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Bioorganic & Medicinal Chemistry Letters 15 (2005) 283–286
Bioorganic & Medicinal Chemistry Letters

Synthesis and biological activity of 3,4-dihydroquinazolines for selective T-type Ca^{2+} channel blockers

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Received 16 September 2004; accepted 28 October 2004
Available online 21 November 2004

Abstract—We have synthesized 3,4-dihydroquinazoline derivatives for the potent and selective T-type Ca^{2+} channel blockers and evaluated for their inhibitory activities against two subtypes T-type Ca^{2+} channels and N-type Ca^{2+} channels. Among them, **5b** (**KYS05044**, $IC_{50} = 0.56 \pm 0.10\,\mu M$) was identified as potent T-type Ca^{2+} channel blocker with in vitro selectivity profile at meaningful level (T/N-type, SI = >100).

Native calcium channels have been classified by their electrophysiological and pharmacological properties as T-, L-, N-, P/Q- and R-types. L-, N-, P/Q- and R-type (or high voltage-activated) channels are activated at more positive potentials and display diverse kinetics and voltage-dependent properties.[1–3] High voltage-activated channels are activated by relatively strong membrane depolarization and permit Ca^{2+} influx in response to action potentials. Consequential secondary actions include neurotransmitter release. Thus, these channels establish a major link between neuronal excitability and synaptic transmission. For these reasons, high voltage-activated channels have been the focus of both acute and persistent pain transmission studies.[4] T-type (or low voltage-activated) channels describe a broad class of molecules that transiently activate at negative potentials and are highly sensitive to changes in resting potential. T-type channels are strongly associated with the generation of rhythmical firing patterns in the mammalian CNS.[5–7] Furthermore, many reports have suggested that T-type channels are implicated in pathogenesis of epilepsy and neuropathic pain.[8–11] In spite of these many researches, however, investigation of the role of T-type channels in physiological processes was limited by two factors: a lack of potent and selective T-type channel blockers and a lack of information about T-type channels at the molecular level. Until recently, three genes encoding T-type Ca^{2+} channel-pore-forming subunits were identified and designated $Ca_v3.1$ (α_{1G}), $Ca_v3.2$ (α_{1H}) and $Ca_v3.3$ (α_{1I}).[12–15] However, only limited progress has been made to date in the quest to identify both potent and selective compounds except kurtoxin ($IC_{50} = 15$ nM) and mibefradil ($IC_{50} = 1\,\mu M$) for T-type channel blockade.[16–18] Therefore, we sought to discover a novel small molecular compound with high potency and selectivity for T-type channels. As part of our recent efforts, the initial lead compounds, 3,4-dihydroquinazoline derivatives (**KYS05001**, **KYS05041** and **KYS05042**) with IC_{50} values of sub-micromolar concentration were identified by random screening of a chemical library and SAR study as shown in Figure 1.[19,20] Herein, we report the identification and synthesis of 3,4-dihydroquinazoline derivatives as potent blockers with high selectivity for T-type Ca^{2+} channels. Our primary goal was to optimize the potency and channel selectivity of these compounds focusing mainly on introduction of biphenyl and ω-aminoalkyl groups at the 2- or 3-position, respectively.

Keywords: T-type Ca^{2+} channel; Mibefradil; 3,4-Dihydroquinazolines; Channel selectivity; Blockers.
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doi:10.1016/j.bmcl.2004.10.078

