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Modification, Characterization and Biological Effectiveness of Anti–flu Drug

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Abstract

In recent decades, current flu treatments faced major challenges. Accordingly, there is a huge need for improved access, new drugs, and faster care. It explores promising options like natural compounds and advanced antivirals. Enhancing vaccines and case tracking are key to fighting future flu outbreaks. Moreover, highlighting Amantadine's role as an antiviral and Parkinson's treatment, noting resistance issues and side effects. It also outlines improved, eco–friendly synthesis methods for safer and more efficient production. The condensation of Amantadine with 3–formylchromone, forming the targeted Schiff base, which shows spectroscopic agreement and computational promise against H5N1, supporting further lab testing as a potential treatment for resistant strains.

Key Words:

Antiviral Drugs, Anti–flu drugs, Amantadine, Molecular docking

1. Introduction

Viral Infections

Due to medication resistance, the 2009 H1N1 (influenza A virus, which causes respiratory infections in humans and animals.) pandemic revealed the shortcomings of existing influenza treatments, emphasizing the need for combination therapy, new broad-spectrum antivirals, and better drug delivery (1). Additionally, the study found that underreporting of influenza-like illness was caused by low rates of healthcare seeking, especially among high-risk populations. It highlights the need for better surveillance, more awareness, and timely treatment to prevent the spread of viruses and improve pandemic response (2). Antiviral medications are crucial for prevention because influenza viruses kill more than 500,000 people each year and immunizations don't always work. Notwithstanding the effectiveness of neuraminidase inhibitors, resistance to oseltamivir and adamantanes has surfaced, highlighting the necessity of ongoing resistance monitoring in conjunction with the creation of novel antiviral medications and combination treatments (3). In this regard, researchers have created a network of virus-host protein interactions, identifying GBF1 and JAK1 as host factors influencing influenza virus replication. These findings position them as potential antiviral targets and demonstrate the value of virus-host interactome screens in guiding antiviral drug development. This study has identified over 1,000 host variables that interact with influenza virus proteins and impact viral replication (4). The importance of rapid antiviral deployment is particularly evident in remote areas, such as a Canadian city where access to critical care is limited. A study aimed at evaluating the effectiveness of antiviral treatment and prevention for close contacts suggested that targeted treatment alone may not be as effective. Instead, public health programs in remote areas should prioritize swift distribution of antiviral drugs to sick individuals and broader populations (5). To better estimate the global mortality burden from influenza, another study assessed influenza-associated excess respiratory mortality in mainland China from the 2010–11 to 2014–15 seasons. The findings revealed that influenza significantly increased respiratory mortality, particularly among individuals aged 60 and older. Improved surveillance and targeted public health initiatives are crucial for more accurate assessments and for reducing the disease burden (6). Every year, influenza generates epidemics that have high rates of morbidity and death. Treatment involves the use of three medication classes: RNA polymerase inhibitors, neuraminidase inhibitors, and M2 inhibitors. M2 inhibitors are no longer advised because of resistance. Only in Japan is the efficacy of the sole RNA polymerase inhibitor, approved. Although there is evidence to support the early use of neuraminidase inhibitors, in some groups, their usage is still not ideal. This study covers the molecular basis of flu, disease progression, and prevention and treatment strategies. Influenza is caused by influenza A viruses and develops seasonal infections and rare pandemics. Influenza can cause serious

