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An Overview of ADAR Inhibitors: Mechanisms, Applications and Future Directions

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ABSTRACT

Adenosine deaminases acting on RNA (ADARs) are enzymes responsible for converting adenosine to inosine in double-stranded RNA through a process known as A-to-I editing. This modification is essential for regulating RNA function, impacting key processes such as immune response, gene expression, and RNA stability. ADARs, especially ADAR1, have been linked to several diseases, including cancer, viral infections, and autoimmune disorders, making them promising targets for therapeutic development. Currently, no FDA-approved drugs are specifically marketed as ADAR inhibitors. This review offers a brief analysis of known ADAR inhibitors, with a focus on their mechanisms of action, structural properties, and potential applications. We explore a range of inhibitors, including small molecules, peptide-based inhibitors, natural compounds, and RNA-based inhibitors, and discuss their ability to selectively modulate ADAR activity. The review also addresses the therapeutic implications of these inhibitors in cancer, viral infections, and inflammatory diseases. In addition, we examine future directions for developing more selective and potent ADAR inhibitors, emphasizing both the challenges and opportunities in the field. By consolidating current findings and identifying existing gaps, this review aims to enhance the understanding and therapeutic potential of ADAR inhibition.

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1. Introduction

1.1 Overview of ADARs:

Adenosine deaminases acting on RNA (ADARs) are a family of enzymes that catalyze the deamination of adenosine to inosine in double-stranded RNA (dsRNA), a process known as A-to-I RNA editing.[1] This modification is essential for altering the coding potential of RNA molecules, thereby influencing their structure, stability, and function.[2] By converting adenosine (A) to inosine (I), ADARs create a modified base that behaves like guanosine during translation, thereby diversifying the proteome without changing the underlying DNA sequence.[3] This RNA editing process plays a critical role in various cellular processes, including the regulation of gene expression[4], RNA splicing[5], and immune response modulation[6].

The ADAR family consists of three isoforms: ADAR1, ADAR2, and ADAR3, each with distinct roles. ADAR1 is expressed in both the nucleus and cytoplasm and exists as two isoforms, p110 and p150, which differ in structure and function. All ADAR family proteins share a common domain architecture, including one or more dsRNA-binding domains (dsRBDs) and a catalytic deaminase domain.[7-9] In humans, ADAR1 isoforms also contain Z-DNA-binding domains. The Z-alpha domain is a nucleic acid-binding domain that specifically binds double-stranded RNA (dsRNA) and is important for ADAR's RNA-editing activity. The Z-beta domain, found in the constitutive ADAR1 p110 isoform, is structurally similar to the Z-alpha domain but does not bind Z-DNA or dsRNA directly. It plays a supporting role in stabilizing the protein structure and interactions with other components. The constitutive ADAR1 p110 isoform includes a Zβ domain, which resembles Zα domains but does not bind Z-DNA, while the interferon-inducible ADAR1 p150 isoform features a Zα domain with a nuclear export signal (NES). ADAR3, a cat-

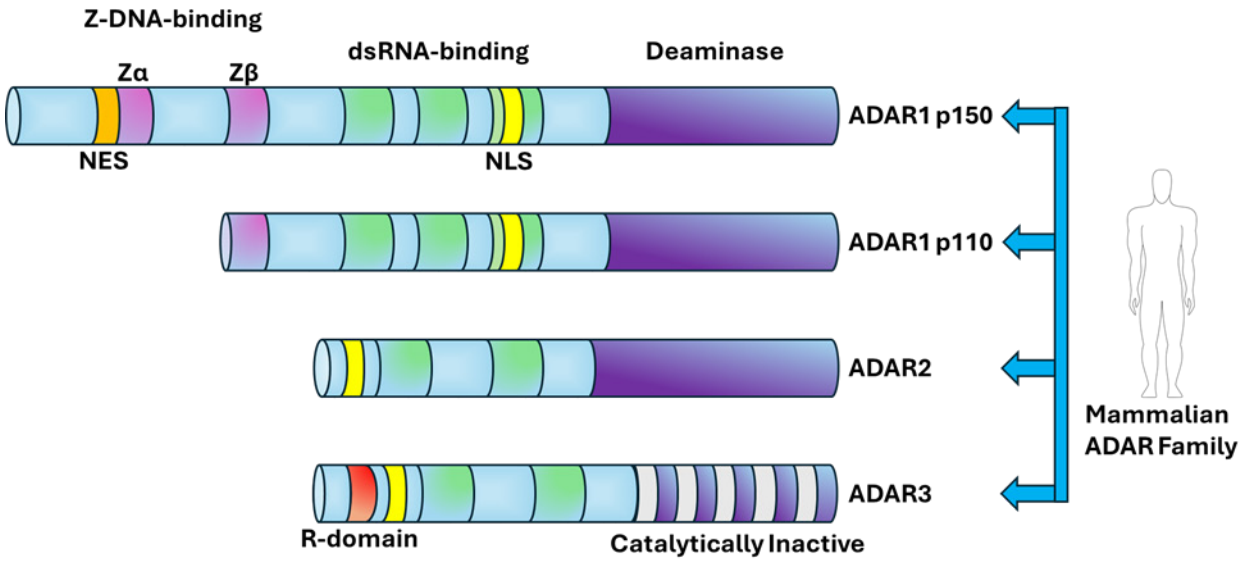


Figure 1. Domain architecture of mammalian

alytically inactive member, contains an additional R-domain (Fig.1).[8-11]

ADAR1 plays a key role in immune regulation, particularly in modulating the immune response to viral infections and maintaining self-tolerance. ADAR1 p150, with its Zα domain, helps suppress innate immune pathways by editing viral and host RNA structures, preventing recognition by pattern recognition receptors like MDA5. ADAR2, predominantly localized in the nucleus, edits RNA substrates involved in neuronal function, such as neurotransmitter receptors. ADAR3, primarily expressed in the brain, is believed to regulate neuronal RNA editing, though its exact functions remain unclear.[10-12]

ADAR family members. The ADAR family proteins share a common structure with multiple dsRNA-binding domains (dsRBDs) and a catalytic deaminase domain. In humans, ADAR1 isoforms also include Z-DNA-binding domains, with ADAR1 p110 containing a Zβ domain and ADAR1 p150 having a Zα domain with a nuclear export signal (NES). ADAR3, a catalytically inactive member, also features an R-domain. Abbreviations: NES, nuclear export signal; NLS, nuclear localization signal; R-domain, arginine-rich domain. Figure adapted from [[13]], with modifications.

