

RESEARCH MONOGRAPH . COMPOUND PROFILE

LDN-193189

A pyrazolopyrimidine-scaffold small molecule derived from dorsomorphin structure-activity optimization, selectively inhibiting BMP type I receptor kinases ALK2 and ALK3 at low-nanomolar potency with 200-fold selectivity over TGF-beta signaling, and widely adopted as a research tool for BMP pathway dissection, directed stem cell differentiation, and preclinical modeling of fibrodysplasia ossificans progressiva and diffuse intrinsic pontine glioma.

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ABSTRACT

LDN-193189 (DM-3189; CAS 1062368-24-4) is a cell-permeable, small-molecule inhibitor of bone morphogenetic protein (BMP) type I receptor serine/threonine kinases, developed through structure-activity relationship optimization of dorsomorphin at the Brigham and Women's Hospital and Harvard Medical School laboratories of Paul B. Yu. The compound inhibits activin receptor-like kinase 2 (ALK2, also designated ACVR1) with an IC₅₀ of approximately 5 nM and activin receptor-like kinase 3 (ALK3, also designated BMPRI1A) with an IC₅₀ of approximately 30 nM, while exhibiting approximately 200-fold selectivity for BMP receptors over the transforming growth factor-beta (TGF-beta) type I receptor ALK5 and negligible activity against ALK4 and ALK7 at concentrations below 500 nM. The mechanism of action involves competitive binding at the ATP-binding pocket of BMP type I receptor kinases, preventing receptor autophosphorylation and blocking downstream phosphorylation of the canonical signaling effectors Smad1, Smad5, and Smad8. In addition to canonical Smad pathway inhibition, LDN-193189 suppresses BMP-induced non-canonical signaling through the p38 mitogen-activated protein kinase (MAPK) and Akt (protein kinase B) pathways, providing broader coverage of BMP-dependent intracellular signaling than Smad-selective interventions alone.

The compound originated from the 2008 Cuny, Yu, and colleagues structure-activity relationship study that optimized the pyrazolo[1,5-a]pyrimidine scaffold of dorsomorphin, a compound itself discovered in 2008 by Yu et al. through a phenotypic zebrafish embryo screen of approximately 7,500 bioactive

compounds for agents that could dorsalize the developing embryo and thereby phenocopy loss-of-function mutations in the BMP signaling pathway. Dorsomorphin, while the first small-molecule BMP pathway inhibitor identified, exhibited substantial off-target activity against AMP-activated protein kinase (AMPK), vascular endothelial growth factor receptor 2 (VEGFR2), and platelet-derived growth factor receptor (PDGFR), limiting its utility as a selective BMP pathway probe. LDN-193189 retained the central pyrazolopyrimidine core but incorporated a quinoline moiety and a piperazinylphenyl substituent that conferred markedly improved potency and selectivity for BMP type I receptors over the off-target kinases that compromised dorsomorphin's pharmacological profile.

LDN-193189 has become one of the most widely used pharmacological tools for interrogating BMP signaling in developmental biology, stem cell biology, and disease modeling. In stem cell biology, the compound is a core component of the dual SMAD inhibition protocol (in combination with the TGF-beta/Activin/Nodal inhibitor SB431542) developed by Chambers et al. (2009) for the efficient directed differentiation of human embryonic stem cells (hESCs) and induced pluripotent stem cells (iPSCs) toward neuroectodermal and neural progenitor fates, a protocol that has become the standard method for generating central nervous system neurons from human pluripotent stem cells. In disease modeling, the compound has been extensively characterized in preclinical models of fibrodysplasia ossificans progressiva (FOP), a rare genetic disorder caused by gain-of-function mutations in the ACVR1/ALK2 gene that produce constitutive BMP pathway activation and progressive heterotopic ossification of soft tissues. Treatment of mice expressing constitutively active ALK2 with LDN-193189 reduced heterotopic

bone formation in the landmark Yu et al. (2008) Nature Medicine study. The compound has also demonstrated preclinical efficacy in orthotopic xenograft models of diffuse intrinsic pontine glioma (DIPG) harboring ACVR1 mutations, extending survival in treated animals. Additional research applications span iron metabolism and hepcidin regulation (through BMP6-dependent Smad signaling in hepatocytes), pulmonary arterial hypertension modeling, chondrogenesis and osteogenesis research, and cancer biology.

LDN-193189 has not entered human clinical trials and is not a registered medicine in any jurisdiction. The compound is classified as a research tool and is supplied by multiple commercial vendors (Selleck Chemicals, MedChemExpress, Cayman Chemical, Tocris, Sigma-Aldrich, Stemgent/REPROCELL, and others) as the free base or hydrochloride salt at greater than 98 percent purity. In vivo pharmacokinetic characterization in mice has demonstrated oral bioavailability and brain penetration sufficient for central nervous system target engagement, though metabolic liabilities including aldehyde oxidase-dependent metabolism and generation of reactive piperazinyl iminium intermediates have been identified as concerns for clinical translation and have motivated the development of next-generation analogs (LDN-212854, LDN-214117, ML347, DMH1, K02288) with improved selectivity or metabolic profiles. This monograph reviews the chemistry, synthesis, and structural pharmacology of LDN-193189; the molecular mechanism of BMP type I receptor kinase inhibition; the preclinical pharmacology across FOP, DIPG, stem cell, iron metabolism, and cancer applications; the pharmacokinetic and metabolic characterization; sourcing, reconstitution, and handling; stack interactions with other signaling pathway modulators; the adverse-event and safety

signal from preclinical studies; and a comparative assessment of five alternative BMP pathway inhibitors against LDN-193189 on five competency standards.

1. Compound Identification

1.1 Names, synonyms, and registry identifiers

Preferred compound name: LDN-193189.

Synonyms and development codes: DM-3189 (dorsomorphin-3189, the internal designation from the dorsomorphin optimization series); LDN193189 (no hyphen, used interchangeably in some vendor catalogs and publications). The "LDN" prefix refers to the Longwood medical area / Harvard developmental therapeutics program designation under which the compound was synthesized.

Chemical Abstracts Service registry numbers: 1062368-24-4 (free base); 1435934-00-1 (monohydrochloride salt); 1062368-62-0 (dihydrochloride salt). The free base is the form used in most in vitro applications; the hydrochloride salts are supplied for improved aqueous handling and in vivo administration.

International Union of Pure and Applied Chemistry name: 4-[6-[4-(piperazin-1-yl)phenyl]pyrazolo[1,5-a]pyrimidin-3-yl]quinoline.

PubChem CID: 11214940 (free base); 54613581 (monohydrochloride).

