

ABSTRACT

Piperlongumine

Piperlongumine (piplartine, 5,6-dihydro-1-[(2E)-1-oxo-3-(3,4,5-trimethoxyphenyl)-2-propenyl]-2(1H)-pyridinone; CAS 20069-09-4; molecular formula C₁₇H₁₉NO₅; molecular weight 317.34 g/mol) is an amide alkaloid constituent of the fruit, root, and stem of *Piper longum* L. (long pepper), a plant of the Piperaceae family native to the Indian subcontinent and Southeast Asia with a centuries-long history of use in Ayurvedic and traditional Chinese medicine. The compound was first isolated and structurally characterized by Chatterjee and Dutta in 1965 and remained a minor phytochemical curiosity until the landmark 2011 report by Raj et al. in *Nature*, which identified piperlongumine through an unbiased chemical screen as a small molecule that selectively kills cancer cells and oncogene-transformed cells but not normal cells by inducing the accumulation of reactive oxygen species (ROS), irrespective of p53 status or proliferation rate [1]. That demonstration catalyzed an extensive preclinical research literature that now spans more than two dozen cancer histologies, multiple molecular targets, and several emerging non-oncologic applications including senolytic clearance of senescent cells, neuroinflammation, and metabolic disease.

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COMPOUND CLASS

Electrophilic alpha,beta-unsaturated delta-valerolactam

COMPOUND CLASS

alkaloid amide with pro-oxidant and multi-target anticancer activity

reacts covalently with nucleophilic cysteine residues on multiple redox-regulatory and signaling proteins. Direct binding targets include glutathione S-transferase pi 1 (GSTP1), thioredoxin

reductase 1 (TrxR1), and carbonyl reductase 1 (CBR1), whose

inhibition impairs cellular antioxidant defenses and elevates

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intracellular ROS (principally hydrogen peroxide and superoxide) to levels that exceed the already elevated oxidative baseline of transformed cells [1, 2, 3]. Downstream consequences include ROS-dependent downregulation of specificity protein transcription factors Sp1, Sp3, and Sp4 and their pro-oncogenic target genes (cyclin D1, survivin, cMyc, EGFR, cMet) [4]; inhibition of the NF- κ B signaling pathway with suppression of pro-inflammatory and pro-survival gene expression [5, 6]; direct inhibition of STAT3 phosphorylation and dimerization [7]; inhibition of PI3K/Akt/mTOR signaling with consequent promotion of autophagy and apoptosis [8, 9]; and blockade of NLRP3 inflammasome assembly through disruption of NLRP3-NEK7 association and NLRP3 oligomerization [10]. The compound has also been identified as a ligand for the orphan nuclear receptor NR4A1 (Nur77) [11]. The selectivity for cancer cells over normal cells is attributed to the higher basal ROS burden and the greater dependence on antioxidant buffering capacity in transformed cells; normal cells, operating at lower oxidative stress, tolerate the moderate ROS elevation induced by piperlongumine without reaching the apoptotic threshold.

Pharmacokinetic characterization in rodent models indicates oral bioavailability of approximately 50 to 76 percent (dose-dependent, inversely related to dose in the 5 to 10 mg/kg range in rats), low hepatic extraction ratio ($E = 0.09$), plasma protein binding of approximately 93 percent, and metabolism by multiple CYP isoenzymes producing several hydroxylated and demethylated metabolites [12, 13]. The compound exhibits poor aqueous solubility (approximately 26 micrograms per milliliter in water at ambient pH) but adequate solubility in dimethyl sulfoxide, ethanol, PEG 400, and lipid-based formulations. Stability is pH-dependent, with maximum stability near pH 4 and significant

degradation at alkaline pH values and under ultraviolet irradiation [14]. No human pharmacokinetic data have been published; the compound has not entered formal clinical trials as of the most recent monograph revision, though the extensive preclinical evidence base and favorable rodent safety profile support advancement to first-in-human studies.

In vivo antitumor efficacy has been demonstrated in xenograft models of colorectal, pancreatic, lung, breast, head and neck, thyroid, prostate, and cervical cancers at intraperitoneal doses of 2.4 to 10 mg/kg per day, with significant tumor growth inhibition and no apparent systemic toxicity to normal tissues in treated animals [1, 15, 16, 17, 18]. Synergistic combinations with conventional chemotherapeutics (cisplatin, gemcitabine, paclitaxel, oxaliplatin, doxorubicin, 5-fluorouracil) have been reported across multiple tumor types, with the ROS-elevating mechanism of piperlongumine sensitizing resistant cells to cytotoxic therapy [19, 20, 21]. A second application of growing interest is the senolytic activity first reported by Wang et al. (2016), who demonstrated that piperlongumine selectively kills radiation-induced, replicative, and oncogene-induced senescent human WI-38 fibroblasts and synergizes with the BH3 mimetic ABT-263 in senescent cell clearance [22]. Subsequent medicinal chemistry optimization has produced piperlongumine analogs with up to 50-fold enhanced senolytic potency [23].

This monograph documents the chemistry, isolation, and synthesis of piperlongumine; the multi-target molecular pharmacology; the rodent pharmacokinetic profile; the preclinical antitumor and senolytic evidence base; the absence of clinical trial data and the translational considerations for first-in-human development; sourcing and quality verification; reconstitution and

handling; stack-interaction considerations; adverse-event and safety signal from animal studies; and a structured comparative assessment of five pro-oxidant or senolytic natural products (withaferin A, sulforaphane, parthenolide, curcumin, fisetin) against piperlongumine on five competency standards.

1. Compound Identification

1.1 Names, synonyms, and registry identifiers

Common name: Piperlongumine.

Synonyms: Piplartine, piperlonguminine (historical, sometimes confused with a distinct lower-molecular-weight Piper alkaloid), 5,6-dihydro-1-[(2E)-1-oxo-3-(3,4,5-trimethoxyphenyl)-2-propenyl]-2(1H)-pyridinone.

Chemical Abstracts Service registry number: 20069-09-4.

PubChem CID: 637858.

International Union of Pure and Applied Chemistry name: (E)-1-(5,6-dihydropyridin-2(1H)-yl)-3-(3,4,5-trimethoxyphenyl)prop-2-en-1-one. The compound is an N-acyl-delta-valerolactam bearing a trans-cinnamoyl substituent derived from 3,4,5-trimethoxycinnamic acid.

