

RESEARCH MONOGRAPH . COMPOUND PROFILE

Bronchogen

A synthetic tetrapeptide (H-Ala-Asp-Glu-Leu-OH; ADEL) developed at the Saint Petersburg Institute of Bioregulation and Gerontology as an ultrashort peptide bioregulator with bronchopulmonary tissue-specific proliferative, anti-inflammatory, and epithelial-regenerative activity targeting bronchial epithelium, ciliated cell restoration, and respiratory mucosal barrier function.

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ABSTRACT

Bronchogen (H-Ala-Asp-Glu-Leu-OH; ADEL tetrapeptide; molecular formula $C_{18}H_{30}N_4O_9$; molecular weight 446.45 g/mol) is a synthetic tetrapeptide bioregulator developed by Vladimir Khavinson and colleagues at the Saint Petersburg Institute of Bioregulation and Gerontology as the bronchopulmonary-specific member of the Khavinson ultrashort peptide bioregulator family [1, 2]. The compound belongs to a class of synthetic two-to-seven-residue peptide sequences modeled on tissue-specific peptide fragments isolated from mammalian organ extracts, and is designated as the respiratory system bioregulator within this peptide family. Bronchogen is structurally related to but distinct from the other Khavinson tetrapeptides that share the Ala-Glu-Asp tripeptide core (Cardiogen, Cortagen, Epithalon); the ADEL sequence carries a different arrangement of the acidic residues (Asp at position two, Glu at position three) and a hydrophobic leucine residue at the C-terminus, a configuration that determines bronchial tissue specificity within the Khavinson classification system [3, 4]. The principal molecular mechanism of Bronchogen, characterized through molecular modeling, cell culture, organotypic bronchial tissue studies, and animal models of obstructive lung disease, is epigenetic regulation of gene expression through direct interaction of the tetrapeptide with double-stranded DNA and with histone proteins, producing chromatin remodeling and reactivation of transcriptional programs in bronchial epithelial cells [5, 6, 7]. The bronchopulmonary activity, characterized in organotypic lung tissue cultures from young and aged rats, human bronchial epithelial cell cultures across multiple passages, and nitrogen

dioxide-induced chronic obstructive pulmonary disease (COPD) rat models, includes stimulation of bronchial epithelial cell proliferation and differentiation with upregulation of differentiation markers in aging cell cultures [8, 9], regulation of Ki67, Mcl-1, p53, CD79, and endothelial nitric oxide synthase (NOS-3) protein expression in human bronchial epithelium [5], restoration of normal ciliated epithelial architecture with reduction of goblet cell hyperplasia and squamous metaplasia in COPD models [10], normalization of proinflammatory cytokine profiles and neutrophilic inflammation in bronchoalveolar lavage fluid [10, 11], and enhancement of secretory immunoglobulin A and surfactant protein B production indicating recovery of respiratory mucosal barrier and surfactant function [11]. No formal pharmacokinetic studies have been published for Bronchogen as the isolated synthetic ADEL tetrapeptide. As a linear tetrapeptide with unprotected termini, the compound is expected to undergo rapid proteolytic degradation by aminopeptidases and carboxypeptidases in plasma and gastrointestinal fluid; however, molecular modeling studies have demonstrated that ultrashort peptides are substrates of the proton-coupled oligopeptide transporter (PEPT1/PEPT2) family carriers, supporting intestinal absorption and cellular uptake through active transport mechanisms [12, 13]. No human clinical trials have been published. The compound is not approved by the United States Food and Drug Administration, the European Medicines Agency, or any major Western regulatory authority. Bronchogen is registered in the Russian Federation as a biologically active additive and is commercially available there in capsule formulations. It is supplied internationally as a research-grade lyophilized peptide by multiple peptide synthesis vendors at greater than 95 percent purity by high-performance liquid

chromatography. This monograph reviews the chemistry, synthesis, and structural characterization of Bronchogen; the discovery and development history within the Khavinson bioregulatory peptide program; the molecular pharmacology including peptide-DNA binding, histone interaction, and bronchial gene expression modulation; the pharmacokinetic considerations for ultrashort peptides; the preclinical pharmacology across bronchial, inflammatory, and aging cell models; the clinical evidence base (absent); sourcing and quality verification; reconstitution and handling; stack interactions and combinations; adverse events and safety signal; and a comparative assessment of five bronchopulmonary or respiratory-protective peptide candidates (Chonluten, GHK-Cu, BPC-157, Thymalin, N-acetylcysteine) against Bronchogen on five competency standards.

1. Compound Identification

1.1 Names, synonyms, and registry identifiers

International Nonproprietary Name: Not assigned. The compound has not received an INN designation from the World Health Organization.

Designated name: Bronchogen. The name is a proprietary designation within the Khavinson peptide bioregulator nomenclature system and is used across the Russian-language and international research literature to identify the ADEL tetrapeptide in its bronchopulmonary bioregulator application. The name is occasionally transliterated from the Russian as Bronkhogen.

One-letter amino acid code: ADEL (Ala-Asp-Glu-Leu).

Three-letter amino acid code: L-Ala-L-Asp-L-Glu-L-Leu.

Full chemical name: L-alanyl-L-aspartyl-L-glutamyl-L-leucine. Alternate designations in the peptide chemistry literature include H-Ala-Asp-Glu-Leu-OH (indicating unprotected N-terminal alpha-amino and C-terminal carboxyl groups).

Chemical Abstracts Service registry number: Not uniquely assigned as a pharmaceutical entity at the time of monograph preparation. The tetrapeptide sequence Ala-Asp-Glu-Leu is a defined sequence within general peptide nomenclature but has not received a unique CAS number in the manner of small-molecule pharmaceuticals. Investigators should reference the compound by its full sequence (H-Ala-Asp-Glu-Leu-OH or ADEL) and by its molecular formula for unambiguous identification.

Related compound: Chonluten (H-Glu-Asp-Leu-OH; EDL tripeptide) is a structurally related Khavinson tripeptide bioregulator also designated for bronchopulmonary tissue. Chonluten shares the Asp-Glu-Leu motif with Bronchogen but in reversed order (Glu-Asp-Leu versus Asp-Glu-Leu) and lacks the N-terminal alanine residue. The two compounds are distinct molecular entities with partially overlapping tissue specificity claims within the Khavinson system [14].

1.2 Structural class and chemistry

Bronchogen is an ultrashort linear tetrapeptide composed of four alpha-amino acid residues joined by standard alpha-peptide bonds. The structural class places it within the Khavinson ultrashort peptide bioregulator family, a series of synthetic two-to-seven-residue peptides designed as minimal pharmacophore units of tissue-specific polypeptide complexes isolated from mammalian organs by acid-pepsin hydrolysis and ultrafiltration [3, 4]. Bronchogen is distinguished within this family by the combination of a hydrophobic neutral residue (alanine) at the N-terminus, two acidic residues (aspartic acid at position two and glutamic acid at position three), and a branched-chain hydrophobic residue (leucine) at the C-terminus. This arrangement produces two charge centers at physiological pH (the deprotonated side-chain carboxylates of aspartate and glutamate) and a single positive charge center (the protonated N-terminal alpha-amino group), resulting in a net negative charge at physiological pH.

Molecular formula: C₁₈H₃₀N₄O₉.

Molecular weight: 446.45 g/mol (calculated from the protonated free peptide form).

Isoelectric point: Approximately 3.0 (calculated), reflecting the dominance of two acidic side-chain carboxylates (aspartic acid pK_a approximately 3.9; glutamic acid pK_a approximately 4.1) and the C-terminal carboxyl (pK_a approximately 2.1) against the single basic N-terminal amino group (pK_a approximately 9.7). At physiological pH (7.4), the compound carries a net negative charge of approximately minus 2, with both side-chain carboxylates and the C-terminal carboxyl deprotonated and the N-terminal amino group protonated.

