

SAN711, a novel GABA_A α₃ Receptor Subtype preferring Positive Allosteric Modulator for the treatment of Neuropathic Pain and Pruritus

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Introduction:

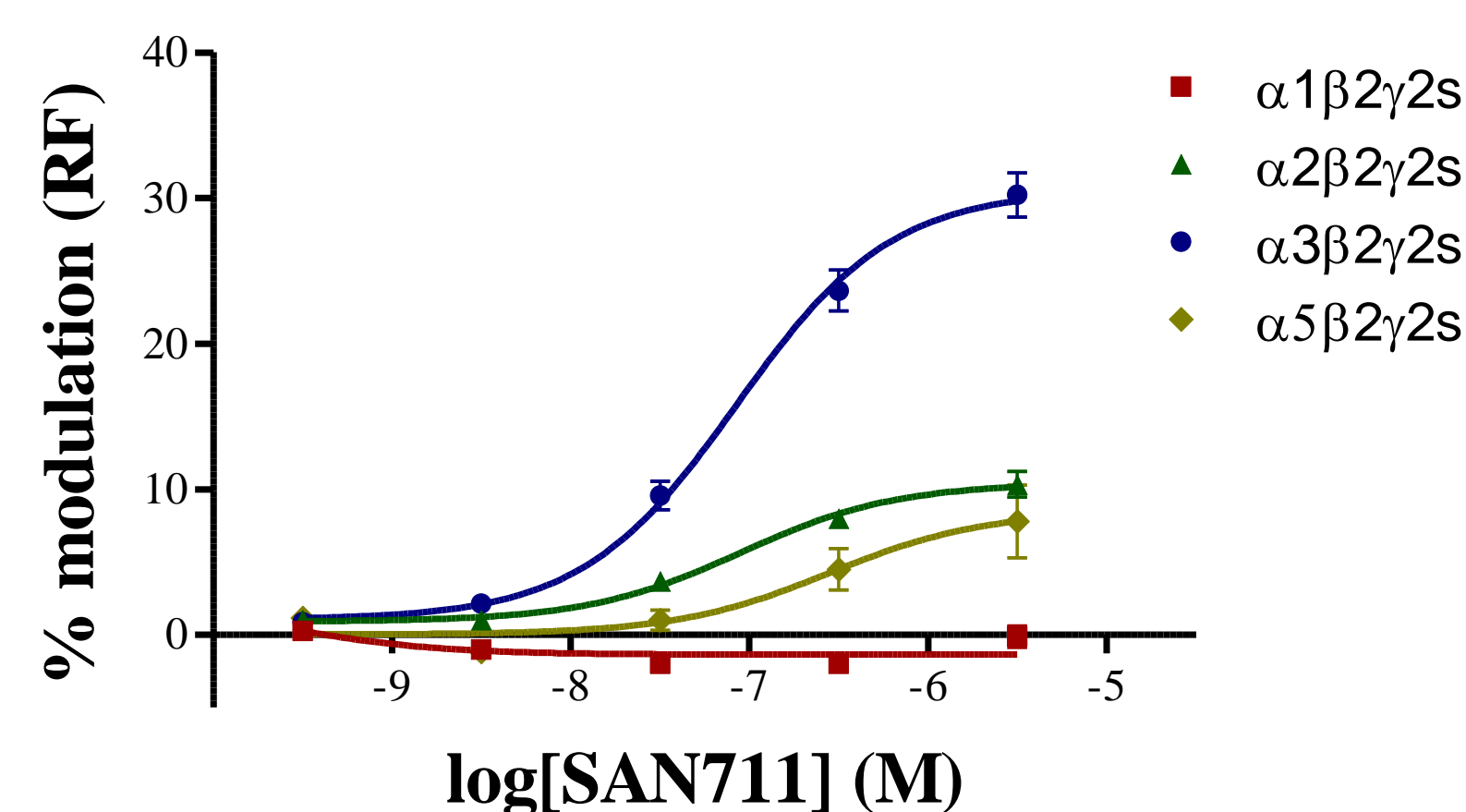
GABA is the main inhibitory neurotransmitter in the CNS including lamina-II of the spinal cord dorsal horn, where nociceptive fibres terminate. The inhibitory neurotransmission in the spinal cord is of great importance in pain transmission and enhancement of inhibition leads to analgesia (1). The GABA_A receptors are ligand gated, heteropentameric channels containing α, β and γ subunits. The majority of GABA_A receptors present in the CNS contain two α, two β, and one γ subunit (2). Recent studies using mice with point mutations rendering the different α subunits insensitive to diazepam, suggest that α₂ and α₃ subunits mediate the analgesic effects of benzodiazepines (3). This is supported by pharmacological studies showing analgesic effects of selective positive modulators of α_{2/3} containing GABA_A receptors in preclinical pain models (4). Further, lack of GABAergic interneuron mediated inhibition in the spinal cord has been shown to be responsible for chronic itch in Bhlhb5 mutant mice (5) suggesting potential therapeutic activity by enhancement of spinal inhibition. Here we report analgesic and antipruritic activity of SAN711, a novel GABA_A α₃ receptor preferring positive allosteric modulator, in preclinical models of neuropathic pain and pruritus.

