Characterisation of GABA2/3 positive allosteric modulators (PAMs) in tests of anxiety and side effect

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INTRODUCTION

Benzodiazepines (BZs), exemplified by diazepam (DZP) have been effective clinical treatments for forms of epilepsy and anxiety for decades, although issues such as sedation, tolerance and dependence restrict their value. BZs exert their effects via non-selective modulation of $GABA_A$Rs containing either an α1, α2, α3 or α5 subunit and the emergence of $GABA_A$R subtype selective PAMs, notably for α2- and α3-containing subtypes (α2/α3-$GABA_A$R PAMs) represents an opportunity to provide therapeutic improvement both in terms of efficacy and tolerability, side-effect profile. The present study was designed to profile the α2/α3-$GABA_A$R PAMs darigabat (CVL-865), and TPA-023B (ENX-102) and the α3-$GABA_A$R-preferring PAM ACP-711 (formerly SAN711) in the conditioned emotional response (CER) test of anxiety, and the 5-choice serial reaction time task (5-CTT) to study attention and reaction time. Since ACP-711 has not been described in detail, we also sought to characterise this novel compound.

METHODS

Animals and Housing

Male Sprague Dawley (CER) or Long Evans (5-CSRTT) rats, (source: Charles River, St. Constant, QC, Canada) served as test subjects. All rats were singly housed and food restricted. In-life studies were approved by an Institutional animal care committee according to guidelines established by the CCAC.

Drugs

Diazepam (source: Sandoz), Darigabat, TPA023B and ACP-711 (source: Draig Therapeutics, Cardiff, UK).

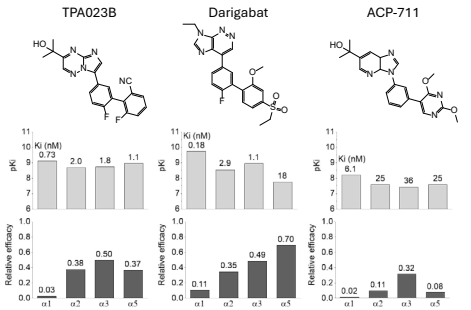
CER procedure

Rats trained to lever press for food reward on a variable interval 40s schedule (VI40) (test chambers: Med Associates Inc. VT, USA). Daily sessions were 40min duration with 2 periods of a 2min conditioning stimulus (CS) consisting of a light/tone. The 2min CS was terminated by a 0.5s unavoidable footshock (0.3mA intensity). On drug test days, and occasional training days, footshock was not delivered. The number of lever presses recorded during the two 2min CS periods (A), and the number of lever presses recorded in the 2min periods immediately prior to the two CS periods (B) were measured and a suppression ratio (SR) was calculated using the following formula: SR = (A) / (A+B). SR of 0 reflects no responding during the CS periods, and SR of 0.5 reflects equivalent responding during both the CS and 2min period prior to CS. An anxiolytic effect is characterised by increased SR (see Higgins et al, 2009 for more detailed methods).

5-choice procedure

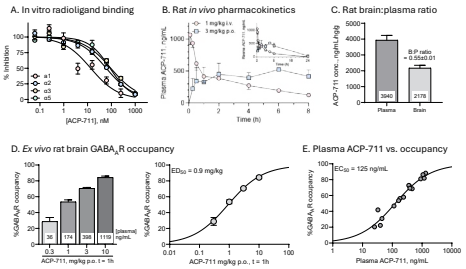
Test subjects trained to asymptotic performance in the 5-CSRTT to a final stimulus duration (SD) of 0.75s, 5s inter-trial interval (ITI), 5s limited hold (LH), 100 trials per session. Target performance levels under these conditions were in the range of >80% accuracy, <20% omissions. Once performance stable, test challenge and drug testing began.

Compounds – Structure and *In vitro* Properties



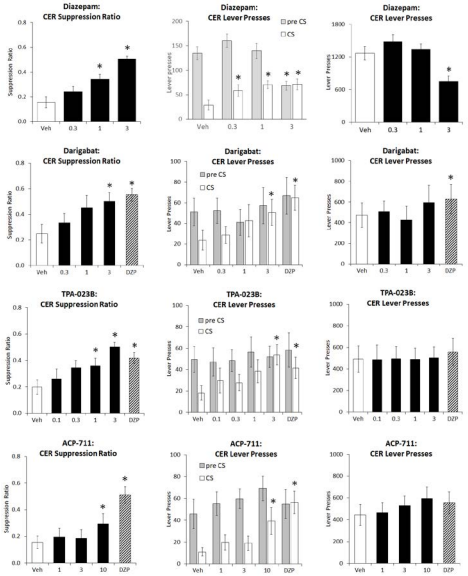
Comparison of the chemical structures and in vitro affinity (Ki values) and relative efficacy (relative to a non-selective benzodiazepine, such as diazepam or chlordiazepoxide, which by definition has a relative efficacy at each subtype of 1.0) at each of the four different GABAAR subtypes for TPA023B, Darigabat and ACP-711. Data for TPA023B and Darigabat are from Atack et al., 2011 and Nickolls et al., 2018, respectively. For ACP-711 the binding data is from figure below while the efficacy data is extrapolated by visual inspection from data presented in Patent WO 2020/053377 A1).

