Disruption of Kv1.1 N-type inactivation by novel small molecule inhibitors (disinactivators)

Qiang Lu, Joseph Peevey, Flora Jow, Michael M. Monaghan, Grace Mendoza, Howard Zhang, Jerome Wu, Callain Y. Kim, James Bicksler, Lynn Greenblatt, Stephen S. Lin, Wayne Childers and Mark R. Bowlby

aDiscovery Neuroscience, Wyeth Research, CN 8000, Princeton, NJ 08543-8000, USA
bChemical and Screening Sciences, Wyeth Research, CN 8000, Princeton, NJ 08543-8000, USA

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Abstract—Kv1.1 channels are expressed in many regions of the brain and spinal cord [Monaghan, M. M.; Trimmer, J. S.; Rhodes, K. J. J. Neurosci. 2001, 21, 5973; Rasband, M. N.; Trimmer, J. S. J. Comp. Neurol. 2001, 429, 166; Trimmer, J. S.; Rhodes, K. J. Ann. Rev. Physiol. 2004, 66, 477]. When expressed alone, they produce a delayed rectifier slowly inactivating type current that contributes to hyperpolarizing the neuron following depolarization. In the hippocampus Kv1.1 is co-expressed with Kvβ1 (and other β subunits), which converts Kv1.1 into a transient, fast inactivating current, reducing its ability to hyperpolarize the cell and thus increasing neuronal excitability. To reduce neuronal excitability, screening for compounds that prevent inactivation of Kv1.1 channels by Kvβ1 was performed using a yeast two-hybrid screen. A variety of compounds were discovered in this assay and subsequently determined to disrupt inactivation of the ionic currents, and hence were termed ‘disinactivators’. Several of these disinactivators also inhibited pentyleneetetrazole-induced seizures (PTZ) in mice. Compounds were found to act by several mechanisms to prevent Kvβ1 inactivation of Kv1.1 channels, including enhancement of Ca2+ release/influx and by direct mechanisms. Two structural classes were identified that act on a Kvβ1N70-Kv1.1 chimera where the N-terminal 70 amino acids of Kvβ1 were attached to the N-terminus of Kv1.1. It is likely that these disinactivators act directly on the Kvβ1 N-terminus or its receptor site on Kv1.1, thus preventing it from blocking Kv1.1 channels. Compounds acting by this mechanism may be useful for reducing neuronal hyperexcitability in diseases such as epilepsy and neuropathic pain.

Keywords: Voltage-gated K+ channels; Inactivation; Fluorescent imaging plate reader (FLIPR); Electrophysiology.

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1. Introduction

Voltage-gated (Kv) K+ channels are integral transmembrane proteins found in all excitable cells. The primary purpose of these channels is to open upon membrane depolarization, allowing potassium ions to exit, and repolarize the cell. After opening, many voltage-gated K+ channels rapidly close through a process called inactivation. In Kv1.1 potassium channels, fast inactivation occurs when the cytoplasmic N-terminus of an associated β subunit (Kvβ1) enters the channel cavity and blocks the pore and thus is termed N-type inactivation. Kv1.1 channels are highly expressed in hippocampal neurons where they play an important role in regulating action potential firing. Activation of Kv1.1 channels terminates action potentials by hyperpolarizing the neuron, while inactivation of Kv1.1 channels prolongs action potential duration and increases neuronal excitability. Increased Kv1.1/Kv1.2 channel expression has also been demonstrated in spinal cord white matter after spinal cord injury in the rat, which may contribute to altered neuronal excitability in this tissue.

Kvβ1 and Kvβ1.3 are splice variants of the same gene differing only at the N-terminus. Kvβ1 is expressed primarily in brain and inactivates only Kv1.1 channels. Kvβ1.3 is expressed in heart cells and inactivates Kv1.4 and Kv1.5, but not Kv1.1, channels.