complications such as pneumonia and bacterial co-infections, and current antivirals are effective early but limited by the virus's genetic instability. New therapies that target the host's pulmonary response are required to improve viral clearance and reduce complications (7). Antiviral treatments and vaccination are both necessary for treating severe influenza. The effectiveness and resistance of current medications are limited. Crystallography (is a technique used to study the 3D structure of molecules (like viruses or proteins) to help design effective drugs.) is helping to create new agents that show potential, such polymerase inhibitors and anti-hemagglutinin antibodies. In the upcoming ten years, these developments could significantly impact how influenza is treated (8). Additionally, zinc has been shown to support the immune system, offer protection against respiratory viruses, and potentially improve the course of viral diseases. However, further research is necessary, particularly regarding the relationship between zinc and vaccines (9). Influenza Due to medication resistance and inefficient vaccine development, existing vaccinations and antivirals are insufficient to fight viruses, which constitute a danger to human health. Innovative strategies are required considering the H5N1 avian flu pandemic danger. One possible strategy for creating broad-spectrum antiviral therapies is to target host components essential for virus replication (10). Despite these concerns, vaccination remains a key preventive measure. A nasal influenza vaccine offers an injection-free alternative that stimulates an immune system response. It is suitable for individuals aged two years and older and, being made from live but weakened influenza viruses, does not cause disease (11).

Comparative efficacy of antiviral treatments

This study explores various antiviral compounds and their effectiveness against influenza and other viral infections. The primary aim was to identify new antiviral compounds that specifically inhibit the replication of influenza A and B viruses. Researchers focused on small molecules that interfere with the interaction between the PB1 and PA subunits of the influenza virus polymerase, which plays a critical role in viral replication. These findings could lead to the development of more targeted flu medications (12). In addition, the study highlighted that EGCG, a compound found in green tea, can prevent the flu virus from evading the immune system by blocking the interaction between the NS1A protein and dsRNA. This discovery may pave the way for new antiviral treatments for influenza in the future (13). Neuraminidase inhibitors, such as oseltamivir and zanamivir, are commonly used to treat and prevent influenza. While they reduce symptoms in adults, they are less effective in children with asthma, and their impact on complications like pneumonia remains uncertain. Moreover, oseltamivir has more side effects compared to the safer zanamivir, and clinical evidence does not fully support their expected action in all populations (14). During the 2009–10 H1N1 pandemic, these inhibitors were widely used, though it remains unclear how they

influenced mortality rates. To further understand their effectiveness, a meta-analysis was performed to assess their association with mortality in hospitalized patients infected with the H1N1pdm09 virus (15). In general, antivirals are crucial for both preventing and treating influenza infections, alongside vaccines. For influenza, they work by blocking the spread and reproduction of the virus. Modified nucleosides, which are effective against smallpox and Hepatitis B, may also play a role in future COVID-19 therapies by targeting viral polymerases and preventing replication (16). In contrast to oseltamivir, favipiravir is another antiviral drug that works by blocking viral RNA replication. Favipiravir exhibits wide efficacy against dangerous RNA viruses, including the rabies virus, by preventing viral mutations. It achieves this by severing the viral RNA chain, which lowers the viral load and cures fatal infections in animals (17). Additionally, baloxavir marboxil is an influenza drug that shortens symptom duration and lowers viral load quickly. In trials, baloxavir reduced symptoms by up to 28.2 hours and worked as effectively as oseltamivir, with a notable decrease in viral load within just one day. Interestingly, flu viruses showed a small ability to fix mutations added to the PAC (Polymerase Acidic Protein) or PB1N (shorter version of the PB1 protein found in influenza viruses) ends, suggesting that escape mutations in these areas are rare (18). A 2024 review found that antivirals such as zanamivir, oseltamivir, laninamivir, and baloxavir effectively reduced the risk of symptomatic flu in high-risk individuals after exposure. However, they were less effective in low-risk groups, and amantadine showed no significant preventive benefit in reducing influenza symptoms or complications (19). Influenza A and B viruses are responsible for annual outbreaks, with oseltamivir helping to reduce deaths by blocking the virus. However, resistance to some strains is becoming a growing concern, emphasizing the need for new treatments (20). Furthermore, LH (Luteinizing Hormone) could be helpful in inhibiting or controlling COVID-19, suggesting it as a potential new treatment option (21). Influenza, a respiratory illness in humans and poultry, is caused by viruses from animals such as birds and bats. The virus undergoes antigenic changes, which require constant updates to vaccines. This highlights the pressing need for the development of new treatments. Research is currently exploring the possibility of a universal flu vaccine and investigating new immune-modulatory therapies. The emergence of novel strains like H1N1, H5N1, and H7N9 presents challenges in the ongoing battle against the virus (22). During the 2013–2014 flu season, universal immunization was advised for children, with no preference for either trivalent or the recently approved quadrivalent vaccines. Healthcare professionals were encouraged to promote vaccination and early antiviral therapy to reduce influenza morbidity and mortality (23). This study also assessed the accessibility and use of influenza vaccines and antivirals in Africa, focusing on high-risk populations. Despite their availability in many

