CTP-354: A novel deuterated subtype-selective GABA(A) modulator for treatment of neuropathic pain, spasticity and anxiety disorders

Julie F. Liu[1], Scott Harbeson[1], Vinita Uttamsingh[1], Arturo J. Morales[2], Sophia Nguyen[1], Gary Bridson[1], Changfu Cheng[1], Ara Aslanian[1], Lijun Wu[1]

[1]Concert Pharmaceuticals, Inc. [2]Currently at Novartis Institutes for Biomedical Research

www.concertpharma.com
Lexington, MA 02421

Abstract

BACKGROUND:

$GABA_A$ receptors are a family of ligand-gated chloride channels that function as inhibitory neurotransmitter receptors in the CNS. The $GABA_A$ receptor is a pentameric protein with subtypes composed of α, β, and γ subunits. Typical benzodiazepines activate the receptor in a non-selective manner, binding to an allosteric site at the interface of a γ subunit and either an α1, α2, α3 or α5 subunit. Mutational studies in mice indicate that the sedative[1], ataxic[2], and dependence[3] effects of benzodiazepines are mediated by α1 subtypes. Agonism at the α2 and α3 subtypes is believed to be associated with anxiolytic[4], analgesic[5a,5b], and spasmolytic[6] activities, whereas α5 subtype activity is believed to have cognitive effects[7].

Concert has prepared CTP-354, a non-benzodiazepine, as a promising subtype-selective $GABA_A$ modulator for neuropathic pain, spasticity and anxiety disorders. CTP-354 is an analog of L-838417, which was evaluated preclinically as part of Merck's research effort towards subtype-selective $GABA_A$ anxiolytics with reduced sedation and ataxia. L-838417 was reported to possess a particularly attractive subtype-selective $GABA_A$ pharmacologic profile[2], with partial agonism at α2 and α3 and antagonism at α1. However, despite its desirable $GABA_A$ subtype selectivity, publications from Merck indicated that L-838417 possessed a poor preclinical pharmacokinetic profile and therefore was not advanced into clinical development[8a-c]. Recently, L-838417 was reported to be efficacious in preclinical models of inflammatory and neuropathic pain[5a], and to exhibit strong muscle relaxant effects[9], further expanding the potential therapeutic utility of the compound beyond anxiolysis.

CTP-354 was designed to overcome the poor PK of L-838417 by incorporating deuterium atoms in place of hydrogen at key positions. Deuterium effects on metabolism are unpredictable, even when deuterium is inserted at a known site of metabolic oxidation. In select cases, however, deuterium substitution can significantly improve a drug's metabolic properties while preserving its pharmacological activity. Concert has designed and synthesized a number of novel deuterated L-838417 analogs with enhanced metabolic stability. We have compared our precision-deuterated analogs to L-838417 with respect to *in vitro* metabolic stability and *in vivo* pharmacokinetics, and CTP-354 has been selected as our lead compound. We have progressed CTP-354 into animal models of sedation/ataxia and neuropathic pain.

METHODS:

CTP-354 and L-838417 were compared in *in vitro* and *in vivo* DMPK assays and pharmacology studies. *In vitro* assessment of metabolic stability was conducted in Sprague-Dawley (SD) rat liver microsomes (RLM, 2 mg/mL) and human liver microsomes (HLM, 2 mg/mL) over 30 min at a compound concentration of 0.25 μM. The PK parameters of CTP-354 and L-838417 were compared in discrete-dose studies in male SD rats (n=8, 1 mg/kg) and in male beagle dogs (n=4, cross-over study, 15 mg/kg) dosed orally. CTP-354 and L-838417 were evaluated at 10 μM in the *in vitro* Ricerca LeadProfilingScreen®, a standard selectivity screen of 68 primary targets including GPCRs, ion channels, CNS transporters, and enzymes. CTP-354 was assessed in a rat rotarod model at oral doses up to and including 100 mg/kg to ascertain sedation/ataxia liability via latency to fall from rod. The Chung model, a sciatic nerve ligation model of neuropathic pain, was performed in the SD rat to compare CTP-354 and L-838417 at oral doses up to 10 mg/kg. Von Frey fibers were used to assess paw withdrawal response for the affected paw and contralateral paw. A follow-up study compared doses of CTP-354 up to 100 mg/kg versus gabapentin at 100 mg/kg as a standard-of-care positive control.