SAN711 binds non-selectively to different GABA_A receptors subtypes in ³H-flumazenil binding

Binding	K _i (nM)
³ H-Flumazenil (cortex)	4.7
³ H-Flumazenil α ₃ β ₃ γ ₂	15
³ H-Flumazenil α ₅ β ₃ γ ₂	15

SAN711 displays non-selective binding affinity to GABA_A α₁ (cortex) vs α₃ receptors. The *in vitro* binding assay for GABA_A α₁ receptor subtype was performed using isolated membranes from rat cerebral cortex. GABA_A α₃- and α₅-receptor binding was determined using HEK293 cells stably expressing α₃β₃γ₂ and α₅β₃γ₂ receptor constructs, respectively. Clonazepam (1 μM) was used to determine non-specific binding.

SAN711 shows functional preference for α₃ containing GABA_A receptors in oocyte electrophysiological recordings



SAN711 shows a unique functional subtype-selective efficacy profile on GABA_A receptors in oocyte two-electrode voltage clamp recordings. SAN711 display a preferential potentiation of GABA-mediated currents in GABA_A-α₃ containing receptors, a minor activation (less than 10%) of GABA_A-α_{2/5} subunits and no activation of GABA_A-α₁ containing receptors. For each experimental data set, GABA was dissolved in an oocyte ringer solution in a concentration (0.5-3 μM) giving rise to EC₁₀₋₂₀ elicited currents for a given GABA_A receptor subtype combination. Peak currents were read and normalized to a maximal effective concentration of diazepam, where after data points were fitted to the empirical Hill equation by non-linear regression, n=3-14.

Plasma and brain free fraction of SAN711

Free fraction		
Plasma	Mouse	16%
	Rat	19%
Brain	Rat	7%

SAN711 is abundantly free in the plasma and brain to exert pharmacological effects. SAN711 was incubated with relevant plasma or brain homogenate in RDD device (Pierce) using standard protocols

SAN711 has low clearance in human hepatocytes

Hepatocyte clearance	
Species	Cl _{int} in vivo (l/h/kg)
Dog	0.82
Human	0.27
Rat	0.52
Mini-pig	0.43

SAN711 was incubated in relevant hepatocyte suspension using standard protocols. The observed clearance was converted to Cl_{int} in vivo using the well-stirred model.

SAN711 has a favourable pharmacokinetic profile in rats with high exposure and long t_{1/2}

Route	Dose (mg/kg)	AUC _(0-8h) (h*ng/ml)	C ₀ (ng/ml)	t _{1/2} (h)	Cl (l/h/kg)	V _z (l/kg)	B/P
IV	0.5	985	863	4.3	0.38	2.4	0.5