Drugs that prevent inactivation of Kv1.1 channels in the brain might be useful for treating diseases with aberrant neuronal hyperexcitability, such as epilepsy and neuropathic pain. Unlike existing anticonvulsants, these drugs would not preclude neurons from responding to an
excitatory stimulus and generating an action potential, but instead would only reduce their repetitive firing.

Having successfully used a yeast two-hybrid assay to find small molecule inhibitors of protein–protein interactions in a calcium channel,\textsuperscript{16} we designed another yeast two-hybrid (YTH) inverse selection (rescue) assay to identify compounds that block the interaction between Kvβ1 and the 16 amino acids comprising the S4–S5 cytoplasmic linker of human Kv1.1.\textsuperscript{17} These compounds rescued yeast cells growing on cyclohexamide-containing media and eliminated N-type inactivation in potassium currents produced by Kv1.1 and Kvβ1 expressed in CHO cells or \textit{Xenopus} oocytes, and thus were named ‘disinactivators’.\textsuperscript{18} A previous study has described an erhstatan analog that disinactivated both Kv1.4 and Kv1.1/Kvβ1, independently of cysteine oxidation or tyrosine kinase inhibition.\textsuperscript{19}

To assess the specificity of disinactivators for Kv1.1/Kvβ1, we tested their activity on two other inactivating potassium channels, Kv1.4 and Kv4.2, and for their ability to increase intracellular calcium, a mechanism reported to remove inactivation in Kv1.1/Kvβ1.\textsuperscript{20} The compounds were also evaluated against a Kv1.1/β1 N-terminus chimeric channel, in which the Kvβ1 inactivating ball-and-chain machinery (N1-70 amino acids) was tethered to the Kv1.1 subunit. Using the chimera, we were able to identify four disinactivators that appear to act on the N-terminus of Kvβ1 or its receptor site in Kv1.1. All of the disinactivators prevented PTZ-induced seizures in vivo, regardless of their mechanism of action.

2. Results

When expressed alone in \textit{Xenopus} oocytes, Kv1.1α subunits produce non-inactivating voltage activated K\textsuperscript{+} currents (Fig. 1A), while co-injection of Kvβ1 with Kv1.1 results in a rapidly inactivating current (Fig. 1B). This fast inactivation is caused by the Kv1.1 channel being blocked by the inactivation peptide of the N-terminus of Kvβ1.\textsuperscript{6} To discover small molecules that interfere with this inactivation process, a YTH screen was performed where full length Kvβ1 was linked to the GAL4 DNA binding domain and the S4–S5 linker of Kv1.1 (amino acids 313–328, GenBank Accession No. L02750) was linked to the GAL4 activation domain. Interactions between Kvβ1 and Kv1.1 bring the activation and binding domains together and trigger the transcription of CYH2 gene, preventing growth of yeast colonies on media containing cyclohexamide.\textsuperscript{16} Drugs that block the Kvβ1–Kv1.1 linker interaction prevent the CYH2 gene from being transcribed and allow yeast to grow. Screening approximately 500,000 random compounds from the Wyeth collection resulted in 18 reproducible, dose-dependent hits. As an example, one of the disinactivating compounds discovered in this YTH screen, compound 1, prevented the inactivation of Kv1.1 by Kvβ1, restoring a Kv1.1-like, non-inactivating current (Fig. 1C). Compound 1 showed only modest enhancement of the peak current, but it slowed the rate of inactivation and significantly increased the residual non-inactivated current at the end of the 200 ms depolarization pulse. These effects of compound 1 were reversible upon washing (Fig. 1C). Other disinactivators had similar effects on these currents in oocytes. A summary of the compounds tested in each of the assays used after the initial YTH screen is given in Table 1.
The specificity of disinactivators for inactivation of Kv1.1/Kvβ1 channels was evaluated by testing the compounds on two other inactivating potassium channels, Kv1.4 and Kv4.2, which are also expressed in brain. Kv1.4 inactivates by a similar N-type mechanism, but unlike Kv1.1/Kvβ1, it is the N-terminus of the Kv1.4α subunit itself that inactivates the channel. Although Kv4.2 inactivates with a similar time course to Kv1.1/Kvβ1, it does so by a different mechanism, termed ‘V-type’ inactivation by some. Most of the compounds tested had no effect on Kv1.4 inactivation, as illustrated by compound 2 (Fig. 2A). However, two compounds did show a small transient disinactivation of Kv1.4 currents, illustrated by compound 3 (Fig. 2B). The transient nature and small amplitude of the disinactivation clearly separated this effect from that observed on Kv1.1/Kvβ1.