Physical properties: White to off-white lyophilized powder or crystalline solid. Freely soluble in water, phosphate-buffered saline, and aqueous buffers at neutral to slightly alkaline pH. Sparingly soluble in apolar organic solvents. Hygroscopic. Stable as a lyophilized solid at minus 20 degrees Celsius for at least 24 months when protected from moisture.

Stereochemistry: All four residues are in the L-configuration. No chiral inversion is expected during standard solid-phase or solution-phase peptide synthesis. Investigators should confirm L-stereochemistry by chiral amino acid analysis after acid hydrolysis or by comparison of chromatographic retention time to synthetic standards.

1.3 Synthesis

Bronchogen is synthesized by standard solid-phase peptide synthesis (Fmoc chemistry) or by solution-phase peptide synthesis. The solid-phase approach proceeds from a leucine-preloaded resin (Wang resin or 2-chlorotrityl chloride resin with Fmoc-Leu-OH), with sequential coupling of Fmoc-Glu(OtBu)-OH, Fmoc-Asp(OtBu)-OH, and Fmoc-Ala-OH using standard activating reagents (HBTU, HATU, or DIC/HOBt). Deprotection of the Fmoc group at each step is carried out with 20 percent piperidine in dimethylformamide. Final cleavage from the resin and simultaneous removal of side-chain protecting groups (the OtBu groups from glutamic acid and aspartic acid) is accomplished with trifluoroacetic acid in the presence of scavengers (triisopropylsilane, water). The crude peptide is purified by reversed-phase high-performance liquid chromatography (C18 column, water-acetonitrile gradient with 0.1 percent trifluoroacetic acid) to greater than 95 percent purity. Lyophilization of the purified fraction yields the final product as the trifluoroacetate salt or, after counterion exchange, as the acetate salt. The solution-phase synthesis proceeds by sequential condensation of the four amino acids using carbodiimide or active ester chemistry with appropriate side-chain protection. The synthesis is straightforward; the four-residue chain length and the absence of sterically demanding or aggregation-prone coupling steps result in high coupling efficiencies and overall yields typically exceeding 50 to 65 percent after purification [15].

2. Abstract

Bronchogen (H-Ala-Asp-Glu-Leu-OH; ADEL) is a synthetic tetrapeptide bioregulator developed at the Saint Petersburg Institute of Bioregulation and Gerontology by Vladimir Khavinson and colleagues as the bronchopulmonary-specific member of a family of ultrashort peptide bioregulators designed to recapitulate the tissue-specific regulatory activity of organ-derived polypeptide complexes [1, 2, 3]. The compound is classified as the respiratory system bioregulator within the Khavinson peptide nomenclature, a designation based on the tissue specificity demonstrated in organotypic culture experiments in which each tetrapeptide stimulated proliferation selectively in its corresponding tissue type [16].

The tissue specificity of Bronchogen was formally established in the Zakutskii et al. (2006) organotypic tissue culture study, in which four tetrapeptide bioregulators (Bronchogen, Cardiogen, Prostamax, and Pancragen) were applied to organotypic tissue cultures of explants from lungs, heart, prostate, and pancreas of young (3-week-old) and aged (18-month-old) rats [16]. Bronchogen stimulated proliferation selectively in lung tissue explants from both age groups at an effective concentration of 0.05 ng/mL, corresponding to a molar concentration of approximately 10^{-10} to 10^{-11} molar, a range characteristic of the Khavinson ultrashort peptide bioregulators and substantially below conventional pharmacological dose ranges for peptide therapeutics.

The compound operates through a multi-level molecular mechanism. At the epigenetic level, Bronchogen and structurally related Khavinson ultrashort peptides have been demonstrated to penetrate cell nuclei and interact directly with double-stranded DNA in gene promoter regions and with histone proteins, producing chromatin decondensation and altered transcriptional availability of tissue-specific gene loci [5, 6, 7]. Fluorescein-labeled Bronchogen (ADEL) was shown to penetrate HeLa cell nuclei and to interact preferentially with DNA sequences containing CNG trinucleotide motifs, with particular selectivity for CTG-containing sequences, suggesting a sequence-specific component to the peptide-DNA interaction [6]. The Monaselidze et al. (2011) differential scanning microcalorimetry study demonstrated that Bronchogen increased the melting temperature of calf thymus and

mouse liver DNA by 3.1 degrees Celsius at specific concentrations, confirming direct DNA stabilization through binding to both strands without base-pair preference [7].

At the cellular level, the principal characterized activities of Bronchogen in bronchial tissue include stimulation of bronchial epithelial cell proliferation and differentiation, regulation of proteins involved in cell cycle control, apoptosis, and endothelial function, and enhancement of differentiation markers in aging cell cultures. The Khavinson et al. (2014) study in human bronchial epithelial cell cultures demonstrated that ADEL regulated expression of Ki67 (proliferation marker), Mcl-1 (anti-apoptotic BCL-2 family member), p53 (tumor suppressor and apoptosis regulator), CD79 (differentiation marker), and NOS-3 (endothelial nitric oxide synthase), with effects more pronounced in late-passage (aged) cell cultures [5]. The Khavinson et al. (2012) study confirmed tissue-specific stimulation of differentiation factors by Bronchogen in bronchial epithelial cells, with the effect more pronounced in aged cultures, supporting a geroprotective mechanism of action [8].

In animal models of obstructive lung disease, Bronchogen demonstrated substantial regenerative and anti-inflammatory activity. The Kuzubova et al. (2015) study in a nitrogen dioxide-induced COPD rat model reported that one month of Bronchogen treatment produced restoration of normal bronchial epithelial architecture including recovery of ciliated cells, reduction of goblet cell hyperplasia and squamous metaplasia, decreased lymphocytic infiltration and emphysema, enhanced secretory immunoglobulin A production, and normalization of proinflammatory cytokine profiles in bronchoalveolar lavage fluid [10]. The Titova et al. (2017) follow-up study in the same COPD model confirmed decreased neutrophilic inflammation with normalization of cellular composition and restoration of surfactant protein B levels, indicating recovery of both mucosal immune-barrier function and surfactant homeostasis [11].

All published Bronchogen research is preclinical. No human clinical trials of any design have been published in peer-reviewed literature. No pharmacokinetic data from human subjects are available. The compound is not approved for therapeutic use by any Western regulatory authority. It is registered in the Russian Federation as a biologically active additive and is commercially available there in oral capsule formulations. The entirety of the published preclinical evidence originates from the Khavinson research network at the Saint Petersburg Institute of Bioregulation and Gerontology and affiliated Russian laboratories. No independent replication by any Western research group has been published for Bronchogen specifically,

though the broader Khavinson peptide-DNA interaction framework has been partially replicated in Italian laboratories [17]. Investigators considering Bronchogen for research applications should weight the evidence base accordingly.

3. Discovery and Development History

3.1 Origin

Bronchogen was developed at the Saint Petersburg Institute of Bioregulation and Gerontology (formerly the Military Medical Academy laboratory of bioregulators) under the direction of Vladimir Khavinson as part of a systematic program to identify ultrashort peptide sequences with tissue-specific bioregulatory activity [1, 2]. The Khavinson bioregulatory peptide program began in the late 1970s and early 1980s with the extraction, fractionation, and characterization of polypeptide complexes from mammalian organ extracts (thymus, pineal gland, brain cortex, blood vessels, heart, prostate, pancreas, bronchi, and other organs). These polypeptide complexes, obtained by acid-pepsin hydrolysis and ultrafiltration, were designated "Cytomedins" and demonstrated tissue-specific biological activity in animal models and, subsequently, in clinical applications within the Soviet and Russian healthcare systems [18, 19].

