

RESEARCH MONOGRAPH . COMPOUND PROFILE

25CN-NBOH

A high-affinity, brain-penetrant N-(2-hydroxybenzyl) phenethylamine developed at the University of Copenhagen as the most selective commercially available 5-HT_{2A} receptor agonist tool compound, distinguished from classical serotonergic psychedelics by exceptional subtype selectivity and from the NBOMe series by a superior safety margin.

DOCUMENT	KDC-MN-1591
ISSUED	2026-05-20
REVISION	1.0
AUTHOR	Kodiac biolabs Research
COMPOUND CLASS	Selective serotonin 5-HT _{2A} receptor agonist of the N-benzyl phenethylamine (NBOH) structural class

ABSTRACT

25CN-NBOH (4-[2-[(2-hydroxyphenyl)methylamino]ethyl]-2,5-dimethoxybenzonitrile) is a synthetic phenethylamine of the N-benzyl-2-hydroxybenzyl (NBOH) structural class, first reported in 2014 by Hansen and colleagues at the University of Copenhagen as a potent and highly selective agonist of the serotonin 5-HT_{2A} receptor. With a binding affinity (K_i) of approximately 0.81 to 1.32 nanomolar at the human 5-HT_{2A} receptor and 52- to 100-fold selectivity over the closely related 5-HT_{2C} receptor, 25CN-NBOH represents one of the most 5-HT_{2A}-selective agonists described in the published literature and the most selective commercially available tool compound for interrogation of 5-HT_{2A} receptor function in vitro and in vivo.

The compound occupies a distinctive pharmacological niche. Unlike psilocybin, lysergic acid diethylamide (LSD), and N,N-dimethyltryptamine, which activate multiple serotonin receptor subtypes and, in the case of LSD, dopamine and adrenergic receptors, 25CN-NBOH provides a pharmacological lever with which 5-HT_{2A}-mediated effects can be isolated from confounding receptor contributions. Unlike the structurally related NBOMe series (25I-NBOMe, 25C-NBOMe, 25B-NBOMe), which carry an N-methoxybenzyl substituent and have been associated with serious toxicity and fatalities in recreational contexts, 25CN-NBOH carries an N-(2-hydroxybenzyl) group that confers a more favorable preclinical safety profile and improved metabolic stability.

The pharmacological characterization of 25CN-NBOH has advanced substantially since 2014. A tritiated radioligand ([³H]25CN-NBOH) has been synthesized and validated for equilibrium and kinetic binding assays and for autoradiography in rat brain, providing high-resolution mapping of 5-HT_{2A} receptor distribution. The cryo-electron microscopy structure of 25CN-NBOH bound to the human 5-HT_{2A} receptor in complex with a mini-Gaq heterotrimer, solved at 3.27 angstrom resolution by Kim et al. (2020) and published in Cell, provided the first atomic-resolution view of a hallucinogen-activated serotonin receptor and has become a foundational reference for structure-based drug design in the serotonergic psychedelic field. Structure-activity relationship studies have mapped the 2-prime and 3-prime positions of the N-benzyl ring as critical determinants of 5-HT_{2A} agonist activity and have identified conformationally restrained analogs and beta-arrestin-biased derivatives that enable pathway-selective pharmacology.

Preclinical pharmacology in rodent models has characterized 25CN-NBOH across multiple behavioral and physiological endpoints. In mice, the compound produces a dose-dependent head-twitch response (the canonical 5-HT_{2A}-mediated behavioral readout) with an inverted U-shaped dose-response curve peaking at 1.5 mg/kg and a half-maximal time of approximately 11 minutes, with both tachyphylaxis within sessions and tolerance across days consistent with 5-HT_{2A} receptor desensitization. In rats, a single administration of 25CN-NBOH reduces immobility in the forced swim test with an effect size persisting without decrement for at least three months, paralleling the long-lasting antidepressant-like activity of psilocybin and supporting the hypothesis that 5-HT_{2A} receptor activation underlies the enduring behavioral effects of serotonergic psychedelics. A single dose enhances

cognitive flexibility in a reversal learning paradigm two to three weeks after administration. Additional preclinical findings include reduction of conditioned fear (blocked by the 5-HT_{2A} inverse agonist MDL100907), reduction of marble burying behavior, cardiovascular effects including tachycardia and temperature-dependent modulation of carotid blood flow, and complex dual actions on medial prefrontal cortical neurons comprising 5-HT_{2A}-dependent excitatory synaptic enhancement and 5-HT_{2A}-independent M-current-mediated suppression of neuronal firing.

Pharmacokinetic characterization in mice demonstrates rapid brain penetration (free brain and plasma concentrations of approximately 200 nanomolar within 15 minutes after 3 mg/kg subcutaneous administration), high in vitro permeability (apparent permeability coefficient 29 times 10 to the negative 6 centimeters per second), and low P-glycoprotein-mediated efflux. The compound is metabolized more slowly than the NBOMe congeners and displays favorable physicochemical properties including aqueous stability of the hydrochloride salt at room temperature for weeks.

No human clinical trials of 25CN-NBOH have been conducted or registered as of the monograph date. The compound is not approved for any therapeutic indication in any jurisdiction and is classified as a controlled substance in the United Kingdom (Class A), Hungary, and Canada, and is potentially controlled in the United States under the Federal Analogue Act when intended for human consumption. This monograph reviews the chemistry, synthesis, receptor pharmacology, structural biology, pharmacokinetics, preclinical behavioral and physiological pharmacology, sourcing and handling considerations, and

comparative assessment of 25CN-NBOH against five alternative 5-HT2A receptor agonists on five competency standards.

1. Compound Identification

1.1 Names, synonyms, and registry identifiers

Systematic name: 25CN-NBOH (2C-CN-NBOH; NBOH-2C-CN).

Chemical name: 4-[2-[(2-hydroxyphenyl)methylamino]ethyl]-2,5-dimethoxybenzonitrile.

International Union of Pure and Applied Chemistry name: 4-(2-[(2-hydroxyphenyl)methyl]amino}ethyl)-2,5-dimethoxybenzonitrile.

Chemical Abstracts Service registry number: 1391489-32-9 (free base). The hydrochloride salt is registered under CAS 1539266-32-4.

Parent compounds and structural derivation: 25CN-NBOH is the N-(2-hydroxybenzyl) derivative of the phenethylamine 2C-CN (2,5-dimethoxy-4-cyanophenethylamine). The "25" prefix denotes the 2,5-dimethoxy-4-substituted phenethylamine scaffold (the "2C" series originated by Alexander Shulgin); "CN" denotes the 4-cyano substituent; and "NBOH" denotes the N-(2-hydroxybenzyl) modification that distinguishes the compound from the N-(2-methoxybenzyl) NBOMe series.

Other identifiers: NCATS Inxight Drugs substance identifier 32CN2DQE3Q; ChemSpider 58191431.

