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REVIEW

Anti-influenza drugs targeting trimeric RNA polymerase complex: From development to clinics

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Drug resistance;
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Abstract The rapid evolution of influenza viruses, driven by high mutation rates and cross-species transmission, underscores the importance of discovering antivirals with novel mechanisms of action and distinct resistance profiles. The influenza virus RNA polymerase, a highly conserved heterotrimeric complex, comprises polymerase basic protein 1 (PB1), polymerase basic protein 2 (PB2), and polymerase acidic protein (PA) in influenza A and B viruses, or polymerase 3 protein (P3) in influenza C and D viruses. This complex is essential for viral genome replication and transcription, rendering it a critical target for antiviral intervention. Over the past two decades, research on influenza polymerase (FluPol) has advanced from fundamental studies to drug development and clinical application. By 2025, six FluPol-targeting drugs have received regulatory approval: the PA inhibitors baloxavir marboxil, suraxavir marboxil, seloxavir marboxil, and pixavir marboxil; the PB1 inhibitor favipiravir; and the PB2 inhibitor onradivir, with several additional candidates progressing to clinical research. This review summarizes the structure and function of influenza polymerase and the mechanisms of action of different inhibitors,

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highlighting the discovery and clinical effectiveness of the newly approved FluPol-targeting drugs. It addresses the potential of FluPol inhibitors against highly pathogenic avian influenza and the challenges posed by resistance mutations.

1. Introduction

Influenza, commonly known as the flu, is a highly contagious respiratory disease which occurs seasonally in epidemic waves and, on occasion, in pandemic proportions[1,2]. Unlike the common cold, influenza is distinguished by pronounced seasonality and a higher frequency of severe symptoms, including high fever accompanied by chills. Severe infections may trigger a cytokine storm, leading to systemic inflammatory responses and complications such as acute respiratory distress syndrome, shock, encephalopathy, and multiple organ dysfunction that can result in death. Influenza remains a major global public health concern, responsible for annual epidemics and periodic pandemics that contribute to considerable morbidity and mortality[3]. The impact is especially severe among vulnerable groups such as young children, the elderly, pregnant women, and individuals with underlying medical conditions. According to the World Health Organization estimates, seasonal influenza epidemics affect up to 15% of the global population and cause approximately 1 billion infections worldwide annually, resulting in 3−5 million severe cases and 290,000−650,000 deaths. Influenza pandemics can have an significant greater impact on the broader population[4]. Historically, four pandemics−A(H1N1) in 1918, A(H2N2) in 1957, A(H3N2) in 1968, and A(H1N1) in 2009−collectively caused more than 50 million deaths worldwide[5,6]. In the 21st century, newly emerging and highly virulent strains have triggered additional pandemics and outbreaks, including the H1N1 pandemic in 2009[7], H5N1 transmission in 2011, and H7N9 outbreak in East China in 2013, all of which raised significant global concern[8,9].

The causative agent of influenza is classified primarily into influenza A and B viruses, with types C and D being less prevalent[2]. Seasonal influenza in humans is mainly caused by the H1N1 and H3N2 subtypes of influenza A virus (IAV) and the Yamagata and Victoria lineages of influenza B virus (IBV)[10]. Influenza A virus, characterized by its high mutability, rapid genetic evolution through antigenic drift and shift, and zoonotic transmission capacity, drives the emergence of pandemic strains[11,12]. These evolutionary processes frequently result in significant changes in hemagglutinin (HA) and neuraminidase (NA) surface glycoproteins. For example, the 2009 influenza pandemic was caused by a novel H1N1 IAV reassortant virus that originated in swine, producing viruses distinct from existing seasonal strains and against which pre-existing antibodies offered limited protection[13,14].

Small-molecule antivirals are essential for the clinical management of influenza, particularly among high-risk populations or during outbreaks. Current antiviral agents targeting the influenza virus-encoded proteins include NA inhibitors (oseltamivir, zanamivir, peramivir), M2 ion channel blockers (amantadine, rimantadine), and RNA-dependent RNA polymerase complex inhibitors[15]. Despite the availability of multiple agents, the extensive clinical use of these drugs exerts significant selective pressure on the virus, leading to the emergence and dissemination of resistant strains. For instance, resistance to adamantanes emerged rapidly after their introduction. Today, M2 mutations that confer this resistance are widespread in circulating influenza A viruses, so these drugs are no longer recommended[16,17]. Notably, over 95% of adamantane-resistant cases involve an S31N point mutation in the M2 protein. Recent studies have reported novel M2 inhibitors designed to overcome this specific M2-S31N resistance[18,19]. Resistance to the NA inhibitor oseltamivir became widespread among influenza A(H1N1) strains shortly before the 2009 pandemic[6,20]. More recently, although less frequent, resistance to NA inhibitors has also been reported, with sporadic outbreaks caused by oseltamivir-resistant H1N1 or H3N2 variants[21,22].

The influenza polymerase (FluPol) is a ∼260 kDa heterotrimer composed of subunits PB1 (polymerase basic protein 1), PB2 (polymerase basic protein 2), and PA (polymerase acidic protein)[23,24]. Small-molecule inhibitors targeting key functional pockets within these subunits (PA, PB1, and PB2) constitute a novel and highly promising class of antivirals, and are currently at the forefront of influenza drug discovery and development[25-27]. Several agents have been approved or are in advanced stages of clinical evaluation (Table 1). While inhibitors targeting different FluPol subunits share some similarities, they exhibit distinct mechanisms of action, resistance profiles, and clinical potential[28]. The PB1 inhibitor favipiravir was approved in 2014, followed by the PA inhibitor baloxavir marboxil in Japan in 2018 (Fig. 1). More recently, PA inhibitor suraxavir marboxil, seloxavir marboxil and pixavir marboxil were approved by the National Medical Products Administration (NMPA) of China in April, July and December 2025, respectively. Although Johnson & Johnson discontinued development of its PB2 inhibitor pimodivir, a more potent PB2 inhibitor, onradivir, successfully advanced and was approved in China in May 2025. The development of influenza virus polymerase inhibitors represents a significant milestone in influenza treatment over the past decade. In recent years, several reviews have summarized FluPol as therapeutic target and their inhibitors[29-32]. Foundational reviews include Hou et al.[29], which emphasized preclinical inhibitor design strategies for viral ribonucleoprotein (vRNP) proteins, and Liu et al.[30], which highlighted emerging technologies such as artificial intelligence (AI)-driven screening and proteolysis-targeting chimeras (PROTACs) for influenza drug discovery. In contrast, this review concentrates on connecting late-stage clinical outcomes with early drug design from a clinical-translational perspective. We examine the mechanisms of action, antiviral activity, and clinical outcomes of clinically approved FluPol inhibitors and those in active development, highlighting the challenges posed by resistance mutations. We also assess the prospects for FluPol inhibitors against zoonotic and emerging influenza strains.

Table 1 Influenza inhibitors targeting the polymerase complex in various stages of clinical development.

Action principle	Modulators	Status	Company	Key registered clinical trials
PA inhibitor	Baloxavir marboxil	Approved	Shionogi & Co.	CTR20192253 NCT03629184 NCT02949011 NCT02954354
	Suraxavir marboxil (GP-681)	Approved	Qingfeng Pharma group, Ginkgo Pharmaceutical Co., Ltd.	CTR20242480 CTR20242984 CTR20243107 NCT04729764 NCT04736758 NCT06589635
	Seloxavir (ZX-7101A)	Approved	Zenshine Pharmaceuticals	CTR20221729 CTR20212778 CTR20242819 CTR20233060 NCT06099873
	Pixavir marboxil (TG-1000)	Approved	Joincare Pharmaceutical group, Taijing Biotechnology	CTR20232723 CTR20202536 NCT04706468 CTR20231822 CTR20201234
	Deunoxavir Marboxil (ADC-189)	NDA	AnDiConBio	NCT06342921 NCT06485401 CTR20230137 CTR20242291 CTR20240923 CTR20234293
	AL-794 (JNJ-6415580)	Discontinued in phase II	Alios BioPharma/Johnson & Johnson	NCT03411421 NCT03126097 NCT02588521 NCT02888327 NCT02877160
	WXSH-0208	Phase III	Cisen Pharmaceutical	CTR20233250 CTR20222526
PB1 inhibitor	Favipiravir	Approved	Toyama chemical Industry Co., Ltd., Toyama University	NCT01728753 NCT05041907 NCT1068912 NCT01728753
PB2 inhibitor	Pimodivir (JNJ-63623872/ VX-787)	Discontinued in phase III	Vertex Pharmaceuticals, Johnson & Johnson	NCT02342249 NCT02532283
	CC-42344	Phase II	Cocrystal Pharma	NCT06160531 NCT05202379
	Onradivir (ZSP1273)	Approved	Guangzhou lab Raynovent Biotech Co., Ltd.	NCT04024137 NCT06160531 NCT05202379 NCT05856513 NCT03679143 NCT04683406 CTR20191373

2. Structure of influenza viruses

Influenza viruses belong to the Orthomyxoviridae family and have a single-stranded, negative-sense, 8-segmented RNA genome[33]. The mature influenza virion is an enveloped, roughly spherical particle measuring 80−120 nm in diameter (Fig. 2A). It is comprised of a host-derived lipid bilayer, matrix proteins, and genomic RNA associated with viral proteins. Embedded within the lipid envelope are two major glycoproteins. HA mediates viral attachment and entry by binding to sialic acid residues on the host cell surface and promoting membrane fusion, whereas NA facilitates the release of progeny virions by cleaving sialic acids from host glycoproteins[34]. Beneath the envelope, matrix proteins M1 and M2 shape the virion and enclose its core. M1 lines the interior of the envelope, providing structural integrity and playing a crucial role in virion assembly and budding. M2 forms a proton channel within the viral membrane, enabling the acidification of the virion interior and genome uncoating after endocytosis, which is a prerequisite for viral replication. High-resolution X-ray crystal structures of the M2 proton channel have revealed detailed insights into its gating mechanism and drug-binding sites[35-37]. Encased by the matrix layer, the viral core contains eight RNA segments (vRNA). Each segment is associated with multiple nucleoproteins (NP) that protect the RNA and facilitate its packaging[38]. Both the 3′ and 5′ termini of each segment are bound by the trimeric FluPol complex[39,40]. The tripartite FluPol complex is conserved across influenza A, B, C, and D viruses, with influenza C and D encoding a homologous P3 protein that functionally replaces PA. Together, the RNA, NP, and polymerase assemble into viral ribonucleoproteins (Fig. 2B), the functional