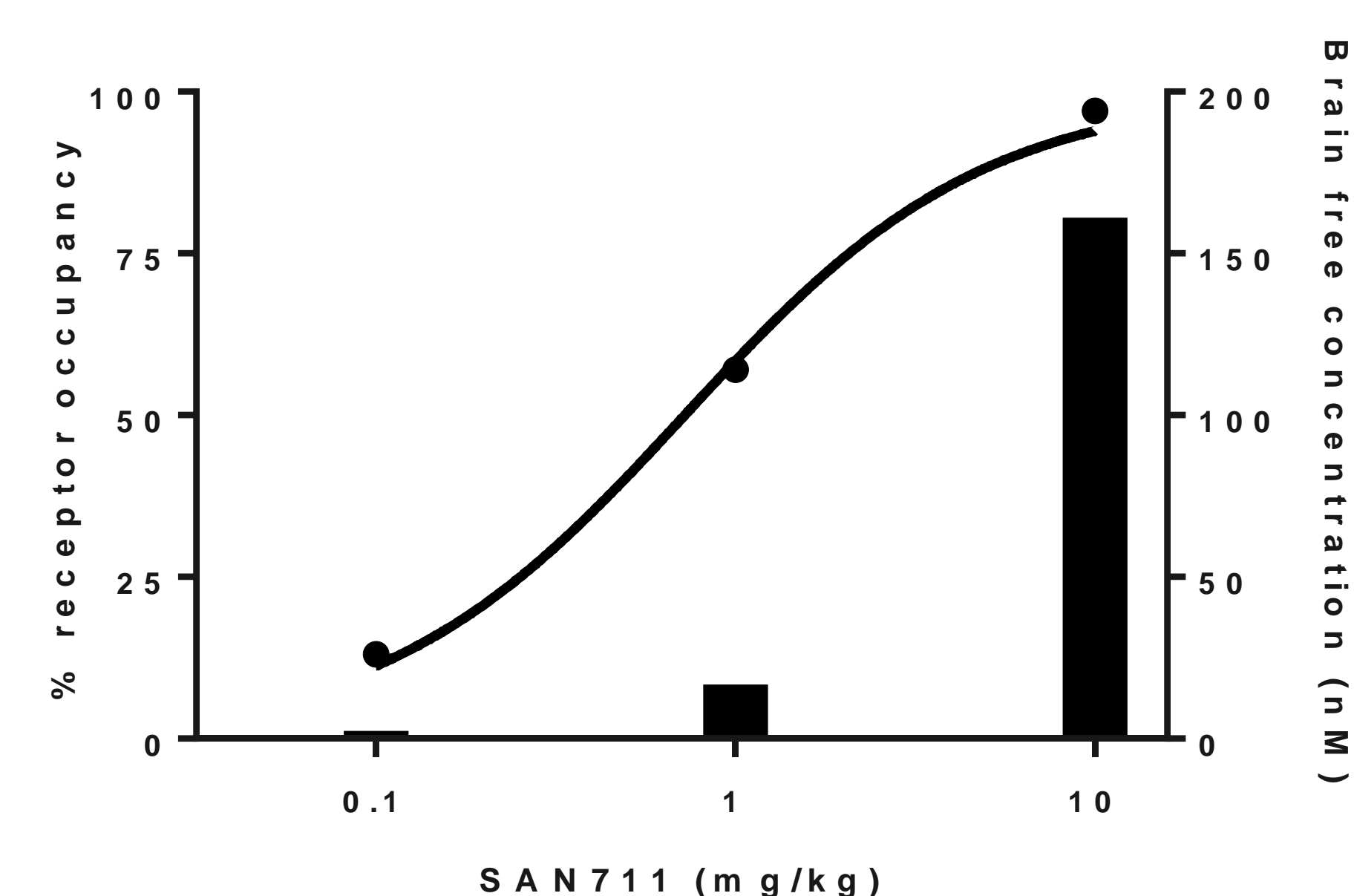
SAN711 was dosed in a clear solution of 30% HP-Beta-CD and 5% DMSO. All calculation were done in WinNonLin. SAN711 show long half-life and low clearance in rat, which is in line with the clearance predicted by rat hepatocytes.

