

SS-20

A synthetic Szeto-Schiller tetrapeptide (Phe-D-Arg-Phe-Lys-NH₂) that selectively concentrates on the inner mitochondrial membrane through electrostatic and hydrophobic interactions with cardiolipin, restoring electron transport chain coupling efficiency and ATP synthesis under ischemic, oxidative, and age-related stress without direct free-radical scavenging, thereby dissociating mitochondrial protection from antioxidant chemistry and establishing cardiolipin modulation as the operative therapeutic mechanism of the SS peptide class.

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ABSTRACT

SS-20 (SBT-20; H-Phe-D-Arg-Phe-Lys-NH₂; CAS 736992-19-1; molecular weight 595.75; molecular formula C₃₀H₄₅N₉O₄) is a cell-permeable, mitochondria-targeted synthetic tetrapeptide of the Szeto-Schiller (SS) class, developed at the Department of Pharmacology at Weill Cornell Medical College by Hazel H. Szeto and colleagues as a structural analog of SS-31 (elamipretide) that retains mitochondrial targeting and cardiolipin binding but lacks the 2',6'-dimethyltyrosine (Dmt) residue responsible for intrinsic reactive oxygen species scavenging in SS-31 [1, 2]. The deliberate substitution of phenylalanine for Dmt at position 1 eliminates the phenolic hydroxyl group that confers direct radical-scavenging capacity, making SS-20 an indispensable mechanistic control compound that has proven essential for establishing that cardiolipin interaction, rather than antioxidant chemistry, is the operative therapeutic mechanism of the SS peptide class [3, 4]. SS-20 carries a net 3⁺ charge at physiological pH and concentrates approximately 1000-fold on the inner mitochondrial membrane (IMM), where it binds the tetra-acyl dianion cardiolipin through electrostatic interactions between its basic residues (D-Arg, Lys) and the cardiolipin phosphate head groups, with its aromatic phenylalanine residues inserting into the hydrophobic acyl chain region [5, 6]. This binding modulates the interaction between cardiolipin and cytochrome c, promoting the electron carrier function of cytochrome c over its peroxidase activity, thereby improving mitochondrial electron transport chain coupling efficiency, increasing ATP synthesis per unit oxygen consumed, and reducing mitochondrial reactive oxygen species generation as a downstream consequence of improved coupling rather than

through direct scavenging [3, 4]. In preclinical models, SS-20 has demonstrated efficacy comparable to SS-31 in protecting against 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced dopaminergic neurotoxicity in mice at 4 mg/kg intraperitoneal, with complete preservation of tyrosine hydroxylase-immunoreactive neurons in the substantia nigra pars compacta and 40 percent attenuation of striatal dopamine depletion [7]. In renal ischemia-reperfusion models, pretreatment with SS-20 extended warm ischemia tolerance in rat kidneys from 30 to 45 minutes, preserved cristae architecture on electron microscopy, restored tissue ATP levels, and reduced apoptosis, cytoskeletal breakdown, and interstitial fibrosis [8]. In cardiac ischemia-reperfusion, intravenous SS-20 (SBT-20) at 0.3 and 3.0 mg/kg/hour reduced myocardial infarct size by 20 percent relative to saline control in a rat coronary occlusion model, outperforming the reported 11 percent reduction achieved by elamipretide (MTP-131) in comparable protocols [9]. In pressure-overload heart failure induced by transverse aortic constriction (TAC) in mice, SS-20 produced partial but significant attenuation of cardiac hypertrophy and improvement in fractional shortening, with preferential protection of actin cytoskeletal pathways over mitochondrial and metabolic pathways in global proteomic analysis, a pattern distinct from the broader mitochondrial proteomic protection provided by SS-31 [10]. In chronic renal failure induced by 5/6 nephrectomy in mice, SBT-20 at 5 mg/kg intraperitoneal reduced inflammatory cytokines (interleukin-1-beta, interleukin-6, tumor necrosis factor alpha), normalized NF-kappaB signaling, restored mitochondrial membrane potential, and improved serum creatinine, blood urea nitrogen, and creatinine clearance [11]. The compound has not entered human clinical trials; all pharmacological characterization is preclinical.

SS-20 is not approved by any regulatory authority for any indication. It is supplied as a research-grade synthetic peptide by multiple chemical suppliers at greater than 98 percent purity by high-performance liquid chromatography and is used principally as a mechanistic tool to dissect the relative contributions of cardiolipin binding and antioxidant activity within the SS peptide class, and as a candidate therapeutic lead for ischemia-reperfusion injury, neurodegenerative disease, and chronic kidney disease in settings where direct radical scavenging may not be the desired pharmacological intervention.

1. Compound Identification

1.1 Names, synonyms, and registry identifiers

Primary designation: SS-20.

Alternative designations: SBT-20, SBT-020. The SBT prefix (Stealth BioTherapeutics) was adopted during commercial development of the SS peptide class by Stealth BioTherapeutics (formerly Stealth Peptides, Inc.), the company founded to advance SS-31 (elamipretide) and related analogs into clinical development. SS-20 retains both the original academic designation (SS-20, from the Szeto-Schiller naming convention) and the commercial designation (SBT-20) in published literature [1, 9, 11].

Chemical Abstracts Service registry number: 736992-19-1.

Sequence: H-Phe-D-Arg-Phe-Lys-NH₂ (single-letter code: f-r-f-K-NH₂, where lowercase denotes D-configuration). The compound is a C-terminally amidated tetrapeptide with the alternating aromatic-basic-aromatic-basic (phi-B-phi-B) motif characteristic of the Szeto-Schiller class [5, 6].

1.2 Structural class and chemistry

SS-20 is a synthetic, cell-permeable, mitochondria-targeted tetrapeptide belonging to the Szeto-Schiller (SS) peptide class, a family of short aromatic-cationic peptides originally developed from opioid peptide analgesic research at Weill Cornell Medical College [3]. The structural class is defined by the alternating aromatic-basic residue motif, the inclusion of at least one D-amino acid for proteolytic stability, and C-terminal amidation [5].

Molecular formula: C₃₀H₄₅N₉O₄.

Exact mass: 595.36 g/mol (monoisotopic).

Molecular weight: 595.75 g/mol.

Net charge at physiological pH: 3+ (contributed by the guanidinium group of D-arginine, the epsilon-amino group of lysine, and the alpha-amino terminus).

Physical properties: White to off-white lyophilized solid or crystalline powder. Freely soluble in water, dimethyl sulfoxide, and aqueous buffers at physiological pH. Soluble in dimethyl sulfoxide at concentrations up to 10 millimolar [12]. Sparingly soluble in apolar organic solvents.

Stereochemistry: SS-20 contains one D-amino acid (D-arginine at position 2). The remaining residues (Phe-1, Phe-3, Lys-4) are in the natural L-configuration. The D-arginine confers resistance to serum and tissue aminopeptidases and carboxypeptidases, extending the metabolic half-life relative to an all-L analog [3, 5].

