

RESEARCH MONOGRAPH . COMPOUND PROFILE

# Polygalasaponin F

*An oleanane triterpenoid saponin isolated from Polygala japonica Houtt. with demonstrated neuroprotection against ischemic and excitotoxic injury, long-term potentiation enhancement via NMDA receptor activation, and anti-neuroinflammatory activity through TLR4-PI3K/AKT-NF- $\kappa$ B and TXNIP/NLRP3 pathway modulation.*

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## ABSTRACT

Polygalasaponin F (PGSF) is an oleanane-type triterpenoid saponin originally isolated from the aerial parts of *Polygala japonica* Houtt., a perennial herb of the Polygalaceae family used in traditional East Asian medicine for its sedative, expectorant, and cognition-enhancing properties. The compound has a molecular formula of C<sub>53</sub>H<sub>86</sub>O<sub>23</sub> (molecular weight 1091.2 g/mol) and bears a presqualene-derived oleanane aglycone substituted with a 3-O-beta-D-glucopyranosyl unit and a C-28 ester-linked trisaccharide chain composed of glucopyranose, rhamnopyranose, and xylopyranose residues. PGSF has emerged as a compound of substantial preclinical research interest across multiple neuroprotective and anti-inflammatory domains, supported by a growing body of in vitro and in vivo evidence published principally between 2012 and 2025.

The pharmacological profile of PGSF is characterized by multi-target activity converging on neuronal survival, synaptic plasticity, and neuroinflammatory suppression. In adult rat hippocampal slices, PGSF at 1 to 10 micromolar induces sustained long-term potentiation (LTP) in the dentate gyrus through activation of N-methyl-D-aspartate receptors (NMDARs), with downstream phosphorylation of NR2B, calcium/calmodulin-dependent protein kinase II (CaMKII), extracellular signal-regulated kinase (ERK), and cyclic AMP response element-binding protein (CREB), placing it among a small number of natural saponins with direct electrophysiological evidence of synaptic strengthening. In cultured hippocampal neurons exposed to glutamate excitotoxicity, PGSF produces concentration-dependent

neuroprotection by attenuating cytosolic calcium overload and modulating NMDAR subunit expression, specifically preserving NR2A while limiting excess NR2B-mediated calcium influx. In oxygen-glucose deprivation and reoxygenation (OGD/R) models of ischemic injury using PC12 cells and primary cortical neurons, PGSF activates the PI3K/Akt survival signaling pathway, upregulates the Bcl-2/Bax ratio, and suppresses caspase-3 activation to inhibit apoptosis.

The anti-inflammatory pharmacology of PGSF operates through at least two characterized signaling cascades. In lipopolysaccharide-stimulated BV-2 microglial cells, PGSF suppresses tumor necrosis factor alpha (TNF-alpha) release through inhibition of the toll-like receptor 4 (TLR4) to phosphoinositide 3-kinase (PI3K) to protein kinase B (AKT) to nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kB) signaling axis. In middle cerebral artery occlusion (MCAO) rat models of focal ischemia and reperfusion, PGSF ameliorates neurological deficit, reduces infarct volume, and inhibits the thioredoxin-interacting protein (TXNIP) to NOD-like receptor pyrin domain-containing 3 (NLRP3) inflammasome signaling pathway. More recent studies (2024 to 2025) have identified additional mechanistic targets: PGSF downregulates the Na-K-2Cl cotransporter 1 (NKCC1) through enhanced DNA methylation, thereby reducing blood-brain barrier disruption and cerebral edema following ischemia-reperfusion, and PGSF alleviates cerebral ischemia-reperfusion injury through inhibition of excessive mitophagy, preserving mitochondrial membrane potential and reducing mitochondrial reactive oxygen species accumulation.

No clinical trials of PGSF in humans have been conducted. The compound remains in the preclinical investigational phase, with the entirety of the evidence base derived from in vitro cell culture systems and rodent models of cerebral ischemia, glutamate excitotoxicity, and neuroinflammation. Pharmacokinetic data specific to PGSF in any species are not available in published form; general considerations for triterpenoid saponin bioavailability (gastrointestinal hydrolysis of glycosidic bonds, limited oral absorption of intact saponin, hepatic first-pass metabolism) apply and represent a translational barrier that has not been formally addressed. The compound is available from multiple research chemical suppliers at greater than 95 percent purity for in vitro and in vivo research applications. This monograph reviews the chemistry, isolation, and structural characterization of PGSF; the multi-target molecular pharmacology across NMDA receptor, PI3K/Akt, TLR4/NF- $\kappa$ B, TXNIP/NLRP3, NKCC1, and mitophagy pathways; the preclinical evidence base in ischemic stroke, glutamate excitotoxicity, and neuroinflammation models; sourcing and quality verification considerations; reconstitution and handling; stack-interaction implications; adverse events and safety signals from animal studies; and a comparative assessment of five neuroprotective Polygala-derived saponins against PGSF on five competency standards.

# 1. Compound Identification

## 1.1 Names, synonyms, and registry identifiers

**Common name:** Polygalasaponin F.

**Abbreviation:** PGSF (used throughout the preclinical pharmacology literature).

**Chemical Abstracts Service registry number:** 882664-74-6.

**International Union of Pure and Applied Chemistry name:** (2beta,3beta,4alpha)-3-(beta-D-glucopyranosyloxy)-2,23-dihydroxy-olean-12-en-28-oic acid O-beta-D-xylopyranosyl-(1->4)-O-6-deoxy-alpha-L-mannopyranosyl-(1->2)-beta-D-glucopyranosyl ester.

**Source organism:** *Polygala japonica* Houtt. (Polygalaceae). The compound was originally isolated from the aerial parts of the plant and has subsequently been identified in root material of related *Polygala* species.

**Development codes:** None. The compound has not entered formal pharmaceutical development and carries no sponsor-assigned development code.

## 1.2 Structural class and chemistry

Polygalasaponin F is an oleanane-type triterpenoid saponin. The structural class is defined by the pentacyclic oleanane aglycone (derived biosynthetically from oxidosqualene cyclization through beta-amyrin), which serves as the core scaffold for a large family of plant secondary metabolites distributed across the Polygalaceae, Leguminosae, Caryophyllaceae, and other angiosperm families [1, 2].

**Molecular formula:** C<sub>53</sub>H<sub>86</sub>O<sub>23</sub>.

**Molecular weight:** 1091.2 g/mol.

**Physical properties:** White to off-white amorphous powder. Soluble in methanol, ethanol, and dimethyl sulfoxide; sparingly soluble in water; poorly soluble in apolar organic solvents such as hexane and chloroform.

**Structural features:** The aglycone bears hydroxyl groups at C-2 and C-23 and a carboxylic acid at C-28. The C-3 hydroxyl is glycosylated with a single beta-D-glucopyranose unit (3-O-glucoside). The C-28 carboxylic acid is esterified with a linear trisaccharide chain consisting of beta-D-glucopyranose linked to 6-deoxy-alpha-L-mannopyranose (rhamnose) at the 2-position and beta-D-xylopyranose at the 4-position of the rhamnose. This bidesmosidic substitution pattern (glycosylation at both C-3 and C-28) is characteristic of the oleanane saponins from *Polygala* species and distinguishes PGSF from monodesmosidic congeners such as senegenin and tenuifolin, which bear aglycone modifications without ester-linked sugar chains [2, 3].

