

## RESEARCH MONOGRAPH . COMPOUND PROFILE

# Zelquistinel

*A third-generation, orally bioavailable spirocyclic beta-lactam NMDA receptor modulator developed from the rapastinel peptidomimetic platform, distinguished by nanomolar positive allosteric modulation of NR2A-, NR2B-, and NR2C-containing NMDA receptors through reduction of calcium-dependent inactivation, producing rapid and sustained antidepressant-like effects without psychotomimetic liability.*

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| COMPOUND CLASS | Novel positive allosteric modulator of the N-methyl-D-aspartate receptor acting at a unique extracellular binding site independent of the glycine co-agonist site |

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## ABSTRACT

Zelquistinel (GATE-251, formerly AGN-241751) is a novel, orally bioavailable, small-molecule positive allosteric modulator of the N-methyl-D-aspartate (NMDA) glutamate receptor, currently in Phase 2b clinical development for major depressive disorder (MDD) under the sponsorship of Syndeio Biosciences (operating as Gate Neurosciences). The compound is the third-generation successor to the tetrapeptide rapastinel (GLYX-13) and the dipeptide apimostinel (NRX-1074), sharing a common mechanism of NMDA receptor positive modulation but replacing the peptide scaffold with a spirocyclic 2,5-diazaspiro[3.4]octane (beta-lactam) core that confers oral bioavailability approaching 100 percent in preclinical species, a plasma half-life of approximately 1.2 to 2.1 hours, and approximately 1000-fold greater potency than rapastinel on a weight basis.

Zelquistinel binds to a unique extracellular site on the NMDA receptor that is distinct from the glutamate agonist site, the glycine co-agonist site, the phencyclidine channel-blocking site, and the polyamine modulatory site. The compound does not displace radioligands at any of these four canonical sites and shows no significant activity across a panel of 80 receptors, ion channels, and monoamine transporters. Mechanistically, zelquistinel acts through a long-distance allosteric pathway: extracellular binding reduces intracellular calcium-dependent inactivation (CDI) of the NMDA receptor channel, thereby enhancing NMDA receptor current in an activity-dependent manner. This mechanism has been demonstrated through experiments showing that replacement of the intracellular

calcium chelator EGTA with the fast chelator BAPTA abolishes zelquistinel potentiation, and that removal of the NR1 C-terminal intracellular domain or infusion of calmodulin-blocking peptide similarly eliminates the modulatory effect. Subtype selectivity studies reveal potent enhancement at NR2A-containing (EC50 approximately 9.9 nM) and NR2C-containing (EC50 approximately 9.7 nM) receptors, with a larger ceiling enhancement at NR2B-containing receptors (EC50 approximately 35.0 nM) and no effect at NR2D-containing receptors.

The pharmacological profile produces a characteristic biphasic, inverted-U dose-response relationship: potentiation of NMDA receptor-mediated calcium influx at concentrations of 0.3 to 60 nM and mild inhibition at concentrations exceeding 100 nM. In preclinical behavioral models, single oral doses of zelquistinel at 30 micrograms per kilogram produce rapid antidepressant-like effects in the forced swim test (onset within one hour) that are sustained for more than seven days, and rescue social approach behavior in the chronic social defeat model at efficacy comparable to ketamine at 10 mg/kg subcutaneous. The compound enhances long-term potentiation (LTP) in both medial prefrontal cortex and hippocampal Schaffer collateral-CA1 pathways, with metaplastic enhancement persisting for at least two weeks after a single dose. Critically, zelquistinel produces no motor impairment on the rotarod test at doses 100-fold above the antidepressant-effective dose and no psychotomimetic symptoms as measured by validated clinical scales in human subjects.

In Phase 1 clinical evaluation, single ascending oral doses from 100 micrograms to 50 mg were well tolerated in 60 healthy volunteers, with dose-proportional pharmacokinetics, rapid absorption, significant cerebrospinal fluid penetration, and no

clinically significant adverse events, vital sign changes, electrocardiographic abnormalities, or psychotomimetic symptoms. A Phase 2a exploratory trial in 251 patients with major depressive disorder demonstrated statistically significant reductions in Montgomery-Asberg Depression Rating Scale (MADRS) scores at the two highest once-weekly oral doses (reductions of 9.5 and 10.6 points versus 7.7 points with placebo at week three). The U.S. Food and Drug Administration granted Fast Track designation for zelquistinel in MDD in July 2018. As of the date of this monograph, a Phase 2b confirmatory trial is actively recruiting under Syndeio Biosciences sponsorship. Additional preclinical research has demonstrated durable relief of core behavioral deficits in three mouse models of autism spectrum disorder, and dose-dependent reversal of phencyclidine-induced hyperlocomotion, expanding the potential therapeutic scope beyond depression.

This monograph reviews the chemistry, structural class, and synthesis of zelquistinel; the dual extracellular-intracellular allosteric mechanism in molecular and electrophysiological detail; the comprehensive preclinical and human pharmacokinetic record; the clinical evidence base in major depressive disorder; sourcing and quality verification considerations for research applications; reconstitution and handling; stack-interaction implications; adverse-event signal; and a comparative assessment of five NMDA receptor-targeting antidepressant candidates (rapastinel, apimostinel, esketamine, REL-1017, and AV-101) against zelquistinel on five competency standards (novelty, effect size, promising potential, side-effect profile, and overall validation). The compound is not approved by any regulatory authority for any indication. It is available as a research-grade

preparation from chemical suppliers; investigators should obtain analytical confirmation of identity and purity on every lot.

# 1. Compound Identification

## 1.1 Names, synonyms, and registry identifiers

**International Nonproprietary Name:** Zelquistinel (recommended).

**Development codes:** GATE-251 (Gate Neurosciences / Syndeio Biosciences, current); AGN-241751 (Allergan / AbbVie, former). The development code AGN-241751 is the canonical identifier in the primary pharmacology literature, including the seminal Bhatt et al. (2022) characterization in the International Journal of Neuropsychopharmacology [1]. The GATE-251 designation was adopted following transfer of development rights to Gate Neurosciences.

**Chemical Abstracts Service registry number:** 2151842-64-5.

**UNII:** 6P3S19V73H.

**International Union of Pure and Applied Chemistry name:** tert-butyl (4S)-2-[(2S,3R)-1-amino-3-hydroxy-1-oxobutan-2-yl]-3-oxo-2,5-diazaspiro[3.4]octane-5-carboxylate.

## 1.2 Structural class and chemistry

Zelquistinel is a spirocyclic beta-lactam peptidomimetic. The structural class places it within the 2,5-diazaspiro[3.4]octane family, a small group of constrained beta-lactam scaffolds designed as conformationally restricted mimetics of peptide backbone geometry. The compound was designed as a non-peptide replacement for the tetrapeptide rapastinel (threonyl-prolyl-prolyl-threoninamide) and the dipeptide apimostinel (NRX-1074), retaining the NMDA receptor modulatory activity of these peptide predecessors while replacing the metabolically labile peptide bonds with a rigid spirocyclic core that confers oral bioavailability and metabolic stability [1, 2].

