

RESEARCH MONOGRAPH . COMPOUND PROFILE

# PRE-084

*A phencyclidine-derived sigma-1 receptor agonist developed as a pharmacological tool compound, distinguished by high selectivity over sigma-2 and PCP receptors and by a broad preclinical literature spanning neuroprotection in motor neuron disease, stroke, Parkinson's disease, cognitive impairment, and antidepressant activity.*

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| COMPOUND CLASS | Selective sigma-1 receptor agonist derived from phencyclidine with neuroprotective, nootropic, and neurotrophic activity |

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## ABSTRACT

PRE-084 (2-(4-morpholino)ethyl 1-phenylcyclohexane-1-carboxylate) is a selective agonist of the sigma-1 receptor (sigma-1R) originally identified in 1991 by Su and colleagues at the National Institute on Drug Abuse through a systematic structure-activity program that sought to separate sigma receptor affinity from phencyclidine (PCP) receptor binding in the parent cyclohexylamine scaffold [1]. The compound binds the sigma-1 receptor with an IC<sub>50</sub> of approximately 44 nM in radioligand displacement assays and exhibits selectivity ratios exceeding 2000-fold over the PCP binding site (IC<sub>50</sub> greater than 100,000 nM) and approximately 300-fold over the sigma-2 receptor subtype (IC<sub>50</sub> approximately 13,091 nM), establishing it as one of the most widely used pharmacological tools for interrogating sigma-1 receptor function in vitro and in vivo [1, 2]. The sigma-1 receptor itself is a ligand-regulated chaperone protein resident at the mitochondria-associated endoplasmic reticulum membrane (MAM), where it modulates calcium signaling through interactions with inositol 1,4,5-trisphosphate receptors, regulates protein folding and endoplasmic reticulum stress responses, and participates in diverse signal transduction cascades including the NF-kappaB, ERK/CREB, and protein kinase C pathways [3, 4]. PRE-084 has not been advanced to human clinical trials and carries no regulatory approval in any jurisdiction; its utility is exclusively as a research-grade pharmacological probe. The preclinical literature on PRE-084 is nonetheless substantial and spans multiple therapeutic domains. In amyotrophic lateral sclerosis, daily administration of PRE-084 to SOD1-G93A transgenic mice from eight weeks of age preserved spinal

motoneuron survival, maintained compound muscle action potential amplitudes, improved locomotion, and extended overall survival, with neuroprotective effects attributed to protein kinase C-mediated phosphorylation of the NMDA receptor NR1 subunit and reduction of microglial reactivity [5, 6]. Comparable neuroprotection was demonstrated in the wobbler mouse model of motor neuron disease not linked to SOD1 mutation, broadening the mechanistic generalizability [7]. In ischemic stroke models, PRE-084 at 5 mg/kg intraperitoneally reduced infarct volume and neurological deficit scores after embolic middle cerebral artery occlusion in rats, with the mechanism involving suppression of pro-inflammatory cytokines (interleukin-1 beta, tumor necrosis factor alpha) and enhancement of anti-inflammatory cytokines [8]. In Parkinson's disease models, PRE-084 administration normalized motor dysfunction and prevented dopaminergic neuron loss in both 6-hydroxydopamine and MPTP paradigms through sigma-1 receptor-mediated promotion of PINK1/Parkin mitophagy and enhancement of dopamine transporter expression [9, 10]. Cognitive and nootropic effects have been characterized across multiple paradigms: PRE-084 attenuated MK-801-induced amnesia, amyloid-beta peptide-induced learning impairment, and spatial learning deficits in aged rats, with mechanisms involving upregulation of NMDA receptor expression in the hippocampus and activation of the ERK/CREB/BDNF signaling axis [11, 12, 13]. Antidepressant-like activity has been demonstrated in the forced swim test in multiple mouse strains at doses of 30 to 60 mg/kg, with enhanced efficacy in amyloid-beta-treated animals [14, 15]. Additional preclinical applications include cardioprotection in myocardial ischemia-reperfusion injury, neuroprotection in perinatal excitotoxic brain injury, glial modulation in spinal muscular atrophy, protection against Huntington's disease-

associated cellular degeneration through NF-kappaB-mediated calpastatin upregulation, and attenuation of sepsis-associated encephalopathy [16, 17, 18, 19, 20]. Pharmacokinetic characterization in mice after intraperitoneal administration at 10 mg/kg reveals rapid central nervous system penetration (brain concentration 773.6 ng/g at five minutes), a plasma elimination half-life of approximately 195 minutes, and stability in biological matrices for at least 24 hours [2]. The compound is supplied as the hydrochloride salt by multiple research chemical vendors at greater than 98 percent purity and is reconstituted in aqueous solution or dimethyl sulfoxide for experimental use. This monograph reviews the chemistry, synthesis, and selectivity of PRE-084; the sigma-1 receptor chaperone pharmacology in molecular detail; the pharmacokinetic profile; the full preclinical evidence base across neurodegenerative, cerebrovascular, cognitive, affective, and cardioprotective domains; sourcing and quality verification; reconstitution and handling; stack interaction considerations; adverse event and safety signal from preclinical data; and a comparative assessment of five sigma-1 receptor candidates (SA4503/cutamesine, ANAVEX2-73/blarcamesine, pridopidine, igmesine, and fluvoxamine) against PRE-084 on five competency standards.

# 1. Compound Identification

## 1.1 Names, synonyms, and registry identifiers

**Systematic name:** PRE-084.

**Chemical name:** 2-(4-Morpholino)ethyl 1-phenylcyclohexane-1-carboxylate. The name PRE-084 derives from its position in the structure-activity series developed by Su and colleagues at the Addiction Research Center of the National Institute on Drug Abuse, where "PRE" designates the phencyclidine-related ester series and "084" the compound number within that series [1].

**Development codes:** PRE-084 (Su et al., 1991). This is the canonical identifier in essentially all published pharmacology literature. No international nonproprietary name has been assigned.

**Chemical Abstracts Service registry numbers:** 138847-85-5 (hydrochloride salt), 75136-54-8 (free base). The hydrochloride salt is the form supplied by essentially all commercial research chemical vendors and is the form used in the majority of published preclinical studies.

**International Union of Pure and Applied Chemistry name:** 2-(4-morpholinyl)ethyl 1-phenylcyclohexane-1-carboxylate. Alternative systematic designations include 2-(morpholin-4-yl)ethyl 1-phenylcyclohexane-1-carboxylate and 2-(4-morpholino)ethyl 1-phenylcyclohexanecarboxylate.

**PubChem CID:** 126402.

**UNII:** 74BW8WG2MQ.

**IUPHAR/BPS ID:** 6678.

## 1.2 Structural class and chemistry

PRE-084 is a cyclohexane carboxylate ester bearing a phenyl substituent at the 1-position and a 2-(4-morpholino)ethyl group as the alcohol moiety of the ester bond. The structural class places it within the phencyclidine-derived sigma receptor ligand family, a group of compounds systematically explored by Su and colleagues

through modifications of the PCP scaffold designed to dissociate sigma receptor affinity from NMDA/PCP channel binding [1]. The key structural modification that confers sigma selectivity is the insertion of a carboxylethylene bridge between the cyclohexyl ring and the amine nitrogen, replacing the direct nitrogen-to-ring bond present in phencyclidine. This insertion produces negligible PCP receptor affinity while preserving high sigma receptor potency.

The compound contains the three pharmacophoric elements characteristic of sigma receptor ligands as defined by the Glennon pharmacophore model: (1) a hydrophobic cluster (the phenylcyclohexane ring system), (2) an amine group (the morpholine nitrogen), and (3) an intermediate chain linking the two (the ethyl ester bridge) [21].

**Molecular formula:** C<sub>19</sub>H<sub>27</sub>NO<sub>3</sub> (free base); C<sub>19</sub>H<sub>28</sub>ClNO<sub>3</sub> (hydrochloride salt).

**Exact mass:** 317.1991 g/mol (free base, monoisotopic).

**Molecular weight:** 317.43 g/mol (free base); 353.88 g/mol (hydrochloride salt).

**Physical properties:** White to off-white crystalline powder as the hydrochloride salt. Soluble in water, methanol, dimethyl sulfoxide, and ethanol. Sparingly soluble in apolar organic solvents.

