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A review of Influenza A virus and relevant drugs

YeeNok Wong

Changsha WES Academy, Changsha, 410199, China

yinuowangmark@126.com

Abstract. Influenza A virus is a verry common virus around the world, where thousands of people will be infected every month. This is why it is important to have drugs that are responsible for curing influenza A virus. In this review, the article will mainly focus on the synthesis and the binding mechanisms of oseltamivir and amantadine hydrochloride with influenza A virus. Apart from they are both used in curing the infection of Influenza A virus, another similarity of these two drugs is they both have some problems in different aspect. For instance, oseltamivir phosphate has a really complex synthesizing process comparing to most of the drugs we can see in the market. The problem of amantadine hydrochloride is even bigger. It will cause several sever negative effects on the human body including damage on DNA and effects on mitotic cell cycle.

Keywords: Influenza A virus, Oseltamivir, Amantadine

1. Introduction

Influenza, a name that nearly everyone has heard about, is one of the commonest diseases in the human history. It will infect at least several thousands of people every month. According to the research [1]. , about 9 million people in the USA had been infected by influenza during 2021-2022. Base on the data of WHO [2]. 110,000 people had been infected by influenza from January the first, 2023 to the twenty-third of July, 2023. In the history, the biggest influenza outbreak occurred in the 1918, more than 20 million people die in the influenza pandemic [3].

Generally, Influenza in human is caused by Influenza A and B virus, though type C and D virus also existed in the nature. Symptoms of the disease usually happened in the respiratory system, including sore throat, runny nose, cough, headache, muscle pain and fatigue to severe, mainly happening in the upper respiratory tract. In other cases, the lower respiratory tract will also be infected by influenza virus or secondary bacterium, causing pneumonia in the patient. The virus can also affect heart, central nervous system and other organ systems. A big outbreak of Influenza causing a pandemic will occurs every 10-50 years, which were mostly led by the mutation of the virus [4].

2. Structure Of Influenza A Virus

The figure (Fig.1) depicts the structure of an Influenza A virus. All Influenza viruses are enveloped virus, containing a lipid envelope. The Genome of Influenza A and B virus split into eight RNA that are negative sense and single stranded. They will form eight rod-shaped RNPs. The RNP can be divided into three parts, the viral RNA, the nucleon protein and the viral polymerase make up of PA, PB1, PB2 and NP. On the other hand, influenza C and D virus only have seven RNPs [4]. The function

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of the RNP includes transcription and intracellular transport of viral RNA, directing viral RNA replication, viral genome packaging and gene reassortment [5]. Other structures of the Influenza A virus include six different types of proteins, including nonstructural protein (NS1), nuclear export protein (NS2), matrix protein (M1), membrane protein (M2) and two types of viral glycoproteins which are hemagglutinin (HA) and Neuraminidase (NA).

