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RESEARCH ARTICLE

Targeting Hsp90 with cemdomespib reduces seizure burden and alters disease course in preclinical epilepsy models

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Abstract

Objective: Epilepsy is a chronic neurological disorder characterized by recurrent seizures and frequent cognitive and psychiatric comorbidities. Although current antiseizure medications provide symptomatic relief, they fail to prevent or modify epileptogenesis. Heat shock protein 90 (Hsp90) is increasingly recognized as a regulator of neuroinflammatory and oxidative stress pathways implicated in seizure generation and disease progression. Here, we investigated the therapeutic potential of cemdomespib, a novel and selective Hsp90 inhibitor, across complementary preclinical models of epilepsy.

Methods: In vitro, cemdomespib was evaluated in the low-magnesium model of epileptiform activity for its effects on neuronal calcium dynamics, mitochondrial membrane stability, and reactive oxygen species (ROS) generation. In vivo, acute seizure protection was assessed in the pentylenetetrazol (PTZ) model, and antiepileptogenic efficacy was tested in the kainic acid-induced status epilepticus (KA-SE) model using chronic video-electrocorticographic recordings. Behavioral outcomes relevant to epilepsy-associated comorbidities, including anxiety-like behavior and exploratory activity, were also examined.

Results: Cemdomespib reduced epileptiform calcium oscillations, stabilized mitochondrial membrane potential, and suppressed ROS generation in vitro. In the PTZ model, 45% of pretreated animals were protected from seizures, and those that seized exhibited reduced severity, shorter duration, and delayed onset. In the KA-SE model, cemdomespib significantly mitigated the severity of SE and reduced the emergence of spontaneous recurrent seizures during the chronic phase, as evidenced by lower seizure frequency, decreased cumulative seizure burden, and prolonged latency to seizure onset. Furthermore, treated animals demonstrated improved anxiety-like behavior and enhanced exploratory activity.

Significance: Cemdomespib confers both acute seizure protection and long-term suppression of epileptogenesis, likely through Hsp90-dependent regulation of mitochondrial integrity and redox signaling. These findings highlight Hsp90

inhibition as a promising therapeutic strategy for seizure control while also mitigating the progression of epileptogenesis and its associated neurobehavioral impairments.

KEYWORDS

cemdomespib, epilepsy, Hsp90 inhibitor, kainic acid, pentylenetetrazol

1 | INTRODUCTION

Epilepsy is one of the most prevalent neurological disorders, affecting more than 65 million individuals worldwide.¹ Despite the availability of various antiseizure medications (ASMs), one third of patients do not respond to currently available ASMs and continue to experience seizures.^{2–4} Currently available ASMs primarily provide symptomatic relief by suppressing seizures but often fail to target the underlying mechanisms. Moreover, these medications are often associated with significant adverse effects.⁵ Given the lack of therapies that prevent the onset or progression of epilepsy, there is an urgent need for novel treatment strategies with antiepileptogenic potential to halt or modify the course of disease development.⁶

Many cases of epilepsy are acquired following brain insults, such as prolonged seizures, traumatic brain injury, or stroke.^{7–9} The process leading from the initial injury to the development of chronic, spontaneous seizures, termed epileptogenesis, involves complex molecular and structural remodeling in the brain over a latent period that may span weeks to years.^{10,11} Among the key drivers of this process, oxidative stress plays a crucial role in promoting neuronal injury, network hyperexcitability, and disease progression.^{12,13} Seizures and associated brain insults result in excessive production of reactive oxygen species (ROS), causing mitochondrial dysfunction, lipid peroxidation, and protein oxidation.^{14,15}

Heat shock protein 90 (Hsp90), a molecular chaperone that is upregulated during cellular stress, plays a central role in maintaining a pro-oxidative and pro-inflammatory environment. It stabilizes multiple client proteins involved in ROS production, including NADPH oxidase 2 (NOX2), whose upregulation and pathogenic role in epileptogenesis have been demonstrated in both experimental and clinical settings.^{16–19} Moreover, Hsp90 suppresses antioxidant signaling pathways, notably the nuclear factor erythroid 2-related factor 2 pathway,^{20–22} which is predominantly expressed in neurons and shows dynamic regulation across different seizure models^{23,24} with promising therapeutic efficacy.^{25,26} Through these mechanisms, Hsp90 supports the pathological processes implicated in epileptogenesis, further underscoring the therapeutic potential of

Key points

- Cemdomespib decreases neuronal calcium oscillations, lowers ROS generation, and preserves mitochondrial function in vitro.
- In acute seizure model, cemdomespib reduces seizure severity, shortens duration, and delays seizure onset.
- Cemdomespib lowers spontaneous seizure frequency and burden and prolongs latency after kainic acid-induced status epilepticus.
- Cemdomespib treatment improves anxiety-like behavior and increases exploratory activity in epileptic animals.

targeting Hsp90 to restore redox balance and limit disease progression.

Previous reports have also confirmed the upregulation of Hsp90 both in the hippocampus of patients and in experimental models of temporal lobe epilepsy (TLE), particularly in neurons and glial cells of the dentate gyrus and CA1 region.^{27,28} Moreover, targeting Hsp90 has shown neuroprotective and antiseizure effects in various neurological disease models as well as in epilepsy models.²⁹ For instance, 17-N-allylamino-17-demethoxygeldanamycin (17-AAG), an early generation Hsp90 inhibitor, reduced seizure frequency in TLE models but was limited by toxicity.²⁸ Newer inhibitors, such as HSP990, exhibit higher potency than 17-AAG and demonstrate seizure suppression in both acute and chronic models of TLE and cognitive benefits, but show limited brain penetration and dose-limiting neurotoxicity, raising concerns about their translational potential.³⁰

Therefore, there is an urgent need to identify next-generation Hsp90 inhibitors with improved pharmacokinetics and safety profiles for use in neurological disorders. In this study, we investigated cemdomespib, a second-generation, brain-penetrant, small-molecule Hsp90 modulator with demonstrated neuroprotective effects in preclinical models of diabetic neuropathy and other central nervous system (CNS) injuries.³¹ Here, we evaluated the efficacy of cemdomespib in rat models of seizures and

epilepsy to determine its potential antiseizure and antiepileptogenic effects, addressing current limitations of Hsp90-targeted therapeutics.

The present study aimed to evaluate the therapeutic potential of cemdomespib using complementary *in vitro* and *in vivo* epilepsy models and assess its pharmacokinetic properties. This work examines whether early intervention with cemdomespib after status epilepticus (SE) can influence subsequent seizure outcomes and associated behavioral impairments, thereby establishing a framework for its further development as a candidate therapy targeting epileptogenesis.

2 | MATERIALS AND METHODS

2.1 | Primary cell culture and live-cell imaging

Primary mixed cortical cultures were prepared from postnatal day 0 (P0)–P1 Sprague Dawley rat pups as previously described.³² Cells were plated on poly-L-lysine-coated coverslips and maintained in Neurobasal-A with B-27; experiments were performed at 13–17 days *in vitro*. Full culture conditions and ethical approvals are provided in the Supplementary Methods: [Appendix S1](#).

