

RESEARCH MONOGRAPH . COMPOUND PROFILE

Hydroxynorketamine

A hydroxylated norketamine metabolite identified as a principal mediator of ketamine's rapid antidepressant effects, distinguished from the parent compound by the absence of NMDA receptor blockade at therapeutic concentrations, lack of dissociative and abuse-related properties, and a novel mechanism converging on AMPA receptor potentiation, BDNF-TrkB signaling, and mGlu2 receptor-dependent glutamatergic plasticity.

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ABSTRACT

Hydroxynorketamine (HNK) refers to a family of twelve stereoisomeric metabolites of the dissociative anesthetic and rapid-acting antidepressant ketamine, formed by cytochrome P450-mediated hydroxylation of the intermediate metabolite norketamine at the 4-, 5-, or 6-position of the cyclohexane ring. The (2R,6R)-hydroxynorketamine stereoisomer ((2R,6R)-HNK) has emerged as the compound of greatest pharmacological interest following the seminal 2016 report by Zanos et al. in *Nature* demonstrating that this metabolite is both necessary and sufficient for the antidepressant actions of ketamine in rodent models, while lacking the anesthetic, dissociative, psychotomimetic, and abuse-related properties of the parent compound. The molecular pharmacology of (2R,6R)-HNK is distinct from ketamine: at concentrations relevant to antidepressant activity, (2R,6R)-HNK does not produce meaningful inhibition of N-methyl-D-aspartate (NMDA) receptors, but instead potentiates alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor-mediated glutamatergic transmission through a presynaptic mechanism that increases glutamate release probability and enhances synaptic plasticity in hippocampal and prefrontal cortical circuits. Downstream consequences include activity-dependent release of brain-derived neurotrophic factor (BDNF), activation of tropomyosin receptor kinase B (TrkB) signaling, stimulation of the mechanistic target of rapamycin complex 1 (mTORC1) pathway, and rapid synaptogenesis in the medial prefrontal cortex. Additional mechanistic contributions involve metabotropic glutamate receptor subtype 2 (mGlu2) receptor-dependent signaling and antagonism of alpha-7 nicotinic acetylcholine receptors controlling presynaptic function and neuroplasticity gene expression. Pharmacokinetically, (2R,6R)-HNK is formed in vivo from (R)-ketamine through sequential N-demethylation (predominantly CYP2B6 and CYP3A4) to (R)-norketamine and

subsequent 6-hydroxylation (predominantly CYP2B6 and CYP2A6). The metabolite is brain-penetrant following peripheral administration, with rapid onset of central nervous system exposure. A Phase 1 clinical trial (NCT04711005) published by Raja et al. in 2024 demonstrated excellent safety and tolerability of intravenous (2R,6R)-HNK at doses from 0.1 to 4 mg/kg in healthy volunteers, with no dissociative effects, no serious adverse events, and a pharmacokinetic profile consistent with once-daily or intermittent dosing. Phase 2 trials in treatment-resistant depression (NCT06511908) and Phase 1/2 trials in neuropathic pain (NCT05864053) and obsessive-compulsive disorder (NCT06575075) are currently enrolling. Preclinical evidence across multiple rodent models demonstrates antidepressant-like, anxiolytic, analgesic, and anti-inflammatory effects, with effect sizes comparable to or exceeding those of the parent ketamine in several paradigms and duration of action extending well beyond the plasma residence time, consistent with a synaptic plasticity-dependent rather than receptor occupancy-dependent mechanism. This monograph reviews the chemistry, stereochemistry, and metabolic origin of hydroxynorketamine; the molecular pharmacology at AMPA, NMDA, mGlu2, alpha-7 nicotinic, and opioid receptors; the comprehensive pharmacokinetic record; the preclinical evidence base across depression, pain, anxiety, and substance use disorder models; the emerging clinical evidence from Phase 1 and ongoing Phase 2 trials; sourcing and quality verification; reconstitution and handling; stack interactions and combinations; adverse events and safety signal; and a comparative assessment of five rapid-acting antidepressant candidates against hydroxynorketamine on five competency standards.

1. Compound Identification

1.1 Names, synonyms, and registry identifiers

Preferred name: Hydroxynorketamine (HNK). The abbreviation HNK is the standard identifier in the pharmacology literature and is used throughout this monograph.

Specific stereoisomer of primary interest: (2R,6R)-Hydroxynorketamine ((2R,6R)-HNK). Also referred to in development contexts as RR-HNK.

Related stereoisomers: (2S,6S)-Hydroxynorketamine ((2S,6S)-HNK) is the enantiomer of (2R,6R)-HNK and is formed from (S)-ketamine metabolism. Additional stereoisomers include 4-hydroxynorketamine and 5-hydroxynorketamine isomers, each with (R) and (S) configurations at the 2-position, yielding a total of twelve distinct HNK stereoisomers identified in human and rodent metabolism [1, 2].

Chemical Abstracts Service registry numbers: 81395-70-2 (6-hydroxynorketamine, racemic free base); 1430202-69-9 ((2R,6R)-hydroxynorketamine hydrochloride). The hydrochloride salt of (2R,6R)-HNK is the form supplied by most commercial research vendors and the form used in the Phase 1 clinical trial.

International Union of Pure and Applied Chemistry name: (2R,6R)-2-amino-2-(2-chlorophenyl)-6-hydroxycyclohexan-1-one. The general class name for the 6-hydroxylated isomers is 2-amino-2-(2-chlorophenyl)-6-hydroxycyclohexanone, with stereochemistry at C2 and C6 specified by prefix.

Development identifiers: The compound has been advanced through clinical development under the auspices of the National Center for Advancing Translational Sciences (NCATS) at the United States National Institutes of Health, in collaboration with the National Institute of Mental Health (NIMH), the National Institute on Aging (NIA), and the University of Maryland School of Medicine (the Zanos and Gould laboratories) [3].

1.2 Structural class and chemistry

Hydroxynorketamine belongs to the arylcyclohexylamine structural class, sharing the 2-(2-chlorophenyl)cyclohexanone core with ketamine, norketamine, and dehydronorketamine. The compound is distinguished from norketamine by a hydroxyl group at the 6-position of the cyclohexane ring and from ketamine by the absence of the N-methyl group on the amine nitrogen.

Molecular formula: C₁₂H₁₄ClNO₂ (free base).

Exact mass: 227.0713 g/mol (free base, monoisotopic).

Molecular weight: 227.69 g/mol (free base); 264.15 g/mol (hydrochloride salt).

Physical properties: White to off-white crystalline powder as the hydrochloride salt. Soluble in water and dimethyl sulfoxide (DMSO); sparingly soluble in ethanol. The hydrochloride salt displays high aqueous solubility, a property that facilitates both parenteral and oral formulation for research applications.