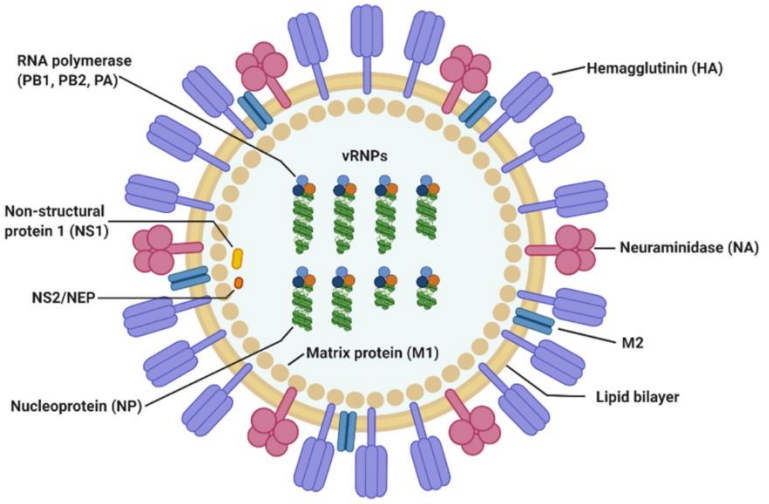

Figure 1. Influenza A virus

3. Replication Of Influenza A Virus

Generally, the epithelial cells of the respiratory tract are where the replication of the influenza A virus occurs, mediated by the viral HA. The HA have two functions, which are the binding process between the virus and the sialic acids at the cellular surface and hemagglutinate during influenza A virus' incubation in the red blood cells. An endosome will take in the influenza A virus after the binding process and the endosome will be trafficked and acidified. The process will cause a change in the HA. This will lead to the fusion between the viral envelope and the endosome. After the fusion, the viral RNPs will be released into the cytoplasm of the infected cell. The transcription and replication of the viral RNA will occur at the viral polymerase complex on the viral RNPs. The HA have a cleavage site which will do the tissue tropism of the virus. The extracellular protease can determine the cleavage site of the influenza A virus. The NS1 will stop the antiviral response of the host by regulating the cellular process in the cell. When the infection of the virus is nearly completed, the M1 and NEP of the virus will move to the nucleus and bund with the viral RNPs. The MA and NEP will interact with the endosomes to move into the membrane and send into the viral RNPs. This will allow them to mediate the export to the cytoplasm. The new virus will be reproduced, The NA will stop the new virus' HA bind with the sialic acid. The replication of the virus will finally lead to the death of the host cell companied with pathological implication [4].

4. Drugs For Influenza A Treatment

The drugs that we usually use to treat influenza include Tamiflu (Oseltamivir), Amantadine hydrochloride, Rimantadine, Zanamivir, Peramivir and Baloxavir marboxil, showed in the figure (Fig.2) below.

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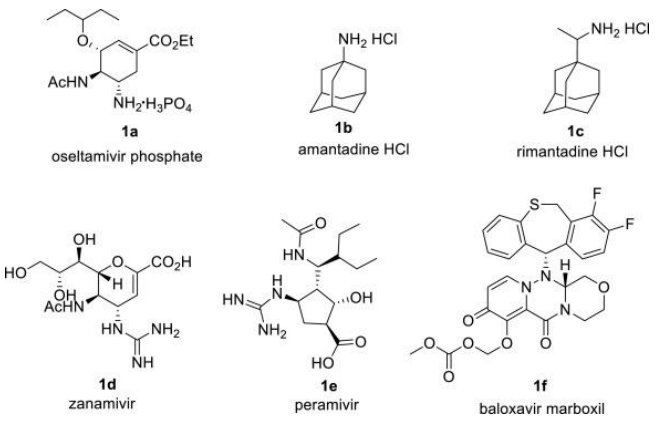

Figure 2. Drugs for influenza treatment [6].

5. Synthesis Of Oseltamivir Phosphate And Oseltamivir Carboxylate

Among all of these drugs, Tamiflu was used the most frequent after it has been approved by FDA in the 1999.

The Tamiflu showed above (Fig.2 1a) wasn't the original molecule developed by Gilead Science. The first candidate of discovered by Gilead Science was oseltamivir carboxylate, which was the final product in Fig.3.

The starting material used by Gilead Science to synthesis oseltamivir carboxylate was (-)-shikimic acid.

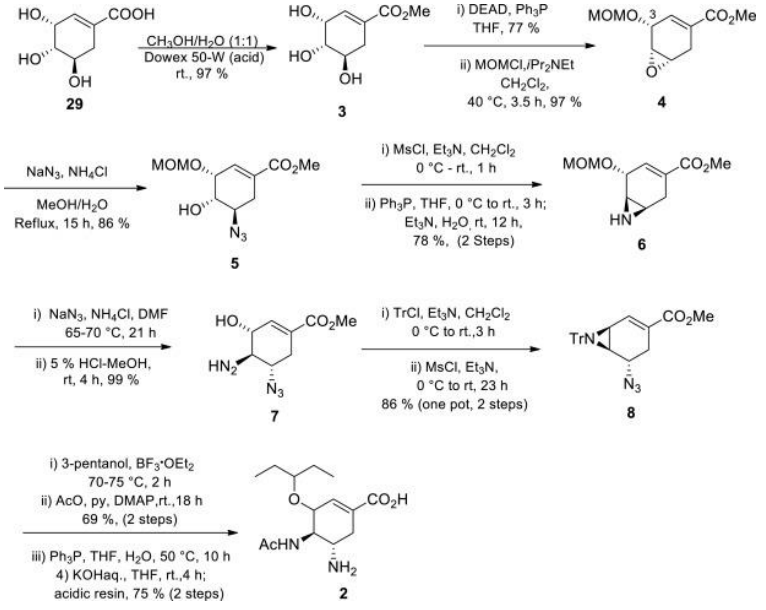

Figure 3. Gilead Sciences' synthetic route of the Oseltamivir carboxylate development [6].

