Targeting Influenza Virus RdRp PB2 Subunit: Advances in Inhibitor Development for Antiviral Therapy

Qianhao Qin*

School of Applied Chemistry, Capital Normal University, Beijing, 100048, China

*Corresponding author: qinqianhao65@ldy.edu.rs

Abstract. Influenza remains a significant global health challenge, causing seasonal outbreaks and occasional pandemics. Current antiviral treatments are often limited by the virus's rapid mutation and the emergence of drug resistance. The RNA-dependent RNA polymerase (RdRp) complex, composed of PA, PB1, and PB2 subunits, is crucial for viral replication, with PB2 playing a key role in binding host pre-mRNA during viral RNA synthesis. Targeting PB2 has become an attractive strategy for developing novel antivirals. In recent years, several small-molecule inhibitors have been identified that specifically disrupt PB2's function, offering a promising approach to halting viral replication. This review explores the discovery and development of PB2 inhibitors, focusing on their structure-activity relationships and mechanisms of action. It also evaluates their effectiveness against various influenza strains, including drug-resistant variants, and highlights challenges in advancing PB2-targeted therapies. By analyzing current progress, this review aims to provide insights for future research in developing more effective antiviral strategies against influenza.

Keywords: Influenza virus; RNA-dependent RNA polymerase; PB2 subunit; Influenza polymerase inhibitors.

1. Introduction

Influenza remains a critical global health concern due to its high transmissibility and the significant burden it imposes through seasonal epidemics and occasional pandemics. The influenza virus, characterized by its rapid mutation rate, poses a continuous challenge to existing treatment strategies, necessitating the development of novel antiviral approaches. Central to the influenza virus's lifecycle is the RNA-dependent RNA polymerase (RdRp) complex, which is indispensable for viral RNA replication and transcription. This complex is composed of three subunits: PA, PB1, and PB2. Among these, the PB2 subunit is particularly crucial as it facilitates the recognition and binding of host pre-mRNA, a step essential for the synthesis of viral mRNA.

Recent research has spotlighted PB2 as a promising target for antiviral drug development due to its fundamental role in viral replication. Targeting the PB2 subunit offers a strategic approach to disrupting viral RNA synthesis and inhibiting viral proliferation. This review aims to provide a comprehensive overview of the influenza virus RdRp, with a focused examination of the PB2 subunit. It will detail the design and discovery of small-molecule PB2 inhibitors, analyze their structure-activity relationships, and elucidate the mechanisms through which these inhibitors impair viral replication. Additionally, the review will assess the antiviral efficacy of these inhibitors against various influenza strains, including those resistant to existing treatments, and address the challenges and limitations associated with PB2-targeted therapies. By synthesizing current research, this review seeks to underscore the advancements in PB2 inhibitor development and propose future directions for enhancing antiviral treatment strategies targeting this pivotal viral component.

2. Key Basics

2.1. Influenza

Seasonal influenza (commonly known as the flu) is an acute viral infection that is easily transmitted between individuals. The influenza A virus is highly contagious and often leads to global pandemics. According to data from the World Health Organization, respiratory diseases caused by

seasonal influenza are estimated to result in approximately 3 to 5 million cases of severe illness and 290,000 to 650,000 deaths annually (Figs.1 and 2). It is estimated that the number of influenza-related respiratory deaths each year is higher than the figures reported by WHO, including deaths related to both respiratory and circulatory systems. The most effective way to prevent influenza virus infection is through the use of influenza vaccines, but licensed vaccines may provide insufficient protection against seasonal influenza and need to be updated annually to match circulating strains of the virus [1,2].