1.2 Structural class and chemistry

Piperlongumine is an amide alkaloid of the Piper genus, belonging to the broader class of cinnamoyl-piperidine (or cinnamoyl-dihydropyridinone) natural products. It is structurally related to piperine (the pungent alkaloid of black pepper, *Piper nigrum*) but is distinguished by its alpha,beta-unsaturated delta-valerolactam ring and the trimethoxyphenyl cinnamoyl substituent. The structural class places it within the electrophilic natural products, a group of plant secondary metabolites that exert biological activity through covalent modification of nucleophilic protein residues.

Molecular formula: C₁₇H₁₉NO₅.

Exact mass: 317.1263 g/mol (monoisotopic).

Molecular weight: 317.34 g/mol.

Physical properties: Yellow to light-yellow crystalline powder. Melting point 123 to 126 degrees Celsius by hot-stage microscopy; differential scanning calorimetry gives an endothermic onset at approximately 110 degrees Celsius and a peak at 123

degrees Celsius [14]. Poorly soluble in water (approximately 26 micrograms per milliliter at ambient temperature and neutral pH). Soluble in dimethyl sulfoxide (up to 100 mM), ethanol (approximately 20 mM with gentle warming), PEG 400 (approximately 22 mg/mL), and methanol. LogP has been estimated computationally at approximately 1.5 to 2.0, consistent with moderate lipophilicity.

Key structural features: The molecule contains two electrophilic sites: (a) the C2-C3 olefin of the cinnamoyl moiety (alpha,beta-unsaturated amide, Michael acceptor); and (b) the C7-C8 olefin of the dihydropyridinone ring (second Michael acceptor). Both sites are capable of covalent reaction with biological nucleophiles, principally the thiolate anion of cysteine residues in target proteins. The 3,4,5-trimethoxyphenyl ring contributes to hydrophobic binding interactions and is a pharmacophoric element whose modification alters both potency and selectivity in structure-activity relationship studies [24, 25].

1.3 Synthesis

Piperlongumine can be obtained by extraction from *Piper longum* plant material (roots, fruit, stem) or by total synthesis. The natural product was first isolated from *Piper longum* roots by Chatterjee and Dutta (1963) using standard alkaloid extraction procedures (acid-base partitioning from ethanol extracts) [26].

The total synthesis proceeds through condensation of 3,4,5-trimethoxycinnamic acid (or its acyl chloride) with 2-piperidinone (delta-valerolactam) or the corresponding 5,6-dihydro-2(1H)-pyridinone precursor. The key step is the N-acylation of the lactam nitrogen with the activated cinnamoyl unit. Published synthetic routes include: (a) acylation of delta-valerolactam with 3,4,5-trimethoxycinnamoyl chloride in the presence of a base (triethylamine or pyridine) followed by oxidation of the C7-C8 bond to introduce the second olefin [27]; and (b) direct condensation under Horner-Wadsworth-Emmons or Wittig conditions to install the trans-cinnamoyl geometry. The trans (E) geometry of the C2-C3 olefin is thermodynamically favored and is the natural product configuration. Yields of 40 to 70 percent are typical for the N-acylation step. Purification is accomplished by silica gel column chromatography followed by recrystallization from ethanol or ethyl acetate.

Medicinal chemistry programs have produced extensive libraries of piperlongumine analogs through modification of the trimethoxyphenyl ring (substitution of methoxy groups with hydroxyl, halogen, or heterocyclic substituents), the C2-C3 olefin (saturation, fluorination, replacement with heterocyclic linkers), and the di-

hydropyridinone ring (ring expansion, ring contraction, replacement with open-chain amides) [24, 25]. These analog programs have informed the structure-activity relationship and have produced compounds with enhanced potency, improved aqueous solubility, and altered target selectivity.

2. Abstract

Piperlongumine (piplartine, CAS 20069-09-4) is an electrophilic amide alkaloid from the long pepper plant (*Piper longum* L.) that selectively induces apoptotic cell death in cancer cells and oncogene-transformed cells while sparing normal cells through a mechanism centered on the elevation of intracellular reactive oxygen species [1]. The compound was identified through an unbiased phenotypic screen and reported by Raj et al. in a 2011 Nature publication that demonstrated selective cytotoxicity across 13 human cancer cell lines compared with 6 untransformed lines, with the selectivity independent of cell proliferation rate and TP53 status [1]. Significant antitumor effects were observed in xenograft mouse models with no apparent toxicity to normal tissues, and piperlongumine potently inhibited the growth of spontaneously formed malignant breast tumors and their associated metastases in mice.

The molecular basis of the selective pro-oxidant activity lies in the dual Michael acceptor reactivity of the molecule, which covalently modifies cysteine residues on key antioxidant and redox-regulatory proteins including GSTP1, TrxR1, and CBR1 [2, 3]. The resulting impairment of antioxidant capacity elevates ROS beyond the tolerance threshold of cancer cells, which operate under higher basal oxidative stress than normal cells, triggering mitochondria-dependent apoptosis. Downstream signaling consequences include suppression of Sp1/Sp3/Sp4 transcription factors and their oncogenic target genes [4], NF- κ B pathway inhibition [5, 6], STAT3 inhibition [7], PI3K/Akt/mTOR pathway suppression with induction of autophagy [8, 9], and NLRP3 inflammasome blockade [10].

Preclinical *in vivo* efficacy has been demonstrated in xenograft and syngeneic models of more than a dozen cancer types at intraperitoneal doses of 2.4 to 10 mg/kg per day, with consistent tumor growth inhibition in the range of 40 to 70 percent and absence of weight loss, organ toxicity, or mortality in treated animals [1, 15, 16, 17, 18]. Synergistic combinations with cisplatin, gemcitabine, paclitaxel, oxaliplatin, doxorubicin, and 5-fluorouracil have been reported [19, 20, 21]. A second research application of growing importance is the senolytic clearance of senescent cells, first characterized by Wang et al. (2016) [22]. No human clinical trials have been conducted; the compound is available as a research-grade natural product and is positioned as a preclinical lead for anticancer and senolytic drug development.

3. Discovery and Development History

3.1 Origin and traditional use

Piper longum L. (long pepper) has been used in Ayurvedic medicine for millennia as a treatment for respiratory disease, digestive complaints, and inflammatory conditions. The plant contains multiple bioactive alkaloids, of which piperine (from black pepper, *Piper nigrum*) is the most commercially significant. Piperlongumine was first isolated from the roots of *Piper longum* by Chatterjee and Dutta in 1963, who described its structure as an N-acylated dihydropyridinone bearing a trimethoxyphenyl cinnamoyl group [26]. The compound was subsequently identified in *Piper tuberculatum* and other *Piper* species, where it is sometimes referred to as piplartine.