The procedure started with (-)-Shikimic acid derivative (Fig.3, 3) in Mitsunobu conditions, which selectively activated the OH-group at the fifth carbon because the third carbon is protected by the MOM acetal group to afford an epoxide (Fig.3, 4). Epoxide was than treated with azide chemistry, selective aziding the fifth carbon to allow it to afford the azido alcohol (Fig.3, 5). Then the azido alcohol was treated using mesylation and azide reduction to produce aziridine (Fig.3, 6). Azide chemistry was used again to selectively activated the OH-group on the fifth carbon, of the aziridine 6, producing an amino alcohol (Fig.3, 7). The amino alcohol's amino functionality is then protected by a trityl group, followed by the treatment of mesylation of the hydroxyl group to synthesized Aziridine (Fig.3, 8). The Aziridine is then treated with the Lewis acid catalyst boron trifluoride etherate and the

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product amine will be treated with acetylation to produce an amido ether corresponded to the amido ether. A reduction reaction will then be used to treat the azide group on the amido ether. In the following process the methyl ester is then treated in a basic condition and hydrolyzed into oseltamivir carboxylate [6].

Nonetheless, Oseltamivir carboxylate haven't been chosen as the drug been sold. Oseltamivir carboxylate had replaced the position of oseltamivir phosphate. According to Kim et al [7]. this is due to the difference in the potent invitro and invivo activities, the good oral bioavailability of Tamiflu (oseltamivir phosphate) compared to oseltamivir carboxylate, the scarcity of the starting material and the difference in the safety of the process of synthesis.

In the late 1990s, one of the biggest limitations for the synthesis of oseltamivir carboxylate is the scarcity of (-)-shikimic acid due to the lack of techniques used in extraction and purification of (-)-shikimic acid. The other limitation is the synthesis route has been limited to milligram scale due to the intermediate have a possibility of explosion. A substitute, oseltamivir phosphate, also known as Tamiflu in present, was then developed by Gilead scientist to replace oseltamivir carboxylate. They chose (-)-quinic acid, a more common material to produce oseltamivir phosphate in a multi-gram scale. The synthesis process includes 12 steps in total. By this process, a product of 4.4% yield of oseltamivir phosphate. Though the yield of oseltamivir phosphate is relatively low, it still been successfully used in producing oseltamivir phosphate in kilogram quantities and the potential safety hazard has been solved. This is the reason why oseltamivir phosphate has replaced oseltamivir carboxylate.

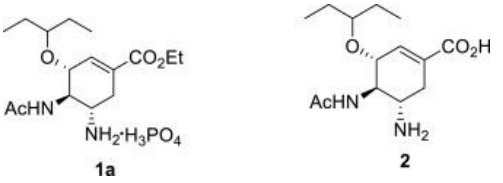

Figure 4. Oseltamivir phosphate (1a) and oseltamivir carboxylate (2) [6].

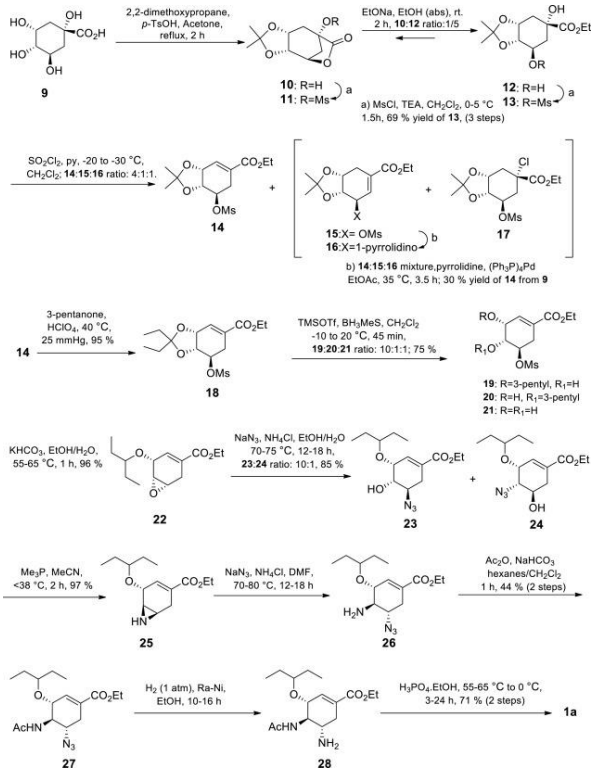

Figure 5. The process of large-scale synthesis of Tamiflu [6].

