Recent Advances in Anti-Influenza Agents with Neuraminidase as Target

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Abstract: Neuraminidase (NA) is the major surface glycoprotein of the influenza virus. It has been considered a suitable target for designing agents against influenza viruses. Rational drug design of NA inhibitors is now in the clinic and is effective for the treatment of influenza. Recently, research of structure-based NA inhibitors is becoming an interesting field, leading to a breakthrough in the control of influenza. Here we review a series of neuraminidase inhibitors and the recent progress in this field.

Keywords: Influenza, influenza virus, neuraminidase (NA), neuraminidase inhibitors (NAIs), sialic acid (SA), anti-influenza agents, antiviral, advances.

1. INTRODUCTION

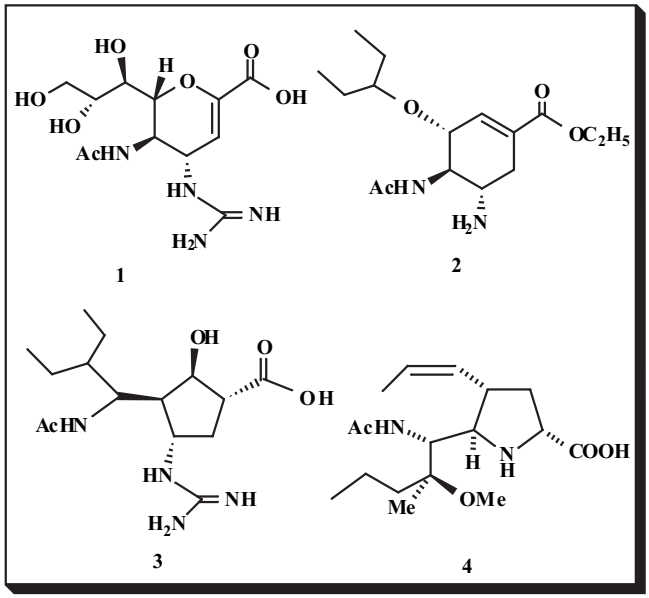
1.1. Influenza

Influenza, commonly known as flu, is a major factor affecting health and economic costs. 20% of children and 5% of adults worldwide suffer from symptomatic influenza A or B each year [1]. About 120 million people in North America, Europe and Japan are infected each year. Influenza infection has been the cause of some of the worst epidemics in the human history. The pandemic of 1918-1919 was responsible for the death of 20 million people [2]. It causes a broad range of illnesses, from symptomless infection through various respiratory syndromes, disorders affecting the lung, heart, brain, liver, kidneys and muscles to secondary bacterial pneumonia. In general, influenza is an acute viral infection of the upper respiratory tract, accompanied by fever, headache, myalgia, prostration, coryza, sore throat and cough. It is typically caused by influenza A and B viruses [3].

Most influenza infections are spread by virus-laden respiratory droplets containing several microns in diameter that are expelled during coughing and sneezing. Fomites represent another mode of transmission. Occasionally, influenza is transmitted to people by pigs or birds [4]. The number of outbreaks of avian influenza which has been reported in Hong Kong, South Korea, Japan, Thailand, Indonesia, China, United States of America, Canada, South Africa, and Malaysia seems to be increasing over the last 5 years. Moreover, a growing number of human cases of avian influenza, in some cases fatal, have paralleled the outbreaks in commercial poultry. There is a great concern about the possibility of a new virus subtype with pandemic potential that could emerge from these outbreaks [5].

Despite considerable knowledge of viral infectivity, current therapeutic measures could not control the disease. Vaccination has provided only a limited control because of the tendency of the virus to mutate to escape the immune system. So the vaccines must be reformulated each year because of the high antigenic drift. Options for the therapeutic treatment of influenza are Amantadine and Rimantadine, which act by interfering with the M2 protein ion channel function that is found only in influenza virus A. Besides the insensitivity of influenza virus B, also the clinical use of these agents is limited because of the rapid emergence of resistance [6].

The influenza virus neuraminidase inhibitors (NAIs) have currently emerged as promising therapeutics for the treatment of influenza [7]. Two such inhibitors of influenza, Relenza **1** (Zanamivir-ZMV by Glaxo Wellcome/Biota) and Tamiflu **2** (Oseltamivir-OMV by Hoffman-La Roche/Gilead), further underscore the importance of NA as a valid anti-influenza drug target [8]. Another two NA inhibitors, RWJ-270201 **3** (BCX-1812) [9] and A-315675 **4** [10] are undergoing phase III trials in North America and Europe.



Because of the essential role of NA in influenza virus replication and the highly conserved enzyme active site in influenza viruses A and B, most interest has been focused on the development of selective inhibitors of this enzyme. In recent years, tremendous progress has been made in the discovering of this new class of anti-influenza agents [11].

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1.2. Influenza Virus

Influenza viruses are negatively stranded RNA viruses with a segmented genome. They are members of the orthomyxoviride family and are further classified into three distinct types—A, B and C. The human population appears to be most affected by types A and B [12]. Influenza viruses B and C affect humans, whereas influenza virus A circulates in a wide range of avian and mammalian hosts. The virus infects by attachment to host cells followed by endocytosis and fusion of the viral and endosomal membranes [13].

