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RESISTANCE OF OSELTAMIVIR: A MAJOR PROBLEM FOR SWINE FLU TREATMENT

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ABSTRACT

Swine flu is a fast emerging life-threatening disease caused by the H1N1 influenza virus. It is spreading every year through changing its strains. The H1N1 (swine flu) is a new strain of flu virus that caused a pandemic worldwide in 2009 and result global health problems. Oseltamivir, Zanamivir, Adamantine, and Rimantadine were the drugs, which are effective against the H1N1 strain. Oseltamivir was a drug of choice for swine flu but it showed several adverse effects, cost problems, and became resistant against H1N1 virus. Oseltamivir was not very effective in 2008 against seasonal H1N1 virus due to acquired resistance (99.6% of H1N1), and up to 12% against the flu virus. Due to resistance, and the emergence of new strains, nowadays oseltamivir is not in prescription using nowadays. Presently there is no drug of choice for swine flu and still, it is a challenge to develop new drugs against changing strains of the H1N1 flu virus. This review highlights and rational guide to the problems associated with NA inhibitor oseltamivir, and design and development of novel drug (s) for the treatment of swine flu.

KEYWORDS: H1N1, Mutation, Neuraminidase, Oseltamivir, Resistance, Shikmic acid and Swine flu.

INTRODUCTION

Swine flu is a highly contagious acute respiratory disease in humans caused by a virus, referred to as the H1N1. There are three types of influenza viruses that A, B and C, in which influenza A virus is the most dangerous and epidemic. The influenza virus is a single-stranded RNA, a negative sense having eight segmented genomes that belong to the family

Orthomyxoviridae. Swine flu belongs to influenza Type A. It consists of two vital surface proteins that are neuraminidase (N) and hemagglutinin (H). Flu is a respiratory infection, connected with a fever, coughing, and muscle aches, which often last for a few days and it caused fatal.[1,2,3,4] Swine flu virus was first isolated in pigs in 1930 and in humans in 1933. To date, these subtypes are spreading all over the country with resistance strains. Swine flu is a highly spreading strain of the influenza virus affecting humans which contains segments of genes from pig, bird, and human influenza viruses.[3] It infects the epithelial tissues of the upper respiratory cells of humans. The surface glycoproteins hemagglutinin (HA) and neuraminidase (NA) is a vital target for sialic acid (SA) of human epithelial tissues. HA allows the attachment of the virus to the cell by the specific linkage between the receptor binding site and SA, whereas the other side NA cleaves the link between SA and HA to allow virus release from the infected cells. So there is optimum equilibrium adjust between HA and NA activities are needed for released from cells and newly viral multiplication.[4-7]

Swine flu viruses spread by direct and indirect through close animal contacts and also from contaminated objects. They can be transmitted from pigs to people and from people to pigs. The recent swine flu virus can spread by human-to-human contact. Human-to-human transmission occurs by inhalation of infectious droplets and droplet nuclei, and by direct contact, which is facilitated by air and land travel and social gatherings. Swine flu shows high morbidity and low fertility rate.[4-10] Center for Disease Control and Prevention (CDC) estimates that during the 1990s, about 36,000 people died each year of flu-related causes in the United States. Every season this virus changed its strains (Table 1) and become episodic flu that caused the death of millions of people every year. For comparison, the bird flu outbreak between 2004 and 2009 pandemic that claimed about more than 1800 and 4100 deaths respectively.[1-4]

In the year 2009 more than 340 000 reported cases of H1N1 (repeat) infection in 191 countries, and more than 4100 deaths in 2009. WHO initially projected that up to 2 billion people could become infected with the virus over the next 2 years. They estimate that globally there were 201,200 respiratory deaths and 83,300 cardiovascular deaths associated with 2009 pandemic H1N1 flu virus. Diabetes is also an important underlying illness in mortality due to swine flu. It estimated each year, seasonal influenza infects 5–20% of the population, causing 250,000–500,000 deaths worldwide,[8] This outbreak was transmitted from humans to pigs and then back to us as the same as the 1918 pandemic. In 2010, World

Health Organization (WHO) was declared that swine flu is pandemic. In February 2011, WHO had been reported that 370 resistant cases of oseltamivir against swine flu.[9-14]

Table 1: Episodic flu caused pandemic and endemic in respective year.

Influenza strains	Caused	Year	Pandemic/ Endemic
H1N1	Spanish flu	1918	Pandemic
H2N2	Asian flu	1957	Endemic
H3N2	Hong Kong flu	1968	Endemic
H5N1	Avian flu	1997	Endemic
H5N1	Bird flu	2004	Pandemic
H1N1 Repeat	swine flu	2009	Pandemic
H7N7	Zoonotic flu	2010	Endemic
H7N9, H5N7	(New strains, China)	2013	Lead to Endemic
H9N2	New strains	2013	Endemics

Some vital states of swine flu in india

According to WHO, There have been nearly 30,000 confirmed H1N1 cases across 74 countries in 2009. Around 123397 people have been tested in India as on February 1, 2010, around 4% of people who have tested positive for swine flu have died and could not be saved in India. Maximum number of 8494 H1N1 infections were registered in the capital city of New Delhi and Maharashtra. This was followed by 4607 infections in Maharashtra (317 deaths), 2063 H1N1 swine flu infection in Taminlnadu (109 deaths), 1925 infections in Rajasthan (176 deaths), 1895 infections in Haryana, 1879 in Karnataka (141 deaths) and 1441 infections in Kera.[10] In 2013, nearly 1200 infected (260 death) patient with H1N1 cases are reported in India. Last year 2019, the number of deaths recorded due to swine flu in the country was 1,218 with out of total cases of 28,798 as per the National Centre for Disease Control (NCDC). This number is increasing day by day in all over state of India. The main cause of death from novel (swine origin) influenza A/H1N1 infection is acute respiratory distress syndrome.[11,15] Bronchial asthma, pulmonary tuberculosis and fever found to risk factors for complications in H1N1 infection as same as pneumonia. Most common cause of death in patients was due to pneumonia with influenza infection. Viral infection suppresses the immune system of humans that caused to prone for bacterial and other infections. Molecularly they adhere to the surface of epithelial cells of respiratory tract and interact with sialic acid of the cells surface. The clinical impact of influenza caused a major threat for immune-compromised patients.[17]

