AG18051
AND THE Aβ–ABAD COMPLEX

Disrupting the Intramitochondrial Amyloid-β Toxicity Bridge — Pharmacology of HSD17B10, mt-RNase P, and the Lustbader Axis

Lustbader · Yan · Stern · Wu · Kissinger · Takuma · Yao

in dialogue with the frentizole-derivative and ABAD-DP decoy-peptide programs

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ONS Pharmacology Series — companion monograph to J-147 and CMS121. Where J-147 modulates mitochondrial function and CMS121 restricts lipid-peroxidation substrate, AG18051 disrupts the intramitochondrial amyloid-β toxicity complex — the only direct engagement of amyloid pathology in the series.

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Abstract

AG18051 — 2-[(2-methoxy-5-methylphenyl)amino]-1-phenyl-1H-benzimidazole — is a small-molecule chemical-biology probe that engages the mitochondrial-matrix enzyme **ABAD** (amyloid-β binding alcohol dehydrogenase, encoded by *HSD17B10* and also known as 17β-hydroxysteroid dehydrogenase type 10) through an unusual mechanism: it forms a covalent adduct with the *reduced* nicotinamide of the enzyme's NAD cofactor, locking the catalytic cleft in an inactive configuration and disrupting the geometry of the loop region through which ABAD binds amyloid-β. The resulting destabilization of the Aβ–ABAD complex — first characterized as a pathologically active intramitochondrial toxicity bridge by Yan, Stern, Lustbader, Wu, and colleagues (Lustbader et al., 2004) — is the property that makes AG18051 a structurally privileged probe of one of the most direct molecular interfaces between amyloid pathology and mitochondrial dysfunction in Alzheimer's disease.

This monograph develops three claims. *First*, the Aβ–ABAD interaction is a uniquely tractable pharmacological target because the location of the pathology — inside the mitochondrial matrix — is largely inaccessible to the antibody and large-molecule therapeutics that have dominated the antiamyloid clinical landscape. A small molecule capable of crossing the plasma membrane, the cytoplasm, and the mitochondrial double membrane can engage Aβ at one of its principal sites of pathological action, in a manner that antibody therapeutics structurally cannot. *Second*, ABAD has an essential basal function as a scaffold subunit of the mitochondrial RNase P (mt-RNase P) complex — a function structurally distinct from its catalytic dehydrogenase activity and from its Aβ-binding interface. This scaffold function defines an intrinsic therapeutic window: an inhibitor that engages the NAD-binding cleft (and therefore the SDR catalysis and the Aβ binding) does not impair mt-RNase P-mediated pre-tRNA processing, because the scaffold function is geometrically segregated from the catalytic site. *Third*, the AG18051 co-crystal structure with ABAD (Kissinger et al., 2004) provides an atomic-resolution roadmap that defines a target-class strategy for successor compounds — reversible, non-covalent, brain-penetrant Aβ–ABAD disruptors whose translational profile is more amenable to chronic dosing than the covalent AG18051 parent.

We trace ABAD biology from its identification as a multifunctional SDR family enzyme through its characterization as the principal binding partner of intramitochondrial Aβ; we detail the downstream consequences of Aβ–ABAD complex formation (complex IV inhibition, mitochondrial ROS, cyclophilin-D-mediated mPTP opening, cytochrome c release); we describe the AG18051 chemistry, the covalent NAD-adduct mechanism, and the disruption of the Aβ–ABAD complex in cellular and biochemical assays; and we situate the compound class within the broader landscape of Aβ–ABAD disruptors that includes the ABAD-decoy peptide (ABAD-DP) of Lustbader et al. and the frentizole-derivative class developed by the Yao, Du, Marques, and Lim groups. The monograph is a companion to the J-147 and CMS121 papers in the ONS Pharmacology Series: where J-147 modulates mitochondrial *function* through ATP

synthase α-subunit engagement and CMS121 restricts the *substrate* of lipid peroxidation through FASN inhibition, AG18051 protects mitochondrial *integrity* by directly disrupting the intramitochondrial amyloid-β toxicity bridge.

Keywords: *AG18051 · ABAD · HSD17B10 · 17β-HSD10 · amyloid-β · Aβ–ABAD complex · intramitochondrial amyloid · mitochondrial RNase P · TRMT10C · complex IV · cyclophilin D · mPTP · covalent inhibitor · NAD adduct · frentizole · ABAD-DP decoy peptide · Lustbader · Yan · Stern · Kissinger · Alzheimer's disease*

1. Introduction The Intramitochondrial Amyloid Problem

The antiamyloid clinical landscape of the past two decades has been organized almost entirely around *extracellular* Aβ. The amyloid immunotherapies, the BACE inhibitors, the γ-secretase modulators, and the aggregation inhibitors have each, in different ways, sought to reduce the burden of Aβ in the extracellular compartment — in plaques, in the parenchymal interstitium, and in the cerebrospinal fluid. The clinical results of this program have been documented elsewhere and need not be re-litigated here. What matters for the present monograph is one specific limitation of the extracellular-Aβ paradigm: it has been agnostic to, and largely incapable of engaging, the *intracellular* fraction of Aβ that accumulates inside vulnerable neurons and that — in a body of work largely outside the antiamyloid mainstream — has been characterized as a principal mediator of the mitochondrial dysfunction characteristic of Alzheimer's disease.

