PRP Pharmacology Research & Perspectives
Open Access

REVIEW

Preclinical pharmacology and pharmacokinetics of CERC-301, a GluN2B-selective *N*-methyl-D-aspartate receptor antagonist

Rachel Garner[1], Shobha Gopalakrishnan[1], John A. McCauley[2], Rodney A. Bednar[2], Stanley L. Gaul[2], Scott D. Mosser[2], Laszlo Kiss[2], Joseph J. Lynch[2], Shil Patel[2], Christine Fandozzi[2], Armando Lagrutta[2], Richard Briscoe[2], Nigel J. Liverton[2], Blake M. Paterson[1,3], James J. Vornov[1,3] & Reza Mazhari[1,3,]

[1]Cerecor Inc., Baltimore, Maryland
[2]Merck Sharp & Dohme Corp.,West Point, Pennsylvania
[3]Johns Hopkins University School of Medicine, Baltimore, Maryland

Keywords
Depression, GluN2B, major depressive disorder, NMDA antagonist

Correspondence
Reza Mazhari, Cerecor Inc., 400 East Pratt Street, Suite 606, Baltimore, MD 21202. Tel: (410) 522-8707; Fax: (410) 558-6696; E-mail: mazhari.rez@gmail.com

Received: 7 October 2015; Accepted: 19 October 2015

Pharma Res Per, 3(6), 2015, e00198, doi: 10.1002/prp2.198

doi: 10.1002/prp2.198

Abstract

The preclinical pharmacodynamic and pharmacokinetic properties of 4-methylbenzyl (3S, 4R)-3-fluoro-4-[(Pyrimidin-2-ylamino) methyl] piperidine-1-carboxylate (CERC-301), an orally bioavailable selective *N*-methyl-D-aspartate (NMDA) receptor subunit 2B (GluN2B) antagonist, were characterized to develop a translational approach based on receptor occupancy (RO) to guide CERC-301 dose selection in clinical trials of major depressive disorder. CERC-301 demonstrated high-binding affinity (K_i, 8.1 nmol L^{-1}) specific to GluN2B with an IC_{50} of 3.6 nmol L^{-1} and no off-target activity. CERC-301 efficacy was demonstrated in the forced swim test with an efficacy dose (ED_{50}) of 0.3–0.7 mg kg^{-1} (RO, 30–50%); increase in locomotor activity was observed at ED_{50} of 2 mg kg^{-1}, corresponding to an RO of 75%. The predicted 50% RO concentration (Occ_{50}) in humans was 400 nmol L^{-1}, similar to that predicted for rat, dog, and monkey (300, 200, and 400 nmol L^{-1}, respectively). Safety pharmacology and neurotoxicity studies raised no specific safety concerns. A first-in-human study in healthy males demonstrated a dose-proportional pharmacokinetic profile, with T_{max} of ~1 h and $t_{1/2}$ of 12–17 h. Based on the preclinical and pharmacodynamic data, doses of ≥8 mg in humans are hypothesized to have an acceptable safety profile and result in clinically relevant peak plasma exposure.

Abbreviations

AUC, area under the curve; BDI, beck depression inventory; CERC-301, 4-methylbenzyl (3S, 4R)-3-fluoro-4-[(Pyrimidin-2-ylamino) methyl] piperidine-1-carboxylate; CHO, Chinese hamster ovary; CL_p, total plasma clearance; C_{max}, maximum plasma concentration of drug; CNS, central nervous system; CP-101,606, (1S,2S)-1-(4-hydroxyphenyl)-2-(4-hydroxy-4-phenylpiperidino)-1-propanol; D5W, 5% dextrose in water; DMSO, dimethylsulfoxide; ED_{50}, efficacy dose; FLIPR, fluorometric imaging plate reader; GluN2B, NMDA receptor subunit 2B; HAMD-17/HDRS-17, hamilton depression rating scale; HEPA, high-efficiency particulate air; hERG, human ether-a-go-go-related gene; IC_{50}, half maximal inhibitory concentration; IV, intravenous; LC/MS/MS, liquid chromatography with tandem mass spectrometry; MC, methylcellulose; MDD, major depressive disorder; MK-801, (5S,10R)-(+)-5-methyl-10,11-dihydro-5H-dibenzo-[a,d]cyclohepten-5,10-imine maleate; NBF, neutral buffered formalin; NMDA, *N*-methyl-D-aspartate; Occ_{50}, 50% receptor

occupancy concentration; PEG, polyethylene glycol; PEI, polyethyleminine; PK, pharmacokinetic; PO, per os; Ro 25-6981, (*R*-(*R**,*S**)-a-(4-hydroxyphenyl)-b-methyl-4-(phenylmethyl)-1-piperidine propanol; Ro 63-1908, 1-[2-(4-hydroxy-phenoxy)-ethyl]-4-(4-methyl-benzyl)-piperidin-4-ol; RO, receptor occupancy; SLS, sodium lauryl sulfate; T_{max}, time to C_{max}; TRD, treatment-resistant depression; Vd_{ss}, volume of distribution at steady state.

Introduction

Evidence suggests that the glutamatergic system plays a significant role as a primary mediator of psychiatric pathology (Mathews et al. 2012; Sanacora et al. 2012). Glutamate is the primary neurotransmitter in the mammalian central nervous system (CNS), and glutamate receptors, such as the *N*-methyl-D-aspartate (NMDA) receptors, mediate neurotransmission across excitatory (and inhibitory) synapses. The NMDA receptor has been a target for therapeutic intervention for many years due to its implication in the pathophysiology of several disease states, including Parkinson's disease, stroke, neuropathic pain, epilepsy, and Alzheimer's disease. There is a growing body of evidence supporting the hypothesis that NMDA receptors are involved in the pathology of depression (Duman and Li 2012; Musazzi et al. 2013), and the NMDA receptor has recently become a therapeutic target for depression (Steece-Collier et al. 2000; Skolnick et al. 2009; Duty 2012; Ibrahim et al. 2012; Machado-Vieira et al. 2012; Schneider 2013).

N-methyl-D-aspartate receptors are homo- and hetero-oligomeric ligand-gated cation channels comprising varying combinations of three different subunits: GluN1, GluN2, and GluN3 (Dingledine et al. 1999; Karakas and Furukawa 2014). There are four types of GluN2 subunits (A to D), which determine the pharmacological properties of the NMDA receptor, with further contributions from alternative splicing of GluN1 as well as the more recently discovered subunits GluN3A and GluN3B. The subunits exhibit differential expression in the brain. GluN1 and GluN2A are ubiquitous, whereas GluN2B is restricted to the forebrain and spinal cord.

N-methyl-D-aspartate receptors are being increasingly studied as therapeutic targets in major depressive disorder (MDD). Ketamine, a nonselective NMDA receptor antagonist mainly used as a dissociative anesthetic, has been shown to induce rapid (within 24 h) antidepressant effects at subanesthetic doses (often administered intravenously at 0.5 mg kg^{-1} for 40 min) and the responses are sustained for several days after a single infusion (Berman et al. 2000; Zarate et al. 2006; DiazGranados et al. 2010; Valentine et al. 2011; Sanacora et al. 2012; Howland 2013).