Stereochemistry: Hydroxynorketamine contains two stereocenters at C2 and C6 of the cyclohexanone ring. The (2R,6R) and (2S,6S) trans-diastereomers are the pharmacologically active forms of greatest interest. The cis-diastereomers ((2R,6S) and (2S,6R)) are minor metabolic products with substantially lower pharmacological activity [2]. Investigators acquiring research-grade HNK should confirm stereochemical identity and enantiomeric purity by chiral chromatography, as the pharmacological profile differs markedly between stereoisomers.

1.3 Synthesis

The synthesis of (2R,6R)-hydroxynorketamine can be accomplished through chemical synthesis from commercially available starting materials or through metabolic generation from (R)-ketamine or (R)-norketamine. The chemical synthesis involves stereoselective hydroxylation of the cyclohexanone ring of norketamine at the 6-position with retention of the trans-relationship between the 2-amino and 6-hydroxyl substituents. Reported synthetic routes employ chiral resolution, asymmetric hydroxylation, or derivatization of enantiopure norketamine intermediates [4]. A United States patent (US 11,377,416) describes crystalline forms of hydroxynorketamine suitable for pharmaceutical development, including polymorphic characterization, stability data, and formulation approaches. Industrial and clinical-grade synthesis is conducted under the direction of NCATS for the ongoing clinical trial program. Research-grade material is available from multiple commercial suppliers (Sigma-Aldrich, Tocris Bioscience, Bio-Techne, Cayman Chemical, MedChemExpress) at greater than 98 percent purity by HPLC as the hydrochloride salt.

2. Abstract

Hydroxynorketamine (HNK) is a family of twelve stereoisomeric metabolites of the dissociative anesthetic and rapid-acting antidepressant ketamine, formed by hepatic cytochrome P450-mediated hydroxylation of the intermediate metabolite norketamine. The (2R,6R)-hydroxynorketamine stereoisomer ((2R,6R)-HNK) was identified by Zanos et al. (2016) as a principal mediator of ketamine's rapid antidepressant effects in rodent behavioral models, producing sustained antidepressant-like activity in the forced swim test, learned helplessness paradigm, and chronic social defeat stress model without the anesthetic, dissociative, psychotomimetic, or abuse-related properties of ketamine [5]. This seminal finding, published in *Nature*, challenged the prevailing hypothesis that NMDA receptor antagonism is the primary mechanism of ketamine's antidepressant action and initiated an extensive research program that has now advanced to Phase 2 clinical trials.

The molecular pharmacology of (2R,6R)-HNK is characterized by potentiation of AMPA receptor-mediated glutamatergic transmission, activity-dependent BDNF release, TrkB-mTORC1 signaling activation, and rapid synaptogenesis in the medial prefrontal cortex and hippocampus [6, 7, 8]. The compound acts through mGlu2 receptor-dependent signaling pathways and through antagonism of alpha-7 nicotinic acetylcholine receptors to control presynaptic glutamate release and neuroplasticity gene expression [9, 10]. At concentrations relevant to antidepressant activity, (2R,6R)-HNK does not produce meaningful NMDA receptor inhibition, distinguishing its mechanism from that of ketamine, esketamine, and other NMDA receptor antagonists [11].

Pharmacokinetically, (2R,6R)-HNK is formed *in vivo* from (R)-ketamine through sequential hepatic metabolism: N-demethylation by CYP2B6 and CYP3A4 to (R)-norketamine, followed by 6-hydroxylation by CYP2B6 and CYP2A6. The metabolite is brain-penetrant, achieving central nervous system concentrations sufficient for pharmacological activity following peripheral administration in rodents and following intravenous infusion in humans [2, 12].

The Phase 1 clinical trial (NCT04711005), published by Raja et al. (2024), demonstrated safety and tolerability of intravenous (2R,6R)-HNK at doses from 0.1 to 4 mg/kg in healthy volunteers through single ascending dose and multiple ascending dose designs. No dissociative effects, no serious adverse events, and no dose-limiting toxicities were observed [12]. Phase 2 trials in treatment-resistant depression (NCT06511908), neuropathic pain (NCT05864053), and obsessive-compulsive disorder (NCT06575075) are currently enrolling or in progress.

Preclinical evidence demonstrates antidepressant-like, anxiolytic, analgesic, and anti-inflammatory effects across multiple rodent models, with effect duration extending well beyond the plasma elimination half-life, consistent with a synaptic plasticity-dependent mechanism rather than a receptor occupancy-dependent mechanism [2, 13]. The compound lacks self-ad-

ministration behavior, conditioned place preference, and locomotor sensitization in rodent models of abuse liability, supporting a favorable safety profile relative to the parent ketamine [5, 14].

3. Discovery and Development History

3.1 Origin and metabolic identification

The hydroxynorketamines were first identified as metabolites of ketamine in the 1970s and 1980s during the early characterization of ketamine metabolism by Woolf and Adams (1987) and by subsequent investigators using gas chromatography-mass spectrometry and liquid chromatography-tandem mass spectrometry methods [15]. Twelve distinct HNK stereoisomers were catalogued, distinguished by the position of cyclohexane ring hydroxylation (4-, 5-, or 6-position) and the stereochemistry at the C2 and C6 stereocenters. The 6-hydroxynorketamines, particularly (2R,6R)-HNK and (2S,6S)-HNK, were identified as the major circulating HNK metabolites in human plasma following intravenous ketamine infusion [1, 2].

For approximately three decades following their identification, the hydroxynorketamines were considered pharmacologically inactive terminal metabolites of ketamine with no intrinsic biological activity. This assumption was based on the low affinity of HNK for the NMDA receptor (the presumed target of ketamine's therapeutic and adverse effects) and the absence of anesthetic or dissociative activity in early animal studies.

3.2 The Zanos et al. (2016) discovery

The transformative finding was reported by Zanos et al. in *Nature* in May 2016, from a collaboration among the University of Maryland School of Medicine, the National Institute of Mental Health, and the National Institute on Aging [5]. The study demonstrated three principal findings. First, metabolism of ketamine to (2R,6R)-HNK was necessary for the antidepressant actions of (R)-ketamine in the forced swim test and learned helplessness model in mice; when metabolism was blocked by deuterium substitution at the C6 position (producing d2-ketamine, which resists hydroxylation), the antidepressant effect was abolished. Second, direct administration of (2R,6R)-HNK was sufficient to produce rapid and sustained antidepressant-like effects in multiple behavioral paradigms. Third, (2R,6R)-HNK did not produce the dissociative, anesthetic, hyperlocomotive, or self-administration behaviors characteristic of ketamine, indicating a fundamentally different pharmacological profile despite the structural relationship.

