

RESEARCH MONOGRAPH . COMPOUND PROFILE

Livagen

A Khavinson-class synthetic tetrapeptide (Lys-Glu-Asp-Ala) developed at the St. Petersburg Institute of Bioregulation and Gerontology as a liver-targeted bioregulator, distinguished by its capacity to induce chromatin decondensation in aged cells, inhibit enkephalin-degrading enzymes in human serum, and restore hepatocyte protein synthesis in senescent tissue.

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ABSTRACT

Livagen (Lys-Glu-Asp-Ala; single-letter code KEDA) is a synthetic tetrapeptide bioregulator developed by Vladimir Khavinson and colleagues at the St. Petersburg Institute of Bioregulation and Gerontology as a liver-targeted member of the broader Khavinson short-peptide bioregulator family. The compound is characterized by three principal pharmacological activities studied across cell culture, animal, and limited human research: (1) epigenetic chromatin remodeling through induction of deheterochromatinization of pericentromeric structural heterochromatin and facultative heterochromatin in lymphocytes and hepatocytes from aged organisms, with consequent reactivation of ribosomal genes and euchromatic loci silenced during aging; (2) potent inhibition of enkephalin-degrading serum peptidases (IC₅₀ approximately 20 micromolar), exceeding the inhibitory potency of established peptidase inhibitors including puromycin, leupeptin, and D-phenylalanyl-alanyl-arginine-p-nitroanilide, without direct interaction with mu or delta opioid receptors; and (3) restoration of protein synthesis rhythms and metabolic function in hepatocyte cultures from aged rats to levels characteristic of young animals at nanomolar concentrations.

The chromatin-remodeling mechanism has been demonstrated in cultured lymphocytes from human subjects aged 75 to 88 years, in which Livagen induced activation of ribosomal genes, decondensation of pericentromeric heterochromatin of chromosomes 1, 9, and 16, and release of genes repressed through age-related condensation of euchromatic regions. This deheterochromatinization effect is shared with other Khavinson

bioregulator peptides (Vilon, Epitalon, Cortagen) but appears tissue-preferential for hepatic and lymphoid lineages at the concentrations studied. Molecular modeling studies of the broader Khavinson peptide class suggest that short peptides interact with the nucleosome, histone proteins, and double-stranded DNA through steric and electrostatic complementarity, altering histone modification patterns and the accessibility of regulatory regions to transcription factors.

In experimental models of liver pathology (acute and chronic hepatitis, liver fibrosis), the KEDA tetrapeptide demonstrated hepatoprotective and immunoprotective effects including normalization of total bilirubin, cholesterol, alanine aminotransferase, and aspartate aminotransferase levels; stimulation of tissue repair; and decreased destructive dystrophic processes in liver stroma. The maximal hepatoprotective effect was observed in aged animals, consistent with the bioregulator hypothesis that these peptides primarily restore age-depleted signaling. In aged rat hepatocyte cultures, Livagen restored circadian rhythms of protein synthesis to patterns characteristic of young specimens at nanomolar concentrations. A separate line of investigation demonstrated that oral administration of Livagen for two weeks modulated digestive enzyme activity in rats in an age-dependent manner, reducing enzyme activity in young animals while increasing it in old animals toward levels observed in young controls.

The compound is resistant to hydrolysis by small intestinal peptidases and is not degraded to a measurable extent by the peptide hydrolases of the small intestine. Formal pharmacokinetic characterization meeting Western regulatory standards has not been published. The compound is not approved by any major

Western regulatory authority (FDA, EMA) and is not registered on ClinicalTrials.gov. The primary research literature originates from Russian and Georgian institutions, and independent Western replication of key findings remains limited. This monograph reviews the chemistry, epigenetic pharmacology, hepatoprotective and digestive enzyme evidence, the limited pharmacokinetic record, sourcing and quality considerations, and a comparative assessment of five alternative hepatoprotective or epigenetic bioregulator compounds against Livagen on five competency standards.

1. Compound Identification

1.1 Names, synonyms, and registry identifiers

Common name: Livagen.

Alternative transliterations: Lyvagen, Livagene.

Proposed International Nonproprietary Name: None assigned. The compound is identified in the research literature exclusively as Livagen or as the tetrapeptide KEDA.

Research names: Livagen. The name derives from the Latin root for liver combined with a suffix convention used across the Khavinson bioregulatory peptide series (compare Vilon, Epitalon, Cortagen, Prostamax, Ovagen).

Chemical name (peptide sequence): L-lysyl-L-glutamyl-L-aspartyl-L-alanine.

Single-letter amino acid code: KEDA.

Three-letter amino acid code: Lys-Glu-Asp-Ala.

Chemical Abstracts Service registry numbers: 195875-84-4 (primary); 402856-42-2 (deprecated).

PubChem Compound Identifier: 87919683.

International Union of Pure and Applied Chemistry name: (4S)-5-[[[(2S)-3-carboxy-1-[[[(1S)-1-carboxyethyl]amino]-1-oxopropan-2-yl]amino]-4-[[[(2S)-2,6-diaminohexanoyl]amino]-5-oxopentanoic acid.

Development context: The compound was developed as a synthetic analog of peptide fragments isolated from young animal liver tissue at the St. Petersburg Institute of Bioregulation and Gerontology under the direction of Professor Vladimir Khavinson. It is classified within the Khavinson bioregulator peptide family as the liver-targeted synthetic tetrapeptide, complementing the natural liver polypeptide extract Svetinorm (also designated Ventvil) [1, 2].

1.2 Structural class and chemistry

Livagen is a linear, unbranched tetrapeptide composed of four L-amino acid residues linked by three peptide bonds. The structural class places it within the Khavinson short-chain bioregulator peptides, a family of synthetic di-, tri-, and tetrapeptides developed as tissue-specific gene-expression modulators. Other members of this family include Epitalon (Ala-Glu-Asp-Gly; pineal gland), Vilon (Lys-Glu; thymus), Cortagen (Ala-Glu-Asp-Pro; central nervous system), and Cardiogen (Ala-Glu-Asp-Arg; cardiac tissue) [3, 4].

Molecular formula: C₁₈H₃₁N₅O₉.

Molecular weight: 461.47 g/mol (calculated for the free acid form).

Isoelectric point: Approximately 3.8 to 4.1, reflecting the net negative charge contributed by the glutamic acid and aspartic acid side chains at physiological pH, partially offset by the positively charged lysine epsilon-amino group.

Net charge at physiological pH (7.4): Approximately minus one. The lysine epsilon-amino group (pK_a approximately 10.5) is protonated and positively charged; the glutamic acid gamma-carboxyl (pK_a approximately 4.1) and the aspartic acid beta-carboxyl (pK_a approximately 3.9) are deprotonated and negatively charged; the C-terminal alanine carboxyl (pK_a approximately 2.3) is deprotonated and negatively charged; the N-terminal alpha-amino group (pK_a approximately 8.0) is partially protonated at physiological pH.

Physical properties: White to off-white lyophilized powder. Freely soluble in water and in aqueous buffered solutions at physiological pH. Soluble in dimethyl sulfoxide. Sparingly soluble in apolar organic solvents. The compound is expected to be hygroscopic owing to the multiple ionizable side chains and the hydrophilic character of the peptide backbone.

Stereochemistry: All four residues are the naturally occurring L-configuration. Investigators acquiring research-grade Livagen should confirm stereochemical purity, as racemization of the aspartyl residue is a known degradation pathway for aspartate-containing peptides under acidic or elevated-temperature storage conditions.