**SMILES:** O=C(OCCN1CCOCC1)C2(c3ccccc3)CCCCC2.

**Stereochemistry:** PRE-084 does not contain a conventional stereocenter. The quaternary carbon at the 1-position of the cyclohexane ring bears four different substituents in the formal sense (phenyl, carboxylate ester, and two segments of the cyclohexane ring), but the compound as described in the original Su et al. publication and as supplied commercially is the racemic mixture at the cyclohexane ring conformation level. No enantiomeric resolution studies have been published for PRE-084, and the compound is used as the racemic form in all published pre-clinical work.

## 1.3 Synthesis

The synthesis of PRE-084 as described by Su et al. (1991) proceeds through esterification of 1-phenylcyclohexane-1-carboxylic acid with 2-(4-morpholino)ethanol [1]. The 1-phenylcyclohexane-1-carboxylic acid precursor is prepared by standard methods: Grignard reaction of phenylmagnesium bromide with cyclohexanone fol-

lowed by Jones oxidation, or alternatively by carboxylation of 1-phenylcyclohexyl lithium or by catalytic hydrogenation of diphenylacetic acid derivatives. The esterification is accomplished by carbodiimide-mediated coupling (1,3-dicyclohexylcarbodiimide or 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide with 4-dimethylaminopyridine catalysis) or by acid chloride formation (thionyl chloride treatment of the carboxylic acid followed by reaction with the morpholino ethanol). The product is purified by column chromatography or recrystallization and characterized by nuclear magnetic resonance spectroscopy, mass spectrometry, and high-performance liquid chromatography. The hydrochloride salt is prepared by treatment with hydrogen chloride in diethyl ether or dioxane followed by recrystallization from ethanol or isopropanol. Yields are typically 60 to 80 percent for the esterification step. The synthesis is straightforward and does not require specialized equipment; research-grade PRE-084 is manufactured at scale by multiple chemical suppliers.

## 2. Abstract

PRE-084 (2-(4-morpholino)ethyl 1-phenylcyclohexane-1-carboxylate) is a selective sigma-1 receptor agonist identified in 1991 from a structure-activity series of phen-cyclidine-derived compounds and established over the subsequent three decades as one of the most extensively characterized pharmacological tools for sigma-1 receptor research [1]. The compound binds the sigma-1 receptor with an IC<sub>50</sub> of 44 nM in the [3H](#)-pentazocine displacement assay and demonstrates greater than 2000-fold selectivity over the PCP/NMDA binding site and approximately 300-fold selectivity over the sigma-2 receptor subtype, a selectivity profile that has made it the reference agonist for in vivo sigma-1 receptor pharmacology across a wide range of disease models [1, 2].

The sigma-1 receptor is a 223-amino-acid integral membrane chaperone protein localized primarily at the mitochondria-associated endoplasmic reticulum membrane, where it forms a complex with the endoplasmic reticulum chaperone BiP (immunoglobulin heavy-chain binding protein, GRP78) under resting conditions [3]. Agonist binding promotes dissociation of the sigma-1R/BiP complex, liberating sigma-1R to chaperone client proteins including inositol 1,4,5-trisphosphate receptors (regulating calcium efflux from the endoplasmic reticulum), voltage-gated ion channels, and components of the protein quality control machinery [3, 4]. Through these interactions, sigma-1R activation modulates endoplasmic reticulum stress, mitochondrial function, calcium homeostasis, neurotrophic factor expression, and inflammatory signaling. The breadth of these downstream effects accounts for the diversity of preclinical applications documented for PRE-084.

The preclinical evidence base for PRE-084 spans five principal therapeutic domains. In motor neuron disease, PRE-084 extended survival and preserved motoneuron function in the SOD1-G93A transgenic mouse model of amyotrophic lateral sclerosis and in the wobbler mouse, with effects mediated through PKC-dependent NMDA receptor modulation, GDNF upregulation, and microglial polarization [5, 6, 7]. In cerebrovascular disease, PRE-084 reduced infarct volume and neurological deficit after embolic stroke through anti-inflammatory cytokine modulation and blood-brain barrier stabilization [8]. In Parkinson's disease, PRE-084 normalized motor function and preserved dopaminergic neurons in 6-OHDA and MPTP models through mitophagy promotion and dopamine transporter stabilization [9, 10]. In

cognitive impairment, PRE-084 reversed pharmacological and age-related amnesia through NMDA receptor potentiation and ERK/CREB/BDNF signaling [11, 12, 13]. In affective disorders, the compound demonstrated antidepressant-like activity in the forced swim test across multiple mouse strains [14, 15].

PRE-084 has not been advanced to human clinical trials. It carries no regulatory approval in any jurisdiction and is classified exclusively as a research chemical. The compound is supplied by multiple vendors (Sigma-Aldrich, Tocris, Cayman Chemical, Selleckchem, others) at greater than 98 percent purity as the hydrochloride salt. Pharmacokinetic characterization in mice demonstrates rapid central nervous system penetration after intraperitoneal administration, with brain concentrations exceeding plasma concentrations at the earliest measured time point (five minutes) and a plasma elimination half-life of approximately 195 minutes [2].

This monograph reviews the complete pharmacological profile of PRE-084 in the context of sigma-1 receptor biology, the full preclinical evidence base, practical considerations for research use, and a comparative assessment against five sigma-1 receptor ligands that have advanced further toward or into clinical development.

## 3. Discovery and Development History

### 3.1 Origin

PRE-084 was identified in 1991 by Tsung-Ping Su, Xian-Zhi Wu, Edward J. Cone, Kumud Shukla, Tina M. Gund, Amy L. Dodge, and Donald W. Parish at the Addiction Research Center of the National Institute on Drug Abuse (NIDA), part of the National Institutes of Health in Baltimore, Maryland [1]. The work was published in the *Journal of Pharmacology and Experimental Therapeutics* under the title "Sigma compounds derived from phencyclidine: identification of PRE-084, a new, selective sigma ligand." The research program was motivated by the recognition that phencyclidine, a dissociative anesthetic acting primarily at the NMDA receptor ion channel, also possessed high affinity for sigma receptors, but that the structural requirements for binding to these two sites were distinct and could be separated through systematic chemical modification.

### 3.2 Structure-activity exploration

The Su et al. program inserted methylene, ethylene, and carboxylethylene spacers between the cyclohexyl ring and the amine nitrogen of the phencyclidine scaffold. Methylene and ethylene insertions decreased affinity at the PCP/NMDA binding site while increasing affinity at sigma receptors. The carboxylethylene insertion (producing the ester linkage characteristic of PRE-084) produced compounds with negligible PCP receptor affinity ( $IC_{50}$  greater than 100,000 nM) while retaining high sigma receptor potency ( $IC_{50}$  44 nM for PRE-084) [1]. This finding confirmed that sigma receptors and PCP receptors have distinctly different structural requirements and provided a new chemical series for synthesizing sigma-selective ligands.

### 3.3 Establishment as a reference tool compound

Following its publication, PRE-084 was adopted rapidly as a reference sigma-1 receptor agonist in academic pharmacology laboratories worldwide. The compound complemented the existing sigma receptor ligand toolkit, which at the time consisted principally of (+)-pentazocine (a sigma-1 agonist with opioid receptor cross-reactivity), haloperidol (a sigma-1 antagonist with extensive dopamine receptor ac-

tivity), and BD-1047 and BD-1063 (sigma-1 antagonists developed by the same NIDA group). The high sigma-1 selectivity of PRE-084 over PCP, opioid, dopamine, and other monoamine receptors made it particularly suitable for in vivo studies where receptor selectivity determines the interpretability of behavioral and neuropathological outcomes.

### 3.4 Expansion of the preclinical literature

The preclinical literature on PRE-084 expanded substantially beginning in the early 2000s with the work of Maurice and colleagues on cognitive and antidepressant applications [11, 14], and accelerated markedly after 2005 with the motor neuron disease studies from the Navarro laboratory at the Universitat Autònoma de Barcelona [22, 5] and the Bhatt/Bhardwaj stroke studies [8]. A 2020 review by Herrando-Grabulosa and colleagues comprehensively catalogued the preclinical applications of PRE-084 across neurodegenerative, cerebrovascular, cognitive, affective, and cardioprotective domains, consolidating PRE-084 as the single most extensively characterized selective sigma-1 receptor agonist in the research literature [2]. As of the monograph revision date, PRE-084 has not been advanced to human clinical investigation. The compound remains exclusively a research tool; clinical translation of the sigma-1 receptor agonist mechanism has been pursued through other compounds in the class, notably SA4503 (cutamesine), ANAVEX2-73 (blarcamesine), pridopidine, and igmesine, each of which has entered human clinical trials for neurological or psychiatric indications.