The Zanos et al. findings challenged the prevailing NMDA receptor inhibition hypothesis of ketamine's antidepressant mechanism and proposed an alternative model in which the therapeutic effect is mediated through an NMDA receptor-independent, AMPA receptor-dependent mechanism driven by the HNK metabolite [5].

3.3 Subsequent mechanistic investigation

The years following the 2016 discovery produced an extensive mechanistic literature. Zanos et al. (2019) demonstrated that (2R,6R)-HNK exerts antidepressant actions through mGlu2 receptor-dependent signaling, with mGlu2 receptor knockout or mGlu2 receptor antagonist pretreatment abolishing the antidepressant-like behavioral effects [9]. Bhatt et al. (2024) identified alpha-7 nicotinic acetylcholine receptor antagonism as a key mechanism by which HNK controls presynaptic glutamate release and nuclear translocation of phosphorylated CREB, with genetic deletion of the *Chrna7* gene fully abolishing HNK-induced synaptic and nuclear signaling [10]. Fukumoto et al. (2019) demonstrated that activity-dependent BDNF signaling through TrkB is required for the antidepressant actions of (2R,6R)-HNK, placing BDNF release downstream of AMPA receptor activation in the mechanistic cascade [8].

3.4 Controversy and replication

The Zanos et al. findings were not universally replicated. Shirayama and Hashimoto (2018) reported that (2R,6R)-HNK failed to produce antidepressant-like effects in a rat learned helplessness model and argued that (R)-ketamine itself, rather than its metabolite, was responsible for the antidepressant action [16]. Yang et al. (2017) similarly reported failure to replicate antidepressant effects of (2R,6R)-HNK in the chronic social defeat stress model in mice [17]. The discrepancies have been attributed to differences in species, strain, stress model, dose, route of administration, and timing of behavioral assessment. The balance of the published literature, including multiple independent replications by the Zanos, Gould, Bhatt, and other laboratories, supports the antidepressant activity of (2R,6R)-HNK in rodent models, while acknowledging that the magnitude and consistency of the effect vary across experimental conditions [2, 18].

3.5 Clinical translation

NCATS, in collaboration with NIMH and the University of Maryland, advanced (2R,6R)-HNK through IND-enabling studies, including comprehensive nonclinical toxicology, safety pharmacology (cardiovascular, respiratory, and central nervous system), and formulation development [3]. The compound entered Phase 1 clinical evaluation in 2021 (NCT04711005), with results published in 2024 demonstrating safety and tolerability in healthy volunteers [12]. Phase 2 clinical trials for treatment-resistant depression, neuropathic pain, and obsessive-compulsive disorder were initiated in 2024 and 2025 and are currently enrolling [19, 20, 21].

4. Mechanism of Action

(2R,6R)-Hydroxynorketamine is a multi-target ligand whose principal molecular activities converge on the enhancement of glutamatergic synaptic transmission and the induction of rapid synaptic plasticity in corticolimbic circuits. The mechanism is fundamentally distinct from that of the parent compound ketamine, which acts primarily as an open-channel blocker of NMDA receptors.

4.1 AMPA receptor potentiation

The earliest and most consistently replicated mechanistic finding is that (2R,6R)-HNK potentiates AMPA receptor-mediated excitatory postsynaptic currents in hippocampal and prefrontal cortical neurons [5, 6]. Electrophysiological studies in hippocampal brain slices demonstrate that (2R,6R)-HNK rapidly increases the amplitude of AMPA receptor-mediated excitatory postsynaptic potentials through a presynaptic mechanism that enhances glutamate release probability. The AMPA receptor antagonist NBQX blocks the antidepressant-like behavioral effects of (2R,6R)-HNK in the forced swim test and learned helplessness model, confirming the AMPA receptor dependence of the behavioral effect [5].

The potentiation of AMPA receptor-mediated transmission is the upstream initiating event in a signaling cascade that leads to activity-dependent BDNF release, TrkB activation, and mTORC1-dependent synaptogenesis.

4.2 NMDA receptor interaction

The relationship between (2R,6R)-HNK and the NMDA receptor is complex and has been the subject of sustained debate. At concentrations relevant to antidepressant activity in rodents (low micromolar range in brain tissue), (2R,6R)-HNK does not produce meaningful NMDA receptor inhibition in electrophysiological recordings from hippocampal neurons [11]. Bhatt et al. (2017) confirmed that antidepressant-relevant concentrations of (2R,6R)-HNK do not block NMDA receptor function in vitro. However, Bhatt et al. (2020) and Suzuki et al. (2017) demonstrated that at higher concentrations (IC₅₀ approximately 46 micromolar at pH 7.2), (2R,6R)-HNK can inhibit NMDA receptor currents by binding to closed (resting) receptors with low affinity and weak voltage dependence [22, 23]. This closed-channel inhibition is mechanistically distinct from the open-channel block produced by ketamine and is unlikely to contribute to the therapeutic effects at pharmacologically relevant concentrations. A 2026 preprint from Bhatt et al. identified presynaptic NMDA receptors on hippocampal mossy fiber terminals as mediating rapid BDNF release by both ketamine and hydroxynorketamine, suggesting that a selective presynaptic NMDA receptor interaction may contribute to the mechanism [24].

4.3 mGlu2 receptor-dependent signaling

Zanos et al. (2019) demonstrated that (2R,6R)-HNK exerts antidepressant actions through a mechanism that converges with mGlu2 receptor signaling [9]. The mGlu2 receptor antagonist LY341495 occluded the antidepressant-like effects of (2R,6R)-HNK in the forced swim test, and mGlu2 receptor knockout mice did not respond to (2R,6R)-HNK treatment. The proposed mechanism is that (2R,6R)-HNK decreases mGlu2 receptor-mediated inhibition of presynaptic glutamate release, thereby disinhibiting glutamatergic transmission and enhancing AMPA receptor activation. This mechanism is functionally convergent with, though molecularly distinct from, the AMPA receptor potentiation described in Section 4.1.

4.4 Alpha-7 nicotinic acetylcholine receptor antagonism

Bhatt et al. (2024) identified the alpha-7 nicotinic acetylcholine receptor (alpha-7 nAChR) as a molecular target mediating HNK's control of presynaptic function and gene expression [10]. In this study, (2R,6R)-HNK but not ketamine acted through alpha-7 nAChR antagonism to induce rapid inhibition of glutamate release by reducing the release competence of synaptic vesicles and to promote nuclear translocation of phosphorylated CREB that controls expression of neuroplasticity genes including Arc and Homer1a. Selective antagonism of alpha-7 nAChR or genetic deletion of the Chrna7 gene fully abolished HNK-induced synaptic and nuclear signaling. This finding positions alpha-7 nAChR antagonism as a mechanism that distinguishes HNK from ketamine at the molecular level.

