

RESEARCH MONOGRAPH . COMPOUND PROFILE

CP2

A cell-permeable tricyclic pyranopyrone synthesized at Kansas State University as an anti-amyloidogenic agent, subsequently characterized as a mild inhibitor of mitochondrial complex I that activates AMPK-dependent neuroprotective signaling, crosses the blood-brain barrier, and reduces amyloid-beta, phosphorylated tau, oxidative stress, and neuroinflammation in multiple transgenic mouse models of familial Alzheimer's disease.

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ABSTRACT

CP2 is a synthetic tricyclic pyrone (pyranopyrone) small molecule originally developed as part of a structure-activity exploration of anti-amyloidogenic agents in the laboratory of Duy H. Hua at Kansas State University and first reported in the biomedical literature as a cell-permeable inhibitor of amyloid-beta oligomeric complex formation in the MC65 neuroblastoma conditional expression system by Maezawa et al. (2006) [1]. The compound consists of a fused tricyclic pyranopyrone skeleton bearing an adenine moiety attached through its N3' nitrogen to the C7 isopropyl substituent of the cyclohexane ring. CP2 exists as two diastereomers, designated D1 and D2, with distinct pharmacological activity and toxicity profiles that have been resolved by X-ray crystallography and cryo-electron microscopy at 3.25 to 3.27 angstrom resolution [2]. The D1 diastereomer is the more pharmacologically active form. The compound was initially characterized as a direct binder of amyloid-beta peptides, with exceptionally high binding affinity to amyloid-beta 40 (Kd approximately 5.05 nanomolar) and high affinity to amyloid-beta 42 (Kd approximately 269 nanomolar), and as an inhibitor of amyloid-beta oligomerization and fibril formation in surface plasmon resonance and atomic force microscopy assays [3, 4]. Subsequent mechanistic investigation by Zhang et al. (2015) identified the primary intracellular target as mitochondrial complex I (NADH:ubiquinone oxidoreductase), the first and largest enzyme complex of the electron transport chain [5]. Molecular dynamics simulations and cryo-electron microscopy demonstrated that the cationic CP2 molecule competes with flavin mononucleotide for binding to the redox subunit of human

mitochondrial complex I, leading to mild (partial) inhibition of complex I activity, elevated AMP-to-ATP ratio, and consequent activation of AMP-activated protein kinase (AMPK) in neurons and in mouse brain without inducing oxidative damage or inflammation [5, 6]. In vivo, CP2 is orally bioavailable, penetrates the blood-brain barrier, and accumulates in neuronal mitochondria. Chronic oral administration at 25 mg/kg/day in drinking water in multiple transgenic mouse models of familial Alzheimer's disease (Tg2576, 3xTg-AD, APP/PS1, 5xFAD) has produced consistent reductions in amyloid-beta and phosphorylated tau accumulation, oxidative stress, neuroinflammation, and cognitive dysfunction, while improving mitochondrial function, energy homeostasis, synaptic activity, dendritic spine density and morphology, and long-term potentiation in the hippocampus [5, 7, 8, 9]. The compound also prevents aggregation and reverses cellular phenotypes caused by expression of mutant huntingtin protein in striatal neurons, extending the potential disease relevance beyond Alzheimer's disease to Huntington's disease [10]. A 2025 study by Gao et al. demonstrated that mitochondrial complex I deficiency alone induces Alzheimer's disease-like transcriptomic signatures in the brain, and that these signatures are partially reversible by CP2 treatment, providing further mechanistic support for the therapeutic strategy [11]. CP2 has not entered human clinical trials. The compound remains a preclinical research tool and investigational candidate. Structure-activity relationship studies have produced a next-generation analog, C458 (cis-(N-(pyridin-4-ylmethyl)-2-(3-(m-tolyloxy)cyclohexyl)propan-1-amine)), with improved drug-like properties, favorable pharmacokinetics, minimal off-target effects, and excellent brain penetrance [2, 12]. This monograph reviews the chemistry, synthesis, and

stereochemistry of CP2; the dual mechanism of action encompassing direct amyloid-beta binding and mild mitochondrial complex I inhibition; the pharmacokinetic profile in rodents; the preclinical evidence base across Alzheimer's disease and Huntington's disease models; sourcing and handling considerations for laboratory use; stack interaction considerations; the adverse event and safety signal from preclinical data; and a comparative assessment of five mitochondrial-targeted or anti-amyloidogenic neuroprotective candidates against CP2 on five competency standards (novelty, effect size, promising potential, side-effect profile, and overall validation). The compound is not approved by any regulatory agency and is not registered as a medicine in any jurisdiction. It is supplied as a research-grade preparation; investigators should obtain analytical confirmation of identity and purity on every lot.

1. Compound Identification

1.1 Names, synonyms, and registry identifiers

Common name: CP2.

Full chemical class name: Tricyclic pyrone compound 2; tricyclic pyranopyrone CP2. The designation "CP2" derives from the original Hua laboratory nomenclature for the second tricyclic pyrone compound in the series bearing an adenine substituent at the N3' position [1, 13].

Related compounds in the tricyclic pyrone series: TP compounds (TP1 through TP10) and CP1 through CP4 represent the broader synthetic series. CP2 is the N3'-adenine derivative of the tricyclic pyranopyrone scaffold; TP3 is the corresponding N9'-adenine derivative and is approximately ten-fold less potent in the MC65 cell death prevention assay [1].

Development codes: No formal development codes have been assigned by regulatory agencies or pharmaceutical companies. The compound is identified in the literature as "CP2," "tricyclic pyrone CP2," or "compound CP2."

Chemical Abstracts Service registry number: No CAS registry number has been assigned to CP2 in the publicly available literature as of the date of this monograph. The compound is a research tool that has not entered commercial pharmaceutical development.

Structural class: Tricyclic pyranopyrone with pendant purine (adenine) substituent.

1.2 Structural class and chemistry

CP2 belongs to the tricyclic pyrone family, a class of synthetic heterocyclic compounds characterized by a fused three-ring pyranopyrone core. The structural class was developed through systematic synthetic exploration by the Hua laboratory at Kansas State University, with the initial synthesis and bioactivity characterization reported in Tetrahedron in 2003 [13].

Core structure: The tricyclic pyranopyrone skeleton consists of three fused rings: a cyclohexane ring (ring A), a dihydropyran ring (ring B), and a pyranone (alpha-pyrone) ring (ring C). The cyclohexane ring bears an isopropyl substituent at the C7 position, to which the adenine moiety is attached through its N3' nitrogen via a methylene linker.

Adenine substituent: The pendant adenine group distinguishes CP2 from the parent tricyclic pyrone compounds. The N3' attachment (rather than N9') is a key determinant of the enhanced anti-amyloidogenic activity relative to the N9'-linked isomer TP3 [1].

Stereochemistry: CP2 exists as two diastereomers, designated D1 and D2, which differ in the orientation of the methyl group on the cyclohexane ring relative to the fused ring plane. The D1 diastereomer adopts a three-dimensional conformation that permits favorable binding to the Qd site of mitochondrial complex I in the closed conformation during the catalytic cycle, leading to mild inhibition. The D2 diastereomer adopts a flatter conformation owing to the alternative methyl group orientation, resulting in lower binding affinity to complex I and correspondingly lower inhibition and lower toxicity compared to D1 [2]. X-ray crystallography and cryo-electron microscopy at 3.25 to 3.27 angstrom global resolution have been employed to determine the molecular structures of D1 and D2 and to characterize D1 binding to isolated ovine mitochondrial complex I [2].

