DOI 10.5012/bkcs.2010.31.9.2451

Multi-Functional 3,4-Dihydroquinazoline Derivative as T-Type Calcium Channel Blocker: Pharmacokinetics and Anti-Tremor Activity

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Received May 25, 2010, Accepted June 30, 2010

Key Words: 3,4-Dihydroquinazoline, T-type calcium channel blocker, Pharmacokinetic parameters, Anti-tremor activity

The pharmacology of T-type calcium channels is in complex because many drugs have been found to block T-type currents.[1-3] Unfortunately, none of these compounds has high selectivity for these channels. Mibefradil has been marketed worldwide for the treatment of hypertension and angina for a short period before it was withdrawn due to its pharmacokinetic and pharmacodynamic interactions with some other drugs such as terfenadine, astemizole, cisapride, cyclosporine, tricyclic antidepressants.[4] Mibefradil binds to skeletal muscle L-type calcium channels and brain voltage-gated sodium channels with dissociation constants of 2.3 and 17 nM, respectively.[5] It also can block potassium and chloride channels.[6] Obviously, this makes it not an ideal tool for *in vitro* or *in vivo* studies on T-type channels. Therefore, more potent and selective blockers are required to study the fundamental function of T-type channel and the related pathophysiological diseases such as epilepsy, neuronal pain, hypertension, congestive heart failure, and cancer.[7-8] Recently, our group have reported the identification of 3,4-dihydroquinazoline derivatives as a novel scaffold, which are potent and selective T-type calcium blockers.[9-10] These compounds also exhibited the anti-cancer activity *in vitro via* cell-cycle arrest mechanism.[11-12]

As a continuous work, three compounds (**1-3**) with the highest T-channel channel selectivity (No blocking against N-type channel) were selected among the chemical library of 3,4-dihydroquinazoline and evaluated for the blocking effect on the hERG potassium channel,[13-14] which is known for its contribution to the electrical activity of the heart that coordinates the heart's beating: both of % inhibitions at 10 μM concentration and the molar concentrations of compounds needed to produce 50% inhibition of peak currents (IC_{50}) were measured by the whole-cell patch-clamp method.[15] The data were summarized in Table 1 with mibefradil as a positive control for comparison. Based on the IC_{50} data in Table 1, **1-3** compounds exhibited low selectivity over hERG channel (T-type/hERG ratio = 3.43, 4.39 and 7.56, respectively) but higher than mibefradil (1.04). This result means that these compounds can distinguish N-type from T-type calcium channel perfectly but not hERG potassium channel effectively. Furthermore, the pharmacokinetic parameters of all compounds were evaluated after single oral dose (20 mg/kg) in the rat and summarized in Table 2. These data demonstrate that compounds **1** and **3** exhibit higher volume of distribution but faster plasma clearance than compound **2**. It is inferred that compound **2** has both proper absorption in gastrointestinal system (C_{max} and AUC) and metabolic stability ($t_{1/2}$ = 1.6 h) based on these parameters. In addition, we found the 22% oral bioavailability of compound **2** particularly gratifying when compared with the poor oral bioavailability of another two compounds.

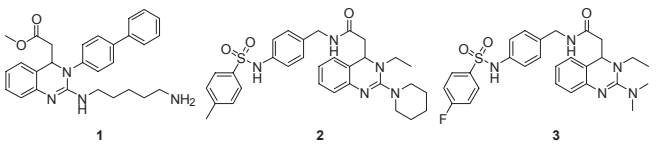

Figure 1. Selected 3,4-dihydroquinazoline derivatives.

Of the three T-type calcium channels, the $Ca_v3.1$ (α_{1G}) and $Ca_v3.3$ (α_{1I}) subtypes are primarily expressed in the brain, while $Ca_v3.2$ (α_{1H}) has a broader central and peripheral expression.[16-18] In addition, T-type channels are highly expressed in the thalamus and cortex and play important roles in thalamocortical signaling.[19] Recent reports from some laboratories have disclosed