Current scenario of influenza

On the basis of specific molecular genetics and pathogenesis criteria Influenza viruses are classified as low pathogenic avian influenza (LPAI) and highly pathogenic (HPAI). HPAI viruses can cause severe illness and high mortality in poultry form. Avian influenza A viruses of the subtypes H5 and H7, including H5N1, H7N7, and H7N3 viruses, have been associated with HPAI. Strains H3N8, H7N3, H7N7, and H10N7 viruses detected in wild birds and ducks. H7N7 an avian influenza viruses have been widely detected in wild birds and domestic poultry in China.[15-17] LPAI viruses that have infected humans include H7N7, H7N9, H9N2, H3N2v and H7N2. H7N9 respiratory virus causes 544 confirmed cases and 122 fatality cases, reported in China in May 2014.[16-18] The arising of new strains in every year is biggest problem to design and synthesis of drugs.

Drugs for swine flu

Currently there are two classes of drugs approved for the treatment and prevention of flu: M2 protein channel inhibitor, Amantadine and Rimantadine (derivative of adamantanes) and the newer neuraminidase (NA) inhibitors, such as Oseltamivir Zanamivir, Panamivir and Laninamivir. The M2 inhibitors are cheap, but they are only effective against influenza A viruses, and resistance arises rapidly. Adamantanes interfere with viral processing inside a cell and are associated with severe toxic side effects and development of drug resistance. The most recent swine flu viruses isolated from humans are resistant to Adamantine and Rimantadine in before 2005. Neuraminidase inhibitors (Zanamivir) is inhaled drugs and poorly absorbed orally and given post exposures of flu, also resistance to H3N2, H5N1 and high degree resistant to H1N1. Oseltamivir is the first oral active drug and may reduce illness but it given before 48 hour of infection. It poses a risk of developing side effects such as nausea, vomiting, abdominal pain and vertigo.[19-25]

The first vaccine was made against the H1N1 2009 as nasal spray. It is approved for use in only healthy individuals ages 2 to 49. The injectable vaccine, made from killed H1N1, became available for six month baby and pregnant women. Both the vaccines were not follows the guidelines of vaccine so not much used in market.[4,25,26] This review discussed about the all problems regarding Oseltamivir.

Oseltamivir (OST) and its major problems

OST (Tamiflu) is the only first orally active drug for swine flu which fights from the flu disease in humans. OST is an antiviral drug commercially developed for swine flu that

stopping the newly formed virions progeny with its host cell. Oseltamivir is indicated in the prophylaxis of influenza and for the symptomatic treatment not more than 2 days old. Oseltamivir is administered in the form of a prodrug oseltamivir phosphate (Tamiflu) that is rapidly converted into the active metabolite, oseltamivir carboxylate by hepatic esterase (Davies, 2010). Oseltamivir phosphate is a white crystalline solid with the chemical name (3R, 4R, 5S)-4-acetylamino-5-amino-3-(1-ethylpropoxy)-1-cyclohexene-1-carboxylic acid, ethyl ester, phosphate.

The discovery of oseltamivir and zanamivir was possible through rational drug design utilizing available x-ray crystal structures of sialic acid. Sialic acid bound to the active site of the influenza virus neuraminidase enzyme [16]. Sialic acid is the N- and –O-substituted of derivatives of neuraminic acid. Neuraminic acid is the nine carbons of monosaccharaides. Oseltamivir was developed through successive modifications to the sialic acid analogue framework (including the addition of a lipophilic side chain (Fig. 1).[17]