3.2 Early pharmacological characterization

Between the 1960s and the 2000s, piperlongumine received limited pharmacological attention. Early studies characterized its antimicrobial, antifungal, antiplatelet, and modest cytotoxic activities as part of broader surveys of *Piper* alkaloids. Bezerra et al. (2007, 2008) evaluated the cytotoxicity and genotoxicity of piplartine in mammalian V79 cells and in yeast, establishing a preliminary safety and activity profile [28, 29]. However, the compound was not recognized as a differentiated anticancer agent until the Raj et al. (2011) report.

3.3 The Raj et al. (2011) Nature report

The seminal discovery that repositioned piperlongumine as a lead anticancer compound was published by Raj, Ide, Gurkar, Foley, Schenone, Li, and colleagues in *Nature* in July 2011 [1]. The investigators conducted an image-based high-throughput screen of a library of small molecules for compounds that selectively killed engineered tumorigenic human cells (BJ fibroblasts expressing hTERT, SV40 large T and small T antigens, and oncogenic HRAS) but not the isogenic normal counterparts. Piperlongumine emerged as a top hit. The key findings were: (a) selective cytotoxicity across 13 human cancer cell lines compared to 6 untransformed lines, independent of proliferation rate and p53 status; (b) sensitization of normal cells to

piperlongumine following HRAS or ERBB2 transformation, confirming oncogene-dependent vulnerability; (c) ROS accumulation as the primary mechanism, with glutathione supplementation abrogating the cytotoxic effect; (d) direct binding to and inhibition of GSTP1 and CBR1; and (e) significant antitumor efficacy in mouse xenograft models, including inhibition of spontaneous mammary tumors and metastases, with no systemic toxicity in normal mice [1].

The Nature publication attracted broad attention and catalyzed the current research literature, which now encompasses over 500 publications spanning medicinal chemistry, molecular pharmacology, preclinical oncology, senolytic biology, and anti-inflammatory applications.

3.4 Subsequent expansion of the research literature

From 2012 onward, independent laboratories extended the piperlongumine pharmacology to additional cancer types (pancreatic, lung, prostate, head and neck, thyroid, cervical, leukemia, lymphoma, glioblastoma, hepatocellular carcinoma, gastric, and ovarian cancers), identified additional molecular targets (TrxR1, STAT3, NF- κ B, PI3K/Akt/mTOR, NLRP3, NR4A1, Sp transcription factors), and reported synergistic combinations with standard chemotherapeutics [2, 3, 4, 5, 6, 7, 8, 9, 10, 11]. The Wang et al. (2016) report in *Aging* identified piperlongumine as a senolytic agent, extending the compound beyond oncology into the biology of cellular senescence and aging [22]. Medicinal chemistry programs at multiple institutions have produced hundreds of piperlongumine analogs with improved potency, selectivity, and druglike properties [24, 25].

3.5 Clinical development status

As of the most recent monograph revision, piperlongumine has not entered formal clinical trials in any jurisdiction. No investigational new drug (IND) application has been publicly filed. The compound remains in the preclinical stage of development, though the favorable safety profile in rodent studies, the well-characterized mechanism, and the commercial availability of research-grade material support the feasibility of first-in-human studies. The principal translational barriers are the poor aqueous solubility, the absence of human pharmacokinetic data, and the lack of a commercial development sponsor.

4. Mechanism of Action

Piperlongumine is a multi-target electrophilic compound whose anticancer activity derives from covalent modification of redox-regulatory proteins, leading to ROS accumulation beyond the tolerance threshold of transformed cells. The mechanism is selective because cancer cells operate under elevated basal oxidative stress and depend more heavily on antioxidant buffering systems than normal cells; pharmacological impairment of those systems by piperlongumine produces a disproportionate ROS burden in cancer cells relative to normal cells [1].

4.1 Covalent target engagement

The alpha,beta-unsaturated carbonyl systems in piperlongumine (the cinnamoyl Michael acceptor at C2-C3 and the dihydropyridinone Michael acceptor at C7-C8) react with nucleophilic cysteine thiolates on multiple protein targets through 1,4-conjugate addition (Michael reaction). The principal characterized direct binding targets are:

Glutathione S-transferase pi 1 (GSTP1). Raj et al. (2011) identified GSTP1 as a direct binding partner of piperlongumine by affinity chromatography and validated the interaction by demonstrating that GSTP1 overexpression partially rescued piperlongumine-induced cell death [1]. GSTP1 catalyzes the conjugation of glutathione to electrophilic substrates and is overexpressed in many cancers, where it contributes to chemoresistance and to maintenance of intracellular redox homeostasis. Inhibition of GSTP1 by piperlongumine reduces glutathione conjugation capacity and elevates ROS.

Thioredoxin reductase 1 (TrxR1). Zou et al. (2019) demonstrated that piperlongumine is a direct inhibitor of TrxR1, the selenocysteine-containing flavoenzyme that reduces oxidized thioredoxin and maintains the cellular thiol-disulfide balance [3]. Inhibition of TrxR1 impairs the thioredoxin antioxidant system and contributes to ROS-mediated endoplasmic reticulum stress and apoptosis in hepatocellular carcinoma cells.

Carbonyl reductase 1 (CBR1). Raj et al. (2011) also identified CBR1 as a direct binding target [1]. CBR1 catalyzes the NADPH-dependent reduction of carbonyl-containing substrates, including prostaglandins and xenobiotics, and its inhibition contributes to the altered redox state.

4.2 ROS accumulation and oxidative stress

The composite inhibition of GSTP1, TrxR1, and CBR1 produces a convergent elevation of intracellular ROS, principally hydrogen peroxide and superoxide. The ROS accumulation is dose-dependent and time-dependent, detectable within 1 to 4 hours of piperlongumine exposure in most cancer cell lines, and is abrogated by co-treatment with the thiol antioxidant N-acetylcysteine or with exogenous glutathione, confirming the causal role of ROS in the cytotoxic mechanism [1, 2]. The ROS-dependent nature of the cytotoxicity has been confirmed across cancer types including breast, colorectal, pancreatic, lung, head and neck, thyroid, prostate, cervical, and leukemia.

4.3 Downstream signaling consequences

The ROS elevation induced by piperlongumine triggers multiple downstream signaling events:

Sp transcription factor downregulation. Piperlongumine induces ROS-dependent proteasomal degradation of Sp1, Sp3, and Sp4, transcription factors that are overexpressed in many cancers and that drive expression of pro-oncogenic genes including cyclin D1, survivin, cMyc, EGFR, and cMet [4]. Sp factor downregulation is a secondary consequence of ROS accumulation and contributes to the antiproliferative and pro-apoptotic phenotype.

