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Review

Therapeutic Potential of ACMSD Inhibitors in NAD$^+$ Deficient Diseases

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Abstract: Nicotinamide adenine dinucleotide (NAD$^+$) is one of the most essential coenzymes that is widely distributed in human tissues. However, with the progress of aging, the NAD$^+$ level gradually decreases, thus impacting the metabolic dynamics and heightening susceptibility to various pathologies. Increasing NAD$^+$ levels are expected to delay aging and improve age-related degenerative diseases. Amino–carboxylic semialdehyde dehydrogenase (ACMSD) is a key enzyme involved in the de novo synthesis of NAD$^+$. It reduces the intermediate products of the de novo synthesis pathway by catalyzing the degradation of α-amino-β-carboxyethylglutamic acid-ε-semialdehyde (ACMS), thus reducing the production of NAD$^+$. Genetic and pharmacological inhibition of ACMSD has been demonstrated to increase NAD$^+$ levels in vitro and in vivo, thus making it a potential target for the treatment of NAD$^+$-deficient diseases. In this mini-review, we detail the molecular mechanisms regulated by ACMSD. We also discuss the potential efficacy and progress of ACMSD inhibitors in treating aging and age-related diseases.

Keywords: nicotinamide adenine dinucleotide; amino–carboxylic semialdehyde dehydrogenase; de novo synthesis pathway; inhibitors; degenerative diseases

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1. Introduction

Nicotinamide adenine dinucleotide (NAD$^+$) is ubiquitously present within human cells and serves as an indispensable coenzyme [1]. It participates in a myriad of physiological processes and catalytic reactions, playing a pivotal role in cellular homeostasis and maintenance. Since its discovery in 1904 [2], NAD$^+$ has been the subject of continuous scientific investigation. The multifaceted functions of NAD$^+$ have meticulously summarized in previous reviews, including metabolic pathways, redox reactions, DNA repair, genetic stability, and epigenetic modulation, highlighting the paramount importance of NAD$^+$ for overall systemic health and equilibrium [3–5].

Under physiological conditions, NAD$^+$ undergoes perpetual synthesis, degradation, and recycling within cells to uphold a stable intracellular NAD$^+$ pool. However, during the aging process, this equilibrium shifts to a net depletion of NAD$^+$ due to increased consumption relative to production [5,6]. This decline significantly impacts metabolic dynamics and heightens susceptibility to various pathologies. Reduced NAD$^+$ levels have been implicated in diverse diseases, including cardiovascular disorders, malignancies, age-related metabolic perturbations, organ fibrosis, and neurodegenerative conditions [4,7–9].

NAD^+ metabolic imbalance consistently manifests during aging and various age-related diseases [10]. Consequently, researchers have proposed that restoring NAD^+ levels could potentially retard aging and ameliorate age-associated ailments [7]. However, relying solely on methods like exercise or caloric restriction yields only modest increases in NAD^+ and falls short of meeting physiological demands. Moreover, due to its substantial molecular weight and inherent instability, direct NAD^+ supplementation remains inefficiently absorbed by the body. Consequently, attention has shifted toward supplementing NAD^+ precursors or modulating enzymes involved in its biosynthesis and degradation [11–14]. Notable candidates include nicotinamide riboside (NR), nicotinamide mononucleotide (NMN), and nicotinamide phosphoribosyltransferase (NAMPT), all aimed at elevating NAD^+ levels. Existing clinical trials have demonstrated the safety and efficacy of the short-term administration of these compounds for augmenting intracellular NAD^+ levels [5,8]. These findings underscore the pathophysiological significance and therapeutic potential of NAD^+ regulation.

Amino–carboxylic semialdehyde dehydrogenase (ACMSD) emerges as a critical enzyme in de novo NAD^+ synthesis [15,16]. It exhibits high expression in the liver and kidneys [17]. ACMSD catalyzes the degradation of α-amino-β-carboxyethylglutamic acid-ε-semialdehyde (ACMS), thereby reducing intermediate products in the de novo NAD^+ synthesis pathway and subsequently limiting overall NAD^+ production. Reducing ACMSD activity enhances de novo NAD^+ synthesis [18,19]. Transgenic mice expressing human ACMSD exhibit reversible, mild-to-severe NAD^+ deficiency, characterized by reduced body weight, decreased adiposity, and impaired locomotor abilities [20]. In liver cells, ACMSD silencing promotes NAD^+ generation, leading to improved mitochondrial function and inhibition of cellular apoptosis. Genetic or pharmacological ACMSD inhibition enhances NAD^+ levels in animal liver, kidney, and brain tissues, conferring protective effects against acute kidney injury and non-alcoholic fatty liver disease [18]. Consequently, ACMSD inhibition emerges as a potent strategy for replenishing NAD^+ levels and enhancing cellular health.