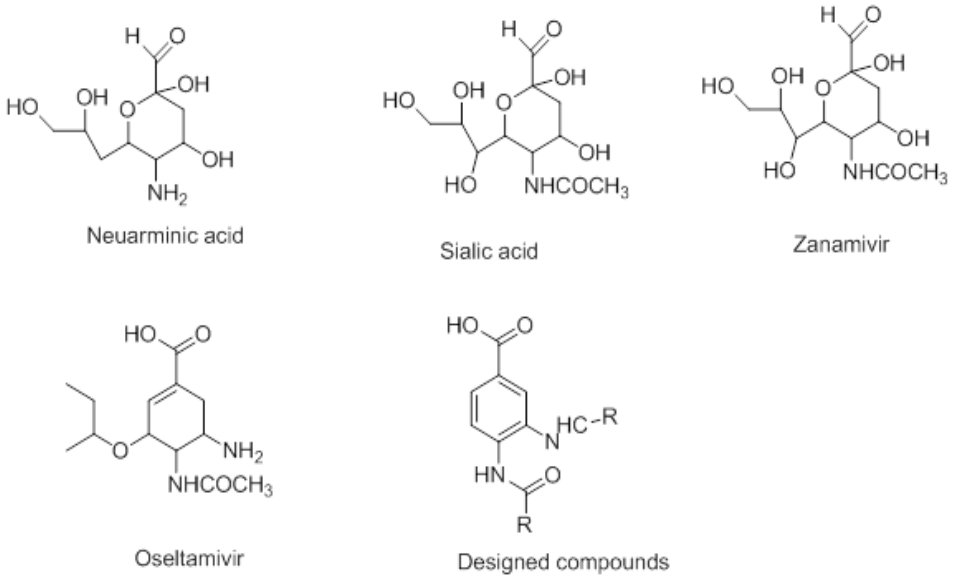
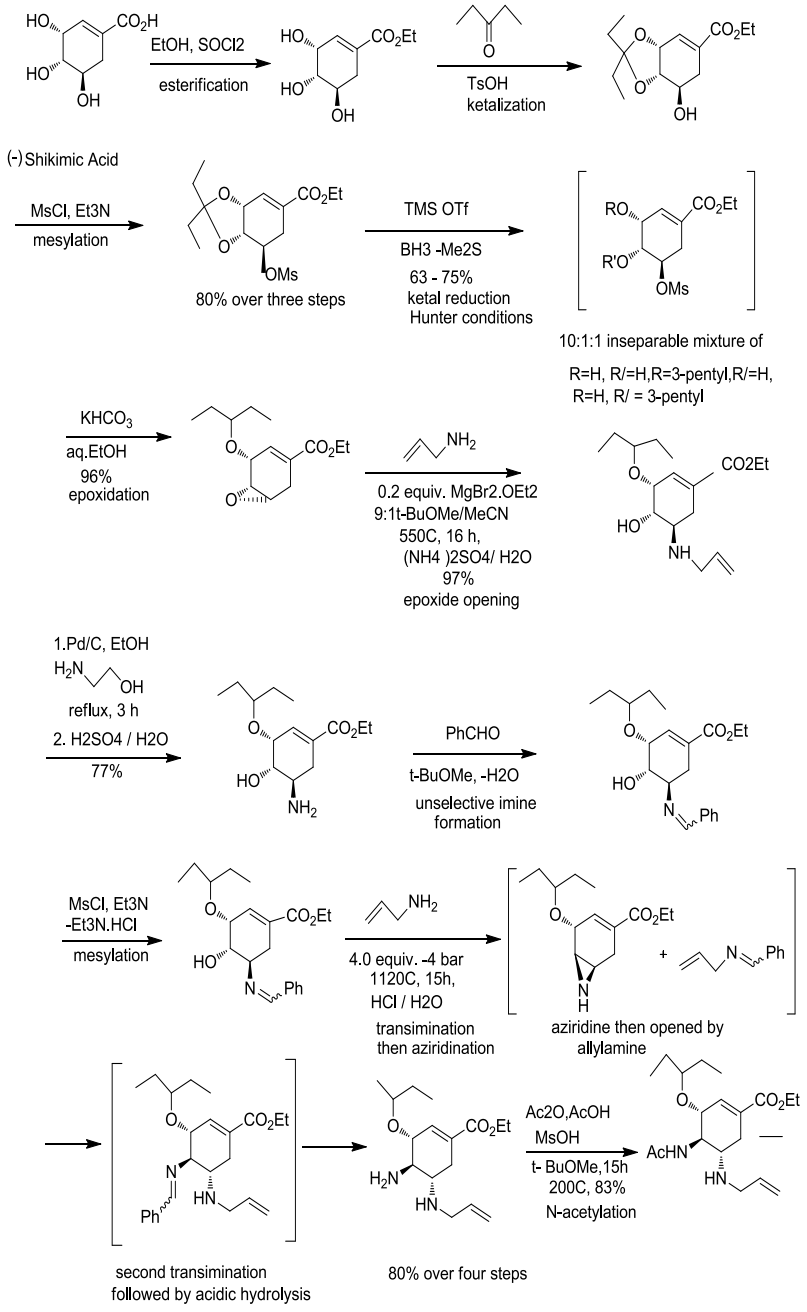


Figure 1: NA inhibitors and designed compounds.

OST mainly synthesized from rare natural plants that is Chinese star anise. Shikimic acid is the main active product of this herb which is the precursor of oseltamivir. OST is sometimes very hard to collect from its sources.[18] The shikimic acid is extracted from the seeds of Chinese star anise in a ten-stage extraction process. Thirteen grams of star anise make 1.3 grams of shikimic acid, which can be made only 10 oseltamivir capsules (75 mg). The sudden demand for shikimic acid for the production of OST, it increases the price and scarcity of shikimic acid. The scarcity of shikimic acid make a major obstacle for the

production of OST. During 2005 in pandemic flu, huge demand of surrounding avian flu, and there were globally resulting in a shortage of Tamiflu.[32] So it will be necessary to take an alternative and simple approach for the synthesis of new drugs against the flu.

The current production method involves a number of reaction steps (Fig. 2). Multiple steps synthesis is also give very complex and costlier for production of Tamiflu.[18] According to Roche synthesis of Tamiflu is summarized graphically below (Fig. 2).[32] This multistep synthesis involves complex reaction and free radicals compounds. It is very difficult to isolated and synthesis of OST from academic style manufacturing process because academic has been too many chromatography techniques, and solvent process involved.



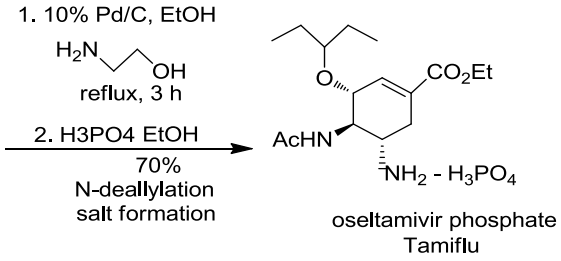


Figure 2: Synthesis of oseltamivir from Shikimic acid which involves sixteen steps.

