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REVIEW

Mechanistic insights and therapeutic advances in antiviral strategies against African swine fever virus

Huihan Shao[†], **Quanjie Li**[*,†], **Shan Cen**[*]

Institute of Medicinal Biotechnology, Chinese Academy of Medical Science, Beijing 100050, China

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Abstract African swine fever virus (ASFV) causes a highly contagious and lethal disease in domestic and wild pigs, posing a significant threat to global swine production. The lack of effective vaccines or antiviral therapies underscores the urgent need for alternative intervention strategies. In recent years, notable progress has been made in developing antiviral agents that target both viral components and host-dependent pathways. This review provides a comprehensive summary of recent advances in ASFV antiviral research, with a focus on therapeutic interventions aimed at viral-encoded proteins and host factors, particularly small-molecule inhibitors and their mechanisms of action. In addition, the review highlights target-agnostic and multifaceted antiviral strategies, including physical inactivation, early life cycle disruption, and gene-level interference, which act through broad or partially understood mechanisms. By integrating mechanistic insights with emerging therapeutic approaches, this review supports the rational design of antiviral interventions against ASFV and informs the subsequent exploration of candidate drugs with clinical translational potential.

*Corresponding authors.
E-mail addresses: quanjie.li@imb.pumc.edu.cn (Quanjie Li), shancen@imb.pumc.edu.cn (Shan Cen).
[†]These authors made equal contributions to this work.

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1. Introduction

African swine fever (ASF) is a highly contagious and lethal disease that affects both domestic pigs and wild suids, caused by the African swine fever virus (ASFV)[1−3]. Since its introduction into Europe and Asia, ASF has caused huge economic losses and poses a severe threat to global food security[4,5]. Although current measures for controlling ASF primarily rely on early detection and culling of infected animals, sustainable long-term strategies are still lacking. Vaccination is widely known as a key component of infectious disease control. However, recent evidence has raised concerns regarding the safety and efficacy of the two live-attenuated vaccines approved in Vietnam, NAVET-ASFVAC (ASFV-G-ΔI177L) and AVAC ASF LIVE (ASFV-G-ΔMGF)[6,7]. A key limitation lies in the potential for complex interactions between vaccine-derived and circulating field strains, which may confound immunological outcomes under field conditions. Moreover, considering a potentially increasing viral mutation rate, the emergence of novel variants with altered phenotypic characteristics could further undermine the protective efficacy of existing vaccines[8]. These challenges underscore the pressing need to advance alternative or adjunct therapeutic strategies, including both direct-acting antivirals and host-targeting agents, as part of an integrated framework for ASFV control.

ASFV is a large, enveloped, double-stranded DNA virus and the sole member of the Asfarviridae family[9]. It exhibits a complex icosahedral morphology with a multilayered structure and encodes over 150 proteins involved in genome replication, virion assembly, immune evasion, and host manipulation[10−12]. The replication cycle of ASFV is a highly orchestrated process that occurs predominantly in the cytoplasm of infected cells[13]. Viral particles enter cells *via* clathrin-mediated endocytosis, macropinocytosis, or efferocytosis, and are subsequently trafficked along microtubules within endosomal vesicles to perinuclear regions. The acidic environment of late endosomes promotes partial disassembly of the viral capsid and facilitates fusion between the viral inner envelope and the endosomal membrane, leading to the release of viral cores into the cytoplasm[14]. Early transcription is initiated within partially uncoated viral cores and primarily generates transcripts encoding proteins necessary for viral DNA replication[15]. Once DNA replication commences in the cytoplasm, the transcriptional program shifts to late-phase transcription, which produces structural and morphogenetic proteins required for virion assembly[16]. Viral morphogenesis and assembly occur in perinuclear cytoplasmic sites known as viral factories[17]. Following assembly, mature intracellular virions are transported along the microtubule network to the plasma membrane, where they are released either by budding through the membrane or *via* apoptotic bodies[18].

Given the complexity of the ASFV replication cycle and its extensive reliance on host cellular machinery, multiple stages of the viral life cycle present promising opportunities for therapeutic intervention[1]. Several virus-encoded proteins, including those involved in genome replication, transcriptional regulation, and virion morphogenesis, serve as direct antiviral targets due to their essential roles in viral propagation[19]. At the same time, ASFV hijacks numerous host pathways, such as lipid metabolism, cytoskeletal transport, protein folding systems, and innate immune signaling, to support its replication and evade immune responses[20]. In addition to classical target-based strategies, increasing attention has been directed toward diverse, target-agnostic approaches. These include naturally derived viral inactivation agents such as natural oil blend formulations (NOBFs)[21,22], as well as materials-based antiviral technologies such as silver nanoparticles (AgNPs)[23], which exhibit broad-spectrum virucidal activity through physicochemical interactions. Furthermore, gene-targeting platforms such as RNA interference (RNAi)[24] and CRISPR-Cas systems[25] offer tools for sequence-specific inhibition of viral gene expression. Collectively, these emerging strategies provide alternative pathways for effective ASFV control.

In this review, we systematically summarize mechanism-based antiviral strategies against ASFV. Recent advances in antiviral research can be broadly classified into three principal domains, namely inhibitors that directly target essential viral proteins, host-targeted antiviral interventions, and emerging technologies and materials with demonstrated or potential antiviral activity. Fig. 1 illustrates the ASFV life cycle, highlighting both viral and host targets, as well as their respective small-molecule inhibitors. By integrating mechanistic insights with emerging therapeutic approaches, this review supports the rational design of antiviral interventions against ASFV and provides a foundation for the discovery and development of candidate agents with clinical translational potential.

2. Inhibitors targeting key viral proteins

The ASFV genome encodes over 60 structural proteins and 100 non-structural proteins, many of which have no close homologs in host cells, making them compelling and selective targets for direct-acting antiviral intervention[19]. The currently reported direct-acting antivirals targeting ASFV primarily focus on several key viral proteins essential for viral replication. These include the multi-subunit RNA polymerase complex (RNAP)[26], which is essential for early gene expression, and ribonucleotide reductase (RNR)[27], responsible for nucleotide supply. The RNA helicase D1133L[28] and B-family DNA polymerase (DNAPol)[29] are critical for genome replication. Topoisomerase II (Topo II)[30−33] and the histone-like DNA-binding protein pA104R[34−37] are involved in topological control and genome packaging. DNA Polymerase X (Pol X)[38] facilitates post-replicative base-excision repair. Final, the cysteine protease pS273R[39] is crucial for late proteolytic maturation. These viral proteins are essential for distinct and sequential stages of the ASFV life cycle. Accordingly, direct inhibition of these essential viral components represents a promising strategy for therapeutic development against ASFV. This section reviews the structural and mechanistic features of these key viral targets and highlights representative small-molecule inhibitors identified to modulate their activity (Table 1).

2.1. Inhibitors targeting ASFV RNA polymerase (RNAP)

Unlike many DNA viruses that depend on host nuclear transcription machinery, ASFV replicate exclusively in the cytoplasm of infected cells[80]. Consequently, ASFV encodes a self-sufficient viral transcription system, capable of producing fully functional, capped and polyadenylated mRNAs independently of host nuclear factors[16]. A central component of this machinery is the ASFV RNAP, which catalyzes the transcription of viral genes throughout the infection cycle[16,42]. Recent high-resolution structural studies have revealed that ASFV RNAP adopts the canonical crab claw-like conformation characteristic of double-psi β-barrel (DPBB) RNAPs, which are conserved across archaea and eukaryotes[26,42].

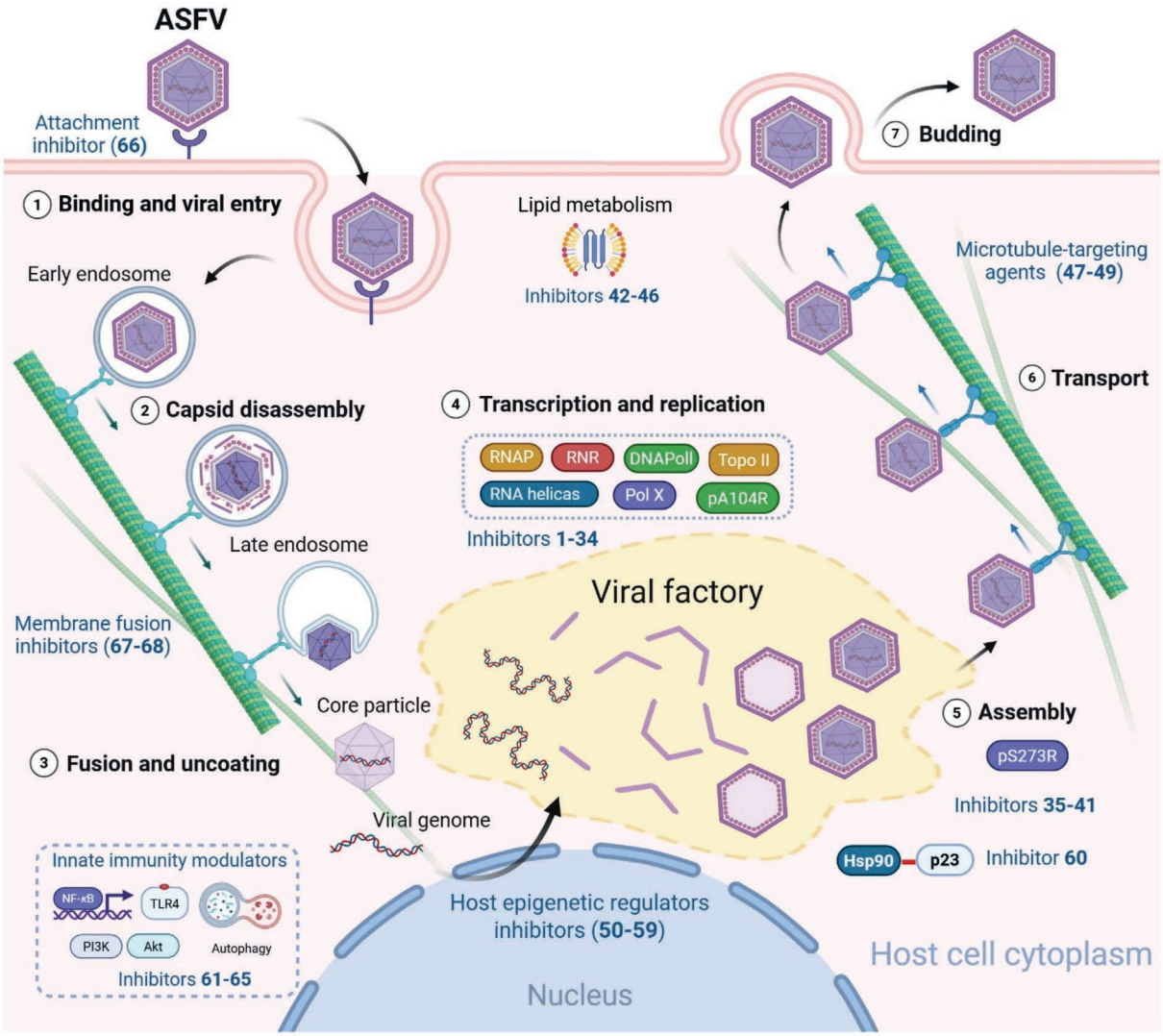

Figure 1 The ASFV life cycle and reported inhibitors targeting key stages of infection. Upon entering host cells *via* multiple pathways, ASFV particles are transported along microtubules to perinuclear viral factories. Acidification in late endosomes triggers capsid disassembly and uncoating, releasing the viral core into the cytoplasm. The viral genome then replicates within the perinuclear factory, and mature virus particles are assembled using modified endoplasmic reticulum membranes. These particles are subsequently released through budding or apoptotic body-mediated processes. The antiviral compounds indicated in the figure target critical stages of the viral lifecycle, including entry, trafficking, replication, assembly, and modulation of host immune responses. For clarity, the compounds are numbered in the Figure, with their corresponding details outlined in Tables 1 and 2 Figure was created in BioRender. Li, Q. (2025), https://BioRender.com/110b9qq.

The core enzyme comprises eight subunits, among which subunit 1 and subunit 2 form the principal scaffold (Fig. 2A). Each contributes a DPBB domain, together constituting the catalytic center at their interface. This active site harbors a catalytically essential magnesium ion (Mg^{2+}, site A), which is precisely coordinated by a conserved aspartate triad (D457, D459, D461) within the canonical NADFDGD motif (Fig. 2A), mirroring the configuration seen in eukaryotic RNA polymerase II (RNAPII). These findings highlight the evolutionary conservation of ASFV RNAP and underscore its essential role in viral transcription, making it an attractive target for antiviral intervention[26].

Several classes of compounds have been evaluated for their inhibitory effects on the ASFV RNAP, revealing diverse mechanisms of action (Fig. 2B−F). Natural antibiotics of the rifamycin class were among the first agents reported to suppress ASFV replication. A series of rifamycin derivatives have been tested for their ability to inhibit ASFV RNAP activity[40]. Among them, AF/ABDP exhibited the strongest effect, reducing RNAP activity to 0.03 relative to control, followed by 3-formyl Rifamycin (0.35), Rifampicin (0.50), *N*-demethyl Rifampicin (0.55), Rifamycin (0.57). Among these compounds, Rifampicin (rifampin), a semi-synthetic derivative of rifamycin, was among the first compounds reported to suppress ASFV replication. In PK-15 cells, it reduced viral titers by 1−5 logs at concentrations up to 200 μg/mL, primarily by disrupting early-stage cytoplasmic RNA synthesis[41]. Earlier work by Salas et al. reported that rifampicin at 200 μg/mL (243 μmol/L) reduced ASFV RNAP activity to approximately 50% of control levels[40]. However, recent biochemical assays using purified recombinant ASFV RNAP showed no measurable inhibition at 100 μmol/L[42], suggesting a concentration-dependent

Table 1 Summary of antiviral agents that act on key viral protein targets.