Live-cell experiments were performed in hydroxyethylpiperazine ethane sulfonic acid-buffered artificial cerebrospinal fluid (aCSF; pH 7.4); epileptiform activity was induced by omitting Mg^{2+} from the aCSF. Cultures were pretreated with the Hsp90 inhibitor cemdomespib (10 – 50 nmol·L⁻¹) or vehicle for 1 h prior to imaging (dosing and vehicle details are provided in Supplementary Methods: [Appendix S1](#)).

Intracellular Ca^{2+} oscillations, mitochondrial membrane potential ($\Delta\psi_m$), and ROS were assessed using Fura-2 acetoxymethyl, Rhodamine-123, and dihydroethidium (DHE), respectively, and recorded by live-cell epifluorescence imaging. Fluorescence signals were baseline-normalized; acquisition parameters, normalization procedures, and statistical analyses are described in Supplementary Methods: [Appendix S1](#).

2.2 | Pharmacokinetic profiling

Pharmacokinetic studies were performed in male rats following single intraperitoneal doses of cemdomespib (3 and 10 mg/kg) and a single oral dose (10 mg/kg). At predefined time points, animals ($n = 3$ per time point) were anesthetized, and blood and brain were collected and stored at -80°C . Cemdomespib concentrations in plasma and brain homogenates were quantified by a validated liquid

chromatography–tandem mass spectrometry (LC-MS/MS) assay (calibration ranges: plasma, 10–4000 ng/mL; brain, 1–500 ng/mL; lower limit of quantification = 1 ng/mL). Full details of extraction, LC-MS/MS parameters, and data analysis procedures are provided in Supplementary Methods: [Appendix S1](#).

2.3 | Animal model and experiments

Male Sprague Dawley rats (150–200 g) were used in all *in vivo* experiments. All procedures were approved by the Hebrew University Institutional Animal Care and Use Committee (approval no. MD-20-16254-5) and conformed to Association for Assessment and Accreditation of Laboratory Animal Care International guidelines. Acute generalized seizures were induced using a two-dose subcutaneous pentylenetetrazol (PTZ) protocol (50 mg/kg then 30 mg/kg), and SE was induced by single dose intraperitoneal kainic acid (KA); seizures were scored using the modified Racine scale.³³ Animals were implanted with electrocorticographic (ECoG) transmitters and continuously monitored by wireless video-ECoG. Only animals that exhibited continuous electrographic SE for 2 h—measured from the onset of SE rather than from KA injection—were included in the chronic study. Cemdomespib (10 mg/kg ip) or vehicle was administered according to the experimental paradigm (single pre-PTZ dose or twice daily for 2 weeks in the KA model). Seizure detection used automated ECoG-based algorithms with blinded manual review; details of surgical procedures, dosing regimens, telemetry settings, behavioral scoring, and ECoG analysis are provided in Supplementary Methods: [Appendix S1](#).

2.4 | Behavioral assessment

At 8 weeks post-SE, animals underwent open field and elevated plus maze testing on separate days (≥ 24 h apart) in a dim, quiet room. Behavior was recorded by overhead camera and analyzed with a semiautomated DeepLabCut pipeline for locomotion and anxiety-related measures (total distance, center time, arm entries, percentage time in open arms). Full apparatus dimensions, habituation procedures, tracking/analysis parameters, cleaning protocols, and video analysis scripts are provided in Supplementary Methods: [Appendix S1](#).³⁴

2.5 | Statistical analysis

All analyses were performed in GraphPad Prism 9.3.1. Investigators were blinded to treatment conditions.

Data are expressed as mean $\pm$ SEM, with n denoting the number of individual animals or independent biological replicates (see figure legends). Two-group comparisons used unpaired Student t -test or Mann–Whitney test; multiple groups were analyzed by one-way analysis of variance (ANOVA) with Tukey or Sidak post hoc tests. Two-way ANOVA with Tukey or Dunnett post hoc test assessed interactions between treatment and time or other two-factor designs. Longitudinal seizure frequency data were analyzed using a generalized log-linear mixed-effects model with an autoregressive covariance structure and fixed effects for the treatment group, time (weeks), and their interaction. Statistical significance was set at $p < .05$ for all analyses. Group sizes were based on prior experience with variability in similar experimental paradigms, and the number of animals included in each experiment is detailed in the corresponding figure legend.

3 | RESULTS

3.1 | Cemdomespib attenuates low-magnesium-induced epileptiform activity, mitochondrial depolarization, and ROS accumulation in mixed cortical cultures

Low-Mg²⁺-induced epileptiform activity is an established in vitro model of NMDA-receptor-driven synchronous Ca²⁺ transients that reproduce seizurelike activity, mitochondrial dysfunction, and oxidative stress implicated in epilepsy and neurodegeneration.^{35,36} Persistent neuronal hyperactivity promotes mitochondrial depolarization and excessive ROS production,³⁷ yet effective strategies to mitigate these effects remain limited. Cemdomespib, a novel Hsp90 inhibitor, has reported neuroprotective effects in neurodegenerative diseases.³⁸ Here, we investigated whether cemdomespib could suppress low-Mg²⁺-induced network hyperactivity, mitochondrial depolarization, and ROS generation in primary cortical cultures. Mixed cortical neuronal cultures were exposed to Mg²⁺-free aCSF with or without pretreatment (10–50 nmol·L⁻¹).

Mg²⁺-free aCSF induced robust synchronous Ca²⁺ oscillations, confirmed by Fura-2 ratio (Figure 1A) at $2.3 \pm .3$ spikes/min (Figure 1C). Interestingly, pretreatment with cemdomespib significantly inhibited Ca²⁺ oscillation frequencies (Figure 1B) up to $.5 \pm .1$ spikes/min (Figure 1C) in a dose-dependent manner, with 20 nmol·L⁻¹ cemdomespib showing the highest efficacy (Figure 1C), further confirmed by the corresponding decrease in coastline $1.3 \pm .1$ arbitrary units (Figure 1D), reflecting the overall network activity.

Prolonged seizure-like activity induces progressive mitochondrial depolarization ($\Delta\psi_m$) via substrate depletion and pathological opening of the mitochondrial permeability transition pore, increasing neuronal vulnerability to oxidative stress and excitotoxicity.^{39,40} Mg²⁺-free aCSF caused a time-dependent increase in $\Delta\psi_m$ at 10, 20, and 30 min postexposure (Figure 1E). In contrast, preincubation with cemdomespib (20 nmol·L⁻¹, 1 h) significantly stabilized the mitochondrial membrane potential and attenuated depolarization (Figure 1E), which is typically observed under low-Mg²⁺ stress. Quantitative analysis confirmed that cemdomespib-treated neurons exhibited significantly preserved $\Delta\psi_m$ at all examined time points compared to the controls (Figure 1F). These data indicate that cemdomespib maintains mitochondrial integrity during prolonged neuronal hyperactivity, suggesting its potential to prevent hyperactivity-dependent mitochondrial dysfunction and reduce neuronal vulnerability associated with epileptogenesis.