RESULTS:

In vitro metabolic stability assessment in RLM and in HLM showed CTP-354 was highly stabilized versus L-838417. *In vivo* oral PK studies comparing CTP-354 to L-838417 in the rat and dog demonstrated a 3- to 4-fold increase in exposure for CTP-354. In the Ricerca selectivity screen CTP-354 and L-838417 showed binding to the benzodiazepine site of the $GABA_A$ receptor, as expected, with no significant off-target activities. CTP-354 was well tolerated in the rat rotarod model at oral doses up to and including 100 mg/kg. In the rat Chung model of neuropathic pain CTP-354 was efficacious and demonstrated a significantly prolonged pharmacodynamic effect versus L-838417 at the 10 mg/kg dose. In the second Chung model study, with doses up to 100 mg/kg, CTP-354 showed a dose response and demonstrated equivalent efficacy to gabapentin with a superior duration of effect.

CONCLUSIONS:

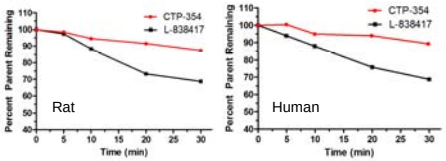
CTP-354 has shown markedly improved metabolic stability relative to L-838417 in both *in vitro* and *in vivo* DMPK assessments. CTP-354 has been evaluated in rodent *in vivo* efficacy models assessing sedation/ataxia and amelioration of neuropathic pain, demonstrating a lack of sedative/ataxic effects at doses which afford excellent pain protection. CTP-354 has demonstrated equivalent efficacy to gabapentin in the Chung model with an enhanced duration of effect. Based on this favorable profile, CTP-354 has been selected as a development candidate and is undergoing IND-enabling non-clinical toxicology and safety pharmacology studies.

For further information

Please contact *jliu@concertpharma.com*

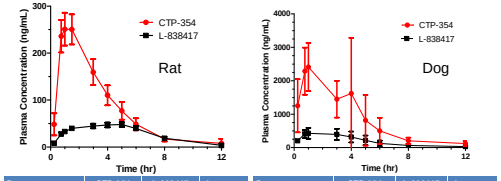
CTP-354 Contains 9 Key Deuterium Atoms

CTP-354 Shows Enhanced Metabolic Stability In Rat And Human Liver Microsomes vs. L-838417



Disappearance of parent compounds (0.25 μM) vs. time in the presence of rat liver microsomes and human liver microsomes (2 mg/mL).

Enhanced *in vitro* Stability Translates To 3- to 4-Fold Increase In Exposure (AUC) Of CTP-354 vs. L-838417 In Rat And Dog



Parameters	CTP-354	L-838417	Increase
$t_{1/2}$ (hr)	1.76 ± 0.78	1.74 ± 0.21	--
C_{max} (ng/mL)	261 ± 34	55 ± 11	4.7x
AUC_{inf} (hr*ng/mL)	1007 ± 114	347 ± 86	2.9x

Parameters	CTP-354	L-838417	Increase
$t_{1/2}$ (hr)	3.53 ± 2.54	2.74 ± 0.63	1.3x
C_{max} (ng/mL)	2587 ± 1004	492 ± 139	5.3x
AUC_{inf} (hr*ng/mL)	10882 ± 4741	2410 ± 932	4.5x

PO discrete dosing of CTP-354 and L-838417 (0.5% methyl cellulose) in rats (n=8) at 1 mg/kg and in dogs (n=4; crossover study) at 15 mg/kg.

