Synthesis and Pharmacological Evaluation of 3-\{(4-Oxo-4H-pyrido[3,2-e][1,3]thiazin-2-yl)(phenyl)amino\}propanenitrile Derivatives as Orally Active AMPA Receptor Antagonists

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In our search for novel orally active \(\alpha\)-amino-3-hydroxy-5-methyl-4-isoxazolopropionic acid (AMPA) receptor antagonists, we found that conversion of an allyl group in the lead compound 2-allyl(4-methylphenyl)amino-4H-pyrido[3,2-e][1,3]thiazin-4-one (4) to a 2-cyanoethyl group significantly increased inhibitory activity against AMPA receptor-mediated kainate-induced toxicity in rat hippocampal cultures. Here, we synthesized 10 analogs bearing a 2-cyanoethyl group and administered them to mice to evaluate their anticonvulsant activity in maximal electroshock (MES)- and pentylenetetrazol (PTZ)-induced seizure tests, and their effects on motor coordination in a rotarod test. 3-{(4-Oxo-4H-pyrido[3,2-e][1,3]thiazin-2-yl)[4-trifluoromethoxy]phenyl}amino)propanenitrile (25) and 3-{[2,2-difluoro-2H-1,3-benzodioxol-5-yl)-\(\alpha\)-amino-3-hydroxy-5-methyl-4-isoxazolopropionic acid receptor antagonist (27) exhibited potent anticonvulsant activity in both seizure tests and induced minor motor disturbances as indicated in the rotarod test. The protective index values of 25 and 27 for MES-induced seizures (10.7 and 12.0, respectively) and PTZ-induced seizures (6.0 and 5.6, respectively) were considerably higher compared with those of YM928 (5) and talampamil (1).

Key words kainate-induced neurotoxicity; anticonvulsant activity; protective index; \(\alpha\)-amino-3-hydroxy-5-methyl-4-isoxazolopropionic acid receptor antagonist

Introduction
Epilepsy, one of the most common neurological disorders, affects approximately 65 million people worldwide and is characterized by recurrent unprovoked seizures caused by an imbalance between excitatory and inhibitory neurotransmission. Although more than 40 antiepileptic drugs (AEDs) are currently on the market, they are ineffective for controlling symptoms in approximately one-third of patients, and third-generation AEDs, first marketed in the 1980s, have not decreased the proportion of intractable patients. Moreover, AED therapies are frequently associated with adverse effects on the central nervous system (CNS) such as sedation, motor disturbances, cognitive dysfunction, and psychiatric effects, as well as idiosyncratic and other adverse effects, which seriously impair quality of life. Therefore, more effective and safer AEDs must be developed.

Most available AEDs have multiple and complementary mechanisms of action, which can be categorized as blockade of voltage-dependent Na\(^+\) and/or Ca\(^{2+}\) channels, potentiation of \(\gamma\)-aminobutyric acid (GABA)-mediated inhibitory neurotransmission, and reduction of glutamate-mediated excitatory neurotransmission. Glutamate is a major neurotransmitter in the vertebrate CNS and plays an essential role in fast excitatory neurotransmission via the activation of ionotropic glutamate receptors including \(N\)-methyl-D-aspartate (NMDA), \(\alpha\)-amino-3-hydroxy-5-methyl-4-isoxazolopropionic acid (AMPA), and kainate receptors. The former two subtypes are most abundant, and the effects of their antagonists in seizure animal models have been well investigated. In studies employing a rat kindling model of complex partial seizures, selective NMDA receptor antagonists exert only weak anticonvulsant effects with marked behavioral side effects such as hyperactivity and stereotypes. Consistent with these results, the selective NMDA receptor antagonist d-CPP-ene failed to suppress intractable complex partial seizures in a clinical trial. In contrast, selective AMPA receptor antagonists have broad-spectrum anticonvulsant activity in seizure animal models, including the rat kindling model, and do not cause cognitive dysfunction or psychiatric effects associated with NMDA receptor antagonists. Compounds such as the noncompetitive antagonists talampanel (1) and perampanel (2) as well as the competitive antagonist selurampanel (3) were developed to treat epilepsy, with perampanel (2) being the first AMPA receptor antagonist introduced to the market in 2012. However, despite intense efforts to develop AMPA receptor antagonists, few such drug candidates are currently in clinical development.

In our search for a novel class of orally active AMPA receptor antagonists, we identified 2-allyl(4-methylphenyl)-amino-4H-pyrido[3,2-e][1,3]thiazin-4-one (4) as a lead compound that inhibits AMPA receptor-mediated kainate-induced toxicity in rat hippocampal cultures. A subsequent structure–activity relationship (SAR) study of the substituted phenyl ring attached to the 2-amino group led to the discovery of the selective and noncompetitive AMPA receptor antagonist 2-[(4-chlorophenyl)(methyl)amino]-4H-pyrido[3,2-e][1,3]thiazin-4-one (YM928) (5) and its analogs. Oral administration of 5 resulted in a broad spectrum of anticonvulsant activity in various seizure animal models.