We and others have established elevated levels of ROS accumulation during in vitro epileptiform activity induced either by low Mg²⁺^{19,32,36,41} or by treatment with proepileptic drugs such as picrotoxin and 4-aminopyridine.¹⁷ First, we confirmed the elevated level of ROS accumulation during low-Mg²⁺-induced epileptiform activity over the course of imaging using DHE fluorescence. Moreover, cemdomespib significantly inhibited the increase in ROS accumulation compared to the control condition, from 380% to 184% and from 589% to 270% at 10 and 20 min, respectively (Figure 1G,H).

Together, these results indicate that cemdomespib confers robust protection against low-Mg²⁺-induced epileptiform activity, preserves mitochondrial function, and attenuates oxidative stress by inhibiting ROS accumulation, supporting the therapeutic potential of Hsp90 inhibition in neuronal hyperexcitability and excitotoxicity.

3.2 | Pharmacokinetic profile of cemdomespib following intraperitoneal and oral administration

Next, we characterized cemdomespib pharmacokinetics to select dosing strategies and assess plasma and brain concentration–time profiles in vivo, optimizing preclinical efficacy. Both intraperitoneal and oral routes were examined due to their differing pharmacokinetic properties. Adult rats received a single dose (intraperitoneal: 3 or 10 mg/kg; oral: 10 mg/kg), with plasma and brain samples collected over 6 h. Cemdomespib levels were measured by LC-MS/MS. Intraperitoneal administration produced rapid, dose-dependent plasma increases (peaks: ~ 5463 μ g/L at 10 mg/kg, ~ 2300 μ g/L at 3 mg/kg; Figure 2A), whereas oral

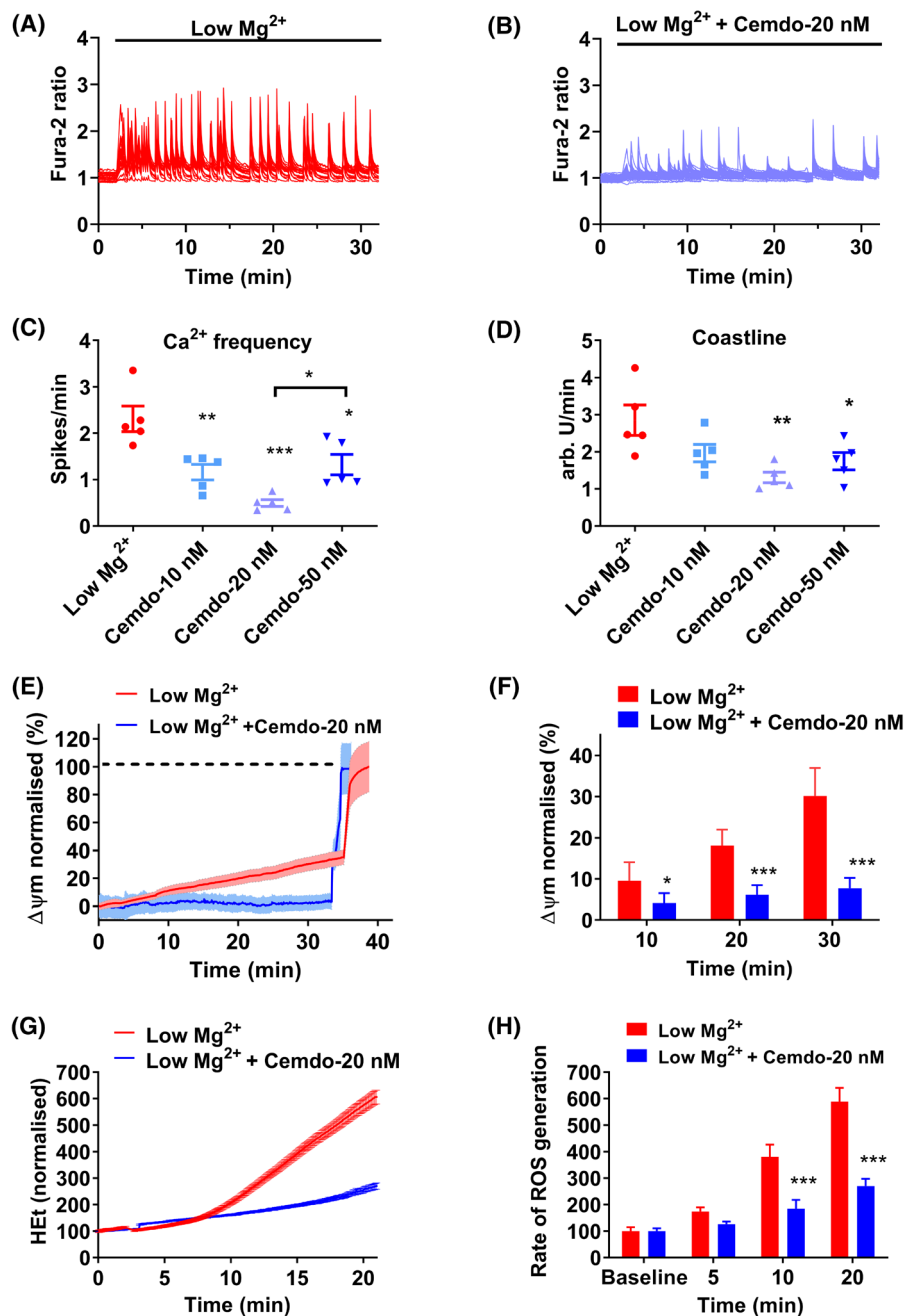


FIGURE 1 Cemdomespib attenuates low-magnesium model of epileptiform activity and associated mitochondrial depolarization, and reactive oxygen species (ROS) accumulation in mixed cortical culture. (A, B) Representative traces of Fura-2 acetoxymethyl calcium imaging showing synchronized Ca^{2+} oscillation activity following low- Mg^{2+} artificial cerebrospinal fluid condition either alone (A) or following pretreatment with cemdomespib (Cemdo; 20 nmol·L⁻¹; B). (C, D) Effect of cemdomespib (10, 20, 50 nmol·L⁻¹) on low- Mg^{2+} -induced synchronized Ca^{2+} oscillation frequency (spikes/min; C) and on the coastline (D). (E) Time course of mitochondrial membrane potential ($\Delta\psi\text{m}$) changes during low- Mg^{2+} -induced epileptiform activity with and without pretreatment (20 nmol·L⁻¹) pretreatment, measured by Rhodamine-123 fluorescence, normalized to carbonyl cyanide-p-trifluoromethoxyphenylhydrazone (FCCP)-induced maximal depolarization. (F) Quantification of $\Delta\psi\text{m}$ depolarization over time during low- Mg^{2+} -induced epileptiform activity following pretreatment with and without cemdomespib (20 nmol·L⁻¹) pretreatment. (G) Time-dependent accumulation of ROS during low- Mg^{2+} -induced epileptiform activity following pretreatment with and without cemdomespib, measured by dihydroethidium (HET) fluorescence. (H) Quantification of ROS generation rates at the indicated time points revealed a significant reduction in oxidative stress in the cemdomespib-pretreated group during epileptiform activity. $n = 3$ independent experiments, two coverslips per experiment, 50–90 neurons analyzed per coverslip. Data are presented as mean $\pm$ SEM. Statistical significance was determined using one-way or two-way analysis of variance followed by Bonferroni post hoc tests. * $p < .05$, ** $p < .01$, *** $p < .001$. arb. U, arbitrary units.