In the cyclohexadiione series exemplified by compounds 1 and 5, however, significant disinactivation occurred, resulting in similar IC₅₀ in the two channels (Table 2). None of the compounds tested at 30 µM had any effect on Kv4.2 inactivation (data not shown).

Inactivation of Kv1.1/Kvβ1 channels can be altered (reduced) by several biological processes, including increases in intracellular calcium. The effect of Ca²⁺ in this system is illustrated in Figure 3A, where the effect of the Ca²⁺ ionophore A23187 is shown to transform fast inactivation of Kv1.1/Kvβ1 into slowly inactivating current reminiscent of the effect of disinactivator compounds. To test if disinactivator compounds act by increasing intracellular Ca²⁺, a FLIPR-based calcium sensitive assay was used to identify disinactivators that

Table 1. Summary of compound activity in various assays

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Measurement</th>
<th>Number of compounds tested</th>
<th>Number of actives</th>
</tr>
</thead>
<tbody>
<tr>
<td>TEVC (oocytes)</td>
<td>Disinactivation on Kv1.1α + Kvβ1</td>
<td>847</td>
<td>84</td>
</tr>
<tr>
<td>FLIPR (TSA201 cells)</td>
<td>Calcium mobilization</td>
<td>84</td>
<td>19</td>
</tr>
<tr>
<td>TEVC (oocytes)</td>
<td>Disinactivation on Kv1.1/βN chimera</td>
<td>65</td>
<td>4</td>
</tr>
<tr>
<td>PTZ mouse model</td>
<td>Effective on seizures</td>
<td>89</td>
<td>46</td>
</tr>
</tbody>
</table>

Compounds were obtained from yeast based primary screening, structure similarity searches, and new syntheses. Actives in the TEVC assays were defined as compounds that showed greater than 50% disinactivation at 50 µM. In the FLIPR assay, any compound at 50 µM or less that increased fluorescent changes greater than 25% of that induced by 10 µM A23187 was considered active. Compounds were considered effective in the PTZ model if they reduced the number of animals having seizures compared to control.

Table 2. Disinactivation activity in oocytes expressing each of the four different channels

<table>
<thead>
<tr>
<th>Compound</th>
<th>Kv1.1/β1 (n)</th>
<th>Kv1.4 (n)</th>
<th>Kv1.1/βN chimera (n)</th>
<th>Kv1.1 % change (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4.3 (7)</td>
<td>2.3 (3)</td>
<td>16.7 (8)</td>
<td>n/e (5)</td>
</tr>
<tr>
<td>2</td>
<td>18.5% (3), 40% at 200 µM (5)</td>
<td>n/e (3)</td>
<td>n/e (8)</td>
<td>—</td>
</tr>
<tr>
<td>3</td>
<td>45% at 150 µM (3)</td>
<td>5% (3)</td>
<td>n/e (3)</td>
<td>—</td>
</tr>
<tr>
<td>4</td>
<td>96.3% (3)</td>
<td>—</td>
<td>n/e (3)</td>
<td>—</td>
</tr>
<tr>
<td>5</td>
<td>7.1 (9)</td>
<td>1.3 (3)</td>
<td>8.9 (5)</td>
<td>n/e (3)</td>
</tr>
<tr>
<td>6</td>
<td>52.5% (5)</td>
<td>—</td>
<td>34% (6)</td>
<td>n/e (3)</td>
</tr>
<tr>
<td>7</td>
<td>18.8% (3)</td>
<td>—</td>
<td>37% (9)</td>
<td>n/e (5)</td>
</tr>
</tbody>
</table>

