A Comprehensive Review of Anti-Influenza Therapeutics

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Abstract: Influenza viruses cause significant global morbidity and mortality. Currently approved anti-influenza drugs include adamantanes, neuraminidase inhibitors and polymerase inhibitors that target different stages of viral life cycle. Adamantanes such as rimantadine and amantadine inhibit the viral M2 ion channel protein. Oseltamivir, zanamivir, peramivir and laninamivir are neuraminidase inhibitors that bind and block the enzymatic activity of neuraminidase. Baloxavir marboxil and favipiravir are Cap-dependent endonuclease and RNA polymerase inhibitors respectively. These drugs are commonly quantified using chromatographic and spectrophotometric methods. Moreover, newer drugs such as verdinexor inhibiting viral mRNA export and ASN2 targeting cap-snatching endonuclease are under development. Advances have been made in delivery using nanoparticles, pulmonary and intranasal routes. Point-of-care molecular diagnostics aid rapid diagnosis. However, drug resistance and adverse effects persist as challenges. Future directions involve host-targeting drugs, vaccines inducing broad immunity and resistance-proof formulations. This review discusses about the approved and emerging anti-influenza therapies with analytical and delivery aspects along with ongoing efforts to address existing limitations.

Keywords: Anti-influenza drugs; Analytical methods; Drug delivery; Drug resistance; Point-of-care diagnostics.

1. Introduction

Influenza viruses cause seasonal epidemics and occasional pandemics that have significant health, social and economic impacts worldwide. [1-3] The influenza virus belongs to the Orthomyxoviridae family and is classified into types A, B, C and D based on antigenic differences in viral nucleoproteins and matrix proteins. Among these, influenza A and B are the major cause of seasonal flu epidemics in humans. Influenza A viruses further differentiate into subtypes based on two surface glycoproteins: hemagglutinin (HA) and neuraminidase (NA). [4] The 18 HA and 11 NA subtypes identified so far in birds and some other species can reassort to give rise to novel strains with pandemic potential. The global burden of seasonal influenza is substantial with an estimated 1 billion cases, 3-5 million cases of severe illness and 290,000-650,000 deaths annually according to WHO. Influenza pandemics have occurred irregularly throughout history, caused by the emergence of an entirely new HA subtype in humans to which population immunity is largely absent. The most notorious pandemics were the 1918 Spanish flu (H1N1) causing an estimated 20-50 million deaths, the 1957 Asian flu (H2N2) and the 1968 Hong Kong flu (H3N2). The 2009 H1N1 pandemic was relatively mild but highlighted ongoing pandemic risks. Influenza viruses also pose a significant threat in immunocompromised patients and the elderly due to increased risk of severe outcomes. [5]

The immunity induced by previous infections or vaccinations wanes over time owing to antigenic drift and shift in circulating strains necessitating annual vaccination. [6] Although vaccination is the cornerstone of prevention, the supply chain vulnerabilities during the COVID-19 pandemic underscored the need for effective therapeutics as part of pandemic preparedness. Currently licensed antiviral drugs target different stages of the virus life cycle including penetration, uncoating, replication, assembly and budding. Adamantanes like rimantadine and amantadine block the M2 ion channel whereas neuraminidase inhibitors (NAIs) including oseltamivir, zanamivir and peramivir inhibit the neuraminidase enzyme activity. Baloxavir marboxil is a relatively new endonuclease inhibitor that mechanistically differs from existing drugs. [7]

While effective against susceptible strains when administered early, antiviral resistance mediated via mutations, particularly to adamantanes and earlier NAIs limits their clinical utility now. There is an urgent need for new antiviral mechanisms, host-targeting agents and resistance-proof formulations. [8] Promising drug candidates in clinical development include verdinexor inhibiting viral mRNA export, ASN2 targeting cap-snatching endonuclease and favipiravir, a broad-spectrum polymerase inhibitor. Novel agents

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may also act on host factors hijacked by the virus. Inhalational and intranasal delivery approaches are being explored to achieve higher lung concentrations. Rapid point-of-care diagnostics play a key role in timely treatment decisions. However, cost and infrastructure challenges limit widespread availability, especially in resource-poor settings. Host biomarkers may predict disease severity early but require validation. In vitro and in vivo analyses employ standard techniques like chromatography, spectroscopy as well as cell culture and animal infection models. Biosafety concerns necessitate the use of alternative surrogates like influenza virus-like particles or minigenome systems. [9-11] This review summarizes approved anti-influenza drugs, analytical methods for quality control and key advances. It also identifies current challenges and outlines strategies to develop more effective, accessible and pandemic-ready therapeutics through innovative science and global cooperation in the face of continued influenza threats. Strategic investments in antiviral research are imperative to curtail both seasonal and pandemic impacts.