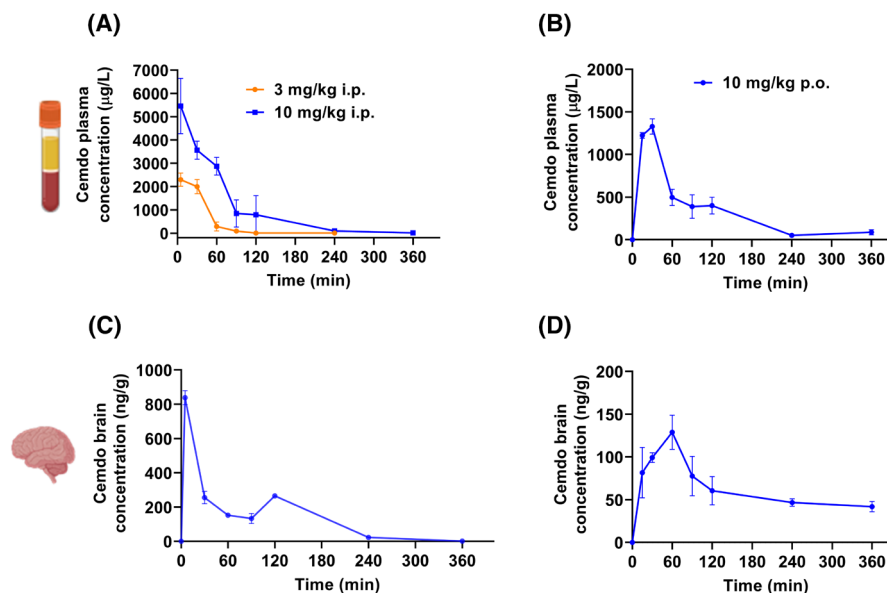


FIGURE 2 Pharmacokinetic profile of cemdomespib (Cemdo) in plasma and brain following intraperitoneal and oral administration. (A, B) Plasma concentration–time curves of cemdomespib following a single intraperitoneal injection at 3 and 10 mg/kg (A), and a single oral dose of cemdomespib (10 mg/kg; B). (C, D) Brain concentrations of cemdomespib over time after 10-mg/kg administration either through intraperitoneal (C) or oral dosing (D). Drug levels were quantified using liquid chromatography–tandem mass spectrometry in plasma (µg/L) and brain tissue (ng/g) at the indicated time points. Data are presented as mean $\pm$ SEM. $n = 3$ animals per timepoint. Cemdomespib demonstrated dose-dependent systemic exposure and measurable brain penetration following both routes of administration.

dosing at 10 mg/kg yielded lower plasma levels (1328 µg/L at 30 min; [Figure 2B](#)), suggesting reduced oral bioavailability. Importantly, the brain concentration profiles confirmed effective CNS penetration ([Figure 2C,D](#)). Following 10 mg/kg ip, cemdomespib was detected in the brain within 5 min, reaching ~838 ng/g, and remaining above 100 ng/g for up to 6 h ([Figure 2C](#)). In contrast, oral dosing also achieved brain concentrations up to 129 ng/g at 1 h, which then gradually declined ([Figure 2D](#)). These findings confirm that cemdomespib exhibits favorable CNS bioavailability, especially via intraperitoneal delivery, supporting its use in preclinical seizure and epilepsy models that require brain-targeted effects.

3.3 | Pretreatment of cemdomespib protects from PTZ-induced seizure susceptibility

Next, we validated the anticonvulsive efficacy of cemdomespib in vivo using a PTZ-induced seizure model. Animals were pretreated with cemdomespib (10 mg/kg ip) or vehicle 30 min prior to the first PTZ injection, and seizure behavior was scored using the modified Racine scale ([Figure 3A](#)). Pretreatment with cemdomespib significantly reduced the proportion of animals exhibiting seizures compared to the vehicle group (vehicle: 9/9, cemdomespib: 5/9, Fisher exact test: $p = .0412$; [Figure 3B](#)),

indicating decreased seizure susceptibility. Furthermore, cemdomespib pretreatment significantly increased seizure latency (vehicle: 16 ± 3 min, cemdomespib: 50 ± 11 min, $p = .0018$; [Figure 3C](#)), demonstrating a significant delay in seizure onset. Moreover, animals pretreated with cemdomespib experienced a lower duration of seizure (vehicle: 56 ± 10 s, cemdomespib: 19 ± 3 s, $p = .0191$; [Figure 3D](#)) than vehicle-treated animals, reflecting the mitigation of the seizure burden. In addition, the maximum seizure severity experienced by each animal was significantly lower in cemdomespib-pretreated animals (vehicle: $5.9 \pm .1$, cemdomespib: $4.2 \pm .5$, $p = .0050$; [Figure 3E](#)) than that in the vehicle group, highlighting its anticonvulsive efficacy in reducing seizure intensity. These results demonstrate that pretreatment with cemdomespib confers significant protection against PTZ-induced seizures by increasing the latency to seizure onset and decreasing both seizure duration and severity, supporting the anticonvulsive potential of cemdomespib and presenting it as an effective therapeutic candidate for seizure control.

3.4 | Pretreatment with cemdomespib attenuates seizure severity in the KA-induced SE model

To assess cemdomespib's antiseizure efficacy under sustained excitotoxic stress, we used a systemic KA-induced