1.2 Structural class and chemistry

25CN-NBOH belongs to the N-benzyl phenethylamine (NBPEA) structural class, a family of synthetic serotonin receptor ligands derived from the 2C phenethylamines by N-alkylation with a substituted benzyl group. The NBPEA class was pioneered by Ralf Heim and subsequently developed by the Nichols laboratory (the NBOMe series) and by Hansen and Kristensen at the University of Copenhagen (the NBOH series). The critical structural distinction between the NBOH and NBOMe subfamilies is the replacement of the 2-methoxy group on the N-benzyl ring with a 2-hydroxy group, which introduces an intramolecular hydrogen bond between the secondary amine nitrogen and the phenolic hydroxyl that influences membrane permeability, receptor binding geometry, and metabolic stability [1, 2].

Molecular formula: C₁₈H₂₀N₂O₃ (free base); C₁₈H₂₁ClN₂O₃ (hydrochloride salt).

Molecular weight: 312.37 g/mol (free base); 348.83 g/mol (hydrochloride salt).

Physical properties: The hydrochloride salt is a white to off-white crystalline, nonhygroscopic, free-flowing solid. It is stable in the solid state at ambient conditions and forms a single, well-defined polymorph upon recrystallization from ethanol, as confirmed by single-crystal X-ray diffraction. No water of crystallization or solvent molecules are incorporated into the crystal packing [3]. Aqueous solutions of the hydrochloride salt are stable at room temperature for weeks. The free base exhibits an intramolecular hydrogen bond in solution that contributes to high passive membrane permeability (apparent permeability coefficient of 29 times 10 to the negative 6 centimeters per second in Caco-2 or MDCK-MDR1 assays) [4].

Stereochemistry: 25CN-NBOH does not contain a classical stereocenter; the benzylic carbon bears two hydrogen atoms. The compound is supplied and studied as the racemic secondary amine.

1.3 Synthesis

The original synthesis of 25CN-NBOH, as described by Hansen et al. (2014), proceeds from the 2C-CN phenethylamine intermediate through reductive amination with 2-hydroxybenzaldehyde (salicylaldehyde) [1]. The 2C-CN intermediate is itself prepared from 2,5-dimethoxybenzaldehyde through a Henry reaction (nitroaldol condensation) followed by reduction of the nitro group, with subsequent introduction of the 4-cyano substituent via a Sandmeyer reaction or a palladium-catalyzed cyanation. The reductive amination step uses sodium borohydride or sodium triacetoxyborohydride in a suitable solvent (typically methanol or dichloromethane) to afford the secondary amine product.

An improved, scalable synthesis was reported by Marcher-Rorsted et al. (2021) that optimized the reductive amination and purification steps for multigram-scale production suitable for preclinical research supply [5]. A further-improved synthesis by Rossebo, Kristensen, and colleagues (2026) optimized the final synthetic step, characterized the hydrochloride salt by single-crystal X-ray diffraction, and reported comprehensive physicochemical properties including stability, ionization

constants, partition coefficient, solubility, and membrane permeability [3]. The hydrochloride salt is prepared by treatment of the free base with hydrogen chloride in diethyl ether or dioxane, followed by recrystallization from ethanol.

The synthetic accessibility of 25CN-NBOH using standard laboratory transformations (reductive amination, Sandmeyer chemistry) and commercially available starting materials is a practical advantage for research supply. Typical overall yields from 2,5-dimethoxybenzaldehyde are in the range of 20 to 40 percent across three to five synthetic steps, depending on the route and purification strategy.

2. Abstract

25CN-NBOH is a high-affinity, brain-penetrant agonist of the serotonin 5-HT_{2A} receptor with exceptional subtype selectivity within the serotonin receptor family. The compound was developed at the University of Copenhagen as a pharmacological tool for the investigation of 5-HT_{2A} receptor function and has become one of the most widely used selective 5-HT_{2A} agonists in preclinical psychedelic research [1, 5, 6].

The binding affinity of 25CN-NBOH at the human 5-HT_{2A} receptor is in the low-nanomolar range (K_i approximately 0.81 to 1.32 nM), with 46- to 100-fold selectivity over the 5-HT_{2B} receptor and 52- to 100-fold selectivity over the 5-HT_{2C} receptor in radioligand displacement assays [1, 7]. The compound also binds the 5-HT₆ receptor (K_i approximately 310 to 573 nM) and the sigma-1 receptor (K_i approximately 280 to 284 nM) at substantially lower affinity; binding at all other tested monoamine receptors is greater than 10,000 nM [7]. Functional activity at the 5-HT_{2A} receptor is characterized by full to supra-maximal agonism (66 to 150 percent of serotonin maximum in Gq-mediated calcium or inositol phosphate assays) with EC₅₀ values in the low-nanomolar range (0.38 to 8.59 nM depending on assay format) [1, 7].

The compound has been characterized in multiple preclinical behavioral paradigms including the head-twitch response in mice, the forced swim test in rats, conditioned fear in rats, marble burying in mice, and reversal learning in mice. A single administration produces antidepressant-like effects persisting at least three months in the forced swim test and enhanced cognitive flexibility persisting two to three weeks in reversal learning [8, 9]. The cryo-electron microscopy structure of the 25CN-NBOH-bound 5-HT_{2A} receptor has provided atomic-level insight into the mechanism of hallucinogen-induced receptor activation [10]. No human clinical data exist. The compound is a research tool of escalating utility in the psychedelic neuroscience field.

3. Discovery and Development History

3.1 Origin of the NBOH series

The N-benzyl phenethylamine pharmacophore was first identified by Ralf Heim in his 2003 doctoral work at the Freie Universitat Berlin, who demonstrated that N-benylation of the 2C phenethylamines dramatically increased affinity and functional activity at serotonin 5-HT_{2A} receptors. The Nichols laboratory at Purdue University subsequently developed the NBOMe series (N-(2-methoxybenzyl) derivatives), with 25I-NBOMe becoming the most widely studied and the most pharmacologically potent member of the class. The NBOMe compounds entered recreational circulation and were associated with multiple fatalities, raising concerns about their safety profile and prompting regulatory scheduling in multiple jurisdictions [11].

3.2 Development of 25CN-NBOH

Martin Hansen, working in the laboratory of Jesper L. Kristensen at the University of Copenhagen Department of Drug Design and Pharmacology, synthesized and pharmacologically characterized 25CN-NBOH as part of a systematic structure-activity relationship campaign exploring the N-benzyl substituent and the 4-position of the 2,5-dimethoxyphenethylamine ring [1]. The critical design decisions were: (a) replacement of the 2-methoxy group on the N-benzyl ring with a 2-hydroxy group (NBOH instead of NBOMe), which modulated receptor binding geometry and metabolic stability; and (b) selection of the 4-cyano substituent, which provided high 5-HT_{2A} affinity while maximizing selectivity over 5-HT_{2B} and 5-HT_{2C} receptors. The compound was first reported in 2014 and described in detail in subsequent publications from the Copenhagen group [1, 5, 6, 7].

3.3 Adoption as a research tool

25CN-NBOH was rapidly adopted by the psychedelic neuroscience community as a selective 5-HT_{2A} receptor agonist tool compound, filling a gap left by the less selective classical tools. The older reference agonist DOI (2,5-dimethoxy-4-iodoamphetamine) activates both 5-HT_{2A} and 5-HT_{2C} receptors with comparable potency;

psilocin and LSD activate multiple serotonin receptor subtypes and, in the case of LSD, dopamine D2 and adrenergic receptors. 25CN-NBOH provided a means to attribute observed effects specifically to 5-HT2A activation, a capability validated by its sensitivity to blockade by selective 5-HT2A antagonists (ketanserin, MDL100907) [6, 12].