1.2 Structural class and chemistry

LDN-193189 is a nitrogen-containing heteroaromatic small molecule built on a pyrazolo[1,5-a]pyrimidine core scaffold. The compound belongs to the dorsomorphin-derived series of BMP receptor kinase inhibitors, a class of ATP-competitive type I receptor serine/threonine kinase inhibitors that emerged from zebrafish phenotypic screening and subsequent medicinal chemistry optimization [1, 2].

Molecular formula: C₂₅H₂₂N₆ (free base); C₂₅H₂₃ClN₆ (monohydrochloride).

Exact mass: 406.1906 g/mol (free base, monoisotopic).

Molecular weight: 406.48 g/mol (free base); 442.94 g/mol (monohydrochloride salt).

Physical properties: The free base is a white to off-white to pale yellow solid. The compound is soluble in dimethyl sulfoxide (DMSO) at concentrations up to approximately 10 mM (approximately 4 mg/mL) and in dimethylformamide at approximately 1 mg/mL. The free base is sparingly soluble in aqueous solutions at neutral pH; the hydrochloride salt has improved but still limited aqueous solubility. The compound is cell permeable, consistent with its moderate lipophilicity and neutral nitrogen heterocyclic scaffold.

Structural features: The molecule comprises three principal domains connected through a planar, conjugated framework: (1) a quinoline ring at one terminus, which occupies the adenine-binding pocket of the kinase ATP site; (2) a central pyrazolo[1,5-a]pyrimidine core, which provides the scaffold rigidity and defines the orientation of the terminal groups within the kinase active site; and (3) a 4-(piperazin-1-yl)phenyl group at the opposite terminus, which extends into the solvent-exposed region of the kinase and contributes to selectivity discrimination among receptor subtypes. The piperazine nitrogen is the principal site of protonation under physiological conditions and is the basis for salt formation with hydrochloric acid.

1.3 Synthesis

The synthesis of LDN-193189 follows the general synthetic strategy for pyrazolo[1,5-a]pyrimidine-based BMP receptor inhibitors described in the Cuny et al. (2008) structure-activity relationship study [2]. The published approach proceeds through condensation of an appropriately substituted 3-aminopyrazole with a 1,3-biselectrophilic partner (typically a beta-ketoester or malonaldehyde derivative) to construct the pyrazolopyrimidine core, followed by palladium-catalyzed cross-coupling reactions (Suzuki or Buchwald-Hartwig coupling) to install the quinoline and piperazinylphenyl substituents at the 3- and 6-positions of the bicyclic core, respectively [2, 3]. The final compound is purified by column chromatography and/or recrystallization and characterized by nuclear magnetic resonance spectroscopy (¹H and ¹³C), mass spectrometry, and high-performance liquid chromatography. The hydrochloride salt is prepared by treatment of the free base with hydrogen chloride in an appropriate solvent (diethyl ether or dioxane) followed by filtration or recrystallization. Commercial preparations are supplied at greater than 98 percent purity by HPLC with certificates of analysis documenting identity by mass spectrometry and NMR.

2. Abstract

LDN-193189 (DM-3189) is a potent, selective, cell-permeable small-molecule inhibitor of BMP type I receptor serine/threonine kinases ALK2 (ACVR1) and ALK3 (BMPR1A), developed through medicinal chemistry optimization of dorsomorphin, the first pharmacological inhibitor of the BMP signaling pathway [1, 2]. The compound binds competitively at the ATP-binding pocket of BMP type I receptor kinases with IC₅₀ values of approximately 5 nM for ALK2 and 30 nM for ALK3, blocks phosphorylation of the canonical BMP signaling effectors Smad1/5/8, and suppresses non-canonical BMP-dependent p38 MAPK and Akt signaling [4]. Selectivity for BMP receptors over TGF-beta type I receptor ALK5 is approximately 200-fold, and activity against ALK4, ALK5, and ALK7 is negligible at concentrations below 500 nM [5].

The compound emerged from the convergence of two 2008 publications from the Yu laboratory: the identification of dorsomorphin through a zebrafish embryo phenotypic screen of approximately 7,500 compounds for agents that dorsalize the developing vertebrate body axis [1], and the Cuny et al. structure-activity relationship study that optimized the pyrazolo[1,5-a]pyrimidine scaffold of dorsomorphin to produce LDN-193189 with markedly improved potency, selectivity, and reduced off-target kinase activity [2]. Dorsomorphin inhibited BMP signaling but also potently inhibited AMPK, VEGFR2, and PDGFR, limiting its value as a selective BMP pathway probe; LDN-193189 eliminated or substantially reduced these off-target activities.

LDN-193189 has been adopted as one of the principal pharmacological tools for BMP pathway interrogation across multiple fields. In stem cell biology, it is a core component of the dual SMAD inhibition protocol (paired with the TGF-beta/Activin/Nodal pathway inhibitor SB431542) that efficiently directs human pluripotent stem cells toward neuroectodermal fates [6]. In rare disease research, LDN-193189 reduced heterotopic ossification in mouse models of fibrodysplasia ossificans progressiva (FOP) driven by constitutively active ACVR1/ALK2 mutations, providing the first pharmacological proof of concept for BMP type I receptor kinase inhibition as a therapeutic strategy in this devastating genetic disorder [7]. In neuro-oncology, the compound extended survival in orthotopic xenograft models of ACVR1-mutant diffuse intrinsic pontine glioma (DIPG) [8]. Additional preclinical applications include modulation of hepcidin expression through hepatic BMP6-

Smad signaling (relevant to iron metabolism disorders), investigation of BMP pathway contributions to pulmonary arterial hypertension, chondrogenesis and osteogenesis research, and cancer biology.

The compound has not entered human clinical trials. Metabolic characterization has identified aldehyde oxidase-dependent metabolism and generation of reactive piperazinylium intermediates as liabilities for clinical development, findings that have motivated the synthesis of next-generation analogs with improved metabolic stability and subtype selectivity [9]. LDN-193189 remains the benchmark BMP pathway inhibitor for research applications and the reference compound against which newer BMP inhibitors are evaluated.