N-methyl-D-aspartate receptor antagonists specific for GluN2B (e.g., ifenprodil) are believed to act as allosteric modulators of the receptor, binding at the interface of the GluN1/GluN2B amino-terminal domain (Bhatt et al. 2013; Karakas and Furukawa 2014). Compounds specific for the GluN2B receptor have recently been shown to provide rapid, improvement in patients with severe depression (Preskorn et al. 2008; Ibrahim et al. 2012). The investigational drug, CP-101,606, an NMDA-GluN2B receptor antagonist, was shown to have antidepressant effects in previously treatment-resistant depression (TRD) patients (Preskorn et al. 2008). CERC-301 (Fig. 1) is an orally bioavailable, potent selective antagonist of NMDA-GluN2B (or NR2B) initially developed for Parkinson's disease (Addy et al. 2009). A pilot study of CERC-301 in patients with TRD showed significant antidepressant effects in the 17-item Hamilton Depression Rating Scale (HDRS-17) and Beck Depression Inventory (BDI) (Ibrahim et al. 2012). CERC-301 is currently in clinical development as adjunctive treatment in subjects with MDD with recent active suicidal ideation who have not responded to current antidepressant therapy. The objective of this study is to characterize the preclinical efficacy, pharmacodynamic, and pharmacokinetic (PK) properties of CERC-301 as a novel oral GluN2B-specific antidepressant.

Materials and Methods

Materials

CERC-301 or 4-methylbenzyl (3S, 4R)-3-fluoro-4-[(Pyrimidin-2-ylamino) methyl] piperidine-1-carboxylate (previously known as MK-0657), Compound-1 or *N*-(3-chlorobenzyl)-4-iodobenzimidamide (a nonspecific NMDA receptor binder) (Claiborne et al. 2000), [^{3}H] Compound-2 or [^{3}H]-[(*E*)-*N*1-(2-methoxybenzyl)-cinnamamidine] (a GluN2B ligand) (Kiss et al. 2005), [^{3}H]

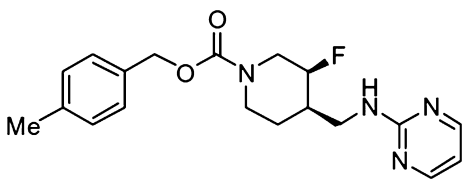

Figure 1. Structure of CERC-301.

Compound-3 or [^{3}H]-*N*-(3,5-dichlorobenzyl)-4-(fluoromethoxy)benzimidamide (a GluN2B ligand) (Hamill et al. 2005), Compound-4 or benzyl 4-((pyridin-4-ylamino) methyl)piperidine-1-carboxylate (a GluN2B antagonist) (Liverton et al. 2007), and [^{14}C]CERC-301 (with ^{14}C incorporated at the C-2 carbon of the pyrimidine ring) were synthesized at Merck Research Laboratories (West Point, PA). All other bulk chemicals were obtained from Sigma Chemical Company (St. Louis, MO).

Cells

Mouse L(tk-) cells expressing the cloned human GluN2B receptor were prepared as previously described (Kiss et al. 2005). Slices of human temporal cortex were obtained from National Disease Research Interchange (Philadelphia, PA) or Analytical Biological Services (Wilmington, DE).

Animals

Sprague–Dawley rats (Crl:CD®(SD)IGS BR) were purchased from Charles River Laboratories (Raleigh, NC and Portage, MI) or Harlan (Indianapolis, IN). Animals were individually housed in stainless steel cages in environmentally controlled high-efficiency particulate air (HEPA)-filtered rooms with a 12-h light/dark cycle and standard diets based on industry standards were supplied. All animal studies were conducted in accordance with the Guide for the Care and Use of Laboratory Animals as adopted and promulgated by the U.S. National Institutes of Health.

In vitro pharmacology assays

Affinity of CERC-301 for NMDA-GluN2B receptors

Radioligand binding assays were performed at room temperature (or 37°C) as described previously (Kiss et al. 2005; Liverton et al. 2007). Analogous binding experiments, as described in Appendix S1 for the cloned human receptor expressed in L(tk-) cells, were also performed using whole brain homogenate (rat), frontal cortex homogenate (dog and rhesus monkey), and temporal cortex homogenate (human). More detailed description could be found in the Appendix S1.

Functional activity and selectivity of CERC-301 for NMDA receptors

The inhibition of calcium influx into L(tk-) cells expressing either GluN1a/GluN2B or GluN1a/GluN2A human receptors was measured to determine the IC_{50} of CERC-301 inhibition of NMDA receptor functions, as previously described in detail (Kiss et al. 2005; see also Appendix S1).

Electrophysiology studies of CERC-301

An in vitro electrophysiological assay with cloned human NMDA-GluN2B receptors was used to determine the binding and dissociation kinetics as well as the potency of CERC-301 (Kiss et al. 2005; see also Appendix S1).

Counterscreening profile of CERC-301

CERC-301 was evaluated in a battery of standard receptor-binding and enzyme assays (MDS Pharma, Taipei, Taiwan). CERC-301 was tested in vitro at 10, 30, or 100 μmol L^{-1} (~3584, 10752, or 35840 ng mL^{-1}, respectively) for its ability to compete with reference ligands to evaluate possible undetected pharmacological activities (>150 receptors and enzymes, including sigma-type opioid receptors, were investigated).

Nonclinical pharmacokinetics

Plasma protein binding

Rat, dog, rhesus monkey, and human plasma samples (3 mL, $N = 3$) were incubated with 2 and 20 μmol L^{-1} [^{14}C]CERC-301 at 37°C for 30 min in a shaking water bath. Following incubation, standard ultracentrifugation methodology was used to determine the percentage of drug unbound (Pacifici and Viani 1992).

Nonclinical safety

Neurotoxicity in rats

Four groups of 24 Sprague–Dawley rats (12/sex) were given single doses of vehicle (0.5% methylcellulose [MC] and 0.02% sodium lauryl sulfate [SLS] in deionized water) or CERC-301 at 10, 30 or 100 mg kg^{-1} by oral gavage at a dose volume of 10 mL kg^{-1}. An additional group of 12 male rats was given single doses of MK-801 (a noncompetitive antagonist of the NMDA receptor; positive control) at 10 mg kg^{-1} by subcutaneous injection at a dose volume of 2 mL kg^{-1}. Six rats per sex in each group were terminated and necropsied at 4 to 6 h postdose, and the remaining rats in each group were terminated and necropsied 3 days postdose (on Day 4). In-life observations and measurements included body weight and clinical observations. At termination, rats were anesthetized and perfusion fixed. At necropsy, the brain was collected for histopathological evaluation.

Animals in CERC-301 and MK-801 assessment groups were terminated at the scheduled necropsy intervals (4–6 h postdose or Day 4). All animals were anesthetized with an isoflurane/oxygen mixture and perfused via the left cardiac ventricle with heparinized 0.001% sodium nitrite in saline. The saline wash was followed by perfusion of 10% neutral buffered formalin (NBF). Brains were harvested, weighed, and stored in 10% NBF.

The brain was sectioned into 2 mm coronal sections to produce multiple sections in three blocks for each animal. The following brain regions were stained: neocortex, paleocortex, basal nuclei, limbic system, thalamus/hypothalamus, midbrain regions, cerebellum, pons region, and medulla oblongata. All brain sections from all animals sacrificed 4 to 6 h after dosing and all animals sacrificed 3 days after dosing were embedded in paraffin, sectioned at 5 μm, stained with hematoxylin and eosin and examined microscopically. For rats sacrificed on Day 4 (3 days after dosing), serial sections from Blocks 1 and 2 were stained with Fluoro-Jade B (a stain increasing the sensitivity of evaluating the brain for neuronal degeneration) and glial fibrillary acidic protein (a stain for astrocyte reactions) and examined microscopically. Three additional groups of rats (four males and three females per group) were orally dosed in the same manner with CERC-301, and 24-h serial blood samples were obtained and analyzed for CERC-301 plasma concentrations and evaluated for systemic exposure.