2. Approved anti-influenza drugs

Currently, three classes of antiviral drugs are approved globally for treating influenza - adamantanes, neuraminidase inhibitors and polymerase inhibitors. They target specific viral proteins and inhibit different stages of the viral life cycle.

2.1. Adamantanes

Adamantanes were the first class approved in the 1960s but now have limited clinical utility due to widespread resistance. The two adamantane drugs - rimantadine and amantadine work by blocking the influenza A M2 ion channel protein. [12-14] Rimantadine was approved in 1994 for influenza A therapy and chemically resembles amantadine in structure (Table 1). It is administered orally as hydrochloride salt with common gastrointestinal side effects. Rimantadine's absorption is 70-80% with a half-life of 12-17 hours and it is metabolized via N-demethylation and hydroxylation. Various analytical techniques including UV-Vis spectrophotometry, GC and HPLC have been used for quantification. Amantadine was licensed in 1976 also for influenza A. It exists as a racemic mixture but the S(+) enantiomer alone possesses antiviral activity. Amantadine is well absorbed orally with a bioavailability around 80% and plasma half-life of 12-17 hours. [15] It crosses the blood-brain barrier and is excreted renally. Amantadine is increasingly used off-label for Parkinson's disease treatment to reduce dyskinesia side effects. However, usage is now curtailed due to high adamantane resistance (>90%) observed globally.

Table 1. Properties of Adamantenes

<table>
<thead>
<tr>
<th>Drug</th>
<th>Chemical Structure</th>
<th>Approval Year</th>
<th>Mechanism of Action</th>
<th>Administration Route</th>
<th>Pharmacokinetics</th>
<th>Resistance</th>
<th>Clinical Use</th>
<th>Adverse Effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rimantadine</td>
<td><img src="image" alt="H2N" /></td>
<td>1994</td>
<td>Blocks M2 ion channel protein preventing viral RNA release into host cell</td>
<td>Oral</td>
<td>Well absorbed orally, t1/2 12-17 hrs, metabolized via N-demethylation and hydroxylation</td>
<td>M2 gene mutations confer resistance</td>
<td>Influenza A treatment</td>
<td>GI disturbances, insomnia</td>
</tr>
<tr>
<td>Amantadine</td>
<td><img src="image" alt="NH2" /></td>
<td>1976</td>
<td>Blocks M2 ion channel protein and inhibits virus assembly during replication</td>
<td>Oral, may be administered parenterally for severe cases</td>
<td>Well absorbed orally (~80%), t1/2 12-17 hrs, crosses blood-brain barrier, renally excreted</td>
<td>M2 gene mutations confer resistance</td>
<td>Influenza A treatment, used off-label for Parkinson's disease dyskinesia</td>
<td>GI disturbances, CNS effects like insomnia</td>
</tr>
</tbody>
</table>

2.2. Neuraminidase inhibitors

Neuraminidase is a viral surface glycoprotein key to influenza infection and transmission by facilitating viral spread. The four approved neuraminidase inhibitor drugs are effective against both influenza A and B viruses with low reported resistance.
Oseltamivir was the first oral neuraminidase inhibitor licensed in 1999 and is administered as phosphate salt (Table 2). It behaves as a prodrug activated to the active metabolite oseltamivir carboxylate with 32-37% oral bioavailability. The 4-6 hour plasma half-life leads to regular dosing over 5 days. Oseltamivir is primarily excreted unchanged in urine and pharmacokinetics are altered in renal impairment requiring dosage adjustments. It is well tolerated and associated with nausea, vomiting, headache as common side effects. Various analytical methods like HPLC, mass spectrometry and UV-Vis spectrophotometry help quantification. [15]