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6. The Binding Process Of Oseltamivir Phosphate And Influenza A Virus

The binding process of oseltamivir phosphate with influenza virus can be divided into 10 different states (Fig.6).

The first state is the metastable state S1. In the S1 state, the oseltamivir phosphate molecules are dissolved in solvent, and the distance between the oseltamivir phosphate and the surface of the viruses' NA was more than 5Å. The second state will be the metastable state S0. In the S0 state, the oseltamivir mainly stayed in two regions, namely S0A and S0B, they make up 33.5% and 14.5% of the metastable state S0 respectively. Oseltamivir phosphate stayed in the 2° site during S0A. N400, S367 and W403 will be connected by H-Bonds. Hydrophobic interaction will occur with N400, S367 and S370. In the second part, S0B, oseltamivir phosphate can be found inside the electrostatic funnel. Various conformations will be adopted by oseltamivir phosphate, which includes the interaction between it and P326, R327, G343, N344, N346, N347, G348, and A369.The third state of the process is the metastable state S5. In the S5 state, the oseltamivir phosphate will stay at the N1-type NA's antigen epitope. the 250 and 270 loop and the partial β sheets will form a pocket that is not deep to carry the oseltamivir phosphate. Hydrophobic interaction will occur between oseltamivir phosphate and P249, A250, K273 and I275.The upcoming state will be the metastable state S6. In the S6 state, the oseltamivir phosphate will point its carboxylate moiety toward the direction of the 1° site's center. The oxygen of the carboxylate group will react with R152's guanidine group on the 150 loop to form two H-bonds and a H-bond will be formed with N198's amide group by the acetyl group. Hydrophobic interaction will occur due to the pentyl group in the oseltamivir phosphate and S153's side chain. The fifth state is the metastable state S2. In the S2 state, the oseltamivir phosphate will have an inclined posture while going into the 1° site. H-bonds will be formed between oseltamivir's carboxylate group and R292 and R371's side chains. The sixth state is the metastable state S3. In the S3 state, the oseltamivir will not maintain its crystal structure, but the oseltamivir's carboxylate group and pentyl group will be lifted up to face the direction of the solvent. This is due to the H-bonds, which make the structure of oseltamivir phosphate be stabilized, has been removed between the carboxylate group and pentyl group and R292 and R371's side chain. For replacement, H-bonds will be formed between oseltamivir phosphate's guanidine group and carboxylate group with R152 and D151, E119 respectively. Hydrophobic interactions will occur between R224 and W178 and oseltamivir phosphate's pentyl group and acetyl group respectively. The seventh state is the metastable state S4. In the S4 state, the oseltamivir phosphate's acetyl group will react with R118's guanidine group to form a H-bond between them. The oseltamivir phosphate's carboxylate group will react with both R292 and R371's guanidine group and form two H-bonds at the same time. Hydrophobic interaction will occur between oseltamivir phosphate's pentyl group and R371 and W403' side chains & oseltamivir phosphate's acetyl group and R430's side chain. The next state will be the metastable state S7. In the S7 state, the oseltamivir phosphate's acetyl group is still at the outside of the 1° site due to the R152 did not enclose the second half of oseltamivir phosphate. Oseltamivir phosphate's carboxylate group will react with R292's guanidine group and R371's guanidine group to form a H-bond and a bidentate H-bond respectively. Oseltamivir's protonated amino group will react with D151's carboxylate group to form another H-bond and Hydrophobic interaction will occur due to the reaction between oseltamivir phosphate's pentyl group and N294, A246 and R292's side chain. The ninth state is the metastable state S8. In the S8 state, the oseltamivir phosphate's structure only have one difference when comparing to S9, which is the final state of the binding process. The only difference is that the water-mediated H-bond formed by the reaction between the oseltamivir phosphate's carboxylate group and the D151's side chain. The last state is the stable binding state S9. In the S9 state, there is a distance approximately 2 Å between oseltamivir phosphate's root mean square deviation and the crystal structure of oseltamivir phosphate. A H-bond, a bidentate H-bond, a H-bond and several H-bonds are formed between the oseltamivir phosphate's acetyl group and R152's guanidine group, oseltamivir phosphate's carboxylate group and R371's guanidine group, the carboxylate group with R292's guanidine group and the oseltamivir phosphate's protonated amino group and E119 and D151's side chains respectively. Hydrophobic interaction will occur due to the reaction between oseltamivir