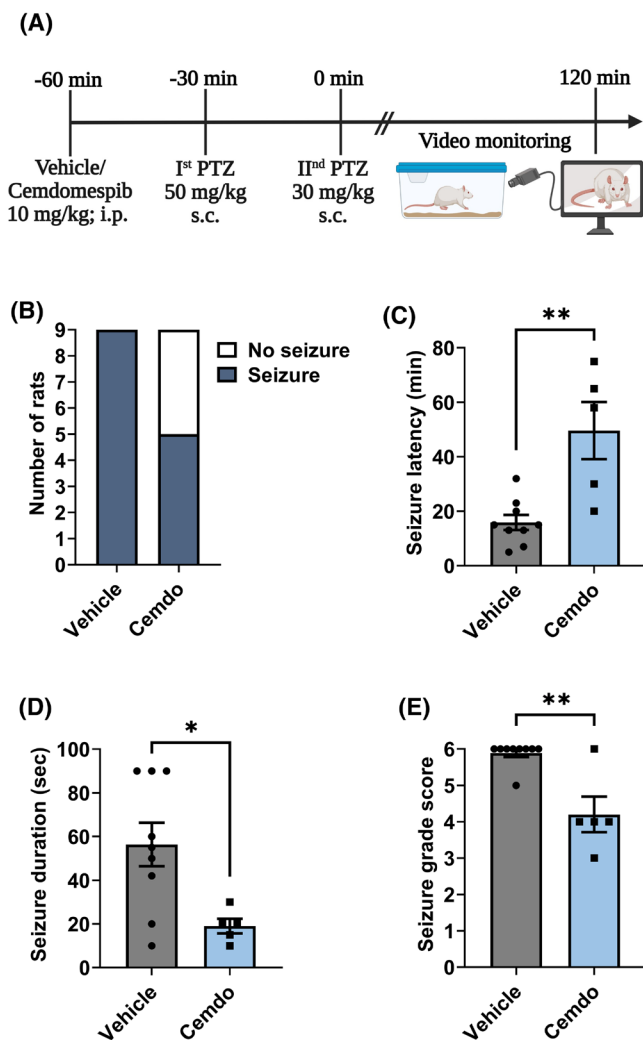


FIGURE 3 Pretreatment with cemdomespib attenuates seizure susceptibility and severity in a pentylenetetrazol (PTZ)-induced seizure model. (A) Schematic illustration of the experimental timeline and setup. Cemdomespib (Cemdo; 10 mg/kg ip) was administered 30 min prior to the first subcutaneous PTZ injection (50 mg/kg), followed by a second PTZ dose (30 mg/kg) 30 min later. Seizure activity was monitored and scored behaviorally. (B) Proportion of animals exhibiting seizures in the vehicle- and cemdomespib-pretreated groups. (C) Pretreatment with cemdomespib significantly increased seizure latency compared to that with the vehicle following PTZ administration. (D) Seizure duration was significantly reduced in cemdomespib-pretreated animals following PTZ administration. (E) Maximum seizure severity (scored using the modified Racine scale) experienced by each animal in the cemdomespib- and vehicle-pretreated groups following PTZ administration. $n = 9$ animals per group. Data are presented as mean $\pm$ SEM. Statistical significance was determined using an unpaired t -test. * $p < .05$, ** $p < .01$.

SE model, which mimics prolonged limbic seizures and models TLE.⁴² Based on prior in vitro and PTZ results, we tested whether cemdomespib pretreatment could reduce KA-induced seizure severity and total seizure burden.

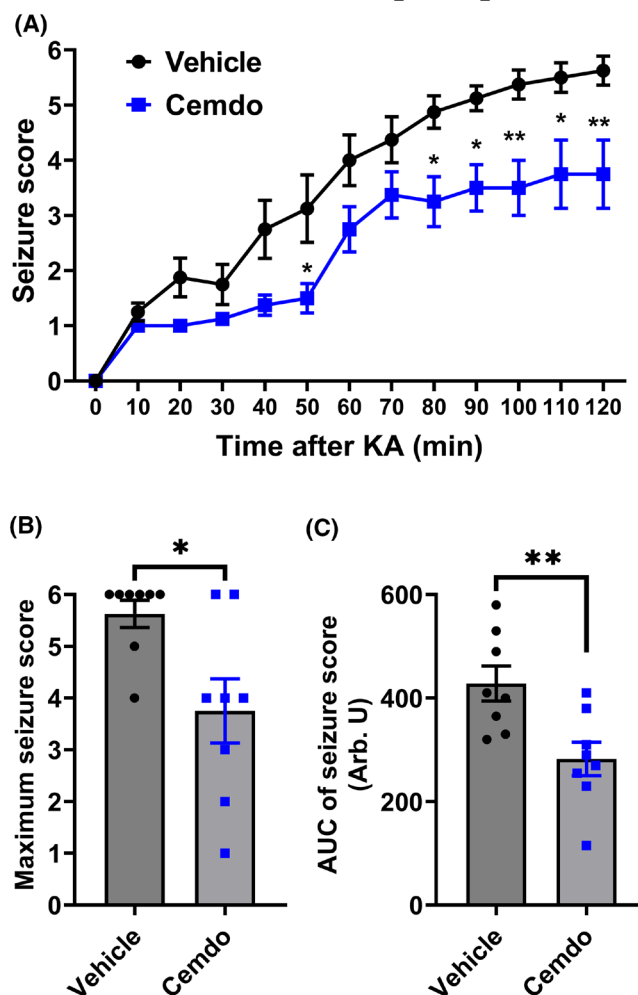


FIGURE 4 Pretreatment with cemdomespib (Cemdo) attenuates seizure severity following kainic acid (KA)-induced status epilepticus. (A) Time course of seizure progression following a single intraperitoneal injection of KA, over 120 min after cemdomespib and vehicle pretreatment. (B) Maximum seizure severity experienced by each animal following KA injection in the cemdomespib or vehicle pretreatment group. (C) Total seizure burden, quantified as the area under the curve (AUC) of seizure scores over time in both the cemdomespib and vehicle pretreatment groups followed by KA administration. $n = 8$ animals per group. Data are presented as mean $\pm$ SEM. Statistical significance was determined using two-way repeated measures analysis of variance (A) and unpaired t -tests (B, C). * $p < .05$, ** $p < .01$. Arb. U, arbitrary units.

Rats received cemdomespib (10 mg/kg ip) or vehicle 30 min before a single KA injection, and seizures were scored every 10 min for 120 min using the Racine scale. Vehicle-pretreated animals exhibited a continuous increase in seizure severity over time (Figure 4A), whereas cemdomespib-pretreated animals demonstrated a significant reduction in seizure scores beginning at 50 min post-KA injection (Figure 4A). Moreover, cemdomespib-pretreated animals experienced significantly lower

seizure severity compared to the vehicle group (vehicle: $5.6 \pm .3$, cemdomespib: $3.8 \pm .6$, $p = .0275$; [Figure 4B](#)), suggesting the protective effect of cemdomespib pretreatment against seizure severity. Furthermore, the total seizure burden, quantified as the area under the curve of seizure scores, was significantly reduced in cemdomespib-treated animals compared to vehicle controls (vehicle: 428 ± 34 , cemdomespib: 282 ± 32 , $p = .0075$; [Figure 4C](#)), reflecting an overall attenuation of the progression toward SE. These findings demonstrate that acute pretreatment with cemdomespib significantly suppresses seizure severity and total seizure burden in the KA-induced SE model, complementing its efficacy in the PTZ model and supporting its potential as a broad-spectrum therapeutic candidate for epilepsy.

3.5 | Cemdomespib therapy prevents epilepsy development and suppresses spontaneous seizure burden following SE

To determine whether cemdomespib has disease-modifying potential beyond acute anticonvulsive effects, we tested its efficacy in the KA-induced SE model, which recapitulates the key features of acquired TLE, including delayed spontaneous recurrent seizures following an initial SE insult.⁴³ Animals were implanted with ECoG transmitters to monitor seizure activity 24/7 using wireless telemetry (see [Section 2](#)). One week postsurgery, animals were administered a single intraperitoneal KA injection to induce SE. Animals were randomized to receive cemdomespib (10 mg/kg) or vehicle twice daily, intraperitoneally for 2 weeks ([Figure 5A](#)) under continuous video-ECoG monitoring for the next 15 weeks after SE. Weekly seizure frequency confirmed a progressive increase in spontaneous recurrent seizures in vehicle-treated animals following SE over 15 weeks, whereas cemdomespib-treated animals exhibited significantly lower seizure frequency throughout the same period ([Figure 5B](#)). Early intervention with cemdomespib for 2 weeks following SE exerted a sustained inhibitory effect on spontaneous recurrent seizure activity, which delayed the course of epilepsy development throughout the monitoring duration, indicating its antiepileptogenic efficacy. The cumulative seizure burden, determined as the total number of seizures experienced by each animal, confirmed that cemdomespib-treated animals experienced a significantly lower seizure burden than the vehicle group (vehicle: 163 ± 29 , cemdomespib: 22 ± 9 , $p = .0003$; [Figure 5C](#)). The Kaplan–Meier plot revealed that a higher proportion of cemdomespib-treated animals remained seizure-free over 15 weeks of monitoring compared to vehicle-treated animals (vehicle: 10% vs.

cemdomespib: 40%; [Figure 5D](#)), and latency to the first spontaneous seizure was significantly increased in the cemdomespib group (vehicle: 8 ± 1 days, cemdomespib: 25 ± 3.6 days, $p = .0001$; [Figure 5E](#)), suggesting delayed epilepsy development.