No.	Name	Category	Target	Antiviral activity (against ASFV unless otherwise noted)	Development stage (in ASFV)	Ref.
1	AF/ABDP	Rifamycin derivative	ASFV RNAP	Reduces RNAP relative activity to 0.03 at 200 μg/mL	*In vitro*	40
2	3-Formyl Rifamycin	Rifamycin derivative	ASFV RNAP	Reduces RNAP relative activity to 0.35 at 200 μg/mL	*In vitro*	40
3	Rifampicin	Rifamycin derivative	ASFV RNAP	Reduces RNAP relative activity to 0.50 and viral titers by 1−5 logs at 200 μg/mL (PK-15 cells)	*In vitro* (repurposed drug)	40,41
4	*N*-demethyl Rifampicin	Rifamycin derivative	ASFV RNAP	Reduces RNAP relative activity to 0.55 at 200 μg/mL	*In vitro*	40
5	Rifamycin	Rifamycin antibiotic	ASFV RNAP	Reduces RNAP relative activity to 0.57 at 200 μg/mL	*In vitro* (repurposed drug)	40
6	Actinomycin D	Actinomycin antibiotic	ASFV RNAP	Effective at 100 μmol/L (against RNAP activity)	*In vitro* (repurposed drug)	42
7	EDTA	Metal chelator	ASFV RNAP	Effective at 50 mmol/L (against RNAP activity)	*In vitro* (repurposed drug)	42
8	Triapine	Thiosemicarbazone	ASFV RNR	IC_{50} = 0.44 μmol/L, CC_{50} = 158.3 μmol/L (PAMs)	*In vitro*	43
9	Cyproheptadine hydrochloride	Piperidine derivative	ASFV D1133L	Reduces ASFV genomic DNA copy number by > 50% at 18 μmol/L (PAMs)	*In vitro* (repurposed drug)	28
10	IDU	Nucleoside analogue	ASFV DNAPol	Effective at 100 μg/mL (Vero)	*In vitro* (repurposed drug)	44,45
11	(*S*)-HPMPA	Nucleotide analogue	ASFV DNAPol	MIC_{50} = 0.01 μg/mL, SI = 15000 (Vero)	*In vitro*	46−48
12	(*S*)-cHPMPA	Nucleotide analogue	ASFV DNAPol	MIC_{50} = 0.2 μg/mL, SI = 2500 (Vero)	*In vitro*	49
13	(*S*)-HPMPDAP	Nucleotide analogue	ASFV DNAPol	MIC_{50} = 0.5 μg/mL, SI = 1400 (Vero)	*In vitro*	49
14	C-c^{3}Ado	Nucleotide analogue	ASFV DNAPol	MIC_{50} = 0.025 μg/mL, SI = 8000 (Vero)	*In vitro*	46
15	cHPMPC	Nucleotide analogue	ASFV DNAPol	IC_{50} = 0.11−0.59 μmol/L, SI = 126−593 (PAMs)	*In vitro* and *in vivo* (repurposed drug)	50
16	Cytarabine hydrochloride	Nucleotide analogue	ASFV DNAPol	IC_{50} = 0.23 μmol/L, CC_{50} = 229.6 μmol/L (PAMs)	*In vitro*	43
17	Brincidofovir	Lipid-conjugated nucleotide analogue	ASFV DNAPol	IC_{50} = 2.76 nmol/L, CC_{50} = 58 μmol/L (PAMs)	*In vitro* and *in vivo*	51
18	*m*-AMSA	Aminoacridine derivative	ASFV Topo II	IC_{50} = 1 μmol/L (24 h), 0.4 μmol/L (48 h), CC_{50} = 23.6 μmol/L (24 h), 16.8 μmol/L (48 h) (PAMs)	*In vitro* (repurposed drug)	52
19	Doxorubicin	Anthracycline antibiotic	ASFV Topo II	Active at concentration >1 μmol/L	*In vitro*	32
20	Genistein	Isoflavone derivative (natural product)	ASFV Topo II	IC_{50} = 4.403 μmol/L (PAMs)	*In vitro*	53,54
21	Arctiin	Lignan glycoside (natural product)	ASFV Topo II	IC_{50} = 4.139 μmol/L (PAMs)	*In vitro*	54
22	Enrofloxacin	Fluoroquinolone antibiotic	ASFV Topo II	MIC = 100 μg/mL (induces viral DNA breakage)	*In vitro* (repurposed drug)	55,56
23	Grepafloxacin	Fluoroquinolone antibiotic	ASFV Topo II	MIC = 100 μg/mL (induces viral DNA breakage)	*In vitro* (repurposed drug)	55
24	Balofloxacin	Fluoroquinolone antibiotic	ASFV Topo II	MIC = 100 μg/mL (induces viral DNA breakage)	*In vitro* (repurposed drug)	55
25	Tosufloxacin	Fluoroquinolone antibiotic	ASFV Topo II	MIC = 100 μg/mL (induces viral DNA breakage)	*In vitro* (repurposed drug)	55
26	Gatifloxacin	Fluoroquinolone antibiotic	ASFV Topo II	MIC = 100 μg/mL (induces viral DNA breakage)	*In vitro* (repurposed drug)	55
27	Garenoxacin	Fluoroquinolone antibiotic	ASFV Topo II	MIC = 100 μg/mL (induces viral DNA breakage)	*In vitro* (repurposed drug)	55
28	Coumermycin A1	Hydroxycoumarin antibiotic	ASFV Topo II	Effective at micromolar concentration	*In vitro*	40
29	SD1	Stilbene derivatives	ASFV pA104R	IC_{50} = 2.93 μmol/L, CC_{50} = 102 μmol/L (PAMs)	*In vitro*	57

(*continued on next page*)

Table 1 (*continued*)

No.	Name	Category	Target	Antiviral activity (against ASFV unless otherwise noted)	Development stage (in ASFV)	Ref.
30	SD4	Stilbene derivatives	ASFV pA104R	IC_{50} = 0.48 μmol/L, CC_{50} = 263 μmol/L (PAMs)	*In vitro*	57
31	Thonningianin A	Ellagitannin (natural product)	ASFV pA104R	IC_{50} = 0.068 μmol/L, CC_{50} = 17.1 μmol/L (PAMs)	*In vitro*	34
32	Resveratrol	Polyphenolic stilbenoid (natural product)	ASFV pA104R	IC_{50} = 1 μg/mL, CC_{50} = 30 μg/mL (Vero)	*In vitro*	58
33	Oxyresveratrol	Polyphenolic stilbenoid (natural product)	ASFV pA104R	IC_{50} = 10 μg/mL, CC_{50} = 50−75 μg/mL (Vero)	*In vitro*	58
34	Fostamatinib	Splenic tyrosine kinase inhibitor	ASFV PolX	Inhibits PolX activity by up to 90% at 12.5 μmol/L	*In vitro* (repurposed drug)	59
35	E-64	Epoxysuccinate class of inhibitors	ASFV pS273R	Effective at 4 mmol/L (against pS273R activity)	*In vitro*	60
36	ZW60	Peptidomimetic aldehydes inhibitors	ASFV pS273R	IC_{50} = 0.900 μmol/L (against pS273R activity)	*In vitro*	39
37	ZW30	Peptidomimetic aldehydes inhibitors	ASFV pS273R	IC_{50} = 2.605 μmol/L (against pS273R activity)	*In vitro*	39
38	ZW22	Peptidomimetic aldehydes inhibitors	ASFV pS273R	IC_{50} = 3.440 μmol/L (against pS273R activity)	*In vitro*	39
39	Myricetin	Natural flavonoid	ASFV pS273R	IC_{50} = 8.4 μmol/L (against pS273R activity)	*In vitro*	61
40	Myricitrin	Natural flavonoid	ASFV pS273R	IC_{50} = 2.7 μmol/L (against pS273R activity)	*In vitro*	61
41	OPTX-1	Defensin-like peptide toxin	ASFV pS273R	Effective at 5−10 μmol/L (PAMs), K_i = 0.821 μmol/L (against pS273R activity)	*In vitro*	62

effect. Although ASFV RNAP shares overall architectural similarity with eukaryotic RNAPII, cryo-EM analysis has revealed that it lacks the canonical rifampicin-binding pocket found in the β subunit of bacterial RNAP. This structural divergence likely underlies the intrinsic resistance of ASFV RNAP to rifampicin at low micromolar concentrations. In addition to rifamycin-based inhibitors, actinomycin D (Fig. 2G), a classical DNA-intercalating agent, has also been shown to significantly inhibit ASFV RNAP activity at a concentration of 100 μmol/L. By intercalating into the DNA double helix, actinomycin D disrupts the integrity of the DNA template and blocks transcription elongation, thereby impairing viral RNA synthesis[42]. Furthermore, EDTA (Fig. 2H), a metal ion chelator, inhibits ASFV RNAP activity at 50 mmol/L by sequestering the essential catalytic Mg^{2+} ion, which is required for coordination within the active site of the polymerase[42]. Due to their limited selectivity and the requirement for non-physiological concentrations, these compounds are regarded as *in vitro* mechanistic controls rather than as translatable anti-ASFV leads.

Despite the limited availability of potent compounds, recent cryo-EM structures of ASFV RNAP provide a foundation for rational inhibitor development. Notably, the resolved open and closed clamp conformations delineate a conserved transcriptional cycle and offer a structural basis for designing conformation-selective allosteric modulators.

2.2. Inhibitors targeting ASFV ribonucleotide reductase (RNR)

In addition to blocking early transcription through RNAP inhibition, recent studies have also focused on disrupting the metabolic underpinnings of viral genome replication by targeting enzymes involved in nucleotide biosynthesis (Fig. 3A). A representative strategy involves the indirect targeting of auxiliary enzyme systems that facilitate dNTP biosynthesis[81]. Among them, RNR plays a central role by catalyzing the conversion of ribonucleotides to dNTPs, which serve as essential precursors for viral DNA replication[27,82]. As the rate-limiting step in dNTP production, RNR constitutes a strategic upstream metabolic target for antiviral intervention.

Li et al.[43] employed high-throughput screening of an FDA-approved drug library and identified Triapine (Fig. 3B) as a potential inhibitors of ASFV infection. In PAMs, Triapine exhibited dose-dependent inhibition of ASFV replication across a concentration range of 0.1 to 10 μmol/L, with an IC_{50} of 0.44 μmol/L. This value is significantly lower than its cytotoxic concentration (CC_{50} = 158.3 μmol/L, resulting in a favorable selectivity index (SI > 300) and indicating a strong antiviral safety margin. RNR consists of a large subunit (pF778R, RRls) and a small subunit (pF334L, RRss), the latter harboring an iron-dependent catalytic center essential for enzymatic activity[83]. Mechanistically, molecular docking analyses indicates that Triapine stably coordinates the Fe^{2+} at the catalytic center of ASFV RNR, thereby disrupting RNR function and reducing intracellular dNTP levels[43]. As a potent RNR inhibitor under phase-III oncology evaluation, Triapine exhibits favorable clinical safety and pharmacokinetics, supporting its translational potential as anti-ASFV agent.

2.3. Inhibitors of ASFV RNA helicases D1133L

Targeting the viral RNA helicase offers a complementary strategy to disrupt nucleotide synthesis through ASFV RNR inhibition, as it impairs the RNA duplex unwinding necessary for transcription and replication. ASFV encodes five putative superfamily 2 RNA helicases (Q706L, QP509L, A859L, D1133L, and B962L), which

Table 2 Summary of antiviral agents that act on key viral protein targets.

No.	Name	Category	Targeted host factor/ Pathway	Antiviral activity (against ASFV unless otherwise noted)	Development stage (in ASFV)	Ref.
42	Lauryl gallate (LG)	Natural antioxidant	Lipid metabolic pathways	IC_{50} = 1 μmol/L (Vero), IC_{50} = 0.4 μmol/L (COS); EC_{50} = 2−3 μmol/L (PAMs), CC_{50} = 3 0 μmol/L (PAMs)	*In vitro*	63,64
43	Valproic acid (VPA)	Fatty acid derivative	Lipid metabolic pathways	IC_{50} = 2.5−3 mmol/L	*In vitro* (repurposed drug)	63
44	Cerulenin (CRL)	Antifungal antibiotic	Lipid metabolic pathways	IC_{50} = 3−9 μmol/L	*In vitro*	63
45	Theaflavin (TF)	Antioxidant polyphenols (natural product)	Activates the AMPK pathway	Effective at 80 μmol/L	*In vitro*	65
46	Brequinar (BQR)	Squinolinecarboxylic acid analogue	Inhibition of pyrimidine biosynthesis/Induction of ferroptosis	EC_{50} = 2.83 μmol/L, CC_{50} > 100 μmol/L (Vero); EC_{50} = 21.95 μmol/L, CC_{50} = 451.8 μmol/L (PAMs)	*In vitro*	66,67
47	Apigenin	Plant-derived flavonoid	Microtubule networks	Effective at 50 μmol/L (Vero)	*In vitro*	68
48	Genkwanin	Plant-derived flavonoid	Microtubule networks	IC_{50} = 2.9 μmol/L, SI = 205.2 (Vero)	*In vitro*	69
49	Compound **6b**	Carbohydrazide derivative	Microtubule networks	IC_{50} = 19.5 μmol/L, CC_{50} > 500 μmol/L (Vero)	*In vitro*	70
50	NaPB	Short-chain fatty acid butyrate	HDAC	Synergistically inhibits ASFV with enrofloxacin (25 μg/mL) at 2.5 mmol/L	*In vitro* (repurposed drug)	71
51	ARV-825	PROTAC molecule	BET/BRD4	Effective at 0.25−1 μmol/L, CC_{50} = 10.11 μmol/L (PAMs)	*In vitro*	72
52	ZL0580	BRD4-specific inhibitors	BET/BRD4	Effective at 10 μmol/L, CC_{50} = 25.3 μmol/L (PAMs)	*In vitro*	72
53	I-BET-762	Pan-BET inhibitors	BET/BRD4	Effective at 10 μmol/L, CC_{50} = 35.86 μmol/L (PAMs)	*In vitro*	72
54	PLX51107	Pan-BET inhibitors	BET/BRD4	Effective at 5 μmol/L, CC_{50} = 19.37 μmol/L (PAMs)	*In vitro*	72
55	3-Deazaneplanocin A	Adenosine analog	AdoHcy hydrolase	EC_{50} = 0.01 μg/mL, CC_{50} = 30 μg/mL, SI = 3000 (Vero)	*In vitro*	73
56	c^3DHCaA	Adenosine analog	AdoHcy hydrolase	EC_{50} = 0.08 μg/mL, CC_{50} = 200 μg/mL, SI = 2500 (Vero)	*In vitro*	73
57	4′β-Vinyl-DHCaA	Adenosine analog	AdoHcy hydrolase	EC_{50} = 0.10 μg/mL, CC_{50} = 200 μg/mL, SI = 2000 (Vero)	*In vitro*	73
58	6′β-Fluoroaristeromycin	Adenosine analog	AdoHcy hydrolase	EC_{50} = 0.008 μg/mL, CC_{50} = 10 μg/mL, SI = 1250 (Vero)	*In vitro*	73
59	MDL 28842	Adenosine analog	AdoHcy hydrolase	EC_{50} = 0.03 μg/mL, CC_{50} = 20 μg/mL, SI = 667 (Vero)	*In vitro*	73
60	Ailanthone (AIL)	Triterpenoid derivative (natural product)	HSP90−p23 interaction	IC_{50} = 15 nmol/L, SI > 41 (PAMs); IC_{50} = 12 nmol/L (Vero-E6)	*In vitro*	74
61	BAY11-7082	Synthetic vinyl sulfone derivatives	Inhibition of NF-κB pathway	Effective at 10 μmol/L (PAMs)	*In vitro*	75
62	Dihydromyricetin (DHM)	Natural flavonoid	Inhibition of TLR4-mediated pyroptosis pathway	IC_{50} = 20.06 μmol/L, CC_{50} = 532.1 μmol/L, SI = 26.53 (PAMs)	*In vitro*	76
63	Tetrandrine (TET)	Natural bis-benzylisoquinoline alkaloid	Inhibition of PI3K/Akt pathway	IC_{50} = 2.2 μmol/L (Vero), IC_{50} = 3.0 μmol/L (PAMs)[77]; IC_{50} = 0.45 μmol/L, CC_{50} = 12.12 μmol/L (PAMs)[78]; IC_{50} = 1.15 μmol/L, CC_{50} = 11.1 μmol/L (BMDMs); IC_{50} = 0.87 μmol/L, CC_{50} = 26.74 μmol/L (MA104)	*In vitro*	77,78
64	Berbamine	Natural bis-benzylisoquinoline alkaloid	Reduces the production of proinflammatory cytokines	IC_{50} = 8.4 μmol/L (Vero), IC_{50} = 11.6 μmol/L (PAMs)	*In vitro*	77
65	Kaempferol	Natural flavonoids	Induces autophagy	IC_{50} = 2.2 μg/mL (7.7 μmol/L), CC_{50} = 93.1 μg/mL (325.3 μmol/L), SI = 42.3 (Vero)	*In vitro*	79