The parent polypeptide complex from which Bronchogen was derived is the bronchial tissue extract, a preparation analogous to the cortexin (cerebral cortex extract), thymalin (thymic extract), and epithalamin (pineal extract) preparations that preceded the identification of their minimal peptide sequences. The bronchial polypeptide complex was administered clinically in the Russian Federation for chronic obstructive pulmonary disease, chronic bronchitis, and other respiratory conditions before the minimal active peptide sequence was identified.

3.2 Identification of the ADEL sequence

The second phase of the Khavinson program, beginning in the 1990s and continuing through the 2000s, involved identification of the minimal bioactive peptide sequences within the polypeptide complexes. Using iterative fractionation, amino acid analysis, and systematic synthesis of candidate sequences, the Khavinson laboratory identified a series of di-, tri-, and tetrapeptide sequences that retained the tissue specificity of their parent polypeptide complexes. Bronchogen (ADEL) was

identified as the bronchopulmonary-specific tetrapeptide bioregulator through this process, representing the minimal peptide sequence from bronchial tissue extracts that maintained lung-specific proliferative and cytoprotective activity [3, 4].

A parallel identification effort produced the related tripeptide Chonluten (EDL; Glu-Asp-Leu), also designated as a bronchopulmonary bioregulator within the Khavinson nomenclature. Chonluten represents a shorter active sequence with partially overlapping but not identical tissue-specificity claims. The relationship between the tetrapeptide and tripeptide bronchopulmonary bioregulators has not been systematically characterized in published comparative studies; the Khavinson literature presents them as complementary rather than competing entities within the respiratory bioregulator class.

3.3 Relationship to the broader Khavinson peptide family

Bronchogen occupies a defined position within the Khavinson ultrashort peptide bioregulator family, though its sequence relationship to the other family members is distinct from the pattern of the Ala-Glu-Asp core tetrapeptides. The principal Ala-Glu-Asp core family members are Cardiogen (Ala-Glu-Asp-Arg; cardiac), Cortagen (Ala-Glu-Asp-Pro; cortical/nervous system), and Epithalon (Ala-Glu-Asp-Gly; pineal/neuroendocrine). Bronchogen (Ala-Asp-Glu-Leu) differs from this core family in that the aspartate and glutamate residues are in reversed order relative to the Ala-Glu-Asp core, and the C-terminal residue is the hydrophobic leucine rather than a basic or polar residue [4]. This sequence distinction places Bronchogen in a partially separate structural subclass within the Khavinson family, sharing the N-terminal alanine and the acidic dipeptide character but not the precise Ala-Glu-Asp sequence motif. The structural divergence may contribute to the distinct tissue specificity (bronchopulmonary rather than cardiac, cortical, or pineal) through differential DNA sequence recognition, histone-binding affinity, or cellular uptake kinetics.

3.4 Tissue-specificity demonstration

The formal demonstration of bronchopulmonary tissue specificity was reported by Zakutskii et al. (2006) and is cross-referenced in the Chalisova et al. series of organotypic tissue culture studies that established the tissue-specificity paradigm for the Khavinson tetrapeptide family [16]. In these experiments, explants from lungs, heart, prostate, and pancreas of young and aged rats were cultured in the presence

of Bronchogen, Cardiogen, Prostamax, or Pancragen at concentrations ranging from 0.005 to 50 ng/mL. Each tetrapeptide produced statistically significant stimulation of proliferation (measured as the ratio of the explant growth zone area to the initial explant area) exclusively in its corresponding tissue type. Bronchogen stimulated lung tissue proliferation at an optimal concentration of 0.05 ng/mL in both young and aged animals. The other three tetrapeptides produced no significant effect on lung tissue at any concentration tested. This cross-tissue selectivity experiment is the principal evidence for the tissue-specificity claim of the Khavinson tetrapeptide system and has been cited across all subsequent Bronchogen publications as the foundational tissue-targeting demonstration.

3.5 Regulatory status

Bronchogen is registered in the Russian Federation as a biologically active additive (biologicheskii aktivnaya dobavka, BAD), a regulatory category analogous to a dietary supplement under United States law. The compound is not classified as a medicine (lekarstvennoye sredstvo) in the Russian Federation and has not undergone the registration process required for pharmaceutical products. It is commercially available in Russia in oral capsule formulations marketed for respiratory health maintenance. The compound is not registered, approved, or reviewed by the United States Food and Drug Administration, the European Medicines Agency, Health Canada, the Australian Therapeutic Goods Administration, or any other major Western regulatory authority.

4. Mechanism of Action

Bronchogen is characterized as a multi-level regulatory agent acting through epigenetic, transcriptional, and cellular mechanisms to modulate bronchial epithelial cell behavior. The proposed mechanisms, characterized through molecular modeling, biophysical studies, cell culture experiments, and animal models, encompass direct DNA interaction, histone binding, gene expression modulation, and downstream effects on bronchial epithelial proliferation, differentiation, apoptosis, and inflammatory signaling.

4.1 Peptide-DNA interaction

The Fedoreyeva et al. (2011) study demonstrated that fluorescein-labeled Bronchogen (ADEL) penetrated HeLa cell nuclei within hours of extracellular application, localizing to chromatin structures visible by confocal microscopy [6]. In vitro binding studies using fluorescence quenching methodology showed that Bronchogen interacted preferentially with deoxyribooligonucleotides containing CNG trinucleotide motifs (where N is any nucleotide), with particular selectivity for CTG-containing sequences. The CNG motif is a site of cytosine methylation by DNA methyltransferases, and the peptide binding was shown to depend on the methylation status of the DNA substrate. This methylation-sensitive DNA binding is proposed as one molecular basis for the tissue-specific gene regulatory activity, wherein differential methylation patterns across tissue types determine which gene promoter regions are accessible to the tetrapeptide [6, 20].

The Khavinson et al. (2014) study extended the DNA-binding characterization to bronchial epithelial cells specifically, reporting that ADEL bound DNA through the major groove and activated genes involved in epithelial differentiation [5]. The major-groove binding mode is consistent with the acidic peptide interacting with the electropositive major groove surface through a combination of hydrogen bonding (from the amide backbone and the carboxylate side chains) and hydrophobic interactions (from the alanine and leucine residues) with the base-pair edges exposed in the major groove.

4.2 DNA stabilization

The Monaselidze et al. (2011) differential scanning microcalorimetry study provided biophysical characterization of the Bronchogen-DNA interaction [7]. Bronchogen increased the melting temperature of DNA from calf thymus and mouse liver by 3.1 degrees Celsius in a narrow concentration range, demonstrating direct thermal stabilization of the double helix. The stabilization was concentration-dependent but saturated above a threshold concentration, consistent with a limited number of binding sites on the DNA molecule. Analysis of the melting curves indicated that Bronchogen bound to both DNA strands without preference for adenine-thymine or guanine-cytosine base pairs, with binding characterized by the authors as "strong and occasional" (high affinity at a limited number of sites rather than low-affinity coating of the DNA surface) [7]. The Monaselidze et al. (2011) companion study in Georgian Medical News extended these findings to chromatin thermostability, reporting that the tetrapeptide influenced the thermal properties of chromatin in a manner consistent with modulation of histone-DNA interactions [21].

4.3 Gene expression regulation in bronchial epithelium

The Khavinson et al. (2014) study in human bronchial epithelial cell cultures across multiple passages (representing early, middle, and late stages of replicative aging) is the principal characterization of Bronchogen's gene-regulatory activity in its target tissue [5]. The study reported that ADEL at nanomolar to picomolar concentrations regulated the expression of the following proteins:

Ki67: Proliferation marker expressed in all active phases of the cell cycle (G1, S, G2, M) but absent in quiescent (G0) cells. ADEL increased Ki67 expression in late-passage bronchial epithelial cells, indicating reactivation of proliferative capacity in aging cells.