Influenza virus particles are highly pleomorphic (variable), mostly spherical or ovoid, 80-120nm diameter, but many forms occur, including long filamentous particles (up to 2000nm long x 80-120nm diameter). Different strains of virus vary in their tendency to form filaments; this property maps to the matrix protein [14].

There are two major surface antigenic proteins, hemagglutinin (HA) and neuraminidase (sialidase, *N*-acetylneuraminate glycohydrolase, NA), and their functions in the infective cycle of influenza have been well understood. These membrane glycoproteins are seen as "spikes" covering the surface of the virus particle, with an average of about 500 HA and 100 NA "spikes" on each virion [15]. In addition to these two major surface proteins, influenza virus A has a small hydrophobic protein (M2), which functions as an ion channel [16] (Fig. **1**). Type B influenza virus contains an analogous protein, designated NB, which is encoded by a second reading frame on the NA gene.

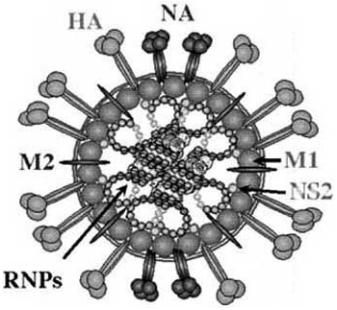


Fig. (1). Schematic representation of influenza A virion(http://www-micro.msb.le.ac.uk/3035/Orthomyxoviruses.html).

The influenza virus undergoes frequent and rapid mutations in its surface antigens. It is this characteristic of the virus that limits the efficacy of influenza vaccines, and until recently, has hindered the development of efficacious specific anti-influenza agents. The most serious thing is the fact that a major antigenic transformation results in a pandemic strain of the virus.

2. THE STRUCTURE AND FUNCTION OF NEURAMINIDASE

2.1. Structure of Neuraminidase

Influenza virus NA (EC 3.2.1.18) is a major surface glycoprotein of the influenza viruses A and B (about 50 copies per virion). It is a homotetramer consisting of cytoplasm and transmembrane domains, the thin stalk and the globular head. NA is a tetramer consisting of four identical disulfide linked subunits (Mr 60 kDa) and extends ~60 Å from the viral membrane *via* a long thin stalk [17]. The X-ray crystallographic determination of influenza virus NA has been resolved to 2.9 Å (Fig. **2**), and backbone chain tracing of the monomer shows a β-sheet propeller topology with an approximate six-fold symmetry passing through the center of each subunit [18].

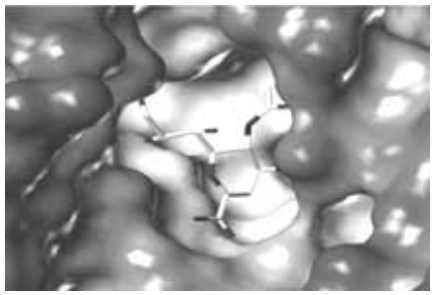


Fig. (2). Influenza virus NA binding with ZMV (docked by sybyl).

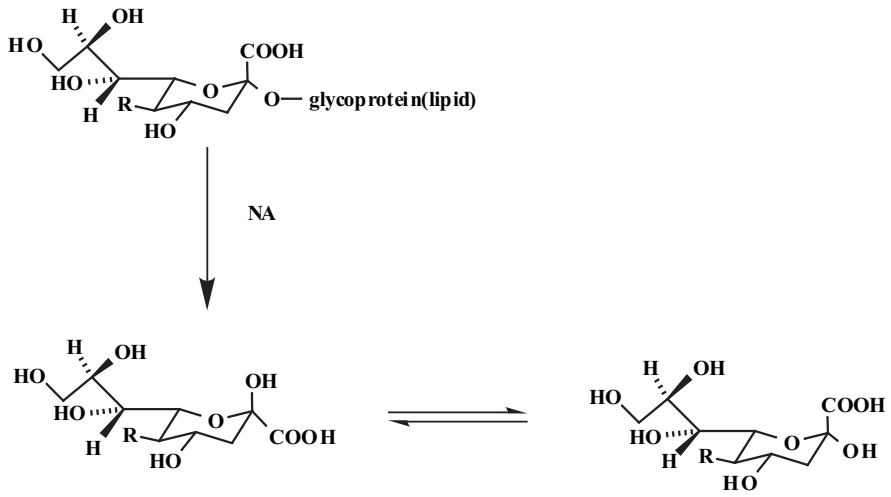
X-ray crystallography revealed the positions both of enzyme active sites and antigenic epitopes of NA [19]. The NA molecule is orientated rather unusually from a glycoprotein; it's N-terminus being anchored in the viral membrane. After establishing the three-dimensional structure of influenza virus NA, the positions on the molecule of the catalytic site could be identified. The molecule has a box-shaped head, with a unique folding pattern. Each monomer contains six sheets and four polypeptide strands [20]. The catalytic site of the NA has been located by different Fourier analyses of crystals soaked in SA. The site is surrounded by 14 conserved charged residues and contains three hydrophobic residues-Tyr, Trp and Leu.