This review will delve into the regulatory mechanisms of ACMSD regarding NAD^+ metabolism and explore its potential in treating aging-related diseases, particularly those affecting the kidneys and liver.

2. NAD^+ Function and Deficiency

NAD^+ is a crucial cofactor involved in numerous metabolic pathways within cells [21–23]. It serves as a hydrogen acceptor and donor, facilitating the transfer of electrons during redox reactions, which are essential for energy production through processes like glycolysis, the Krebs cycle, and oxidative phosphorylation. NAD^+ accepts electrons from metabolic processes and is reduced to NADH. The interconversion between these states is pivotal for driving ATP synthesis, the primary energy currency of cells. NAD^+ is also involved in various biosynthetic pathways, including the synthesis of fatty acids, cholesterol, and amino acids.

NAD^+'s versatility extends to its role in regulating gene expression, DNA repair, and maintaining cellular health [22,23]. It serves as a cofactor in the enzyme poly(ADP-ribose) polymerase (PARP), which is involved in the repair of DNA damage caused by oxidative stress, ultraviolet radiation, and other environmental factors [24,25]. NAD^+ also participates in CD38-mediated signaling, regulating cellular immune and inflammatory responses [26–28]. Furthermore, NAD^+ is integral to longevity pathways, particularly those involving sirtuins, a family of proteins that rely on NAD^+ for their activation and mediate metabolic adaptations and stress resistance. Thus, NAD^+ is a crucial component in the production of these long-lived proteins that contribute to maintaining our body's health [29–31].

Deficiencies in NAD^+ can lead to a range of diseases, including aging, neurodegenerative disorders, and metabolic disorders [4,8,9,32]. Study has indicated a decline of NAD^+ levels with older age, leading to a decrease in cellular energy production and an increase in oxidative stress. This can contribute to the development of age-related diseases such as Alzheimer's disease and Parkinson's disease [5,31]. NAD^+ deficiency has been implicated as a contributing factor in neurodegenerative disorders, such as Huntington's disease and amyotrophic lateral sclerosis (ALS). These disorders are characterized by the degeneration of nerve cells in the brain and spinal cord, leading to progressive loss of motor function.

Reducing NAD^+ levels could lead to a range of metabolic diseases, including diabetes, obesity, hyperlipidemia, and fatty liver disease [3,32]. NAD^+ insufficiency disorders mitochondrial functions, characterized by reduced energy production and impaired organ function. These might manifest as muscle weakness, and cardiovascular issues. Additionally, metabolic syndromes such as type 2 diabetes mellitus (T2DM), characterized by insulin resistance and abnormal glucose metabolism, have been linked to low NAD^+ levels. NAD^+ deficiency can also affect the function of liver and kidney, which are important metabolic organs responsible for breaking down and removing toxins and waste from the body. When NAD^+ levels are reduced, the metabolic function is disordered, leading to lipid and ROS accumulation, and mitochondrial function impairment, which in turn triggers liver and kidney disease, including hepatitis, cirrhosis, and kidney failure [33–35].

Taken together, due to the critical role of NAD^+ in maintaining cell function and metabolism, increasing NAD^+ levels is considered a potential therapeutic strategy [36]. Increasing NAD^+ levels in the body through medication or dietary interventions may help slow down the aging process and the progression of related diseases.

3. NAD^+ Synthesis and Consumption

NAD^+ is widely distributed within human cells and participates in thousands of biological catalytic reactions [3–5]. Understanding and manipulating NAD metabolism holds promise for therapeutic interventions in these disorders, aiming to restore metabolic balance and promote cellular health.

Under normal physiological conditions, NAD^+ undergoes continuous synthesis, breakdown, and recycling within cells to maintain a stable intracellular NAD^+ level [32,37]. The schematic of the NAD^+ synthesis and consumption pathway is shown in Figure 1. However, an imbalance between NAD^+ synthesis and consumption leads to a decline in NAD^+ levels, contributing to aging and degenerative pathologies. In this section, we will briefly outline the synthesis and consumption pathways of NAD^+ to provide a conceptual basis for regulating NAD^+ levels.

The synthesis of NAD^+ involves a complex process, primarily comprising three pathways, including the Preiss–Handler pathway, the de novo synthesis pathway, and the salvage synthesis pathway [3,23,37]. Each of the pathways utilizes distinct NAD^+ precursors. Notable precursors include nicotinic acid (NA), tryptophan (Trp), nicotinamide (NAM), and nicotinamide riboside (NR). These precursors can either be obtained from the diet (e.g., NA, Trp, NR) or recycled from NAD^+ degradation products (e.g., NAM). They appear in different synthesis pathways and can be interconverted (Figure 1).