Mcl-1: Anti-apoptotic member of the BCL-2 protein family that sequesters pro-apoptotic BH3-only proteins and prevents mitochondrial outer membrane permeabilization. ADEL upregulated Mcl-1 expression, suggesting an anti-apoptotic mechanism that protects bronchial epithelial cells from programmed cell death during aging and pathological stress.

p53: Tumor suppressor protein and master regulator of the DNA damage response, cell cycle arrest, and apoptosis. ADEL modulated p53 expression in a context-dependent manner, with the direction and magnitude of effect depending on the passage number of the cell culture. The modulation of p53 is consistent with a regulatory rather than a simple suppressive mechanism, adjusting the apoptotic threshold rather than eliminating apoptotic capacity.

CD79: Cell surface glycoprotein involved in cellular differentiation. ADEL regulated CD79 expression in bronchial epithelial cells, supporting its role as a differentiation-modulating agent.

NOS-3 (endothelial nitric oxide synthase): Enzyme responsible for constitutive production of nitric oxide from L-arginine. ADEL upregulated NOS-3 expression in bronchial epithelial cells, a finding with implications for mucosal blood flow, bronchodilation, and antimicrobial defense in the respiratory tract (nitric oxide is a key mediator of airway smooth muscle relaxation and a component of innate immune defense against respiratory pathogens).

The gene-regulatory effects were consistently more pronounced in late-passage (aged) cell cultures than in early-passage (young) cultures, supporting the Khavinson thesis that ultrashort peptide bioregulators act preferentially on cells with age-related decline in gene expression and that their primary utility is geroprotective restoration of youthful gene expression patterns rather than pharmacological augmentation beyond physiological levels [5, 8].

4.4 Epigenetic framework

The Ashapkin et al. (2015) review characterized the broader epigenetic framework within which Bronchogen and other Khavinson ultrashort peptides operate [20]. The proposed model posits that age-related changes in DNA methylation patterns (global hypomethylation with localized hypermethylation at specific promoter regions) alter the accessibility of CNG-containing regulatory sequences to ultrashort peptides. In young cells, normal methylation patterns restrict peptide binding to a defined set of tissue-appropriate gene promoters. In aged cells, altered methylation creates new binding opportunities, and the exogenous addition of the appropriate tissue-specific peptide restores a transcriptional profile closer to the young-cell state. The Khavinson et al. (2021) systematic review extended this framework, re-

viewing evidence across multiple ultrashort peptides including Bronchogen and proposing a unified model of peptide-mediated gene expression regulation through DNA and histone interactions [22].

4.5 Tissue-specific differentiation

The Khavinson et al. (2012) study examined the expression of differentiation-related transcription factors in cultured human cells from different tissue types and demonstrated that Bronchogen stimulated expression of differentiation markers specifically in bronchial epithelial cells but not in pancreatic cells, vascular endothelial cells, or other non-target tissues [8]. The tissue-specific differentiation effect was more pronounced in late-passage (aging) cell cultures, where baseline differentiation marker expression was reduced relative to early-passage cultures. This finding supports the selectivity of Bronchogen for bronchial tissue at the cellular level and is consistent with the organotypic tissue culture results from the Zakutskii et al. (2006) study [16].

5. Pharmacokinetics and Routes of Administration

5.1 General considerations for ultrashort peptides

No formal pharmacokinetic studies have been published for Bronchogen as the isolated synthetic ADEL tetrapeptide in any species. The pharmacokinetic properties of the compound must therefore be inferred from the general characteristics of ultrashort peptides and from the limited published data on structurally related Khavinson tetrapeptides.

Linear tetrapeptides with unprotected N-terminal and C-terminal groups are expected to undergo rapid proteolytic degradation in biological fluids. Aminopeptidases (particularly aminopeptidase N/CD13 and aminopeptidase A) cleave the N-terminal alanine residue. Carboxypeptidases cleave the C-terminal leucine residue. Dipeptidyl peptidases (DPP-IV and related enzymes) may cleave the N-terminal dipeptide Ala-Asp. The combined action of these ubiquitous exopeptidases produces a predicted plasma half-life on the order of minutes for the intact tetrapeptide. The degradation products (individual amino acids and short fragments) are nutritionally and pharmacologically inert at the picomolar to nanomolar concentrations relevant to the bioregulatory activity.

5.2 Intestinal absorption

The Khavinson laboratory has characterized intestinal absorption of ultrashort peptides through the PEPT1 (SLC15A1) and PEPT2 (SLC15A2) proton-coupled oligopeptide transporters [12, 13]. These transporters accept di- and tripeptides as substrates and have been shown in molecular modeling studies to accommodate tetrapeptides in extended conformations. The PEPT1 transporter is expressed at high density on the apical brush border membrane of enterocytes throughout the small intestine and provides a mechanism for intact or partially degraded tetrapeptide to cross the intestinal epithelium by active transport rather than by passive paracellular diffusion. The Khavinson et al. (2022) study on transport of ultrashort peptides through POT and LAT carriers provided additional evidence for active cellular uptake mechanisms for peptides of this size class [13].

The oral bioavailability of Bronchogen has not been quantified. The commercial formulation in Russia is an oral capsule, implying sufficient oral bioactivity to produce the claimed biological effects, though the mechanistic basis for this could include local gastrointestinal effects, absorption of intact or partially degraded peptide through PEPT1/PEPT2, or absorption of component amino acids followed by intracellular reassembly (a mechanism that has not been demonstrated for exogenous peptide sequences and is considered speculative).

5.3 Cellular uptake and nuclear penetration

The Fedoreyeva et al. (2011) study demonstrated that fluorescein-labeled Bronchogen penetrated cell membranes and localized to nuclei in HeLa cells [6]. The mechanism of membrane penetration for a tetrapeptide of this size (molecular weight approximately 446 g/mol) could involve the PEPT2 transporter (expressed on many cell types in addition to renal tubular cells), passive diffusion (feasible for molecules below approximately 500 g/mol with appropriate lipophilicity), or endocytic uptake. The nuclear localization, demonstrated by confocal microscopy, supports the proposed mechanism of direct DNA interaction within intact cells and is consistent with passive nuclear pore transit for molecules of this size (the nuclear pore complex permits passive diffusion of molecules below approximately 40 kilodaltons) [6].

5.4 Routes of administration

Three routes have been used or proposed for Bronchogen administration:

Oral: The commercial formulation in the Russian Federation is an oral capsule, typically containing 200 micrograms of synthetic ADEL peptide. Oral administration is the primary route for the marketed biologically active additive product. The effective oral dose reported in Russian product literature is 200 to 400 micrograms (one to two capsules) taken one to two times daily.

Sublingual: Some Russian product formulations offer sublingual administration, which would bypass first-pass hepatic metabolism and potentially improve the fraction of intact tetrapeptide reaching systemic circulation. No formal comparison of sublingual versus oral bioavailability has been published.

Parenteral: Intramuscular or subcutaneous injection of the reconstituted lyophilized peptide has been used in research settings and in some Russian clinical protocols for the parent bronchial polypeptide complex. No formal parenteral pharmacokinetic data for the synthetic ADEL tetrapeptide have been published.

5.5 Special populations

No data are available on the pharmacokinetics of Bronchogen in any special population (renal impairment, hepatic impairment, pediatric, geriatric, pregnancy, lactation). Given the absence of formal pharmacokinetic characterization in healthy adults, the lack of special-population data is expected. Investigators should treat the complete absence of human pharmacokinetic data as a fundamental research limitation.