Furthermore, Nissl staining of CA3 and CA1 subfields showed visibly preserved neuronal architecture in cemdomespib-treated rats compared with vehicle controls. Quantification confirmed significantly higher neuronal densities in both CA3 and CA1 following cemdomespib therapy ([Figure S1](#)). These findings demonstrate that post-SE administration of cemdomespib confers long-term protection against the development and progression of spontaneous seizures, supporting the potential of this approach as a disease-modifying therapy for acquired epilepsy.

3.6 | Cemdomespib therapy improves locomotor activity and partially rescues anxiety-like behavior following epilepsy development

Cognitive comorbidities, such as impaired locomotion and anxiety-like behavior, are common consequences of chronic epilepsy.^{44,45} We evaluated the effect of cemdomespib on these behaviors 8 weeks after SE (5 weeks after cemdomespib therapy withdrawal), corresponding to the early phase of chronic epilepsy. In the open field test, vehicle-treated animals showed hypo-locomotor activity, as indicated by significantly reduced distance traveled compared to sham controls ([Figure 6B](#), $p < .0001$), whereas cemdomespib-treated animals showed a marked improvement in locomotor activity, with significantly greater total distance traveled ($p < .001$ vs. vehicle), further supported by the representative track paths ([Figure 6A](#)). Furthermore, cemdomespib-treated animals spent a significantly higher percentage of time in the center of the arena ($p < .01$; [Figure 6C](#)) than vehicle-treated animals, suggesting reduced anxiety-like behavior. These results indicate that the cemdomespib-treated animals were less anxious and exhibited greater exploratory drive than their vehicle-treated counterparts. We further validated this anxiolytic effect using the elevated plus maze test. Vehicle-treated animals exhibited a significantly reduced number of entries into the open arms (sham: 11 ± 1.9 vs. vehicle: $3.3 \pm .9$, $p = .0004$; [Figure 6E](#)) and spent less time in them (sham: 17.3 ± 2.4 s vs. vehicle: 6.5 ± 1.9 s; [Figure 6F](#)) than sham animals, reflecting increased anxiety. In contrast, cemdomespib-treated animals demonstrated a significant increase in open arm entries

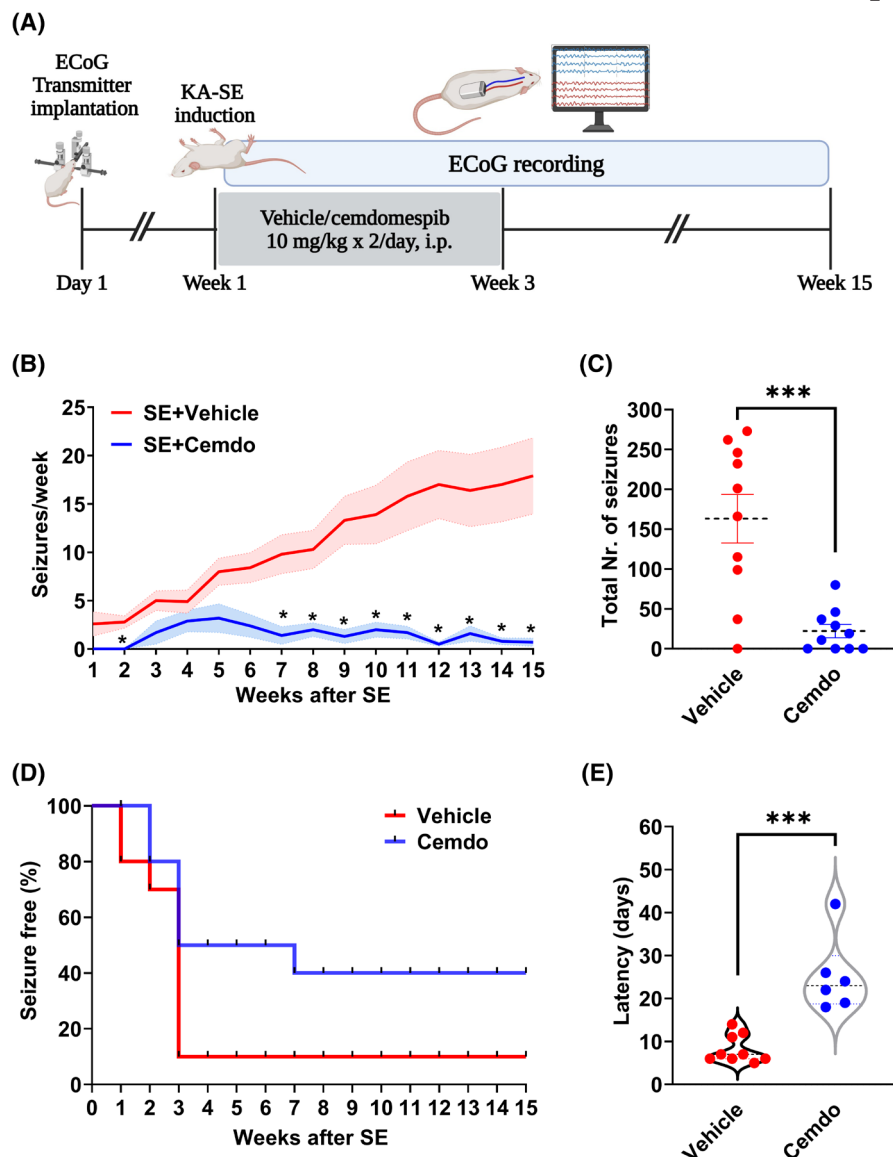


FIGURE 5 Cemdomespib (Cemdo) therapy prevents the development of epilepsy and reduces the seizure burden. (A) Schematic illustration of the experimental setup and timeline. (B) Seizure frequency per week in vehicle (red) and cemdomespib-treated (blue) animals over a 15-week period after status epilepticus (SE). (C) Total number of seizures experienced by each animal in the vehicle- and cemdomespib-treated groups over 15 weeks. (D) Kaplan–Meier plot showing the percentage of animals experiencing seizure-free weeks in the vehicle- and cemdomespib-treated groups over 15 weeks. (E) Latency to the first spontaneous seizure in the cemdomespib and vehicle treatment groups after SE. $n = 10$ animals per group. Data are presented as mean $\pm$ SEM. Statistical significance was determined by generalized log-linear mixed model, incorporating the random effect of the animal (autoregressive covariance) and fixed effects of treatment group, time followed by Sidak post hoc test (B) or by unpaired t -test (C–E). * $p < .05$, *** $p < .001$. ECoG, electrocorticographic; KA, kainic acid.