**Stereochemistry:** The aglycone possesses the (2beta,3beta,4alpha) configuration at the A-ring stereocenters, consistent with the natural oleanane series. The sugar residues retain their natural D- or L-configurations. Stereochemical assignment was established by the original isolation group through a combination of nuclear magnetic resonance spectroscopy (<sup>1</sup>H-NMR, <sup>13</sup>C-NMR, DEPT, COSY, HMQC, HMBC, NOESY), high-resolution mass spectrometry, and acid hydrolysis with sugar identification by comparison to authentic standards [1].

## 1.3 Isolation and characterization

Polygalasaponin F was first reported as one of five new triterpenoid saponins (polygalasaponins E, F, G, H, and J) isolated from the aerial parts of *Polygala japonica* Houtt. collected in China [1]. The isolation procedure involved methanol extraction of the dried plant material, partition between water and n-butanol, and sequential chromatographic purification by silica gel column chromatography, octadecyl silica (ODS) column chromatography, and preparative high-performance liquid chromatography (HPLC). Structure elucidation was accomplished by spectroscopic analysis and confirmed by acid hydrolysis with identification of the component sugars (glucose, rhamnose, xylose) by thin-layer chromatography comparison to standards and by optical rotation measurement.

Subsequent isolation studies have confirmed the presence of PGSF in root material of *Polygala japonica* and have extended the phytochemical profile of the species to include approximately 86 characterized secondary metabolites, with triterpenoid saponins constituting the largest and most pharmacologically active chemical class [4, 5]. PGSF has also been reported in *Polygala tenuifolia* Willd. (the

source of the traditional Chinese medicine Yuan Zhi), although the predominant saponins of *P. tenuifolia* are polygalasaponin XXXII, onjisaponin B, and tenuifolin rather than PGSF.

## 2. Abstract

Polygalasaponin F (PGSF; CAS 882664-74-6) is an oleanane-type triterpenoid saponin isolated from *Polygala japonica* Houtt. with a molecular weight of 1091.2 g/mol and a bidesmosidic glycosylation pattern comprising a 3-O-glucoside and a C-28 ester-linked trisaccharide. The compound has been the subject of a growing pre-clinical research literature that characterizes multi-target neuroprotective, cognition-enhancing, and anti-neuroinflammatory activities converging on neuronal survival and synaptic plasticity.

The cognition-relevant pharmacology of PGSF centers on the induction of long-term potentiation (LTP) in the adult rat hippocampal dentate gyrus at 1 to 10 micromolar concentrations through NMDA receptor activation, with demonstrated downstream engagement of CaMKII, ERK, and CREB phosphorylation cascades [6]. This electrophysiological demonstration places PGSF among a limited number of plant-derived saponins with direct evidence of synaptic strengthening in intact hippocampal circuits and provides a mechanistic basis for the cognition-improving properties historically attributed to *Polygala* preparations in traditional East Asian pharmacopoeias.

The neuroprotective pharmacology of PGSF spans three principal domains. First, against glutamate excitotoxicity in cultured hippocampal neurons, PGSF produces concentration-dependent cytoprotection by attenuating calcium overload and modulating NMDA receptor subunit expression [7]. Second, against ischemic injury modeled by oxygen-glucose deprivation and reoxygenation (OGD/R), PGSF activates the PI3K/Akt survival pathway, increases the Bcl-2/Bax ratio, and inhibits caspase-3 activation [8]. Third, in the middle cerebral artery occlusion (MCAO) rat model of focal cerebral ischemia-reperfusion, PGSF reduces neurological deficit scores and infarct volumes through inhibition of the TXNIP/NLRP3 inflammasome pathway [9] and through downregulation of NKCC1 via enhanced DNA methylation, reducing blood-brain barrier disruption and cerebral edema [10].

The anti-inflammatory pharmacology is mediated through inhibition of the TLR4-PI3K/AKT-NF- $\kappa$ B signaling axis in microglial cells, suppressing TNF- $\alpha$  and other proinflammatory cytokine release [11]. A 2025 study has additionally demon-

strated that PGSF alleviates cerebral ischemia-reperfusion injury through inhibition of excessive mitophagy, preserving mitochondrial membrane potential and reducing mitochondrial reactive oxygen species [12].

No clinical trials of PGSF in humans have been reported. The compound has not entered formal pharmaceutical development. The entire evidence base is preclinical, derived from in vitro cell culture systems (PC12 cells, primary cortical and hippocampal neurons, BV-2 microglial cells, hippocampal slice preparations) and rodent models (MCAO in rats). Pharmacokinetic parameters specific to PGSF have not been published; general considerations for oral triterpenoid saponin bioavailability (gastrointestinal glycosidic hydrolysis, limited intestinal absorption of intact high-molecular-weight saponins, hepatic metabolism) apply as translational constraints. The compound is commercially available from research chemical suppliers at greater than 95 percent purity.

## 3. Discovery and Development History

### 3.1 Ethnobotanical context

*Polygala japonica* Houtt. is a perennial herbaceous plant of the family Polygalaceae, distributed throughout East and Southeast Asia including China, Japan, Korea, and Vietnam. The plant has been used in traditional Chinese medicine, Japanese Kampo medicine, and Korean traditional medicine as a sedative, expectorant, and cognition-enhancing agent. The closely related species *Polygala tenuifolia* Willd. is the source of Yuan Zhi (literally "Vast Will"), one of the 50 fundamental herbs of traditional Chinese medicine and the third most frequently used single herb in clinical cognitive enhancement prescriptions in Chinese medical practice [13, 14]. The traditional indications for *Polygala* species preparations include insomnia, anxiety, memory impairment, cough with excessive phlegm, and mental fatigue.

The pharmacological basis for these traditional applications has been attributed to the triterpenoid saponin content of the *Polygala* species, which constitutes the largest and most bioactive chemical class among the approximately 86 characterized secondary metabolites of *P. japonica*. The saponins share the oleanane backbone and vary in their glycosylation patterns, hydroxylation positions, and the presence or absence of acyl substituents [4, 5].

### 3.2 Isolation and initial characterization

Polygalasaponin F was first reported as a novel triterpenoid saponin in a phytochemical investigation of the aerial parts of *Polygala japonica*, published alongside polygalasaponins E, G, H, and J [1]. The isolation was motivated by the ethnopharmacological record of *Polygala* species and by the preceding identification of other bioactive saponins from the roots of *P. japonica*, including polygalasaponins XXVIII through XXXII [15]. The structural characterization established the bidesmosidic oleanane framework and placed PGSF within the growing inventory of *Polygala* triterpenoid saponins, which by 2020 numbered over 40 structurally characterized compounds across *P. japonica*, *P. tenuifolia*, *P. aureocauda*, and other species [2].

### 3.3 Pharmacological characterization

The first pharmacological study of PGSF as an individual compound (rather than as a component of a crude saponin fraction) was the 2012 report by Cao and colleagues demonstrating induction of long-term potentiation in the adult rat hippocampal dentate gyrus via NMDA receptor activation [6]. This study established PGSF as a compound of specific pharmacological interest distinct from the crude *Polygala* saponin fractions that had previously been studied for anxiolytic and sedative-hypnotic activities.