regions, the use of these treatments remains limited. Expanding vaccine coverage requires securing funding and raising awareness of the local disease burden to improve access and utilization (24). Most of the vaccines used to treat influenza rely on a manufacturing process that depends on eggs, thus containing some quantity of the hormone ovalbumin, as seen in the Fluda vaccine. However, there are alternative vaccines, such as Flucelvax, that are free from egg-based ingredients and rely on cell culture for production, making them safer for individuals with egg allergies (25).Vaccination is considered the most effective method of combating pandemic and epidemic influenza, as it stimulates antibody responses to neutralize the hemagglutinin (HA) protein, a key component of the virus (26).Since the flu virus changes over time, annual updates to the vaccine are necessary to maintain effectiveness and ensure protection against circulating strains (27).The study further evaluated the effectiveness of oseltamivir and makeover in Russia (2010–2011), where both antivirals were found to be effective. Umifenovir remained active against oseltamivir-resistant strains, and early treatment within 48 hours reduced illness duration by 2–3 days and lowered pneumonia incidence, highlighting the importance of early intervention in managing resistant infections (28). The effectiveness of five antiviral medications—Ribavirin, Acyclovir, and three Interferon drugs—against the Herpes simplex virus was also assessed in this study. Ribavirin and Acyclovir were found to be more effective than interferon medications, with significant interactions observed within each drug group but not between the groups, suggesting the potential for enhancing antiviral combinations for improved treatment outcomes (29). In conclusion, this study highlights the importance of continued research into antiviral drugs and vaccines to combat influenza and other viral infections. While current antivirals offer critical support in infection prevention and treatment, new therapies, such as modified nucleosides and immune-modulatory treatments, offer promising potential for future flu and viral disease management. Integrating these new treatments with updated vaccines can significantly reduce the global burden of influenza, especially in high-risk populations, and improve overall public health outcomes (30).

Challenges in antiviral Treatments

The primary aim of this study was to identify new antiviral compounds capable of specifically inhibiting the replication of influenza A and B viruses. To achieve this, researchers focused on small molecules that disrupt the interaction between the PB1 and PA subunits of the viral nature plication (31). Additionally, the study explored strategies to block the influenza virus from evading the immune system. Specifically, researchers targeted the viral NS1A protein, which binds to double-stranded RNA (dsRNA) to shield the virus from immune detection. After screening multiple compounds, they identified epigallocatechin gallate (EGCG), a natural polyphenol found in green tea