In vivo pharmacology

Correlation of GluN2B receptor occupancy with plasma drug levels

CERC-301 was administered to rats, dogs, and rhesus monkeys, as described in detail in Appendix S1 and previously elsewhere (Liverton et al. 2007). The RO was also determined in rats following IV administration of [^{3}H] Compound-3. The relationship between plasma concentrations and brain RO was evaluated 15 min after IV and 60 min after oral (PO) dosing of CERC-301, as described in Appendix S1.

Acute depression model

Forced swim test. Young, adult, male Sprague–Dawley rats were randomly assigned across the treatment groups and were administered vehicle (0.5% MC/0.02% SLS), the reference compound desipramine (20 mg kg^{-1}; a tricyclic antidepressant; Sigma, Lot# 078K1326) dissolved in sterile water, or CERC-301 (0.1, 0.3, 1, 3, 10, and 30 mg kg^{-1}) suspended in 0.5% MC/0.02% SLS, twice on Day 1 (after habituation; ~24 h prior to test, and prior to dark cycle) and once on Day 2 (30-min pretest for desipramine and 45-min pretest for CERC-301 and vehicle).

Each Forced Swim chamber was constructed of clear acrylic (height, 40 cm; diameter, 20.3 cm). Rats were subjected to a predose swim test of one 15-min session in cylinders containing water at 23°C $\pm$ 1°C, followed approximately 24 h later by the experimental 5-min session. The water level was 16 cm deep during habituation and 30 cm deep during the test. Immobility, climbing, and swimming behaviors were recorded every 5 sec for a total of 60 counts per subject. When an animal was unable to maintain a posture with its nose above water, it was immediately removed from the water and eliminated from the study. Blood was collected at the completion of swim test procedures and plasma was analyzed for CERC-301 concentrations.

Locomotor assay. To confirm that the effect of CERC-301 in the forced swim test was not due to a general increase in activity levels, rats were subjected to a locomotor assay following oral CERC-301 administration. Adult male Sprague–Dawley rats (N = 42) were randomly assigned across the treatment groups (vehicle or CERC-301 at 0.1, 0.3, 1, 3, 10, and 30 mg kg^{-1}; N = 6/group). Locomotor activity was assessed during the light cycle in photocell-monitored cages (Hamilton Kinder, San Diego, CA). Each cage consisted of a standard plastic rat cage (24 $\times$ 45.5 cm) surrounded by a stainless steel frame. Infrared photocell beams were located across the long axis of the frame to measure the ambulatory distance traveled. A second set of beams was placed above the floor and was used to measure rearing activity. Photocell beam interruptions were recorded by a computer system. Filter tops were placed on top of the test enclosures during testing. Rats were administered either vehicle or test compound via oral gavage twice on Day 1 (approximately 24 h before the test and prior to dark cycle) and once on Day 2 (45 min prior to placing in the locomotor cages for a 60-min test). Locomotor activity was captured in 5-min bins.

Hemodynamic effects in telemetered rats

To determine the systemic hemodynamic effects, CERC-301 was administered as a single oral gavage dose to six (n = 6) chronically telemetered rats (implantation at least 7 days prior to the dosing day) at doses of 0.3, 0.6, 1, 3, and 10 mg kg^{-1} and systemic blood pressure and heart rate values were recorded. The hemodynamic effects were compared to MK-801 at a dose of 0.2 mg kg^{-1} given intravenously (in 0.9% saline). In each animal, a single oral gavage dose of CERC-301 or vehicle (0.5% MC/

0.02% SLS) was administered (volume: 5 mL kg^{-1}). A 24-h recording was performed prior to dosing (vehicle alone) and after each oral dose. In another set of studies, CERC-301 (1 mg kg^{-1}) was administered in combination with atenolol (β1-adrenergic blocker, 1 mg kg^{-1}, IV bolus) and prazosin (α1-adrenergic receptor antagonist, 200 μg kg^{-1}, IV bolus) to elucidate the underlying mechanism of hypertension. Data were analyzed and compared to baseline, with correction for 24-h predose vehicle control.

First-in-human pharmacokinetic study

The study protocol was approved by the Institutional Review Board and written informed consent was obtained from all subjects. Twenty-four healthy, young male subjects were assigned to 1 of 3 sequential treatment panels (A, B, and C). For each panel of eight subjects, two subjects received placebo and six subjects were administered single ascending oral doses of CERC-301 with a minimum 7-day washout between each dose: Panel A (0.1, 0.2, 0.5, 1, and 2 mg); Panel B (2, 4, 8, and 15 mg, and 4 mg with food); and Panel C (15 and 20 mg). Blood samples were collected pre-dose and 0.5, 1, 1.5, 2, 3, 4, 6, 9, 12, 18, 24, 30, 48, and 72 h postdose. Plasma samples were analyzed for CERC-301 concentrations using reversed phase high-performance liquid chromatography with tandem mass spectrometric detection (Merck Research Laboratories). The analytical range was 0.5 to 500 nmol L^{-1} (0.180 to 180 ng mL^{-1}).

Data analysis

Receptor binding

The apparent inhibition constant (K_i), the maximum percentage inhibition (%I_{max}), the minimum percentage inhibition (%I_{min}), and the hill slope (nH) were determined by nonlinear least squares fitting of the bound radioactivity (CPM bound) to the equation below.

$$\text{CPM Bound} = \frac{(\text{SB})(\%I_{max} - \%I_{min}) \div 100}{\left(1 + ([\text{Drug}] \div (K_i(1 + [\text{Merck} - 2] \div K_D)))\right)^{nH}} + \text{NSB} + (\text{SB})(100 - \%I_{max}) \div 100,$$

where K_D is the apparent dissociation constant for the radioligand for the receptor as determined by a hot saturation experiment, and SB is the specifically bound radioactivity determined from the difference of total binding (TB) and nonspecific binding (NSB) control wells (Mosser et al. 2003). Saturation experiments to determine the K_D of the radio ligand were analyzed using the KELL software from Biosoft (Cambridge, United Kingdom).

The IC_{50} values in the FLIPR-based functional assay were determined by nonlinear least squares fitting of the endpoint fluorescence values to the equation below.

$$\text{EndpointFluorescence} = \frac{Y_{max} - Y_{min}}{1 + \left(\frac{[\text{Drug}]}{IC_{50}}\right)^{nH}} + Y_{min},$$

where Y_{min} is the average endpoint fluorescence of the control wells containing 1 μmol L^{-1} [^{3}H]Compound-2 and Y_{max} is the average endpoint fluorescence of wells containing 0.1% DMSO in assay buffer.

Electrophysiology

Antagonist on-rates, which were used to calculate k_{on}, were estimated from steady state current recordings obtained with different antagonist concentrations. Off-rates, which were used to calculate k_{off}, were obtained from recordings in which the current was allowed to recover to the control amplitude following a short (5 min, long enough to reach a steady state inhibition) exposure to a high concentration of an antagonist. k_{on} and k_{off} were used to calculate K_D ($\frac{k_{on}}{k_{off}} = \frac{1}{K_D}$).

Pharmacokinetic parameters and predictive modeling

Pharmacokinetic parameters (AUC, C_{max}, T_{max}, CL_p, Vdss, $t_{1/2}$) were obtained from the CERC-301 plasma concentration-time profiles using noncompartmental methods (WinNonlin®: Princeton, NJ, USA).