2.2. Function of Neuraminidase (NA)

NA cleaves the α-glycosidic bond between SA and the glycoconjugates, and thus destroys receptors to which influenza virus binds [21]. The NA substrate specificity with respect to the SA species and the type of SA linkage to the glycoconjugates vary among different viruses. The NA activity is particularly important at the late stages of infection. It has been suggested that the enzyme is essential in the release of virion away from infected cells [22], as well as assisting the movement of the virus through the mucus in the respiratory tract and also reducing the propensity of the virus particles to aggregate [23]. It is believed that the enzyme facilitates the virus access to epithelial cells by mucus degradation. A potential "side effect" of the NA activity might be the interference with the virus attachment to cells due to destruction of receptors [24]. The receptor-binding activities of the NA counteract each other at several distinct stages of virus infection.

At the molecular level, NA cleaves terminal α-ketosidically linked SA residues (Scheme **1**) leading to the formation of the proposed endocyclic sialosyl cation transition-state intermediate (**5**), which is subsequently released as α-Neu5Ac [25]. The cationic intermediate (**5**) is released as the α-anomer [26].

As with hemagglutinin (HA), the presence of NA as a surface glycoprotein and its intimate involvement in the infective process of influenza, makes it an attractive drug design target. Early attempts at the development of



(Scheme 1)

Scheme 1.

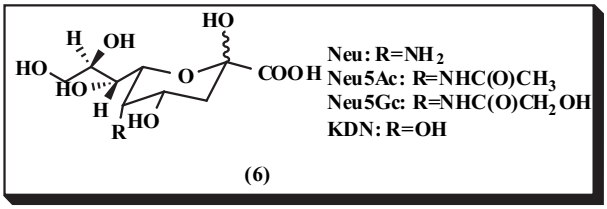
inhibitors of influenza virus NA resulted in oxamic acid derivatives being studied [27], wherein their activity was rationalized by comparison with SA using the carboxylate moiety as a common structural feature. Several compounds were subsequently investigated as NA inhibitors based on the results of random screening poor potency, lack of selectivity, or lack of *in vivo* activity. The successful strategy that has resulted in the development of potent substrate-based influenza virus NA inhibitors relies on several factors: the information from the X-ray crystallographic studies of influenza virus NA; advances in computational chemistry and therefore rational drug design techniques; and an understanding of the enzyme mechanism.

3. NEURAMINIDASE INHIBITORS AS ANTI-INFLUENZA AGENTS

Influenza virus NA is an antiviral target for structure-based drug design (SBDD) because of its essential role in cleaving SA residues from glycoconjugates and facilitating release of virions from infected cells [28]. The activity site of NA is highly conserved across all influenza virus A and B strains, rendering broad-spectrum, anti-influenza agents possible. Indeed, the effectiveness of NA inhibitors as anti-influenza agents has been demonstrated both in animal models and in human clinical trials.

3.1. Sialic Acid (SA)

Sialic acids (SA) are the natural substrates of NA. Sialic acids were first isolated in the 1930's, in one case from a salivary gland mucin from which the name "sialic acid" was derived. This is a slightly different compound, isolated from a brain ganglioside, containing both an acid and an amine [29]. The general structure of the sialic acids was elucidated in the 1950's. The term sialic acids now encompasses all derivatives of 3-deoxy-D-*glycero*-D-*galacto*-2-nonulosonic acid, while neuraminic acid means the unsubstituted derivative with an amino substituent at C-5, 5-amino-3,5-dideoxy-D*glycero*-D-*galacto*-2-nonulosonic acid (**6**).



2-Deoxy-2,3-didehydro-Neu5Ac (Neu5Ac2en) is found as a component of normal serum, urine and saliva . The 2-deoxy-2,3-didehydro sialic acids are generally found to be inhibitors of NA [30].

The biological functions of SA are derived both from their physical properties and from their natural position as the terminal residue on cell-surface glycoconjugates [31]. They appear to have three broad areas of involvement: mediation of interactions based on their charge, masking of underlying glycoconjugate structures, and acting as receptors for binding and adhesion. The physical nature of SA, in particular their charge, renders them a role in cell-to-cell contact interactions. SA residues also function to mask recognition sites. For example, gradual desialylation of erythrocytes with aging leads to the unmasking of galactose residues.

SA-recognizing proteins such as the NA of pathogenic organisms, and the receptor binding proteins of cells and microorganisms can be used as targets for designing potential therapeutic agents. A variety of SA-containing inhibitors of influenza virus NA from different animal sources, ranging from sera, urine and meconium to egg white and edible nests, have been described since the late 1940's [32]. The inhibitors of this type of glycoproteins or proteoglycans bind to the virus via HA-SA interactions, thus, competitively prevent HA binding to receptors on erythrocytes or susceptible cells. Besides directly blocking receptor-binding sites of the HA, the inhibitors can create steric obstacles to the polyvalent interaction of the virus with cells. In addition, binding to inhibitors can lead to aggregation of virus-cell interaction and reduce infectivity.