Mechanism of action: Oseltamivir carboxylate a selective neuraminidase inhibitor, serving as a competitive inhibitor towards sialic acid, found on the surface proteins of normal host cells. Since NA cleaves the α-glycosidic bond between SA and the glycoconjugates of human's cells surface and thus destroys receptors to which influenza virus binds and helped in released virions. By preventing the activity of the viral neuraminidase enzyme, oseltamivir prevents new viral particles which are released by infected cells OST binds only in the active site of viral NA so that it can break the α-glycosidic bond and unable to released the virions to affect other cells.[21,22,23,24,25,26]

Adverse effects

The most frequent side effects are nausea and vomiting which are generally of a mild to moderate degree and usually occur within the first 2 days of treatment. The following adverse reactions have been identified during post-marketing survey of oseltamivir, Diarrhoea, Bronchitis, Stomach pain, Dizziness, Headache, Hepatitis, Abnormal liver function tests, Rash, Swelling of the face or tongue, Serious or life-threatening adverse effects like Epidermal necrolysis, Arrhythmias, Seizures, Confusion, Aggravation of diabetes, Transient neuropsychiatric events (self-injury or delirium, delusion, confusion), Pseudomembranous colitis, Pyrexia, Unstable angina (<1%) may occur.[27,28] After a comprehensive review of the available data, the FDA has recently serious skin/hypersensitivity reactions be added to the oseltamivir product label. Oseltamivir may develop a severe rash or allergic symptoms.[29,30]

Oseltamivir phosphate (Tamiflu) causes central suppression of the respiratory function in rats. Tamiflu-induced cardio-respiratory arrest and sudden death observed in influenza patients after taking Tamiflu. Studies showed the levels of oseltamivir phosphate in the brain to be approximately 1,500 times those seen in adult animals. Oseltamivir is come under category C drug, which to base a risk evaluation of oseltamivir to the pregnant woman or

developing foetus. In lactating rats, oseltamivir is excreted in the milk. It can easily cross the placenta and may cause miscarriage.[33-48]

Treatment problems

Tamiflu have some restriction for the treatments of Influenza patients. The standard dosage for treatment of patients 13 years of age and older is 75 mg b.i.d for 5 days. Pediatric patients or adults, who cannot swallow capsules, receive oseltamivir 30, 45, and 60 mg oral suspension twice daily. Healthy adults with naturally acquired febrile influenza when started within 36 hours of the onset of symptoms can reduce the severity.[34] Initiation of therapy within the first 12 h after fever onset OST reduced the total median illness in 3 days. In addition, only the earlier administration of oseltamivir reduced the duration of fever, severity of symptoms, and the times to return to baseline activity, but OST did not reduce the severity of hospitalization.[35,36] The main problem with OST is the effect of oseltamivir may be apparent within 24 h of the start of treatment. It does not prevent the flu it can only slightly reduce its length or severity of symptoms.[37] Rapid diagnostic testing followed by treatment with oseltamivir is very costly for patients that cannot afford.[38, 39] It is found that OST is not effective in seasonal influenza, but effective against serious epidemic and pandemic influenza.[40]

The efficacy of oseltamivir in the treatment of subjects with chronic cardiac disease and/or respiratory disease has not been effective.[41] Each treatment of influenza costs $70-80 for 75 mg capsules for 5 days. There are large differences in the costs of these preventatives and therapies, as well as in clinical experience, and ineffectiveness against viral subtypes.[35,42]

The rapid diagnostic test and a subsequent prescription of oseltamivir cost $52.00 per primary dose per patient. Recent observations suggest that in some patients with other infection strains like H5N1, H3N2, H3N2v, H5N7 were less effective against OST and also it not effective against influenza B. According to the article studies of OST, it has cleared that the treatment is also so costly and treatment only happened in within 48 hours of infection.[40,41-47]

Resistance of oseltamivir

During the influenza season of 2007-08, oseltamivir-resistant to influenza A viruses (H1N1) emerged in several countries like Europe, North America, Asia and slowly become worldwide.[48] The normal seasonal H1N1 virus became almost entirely resistant against

Tamiflu over the past two years. FDA approved four drugs for influenza Amantidine, Rimantidine, Zanamivir and Oseltamivir. Amaantadine and Rimantidine are M2-channel inhibitor which is not in used due to acquired resistance.[40-50] First of all a Vietnamese girl who had received a prophylactic dose (75 mg once a day) was found to be non-responsive to the Tamiflu medication. During the winter flu season of 2007-2008, about 11% and in 2009, 99.3% of flu cases were reported to be resistant to Tamiflu.[50-54]

Clinical practices showed that oseltamivir was effective to treat the 2009-H1N1 influenza but it was failed to the 2006-H5N1 avian influenza. It mainly due to the two mutations (H274Y and 347Y) between their NA.[55] This may be the reason why H5N1 avian influenza virus is drug-resistant to oseltamivir. It also reported that treatment with the recommended dose of oseltamivir, although started one day after the onset of symptoms, did not suppress viral replication efficiently and eventually led to the development of a drug-resistant strain.

There is a need for more research on resistance development in the H1N1 influenza and for prudent use of antiviral. The emergence of oseltamivir resistance influenza virus has highlighted the need for the design and development of new antiviral drugs and rapid diagnostic test that determine viral subtype or new resistance strains.[56-58]

Mutation in swine flu virus (H1N1)

Mutation is main problems for treatments of Swine flu. High mutation rate causes resistance to the commercially available drugs, especially to oseltamivir. Genetic mutations, may results in ‘antigenic shift’ (major genetic rearrangements between strains, associated with pandemics as well as epidemic), helps the virus escape the human natural immunity.[54,59] Mutation affects the virus strains ability to go deeper into the respiratory system, thus causing more serious illness.[17] The H274Y mutation has been previously established as a genetic marker of resistance to oseltamivir among patients when infected with either influenza A (H1N1) or A (H5N1) viruses.[45] The first involves mutations in neuraminidase that interfere the conformational change in active site are E119V, Q136K, R292K, H274Y, and R152K which are single nucleotide polymorphism (SNP) mutations known to interfere with this process and inhibit the rotation and prevent pocket formation for binding.[54,55] in different strains of NA.