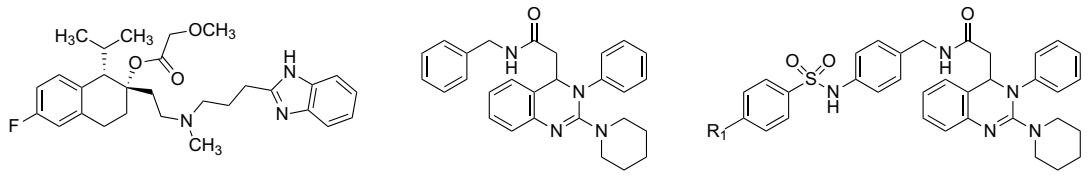
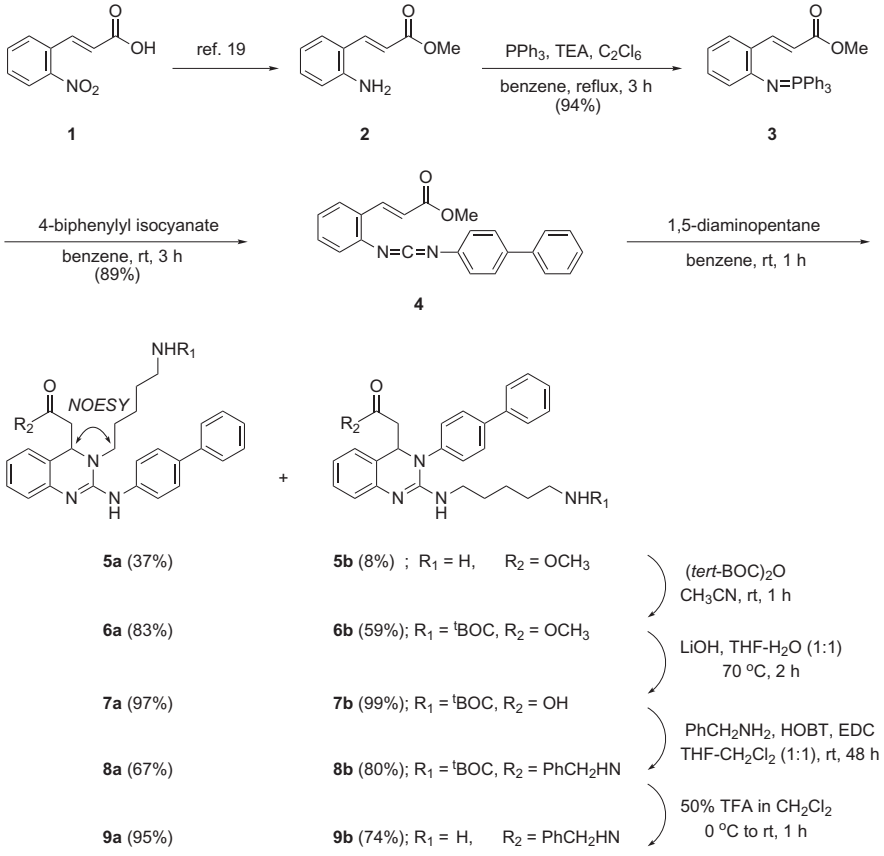


Figure 1. Structures of some T-type Ca^{2+} channel blockers.

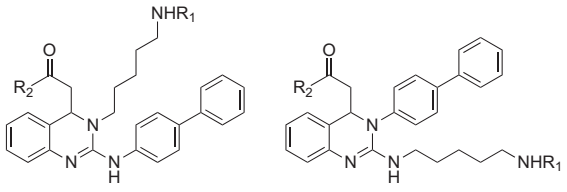
The 3,4-dihydroquinazoline derivatives (**5**–**9**) containing both biphenyl and ω-aminoalkyl groups were derived from the intermolecular aza-Wittig reaction as shown in Scheme 1. The iminophosphorane derivative (**3**) was directly prepared in 94% yield by the Appel's method (PPh_3–C_2Cl_6–Et_3N reagent system) from methyl 2-aminocinnamate (**2**),[21] which was derived from commercially available 2-nitrocinnamic acid (**1**) using a procedure earlier.[19]

In the next step, the intermolecular aza-Wittig reaction of iminophosphorane (**3**) with 4-biphenylyl isocyanate (1.5 equiv) in dry benzene at room temperature for 3 h, followed by the reaction with 1,5-diaminopentane (2.0 equiv) at room temperature for 1 h afforded the 3,4-dihydroquinazoline derivative (**5a** and **5b**) as a mixture of regioisomers in 45% yield via tandem intramolecular conjugate addition. Two regioisomers (**5a**:**5b** = 4.6:1) could be separated by silica gel column chromatography (using a small amount of ammonia solution) and their structures could be completely elucidated by ^1H NMR technique such as NOESY (the data not shown here), respectively, as illustrated in Scheme 1. Then, each ω-amino group of compounds **5a** and **5b** was protected with di-*tert*-butoxycarbonate to afford the carbamate **6a** and **6b** in 83% and 59% yield, respectively. The hydrolysis of compounds **6a** and **6b** with LiOH at 70 °C provided the corresponding carboxylic compounds **7a** and **7b** in quantitative yield. Coupling of **7a** and **7b** with benzylamine in the presence of EDC and HOBT afforded the respective benzyl amides **8a** and **8b** in 67% and 80% yield, respectively.[22] Finally, deprotection of *tert*-butoxy group was carried out by 50% TFA in CH_2Cl_2 to provide the corresponding 3,4-dihydroquinazoline derivatives (**9a** and **9b**) containing ω-aminoalkyl and biphenyl groups in 95% and 74% yields.