Our alternative approach to enhance inhibitory activity...
against kainate-induced neurotoxicity focused on the structural modification of the allyl group in 4. In previous SAR studies of 1-phenyl-1,2,3,4-tetrahydroisoquinoline derivatives such as 6 (Fig. 3), a noncompetitive AMPA receptor antagonist, Gitto et al. suggested that the acetyl group at the 2-position, which may function as a hydrogen bond acceptor, positively influences AMPA receptor recognition and enhances anticonvulsant activity in some cases.27,28) Based on this finding and the structural similarities between 4 and 6, we designed novel 4H-pyrido[3,2-e][1,3]thiazin-4-one derivatives bearing a cyano group, which possesses hydrogen bond acceptor ability, on an alkyl substituent attached to the 2-amino group. Here, we describe the synthesis and pharmacological evaluation of 10 3-[(4-oxo-4H-pyrido[3,2-e][1,3]thiazin-2-yl)(phenyl)amino]propanenitrile derivatives as novel AMPA receptor antagonists. Four of these compounds demonstrated marked anticonvulsant activity in maximal electroshock (MES)- and pentylenetetrazol (PTZ)-induced seizure tests (MES and PTZ tests). Moreover, two of these compounds induced considerably less motor disturbances as demonstrated in a rotarod test compared with YM928 (5) and talampanel (1).

Chemistry
3-[(4-Oxo-4H-pyrido[3,2-e][1,3]thiazin-2-yl)(phenyl)amino]propanenitrile derivatives 18–27 were synthesized by adding the appropriate 3-anilinopropanenitriles to 2-chloronicotinoyl isothiocyanate, which was prepared from 2-chloronicotinoyl chloride (17) (48–87% yields)22,29) (Chart 1). The 3-anilinopropanenitriles used here were synthesized by aza-Michael addition of anilines to acrylonitrile. In this reaction, we examined the effects of catalysts such as Dowex® 50WX4; montmorillonite K10; molecular sieves 4A; Proton sponge; and acidic, neutral, and basic aluminas (data not shown). Among these, acidic and neutral aluminas exhibited sufficient catalytic activity and afforded the desired product in acceptable yields without the need for further optimization of the reaction conditions.30)

Results and Discussion
We first examined the effect of a 2-cyanoethyl group attached to the 2-amino group of the 4H-pyrido[3,2-e][1,3]thiazin-4-one ring on inhibitory activity against kainate-induced neurotoxicity. As shown in Table 1, introduction of the 2-cyanoethoxy group (20) led to a 5-fold increase in inhibitory activity compared with allyl (4) or methyl (7) groups. Encouraged by these results, we next evaluated the pharmacological activity of 3-[(4-oxo-4H-pyrido[3,2-e][1,3]thiazin-2-yl)(phenyl)amino]propanenitrile derivatives 18–27 containing different substituents on the phenyl ring. The results of a SAR study of corresponding N-methyl and N-ethyl analogs such as YM928 (5)22) suggested that compounds 18–27 should retain inhibitory activity with IC50 values less than 10µM. Anticonvulsant activity was assessed in mice subjected to MES and PTZ tests, and the data are presented as the ED50.
The effects of these compounds on motor coordination were evaluated using the rotarod test, and the data are presented as the median toxic dose (TD$_{50}$). These tests are widely used in primary screens for novel AEDs.$^{31,32}$ The protective index (PI), defined as TD$_{50}$/ED$_{50}$, was calculated for both seizure tests. The results of these pharmacological evaluations for 18–27 and the N-methyl and N-ethyl analogs 5 and 8–16 are summarized in Table 2.

In the MES test of the N-methyl and N-ethyl analogs 5 and 8–16, the 4-chlorophenyl derivative YM928 (5) exhibited the highest anticonvulsant activity (ED$_{50}$, 7.4 mg/kg) following oral administration.$^{24}$ The anticonvulsant activity of the 4-fluorophenyl (8), 4-bromophenyl (9),$^{22}$ and 4(trifluoromethyl)phenyl (10) derivatives were 2-, 3-, and >4-fold lower compared with that of 5, respectively. The 2,3-dihydro-1H-inden-5-yl derivative 11 exhibited anticonvulsant activity in half of the animals after intraperitoneal administration of 30 mg/kg, indicating that the anticonvulsant activity of 11 following oral administration should be much lower compared with that of 5. Moreover, the 3-methoxyphenyl (12) and 4-methoxyphenyl (13, 14) derivatives exhibited little or no anticonvulsant effects following intraperitoneal administration of 30 mg/kg. The benzylic methylene groups of 11 and the methoxy groups of 12–14 can be converted to benzylic hydroxyl and phenolic hydroxyl groups, respectively, through CYP metabolism. Our previous report revealed that the hydrophobic interactions of 4- (and 3-) substituents on the phenyl ring play an important role in the inhibition of kainate-induced neurotoxicity.$^{25}$ Conversion of 11–14 to metabolites bearing a hydrophilic hydroxyl group at the 3- or 4-position may explain their weak anticon-