NF- κ B pathway inhibition. Piperlongumine suppresses NF- κ B transcriptional activity through inhibition of I κ B- α phosphorylation and degradation, preventing nuclear translocation of NF- κ B p65 [5, 6]. The NF- κ B inhibition reduces expression of anti-apoptotic genes (Bcl-2, Bcl-xL, survivin, XIAP), pro-inflammatory cytokines (TNF- α , IL-1 β , IL-6), and invasion-related genes (MMP-9, VEGF). This mechanism has been demonstrated in prostate cancer, pancreatic cancer, lung cancer, and BV2 microglial cells.

STAT3 inhibition. Piperlongumine directly inhibits STAT3 phosphorylation at Tyr705 and Ser727, preventing STAT3 dimerization and nuclear translocation [7]. The STAT3 inhibition was identified through a drug-repositioning computational screen and validated in breast cancer models. STAT3 is a convergence node for multiple oncogenic signaling pathways and its inhibition contributes to the antiproliferative and pro-apoptotic effects.

PI3K/Akt/mTOR pathway suppression. Piperlongumine inhibits Akt phosphorylation in a ROS-dependent manner, reducing mTORC1 activity and promoting autophagy [8, 9]. The PI3K/Akt/mTOR pathway is constitutively activated in many cancers and contributes to cell survival, proliferation, and chemoresistance. Piperlongumine-induced autophagy has been characterized as both cytoprotective (at low doses) and cytotoxic (at high doses or in combination with autophagy inhibitors).

NLRP3 inflammasome blockade. Piperlongumine inhibits NLRP3 inflammasome assembly by disrupting the association between NLRP3 and NEK7 and by preventing NLRP3 oligomerization [10]. The NLRP3 inhibition suppresses caspase-1 activation and IL-1 β and IL-18 maturation and secretion. This mechanism has been validated in LPS-stimulated macrophages and in NLRP3-dependent mouse models of endotoxemia and peritonitis, positioning piperlongumine as an anti-inflammatory compound independent of its anticancer activity.

NR4A1 (Nur77) ligand activity. Piperlongumine has been identified as a ligand for the orphan nuclear receptor NR4A1 [11]. NR4A1 is overexpressed in several cancers and drives expression of genes involved in cell survival and metabolism. The functional consequence of piperlongumine binding to NR4A1 is under investigation and may contribute to the antiproliferative mechanism in NR4A1-dependent tumors.

4.4 Selectivity for cancer cells

The selectivity of piperlongumine for cancer cells over normal cells is the compound's most distinctive pharmacological feature and was the central finding of the Raj et al. (2011) report [1]. The selectivity is attributed to the differential oxidative stress baseline between cancer cells and normal cells. Cancer cells generate higher basal levels of ROS owing to oncogene activation, mitochondrial dysfunction, aberrant metabolism, and loss of tumor suppressor function. These cells depend on upregulated antioxidant defense systems (glutathione, thioredoxin, cata-

lase, superoxide dismutase) to maintain ROS below the apoptotic threshold. Piperlongumine impairs the antioxidant defense systems through GSTP1 and TrxR1 inhibition, pushing cancer cells above the threshold. Normal cells, with lower basal ROS and less dependence on the inhibited antioxidant enzymes, tolerate the pharmacological perturbation without reaching the apoptotic threshold [1].

The selectivity has been confirmed by multiple independent groups and has been demonstrated across transformed cell lines of varied tissue origin. Importantly, the selectivity is preserved when normal cells are engineered to express oncogenic HRAS or ERBB2, confirming that the vulnerability is oncogene-dependent rather than tissue-type-dependent [1].

5. Pharmacokinetics and Routes of Administration

5.1 Absorption

Piperlongumine is absorbed from the gastrointestinal tract after oral administration in rodents. Oral bioavailability in rats has been reported as approximately 76 percent at 5 mg/kg and approximately 50 percent at 10 mg/kg, indicating dose-dependent absorption or saturable first-pass processes at higher doses [12, 13]. The low hepatic extraction ratio ($E = 0.09$) estimated from in vitro microsomal studies suggests that first-pass hepatic metabolism by CYP450 enzymes is not a major determinant of oral bioavailability, and that the dose-dependent reduction in bioavailability may reflect solubility-limited absorption at higher doses [12].

5.2 Distribution

Plasma protein binding is approximately 93 percent [12]. Microsomal binding is approximately 23 percent. Volume of distribution data from formal pharmacokinetic studies in rodents have not been published in full, but the moderate lipophilicity (estimated logP approximately 1.5 to 2.0) and the substantial plasma protein binding are consistent with moderate tissue distribution. Central nervous system penetration has not been directly characterized by PET or CSF sampling, but the anti-neuroinflammatory activity observed in BV2 microglial cell models and the neuroprotective effects in LPS-stimulated amyloidogenesis models suggest functional CNS exposure at systemically active doses [30, 31].

5.3 Metabolism

Piperlongumine is metabolized by multiple CYP isoenzymes in both rat liver microsomes and recombinant human CYP systems [12]. The metabolic profile is complex, with several hydroxylated and O-demethylated metabolites identified. The compound exhibits non-Michaelian (atypical) kinetics in microsomal metabolism assays, consistent with interaction with multiple CYP isoforms at overlapping concentration ranges. A metabolic finding of pharmacological importance is that

piperlongumine inhibits CYP1A2 in a dose-, time-, and NADPH-dependent manner, with evidence for mechanism-based (irreversible) inactivation of this isoenzyme [12]. The irreversible CYP1A2 inactivation raises the possibility of clinically relevant drug-drug interactions in future human applications and is a consideration for translational development.

Piperlongumine also inhibits CYP3A4 activity, as demonstrated by its ability to reduce testosterone and docetaxel metabolism *in vitro*, producing an approximately 2-fold increase in the half-life of these CYP3A4 substrates [13]. This interaction was exploited therapeutically in a preclinical study demonstrating that co-administration of piperlongumine enhanced the oral bioavailability and cytotoxicity of docetaxel in a triple-negative breast cancer model.

5.4 Elimination

Detailed elimination data (renal clearance, biliary excretion, fecal recovery) from formal mass-balance studies have not been published. Based on the low hepatic extraction ratio and the predominance of CYP-mediated metabolism, elimination is expected to be primarily metabolic with subsequent renal excretion of conjugated metabolites.