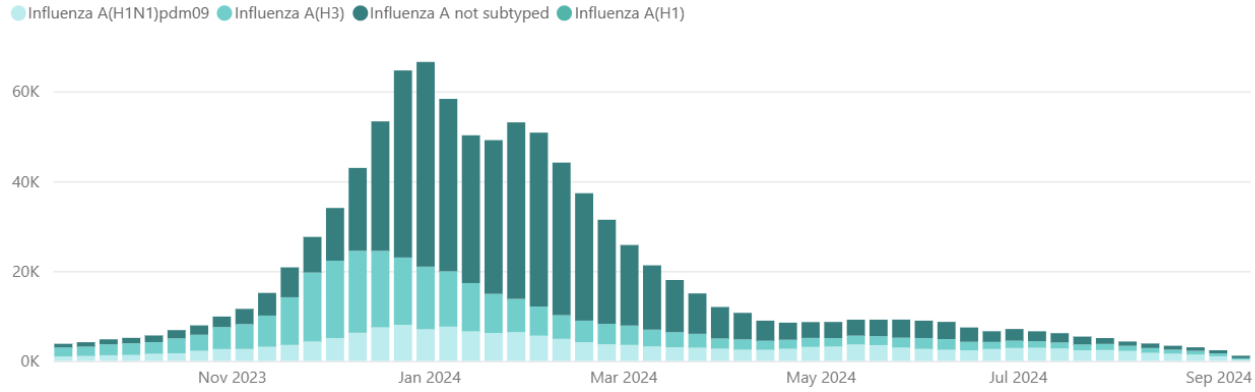


Fig. 1 Global influenza incidence since Sep. 2023 [3].

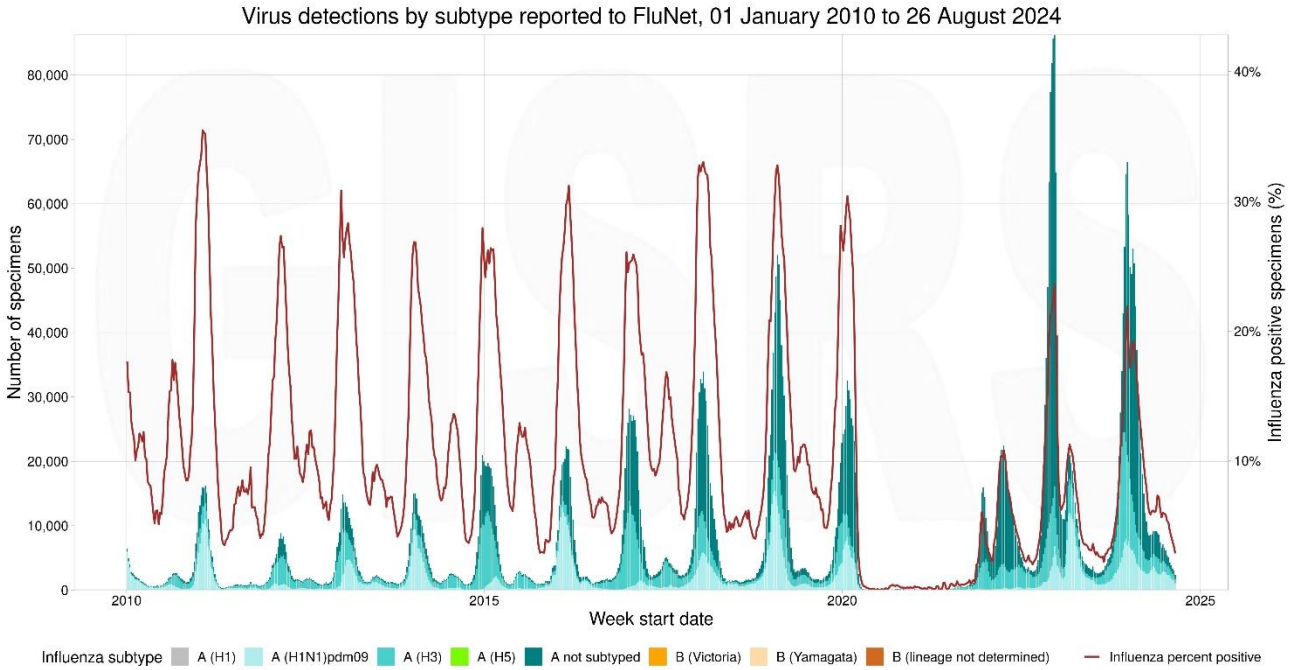


Fig. 2 Total number of influenza virus infections worldwide (2010-2024) [3].