6. Preclinical Pharmacology

6.1 Organotypic tissue culture studies

The foundational preclinical characterization of Bronchogen is the organotypic tissue culture work demonstrating bronchopulmonary tissue specificity. The Zakutskii et al. (2006) study established that Bronchogen at 0.05 ng/mL (approximately 0.1 nanomolar) stimulated proliferation of lung tissue explants from both young (3-week-old) and aged (18-month-old) rats, measured as the ratio of growth zone area to initial explant area [16]. The stimulatory effect was statistically significant compared to untreated controls and was selective for lung tissue; heart, prostate, and pancreatic tissue explants showed no response to Bronchogen at any concentration tested. The effective concentration range of 0.005 to 0.5 ng/mL is two to three orders of magnitude below conventional peptide hormone concentrations and is consistent with the picomolar activity range reported across the Khavinson ultrashort peptide family.

6.2 Human bronchial epithelial cell culture studies

The Khavinson et al. (2014) study in human bronchial epithelial cells (primary cultures passaged through early, middle, and late stages of replicative senescence) is the most detailed in vitro characterization of Bronchogen's cellular pharmacology [5]. The principal findings, as detailed in Section 4.3, are the regulation of Ki67, Mcl-1, p53, CD79, and NOS-3 protein expression, with effects more pronounced in late-passage cells. The study additionally reported that ADEL activated gene expression programs associated with epithelial differentiation, assessed by microarray or quantitative polymerase chain reaction analysis of bronchial epithelial gene sets. The compound bound to specific DNA regions within the bronchial epithelial cell genome through the major groove, as characterized by molecular modeling and binding assays [5].

The Khavinson et al. (2012) tissue-specific differentiation study extended the in vitro characterization by demonstrating that Bronchogen increased expression of differentiation-related transcription factors specifically in bronchial epithelial cells

but not in other cell types [8]. The effect was geroprotective in character, restoring differentiation marker expression in aged cell cultures toward levels observed in young cultures.

6.3 COPD animal models

The Kuzubova et al. (2015) study in a nitrogen dioxide-induced COPD rat model is the principal *in vivo* pharmacology study of Bronchogen [10]. Chronic obstructive pulmonary disease was induced by 60-day intermittent exposure to nitrogen dioxide, producing the characteristic pathological changes: goblet cell hyperplasia, squamous metaplasia of bronchial epithelium, loss of ciliated cells, lymphocytic infiltration, and emphysematous changes in alveolar architecture. Following one month of Bronchogen treatment (dose not specified in the English-language abstract; the treatment course is described as one month of tetrapeptide administration), the study documented:

Epithelial restoration: Recovery of ciliated epithelial cells with reduction of goblet cell hyperplasia and squamous metaplasia. The restoration of ciliated cells is functionally significant because the mucociliary escalator (coordinated ciliary beating that transports mucus and trapped particulates from the lower airways to the pharynx) is the principal mechanical clearance mechanism of the respiratory tract and is severely compromised in COPD.

Inflammatory resolution: Reduction of lymphocytic infiltration in the bronchial wall and normalization of proinflammatory cytokine profiles in bronchoalveolar lavage fluid. The normalization of the inflammatory milieu suggests that Bronchogen acts through anti-inflammatory mechanisms in addition to its proliferative and differentiative effects.

Immune barrier recovery: Enhancement of secretory immunoglobulin A (sIgA) production. sIgA is the principal immunoglobulin of the respiratory mucosal surface and provides first-line adaptive immune defense against respiratory pathogens. Its restoration indicates functional recovery of the mucosal immune barrier.

The Titova et al. (2017) follow-up study in the same COPD model confirmed and extended these findings [11]. The study reported decreased activity of neutrophilic inflammation with normalization of cellular composition in bronchoalveolar lavage fluid, restoration of bronchial tissue structure assessed by histological exam-

ination, increased sIgA levels, and recovery of surfactant protein B levels. Surfactant protein B is an essential component of the pulmonary surfactant system that reduces surface tension at the alveolar air-liquid interface and prevents alveolar collapse during expiration. Its recovery indicates restoration of type II alveolar pneumocyte function, extending the demonstrated target-cell repertoire of Bronchogen beyond bronchial epithelial cells to include the surfactant-producing cells of the alveolar compartment.

6.4 Cross-kingdom activity in plant systems

The Fedoreyeva et al. (2017) study demonstrated that Bronchogen (ADEL) influenced growth and differentiation in *Nicotiana tabacum* (tobacco) callus cultures and regenerants at concentrations of 10^{-7} to 10^{-9} molar [23]. The peptide increased callus growth and stimulated leaf formation in plant regenerants, and regulated expression of CLE (CLAVATA3/embryo surrounding region-related), KNOX1 (KNOTTED1-like homeobox), and GRF (growth-regulating factor) family genes. These gene families regulate meristematic activity, organ differentiation, and developmental patterning in plants. The cross-kingdom activity of an animal-derived ultrashort peptide in plant systems is remarkable and suggests that the peptide-DNA interaction mechanism operates through a conserved molecular recognition process (CNG motif binding) that transcends the animal-plant kingdom boundary. The authors interpreted the findings as evidence that ultrashort peptides function through a fundamental, evolutionarily conserved epigenetic mechanism rather than through classical receptor-ligand pharmacology [23].

7. Clinical Evidence Base

7.1 Human clinical trials

No human clinical trials of Bronchogen have been published in peer-reviewed literature indexed in PubMed, EMBASE, Web of Science, or the Cochrane Central Register of Controlled Trials. No entries for Bronchogen, ADEL tetrapeptide, or Ala-Asp-Glu-Leu appear in ClinicalTrials.gov, the EU Clinical Trials Register, or the Russian Federation State Registry of Clinical Trials (GRLS). The compound has not been submitted for regulatory review as a pharmaceutical product in any jurisdiction.

7.2 Russian clinical use

The parent bronchial polypeptide complex (the organ extract preparation from which the ADEL tetrapeptide sequence was derived) has been used clinically in the Russian Federation for chronic obstructive pulmonary disease, chronic bronchitis, and related respiratory conditions under the regulatory framework for biologically active additives. Published clinical reports for the parent complex exist in the Russian-language literature but have not been independently verified by Western investigators and do not meet the evidentiary standards of Western regulatory agencies. The synthetic ADEL tetrapeptide (Bronchogen) has been marketed in Russia as a biologically active additive for respiratory health maintenance, with anecdotal clinical reports but no formal clinical trial publications.

7.3 Evidence limitations

The absence of human clinical data is the most significant limitation of the Bronchogen evidence base. The published preclinical evidence, while internally consistent across organotypic culture, human cell culture, and animal model systems, has not been tested in human subjects under controlled conditions. Additionally, the entirety of the preclinical evidence originates from a single research network (the Khavinson group and affiliated Russian laboratories), and inde-

pendent replication by Western research groups has not been published for Bronchogen specifically. Investigators should weight the evidence base with these limitations in mind.

8. Sourcing and Quality Verification

Bronchogen is supplied by multiple peptide synthesis vendors as a research-grade lyophilized peptide at greater than 95 percent purity by high-performance liquid chromatography. Sources include specialty peptide suppliers (Khavinson Peptides, CosmicNootropic, and various Russian and Chinese peptide synthesis companies). The compound is also available as a consumer product (oral capsules) from Russian manufacturers under the biologically active additive regulatory category.

Pharmaceutical-grade Bronchogen does not exist in the sense of a regulated pharmaceutical product manufactured under Good Manufacturing Practice conditions; the compound has not been approved as a medicine in any jurisdiction. The highest quality research-grade material is obtained from reputable peptide synthesis vendors that provide analytical documentation.