In the Preiss–Handler pathway, the dietary NA (a form of vitamin B3) is converted to NAD^+ in three step. Firstly, NA is converted to nicotinic acid mononucleotide (NaMN) through the action of nicotinic acid phosphoribosyltransferase (NAPRT), and then to NaAD (nicotinic acid adenine dinucleotide) catalyzed by NaMN transferase (NMNAT), and then completed by the transformation of $NaAD^+$ into NAD^+ by NAD^+ synthase (NAD syn).

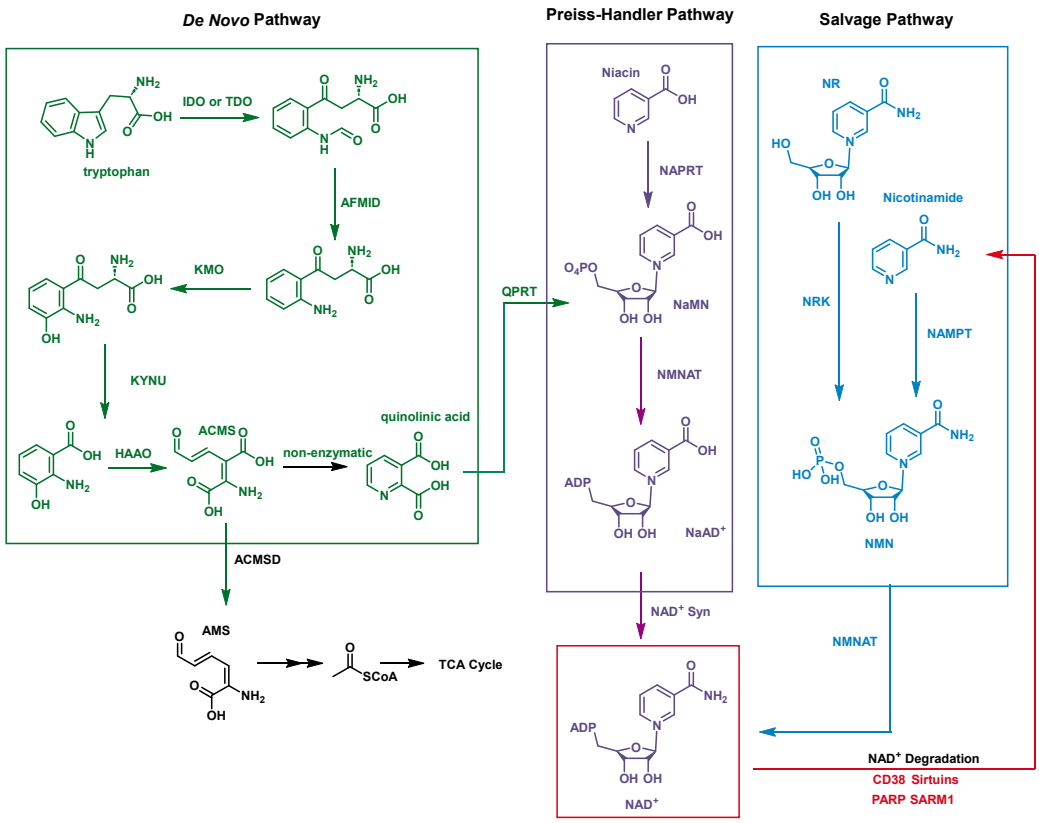


Figure 1. The schematic of nicotinamide adenine dinucleotide (NAD^+) synthesis and consumption.

NAM is generated as a by-product in NAD^+-consuming enzymatic processes (e.g., sirtuins, PARPs, and cADPR synthases), and is recycled in the salvage pathway to maintain intracellular NAD levels. In this path, NAMPT recycles NAM into nicotinamide mononucleotide (NMN), which is a rate-limiting enzyme in this pathway. Then, NMN is converted into NAD^+ through the action of NMNATs.

Trp is the starting molecule that initiates the de novo synthesis pathway. It is metabolized through a series of enzymatic reactions to form quinolinic acid, which is then phosphoribosylated to form NaMN, and is converted to NAD^+ following the same subsequent steps as in the Preiss–Handler pathway. Each of these pathways plays a crucial role in maintaining adequate levels of NAD^+ within cells, ensuring the proper function of various metabolic processes and cellular machinery.