SAN711 displays dose linearity and good brain exposure

Dose (mg/kg)	AUC _(0-24h) (h*ng/ml)	C _{max} (ng/ml)	T _{max} (h)	B/P 3h
1	1134*	163	4	0.4
3	8005	552	6	0.5
10	34528	2267	8	0.5
30	98537	6591	4	0.6

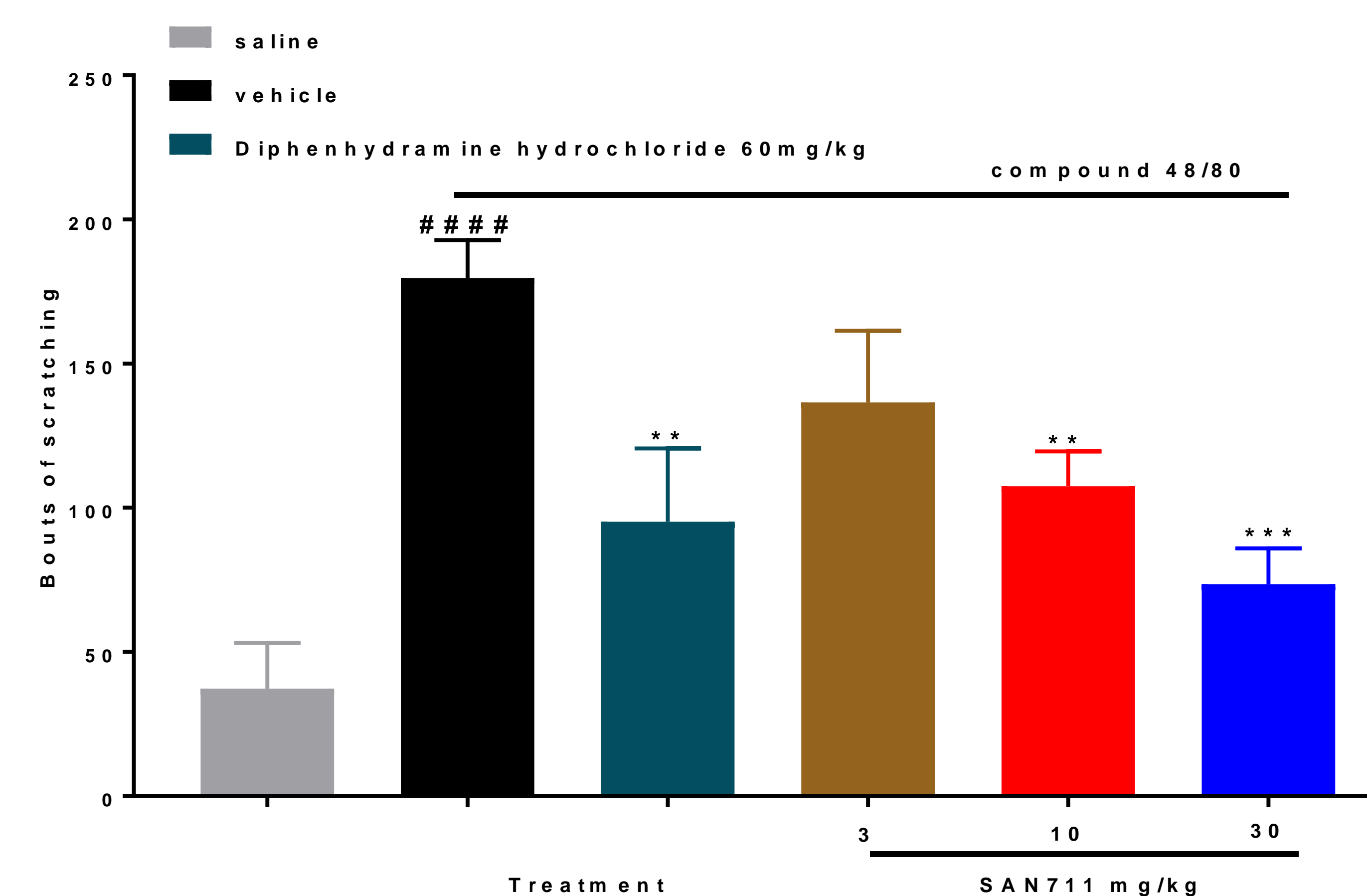
*AUC calculated 0-8h, concentration after 8h was 149 ng/ml. SAN711 was dosed in a clear solution of 30% HP-Beta-CD and 5% DMSO. All calculation were done in WinNonLin. SAN711 show dose linearity (C_{max}), high plasma and good brain exposure.