1.3 Synthesis

Livagen is synthesized by standard solid-phase peptide synthesis (SPPS) using fluorenylmethyloxycarbonyl (Fmoc) chemistry on a resin-bound C-terminal alanine [5]. The synthesis proceeds from C-terminus to N-terminus: the Fmoc-Ala-OH residue is coupled to a Wang resin or Rink amide resin (for the free acid or amide C-terminus respectively); sequential deprotection and coupling of Fmoc-Asp(OtBu)-OH, Fmoc-Glu(OtBu)-OH, and Fmoc-Lys(Boc)-OH completes the tetrapeptide chain. Coupling reagents are typically HBTU (O-benzotriazol-1-yl-N,N,N',N'-tetramethyluronium hexafluorophosphate) or HATU (1-[bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxide hexafluorophosphate) with N,N-diisopropylethylamine as base in N,N-dimethylformamide. Cleavage from resin and simultaneous side-chain deprotection is achieved with a trifluoroacetic acid/triisopropylsilane/water cocktail (typically 95:2.5:2.5 v/v/v). The crude peptide is purified by preparative reversed-phase high-performance liquid chromatography on a C18 column with acetonitrile/water/trifluoroacetic acid gradient. Identity is confirmed by electrospray ionization mass spectrometry (expected $[M+H]^+ = 462.22$) and by amino acid analysis. Purity of greater than 95 percent by analytical HPLC is standard for research-grade material; greater than 98 percent is achievable with additional chromatographic purification. The compound is lyophilized from dilute acetic acid or from water and stored as a dry powder. The synthesis is straightforward and high-yielding for a tetrapeptide; industrial-scale production presents no unusual challenges.

2. Abstract

Livagen (KEDA; Lys-Glu-Asp-Ala; CAS 195875-84-4) is a synthetic tetrapeptide bioregulator developed by Vladimir Khavinson at the St. Petersburg Institute of Bioregulation and Gerontology as the defined active component of the liver polypeptide complex Ventvil [1, 2]. The compound belongs to the Khavinson class of ultrashort peptide bioregulators, a family of synthetic sequences that are proposed to act as epigenetic regulators through direct interaction with chromatin components (histone proteins and DNA) to modulate gene expression in a tissue-specific manner [3, 4].

The principal characterized biological activity of Livagen is chromatin decondensation. In the seminal Khavinson and Lezhava (2002) study, treatment of cultured lymphocytes from elderly donors (aged 75 to 88 years) with Livagen induced activation of ribosomal genes in nucleolus organizer regions, decondensation of pericentromeric structural heterochromatin on chromosomes 1 and 9, and release of genes repressed by age-related facultative heterochromatin condensation [6]. This deheterochromatinization effect was confirmed in a subsequent five-peptide comparative study in the same donor age group [7] and extended in a 2006 study demonstrating that Livagen, Epitalon, and Vilon activate chromatin and reactivate ribosomal genes in lymphocytes from elderly individuals [8].

A second characterized activity is inhibition of enkephalin-degrading enzymes (enkephalinases) in human serum. Kost, Sokolov, Gabaeva, and colleagues (2003) demonstrated that Livagen inhibits the hydrolysis of tritium-labeled leucine-enkephalin by serum enkephalinases with an IC₅₀ of approximately 20 micromolar, a potency exceeding the reference inhibitors puromycin, leupeptin, and D-PAM [9]. The compound does not bind mu or delta opioid receptors in rat brain membrane fractions, indicating that the mechanism operates through preservation of endogenous opioid signaling rather than through direct receptor activation.

The third characterized activity is hepatoprotection. The comprehensive 2020 review by Trofimova and Khavinson evaluated the hepatoprotective properties of Livagen and the parent liver polypeptide complex Ventvil across animal models of fibrosis, acute hepatitis, and chronic hepatitis [2]. Both preparations normalized immune and antioxidant status, restored liver enzyme levels, reduced inflamma-

tory infiltration, and promoted hepatocyte regeneration. The hepatoprotective and immunoprotective effects were maximal in aged animals, consistent with the chromatin-decondensation mechanism as the molecular basis for the hepatoprotective activity.

No formal pharmacokinetic studies have been published. No human clinical trials of the synthetic KEDA tetrapeptide have been registered. The compound is not approved by any Western regulatory authority and is available exclusively as a research-grade preparation. This monograph documents the complete research record of Livagen across all characterized activities and provides a comparative assessment of five bioregulatory and hepatoprotective candidates against Livagen on five competency standards.

3. Discovery and Development History

3.1 Origin and conceptual framework

The Khavinson peptide bioregulator program originated in the 1970s at the Military Medical Academy in Leningrad (now St. Petersburg), where Vladimir Khavinson and colleagues began investigating polypeptide extracts from animal organs as potential geroprotective agents [3, 10]. The foundational concept of this program is that short peptide fragments (two to four amino acids), derived from or modeled on tissue-specific protein hydrolysates, function as endogenous gene-expression regulators that maintain tissue homeostasis and whose depletion during aging contributes to age-related functional decline. The program advanced from crude tissue extracts (termed "cytomedins") to identification of the minimal active peptide sequences within those extracts, yielding a family of synthetic ultrashort peptides of two to four amino acid residues [3, 4].

3.2 Identification of the KEDA sequence

Livagen was identified in approximately 1998 as the minimal active tetrapeptide sequence derived from liver tissue polypeptide extracts [1, 10]. The parent extract, designated Ventvil (later commercialized as the dietary supplement Svetinorm), is a polypeptide complex extracted from calf liver containing a heterogeneous mixture of peptides and small proteins under 10,000 daltons. Systematic fractionation and activity-guided isolation identified the KEDA sequence as a principal bioactive component with hepatoprotective and immunomodulatory properties. The synthetic tetrapeptide was designated "Livagen" (from the Latin root for liver combined with a suffix indicating gene activation) to distinguish it from the natural polypeptide complex [1, 2].

3.3 Key experimental milestones

The first major published demonstration of Livagen bioactivity in a Western-indexed journal was the Khavinson and Lezhava (2002) report in the *Bulletin of Experimental Biology and Medicine*, conducted in collaboration with investigators at Tbilisi State University and the Andronikashvili Institute of Physics [6]. This

study documented chromatin activation in lymphocytes from elderly human subjects (aged 75 to 88 years) following in vitro Livagen exposure and provided the first human-tissue evidence for the deheterochromatinization mechanism.

Subsequent publications extended the characterization to: enkephalin-degrading enzyme inhibition in human serum (Kost et al., 2003) [9]; hepatocyte protein synthesis restoration in aged rat cultures (Brodskii, Khavinson et al., 2004) [11]; digestive enzyme modulation in rats of different ages (Timofeeva et al., 2005) [12]; comparative chromatin effects of five Khavinson peptides (Khavinson, Lezhava, Malinin, 2004) [7]; chromatin reactivation with anti-aging peptides (Lezhava, Monaselidze et al., 2006) [8]; and comprehensive hepatoprotective characterization in experimental liver pathology models (Trofimova and Khavinson, 2020) [2].

3.4 Vladimir Khavinson and the bioregulator program

Vladimir Khavinson (1946 to 2024) was the originator and principal investigator of the peptide bioregulator program. He held the position of Director of the St. Petersburg Institute of Bioregulation and Gerontology, was a corresponding member of the Russian Academy of Sciences, and was the author or co-author of more than 700 publications on peptide bioregulation [3, 10]. Six peptide-based pharmaceutical preparations and 64 peptide dietary supplements were introduced into clinical practice in Russia under his direction. Khavinson passed away in January 2024. The research program continues through collaborators at the St. Petersburg Institute and at international partner institutions.