2.2. Other Treatments for Influenza Virus

The main methods for preventing and controlling influenza include vaccination, antiviral drugs, and supportive treatment. Influenza vaccination is the most effective means of preventing infection. Each year, the vaccine composition is updated according to circulating viral strains to reduce infection rates, severe cases, and mortality, especially providing crucial protection for high-risk groups such as the elderly, young children, and individuals with chronic diseases. Additionally, supportive treatment is used to alleviate symptoms and promote recovery, including the use of antipyretics, cough suppressants, and fluid therapy, as well as oxygen therapy or mechanical ventilation in severe cases. These two approaches play a key role in influenza prevention and control, particularly in addressing viral mutations and antiviral drug resistance.

Despite the importance of vaccination and supportive treatment in influenza control, both have certain limitations. First, the effectiveness of vaccination depends on how well the annual vaccine matches the circulating strains. Rapid viral mutations (antigenic drift) can reduce the protective effect of the vaccine. Moreover, the immune response to the vaccine in elderly individuals and those with weakened immune systems may be insufficient to provide adequate protection. On the other hand, supportive treatment only relieves symptoms without directly inhibiting viral replication or preventing the spread of influenza. In severe cases, supportive treatment depends on medical resources, such as oxygen therapy and mechanical ventilation, which increase the healthcare burden. This is particularly concerning during large-scale influenza outbreaks, which may overwhelm healthcare systems. These limitations indicate that while vaccines and supportive treatment are crucial in influenza control, the development of more effective antiviral drugs is still necessary [4].

2.3. Representative Antiviral Drugs Used Clinically for Influenza

M2 Ion Channel Inhibitors: For example, Amantadine. This class of drugs is effective only against influenza A and not influenza B. Due to the widespread resistance of current circulating strains to Amantadine, this drug is no longer recommended for clinical use [5].

Neuraminidase Inhibitors: Such as Oseltamivir. These are currently the frontline treatments for influenza. Neuraminidase inhibitors have a moderate impact on the severity and duration of influenza symptoms. Oseltamivir is widely used to treat seasonal influenza virus infections but must be administered within 48 hours of infection to achieve satisfactory results [6]. However, resistant strains to Oseltamivir have emerged, reducing its therapeutic efficacy [7].

Hemagglutinin Inhibitors: A typical example is Arbidol, developed by the former Soviet Union's research center. This drug is available only in Russia and mainland China.

RdRp Inhibitors: Two drugs in this category are available. One is Favipiravir, a PB1 inhibitor, which is approved only in Japan and mainland China for limited indications. The other is Baloxavir marboxil, a PA inhibitor (i.e., cap-dependent endonuclease inhibitor), which is the first antiviral with an innovative mechanism of action in the past 20 years [8]. However, resistant strains to Baloxavir marboxil were discovered shortly after its market release, and there are concerns about its safety [9,10].

3. PB2 Inhibitors of Influenza Virus

3.1. The PB2 Subunit of the Influenza Virus

When receiving the paper, we assume that the corresponding authors grant us the copyright to use the paper for the book or journal in question. When receiving the paper, we assume that the corresponding authors grant us the copyright to use.

The RdRp of the influenza virus is a heterotrimeric protein composed of three subunits: polymerase acid (PA), polymerase basic 1 (PB1), and polymerase basic 2 (PB2). This enzyme plays a crucial role in the transcription and replication of the influenza virus genome [11]. The X-ray diffraction structure of the human influenza A virus polymerase complex at 3.31 Å resolution is shown in Fig. 3 (PDB No: 6QNW) [12].

