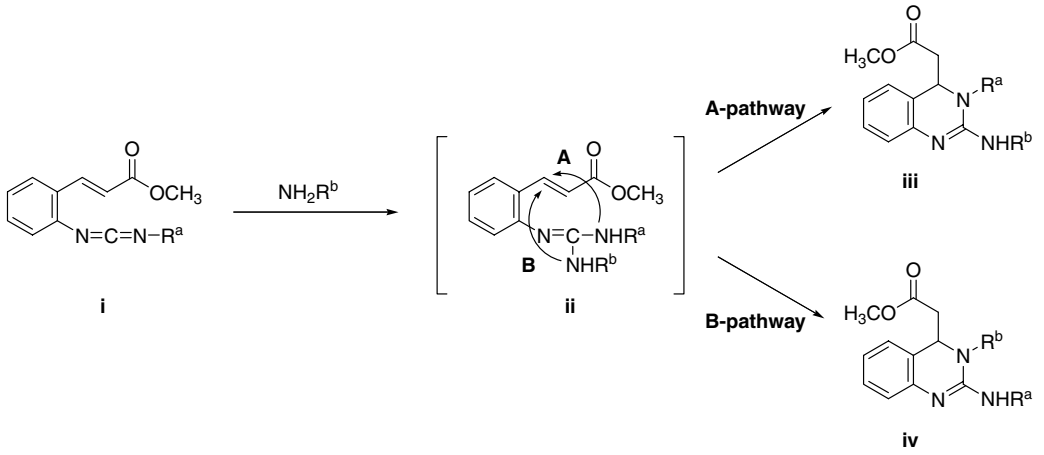


Figure 2. Formation of two regioisomers due to a primary amine nucleophile.

molecule and/or a hydrophobic residue is introduced into its terminal amine group (Fig. 3). Such implication prompted us to design the subsequent modification of compounds **KYS05050**. Herein, we report on the synthesis and brief structure–activity relationship (SAR) of target compounds as **KYS05050** analogues (Fig. 1).

The key intermediate, *N*-methyl-*N*-(5-pyrrolidinopentyl)amine (**6**), was prepared starting from 1,5-dibromopentane **1** (Scheme 1). Compound **1** was treated with potassium phthalimide to afford mono-phthalimide **2**,[12] which was treated with pyrrolidine to provide compound **3** under the basic condition (K_2CO_3, EtOH). Deprotection of phthalimide group of compound **3** via Gabriel condition afforded a free amine **4**.[13,14] Protection of amine group of compound **4** with *tert*-butyloxycarbonyl group followed by reduction using lithium aluminum hydride provided the key intermediate **6** as a secondary amine.[15]

This intermediate **6** was coupled with cinnamate-derived carbodiimide **8** to provide 3,4-dihydroquinazoline derivative **9** as a single regioisomer via tandem nucleophilic addition and intramolecular conjugate addition. Finally, hydrolysis of compound **9** with LiOH followed by coupling reaction of compound **10** with benzylamine, EDC, and HOBt afforded the target compound **11** by using our previous procedure (Scheme 2).[10]

The in vitro calcium channel blocking activities of 3,4-dihydroquinazoline derivatives (**9a**–**b** and **11a**–**b**) were determined in T-type channels stably expressed in *Xenopus* oocytes (α_{1H}) and HEK293 cells (α_{1G}). First, all compounds were evaluated for their blocking effects on α_{1H} T-type calcium channels expressed in *Xenopus* oocytes by a two-electrode voltage clamp method at 100 μM concentration.[16] They were again re-evaluated for the blocking effects on α_{1G} T-type calcium channels expressed in HEK293 cells at 10 μM concentration by whole-cell patch clamp methods.[17] The molar concentrations (IC_{50}) of test compounds required to produce 50% inhibition of α_{1G} T-type currents were determined from fitting raw data into dose–response curves. Their in vitro blocking data are summarized in Table 1 and the data of parent compound (**KYS05044** and **KYS05050**) were also inserted for comparison. New synthetic compounds showed high blocking activities against α_{1H} T-type calcium channel (*Xenopus* oocyte) except for compound **9a** (ca. 48%), which has a monophenyl group at 3-position and methyl ester at 4-position of 3,4-dihydroquinazoline ring. Among them, compound **11b** possessing a biphenyl group at 3-position and a benzyl amide group at 4-position was found to be most active (96%) and also comparable to the parent compound **KYS05044** (97%). Against α_{1G} T-type calcium channel (HEK293 cell), additionally, compound **9a** and **11a** containing a simple phenyl group at 3-position