3. Discovery and Development History

3.1 Origin: the dorsomorphin zebrafish screen

The discovery of LDN-193189 traces directly to the identification of dorsomorphin (compound C, BML-275) by Yu, Peterson, and colleagues at Massachusetts General Hospital and Harvard Medical School in 2008 [1]. The Yu laboratory screened a library of approximately 7,500 small molecules with known bioactivity for compounds that could dorsalize the developing zebrafish embryo, phenocopying loss-of-function mutations in BMP pathway components. One compound, subsequently named dorsomorphin, reproducibly induced a dorsalized body axis phenotype characterized by expansion of dorsal markers and suppression of ventral markers, effects consistent with inhibition of BMP signaling during gastrulation. Dorsomorphin was shown to inhibit BMP type I receptor kinases (predominantly ALK2, ALK3, and ALK6) and to block Smad1/5/8 phosphorylation in cell-based assays. The compound also suppressed BMP-dependent iron metabolism signaling through hepcidin regulation, demonstrating the breadth of BMP pathway involvement in vertebrate physiology [1].

However, dorsomorphin exhibited substantial off-target activity against AMP-activated protein kinase (AMPK, the original pharmacological target for which the compound was commercially available as "compound C"), vascular endothelial growth factor receptor 2 (VEGFR2), platelet-derived growth factor receptor (PDGFR), and several other kinases [2, 4]. These off-target activities limited the compound's utility as a selective probe for BMP signaling and motivated a medicinal chemistry optimization campaign.

3.2 Structure-activity relationship optimization

The Cuny, Yu, and colleagues structure-activity relationship study, published in *Bioorganic and Medicinal Chemistry Letters* in 2008, systematically varied the three principal domains of the dorsomorphin pyrazolo[1,5-a]pyrimidine scaffold to identify analogs with improved BMP receptor potency, enhanced selectivity over off-target kinases, and retained cell permeability [2]. The optimization focused on three regions: the adenine-pocket-binding group (position 3 of the pyrazolopyrimi-

dine, where a quinoline moiety was incorporated), the solvent-exposed group (position 6, where a piperazinylphenyl substituent was installed), and the central bicyclic core. LDN-193189 emerged from this series as a compound with approximately 5 nM IC₅₀ against ALK2 (a substantial improvement over dorsomorphin), approximately 30 nM IC₅₀ against ALK3, approximately 200-fold selectivity over ALK5, and markedly reduced activity against AMPK, VEGFR2, and PDGFR [2, 5].

3.3 Proof of concept in fibrodysplasia ossificans progressiva

The first major *in vivo* validation of LDN-193189 came in the Yu et al. (2008) study published in *Nature Medicine*, which demonstrated that pharmacological inhibition of BMP type I receptor kinases with LDN-193189 reduced heterotopic ossification in a mouse model of FOP expressing constitutively active ALK2 [7]. This study provided the first pharmacological proof of concept for treating FOP through BMP receptor kinase inhibition and established LDN-193189 as a tool compound for *in vivo* BMP pathway studies. The compound demonstrated oral bioavailability in mice and was tolerated at doses up to 35 mg/kg without severe toxicity.

3.4 Adoption as a standard research tool

Following the FOP proof-of-concept study, LDN-193189 was rapidly adopted across multiple research fields. The Chambers et al. (2009) dual SMAD inhibition protocol for neural differentiation of human pluripotent stem cells, published in *Nature Biotechnology*, established LDN-193189 (in combination with SB431542) as the standard method for directed neuroectodermal differentiation and became one of the most cited protocols in stem cell biology [6]. Subsequent studies extended the compound's research applications to DIPG [8], iron metabolism [10], pulmonary arterial hypertension [11], chondrogenesis [12], myoblast differentiation [13], and cancer biology [14, 15]. The compound is now supplied by more than a dozen commercial vendors and is referenced in thousands of published studies.

3.5 Next-generation analog development

Recognition of LDN-193189's metabolic liabilities and its pan-BMP-receptor (rather than ALK2-selective) inhibition profile motivated continued medicinal chemistry optimization from the Yu and Bhatt laboratories, producing a series of next-genera-

tion analogs. LDN-212854 (2013) was developed as an ALK2-biased inhibitor with improved selectivity over ALK3 and ALK5 [16]. LDN-214117 demonstrated enhanced ALK2 selectivity and improved in vivo properties in DIPG models [8]. ML347 was deposited as a Molecular Libraries Probe Centers Network (MLPCN) probe compound with ALK2-versus-ALK3 selectivity [3]. These analogs have not displaced LDN-193189 from its position as the standard research tool but have provided improved pharmacological tools for applications requiring subtype-selective BMP receptor inhibition.

4. Mechanism of Action

LDN-193189 is a type I receptor serine/threonine kinase inhibitor that acts through competitive occupation of the ATP-binding pocket of BMP type I receptors. The compound blocks both canonical and non-canonical BMP signaling cascades.

4.1 BMP signaling pathway overview

The bone morphogenetic protein (BMP) signaling pathway is a branch of the transforming growth factor-beta (TGF-beta) superfamily signaling system. BMP ligands (BMP2, BMP4, BMP6, BMP7, BMP9, BMP10, and others) signal through heteromeric receptor complexes composed of type I and type II serine/threonine kinase receptors. The type I receptors relevant to BMP signaling include ALK1, ALK2 (ACVR1), ALK3 (BMPR1A), and ALK6 (BMPR1B). Upon BMP ligand binding, the constitutively active type II receptor (BMPR2, ACVR2A, or ACVR2B) phosphorylates the glycine-serine (GS) domain of the type I receptor, activating its kinase domain. The activated type I receptor then phosphorylates the receptor-regulated Smad proteins (Smad1, Smad5, and Smad8, collectively designated R-Smads or BR-Smads), which complex with the common mediator Smad4 and translocate to the nucleus to regulate transcription of BMP-responsive target genes [17].

4.2 ATP-competitive kinase inhibition

LDN-193189 binds the ATP-binding pocket of BMP type I receptor kinases in a manner competitive with ATP. Structural and biochemical studies indicate that the quinoline moiety of LDN-193189 occupies the adenine-binding subpocket, forming hydrogen bonds with the hinge region of the kinase domain, while the pyrazolopyrimidine core provides shape complementarity with the hydrophobic pocket and the piperazinylphenyl group extends into the solvent-exposed region adjacent to the kinase active site [16, 18]. The binding affinity hierarchy is ALK2 (IC₅₀ approximately 5 nM) greater than ALK3 (IC₅₀ approximately 30 nM), with weaker inhibition of ALK6 (BMPR1B) and negligible activity against ALK4, ALK5, and ALK7 at concentrations below 500 nM [2, 5]. This selectivity profile distinguishes LDN-193189 from dorsomorphin, which inhibits ALK2 and ALK3 but also potently inhibits AMPK and VEGFR2.