(constituting 5–15% of its dry weight), as the most effective inhibitor. This discovery holds promise for developing new antiviral drugs against influenza. (32). Currently, public health organizations rely heavily on neuraminidase inhibitors (NIs), such as oseltamivir and zanamivir, for managing seasonal and pandemic influenza. While these drugs reduce symptoms and prevent infection in adults, their efficacy is limited in children with asthma, and their impact on severe outcomes like pneumonia remains unclear. Moreover, oseltamivir is associated with more adverse effects compared to zanamivir, which has a better safety profile. However, clinical data do not fully support their proposed mechanisms of action (33). During the 2009–10 H1N1 pandemic, NIs were widely used, yet their effect on mortality rates remains uncertain. A meta-analysis was conducted to evaluate their association with mortality in hospitalized H1N1pdm09 patients. (34) Overall, antivirals—when combined with vaccination —play a crucial role in infection prevention and treatment. For instance, neuraminidase inhibitors hinder viral spread, while polymerase inhibitors (such as modified nucleosides used against smallpox and hepatitis B) block viral replication. Interestingly, research suggests these polymerase inhibitors could also be repurposed for COVID-19 treatment (ACS Medicinal Chemistry Letters) (35). In contrast to oseltamivir, favipiravir—an antiviral that blocks viral RNA replication—exhibits broad-spectrum activity against dangerous RNA viruses, including rabies. It works by inducing RNA chain termination, reducing viral load, and curing lethal infections in animal models (36). Another promising drug, baloxavir marboxil (a cap-dependent endonuclease inhibitor), was tested in clinical trials. In Phase II, it shortened symptom duration by 23.4–28.2 hours compared to placebo, while Phase III demonstrated a reduction to 53.7 hours (versus 80.2 hours for placebo). Notably, baloxavir showed comparable efficacy to oseltamivir and rapidly decreased viral load within 24 hours (37). Furthermore, influenza viruses displayed limited ability to repair mutations in the PAC or PB1N protein regions, making drug-resistant escape mutations rare in these areas (38). A systematic review and meta-analysis (August 2024) assessed post-exposure antiviral prophylaxis against influenza. The results showed that zanamivir, oseltamivir, laninamivir, and baloxavir significantly reduced symptomatic influenza in high-risk individuals, though their effect was less pronounced in low-risk groups. On the other hand, amantadine showed no significant preventive benefit. (39). Despite the effectiveness of oseltamivir in reducing influenza-related deaths, emerging resistant strains highlight the urgent need for new treatments. (40). Interestingly, luteinizing hormone (LH) has shown potential in inhibiting or controlling COVID-19, suggesting it could be repurposed for influenza therapy (41). Both humans and poultry are susceptible to influenza, a respiratory illness originating from animal reservoirs (e.g., birds, bats). Due to the virus's

antigenic variations, vaccines require frequent updates, and the limited availability of effective drugs underscores the need for new vaccines and therapeutics. Ongoing research explores a universal flu vaccine and immune–modulatory therapies (42). During the 2013–14 flu season, universal vaccination was recommended for children using either trivalent or quadrivalent vaccines. To reduce morbidity and mortality, healthcare providers must promote vaccination and early antiviral treatment. (43). Accessibility of Vaccines and Antivirals in Africa This study also evaluated the accessibility and usage of influenza vaccines and antivirals in Africa, particularly among high–risk populations. Although these treatments are available in many African countries, their utilization remains limited. To improve vaccine uptake, increased funding and awareness of local disease burden are essential. (44). Most influenza vaccines (e.g., Flu Laval) are egg–based, containing traces of ovalbumin. However, cell–culture–derived vaccines (e.g., Flucelvax) are egg–free, offering an alternative for egg–allergic individuals (45). Vaccination as the Primary Defense Vaccination remains the most effective strategy against epidemic and pandemic influenza, as it induces antibody responses targeting the hemagglutinin (HA) protein. Nevertheless, annual updates are necessary due to the virus's antigenic drift (46). A study in Russia (2010–2011) compared oseltamivir and umifenovir, finding both effective against influenza, with umifenovir retaining activity against oseltamivir–resistant strains. Early treatment (within 48 hours) reduced illness duration by 2–3 days and lowered pneumonia incidence, emphasizing its role in managing resistant infections. (47). Finally, a separate study evaluated five antivirals (ribavirin, acyclovir, and three interferons) against herpes simplex virus (HSV). The findings revealed that ribavirin and acyclovir outperformed interferons, with significant intra–group interactions but no cross–group synergies, suggesting optimized combination therapies could enhance antiviral efficacy (48).