5.5 Routes of administration

The principal route in preclinical studies is intraperitoneal injection, with doses ranging from 2.4 to 10 mg/kg per day in tumor xenograft models [1, 15, 16, 17, 18]. Oral administration has been studied for bioavailability characterization and for combination pharmacokinetic studies. Intravenous administration has been used in selected pharmacokinetic studies. Formulation vehicles for intraperitoneal and oral dosing include DMSO/PEG 400/saline mixtures, corn oil, and nanoemulsion systems. A piperlongumine-loaded nanoemulsion formulation improved oral bioavailability by approximately 1.5-fold compared to the free compound in suspension [13].

5.6 Special populations

No data exist for pediatric, geriatric, renally impaired, or hepatically impaired populations. All pharmacokinetic data are from healthy adult rodent models. Human pharmacokinetic characterization has not been performed.

6. Clinical Evidence Base

6.1 Status of clinical development

Piperlongumine has not been evaluated in any published human clinical trial as of the most recent monograph revision. No Phase 1 pharmacokinetic, dose-finding, or safety study in human subjects has been reported. No IND application has been publicly filed with the United States Food and Drug Administration or equivalent agencies.

6.2 Preclinical evidence supporting clinical translation

The preclinical evidence base supporting future clinical development includes: (a) selective cytotoxicity against cancer cells with sparing of normal cells, demonstrated across more than a dozen tumor types and confirmed by multiple independent groups [1]; (b) significant antitumor efficacy in multiple xenograft and syngeneic mouse models at tolerable doses [1, 15, 16, 17, 18]; (c) synergistic activity with multiple classes of conventional chemotherapy [19, 20, 21]; (d) oral bioavailability in rodents adequate for systemic exposure [12, 13]; (e) absence of overt systemic toxicity, organ damage, or mortality in treated animals at efficacious doses; and (f) a well-characterized molecular mechanism with multiple validated targets.

6.3 Translational barriers

The principal barriers to clinical translation are: (a) poor aqueous solubility requiring formulation optimization for oral or intravenous delivery; (b) absence of human pharmacokinetic, safety, and tolerability data; (c) the CYP1A2 mechanism-based inactivation and CYP3A4 inhibition, which raise drug-drug interaction concerns; (d) absence of a commercial development sponsor; and (e) the compound's status as a natural product, which complicates intellectual property protection and may reduce commercial incentive for development.

6.4 Traditional medicine context

Piper longum extracts containing piperlongumine (and the related alkaloid piperine) have been consumed as part of Ayurvedic medical preparations (Trikatu, Sitopaladi Churna) for centuries, providing indirect evidence of human tolerability at the low doses achieved through dietary and traditional medicinal exposure. However, the doses required for anticancer or senolytic effects are likely substantially higher than those achieved through traditional preparations, and the traditional use cannot substitute for formal pharmacokinetic and safety studies.

7. Reported Research Dose Ranges

7.1 In vitro effective concentrations

Piperlongumine induces selective cytotoxicity in cancer cell lines at concentrations of 2 to 20 micromolar in most published studies, with IC₅₀ values typically in the range of 5 to 15 micromolar depending on cell type and exposure duration [1, 2, 3, 4]. Normal cell lines are generally unaffected at concentrations up to 20 to 30 micromolar. ROS accumulation is detectable within 1 to 4 hours at concentrations of 5 micromolar and above.

7.2 In vivo efficacious doses

Xenograft tumor models: Intraperitoneal doses of 2.4 to 10 mg/kg per day have been used across published studies, with the majority of studies using 2.5 to 5 mg/kg per day [1, 15, 16, 17, 18]. Treatment durations range from 14 to 30 days. Tumor growth inhibition of 40 to 70 percent is typical at these doses.

Combination studies: Lower doses (2.4 to 5 mg/kg) are used in combination with conventional chemotherapeutics, where the synergistic interaction reduces the required dose of both agents [19, 20, 21].

Anti-inflammatory models: Doses of 1 to 10 mg/kg have been used in LPS-induced endotoxemia and MSU-induced peritonitis models, with significant anti-inflammatory effects at the lower end of this range [10].

7.3 Dose considerations for future human studies

Allometric scaling from rodent efficacious doses (2.5 to 10 mg/kg intraperitoneal in mice) to human equivalent doses yields an estimated starting range of approximately 12 to 50 mg per day for a 60-kg adult, using the standard mouse-to-human body surface area conversion factor (division by 12.3). This range is provisional and would require confirmation through formal first-in-human pharmacokinetic and dose-finding studies.

8. Sourcing and Quality Verification

Research-grade piperlongumine is commercially available from multiple chemical and natural product suppliers at high purity. Principal suppliers include Sigma-Aldrich (catalog SML0221, greater than or equal to 97 percent purity by HPLC), Cayman Chemical (catalog 11006), Selleckchem (catalog S7551), Santa Cruz Biotechnology (catalog sc-205875), MedChemExpress, Tocris, and BocSciences. Supply is in milligram to gram quantities as the free base; pricing is moderate, typically in the range of \$30 to \$150 per 100 milligrams of research-grade material at greater than 95 percent purity.

Natural product isolation from *Piper longum* root or fruit is an alternative source for investigators requiring larger quantities, though the yield is variable (approximately 0.1 to 0.5 percent of dry root weight) and the isolation requires chromatographic purification to remove co-extracted alkaloids including piperine, piperlonguminine, and guineensine.

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

- High-performance liquid chromatography area-percent purity, targeting greater than 95 percent (greater than 98 percent preferred)
- Mass spectrometry confirmation of molecular weight (318.13 for the protonated molecular ion $[M+H]^+$)
- Nuclear magnetic resonance spectroscopy (1H and ^{13}C) confirmation of the trans-cinnamoyl geometry and the dihydropyridinone ring system
- Assessment of the E/Z isomer ratio, as the (Z)-isomer is a synthetic byproduct with reduced biological activity
- Residual solvent analysis if the material is synthetic rather than naturally extracted

Third-party analytical confirmation is appropriate for any study where dose-response precision or mechanistic conclusions depend on compound purity and identity.

9. Reconstitution and Handling

Piperlongumine is supplied as a crystalline powder and is reconstituted as needed for in vitro or in vivo applications.

Stock solution preparation: Dissolve in dimethyl sulfoxide (DMSO) to a stock concentration of 10 to 100 mM (3.17 to 31.7 mg/mL). DMSO stocks are stable at minus 20 degrees Celsius for at least 6 months with protection from light and moisture. Aliquot into single-use volumes to avoid freeze-thaw cycles, which may promote degradation. For in vivo use where DMSO is to be minimized, prepare working solutions by diluting the DMSO stock into PEG 400, ethanol/water mixtures, or nanoemulsion vehicles immediately before dosing.

