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ORIGINAL INVESTIGATION

Pharmacological profile of a 17β-heteroaryl-substituted neuroactive steroid

Derk J. Hogenkamp · Minhtam B. Tran · Ryan F. Yoshimura · Timothy B. Johnstone · Richard Kanner · Kelvin W. Gee

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Abstract
Rationale In order to improve upon the pharmacological properties of the neuroactive steroid ganaxolone, it was used as the starting point in the design of novel neurosteroids that replace the 17β-acetyl side chain with an isoxazole bioisostere.
Objectives UCI-50027 (3-[3α-hydroxy-3β-methyl-5α-androstan-17β-yl]-5-(hydroxymethyl)isoxazole) was designed as an orally active neuroactive steroid specifically targeted at the gamma-aminobutyric acid(A) receptor ($GABA_AR$).
Methods UCI-50027 was tested in vitro in *Xenopus* oocytes expressing human $GABA_ARs$ and in vivo as an anticonvulsant, for ataxic effects and for anxiolytic activity.
Results In vitro, UCI-50027 dose-dependently enhanced the activity of GABA at human $\alpha_1\beta_2\gamma_{2L}$, $\alpha_2\beta_1\gamma_{2L}$, and $\alpha_4\beta_3\delta$ $GABA_ARs$. Consistent with its action as a positive allosteric modulator (PAM), it had no direct activity in the absence of GABA. UCI-50027 protected against acute pentylenetetrazol (PTZ)-induced convulsions with an ED_{50} of 6 mg/kg p.o. In the rotarod (RR) paradigm in mice, the AD_{50} (the ataxic dose where half of the animals fail the RR test) was found to be 38 mg/kg p.o., giving a therapeutic index (TI = RR AD_{50}/PTZ ED_{50})~6 versus 2.8 for ganaxolone. In the mouse-elevated plus maze (EPM) model for anxiety, UCI-50027 showed a minimum effective dose (MED) ≤0.3 mg/kg p.o. Thus, the TI (TI = RR AD_{50}/EPM MED) for the compound as an anxiolytic is ≥127 versus 3.3 for ganaxolone.
Conclusions UCI-50027 is an orally active neuroactive steroid with pharmacological activity consistent with a $GABA_AR$ PAM that has an improved separation between anticonvulsant/anxiolytic and rotarod effects, potent activity as an anticonvulsant and anxiolytic when compared to ganaxolone.

Keywords Anticonvulsant · Anxiolytic · $GABA_AR$ · Neuroactive steroid · Positive allosteric modulator

Abbreviations

ACN	Acetonitrile
AD_{50}	Ataxogenic half-maximal dose where half of the mice fail the RR assay
ANOVA	Analysis of variance
BZ	Benzodiazepine
CNS	Central nervous system
DMRM	Daughter multiple reaction monitoring
DMSO	Dimethyl sulfoxide
EC_{10}	Concentration that evokes 10 % of the maximum response
EC_{50}	Concentration eliciting half the maximum response
EC_{100}	Concentration that evokes a maximum response
ED_{50}	Effective dose of drug at which half of the animals respond
EPM	Elevated plus maze
$GABA_AR$	Gamma-aminobutyric acid(A) receptor
Ganaxolone	3α-hydroxy-3β-methyl-5α-pregnan-20-one
HPβCD	2-Hydroxypropyl-β-cyclodextrin
HPLC	High performance liquid chromatograph
IACUC	Institutional Animal Care and Use Committee
LC/MS	Liquid chromatography/mass spectrometry

D. J. Hogenkamp · M. B. Tran · R. F. Yoshimura · T. B. Johnstone · K. W. Gee (✉)
Department of Pharmacology, School of Medicine, University of California, Irvine, Irvine, CA 92697, USA
e-mail: kwgee@uci.edu

R. Kanner
Anvyl LLC, 18092 Sky Park South, Suite F, Irvine, CA 92614, USA

LGIC	Ligand-gated ion channel
MED	Minimum effective dose
MTBE	Methyl *tert*-butyl ether
PAM	Positive allosteric modulator
PD	Pharmacodynamic
PK	Pharmacokinetic
PTZ	Pentylenetetrazol
RR	Rotarod
SAR	Structure-activity relationship
SEM	Standard error of the mean
UCI-50027	3-[3α-hydroxy-3β-methyl-5α-androstan-17β-yl]-5-(hydroxymethyl)isoxazole
UCI-50031	(S)-3-[3α-hydroxy-3β-methyl-5α-androstan-17β-yl]-5-(1-hydroxyethyl)isoxazole