NAD^+ serves as a crucial coenzyme in cellular redox reactions and also acts as a substrate for various enzymes involved in signal transduction. Unlike redox reactions, these enzymes facilitate NAD^+ breakdown metabolism, earning them the label of NAD^+-consuming enzymes. Noteworthy NAD^+-consuming enzymes include CD38, poly(ADP-ribose) polymerases (PARPs), sirtuins, SARM1, etc. [23]. CD38 is an extracellular enzyme primarily expressed in immune cells, which is closely associated with cellular immune responses and inflammatory states. CD38 expression rises with aging and increased inflammation, leading to a decline in NAD^+ levels, and is considered a key factor in NAD^+ degradation [26–28]. PARPs are crucial intracellular DNA repair enzymes. When DNA damage occurs, PARPs are activated for participating in DNA repair and maintaining genomic stability. PARPs are activated to consume NAD^+, and are labeled as NAD^+-consuming enzymes [24,25]. Sirtuins constitute a protein deacetylase family comprising seven members (SIRT1-SIRT7) [38–40]. They regulate protein function through deacetylation processes, and play essential roles in cellular metabolism, stress responses, and the aging process. SARM1 primarily functions in neural tissues, especially during the regressive process after

axonal injury. The activation of SARM1 leads to substantial degradation of NAD^+, affecting neuronal cell survival and function [29–31]. In summary, these NAD^+-consuming enzymes are crucial in maintaining cellular balance while significantly impacting overall health. Following NAD^+ consumption by these enzymes, the resulting NAM can re-enter the NAD^+ synthesis pathway as a precursor, completing a dynamic recycling process.

Overall, these pathways ensure the maintenance of cellular NAD^+ levels and play critical roles in metabolism, DNA repair, and overall cellular health. Understanding their interconnections and regulation is essential for maintaining NAD^+ homeostasis under various growth conditions. The subsequent sections of this review will focus on how ACMSD regulates NAD^+ levels via the de novo synthesis pathway.

4. ACMSD Decreases NAD^+ Level

The de novo synthesis pathway, also known as the kynurenine pathway or the tryptophan-to-quinolinic acid pathway, converts dietary-derived tryptophan into NAD^+ [18,41,42]. Specifically, tryptophan is catalyzed by either indoleamine 2,3-dioxygenase (IDO) or tryptophan 2,3-dioxygenase (TDO) to form N-formylkynurenine, which subsequently undergoes a series of enzymatic reactions to yield ACMS. ACMS serves as an unstable intermediate in the de novo NAD^+ synthesis pathway (Figure 1). The spontaneous cyclization of ACMS generates quinolinic acid (QA), which is further converted to nicotinic acid mononucleotide (NAMN) catalyzed by quinolinate phosphoribosyltransferase (QPRT). Subsequently, NAMN is transformed into NAD^+ under the influence of nicotinamide mononucleotide adenylyltransferase (NMNAT). Enhanced levels of QA and QPRT correlate with higher NAD^+ levels, demonstrating the promoting effect of the de novo synthesis pathway on NAD^+ levels [43]. However, beyond spontaneous cyclization to QA, ACMS can also undergo decarboxylation by ACMSD, resulting in the formation of aminocarboxymuconate semialdehyde (AMS). AMS subsequently converts to acetyl coenzyme A (acetyl-CoA) and enters the tricarboxylic acid (TCA) cycle. Therefore, ACMSD inhibition prevents the spontaneous cyclization of ACMS, serving as a critical enzyme that determines whether tryptophan-derived metabolites enter the TCA cycle or contribute to the NAD^+ biosynthesis.

By reducing the flux of tryptophan to NAD^+, ACMSD influences NAD^+ biosynthesis. Research has demonstrated a close relationship between ACMSD activity and NAD^+ levels. As early as 1999, Shin et al. discovered that endogenous regulation of ACMSD activity could alter liver NAD^+ levels [43]. More recently, Palzer et al. developed a transgenic mouse model overexpressing human ACMSD, which exhibited dietary dependence on nicotinic acid [20]. In a nicotinic acid-deficient diet, these mice showed significantly reduced blood NAD^+ levels, along with decreased NAD^+ content in the liver, kidneys, spleen, and brain. These mice displayed NAD^+ deficiency symptoms, including weight loss, reduced body fat, and impaired physical activity. Additionally, hepatic acetyl-CoA levels increased by approximately 50% due to hACMSD overexpression, suggesting ACMSD's promotion of ACMS entry into the TCA cycle. These findings underscore ACMSD's role in inhibiting tryptophan metabolite entry into the de novo NAD^+ synthesis pathway, leading to decreased NAD^+ levels.

ACMSD has the potential to reduce NAD^+ biosynthesis, making its inhibition a promising avenue for increasing NAD^+ levels. Notably, the complete inhibition of ACMSD homologs in *C. elegans* elevated NAD^+ levels in a tryptophan-dependent manner, simultaneously enhancing mitochondrial function and extending lifespan [18]. In human primary liver cells, ACMSD shRNA or ACMSD inhibitors upregulated cellular NAD^+ levels, increased mtDNA content, and improved cellular oxygen consumption and ATP generation, indicating enhanced mitochondrial function. Furthermore, ACMSD silencing reduced

triglyceride accumulation and cell apoptosis in liver cells. In vivo studies demonstrated that pharmacological ACMSD inhibition increased NAD^+ levels in mouse liver, kidney, and brain, consistent with ACMSD's tissue distribution. Moreover, ACMSD inhibition improved kidney and liver function in acute kidney injury (AKI) and metabolic dysfunction-associated steatotic liver disease (MASLD, used to be called NAFLD) animal models, suggesting protective effects on these organs.