**Molecular formula:** C<sub>15</sub>H<sub>25</sub>N<sub>3</sub>O<sub>5</sub>.

**Molecular weight:** 327.38 g/mol.

**Physical properties:** White to off-white crystalline solid or lyophilized powder. Soluble in water, dimethyl sulfoxide, and common aqueous buffers at research-relevant concentrations. The compound contains a tert-butyloxycarbonyl (Boc) protecting group on the pyrrolidine nitrogen of the diazaspiro ring, a beta-lactam carbonyl, and a threonine-derived side chain bearing a primary amide and secondary hydroxyl.

**Stereochemistry:** Zelquistinel contains three stereocenters: the (4S) configuration at the spiro junction, the (2S) configuration at the alpha-carbon of the threonine-derived side chain, and the (3R) configuration at the hydroxyl-bearing beta-carbon. The absolute configuration is critical for NMDA receptor modulatory activity; investigators acquiring research-grade zelquistinel should confirm stereochemical integrity by chiral chromatography or optical rotation.

## 1.3 Synthesis

The published medicinal chemistry of zelquistinel proceeds from the Aptinyx peptidomimetic design platform, which explored constrained spirocyclic replacements for the Pro-Pro dipeptide core of rapastinel [1, 2]. The key synthetic steps involve: (a) construction of the 2,5-diazaspiro[3.4]octane ring system through intramolecular cyclization of a suitably functionalized pyrrolidine precursor bearing a beta-amino acid side chain; (b) installation of the beta-lactam ring through a [2+2] or stepwise cyclization strategy; (c) coupling of the threonine-derived amide side chain to the beta-lactam nitrogen through standard peptide coupling reagents; and (d) Boc protection of the pyrrolidine nitrogen. The final product is purified by preparative high-performance liquid chromatography or recrystallization and characterized by nuclear magnetic resonance spectroscopy, high-resolution mass spectrometry, and chiral chromatographic analysis. The compound is typically supplied as the free base; salt forms have not been reported in the public literature. Detailed synthetic procedures and structure-activity relationship data from the Aptinyx medicinal chemistry program remain proprietary.

## 2. Abstract

Zelquistinel (GATE-251, AGN-241751) is a third-generation, orally bioavailable NMDA receptor positive allosteric modulator that emerged from the Aptinyx peptidomimetic design platform as a non-peptide successor to rapastinel and apimostinel [1, 2]. The compound operates through a mechanism that is fundamentally distinct from the NMDA receptor antagonism of ketamine and esketamine: rather than blocking the ion channel pore, zelquistinel binds to a unique extracellular site and enhances NMDA receptor function by reducing intracellular calcium-dependent inactivation (CDI), thereby potentiating activity-dependent glutamatergic neurotransmission and long-term synaptic plasticity [1, 3].

The pharmacological profile is characterized by nanomolar potency at NR2A-, NR2B-, and NR2C-containing NMDA receptor subtypes (EC<sub>50</sub> values of 9.9, 35.0, and 9.7 nM, respectively), absence of activity at NR2D-containing receptors, and a biphasic inverted-U dose-response with potentiation at 0.3 to 60 nM and mild inhibition above 100 nM [1, 3]. In preclinical behavioral models, single oral doses at 30 micrograms per kilogram produce rapid (within one hour) and sustained (more than seven days) antidepressant-like effects in the forced swim test, rescue social approach behavior in the chronic social defeat model, and dose-dependently reverse phencyclidine-induced hyperlocomotion [1]. The compound enhances long-term potentiation in medial prefrontal cortex and hippocampal circuits with metaplastic effects persisting for at least two weeks after a single administration [1].

Pharmacokinetics in the rat are favorable: oral bioavailability approaches 100 percent, plasma half-life is approximately 1.2 to 2.1 hours, and central nervous system penetration yields cerebrospinal fluid concentrations approximately 30 percent of plasma concentrations [1]. In Phase 1 human studies, single ascending oral doses from 100 micrograms to 50 mg were well tolerated, with dose-proportional pharmacokinetics, rapid absorption, significant cerebrospinal fluid exposure, no psychotomimetic symptoms, and no clinically significant adverse events [4]. A Phase 2a exploratory trial in 251 patients with MDD demonstrated statistically and clinically significant reductions in MADRS depression scores at the two highest once-weekly oral doses compared to placebo [5]. The FDA granted Fast Track designation in July 2018, and as of May 2026, a Phase 2b confirmatory trial is actively recruiting [6].

Additional preclinical research has demonstrated durable relief of core behavioral deficits (social interaction deficits and stereotypic behaviors) across three mouse models of autism spectrum disorder (Shank3-knockout, Fmr1-knockout, and in utero valproate exposure), suggesting potential therapeutic applications beyond depression [7]. The compound exhibits no motor impairment at doses 100-fold above the antidepressant threshold and no psychotomimetic liability in either pre-clinical or clinical assessment [1, 4].

## 3. Discovery and Development History

### 3.1 Origin: the rapastinel platform

The development of zelquistinel traces to the discovery of rapastinel (GLYX-13), a tetrapeptide (threonyl-prolyl-prolyl-threoninamide) identified in the laboratory of Joseph Bhatt and colleagues at Naurex, Inc. as a functional partial agonist at the glycine site of the NMDA receptor [8, 9]. Rapastinel demonstrated rapid and sustained antidepressant-like effects in rodent models and produced significant improvements in depression scores in a Phase 2 clinical trial in patients with MDD who had an inadequate response to antidepressant therapy [10]. Rapastinel received FDA Breakthrough Therapy designation in 2016, representing the first positive allosteric modulator of the NMDA receptor to advance to late-stage clinical development for depression.

However, rapastinel had significant pharmaceutical limitations: as a tetrapeptide, it required intravenous administration (no oral bioavailability) and had a short plasma half-life. These limitations drove the development of second-generation (apimostinel, NRX-1074) and third-generation (zelquistinel) successors with improved drug-like properties.

### 3.2 Aptinyx and the peptidomimetic platform

Naurex was acquired by Allergan in 2015. The research platform, including the structure-activity relationship data and the medicinal chemistry team, was spun out into a new company, Aptinyx, Inc., which entered a collaborative research agreement with Allergan to develop next-generation NMDA receptor modulators [2]. The Aptinyx medicinal chemistry program systematically explored constrained cyclic and spirocyclic replacements for the Pro-Pro dipeptide core of rapastinel, ultimately identifying the 2,5-diazaspiro[3.4]octane (spirocyclic beta-lactam) scaffold as an optimal peptidomimetic that retained NMDA receptor modulatory activity while conferring oral bioavailability, metabolic stability, and approximately 1000-fold greater potency than rapastinel [1].