Natural antiviral compounds

Researchers are looking into the potential of natural plant components including triterpenoids and flavonoids to combat viruses like RSV (a virus that causes lung infections), and HSV–1(the virus that causes cold sores). Bamboo's tricin is more effective than popular antiviral drug ganciclovir at combating HCMV, (a virus that poses a threat to immunocompromised individuals and neonates). Bacterial genistein also exhibits potential against lethal arenaviruses. (49). Similarly, aloe polysaccharides (APS), which are isolated from aloe vera leaves, can stop the H1N1 influenza virus from spreading. APS lessened the lung damage brought on by the infection in a mouse study using the PR8 H1N1 virus (PR8 strain of H1N1 virus) (50). other natural substances have also demonstrated strong potential against viruses. For example, compounds like andrographolide (found in a Chinese plant), sesame (found in sesame seeds and oil), and hesperidin (found in citrus) show a strong ability to bind to the coronavirus, suggesting

they may offer a safer and less harmful alternative to traditional antiviral medications (51). In addition to these, other natural compounds include, quinic acid—an organic acid found in tobacco and coffee beans. Oseltamivir and other effective antiviral medications have been aided by polyalcohol leaves, carrot leaves, apples, peaches, pears, and shikimic acid (found in Chinese star anise) (52).

Advances in antiviral compounds

Nanotechnology can fight influenza by using nanoparticles to deliver antivirus drugs, work as vaccines, or directly stop the virus, its useful tool for improving treatments (53). Building on the potential of innovative treatments, this review offers an updated analysis of the antiviral activity of plants and their isolated bioactive compounds. The authors examine various plant species and their compounds, which show promising effects against a wide array of viruses, including influenza, HIV, and herpes simplex virus (54). In addition to plant-based therapies, silver nanoparticles (Ag NPs) can help stop viruses from spreading in the air and can be used in healthcare to protect people from infections especially considering the COVID-19 pandemic (55). However, despite these advancements, viral resistance remains a significant challenge. This study highlights the emergence of drug-resistant monkeypox clusters in the United States, discussing the implications for current antiviral strategies and the need for new treatments. It emphasizes the importance of antiviral stewardship and the development of broader-spectrum antiviral agents. (56) To tackle such resistance effectively, this review discusses current FDA-approved influenza antivirals, including amantadine, and explores innovative therapeutic approaches such as combination therapies and targeted protein degradation. The paper highlights the challenges of drug resistance and the need for new antiviral strategies (57). Influenza A virus subtype H5N1 (A/H5N1) constitutes a specific subtype of the influenza A virus, which is responsible for the pathology known as avian influenza (commonly designated as "avian flu"). This virus is enzootic (sustained within the population) among numerous avian species, and it is also panzootic (impacting a variety of animal species across extensive geographical ranges). H5N1 poses significant threats to both human and animal health due to its high contagiousness and fatality rates (58). The development of effective antiviral drugs, such as amantadine, is critical to combat this virus. However, the emergence of drug-resistant strains, necessitates the use of advanced computational tools like molecular docking to design and optimize potent inhibitors. This response provides a comprehensive overview of molecular docking software tools and their applications in the design of amantadine derivatives against resistant H5N1 strain (59).

2. The Theoretical Framework

Targeting the virus or host cell factors are the two primary tactics used in the creation of antiviral medications, which are crucial treatments for viral infections. Inhibitors of viral attachment and entrance, uncoating inhibitors, and enzyme inhibitors like

polymerase, protease, and integrase inhibitors are among the medications that specifically target viruses. Acyclovir, tenofovir, ritonavir, and raltegravir are some of the most widely used and potent antiviral medications; in fact, some of these medications were among the best-selling ones during the previous ten years. Effective therapies are still lacking for several viral illnesses, though. Antiviral drugs work by blocking the creation of viral DNA or RNA or by using interferons to increase a cell's resistance to infection. The antiviral medications currently in use are highlighted in this study along with their modes of action, including those created to combat SARS-CoV-2 (COVID-19) (60).