ADARs bind to double-stranded RNA regions, often formed by complementary RNA sequences or secondary structures like stem loops. The catalytic domain facilitates the deamination of adenosine to inosine.[8, 9] The specificity of ADARs is determined by their interaction with RNA substrates, and their editing activity is guided by the structure of the dsRNA. ADARs typically edit repetitive sequences, including inverted Alu repeats, and can also modify RNA involved in immune responses, such as interferon receptors and cytokines. This editing fine-tunes gene expression and RNA processing.[14]

1.2 Importance of ADAR Inhibition:

ADARs play a critical role in regulating RNA editing, which is central to various diseases (Table 1).[8, 15-20] In cancer, ADAR1 is often overexpressed, aiding immune evasion and promoting cancer cell survival by editing viral or self-RNA to prevent immune detection, allowing tumor growth and metastasis.[21] ADAR1 also alters RNA structures that suppress immune responses, such as the activation of MDA5, a pattern recognition receptor. Inhibiting ADAR1 could enhance anti-tumor immunity, making it a promising cancer immunotherapy target.[22]

In autoimmune diseases, dysregulated ADAR1 activity can lead to aberrant immune activation, with mutations in ADAR1 associated with conditions like Aicardi-Goutières syndrome (AGS), where improper RNA editing causes the immune system to attack self-RNA.[22] ADARs are also involved in viral infections, where they play a dual role: controlling viral replication through RNA editing while also enhancing viral persistence by reducing immune detection, reflecting the complex relationship between ADARs and host-pathogen interactions.[23]

Table 1. Disorders linked to human ADARs adapted from [8] with some differences.

Disease	Edited transcripts	ADAR
Amyotrophic lateral Sclerosis (ALS)	*GRIA2*	ADAR2
Aicardi-Goutiéres Syndrome (AGS)	-	ADAR1
Chronic myeloid leukemia (CML)	*PU.1 (Spi1)*	ADAR1
Dyschromatosis Symmetrica Hereditaria (DSH)	-	ADAR1
Glioblastoma (GBM)	*CDC14B*	ADAR2
	pri-miR-221/222	ADAR2
	Pri-miR-21	ADAR2
	miR-376a-5p	ADAR2
	GRIA2	ADAR2
Hepatocellular carcinoma (HCC)	*AZIN1*	ADAR1
	FLNB	ADAR1
	COPA	ADAR2
	pre-miR-214	ADAR2
Prostate cancer	*PRUNE2/PCA3*	ADAR1/ADAR2

The need for ADAR inhibitors is growing as these enzymes are implicated in a wide range of diseases, particularly those involving immune dysregulation, viral persistence, and cancer progression.[16, 21-23] Inhibiting ADARs offers potential benefits in reversing immune suppression in tumors, correcting immune activation in autoimmune diseases, and limiting viral replication. Selective ADAR inhibitors are essential to achieve these therapeutic effects, as they could prevent immune evasion in cancer, restore immune tolerance in autoimmune diseases, and provide new strategies for viral infections. However, developing ADAR inhibitors is challenging due to the enzymes' complex RNA recognition and broad biological roles. Current research focuses on creating selective inhibitors that can modulate these processes without causing adverse effects, potentially leading to innovative treatments in disease areas with limited therapies.

2. Strategies for Inhibiting ADARs in Therapeutic Applications

Inhibiting ADARs can be achieved through various strategies that target different aspects of their function (Fig. 2). One approach is to inhibit the deaminase activity of ADARs using small molecule inhibitors, such as nucleoside analogs (e.g., 8-azanebularine), which selectively block the conversion of adenosine to inosine in RNA.[24] Another strategy involves disrupting RNA binding, preventing ADARs from interacting with their RNA substrates. Additionally, protein-protein interaction inhibitors can be used to block the association of ADARs with other cellular proteins that facilitate their activity. Depletion of ADARs through targeted degradation mechanisms like PROTACs (proteolysis-targeting chimeras) is another promising approach.[25] Furthermore, modulation of ADAR pre-mRNA splicing can alter the expression of specific ADAR isoforms, particularly ADAR1p150, to selectively target immune evasion without affecting normal tissue function.[26] Lastly, elevating endogenous dsRNA levels through inhibition of DNA methyltransferases or other RNA-processing enzymes can saturate ADAR's editing capacity, thus promoting immune activation.[25] Together, these strategies offer diverse and potentially complementary ways to modulate ADAR activity in various therapeutic contexts, such as cancer and autoimmune diseases.[27]