### Table 1. Inhibitory Activity of 2-[(4-Methylphenyl)amino]-4H-pyrido[3,2-e][1,3]thiazin-4-one Derivatives against Kainate-Induced Neurotoxicity

<table>
<thead>
<tr>
<th>Compound</th>
<th>R</th>
<th>Anti-kainate toxicity IC$_{50}$ (μM)$^{a}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>Allyl</td>
<td>9.0$^{a}$</td>
</tr>
<tr>
<td>7</td>
<td>Me</td>
<td>10$^{b}$</td>
</tr>
<tr>
<td>20</td>
<td>(CH$_2$)$_2$CN</td>
<td>1.8</td>
</tr>
<tr>
<td>Talampanel (1)</td>
<td></td>
<td>6.0$^{c}$</td>
</tr>
</tbody>
</table>

*a) Concentration required for 50% inhibition. b) Ref. 22.

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### Table 2. Effects of 2-Amino-4H-pyrido[3,2-e][1,3]thiazin-4-one Derivatives in MES, PTZ, and Rotarod Tests in Mice$^{a}$

<table>
<thead>
<tr>
<th>Compound</th>
<th>R1 R2</th>
<th>MES test ED$_{50}$ (mg/kg, p.o.$^{a}$)</th>
<th>PTZ test ED$_{50}$ (mg/kg, p.o.$^{a}$)</th>
<th>Rotarod test TD$_{50}$ (mg/kg, p.o.$^{a}$)</th>
<th>PI$^{a}$ MES, PTZ</th>
</tr>
</thead>
<tbody>
<tr>
<td>YM928 (5)$^{b}$</td>
<td>4-Cl</td>
<td>Me</td>
<td>7.4 (5.9–9.1)$^{b}$</td>
<td>9.6 (7.6–12.0)$^{b}$</td>
<td>22.5 (19.5–26.1)$^{b}$</td>
</tr>
<tr>
<td>8$^{c}$</td>
<td>4-F</td>
<td>Me</td>
<td>15.3 (11.7–19.0)$^{c}$</td>
<td>35.0 (31.0–38.6)</td>
<td>33.7 (26.5–40.3)</td>
</tr>
<tr>
<td>9$^{d}$</td>
<td>4-Br</td>
<td>Me</td>
<td>23.1 (16.2–36.0)$^{d}$</td>
<td>27.2 (19.8–34.3)</td>
<td>83.3 (62.3–179.3)</td>
</tr>
<tr>
<td>10$^{e}$</td>
<td>4-CF$_3$</td>
<td>Me</td>
<td>4/10$^{e}$</td>
<td>10/10$^{e}$</td>
<td>45</td>
</tr>
<tr>
<td>11$^{f}$</td>
<td>3,4-(CH$_2$)$_2$CN</td>
<td>Me</td>
<td>5/10$^{f}$</td>
<td>30</td>
<td></td>
</tr>
<tr>
<td>12$^{g}$</td>
<td>3-OMe</td>
<td>Et</td>
<td>1/10$^{g}$</td>
<td>30</td>
<td></td>
</tr>
<tr>
<td>13$^{h}$</td>
<td>4-OMe</td>
<td>Me</td>
<td>0/10$^{h}$</td>
<td>30</td>
<td></td>
</tr>
<tr>
<td>14$^{i}$</td>
<td>4-OMe</td>
<td>Et</td>
<td>0/10$^{i}$</td>
<td>30</td>
<td></td>
</tr>
<tr>
<td>15$^{j}$</td>
<td>4-OCF$_3$</td>
<td>Me</td>
<td>25.2 (22.1–28.3)$^{j}$</td>
<td>96.5 (72.9–122.4)</td>
<td>125.2 (103.5–152.1)</td>
</tr>
<tr>
<td>16$^{k}$</td>
<td>3,4-OCH$_2$NO–</td>
<td>Et</td>
<td>38.9 (24.4–60.3)$^{k}$</td>
<td>117.4 (85.5–152.8)</td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>4-F</td>
<td>(CH$_2$)$_2$CN</td>
<td>10.9 (8.1–14.4)</td>
<td>28.7 (21.2–38.9)</td>
<td>48.2 (41.9–54.6)</td>
</tr>
<tr>
<td>19</td>
<td>4-Br</td>
<td>(CH$_2$)$_2$CN</td>
<td>4/10$^{l}$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>4-Me</td>
<td>(CH$_2$)$_2$CN</td>
<td>0/10$^{m}$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>21</td>
<td>4-CF$_3$</td>
<td>(CH$_2$)$_2$CN</td>
<td>14.5 (11.3–17.8)</td>
<td>37.5 (23.3–75.4)</td>
<td>17.1 (13.0–21.7)</td>
</tr>
<tr>
<td>22</td>
<td>3-OMe</td>
<td>(CH$_2$)$_2$CN</td>
<td>1/10$^{n}$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>3-F,4-OMe</td>
<td>(CH$_2$)$_2$CN</td>
<td>0/10$^{o}$</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$^{a}$ Mice were tested 60 min after oral administration of compounds unless otherwise indicated in braces in minutes. b) ED$_{50}$ and TD$_{50}$ values were calculated using the probit method (95% confidence intervals are shown in parentheses). c) Protective index (PI) values, defined as TD$_{50}$/ED$_{50}$, were calculated for MES- and PTZ-induced seizures. d) Ref. 24. e) Ref. 22. f) Number of animals responding out of the total number of animals tested. g) Oral administration of 30 mg/kg. h) Oral administration of 100 mg/kg. i) Intraperitoneal administration of 30 mg/kg.
vulsant activity. This hypothesis is supported by findings that 15, which bears a metabolically stable trifluoromethoxy group, exhibited good anticonvulsant activity, which was 3-fold lower compared with that of 5 following oral administration.\textsuperscript{23} The 2H-1,3-benzodioxol-5-yl derivative 16, one of the most potent inhibitors of kainate-induced neurotoxicity (IC\textsubscript{50}, 0.40 \textmu M), exhibited relatively weak anticonvulsant activity, presumably because of its susceptibility to CYP metabolism.\textsuperscript{23}

