

THE SAFETY PHARMACOLOGY PROFILE OF EPM301, A NOVEL CANNABINOID DERIVATIVE

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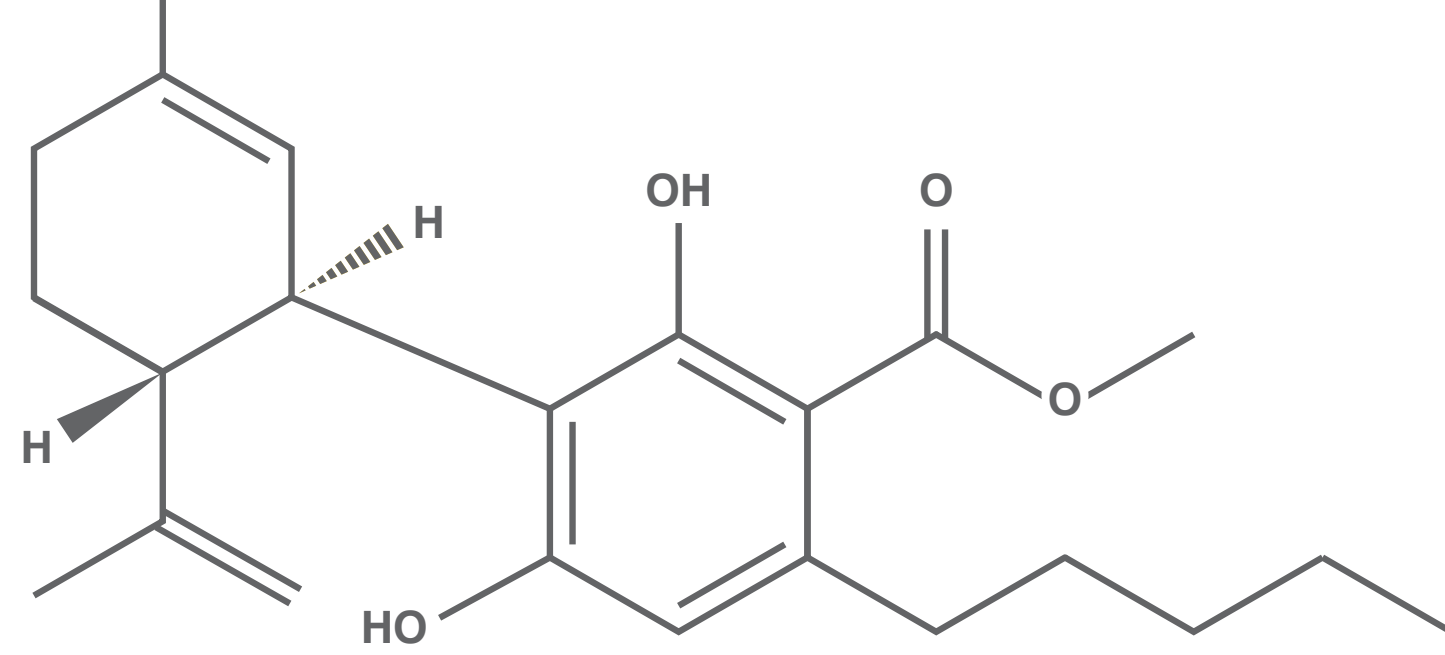
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- EPM301 is a novel cannabidiol (CBD) analog currently in development by EPM Therapeutics as a potential oral treatment for Prader-Willi Syndrome (PWS).
- Early animal studies suggest EPM301 may address hyperphagia, in addition to anxiety and daytime sleepiness in PWS patients.

EPM301



- EPM301 administration (20 and 40 mg/kg/day, intraperitoneally) was shown to significantly reduce body weight and food intake in high-fat diet-fed *Magel2* null mouse model of PWS.¹
- The effects of EPM301 on the central nervous system, respiratory function, and cardiovascular risk were evaluated as part of the IND-enabling program.