Introduction

An accelerating interest in the development of gamma-aminobutyric acid(A) receptor ($GABA_AR$)-active neurosteroids as novel therapeutic agents has occurred in recent years. This has been stimulated, in part, by reports of the clinical efficacy of the synthetic neurosteroid ganaxolone (3α-hydroxy-3β-methyl-5α-pregnan-20-one, Fig. 1) as a treatment for temporal lobe epilepsies (Nohria et al. 2010; Bialer et al. 2013) and infantile spasms (Tsao 2009). Moreover, it is being tested beyond the indication of epilepsy in clinical trials for fragile X syndrome, post-traumatic stress disorder, and smoking cessation (Bialer et al. 2013). The discovery and clinical development of ganaxolone as well as other $GABA_AR$-active neurosteroids were pioneered by our group at CoCensys Inc. in the 1990s (Monaghan et al. 1999). Up to that point, the only other neurosteroid that had been available for clinical use was the anesthetic alphaxolone (Swerdlow et al. 1971). Today, it is no longer used clinically but has some applications in veterinary practice. During the early 1990s, we recruited Robert Purdy to CoCensys as one of the first consultants to help us with development of the pregnane-based neurosteroids as drugs for the treatment of epileptic disorders. He became an integral part of the team that was instrumental in moving several neurosteroids including ganaxolone into clinical testing. After CoCensys was acquired by Purdue Pharma, the rights to develop ganaxolone were acquired from Purdue Pharma by Marinus Inc. who now sponsors its further clinical development. Although we have since moved on to other therapeutic targets, we have always had fond memories of Bob and a special affinity to the neurosteroids. Thus, in honor of Robert Purdy, we describe here the pharmacological profile of a novel 17β-heteroaryl-substituted neuroactive steroid and compare it to ganaxolone, our first effort, in the development of therapeutic neurosteroids.

Ganaxolone has a number of therapeutically desirable attributes that distinguish it from other positive allosteric modulators (PAMs) of $GABA_ARs$ that may render it an effective treatment for complex partial seizures (Reddy and Rogawski 2012). We were interested in whether the efficacy and benign side-effect profile of ganaxolone could be retained or improved upon by creating a novel series of neurosteroids that have structural similarity to ganaxolone with the exception of isoxazole substitution in place of the 17β-acetyl group in ganaxolone. Isoxazoles are known bioisosteres for ketones, and it was hoped an isoxazole would maintain activity at $GABA_ARs$ while improving on the duration of action of ganaxolone. Specifically, we describe the preliminary preclinical pharmacological properties of 3-[3α-hydroxy-3β-methyl-5α-androstan-17β-yl]-5-(hydroxymethyl)isoxazole also known as UCI-50027 (Fig. 1).

Methods

Drugs Ganaxolone, UCI-50027, and (S)-3-[3α-hydroxy-3β-methyl-5α-androstan-17β-yl]-5-(1-hydroxyethyl)isoxazole (UCI-50031) were synthesized in our lab using methods reported in the literature. Ganaxolone was synthesized as

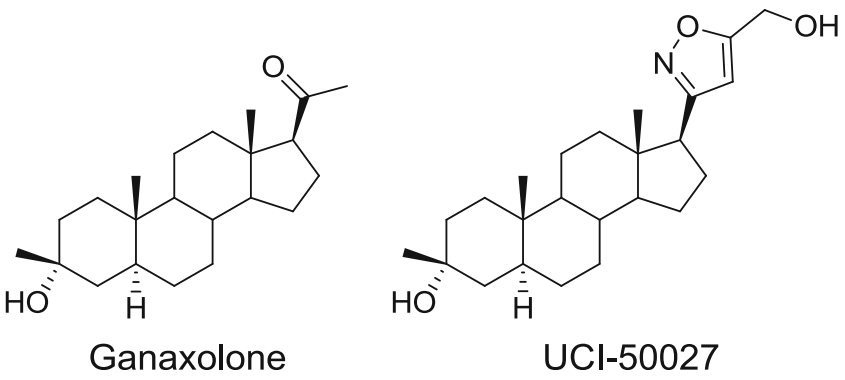

Fig. 1 Structures of ganaxolone and UCI-50027

described previously (Hogenkamp et al. 1997). UCI-50027 and UCI-50031 were synthesized from ganaxolone in five steps (Hogenkamp 2013). Reaction of ganaxolone with Br_2/NaOH/1,4-dioxane gave the expected 17β-carboxylic acid. Reduction to the alcohol with lithium aluminum hydride in refluxing tetrahydrofuran followed by oxidation with pyridinium chlorochromate afforded the 17β-aldehyde. Addition of hydroxylamine hydrochloride in EtOH in the presence of base then gave the corresponding oxime. The oxime was converted to UCI-50027 by reaction with *N*-chlorosuccinimide and addition of excess propargyl alcohol and base. Addition of (S)-(−)-3-butyn-2-ol was conducted similarly, giving UCI-50031. For electrophysiology experiments, neurosteroids were first dissolved in dimethyl sulfoxide (DMSO) to 10 mM and diluted in Ringer's salt solution (0.1 % total DMSO final solution). For p.o. administration, UCI-50027 and ganaxolone were dissolved in 20 and 45 % 2-hydroxypropyl-β-cyclodextrin (HPβCD), respectively. Under these conditions, maximum solubilities were 20 and 52 mM for ganaxolone and UCI-50027, respectively.

Animals Male CD1 mice (24–28 g, Harlan Labs, Los Angeles, CA) or male Sprague–Dawley rats (250–350 g, Harlan Labs) were used in all in vivo studies. Animals were housed under a 12:12 h light:dark cycle starting at 0630 hours and tested according to U.C. Irvine Institutional Animal Care and Use Committee (IACUC) approved protocols. Oocytes were obtained from *Xenopus laevis* frogs using procedures approved and monitored by the IACUC.