Subsequent pharmacological studies expanded the characterized mechanisms in a logical progression: the 2015 report on TLR4-PI3K/AKT-NF- $\kappa$ B-mediated TNF- $\alpha$  suppression in microglial cells [11]; the 2020 report on PI3K/Akt-mediated neuroprotection against OGD/R injury [8]; the 2021 report on glutamate excitotoxicity protection via NMDAR modulation [7]; the 2023 report on TXNIP/NLRP3 inflammasome inhibition in the MCAO stroke model [9]; the 2024 report on NKCC1 targeting and blood-brain barrier protection [10]; and the 2025 report on mitophagy inhibition in cerebral ischemia-reperfusion [12].

### 3.4 Current development status

PGSF has not entered formal pharmaceutical development. No investigational new drug application has been filed. No clinical trials have been conducted or registered. The compound remains in the preclinical investigational phase, with active research conducted principally by Chinese academic laboratories. The trajectory is consistent with early-stage natural product pharmacology in which a structurally characterized plant metabolite has demonstrated multi-target activity in cell-based and animal models but has not yet been advanced through formal preclinical safety pharmacology, toxicology, or pharmacokinetic optimization required for clinical translation.

## 4. Mechanism of Action

Polygalasaponin F exhibits multi-target pharmacological activity affecting neuronal survival, synaptic plasticity, and neuroinflammatory signaling. The characterized mechanisms converge on the central nervous system and operate at functionally relevant concentrations in the low-micromolar range demonstrated in vitro and at systemic doses of 10 to 40 mg/kg in rodent models in vivo.

### 4.1 NMDA receptor modulation and long-term potentiation

The cognition-relevant mechanism of PGSF was characterized by Cao et al. (2012) using in vivo electrophysiological recording in adult rat hippocampal dentate gyrus [6]. Intracerebroventricular administration of PGSF at 1 and 10 micromolar produced a long-lasting, dose-dependent increase in population spike (PS) amplitude, the electrophysiological hallmark of long-term potentiation. The effect was completely abolished by pre-injection of the NMDA receptor antagonist MK-801 (dizocilpine), confirming that PGSF-induced LTP requires functional NMDA receptor activation. Pre-injection of the CaMKII inhibitor KN93 similarly abolished the LTP, establishing the dependence on calcium/calmodulin-dependent protein kinase II.

Biochemical analysis demonstrated that phosphorylation of NMDA receptor subunit NR2B, CaMKII, ERK1/2, and CREB in hippocampal tissue was significantly elevated 5 to 60 minutes after LTP induction by PGSF [6]. The temporal pattern of kinase activation (NR2B phosphorylation peaking at 5 minutes, CaMKII at 5 to 15 minutes, ERK at 15 to 30 minutes, CREB at 30 to 60 minutes) is consistent with the canonical NMDAR-CaMKII-ERK-CREB signaling cascade that underlies activity-dependent synaptic strengthening and long-term memory formation.

The mechanism distinguishes PGSF from NMDA receptor antagonists (such as memantine) and from acetylcholinesterase inhibitors (such as donepezil) by its capacity to directly enhance, rather than merely protect, synaptic plasticity. The functional implication is that PGSF may operate as a cognitive enhancer through positive modulation of glutamatergic transmission in the hippocampus, in addition to its neuroprotective properties.

## 4.2 Neuroprotection against glutamate excitotoxicity

In cultured primary rat hippocampal neurons, PGSF produces concentration-dependent protection against glutamate-induced cytotoxicity [7]. The neuroprotective mechanism involves attenuation of glutamate-induced cytosolic calcium overload, the proximate cause of excitotoxic neuronal death. PGSF partially blocks excess NMDA receptor activity without eliminating physiological NMDA receptor function, a selectivity profile that is pharmacologically consistent with the dual observation that PGSF can both enhance LTP (at physiological NMDAR activation levels) and protect against excitotoxicity (at pathological NMDAR overactivation levels).

At the molecular level, PGSF inhibits the glutamate-induced downregulation of NMDA receptor subunit NR2A and modulates the NR2A/NR2B subunit ratio in favor of NR2A [7]. NR2A-containing NMDA receptors are associated with pro-survival signaling (CREB phosphorylation, brain-derived neurotrophic factor expression), whereas NR2B-containing receptors are associated with excitotoxic cell death signaling (activation of neuronal nitric oxide synthase, p38 MAPK). The PGSF-mediated shift in subunit composition toward NR2A therefore represents a mechanistically coherent basis for both the neuroprotective and the LTP-enhancing activities.

## 4.3 PI3K/Akt survival signaling pathway

In oxygen-glucose deprivation and reoxygenation (OGD/R) models of ischemic neuronal injury, PGSF activates the phosphoinositide 3-kinase (PI3K)/protein kinase B (Akt) survival signaling pathway [8]. OGD/R treatment of PC12 cells and primary rat cortical neurons reduced cell viability, decreased the Bcl-2/Bax protein ratio, and elevated caspase-3 levels. PGSF treatment prevented the viability reduction, upregulated Bcl-2/Bax, downregulated caspase-3, and increased the ratio of phosphorylated Akt to total Akt. The PI3K inhibitor LY294002 blocked the PGSF-mediated neuroprotection, confirming the pathway dependence [8].

The PI3K/Akt pathway is a canonical pro-survival signaling cascade that phosphorylates and inactivates pro-apoptotic substrates (Bad, caspase-9, glycogen synthase kinase 3-beta) and activates anti-apoptotic transcription (NF-kB pro-survival gene expression, mTOR-mediated translation). The PGSF-mediated activation of this pathway in the ischemic context is mechanistically complementary to the NMDAR-dependent LTP enhancement in the synaptic plasticity context, as both converge on neuronal health and function.

## 4.4 TLR4-PI3K/AKT-NF- $\kappa$ B anti-inflammatory signaling

In lipopolysaccharide (LPS)-stimulated BV-2 microglial cells, PGSF suppresses the release of tumor necrosis factor alpha (TNF-alpha) and other proinflammatory cytokines through inhibition of the toll-like receptor 4 (TLR4) signaling cascade [11]. The mechanism involves suppression of AKT phosphorylation and inhibition of NF- $\kappa$ B nuclear translocation, the transcriptional endpoint that drives expression of TNF-alpha, interleukin-1 beta (IL-1beta), interleukin-6 (IL-6), and other inflammatory mediators.

Pharmacological validation using specific inhibitors of TLR4, PI3K, and I $\kappa$ B-alpha phosphorylation demonstrated that each node of the TLR4-PI3K/AKT-NF- $\kappa$ B cascade is required for LPS-induced TNF-alpha release, and that PGSF reproduces the inhibitory profile of these pharmacological tools on TNF-alpha release, AKT phosphorylation, and NF- $\kappa$ B translocation [11]. The anti-inflammatory mechanism operates in microglial cells, the resident immune cells of the central nervous system, positioning PGSF as an anti-neuroinflammatory agent relevant to the inflammatory component of ischemic stroke, neurodegenerative disease, and chronic neuroinflammatory conditions.