4.3 Canonical Smad pathway inhibition

The principal pharmacological consequence of BMP type I receptor kinase inhibition by LDN-193189 is the blockade of Smad1/5/8 phosphorylation. In the canonical pathway, BMP ligand binding to the receptor complex leads to type II receptor-mediated phosphorylation of the type I receptor GS domain, which in turn phosphorylates Smad1/5/8 at the C-terminal SSXS motif. LDN-193189 prevents this step by occupying the ATP-binding pocket of the type I receptor and preventing its catalytic activity. The downstream consequence is the abrogation of Smad1/5/8 nuclear translocation, loss of transcriptional activation at BMP-responsive elements (BREs), and suppression of BMP target gene expression including *Id1*, *Id2*, *Id3*, *Smad6*, *Smad7*, and *hepcidin* [4, 7, 10].

4.4 Non-canonical pathway inhibition

In addition to the canonical Smad pathway, BMP type I receptors activate non-Smad signaling cascades, including the p38 MAPK pathway (through TAK1-TAB1 complex formation) and the Akt/PI3K pathway. The Boergermann et al. (2010) study in C2C12 cells demonstrated that LDN-193189 inhibits BMP-induced phosphorylation of p38, ERK1/2, and Akt, indicating that the compound blocks both canonical and non-canonical BMP signaling outputs from the type I receptor [4]. This broader inhibition profile is pharmacologically relevant because non-Smad BMP signaling contributes to cellular responses (proliferation, migration, survival) that are not captured by Smad pathway measurements alone.

4.5 Selectivity and off-target considerations

The 200-fold selectivity of LDN-193189 for BMP type I receptors over ALK5 (the TGF-beta type I receptor) permits its use at concentrations that effectively suppress BMP signaling without materially affecting TGF-beta/Activin/Nodal pathway signaling [2, 5]. However, at higher concentrations (above 500 nM to 1 micromolar), partial inhibition of ALK4, ALK5, and ALK7 has been reported, and investigators should use the minimum effective concentration for their assay to maintain pathway selectivity. The compound retains modest activity against certain non-receptor kinases at higher concentrations, though the off-target kinase profile is substantially cleaner than that of dorsomorphin.

5. Pharmacokinetics

5.1 Absorption and bioavailability

LDN-193189 demonstrates oral bioavailability in mice, as established in the Yu et al. (2008) FOP study and confirmed in subsequent in vivo studies [7, 8]. Quantitative oral bioavailability values have not been published in detail in peer-reviewed literature, but the compound achieves pharmacologically active systemic concentrations after oral dosing at 6 to 35 mg/kg in mice, sufficient to suppress Smad1/5/8 phosphorylation in target tissues including bone, muscle, and brain.

5.2 Distribution

The compound has adequate tissue distribution for preclinical pharmacology studies. LDN-193189 demonstrates brain penetration, a property that has been exploited in DIPG xenograft models where the compound extended survival of mice bearing orthotopic intracranial tumors [8]. The brain penetration is consistent with the compound's moderate molecular weight (406 Da), moderate lipophilicity, and absence of strong P-glycoprotein efflux liability. Volume of distribution data in preclinical species have not been comprehensively published.

5.3 Metabolism

The metabolic fate of LDN-193189 has been characterized in vitro and represents a significant concern for clinical translation. The Smil et al. (2020) study from the Bhatt and Yu laboratories identified that LDN-193189 undergoes significant metabolism through aldehyde oxidase (AO), a molybdenum-cofactor-dependent cytosolic enzyme that is highly expressed in human liver but has lower activity in mouse liver [9]. The piperazinyl moiety of LDN-193189 generates reactive iminium intermediates during oxidative metabolism, and the resulting aniline metabolites raise potential drug safety concerns regarding genotoxicity and idiosyncratic toxicity [9]. These metabolic findings are important for interpreting species differences in pharmacokinetics (mouse AO activity is lower than human, so mouse PK may over-

estimate human metabolic stability) and have been a principal driver of the next-generation analog programs that sought to replace the piperazine moiety with metabolically more stable groups.

CYP450-mediated metabolism has been reported but is not the dominant clearance pathway. The compound is not a characterized substrate or inhibitor of specific CYP isoforms in published literature at the level of detail available for registered medicines.

5.4 Elimination

Elimination data for LDN-193189 in preclinical species are limited. The compound is cleared predominantly through hepatic metabolism (AO-mediated and CYP-mediated pathways) rather than renal excretion, consistent with its lipophilic character and low aqueous solubility. Plasma half-life data in mice have not been systematically published in peer-reviewed form, though the dosing schedules used in published in vivo studies (typically once or twice daily) are consistent with a half-life permitting pharmacologically relevant receptor occupancy over the dosing interval.

5.5 Routes of administration

LDN-193189 has been administered by multiple routes in preclinical studies:

Oral gavage: The most common in vivo route. Doses of 2.5 to 35 mg/kg once or twice daily in mice have been used across FOP, DIPG, and iron metabolism studies [7, 8]. The compound is formulated for oral administration in vehicle solutions containing DMSO, polyethylene glycol, and/or methylcellulose suspensions.

Intraperitoneal injection: Used in some preclinical studies at doses of 2.5 to 25 mg/kg. Intraperitoneal dosing achieves rapid systemic exposure and has been employed in acute BMP pathway inhibition experiments.

In vitro / cell culture: The most common application. Typical working concentrations are 100 nM to 500 nM for BMP Smad1/5/8 pathway suppression in cell-based assays, and 100 to 200 nM in dual SMAD inhibition protocols for stem cell neural differentiation [6].

6. Preclinical Pharmacology

6.1 Fibrodysplasia ossificans progressiva

The foundational preclinical application of LDN-193189 is in FOP, a rare autosomal dominant genetic disorder (prevalence approximately 1 in 2 million) caused by heterozygous gain-of-function mutations in ACVR1/ALK2, most commonly the R206H substitution. The mutant ALK2 receptor is constitutively active or hyperresponsive to BMP ligands, driving progressive heterotopic ossification of skeletal muscle, tendons, ligaments, and other soft connective tissues [19].

The Yu et al. (2008) Nature Medicine study demonstrated that LDN-193189 inhibited Smad1/5/8 phosphorylation in tissues expressing constitutively active ALK2 and reduced the extent of ectopic bone formation in a transgenic mouse model of FOP [7]. Treatment was tolerated at doses up to 35 mg/kg daily, with initial body weight loss that stabilized over the treatment course. The study established the pharmacological proof of concept for BMP type I receptor kinase inhibition in FOP and stimulated the development of clinical-stage ALK2 inhibitors (palovarotene, garetosmab, saracatinib, BLU-782) that have subsequently entered human clinical trials for this indication.