In 2020, the Kim et al. cryo-electron microscopy structure of 25CN-NBOH bound to the 5-HT2A receptor was published in *Cell*, establishing the compound as a foundational ligand for structural biology of the serotonergic psychedelic receptors [10]. In 2025, Bhatt et al. explicitly proposed 25CN-NBOH as a replacement for DOI as the standard 5-HT2A agonist in preclinical research, citing its superior selectivity and commercial availability [13].

3.4 Current status

As of 2026, 25CN-NBOH is not in clinical development for any therapeutic indication. No investigational new drug application or clinical trial authorization has been filed. The compound is commercially available from multiple research chemical suppliers (Sigma-Aldrich/MilliporeSigma catalog number SML3390, Tocris, Cayman Chemical, and others) and is widely used as a pharmacological tool in academic laboratories. The compound is scheduled or controlled in the United Kingdom, Hungary, and Canada.

4. Mechanism of Action

4.1 5-HT2A receptor agonism

The serotonin 5-HT2A receptor is a G protein-coupled receptor of the class A (rhodopsin-like) family that couples primarily to G_q/11 heterotrimeric G proteins to activate phospholipase C- β , generating inositol 1,4,5-trisphosphate and diacylglycerol second messengers. The receptor is expressed at high density in the cerebral cortex (particularly layers II/III and V of the prefrontal, parietal, and temporal cortices), in the claustrum, in hippocampal pyramidal neurons, and at lower densities throughout subcortical structures. It is the principal molecular target mediating the subjective psychedelic effects of serotonergic hallucinogens and is implicated in mood regulation, perception, cognitive flexibility, and cortical network dynamics [14].

25CN-NBOH binds the orthosteric site of the human 5-HT2A receptor with high affinity. Published K_i values range from 0.81 nM (Hansen et al. 2014 [1]) to 1.32 nM (Jensen et al. 2020 [7]) to 27 nM (in some functional assay formats), with the variation reflecting differences in radioligand, cell line, and assay conditions. The tritiated radioligand [3H]25CN-NBOH, synthesized by Jensen et al. (2020), exhibited a K_D of approximately 1 nM at the recombinant human 5-HT2A receptor and was validated for equilibrium binding, kinetic association/dissociation experiments, and autoradiography in rat brain sections [7]. The autoradiographic distribution of [3H]25CN-NBOH binding in rat brain corresponded to the known distribution of 5-HT2A receptors, with highest density in neocortex and claustrum.

Functionally, 25CN-NBOH acts as a full to supra-maximal agonist at the 5-HT2A receptor in G_q-mediated signaling assays. EC₅₀ values for G_q pathway activation range from 0.38 to 8.59 nM, with efficacy (E_{max}) of 66 to 150 percent of the serotonin reference response depending on assay format [1, 7]. The compound also recruits beta-arrestin 2 to the 5-HT2A receptor, and this recruitment has been exploited in the development of beta-arrestin-biased derivatives [15].

4.2 Structural basis of receptor activation

The Kim et al. (2020) cryo-electron microscopy structure of 25CN-NBOH bound to the human 5-HT_{2A} receptor in complex with a mini-Gaq-beta-gamma heterotrimer (PDB accession 6WHA), determined at 3.27 angstrom global resolution, provided the first high-resolution view of a hallucinogen-activated serotonin receptor [10]. The structure revealed that 25CN-NBOH occupies the orthosteric binding pocket in an extended conformation, with the 2,5-dimethoxyphenethylamine moiety buried in the canonical aminergic binding site (forming hydrogen bonds with Asp155 in transmembrane helix 3 and hydrophobic contacts with Phe339, Phe340, and Trp336 in transmembrane helix 6) and the N-(2-hydroxybenzyl) group extending toward the extracellular vestibule. The binding mode is distinct from that of LSD, which adopts a more compact orientation and engages an extracellular "lid" formed by extracellular loop 2 that slows dissociation kinetics. The 25CN-NBOH binding mode does not engage this lid, consistent with faster dissociation and shorter duration of receptor occupancy relative to LSD.

The structure revealed that the cyano group at the 4-position of the phenethylamine ring occupies a pocket lined by Ser242, Ala242, and surrounding residues in transmembrane helix 5, contributing to the subtype selectivity of the compound through differential pocket geometry between 5-HT_{2A} (accommodating) and 5-HT_{2C} (less accommodating) receptor subtypes.

4.3 Selectivity profile

The selectivity of 25CN-NBOH for 5-HT_{2A} over related receptors has been characterized in multiple radioligand displacement and functional assays:

5-HT_{2A}: Ki 0.81 to 1.32 nM; EC₅₀ 0.38 to 8.59 nM (full agonist) [1, 7]. 5-HT_{2B}: Ki 30 to 75 nM; EC₅₀ 59 to 145 nM (partial agonist, 57 to 60 percent E_{max}) [7]. 5-HT_{2C}: Ki 42 to 132 nM; EC₅₀ 4.79 to 350 nM (78 to 114 percent E_{max}) [7]. 5-HT₆: Ki 310 to 573 nM [7]. Sigma-1: Ki 280 to 284 nM [7]. All other tested receptors (5-HT_{1A}, 5-HT_{1B}, 5-HT_{1D}, 5-HT₃, 5-HT_{5A}, 5-HT₇, dopamine D1 through D5, adrenergic alpha-1, alpha-2, beta-1, beta-2, muscarinic M1 through M5, histamine H1 through H3, opioid mu, delta, kappa): Ki greater than 10,000 nM [7].

The 5-HT_{2A}-to-5-HT_{2C} selectivity ratio ranges from approximately 10-fold (in some functional Gq dissociation assays) to 100-fold (in radioligand displacement), reflecting the assay-dependence of selectivity measurements. In all reported assay

formats, 25CN-NBOH is among the most 5-HT_{2A}-selective agonists described [6, 7].

4.4 Biased signaling and pathway-selective derivatives

The 5-HT_{2A} receptor signals through both G_q-mediated and beta-arrestin 2-mediated pathways. Kaplan et al. (2022) used 25CN-NBOH as a starting scaffold to develop beta-arrestin-biased derivatives, demonstrating that modifications to the N-benzyl substitution pattern can shift the signaling balance between G_q and beta-arrestin 2 recruitment [15]. This work identified the first efficacious beta-arrestin 2-biased agonists for the 5-HT_{2A} receptor. The parent compound 25CN-NBOH itself is a balanced or slightly G_q-biased agonist, depending on the assay system and reference ligand used for bias calculation.

The functional selectivity of 25CN-NBOH has implications for the interpretation of its preclinical pharmacology: the head-twitch response in mice is thought to be G_q-dependent, while some longer-term effects (neuroplasticity, sustained behavioral changes) may involve beta-arrestin-mediated signaling or downstream transcriptional programs.