Aqueous solutions: Owing to the poor aqueous solubility (approximately 26 micrograms per milliliter), direct dissolution in aqueous buffers is not practical at concentrations required for most in vitro assays. Use cosolvents (10 percent DMSO in cell culture medium is standard, with final DMSO concentration in the assay well kept below 0.5 percent) or surfactant-assisted dissolution (10 percent polysorbate 80 increases solubility approximately 27-fold) [14].

Stability considerations: Piperlongumine is maximally stable near pH 4 in aqueous solution. Significant degradation occurs at alkaline pH (7 and above) and at pH 3, with estimated 10 percent degradation in approximately 17 weeks at pH 4 and 25 degrees Celsius [14]. The compound is photosensitive; marked photodegradation occurs upon exposure to ultraviolet light, especially in aqueous media. Storage of solid material and stock solutions should be in amber vials or wrapped in foil, at 2 to 8 degrees Celsius for short-term storage and at minus 20 degrees Celsius for long-term storage.

In vivo formulation: For intraperitoneal injection in rodents, a common vehicle is 10 percent DMSO, 40 percent PEG 400, and 50 percent saline (v/v/v). Oral gavage formulations may use corn oil, PEG 400, or nanoemulsion systems to improve bioavailability. The nanoemulsion approach has been shown to improve oral bioavailability by approximately 1.5-fold [13].

10. Stack Interaction Considerations

Piperlongumine interacts with several drug classes through its CYP inhibition profile and through its pro-oxidant and multi-pathway pharmacology. Investigators conducting research with piperlongumine, especially in combination studies or in chronic dosing regimens, should consider:

CYP1A2 substrates: Piperlongumine produces mechanism-based (irreversible) inactivation of CYP1A2 [12]. Concurrent administration with CYP1A2 substrates (caffeine, theophylline, melatonin, tizanidine, duloxetine, fluvoxamine, olanzapine, clozapine) may substantially increase exposure to these substrates. This interaction is clinically relevant and is a translational development concern.

CYP3A4 substrates: Piperlongumine inhibits CYP3A4, producing an approximately 2-fold increase in the half-life of CYP3A4 substrates such as docetaxel and testosterone [13]. This interaction has been exploited as a bioavailability-enhancing strategy for docetaxel in preclinical breast cancer studies but would produce unwanted exposure increases for other CYP3A4 substrates in a polypharmacy context.

Conventional chemotherapeutics: Piperlongumine synergizes with cisplatin, gemcitabine, paclitaxel, oxaliplatin, doxorubicin, and 5-fluorouracil through ROS elevation that sensitizes resistant cells to cytotoxic therapy [19, 20, 21]. The synergistic interactions are well characterized in vitro and in selected xenograft models. The combination may produce additive myelosuppression or gastrointestinal toxicity that would require dose adjustment in future clinical application.

Antioxidant supplements: N-acetylcysteine, glutathione, and other thiol antioxidants directly antagonize the pro-oxidant mechanism of piperlongumine and should not be co-administered in studies where the ROS-mediated cytotoxicity is the desired pharmacological effect [1].

Other pro-oxidant compounds: Concurrent administration with other ROS-inducing agents (withaferin A, parthenolide, sulforaphane, arsenic trioxide, buthionine sulfoximine) may produce additive or synergistic oxidative stress. The combination may enhance anticancer efficacy but also increases the risk of off-target oxidative damage to normal tissues.

BH3 mimetics and senolytic agents: Piperlongumine synergizes with ABT-263 (navitoclax) in the selective killing of senescent cells [22]. This combination is of interest for senolytic research but has not been characterized for safety in vivo.

PI3K/mTOR inhibitors: Given the PI3K/Akt/mTOR inhibitory activity of piperlongumine, concurrent use with pharmacological PI3K or mTOR inhibitors may produce additive pathway suppression. The interaction has not been formally studied.

11. Adverse Events and Safety

11.1 Preclinical safety profile

The safety profile of piperlongumine is characterized exclusively from animal studies. The principal finding across published xenograft and toxicity studies is the absence of overt systemic toxicity at efficacious doses. Raj et al. (2011) reported no weight loss, behavioral changes, or organ pathology in mice treated with piperlongumine at tumor-inhibiting doses [1]. Multiple subsequent xenograft studies have confirmed this finding at intraperitoneal doses of 2.4 to 10 mg/kg per day for treatment periods of 14 to 30 days [15, 16, 17, 18].

11.2 Potential safety concerns from mechanism

Despite the favorable acute safety profile in rodents, several mechanistic features warrant monitoring in future translational development:

CYP inactivation. The mechanism-based (irreversible) inactivation of CYP1A2 is of concern because of its permanent nature; recovery requires de novo synthesis of the enzyme [12]. Chronic exposure to piperlongumine could produce cumulative CYP1A2 inactivation with progressive increase in exposure to CYP1A2 substrates. This is a potential hepatotoxicity and drug-interaction concern that would require monitoring in first-in-human studies.

Oxidative stress in sensitive tissues. While the selective pro-oxidant mechanism spares normal cells in short-term exposures, chronic administration at high doses could potentially overwhelm the antioxidant capacity of oxidatively vulnerable normal tissues (cardiac muscle, neurons, hepatocytes). Long-term toxicity studies have not been published.

Reproductive toxicity. No reproductive or developmental toxicity studies have been reported. The compound's pro-oxidant mechanism and its effects on rapidly dividing cells suggest a theoretical concern for embryotoxicity.

11.3 Observed adverse findings in animal studies

No consistent adverse findings have been reported across the published literature. Individual studies have noted transient reduction in food intake at higher doses (10 mg/kg and above), but without corresponding weight loss or morbidity. Hematological, hepatic, and renal function parameters have been reported as normal in studies that assessed these endpoints.

11.4 Safety in the context of traditional use

Long pepper preparations containing piperlongumine have been consumed in Ayurvedic practice for centuries at traditional doses, providing indirect evidence of tolerability at low exposures. However, the anticancer and senolytic doses likely exceed traditional exposure levels substantially, and the traditional safety record cannot be extrapolated to pharmacological dosing.