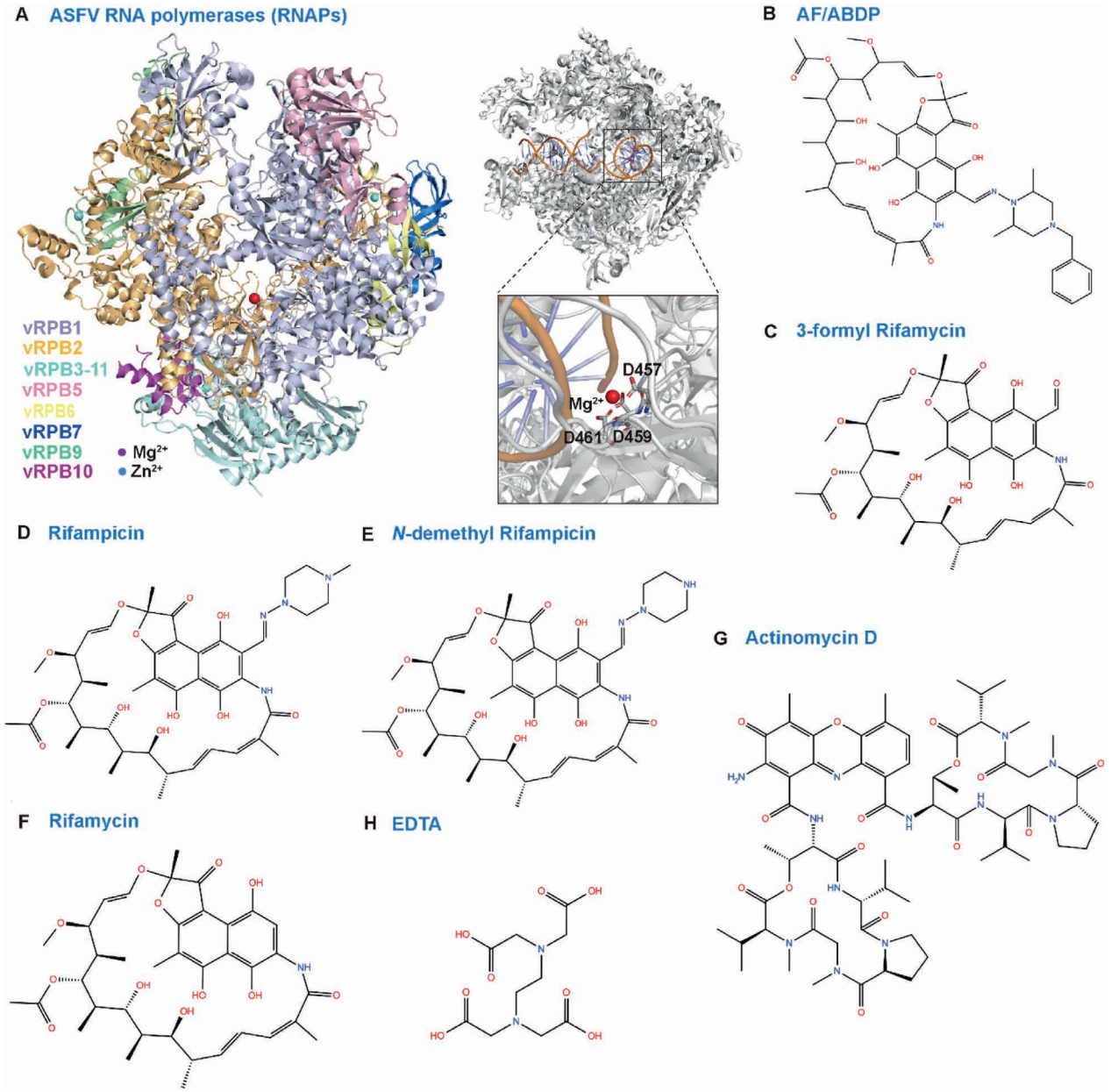

Figure 2 Tertiary structure of the ASFV RNAP and chemical structures of representative protease inhibitors. (A) Three-dimensional structure of ASFV RNAP core complex (PDB ID: 8XXP, left), elongation complex (PDB ID: 8XX4, top right), and catalytic center (from 8XX4, bottom right). The protein structure images were generated using PyMOL (version 3.1.4), based on the corresponding PDB files; (B−H) Chemical structures of representative small-molecule inhibitors targeting ASFV RNAP, including AF/ABDP, 3-formyl Rifamycin, Rifampicin, *N*-demethyl Rifampicin, Rifamycin, Actinomycin D, and EDTA. The compound structures were drawn using the 2D Sketcher in the academic version of Maestro (version 14.5).

are ATP-dependent enzymes involved in nucleic acid metabolic processes, including DNA replication, repair, and transcription[84]. Among them, D1133L is a large helicase-like protein (∼3402 bp) structurally homologous to the vaccinia virus D6R, and is predicted to play a critical role in ASFV transcription initiation[85]. Structurally, it comprises an N-terminal domain, a central catalytic core, and a C-terminal domain. The core region contains two RecA-like subdomains responsible for nucleic acid strand separation and harbors conserved DEAH-box motifs essential for ATPase activity (Fig. 3C).

Targeting ASFV D1133L, Cui et al.[28] performed a structure-based virtual screening of FDA-approved drugs and identified cyproheptadine hydrochloride (Periactin), a well-known antihistamine, as the most potent hit (Fig. 3D). Molecular docking predicted a strong binding affinity between Periactin and D1133L (−11.3 kcal/mol), indicating potential direct interaction with the viral helicase. Subsequent *vitro* validation demonstrated that Periactin significantly inhibited ASFV replication in both bone marrow-derived macrophages and PAMs. At 18 μmol/L, it reduced viral genomic DNA levels by over 50% and markedly suppressed the production of infectious virions. Mechanistic

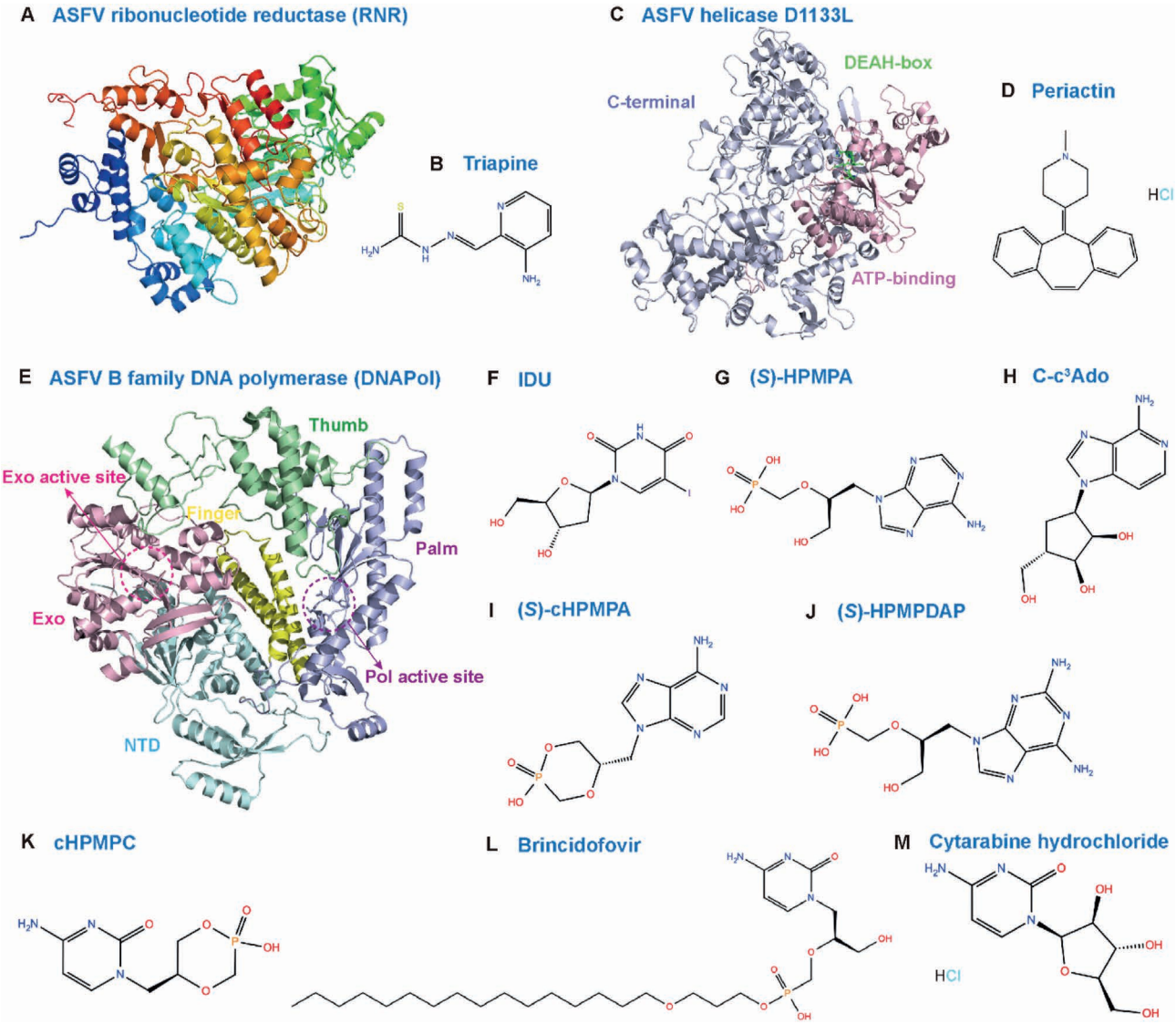

Figure 3 Tertiary structures of ASFV RNR, RNA helicases D1133L, and DNAPol, along with their representative small-molecule inhibitors. (A) Predicted tertiary structure of ASFV RNR, generated using AlphaFold3. (B) Chemical structure of Triapine, an inhibitor targeting ASFV RNR. (C) Predicted tertiary structure of ASFV RNA helicase D1133L, generated using AlphaFold3. (D) Chemical structure of cyproheptadine hydrochloride, an inhibitor targeting ASFV D1133L. (E) Three-dimensional structure of ASFV DNAPol (PDB ID: 8YWG). The protein structure images were generated using PyMOL (version 3.1.4), based on the corresponding PDB files. (F−M) Chemical structures of representative inhibitors targeting ASFV DNAPol, including IDU, (*S*)-HPMPA, C-c3Ado, (*S*)-cHPMPA, (*S*)-HPMPDAP, cHPMPC, Brincidofovir and cytarabine hydrochloride. The compound structures were drawn using the 2D Sketcher in the academic version of Maestro (version 14.5).

studies demonstrated that Periactin downregulated the transcription and translation of key ASFV genes, including the early gene P30 (CP204L) and late gene P72 (B646L), with up to 95% inhibition observed at concentrations above 12 μmol/L. Notably, Periactin exhibited low cytotoxicity in PAMs, maintaining over 70% cell viability at concentrations up to 50 μmol/L. These findings, combined with its clinical application[86], particularly as an appetite stimulant in both veterinary and human medicine, supports its potential for repurposing as an anti-ASFV therapeutic candidate.

2.4. *Inhibitors of ASFV B family DNA polymerase (DNAPol)*

Following transcriptional regulation mediated by viral helicases, the next critical step in the ASFV lifecycle is the replication of its DNA genome. This process is mediated by the ASFV DNAPol, an essential enzyme that shares structural and functional features with eukaryotic replicative polymerases[87]. ASFV DNAPol adopts a classical right-handed configuration composed of palm, fingers, and thumb subdomains (Fig. 3E) and is presumed to contain $3' \rightarrow 5'$ exonuclease proofreading activity to ensure replication accuracy[29]. DNAPol is one of the most conserved replication-associated proteins across ASFV genotypes, highlighting its essential role in the viral life cycle and its potential as an antiviral target. Several nucleoside analogs have been identified that inhibit ASFV DNAPol activity by mimicking natural nucleotides and interfering with viral DNA synthesis (Fig. 3F−M). These compounds either terminate chain elongation or disrupt DNA polymerase function through direct interactions with catalytic residues.

Among classical DNA synthesis inhibitors, IDU was one of the earliest compounds shown to inhibit ASFV. At a concentration of 100 μg/mL, IDU completely suppressed viral antigen and inclusion body formation, and could induce persistent infection models in Vero cells[44,45]. Another highly potent inhibitor is (*S*)-HPMPA, which exhibits an IC_{50} of 0.01 μg/mL and a remarkable SI of 15,000. It acts *via* multi-site inhibition by blocking viral DNA synthesis and late-stage protein expression, such as IP-73[46−48]. Similarly, C-c^{3}Ado demonstrated strong efficacy (IC_{50} = 0.025 μg/mL, SI = 8000), attributed to its unique structure and high specificity[46]. Related analogs, including (*S*)-cHPMPA (IC_{50} = 0.2 μg/mL, SI = 2500) and (*S*)-HPMPDAP (IC_{50} = 0.5 μg/mL, SI = 1400), further expand the repertoire of nucleoside-based inhibitors with distinct structural features[49].

Several preclinical or investigational drugs against DNA viruses have also demonstrated anti-ASFV activity. Cyclic cidofovir (cHPMPC), a nucleotide analogue, exhibits broad-spectrum efficacy against four ASFV genotypes, with submicromolar inhibitory activity (IC_{50} = 0.11−0.59 μmol/L) and a high selectivity index (SI = 126−593)[50]. *In vitro* and *in vivo* studies indicate that cHPMPC not only possesses extracellular virucidal activity but also effectively suppresses viral DNA replication, protein expression, and the formation of viral factories, suggesting strong cross-genotype antiviral potential and clinical translational value[50]. Brincidofovir, the lipid-conjugated prodrug of cidofovir, was identified *via* kinase inhibitor library screening in PAMs and demonstrated >90% inhibition of ASFV infection. It exhibits nanomolar potency (IC_{50} = 2.76 nmol/L) with low cytotoxicity (CC_{50} = 58 μmol/L), yielding an exceptionally high selectivity index[51]. Time-of-addition assays revealed that brincidofovir primarily exerts its antiviral effect during the post-entry stage of the viral life cycle, implicating its interference with viral genome replication or downstream protein synthesis[51]. Kuai et al.[29] performed molecular docking based on the solved structure of the ASFV DNAPol. The active metabolite CDVpp (cidofovir diphosphate) was predicted to occupy the catalytic site and form hydrogen bonds with the backbones of S520 and L521 and the side chains of R593 and K635, contacts that also stabilize the incoming dNTP, indicating that it is a competitive substrate. Together, these findings highlight the clinical promise of cidofovir-based analogues as broad-spectrum inhibitors targeting ASFV DNA replication.

In addition to these classical nucleoside analogs, cytarabine hydrochloride, a cytidine analog used in chemotherapy, has also demonstrated promising activity against ASFV. After intracellular activation, cytarabine is incorporated into viral DNA during replication, leading to premature chain termination. Molecular docking suggests that the active form of cytarabine interacts with key catalytic residues of ASFV DNAPol, including R589, K593, and K631, thereby interfering with its polymerase activity. In ASFV-infected PAMs, cytarabine exhibited potent antiviral activity (IC_{50} = 0.23 μmol/L; CC_{50} = 229.6 μmol/L), yielding a favorable SI > 300[43]. Collectively, these findings highlight the potential of nucleoside analogs in targeting ASFV DNAPol, offering a broad chemical space for the development of highly selective and effective antiviral agents.

2.5. *Inhibitors targeting ASFV topoisomerase II (topo II)*

In addition to DNAPol-mediated genome synthesis, ASFV relies on Topo II, encoded by the ASFV ORF P1192R, to resolve topological stress during replication. Notably, ASFV Topo II is the only known type II topoisomerase expressed by a mammalian-infecting virus[33] and plays a central role in viral genome replication and the regulation of DNA topology. Structural analysis of ASFV Topo II revealed that its ATPase region adopts a characteristic heart-shaped dimeric architecture, comprising two highly conserved domains: the N-terminal GHKL ATP-binding fold and the C-terminal transducer domain (Fig. 4A). This configuration closely resembles that of eukaryotic Topo IIA, indicating that the mechanism by which ASFV Topo II facilitates DNA relaxation *via* ATP hydrolysis is evolutionarily conserved[30,31,52].

Among the inhibitors tested against ASFV Topo II, *m*-AMSA (Fig. 4B), exhibited potent and specific activity. Structural studies revealed that *m*-AMSA intercalates into DNA at two sites within the ASFV Topo II−DNA complex: the DNA cleavage site (Site 1) and a pre-cleavage site (Site 2)[52]. Stronger binding was observed at Site 2, where its methanesulfone-m-anisidide group anchors into a hydrophobic pocket formed by residues L470, G471, G472, and V473, stabilizing the complex and blocking DNA cleavage (Fig. 4A). Biochemical analysis showed that *m*-AMSA inhibits Topo II-mediated DNA relaxation in a dose-dependent manner by inducing both single- and double-strand breaks. In PAMs, cytotoxicity assays revealed CC_{50} values of approximately 23.6 μmol/L at 24 h and 16.8 μmol/L at 48 h. At a non-toxic concentration of 10 μmol/L, *m*-AMSA significantly suppressed ASFV replication, reducing viral DNA levels by over 97% at 24 h and over 99% at 48 h, with corresponding IC_{50} values of 1 and 0.4 μmol/L[52].

