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Review Article

Neuraminidase Inhibitors – For Effective Treatment of Influenza

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ABSTRACT
Influenza or simple 'flu' is a very common virus mediated infection in upper respiratory tract caused by Flu viruses, belonging to the *Orthomyxoviridae* family, are generally classified as A, B, and C, on the basis of the antigenic differences of their nuclear and matrix proteins. This causes pandemic in eastern world in majority with few other cases of western globe at current. "The Spanish Influenza Pandemic of 1918" which claimed about 50 million people worldwide was the best scientific documented evidence, followed by "Hongkong Disaster" of 1968 which killed about 70000 people in Asia and 34000 people in United States of America. With the drug design researches Sialic acid based, Cyclohexane based & benzoic acid based designed drugs were marketed successfully. Six drugs are currently available for the treatment or prophylaxis of influenza infections: the adamantanes (AM) (amantadine and rimantadine) and the newer class of neuraminidase inhibitors (NAI) (zanamivir, Laninamivir, Peramivir and oseltamivir). But with the passage of time efficacy of drugs are reducing due to genotypic, phenotypic and clinical resistance. It can be used therapeutically for prophylactic use in children, who suffers largely in winters & breast feeding mothers. NAIs are category C drugs which makes them not cent percent fit for safe administration and lack of clinical data with large variability in obtained results restricts its frequent use. For complete eradication better drug design, drug discovery, synthetic & drug development research approaches are needed to develop cost effective formulations along with necessary improvement in primary health measures.
Keywords: Neuraminidase Inhibitors, Influenza, Oseltamivir, Drug design, Drug synthesis, Tamiflu resistance.

INTRODUCTION

Influenza or simple 'flu' is a very common virus mediated infection in upper respiratory tract caused by Flu viruses, belonging to the *Orthomyxoviridae* family, and are classified as A, B, and C, on the basis of the antigenic differences of their nuclear and matrix proteins that significantly caused morbidity and mortality across the globe [1]. All civilizations had come across this disease for centuries and are the most widespread suffering for present day population across the globe too. Although humans had faced many influenza pandemics year by year, but the best ever documentation of influenza disaster was "The Spanish Influenza Pandemic of 1918" which claimed about 50 million people worldwide [2]. In the peak scientific era of 1960s when infectious disease prevention missions were rising at their greatest pace, then appeared the "Hongkong Disaster" of 1968 which killed about 70000 people in Asia and 34000 people in United States of America [3, 4]. Influenza epidemics occur almost every winter and are associated with considerable morbidity and mortality. All age groups are susceptible, but increasing age, certain chronic medical conditions, and residential care increase the risk of complications and death. Two interventions can lessen the impact of flu: immunization with inactivated vaccines and treatment and prophylaxis with antivirals [5].

Six drugs are currently available for the treatment or prophylaxis of influenza infections: the adamantanes (amantadine and rimantadine) and the newer class of neuraminidase inhibitors (zanamivir, Laninamivir, Peramivir and oseltamivir). The adamantanes interfere with viral uncoating inside the cell. They are effective only against influenza A and are associated with several toxic effects and with rapid emergence of drug-resistant variants [6]. Adamantane-resistant isolates of influenza A are genetically stable, can be transmitted to susceptible contacts, are as pathogenic as wild-type virus isolates, and can be shed for prolonged periods in immunocompromised patients taking the drug. This potential for the development of resistance especially limits the use of the adamantanes for the treatment of influenza, although the drugs still have a place in planning for prophylaxis during an epidemic [6, 7].

The neuraminidase inhibitors zanamivir and oseltamivir interfere with the release of progeny influenza virus from infected host cells, a process that prevents infection of new host cells and thereby halts the spread of infection in the respiratory tract. Since replication of influenza virus in the respiratory tract reaches its peak between 24 and 72 hours after the onset of the illness, drugs such as the neuraminidase inhibitors that act at the stage of viral replication must be administered as early as possible [7]. In contrast to the adamantanes, the neuraminidase inhibitors are associated with very little toxicity and are far less likely to promote the development of drug-resistant influenza [7, 8].

Influenza virus contains two highly variable envelope of glycoproteins, hemagglutinin (HA) and neuraminidase (NA) [7]. Swine influenza is known to be caused by the influenza A subtypes H1N1, H1N2, H3N1, H3N2 and H2N3. In pigs, three influenza A virus subtypes (H1N1, H3N2, and H1N2) are the most common strains worldwide [9]. In the United States, the H1N1 subtype was exclusively prevalent among the swine populations before 1998; however, since late August 1998, H3N2 subtypes have been isolated from the pigs [9, 10]. As of 2004, H3N2 virus isolates from US swine and turkey stocks were triple reassortants, containing genes from human (HA, NA, and PB1), swine (NS, NP, and M), and avian (PB2 and PA) lineages [9].

Mechanism of Influenza Virus Replication

For Influenza virus to be infective, its haemagglutinin must first bind with sialic acid glycoconjugates, the putative receptor on the host cell for the virus [6, 7]. Binding of the haemagglutinin allows the virus to penetrate the plasma cell membrane, uncoat and enter the cytoplasm. Viral RNA strands replicate in the nucleus and new virus particles are produced [1, 7]. Neuraminidase is a glycohydrolase enzyme that is responsible for cleavage of terminal sialic acid residues from carbohydrate moieties on the surface of host cells and the influenza virus envelope. Neuraminidase is responsible for the release of newly formed virus from the surface of infected cells and aids the motility of virus through the mucous lining of the respiratory tract [7, 11].