CTP-354 Retains Binding Affinity And Specificity To Benzodiazepine Site Of $GABA_A$ Receptor

Receptor	Radiolabeled Ligand	% Inhibition @ 10 μM		Note
		CTP-354	L-838417	
(1) Rat, $GABA_A$	Flunitrazepam (PAM Binding Site)	101%	102%	Benzodiazepine binding site
(2) Rat, $GABA_A$	Muscimol (GABA binding site)	-8%	4%	GABA ligand binding site

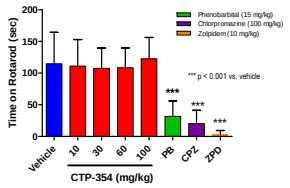
Wistar rat brain minus cerebellum. (1) Ligand: ^{3}H-flunitrazepam (1 nM). Incubation time/temp/buffer: 60 min @ 25°C; 50 mM phosphate, pH 7.4. (2) Ligand: ^{3}H-muscimol (1 nM). Incubation time/temp/buffer: 10 min @ 4°C; 50 mM Tris-HCl, pH 7.4.

CTP-354 Retains *in vitro* $GABA_A$ Pharmacologic Activity of L-838417

Receptor Subtype	Format	% Maximal Potentiation of GABA EC_{20} Current		EC_{50}	
		CTP-354	L-838417	CTP-354	L-838417
α2β2γ2	Transient HEK293	145%	137%	0.94 nM	0.69 nM
α5β2γ2	Stable CHO	135%	140%	0.47 nM	0.40 nM

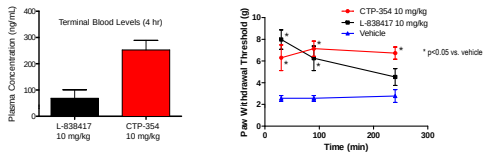
A FLIPR-based membrane potential assay was used to test CTP-354 and L-838417 for positive allosteric modulation activity in two human $GABA_A$ channels. Channels were activated by GABA (EC_{20}, 1 μM), and the ability of compounds to potentiate this signal was assessed. Data are normalized to the mean signal from 1 μM GABA, which is set at 100%. Compounds were tested at 0.1, 0.33, 1, 3.3, 10, 33, 100, and 330 nM, n=4.

CTP-354 Tolerability Demonstrated in Rat Rotarod Model

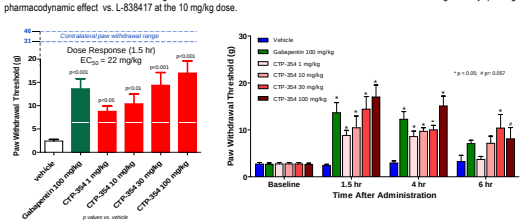


CTP-354 had no sedative/ataxic effects on rat rotarod performance at doses up to 100 mg/kg compared to three positive controls

CTP-354 Shows Sustained Efficacy vs. L-838417 and Gabapentin in Chung Neuropathic Pain Rat Models



CTP-354 was efficacious in the Chung model. CTP-354 demonstrated sustained blood levels and a significantly prolonged pharmacodynamic effect vs. L-838417 at the 10 mg/kg dose.



CTP-354 demonstrated an excellent dose response upon oral dosing up to 100 mg/kg, with an EC_{50} of 22 mg/kg. CTP-354 exhibited equivalent efficacy to the standard-of-care pain drug gabapentin and provided a superior duration of action, with efficacy out to 6 hours.

Conclusions

Deuterium substitution confers metabolic stability:

- CTP-354 exhibited markedly improved metabolic stability relative to L-838417 *in vitro* in liver microsomes from human and from rat
- CTP-354 showed a 3- to 4-fold increase in exposure relative to L-838417 in oral dosing of rat and dog

Deuterium substitution does not change the intrinsic pharmacology:

- In a selectivity screen with 68 targets, both CTP-354 and L-838417 showed the expected binding to the benzodiazepine site of the $GABA_A$ receptor with no significant off-target activities
- CTP-354 retained the *in vitro* $GABA_A$ pharmacological activity of L-838417 in the subtypes tested

CTP-354 was well tolerated and efficacious in *in vivo* animal models:

- CTP-354 demonstrated a lack of sedative/ataxic effects in the rat rotarod model at doses which afforded excellent pain protection in the rat Chung model
- CTP-354 exhibited a PK/PD effect *versus* L-838417 in the rat Chung model, with the improved exposure of CTP-354 leading to an enhanced duration of action
- CTP-354 showed equivalent efficacy to standard-of-care pain drug gabapentin in the rat Chung model with a superior duration of effect

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