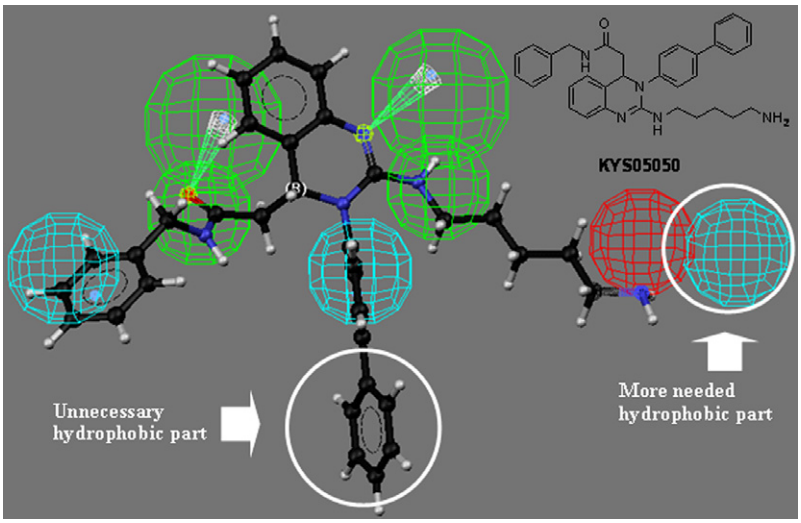
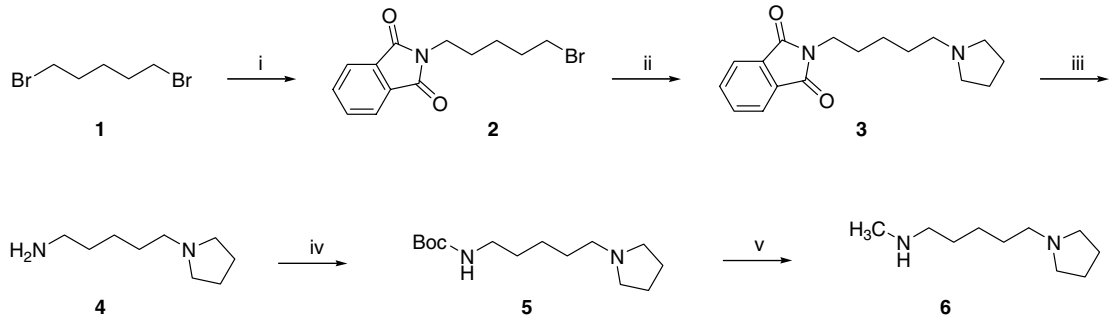
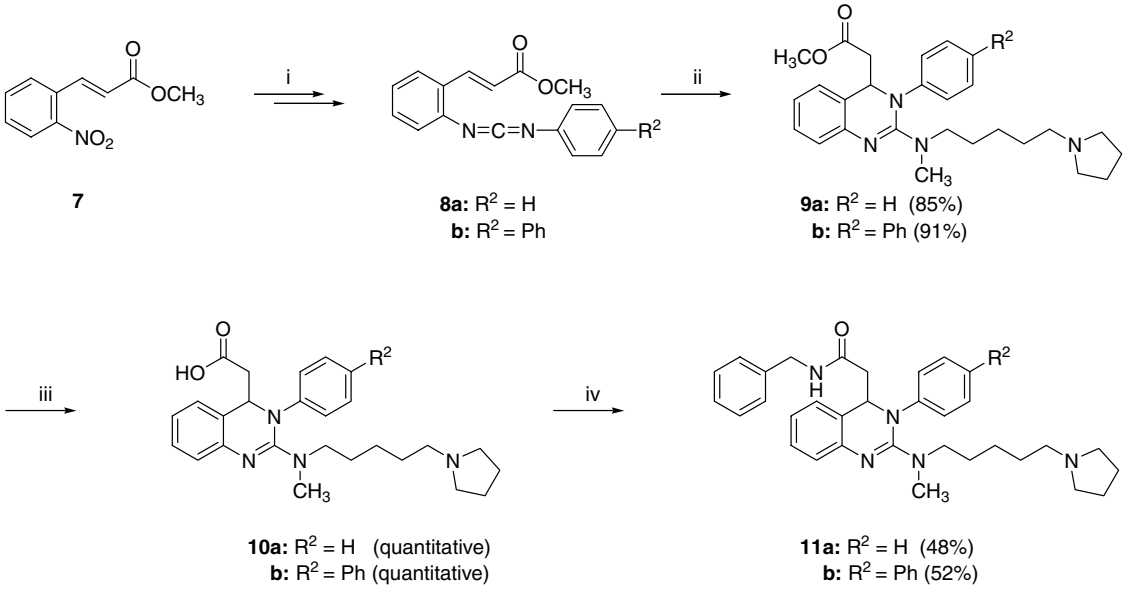