The resulting compound, designated AGN-241751, was advanced by Allergan into preclinical development and subsequently into clinical trials.

### 3.3 Allergan clinical development and FDA Fast Track designation

Allergan advanced zelquistinel (AGN-241751) through IND-enabling studies and into Phase 1 clinical evaluation. On July 23, 2018, the FDA granted Fast Track designation for the development of zelquistinel as an investigational new treatment for major depressive disorder [11]. This designation followed rapastinel's own Breakthrough Therapy designation and reflected the agency's recognition of the unmet medical need for rapid-acting antidepressants and the promising preclinical and early clinical signal for the NMDA receptor modulation mechanism.

In 2019, Allergan completed an exploratory Phase 2a clinical trial of once-weekly oral zelquistinel in patients with MDD [5]. Results demonstrated statistically significant reductions in MADRS depression scores at the two highest doses compared to placebo.

Concurrently, in March 2019, Allergan reported that rapastinel had failed to meet its primary and key secondary endpoints in three Phase 3 adjunctive MDD studies (RAP-MD-01, -02, -03) enrolling a total of 1,510 patients, and an interim analysis of a fourth relapse-prevention study (RAP-MD-04) suggested that study would also not meet its endpoints [12]. The rapastinel Phase 3 failure did not directly implicate zelquistinel, as the two compounds, despite sharing a common receptor target, have distinct pharmacological properties (oral vs. intravenous, peptidomimetic vs. peptide, different subtype selectivity profiles, and potentially different receptor binding sites). However, the failure raised questions about the broader translatability of NMDA receptor positive modulation as an antidepressant mechanism.

### 3.4 Transfer to Gate Neurosciences and current development

Following AbbVie's acquisition of Allergan in 2020, the zelquistinel program was deprioritized within the larger AbbVie portfolio. Gate Neurosciences, a precision medicine biotechnology company founded in 2019 and headquartered in Indianapolis, acquired the zelquistinel program along with the apimostinel and rapastinel programs from AbbVie [13]. Gate emerged from stealth mode in August 2022 following completion of 25 million dollars in financing from life science investors. The company also acquired exclusive rights to preclinical mGlu2/3 receptor antagonists from Eli Lilly for sleep disorder applications.

Gate Neurosciences operates through its subsidiary Syndeio Biosciences. Under the GATE-251 designation, zelquistinel entered Phase 2b clinical development for MDD, with the confirmatory trial registered as NCT06547489 and actively recruiting as of the date of this monograph [6]. The Phase 2b trial is a randomized, double-blind, placebo-controlled study evaluating multiple dose levels in adults with MDD, with collaboration from Beacon Biosignals for EEG biomarker integration.

## 4. Mechanism of Action

Zelquistinel is a positive allosteric modulator of the NMDA receptor that operates through a mechanism fundamentally distinct from both the NMDA receptor antagonists (ketamine, esketamine, memantine, REL-1017) and the glycine-site partial agonists (D-cycloserine). The compound binds to a unique extracellular site, modulates intracellular calcium-dependent inactivation through a long-distance allosteric mechanism, and produces activity-dependent enhancement of NMDA receptor current and downstream synaptic plasticity [1, 3].

### 4.1 Binding site characterization

Comprehensive radioligand displacement and receptor panel screening have established that zelquistinel does not interact with the four canonical NMDA receptor binding sites [1]. Specifically: (a) zelquistinel does not displace [3H]CGP 39653 at the glutamate agonist site; (b) it does not displace [3H]TCP or [3H]MK-801 at the phenylcyclidine (PCP) channel-blocking site; (c) it does not displace [3H]ifenprodil at the polyamine/NR2B modulatory site; and (d) co-application of the glycine-site antagonist MDL 105,519 at 10 micromolar does not prevent zelquistinel enhancement of NMDA-induced calcium responses, confirming independence from the glycine co-agonist site. Screening across a panel of 80 receptors, ion channels, and monoamine transporters revealed no significant off-target binding [1].

The extracellular location of the binding site was established by the Bhatt et al. (2024) study, which demonstrated that zelquistinel modulation is abolished by removal of the NR1 intracellular C-terminus, by intracellular infusion of a calmodulin-blocking peptide, and by replacement of the slow intracellular calcium chelator EGTA with the fast chelator BAPTA [3]. These findings establish a long-distance allosteric mechanism: zelquistinel binds extracellularly and transduces its effect through conformational changes that propagate to the intracellular domain, where calcium-calmodulin-dependent inactivation (CDI) of the NMDA receptor channel is reduced.

## 4.2 Calcium-dependent inactivation modulation

Calcium-dependent inactivation (CDI) is a physiological negative-feedback mechanism by which intracellular calcium, acting through calmodulin bound to the C0 and C1 domains of the NR1 subunit intracellular tail, reduces NMDA receptor channel open probability and thereby limits calcium influx during sustained or repetitive activation [3, 14]. CDI serves as a neuroprotective mechanism against excitotoxicity but also constrains the magnitude and duration of NMDA receptor-mediated synaptic transmission.

Zelquistinel reduces CDI, effectively releasing the brake on NMDA receptor current during physiological activation. The consequence is enhancement of NMDA receptor-mediated excitatory postsynaptic currents (EPSCs) and facilitation of the coincidence-detection function of the NMDA receptor that underlies long-term potentiation (LTP) and other forms of Hebbian synaptic plasticity. Critically, because the mechanism operates on CDI rather than on channel gating directly, the enhancement is activity-dependent: zelquistinel potentiates NMDA receptor currents only during ongoing glutamatergic transmission and does not produce tonic receptor activation in the absence of presynaptic glutamate release [1, 3]. This activity dependence is the mechanistic basis for the absence of excitotoxicity, seizure liability, and psychotomimetic effects observed at doses far exceeding the therapeutic range.

## 4.3 NMDA receptor subtype selectivity

Electrophysiological characterization in HEK293 cells expressing defined NMDA receptor subunit combinations has revealed the following subtype selectivity profile [3]:

NR2A-containing receptors (NR1/NR2A):  $EC_{50} = 9.9 \pm 0.5$  nM. Zelquistinel potently enhances NMDA receptor current at this subtype, which predominates at mature cortical and hippocampal synapses and mediates the majority of activity-dependent synaptic plasticity relevant to cognitive function and antidepressant mechanisms.

NR2B-containing receptors (NR1/NR2B):  $EC_{50} = 35.0 \pm 0.7$  nM. Enhancement is potent with a larger ceiling (maximum enhancement) than at NR2A-containing receptors. NR2B-containing receptors are enriched at extrasynaptic and perisynaptic

sites and are implicated in stress-related synaptic dysfunction and depression pathophysiology; the larger ceiling enhancement at this subtype may contribute to the antidepressant efficacy.

NR2C-containing receptors (NR1/NR2C):  $EC_{50} = 9.7 \pm 0.6$  nM. Potent enhancement comparable to NR2A. NR2C-containing receptors are expressed in cerebellar granule cells and in thalamic relay neurons.