Receptor occupancy calculations and predictive modeling

Predicted receptor occupancy from nonclinical studies. Occ_{50} in the plasma was predicted utilizing the apparent inhibition constant (K_i) obtained from RO studies and fraction of CERC-301 unbound (f_u) obtained from plasma protein binding studies:

$$\text{Predicated } Occ_{50} = \frac{K_i \text{ at } 37^\circ\text{C}}{f_u \text{ at } 37^\circ\text{C}}$$

Percent RO versus time profiles were modeled using human plasma concentration-time data and predicted human Occ_{50} according to the equation below.

$$\%\text{RO} = \frac{C_t}{C_t + \frac{K_i}{f_u}} \times 100$$

Results

In vitro pharmacology

Affinity of CERC-301 for NMDA-GluN2B receptors

CERC-301 potently inhibited radioligand ([^{3}H]Compound-2) binding to human NMDA- GluN1a/GluN2B receptors expressed in L(tk-) cells as well as brain tissue homogenates from all tested species (rat, dog, rhesus monkey, human). The binding affinity of CERC-301 determined using human temporal cortex homogenate yielded K_i values of 3.1 nmol L^{-1} (0.0031 μmol L^{-1}) and 8.1 nmol L^{-1} (0.0081 μmol L^{-1}) at room temperature and 37°C, respectively. Findings in other species were consistent with the human data (Table 1).

Functional activity and selectivity of CERC-301 for NMDA receptors

CERC-301 inhibited calcium influx into agonist-stimulated NMDA-GluN1a/GluN2B L(tk-) cells with an IC_{50} of 3.6 nmol L^{-1} but had no effect on calcium influx into agonist-stimulated GluN1a/GluN2A cells at concentrations up to 30,000 nmol L^{-1} (30 μmol L^{-1}).

Electrophysiology studies of CERC-301 effect on NMDA-GluN2B receptors

The potency of CERC-301 was measured in the in vitro GluN2B Antagonist Voltage-Clamp Assay. The observed on-rates (Fig. 2A) and off-rates (Fig. 2B) of CERC-301 were measured in the presence of 10 μmol L^{-1} glutamate and 10 μmol L^{-1} glycine. These experiments demonstrated k_{on} and k_{off} rates of 1.3 $\times$ 10^5 mol L^{-1} s^{-1} and ~2 $\times$ 10^{-5} s^{-1}, respectively, and K_D of approximately 0.15 nmol L^{-1}.

Counter Screening of CERC-301

CERC-301 exhibited no remarkable activity when tested in enzyme and radioligand binding assays at concentrations equal to and greater than 10 μmol L^{-1} (~3584 ng mL^{-1}). CERC-301 exhibited at least 1000 $\times$ selectivity for the GluN2B receptor versus all targets tested, including the hERG potassium channel. CERC-301 also exhibited minimal activity against sigma-type receptors at 10 μmol L^{-1} (sigma-1, sigma-2, and nonspecific).

Table 1. Binding affinities, K_i (nmol L^{-1}), of CERC-301 to expressed human GluN1a/GluN2B receptors in L(tk-) membranes and to brain tissue homogenates.

Species	4°C	Room temp.	37°C
Human GluN1a/GluN2B in L(tk-)	1.8	4.9	31
Human Temporal Cortex	–	3.1	8.1
Rhesus Frontal Cortex	–	3.1	14
Dog Frontal Cortex	–	3.2	13
Rat Brain Homogenate	–	3.3	14

Values are geometric means.

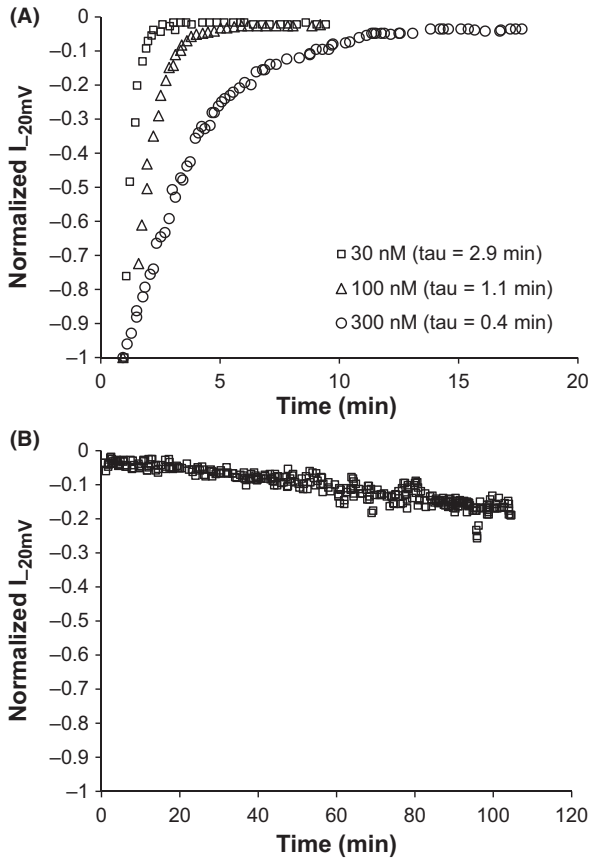

Figure 2. (A) Time course of *N*-methyl-D-aspartate (NMDA) receptor inhibition by CERC-301 at three concentrations of 30, 100, and 300 nmol L^{-1}. Drug was applied at time = 0 min. Mean current amplitudes (measured as the peak current during the −20 mV step) measured before and during the exposure to drug (concentrations indicated on plot) are plotted versus time. Current amplitudes were normalized to the peak current evoked in the absence of drug. (B) Dissociation experiments: time course of recovery from CERC-301 inhibition. Currents were normalized to control current amplitude in the presence of 10 μmol L^{-1} glutamate and 10 μmol L^{-1} glycine.

Nonclinical pharmacokinetics

Plasma protein binding

CERC–301 exhibited a high degree of concentration-independent plasma protein binding at 37°C in rats (89.6%), dogs (97.2%), monkeys (96.9%), and humans (97.7%).

Nonclinical safety

Neurotoxicity in rats

In rats, single doses of CERC-301 (10, 30, and 100 mg kg^{-1}) and vehicle control did not produce vacuolation or necrosis in all examined regions of the brain. At these doses, mean C_{max} was approximately 4, 14, and 26 μmol L^{-1} (1433, 5018, and 9319 ng mL^{-1}), respectively. By contrast, all of the MK-801 (10 mg kg^{-1})-dosed animals had vacuolation and necrosis in cingulate gyrus neurons, consistent with previous reports (Fix et al. 1995). At the 4–6 h time point, the animals treated with MK–801 (six males; Group 5) all had numerous vacuolated neurons in cortical layers 3 and 4 in the cingulate gyrus region of the cerebral cortex. Affected neurons were characterized by numerous, tightly packed, somewhat distinct, vacuoles filling the cytoplasm. On Day 4, all the animals treated with MK-801 (six males; Group 5) had necrotic neurons (visualized using Fluoro-Jade B stain) in cortical layers 3 and 4 in the cingulate gyrus region of the cerebral cortex, consistent with previous reports (Fix et al. 1995). In the MK-801-treated animals, sections stained (immunohistochemically) for glial fibrillary acidic protein showed a very slight increase in staining in the region of the cingulate gyrus.

