

RESEARCH MONOGRAPH . COMPOUND PROFILE

CAD-31

A synthetic trifluoroacetyl hydrazone derived from the curcumin scaffold via four generations of phenotypic optimization at the Salk Institute, selected for combined neuroprotective and neurogenic activity in human neural precursor cells, with preclinical efficacy in transgenic Alzheimer's disease and accelerated-aging mouse models through AMPK-dependent modulation of fatty acid metabolism, inflammation, and acetyl-CoA homeostasis.

DOCUMENT	KDC-MN-1650
ISSUED	2026-05-22
REVISION	1.0
AUTHOR	Kodi <i>ac</i> biolabs Research
COMPOUND CLASS	Trifluoroacetyl hydrazone geroneuroprotector with AMPK-mediated neuroprotective and neurogenic activity

RESEARCH USE ONLY

ABSTRACT

CAD-31 (CAD-031; CAS 2071209-49-7) is a synthetic trifluoroacetyl hydrazone (molecular weight 404.31, C₁₈H₁₄F₆N₂O₂) derived from the curcumin scaffold through four generations of phenotypic optimization at the Cellular Neurobiology Laboratory of the Salk Institute for Biological Studies. The compound was selected from a library of more than 200 derivatives of the parent compound J147 on the basis of superior potency in a human embryonic stem cell-derived neural precursor cell (hNPC) neurogenesis assay and retention of broad neuroprotective activity across six cell culture models of age-associated brain toxicity [1, 2]. In assays for trophic factor withdrawal, oxidative stress (oxygenotoxicity), in vitro ischemia, extracellular and intracellular amyloid-beta toxicity, and BDNF-like neurotrophic activity, CAD-31 demonstrates EC₅₀ values in the range of 12 to 95 nanomolar, comparable to J147 in most assays and superior in the trophic factor withdrawal model [1]. Neurogenesis markers in human neural precursor cells (nestin, Pax6, doublecortin, Ki67) are elevated 2.6- to 5.2-fold relative to J147 at matched concentrations, representing the distinctive pharmacological advance of CAD-31 within the compound series [2]. The molecular mechanism of CAD-31 converges on the AMP-activated protein kinase (AMPK) signaling cascade. CAD-31 activates AMPK by phosphorylation at threonine 172 and inhibits the downstream target acetyl-CoA carboxylase 1 (ACC1) by phosphorylation at serine 79, resulting in elevated acetyl-CoA levels, reduced free fatty acid synthesis, and increased ketone body availability in the brain [1, 6]. This metabolic reprogramming is accompanied by reductions in inflammatory markers (vascular

cell adhesion molecule, receptor for advanced glycation endproducts, clusterin) and increases in synaptic proteins (drebrin, activity-regulated cytoskeleton-associated protein) in hippocampal tissue of transgenic Alzheimer's disease mice [1]. In the therapeutic APP^{swe}/PS1^{deltaE9} transgenic mouse model of Alzheimer's disease, CAD-31 administered orally at approximately 10 mg/kg/day to symptomatic 10-month-old mice for three months rescued hippocampus-dependent memory deficits in fear conditioning, Morris water maze, and elevated plus maze paradigms to wild-type control levels [1]. In rapidly aging SAMP8 mice, CAD-31 extended median lifespan by approximately 30 percent when administered in the final quarter of life, with corresponding preservation of youthful gene, protein, and metabolite expression profiles in the brain [6]. Pharmacokinetic characterization in rats demonstrates brain penetrance with a brain-to-plasma ratio of 2.8 at eight hours after oral gavage at 20 mg/kg, and maximum brain concentrations approximately tenfold higher than the in vitro EC₅₀ values [1]. Preclinical safety screening shows no acute toxicity at 2 g/kg, no activity in hERG, Ames, or micronucleus assays, and no inhibition of five major cytochrome P450 enzymes at concentrations more than tenfold above the effective range [1]. No formal molecular target has been identified for CAD-31; the parent compound J147 binds the alpha-F1 subunit of mitochondrial ATP synthase (ATP5A), and CAD-31 likely engages the same or a closely related target given the shared downstream AMPK activation and neuroprotective phenotype [7, 8]. CAD-31 has not entered human clinical trials. The parent compound J147 completed a Phase 1 safety study (NCT03838185), and the related compound CMS121 has received National Institutes of Health funding for Investigational New Drug studies [5]. This monograph reviews the chemistry and synthesis lineage,

molecular pharmacology, preclinical pharmacokinetics, in vivo efficacy in transgenic Alzheimer's disease and accelerated-aging models, the safety profile, and a comparative assessment of five compounds in the geroneuroprotector and Alzheimer's disease therapeutic landscape against CAD-31. The compound is available as a research-grade preparation from multiple chemical suppliers; it has no approved medical indication in any jurisdiction.

1. Compound Identification

1.1 Names, synonyms, and registry identifiers

Laboratory designation: CAD-31 (also written CAD-031 or CAD31). The designation was assigned by the Cellular Neurobiology Laboratory at the Salk Institute for Biological Studies during the fourth-generation optimization of the curcumin-derived neuroprotective compound series [1, 2]. CAD-31 is the canonical identifier in all published pharmacological literature.

Chemical Abstracts Service registry number: 2071209-49-7 (free base).

International Union of Pure and Applied Chemistry name: (E)-N-(2,4-dimethylphenyl)-2,2,2-trifluoro-N'-(3-(trifluoromethoxy)benzylidene)acetohydrazide.

No International Nonproprietary Name has been assigned; the compound has not entered regulatory development as a drug substance. No marketed names, brand names, or generic names exist. The compound is identified exclusively by its laboratory designation across all published literature and chemical supplier catalogs.

Other identifiers: SMILES: Cc1ccc(N(N=Cc2cccc(OC(F)(F)F)c2)C(=O)C(F)(F)F)c(C)c1. InChIKey: VDYRRQYRYZTLJV-MRUKODCESA-N.

1.2 Structural class and chemistry

CAD-31 is a trifluoroacetyl hydrazone, a subclass of acylhydrazide characterized by a carbon-nitrogen double bond (hydrazone C=N) bearing a trifluoroacetyl group on the nitrogen adjacent to the aryl substituent. The compound contains two aryl rings connected through a hydrazone linker with an acyl cap: a 2,4-dimethylphenyl group bearing the trifluoroacetyl nitrogen and a 3-(trifluoromethoxy)benzylidene group providing the aldimine carbon. The (E)-configuration of the hydrazone C=N bond is the thermodynamically stable isomer and the form used in all published studies [1].

Molecular formula: C₁₈H₁₄F₆N₂O₂.

Molecular weight: 404.31 g/mol (free base, monoisotopic).

The compound belongs to a series of curcumin-derived neuroprotective agents developed at the Salk Institute, each member defined by modifications to the hydrazone pharmacophore. The structural features that distinguish CAD-31 from its parent J147 (1-(2,4-dimethylphenyl)-2-(3-methoxybenzylidene)hydrazine, molecular weight 351.35) are the replacement of the 3-methoxyphenyl ring with a 3-(trifluoromethoxy)phenyl ring and the trifluoroacetyl group on the hydrazide nitrogen [2, 4]. The trifluoromethoxy substitution confers metabolic stability by eliminating the CYP-labile methoxy group that represents a principal metabolic soft spot in J147 [2].

Stereochemistry: CAD-31 contains no stereogenic centers. The sole geometric isomerism arises from the hydrazone C=N bond; the compound is supplied and studied as the (E)-isomer. Enantiomeric purity considerations are not applicable.

Physical properties: White to off-white solid (powder form). Formal solubility, melting point, and partition coefficient data have not been published in the peer-reviewed literature. The compound is administered in corn oil by oral gavage in rodent pharmacokinetic studies and is mixed in standard rodent chow at 200 parts per million for chronic feeding studies, indicating adequate miscibility in lipid matrices [1]. Solubility in dimethyl sulfoxide is sufficient for in vitro cell culture studies at concentrations up to 10 micromolar [1, 2].

1.3 Synthesis lineage

CAD-31 is the product of four sequential optimization stages originating from the natural product curcumin (diferuloylmethane). The optimization was driven entirely by phenotypic screening against cell-based assays recapitulating age-associated brain toxicities, rather than by optimization against a single molecular target [1, 2].

Stage A: Curcumin to CNB-001 (2008). Liu et al. at the Salk Institute synthesized CNB-001 by hybridizing the curcumin scaffold with cyclohexyl bisphenol A, a compound with neurotrophic activity distinct from curcumin. The resulting pyrazole derivative incorporated neuroprotective and neurotrophic activities of both parent molecules and was broadly neuroprotective in cell culture assays for trophic factor withdrawal, oxidative stress, excitotoxicity, glucose starvation, and amyloid toxicity [3].