12. Comparable Compounds Assessment

This section presents a structured comparative assessment of five pro-oxidant, anti-inflammatory, or senolytic natural products against piperlongumine, evaluated against five competency standards: novelty, effect size, promising potential (current development state and trajectory), side-effect profile (preclinical or clinical), and overall validation. The five comparators were selected as the principal natural products with substantive pharmacological characterization in the overlapping domains of ROS-mediated anticancer activity, NF-kB inhibition, and senolytic function.

12.1 Withaferin A

Source and mechanism: Withaferin A is a steroidal lactone (withanolide) isolated from *Withania somnifera* (ashwagandha). It induces ROS accumulation, inhibits NF-kB signaling, suppresses Akt/mTOR, and binds to vimentin intermediate filaments, producing anti-proliferative, pro-apoptotic, and anti-metastatic effects across multiple cancer types [32]. Like piperlongumine, withaferin A contains an electrophilic α,β -unsaturated carbonyl that reacts with cysteine residues on target proteins.

Novelty: Moderate. The withanolide class is well established in the natural product pharmacology literature. The vimentin-binding mechanism is distinctive and differentiates withaferin A from other pro-oxidant natural products.

Effect size: Withaferin A produces significant tumor growth inhibition in xenograft models at doses of 2 to 8 mg/kg, comparable in magnitude to piperlongumine. IC₅₀ values in cancer cell lines are typically 1 to 10 micromolar, somewhat lower (more potent) than piperlongumine in direct comparisons.

Promising potential: Moderate. Multiple Phase 1 and Phase 2 clinical trials of ashwagandha extracts (standardized to withanolide content) have been conducted for stress, anxiety, and athletic performance endpoints. No formal oncology clinical trials of purified withaferin A have been published, though the compound is in active preclinical development for breast cancer and glioblastoma.

Side-effect profile: Ashwagandha extracts are generally well tolerated in human clinical studies at doses up to 600 mg per day. Purified withaferin A has produced hepatotoxicity in rodents at high doses (above 10 mg/kg), a concern not observed with piperlongumine at comparable doses.

Overall validation: Moderate. The preclinical evidence base is substantial and the compound benefits from the broader ashwagandha clinical literature, but purified withaferin A has not been tested in oncology clinical trials.

Comparison to piperlongumine: Both compounds are electrophilic pro-oxidant natural products with overlapping but non-identical target profiles. Withaferin A has higher intrinsic potency in some cell lines and the additional vimentin target. Piperlongumine has superior cancer selectivity data (the Raj et al. normal-versus-cancer panel), a cleaner rodent safety profile, and the established senolytic activity that withaferin A lacks. The two compounds could be complementary in combination studies.

12.2 Sulforaphane

Source and mechanism: Sulforaphane is an isothiocyanate derived from glucoraphanin in cruciferous vegetables (broccoli, broccoli sprouts). It activates the Nrf2 transcription factor (the master regulator of the antioxidant response element), producing a biphasic effect: at lower concentrations, Nrf2 activation upregulates cytoprotective antioxidant enzymes; at higher concentrations, the electrophilic reactivity produces ROS accumulation and cytotoxicity [33]. Sulforaphane also inhibits histone deacetylases (HDACs) and NF- κ B signaling.

Novelty: Low. Sulforaphane is one of the most extensively studied chemopreventive natural products, with research spanning three decades. The mechanism is well characterized and the compound is widely available as a dietary supplement.

Effect size: Modest in oncology. IC₅₀ values in cancer cell lines are typically 5 to 50 micromolar, higher (less potent) than piperlongumine in most direct comparisons. In vivo tumor growth inhibition is generally less pronounced than piperlongumine at comparable doses. The chemopreventive (rather than therapeutic) application is better supported by the evidence.

Promising potential: Moderate to high for chemoprevention; low for therapeutic oncology. Multiple clinical trials have evaluated sulforaphane or broccoli sprout extracts for prostate cancer chemoprevention, with mixed results. The compound is

widely available as a dietary supplement with established human safety data.

Side-effect profile: Excellent. Sulforaphane is consumed regularly as a dietary component. Clinical studies report mild gastrointestinal symptoms (bloating, flatulence) as the principal adverse events. No serious adverse events have been reported at studied doses.

Overall validation: High for chemoprevention research; moderate for therapeutic oncology. The clinical evidence base is broader than piperlongumine's but is directed at a different (preventive rather than therapeutic) application.

Comparison to piperlongumine: Sulforaphane is less potent as a cytotoxic agent but has a more advanced clinical profile and a more favorable safety record. Piperlongumine has superior cancer selectivity, higher potency, and the senolytic activity that sulforaphane lacks. The mechanisms are partially overlapping (both are electrophilic, both modulate NF-kB) but functionally distinct (sulforaphane is primarily Nrf2-activating; piperlongumine is primarily ROS-elevating). The two are more complementary than competitive.

12.3 Parthenolide

Source and mechanism: Parthenolide is a sesquiterpene lactone from the feverfew plant (*Tanacetum parthenium*). It inhibits NF-kB by covalently modifying cysteine 38 of the p65 subunit, generates ROS, depletes glutathione, and selectively kills cancer stem cells and leukemia stem cells [34]. Like piperlongumine, parthenolide contains an electrophilic α,β -unsaturated carbonyl (in the α -methylene- γ -butyrolactone ring) that is the pharmacophoric element for covalent target engagement.

Novelty: Moderate. The NF-kB-targeting sesquiterpene lactone mechanism is well established. The selective leukemia stem cell killing activity, first reported by Guzman et al. (2005), is a distinctive finding [34].

Effect size: Parthenolide produces significant cytotoxicity in leukemia cell lines and primary acute myeloid leukemia blasts at concentrations of 2 to 10 micromolar, comparable to piperlongumine. In vivo efficacy has been limited by poor pharmacokinetics (low oral bioavailability, rapid metabolism). The water-soluble analog dimethylamino-parthenolide (DMAPT) was developed to address these limitations and has advanced to clinical evaluation.

Promising potential: Moderate. DMAPT entered a Phase 1 clinical trial for acute myeloid leukemia and other hematologic malignancies. The parent compound parthenolide has pharmacokinetic limitations that restrict its clinical utility.

Side-effect profile: Feverfew extracts are generally well tolerated; oral parthenolide at dietary supplement doses produces mouth ulceration and gastrointestinal symptoms. Safety data at pharmacological doses are limited.

Overall validation: Moderate. The preclinical evidence is strong, particularly for leukemia stem cells, but the clinical development has been hampered by pharmacokinetic limitations. DMAPT represents an attempt to overcome these limitations.