2-Electrode voltage clamp oocyte electrophysiology Human $GABA_A$ receptor subunit (α_1, α_2, α_3, β_1, β_2, β_3, γ_2, δ) cDNA clones were provided by CoCensys Inc. (Irvine, CA). Preparation, microinjection, and maintenance of oocytes were as previously described (Ng et al. 2007). Individual oocytes were injected with 0.005–50 ng of each subunit mRNA as follows (ratio of subunits in parentheses): $GABA_A$ receptor subunit combinations $\alpha_{1,2,\text{ or }3}$; $\beta_{1,2,\text{ or }3}$; γ_{2L}; and δ (5:1:1). Stage IV–V oocytes were plucked from ovary membranes and defolliculized with collagenase type IA (Worthington's) for 45 min and rinsed 10 times with Ringer's salt solution. cRNA was injected at 50 nL. Oocytes were tested 3–28 days after injection (n=3–7 per compound) in Ringer's salt solution by linear drug application method using electrodes with 1–2 mohm tip resistance. Changes in membrane current were passed through a pre-amp, then through a T200 patch amplifier (Axon Instruments, Sunnyvale, CA), with a bandpass filter of 2 kHz. pClamp software (Axon Instruments) was used to monitor, record, and analyze data. All compounds were tested with a 30-s pretreatment prior to co-application with EC_{10} (concentration of GABA that evokes 10 % of the maximum response) GABA for the control response. The GABA EC_{10} was determined in each individual oocyte expressing the receptor subtype of interest. For example, the EC_{10} has a range of 3×10^{-7} to 10^{-6} M at $\alpha_1\beta_1\gamma_2$ and 3×10^{-6} to 10^{-5} M at $\alpha_1\beta_2\gamma_2$ isoforms. The EC_{100} was 10^{-3} M. Responses in presence of test compound were calculated as percent modulation above control. Concentration-response curves were fitted by nonlinear regression analysis on Prism 4.0 (GraphPad, San Diego, CA) for percent maximal stimulation, EC_{50} (concentration eliciting half the maximum response) values and their 95 % confidence limits. In cases where 0 % modulation was not defined, the bottom of the concentration-response curve was constrained to zero. However, when several concentrations tested resulted in a well-defined 0 % response, then constraining to zero was unnecessary. Percent stimulation corresponding to brain levels of the compounds tested was extrapolated from these concentration-response curves.

Pharmacokinetic studies, tissue extraction, and sample preparation Blood was removed at various time points after drug administration via cardiac puncture under halothane anesthesia and centrifuged at 1,000×*g* for 6 min to separate the plasma. After euthanization, brains were perfused with saline and removed. Brain and plasma samples were stored at −20 °C until processed for extraction. Of the plasma, 100 μL was pipetted into a 1.7-micro centrifuge tube. Forty microliters of internal standard (UCI50031) and 40 μL of methanol to compensate for methanol used in the standard curve samples were added. The sample was then extracted with 0.9 mL of methyl *tert*-butyl ether (MTBE) by vortexing the solution for 1 min. The sample was then centrifuged for 1 min at 10,000 RPM. Of the MTBE, 0.8 mL was removed from the tube and transferred to a 2-mL vial. The sample was then evaporated using a SpeedVac (Thermo) for 15 min at 37 °C at ~10 Torr. The residue was then reconstituted with 150 μL of 0.1 % ammonium formate:methanol 50:50. Approximately 150 mg of accurately weighed frozen brain tissue was placed into a test tube, and 0.1 % formic acid in water was added so that the tissue concentration was 100 mg/mL. The tissue was homogenized with a tissue homogenizer (Model 985–370 Biosec Products Inc.) for about 20 s. The solution was centrifuged for 1 min at 3,450 RPM and 1 mL of solution removed. Of the internal standard, 20 μL was added of UCI50031 to ~yield a concentration of 120 ng/mg. Of the MeOH, 40 μL was added to compensate for MeOH added in the standard curve preparation. Of the sample, 0.3 μL was then extracted using Strata-X 33 μm polymeric sorbent 30 mg/mL (Phenomenex) with 0.45 μL of 1.0 % formic acid in methanol.

Pharmacokinetic studies, analysis Samples were analyzed using a Zorbax SB-C8 Rapid Resolution 4.6×150 mm, 3.5 μm column (Agilent) with 82:18 MeOH:0.1 % ammonium formate buffer mobile phase at a flow rate of 0.7 mL/min

with an Agilent 1100/1200 HPLC and an Agilent 6410B QQQ Mass Spectrometer. Steroids were quantified using DMRM with 389>342 for UCI-50027 and 403>345 and 403>140 for UCI-50031. The mass spectrometer parameters were as follows: FV 170, CE 45, and CAV 7 with positive ion monitoring. Data collection and analysis were performed using Agilent Mass Hunter Software Version 4.0.