Intracellular Aβ is real, and it is biochemically distinct from extracellular Aβ in several respects relevant to pharmacology. *First*, it accumulates inside neurons in transgenic AD mouse models and in postmortem human AD brain at concentrations in the low-nanomolar to low-micromolar range — substantially lower than the local concentrations achieved within mature extracellular plaques, but high enough to support sub-saturating engagement of high-affinity binding partners. *Second*, a fraction of the intracellular Aβ pool localizes to mitochondria, where it has been visualized by immunoelectron microscopy in the matrix compartment in proximity to inner-membrane structures. *Third*, the intramitochondrial fraction does not remain in free solution but binds tightly to a specific protein partner — ABAD — in a complex whose formation triggers a cascade of mitochondrial dysfunction that operates independently of, and in addition to, the synaptic and dendritic toxicity of extracellular Aβ oligomers.

The strategic implication is that an antiamyloid pharmacology directed only at the extracellular compartment cannot, in principle, engage the intramitochondrial mechanism. A complete pharmacology of amyloid toxicity requires, in addition to extracellular Aβ clearance or production reduction, a small-molecule strategy that engages the intramitochondrial Aβ–ABAD complex at the

site of its pathological action. AG18051 is, on the available chemical-biology evidence, the lead probe compound for this strategy, and the Aβ–ABAD complex is its target. This monograph is the first in the ONS Pharmacology Series to address the *amyloid axis* directly — through the unique structural and pharmacological window provided by ABAD.

2. ABAD A Multifunctional Mitochondrial-Matrix Enzyme

2.1 Identification and Nomenclature

ABAD was identified in the mid-1990s in the laboratory of Shi Du Yan and David Stern at Columbia University in the course of work aimed at identifying intracellular binding partners of amyloid-β. Yeast two-hybrid screening of brain cDNA libraries with Aβ as bait yielded a clone encoding a previously characterized mitochondrial enzyme — type 10 17β-hydroxysteroid dehydrogenase, encoded by the X-linked gene *HSD17B10* and a member of the short-chain dehydrogenase/reductase (SDR) superfamily. The enzyme was renamed *amyloid-β binding alcohol dehydrogenase* (ABAD) to reflect this newly characterized Aβ-binding function, and it has subsequently been referred to under both names — HSD17B10 in the steroid metabolism and clinical genetics literature, ABAD in the Alzheimer pharmacology literature, and 17β-HSD10 in some biochemistry contexts.

The enzyme is a homotetramer of ~27-kDa subunits arranged with D_2 symmetry, each subunit containing a Rossmann-fold NAD-binding domain and a substrate-binding pocket. The active site occupies the cleft between the two domains, with the catalytic Tyr168 and Ser155 residues positioned for hydride transfer from substrate to the nicotinamide C4 of the NAD cofactor. The enzyme resides almost exclusively in the mitochondrial matrix, where it is imported through an N-terminal mitochondrial targeting sequence that is cleaved upon import. Subcellular fractionation, immuno-electron microscopy, and biochemical purification have consistently localized the protein to the matrix compartment with negligible cytoplasmic or intermembrane-space pools.

2.2 Three Distinct Functions

ABAD is multifunctional in a strict biochemical sense — three distinct functions, each catalytically or structurally independent of the others, reside in the same protein. Understanding the three functions, and the structural relationships among them, is essential to understanding how a pharmacological intervention can engage one without abolishing the others.

Function one — short-chain dehydrogenase catalysis. ABAD catalyzes the NAD-dependent oxidation of multiple small-molecule substrates, including 17β-hydroxysteroids (the original basis of the HSD17B10 designation), 3-hydroxyacyl-CoA intermediates of mitochondrial fatty acid β-oxidation

(where ABAD performs the third step of the β-oxidation spiral on short- and medium-chain substrates), the 2-methyl-3-hydroxybutyryl-CoA intermediate of isoleucine catabolism, and a set of bile acid intermediates. The substrate breadth is unusual for an SDR family enzyme and reflects the relatively permissive geometry of the ABAD substrate-binding pocket. Catalysis proceeds through the canonical SDR mechanism: substrate hydroxyl binds and orients in the substrate pocket, the catalytic Tyr168 abstracts the proton, the C-H hydride transfers to NAD C4, and the oxidized substrate and reduced NADH are released.

Function two — mitochondrial RNase P scaffold. ABAD is a structural subunit of the mitochondrial ribonuclease P (mt-RNase P) complex, the enzyme responsible for 5′ processing of all 22 mitochondrial pre-tRNAs from the polycistronic mitochondrial primary transcripts. mt-RNase P is a three-subunit protein complex (in contrast to the RNA-containing nuclear RNase P) consisting of TRMT10C (the methyltransferase subunit that contributes catalytic function), MRPP3 (the metallonuclease subunit that performs the 5′ cleavage), and ABAD (the third subunit, contributing structural scaffolding and substrate recognition). The ABAD function within mt-RNase P is *not* its SDR catalytic activity — point mutations that ablate SDR catalysis preserve mt-RNase P function, and point mutations that disrupt mt-RNase P assembly preserve SDR catalysis. The two functions are structurally and biochemically separable, and they reside in geometrically distinct regions of the protein: the SDR catalytic activity at the NAD-binding cleft, the mt-RNase P scaffold function at a surface that includes residues distinct from the catalytic cleft.

Function three — amyloid-β binding. ABAD binds Aβ with high affinity (Kd in the low nanomolar range) at a third interface that overlaps partially with the NAD-binding cleft and includes the loop region between the cleft and the substrate-binding pocket. The Aβ-binding function is what defines the enzyme as ABAD (rather than merely HSD17B10), and it is the function that makes the enzyme a pharmacological target in Alzheimer's disease.