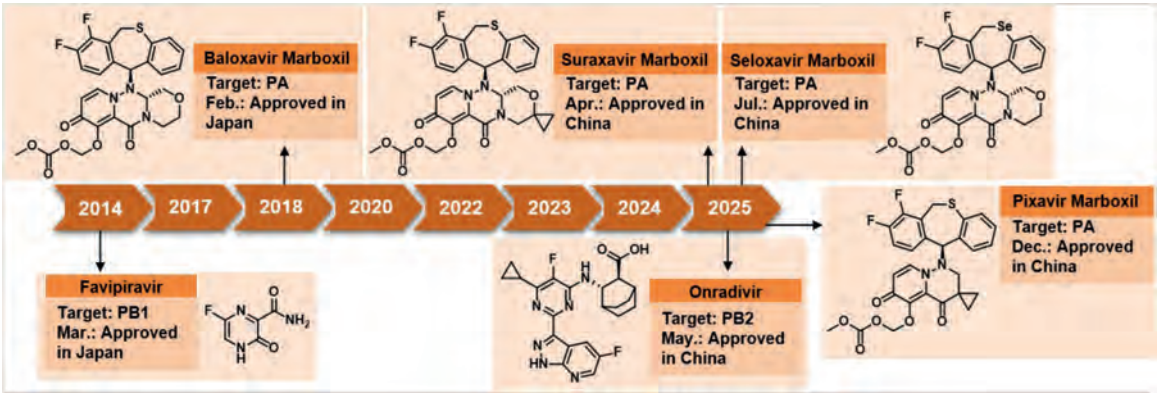


Figure 1 Structure and timeline of approved anti-influenza drugs targeting the polymerase complex.

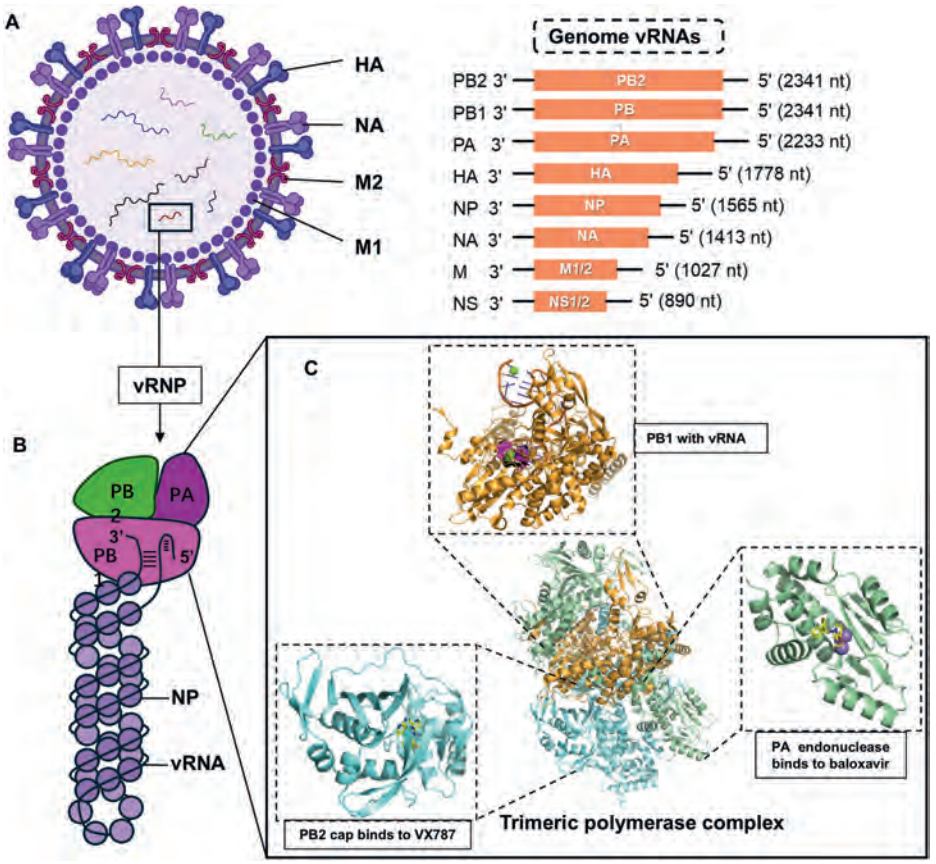


Figure 2 Schematic representation of influenza virus particle (A) and viral ribonucleoprotein complex (B). (C) The structure of trimeric influenza RNA polymerase and the inhibitor binding sites on PA, PB1, or PB2. The complete heterotrimeric RNA polymerase X-ray structure (PDB code 6QNW) is shown: PA in pale green, PB1 in bright orange, PB2 in aquamrine. PA endonuclease binds with BXA. (PDB code 6FS6). PB1 with vRNA (the two Mg^{2+} at RNA polymerase catalytic site were shown in green balls, and RNA around the catalytic site was highlighted in purple, PDB code 6SZV). PB2 cap binds to VX787 (PDB code 6EUV).

units that drive transcription and replication of the influenza genome.

3. FluPol mediates the replication and transcription

The life cycle of the influenza virus begins with viral entry, during which HA on the viral surface binds to sialic acid receptors and mediates virus−host membrane fusion. After entry into the host cells, the M2 proton channel acidifies the viral interior, triggering uncoating and releasing the vRNPs into the cytoplasm, which are subsequently trafficked to the nucleus. Once inside the nucleus, FluPol performs transcription and replication of the viral genome through distinct primed and unprimed RNA synthesis mechanisms, respectively[41,42]. The life cycle concludes with the assembly of viral particle, budding from the host cell membrane, followed by the release of progeny virions mediated by NA, completing the infection process.

Since viral protein production relies on the host's translation machinery, influenza mRNAs require a 5′ cap and a 3′ poly(A) tail, both of which are necessary for recognition by the host protein translation machinery. Transcription of negative-sense vRNAs into mRNAs occurs through a primer-dependent mechanism known as the 'cap-snatching'[43]. At pre-initiation, the cap-binding domain of PB2 binds short capped oligonucleotides (10−15 nucleotides) derived from host RNA polymerase II (RNAP II) transcripts (host pre-mRNAs), which facilitates the PA endonuclease cleavage of host mRNAs at defined positions 10−15 nucleotides from the 5′ cap[44]. Subsequently, these capped oligonucleotide primers are transferred into the PB1 active site through an approximately 60° conformational rotation of the PB2 cap-binding domain, thereby initiating viral mRNA synthesis. During termination, FluPol stutters on a 5′-proximal oligo(U) motif of each vRNA segment to add a poly(A) tail—a stuttering process that differs from canonical host polyadenylation[45]. Following mRNA release, FluPol recycles back into the pre-initiation state. The newly synthesized viral mRNAs, which carry host-derived 5′ caps and newly synthesized poly(A) tails, resemble host mRNAs and are processed similarly to host pre-mRNAs before being exported to the cytoplasm for translation.

In contrast, Replication proceeds only after transcription and the accumulation of sufficient viral proteins. It occurs *via* primer-independent *de novo* initiation on viral RNA templates, producing full-length complementary RNA (cRNA), which is subsequently assembled into a complementary ribonucleoprotein (cRNP), a structure similar to vRNP. cRNA then serves as the template for synthesizing progeny vRNA[46]. Notably, the influenza polymerase lacks proofreading activity, resulting in a high mutation rate that is critical for the virus's evolutionary adaptation and the rapid emergence of drug-resistant variants. The switch between transcription and replication involves conformational rearrangements

and polymerase dimerization or oligomerization, which collectively facilitate efficient cRNA synthesis. Recent cryo-electron microscopy (cryo-EM) studies have captured asymmetric dimers of influenza polymerase bound to host ANP32A, likely representing a replication platform for the viral genome, in which one FluPol molecule functions as the replicase while the other initiates assembly of the nascent replication product[47]. Nascent cRNA and vRNA are immediately bound by an encapsidating polymerase as their 5′ end emerges from the active site of the replicating polymerase, ensuring their prompt incorporation into cRNPs or vRNPs[48]. These cRNP and vRNP complexes are critical for preventing the release of naked viral RNA, thereby minimizing the risk of triggering host innate immune responses. Progeny vRNPs then engage in secondary transcription and replication or, later in infection, are exported from the nucleus and incorporated into progeny virions[49].

Throughout RNA synthesis, FluPol remains tightly bound to the 5′ end of the viral RNA template, and the RNA templates fold into characteristic corkscrew structures *via* complementary base pairing at their 5′ and 3′ ends. FluPol adopts distinct states during transcription and replication: a monomeric polymerase transcribes viral mRNA, whereas a dimerized polymerase associated with the vRNP complex is recruited for genome replication. High-resolution FluPol structures determined by X-ray crystallography and cryo-EM have revealed its remarkable conformational flexibility[50]. The complex features a relatively stable core formed by the PA C-terminal domain (PA-C), PB1, and the N-terminal domain of PB2 (PB2-N), while the peripheral PA N-terminal domain (PA-N) and PB2 C-terminal domain (PB2-C) are flexibly connected and can rearrange depending on the polymerase's functional state (Fig. 2C)[51]. The precise interactions of PA, PB1, and PB2 are essential for enzymatic activity and viral replication[52]. The polymerase operates through coordinated interplay among its domains and subunits, transitioning between multiple conformational states and oligomeric forms to regulate transcription and replication.