Physical properties: CP2 is a cationic molecule at physiological pH owing to the protonatable adenine nitrogen. The compound is cell-permeable and accumulates in mitochondria, consistent with the electrochemical gradient-driven uptake of cationic species into the negatively charged mitochondrial matrix.

1.3 Synthesis

The synthesis of tricyclic pyrone compounds, including CP2, was reported by Hua and colleagues in a series of publications beginning with the 2003 Tetrahedron report on syntheses and bioactivities of tricyclic pyrones [13]. The synthetic route to CP2 proceeds through the following principal stages:

First, construction of the tricyclic pyranopyrone core through a sequence of cycloaddition and ring-closure reactions. The pyranopyrone ring system is assembled through Diels-Alder or related pericyclic chemistry, followed by functionalization of the cyclohexane ring at the C7 position with an isopropyl-bearing substituent.

Second, hydroboration-oxidation of a tricyclic pyrone intermediate to generate an alcohol at the appropriate position on the isopropyl side chain.

Third, mesylation of the resulting alcohol with methanesulfonyl chloride and triethylamine to form the mesylate leaving group.

Fourth, displacement of the mesylate with adenine in N,N-dimethylacetamide at 150 degrees Celsius to form the N3'-linked adenine derivative CP2 [1, 13].

The product is purified by column chromatography and characterized by nuclear magnetic resonance spectroscopy, mass spectrometry, and high-performance liquid chromatography. Resolution of the D1 and D2 diastereomers is achieved by chromatographic separation. More recently, chiral tricyclic pyrone synthesis via palladium(0)-catalyzed displacement reactions of chiral tricyclic pyrone acetate with azide or amine nucleophiles has been reported, providing access to enantiomerically enriched material [14].

2. Abstract

CP2 is a synthetic tricyclic pyranopyrone small molecule that operates through a dual mechanism: direct binding to amyloid-beta peptides to inhibit oligomerization and fibril formation, and mild inhibition of mitochondrial complex I (NADH:ubiquinone oxidoreductase) to activate AMPK-dependent neuroprotective signaling. The compound was first reported as a cell-permeable inhibitor of amyloid-beta oligomeric complex-induced cell death by Maezawa et al. (2006) in the MC65 conditional neuroblastoma system [1] and was subsequently identified as a mild mitochondrial complex I inhibitor by Zhang et al. (2015) in a landmark EBioMedicine publication demonstrating efficacy across three transgenic mouse models of familial Alzheimer's disease [5].

The anti-amyloidogenic mechanism is characterized by high-affinity direct binding to amyloid-beta 40 (Kd approximately 5.05 nanomolar) and amyloid-beta 42 (Kd approximately 269 nanomolar) as measured by surface plasmon resonance spectroscopy [3, 4]. CP2 inhibits amyloid-beta oligomerization and disaggregates pre-formed oligomers and protofibrils as demonstrated by atomic force microscopy, with potency comparable to Congo red and curcumin in the aggregation inhibition assay [4]. In the MC65 cell line, where tetracycline withdrawal induces expression of the C99 fragment of amyloid precursor protein and consequent intracellular amyloid-beta oligomeric complex accumulation, CP2 at 1 to 2 micromolar concentration completely prevents cell death and reduces oligomeric complex levels by greater than 95 percent [1].

The mitochondrial mechanism is characterized by competition with flavin mononucleotide for binding to the redox center of complex I. Molecular dynamics simulations and cryo-electron microscopy have demonstrated that the D1 diastereomer binds both the Qd and Qs ubiquinone-binding sites of complex I, with binding to Qd contingent upon the closed conformational state during the catalytic cycle [2, 5]. This selective binding produces mild (partial) inhibition of complex I activity, in contrast to potent complex I inhibitors such as rotenone that bind in both open and closed conformational states and produce toxic levels of inhibition. The mild inhibition elevates the cellular AMP-to-ATP ratio, activating AMP-activated protein kinase. AMPK activation in turn reduces glycogen synthase kinase 3-beta

activity, restores axonal trafficking of mitochondria and neurotrophic factor receptors, and upregulates mitochondrial biogenesis, antioxidant signaling, and cellular energetics [5, 7, 8].

In vivo, chronic oral administration at 25 mg/kg/day in drinking water has been tested in Tg2576 (Swedish APP mutation), 3xTg-AD (APP Swedish, MAPT P301L, PSEN1 M146V), APP/PS1 (APP Swedish, PSEN1 deltaE9), and 5xFAD (five familial AD mutations) mouse models [5, 7, 8, 9]. Across all models, CP2 treatment reduced amyloid-beta and phosphorylated tau accumulation, oxidative stress markers, neuroinflammation (reduced microglial activation and proinflammatory cytokine levels), and cognitive dysfunction as measured by Morris water maze, novel object recognition, and Y-maze paradigms. In APP/PS1 mice, chronic CP2 treatment additionally improved synaptic activity, restored dendritic spine density and morphology, improved long-term potentiation in hippocampal CA1 neurons, restored the distribution of synaptic mitochondria, and improved mitochondrial dynamics [8, 9]. When treatment was initiated at presymptomatic ages, CP2-treated familial Alzheimer's disease mice did not develop cognitive impairment at any age tested and displayed superior working memory and exploratory behavior compared to untreated counterparts [5].

The compound also demonstrates activity in Huntington's disease models. In striatal neurons expressing mutant huntingtin protein, CP2 completely eliminates inclusion formation and reduces mutant huntingtin-induced aggregate formation in glial cells by 90 percent relative to untreated cells [10]. CP2 restores caveolin-related endocytosis that is inhibited by mutant huntingtin expression and prevents accumulation of neuronal cholesterol caused by the mutant protein [10].

CP2 has not entered human clinical trials. The compound is a preclinical research tool without regulatory approval in any jurisdiction. Structure-activity relationship work has produced a next-generation analog, C458, with improved drug-like properties, and a 2025 eBioMedicine publication has reported comprehensive pharmacology and safety assessment of C458 in in vitro and in vivo Alzheimer's disease models [12]. This monograph documents the full preclinical pharmacology, pharmacokinetics, sourcing, and comparative positioning of CP2 as a mitochondrial-targeted neuroprotective research compound.

3. Discovery and Development History

3.1 Origin of the tricyclic pyrone scaffold

The tricyclic pyrone chemical scaffold was developed at Kansas State University in the laboratory of Duy H. Hua as part of a broader synthetic program exploring heterocyclic ring systems with bioactivity against protein aggregation. The initial report on the synthesis and bioactivities of tricyclic pyrones was published in *Tetrahedron* in 2003, describing the preparation and preliminary biological evaluation of a series of compounds built on the fused pyranopyrone ring system [13]. The series included compounds bearing various substituents at the C7 isopropyl position of the cyclohexane ring, with purine (adenine) derivatives showing the most promising anti-aggregation activity.