Stage B: CNB-001 to CNB-023. Structure-activity relationship optimization of CNB-001 yielded CNB-023, with improved potency in the same neuroprotection assays. The specific structural modifications from CNB-001 to CNB-023 have not been described in detail in the published literature.

Stage C: CNB-023 to J147 (2011). Chen et al. advanced the series through large-scale diversity synthesis from the CNB-023 scaffold. J147 was selected from the resulting compound library for exceptional potency across six neuroprotection assays (EC₅₀ values of 10 to 200 nanomolar), good medicinal chemistry properties (molecular weight 351, cLogP 4.5, total polar surface area 41.9 square angstroms), and classification as high brain-penetrant in MDCK permeability assays [4]. J147 was subsequently validated in multiple transgenic Alzheimer's disease mouse models and in normal rodent cognition assays [4, 14].

Stage D: J147 to CAD-31 (2016). Prior et al. extended the optimization objective from neuroprotection alone to the combination of neuroprotection and neurogenesis, on the rationale that Alzheimer's disease involves both neuronal death and failure of endogenous neural stem cell replacement [2]. A library of more than 200 J147 derivatives was synthesized using structure-activity relationship driven iterative chemistry, with the metabolically labile methoxy and methyl groups on the phenyl rings replaced by isosteric groups including trifluoromethoxy, fluoro, and chloro substituents [2]. Compounds were screened in a human embryonic stem cell-derived neural precursor cell assay for markers of neurogenesis (nestin, Sox1, Sox2, Pax6, doublecortin, Ki67), with concurrent confirmation of neuroprotective activity in the MC65 and HT22 assays. Four compounds (CAD-031, CAD-044, CAD-053, CAD-057) met both neurogenic and neuroprotective criteria. CAD-031 was advanced as the lead on the basis of the highest potency in the hNPC assay and the greatest metabolic stability in microsomal and plasma stability assessments [2].

2. Abstract

CAD-31 (CAD-031; CAS 2071209-49-7) is a trifluoroacetyl hydrazone derived from curcumin through four generations of medicinal chemistry optimization at the Salk Institute for Biological Studies. The compound was developed under a phenotypic screening paradigm that selects for simultaneous neuroprotection and neurogenesis, rather than affinity at a single molecular target [1, 2]. The optimization trajectory proceeded from curcumin to the pyrazole hybrid CNB-001 [3], to the potent neuroprotective compound J147 [4], and finally to CAD-31, which was selected from a library of more than 200 J147 derivatives on the basis of superior neurogenic potency in human embryonic stem cell-derived neural precursor cells and high metabolic stability [2].

The pharmacological profile of CAD-31 encompasses six distinct neuroprotection assays with EC₅₀ values of 12 to 95 nanomolar [1]; neurogenesis markers elevated 2.6- to 5.2-fold relative to J147 in human neural precursor cells [2]; activation of the AMPK/ACC1 signaling cascade with consequent reductions in free fatty acids and increases in acetyl-CoA and ketone bodies in brain tissue [1, 6]; and reductions in inflammatory markers (VCAM, RAGE, clusterin) with concurrent increases in synaptic proteins (drebrin, Arc) in the hippocampus of transgenic Alzheimer's disease mice [1]. In vivo, CAD-31 rescues hippocampus-dependent memory deficits in symptomatic APP^{swe}/PS1^{deltaE9} transgenic mice treated at 10 months of age for three months and extends the median lifespan of rapidly aging SAMP8 mice by approximately 30 percent when administered in the final quarter of life [1, 6].

The compound is brain-penetrant (brain-to-plasma ratio 2.8 at eight hours after oral gavage) and demonstrates a clean preclinical safety profile across standard Investigational New Drug screens [1]. No formal molecular target has been identified for CAD-31; the parent J147 targets mitochondrial ATP synthase (ATP5A), and the shared downstream signaling suggests a related target engagement [7, 8]. CAD-31 has not entered human clinical trials and has no approved indication in any jurisdiction. The published literature is concentrated in approximately 12 primary publications from the developing laboratory; the evidence base reflects this concentration.

3. Discovery and Development History

3.1 Origin and screening paradigm

CAD-31 was developed at the Cellular Neurobiology Laboratory of the Salk Institute for Biological Studies in La Jolla, California, under the direction of David Schubert and Pamela Maher. The compound emerged from a drug discovery paradigm that departs from the target-based approach that has dominated Alzheimer's disease drug development for the past three decades. Rather than selecting compounds for affinity at a single molecular target (amyloid-beta, tau, acetylcholinesterase, or specific kinases), the Schubert laboratory employed phenotypic screening against multiple cell-based assays designed to recapitulate the toxicities associated with the aging brain: energy depletion, oxidative stress, loss of trophic support, proteotoxicity, and inflammation [4, 5].

The rationale, articulated in the 2019 Trends in Pharmacological Sciences review introducing the "geroneuroprotector" concept, holds that age is the greatest risk factor for Alzheimer's disease, that the aging brain is subject to a convergence of multiple toxic insults rather than a single molecular lesion, and that compounds selected for efficacy against multiple age-associated toxicities are more likely to have therapeutic value than compounds selected for single-target affinity [5]. The geroneuroprotector class, as defined by Schubert and colleagues, comprises compounds that simultaneously meet the criteria for geroprotectors (slowing biological aging in model organisms and extending lifespan) and neuroprotectors (protecting the brain from disease-associated degeneration) [5].

3.2 Synthetic lineage

The four-stage synthetic lineage from curcumin to CAD-31 is described in Section 1.3. The critical conceptual advance at each stage was a shift in the primary selection criterion: CNB-001 was selected for broad neuroprotection [3]; J147 was selected for potency, oral bioavailability, and brain penetrance [4]; and CAD-31 was selected for the dual properties of neuroprotection and neurogenesis in human neural precursor cells [2]. This evolution reflects the laboratory's progressive un-

derstanding that a disease-modifying Alzheimer's agent would need to both protect existing neurons from ongoing toxicity and stimulate the replacement of neurons already lost.

3.3 Preclinical characterization

Daugherty et al. (2017) published the definitive preclinical characterization of CAD-31, reporting neuroprotective EC50 values in six cell-based assays (12 to 95 nanomolar), pharmacokinetic properties (brain-to-plasma ratio of 2.8; peak brain and plasma concentrations at steady state in mice fed 200 ppm in chow), preliminary toxicology (clean in hERG, Ames, micronucleus, CYP, and kinase panels at 10 micromolar), and in vivo efficacy in 10-month-old APPswe/PS1deltaE9 transgenic mice treated for three months [1]. The study additionally reported transcriptomic profiling (730 differentially expressed genes in treated AD mice) and metabolomic profiling (plasma and brain cortex) identifying the AMPK/ACC1 fatty acid metabolism axis as the dominant pharmacological pathway [1].

3.4 Aging and lifespan studies

Currais et al. (2021) applied CAD-31 as a chemical biology probe to identify molecular pathways shared between aging and Alzheimer's disease, testing the compound in the rapidly aging SAMP8 mouse model. CAD-31 extended the median lifespan of SAMP8 mice by approximately 30 percent when administered in the final quarter of life, and reduced molecular markers of mitochondrial aging in the brain [6]. The compound activated AMPK and inhibited ACC1 in both HT22 nerve cells and primary neurons, with inhibition of ACC1 accompanied by a decrease in free fatty acids in the brains and plasma of SAMP8 mice [6]. Network analysis identified acetyl-CoA as a central metabolite in CAD-31's mechanism of action [6]. This lifespan extension, combined with the cognitive rescue in transgenic Alzheimer's disease mice, positioned CAD-31 as both a disease-modifying candidate and a geroprotector, fulfilling the geroneuroprotector definition proposed in the 2019 review [5, 6].

3.5 Current development status

CAD-31 has not entered human clinical development. No Investigational New Drug application has been filed for CAD-31, no clinical trial registry entry exists, and no regulatory submission has been made in any jurisdiction. The parent compound

J147 completed a Phase 1 safety study in healthy volunteers (ClinicalTrials.gov identifier NCT03838185); the trial is listed as completed, but results have not been published in a peer-reviewed format as of the present monograph revision [12]. The related compound CMS121, a fisetin-derived geroneuroprotector from the same laboratory that shares the AMPK/ACC1 mechanism, has received National Institutes of Health funding for Investigational New Drug-enabling studies [5]. The path from CAD-31 to clinical evaluation is therefore informed by, but behind, the development trajectories of J147 and CMS121.

4. Molecular Pharmacology

4.1 Neuroprotective activity

The neuroprotective activity of CAD-31 was characterized in six distinct cell culture assays designed to recapitulate toxicities observed in the aging brain. Daugherty et al. (2017) reported the following EC₅₀ values in nanomolar: trophic factor withdrawal in primary cortical neurons, 18 nM (compared to 35 nM for J147); BDNF-like neurotrophic activity in HT22 hippocampal neurons, 95 nM (J147: 74 nM); oxidative stress via glutamate-induced oxytosis in cortical neurons, 20 nM (J147: 6 nM); in vitro ischemia in HT22 cells, 47 nM (J147: 14 nM); extracellular amyloid-beta toxicity in primary hippocampal neurons, 27 nM (J147: 15 nM); and intracellular amyloid-beta toxicity in the MC65 human CNS nerve cell line, 12 nM (J147: 10 nM) [1].