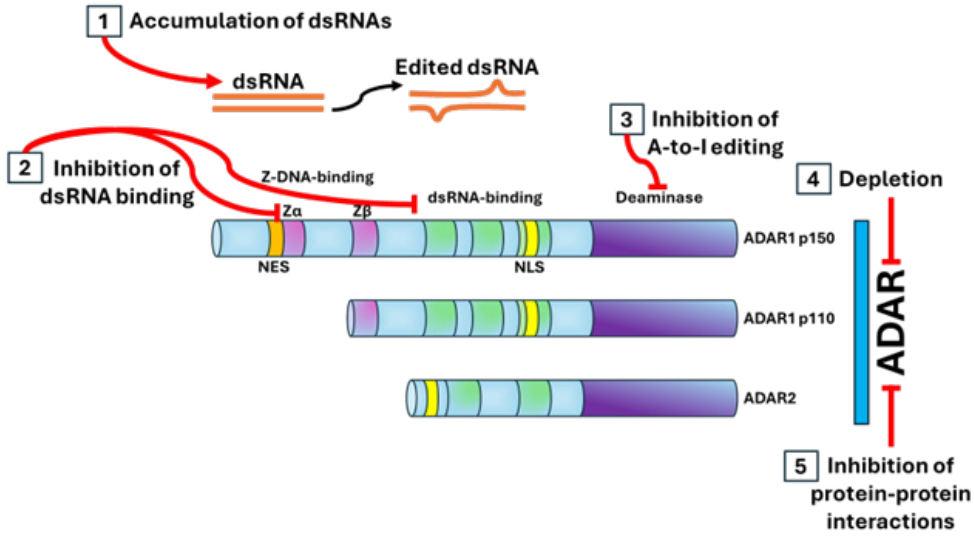


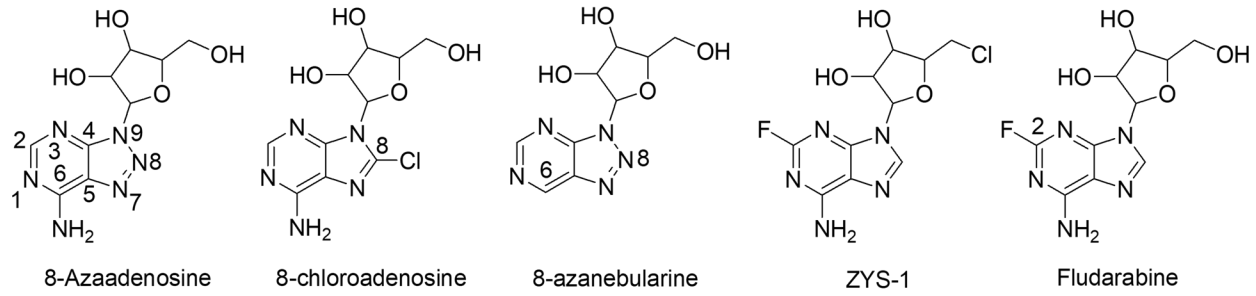
Figure 2. Potential Strategies for Inhibiting ADARs.

3. Reported ADAR-inhibiting compounds

3.1 Purine Derivatives

Purine derivative inhibitors are designed to interfere with the ADAR enzyme's catalytic activity by mimicking natural substrates or interacting with specific regions of the enzyme to block its RNA-editing function.

The mechanism of action of 8-Azaadenosine and 8-Chloroadenosine revolves around their structural similarity to adenosine, the natural substrate of ADAR.[28] 8-Azaadenosine and 8-Chloroadenosine are two modified purine nucleosides that share a similar core structure to adenosine, but they differ in the substitution at the 8-position of the purine ring (Fig. 3). 8-azaadenosine has a nitrogen atom at the 8-position, while 8-chloroadenosine has a chlorine atom at the same position. These substitutions increase the compound's affinity for the active site of ADAR, allowing it to bind more tightly than adenosine itself.



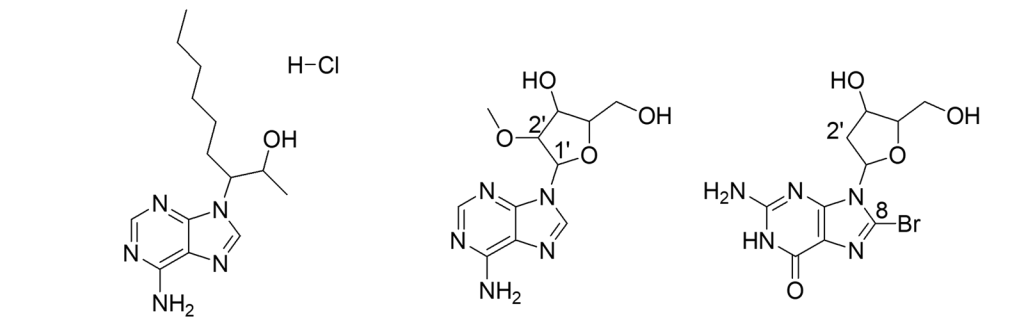


Figure 3. Structures of Purine derivatives reported as ADAR inhibitors.

As a result, 8-azaadenosine competes with adenosine for binding to ADAR's active site. Another research showed that 8-Chloroadenosine inhibits cell growth in breast cancer cells by inducing G1 cell cycle arrest and apoptosis. It activates p53/p21 signaling by downregulating ADAR1 protein, leading to growth inhibition. The RNA-binding domain of ADAR1 plays a very important role in the process instead of the RNA-editing activity.[9, 29-32] However, another study stated that these two compounds are not selective inhibitors of ADAR.[28]

8-azanebularine (8-azaN) is a nucleoside analog that inhibits ADARs by mimicking the intermediate reaction of the ADAR-catalyzed deamination process. ADAR enzymes typically catalyze the deamination of adenosine to inosine in RNA, which involves the displacement of a leaving group (a portion of the molecule that is displaced or 'leaves' during a chemical reaction, usually because it is stabilized after departure). When incorporated into RNA duplexes, 8-azaN is recognized by ADAR and undergoes hydration at the C6 position of the purine ring, similar to the natural substrate (Fig. 3). However, due to the nitrogen substitution at the 8-position of the purine ring, the resulting product lacks a suitable leaving group required for the deamination reaction to proceed. This structural modification traps the enzyme in a non-productive state, preventing the normal conversion of adenosine to inosine and effectively inhibiting ADAR activity.[24]

Fludarabine is a fluorinated purine analog, structurally similar to adenosine but with a fluorine atom at the 2-position of the ribose ring. This fluorine substitution increases the stability of the compound, making it less prone to degradation. Fludarabine interferes with nucleoside metabolism, competing with adenosine for incorporation into RNA and thereby disrupting RNA editing by ADAR. It likely impairs the enzyme's activity by preventing normal RNA processing.[33]

ZYS-1 is a novel small-molecule inhibitor developed to target ADAR1, an RNA-editing enzyme implicated in cancer progression, including prostate cancer (PCa). ZYS-1 inhibits ADAR1's deaminase activity, preventing A-to-G RNA editing, which plays a key role in regulating gene expression and cellular processes. The compound shows potent anti-cancer activity by reducing tumor cell proliferation, invasion, and metastasis, as well as inducing apoptosis. ZYS-1 targets MTDH, an oncogenic protein, by disrupting its ADAR1-mediated editing, leading to decreased MTDH protein levels without affecting mRNA stability. This results in translational shutdown and impaired tumor cell functions. ZYS-1 also modulates oncogenic pathways and immune responses, further enhancing its therapeutic potential. It reduces ADAR1 protein levels through an m6A-YTHDF1-dependent mechanism, independent of proteasomal degradation. Structural studies revealed that ZYS-1 binds to ADAR1 at critical sites, providing insights into its high affinity and inhibitory potency. Overall, ZYS-1 offers a promising strategy for treating PCa and other cancers by targeting epitranscriptomic RNA editing, with a favorable safety profile and potential for use in combination therapies.[34]