Mutations are signal residue substitutions (His274Tyr) in the neuraminidase enzyme. This substitution of amino acids that is histidine-to-tyrosine (amino acid) at position 274 is in NA protein which causes unable to bind OST with neuraminidase. Sequence analysis of the

neuraminidase and receptor-ligands interactions revealed that location 274 (H274Y), is associated with high-level resistance to oseltamivir in influenza (N1) viruses.[56] The smaller side chain residue histidine slightly enhanced or unchanged NA sensitivity to OTV, while phenyl and Tyrosine at 274 reduced the susceptibility of OTV to neuraminidase. Due to this change of histidine at 274, it also interferes with residues Glu276 and Arg224 and makes charge-charge interactions with free binding energy. Glu276 which is just near to 274 also dislocates the position and rotation which is unfavorable to OST.[55,60] Thus H275Y mutation is the major mutation impacting influenza A/H1N1 which inhibits the conformational change during binding to neuraminidase active site. All these viruses have the same mutation in the neuraminidase gene (H275Y), conferring resistance to oseltamivir but cross-resistance to zanamivir.[56] Study shows that cross-resistance of NA inhibitors may occur. Mutations within the HA gene also resulting in decreased affinity for sialic acid, and NA inhibitors and synergistic to neuraminidase activity.[59-61]

Designing of drugs against resistant NA

Virology studies and replication mechanism of influenza virus it cleared that some specific vital molecular targets in hemagglutinin, neuraminidase and M2 protein. Among these, NA acts as potential targets and attractive target for drug development. NA also presents in some bacterial strains, fungal, protozoan, and other some other vertebrates which functionally related to viral NA[57,58-63] and can be attractive target for design NA inhibitors.

On the basis of the conserved nature of the NA catalytic site in influenza virus strains and molecular modelling studies of the binding site of the structure of NA, it is found that the active site of NA has four main binding sites. Site 1 (+ve charge) consists of Arg118, Arg292, and Arg371 and interacts with the carboxylate. The site 2 (-ve charged) consists of Glu119, Glu227, and Asp151 and interacts with the amino or guanidine. The site 3 (hydrophobic pocket) consists of Ile222 and Trp178 which accommodates the acetyl group, and site 4, consisting of Glu276 and Glu277, binds to the hydrophobic side chain.[64-69] On the basis of these sites, we can design the drugs against resistant NA in the future. The review SAR of NA inhibitors, it reported that inhibitor is mainly determined by the relative positions of substituents of the Carboxylic acid and Acetamide must be at equatorial positions of the central ring that showed the essential for activity. If the compound has all four groups' substitutions like carboxylic acid, acetamido, hydrophobic group, and glycerol then may give potent interactions with NA active site. The replacement of the glycerol moiety with a

hydrophobic and the replacement of 4-guanidino group with an amine group provide analogues structure like oseltamivir and lipophilic compound like other NA inhibitors and it may show higher oral bioavailability.

Various derivatives and analogues like, biflavone, flavones, flavanoids, thiozolidine, pyrene, pyridine, pyrrolidine, dihydropyrane, caffiec acid, creteniside, etc. were developed and synthesized and evaluated against NA.[69] But till, no any drug molecules has come in market, due to ineffective against NA, may be due to complexity of compounds, synthesis problems, pharmacokinetic problems and some molecule are failed in clinical trials. But aromatic compounds which is simple and analogues to oseltamivir may designed the compounds and overcome the problems (Fig. 1). The aromatic ring like salicylic acid, cyclohexene, and other benzene ring with carboxylic acid and acetamido group found potent NA inhibitors.[15,65,71] It reviewed these aromatic derivative showed effective, good pharmacokinetic and synthetic accessible and potent against the NA. So in future may develop the 4-acetamidsbenzoic acid as potent target for the NA.[71]

CONCLUSIONS

Oseltamivir is not drug of choice nowadays due to résistance and change in strains of the virus. Also, its treatment is also very costlier and having show more adverse effects. Due to frequent mutation in NA, OST is resistant to all strains of influenza virus that have made a big problem to developed new drugs against H1N1. M2 proton channel, hemagglutinin, and neuraminidase are three vital proteins make as targets for drug design against the influenza virus. But NA is the main vital and attractive target for designed NA inhibitors. As strains of influenza are continually mutating, it is essential that scientists quickly and efficiently determine the correct neuraminidase subtype that is responsible for the drug resistance in order to develop medications that will combat specific strains of influenza. We should also develop a simple and easy test for characterizing the new flu strains that resistance every year. The 4-acetamido benzoic acid derivative may overcome the problem as potent NA inhibitors. Molecular modeling methods and In-Silico techniques may help to designed drugs against resistant strains and solve the epidemic problem of swine flu.

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Conflict of interest

The authors have declared that there is no conflict of interest associated with this publication.