The structural relationships among the three functions are critical to the therapeutic-window argument developed in Section 4. A small molecule that occupies the NAD-binding cleft inhibits the SDR catalytic activity (function one) and disrupts the Aβ-binding interface (function three), because the latter geometrically overlaps with the former. The same small molecule does not impair the mt-RNase P scaffold function (function two), because the scaffold surface is structurally segregated from the catalytic cleft. The therapeutic window is, in structural terms, *intrinsic to the protein architecture* — not dependent on pharmacokinetic dose titration.

2.3 The Essentiality of ABAD — HSD10 Disease

The clinical evidence for ABAD's essentiality is the X-linked metabolic disorder formerly known as 2-methyl-3-hydroxybutyric aciduria, now designated **HSD10 disease**. Hemizygous loss-of-function mutations in *HSD17B10* in males cause a severe neurometabolic syndrome with progressive neurodegeneration in early childhood, optic atrophy, cardiomyopathy, and elevated urinary excretion of the isoleucine catabolic intermediate 2-methyl-3-hydroxybutyrate. The disease was initially attributed to the loss of the isoleucine β-oxidation function of ABAD, but subsequent work has established that the principal pathology — the early-onset neurodegeneration — is in fact attributable to loss of the *mt-RNase P scaffold function*, not the SDR catalytic function. Patients with mutations that selectively impair mt-RNase P assembly without affecting SDR catalysis show the full clinical phenotype; patients with mutations that impair only the SDR catalytic activity show a much milder phenotype dominated by the metabolic findings.

This clinical-genetic dissociation establishes, in human disease, the structural separability of the three ABAD functions. It also establishes a critical safety parameter for ABAD-directed pharmacology: any small molecule that inhibits the *catalytic* function of ABAD, without engaging the mt-RNase P scaffold function, may be safely administered at doses sufficient to fully inhibit the SDR catalysis. The mt-RNase P scaffold function is the load-bearing essential function whose impairment causes the neurodegenerative phenotype of HSD10 disease; the SDR function is dispensable in this clinical sense.

3. The A ABAD Complex

3.1 Discovery and Initial Characterization

The Aβ–ABAD interaction was characterized through a sequence of papers from the Yan and Stern laboratories beginning in the late 1990s and culminating in the landmark Lustbader et al. (2004) report in *Science*. The key observations, in approximate chronological sequence, were these.

Yeast two-hybrid screening identified ABAD (then HSD17B10) as a high-affinity Aβ-binding partner in brain cDNA libraries. The interaction was validated biochemically through coimmunoprecipitation of ABAD with Aβ from cellular lysates and from postmortem AD brain tissue, with corresponding negative controls in non-AD tissue confirming specificity. The binding affinity, determined by surface plasmon resonance and isothermal titration calorimetry, was in the low-nanomolar range — sufficient for significant complex formation at the intramitochondrial Aβ concentrations measured in AD models.

Immunoelectron microscopy localized the Aβ–ABAD complex to the mitochondrial matrix in transgenic AD mouse brain, with negligible complex in non-mitochondrial compartments and absent complex in non-transgenic controls. Subcellular fractionation studies independently confirmed the matrix localization. Together, these observations established that the Aβ–ABAD interaction occurs at the site of its presumed pathological consequence — within the mitochondrial matrix — rather than in another compartment with subsequent translocation.

The Lustbader 2004 *Science* paper consolidated these findings and added two new lines of evidence. *First*, it demonstrated that overexpression of ABAD in cells exposed to extracellular Aβ produces a synergistic exacerbation of cellular pathology — elevated mitochondrial ROS, reduced membrane potential, impaired complex IV activity, and increased markers of apoptosis — beyond what either ABAD overexpression or Aβ exposure alone would produce. *Second*, it demonstrated that a peptide based on the ABAD residues critical for Aβ binding (the **ABAD-decoy peptide**, ABAD-DP) competitively displaces Aβ from ABAD in vitro and rescues the mitochondrial pathology in cellular and transgenic mouse models. The ABAD-DP result was the first pharmacological proof-of-principle that the Aβ–ABAD complex could be therapeutically disrupted and that doing so produced measurable benefit.

3.2 The Pathological Cascade Downstream of Complex Formation

Aβ–ABAD complex formation triggers a sequence of mitochondrial dysfunction that has been characterized through a combination of biochemistry, cellular pharmacology, and transgenic mouse studies (Lustbader et al., 2004; Takuma et al., 2005; Yan & Stern, 2005; and subsequent work). The proximate consequences are five.

Loss of ABAD SDR catalytic function. Aβ binding to the loop region between the NAD-binding cleft and the substrate-binding pocket alters the catalytic geometry of the active site. The Tyr168 catalytic residue is displaced from its productive orientation, the NAD cofactor occupancy is altered, and substrate turnover is markedly reduced. Functionally, this means that the metabolic functions of ABAD — short-chain fatty acid β-oxidation, isoleucine catabolism, 17β-hydroxysteroid handling — are impaired in mitochondria of cells with substantial Aβ–ABAD complex burden.

Generation of superoxide and lipid peroxidation. The Aβ-bound conformation of ABAD shifts the enzyme into a state in which the partially reduced NAD cofactor leaks electrons to molecular oxygen, generating superoxide. The superoxide generation is proximal to the inner mitochondrial membrane and the matrix-facing PUFA-containing phospholipids; it drives local lipid peroxidation, generates the reactive carbonyl 4-hydroxynonenal, and propagates damage to mitochondrial inner-membrane proteins.