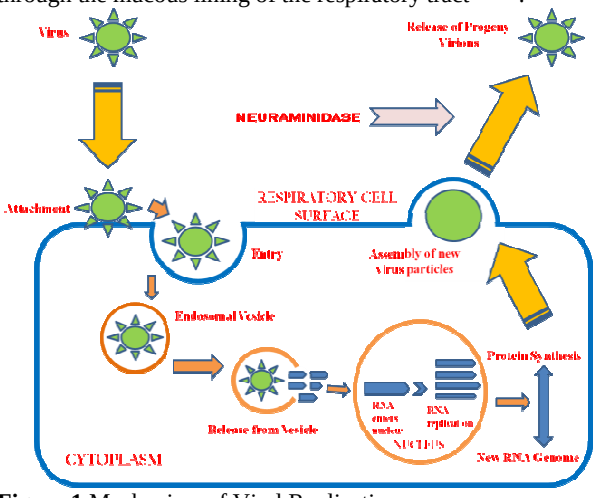


Figure 1 Mechanism of Viral Replication

Neuraminidase Crystal Structure

Analysis of these structures has revealed some common structural features among all influenza NAs: (1) the NA active site contains some well-formed, large and relatively rigid pockets; (2) all residues making direct contact with the substrate are strictly conserved and interact in a similar fashion with both substrate and inhibitor molecules; and (3) unlike the active site of most other enzymes, the NA active site contains an unusually large number of polar or charged residues, suggesting that electrostatic interactions might play a critical role for any successful inhibitors [12, 13]. The NA active site can be further divided into three major binding pockets based on the crystal structures, which could be explored for improving inhibitor binding. Pocket 1 is formed by Glu-276, Glu- 277, Arg-292 and Asn-294. This pocket interacts with the glycerol moiety of sialic acid. Pocket 2 is surrounded on one side by Ala-246, on the other side by Ile-222 and an Arg-224 side-chain forms the bottom. All three residues are highly conserved and could provide hydrophobic interactions with potential inhibitors. The cyclohexene series of inhibitors do bind in this pocket and form favorable hydrophobic interactions with the residues present. Glu-119, Asp-151, Arg-152, Trp-178, Ser-179, Ile-222 and Glu-227 of NA form pocket 3 and interact with both the C-4 hydroxyl and N-acetyl groups of sialic acid. This pocket is very large, becomes deeply buried upon inhibitor binding and is not fully utilized by sialic acid [12, 14].

Structure of Neuraminidase

Neuraminidase is a mushroom-shaped tetrameric protein, anchored to the viral membrane by a single hydrophobic sequence of some 29 amino acids near the N-terminus. Treatment of virions with pronase liberates a 200-kDa protein containing 4 identical and glycosylated polypeptides that have all of the antigenic and enzymatic properties of membrane-bound neuraminidase [13]. This protein has been crystallized and its structure determined by X-ray diffraction. The structure displays a symmetrical folding pattern of six 4-stranded anti-parallel 0-sheets arranged like the blades of a propeller. The central and first strand of each sheet is parallel to the propeller axis and the outermost strand is nearly perpendicular to it, giving each sheet the characteristic twist [15]. The outermost strand of 1 sheet is then connected to the central strand of the following sheet. These connecting loops, on the upper surface of the enzyme with respect to its orientation on the viral membrane, contain many of the antigenically and enzymatically important amino acids. The 4 identical subunits are arranged with circular 4-fold symmetry, and the enzyme active site is located centrally on each subunit directly over the 6 central & strands of each sheet [16]. The site is a deep pocket, and amino acid which line and surround the walls of the pocket are invariance all strains of influenza virus characterized so far. These active sites are associated, strain-invariant amino acids are of 2 types. Some of them are functional in the sense that they are in direct contact with bound substrate, and some provide only a structural framework for the functional residues [15, 16].

Individual Drugs

Oseltamivir

Oseltamivir phosphate [Ethyl (3*R*, 4*R*, 5*S*)-4-(acetylamino) -5-amino -3- (1-ethylpropoxy) cyclohex-1-ene-1-carboxylate] is a prodrug of oseltamivir carboxylate, which is an inhibitor of neuraminidase that is essential in the replication of influenza A and B viruses [6]. Oral oseltamivir is well absorbed and reaches peak serum concentrations in 1 hour. Bioavailability of oseltamivir phosphate is at least 75%. The prodrug oseltamivir phosphate undergoes extensive hepatic metabolism via ester hydrolysis. More than 99% of active oseltamivir carboxylate is excreted renally [6]. Oseltamivir carboxylate, the active drug metabolite, selectively blocks viral neuraminidase, thereby preventing the release of virus from infected cells. Oseltamivir is approved for the treatment of children (≥1 year) and adults with influenza A or B viral infections. Treatment should start within 48 hours of disease onset and continue for 5 days [17]. Oseltamivir is as effective as the other neuraminidase inhibitor, zanamivir, in reducing the febrile period during infection with influenza A (H1N1), influenza A (H3N2), and influenza B virus. Oseltamivir is also used for post exposure prophylaxis against influenza A and B, including pandemic strains [17].

For this indication, oseltamivir should be started within 48 hours of exposure and continued daily for at least 10 days or for up to 6 weeks during an outbreak. A systematic review reported no statistically significant difference between oseltamivir and zanamivir prophylaxis for preventing symptomatic influenza among immune-competent adults [18, 19]. The most common adverse effects of oseltamivir are nausea, vomiting, diarrhea, abdominal pain, insomnia, and vertigo. Neuropsychiatric adverse effects include delirium, abnormal behavior, and hallucinations [20].

Estimation of Oseltamivir

One of the method include direct UV measurements, oxidative coupling with MBTH23, formation of complex with Fe (III) chloride and potassium ferricyanide, formation of ion-pair associates with bromocresol green, bromocresol purple, congo red and bromochlorophenol blue. The proposed method is based on the oxidation of OSP by potassium permanganate in alkaline medium resulting in formation of bluish green colored manganate ion, which exhibits maximum absorbance at 635 nm [21].

Zanamivir

Zanamivir is an inhaled neuraminidase inhibitor that is used for the treatment and prophylaxis of Influenza A and B viruses. Zanamivir is not bioavailable by oral administration since it is poorly absorbed. Inhaled zanamivir produces high concentrations in the respiratory tract where influenza virus infection occurs [7]. The absorbed drug is not metabolized and is excreted unchanged in the urine, while the unabsorbed drug is excreted in the feces. The mechanism of action of zanamivir is similar to oseltamivir, by inhibiting neuraminidase, which is essential for release of newly formed viral particles from infected cells [6, 11]. For treatment, zanamivir is given by inhalation twice daily for 5 days, with the therapy begun within 48 hours after symptom onset. Inhaled zanamivir is well tolerated. Acute bronchospasm with decline in respiratory function has been reported; a bronchodilator should be available if given as treatment for patients with underlying pulmonary disease [5, 22].