In summary, maintaining NAD^+ levels is crucial for regulating cellular aging and maintaining normal organism function. NAD^+ levels are modulated by various factors, including different synthesis and consumption pathways. ACMSD, as a key enzyme in the de novo NAD^+ synthesis pathway, inhibits NAD^+ biosynthesis, thereby influencing overall NAD^+ levels. Consequently, ACMSD holds promise as a therapeutic target for aging delay and improving degenerative conditions.

5. ACMSD Inhibitors

NAD^+ levels decrease in patients with advanced liver and kidney diseases, highlighting the role of maintaining NAD^+ levels in improving liver and kidney health [9]. ACMSD is highly expressed in the liver and kidneys, and inhibiting ACMSD specifically promotes NAD^+ production in these organs, thereby improving mitochondrial dysfunction [17,19]. Consequently, ACMSD inhibition emerges as a strategy to replenish NAD^+ levels [18,19,44,45]. These inhibitors are being explored for diseases related to NAD^+ dysregulation, especially for liver and kidney diseases where ACMSD is highly expressed.

Camel whey protein (CWP) is a nutritional supplement that has shown advantages in improving the biochemical and histopathology associated with diabetes [34,46]. A recent study has identified the potential role of ACMSD in mediating the improvement of CWP in diabetic liver injury [47]. It was found that the expression of ACMSD was significantly up-regulated in the liver of T2DM rats, but it was significantly down-regulated after CWP intervention, along with the up-regulation of NAD^+ and ATP contents. CWP alone significantly inhibited the expression of ACMSD and protected hepatocytes from glucose/palmitic acid (HG/PA)-induced apoptosis, reducing lipid accumulation and ROS production. Therefore, CWP is a natural ACMSD inhibitor for mitigating liver injury in T2DM rats by inhibiting ACMSD expression, promoting NAD^+ resynthesis, and enhancing mitochondrial function. The results provide new evidence for ACMSD as a key target for CWP to ameliorate diabetic liver injury.

It was also discovered that di(2-ethylhexyl) phthalate (DEHP) and its metabolite mono(2-ethylhexyl) phthalate (MEHP) are reversible ACMSD inhibitors [44]. MEHP inhibits human, mouse, and rat ACMSD in a dose-dependent manner. When MEHP reaches a concentration of 3 mM, it can inhibit human ACMSD activity by up to 90%. Oral administration of MEHP or DEHP in rats promotes the accumulation of QA and NAM metabolites in cells, leading to increased excretion of QA and NAM in urine. This suggests that ACMSD inhibition disrupts the conversion of tryptophan to acetyl-CoA and redirects tryptophan metabolism toward the de novo NAD^+ synthesis pathway. However, these aforementioned compounds lack sufficient selectivity and efficiency, thus limiting their clinical application.

In recent years, the study of ACMSD protein structure has facilitated the design and development of ACMSD inhibitor (Figure 2a). ACMSD is catalytically inactive in the monomer form and active in the homodimer form, because the neighboring subunit contributes one of the two substrate-binding arginine residues. Most of the inhibitors are found to bind the active site of ACMSD through the coordination of the carboxylate group with the zinc ion. For example, scientists resolved the crystal structures of ACMSD complexes with inhibitors, such as 1,3-dihydroxyacetonephosphate (DHAP) [15] and pyridine-2,6-dicarboxylic acid (PDC) (Figure 2b) [45]. In both of the crystal structures,

DHAP and PDC are located near the zinc center. The negatively charged carboxylic groups are both recognized by the Arg47 residue, emerging as a crucial intermolecular interaction for ligand recognition in ACMSD. Recently, a Food and Drug Administration (FDA)-approved drug, diflunisal, has been found to inhibit ACMSD with IC_{50} of 13.5 μM. And the complex structure revealed a new ligand-binding mode [48]. Like the previously reported inhibitors, diflunisal also contains a carboxylate group. However, it interacts with two different active-site arginines of ACMSD (Arg51 and Arg247) without coordinating the catalytic zinc ion, and inhibits ACMSD activity through competitive inhibition.