## 4. Mechanism of Action

PRE-084 is a selective agonist of the sigma-1 receptor. The molecular pharmacology of the compound is understood through the lens of sigma-1 receptor biology, which has been substantially elucidated over the three decades since the receptor was cloned by Hanner et al. in 1996 [3].

### 4.1 Sigma-1 receptor molecular biology

The sigma-1 receptor is a 223-amino-acid integral membrane protein encoded by the SIGMAR1 gene on human chromosome 9p13.3. It is not a member of the G-protein-coupled receptor superfamily, nor of the ligand-gated ion channel superfamily, nor of the nuclear receptor superfamily. It is a unique, single-pass transmembrane chaperone protein with no structural homology to any other mammalian protein except for its approximately 30 percent sequence identity to the sterol isomerase ERG2p of yeast [3, 23].

The sigma-1 receptor is localized primarily at the mitochondria-associated endoplasmic reticulum membrane (MAM), a specialized subdomain of the endoplasmic reticulum that is physically tethered to the outer mitochondrial membrane and serves as a platform for calcium transfer, lipid biosynthesis, and autophagosome formation. Under resting conditions, the sigma-1 receptor forms a complex with the endoplasmic reticulum chaperone BiP (GRP78). Upon agonist binding, sigma-1R dissociates from BiP and becomes available to chaperone client proteins, including the type 3 inositol 1,4,5-trisphosphate receptor (IP3R3) at the MAM, thereby enhancing calcium efflux from the endoplasmic reticulum to the mitochondria and modulating mitochondrial bioenergetics [3, 4].

The receptor is expressed broadly across the central nervous system, with highest density in motoneurons of the spinal cord (particularly at cholinergic C-bouton postsynaptic densities), hippocampal pyramidal neurons, cortical neurons, cerebellar Purkinje cells, and substantia nigra dopaminergic neurons. Peripheral expression includes immune cells (macrophages, T lymphocytes), hepatocytes, and cardiac myocytes [4].

## 4.2 PRE-084 binding and selectivity

PRE-084 binds the sigma-1 receptor with an IC<sub>50</sub> of 44 nM in the [3H](#)-pentazocine radioligand displacement assay in guinea pig brain homogenate, the standard binding assay for sigma-1 receptor pharmacology [1]. Updated affinity determinations using purified recombinant sigma-1 receptor preparations have reported K<sub>i</sub> values in the range of 2 to 5 nM, consistent with the original IC<sub>50</sub> after correction for radioligand concentration and assay conditions [2].

Selectivity over other receptor systems is the defining pharmacological feature of PRE-084:

- Sigma-2 receptor: IC<sub>50</sub> approximately 13,091 nM (selectivity ratio approximately 300-fold) [2].
- PCP/NMDA channel binding site: IC<sub>50</sub> greater than 100,000 nM (selectivity ratio greater than 2000-fold) [1].
- Dopamine D1, D2, D3, D4 receptors: IC<sub>50</sub> greater than 10,000 nM [1].
- Opioid receptors (mu, kappa, delta): IC<sub>50</sub> greater than 10,000 nM [1].
- Serotonin 5-HT1A, 5-HT2A, 5-HT3 receptors: IC<sub>50</sub> greater than 10,000 nM [1].
- Alpha-1 and alpha-2 adrenergic receptors: IC<sub>50</sub> greater than 10,000 nM [1].
- Muscarinic M1, M2 receptors: IC<sub>50</sub> greater than 10,000 nM [1].

This selectivity profile is the basis for the widespread use of PRE-084 as a tool compound: behavioral and neuropathological effects observed at standard doses (typically 0.1 to 10 mg/kg in rodents) can be attributed to sigma-1 receptor activation with high confidence, provided that the standard pharmacological controls (reversal by sigma-1 antagonists such as BD-1047, NE-100, or BD-1063) are applied.

## 4.3 Downstream signaling cascades

Sigma-1 receptor activation by PRE-084 initiates several downstream signaling cascades relevant to the observed preclinical pharmacology:

**Calcium signaling.** Dissociation of the sigma-1R/BiP complex at the MAM enhances IP3R3-mediated calcium transfer from the endoplasmic reticulum to the mitochondrial matrix, supporting oxidative phosphorylation and ATP generation. This mechanism is particularly relevant to motoneuron physiology, where mitochondrial calcium buffering is critical to sustained firing capacity [4, 5].

**Protein kinase C activation.** PRE-084 induces PKC-specific phosphorylation of the NR1 subunit of the NMDA receptor at serines 896 and 897, potentiating NMDA receptor-mediated calcium influx and enhancing long-term potentiation in hippocampal circuits [5, 22]. This phosphorylation event is the principal molecular mechanism advanced for the procognitive and neuroprotective effects in motor neuron disease models.

**ERK/CREB/BDNF pathway.** In hippocampal and cortical neurons, sigma-1R activation by PRE-084 stimulates extracellular signal-regulated kinase (ERK) phosphorylation, cAMP response element-binding protein (CREB) activation, and consequent upregulation of brain-derived neurotrophic factor (BDNF) transcription and secretion [13]. This cascade is the principal mechanism for the procognitive effects in amnesic and aged-animal models.

**NF-kappaB pathway.** In models of Huntington's disease, PRE-084 activates NF-kappaB signaling, increasing cellular antioxidant defenses and upregulating calpastatin (an endogenous inhibitor of the calcium-dependent protease calpain), thereby counteracting the proteotoxic stress induced by mutant huntingtin aggregates [19].

**GDNF and neurotrophic factor expression.** PRE-084 increases the expression of glial cell line-derived neurotrophic factor (GDNF) in glial cells surrounding damaged motoneurons, contributing to neuroprotective and neurorestorative effects in spinal cord injury and motor neuron disease models [22, 5].

**Endoplasmic reticulum stress modulation.** By chaperoning misfolded proteins and stabilizing calcium homeostasis at the MAM, sigma-1R activation by PRE-084 reduces markers of the unfolded protein response (UPR) including CHOP, ATF4, and phosphorylated eIF2alpha. This is the principal mechanism for the neuroprotective effects in endoplasmic reticulum stress-driven neurodegeneration [2, 6].

**Anti-inflammatory signaling.** PRE-084 suppresses pro-inflammatory cytokine release (interleukin-1 beta, tumor necrosis factor alpha, interleukin-6) and enhances anti-inflammatory cytokine expression (interleukin-10) through modulation of microglial polarization from the M1 (pro-inflammatory) to the M2 (neuroprotective) phenotype [8, 20]. This mechanism parallels the cholinergic anti-inflammatory pathway and is relevant to stroke, sepsis, and chronic neuroinflammatory conditions.

**Mitophagy and autophagy modulation.** In Parkinson's disease models, PRE-084 promotes PINK1/Parkin-mediated mitophagy, facilitating the clearance of damaged mitochondria in dopaminergic neurons and reducing oxidative stress and apoptosis [10].

## 4.4 Composite pharmacology

The composite pharmacology of PRE-084 is therefore that of a single-target ligand (sigma-1 receptor) whose target protein functions as a pleiotropic signaling hub. The diversity of downstream effects (calcium regulation, NMDA receptor modulation, neurotrophic factor expression, endoplasmic reticulum stress management, anti-inflammatory signaling, autophagy regulation) arises not from multi-receptor binding but from the position of the sigma-1 receptor at the intersection of multiple intracellular signaling networks. This characteristic accounts for the breadth of the preclinical literature and for the observation that PRE-084 produces beneficial effects in disease models with disparate pathophysiologies (motor neuron degeneration, ischemic stroke, dopaminergic cell loss, cognitive impairment, affective dysfunction).

# 5. Pharmacokinetics and Routes of Administration

## 5.1 Absorption and distribution

Formal human pharmacokinetic data for PRE-084 do not exist, as the compound has not been administered to human subjects. Pharmacokinetic characterization has been performed in mice after intraperitoneal administration, the route used in the majority of preclinical studies.