LDN-193189 inhibits all three BMP type I receptors (ALK2, ALK3, ALK6) rather than selectively targeting the FOP-mutant ALK2, which means that therapeutic BMP signaling through wild-type ALK3 and ALK6 is also suppressed. This pan-BMP-receptor inhibition is a recognized limitation for chronic FOP therapy and was a principal motivation for developing ALK2-selective inhibitors [16].

6.2 Diffuse intrinsic pontine glioma

Approximately 25 percent of DIPG tumors harbor somatic gain-of-function mutations in ACVR1/ALK2 (typically R206H, G328V, G328E, G356D), the same gene mutated in FOP but in the context of a pediatric brainstem malignancy [20]. The Carvalho et al. (2019) study in Communications Biology demonstrated that LDN-193189 and the related compound LDN-214117 displayed beneficial effects in preclinical models of ACVR1-mutant DIPG [8]. In orthotopic xenograft models using patient-derived DIPG cells harboring ACVR1 R206H mutations, treatment with LDN-

193189 at 25 mg/kg for 28 days extended progression-free survival by approximately 15 days compared to vehicle controls. The compound demonstrated adequate oral bioavailability and brain penetration to achieve target engagement in the brainstem.

6.3 Stem cell differentiation (dual SMAD inhibition)

The most widely cited application of LDN-193189 in the research literature is the dual SMAD inhibition protocol for directed neural differentiation of human pluripotent stem cells. The Chambers et al. (2009) study demonstrated that simultaneous inhibition of BMP signaling (with LDN-193189 or the related compound Noggin) and TGF-beta/Activin/Nodal signaling (with SB431542) converts human ESCs and iPSCs to PAX6-positive central nervous system neural precursors within 11 days of differentiation, at efficiencies exceeding 80 percent [6]. The mechanistic basis is the suppression of two anti-neuralizing signals: BMP signaling, which drives epidermal and mesodermal fates, and TGF-beta/Activin/Nodal signaling, which maintains pluripotency and promotes endodermal differentiation. By inhibiting both pathways, dual SMAD inhibition recapitulates the "default model" of neural induction in which ectodermal cells adopt a neural fate in the absence of instructive BMP and Nodal signals.

The dual SMAD inhibition protocol has been extended to the generation of specific neuronal subtypes (cortical neurons, motor neurons, dopaminergic neurons, nociceptors) through the addition of downstream patterning factors (SHH agonists, retinoic acid, Wnt modulators, DAPT) and has become the standard first step in virtually all human pluripotent stem cell neural differentiation protocols [21, 22].

6.4 Iron metabolism and hepcidin regulation

BMP6 is a key physiological regulator of hepcidin expression in hepatocytes, acting through BMP type I receptors and the Smad1/5/8 pathway to activate hepcidin transcription in response to iron loading [10, 23]. LDN-193189 completely reverses BMP6-induced hepcidin upregulation and Smad1/5/9 phosphorylation in cell-based assays. The original Yu et al. (2008) dorsomorphin study demonstrated that BMP pathway inhibition suppressed hepcidin expression in zebrafish, establishing the connection between BMP signaling and iron homeostasis [1]. LDN-193189 has been

used as a pharmacological tool to dissect BMP-dependent and BMP-independent regulation of hepcidin in models of iron overload, iron deficiency, and the anemia of inflammation.

6.5 Cancer biology

BMP signaling plays context-dependent roles in cancer, acting as a tumor suppressor in some settings and as a promoter of tumor growth, invasion, and metastasis in others. LDN-193189 has been used to interrogate BMP pathway contributions in multiple cancer types. Owens et al. (2019) demonstrated that LDN-193189 enhanced metastasis development in a mouse model of breast cancer, with BMP pathway inhibition promoting dissemination of ZNF217-overexpressing cells [14]. This finding underscores the context-dependent and potentially deleterious consequences of systemic BMP pathway inhibition in cancer and highlights the importance of understanding BMP pathway function in the specific tumor context before considering therapeutic applications.

6.6 Myostatin/GDF8 signaling

Sako et al. (2010) demonstrated that dorsomorphin and LDN-193189 inhibit myostatin (GDF8) signaling and promote functional myoblast differentiation [13]. Myostatin signals through ALK4 and ALK5 in addition to ActRIIB, and the inhibition by LDN-193189 at the concentrations used in this study likely reflects cross-reactivity with ALK4/ALK5 at higher concentrations rather than the BMP-selective mechanism. This application illustrates the importance of maintaining concentration discipline when using LDN-193189 to ensure BMP pathway selectivity.

7. Clinical Evidence Base

LDN-193189 has not entered human clinical trials. The compound is classified exclusively as a research tool and has no regulatory filings, investigational new drug applications, or clinical trial registrations in any jurisdiction. The absence of clinical data reflects several factors: the metabolic liabilities identified in preclinical characterization (aldehyde oxidase-dependent metabolism, reactive metabolite formation); the availability of more advanced clinical-stage compounds for the primary therapeutic indication of FOP (palovarotene, an RARgamma agonist approved by the FDA in 2023 for FOP; garetosmab, an anti-Activin A antibody; saracatinib, a Src/ABL kinase inhibitor repurposed for FOP); and the compound's primary positioning as a research tool rather than a clinical candidate.

The clinical evidence base for BMP pathway inhibition in human disease is therefore derived from other compounds, and LDN-193189's contribution to the field is principally as the pharmacological tool that established preclinical proof of concept and enabled the mechanistic studies that supported clinical development of subsequent agents.

8. Sourcing and Quality Verification

LDN-193189 is supplied by multiple commercial vendors as a research-grade preparation. The compound is not a registered pharmaceutical product and is not available in pharmaceutical-grade formulations.

Principal vendors: Selleck Chemicals, MedChemExpress, Cayman Chemical, Tocris Bioscience (Bio-Techne), Sigma-Aldrich (Merck), Stemgent/REPROCELL, APExBIO, GlpBio, Cellagen Technology, BioGems, Abcam, MedKoo Biosciences, and others. Supply is in milligram quantities (typically 5 mg, 10 mg, 25 mg, or 50 mg) as the free base or hydrochloride salt at greater than 98 percent purity by HPLC. Pricing is moderate, typically in the range of \$50 to \$300 per 10 mg vial depending on vendor and quantity.