Complex IV inhibition. The complex IV (cytochrome c oxidase) inhibition characteristic of AD mitochondria has been mechanistically linked to Aβ–ABAD-derived oxidative damage. Complex

IV subunits I, II, and IV — particularly the heme-bearing subunit I — are susceptible to oxidative modification by Aβ–ABAD-derived ROS, and the resulting reduction in complex IV activity impairs the terminal step of the electron transport chain. The complex IV impairment has the secondary consequence of further elevating mitochondrial ROS (because the electron transport chain becomes more reduced and propensity for upstream electron leak increases), producing a feed-forward amplification of the oxidative cascade.

Cyclophilin-D-mediated mPTP opening. Sustained Aβ–ABAD complex burden, combined with the elevated mitochondrial calcium and ROS that follows, lowers the threshold for opening of the mitochondrial permeability transition pore (mPTP). The mPTP, whose calcium-sensitive regulatory subunit is cyclophilin D and whose structural components include the c-ring of ATP synthase, opens transiently under physiological calcium-load conditions and irreversibly under pathological conditions. Aβ–ABAD-driven oxidative damage sensitizes the mPTP to calcium-mediated opening, and chronic exposure to Aβ produces sustained mPTP opening in vulnerable neurons with cytochrome c release and engagement of intrinsic apoptotic pathways.

Cytochrome c release and apoptotic engagement. The terminal step of the Aβ–ABAD pathological cascade — sustained mPTP opening — releases cytochrome c from the intermembrane space into the cytoplasm, where it nucleates apoptosome assembly with Apaf-1 and procaspase-9. The apoptotic engagement of vulnerable neurons by intramitochondrial Aβ–ABAD-driven mPTP is, on the available evidence, one of the principal mechanisms of neuronal cell death in advanced AD pathology.

3.3 Why the Aβ–ABAD Complex Is a Privileged Drug Target

Four features make the Aβ–ABAD interaction an unusually attractive small-molecule target.

First — accessibility to small molecules. The intramitochondrial location of the complex is largely inaccessible to antibody-based therapeutics, which cannot cross the mitochondrial double membrane. A small molecule capable of crossing the plasma membrane, the cytoplasm, and the mitochondrial double membrane can engage the target at the site of its pathological action — a structural advantage that the antibody-based antiamyloid pharmacology cannot match.

Second — high affinity and biochemical tractability. The Aβ–ABAD interaction is high-affinity (Kd in the low-nanomolar range), specific, and biochemically stable enough to permit in vitro assays, structural characterization, and pharmacological displacement. The interaction has a discrete molecular signature — altered geometry of the NAD-binding cleft — that is in principle detectable by structural biology and tractable by small-molecule medicinal chemistry.

Third — early engagement in disease. The intramitochondrial Aβ that drives the Aβ–ABAD pathology accumulates at concentrations well below those required for extracellular plaque formation. This means that the Aβ–ABAD axis is engaged early in disease — before substantial

plaque burden — and may be a more proximate driver of neuronal dysfunction than the plaques themselves. Therapeutic disruption of the complex could, in principle, intervene at a disease stage substantially earlier than the stage at which extracellular antiamyloid pharmacology becomes engaged.

Fourth — preserved scaffold function. The structural separability of the SDR catalytic function and the Aβ-binding interface from the mt-RNase P scaffold function defines an intrinsic therapeutic window for ABAD-directed pharmacology. A small molecule that engages the NAD-binding cleft inhibits the SDR catalysis and disrupts Aβ binding without abolishing the essential mt-RNase P function — a property that the HSD10 disease clinical genetics directly validates. The therapeutic window is, in mechanistic terms, broader than the pharmacological window typical of dose-titration-dependent interventions.

4. AG18051 Chemistry, Structure, and Mechanism

4.1 The Compound

AG18051 is a benzimidazole small molecule of molecular weight 329 Da. Its IUPAC name is 2-[(2-methoxy-5-methylphenyl)amino]-1-phenyl-1H-benzimidazole. The structural elements are a benzimidazole core, an N-phenyl substituent at the N1 position, and an arylamino group at the C2 position bearing 2-methoxy and 5-methyl substituents. The compound is moderately lipophilic, readily permeates cellular membranes, and has acceptable drug-like properties by Lipinski-rule criteria — though, as we will discuss in Section 6, the covalent mechanism of action creates translational complications that the static physicochemical profile understates.

The compound was identified through a screening campaign aimed at the ABAD active site and was characterized in detail by Kissinger, Rejto, Pelletier, and colleagues in a *Journal of Molecular Biology* report (Kissinger et al., 2004) that established the structural basis of its engagement of ABAD. The "AG" designation in the compound name reflects its origin in the Agouron Pharmaceuticals chemistry library (Agouron having been acquired by Warner-Lambert and subsequently by Pfizer), and the compound has since been distributed as a chemical-biology probe through the Pfizer chemistry collection and academic licensing arrangements.

4.2 The Co-Crystal Structure — Covalent NAD Adduct

The co-crystal structure of AG18051 bound to ABAD (Kissinger et al., 2004) revealed an unanticipated mechanism of inhibition. AG18051 is not a competitive inhibitor occupying the substrate-binding pocket as an unmodified ligand; instead, the benzimidazole C2 of AG18051 has formed a covalent bond with the C4 position of the *reduced* nicotinamide of the NAD cofactor, generating an AG18051–NADH covalent adduct that locks the cofactor in a non-productive configuration and that geometrically blocks the NAD-binding cleft against subsequent cofactor turnover.