After intraperitoneal injection at 10 mg/kg in mice, PRE-084 hydrochloride achieves rapid systemic and central nervous system exposure [2]:

- Maximum plasma concentration (C<sub>max</sub>): 659.0 plus or minus 117.1 ng/mL.
- Time to maximum plasma concentration (T<sub>max</sub>): 5 minutes.
- Brain concentration at T<sub>max</sub>: 773.6 plus or minus 26.8 ng/g.
- Spinal cord concentration at T<sub>max</sub>: 871.5 plus or minus 77.3 ng/g.

The brain-to-plasma ratio at the earliest measured time point exceeds 1.0, indicating rapid and substantial central nervous system penetration. The spinal cord concentration exceeds both brain and plasma concentrations, consistent with the high density of sigma-1 receptor expression in spinal motoneurons and relevant to the motor neuron disease applications. The rapid T<sub>max</sub> (five minutes after intraperitoneal injection) reflects the small molecular weight, moderate lipophilicity, and absence of significant efflux transporter-mediated exclusion from the central nervous system.

## 5.2 Metabolism and elimination

The plasma elimination half-life in mice after intraperitoneal administration at 10 mg/kg is 195.5 plus or minus 17.5 minutes (approximately 3.3 hours) [2]. The compound is stable in biological matrices for at least 24 hours under standard assay conditions.

Formal metabolic pathway characterization (identification of metabolizing CYP isoforms, characterization of metabolites) has not been published for PRE-084. The ester bond in the molecular structure is susceptible to hydrolysis by plasma and tissue esterases, and ester cleavage to 1-phenylcyclohexane-1-carboxylic acid and 2-(4-morpholino)ethanol is a plausible primary metabolic route. Hepatic cytochrome P450-mediated oxidation of the morpholine ring or the cyclohexane ring represents a secondary metabolic pathway. No active metabolites have been identified.

## 5.3 Routes of administration

Intraperitoneal injection is the route used in the majority of published preclinical studies. Subcutaneous administration has been used in selected studies (notably the spinal muscular atrophy work by Cervero et al. at 0.25 mg/kg/day) [18]. Intravenous administration has been reported in stroke models [8]. Oral bioavailability has not been formally characterized in published reports, and oral dosing is uncommon in the PRE-084 literature.

For in vitro studies, PRE-084 is applied directly to cell culture media at concentrations typically ranging from 1 nM to 100 micromolar, with effective concentrations for neuroprotection in the range of 10 nM to 10 micromolar depending on the cell type and the assay endpoint.

## 5.4 Dose ranges in preclinical studies

Preclinical dose ranges vary across disease models and species:

- Motor neuron disease (SOD1-G93A mice): 0.25 mg/kg/day subcutaneous [5].
- Motor neuron disease (wobbler mice): 0.25 mg/kg/day [7].
- Spinal muscular atrophy (Smn2B<sup>-/-</sup> mice): 0.25 mg/kg/day subcutaneous [18].
- Embolic stroke (rats): 5 mg/kg intraperitoneal [8].
- Parkinson's disease (6-OHDA mice): 0.3 to 1.0 mg/kg intraperitoneal [9].
- Parkinson's disease (MPTP mice): 1.0 mg/kg intraperitoneal [10].
- Perinatal brain injury (neonatal mice): 0.1 to 10 micrograms per gram body weight, single intraperitoneal injection [17].
- Cognitive impairment (aged rats): 0.3 to 1.0 mg/kg subcutaneous [12].
- Cognitive impairment (diabetic rats): dosing through ERK/CREB pathway studies [13].

- Antidepressant activity (mice): 30 to 60 mg/kg intraperitoneal [14, 15].
- Myocardial ischemia-reperfusion (rats): 1 mg/kg intraperitoneal [16].

The effective dose range across these applications spans approximately three orders of magnitude (0.1 micrograms per gram to 60 mg/kg), reflecting differences in target organ accessibility, disease model severity, and endpoint sensitivity. The motor neuron disease and Parkinson's disease applications generally employ lower doses (0.25 to 1.0 mg/kg), while the antidepressant applications require substantially higher doses (30 to 60 mg/kg), a pattern that may reflect differences in the sigma-1 receptor occupancy required for each therapeutic effect or differences in the downstream signaling thresholds for motor, dopaminergic, and serotonergic circuits.

## 6. Preclinical Pharmacology

### 6.1 Amyotrophic lateral sclerosis

The motor neuron disease application of PRE-084 represents the most extensively characterized preclinical domain and the strongest mechanistic case for sigma-1 receptor agonism as a therapeutic strategy.

Mancuso et al. (2012) reported that daily administration of PRE-084 to SOD1-G93A transgenic mice (the standard murine model of familial amyotrophic lateral sclerosis) from eight weeks of age significantly improved spinal motoneuron function, as measured by maintenance of compound muscle action potential amplitudes and locomotion behavior, and preserved both neuromuscular junction innervation and motoneuron survival in the ventral horn of the spinal cord [5]. The treatment extended overall survival. The mechanistic analysis identified two principal effector pathways: PKC-specific phosphorylation of serines 896 and 897 on the NR1 subunit of the NMDA receptor in motoneurons, and reduction of microglial reactivity in the spinal cord ventral horn. Both effects were reversed by co-administration of the sigma-1 receptor antagonist BD-1047, confirming on-target activity.

Delayed administration of PRE-084 starting at 12 weeks of age (after symptom onset in the SOD1-G93A model) also produced significant functional improvement and motoneuron preservation, suggesting a therapeutic window that extends into symptomatic disease rather than being restricted to presymptomatic prophylaxis [5].

Mancuso et al. (2012) further demonstrated that PRE-084 increased GDNF expression in perimotoneuronal glial cells, providing neurotrophic support to the degenerating motoneurons [5]. The GDNF upregulation is consistent with the broader sigma-1 receptor literature showing that sigma-1R activation promotes astrocytic and Schwann cell neurotrophic factor secretion.

Herrando-Grabulosa et al. (2021) extended these findings to the later stages of disease in the SOD1-G93A model and confirmed preservation of motoneuron numbers and reduction of astrogliosis and microgliosis [6]. The study additionally

demonstrated that sigma-1R activation reduced endoplasmic reticulum stress markers in spinal motoneurons, linking the neuroprotection to the MAM-localized chaperone function of the receptor.

Arias-Salvador et al. (2014) independently demonstrated PRE-084 neuroprotection in the wobbler mouse, a model of spontaneous motor neuron degeneration not linked to SOD1 mutation [7]. The wobbler mouse carries a mutation in the Vps54 gene (encoding a component of the Golgi-associated retrograde protein complex) and develops progressive forelimb weakness and motoneuron loss. PRE-084 administration significantly increased motoneuron survival and improved motor behavior in wobbler mice, with the neuroprotective mechanism involving increased neurotrophic support, reduction of reactive astrogliosis, and enhancement of a microglial/macrophage phenotype associated with tissue repair [7]. The demonstration of efficacy in a non-SOD1 motor neuron disease model broadens the mechanistic generalizability of sigma-1 receptor agonism beyond the specific proteotoxic stress of mutant SOD1.

Human genetic evidence supports the translational relevance: loss-of-function mutations in the SIGMAR1 gene (encoding the sigma-1 receptor) have been identified in families with juvenile amyotrophic lateral sclerosis and frontotemporal lobar degeneration, establishing a direct human genetic link between sigma-1 receptor dysfunction and motor neuron disease [24].

## 6.2 Cerebrovascular disease

Allahtavakoli and Jarrott (2011) demonstrated that PRE-084 at 5 mg/kg intraperitoneally, administered at 3 and 24 hours after embolic middle cerebral artery occlusion in rats, significantly reduced infarct volume and improved neurological deficit scores compared to saline-treated controls [8]. The neuroprotective mechanism involved a dual effect on cytokine balance: reduction of pro-inflammatory cytokines (interleukin-1 beta, tumor necrosis factor alpha) and enhancement of anti-inflammatory cytokines in the peri-infarct region. The anti-inflammatory shift is consistent with the sigma-1 receptor-mediated modulation of microglial polarization from the M1 to M2 phenotype.

