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

So Hyung Lee, Soo Yeon Jung, Hang Ah Park, Han Byul Kang, Jungahn Kim, Dong Joon Choo, Adrian Handforth, and Jae Yeol Lee*

Research Institute for Basic Sciences and Department of Chemistry, College of Sciences, Kyung Hee University, Seoul 130-701, Korea. E-mail: fly@khu.ac.kr

*Neurology Service, Veterans Affairs Greater Los Angeles Healthcare System, Los Angeles, California, USA

Received May 25, 2010, Accepted June 30, 2010

Key Words: 3,4-Dihydroquinazoline, T-type calcium channel blocker, Pharmacokinetic parameters, Antitremor 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 Mibefradil has been marketed worldwide for the treatment of hypertension and angina for a short period before it was withdrawn due to its pharmacokinetic and pharmacodynamic interactions with some other drugs such as terfenadine, astemizole, cisapride, cyclosporine, tricyclic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.5,7 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.8-10 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,13-15 which is known for its contribution to the electrical activity of the heart that coordinates the heart’s beating: both of % inhibition at 10 µM and the molar concentrations of compounds needed to produce 50% inhibition of peak currents (IC50) 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 IC50 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 (Cmax and AUC) and metabolic stability (t1/2 = 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.

Of the three T-type calcium channels, the Ca,3.1 (α1c) and Ca,3.3 (α1d) subtypes are primarily expressed in the brain, while Ca,3.2 (α1h) has a broader central and peripheral expression.16,17 In addition, T-type channels are highly expressed in the thalamus and cortex and play important roles in thalamocortical signaling.18 Recent reports from some laboratories have disclosed

![Figure 1. Selected 3,4-dihydroquinazoline derivatives.](image_url)

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

<table>
<thead>
<tr>
<th>Compound</th>
<th>T-type (α1c)*</th>
<th>N-type (α1h)*</th>
<th>hERG</th>
<th>T-type (α1c)*</th>
<th>hERG</th>
<th>Ratio IC50 (hERG)/IC50 (T-type)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>82.5 ± 0.7</td>
<td>No blocking</td>
<td>83.0 ± 2.6</td>
<td>0.56 ± 0.10</td>
<td>1.92 ± 0.44</td>
<td>3.43</td>
</tr>
<tr>
<td>2</td>
<td>91.3 ± 0.6</td>
<td>No blocking</td>
<td>70.3 ± 2.6</td>
<td>0.96 ± 0.22</td>
<td>4.21 ± 0.60</td>
<td>4.39</td>
</tr>
<tr>
<td>3</td>
<td>62.7 ± 2.3</td>
<td>No blocking</td>
<td>23.8 ± 1.4</td>
<td>4.10 ± 1.08</td>
<td>31.0 ± 3.35</td>
<td>7.56</td>
</tr>
<tr>
<td>Mibefradil</td>
<td>95.9 ± 1.7</td>
<td>67.6 ± 1.2</td>
<td>-</td>
<td>1.34 ± 0.40</td>
<td>1.40 ± 0.29</td>
<td>1.04</td>
</tr>
</tbody>
</table>

*Value was determined from dose-response curve and obtained from three independent experiments; expressed in HEK293 cell; human cardiac potassium channel.
Table 2. Pharmacokinetic parameters of 3,4-dihydroquinazoline compounds$^{\text{a,b,c}}$

<table>
<thead>
<tr>
<th>Compound</th>
<th>Vd/F (L/kg)</th>
<th>T(_{\text{max}}) (min)</th>
<th>C(_{\text{max}}) (ng/mL)</th>
<th>t(_{1/2}) (min)</th>
<th>CI/F (mL/min/kg)</th>
<th>MRT (min)</th>
<th>AUC (ng-hr/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>502.4 ± 225.0</td>
<td>10 ± 0.0</td>
<td>60.2 ± 9.2</td>
<td>164.5 ± 111.0</td>
<td>2740.4 ± 1398.0</td>
<td>135.8 ± 52.3</td>
<td>114.43 ± 29.6</td>
</tr>
<tr>
<td>2</td>
<td>129.4 ± 15.6</td>
<td>120 ± 0.0</td>
<td>105.0 ± 12.3</td>
<td>97.6 ± 10.4</td>
<td>923.6 ± 134.3</td>
<td>183.3 ± 5.3</td>
<td>348.0 ± 48.4</td>
</tr>
<tr>
<td>3</td>
<td>483.3 ± 189.5</td>
<td>120 ± 0.0</td>
<td>34.4 ± 5.9</td>
<td>75.4 ± 4.3</td>
<td>4500.6 ± 2000.5</td>
<td>169.2 ± 11.6</td>
<td>79.3 ± 35.2</td>
</tr>
</tbody>
</table>

$^{\text{a}}$After single oral injection (20 mg/kg); $^{\text{b}}$bioavailability (F%): 3% for 1; 22% for 2; 4% for 3; $^{\text{c}}$the parameters were calculated using WinNonlin (Ver. 1.1) program.

Table 3. Suppression effect of compound 2 on two tremor mouse models$^{\text{d}}$

<table>
<thead>
<tr>
<th>Compound</th>
<th>Harmaline-induced tremor model (post ip injection (10 mg/kg))</th>
<th>Genetic tremor model$^{\text{e}}$ (30 min post ip injection)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>20 - 40 min</td>
<td>40 - 60 min</td>
</tr>
<tr>
<td>2</td>
<td>44.7%</td>
<td>55.3%</td>
</tr>
<tr>
<td></td>
<td>58.0%</td>
<td>82.2%</td>
</tr>
</tbody>
</table>

$^{\text{d}}$P values less than 0.05 were considered significant; $^{\text{e}}$GABA_A receptor α1 subunit-null mouse model.

that selective T-type calcium channel blockers show in vivo efficacy in epilepsy and tremor models.\(^{20-22}\) 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 pharmaco-kinetic 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).

References

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