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RESEARCH PAPER

# Allosteric modulation of sigma-1 receptors elicits anti-seizure activities

Lin Guo[1]*, Yanke Chen[1]*, Rui Zhao[1], Guanghui Wang[1], Eitan Friedman[2], Ao Zhang[3] and Xuechu Zhen[1]

[1]*Jiangsu Key Laboratory of Translational Research and Therapy for Neuropsycho-Disorders and Department of Pharmacology, College of Pharmaceutical Sciences, Soochow University, Suzhou, Jiangsu Province, China,* [2]*Department of Pharmacology and Neuroscience, School of Medicine at CCNY, City University of New York, New York, NY, USA, and* [3]*Department of Medicinal Chemistry, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai, China*

**Correspondence**
Xuechu Zhen, Jiangsu Key Laboratory of Translational Research and Therapy for Neuropsycho-Disorders and Department of Pharmacology, College of Pharmaceutical Sciences, Soochow University, 199 Ren'ai Road, Suzhou, Jiangsu Province 215123, China. E-mail: zhenxuechu@suda.edu.cn

*Lin Guo and Yanke Chen equally contribute to the work.

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## BACKGROUND AND PURPOSE

Application of orthosteric sigma-1 receptor agonists as anti-seizure drugs has been hindered by questionable efficacy and potential adverse effects. Here, we have investigated the anti-seizure effects of the novel and potent allosteric modulator of sigma-1 receptors, SKF83959 and its derivative SOMCL-668 (3-methyl-phenyl-2,3,4,5-tetrahydro-1H-benzo[d]azepin-7-ol).

## EXPERIMENTAL APPROACH

The anti-seizure effects of SKF83959 were investigated in three mouse models, maximal electroshock seizures, pentylenetetrazole-induced convulsions and kainic acid-induced 'status epilepticus'. Also, in rats, the cortical epileptiform activity induced by topical application of picrotoxin was recorded in electrocorticograms. In rat hippocampal brain slices, effects of the drugs on the high potassium-evoked epileptiform local field potentials were studied. Anti-seizure activities of SOMCL-668, a newly developed sigma-1 receptor selective allosteric modulator, were also investigated.

## KEY RESULTS

SKF83959 (20, 40 mg·kg$^{-1}$) exhibited anti –seizure actitity in the three mouse models and reduced the cortical epileptiform activity without alteration of spontaneous motor activity and motor coordination. These effects were blocked by the sigma-1 receptor antagonist BD1047, but not the dopamine $D_1$ receptor antagonist SCH23390. SKF83959 alone did not directly inhibit the epileptiform firing of CA3 neurons induced by high potassium in hippocampal slices, but did potentiate inhibition by the orthosteric sigma-1 receptor agonist SKF10047. Lastly, a selective sigma-1 receptor allosteric modulator SOMCL-668, which does not bind to dopamine receptors, exerted similar anti-seizure activities.

## CONCLUSIONS AND IMPLICATIONS

SKF83959 and SOMCL-668 displayed anti-seizure activities, indicating that allosteric modulation of sigma-1 receptors may provide a novel approach for discovering new anti-seizure drugs.

## Abbreviations

aCSF, artificial CSF; ECoG, electrocorticogram; KA, kainic acid; MEST, maximal electroshock seizure threshold; PTZ, pentylenetetrazole; SKF10047, (2R,6R,11R)-6,11-dimethyl-3-prop-2-en-1-yl-1,2,3,4,5,6-hexahydro-2,6-methano-3-benzazocin-8-ol; SKF83959, 3-methyl-6-chloro-7,8-hydroxy-1-(3-methylphenyl)-2,3,4,5-tetrahydro-1H-3-benzazepine; SOMCL-668, 3-methyl-phenyl-2,3,4,5-tetrahydro-1H-benzo[d]azepin-7-ol; VPA, valproic acid

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These Tables list key protein targets and ligands in this article which are hyperlinked to corresponding entries in http://www.guidetopharmacology.org, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY (Pawson *et al.*, 2014) and are permanently archived in the Concise Guide to PHARMACOLOGY 2013/14 ([a,b]Alexander *et al.*, 2013a,b).

## Introduction

Epilepsy is a common debilitating disease, which occurs in 3% of the world population (Sander, 2003; Blume, 2006). The characteristic symptom of epilepsy patients is recurrent and unpredictable seizures. Thus, control of seizures is the primary goal in medicating patients with epilepsy. Epileptic seizures result from abnormal, excessive or hypersynchronous neuronal activity in brain. Most currently available anti-seizure drugs act by either altering the activities of ion channels (such as $Na^+$ channel blockers) or by enhancing GABA-mediated synaptic inhibition (Brodie *et al.*, 2011).

The sigma-1 receptor is an intracellular chaperone protein (molecular weight: 25–30 kD), which is widely distributed in brain and in peripheral organs. Unlike other chaperones, its activity can be modulated by a number of ligands, such as SKF10047, dextrorphan and phenytoin (Su *et al.*, 2010). Although the role of sigma-1 receptors in the pathophysiology of epilepsy has not been fully established, some sigma-1 receptor agonists have shown anti-seizure activities. For instance, dextrorphan and carbetapentane can ameliorate 'status epilepticus' induced by kainic acid (KA; Kim *et al.*, 2001; 2003a) and racemic (+/−)-pentazocine antagonized electrical tonic convulsions in mice (Khanna *et al.*, 1998).

Despite these advances, the use of orthosteric agonists of sigma-1 receptors in the treatment of seizures is limited. Compounds with high-affinity for sigma-1 receptors, such as SKF10047 or dextromethorphan, are reported to induce ataxia or hyper-locomotion in mice, which may be due to excessive activation of sigma-1 receptors (Jerram *et al.*, 1996; Chou *et al.*, 1999). In addition, those drugs that bind to sigma-1 receptors and are already in clinical use, such as pentazocine, dextromethorphan and fluoxetine, exhibit poor selectivity for this type of receptor (Maurice and Su, 2009). Thus, these disadvantages prevent the orthosteric agonists of sigma-1 receptors from being used in the treatment of seizures.

Unlike orthosteric receptor agonists, allosteric receptor modulators elicit their action only in the presence of endogenous or exogenous agonists, which augments the functional selectivity of the respective agonist. Thus, it is believed that allosteric modulation may provide a better approach in terms of selectivity and physiological relevance, compared with an orthosteric receptor agonist (Soudijn *et al.*, 2004).

The atypical dopamine $D_1$ receptor agonist SKF83959 has been reported to elicit many $D_1$ receptor-independent biological responses (Yu *et al.*, 2008; Chu *et al.*, 2010; 2011; Fang *et al.*, 2013) and was recently identified as a potent σ-1 receptor allosteric modulator (Guo *et al.*, 2013). Until now, the potential role of allosteric modulators of the σ-1 receptor in treating seizures has not been studied. In the present study, we have used several different animal models to assess the anti-seizure effects of SKF83959. The results indicated that SKF83959 effectively ameliorated seizures induced by electrical stimulation, pentylenetetrazol (PTZ) and KA and the cortical epileptiform activity induced by picrotoxin, a $GABA_A$ receptor antagonist. We further demonstrated that the anti-seizure effects of SKF83959 and a specific sigma-1 receptor allosteric modulator, SOMCL-668, were mediated by modulating sigma-1 receptors.

## Methods

### *Animals*

All animal care and experimental protocols were approved by the Institutional Animal Care and Use Committee of Soochow University and were in compliance with the Guidelines for the Care and Use of Laboratory Animals (Chinese-National-Research-Council, 2006) and with the 'ARRIVE' guidelines (Animals in Research: Reporting In Vivo Experiments). All studies involving animals are reported in accordance with the ARRIVE guidelines for reporting experiments involving animals (Kilkenny *et al.*, 2010; McGrath *et al.*, 2010). A total of 1010 animals were used in the work described here.

Nine hundred male C57BL/6J mice (6–8 weeks, 18–22 g), 90 adult male Sprague-Dawley rats (6–8 weeks, 170–190 g) and 20 juvenile Sprague-Dawley rats (2–3 weeks, 25–45 g) were purchased from the Shanghai SLAC Laboratory Animal Co. Ltd (Shanghai, China). The animals were housed in plastic cages (adult mice and rats: 5 per cage; juvenile rats: a litter per cage) with metal covers in specific pathogen free conditions (temperature: 21 ± 1°C, air exchange every 20 min), were allowed free access to a standard rodent diet

and water and maintained on a 12 h/12 h light–dark schedule (lights-on at 07:00). All animals were tested during the diurnal phase between 09:00 and 18:00. After the experiments, the animals were killed by $CO_2$ exposure for 5 min.

## Maximal electroshock seizure threshold (MEST) test in mice

Mice were randomly divided into several groups. Groups of 10 mice were injected intraperitoneally (i.p.) with SKF83959 (2–40 mg·kg$^{-1}$), SOMCL-668 (40 mg·kg$^{-1}$) or SKF10047 (20 mg·kg$^{-1}$), respectively, 40 min prior to the implementation of the MEST test. Phenytoin (60 mg·kg$^{-1}$) and VPA (200 mg·kg$^{-1}$) were injected 120 min and 20 min respectively before testing, to ensure their maximal effects. Each group of mice was tested only once.

For the MEST test, all mice received electrical stimulation (0.250 s in duration; 50 Hz sine wave) via saline-moistened auricle electrodes. Successful generalized tonic seizure was considered when mice exhibited tonic hindlimb extension at 180° angle to the torso 1 min after stimulation (Socala *et al.*, 2010). The maximal electroconvulsive threshold was determined using the 'up and down' method (Lichtman, 1998). Briefly, the first animal was shocked using the expected/estimated $CC_{50}$ current value. If tonic seizure was not induced, the next animal received a shock 1 mA higher than the previous mouse. Otherwise, the next animal received a shock 1 mA lower. This procedure was followed for all animals within a given treatment group. The $CC_{50}$ values (the threshold current level inducing hindlimb extension in 50% of animals) were then calculated.

In some experiments, the dopamine $D_1$ receptor antagonist (SCH23390) or the sigma-1 receptor antagonist (BD1047) that was found not to alter seizure threshold was used to test the involvement of the corresponding receptor in the anti-seizure effects of SKF83959 or SOMCL-668. Twenty minutes before the injection of SKF83959 (40 mg·kg$^{-1}$), mice were pretreated i.p. with 1 mg·kg$^{-1}$ SCH23390 or 1 mg·kg$^{-1}$ BD1047.