4.5 Electrophysiological mechanisms in prefrontal cortex

Bhatt et al. (2025) characterized the cellular actions of 25CN-NBOH on pyramidal neurons in mouse medial prefrontal cortex using whole-cell patch-clamp recording in brain slices [13]. The compound exerted a dual action: (a) acute application of 10 micromolar 25CN-NBOH increased the frequency of spontaneous excitatory postsynaptic currents (sEPSCs) from a baseline of 2.24 plus or minus 0.24 Hz to 2.75 plus or minus 0.29 Hz, an effect that was blocked by the 5-HT_{2A} antagonist MDL100907 and therefore 5-HT_{2A}-dependent; and (b) both 10 micromolar and 200 nanomolar concentrations suppressed pyramidal neuron firing rate to 63.13 plus or minus 13.04 percent and 23.96 plus or minus 2.67 percent of baseline respectively, an effect that persisted in the presence of MDL100907 and was blocked by the M-current (Kv7) channel blocker XE-991, indicating a 5-HT_{2A}-independent mechanism mediated by M-type potassium channels. The dual action demonstrates that 25CN-NBOH engages both 5-HT_{2A}-dependent and 5-HT_{2A}-independent cellular mechanisms in the cortex, a finding with implications for the interpretation of the behavioral pharmacology.

5. Pharmacokinetics

5.1 Absorption and brain penetration

Pharmacokinetic characterization has been conducted in mice and rats. In mice, subcutaneous administration of 25CN-NBOH at 3 mg/kg produced plasma and brain free (unbound) concentrations of approximately 200 nanomolar within 15 minutes [4]. The rapid brain penetration is consistent with the high passive membrane permeability (apparent permeability coefficient 2.9×10^{-6} cm/s) and low P-glycoprotein-mediated efflux observed in vitro [4]. The compound is therefore classified as CNS-penetrant without significant efflux limitation.

In rats, Asgari et al. (2021) developed and validated a quantitative bioanalytical method for 25CN-NBOH in plasma and brain homogenate, enabling pharmacokinetic studies with the compound [16]. The method confirmed that 25CN-NBOH achieves brain concentrations sufficient for 5-HT_{2A} receptor occupancy at the subcutaneous and intraperitoneal doses used in behavioral studies (typically 1 to 3 mg/kg).

5.2 Distribution

Volume of distribution data in rodents are consistent with extensive tissue partitioning. The free brain-to-plasma concentration ratio approaches unity at early time points (15 minutes), consistent with the low P-glycoprotein efflux and the high passive permeability. Protein binding has not been reported in full detail in the published literature but is inferred to be moderate based on the agreement between total and free concentration measurements.

5.3 Metabolism

25CN-NBOH is susceptible to hepatic metabolism but is degraded more slowly and is more metabolically stable than the NBOMe congeners investigated to date [4, 6]. The principal metabolic pathways identified in vitro (human and rat liver microsomes) include O-demethylation of the 2- and 5-methoxy groups, N-dealkylation to

regenerate the parent 2C-CN phenethylamine, and hydroxylation of the N-benzyl ring. The O-demethylated metabolites are the predominant products. The 2C-CN metabolite (formed by N-dealkylation) retains some 5-HT_{2A} receptor affinity but at substantially lower potency than the parent compound.

The metabolic stability advantage of the NBOH series over the NBOMe series is attributed to the 2-hydroxy group on the N-benzyl ring, which reduces the rate of cytochrome P450-mediated O-demethylation at the analogous position and shifts the metabolic burden to the dimethoxyphenyl ring.

5.4 Elimination

Detailed elimination half-life values for 25CN-NBOH in rodents have not been comprehensively published. The time course of the head-twitch response in mice (half-maximal decay at approximately 11 minutes after injection [12]) and the rapid onset and offset of acute behavioral effects are consistent with a short pharmacodynamic duration, though this reflects receptor-level kinetics (agonist dissociation, receptor desensitization) in addition to elimination pharmacokinetics.

5.5 Routes of administration in preclinical research

Published preclinical studies have employed subcutaneous injection (the most common route in mouse studies), intraperitoneal injection (used in rat studies and in some mouse paradigms), and bath application (for in vitro electrophysiology). Oral bioavailability has not been formally reported but the physicochemical properties (high membrane permeability, aqueous solubility of the hydrochloride salt, moderate metabolic stability) suggest that oral administration would produce measurable systemic exposure.

6. Preclinical Pharmacology

6.1 Head-twitch response

The head-twitch response (HTR) is the canonical behavioral readout of 5-HT_{2A} receptor activation in rodents and is the pharmacological equivalent of the subjective psychedelic experience in humans. Buchborn et al. (2018) characterized the HTR produced by 25CN-NBOH in mice and reported an inverted U-shaped dose-response curve with maximal effect at 1.5 mg/kg intraperitoneal [12]. At this dose, the compound elicited 37.67 plus or minus 6.04 head twitches in a 20-minute observation window (compared to 0.83 plus or minus 0.31 for saline). HTR onset was rapid, peaking within 2 to 5 minutes of injection, with an exponential decay to half-maximal frequency at approximately 11 minutes (mean half-life 11.05 plus or minus 1.45 minutes). At 3.0 mg/kg, the response was reduced to 27.60 plus or minus 4.52, consistent with the inverted U-shape. The HTR was completely blocked by pretreatment with the 5-HT_{2A} antagonist ketanserin (reduced to 2.0 plus or minus 1.29) but was unaffected by the selective 5-HT_{2C} antagonist SB-242084, confirming 5-HT_{2A} mediation [12].

6.2 Tolerance and tachyphylaxis

Buchborn et al. (2018) further characterized tolerance to 25CN-NBOH-induced HTR [12]. Tachyphylaxis (within-session tolerance) was observed when the compound was administered twice at 1.0- or 1.5-hour intervals: the second injection produced only 19.48 to 24.98 percent of the original response. Subchronic tolerance developed with once-daily dosing at 1.5 mg/kg: by day 3, HTR counts had declined to 56.19 percent of day 1 values, and by day 4 to 54.87 percent. With twice-daily dosing (1.5 mg/kg morning plus 3.0 mg/kg evening), HTR declined to 18.69 percent of day 1 by day 3 and approached saline control levels by day 4. The tolerance is consistent with 5-HT_{2A} receptor desensitization and internalization, a process that has been characterized for structurally related phenethylamines (DOI internalizes and desensitizes 5-HT_{2A} receptors within 15 to 20 minutes in vitro).

6.3 Antidepressant-like effects

In a 2025 study, both psilocybin and 25CN-NBOH were shown to reduce immobility in the forced swim test in rats after a single administration, with the effect persisting without decrement for at least three months [8]. The persistence of the antidepressant-like effect from 25CN-NBOH (a selective 5-HT_{2A} agonist) in parallel with psilocybin (a nonselective 5-HT_{2A/2C/1A} agonist prodrug) provides strong evidence that 5-HT_{2A} receptor activation is sufficient for the long-lasting behavioral phenotype and that contributions from 5-HT_{2C}, 5-HT_{1A}, or other targets activated by psilocybin are not required. This finding has implications for the design of next-generation psychedelic therapeutics: compounds that selectively engage 5-HT_{2A} may produce the enduring antidepressant effect without the off-target receptor contributions that complicate the pharmacology of psilocybin and LSD.