(cemdomespib: $8.1 \pm .8$, $p = .0154$; Figure 6E), whereas the time spent in the open arms showed a partial improvement (cemdomespib: 10.4 ± 1.5 s, $p = .115$; Figure 6F). These findings were further supported by representative heatmaps of spatial exploration across elevated plus maze arms (Figure 6D), which showed enhanced open-arm navigation in the cemdomespib group. Together, these findings suggest that cemdomespib treatment improves locomotor performance and partially reverses anxiety-like behavior associated

with epilepsy development, even several weeks after therapy cessation.

4 | DISCUSSION

Epilepsy remains a major clinical challenge, with one third of patients exhibiting resistance to available ASMs.² The failure of current ASMs to prevent the development of epilepsy or modify disease progression has

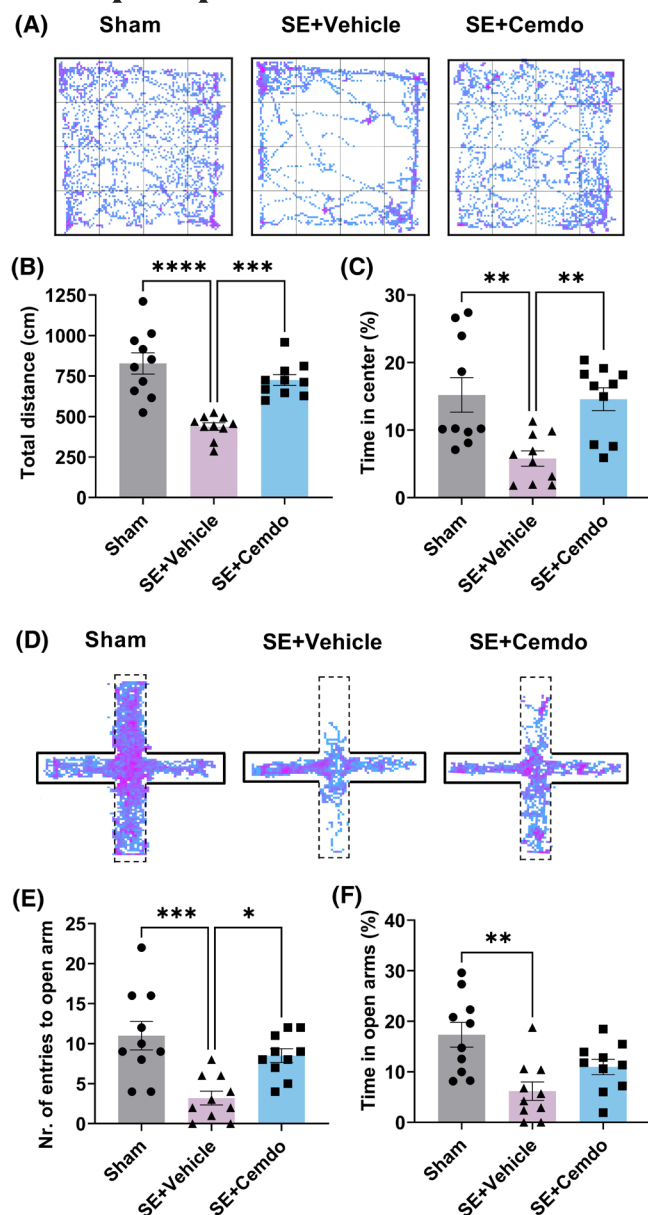


FIGURE 6 Cemdomespib (Cemdo) therapy improves locomotor activity and partially rescues anxiety-like behavior associated with the development of epilepsy. (A) Representative heatmaps showing the track path of sham, vehicle-treated, and cemdomespib-treated animals in an open field arena at the 8th week after status epilepticus (SE; 5th week after therapy withdrawal). (B) Total distance traveled by sham, vehicle-treated, and cemdomespib-treated animals. (C) Percentage of time spent in the center zone of the open field arena. (D) Representative heatmaps showing spatial exploration patterns of sham, vehicle-treated, and cemdomespib-treated animals across the arms of the elevated plus maze. (E) Number of entries into the open arms by sham, vehicle-treated, and cemdomespib-treated animals. (F) Percentage of time spent in the elevated plus maze open arms. $n = 10$ animals per group. Data are presented as mean $\pm$ SEM. Statistical significance was determined using one-way analysis of variance with Tukey post hoc test. * $p < .05$, ** $p < .01$, *** $p < .001$, **** $p < .0001$.

necessitated the search for novel therapeutic strategies that can target the underlying mechanisms of epileptogenesis.^{3,4} One emerging target is Hsp90, a ubiquitous molecular chaperone involved in the regulation of protein folding, degradation, and stabilization under both physiological and pathological conditions.^{46,47} Hsp90 has been implicated in the pathogenesis of various neurodegenerative diseases and has been shown to modulate cellular stress responses, inflammation, and mitochondrial function.^{29,46,48} However, its role in epilepsy remains unclear. Here, we provide a comprehensive evaluation of cemdomespib, a selective Hsp90 inhibitor, demonstrating its anticonvulsant, neuroprotective, and antiepileptogenic efficacies in both in vitro and in vivo models of epilepsy.

To establish a clear link between mechanism and translation, we deliberately employed three complementary experimental systems: primary cortical neurons, PTZ-induced acute seizure, and KA-induced SE models. The in vitro assay enabled precise characterization of cemdomespib's redox-modulatory effects and mitochondrial protection under controlled conditions, whereas the PTZ-induced acute seizure paradigm provided a standardized platform for quantifying acute anticonvulsant efficacy and optimizing dosing with minimal mortality. Findings from these acute studies guided treatment parameters in the KA-SE model, which was subsequently used to evaluate long-term antiepileptogenic and behavioral outcomes. This integrative design allowed us to bridge molecular mechanisms with disease-modifying effects.

We first utilized a well-established low-Mg²⁺ in vitro model, which induces NMDA receptor-mediated hyperexcitability and mimics epileptiform activity.^{35,36,49} Cemdomespib pretreatment significantly reduced calcium oscillations and overall network hyperactivity, indicating suppression of epileptiform discharges (Figure 1A–D). These findings are consistent with previous reports suggesting that modulation of NMDA receptor function and intracellular calcium dynamics can attenuate epileptiform activity.^{50,51} Moreover, persistent neuronal hyperexcitability impairs mitochondrial function and promotes oxidative stress, contributing to epileptogenesis.^{37,39–40} Primary cortical neurons were selected to maintain continuity with our previous mechanistic investigations into oxidative stress regulation during epileptiform activity.^{32,52} These cultures exhibit highly synchronized bursting and reproducible redox dynamics suitable for assessing pharmacological modulation. Nonetheless, given the hippocampus's central role in TLE, future studies will incorporate hippocampal neuronal cultures to validate and extend these findings. In our previous investigations, we highlighted the elevated level of mitochondrial depolarization and ROS accumulation

during epileptiform activity induced by either low Mg^{2+} or cotreatment with proepileptic drugs such as picrotoxin and 4-aminopyridine.^{17,19,32,41} We observed that cemdomespib not only preserved the mitochondrial membrane potential and inhibited depolarization (Figure 1E,F) but also attenuated ROS accumulation (Figure 1G,H) during epileptiform activity. These effects are consistent with the hypothesis that Hsp90 promotes a prooxidative environment by stabilizing pro-oxidant proteins^{53,54} and interfering with antioxidant pathways.^{55,56} Inhibition of Hsp90 stabilizes client proteins, such as NOX2, which are central ROS producers during seizures and exert an antioxidant effect.^{19,32} Targeting Hsp90 under oxidative conditions may prevent its upregulation and subsequent response. Previous reports have highlighted that Hsp90 inhibitors and antioxidants, such as 7,8-dihydroxy-4-methylcoumarin, achieve this by activating Nrf2 and reducing oxidative stress.⁵⁷ Together, our *in vitro* data position cemdomespib as a potent neuroprotective agent that mitigates three critical hallmarks of seizure pathology: hyperexcitability, mitochondrial dysfunction, and oxidative stress.