Structural distinction from SS-31: SS-20 differs from SS-31 (D-Arg-Dmt-Lys-Phe-NH₂) at three of four positions. SS-31 contains 2',6'-dimethyltyrosine (Dmt) at position 2, which provides a phenolic hydroxyl group capable of hydrogen-atom transfer to hydroxyl and peroxy radicals. SS-20 substitutes L-phenylalanine at positions 1 and 3 and lacks any phenolic hydroxyl group, eliminating intrinsic radical-scavenging activity while preserving the aromatic-cationic pharmacophore required for membrane penetration and cardiolipin binding [5, 6]. The residue order also differs: SS-20 is Phe-D-Arg-Phe-Lys-NH₂, whereas SS-31 is D-Arg-Dmt-Lys-Phe-NH₂.

1.3 Synthesis

SS-20 is prepared by standard 9-fluorenylmethoxycarbonyl (Fmoc) solid-phase peptide synthesis on Rink amide resin, which provides the C-terminal amide upon cleavage [13]. The synthesis proceeds from the C-terminus to the N-terminus in four coupling steps: Fmoc-Lys(Boc)-OH, Fmoc-Phe-OH, Fmoc-D-Arg(Pbf)-OH, and Fmoc-Phe-OH. Each coupling employs a carbodiimide or uronium activating agent (typically HBTU or HATU) with diisopropylethylamine in N,N-dimethylformamide. Fmoc removal between couplings uses 20 percent piperidine in dimethylformamide. Global side-chain deprotection and cleavage from resin are accomplished with trifluoroacetic acid containing triisopropylsilane and water as scavengers (typically 95:2.5:2.5 v/v/v for 2 to 3 hours). The crude peptide is precipitated in cold diethyl ether, dissolved in water or dilute acetic acid, and purified by preparative reversed-phase high-performance liquid chromatography on a C18 column with an acetonitrile-water gradient containing 0.1 percent trifluoroacetic acid. The purified peptide is lyophilized to yield a white powder. Identity is confirmed by electrospray ionization mass spectrometry (expected [M+H]⁺ 596.36) and amino acid analysis. Purity is assessed by analytical reversed-phase HPLC (greater than 98 percent area by ultraviolet detection at 220 nanometers). The trifluoroacetate counterion may be

exchanged for acetate or hydrochloride by ion-exchange chromatography or repeated lyophilization from dilute hydrochloric acid if the application requires a specific salt form. Industrial synthesis achieves the compound in milligram to multi-gram quantities with typical yields of 60 to 80 percent after purification.

2. Abstract

SS-20 (SBT-20; H-Phe-D-Arg-Phe-Lys-NH₂) is a synthetic mitochondria-targeted tetrapeptide of the Szeto-Schiller class that selectively concentrates on the inner mitochondrial membrane through high-affinity binding to the anionic phospholipid cardiolipin [3, 5, 6]. The compound was developed at Weill Cornell Medical College by Hazel H. Szeto and colleagues as a deliberate structural variant of the lead clinical compound SS-31 (elamipretide, MTP-131, Bendavia, now marketed as Forzinity for Barth syndrome), in which the radical-scavenging 2',6'-dimethyltyrosine residue of SS-31 is replaced by phenylalanine, eliminating intrinsic antioxidant activity while preserving the aromatic-cationic pharmacophore required for cell penetration and cardiolipin interaction [1, 2, 3].

The pharmacological significance of SS-20 is dual. First, as a mechanistic probe, the compound has been essential for demonstrating that the therapeutic activity of the SS peptide class derives from modulation of cardiolipin-cytochrome c interactions on the inner mitochondrial membrane rather than from direct reactive oxygen species scavenging [3, 4]. This distinction has shaped the mechanistic understanding of the entire class. Second, as a therapeutic candidate in its own right, SS-20 has demonstrated robust efficacy across multiple preclinical disease models: complete neuroprotection against MPTP-induced dopaminergic neuron loss in a Parkinson's disease model [7]; extension of warm ischemia tolerance from 30 to 45 minutes in rat kidneys with preservation of cristae architecture and restoration of ATP synthesis [8]; 20 percent reduction in myocardial infarct size in two independent rat ischemia-reperfusion models [9]; partial attenuation of pressure-overload-induced heart failure with selective protection of cytoskeletal proteome pathways [10]; and normalization of inflammatory cytokines, oxidative stress markers, and renal function in chronic kidney disease [11].

The mechanism of action centers on the modulation of cardiolipin on the inner mitochondrial membrane. SS-20 binds cardiolipin through electrostatic attraction between its cationic residues and the cardiolipin phosphate head groups and through hydrophobic partitioning of its phenylalanine side chains into the lipid acyl chain region [5, 6]. This binding optimizes the interaction between cardiolipin and cytochrome c, favoring the electron carrier conformation of cytochrome c over the peroxidase conformation that is promoted by pathological cardiolipin peroxi-

dation in ischemia, aging, and mitochondrial disease [3, 4]. The downstream consequences are improved electron transport chain coupling efficiency, increased ATP synthesis per unit oxygen consumed, and reduced mitochondrial superoxide and hydrogen peroxide generation as a secondary effect of improved coupling rather than through direct radical interception.

SS-20 has not entered human clinical trials. All pharmacological and safety data are preclinical. The compound is not approved by any regulatory authority. It is available from multiple commercial peptide suppliers (Probechem Biochemicals, MedChemExpress, custom synthesis laboratories) at research-grade purity (greater than 98 percent by HPLC) and is used principally in academic and preclinical research as a mechanistic dissection tool and as a candidate for ischemia-reperfusion injury, neurodegenerative disease, and chronic kidney disease.

3. Discovery and Development History

3.1 Origin in opioid peptide research

The Szeto-Schiller (SS) peptide class was discovered serendipitously during structure-activity studies of opioid peptide analgesics conducted by Hazel H. Szeto and Peter W. Schiller in the late 1990s and early 2000s [3]. The original compound in the series, SS-02 (H-Dmt-D-Arg-Phe-Lys-NH₂), was synthesized as a mu-opioid receptor agonist. Unexpectedly, this highly polar, water-soluble tetrapeptide demonstrated potent central nervous system analgesic activity after subcutaneous administration to rodents, suggesting efficient blood-brain barrier penetration despite its hydrophilic character and 3+ net charge at physiological pH [3].

Subsequent confocal fluorescence microscopy studies using a dansyl-labeled analog revealed that SS-02 accumulated selectively in mitochondria at concentrations approximately 1000-fold above the extracellular concentration, with localization to the inner mitochondrial membrane rather than the mitochondrial matrix [3, 4]. This finding was unexpected because prior mitochondria-targeted cations (such as the triphenylphosphonium-conjugated compounds MitoQ and SkQ1) accumulate in the mitochondrial matrix driven by the membrane potential across the inner mitochondrial membrane. The SS peptide localization to the inner membrane itself, rather than the matrix, suggested a distinct targeting mechanism.

3.2 Design of SS-20 as a non-scavenging analog

Recognizing the potential of mitochondria-targeted peptides as therapeutic agents, Szeto and colleagues designed analogs that would retain mitochondrial targeting but lack opioid receptor affinity. SS-31 (D-Arg-Dmt-Lys-Phe-NH₂) and SS-20 (Phe-D-Arg-Phe-Lys-NH₂) were the two principal non-opioid analogs [1, 3]. SS-31 retained the Dmt residue and therefore preserved direct radical-scavenging capacity through its phenolic hydroxyl group. SS-20 replaced Dmt with phenylalanine at position 1, eliminating the phenolic group and thereby eliminating intrinsic antioxidant activity while preserving the aromatic-cationic structural motif [3, 5].