EHNA (Erythro-9-(2-hydroxy-3-nonyl)adenine is primarily known as an inhibitor of adenosine deaminase (ADA), and in this context, it was found to preferentially inhibit the RNA editing activity of ADAR2. Specifically, EHNA inhibited editing at sites C and D of the 5-HT2CR mRNA without affecting editing at sites A and B or the editing of other genes. The 5-HT2CR mRNA undergoes editing at these five nucleotide positions (sites A-E), which are located in the sequence encoding the second intracellular loop of the 5-HT2C receptor. These editing sites allow for the generation of multiple mRNA variants and protein isoforms of the receptor, influencing its G-protein coupling efficiency. Sites A and B are edited by ADAR1, which typically modifies the adenosine residues at these positions to inosine. Sites C and D, however, are edited by ADAR2, and EHNA selectively inhibited editing at these sites, suggesting that it acts specifically on ADAR2. Interestingly, EHNA did not affect the editing of GluR2 mRNA at the Q/R site, which is also edited by ADAR2. This selectivity suggests that EHNA's inhibitory effects might depend on the specific characteristics of the RNA editing sites, in addition to its selectivity for ADAR2. The study highlights EHNA as a useful pharmacological tool to investigate the physiological roles of RNA editing at specific sites, especially concerning psychiatric disorders, as alterations in 5-HT2CR mRNA editing have been implicated in conditions like depression and anxiety.[35]

The methylation of the 2′-OH group of adenosine significantly inhibits the deamination reaction catalyzed by ADAR-2. This modification involves the addition of a methyl group to the 2′-hydroxyl group of the ribose sugar in the RNA, which is typically in-

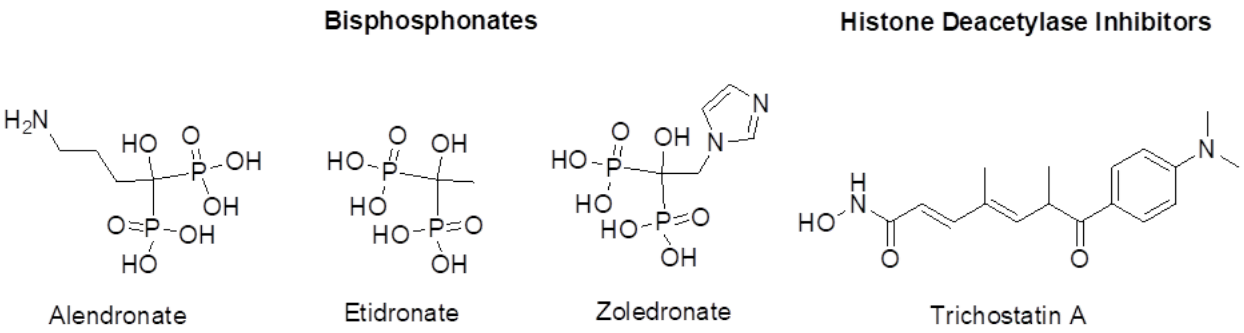


Figure 4. Structures of FDA-approved drugs that are reported for potential ADAR inhibition.

volved in hydrogen bonding and interactions with the enzyme.

The mechanism behind this inhibition is likely due to the steric hindrance caused by the bulky methyl group, which prevents the enzyme from properly interacting with the substrate. The 2′-hydroxyl group plays a crucial role in the enzyme-substrate binding, and the methyl group at this position increases the bulkiness of the RNA, making it difficult for ADAR-2 to properly accommodate the substrate in its active site. As a result, the enzyme's ability to catalyze the conversion of adenosine to inosine (the hallmark of the ADAR-mediated deamination reaction) is significantly reduced. This suggests that ADAR-2 has a limited tolerance for modifications at the 2′-OH position, and methylation at this site disrupts its catalytic efficiency.[36]

8-Bromo-2′-deoxyguanosine (8-bromo dG) is a modified nucleoside where a bromine atom is substituted for the hydrogen at the 8-position of the guanine base (Fig. 3). This structural modification impacts the normal function of guanine in nucleic acids, altering its behavior in base pairing and its interactions with enzymes like ADAR. The substitution of the bromine atom at the 8-position disrupts the normal hydrogen bonding capacity of the base. This forces 8-bromo dG to adopt a *syn conformation*, which is an unnatural orientation for guanine in a double-stranded RNA molecule. The syn conformation refers to the positioning of the base where the 8-position is flipped toward the sugar-phosphate backbone of the nucleic acid, rather than the usual anti-conformation where the base projects away. In a typical Watson-Crick base pairing, guanine pairs with cytosine, but in this modified form, the syn-conformation prevents proper base pairing with other nucleotides, such as cytosine or guanine. In the context of ADAR, 8-bromo dG is inhibitory because it cannot form a stable hydrogen bond with the Hoogsteen face of a guanine base in the 5′-G position. As a result, ADAR cannot properly recognize and edit RNA at this position, thereby hindering its normal deaminase function. This renders 8-bromo dG ineffective in supporting ADAR-mediated RNA editing and illustrates how its altered structure can inhibit enzyme activity.[37]

3.2 FDA-approved drugs for other uses that inhibit ADAR

Numerous compounds have demonstrated potential in inhibiting ADAR, yet there are currently no FDA-approved drugs specifically targeting ADAR. This section highlights several FDA-approved drugs, originally designed for other indications, that have shown evidence of inhibiting ADAR activity.

Trichostatin A (TSA) is primarily known as a histone deacetylase (HDAC) inhibitor, with its structure featuring a benzene ring linked to an amide group and a hydroxamic acid functional group, which allows it to bind to zinc ions in the active sites of HDACs (Fig. 4).[38] While TSA's primary mechanism of action is through inhibiting HDACs, which results in increased histone acetylation and a more open chromatin structure, it may also influence ADAR1 activity indirectly. TSA's epigenetic effects could alter gene expression, potentially modulating the levels of ADAR1 or other RNA-editing enzymes. Although TSA is not a direct inhibitor of ADAR1, its hydroxamic acid group might interact with the zinc ions in ADAR1's catalytic site, potentially disrupting the enzyme's ability to catalyze the deamination of adenosine to inosine in RNA.[39]

A screening of 2,627 compounds identified potential inhibitors targeting the Zα/Z-RNA complex of ADAR1, specifically the p150 isoform, which plays a critical role in immune responses, viral infections, and cancer. The study focused on the Zα domain of ADAR1, which is essential for its catalytic activity in RNA editing, particularly the A-to-I editing process.