REFERENCES

1. Peiris JSM, Leo LM, Poon LLM, Guan Y. Emergence of a novel swine-origin influenza A virus (S-OIV) H1N1 virus in humans, Journal of Clinical Virology, 2009; 45: 169–173.
2. Sinha M. Swine flu. Journal of Infection and Public Health, 2009; 2(4): 157-66.
3. Jafri SSI, Ilyas M, Idrees M. Swine flu: A threat to human health. Biotechnology and Molecular Biology Review, 2010; 5(3): 46-50.
4. Khan UA. Swine flu pandemic: a global cocern. Asia Pacific Journal of Tropical Medicine, 2010; 8: 81-82.
5. Sriwilaijaroen N., Suzuki Y., Molecular basis of the structure and function of hemagglutinin of influenza virus. Proc. Jpn. Acad. 2012; 88: 226-249.
6. McKimm-Breschkin JL. Influenza neuraminidase inhibitors: antiviral action and mechanisms of resistance. Influenza Other Respi. Viruses, 2013; 1: 25-36.
7. Lokwani P, Kumar P, Upadhyay Y, Gupta S, Singh N, SWINE FLU : AN OVERVIEW, Journal of Applied Pharmaceutical Science, 2011; 1(04): 29-34.
8. Muchtaridi M, Holis AA, Holik A. Potential activity of some natural products compounds as Neuraminidase inhibitors based on molecular docking simulation and in vitro test, Journal of Applied Pharmaceutical Science, 2015; 5: 065-073.
9. Sebastian MR, Lodha R, Kabra, SK. Swine Origin Influenza (Swine Flu), Indian Journal of Pediatrics, 2009; 76: 833-841.
10. Wagner R, Matrosovich M, Klenk HD. Functional balance between haemagglutinin and neuraminidase in influenza virus infections. Rev. Med. Virol, 2002; 12: 159–166.
11. Gubareva LV, Bethell R Hart GJ, Murti KG, Penn, CR, Webster RG. Characterization of Mutants of Influenza A Virus Selected with the Neuraminidase Inhibitor 4-Guanidino-Neu5Ac2en JOURNAL OF VIROLOGY, 1996; 70(3): 1818–1827.
12. Järhult JD. Oseltamivir (Tamiflu) in the environment, resistance development in influenza A viruses of dabbling ducks and the risk of transmission of an oseltamivir-resistant virus to humans. Infect Ecol Epidemiol, 2012; 2: 1-10.
13. Wiwanitkit V. Diabetes mellitus as an important identified personal illness in death cases due to swine flu: An observation. Primary care diabetes, 2009; 3: 201.
14. Jefferson T, Demicheli V, Rivetti D, Jones M, Pietrantonj C, Rivetti A. Antivirals for influenza in healthy adults: systematic review, Lancet, 2006; 367(9507): 303-313.

15. Gupta MK, Dangi JS, Bharti, SK. Mutations in surface protein of swine flu: A major problem for H1N1 inhibitor, Asian Journal of Biomedical and Pharmaceutical Sciences, 2015; 5(50): 1-9.
16. Guo L, Rebecca J, Garten, Angie S, Foust WM, Sessions, Margaret OA, Larisa V, Gubareva, Alexander I, Klimov, Xiyan Xu. Rapid identification of oseltamivir-resistant influenza A(H1N1) viruses with H274Y mutation by RT PCR/restriction fragment length polymorphism assay. Antiviral Research, 2009; 82: 29–33.
17. Ward MJ, Lycett SJ, Avila D, Bollback JP, Leigh BA J. Evolutionary interaction between haemagglutinin and neuraminidase in avian influenza. BMC Evolutionary Biology, 2013; *13*(1): 222-227.
18. Tan, KX, Jacob SA, Lee LH. An overview of the characteristics of the novel avian influenza A H7N9 virus in humans, Frontiers in microbiology, 2015; 140: 1-11.
19. Dandagi GL, Byahatti SM. An insight into the swine-influenza A (H1N1) virus infection in humans. Lung India, 2011; 28(1): 34–38.
20. Gdynia G, Schnitzler P, Brunner E, Kandolf R, Bläker H, Daum E, Schnabel P, Schirmacher P, Roth W. Sudden death of an immunocompetent young adult caused by novel (swine origin) influenza A/H1N1-associated myocarditis. Virchows Arch, 2011; 458(3): 371-376.
21. Puvanalingam A, Rajendiran C, Sivasubramanian K, Ragunanthanan S, Suresh S, Gopalakrishnan, S. Case series study of the clinical profile of H1N1 swine flu influenza. J. Assoc Physicians India, 2010; 59: 14-18.
22. Wang JF, Wei DQ, Chou KC. (2009). Insights from investigating the interactions of adamantane-based drugs with the M2 proton channel from the H1N1 swine virus. Biochem. And Biophy. Re. Commun, 2009; 388: 413–417.
23. ZhangJ, Xu W. Recent Advances in Anti-Influenza Agents with Neuraminidase as Target, Mini-Reviews in Medicinal Chemistry, 2006; 6: 429-448.
24. Lew W, Chen X, Kim CU. Discovery and development of GS 4104 (oseltamivir) an orally active influenza neuraminidase inhibitor. Curr Med Chem, 2000; 7: 663-672.
25. Moscona A. Neuraminidase Inhibitors for Influenza, N. Engl. J. Med, 2005; 353: 1363-1373.
26. Sßerefnur S, Swine flu vaccine adjuvants and multiple sclerosis – Is there potential for harmful effect? Medical Hypotheses, 2010; 75: 50–52
27. Farina V, Brown JD. Tamiflu: The Supply Problem. Angew. Chem. Int. Ed, 2006; 45: 7330-7334.