In addition to *m*-AMSA, several other compounds have exhibited inhibitory activity against ASFV-Topo II. Doxorubicin (Fig. 4C), a classical anticancer drug, significantly inhibits the catalytic activity of ASFV-Topo II at concentrations as low as 1 μmol/L and induces DNA cleavage at higher concentrations, consistent with the dual mechanism of a typical topoisomerase poison[32]. Genistein (Fig. 4D), a naturally occurring isoflavone, competitively binds the ATP-binding site of ASFV-topo II by targeting key residues such as N144, V146, G147, and L148, thereby blocking its ATP-dependent catalytic activity. Single cell electrophoresis analysis confirmed that genistein induces fragmentation of the ASFV genome. *In vitro* assays demonstrated effective antiviral activity, with IC_{50} values of 13 μmol/L in Vero cells and 4.403 μmol/L in PAMs[53,54]. Similarly, Arctiin (Fig. 4E), a natural compound derived from *Bacillus subtilis* metabolites, also targets the ATP-binding pocket of ASFV Topo II and exhibited antiviral activity in PAM cells (IC_{50} = 4.139 μmol/L) without detectable cytotoxicity. Preliminary *in vivo* studies using oral administration further support its antiviral potential[54].

Additionally, several fluoroquinolone antibiotics have shown potential for repurposing as ASFV-Topo II inhibitors. Mottola et al.[55] screened 30 fluoroquinolones and identified six compounds (Enrofloxacin, Grepafloxacin, Balofloxacin, Tosufloxacin, Gatifloxacin, and Garenoxacin) (Fig. 4F−K) with potent anti-ASFV activity. After 7 days of treatment, ASFV-infected cells became PCR-negative for the A238L gene. Moreover, no residual infectivity was detected in the culture supernatants. Among them, enrofloxacin (100 μg/mL) induced marked DNA fragmentation as evidenced by TUNEL assay within 1 h of treatment[56]. Coumermycin A1 (Fig. 4L), a classical Topo II inhibitor, was found to inhibit ASFV Topo II activity at approximately 1 μmol/L. In addition, it effectively suppressed ASFV RNA synthesis *in vitro* at concentrations comparable to those used in studies targeting eukaryotic topo II, suggesting its potential to interfere with ASFV transcription through Topo II inhibition[40].

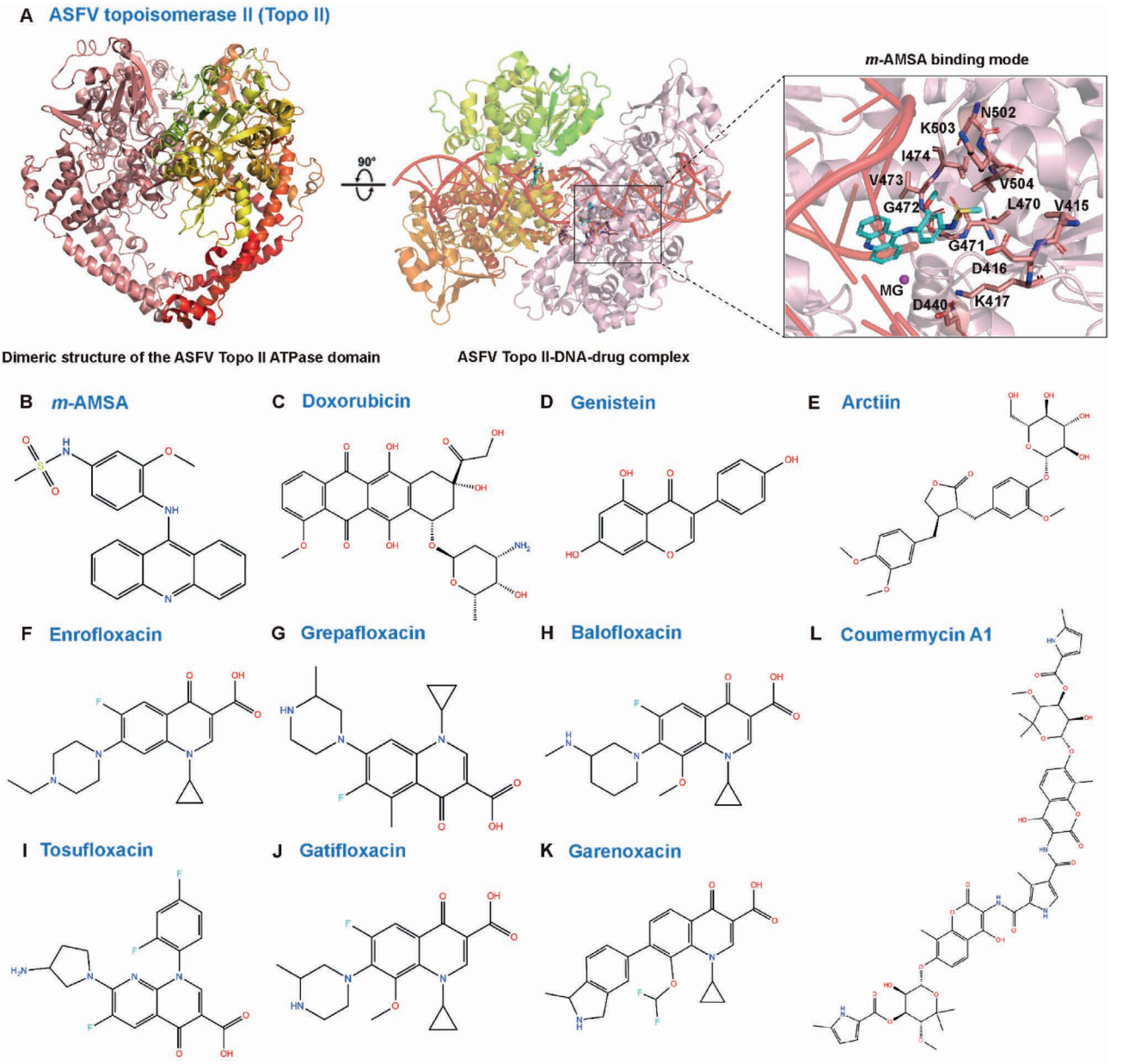

Figure 4 Tertiary structure of the ASFV Topo II and chemical structures of representative inhibitors. (A) Three-dimensional structure of ASFV Topo II dimer (PDB ID: 8KGO) and its complex with DNA and the inhibitor *m*-AMSA (PDB ID: 8J9X). The protein structure images were generated using PyMOL (version 3.1.4), based on the corresponding PDB files; (B−L) Chemical structures of representative small-molecule inhibitors targeting ASFV Topo II, including *m*-AMSA, Doxorubicin, Genistein, Arctiin, Enrofloxacin, Grepafloxacin, Balofloxacin, Tosufloxacin, Gatifloxacin, Garenoxacin, and Coumermycin A1. The compound structures were drawn using the 2D Sketcher in the academic version of Maestro (version 14.5).

2.6. Inhibitors targeting the DNA-binding protein pA104R

ASFV encodes a DNA-binding protein pA104R that acts in conjunction with Topo II to facilitate DNA folding and supercoiling, thereby ensuring the proper packaging of nucleoid-like structures and promoting efficient late-stage transcription[35]. pA104R is the only known histone-like protein encoded by a eukaryotic DNA virus, and is structurally and functionally homologous to the bacterial HU/IHF family of nucleoid-associated proteins[88]. In solution, pA104R forms a homodimer comprising an α-helix-rich region (AHR) and a β-strand-enriched DNA-binding region (BDR)[34,57] (Fig. 5A). It binds both single- and double-stranded DNA in an ATP-independent manner, primarily through electrostatic interactions between the negatively charged DNA backbone and positively charged residues in the BDR. Key residues involved in DNA binding include R69, H72, K92, R94, and K97, with K92, R94, and K97 contributing most significantly[57]. pA104R is highly conserved across ASFV genotypes and has been recognized as a novel virulence determinant[37]. As a result, multiple studies have focused on disrupting the pA104R−DNA interaction, leading to the identification of small-molecule inhibitors with validated anti-ASFV activity.

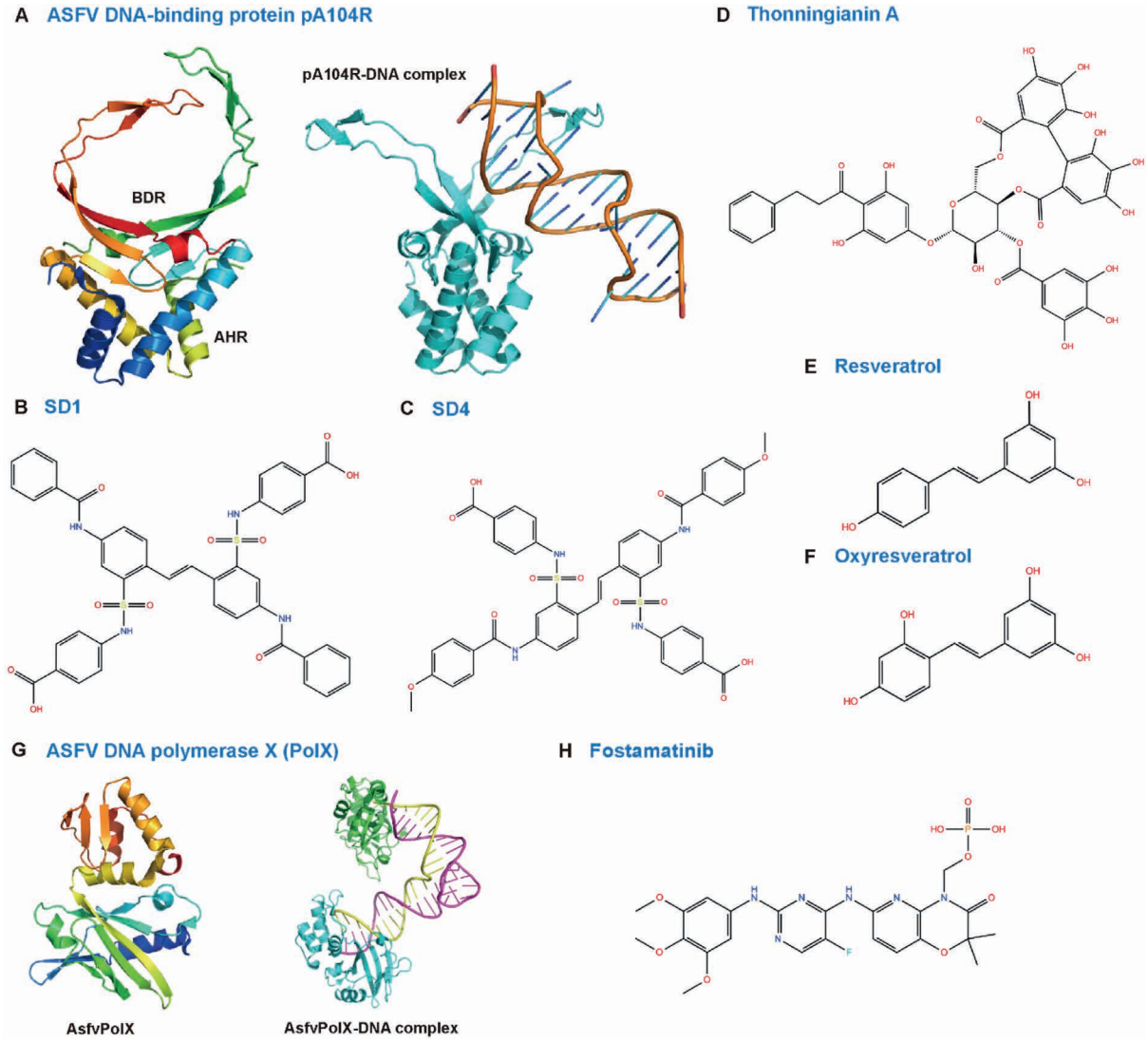

Figure 5 Tertiary structures of ASFV pA104R and PolX, along with their respective small-molecule inhibitors. (A) Crystal structure of ASFV pA104R (PDB ID: 9JS5) and its complex structure with DNA (PDB ID: 6LJB. The protein structure images were generated using PyMOL (version 3.1.4), based on the corresponding PDB files. (B−F) Chemical structures of representative small-molecule inhibitors targeting pA104R, including SD1, SD4, Thonningianin A, resveratrol, and oxyresveratrol. (G) Crystal structure of ASFV PolX (PDB ID: 1JQR) and its complex with DNA (PDB ID: 5XMA). (H) Chemical structure of fostamatinib, an inhibitor targeting ASFV PolX. The compound structures were drawn using the 2D Sketcher in the academic version of Maestro (version 14.5).

Among synthetic compounds, stilbene-derived inhibitors such as SD1 and SD4 (Fig. 5B and C) have shown selective binding to different regions of pA104R. SD1 interacts with the base of the BDR, while SD4 targets the arm region, effectively blocking the protein's interaction with viral DNA. Notably, SD4 exhibited superior inhibitory potency (IC_{50} = 0.48 μmol/L) and lower cytotoxicity (CC_{50} = 263 μmol/L), making it a favorable lead compound for further development[57]. In addition, natural products have shown great promise. Thonningianin A (Fig. 5D), a polyphenolic compound of natural origin, has been demonstrated to significantly disrupt pA104R−DNA binding. It forms hydrogen bonds with key DNA-interacting residues on pA104R (H100, K92, R94, and K97), thereby inhibiting its function. Thonningianin A suppresses ASFV replication in PAMs at extremely low concentrations (IC_{50} = 0.068 μmol/L) and displays excellent safety and selectivity (SI = 251)[34].

Furthermore, natural stilbenol compounds such as resveratrol and oxyresveratrol (Fig. 5E and F) have demonstrated potent antiviral activity against ASFV *in vitro*. Resveratrol completely inhibited viral replication at a non-cytotoxic concentration of 5 μg/mL, with an IC_{50} of 1 μg/mL and a CC_{50} of 30 μg/mL. Oxyresveratrol reduced viral replication by over 99% at 30 μg/mL, with an IC_{50} of 10 μg/mL and a CC_{50} exceeding 50 μg/mL, indicating a favorable therapeutic window. Both resveratrol and oxyresveratrol have been shown to interfere with ASFV factory formation, a crucial step in late-stage replication. While their precise mechanism in ASFV is yet to be fully understood, given that stilbenes inhibit the HU protein of *Mycobacterium tuberculosis*, a nucleoid-associated DNA-binding protein, it is likely that their effect on ASFV assembly involves interference with the DNA-binding activity of pA104R[58].

In summary, small-molecule inhibitors targeting the pA104R−DNA interface represent a promising antiviral strategy.

Compounds such as thonningianin A and SD4, characterized by well-defined mechanisms and potent antiviral activity, warrant further investigation and optimization.

2.7. Inhibitors targeting ASFV DNA polymerase X

Early ASFV genome replication events in the nucleus activate DNA damage response pathway. ASFV Pol X, a member of the X-family DNA polymerases, plays a crucial role in the repair of mutated viral DNA resulting from oxidative damage during ASFV replication. As an error-prone polymerase lacking a 3′−5′ exonuclease proofreading activity[89], ASFV PolX catalyzes the gap-filling reaction during viral genome repair *via* the base excision repair pathway[38,90]. Unlike other DNA polymerases, ASFV PolX consists of only a palm and a C-terminal subdomain. The N-terminal palm domain is composed of several β-sheets and three α-helices, one of which is unique to ASFV PolX and contributes to single-nucleotide gap DNA binding through electrostatic interactions with DNA *via* Lys residues. Its compact structure, well-defined active site, and essential roles in viral replication (Fig. 5G) make ASFV PolX an attractive target for structure-based antiviral drug design.