Table 1. Channel selectivity data of 3,4-dihydroquinazoline compounds

Compound	Patch-Clamp Assay (%inhibition at 10 μM)			Patch-Clamp Assay (IC_{50}: μM)[a]		Ratio IC_{50} (hERG)/IC_{50} (T-type)
	T-type (α_{1G})[b]	N-type (α_{1B})[b]	hERG[c]	T-type (α_{1G})[b]	hERG[c]	
1	82.5 ± 0.7	No blocking	83.0 ± 2.6	0.56 ± 0.10	1.92 ± 0.44	3.43
2	91.3 ± 0.6	No blocking	70.3 ± 2.6	0.96 ± 0.22	4.21 ± 0.60	4.39
3	62.7 ± 2.3	No blocking	23.8 ± 1.4	4.10 ± 1.08	31.0 ± 3.35	7.56
Mibefradil	95.9 ± 1.7	67.6 ± 1.2	-	1.34 ± 0.49	1.40 ± 0.29	1.04

[a]Value was determined from dose-response curve and obtained from three independent experiments; [b]expressed in HEK293 cell; [c]human cardiac potassium channel.

Table 2. Pharmacokinetic parameters of 3,4-dihydroquinazoline compounds[a,b,c]

Compound	Vd/F (L/kg)	T_{max} (min)	C_{max} (ng/mL)	$t_{1/2}$ (min)	Cl/F (mL/min/kg)	MRT (min)	AUC (ng·hr/mL)
1	502.4 ± 225.0	10 ± 0.0	60.2 ± 9.2	164.5 ± 111.0	2740.4 ± 1398.0	135.8 ± 52.3	114.43 ± 29.6
2	129.4 ± 15.6	120 ± 0.0	105.0 ± 12.3	97.6 ± 10.4	923.6 ± 134.3	183.3 ± 5.3	348.0 ± 48.4
3	483.3 ± 189.5	120 ± 0.0	34.4 ± 5.9	75.4 ± 4.3	4500.6 ± 2000.5	169.2 ± 11.6	79.3 ± 35.2

[a]After single oral injection (20 mg/kg); [b]bioavailability (F%): 3% for **1**; 22% for **2**; 4% for **3**; [c]the parameters were calculated using WinNonlin (Ver. 1.1) program.

Table 3. Suppression effect of compound **2** on two tremor mouse models[a]

Compound	harmaline-induced tremor model [post ip injection (10 mg/kg)]		genetic tremor model[b] [30 min post ip injection]	
	20 - 40 min	40 - 60 min	10 mg/kg	20 mg/kg
2	44.7%	55.3%	58.0%	82.2%

[a]P values less than 0.05 were considered significant; [b]$GABA_A$ receptor α1 subunit-null mouse model.

that selective T-type calcium channel blockers show *in vivo* efficacy in epilepsy and tremor models.[20-21] Based on these reports, therefore, our 3,4-dihydroquinazoline compound, in particular compound **2** which has both proper selective/potent T-type channel blocking effect and pharmacokinetic profile, was evaluated for the anti-tremor activity using two mouse model: harmaline-induced tremor mouse model[22] and $GABA_A$ receptor α1 subunit-null mouse model.[23] It has been well known that harmaline, a fluorescent psychoactive indole alkaloid from the group of harmala alkaloids, induces tremor in animals.[24] Thus, harmaline in saline (20 mg/kg) was injected subcutaneously in order to induce tremor in male ICR mouse. After 15 min when tremor had fully developed, compound **2** in 10% DMSO/saline solution was injected intraperitoneally. Then, the tremor-related motion data was obtained for five successive 20-min epochs and summarized in Table 3. As a result, compound **2** suppressed harmaline-induced tremor by 44.7% and 53.3% at 20 - 40 and 40 - 60 min after injection respectively.

In the case of $GABA_A$ receptor α1 subunit-null mouse model which exhibits postural and kinetic tremor and motor incoordination that is characteristic of essential tremor disease, the tremor-related motion data was obtained four times at a specified time after compound treatment and summarized in Table 3. As a result, compound **2** suppressed tremor by 58.0% at 10 mg/kg dose and by 82.2% at 20 mg/kg dose, respectively, 30 min post injection. This result suggests that 3,4-dihydroquinazoline compound would be developed as potential therapeutics for the tremor.

In summary, 3,4-dihydroquinazoline derivative (**2**) with proper T-type channel selectivity/activity and oral pharmacokinetic profile was evaluated for anti-tremor activity. This compound suppressed tremor in two tremor animal models effectively. This suggests that 3,4-dihydroquinazoline compound has considerable potential as an anti-tremor agent together with the previously reported anti-cancer agent.[11-12]

Acknowledgments. This research was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (2009-0088135).

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