3.5 Regulatory and commercial status

Livagen has not been submitted for regulatory approval in any Western jurisdiction. The compound is not approved by the United States Food and Drug Administration, the European Medicines Agency, the United Kingdom Medicines and Healthcare products Regulatory Agency, or the Therapeutic Goods Administration of Australia. In Russia, certain Khavinson peptide bioregulators (Thymalin, Epithalamin, Cortexin, Retinalamin, Prostatilen) have been registered as pharmaceutical preparations; Livagen has not achieved pharmaceutical registration and is classified as a dietary supplement or biologically active additive in the Russian regulatory framework [3]. Outside Russia, the compound is sold by re-

search peptide suppliers as a research-use-only material and by some supplement vendors as an unregulated product. Commercial preparations are typically supplied as lyophilized powder in 10 mg or 20 mg vials or as oral capsules.

4. Mechanism of Action

Livagen is proposed to operate through multiple molecular mechanisms. The three principal characterized activities are chromatin decondensation (the primary and most extensively studied mechanism), enkephalinase inhibition, and restoration of hepatocyte biosynthetic function. A fourth proposed mechanism, direct interaction of the peptide with DNA promoter sequences, is supported by molecular modeling and by analogy with other Khavinson peptides but has not been directly demonstrated for the KEDA sequence.

4.1 Chromatin decondensation (deheterochromatinization)

The defining molecular activity of Livagen is the induction of chromatin decondensation in aged cells [6, 7, 8]. Progressive chromosome heterochromatinization (condensation of both constitutive and facultative chromatin regions) is a recognized feature of cellular aging. This condensation leads to inactivation of genes that were functionally active in younger cells, contributing to the decline in protein synthesis, metabolic activity, and regenerative capacity observed in aged tissues.

In cultured lymphocytes from human subjects aged 75 to 88 years, Livagen induced three distinct chromatin modifications: (a) activation of ribosomal genes (nucleolar organizer regions), as assessed by silver staining of active ribosomal DNA; (b) decondensation of pericentromeric structural heterochromatin of chromosomes 1, 9, and 16, assessed by C-banding techniques; and (c) release of genes repressed through age-related condensation of euchromatic regions (deheterochromatinization of facultative heterochromatin) [6, 7]. These effects were consistent across multiple donor lymphocyte preparations and were reproducible with other Khavinson peptides (Vilon, Epitalon) acting on different chromosomal targets [7, 8].

A comparative study of five Khavinson peptides (Vilon, Epitalon, Livagen, Prostamax, Cortagen) in lymphocytes from elderly subjects demonstrated that all five peptides activated ribosomal genes through decondensation of nucleolus organizer regions [7]. However, only Livagen and Epitalon additionally induced deheterochromatinization of pericentromeric structural heterochromatin on chromosomes 1 and 9, an effect not produced by Vilon, Prostamax, or Cortagen. This differential activity pattern indicates structural specificity within the bioregulator class

and suggests that the KEDA and AEDG sequences share a chromatin-remodeling capacity at constitutive heterochromatin that shorter or structurally distinct peptides do not possess.

4.2 Proposed DNA and histone interaction mechanism

The broader Khavinson research program has proposed that ultrashort peptides (two to four amino acid residues) penetrate cell nuclei and interact directly with DNA and histone proteins to modulate gene expression [3, 4, 13]. Fluorescence microscopy studies with labeled peptides have demonstrated nuclear localization of Khavinson peptides in human cell cultures [13]. Systematic analysis of dipeptide binding to all possible double-stranded DNA tetranucleotide sequences identified 57 low-energy complexes with high selectivity for specific DNA sequences [4]. For the related tetrapeptide Epitalon (AEDG), molecular models demonstrated preferential interaction with linker histone H1/6 (binding energy approximately minus 64.51 kcal/mol) and H1/3 (approximately minus 56.49 kcal/mol) [4].

For the KE dipeptide (Lys-Glu, the first two residues of the Livagen sequence and the sequence of the related bioregulator Vilon), selective binding to the TCGA double-stranded DNA sequence in the promoter region of the APG5L gene was demonstrated, with the intact dipeptide showing greater binding affinity than the individual amino acid components [4, 13]. Short peptides were also shown to interact with histone proteins H1, H2B, H3, and H4 in the region of peptide-binding motifs at the N-terminal sections of the histones [4].

While specific molecular modeling data for the full KEDA tetrapeptide have not been published in equivalent detail, the shared amino acid composition (both KEDA and AEDG contain glutamic acid and aspartic acid residues that contribute anionic character) supports analogous DNA and histone interaction mechanisms. The acidic residues are proposed to destabilize histone-DNA interactions through electrostatic repulsion with the negatively charged phosphodiester backbone, while the basic lysine residue stabilizes alternative chromatin configurations through electrostatic attraction [4]. The net effect at the chromatin level is proposed to be a selective loosening of histone-DNA interactions at specific loci, favoring the transition from heterochromatin to euchromatin.

4.3 Enkephalinase inhibition

Kost, Sokolov, Gabaeva, and colleagues (2003) characterized Livagen as an inhibitor of enkephalin-degrading enzymes (enkephalinases) in human blood serum [9]. Enkephalinases are a heterogeneous group of peptidases (including aminopeptidase N, dipeptidyl peptidase III, angiotensin-converting enzyme, and neutral endopeptidase/neprilysin) that hydrolyze endogenous opioid pentapeptides (leucine-enkephalin and methionine-enkephalin) in the circulation and in tissues.

In the *in vitro* assay, Livagen inhibited the hydrolysis of tritium-labeled leucine-enkephalin ([³H]-Leu-enkephalin) by human serum enkephalinases with an IC₅₀ of approximately 20 micromolar [9]. This potency exceeded that of the reference peptidase inhibitors puromycin, leupeptin, and D-PAM (D-phenylalanyl-alanyl-arginine-p-nitroanilide) in the same assay system. The companion peptide Eptalon (AEDG) also inhibited serum enkephalinases but with substantially lower potency (IC₅₀ approximately 500 micromolar).

Critically, neither Livagen nor Eptalon bound to mu or delta opioid receptors in rat brain membrane preparations in radioreceptor assays at concentrations up to 100 micromolar [9]. This indicates that the mechanism operates through preservation of endogenous enkephalin signaling (by preventing enkephalin degradation) rather than through direct opioid receptor agonism. The functional consequence is potentiation of endogenous analgesic and immunomodulatory opioid peptide signaling without the dependence, tolerance, or respiratory depression risks associated with direct opioid receptor agonists.

The relationship between the enkephalinase inhibition mechanism and the chromatin decondensation mechanism has not been established. They may represent independent activities of the KEDA sequence operating through distinct molecular targets at different cellular compartments (nuclear for chromatin effects, extracellular/membrane-associated for enkephalinase inhibition).

4.4 Hepatocyte protein synthesis restoration

Brodskii, Khavinson, and colleagues (2004) demonstrated that Livagen restores protein synthesis rates and circadian (approximately one-hour period) biosynthetic rhythms in aged hepatocytes [11]. In monolayer cultures of rat hepatocytes from animals of varying ages (1 to 24 months), Livagen at nanomolar concentrations increased the overall level of protein synthesis and enhanced the amplitude

of the circumborhalian protein synthesis rhythm. The greatest effect was observed in hepatocytes from old animals, where protein synthesis rates approached those characteristic of young specimens. Epitalon (Ala-Glu-Asp-Gly), tested in the same experimental system, produced no measurable change in hepatocyte protein synthesis, supporting the tissue-specificity hypothesis of the bioregulator class [11].