4.5 BDNF-TrkB-mTORC1 signaling cascade

The downstream signaling cascade from AMPA receptor activation to synaptogenesis proceeds through the following established steps [7, 8]. AMPA receptor-mediated sodium and calcium influx triggers activity-dependent vesicular release of BDNF from the postsynaptic neuron. BDNF binds TrkB receptors, activating the MEK-ERK and PI3K-Akt signaling cascades. These converge on mTORC1, which stimulates local translation of synaptic proteins including PSD-95, GluA1, and synapsin. The result is rapid formation of new dendritic spines and functional synapses in layer V pyramidal neurons of the medial prefrontal cortex, a structural correlate of the antidepressant response. Fukumoto et al. (2019) confirmed that BDNF signaling through TrkB is required for the antidepressant actions of (2R,6R)-HNK: BDNF Val66Met knockin mice (which have impaired activity-dependent BDNF release) and conditional BDNF knockout mice did not respond to (2R,6R)-HNK treatment [8].

4.6 Opioid receptor interactions

Bhatt et al. (2021) examined the interaction of (2R,6R)-HNK with mu and kappa opioid receptors and found that the compound shows low-affinity binding to both receptor types [25]. However, at pharmacologically relevant concentrations, (2R,6R)-HNK does not activate opioid receptors and does not produce opioid-like behavioral effects. The kappa opioid receptor an-

tagonist nor-binaltorphimine did not block the antidepressant-like effects of (2R,6R)-HNK in mice, and naltrexone pretreatment did not attenuate the behavioral effects of HNK, in contrast to its attenuation of ketamine's effects [26]. These findings support the conclusion that the antidepressant mechanism of (2R,6R)-HNK does not involve opioid receptor activation.

4.7 Composite mechanism summary

The composite mechanism of (2R,6R)-HNK antidepressant action involves: (a) reduction of mGlu2 receptor-mediated presynaptic inhibition and α -7 nAChR-mediated presynaptic regulation, leading to enhanced glutamate release; (b) potentiation of postsynaptic AMPA receptor-mediated transmission; (c) activity-dependent BDNF release and TrkB-mTORC1 signaling activation; and (d) rapid synaptogenesis in prefrontal cortical and hippocampal circuits. This mechanism is NMDA receptor-independent at therapeutic concentrations, distinguishing (2R,6R)-HNK from ketamine, esketamine, and other NMDA receptor-based antidepressant strategies.

5. Pharmacokinetics and Routes of Administration

5.1 Metabolic formation from ketamine

Hydroxynorketamine is formed *in vivo* through a two-step metabolic sequence from ketamine [1, 2]. The first step is N-demethylation of ketamine to norketamine, catalyzed predominantly by hepatic CYP2B6 and CYP3A4, with minor contributions from CYP2C9 and CYP2C19. N-demethylation accounts for approximately 80 percent of ketamine metabolism in humans. The second step is hydroxylation of norketamine at the 6-position of the cyclohexane ring, catalyzed predominantly by CYP2B6 and CYP2A6, to yield 6-hydroxynorketamine. This second step accounts for approximately 15 percent of the ketamine dose as circulating HNK metabolites.

The stereochemistry of HNK formation is determined by the stereochemistry of the parent ketamine enantiomer: (R)-ketamine produces (R)-norketamine, which is hydroxylated to yield (2R,6R)-HNK (the *trans*-isomer) as the major product; (S)-ketamine produces (S)-norketamine, which yields (2S,6S)-HNK. Following racemic ketamine infusion, both (2R,6R)-HNK and (2S,6S)-HNK are detected in human plasma, with (2R,6R)-HNK and (2S,6S)-HNK being the major circulating HNK species [1].

5.2 Pharmacokinetics of exogenous (2R,6R)-HNK

The Phase 1 clinical trial (NCT04711005) characterized the pharmacokinetics of intravenous (2R,6R)-HNK in healthy volunteers through a six-level single ascending dose (SAD) study at 0.1, 0.25, 0.5, 1, 2, and 4 mg/kg and a two-level multiple ascending dose (MAD) study at 1 and 2 mg/kg [12]. The compound demonstrated dose-proportional pharmacokinetics across the SAD dose range. Peak plasma concentrations were achieved at the end of the intravenous infusion (approximately 40 minutes), with subsequent biphasic decline. The terminal elimination half-life was consistent with once-daily or intermittent dosing. Specific numerical pharmacokinetic parameters from the Phase 1 trial (*C*_{max}, AUC, clearance, volume of distribution) have been reported by Raja et al. (2024) and are consistent with the preclinical pharmacokinetic profile [12].

5.3 Brain penetration

Preclinical studies in mice and rats have consistently demonstrated that (2R,6R)-HNK and (2S,6S)-HNK readily penetrate the blood-brain barrier following intraperitoneal, subcutaneous, and intravenous administration [2, 27]. Brain-to-plasma ratios vary by species and route of administration but are sufficient to achieve pharmacologically active concentrations in hip-

pocampus, prefrontal cortex, and other corticolimbic regions. The brain penetrance of all twelve HNK stereoisomers has been characterized, with robust differences in peak brain concentrations and exposures among the isomers [27].

5.4 Metabolism and elimination

The metabolic fate of exogenously administered (2R,6R)-HNK has been characterized in pre-clinical studies. The compound is subject to glucuronidation and renal excretion as the primary elimination pathway. Hepatic oxidative metabolism is a minor route compared to the parent ketamine, consistent with the terminal metabolite positioning of HNK in the ketamine metabolic pathway. The compound does not undergo significant back-conversion to nor-ketamine or ketamine [2].

5.5 Routes of administration

Intravenous infusion: The route used in the Phase 1 clinical trial and in ongoing Phase 2 trials. Intravenous (2R,6R)-HNK at doses of 0.1 to 4 mg/kg delivered over 40 minutes produced dose-proportional exposure and was well tolerated [12].

Intraperitoneal injection: The principal route in preclinical rodent studies. Doses of 10 to 50 mg/kg intraperitoneal in mice produce robust antidepressant-like, anxiolytic, and analgesic effects [5, 13].

Oral administration: Preclinical studies have demonstrated oral bioavailability of (2R,6R)-HNK in rodents, supported by the high aqueous solubility of the hydrochloride salt. A United States patent describes crystalline forms with pharmaceutical-grade oral bioavailability characteristics [4]. Oral formulation for clinical use has not been publicly reported but is plausible given the physicochemical properties.

Subcutaneous injection: Evaluated in preclinical models; effective and well tolerated in rodents.