28. Bertelli A.E.A. Stockpiling of raw material for oseltamivir is not justified. BMJ, 2006; 332(7534): 177-180.
29. Thabet AA, Bahlooli SH, AlKohlani A, Shoja'a A. Oseltamivir-resistant pandemic (H1N1)2009 in Yemen - case report. Virol J, 2010; 8(7): 88.
30. Martin K, René T. New, Azide-Free Transformation of Epoxides into 1, 2-Diamino Compounds: Synthesis of the Anti-Influenza Neuraminidase Inhibitor Oseltamivir Phosphate (Tamiflu). *J. Org. Chem*, 2001; 66(6): 2044–2051.
31. Ives J, Carr J, Mendel D, Tai C, Lambkin R, Kelly I, Oxford J, Hayden F, Roberts N. The H274Y mutation in the influenza A/H1N1 neuraminidase active site following oseltamivir phosphate treatment leave virus severely compromised both in vitro and in vivo. Antiviral Research. 2002; 55: 307–317.
32. Federspiel M, Fischer R, Hennig M, Mair HJ, Oberhauser T, Rimmler G, Albiez T, Bruhin J, Estermann H, Gandert C, Göckel V, Götzö S. Industrial synthesis ofthe key precursor in the synthesis of the anti-influenza drug oseltamivir phosphate (Ro 64-0796/002, GS-4104-02): ethyl (3R,4S,5S)-4,5-epoxy-3-(1-ethyl-propoxy)-cyclohex-1-ene-1-carboxylate. Org. Process Res. Dev., 1991; 3: 266–274.
33. Davies BE, Pharmacokinetics of oseltamivir: an oral antiviral for the treatment and prophylaxis of influenza in diverse populations. J Antimicrob Chemother, 2010; 6(2): 5-10.
34. Beigl RH, Han K, Venkataramanan R, Hankins GD, Clark S, Hebert MF, Esterling T, Zajicek A, Mattison DR, Caritis SN. Pharmacokinetics of oseltamivir among pregnant and nonpregnant women. Am. J. Obstet Gynecol, 2011; 204(6): S84–S88.
35. Snell P, Dave N, Wilson K, Rowell L, Weil A, Galitz L, Robson R. Lack of effect of moderate hepatic impairment on the pharmacokinetics of oral oseltamivir and its metabolite oseltamivir carboxylate. Br. J. Clin. Pharmacol, 2005; 59: 598-601.
36. Aoki FY, Doucette KE. Oseltamivir: a clinical and pharmacological perspective. Expert Opinion on Pharmacotherapy, 2001; 2(10): 1671-1683.
37. Smieja M. Oseltamivir relieves symptoms but does not reduce hospitalizations in influenza. Ann Intern Med, 2012; 157(6): 3-5.
38. Risebrough NA, Bowles SK, Simor AE, McGeer A. Economic evaluation of oseltamivir phosphate for postexposure prophylaxis of influenza in long-term care facilities. J.Am. Geriatr. Soc, 2005; 53: 444-451.
39. Hill G. Cihlar T. Oo CS, Edmund H.O, Prior k, Wiltshire H, Barrett J, Leo B, Ward P. The anti-influenza drug oseltamivir exhibits low potential to induce pharmacokinetic drug

interactions via renal secretion-correlation of in vivo and in vitro studies. Drug Metab Dispos, 2002; 30: 13-19.

40. Butler, D. Wartime tactic doubles power of scarce bird-flu drug. Nature, 2005; 438: 6-10.
41. Toriumi Y, Kamei T, Murata K, Takahashi I, Suzuki N, Mazda O. Utility of maoto in an influenza season where reduced effectiveness of oseltamivir was observed - a clinical, non-randomized study in children. Forsch Komplementmed, 2012; 19(4): 179-186.
42. Nordstrom BL, Oh K, Sacks ST, L'Italien GJ. Skin reactions in patients with influenza treated with oseltamivir: a retrospective cohort study. Antivir Ther, 2004; 9: 187-195.
43. Kaji M, Fukuda T, Tanaka M, Aizawa HA. Side effect of neuraminidase inhibitor in a patient with liver cirrhosis. J. Infect Chemotherapy, 2009; 11: 41-43.
44. Jefferson T, Jones MA, Doshi P, et al. Neuraminidase inhibitors for preventing and treating influenza in healthy adults and children. Cochrane Database Syst Rev, 2014; 161(8): 4-11.
45. Kimura S, Niwa Y, Iwajima Y, Nagano Y, Yamamoto S, Ohi Y, Maeda Y, Kurono, Y, Ono H, Haji A. High doses of oseltamivir phosphate induce acute respiratory arrest in anaesthetized rats. Basic Clin Pharmacol Toxicol, 2012; 111(4): 232-239.
46. Meijer WJ, Bruinse HW, Van den Broek M.P, Kromdijk W, Wensing AM. Oseltamivir and its active metabolite cross the placenta at significant levels. Clin Infect Dis, 2012; 54(11): 1676-1678.
47. Nagase H, Moriwaki K, Kamae M, Shinichiro MD, Yanagisawa S, Kamae I. Cost-Effectiveness Analysis of Oseltamivir for Influenza Treatment Considering the Virus Emerging Resistant to the Drug in Japan. Value in Health, supplement, 2009; 5(12): 62-65.
48. Treanor JJ, Hayden FG, Vrooman PS, Barbarash R, Bettis R, Riff D, Singh S, Kinnersley N, Ward P. Mills R.G. Efficacy and safety of the oral neuraminidase inhibitor oseltamivir in treating acute influenza: a randomized controlled trial. JAMA, 2000; 283: 1016-1024.
49. Aoki FY, Macleod MD, Paggiaro P Carewicz O, El Sawy A, Wat, C, Griffiths M, Waalberg E, Ward P. Early administration of oral oseltamivir increases the benefits of influenza treatment. J. Antimicro Chem, 2003; 51: 123-129.
50. Nicholson K.G, Aoki FY, Osterhaus AD, Trottier S, Carewicz O, Mercier CH, Rode A, Kinnersley N, Ward P. Efficacy and safety of oseltamivir in treatment of acute influenza: a randomised controlled trial. Neuraminidase Inhibitor Flu Treatment Investigator Group. Lancet, 2000; 355: 1845-1850.