NR2D-containing receptors (NR1/NR2D): No effect. The absence of NR2D modulation is notable because NR2D-containing receptors mediate tonic extrasynaptic NMDA receptor currents implicated in excitotoxicity and because the NMDA receptor antagonist REL-1017 (esmethadone) preferentially targets this subtype.

## 4.4 Dose-response characteristics

Zelquistinel exhibits a biphasic, inverted-U dose-response relationship in both in vitro and in vivo assays [1]. In cortical neuronal cultures, potentiation of NMDA-induced intracellular calcium influx is observed at concentrations of 0.3 to 60 nM, with peak potentiation at approximately 10 to 30 nM. At concentrations exceeding 100 nM, mild inhibition (approximately 15 to 20 percent) of NMDA receptor-mediated responses is observed. This biphasic profile is characteristic of allosteric modulators operating at conformationally coupled sites and has important implications for dose selection: the therapeutic window lies within the potentiation phase, and suprapharmacological dosing may reduce rather than enhance efficacy.

In vivo, the inverted-U relationship translates to a therapeutic dose range centered at approximately 30 micrograms per kilogram (oral) in rodents, with reduced efficacy at doses above 100 micrograms per kilogram [1]. The estimated cerebrospinal fluid concentration at the therapeutic dose of 30 micrograms per kilogram is approximately 2.56 ng/mL (8 nM), consistent with the in vitro potentiation range [1].

## 4.5 Downstream consequences: synaptic plasticity

The enhancement of NMDA receptor function by zelquistinel produces robust facilitation of long-term potentiation (LTP) in both the medial prefrontal cortex (mPFC) and the hippocampal Schaffer collateral-CA1 pathway [1].

In acute mPFC slices, zelquistinel at 60 to 250 nM significantly enhances LTP magnitude compared to vehicle control. In vivo, a single oral dose of 300 micrograms per kilogram enhances hippocampal Schaffer collateral-CA1 LTP for at least two weeks, with peak enhancement at one week; mPFC LTP enhancement peaks at 24 hours and is sustained for at least one week [1]. These metaplastic effects (enhanced capacity for LTP induction at a time point long after the drug has been eliminated from plasma) are consistent with transcriptional and translational remodeling of the synaptic plasticity machinery and represent the proposed mechanistic basis for the sustained antidepressant-like effects observed after single-dose administration. Transcriptome profiling of mPFC tissue 24 hours after a single 30 micrograms per kilogram oral dose revealed significant changes in synaptic plasticity pathway gene expression [1].

## 5. Pharmacokinetics and Routes of Administration

### 5.1 Preclinical pharmacokinetics (rat)

Zelquistinel demonstrates favorable oral pharmacokinetic properties in the rat, consistent with the design objective of replacing the peptide backbone of rapastinel with an orally bioavailable small molecule [1].

After intravenous administration at 2 mg/kg: plasma half-life ( $t_{1/2}$ ) = 1.21  $\pm$  0.14 hours; volume of distribution at steady state ( $V_{ss}$ ) = 0.57  $\pm$  0.06 L/kg; clearance (CL) = 11.2  $\pm$  0.55 mL/min/kg.

After oral administration at 10 mg/kg: plasma half-life = 2.06  $\pm$  0.66 hours; time to peak plasma concentration ( $T_{max}$ ) = 0.50  $\pm$  0.0 hours; clearance (CL/F) = 9.05  $\pm$  0.70 mL/min/kg; oral bioavailability = approximately 100 percent.

Peak plasma, cerebrospinal fluid (CSF), and brain concentrations at 10 mg/kg oral: plasma  $C_{max}$  = 5,991  $\pm$  545 ng/mL; CSF  $C_{max}$  = 1,470  $\pm$  775 ng/mL; brain  $C_{max}$  = 1,013  $\pm$  560 ng/g. The CSF/plasma ratio is approximately 0.30, and the brain/plasma ratio is approximately 0.16, indicating significant central nervous system penetration [1].

At the pharmacologically active oral dose of 30 micrograms per kilogram, the estimated CSF  $C_{max}$  is approximately 2.56 ng/mL (approximately 8 nM), which falls within the in vitro potentiation range of 0.3 to 60 nM [1].

### 5.2 Human pharmacokinetics (Phase 1)

In the Phase 1 single ascending dose study in healthy volunteers, zelquistinel (GATE-251) was rapidly absorbed from oral solution, with dose-proportional increases in  $C_{max}$  and AUC across the 100 microgram to 50 mg dose range [4]. Plasma half-life was reported as approximately 0.5 hours across doses, substantially shorter than the rat half-life, though this value may reflect the limitations of sampling intervals in a first-in-human study. No gender-associated differences in pharmacokinetic parameters were observed.

Cerebrospinal fluid pharmacokinetics were characterized in a dedicated CSF-sampling cohort (Part B: 12 zelquistinel, 4 placebo; doses of 1 mg fasted, 1 mg fed, and 10 mg fasted). Significant CSF transport was documented, with a CSF T<sub>max</sub> of approximately 4 hours (delayed relative to plasma T<sub>max</sub>), confirming central nervous system exposure at clinically relevant doses [4].

A food-effect analysis (Part C: 6 subjects at 1 mg with high-fat meal) demonstrated that a high-fat meal reduced plasma C<sub>max</sub> but did not affect plasma AUC. Notably, CSF AUC appeared to be enhanced by a high-fat meal, though the small cohort size limits the precision of this estimate [4].

## 5.3 Routes of administration

Zelquistinel is administered orally. The compound was formulated as an oral solution for Phase 1 studies and is anticipated to be developed as an oral dosage form (capsule or tablet) for Phase 2b and subsequent registration studies. Intravenous administration is feasible in research settings but is not the intended clinical route and does not confer pharmacological advantages over oral administration given the near-complete oral bioavailability.

## 5.4 Metabolism

Detailed human metabolic pathway data have not been published in peer-reviewed literature as of the date of this monograph. The short plasma half-life in humans suggests rapid hepatic metabolism, possibly involving hydrolysis of the beta-lactam ring, deprotection of the Boc group, or oxidative metabolism of the pyrrolidine ring. Cytochrome P450 isoform involvement has not been publicly characterized. Investigators combining zelquistinel with CYP inhibitors or inducers should monitor for pharmacokinetic interactions pending publication of definitive metabolism data.

## 6. Clinical Evidence Base

### 6.1 Phase 1: single ascending dose study

The Phase 1 study enrolled 60 healthy volunteers across three parts [4]:

Part A (dose escalation): 42 zelquistinel recipients and 14 placebo controls received single ascending oral doses of 100 micrograms, 1 mg, 3 mg, 10 mg, 25 mg, or 50 mg as oral solution under fasted conditions.