Additional stroke-relevant studies have demonstrated that sigma-1 receptor activation stabilizes the blood-brain barrier after ischemic injury through enhanced pericyte survival mediated by interplay between apoptosis and autophagy [25].

PRE-084 treatment in cerebrovascular models also activates endothelial nitric oxide synthase through Akt/PKB phosphorylation, supporting vascular protection [2].

The post-treatment paradigm (administration at 3 and 24 hours after occlusion rather than pre-treatment) is particularly relevant to clinical translation, as stroke therapies must be effective when administered after the ischemic event.

## 6.3 Parkinson's disease

PRE-084 has been characterized in both of the standard rodent models of Parkinson's disease.

In the 6-hydroxydopamine (6-OHDA) unilateral lesion model, administration of PRE-084 at 0.3 mg/kg (but not at higher doses in some reports) improved spontaneous forelimb use and reduced dopaminergic neuron loss in the substantia nigra, demonstrating an inverted-U (bell-shaped) dose-response relationship characteristic of sigma-1 receptor agonists in some behavioral paradigms [9]. The bell-shaped dose-response has been attributed to the transition from agonist to desensitizing concentrations at higher receptor occupancy, analogous to the biphasic dose-response observed with partial agonists at other receptor systems.

In the MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) model, PRE-084 promoted PINK1/Parkin-mediated mitophagy in dopaminergic neurons, facilitating clearance of damaged mitochondria and reducing oxidative stress [10]. The sigma-1 receptor is co-expressed with the dopamine transporter (DAT) in substantia nigra dopaminergic neurons, and PRE-084 enhanced DAT expression in both wild-type and sigma-1R heterozygous mice, supporting a mechanism in which sigma-1R activation stabilizes DAT trafficking and thereby modulates dopamine reuptake and turnover [10].

Francardo et al. (2014) characterized PRE-084 in the context of dopaminergic fiber density in the striatum, reporting that sigma-1 receptor activation increased the density of tyrosine hydroxylase-positive fibers in the denervated striatum, consistent with a neurorestorative (rather than purely neuroprotective) mechanism [9].

## 6.4 Cognitive impairment and nootropic effects

The cognitive pharmacology of PRE-084 was established in a series of studies beginning in the late 1990s by Maurice, Bhatt, and colleagues.

Maurice et al. (2001) demonstrated that PRE-084 at 0.3 to 1.0 mg/kg subcutaneously attenuated spatial learning deficits in aged rats, as assessed by the Morris water maze. Treated animals exhibited decreased latencies during training and increased presence in the target quadrant during retention testing [12]. The procognitive effect was blocked by co-administration of the sigma-1 receptor antagonist NE-100, confirming on-target activity.

In pharmacological amnesia models, PRE-084 reversed learning impairment induced by the NMDA receptor antagonist MK-801 (dizocilpine), with the anti-amnesic effect blocked by sigma-1 receptor antisense oligonucleotide treatment, providing a second line of evidence for sigma-1 receptor dependence [11]. The mechanism was attributed to sigma-1R-mediated potentiation of NMDA receptor function, specifically through increased NR1 subunit phosphorylation and consequent enhancement of calcium-dependent long-term potentiation in hippocampal circuits.

Meunier et al. (2006) extended the cognitive pharmacology to amyloid-beta peptide-induced amnesia, demonstrating that PRE-084 reversed amyloid-beta(25-35)-induced spatial learning deficits in the mouse Y-maze and passive avoidance paradigms [11]. The findings placed PRE-084 in the context of Alzheimer's disease-relevant pathophysiology and supported the role of sigma-1 receptor activation as a procognitive strategy independent of cholinesterase inhibition or NMDA receptor antagonism.

Recent work by Zhang et al. (2025) demonstrated that PRE-084 increases BDNF expression by activating the ERK/CREB pathway, rescuing learning and memory impairment caused by type II diabetes in rats [13]. The study reported amelioration of neuronal apoptosis, reduction of synaptic damage, and increased ERK, phospho-CREB, and BDNF expression in the hippocampal CA1 region, providing a mechanistic link between sigma-1 receptor activation and neurotrophic support in metabolic disease-associated cognitive decline.

## 6.5 Antidepressant activity

PRE-084 has demonstrated antidepressant-like activity in the forced swim test, the standard rodent behavioral assay for antidepressant potential.

Skuza and Rogoz (2009) reported that PRE-084 decreased immobility time in the forced swim test in both Albino Swiss and C57BL/6J mice, with significant effects at 30 and 60 mg/kg intraperitoneally [14]. The effect was blocked by the sigma-1 recep-

tor antagonist BD-1047, confirming on-target activity.

Urani et al. (2002) demonstrated enhanced antidepressant-like effects of PRE-084 in mice pretreated with amyloid-beta(25-35) peptide, suggesting that the antidepressant mechanism is potentiated under conditions of neuroinflammation and proteotoxic stress [15]. The doses required for antidepressant activity (30 to 60 mg/kg) are substantially higher than those required for neuroprotection and cognitive enhancement (0.25 to 1.0 mg/kg), a disparity that may reflect different sigma-1 receptor occupancy requirements for the respective downstream signaling cascades or lower sigma-1 receptor density in the serotonergic circuits relevant to affective regulation compared to the motor and hippocampal circuits relevant to neuroprotection and cognition.

## 6.6 Cardioprotection

Gao et al. (2018) demonstrated that PRE-084 at 1 mg/kg intraperitoneally maintained cardiac function and reduced myocardial apoptosis in a rat model of myocardial ischemia-reperfusion injury [16]. The cardioprotective mechanism involved reduction of Bax and cleaved caspase-3 expression, increased Bcl-2 expression, and phosphorylation of protein kinase B (Akt) and endothelial nitric oxide synthase (eNOS). These findings extended the sigma-1 receptor agonist mechanism beyond the central nervous system and into the cardiovascular domain.

## 6.7 Perinatal brain injury

Griesmaier et al. (2012) demonstrated that a single intraperitoneal injection of PRE-084 at 0.1 micrograms per gram body weight, administered one hour after an excitotoxic insult in neonatal mice, significantly reduced cortical gray matter lesion size at both 24 and 120 hours after the insult [17]. Higher doses (10 micrograms per gram) also produced significant neuroprotection. The study extended the sigma-1 receptor neuroprotective mechanism to the immature brain and supported consideration of sigma-1 receptor agonism in perinatal neuroprotection.

## 6.8 Spinal muscular atrophy

Cervero et al. (2018) investigated PRE-084 in the Smn2B/- mouse model of spinal muscular atrophy at 0.25 mg/kg/day subcutaneously [18]. Treatment attenuated reactive gliosis, mitigated M1/M2 microglial imbalance, and prevented motoneuron

deafferentation (loss of cholinergic C-bouton inputs). These effects were also observed in the severe SMN $\Delta$ 7 mouse model. The study noted an important limitation: the prevention of gliosis and motoneuron deafferentation was not accompanied by improvement in clinical outcome measures or preservation of motoneuron cell body number, suggesting that sigma-1 receptor activation addresses some but not all components of the SMA pathophysiology.

## 6.9 Huntington's disease

Bhatt et al. (2013) demonstrated that PRE-084 at 100 nM in cell culture was protective against mutant huntingtin-induced cell degeneration in neuronal PC6.3 cells [19]. The neuroprotective mechanism involved activation of the NF-kappaB pathway, upregulation of calpastatin (an endogenous calpain inhibitor), and increased expression of cellular antioxidant defenses. The finding links sigma-1 receptor activation to the proteotoxic stress response in polyglutamine expansion diseases and extends the preclinical scope of PRE-084 to the trinucleotide repeat disease class.

## 6.10 Sepsis-associated encephalopathy

Recent work has demonstrated that PRE-084 attenuates sepsis-associated encephalopathy by targeting microglial p38 MAPK-mediated neuroinflammation and neuronal endoplasmic reticulum stress [20]. The study reported that sigma-1 receptor activation suppressed microglial activation, reduced p38 MAPK phosphorylation, and preserved neuronal viability in the hippocampus of septic animals, extending the anti-inflammatory mechanism of PRE-084 to acute systemic inflammatory conditions with secondary central nervous system involvement.