Zanamivir was approved in 1999 as an inhalational powder for maximum drug delivery to infection sites in the respiratory tract (Table 2). Its epithelial lining targeting and rapid 8-hour plasma half-life necessitates twice daily administration over 5 days. Zanamivir exhibits potent activity as demonstrated by cell-based assays and animal models. Clinical efficacy was observed in reducing influenza symptoms when administered within 48 hours. Adverse effects are typically mild but include bronchospasm in asthmatics requiring pre-screening. In contrast, intravenous peramivir has extended plasma elimination half-life of 12-17 hours allowing single dose administration under emergency use authorization granted during 2009 H1N1 pandemic. Peramivir is 73% protein bound with renal and non-renal clearance pathways metabolically converted to inactive peramivir glucuronide (Table 2). Laninamivir octanoate was licensed in 2010 in Japan as the prodrug laninamivir administered via inhalation. Slow cleavage into active laninamivir over 1-2 weeks helps sustain antiviral concentrations (Table 2). This long-acting single dose property mitigates issues with patient adherence to multiple daily dosing. [12, 13]

Table 2. Properties of Neuraminidase inhibitors

<table>
<thead>
<tr>
<th>Drug</th>
<th>Structure</th>
<th>Dosage Form</th>
<th>Mechanism of Action</th>
<th>Route of Administration</th>
<th>Pharmacokinetics</th>
<th>Indications</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oseltamivir</td>
<td><img src="image1" alt="Oseltamivir Structure" /></td>
<td>Oral capsule (prodrug)</td>
<td>Inhibits viral neuraminidase, preventing viral release from host cells</td>
<td>Oral</td>
<td>Prodrug activated to active metabolite with 30-40% oral bioavailability, half-life 6-10 hours</td>
<td>Influenza A and B treatment/prophylaxis</td>
</tr>
<tr>
<td>Zanamivir</td>
<td><img src="image2" alt="Zanamivir Structure" /></td>
<td>Inhalation powder (prodrug)</td>
<td>Inhibits viral neuraminidase, preventing viral release from host cells</td>
<td>Inhalation via dry powder inhaler</td>
<td>Poor oral bioavailability, half-life 2.5-5 hours</td>
<td>Influenza A and B treatment/prophylaxis</td>
</tr>
<tr>
<td>Laninamivir</td>
<td><img src="image3" alt="Laninamivir Structure" /></td>
<td>Inhalation powder (prodrug)</td>
<td>Inhibits viral neuraminidase, preventing viral release from host cells</td>
<td>Inhalation as octanoate prodrug</td>
<td>Prodrug slowly converted to active form over 1-2 weeks, allowing single-dose regimen</td>
<td>Influenza A and B treatment</td>
</tr>
<tr>
<td>Peramivir</td>
<td><img src="image4" alt="Peramivir Structure" /></td>
<td>Intravenous injection</td>
<td>Inhibits viral neuraminidase, preventing viral release from host cells</td>
<td>Intravenous</td>
<td>High plasma protein binding (&gt;90%), half-life 20-30 hours</td>
<td>Influenza A and B treatment</td>
</tr>
</tbody>
</table>

2.3. Polymerase inhibitors

Baloxavir marboxil is a first-in-class antiviral approved in 2018 that selectively inhibits endonuclease activity of influenza virus polymerases. It is orally administered as an inactive prodrug metabolized to baloxavir acid, the active form through carboxylesterase hydrolysis (Table 3). Notable side effects with baloxavir include vomiting, diarrhea and bronchitis. It exhibits efficacy against both adamantane-resistant and oseltamivir-resistant influenza strains. [12, 13]

Favipiravir is an investigational polymerase inhibitor converted inside cells to the pharmacologically active form favipiravir ribofuranosyl-5'-triphosphate and incorporated into viral RNA chains, halting influenza replication. Favipiravir is being evaluated for pandemic preparedness and has shown promise against viruses like ebola and COVID-19. However, further research is required to determine its clinical potential fully (Table 3). [9-11]
Table 3. Properties of Polymerase inhibitors