Choi et al.[59] have explored the integration of molecular docking and machine learning to identify novel inhibitors of ASFV PolX. By targeting the conserved 5′-phosphate (5′-P) binding site and analyzing multiple crystal structures, researchers applied advanced scoring calibration to account for structural diversity and improve hit reliability. Through integrative virtual screening, fostamatinib (Fig. 5H), an oral spleen tyrosine kinase inhibitor with well-established pharmacokinetics and clinical safety, emerged as a promising candidate for drug repurposing. *In vitro* polymerase activity assays confirmed that fostamatinib could significantly inhibit ASFV PolX catalytic activity in a dose-dependent manner, achieving up to 90% inhibition at 12.5 μmol/L[59].

Notably, although previous studies have shown that Brincidofovir (Fig. 2L) inhibits ASFV replication by interfering with viral DNA polymerase activity and destabilizing viral DNA. Molecular docking analyses by Guo et al.[51] further suggest that Brincidofovir may competitively and covalently bind to the R42 site of ASFV PolX, implicating a potential role in disrupting DNA repair or terminal gap-filling synthesis. Given the exceptionally potent antiviral activity observed in cellular assays, the inhibition of ASFV PolX alone is unlikely to fully account for its broad-spectrum efficacy. These findings indicate that Brincidofovir may exert its antiviral effect through multiple mechanisms, potentially involving additional viral or host targets. Further experimental validation is necessary to elucidate its precise mechanism.

2.8. Inhibitors of the viral cysteine protease pS273R

ASFV encodes cysteine protease, pS273R, which plays a pivotal role in the viral life cycle[91]. It is responsible for processing the viral polyprotein precursors pp220 and pp62, thereby generating structural proteins essential for viral core shell assembly. Structural studies have revealed that pS273R consists of two distinct domains: an N-terminal arm domain encompassing residues M1 to N83, and a core domain spanning residues N84 to A273 (Fig. 6A, left). The core domain harbors conserved catalytic residues, including cysteine and histidine, and its active-site pocket positions the catalytic triad (C232−H168−N187) in an orientation favorable for substrate cleavage (Fig. 6A, right)[39]. These structural and functional features establish pS273R as a promising therapeutic target for the development of ASFV-specific protease inhibitors.

Several small-molecule inhibitors targeting pS273R have shown promising progress (Fig. 6B−G). E-64, a well-known covalent inhibitor of cysteine proteases, binds to pS273R by forming a covalent C−S bond between its C14 carbon and the active site residue C232. The compound is stabilized in the active site through seven hydrogen bonds and effectively blocks the cleavage of pp62. At a concentration of 4 mmol/L, E-64 not only inhibits the enzymatic activity of pS273R but also induces the expression of immune-related cytokines, while exhibiting no significant cytotoxicity. These findings indicate that E-64 possesses both biosafety and antiviral potential[60]. Based on the structural features of the pS273R active site, Li et al.[39] designed the peptidomimetic aldehyde inhibitor ZW60 to mimic the consensus cleavage sites of the natural substrate, thereby disrupting ASFV polyprotein maturation. ZW60 forms a reversible covalent bond between its P1 aldehyde and the catalytic residue C232, while optimized small-volume polar and hydrophobic groups at P1−P4 enhance non-covalent interactions. Molecular docking revealed that ZW60 forms hydrogen bonds with residues L87, N89, N158, H168, W169, and Y121, and engages in hydrophobic interactions with F111 and M113. In contrast, control compounds ZW22 and ZW30, which bear bulkier side chains at the P1 and P2 positions, showed impaired hydrogen bonding with W169 and L87. Consistent with these findings, ZW60 exhibited the most potent inhibitory activity (IC_{50} = 0.900 ± 0.037 μmol/L), surpassing ZW30 (IC_{50} = 2.605 ± 0.145 μmol/L) and ZW22 (IC_{50} = 3.440 ± 0.144 μmol/L, highlighting the importance of steric compatibility for further optimization.

In addition to synthetic molecules, several natural products have demonstrated significant potential. Using a fluorescence resonance energy transfer-based screening system, Jo et al.[61] evaluated the inhibitory activity of ten flavonoid subclasses against pS273R[61]. Their results identified flavonols as the preferred scaffold, with activity largely dependent on the synergistic presence of a C3-hydroxyl group and a 3′,4′,5′-trihydroxy substitution pattern on the B-ring. Selective glycosylation at the C3 position further enhanced potency. For example, myricetin (IC_{50} = 8.4 μmol/L) showed superior activity compared to related flavonoids, underscoring the importance of the C3-OH. Its glycosylated derivative, myricitrin, exhibited greater inhibitory effects on protease activity (IC_{50} = 2.7 μmol/L), suggesting that glycosylation may enhance the antiviral properties of these compounds.

Furthermore, the defensin-like peptide OPTX-1 (Fig. 6H), derived from the soft tick *Ornithodoros papillipes*, demonstrated significant inhibitory activity against pS273R (K_i = 0.821 ± 0.526 μmol/L) and effectively suppressed ASFV replication in PAMs at concentrations of 5−10 μmol/L[62]. Docking studies reveal that peptide ligands are primarily recognized through a hydrogen-bond network within the active site of the pS273R core domain. Specifically, OPTX-1 engages in hydrogen bonding with key residues T164, S192, R224, and Q229. Mechanistically, OPTX-1 acts as a competitive inhibitor by binding to the active site of pS273R and disrupting its proteolytic function.

3. Host-targeted antiviral strategies against ASFV

While direct inhibition of viral proteins remains a central strategy in antiviral drug development, this approach can be limited by the emergence of resistant viral variants and the structural variability of viral targets. As a complementary strategy, increasing attention has been focused on targeting host factors that are essential for

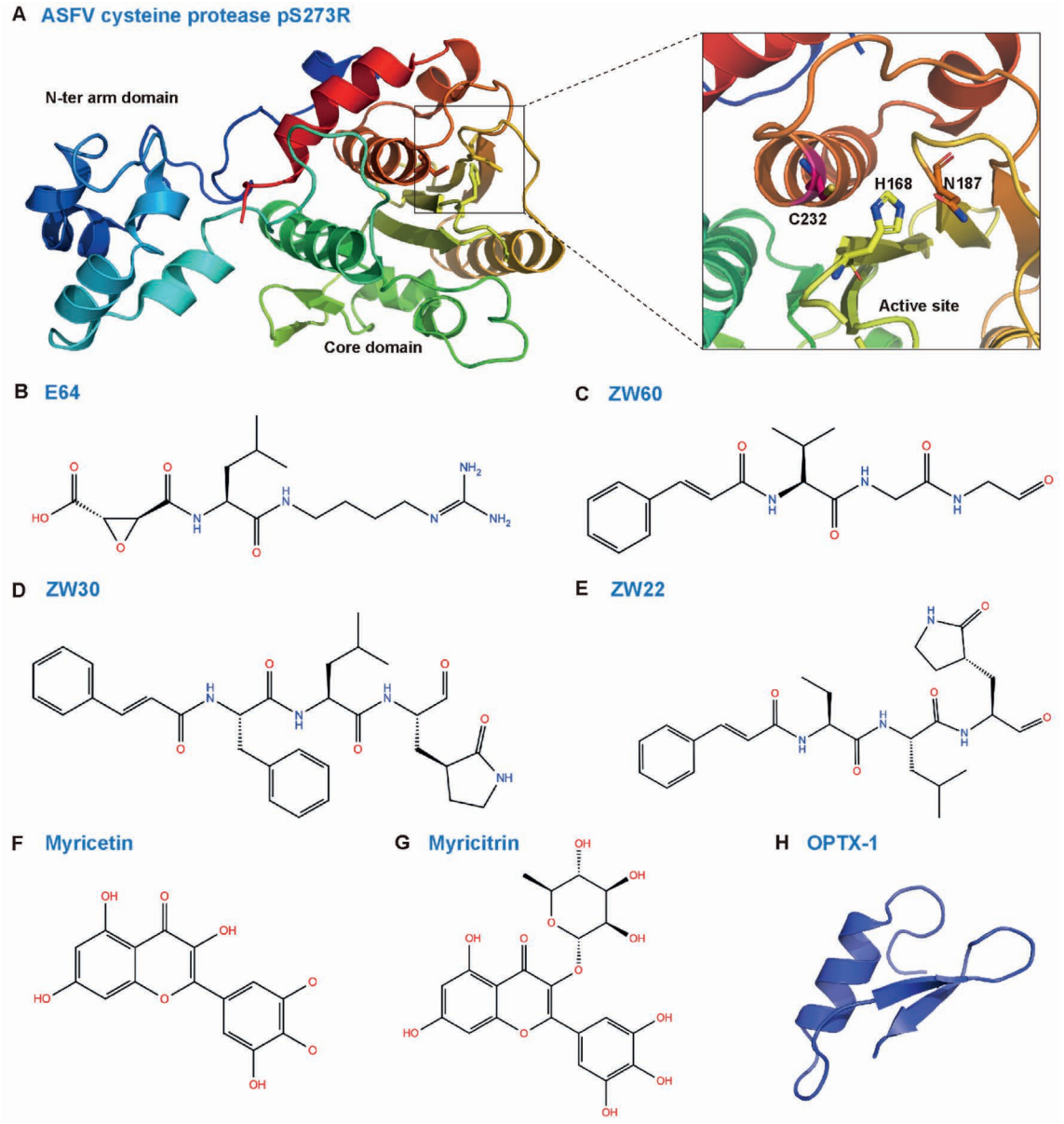

Figure 6 Tertiary structure of the ASFV cysteine protease pS273R and chemical structures of representative protease inhibitors. (A) Crystal structure of the ASFV cysteine protease pS273R, with the catalytic center prominently highlighted (PDB ID: 6LJB). The protein structure images were generated using PyMOL (version 3.1.4), based on the corresponding PDB files. (B−G) Chemical structures of representative small-molecule inhibitors targeting pS273R, including E64, ZW60, ZW30, ZW22, myricetin, and myricitrin. The compound structures were drawn using the 2D Sketcher in the academic version of Maestro (version 14.5). (H) Predicted 3D structure of the peptide inhibitor OPTX-1, generated using AlphaFold 3.

viral replication and genetically more stable. These host-directed therapies aim to disrupt critical virus−host interactions or modulate cellular pathways exploited by ASFV during infection, thereby offering broader antiviral efficacy and a reduced likelihood of resistance. The following section provides an overview of recent advances in host-targeted inhibitors with demonstrated or potential activity against ASFV (Table 2).

3.1. Intervention in lipid metabolism and membrane remodeling

In the context of host-targeted antiviral strategies against ASFV, modulation of lipid metabolism and membrane remodeling has emerged as a promising therapeutic approach. ASFV relies extensively on host lipid metabolic pathways to support essential stages of its life cycle, including envelope formation, replication complex assembly, and virion release[92,93]. Several small-molecule inhibitors targeting these processes can effectively suppress ASFV replication *via* well-defined molecular mechanisms, highlighting their potential for advancement as antiviral drug candidates (Fig. 7A−E).

Classic lipid synthesis inhibitors, including lauryl gallate (LG), valproic acid (VPA), and cerulenin (CRL), have demonstrated significant anti-ASFV activity by targeting host lipid metabolism pathways in Vero and COS cells. According to the study by de León et al.[63], VPA (IC_{50} = 2.5−3 mmol/L) and CRL (IC_{50} = 9 μmol/L) exhibit potent antiviral effects against the

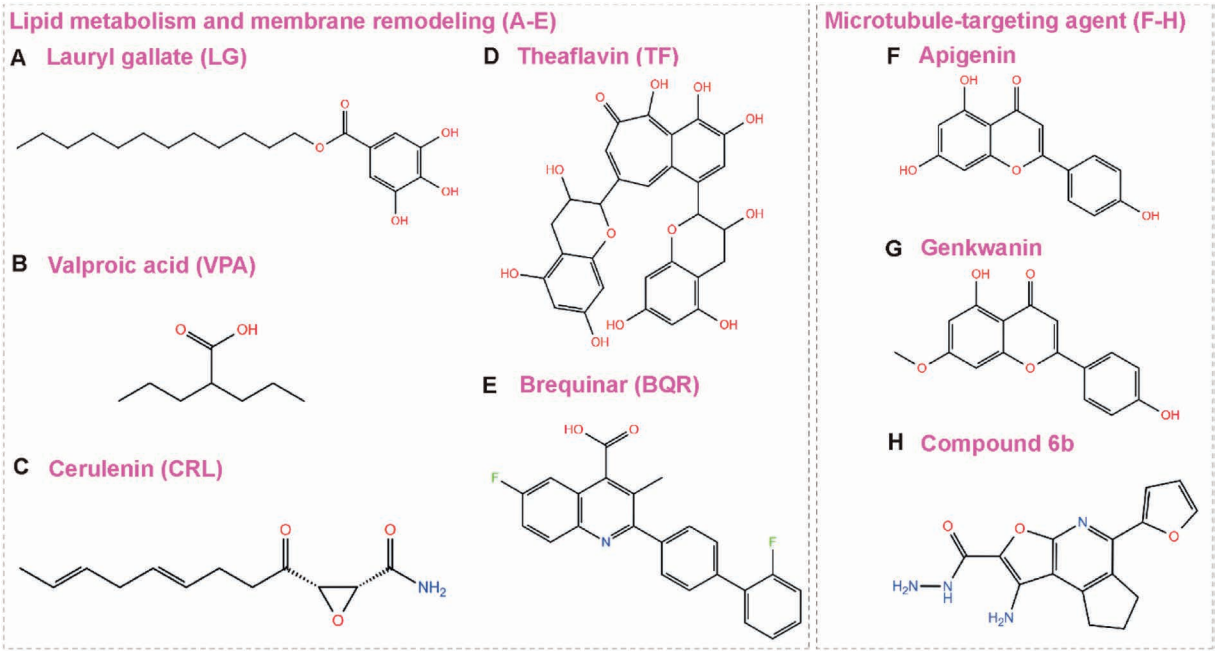

Figure 7 2D Structures of representative small-molecule inhibitors targeting host lipid Metabolism and microtubule dynamics. (A−E) Representative inhibitors that interfere with lipid metabolism and membrane remodeling. (F−H) Microtubule-targeting agents that disrupt cytoskeletal dynamics and intracellular trafficking. The compound structures were drawn using the 2D Sketcher in the academic version of Maestro (version 14.5).

ASFV Ba71V strain, particularly during the early phase of infection. These compounds are believed to interfere with lipid biosynthesis or membrane remodeling, thereby disrupting viral entry or early replication events. Interestingly, the antiviral activity of LG has been reported at different stages of the viral life cycle. Hurtado et al.[64] observed that LG primarily acts during the early stage of ASFV infection. In a Vero cell model, pre-treatment with LG 1 h prior to viral adsorption completely inhibited virus production within a single replication cycle, indicating that LG may block viral attachment or membrane fusion. Subsequent studies in PAMs further confirmed its efficacy, with an EC_{50} of 2−3 μmol/L and a CC_{50} of 30 μmol/L. In contrast, de León et al. [63] reported that LG exerts its antiviral effect mainly during the late stage of the ASFV life cycle, potentially by interfering with virion assembly or release. In this context, LG exhibited potent inhibitory activity, with IC_{50} values of 1 μmol/L in Vero cells and 0.4 μmol/L in COS cells.

Natural polyphenol theaflavin (TF) has also been validated for anti-ASFV efficacy. TF treatment greatly reduced viral p30 protein expression in a dose-dependent manner. At 80 μmol/L, TF lowered progeny virus titers by approximately 5 $\log_{10}$ units and suppressed *B646L* mRNA levels by over 90%[65]. Mechanistically, TF activates the host AMPK pathway, leading to the downregulation of lipid synthesis-related genes and a subsequent decrease in intracellular cholesterol and triglyceride levels, thereby inhibiting viral replication. This effect is mimicked by the AMPK agonist MK8722 and partially reversed by AMPK inhibitors, supporting a causal role for AMPK signaling. Overall, TF emerges as a promising lead targeting the AMPK-lipid metabolism axis against ASFV.