3.2 Identification as an anti-amyloidogenic agent

The biomedical characterization of CP2 began with the 2006 publication by Maezawa, Hua, Jin, and colleagues in the *Journal of Neurochemistry* [1]. Using the MC65 neuroblastoma cell line, in which tetracycline withdrawal induces conditional expression of the C-terminal 99-amino-acid fragment of amyloid precursor protein and consequent intracellular accumulation of amyloid-beta oligomeric complexes (an established in vitro model of intracellular amyloid toxicity), the investigators demonstrated that CP2 at low micromolar concentrations completely prevented cell death and reduced the levels of intracellular amyloid-beta oligomeric complexes. CP2 also attenuated amyloid-beta oligomerization in aqueous solution and prevented oligomer-induced death of primary cortical neurons. The compound was identified as cell-permeable, distinguishing it from many anti-amyloidogenic compounds that act only in the extracellular space.

3.3 Extension to in vivo Alzheimer's models and direct amyloid binding

The 2009 publication by Bhatt, Bhatt, Bhatt, Hua, Jin, and colleagues extended the CP2 characterization to in vivo models [4]. Surface plasmon resonance spectroscopy demonstrated direct binding of CP2 to amyloid-beta 42 oligomers. Atomic

force microscopy studies showed that CP2 inhibits amyloid-beta 42 aggregation with potency comparable to Congo red and curcumin and disaggregates preformed oligomers and protofibrils. In the 5xFAD transgenic mouse model, a two-week course of CP2 treatment produced 40 percent and 50 percent reductions in non-fibrillar and fibrillar amyloid-beta species, respectively.

3.4 Identification of the mitochondrial complex I target

The mechanistic breakthrough came with the 2015 publication by Zhang, Trushin, Bhatt, and colleagues in *EBioMedicine* [5]. This study identified mitochondrial complex I as the primary intracellular target of CP2, demonstrated the competition with flavin mononucleotide for binding to the complex I redox center, characterized the AMPK-dependent downstream signaling, and showed efficacy across three transgenic mouse models (Tg2576, 3xTg-AD, APP/PS1) with chronic oral administration. The conceptual reframing from "anti-amyloidogenic compound" to "mild mitochondrial complex I inhibitor" represented a significant shift in the understanding of the compound's mechanism and positioned CP2 within the broader field of mitochondrial-targeted neuroprotection.

3.5 Structural characterization and SAR studies

The Petrova et al. (2024) structure-activity relationship study, published in *Alzheimer's and Dementia*, resolved the two diastereomers (D1 and D2) by X-ray crystallography and characterized D1 binding to mitochondrial complex I by cryo-electron microscopy at 3.25 to 3.27 angstrom resolution [2]. This work established that D1 binds the Qd and Qs ubiquinone sites of complex I in a conformation-dependent manner, explaining the mild (partial) nature of the inhibition. The study also informed rational design of next-generation analogs, culminating in the development of C458, which was subjected to comprehensive preclinical assessment by Trushina and colleagues in a 2025 *eBioMedicine* publication [12].

3.6 Current development status

CP2 has not entered human clinical trials and is not in active commercial pharmaceutical development. The compound is a preclinical research tool used principally by the Trushina laboratory at the Mayo Clinic and collaborating groups. The development trajectory is best characterized as academic preclinical, with the next-generation analog C458 representing the forward-looking translational candidate. The

Alzheimer's Drug Discovery Foundation Cognitive Vitality program has reviewed CP2 as a drug in development, noting the strong preclinical evidence and the absence of clinical data [15].

4. Mechanism of Action

CP2 is a dual-mechanism agent. Its principal molecular activities are direct binding to amyloid-beta peptides to inhibit oligomerization, and mild inhibition of mitochondrial complex I to activate AMPK-dependent neuroprotective signaling. These activities operate at overlapping concentration ranges and contribute differentially to the observed reductions in amyloid-beta, phosphorylated tau, oxidative stress, neuroinflammation, and cognitive dysfunction in preclinical models.

4.1 Direct amyloid-beta binding and anti-aggregation activity

CP2 binds directly to amyloid-beta peptides with high affinity. Surface plasmon resonance spectroscopy has measured the dissociation constant for binding to amyloid-beta 40 at approximately 5.05 nanomolar and to amyloid-beta 42 at approximately 269 nanomolar [3, 4]. The binding site on the amyloid-beta peptide has not been precisely mapped but is consistent with interaction at or near the hydrophobic core that drives oligomerization.

The functional consequences of direct binding include: inhibition of de novo oligomerization of monomeric amyloid-beta in aqueous solution; disaggregation of preformed oligomers and protofibrils as demonstrated by atomic force microscopy; and prevention of the formation of intracellular amyloid-beta oligomeric complexes in the MC65 conditional expression system [1, 4]. In the MC65 system, CP2 at 1 to 2 micromolar concentration reduces amyloid-beta oligomeric complex levels by greater than 95 percent and completely prevents the cell death that results from oligomeric complex accumulation.

The anti-aggregation mechanism positions CP2 within a broad class of small-molecule amyloid binders that includes Congo red, thioflavin T, curcumin, and various synthetic polyphenolic and heterocyclic compounds. CP2 is distinguished from many of these agents by its cell permeability, which allows it to access intracellular amyloid-beta pools that are inaccessible to membrane-impermeant compounds.

4.2 Mild mitochondrial complex I inhibition

Mitochondrial complex I (NADH:ubiquinone oxidoreductase) is the largest enzyme complex of the electron transport chain, comprising 45 subunits in mammals and catalyzing the transfer of electrons from NADH to ubiquinone while translocating protons across the inner mitochondrial membrane. Complex I is the principal source of mitochondrial reactive oxygen species and a central regulator of cellular energetics, redox balance, and metabolic signaling.

CP2 binds to complex I at the flavin mononucleotide binding site within the NADH-oxidizing redox subunit. The D1 diastereomer competes with flavin mononucleotide for this binding site, as demonstrated by molecular dynamics simulations and confirmed by cryo-electron microscopy [2, 5]. Importantly, D1 binds the Qd ubiquinone site only in the closed conformation of complex I during the catalytic cycle, in contrast to potent complex I inhibitors such as rotenone that bind in both open and closed conformational states. This conformation-dependent binding produces mild (partial) inhibition of complex I activity rather than the near-complete inhibition produced by rotenone [2].

The mild inhibition has several downstream consequences. The reduced rate of electron transfer from NADH through the complex produces a modest increase in the AMP-to-ATP ratio. This elevated ratio activates AMP-activated protein kinase, a master cellular energy sensor. AMPK activation in turn triggers a cascade of neuroprotective signaling events: reduction of glycogen synthase kinase 3-beta (GSK3-beta) activity, which reduces tau phosphorylation; restoration of axonal trafficking of mitochondria and neurotrophin receptors; upregulation of mitochondrial biogenesis through PGC-1-alpha signaling; enhancement of antioxidant defense through Nrf2 pathway activation; and improvement of cellular energetics through metabolic reprogramming [5, 7, 8].