Mouse brain receptor occupancy and brain free concentration of SAN711 in ³H-Flumazenil binding assay



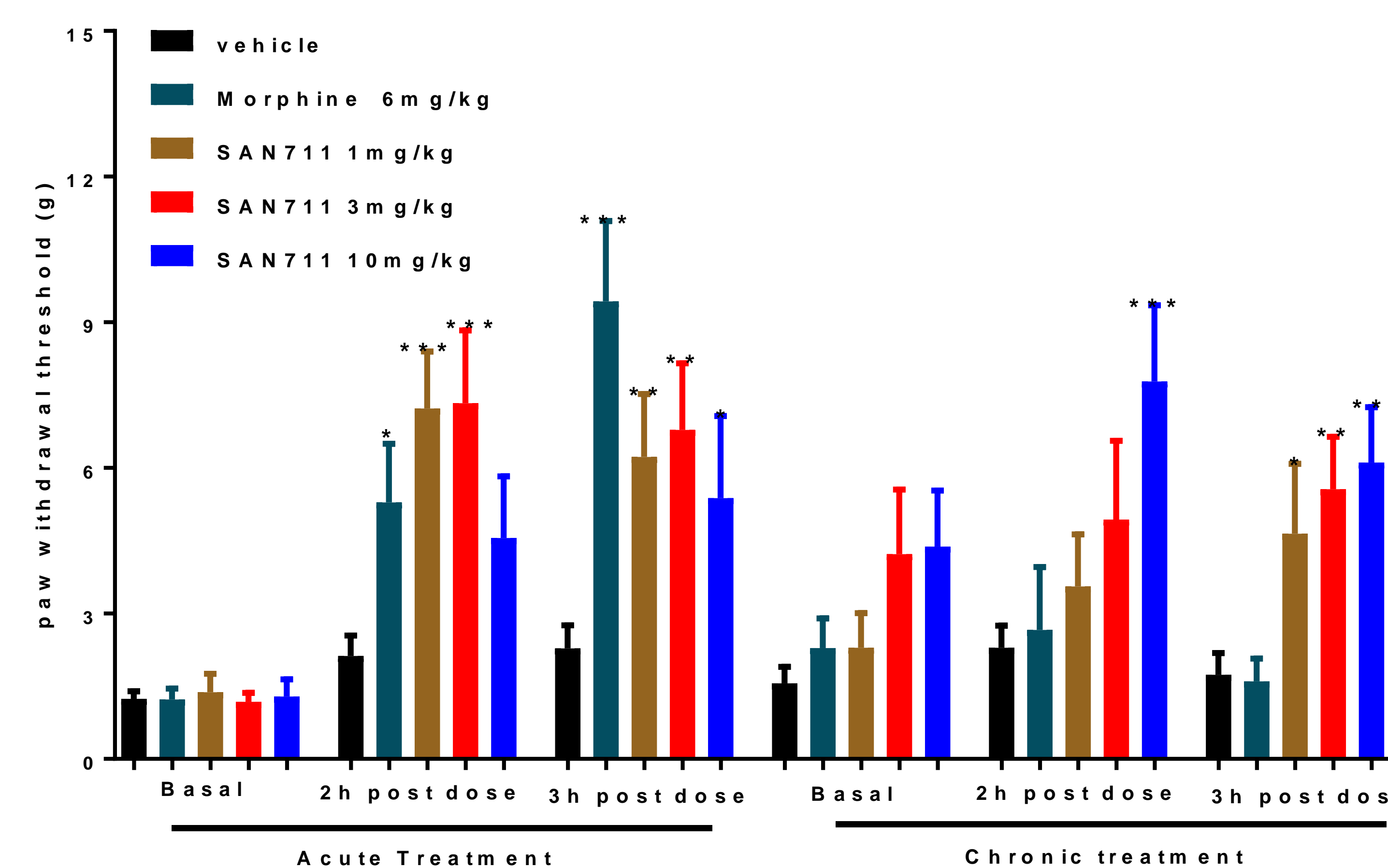
Full receptor occupancy (100%) was reached at a dose of 10 mg/kg (p.o.) of SAN711. The ED₅₀ was reached at 0.72 mg/kg corresponding to a free brain concentration of 16 nM. Receptor occupancy levels of SAN711 was measured by displacement of ³H-Flumazenil in mice brain. Clonazepam (3 mg/kg, i.p.) was used to define non-specific binding.