In addition, Brequinar (BQR), a dihydroorotate dehydrogenase (DHODH) inhibitor, exerts dual antiviral effects against ASFV through two distinct host-directed mechanisms[66]. First, it blocks *de novo* pyrimidine biosynthesis, leading to depletion of intracellular nucleotide pools required for viral replication[67]. This mechanism is supported by uridine-rescue experiments, in which exogenous uridine greatly reduces the antiviral activity of BQR. In addition, BQR induces ferroptosis, a regulated form of cell death associated with iron accumulation and lipid peroxidation, which further restricts viral propagation. Treatment with BQR increases total and mitochondrial Fe^{2+} levels and lipid peroxides, as shown by FerroOrange, Mito-FerroGreen, and Liperfluo staining. The partial restoration of viral replication by ferrostatin-1 further supports the involvement of ferroptosis in the antiviral activity of BQR. Notably, uridine does not reverse these ferroptotic changes, indicating that pyrimidine depletion and ferroptosis are independent but complementary pathways involved in the antiviral effect of BQR. *In vitro*, BQR exhibits potent anti-ASFV activity with low cytotoxicity. In PAMs, it shows a CC_{50} of 451.8 μmol/L and an EC_{50} of 21.95 μmol/L (SI = 20.58). In Vero cells, BQR displays greater potency, with an IC_{50} of 2.83 and $CC_{50} > 100$ μmol/L, indicating a high selectivity index[66,67]. Collectively, these studies highlight a framework for combining nucleotide biosynthesis blockade with modulation of lipid peroxidation-driven cell death, offering a promising direction for host-directed intervention focused on metabolic and membrane dynamics.

3.2. *Microtubule-targeting agents*

ASFV relies on microtubule-mediated intracellular transport throughout its life cycle, including viral entry, trafficking, assembly, and egress[94]. Following internalization and uncoating, the exposed viral core is transported *via* dynein-mediated retrograde movement along microtubules to the perinuclear microtubule-organizing center, where viral factories are established to facilitate genome replication and virion assembly. In the late phase of

infection, mature particles are recognized as kinesin cargo and undergo microtubule-dependent anterograde transport from perinuclear assembly sites to the plasma membrane for secondary envelopment and release. Disruption of these processes by microtubule-targeting compounds has demonstrated broad-spectrum antiviral potential. Recent studies have shown that various small molecules, including natural flavonoids and their derivatives (Fig. 7F−H), can inhibit ASFV replication by modulating microtubule dynamics, providing strong support for host-directed antiviral strategies.

Apigenin, a natural flavonoid, exhibits potent antiviral activity against ASFV, with significant reduction of viral titers observed in infected cells at a concentration of 50 μmol/L[68]. This effect is most pronounced when apigenin is administered within 1 h post-infection, resulting in a reduction of viral titer by over 99.99%, marked inhibition of virus-specific protein expression, and disruption of viral factory formation[68]. Choudhury et al.[95] reported that apigenin directly binds to tubulin and inhibits tubulin polymerization. Given that ASFV replication relies on the host cell microtubule network for virion assembly and intracellular transport, these findings suggest that apigenin may interfere with ASFV replication by disrupting microtubule polymerization and impairing the reorganization of the cytoskeletal framework required for efficient viral trafficking. Genkwanin, a methylated derivative of Apigenin, offers improved aqueous solubility and cellular efficacy. It significantly inhibits ASFV replication at 2.9 μmol/L, with SI of 205.2, and markedly reduces viral DNA synthesis and protein expression. Time-of-addition studies suggest that Genkwanin interferes with both viral entry and release, indicating its potential as a microtubule-dependent antiviral agent, although its precise molecular target remains to be elucidated[69]. Compound **6b,** a novel microtubule-targeting molecule identified through structure-based virtual screening of the colchicine-binding domain of tubulin, also shows potent anti-ASFV activity. In Vero cells, 6b inhibits ASFV infection in a dose-dependent manner (IC_{50} = 19.5 μmol/L, CC_{50} > 500 μmol/L), reflecting low cytotoxicity. Mechanistic studies demonstrate that compound **6b** interferes with multiple stages of the ASFV life cycle, including viral attachment, internalization, and egress. Further investigations reveal that **6b** promotes microtubule polymerization within cells, functioning as a microtubule-stabilizing agent rather than a conventional destabilizer[70]. These findings underscore the therapeutic potential of microtubule-targeting compounds as part of host-directed antiviral strategies against ASFV.

3.3. *Host transcriptional and epigenetic regulators*

ASFV relies on host transcriptional machinery and epigenetic environment to support its gene expression and replication[96,97]. Among various epigenetic modifications, histone lysine acetylation plays a central role in regulating chromatin accessibility and transcriptional activity during infection[98]. Recent studies have revealed that ASFV can exploit multiple host epigenetic regulatory pathways, notably those involving histone deacetylases (HDACs) and the bromodomain and extraterminal domain (BET) protein family, to reprogram the host chromatin landscape in ways that promote viral replication.

HDACs catalyze the removal of acetyl groups from histone lysine residues, leading to chromatin condensation and transcriptional silencing of host antiviral genes. Simões et al.[99] reported that ASFV recruits HDAC1, HDAC2, and HDAC3 to viral factories to induce nuclear heterochromatinization, thereby repressing host immune and apoptotic pathways. In line with this mechanism, Frouco et al.[71] evaluated several HDAC inhibitors and identified sodium phenylbutyrate (NaPB) (Fig. 8A), as a potent antiviral agent. NaPB completely suppressed ASFV replication in Vero cells at 5 mmol/L without detectable cytotoxicity, markedly

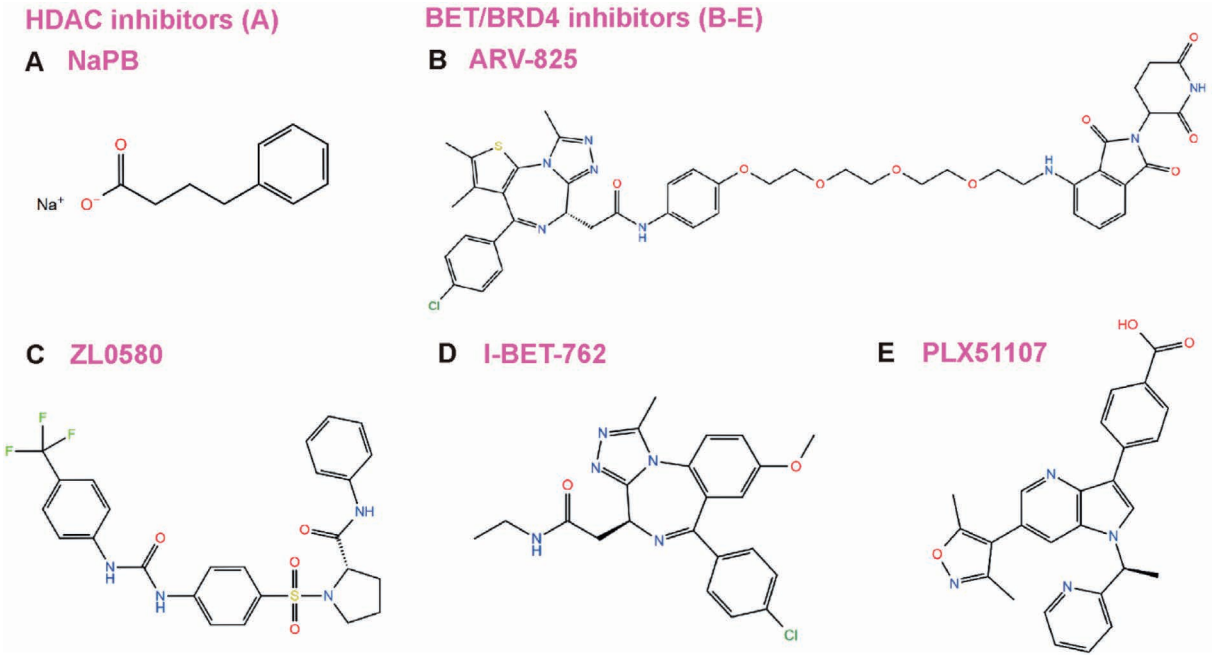

Figure 8 2D Structures of representative epigenetic modulators targeting host chromatin regulation. (A) HDAC inhibitor NaPB; (B−E) BET/BRD4 inhibitors that block acetyl-lysine recognition and transcriptional activation. The compound structures were drawn using the 2D Sketcher in the academic version of Maestro (version 14.5).

reducing the expression of structural proteins Vp72 and Vp54. Mechanistic studies further showed that ASFV infection induces hypoacetylation of histone H3 at lysines 9 and 14, which is associated with chromatin compaction and transcriptional repression. Treatment with NaPB effectively reversed this virus-induced hypoacetylation, restored an open chromatin state, and reactivated host gene expression programs unfavorable to viral replication. Furthermore, NaPB exhibited synergistic antiviral activity when combined with enrofloxacin (25 μg/mL), a topoisomerase II inhibitor. The combination completely abolished ASFV replication at a sub-effective concentration of NaPB (2.5 mmol/L), whereas either agent alone led to only a partial reduction in viral titers.

In parallel, the BET protein family, including BRD2, BRD3, BRD4, and BRDT, acts as a group of epigenetic readers that recognize acetylated histones through their two bromodomains (BD1 and BD2), thereby facilitating the recruitment of transcription factors and promoting transcriptional activation and chromatin remodeling. Recent studies have shown that ASFV hijacks BRD4 by redirecting it from host chromatin to the viral genome, thereby enhancing viral replication and gene transcription[100]. Zhao et al.[72] evaluated 12 BET inhibitors in PAMs and identified four representative compounds, including ARV-825, ZL0580, I-BET-762, and PLX51107 (Fig. 8B−E), potently suppressed ASFV replication within low-toxicity ranges. The corresponding CC_{50} values were 10.11, 25.3, 35.86, and 19.37 μmol/L, respectively, with maximal antiviral testing concentrations of 1, 10, 10, and 5 μmol/L. Mechanistically, I-BET-762 and PLX51107 act as pan-BET inhibitors by occupying the acetyl-lysine recognition pocket of bromodomains, thereby preventing their binding to acetylated histones and blocking transcriptional activation required for ASFV replication. ZL0580 displays strong selectivity for the BD1 domain of BRD4 (approximately 6.6-fold higher than BD2) and has been shown in HIV systems to inhibit transcription by disrupting BRD4-mediated assembly. ARV-825 functions as a BRD4-targeting PROTAC that induces cereblon-mediated proteasomal degradation, effectively removing this essential transcriptional coactivator. The established clinical utility of BET inhibitors in oncology further supports their translational potential in ASFV control.

3.4. *S-Adenosylhomocysteine (AdoHcy) hydrolase inhibitors*

AdoHcy hydrolase is a mechanistically distinct host target for anti-ASFV therapy. This enzyme catalyzes the hydrolysis of AdoHcy, a byproduct generated during *S*-adenosylmethionine-dependent methylation reactions and plays a critical role in maintaining intracellular methylation homeostasis[101]. Inhibition of AdoHcy hydrolase leads to the accumulation of AdoHcy, which functions as a potent feedback inhibitor of various methyltransferases. This accumulation disrupts key methylation-dependent processes, particularly the 5′-cap methylation of viral mRNAs, ultimately impairing viral gene expression and replication[102].

Several adenosine analogs (Fig. 9A−E) designed to inhibit AdoHcy hydrolase have demonstrated potent anti-ASFV activity with favorable selectivity profiles *in vitro*, making them promising candidates for host-targeted antiviral development[73]. Among

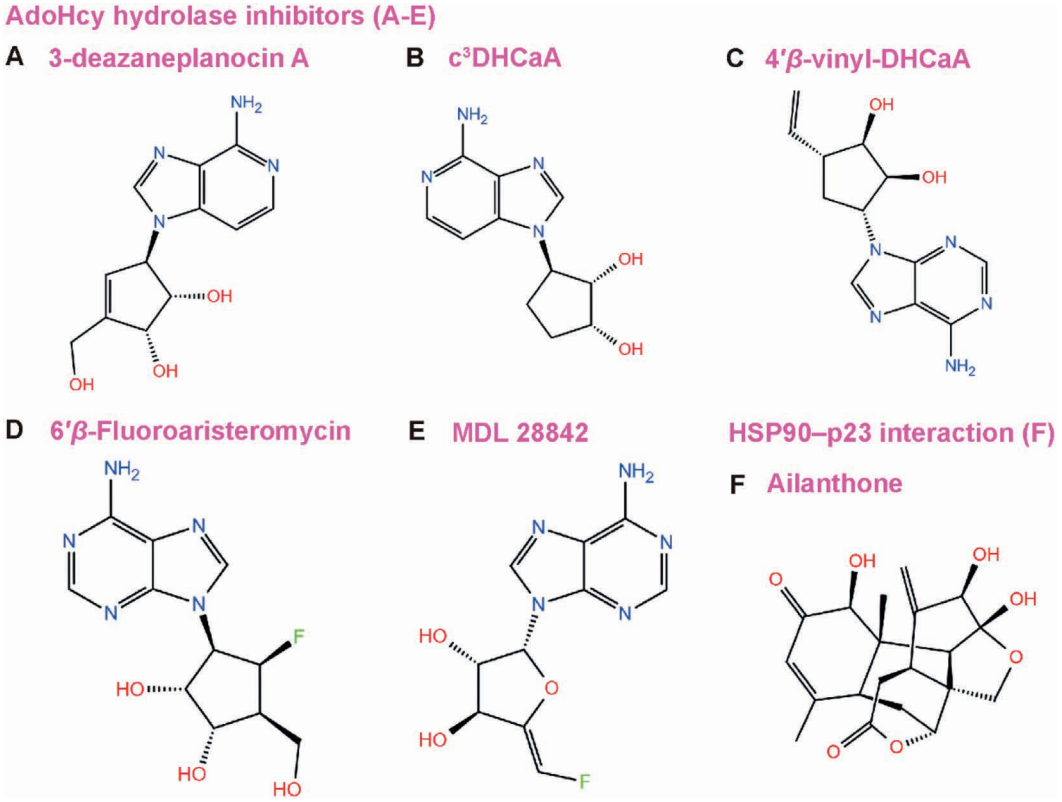

Figure 9 2D Structures of representative small-molecule inhibitors targeting host metabolic and chaperone pathways. (A−E) AdoHcy inhibitors that disrupt methylation-dependent nucleotide metabolism; (F) Molecular chaperone inhibitor targeting the HSP90−p23 interaction, thereby interfering with chaperone-assisted viral protein maturation. The compound structures were drawn using the 2D Sketcher in the academic version of Maestro (version 14.5).

them, 3-deazaneplanocin A is the most potent compound identified to date. In Vero cells, it exhibits an EC_{50} of 0.01 μg/mL, a CC_{50} of 30 μg/mL, and a SI of 3000, reflecting an exceptional therapeutic window. Other structurally related adenosine analogs with notable anti-ASFV activity include: c^3DHCaA (EC_{50} = 0.08 μg/mL, CC_{50} = 200 μg/mL, SI = 2500), 4′β-vinyl-DHCaA (EC_{50} = 0.1 μg/mL, CC_{50} = 200 μg/mL, SI = 2000), 6′β-fluoroaristeromycin (EC_{50} = 0.008 μg/mL, CC_{50} = 10 μg/mL, SI = 1250), and MDL 28842 (EC_{50} = 0.03 μg/mL, CC_{50} = 20 μg/mL, SI = 667)[73]. Structure−activity relationship analysis shows that 3-deaza modification greatly improves selectivity, likely by altering the electronic properties of the base. Both 3-deazaneplanocin A and c^3DHCaA display much higher selectivity indices compared to Neplanocin A (SI = 66). Within the carbocyclic dihydroxycyclopentyl scaffold, incorporation of either a 4′-vinyl or 6′-fluoro substituent leads to marked enhancements in antiviral potency and selectivity. In contrast, compounds bearing a 5′-fluoro group combined with unsaturation, such as MDL-28842, show only moderate selectivity. These compounds act as competitive inhibitors of AdoHcy hydrolase by mimicking the adenosine scaffold, thereby blocking AdoHcy catabolism and leading to its intracellular accumulation, which in turn disrupts viral mRNA methylation and ultimately impairs ASFV gene expression and replication.