4. Structure and inhibitors of the PA subunit

The PA subunit (~25 kD) of FluPol comprises an N-terminal cap-dependent endonuclease (CEN, residues 1−196) domain and a larger C-terminal domain (Fig. 3)[53]. The PA C-terminal domain tightly associates with the N-terminal segment of PB1, stabilizing the trimeric polymerase complex and facilitating functional integration. During transcription initiation, PA undergoes conformational changes that reposition capped RNA fragments to the PB1 catalytic site. Influenza CEN belongs to the PD-(D/E)XK nuclease superfamily and features a metal-dependent active site[54]. Conserved catalytic residues, including His41, Glu80, Asp108, and Glu119, coordinate two essential divalent metal ions (Mg^{2+} or Mn^{2+}). PA inhibitors act by chelating these divalent metal ions within the CEN active site, thereby blocking host RNA cleavage and the production of capped primers. Currently, three PA inhibitors have been approved for marketing, and four others are in clinical development.

4.1. *Baloxavir marboxil (BXM)*

Baloxavir marboxil (BXM), the first clinically approved PA inhibitor, was approved in Japan in February 2018 for the treatment of influenza A and B virus infections in patients aged 12 years and older[55]. Subsequently, it received approval in the United States in October 2018 and later in Europe and China in 2021. BXM was discovered by Shionogi & Co., Ltd. and is being further developed and commercialized globally in collaboration with the Roche Group. Core patents by Kahashi and his colleagues was filled to protect the intellectual property of BXM and related deriratives[56]. The rational design of baloxavir acid (BXA), the active form of BXM, was inspired by the similarity in metal coordination between the human immunodeficiency virus (HIV) integrase domain and the influenza virus PA endonuclease. BXA contained a two-metal-chelating privilege tricyclic pharmacophore, resembling that of dolutegravir, an approved HIV integrase inhibitor[55,57]. However, compounds containing metal-chelating groups often exhibit low oral bioavailability due to poor membrane permeability. In oral pharmacokinetic (PK) tests with rats and monkeys, baloxavir acid demonstrated limited bioavailability. Using the prodrug strategy of esterification yields the methoxy(methyl)carbonate prodrug BXM with enhanced oral absorption of BXA[58]. Following oral administration, BXM is rapidly metabolized primarily by the enzyme arylacetamide deacetylase (AADAC) in the intestinal lumen, epithelium, liver and blood, and converted into the active form BXA (Fig. 4A)[59].

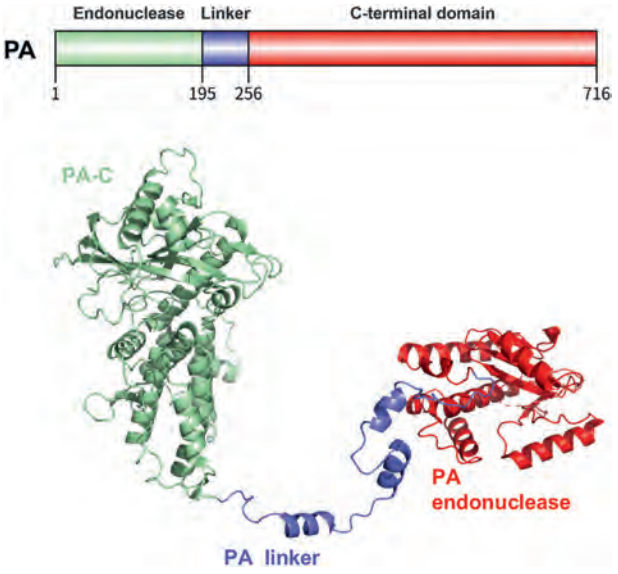


Figure 3 Schematics and structure of the PA subunit (PDB code 6QNW).

In the cocrystal structure of BXA bound to the PA endonuclease. BXA adopts a characteristic butterfly-shaped conformation: one "wing" comprises a polar, metal-chelating oxazino-pyrido-triazin-dione head group, which tridentate chelates the two metal ions in the enzyme active site *via* three oxygen atoms; the other "wing" consists of a hydrophobic difluoro-dihydro-dibenzothiepin-cycloheptane tail moiety, which nestles within a V-shaped pocket and engages the surrounding residues through van der Waals interactions (Fig. 4C and D)[58]. Conserved I38 intimately packs into the V of the tail-group identically in both FluA and FluB, making tight and extensive van der Waals contacts with baloxavir and its mutation to the polar threonine would significantly impact binding affinity as observed.

The recommended dose of BXM is weight-based and is administered as a single dose of 40 mg for adult patients weighing under 80 kg and 80 mg for those weighing 80 kg or more. Its single-dose administration facilitates patient compliance. Adverse

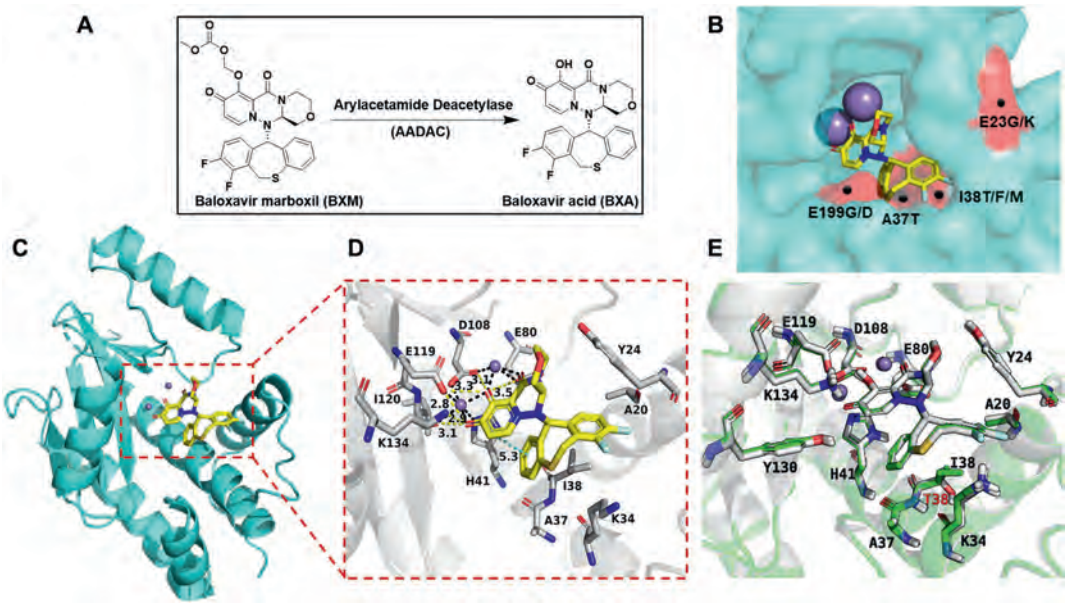


Figure 4 (A) Conversion of prodrug BXM and its active metabolite BXA, which is hydrolyzed by arylacetamide deacetylase. (B) Resistance mutation sites of BXA were highlight in red and black dot (PDB: 6FS6). (C, D) The binding mode of BXA to PA (H3N2) subunit. Yellow sticks represent BXA, gray sticks represent amino acid residues, black dashed lines indicate coordination bonds formed between Mn^{2+} and BXA as well as the amino acid residues, yellow dashed lines represent hydrogen bonds, and blue dashed lines indicate $\pi-\pi$ interactions. (E) The superimposed structure of the BXA binding site in wt I38 PA and T38 PA (PA I38T (PDB: 6FS7) is shown (structures are depicted in green for I38 PA bound to BXA and in white for T38 PA bound to BXA).

events included diarrhea, bronchitis, nasopharyngitis, headache, and nausea[60]. In rare cases, hemorrhagic complications, liver dysfunction, rhabdomyolysis, and life-threatening cardiorespiratory events were reported[61]. Phase II/III trials demonstrated a median reduction of 26.5 h in time to symptom alleviation (53.7 h *vs*. 80.2 h) and a 17.5-h faster fever resolution compared with the placebo[62]. In high-risk influenza patients, the time to symptom resolution was shortened by 29.1 h (73.2 h *vs*. 102.3 h). In a post-exposure prophylaxis trial, treatment with baloxavir reduced transmission from index patients to household contacts by 86% (adjusted incidence, 1.9% *vs*. 13.6%)[63,64]. These findings are particularly significant because studies on NA inhibitors did not provide comparable transmission-blocking evidence[65].

The use of BXM is accompanied by the emergence of resistance mutations due to the highly mutagenic nature of influenza. In cell culture, influenza A virus strains with reduced sensitivity to baloxavir have been observed after continuous passaging under gradually increasing drug concentrations. Amino acid substitutions adjacent to the BXA binding pocket of the PA protein—including I38T (in A/H1N1, A/H3N2 and IBV), E23 K/R and I38 F/N in A/H1N1; E23 G/K, A37T, I38M and E199G in A/H3N2 (Fig. 4B)—reduce the susceptibility of influenza A virus to BXA[66]. The most prevalent I38T mutation reduces susceptibility by approximately 30- to 50-fold in influenza A viruses and by 7-fold in influenza B viruses, as compared by EC_{50}[58]. This was consistent with another report that binding of BXA to purified I38T mutant PA-N endonuclease showed a 27-fold reduction in affinity as determined by spectral shift methods[67]. Cocrystal structural analysis reveals BXA binds in an almost identical manner, with the primary difference localized to the I/T side-chain, where the more polar threonine diminishes the van der Waals interaction with BXA (Fig. 4E). Additionally, a comparison of the unbound and BXA-bound T38 PA structures shows conformational changes in both the side-chains of T38 and neighbouring M34, resulting in an altered compound hydrophobic pocket. Thus, BXA binding to the T38 variant requires local induced-fit rearrangements, whereas binding to wild-type PA does not[58]. The structure of baloxavir bound to the PA-E119D mutant PA (PDB: 7KBC) is also very similar to that of the wild-type PA. However, the shorter aspartic acid weakens the interaction with the oxazino-pyridotriazin-dione head-group of BXA, which may explain the reduced susceptibility associated with this mutation[68].