SAN711 dose dependently reduced compound 48/80 induced scratching in mice



SAN711 dose dependently reduced compound 48/80 induced scratching behavior in CD-1 male mice. Compound 48/80, injected in 50μL subcutaneously at the nape of neck, induced a marked and significant increase in bouts of scratching as compared to vehicle injected mice. The histaminergic H1 antagonist, Diphenhydramine hydrochloride was used as reference and administered orally 60 min before Compound 48/80 while SAN711 was administered orally 30 min prior to compound 48/80 administration. #### p<0.0001 vs. Saline; ***p<0.001, **p<0.01, vs vehicle+compound 48/80, One-way ANOVA Fisher's LSD post hoc test, n=7-9.

SAN711 reverses the mechanical allodynia induced by CCI without development of tolerance after chronic treatment

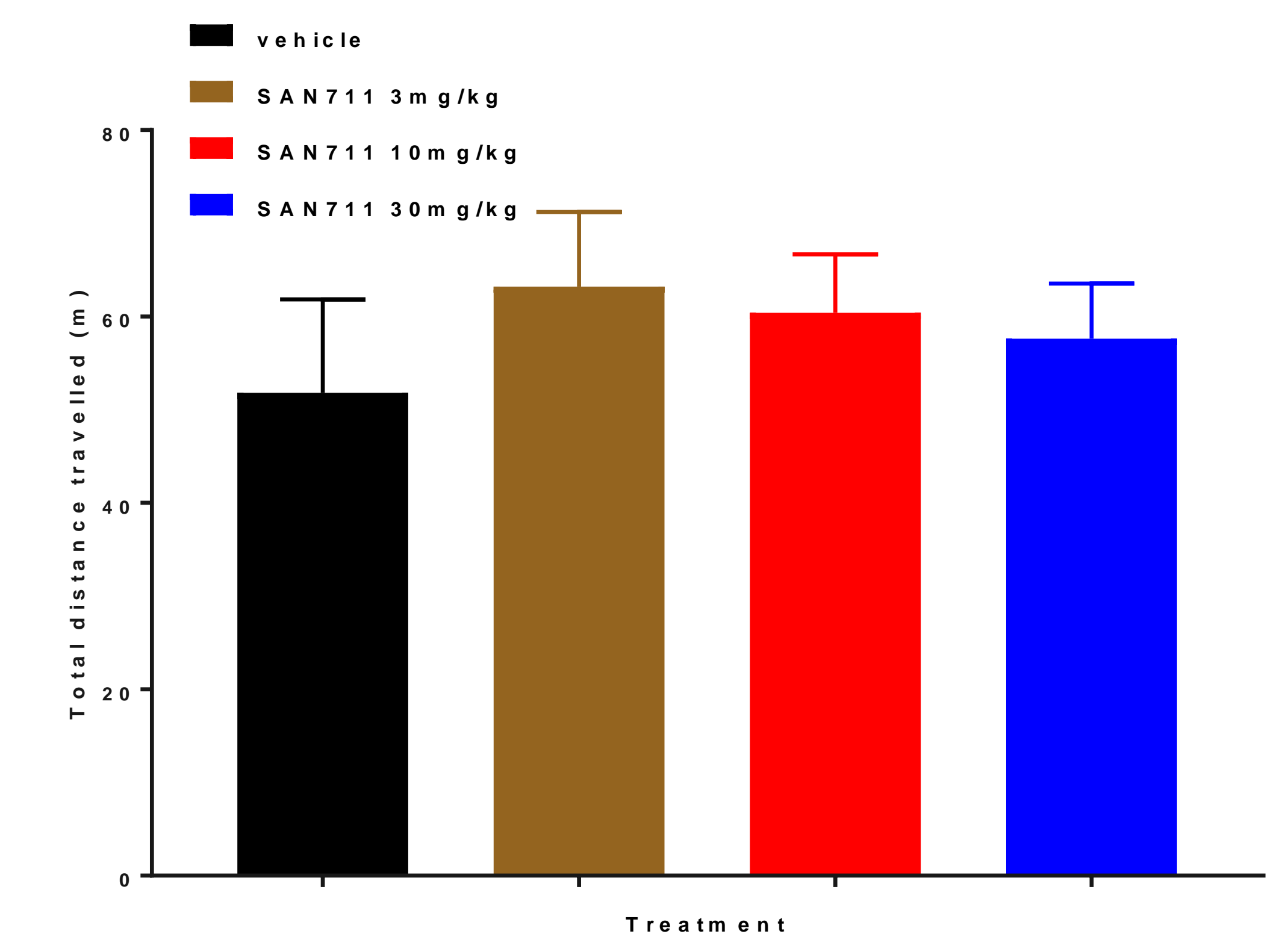