The search for inhibitors of influenza virus NA has undergone three phases. The first phase is attributable to Edmond, who discovered that N-substituted oxamic acids had enzyme-inhibitory properties, and had antiviral activity when incubated with virus on portions of egg membrane [33]. The second phase begins with the synthesis of Neu5Ac2en (**5**) by Meindl and Tuppy [34]. This compound is an analog of transition state in the reaction catalyzed by NA, the cleavage of N-acetyl neuraminidase (SA) (**6**) from glycoconjugates. The third phase springs from the description of the three-dimensional structure of the NA and the realization that the catalytic site of the protein is indeed a strain-invariant feature which might be exploited to develop strain-independent therapy [35].

3.2. Active Site of Neuraminidase (NA)

The NA active site is highly polar, with ten Arg, Asp and Glu residues and four hydrophobic residues [36]. To facilitate the discussion of the inhibitor binding modes, the active site is divided into 5 regions: S1-S5. These subsites are diagrammed and numbered in counterclockwise fashion using the crystal structure of the substrate-based inhibitor DANA (dehydrodeoxy-N-acetylneuraminic acid) bound to the active site [37] (Fig. **3**).

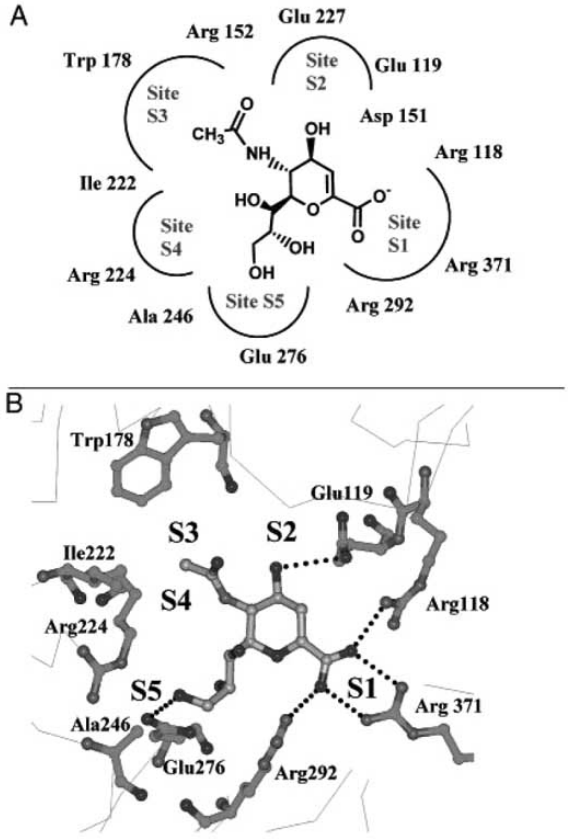


Fig. (3). Active site of NA (reference 36).

Site S1 is comprised of three residues: Arg118, Arg292 and Arg371. It provides a positively charged electrostatic and hydrogen-binding environment for anionic substituents from the inhibitor, such as carboxylate. Site S2 is a negatively charged region of the active site and bound by Glu119 and Glu227. Site S3 contains a small hydrophobic region formed from the side chains of Trp178 and Ile222 adjacent to a polar region provided by the side chain of Arg152 and a bound water molecule. Site S4 is not occupied by any portion of the DANA inhibitor and is primarily a hydrophobic region derived from the side chains of Ile222, Ala246 and the hydrophobic face of Arg224. Site S5 is a region of mixed polarity and is comprised of the carboxylate of Glu276 and the methyl of Ala246. As shown below and by others [38], Glu276 can exist in an alternative gauche conformation with its carboxylate ion-pair with Arg224. When Glu276 exists in this conformation, its methylenes join with Ala246 to create a hydrophobic pocket within S5. An additional important active site residue is Asp151, which is not formally a part of the subsites. While the carboxylate of this residue does neither make direct contact with DANA nor with the inhibitors having been discovered, Asp151 is believed to play a critical role in the catalysis by polarizing the scissile glycosidic linkage.

3.3. Development of Neuraminidase Inhibitors as Anti-influenza Agents.

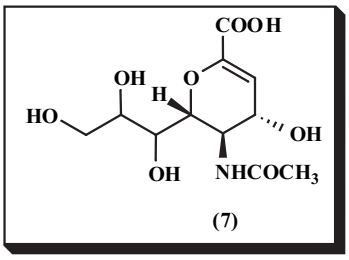
Paless and Schulman (1977) were the first virologists to exploit NA as a target for chemical inhibitors [39]. Essentially the key molecule-Neu5Ac2en (**5**) was a dehydrated SA derivative that mimicked the geometry of the transition state during the enzymatic reaction. In a retrospective follow up study, positive results of intranasal or aerosol administration of this drug is observed in the animal models.

In the USA, Kilbourne suggested that a NA vaccine could have benefits compared with the conventional mixture of NA. In Australia, the Laver and Colman research teams crystallized the NA protein [40].

We will now introduce the NA inhibitors according to the structure.