Comparison to piperlongumine: Both compounds are electrophilic natural products that inhibit NF- κ B and generate ROS. Parthenolide has a stronger evidence base in leukemia stem cell biology; piperlongumine has broader solid-tumor data and superior pharmacokinetic properties (higher oral bioavailability, lower hepatic extraction). The piperlongumine selectivity data (cancer versus normal cell panels) are more comprehensive than those available for parthenolide.

12.4 Curcumin

Source and mechanism: Curcumin is a diarylheptanoid polyphenol from turmeric (*Curcuma longa*). It inhibits NF- κ B, STAT3, and multiple kinase pathways, generates ROS at high concentrations, and has anti-inflammatory, anti-oxidant, and anti-proliferative activities [35]. The compound has been studied in over 200 clinical trials across oncology, inflammatory disease, metabolic disease, and neurodegenerative disease.

Novelty: Low. Curcumin is the single most studied natural product in cancer pharmacology. The mechanism is broadly characterized but the compound suffers from poor bioavailability, rapid metabolism, and concerns about assay interference (the "pan-assay interference compounds" or PAINS designation).

Effect size: Modest in oncology. IC₅₀ values in cancer cell lines are typically 10 to 50 micromolar, higher than piperlongumine. In vivo anticancer efficacy has been inconsistent, with many studies using bioavailability-enhanced formulations. Clinical trials in oncology have produced mixed results, with no definitive demonstration of single-agent anticancer efficacy in a randomized controlled trial.

Promising potential: Low for therapeutic oncology (despite extensive clinical investigation); moderate for anti-inflammatory and chemopreventive applications. The poor bioavailability and the PAINS concerns have reduced enthusiasm for curcumin as a drug development candidate.

Side-effect profile: Curcumin is consumed as a dietary spice and is generally well tolerated at doses up to 8 g per day in clinical studies. Gastrointestinal symptoms (nausea, diarrhea) are the principal adverse events. Rare hepatotoxicity has been reported with high-dose supplementation.

Overall validation: Low to moderate for oncology despite the enormous volume of published research. The gap between preclinical promise and clinical delivery has been a persistent challenge for curcumin.

Comparison to piperlongumine: Curcumin has a vastly larger research literature and more extensive clinical safety data, but its therapeutic utility in oncology is undermined by poor bioavailability and inconsistent clinical efficacy. Piperlongumine has superior potency, higher oral bioavailability, a cleaner selectivity profile (the Raj et al. cancer-versus-normal data), and is not a PAINS compound. As a drug development candidate for anticancer applications, piperlongumine is better positioned despite the smaller evidence base.

12.5 Fisetin

Source and mechanism: Fisetin (3,3',4',7-tetrahydroxyflavone) is a dietary flavonoid found in strawberries, apples, and onions. It has been identified as a senolytic compound that selectively kills senescent cells, in addition to having anti-inflammatory, antioxidant, and anti-proliferative activities [36]. The senolytic mechanism involves inhibition of the PI3K/Akt/mTOR pathway and of the BCL-2 family of anti-apoptotic proteins in senescent cells. Fisetin is the only natural product besides piperlongumine to have been identified as a senolytic in the original dasatinib/quercetin screening paradigm.

Novelty: Moderate. The dual senolytic and anticancer activity is a distinctive positioning. The compound is a dietary flavonoid with established safety.

Effect size: Fisetin produces moderate senolytic activity with selectivity for senescent over non-senescent cells. Anticancer IC₅₀ values are typically 10 to 100 micromolar, lower potency than piperlongumine. In a direct comparison study,

piperlongumine was more potent and more selective as a senolytic agent than fisetin [22, 36].

Promising potential: Moderate to high. A Phase 2 clinical trial of fisetin in older adults (the AFFIRM trial) has been conducted at the Mayo Clinic to evaluate senolytic effects on markers of frailty and inflammation. Preliminary results have been presented. The compound benefits from GRAS (generally recognized as safe) status as a dietary component.

Side-effect profile: Excellent. Fisetin is a dietary component consumed regularly without known adverse effects. Clinical studies report no serious adverse events at doses up to 20 mg/kg per day.

Overall validation: Moderate. The clinical senolytic data are preliminary but the compound is the most clinically advanced natural product senolytic.

Comparison to piperlongumine: Both compounds have senolytic activity, but piperlongumine is more potent and more selective in preclinical assays [22]. Fisetin has a superior clinical development trajectory (clinical trial data exist) and a more favorable safety profile (dietary component, GRAS status). Piperlongumine has the advantage of broader and more potent anticancer activity. The two compounds may be complementary in senolytic research, with fisetin providing the clinical validation bridge and piperlongumine providing the higher preclinical potency.

12.6 Comparator summary

The five comparators occupy distinct positions in the pro-oxidant anticancer and senolytic pharmacology space. **Withaferin A** is the most potent pro-oxidant comparator with the additional vimentin target, but lacks the cancer selectivity data and the senolytic activity of piperlongumine. **Sulforaphane** is the most clinically advanced chemopreventive agent with the best safety profile, but is less potent as a cytotoxic agent and lacks senolytic activity. **Parthenolide** has distinctive leukemia stem cell data but is limited by poor pharmacokinetics; its water-soluble analog DMAPT is the more developable form. **Curcumin** has the largest research literature but the weakest clinical translation, undermined by poor bioavailability and PAINS concerns. **Fisetin** is the most clinically advanced senolytic comparator with established human safety, but is less potent than piperlongumine in both anticancer and senolytic assays.

Piperlongumine occupies a distinctive position in this comparator landscape: it is the only compound with a landmark publication (Raj et al. 2011, Nature) demonstrating systematic cancer selectivity across a panel of transformed versus normal cell lines [1]; it has the broadest target engagement profile (GSTP1, TrxR1, CBR1, STAT3, NF- κ B, PI3K/Akt/mTOR, NLRP3, NR4A1); it has dual oncologic and senolytic potential; and it has favorable rodent pharmacokinetics with oral bioavailability of 50 to 76 percent. Its principal weakness relative to the comparators is the absence of any human clinical data, a gap that limits the translational confidence and that positions fisetin and sulforaphane ahead of piperlongumine in the clinical development timeline.

Investigators considering piperlongumine for anticancer or senolytic research should expect selective cytotoxicity against cancer and senescent cells at concentrations of 5 to 15 micromolar, should account for the CYP inhibition profile in combination studies, and should consider the comparator landscape as the realistic context for translational expectations. The compound's multi-target electrophilic mechanism, the favorable selectivity and safety data, and the commercial availability of research-grade material support its continued use as a preclinical research tool and as a lead compound for medicinal chemistry optimization.

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