Acute treatment with SAN711 reversed the mechanical allodynia in rats subjected to CCI lesions with a minimal effective dose less than or equal to 1 mg/kg after oral administration. After 7 days of chronic treatment, a significant analgesic effect of all 3 doses were maintained, while the effect of morphine (6 mg/kg) was completely lost. Morphine was administered subcutaneously. Chronic constriction injury (CCI) in male Sprague Dawley's rats was performed as described by Bennette and Xie's, (1998). The animals were tested after 14 days of surgery. ***p <0.001, **p<0.01, *p<0.05 vs vehicle, Two-way ANOVA Fisher's LSD posttest, n=7-9.

SAN711 demonstrates good PK-PD correlation

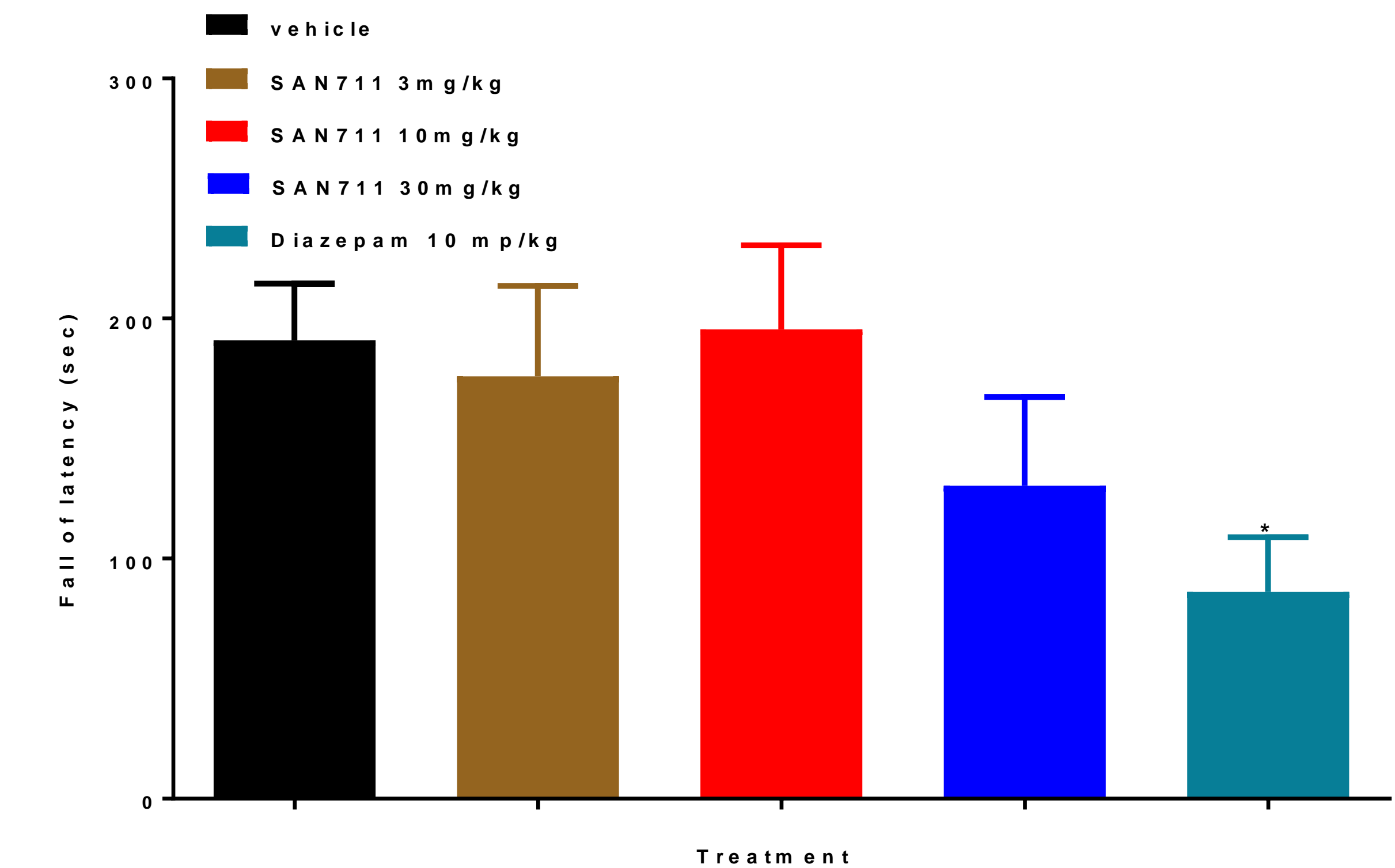
Sr. No	Assay	K _i (nM)	ED50 (mg/kg)	Minimum Effective dose (mg/kg)	Free Brain concentration (nM)
1	α ₃ β ₃ γ ₂ - in vitro binding	15			
2	In vivo binding in mouse brain		0.72		16
3	Chronic constriction injury in rats			1	18
4	Itching model in mice			10	160
5	Rota rod in rats			>30	>494
6	Explorative motility in rats			>30	>494