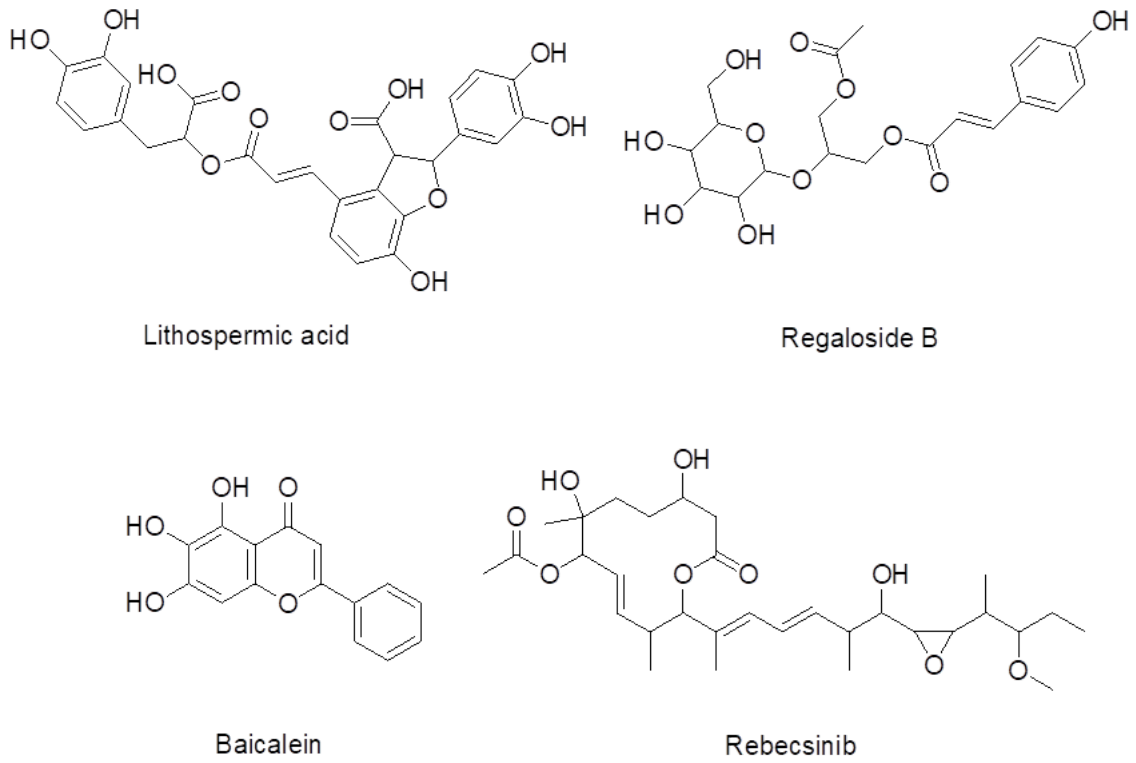


Figure 5. Structures of some phenolic compounds and plant metabolites reported as ADAR inhibitors.

This Zα domain interacts with Z-RNA and Z-DNA, modulating ADAR1's activity and influencing editing patterns. Three compounds were selected for their potential to inhibit the Zα domain of ADAR1: alendronate, etidronate, and zoledronate (Fig. 4). These drugs were chosen based on their molecular interactions with key residues (such as Lys169, Lys170, Asn173, and Tyr177) in the Zα/Z-RNA and Zα/Z-DNA complexes. The molecular interactions, particularly the strong hydrogen bonding with Arg174 in the Zα/Z-RNA complex, suggest that zoledronate may be a potent inhibitor, potentially blocking the catalytic activity of ADAR1 in A-to-I RNA editing. The study proposes that these drugs, which are FDA-approved for other uses, could be repurposed to inhibit ADAR1 activity, particularly in diseases such as viral infections and cancer where ADAR1 is overexpressed. Specifically, zoledronate, alendronate, and etidronate could be considered for combination therapies aimed at treating cancers with high ADAR1 expression, such as breast cancer, lung cancer, and liver cancer, where ADAR1 contributes to cancer cell proliferation, progression, and immune evasion.[40]

3.3 Phenolic Compounds and Plant Metabolites

This section highlights the potential of phenolic compounds and plant metabolites as ADAR inhibitors, particularly those targeting the enzyme's Zα domain.

Through High-throughput virtual screening, several potential inhibitors of ADAR1 were identified, with a focus on compounds targeting the Zα domain, which plays a crucial role in the enzyme's catalytic activity. Through molecular docking and screening of over 100,000 compounds, including natural products and small molecules, several promising inhibitors were discovered. Among them, Lithospermic acid and Regaloside B showed significant binding affinities to the Zα domain of ADAR1, with Lithospermic acid displaying a binding affinity of KD 7.56×10^{-5} M and Regaloside B showing KD 7.523×10^{-3} M. These compounds, along with others such as baicalein, sodium camptothecin, and lithospermic acid, have the potential to inhibit ADAR1 activity and thus modulate RNA editing processes involved in diseases like cancer and viral infections (Fig. 5). Molecular dynamics simulations further confirmed the binding stability and energy profiles of these compounds, sup-

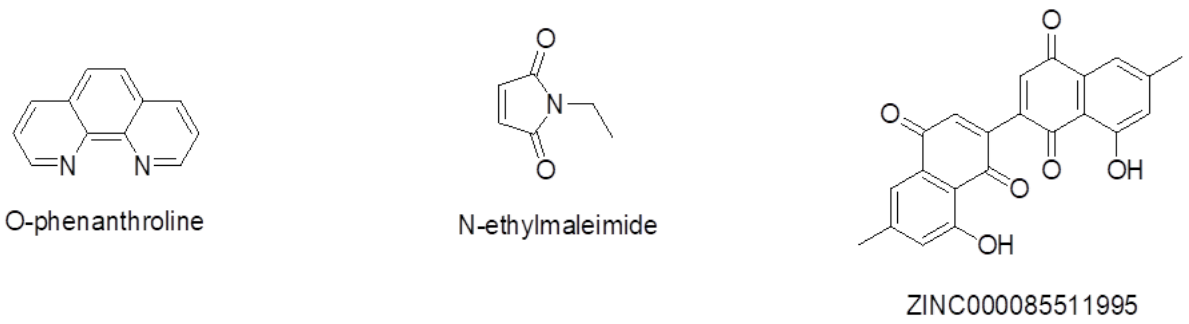


Figure 6. Structures of O-phenanthroline, N-ethylmaleimide (NEM), and potential ADAR2 inhibitors identified via molecular docking.

porting their potential as ADAR1 inhibitors. These findings open the door for further experimental validation of these compounds and their application in ADAR1-targeted therapies.[41]