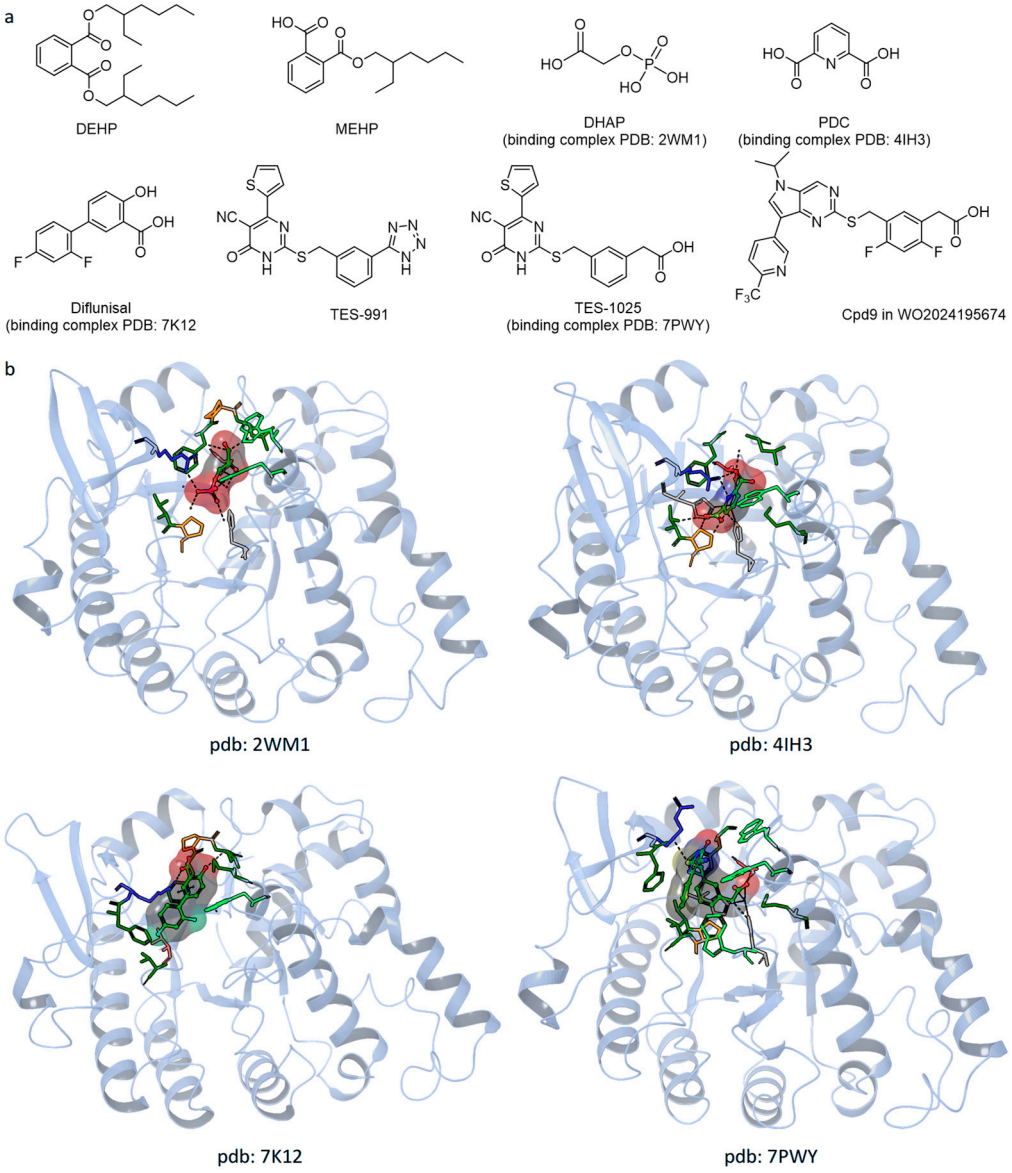


Figure 2. (**a**). Chemical structures of amino–carboxylic semialdehyde dehydrogenase (ACMSD) inhibitors. (**b**). Inhibitor-binding complexes of 1,3-dihydroxyacetonephosphate (DHAP) (pdb: 2WM1), pyridine-2,6-dicarboxylic acid (PDC) (pdb: 4IH3), Diflunisal (pdb: 7K12) and TES-1025 (pdb: 7PWY).

These efforts provide insights into the inhibition mechanisms. However, the low activity and selectivity of these compounds limit their therapeutic potential and clinical application. Scientists are working to develop more potent and selective ACMSD inhibitors. EA Pharma Co., Ltd. has prepared and tested nitrogen-containing heterocyclic derivatives functioning as inhibitors of ACMSD. They are described as potentially beneficial for the treatment of aging, inflammatory disorders, metabolic and mitochondrial diseases, neu-

rodegeneration, and renal disorders. An exemplified compound (Cpd 9) inhibited human ACMSD activity (IC50 < 0.01 μM) in absorbance-based assays (WO 2024195674).