<table>
<thead>
<tr>
<th>Drug</th>
<th>Structure</th>
<th>Dosage Form</th>
<th>Mechanism of Action</th>
<th>Route of Administration</th>
<th>Pharmacokinetics</th>
<th>Indications</th>
<th>Advantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baloxavir marboxil</td>
<td>![Image]</td>
<td>Oral tablets (prodrug)</td>
<td>Inhibits cap-dependent endonuclease, blocking viral mRNA synthesis</td>
<td>Oral</td>
<td>Prodrug converted to active form with good oral bioavailability, half-life ~80 hours</td>
<td>Influenza A and B treatment</td>
<td>Single-dose regimen, effective against oseltamivir-resistant strains</td>
</tr>
<tr>
<td>Favipiravir</td>
<td>![Image]</td>
<td>Oral tablets</td>
<td>Inhibits viral RNA-dependent RNA polymerase, preventing viral replication</td>
<td>Oral</td>
<td>Good oral bioavailability, half-life 4-5 hours</td>
<td>Influenza A and B treatment</td>
<td>Active against wide range of influenza strains, including resistant strains</td>
</tr>
</tbody>
</table>

3. Analytical techniques for drug quantification

A variety of techniques are used for quantification of anti-influenza drugs in pharmaceutical formulations (Table 4), biological matrices and environment samples for purposes of quality control, bioanalysis and residue detection. Chromatographic methods such as HPLC, UPLC and LC-MS/MS are widely applied given their high sensitivity and ability to separate analytes from complex matrices. HPLC coupled with UV/fluorescence detection helps quantify M2 channel inhibitors at low μg/mL levels in tablets and plasma. Derivatization aids amantadine detection at sub-ng levels in tissues and urine. LC-MS/MS affords greater selectivity and precision in quantification of oseltamivir, zanamivir and peramivir at sub-ng/mL levels in biosamples. [9-11]

Gas chromatography remains useful for volatile adamantanes after derivatization, attaining picogram sensitivity in water and air. Fourier transform-infrared spectroscopy aids rapid tablet assay without organic solvents. Voltammetry and amperometric biosensors show potential for point-of-care quantification of peramivir and laninamivir. Vibrational spectroscopic techniques like UV-Vis, FTIR, Raman are employed for quantitative structure-activity analyses and fingerprint identification. NMR spectroscopy coupled to multivariate statistics enables rapid characterisation and quantification of mixtures. Titrimetric, spectrophotometric and spectrofluorimetric methods feature in high-throughput screening of drug libraries. Cell culture-based luminescent, colorimetric and fluorescent assays are used to determine 50% effective concentrations and toxicity profiles. Flow cytometry and confocal/live cell imaging permit mechanism understanding. Animal models evaluate pharmacokinetics, tissue distribution, toxicity and therapeutic efficacy. [9-11] Molecular docking, drug-protein interactions and virus yield reduction assays provide insights into mechanisms of resistance.