5.6 CYP2B6 polymorphism considerations

Because the formation of HNK from ketamine depends on CYP2B6 activity, and because CYP2B6 is a polymorphic enzyme (with clinically relevant loss-of-function alleles including CYP2B6*6, *16, and *18), the in vivo exposure to HNK following ketamine administration varies among individuals as a function of CYP2B6 genotype [28]. This polymorphism is relevant to the interpretation of ketamine clinical trial data (differential HNK formation may contribute to inter-individual variability in ketamine antidepressant response) but is not directly relevant to the pharmacokinetics of exogenously administered (2R,6R)-HNK.

6. Preclinical Pharmacology

6.1 Antidepressant-like effects

(2R,6R)-HNK produces rapid and sustained antidepressant-like effects in multiple preclinical paradigms. In the forced swim test, (2R,6R)-HNK at 10 mg/kg intraperitoneal reduces immobility within 1 hour of administration, with effect duration extending to at least 24 hours and in some reports to 3 days [5]. In the learned helplessness model, (2R,6R)-HNK reverses escape deficits following exposure to uncontrollable stress [5]. In the chronic social defeat stress model, (2R,6R)-HNK reverses social avoidance and anhedonia (measured by sucrose preference) [18]. In the novelty-suppressed feeding paradigm, (2R,6R)-HNK reduces the latency to feed in a novel environment, an anxiolytic and antidepressant-relevant readout. The sustained duration of effect (extending well beyond the plasma elimination half-life) is consistent with a synaptic plasticity-dependent mechanism rather than a receptor occupancy-dependent mechanism [2].

6.2 Anxiolytic effects

(2R,6R)-HNK reduces anxiety-like behavior in the elevated plus maze, open field test, and novelty-suppressed feeding paradigms in rodents [18]. The anxiolytic effects are observed at doses that also produce antidepressant-like effects, suggesting a common underlying mechanism involving glutamatergic plasticity in corticolimbic circuits.

6.3 Analgesic effects

Preclinical evidence across multiple pain models demonstrates that (2R,6R)-HNK produces potent and sustained analgesic effects [13, 29, 30]. In a model of localized inflammatory pain (complete Freund's adjuvant), (2R,6R)-HNK reversed mechanical hypersensitivity with onset less than 1 hour and duration exceeding 24 hours, an effect comparable to the NSAID carprofen [29]. In murine models of acute pain (hot plate, acetic acid writhing), (2R,6R)-HNK produced dose-dependent analgesia [30]. In a neuropathic pain model (spared nerve injury), (2R,6R)-HNK reversed mechanical allodynia. A 2026 study confirmed the compound as a novel analgesic in multiple animal models of pain, including inflammatory, neuropathic, and visceral paradigms [31]. Notably, the analgesic effects lack the sedative, dissociative, and abuse-related properties of the parent ketamine.

6.4 Anti-inflammatory effects

(2R,6R)-HNK has demonstrated anti-inflammatory activity in preclinical models, consistent with the known anti-inflammatory actions of AMPA receptor-dependent BDNF signaling and with the alpha-7 nAChR-mediated regulation of neuroinflammatory gene expression [10]. The anti-inflammatory mechanism may contribute to both the analgesic and antidepressant effects, given the established role of neuroinflammation in chronic pain and in the pathophysiology of major depressive disorder.

6.5 Substance use disorder models

(2R,6R)-HNK has been evaluated in rodent models of opioid and alcohol use disorder. Shaffer et al. (2025) reported that (2R,6R)-HNK prevents opioid abstinence-related negative affect and stress-induced reinstatement of morphine-seeking behavior in mice [32]. Additional studies have demonstrated that (2R,6R)-HNK facilitates extinction and prevents emotional impairment in morphine-abstinent mice [33]. These findings position HNK as a candidate for adjunctive treatment in substance use disorders, though clinical evidence is absent.

6.6 Absence of abuse liability

(2R,6R)-HNK does not produce conditioned place preference, does not support self-administration, does not induce locomotor sensitization, and does not substitute for ketamine or phencyclidine in drug discrimination assays in rodents [5, 14]. The absence of abuse-related behaviors is a critical differentiator from the parent ketamine and from esketamine and supports the favorable safety profile for clinical development.

7. Clinical Evidence Base

7.1 Phase 1 clinical trial

The Phase 1 clinical trial (NCT04711005) was a randomized, double-blind, placebo-controlled study conducted at the NIH Clinical Center, evaluating the safety, tolerability, pharmacokinetics, and pharmacodynamics of intravenous (2R,6R)-HNK in healthy volunteers [12]. The study design included a single ascending dose (SAD) component at six dose levels (0.1, 0.25, 0.5, 1, 2, and 4 mg/kg) and a multiple ascending dose (MAD) component at two dose levels (1 and 2 mg/kg). Results, published by Raja et al. in *Clinical Pharmacology and Therapeutics* in 2024, demonstrated:

- No serious adverse events at any dose level
- No dissociative effects at any dose level, as measured by the Clinician-Administered Dissociative States Scale (CADSS)
- No anesthetic effects at any dose level
- No clinically significant changes in vital signs, electrocardiographic parameters, or laboratory values
- Dose-proportional pharmacokinetics in the SAD component
- Acceptable tolerability in the MAD component

The most common adverse events were mild and transient: dizziness, somnolence, and headache. No dose-limiting toxicities were identified. The pharmacodynamic assessment included electroencephalographic biomarkers and subjective mood measures, though the study was not powered for efficacy in a healthy volunteer population [12].

7.2 Ongoing Phase 2 trials

Treatment-resistant depression (NCT06511908): A Phase 2, randomized, double-blind, placebo-controlled, crossover study evaluating the efficacy, safety, and pharmacokinetics of intravenous (2R,6R)-HNK in adults with treatment-resistant major depressive disorder. The primary endpoint is change in depression severity scores. This trial represents the first controlled efficacy assessment of (2R,6R)-HNK in a psychiatric population [19].

Neuropathic pain (NCT05864053): A Phase 1/2, randomized, double-blind, three-way crossover study comparing a single infusion of (2R,6R)-HNK (0.5 mg/kg), ketamine (0.5 mg/kg), and saline placebo on pain and quality of life outcomes in adults with chronic neuropathic pain of the extremities. The study began enrolling in September 2024 with an estimated completion date of December 2026 [20].

Obsessive-compulsive disorder (NCT06575075): A clinical trial evaluating (2R,6R)-HNK in obsessive-compulsive disorder, consistent with preclinical evidence of anxiolytic effects and with the broader hypothesis that glutamatergic plasticity-enhancing compounds may benefit obsessive-compulsive spectrum disorders [21].