In vivo pharmacology

Correlation of GluN2B receptor occupancy with plasma drug levels

In vivo RO studies in rat demonstrated that Occ_{50} correlated with total plasma drug levels of 500 nmol L^{-1} (0.5 μmol L^{-1}). Ex vivo RO studies in rat, dog, and monkey demonstrated that Occ_{50} correlated with total plasma drug levels of 300, 200, and 400 nmol L^{-1} (0.3, 0.2 and 0.4 μmol L^{-1}), respectively.

The predicted Occ_{50} in humans, which was similar to that predicted for rat, dog, and monkey, was 400 nmol L^{-1} (0.4 μmol L^{-1}, 143 ng mL^{-1}) based on the intrinsic affinity of CERC-301 at 37°C for GluN2B receptors in human temporal cortex and relative free fraction at 37°C of CERC-301 in human plasma (2.3%; this value was used for PK predictive modeling) (see Table 2).

Acute depression model

Forced swim test. CERC-301 (1, 3, 10, and 30 mg kg^{-1}) significantly decreased immobility frequency ($P < 0.001$) and significantly increased swimming behavior ($P < 0.01$ for 1, 3, and 30 mg kg^{-1}; $P < 0.05$ for 10 mg kg^{-1}) compared to the vehicle control (Fig. 3), but did not affect climbing behavior except at the dose of 3 mg kg^{-1} ($P < 0.05$). Desipramine (20 mg kg^{-1}) significantly decreased immobility ($P < 0.001$) and significantly increased climbing behavior ($P < 0.01$) compared to the vehicle control, with no change in swimming behavior. CERC-301 plasma levels were approximately 15, 120, 390, 1420, 4700, and 14,110 nmol L^{-1} (0.015, 0.120, 0.390, 1.42, 4.7, and 14.11 μmol L^{-1}) at the time of sampling, corresponding to approximately 5, 29, 56, 83, 94, and 98% RO, respectively, in rats. The ED_{50} for increase in frequency of swimming and decrease in immobility were ~0.3 and 0.7 mg kg^{-1}, respectively, corresponding to RO of ~30 and 50%.

Table 2. Comparison of predicted receptor occupancies with the experimental observed values.

Species	GluN2B-binding K_i (nmol L^{-1} @ 37°C)	Plasma free fraction at 37°C (%)	Occ_{50} predicted (plasma, μmol L^{-1})	Occ_{50} observed (plasma, μmol L^{-1})
Rat	14	10	0.1	0.3
Dog	13	2.8	0.5	0.2
Rhesus	14	3.1	0.5	0.4
Human	8[1]	2.3	0.4	–

[1]Binding to human temporal cortex.

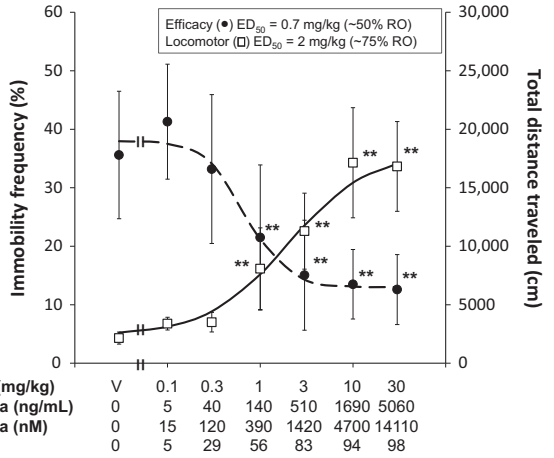

Figure 3. In-vivo efficacy and potential central nervous system (CNS) side effects of CERC-301 when orally administered in rats. Efficacy is depicted by a reduction in immobility frequency (filled circles; left axis) during the forced swim test. Potential CNS side effect is depicted by an increase in total distance traveled (open squares; right axis) as a function of dose. A dose-response curve (solid and dashed lines) was fitted to each mean dataset to calculate efficacious dose (ED_{50}), corresponding CERC–301 plasma concentration (in ng mL^{-1} and nmol L^{-1}), and the estimated receptor occupancy (RO). Data are presented as mean ± SD; $^{**}P < 0.01$. V, vehicle.

Locomotor assay. CERC-301 (1, 3, 10, and 30 mg kg^{-1}) significantly increased distance traveled ($P < 0.01$ for 1 and 3 mg kg^{-1}; $P < 0.001$ for 10 and 30 mg kg^{-1}) compared to vehicle control during the first 5 min of testing (timing correlates with time of forced swim test). CERC-301 (1, 3, 10, and 30 mg kg^{-1}) significantly increased total distance traveled ($P < 0.01$ for 1 mg kg^{-1}; $P < 0.001$ for 3, 10, and 30 mg kg^{-1}) compared to vehicle control summed over the 60-min test. The ED_{50} for increase in locomotor activity was ~2 mg kg^{-1}, translating to an RO of ~75%, which is higher than the ED_{50} for increase in frequency of swimming and decrease in immobility. No locomotor effects were observed for the 0.1 and 0.3 mg kg^{-1} dose groups (Fig. 3).

Hemodynamic study in telemetered rats. Single oral administration of CERC-301 increased arterial blood pressure transiently in conscious rats in a dose-dependent manner between doses of 0.3^{-1} mg kg^{-1} ($ED_{50} \approx 0.7$ mg kg^{-1}), and this effect plateaued at doses 1-10 mg kg^{-1} (Fig. 4). The peak effect was observed 1-2 h postdose (Fig. 4A), consistent with PK profile of CERC-301 in rats. The magnitude of change in hemodynamics with CERC-301 was less than that of MK-801 at