EPM301 (100, 250, or 500 mg/kg) had no effects on the central nervous system of male Wistar rats²

METHODS

- To evaluate the effect of EPM301 on the central nervous system (CNS), 24 male Wistar Han rats were randomly assigned to four groups. Each group was administered a single dose of the following via oral gavage at a dose volume of 10 mL/kg:
 - Group 1 (control; n=6): vehicle
 - Group 2 (n=6): 100 mg/kg EPM301
 - Group 3 (n=6): 250 mg/kg EPM301
 - Group 4 (n=6): 500 mg/kg EPM301.
- Body weights for all animals were recorded once during the pre-dose phase, once prior to dosing on Day 1, Day 2 and on Day 7 (the end of the study).
- A battery of behavioural tests and observations (Modified Irwin Testing) was carried out on all animals at pre-dose, and at 1, 2, 4 and 24 hours post-dose. At the end of each observation period, the rectal temperature of each animal was measured, followed by an assessment of locomotor activity and rearing.
 - Subjective assessment: the majority of the tests were assessed using a mild, moderate, or marked severity; some tests were scored as present (e.g., discharge, aggression to cage mate), absent (e.g., pinna response, palpebral reflex), or abnormal (e.g., waxy rigidity, pupillary response).
 - Body temperature measurement: a thermocouple, attached to an electric thermometer, was inserted into the rectum to a constant depth of 3 cm to record body temperature.
 - Locomotor activity and rearing assessment: spontaneous locomotor and rearing activity was measured in terms of number of squares crossed (locomotor activity) and number of rearing occasions (supported or unsupported) during a 2 min period.

Modified Irwin Testing

Home Cage Observations	Arena Benchtop Assessments	Hand-Held Observations
Restlessness (E) Aggression to Cage Mate (E) Respiration (A) Excretion (U)	Alertness (E) Posture (P) Gait (N) Tail(N) Behaviour – Stereotype (A) Behaviour – Other (A) Involuntary Movements (N) Auditory Startle Response (S) Approach Response (S)	Reactivity to Handling (E) Vocalization (E) Body Tone (P) Extensor Thrust (N) Lacrimation (U) Eyes (P) Eye Closure (N) Salivation (U) Discharge (U) Skin Color (P) Pelage (P)
Elicited Behaviours	Body Temperature (U)	Locomotor Activity
Visual Response (S) Waxy Rigidity (N) Grip Strength (N) Pinna Response (S) Palpebral Reflex (U) Pupil Status (U) Pupil Response (A) Righting Reflex (N) Proprioception (N) Pain Response (S)		Number of Squares Crossed (A) Number of Rears (A) Body Tone (P) Extensor Thrust (N)

A = Central nervous system activity; E = Central nervous system excitability; N = neuromuscular; P = Physiological; S = Sensorimotor; U = Autonomic.

RESULTS

Following a single oral gavage administration of EPM301 to male rats at doses of 100, 250, or 500 mg/kg:

Modified Irwin Testing	No abnormal observations were recorded at any timepoint (pre-dose and 1, 2, 4, and 24 hours post-dose) and at all EPM301 doses tested.
CNS Activity CNS Excitability Sensorimotor Neuromuscular Autonomic Physiological Locomotor & Rearing Activity	
Rectal Body Temperature	No statistically significant changes were recorded at any timepoint (pre-dose and 1, 2, 4, and 24 hours post-dose) and at all EPM301 doses tested.
Body Weight	No statistically significant changes were recorded at any timepoint (pre-dose phase, once prior to dosing on Day 1, Day 2 and Day 7) and at all EPM301 doses tested.