Drug Interactions

Zanamivir has very low interaction with other drugs and is considered as a safer drug for both prophylactic and pregnancy state use [23]. Animal models did not show any significant changes in expression of hepatic cytochrome isoenzymes. Zanamivir has no effect on the metabolism of the cytochrome substrates bufuralol, chlorzoxazone, coumarin, ethoxyresorufin, mephenytoin, midazolam, phenacetin and tolbutamide [7].

PERAMIVIR

Peramivir (C15H28N4O4) is a neuraminidase inhibitor developed by BioCryst Pharmaceuticals Inc. Its absolute configuration has been determined crystallographically from a complex of the molecule with neuraminidase (influenza A). The compound was tested as an influenza A/B and an avian-influenza (H5N1) drug. Peramivir can be crystallized from methanol/water mixtures either as a dihydrate (needle-like crystals) of hitherto unknown structure or as a trihydrate (distorted octahedra), the latter being used as the pharmaceutical active form [24].

Laninamivir

Laninamivir is a potent NA for various viruses. Its chemical structure is (2R, 3R, 4S)-3-(acetamido)-2-[(1R, 2R)-2, 3-dihydroxy-1-Methoxypropyl]-4-guanidino-3, 4-dihydro-2H-pyran-6-carboxylic acid. It is discovered by Daiichi Sankyo Company Limited. It is used for H1N1, H2N2, H5N1, H3N2 viral infection [25].

Pharmacokinetics

Zanamivir is not bioavailable orally and is marketed as a dry powder for inhalation. It is delivered directly to the respiratory tract through an inhaler that holds small pouches of the drug [1]. Zanamivir is highly concentrated in the respiratory tract; 10 to 20 percent of the active compound reaches the lungs, and the rest is deposited in the oropharynx. Five to 15 percent of the total dose is absorbed and excreted in the urine, resulting in a bioavailability of 2 percent, a feature that is potentially advantageous in situations in which a systemic drug is undesirable [1, 7]. Oseltamivir is available as a capsule or powder for liquid suspension with good oral bioavailability. It is readily absorbed from the gastrointestinal tract, is converted by hepatic esterases to the active form of the compound (oseltamivir carboxylate), and is widely distributed in the body. The half-life is 6 to 10 hours. The drug is excreted primarily through the kidneys; thus, dosing must be modified in patients with renal insufficiency. Oseltamivir achieves high plasma levels and thus can act outside the respiratory tract [1, 6].

Table 1 Description of Properties of Neuraminidase Inhibitors

	OSELTAMIVIR	**ZANAMIVIR**	**PERAMIVIR**	**LANINAMIVIR**
Molecular Formula	$C_{16}H_{28}N_2O_4$	$C_{12}H_{20}N_4O_7$	$C_{15}H_{28}N_4O_4$	$C_{13}H_{22}N_4O_7$
Molar Mass	312.4	332.3	328.4	346.34
Appearance	White powder	White to off-white Crystalline powder	White to off-white powder	White Crystalline powder
Solubility in water	Highly soluble	Soluble	Freely soluble	Highly soluble
Melting Point (in °C)	190-192	250-256	170-172	224-228
pKa_1	7.75	3.82	4.2	3.68
Density (in $g/cm^{3)}$ (at 20°C)	1.22	1.75	1.39	1.61

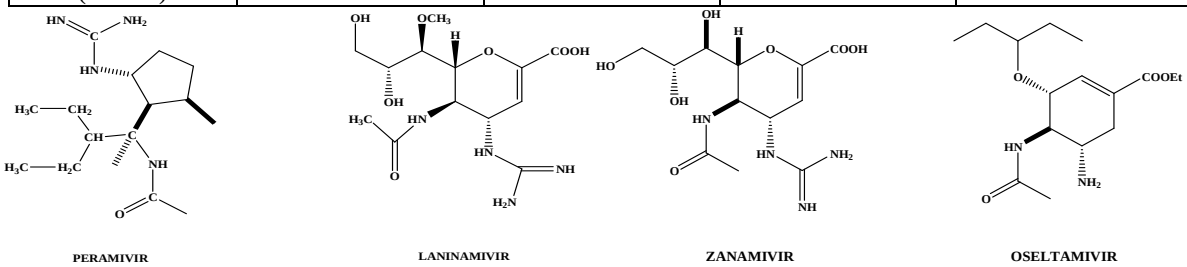


Figure 2 Structures of Peramivir, Lanamivir, Zanamivir and Oseltamivir

Efficacy of Neuraminidase Inhibitors as Prophylactic Agent

In prophylaxis models, oseltamivir was effective in preventing experimental influenza virus infection in healthy volunteers when treatment began prior to a virus challenge. Oseltamivir, 75 mg bid for 5 days, administered to otherwise healthy adults with naturally acquired febrile influenza when started within 36 hours of the onset of symptoms, reduced the duration of the disease by up to 1.5 days and the severity of illness by up to 38 %. In addition, oral administration of oseltamivir reduced the levels of inflammatory mediators (cytokines and chemokines) produced in response to the experimental viral infection when treatment was initiated a day after infection. Inhaled zanamivir reduces the median time to alleviation of major influenza symptoms by up to 2.5 days if taken within 48 h of symptom onset. These benefits appear to be particularly marked in severely ill patients and in individuals≥ 50 years of age, who have underlying illnesses, or who are considered high risk. Patients with a lower temperature or less severe symptoms appear to derive less benefit from treatment with zanamivir. [26].