3.5. *Molecular chaperone inhibitors*

Viruses depend entirely on host machinery for protein synthesis, genome replication, and virion assembly. The production of viral proteins places unique demands on the host proteostasis networks, requiring rapid and accurate folding of multifunctional, aggregation-prone proteins[103]. HSP90 is a highly conserved chaperone in eukaryotic cells and supports the folding and maturation of hundreds of client proteins. The cochaperone p23 binds the N-terminal region of HSP90 and stabilizes the closed conformation, which promotes client maturation and stability[104]. In ASFV, the HSP90−p23 complex has been identified as a key host factor essential for viral replication. Targeting this chaperone machinery is a feasible host-directed antiviral strategy.

Using a luciferase-based screening system driven by the p72 promoter, Zhang et al.[74] identified Ailanthone (AIL) (Fig. 9F), a quassinoid derived from *Ailanthus altissima*, as a potent and low-toxicity candidate inhibitor against ASFV. In PAMs, AIL inhibited ASFV-eGFP replication with an IC_{50} of 15 nmol/L, and a CC_{50} greater than 613 nmol/L, corresponding to a high selectivity index (SI > 41). Comparable antiviral potency was observed in Vero-E6 cells, with an IC_{50} of 12 nmol/L. Mechanistically, AIL has been reported to bind p23, disrupt the interaction between p23 and HSP90, and promote degradation of HSP90 client proteins[74]. Consistently, overexpression of p23 in the p72-luciferase reporter assay partially rescued the inhibitory effect of AIL. These findings indicate that the HSP90−p23 interaction represents a critical host dependency for ASFV replication and a promising target for host-directed antiviral intervention.

3.6. *Host innate immune-modulating small-molecule inhibitors*

Host innate immune responses play a pivotal role in ASFV replication and pathogenesis[105,106]. ASFV engages and manipulates immune regulatory pathways, including pro-inflammatory signaling and programmed cell death, to facilitate immune evasion and enhance viral propagation[107,108]. As a result, targeting these host pathways using small-molecule modulators has

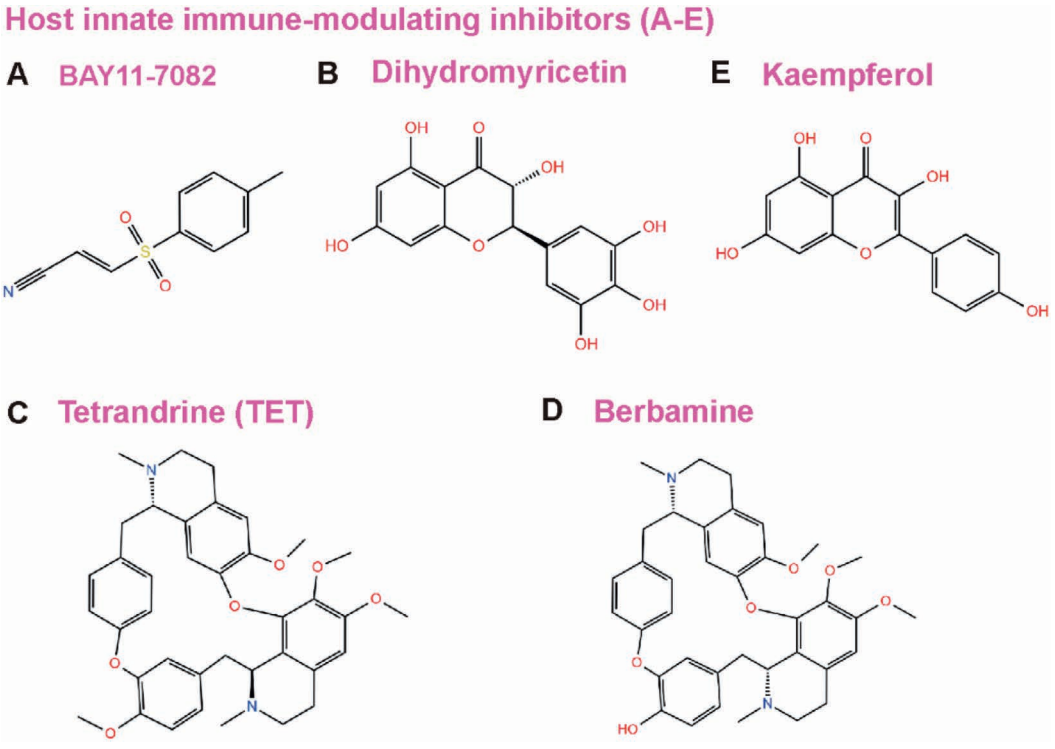

Figure 10 2D Structures of representative host innate immune-modulating small-molecule Inhibitors. (A−D) Small-molecule inhibitors targeting inflammatory signaling pathways; (E) Autophagy pathway activator that enhances host antiviral responses. The compound structures were drawn using the 2D Sketcher in the academic version of Maestro (version 14.5).

emerged as a promising antiviral strategy. An increasing number of small-molecule inhibitors and natural compounds have been identified that effectively modulate these pathways and suppress ASFV infection, highlighting the therapeutic potential of host-targeted immune regulation.

3.6.1. Small-molecule inhibitors targeting inflammatory pathways

Excessive or dysregulated inflammatory responses during viral infection can contribute to host tissue damage and facilitate viral replication. Accordingly, targeting key inflammatory signaling pathways has emerged as a promising strategy for host-directed antiviral intervention. ASFV activates the NF-κB and TLR4 pathways to induce the production of inflammatory cytokines and promote a cellular environment conducive to viral replication[109].

BAY11-7082 (Fig. 10A), a classical NF-κB pathway inhibitor, suppresses ASFV-induced inflammatory responses and viral replication in PAMs. ASFV infection activates the NF-κB pathway, as evidenced by increased levels of phosphorylated p65, phosphorylated IκB, MyD88, and elevated expression of pro-inflammatory cytokines IL-1β and IL-8. Treatment with 10 μmol/L BAY11-7082 significantly attenuates this inflammatory response by inhibiting NF-κB activation and reducing cytokine expression. At the same time, BAY11-7082 decreases transcription of the viral structural gene B646L, downregulates the early viral protein p30, and lowers progeny virus titers, indicating its dual role in modulating host inflammation and restricting ASFV replication[75].

Dihydromyricetin (DHM) (Fig. 10B), a natural flavonoid, exhibits potent anti-ASFV activity with an EC_{50} of 20.06 μmol/L, a CC_{50} of 532.1 μmol/L, and a selectivity index of 26.53. Mechanistically, DHM modulates the TLR4−MyD88−MAPK−NF-κB signaling pathway, suppresses the reactive oxygen species accumulation, and inhibits NLRP3 inflammasome activation, thereby reducing pyroptosis[76]. Functional validation shows that the TLR-4 agonist RS09 partially reverses the antiviral effect of DHM, whereas the TLR4 inhibitor resatorvid and TLR4-targeted siRNA further enhances its inhibitory activity. These results support that DHM inhibits ASFV mainly by modulating TLR4-mediated inflammation and pyroptosis, highlighting TLR4 signaling as a promising host-directed antiviral target[76].

Tetrandrine (TET) (Fig. 10C), a bis-benzylisoquinoline alkaloid, exhibits potent antiviral activity with IC_{50} values of 2.2 μmol/L in Vero cells and 3.0 μmol/L in PAMs[77]. Mechanistically, TET the PI3K/Akt signaling pathway and suppresses macropinocytosis-mediated endocytosis, thereby blocking viral entry[78]. In addition to impairing viral entry, emerging evidence suggests that TET also modulates host inflammatory responses. Specifically, Jackman reported that TET downregulates early-phase expression of proinflammatory cytokines such as TNF-α and IL-6, thereby dampening excessive inflammatory signaling that may facilitate viral replication. Similarly, berbamine (Fig. 10D), another natural anti-inflammatory compound, demonstrates comparable antiviral potency and anti-inflammatory capacity, with IC_{50} values of 8.4 μmol/L in Vero cells and 11.6 μmol/L in PAMs[77]. By suppressing virus-induced inflammatory cytokine responses, tetrandrine and berbamine demonstrate their potential as host-directed antiviral agents targeting inflammatory pathways in ASFV infection.

3.6.2. Autophagy pathway activators

Autophagy is an evolutionarily conserved catabolic process that maintains cellular homeostasis through the degradation and recycling of intracellular components. In addition to its fundamental physiological functions, autophagy acts as an intrinsic antiviral defense mechanism by targeting invading pathogens for lysosomal degradation[110]. Pharmacological activation of autophagy has thus emerged as a promising host-directed antiviral strategy against ASFV[111].

Kaempferol (Fig. 10E), a naturally occurring plant flavonoid, has been identified as a potent autophagy activator with anti-ASFV activity[79]. In a cell-based screen of 90 flavonoids, kaempferol demonstrated the strongest inhibitory effect against the ASFV Ba71V strain in Vero cells. At 20 μg/mL (∼70 μmol/L), kaempferol reduces viral titer by 1.26 $\log_{10}$, with an IC_{50} of 2.2 μg/mL (7.7 μmol/L) and a SI of 42.3, reflecting excellent antiviral specificity and low cytotoxicity[79]. Time-of-addition assays revealed that kaempferol interferes with both entry and post-entry stages of the viral replication cycle. Mechanistically, kaempferol was shown to induce autophagy in ASFV-infected cells, and its antiviral activity was partially reversed by co-treatment with autophagy inhibitors, confirming the involvement of autophagy as a key antiviral mechanism[79].

3.7. Endogenous innate immune restriction factors against ASFV

While Section 3.6 focuses on small-molecule inhibitors that modulate host innate immune signaling pathways, this section emphasizes the endogenous effectors of the innate immune system, namely interferons (IFNs) and their downstream interferon-stimulated genes (ISGs), which function as intrinsic antiviral defenses against ASFV[105]. The interferon system, particularly type I and type III IFNs, plays a pivotal role in initiating antiviral responses[112], and the induction of ISGs forms a key mechanism for limiting ASFV replication and spread.

In porcine-derived cells, both bovine IFN-α11 and porcine IFN-γ significantly inhibit ASFV replication in monocytes and alveolar macrophages[113]. Notably, IFN-γ demonstrates the strongest inhibitory effect in macrophages, accompanied by a marked reduction in the synthesis of late viral proteins, suggesting a mechanism linked to the suppression of viral DNA polymerase activity. In Vero cells, human IFN-α and IFN-γ also exhibit synergistic antiviral effects, with the ability to clear both lytic and persistent ASFV infections[114].

Interferon stimulation leads to the activation of multiple host restriction factors, which exert broad-spectrum antiviral effects against ASFV. Although Myxovirus resistance protein A (MxA) is classically known for its role in inhibiting RNA viruses, it has also been shown to be effective against ASFV. Overexpression of MxA results in a 100-fold reduction in ASFV replication and inhibition of late viral protein synthesis. Immunolocalization studies reveal that MxA accumulates around ASFV viral factories, suggesting that its antiviral activity may involve interference with viral assembly or morphogenesis[115]. Another group of key IFN-induced restriction factors, the interferon-induced transmembrane proteins (IFITMs), particularly IFITM1, 2, and 3, suppress ASFV infection by disrupting the fusion between the virus and endosomal membranes. Among them, IFITM2 has demonstrated the most potent inhibitory effect, blocking viral endocytosis, uncoating, and cholesterol efflux, thereby preventing release of the viral core into the cytoplasm[116].

These findings provide a theoretical basis for the development of immune-based antiviral strategies. Future research may further integrate these host factors with small-molecule antivirals and

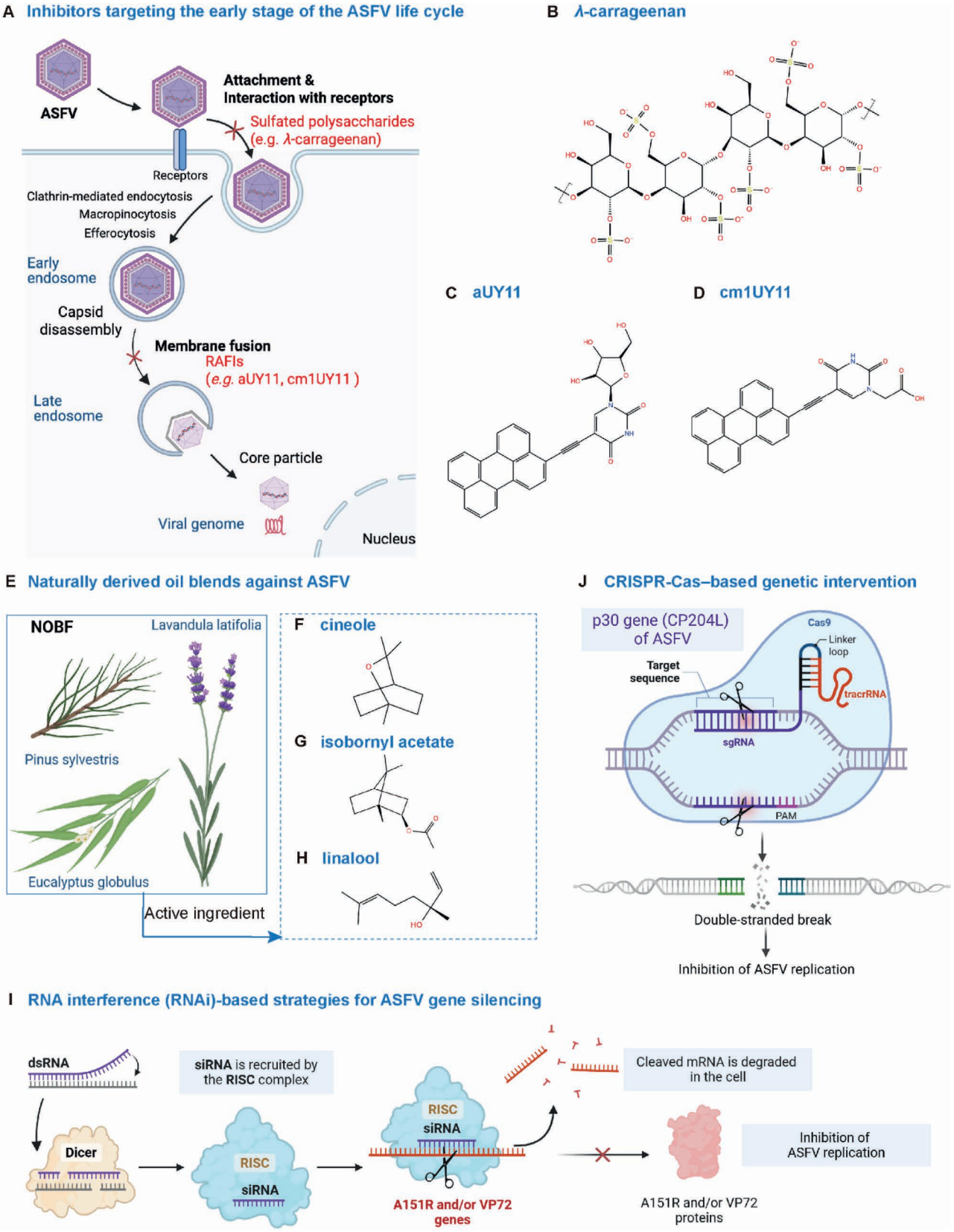

Figure 11 Target-agnostic and multifaceted antiviral strategies against ASFV. (A) Inhibitors targeting the early stage of the ASFV life cycle; (B) Chemical structure of lambda-carrageenan, a potent inhibitor of viral attachment. (C, D) Chemical structure of aUY11 and cm1UY11, rigid amphipathic fusion inhibitors targeting viral membrane fusion; (E) NOBF, a blend of essential oils from *Eucalyptus globulus*, *Pinus sylvestris*, and *Lavandula latifolia* in equal proportions (1:1:1). (F−H) Chemical structures of key antiviral constituents within NOBF. (I) Schematic

Table 3 Summary of antiviral targeting the early stage of the ASFV life cycle.