3.3.1. Neuraminidase Inhibitors Based on Sialic Acid

Analogs of SA, such as 2,3-didehydro-2-deoxy-N-acetylneuraminic acid (**7**) (DANA) are known to inhibit neuraminidase *in vitro* with a *Ki* value of 0.004mM [41].



(7)

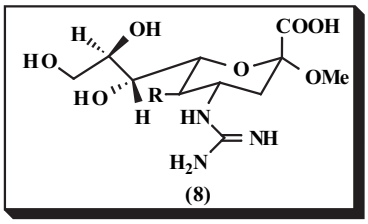
The replacement of hydroxyl group in DANA with a guanidine group resulted in discovering of ZMV (**1**), which is a potent inhibitor of influenza A and B [42]. It was approved by FDA in 1999 and is used as an inhaler, which may be inconvenient for the children and elderly people. ZMV is more effective in preventing culture-position influenza or for treatment of culture-positive influenza in febrile individuals. The treatment is more effective if initiated within 30 hours of symptom onset in febrile individuals. But ZMV can only be delivered by inhalation because of its low oral bioavailability, small volume of distribution, and rapid elimination.

Another compound-OMV (**2**) was also approved by FDA in 1999 as an oral drug [43]. It is the ethyl ester prodrug of GS4071, a selective inhibitor of influenza A and B. This compound is also very effective both for influenza viruses A and B, but has been reported to have some side effects. OMV is indicated for the treatment of uncomplicated acute illness due to influenza and prevention of influenza. It is safe and efficacious for the treatment of influenza in medically high risk individuals or for prevention of complications due to influenza. OMV is approved as a prophylactic agent in adults and adolescents 13 years and older.

Evidence supports the use of ZMV and OMV in the treatment of influenza; however, additional studies are needed to clarify their utility and tolerability in pediatric and high-risk patients, as well as their utility in the prevention of influenza.

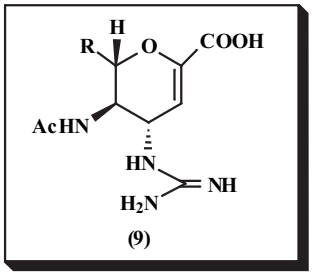
After NA inhibitors, ZMV (**1**) and OMV (**2**) were approved by FDA as anti-influenza drugs; synthesis of Neu5Ac2en (**5**) analogs, which are thought to be transition-state analogs [44] of the enzyme reaction, has drawn considerable attention for NA inhibitors over the past decade. Several research groups reported the synthesis and NA inhibitory activities of analogs related to ZMV (**1**) and OMV (**2**) and demonstrated that four substituents of guanidine, amino group, carboxylic acid, acetamide, and glycerol side chain (especially the 8- and 9- hydroxyl groups) on the dihydropyran ring made important contributions to their binding activities to the NA.

In 1995, Silvan Clccotosto and Mark von Itzstein [45] found that 4-deoxy-4-guanidino-Neu5Acα2Me (**8**) is a high affinity ligand for NA. And the synthesis of compound (**8**) was achieved from 4-azido-4-deoxy-Neu5Ac2en. The increase in affinity is proposed to come from salt bridge formation between the guanidino group and a highly conserved binding pocket residue.



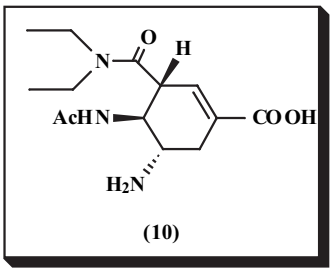
(8)

In the same year, John Scheigetz and his colleagues [46] found that 4-guanidino derivative (**9**) of SA have potential NA inhibitory activity both *in vivo* and *in vitro*.



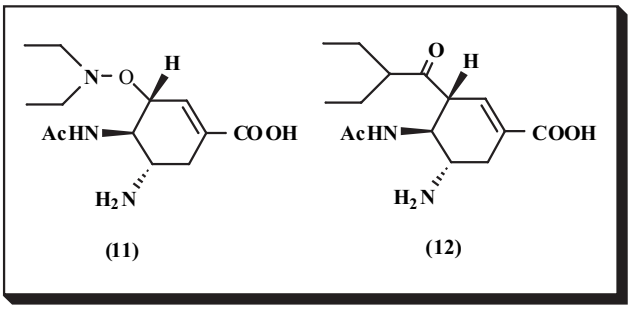
(9)

In the search for further clinical candidate for election of anti-influenza agents, a number of reports have also documented other potent NA inhibitors. In 1998, Smith and his colleagues [47] synthesized 4-*H*-pyran-6-carboxamide compounds (**10**). These compounds resemble OMV (**2**), which contain a substituent capable of forming hydrophobic interactions with NA. However, unlike OMV (**2**), these compounds show remarkable selectivity for influenza virus A NA. It is speculated that this difference might be attributed to the greater conformation flexibility of the pentyl ether side chain, enabling the inhibitors to adopt a favorable binding conformation within the influenza virus B enzyme which was inaccessible to the relatively rigid amide.