The neuroprotective potency of CAD-31 is thus broadly comparable to J147 across assay systems, with a modest improvement in the trophic factor withdrawal assay (approximately twofold) and modestly lower potency in the oxytosis and ischemia assays. The consistent nanomolar activity across six mechanistically distinct toxicity models supports a multi-target or pathway-level mechanism of neuroprotection rather than blockade of a single receptor or enzyme. Prior et al. (2016) confirmed the neuroprotective activity of CAD-31 in the MC65 and HT22 assays during the compound selection phase, reporting that compounds with trifluoromethoxy substitutions on the B-ring showed improved neuroprotective activity relative to the methoxy-substituted parent [2].

4.2 AMPK/ACC1/acetyl-CoA signaling cascade

The dominant molecular pathway engaged by CAD-31 is the AMP-activated protein kinase (AMPK) signaling cascade. Daugherty et al. (2017) demonstrated that CAD-31 enhances AMPK phosphorylation at threonine 172 in the hippocampus of both wild-type and APP^{swe}/PS1^{deltaE9} transgenic mice after three months of oral administration at 200 ppm in chow [1]. Activated AMPK phosphorylates the downstream target acetyl-CoA carboxylase 1 (ACC1) at serine 79, inhibiting ACC1's cat-

alytic conversion of acetyl-CoA to malonyl-CoA; the resulting shift in metabolic flux preserves acetyl-CoA for mitochondrial oxidative metabolism and ketone body synthesis while reducing de novo fatty acid synthesis [1].

In the APPswe/PS1deltaE9 mice, the AMPK/ACC1 axis was dysregulated at baseline: phosphorylation of ACC1 was depressed in untreated AD mice relative to wild-type controls. CAD-31 treatment restored and substantially increased ACC1 phosphorylation in AD mice while modestly reducing it in wild-type mice, a pattern consistent with normalization of a disease-disrupted pathway rather than simple pharmacological activation [1].

Currais et al. (2021) independently confirmed that CAD-31 activates AMPK and inhibits ACC1 in HT22 nerve cells and in primary cortical neurons, establishing that the pathway engagement observed in vivo is reproduced in isolated neural cell systems [6]. The inhibition of ACC1 was accompanied by a decrease in free fatty acids in the brains and plasma of SAMP8 mice, and network analysis identified acetyl-CoA as a central metabolite in CAD-31's mechanism of action [6].

The AMPK/ACC1/acetyl-CoA mechanism is shared across the geroneuroprotector compound family. Currais et al. (2019) demonstrated that CMS121 and J147, despite different structures and initial molecular targets, both increase acetyl-CoA levels in cell culture and in SAMP8 mice through AMPK-dependent inhibition of ACC1 [8]. The convergence of three structurally distinct compounds (J147, CMS121, and CAD-31) on the same metabolic pathway through AMPK activation suggests that AMPK/ACC1 signaling is a critical node for neuroprotective and geroprotective efficacy in the aging brain [6, 8].

4.3 Relationship to J147's ATP synthase target

The molecular target of J147, the parent compound of CAD-31, was identified by Goldberg et al. (2018) as the alpha-F1 subunit of mitochondrial ATP synthase (ATP5A). J147 binds ATP5A and produces saturating partial inhibition of ATP synthase activity (23.6 percent inhibition at saturation) with an EC50 of 20 nanomolar [7]. This partial inhibition increases mitochondrial membrane potential, elevates cytosolic calcium, and activates calcium/calmodulin-dependent protein kinase kinase beta (CAMKK2), which in turn phosphorylates AMPK at threonine 172 [7]. The pathway therefore runs: J147 binds ATP5A, partial inhibition of ATP synthase, increased mitochondrial membrane potential, calcium mobilization, CAMKK2 activation, AMPK phosphorylation, ACC1 inhibition, acetyl-CoA preservation [7, 8].

No equivalent target identification has been published for CAD-31. The formal molecular target of CAD-31 remains unknown. However, the shared downstream signaling (AMPK activation, ACC1 inhibition, acetyl-CoA elevation) and the structural relationship to J147 (CAD-31 differs from J147 only in the trifluoromethoxy substitution on the B-ring and the trifluoroacetyl group on the hydrazide nitrogen) make it plausible that CAD-31 engages ATP5A or a closely related mitochondrial target. Investigators should note that this is an inference from shared pharmacology rather than a demonstrated binding event; the formal target of CAD-31 is an open question [6, 7].

4.4 Neurogenic activity

The neurogenic activity of CAD-31 is the distinctive pharmacological property that differentiates it from J147 and the other compounds in the series. Prior et al. (2016) demonstrated that CAD-31 at 100 nanomolar for seven days in human embryonic stem cell-derived neural precursor cells (hNPCs, derived from Hues6 and H9 lines) elevated markers of neurogenesis relative to J147 at matched concentrations: nestin (self-renewal marker) was increased 2.6-fold, Pax6 (neural progenitor marker) 5.1-fold, doublecortin (DCX; neuronal restriction marker) 3.0-fold, and Ki67 (proliferation marker) 5.2-fold [2]. These fold increases were measured by quantitative PCR relative to J147-treated cells, with fibroblast growth factor-2 as the positive control for proliferation [2].

The neurogenic activity was confirmed in vivo. In APPswe/PS1deltaE9 mice treated with CAD-31 for three months, the number of BrdU-positive cells (marking recently dividing cells) was increased in the granule cell layer of the dentate gyrus, and the number of BrdU-positive/DCX-positive double-labeled cells (marking newly generated neurons) was significantly increased in both wild-type and AD mice treated with CAD-31 relative to untreated controls [2]. At the protein level, Daugherty et al. (2017) confirmed that DCX expression was elevated in the hippocampus of CAD-31-treated AD mice [1].

4.5 Anti-inflammatory effects

CAD-31 reduces multiple markers of brain inflammation in the APPswe/PS1deltaE9 transgenic mouse model. Daugherty et al. (2017) reported that three months of CAD-31 treatment at 200 ppm in chow reduced hippocampal protein levels of three inflammation-associated markers: vascular cell adhesion molecule (VCAM), which

is induced by reactive oxygen species and is elevated in Alzheimer's disease patients; receptor for advanced glycation endproducts (RAGE), which promotes neuroinflammation and is elevated in Alzheimer's disease brain; and clusterin, a stress-induced chaperone molecule that serves as a biomarker for inflammation in Alzheimer's disease [1]. VCAM and RAGE were reduced to or below wild-type control levels; clusterin was significantly reduced but not fully normalized [1].

Transcriptomic analysis of untreated AD mice relative to wild-type controls identified cytokine-cytokine receptor interactions, tumor necrosis factor signaling, and Toll-like receptor signaling as the dominant upregulated pathways. CAD-31 treatment reversed this inflammatory gene expression signature, consistent with the observed reductions in protein-level inflammation markers [1]. The anti-inflammatory mechanism is attributed in part to AMPK activation, which inhibits nuclear factor-kappa-B (NF-kappaB) activity and thereby suppresses proinflammatory cytokine transcription [1].

4.6 Metabolic reprogramming

CAD-31 produces a characteristic metabolic reprogramming in both brain cortex and plasma. Metabolomic profiling of wild-type and APP^{swe}/PS1^{deltaE9} mice treated for three months identified the following principal changes: increased ketone body levels (including 3-hydroxybutyrate), decreased long-chain free fatty acids, increased acyl-carnitine species (reflecting enhanced mitochondrial fatty acid beta-oxidation), and modified sphingolipid profiles [1]. The top metabolic pathways modified by CAD-31 in the plasma of wild-type mice were ketone bodies, long-chain fatty acids, acyl carnitines, and sphingolipids [1]. In brain cortex, the pattern was consistent: long-chain fatty acids and monoacylglycerols were decreased, while acetyl-CoA levels were elevated [1].

The metabolic signature is mechanistically coherent with the AMPK/ACC1 pathway: inhibition of ACC1 blocks the conversion of acetyl-CoA to malonyl-CoA, reducing de novo fatty acid synthesis and increasing the availability of acetyl-CoA for the tricarboxylic acid cycle, for ketogenesis, and for histone acetylation [1, 8]. The increase in acyl-carnitines reflects enhanced shuttling of existing fatty acids into mitochondria for beta-oxidation, a compensatory shift in energy metabolism that maintains ATP production while diverting carbon flux away from lipid synthesis [1, 8].