## 4.5 TXNIP/NLRP3 inflammasome inhibition

In the middle cerebral artery occlusion (MCAO) rat model of focal cerebral ischemia-reperfusion injury, PGSF ameliorates neurological deficit and reduces infarct volume through inhibition of the thioredoxin-interacting protein (TXNIP)/NOD-like receptor pyrin domain-containing 3 (NLRP3) inflammasome signaling pathway [9]. The TXNIP/NLRP3 axis is a redox-sensitive inflammatory amplification cascade: ischemia-reperfusion generates reactive oxygen species (ROS) that dissociate TXNIP from thioredoxin, freeing TXNIP to activate the NLRP3 inflammasome, which in turn cleaves pro-interleukin-1 beta and pro-interleukin-18 into their mature, secreted inflammatory forms and activates gasdermin D-mediated pyroptotic cell death.

PGSF inhibits both the TXNIP upregulation and the NLRP3 inflammasome assembly in the ischemic penumbra, reducing inflammatory cytokine production and pyroptotic neuronal death [9]. The mechanism was validated in vitro using OGD/R-induced neuronal injury models, confirming that PGSF increases damaged neuron survival, reduces apoptosis and ROS production, and inhibits the TXNIP/NLRP3 pathway at the cellular level.

## 4.6 NKCC1 downregulation and blood-brain barrier protection

Elevated expression of the Na-K-2Cl cotransporter 1 (NKCC1) following cerebral ischemia-reperfusion disrupts the blood-brain barrier (BBB) and produces vasogenic cerebral edema. PGSF downregulates NKCC1 expression through enhancement of DNA methylation at the NKCC1 promoter region, a novel epigenetic mechanism for neuroprotection [10]. The reduction in NKCC1-mediated chloride and water influx preserves BBB integrity and reduces cerebral edema in the MCAO model. This epigenetic mechanism is distinct from the kinase-mediated signaling pathways described above and represents an additional pharmacological dimension of PGSF activity.

## 4.7 Mitophagy regulation

A 2025 study demonstrated that PGSF alleviates cerebral ischemia-reperfusion injury through inhibition of excessive mitophagy [12]. Cerebral ischemia-reperfusion produces oxidative stress that disrupts mitochondrial function; the resulting mitophagy response can be either protective (removal of damaged mitochondria) or deleterious (excessive clearance of functional mitochondria). PGSF modulates this balance by inhibiting excessive mitophagy, preserving mitochondrial membrane potential, and reducing mitochondrial ROS accumulation. The PGSF-treated animals showed attenuated apoptosis both in vivo and in vitro, with preserved mitochondrial integrity as the proposed upstream mechanism [12].

## 4.8 Composite pharmacology

The composite pharmacology of PGSF is multi-target and convergent on neuronal survival and function. The principal targets (NMDA receptor modulation, PI3K/Akt activation, TLR4/NF- $\kappa$ B inhibition, TXNIP/NLRP3 suppression, NKCC1 downregulation, and mitophagy regulation) operate through mechanistically independent pathways but produce a coherent phenotypic outcome: protection of neurons against ischemic, excitotoxic, and inflammatory injury with simultaneous enhancement of synaptic plasticity. This multi-target profile is characteristic of complex natural product pharmacology and is both a strength (pharmacological resilience against single-pathway escape) and a translational challenge (difficulty in identifying a single registerable mechanism for clinical development).

## 5. Pharmacokinetics

### 5.1 General considerations

Pharmacokinetic parameters specific to Polygalasaponin F have not been published in any species. The following considerations are derived from the general pharmacokinetic literature on triterpenoid saponins, including structurally related compounds from Polygala and other plant genera, and from the in vivo dosing protocols used in the PGSF pharmacology studies.

### 5.2 Absorption

Triterpenoid saponins as a class exhibit limited oral bioavailability, typically in the range of 1 to 10 percent for high-molecular-weight bidesmosidic glycosides [16]. The primary barriers to oral absorption are the large molecular weight (PGSF at 1091.2 g/mol substantially exceeds the conventional 500 g/mol threshold for oral drug candidates), the high polarity and hydrogen bond donor/acceptor count imposed by the sugar residues, and the susceptibility of glycosidic bonds to hydrolysis by intestinal beta-glucosidases and gut microbiota glycosidases. Acid hydrolysis in the stomach and enzymatic hydrolysis in the small intestine can cleave the sugar chains to release the aglycone (presenegenin or a related oleanane acid), which has improved membrane permeability but different pharmacological properties than the intact saponin.

The published in vivo PGSF studies have used intraperitoneal injection (10 to 40 mg/kg in rats) or intracerebroventricular injection (1 to 10 micromolar) rather than oral gavage, consistent with the anticipated low oral bioavailability and the investigator decision to bypass the gastrointestinal barrier for mechanistic pharmacology studies.

### 5.3 Distribution

No tissue distribution data specific to PGSF are available. For structurally related Polygala saponins, limited central nervous system penetration has been inferred from the observation that systemic (intraperitoneal) administration produces cen-

tral pharmacological effects, although the contribution of the intact saponin versus hydrolysis products to central activity has not been formally resolved. The high molecular weight and polarity of PGSF predict limited passive diffusion across the blood-brain barrier; however, the NKCC1-mediated BBB-protective activity of PGSF [10] implies that at least some fraction of the administered compound reaches the cerebrovascular endothelium.

## 5.4 Metabolism

Triterpenoid saponins undergo extensive metabolism, principally through sequential deglycosylation by intestinal and hepatic glycosidases, followed by phase I oxidation (cytochrome P450-mediated hydroxylation of the oleanane ring system) and phase II conjugation (glucuronidation, sulfation) of the aglycone and its metabolites [16]. The specific CYP isoforms responsible for PGSF metabolism have not been identified. The metabolic fate of the trisaccharide ester chain at C-28 (which is susceptible to esterase-mediated hydrolysis in addition to glycosidase activity) has not been characterized.

## 5.5 Elimination

No elimination data specific to PGSF are available. For triterpenoid saponins generally, biliary excretion of conjugated aglycone metabolites and renal excretion of polar conjugates are the principal elimination pathways.

## 5.6 Routes of administration in research

Three routes have been employed in published PGSF studies: intracerebroventricular injection for electrophysiological studies (1 to 10 micromolar in artificial cerebrospinal fluid), intraperitoneal injection for in vivo neuroprotection studies (10 to 40 mg/kg in rats), and direct addition to cell culture medium for in vitro studies (1 to 100 micromolar). No oral dosing studies with pharmacokinetic endpoints have been published.

## 5.7 Translational implications

The absence of pharmacokinetic data represents a significant gap in the translational readiness of PGSF. Investigators planning in vivo studies should consider pharmacokinetic characterization as a priority, including oral and parenteral

bioavailability determination, plasma protein binding, tissue distribution (with emphasis on brain penetration), metabolite identification, and elimination kinetics. The development of prodrug or nanoparticle delivery strategies to improve oral bioavailability may be necessary for clinical translation, consistent with the broader challenge facing high-molecular-weight saponin drug candidates.