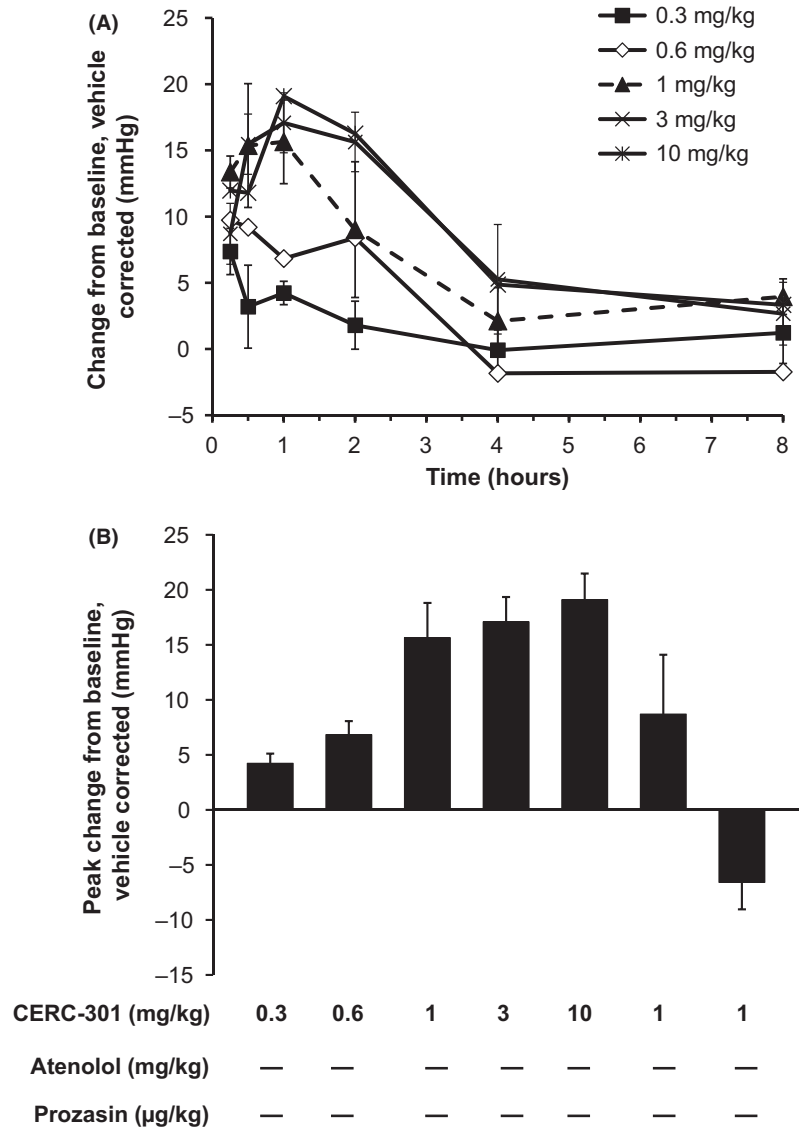

Figure 4. Effects of a single oral dose of CERC-301 on systolic blood pressure. (A) Vehicle subtracted mean changes from time-controled baseline is shown. (B) Peak change from baseline, vehicle subtracted is shown for each dose of CERC-301, with or without atenolol and prozasin. All data represented as mean ± SEM, with $N = 6$ for each group.

0.2 mg kg^{-1} (e.g., +36 mmHg in systolic blood pressure). The hemodynamic changes during the first 3.5 h post administration of CERC-301 were linearly correlated with the level of activity (as estimated from telemetry recordings); in particular, activity was a predictor of HR ($R^2 = 0.67$), and therefore, the excitatory effects of CERC-301 may explain the observed increases in HR (particularly at the higher dose levels) in rats. This study also demonstrated that α1- and/or β1-AR blockade may provide protection from CERC-301 mediated increases in blood pressure (Fig. 4B) and heart rate (data not shown), respectively.

Human pharmacokinetic study

CERC-301 was rapidly absorbed (Fig. 5A) with mean T_{max} within 1 h postdose across all fasted dose levels and terminal elimination half-life ranging from approximately 12 to 17 h over the 4- to 20-mg dose range. C_{max} and AUC behaved in a dose-proportional manner over the dose range studied. Dosing in the fed state led to an approximate 1-h delay in T_{max} and an approximate 56% decrease in C_{max}, but the overall extent of absorption (AUC) was not affected (Fig. 5B, Table 3).

Multiple-dose PK simulations based on these single oral dose data indicate that approximately 90% of steady state should be achieved within 3 days following once-daily oral dosing.

Discussion

A series of in vitro and in vivo pharmacology studies demonstrated that CERC-301 is a high-affinity NMDA receptor antagonist that is selective for the GluN2B

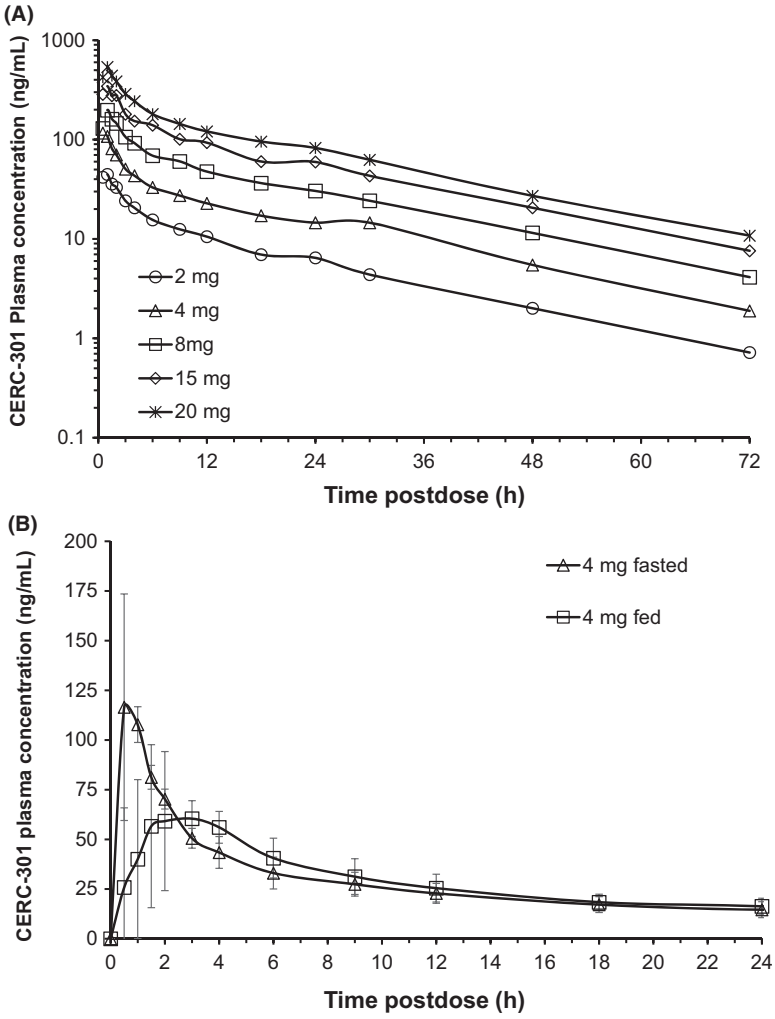

Figure 5. Plasma CERC-301 concentration-time profile in healthy male subjects. (A) Single doses of 2 to 20 mg fasted. (B) Comparison of fasted versus fed at 4 mg dose. Data are presented as mean ± SD.

Table 3. CERC-301 plasma pharmacokinetic parameters in healthy male volunteers.

Parameter (unit)	CERC-301 2 mg (N = 12)	CERC-301 4 mg (N = 6)	CERC-301 4 mg (fed) (N = 6)	CERC-301 8 mg (N = 6)	CERC-301 15 mg (N = 12)	CERC-301 20 mg (N = 6)
C_{max} (ng mL^{-1})	51.47 (16.96)	140.59 (22.27)	80.00 (18.46)	211.93 (42.70)	385.21 (99.15)	590.22 (87.09)
T_{max} (h)	1.08 (0.42)	0.67 (0.26)	2.00 (1.30)	0.92 (0.20)	1.04 (0.33)	1.00 (0.32)
AUC_{0-t} (h*ng mL^{-1})	397.64 (83.40)	1063.42 (196.97)	1080.32 (252.37)	2151.14 (442.25)	3521.08 (702.88)	5593.55 (874.71)
$AUC_{0-\infty}$ (h*ng mL^{-1})	493.25 (101.99)	1110.27 (200.68)	1137.98 (261.23)	2255.09 (473.43)	4396.45 (966.26)	5909.82 (1173.62)
$t_{½}$ (h)	16.14 (3.12)	16.92 (1.88)	17.31 (2.20)	16.96 (2.10)	16.79 (4.67)	15.90 (5.91)

Data are presented as mean (SD). AUC, area under the curve; C_{max}, maximum plasma concentration of drug; T_{max}, time to C_{max}; $t_{1/2}$, half-life.

subunit and devoid of effects on other receptors or enzymes at exposures at or above predicted Occ50. The correlation of CERC-301 plasma concentrations with RO was investigated in rat, dog, and rhesus monkey in order to provide a basis for the prediction of human plasma levels that would correlate with Occ_{50}. These results were consistent with CERC-301 being rapidly brain-penetrant by passive diffusion and CERC-301 free plasma levels being equal to free brain levels. The experimentally determined occupancies in rat, dog, and rhesus monkey are in agreement with the predicted values, thus providing a means to predict RO in humans utilizing measured plasma levels. The predicted plasma concentration required for Occ_{50} in humans is 0.4 μmol L^{-1} (143 ng mL^{-1}) based on the intrinsic binding affinity (K_i) of CERC-301 observed for human temporal cortex and the proportion of unbound drug in human plasma.