In clinical trials, influenza A and B virus strains carrying amino acid mutations at sites associated with reduced baloxavir sensitivity, as identified in cell culture, were detected after BXM treatment. Among adult and adolescent subjects diagnosed with influenza virus infection, the incidence of post-treatment point mutations conferring decreased baloxavir sensitivity was 5% for A/H1N1 and 11% for A/H3N2, respectively. However, in children under 12 years of age, the incidence of post-treatment BXM-resistant mutations was substantially higher. Four clinical trials involving children younger than 12 showed overall incidences of 17% and 18% for influenza A/H1N1 and A/H3N2 infections, respectively. The highest incidence of post-treatment resistance was observed in children under 5 years of age, reaching 24% (A/H1N1) and 65% (A/H3N2). Resistance rates remained low for influenza B virus infections across all age groups[60,69]. Due to this concern, BXM is not indicated for influenza in persons younger than 5 years of age. In a more recent four-season, multicenter study conducted from 2018 to 2019 to 2023—2024 conducted in Japan, pediatric patients younger than 19 years with influenza A treated with baloxavir were evaluated; PA variants were detected in 13.8% of case for A(H3N2) and 5.1% for A(H1N1)[70]. Resistant mutations can arise rapidly during the early stages of treatment and pose a risk of transmission, underscoring the importance of molecular-level surveillance and ongoing resistance

monitoring[71,72]. Global susceptibility analysis from 2020 to 2023 revealed low global frequencies (~0.1%) of seasonal influenza viruses harbored PA substitutions associated with reduced susceptibility to BXM[73]. In Japan, where BXM is most commonly used, the frequency was 3.3% in 2022−2023 and 4.5% in 2018−2019[74]. It has been noted that the use of baloxavir in children younger than 12 years may contribute to the higher resistant mutations rate in Japan[74].

4.2. *Suraxavir marboxil (GP-681)*

Suraxavir marboxil, jointly developed by Qingfeng Pharma Group and Ginkgo Pharmaceutical Co., Ltd., is the second approved PA inhibitor for the treatment of uncomplicated influenza A and B in patients aged 12 years and older. It is recommended to take a single 40 mg dose to patients weighing no less than 40 kg. Structurally, it is analogous to BXM, differing primarily by a cyclopropyl-fused spirocycle at the 6-position of the morpholine moiety. This modification enhances antiviral potency and opens access to novel spirocyclic chemotypes in relatively unencumbered patent space, supporting inventiveness and novelty of the patent claims. After oral administration, suraxavir marboxil is metabolized by esterases into its active form, suraxavir (GP1707D07). Suraxavir exhibits antiviral activity against various laboratory influenza virus strains, including influenza A (H1N1, H3N2), influenza B (B/Lee/40), and avian influenza viruses (H5N1, H5N6, H6N1, H7N4, H7N9, H9N2, H10N8), with EC_{50} values ranging from 0.22 nmol/L to 16.82 nmol/L. In the MDCK cell virus plaque reduction assay, suraxavir showed EC_{50} ranges of 0.36−3.33, 0.29−0.32, and 3.04−3.14 nmol/L against influenza A H1N1, influenza A H3N2, and influenza B viruses isolated clinically in China, respectively (data from Drug Insert Sheet).

Phase I clinical trial (NCT04729764) in healthy subjects demonstrated that doses ranging from 20 to 80 mg were clinically tolerable. The primary active metabolite, suraxavir, appeared rapidly in plasma, with a median T_{max} of approximately 3.0 to 4.5 h and an elimination half life ($T_{1/2}$) of 58−75.9 h. The peak concentration (C_{max}, ranging from 23.6 ng/mL at 20 mg to 110 ng/mL at 80 mg) and area under the curve (AUC, from 1639.6 ng h/mL to 5290.6 ng h/mL) of the active metabolite suraxavir increased with dose escalation; however, the PK characteristics were not dose-proportional[75]. In a Phase II study (NCT04736758) involving 245 patients aged 18−65 (predominantly influenza B, 99.5%), single oral doses of 20 mg and 40 mg shortened median symptom relief times to 50.0 h and 46.1 h, respectively, compared to 82.3 h in the placebo group ($P < 0.0001$)[76]. Viral loads decreased by 2.14 and 1.48 $\log_{10}$ $TCID_{50}$/mL after 24 h in the treatment group, *versus* 0.69 $\log_{10}$ in the placebo. No serious adverse events or PA I38T resistance-associated mutations were observed.

During the 2022−2023 influenza season in the Northern Hemisphere, a total of 588 participants aged 5−65 years were randomized and treated (ClinicalTrials.gov registration: NCT03787459). The predominant influenza virus strains in this study were influenza A H3N2 subtype (60.15%), followed by influenza A H1N1 subtype (39.47%) and influenza B (0.38%). Patients were stratified based on age (<18 years or >18 years) and randomized in a 2:1 ratio to receive either 40 mg of the drug or placebo. The study found that the median time to alleviation of influenza symptom (TTAS) was significantly shorter in suraxavir marboxil 40 mg group (41.95 h) compared to the placebo group (63.00 h), with a difference of approximately 20.3 h. Viral load declined by 2.2 $\log_{10}$ RNA copies/mL within 24 h (*versus* 1.3 $\log_{10}$ in placebo)[77]. Treatment-emergent resistance mutations at PA residue I38T were rare, occurring in 0.7% of H1N1pdm and 0.9% of H3N2 cases.

4.3. *Seloxavir marboxil (ZX-7101A)*

Seloxavir marboxil, developed by Zenshine Pharmaceuticals, is a selenium-containing CEN inhibitor that received approval in China in July 2025, three months after the approval of suraxavir marboxil[78]. The recommended regimen is a single 80 mg oral dose for adults. According to a strategic cooperation agreement in 2023, Jumpcan Pharmaceutical holds exclusive rights for the promotion and sales of seloxavir marboxil in mainland China. Selenium's unique chemical properties confer distinctive pharmacological and physicochemical of organoselenium compounds. Replacing sulfur with selenium in drug scaffolds often enhances biological activity because selenium is more polarizable than sulfur, has more loosely held outer valence electrons, and greater nucleophilicity. Furthermore, selenium may improve cellular uptake and biodistribution, as well as modulate redox balance through both antioxidant and pro-oxidant mechanisms[79]. Like other PA inhibitors, seloxavir marboxil is a marboxil prodrug that is rapidly converted to the active metabolite seloxavir by carboxylesterases after oral administration. Preclinical studies in MDCK cells demonstrated seloxavir's inhibition of multiple influenza virus subtypes, including seasonal strains (H1N1, H3N2) and avian viruses (H7N9, H9N2). The EC_{50} for influenza A strains ranged from 0.30 to 1.6 nmol/L, and the EC_{50} for influenza B strains ranged from 8.3 to 42 nmol/L (data from Drug Insert Sheet). In a Phase I clinical trial, single oral doses of seloxavir marboxil (40−320 mg) were rapidly converted into the active ingredient seloxavir, with T_{max} of approximately 3−4 h in all dose cohorts. Seloxavir had a $T_{1/2}$ of 83.01−125.55 h and AUC_{0-t} of 7183.6−35,671.8 ng h/mL. Food intake reduced the peak concentration and overall exposure of drug. Under fasting conditions, C_{max} and AUC_{0-t} were 1.73-fold and 1.78-fold of those under fed conditions, respectively[80]. Seloxavir is not a substrate of CYP450 enzymes and is not metabolized by CYP450 enzymes. It is primarily metabolized by UDP-glucuronosyltransferase 2B7 (UGT2B7). In a human mass-balance study following a single oral 80 mg dose of [^{14}C]-labeled seloxavir, the glucuronide conjugate of seloxavir accounted for 9.04% and seloxavir itself accounted for 88.63% of the total plasma radioactivity exposure.

In Phase II/III clinical trials (CTR20221729), 900 patients aged 18 to 64 with uncomplicated influenza were recruited and randomized to either the seloxavir marboxil group or the placebo group; the primary endpoint was the time to alleviation of all influenza symptoms, while secondary endpoints included virological markers and safety indicators. The 80 mg seloxavir marboxil group had a TTAS of 34.7−39.4 h *versus* 62.9−63.6 h in the placebo group, which was approximately 30% shorter in the treatment group. Median time to viral RNA negativity was 41.4 h for the seloxavir 80 mg group, which was significantly shorter than the 90.8 h observed in the placebo group[81]. Notably, a PA-E18G mutation conferring cross-resistance to seloxavir and baloxavir was identified at an incidence of 1.7% (6/359), highlighting its shared resistance profile to baloxavir[78].

4.4. *Pixavir marboxil (TG-1000)*

PA inhibitor pixavir marboxil was developed by Taijing Biotechnology and licensed to Joincare Pharmaceutical Group for

development and commercialization in Chinese mainland, Hong Kong, and Macau. In a Phase I clinical trial, the single ascending dose (10−160 mg) study demonstrated that the half-life of the active metabolite TG-0527 remained stable at 33.8−39.4 h, with a dose-independent T_{max} of 3−6 h. Food intake reduced the peak concentration by about 41% but had minimal impact on overall exposure[82]. A Phase III study (CTR20232723) in patients aged 12 and older demonstrated a statistically significant reduction of 27 h in median symptom relief time of pixavir marboxil compared to placebo (60.9 h *vs*. 87.9 h), with comparable adverse event profiles primarily involving mild gastrointestinal reactions. Pixavir marboxil was approved as a New Drug Application (NDA) to the NMPA in Dec 30, 2025.