Rebecsinib is a selective small-molecule inhibitor that targets splicing-mediated ADAR1 activation. It specifically inhibits the ADAR1p150 isoform, which is implicated in the generation of cancer stem cells (CSCs) and therapeutic resistance in various malignancies. Rebecsinib has been shown to inhibit leukemia stem cell (LSC) self-renewal and prolong survival in humanized LSC mouse models while sparing normal hematopoietic stem and progenitor cells (HSPCs). Additionally, pre-IND studies (which are research conducted before filing an Investigational New Drug application to the FDA) indicate that Rebecsinib has favorable toxicokinetic and pharmacodynamic properties, making it a promising candidate for further development as an ADAR1p150 antagonist in cancer therapy.[26]

3.4 Transition Metal Compounds, Electrophiles, and Naphthoquinones

O-phenanthroline is a heterocyclic compound that features a phenanthroline core, composed of three fused benzene rings with nitrogen atoms at positions 1 and 10, forming a bipyridine-like structure (Fig. 6). One of the nitrogen atoms in O-phenanthroline is part of a bidentate chelating system, allowing it to interact with metal ions. The key structural features of O-phenanthroline include a highly conjugated planar aromatic ring system, with nitrogen atoms at the 1st and 10th positions elating metal ions required for their catalytic activity. ADAR enzymes, especially ADAR1 and ADAR2, depend on zinc or copper ions to stabilize their structure and facilitate the deamination process, in which adenosine (A) in RNA is converted to inosine (I). By binding tightly to these metal ions in the active site of ADARs, O-phenanthroline prevents the ions from binding to the enzyme, thereby inhibiting its catalytic function. This inhibition blocks RNA editing, which is essential for processes like gene expression regulation, and RNA splicing, and essential for coordinating metal ions, and the ability to chelate metal ions, particularly zinc and copper. These metal ions are critical for the function of many enzymes, including ADARs. O-phenanthroline inhibits ADAR enzymes primarily by cellular stress responses.[12, 29, 42]

N-ethylmaleimide (NEM) consists of a maleimide core, which is a five-membered ring structure containing a carbonyl group (C=O) and a double bond (C=C). The maleimide ring is substituted with an ethyl group (-CH2CH3) at the nitrogen atom, making it more hydrophobic and able to penetrate cell membranes. The maleimide structure is highly electrophilic, with the carbonyl carbon (C=O) being reactive toward thiol groups (-SH) on cysteine residues in proteins (Fig. 6). This planar structure allows NEM to efficiently interact with these thiol groups in enzymes. N-ethylmaleimide acts as an electrophilic alkylating agent that specifically reacts with thiol groups in cysteine residues, which are crucial for the catalytic activity or structural stability of enzymes like ADAR. When NEM interacts with a thiol group, it forms a covalent bond, inactivating the enzyme by blocking the active site or altering its conformation. In ADAR enzymes, this modification prevents the enzyme from performing its RNA editing function, specifically the deamination of adenosine to inosine. This inhibition can have significant biological effects, particularly in studying the enzyme's role in cellular processes.[12, 42-44]

Through molecular docking simulations of natural compounds derived from Chinese flora and fauna, this study identified several potential in-

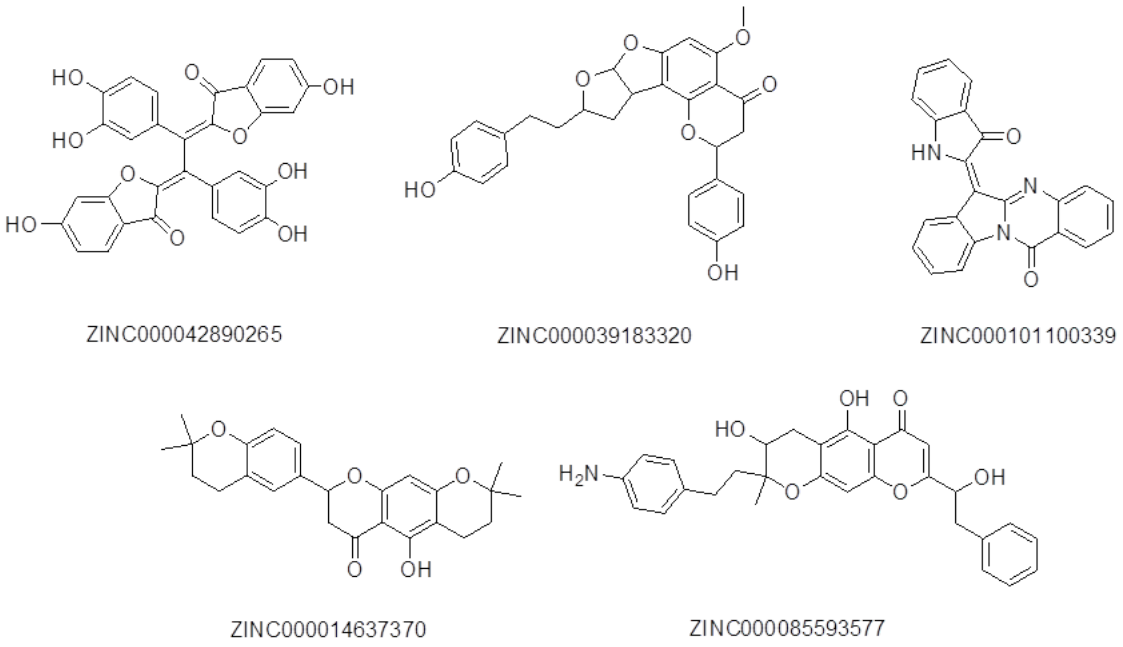


Figure 7. Structures of five potential ADAR2 inhibitors identified from a traditional Chinese medicine library through molecular docking and dynamics simulations.

hibitors of ADAR2. Among the top compounds, ZINC000085511995 demonstrated the highest binding affinity to ADAR2 (-1068.26 kJ/mol) compared to the known binder IHP (-873.873 kJ/mol), making it a promising lead for further investigation. Other compounds containing structural motifs such as naphthoquinone, indole, furanocoumarin, and benzofuran were also identified, suggesting their potential to inhibit ADAR2. Notably, compounds with indole moieties, such as serotonin and tryptophan, are known for their beneficial effects on mood, sleep, and digestion, while naphthoquinone derivatives like Vitamin K have been linked to cognitive function improvement. These findings highlight the potential of these compounds as ADAR2 inhibitors, warranting further experimental studies and medicinal chemistry efforts to explore their inhibitory activity and therapeutic potential.[45]