Neuraminidase Inhibitors Use For Pediatrics

Influenza attack rates often exceed 40% in preschool children and 30% in school age children. School age children are the main source of spread of influenza into households [27]. Children and old age people are the greatest sufferers of Influenza, due to reduced immunity and exposure. Vaccination is the prime step for prevention [28]. Treatment of influenza in children with zanamivir and oseltamivir provided a more rapid resolution of symptoms and resolution of illness generally (resolution of symptoms and fever and return to school or normal activity) by between 0.5 to 1.5 days [29].

Use of Neuraminidase Inhibitors in Pregnant & Breastfeeding Mothers

The current medical practice insists prevention rather than treatment. There are only a handful of evidences of long term pharmacotherapy for pregnant & breastfeeding mothers. Many trials had been conducted across the world but no sure data on a common platform for global acceptance has been ever established [20]. Oseltamivir is a Category C drug [30] that reflects potentiality to cause foetal damage. It is also secreted in breast milk. From one of the accepted study following points have been established-

- Pregnant women and infants are at high risk of influenza related complications.
- Limited data suggest that oseltamivir is not a major human teratogen.
- Because of more data about its safety in pregnancy, the use of oseltamivir is preferred over zanamavir during pregnancy.
- Oseltamivir and zanamivir are considered to be compatible with breastfeeding [31].

Development of Resistance

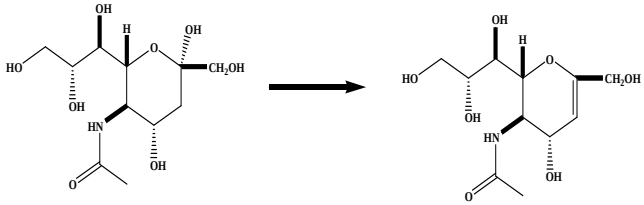
There are generally three levels of antiviral resistance according to the way that resistance can be detected or inferred:

- Genotypic resistance (detecting through sequencing of the viral genome and identification of mutations previously associated with certain level of drug resistance)
- Phenotypic resistance (resistance of the virus to drugs is tested *in vitro* (not in living systems) by measuring viral replication at a different drug concentrations (IC50).
- Clinical resistance (based on animals (ferret or mice) and human patients and measuring or observing the actual response to treatment with antiviral [30].

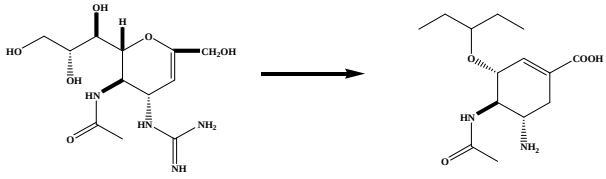
Resistance to neuraminidase inhibitors may be due to mutations in haemagluglutinin, which often confers resistance to both zanamivir and oseltamivir, while mutation in neuraminidase may render oseltamivir ineffective but retains susceptibility to zanamivir. Some mutants had amino acid sequence changes in the hemagglutinin and none in the neuraminidase, while others had changes in the neuraminidase but not in the hemagglutinin. It is thought that the hemagglutinin mutants had a reduced capacity to bind to sialic acid receptors, and so had little need for these to be destroyed by neuraminidase for the virus to escape [32].

Drug Design Approaches

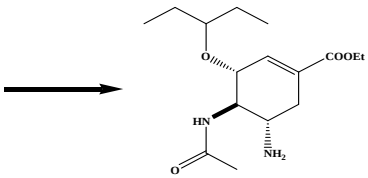
The drug design approaches include mainly three type of discovery method/approach.



Sailic Acid [a] DANA [b]



Zanamivir [c] GS-4071 [d]



Oseltamivir [e]

Sialic Acid-Based Influenza Neuraminidase Inhibitors

X-ray crystal studies for structures of sialic acid and its analogues bound with neuraminidase, inhibitors **[c]** and **[e]** were rationally designed and synthesized. Compounds **[c]** and **[e]** exhibited potent neuraminidase inhibitory activity with Ki values of 10-8 M and 10-10 M, respectively. Consistent with its potent *in vitro* inhibitory activity, **[e]** (zanamivir) exhibited potent antiviral activity against a variety of influenza A and B strains in cell culture and demonstrated *in vivo* efficacy in the influenza infected animal models via intranasal administration, is approved for the treatment of influenza infection in the U.S., Europe and Australia and is administered via inhalation [12].

The unsaturated Neu5Ac derivative 2‑deoxy‑2, 3-didehydro‑*N*-acetylneuraminic acid, a micromolar inhibitor of influenza virus sialidase, has provided the most potent inhibitor core template [33]. Structure-based drug design methods have played a critical role in the discovery of NA inhibitors. By exploiting the crystal structure of NA–inhibitors complexes, many compounds were successfully optimized and modified based on the characters of charge and shape in binding site [34].

Inhibitor Design Based on a Cyclohexene Scaffold

In the search for potent, orally active neuraminidase inhibitors, an approach based on a cyclohexene scaffold was investigated. The double bond of the cyclohexene ring was considered as an isostere of the flat oxonium cation. The cyclohexene ring is expected to be chemically and enzymatically stable and provide a stable template for chemical modifications of substituents for optimizing biological activity. X-ray crystallographic studies of sialic acid and related analogues bound to neuraminidase revealed that the C-7 hydroxyl of the glycerol side chain does not interact with amino acid residues in the neuraminidase active site. Taking this into consideration, an oxygen atom was incorporated as a replacement for the C-7 hydroxy methylene unit. In addition, this would allow for the preparation of a variety of alkyl side chain analogues. Although the C-8 and C-9 hydroxyl groups of the sialic acid glycerol side chain in the neuraminidase complex form a bidentate interaction with Glu 276, X-ray crystal structures indicate that the C-9 carbon of the sialic acid glycerol side chain makes hydrophobic contacts with the hydrocarbon chain of Arg 224. Therefore, optimization of this hydrophobic interaction was systematically investigated [12].