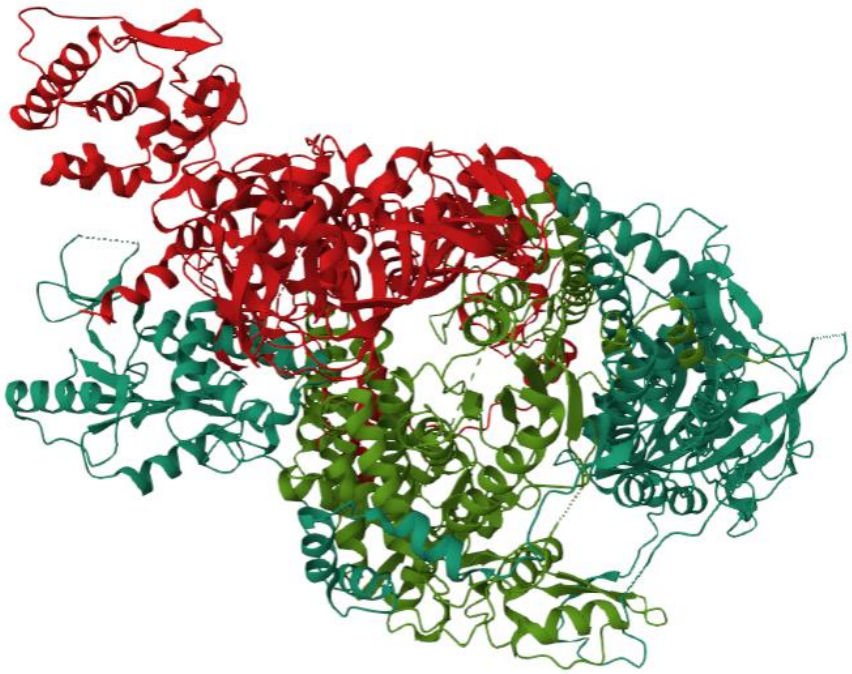


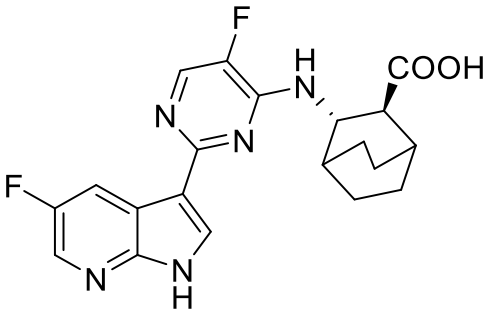
Fig. 3 X-ray diffraction structure of the influenza A virus polymerase. Note that the dark green represents the PB1 subunit, red represents the PB2 subunit, and cyan represents the PA subunit [13].

RNA polymerase is a key enzyme in the replication of influenza A virus, and the PB2 subunit of the influenza RNA-dependent RNA polymerase (RdRp) has been identified as a promising target for influenza treatment. The PB2 subunit is a compact, orderly α-β fold characterized by a cap-binding domain, which is distinct from other host cap-binding proteins. This domain is capable of recognizing the 5' cap of host cell mRNA, which is crucial for the effective translation of the viral genome [14].

3.2. Research Progress on PB2 Inhibitors

3.2.1 Pimodivir

Among the PB2 inhibitors developed so far, the most influential drug is Pimodivir (Vx-787, JNJ-63623872) (Fig. 4), an oral PB2 inhibitor developed by Vertex Pharmaceuticals for the treatment of influenza A. Pimodivir was first reported in 2014 [15]. In March 2017, the U.S. Food and Drug Administration (FDA) approved Pimodivir for emergency use in treating influenza A infections in hospitalized patients or those at high risk for complications.



Vx-787

Fig. 4 Structure of Pimodivir. (Picture credit: Original)

Pimodivir is primarily composed of three components: an indole nitrogen, a pyridine ring, and a bicyclic octane structure. It has good activity against the influenza A virus, including viruses resistant to M2 inhibitors and viruses resistant to NA inhibitors, but is basically inactive against the influenza

B virus. The bicyclic octane structure has been the most extensively studied. Farmer et al. demonstrated that substituting the bicyclic octane with various substituents revealed that tert-butyl is an excellent substituent. Among these, compound 4 showed the best activity (Fig. 5) [16].