## PTZ-induced convulsion in mice

Mice were treated i.p. with SKF83959 (2–40 mg·kg$^{-1}$), SOMCL-668 (40 mg·kg$^{-1}$), SKF10047 (20 mg·kg$^{-1}$) or saline, 40 min before an injection of $CD_{97}$ dose (80 mg·kg$^{-1}$, s.c.) of PTZ. VPA (200 mg·kg$^{-1}$) was injected 20 min before testing. Immediately after the injection of PTZ, the mice were placed in clear polyvinyl chloride boxes and their behaviour observed via a video-based computer system for 60 min. For each mouse, latencies of onset of three separate events were recorded: clonic convulsion, generalized tonic-clonic convulsion (GTCS) and death as previously described (Hill *et al.*, 2012). Seizure severity was scored as follows: 0, normal behaviour; 1, isolated myoclonic jerks; 2, atypical clonic seizure; 3, fully developed bilateral forelimb clonus; 4, tonic–clonic seizure with suppressed tonic phase; 5, fully developed tonic–clonic seizure.

## KA-induced status epilepticus

SKF83959 (2–40 mg·kg$^{-1}$), SOMCL-668 (40 mg·kg$^{-1}$), SKF10047 (20 mg·kg$^{-1}$) or saline was given i.p. to mice, respectively, 40 min prior to the injection of KA (30 mg·kg$^{-1}$, i.p.). VPA (300 mg·kg$^{-1}$) was injected 20 min before testing. Mice were observed continuously for 3 h and monitored for time of seizure onset, as well as seizure severity and duration. Severity of seizures was rated according to the scales proposed previously (McLin and Steward, 2006), as follows: level 1, not moving; level 2, stretched posture with straight and rigid tail; level 3, repetitive head bobbing, rearing into a sitting position with forepaws resting on belly; level 4, rearing and falling clonic seizures, jumping and running clonus; level 5, continuous level 4; level 6, body clonus, no longer using limbs to maintain posture, usually precursor to death. The maximal rating was used to define the severity of the seizure for each mouse. Seizure duration was measured as the time from the appearance of level 2 to level 5.

## Electrocorticogram (ECoG) recordings

Experiments were carried out on adult male Sprague-Dawley rats. Under urethane anaesthesia (1.5–1.7 g·kg$^{-1}$, i.p.), the head of the animal was secured in a stereotaxic instrument and body temperature was maintained at 37°C by means of a heating blanket and monitored by a rectal thermistor. During the experiments, the heart rate was maintained more than 300 min$^{-1}$, the respiratory rate between 65 and 90 min$^{-1}$, and the tail systolic pressure more than 10 kPa. Centering at the point which lay at bregma −1 mm, lateral +3 mm, a 3 mm diameter skull was removed to expose the somatosensory cortex, and the pia mater was kept intact. A glass microelectrode filled with 2 M NaCl (resistance of approximately 5 MΩ) was attached tightly to the pia mater. The reference silver wire electrode was attached to the nasal bone. A saline solution containing 100 μM picrotoxin was dripped on the surface of the pia mater to induce epilepiform activity. The ECoG was acquired using JL-H2003 microelectrode amplifier (Shanghai Jialong Educational Instrument Factory, Shanghai, China) with a band pass filter of 0.32–200 Hz and amplification of 200×, and data were collected online, through SMUP-U4 Biosignal Processing System (developed by Fudan University, Shanghai, China) and stored in a computer for off-line analysis. After recording, the animals were still kept unconscious, then were killed by $CO_2$ exposure for 5 min.

## High $K^+$ induced epileptiform activity in hippocampal slices

Brain slices were prepared from Sprague-Dawley rats (2–3 weeks old) following the procedures described previously (Zhang *et al.*, 2011). Briefly, rats were anaesthetized with 20% chloral hydrate prior to decapitation. The brains were rapidly transferred into chilled and oxygenated artificial CSF (aCSF), which had the following composition (in mM): 125 NaCl, 2.5 KCl, 1 $MgCl_2$, 2.4 $CaCl_2$, 1.25 $NaH_2PO_4$, 11 glucose and 26 $NaHCO_3$. Hippocampal slices (350 μm) were cut using a M752 motorized advance vibroslice (Campden Instruments Ltd., Lafayette, IN, USA) and incubated at 37°C in oxygenated aCSF for at least 1 h. The slices were then transferred to a chamber filled with 35°C oxygenated high potassium ($K^+$: 8.5 mM) aCSF to induce spontaneous, synchronous discharge of hippocampal pyramidal neurons. Local field potential was recorded in CA3 pyramidal neuronal cell bodies under a DIC upright microscope (BX51WI, Olympus, Tokyo, Japan) using a HEKA EPC-10 patch clamp amplifier (HEKA Instruments Inc., Lambrecht/Pfalz, Germany). Recording electrodes (resistance of 4–5 MΩ) were pulled from borosilicate glass pipettes with

the Flaming/Brown micropipette puller (Model P-97, Sutter Instrument, Novato, CA, USA), and were filled with aCSF. Data acquisition and analysis were performed by the PATCHMASTER software (Version 2.64, HEKA Instruments Inc.). Signals were filtered at 2 kHz and sampled at 10 kHz. Drugs were delivered through perfusion.

### Locomotion and motor coordination

Assessment of locomotion was conducted with a video-based computer system (Shanghai Jiliang Company, Shanghai, China). The locomotion apparatus contained four chambers, each of which was sound-attenuated and illuminated by diffuse white light (about 10 lux) at the centre. Each mouse was placed into the central quadrant of the open field and allowed to freely explore the arena for 120 min. After each trial, the entire apparatus was cleaned with 30% (v/v) ethanol and thoroughly dried. Cameras located above each chamber recorded all activities with an automated video tracking system (Jiliang Software, Shanghai, China), and the total moving distance was calculated.

Motor coordination was tested using an accelerating rotating rod system. The rotating rod was equipped with a surface-striated axis (with a diameter of 3.5 cm) and was 10 cm above the table surface. For each trial, a mouse was placed on the inactive rod, with the head pointed in the direction opposite to that of rod motion. The rod was accelerated to 50 rotations per minute over 1 min in a stepwise manner. All mice were trained six times every day for 3 days. Only the mice that kept clinging to the rod for more than 30 s were chosen. In the fourth day, SKF83959 and other drugs were injected, using the same doses and times as in the seizure tests, described above. The latencies (the time from commencement of rotation to the time when a mouse fell from the rod) were recorded. For each animal, the mean of latencies in three measurements was taken for evaluating the animal's motor function.

### Sigma-1 receptor binding assay

Binding to sigma-1 receptors was assayed as described earlier (Guo *et al.*, 2013). Briefly, synaptosomes were diluted with Krebs solution ((NaCl 118 mM, KCl 4.7 mM, $CaCl_2$ 2.5 mM, $MgSO_4$ 1.2 mM, $KH_2PO_4$ 1.2 mM, $NaHCO_3$ 25 mM, glucose 11.1 mM, pH 7.2–7.4). The binding assay buffer consisted of 20 µL brain homogenate (the final protein concentration: 2 mg $mL^{-1}$) was incubated with 20 µL $^3$H(+)-pentazocine (final concentration 0.1–60 nM), 20 µL SOMCL-668 (10 µM) and 140 µL Krebs solution. In the control tubes, 20 µL SOMCL-668 was replaced with an equal volume of Krebs solution. BD1047 10 µM was used for testing non-specific binding. After incubation for 2.5 h at 30°C, the bound radioligands were separated and collected on Whatmann GF/B glass fibre filters by a Brandel vacuum harvester. Radioactivity was measured with a liquid scintillation spectrometer (PerkinElmer).

### Data analysis

Seizure scores were expressed as medians, with interquartile ranges. Other data were expressed as mean ± SEM. Seizure scores were assessed using the Kruskal–Wallis test, followed by Dunnett's *post hoc* test for multiple comparisons. Other data were evaluated using one-way ANOVA and Dunnett's *post hoc* test. In some experiments, a two-way ANOVA followed by Bonferroni's *post hoc* test were employed. Statistical significance level was set at $P < 0.05$. Data were analysed using the GraphPad Prism software Version 5.0 (GraphPad Software, Inc. La Jolla, CA, USA).

### Materials

(+/−)-SKF83959, BD1047 (N′-[2-(3,4-dichlorophenyl)ethyl]-N,N,N′-trimethylethane-1,2-diamine), kainic acid (KA), phenytoin, valproic acid (VPA), picrotoxin and SKF10047 were obtained from Sigma-Aldrich Co. (St. Louis, MO, USA). $^3$H(+)-pentazocine (1250 GBq·$mmol^{-1}$) was purchased from PerkinElmer Co. (Waltham, MA, USA). SOMCL-668 was synthesized in the Synthetic Organic and Medicinal Chemistry Laboratory, Shanghai Institute of Materia Medica, Chinese Academy of Sciences. Pentylenetetrazol (PTZ) was purchased from the Aladdin Reagents Company (Shanghai, China) and was directly dissolved in 0.9% saline solution. Other compounds were initially dissolved in DMSO and diluted with saline solution to produce a final concentration of DMSO that was less than 0.5%. All drugs were freshly prepared prior to use. Injection volume (10 mL·$kg^{-1}$) was kept constant. The dosage selection and injection time for the different compounds were based on preliminary experiments and pharmacokinetic considerations.

## Results

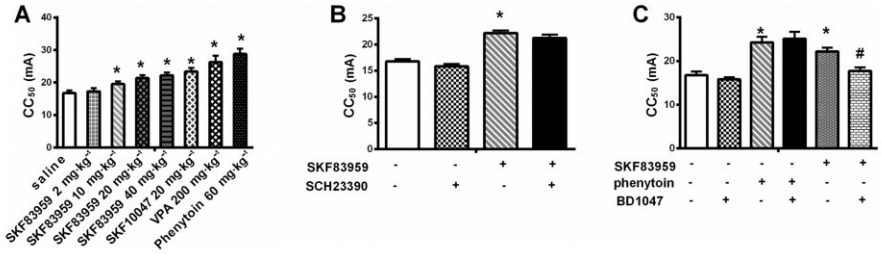
### SKF83959 significantly elevates the threshold for generalized tonic seizures in the MEST test

VPA is a widely used anti-convulsive drug in clinical practice, which antagonizes a broad spectrum of seizures, including partial seizure and general clonic-tonic seizures (Gerstner *et al.*, 2008; Vajda and Eadie, 2014). In preclinical studies for evaluating anti-seizure drugs, VPA is active against PTZ and maximal electroshock seizures. Thus, we used VPA as a reference compound in our study. Compared with VPA, phenytoin is effective only against seizures induced by electric stimulation (Vohora *et al.*, 2010; Vajda and Eadie, 2014). As phenytoin was the first allosteric modulator of sigma-1 receptors to be described, we also, at the same time, examined the anti-seizure activity of phenytoin in the MEST test.