In the MES test of the N-(2-cyanoethoxy) analogs 18–27, the 4-fluorophenyl derivative 18 exhibited anticonvulsant activity comparable to that of the corresponding N-methyl analog 8, and the 4-bromophenyl derivative 19 exhibited slightly less anticonvulsant activity than the corresponding N-methyl analog 9. The 4-methylphenyl derivative 20 showed no detectable effect following oral administration of 30 mg/kg, suggesting that it may be metabolically converted to a benzylic alcohol derivative, as discussed for 11. The 4-(trifluoromethyl)phenyl derivative 21 exhibited >2-fold higher anticonvulsant activity than the corresponding N-methyl analog 10. Consistent with the results for the N-methyl and N-ethyl analogs 12–14, the 3-methoxyphenyl (22) and 4-methoxyphenyl (23) derivatives exhibited little or no anticonvulsant effects following oral administration of 30 mg/kg. Compound 23 showed poor metabolic stability in mouse liver microsomes (intrinsic clearance (CL\textsubscript{int}), >600 mL/min/kg). Introduction of a fluorine atom at the 3-position of the phenyl ring of 23 (24) did not enhance anticonvulsant activity, suggesting that the fluorine atom may not inhibit metabolism of the adjacent methoxy group. The 4-(trifluoromethoxy)phenyl derivative 25 exhibited improved metabolic stability (CL\textsubscript{int}, 111 mL/min/kg) compared with 23 and retained an anticonvulsant effect comparable to that of the corresponding N-methyl analog 15. In contrast to the corresponding N-ethyl analog 16, the 2H-1,3-benzodioxol-5-yl derivative 26 had no detectable effect following oral administration of 30 mg/kg. While the metabolic stability of 26 in mouse liver microsomes was poor (CL\textsubscript{int}, >600 mL/min/kg), introduction of two fluorine atoms at the methylenedioxy moiety (27) greatly improved metabolic stability\textsuperscript{33} (CL\textsubscript{int}, <78.8 mL/min/kg) and caused a dramatic increase in anticonvulsant activity.

In a 12-h time course study of the anticonvulsant activity of YM928 (5) in the MES test, we estimated that the time of peak effect (TPE) would be 60 min after oral administration (data not shown). A comparable effect was observed 45 min after oral administration (ED\textsubscript{50}, 13.3 mg/kg; 95% confidence interval, 10.4–16.6 mg/kg). The effects of the other compounds following oral administration were evaluated after 60 min or 45 min without estimating their respective TPE. The fact that the activity of 26 after 60 min was much weaker than that of 16 after 45 min suggests that these compounds may have short-lasting effects with TPEs less than 45 min. Therefore, the peak effect of each compound needs to be evaluated for a more precise understanding of their SAR.

Next, the N-methyl analogs YM928 (5), 8, 9, and 15, and the N-(2-cyanoethoxy) analogs 18, 21, 25, and 27, which exhibited good to excellent anticonvulsant activity in the MES test with ED\textsubscript{50} values less than 30 mg/kg, were evaluated in the PTZ test. The activity of 5\textsuperscript{33} and 9 in the PTZ test was comparable to that in the MES test. The activity of the other six compounds was 2–4-fold lower in the PTZ test compared with the MES test. The activity of talampanel (1) was 6-fold lower in the PTZ test compared to the MES test. These results suggest that the 2-amino-4H-pyrido[3,2-e][1,3]thiazin-4-one derivatives tend to maintain relatively similar anticonvulsant activity in MES and PTZ tests compared with talampanel (1).