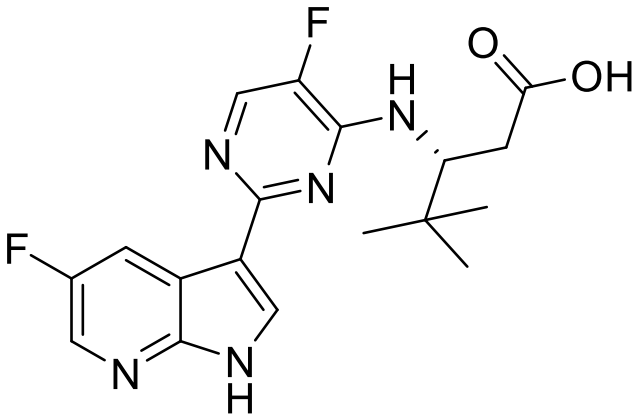


Fig. 5 Tert-butyl substituted small molecule inhibitor. (Picture credit: Original)

3.2.2 Baloxavir

VX-657 (Fig. 6), also known as baloxavir marboxil, is primarily a PA (polymerase acidic) inhibitor rather than a PB2 inhibitor like Pimodivir. It features a distinct chemical structure optimized for inhibiting the PA subunit of the influenza virus RNA-dependent RNA polymerase. The drug was designed to interfere with the endonuclease activity of the PA subunit, which is crucial for viral RNA synthesis.

Baloxavir marboxil's structure includes a unique combination of functional groups that enhance its binding affinity and specificity for the PA subunit. The drug is known for its novel mechanism of action compared to traditional neuraminidase inhibitors and has been shown to be effective against a broad range of influenza strains, including those resistant to other treatments [17,18].

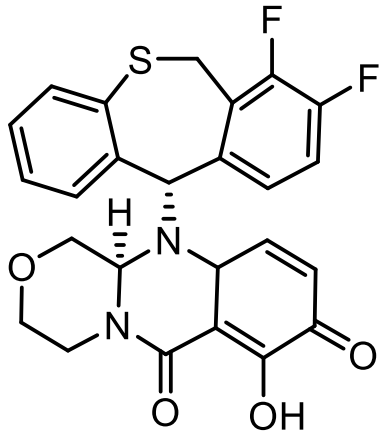


Fig. 6 Baloxavir (Picture credit: Original)

3.2.3 ZSP1273

Guangdong Zhongsheng Ruichuang Biotechnology Co., Ltd. in China has developed a new PB2 inhibitor, ZSP1273, by replacing the indole ring in Pimodivir with a pyrazole and pyridine ring. This drug has completed Phase III clinical trials for treating influenza A virus. ZSP1273 exhibits high target selectivity and has demonstrated in vitro antiviral activity against influenza virus that is 1,000 times more effective than the marketed drug Oseltamivir (Fig. 7) [19]. The application for its market approval is currently under review.

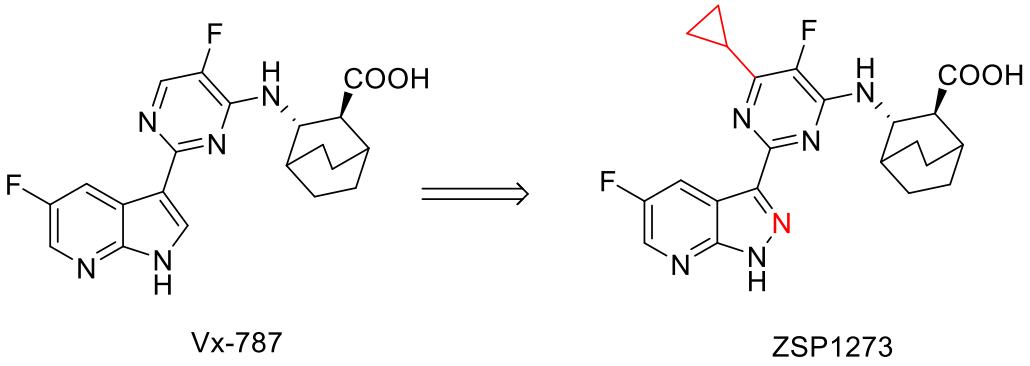


Fig. 7 Development of ZSP1273 from Pimodivir as the lead compound. (Picture credit: Original)