The molecular mechanism linking the KEDA sequence to increased hepatocyte protein synthesis has not been definitively established. The chromatin decondensation mechanism, particularly the activation of ribosomal genes through decondensation of nucleolus organizer regions, provides a plausible upstream explanation: increased ribosomal gene transcription would increase ribosome biogenesis and therefore increase the protein synthesis capacity of the cell.

4.5 Digestive enzyme modulation

Oral administration of Livagen for two weeks modulated digestive enzyme activity (invertase, maltase, aminopeptidases) in the gastrointestinal tract and in non-digestive organs (liver, kidney) of rats in an age-dependent manner [12]. In young animals, enzyme activity decreased; in old animals, enzyme activity increased toward levels characteristic of young controls. This bidirectional, normalizing effect is consistent with a gene-regulatory mechanism rather than direct enzymatic activation or inhibition, as a direct activator or inhibitor would be expected to produce unidirectional effects regardless of the baseline enzyme level [12].

4.6 Resistance to peptidase hydrolysis

Livagen is resistant to hydrolysis by the peptide hydrolases of the small intestine. The Timofeeva et al. (2005) study reported that small intestinal peptidases did not hydrolyze Livagen "even to a small extent," though the peptide reduced glycyl-L-leucine dipeptidase activity in the small intestine by approximately 50 percent under in vitro conditions [12]. This resistance to intestinal proteolysis is unusual for a small linear peptide and may reflect the specific amino acid composition (the glutamic acid and aspartic acid side chains may sterically or electrostatically hinder enzymatic access to the peptide backbone) or the formation of intramolecular salt bridges that stabilize the peptide against hydrolysis.

5. Pharmacokinetics and Routes of Administration

5.1 Absorption

Formal pharmacokinetic characterization of Livagen in humans or animals meeting international regulatory standards (FDA, EMA, ICH guidelines) has not been published. The available pharmacokinetic information is derived from indirect evidence in animal studies and from observed biological activity following different routes of administration.

Oral bioavailability has not been quantified. However, the Timofeeva et al. (2005) demonstration that oral administration of Livagen for two weeks produced measurable biological effects (age-dependent modulation of digestive enzyme activity) in rats supports functionally relevant oral absorption [12]. The reported resistance of the peptide to small intestinal peptidase hydrolysis [12] is consistent with at least partial oral bioavailability, an unusual property for a small linear peptide. Subcutaneous administration has been employed in research settings and is reported to provide gradual absorption into systemic circulation.

5.2 Distribution

Volume of distribution has not been formally characterized. As a small, hydrophilic tetrapeptide (molecular weight 461.47 g/mol), Livagen is expected to distribute widely in extracellular fluid and to enter cells through passive diffusion or active transport mechanisms. The demonstrated nuclear localization of related Khavinson peptide effects (chromatin remodeling in lymphocyte and hepatocyte nuclei) implies that the compound or its active fragments traverse the cell membrane and the nuclear envelope to reach chromosomal targets [6, 7, 13]. Plasma protein binding has not been characterized.

5.3 Metabolism

The primary metabolic pathway for small peptides is enzymatic hydrolysis by tissue and circulating peptidases. The demonstrated resistance of Livagen to small intestinal peptidases [12] and the potent inhibition of serum enkephalin-degrading peptidases [9] suggest that the compound may exhibit slower metabolic clearance than would be predicted for a typical linear tetrapeptide. However, formal metabolic stability studies in liver microsomes, hepatocyte cultures, or in vivo pharmacokinetic models have not been published. As an unmodified L-amino acid tetrapeptide, Livagen is expected to be susceptible to degradation by plasma and tissue aminopeptidases, carboxypeptidases, and endopeptidases. The plasma half-life of unmodified short peptides in general is typically on the order of minutes to low tens of minutes, though specific data for KEDA are unavailable [14].

5.4 Elimination

Elimination kinetics (half-life, clearance, routes of excretion) have not been formally characterized. By analogy with other short peptides of similar molecular weight and amino acid composition, renal filtration and peptidase-mediated hydrolysis are the expected elimination pathways. The compound is expected to be metabolized entirely to constituent amino acids (lysine, glutamic acid, aspartic acid, alanine), which enter normal amino acid metabolism.

5.5 Routes of administration

Three principal routes have been employed in research settings: subcutaneous injection (the most commonly cited research route), oral administration (demonstrated to produce biological effects in rat studies [12]), and in vitro application to cell cultures. Intravenous administration has not been specifically reported for Livagen in the published literature. The compound is typically reconstituted from lyophilized powder in bacteriostatic water or normal saline for subcutaneous administration and is supplied as a dry powder for oral dissolution.

5.6 Special populations

No specific pharmacokinetic data are available for pediatric, geriatric, hepatically impaired, or renally impaired populations. The demonstrated age-dependent biological effects (greater efficacy in aged versus young animals and tissues [2, 11, 12])

suggest that the pharmacokinetic or pharmacodynamic profile may differ between age groups, but the relative contributions of pharmacokinetic (altered peptide metabolism in aging) and pharmacodynamic (greater substrate for chromatin reactivation in aged chromatin) factors have not been distinguished.

6. Preclinical Pharmacology

6.1 Hepatoprotective activity in experimental liver pathology

The Trofimova and Khavinson (2020) comprehensive review evaluated the hepatoprotective properties of both the liver polypeptide complex (Ventvil/Svetinorm) and the synthetic KEDA tetrapeptide (Livagen) across multiple experimental models [2].

In acute experimental toxic hepatitis (induced by carbon tetrachloride or other hepatotoxins), the KEDA peptide demonstrated protective and therapeutic effects including normalization of serum total bilirubin and cholesterol levels, reduction of elevated alanine aminotransferase (ALT) and aspartate aminotransferase (AST) to near-normal levels, stimulation of tissue repair processes, and decreased destructive dystrophic changes in liver stroma [2].

In chronic hepatitis models, both the polypeptide complex and the KEDA tetrapeptide normalized immune status parameters (lymphocyte subpopulations, immunoglobulin levels) and antioxidant status (superoxide dismutase activity, lipid peroxidation markers) while restoring liver function markers [2]. The effects of the synthetic tetrapeptide and the natural polypeptide complex were concordant, supporting the conclusion that the KEDA sequence reproduces the essential hepatoprotective activity of the full liver polypeptide extract.

In liver fibrosis models, Livagen reduced collagen deposition and fibrotic tissue formation, though the magnitude and reproducibility of this effect across different fibrosis induction protocols have not been extensively characterized in published studies [2].

The maximal hepatoprotective and immunoprotective effects were consistently observed in aged animals, supporting the bioregulator hypothesis that these peptides primarily restore age-depleted signaling rather than introducing pharmacological effects de novo [2].

6.2 Hepatocyte protein synthesis

Brodskii, Khavinson, and colleagues (2004) characterized the effect of Livagen on circadian rhythms of protein synthesis in cultured hepatocytes isolated from rats of different ages [11]. Hepatocytes from aged rats exhibited diminished amplitude and altered periodicity of protein synthesis oscillations compared to hepatocytes from young animals. Addition of Livagen at nanomolar concentrations to aged hepatocyte cultures restored the amplitude and periodicity of protein synthesis rhythms toward patterns observed in young hepatocyte cultures. The effect was most pronounced in cells from the oldest animals, consistent with the age-dependent efficacy pattern observed across the Livagen evidence base [11].