3.5 Other compounds with ADAR inhibiting activity

Molecular docking and dynamics simulations identified five promising ADAR2 inhibitors from a traditional Chinese medicine library. The compounds demonstrated higher binding affinity to ADAR2 than the control, 8-azanebularine, by targeting key residues in the RNA binding loop, including Lys350, Cys377, Glu396, Cys451, Arg455, Ser486, Gln488, and Arg510. The five identified compounds (Fig. 7) - ZINC000042890265, ZINC000039183320, ZINC000101100339, ZINC000014637370, and ZINC000085593577, showed promising binding properties, minimal toxicity, and strong binding to the serotonin 2C receptor (5-HT2CR), suggesting potential for treating both RNA editing-related disorders and serotonin receptor-related conditions. These findings open avenues for further experimental validation to assess their therapeutic potential in neurological disorders, cancers, viral infections, and more.[46]

3.6 Peptoid and Peptide Inhibitors

Peptoid and peptide inhibitors are designed to interfere with ADAR's ability to recognize and bind to RNA substrates. These compounds are often used to disrupt the enzyme's RNA-editing activity by preventing the enzyme from interacting with its natural RNA targets.[47, 48]

Peptides Derived from ADAR dsRBDs mimic the double-stranded RNA-binding domains (dsRBDs) of ADAR enzymes. These peptides bind to double-stranded RNA, effectively sequestering the enzyme and preventing it from interacting with its natural RNA substrates. By mimicking the dsRBDs, these peptides act as competitive inhibitors of ADAR's RNA-binding activity, reducing RNA editing. Anoth-

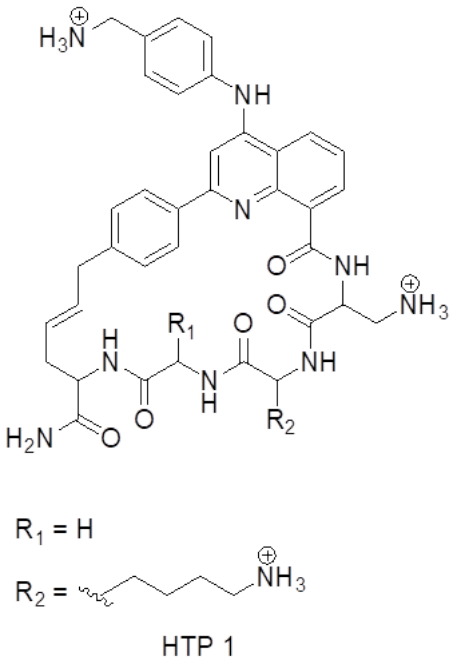


Figure 8. Structure of HTP 1, a macrocyclic helix-threading peptide.

er ADAR inhibitor described in the study is HTP 1, a macrocyclic helix-threading peptide (Fig. 8). This compound selectively binds to a specific site near the edited adenosines on the pre-mRNA for the 5-HT2c receptor (5-HT2cR). HTP 1 was shown to inhibit the in vitro RNA editing reaction catalyzed by ADAR2, with the inhibition being substrate-selective. Specifically, editing on the 5-HT2cR pre-mRNA was inhibited more effectively than on a control RNA with diminished binding to the compound. HTP 1 represents the first demonstration of substrate-selective inhibition of RNA editing by an RNA-binding small molecule. The compound binds to RNA secondary structures and has the potential for use as a molecular probe for studying RNA editing and as a therapeutic lead. Further optimization of this compound for better binding affinity and selectivity could help in modulating RNA editing levels, which may be relevant in conditions like severe depression, Prader-Willi syndrome, and responses to interferon therapy.[49]

Peptoid Mimetics are synthetic molecules that resemble peptides but have a modified backbone structure. This modification enhances their stability and selectivity compared to traditional peptides. Peptoids mimic the RNA-binding domains of ADAR, binding directly to the enzyme's active site or to the RNA substrate itself. By preventing enzyme-substrate interaction, peptoids inhibit ADAR's RNA-editing activity.[50, 51]

3.7 RNA-Based Inhibitors

RNA-based inhibitors target ADAR enzymes either directly or indirectly by preventing their interaction with RNA substrates. Aptamers are short RNA or DNA molecules that bind to ADAR's catalytic or RNA-binding domains, acting as competitive inhibitors and blocking its RNA-editing activity. Decoy RNAs mimic ADAR's natural substrates, competing for binding and inhibiting its function. Modified duplex RNAs, which are chemically altered to improve stability and binding affinity, prevent ADAR from interacting with endogenous RNA targets, thus inhibiting RNA editing. Short interfering RNAs (siRNAs) reduce ADAR levels or activity, further inhibiting RNA editing. Double-stranded RNA mimetics, such as dsRNA analogs, bind to ADAR, blocking its interaction with authentic RNA molecules.[52]

Recent research has demonstrated how ribose modification, such as Locked Nucleic Acids (LNA) and 4′-C-methylation, can act as potent ADAR inhibitors. LNA modifications at positions −1 and −2 completely block ADAR activity, while 4′-C-methylation at position −2 also inhibits the enzyme. These modifications alter the guide strand's binding properties, preventing ADAR from deaminating target RNA. This research offers insights into ADAR deamination mechanisms and guides the design of selective inhibitors for therapeutic RNA editing applications.[53]

4. Therapeutic Applications of ADAR Inhibitors

ADAR (adenosine deaminase acting on RNA) inhibitors have significant applications in various fields, ranging from cancer therapy to drug discovery. By targeting RNA-editing mechanisms, ADAR inhibitors hold promise for altering gene expression, controlling immune responses, and preventing viral replication. This section explores the therapeutic potential of ADAR inhibitors, their role in research, and their use in drug discovery and development.

4.1. Therapeutic Potential

ADAR1 plays a key role in cancer progression and treatment resistance, with its overexpression linked to poor prognosis due to its regulation of immune evasion and apoptosis-related genes.[19] Inhibiting ADAR1 in cancers like melanoma, glioblastoma, and breast cancer can enhance immune surveillance and reduce tumor growth by altering the tumor microenvironment.[20] ADAR1 also contributes to therapy resistance, and its inhibition can increase sensitivity to treatments like chemotherapy and immunother-

apies.[15, 27] In viral infections, inhibiting ADAR enzymes can disrupt RNA virus replication, such as hepatitis C and HIV, by targeting their immune evasion mechanisms.[18] ADAR inhibition may also help restore immune function in autoimmune diseases like lupus and rheumatoid arthritis by modulating immune responses and reducing tissue damage.[17]

5. Challenges in ADAR Inhibition

While ADAR inhibitors hold great promise as therapeutic agents, there are several challenges in their development. These challenges include achieving selectivity, ensuring effective delivery and bioavailability, and addressing potential resistance mechanisms. Each of these issues must be carefully considered to maximize the therapeutic potential of ADAR inhibitors while minimizing side effects and complications.