4.5. Deunoxavir Marboxil (ADC-189)

Deunoxavir Marboxil was developed by AnDiConBio and was submitted an NDA to the NMPA in 2025. Preclinical data have demonstrated broad-spectrum inhibition against influenza A (H1N1, H3N2), influenza B (Victoria and Yamagata lineages), and highly pathogenic avian influenza strains such as H5N1, with potent EC_{50} values in the nanomolar range. In a Phase II/III trial involving adults and adolescents with uncomplicated influenza (NCT06507813), a single weight-adjusted oral dose (45−90 mg) shortened the median relief time for seven core influenza symptoms to 50 h, representing a 26.5% improvement compared to the placebo (68 h)[83].

4.6. AL-794 (ALS-033794/JNJ-6415580)

AL-794 is an ester prodrug developed by Alios BioPharma/ Johnson & Johnson, which is rapidly converted into ALS-033719 *in vivo*. It binds to the PA endonuclease domain and has demonstrated efficacy against influenza A and B. Phase I trials documented dose-proportional pharmacokinetics[84,85]. Adverse events were mostly mild and transient, with the most frequent events reported being headaches and lightheadedness. In a human challenge study, healthy volunteers were inoculated with the A(H3N2) virus and received oral doses of AL-794 at either 50 or 150 mg twice daily for 5 days, starting 12 h post-infection, or received a placebo. Dose-dependent antiviral activity was observed, with a greater reduction in viral load, symptoms, and mucus weight at the 150-mg dose, compared with the 50-mg dose, and no safety concerns were reported[71]. However, development was discontinued due to pharmacokinetic variability influenced by food intake and gender, and inability to achieve a competitive single-dose regimen, particularly in comparison with baloxavir.

4.7. WXSH0208

A series of macrocyclic PA inhibitors was reported by WuXi AppTec Co., Ltd.[86]. Compound **8** inhibited laboratory strain A/PR/8/34 (H1N1) strain with an EC_{50} of 7.2 nmol/L and showed an EC_{50} of 66 nmol/L against the baloxavir-resistant strains. It also exhibited broad inhibitory activity against a panel of H1N1, H3N2 and influenza B virus (Victoria/Yamagata) with EC_{50} values ranging from 0.9 to 8.5 nmol/L. Preclinical mouse models revealed that the carbonate prodrug **7** exerted dose-dependent antiviral efficacy in mice that received twice daily treatment for three days, starting at 48 h post-inoculation. This compound was subsequently selected for further clinical evaluation.

PA inhibitor WXSH0208, developed by Cisen Pharmaceutical, demonstrated linear pharmacokinetics and a $T_{1/2}$ of 14.7 h in an ascending single-dose study involving healthy participants. Preclinical results of WXSH0208-4 (the active form of WXSH0208) showed that the EC_{50} of 4.42−7.17 nmol/L against a panel of different strains of Flu A and FluB while baloxavir was 0.74−17.74 nmol/L in parallel[86]. In a placebo-controlled Phase II trial in adult outpatients with uncomplicated influenza A and B virus infections (Clinical trials registration: CTR20233250), WXSH0208 was administered once daily at doses of 10, 20, or 30 mg for 5 days. The median time to viral load negativity was 49.3, 48.0, and 48.2 h for the 3 ascending dose regimens, compared with 95.6 h in the placebo group[86]. A multicenter Phase III trial with larger cohorts was initiated.

It is worth mentioning that all PA inhibitors above bind to similar sites. Among them, resistance to BXM—mainly caused by amino acid substitutions adjacent to the binding site—has been extensively studied *in vitro* and in clinical settings. Post-treatment point mutations associated with reduced susceptibility to baloxavir were observed at a relatively low incidence in adults and adolescents, while at a higher incidence in children[64]. With the two PA inhibitors approved in 2025, the PA-I38T resistance mutation, which is cross-resistant to baloxavir emerged during suraxavir treatment in a phase III trial[69]. Additionally, the PA-E18G mutation—implicated in cross-resistance to both suraxavir and baloxavir—was detected in seloxavir marboxil treated patients, underscoring a shared resistance profile among the PA inhibitors[70]. No comparative studies of resistance incidence between children and adults with suraxavir or seloxavir have been reported, and the resistance incidence still needs to be observed in post-approval studies. To date, the emergence of variants with reduced susceptibility to the other clinical-stage PA candidates has not been reported. However, experience with other anti-influenza agents suggests that development of resistance appears to be an inevitable consequence of therapeutic use. Whether the stronger antiviral potency of PA inhibitors will translate into a lower risk of resistant variants remains to be determined.

5. Structure and inhibitors of the PB1 subunit

PB1 forms the catalytic core of the FluPol responsible for RNA chain initiation and elongation during both transcription and replication[87]. It exhibits a canonical right-hand-like polymerase architecture comprising fingers, palm, and thumb domains characteristic of polymerases (Fig. 5)[43]. PB1 interacts with the PB2 N-terminal domain to form a central cavity within the polymerase, which houses the catalytic active site and serves as a hub for the entry of nucleotide triphosphates (NTPs) and the RNA template, while permitting the exit of the template and nascent RNA product[88]. Within the PB1 active site, several conserved motifs coordinate magnesium ions required for phosphodiester bond formation, enabling integration of nucleoside triphosphates into the growing RNA chain. PB1 engages the 3′ and 5′ termini of the viral RNA template within an internal cavity, maintaining a closed conformation that promotes the processivity of RNA synthesis[89]. Its N-terminal domain interacts intimately with PA's C-terminus, while its C-terminal region associates with the PB2 N-terminus, enabling communication between primer acquisition and RNA polymerization activities[90,91]. PB1 is targeted by nucleotide analog antiviral agents, including favipiravir and EIDD-2801(molnupiravir)[92].

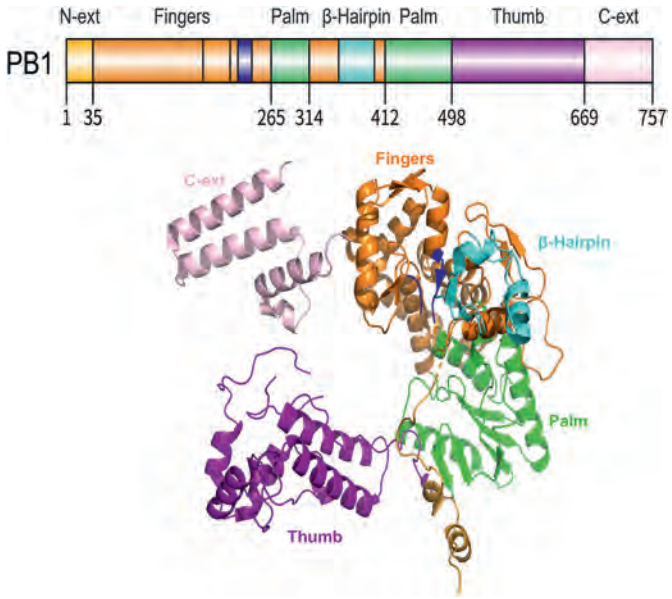
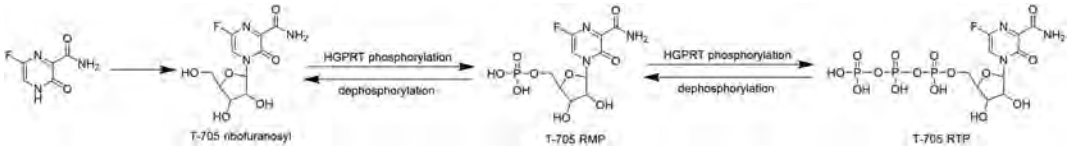


Figure 5 Schematics and structure of PB1 subunit (PDB code 6QNW).

5.1. Favipiravir

Favipiravir (T-705) is a substituted pyrazine compound developed initially in 1999 through a collaboration between Toyama University and Toyama Chemical Industry Co., Ltd. (now Fujifilm Toyama Chemical) in Japan[93,94]. It was first approved in Japan in 2014 for influenza pandemic preparedness and subsequently for the treatment of influenza viruses resistant to other antiviral drugs[95]. Favipiravir exhibits strong inhibitory activity against many subtypes of influenza virus, with EC_{50} values ranging from 0.19 to 22.5 μmol/L[96]. The reduction of its antiviral activity upon addition of purines—but not pyrimidines—suggests that favipiravir functions similarly to purines or purine nucleosides during viral RNA replication[93]. Although the chemical structure of favipiravir differs significantly from that of purine nucleoside bases, HPLC radioactivity elution profiles demonstrate that cellular enzymes recognize T-705 as a nucleoside base and convert it into phosphorylated metabolites. Intracellularly, favipiravir is converted to its ribosyl-5′-monophosphate form (T-705 RMP, Scheme 1) by hypoxanthine-guanine phosphoribosyltransferase[97]. Further phosphorylation steps yield the pharmacologically active triphosphate form (favipiravir-RTP or T-705 RTP). Favipiravir-RTP is efficiently recognized by viral polymerase and incorporated into nascent viral RNA strands, particularly mimicking guanine and, to a lesser extent, adenine nucleotides. This incorporation leads to the accumulation of transition mutations, inducing lethal mutagenesis that disrupts viral replication and generates nonviable progeny virus. In addition to influenza, favipiravir exhibits broad-spectrum antiviral activity against various RNA viruses—including flavi-, alpha-, filo-, bunya-, arena-, noroviruses—many of which are neglected and (re)emerging pathogens with no specific treatment[98]. These unique anti-viral profiles make favipiravir a potentially drug for specifically untreatable RNA viral infections. It was off-label used for diseases like Ebola and Lassa fever[99,100]; nevertheless, its clinical efficacy to treat Ebola has not been demonstrated[101]. During the COVID-19 pandemic, favipiravir was repurposed and received emergency use authorization in several countries; however, its efficacy in the treatment of SARS-CoV-2 infection was controversial, due to suboptimal antiviral activity and the very high dosages required (often between 1600 and 3600 mg/day), which raise concerns regarding potential toxicity[102].