Goldberg et al. (2022) demonstrated that J147 reduces blood plasma free fatty acid levels through hepatic AMPK/ACC1 activation, and specifically noted that CAD-31 was capable of reducing several free fatty acid species in the plasma of wild-type C57BL/6 mice, confirming the systemic metabolic effects beyond the brain [9].

4.7 Selectivity and off-target profile

CAD-31 demonstrates a clean selectivity profile in standard preclinical off-target panels. At 10 micromolar (a concentration more than 100-fold above the neuroprotective EC₅₀ values), CAD-31 showed no interaction with any protein in a lead profile screen panel of 60 brain ion channels, transporters, and receptors, and no inhibition of any protein kinase in a panel of 352 kinases [1]. The compound did not block the activities of five major cytochrome P450 enzymes (CYP1A2, CYP2C9, CYP2C19, CYP2D6, CYP3A4) at 10 micromolar [1]. These data indicate that the pharmacological activity of CAD-31 is unlikely to arise from direct interaction with conventional drug targets (G-protein-coupled receptors, ligand-gated ion channels, kinases, or metabolic enzymes) and is instead attributable to modulation of cellular metabolic pathways, consistent with the AMPK/ACC1 mechanism [1].

5. Pharmacokinetics

5.1 Brain penetrance and distribution

CAD-31 is brain-penetrant after oral administration. Daugherty et al. (2017) reported pharmacokinetic data from rats administered 20 mg/kg by oral gavage in corn oil: the brain-to-plasma ratio at eight hours post-dose was 2.8, and the maximum brain concentration was approximately tenfold higher than the EC50 values in the cell culture neuroprotection assays [1]. This ratio indicates preferential brain accumulation, a property that supports central nervous system target engagement at achievable oral doses.

In mice maintained on chow containing 200 ppm CAD-31 (approximately 10 mg/kg/day based on standard food consumption estimates), steady-state plasma concentrations reached a peak of 82 nanomolar and brain concentrations reached a peak of 53 nanomolar when measured in ad libitum-fed animals [1]. Both the plasma and brain concentrations are within the range of the in vitro EC50 values (12 to 95 nanomolar across assays), confirming that the oral dose used in the chronic efficacy studies achieves pharmacologically relevant concentrations [1].

5.2 Metabolic stability

CAD-31 was selected in part for metabolic stability superior to J147 and to other derivatives in the library. Prior et al. (2016) reported that CAD-31 was the most stable compound in microsomal and plasma stability assessments among the four qualifying derivatives (CAD-031, CAD-044, CAD-053, CAD-057) [2]. The improved stability is attributed to the trifluoromethoxy substitution on the B-ring, which replaces the CYP-labile methoxy group of J147 with a metabolically resistant fluorinated ether [2].

No published data on the specific metabolic pathways of CAD-31 are available. No CYP isoform has been identified as the primary metabolizing enzyme for CAD-31. The compound does not inhibit five major CYP enzymes at 10 micromolar, suggesting a low risk of CYP-mediated drug-drug interactions at concentrations relevant to in vivo pharmacology [1].

5.3 Comparison to parent compound J147

The pharmacokinetic profile of CAD-31 has not been characterized to the same depth as that of J147. For comparative context: J147 has an oral bioavailability of approximately 28 percent in mice and a plasma elimination half-life of approximately 2.5 hours after oral administration [13]. J147 is metabolized to five oxidation products (identified in mouse and human microsomes), none of which showed hazardous potential [13]. The hydrazone scaffold of J147 remained intact during metabolism, with no hydrolysis of the amide or trifluoroacetamide bonds observed [13].

The improved microsomal stability of CAD-31 relative to J147 [2] suggests that the oral bioavailability and elimination half-life of CAD-31 may be modestly superior to those of J147, but formal comparative pharmacokinetic studies have not been published.

5.4 Limitations of the pharmacokinetic record

Investigators should note several significant gaps in the pharmacokinetic characterization of CAD-31. Formal oral bioavailability has not been reported. Plasma elimination half-life has not been published. The volume of distribution, plasma protein binding, and renal clearance fraction are unknown. No human pharmacokinetic data exist. The available data are limited to the rat brain-to-plasma ratio at a single time point and the steady-state mouse tissue concentrations at a single dietary dose level [1]. Investigators planning pharmacokinetic studies with CAD-31 should design dose-finding experiments that characterize the time-concentration profile across multiple doses and time points.

6. Preclinical Pharmacology

6.1 In vitro neuroprotection

The six-assay neuroprotection panel is described in Section 4.1. CAD-31 demonstrates nanomolar EC50 values (12 to 95 nM) across assays for trophic factor withdrawal, oxidative stress, metabolic stress, and amyloid proteotoxicity in primary cortical neurons, hippocampal neurons, and established cell lines (HT22, MC65) [1]. The breadth of activity across mechanistically distinct toxicity models is the signature of the phenotypic screening paradigm: CAD-31 was selected to protect against the convergence of insults characteristic of the aging brain, rather than to block a single death pathway.

6.2 APPswe/PS1deltaE9 transgenic mouse model (therapeutic paradigm)

The definitive in vivo efficacy study for CAD-31 was a therapeutic-paradigm trial in the APPswe/PS1deltaE9 (line 85) transgenic mouse model of familial Alzheimer's disease. Study design: 10-month-old mice (already symptomatic with established amyloid pathology and cognitive deficits) were administered CAD-31 at 200 ppm in standard mouse chow (approximately 10 mg/kg/day) for three months, with sacrifice at 13 months. Control groups included age-matched untreated wild-type littermates and untreated AD transgenic mice. Ten mice per group were used [1].

Behavioral outcomes: CAD-31 treatment rescued cognitive deficits across three hippocampus-dependent behavioral paradigms. In the elevated plus maze, AD mice showed pathological disinhibition (increased time on open arms), a phenotype completely rescued to age-matched wild-type levels by CAD-31 treatment [1]. In contextual fear conditioning, AD mice showed significantly reduced freezing in response to the conditioned context (indicating impaired hippocampal memory); CAD-31 treatment increased freezing time to wild-type control levels [1]. In the Morris water maze, AD mice showed prolonged escape latency on the hidden platform trial; CAD-31 significantly reduced escape latency to control levels [1]. Prior et

al. (2016) additionally reported that CAD-31 restored the recognition index in the hippocampus-dependent spatial pattern separation test in the same mouse model [2].

Inflammatory markers: Three inflammation-associated proteins were measured in hippocampal tissue by western blotting. Vascular cell adhesion molecule (VCAM), receptor for advanced glycation endproducts (RAGE), and clusterin were all significantly reduced in CAD-31-treated AD mice relative to untreated AD mice. VCAM and RAGE were reduced to or below wild-type levels; clusterin was significantly reduced but not normalized to control levels [1].

Synaptic and neurogenic markers: The synaptic proteins drebrin and activity-regulated cytoskeleton-associated protein (Arc) were increased in the hippocampus of CAD-31-treated AD mice relative to untreated AD mice [1]. The neurogenesis marker doublecortin (DCX) was elevated in CAD-31-treated mice [1]. The oligomycin sensitivity-conferring protein (OSCP), a synapse-associated mitochondrial protein reduced in Alzheimer's disease, was increased by CAD-31 treatment in both wild-type and AD mice [1].

Protein aggregation: Accumulation of RIPA-insoluble ubiquitinated proteins in the hippocampus (a marker of age-associated proteostasis failure) was reduced by CAD-31 in both wild-type and AD mice [1].

Amyloid pathology: In a notable negative finding, CAD-31 treatment did not reduce the number or size of amyloid plaques in the brains of treated AD mice [1]. The cognitive rescue therefore occurred through mechanisms independent of amyloid clearance, consistent with the compound's primary action on metabolism, inflammation, and synaptic integrity rather than on amyloid-beta processing or degradation.

Body weight and tolerability: Mouse body weights and food consumption were measured weekly throughout the three-month treatment period; no significant differences between groups were observed [1]. The open-field locomotor assay showed no differences between groups, indicating absence of overt neurological toxicity or sedative effects [1].

6.3 SAMP8 accelerated-aging model

Currais et al. (2021) tested CAD-31 in the senescence-accelerated mouse prone 8 (SAMP8) model, a strain that exhibits accelerated aging with a median lifespan of approximately 15 months. CAD-31 was administered in chow beginning in the final quarter of the SAMP8 lifespan and extended the median lifespan by approximately 30 percent [6]. The treatment reduced changes in gene, protein, and small-molecule expression associated with mitochondrial aging in the brain, maintaining a more youthful molecular phenotype [6].

The SAMP8 findings are mechanistically coherent with the APPswe/PS1deltaE9 results: AMPK activation, ACC1 inhibition, decreased free fatty acids, and preservation of mitochondrial function are the shared pathways. The critical additional finding from the SAMP8 study is the lifespan extension, which positions CAD-31 as a geroprotector with effects on overall organismal aging beyond the Alzheimer's disease phenotype [6].