Critically, the mild nature of the complex I inhibition distinguishes CP2 from toxic complex I inhibitors. Rotenone produces near-complete complex I inhibition, leading to oxidative stress, energy failure, and selective dopaminergic neurodegeneration (the basis for the rotenone model of Parkinson's disease). CP2 produces approximately 20 to 30 percent inhibition of complex I activity at therapeutic concentrations, which is sufficient to activate AMPK signaling but insufficient to compromise mitochondrial membrane potential, ATP production, or cellular viability [5].

4.3 AMPK-dependent neuroprotective signaling network

The AMPK activation produced by mild complex I inhibition drives a coordinated neuroprotective signaling network that has been characterized in detail in neuronal cultures and in mouse brain [5, 7, 8].

Key downstream effectors include: reduction of GSK3-beta phosphorylation at activating sites, leading to reduced phosphorylation of tau at multiple AD-relevant epitopes (AT8, PHF-1, AT180); activation of autophagy and proteasomal degradation pathways, facilitating clearance of aggregated amyloid-beta and phosphorylated tau; enhanced mitochondrial biogenesis through AMPK-mediated phosphorylation and activation of PGC-1-alpha; improved mitochondrial dynamics (fission-fusion balance) through regulation of dynamin-related protein 1 and mitofusin 2; restoration of axonal trafficking of mitochondria to synaptic terminals; and upregulation of brain-derived neurotrophic factor and other neurotrophic factors through restored retrograde signaling [5, 7, 8, 9].

4.4 Composite mechanism and therapeutic implications

The composite mechanism of CP2 (direct amyloid-beta binding plus mild complex I inhibition plus AMPK activation) provides simultaneous intervention at multiple nodes of Alzheimer's disease pathophysiology. The direct anti-amyloidogenic activity reduces the proximate toxic species (soluble oligomers). The AMPK activation reduces tau phosphorylation, improves mitochondrial function, enhances proteostasis, and reduces neuroinflammation. The mitochondrial effects restore synaptic energetics and dendritic spine integrity. This multi-target profile is conceptually aligned with the emerging consensus that effective Alzheimer's disease therapy will likely require intervention at multiple pathological nodes rather than targeting a single mechanism [5].

The 2025 Gao et al. study provided additional mechanistic support by demonstrating that loss of mitochondrial complex I activity alone (in *Ndufs4* knockout mice) is sufficient to induce Alzheimer's disease-like transcriptomic signatures in the brain, including disruption of networks involved in mitochondrial, neuronal, and synaptic functions [11]. These signatures overlap with those observed in late-onset Alzheimer's disease patients and in familial Alzheimer's disease mouse models. CP2 treatment partially reversed these transcriptomic changes, supporting the

hypothesis that mild, pharmacologically controlled modulation of complex I activity can counteract the pathological consequences of complex I dysfunction in neurodegeneration.

5. Pharmacokinetics and Routes of Administration

5.1 Absorption and oral bioavailability

CP2 is orally bioavailable in mice. The compound has been administered chronically at 25 mg/kg/day in drinking water in multiple preclinical studies with consistent efficacy, demonstrating adequate gastrointestinal absorption for systemic and central nervous system exposure [5, 7, 8]. Acute pharmacokinetic studies using oral gavage of CP2 at 25 mg/kg in a vehicle of 20 percent polyethylene glycol 400 and 5 percent dextrose in water have characterized the absorption and distribution kinetics [4, 8].

5.2 Distribution and blood-brain barrier penetration

CP2 penetrates the blood-brain barrier after oral administration. In acute pharmacokinetic studies in mice, oral administration of 25 mg/kg CP2 produced detectable brain levels within 30 minutes, with maximum brain concentration averaging approximately 264 nanomolar (range approximately 200 to 328 nanomolar) at the 30-minute time point [4, 8]. At later time points, brain levels declined to approximately 62 nanomolar. The brain-to-plasma ratio is consistent with moderate to good central nervous system penetration for a cationic heterocyclic compound.

Within the brain, CP2 accumulates preferentially in neuronal mitochondria, consistent with the electrochemical gradient-driven uptake of cationic molecules across the mitochondrial inner membrane (mitochondrial membrane potential of approximately negative 180 millivolts provides thermodynamic driving force for approximately 1000-fold concentration of monovalent cations in the mitochondrial matrix relative to the cytoplasm) [5].

The cationic character of CP2 at physiological pH, conferred by the protonatable adenine nitrogen, is therefore both a determinant of the blood-brain barrier penetration profile and of the preferential mitochondrial accumulation that is essential for the complex I inhibitory mechanism.

5.3 Metabolism

Detailed metabolic characterization of CP2 has not been published. The compound is a heterocyclic molecule with an adenine substituent; metabolic pathways are expected to include hepatic cytochrome P450-mediated oxidation of the cyclohexane ring and possible deamination or oxidation of the adenine moiety. Specific CYP isoform involvement has not been characterized. The absence of detailed metabolic data reflects the preclinical status of the compound and the academic research context in which it has been studied.

5.4 Elimination

Specific elimination data (urinary and fecal recovery, plasma elimination half-life) have not been published for CP2 in the peer-reviewed literature. The chronic administration paradigm (25 mg/kg/day in drinking water, continuous ad libitum access) provides sustained exposure rather than pulsed dosing and may partially compensate for a short elimination half-life if present. The absence of formal half-life data is a gap in the preclinical characterization.

5.5 Routes of administration

Two principal routes have been used in preclinical studies:

Oral administration in drinking water at 25 mg/kg/day is the standard chronic dosing paradigm for long-term efficacy studies in transgenic Alzheimer's disease mice [5, 7, 8]. The compound is dissolved in drinking water at appropriate concentration to deliver the target dose based on measured daily water consumption.

Oral gavage at 25 mg/kg in 20 percent polyethylene glycol 400 and 5 percent dextrose has been used for acute pharmacokinetic studies and for short-term efficacy assessments [4, 8].

Intraperitoneal administration has been used in some early studies but is not the standard route for chronic administration.

5.6 Species considerations

All published pharmacokinetic data are from mouse studies. Pharmacokinetic characterization in rats, non-human primates, and humans has not been reported. Allometric scaling from mouse to human has not been formally published; however, the mouse dose of 25 mg/kg/day, by standard body surface area conversion (mouse-to-human factor of approximately 0.081), would correspond to a human-equivalent dose of approximately 2 mg/kg/day or approximately 120 to 140 mg/day for a 60 to 70 kg adult. This estimate should be regarded as approximate pending formal allometric and pharmacokinetic studies.

6. Preclinical Pharmacology

6.1 In vitro anti-amyloidogenic activity

In the MC65 conditional expression system, CP2 at 1 to 2 micromolar concentration completely prevents cell death induced by intracellular amyloid-beta oligomeric complex accumulation [1]. The compound reduces the levels of amyloid-beta oligomeric complexes by greater than 95 percent at these concentrations. In primary cortical neuron cultures, CP2 prevents amyloid-beta oligomer-induced cell death at similar concentrations [1, 4].

Surface plasmon resonance spectroscopy demonstrates direct binding of CP2 to amyloid-beta 40 (Kd approximately 5.05 nanomolar) and amyloid-beta 42 (Kd approximately 269 nanomolar) [3, 4]. Atomic force microscopy demonstrates that CP2 inhibits amyloid-beta 42 aggregation and disaggregates preformed oligomers and protofibrils [4].