Scheme 1. Synthesis of 3,4-dihydroquinazoline derivatives.

Table 1. In vitro calcium channel blocking effects of 3,4-dihydroquinazolines (**5**, **6**, **8** and **9**)



Compounds (library code)	R_1	R_2	*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 (10 μM)[a]	IC_{50} (μM)[b]	% Inhibition (10 μM)[a]	
5a (KYS05043)	H	CH_3O	94.6	84.1 ± 1.6	0.30 ± 0.09	7.5 ± 0.7	11.2
5b (KYS05044)	H	CH_3O	**97.0**	**82.5 ± 0.7**	**0.56 ± 0.10**	**No blocking**[c]	**>100**
6a (KYS05045)	^{t}BOC	CH_3O	96.5	88.5 ± 0.4	0.37 ± 0.08	94.9 ± 1.7	0.9
6b (KYS05046)	^{t}BOC	CH_3O	92.2	86.5 ± 0.5	0.68 ± 0.18	98.6 ± 1.3	0.9
8a (KYS05047)	^{t}BOC	$PhCH_2NH$	91.2	88.5 ± 0.6	0.17 ± 0.03	30.1 ± 1.1	2.9
8b (KYS05048)	^{t}BOC	$PhCH_2NH$	84.2	88.3 ± 1.5	0.16 ± 0.02	16.6 ± 0.7	5.3
9a (KYS05049)	H	$PhCH_2NH$	97.0	91.8 ± 1.9	0.14 ± 0.01	15.2 ± 2.4	6.0
9b (KYS05050)	H	$PhCH_2NH$	80.1	83.8 ± 1.4	0.13 ± 0.01	8.3 ± 1.8	10.1
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 that the inhibition was less than 1%.

The in vitro calcium channel blocking activities of each pair of 3,4-dihydroquinazoline derivatives were determined in T-type channels stably expressed in *Xenopus* oocytes (α_{1H}) and HEK293 cells (α_{1G}). As preliminary assays, all synthetic compounds (100 μM) were evaluated for their inhibitory effects on α_{1H} T-type Ca^{2+} channels expressed in *Xenopus* oocytes by a two-electrode voltage clamp method.[23] The compounds were again re-evaluated for the blocking effects on α_{1G} T-type Ca^{2+} channels expressed in HEK293 cells at 10 M concentration by whole-cells patch clamp methods.[24] 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. In vitro blocking effects of all pairs of 3,4-dihydroquinazoline derivatives except compounds **7a** and **7b** are summarized in Table 1.

Against the α_{1H} T-type Ca^{2+} channel (*Xenopus* oocyte), most pairs of compounds showed high inhibitory activities (% inhibition in 80.1–97.0 range) except carboxylic acid compound pair **7a** (37.4%) and **7b** (77.3%) compared to mibefradil (86%), a reference compound. This inhibitory trend was very interesting compared with our initial work, which showed that compounds having a benzyl amide group at 4-position of parent ring generally showed more potent efficacies than compounds possessing a simple ester.[19] This biological result implies that the present series of compounds may have a different binding pattern on α_{1H} T-type channels from that of our initial compound.

Next, all pairs of compounds, except compounds **7a** and **7b**, were re-evaluated in HEK293 cells (α_{1G}) at lower concentration (10 μM). Together with the primary inhibitory activity, our experimental results are summarized as follows. First, their profiles had a similar inhibitory trend to those against *Xenopus* oocyte (α_{1H}) with values of 82.5–91.8% inhibition range. Second, the compound **9a** (**KYS05049**), the most potent compound against the α_{1H} T-type Ca^{2+} channel (*Xenopus* oocyte) was nearly equipotent (91.8 ± 1.9% inhibition) comparable to mibefradil (95.9 ± 1.7% inhibition) against the α_{1G} T-type Ca^{2+} channel (HEK293 cell). With respect to the IC_{50} values, however, all pairs of compounds showed more potent efficacy (IC_{50} value 0.13 ± 0.01 to 0.68 ± 0.18 μM range) than mibefradil (IC_{50} = 1.34 ± 0.49 μM) and also each pair showed similar IC_{50} values against the α_{1G} T-type Ca^{2+} channel (HEK293 cell). Compared to mibefradil, one pair of compounds **9a** and **9b** in this series was most potent (IC_{50} = 0.14 ± 0.01 and 0.13 ± 0.01 μM) showing a 10-fold increase in potency, as well as the other pair of compounds **8a** and **8b** (IC_{50} = 0.17 ± 0.03 and 0.16 ± 0.02 μM), as shown in Table 1. The above two pairs of compounds were shown to possess the common benzyl amide group at 4-position, which consistent with our previous research result.[19,20]