## 6. Preclinical Pharmacology

### 6.1 Long-term potentiation enhancement

Cao et al. (2012) demonstrated that intracerebroventricular administration of PGSF (1 and 10 micromolar) in adult rats produced a sustained increase in population spike amplitude in the hippocampal dentate gyrus, with the effect lasting throughout the 60-minute recording period [6]. The LTP was dose-dependent, with 10 micromolar producing approximately twice the PS amplitude increase observed at 1 micromolar. Pre-injection of MK-801 (0.1 mg/kg intraperitoneal) completely blocked the LTP induction, confirming NMDA receptor dependence. Pre-injection of KN93 (10 micromolar intracerebroventricular) similarly abolished the response, confirming CaMKII dependence. Western blot analysis of hippocampal tissue harvested at 5, 15, 30, and 60 minutes after LTP induction demonstrated time-dependent increases in phosphorylation of NR2B (Tyr1472), CaMKII (Thr286), ERK1/2 (Thr202/Tyr204), and CREB (Ser133) [6].

### 6.2 Glutamate excitotoxicity protection

PGSF pretreatment of primary rat hippocampal neurons exposed to glutamate (100 micromolar, 24 hours) produced concentration-dependent neuroprotection as measured by cell viability assays [7]. PGSF at 10 micromolar reduced glutamate-induced cell death by approximately 40 percent. Calcium imaging demonstrated that PGSF attenuated the glutamate-induced rise in intracellular calcium concentration. Immunoblotting showed that PGSF preserved NR2A subunit expression and modulated the NR2A/NR2B ratio, shifting the balance from the excitotoxic NR2B-dominant state toward the pro-survival NR2A-dominant state. These findings established that PGSF modulates NMDA receptor pharmacology bidirectionally: enhancing physiological NMDA receptor function (LTP) while attenuating pathological overactivation (excitotoxicity) [7].

## 6.3 Oxygen-glucose deprivation and reoxygenation injury

Xie et al. (2020) reported that PGSF inhibited neuronal apoptosis induced by OGD/R in PC12 cells and primary rat cortical neurons through the PI3K/Akt pathway [8]. OGD was achieved by incubation in glucose-free medium under nitrogen atmosphere for 2 to 4 hours, followed by reoxygenation in normal medium for 24 hours. PGSF treatment (applied during the reoxygenation phase) increased cell viability in a concentration-dependent manner, with significant effects observed at 5 to 20 micromolar. The anti-apoptotic mechanism was confirmed by upregulation of Bcl-2/Bax ratio, downregulation of cleaved caspase-3, and increased phosphorylation of Akt (Ser473). The PI3K inhibitor LY294002 (10 micromolar) abolished the PGSF-mediated neuroprotection, confirming pathway specificity [8].

## 6.4 Middle cerebral artery occlusion model

In a 2023 study, PGSF was administered to rats subjected to MCAO (90 minutes of occlusion followed by reperfusion) at doses of 10 to 40 mg/kg by intraperitoneal injection [9]. PGSF treatment reduced neurological deficit scores, decreased cerebral infarct volume (measured by TTC staining), and attenuated histological markers of neuronal injury in the ischemic penumbra. The molecular mechanism was characterized as inhibition of the TXNIP/NLRP3 inflammasome signaling pathway, with PGSF reducing TXNIP protein expression, NLRP3 inflammasome assembly, caspase-1 activation, and mature IL-1 $\beta$  and IL-18 release in the ischemic hemisphere. ROS levels were concurrently reduced, consistent with the upstream role of oxidative stress in TXNIP dissociation from thioredoxin [9].

## 6.5 Blood-brain barrier protection and NKCC1

A 2024 study identified NKCC1 as a therapeutic target of PGSF in cerebral ischemia-reperfusion injury [10]. NKCC1 is a sodium-potassium-chloride cotransporter expressed on cerebrovascular endothelial cells and astrocyte endfeet; its upregulation following ischemia drives chloride and water influx that disrupts BBB integrity and produces vasogenic edema. PGSF downregulated NKCC1 expression through enhancement of DNA methylation at the NKCC1 gene promoter. The epigenetic mechanism was validated by methylation-specific PCR and by the observation that the DNA methyltransferase inhibitor 5-azacytidine reversed the PGSF-mediated

NKCC1 suppression. The functional consequence was reduced Evans blue extravasation (a marker of BBB permeability) and reduced brain water content in MCAO rats treated with PGSF [10].

## 6.6 Mitophagy modulation

A 2025 study published in *Metabolic Brain Disease* investigated the role of mitophagy regulation in the neuroprotective activity of PGSF [12]. In both MCAO rats and OGD/R-treated neurons, cerebral ischemia-reperfusion produced excessive mitophagy characterized by increased PINK1/Parkin pathway activation and accelerated mitochondrial clearance. PGSF treatment inhibited the excessive mitophagy, preserved mitochondrial membrane potential (measured by JC-1 staining), reduced mitochondrial ROS (measured by MitoSOX), and attenuated apoptosis. The study proposed that PGSF achieves neuroprotection in part by maintaining the balance between protective and deleterious mitophagy, preventing the excessive mitochondrial clearance that can deplete the organelle pool below the threshold required for neuronal survival [12].

## 6.7 Anti-inflammatory activity in microglial cells

Xie et al. (2015) characterized the anti-inflammatory mechanism of PGSF in LPS-stimulated BV-2 microglial cells [11]. LPS (1 microgram/mL) treatment induced TNF- $\alpha$  release, AKT phosphorylation, and NF- $\kappa$ B p65 nuclear translocation. PGSF pretreatment (5 to 20 micromolar) suppressed all three endpoints in a concentration-dependent manner. Comparative experiments with specific inhibitors of TLR4 (CLI-095), PI3K (LY294002), and I $\kappa$ B- $\alpha$  phosphorylation (BAY 11-7082) demonstrated that PGSF reproduced the inhibitory profile of these pharmacological tools, confirming that the TLR4-PI3K/AKT-NF- $\kappa$ B axis is the primary anti-inflammatory signaling pathway modulated by PGSF [11].

## 6.8 Anxiolytic and sedative-hypnotic activities

Crude polygalasaponin fractions from *Polygala* species (including *P. japonica* and *P. tenuifolia*) have demonstrated anxiolytic and sedative-hypnotic activities in mice at oral doses of 40 to 160 mg/kg, with effects demonstrated in the elevated plus maze, open field, hole-board, and sodium pentobarbital-induced hypnosis tests [17, 18]. These activities have been attributed to the saponin components collectively rather than to PGSF specifically. The polygalasaponin fractions increased open arm time

in the elevated plus maze (anxiolytic effect), increased head-dip frequency in the hole-board test (exploratory behavior), prolonged pentobarbital-induced sleep duration, and shortened sleep latency. Behavioral pharmacology studies have also demonstrated dopamine and serotonin receptor antagonist properties of polygalasaponins in vivo, suggesting a neuroleptic-like pharmacological component [19]. The contribution of PGSF as an individual compound to these behavioral effects has not been isolated from the crude fraction data.

## 7. Clinical Evidence Base

No clinical trials of Polygalasaponin F in humans have been conducted or registered in any clinical trial registry (ClinicalTrials.gov, Chinese Clinical Trial Registry, EU Clinical Trials Register) as of the monograph preparation date. The compound has not been the subject of an investigational new drug application in any jurisdiction. No human pharmacokinetic, safety, tolerability, or efficacy data are available.

The clinical evidence for Polygala-derived compounds in cognitive and neurological applications is limited to crude Polygala extract preparations studied in traditional medicine contexts and to the related compound tenuifolin (the aglycone metabolite of several Polygala saponins), which has been the subject of small exploratory studies in cognitive impairment associated with aging. These studies are not directly applicable to PGSF as an individual chemical entity.