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phosphate's pentyl group and A246, I222, W178, E276 and R224 and between the reaction of oseltamivir phosphate's acetyl group and I222 andW178. A water-mediated H-bond is formed between the reaction of the crystal structure of oseltamivir phosphate and R118, where a carboxylate group and a side chain will react [8].

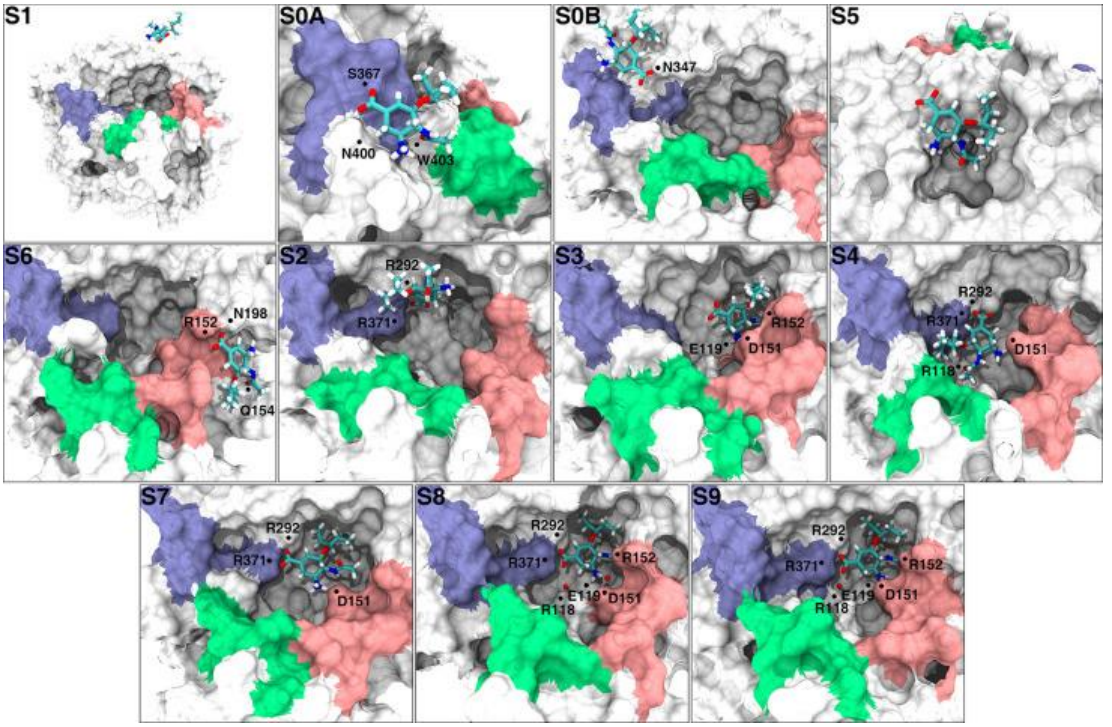

Figure 6. The binding process of oseltamivir phosphate with the Neuraminidase of Influenza A virus from S0 to S9 [8].

7. Synthesis Of Amantadine Hydrochloride

The other drug discussed in this review article is amantadine hydrochloride. Apart from treating Influenza A virus, it can also be used to alleviate Parkinson's symptoms in the early stage. The drug was first reported by Davies et al [9]. in 1964. The drug is first approved by the Food and Drug administration in 1966 to use in prevention of respiratory disease caused by influenza A virus [10].