Subsequently, the eight compounds evaluated in the PTZ test and compound 16 were assessed in the rotarod test. Oral administration of YM928 (5) markedly induced motor disturbances (TD\textsubscript{50}, 22.5 mg/kg),\textsuperscript{24} with PI values of 3.0 and 2.3 for MES- and PTZ-induced seizures, respectively. The PI values of the N-methyl and N-ethyl analogs 8, 9, 15, and 16 for MES-induced seizures were comparable to that of 5. Of the series of N-(2-cyanoethoxy) analogs, the 4-(trifluoromethyl)phenyl derivative 21 induced motor disturbances at a dose close to its ED\textsubscript{50} for MES-induced seizures. The PI values of the 4-fluorophenyl (18) and 4-(trifluoromethoxy)phenyl (25) derivatives for MES-induced seizures were 2-fold higher than those of their corresponding N-methyl analogs 8 and 15, respectively. Further, the PI value of 25 (10.7) was >3-fold higher compared with that of 5. Moreover, the PI value of the 2,2-difluoro-2H-1,3-benzodioxol-5-yl derivative 27 for MES-induced seizures (12.0) was the highest among the 2-amino-4H-pyrido[3,2-e][1,3]-thiazin-4-one derivatives, which was 4-fold higher compared with that of 5. In addition, the PI values of 25 and 27 for PTZ-induced seizures (6.0 and 5.6, respectively) were >2-fold higher compared with that of 5. In contrast, talampanel (1) markedly induced motor disturbances, with PI values of 2.8 and 0.5 for MES- and PTZ-induced seizures, respectively, which were similar or lower compared with those of 5.

Finally, we evaluated the brain penetration of compound 27 by measuring plasma and brain concentrations upon administration to mice (Table 3). After an oral administration of 15 mg/kg of 27, concentrations in plasma and brain at 60 min were 1450 ng/mL and 1720 ng/g, respectively. K\textsubscript{p,brain}, defined as the brain-to-plasma concentration ratio, was 1.2, confirming that 27 possesses adequate blood–brain barrier permeability for evaluation in CNS pharmacological studies.

Representative AMPA receptor antagonists such as NBQX,\textsuperscript{34,35} tezampanel,\textsuperscript{36,37} GYK152466,\textsuperscript{34,35,37,38} CP-465022,\textsuperscript{39} and perampanel (2)\textsuperscript{40,41} induce sedation or motor disturbances at doses close to those required for anticonvulsant effects in animals. Therefore, development of AMPA receptor antagonists with reduced CNS-depressant effects remains a challenge.\textsuperscript{42–45} Our present results suggest that compounds 25 and 27 may be safer due to their lower CNS-depressant effects compared to representative AMPA receptor antagonists. To confirm this hypothesis, the pharmacological profiles of these AMPA receptor antagonists should be assessed under the same experimental conditions.

**Conclusion**

Our SAR study of the alkyl substituents attached to the 2-amino group of 4H-pyrido[3,2-e][1,3]thiazin-4-one derivatives to determine their AMPA receptor antagonist activity showed that introduction of a 2-cyanoethyl group

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### Table 3. Plasma and Brain Concentrations of 27 after Oral Administration to Mice

<table>
<thead>
<tr>
<th>Dose (mg/kg)</th>
<th>Time (min)</th>
<th>Plasma (ng/mL)</th>
<th>Brain (ng/g)</th>
<th>K\textsubscript{p,brain}</th>
<th>a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>60</td>
<td>1450</td>
<td>1720</td>
<td>1.2</td>
<td></td>
</tr>
</tbody>
</table>

a) Brain-to-plasma concentration ratio.
(20), compared with allyl (4) and methyl (7) groups, significantly increased inhibitory activity against kainate-induced toxicity in primary rat hippocampal cultures. Here, among a series of N-(2-cyanoethyl) analogs, 3-{[4-fluorophenyl](4-oxo-4H-pyrido[3,2-e][1,3]thiazin-2-yl)-amino}propanenitrile (18), 3-{[4-fluorophenyl]pyrido[3,2-e][1,3]-thiazin-2-yl)[4-trifluoromethyl]phenyl]amino}propanenitrile (21), and 3-{[4-oxo-4H-pyrido[3,2-e][1,3]thiazin-2-yl]-[4-(trifluoromethoxy)phenyl]amino}propanenitrile (25) exhibited similar or more potent anticonvulsant activity in the MES test than their corresponding N-methyl analogs 8, 10, and 15, respectively. In addition, 3-{[(2,2-difluoro-2H,1,3-benzodioxol-5-yl)(4-oxo-4H-pyrido[3,2-e][1,3]thiazin-2-yl)-amino}propanenitrile (27) exerted anticonvulsant activity comparable to that of 18, 21, and 25. Compounds 18, 21, 25, and 27 exhibited 2-3-fold lower anticonvulsant activity in the PTZ test compared with the MES test. Further, the PI values of 25 and 27 for MES-induced seizures (10.7 and 12.0, respectively) and PTZ-induced seizures (6.0 and 5.6, respectively) were comparable to that of 18.