The design of SS-20 was motivated by the need to dissect the mechanistic contributions of mitochondrial targeting, cardiolipin binding, and antioxidant activity within the SS peptide pharmacology. If SS-31's therapeutic effects were primarily attributable to direct radical scavenging, SS-20 would be expected to show substantially reduced efficacy. If, alternatively, the effects were primarily attributable to cardiolipin modulation, SS-20 would be expected to show comparable efficacy.

3.3 Demonstration of cardiolipin as the operative target

The critical experiments establishing SS-20 as a productive therapeutic agent came from multiple laboratories between 2009 and 2022. Yang et al. (2009) demonstrated that SS-20, despite lacking radical-scavenging capacity, provided neuroprotection against MPTP toxicity equivalent to SS-31, establishing that antioxidant activity was not required for the neuroprotective mechanism [7]. Szeto and Birk (2014) synthesized these findings in a comprehensive review establishing cardiolipin interaction as the unifying mechanism for the SS peptide class [3]. Birk et al. (2014) demonstrated that SS-20 pretreatment extended warm ischemia tolerance in rat kidneys [8]. Mitchell et al. (2022) provided the definitive biophysical characterization, showing that SS-20 binds cardiolipin-containing membranes and adopts compact reverse-turn conformations in the membrane-bound state [5].

3.4 Commercial development context

Stealth BioTherapeutics (formerly Stealth Peptides, Inc.; Needham, Massachusetts) was founded to advance the SS peptide class into clinical development. The company's clinical pipeline has focused exclusively on SS-31 (designated elamipretide, MTP-131, and subsequently Bendavia). Elamipretide received FDA accelerated approval in September 2025 under the trade name Forzinity for the treatment of Barth syndrome, a rare mitochondrial cardiomyopathy caused by mutations in the tafazzin gene [14]. SS-20 (SBT-20) was retained as a preclinical research compound and has not entered clinical development. The compound is designated SBT-20 in the Stealth BioTherapeutics nomenclature.

3.5 Current status

SS-20 remains a preclinical research compound with no active clinical development program. Its primary value in the research literature is as a mechanistic control that has been essential for establishing the cardiolipin-binding mechanism of the

SS peptide class. The compound has also generated an independent preclinical efficacy literature supporting potential therapeutic application in ischemia-reperfusion injury of the heart and kidney, neurodegenerative disease, and chronic kidney disease.

4. Mechanism of Action

SS-20 is a single-mechanism compound whose therapeutic activity derives from modulation of cardiolipin on the inner mitochondrial membrane. Unlike its structural analog SS-31, which combines cardiolipin modulation with direct radical scavenging, SS-20 operates exclusively through the cardiolipin-binding mechanism, making it the cleanest pharmacological probe for this target within the SS peptide class.

4.1 Cardiolipin binding and membrane interaction

Cardiolipin (1,3-bis(sn-3'-phosphatidyl)-sn-glycerol) is a tetra-acyl dianion phospholipid unique to the inner mitochondrial membrane, where it constitutes approximately 15 to 20 percent of total phospholipid content [15]. Cardiolipin is not present in significant quantities in any other cellular membrane, a feature that accounts for the selective mitochondrial targeting of SS-20. The phospholipid plays essential structural and functional roles: it is required for the assembly and activity of respiratory chain complexes I, III, IV, and V (ATP synthase), for the formation of respiratory supercomplexes, and for the optimal function of the adenine nucleotide translocase [15, 16].

SS-20 binds cardiolipin-containing membranes through two complementary interactions [5, 6]. First, electrostatic attraction between the cationic residues (D-Arg guanidinium, Lys epsilon-amino, N-terminal amino) and the anionic phosphate head groups of cardiolipin positions the peptide at the membrane surface. Second, the aromatic phenylalanine side chains at positions 1 and 3 partition into the hydrophobic acyl chain region of the bilayer, anchoring the peptide in the membrane. Biophysical studies by Mitchell et al. (2022) using molecular dynamics simulations and nuclear magnetic resonance spectroscopy demonstrated that SS-20 adopts a compact reverse-turn conformation in the membrane-bound state, distinct from the relatively disordered extended conformation it adopts in aqueous solution [5]. The lipid-to-peptide stoichiometry for cardiolipin-containing membranes is approximately 1.4 to 1.5 dianionic lipids per peptide molecule, indicating that each SS-20 molecule associates with approximately one to two cardiolipin molecules on average [6].

4.2 Modulation of cytochrome c-cardiolipin interaction

The principal molecular target of SS-20 is the interaction between cardiolipin and cytochrome c on the outer leaflet of the inner mitochondrial membrane [3, 4]. Under normal physiological conditions, cytochrome c is loosely associated with the outer surface of the inner mitochondrial membrane through electrostatic interactions with cardiolipin, and it functions as a mobile electron carrier shuttling electrons from Complex III (cytochrome bc1) to Complex IV (cytochrome c oxidase). In this electron carrier conformation, the heme iron of cytochrome c is hexacoordinate, with Met-80 serving as the sixth axial ligand.

Under pathological conditions (ischemia, oxidative stress, aging, mitochondrial disease), cardiolipin undergoes peroxidation and structural alterations that tighten the hydrophobic interaction between cardiolipin acyl chains and cytochrome c [3, 4]. This tighter binding disrupts the Met-80 axial ligation and converts cytochrome c from an electron carrier into a cardiolipin peroxidase, generating a positive feedback loop: peroxidized cardiolipin promotes the peroxidase conformation of cytochrome c, which generates more cardiolipin peroxidation, further disrupting electron transport chain function and amplifying reactive oxygen species generation.

SS-20 interrupts this pathological cycle by binding cardiolipin and modulating its interaction with cytochrome c, favoring the electron carrier conformation over the peroxidase conformation [3, 4]. The downstream consequences are restoration of normal electron flow through the respiratory chain, improved coupling efficiency (increased ATP per oxygen consumed), and reduced mitochondrial superoxide and hydrogen peroxide generation. Critically, the reduction in reactive oxygen species is a secondary consequence of improved electron transport coupling rather than a direct radical-scavenging event, a distinction that SS-20, lacking any radical-scavenging moiety, has been essential for establishing.

4.3 Mitochondrial bioenergetic effects

The functional consequences of SS-20 binding to cardiolipin include restoration of mitochondrial state 3 (ADP-stimulated) respiration, improved respiratory control ratio (the ratio of state 3 to state 4 oxygen consumption), increased ATP synthesis rate, and reduced proton leak across the inner mitochondrial membrane [3, 8]. In stressed cells (C2C12 muscle cells challenged with 500 micromolar hydrogen peroxide), treatment with 1 or 10 micromolar SS-20 significantly improved maximal mito-

chondrial respiration, producing effects comparable to the reactive oxygen species scavenger N-acetylcysteine [9]. In isolated mitochondria, SS-20 treatment significantly reduced succinate-driven reactive oxygen species production, particularly through inhibition of reverse electron transfer from Complex II to Complex I [9].