The threshold for eliciting tonic hindlimb extension was assessed in mice. Drugs that are effective against tonic hindlimb extension induced by electroshock are often effective against partial and tonic-clonic seizures in humans. As shown in Figure 1A, the average basal threshold for mice was 16.7 ± 0.5 mA. As expected, VPA, phenytoin and SKF10047 (sigma-1 receptor agonist) raised seizure thresholds in the MEST test. Moreover, administration of SKF83959 10–40 mg·$kg^{-1}$ significantly (one-way ANOVA, $P < 0.05$, Figure 1A) elevated seizure thresholds.

### SKF83959 prolongs the latency to clonus, generalized tonic–clonic seizure in the PTZ-induced seizure model

PTZ-induced seizure is another widely used model for evaluating anti-seizure drugs. Drugs that inhibit PTZ-induced sei-




## Figure 1

Effect of SKF83959 on seizure threshold ($CC_{50}$) in mice in the MEST test. (A) Effect of increasing doses of SKF83959 on $CC_{50}$. SKF83959 was administered i.p. 40 min prior to testing, phenytoin 120 min before testing, VPA 20 min before testing. The $CC_{50}$ values were obtained using the 'up and down' method. Data were presented as mean ± SEM and analysed using one-way ANOVA followed by Dunnett's *post hoc* test. *$P < 0.05$, compared with the saline-treated group. (B) Effect of 1 mg·kg$^{-1}$ SCH23390 on the anti-seizure activity of SKF83959. (C) Effect of 1 mg·kg$^{-1}$ BD1047 on the anti-seizure activity of SKF83959. BD1047 or SCH23390 was given i.p. 60 min before testing. Each group consisted of 10 animals. Data were expressed as mean ± SEM and analysed using two-way ANOVA followed by Bonferroni's *post hoc* test. *$P < 0.05$, compared with the saline treated group; $^{\#}P < 0.05$, compared with the SKF83959 alone treated group.

zures are usually effective against clonic seizures in humans. We found that SKF83959, SKF10047 and VPA prolonged the latencies of clonic seizure and GTCS (one-way ANOVA, $P < 0.05$; Figure 2A, 2B), survival time (one-way ANOVA, $P < 0.05$; Figure 2C), and significantly lowered seizure scores (Kruskal–Wallis test, $P < 0.05$; Figure 2D).

Despite the delay in seizure latency, SKF83959 did not reduce the incidence of seizure ($\chi^2$ test, $P < 0.05$; Supporting Information Fig. S1A). SKF83959 40 mg·kg$^{-1}$, however, lowered mortality ($\chi^2$ test, $P < 0.05$; Supporting Information Fig. S1B). In this regard, 20 mg·kg$^{-1}$ SKF10047 demonstrated similar activity to 40 mg·kg$^{-1}$ SKF83959 (Supporting Information Fig. S1A, S1B). In contrast, VPA 200 mg·kg$^{-1}$ completely protected mice from GTCS ($P < 0.05$; Supporting Information Fig. S1A, S1B), and all the mice in this group survived.

## *SKF83959 significantly ameliorates status epilepticus induced by KA*

Status epilepticus requires intensive care in clinical practice and is often resistant to traditional anti-seizure drugs. We confirmed that VPA did not modify status epilepticus induced by KA. In contrast, both SKF83959 (20, 40 mg·kg$^{-1}$) and SKF10047 (20 mg·kg$^{-1}$) prolonged the latency to seizures (one-way ANOVA, $P < 0.05$; Figure 3A), and shortened the duration of seizure (one-way ANOVA, $P < 0.05$; Figure 3C). SKF83959 40 mg·kg$^{-1}$ also lowered the average severity of seizure (Kruskal–Wallis test, $P < 0.05$; Figure 3B). Moreover, treatment with SKF83959 or SKF10047 decreased mortality ($\chi^2$ test, $P < 0.05$; Supporting Information Fig. S2A) and SKF83959 40 mg·kg$^{-1}$ reduced seizure incidence significantly ($\chi^2$ test, $P < 0.05$; Supporting Information Fig. S2B), which was different from that observed in the PTZ seizure model.

## *The activation of sigma-1 receptors contributes to the anti-seizure action of SKF83959*

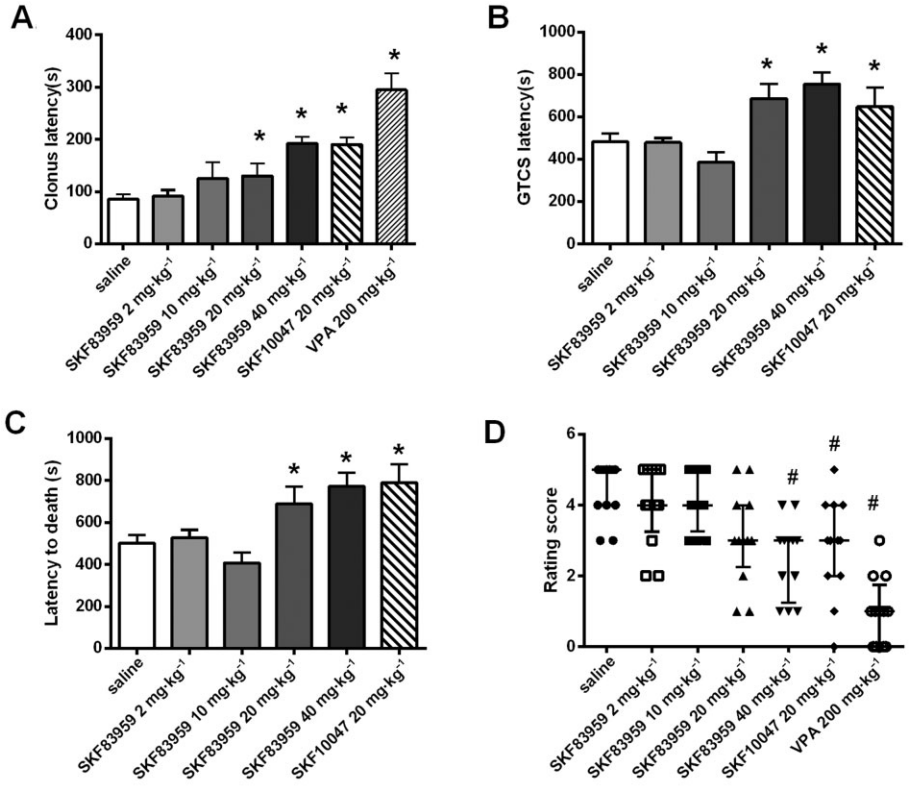
Although SKF83959 was recently identified as a potent sigma-1 receptor allosteric modulator (Guo *et al.*, 2013), it has long been known to be an atypical dopamine $D_1$ receptor agonist. We thus examined whether the $D_1$ or sigma-1 receptors were involved in the anti-seizure effect of SKF83959. In preliminary experiments, we found that the sigma-1 receptor antagonist BD1047 or the $D_1$ receptor antagonist SCH23390 at doses that are sufficient to block the respective receptors (1 mg·kg$^{-1}$, each) did not affect seizure thresholds.

As shown in Figure 1B and 1C, pretreatment with BD1047, but not with SCH23390, restored the SKF83959-elevated seizure thresholds to the basal level in the MEST test. Similarly, in the PTZ seizure model, BD1047, but not SCH23390, attenuated SKF83959-induced increases in latencies to seizures (clonus and GTCS) and survival time (two-way ANOVA, $P < 0.05$; Figure 4A, 4B, 4C). Likewise, BD1047 (1 mg·kg$^{-1}$) also attenuated the anti-seizure activity of SKF83959 in the KA-induced status epileptics model. Pretreatment with BD1047 significantly suppressed the prolongation of the latency to seizure onset (two-way ANOVA, $P < 0.05$; Figure 5A), the decrease in seizure severity (two-way ANOVA, $P < 0.05$; Figure 5B) and the shortening of seizure duration (two-way ANOVA, $P < 0.05$; Figure 5C). Taken together, the data clearly indicated that SKF83959 elicits its anti-seizure action via the activation of sigma-1 receptors and not via $D_1$ dopamine receptors.

It should be noted that the anti-seizure activity of phenytoin seems not to depend on the activation of sigma-1 receptors because combined treatment with phenytoin and BD1047 did not alter seizure threshold, compared with treatment with phenytoin alone (one-way ANOVA, $P < 0.05$; Figure 1C).

## *SKF83959 reduces the epileptiform activity in rat neocortex* in vivo

To further study the mechanism by which SKF83959 exerted its anti-seizure activity, we examined the effect of SKF83959 on cortical epileptiform activity. As shown in Figure 6A, topical application of 100 μM picrotoxin generated epileptiform spikes and the mean values of the latency to the first epileptiform spike and of spike frequency are shown in




## Figure 2

The anti-convulsive effects of SKF83959 in the PTZ-induced seizure model in mice. Bar graphs showing the effects of SKF83959 on (A) latency to clonus, (B) latency to tonic-clonic seizure, (C) survival time and (D) the seizure scores in C57BL/6J mice. Animals were injected i.p. with saline, 2 mg·kg$^{-1}$ SKF83959, 10 mg·kg$^{-1}$ SKF83959, 20 mg·kg$^{-1}$ SKF83959, 40 mg·kg$^{-1}$ SKF83959 or 20 mg·kg$^{-1}$ SKF10047 40 min before the injection of PTZ (80 mg·kg$^{-1}$, s.c.). 200 mg·kg$^{-1}$ VPA was given before the injection of PTZ. Seizures were monitored for 60 min after PTZ treatment. Data in latencies (A, B, C) were expressed as mean ± SEM. Rating scores (D) were expressed as medians with interquartile ranges. *$P < 0.05$, compared with the saline-treated group; one-way ANOVA with a *post hoc* Dunnett's test. $^{\#}P < 0.05$, compared with the saline treated group; Kruskal–Wallis one-way ANOVA. $n = 12$ for each group.