A series of ACMSD inhibitors based on the co-crystal structure of ACMSD and its ligands have been developed, including TES-991 and TES-1025, which exhibit potent inhibitory activity against ACMSD (with IC_{50} values of 3 nM and 13 nM, respectively) [19,49]. TES-1025 inhibits ACMSD through multiple functional groups. The carboxyl group coordinates with the Zn^{2+} metal ion and forms H-bonds with Trp191, while interacting with Asp291 through a bridging water molecule. The pyrimidine nitrogen forms charge-charge interactions with a positively charged nitrogen of Arg47. And the 2-thienyl ring fits into a hydrophobic cavity generated by Trp176, Phe46, Met180, and Trp191. This strong network of interactions is consistent with the experimentally observed high affinity and potency of the ligand. Pharmacokinetic/pharmacodynamic studies show that TES-1025 significantly reduces enzyme activity in mouse kidneys after oral administration at a dose of 5 mg/kg. Furthermore, continuous feeding of mice with 15 and 30 mg/kg/day of TES-1025 for 10 days significantly increases NAD^+ levels in liver extracts, indicating enhanced NAD^+ biosynthesis through ACMSD inhibition. The protective effects of TES991 and TES-1025 in liver and kidney diseases are also being explored. In vitro, both TES991 and TES-1025 significantly increase NAD^+ levels in human primary hepatocytes, enhance mitochondrial function, and protect against apoptosis induced by high-dose fatty acids. TES-1025, which is the best inhibitor for kidney exposure, increases NAD^+ content in human HK-2 renal cells and attenuating cisplatin-induced renal cell apoptosis. Overall, ACMSD inhibitors play a protective role by enhancing NAD^+ levels in liver and kidney cells.

In vivo studies have also demonstrated the protective effects of these inhibitors on the kidneys and liver. Acute kidney injury (AKI) is a common and high-risk critical condition in hospitalized patients, with over 2 million deaths worldwide annually [50,51]. Research indicates that impaired de novo NAD^+ synthesis from tryptophan is associated with AKI [33,52]. In models of cisplatin-induced AKI and ischemia–reperfusion AKI (IR-AKI), TES-1025 (15 mg/kg/day) treatment mitigates the decline in renal NAD^+ levels, maintains glomerular filtration rate, and prevents kidney structural and functional damage caused by AKI [18]. Metabolic dysfunction-associated steatotic liver disease (MASLD, used to be called NAFLD) is the most common liver disease worldwide, often leading to progressive liver damage known as metabolic dysfunction-associated steatohepatitis (MASH, used to be called NASH) [53,54]. In a mouse model of MASLD induced by methionine–choline-deficient (MCD) diet, TES-991 (15 mg/kg/day, prophylactically) reverses the decrease in NAD^+ levels caused by the MCD diet, prevents liver lipid accumulation, reduces inflammation, and improves MASLD symptoms in the model mice [18].

These studies represent the first application of ACMSD inhibitors in disease models, demonstrating promising results by suppressing the progression of AKI or MASLD. Subsequently, OrsoBio also reported relevant research on ACMSD inhibition [55]. TLC-065, part of the ACMSD inhibitor portfolio acquired by OrsoBio, is currently under investigation for its ability to replenish NAD^+ levels and improve mitochondrial function. In primary mouse hepatocytes, TLC-065 achieves a lower dose to enhance cellular NAD^+ levels, compared to nicotinamide riboside (NR). It resists endoplasmic reticulum stress induced by palmitate salts, with reduced reactive oxygen species (ROS) production and cell apoptosis. In a Western diet (WD)-induced MASLD/MASH model, dietary intake of TLC-065 at 25 mg/kg mitigates MASLD/MASH symptoms, reducing liver inflammation and fibrosis. Gene expression analysis in mouse liver further elucidates the mechanism of action of TLC-065. Compared to chow diet (CD)-fed mice, WD upregulates genes related to fibrosis and inflammation, while downregulating genes associated with mitochondrial function. TLC-065 reverses the transcriptomic changes induced by WD in the liver, thereby

counteracting liver fibrosis and inflammation and enhancing mitochondrial function. Additionally, researchers simulate the protective effect of TLC-065 against hepatic steatosis using human liver organoids derived from induced pluripotent stem cells (iPSC-HLOs). As expected, steatosis-induced HLOs (sHLOs) express higher levels of ACMSD. TLC-065 treatment reverses the gene features of fibrosis and mitochondrial dysfunction induced by steatosis. In addition to TLC-065, the ACMSD inhibitor program includes a range of proprietary compounds. These compounds are being evaluated for their potential in treating metabolic and inflammatory liver and kidney disorders.