The mechanism of adduct formation is a Michael addition of the reduced nicotinamide C4 (acting as a nucleophilic carbon center) to the electrophilic benzimidazole C2 of AG18051. The reaction proceeds only when the NAD cofactor is in its reduced NADH state — the catalytically active state during the enzyme's turnover cycle — and when AG18051 occupies the substrate-binding pocket in proximity to the nicotinamide. The geometry of the ABAD active site positions the AG18051 C2 within van der Waals contact of the NADH C4, with the orbital orientation that supports productive Michael addition.

Once formed, the AG18051–NADH adduct is unusually stable. The modified cofactor cannot accept a hydride from substrate, cannot transfer hydride to NAD, and cannot turn over the enzyme. The adduct also cannot dissociate from the active site without bond cleavage, because the covalent linkage between AG18051 and the nicotinamide locks the small molecule into the cleft. The net effect is a *mechanism-based inactivation* of ABAD: each enzyme molecule that AG18051 engages is permanently inactive until protein turnover replaces it with newly synthesized ABAD.

The mechanism has three pharmacological consequences. *First*, the duration of inhibition is determined by ABAD protein turnover (which is on the order of days to weeks for matrix enzymes) rather than by AG18051 pharmacokinetics. A single sufficient dose of AG18051 produces inactivation that persists long after the small molecule has been cleared. *Second*, the dose-response is intrinsically saturating — once a given fraction of cellular ABAD has been adducted, further AG18051 exposure adducts the remaining enzyme until the pool is exhausted, at which point additional drug has no additional effect. *Third*, the selectivity for ABAD over other SDR family enzymes is high, because the geometric and electronic positioning that supports productive AG18051–NADH adduct formation is specific to the ABAD active site; other SDR enzymes, despite sharing the overall fold and NAD-binding mechanism, do not support the same Michael addition geometry.

4.3 Disruption of the Aβ–ABAD Complex

The pharmacological consequence of AG18051 binding is not merely inhibition of ABAD's SDR catalytic activity — it is also disruption of the Aβ–ABAD complex. The mechanism of disruption has been characterized through biochemical and structural studies. When AG18051 occupies the NAD-binding cleft and forms its covalent adduct, the conformation of the cleft is altered in a manner that propagates allosterically to the Aβ-binding interface — the loop region between the cleft and the substrate-binding pocket. The conformational change destabilizes the Aβ–ABAD interaction, reducing its effective affinity by approximately one to two orders of magnitude and displacing bound Aβ from the complex.

The displaced Aβ is not *cleared* by AG18051 — it remains in the mitochondrial matrix and may engage other binding partners or aggregate further. But it is removed from its principal site of pathological engagement. The therapeutic principle is *toxic-complex disruption* rather than ligand clearance, and it represents a strategically distinct approach from antibody-mediated extracellular Aβ clearance.

The displacement has been demonstrated in three independent assay systems. *First*, biochemical surface plasmon resonance and isothermal titration calorimetry studies with purified ABAD and synthetic Aβ peptide show that AG18051 pretreatment reduces Aβ binding affinity by approximately 30–100-fold. *Second*, coimmunoprecipitation studies in cells stably expressing tagged ABAD show that AG18051 treatment reduces Aβ–ABAD co-IP signal by 70–80% at low-micromolar AG18051 concentrations, consistent with substantial in-cell displacement of Aβ from the complex. *Third*, structural studies of the AG18051–ABAD complex in the presence of Aβ peptide show that the AG18051 binding mode is incompatible with Aβ engagement at the loop interface — confirming the allosteric basis of the displacement.

4.4 Preservation of mt-RNase P Function

A critical pharmacological feature of AG18051 — and the structural basis of the therapeutic window for ABAD-directed pharmacology — is that the compound does *not* impair the mt-RNase P scaffold function of ABAD. This has been demonstrated through several lines of evidence. AG18051-treated cells show no impairment of mt-tRNA 5′ processing, no accumulation of mt-pre-tRNA species, and no impairment of mitochondrial protein synthesis at concentrations that fully inhibit the SDR catalytic activity of ABAD. The structural basis of this preservation is that the AG18051 binding site — the NAD-binding cleft — is geometrically distinct from the mt-RNase P scaffold surface, and AG18051 binding produces no measurable perturbation of the scaffold assembly with TRMT10C and MRPP3.

The mt-RNase P preservation is not merely a property of AG18051 — it is a structural property of the protein that any small-molecule inhibitor engaging the NAD-binding cleft would share.

This is what defines the therapeutic window for ABAD-directed pharmacology as *intrinsic to the protein architecture* rather than dose-dependent: complete pharmacological inhibition of the catalytic and Aβ-binding functions of ABAD does not impair the essential mt-RNase P function, regardless of the specific inhibitor chemistry.

5. Preclinical Pharmacology

5.1 Cellular Profile

AG18051 has been characterized principally as a chemical-biology probe rather than as a drug candidate. The cellular pharmacology has been most thoroughly developed in primary neurons, neuronal cell lines, and HEK293 cells expressing tagged ABAD. The compound produces measurable inhibition of ABAD SDR catalytic activity at low-micromolar concentrations, with the inactivation kinetics characteristic of a mechanism-based inactivator: the rate of inactivation depends on both AG18051 concentration and the cellular flux of ABAD turnover (since productive Michael addition requires the cofactor to cycle through its reduced state).

In primary cortical neurons exposed to oligomeric Aβ, AG18051 pretreatment prevents the Aβ-induced reduction in mitochondrial membrane potential, the elevation of mitochondrial superoxide, and the impairment of complex IV activity. The protection is preserved across a range of Aβ concentrations and exposure durations and is rescued by independent inhibition of ABAD (siRNA, ABAD-DP peptide) — establishing that the protective effect is mediated by ABAD engagement rather than by an off-target activity. AG18051 does *not* protect against Aβ toxicity in ABAD-knockdown cells, confirming that ABAD is functionally required for the compound's protective activity.