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

- High-performance liquid chromatography area-percent purity, ideally greater than 95 percent (research grade) or greater than 98 percent (high-purity research grade)
- Mass spectrometry confirmation of molecular weight (447.45 +/- 0.01 for the protonated peptide [M+H]⁺; 446.45 for the neutral form)
- Amino acid analysis after acid hydrolysis to confirm the correct amino acid composition (Ala:Asp:Glu:Leu in a 1:1:1:1 molar ratio)
- Chiral amino acid analysis or comparison of chromatographic retention time to confirm all-L stereochemistry
- Residual solvent analysis and trifluoroacetic acid content determination (relevant for peptides purified by reversed-phase HPLC with trifluoroacetic acid-containing mobile phases)
- Endotoxin testing (limulus amebocyte lysate or recombinant Factor C assay) for material intended for cell culture or injection applications

Third-party analytical laboratories (Janoshik Analytical, Anabolic Lab, others) provide independent confirmation of vendor certificates of analysis. The research-grade ultrashort peptide market is less well established than the small-molecule or

long-peptide markets, and lot-to-lot variability is observed; on-receipt analytical confirmation is appropriate for any investigation where dose-response precision is important.

9. Reconstitution and Handling

Bronchogen as a lyophilized or crystalline powder is freely soluble in water at room temperature. For research applications, the compound is reconstituted in sterile water for injection, bacteriostatic water for injection (for multi-use vials), or phosphate-buffered saline at the desired concentration.

Stock solution preparation: Typical stock concentrations range from 1 mg/mL to 10 mg/mL in aqueous solution. The compound dissolves rapidly without heating or sonication. For in vitro applications at picomolar to nanomolar working concentrations, serial dilution from a concentrated stock is necessary to achieve accurate pipetting volumes.

Storage: The lyophilized solid should be stored at minus 20 degrees Celsius, protected from moisture, and allowed to equilibrate to room temperature before opening the container (to prevent condensation-mediated hydrolysis). Reconstituted solutions should be stored refrigerated (2 to 8 degrees Celsius) and used within 14 to 30 days for bacteriostatic water reconstitutions or within 24 hours for sterile water reconstitutions. Aliquoting of stock solution for single-use is recommended to minimize freeze-thaw cycles.

Stability: Linear tetrapeptides are generally stable in aqueous solution at neutral pH and refrigerated temperature for weeks to months. The principal degradation pathway is hydrolysis of the peptide bonds, which is slow at neutral pH and refrigerated temperature but accelerated at extremes of pH and at elevated temperature. The Asp-Glu bond is the most susceptible to acid-catalyzed hydrolysis (aspartyl peptide bonds are known lability sites in peptide chemistry). Protection from light is reasonable practice but not strictly required for peptides without aromatic or photosensitive residues; Bronchogen contains no tryptophan, tyrosine, or other chromophoric residues and is not photosensitive.

For cell culture applications: The peptide should be dissolved in sterile, endotoxin-free water or buffer, sterile-filtered through a 0.22-micrometer membrane if prepared from non-sterile solid, and added to culture medium at the desired final concentration. Working concentrations in the published Khavinson literature range from 0.005 to 50 ng/mL (approximately 0.01 to 100 nanomolar), with optimal effects typically observed at 0.05 ng/mL (approximately 0.1 nanomolar) [16].

10. Stack Interaction Considerations

Bronchogen is a short peptide with no characterized receptor-level pharmacology at conventional drug targets (G-protein-coupled receptors, ion channels, nuclear hormone receptors, kinases). Its mechanism of action (direct DNA binding and epigenetic gene regulation) does not involve the classical drug-target interactions that underlie most pharmacodynamic drug-drug interactions. Accordingly, the stack interaction profile is expected to be minimal at the dose levels used in research.

CYP enzyme interactions: As a tetrapeptide degraded by aminopeptidases and carboxypeptidases rather than by cytochrome P450 enzymes, Bronchogen is not expected to produce CYP-mediated drug-drug interactions. It is not a substrate, inhibitor, or inducer of CYP1A2, CYP2C9, CYP2C19, CYP2D6, CYP3A4, or other major drug-metabolizing CYP isoforms.

Other Khavinson peptides: Concurrent administration of Bronchogen with other Khavinson tetrapeptide bioregulators (Cardiogen, Cortagen, Epithalon, Pancragen, Prostamax) is practiced in the Russian biologically active additive market, where multi-peptide bioregulator regimens are commonly used. The Khavinson literature does not report adverse interactions between concurrent tetrapeptide bioregulators. The theoretical concern is that multiple peptides competing for the same DNA-binding sites or the same PEPT1/PEPT2 transport mechanisms could produce either additive or antagonistic effects depending on the relative affinities and concentrations. This interaction has not been characterized experimentally.

Mucolytics and bronchodilators: Concurrent use of Bronchogen with N-acetylcysteine, carbocysteine, ambroxol, or other mucolytics is plausible in the context of respiratory research. No pharmacodynamic interactions are predicted. Concurrent use with inhaled bronchodilators (beta-2 agonists, anticholinergics) or inhaled corticosteroids is similarly not expected to produce interactions, though the anti-inflammatory effects of Bronchogen could theoretically complement the anti-inflammatory effects of inhaled corticosteroids through different mechanisms.

Anti-inflammatory agents: The anti-inflammatory effects of Bronchogen in the COPD rat model (reduction of neutrophilic inflammation, normalization of cytokine profiles) could be additive with systemic anti-inflammatory agents (corticoids).

teroids, non-steroidal anti-inflammatory drugs, phosphodiesterase-4 inhibitors). The nature of any additive effect has not been characterized.

Immunomodulatory peptides: Concurrent use with other immunomodulatory peptides (thymalin, thymogen, immunofan) is practiced in Russian clinical protocols. The theoretical concern is additive immunostimulation, though the characteristically low concentrations of the Khavinson ultrashort peptide bioregulators and the absence of direct immune receptor agonism make clinically significant additive immunostimulation unlikely.

Investigators combining Bronchogen with other research compounds should approach novel combinations with appropriate caution and titration, recognizing that the absence of characterized interactions reflects the absence of formal interaction studies rather than a demonstrated absence of interaction potential.

11. Adverse Events and Safety

11.1 Animal toxicology

No formal toxicology studies of Bronchogen (as the isolated synthetic ADEL tetrapeptide) have been published in peer-reviewed literature. The compound is composed of four naturally occurring L-amino acids joined by standard peptide bonds, and the degradation products are nutritionally common amino acids (alanine, aspartic acid, glutamic acid, leucine). The expected systemic toxicity of a tetrapeptide at the picomolar to nanomolar effective concentrations reported in the Khavinson literature is negligible.

The parent bronchial polypeptide complex (from which the ADEL sequence was derived) was characterized in Soviet-era preclinical toxicology studies that reportedly demonstrated an absence of acute or chronic toxicity at multiples of the therapeutic dose. These studies are not accessible in Western bibliographic databases and have not been independently verified.

11.2 Clinical safety

No adverse events have been reported in the published preclinical literature for Bronchogen. The absence of adverse-event data reflects the absence of formal clinical trials rather than a demonstrated favorable safety profile. The Russian commercial product literature for Bronchogen capsules reports no contraindications other than known hypersensitivity to the peptide components and pregnancy/lactation (as a precautionary exclusion in the absence of reproductive toxicology data).

11.3 Theoretical safety considerations

The principal theoretical safety considerations for Bronchogen are:

Proliferative stimulation: The compound stimulates cell proliferation in bronchial epithelial cells. In the context of pre-existing bronchial neoplasia or pre-neoplastic lesions (bronchial dysplasia, carcinoma in situ), exogenous proliferative stimulation could theoretically promote neoplastic progression. The Khavinson literature addresses this concern by noting that the proliferative effect is accompa-

nied by upregulation of differentiation markers and modulation of p53, suggesting that the proliferative program is coupled to differentiation rather than to undifferentiated proliferation. This argument has not been tested in a tumor-bearing animal model for Bronchogen specifically.

Immunomodulation: The restoration of sIgA production and the normalization of inflammatory cytokine profiles in COPD models indicate immunomodulatory activity. In the context of immunosuppressed patients or patients with autoimmune respiratory disease, the immunomodulatory effects could have unpredictable consequences.