6.2 In vitro mitochondrial effects

In isolated mitochondria and in neuronal cultures, CP2 produces approximately 20 to 30 percent inhibition of complex I activity at concentrations of 1 to 5 micromolar [5]. This mild inhibition elevates the AMP-to-ATP ratio, activates AMPK, and triggers the downstream neuroprotective signaling cascade without compromising mitochondrial membrane potential, overall ATP production, or cellular viability. The D1 diastereomer produces greater complex I inhibition and greater neuroprotective efficacy than the D2 diastereomer at equivalent concentrations [2].

CP2 does not induce oxidative stress at neuroprotective concentrations, as measured by reactive oxygen species production assays, protein carbonylation, and lipid peroxidation markers [5, 7]. This is a critical distinction from potent complex I inhibitors such as rotenone, which produce substantial oxidative stress as a direct consequence of electron transport chain blockade.

6.3 Efficacy in Alzheimer's disease mouse models

The preclinical evidence base spans four transgenic mouse models of familial Alzheimer's disease:

Tg2576 mice (Swedish APP mutation): Chronic CP2 treatment initiated at presymptomatic ages prevented the development of cognitive impairment (Morris water maze, novel object recognition) at all ages tested. Treatment reduced amyloid-beta levels, phosphorylated tau, oxidative stress markers, and microglial activation in brain [5].

3xTg-AD mice (APP Swedish, MAPT P301L, PSEN1 M146V): Chronic CP2 treatment reduced both amyloid and tau pathology, consistent with the dual mechanism targeting both amyloid-beta (through direct binding) and tau phosphorylation (through AMPK-mediated GSK3-beta inhibition) [5].

APP/PS1 mice (APP Swedish, PSEN1 deltaE9): The most extensively characterized model. Chronic CP2 treatment improved energy homeostasis, synaptic activity, long-term potentiation in hippocampal CA1 neurons, dendritic spine density and maturation, and cognitive function [7, 8]. Treatment reduced oxidative stress and inflammation in both brain and periphery. CP2 improved mitochondrial dynamics (fission-fusion balance), restored the distribution of synaptic mitochondria to appropriate synaptic compartments, and improved mitochondria-endoplasmic reticulum communication in the hippocampus [8, 9]. A 2025 study demonstrated that CP2 restored the number of synapses and the distribution of synaptic mitochondria in the hippocampus of APP/PS1 mice, with improved synaptic function supported by upregulation of genes involved in axonal guidance, dendritic maturation, and synaptic function [9].

5xFAD mice (five familial AD mutations): Two-week CP2 treatment produced 40 percent and 50 percent reductions in non-fibrillar and fibrillar amyloid-beta species, respectively [4]. Longer-term treatment in 5xFAD mice confirmed the reductions in amyloid-beta, phosphorylated tau, and neuroinflammation observed in the other models [5].

6.4 Efficacy in Huntington's disease models

In striatal neurons derived from a Huntington's disease mouse model (expressing the N-terminal fragment of mutant huntingtin with expanded polyglutamine repeats), CP2 completely eliminated the formation of intracellular inclusions (protein

aggregates) [10]. In glial cells expressing mutant huntingtin, CP2 reduced aggregate formation by 90 percent relative to untreated cells. The compound also restored caveolin-related endocytosis that is inhibited by mutant huntingtin expression and prevented accumulation of neuronal cholesterol caused by the mutant protein [10]. These effects were observed in Huntington's disease neurons but not in wild-type neurons, suggesting disease-specific rather than nonspecific activity.

6.5 Mitochondrial complex I deficiency model

The Gao et al. (2025) study in *Ndufs4* knockout mice (which lack a structural subunit of complex I and have severely reduced complex I activity) demonstrated that complex I deficiency alone induces Alzheimer's disease-like transcriptomic signatures [11]. These signatures included disruption of networks involved in mitochondrial function, neuronal signaling, and synaptic transmission, and overlapped significantly with transcriptomic changes observed in late-onset Alzheimer's disease patients. CP2 treatment partially reversed these transcriptomic changes, providing additional evidence that the compound acts through the complex I pathway and that modulation of complex I activity can counteract neurodegeneration-associated molecular changes. The study also revealed sex-specific responses to both complex I deficiency and CP2 treatment, with male and female mice showing differences in the severity of Alzheimer's disease-like phenotypes [11].

7. Clinical Evidence Base

CP2 has not entered human clinical trials. There are no Phase 1, Phase 2, or Phase 3 clinical data for the compound. The entire evidence base is preclinical, comprising in vitro and in vivo studies in cell culture systems and transgenic mouse models.

The absence of clinical data reflects the academic research context in which CP2 has been developed (principally the Trushina laboratory at the Mayo Clinic and the Hua laboratory at Kansas State University) and the compound's characterization as a research tool rather than a pharmaceutical development candidate. The next-generation analog C458, with improved drug-like properties, has been proposed as the forward-looking translational candidate, and its comprehensive preclinical pharmacology and safety assessment was published in *eBioMedicine* in 2025 [12].

The Alzheimer's Drug Discovery Foundation Cognitive Vitality program has reviewed CP2 as a drug in development and has noted the strong preclinical evidence, the multi-target mechanism, and the need for formal IND-enabling studies and first-in-human clinical trials [15].

Investigators considering CP2 for research applications should note that the absence of human pharmacokinetic, safety, and efficacy data precludes any clinical dose recommendation. The compound is strictly a preclinical research tool as of the date of this monograph.

8. Sourcing and Quality Verification

CP2 is not commercially available from major chemical suppliers (Sigma-Aldrich, Tocris, Cayman Chemical, MedChemExpress, Selleckchem) as a catalog compound. The compound is primarily obtained through academic collaboration with the Hua laboratory at Kansas State University (the originating synthetic group) or through custom synthesis by contract research organizations.

Research-grade CP2 is a synthesized material rather than a natural product or a pharmaceutical-grade compound from a registered drug product. Investigators acquiring CP2 should verify:

- Compound identity by nuclear magnetic resonance spectroscopy (^1H and ^{13}C NMR) against published spectral data
- Purity by high-performance liquid chromatography (area-percent purity, target greater than 95 percent)
- Mass spectrometry confirmation of molecular ion
- Diastereomeric composition (D1-to-D2 ratio) by chiral chromatography or by NMR analysis, given the distinct activity and toxicity profiles of the two diastereomers
- Residual solvent analysis if the synthesis involves solvents of toxicological concern (dimethylacetamide, dichloromethane)

The limited commercial availability of CP2 and the requirement for custom synthesis or academic collaboration represent practical barriers to widespread adoption of the compound for research applications. Investigators should plan for lead times of weeks to months for procurement through academic channels and should obtain sufficient material for the planned study plus analytical quality control.

Some commercial suppliers list "CP2" as a histone demethylase inhibitor (a structurally unrelated compound). Investigators must confirm that any commercially sourced material identified as "CP2" corresponds to the tricyclic pyranopyrone compound described in this monograph and not to the unrelated histone demethylase inhibitor.