4.4 Membrane electrostatic modulation

Recent biophysical work has demonstrated that SS peptides, including SS-20, modulate the surface electrostatic potential of cardiolipin-containing membranes [6]. The binding of the 3+ charged peptide to the anionic membrane surface reduces the local negative surface potential, which may influence the assembly and activity of cardiolipin-dependent membrane proteins including respiratory chain complexes and the adenine nucleotide translocase. This electrostatic mechanism is independent of the cytochrome c-cardiolipin interaction and may contribute to the broader mitochondrial protective effects observed with SS-20 treatment.

4.5 Absence of opioid receptor activity

Unlike the parent compound SS-02 (H-Dmt-D-Arg-Phe-Lys-NH₂), which possesses high-affinity mu-opioid receptor agonist activity, SS-20 has negligible affinity for mu, delta, and kappa opioid receptors [1, 3]. This was a deliberate design feature: the replacement of Dmt with Phe at position 1 eliminates the pharmacophoric elements (the phenolic hydroxyl and the N-terminal amino-aromatic spatial relationship) required for opioid receptor recognition. Investigators using SS-20 in preclinical models therefore need not account for opioid-mediated confounds in the interpretation of analgesic, neuroprotective, or cardiovascular endpoints.

4.6 Absence of intrinsic radical-scavenging activity

SS-20 does not scavenge hydroxyl radicals, superoxide anion, peroxy radicals, or peroxynitrite in cell-free radical-generating assays [3, 7]. The absence of radical-scavenging activity has been confirmed by comparison with SS-31, which scavenges these species through hydrogen-atom transfer from the phenolic hydroxyl of Dmt, and with SS-02, which shares the Dmt radical-scavenging capacity [3]. The reductions in mitochondrial reactive oxygen species observed with SS-20 treatment in intact cells and in vivo are therefore attributable entirely to improved electron transport chain coupling efficiency rather than to direct radical interception.

5. Pharmacokinetics and Routes of Administration

5.1 Absorption and distribution

Formal pharmacokinetic studies of SS-20 in animals have not been published in the detail available for SS-31 (elamipretide). The pharmacokinetic properties of SS-20 are therefore estimated from the class pharmacology of the SS peptides and from the pharmacokinetic data available for SS-31.

SS-20 is a highly polar, water-soluble tetrapeptide with a net 3+ charge at physiological pH. Despite this polarity, the compound demonstrates rapid cellular uptake and efficient blood-brain barrier penetration, as demonstrated by the neuroprotective efficacy of intraperitoneally administered SS-20 in the central nervous system MPTP model [7]. Cellular uptake does not require specific membrane transporters and is not dependent on mitochondrial membrane potential, distinguishing the SS peptides from the triphenylphosphonium-conjugated mitochondria-targeted compounds [3].

For the structurally related SS-31 (elamipretide), subcutaneous administration achieves approximately 92 percent bioavailability, with peak plasma concentrations reached within 1 to 2 hours and an elimination half-life of approximately 4 hours [17]. SS-20 is expected to have broadly similar pharmacokinetic behavior given the shared aromatic-cationic structural motif, similar molecular weight, and similar charge state, though the absence of the Dmt hydroxyl may modestly affect metabolic clearance pathways.

The volume of distribution of SS peptides is large, consistent with rapid tissue distribution and the 1000-fold concentration on the inner mitochondrial membrane [3]. Tissue distribution studies with fluorescently labeled SS peptide analogs have demonstrated accumulation in heart, kidney, brain, skeletal muscle, and liver, consistent with the mitochondrial density of these tissues.

5.2 Metabolism and elimination

The D-arginine at position 2 confers substantial resistance to aminopeptidases and endopeptidases, extending the metabolic half-life relative to all-L peptide analogs [3, 5]. The C-terminal amidation protects against carboxypeptidases. The principal metabolic pathways are expected to include renal clearance of intact peptide and cleavage by tissue peptidases at sites flanking the D-amino acid, with the D-Arg-containing fragments resistant to further degradation.

For SS-31, clinical data indicate well-tolerated intravenous infusion over a wide dose range (0.01 to 0.25 mg/kg/hour over 4 hours) in healthy human volunteers [17]. Renal excretion contributes to clearance; hepatic cytochrome P450 metabolism is not expected to contribute significantly to clearance of a tetrapeptide that lacks hepatic drug metabolizing enzyme substrates.

5.3 Routes of administration

SS-20 has been administered by the following routes in published preclinical studies:

Intraperitoneal: 4 mg/kg in the MPTP neuroprotection model (Yang et al. 2009) [7]; 5 mg/kg in the chronic renal failure model (Sun et al. 2020) [11].

Intravenous: 0.3 mg/kg/hour and 3.0 mg/kg/hour continuous infusion via jugular vein catheter in the rat cardiac ischemia-reperfusion model (Dai et al. 2016) [9].

Subcutaneous: The route used for SS-31 clinical trials; SS-20 is expected to be suitable for subcutaneous administration based on class pharmacology.

In vitro: 1 to 10 micromolar in cell culture systems (C2C12 muscle cells, HK-2 human kidney proximal tubular cells) [9, 11].

5.4 Special populations

No special population pharmacokinetic data exist for SS-20. The compound has not been studied in humans. Preclinical models have used young adult female rats (Sprague Dawley, approximately 200 grams) for cardiac ischemia-reperfusion [9], male C57BL/6 mice for chronic renal failure and MPTP models [7, 11], and both male and female rodents in the renal ischemia model [8].

6. Preclinical Pharmacology

6.1 Neuroprotection in the MPTP Parkinson's disease model

Yang et al. (2009) evaluated SS-20 and SS-31 in the MPTP model of Parkinson's disease in C57BL/6 mice [7]. MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) is metabolized to the mitochondrial Complex I inhibitor MPP⁺ in astrocytes and selectively destroys dopaminergic neurons in the substantia nigra pars compacta, producing a parkinsonian syndrome in mice. The study used a moderate-exposure paradigm: three doses of 10 mg/kg MPTP administered intraperitoneally at 2-hour intervals, producing approximately 44 percent striatal dopamine depletion in control animals.

SS-20 was administered at 4 mg/kg intraperitoneally 30 minutes before each MPTP dose and again 1 and 12 hours after the final MPTP injection. At this dose, SS-20 attenuated 40 percent of the MPTP-induced striatal dopamine depletion and produced complete preservation of tyrosine hydroxylase-immunoreactive neurons in the substantia nigra pars compacta. The metabolites 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) were similarly protected. The efficacy of SS-20 was equivalent to that of SS-31 at the same dose, despite the absence of radical-scavenging capacity in SS-20 [7].

Mechanistic studies demonstrated that both SS-20 and SS-31 preserved mitochondrial ATP production and oxygen consumption in MPP⁺-treated cells, prevented MPP⁺-induced mitochondrial swelling, and blocked cytochrome c release [7]. The equal efficacy of the non-scavenging analog SS-20 was the first demonstration that antioxidant activity was not required for SS peptide-mediated neuroprotection, establishing cardiolipin modulation as the operative mechanism.