EPM301 (100, 250, or 500 mg/kg) did not cause any changes in the respiratory rate, tidal volume, or minute volume of male Wistar rats²

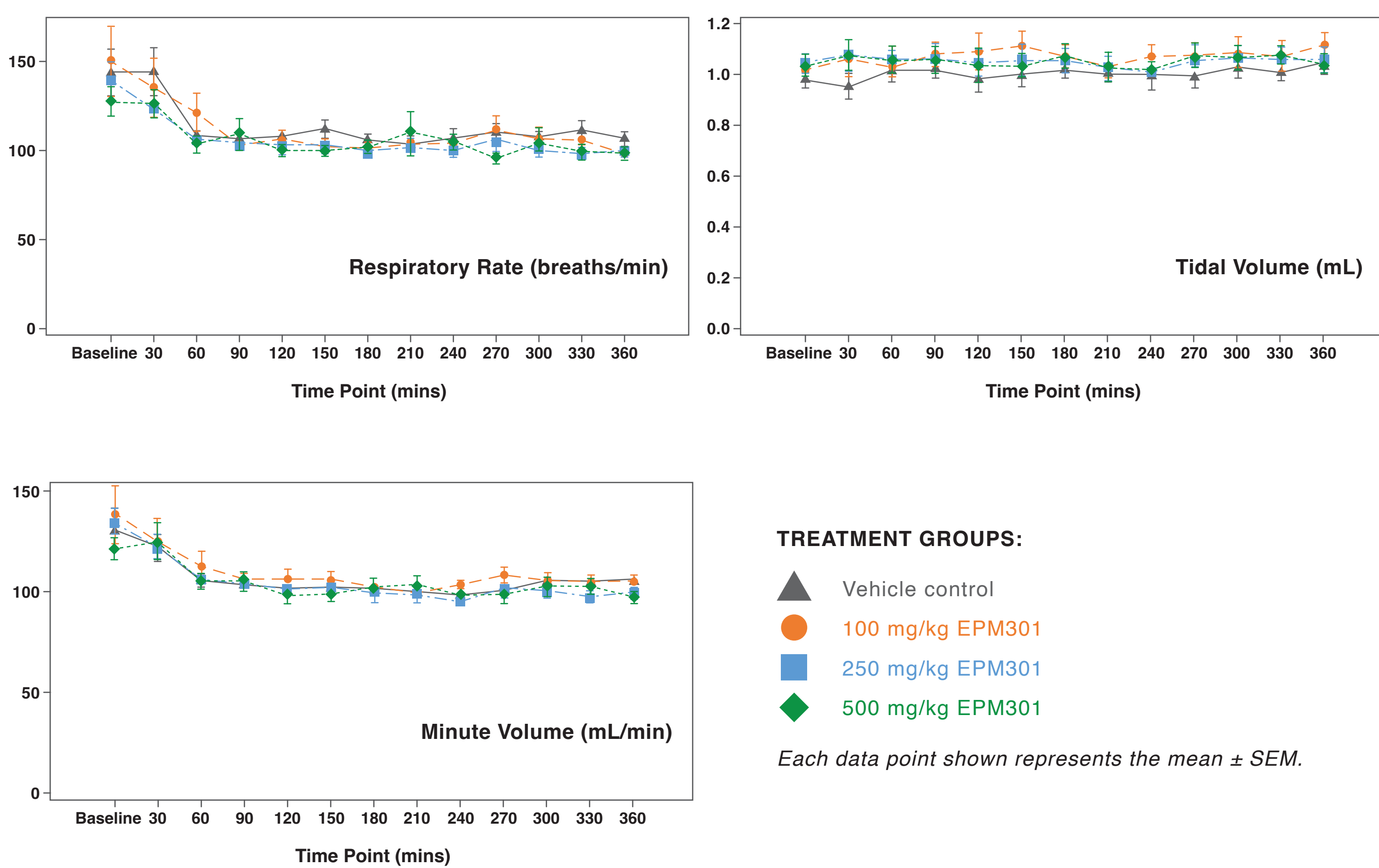
METHODS

- The effect of EPM301 on respiratory function was evaluated in 32 male Wistar Han rats, which were randomly assigned into four groups. Each group received a single dose of the following via oral gavage at a dose volume of 10 mL/kg:
 - Group 1 (control; n=8): vehicle
 - Group 2 (n=8): 100 mg/kg EPM301
 - Group 3 (n=8): 250 mg/kg EPM301
 - Group 4 (n=8): 500 mg/kg EPM301.
- Body weights for all animals were recorded at least once during the pre-dose phase, and prior to dosing on Day 1.
- The respiratory study was conducted over 2 days. Sixteen rats (four from each group) were examined on each day.
 - Respiratory function was assessed on freely moving conscious animals using whole-body, bias flow plethysmography.
 - Respiratory parameters (respiratory rate, tidal volume and minute volume) were derived from the changes in pressure associated with the warming and humidification of the air breathed in by the animal, which was monitored by specific probes located within the plethysmograph chambers.
 - On a day prior to dosing, all animals were habituated to the plethysmographs on one occasion for approximately 2 hours.
 - Pre-dose recording*: on the day of dosing, animals were placed into the plethysmographs for recording of respiratory parameters for at least 60 minutes. Animals were then removed from the chambers and returned to their home cages.
 - Dosing: Animals were then dosed (a.m.).
 - Post-dose recording*: immediately after the end of dosing (a.m.), animals were placed back into the plethysmographs, where they could freely move around, to continuously record respiratory parameters for approximately 6 hours post-dose (undisturbed recording).