History of amantadine

Amantadine, developed in 1964, was the first antiviral for humans, effective against influenza A when given within 48 hours of symptoms. It is used at a dose of 100-150 mg for those with renal insufficiency. A major limitation is rapid viral resistance due to changes in the M2 protein, though resistant viruses are less virulent. It is preferred over neuraminidase inhibitors due to quick diagnostic kits for influenza (61). In addition to its antiviral use, amantadine, developed for influenza A, also treats extrapyramidal symptoms, Parkinson's disease, and is used off-label for brain injury and multiple sclerosis (MS). It requires dose adjustments in renal impairment and may cause side effects like dry mouth, nausea, and dizziness. Serious effects include confusion and hallucinations. Caution is advised in older adults, pregnancy, and with other dopamine/NMDA drugs (62).

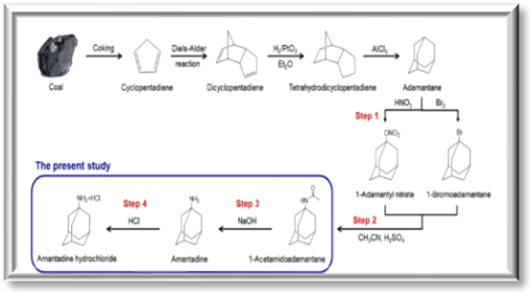
The Mechanism of action of amantadine

Stage 1: Coal as the Source: Products such as "cyclopentadiene" are produced by "coal-coking processes", which employ coal. Cyclopentadiene can self-polymerize into "dicyclopentadiene", which is subsequently hydrogenated and catalyst-assisted to produce "adamantane".

Stage 2: Adamantane to Amantadine Conversion:

Two primary conventional conversion techniques are as follows: The process of "Nitration" yields adamantly nitrate. The second step, "bromination", yields 1-bromo adamantane, which is more widely used but sensitive and not suited for long-term storage. The "Ritter reaction" involving acetonitrile and sulfuric acid is then used to transform these intermediates into "acetamido adamantane".

Stage 3: Making Amantadine and Its Salt "Amantadine" is made by "basic hydrolysis" with sodium hydroxide. To increase amantadine's water solubility and pharmacological effectiveness, it is subsequently transformed into "amantadine hydrochloride (HCl)".



One unusual source of valuable medicinal ingredients is coal. Beginning with cyclopentadiene, several chemical changes

are required to fully convert coal to amantadine.

Amantadine. HCl is best absorbed when it is in its salt form and is used to treat the early signs of Parkinson's disease (63). Researchers found that amantadine hydrochloride had no significant effect on dopamine or noradrenaline levels in the rat brain, even after nine days of daily use. It also did not alter the rate at which these neurotransmitter levels decreased. In vitro, amantadine caused little dopamine release and only inhibited dopamine uptake at high concentrations. These findings suggest that dopaminergic processes are not involved in amantadine's anti-Parkinsonian effects (64). In terms of its synthesis, acetamido adamantane can be hydrolysed in excess NaOH at pH > 13 and 100°C for 24 hours to produce amantadine, with sodium acetate as a by-product. The process achieves complete hydrolysis, with no residual reactant detected in the resulting mixture (mother liquor) by 1H NMR spectrum (65). Alternatively, amantadine can be synthesized through the Ritter reaction, where acetamido adamantane is converted with acetonitrile in the presence of sulfuric acid (aquas) into *N*-(1-adamantyl) acetamide, also known as 1-acetamidoadamantane. This is then hydrolysed using aq. NaOH to produce amantadine and sodium acetate as a byproduct (66). Amantadine can also be converted into various salts, including Amantadine. HCl, to enhance its water solubility and bioavailability. Amantadine. HCl is one of the most used salts due to the strong counterions and low PKa of hydrochloric acid, with nearly 50% of Amantadine salts being HCl salts. Additionally, Amantadine can be separated with water vapor through steam distillation due to its insolubility and sublimability. However, a problem arises when some of Amantadine's vapor crystallizes and deposits on the condenser tube walls, particularly at the joint, if the condenser temperature is not high enough (67). To synthesize Amantadine. HCl more efficiently, a Ritter-type reaction is employed using a microwave method. This two-step process yields 71% and is more environmentally friendly, as it enhances efficiency, reduces reaction time, and minimizes the use of hazardous chemicals (68).

