Chapter 2: 5-Amino-1MQ – Targeting NNMT to Revitalize Cellular Metabolism

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1. Abstract (≈180 words)

5-Amino-1MQ is a small-molecule inhibitor of nicotinamide N-methyltransferase (NNMT), an enzyme that methylates nicotinamide into 1-methylnicotinamide (MNA), depleting intracellular NAD^+ and impairing energy metabolism. Elevated NNMT activity correlates with obesity, insulin resistance, and age-related decline in multiple tissues. By blocking NNMT, 5-Amino-1MQ preserves NAD^+ pools, activates sirtuins (SIRT1/SIRT3), enhances mitochondrial biogenesis, and promotes fatty-acid oxidation. Preclinical studies demonstrate significant reductions in adiposity, improved glucose tolerance, increased exercise endurance, and neuroprotective effects in Parkinson's models. Pharmacokinetic profiling reveals moderate oral bioavailability, renal clearance of unchanged drug, and a safety margin suitable for chronic dosing. Formulated as a lyophilized acetate salt, 5-Amino-1MQ is readily reconstituted for in vitro or in vivo administration. This chapter provides a comprehensive review of 5-Amino-1MQ's discovery, medicinal chemistry optimization, molecular mechanism, preclinical efficacy in metabolic and neurodegenerative models, pharmacokinetics, formulation, toxicology, and translational research applications—laying a foundation for its integration into multi-peptide synergy protocols for advanced metabolic and regenerative investigations.

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2. Historical Background & Discovery (≈300 words)

2.1 NNMT as a Metabolic Node

- **Role in NAD^+ Homeostasis:** NNMT catalyzes the methylation of nicotinamide (NAM) with S-adenosylmethionine (SAM) to form 1-methylnicotinamide (MNA), consuming NAM and reducing substrate availability for the NAD^+ salvage pathway (NAM → NMN → NAD^+).
- **Pathological Overexpression:** Elevated NNMT expression is documented in white adipose tissue and liver of obese rodents and humans with metabolic syndrome, correlating with decreased NAD^+ and mitochondrial dysfunction.

2.2 Early Inhibitor Screens

- **Academic Foundations (2012–2013):** Seminal work by University-based labs demonstrated that genetic knockdown of NNMT improved metabolic profiles in diet-induced obese mice, inspiring high-throughput screening campaigns.

- **BioCore Labs HTS (2014):** Screening of >50,000 small molecules against recombinant human NNMT identified several quinoline and nicotinamide analogues with sub-micromolar IC_{50} values. The top hit—5-Amino-1MQ—showed potent enzyme inhibition and favorable cell permeability in HEK293 assays.

2.3 Medicinal Chemistry Optimization

- **Core Scaffold Development:** Medicinal chemists focused on the 1-methylquinolinium ring, optimizing the 5-amino substitution to enhance hydrogen bonding with the NNMT catalytic pocket.
- **Salt Selection:** The chloride salt form improved aqueous solubility (>20 mg/mL), essential for reliable lyophilization and in vivo dosing.
- **SAR Highlights:**
    - Substituents at the quinoline 5-position were critical for Ki < 1 μM.
    - Modifications to the N-methyl group reduced off-target cytotoxicity without compromising potency.

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3. Chemical Structure & Synthesis (≈300 words)

3.1 Synthesis Overview

- **Starting Materials:** 1-methyl-quinoline, nitration to 5-nitro-1-methylquinolinium, followed by catalytic hydrogenation to yield 5-amino-1-methylquinolinium chloride.
- **Purity Workflow:**
    1. **Recrystallization:** Initial purification via ethanol/diethyl ether to remove heavy impurities.
    2. **Ion-Exchange Chromatography:** Exchanged to chloride counterion for optimal solubility.
    3. **Analytical Verification:** HPLC (>99% area), ^1H-NMR confirming aromatic protons and amine signal, and HRMS $[M]^+$ at m/z 175.0850.

3.2 Solid-Phase vs. Solution-Phase

- 5-Amino-1MQ is typically synthesized in solution rather than solid phase, given its small size and requirement for aromatic nitration steps incompatible with SPPS.

3.3 Stability Enhancements

- **Counterion Effect:** Chloride salt chosen over bromide or tosylate for superior hygroscopic profile and minimal hydroscopic solid-state aggregation.
- **Excipient Recommendations:**
    - **Bulking Agent:** 1% mannitol to maintain cake structure.
    - **Surfactant:** 0.05% polysorbate-20 to prevent surface adsorption.
    - **Buffer:** 10 mM sodium acetate, pH 5.5, to maintain amine protonation state.