Safety concerns, particularly regarding teratogenicity and embryotoxicity, restrict favipiravir's use in pregnant women and children. Common adverse effects include mild gastrointestinal symptoms and asymptomatic hyperuricemia, with serious adverse events occurring in fewer than 1% of cases[103]. Consequently, the clinical application of favipiravir is largely restricted to treating novel or reemerging pandemic influenza infections when alternative antivirals are ineffective. Favipiravir demonstrated synergistic effects with NA inhibitors (oseltamivir, peramivir, zanamivir, laninamivir)[104,105]. Clinical data suggest that combination therapies can accelerate viral clearance and clinical recovery in patients with severe influenza. For instance, in hospitalized patients over 18 years old with severe influenza, coadministration of favipiravir and oseltamivir significantly



Scheme 1 The activation of favipiravir: Favipiravir initial conversion to the ribosyl-5′-monophosphate form (T-705 RMP) catalyzed by hypoxanthine-guanine phosphoribosyltransferase (HGPRT). Further phosphorylations produce the active favipiravir-RTP (T-705 RTP).

reduced viral positivity rates by day 10 compared with oseltamivir monotherapy[106].

Influenza viruses resistant to favipiravir have been identified following 10 *in vitro* passages in the presence of favipiravir[107,108]. Two notable resistance-associated mutations involve a lysine-to-arginine substitution at position 229 (K229R) in PB1, which confers an approximately 31-fold reduction in drug susceptibility, and a compensatory P653L mutation in PA that restores polymerase activity, thus causing favipiravir resistance. Transmission studies in ferrets demonstrated the ability of these mutant viruses to spread, although their prevalence declined over time[109]. Importantly, these resistance mutations have not been identified in natural viral isolates, and clinical studies to date have not reported the emergence of favipiravir-resistant influenza strains, suggesting that it has a high barrier to resistance development.

6. Structure and inhibitors of the PB2 subunit

The PB2 subunit of FluPol displays a modular domain architecture, divisible into an N-terminal one-third (PB2-N) and a C-terminal two-thirds (Fig. 6). C-terminal encompasses the midlink region (which further divided into mid, cap-binding, and linker), as well as the 627, and nuclear localization signal (NLS) domains. The specialized cap-binding domain is essential for recognizing the 7-methylguanosine cap structures of host mRNAs and for sequestrating capped host RNA transcripts, which are then cleaved by PA endonuclease to generate primers for viral mRNA synthesis[44,110]. The flexible nature of PB2 allows it to undergo substantial conformational rearrangements during the viral transcription cycle, particularly to position capped RNA primers into the PB1 catalytic site following cap-snatching. Such plasticity is also critical for the rational design of polymerase inhibitors.

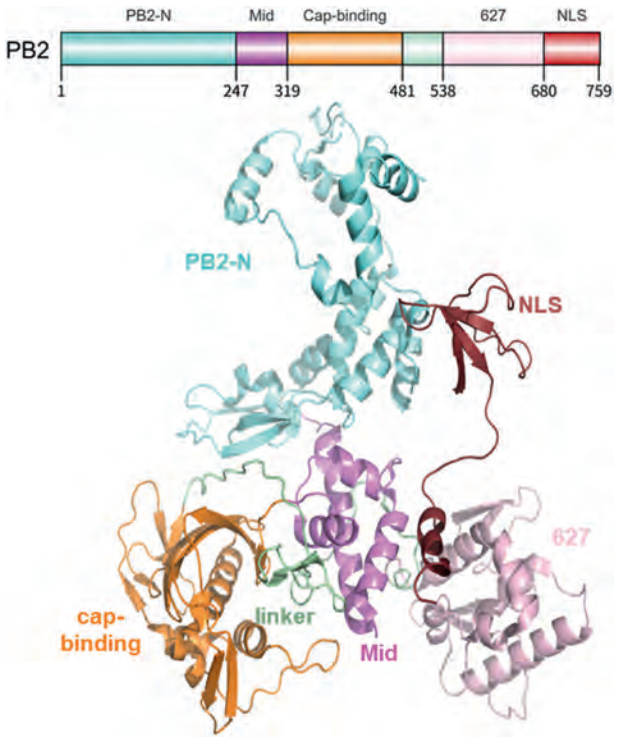


Figure 6 Schematics and structure of PB2 subunit (PDB code 6QNW).

6.1. *Pimodivir (JNJ-63623872/VX-787)*

Oral PB2 inhibitor pimodivir was initially developed by Vertex Pharmaceuticals and later acquired by Janssen Pharmaceuticals[111]. It exhibits potent activity against influenza A viruses, including avian strains with pandemic potential, such as A(H5N6) and A(H7N9), as well as strains resistant to M2 inhibitors and NA inhibitors, but shows no activity against influenza B viruses[112]. The chemical structure of pimodivir can be divided into three functional moieties: an indazole moiety, a pyrimidine ring, and a bicyclic octane carboxylic acid. Pimodivir binds specifically to the cap-binding and midlink domains of the PB2 subunit. X-ray crystallography elucidated the key interactions between pimodivir and PB2 (Fig. 7)[113]. The carboxylic acid of pimodivir forms a salt bridge with the side chain of R355 and a hydrogen bond to N510 in the PB2long (H3N2 PB2 residues 241−741)/pimodivir complex, while in the PB2cap (H3N2 PB2 residues 318−483)/pimodivir complex[44], this moiety is solvent-exposed and forms water-mediated interactions with neighboring basic residues. The pyrimidine ring forms $\pi-\pi$ stacking with Phe323, Phe404, and His357, and the indazole (pyrrole) ring also stacks with His357. A hydrogen-bond network is formed between the carbonyl oxygen of Glu361 and the pyrrole nitrogen, as well as the amino proton of Lys376 and the pyrimidine nitrogen. Beyond the known "cutting" and "priming" conformations, the polymerase exists in an "apo" state, characterized by rearrangements in the PB2 subunit that leads to transcriptional inactivity[114]. Pimodivir inhibits transcription both by competitively occupying the cap-binding site and by stabilizing this inactive "apo" form[115]. This stabilization involves introducing a hydrogen bond network between the cap-binding and midlink domains, thereby perturbing the conformational equilibrium in favor of the inactive state. As a result, pimodivir suppresses viral transcription *via* a dual mechanism: first, by targeting the cap-binding domain of PB2 to competitively block interaction with the 5′ cap structure (m^7GTP) of host mRNA; second, by stabilizing the polymerase's transcriptionally inactive "apo" conformation through interaction with the midlink domain, further suppressing polymerase activity.

Pimodivir binds A/H3N2 cap-midlink double domain with a K_D value of 2.4 nmol/L, whereas its binding to B/Memphis cap-midlink double domain is substantially weaker (K_D = 1.2 μmol/L), *i.e.*, roughly 500-fold reduced affinity[113]. VX-787N is a closely related analogue of pimodivir that contains a 5-fluoro-1*H*-pyrazolo[3,4-*b*]pyridine moiety in place of the 5-fluoro-1*H*-pyrrolo[2,3-*b*]pyridine group. A cocrystal structure of VX-787N with the IBV cap-binding domain (PDB: 6EUX) shows an overall binding pose similar to that of pimodivir with IAV PB2 (Fig. 7D)[113]. In the influenza B complex, the inhibitor's exocyclic fluorine lies close to the carbonyl oxygen of Val512, whereas in influenza A, the corresponding oxygen engages the inhibitor's amide *via* a hydrogen bond. The most notable difference is that Gln325 in IBV replaces Phe323 in IAV, abolishing the strong $\pi-$stack interaction with the inhibitor's pyrimidine ring. The loss of these key contacts explains the markedly reduced affinity of pimodivir to IBV PB2 and the consequent loss of inhibitory activity.

In Phase II trials, compared with the placebo group, administering pimodivir at 600 mg twice daily resulted in a significant reduction in viral shedding duration and symptom duration in influenza A infection[116,117]. Dose-dependent adverse events, including diarrhea and neutropenia, were observed in clinical studies. However, in Phase III clinical trials, pimodivir did not