Guillemont's research team modified Pimodivir by replacing the fluorine atom at position 5 with fluorine atoms at positions 4 and 6, resulting in compound 15 (Fig. 8) [20]. This compound has been evaluated as an effective and metabolically stable influenza virus inhibitor, demonstrating favorable oral pharmacokinetic properties and in vivo efficacy. The cell-based assay for antiviral activity in canine kidney cells showed that compound 15 has IC50 values of 11 nmol/L for H1N1 (A/Puerto Rico/8/34) and 14 nmol/L for H3N2 (A/Virginia/3/1975), with selectivity indexes (SI) of 145 and 114, respectively. The use of PEG400 as a carrier confirmed that the molecule has good stability in liver microsomes (clearance rate < 7.7 μL/(min·mg)$^{-1}$ protein). Furthermore, compound 15 did not show significant metabolism in human liver cells, either with or without the presence of raloxifene (an aldehyde oxidase inhibitor), indicating that it is not a substrate of aldehyde oxidase. This is a novel and interesting finding, as the molecule's indole nitrogen at position 2 is not protected but still avoids aldehyde oxidase metabolism, offering an advantage over previously described inhibitors.

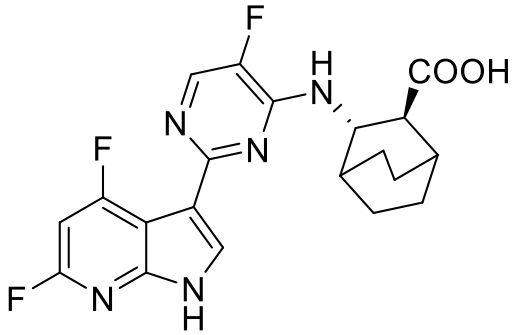


Fig. 8 4,6-Difluoro indole derivatives. (Picture credit: Original)

4. Conclusion

This review provides an in-depth examination of recent research on the RNA-dependent RNA polymerase (RdRp) of the influenza virus, with a particular emphasis on the PB2 subunit. The key finding is that PB2 plays a crucial role in viral replication and transcription, making it an attractive target for antiviral drug development. Several PB2 inhibitors have been identified, demonstrating potent antiviral activity by disrupting the interaction between the PB2 subunit and the viral RNA. The review highlights the discovery of these inhibitors, their structure-activity relationships, and their mechanisms of action, including their ability to inhibit RNA synthesis in various influenza strains, including drug-resistant variants.

The impact of this research is significant, as it addresses the urgent need for new antiviral strategies amid the limitations of existing treatments, such as drug resistance and limited efficacy against emerging strains. By focusing on the PB2 subunit, the review underscores its potential as a promising therapeutic target, offering a new avenue for the development of more effective influenza treatments.

However, the review also identifies several challenges and limitations. Despite progress, PB2 inhibitors face obstacles in terms of optimizing their pharmacokinetics, reducing toxicity, and

ensuring broad-spectrum activity across different influenza subtypes. Furthermore, the emergence of resistance to PB2 inhibitors remains a concern that warrants continuous monitoring.

Looking ahead, the review suggests future research should focus on overcoming these limitations, including the design of novel PB2 inhibitors with improved efficacy and safety profiles. Additionally, exploring combination therapies with existing antiviral drugs may enhance treatment outcomes and reduce the risk of resistance development. The review concludes by emphasizing the need for continued efforts to fully exploit the therapeutic potential of PB2-targeted antivirals.

References

[1] Liao Hui, Li Yinyan, Yu Luqiang, et al. Design, synthesis and structure-activity relationship of dihydrobenzoquinolines as novel inhibitors against influenza A virus. European Journal of Medicinal Chemistry, 2022, 244: 114799.

[2] Zhou Zhen, Liu Tian, Zhang Jun, et al. Influenza A virus polymerase: an attractive target for next-generation anti-influenza therapeutics. Drug Discovery Today, 2018, 23(3): 503-518.