Recently, the causal relationship between DNA damage and MASLD/MASH has been explored in further detail, providing a new perspective for understanding the disease mechanism [56]. Correlation analysis has linked liver transcriptome signatures of DNA damage to liver disease phenotypes. The gene enrichment analysis showed that the treatment with the ACMSD inhibitor significantly downregulates the DNA repair genes and their associated pathways in hepatocytes. In addition, it reduced cellular oxidative stress, apoptosis, and ER stress due to doxorubicin-induced DNA damage and lipid toxicity. In iPSC-derived liver organoid models, the ACMSD inhibitor could reduce the accumulation of DNA damage markers p-H2A.X and p-53BP1, thus alleviating genomic stress. These findings support the effectiveness of ACMSD inhibition in reducing DNA damage and protecting genomic stability, underscoring the therapeutic potential of ACMSD inhibitors in MASLD/MASH.

To date, researchers have developed several ACMSD inhibitors to modulate cellular NAD^+ levels (Table 1). Preclinical data demonstrate that these molecules effectively promote NAD^+ synthesis in mouse models and exhibit good tolerability. They hold promise for improving mitochondrial dysfunction, metabolic disorders, and kidney diseases associated with ACMSD dysregulation or reduced NAD^+ levels. However, these studies are still in the preclinical stage, and the therapeutic effects of ACMSD inhibitors in humans await further validation through additional research.

Table 1. Amino–carboxylic semialdehyde dehydrogenase (ACMSD) inhibitors.

Inhibitor	Potency	In Vivo	Disease	Ref.
DEHP MEHP	Inhibition rate: >90% (3 mM)	Increasing urinary excretion of QA and NAM in rats	-	[44]
DHAP	-	-	-	[15]
PDC	Ki: 15.2 ± 0.5 μM	-	-	[45]
Diflunisal	ACMSD IC_{50}: 13.5 μM	-	-	[48]
TES-991	ACMSD IC_{50}: 3 nM	Upregulating the NAD^+ level in liver, protecting against hepatic lipid accumulation and attenuating inflammation in MCD-induced MASLD mouse model	MASLD	[18,19]
TES-1025	ACMSD IC_{50}: 13 nM	Protecting from structural and functional renal damage inflicted by both cisplatin-induced AKI and IR-AKI	AKI	[18,19,49]
TLC-065	EC_{50}: 63.23 nM (NAD^+ increasing)	Increasing hepatic NAD^+ and significantly reducing inflammation and fibrosis in MASLD/MASH mice and iPSC-derived sHLOs	MASLD	[55,56]
Cpd 9 (EA Pharma)	ACMSD IC_{50} < 0.01 μM	-	WO 2024195674	

6. Conclusions

NAD^+, as one of the most critical metabolites in the human body, maintains a dynamic equilibrium through a steady state of biosynthesis, consumption, and recycling at both cellular and systemic levels. However, as individuals age, the intracellular NAD^+ content decreases, leading to organ and tissue dysfunction. Elevating NAD^+ levels holds promise for slowing down aging and improving age-related degenerative diseases.

ACMSD, which is highly expressed in the liver and kidneys, stands at a branch point in the de novo NAD^+ synthesis pathway. It acts as a key enzyme that negatively regulates NAD^+ production by controlling the conversion of tryptophan to NAD^+. Genetic and pharmacological inhibition of ACMSD can promote NAD^+ generation, making it an attractive therapeutic target in NAD^+ deficient disease, especially kidney and liver disease where ACMSD is highly expressed. Preclinical data on certain ACMSD inhibitors have already demonstrated their protection of liver and kidney. ACMSD inhibitors enhance NAD^+ production and reduce liver inflammation and fibrosis, particularly in the context of high-fat diet-induced MASLD. Additionally, ACMSD inhibition in the kidneys protects against structural and functional damage in various AKI models.

In summary, ACMSD inhibitors hold promise as potential therapeutic targets for liver and kidney diseases. However, studies are still in the early stages, and there is currently no clinical data supporting the protective effects of ACMSD inhibition in humans. Furthermore, the dynamic balance of NAD^+ levels involve complex processes, including multiple biosynthetic and degradation pathways. Therefore, evaluating the efficiency of promoting NAD^+ synthesis through a single pathway requires further investigation. Additionally, researchers must consider the relationship of ACMSD and other key enzymes involved in NAD^+ synthesis, as well as potential combination strategies. In terms of safety, some studies have reported that the accumulation of QA, an intermediate in NAD^+ synthesis, can induce neurotoxicity [57]. And the inhibition of ACMSD promotes QA production. Existing data on ACMSD inhibitors support their safety and tolerability, with no observed neurotoxicity; however, safety risks should still be carefully considered during the development of ACMSD inhibitors.

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