Characterisation of ACP-711 (SAN711)



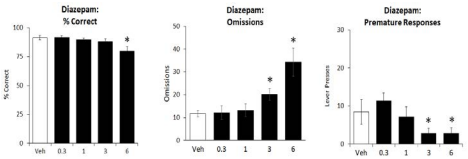
In vitro and *in vivo* characterisation of ACP-711 (formerly SAN711). **A.** Binding affinity of ACP-711 at human recombinant $GABA_A$Rs with either an α1β3γ2, α2β3γ2, α3β3γ2, or α5β3γ2 composition. **B.** Rat plasma pharmacokinetics. **C.** Rat brain:plasma ratio of 10 mg/kg i.p. ACP-711. The brain:plasma ratio averaged across animals was 0.55. **D.** Ex vivo rat brain $GABA_A$R occupancy following 0.3, 1, 3 and 10 mg/kg p.o. ACP-711. The dose giving 50% $GABA_A$R occupancy (ED_{50}) being estimated at 0.7 mg/kg. **E.** By plotting the $GABA_A$R occupancy and corresponding plasma drug concentration for each individual animal, it was estimated that the plasma drug concentration required to give 50% occupancy (the EC_{50}) was 125 ng/mL.

RESULTS – Conditioned Emotional Response



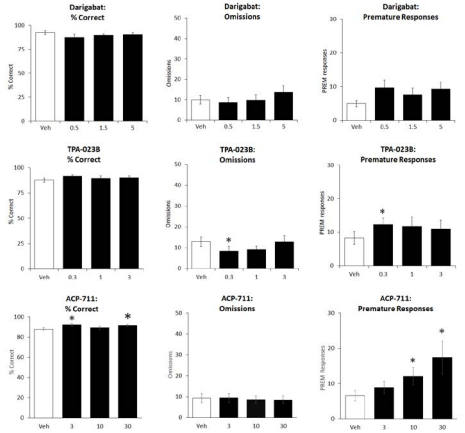
Effect of Diazepam (0.3-3 mg/kg IP; 30min ptt.), DarigaXXbat (0.3-3 mg/kg IP; 60min ptt.), TPA-023B (0.1-3 mg/kg oral; 60min ptt.), ACP-711 (1-10 mg/kg oral; 60min ptt.) on CER. Data presented as mean±SEM for (A) Suppression ratio, (B) number of lever press during 2min pre-CS and 2min CS, (C) total lever press recorded over 40min test session. DZP (2 mg/kg IP) was included as reference control. * P<0.05 vs. Vehicle (Dunnetts test following ANOVA).

RESULTS – 5-Choice Serial Reaction Time Task



Effect of Diazepam (0.3-6 mg/kg IP; 30min ptt.) on 5-choice task performance. Data presented as mean±SEM for (A) Total % correct, (B) omissions, (C) Premature responses. * P<0.05 vs. Vehicle (Dunnetts test following ANOVA).

RESULTS – 5-Choice Serial Reaction Time Task



Effect of Darigabat (0.5-5 mg/kg IP; 60min ptt.), TPA-023B (0.3-3 mg/kg oral; 60min ptt.), ACP-711 (3-30 mg/kg oral; 60min ptt.) on 5-choice task performance. Data presented as mean±SEM for (A)Total % correct, (B) omissions, (C) Premature responses. * P<0.05 vs. Vehicle

CONCLUSIONS

- **TPA023B, Darigabat and ACP-711 each have subtle differences in their GABAAR subtype affinity and intrinsic efficacy.**
- **While each GABA PAM had a significant positive effect on SR, these differences translated into marked differences in their anxiolytic potencies in the CER with TPA023B, Darigabat and ACP-711 having respective minimal effective doses of 0.3, 3 and 10 mg/kg p.o.**
- **In the 5-CSRTT. DZP impaired performance measured as omissions, slower response speed and accuracy at doses marginally higher than those required for anxiolysis. In contrast, TPA023B, darigabat and ACP-711 showed better tolerability with trends to improvement on some measures of attention.**
- **The present data support the principle of GABA2/3 selective PAMs as treatments for anxiety disorders.**

REFERENCES

1. Atack J.R et al (2011) J Psychopharmacol. 25(3):329-44.
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3. Higgins G.A. et al (2009) Eur J Pharmacol. 624(1-3):1-9.