Compound effects expressed as an EC₅₀ in µM or percent disinactivation at 50 µM, unless otherwise noted. Concentrations all expressed as micromolar. Number of cells tested is expressed as (n). n/e = no effect.
increase intracellular calcium in tsa201 cells, a cell line which shows a robust Ca\(^{2+}\) signal in a non-K\(_v\) channel dependent manner. Of the 84 disinactivators examined, 19 generated increases in intracellular calcium (Table 1). One of these disinactivators, compound 4 (at 50 \(\mu\)M), elevated intracellular Ca\(^{2+}\) to a similar magnitude as that observed with A23187 (10 \(\mu\)M) (Fig. 3B). Pre-loading cells with the calcium chelator, BAPTA-AM, eliminated these effects, indicating that the increase in fluorescence was not due to autofluorescence of the compound.

Kv\(_{\beta}1\) is one of three splice variants generated from the Kv\(_{\beta}1\) gene. All three splice variants share the same C-terminal core region and differ only in their N-termini.\(^{12-15}\) All three Kv\(_{\beta}1\) splice variants induce fast inactivation on the Kv\(_{\beta}1\) subunits, while Kv\(_{\beta}2\) and Kv\(_{\beta}3\) do not. The C-terminal core contains the region that interacts with the T1 domain of the Kv\(_{\beta}1\) subunit and is conserved in the Kv\(_{\beta}2\) and Kv\(_{\beta}3\) genes.\(^{24}\) Compounds that block the association of the Kv\(_{\beta}1\) C-terminal core with the T1 domain should also prevent inactivation of Kv\(_{\beta}1\) channels by Kv\(_{\beta}1\), but they may also disrupt the interaction of Kv\(_{\beta}2\) and Kv\(_{\beta}3\) with their appropriate targets. To distinguish disinactivators that act specifically on the N-terminus of Kv\(_{\beta}1\), we generated a Kv\(_{\beta}1\)/Kv\(_{\beta}1\) N-terminal chimera channel, in which the Kv\(_{\beta}1\) inactivating N-terminus was tethered to the Kv\(_{\beta}1\) subunit (Fig. 4A). Upon assembly into a tetramer, the channel complex should contain four Kv\(_{\beta}1\) N-termini, corresponding to the 1\(\alpha\)-1\(\beta\) subunit stoichiometry present in native cells that contain those subunits.\(^{25}\) As expected, oocytes injected with mRNA encoding the chimera gave rise to fast inactivating K\(^{+}\) currents (Fig. 4B). The current–voltage relation was similar to that obtained from Kv\(_{\beta}1\) alone (Fig. 4C), and the inactivation kinetics resembled those recorded from oocytes expressing separate Kv\(_{\beta}1\) and Kv\(_{\beta}1\) subunits. The fraction of inactivated current at the end of the voltage pulse was larger for the chimera (and Kv\(_{\beta}1\)) than with the separate expression of Kv\(_{\beta}1\) and Kv\(_{\beta}1\), indicating either a more rapid dissociation of the N-terminal blocker or that some fraction of the Kv\(_{\beta}1\) channels was not associated with Kv\(_{\beta}1\) in the latter case.