Experimental

Melting points were determined using a Yanaco MP-S3 melting point apparatus or a TA DSC Q2000 differential scanning calorimeter. 1H-NMR spectra were recorded on a JEOL JNM EX400, a Varian VNS 400, or a Bruker Avance III HD 500 spectrometer. 13C-NMR spectra were recorded on a Bruker Avance III HD 500 spectrometer. Chemical shifts are expressed in δ (ppm) values using tetramethylsilane as an internal standard (NMR descriptions: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; and br, broad peak). Mass spectra were recorded on a Waters UPLC/SQD- LC/MS system or an Agilent HP 5970 MSD spectrometer. Elemental analyses were conducted using a Yanaco MT-5 microanalyzer (C, H, N) and a Yokogawa IC-7000S ion chromatographic analyzer (halogen and S). The animal experimental procedures were approved by the Corporate Animal Ethical Committee.

3-{[4-Fluorophenyl]amino}propanenitrile. A mixture of 4-fluoroaniline (5.56 g, 50.0 mmol), acrylonitrile (8.00 g, 151 mmol), and alumina (2.00 g, 19.6 mmol) was heated at 40°C for 10 min. After cooling, 4-fluoroaniline (5.56 g, 50.0 mmol), acrylonitrile (8.00 g, 125 mmol), and alumina (2.00 g, 19.6 mmol) was heated at 40°C for 10 min. After cooling, a mixture of 3-{[4-bromophenyl]amino}propanenitrile instead of 3-{[4-fluorophenyl]amino}propanenitrile was prepared from 17 in the same manner as described for 18, using 3-{[4-bromophenyl]amino}propanenitrile instead of 3-{[4-fluorophenyl]amino}propanenitrile. Off-white solid, 87% yield, mp 190°C (95% ethanol). 1H-NMR (500 MHz, CDCl3) δ: 8.67 (1H, dd, J = 7.9, 1.9 Hz), 8.61 (1H, dd, J = 4.7, 1.9 Hz), 7.44–7.49 (2H, m), 7.42 (1H, dd, J = 8.0, 4.6 Hz), 7.07–6.93 (2H, m), 7.33–7.37 (2H, m), 3.42 (2H, t, J = 6.4 Hz), 2.99 (2H, t, J = 6.4 Hz). 13C-NMR (125 MHz, CDCl3) δ: 169.42, 165.59, 155.58, 153.07, 138.24, 138.17, 134.14, 130.88, 124.97, 123.74, 119.81, 117.51, 48.32, 16.75. MS (ESI) m/z: 387, 389 [M+H]+. Anal. Calc. for C_{16}H_{11}N_{4}O_{3}: C, 58.89; H, 3.40; N, 17.17; S, 9.83; F, 5.82. Found: C, 58.79; H, 3.29; N, 17.06; S, 9.93; F, 5.74.

3-{[4-Bromophenyl]amino}propanenitrile (19) The title compound was prepared from 17 in the same manner as described for 18, using 3-{[4-bromophenyl]amino}propanenitrile instead of 3-{[4-fluorophenyl]amino}propanenitrile. Off-white solid, 87% yield, mp 190°C (95% ethanol). 1H-NMR (500 MHz, CDCl3) δ: 7.27–7.32 (2H, m), 6.48–6.53 (2H, m), 4.02 (1H, brs), 3.50 (2H, t, J = 6.4 Hz), 2.63 (2H, t, J = 6.5 Hz). MS (ESI) m/z: 225 [M+H]+.

3-{[4-(4-Methylphenyl)amino]propanenitrile (20) A mixture of p-toluidine (6.77 g, 63.2 mmol), acrylonitrile (10.1 g, 190 mmol), and alumina (2.50 g, 24.5 mmol) was heated at 60°C in a screw-capped tube for 3 h. After cooling, the mixture was diluted with chloroform, and alumina was filtered off. The filtrate was successively washed with 1M HCl, saturated aqueous NaHCO3 solution, and brine, dried over anhydrous MgSO4, and concentrated in vacuo. The residue was recrystallized from 95% ethanol to yield the title compound (0.90 g, 20%). 1H-NMR (500 MHz, CDCl3) δ: 7.27–7.32 (2H, m), 6.48–6.53 (2H, m), 4.02 (1H, brs), 3.50 (2H, t, J = 6.4 Hz), 2.63 (2H, t, J = 6.5 Hz). MS (ESI) m/z: 225 [M+H]+.