6.3 Digestive enzyme modulation

Timofeeva, Khavinson, Malinin, Nikitina, and Egorova (2005) characterized the effect of oral Livagen administration (two weeks duration) on digestive enzyme activity in the gastrointestinal tract and in non-digestive organs (liver, kidney) of rats at different ages [12]. The key finding was a bidirectional, age-dependent modulation: enzyme activity (including invertase, maltase, and aminopeptidases) decreased in young animals and increased in old animals. In old rats, post-Livagen enzyme activity levels approached those of young control animals in the majority of measured parameters. This normalizing effect is consistent with a regulatory mechanism that restores age-altered gene expression rather than a direct enzymatic effect [12].

Notably, Livagen itself was not hydrolyzed by small intestinal peptide hydrolases, even to a small extent, indicating substantial resistance to luminal proteolysis [12]. This finding is unusual for an unmodified tetrapeptide and suggests structural features of the KEDA sequence that confer proteolytic stability in the intestinal environment.

6.4 Chromatin activation in human lymphocytes ex vivo

The chromatin decondensation studies, while using human cells (lymphocytes from elderly donors), were conducted ex vivo and are therefore classified here as translational preclinical evidence rather than clinical efficacy trials [6, 7, 8].

The Khavinson and Lezhava (2002) study cultured peripheral blood lymphocytes from donors aged 75 to 88 years with and without Livagen [6]. Cytogenetic analysis demonstrated statistically significant activation of ribosomal genes, decondensation of pericentromeric heterochromatin on chromosomes 1 and 9, and release of age-repressed euchromatic genes.

The Khavinson, Lezhava, and Malinin (2004) study extended these findings across five Khavinson peptides in lymphocytes from elderly subjects, demonstrating that all five peptides activated ribosomal genes but that Livagen and Epitalon uniquely induced deheterochromatinization of pericentromeric structural heterochromatin [7].

The Lezhava, Monaselidze, and colleagues (2006) study confirmed that Livagen, Epitalon, and Vilon activate chromatin in lymphocytes from elderly individuals through deheterochromatinization and demonstrated that the anti-mutagenic and protective effects of these peptides extended to heavy-metal-induced genotoxicity contexts, where Livagen reduced the frequency of chromosomal aberrations in lymphocytes exposed to nickel, cobalt, and zinc ions [8].

6.5 Enkephalinase inhibition in human serum in vitro

The Kost et al. (2003) study measured the rate of [3H]-Leu-enkephalin hydrolysis by human serum peptidases in the presence and absence of Livagen at concentrations from 1 to 100 micromolar [9]. Livagen inhibited enkephalinase activity in a concentration-dependent manner with an IC₅₀ of approximately 20 micromolar. This potency placed Livagen above puromycin, leupeptin, and D-PAM on the inhibitory potency scale for serum enkephalinases. Livagen itself did not bind to mu or delta opioid receptors at concentrations up to 100 micromolar, confirming that the opioid-related effects are indirect (through preservation of endogenous ligands rather than direct receptor activation) [9].

6.6 Nucleolar organizer region activity in cardiomyopathy

Livagen, alone and in combination with cobalt ions, was tested on lymphocytes from patients with hypertrophic cardiomyopathy and their relatives [15]. The combined treatment increased the frequency of large nucleolar organizer regions and boosted the associative activity of acrocentric chromosomes in both patients and relatives. The authors proposed that Livagen induces decondensation of hete-

rochromatinized chromatin, potentially releasing inactivated genes relevant to the cardiomyopathy phenotype. This study extends the chromatin remodeling activity of Livagen beyond the hepatocyte and general lymphocyte context to a specific disease population.

7. Clinical Evidence Base

7.1 Absence of registration-quality clinical trials

No randomized, controlled, registration-quality clinical trials of Livagen have been conducted in any jurisdiction. No clinical trials of Livagen are registered in ClinicalTrials.gov, the EU Clinical Trials Register, the ISRCTN Registry, or equivalent international registries. The human evidence for Livagen is limited to ex vivo lymphocyte studies from elderly donors and to observational reports within the Russian clinical literature.

7.2 Ex vivo human lymphocyte studies

The principal human evidence comprises the chromatin decondensation studies in lymphocytes from elderly donors [6, 7, 8]. These studies demonstrated that Livagen produces measurable epigenetic changes in human cells, confirming that the mechanism observed in animal models is relevant to human biology. However, the studies were conducted on isolated cells in culture and do not constitute evidence of clinical efficacy or safety in intact human subjects.

7.3 Clinical use of the parent preparation

The parent liver polypeptide complex Ventvil (Svetinorm) has been used in Russian gerontological clinical practice for age-related liver dysfunction, metabolic syndrome, and as a hepatoprotective agent during polypharmacy in elderly patients [1, 2]. Russian-language clinical reports describe tolerability and improvements in liver function tests in patients with chronic hepatitis and cirrhosis, but these reports have not been published in English-language peer-reviewed journals with standard Western clinical trial methodology (randomization, blinding, placebo control, intention-to-treat analysis, independent monitoring).

7.4 Tolerability reports

Reports from clinical use in Russian and Eastern European clinical settings describe tolerability of Khavinson peptide bioregulators without serious adverse events [2]. Mild injection-site reactions (redness, soreness, minor swelling) are the most frequently reported side effects, typically resolving within 24 to 48 hours. However, these reports lack the systematic adverse-event collection and reporting methodology required for regulatory assessment.

7.5 Evidence assessment

The absence of controlled human clinical trials represents the most significant limitation of the Livagen evidence base. All published research originates from the Khavinson laboratory and from its immediate collaborators (principally Lezhava and colleagues in Tbilisi); no independent Western laboratory has published a replication of any Livagen finding. Investigators should weight the evidence accordingly and should not extrapolate preclinical and ex vivo findings to clinical efficacy or safety conclusions.

8. Sourcing and Quality Verification

Research-grade Livagen (synthetic KEDA tetrapeptide) is supplied by multiple peptide research suppliers at purities of 95 to 99 percent by HPLC. Suppliers include Peptide Sciences, Biotech Peptides, Limitless Life Nootropics, BioLongevity Labs, Core Peptides, and others. The compound is supplied as a lyophilized powder, typically in vial quantities of 10 to 20 milligrams. Pricing is moderate for a synthetic tetrapeptide, typically in the range of \$30 to \$100 per 20 milligrams of research-grade material at the time of monograph preparation.

The parent liver polypeptide complex Svetinorm is available as an oral dietary supplement (capsules containing approximately 200 micrograms of liver-derived peptide fraction per capsule) through Russian and international online distributors.

No pharmaceutical-grade reference standard exists for Livagen. No pharmacopoeial monograph has been published by the United States Pharmacopeia, the European Pharmacopoeia, or the Japanese Pharmacopoeia.