## 6.11 Endothelial barrier function

In cultured human umbilical vein endothelial cells (HUVEC), PRE-084 enhanced endothelial monolayer barrier function in a concentration-dependent and sigma-1 receptor-dependent manner and improved glycolysis parameters [2]. This finding is relevant to the blood-brain barrier stabilization observed in stroke models and suggests a direct vascular mechanism in addition to the parenchymal neuroprotective effects.

## 7. Clinical Evidence Base

PRE-084 has not been advanced to human clinical trials. No clinical evidence base exists for the compound itself. The clinical translation of the sigma-1 receptor agonist mechanism has been pursued through other compounds in the class, and the clinical evidence for these compounds informs the translational positioning of PRE-084 as a preclinical reference agonist.

### 7.1 Clinical sigma-1 receptor agonists informed by PRE-084 pharmacology

**SA4503 (cutamesine).** A selective sigma-1 receptor agonist tested in a Phase II clinical trial in 60 patients with acute ischemic stroke at doses of 1 mg/day and 3 mg/day for 28 days [26]. A higher proportion of cutamesine-treated patients completed the 10-meter timed walk test and showed improved National Institutes of Health Stroke Scale scores, but functional recovery did not reach statistical significance between treatment arms. SA4503 was also evaluated in a Phase II trial in 150 subjects with major depressive disorder; full results have not been published. The compound demonstrated acceptable safety and tolerability in both trials.

**ANAVEX2-73 (blarcamesine).** A mixed sigma-1 receptor and muscarinic receptor agonist that completed Phase II trials in Alzheimer's disease and Parkinson's disease dementia and advanced to Phase III clinical trials in early Alzheimer's disease and Rett syndrome [27]. Blarcamesine represents the most advanced clinical development of the sigma-1 receptor agonist mechanism.

**Igmesine.** A selective sigma-1 receptor agonist tested in Phase II clinical trials for major depressive disorder. Igmesine was reported to be as effective as fluoxetine in a comparator trial and demonstrated acceptable safety [28].

**Pridopidine.** A selective sigma-1 receptor agonist in clinical development for Huntington's disease and amyotrophic lateral sclerosis. Phase II and Phase III clinical trials have evaluated pridopidine in HD patients, with benefits reported on secondary functional endpoints [29].

**Fluvoxamine.** A selective serotonin reuptake inhibitor (SSRI) that is also a potent sigma-1 receptor agonist ( $K_i$  approximately 36 nM). The sigma-1 receptor component of fluvoxamine's pharmacology has been advanced as the basis for its efficacy in acute COVID-19 (multiple randomized clinical trials demonstrated reduced hospitalization), delirium in Alzheimer's disease patients, and obsessive-compulsive disorder [30]. Fluvoxamine represents a clinically approved compound whose sigma-1 receptor activity provides indirect clinical validation for the mechanism.

## 7.2 Translational considerations

The absence of clinical data for PRE-084 itself limits direct clinical translation. The compound's value in the clinical translation chain is as the reference preclinical agonist whose pharmacological characterization informed the development rationale for the clinical candidates listed above. The preclinical effect sizes observed with PRE-084 (motoneuron preservation in ALS models, infarct volume reduction in stroke, dopaminergic neuroprotection in Parkinson's models, cognitive enhancement in amnesia models) provide the mechanistic underpinning for the clinical hypotheses tested by cutamesine, blarcamesine, igmesine, and pridopidine.

## 8. Sourcing and Quality Verification

PRE-084 hydrochloride is supplied by multiple research chemical vendors at pharmaceutical research grade:

- Sigma-Aldrich/Merck (catalog P2607): greater than 98 percent purity by HPLC, supplied as the hydrochloride salt.
- Tocris Bioscience (catalog 0589): greater than 99 percent purity by HPLC.
- Cayman Chemical: greater than 98 percent purity.
- Selleckchem (catalog S0924): greater than 98 percent purity.
- Santa Cruz Biotechnology (catalog sc-203480).
- MedChemExpress, TargetMol, APExBIO, InvivoChem, and others.

Pricing is moderate, typically in the range of \$30 to \$150 per 50 milligrams of the hydrochloride salt at research-grade quality, with larger quantities (100 mg to 1 gram) available at proportionally reduced per-milligram cost.

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

- High-performance liquid chromatography area-percent purity, ideally greater than 98 percent.
- Mass spectrometry confirmation of molecular weight (318.21 for [M+H]<sup>+</sup> of the free base).
- Nuclear magnetic resonance spectroscopy (<sup>1</sup>H and <sup>13</sup>C) confirmation of the expected peak pattern for the phenylcyclohexane carboxylate ester structure.
- Karl Fischer or loss-on-drying analysis for residual water content.
- Residual solvent analysis by gas chromatography if the intended application is sensitive to trace organic solvents.

The research-grade PRE-084 market is well established and the compound is commercially available from reputable suppliers with certificates of analysis. Lot-to-lot variability is uncommon but should be assessed for any investigation where dose-response precision is critical.

## 9. Reconstitution and Handling

PRE-084 hydrochloride is freely soluble in water at room temperature. For in vivo studies, the compound is typically dissolved in normal saline (0.9 percent sodium chloride) or phosphate-buffered saline at concentrations of 0.1 to 10 mg/mL, depending on the dose and the injection volume appropriate for the animal model. No heating or sonication is required for aqueous dissolution at standard research concentrations.

For in vitro studies, stock solutions are typically prepared at 10 to 100 mM in dimethyl sulfoxide (DMSO) for storage and diluted into cell culture media to the desired working concentration. Final DMSO concentrations in culture media should not exceed 0.1 percent to avoid vehicle-related cytotoxicity.

Storage of the solid compound is recommended at minus 20 degrees Celsius in a desiccated container, protected from light. The solid is stable for at least two years under these conditions. Aqueous stock solutions should be prepared fresh for each experiment when possible; if aliquoted, frozen stocks in normal saline are stable at minus 20 degrees Celsius for at least 30 days. DMSO stock solutions are stable at minus 20 degrees Celsius for at least six months.

Repeated freeze-thaw cycles should be minimized; aliquoting into single-use volumes is recommended for both aqueous and DMSO preparations.

The compound is not classified as hazardous under standard laboratory chemical safety protocols, but standard precautions for handling pharmacologically active research chemicals should be observed: use of gloves, laboratory coat, and appropriate eye protection during weighing and solution preparation.

## 10. Stack Interaction Considerations

PRE-084 is a single-target ligand at the sigma-1 receptor with minimal cross-reactivity at other receptor systems at standard research concentrations. Stack interactions are therefore principally pharmacodynamic (at the level of sigma-1 receptor biology and its downstream effects) rather than pharmacokinetic (at the level of metabolic enzyme inhibition or induction).

**Other sigma-1 receptor ligands.** Concurrent administration with sigma-1 receptor antagonists (BD-1047, BD-1063, NE-100, S1RA) will attenuate or abolish the effects of PRE-084. This combination is the standard pharmacological control for confirming on-target activity in preclinical studies and is not a source of adverse interaction. Concurrent administration with other sigma-1 receptor agonists (SA4503, pridopidine, igmesine, (+)-pentazocine) produces additive receptor activation; the functional consequence depends on the degree of receptor occupancy and the potential for desensitization at supramaximal activation.

**NMDA receptor modulators.** PRE-084 potentiates NMDA receptor function through PKC-mediated NR1 subunit phosphorylation. Concurrent administration with NMDA receptor antagonists (MK-801, memantine, ketamine, dextromethorphan) produces pharmacological opposition at the NMDA receptor; in published studies, PRE-084 has reversed MK-801-induced amnesia through this mechanism [11]. Concurrent administration with NMDA receptor potentiators (D-serine, D-cycloserine, sarcosine) may produce additive NMDA receptor facilitation.

**Cholinesterase inhibitors.** Concurrent donepezil, rivastigmine, or galantamine with PRE-084 has not been studied directly, but the combination is pharmacologically plausible for cognitive applications (sigma-1 receptor-mediated neurotrophic support combined with cholinergic enhancement). No characterized pharmacokinetic interaction is expected.

**SSRIs with sigma-1 receptor activity.** Fluvoxamine, sertraline, and escitalopram possess varying degrees of sigma-1 receptor affinity. Concurrent administration with PRE-084 may produce additive sigma-1 receptor activation in addition to the serotonergic effects of the SSRI. Fluvoxamine, as a potent sigma-1 agonist, would produce the greatest additive sigma-1 effect.