3-{[4-(4-Methylphenyl)]amino}propanenitrile (20). A mixture of p-toluidine (6.77 g, 63.2 mmol), acrylonitrile (10.1 g, 190 mmol), and alumina (2.50 g, 24.5 mmol) was heated at 60°C in a screw-capped tube for 3 h. After cooling, the mixture was diluted with chloroform, and alumina was filtered off. The filtrate was successively washed with 1M HCl, saturated aqueous NaHCO3 solution, and brine, dried over anhydrous MgSO4, and concentrated in vacuo. The residue was recrystallized from 95% ethanol to yield the title compound (0.90 g, 20%). 1H-NMR (500 MHz, CDCl3) δ: 7.27–7.32 (2H, m), 6.48–6.53 (2H, m), 4.02 (1H, brs), 3.50 (2H, t, J = 6.4 Hz), 2.63 (2H, t, J = 6.5 Hz). MS (ESI) m/z: 225 [M+H]+.

3-{[4-(4-Methylphenyl)]amino}propanenitrile (20). A mixture of p-toluidine (6.77 g, 63.2 mmol), acrylonitrile (10.1 g, 190 mmol), and alumina (2.50 g, 24.5 mmol) was heated at 60°C in a screw-capped tube for 3 h. After cooling, the mixture was diluted with chloroform, and alumina was filtered off. The filtrate was successively washed with 1M HCl, saturated aqueous NaHCO3 solution, and brine, dried over anhydrous MgSO4, and concentrated in vacuo. The residue was recrystallized from 95% ethanol to yield the title compound (0.90 g, 20%). 1H-NMR (500 MHz, CDCl3) δ: 7.27–7.32 (2H, m), 6.48–6.53 (2H, m), 4.02 (1H, brs), 3.50 (2H, t, J = 6.4 Hz), 2.63 (2H, t, J = 6.5 Hz). MS (ESI) m/z: 225 [M+H]+.
Compounds 21–27 were prepared in a similar manner.

3-[4-(4-Oxo-4H-pyrido[3,2-e][1,3]thiazin-2-yl)amino]propanenitrile (23) White solid, 48% yield, mp 164°C (95% ethanol). 1H-NMR (400 MHz, CDCl3) δ: 8.67 (1H, dd, J = 8.0, 1.8 Hz), 8.60 (1H, dd, J = 4.6, 1.7 Hz), 7.47 (1H, t, J = 8.1 Hz), 7.41 (1H, dd, J = 7.9, 4.6 Hz), 7.07–7.11 (1H, m), 6.99–7.02 (1H, m), 6.97 (1H, t, J = 2.2 Hz), 4.35 (2H, t, J = 6.5 Hz), 3.87 (3H, s), 2.98 (2H, t, J = 6.6 Hz). 13C-NMR (125 MHz, CDCl3) δ: 169.63, 166.50, 161.80, 161.33, 155.95, 152.94, 140.04, 138.19, 131.52, 132.54, 120.85, 119.89, 117.59, 117.0, 114.43, 55.69, 48.16, 16.68. MS (ESI) m/z: 377 [M + H]⁺. Anal. Calcd for C17H13N4O2SF: C, 52.54; H, 2.95; N, 14.89; S, 8.52; F, 15.14. Found: C, 54.19; H, 3.10; N, 14.84; S, 8.61; F, 15.35.

3-[3-Methoxyphenyl]-4-(4-oxo-4H-pyrido[3,2-e][1,3]thiazin-2-yl)aminopropanenitrile (22) Off-white solid, 78% yield, mp 139°C (95% ethanol). 1H-NMR (400 MHz, CDCl3) δ: 8.67 (1H, dd, J = 8.0, 1.8 Hz), 8.60 (1H, dd, J = 4.6, 1.7 Hz), 7.47 (1H, t, J = 8.1 Hz), 7.41 (1H, dd, J = 7.9, 4.6 Hz), 7.07–7.11 (1H, m), 6.99–7.02 (1H, m), 6.97 (1H, t, J = 2.2 Hz), 4.35 (2H, t, J = 6.5 Hz), 3.87 (3H, s), 2.98 (2H, t, J = 6.6 Hz). 13C-NMR (125 MHz, CDCl3) δ: 169.63, 166.50, 161.80, 161.33, 155.95, 152.94, 140.04, 138.19, 131.52, 132.54, 120.85, 119.89, 117.59, 117.0, 114.43, 55.69, 48.16, 16.68. MS (ESI) m/z: 339 [M + H]⁺. Anal. Calcd for C17H13N4O2S: C, 60.34; H, 4.17; N, 16.56; S, 9.48. Found: C, 60.48; H, 4.19; N, 16.63; S, 9.51.

3-[4-Methoxyphenyl]-4-(4-oxo-4H-pyrido[3,2-e][1,3]thiazin-2-yl)aminopropanenitrile (24) Pale yellow solid, 79% yield, mp 182°C (95% ethanol). 1H-NMR (400 MHz, CDCl3) δ: 8.66 (1H, dd, J = 7.9, 1.9 Hz), 8.60 (1H, dd, J = 4.6, 2.0 Hz), 7.40 (1H, dd, J = 7.9, 4.6 Hz), 7.32–7.37 (2H, m), 7.03–7.08 (2H, m), 4.32 (2H, t, J = 6.6 Hz), 3.89 (3H, s), 2.96 (2H, t, J = 6.7 Hz). 13C-NMR (125 MHz, CDCl3) δ: 169.63, 166.50, 162.00, 156.01, 152.90, 138.17, 131.58, 130.43, 130.50, 119.83, 117.58, 115.89, 55.71, 48.26, 16.61. MS (ESI) m/z: 339 [M + H]⁺. Anal. Calcd for C17H14N4O2S: C, 60.34; H, 4.17; N, 16.56; S, 9.48. Found: C, 60.35; H, 4.10; N, 16.47; S, 9.52.