Quality verification: Investigators should confirm compound identity and purity on receipt through:

- HPLC area-percent purity confirmation (greater than 98 percent)
- Mass spectrometry confirmation of molecular weight (407.20 [M+H]⁺ for the free base)
- Nuclear magnetic resonance spectroscopy (¹H NMR) for structural confirmation, particularly verification of the quinoline, pyrazolopyrimidine, and piperazine proton resonances
- Comparison of biological activity (IC₅₀ for Smad1/5/8 phosphorylation inhibition in a standard cell line such as C2C12 treated with BMP4) against published values as a functional quality control

Third-party analytical verification is generally not required for research-grade LDN-193189 from established vendors, as the compound is well characterized and the commercial market is mature. However, investigators conducting quantitative dose-response studies or in vivo pharmacology experiments should independently verify purity and potency on each lot.

9. Reconstitution and Handling

9.1 Stock solution preparation

LDN-193189 free base or hydrochloride is reconstituted in DMSO to prepare concentrated stock solutions. A 10 mM stock solution is prepared by dissolving the compound in DMSO at a concentration of approximately 4.06 mg/mL (free base) or 4.43 mg/mL (monohydrochloride). If precipitate is observed on initial reconstitution, gentle warming to 37 degrees Celsius for 2 to 5 minutes with periodic vortexing is recommended. The resulting stock solution should be clear and colorless to faintly yellow.

9.2 Working concentrations

For cell culture applications, the DMSO stock solution is diluted into culture medium to achieve the desired working concentration. Typical working concentrations are:

- **Dual SMAD inhibition (neural differentiation):** 100 to 200 nM (in combination with 10 micromolar SB431542) [6]
- **BMP signaling suppression in cell-based assays:** 100 to 500 nM
- **Complete Smad1/5/8 phosphorylation blockade:** 500 nM to 1 micromolar (note: at concentrations above 500 nM, partial cross-reactivity with ALK4/ALK5 may occur)

The final DMSO concentration in cell culture medium should not exceed 0.1 percent (v/v) to minimize solvent toxicity. Vehicle controls with equivalent DMSO concentration should be included in all experiments.

9.3 Storage and stability

Powder storage: Store at minus 20 degrees Celsius or at 2 to 8 degrees Celsius, protected from light and moisture. The compound is stable as a dry powder for at least 2 years under these conditions.

DMSO stock solution storage: Aliquot reconstituted stock solutions into single-use volumes and store at minus 20 degrees Celsius. Stock solutions are stable for at least 6 months when stored as directed. Avoid repeated freeze-thaw cycles; aliquoting into single-use volumes is strongly recommended.

Working solution stability: Diluted working solutions in cell culture medium should be prepared fresh for each experiment or used within 24 hours of preparation. LDN-193189 is susceptible to degradation in aqueous solution at physiological pH over extended periods.

9.4 In vivo formulation

For in vivo administration in mice, LDN-193189 is typically formulated in vehicle solutions containing DMSO (5 to 10 percent), polyethylene glycol 400 (30 to 40 percent), and sterile saline or water (balance). Methylcellulose (0.5 percent) suspensions have also been used for oral gavage administration. The compound should be formulated fresh before each dosing session or within 24 hours if stored at 2 to 8 degrees Celsius.

10. Stack Interactions and Combinations

LDN-193189 is most commonly used in combination with other signaling pathway modulators in stem cell differentiation and disease modeling applications. The principal pharmacological interactions to consider are:

SB431542 (TGF-beta/Activin/Nodal pathway inhibitor): The most common combination partner. SB431542 inhibits ALK4, ALK5, and ALK7 (the TGF-beta/Activin/Nodal type I receptors) at approximately 10 micromolar and is pharmacologically complementary to LDN-193189, which inhibits ALK2 and ALK3 (the BMP type I receptors). The combination provides dual SMAD inhibition (Smad1/5/8 through LDN-193189 and Smad2/3 through SB431542) and is the basis of the Chambers et al. (2009) neural differentiation protocol [6]. No pharmacokinetic interaction exists between the two compounds; the interaction is purely pharmacodynamic (additive pathway suppression at different nodes of the TGF-beta superfamily signaling network).

CHIR99021 (GSK3-beta inhibitor / Wnt pathway activator): Commonly combined with LDN-193189 and SB431542 in neural crest and caudal neural progenitor differentiation protocols. CHIR99021 activates canonical Wnt signaling, which cooperates with BMP inhibition and TGF-beta inhibition to specify posterior neural fates [22].

SU5402 (FGFR inhibitor) and DAPT (gamma-secretase/Notch inhibitor): Used in combination with LDN-193189 and SB431542 in the Chambers et al. (2012) accelerated nociceptor differentiation protocol. The four-inhibitor combination (LSB plus SU5402 plus CHIR99021 plus DAPT) drives rapid differentiation of human pluripotent stem cells to nociceptor sensory neurons within 10 days [22].

Noggin (recombinant BMP antagonist protein): Noggin is a physiological BMP antagonist that binds BMP ligands extracellularly and prevents receptor engagement. LDN-193189 is frequently used as a small-molecule replacement for Noggin in dual SMAD inhibition protocols because of its lower cost, batch-to-batch consistency, and ease of handling compared to recombinant protein. The two agents inhibit BMP signaling at different levels (receptor kinase versus ligand sequestra-

tion), and their combination provides more complete BMP pathway suppression than either alone, though this is rarely necessary in standard differentiation protocols.

CYP and AO substrates or inhibitors: Because LDN-193189 is metabolized in part by aldehyde oxidase and CYP enzymes, in vivo combinations with strong AO inhibitors (raloxifene, menadione) or CYP inhibitors could alter its pharmacokinetic profile. These interactions are not characterized in published studies and are relevant principally for in vivo research applications.

BMP ligand concentrations: In cell culture assays where exogenous BMP ligands (BMP2, BMP4, BMP6, BMP7) are present, the effective LDN-193189 concentration required for pathway suppression may increase with increasing BMP ligand concentration, because the compound acts at the receptor level rather than by neutralizing the ligand. Investigators should titrate LDN-193189 concentration against their specific BMP ligand conditions.

11. Adverse Events and Safety Signal

11.1 Preclinical tolerability

LDN-193189 has been characterized for tolerability in mouse studies at doses ranging from 2.5 to 35 mg/kg administered orally or intraperitoneally. At doses up to 25 mg/kg daily for 28 days, the compound was generally well tolerated, with no severe toxic effects and no significant body weight loss reported in most studies [8]. At 35 mg/kg, initial body weight loss was observed that stabilized over the treatment course [7]. No comprehensive formal toxicology studies (GLP-compliant single-dose and repeat-dose toxicology, genotoxicity, reproductive toxicity, carcinogenicity) have been conducted, consistent with the compound's status as a research tool rather than a clinical candidate.