(10)

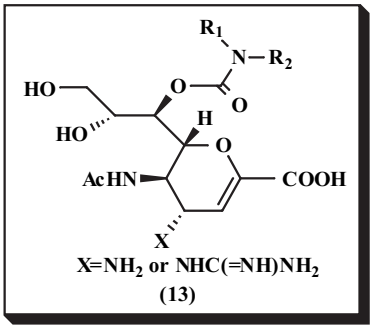
One year latter, they reported the synthesis and biological activity of the dihydropyran analogs (**11**) and (**12**) containing 6-substituent with greater conformational flexibility [48]. The results of NA inhibitory activity demonstrate that despite possessing 6-substituent with greater conformational flexibility, compounds (**11**) and (**12**) all resemble the carboxamide (**10**), in which they are highly selective inhibitors of influenza virus A. But the compound (**11**) shows surprisingly low NA inhibitory activity. It is apparent that a simple argument based on a rigid conformation cannot explain the difference in NA selectivity observed between the dihydropyran carboxamides and the cyclohexene.



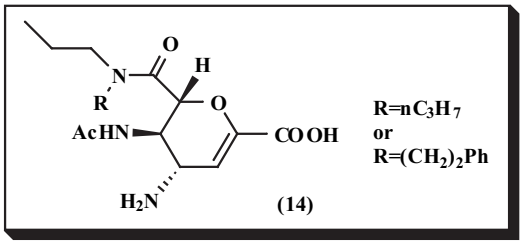
(11) (12)

In 1998, another group led by Tayler [49] reported that the binding of ligands containing tertiary amide groups is accompanied by the formation of an intramolecular planar salt bridge between two amino acid residues in the active site of the NA. It is proposed that the unexpected strong binding of inhibitors is a result of the burial of hydrophobic surface area and salt-bridge formation in an environment of low dielectric. In NA from influenza virus A, binding of the carboxamide moiety and salt bridge formation have only a minor effect on the positions of the surrounding residues, whereas in influenza virus B in NA, significant distortion of the protein is observed. The results suggest that the decreased affinity in NA from influenza virus B is directly correlated with the small changes that occur in the amino acid residue interactions accompanying ligand binding. Molecular dynamics calculations have shown that the tendency for salt-bridge formation is greater in influenza virus A NA than influenza virus B NA and that this tendency is a useful descriptor for the prediction of inhibitor potency.

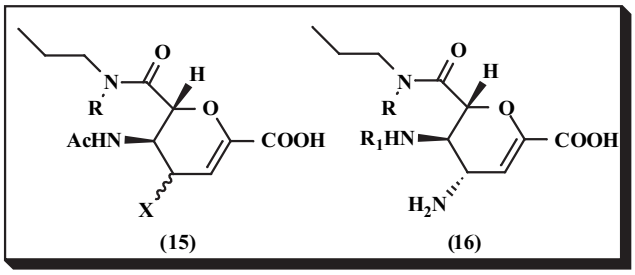
In 1999, Andrews [50] *et al* synthesized a series of analogs (**13**) of 4-guanidino-Neu5Ac2en (ZMV) containing carbamate substituent at the 7-hydroxy position. They carried out studies in which the glycerol side chain had been truncated in a stepwise fashion, confirming that the interaction which the 8,9-diol made with the conserved Glu276 residues offered an important contribution to overall binding of SA derivatives to NA. The X-ray structures of SA and ZMV bound to NA show that the 7-hydroxyl group makes no direct interactions with the protein and is exposed to bulk solvent, indicating that it may be possible to modify the glycerol side chain at this position, whilst retaining the important 8,9-diol moiety. 7-modification is of particular interest since it allows the synthesis of a wide range of ZMV derivatives which have modified physicochemical properties. Several of these compounds possess potent NA inhibitory activity, although none of these compounds described herein is as potent as ZMV; they demonstrate that the 7-position is capable of tolerating groups of greatly differing sizes and chemical functionality, with retention of activity. And they had synthesized a wide diversity of analogs at this position, exemplifying the adaptability of this class of compounds.



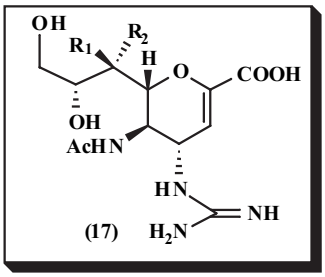
In 2001, Wyatt and coworkers [51] reported their investigations of the 4- and 5-positions of a series of 4-amino-4*H*-pyran-2-carboxylic acid 6-carboxyamides. They obtained potent inhibitors of influenza A NA with remarkable selectivity over the influenza virus B NA when the basic 4-amino substituent was replaced by hydroxyl or even deleted. Modifications at the 5-position exhibited a tight steric requirement, with trifluoroacetamide being optimal. Previously (1998), as part of the SAR investigation of ZMV [52], they reported a series of 4*H*-pyran carboxamide NA inhibitors (**14**). In these compounds the glycerol side chain of ZMV was replaced by a lipophilic carboxamide moiety. Although some of these compounds were more potent inhibitors of influenza virus A NA than ZMV, they were significantly less potent against influenza virus B NA.