3-[2,4-Difluoro-2H-1,3-benzodioxol-5-yl](4-oxo-4H-pyrido[3,2-e][1,3]thiazin-2-yl)aminopropanenitrile (27) White solid, 74% yield, mp 177°C (95% ethanol). 1H-NMR (500 MHz, DMSO-d6, 80°C) δ: 8.68 (1H, dd, J = 4.7, 1.8 Hz), 8.51 (1H, dd, J = 7.8, 1.8 Hz), 7.72 (1H, dd, J = 2.1 Hz), 7.61 (1H, dd, J = 8.4 Hz), 7.56 (1H, dd, J = 7.8, 4.6 Hz), 7.46 (1H, dd, J = 8.4, 2.1 Hz), 4.29 (2H, t, J = 6.7 Hz), 2.96 (2H, t, J = 6.7 Hz). 13C-NMR (125 MHz, CDCl3) δ: 169.37, 165.94, 155.48, 153.14, 144.98, 144.79, 138.29, 134.75, 133.95, 131.89, 129.83, 125.66, 123.84, 119.77, 115.99, 111.15, 11.00, 48.62, 16.77. MS (ESI) m/z: 389 [M + H]⁺. Anal. Calcd for C17H14N4O2SF2: C, 52.58; H, 2.60, N, 14.43; S, 8.26; F, 9.78. Found: C, 52.65; H, 2.55; N, 14.27; S, 8.11; F, 9.86.

Inhibition of Kainate-Induced Toxicity in Primary Rat Hippocampal Cultures Hippocampal cell cultures were prepared from embryonic day 18–20 Wistar rats were used after culturing for 8 or 9 d in vitro. The cells were simultaneously treated with test compounds and 300 μM kainate. Neuronal cell injury was quantitatively assessed by measuring the release of lactate dehydrogenase (LDH) into the extracellular fluid from damaged or destroyed cells 24 h after kainate exposure. LDH activity was measured using an LDH assay kit in a 7250 Automatic Analyzer (Hitachi, Japan). A single experiment for each compound was performed in triplicate.

MES-Induced Seizures Male ICR mice were stimulated with corneal electrodes using a supraphosphorescent current (50Hz, 50mA, 0.2s). The electrodes were placed in the 0.9% sodium chloride solution before application. Tonic hind limb extension (limb extension exceeding a 90° angle to the plane of the body) was used as the criterion of convulsion. Compounds were administered orally 60 min before the stimulus unless otherwise noted in Table 2. ED50 values, the dose that prevents tonic hind limb seizures in 50% of animals, and 95% confidence intervals were calculated using the probit method (α = 9–10 per group).

PTZ-Induced Seizures PTZ (100 mg/kg) was injected subcutaneously 60 min after the oral administration of compounds. Male ICR mice were observed for 30 min after injection, and clonic seizure, tonic seizure, tonic extension, and death were monitored. ED50 values and 95% confidence inter-

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vals of clonic seizure were calculated using the probit method (n = 10–12 per group).

**Rotarod Performance** Male ICR mice that remained on a rotarod apparatus revolving at 5 rpm for 120 s were selected for testing. Compounds were administered orally, and rotarod performance was restested 60 min later unless otherwise noted in Table 2. Mice that did not remain on the apparatus for 60 s in three trial sessions were considered motor-impaired. The number of motor-impaired mice was determined, and TD50 values and 95% confidence intervals were calculated using the probit method (n = 3–16 per group).

**In Vitro Liver Microsomal Stability** To estimate stability against mouse hepatic CYPs, test compounds (0.2 µM) were incubated with pooled male CD1 mouse liver microsomes (0.2 mg protein/mL) in the presence of reduced nicotinamide adenine dinucleotide phosphate (1 mM) and ethylenediaminetetraacetic acid (0.1 mM) in pH 7.4 phosphate buffer (100 mM) at 37°C. Incubations were conducted for 0 and 30 min. The 

**Plasma and Brain Concentrations of Compound 27 after Oral Administration** Plasma and brain samples were collected from male ICR mice 60 min after oral administration of 27 at 15 mg/kg (n = 2). Brain samples were homogenized with phosphate-buffered saline (20% w/v). The test compound in plasma and brain homogenate samples was extracted by deproteination with acetonitrile and analyzed using LC/MS/MS.

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**Conflict of Interest** The authors declare no conflict of interest.

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