Benzoic acid based neuraminidase inhibitors

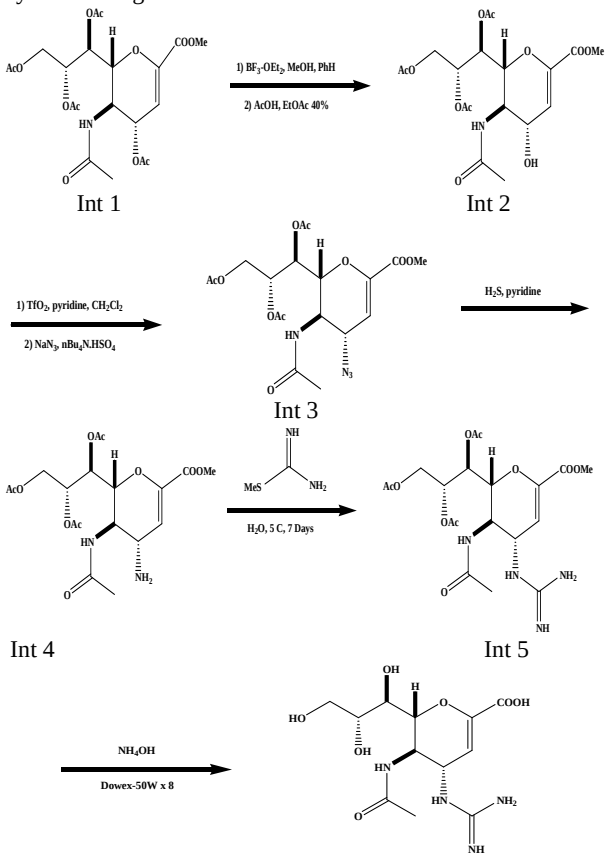
The approach of using the benzene ring as a template was based on the observation that in Neu5Ac2en–NA crystal structure, all substituents attached to the dihydropyrane ring are positioned in an equatorial orientation. Therefore, replacement of the dihydropyrane ring with the benzene ring was predicted to generate minimal disturbance for the overall binding mode. The benzoic acid analogue without the glycerol side chain showed similar NA inhibitory activity comparable to that of Neu5Ac2en. The X-ray crystal structure of bound to NA revealed that the guanidine group in oriented into the glycerol binding pocket. Addition of another guanidine group resulted in much reduced NA inhibitory activity [12].

SYNTHESIS OF SELECTED DRUGS [35-44]

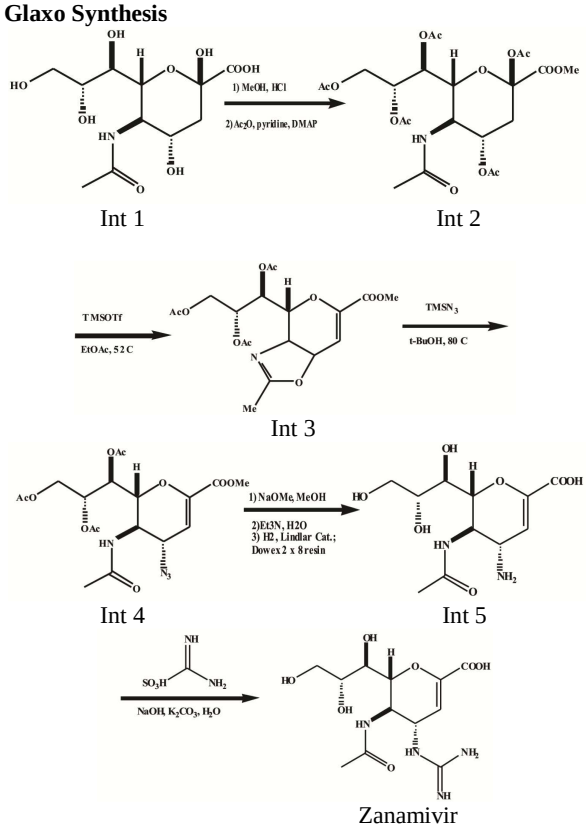
Zanamivir

Biota Synthesis

Methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-2,3,5-trideoxy-D-glycero-D-galacto-non-2-enopryano sonate (Int 1) was treated with $BF_3.Et_2O$ and methanol followed by acetic acid and water to affect a selective deprotection of the C-4 O-acetyl on the sialic acid template to give (Int 2). The alcohol was converted to the triflate and then displaced with sodium azide to afford (Int 3). Azide (Int 3) was reduced with hydrogen sulfide in pyridine to give the corresponding amine (Int 4). The guanidine was introduced by reacting (Int 4) with S-methylisothiourea in water. Finally, (Int 5) was saponified by passing it through a column of Dowex 50W X 8 eluting with 1.5 M ammonium hydroxide to give zanamivir.



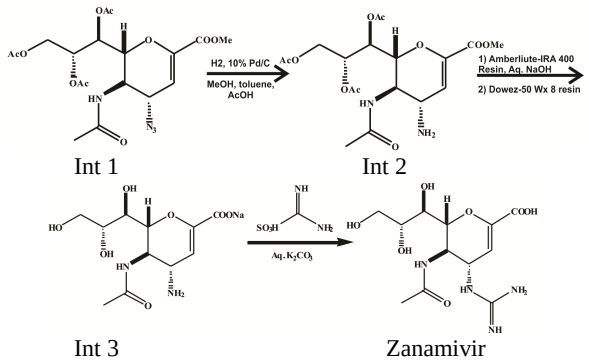
Glaxo Synthesis



The Glaxo synthesis of zanamivir started with the esterification of commercially available N-acetyl-neuraminic acid (Int 1) with methanolic HCl to give the methyl ester. Global acetylation of all the hydroxyl groups with acetic anhydride in pyridine with catalysis by 4-(dimethylamino) pyridine (DMAP) led to the penta-acetoxy compound (Int 2). Treatment of (Int 2) with trimethylsilyl triflate in ethyl acetate at 52°C introduced the oxazoline as well as the 2, 3-double bond to provide (Int3). Addition of trimethysilyl azide to the activated allylic oxazoline group led to the stereoselective introduction of azide at the C-4 position to get (Int 4). The acetate protecting groups were removed with catalytic sodium methoxide in methanol, resulting in a compound with improved water solubility. The methyl ester was then hydrolyzed using triethylamine in water and the azide was hydrogenated in the presence of Lindlar's catalyst to afford the triethylamine salt of the free amine, which was desalted to give amino acid (Int 5). Finally, treatment of (Int 5) with three equivalents of aminoiminomethanesulfonic acid in the presence of aqueous potassium carbonate introduced the guanidine, and afforded crystalline zanamivir after ion-exchange chromatography.