Next, these compounds were screened against α_{1B} N-type Ca^{2+} channels (high voltage-activated Ca^{2+} channels) stably expressed in HEK293 cells for the evaluation of ion channel selectivity. The inhibitory activity data of these compounds were also summarized in Table 1. Under our assay condition, mibefradil showed lower selectivity for α_{1G} T-type channel (T/N-type channel = 1.4), which is consistent with the recent reported studies.[25] As shown in Table 1, most pairs of compounds, except compounds **5a** and **5b**, showed similar and less inhibitory activities against α_{1B} N-type Ca^{2+} channels, although there is about a twofold difference with respect to the blocking effect of each other. Compounds **6a** (94.9 ± 1.7% inhibition) and **6b**

(98.6 ± 1.3% inhibition) in these pairs showed more potent efficacy against N-type than T-type channels and thus less selectivity for T-type channels (T/N = 0.9-fold, respectively). Meanwhile, compound **9a** (**KYS05049**), the most potent compound against two isoforms of T-type Ca^{2+} channel sub-family, showed low selectivity over N-type channels by only 6.0-fold. Also, compounds **9b** (**KYS05050**), **8a** and **8b**, the most potent compounds against T-type channels, did not show the favorable selectivity for T-type channels by 10.1-, 2.9- and 5.3-fold, respectively. However, compound **5b** (**KYS05044**) possessing ω-aminoalkyl group at 3-position and methyl ester group at 4-position blocked little the current of N-type channels (less than 1%) and thus exhibited the highest selectivity for T-type over N-type channel (T/N = >100), even though compound **5b** (IC_{50} = 0.56 ± 0.10 μM) was more potent than mibefradil (IC_{50} = 1.34 ± 0.49 μM) by only 2.4-fold. More importantly, all compounds, including compound **5b**, had no cytotoxicity on HEK293 cells at 10 μM concentration as confirmed using MTT assay method (data not shown). Therefore, it is likely that compound **5b** would really be regarded as a new promising lead compound for selective T-type Ca^{2+} channel blocker with respect to the IC_{50} value and channel selectivity.[26]

In conclusion, new series of 3,4-dihydroquinazoline derivatives containing both biphenyl and ω-aminoalkyl groups as a continuous research for a novel selective T-type Ca^{2+} channel blocker were prepared and evaluated for the blocking effects against two isoforms of T-type Ca^{2+} channel subfamily and N-type Ca^{2+} channel. In vitro results demonstrated that compound **5b** in this series exhibited both favorable potency and highest selectivity for T-type Ca^{2+} channel in comparison with mibefradil. Therefore, the discovery of compound **5b** (**KYS05044**), a novel and selective T-type Ca^{2+} channel blocker, is expected to provide impetus for the development of new T-type Ca^{2+} channel drugs as well as the research in the field of electrophysiology.[26] Encouraged by these findings, further evaluation of pharmacokinetics profiles and neuroprotective properties are in progress.

Acknowledgements

This study was supported by Vision 21 Program from Korea Institute of Science and Technology (2E18000-04-024).

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26. Spectral data of the selected compound: **5b** (**KYS05044**): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.58–6.91 (13H, m, Ph), 5.10 (1H, t, J = 7.1 Hz, –CO–CH_2–C*H*–N–), 3.66 (3H, s, CH_3O–), 3.46–3.30 (2H, m, –N–CH_2–CH_2–), 2.87 (1H, dd, J = 7.1, 14.9 Hz, –CO–C*H*–CH–N–), 2.74–2.65 (3H, m, –CO–C*H*–CH–N– and –CH_2–CH_2–NH_2), 1.78–1.27 (6H, m, –CH_2–C_3H_6–CH_2–); ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.5, 147.1, 141.1, 137.0, 135.4, 129.1, 128.9, 128.5, 127.0, 126.9, 125.7, 123.3, 122.3, 115.1, 56.2, 52.1, 47.8, 41.7, 39.1, 32.3, 28.3, 25.0, 24.1; ES-MS (*m*/*z*, M + 1) 457; HRMS (FAB, M + 1) calcd for $C_{28}H_{33}N_4O_2$ 457.2604, found 457.2608.