6.2 Renal ischemia-reperfusion and warm ischemia tolerance

Szeto and colleagues demonstrated that pretreatment with SS-20 thirty minutes before warm ischemia in rat kidneys increased the tolerable ischemia duration from 30 to 45 minutes, a clinically meaningful extension with direct implications for partial nephrectomy and renal transplantation surgical windows [8]. SS-20 reduced cytoskeletal breakdown (loss of actin polarity and brush border disruption) and cell swelling after 45-minute ischemia. Transmission electron microscopy demonstrated that SS-20-treated kidneys maintained normal mitochondrial structure with preserved cristae architecture, whereas control kidneys showed severe mitochondrial matrix swelling and cristae loss. Tissue ATP levels were restored within 1 hour of reperfusion in SS-20-treated kidneys. Long-term outcomes included reduced apoptosis, reduced oxidative stress markers, reduced inflammation, and reduced interstitial fibrosis [8].

The ability of SS-20, a non-scavenging compound, to boost ATP synthesis during ischemia and improve coupling efficiency was interpreted as evidence that the therapeutic target in renal ischemia-reperfusion injury is the cardiolipin-cytochrome c interaction rather than direct radical interception.

6.3 Cardiac ischemia-reperfusion

Dai et al. (2016) evaluated SBT-20 (SS-20) in two models of rat myocardial ischemia-reperfusion [9].

In vivo model: Female Sprague Dawley rats underwent 30 minutes of left coronary artery occlusion followed by 3 hours of reperfusion. SBT-20 was administered intravenously via jugular vein catheter beginning 5 minutes after coronary occlusion and continuing for 1 hour into reperfusion. Two dose groups were studied: low dose (0.3 mg/kg/hour, n=15) and high dose (3.0 mg/kg/hour, n=15), compared to saline control (n=15). Infarct size (percentage of risk area) was 77.6 plus or minus 2.6 percent in saline controls, 62.1 plus or minus 4.4 percent in the low-dose group ($p=0.001$ versus control), and 64.0 plus or minus 4.9 percent in the high-dose group ($p=0.001$ versus control), representing approximately 20 percent relative reduction in infarct size at both doses [9]. Notably, SBT-20 did not reduce the no-reflow area (a measure of microvascular obstruction), suggesting that the protective mechanism operates at the cardiomyocyte mitochondrial level rather than at the vascular level.

Ex vivo model: Isolated rat hearts subjected to 20 minutes of global ischemia followed by 120 minutes of reperfusion were treated with 1 micromolar SBT-20 at the onset of reperfusion. Infarct size was 17.2 plus or minus 2.8 percent in SBT-20-treated hearts versus 43.3 plus or minus 2.6 percent in controls (p less than 0.05), a 60.3 percent relative reduction [9].

Cellular studies: In C2C12 muscle cells stressed with 500 micromolar hydrogen peroxide, SBT-20 at 1 and 10 micromolar significantly improved maximal mitochondrial respiration and significantly reduced reactive oxygen species production from succinate-driven reverse electron transfer [9].

The authors noted that the 20 percent in vivo infarct size reduction with SBT-20 compared favorably to the approximately 11 percent reduction reported for elamipretide (MTP-131, SS-31 acetate salt) in comparable ischemia-reperfusion protocols, though direct cross-study comparisons must be interpreted with caution.

6.4 Pressure-overload heart failure

Dai et al. (2013) evaluated SS-20 and SS-31 in a mouse model of pressure-overload heart failure induced by transverse aortic constriction (TAC) [10]. Using label-free shotgun proteomics, the study identified 538 proteins significantly altered after TAC, mapping to 53 pathways including actin cytoskeleton, mitochondrial function, intermediate metabolism, glycolysis/gluconeogenesis, and the citrate cycle.

Concomitant treatment with SS-31 ameliorated congestive heart failure phenotypes and mitochondrial damage, with 84 percent protection of mitochondrial protein changes and 69 percent protection of nonmitochondrial protein changes. SS-20 had more modest effects: fractional shortening decline was significantly ameliorated by SS-20 but less completely than by SS-31. Proteomic analysis revealed that SS-20 preferentially protected actin cytoskeletal pathways, whereas mitochondrial and metabolic pathways were not significantly protected [10]. This differential proteomic pattern is consistent with the absence of direct radical-scavenging activity in SS-20: the actin cytoskeleton pathways may be protected through a mechanism downstream of cardiolipin stabilization and improved bioenergetics, while the full mitochondrial proteomic protection achieved by SS-31 may additionally require direct radical interception within the mitochondrial matrix.

6.5 Chronic renal failure

Sun et al. (2020) evaluated SBT-20 in a mouse model of chronic renal failure induced by 5/6 nephrectomy (5/6NX) in C57BL/6 mice [11]. SBT-20 at 5 mg/kg intraperitoneal injection prevented the elevated expression of inflammatory cytokines (interleukin-1-beta, interleukin-6, NF-kappaB1, NF-kappaB2) and reduced NF-kappaB-p65 and tumor necrosis factor alpha levels. Oxidative stress markers (reactive oxygen species production, HIF-1-alpha, HO-1) were normalized, and the antioxidant enzymes SOD1 and SOD2 were restored toward baseline levels.

Mitochondrial function studies demonstrated that SBT-20 restored mitochondrial membrane potential, recovered elongated mitochondrial morphology (indicating normalized mitochondrial dynamics), downregulated the fission protein Drp1, and upregulated the fusion protein Mfn2 [11]. The normalization of mitochondrial dynamics (balanced fusion and fission) is a downstream consequence of improved bioenergetics and reduced mitochondrial stress.

Renal functional endpoints improved: serum creatinine, blood urea nitrogen, fractional sodium excretion, and creatinine clearance were all significantly improved relative to untreated 5/6NX controls [11]. In vitro validation in HK-2 human kidney proximal tubular cells at 1 micromolar SBT-20 confirmed the mitochondrial protective mechanism.

6.6 Contrast-induced and other nephrotoxicity models

The Szeto laboratory has reported that SS-20 protects against contrast agent-induced kidney injury in rodent models, consistent with the renal ischemia-reperfusion protection mechanism [3]. The cardiolipin-binding mechanism provides a unifying explanation for renal protection across multiple insult types (ischemia-reperfusion, chronic renal failure, nephrotoxin exposure), each of which converges on mitochondrial dysfunction in proximal tubular epithelial cells.

7. Clinical Evidence Base

SS-20 has not been evaluated in any human clinical trial. No clinical efficacy, safety, or pharmacokinetic data exist in humans. All evidence is preclinical and is described in sections 5 and 6.

The clinical evidence base for the SS peptide class derives entirely from SS-31 (elamipretide), which has completed multiple Phase 2 and Phase 3 clinical trials across indications including Barth syndrome, primary mitochondrial myopathy, heart failure with preserved ejection fraction (HFpEF), heart failure with reduced ejection fraction (HFrEF), age-related macular degeneration, and acute kidney injury associated with renal artery stenosis [14, 17, 18]. Elamipretide received FDA accelerated approval in September 2025 under the trade name Forzinity for the treatment of Barth syndrome [14]. The clinical success of SS-31 validates the cardiolipin-targeting mechanism shared with SS-20 and provides a translational framework for evaluating whether SS-20's distinct pharmacological profile (cardiolipin binding without radical scavenging) might offer advantages in specific clinical contexts.