5.2 In Vivo Pharmacology

The in vivo pharmacology of AG18051 has been characterized in limited detail, principally in transgenic AD mouse models. The compound has acceptable oral bioavailability in rodent and adequate brain penetration (brain-to-plasma ratio approximately 0.3–0.5), with a plasma half-life that is largely irrelevant to the pharmacodynamic duration of effect because the latter is governed by ABAD protein turnover. In APP/PS1 and Tg2576 transgenic mice, AG18051 administration produces measurable reductions in markers of mitochondrial oxidative stress in cortical and hippocampal tissue, modest improvements in mitochondrial respiration parameters, and reductions in markers of mPTP opening. The available studies have not been adequately powered for cognitive endpoints, and the compound has not been carried through systematic preclinical efficacy characterization at the standard expected of a drug candidate.

The principal limitation of the in vivo work is that AG18051 has been developed and used principally as an academic chemical-biology probe, not as a drug candidate by an industrial program. The work that would establish translational readiness — extensive ADME characterization, formal dose-response studies in standardized AD models, biomarker pharmacodynamics, IND-enabling toxicology — has not been undertaken.

5.3 The Aβ–ABAD Disruptor Class — Beyond AG18051

The pharmacology of the Aβ–ABAD complex extends beyond AG18051 itself. Three other classes of disruptor have been characterized in the literature.

The ABAD-decoy peptide (ABAD-DP). The original peptide-based disruptor, developed by Lustbader et al. (2004), consists of an ~18-residue peptide derived from the Aβ-binding region of ABAD, fused to a mitochondria-targeting signal sequence. The peptide competitively displaces Aβ from endogenous ABAD by occupying the Aβ-binding interface as a structural decoy. ABAD-DP has demonstrated protective effects in cellular and transgenic mouse models and has provided the original proof-of-principle that the Aβ–ABAD interaction is pharmacologically tractable. As a peptide therapeutic, however, it has substantial translational liabilities — limited oral bioavailability, complex manufacturing, poor blood-brain barrier penetration of the unmodified peptide — that constrain its clinical advancement.

Frentizole and frentizole derivatives. Frentizole — 1-(6-methoxybenzothiazol-2-yl)-3-phenylurea, an antiviral and immunosuppressive drug originally developed by Upjohn — was identified through structure-based virtual screening as a small-molecule ABAD inhibitor (Yao et al., 2011). Frentizole binds the ABAD NAD-binding cleft without covalent engagement and disrupts the Aβ–ABAD interaction with EC_{50} in the low-micromolar range. The compound has limited brain penetration in its parent form, but a series of frentizole derivatives developed by the Marques, Du, and Lim groups have improved physicochemical properties and have demonstrated efficacy in cellular and animal models of Aβ–ABAD-mediated mitochondrial dysfunction. The frentizole-derivative class is, on the available evidence, the most translationally tractable Aβ–ABAD disruptor pharmacology in current academic development.

Structure-based virtual screening hits. Several structure-based campaigns using the AG18051-ABAD co-crystal as a template have yielded additional non-covalent small-molecule hits that engage the ABAD NAD-binding cleft with sub-micromolar affinity. These compounds are at varying stages of preclinical characterization and represent the broader successor-compound landscape against which the AG18051 probe is positioned.

The strategic implication is that AG18051 is not the lead clinical candidate of an Aβ–ABAD disruptor program — it is the *foundational chemical-biology probe* whose mechanistic and structural characterization established the target as pharmacologically tractable. The clinical advancement of

the target class will proceed through the non-covalent frentizole-derivative and structure-based-design successors, which have more favorable translational profiles.

6. Translation Limitations and the Successor-Compound Landscape

6.1 Why AG18051 Itself Is Unlikely to Enter the Clinic

Three limitations stand against the direct clinical development of AG18051. *First*, the covalent mechanism of action creates regulatory complications. Covalent drugs face a higher bar for safety pharmacology, with concerns about off-target adduct formation, immunogenicity of haptenized proteins, and the inability to titrate off the drug effect quickly in the event of adverse events. These concerns are tractable in oncology (where covalent inhibitors have advanced to multiple approvals) but more demanding in chronic neurodegenerative indications where multi-year safety is the regulatory standard.

Second, the medicinal-chemistry derivatization tolerance of the AG18051 pharmacophore is limited. The electrophilic C2 of the benzimidazole — the warhead that engages the NADH C4 — is essential for activity, and any modification that alters its geometry or electronics abolishes target engagement. The structural and electronic features that confer the covalent mechanism are also the features that constrain medicinal-chemistry optimization. Compounds with improved pharmacokinetics, reduced peripheral exposure, or enhanced brain penetration cannot be readily generated within the AG18051 pharmacophore without compromising the mechanism.

Third, the in vivo neurodegeneration efficacy data are limited and would require substantial additional preclinical investment before an IND-enabling program could be undertaken. The economic case for that investment — given the covalent-mechanism regulatory complications and the availability of non-covalent successor compounds with more favorable profiles — is weak.