Part B (CSF pharmacokinetics): 12 zelquistinel and 4 placebo recipients received doses of 1 mg (fasted), 1 mg (fed), or 10 mg (fasted) with serial cerebrospinal fluid sampling via indwelling lumbar catheter.

Part C (food effect): 6 zelquistinel recipients received 1 mg with a high-fat meal.

Safety findings: No serious adverse events were reported. Treatment-related adverse events were limited to one headache (1 mg group, Part A), one lumbar puncture syndrome and one attention disturbance (10 mg group, Part B). No clinically significant changes in plasma chemistry, hematology, urinalysis, vital signs, or electrocardiographic parameters were observed. No psychotomimetic symptoms were detected on the Columbia Suicide Severity Rating Scale (C-SSRS), Brief Psychiatric Rating Scale positive symptom subscale (BPRS+), or Clinician-Administered Dissociative States Scale (CADSS) at any dose [4].

Pharmacodynamic findings: Zelquistinel increased resting-state alpha electroencephalographic (EEG) power in a dose-dependent pattern, with an inverted-U relationship at higher doses, indicating effective central NMDA receptor modulation at clinically well-tolerated doses [4].

### 6.2 Phase 2a: exploratory trial in major depressive disorder

Allergan completed an exploratory Phase 2a clinical trial of once-weekly oral zelquistinel in patients with major depressive disorder in 2019 [5]. The trial enrolled 251 patients. At week three, the two highest doses studied produced reductions in MADRS total score of 9.5 and 10.6 points, compared to 7.7 points with

placebo. The separation from placebo was reported as statistically and clinically significant. The once-weekly dosing regimen was selected on the basis of the sustained preclinical antidepressant-like effects (more than seven days after a single dose) and is a distinguishing feature of the zelquistinel clinical program, contrasting with the daily or twice-daily dosing required for conventional monoaminergic antidepressants and the twice-weekly clinical administration schedule of esketamine (Spravato).

## 6.3 Phase 2b: confirmatory trial (ongoing)

A Phase 2b randomized, double-blind, placebo-controlled confirmatory trial (NCT06547489) is actively recruiting as of the date of this monograph [6]. The trial is evaluating multiple oral dose levels of zelquistinel (GATE-251) in adults with MDD. The trial includes integration of EEG biomarkers through a collaboration with Beacon Biosignals, reflecting Gate Neurosciences' precision medicine strategy of identifying early pharmacodynamic biomarkers of treatment response. Topline results were initially anticipated in mid-2024; the timeline appears to have extended, with recruitment ongoing through 2026.

## 6.4 Preclinical indications beyond depression

**Autism spectrum disorder:** Bhatt et al. (2024) demonstrated that zelquistinel durably relieves core behavioral deficits in three mouse models of autism spectrum disorder [7]. In Shank3-delta-exon-13-16 knockout mice, single oral doses of zelquistinel rescued social interaction deficits and stereotypic behaviors in a dose-response manner. Chronic intraperitoneal administration promoted long-lasting relief of autistic-like behavioral features. Subchronic oral mid-dose treatment demonstrated durable effects in Shank3-knockout, Fmr1-knockout (the Fragile X model), and in utero valproate-exposed mice. Carry-over effects were best maintained in the Fmr1 null mouse model, with social parameters remaining fully recovered two weeks after treatment withdrawal [7]. These findings suggest potential therapeutic applications in neurodevelopmental disorders characterized by NMDA receptor hypofunction.

**Phencyclidine-induced hyperlocomotion:** Zelquistinel at 30 to 300 micrograms per kilogram oral dose-dependently reversed phencyclidine (2.5 mg/kg subcutaneous)-induced hyperactivity in mice, with significant inhibition at both 30 and 300

micrograms per kilogram [1]. This model is relevant to both the positive symptoms of schizophrenia and to the broader understanding of NMDA receptor hypofunction states.

## 7. Reported Research Dose Ranges

### 7.1 Preclinical doses (rodent)

Antidepressant-like effects (forced swim test, chronic social defeat): 0.1 to 100 micrograms per kilogram oral, with optimal efficacy at 30 micrograms per kilogram. No sex differences at the 30 micrograms per kilogram dose [1].

Synaptic plasticity enhancement (LTP): 10 to 300 micrograms per kilogram oral for in vivo metaplasticity studies; 60 to 250 nM for acute slice electrophysiology [1].

Autism spectrum disorder models: Dose-response characterization in Shank3-knockout mice; subchronic oral mid-dose treatment across three models [7].

Phencyclidine reversal: 30 to 300 micrograms per kilogram oral [1].

Motor safety assessment (rotarod): No impairment at 10 mg/kg oral (approximately 333-fold above the antidepressant-effective dose) [1].

### 7.2 Clinical doses (human)

Phase 1 single ascending dose: 100 micrograms, 1 mg, 3 mg, 10 mg, 25 mg, 50 mg oral solution [4].

Phase 2a exploratory: Once-weekly oral administration; specific dose levels have not been fully disclosed in peer-reviewed publications, but the two highest doses produced clinically significant MADRS separation from placebo [5].

Phase 2b confirmatory: Multiple oral dose levels under evaluation; specific doses are defined in the trial protocol (NCT06547489) [6].

Investigators acquiring research-grade zelquistinel should note the inverted-U dose-response relationship and the very low effective doses (micrograms per kilogram range in rodents), which necessitate precise analytical confirmation of compound mass and careful gravimetric or volumetric dose preparation.

## 8. Sourcing and Quality Verification

Research-grade zelquistinel is supplied by multiple chemical and pharmaceutical research suppliers (MedChemExpress, BenchChem, BOC Sciences, MedKoo, Smolecule, and others) at greater than 98 percent purity by HPLC [15, 16]. Supply is in milligram quantities as the free base. Pricing is moderate to high, consistent with a compound in active clinical development with limited synthetic-scale availability.

Pharmaceutical-grade zelquistinel is not commercially available, as the compound has not received regulatory approval for any indication. Clinical trial material is manufactured under GMP conditions by the sponsor (Syndeo Biosciences / Gate Neurosciences) and is not available for independent research procurement.

Investigators should confirm compound identity and purity on every research-grade lot through:

- High-performance liquid chromatography area-percent purity, ideally greater than 98 percent
- High-resolution mass spectrometry confirmation of molecular weight (exact mass 327.1794 for C<sub>15</sub>H<sub>25</sub>N<sub>3</sub>O<sub>5</sub> [M+H]<sup>+</sup> = 328.1872)
- Nuclear magnetic resonance spectroscopy (1H and 13C) confirmation of structural identity, with particular attention to the beta-lactam carbonyl and the spirocyclic junction signals
- Chiral chromatography or optical rotation confirmation of stereochemical integrity at all three stereocenters
- Karl Fischer or loss-on-drying analysis for residual water content

Third-party analytical laboratories provide independent confirmation of vendor certificates of analysis. Given the nanomolar potency of zelquistinel and the inverted-U dose-response, even modest impurities or stereochemical degradation could meaningfully affect pharmacological results in research applications.