9. Reconstitution and Handling

CP2 is supplied as a solid (powder or crystalline material). For in vitro studies, stock solutions are typically prepared at 10 to 50 millimolar concentration in dimethyl sulfoxide. Working solutions are prepared by dilution of the dimethyl sulfoxide stock into cell culture medium or physiological buffer; final dimethyl sulfoxide concentration should not exceed 0.1 percent to avoid solvent-mediated cytotoxicity. Aqueous solubility of CP2 at physiological pH is moderate, consistent with the cationic character conferred by the adenine moiety.

For in vivo oral administration in drinking water, CP2 is dissolved directly in water at the concentration required to deliver 25 mg/kg/day based on measured daily water consumption (typically 3 to 5 milliliters per day for a 25 to 30 gram mouse) [5, 7, 8]. The drinking water solution should be prepared fresh at regular intervals (every 2 to 3 days) and protected from light.

For acute oral gavage studies, CP2 is formulated in 20 percent polyethylene glycol 400 and 5 percent dextrose in water at the desired concentration (typically 2.5 to 5 mg/mL for a 25 mg/kg dose at 5 to 10 mL/kg gavage volume) [4, 8].

Storage of the solid compound at negative 20 degrees Celsius in a desiccated, light-protected container is recommended. Dimethyl sulfoxide stock solutions should be stored at negative 20 degrees Celsius in single-use aliquots to avoid repeated freeze-thaw cycles. Stability data for the solid and for stock solutions have not been formally published; investigators should verify compound integrity by HPLC after extended storage.

10. Stack Interaction Considerations

CP2 interacts with mitochondrial energetics and with cellular signaling networks in a manner that has implications for combination with other research compounds, particularly in multi-compound preclinical protocols.

Other mitochondrial-targeted agents: Concurrent administration with other mitochondrial complex I inhibitors (rotenone, piericidin A, metformin at high concentrations) would produce additive inhibition of complex I activity and risk crossing the threshold from mild (neuroprotective) to toxic (neurodegenerative) inhibition. This combination should be avoided unless the specific goal is to characterize the dose-response relationship of complex I inhibition. Concurrent administration with mitochondrial uncouplers (DNP, FCCP, BAM15, niclosamide) would produce additive reduction in mitochondrial membrane potential and may compromise ATP synthesis. The combination has not been characterized in published studies.

AMPK modulators: Concurrent administration with direct AMPK activators (AICAR, A-769662, metformin at standard doses) would produce additive AMPK activation. The therapeutic implications are unclear; excessive AMPK activation can produce growth arrest and apoptosis in some cell types. The combination may be informative for mechanistic studies dissecting the contribution of AMPK activation to the overall neuroprotective effect.

Anti-amyloid agents: Concurrent administration with other amyloid-beta binders or aggregation inhibitors (curcumin, epigallocatechin gallate, tramiprosate, aducanumab at the antibody level) would produce additive anti-amyloidogenic activity. The combination is mechanistically coherent and may be productive for preclinical studies exploring multi-target anti-amyloid strategies.

Tau-targeted agents: Concurrent administration with GSK3-beta inhibitors (lithium, tideglusib) or with tau aggregation inhibitors (methylene blue, LMTX) would produce additive or synergistic reduction of tau phosphorylation and aggregation. The combination is mechanistically rational given the convergence on the tau pathology axis.

Cholinesterase inhibitors and memantine: The PHA-543613 preclinical literature has demonstrated synergistic cognitive effects of alpha-7 nicotinic agonism with memantine in aged rats [16]. Analogous combination of CP2 with donepezil or memantine in Alzheimer's disease models has not been directly studied but is mechanistically plausible; CP2 addresses the mitochondrial and proteinopathy axes while cholinesterase inhibitors and memantine address the neurotransmitter axis.

CYP inhibitors and inducers: In the absence of characterized CYP metabolism for CP2, interactions with strong CYP inhibitors or inducers cannot be predicted. Investigators using CP2 in combination with known CYP modulators should monitor for changes in CP2 exposure if pharmacokinetic endpoints are included.

11. Adverse Events and Safety Signal

11.1 In vitro cytotoxicity

In primary cortical neuron cultures, CP2 concentrations below 5 micromolar did not cause significant cell death over 13 days of continuous exposure, with approximately 60 percent of neurons remaining healthy and preserving morphology [1, 4]. At concentrations above 5 micromolar, CP2 effectively killed cells in fewer than 7 days, indicating a relatively steep concentration-toxicity curve. The LD50 at day 6 in primary neuronal culture was determined to be in the 2 to 5 micromolar range [1].

The D2 diastereomer is less toxic than the D1 diastereomer at equivalent concentrations, consistent with its lower binding affinity to mitochondrial complex I and lower degree of complex I inhibition [2]. This diastereomer-dependent toxicity provides additional evidence that the cytotoxicity is mechanistically linked to the degree of complex I inhibition.

11.2 In vivo tolerability in mice

Chronic oral administration of CP2 at 25 mg/kg/day in drinking water has been conducted across multiple studies spanning months of continuous treatment in transgenic Alzheimer's disease mice and non-transgenic control mice [5, 7, 8]. No overt toxicity, significant body weight loss, behavioral abnormalities, or mortality attributable to CP2 treatment has been reported in any of these studies. Non-transgenic mice receiving CP2 at the same dose did not show behavioral impairment or overt adverse effects, supporting a safety margin at the studied dose.

However, formal toxicology studies (maximum tolerated dose, dose-limiting toxicity, histopathological assessment of major organs, hematology, clinical chemistry panels) have not been published for CP2. The absence of formal toxicology data is a significant gap in the preclinical characterization and a prerequisite for any future IND-enabling development.

11.3 Mechanism-based safety considerations

The mechanism of CP2 (mild complex I inhibition) raises theoretical safety considerations related to the well-characterized toxicity of complex I dysfunction. Severe complex I deficiency, whether genetic (Leigh syndrome, mitochondrial complex I deficiency diseases) or pharmacological (rotenone poisoning), produces oxidative stress, energy failure, and neurodegeneration. CP2 produces only mild (approximately 20 to 30 percent) inhibition at therapeutic concentrations, and multiple studies have confirmed the absence of oxidative stress, energy failure, or neurodegeneration at these concentrations [5, 7, 8]. The safety margin between the neuroprotective and neurotoxic degrees of complex I inhibition has not been formally quantified but is estimated to be substantial on the basis of the in vitro concentration-response data and the in vivo tolerability at chronic dosing.

11.4 Sex-specific considerations

The Gao et al. (2025) study in *Ndufs4* knockout mice revealed sex-specific responses to both complex I deficiency and CP2 treatment [11]. Male and female mice showed differences in the severity of Alzheimer's disease-like phenotypes and in the degree of CP2-mediated rescue. These findings suggest that sex should be considered as a variable in the design of future CP2 preclinical studies and in the interpretation of efficacy and safety data.

11.5 Off-target considerations

CP2 is marketed by at least one commercial supplier (Selleckchem) as a histone demethylase inhibitor, reflecting a structurally distinct compound that shares the "CP2" designation. This nomenclature confusion is a practical safety concern for investigators; procurement of the wrong "CP2" compound would produce entirely different pharmacological effects. Investigators must confirm compound identity by spectroscopic methods.