The absence of clinical data places PGSF at a Technology Readiness Level equivalent to late preclinical characterization. Clinical translation would require completion of formal preclinical safety pharmacology (cardiovascular, respiratory, central nervous system safety assessment), repeat-dose toxicology in two species, genotoxicity testing, pharmacokinetic characterization in multiple species with allometric scaling, and development of a formulation with adequate bioavailability for the intended route of administration.

## 8. Sourcing and Quality Verification

Polygalasaponin F is available from multiple research chemical suppliers for in vitro and in vivo research applications. Representative suppliers include Cayman Chemical (catalog number 30064), MedChemExpress, Selleck Chemicals, AdooQ Bioscience, PhytoLab (phyproof reference substance catalog number 83894), Sigma-Aldrich (catalog number PHL83894), and Biomol. Typical supply quantities range from 1 mg to 25 mg at prices ranging from approximately \$80 to \$300 per milligram depending on quantity and supplier.

**Purity standards:** Research-grade PGSF is supplied at greater than 95 percent purity by HPLC (area percent at 210 nm or 254 nm UV detection). PhytoLab supplies PGSF as a certified phyproof reference substance with a certificate of analysis documenting identity, purity, and water content, suitable for use as an analytical standard in phytochemical quality control.

**Identity confirmation:** Investigators should confirm compound identity on every lot through:

- High-performance liquid chromatography with UV detection, comparing retention time and UV spectrum to an authenticated reference standard or to published spectroscopic data
- High-resolution mass spectrometry confirming the molecular ion at  $m/z$  1091.5  $[M-H]^-$  (negative mode electrospray ionization) or  $m/z$  1093.6  $[M+H]^+$  (positive mode)
- Nuclear magnetic resonance spectroscopy ( $^1H$ -NMR and  $^{13}C$ -NMR in pyridine- $d_5$  or methanol- $d_4$ ) for complete structural confirmation when required for mechanistic pharmacology studies

**Purity verification:** HPLC area-percent purity determination at 210 nm is recommended. Purity below 95 percent may indicate the presence of structurally related saponin congeners (polygalasaponins E, G, H, J, or hydrolysis products) that could confound dose-response interpretation.

**Water content:** Karl Fischer titration or thermogravimetric analysis for residual water determination is recommended for precise dose preparation.

**Botanical source verification:** PGSF can also be obtained by direct isolation from *Polygala japonica* plant material, though this route is impractical for most pharmacological studies given the low yield and the chromatographic complexity of the crude saponin fraction.

## 9. Reconstitution and Handling

Polygalasaponin F is supplied as a white to off-white amorphous powder. The compound is hygroscopic and should be stored desiccated at -20 degrees Celsius under nitrogen or argon atmosphere for long-term storage. Short-term storage at 2 to 8 degrees Celsius in a sealed container with desiccant is acceptable for periods up to 4 weeks.

**Stock solution preparation for in vitro studies:** Dissolve PGSF in dimethyl sulfoxide (DMSO) at a concentration of 10 to 50 mM (approximately 11 to 55 mg/mL) to prepare a concentrated stock solution. DMSO stock solutions should be aliquoted into single-use volumes and stored at -20 degrees Celsius. Avoid repeated freeze-thaw cycles; stability data for PGSF in DMSO through multiple freeze-thaw cycles have not been published, and degradation of the labile ester-linked C-28 trisaccharide should be assumed as a risk.

**Working solution preparation for cell culture:** Dilute the DMSO stock solution into cell culture medium to achieve the desired final concentration (typically 1 to 100 micromolar for in vitro pharmacology studies). The final DMSO concentration should not exceed 0.1 percent (v/v) to avoid solvent-related cytotoxicity. PGSF is sparingly soluble in aqueous media at concentrations above approximately 100 micromolar; sonication or brief warming (37 degrees Celsius, 5 minutes) may improve dissolution.

**Working solution preparation for in vivo studies:** For intraperitoneal injection in rodents, PGSF may be dissolved in normal saline with up to 5 percent DMSO or in a saline-based vehicle containing 5 percent polyethylene glycol 400 and 5 percent Tween 80 to improve solubility. Filter-sterilize (0.22 micrometer) before injection.

**Intracerebroventricular preparation:** Dissolve PGSF in artificial cerebrospinal fluid at the desired concentration (1 to 10 micromolar). Filter-sterilize and use within 24 hours of preparation.

**Light sensitivity:** No data on photostability are available. Routine light protection (amber vials, foil wrapping) is recommended as a precautionary measure for saponin compounds with UV-absorbing chromophores.

**Compatibility:** PGSF is compatible with standard cell culture plasticware (polystyrene, polypropylene). Glass containers are preferred for stock solutions to minimize surface adsorption.

# 10. Stack Interactions and Combinations

The multi-target pharmacology of PGSF creates multiple potential interaction surfaces with other research compounds and pharmacological tools. The following considerations apply to in vitro and in vivo research applications.

**NMDA receptor modulators:** Concurrent administration of PGSF with NMDA receptor antagonists (MK-801, memantine, ketamine, AP5) will block the LTP-enhancing activity of PGSF, as demonstrated by the Cao et al. (2012) MK-801 blocking experiment [6]. Conversely, concurrent administration with NMDA receptor co-agonists (D-serine, glycine) or glycine site modulators may potentiate the PGSF-mediated LTP. NMDA receptor positive allosteric modulators (such as GNE-6901) have not been studied in combination with PGSF but represent a logical combination for synaptic plasticity research.

**PI3K/Akt pathway modulators:** Concurrent administration with PI3K inhibitors (LY294002, wortmannin) will block the anti-apoptotic activity of PGSF, as demonstrated in the Xie et al. (2020) study [8]. Concurrent administration with Akt activators (SC79) may produce additive neuroprotective effects. Investigators using PGSF as a neuroprotective agent should avoid concurrent use of PI3K or mTOR inhibitors (rapamycin, everolimus) that would counteract the survival signaling.

**Anti-inflammatory compounds:** Concurrent administration with other TLR4 antagonists (CLI-095, eritoran) or NF- $\kappa$ B inhibitors (BAY 11-7082, PDTC) may produce additive anti-inflammatory effects. The combination with dexamethasone or other glucocorticoids has not been characterized but is expected to produce additive suppression of microglial activation. The combination with non-steroidal anti-inflammatory drugs (which operate through cyclooxygenase inhibition rather than TLR4/NF- $\kappa$ B pathways) is mechanistically complementary.

**Other Polygala saponins:** Concurrent administration with structurally related Polygala saponins (polygalasaponin XXXII, tenuifolin, onjisaponin B, senegenin) is expected to produce additive or potentially synergistic neuroprotective effects, given the shared oleanane scaffold and partially overlapping but distinct mecha-

nisms of action. Combination studies with other Polygala saponins have not been published for PGSF specifically but are a logical line of investigation for evaluating the pharmacological basis of crude Polygala extract efficacy.

**CYP enzyme interactions:** The CYP enzyme inhibition or induction profile of PGSF has not been characterized. Investigators should exercise caution when combining PGSF with compounds that are sensitive CYP substrates or potent CYP inhibitors until interaction data are available.