7.3 Evidence from ketamine clinical trials

Indirect clinical evidence for HNK's role in ketamine's antidepressant effect comes from pharmacokinetic-pharmacodynamic analyses of ketamine clinical trials. Multiple studies have reported correlations between plasma (2R,6R)-HNK concentrations and antidepressant response following ketamine infusion, supporting the hypothesis that HNK formation contributes to ketamine's clinical efficacy [1, 34]. However, the correlational nature of these analyses does not establish causality, and the ongoing Phase 2 trials with exogenous (2R,6R)-HNK will provide the definitive test of whether the metabolite produces antidepressant effects in humans independent of the parent compound.

8. Sourcing and Quality Verification

Research-grade (2R,6R)-hydroxynorketamine hydrochloride is supplied by multiple chemical and pharmaceutical research suppliers at greater than 98 percent purity by HPLC. Principal suppliers include:

- Sigma-Aldrich (catalog SML1873): (2R,6R)-hydroxynorketamine, greater than or equal to 98 percent purity by HPLC
- Tocris Bioscience / Bio-Techne (catalog 6094): (2R,6R)-hydroxynorketamine hydrochloride
- Cayman Chemical (catalog 19603): (2R,6R)-hydroxynorketamine hydrochloride, CAS 1430202-69-9
- MedChemExpress, Invivochem, and additional specialty suppliers

The (2S,6S)-HNK enantiomer is also available from Tocris Bioscience (catalog 6095) and other suppliers for comparative pharmacological studies.

Pricing is moderate to high relative to common pharmacological tools, reflecting the stereoselective synthesis requirements; typical pricing is in the range of \$100 to \$500 per 10 milligrams at the time of monograph preparation.

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

- High-performance liquid chromatography area-percent purity (greater than 98 percent)
- Mass spectrometry confirmation of molecular weight (expected [M+H]⁺ for free base: 228.08)
- Chiral chromatography confirmation of enantiomeric purity (greater than 99 percent enantiomeric excess of the (2R,6R) form, since the (2S,6S) enantiomer has distinct pharmacological activity)
- Certificate of analysis from the vendor, with batch-specific data

Clinical-grade (2R,6R)-HNK for the ongoing clinical trial program is manufactured under Current Good Manufacturing Practice (cGMP) conditions under the direction of NCATS. This material is not commercially available; investigators conducting clinical research should coordinate with NCATS for supply.

9. Reconstitution and Handling

(2R,6R)-Hydroxynorketamine hydrochloride is soluble in water and in DMSO. For in vitro studies, stock solutions are typically prepared at 10 to 100 mM in DMSO and diluted to working concentrations in physiological buffer. For in vivo studies in rodents, the compound is dissolved in normal saline (0.9 percent sodium chloride) at concentrations of 1 to 10 mg/mL for intraperitoneal or intravenous injection. The high aqueous solubility of the hydrochloride salt facilitates direct dissolution in saline without the need for organic co-solvents.

For DMSO stock solutions intended for in vivo use, the DMSO stock can be mixed with PEG300, Tween 80, and sterile water to produce injectable formulations compatible with rodent pharmacokinetic studies.

Storage of the solid compound is recommended at -20 degrees Celsius, protected from light and moisture. Aqueous solutions should be prepared fresh for each experiment; if storage is necessary, aliquoting and freezing at -20 degrees Celsius is recommended. DMSO stock solutions are stable for at least 3 months at -20 degrees Celsius. Batch-specific molecular weights may vary due to hydration state; investigators should consult the certificate of analysis for each lot.

The compound is supplied in milligram quantities (1, 5, 10, 25 mg vials) from most commercial suppliers. Gram-scale availability may require custom synthesis or coordination with specialty chemical manufacturers.

10. Stack Interactions and Combinations

Investigators conducting research with (2R,6R)-HNK, particularly in combination pharmacology studies or in subjects receiving concurrent medications, should consider the following interaction categories.

AMPA receptor modulators: Concurrent positive allosteric modulators of AMPA receptors (e.g., CX-546, LY451646, aniracetam) would be expected to produce additive or synergistic potentiation of glutamatergic transmission. The AMPA receptor antagonist NBQX blocks the antidepressant-like effects of (2R,6R)-HNK and should not be co-administered in efficacy studies [5].

NMDA receptor antagonists: Concurrent ketamine, memantine, or other NMDA receptor antagonists do not share the molecular mechanism of (2R,6R)-HNK at therapeutic concentrations. Co-administration produces additive rather than synergistic effects in some preclinical paradigms. Clinical co-administration of ketamine and (2R,6R)-HNK has not been studied and is not recommended outside a controlled research setting.

mGlu2 receptor ligands: The mGlu2 receptor antagonist LY341495 occludes the antidepressant-like effects of (2R,6R)-HNK [9]. mGlu2/3 receptor agonists (e.g., LY379268, pomaglumetad) would be expected to oppose the mechanism of HNK and should be considered as pharmacological antagonists in combination studies.

Serotonergic antidepressants: Selective serotonin reuptake inhibitors (SSRIs), serotonin-norepinephrine reuptake inhibitors (SNRIs), and other serotonergic antidepressants do not share the molecular mechanism of (2R,6R)-HNK. Preclinical combination studies have not identified synergistic toxicity. In the ongoing Phase 2 depression trial, (2R,6R)-HNK is administered as adjunctive therapy to an oral antidepressant [19].

CYP2B6 and CYP2A6 substrates/inhibitors: Although CYP2B6 and CYP2A6 are the principal enzymes forming HNK from norketamine, this interaction is relevant only to *in vivo* HNK formation from ketamine, not to exogenous HNK administration. Exogenous (2R,6R)-HNK is not a significant CYP substrate and is unlikely to produce CYP-mediated drug-drug interactions at therapeutic doses.

Opioids: (2R,6R)-HNK does not activate opioid receptors at pharmacologically relevant concentrations [25, 26]. Concurrent opioid therapy is unlikely to produce pharmacodynamic synergy or antagonism with the antidepressant mechanism of HNK, though the analgesic effects of HNK may provide additive benefit in pain management contexts.

Alpha-7 nicotinic receptor ligands: Because (2R,6R)-HNK acts as an antagonist at alpha-7 nAChR [10], concurrent alpha-7 agonists (e.g., varenicline at alpha-7, GTS-21, tropisetron) may oppose or modulate the HNK mechanism. Concurrent alpha-7 positive allosteric modulators

(e.g., AVL-3288) would interact at the same receptor through a different binding site.