No.	Name	Category	Mechanism	Antiviral activity (against ASFV unless otherwise noted)	Development stage (in ASFV)	Ref.
66	lambda-carrageenan	Sulfated polysaccharides	Inhibits viral attachment	EC_{50} = 25 μg/mL, CC_{50} = 3000 μg/mL (Vero)	*In vitro*	117
67	aUY11	Rigid amphipathic fusion inhibitors	Blocks fusion of viral and endosomal membranes	IC_{50} = 1.6 μmol/L, SI = 78.8 (Vero)	*In vitro*	119
68	cm1UY11	Rigid amphipathic fusion inhibitors	Blocks fusion of viral and endosomal membranes	IC_{50} = 2.3 μmol/L, SI = 69.7 (Vero)	*In vitro*	119

vaccine platforms to establish a more comprehensive and robust antiviral defense system against ASFV.

4. Target-agnostic and multifaceted antiviral strategies against ASFV

While previous sections have focused on inhibitors that directly target viral or host proteins essential for ASFV replication, increasing attention has been directed toward alternative strategies that do not rely on defined molecular targets. These target-agnostic and multifaceted approaches encompass a diverse array of interventions, including physical inactivation, early-stage life cycle disruption, and gene-level interference, that act through broad or poorly defined mechanisms (Fig. 11). These modalities expand the antiviral arsenal beyond classical protein-targeted paradigms and provide versatile tools for integrated ASFV prevention and control.

4.1. Inhibitors targeting the early stage of the ASFV life cycle

The early phase of ASFV infection, including viral adsorption, membrane fusion, and initiation of replication, is critical for establishing successful infection[1,15]. Several compounds (Fig. 11A) with undefined molecular targets but well-characterized stage-specific activities have shown promise in disrupting these early steps, offering a non-classical yet mechanistically clear approach to ASFV control.

Sulfated polysaccharides, such as lambda-carrageenan, pentosan polysulfate, and kappa carrageenan, exhibit potent antiviral activity primarily by inhibiting viral attachment. Among them, lambda-carrageenan (Fig. 11B) demonstrated the strongest efficacy with an EC_{50} of 25 μg/mL, CC_{50} of 3000 μg/mL, and a SI of 120 in Vero cells[117]. Similarly, studies have shown that aqueous extracts from *P. cruentum*, *C. autotrophica*, and *Ellipsoidon* sp. exhibit significant, dose-dependent inhibitory effects on ASFV replication *in vitro*. The antiviral activity of these marine microalgal extracts is likely mediated by sulfated polysaccharides that interfere with early stages of the viral life cycle[118]. However, the precise molecular targets and underlying mechanisms remain to be fully elucidated.

Rigid amphipathic fusion inhibitors, such as aUY11 and cm1UY11 (Fig. 11C and D), represent a novel class of nucleoside-derived antiviral agents that act by targeting viral membrane fusion. These compounds integrate into the viral envelope and prevent fusion with host endosomal membranes, thereby effectively blocking viral entry. In Vero cells, both aUY11 and cm1UY11 exhibited potent, dose-dependent antiviral activity, with IC_{50} values of 1.6 and 2.3 μmol/L, and SI of 78.8 and 69.7, respectively[119] (Table 3). As two of the few small molecules currently known to directly interfere with early ASFV infection events and viral membrane fusion, aUY11 and cm1UY11 represent lead compounds for the development of novel mechanism-based antiviral agents targeting ASFV.

4.2. Inhibitory effects of naturally derived oil blends against ASFV

The Natural Oil Blend Formulation (NOBF) is a volatile plant-derived mixture composed of essential oils extracted from *Eucalyptus globulus*, *Pinus sylvestris*, and *Lavandula latifolia* (Fig. 11E). Recent studies have demonstrated that NOBF exhibits significant anti-ASFV activity both *in vitro* and *in vivo*[21,22].

In PAMs, NOBF, when diluted at 1:13 (approximately 0.000625 mL), effectively inhibited replication or directly inactivated high-titer ASFV strains (10^5 HAD_{50}/mL) without observable cytotoxicity[21]. Quantitative real-time PCR revealed consistently high C_t values in treated samples, indicating suppression of viral replication. In parallel, the infectious viral load in the culture supernatant was markedly reduced, suggesting that NOBF may act by directly disrupting viral particles or by interfering with virus−host cell interactions at early stages of infection[21]. More importantly, *in vivo* prophylactic administration of NOBF *via* drinking water (80 mL/ton) significantly reduced ASFV infection and transmission in swine[22].

Mechanistic studies have shown that NOBF exhibits multifaceted antiviral activity against ASFV. These include direct viral inactivation, disruption of early virus−host cell interactions, inhibition of viral replication kinetics, and enhancement of host immune responses. Gas chromatography (GC) analysis identified several key antiviral constituents within NOBF, namely cineole, linalool, and isobornyl acetate (Fig. 11F−H)[21]. These compounds are well-recognized for their membrane-disruptive, anti-inflammatory, and immunomodulatory properties, which may act synergistically to confer the observed antiviral effects. Given its low cytotoxicity, broad-spectrum antiviral potential, and demonstrated field efficacy, NOBF represents a promising natural antiviral

representation of RNAi-based strategies for silencing ASFV genes; (J) Overview of CRISPR-Cas-based genetic intervention strategies against ASFV. Panels A, E, I and J of Figure were created in BioRender. Li, Q. (2025) https://BioRender.com/110b9qq.

candidate for integration into ASFV prevention and control strategies.

4.3. RNA interference (RNAi)-based strategies for ASFV gene silencing

RNAi is a conserved post-transcriptional gene silencing mechanism in eukaryotic cells, mediated by small interfering RNAs (siRNAs) that direct the sequence-specific degradation of target mRNAs[120]. In a recent study, siRNAs were designed to target two critical ASFV genes: the major capsid protein gene VP72 (B646L) and A151R, a gene of unknown function but essential for viral replication (Fig. 11I). Silencing of these genes using siRNA resulted in a substantial reduction in viral titers, by up to 4 $\log_{10}$, and a corresponding 3 $\log_{10}$ decrease in viral mRNA levels, demonstrating that RNAi can effectively interfere with multiple stages of the ASFV replication cycle[24]. Although the combination of multiple siRNAs did not significantly improve efficacy over single agents, individual, highly potent siRNAs were sufficient to achieve robust viral suppression. These findings highlight the potential of siRNA-based strategies for efficient, sequence-specific inhibition of ASFV, and provide a strong theoretical foundation for the development of RNAi-based antiviral therapeutics targeting large DNA viruses.

4.4. CRISPR-Cas-based genetic intervention against ASFV

The CRISPR-Cas9 system, a precise and efficient genome-editing platform, has recently gained attention as a potential antiviral tool[121,122]. Hübner et al.[25] first demonstrated its efficacy by engineering a wild boar lung cell line to stably express Cas9 endonuclease and a guide RNA (gRNA) targeting codons 71−78 of the ASFV p30 gene (CP204L), an early structural protein involved in viral entry and immune evasion (Fig. 11J). CRISPR-mediated cleavage of this region led to complete abrogation of viral plaque formation and a $\sim$4 $\log_{10}$ reduction in viral titer, indicating potent and sequence-specific suppression of viral replication. Importantly, stable Cas9-gRNA expression did not compromise host cell viability, and antiviral activity was maintained over at least 50 passages, highlighting its genetic stability and biosafety. However, this single site targeting strategy was prone to escape mutations at the gRNA binding site, limiting its effectiveness against variant ASFV strains.

To address this limitation, Zheng et al.[122] conducted a comprehensive comparative analysis of all publicly available ASFV genomes and designed a multiplexed CRISPR-Cas9 system incorporating six gRNAs targeting nine conserved loci across the viral genome. The system was shown to effectively suppress ASFV replication *in vitro*. Furthermore, the researchers generated germline-edited pigs that constitutively express the multiplexed CRISPR-Cas9 components. In infection studies, these transgenic pigs exhibited delayed onset of ASF symptoms, suggesting a partial inhibitory effect on viral replication. However, no significant survival advantage was observed under high-dose viral challenge, likely due to insufficient *in vivo* expression of Cas9 and gRNAs. These findings underscore the importance of optimizing expression efficiency and gRNA delivery to enhance antiviral efficacy *in vivo*.

Collectively, these studies highlight the potential of CRISPR-Cas-based strategies for ASFV control while emphasizing the necessity for multi-site targeting and transgenic system refinement to overcome viral escape and achieve effective protection *in vivo*.

4.5. Antiviral potential of silver nanoparticles (AgNPs) against ASFV

With the growing application of nanomaterials in biomedicine, AgNPs have emerged as promising broad-spectrum antimicrobial agents[123,124]. Dung et al.[23] systematically evaluated AgNPs in the context of ASFV, highlighting their dual utility in cell-based viral inhibition and environmental disinfection. In a PAM model, AgNPs at a low concentration of 0.78 ppm completely inhibited ASFV infection at a viral load of 10^3 HAD_{50}, without inducing detectable cytotoxicity—demonstrating both high potency and safety. Additionally, application of 25 ppm AgNPs in pig housing environments significantly reduced microbial contamination, supporting their feasibility as a field-scale disinfectant[23]. Although the precise antiviral mechanism of AgNPs against ASFV remains to be elucidated, prior studies on other viruses suggest multiple non-specific modes of action: disruption of viral envelopes, inhibition of virus−receptor interactions, interference with viral entry, and potential activation of innate immune responses. In summary, AgNPs offer a non-specific, material-based antiviral strategy with demonstrated efficacy at both the cellular and environmental levels. Their favorable safety profile, practical applicability, and mechanistic versatility position them as promising adjuncts in integrated ASFV control frameworks.

5. Conclusions

ASFV is a complex large double-stranded DNA virus for which no effective vaccines or targeted antiviral therapies are currently available. In recent years, substantial progress has been made in the identification and development of small-molecule inhibitors targeting essential viral proteins, such as pS273R protease, pA104R, DNA polymerase, and enzymes involved in viral genome replication and transcription. Parallel efforts have also focused on host-directed strategies, such as the modulation of lipid metabolism, microtubule dynamics, innate immune signaling, molecular chaperones, and transcriptional regulators. Beyond target-specific agents, target-agnostic and multifunctional strategies, including inhibitors acting during early stages of the viral life cycle, gene-silencing platforms, and virucidal agents such as silver nanoparticles, provide complementary avenues for ASFV control. Integrating these diverse antiviral modalities through rational combination strategies holds promise for enhancing therapeutic efficacy, overcoming resistance, and advancing the development of effective countermeasures against ASFV. Collectively, advances in mechanism-based, multidimensional antiviral approaches are expected to facilitate the development of more effective and durable interventions against ASFV.

Recent advances in structural biology have provided a critical foundation for structure-based drug discovery. In recent years, high-resolution structures of several essential viral proteins encoded by ASFV have been resolved, including the ASFV RNAP, DNAPol, PolX, Topo II, pA104R, and pS273R. These structural insights have not only revealed the molecular mechanisms underlying viral genome replication, transcription, and host immune evasion, but also uncovered druggable pockets and key catalytic or interaction residues that can be exploited for rational inhibitor design. As more functionally critical viral proteins are identified and structurally characterized, structure-based approaches such as virtual screening[125], molecular docking[126], and fragment-based drug design will play an increasingly pivotal role

in guiding the discovery and optimization of ASFV-targeted therapeutics. The integration of structural biology with medicinal chemistry and virological validation represents a promising direction for accelerating the development of effective antiviral agents against ASFV.

Among the structural and non-structural proteins of ASFV, the major capsid protein p72 has attracted particular attention due to its central role in viral assembly and particle stability[127]. Encoded by the B646L gene and accounting for ~31%−33% of virion mass, p72 is the most abundant structural protein and the dominant antigen in infected pigs. Structurally, it adopts a double jelly-roll fold, assembles into pseudo-hexameric capsomers, and forms stable trimeric spikes (~85 Å) whose propeller-like top likely mediates receptor engagement[128]. Proper folding of p72 requires the viral chaperone pB602L, suggesting that disruption of this interaction could represent a potential therapeutic strategy. While p72 is widely exploited for molecular epidemiology, diagnostics, and vaccine development, no direct antivirals have yet been reported[129]. Nevertheless, advances in targeting capsid proteins of other viruses provide valuable precedents. For example, the HIV capsid inhibitor lenacapavir binds the NTD−CTD interface and allosterically blocks multiple replication steps, while PF74-derived inhibitors exploit the same site[130−132]. In HBV, core protein modulators perturb dimer−dimer hydrophobic interactions, leading to aberrant or empty capsids and suppression of replication and cccDNA formation, with discovery driven by high-throughput and virtual screening and scaffold modification[133,134]. These strategies highlight the "druggability" of viral capsid proteins and offer a framework for ASFV. Future research should apply structure-guided design and allosteric modulation to p72, focusing on its trimeric assembly interface and dependence on pB602L, with the goal of disrupting capsid formation or host interactions to suppress viral replication.

Host-targeted anti-ASFV strategies present a critical challenge in balancing antiviral efficacy with host safety[135−137]. The cellular pathways exploited by ASFV, including lipid metabolism, microtubule-based transport, molecular chaperone systems (*e.g.*, HSP90−p23), epigenetic and transcriptional regulators, as well as nucleotide biosynthesis, are integral to host physiology and homeostasis. Therapeutic interventions must therefore minimize mechanism-based (on-target) toxicity while preserving essential host functions[138]. Specifically, inhibition of lipid metabolism may disrupt hepatic lipid regulation and perturb steroid hormone homeostasis[139]. Agents that affect microtubule dynamics can impair normal proliferation of host cells, which may result in neurotoxicity, bone marrow suppression, and gastrointestinal injury[140]. Modulation of chromatin remodeling or transcriptional activity can lead to abnormal patterns of gene expression that may cause pathological consequences. Inhibitors of DHODH, a key enzyme involved in pyrimidine biosynthesis, may interfere with nucleotide metabolism and affect immune, hematopoietic, metabolic, neurological, and reproductive functions if they lack adequate selectivity[141]. These safety considerations highlight the need for systematic evaluation of mechanistic biological function and intracellular targeting selectivity in host targeted anti-ASFV therapies, with particular attention to selectivity against human and porcine homologs and the potential risk of disrupting physiological homeostasis. From a translational perspective, combining host targeted agents with direct-acting antivirals can reduce individual drug exposure and systemic toxicity, allow partial modulation of host pathways while preserve tolerability and overall antiviral efficacy.

Looking ahead, the development of multi-target antiviral strategies against ASFV holds promise for enhancing therapeutic efficacy and preventing resistance by simultaneously modulating distinct viral or host pathways across multiple stages of the viral life cycle. A representative example is the combination of the histone deacetylase inhibitor NaPB and the topoisomerase II inhibitor enrofloxacin, which has demonstrated synergistic antiviral activity *in vitro*[71]. This synergy is likely driven by functional crosstalk between epigenetic regulation and DNA topology control. In ASFV-infected Vero cells, co-treatment with sub-effective doses of NaPB (2.5 mmol/L) and enrofloxacin (25 μg/mL) resulted in complete inhibition of viral replication, whereas monotherapy with either agent reduced viral titers by only ~1.6−2.2 log. Notably, no infectious progeny virus was detected in the combination group, indicating a profound blockade of the viral life cycle. These findings highlight the therapeutic potential of multi-target combination regimens, while also emphasizing the need for careful optimization of pharmacodynamic synergy, pharmacokinetic compatibility, and overall safety to ensure clinical viability.

In summary, the ongoing efforts to combat ASFV have led to the identification of a wide range of viral and host targets, supporting the development of various antiviral strategies, including small-molecule inhibitors, gene-silencing technologies, and virucidal agents. At the same time, advances in structural biology have enabled structure-based drug design by revealing key mechanistic details and supporting the rational development of targeted therapies. Together, these developments highlight the potential of mechanism-based and multidimensional approaches to improve the effectiveness and durability of antiviral interventions against ASFV.

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Author contributions

Quanjie Li was responsible for literature collection, data integration, Figure preparation, original draft writing, and manuscript review and editing; Huihan shao participated in literature collection, data consolidation, and manuscript proofreading; Shan Cen revised the manuscript and provided overall supervision. All authors have read and approved the final version of the manuscript.

Conflicts of interest

The authors declare no conflicts of interest.

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