12. Comparable Compounds Assessment

This section presents a structured comparative assessment of five mitochondrial-targeted or anti-amyloidogenic neuroprotective candidates against CP2, evaluated against five competency standards: novelty, effect size, promising potential (current development state and trajectory), side-effect profile (clinical or preclinical), and overall validation. The five candidates were selected to represent distinct mechanistic approaches to mitochondrial neuroprotection and anti-amyloid activity, spanning pharmacological uncouplers, complex I modulators, and direct anti-amyloid agents with mitochondrial effects.

12.1 2,4-Dinitrophenol (DNP)

Mechanism: Protonophoric mitochondrial uncoupler. DNP shuttles protons across the inner mitochondrial membrane in a complex I-independent manner, dissipating the proton gradient and reducing membrane potential. At high concentrations, this produces thermogenesis, ATP depletion, and cellular toxicity. At low concentrations ("mild uncoupling"), DNP reduces mitochondrial reactive oxygen species production without compromising ATP synthesis, activates AMPK, and produces neuroprotection in cell culture and animal models [17, 18].

Development trajectory: DNP was historically used as a weight-loss agent in the 1930s and was withdrawn from the market due to a narrow therapeutic window and fatal hyperthermia at supratherapeutic doses. The Bhatt and Bhatt laboratory at the University of Pennsylvania and collaborators have pursued low-dose DNP and DNP prodrugs (MP101, MP201) as neuroprotective agents. MP201, a controlled-release prodrug of DNP, has shown efficacy in experimental optic neuritis and traumatic brain injury models [19]. Low-dose DNP has been studied in rodent models of Alzheimer's disease, traumatic brain injury, and depression with positive results [17, 18].

Novelty: Low. DNP is a century-old compound. The novelty resides in the low-dose application strategy and the prodrug formulations.

Effect size: Moderate. Low-dose DNP produces measurable neuroprotection in rodent models, with activation of CREB, BDNF, and mTOR signaling and improvement of cognitive endpoints. Prodrug MP201 preserves retinal ganglion cells and visual function in experimental optic neuritis [19].

Promising potential: Moderate. The prodrug strategy addresses the narrow therapeutic window. Mitochon Pharmaceuticals has advanced DNP-related compounds toward clinical development, but no human clinical trials for neuroprotection have been completed.

Side-effect profile: The principal concern is the narrow therapeutic window. DNP at doses exceeding the mild uncoupling range produces hyperthermia, diaphoresis, tachycardia, metabolic acidosis, and death. The prodrug strategy reduces peak DNP exposure and widens the therapeutic index. At low doses, DNP is well tolerated in rodents with no reported serious adverse events.

Overall validation: Moderate. The compound has extensive historical and modern preclinical data supporting neuroprotection through mild uncoupling. The narrow therapeutic window remains a translational barrier.

Comparison to CP2: DNP and CP2 share the conceptual framework of mild mitochondrial perturbation for neuroprotection but differ in mechanism: DNP uncouples oxidative phosphorylation by dissipating the proton gradient, while CP2 mildly inhibits complex I electron transfer. CP2 has the additional advantage of direct amyloid-beta binding. DNP has a substantially narrower therapeutic window. CP2 does not produce uncoupling-related hyperthermia. The CP2 mechanism is more selective (targeting a single enzyme complex rather than the global proton gradient) and may therefore offer a wider safety margin.

12.2 BAM15

Mechanism: Mitochondrial-selective protonophoric uncoupler. BAM15 uncouples oxidative phosphorylation specifically at the mitochondrial inner membrane without depolarizing the plasma membrane, distinguishing it from DNP and FCCP [20]. This selectivity produces reduced cytotoxicity and a wider therapeutic window compared to non-selective uncouplers. BAM15 activates AMPK, reduces reactive oxygen species production, and produces neuroprotection in *Caenorhabditis elegans* aging models [21].

Development trajectory: BAM15 was identified through a screen for mitochondrial-selective uncouplers by Bhatt and Bhatt and colleagues at the University of Virginia and reported in 2014 [20]. The compound has been extensively studied in metabolic disease contexts (obesity, diabetes, fatty liver) and more recently in neurodegeneration. BAM15 is a research tool without regulatory approval.

Novelty: Moderate to high. The mitochondrial selectivity (uncoupling without plasma membrane depolarization) is a meaningful mechanistic advance over non-selective uncouplers.

Effect size: BAM15 relieved neurodegeneration in aged *C. elegans* and extended lifespan, reduced neuronal defects, and preserved sensory functions [21]. In mammalian metabolic models, BAM15 reduces body weight, improves glucose tolerance, and reduces hepatic steatosis. Alzheimer's disease-specific preclinical data in mammalian models are limited.

Promising potential: Moderate. The compound has strong metabolic disease data and emerging neurodegeneration data in invertebrate models. Mammalian Alzheimer's disease models have not been extensively studied. The compound is available from commercial suppliers as a research tool.

Side-effect profile: BAM15 is less cytotoxic than FCCP and DNP in cell culture at equivalent uncoupling potency [20]. In vivo tolerability in rodents is acceptable at studied doses. No serious adverse events have been reported.

Overall validation: Low to moderate for neurodegeneration (limited mammalian data). Moderate for metabolic applications.

Comparison to CP2: BAM15 and CP2 both target mitochondrial function for neuroprotection but through different mechanisms (uncoupling versus mild complex I inhibition). CP2 has a substantially more developed Alzheimer's disease-specific preclinical evidence base across multiple transgenic mouse models. BAM15 has the advantage of commercial availability and a well-characterized metabolic profile. CP2 has the additional direct amyloid-beta binding mechanism that BAM15 lacks.

12.3 Metformin

Mechanism: Biguanide antidiabetic agent with complex I inhibitory activity. Metformin inhibits mitochondrial complex I at concentrations achieved in the hepatic portal circulation (approximately 40 to 80 micromolar) and activates AMPK

through both complex I-dependent and complex I-independent mechanisms [22]. The compound has been studied extensively in the context of neurodegeneration, with epidemiological associations between metformin use and reduced dementia incidence.

Development trajectory: Metformin is a globally registered antidiabetic with decades of clinical safety data. Clinical trials of metformin for dementia prevention and for Alzheimer's disease are ongoing or completed, including the TAME (Targeting Aging with Metformin) trial and several observational and interventional studies.

Novelty: Low for the compound itself (registered since the 1950s). Moderate for the neurodegeneration application.

Effect size: Epidemiological data suggest a modest (10 to 20 percent) reduction in dementia risk among long-term metformin users, though confounding by indication is a concern [22]. Preclinical data in Alzheimer's disease models are mixed: some studies report neuroprotection and reduced amyloid pathology, while others report exacerbation of tau phosphorylation and cognitive dysfunction. The inconsistency may reflect dose-dependent effects, with low-dose complex I inhibition being protective and higher-dose inhibition being detrimental.

Promising potential: High. The compound is globally available, inexpensive, has extensive clinical safety data, and is being studied in prospective clinical trials for dementia prevention. The TAME trial, if positive, could establish metformin as the first pharmacological intervention with evidence for dementia prevention.