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

- Reversed-phase high-performance liquid chromatography area-percent purity, ideally greater than 98 percent
- Electrospray ionization mass spectrometry confirmation of molecular weight (expected $[M+H]^+$ at m/z 462.2)
- Amino acid analysis confirming the expected Lys:Glu:Asp:Ala ratio of 1:1:1:1
- Residual trifluoroacetic acid or acetate counterion quantification (peptides purified by reverse-phase HPLC with trifluoroacetic acid-containing mobile phases typically retain trifluoroacetate as the counterion, which may produce artifacts in biological assays at high peptide concentrations)
- Karl Fischer or loss-on-drying analysis for residual water content
- Endotoxin testing (Limulus amoebocyte lysate assay) for lots intended for parenteral research applications
- Residual solvent analysis by gas chromatography for common synthesis solvents (dimethylformamide, dichloromethane, acetonitrile)

Third-party analytical laboratories (Janoshik Analytical and others) provide independent confirmation of vendor certificates of analysis. Lot-to-lot variability in purity, counterion content, and residual solvent profile should be expected, and on-receipt analytical confirmation is appropriate for any investigation where dose-response precision is important.

9. Reconstitution and Handling

Livagen is supplied as a lyophilized powder and is reconstituted in aqueous solvent prior to use. The compound is freely soluble in water at room temperature owing to its multiple ionizable side chains.

Reconstitution for parenteral research use: Reconstitute the lyophilized powder with bacteriostatic water for injection (containing 0.9 percent benzyl alcohol) to the desired concentration. A typical reconstitution volume for a 20 mg vial is 1 to 2 milliliters, producing a stock concentration of 10 to 20 mg/mL. The solution should be clear and colorless; discard if turbid or discolored.

Storage of lyophilized material: Store at minus 20 degrees Celsius for long-term storage or at 2 to 8 degrees Celsius for storage periods of up to several months. Protect from moisture. The lyophilized peptide is stable at ambient temperature for limited periods during shipping but should be transferred to refrigerated or frozen storage on receipt.

Storage of reconstituted solution: Store at 2 to 8 degrees Celsius. Use within 14 to 30 days of reconstitution when bacteriostatic water is used as the reconstitution vehicle; use within 24 hours when sterile water for injection (without preservative) is used. Avoid repeated freeze-thaw cycles; aliquot stock solutions for single-use applications where practical.

For in vitro research: Stock solutions may be prepared in phosphate-buffered saline, cell culture medium, or dimethyl sulfoxide. Given the aqueous solubility of the peptide, DMSO is generally unnecessary but may be used for preparation of concentrated stocks. Working concentrations in the published literature range from nanomolar (hepatocyte protein synthesis studies) to micromolar (enkephalinase inhibition studies, IC₅₀ approximately 20 micromolar).

Light sensitivity: The peptide does not contain aromatic amino acid residues with significant ultraviolet absorption (no tryptophan or tyrosine); photodegradation is therefore not expected to be a significant concern. Routine light protection during storage is reasonable practice but is not critical.

Sterility: Research-grade lyophilized peptides are not typically supplied as sterile products. For parenteral research applications, reconstituted solutions should be passed through a 0.22 micrometer sterile syringe filter prior to use.

Compatibility: The compound is compatible with normal saline (0.9 percent sodium chloride), phosphate-buffered saline, and standard cell culture media. The acidic isoelectric point (approximately 3.8 to 4.1) means that the peptide carries a net negative charge at physiological pH and is unlikely to adsorb significantly to glass or polypropylene surfaces.

Stability considerations: Aspartate-containing peptides are susceptible to aspartyl residue racemization and to aspartimide formation (cyclization of the aspartyl side chain to the backbone nitrogen) under acidic conditions and at elevated temperatures. Storage of reconstituted solutions at neutral pH and at refrigerated temperatures minimizes these degradation pathways.

10. Stack Interactions and Combinations

No formal drug interaction studies have been conducted with Livagen. The following considerations are based on the characterized mechanisms of action and on general pharmacological principles for peptide compounds.

Other Khavinson peptide bioregulators: Livagen is frequently used in multi-peptide bioregulator protocols in which tissue-specific peptides are administered concurrently or sequentially (for example, Livagen for hepatoprotection combined with Epitalon for pineal/telomerase activation, Vilon for thymic immunomodulation, and Cortagen for neuroprotection) [3]. No pharmacokinetic or pharmacodynamic interaction data exist for these combinations. The chromatin-decondensation mechanism is shared across the class [7], and concurrent administration of multiple chromatin-decondensing peptides could theoretically produce additive or excessive deheterochromatinization; the consequences of this for genomic stability have not been evaluated.

Svetinorm (liver polypeptide complex): The natural liver polypeptide extract from which Livagen was derived contains multiple peptide fragments including the KEDA sequence. Concurrent administration of synthetic Livagen and natural Svetinorm would be expected to produce additive hepatoprotective effects. The combination has been described in clinical practice protocols but has not been evaluated in controlled comparative studies [2].

Enkephalinase inhibitors and opioid system modulators: Livagen inhibits enkephalin-degrading enzymes at micromolar concentrations [9]. Concurrent use with other enkephalinase inhibitors (sacubitril, thiorphan, phosphoramidon) could produce additive inhibition of enkephalin degradation and elevated endogenous opioid tone. Concurrent use with exogenous opioid agonists (morphine, fentanyl, oxycodone) could theoretically augment opioid effects through the combination of direct receptor activation and reduced endogenous opioid degradation.

Hepatoprotective agents: Concurrent use with conventional hepatoprotective agents (silymarin, ursodeoxycholic acid, S-adenosylmethionine, N-acetylcysteine, glycyrrhizin) has not been studied. The mechanisms are likely complementary

(Livagen acts through chromatin remodeling and protein synthesis restoration; conventional hepatoprotectives act through antioxidant, anti-inflammatory, and cytoprotective mechanisms). No pharmacokinetic interaction is expected given the absence of shared metabolic pathways.

Immunosuppressive agents: Livagen has immunomodulatory properties and induces chromatin changes in lymphocytes [6, 7]. Concurrent use with immunosuppressive agents (cyclosporine, tacrolimus, mycophenolate, corticosteroids) could theoretically produce opposing effects on immune cell gene expression.

Epigenetic modifiers: Concurrent administration with pharmacological epigenetic modifiers (histone deacetylase inhibitors such as vorinostat or romidepsin, DNA methyltransferase inhibitors such as azacitidine or decitabine) could theoretically produce additive chromatin-remodeling effects. This combination has not been studied and would require careful evaluation of genomic stability implications.

Protease inhibitors: Given that Livagen inhibits peptidases (enkephalinases) and is itself a substrate for peptidases, concurrent use with broad-spectrum protease inhibitors could alter both the pharmacodynamic effect of Livagen (reduced need for enkephalinase inhibition) and the pharmacokinetic profile of Livagen (reduced degradation, potentially increased exposure).

11. Adverse Events and Safety Signal

11.1 Published safety data

No systematic safety assessment of Livagen has been published. The available safety information is limited to incidental reports within efficacy studies and to general tolerability statements in review articles.

In the ex vivo lymphocyte studies, no cytotoxic effects were reported at the concentrations used for chromatin activation experiments [6, 7, 8]. In animal hepatitis and fibrosis models, no treatment-related adverse effects were reported at the doses used for hepatoprotective studies [2]. In the digestive enzyme study, no adverse effects were reported during the two-week oral administration period in rats of varying ages [12]. The absence of direct opioid receptor binding [9] eliminates the theoretical risks of respiratory depression, physical dependence, and tolerance that are associated with direct opioid receptor agonists.

11.2 Reported adverse events from uncontrolled use

Reports from uncontrolled commercial and community use (not from formal clinical studies) describe the following adverse events:

- Mild injection-site reactions (redness, soreness, minor swelling) following subcutaneous administration, typically resolving within 24 to 48 hours
- Occasional headache during initial days of administration, potentially related to enkephalinase inhibition altering endogenous opioid peptide dynamics [9]
- Mild gastrointestinal disturbance in fewer than 5 percent of recipients, consistent with the digestive enzyme-modulating activity of the peptide [12]
- Isolated reports of anxiety, cardiac palpitations, skin irritation, and pruritus in individual users; causality cannot be established from uncontrolled reports

No hepatotoxicity, nephrotoxicity, or immunosensitization has been reported in available surveillance data [2].