Epigenetic modification: The proposed mechanism of DNA binding and chromatin remodeling, if operative in human subjects, raises theoretical questions about the specificity and reversibility of the epigenetic changes. The Khavinson literature characterizes the effects as restorative (returning aged gene expression patterns toward youthful states) rather than as novel epigenetic modifications, but the distinction between restoration and novel modification has not been formally demonstrated at the genome-wide level.

11.4 Pregnancy and lactation

No reproductive or developmental toxicology data are available. The Russian commercial product literature lists pregnancy and lactation as contraindications as a precautionary measure. Investigators should not use Bronchogen in research involving pregnant or lactating subjects without appropriate preclinical safety data.

12. Comparable Compounds Assessment

This section presents a structured comparative assessment of five bronchopulmonary or respiratory-protective candidates against Bronchogen, evaluated against five competency standards: novelty, effect size, promising potential (current clinical state and trajectory), side-effect profile (clinical or in vitro), and overall validation. The five candidates were selected to span the spectrum from structurally related Khavinson peptides to established Western respiratory therapeutics, providing context for the positioning of Bronchogen within the broader respiratory pharmacology landscape.

12.1 Chonluten (EDL; H-Glu-Asp-Leu-OH)

Mechanism: Synthetic tripeptide bioregulator of the Khavinson family, also designated as a bronchopulmonary bioregulator. The EDL sequence shares the acidic dipeptide and C-terminal leucine motifs with Bronchogen but in a different arrangement (Glu-Asp-Leu versus Ala-Asp-Glu-Leu) and lacks the N-terminal alanine [14]. Proposed mechanism is identical to Bronchogen: direct DNA binding at CNG motifs, chromatin remodeling, and tissue-specific gene expression regulation. Chonluten has been studied for anti-inflammatory effects in monocyte/macrophage cell lines, where it inhibited TNF and IL-6 expression stimulated by lipopolysaccharide [17].

Development trajectory: Developed at the same institution as Bronchogen. Registered in the Russian Federation as a biologically active additive for respiratory applications. The published literature for Chonluten is comparable in size to Bronchogen, with studies from the Khavinson network and a 2022 Italian collaborative study demonstrating anti-inflammatory effects in human THP-1 monocyte/macrophage cells [17]. No clinical trials published.

Novelty: Low. The mechanism is identical to Bronchogen within the Khavinson peptide framework. The tripeptide versus tetrapeptide distinction has not been characterized for differential pharmacology.

Effect size: Preclinical only. Anti-inflammatory effect sizes in the Avolio et al. (2022) THP-1 cell study are comparable to other Khavinson peptides tested in the same system [17].

Promising potential: Low. No clinical development. Evidence base from limited research groups. The Italian collaborative work provides partial independent validation of anti-inflammatory activity outside the Khavinson network.

Side-effect profile: No safety data. Theoretical profile identical to Bronchogen given the structural similarity and identical proposed mechanism.

Overall validation: Low. Preclinical evidence from a small number of research groups. Partial independent replication of anti-inflammatory effects.

Comparison to Bronchogen: Chonluten is the most structurally and mechanistically related compound to Bronchogen in this comparator panel. The key distinction is the tripeptide versus tetrapeptide size; the Khavinson literature does not clearly establish whether the tetrapeptide or the tripeptide is the superior bronchopulmonary bioregulator. Chonluten has the advantage of partial independent validation from a Western laboratory (Avolio et al. 2022). Bronchogen has the advantage of more detailed in vivo pharmacology data (the COPD rat model studies) and more extensive characterization of bronchial gene expression modulation.

12.2 GHK-Cu (Copper tripeptide-1; Gly-His-Lys copper complex)

Mechanism: Endogenous tripeptide-copper complex with tissue-repair, anti-inflammatory, and gene-expression-modulatory activity. GHK-Cu has been demonstrated to modulate expression of approximately 4,000 human genes, including genes involved in tissue remodeling, anti-inflammatory signaling, antioxidant defense, and anti-fibrotic pathways [24, 25]. Among the modulated genes, a substantial fraction are lung-relevant, including genes involved in alveolar repair, matrix metalloproteinase regulation, and inflammatory cytokine suppression. The copper-dependent gene-expression-modulatory mechanism is fundamentally different from the Khavinson peptide-DNA interaction model but shares the conceptual framework of peptide-mediated gene expression regulation.

Development trajectory: GHK-Cu has been studied for over four decades since its discovery by Loren Pickart. Extensive preclinical evidence from multiple independent research groups. Clinical use in dermatological applications (wound healing, anti-aging skin care) is well established. Pulmonary applications are supported by gene expression studies demonstrating modulation of lung-relevant gene networks, but dedicated pulmonary preclinical or clinical studies are limited.

Novelty: Moderate. The copper-dependent mechanism is distinct from the Khavinson direct DNA-binding model. The endogenous origin (GHK is a naturally occurring plasma peptide whose concentration declines with age) provides a different biological framework than the synthetic Khavinson peptides.

Effect size: Substantial for dermatological and wound-healing applications with established clinical efficacy. Lung-specific effects are characterized at the gene expression level but not at the tissue or clinical level.

Promising potential: Moderate. Well-established commercial and clinical use in dermatology. Lung-specific development is not a current priority but the gene expression data are promising.

Side-effect profile: Clinical safety data available from decades of dermatological use. Generally well tolerated. The copper component introduces considerations around copper toxicity at high doses but is not a concern at the microgram to milligram doses used in practice.

Overall validation: Moderate to high for dermatological applications. Low for pulmonary-specific applications.

Comparison to Bronchogen: GHK-Cu has a vastly larger and more independently replicated evidence base than Bronchogen overall, but the lung-specific evidence for both compounds is limited. GHK-Cu has the advantage of established clinical use in a non-pulmonary indication (dermatology), providing a safety foundation that Bronchogen lacks. Bronchogen has the advantage of demonstrated bronchial tissue specificity in organotypic culture and in the COPD rat model, whereas GHK-Cu's lung relevance is inferred from gene expression data rather than from direct pulmonary tissue studies.

12.3 BPC-157 (Body Protection Compound-157)

Mechanism: Synthetic pentadecapeptide (15 amino acids) derived from human gastric juice, characterized as a cytoprotective agent with activity across multiple organ systems including the gastrointestinal tract, musculoskeletal system, cardiovascular system, and central nervous system [26, 27]. The mechanism involves up-regulation of vascular endothelial growth factor (VEGF), activation of nitric oxide pathways, modulation of dopaminergic and serotonergic signaling, and promotion of angiogenesis and tissue repair. Pulmonary effects have been reported in some studies, including protection against pulmonary hypertension and lung injury in animal models.

Development trajectory: Extensive preclinical evidence base from the Sikiric laboratory at the University of Zagreb and from a growing number of independent research groups. Phase 2 clinical trials have been conducted for inflammatory bowel disease (oral administration) and for musculoskeletal injuries (parenteral administration). The compound has not been specifically developed for pulmonary indications.

Novelty: Low for the general cytoprotective mechanism; moderate for the multi-organ applicability that extends to pulmonary tissue.

Effect size: Substantial in preclinical models across multiple tissue types. Pulmonary-specific effect sizes derive from a smaller number of studies than the gastrointestinal or musculoskeletal evidence base.

Promising potential: Moderate. Active research interest. Wide commercial availability. Clinical development for pulmonary applications specifically is not prioritized.

Side-effect profile: Preclinical safety data suggest favorable tolerability. Limited human safety data from clinical trials for non-pulmonary indications. No serious adverse events reported.

Overall validation: Moderate. Extensive preclinical evidence, though primarily from a limited number of research groups for the pulmonary applications. Clinical validation is emerging for non-pulmonary indications.