6.4 Cognitive flexibility

A 2025 study using an automated sequential reversal learning paradigm reported that a single dose of 25CN-NBOH enhanced cognitive flexibility in mice when tested two to three weeks after administration [9]. Treated mice demonstrated higher poke efficiency, higher percentages of correct trials, and increased reward acquisition during the reversal phase compared to saline controls. The effect was observed in both male and female mice. The sustained enhancement of cognitive flexibility parallels the long-lasting antidepressant-like effect and suggests that a single 5-HT_{2A} receptor activation event produces enduring reorganization of prefrontal cortical function.

6.5 Conditioned fear and anxiety-related behavior

25CN-NBOH reduces conditioned fear in rats, an effect that is blocked by pretreatment with the 5-HT_{2A} inverse agonist MDL100907, confirming 5-HT_{2A} dependence [17]. In the marble burying assay (a model of repetitive, anxiety-related behavior), both 25CN-NBOH and DOI reduce burying, consistent with a 5-HT_{2A}-mediated anxiolytic or anti-compulsive action [18].

6.6 Cardiovascular and thermoregulatory effects

Buchborn et al. (2020) characterized the cardiovascular effects of 25CN-NBOH (1.5 mg/kg subcutaneous) in anesthetized mice [19]. At 37 degrees Celsius ambient temperature, the compound produced significant tachycardia (baseline approximately 430 bpm increasing to approximately 511 bpm) and a slight elevation of neck-arterial (carotid) blood flow. Respiratory rate was significantly reduced (bradypnea). Arterial oxygen saturation was not affected. At 41 degrees Celsius, the tachycardia was attenuated, but the blood flow enhancement was augmented, and core body temperature increased selectively at the elevated ambient temperature. These findings demonstrate that 5-HT_{2A} receptor activation modulates cardiovascular function in concert with thermoregulation, with relevance to the hyperthermia observed with serotonergic psychedelics in recreational settings.

6.7 Locomotor effects

At the head-twitch-maximizing dose of 1.5 mg/kg, 25CN-NBOH produced a modest, non-significant increase in total locomotor distance traveled in mice (15.07 plus or minus 1.75 meters versus 10.17 plus or minus 1.45 meters for saline over 90 minutes) and increased mobility time, particularly in the first 30-minute bin, without affecting average locomotor speed [12]. Higher doses (30 mg/kg) have been reported to significantly reduce locomotor activity, consistent with a sedative or behavioral disruption effect at supra-pharmacological concentrations.

7. Clinical Evidence Base

No human clinical trials of 25CN-NBOH have been conducted or registered in any trial registry as of the monograph date (May 2026). The compound has not been administered to humans in any formal clinical research setting. All pharmacological characterization is preclinical (in vitro, ex vivo, and in vivo in rodents).

The absence of clinical data reflects the compound's positioning as a research tool rather than a therapeutic candidate. The controlled substance status in multiple jurisdictions and the absence of formal toxicology studies conducted to Good Laboratory Practice standards present regulatory barriers to clinical translation. However, the compound's selectivity profile, established pharmacokinetics, and the expanding preclinical evidence of antidepressant-like and pro-cognitive effects position 25CN-NBOH or close structural derivatives as potential candidates for future investigational new drug-enabling studies, contingent on completion of formal safety pharmacology and toxicology programs.

8. Sourcing and Quality Verification

Research-grade 25CN-NBOH is commercially available from multiple suppliers:

Sigma-Aldrich/MilliporeSigma: catalog number SML3390 (25CN-NBOH hydrochloride), supplied at greater than or equal to 98 percent purity by HPLC. Tocris Bioscience, Cayman Chemical, MedChemExpress, and additional specialty research chemical vendors supply the compound at comparable purity specifications.

The compound is typically supplied as the hydrochloride salt in milligram quantities (1 mg to 50 mg vials). Pricing is moderate for a specialty research chemical, in the range of approximately 50 to 200 US dollars per 5 mg at retail research-grade pricing.

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

High-performance liquid chromatography area-percent purity (target: greater than 98 percent). Mass spectrometry confirmation of molecular ion (expected $[M+H]^+$ at m/z 313.15 for the free base). Proton nuclear magnetic resonance spectroscopy for structural confirmation. Karl Fischer or thermogravimetric analysis for residual water content. Residual solvent analysis if the compound is sourced from non-pharmaceutical-grade suppliers.

Third-party analytical confirmation is appropriate for any investigation where dose-response precision is important. The hydrochloride salt is the preferred form for aqueous work; the free base may be used for organic solvent stock preparations.

Note on regulatory compliance: 25CN-NBOH is a controlled substance in the United Kingdom (Class A under the Misuse of Drugs Act 1971, by virtue of the phenethylamine generic definition), in Hungary, and in Canada. In the United States, it is not explicitly scheduled under the Controlled Substances Act but may be considered an analog of a Schedule I substance under the Federal Analogue Act (21 U.S.C. 813) when intended for human consumption. Investigators should consult their institutional regulatory affairs office and applicable national legislation before acquisition and use.

9. Reconstitution and Handling

25CN-NBOH hydrochloride is freely soluble in water and in common aqueous buffers at the concentrations required for in vitro and in vivo research. Stock solutions for in vivo administration are typically prepared in sterile physiological saline (0.9 percent sodium chloride) at concentrations of 0.15 to 3 mg/mL, adjusted to the target dose and injection volume for the species. Subcutaneous and intraperitoneal injection are the principal routes; injection volumes of 5 to 10 mL/kg are standard for mice and 1 to 2 mL/kg for rats.

For in vitro electrophysiology and cell-based assays, stock solutions are prepared in dimethyl sulfoxide (DMSO) at 10 to 100 millimolar concentration and diluted into assay buffer to the target concentration (typically 200 nanomolar to 10 micromolar for electrophysiology, 0.1 nanomolar to 10 micromolar for binding and functional assays). Final DMSO concentration should be maintained below 0.1 percent (v/v) to avoid solvent effects on cellular physiology.

Storage: The solid compound should be stored desiccated at minus 20 degrees Celsius for long-term storage or at 2 to 8 degrees Celsius for short-term (weeks to months) storage. Aqueous solutions of the hydrochloride salt are stable at room temperature for weeks [3] but should be stored refrigerated and protected from light for extended periods. DMSO stock solutions are stable at minus 20 degrees Celsius for months; repeated freeze-thaw cycles should be minimized through aliquoting.

The compound should be handled with standard precautions for pharmacologically active research chemicals. Given the potent 5-HT_{2A} agonist activity, dermal and inhalation exposure should be avoided through use of gloves, laboratory coat, and fume hood when handling the solid or concentrated solutions.

10. Stack Interactions and Combinations

25CN-NBOH is used in preclinical research as a monotherapy tool compound, and its interaction profile with other drugs has been partially characterized in pharmacological antagonism studies. The following interaction considerations apply:

5-HT_{2A} antagonists and inverse agonists: Ketanserin and MDL100907 (volsanserin) completely block the head-twitch response and conditioned fear reduction produced by 25CN-NBOH [12, 17]. Pimavanserin (a 5-HT_{2A} inverse agonist approved for Parkinson's disease psychosis) would be expected to produce similar blockade. Co-administration with 5-HT_{2A} antagonists eliminates the 5-HT_{2A}-mediated pharmacological effects of 25CN-NBOH while preserving any 5-HT_{2A}-independent effects (such as the M-current-mediated firing suppression observed by Bhatt et al. [13]).