The absence of clinical development for SS-20 reflects commercial rather than scientific considerations: Stealth BioTherapeutics concentrated its clinical development resources on SS-31, which combines cardiolipin modulation with radical scavenging and was considered the more broadly applicable compound. Whether SS-20's absence of radical scavenging offers advantages in specific disease contexts (for example, in conditions where reactive oxygen species serve essential signaling functions that should not be suppressed by a scavenger) remains an open research question.

8. Sourcing and Quality Verification

SS-20 is available as a research-grade synthetic peptide from multiple commercial suppliers:

Probechem Biochemicals: Supplies SBT-20 (SS-20) as a solid powder at greater than 98 percent purity by HPLC, with molecular weight confirmation at 595.75 [12].

MedChemExpress: Supplies SBT-20 at research-grade purity.

Custom peptide synthesis laboratories: Any laboratory equipped for Fmoc solid-phase peptide synthesis can produce SS-20 to specification from the published sequence (H-Phe-D-Arg-Phe-Lys-NH₂). Custom synthesis is appropriate when specific salt forms (acetate, hydrochloride, trifluoroacetate-free), isotopic labeling, or fluorescent conjugation are required.

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 at 220 nanometers ultraviolet detection
- Electrospray ionization mass spectrometry confirmation of molecular weight (expected [M+H]⁺ 596.36 for the protonated monoisotopic mass; expected average mass [M+H]⁺ 596.75)
- Amino acid analysis to confirm the Phe:D-Arg:Lys ratio of 2:1:1
- Chiral amino acid analysis or chiral chromatography to confirm D-configuration of arginine (critical, as L-Arg substitution would eliminate proteolytic stability)
- Residual trifluoroacetic acid analysis if the application is sensitive to TFA content (counterion from HPLC purification); TFA-free preparations should be specified at order
- Endotoxin testing (Limulus amoebocyte lysate assay) for in vivo animal studies

The research-grade SS-20 market is small relative to the SS-31 market; availability from off-the-shelf suppliers may be intermittent, and custom synthesis may be required for larger quantities. Pricing for research-grade material is moderate to

high, in the range of \$100 to \$500 per 5 milligrams depending on purity grade and supplier.

9. Reconstitution and Handling

SS-20 as a lyophilized powder is stable at minus 20 degrees Celsius for at least 12 months and at 2 to 8 degrees Celsius for at least 6 months [12]. The compound should be stored desiccated and protected from moisture. Light protection is prudent but not strictly required for a compound lacking chromophores beyond the phenylalanine aromatic ring.

Aqueous reconstitution: SS-20 is freely soluble in water, phosphate-buffered saline, normal saline, and other aqueous buffers at physiological pH. Stock solutions for in vivo use are typically prepared at 1 to 10 mg/mL in sterile normal saline or sterile water for injection. For in vitro use, stock solutions at 10 millimolar (5.96 mg/mL) in water or dimethyl sulfoxide are standard [12].

Dimethyl sulfoxide reconstitution: For applications requiring higher stock concentrations or for storage as frozen aliquots, SS-20 can be dissolved in dimethyl sulfoxide at concentrations up to 10 millimolar. DMSO stock solutions are stable at minus 80 degrees Celsius for at least 6 months and at minus 20 degrees Celsius for at least 6 months [12].

Working solutions: Aqueous working solutions should be prepared fresh on the day of use or stored at 2 to 8 degrees Celsius for no longer than 7 days. The peptide does not contain disulfide bonds or other oxidation-sensitive functional groups (the phenylalanine side chains are oxidatively stable), but microbial contamination of aqueous solutions limits storage duration in the absence of bacteriostatic agents.

Aliquoting: Freeze-thaw cycles should be minimized. Single-use aliquots of stock solution at the target concentration in appropriate vehicle are recommended for both in vivo and in vitro applications. If frozen aliquots are necessary, DMSO-based stocks tolerate freeze-thaw more robustly than aqueous stocks.

Filtration: Sterilize aqueous solutions by filtration through 0.22-micrometer polyethersulfone or polyvinylidene fluoride syringe filters. The peptide does not adsorb significantly to standard membrane filter materials at the concentrations used in preclinical research.

10. Stack Interaction Considerations

SS-20 is a preclinical research compound with no approved therapeutic indication and no established drug-drug interaction profile in humans. The following considerations are derived from the known pharmacology and from class interactions observed with SS-31 and related mitochondria-targeted compounds.

Other mitochondria-targeted compounds: Concurrent administration with SS-31 (elamipretide) produces additive or competitive binding at the cardiolipin site on the inner mitochondrial membrane. The combination has been studied in the TAC heart failure model (Dai et al. 2013) but not as a deliberate co-administration; rather, SS-20 and SS-31 were studied as separate treatment arms [10]. Concurrent use with triphenylphosphonium-conjugated compounds (MitoQ, SkQ1, MitoTEMPO) produces concurrent but mechanistically independent mitochondrial effects: the TPP compounds accumulate in the mitochondrial matrix driven by membrane potential, while SS-20 resides on the inner membrane. The combination is pharmacologically coherent but has not been studied systematically.

Electron transport chain inhibitors: Compounds that inhibit specific respiratory chain complexes (rotenone, Complex I; antimycin A, Complex III; cyanide, Complex IV) will counteract the bioenergetic improvements produced by SS-20. Investigators studying SS-20 mechanism should be aware that concomitant respiratory chain inhibitors will confound the bioenergetic readouts.

Calcineurin inhibitors (cyclosporine A, tacrolimus): Cyclosporine A inhibits the mitochondrial permeability transition pore through binding to cyclophilin D. SS-20 does not directly inhibit the permeability transition pore [3], and the two mechanisms are independent. Combination may produce additive mitochondrial protection in ischemia-reperfusion models.

Antioxidants: Direct antioxidants (N-acetylcysteine, vitamin E, vitamin C, glutathione precursors) produce mechanistically independent effects. N-acetylcysteine was used as a positive control comparator for SS-20's effects on maximal mitochondrial respiration in the Dai et al. (2016) cellular studies [9]. The combination is pharmacologically coherent.

Opioids: SS-20 has negligible opioid receptor affinity and does not interact with opioid agonists or antagonists at the receptor level [3]. No pharmacodynamic or pharmacokinetic interaction is expected.

CYP450 substrates and inhibitors: As a tetrapeptide, SS-20 is not expected to be a substrate for or inhibitor of hepatic cytochrome P450 enzymes. No CYP-mediated drug interactions are anticipated.

11. Adverse Events and Safety Signal

11.1 Preclinical safety

No formal toxicology or safety pharmacology studies of SS-20 have been published. The safety profile is inferred from the preclinical pharmacology studies (which reported no treatment-related adverse effects at the studied doses) and from the class safety data available for SS-31 (elamipretide), which has been extensively characterized in both preclinical and clinical safety studies.

In the Dai et al. (2016) cardiac ischemia-reperfusion study, hemodynamic monitoring revealed that the high-dose SBT-20 group (3.0 mg/kg/hour) showed significantly lower systolic and diastolic blood pressure at 1 to 2 hours post-reperfusion compared to the low-dose group, suggesting a potential dose-dependent hypotensive effect [9]. Heart rate and body temperature were comparable across all treatment groups. No other treatment-related adverse effects were reported.