11.3 Critical safety data gaps

The following safety assessments have not been conducted for Livagen:

- Dose-escalation and maximum tolerated dose studies in any species
- Formal single-dose and repeat-dose toxicology studies under Good Laboratory Practice conditions
- Reproductive and developmental toxicity assessment
- Genotoxicity assessment (Ames test, chromosomal aberration, micronucleus)
- Carcinogenicity assessment
- Drug interaction studies (in vitro CYP inhibition, P-glycoprotein interaction, plasma protein binding displacement)
- Formal pharmacokinetic studies (ADME characterization)
- QT interval assessment (hERG channel, telemetry)
- Immunogenicity assessment

The absence of these standard safety evaluations represents a fundamental limitation of the current evidence base.

11.4 Theoretical safety concerns

Genomic stability: The most significant theoretical safety concern for Livagen is the consequence of sustained chromatin decondensation on genomic stability [16]. Heterochromatin serves structural and functional roles in maintaining chromosome integrity, including centromere function, telomere protection, and suppression of transposable element mobilization. Sustained or excessive deheterochromatinization could theoretically compromise these protective functions, leading to chromosomal instability, transposable element activation, or inappropriate gene expression. The available studies have demonstrated anti-mutagenic (rather than mutagenic) effects of Livagen [8], and the Khavinson bioregulator research program has published long-term animal survival studies showing no increase in tumor incidence with chronic peptide bioregulator administration [3, 10]. However, long-term studies of genomic stability under chronic Livagen exposure have not been conducted.

Immunomodulation risks: The immunomodulatory profile raises theoretical concerns about exacerbation of autoimmune conditions or inappropriate immune activation in individuals with pre-existing autoimmune disease. No data exist on

Livagen use in autoimmune contexts.

Proteolytic degradation products: As a tetrapeptide composed of naturally occurring L-amino acids, Livagen is degraded to constituent amino acids by normal peptidase activity. These amino acids are endogenous metabolites and are not expected to produce toxicity. No unique or reactive metabolites are predicted.

11.5 Pregnancy and lactation

No reproductive or developmental toxicity data are available. Use in pregnancy and lactation cannot be recommended on the basis of current evidence.

12. Comparable Compounds Assessment

This section presents a structured comparative assessment of five alternative hepatoprotective or peptide-based bioregulatory compounds against Livagen, evaluated on five standards: novelty, effect size, promising potential (current trajectory), side-effect profile, and overall validation. The comparators were selected to represent the range of hepatoprotective approaches spanning conventional pharmacology, peptide bioregulation, and natural product chemistry.

12.1 Epitalon (Ala-Glu-Asp-Gly, AEDG)

Mechanism: Synthetic tetrapeptide bioregulator of pineal origin. Proposed mechanisms include telomerase activation (hTERT induction in human somatic cells), melatonin secretion restoration, and chromatin decondensation [3, 17, 18]. Epitalon is the most extensively studied Khavinson peptide and the compound that attracted the greatest international attention to the bioregulator class.

Novelty: Low within the Khavinson peptide class (same structural concept as Livagen). Moderate in the broader pharmacological landscape owing to the proposed telomerase activation mechanism, which is distinct from conventional hepatoprotective approaches.

Effect size: In vitro telomerase activation in human fetal fibroblasts produced measurable increases in telomere length [18]. Chromatin decondensation in elderly lymphocytes is comparable in magnitude to Livagen [7]. Animal longevity studies report increases in mean and maximum lifespan in mice and rats [17]. However, Epitalon produced no measurable change in hepatocyte protein synthesis in the same experimental system where Livagen showed significant activity [11], confirming organ-specificity that limits its relevance as a hepatoprotective comparator.

Promising potential: Moderate. The telomerase-activation and longevity data have generated substantial research interest. No Western-designed clinical trials have been completed.

Side-effect profile: No adverse effects reported in published studies. The telomerase-activation mechanism raises theoretical oncogenesis concerns, though no carcinogenicity has been reported.

Overall validation: Moderate. The most validated compound in the Khavinson class, with the largest body of published research. Independent Western replication remains limited.

Comparison to Livagen: Epitalon shares the chromatin-decondensation mechanism with Livagen and has additional telomerase-activation activity that Livagen does not possess. Livagen has hepatocyte-specific activity and enkephalinase-inhibition activity that Epitalon does not possess. The two compounds are mechanistically complementary; Epitalon is not a hepatoprotective alternative.

12.2 Vilon (Lys-Glu, KE)

Mechanism: Synthetic dipeptide thymic bioregulator. Principal characterized activities include thymic function restoration, immunomodulation, and chromatin decondensation in aged lymphocytes [7, 19].

Development trajectory: Vilon was identified as the minimal active peptide of the thymic polypeptide complex Thymalin. It has been studied in immunosenescence models, chronic infection models, and lymphocyte culture systems.

Novelty: Low. The dipeptide structure is the simplest possible bioregulator, and the immunomodulatory mechanism through thymic function restoration is a well-established concept within the Khavinson framework.

Effect size: Vilon induced chromatin decondensation in lymphocytes from elderly subjects with magnitudes comparable to Livagen in the Khavinson et al. (2004) comparative study, though Vilon did not induce deheterochromatinization of pericentromeric structural heterochromatin (an effect unique to Livagen and Epitalon among the five tested peptides) [7]. Immunomodulatory effects in aged animal models include normalization of T-lymphocyte subpopulations and restoration of thymic cellularity.

Promising potential: Low to moderate. The compound has a smaller research literature than Epitalon or Livagen and has not been advanced through formal clinical development.

Side-effect profile: Minimal reported adverse events. The dipeptide structure (Lys-Glu) is composed of common dietary amino acids and is expected to have a favorable safety profile, though this has not been formally demonstrated.

Overall validation: Low to moderate. Limited by the small evidence base and absence of independent replication.

Comparison to Livagen: Vilon shares the Lys-Glu sequence (the first two residues of Livagen's KEDA sequence), raising the question of whether Livagen's hepatoprotective activity requires the full tetrapeptide or whether the Lys-Glu fragment contributes substantially. The comparative chromatin study demonstrated that Vilon activates ribosomal genes but does not induce pericentromeric heterochromatin decondensation [7], suggesting that the additional Asp-Ala residues confer both tissue specificity and enhanced chromatin-remodeling scope.

12.3 BPC-157 (Body Protection Compound-157)

Mechanism: Synthetic pentadecapeptide (15 amino acids) derived from human gastric juice proteins. Principal characterized activities include gastrointestinal mucosal protection, wound healing, angiogenesis modulation, and hepatoprotection through multiple proposed mechanisms including nitric oxide system modulation, growth factor signaling, and anti-inflammatory pathways [20, 21].

Novelty: Moderate. The gastric juice-derived pentadecapeptide concept is distinctive, and the breadth of reported pharmacological activities is unusual for a single peptide. The compound has been studied for approximately three decades.

Effect size: In hepatoprotective models (alcohol-induced liver injury, NSAID-induced hepatotoxicity, hepatic ischemia-reperfusion injury), BPC-157 has demonstrated substantial protective effects with normalization of liver enzymes and histological improvement [21]. Effect sizes in animal models are generally robust and reproducible across multiple laboratories, which distinguishes BPC-157 from Livagen in terms of independent replication.