11.2 Mechanism-based safety concerns

Several mechanism-based adverse effects are predicted from systemic BMP pathway inhibition:

Musculoskeletal effects: BMP signaling is a physiological promoter of muscle hypertrophy and maintenance. Chronic inhibition of BMP signaling with LDN-193189 or related compounds could promote skeletal muscle atrophy, a concern that has been raised but not systematically characterized in published literature [13].

Iron metabolism disruption: BMP6-Smad signaling is the principal regulator of hepcidin expression in hepatocytes, and hepcidin is the master regulator of systemic iron homeostasis. Chronic BMP pathway inhibition with LDN-193189 suppresses hepcidin expression, which would be expected to increase dietary iron absorption and potentially predispose to iron overload with prolonged treatment [1, 10].

Skeletal development effects: BMP signaling is essential for normal skeletal development and bone homeostasis. Chronic inhibition could impair bone formation, fracture healing, and growth plate function, particularly in juvenile animals.

Cardiovascular effects: BMP9 and BMP10 signaling through ALK1 contributes to vascular homeostasis and endothelial function. While LDN-193189 has relatively low activity against ALK1 at standard concentrations, high-dose or chronic exposure could affect vascular BMP signaling [11].

Cancer risk modulation: The Owens et al. (2019) finding that LDN-193189 enhanced metastasis development in a breast cancer mouse model highlights the potential for BMP pathway inhibition to promote tumor dissemination in certain cancer contexts [14]. Systemic BMP pathway inhibition should be considered with caution in any setting where occult or active malignancy may be present.

11.3 Metabolic safety concerns

The Smil et al. (2020) metabolic characterization identified that the piperazine moiety of LDN-193189 generates reactive iminium intermediates during aldehyde oxidase-mediated metabolism, and that aniline metabolites are formed [9]. Reactive iminium species can covalently modify proteins and DNA, raising theoretical concerns about idiosyncratic drug reactions and genotoxicity. These metabolic findings are a principal reason that LDN-193189 has not been advanced to clinical development and have motivated the development of next-generation analogs with metabolically more stable substituents.

11.4 Reproductive and developmental toxicity

No formal reproductive or developmental toxicity studies of LDN-193189 have been published. Given the essential role of BMP signaling in embryogenesis, gastrulation, neural tube formation, limb development, and organogenesis, the compound should be considered potentially teratogenic and should not be administered to pregnant animals without explicit experimental justification and institutional review.

12. Comparable Compounds Assessment

This section presents a structured comparative assessment of five alternative BMP pathway inhibitors against LDN-193189, evaluated against five competency standards: novelty, effect size (potency and efficacy in published assays), promising potential (current development trajectory and research adoption), side-effect profile (preclinical or theoretical), and overall validation (depth and breadth of published characterization). The five comparators were selected as the principal small-molecule BMP pathway inhibitors with substantial published characterization in the research literature.

12.1 Dorsomorphin (Compound C, BML-275)

Mechanism: ATP-competitive inhibitor of BMP type I receptor kinases ALK2, ALK3, and ALK6, with substantial off-target activity against AMPK, VEGFR2, and PDGFR [1, 4].

Development trajectory: Dorsomorphin was the first small-molecule BMP pathway inhibitor, identified in the Yu et al. (2008) zebrafish phenotypic screen [1]. It is the direct precursor of LDN-193189 and remains commercially available as a research tool. However, its poor selectivity has led to its displacement by LDN-193189 in most BMP pathway applications.

Novelty: High at the time of discovery (2008), as the first pharmacological BMP pathway inhibitor. Low relative to LDN-193189 in the current landscape.

Effect size: Comparable BMP pathway inhibition to LDN-193189 but at higher concentrations and with confounding off-target effects. The IC₅₀ against ALK2 in cell-based assays is approximately 0.5 to 1 micromolar, roughly 100-fold less potent than LDN-193189.

Promising potential: Low. The compound has been largely superseded by LDN-193189 and next-generation analogs for BMP-selective applications. It retains utility as an AMPK inhibitor (its original commercial application) and as a historical reference compound.

Side-effect profile: More pronounced than LDN-193189 owing to off-target AMPK, VEGFR2, and PDGFR inhibition. AMPK inhibition produces metabolic consequences (altered glucose metabolism, lipid metabolism) that confound BMP-specific studies [4].

Overall validation: High. The most extensively published BMP pathway inhibitor in terms of total citations, but most recent studies prefer LDN-193189 for BMP-selective applications.

Comparison to LDN-193189: LDN-193189 is superior in potency, selectivity, and pharmacological interpretability. Dorsomorphin is appropriate only when AMPK inhibition is specifically desired or when historical continuity with earlier studies is required.

12.2 DMH1 (4-[6-[4-(1-methylethoxy)phenyl]pyrazolo[1,5-a]pyrimidin-3-yl]quinoline)

Mechanism: Selective BMP type I receptor kinase inhibitor derived from the same pyrazolopyrimidine scaffold as LDN-193189, with the piperazinylphenyl solvent-exposed group replaced by an isopropoxyphenyl ether. This modification eliminates activity against VEGFR2 and produces enhanced kinome-wide selectivity [25].

Development trajectory: Reported by Hao et al. (2010) as a dorsomorphin analog with improved selectivity. The compound has been adopted as a research tool for BMP pathway studies where VEGFR2 cross-reactivity is a concern.

Novelty: Low. The mechanism is identical to LDN-193189; the innovation is the improved selectivity profile.

Effect size: Biochemical ALK2 IC₅₀ of approximately 107 nM, substantially less potent than LDN-193189 (5 nM). In cell-based assays, DMH1 shows reduced potency compared to LDN-193189, suggesting diminished cell permeability or altered intracellular target engagement [25].

Promising potential: Moderate. DMH1 occupies a niche for applications where VEGFR2 cross-reactivity of LDN-193189 (modest but nonzero) is a concern, but its lower potency limits its appeal for most applications.

Side-effect profile: Theoretically cleaner than LDN-193189 owing to improved kinase selectivity, but the reduced potency necessitates higher working concentrations that may partially offset the selectivity advantage.

Overall validation: Moderate. Published in a substantial number of studies, though fewer than LDN-193189.

Comparison to LDN-193189: DMH1 offers improved selectivity at the cost of significantly reduced potency. LDN-193189 is preferred for most applications; DMH1 is appropriate when VEGFR2 pathway contributions must be rigorously excluded.

12.3 LDN-212854

Mechanism: ALK2-biased BMP type I receptor kinase inhibitor developed by Mohedas et al. (2013) from the Bhatt and Yu laboratories. The compound retains potent ALK2 inhibition while exhibiting substantially reduced activity against ALK3 (BMPRI1A) and improved selectivity over ALK5 [16].