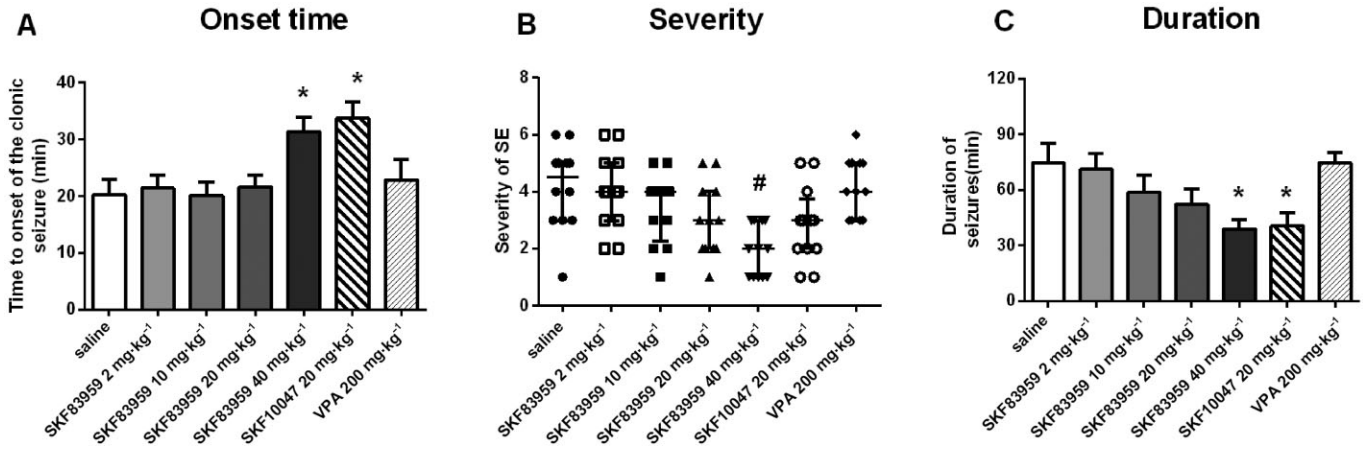
Figures 6B and Figure 6C). When SKF83959 (40 mg·kg$^{-1}$) was administered 40 min prior to picrotoxin, the latency to the first epileptiform spike was significantly increased (one-way ANOVA, $P < 0.05$, $n = 8$; Figure 6B) and firing frequency was markedly reduced (one-way ANOVA, $P < 0.05$, $n = 8$, Figure 6C). The sigma-1 receptor antagonist, BD-1047 (1 mg·kg$^{-1}$) blocked the effects of SKF83959 on the latency and firing frequency of ECoG (two-way ANOVA, $P < 0.05$, $n = 8$, Figure 6B and 6C). However, SCH23390 did not change these inhibitory activities of SKF83959.

We next examined the effect of SKF83959 on the epileptiform discharge induced by high $K^+$ aCSF ($K^+$, 8.5 mM) in hippocampal slices. As shown in Figure 7A, perfusion of slices with high-$K^+$ aCSF produced an epileptiform discharge in CA3 regions. The epileptiform activity was attenuated by the addition of the selective sigma-1 receptor agonist, SKF10047, to the perfusion media (one-way ANOVA, $P < 0.05$, Figure 7B). SKF83959 (1 μM) alone did not change the epileptiform activity; however, as expected, it potentiated the anti-epileptiform activity of SKF10047. As shown in Figure 7B, SFK83959 (1 μM) together with SKF10047 (0.1 μM) suppressed the firing frequency to a greater extent than did SKF10047 (0.1 μM) alone (two-way ANOVA, $P < 0.05$). In addition, after 15 min of washing with high-$K^+$ aCSF, the firing activity returned to control levels (Figure 7B).

### *SKF83959 did not alter motor coordination and spontaneous locomotion*

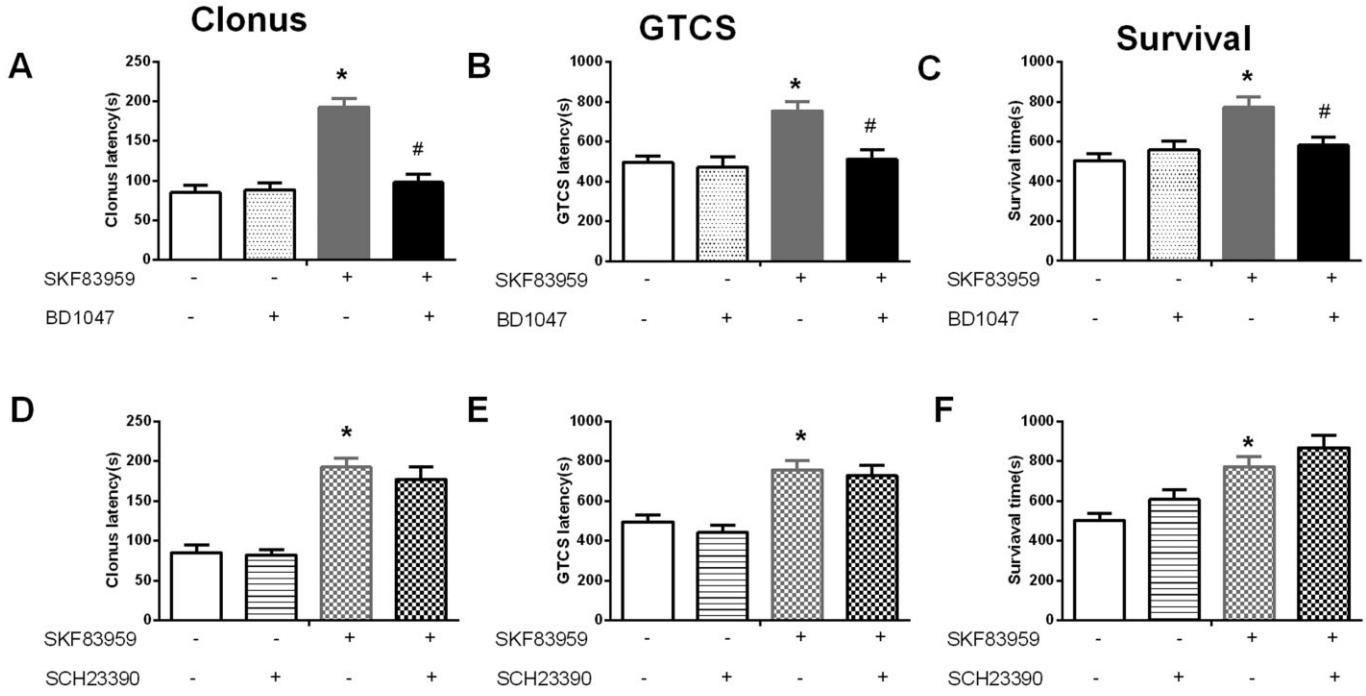
We also checked whether SKF83959 altered spontaneous activity and motor coordination in mice. As shown in Figure 8A, administration of SKF83959 did not produce a significant change in the rotarod test (one-way ANOVA, $P > 0.05$). This was also true for the locomotion as measured by the distance travelled every 10 min (two-way ANOVA, $P > 0.05$; Figure 8B). These results indicated that SKF83959 did not impair motor performance at doses that exert anti-seizure actions.

Motor coordination was, however, affected by SK10047 and VPA. SKF10047 (20 mg·kg$^{-1}$) and VPA (200 mg·kg$^{-1}$) decreased the falling latency of mice in the rotarod test (one-way ANOVA, $P < 0.05$; Figure 8A); VPA, in addition, inhibited spontaneous locomotion in mice (two-way ANOVA, $P < 0.05$; Figure 8B).




## Figure 3

Effects of SKF83959 on status epilepticus induced by KA. Bar graphs show the effects of SKF83959 on the latencies to 'status epilepticus' following the injection of 30 mg·kg$^{-1}$ KA. (A) Represents latencies to seizure, (B) seizure scores and (C) the seizure duration. C57BL/6J mice were injected i.p. with saline, 2 mg·kg$^{-1}$ SKF83959, 10 mg·kg$^{-1}$ SKF83959, 20 mg·kg$^{-1}$ SKF83959, 40 mg·kg$^{-1}$ SKF83959, 20 mg·kg$^{-1}$ SKF10047, respectively, 40 min before the injection of KA. 300 mg·kg$^{-1}$ VPA was given before the injection of KA. Seizures were monitored for 3 h after treatment with KA. Latency (A) and duration (C) of seizures were expressed as Mean ± SEM. Severity of seizure activity (B) was expressed as median with interquartile range. *$P < 0.05$, compared with the saline-treated group (one-way ANOVA with a *post hoc* Dunnett's test). $^{\#}P < 0.05$, compared with the saline treated group (Kruskal–Wallis one-way ANOVA). $n = 12$ for each group.




## Figure 4

Effect of BD1047 or SCH23390 on the anti-seizure activity of SKF83959 in the PTZ-induced seizure model in mice. BD1047 1 mg·kg$^{-1}$ or SCH23390 1 mg·kg$^{-1}$ was given i.p. 60 min before testing. SKF83959 (40 mg·kg$^{-1}$) was administered 40 min prior to testing. Data were expressed as mean ± SEM and analysed using two-way ANOVA followed by Bonferroni's *post hoc* test. *$P < 0.05$, compared with the saline group; $^{\#}P < 0.05$, compared with the SKF83959-treated group. $n = 10$ for each group.