Mouse EPM Mice were group housed and handled daily for 3 days prior to testing in the elevated plus maze (EPM; Coulbourn Instruments). The EPM paradigm was performed as previously described (Yoshimura et al. 2013). Briefly, testing was conducted in a dimly lit (2 lx) room, with two 60-W bulbs pointed at the ceiling near the open arms (4 ft above the maze, 400 lx at the surface of the maze). The maze was cleaned between each run. All compounds were tested at times that correspond to peak brain concentrations of test drug. Automated counting of time spent in the open arms of the maze was achieved by using Med Associates (St. Albans, Vermont) MedPC-IV program. Data were analyzed with GraphPad Prism 4.0 for statistical significance by one-way ANOVA with Dunnett's multiple comparison post hoc test.

Mouse RR The rotarod (RR) assay was conducted as previously described (Gee et al. 2010). Briefly, naïve mice were trained on a RR (Columbus Instruments, Columbus, OH) in four sessions (6–15 RPM) over 2 days to successfully complete the 2-min trial prior to final testing (6 RPM). On day 3, the mice were administered with a compound and tested over a period of 360 min at various intervals. The percentage of animals remaining on the RR throughout each 2-min trial was recorded. The results that coincided with the time of peak effect were analyzed by the method of Litchfield and Wilcoxon (Litchfield and Wilcoxon 1949) to determine the AD_{50} (ataxogenic half-maximal dose where half of the mice fail the RR assay).

Mouse anticonvulsant activity Anticonvulsant activity against pentylenetetrazol (PTZ) measured as previously described (Hawkinson et al. 1998). Briefly, mice pretreated (10 min) with various doses of ganaxolone, UCI-50027, or vehicle were observed for a period of 45–60 min after the injection of PTZ (Sigma-Aldrich, 90 mg/kg s.c.). The dose of PTZ used was previously determined to be the dose producing convulsions in 97 % of animals (CD_{97}). A clonic seizure was defined as forelimb clonus of ≥3 s duration. The number of animals with tonic/clonic convulsions was recorded. The dose that protected half of the animals from seizures was determined from the dose-response for each compound by the method of Litchfield and Wilcoxon and designated the ED_{50} (effective dose of drug at which half of the animals respond).

Results

Positive allosteric modulation of $GABA_A$Rs expressed in *Xenopus* oocytes

The effects of ganaxolone and UCI-50027 on $GABA_A$R-mediated currents were measured in *Xenopus* oocytes expressing different $GABA_A$R subtypes. Consistent with their activities as PAMs, neither compound showed activity in the absence of GABA under the conditions used. Representative current traces showing the concentration-dependent effect of UCI-50027 on EC_{10} GABA-induced currents mediated by $\alpha_1\beta_2\gamma_{2L}$ $GABA_A$ receptors are shown in Fig. 2a. In the presence of an EC_{10} GABA concentration, UCI-50027 potentiates GABA currents in a concentration-dependent manner (Fig. 2b). The in vitro activity of UCI-50027 on $GABA_A$Rs expressed in oocytes is compared to ganaxolone (Carter et al. 1997) in Table 1. Both compounds showed robust modulation of GABA EC_{10} currents in oocytes expressing $\alpha_1\beta_2\gamma_{2L}$ $GABA_A$Rs, although UCI-50027 was sixfold less potent. Both compounds were more potent as modulators of $\alpha_2\beta_1\gamma_{2L}$ $GABA_A$Rs. The EC_{50}'s at the $\alpha_2\beta_1\gamma_{2L}$

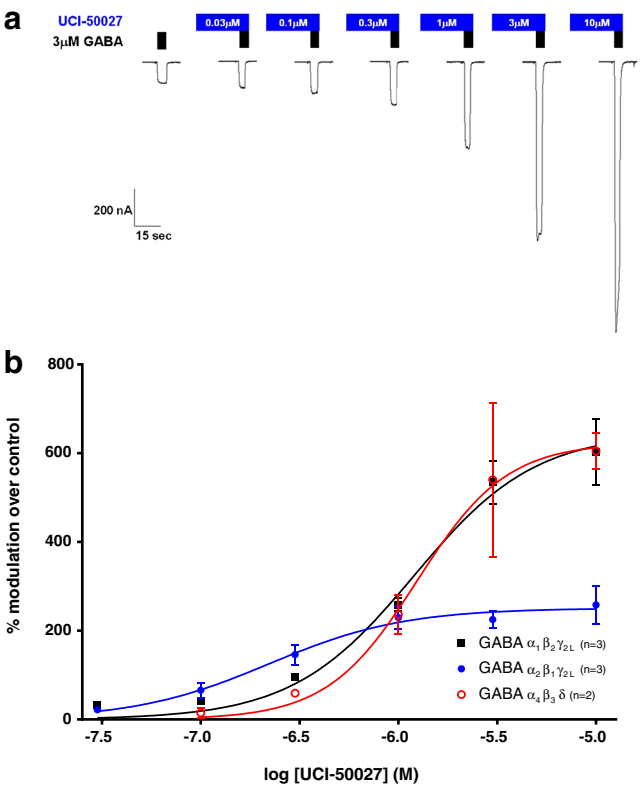

Fig. 2 **a** Representative current traces showing the concentration-dependent effect of UCI-50027 on EC_{10} GABA induced currents mediated by $\alpha_1\beta_2\gamma_{2L}$ $GABA_A$ receptors expressed in *Xenopus* oocytes. **b** Concentration-response curves for UCI-50027 modulation of GABA EC_{10} currents in *Xenopus* oocytes expressing $\alpha_1\beta_2\gamma_{2L}$, $\alpha_2\beta_1\gamma_{2L}$, and $\alpha_4\beta_3\delta$ $GABA_A$Rs