Uses

Extended-release Amantadine is the only approved treatment for levodopa-induced dyskinesia in Parkinson's disease, demonstrating efficacy in reducing motor Symptoms and shutdowns. Due to its diverse pharmacological properties, it represents a complementary treatment option to levodopa therapy, but further studies are needed on its use in early disease Stages and other movement disorders (69). Despite its effectiveness in treating movement disorders such as levodopa-induced dyskinesia in Parkinson's disease, its role in the treatment of hepatitis does not appear promising. Studies have shown its ineffectiveness as a primary treatment for this disease, as adding 400 mg of Amantadine daily to interferon did not improve the viral response (70). Despite this, Amantadine remains an effective antiviral

against influenza An and helps alleviate the symptoms of early Parkinson's disease (71). In this context, a more efficient method for preparing Amantadine has been improved by reacting adamantine with *N*–(1–adamanthyl) acetamide, achieving higher yields of up to 67% compared to conventional methods that achieved lower yields. This makes the process more environmentally friendly and feasible for large–scale Production (72). However, Amantadine serves as an effective antiviral in treating influenza A, in addition to its use in treating early–stage Parkinson's symptoms (73). In line with these diverse advances, methods for producing Amantadine have begun to become more efficient and sustainable. A new method has been introduced that converts adamantine to *N*–(1–adamanthyl) acetamide in just two steps, increasing yields to 67% compared to conventional methods, which ranged from 45–58%. This method also eliminates the use of Harmful chemicals, allowing for greater yield and greater Potential for large–scale production for industrial use (74). It is worth noting that using Amantadine with some other medications requires caution, such as Dayside (which contains hydrochlorothiazide and triamterene), as it leads to a reduction in Amantadine excretion from the body, which increases its concentration in the plasma and increases the risk of toxicity, which requires close monitoring when using together (75).

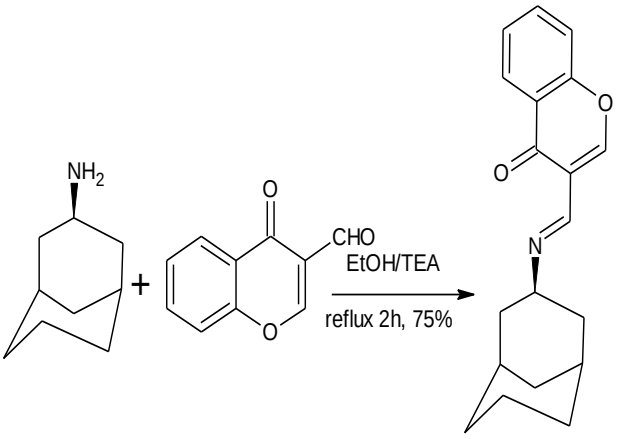
Effect of Amantadine

In addition to its therapeutic use, studies have shown that Amantadine directly affects cells, reducing short–circuit current in a manner like that of clotrimazole, and increasing cell area and height without causing cell death (76). Since these cellular effects may be related to drug concentrations within the body, a precise, one–step technique has recently been developed to determine Amantadine concentrations in plasma and urine, facilitating monitoring of its effects and a more precise understanding of its mechanism (77). Diuretic variability also affects Amantadine excretion from the body; diuretic variability reduces excretion, while diuretic variability accelerates its elimination (78). This highlights the importance of monitoring tobacco acidity when using Amantadine, as its activity can lead to toxic accumulation, and must avoid Amantadine during breastfeeding because of its potential negative effect on lactation (79).

3.Methods of Research and the tools used

Chemical synthesis

Condensation of bicycle[3.3.1]nonan–3–amine (Amantadine in pure form was obtained from Sigma Aldrich, Darmstadt, Germany) with 3–formylchromone (80) in boiling ethanol containing a catalytic amount of triethylamine as a base afforded 3–[(*E*)–(bicycle[3.3.1]non–3–ylimino)methyl]–4*H*–chromen–4–one (Schiff base) 3 in good yield.