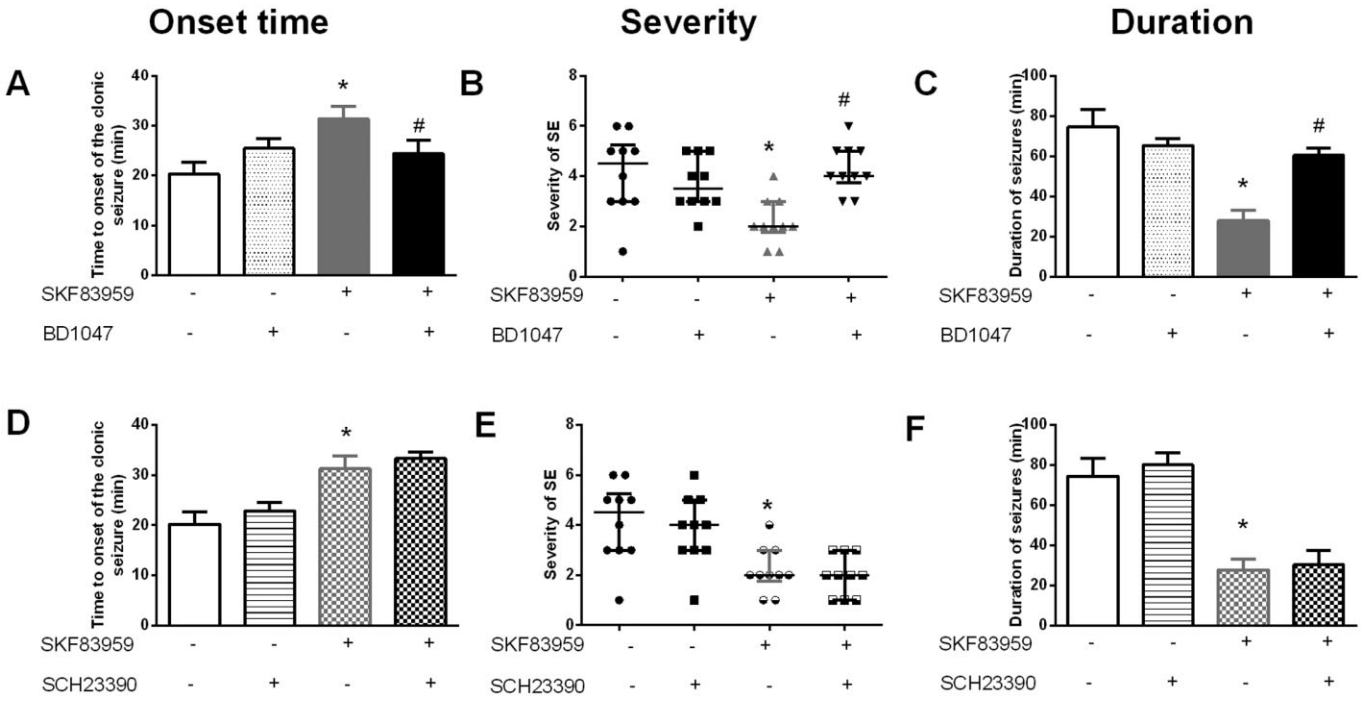



**Figure 5**

Effect of BD1047 or SCH23390 on the anti-seizure activity of SKF83959 in the KA-induced status epilepticus model in mice. BD1047 (1 mg·kg$^{-1}$) or SCH23390 (1 mg·kg$^{-1}$) was given 60 min before the treatment of KA. SKF83959 (40 mg·kg$^{-1}$, i.p.) was administered 40 min prior to the treatment of KA. KA (30 mg·kg$^{-1}$) was given i.p. Latencies to seizure (A, D) and seizure durations (C, F) were expressed as mean ± SEM, and severities (B, E) were expressed as median with interquartile range. *$P < 0.05$, compared with the saline group; $^{\#}P < 0.05$, compared with the single dose SKF83959-treated group. Data were analysed using two-way ANOVA followed by Bonferroni's *post hoc* test. $n = 10$ for each treatment group.

### *SOCML-668, a newly identified selective sigma-1 receptor allosteric modulator, displayed anti-convulsive activities*

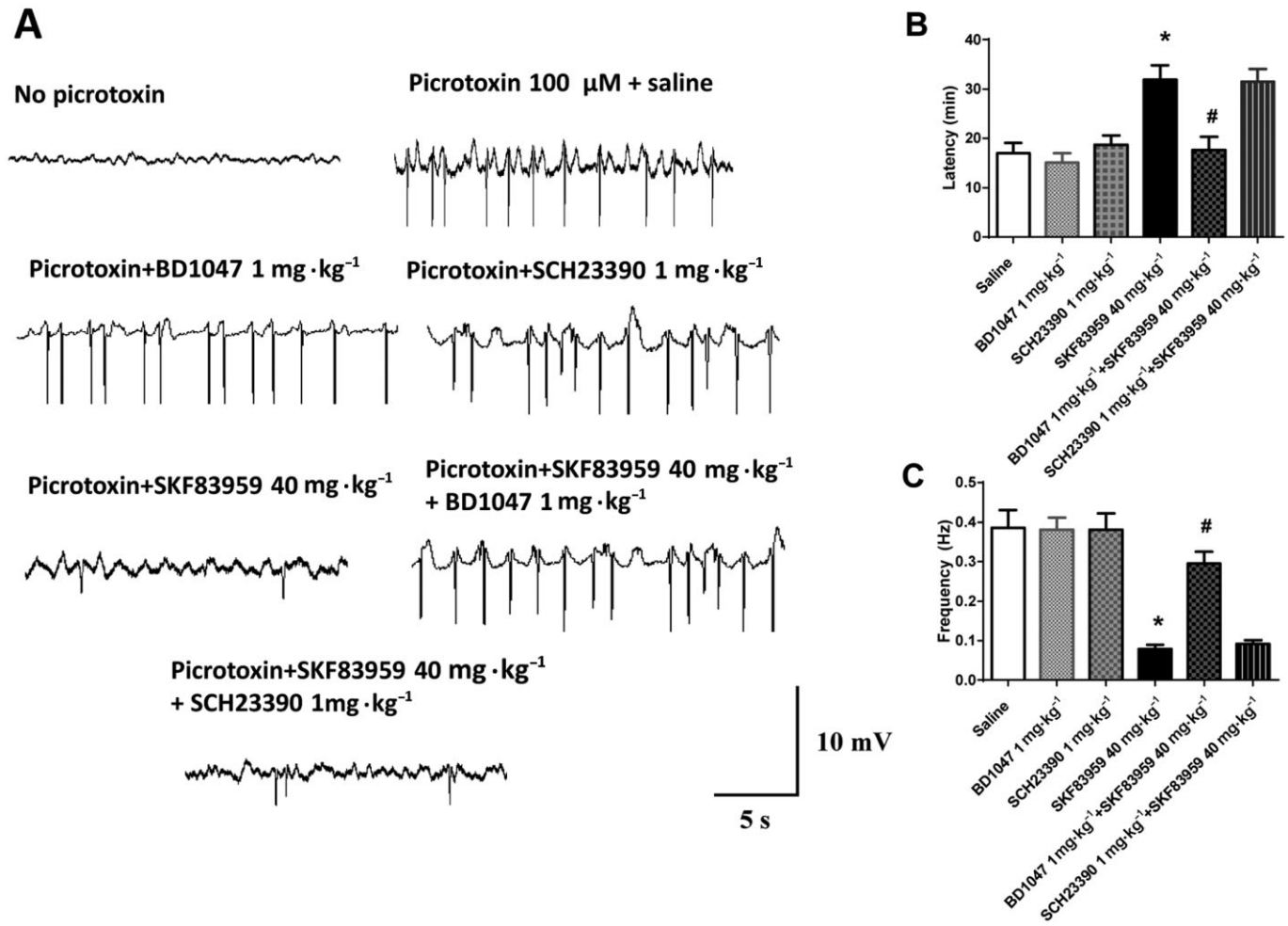
To further establish the role of sigma-1 receptors in anti-seizure activity and exclude the potential involvement of other receptors ($D_1$, $D_2$, 5-$HT_{1A}$, etc), we synthesized several chemical derivatives of SKF83959 in order to find a selective sigma-1 receptor allosteric modulator. One of these compounds, SOMCL-668, did not exhibit affinities for human $D_1$, $D_2$, $D_3$, 5-$HT_{1A}$, 5-$HT_{2A}$ receptors (Zhang *et al.*, 2014), but showed a potent allosteric modulating activity on sigma-1 receptors. As shown in Figure 9, SOMCL-668 100 μM shifted the saturation curve toward the left, and significantly decreased the $K_D$ (dissociation constant of the radioligand) value (3.91 ± 0.35 nM vs. 7.24 ± 1.03 in control, $P < 0.05$, *t*-test). However, it did not change the value of $B_{max}$ (maximal number of receptors labelled, pmol mg$^{-1}$ protein; 2.92 ± 0.06 nM vs. 3.11 ± 0.10 nM in control, $P > 0.05$, *t*-test). SOMCL-668 also failed to alter the binding of $^3$H-progesterone to sigma-1 receptors (data not shown), indicating ligand selectivity, which is an intrinsic trait for allosteric modulators.

We then examined the anti-seizure activity of SOMCL-668 and found that SOMCL-668 (40 mg·kg$^{-1}$) raised the seizure threshold in the MEST test (Figure 10A and B), and prolonged the latencies to the clonus (Supporting Information Fig. S3A) and GTCS (Supporting Information Fig. S3B), as well as the survival time (Supporting Information Fig. S3C) in the PTZ-induced seizure model. In addition, SOMCL-668 prolonged the latency to seizure (Supporting Information Fig. S4A), lowered the average severity of seizure (Supporting Information Fig. S4B), and shortened the duration of seizure (Supporting Information Fig. S4C) in the KA-induced 'status epilepticus'. Moreover, the sigma-1 receptor antagonist, BD1047, but not the $D_1$ receptor antagonist SCH23390 (Figure 10, Supporting Information Figs S3D, E, F and S4D, E, F) abolished the anti-seizure activity of SOMCL-668, indicating that its effects were dependent on sigma-1 receptors.

We further examined the effect of SOMCL-668 on the anti-seizure activity of SKF83959 using the MEST test. As shown in Figure 10C, the individual values of $EC_{50}$ for SOMCL-668 and SKF83959 were very similar. Isobolographic analysis showed the experimental $ED_{50}$ of mixtures of these compounds ($ED_{50\text{-mix}}$) was not different from the theoretical value ($ED_{50\text{-add}}$; $P > 0.05$, Student's *t*-test) at any ratio tested (SOMCL-668: SKF83959 = 1:3, 1:1 or 3:1), which indicated that SOMCL-668 had an additive effect on SKF83959, and further supported that allosteric modulation of sigma-1 receptors is a novel approach to the treatment of convulsions.