Table 1 In vitro activity of ganaxolone versus UCI-50027 in oocytes expressing $\alpha_1\beta_2\gamma_{2L}$ and $\alpha_2\beta_1\gamma_{2L}$ $GABA_AR$ subtypes

Compound	$\alpha_1\beta_2\gamma_{2L}$ $GABA_AR$ EC_{50} (μM)	$\alpha_1\beta_2\gamma_{2L}$ $GABA_AR$ Max Mod (%)	$\alpha_2\beta_1\gamma_{2L}$ $GABA_AR$ EC_{50} (μM)	$\alpha_2\beta_1\gamma_{2L}$ $GABA_AR$ Max Mod (%)
Ganaxolone	0.2	800	0.1	700
UCI-50027	1.2 (0.8–1.9)	650	0.2 (0.1–0.4)	250

Data for ganaxolone are from Carter et al. 1997. Potentiation of GABA EC_{10} currents was performed as described in Methods section, and the EC_{50} (concentration of steroid giving half the maximum response followed by the 95 % confidence limits in parentheses) and the maximum modulation (Max Mod) were determined

subtype were 0.1 μM for ganaxolone and 0.2 μM for UCI-50027 (Table 2). Unlike ganaxolone, which modulated $\alpha_1\beta_2\gamma_{2L}$ and $\alpha_2\beta_1\gamma_{2L}$ $GABA_ARs$ to a similar extent, UCI-50027 showed only modest maximum modulation (250 %) of $\alpha_2\beta_1\gamma_{2L}$ $GABA_ARs$.

Anxiolytic activity Ganaxolone and UCI-50027 were tested for anxiolytic activity in the elevated plus maze (EPM) in mice. Significant increases in time spent in the open arm of the maze versus vehicle were recorded as anxiolytic activity. The minimum effective dose (MED) for ganaxolone orally in 45 % HPβCD was found to be 20 mg/kg. Because of improved solubility, UCI-50027 could be dosed in 20 % HPβCD. In this vehicle, the MED for orally dosed UCI-50027 in the EPM paradigm was ≤0.3 mg/kg. Dose responses for ganaxolone and UCI-50027 in the EPM paradigm are given in Fig. 3a and b, respectively.

Anticonvulsant activity Ganaxolone and UCI-50027 were tested prophylactically (30 min pretreatment) for anticonvulsant activity against the chemical convulsant PTZ (90 mg/kg s.c.). Ganaxolone's oral ED_{50} (dosed in 45 % HPβCD) was found to be 23 mg/kg. UCI-50027, dosed orally in 20 % HPβCD, exhibited an ED_{50} of 6 mg/kg.

Rotarod assay The steroids were evaluated for CNS depressant effects in the mouse rotarod (RR) assay. Ganaxolone and UCI-50027 were dosed orally in 45 and 20 % HPβCD, respectively. The AD_{50}'s (dose at which half of the animals failed the test) at time of peak effect were 65 mg/kg for ganaxolone and 38 mg/kg for UCI-50027.

The therapeutic index for UCI-50027 and ganaxolone

Comparing the MEDs of UCI-50027 and ganaxolone as anxiolytics in the mouse EPM to their AD_{50}'s in the RR paradigm, a therapeutic index (TI) for each compound can be calculated. For UCI-50027, the TI (RR AD_{50}/EPM MED) is 38/0.3 or ≥127. In the case of ganaxolone, the TI as an anxiolytic is 65/20 or 3.3. The TIs for the compounds as anticonvulsants can be determined similarly, resulting in a TI for UC-50027 (RR AD_{50}/PTZ ED_{50})~6 and ganaxolone~3.

In order to determine if these differences in TI are the result of poor absorption for UCI-50027 at higher doses versus ganaxolone, pharmacokinetic (PK) studies were undertaken with UCI-50027. At a dose of 3 mg/kg orally in mouse (20 % HPβCD vehicle), the maximum levels of UCI-50027 were determined by LC/MS to be 775± 45 ng/mL (~2.0 μM) in plasma and 1,318±660 ng/mL (~3.4 μM) in brain at 30 min. At 20 mg/kg orally in mouse, levels in plasma were 1,410±1,080 and 1,206± 785 ng/mL (3.6 and 3.1 μM) at 30 and 90 min, respectively. The corresponding levels in brain were 2,250±