**NLRP3 inflammasome modulators:** Concurrent administration with other NLRP3 inhibitors (MCC950, OLT1177, dapansutrile) may produce additive inhibition of inflammasome-mediated inflammation. The combination has not been studied and represents a potential research direction for optimizing anti-inflammatory neuroprotection in stroke models.

# 11. Adverse Events and Safety Signal

## 11.1 Acute toxicity

The oral median lethal dose (LD<sub>50</sub>) of crude polygalasaponin fractions from *Polygala* species in mice has been reported at approximately 3.95 g/kg, with a zero-percent lethal dose of 2.6 g/kg [17]. These values are derived from mixed saponin fractions rather than from purified PGSF; the acute toxicity of PGSF as an individual compound has not been independently determined. The LD<sub>50</sub> value suggests a wide margin between pharmacologically active doses (40 to 160 mg/kg for crude fractions producing behavioral effects) and lethal doses, consistent with a generally favorable acute safety profile.

## 11.2 Preclinical tolerability

In the published in vivo PGSF studies using intraperitoneal doses of 10 to 40 mg/kg in rats, no adverse events or toxicity signals have been explicitly reported [9, 10, 12]. The MCAO studies included neurological deficit scoring that would have captured gross neurological adverse effects; none were attributed to PGSF treatment. Body weight, feeding behavior, and general condition were not systematically reported across all studies, and formal tolerability endpoints (clinical chemistry, hematology, organ histopathology) have not been published.

## 11.3 Gastrointestinal considerations

Triterpenoid saponins as a class are associated with gastrointestinal irritation, including mucosal damage, hemolysis of red blood cells (through interaction with cholesterol in cell membranes), and alterations in intestinal permeability at high doses [16]. These class effects have not been specifically evaluated for PGSF but should be considered as a potential risk at oral doses that deliver significant intact saponin to the gastrointestinal mucosa.

## 11.4 Hemolytic potential

Saponins are characterized by their capacity to hemolyze red blood cells in vitro through interaction with cholesterol in the erythrocyte membrane. The hemolytic index of PGSF has not been published. Investigators should avoid intravenous bolus administration of high-concentration PGSF preparations until the hemolytic potential is characterized.

## 11.5 Reproductive and developmental toxicity

No reproductive or developmental toxicity data are available for PGSF or for purified *Polygala* saponin fractions.

## 11.6 Genotoxicity

No genotoxicity data (Ames test, micronucleus assay, chromosomal aberration) are available for PGSF.

## 11.7 Safety data gaps

The safety characterization of PGSF is minimal and confined to the absence of reported adverse events in small-group (typically 6 to 10 animals per group) mechanistic pharmacology studies. Formal preclinical safety pharmacology (cardiovascular, respiratory, CNS safety), repeat-dose toxicology, genotoxicity, and reproductive toxicity have not been conducted. These studies would be required before any clinical translation.

## 12. Comparable Compounds Assessment

This section presents a structured comparative assessment of five neuroprotective Polygala-derived saponins against Polygalasaponin F, evaluated against five competency standards: novelty of mechanism, effect size in preclinical models, promising potential (current research trajectory), side-effect profile (preclinical), and overall validation. The five comparators were selected as the principal characterized Polygala saponins with substantive neuroprotective or cognition-enhancing pharmacology programs.

### 12.1 Polygalasaponin XXXII (PGS32)

**Source and structure:** Triterpenoid saponin isolated from the roots of *Polygala tenuifolia* Willd. Oleanane-type aglycone with a glycosylation pattern differing from PGSF in the sugar chain composition and attachment positions [20].

**Mechanism:** PGS32 improves hippocampal-dependent learning and memory in rodents. The characterized mechanisms include enhancement of synaptic function, activation of CREB phosphorylation, and modulation of brain-derived neurotrophic factor (BDNF) expression. PGS32 attenuates scopolamine-induced cognitive impairment in mice through mechanisms involving cholinergic neurotransmission and synaptic plasticity [20].

**Novelty:** Moderate. The cholinergic and synaptic plasticity mechanisms are well established in the cognition enhancement literature. PGS32 does not possess the NMDA receptor-mediated LTP induction that distinguishes PGSF.

**Effect size:** Significant cognitive improvement in scopolamine-induced amnesia and aged-mouse models. Effect sizes in Morris water maze and novel object recognition paradigms are comparable to other Polygala saponins.

**Promising potential:** Moderate. Active research continues, principally in Chinese academic laboratories. The compound benefits from being derived from *P. tenuifolia*, which has a more extensive pharmacological and ethnopharmacological literature than *P. japonica*.

**Side-effect profile:** No specific adverse events reported in published studies. Class-typical saponin gastrointestinal and hemolytic considerations apply.

**Overall validation:** Moderate. The cognitive pharmacology literature is more extensive than that for PGSF, with multiple behavioral paradigms studied. The molecular mechanisms are less precisely characterized than the multi-pathway pharmacology of PGSF.

**Comparison to PGSF:** PGS32 has a more developed cognitive enhancement literature, while PGSF has more precisely characterized molecular mechanisms across a broader range of neuroprotective targets. PGSF's NMDA receptor-dependent LTP induction and its ischemic stroke pharmacology (TXNIP/NLRP3, NKCC1, mitophagy) are mechanistic dimensions that PGS32 lacks.

## 12.2 Tenuifolin

**Source and structure:** Tenuifolin is the aglycone prosapogenin produced by partial hydrolysis of several *Polygala tenuifolia* saponins, including onjisaponin B. It retains the oleanane scaffold with reduced glycosylation relative to the intact parent saponins [21].

**Mechanism:** Tenuifolin counteracts neurotoxicity induced by amyloid-beta peptides (A $\beta$ <sub>25-35</sub>) in vitro and in vivo. The mechanisms include inhibition of A $\beta$  aggregation, anti-oxidant activity, anti-inflammatory signaling, and enhancement of central cholinergic transmission. Tenuifolin has also been reported to promote neurogenesis in the hippocampal dentate gyrus [21].

**Novelty:** Low to moderate. Anti-A $\beta$  and cholinergic mechanisms are extensively studied in the Alzheimer's disease pharmacology field.

**Effect size:** Moderate neuroprotection against A $\beta$ -induced toxicity in cell culture. In vivo cognitive improvement in A $\beta$ -injected and transgenic Alzheimer's disease mouse models.

**Promising potential:** Moderate. Tenuifolin benefits from being a metabolic product of multiple parent saponins and from the extensive *P. tenuifolia* research literature. Its improved bioavailability relative to intact saponins (lower molecular weight, reduced polarity after deglycosylation) is a translational advantage.

**Side-effect profile:** No specific adverse events reported. Improved gastrointestinal tolerability relative to intact saponins is expected from the reduced hemolytic and mucosal-irritant potential of the deglycosylated form.

**Overall validation:** Moderate to high within the Polygala saponin class. The Alzheimer's disease pharmacology literature for tenuifolin is more extensive than that for PGSF.

**Comparison to PGSF:** Tenuifolin has superior translational characteristics (lower molecular weight, better predicted bioavailability, anti-Abeta mechanism relevant to an established clinical target). PGSF has mechanistic advantages in the ischemic stroke domain (TXNIP/NLRP3, NKCC1, mitophagy) and the direct LTP enhancement that tenuifolin lacks.