Separate studies of the compound family established that J147 extends lifespan in *Drosophila melanogaster* by 9.5 to 12.5 percent [7], and that both J147 and CMS121 slow age-associated transcriptomic and metabolomic drift in SAMP8 mice [8, 11]. The convergence of lifespan and healthspan benefits across the geroneuro-protector compound family supports the hypothesis that targeting age-associated mitochondrial and metabolic dysfunction provides broader benefits than targeting amyloid-beta alone.

6.4 Neurogenesis in vivo

Prior et al. (2016) assessed neurogenesis in vivo using BrdU incorporation in APPswe/PS1deltaE9 and wild-type mice treated with CAD-31. The number of BrdU-positive cells was increased after CAD-31 administration in the granule cell layer of the dentate gyrus in AD mice [2]. Both wild-type and AD mice treated with CAD-31 displayed a significant increase in the number of BrdU-positive/doublecortin-positive double-labeled cells compared to mice fed normal chow, demonstrating that CAD-31 stimulates the generation of new neurons in vivo and that this effect is not limited to the diseased brain [2].

6.5 Transcriptomic and metabolomic profiling

Transcriptomics: RNA-sequencing of hippocampal tissue from the therapeutic APPswe/PS1deltaE9 study revealed 29 significantly changed genes in wild-type mice treated with CAD-31 and 730 significantly changed genes in AD mice treated with CAD-31 [1]. KEGG pathway analysis of the AD-specific drug effect identified oxidative phosphorylation, long-term potentiation, AMPK signaling, and Alzheimer's disease-related pathways as the dominant enriched categories [1]. Genes associated with ATP synthesis were upregulated by CAD-31, consistent with the metabolic reprogramming evident in the metabolomic data [1].

Metabolomics: Small-molecule metabolomic profiling of brain cortex and plasma identified the pathway-level changes described in Section 4.6: increased ketone bodies, decreased long-chain fatty acids, increased acyl-carnitines, elevated acetyl-CoA, and modified sphingolipid profiles [1]. The metabolomic signature in CAD-31-treated mice is consistent with AMPK-driven suppression of de novo lipogenesis and enhancement of mitochondrial fatty acid oxidation.

6.6 Translational considerations

The preclinical efficacy of CAD-31 has been demonstrated in two complementary mouse models: a transgenic amyloid-overexpression model (APPswe/PS1deltaE9) and an accelerated-aging model (SAMP8). The therapeutic paradigm (treatment initiated after symptom onset at 10 months in the AD model and in the final quarter of life in the SAMP8 model) is clinically relevant, as human patients typically present after substantial neurodegeneration has occurred. The cognitive rescue in the AD model without amyloid plaque reduction is noteworthy: it suggests that CAD-31's benefits derive from metabolic and inflammatory modulation rather than from amyloid clearance, a mechanism distinct from the anti-amyloid antibodies that have dominated recent Alzheimer's drug development.

Investigators should note that the efficacy data derive from a single laboratory, that replication by independent groups has not been published, and that the two mouse models represent different aspects of Alzheimer's disease pathophysiology (familial amyloid overexpression versus accelerated general aging) without fully modeling sporadic late-onset Alzheimer's disease.

7. Clinical Evidence Base

CAD-31 has not been administered to human subjects. No clinical trial, at any phase, has been registered or conducted with CAD-31 as of the present monograph revision. The compound's evidence base is entirely preclinical, as described in Sections 4 through 6.

For contextual framing of the clinical development trajectory of the geroneuro-protector compound family, the following points are noted:

J147 (parent compound). A Phase 1 safety, tolerability, and pharmacokinetics study of J147 in healthy adult volunteers was completed under ClinicalTrials.gov identifier NCT03838185. The trial is listed as completed; peer-reviewed results have not been published as of the present monograph revision [12].

CMS121 (related compound). CMS121, a fisetin-derived geroneuroprotector from the same laboratory that shares the AMPK/ACC1 mechanism, has received National Institutes of Health funding for Investigational New Drug-enabling studies as of the 2019 review [5]. Clinical trial initiation for CMS121 has not been reported.

Implications for CAD-31. The clinical development of CAD-31 is contingent on completion of Investigational New Drug-enabling studies (good laboratory practice toxicology, formal pharmacokinetics, manufacturing characterization) that have not been publicly described. The timeline for clinical evaluation of CAD-31 is uncertain. Investigators interested in the clinical translation of the geroneuroprotector mechanism may monitor the J147 and CMS121 programs for informative safety and pharmacokinetic data that would inform CAD-31 clinical trial design.

Investigators should treat CAD-31 as a research-use-only compound without human safety or efficacy data. All pharmacological parameters described in this monograph derive from cell culture and rodent studies. Application of these parameters to human dosing is speculative and should not be undertaken without formal clinical trial authorization.

8. Sourcing and Quality Verification

CAD-31 is commercially available as a research-grade compound from multiple specialty chemical suppliers. At the time of this monograph revision, the compound is listed by MedKoo Biosciences, Probechem Biochemicals, Invivochem, and AHA BioChem, among others, under CAS number 2071209-49-7 [1, 2]. The compound is supplied as the free base in milligram quantities (typically 5, 10, or 25 milligrams) as a white to off-white solid.

No pharmaceutical-grade or Good Manufacturing Practice-compliant supply of CAD-31 exists, as the compound has not entered clinical development. All commercially available material is research-grade.

Investigators should confirm compound identity and purity on every lot through the following analytical characterization:

- **High-performance liquid chromatography** area-percent purity, ideally greater than 95 percent (the standard threshold for research-grade small molecules)
- **Mass spectrometry** confirmation of molecular weight (expected $[M+H]^+$ of 405.10 for $C_{18}H_{14}F_6N_2O_2 + H$)
- **Nuclear magnetic resonance spectroscopy** (1H and ^{19}F NMR) to confirm the trifluoromethoxy and trifluoroacetyl groups and the (E)-hydrazone configuration
- **Elemental analysis** or coulometric Karl Fischer analysis for residual water content

The compound is a small-molecule synthetic product with no stereocenters, so chiral purity analysis is not required. The (E)/(Z) hydrazone isomer ratio should be confirmed by NMR if the supplier does not provide this specification, as the (Z)-isomer may have different pharmacological activity.

Third-party analytical confirmation of vendor certificates of analysis is appropriate for any investigation where dose-response or mechanism-of-action precision is important. The research-grade CAD-31 market is small and limited to a few specialized suppliers; lot-to-lot variability is plausible and on-receipt analytical confirmation is recommended.

9. Reconstitution and Handling

CAD-31 is supplied as a solid and requires reconstitution for both in vitro and in vivo applications. Formal stability, solubility, and storage data have not been published; the following guidance is derived from the published experimental protocols and from general principles for compounds of this structural class.

In vitro applications: Stock solutions are typically prepared in dimethyl sulfoxide (DMSO) at concentrations of 10 to 100 millimolar. Working concentrations in published cell culture experiments range from 100 nanomolar (neurogenesis assays) to 10 micromolar (safety panel screens) [1, 2]. Final DMSO concentration in culture media should not exceed 0.1 percent to avoid solvent toxicity. Stock solutions in DMSO should be stored at minus 20 degrees Celsius; stability under these conditions has not been formally characterized for CAD-31, but trifluoroacetyl hydrazones are generally stable in anhydrous DMSO for months. Aqueous stock solutions are not recommended due to the hydrophobic character of the compound.

In vivo applications (oral gavage): Daugherty et al. (2017) administered CAD-31 by oral gavage in corn oil at 20 mg/kg for pharmacokinetic studies in rats [1]. Corn oil provides a lipid vehicle appropriate for hydrophobic compounds and achieves adequate dissolution for gavage administration.

In vivo applications (chronic feeding): The published efficacy studies administered CAD-31 mixed in standard rodent chow at 200 ppm, producing an estimated daily intake of approximately 10 mg/kg/day based on standard food consumption [1, 2]. The compound was mixed into LabDiet 5015 or equivalent base chow by the manufacturer. Investigators should verify homogeneity of compound distribution in the chow and confirm stability under storage conditions (typically minus 20 degrees Celsius for compounded chow).

Storage: Solid CAD-31 should be stored at minus 20 degrees Celsius under desiccation, protected from light. The trifluoroacetyl hydrazone linkage is susceptible to hydrolysis under prolonged exposure to aqueous acid; storage under dry conditions is appropriate. Aliquoting of DMSO stock solutions into single-use volumes is recommended to minimize freeze-thaw cycling.

10. Stack Interactions and Combinations

10.1 Cytochrome P450 interactions

CAD-31 does not inhibit five major cytochrome P450 enzymes (CYP1A2, CYP2C9, CYP2C19, CYP2D6, CYP3A4) at 10 micromolar, a concentration more than 100-fold above the pharmacologically active range [1]. This clean CYP profile indicates a low risk of pharmacokinetic drug-drug interactions mediated through CYP inhibition. The CYP enzymes responsible for CAD-31 metabolism have not been identified; investigators combining CAD-31 with strong CYP inhibitors or inducers should monitor for unexpected changes in CAD-31 exposure.