The rat forced swim test data demonstrated that brain GluN2B RO of approximately 30% or higher (corresponding to plasma concentrations of ~120 nmol L^{-1} or higher at the calculated ED_{50}) was associated with pharmacological and antidepressant effects. Although there was overlap between doses with locomotor activity and antidepressant effects, antidepressant effects were seen at doses that demonstrated minimal side effects (i.e., increased activity and movement). Locomotor activity findings were consistent with the CNS safety pharmacology studies in mice. The plasma exposure needed in rats for antidepressant activity (based on forced swim test) was demonstrated to be lower than that required to achieve efficacy with CERC-301 in animal models of pain (10 mg kg^{-1}; >1000 nmol L^{-1}) and Parkinson's disease (~70% RO for 25% improvement in the MPTP monkey model) (data not shown). Taken together, these data suggest a larger therapeutic index for CERC-301 in the treatment of depression versus other potential indications.

Ketamine has shown antidepressant effects after IV dosing of 0.5 mg kg^{-1} over a 40-min period (Berman et al. 2000; Murrough et al. 2013), a dosing regimen that results in peak plasma exposure of approximately 200 ng mL^{-1} (Krystal et al. 1994, 1998) and short half-life of approximately 2.5 h (Mion and Villevieille 2013). Based on its K_i of 1.1 to 2.5 μmol L^{-1} (Bresink et al. 1995; Moaddel et al. 2012), peak exposure of 200 ng mL^{-1}, and human plasma protein binding of 20% (Mion and Villevieille 2013), the estimated RO after a 40-min ketamine infusion of 0.5 mg kg^{-1} is approximately 20–40%. This supports the belief that achieving a transient and relatively low RO% will be sufficient for producing a rapid antidepressant effect (Shaffer et al. 2014), an effect that lasts up to 7 days after ketamine administration (Zarate et al. 2006). This is further supported by positive clinical data in small studies with CP-101,606 and CERC-301 (Preskorn et al. 2008; Ibrahim et al. 2012). A small trial of CERC-301 in patients with TRD showed significant antidepressant effects in the HDRS-17 and BDI at doses up to 8 mg daily (Ibrahim et al. 2012), which corresponds to approximately 50% predicted RO, consistent with nonclinical findings with CERC-301 in rats. Therefore, as ketamine and GluN2B-specific antagonists alter receptor function through different sites of action, it appears that the downstream molecular events leading to antidepressant effects may be similar (Li et al. 2010; Duman and Aghajanian 2012; Duman and Li 2012).

The studies in conscious telemetered rats demonstrates that CERC-301, when given orally, increased arterial blood pressure transiently, and in a dose-dependent manner between 0.3 and 1 mg kg^{-1}, and this effect plateaued at 1–10 mg kg^{-1}. Interestingly, the ED_{50} for blood pressure effects was similar to ED_{50} for the forced swim test. The magnitude of change in hemodynamics with CERC-301 was less than that of MK-801, a broad NMDA receptor antagonist. The findings with MK-801 were consistent with previously published mechanistic data on MK-801 (Lewis et al. 1989). The changes in HR and blood pressure may be partially explained by drug-dependent enhanced movement of rats; dose-dependent movement analysis was also observed in the Locomotor study, and demonstrate a supporting trend at these higher doses. This study also demonstrated that, α1-adernergic blockade may provide protection from CERC-301 mediated increases in blood pressure; however, more studies are required to further elucidate the underlying mechanisms.

Unlike some clinically utilized NMDA receptor antagonists (e.g., ketamine or memantine), CERC-301 showed no evidence of neurotoxicity in rats given single oral doses at up to 100 mg kg^{-1}. This result is consistent with those obtained with other GluN2B-specific receptor antagonists (e.g., CP-101,606 and Ro 63-1908), which have demonstrated a lack of neurotoxicity, and in the case of CP-101,606 paradoxically may have neuroprotective potential in the developing brain (Gill et al. 2002; Lewis et al. 2012). Lack of a nonclinical neurotoxicity signal with GluN2B-specific antagonists, along with the absence of ketamine-like psychotomimetic effects in the clinical setting allows administration of these agents at higher doses to achieve higher receptor occupancies (than what has been predicted herein), should the clinical data necessitate that in the future clinical trials.

Pharmacokinetic data from the first-in-human study with CERC-301 demonstrated mean C_{max} ranging from 0.007 μmol L^{-1} (2.4 ng mL^{-1}) to 1.65 μmol L^{-1} (590.3 ng mL^{-1}) across the 0.1 to 20 mg single dose (fasted) range, with a half-life that makes the drug suitable for once-daily dosing in human subjects.

CERC-301 is a potent orally bioavailable, selective NMDA-GluN2B receptor antagonist with antidepressant effects. Based on the preclinical PK and pharmacodynamic data, chronic 8-mg daily administration in humans is hypothesized to have an acceptable safety profile, result in clinically relevant plasma and brain concentrations, and exhibit rapid onset of antidepressant activity.

Authorship contributions

Bednar, Lynch, Mazhari, Garner, Vornov, Fandozzi, Lagrutta, and Briscoe participated in research design. Gaul, Mosser, Kiss, Patel, Fandozzi, and Briscoe conducted experiments. Liverton, and McCauley contributed to new reagents or analytic tools. Bednar, Kiss, Patel, Fandozzi, Briscoe, Garner, Mazhari, Vornov, and Gopalakrishnan performed data analysis. Bednar, Lynch, Mazhari, Garner, Gopalakrishnan, and Paterson wrote or contributed to the writing of the manuscript.

Disclosures

None declared.

References

Addy C, Assaid C, Hreniuk D, Stroh M, Xu Y, Herring WJ, et al. (2009). Single-dose administration of MK-0657, an NR2B-selective NMDA antagonist, does not result in clinically meaningful improvement in motor function in patients with moderate Parkinson's disease. J Clin Pharmacol 49: 856–864.

Berman RM, Cappiello A, Anand A, Oren DA, Heninger GR, Charney DS, et al. (2000). Antidepressant effects of ketamine in depressed patients. Biol Psychiatry 47: 351–354.

Bhatt JM, Prakash A, Suryavanshi PS, Dravid SM (2013). Effect of ifenprodil on GluN1/GluN2B N-Methyl-D-aspartate receptor gating. Mol Pharmacol 83: 9–21.

Bresink I, Danysz W, Parsons CG, Mutschler E (1995). Different binding affinities of NMDA receptor channel blockers in various brain regions–indication of NMDA receptor heterogeneity. Neuropharmacology 34: 533–540.

Claiborne CF, Liverton NJ, Libby B, Curtis NR, Kulagowski J (2000). Aryl amidines as NMDA NR2B antagonists. PCT Int Appl: WO2000067751.

DiazGranados N, Ibrahim L, Brutsche NE, Newberg A, Kronstein P, Khalife S, et al. (2010). A randomized add-on trial of an N–methyl-D-aspartate antagonist in treatment-resistant bipolar depression. Arch Gen Psychiatry 67: 793–802.

Dingledine R, Borges K, Bowie D, Traynelis SF (1999). The glutamate receptor ion channels. Pharmacol Rev 51: 7–61.

Duman RS, Aghajanian GK (2012). Synaptic dysfunction in depression: potential therapeutic targets. Science 338: 68–72.