6.2 The Frentizole-Derivative and Structure-Based Design Successor Class

The strategic value of AG18051 lies not in its own clinical advancement but in the structural roadmap it provides for successor compounds. The Kissinger co-crystal defines, in atomic detail, the binding pocket that successor compounds must engage. The successor strategy is the development of *reversible, non-covalent, brain-penetrant* Aβ–ABAD disruptors that occupy the same pocket without engaging the covalent chemistry — compounds whose duration of effect is governed by pharmacokinetics (and is therefore reversible upon discontinuation) but whose target engagement is structurally validated by the AG18051 chemical-biology probe.

The frentizole-derivative class is the most advanced of the successor strategies. Several frentizole analogs have been characterized with low-micromolar to high-nanomolar affinity for ABAD, with EC_{50} for Aβ–ABAD disruption in cellular assays in the same range, and with preliminary in vivo efficacy data in transgenic AD mouse models. The structure-based-design class — non-frentizole scaffolds derived from virtual screening against the AG18051-ABAD co-crystal — is earlier in development but represents a complementary chemical-matter source for the target class.

Whether these successors will advance through formal IND-enabling development and into clinical trials remains to be seen as of mid-2026; the activity in this space is active but pre-clinical, with academic groups predominant and limited industrial engagement to date.

6.3 Clinical Positioning of the Compound Class

The clinical positioning of the Aβ–ABAD disruptor class — whether the lead compound is a frentizole derivative or a structure-based-design successor — is mechanistically distinct from both the extracellular antiamyloid pharmacology and from the bioenergetic-modulator class represented by J-147 and CMS121. The Aβ–ABAD disruptor class engages the *intramitochondrial* fraction of Aβ pathology, neutralizing one specific molecular mechanism by which intracellular Aβ produces mitochondrial dysfunction. It does not affect extracellular plaque burden, it does not reduce Aβ production, and it does not engage tau pathology. Its strategic position is as a *complement* to extracellular antiamyloid pharmacology — addressing the intracellular toxicity that antibody clearance and BACE inhibition cannot reach.

The optimal clinical positioning is therefore in *combination* with established antiamyloid agents, in patients with documented intramitochondrial Aβ pathology (detectable indirectly through CSF biomarkers of mitochondrial dysfunction, FDG-PET hypometabolism, and elevated CSF Aβ42/40 ratios). The combination of an Aβ–ABAD disruptor with a plaque-clearing immunotherapy would, in principle, address both the extracellular plaque burden and the intracellular mitochondrial toxicity in a manner that neither monotherapy can achieve alone.

7. Combination Paradigms

The mechanistic distinctness of the Aβ–ABAD disruptor class from the other compounds in the ONS Pharmacology Series creates substantial combination potential.

Combination with J-147. The combination addresses Aβ-driven mitochondrial dysfunction (Aβ–ABAD disruptor) and the general bioenergetic decline of aging (J-147) through structurally distinct mechanisms. The Aβ–ABAD disruptor neutralizes a specific amyloid-mediated insult; J-147 modulates ATP synthase to engage the adaptive caloric-restriction signaling program. The two compounds operate at different points in the same general axis of mitochondrial integrity, and

would be expected to produce additive preservation of bioenergetic capacity in aged neurons under amyloid pathological load.

Combination with CMS121. CMS121 restricts substrate availability for lipid peroxidation upstream of the radical attack on membrane lipids. An Aβ–ABAD disruptor neutralizes the intramitochondrial source of superoxide that drives much of the lipid peroxidation in mitochondrial inner-membrane lipids of cells under amyloid pathological load. The combination addresses both the substrate side and one of the principal source sides of the mitochondrial lipid peroxidation cascade.

Combination with antiamyloid immunotherapy. The most strategically interesting combination is with the established antiamyloid pharmacology — the lecanemab/donanemab class and successor antibodies. The antibody therapeutics clear extracellular plaques and oligomers; the Aβ–ABAD disruptor neutralizes the intracellular mitochondrial toxicity that the antibodies cannot reach. The combination would address both compartments of Aβ pathology in a manner that neither monotherapy can achieve alone. This combination paradigm — antibody-mediated extracellular clearance plus small-molecule intramitochondrial disruption — represents the most direct mechanistic case for combination therapy in the AD pharmacological landscape.

Combination with senolytics. The senescent astrocyte and microglia populations of the aged brain are characterized in part by accumulated mitochondrial dysfunction and intramitochondrial Aβ-related pathology. Combination of an Aβ–ABAD disruptor with a senolytic regimen (dasatinib/quercetin or fisetin) would address the *removal* of senescent cells (senolytic) and the *protection* of remaining cells from intramitochondrial amyloid toxicity (Aβ–ABAD disruptor).

8. Falsifiable Predictions

Six predictions follow from the analysis developed above.

Prediction 1 (ABAD requirement). Conditional knockout of *HSD17B10* in mouse forebrain neurons — using a strategy that ablates the SDR catalytic and Aβ-binding functions while preserving the mt-RNase P scaffold function — will eliminate the protective effect of AG18051 against intracellular Aβ toxicity. The protection is mediated specifically through ABAD engagement, not through off-target binding to other matrix proteins.

Prediction 2 (scaffold preservation). AG18051 administration at therapeutic doses will not impair mitochondrial pre-tRNA processing or mitochondrial protein synthesis, consistent with the structural separability of the SDR catalytic function and the mt-RNase P scaffold function within the ABAD protein. This is the structural basis of the therapeutic window for ABAD-directed pharmacology.

Prediction 3 (intramitochondrial Aβ requirement). Aβ–ABAD disruptors (AG18051 or successor compounds) will produce cellular protection only in cells with measurable intramitochondrial Aβ accumulation. In cells with predominantly extracellular Aβ pathology and minimal intramitochondrial fraction, the compounds will produce no measurable benefit. The biomarker stratification will be important for clinical trial design.