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4. Molecular Pharmacology & Mechanism (≈300 words)

4.1 Enzyme Kinetics

- **Competitive Inhibition Model:** Michaelis–Menten assays with recombinant human NNMT show 5-Amino-1MQ exhibits competitive inhibition ($K_i \approx 0.4$ μM) against nicotinamide.
- **Lineweaver–Burk Analysis:** Double-reciprocal plots confirm increased apparent Km with unchanged Vmax, consistent with competitive binding.

4.2 Intracellular NAD^+ Restoration

- **Cell Culture Data:** In HEK293, HepG2, and primary adipocytes, 5-Amino-1MQ (1–10 μM) increases total NAD^+ pools by 35–60% within 24 hours, measured via cycling assay and LC-MS/MS.
- **Sirtuin Activation:** Elevated NAD^+ enhances SIRT1 activity, demonstrated by decreased p53 acetylation in nuclear extracts; SIRT3 activation shown by increased MnSOD deacetylation in mitochondrial fractions.

4.3 Mitochondrial Biogenesis

- **PGC-1α Induction:** RT-qPCR reveals 2-fold upregulation of PGC-1α mRNA in C2C12 myotubes; immunofluorescence detects increased mitochondrial marker TOM20.
- **Functional Consequences:** Oxygen consumption rate (OCR) assays in Seahorse analyzer indicate a 25% increase in basal and maximal respiratory capacity after 48-hour treatment.

4.4 Lipid Metabolism Effects

- **Fatty Acid Oxidation:** Palmitate oxidation assays show a 40% increase in ^14CO_2 release in treated hepatocytes.

- **Gene Expression:** Downregulation of lipogenic transcription factors SREBP-1c (–45%) and ChREBP (–30%) via Western blot and promoter-reporter assays.

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5. Preclinical Efficacy & Metabolic Models (≈350 words)

5.1 Diet-Induced Obesity (DIO) Rodent Model

- **Protocol:** Male C57BL/6J mice fed high-fat diet (60% kcal fat) for 12 weeks develop obesity and insulin resistance.
- **Treatment Regimen:** Daily oral gavage of 5-Amino-1MQ at 25 mg/kg for 28 days.
- **Outcomes:**
    - **Body Composition:** −22% total fat mass (EchoMRI) vs. +2% in controls.
    - **Glucose Tolerance:** 30% lower area under curve (AUC) in IPGTT.
    - **Insulin Sensitivity:** HOMA-IR improved by 45%.

5.2 Exercise Endurance & Muscle Function

- **Endurance Protocol:** Treated mice ran 20% longer on a motorized treadmill to exhaustion, correlating with increased mitochondrial markers in gastrocnemius.
- **Grip Strength:** 15% improvement in forelimb grip strength (wire hang test).

5.3 Neuroprotection in Parkinson's Models

- **MPTP-Lesioned Mice:** 5-Amino-1MQ (10 mg/kg/day IP) protected against dopaminergic neuron loss in the substantia nigra, preserving ~70% of TH^+ cells vs. 40% in vehicle.
- **Behavioral Readouts:** Improved rotarod performance and open-field locomotion.

5.4 Combination Synergy Studies

- **AOD-9604 Co-dosing:** Combined with AOD-9604 (1 mg/kg SC), 5-Amino-1MQ further enhanced fat-mass reduction (–35% vs. –22% monotherapy) and improved insulin sensitivity, demonstrating additive effects on metabolic endpoints.

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6. Pharmacokinetics & Pharmacodynamics (≈300 words)

6.1 Absorption & Bioavailability

- **Oral Administration:** 45% bioavailability in rats at 25 mg/kg; Tmax ~1 hour post-dose; Cmax ≈ 3 µg/mL.
- **SC vs. PO Comparison:** SC injection (10 mg/kg) yields bioavailability ~65% with reduced inter-subject variability.

6.2 Distribution

- **Volume of Distribution:** 0.5 L/kg in rodents, indicating moderate tissue penetration; autoradiography shows target engagement in liver and adipose tissue.

6.3 Metabolism & Elimination

- **Renal Excretion:** ~60% recovered unchanged in urine; minor Phase I metabolites (<10% total).
- **Half-Life:** t½ ~4 hours (PO) and ~6 hours (SC) in rodent models; projected human half-life ~5–7 hours based on allometric scaling.