Comparison to Bronchogen: BPC-157 has a substantially larger preclinical evidence base than Bronchogen overall, though neither has pulmonary-specific clinical data. BPC-157 operates through a different mechanism (VEGF/NO angiogenesis

and cytoprotection) and lacks the bronchial tissue specificity that Bronchogen demonstrates in organotypic culture. The broader tissue activity of BPC-157 is both an advantage (wider applicability for systemic cytoprotection) and a limitation (less pulmonary specificity).

12.4 Thymalin (thymic polypeptide complex)

Mechanism: Polypeptide complex extracted from calf thymus, containing a mixture of peptide fragments with immunomodulatory activity. Thymalin is the parent polypeptide complex from which the Khavinson dipeptide Vilon (Lys-Glu) and the synthetic thymogen (Glu-Trp) were derived [18, 19]. The mechanism involves modulation of T-cell differentiation and maturation, enhancement of innate immune function, and anti-inflammatory activity through cytokine regulation. Pulmonary relevance arises from the role of immune function in respiratory defense and the clinical application of Thymalin in respiratory infections and chronic lung disease in Russian clinical practice.

Development trajectory: Thymalin has been registered as a pharmaceutical product in the Russian Federation since the 1980s and has been used clinically for immunodeficiency, chronic infections including respiratory infections, and as an adjunct to cancer chemotherapy. The compound has a larger clinical evidence base than any other Khavinson-class product, though the evidence is predominantly from Russian clinical studies not independently replicated by Western investigators.

Novelty: Low. The thymic polypeptide extract concept has been studied for over four decades. The mechanism is conventional immunomodulation.

Effect size: Moderate in Russian clinical studies for immunodeficiency and chronic infection endpoints. Respiratory-specific effect sizes are embedded within the broader immunomodulatory effect and have not been independently characterized.

Promising potential: Low to moderate. The compound is commercially available and clinically used in the Russian Federation but has no Western regulatory approval or clinical development pathway.

Side-effect profile: Russian clinical experience over several decades reports favorable tolerability. The principal adverse events are local injection-site reactions. No serious adverse events have been reported.

Overall validation: Moderate within the Russian clinical framework. Low by Western regulatory and evidentiary standards.

Comparison to Bronchogen: Thymalin has a substantially larger clinical evidence base than Bronchogen (decades of Russian clinical use versus no clinical data). However, Thymalin operates through a fundamentally different mechanism (immune modulation through thymic peptide complex activity) and does not have bronchial tissue-specific effects. The respiratory applications of Thymalin are secondary to its primary immunomodulatory indication, whereas Bronchogen is specifically designed and characterized for bronchopulmonary tissue targeting. The two compounds could theoretically be complementary in a respiratory research context (Thymalin for immune support, Bronchogen for epithelial regeneration).

12.5 N-Acetylcysteine (NAC)

Mechanism: Small-molecule mucolytic and antioxidant (molecular weight 163.19 g/mol) that acts as a precursor to glutathione synthesis (through provision of the rate-limiting cysteine residue), as a direct free-radical scavenger, and as a mucolytic agent (through reduction of disulfide bonds in mucin glycoproteins) [28]. The respiratory applications are well characterized: mucolysis reduces mucus viscosity and improves mucociliary clearance; antioxidant activity protects airway epithelium from oxidative stress; and anti-inflammatory activity (secondary to glutathione restoration and NF-kappaB modulation) reduces neutrophilic airway inflammation.

Development trajectory: NAC has been used clinically for over five decades as a mucolytic agent for chronic bronchitis, COPD, cystic fibrosis, and as an antidote for acetaminophen overdose. It is approved by multiple Western regulatory authorities and is available as a prescription medication and as a dietary supplement. The BRNCUS trial (Bronchitis Randomized on NAC Cost-Utility Study, 2005) and the PANTHEON trial (Placebo-controlled study on efficacy and safety of N-acetylcysteine High dose in Exacerbations of chronic Obstructive pulmonary disease, 2014) are the principal Western randomized controlled trials evaluating NAC in COPD [29, 30].

Novelty: Low. The mechanism is well established and widely understood. No novel pharmacology.

Effect size: The BRONCUS trial reported that NAC at 600 mg daily for 3 years did not reduce the rate of FEV1 decline or exacerbation frequency in COPD patients overall, though post-hoc analysis suggested benefit in patients not receiving inhaled corticosteroids [29]. The PANTHEON trial reported that NAC at 600 mg twice daily for one year reduced exacerbation frequency by 22 percent compared to placebo (risk ratio 0.78; $p = 0.004$) in Chinese COPD patients [30]. Effect sizes are modest but statistically significant in the PANTHEON trial.

Promising potential: Moderate. Established clinical use worldwide. The evidence for COPD exacerbation reduction is supportive but not universally endorsed by clinical guidelines.

Side-effect profile: Well-characterized clinical safety. Principal adverse events are gastrointestinal (nausea, vomiting, diarrhea) at oral doses. Anaphylactoid reactions occur rarely with intravenous administration (principally in the acetaminophen overdose indication). Overall safety profile is favorable.

Overall validation: High. Decades of clinical use, multiple randomized controlled trials, regulatory approval in multiple jurisdictions.

Comparison to Bronchogen: NAC and Bronchogen operate through entirely different mechanisms (mucolysis and glutathione precursor versus epigenetic gene regulation). NAC has an incomparably larger and more rigorous evidence base, regulatory approval, and clinical validation. Bronchogen's theoretical advantage is tissue-specific regenerative activity (epithelial restoration, ciliated cell recovery) that NAC does not produce; NAC primarily addresses mucus clearance and oxidative stress without promoting epithelial regeneration. The two compounds target different aspects of respiratory pathology and are not direct functional substitutes. A research question of interest is whether Bronchogen's epithelial regenerative effects and NAC's mucolytic and antioxidant effects could be complementary in COPD, though this combination has not been studied.

12.6 Comparator summary

The five compounds represent a spectrum of validation levels and mechanistic approaches for respiratory and bronchopulmonary interventions. **N-Acetylcysteine** is the clear leader in validation, with decades of clinical use, multiple randomized controlled trials, and regulatory approval in multiple jurisdictions, though its mechanism (mucolysis and antioxidant activity) does not address the epithelial re-

generation that Bronchogen targets. **BPC-157** and **GHK-Cu** occupy intermediate positions, with substantial preclinical evidence bases (though primarily for non-pulmonary applications) and varying degrees of clinical use. **Thymalin** provides relevant Russian clinical experience in respiratory immunomodulation but operates through a different mechanism and tissue target. **Chonluten** is the most structurally related compound to Bronchogen, sharing the Khavinson ultrashort peptide bioregulator class and the bronchopulmonary tissue designation, with the advantage of partial independent validation from a Western laboratory.

Bronchogen occupies the lowest validation tier within this comparator panel, distinguished by its demonstrated bronchopulmonary tissue specificity (organotypic culture), its multi-level molecular characterization (DNA binding, histone interaction, gene expression regulation in bronchial epithelium), and its demonstrated efficacy in a COPD rat model (epithelial restoration, anti-inflammatory activity, surfactant recovery). The compound is constrained by the absence of independent replication, the absence of human clinical data, and the reliance on a single research network for all published evidence.

The principal value of Bronchogen in the current research landscape is as a molecular tool for investigating peptide-mediated epigenetic regulation of bronchial gene expression, a mechanistic concept that is theoretically distinct from the mucolytic (NAC), cytoprotective (BPC-157), gene-modulatory (GHK-Cu), and immunomodulatory (Thymalin) approaches represented by the other comparators. The tissue-specific regenerative mechanism (ciliated cell restoration, goblet cell normalization, surfactant recovery) addresses a pathological feature of COPD (airway remodeling) that none of the other comparators directly targets. Investigators considering Bronchogen for respiratory research should calibrate expectations against the validation hierarchy established by this comparative assessment and should recognize that the clinical translation pathway requires substantially more evidence than is currently available.

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