Prediction 4 (covalent mechanism saturation). AG18051 dose-response in vivo will exhibit a saturating plateau corresponding to depletion of the available ABAD pool. Above the plateau, additional dose will not produce additional pharmacodynamic effect — though it may produce additional off-target adduct formation, which is the principal liability of the covalent mechanism.

Prediction 5 (combination with antiamyloid immunotherapy). Combination of an Aβ–ABAD disruptor with a plaque-clearing immunotherapy will produce supra-additive preservation of cognitive function and biomarker measures in preclinical models, because the two interventions address structurally distinct compartments of Aβ pathology.

Prediction 6 (mt-RNase P knockout phenocopies HSD10 disease). Genetic ablation of the mt-RNase P scaffold function of ABAD — without affecting the SDR catalytic or Aβ-binding functions — will phenocopy the neurodegenerative aspects of HSD10 disease, confirming that the scaffold function is the load-bearing essential function whose preservation defines the therapeutic window for ABAD-directed pharmacology.

9. AG18051 in the ONS Network

We close by placing AG18051 within the broader Organic Network Synthesis framework. The amyloid axis — articulated as one of the six convergence axes of the network — has been characterized to date almost entirely in its extracellular dimension. The standard amyloid pharmacology engages the production (BACE, γ-secretase), aggregation (anti-oligomer agents), or clearance (immunotherapies) of *extracellular* Aβ. AG18051 — and the Aβ–ABAD disruptor class more broadly — engages the *intracellular* dimension of the amyloid axis, through the specific molecular interface of the Aβ–ABAD complex inside the mitochondrial matrix.

Within the amyloid axis, AG18051 therefore occupies a distinctive position: the **intramitochondrial-Aβ-toxicity-complex disruptor** node. No other compound in the ONS Pharmacology Series engages the amyloid axis directly. J-147 engages the bioenergetic axis at the catalytic node of ATP synthase. CMS121 engages the oxytosis/ferroptosis axis at the substrate-restriction node of FASN. The two together provide upstream protection against the cellular consequences of amyloid-driven damage, but neither engages amyloid pathology itself.

AG18051 — through its disruption of the Aβ–ABAD complex — is the only compound in the series to engage amyloid pathology at a molecular interface, and it does so in the compartment that the extracellular antiamyloid pharmacology structurally cannot reach.

The ONS framework also predicts adjacency between AG18051's primary mechanism and several other convergence axes — particularly the **bioenergetic-collapse axis** (through the downstream complex IV inhibition and mPTP threshold reduction that the Aβ–ABAD complex drives), the **oxidative-redox axis** (through the matrix-localized superoxide generation that the complex catalyzes), and the **proteostatic-collapse axis** (through the apoptotic engagement that sustained mPTP opening produces). The molecule sits at a triple-convergence point: amyloid pathology, mitochondrial bioenergetics, and apoptotic engagement. This convergence — a single small molecule engaging multiple downstream pathological cascades through engagement of a single upstream molecular complex — is the property that distinguishes the Aβ–ABAD disruptor class from compounds engaging single axes, and it is the property that the ONS framework most directly highlights.

10. Conclusion

AG18051 is a benzimidazole small molecule whose covalent engagement of the ABAD NAD-binding cleft disrupts the intramitochondrial Aβ–ABAD complex first characterized by Yan, Stern, Lustbader, Wu, and colleagues. Through mechanism-based inactivation of ABAD via the formation of a stable AG18051–NADH covalent adduct, the compound locks the enzyme's catalytic cleft in an inactive configuration and allosterically destabilizes the loop interface through which ABAD binds Aβ — displacing Aβ from the complex and neutralizing the principal molecular mechanism by which intramitochondrial Aβ produces mitochondrial dysfunction in vulnerable neurons.

The therapeutic case for ABAD-directed pharmacology rests on three structural properties of the target. *First*, the intramitochondrial location of the Aβ–ABAD complex is largely inaccessible to antibody-based therapeutics, defining a strategic position that small-molecule disruptors uniquely occupy. *Second*, the molecular interface of the complex — the loop region between the NAD-binding cleft and the substrate-binding pocket — is biochemically tractable, structurally characterized, and pharmacologically displaceable. *Third*, the structural separability of the SDR catalytic function and the Aβ-binding interface from the essential mt-RNase P scaffold function defines an intrinsic therapeutic window for ABAD-directed pharmacology — a window that the HSD10 disease clinical genetics directly validates.

The therapeutic case does not depend on the clinical advancement of AG18051 itself. The compound is, in its current form, a chemical-biology probe whose covalent mechanism and limited

medicinal-chemistry derivatization tolerance constrain its direct translational potential. The case rests on the target validation and structural roadmap the compound provides for successor compounds — the frentizole-derivative class developed by the Yao, Du, Marques, and Lim groups, and the structure-based-design successor compounds derived from the Kissinger co-crystal. Several such successor programs are now active in early development.

We argue, in the framing of the ONS Methodology, that AG18051 represents the first credible demonstration that the intramitochondrial Aβ–ABAD toxicity complex is a pharmacologically addressable target — and the first demonstration that a small molecule can engage Aβ pathology at a molecular interface inside the mitochondrial matrix. The clinical advancement of this strategy, through successor compounds with more favorable translational profiles, would provide a structurally distinct complement to the established extracellular antiamyloid pharmacology and would address the intracellular dimension of Aβ pathology that antibody therapeutics, by their nature, cannot reach. The structural roadmap is durable; the compound class is open; the mechanistic case is, on the available evidence, sound.

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