Lack of sedative effects of SAN711 on exploratory locomotor activity in rats



SAN711 dosed up to 30 mg/kg, corresponding to a free brain concentration of 494 did not affect exploratory locomotor activity in male Sprague Dawley rats (p > 0.05 two way repeated measures ANOVA with time and dose as factors). SAN711 was administered orally at 3,10 and 30 mg/kg, 10 ml/kg, 120 minutes prior to introducing the rats into novel homecages under dim light conditions. The activity of the rats was automatically registered for 30 minutes (TSE MoTil, Germany).

Lack of motor impairment by SAN711 on rotarod performance in rats



SAN711 dosed up to 30 mg/kg did not impair rats ability to maintain balance on an accelerating rotating rod, measured as latency to fall of the rod. In contrast, the non-selective GABA_A receptor positive modulator, Diazepam, significantly shortened the latency to fall off (p<0.05). SAN711 and diazepam were administered orally 2h and 1h before teststart respectively. Rats were trained on the rota rod for two days at 4-40 rpm for 5 min before evaluating the drug effects on the 3rd day. Rats which failed to run for more than 90 sec after training were not included in the experiment. *p<0.05, vs vehicle, One-way ANOVA Fisher's LSD posttest, n=6-7.

Conclusion:

- SAN711 is a novel high affinity GABA_A positive allosteric modulator with functional preference for α₃-containing receptors and devoid of activity at α₁-containing receptors
- SAN711 displays an attractive pharmacokinetic profile in rats and is stable in human hepatocytes
- SAN711 show robust analgesic effect in the rat CCI model for neuropathic pain at free brain concentrations corresponding to 50% receptor occupancy while anti-pruritic effects are reached at higher receptor occupancies
- The analgesic effect of SAN711 is maintained after chronic treatment, indicating lack of tolerance development
- No adverse effects are evident at free brain concentrations more than 30-fold higher than the free brain concentrations required for analgesic effect
- SAN711 is currently undergoing preclinical development

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