## 9. Reconstitution and Handling

Zelquistinel is supplied as a white to off-white crystalline solid or lyophilized powder. For in vitro research applications, stock solutions are typically prepared at 1 to 10 mM in dimethyl sulfoxide (DMSO) and diluted to working concentrations (0.3 to 250 nM) in assay buffer or recording solution immediately before use. Final DMSO concentration in the assay should not exceed 0.1 percent (v/v) to avoid solvent effects on NMDA receptor function.

For in vivo research applications, the compound is dissolved in sterile saline, water for injection, or buffered aqueous vehicle at concentrations appropriate for the target dose volume (typically 1 to 10 mL/kg in rodent studies). Given the very low effective doses (30 micrograms per kilogram), serial dilution from a concentrated stock may be necessary to achieve accurate dosing; investigators should validate dose preparation by analytical confirmation of the final dosing solution concentration.

Storage of the solid compound is recommended at -20 degrees Celsius, desiccated and protected from light. DMSO stock solutions are stable at -20 degrees Celsius for at least 6 months; aqueous solutions should be used within 24 hours of preparation or stored at 2 to 8 degrees Celsius for short-term use (up to 7 days).

The beta-lactam ring is a potential site of hydrolytic instability under strongly acidic or basic conditions; investigators should avoid extremes of pH in reconstitution and storage.

## 10. Stack Interaction Considerations

Zelquistinel interacts with several drug and compound classes through its NMDA receptor modulatory mechanism and through potential pharmacokinetic interactions. Investigators conducting research with zelquistinel, particularly in combination with other neuroactive compounds, should consider the following:

**NMDA receptor antagonists (ketamine, esketamine, memantine, dextromethorphan, REL-1017):** Concurrent administration of an NMDA receptor positive allosteric modulator and an NMDA receptor antagonist produces pharmacologically antagonistic effects at the receptor level. The net effect depends on the relative concentrations and binding kinetics; at some concentration ratios, the combination may produce net neutral or net inhibitory effects. The combination has not been systematically characterized and is not recommended for research applications unless the specific interaction is the object of study.

**Other NMDA receptor positive modulators (D-cycloserine, D-serine, rapastinel, apimostinel):** Concurrent administration with other NMDA receptor positive modulators may produce additive or supraadditive effects on NMDA receptor current and downstream plasticity, potentially exceeding the physiological range and risking excitotoxicity. The inverted-U dose-response of zelquistinel suggests that co-administration with other positive modulators may shift the effective concentration into the inhibitory phase.

**Glycine site ligands:** The independence of zelquistinel's binding site from the glycine co-agonist site suggests that glycine, D-serine, and glycine transport inhibitors (sarcosine, bitopertin) may produce additive NMDA receptor enhancement through complementary mechanisms. This combination is a plausible research direction but has not been characterized for safety.

**Glutamate transport inhibitors and glutamate release enhancers:** Agents that increase extracellular glutamate concentration (such as glutamate transport inhibitors) would increase NMDA receptor activation and potentially synergize with zelquistinel's CDI-reducing mechanism. The combination carries theoretical excitotoxicity risk.

**AMPA receptor positive modulators (ampakines):** AMPA receptor potentiation increases glutamatergic drive onto NMDA receptor-containing synapses and may produce synergistic enhancement of synaptic plasticity when combined with zelquistinel. The combination has mechanistic rationale but has not been characterized.

**Monoaminergic antidepressants (SSRIs, SNRIs, TCAs, MAOIs):** No characterized pharmacodynamic interaction at the NMDA receptor level. Pharmacokinetic interactions through CYP-mediated metabolism are possible but uncharacterized. The Phase 2a trial evaluated zelquistinel as adjunctive therapy to existing antidepressant treatment, suggesting that the combination is tolerated at the clinical doses studied [5].

**Benzodiazepines and GABAergic agents:** No direct pharmacodynamic interaction at the NMDA receptor. However, GABAergic inhibition may indirectly modulate the expression of zelquistinel-enhanced synaptic plasticity by reducing overall network excitability.

**Ethanol:** Ethanol is an NMDA receptor antagonist at moderate to high concentrations. Concurrent ethanol exposure may attenuate zelquistinel efficacy. Investigators should control for ethanol exposure in preclinical and clinical research protocols.

# 11. Adverse Events and Safety

## 11.1 Preclinical safety

Zelquistinel demonstrates a favorable preclinical safety profile characterized by three principal findings [1]:

**No motor impairment:** The rotarod test in rodents showed no reduction in motor coordination at 10 mg/kg oral, a dose approximately 333-fold above the antidepressant-effective dose of 30 micrograms per kilogram. This contrasts with ketamine and gabapentin, which produce significant motor impairment at doses proximate to their antidepressant-effective ranges.

**No psychotomimetic liability:** The compound does not produce the hyperlocomotion, stereotypy, or prepulse inhibition deficits associated with NMDA receptor antagonists in rodent models. Rather, zelquistinel dose-dependently reverses phencyclidine-induced hyperlocomotion, consistent with a normalizing rather than destabilizing effect on glutamatergic function.

**Wide therapeutic index:** The biphasic dose-response and the absence of motor or behavioral toxicity at doses several hundred-fold above the therapeutic range indicate a substantial safety margin.

## 11.2 Clinical safety (Phase 1)

In the Phase 1 single ascending dose study across 60 healthy volunteers [4]:

No serious adverse events were reported at any dose (100 micrograms to 50 mg oral).

Treatment-related adverse events were rare and mild: one headache (Part A, 1 mg), one lumbar puncture syndrome (Part B, attributable to the CSF sampling procedure rather than the study drug), and one attention disturbance (Part B, 10 mg).

No clinically significant changes in plasma chemistry, hematology, or urinalysis.

No vital sign abnormalities.

No electrocardiographic changes (including no QT interval prolongation).

No psychotomimetic symptoms on C-SSRS, BPRS positive symptom subscale, or CADSS at any dose, from baseline through end of study. This finding is particularly significant given the psychotomimetic liability associated with NMDA receptor antagonists (ketamine, esketamine, PCP) and validates the mechanistic distinction between NMDA receptor positive allosteric modulation and NMDA receptor antagonism.

### 11.3 Clinical safety (Phase 2a)

Detailed adverse-event data from the Phase 2a trial have not been published in peer-reviewed form as of the date of this monograph. The trial was completed and results presented without reported safety signals that would have precluded advancement to Phase 2b. The ongoing Phase 2b trial will provide the most comprehensive human safety dataset for zelquistinel to date.