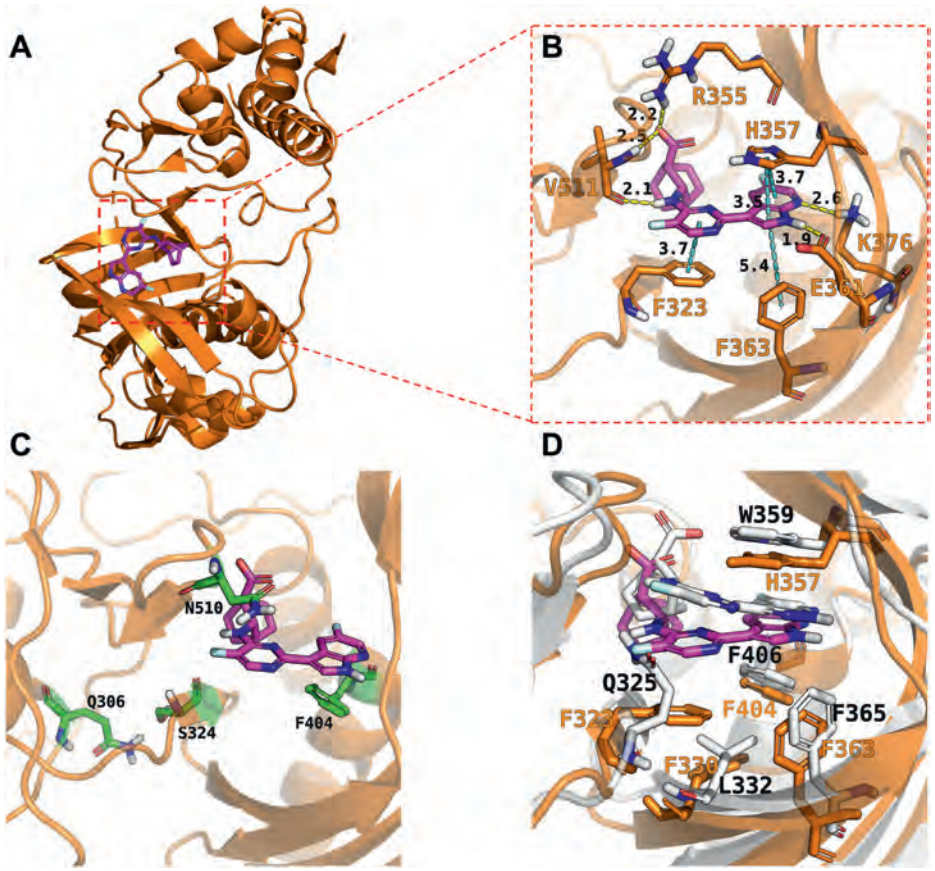


Figure 7 The binding mode of pimodivir to PB2 of IAV (PDB: 6EUV) and PB2 of IBV (PDB: 6EUX) is shown in Cartoons (A) and binding sites (B). (C) Residues liable to potential substitutions associated with resistance to pimodivir are displayed as green sticks: F404 (to Y), S324 (to I/N/R), Q306 (to H), N510 (to T). (D) The alignment and difference of pimodivir binding site of IAV PB2 (residues are depicted in gold and pimodivir in magenta) and IBV PB2 with VX-787N (depicted in white).

demonstrate superior benefits compared to established standard treatment in hospitalized patients with influenza A, exhibiting efficacy nearly equivalent to that of oseltamivir in head-to-head comparisons. Furthermore, the combination of pimodivir with oseltamivir did not demonstrate improved therapeutic efficacy compared to monotherapy[118]. Johnson & Johnson decided to discontinue further development of pimodivir in 2020. Although its clinical development was halted at Phase III, the compound's structure and mechanism of action have inspired the development of other PB2 inhibitors.

Resistance studies have shown that six PB2-resistant viral mutants (Q306H, S324I, S324N, S324I/N/R, F404Y, N510T*, and H357N) exhibit reduced sensitivity to pimodivir (Fig. 7C)[112,119,120]. The mutations caused a 63- to 257-fold increase in EC_{50}, either by introducing steric hindrance or destabilizing the "apo" conformation. For example, F404Y induces steric clashes with the inhibitor's hydroxyl group, while N510T reduces binding affinity by obstructing interactions between the threonine side chain and the inhibitor's fluorine atom. Mutations at S324 disrupt a critical hydrogen-bond network that stabilizes the midlink-cap-binding interface, and Q306H increases rigidity, thereby interfering with inhibitor binding. Additional amino acid substitutions potentially associated with pimodivir resistance, such as F325L, S337P, K376 N/R, T378S, and M431 L/R/V, have also been observed in clinical studies[121,122].

6.2. CC-42344

CC-42344, developed by Cocrystal Pharma through structure-based design, is another innovative PB2 inhibitor that targets the m^7GTP cap-binding site to inhibit influenza A virus transcription. It demonstrates activity against multiple influenza A strains, including H1N1, H3N2, H5N1, and H7N9, with IC_{50} values ranging from 0.1 to 9 nmol/L. Importantly, it remains active against strains resistant to oseltamivir and baloxavir. Multiple formulations, including oral, inhaled, and intravenous, have been evaluated in mice, achieving >60% oral bioavailability and showing no evidence of hepatic or cardiac toxicity. Phase I clinical trials assessing safety and pharmacokinetics in healthy volunteers confirmed good tolerability, with no serious adverse events (NCT05202379)[123,124]. A Phase IIa randomized, double-blind trial is underway to evaluate antiviral efficacy and dose response in influenza viral challenge study involving healthy participants, registered under NCT06160531.

6.3. Onradivir (ZSP1273)

Onradivir is the world's first approved PB2-targeting antiviral for the treatment of influenza A. It was developed jointly by Guangzhou National Laboratory, the First Affiliated Hospital of Guangzhou Medical University (FAHGMU), and Guangdong

Raynovent Biotech Co., Ltd. Our team at the National Center for Respiratory Medicine and the National Clinical Research Center for Respiratory Diseases, both affiliated to FAHGMU, is the primary investigator of phase II and phase III clinical trials. Onradivir potently inhibits PB2 polymerase with an IC_{50} of 0.56 nmol/L and demonstrates antiviral activity against H1N1 and H3N2 strains with EC_{50}s ranging from 0.01 to 0.063 nmol/L, which are substantially lower than those observed for the NA inhibitor oseltamivir and the PA inhibitor baloxavir marboxil[125]. It also shows strong inhibitory activity against oseltamivir-resistant strains, baloxavir-resistant strains (EC_{50} of 0.014−0.028 nmol/L), and against highly pathogenic avian influenza virus strains (EC_{50} of 0.245−0.777 nmol/L). In murine and ferret models, onradivir reduced viral titers and improved survival rates in a dose-dependent manner.

Onradivir binds to the PB2 cap-binding site similar to pimodivir in a substrate-competitive way. The strategy of rigidifying inhibitor-target interactions by introducing a cyclopropyl group at the 6-position of the pyrimidine ring enhances the PB2 target binding affinity and antiviral efficiency (Fig. 8A). The cyclopropyl fragment is a versatile motif that frequently appears in preclinical/clinical drugs, providing conformational restriction, and often favorably modifing pharmacokinetic properties by reducing the P-glycoprotein efflux ratio and increasing metabolic stability[126]. Additionally, inclusion of a cyclopropyl group can create new opportunities for intellectual-property protection[127]. Docking analyses reveal that the cyclopropyl moiety occupies a hydrophobic pocket (Fig. 8B and C). Onradivir forms stronger polar contacts through the pyrazole ring, establishing double hydrogen bonds with Glu361. Additional stabilizing interactions include a salt bridge between the carboxylic acid and Arg355, as well as improved complementarity within the binding pocket due to increased molecular rigidity. This more extensive interaction network confers stronger binding to PB2 and enhanced inhibitory activity. Onradivir also exhibits improved pharmacokinetic properties compared to pimodivir. It exhibited rapid absorption and dose-dependent plasma concentrations without significant food effects or drug accumulation[128]. In healthy adult subjects, a single oral fasting dose of 100−1200 mg of the drug was rapidly absorbed, with median T_{max} between 0.5 and 2 h. In subjects given a single 600 mg dose, the estimated mean AUC_{0-inf} were 20,002.6 ng h/mL, mean C_{max} was 6756.9 ng/mL, and a $T_{1/2}$ was 24.3 h. Onradivir also shows a high serum protein binding ratio of 99.9%. Onradivir is primarily metabolized by UGT1A3, UGT1A9, and UGT2B7, with glucuronidation and monooxygenation the main *in vivo* pathways. Studies on individuals with hepatic impairment indicate that liver dysfunction significantly affects the absorption and elimination, while renal impairment has little impact on onradivir pharmacokinetics[129,130]. Phase I clinical study with crossover treatment of onradivir and oseltamivir suggested no clinically significant drug−drug interaction between the two agents, and no dose adjustment was required for clinical use[131].

In multicentre, double-blind, randomized, placebo-controlled, Phase II clinical studies conducted between Dec 7, 2019, and May 18, 2020 (NCT04024137)[132]. During the COVID-19 pandemic in 2020−2022, influenza activity was temporarily reduced due to the execution of epidemic prevention and control measures[133]. Consequently, we halted recruitment ahead of schedule because the incidence of influenza had markedly declined. As a result, the trial enrolled only 172 participants (43% of the planned 400); most participants were relatively young, with a median age of 22 years. Median time to symptom alleviation was 46.92 h in the 200 mg twice-daily group, 54.87 h in the 400 mg twice-daily group, and 40.05 h in the 600 mg once-daily group. Compared with the placebo group (62.87 h), a reduction in median time to symptom alleviation was observed in all three treatment groups.

As influenza activity returned to pre-pandemic levels in many countries for the 2022−2023 season, we conducted a multicenter, randomized, double-blind, placebo- and active-controlled Phase III trial (NCT04683406) at 68 sites in China from May 12, 2022, to May 16, 2023. The trial evaluated the efficacy and safety of 5 days of oral onradivir *versus* placebo or oseltamivir in adults (18−64 years) with uncomplicated influenza. A total of 750 subjects who tested positive for influenza A by rapid antigen test were randomized 2:1:1 to receive onradivir (600 mg once daily), oseltamivir phosphate capsules (75 mg twice daily), or placebo for 5 days. The primary efficacy endpoint was the time from the first dose to the first observation at which all seven influenza symptoms (cough, sore throat, headache, nasal congestion, fever or chills, muscle or joint pain, and fatigue) were alleviated, and remained improved for at least 24 h. The TTAS was 38.83 h in the onradivir group, which was 39% shorter than the placebo group (63.35 h) and 8% shorter than the oseltamivir group (42.17 h)[134,135]. Onradivir showed faster and greater reductions in viral titres and RNA loads, with median time to viral clearance of 68.65 h compared to both placebo (86.03 h) and oseltamivir (88.62 h), demonstrating superior antiviral activity.