Table 3. Analytical methods reported for Anti-influenza drugs

<table>
<thead>
<tr>
<th>Analytical Technique</th>
<th>Analytical Method</th>
<th>Key Highlights</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>HPLC-UV</td>
<td>Isocratic reversed-phase HPLC with UV detection at 215 nm</td>
<td>Quantification of rimantadine in human plasma with LOQ 10 ng/mL</td>
<td>[16]</td>
</tr>
<tr>
<td>GC-MS</td>
<td>Derivatization with BSTFA, GC-MS in SIM mode</td>
<td>Detection of amantadine in wastewater at pg/L levels, MDL 20 pg/L.</td>
<td>[17]</td>
</tr>
<tr>
<td>LC-MS/MS</td>
<td>Protein precipitation, LC-MS/MS in MRM mode</td>
<td>Simultaneous quantification of oseltamivir, oseltamivir carboxylate in human plasma, LLOQ 0.5 ng/mL.</td>
<td>[18]</td>
</tr>
<tr>
<td>CE-UV</td>
<td>Micellar electrokinetic capillary chromatography with UV detection</td>
<td>Separation and quantification of oseltamivir, oseltamivir carboxylate and metabolites in urine</td>
<td>[19]</td>
</tr>
</tbody>
</table>
HPLC-FL  | Pre-column derivatization with dansyl chloride, HPLC with fluorescence detection  | Quantification of zanamivir in rat plasma, LLOQ 5 ng/mL  | [20]  
LC-MS/MS  | Protein precipitation, LC-MS/MS in MRM mode  | Simultaneous quantification of peramivir, oseltamivir in rat plasma, LLOQ 0.5 ng/mL  | [21]  
HPLC-FL  | Pre-column derivatization with 9-fluorenylmethyl chloroformate, HPLC with fluorescence detection  | Quantification of laninamivir in rat plasma, LLOQ 5 ng/mL  | [22]  
Spectrofluorimetry  | Fluorescence enhancement by β-cyclodextrin complex  | Determination of binding constants and stoichiometry of rimantadine-cyclodextrin complexes  | [23]  
HPLC-ELSD  | Reverse-phase HPLC with evaporative light scattering detection  | Quantification of zanamivir in rat lung tissues, LOQ 0.2 μg/g  | [24]  
LC-MS/MS  | Dilution with acetonitrile, LC-MS/MS in MRM mode  | Quantification of baloxavir, baloxavir carboxylate in human/animal plasma, LLOQ 1 ng/mL  | [25]  
CE-MS  | Capillary electrophoresis coupled to ion trap mass spectrometry  | Separation and detection of oseltamivir, oseltamivir carboxylate and metabolites in urine samples  | [26]  
NMR  | 1H NMR spectroscopy with chemometrics  | Identification and quantification of anti-influenza drugs in seized counterfeit tablets  | [27]  
Biosensor  | Amperometric biosensor with viral neuraminidase immobilized  | Detection of zanamivir binding to neuraminidase, IC50 determination  | [28]  
Voltammetry  | Square wave voltammetry at glassy carbon electrode  | Electroanalytical determination of peramivir in tablets and spiked plasma samples  | [29]  
Raman Spectroscopy  | Surface-enhanced Raman spectroscopy with gold nanoparticles  | Detection and quantification of oseltamivir carboxylate in plasma, LOD 20 nM  | [30]  

4. Drug delivery approaches

Inhaled, intranasal and transdermal routes are being explored to maximize lung and upper airways delivery of anti-influenza drugs. Inhalable dry powders and metered dose suspensions administer zanamivir, laninamivir and peramivir directly to infection sites. Liquid sprays and gels permit intranasal self-administration addressing barriers to inhaler access and compliance.


[22, 23]

5. Point-of-care diagnosis

Timely diagnosis is imperative for early antiviral administration and treatment monitoring. Rapid POC assays help minimize infection spread by expediting lab confirmation results. Traditional cell culture-based assays detecting hemagglutination/cytopathic effects take 1-2 weeks while immunochromatographic assays require 2-4 hours. Lateral flow immunoassays utilize gold/colored nanoparticles conjugated to antibodies against nucleoprotein/matrix protein to visually detect influenza A/B in throat/nose swabs within 15-30 minutes. Color changes indicate positivity compared to controls. However, assay sensitivity depends on viral shedding and antigen levels. Isothermal nucleic acid amplification techniques like RPA, LAMP and NASBA combine reverse transcription and high specific amplification of viral RNA/cDNA within 1 hour without requiring thermal cycling equipment. Fluorescence, turbidity or electrical impedance measurements indicate results.

Microfluidic PCR chips containing all necessary reagents rapidly heat-cool specific gene segments of influenza subtypes/variants within 1 hour. Exhalation breath analysis employing ion mobility spectrometry detects unique ion distributions of volatile organic
compounds from infected patients. Smartphone-based diagnostics integrate compact microscope adapters to read lateral flow/immuno-chromatographic assays or microfluidic PCR using in-built cameras aided by machine vision algorithms. Wireless data transfer enables telemonitoring. However, high sensitivity and multiplexing ability need improvements.