[3] World Health Organization. Virus detections by subtype reported to FluNet. September 14, 2024. Retrieved on September 14, 2024. Retrieved from: https://www.who.int/

[4] Principi Nicola, Esposito Susanna. Drugs for influenza treatment: is there significant news? Frontiers in Pharmacology, 2020, 11: 1-15.

[5] Mills Christina E, Robins James M and Lipsitch Marc. Transmissibility of 1918 pandemic influenza. Nature, 2004, 432(16): 904-906.

[6] Moscona Anne. Neuraminidase inhibitors for influenza. The New England Journal of Medicine, 2005, 353: 1363-1373.

[7] Moscona Anne. Global transmission of oseltamivir-resistant influenza. The New England Journal of Medicine, 2009, 360(10): 953-956.

[8] Feng Jingjing, Guo Fuyuan, Li Ping, et al. Discovery of a macrocyclic influenza cap-dependent endonuclease inhibitor. Journal of Medicinal Chemistry, 2024, 67(4): 2570-2583.

[9] Takashita Emi, Kawakami Chika, Morita Hiroki, et al. Detection of influenza A (H3N2) viruses exhibiting reduced susceptibility to the novel cap-dependent endonuclease inhibitor baloxavir in Japan. Eurosurveillance, 2019, 24(3): 1800698.

[10] Yoshino Ryo, Yasuo Nobuhiro, Sekijima Masakazu. Molecular dynamics simulation reveals the mechanism by which the influenza cap-dependent endonuclease acquires resistance against baloxavir marboxil. Scientific Reports, 2019, 9: 17464.

[11] Chen Fang, Yang Li, Zhai Liang, et al. Methyl brevifolin carboxylate, a novel influenza virus PB2 inhibitor from Canarium Album (Lour.) Raeusch. Chemical Biology & Drug Design, 2020, 96(5): 1280-1291.

[12] Fan Haibo, Walker Andrew P, Carrique Lilia, et al. Structures of influenza A virus RNA polymerase offer insight into viral genome replication. Nature, 2019, 573: 287-290.

[13] Li Xinhong, Xu Yijie, Li Wei, et al. Design, synthesis, biological evaluation, and molecular dynamics simulation of influenza polymerase PB2 inhibitors. Molecules, 2023, 28, 1849.

[14] Chen Wenteng, Shao Jiaan, Ying Zhimin, et al. Approaches for discovery of small-molecular antivirals targeting the influenza A virus PB2 subunit. Drug Discovery Today, 2022, 27(6): 631-638.

[15] Clark Michael P, Ledeboer Mark W, Davies Ian, et al. Discovery of a novel, first-in-class, orally bioavailable azaindole inhibitor (VX-787) of influenza PB2. Journal of Medicinal Chemistry, 2014, 57: 6668–6678.

[16] Farmer Leslie J, Clark Michael P, Boyd Michael J, et al. Discovery of novel, orally bioavailable β-aminoacid azaindole inhibitors of influenza PB2. ACS Medicinal Chemistry Letters, 2017, 8(2): 256-260.

[17] Shinya Kawaoka, Kiso Masaki and Yamayoshi Shuichi. The X-Stand structure of baloxavir marboxil: a new antiviral drug with a novel mechanism of action against influenza A and B virus. Journal of Virology, 2014, 88(12): 7100-7110.

[18] Inoue Kazuhiro and Matsumoto Kazuhiko. Baloxavir marboxil for the treatment of influenza: clinical efficacy and safety profile. The Lancet Infectious Diseases, 2020, 20(5): 539-550.

[19] Chen Xiaoxin, Ma Qinhai, Zhao Manyu, et al. Preclinical study of ZSP1273, a potent antiviral inhibitor of cap binding to the PB2 subunit of influenza A polymerase. Pharmaceuticals, 2023, 16(3): 365-395.

[20] McGowan David C, Balemans Wouter, Embrechts Wouter, et al. Design, synthesis, and biological evaluation of novel indoles targeting the influenza PB2 cap binding region. Journal of Medicinal Chemistry, 2019, 62: 9680-9690.