11. Adverse Events and Safety

11.1 Preclinical safety

Comprehensive nonclinical toxicology studies conducted as part of the IND-enabling package for (2R,6R)-HNK demonstrated no predicted safety pharmacology liabilities across cardiovascular (hERG, telemetered dog), respiratory, and central nervous system endpoints [3, 12]. The compound did not produce seizure activity, did not affect cardiac repolarization at multiples of the clinical dose, and did not cause sedation or motor impairment at antidepressant-effective doses in rodents. Repeat-dose toxicology studies in rodents and non-human primates (as required for IND filing) were completed with acceptable safety margins.

11.2 Phase 1 clinical adverse events

The Phase 1 trial in healthy volunteers (Raja et al. 2024) reported the following adverse event profile [12]:

- No serious adverse events at any dose level (0.1 to 4 mg/kg intravenous)
- No dissociative effects as measured by the Clinician-Administered Dissociative States Scale
- No anesthetic effects
- No psychotomimetic effects
- No dose-limiting toxicities
- Most common treatment-emergent adverse events: dizziness (mild, transient), somnolence (mild, transient), headache (mild)
- No clinically significant changes in vital signs, electrocardiograms, or laboratory values
- No evidence of abuse potential or subjective drug-liking effects

11.3 Comparison to ketamine adverse events

The adverse event profile of (2R,6R)-HNK differs qualitatively from that of the parent ketamine. Ketamine at antidepressant doses (0.5 mg/kg intravenous over 40 minutes) reliably produces dissociation, perceptual disturbances, transient blood pressure elevation, nausea, and subjective drug-liking effects that contribute to its abuse potential and that limit its clinical utility. (2R,6R)-HNK at pharmacologically relevant doses in the Phase 1 trial produced none of these effects [12]. The absence of dissociative, psychotomimetic, and abuse-related adverse events is the principal safety advantage of (2R,6R)-HNK over the parent compound and the primary motivation for its clinical development as an independent therapeutic agent.

11.4 Theoretical safety considerations

As (2R,6R)-HNK enhances glutamatergic transmission through AMPA receptor potentiation, the theoretical risk of excitotoxicity at supratherapeutic doses exists, though no evidence of seizure activity or excitotoxic injury has been observed in preclinical studies at multiples of the therapeutic dose. The mTORC1 activation downstream of BDNF-TrkB signaling raises a theoretical concern about chronic proliferative stimulation, though this pathway is activated transiently and at physiological levels by (2R,6R)-HNK.

11.5 Pregnancy and lactation

Reproductive toxicology data for (2R,6R)-HNK have not been publicly reported. The compound is in early clinical development and should not be administered to pregnant or lactating individuals outside a controlled research setting with appropriate ethical oversight.

12. Comparable Compounds Assessment

This section presents a structured comparative assessment of five rapid-acting antidepressant candidates against (2R,6R)-hydroxynorketamine, 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 glutamatergic or rapid-acting antidepressant agents with substantive clinical or preclinical evidence, prioritizing those that share mechanistic relevance with HNK.

12.1 Esketamine (Spravato)

Mechanism: Potent open-channel blocker of the NMDA receptor; the (S)-enantiomer of ketamine with approximately 4-fold greater NMDA receptor affinity than (R)-ketamine. Administered as an intranasal spray.

Development trajectory: Johnson and Johnson / Janssen advanced esketamine through Phase 3 clinical trials for treatment-resistant depression (TRANSFORM studies) and major depressive disorder with acute suicidality (ASPIRE studies). FDA approval was granted in March 2019 under the brand name Spravato for treatment-resistant depression in adults, in conjunction with an oral antidepressant [35]. Subsequent approvals extended to major depressive disorder with suicidal ideation or behavior.

Novelty: Low. The mechanism is conventional NMDA receptor antagonism, shared with the parent racemic ketamine.

Effect size: Moderate to large. The TRANSFORM-2 Phase 3 trial reported a least-squares mean difference of -4.0 points on the Montgomery-Asberg Depression Rating Scale (MADRS) versus placebo at Day 28, with more pronounced differences at earlier time points (Day 2) [35]. Response rates were approximately 65 to 70 percent in the esketamine arms.

Promising potential: Established. Esketamine is the only FDA-approved rapid-acting antidepressant administered through a non-oral route for treatment-resistant depression. Post-marketing use is extensive but requires administration in a certified healthcare setting with a Risk Evaluation and Mitigation Strategy (REMS) due to dissociation and sedation concerns.

Side-effect profile: The principal limitations are dissociation (61 to 75 percent of patients in clinical trials), sedation, dizziness, nausea, vertigo, blood pressure elevation, and subjective drug effects that necessitate 2-hour post-dose monitoring. Abuse potential has been characterized in Schedule III classification. These adverse effects limit the practical utility of esketamine and are the primary motivation for developing alternatives such as (2R,6R)-HNK.

Overall validation: High. The compound has regulatory approval, extensive Phase 3 data, and post-marketing experience.

Comparison to (2R,6R)-HNK: Esketamine and (2R,6R)-HNK are mechanistically distinct: esketamine is an NMDA receptor antagonist, while (2R,6R)-HNK acts through AMPA receptor potentiation and BDNF-TrkB signaling without meaningful NMDA receptor inhibition. The critical advantage of (2R,6R)-HNK is the absence of dissociative, sedative, and abuse-related effects observed in the Phase 1 trial. The critical advantage of esketamine is the established clinical validation through Phase 3 trials and FDA approval. (2R,6R)-HNK is in Phase 2 development and has not yet demonstrated clinical antidepressant efficacy.

12.2 Arketamine (R-ketamine, PCN-101)

Mechanism: Open-channel blocker of the NMDA receptor; the (R)-enantiomer of ketamine with approximately 4-fold lower NMDA receptor affinity than esketamine. Proposed to act through BDNF-TrkB signaling and synaptogenesis rather than through NMDA receptor blockade, a hypothesis mechanistically convergent with (2R,6R)-HNK.

Development trajectory: Perception Neuroscience (a subsidiary of Atai Life Sciences) advanced arketamine (PCN-101) through Phase 2 clinical trials for treatment-resistant depression, with intravenous formulation [36]. Phase 2 results were reported in 2023 with signals of antidepressant efficacy. Additional development has proceeded in Japan under Otsuka Pharmaceutical. The compound is not FDA-approved.

Novelty: Moderate. While it shares the NMDA receptor antagonist mechanism with ketamine and esketamine, arketamine's proposed NMDA receptor-independent effects distinguish it within the enantiomer pair.

Effect size: In preclinical models, arketamine produces more potent and longer-lasting antidepressant effects than esketamine [37]. Clinical effect sizes from Phase 2 trials are modest, with separation from placebo on depression rating scales.

Promising potential: Moderate. The compound is in Phase 2 development with mixed early results.

Side-effect profile: Arketamine produces fewer dissociative effects than esketamine in preclinical models and in early clinical evaluation, consistent with its lower NMDA receptor affinity [36, 37]. However, it retains some dissociative and psychotomimetic activity at antidepressant doses.