The remaining 65 disinactivators that did not increase intracellular calcium were tested for their ability to prevent inactivation of the Kv\(_{\beta}1\)/\(\beta\)1 N-terminal chimera. While all of the compounds prevented inactivation of Kv\(_{\beta}1\) and Kv\(_{\beta}1\) channels in oocytes, most had weaker or no effect on the Kv\(_{\beta}1\)/\(\beta\)1 N-terminal chimera. For example, compound 2 demonstrated disinactivating activity on Kv\(_{\beta}1\) channels co-expressed with full length Kv\(_{\beta}1\) (Fig. 5A), but did not eliminate fast inactivation of the chimeric channel (Fig. 5B). Only four of the 65 disinactivators tested were able to disinactivate the chimeric channel. None of the four compounds affected Kv\(_{\beta}1\) current alone, consistent with a possible N-terminal site of action for these four compounds (Table 2). Compounds that disinactivate Kv\(_{\beta}1\)/\(\beta\)1, but not the chimera, presumably act via a different site in the Kv\(_{\beta}1\)/\(\beta\)1 channel complex, perhaps by interacting with the non-N-terminal portion of the \(\beta\)1 subunit, such as in or near the NADPH binding site.

The structures of these four ‘specific’ compounds fall into two distinct chemical classes, cyclohexadiones (Fig. 6A) and hydantoinos (Fig. 6B). Structures of the 1,3-dione-2-carboxamide compounds which disinactivate only the Kv\(_{\beta}1\)/\(\beta\)1 channel are illustrated in Fig. 6C, along with the one disinactivator shown to mobilize Ca\(^{2+}\) (compound 4).

The effect of compound 5 on inactivation of the Kv\(_{\beta}1\)/\(\beta\)1 N-terminal chimera current was concentration-dependent (Fig. 7A), and at the highest concentration of 30 \(\mu\)M the residual current at the end of the 200 ms
pulse increased remarkably, whereas the peak current remained relatively unchanged. This is similar to the pattern of disinactivation observed with the Kv1.1/K\(\beta\)1 channel. The effect of compound 5 was reversible and could be washed out within minutes (Fig. 7A). The EC\(_{50}\) for compound 5 on the chimeric channel was determined to be 6.0 \(\mu\)M ± 0.6 \(\mu\)M (Fig. 7B). The full profile of activity for all seven compounds reported here in the four different channels tested is detailed in Table 2.

Finally, the in vivo anticonvulsant activity of the disinactivators was examined. In the PTZ model, compounds 2 and 3 were two of the more potent compounds, with ED\(_{50}\)s of 32 and 66 mg/kg, respectively. Compound 5 had little effect when given at 100 mg/kg ip, but at higher doses of 178 and 300 mg/kg, it prevented seizures from developing in half the animals (Table 3). Compound 1 showed a similar pattern of activity, having an ED\(_{50}\) of 139 mg/kg, while compound 6 blocked nearly all seizures at 100 mg/kg ip. Compound 4 was inactive in the PTZ model, perhaps relating to its ability to elevate intracellular Ca\(^{2+}\) in the in vitro assay.

3. Discussion

Inactivation of voltage-gated potassium channels in neurons shortens the duration of hyperpolarization fol-
Following an action potential and increases the probability of another action potential. The importance of this activity has recently been demonstrated by Schulte et al., where they reported that Lgi1 (leucine-rich glioma inactivated gene 1 protein) associates with Kv1.1 and inhibits its inactivation by Kvβ1. Mutations in the LGI1 gene result in a protein that is defective in preventing Kvβ1-mediated inactivation and is the cause of autosomal dominant lateral temporal lobe epilepsy (ADLTE). Thus, modulating or eliminating inactivation of voltage-gated potassium channels should prolong the resting period and prevent hyperexcitability of neurons, a potentially useful approach for treating epilepsy or pain.

Several mechanisms for modulating N-type inactivation have been reported, including phosphorylation and dephosphorylation, oxidation-reduction reactions, and intracellular calcium. The current study focused on identifying compounds that specifically targeted Kvβ1-mediated inactivation of Kv1.1 channels in the brain. This was accomplished by testing compounds obtained from yeast based primary screening, structure similarity searches, and new syntheses on Kv1.1/Kvβ1 currents. Compounds were identified that blocked inactivation by several different mechanisms or sites of action, apparently including direct and indirect mechanisms.