 - Any unusual behaviour (e.g., animals appearing unsettled in the plethysmographs) or adverse clinical observations noted during the recording periods or upon handling of the animal at the end of recording periods were recorded.

RESULTS

EPM301, administered as a single oral gavage at dose levels of 100, 250, or 500 mg/kg, resulted in:

- no EPM301-related changes in the respiratory rate, tidal volume, or minute volume throughout the duration of the study.
- no EPM301-related clinical observations noted over the course of the study.



EPM301, at the highest tolerated concentration (nominal 0.1118 μ g/mL), did not inhibit *in vitro* hERG ion channel²

The human ether-à-go-go-related gene (hERG) plays an important role in myocardial repolarisation. It encodes the pore-forming subunit of the rapid delayed rectifier potassium (I_{Kr}) channel, and blockage of this channel may cause ventricular arrhythmia.

METHODS

- The effects of EPM301 on the hERG ion channels were assessed by examining the acute *in vitro* effects on the hERG tail current amplitude in human embryonic kidney cells (HEK)-293 stably expressing the hERG ion channel cDNA (HEK-hERG cells).
- The appropriate EPM301 formulation and concentration for the hERG assay was firstly determined through a series of range finding and stability assessment, which was a maximum concentration of 9.5 μ M in HEPES-buffered Physiological Salt Solution (HB-PSS) containing 1% v/v DMSO.
- Electrophysiological assessment setup:
 - A coverslip of the plated HEK-hERG cells was transferred from the cell culture incubator to a recording chamber mounted on the stage of an inverted microscope.
 - The recording chamber was continuously superfused with HB-PSS and all treatment solutions were applied at physiological temperature (35°C to 37°C).
 - Electrophysiological recordings were obtained with an Axopatch 200B amplifier using the Clampex module of the pCLAMP software, and the resulting data were sampled using a Digidata analogue-to-digital converter.
- Groups of HEK-hERG cells were treated as follows:

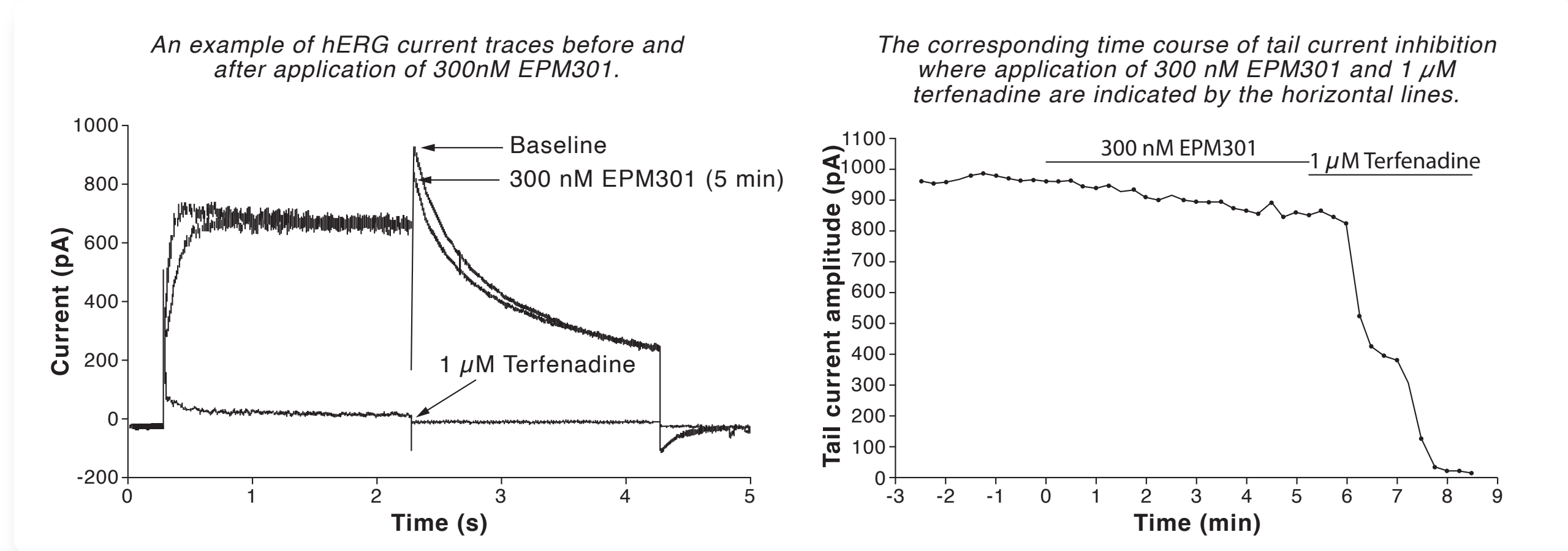
Vehicle Control	1% v/v DMSO in HB-PSS was applied to 4 cells.
Positive Control	Terfenadine (hERG blocker; 50 nM) was applied to 3 cells.
EPM301	- Four higher concentrations were initially assessed (9.5 μM, 5.3 μM, 3 μM, and 1 μM) but were not tolerated in the hERG assay. - One tolerated concentration (nominal 300 nM) was applied to 3 cells.

- Experimental phase:
 - The voltage protocol used to elicit hERG currents was as follows:
 - From holding potential of -80 mV, at 15 seconds intervals, a 2-second voltage step to +20 mV was applied to activate the hERG channels and generate an outward current.
 - A further 2-second step from +20 mV to -50mV elicited a larger outward current, known as the tail current, on which the effect of EPM301 was determined.
 - The cell was then returned to the holding potential and the cycle repeated.
 - As initial electrophysiological assessment with higher EPM concentrations indicated tolerability issues, a nominal 300 nM concentration was established to be tolerated. EPM301 300 nM was applied for 5–6 minutes, which was sufficient to elicit any effect on hERG.

RESULTS

Treatment effects on hERG Tail Current		
	% Baseline (Mean $\pm$ SEM)	Mean changes in tail current
Vehicle control (1% v/v DMSO in HB-PSS)	81.9 $\pm$ 4.5	- 18.1%
Positive control (50 nM Terfenadine)	17.4 $\pm$ 0.8	- 82.6%
EPM301 (300 nM)	106.0 $\pm$ 0.6*	+ 6.0%*

*Time-matched vehicle corrected.



- As EPM301 at the highest tolerated level did not inhibit hERG tail current, an IC₅₀ was not determined but can be assumed to be greater than a nominal concentration of 300 nM.

EPM301 demonstrated a favourable safety pharmacology profile across the non-clinical studies evaluated. Collectively, these findings indicate no apparent adverse effects on key CNS, respiratory, or cardiac electrophysiological parameters under the conditions of these studies and provide supportive evidence for the continued non-clinical development of EPM301 as a potential oral treatment for Prader-Willi syndrome (PWS).

References: 1. Ben-Cnaan E., et al. *Int. J. Mol. Sci.* 2022;23(10):5610-5626. 2. Data on File.

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