In the Yang et al. (2009) MPTP study, no adverse effects of SS-20 at 4 mg/kg intraperitoneal were reported [7]. In the Sun et al. (2020) chronic renal failure study, no adverse effects of SBT-20 at 5 mg/kg intraperitoneal were reported [11].

11.2 Class safety from SS-31 clinical trials

The most comprehensive safety data for the SS peptide class come from SS-31 (elamipretide) clinical trials. In human studies, elamipretide was well tolerated as intravenous infusion over a wide dose range (0.01 to 0.25 mg/kg/hour over 4 hours) in healthy volunteers, with no drug-related adverse events at 0.05 mg/kg/hour for 2 hours [17]. Subcutaneous administration (the route used in most Phase 2 and Phase 3 trials) produced injection site reactions as the principal adverse events: erythema (57 percent), pruritus (47 percent), pain (20 percent), urticaria (20 percent), and irritation (10 percent), most of which were mild and transient [18]. No serious adverse reactions or fatalities attributable to elamipretide have been reported in published clinical trials.

The SS peptide class does not produce effects on normal, healthy mitochondria, even at concentrations substantially above the therapeutic range [3]. This selectivity for stressed or dysfunctional mitochondria is attributed to the pathology-dependent increase in cardiolipin exposure and peroxidation that enhances SS peptide binding and modulation under disease conditions.

11.3 Theoretical safety considerations

Cardiolipin perturbation in normal tissue: Excessive occupancy of cardiolipin binding sites could theoretically disrupt normal respiratory chain function. The preclinical evidence argues against this concern at studied doses, but formal dose-ranging toxicology in healthy animals has not been published for SS-20.

Immunogenicity: As a synthetic tetrapeptide of less than 600 daltons molecular weight, SS-20 is below the typical molecular weight threshold for immunogenicity. No anti-drug antibody responses have been reported for any SS peptide in published preclinical or clinical studies.

Reproductive and developmental toxicity: No reproductive or developmental toxicity data exist for SS-20. Pregnancy and lactation use is not recommended in the absence of safety data.

11.4 Safety summary

The available preclinical evidence and the class safety profile of SS-31 suggest that SS-20 has a favorable safety profile at the doses studied in preclinical models. The principal caution for investigators is the dose-dependent hypotensive effect observed at the high intravenous dose in the cardiac ischemia-reperfusion model, which may reflect a direct vasodilatory effect of the peptide or a consequence of improved cardiac bioenergetics reducing sympathetic tone. Formal toxicology studies are needed before any consideration of human investigation.

12. Comparable Compounds Assessment

This section presents a structured comparative assessment of five mitochondria-targeted or mitochondria-active compounds against SS-20, evaluated against five competency standards: novelty, effect size, promising potential (current clinical state and trajectory), side-effect profile (clinical or preclinical), and overall validation. The five comparators were selected as the principal mitochondria-targeted therapeutic candidates with substantive preclinical or clinical evidence in the indications relevant to SS-20 (ischemia-reperfusion injury, neurodegenerative disease, mitochondrial disease, chronic kidney disease).

12.1 SS-31 (Elamipretide, MTP-131, Bendavia, Forzinity)

Mechanism: Mitochondria-targeted tetrapeptide (D-Arg-Dmt-Lys-Phe-NH₂) that binds cardiolipin on the inner mitochondrial membrane and directly scavenges reactive oxygen species through the 2',6'-dimethyltyrosine phenolic hydroxyl group. Dual mechanism: cardiolipin modulation plus radical scavenging [3, 4].

Novelty: High. SS-31 is the first-in-class cardiolipin-binding peptide and the first mitochondria-targeted compound to achieve regulatory approval (FDA accelerated approval September 2025 for Barth syndrome as Forzinity) [14]. The mechanism of action (cardiolipin modulation rather than matrix antioxidant accumulation) was novel at the time of development.

Effect size: Substantial across preclinical models: near-complete neuroprotection in MPTP models, significant reduction in infarct size in cardiac ischemia-reperfusion, improvement in diastolic function in aged mice, restoration of skeletal muscle ATP energetics within 1 hour in aged mice [7, 10, 17]. Clinical effect sizes in Barth syndrome include improvement in 6-minute walk distance and improvement in muscle strength (the basis for accelerated approval). Phase 2 effect sizes in heart failure (EMBRACE trial) were modest and did not reach primary endpoint in the pivotal Phase 3 trial.

Promising potential: High. FDA-approved for Barth syndrome. Active development for additional indications including primary mitochondrial myopathy and heart failure. The clinical validation of the cardiolipin-targeting mechanism provides a strong translational framework for the entire SS peptide class.

Side-effect profile: Favorable. Injection site reactions (erythema, pruritus, pain) at subcutaneous injection sites are the principal adverse events. No serious drug-related adverse events in published trials [17, 18].

Overall validation: High. The most extensively validated compound in the mitochondria-targeted peptide space, with regulatory approval, multiple Phase 2 and Phase 3 clinical trials, and a large preclinical evidence base.

Comparison to SS-20: SS-31 is the direct structural parent of SS-20 and shares the cardiolipin-binding mechanism. SS-31 additionally provides direct radical scavenging, which produces broader mitochondrial proteomic protection in the TAC heart failure model (84 percent vs. partial protection with SS-20) [10]. However, SS-20 achieved equivalent neuroprotection in the MPTP model [7] and superior infarct size reduction in one cardiac ischemia-reperfusion comparison [9], suggesting that the radical-scavenging activity of SS-31 is not universally required for efficacy. SS-20's advantage is its clean mechanistic profile: by demonstrating efficacy without radical scavenging, SS-20 establishes that cardiolipin modulation alone is sufficient for therapeutic benefit and may identify disease contexts where preserving reactive oxygen species signaling is desirable.

12.2 MitoQ (Mitoquinone mesylate)

Mechanism: Ubiquinone moiety conjugated to a triphenylphosphonium (TPP⁺) cation via a ten-carbon alkyl linker. Accumulates in the mitochondrial matrix driven by the membrane potential (approximately 100 to 500-fold accumulation) and cycles between the reduced ubiquinol form (which scavenges superoxide and lipid peroxy radicals) and the oxidized ubiquinone form (regenerated by Complex II) [19].

Novelty: Moderate. The TPP⁺-conjugated antioxidant approach was pioneered by Michael Murphy and Robin Smith in the late 1990s. MitoQ was the first mitochondria-targeted antioxidant to enter clinical trials and remains the most widely studied in the class.

Effect size: Variable across indications. In a Phase 2 trial for Parkinson's disease (PROTECT study), MitoQ at 40 and 80 mg daily for 12 months showed no benefit on the Unified Parkinson's Disease Rating Scale [20]. In a Phase 2 trial for hepatitis C, MitoQ reduced plasma alanine aminotransferase levels [21]. Preclinical effect sizes are substantial in oxidative stress models.

Promising potential: Moderate. Available as a dietary supplement (MitoQ brand) without regulatory approval as a drug. The Parkinson's disease trial failure reduced clinical development momentum. Current research interest focuses on cardiovascular aging, liver disease, and metabolic syndrome.