Improved Biota Synthesis

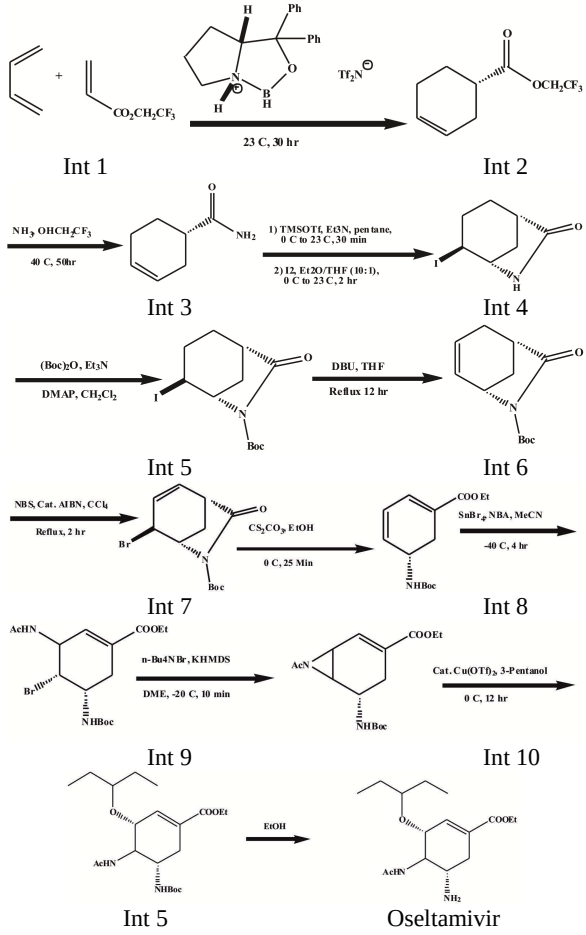
Hydrogenation of (Int 1) in the presence of 10% Pd/C in toluene, methanol, and acetic acid afforded amine (Int 2) in 72% yield. These particular reaction conditions minimized unwanted by-products resulting from acetate migration or over reduction. Hydrolysis of the ester and the acetate protecting groups led to (Int 3), followed by introduction of the guanidine by treatment with aminoimino methane sulfonic acid in the presence of aqueous potassium carbonate to afford zanamivir.



Oseltamivir

Corey Synthesis

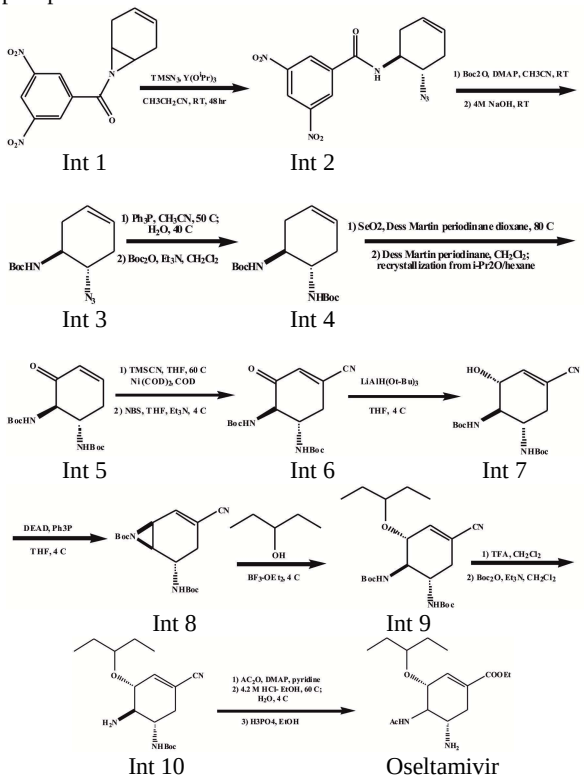
The Corey synthesis began with an asymmetric Diels–Alder reaction between butadiene and 2, 2, 2-trifluoroethyl acrylate in the presence of the S-proline-derived catalyst (Int 1) to form the adduct (Int 2). Ammonolysis of (Int 2) produced amide (Int 3) quantitatively, which underwent iodolactamization using the Knapp protocol to generate lactam (Int 4). N-Acylation of (Int 4) with di-tert-butyl dicarbonate provided the tert-butoxycarbonyl (Boc) derivative (Int 5) in 99% yield. Dehydroiodination of (Int 5) occurred cleanly with 1,8 diazabicyclo [5.4.0] undec-7-ene (DBU) to give (Int 6), which was allylically brominated using N-bromosuccinimide to generate (Int 7) in excellent yield. Treatment of (Int 7) with cesium carbonate in ethanol afforded the diene ethyl ester 66 quantitatively. The diene (Int 8) was converted to the bromodiamide (Int 9) using a novel SnBr4-catalyzed bromoacetamidation with N-bromoacetamide (NBA) in acetonitrile at 240°C. The reaction was completely regio- and stereoselective and the structure was verified by single-crystal X-ray diffraction analysis (of the racemic methyl ester). Cyclization of (Int 9) to the N-acetylaziridine (Int 10) was rapid and efficient using in situ generated tetra-n-butylammonium hexamethyldisilazane. Reaction of (Int 10) with 3-pentanol in the presence of a catalytic amount of cupric triflate at 0°C occurred regioselectively to generate ether (In 11). Finally, removal of the Boc group and salt formation with phosphoric acid in ethanol afforded oseltamivir.