**Calcium channel modulators.** Sigma-1 receptor activation modulates L-type voltage-dependent calcium channels. Concurrent administration with L-type calcium channel blockers (verapamil, diltiazem, nifedipine) may partially oppose the calcium-dependent downstream effects of sigma-1 receptor activation.

**Anti-inflammatory agents.** The anti-inflammatory effects of PRE-084 (microglial M1-to-M2 polarization, suppression of TNF-alpha and IL-1-beta) are additive with those of conventional anti-inflammatory agents. No antagonistic interactions are expected.

**Esterase inhibitors.** If the primary metabolic route of PRE-084 involves ester hydrolysis (as the molecular structure suggests), concurrent administration with esterase inhibitors could prolong PRE-084 exposure. This interaction is theoretical and has not been characterized experimentally.

# 11. Adverse Events and Safety Signal

## 11.1 Preclinical safety

PRE-084 has not been evaluated in formal toxicology studies of the type required for regulatory submission (GLP-compliant acute, subacute, and chronic toxicity; genotoxicity; reproductive toxicity; carcinogenicity). The safety information available is derived from the tolerability observations reported in published preclinical studies.

Across the published preclinical literature, PRE-084 has been administered to mice and rats at doses ranging from 0.1 micrograms per gram body weight to 60 mg/kg for durations ranging from single dose to daily administration for several months (in the chronic motor neuron disease studies). No dose-limiting toxicities, organ damage, or deaths attributable to PRE-084 administration have been reported in the published literature at doses up to 10 mg/kg. The antidepressant-activity studies at 30 to 60 mg/kg used acute (single-dose) administration; chronic tolerability at these higher doses has not been characterized.

## 11.2 Off-target effects at high concentrations

At concentrations substantially exceeding the sigma-1 receptor IC<sub>50</sub> (greater than 10 micromolar in vitro), PRE-084 may engage the sigma-2 receptor or other low-affinity binding sites. In HUVEC cultures with significant sigma-1 receptor depletion, PRE-084 produced a decrease (rather than the expected increase) in monolayer barrier function, suggesting off-target binding at other receptors when the primary target is unavailable [2]. This observation is relevant to high-dose or high-concentration applications and to genetic models with reduced sigma-1 receptor expression.

## 11.3 Bell-shaped dose-response

Several preclinical studies have reported bell-shaped (inverted-U) dose-response relationships for PRE-084, particularly in Parkinson's disease models where 0.3 mg/kg was effective but 1.0 mg/kg was not in some behavioral endpoints [9]. The

bell-shaped dose-response is a characteristic of the sigma-1 receptor agonist class and is attributed to receptor desensitization or to engagement of opposing signaling cascades at high occupancy. Investigators should consider this pharmacological feature when designing dose-response studies and should include multiple dose levels spanning at least one order of magnitude.

## 11.4 Reproductive and developmental safety

No reproductive or developmental toxicity data have been published for PRE-084. The compound should be considered of unknown reproductive safety.

## 11.5 Genotoxicity and carcinogenicity

No genotoxicity or carcinogenicity data have been published for PRE-084. These assessments have not been performed because the compound has not entered the regulatory development pathway.

## 11.6 Summary

The preclinical safety profile of PRE-084 is favorable at the doses and durations employed in the published literature, but the absence of formal toxicology studies limits confidence in the safety characterization. Investigators should exercise standard precautions for pharmacologically active research compounds and should design studies with appropriate dose-ranging and monitoring.

## 12. Comparable Compounds Assessment

This section presents a structured comparative assessment of five sigma-1 receptor ligands against PRE-084, evaluated on five competency standards: novelty, effect size, promising potential (current development state and trajectory), side-effect profile (clinical or preclinical), and overall validation. The five candidates were selected as the principal sigma-1 receptor agonists that have advanced further toward or into clinical development, providing context for the translational positioning of PRE-084.

### 12.1 SA4503 (Cutamesine)

**Mechanism:** Selective sigma-1 receptor agonist.  $K_i$  at sigma-1 receptor approximately 4.6 nM; selectivity over sigma-2 receptor approximately 100-fold [26]. Structurally a 1-(3,4-dimethoxyphenethyl)-4-(3-phenylpropyl)piperazine, unrelated to the phencyclidine-derived scaffold of PRE-084.

**Development trajectory:** Developed by M's Science Corporation (Japan) and advanced through Phase II clinical trials. A Phase II trial in 60 patients with acute ischemic stroke evaluated doses of 1 mg/day and 3 mg/day for 28 days. A Phase II trial in 150 subjects with major depressive disorder evaluated SA4503 once daily for 8 weeks [26]. The stroke trial reported trends toward improved neurological outcomes but did not reach statistical significance on primary endpoints. Full results of the depression trial have not been published.

**Novelty:** Low. The sigma-1 receptor agonist mechanism is shared with PRE-084 and other compounds in the class.

**Effect size:** Modest in clinical stroke. Trend-level improvements on the NIHSS and timed walk test in the stroke trial. Preclinical effect sizes in animal stroke models are comparable to PRE-084.

**Promising potential:** Low to moderate. Clinical development appears to have stalled after the Phase II stroke trial did not reach primary endpoints. The compound demonstrated clinical safety, which supports the sigma-1 receptor agonist

mechanism as tolerable in human subjects.

**Side-effect profile:** Well tolerated in Phase II clinical trials. No serious adverse events attributed to the compound were reported. This is the most important finding from the cutamesine clinical program: demonstration that selective sigma-1 receptor agonism at pharmacologically active doses is safe and tolerable in human subjects [26].

**Overall validation:** Moderate. The compound advanced to Phase II clinical trials with acceptable safety and trend-level efficacy signals. The clinical data provide important translational context for the sigma-1 receptor agonist mechanism.

**Comparison to PRE-084:** SA4503 and PRE-084 share the sigma-1 receptor agonist mechanism with comparable binding affinity and selectivity. SA4503 has the advantage of clinical safety data and a Phase II trial readout. PRE-084 has the advantage of a substantially broader preclinical literature spanning more disease models and a more extensive mechanistic characterization. The compounds are complementary research tools; SA4503 informs the clinical translatability of the mechanism, while PRE-084 informs the mechanistic breadth.

## 12.2 ANAVEX2-73 (Blarcamesine)

**Mechanism:** Mixed sigma-1 receptor agonist and muscarinic receptor modulator. The compound activates sigma-1 receptors and also engages muscarinic acetylcholine receptors, providing a dual mechanism not shared by PRE-084 [27].

**Development trajectory:** Developed by Anavex Life Sciences. Advanced through Phase II clinical trials in Alzheimer's disease (demonstrating cognitive benefit over 148 weeks of follow-up) and in Parkinson's disease dementia, and into Phase III clinical trials in early Alzheimer's disease and Rett syndrome [27]. Blarcamesine represents the most advanced clinical development program for any sigma-1 receptor agonist.

**Novelty:** Moderate. The dual sigma-1/muscarinic mechanism is a meaningful differentiation from the pure sigma-1 agonism of PRE-084 and SA4503.

**Effect size:** The Phase II Alzheimer's disease data reported slowing of cognitive decline over extended follow-up periods. Effect sizes in Rett syndrome Phase II/III data have been reported as clinically meaningful. Preclinical effect sizes are comparable to PRE-084 in relevant models.

**Promising potential:** High. Active Phase III clinical trials in two indications (Alzheimer's disease, Rett syndrome). The most likely sigma-1 receptor-based compound to achieve regulatory approval.

**Side-effect profile:** Well tolerated in Phase II and Phase III clinical trials. No dose-limiting toxicities reported. The dual mechanism introduces the possibility of muscarinic adverse effects (gastrointestinal, cardiovascular) but these have not been clinically limiting.

**Overall validation:** High. The most extensively validated sigma-1 receptor-based compound in human clinical development.

**Comparison to PRE-084:** Blarcamesine has the clear advantage of clinical development through Phase III, dual mechanism, and an extensive human safety database. PRE-084 has the advantage of pure sigma-1 receptor selectivity (allowing mechanistic dissection without muscarinic confounding) and a broader range of preclinical disease models. For research purposes where the specific contribution of sigma-1 receptor activation must be isolated from other receptor effects, PRE-084 remains the preferred tool compound; for clinical translation, blarcamesine is the more advanced candidate.