The disinactivators described here are the first small molecules reported to specifically interfere with the protein–protein interaction of the Kv ball-and-chain N-type inactivation process. They are effective in blocking seizure activity both in vitro (data not shown) and in vivo. Many of the disinactivators are efficacious in the PTZ-induced seizure model, and some are also active in the MES-induced seizure model (data not shown). Small molecule Kv channel disinactivators thus represent a new class of potential anticonvulsant drugs, and as such they may have unique properties and usefulness in multiple diseases of hyperexcitability. However, while many disinactivating compounds were found to be active in anticonvulsant models, some were also found to cause decreased heart rate and hypotension, which could result from disinactivators affecting Kv channels in heart and vascular smooth muscle. Since these channels are also associated with Kvβ1.3 (a splice variant of Kvβ1 differing in the N-terminus), it is possible that many disinactivators could prevent inactivation of these channels as well. By preventing Kvβ1.3 mediated inactivation of their associated Kv1.4 and Kv1.5 channels, these disinactivators might be expected to decrease heart rate and reduce blood pressure. Interestingly, the disinactivators that act on both Kv1.1/Kvβ1 and the Kv1.1/β1 N-terminal chimera did not produce cardiac effects in vivo, suggesting that compounds acting via the N-terminal inactivation domain of the channel may be more specific in their actions.

Using the N-terminal chimera, we were able to identify four disinactivating compounds that do not appear to interact with the C-terminal region of Kvβ1 to prevent inactivation of Kv1.1 channels. Two of these compounds (the cyclohexadiones) were originally identified in the YTH assay, while the two hydantoins were discovered from synthetic chemistry efforts. These four compounds were able to prevent PTZ-induced seizures in mice without affecting heart rate or blood pressure, and thus may represent a new class of potential anticonvulsants that selectively block N-type inactivation of Kv1.1 channels.

4. Conclusion

A novel group of ‘disinactivator’ compounds were discovered which disrupt inactivation of Kv1.1/β1 channels and exhibit anticonvulsant activity in vivo. Two series of disinactivators were found to act directly on the Kvβ1 N-terminus or its receptor site on Kv1.1, while others apparently act via binding to another site(s) on the channel complex or via an indirect mechanism of enhancing Ca2+ release/influx.
5. Experimental

5.1. DNA construction and in vitro mRNA synthesis

Human Kv1.1 (GenBank Accession No. L02750, 34) was amplified with primers: 5′-GGTCGACGGTATCGATGACGGTGATGTCTG-3′ and 5′-GTTAGTGGTACCACTTTAAACATCGGTCAG-3′, then subcloned into the pKSm vector35 at Cla I (5′) and BglII (3′) site, respectively. Human Kvβ1 (GenBank Accession No. X83127, 36) was subcloned into pKSm vector using a similar strategy as Kv1.1. Both constructs were sequenced to confirm their accuracy.

For the Kv1.1/Kvβ1 N-terminal chimera, Kvβ1 was amplified with primers: 5′-CTTTGGGCCCCTCATGATCGATCGATCGATCGATCGATCGATGACGGTGATGTCTG-3′ and 5′-GTTTAGTGGTACCACTTTAAACATCGGTCAG-3′, then subcloned into the pKSm-Kv1.1 construct at XbaI (5′) and ClaI (3′). The resulting construct (Kv1.1-N-β70) encodes a protein that has Kvβ1 amino acids 1–70 at the N-terminus, which is linked, via a Serine, to the whole Kv1.1 sequence at the C-terminus (Fig. 4A).

The above cDNA constructs were linearized with NotI, and purified linear DNA was used as a template for mRNA, which was prepared with in vitro transcription methods37 using the T3 promoter in pKSm.

5.2. Two-electrode voltage clamp

Xenopus oocytes (stage V–VI) were selected and injected with 46 nl of solution containing ~0.5–5 ng mRNA using a Drummond microinjector (Drummond Scientific Co., Broomall, PA). Oocytes were then incubated in ND-96 solution (in mM: 96 NaCl, 2 KCl, 1 MgCl2, 0.3 CaCl2, and 5 Hepes, pH 7.6) for one to four days before recording. Oocytes were injected with Kv1.1 and Kvβ1 RNA at a 1:160 ratio, as determined empirically to produce the greatest amount of inactivation.