Shibashaki Synthesis

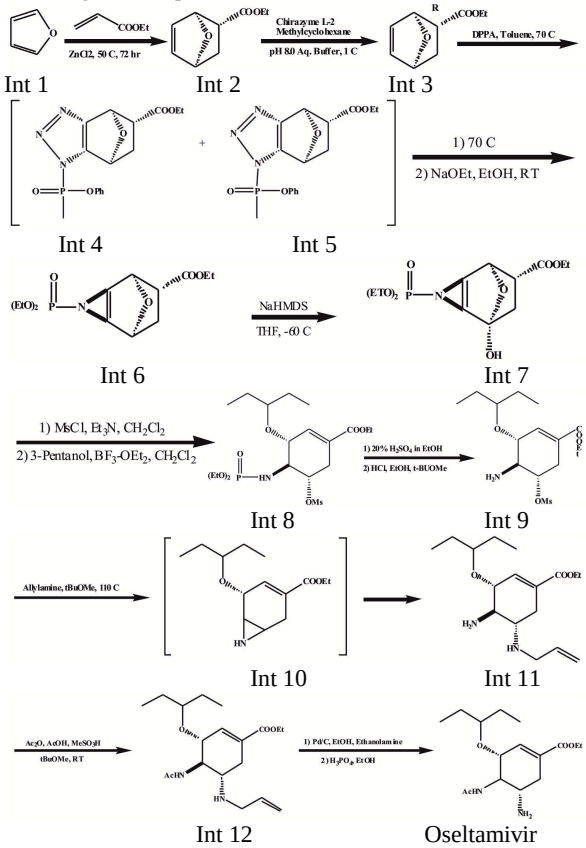
Catalytic desymmetrization of meso-aziridine (Int 1) with trimethylsilyl azide using the rare earth alkoxide Y (OiPr)3 provides (Int 2). Recrystallization from isopropanol enhanced the yield to 99%. The amide (Int 2) was Boc-protected and the N-3, 5-dinitrobenzoyl group was hydrolyzed with sodium hydroxide to afford (Int 3). The azide was reduced with triphenylphosphine and the resulting amine was Boc-protected to give the optically pure C2 symmetric 1, 2-diamine (Int 4). Allylic oxidation of (Int 4) with selenium dioxide in the presence of Dess–Martin periodinane produced a mixture of enone (Int 5) and the corresponding allylic alcohol, which was oxidized with Dess–Martin periodinane to give enone (Int 5) in 68% yield. Trimethylsilyl cyanide was added to (Int 5) in the presence of Ni $(COD)_2$ to give the 1,4-adduct, which was

brominated with N-bromosuccinimide followed by elimination of the bromide with triethylamine to afford g-keto nitrile (Int 6). Diastereoselective reduction of the ketone was accomplished using the bulky aluminum reagent $LiAlH(Ot\text{-}Bu)_3$ to give alcohol (Int 7) with 20 : 1 diastereoselectivity. The aziridine (Int 8) was formed under Mitsunobu conditions and then opened with 3-pentanol in the presence of $BF_3.Et_2O$ to afford (Int 9). The Boc protecting groups were removed by treatment of (Int 9) with trifluoroacetic acid and a Boc protecting group was reintroduced on the sterically less hindered amine to give (Int 10). The unprotected amine was acetylated with acetic anhydride and the nitrile was converted to the ethyl ester in acidic ethanol with concomitant removal of the Boc group to provide oseltamivir. Finally, salt formation with 85% phosphoric acid in ethanol afforded oseltamivir.



Diels-Alder Approach

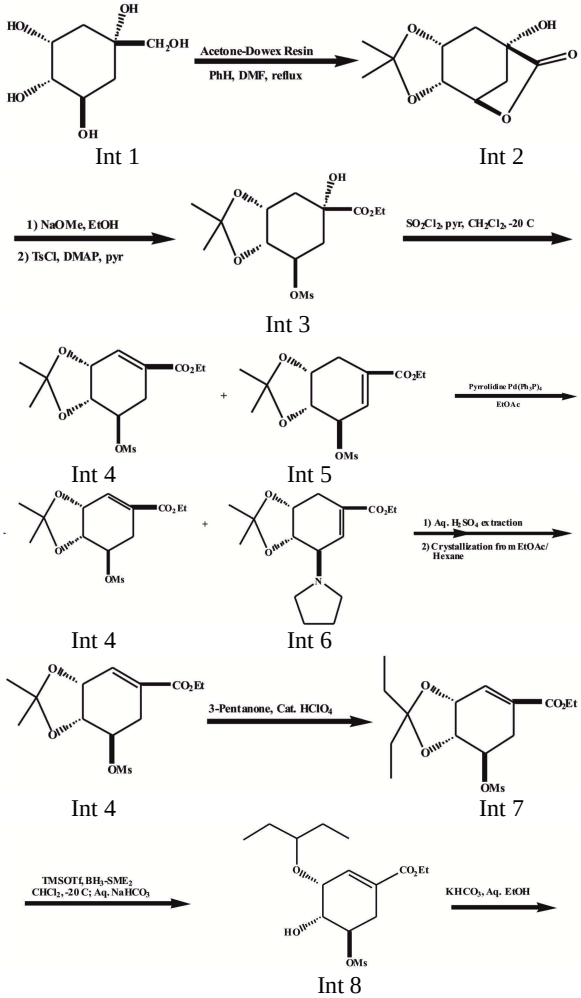
The zinc-catalyzed Diels–Alder reaction between furan (Int 1) and ethyl acrylate was heated at 50°C for 72 h to provide a 9: 1 mixture favoring exo-isomer (Int 2) over the endo-isomer. The endo-isomer was kinetically preferred, but with increased reaction times an equilibrium ratio of 9: 1 was achieved favoring the thermodynamically preferred exo-isomer (Int 2). The optical resolution of rac-43 was achieved via enantioselective ester hydrolysis using Chirazyme L-2 to give (Int 3) in 97% yield after intensive reaction optimization. Another key step in this synthesis was the conversion of (Int 3) to aziridine (Int 6). Thus, [3 + 2]-cycloaddition of (2)-43 with diphenylphosphoryl azide provided a mixture of the exo-triazoles (Int 4) and (Int 5), which with continued heating at 70°C resulted in the thermal extrusion of nitrogen to give the endo- N-diphenylphosphoryl aziridine. A potential explanation for this "inversion" could be that the [3 + 2] cycloaddition reaction produces small equilibrium amounts of the endotriazoles, which may undergo nitrogen extrusion at a much faster rate due to the higher steric strain. Transesterification with sodium ethoxide in the same pot then led to the N-diethylphosphoryl aziridine (Int 6). Treatment of endo-aziridine (Int 6) with sodium hexamethyldisilazane led to smooth ring opening to give cyclohexene (Int 7). After O-mesylation of 47, the N-diethylphosphoryl aziridine was opened with 3-pentanol in the presence of $BF_3.EtO_2$ to afford (Int 8). The phosphoryl amide of (Int 8) was hydrolyzed with 20% sulfuric acid in ethanol and (Int 9) was isolated as the hydrochloride salt. (Int 9) was reacted with four equivalents of allylamine in t-butylmethyl ether at 110°C to give (Int 11) via the aziridine intermediate. The 4-amino group of (Int 11) was selectively acetylated using acetic anhydride (1 equiv.) and methanesulfonic acid (1 equiv.) in acetic acid and t-butylmethyl ether to give (Int 12). Deallylation of (Int 12) using 10% Pd/C in the presence of ethanolamine in refluxing ethanol proceeded to afford oseltamivir.