## Discussion

In this study, we have evaluated the anti-seizure activity of SKF83959, a recently identified potent sigma-1 receptor allosteric modulator (Guo *et al.*, 2013). We found that SKF83959

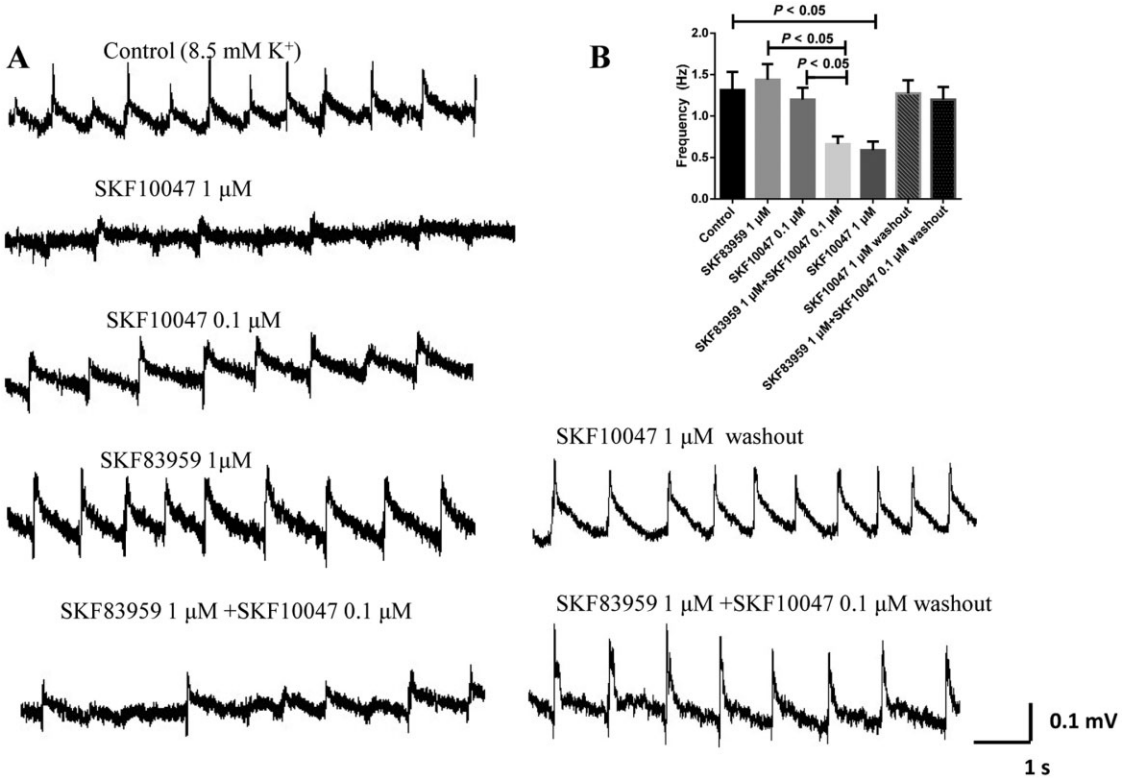



## Figure 6

Effect of SKF83959 on picrotoxin-induced epileptiform acitivity in the rat somatosensory cortex. (A) Representative ECoG showing the epileptiform spikes induced by picrotoxin. (B–C) Bar graphs of pooled data showing the latency to the first spike (B) and the spiking frequency (C). The epileptiform spikes were induced by topical application of 10 μM picrotoxin on the surface of the somatosensory cortex in adult male Sprague-Dawley rats. SKF83959 (40 mg·kg$^{-1}$) was administered 40 min before the application of picrotoxin. BD1047 (1 mg·kg$^{-1}$) or SCH23390 (1 mg·kg$^{-1}$) was given 60 min before the application of picrotoxin. Data were expressed as mean ± SEM and analysed with two-way ANOVA. *$P$ < 0.05, compared with the saline group. $^{\#}P$ < 0.05, compared with the 40 mg·kg$^{-1}$ SKF83959-treated group. $n$ = 8 for each treatment group.

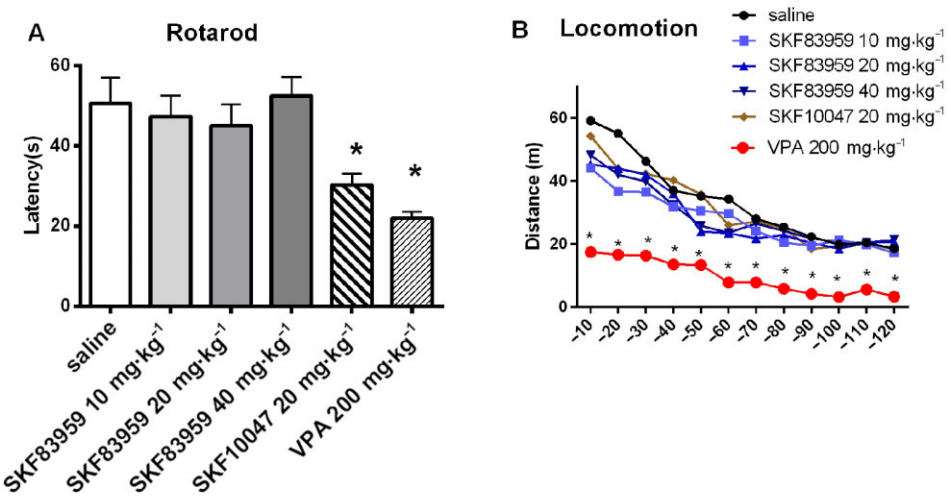
(20, 40 mg·kg$^{-1}$) can effectively ameliorate seizures induced by electrical stimulus, PTZ and KA, without impairing spontaneous locomotor activity or motor coordination in mice. Moreover, the sigma-1 receptor antagonist BD1047, but not the $D_1$ receptor antagonist SCH23390, abolished the anti-seizure activity of SFK83959, indicating that its effects are dependent on the activation of sigma-1 receptors. Stronger support came from the data on SOMCL-668, a newly identified specific sigma-1 receptor allosteric modulator, which elicited effects similar to those of SKF83959 (Figure 10, Supporting Information Figs S3, S4). Therefore, our data clearly demonstrated that allosteric modulation of sigma-1 receptors elicits anti-seizure activity.

Recent reports indicate that sigma-1 receptors may be a promising therapeutic target for a number of neuropsychiatric disorders including epilepsy (Hayashi and Su, 2004; Matsumoto *et al.*, 2004; Meurs *et al.*, 2007; Chevallier *et al.*, 2011; Kourrich *et al.*, 2012). Activation of sigma-1 receptors antagonized experimental seizures such as electrical stimulation-induced tonic seizures, and status epilepticus induced by KA (Kim *et al.*, 2001; 2003b). We also found that the sigma-1 receptor agonist SKF10047 exerted a stronger protective effect than VPA in attenuating KA-induced status epilepticus. However, the use of sigma-1 receptor agonists is hindered because of the wide distribution of the receptor and the functional complexity of this receptor. For instance, SKF10047 induces ataxia in animals while exerting its anti-seizure activity (Jerram *et al.*, 1996). Moreover, some sigma-1 receptor agonists also produce psychotomimetic effects (Maurice and Su, 2009). In this regard, it is conceivable that



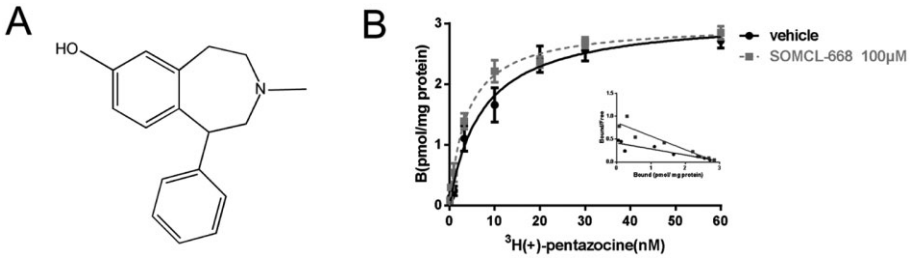

## Figure 7

Effects of SKF83959 on the epileptiform activity induced by high $K^+$ in hippocampal slices. (A) Representative extracellular recordings showing spontaneous epileptiform events in CA3 area. (B) Bar graphs of pooled data showing the firing frequency recorded in the presence and absence of SKF83959 (1 μM), SKF10047 (0.1 and 1 μM) or the combination of SKF83959 (1 μM) with SKF10047 (0.1 μM). Data were expressed as mean ± SEM and analysed with two-way ANOVA. $n = 6$ in each treatment group.



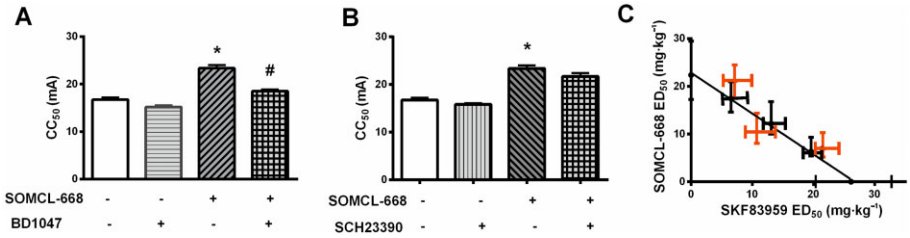

## Figure 8

Effect of SKF83959 on motor coordination (A) and locomotor activity (B) in mice. C57BL/6J mice were treated with saline, SKF83959 (10, 20 and 40 $mg \cdot kg^{-1}$), SKF10047 (20 $mg \cdot kg^{-1}$) or VPA (200 $mg \cdot kg^{-1}$). Motor coordination and locomotor activity were examined as described in methods. Data were expressed as mean ± SEM and analysed via two-way ANOVA. $^*P < 0.05$, compared with the saline group. $n = 10$ in each treatment group.




## Figure 9

The chemical structure of SOMCL-668 (A) and the saturation binding of $^3$H(+)-pentazocine to sigma-1 receptors in brain synaptosomes and the interaction of SOMCL-668 and SKF83959 (B). Inset: the Scatchard plot redrawn from the saturation binding curve. In B, synaptosomes were incubated for 2.5 h at 30°C with $^3$H(+)-pentazocine (0.1–60 nM) in the presence of SOMCL-668 100 μM, or vehicle. BD1047 10 mM was used to define the non-specific binding. Each experiment was repeated three times.