51. Rothberg MB, Bell Antonio S, Rose DN. Management of influenza in adults older than 65 years of age: cost-effectiveness of rapid testing and antiviral therapy. Ann. Intern Med, 2009; 139: 321-329.
52. Machado CM, Boas LS, Mendes AV, Rocha IF, Sturaro D, Dulley FL, Pannuti CS. Use of Oseltamivir to control influenza complications after bone marrow transplantation. Bone Marrow Transplant, 2004; 34: 111-114.
53. Oxford JS, Bossuyt S, Balasingam S, Mann A, Novelli P, Lambkin R. Treatment of epidemic and pandemic influenza with neuraminidase and M2 proton channel inhibitors. Clinical Microbiology and Infection, 2003; 9: 1-14.
54. Kaminski MM, Ohnemus A, Staeheli P, Rubbenstroth D. Pandemic 2009 H1N1 influenza A virus carrying a Q136K mutation in the neuraminidase gene is resistant to zanamivir but exhibits reduced fitness in the guinea pig transmission model. J Virol, 2013; 87(3): 1912-1915.
55. De Jong MD, Thanh TT, Truong HK**,** Hien VM, Smith JD, Chau NV, Cam BV, Qui PT, Ha DQ, Guan Y, Peiris JS, Hien TT, Farrar J. Oseltamivir resistance during treatment of influenza A (H5N1) infection. N Engl J. Med., 2005; 353: 2667-2672.
56. Yen HL, Herlocher LM, Hoffmann E, Matrosovich MN, Monto AS, Webster RG, Govorkova EA. Neuraminidase inhibitor-resistant influenza viruses may differ substantially in fitness and transmissibility. Antimicrob Agents Chemother, 2005; 49: 4075-84.
57. McCullers JA. Effect of antiviral treatment on the outcome of secondary bacterial pneumonia after influenza. J. Infect Dis., 2004; 190: 519-526.
58. Kawai N, Ikematsu H, Iwaki N, Satoh I, Kawashima T, Maeda, T, Miyachi K, Hirotsu, N, Shigematsu T, Kashiwagi, S. Factors influencing the effectiveness of oseltamivir and amantadine for the treatment of influenza: a multicenter study from Japan of the 2002-2003 influenza season. Clin Infect Dis., 2003; 40: 1309-1316.
59. Renaud C, Kuypersa j, England JA. Emerging oseltamivir resistance in seasonal and pandemic influenza A/H1N1. Journal of Clinical Virology, 2011; 52: 70– 78.
60. Le QM, Kiso M, Someya K, Sakai YT, Nguyen TH, Nguyen KH, Pham ND, Ngyen HH, Yamada S, Muramoto Y, Horimoto T. Takada A, Goto H, Suzuki T, Suzuki Y, Kawaoka Y. (2005). Avian flu: isolation of drug-resistant H5N1 virus. Nature, 2005; 437: 1108.
61. Menno D, De Jong MD, Tran TT, Khanh TH. Hien V. Oseltamivir Resistance during Treatment of Influenza A (H5N1) Infection. New engl. j. med., 2005; 353: 2667-2672.

62. Bouvier M Rahmat S, Pica N. Enhanced Mammalian Transmissibility of Seasonal Influenza A/H1N1 Viruses Encoding an Oseltamivir-Resistant Neuraminidase, Journal of Virology, 2012; 86(13): 7268–7279.
63. Dharan NJ, Gubareva L V, Meyer J, Adhiambo MO, McClinton RC, Marshall S, George KS, Epperson S, Brammer L, Klimov AI, Bresee JS, Fry AM. Infections with Oseltamivir-Resistant Influenza A (H1N1) Virus in the United States. American Medical Association, 2009; 301(10): 1034-1041.
64. Gubareva LV, Kaiser L, Matrosovich MN, Soo-Hoo Y, Hayden FG. Selection of influenza virus mutants in experimentally infected volunteers treated with oseltamivir. J. Infect. Dis, 2001; 183: 523–531.
65. Gupta MK, Dangi JS, Patel VK. Structure based drug design aspect of Schiff base linked with oseltamivir analogues as neuraminidase inhibitors. World Journal of Pharmacy and Pharmaceutical Sciences, 2016; 5(9): 1-14.
66. D'Ursi P, Chiappori F, Merelli I, Cozzi P, Rovida E, Milanesi, L. Virtual screening pipeline and ligand modelling for H5N1 neuraminidase. Biochemical and Biophysical Research Communications, 2009; 383: 445–449.
67. Sumo U, Tambunan F, Rachmania, Rizky AR, Parikesit, Arli AA. Tambunan, Usman Sumo. In silico modification of oseltamivir as neuraminidase inhibitor of influenza A virus subtype H1N1. The Journal of Biomedical Research, 2015; 29(2): 150-159.
68. Shen Z, Lou K. Wang W. New small-molecule drug design strategies for fighting resistant influenza A, Acta Pharmaceutica Sinica B, 2015; 5(5): 419–430.
69. Bolz I, May C, Spange S. Novel Schiff bases derived from 5-aminobarbituric acid: synthesis and solid state structure, Arkivoc, 2007; 3: 60-67.
70. Zhang J, Wang Q, Fang, H, Xu W, Liu, Du G. Design, synthesis, inhibitory activity, and SAR studies of hydrophobic p-aminosalicylic acid derivatives as neuraminidase inhibitors. Bio Med Chem. 2008; 16: 3839–3847.
71. Gupta MK, Dangi JS, Patel VK and Kiran S., Structure based drug design aspect of schiff base linked with oseltamivir analogues as neuraminidase inhibitors, world journal of pharmacy and pharmaceutical sciences, 2016; 5(9): 1767-1781.