10.2 Receptor and transporter interactions

CAD-31 showed no interaction with any target in a lead profile screen panel of 60 brain ion channels, transporters, and receptors at 10 micromolar [1]. This result indicates that the compound does not engage conventional drug targets (G-protein-coupled receptors, voltage-gated and ligand-gated ion channels, monoamine transporters) at concentrations relevant to pharmacology. Pharmacodynamic drug-drug interactions through receptor co-occupancy are therefore unlikely.

10.3 AMPK pathway interactions

The principal pharmacodynamic interaction concern for CAD-31 is additive or synergistic AMPK activation when combined with other AMPK-activating compounds. Metformin (a biguanide antidiabetic agent), AICAR (5-aminoimidazole-4-carboxamide ribonucleotide), berberine, and salicylate are all AMPK activators with established pharmacology [16]. Combination of CAD-31 with metformin or other AMPK activators could produce supra-physiological AMPK activation, excessive ACC1 inhibition, and disproportionate shifts in fatty acid and ketone body metabolism. This combination has not been studied and should be approached with caution in research settings; the pharmacological interaction could be either beneficial (additive neuroprotection) or detrimental (metabolic disruption).

10.4 Fatty acid metabolism interactions

CAD-31's primary metabolic effect is suppression of de novo fatty acid synthesis through ACC1 inhibition. Combination with other agents that modulate lipid metabolism (statins, fibrates, omega-3 fatty acid supplements, PCSK9 inhibitors, or experimental ACC inhibitors) could produce additive effects on plasma lipid profiles. The systemic reduction in free fatty acids observed with CAD-31 in mice [1, 9] suggests that investigators using CAD-31 in metabolic research should control for confounding effects on lipid endpoints.

10.5 Ketogenic diet interaction

The metabolic signature of CAD-31 (increased ketone bodies, decreased fatty acid synthesis, elevated acetyl-CoA) overlaps with the metabolic profile of ketogenic diets. Combination of CAD-31 with ketogenic feeding in rodent models could produce additive ketosis; whether this enhances or complicates interpretation of neuroprotective endpoints is an open question for research design.

10.6 Geroneuroprotector combinations

Combination of CAD-31 with other compounds from the same geroneuroprotector family (J147, CMS121, fisetin) has not been studied. Given the convergence of all three synthetic derivatives on the AMPK/ACC1/acetyl-CoA pathway [6, 8], additive effects on metabolic endpoints are expected, but whether the combination produces additive neuroprotection, ceiling effects, or adverse metabolic shifts is unknown.

11. Adverse Events and Safety Signal

11.1 Preclinical safety profile

The safety profile of CAD-31 has been characterized in standard Investigational New Drug-enabling assays. Daugherty et al. (2017) reported the following results at 10 micromolar (a concentration more than 100-fold above the neuroprotective EC₅₀ range): no activity in the hERG cardiac safety assay (indicating absence of hERG potassium channel blockade and low risk of QT prolongation); negative Ames test (indicating absence of mutagenic potential); negative micronucleus assay (indicating absence of clastogenic potential); and no inhibition of five major cytochrome P450 enzymes [1]. The compound did not interact with any target in a panel of 60 brain ion channels, transporters, and receptors, and showed no inhibition of 352 protein kinases [1].

11.2 Acute toxicity

No acute toxicity was observed in mice at a dose of 2 g/kg, a dose approximately 200-fold above the chronic efficacy dose of approximately 10 mg/kg/day [1]. This acute toxicity threshold provides a safety margin greater than 100-fold relative to the pharmacologically effective dose.

11.3 Chronic tolerability

In the three-month chronic feeding study in APP^{swe}/PS1^{deltaE9} and wild-type mice at 200 ppm in chow (approximately 10 mg/kg/day), no adverse effects on body weight, food consumption, or general behavior were observed over the treatment period [1]. The open-field locomotor assay showed no differences between treated and untreated groups, indicating absence of sedation, hyperactivity, or overt neurological effects [1].

11.4 Identified risks and knowledge gaps

Despite the clean preclinical safety profile, several important safety questions remain unanswered:

No human safety data. CAD-31 has not been administered to humans. All safety assessments derive from cell culture assays and rodent studies. Rodent safety does not predict human safety with certainty; species-specific metabolites, receptor interactions, or immune responses may emerge in human studies.

No formal good laboratory practice toxicology. The toxicology data reported in Daugherty et al. (2017) appear to be from screening-level assays rather than from full good laboratory practice (GLP) toxicology studies. Formal GLP 28-day and 90-day repeat-dose toxicology in two species (as required for Investigational New Drug applications) have not been described.

Reproductive and developmental toxicity. No reproductive or developmental toxicology data have been published. The effects of chronic AMPK activation on fertility, embryonic development, and lactation are unknown.

Cardiovascular safety beyond hERG. The clean hERG result at 10 micromolar is reassuring but does not constitute a complete cardiovascular safety assessment. In vivo telemetry cardiovascular studies have not been reported.

Long-term carcinogenicity. No carcinogenicity studies have been described. The compound's promotion of cell proliferation (neurogenesis) raises a theoretical concern about proliferative effects in other tissues, though no evidence of such effects has been reported.

Investigators should treat CAD-31 as a compound in the earliest stages of safety characterization and should design research protocols with appropriate safety monitoring for a compound without established human tolerability.

12. Comparative Assessment

This section presents a structured comparative assessment of five compounds against CAD-31, evaluated against five competency standards: novelty of mechanism, effect size in published models, promising potential (current development trajectory), side-effect profile (preclinical or clinical), and overall validation. The five comparators were selected to represent the geroneuroprotector compound family (J147, CMS121, fisetin), a mechanistically related clinical-stage drug (metformin), and the dominant current approach to Alzheimer's disease treatment (lecanemab).

12.1 J147

Mechanism: Phenotypically selected neuroprotective compound with identified target: the alpha-F1 subunit of mitochondrial ATP synthase (ATP5A). J147 partially inhibits ATP synthase (23.6 percent at saturation, EC50 20 nM), leading to calcium mobilization, CAMKK2 activation, and AMPK/mTOR pathway engagement [7]. The pathway downstream of AMPK (ACC1 inhibition, acetyl-CoA elevation) is shared with CAD-31 [7, 8].

Development trajectory: J147 is the most advanced compound in the geroneuroprotector series. Discovered in 2011 by Chen et al. at the Salk Institute [4], J147 has been validated in multiple transgenic Alzheimer's disease mouse models, in aged normal mice, and in SAMP8 accelerated-aging mice [4, 14, 8]. A Phase 1 safety and pharmacokinetics study in healthy adult volunteers (NCT03838185) has been completed, but results have not been published in peer-reviewed form [12]. Pharmacokinetic characterization reports oral bioavailability of approximately 28 percent and a plasma half-life of approximately 2.5 hours in mice [13].

Novelty: Moderate to high. The identification of ATP synthase as a druggable target for both aging and dementia was a significant finding in the geroneuroprotector field [7]. The phenotypic discovery approach itself is novel relative to the target-based paradigm that dominates Alzheimer's drug development.

Effect size: J147 produces robust cognitive rescue in transgenic Alzheimer's disease mice and extends *Drosophila* lifespan by 9.5 to 12.5 percent [7]. Cognitive effect sizes in mouse models are large (restoration to wild-type levels on multiple behavioral paradigms) but have not been tested in human subjects [4].

Side-effect profile: J147 has a clean preclinical safety profile comparable to CAD-31: no acute toxicity at 2 g/kg, no hERG activity, no mutagenicity, no kinase or receptor hits [4]. No human safety data have been reported.

Overall validation: Moderate. J147 has the broadest preclinical evidence base of any compound in the geroneuroprotector family, with over a dozen rodent model studies, an identified molecular target, and a completed (but unreported) Phase 1 trial. The absence of published Phase 1 results is a significant gap.

Comparison to CAD-31: J147 is the parent compound of CAD-31 and the principal reference comparator. CAD-31 has improved neurogenic potency (2.6- to 5.2-fold on hNPC markers) and improved metabolic stability relative to J147 [2]. J147 has the advantage of an identified molecular target (ATP5A), a broader published preclinical evidence base, and a more advanced clinical development trajectory. Investigators selecting between J147 and CAD-31 should weigh the neurogenesis advantage of CAD-31 against the deeper characterization of J147.

Scores (1-5): Novelty: 4. Effect size: 4. Promising potential: 3. Side-effect profile: 3 (preclinical only). Overall validation: 3.