Figure 3. The mapping of compound **KYS05050** into 6-feature 3D pharmacophore hypothesis: blue color is hydrophobic region; red color is positive ionizable region; green color is hydrogen bonding region.

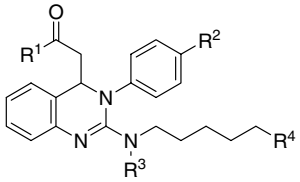


Scheme 1. Reagents and conditions: (i) potassium phthalimide, DMF, rt, 50%; (ii) pyrrolidine, K_2CO_3, NaI, EtOH, reflux, 35%; (iii) NH_2NH_2/H_2O, EtOH, reflux, 81%; (iv) $(^tBOC)_2O$, CH_2Cl_2, rt, 86%; (v) $LiAlH_4$, THF, reflux, 91%.



Scheme 2. Reagents and conditions: (i) Ref. 10; (ii) compound **6**, benzene, rt; (iii) LiOH, THF–H_2O (1:1), 70 °C; (iv) $PhCH_2NH_2$, HOBt, EDC, THF–CH_2Cl_2 (1:1), rt.

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



Compound (library code)	R^1	R^2	R^3	R^4	*Xenopus* oocyte T-type (α_{1H})	HEK293 cell (T-type: α_{1G})		HEK293 cell (N-type: α_{1B})	Selectivity (T/N-type) at 10 μM
					% inhibition (100 μM)	% Inhibition[a] (10 μM)	IC_{50} [b](μM)	% inhibition[a] (10 μM)	
KYS05044	OCH_3	Ph	H	NH_2	97.0	82.5 ± 0.7	0.56 ± 0.10	No blocking[c]	>100
KYS05050	NHBn	Ph	H	NH_2	80.1	83.8 ± 1.4	0.13 ± 0.01	8.3 ± 1.8	10.1
9a (KYS05076)	OCH_3	H	CH_3	—N	47.7	59.5 ± 1.1	5.84 ± 0.44	10.7 ± 2.3	5.6
9b (KYS05079)	OCH_3	Ph	CH_3	—N	88.0	94.9 ± 1.2	0.34 ± 0.04	36.5 ± 0.2	2.6
11a (KYS05077)	NHBn	H	CH_3	—N	82.7	68.1 ± 0.5	4.20 ± 0.20	46.3 ± 0.3	1.5
11b (KYS05080)	NHBn	Ph	CH_3	—N	95.5	88.1 ± 1.7	0.26 ± 0.01	11.7 ± 5.8	7.5
Mibefradil					86.0	95.9 ± 1.7	1.34 ± 0.49	67.6 ± 1.2	1.4

[a] % inhibition value (±SE) was obtained by repeated procedures ($n \geqslant 4$).
[b] IC_{50} value was determined from the dose–response curve.
[c] 'No blocking' means the inhibition was less than 1%.

showed the diminished activity (both of % inhibition and IC_{50} values) irrespective of a kind of 4-position group when compared to parent compounds (**KYS05044** and **KYS05050**). Compound **9b** possessing a biphenyl group at 3-position and a methyl ester group at 4-position was most active and comparable to mibefradil (96%). With respect to IC_{50} values, compound **9b** and **11b** were more or less active than the parent compounds **KYS05044** and **KYS05050**, but they were found to be 4- to 5-fold higher than mibefradil. With respect to the channel selectivity (T/N-type channel), all compounds were less selective than the parent compounds although they still showed the improved selectivity when compared to mibefradil. Therefore, these biological data imply that the biphenyl group may be an essential pharmacophore for the blocking activity of this series compounds opposite to the above 6-feature 3D pharmacophore hypothesis. Additionally, the introduction of methyl and/or hydrophobic residue in terminal area resulted in the decreased channel selectivity although of less effect on the blocking activity.