Molecular modeling

Crystal Structure of a H5N1 influenza virus hemagglutinin with CBS1117 (81). The data was procured from the RCSB Protein Data Bank (PDB). All water molecules were systematically eliminated from the crystallographic structure, and polar hydrogen atoms were incorporated using Auto dock tools (ADT) version 1.5.6. The ADT subsequently exported the processed file in the PDBQT format. In the case of H5N1, the grid box was strategically centered on the conserved catalytic triad and the adjacent amino acid residues that constitute the ubiquitin Binding Domain, along with the amino acid residues that form subsites 1, 2, and 3. The grid dimensions were established at 50 × 50 × 50 Å with points spaced at intervals of 0.5 Å for H5N1, while the grid dimensions for the other structure were set at 40 × 40 × 40 Å with points also separated by 0.5 Å. Docking studied C3 and C4 binding energies, residues, types of interactions, hydrophobic interactions, and their electrostatic interactions of newly synthesized compound during docking studies.

The molecular docking of Schiff base amantadine against resistant H5N1 influenza virus highlights its potential as a therapeutic agent. Molecular docking studies allow for the prediction of binding affinities and interactions between small molecules and viral proteins, which is crucial for developing effective antiviral drugs. Its crucial role through this study was previously predicted computationally by (82) as their study proved the activity of Schiff base on the binding site of neuraminidase protein of H5N1, like established inhibitors like oseltamivir. Besides, Schiff base interacted with binding sites that are less affected by mutations conferring resistance, thus maintaining their efficacy against resistant strains (83). This new compound demonstrated significant antiviral activity, with some exhibiting EC50 (Half maximum effective concentration) values comparable to amantadine (84).

The methodology of Molecular modeling

The methodology involves utilizing molecular docking simulations to analyze the binding affinity and interaction patterns of amantadine with a Schiff base compound, specifically targeting the H5N1 virus's proteins. This process includes preparing the molecular structures of both amantadine and the Schiff base, followed by using docking software to predict their binding poses and affinities, which will provide insights into potential therapeutic strategies against the virus.

The results obtained from these simulations will be further validated through experimental studies to confirm the predicted interactions and assess the efficacy of the combined compounds in inhibiting H5N1

virus activity. These findings could pave the way for developing novel antiviral therapies that enhance the effectiveness of existing treatments, ultimately contributing to better management and prevention of H5N1 infections in affected populations. The integration of computational and experimental approaches will not only enhance our understanding of the virus's mechanisms but also facilitate the identification of critical targets for drug development, leading to more robust therapeutic options.

4.Results of Research

The formation of desired compound was confirmed through spectroscopic data. IR spectrum displayed good evidence for formation Schiff base through absence of NH_2 and formyl absorption bands and presence of C=N band at 1629 cm^{-1}. Also, its H NMR spectrum showed characteristic multiple signal attributed to aliphatic protons at δ 1.08–1.67 ppm, in addition to a distinctive signal at δ 4.93 related to N–CH. Azomethine has a specific singlet signal at δ 7.52, furthermore another singlet signal at 7.62 associated to C2 of chromone ring.

Molecular docking offered a good simulation of Schiff base reaction with amantadine to overcome the resistance of H5N1 strain KU715952.

5. Interpretation of Results

This novel compound revealed strong binding affinity to cell receptors leading to suppress viral diffusion into the cell and the deproteinization process. The mode of action involved structural modifications that enhance hydrogen bonding or hydrophobic interactions with the target protein.

6. Conclusion

Newly synthesized Schiff base amantadine showed potential antiviral activity against H5 N1 strain KU715952 computationally. This recommends the laboratory trial with other biological effects to consider the new synthesized compound a good competitor to overcome H5N1 amantadine resistant strains.

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