12.2 CMS121

Mechanism: Synthetic fisetin derivative with neuroprotective and geroprotective activity. CMS121 converges on the same AMPK/ACC1/acetyl-CoA pathway as J147 and CAD-31, but the initial molecular target differs from J147's ATP synthase and has not been formally identified [8]. CMS121 and J147 both increase acetyl-CoA levels via AMPK-dependent ACC1 inhibition, resulting in neuroprotection and increased acetylation of histone H3K9 in SAMP8 mice [8].

Development trajectory: CMS121 has received National Institutes of Health funding for Investigational New Drug-enabling studies as of 2019 [5]. The compound has been validated in SAMP8 accelerated-aging mice and in APP^{swe}/PS1^{deltaE9} transgenic Alzheimer's disease mice [8, 11]. CMS121 improved learning in the Barnes maze reversal phase in SAMP8 mice and prevented age-associated increases in disinhibition [8].

Novelty: Moderate. CMS121 is derived from the natural product fisetin, which has its own research base. The synthesis optimization improves potency by several orders of magnitude relative to fisetin while maintaining the biological activities [5].

Effect size: CMS121 improved cognitive performance in SAMP8 mice and preserved mitochondrial, metabolic, and transcriptomic homeostasis [8]. *Drosophila* lifespan extension was 8.5 percent [11]. Effect sizes are comparable to J147 and CAD-31 in overlapping models.

Side-effect profile: CMS121 has a preclinical safety profile comparable to J147 and CAD-31 based on published tolerability data in chronic mouse studies [8, 11]. No formal safety pharmacology has been published.

Overall validation: Low to moderate. CMS121 has a smaller published evidence base than J147 but shares the mechanistic validation through the AMPK/ACC1 pathway. IND-enabling studies are underway but clinical evaluation has not begun.

Comparison to CAD-31: CMS121 and CAD-31 converge on the same downstream mechanism (AMPK/ACC1/acetyl-CoA) despite different structural origins (fisetin versus curcumin). CAD-31 has the advantage of demonstrated neurogenic activity and demonstrated lifespan extension (approximately 30 percent in SAMP8), while CMS121 has the advantage of more advanced IND-enabling progress and a natural-product parent (fisetin) with independent clinical interest [5].

Scores (1-5): Novelty: 3. Effect size: 3. Promising potential: 3. Side-effect profile: 3 (preclinical only). Overall validation: 3.

12.3 Fisetin

Mechanism: Fisetin (3,3',4',7-tetrahydroxyflavone) is a naturally occurring flavonoid found in strawberries, apples, and other fruits. Fisetin activates multiple pathways relevant to neuroprotection: it promotes autophagic degradation of phosphorylated tau via TFEB and Nrf2 transcription factors, reduces inflammation through NF-kappaB inhibition, and has senolytic activity (selective clearance of senescent cells) [15]. Fisetin's relationship to the geroneuroprotector program is as the natural-product parent of CMS121, identified through the same phenotypic screening platform at the Salk Institute [5].

Development trajectory: Fisetin is commercially available as a dietary supplement. Currais et al. (2014) demonstrated that oral fisetin maintained cognitive function in APPswe/PS1deltaE9 transgenic mice from 3 to 12 months of age [15]. A Phase 2 clinical trial of fisetin in mild Alzheimer's disease (NCT07279714, FIS-AD) is planned at Sunnybrook Health Sciences Centre with an estimated start date of December 2025 [18]. Several smaller trials of fisetin for senolytic and anti-aging endpoints (NCT06431932, NCT06399809) are also in progress.

Novelty: Low for the molecule (a well-known dietary flavonoid), moderate for the application (senolytic and neuroprotective combination).

Effect size: In the APPswe/PS1deltaE9 model, fisetin prevented the development of learning and memory deficits when administered prophylactically from 3 to 12 months [15]. However, fisetin is approximately 100-fold less potent than CMS121 and the synthetic geroneuroprotectors in cell culture neuroprotection assays, and its oral bioavailability is poor [5].

Side-effect profile: Fisetin has an established safety profile as a dietary supplement. No serious adverse events have been attributed to fisetin supplementation in published human studies. Clinical-dose safety data from formal trials are limited.

Overall validation: Moderate. Fisetin has the broadest accessibility (dietary supplement, no prescription required) and the most diverse research base (senescence, inflammation, cancer, neurodegeneration) of any compound in this comparator set. Its low potency and poor bioavailability are significant limitations for clinical translation to Alzheimer's disease.

Comparison to CAD-31: Fisetin is the upstream natural product in the flavonoid arm of the geroneuroprotector program; CMS121 is the potency-optimized derivative. CAD-31 occupies the analogous position in the curcumin arm (J147 is to curcumin as CMS121 is to fisetin). CAD-31 has dramatically higher potency than fisetin (EC50 values 12-95 nM versus micromolar range), better brain penetrance, and demonstrated lifespan extension. Fisetin has the advantages of human dietary exposure history, broader indication research, and planned clinical trials.

Scores (1-5): Novelty: 2. Effect size: 2 (limited by potency and bioavailability). Promising potential: 3 (clinical trials planned). Side-effect profile: 4 (dietary supplement safety). Overall validation: 3.

12.4 Metformin

Mechanism: Metformin is a biguanide antidiabetic agent that activates AMPK through multiple mechanisms, including inhibition of mitochondrial complex I and modulation of the AMP/ATP ratio. AMPK activation by metformin leads to downstream effects including ACC1 inhibition, enhanced autophagy, and mTOR suppression, producing substantial overlap with the AMPK/ACC1 signaling cascade engaged by CAD-31 [16]. Metformin additionally modulates several hallmarks of aging including epigenetic modifications, mitochondrial function, cellular senescence, and the gut microbiome [16].

Development trajectory: Metformin is the most widely prescribed antidiabetic drug globally and has been used clinically since 1957. The Targeting Aging with Metformin (TAME) trial, a landmark geroscience clinical trial led by Nir Barzilai, aims to demonstrate that metformin can delay the composite outcome of age-associated diseases (cardiovascular disease, cancer, dementia, mortality) in older adults without diabetes [19]. The TAME trial represents the first attempt to obtain FDA recognition of aging as a treatable condition. Observational studies have associated metformin use in diabetic patients with reduced incidence of Alzheimer's disease, though confounding by indication limits interpretation [16].

Novelty: Low for mechanism (AMPK activation by metformin is well established), high for application (the TAME trial's reconceptualization of aging as a therapeutic target).

Effect size: Preclinical evidence for metformin's neuroprotective effects is mixed; some rodent studies show cognitive benefits while others do not, and the doses required for neuroprotection in mice are substantially higher than the antidiabetic doses. The effect size in aging endpoints is expected to be modest, consistent with the incremental-benefit model of geroscience [16].

Side-effect profile: Metformin has a well-established clinical safety profile over decades of human use. Common adverse events are gastrointestinal (nausea, diarrhea, abdominal discomfort), with rare lactic acidosis as the principal serious adverse event. The extensive human safety data represent the principal advantage of metformin over the experimental geroneuroprotectors.

Overall validation: High for the AMPK activation mechanism and for clinical safety; moderate for Alzheimer's disease-specific efficacy, which has not been demonstrated in a randomized controlled trial.

Comparison to CAD-31: Metformin and CAD-31 share the AMPK/ACC1 pathway but differ in almost every other respect. CAD-31 is approximately 1000-fold more potent in neuroprotection assays, is brain-penetrant by design, and has demonstrated neurogenic activity; metformin has 60+ years of human safety data, established clinical infrastructure, and a registration pathway for aging endpoints. Metformin provides the clinical validation for the AMPK mechanism that CAD-31 lacks; CAD-31 provides the potency and brain-targeted pharmacology that metformin lacks.

Scores (1-5): Novelty: 2. Effect size: 2 (for AD-specific endpoints). Promising potential: 4 (TAME trial). Side-effect profile: 5 (decades of clinical data). Overall validation: 4.

12.5 Lecanemab (Leqembi)

Mechanism: Lecanemab is a humanized IgG1 monoclonal antibody directed against soluble amyloid-beta protofibrils. The mechanism is amyloid clearance from the brain through antibody-mediated phagocytosis, representing the anti-amyloid hypothesis of Alzheimer's disease [17]. This mechanism is fundamentally different from CAD-31's metabolic/anti-inflammatory/neurogenic mechanism and provides a useful contrast for positioning CAD-31 in the Alzheimer's disease therapeutic landscape.

Development trajectory: The Clarity AD Phase 3 trial enrolled 1,795 patients with early Alzheimer's disease (mild cognitive impairment or mild dementia with confirmed brain amyloid) in an 18-month randomized, double-blind, placebo-controlled study. Lecanemab received traditional FDA marketing approval in July 2023 following the positive Phase 3 readout [17]. The compound is the first anti-amyloid antibody to achieve traditional (non-accelerated) FDA approval for Alzheimer's disease.

Novelty: Low for mechanism (anti-amyloid antibodies have been in development for over 20 years), moderate for clinical achievement (first traditional approval in the class).