In summary, new **KYS05050** analogues were prepared for promoting the synthetic efficiency and studying a brief structure–activity relationship via the molecular modeling. Among them, compound **11b** (**KYS05080**) was found to be more active and selective than mibefradil although more or less active and selective than their parent compounds.[18] However, it is conceivable that compound **11b** (**KYS05080**) can be further modified to exhibit better blocking activity and selectivity. Further studies to acquire more information about structure–activity relationships are in progress in our laboratory.

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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.2006.10.024.

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18. Spectral data of selected compounds. Compound **6**: ^{1}H NMR (300 MHz, $CDCl_3$) 2.41 (2H, t, J = 6.9 Hz, CH_3–N–CH_2–C_4H_8–), 2.34–2.24 (9H, m, CH_3–N–CH_2–C_4H_8–, –C_4H_8–CH_2-pyrrolidinyl-*H*2, *H*5), 1.63–1.59 (4H, m, pyrrolidinyl-*H*3, *H*4), 1.41–1.31 (4H, m, –N–CH_2–CH_2–CH_2–CH_2–CH_2–), 1.27–1.17 (2H, m, –N–C_2H_4–CH_2–C_2H_4–); ^{13}C NMR (75 MHz, $CDCl_3$) δ 56.7, 54.3, 52.2, 36.7, 30.0, 29.2, 25.5, 23.5; MS(GC) *m*/*z* (relative intensity, %) 170 (M^+, 16), 140(23), 110(31), 84(100), 55(42). Compound **9b** (**KYS05079**): ^{1}H NMR (300 MHz, $CDCl_3$) 7.56–7.39 (6H, m, Ph), 7.34–7.26 (1H, m, Ph), 7.23–7.14 (4H, m, Ph), 7.00–6.88 (2H, m, Ph), 5.16 (1H, dd, J = 10.8 and 4.5 Hz, –CO–CH_2–C*H*–N–), 3.79 (3H, s, CH_3O–), 3.58 (1H, br s, –NH–CH_2–C_4H_8–), 3.16 (1H, br s, –NH–CH_2–C_4H_8–), 2.93–2.85 (4H, m, –CO–CH_2–CH–N–, –NCH_3–C_5H_{10}–), 2.58–2.41 (7H, m, –CO–CH_2–CH–N–, –C_4H_8–CH_2-pyrrolidinyl-*H*2, *H*5), 1.80–1.79 (4H, m, –pyrrolidinyl-*H*3, *H*4), 1.57–1.53 (4H, m, –CH_2–CH_2–CH_2–CH_2–CH_2–), 1.29–1.28 (2H, m, –C_2H_4–CH_2–C_2H_4–); ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.2, 153.6, 145.7, 144.5, 140.6, 137.0, 129.0, 128.7, 128.1, 127.3, 127.0, 125.7, 125.0, 123.2, 122.9, 122.4, 61.5, 56.8, 54.5, 52.2, 50.2, 39.8, 35.7, 29.0, 27.6, 25.4, 23,7. Compound **11b** (**KYS05080**): ^{1}H NMR (300 MHz, $CDCl_3$) 7.58–7.41 (6H, m, Ph), 7.36–7.11 (10H, m, Ph), 7.02–6.89 (2H, m, Ph), 5.33 (1H, dd, J = 9.6 and 5.1 Hz, –CO–CH_2–C*H*–N–), 4.51 (2H, dd, J = 5.4 and 3.3 Hz, Ph–CH_2–NH–), 3.36 (1H, br s), 2.76–2.56 (10H, m, –NCH_3–CH_2–C_4H_8–, –NCH_3–CH_2–, –C_4H_8–CH_2-pyrrolidinyl-*H*2, *H*5, –CO–CH_2–CH–N–), 2.47 (1H, dd, J = 14.4 and 5.1 Hz, –CO–CH_2–CH–N–), 1.80 (4H, br s, –pyrrolidinyl-*H*3, *H*4), 1.52 (4H, br s, –CH_2–CH_2–CH_2–CH_2–CH_2–), 1.35–1.29 (2H, m, –C_2H_4–CH_2–C_2H_4–); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.4, 154.2, 145.7, 144.2, 140.7, 138.7, 136.9, 129.0, 128.8, 128.3, 128.1, 127.6, 127.3, 127.0, 126.5, 125.4, 123.1, 122.6, 122.4, 61.4, 56.6, 54.3, 43.9, 41.9, 35.4, 30.0, 28.1, 27.2, 24.9, 23.5.