5.1. Selectivity and Toxicity

Achieving selective inhibition of ADAR isoforms, such as ADAR1 and ADAR2, is critical because they have distinct RNA targets and roles in different tissues. ADAR1 is more prevalent in the immune system and cancer cells, while ADAR2 is more active in neurons. Selectively inhibiting ADAR1 in cancer or viral infections without affecting ADAR2 in the brain is essential to avoid unintended neurological effects.[54-58] Off-target effects and toxicity concerns are significant when inhibiting ADARs, as these enzymes play key roles in RNA editing across many transcripts. Inhibiting ADAR1 may impair immune responses and the body's ability to combat infections or cancer while inhibiting ADAR2 could affect synaptic function and lead to neurological issues like cognitive dysfunction or seizures.[59, 60] Furthermore, off-target inhibition of other RNA-modifying enzymes or proteins interacting with ADARs could exacerbate toxicity by disrupting cellular homeostasis. Therefore, developing highly selective inhibitors is crucial to minimize toxicity while maintaining therapeutic efficacy.

5.2. Delivery and Bioavailability

A major challenge in developing ADAR inhibitors is effectively delivering them to target tissues or cells, as ADAR enzymes are present in various tissues, including the immune system, brain, liver, and cancerous tissues. To achieve therapeutic effects, inhibitors must reach specific areas where ADAR activity is dysregulated, such as tumors or infected cells, without accumulating in non-target tissues.[59] RNA-based inhibitors, like aptamers or modified duplex RNAs, face additional challenges, including rapid degradation by nucleases and difficulty crossing cell membranes, limiting their bioavailability and effectiveness.[61-63]

To improve bioavailability and tissue specificity, strategies are being explored. For small molecules, enhancing pharmacokinetic properties, such as stability, solubility, and half-life, is crucial.[64, 65] Nanoparticle-based delivery systems, like liposomes and dendrimers, are being studied to protect inhibitors from degradation and ensure targeted release.[66, 67]

For RNA-based inhibitors, chemical modifications (e.g., 2′-O-methylation, phosphorothioate modifications) and the development of conjugates or carriers (such as lipids or peptides) help improve stability, cellular uptake, and protection from degradation.[68-70] Additionally, tissue-specific ligands[71] or antibodies[72] may be used to direct inhibitors to the desired tissues, reducing off-target effects.

5.3. Resistance Mechanisms

Resistance to ADAR inhibitors is a significant concern, especially in chronic diseases like cancer and viral infections. In cancer, one resistance mechanism is the upregulation of ADAR expression, particularly ADAR1, allowing tumor cells to restore RNA editing and evade immune detection, thereby reducing the treatment's effectiveness.[73, 74] Mutations in ADAR may also alter its structure, preventing effective binding by inhibitors or increasing its affinity for RNA substrates. Additionally, tumor cells might use alternative RNA editing pathways or other RNA-modifying enzymes to compensate for reduced ADAR activity.[73-78]

In viral infections, resistance can arise through mutations in viral RNA or changes in ADAR expression. Viruses like HIV or hepatitis C, which rely on ADAR-mediated RNA editing, may evolve mechanisms to bypass inhibition or reduce their dependence on RNA editing for immune evasion.[79-81] Viral proteins interacting with ADAR may also undergo modifications to decrease binding affinity. Furthermore, host cells may upregulate ADAR expression in response to reduced viral replication, compensating for decreased RNA editing activity and allowing the virus to maintain its ability to edit its genome, overcoming the effects of inhibitors.[18, 82-85]

6. Future Perspectives

The potential of ADAR inhibitors in the treatment of various diseases is vast, but there are still numerous challenges and opportunities for progress. As research into RNA editing and its role in health and disease continues to expand, several exciting avenues for the development and application of ADAR inhibitors are emerging. This section explores future perspectives on ADAR inhibitor development, the role of ADAR inhibition in precision medicine, and technological advances that could revolutionize this field.

6.1. New Targets and Inhibitor Development

Efforts to develop more specific and potent ADAR inhibitors focus on achieving selectivity for ADAR1 or ADAR2, which differ in structure, substrate specificity, and tissue distribution. Research into the structural differences between the two isoforms, especially in their catalytic and RNA-binding domains, provides opportunities for designing selective inhibitors. High-throughput screening and small molecule libraries are being used to discover compounds with better binding affinity, stability, and bioavailability. A key goal is developing inhibitors that can target specific tissues, such as tumors or viral infection sites, and cross the blood-brain barrier.[40, 41, 46, 86]

Novel approaches in inhibitor design include combination therapies, where ADAR inhibitors are paired with other treatments like immune checkpoint inhibitors, chemotherapy, or antiviral agents to enhance therapeutic efficacy and overcome resistance mechanisms. For instance, combining ADAR inhibitors with immune checkpoint inhibitors could sensitize tumors, while pairing them with antiviral drugs could target both host RNA editing and viral replication.[27, 81, 87, 88] Another promising strategy involves dual-target inhibitors that block ADAR and related RNA-modifying enzymes, such as the APOBEC family, potentially providing broader therapeutic benefits, particularly in diseases involving multiple RNA-editing enzymes.[89] Additionally, combining ADAR inhibitors with agents that modulate RNA splicing, translation, or other post-transcriptional modifications may offer new therapeutic possibilities.

7. Conclusion

ADAR inhibitors show significant potential as therapeutic agents for cancer, viral infections, autoimmune disorders, and neurological conditions. These enzymes, ADAR1 and ADAR2, regulate RNA through adenosine-to-inosine editing, influencing gene expression, immune responses, and viral replication. Various ADAR inhibitors, including small molecules, peptides, and RNA-based agents, have demonstrated promise in modulating RNA editing. Their therapeutic potential lies in reducing tumor progression, metastasis, and immune evasion in cancer, blocking viral RNA editing to hinder immune evasion, and modulating immune responses in autoimmune diseases.

While ADAR inhibitors are valuable tools for research and genetic editing, challenges include achieving selectivity without disrupting essential cellular functions, improving drug delivery and bioavailability, and addressing potential resistance. The development of more specific inhibitors, along with the integration of ADAR inhibitors into precision medicine and advanced technologies like CRISPR, promises enhanced therapeutic outcomes. Despite current challenges, ADAR inhibitors hold promises for advancing treatments for various diseases.

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