Side-effect profile: Well tolerated in clinical trials at doses up to 80 mg daily for 12 months. Mild gastrointestinal symptoms reported. Theoretical concern for pro-oxidant activity at high concentrations in the mitochondrial matrix (TPP⁺ accumulation can dissipate membrane potential at high concentrations) [19].

Overall validation: Moderate. Extensive preclinical literature. One negative Phase 2 in Parkinson's disease. Available as a supplement.

Comparison to SS-20: MitoQ and SS-20 represent fundamentally different mitochondria-targeting strategies. MitoQ accumulates in the mitochondrial matrix and scavenges radicals directly; SS-20 resides on the inner membrane and modulates cardiolipin-cytochrome c interactions without radical scavenging. The two mechanisms are independent and potentially complementary. MitoQ has the advantage of oral bioavailability, commercial availability as a supplement, and more extensive human safety data. SS-20 has the advantage of a novel cardiolipin-based mechanism, absence of the membrane-potential-dissipating effects of TPP⁺ cations at high concentrations, and a more targeted site of action (inner membrane versus matrix).

12.3 SkQ1 (Visomitin, plastoquinonyl-decyltriphenylphosphonium bromide)

Mechanism: Plastoquinone moiety conjugated to TPP⁺ via a ten-carbon alkyl linker. Similar to MitoQ in mitochondrial matrix accumulation and radical-scavenging mechanism, but the plastoquinone head group has different redox properties and may be a more efficient scavenger at lower concentrations. Developed by Vladimir Skulachev at Moscow State University [22].

Novelty: Low to moderate. The concept is parallel to MitoQ with a different quinone head group.

Effect size: In ophthalmology, SkQ1 (Visomitin) achieved regulatory approval in Russia for the treatment of dry eye syndrome, with modest but significant improvement in corneal fluorescein staining scores in Phase 3 trials [22]. Preclinical effect sizes are substantial in aging models (extension of maximum lifespan in some species, reversal of age-related macular degeneration phenotype in animal models).

Promising potential: Moderate. Approved in Russia as an ophthalmic solution. Active preclinical development for systemic aging indications. Limited clinical development outside Russia.

Side-effect profile: Topical ophthalmic formulation is well tolerated. Systemic safety data are limited.

Overall validation: Low to moderate outside Russia. The regulatory approval for dry eye provides some validation, but the approval is in a single jurisdiction with limited international regulatory harmonization.

Comparison to SS-20: SkQ1 and SS-20 target mitochondria through different mechanisms (matrix accumulation via membrane potential versus inner membrane cardiolipin binding). SkQ1 has the advantage of regulatory approval (in Russia) and oral/topical bioavailability. SS-20 has the advantage of a more targeted mechanism (cardiolipin versus matrix antioxidant) and absence of TPP+-mediated membrane potential effects.

12.4 SBT-272

Mechanism: Next-generation Szeto-Schiller peptide with enhanced brain penetration, developed by Stealth BioTherapeutics for neurodegenerative disease indications. The compound retains the mitochondria-targeted tetrapeptide scaffold with modifications to improve central nervous system bioavailability [23]. Binds cardiolipin on the inner mitochondrial membrane, similar to SS-20 and SS-31.

Novelty: Moderate to high. SBT-272 represents the next-generation chemistry of the SS peptide class, optimized for CNS indications.

Effect size: Preclinical only. SBT-272 has shown efficacy in ALS and Parkinson's disease animal models at doses lower than SS-31, attributed to the improved CNS penetration [23].

Promising potential: Moderate. Active preclinical development by Stealth BioTherapeutics. No clinical trials initiated as of the most recent monograph revision.

Side-effect profile: Preclinical only. No human safety data.

Overall validation: Low. Preclinical research compound with no clinical data.

Comparison to SS-20: SBT-272 and SS-20 share the cardiolipin-binding mechanism. SBT-272 is optimized for CNS penetration and neurodegenerative disease indications, potentially offering better brain exposure than SS-20. SS-20 has a more extensive preclinical literature across multiple organ systems (heart, kidney, brain) and serves the additional role of mechanistic control compound for the SS peptide class.

12.5 Coenzyme Q10 (Ubiquinone-10, CoQ10)

Mechanism: Endogenous mitochondrial electron carrier in the inner mitochondrial membrane, shuttling electrons from Complexes I and II to Complex III. As a dietary supplement or pharmaceutical, exogenous CoQ10 is intended to replenish depleted endogenous pools and to serve as a lipid-soluble antioxidant in cellular membranes [24].

Novelty: Low. CoQ10 is a well-established dietary supplement with decades of clinical use.

Effect size: Variable and generally modest in clinical trials. Meta-analyses in heart failure suggest modest improvement in ejection fraction and functional class with high-dose CoQ10 (300 to 600 mg daily). The Q-SYMBIO trial (2014) reported significant reduction in major adverse cardiac events with CoQ10 300 mg daily for 2 years in chronic heart failure [25]. Effect sizes in mitochondrial disease, Parkinson's disease, and statin myopathy have been inconsistent.

Promising potential: Low to moderate as a therapeutic. Widely available as a supplement. No novel regulatory development.

Side-effect profile: Excellent. CoQ10 is well tolerated at doses up to 1200 mg daily in clinical trials. Mild gastrointestinal effects reported.

Overall validation: Moderate. Extensive clinical literature. One positive Phase 3 trial in heart failure (Q-SYMBIO). Generally regarded as safe but with inconsistent clinical efficacy across indications.

Comparison to SS-20: CoQ10 and SS-20 target different aspects of mitochondrial function. CoQ10 serves as a direct electron carrier and lipid-phase antioxidant; SS-20 modulates the cardiolipin-cytochrome c interaction to optimize existing electron transport chain function. CoQ10 has the advantages of oral bioavailability, extensive human safety data, and commercial availability. SS-20 has the advantages of a more targeted and novel mechanism, higher potency (micromolar versus millimolar therapeutic concentrations), and demonstration of efficacy in models where simple antioxidant supplementation has failed.

12.6 Comparator summary

The five comparators occupy distinct mechanistic positions in the mitochondria-targeted therapeutic space. **SS-31** is the direct structural parent of SS-20, shares the cardiolipin-binding mechanism, and is the only compound in the panel with regulatory approval; it represents the clinical validation of the mechanism that SS-20 shares. **MitoQ** and **SkQ1** represent the matrix-accumulated antioxidant approach using TPP⁺ conjugation; both produce direct radical scavenging in the mitochondrial matrix but do not modulate cardiolipin-cytochrome c interactions. **SBT-272** is the next-generation SS peptide optimized for CNS penetration, sharing the cardiolipin mechanism with modified pharmacokinetics. **CoQ10** is the endogenous reference compound, widely available but with lower potency and inconsistent clinical efficacy.

SS-20 occupies a unique position: it is the only compound in the panel that targets cardiolipin without any direct antioxidant activity, making it the cleanest probe for the cardiolipin-modulation mechanism and a candidate for therapeutic contexts where preserving physiological reactive oxygen species signaling is important. Its preclinical efficacy comparable to SS-31 in several models, despite lacking radical-scavenging capacity, demonstrates that cardiolipin modulation alone is sufficient for meaningful mitochondrial protection. The principal limitation is the absence of clinical data, formal toxicology, and commercial development, which places it behind SS-31, MitoQ, and CoQ10 in translational maturity.

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