Development trajectory: Designed to address the pan-BMP-receptor inhibition profile of LDN-193189, which inhibits both ALK2 and ALK3. ALK2-selective inhibition is therapeutically desirable for FOP (where the disease driver is mutant ALK2 and physiological ALK3 signaling should be preserved) and for ACVR1-mutant DIPG. The compound has been adopted in a growing number of preclinical studies.

Novelty: Moderate. The ALK2-versus-ALK3 selectivity represents a meaningful advance in the pharmacological toolkit for dissecting BMP receptor subtype contributions.

Effect size: Potent ALK2 inhibition (low-nanomolar IC₅₀), with substantially reduced ALK3 inhibition. The ALK2 selectivity has been confirmed by structural biology studies demonstrating the basis for the differential binding [18].

Promising potential: Moderate to high. The compound is positioned as a successor to LDN-193189 for ALK2-selective applications and is gaining adoption in the FOP and DIPG research communities.

Side-effect profile: Theoretically improved over LDN-193189 owing to preservation of ALK3-mediated physiological BMP signaling, which could reduce musculoskeletal and skeletal adverse effects of pan-BMP-receptor inhibition.

Overall validation: Moderate. Published literature is growing but remains smaller than the LDN-193189 corpus. Structural characterization of the binding mode provides mechanistic confidence [18].

Comparison to LDN-193189: LDN-212854 is preferred for applications requiring ALK2-selective BMP pathway inhibition (FOP modeling, ACVR1-mutant DIPG). LDN-193189 remains preferred for applications requiring broad BMP pathway suppression (dual SMAD inhibition for stem cell differentiation, general BMP signaling studies) and for continuity with the established published literature.

12.4 K02288

Mechanism: A structurally distinct BMP type I receptor kinase inhibitor identified from a different chemical scaffold (substituted 2-aminopyridine) by Sanvitale et al. (2013) [26]. K02288 inhibits ALK1, ALK2, ALK3, and ALK6 with low-nanomolar potency and exhibits remarkable selectivity over a panel of approximately 250 human kinases, with a kinome-wide selectivity profile more favorable than that of LDN-193189.

Development trajectory: Developed at the University of Oxford as an alternative BMP pathway inhibitor scaffold. The compound has been adopted for applications where kinome-wide selectivity is paramount.

Novelty: Moderate to high. The distinct chemical scaffold and the superior kinome-wide selectivity profile represent a meaningful structural and pharmacological advance.

Effect size: Low-nanomolar IC₅₀ values against ALK2, ALK3, and ALK6, broadly comparable to LDN-193189 in potency. In cell-based assays, K02288 effectively suppresses BMP-induced Smad1/5/8 phosphorylation at concentrations similar to those required for LDN-193189.

Promising potential: Moderate. The compound has been adopted in a growing but still modest number of published studies. The distinct scaffold may have advantages for medicinal chemistry optimization toward clinical candidates.

Side-effect profile: The superior kinome-wide selectivity predicts fewer off-target kinase-mediated adverse effects than LDN-193189, though formal preclinical safety characterization has not been published.

Overall validation: Low to moderate. The published literature is smaller than that of LDN-193189, and the compound has not been as extensively characterized in in vivo models.

Comparison to LDN-193189: K02288 offers superior kinome-wide selectivity from a distinct chemical scaffold, potentially advantageous for applications where off-target kinase effects are a concern. LDN-193189 retains the advantage of vastly larger published validation, established in vivo protocols, and broader commercial availability.

12.5 ML347

Mechanism: ALK2-selective BMP type I receptor kinase inhibitor developed by Cuny et al. (2012) and deposited as a Molecular Libraries Probe Centers Network (MLPCN) probe compound [3]. ML347 is derived from the same pyrazolo[1,5-a]pyrimidine scaffold as LDN-193189 and inhibits BMP receptors ALK2, ALK3, and ALK6 while exhibiting selectivity over ALK5 and the Smad2/3 pathway.

Development trajectory: Developed specifically as a publicly available probe compound for the biomedical research community through the NIH MLPCN initiative. ML347 has been characterized in cell-based and in vitro kinase assays and deposited with full selectivity profiling data.

Novelty: Low to moderate. The scaffold is shared with LDN-193189; the advance is the ALK2-versus-ALK3 selectivity and the comprehensive public characterization as an MLPCN probe.

Effect size: Potent ALK2 inhibition with reduced ALK3 activity, confirmed in cell-based and biochemical assays. The selectivity profile places ML347 between LDN-193189 (pan-BMP) and LDN-212854 (ALK2-biased) in terms of receptor subtype discrimination.

Promising potential: Low to moderate. The compound is principally a research tool with a specific public-access mandate. It has not been developed toward clinical applications.

Side-effect profile: Not systematically characterized. The ALK2-biased profile predicts a similar or modestly improved safety profile relative to LDN-193189.

Overall validation: Moderate. The MLPCN characterization provides a comprehensive publicly available selectivity dataset, but the total published literature is smaller than that of LDN-193189.

Comparison to LDN-193189: ML347 is preferred for applications requiring public-access probe characterization and for ALK2-selective studies. LDN-193189 retains the advantage of broader validation and more extensive in vivo characterization.

12.6 Comparator summary

The five comparators occupy distinct positions in the BMP pathway inhibitor landscape. **Dorsomorphin** is the historical precursor with poor selectivity, largely superseded by LDN-193189. **DMH1** offers improved kinome selectivity at the cost of reduced potency. **LDN-212854** is the leading ALK2-selective next-generation analog, preferred for FOP and ACVR1-mutant DIPG applications. **K02288** provides superior kinome-wide selectivity from a distinct chemical scaffold. **ML347** is the publicly deposited MLPCN probe with intermediate selectivity characteristics.

LDN-193189 occupies the central position in this comparator landscape. It is the most extensively validated and widely cited BMP pathway inhibitor in the research literature, the standard component of the dual SMAD inhibition protocol for neural differentiation, and the benchmark compound against which all newer BMP inhibitors are compared. Its principal limitations are the pan-BMP-receptor inhibition profile (which does not discriminate ALK2 from ALK3 and ALK6), the aldehyde oxidase-mediated metabolic liability that has prevented clinical development, and the modest but nonzero off-target kinase activity at higher concentrations. For the majority of research applications, LDN-193189 remains the first-choice BMP pathway inhibitor; investigators requiring ALK2 selectivity, improved kinome selectivity, or clinical-grade metabolic stability should consider the next-generation analogs on a case-by-case basis.

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