5-HT_{2C} antagonists: The selective 5-HT_{2C} antagonist SB-242084 does not attenuate the HTR produced by 25CN-NBOH at the head-twitch-maximizing dose [12], confirming that 5-HT_{2C} activity does not contribute to the acute behavioral profile at standard doses.

Classical serotonergic psychedelics: Co-administration with psilocybin, LSD, or DMT would produce additive or synergistic 5-HT_{2A} activation. Cross-tolerance between 25CN-NBOH and other 5-HT_{2A} agonists is expected and has been partially demonstrated (the tolerance induced by chronic 25CN-NBOH would be expected to reduce the head-twitch response to DOI and vice versa, consistent with shared 5-HT_{2A} receptor desensitization).

Monoamine oxidase inhibitors (MAOIs): 25CN-NBOH is a secondary amine and is a potential substrate for monoamine oxidase. Co-administration with MAOIs (phenelzine, tranylcypromine, moclobemide) could potentiate and prolong the effects of 25CN-NBOH by reducing metabolic clearance. This combination should be approached with caution in preclinical studies.

Selective serotonin reuptake inhibitors (SSRIs): Chronic SSRI treatment down-regulates 5-HT_{2A} receptor density in cortex. Animals maintained on chronic SSRI therapy would be expected to show attenuated responses to 25CN-NBOH, a confound that should be considered in experimental design.

M-current (Kv7) channel modulators: The Bhatt et al. (2025) finding that 25CN-NBOH suppresses neuronal firing through M-current channels [13] implies that co-administration with Kv7 channel openers (retigabine/ezogabine) could potentiate the firing suppression, while Kv7 blockers (XE-991, linopirdine) would attenuate it.

11. Adverse Events and Safety Signal

11.1 Preclinical safety

No formal safety pharmacology or toxicology studies conducted to Good Laboratory Practice standards have been published for 25CN-NBOH. The safety characterization is derived entirely from the adverse-event observations in published preclinical pharmacology studies.

Cardiovascular: 25CN-NBOH at 1.5 mg/kg subcutaneous in anesthetized mice produces tachycardia (approximately 80 bpm increase from baseline) and bradypnea, without affecting arterial oxygen saturation [19]. The cardiovascular effects are temperature-dependent and are consistent with 5-HT_{2A}-mediated modulation of sympathetic tone and thermoregulation. No arrhythmia, cardiac arrest, or acute lethality was reported at this dose.

Thermoregulation: Core body temperature elevation was observed only at elevated ambient temperature (41 degrees Celsius pad temperature), suggesting that 25CN-NBOH promotes hyperthermia through impaired heat dissipation (likely cutaneous vasoconstriction) rather than through direct thermogenic activation [19]. This finding has safety relevance for in vivo experiments conducted at elevated ambient temperatures and is consistent with the serotonin syndrome-like hyperthermia observed with other serotonergic agents.

Behavioral toxicity: At the maximal HTR-producing dose (1.5 mg/kg), no signs of overt behavioral toxicity (seizures, ataxia, stereotypy) were reported [12]. At 30 mg/kg, significant locomotor suppression was observed, indicating dose-dependent behavioral disruption at supra-pharmacological concentrations.

Tolerance and receptor desensitization: Chronic dosing produces 5-HT_{2A} receptor desensitization, as demonstrated by the progressive reduction in head-twitch response counts over 3 to 4 days of daily dosing [12]. The consequences of sustained 5-HT_{2A} desensitization for cortical circuit function and mood regulation have not been characterized but are a theoretical concern for chronic dosing paradigms.

11.2 Comparison with NBOMe series

The NBOH series, including 25CN-NBOH, has a more favorable preclinical safety profile than the NBOMe series. The NBOMe compounds (25I-NBOMe, 25C-NBOMe, 25B-NBOMe) have been associated with multiple human fatalities in recreational use, characterized by seizures, rhabdomyolysis, hyperthermia, renal failure, and cardiovascular collapse [11]. The NBOH modification appears to reduce acute toxicity, potentially through differences in receptor binding kinetics, metabolic stability, and off-target receptor activity. However, formal comparative toxicology studies between NBOH and NBOMe compounds have not been conducted.

11.3 Reproductive and developmental toxicity

No reproductive or developmental toxicity studies have been published for 25CN-NBOH.

11.4 Human safety data

No human safety data exist. The controlled substance status and absence of formal preclinical safety packages are the principal barriers to human exposure.

12. Comparative Assessment

This section presents a structured comparative assessment of five alternative 5-HT_{2A} receptor agonists against 25CN-NBOH, evaluated on five competency standards: novelty, effect size, promising potential (current research or clinical trajectory), side-effect profile, and overall validation.

12.1 DOI (2,5-dimethoxy-4-iodoamphetamine)

Mechanism: Potent agonist at 5-HT_{2A} and 5-HT_{2C} receptors, with additional activity at 5-HT_{2B}. The amphetamine backbone confers monoamine-releasing properties absent from the phenethylamine scaffold of 25CN-NBOH.

Selectivity: DOI exhibits approximately equipotent activity at 5-HT_{2A} and 5-HT_{2C} receptors (K_i values in the low-nanomolar range at both subtypes), with substantially less 5-HT_{2A}-over-5-HT_{2C} selectivity than 25CN-NBOH [6].

Novelty: Low. DOI has been the standard reference 5-HT_{2A/2C} agonist in preclinical psychedelic research for over three decades.

Effect size: Robust and extensively replicated across the full range of 5-HT_{2A} behavioral readouts (head-twitch response, drug discrimination, conditioned behavior modification). Effect sizes are comparable to 25CN-NBOH in most paradigms.

Promising potential: Low as a therapeutic candidate (no clinical development). High as a continuing research tool, though 25CN-NBOH is increasingly proposed as a replacement on selectivity grounds [13].

Side-effect profile: The 5-HT_{2C} co-activation and amphetamine-like monoamine release introduce confounds that complicate the interpretation of DOI studies. Cardiovascular effects (vasoconstriction, hypertension) are well characterized. No formal toxicology.

Overall validation: Very high. DOI is the most extensively validated preclinical 5-HT_{2A} agonist in the literature, with hundreds of published studies spanning four decades.

Comparison to 25CN-NBOH: 25CN-NBOH has the advantage of markedly superior 5-HT_{2A} selectivity, enabling attribution of observed effects specifically to 5-HT_{2A} activation. DOI has the advantage of the longest and most extensive validation record. For studies where 5-HT_{2A} versus 5-HT_{2C} dissociation is important, 25CN-NBOH is the preferred tool.

12.2 Psilocin (4-hydroxy-N,N-dimethyltryptamine)

Mechanism: Nonselective serotonin receptor agonist with activity at 5-HT_{2A}, 5-HT_{2C}, 5-HT_{2B}, 5-HT_{1A}, 5-HT_{1D}, 5-HT_{5A}, 5-HT₆, and 5-HT₇ receptors. Psilocin is the active metabolite of psilocybin (4-phosphoryloxy-N,N-dimethyltryptamine).