Hsp90 has been implicated in the pathophysiology of epilepsy, with previous studies reporting its upregulation both in experimental epilepsy models and in the hippocampus of patients with TLE, particularly within neurons and glial cells of the dentate gyrus and CA1 regions.²⁷ Pharmacological inhibition of Hsp90 using 17-AAG exhibited seizure-suppressive effects in mouse models of TLE; however, its clinical advancement has been limited by hepatotoxicity and poor solubility.²⁸ Similarly, HSP990, a second-generation Hsp90 inhibitor, exhibited strong anti-seizure activity in rodent and primate models.³⁰ However, limited blood–brain barrier penetration and pharmacokinetic variability may restrict its clinical application.

In our study, cemdomespib exhibited a favorable pharmacokinetic profile *in vivo*, with intraperitoneal administration leading to rapid and sustained CNS penetration (Figure 2). This property is essential for targeting epileptogenic foci and aligns with the pharmacodynamic characteristics required for the effective treatment of CNS disorders.⁵⁸ Importantly, measurable brain concentrations persisted for several hours post-administration (Figure 2), supporting the suitability of cemdomespib for *in vivo* therapeutic applications.

To assess anticonvulsant efficacy, we employed the PTZ model, which induces seizures via γ -aminobutyric acidergic inhibition and is widely used to evaluate anticonvulsant activity.⁵⁹ Cemdomespib pretreatment significantly reduced seizure incidence, delayed onset, shortened seizure duration, and reduced seizure severity (Figure 3). Additionally, we extended our investigation to the systemic KA model, which closely mimics TLE and induces robust SE and subsequent neurodegeneration.⁴²

We observed that pretreatment with cemdomespib significantly attenuated seizure severity and total seizure burden in this model (Figure 4), supporting its efficacy across chemically distinct seizure paradigms.

Our study highlights the potential of cemdomespib as a disease-modifying therapy for epilepsy, with treatment initiated during the latent period, which is the critical window between the initial SE insult and the manifestation of spontaneous recurrent seizures. This phase, characterized by dynamic functional and structural brain changes,³ offers a unique opportunity for interventions aimed at preventing or delaying the onset of epilepsy; however, few treatments have been successful in this regard.^{60,61} By administering cemdomespib early in the post-SE period, we observed a sustained reduction in spontaneous recurrent seizures over 15 weeks (Figure 5B), as assessed by continuous 24/7 video-ECoG monitoring (Figure 5B). The treated animals exhibited delayed seizure onset and reduced overall seizure burden, and a higher proportion of animals remained seizure-free (Figure 5C–E). This is particularly significant as very few compounds exhibit lasting antiepileptogenic efficacy once SE has been established,^{62–64} suggesting that Hsp90 inhibition during epileptogenesis can delay the pathological processes underlying epilepsy development, likely through the suppression of early seizure-induced oxidative and inflammatory cascades and preservation of neural network integrity.⁶⁵ To extend the molecular understanding of cemdomespib's actions, future studies will employ transcriptomic profiling (bulk and single-cell RNA-seq) to identify downstream gene networks regulated by Hsp90/Hsp70 modulation and their convergence with oxidative stress and inflammatory pathways in epileptogenesis. Such analyses may also uncover biomarkers predictive of therapeutic responsiveness, aiding in the rational design of redox-targeted interventions.

Although Hsp90 modulation has been previously associated with neuroprotective effects, the current study provides several novel insights. First, we demonstrate for the first time that cemdomespib exerts both anticonvulsant and antiepileptogenic actions in the KA-SE model, markedly reducing spontaneous seizure frequency and cognitive impairment. Second, by integrating redox imaging, electrophysiology, and behavioral assays across cellular and *in vivo* systems, we revealed a unified mechanistic framework connecting cemdomespib molecular chaperone modulation with functional seizure suppression. Together, these advances underscore the study's fundamental novelty beyond prior reports of generic Hsp90 inhibition.

In line with core behavioral comorbidities of epilepsy, such as impaired locomotion and anxiety, which are consistently reported in both clinical and experimental settings,^{66,67} we observed that treatment with cemdomespib

improved locomotor activity and partially reversed anxiety-like behaviors (Figure 6). These effects persisted for several weeks after treatment withdrawal, indicating durable recovery of function. Supporting these observations, previous studies have documented that Hsp90 inhibitors can modulate glucocorticoid receptor activity, reduce neuroinflammation, and enhance mitochondrial resilience, mechanisms closely linked to affective and cognitive dysfunction in epilepsy and other neurological disorders.^{65,68}

Importantly, cemdomespib administration reduced hippocampal neuronal loss in the chronic phase, suggesting that delayed molecular injury processes remain responsive to intervention. The preservation of CA3 and CA1 neuronal populations indicates that modulating stress-related pathways may confer lasting structural protection beyond the acute insult. These findings support the potential of cemdomespib to mitigate progressive neurodegeneration associated with epileptogenesis. Collectively, these findings establish cemdomespib as a promising therapeutic candidate with acute and long-term efficacy in epilepsy treatment. By intervening early after the initial insult, cemdomespib not only suppressed seizure activity but also delayed disease progression and improved behavioral outcomes, highlighting its potential to address the critical unmet needs in epilepsy management.

AUTHOR CONTRIBUTIONS

Yara Sheeni: Investigation; visualization; formal analysis; project administration; writing—original draft; writing—review and editing. **Prince Kumar Singh:** Methodology; investigation; formal analysis; writing—original draft; writing—review and editing. **Sereen Sandouka:** Investigation; formal analysis. **Taige Zhang:** Methodology; investigation; formal analysis. **Alina Nemirovski:** Investigation; methodology. **Aseel Saadi:** Investigation; writing—review and editing. **Rhoda Olowe Taiwo:** Investigation. **Matthew C. Walker:** Writing—review and editing. **Tawfeeq Shekh-Ahmad:** Conceptualization; visualization; project administration; funding acquisition; writing—original draft; writing—review and editing.

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CONFLICT OF INTEREST STATEMENT

All authors declare that they have no conflicts of interest related to this work. We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

DATA AVAILABILITY STATEMENT

All data supporting the findings of this study are included in the article. Additional data can be provided by the corresponding author upon reasonable request.

CONSENT FOR PUBLICATION

All authors have read and approved the final manuscript.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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