The method for preparing amantadine hydrochloride (Fig.7) through intermediate N-(1-adamantyl)-acetamide is much simpler compare to the synthesis of oseltamivir phosphate. The starting material of the synthesis process are either adamantine or 1-bromoadamantane. Through the Ritter-type reaction, one of them will be reacted with sulfuric acid to afford N-(1-adamantyl)-acetamide. N-(1-adamantyl)-acetamide will then be deacetylated to obtain 1-amino adamantane. 1-amino adamantane will react with anhydrous hydrochloric acid to afford a 50-58% yield of amantadine hydrochloride. Another synthesis route was discovered in 2022. It requires only two steps and produce a product that have a yield as high as 88%. In this process, 1-bromadamantane was used to produce a 94% yield of N-1-(1-adamantyl)-formamide. N-1-(1-adamantyl)-formamide was than hydrolyzed with aqueous hydrochloric acid to afford amantadine hydrochloride with a yield of 93% [10].

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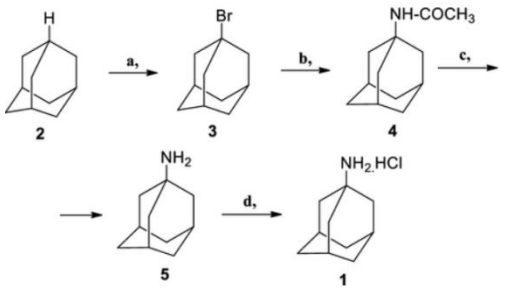

Figure 7. Four steps synthesis of amantadine hydrochloride from adamantine [10].

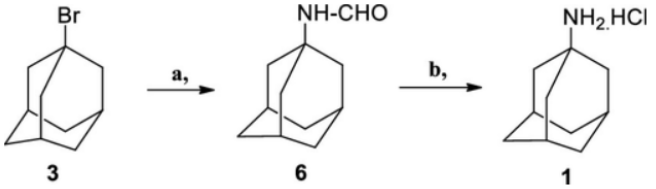

Figure 8. Two-steps synthesis of amantadine hydrochloride from 1-bromoadamantane [10].

8. The Binding Mechanism Of Amantadine Hydrochloride And Influenza A Virus

According to Brison et al [10]. , they have observed the binding mechanism of amantadine hydrochloride and Influenza A virus using human epithelial cell (do not contain N-Methyl-D-aspartic acid receptor) and mouse central nervous system cell (contain N-Methyl-D-aspartic acid receptor). The result show that Amantadine hydrochloride influence the virus replication in both type of cells, which show that it doesn't work based on the existence of N-Methyl-D-aspartic acid receptors. Amantadine will influence the viral replication after the attachment of the virus on to the receptor of the cell. Amantadine hydrochloride will affect the influenza A virus in the endosome at the uncoating stage.

9. Negative Effects Of Amantadine Hydrochloride

However, the CDC does not recommend patients to use amantadine hydrochloride to treat influenza A virus' infection. This is because amantadine hydrochloride has several side effects that can highly influenced the human body when a large doses of it has been intake by patients. According to the studies carry out by Lee et al [12], amantadine hydrochloride can affect bovine cornea endothelial cells' growth, apoptosis and proliferation. The in vitro permeability of endothelium has also been tested. The outcome depicts when the doses of amantadine hydrochloride is at ≤ 20 μM, there will be no impact on the growth of the cell. When the doses of amantadine hydrochloride are at the level of ≥ 50 μM, it will inhibit the growth of the cell. When the level of amantadine reaches ≥ 200 μM, the G1 phase in the mitotic cell cycle will be affected and the proliferation will be attenuate. DNA will be damaged at the level of amantadine hydrochloride reaches ≥ 1000 μM. Finally, at 2000 μM, amantadine hydrochloride will induce apoptosis and will increase the effect on sub-G1 phase.

10. Conclusion

Since the drugs that are designed to treat influenza virus have been invented, the virus has been always mutating, causing us human beings to put in more people and money into the invention of new drugs to treat the mutated virus and also the other new discovered ones, such as Covid-19. Though oseltamivir phosphate and amantadine hydrochloride are still been used in different places around the world, the problem of these two drugs had already appeared. No matter the problem is the complexity of the synthesis process of oseltamivir phosphate when comparing to other drugs or the negative effects caused by the intake of amantadine chloride, they are both a signal showing the importance of inventing and synthesis new drugs responsible for the treatment of influenza virus using new

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technology. The appearance of carriers made by nanomaterials have allow the usage of drugs with problems such as low permeability and some toxicity. This is a new way that we can studied in the future.

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