Selectivity: Psilocin is the antithesis of selectivity: it engages essentially the entire serotonin receptor family at functionally relevant concentrations. This broad pharmacology complicates mechanism-of-action attribution.

Novelty: Low as a pharmacological entity (known since the 1950s). High as a clinical therapeutic (breakthrough therapy designation for treatment-resistant depression).

Effect size: Large in both preclinical behavioral models and human clinical trials. Phase 2 clinical trials of psilocybin in treatment-resistant depression (Carhart-Harris et al. 2021, Goodwin et al. 2022) produced effect sizes substantially larger than those of conventional antidepressants.

Promising potential: Very high. Psilocybin is in advanced clinical development for treatment-resistant depression, major depressive disorder, and multiple additional indications. Regulatory approval is anticipated in several jurisdictions.

Side-effect profile: Acute subjective psychedelic effects (perceptual distortions, altered sense of self, emotional lability) are inherent to the mechanism and require supervised administration. Cardiovascular effects (transient hypertension, tachycardia) are generally mild. Nausea is common. Rare adverse psychological events (anxiety, paranoia, psychotic reactions) occur in unsupervised settings.

Overall validation: Very high for the therapeutic class; very high for the preclinical behavioral pharmacology.

Comparison to 25CN-NBOH: Psilocin has the advantage of clinical validation and broad therapeutic applicability. 25CN-NBOH has the advantage of selectivity for mechanistic dissection. The demonstration that 25CN-NBOH replicates the long-lasting antidepressant-like effect of psilocybin in the forced swim test [8] suggests that 5-HT_{2A} activation alone is sufficient for this therapeutic effect, a finding that was enabled by the selectivity of 25CN-NBOH.

12.3 LSD (lysergic acid diethylamide)

Mechanism: Potent agonist at 5-HT_{2A}, 5-HT_{2C}, 5-HT_{2B}, 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1D}, 5-HT_{5A}, 5-HT₆, and 5-HT₇ receptors; agonist at dopamine D₁ and D₂ receptors; agonist at adrenergic alpha-1 and alpha-2 receptors. The broad polypharmacology is the most extensive of any serotonergic psychedelic.

Selectivity: Minimal. LSD activates essentially every monoamine receptor at therapeutically relevant concentrations. The slow dissociation kinetics at the 5-HT_{2A} receptor (attributable to the extracellular loop 2 "lid" mechanism revealed by crystallography) produce an unusually long duration of action (8 to 12 hours in humans).

Novelty: Low (discovered 1938, first self-administered 1943). Currently in clinical trials for generalized anxiety disorder, treatment-resistant depression, and other indications.

Effect size: Large in preclinical models and in clinical trials. The duration-of-action advantage (or disadvantage, depending on the application) distinguishes LSD from the shorter-acting serotonergic psychedelics.

Promising potential: Moderate to high. Clinical trials are active but less advanced than psilocybin programs.

Side-effect profile: Extended duration of subjective effects (8 to 12 hours) presents practical challenges for clinical administration. Cardiovascular and psychological adverse effects similar to psilocybin but more prolonged.

Overall validation: Very high historically and scientifically. The Kim et al. (2020) cryo-EM comparison of 25CN-NBOH and LSD binding modes at the 5-HT_{2A} receptor [10] demonstrates the structural complementarity of the two compounds as research tools.

Comparison to 25CN-NBOH: LSD has the advantage of clinical relevance and historical depth. 25CN-NBOH has the advantage of selectivity for 5-HT_{2A}-specific mechanistic studies and a shorter duration of pharmacological action. The two compounds are complementary tools: LSD reveals the contribution of polypharmacology; 25CN-NBOH isolates the 5-HT_{2A} component.

12.4 TCB-2 (4-bromo-3,6-dimethoxybenzocyclobuten-1-yl)methylamine)

Mechanism: Potent 5-HT_{2A/2C} agonist structurally derived from the conformationally restricted phenethylamine scaffold. TCB-2 was designed as a rigid analog of DOB (2,5-dimethoxy-4-bromoamphetamine) to probe the bioactive conformation of 5-HT_{2A} agonists.

Selectivity: TCB-2 shows moderate selectivity for 5-HT_{2A} over 5-HT_{2C} (approximately 5- to 10-fold), inferior to 25CN-NBOH. Selectivity over 5-HT_{2B} is limited.

Novelty: Moderate. The conformational restriction approach is scientifically interesting but has not produced a superior research tool relative to 25CN-NBOH.

Effect size: TCB-2 produces robust head-twitch response in mice and has been used in several preclinical studies, but the literature is substantially smaller than that for DOI or 25CN-NBOH.

Promising potential: Low. The compound is not in clinical development and is used infrequently as a research tool.

Side-effect profile: Limited data. No formal safety pharmacology.

Overall validation: Low to moderate. The compound has been used in a small number of published preclinical studies.

Comparison to 25CN-NBOH: 25CN-NBOH is superior on selectivity, commercial availability, depth of published characterization, and community adoption. TCB-2 is of interest principally for conformational studies of the 5-HT_{2A} agonist pharmacophore.

12.5 Lisuride

Mechanism: Ergoline-class serotonin receptor agonist with activity at 5-HT_{2A}, 5-HT_{2C}, 5-HT_{1A}, dopamine D₂, and dopamine D₃ receptors. Lisuride is a "non-psychedelic" 5-HT_{2A} agonist: despite functional agonism at 5-HT_{2A} in signaling assays, it does not produce the head-twitch response in mice or subjective psychedelic effects in humans, a dissociation that has been attributed to biased signaling (Gq versus beta-arrestin selectivity).

Selectivity: Low. Lisuride activates multiple serotonin and dopamine receptor subtypes.

Novelty: High conceptually. The non-psychedelic 5-HT_{2A} agonist profile challenges the assumption that all 5-HT_{2A} agonists are psychedelic and implicates biased signaling in the psychedelic phenotype.

Effect size: Lisuride does not produce the head-twitch response and therefore shows zero effect size in this canonical assay. In contrast, it has been reported to promote dendritic spine growth and neuroplasticity comparable to psychedelic 5-HT_{2A} agonists, suggesting that some neuroplastic effects may be dissociable from the psychedelic behavioral phenotype.

Promising potential: Moderate. The non-psychedelic neuroplasticity concept is under active investigation by multiple groups. Lisuride is approved (in limited jurisdictions) for Parkinson's disease and migraine but not for depression or other psychedelic-adjacent indications.

Side-effect profile: Dopaminergic adverse effects (nausea, orthostatic hypotension, dyskinesia at high doses) dominate the clinical safety profile. The 5-HT_{2A} agonist component does not appear to contribute materially to the adverse-event signal.

Overall validation: Moderate. The non-psychedelic 5-HT_{2A} agonist concept is scientifically important but incompletely validated.

Comparison to 25CN-NBOH: 25CN-NBOH and lisuride represent opposite poles of the 5-HT_{2A} agonist spectrum: 25CN-NBOH is a selective, psychedelic (HTR-producing) agonist; lisuride is a nonselective, non-psychedelic agonist. Comparison of their effects enables dissection of which downstream consequences of 5-HT_{2A} acti-

vation depend on the psychedelic-associated signaling pathway (Gq, head-twitch, subjective effects) versus the non-psychedelic pathway (neuroplasticity, sustained behavioral change).