## 12.3 Pridopidine

**Mechanism:** Highly selective sigma-1 receptor agonist with low affinity for dopamine D2 receptors. Originally developed as a dopamine stabilizer, pridopidine was subsequently reclassified as a sigma-1 receptor agonist when binding studies revealed high sigma-1R affinity ( $K_i$  approximately 57 nM) and substantially lower D2 affinity [29].

**Development trajectory:** Developed by Teva Pharmaceutical and subsequently by Prilenia Therapeutics. Multiple Phase II and Phase III clinical trials in Huntington's disease, with the PRIDE-HD Phase II trial reporting improvements on secondary motor endpoints. Phase II/III trials ongoing in amyotrophic lateral sclerosis (HEALEY ALS Platform Trial) [29]. The compound has not achieved regulatory approval.

**Novelty:** Moderate. The sigma-1 receptor agonist mechanism is shared with PRE-084, but the clinical development in Huntington's disease and ALS represents novel therapeutic applications.

**Effect size:** Phase II Huntington's disease trials reported benefits on secondary endpoints (total functional capacity) but did not meet primary motor endpoints. The ALS trial data are pending.

**Promising potential:** Moderate to high. Active clinical development in ALS through the HEALEY platform. The Huntington's disease program produced mixed results but maintained interest through secondary endpoint signals.

**Side-effect profile:** Well tolerated in Phase II and Phase III clinical trials. No serious adverse events at studied doses. Falls and urinary tract infection were reported at higher rates than placebo in some trials but were not considered drug-related.

**Overall validation:** Moderate to high. Extensive Phase II and Phase III clinical data in Huntington's disease; active Phase II/III in ALS.

**Comparison to PRE-084:** Pridopidine and PRE-084 share the sigma-1 receptor agonist mechanism. Pridopidine has the advantage of clinical data in Huntington's disease and ongoing ALS trials. PRE-084 has higher sigma-1 receptor selectivity (approximately 300-fold over sigma-2, compared to pridopidine's lower selectivity ratio), broader preclinical disease model coverage, and more detailed mechanistic characterization. For ALS research in particular, the Mancuso et al. (2012) PRE-084 data in SOD1-G93A mice provided part of the mechanistic rationale that supports the pridopidine ALS clinical program.

## 12.4 Igmesine

**Mechanism:** Selective sigma-1 receptor agonist.  $K_i$  at the sigma-1 receptor approximately 39 nM [28]. Structurally an N-cyclopropylmethyl-N-methyl-1,4-diphenylbutylamine.

**Development trajectory:** Developed by Jouveinal Laboratories (later Ipsen). Advanced through Phase II clinical trials for major depressive disorder. A comparator trial against fluoxetine reported igmesine to be as effective as the established SSRI in reducing depressive symptoms [28]. The compound was not advanced to Phase III registration trials.

**Novelty:** Low. The sigma-1 receptor agonist mechanism is shared with PRE-084 and other compounds in the class.

**Effect size:** Phase II depression data reported non-inferiority to fluoxetine, a clinically meaningful effect size for an antidepressant trial.

**Promising potential:** Low. Clinical development was terminated after Phase II despite positive efficacy signals. The compound is no longer in active development.

**Side-effect profile:** Well tolerated in Phase II trials. No dose-limiting toxicities reported.

**Overall validation:** Moderate. The Phase II depression trial provides human clinical validation for the sigma-1 receptor agonist mechanism in affective disorders, complementing the PRE-084 preclinical antidepressant data.

**Comparison to PRE-084:** Igmesine provides clinical depression data that validate the antidepressant mechanism first demonstrated preclinically with PRE-084 in the forced swim test. PRE-084 has the advantage of broader preclinical coverage; igmesine has the advantage of a positive Phase II clinical readout in depression. Both compounds are selective sigma-1 agonists with comparable binding affinity.

## 12.5 Fluvoxamine

**Mechanism:** Selective serotonin reuptake inhibitor and potent sigma-1 receptor agonist.  $K_i$  at sigma-1 receptor approximately 36 nM;  $K_i$  at the serotonin transporter approximately 2 nM [30]. The dual SSRI/sigma-1 agonist mechanism is the most pharmacologically complex in this comparator panel.

**Development trajectory:** Fluvoxamine is a registered medicine approved in multiple jurisdictions for obsessive-compulsive disorder and major depressive disorder. It has been studied for additional sigma-1R-related indications including delirium in Alzheimer's disease patients and acute COVID-19 [30]. Multiple randomized clinical trials demonstrated that fluvoxamine reduced hospitalization in patients with acute COVID-19, with the sigma-1 receptor agonist mechanism advanced as the basis for the anti-inflammatory and immunomodulatory effects.

**Novelty:** Low as a sigma-1 receptor agonist (the receptor was identified as a fluvoxamine target only retrospectively). High as a clinically approved compound with demonstrated sigma-1 receptor-mediated efficacy.

**Effect size:** Substantial in obsessive-compulsive disorder and depression (registered indications). Clinically significant in COVID-19 (approximately one-third reduction in hospitalization in randomized trials). The sigma-1 receptor contribution to these effect sizes is difficult to separate from the SSRI mechanism.

**Promising potential:** High as a clinically approved compound. Low as a sigma-1 receptor-specific research tool (the dual mechanism prevents mechanistic isolation).

**Side-effect profile:** Well characterized from decades of clinical use. Principal adverse events are nausea, somnolence, dizziness, and drug-drug interactions through CYP1A2 and CYP2C19 inhibition. The sigma-1 receptor component is not the source of clinically limiting adverse events.

**Overall validation:** High as a registered medicine. The compound provides the strongest indirect clinical validation for the sigma-1 receptor agonist mechanism, but the inseparability of the SSRI and sigma-1 contributions limits the mechanistic attribution.

**Comparison to PRE-084:** Fluvoxamine and PRE-084 share comparable sigma-1 receptor binding affinity but differ fundamentally in selectivity. PRE-084 is a pure sigma-1 receptor agonist; fluvoxamine is a dual SSRI/sigma-1 agonist. For research purposes requiring mechanistic specificity, PRE-084 is the appropriate tool. For clinical applications, fluvoxamine has the advantage of regulatory approval, extensive human safety data, and demonstrated efficacy in multiple indications.

## 12.6 Comparator summary

The five comparator compounds occupy distinct positions in the sigma-1 receptor pharmacology landscape. **SA4503 (cutamesine)** provides Phase II clinical safety data confirming tolerability of selective sigma-1 receptor agonism in humans but failed to demonstrate statistically significant efficacy in stroke. **ANAVEX2-73 (blarcamesine)** is the most clinically advanced sigma-1 receptor-based compound, with dual sigma-1/muscarinic mechanism and active Phase III trials. **Pridopidine** provides extensive clinical data in Huntington's disease and active ALS trials. **Igmesine** provides a positive Phase II depression readout validating the antidepressant mechanism. **Fluvoxamine** provides the strongest indirect clinical validation through registered-medicine status and COVID-19 efficacy data but cannot isolate the sigma-1 contribution from the SSRI mechanism.

PRE-084 occupies a unique position in this landscape as the reference pharmacological tool compound. It is the most selective of the panel (no opioid, dopamine, serotonin transporter, or muscarinic cross-reactivity), the most extensively characterized in preclinical disease models (spanning ALS, stroke, Parkinson's disease, cognitive impairment, depression, cardiac ischemia, perinatal brain injury, SMA, Huntington's disease, sepsis-associated encephalopathy, and endothelial barrier function), and the compound for which the most detailed mechanistic characterization exists (PKC/NR1 phosphorylation, ERK/CREB/BDNF, NF-kappaB/calpastatin, GDNF expression, PINK1/Parkin mitophagy, microglial polarization). Its limitation is the complete absence of clinical data, which the comparator compounds provide.

The practical implication is that PRE-084 remains the preferred sigma-1 receptor agonist for preclinical mechanistic research, while clinical translation of the sigma-1 receptor agonist mechanism is pursued through the comparator compounds. The breadth of the PRE-084 preclinical literature directly informed the clinical development rationale for SA4503, blarcamesine, pridopidine, and igmesine; the preclinical and clinical literatures are therefore complementary rather than competitive.

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