Effect size: In the Clarity AD trial, lecanemab delayed cognitive and functional decline by approximately 27 percent on the CDR-SB (Clinical Dementia Rating Sum of Boxes) over 18 months relative to placebo, corresponding to a slowing of decline

by approximately 5 months [17]. The effect size is statistically significant but clinically modest, and the clinical meaningfulness of the observed difference remains debated in the field.

Side-effect profile: The most common adverse events were infusion-related reactions (24.5 percent), amyloid-related imaging abnormalities with hemosiderin deposits (ARIA-H microhemorrhages, 16.0 percent), and amyloid-related imaging abnormalities with edema (ARIA-E, 13.6 percent) [17]. ARIA events are specific to anti-amyloid antibodies and represent a clinically significant safety concern, particularly in patients on anticoagulant therapy or with cerebral amyloid angiopathy.

Overall validation: High. Lecanemab is the most clinically validated Alzheimer's disease therapeutic introduced in the past two decades, with a completed Phase 3 trial, regulatory approval, and ongoing open-label extension data.

Comparison to CAD-31: Lecanemab and CAD-31 represent fundamentally different approaches to Alzheimer's disease. Lecanemab targets amyloid clearance, while CAD-31 targets metabolic dysfunction, inflammation, and neurogenesis without affecting amyloid plaques [1]. Lecanemab has an established clinical evidence base and regulatory approval; CAD-31 has no human data. The two mechanisms are not mutually exclusive, and combination treatment (amyloid clearance plus metabolic neuroprotection) is a plausible research direction that has not been explored. The modest effect size of lecanemab (approximately 27 percent slowing of decline) leaves substantial room for additive benefit from compounds targeting non-amyloid mechanisms.

Scores (1-5): Novelty: 2. Effect size: 3 (statistically significant, clinically modest). Promising potential: 5 (FDA approved). Side-effect profile: 2 (ARIA risk). Overall validation: 5.

12.6 Comparator summary

STANDARD	J147	CMS121	FISETIN	METFORMIN	LECANEMAB
Novelty	4	3	2	2	2
Effect size	4	3	2	2	3
Promising potential	3	3	3	4	5
Side-effect profile	3	3	4	5	2
Overall validation	3	3	3	4	5

CAD-31 occupies a distinctive position in this comparator landscape. Within the geroneuroprotector family (J147, CMS121, fisetin), CAD-31 is differentiated by its neurogenic activity and its demonstrated lifespan extension in SAMP8 mice. Relative to J147, it sacrifices the identified molecular target (ATP5A) and the broader preclinical evidence base for improved neurogenic potency and metabolic stability. Relative to CMS121, it shares the AMPK/ACC1 mechanism from a different structural origin and has the additional lifespan extension data.

Against the mechanistically related metformin, CAD-31 offers dramatically higher neuroprotective potency and brain-targeted pharmacology, but lacks the decades of human safety data that make metformin a practical clinical candidate. Against lecanemab, CAD-31 represents a complementary rather than competing mechanism: metabolic/anti-inflammatory neuroprotection versus amyloid clearance, with the possibility of additive benefit in combination.

The principal limitation of CAD-31 relative to all comparators except CMS121 is the absence of human data and the concentration of the evidence base in a single laboratory. Independent replication of the preclinical findings, completion of formal IND-enabling studies, and progression to Phase 1 clinical evaluation are the critical development milestones for CAD-31 to advance from a promising preclinical candidate to a validated therapeutic.

13. References

The published literature on CAD-31 is concentrated in approximately 12 primary publications from the Cellular Neurobiology Laboratory at the Salk Institute for Biological Studies, where the compound was developed. The reference list below reflects this concentration; investigators should be aware that the compound's evidence base derives predominantly from a single research group and has not been independently replicated.

1. Daugherty D, Goldberg J, Fischer W, Dargusch R, Maher P, Schubert D. A novel Alzheimer's disease drug candidate targeting inflammation and fatty acid metabolism. *Alzheimers Res Ther.* 2017;9(1):50. PMID: 28709449. DOI: 10.1186/s13195-017-0277-3.
2. Prior M, Dargusch R, Ehren JL, Chiruta C, Maher P, Schubert D. Selecting for neurogenic potential as an alternative for Alzheimer's disease drug discovery. *Alzheimers Dement.* 2016;12(6):678-686. PMID: 27149904.
3. Liu Y, Dargusch R, Maher P, Schubert D. A broadly neuroprotective derivative of curcumin. *J Neurochem.* 2008;105(4):1336-1345. PMID: 18208543.
4. Chen Q, Prior M, Dargusch R, Roberts A, et al. A novel neurotrophic drug for cognitive enhancement and Alzheimer's disease. *PLoS One.* 2011;6(12):e27865.
5. Schubert D, Currais A, Goldberg J, Finley K, Petrascheck M, Maher P. Geroneuroprotectors: effective geroprotectors for the brain. *Trends Pharmacol Sci.* 2019;40(2):73-82.
6. Currais A, Huang L, Petrascheck M, Maher P, Schubert D. A chemical biology approach to identifying molecular pathways associated with aging. *GeroScience.* 2021;43(1):353-365. PMID: 32705410. DOI: 10.1007/s11357-020-00238-5.
7. Goldberg J, Currais A, Prior M, Fischer W, Chiruta C, Ratliff E, Daugherty D, Dargusch R, Finley K, Esparza-Molto PB, Cuezva JM, Maher P, Petrascheck M, Schubert D. The mitochondrial ATP synthase is a shared drug target for aging and dementia. *Aging Cell.* 2018;17(2):e12715. PMID: 29316249.

8. Currais A, Huang L, Goldberg J, Petrascheck M, Ates G, Pinto-Duarte A, Shokhirev MN, Schubert D, Maher P. Elevating acetyl-CoA levels reduces aspects of brain aging. *eLife*. 2019;8:e47866. PMID: 31742554.
9. Goldberg J, Currais A, Ates G, Huang L, Shokhirev M, Maher P, Schubert D. The Alzheimer's disease drug candidate J147 decreases blood plasma fatty acid levels via modulation of AMPK/ACC1 signaling in the liver. *Biomed Pharmacother*. 2022;147:112648. PMID: 35051863.
10. Goldberg J, Currais A, Ates G, Huang L, Shokhirev M, Maher P, Schubert D. Targeting of intracellular Ca²⁺ stores as a therapeutic strategy against age-related neurotoxicities. *NPJ Aging Mech Dis*. 2020;6:10. PMID: 32884834.
11. Kepchia D, Currais A, Dargusch R, Finley K, Schubert D, Maher P. Geroprotective effects of Alzheimer's disease drug candidates. *Aging (Albany NY)*. 2021;13(3):3269-3289. PMID: 33550278.
12. ClinicalTrials.gov. A Study to Evaluate Safety, Tolerability, and Pharmacokinetics of J147 in Healthy Adult Volunteers. Identifier: NCT03838185. First posted: 2019. Status: completed.
13. Current evidence for J147 as a potential therapeutic agent in nervous system disease: a narrative review. 2023. PMCID: PMC10481599.
14. Prior M, Dargusch R, Ehren JL, Chiruta C, Maher P, Schubert D. The neurotrophic compound J147 reverses cognitive impairment in aged Alzheimer's disease mice. *Alzheimers Res Ther*. 2013;5(3):25. PMID: 23673233.
15. Currais A, Goldberg J, Farrokhi C, et al. Modulation of p25 and inflammatory pathways by fisetin maintains cognitive function in Alzheimer's disease transgenic mice. *Aging Cell*. 2014;13(2):379-390.
16. Barzilai N, Crandall JP, Kritchevsky SB, Espeland MA. Metformin as a tool to target aging. *Cell Metab*. 2016;23(6):1060-1065. PMID: 27304507.
17. van Dyck CH, Swanson CJ, Aisen P, et al. Lecanemab in early Alzheimer's disease. *N Engl J Med*. 2023;388(1):9-21.
18. ClinicalTrials.gov. Fisetin Intervention Study in Mild Alzheimer's Disease (FIS-AD). Identifier: NCT07279714. Status: not yet recruiting.
19. Barzilai N, et al. Targeting aging with metformin (TAME). In: *Healthy Ageing and Longevity*. Springer; 2018. PMCID: PMC6230116.

This monograph is provided for research use only. Not for medical, diagnostic, or therapeutic purposes. Not for human consumption. CAD-31 has no approved medical indication in any jurisdiction and has not been administered to humans. The preclinical data described herein derive predominantly from a single research group (the Cellular Neurobiology Laboratory at the Salk Institute for Biological Studies) and have not been independently replicated. Investigators should treat all pharmacological parameters as preliminary pending independent confirmation and formal clinical evaluation. The reference list reflects the concentrated literature base for this preclinical compound; the smaller number of citations relative to monographs on clinically advanced compounds is an honest reflection of the evidence base rather than an editorial choice.