Duman RS, Li N (2012). A neurotrophic hypothesis of depression: role of synaptogenesis in the actions of NMDA receptor antagonists. Phil Trans R Soc B 367: 2475–2484.

Duty S (2012). Targeting glutamate receptors to tackle the pathogenesis, clinical symptoms and levodopa-induced dyskinesia associated with Parkinson's disease. CNS Drugs 26: 1017–1032.

Fix AS, Wozniak DF, Truex LL, McEwen M, Miller JP, Olney JW (1995). Quantitative analysis of factors influencing neuronal necrosis induced by MK-801 in the rat posterior cingulate/retrosplenial cortex. Brain Res 696: 194–204.

Gill R, Alanine A, Bourson A, Buttelmann B, Fischer G, Heitz MP, et al. (2002). Pharmacological characterization of Ro 63-1908 (1-[2-(4-Hydroxy-phenoxy)-ethyl]-4-(4-methyl-benzyl)-piperidin-4-ol), a novel subtype-selective *N*-methyl-D-aspartate antagonist. J Pharmacol Exp Ther 302: 940–948.

Hamill TG, McCauley JA, Burns HD (2005). The synthesis of a benzamidine-containing NR2B-selective NMDA receptor ligand labelled with tritium or fluorine-18. J Labelled Comp Radiopharm 48: 1–10.

Howland RH (2013). Ketamine for the treatment of depression. J Psychosoc Nurs Ment Health Serv 51: 11–14.

Ibrahim L, DiazGranados N, Jolkovsky L, Brutsche N, Luckenbaugh DA, Herring WJ, et al. (2012). A randomized, placebo-controlled, crossover pilot trial of the oral selective NR2B antagonist MK-0657 in patients with treatment-resistant major depressive disorder. J Clin Psychopharmacol 32:551–557.

Karakas E, Furukawa H (2014). Crystal structure of a heterotetrameric NMDA receptor ion channel. Science 344: 992–997.

Kiss L, Cheng G, Bednar B, Bednar RA, Bennett PB, Kane SA, et al. (2005). In vitro characterization of novel NR2B selective NMDA receptor antagonists. Neurochem Int 46: 453–464.

Krystal JH, Karper LP, Seibyl JP, Freeman GK, Delaney R, Bremner JD, et al. (1994). Subanesthetic effects of the noncompetitive NMDA receptor antagonist, ketamine, in humans. Arch Gen Psychiatry 51: 199–214.

Krystal JH, Karper LP, Bennett A, D'Souza DC, Abi-Dargham A, Morrissey K, et al. (1998). Interactive effects of subanesthetic ketamine and subhypnotic lorazepam in humans. Psychopharmacology 135: 213–229.

Lewis SJ, Barres C, Jacob HJ, Ohta H, Brody MJ (1989). Cardiovascular effects of the N-methyl-D-aspartate receptor antagonist MK-801 in conscious rats. Hypertension 13: 759–765.

Lewis B, Wellmann KA, Kehrberg AMH, Carter ML, Baldwin T, Cohen M, et al. (2012). Behavioral deficits and cellular damage following developmental ethanol exposure in rats are attenuated by CP-101,606, an NMDAR antagonist with unique NR2B specificity. Pharmacol Biochem Behav 100: 545–553.

Li N, Lee B, Liu RJ, Banasr M, Dwyer JM, Iwata M, et al. (2010). mTOR-dependent synapse formation underlies the rapid antidepressant effects of NMDA antagonists. Science 329: 959–964.

Liverton NJ, Bednar RA, Bednar B, Butcher JW, Claiborne CF, Claremon DA, et al. (2007). Identification and characterization of 4-Methylbenzyl-4-[(pyrimidine-2-ylamino)methyl] piperidine-1-carboxylate as an orally bioavailable, CNS penetrant NR2B-selective NMDA antagonist. J Med Chem 50: 807–819.

Machado-Vieira R, Ibrahim L, Henter ID, Zarate CA Jr (2012). Novel glutamatergic agents for major depressive disorder and bipolar disorder. Pharmacol Biochem Behav 100: 678–687.

Mathews DC, Henter ID, Zarate CA Jr (2012). Targeting the glutamatergic system to treat major depressive disorder. Drugs 72: 1313–1333.

Mion G, Villevieille T (2013). Ketamine pharmacology: an update (pharmacodynamics and molecular aspects, recent findings). CNS Neurosci Ther 19: 370–380.

Moaddel R, Abdrakhmanova G, Kozak J, Jozwiak K, Toll L, Jimenez L, et al. (2012). Sub-anesthetic concentrations of (R, S)-ketamine metabolites inhibit acetylcholine-evoked currents in α7 nicotinic acetylcholine receptors. Eur J Pharmacol 698: 228–234.

Mosser SD, Gaul S, Bednar B, Koblan KS, Bednar RA (2003). Automation of in vitro dose-inhibition assays utilizing the tecan genesis and an integrated software package to support the drug discovery process. J Lab Autom 8: 54–63.

Murrough JW, Iosifescu DV, Chang LC, Al Jurdi RK, Green CE, Perez AM, et al. (2013). Antidepressant efficacy of ketamine in treatment-resistant major depression: a two-site randomized controlled trial. Am J Psychiatry 170: 1134–1142.

Musazzi L, Treccani G, Mallei A, Popoli M (2013). The action of antidepressants on the glutamate system: regulation of glutamate release and glutamate receptors. Biol Psychiatry 73: 1180–1188.

Pacifici GM, Viani A (1992). Methods of determining plasma and tissue binding of drugs. Pharmacokinetic consequences. Clin Pharmacokinet 23: 449–468.

Preskorn SH, Baker B, Kolluri S, Menniti FS, Krams M, Landen JW (2008). An innovative design to establish proof of concept of the antidepressant effects of the NR2B subunit selective N-methyl-D-aspartate antagonist, CP-101,606, in patients with treatment-refractory major depressive disorder. J Clin Psychopharmacol 28: 631–637.

Sanacora G, Treccani G, Popoli M (2012). Towards a glutamate hypothesis of depression: an emerging frontier of neuropsychopharmacology for mood disorders. Neuropharmacol 62: 63–77.

Schneider LS (2013). Alzheimer disease pharmacologic treatment and treatment research. Continuum (Minneap Minn) 19: 339–357.

Shaffer CL, Osgood SM, Smith DL, Liu J, Trapa PE (2014). Enhancing ketamine translational pharmacology via receptor occupancy normalization. Neuropharmacol 86: 174–180.

Skolnick P, Popik P, Trullas R (2009). Glutamate-based antidepressants: 20 years on. Trends Pharmacol Sci 30: 563–569.

Steece-Collier K, Chambers LK, Jaws-Tsai SS, Menniti FS, Greenamyre JT (2000). Antiparkinsonian actions of CP-101-606, an antagonist of NR2B subunit-containing N-methyl-aspartate receptors. Experiment Neurol 163: 239–243.

Valentine GW, Mason GF, Gomez R, Fasula M, Watzl J, Pittman B, et al. (2011). The antidepressant effect of ketamine is not associated with changes in occipital amino acid neurotransmitter content as measured by [(1)H]-MRS. Psychiatry Res 191: 122–127.

Zarate CA Jr, Singh JB, Carlson PJ, Brutsche NE, Ameli R, Luckenbaugh DA, et al. (2006). A randomized trial of an N-methyl-D-aspartate antagonist in treatment-resistant major depression. Arch Gen Psychiatry 63: 856–864.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Appendix S1. In vitro assays.