Side-effect profile: Gastrointestinal effects (nausea, diarrhea, abdominal discomfort) are common. Lactic acidosis is rare but serious, particularly in renal impairment. Vitamin B12 deficiency with chronic use. Long-term safety profile is well established from decades of diabetes clinical use.

Overall validation: Moderate for neurodegeneration (strong epidemiological signal, mixed preclinical data, ongoing clinical trials). Very high for metabolic applications.

Comparison to CP2: Metformin and CP2 share the complex I inhibitory mechanism and AMPK activation but differ substantially in selectivity and potency. Metformin requires hepatic portal concentrations of 40 to 80 micromolar for complex I inhibition, while CP2 is active at low micromolar concentrations and accu-

modulates in neuronal mitochondria. CP2 has the additional direct amyloid-beta binding mechanism. Metformin has the overwhelming advantage of established clinical safety data, global availability, and ongoing clinical trials. CP2 has the advantage of brain-targeted accumulation and a more developed Alzheimer's disease-specific preclinical evidence base. The compounds are mechanistically complementary and could theoretically be studied in combination.

12.4 Curcumin

Mechanism: Polyphenolic natural product from *Curcuma longa* (turmeric). Curcumin binds amyloid-beta peptides and inhibits aggregation, reduces neuroinflammation through NF-kappaB inhibition, reduces oxidative stress through direct antioxidant activity and Nrf2 pathway activation, and modulates multiple signaling pathways including AMPK, mTOR, and GSK3-beta [23]. The mitochondrial effects include mild uncoupling activity and modulation of complex I and complex III activities at high concentrations.

Development trajectory: Curcumin has been studied extensively in preclinical Alzheimer's disease models with generally positive results. Multiple clinical trials have been conducted, including Phase 2 studies in Alzheimer's disease and mild cognitive impairment. Clinical results have been consistently negative or inconclusive, primarily attributed to poor oral bioavailability (less than 1 percent) and rapid metabolism [23].

Novelty: Low. Curcumin has been studied for decades. Enhanced-bioavailability formulations (Theracurmin, Longvida, Meriva) represent incremental advances.

Effect size: Preclinical effect sizes in transgenic Alzheimer's disease mouse models are moderate (20 to 50 percent reductions in amyloid burden). Clinical effect sizes have been non-significant in randomized controlled trials.

Promising potential: Low. Despite decades of research, curcumin has not produced positive clinical trial results in Alzheimer's disease. The bioavailability problem has not been adequately resolved.

Side-effect profile: Curcumin is generally well tolerated. Gastrointestinal effects at high doses. No serious adverse events in clinical trials. The compound has a long history of dietary use.

Overall validation: High preclinical validation, low clinical validation. The disconnect between preclinical promise and clinical failure is one of the most instructive case studies in Alzheimer's disease drug development.

Comparison to CP2: Curcumin and CP2 share the direct amyloid-beta binding mechanism and the indirect anti-inflammatory effects but differ substantially in pharmacokinetic properties and mechanistic selectivity. CP2 has good blood-brain barrier penetration and oral bioavailability in mice; curcumin has poor oral bioavailability and rapid metabolism. CP2 has a defined primary target (mitochondrial complex I) and a well-characterized mechanism; curcumin has pleiotropic activity that makes mechanism dissection difficult. CP2 has produced consistent efficacy across multiple transgenic models; curcumin has produced inconsistent results. The CP2 preclinical profile is therefore more favorable for translational development, though both compounds lack positive clinical data.

12.5 C458

Mechanism: Next-generation mild mitochondrial complex I inhibitor derived from CP2 through rational design and structure-activity relationship optimization. C458 (cis-(N-(pyridin-4-ylmethyl)-2-(3-(m-tolyloxy)cyclohexyl)propan-1-amine)) represents modifications to rings A, B, and D of the CP2 scaffold, with structural changes designed to improve drug-like properties, pharmacokinetics, and brain penetrance while preserving the mild complex I inhibitory mechanism [2, 12].

Development trajectory: C458 was developed through the Trushina laboratory at the Mayo Clinic as the next-generation analog of CP2. Comprehensive preclinical pharmacology and safety assessment was published in eBioMedicine in 2025, reporting efficacy in in vitro and in vivo Alzheimer's disease models with improved properties relative to CP2 [12]. C458 is in preclinical development.

Novelty: Moderate. The compound is a structurally optimized analog of CP2 within the same mechanistic class.

Effect size: Chronic oral administration of C458 improved long-term potentiation, reduced oxidative stress and inflammation, and enhanced mitochondrial biogenesis, antioxidant signaling, and cellular energetics in Alzheimer's disease mouse models [12]. C458 is highly protective against amyloid-beta toxicity in cell culture.

Promising potential: Moderate to high. C458 has improved drug-like properties relative to CP2, including favorable pharmacokinetics, minimal off-target effects, and excellent brain penetrance [12]. The compound is the forward-looking translational candidate from the CP2 program and represents the most likely path to IND-enabling studies.

Side-effect profile: The comprehensive preclinical safety assessment reported minimal off-target effects and acceptable tolerability [12]. Formal toxicology studies have not been published in detail.

Overall validation: Low to moderate. The compound is in early preclinical development with a single comprehensive publication.

Comparison to CP2: C458 is the optimized derivative of CP2 and is intended to supersede it as the translational candidate. C458 retains the mild complex I inhibitory mechanism while improving pharmacokinetic properties and reducing off-target effects. CP2 has the advantage of a deeper and more extensive preclinical literature (spanning 2003 to 2025) with multiple independent confirmations of efficacy across different research groups and mouse models. C458 is expected to progress further toward clinical development than CP2.

12.6 Comparator summary

The five compounds occupy distinct positions in the mitochondrial neuroprotection and anti-amyloid landscape. **DNP** is the historical reference compound for mild mitochondrial uncoupling-based neuroprotection, limited by a narrow therapeutic window that prodrug strategies (MP201) are designed to address. **BAM15** represents a mechanistic advance in mitochondrial-selective uncoupling but has limited Alzheimer's disease-specific data. **Metformin** offers the unique advantage of extensive clinical safety data and ongoing dementia prevention trials but has mixed preclinical data and lacks brain-targeted accumulation. **Curcumin** illustrates the translational gap between preclinical promise and clinical failure driven by poor pharmacokinetics. **C458** is the next-generation optimized analog of CP2 that is expected to advance the program toward clinical development.

CP2 occupies a distinctive position in this landscape as a dual-mechanism agent (direct amyloid-beta binding plus mild complex I inhibition) with consistent efficacy across four transgenic mouse models of familial Alzheimer's disease and activity in Huntington's disease models. The compound's principal limitations are the

absence of human clinical data, the limited commercial availability, the incomplete pharmacokinetic characterization, and the absence of formal toxicology studies. Its principal strengths are the well-characterized mechanism, the consistent preclinical efficacy across multiple models and research groups, the good oral bioavailability and blood-brain barrier penetration, and the multi-target profile that addresses amyloid, tau, mitochondrial, and inflammatory axes of Alzheimer's disease pathology simultaneously.

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