Table 2 Comparison of the preclinical profile of ganaxolone and UCI-50027

Properties	Ganaxolone	UCI-50027
In vitro activity (EC_{50}) at $\alpha_1\beta_2\gamma_{2L}$ $GABA_ARs$ (μM)	0.2	1.2
In vitro activity (EC_{50}) at $\alpha_2\beta_1\gamma_{2L}$ $GABA_ARs$ (μM)	0.1	0.2
Solubility	20 mM in 45 % HPβCD	52 mM in 20 % HPβCD
Stable formulation	45 % HPβCD	20 % HPβCD
Bioavailability (rat)	F% ND	F%~77 %
In vivo potency (mouse)	EPM MED = 20 mg/kg p.o.	EPM MED ≤0.3 mg/kg p.o.
	PTZ ED_{50} = 23 mg/kg p.o.	PTZ ED_{50} = 6 mg/kg p.o.
Preliminary safety (mouse)	RR AD_{50} = 65 mg/kg p.o.	RR AD_{50} = 38 mg/kg p.o.
	TI as anxiolytic (EPM) = 3.3	TI as anxiolytic (EPM) ≥127
	TI as anticonvulsant = 3	TI as anticonvulsant~6

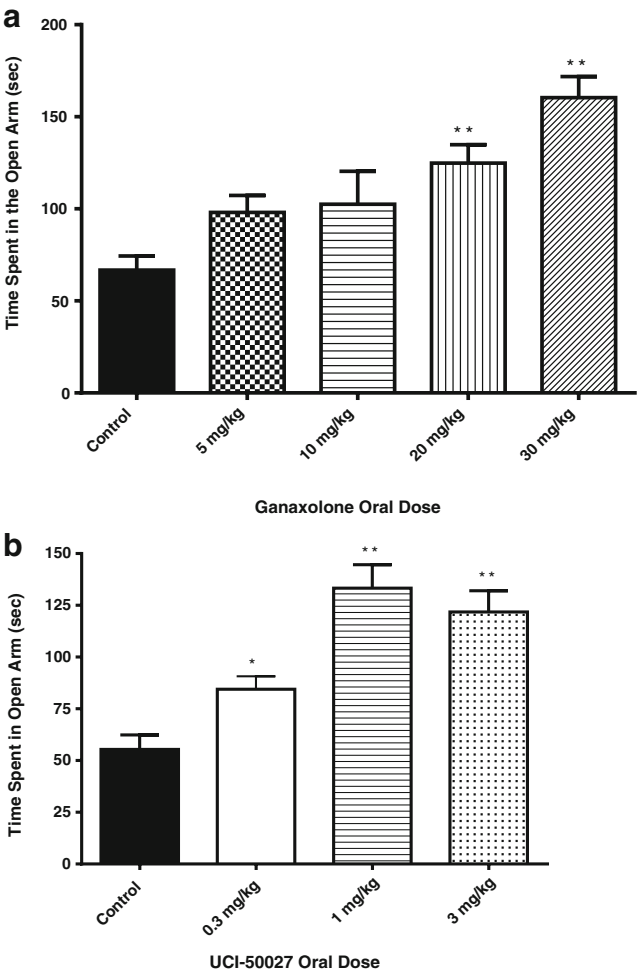

Fig. 3 Effects of ganaxolone (**a**) and UCI-50027 (**b**) on time spent in the open arms in the mouse EPM. Time spent in the open arm compared to vehicle is shown for each compound. Each *bar* represents the mean± S.E.M. time spent in the open arm (n=4–22 animals). Significantly different from vehicle control at *P<0.05 and **P<0.01 after ANOVA and post hoc Dunnett's test

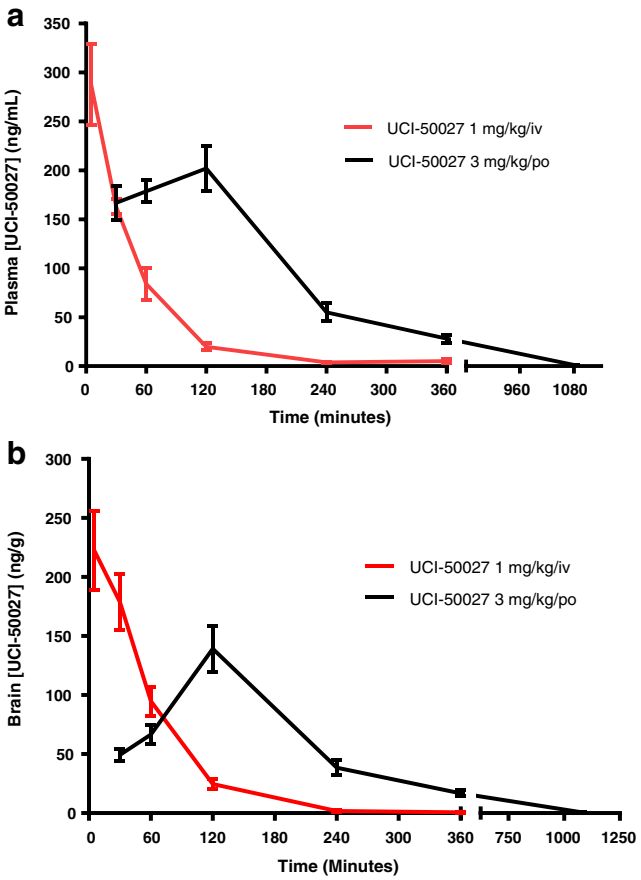

Fig. 4 Time course for plasma (**a**) and brain (**b**) levels of UCI-50027 after 3 mg/kg p.o. and 1 mg/kg i.v. dosing in rats. The oral bioavailability is ~77 %. Each data point represents mean±SEM of levels measured in 4–6 rats