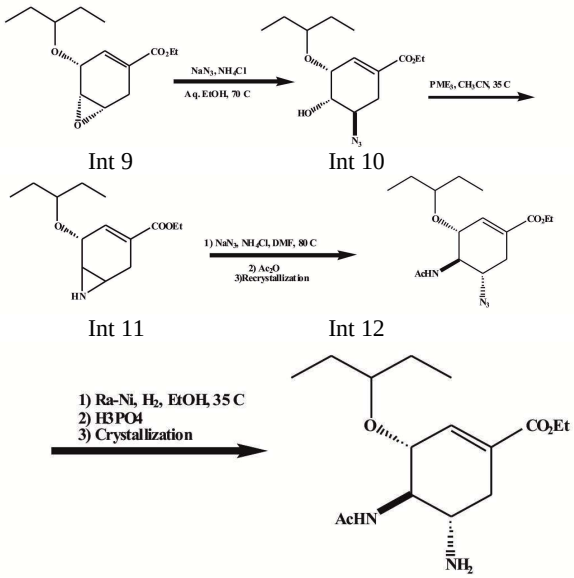
Gilead Synthesis

The synthesis started with the conversion of quinic acid (Int 1) to compound (Int 4). Quinic acid was converted to the acetonide with concomitant lactonization to give (Int 2). The lactone was opened with sodium methoxide to afford the methyl ester and the resulting secondary alcohol was tosylated selectively to provide (Int 3). Dehydration of (Int 3) was accomplished using sulfuryl chloride and pyridine to give a 4: 1 mixture of 1, 2- and 1,6-olefin regioisomers (Int 4) and (Int 5) in 60% yield. It was not possible to separate (Int 4) and (Int 5) by fractional crystallization so the crude mixture was treated with pyrrolidine and catalytic Pd $(Ph_3P)_4$, which led to the selective conversion of (Int 5) to (Int 6). Compound (Int 5) was then removed by aqueous sulfuric acid extraction and the pure 1, 2-olefin isomer (Int 4) was isolated by

crystallization in 42% yield from (Int 3). Transketalization of (Int 4) with 3-pentanone in the presence of catalytic perchloric acid gave (Int 7). The 3, 4-pentylidene ketal was not incorporated at the beginning of the synthesis because the corresponding intermediates were not crystalline. Reductive opening of the 3, 4-pentylidene ketal (Int 7) was accomplished using trimethylsilyl triflate and borane dimethyl sulfide complex to give a 10: 1: 1 mixture of (Int 8), the isomeric pentyl ether and the diol. This crude mixture was treated with potassium bicarbonate in aqueous ethanol followed by heptane extraction to give the crystalline epoxide (Int 9) in 60% yield from (Int 8). Epoxide (Int 9) was heated with sodium azide and ammonium chloride in aqueous ethanol to give a 10: 1 mixture of azido alcohol (Int 10) and its corresponding regioisomer. Reductive cyclization of 24 with trimethylphosphine afforded aziridine (Int 11). Ring-opening of aziridine (Int 11) with sodium azide in the presence of ammonium chloride provided the azidoamine, which was directly acylated with acetic anhydride to afford azidoacetamide (Int 12) in 37% yield after recrystallization. The azide in (Int 12) was reduced using catalytic hydrogenation with Raney nickel in ethanol. Following the removal of the catalyst, 85% phosphoric acid (1 equiv.) was added and oseltamivir was crystallized as the phosphoric acid salt, which was isolated in 71% yield from (Int 12).



Int 1 Int 2 Int 3 Int 4 Int 5 Int 4 Int 6 Int 4 Int 7 Int 8



Int 9 Int 10 Int 11 Int 12 Oseltamivir

CONCLUSION

Influenza virus had affected civilizations from long and long years ago before an actual scientific documentation in 1919 was done. This virus had affected millions across the globe and every year the toll went on increasing in higher percent. With the discovery of neuraminidase inhibitors, often considered as a magic drug for flu in east, the death toll was restricted by its efficacious utility & safety of drugs along with necessary patient care. Although recurrent use of drugs has imparted resistance at present and a global need for new drug or alternative technique had arisen, still, this class remained a drug of global importance and is widely preferred.

Virus had the tendency to undergo resistance which makes it extremely difficult for a single line therapy to continue. H1N1, H3N2, and H1N2 are the most common strains worldwide with which scientist are in combat. For complete eradication better drug design, drug discovery, synthetic & drug development research approaches are needed to develop cost effective formulations. A necessary improvement in primary health measures in developing nations are of prime importance for effective & fast diagnosis of flu and better treatment initiation. Adequate researches must be conducted across institutes to generate sufficient data to ensure safety and efficacy in multiple drug therapy. Safety in pregnant and breastfeeding mothers is necessary to indicate to avoid spontaneous abortion and malformation.

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