### 11.4 Theoretical safety considerations

**Excitotoxicity:** As a positive allosteric modulator of the NMDA receptor, zelquistinel theoretically carries a risk of glutamate-mediated excitotoxicity at excessive doses or in pathological states with already elevated glutamatergic transmission (such as epilepsy or acute stroke). The inverted-U dose-response (transition from potentiation to inhibition above 100 nM) and the activity-dependent mechanism (CDI modulation rather than direct channel activation) provide intrinsic pharmacological safeguards against this risk. However, investigators should exercise caution in epilepsy models or in combination with pro-excitatory agents.

**Seizure liability:** NMDA receptor potentiation could theoretically lower seizure threshold. No seizure activity has been reported in preclinical or clinical studies, but formal seizure liability assessment data have not been published.

**Pregnancy and lactation:** No reproductive or developmental toxicity data have been published. The compound should be considered investigational for all populations.

## 12. Comparable Compounds Assessment

This section presents a structured comparative assessment of five NMDA receptor-targeting antidepressant candidates against zelquistinel, evaluated against five competency standards: novelty, effect size, promising potential (current clinical state and trajectory), side-effect profile, and overall validation. The five candidates were selected as the principal NMDA receptor-targeting compounds with substantive clinical pharmacology programs in depression, spanning positive modulators, antagonists, and prodrug strategies.

### 12.1 Rapastinel (GLYX-13)

**Mechanism:** Tetrapeptide (Thr-Pro-Pro-Thr-NH<sub>2</sub>) positive allosteric modulator of the NMDA receptor, originally characterized as a glycine-site functional partial agonist. The compound enhances NMDA receptor function and long-term potentiation through a mechanism related to but not identical with zelquistinel's CDI modulation [8, 9].

**Development trajectory:** Naurex advanced rapastinel through Phase 2 with positive results in MDD, receiving FDA Breakthrough Therapy designation in 2016. Allergan acquired Naurex in 2015 and advanced rapastinel to four Phase 3 studies (three acute, one relapse prevention) enrolling 1,510 patients with MDD as adjunctive therapy to antidepressant treatment [12].

**Clinical outcome:** In March 2019, Allergan reported that all three acute Phase 3 studies (RAP-MD-01, -02, -03) failed to differentiate rapastinel from placebo on primary and key secondary endpoints. An interim analysis of the relapse-prevention study (RAP-MD-04) also suggested negative results. Rapastinel was well tolerated without psychotomimetic side effects in all Phase 3 studies [12].

**Novelty:** High at the time of discovery (first NMDA receptor positive modulator to reach Phase 3 for depression). Reduced by the subsequent failure.

**Effect size:** Phase 2 signal was promising (significant MADRS improvement). Phase 3 effect size was insufficient to separate from placebo, possibly due to the intravenous route, short half-life, and challenges in the adjunctive MDD trial design.

**Promising potential:** Low. Active development has ceased. The compound is owned by Gate Neurosciences but is not in active clinical trials. It serves as a pharmacological reference compound.

**Side-effect profile:** Excellent. Phase 3 data confirmed no psychotomimetic effects and no clinically significant adverse events beyond injection-site reactions. The favorable safety profile was preserved across 1,510 patients.

**Overall validation:** Moderate. The compound proved the concept that NMDA receptor positive modulation produces rapid antidepressant effects without psychotomimetic liability, but the Phase 3 failure raised questions about the sufficiency of the effect size, the intravenous route, and the adjunctive trial design.

**Comparison to zelquistinel:** Zelquistinel was designed specifically to overcome rapastinel's pharmaceutical limitations: oral bioavailability (versus intravenous), approximately 1000-fold greater potency, and longer plasma half-life. The Phase 3 failure of rapastinel does not directly predict zelquistinel's clinical outcome, as the two compounds differ in route, potency, pharmacokinetics, and potentially in NMDA receptor subtype selectivity. Zelquistinel's Phase 2a signal (MADRS separation from placebo at once-weekly oral dosing) addresses some of the operational and pharmacokinetic limitations that may have contributed to rapastinel's Phase 3 failure.

## 12.2 Apimostinel (NRX-1074, GATE-202)

**Mechanism:** Dipeptide NMDA receptor positive allosteric modulator, approximately 100-fold more potent than rapastinel on a weight basis. Shares the same NMDA receptor binding mechanism as rapastinel and zelquistinel (unique site independent of the glycine co-agonist site) [2].

**Development trajectory:** Developed by Naurex and subsequently by Allergan. Intravenous apimostinel completed a Phase 2a clinical trial for MDD. Now owned by Gate Neurosciences (Syndeio Biosciences) under the designation GATE-202.

**Novelty:** Moderate. Represents an incremental potency improvement over rapastinel within the same mechanistic class.

**Effect size:** Phase 2a data have not been extensively published in peer-reviewed form. The compound produced antidepressant-like effects at lower doses than rapastinel, consistent with its greater potency.

**Promising potential:** Low to moderate. The compound is owned by Gate Neurosciences but is not the lead clinical program (zelquistinel is the lead). Apimostinel retains the limitation of intravenous or parenteral administration (limited oral bioavailability for the dipeptide scaffold).

**Side-effect profile:** No significant safety signals in available Phase 2a data. Expected to share the favorable safety profile of the NMDA receptor positive modulator class.

**Overall validation:** Low to moderate. Limited published clinical data.

**Comparison to zelquistinel:** Apimostinel is the structural and mechanistic intermediate between rapastinel and zelquistinel. Zelquistinel surpasses apimostinel in oral bioavailability, potency (approximately 10-fold greater), and clinical development stage (Phase 2b versus Phase 2a completed).

## 12.3 Esketamine (Spravato)

**Mechanism:** Non-selective, non-competitive NMDA receptor channel blocker (the S-enantiomer of ketamine). Blocks the NMDA receptor ion channel pore at the phencyclidine binding site, producing use-dependent antagonism of NMDA receptor current. The antidepressant mechanism is proposed to involve a paradoxical burst of glutamate release (the "glutamate surge" hypothesis) and subsequent AMPA receptor-mediated BDNF and mTOR pathway activation, rather than direct NMDA receptor enhancement [17].

**Development trajectory:** Developed by Janssen (Johnson and Johnson). Approved by the FDA in March 2019 as Spravato (esketamine nasal spray) for treatment-resistant depression as adjunctive therapy with an oral antidepressant. Subsequently approved as monotherapy for treatment-resistant depression. Regulated under a Risk Evaluation and Mitigation Strategy (REMS) program due to sedation and dissociation risks [17].

**Novelty:** Moderate. The compound is the S-enantiomer of the long-established anesthetic ketamine; the novel aspect is the clinical application in depression and the nasal spray formulation.

**Effect size:** Robust. The TRANSFORM-2 trial demonstrated a mean MADRS change from baseline of -10.3 points versus placebo at week 4, with 22.5 percent remission versus 7.6 percent on placebo [17]. Effect onset is rapid (within hours). The effect size is the strongest among the NMDA receptor-targeting antidepressants evaluated in this panel.