Overall validation: Low to moderate. Phase 2 data exist; Phase 3 data do not.

Comparison to (2R,6R)-HNK: Arketamine is the direct metabolic precursor of (2R,6R)-HNK (via norketamine hydroxylation). Whether arketamine's antidepressant effects are mediated through its own NMDA receptor activity, through conversion to (2R,6R)-HNK, or through both mechanisms remains an open question. (2R,6R)-HNK has the advantage of complete absence of dissociative and abuse-related effects; arketamine retains some of these properties.

12.3 Rapastinel (GLYX-13)

Mechanism: Partial agonist at the glycine site of the NMDA receptor. Modulates NMDA receptor function without producing channel block. Enhances long-term potentiation in hippocampal circuits.

Development trajectory: Allergan (acquired by AbbVie) advanced rapastinel through Phase 2 trials for treatment-resistant depression, with initial positive results (Preskorn et al. 2015) showing antidepressant effects at a single intravenous dose without psychotomimetic side effects [38]. However, three Phase 3 trials (CLARITY studies) failed to meet their primary endpoint, and development was discontinued in 2019 [39].

Novelty: Moderate. The glycine-site partial agonist mechanism is distinct from NMDA receptor channel block and from the AMPA-dependent HNK mechanism.

Effect size: Phase 2 effect sizes were moderate (Cohen's d approximately 0.5 to 0.7). Phase 3 trials showed no significant separation from placebo.

Promising potential: Low. Development is terminated following Phase 3 failure.

Side-effect profile: The principal advantage of rapastinel was the absence of dissociative and psychotomimetic effects, similar to (2R,6R)-HNK. The compound was well tolerated in clinical trials.

Overall validation: Low. Phase 3 failure has materially reduced the credibility of the glycine-site NMDA receptor modulation approach.

Comparison to (2R,6R)-HNK: Both compounds share the attractive safety profile of absent dissociative effects. Rapastinel's failure at Phase 3 is a cautionary lesson for the field; however, the mechanisms are distinct (glycine-site NMDA receptor modulation versus AMPA receptor potentiation), and the failure of one does not predict the outcome of the other.

12.4 Zuranolone (SAGE-217, Zurzuvae)

Mechanism: Neuroactive steroid; positive allosteric modulator of synaptic and extrasynaptic GABA-A receptors. Mechanistically unrelated to the glutamatergic mechanisms of HNK, ketamine, and rapastinel.

Development trajectory: Sage Therapeutics and Biogen advanced zuranolone through Phase 3 trials for major depressive disorder (MDD) and postpartum depression (PPD). FDA approval was granted in August 2023 for postpartum depression (Zurzuvae) as a 14-day oral course [40]. Phase 3 trials in MDD showed mixed results, with two of three trials missing the primary endpoint.

Novelty: High within the rapid-acting antidepressant space. The GABAergic mechanism is orthogonal to the glutamatergic approaches.

Effect size: In the positive Phase 3 PPD trial, zuranolone showed a Hamilton Depression Rating Scale (HAM-D) improvement of -4.0 points versus placebo at Day 15. Effect in MDD was inconsistent across trials.

Promising potential: Moderate. Approved for PPD; mixed evidence in broader MDD.

Side-effect profile: The principal adverse events are somnolence (up to 20 percent), dizziness, and headache. The compound does not produce dissociation or psychotomimetic effects. A driving impairment warning is included in the label.

Overall validation: Moderate. FDA-approved for one indication (PPD) with mixed data in MDD.

Comparison to (2R,6R)-HNK: Zuranolone and (2R,6R)-HNK represent entirely different mechanistic approaches (GABAergic vs. glutamatergic). Zuranolone has the advantage of oral administration and FDA approval (for PPD). (2R,6R)-HNK has the advantage of a mechanism directly connected to the well-validated rapid antidepressant effect of ketamine. The two compounds could theoretically be complementary in combination.

12.5 AV-101 (L-4-Chlorokynurenine)

Mechanism: Prodrug of 7-chlorokynurenic acid, which is an antagonist at the glycine site of the NMDA receptor. Provides selective NMDA receptor modulation through the kynurenine pathway.

Development trajectory: VistaGen Therapeutics (now Bionomics) advanced AV-101 through a Phase 2 trial in treatment-resistant depression (ELEVATE study). The Phase 2 trial, conducted in collaboration with NIMH, failed to separate from placebo on the primary endpoint (HDRS-17) [41]. Development for depression was deprioritized; the compound was subsequently evaluated for neuropathic pain.

Novelty: Moderate. The prodrug approach to glycine-site NMDA receptor antagonism is mechanistically distinct but convergent with the broader NMDA receptor modulation strategy.

Effect size: Phase 2 trial in treatment-resistant depression showed no significant antidepressant effect versus placebo.

Promising potential: Low for depression. Limited ongoing development.

Side-effect profile: The compound was well tolerated in Phase 2, with no dissociative effects and an adverse event profile similar to placebo.

Overall validation: Low. Phase 2 failure for the primary depression indication.

Comparison to (2R,6R)-HNK: Both compounds are well tolerated and lack dissociative effects. AV-101's clinical failure further supports the hypothesis (advanced by Zanos et al.) that NMDA receptor antagonism alone may be insufficient for robust antidepressant efficacy and

that the AMPA receptor-dependent, BDNF-mediated mechanism of (2R,6R)-HNK may represent a more effective therapeutic strategy.

12.6 Comparator summary

The five comparators occupy distinct mechanistic and clinical positions. **Esketamine** is the only FDA-approved comparator with established clinical efficacy, but its dissociative, sedative, and abuse-related adverse effects are the primary motivation for alternatives. **Arketamine** is the metabolic precursor of (2R,6R)-HNK with reduced but not absent side effects and Phase 2 clinical data. **Rapastinel** demonstrated proof-of-concept at Phase 2 but failed at Phase 3. **Zuranolone** represents an orthogonal GABAergic mechanism with FDA approval for postpartum depression. **AV-101** failed at Phase 2 for depression.

(2R,6R)-Hydroxynorketamine occupies a distinctive position: it retains a direct mechanistic link to ketamine's validated antidepressant efficacy (as a metabolite and mediator of the parent compound's effects) while eliminating the dissociative, psychotomimetic, and abuse-related adverse events that limit ketamine and esketamine. The principal uncertainty is clinical: the compound has not yet demonstrated antidepressant efficacy in a controlled Phase 2 trial. The ongoing Phase 2 program in treatment-resistant depression, neuropathic pain, and obsessive-compulsive disorder will determine whether the robust preclinical evidence translates to clinical antidepressant activity in the absence of the parent compound.

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