## Figure 10

Effect of SOMCL-668 on seizure threshold ($CC_{50}$) in mice in the MEST test. (A–B) Effects of BD1047 (A) or SCH23390 (B) on the anti-seizure effect of SOMCL-668. (C) Effect of SOMCL-668 on anti-seizure activity of SKF83959. In A–B, SOMCL-668 40 mg·kg$^{-1}$ was administered i.p. 40 min prior to test. BD1047 1 mg·kg$^{-1}$ or SCH23390 1 mg·kg$^{-1}$ was given i.p. 60 min before testing. Each group consisted of 10 animals. The $CC_{50}$ values were obtained using the 'up and down' method. Data were expressed as mean ± SEM and were analysed using two-way ANOVA followed by Dunnett's *post hoc* test.*$P < 0.05$, compared with the saline treated group; $^{\#}P < 0.05$, compared with the SOMCL-668 sole-treated group. In C, the effect of SOMCL-668 on the anti-seizure activity of SKF83959 was examined in the MEST tests using isobolographic analysis. SOMCL-668 and SKF83959 were mixed at the ratios: 1:3, 1:1 and 3:1. The experimental values of $ED_{50}$ for SOMCL-668 (on the ordinate), SKF83959 (on the abscissa) and the mixtures ($ED_{50\text{-mix}}$, marked with orange blots) were obtained; the theoretical values of mixtures ($ED_{50\text{-add}}$, marked with black dots) was calculated using Equation 2. Data were expressed as mean (95% confidential interval). Difference between $ED_{50\text{-mix}}$ and $ED_{50\text{-add}}$ was examined with Student's *t*-test. The method details were given in Supporting information Appendix S1.

the unwanted effects of orthosteric sigma-1 receptor agonists may be attributed to activation of sigma-1 receptors in other brain areas apart from those involved in the seizure events.

Allosteric receptor modulation represents an important advance in our understanding of the regulation of receptor function. It might provide an alternative approach in regulating the receptor functions other than a selective receptor agonist; positive allosteric receptor modulation is associated with, and dependent upon, agonist stimulation. (Soudijn *et al.*, 2004). Allosteric modulation may be particularly important for receptors, such as σ-1 receptors, whose endogenous ligand(s) has not been identified. SKF83959 and SOMCL-668 are newly identified sigma-1 receptor allosteric modulators with greater potencies than phenytoin, the first identified allosteric modulator of this receptor (Cobos *et al.*, 2005; 2006; Guo *et al.*, 2013). Using SKF83959 and SOMCL-668 as probes, we confirmed the practicability of allosteric modulation of sigma-1 receptors as an alternative for the treatment of convulsive seizures. The advantage of sigma-1 receptor allosteric modulators is further supported by the fact that these modulators do not impair spontaneous motor activity or motor coordination while displaying similar therapeutic efficacies to the selective, orthosteric, receptor agonist SKF10047 (Figure 8).

In this study, we also supplied more evidence for allosteric modulation of SKF83959 on σ-1 receptors in addition to the data of ligand–receptor binding tests (Guo *et al.*, 2013). The function of allosteric modulators depends on the presence of receptor orthodox agonists (Kenakin, 2013). Indeed, we found that SKF83959 alone produced no anti-seizure activity in the hippocampal slice preparations, which is most likely due to the loss of σ-1 receptor endogenous ligand(s) in the isolated hippocampal slice preparations. In addition, the synergic effect of SKF83959 on SKF10047 activity also strongly supported the suggestion that SKF83959 acts as a σ-1 receptor allosteric modulator.

Phenytoin was the first sigma-1 receptor allosteric modulator to be identified (Cobos *et al.*, 2005; 2006). Although it elicited anti-seizure action in our tests (Figure 1), this effect appeared not to be related to the activation of sigma-1

receptors. This may contributes to the low (10%) degree of unbound phenytoin in plasma (concentration about 3.9–7.8 μM) following its administration. At such concentrations the allosteric effect of phenytoin is negligible (Cobos *et al.*, 2005; 2006). At higher phenytoin concentrations, toxic effects are apparent (Tanaka *et al.*, 2013). Moreover, the sigma-1 receptor antagonist, BD1047, did not block the anti-seizure effects of phenytoin (Figure 1C), suggesting that the primary mechanism by which phenytoin exerts its anti-seizure effect is more likely to be through its well-known action on voltage-sensitive $Na^+$ channels located in the neuronal plasma membrane (Tunnicliff, 1996).

As SKF83959 is a well-known atypical $D_1$ receptor agonist (Panchalingam and Undie, 2001; Jin *et al.*, 2003; Zhen *et al.*, 2005), we tested the potential role of $D_1$ dopamine receptors in its anti-seizure action. It seems that the anti-seizure effect of this compound is unlikely to be related to its action on $D_1$ receptors and other monoamine receptors. Firstly, the anti-seizure dose of SKF83959 was far higher than that needed for $D_1$ receptor activation. SKF83959 in doses of 1–2 $mg \cdot kg^{-1}$ is enough for activation of the $D_1$ dopamine receptor (Zhang *et al.*, 2007; 2009), while no anti-seizure effects were observed at this dose range (Figures 1–5). Secondly, the $D_1$ receptor antagonist, SCH23390 (1 $mg \cdot kg^{-1}$), did not alter seizure thresholds in our experimental models. This is in agreement with other studies in which $D_1$ receptors do not play a role in acute seizure models (Weinshenker and Szot, 2002). Moreover, an injection of SKF38393 (0.1–5 $mg \cdot kg^{-1}$) did not increase the susceptibility of mice to pilocarpine-induced seizures (Alam and Starr, 1993). Together with the data concerning a specific sigma-1 allosteric modulator SOMCL-668 (Figures 9, 10 and Supporting Information Figs S3 and S4), we are confident in concluding that allosteric modulation of sigma-1 receptors will elicit potent anti-seizure effects.

In summary, the present data provide the first evidence that allosteric modulation of sigma-1 receptors is a novel therapeutic approach to the treatment of convulsive seizures.

## Acknowledgements

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## Author contributions

L. G. Y. C. and R. Z. carried out the behavioural and electrophysiological studies. L. G., G. W. and X. Z. participated in study design and analysed data. A. Z. supplied compound SOMCL-668 and helped to prepare the paper. L. G. wrote the paper. E. F. and X. Z. revised the paper critically. All authors read and approved the final paper.

## Conflict of interest

The authors state no conflict of interest.

## References

Alam AM, Starr MS (1993). Dopaminergic modulation of pilocarpine-induced motor seizures in the rat: the role of hippocampal D2 receptors. Neuroscience 53: 425–431.

Alexander SPH, Benson HE, Faccenda E, Pawson AJ, Sharman JL, McGrath JC *et al.* (2013a). The Concise Guide to PHARMACOLOGY 2013/14: Overview. Br J Pharmacol 170: 1449–1458.

Alexander SP, Benson HE, Faccenda E, Pawson AJ, Sharman JL, Spedding M *et al.* (2013b). The Concise Guide to PHARMACOLOGY 2013/14: G protein-coupled receptors. Br J Pharmacol 170: 1459–1581.

Blume WT (2006). The progression of epilepsy. Epilepsia 47 (Suppl. 1): 71–78.

Brodie MJ, Covanis A, Gil-Nagel A, Lerche H, Perucca E, Sills GJ *et al.* (2011). Antiepileptic drug therapy: does mechanism of action matter? Epilepsy Behav 21: 331–341.

Chevallier N, Keller E, Maurice T (2011). Behavioural phenotyping of knockout mice for the sigma-1 (sigma(1)) chaperone protein revealed gender-related anxiety, depressive-like and memory alterations. J Psychopharmacol 25: 960–975.

Chinese-National-Research-Council (2006). The guidelines for the care and use of laboratory animals. Available at: http://www.nsfc.gov.cn/nsfc/cen/pfzl/pufanew/20110801_02.pdf

Chou YC, Liao JF, Chang WY, Lin MF, Chen CF (1999). Binding of dimemorfan to sigma-1 receptor and its anticonvulsant and locomotor effects in mice, compared with dextromethorphan and dextrorphan. Brain Res 821: 516–519.

Chu HY, Gu Q, Jin GZ, Hu GY, Zhen X (2010). Electrophysiological effects of SKF83959 on hippocampal CA1 pyramidal neurons: potential mechanisms for the drug's neuroprotective effects. PLoS ONE 5: e13118.

Chu HY, Wu Q, Zhou S, Cao X, Zhang A, Jin GZ *et al.* (2011). SKF83959 suppresses excitatory synaptic transmission in rat hippocampus via a dopamine receptor-independent mechanism. J Neurosci Res 89: 1259–1266.

Cobos EJ, Baeyens JM, Del Pozo E (2005). Phenytoin differentially modulates the affinity of agonist and antagonist ligands for sigma 1 receptors of guinea pig brain. Synapse 55: 192–195.

Cobos EJ, Lucena G, Baeyens JM, Del Pozo E (2006). Differences in the allosteric modulation by phenytoin of the binding properties of the sigma1 ligands [3H](+)-pentazocine and [3H]NE-100. Synapse 59: 152–161.

Fang X, Guo L, Jia J, Jin GZ, Zhao B, Zheng YY *et al.* (2013). SKF83959 is a novel triple reuptake inhibitor that elicits anti-depressant activity. Acta Pharmacol Sin 34: 1149–1155.

Gerstner T, Bell N, Konig S (2008). Oral valproic acid for epilepsy – long-term experience in therapy and side effects. Expert Opin Pharmacother 9: 285–292.

Guo L, Zhao J, Jin G, Zhao B, Wang G, Zhang A *et al.* (2013). SKF83959 is a potent allosteric modulator of sigma-1 receptor. Mol Pharmacol 83: 577–586.

Hayashi T, Su TP (2004). Sigma-1 receptor ligands: potential in the treatment of neuropsychiatric disorders. CNS Drugs 18: 269–284.

Hill AJ, Mercier MS, Hill TD, Glyn SE, Jones NA, Yamasaki Y *et al.* (2012). Cannabidivarin is anticonvulsant in mouse and rat. Br J Pharmacol 167: 1629–1642.

Jerram AH, Smith PF, Darlington CL (1996). The effects of (+)-SKF10047 and ketamine hydrochloride on stereotyped behaviour, locomotor activity and ataxia in guinea pig. Eur J Pharmacol 307: 269–273.