1,483 and 2,160±1,675 ng/mg (5.8 and 5.6 μM), respectively. Comparison of the levels at these two doses shows that absorption is not dose proportional. At the lowest dose (0.3 mg/kg) tested in the EPM, brain levels of UCI-50027 would correspond to ~0.34 μM, assuming that the absorption is linear at these lower doses. At the ED_{50} for UCI-50027 against PTZ-induced seizures (6 mg/kg), the similarly extrapolated levels in the plasma would be ~4.0 μM. The oral biovailability of UCI-50027 in rat was determined by dosing 3 mg/kg p.o. and 1 mg/kg i.v. followed by the determination of plasma and brain levels at various time points (see Fig. 4a, b). Based on these data, the calculated rat oral bioavailability is ~77 %, and the i.v. half-life is ~30 min. Unlike ganaxolone, where rat brain levels are higher than plasma levels, UCI-50027 shows maximum levels that are somewhat lower in brain (~140 ng/g) than in plasma (~200 ng/mL).

Discussion

Like ganaxolone, UCI-50027 is a positive allosteric modulator of $GABA_A$Rs expressed in *Xenopus* oocytes. While less potent in vitro than ganaxolone, UCI-50027 is more potent in vivo both as an anxiolytic and as an anticonvulsant. The MED for UCI-50027 is ≤0.3 mg/kg orally in the mouse EPM paradigm, while ganaxolone has an MED of 20 mg/kg. Similarly, UCI-50027 is more potent as an anticonvulsant than ganaxolone. The ED_{50} for UCI-50027 is 6 mg/kg orally in mouse against PTZ-induced seizures, while ganaxolone has an oral ED_{50} of 23 mg/kg. Further distinguishing UCI-50027 from ganaxolone, the RR AD_{50}'s for the compounds in mice are 38 and 65 mg/kg p.o., respectively. The therapeutic index (TI) for the compounds as anxiolytics (RR AD_{50}/EPM MED) is ≥127 for UCI-50027 and 3.3 for ganaxolone. Most noteworthy is the anxiolytic potency of UCI-50027 which is ~67× greater than that of ganaxolone and accounts for the large TI relative to ganaxolone. This potency is in a similar range to that observed with the clinically used agonist benzodiazepines in the EPM. In contrast, the anticonvulsant potencies of the

two neurosteroids show less than a fourfold difference. Implicit in these observations is the possibility that UCI-50027 may have a greater potential as an anxiolytic than as an anticonvulsant. As anticonvulsants, UCI-50027 has a TI (RR AD_{50}/PTZ ED_{50})~6, while the corresponding TI for ganaxolone is ~3. In both cases, UCI-50027 has a wider safety margin than ganaxolone. PK studies indicated that UCI-50027 shows excellent adsorption orally both in mice and rats. When UCI-50027 was dosed orally at 3 mg/kg in rats, the maximum plasma levels are ~200 ng/mL (0.52 μM), and the maximum brain levels were found to be ~140 ng/g (Fig. 4). At a dose of 10 mg/kg p.o. in rats, ganaxolone is reported to achieve plasma levels of only 37±36 ng/mL (~0.12 μM; Ramu et al. 2001). The improved in vivo potency for UCI-50027 is clearly consistent with its improved oral bioavailability relative to ganaxolone. This offsets the lower in vitro potency of UCI-50027 when compared to that of ganaxolone.

In contrast, it is more difficult to reconcile the improved TIs for UCI-50027 as an anxiolytic and anticonvulsant compared to ganaxolone. At a dose of 3 mg/kg p.o. in mice, maximum brain levels for UCI-50027 were ~3.4 μM, yet no sedative effects in RR performance were noted. Mouse brain levels at 20 mg/kg p.o. were 2,250 and 2,160 ng/mg at 30 and 90 min, respectively, suggesting that poor brain penetration is not a factor in the high TIs observed relative to ganaxolone. $GABA_AR$ PAMs with selectivity for $\beta_{2/3}$- over β_1-subunit-containing receptors have been described as nonsedating anxiolytics (Hogenkamp et al. 2007, Gee et al. 2010). Compounds with limited modulation of β_1-subunit containing $GABA_ARs$ were found to have improved TIs as anxiolytics compared to compounds with greater efficacy as modulators of β_1-subunit containing $GABA_ARs$. UCI-50027 shows β-subunit subtype selectivity with maximum modulation of $\alpha_1\beta_2\gamma_{2L}$-subunit-containing receptors of 760 % and maximum modulation of $\alpha_2\beta_1\gamma_{2L}$-subunit-containing receptors of 250 %. In contrast, ganaxolone has similar maximum modulation at both $\beta_{2/3}$- and β_1-subunit containing $GABA_ARs$ (Carter et al. 1997). Ultimately, the amount of UCI-50027 available at β_1-subunit containing $GABA_ARs$ will need to be determined before the relative contribution of this receptor subtype selectivity to its reduced sedative effects is known. Additionally, a more complete evaluation of the $GABA_AR$ subtype selectivity profile of UCI-50027 may provide clues to its favorable TIs relative to ganaxolone.