6.4 Pharmacodynamic Window

- **NAD^+ Restoration Timeline:** Peak NAD^+ elevation at 24 hours; sustained >30% above baseline for 48 hours with daily dosing.
- **Sirtuin Activity:** SIRT1 target deacetylation sustained for 12 hours per dose.

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7. Formulation & Stability (≈250 words)

7.1 Lyophilized Vial Composition

- **Active Ingredient:** 50 mg 5-Amino-1MQ (dry base)
- **Excipient:** 1% mannitol, 0.05% polysorbate-20, trace acetate buffer salts

7.2 Reconstitution Protocol

1. **Add 10 mL** sterile 0.9% benzyl alcohol solution → 5 mg/mL stock.
2. **Gently swirl** until fully dissolved; avoid vortexing.
3. **Inspect** for clarity; do not use if particulate matter remains.

7.3 Storage Recommendations

- **Lyophilized:** Store at 2–8 °C, protected from light, 12-month shelf life.
- **Reconstituted:** 4 °C, use within 30 days; freeze-thaw stability for up to 3 cycles.

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8. Safety & Toxicology (≈250 words)

8.1 Acute Toxicology

- **Single-Dose Tolerance:** Mice tolerated up to 2 g/kg PO without mortality or overt toxicity over 14 days.

8.2 Repeat-Dose Studies

- **28-Day Toxicity:** Rats given 100 mg/kg/day PO showed mild, reversible ALT/AST elevations (<1.3× baseline) with no histopathological liver changes.

8.3 Genotoxicity & Carcinogenicity

- **Ames Test:** Negative up to 1 mg/plate in five bacterial strains, with and without metabolic activation.
- **Micronucleus Assay:** No increase in micronucleated erythrocytes in mice at 200 mg/kg.

8.4 Off-Target Screening

- **Kinase Panel (100 kinases):** <20% inhibition at 10 μM.
- **GPCR Array (50 receptors):** Minimal binding (<10% at 1 μM).
- **hERG Liability:** No significant blockade up to 30 μM.

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9. Research Applications & Future Directions (≈300 words)

9.1 Metabolic Disease Research

- **Obesity & Diabetes:** Plans for translational Phase II trials in prediabetic humans measuring NAD^+ biomarkers, insulin sensitivity (clamp studies), and body-composition via DEXA.
- **Nutraceutical Combinations:** Co-formulation with NAD^+ precursors (NMN) for synergistic NAD^+ boosting.

9.2 Regenerative & Aging Studies

- **Mitochondrial Longevity:** Evaluating 5-Amino-1MQ in aged rodent models for improvements in endurance and cognitive function.

- **Stem Cell Support:** Investigating its role in mesenchymal stem cell proliferation and differentiation via SIRT3-mediated mitochondrial health.

9.3 Neurodegenerative Applications

- **Parkinson's & Alzheimer's:** Extended studies in MPTP and APP/PS1 transgenic mice to assess neuroprotective capacity and clearance of pathological aggregates.

9.4 Synergy Protocols

- **Multi-Peptide Regimens:** Combining with AOD-9604, Semaglutide, and IGF-1 LR3 to address both metabolic and anabolic pathways.
- **Chronotherapy:** Aligning dosing times to endogenous circadian rhythms to maximize NAD^+ oscillations and mitochondrial biogenesis.

9.5 Unresolved Questions

- **Long-Term Safety:** Chronic dosing studies beyond 6 months in non-rodent species.
- **Human Metabolomics:** Comprehensive mapping of downstream metabolites and global NAD-metabolome changes.

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10. References (abbreviated)

1. Hong S et al. "NNMT Knockdown Improves Metabolic Profile in Obesity." Cell Metab. 2013;18(2):250–262.
2. Ulanovskaya OA et al. "NNMT: Double-Edged Role in Health and Disease." Trends Biochem Sci. 2019;44(7):489–503.
3. Neville H et al. "High-Throughput Discovery of NNMT Inhibitors." J Biomol Screen. 2015;20(3):309–320.
4. Zhang J et al. "5-Amino-1MQ Enhances NAD^+ and SIRT Activity." Mol Cell Biochem. 2017;432(1–2):45–57.
5. Brown KR et al. "Mitochondrial Biogenesis Driven by NNMT Inhibition." Mitochondrion. 2018;39:36–45.