Three years later (2001), they focused on the 4- and 5-positions on the pyran ring, and structures (**15**) and (**16**) had been synthesized. In contrast to the 6-glycerol substituted series related to ZMV, the carboxamide series does not require a basic group in the 4-position for potent NA inhibitory activity. In fact, no functionality at all is required for nanomolar activity against influenza A NA. This surprising finding could be used to design further influenza NA inhibitors. Modifications at the 5-positon demonstrated a very tight steric requirement for this substituent. As with the previous examples of the carboxamides, a significant selectivity for influenza virus A over influenza virus B NA was observed for all the synthesized compounds.

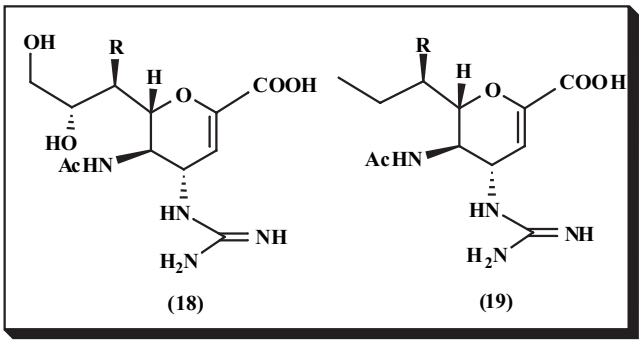


In 2002, a research group led by Honda [53] was interested to find the influence of NA binding of the replacement of the hydroxyl group at the C-7 position of Neu5Ac2en by lipophilic substituents. But, they had found that the chemical synthesis of 7-substitueted-Neu5Ac derivatives from N-acetylneuraminic acid was particularly difficult. In fact, little chemistry had been reported at this position. They attempted to find a reasonable solution to this problem and they focused their attention on the enzyme catalyzed aldol condensation between 4-modified-N-acetylmannosamines and sodium pyruvate in the presence of Neu5Ac aldolase. They synthesized a series of 4-guanidino-7-modified Neu5Ac2en (**17**) from 4-modified-N-acetylmannosamines. The activity assay result suggested that the replacement of the C-7 hydroxyl group by lipophilic substituents would make it easier to access the cell membrane or active site compared to ZMV. A series of ZMV derivatives possessing C-7 substituted glycerol side chains were synthesized using enzyme catalyzed aldol condensation. And some of these compounds exhibited very good inhibitory activity against influenza virus A NA. Furthermore, replacement of the C-7 hydroxyl group of ZMV by small lipophilic substituents (F, OMe, OEt, N_3) improved influenza virus A plaque reduction activity.

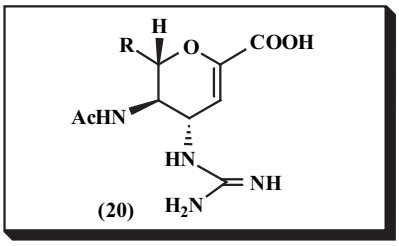


In the same year (2002), Honda *et al.* [54] synthesized a series of 7-alkyl ether derivatives (**18**) related to ZMV using direct alkylation of the C-7 alcohol of SA analogs. Alkyl ether moiety of less than 12 carbons in length showed low nanomolar inhibitory activity against influenza virus A NA. Furthermore, this moiety modification improved influenza virus A plaque reduction activity compared to ZMV. However, removal of the 8,9-diol of the 7-*O*-alkyl

derivatives (**19**) resulted in loss of antiviral potency. This result suggests that 8,9-diol must play an important role in binding with both with influenza virus A and B. These compounds could have modified physicochemical properties which could make them more suitable for systemic delivery. They also demonstrated that 7-modified derivatives related to ZMV possessing relatively small lipophilic substituent such as F, N_3, OMe, and OEt groups showed similar NA inhibitory activity to ZMV. And then they investigated the extensive SAR of a variety of alkyl ether analogs at the C-7 position related to ZMV. Linear alkyl ether chains of less than 12 carbons in length exhibited similar inhibitory activity compared to ZMV against influenza virus A, regardless of the carbon chain length of the alkyl ether. However, the moiety of linear chains of more than 12 carbons in length resulted in a slight decrease in NA inhibitory and plaque reduction activities, probably because of unfavorable steric and electrostatic interaction within the enzyme binding site. Addition of a terminal OH, N_3, NH_2, or NHAc did not significantly affect the affinity. Removal of the 8,9-diol of the C-7 alkyl ether derivatives resulted in a similar activity against influenza virus A compared to ZMV, but relatively little effect on activity against the influenza B NA. Evaluation of a plaque reduction assay demonstrated that 7-alkyl ether derivatives (**18**) related to ZMV were more active than the 8,9-dideoxy derivatives (**19**)..