Conclusions

UCI-50027 has been identified as a novel, orally active, neuroactive steroid that acts as a PAM of $GABA_ARs$. While less potent than ganaxolone in vitro, UCI-50027 is more potent than ganaxolone, both as an anxiolytic and anticonvulsant. This difference can be explained by the improved PK profile of UCI-50027 compared to ganaxolone. UCI-50027 also has larger TIs as an anxiolytic and as an anticonvulsant. This improved TI may be in part the result of the selectivity of UCI-50027 for $\beta_{2/3}$- compared to β_1-subunit containing $GABA_AR$ subtypes. Additional studies are underway to more fully understand the pharmacological profile of UCI-50027 and to expand the SAR around these novel 17β-heteroaryl-substituted androstanes as PAMs of the $GABA_AR$.

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References

Bialer M, Johannessen SI, Levy RH, Perucca E, Tomson T, White HS (2013) Progress report on new antiepileptic drugs: a summary of the Eleventh Eilat Conference (EILAT XI). Epilepsy Res 103:2–30

Carter RB, Wood PL, Wieland S, Hawkinson JE, Belelli D, Lambert JJ, White HS, Wolf HH, Mirsadeghi S, Tahir SH, Bolger MB, Lan NC, Gee KW (1997) Characterization of the anticonvulsant properties of ganaxolone (CCD 1042; 3α-hydroxy-3β-methyl-5α-pregnan-20 one), a selective, high-affinity, steroid modulator of the γ-aminobutyric acidA receptor. J Pharmacol Exp Ther 280:1284–1295

Gee KW, Tran MB, Hogenkamp DJ, Johnstone TB, Bagnera RE, Yoshimura RF, Huang J-C, Belluzzi JD, Whittemore ER (2010) Limiting activity at β1-subunit-containing $GABA_A$ receptor subtypes reduces ataxia. J Pharmacol Exp Ther 332:1040–1053

Hawkinson JE, Acosta-Burruel M, Yang KC, Hogenkamp DJ, Chen JS, Lan NC, Drewe JA, Whittemore ER, Woodward RM, Carter RB, Upasani RB (1998) Substituted 3β-phenylethynyl derivatives of 3α-hydroxy-5α-pregnan-20-one: remarkably potent neuroactive steroid modulators of γ-aminobutyric $acid_A$ receptors. J Pharmacol Exp Ther 287:198–207

Hogenkamp DJ (2013) Novel 17β-heteraryl-substituted steroids as modulators of $GABA_A$ receptors. International Published Application WO 2013/019711 A2, Feb. 7, 2013

Hogenkamp DJ, Tahir SH, Gee KW, Bolger MB et al (1997) Synthesis and in vitro activity of 3α-substituted-3β-hydroxypregnan-20-ones: allosteric modulators of the $GABA_A$ receptor. J Med Chem 40:61–72

Hogenkamp DJ, Johnstone TBC, Huang J-C, Li W-Y, Tran M, Whittemore ER, Bagnera RE, Gee KW (2007) Enaminone amides as novel orally active modulators of $GABA_A$ receptors. J Med Chem 50:3369–3379

Litchfield JT Jr, Wilcoxon FJ (1949) A simplified method of evaluating dose-effect experiments. J Pharmacol Exp Ther 96:99–113

Monaghan EP, McAuley JW, Data JL (1999) Ganaxolone: a novel positive allosteric modulator of the GABA(A) receptor complex for the treatment of epilepsy. Expert Opin Investig Drugs 8:1663–1671

Ng HJ, Whittemore ER, Tran MB, Hogenkamp DJ, Broide RS, Johnstone TB, Zheng L, Stevens KE, Gee KW (2007) Nootropic α7 nicotinic receptor allosteric modulator derived from $GABA_A$ receptor modulators. Proc Natl Acad Sci U S A 104:8059–8064

Nohria V, Tsai J, Shaw K, Rogawski MA, Pieribone VA, Farfel G (2010) Ganaxolone in progress report on new antiepileptic drugs: a summary of the Tenth Eilat Conference (EILAT X). Epilepsy Res 92: 104–107

Ramu K, Lam GN, Chien B (2001) A high performance liquid chromatography-tandem mass spectrometric method for the

determination of pharmacokinetics of ganaxolone in rat, monkey, dog and human plasma. J Chrom B 751:49–59

Reddy DS, Rogawski MA (2012) Neurosteroids-endogenous regulators of seizure susceptibility and role in the treatment of epilepsy. In: Noebels JL, Avoli M, Rogawski MA, Olsen RW, Delgado-Escueta AV (eds) Jasper's basic mechanisms of the epilepsies, 4th edn. National Center for Biotechnology Information, Bethesda, pp 894–1002

Swerdlow M, Chakraborty SK, Zahangir MA (1971) A trial of CT1341. Br J Anaesth 43:1075–1080

Tsao CY (2009) Current trends in the treatment of infantile spasms. Neuropsychiatr Dis Treat 5:289–299

Yoshimura RF, Tran MB, Hogenkamp DJ, Johnstone TBJ, Gee KW (2013) Limited central side effects of a β-subunit subtype selective $GABA_A$ receptor allosteric modulator. J Psychopharmacol. doi:10.1177/0269881113507643