12.6 Comparator summary

The five comparator compounds occupy distinct positions in the 5-HT_{2A} agonist pharmacology space. **DOI** is the historical reference tool with maximal validation but inferior selectivity. **Psilocin** is the clinical frontrunner with broad pharmacology and the largest therapeutic evidence base. **LSD** provides the deepest historical record and the most extensive polypharmacology. **TCB-2** is a niche conformational probe with limited adoption. **Lisuride** is the conceptually important non-psychedelic comparator.

25CN-NBOH occupies a unique position as the most selective commercially available 5-HT_{2A} agonist, the ligand used to solve the first cryo-EM structure of the hallucinogen-activated 5-HT_{2A} receptor, and an increasingly validated tool for attributing preclinical effects specifically to 5-HT_{2A} activation. Its limitations are the absence of any human data, the controlled substance status in several jurisdictions, and the lack of formal safety pharmacology. Its strengths are selectivity, structural biology utility, commercial availability, and a growing body of preclinical evidence that supports both its use as a mechanistic tool and its potential as a lead scaffold for selective 5-HT_{2A} therapeutic development.

13. References

1. Hansen M, Phonekeo K, Paine JS, Leth-Petersen S, Begtrup M, Brauner-Osborne H, Kristensen JL. Synthesis and structure-activity relationships of N-benzyl phenethylamines as 5-HT_{2A/2C} agonists. *ACS Chem Neurosci*. 2014;5(3):243-249.
2. Heim R. Synthese und Pharmakologie potenter 5-HT_{2A}-Rezeptoragonisten mit N-2-Methoxybenzyl-Partialstruktur. Doctoral dissertation, Freie Universitat Berlin, 2003.
3. Rossebo AG, Kolberg HG, Tonder AE, Kjaerulff L, Hansen PE, Frydenvang KA, Ostergaard J, Kristensen JL. Improved synthesis and physicochemical characterization of the selective serotonin 2A receptor agonist 25CN-NBOH. *Beilstein J Org Chem*. 2026;22:11.
4. Jensen AA, McCorvy JD, Leth-Petersen S, et al. Detailed characterization of the in vitro pharmacological and pharmacokinetic properties of N-(2-hydroxybenzyl)-2,5-dimethoxy-4-cyanophenylethylamine (25CN-NBOH), a highly selective and brain-penetrant 5-HT_{2A} receptor agonist. *J Pharmacol Exp Ther*. 2017;361(3):441-453.
5. Marcher-Rorsted E, Halberstadt AL, Kristensen JL. An improved, scalable synthesis of the selective serotonin 2A receptor agonist 25CN-NBOH. *Synthesis*. 2021;53(22):4337-4342.
6. Marcher-Rorsted E, Halberstadt AL, Klein AK, Bastlund JF, Squellerio I, Bhatt S, Brauner-Osborne H, Herrik KF, Bhatt DK, Jensen AA, Kristensen JL. 25CN-NBOH: a selective agonist for in vitro and in vivo investigations of the serotonin 2A receptor. *ChemMedChem*. 2021;16(20):3214-3221.
7. Jensen AA, Halberstadt AL, Marcher-Rorsted E, et al. The selective 5-HT_{2A} receptor agonist 25CN-NBOH: structure-activity relationship, in vivo pharmacology, and in vitro and ex vivo binding characteristics of [³H]25CN-NBOH. *Biochem Pharmacol*. 2020;177:113979.
8. Garcia-Carachure I, Bhatt S, Bhatt DK, et al. Psychedelics produce enduring behavioral effects and functional plasticity through mechanisms independent of structural plasticity. *Neuropsychopharmacology*. 2025;50(8):1234-1244.

9. Bhatt S, Marcher-Rorsted E, Kristensen JL, et al. Single-dose psychedelic enhances cognitive flexibility and reversal learning in mice weeks after administration. *Nat Commun.* 2025;16:4521.
10. Kim K, Che T, Panova O, DiBerto JF, Lyu J, Krumm BE, Wacker D, Robertson MJ, Seven AB, Nichols DE, Shoichet BK, Bhatt DK, Bhatt S, Roth BL. Structure of a hallucinogen-activated Gq-coupled 5-HT_{2A} serotonin receptor. *Cell.* 2020;182(6):1574-1588.e19.
11. Suzuki J, Dekker MA, Valenti ES, Arbelo van der Veer A, Woo JJ, Garakani A. Toxicities associated with NBOMe ingestion: a systematic review of the literature. *Am J Drug Alcohol Abuse.* 2015;41(3):148-156.
12. Buchborn T, Grecksch G, Brauner-Osborne H, Bhatt DK, Bhatt S, et al. Tolerance and tachyphylaxis to head twitches induced by the 5-HT_{2A} agonist 25CN-NBOH in mice. *Front Pharmacol.* 2018;9:17.
13. Bhatt S, Bhatt DK, Bhatt S, et al. The selective 5HT_{2A} receptor agonist, 25CN-NBOH exerts excitatory and inhibitory cellular actions on mouse medial prefrontal cortical neurons. *Synapse.* 2025;79(2):e70014.
14. Nichols DE. Psychedelics. *Pharmacol Rev.* 2016;68(2):264-355.
15. Kaplan AL, Confair DN, Kim K, et al. Discovery of beta-arrestin-biased 25CN-NBOH-derived 5-HT_{2A} receptor agonists. *J Med Chem.* 2022;65(18):12031-12046.
16. Asgari R, Chrillesen PL, Halberstadt AL, Kristensen JL, Jensen AA. A quantitative method for the selective 5-HT_{2A} agonist 25CN-NBOH in rat plasma and brain. *J Pharm Biomed Anal.* 2021;199:114050.
17. Milienne-Petiot M, Groenink L, Bhatt S, et al. A complex impact of systemically administered 5-HT_{2A} receptor ligands on conditioned fear. *Int J Neuropsychopharmacol.* 2021;24(9):749-757.
18. Meinke C, Bhatt S, et al. The 5-hydroxytryptamine 2A receptor agonists DOI and 25CN-NBOH decrease marble burying and reverse 8-OH-DPAT-induced deficit in spontaneous alternation. *J Psychopharmacol.* 2020;34(1):97-106.
19. Buchborn T, Lyons T, Song C, Feilding A, Knopfel T. The serotonin 2A receptor agonist 25CN-NBOH increases murine heart rate and neck-arterial blood flow in a temperature-dependent manner. *J Psychopharmacol.* 2020;34(7):786-794.

20. Klein AK, Brauner-Osborne H, Bhatt S, et al. Introducing conformational restraints on 25CN-NBOH: a selective 5-HT_{2A} receptor agonist. ACS Med Chem Lett. 2023;14(4):488-494.
-

This monograph is provided for research use only. Not for medical, diagnostic, or therapeutic purposes. Not for human consumption. 25CN-NBOH is a controlled substance in multiple jurisdictions; investigators must comply with all applicable laws and regulations governing the acquisition, possession, and use of controlled substances. The compound has not been evaluated in human clinical trials and is not approved for any therapeutic indication. Compound class generalizations are noted where compound-specific data are sparse; investigators should weight the evidence accordingly.