Jin LQ, Goswami S, Cai G, Zhen X, Friedman E (2003). SKF83959 selectively regulates phosphatidylinositol-linked D1 dopamine receptors in rat brain. J Neurochem 85: 378–386.

Kenakin T (2013). Allosteric drugs and seven transmembrane receptors. Curr Top Med Chem 13: 5–13.

Khanna N, Khosla R, Kohli J (1998). Opioid receptor mediated anticonvulsant effect of pentazocine. Indian J Med Sci 52: 1–7.

Kilkenny C, Browne W, Cuthill IC, Emerson M, Altman DG (2010). Animal research: reporting in vivo experiments: the ARRIVE guidelines. Br J Pharmacol 160: 1577–1579.

Kim HC, Jhoo WK, Kim WK, Shin EJ, Cheon MA, Shin CY *et al.* (2001). Carbetapentane attenuates kainate-induced seizures via sigma-1 receptor modulation. Life Sci 69: 915–922.

Kim HC, Bing G, Jhoo WK, Kim WK, Shin EJ, Im DH *et al.* (2003a). Metabolism to dextrorphan is not essential for dextromethorphan's anticonvulsant activity against kainate in mice. Life Sci 72: 769–783.

Kim HC, Shin CY, Seo DO, Jhoo JH, Jhoo WK, Kim WK *et al.* (2003b). New morphinan derivatives with negligible psychotropic effects attenuate convulsions induced by maximal electroshock in mice. Life Sci 72: 1883–1895.

Kourrich S, Su TP, Fujimoto M, Bonci A (2012). The sigma-1 receptor: roles in neuronal plasticity and disease. Trends Neurosci 35: 762–771.

Lichtman AH (1998). The up-and-down method substantially reduces the number of animals required to determine antinociceptive ED50 values. J Pharmacol Toxicol Methods 40: 81–85.

Matsumoto RR, Potelleret FH, Mack A, Pouw B, Zhang Y, Bowen WD (2004). Structure-activity comparison of YZ-069, a novel sigma ligand, and four analogs in receptor binding and behavioral studies. Pharmacol Biochem Behav 77: 775–781.

Maurice T, Su TP (2009). The pharmacology of sigma-1 receptors. Pharmacol Ther 124: 195–206.

McGrath J, Drummond G, McLachlan E, Kilkenny C, Wainwright C (2010). Guidelines for reporting experiments involving animals: the ARRIVE guidelines. Br J Pharmacol 160: 1573–1576.

McLin JP, Steward O (2006). Comparison of seizure phenotype and neurodegeneration induced by systemic kainic acid in inbred, outbred, and hybrid mouse strains. Eur J Neurosci 24: 2191–2202.

Meurs A, Clinckers R, Ebinger G, Michotte Y, Smolders I (2007). Sigma 1 receptor-mediated increase in hippocampal extracellular dopamine contributes to the mechanism of the anticonvulsant action of neuropeptide Y. Eur J Neurosci 26: 3079–3092.

Panchalingam S, Undie AS (2001). SKF83959 exhibits biochemical agonism by stimulating [(35)S]GTP gamma S binding and phosphoinositide hydrolysis in rat and monkey brain. Neuropharmacology 40: 826–837.

Pawson AJ, Sharman JL, Benson HE, Faccenda E, Alexander SP, Buneman OP *et al.*; NC-IUPHAR (2014). The IUPHAR/BPS Guide to PHARMACOLOGY: an expert-driven knowledge base of drug targets and their ligands. Nucl. Acids Res 42 (Database Issue): D1098–D1106.

Sander JW (2003). The epidemiology of epilepsy revisited. Curr Opin Neurol 16: 165–170.

Socala K, Nieoczym D, Rundfeldt C, Wlaz P (2010). Effects of sarcosine, a glycine transporter type 1 inhibitor, in two mouse seizure models. Pharmacol Rep 62: 392–397.

Soudijn W, Van Wijngaarden I, IJzerman AP (2004). Allosteric modulation of G protein-coupled receptors: perspectives and recent developments. Drug Discov Today 9: 752–758.

Su TP, Hayashi T, Maurice T, Buch S, Ruoho AE (2010). The sigma-1 receptor chaperone as an inter-organelle signaling modulator. Trends Pharmacol Sci 31: 557–566.

Tanaka J, Kasai H, Shimizu K, Shimasaki S, Kumagai Y (2013). Population pharmacokinetics of phenytoin after intravenous administration of fosphenytoin sodium in pediatric patients, adult patients, and healthy volunteers. Eur J Clin Pharmacol 69: 489–497.

Tunnicliff G (1996). Basis of the antiseizure action of phenytoin. Gen Pharmacol 27: 1091–1097.

Vajda FJ, Eadie MJ (2014). The clinical pharmacology of traditional antiepileptic drugs. Epileptic Disord 16: 395–408.

Vohora D, Saraogi P, Yazdani MA, Bhowmik M, Khanam R, Pillai KK (2010). Recent advances in adjunctive therapy for epilepsy: focus on sodium channel blockers as third-generation antiepileptic drugs. Drugs Today (Barc) 46: 265–277.

Weinshenker D, Szot P (2002). The role of catecholamines in seizure susceptibility: new results using genetically engineered mice. Pharmacol Ther 94: 213–233.

Yu Y, Wang JR, Sun PH, Guo Y, Zhang ZJ, Jin GZ *et al.* (2008). Neuroprotective effects of atypical D1 receptor agonist SKF83959 are mediated via D1 receptor-dependent inhibition of glycogen synthase kinase-3 beta and a receptor-independent anti-oxidative action. J Neurochem 104: 946–956.

Zhang H, Ma L, Wang F, Chen J, Zhen X (2007). Chronic SKF83959 induced less severe dyskinesia and attenuated L-DOPA-induced dyskinesia in 6-OHDA-lesioned rat model of Parkinson's disease. Neuropharmacology 53: 125–133.

Zhang H, Ye N, Zhou S, Guo L, Zheng L, Liu Z *et al.* (2011). Identification of N-propylnoraporphin-11-yl 5-(1,2-dithiolan-3-yl)pentanoate as a new anti-Parkinson's agent possessing a dopamine D2 and serotonin 5-HT1A dual-agonist profile. J Med Chem 54: 4324–4338.

Zhang J, Huang J, Song Z, Guo L, Cai W, Wang Y *et al.* (2014). Structural manipulation on the catecholic fragment of dopamine D(1) receptor agonist 1-phenyl-N-methyl-benzazepines. Eur J Med Chem 85: 16–26.

Zhang X, Zhou Z, Wang D, Li A, Yin Y, Gu X *et al.* (2009). Activation of phosphatidylinositol-linked D1-like receptor modulates FGF-2 expression in astrocytes via IP3-dependent Ca2+ signaling. J Neurosci 29: 7766–7775.

Zhen X, Goswami S, Friedman E (2005). The role of the phosphatidyinositol-linked D1 dopamine receptor in the pharmacology of SKF83959. Pharmacol Biochem Behav 80: 597–601.

## Supporting information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

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**Figure S1** Effects of SKF83959 on (A) clonus incidence,(B) GTCS incidence and (C) mortality incidence in PTZ-induced seizures. Male C57BL/6J mice were injected (i.p.) with saline, 2 mg·kg$^{-1}$ SKF83959, 10 mg·kg$^{-1}$ SKF83959, 20 mg·kg$^{-1}$ SKF83959, 40 mg·kg$^{-1}$ SKF83959 or 20 mg·kg$^{-1}$ SKF10047 40 min before the injection of PTZ (80 mg·kg$^{-1}$, s.c.). 200 mg·kg$^{-1}$ VPA was given before the injection of PTZ. Seizure incidence was calculated 60 min after PTZ treatment. *$P$ < 0.05, compared with the saline treated group (Fisher's exact probability test). $n$ = 12 for each group.

**Figure S2** Effects of SKF83959 on the seizure (SE) incidence (A) and mortality (B) incidence in KA-induced status epilepticus. C57BL/6J mice were treated (i.p.) with saline, 2 mg·kg$^{-1}$ SKF83959, 10 mg·kg$^{-1}$ SKF83959, 20 mg·kg$^{-1}$ SKF83959, 40 mg·kg$^{-1}$ SKF83959, 20 mg·kg$^{-1}$ SKF10047, respectively, 40 min before the injection of KA. 300 mg·kg$^{-1}$ VPA was given before the injection of KA. The SE and mortality incidences were calculated 3 h after treatment with KA. *$P$ < 0.05, compared with the saline group (Fisher's exact probability test). $n$ = 12 for each treatment group.

**Figure S3** Effect of SOMCL-668 and effects of BD1047 (A,B,C) or SCH23390 (D, E, F) on the anti-seizure activity of SOMCL-668in the PTZ-induced seizure model in mice. SCH23390 1 mg·kg$^{-1}$ or BD1047 1 mg·kg$^{-1}$ was given 60 min before testing. SOMCL-668 (40 mg·kg$^{-1}$) was administered 40 min prior to testing for clonus latency, GTCS latency, and survival time. Data are expressed as mean ± SEM and analysed using two-way ANOVA followed by Bonferroni's *post hoc* test.*$P$ < 0.05, compared with the saline group; $^{\#}P$ < 0.05, compared with the SOMCL-668-treated group. $n$ = 10 for each group.

**Figure S4** Effect of SOMCL-668 and effects of BD1047 (A,B,C) or SCH23390 (D, E, F) on the anti-seizure activity of SOMCL-668 in the kainic acid-induced status epilepticus model in mice. SCH23390 (1 mg·kg$^{-1}$) or BD1047 (1 mg·kg$^{-1}$) was given 60 min before the treatment with kainic acid. SMOCL-668 (40 mg·kg$^{-1}$, i.p.) was administered 40 min prior to the treatment of kainic acid. Kainic acid (30 mg·kg$^{-1}$) was given i.p. Latencies to seizure (A) and seizure durations (C) were expressed as mean ± SEM, and severities (B) were expressed as median with interquartile range. *$P$ < 0.05, compared with the saline group; $^{\#}P$ < 0.05, compared with the single dose SOMCL-668-treated group. Data were analysed using two-way ANOVA followed by Bonferroni's *post hoc* test. $n$ = 10 for each treatment group.

**Appendix S1** Materials and methods, and results.