**Promising potential:** High. The compound is FDA-approved and commercially available. It is the only NMDA receptor-targeting compound with a depression indication approved by any major regulatory authority.

**Side-effect profile:** Significant. The principal adverse events are dissociation (experienced by approximately 40 to 60 percent of patients in clinical trials), dizziness, nausea, sedation, vertigo, and transient increases in blood pressure. The REMS program requires administration in certified healthcare settings with monitoring for at least 2 hours post-dose. The compound has abuse potential and is classified as Schedule III. The side-effect profile is the principal limitation of esketamine and the primary motivation for developing alternative NMDA receptor-targeting antidepressants [17].

**Overall validation:** High. FDA-approved, commercially successful, and supported by an extensive clinical evidence base. The compound validates the NMDA receptor as a therapeutic target in depression.

**Comparison to zelquistinel:** Esketamine and zelquistinel represent fundamentally opposite approaches to NMDA receptor pharmacology in depression. Esketamine blocks the receptor channel (antagonism), producing a secondary glutamate surge and downstream plasticity. Zelquistinel directly enhances the receptor function (positive allosteric modulation), producing activity-dependent plasticity without glutamate surge, dissociation, sedation, or abuse potential. The esketamine safety profile (dissociation, sedation, abuse liability, REMS requirement, healthcare-facility administration) is the principal limitation that zelquistinel's mechanism is designed to avoid. If the Phase 2b trial confirms the Phase 2a signal, zelquistinel would represent a mechanistically differentiated, potentially safer alternative to esketamine, with the additional advantage of oral self-administration and once-weekly dosing.

## 12.4 REL-1017 (Esmethadone)

**Mechanism:** Uncompetitive NMDA receptor channel blocker with preferential activity at NR2D-containing NMDA receptor subtypes. The compound is the dextro-isomer of racemic methadone and is opioid-inactive [18].

**Development trajectory:** Developed by Relmada Therapeutics. Phase 2a results demonstrated rapid, robust antidepressant effects at 25 and 50 mg oral daily doses with clinically meaningful MADRS improvements starting on day 4. Phase 3 results have been mixed [18].

**Novelty:** Moderate. The NMDA receptor channel-blocking mechanism is shared with ketamine and memantine; the NR2D subtype selectivity and opioid inactivity are distinguishing features.

**Effect size:** Phase 2a showed promising MADRS separation from placebo. Phase 3 results have been mixed, with the primary study showing mixed outcomes.

**Promising potential:** Moderate. Phase 3 data are equivocal; the regulatory path is uncertain.

**Side-effect profile:** Favorable compared to ketamine/esketamine. No psychotomimetic effects, no abuse liability (opioid-inactive). No meaningful dissociative effects.

**Overall validation:** Moderate. Phase 2 signal was positive; Phase 3 has not clearly replicated.

**Comparison to zelquistinel:** REL-1017 and zelquistinel target opposite aspects of NMDA receptor function (antagonism versus positive modulation) but share the objective of avoiding the ketamine/esketamine side-effect profile. REL-1017 selectively targets NR2D-containing receptors, which zelquistinel does not modulate, representing complementary rather than competing receptor pharmacology. Zelquistinel's mechanism (activity-dependent enhancement rather than tonic channel blockade) is more mechanistically novel and may offer a more favorable therapeutic index. Both compounds are in clinical development; neither has completed Phase 3.

## 12.5 AV-101 (L-4-Chlorokynurenine)

**Mechanism:** Prodrug of 7-chlorokynurenic acid, a competitive antagonist at the glycine co-agonist site of the NMDA receptor. The prodrug is converted to the active metabolite by kynurenine aminotransferase in astrocytes, providing brain-selective NMDA receptor inhibition [19].

**Development trajectory:** Developed by VistaGen Therapeutics. Phase 2 ELEVATE trial in treatment-resistant depression (adjunctive to antidepressant therapy) failed to meet the primary endpoint; AV-101 did not separate from placebo on MADRS [19]. The compound was well tolerated without psychotomimetic effects or serious adverse events. Development has been deprioritized.

**Novelty:** Moderate. The prodrug approach to brain-selective glycine-site antagonism is a novel drug-delivery concept, though the underlying mechanism (glycine-site antagonism) is well established.

**Effect size:** Phase 2 ELEVATE trial was negative; no significant MADRS separation from placebo. The sponsor suggested that insufficient blood-brain barrier penetration or inadequate active metabolite concentrations may have compromised efficacy.

**Promising potential:** Low. Development is essentially terminated for the depression indication.

**Side-effect profile:** Favorable. No psychotomimetic effects, no serious adverse events in Phase 2.

**Overall validation:** Low. The compound failed in Phase 2 and does not have a clear path to further clinical development in depression.

**Comparison to zelquistinel:** AV-101 and zelquistinel represent different approaches to NMDA receptor modulation in depression: AV-101 antagonizes the glycine co-agonist site (reducing NMDA receptor function), while zelquistinel enhances NMDA receptor function through a glycine-site-independent positive allosteric mechanism. The AV-101 Phase 2 failure reinforces the emerging evidence that simple NMDA receptor antagonism at the glycine site may be insufficient for antidepressant efficacy and supports the mechanistic differentiation of the NMDA receptor positive modulation approach represented by zelquistinel.

## 12.6 Comparator summary

The five comparators occupy distinct positions in the NMDA receptor pharmacology space for depression. **Rapastinel** proved the concept of NMDA receptor positive modulation as an antidepressant mechanism but failed at Phase 3, limited by intravenous route and potentially insufficient pharmacokinetics. **Apimostinel** improved on rapastinel's potency but remains parenteral and underdeveloped. **Esketamine** is the only FDA-approved NMDA receptor-targeting antidepressant, with robust efficacy but significant safety limitations (dissociation, sedation, abuse potential, REMS requirement) that constrain its clinical utility. **REL-1017** offers a more tolerable NMDA receptor antagonist profile through NR2D subtype selectivity but faces uncertain Phase 3 outcomes. **AV-101** failed in Phase 2 with a glycine-site antagonist prodrug strategy.

Zelquistinel occupies a unique position in this landscape: it is the only orally bioavailable NMDA receptor positive allosteric modulator in active clinical development; the only compound in this panel that enhances rather than blocks NMDA receptor function; the only compound that produces its effect through modulation of calcium-dependent inactivation; and the compound with the widest demonstrated therapeutic index (no motor or psychotomimetic effects at doses exceeding 100-fold the antidepressant threshold). The once-weekly oral dosing regimen, if confirmed in Phase 2b, would represent a meaningful practical advantage over esketamine's twice-weekly healthcare-facility administration and over the daily dosing of conventional antidepressants. The principal risk is the rapastinel precedent: the Phase 3 failure of the first-generation NMDA receptor positive modulator raises the question of whether the mechanism can produce effect sizes sufficient for registration. The Phase 2a MADRS signal and the pharmacokinetic improvements over rapastinel are encouraging but not yet definitive.

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