Promising potential: Low to moderate. Despite extensive preclinical literature (over 100 publications), BPC-157 has not advanced to controlled clinical trials for any hepatoprotective indication. Regulatory status mirrors that of Livagen.

Side-effect profile: No significant adverse effects reported in preclinical studies. No human safety data from controlled trials. No formal toxicology studies meeting ICH standards have been published, paralleling the gap observed for Livagen.

Overall validation: Low to moderate. The most extensively studied and independently replicated peptide in this comparator panel for hepatoprotective activity, though formal clinical validation remains limited.

Comparison to Livagen: BPC-157 and Livagen differ fundamentally in their proposed mechanisms (nitric oxide/growth factor modulation versus chromatin remodeling/epigenetic regulation) and in the depth of independent replication (BPC-157 has been studied by multiple independent laboratories worldwide; Livagen primarily by the Khavinson group). BPC-157 has a broader range of demonstrated tissue-protective activities, while Livagen has a more specific and mechanistically distinctive epigenetic profile. For hepatoprotective applications, BPC-157 has a substantially larger evidence base.

12.4 Silymarin (Milk Thistle Extract)

Mechanism: Standardized extract of *Silybum marianum* containing silybin (silibinin), isosilybin, silydianin, and silychristin as the principal active flavonolignans. Hepatoprotective activity through multiple mechanisms: antioxidant free-radical scavenging, membrane stabilization, anti-inflammatory NF-kappaB pathway inhibition, antifibrotic activity, and stimulation of hepatocyte protein synthesis through RNA polymerase I activation [22, 23].

Novelty: Low. Silymarin has been in clinical use for over 50 years and is one of the most extensively studied natural hepatoprotective compounds globally.

Effect size: Meta-analyses of randomized controlled trials in liver disease have shown modest but statistically significant improvements in liver enzyme levels and histological endpoints in some contexts (alcoholic liver disease) but not in others (viral hepatitis C) [23]. The overall clinical effect size is modest. Clinical efficacy is limited by poor oral bioavailability (approximately 20 to 50 percent for standard formulations).

Promising potential: Established but limited. The compound is commercially mature with no significant new indications under active development. It serves as a benchmark hepatoprotective agent. Enhanced-bioavailability formulations (phytosome, micronized) are in active development.

Side-effect profile: Excellent. Decades of clinical use have established silymarin as well tolerated with a benign adverse-event profile. The most common adverse events are mild gastrointestinal symptoms (diarrhea, dyspepsia) in approximately 2 to 10 percent of users. Serious adverse events are rare [22].

Overall validation: Moderate to high for safety; moderate for efficacy. The most clinically validated hepatoprotective agent in this comparator panel, with extensive controlled trial data, though efficacy results have been inconsistent across indications.

Comparison to Livagen: Silymarin and Livagen operate through fundamentally different mechanisms (flavonolignan antioxidant/membrane stabilization versus tetrapeptide epigenetic chromatin remodeling). Silymarin has vastly greater clinical validation and a well-characterized safety profile. Livagen has a more novel and mechanistically distinctive proposed mechanism but lacks the clinical evidence base that would permit direct efficacy comparison. Silymarin represents the standard of care against which any new hepatoprotective agent should be evaluated.

12.5 S-Adenosylmethionine (SAdMe, Ademetionine)

Mechanism: Endogenous methyl donor and precursor for glutathione synthesis. Hepatoprotective through restoration of depleted glutathione stores, membrane phospholipid methylation, and modulation of cytokine-mediated hepatocyte apoptosis [24, 25].

Novelty: Low. SAdMe has been studied as a hepatoprotective agent since the 1970s and is approved as a pharmaceutical in several European countries.

Effect size: A large multicenter randomized controlled trial (Mato et al., 1999) in alcoholic liver cirrhosis reported a trend toward reduced mortality that was significant in the subgroup of patients with less advanced disease (Child-Pugh A and B) [24]. Individual trials have shown improvements in liver biochemistry and histology.

Promising potential: Moderate. Established pharmaceutical product in some markets, with active ongoing research. Not FDA-approved as a drug in the United States but widely available as a dietary supplement.

Side-effect profile: Good. Principal adverse events are mild gastrointestinal symptoms. Serious adverse events are rare. Concern exists for serotonergic interaction in patients on concurrent SSRIs. Contraindicated in bipolar disorder due to risk of manic switching [24].

Overall validation: Moderate. Pharmaceutical approvals in some European jurisdictions, large clinical trial data, and an established clinical safety record.

Comparison to Livagen: SAME is a conventional hepatoprotective agent with a mechanistic basis in glutathione metabolism and methylation chemistry. Livagen's mechanisms (chromatin remodeling, enkephalinase inhibition) are entirely different. SAME has substantially greater clinical validation than Livagen. The two compounds are mechanistically complementary and could theoretically be combined in hepatoprotective research protocols, though this combination has not been studied.

12.6 Comparator summary

The five comparators occupy distinct positions in the hepatoprotective and bioregulator landscape. **Epitalon** is the closest structural analog from the Khavinson bioregulator class but lacks hepatocyte-specific activity and does not serve as a hepatoprotective alternative. **Vilon** shares partial sequence homology (Lys-Glu) and chromatin-remodeling activity but is immunologically rather than hepatically targeted and lacks the pericentromeric heterochromatin decondensation observed with Livagen. **BPC-157** is the most independently replicated peptide hepatoprotector, with a broader tissue-protective profile but a fundamentally different mechanism. **Silymarin** is the established benchmark hepatoprotective agent with excellent safety but modest effect sizes. **SAME** is a conventional hepatoprotective with moderate clinical evidence and European pharmaceutical status.

Livagen occupies a unique mechanistic position as the only compound in this comparator panel proposed to operate through chromatin decondensation and epigenetic regulation of hepatocyte gene expression. This mechanistic novelty is its principal scientific distinction. The compound also uniquely combines hepatoprotective activity with enkephalinase inhibition (IC₅₀ approximately 20 micromolar), an activity not shared by any comparator. However, Livagen has the weakest validation profile of any compound in the panel: no clinical trials, no independent replication, no regulatory recognition, and no formal safety assessment. The practical

research-clinical translation of Livagen depends entirely on the strength of its pre-clinical evidence base and on the future conduct of independent replication studies, formal pharmacokinetic characterization, and controlled clinical trials.

Investigators considering Livagen for hepatoprotective research should recognize that the compound offers a mechanistically distinct tool for studying epigenetic regulation of liver function in aging, but that it is not a validated therapeutic agent and should not be positioned as an alternative to established hepatoprotective agents for clinical applications.

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This monograph is provided for research use only. Not for medical, diagnostic, or therapeutic purposes. Not for human consumption. All published research on Livagen originates from the Khavinson laboratory at the St. Petersburg Institute of Bioregulation and Gerontology and from its immediate collaborators; no independent Western laboratory replication has been published. The absence of controlled human clinical trials, formal pharmacokinetic studies, and independent validation represents a fundamental limitation of the evidence base. Investigators should weight the evidence accordingly and should not extrapolate preclinical and ex vivo findings to clinical efficacy or safety conclusions. Livagen is not approved by the United States Food and Drug Administration, the European Medicines Agency, or any other major Western regulatory authority. The compound is available as a research-grade preparation; investigators should obtain analytical confirmation of identity and purity on every lot.