## 12.3 Senegenin (Tenuigenin)

**Source and structure:** Senegenin is the oleanane aglycone (mother nucleus) of the Polygala saponins, produced by complete acid hydrolysis of the glycosidic bonds. Molecular formula C<sub>30</sub>H<sub>46</sub>O<sub>6</sub>, molecular weight 502.7 g/mol [22].

**Mechanism:** Senegenin is neuroprotective against cognitive dysfunction induced by hepatic ischemia-reperfusion, against Abeta-induced neurotoxicity, and against glutamate excitotoxicity. The mechanisms include antioxidant activity, anti-inflammatory signaling, promotion of neurite outgrowth, and enhancement of cholinergic neurotransmission [22].

**Novelty:** Low. The aglycone represents the pharmacophore core of the entire Polygala saponin family but lacks the glycosylation-dependent activities that distinguish the individual saponins.

**Effect size:** Moderate neuroprotection in multiple in vitro and in vivo models. Effect sizes are generally comparable to but not greater than those of the intact glycosylated saponins.

**Promising potential:** Moderate. Senegenin has the translational advantage of substantially lower molecular weight and improved drug-like properties relative to the intact saponins. However, the loss of glycosylation-dependent receptor interactions may reduce pharmacological potency at certain targets.

**Side-effect profile:** Improved relative to intact saponins; reduced hemolytic potential. Limited toxicological data are available.

**Overall validation:** Moderate. The aglycone has served as a reference pharmacophore for structure-activity relationship studies of the Polygala saponins.

**Comparison to PGSF:** Senegenin has superior drug-like properties (lower molecular weight, improved oral bioavailability). PGSF has superior potency at specific targets (NMDA receptor-dependent LTP, TXNIP/NLRP3, NKCC1) that may depend on the intact glycosylation pattern for receptor recognition and activity.

## 12.4 Onjisaponin B

**Source and structure:** Triterpenoid saponin from the roots of *Polygala tenuifolia*. The sugar chain differs from PGSF; onjisaponin B bears an acyl substituent (cinnamic acid or methoxycinnamic acid ester) on one of the sugar residues [23].

**Mechanism:** Onjisaponin B promotes autophagy and clearance of aggregated proteins (alpha-synuclein, huntingtin, amyloid-beta) in neuronal cell models. The autophagy-promoting mechanism operates through AMPK activation and mTOR inhibition [23]. Additionally, onjisaponin B has anti-inflammatory and neuroprotective activities in stroke models.

**Novelty:** High within the Polygala saponin class. The autophagy-promoting mechanism is mechanistically distinct from the anti-apoptotic and anti-inflammatory mechanisms of PGSF and other Polygala saponins, and targets a different pathological process (protein aggregation rather than ischemic injury).

**Effect size:** Significant clearance of protein aggregates in cell models; moderate neuroprotection in stroke models. The autophagy effect sizes are among the largest reported for Polygala saponins.

**Promising potential:** Moderate to high. The protein aggregation clearance mechanism is highly relevant to neurodegenerative diseases (Parkinson's disease, Huntington's disease, Alzheimer's disease) and represents a differentiated therapeutic concept within the Polygala saponin class.

**Side-effect profile:** No specific adverse events reported in published preclinical studies. The autophagy-promoting mechanism carries a theoretical risk of excessive autophagy at high concentrations, analogous to the excessive mitophagy con-

cern addressed in the PGSF literature.

**Overall validation:** Moderate. The autophagy mechanism is well characterized but the in vivo cognitive and behavioral evidence is less extensive than for PGS32 or tenuifolin.

**Comparison to PGSF:** Onjisaponin B and PGSF have complementary mechanisms. PGSF inhibits excessive mitophagy and promotes neuronal survival through anti-apoptotic pathways; onjisaponin B promotes macroautophagy for aggregate clearance. The combination could theoretically address both ischemic neuronal injury and protein aggregation pathology.

## 12.5 Polygalasaponin XXVIII

**Source and structure:** Triterpenoid saponin isolated from the roots of *Polygala japonica*. Oleanane-type structure with a glycosylation pattern similar to but distinct from PGSF [15].

**Mechanism:** Limited pharmacological characterization. The compound has been identified as a component of *P. japonica* root saponin fractions with demonstrated forced swimming test activity (anti-depressant-like behavioral effect in mice) [1]. Specific molecular mechanisms have not been characterized for polygalasaponin XXVIII as an individual compound.

**Novelty:** Low. The compound has not been sufficiently characterized to establish a novel mechanism.

**Effect size:** Modest anti-depressant-like behavioral effect as part of a crude fraction. Individual compound effect sizes are not available.

**Promising potential:** Low. The compound has not been the subject of focused pharmacological investigation.

**Side-effect profile:** No data available.

**Overall validation:** Low. Minimal pharmacological characterization.

**Comparison to PGSF:** PGSF has substantially more extensive and detailed pharmacological characterization than polygalasaponin XXVIII. The two compounds share the same plant source (*P. japonica*) and structural class but PGSF has been the

subject of at least seven focused pharmacological studies compared to minimal independent investigation of polygalasaponin XXVIII.

## 12.6 Comparator summary

The five comparator Polygala saponins occupy distinct positions in the neuroprotective pharmacology landscape. **Polygalasaponin XXXII** has the most extensive cognitive enhancement literature and benefits from the larger *P. tenuifolia* research base. **Tenuifolin** has the best translational characteristics (lowest effective molecular weight, improved bioavailability prediction, anti-Abeta mechanism). **Senegenin** provides the reference pharmacophore for structure-activity analysis and the most drug-like properties among the comparators. **Onjisaponin B** has the most novel mechanism (autophagy promotion for aggregate clearance) and the most differentiated therapeutic concept. **Polygalasaponin XXVIII** is minimally characterized and serves principally as a structural congener reference.

Polygalasaponin F occupies a unique position in this landscape through three distinguishing features. First, the direct electrophysiological demonstration of NMDA receptor-dependent LTP induction, placing it among a very small number of natural saponins with evidence of synaptic strengthening in intact hippocampal circuits. Second, the most extensively characterized multi-target neuroprotective mechanism within the Polygala saponin class, spanning NMDA receptor modulation, PI3K/Akt activation, TLR4/NF- $\kappa$ B suppression, TXNIP/NLRP3 inflammasome inhibition, NKCC1 epigenetic downregulation, and mitophagy regulation. Third, the most developed ischemic stroke pharmacology program, with in vivo efficacy demonstrated in the MCAO model across multiple mechanistic endpoints.

The principal translational disadvantage of PGSF relative to the comparators is shared by all intact Polygala saponins: the high molecular weight, limited oral bioavailability, and absence of pharmacokinetic and formal safety data. Among the comparators, tenuifolin and senegenin have inherent advantages on this dimension due to their lower molecular weights, while onjisaponin B shares the translational challenges of the intact saponin class.

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*This monograph is provided for research use only. Not for medical, diagnostic, or therapeutic purposes. Not for human consumption. Polygalasaponin F has not been evaluated in clinical trials in any species. All pharmacological data are derived from in vitro cell culture systems and rodent models. Compound class generalizations are noted where compound-specific data are sparse; investigators should weight the evidence accordingly. Investigators should obtain analytical confirmation of identity and purity on every lot. No regulatory authority has approved Polygalasaponin F for any therapeutic indication.*