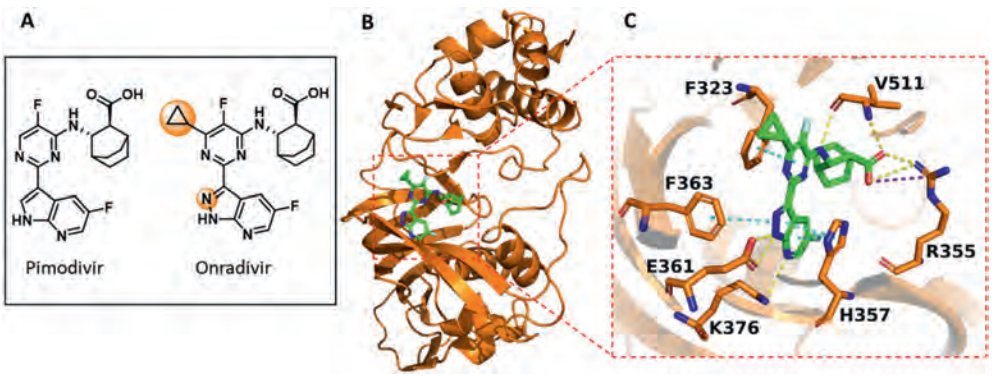


Figure 8 Structure of pimodivir and onradivir (A) and the predicted binding mode of onradivir to the cap binding domain of IAV PB2 (B). Representative structures illustrating key interactions between the onradivir with PB2 (C). Onradivir was docked into PB2 (PDB: 6EUV) and visualized using PyMOL 2.0 software.

In the two clinical trials, the overall incidence of post-treatment amino acid substitutions associated with reduced onradivir susceptibility was 0% (0/76) and 1.6% (3/189), respectively[134,135]. Post-treatment substitutions suspected to be associated with reduced susceptibility to onradivir included N510T, M475I/I554del, and I674M. Compared with pimodivir, onradivir demonstrated a reduced occurrence of variants with decreased susceptibility, and these variants displayed smaller fold changes in EC_{50}. These findings indicate a low risk of resistance development in patients treated with onradivir, suggesting that the rigidified inhibitor-target interactions (*via* cyclopropyl groups) and stronger inhibition may reduce mutational escape. Since the viral protein targets differ, cross-resistance between onradivir and NA inhibitors, M2 proton-channel inhibitors, or PA inhibitors is not expected. As an antiviral agent with a novel mechanism of action against influenza, onradivir remains highly effective against strains resistant to NA inhibitors and PA inhibitors, representing a valuable addition to the therapeutic arsenal against influenza.

7. Perspectives and challenges

The last two decades have witnessed significant advancements in influenza trimeric RNA polymerase complex research, progressing from the elucidation of structural and functional mechanisms to the approval of three types of inhibitors for clinical use. In addition to these six approved drugs, several other drug candidates are in various stages of clinical development. The development of influenza virus RNA polymerase inhibitors holds great promise for expanding the therapeutic arsenal against influenza, particularly in light of increasing NA inhibitor resistance and evolving viral strains. The development of FluPol inhibitors, through both successes and setbacks, yields valuable insights for future drug research. First, the success of structure-based drug design (SBDD) in developing PA and PB2 inhibitors highlights the indispensable role of high-resolution FluPol structural data and the importance of structural biology in modern drug research[51]. A multifaceted strategy integrating structural biology, medicinal chemistry, and pharmacokinetic optimization has proven essential for the successful development of FluPol inhibitors. In the current era of artificial intelligence, integrating AI-driven modeling and prediction tools with structure insight of modulators binds to target holds great potential to accelerate and enhance the design of more effective inhibitors. Second, the strategy of rigidifying inhibitor-target interactions has been effective in both improving antiviral potency and reducing the concurrence of resistance. For example, onradivir, which contains a cyclopropyl moiety, exhibits enhanced activity and a low rate of resistance emergence. Similar effects are observed with suraxavir marboxil, which features spirocyclomethyl groups fused into the tricyclic metal-chelating pharmacophores. Third, the development of the marboxil prodrugs targeting the PA subunit successfully addressed bioavailability challenges, underscoring the importance of early pharmacokinetic profiling in drug development. Early optimization of absorption, distribution, metabolism, and excretion properties is essential in advancing promising compounds toward clinical use.

It is worth noting that, the rise in human infections with highly pathogenic avian influenza (HPAI) and its high fatality rate pose a potential and continuous threat. The avian influenza A(H7N9) virus is a major global public health concern due to its pandemic potential. While evidence supports the therapeutic effects of oseltamivir, available NA inhibitors exhibit limited efficacy in reducing viral titers. Studies have demonstrated the inhibitory effects of baloxavir against A(H7N9) *in vitro* and *in vivo*, including the NA-R292K mutant virus and highly pathogenic avian influenza strains[136]. Recently, highly pathogenic avian influenza A(H5N1) (HA clade 2.3.4.4b) emerged in North America, infecting US dairy cows and subsequently humans, with severe cases requiring hospitalization and fatalities reported[137]. Baloxavir improved disease outcomes in mice after intranasal or ocular infection with H5N1-contaminated cow's milk[138]. Additionally, the PB2 inhibitor onradivir exhibited an IC_{50} below 1 nmol/L against oseltamivir-resistant H7N9 or H5N6 avian influenza strains. These findings suggest that polymerase inhibitors could be valuable for treating severe infections and for pandemic preparedness, particularly in cases involving oseltamivir-resistant viruses. A clinical study we conducted in Southern China involving five critically ill influenza A H5N6 patients treated with baloxavir marboxil showed rapid declines in viral load and cytokine levels. Of these patients, two survived, while three succumbed to comorbidities and secondary bacterial infections[139]. Emerging evidence suggests that the combination of NA inhibitors and polymerase inhibitors may enhance antiviral efficacy; however, the combination therapy still needs further clinical validation. Polymerase inhibitors hold potential for treating HPAI infection and stockpiling for pandemic preparedness. However, their clinical application still needs to be validated through preclinical modeling and clinical observations.

The high sequence variability and mutability of influenza A pose a critical challenge for anti-influenza medications, threatening the long-term efficacy of these drugs. Resistance mutations to baloxavir, pimodivir, or favipiravir have been identified both in laboratory and clinical settings[31]. A typical resistance-associated mutation occurs at the key residue adjacent to the inhibitor binding sites. To date, no cross-resistance mutations affecting two different classes of inhibitors have been reported. Nevertheless, ongoing monitoring of drug susceptibility is essential for public health planning and clinical antiviral recommendations. Systematic surveillance of circulating influenza viruses across multiple seasons and regions is needed to understand the epidemiology and clinical implications of polymerase inhibitor resistance. Combination therapies involving multi-target inhibitors may help delay resistance, though clinical validation remains limited.

Finally, the continued development of novel drugs with distinct mechanisms and resistance profiles, combined with novel drug discovery technology remains a key task in influenza antiviral research. A variety of strategies have been proposed and advanced in recent years[30,140]. Bifunctional molecules that target multiple viral or host factors or that combine antiviral and anti-inflammatory activities, can enhance therapeutic efficacy while mitigating resistance. PROTACs have emerged as a potential strategy that leverages the ubiquitin−proteasome system to degrade viral proteins. The first example in anti-influenza drug design was an oseltamivir-based PROTAC developed by Xu et al.[141]. Compound **8e** effectively degraded the NA protein by using E3 ligase ligands VHL and demonstrated potent antiviral activity against both wild-type H1N1 virus and an oseltamivir-resistant strain. Compound APL-16-5, isolated from the endophytic fungus, was reported to bind simultaneously to the E3 ligase TRIM25 and FluPol PA subunit, promoting TRIM25-mediated ubiquitination of PA and its subsequent proteasomal degradation. As a molecular glue, APL-16-5 showed antiviral activity against IAV (EC_{50} = 0.28 μmol/L) and IBV (EC_{50} = 1.22 μmol/L) and protected mice from lethal influenza A challenge[142]. The outcome of PROTAC action depends on the race

between the rate of PROTAC-mediated degradation and the rate of new target-protein synthesis. The target protein expression level (*i.e.*, the initial "inventory") and its expression rate (*i.e.*, the "replenishment speed") are primary determinants of degradation success. During acute influenza infection, viral proteins are often produced at high levels and replenished rapidly, posing intrinsic challenges for PROTAC-based anti-influenza therapeutics. Therefore, designing degraders with higher degradation potency and a longer *in vivo* half-life may be critical to achieve successful degradation.

In recent years, protein−protein interaction (PPI) inhibitors targeting the heterodimerization interface between the Fulpol complex or virus-host interactions involving FulPol subunits have been reported[52,143,144]. Disrupting these PPIs offers a promising strategy to develop compounds with broad activity and a reduced propensity for resistance[17,145,146]. Among these interfaces, the relatively small but highly conserved hydrophobic interface at PA-C, which forms a deep groove for PB1-N binding, is well-suited for inhibition by small molecules. Targeting the PA-PB1 interface has shown particular promise. One of the most potent inhibitors identified is a cycloheptathiophene derivative, which exhibited EC_{50} values ranging from 0.08 to 0.46 μmol/L against a panel of influenza A and B viruses, including HPAIVs, and showed a high barrier to resistance *in vitro*[147]. More recently, a C-3-substituted oleanolic acid benzyl amide derivative **A5** was reported and showed antiviral EC_{50} values between 0.60 and 1.83 μmol/L, with affinity (K_D) to PA of 0.07 μmol/L as determined by surface plasmon resonance experiments[148]. Although many of the current lead inhibitors are still in the early stages of development and require substantial further optimization, they lay a strong foundation for overcoming resistance and advancing the development of next-generation, broad-spectrum anti-influenza therapeutics targeting FluPol.

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Author contributions

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Conflicts of interest

The authors declare no conflict of interest.

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