During recording, oocytes were impaled with two microelectrodes (0.5–1.0 MΩ) filled with 3 M KCl in a 40 μl recording chamber. The chamber was constantly perfused with ND-96. For voltage-clamp, oocytes were held at −80 mV, and pulsed for 200 ms to various voltages as

![Chemical structures of the disinactivators.](image-url)
indicated, or to +50 mV to test compound effects. The inter-pulse intervals were kept at 3 s.

Currents were recorded in ND-96 solution using a GeneClamp 500 amplifier (Axon Instruments, Union City, CA). All recordings were conducted at room temperature (22 ± 1°C). Data were acquired using Pulse software (HEKA, Southboro, MA), low-pass filtered at 200 Hz, and digitized at 1.0 kHz.

Peak and steady state current (measured as the last 5 ms during 200-ms pulses), $I_{\text{peak}}$ and $I_{\text{ss}}$, respectively, were taken at +50 mV. The percent of disinactivation is calculated as: 100 * $I_{\text{ss}}/I_{\text{peak}}$. All data points are the result of three or more separate experiments and given as mean ± SEM. Data were analyzed and graphs were generated using Origin 5.0 (Microcal Software Inc., Northampton, MA).

5.3. Calcium mobilization assay with FLIPR (fluorescent imaging plate reader)

The ability of disinactivating compounds to increase intracellular calcium was measured with a fluorescent imaging plate reader (FLIPR) assay using tsa201 cells. Tsa201 cells were seeded at a density of 50,000 cells/well in 96-well black clear-bottomed plates. The next day, cells were loaded with 3 µM Fluo-4 AM in Hanks’ balanced saline solution (HBSS) supplemented with 2.5 mM probenecid at 37°C for 60 min. Increases in intracellular calcium result in increases in fluorescence intensity, which was measured before and after adding various solutions. The fluorescence signal is normalized to the background level. Cells were washed twice with calcium-free HBSS and left in 180 µl/well calcium-free HBSS. For FLIPR runs, baseline images were taken for 5 s, followed by addition of 20 µl of compound in HBSS into each well to a final concentration of 50 µM (except for the positive control, A23187, added at 10 µM). The change in fluorescence was measured for another 3 min. and plotted. To exclude autofluorescence, 10 µM BAPTA-AM was added in the wash and final loading solution with all positive compounds, which abolished any increase in intracellular calcium.

5.4. Anticonvulsant models

Male CF-1 mice weighing between 20 and 25 g were maintained on free access to food and water prior to all experiments.

5.4.1. Pentylenetetrazole (PTZ) seizure model. Test compounds had unknown pharmacokinetic/absorption properties in vivo, thus all were solubilized into a 2% Tween 80, 1% methylcellulose, saline solution and administered intraperitoneally (ip) 30 min prior to convulsant treatment. The convulsant, PTZ (Sigma) 85 mg/kg, was delivered subcutaneously and subjects were immediately placed in individual chambers for a 30-min observation period. Treatment groups consisted of eight mice scored for the presence or absence of a clonic seizure. A clonic seizure was defined as both forelimb and hindlimb paddling with loss of righting. Data were compared to the positive control phenytoin in parallel experiments.

5.4.2. Maximal electroconvulsant shock (MES) model. All test compounds were administered ip 30 min prior to
receiving an electroconvulsant shock. Electroconvulsant stimulation was delivered through a pair of ear clips soaked in saline using a standard device (Ugo Basile, Model 7800). The electrical current was 50 mA at a frequency of 50 pulses/s for 0.4 s. Subjects were scored for the presence or absence of a tonic convulsion. A tonic convulsion was defined as full extension of the forelimbs and hindlimbs.

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References and notes