6. Challenges
Existing challenges limit further progress in anti-influenza therapies and diagnostics. Emergence and transmission of drug-resistant strains jeopardize effectiveness of currently available drugs, necessitating novel mechanisms. Resistance also arises due to sub-therapeutic dosing from missed doses non-compliance or improper prescribing. Diagnostic challenges include need for standardized protocols, infrastructure, skilled resources to implement POC devices in field settings worldwide. Ensuring year-round supply chain, quality assurance and robust instrumentation remains difficult in resource-limited areas vulnerable to pandemics. High costs reduce accessibility for mass diagnosis campaigns. Other issues cover lack of pediatric formulations, long-term safety/toxicity data of newer drugs, limited understanding of resistance mechanisms beyond known mutations. Delineating host responses, viral-bacterial interactions and difference in disease manifestations between community and nosocomial settings requires clarity. Strategic global efforts integrating epidemiological and laboratory based surveillance, research partnerships, open-source data sharing and pandemic preparedness models can collectively help address these varied influenza challenges ahead. Strengthening public health systems warrants bolstering R&D capacity particularly in the developing world.

7. Conclusion
Adamantanes, neuraminidase inhibitors and recently introduced polymerase inhibitors represent major pharmacological classes targeting distinct stages of the viral life cycle. Continuous progress is being made through novel delivery approaches, rapid diagnostics, drug combinations and host-targeting agents to enhance clinical efficacy. However, barriers like viral mutations, resistance, lack of pediatric data and global access disparities demand focused research investments. Collaborative efforts are essential to address seasonal epidemics, potential pandemics through cost-effective universal vaccines, resistance-proof drug formulations and pandemic preparedness planning. With continued medical innovation and strengthening of public health infrastructure worldwide, the goal of curbing influenza’s health and economic burden can be realized in the future.

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### Author's short biography

**Mr. Ramakanth Reddy Tetali**

Ramakanth Reddy Tetali is a fourth-year student studying Bachelor of Pharmacy at K. G. R. L College of Pharmacy in Bhimavaram, Andhra Pradesh, India. His interest in pharmacy was sparked during his high school years when he learned about the importance of medicines and how they are developed. Ramakanth is passionate about using his knowledge and skills to help patients. In his spare time, he volunteers at a local community health center, educating people about common illnesses and teaching them healthy lifestyle habits. He also enjoys reading research papers on new drug discoveries and trends in the pharmaceutical industry.

**Miss BNV Sai Durga G**

BNV Sai Durga G is a fourth-year Bachelor of Pharmacy student at K. G. R. L College of Pharmacy in Bhimavaram, Andhra Pradesh. Her interest in the medical field began at a young age from helping care for sick relatives. Sai Durga realized pharmacy allowed her to fulfill her passion for both healthcare and science. After graduation next year, she wishes to pursue a Master's program in clinical pharmacy research. Her goal is to develop effective and affordable drugs, especially for commonly occurring chronic illnesses in India. Sai Durga ultimately aspires to earn a PhD and have a career in academia, where she can educate and train future generations of pharmacists.

**Miss Sharon Pushpa P**

Sharon Pushpa P is a fourth-year B.Pharmacy student at K. G. R. L College of Pharmacy in Bhimavaram, Andhra Pradesh. Her interest in pharmacy was sparked during her school science projects, where she enjoyed learning about medication development. After finishing her degree next year, she wishes to pursue a career in hospital pharmacy management. Sharon believes in the holistic approach of healthcare that addresses both medical and lifestyle factors. She strives to play a part in making quality treatment accessible and affordable to all sections of society.

**Miss Salomi K**

Miss Salomi K is a fourth-year Bachelor of Pharmacy student at K. G. R. L College of Pharmacy in Bhimavaram. After graduating next year, she aims to obtain a Master’s in Public Health to pursue a career as a health administrator. Salomi wishes to work with NGOs and the government to boost preventive healthcare programs in rural India. She strives to use innovative community engagement strategies to increase access to medical facilities and health education nationwide.

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Mr. Edward Raju Gope is an Assistant Professor of Pharmaceutics at K. G. R. L College of Pharmacy in Bhimavaram, Andhra Pradesh. He holds a Master’s degree in Pharmaceutical Analysis. Edward is passionate about educating students in developing effective and industrially applicable pharmaceutical formulations. He constantly strives to make the subject engaging and research-oriented for learners. Edward also encourages collaboration with industries through student projects and facility visits.