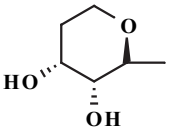
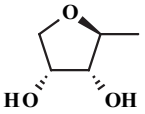
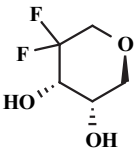
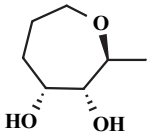
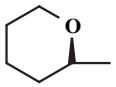
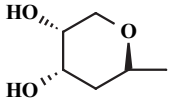
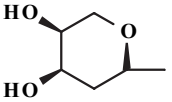
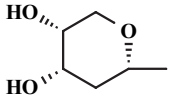
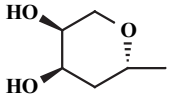


In 2003, Masuda and colleagues [55] designed and synthesized NA inhibitor (**20**) possessing cyclic ether moieties such as tetrahydro-furan-2-yl, tetrahydropyran-2-yl, and oxepan-2-yl groups in place of the glycerol side chain of ZMV. The influenza virus A inhibitory and plaque reduction activities of a range of the bicyclic NA inhibitors are summarized in (Table **1**).



The result suggests that removal of the diol at the C-3' and C-4' positions resulted in reduced inhibitory activity (**20e**). Movement of the diol to the C-4' and C-5' positions from the C-3' and C-4' positions, respectively, showed a significant loss of enzyme inhibitory activity (**20a vs 20f**). This result suggests the position and the stereochemistry of the diol of the tetrahydropyrane side chain must play an important role in binding affinity with NA.

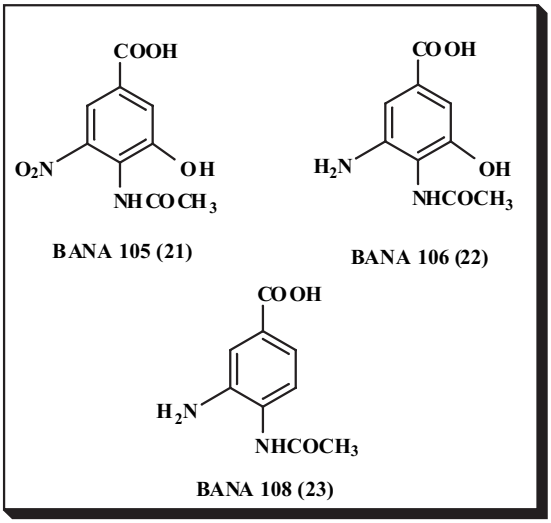
Table 1. NA inhibitory and Plaque Reduction Activities of Bicyclic NA Inhibitors Related to ZMV (Nmol/L) (Reference 55)

Comp.	R	NA inhibitory assay	Plaque reduction assay
ZMV		0.6-12.0(1.0)	0.9-6.6(1.0)
20a		10.3(0.94)	0.6(0.46)
20b		6.1(1.22)	1.4(0.82)
20c		10.7(0.88)	1.4(0.53)
20d		12.4(1.12)	2.2(1.47)
20e		240(4.00)	>100
20f		2.45(22.2)	>100
20g		998(90.7)	>100
20h		370(30.8)	>100
20i		3600(300)	>100

3.3.2. Neuraminidase Inhibitors Containing Benzoic Acid Scaffold

In 1995, Mearek [56] and coworkers found that the crystal structure of influenza virus NA showed that in the active site, 11 residues are universally conserved among all strains known so far. Several potent inhibitors based on the carbohydrate compound 2-deoxy-2,3-didehydro-D-N-acetylneuraminic acid (DANA) have been shown to bind to the conserved active site and to reduce virus infection in animals when administered by nasal spray. But inhibitors of this type were rapidly excreted from physiological systems

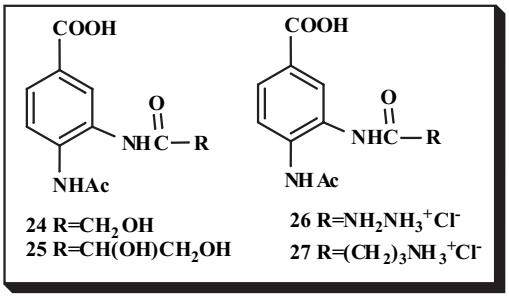
and might not be effective in order to provide a long-time protection. So they designed a new class of specific NA inhibitors which were benzoic acid derivatives (BANA 105,106,108) (**21, 22, 23**) on the basis of the three-dimensional structure of the NA-DANA complex and modeling of derivatives of 4-(acetylamino) benzoic acid in the NA active site.



The aromatic BANA inhibitors occupy the same site as DANA in the active site of NA. But there are some differences in the orientation of the ring and the side-chain groups. Substituents of benzene ring are well defined within the density.

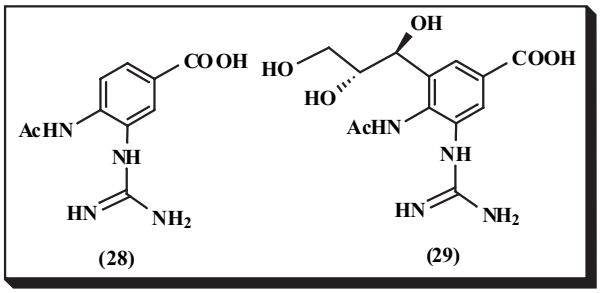
The affinity of these three BANA inhibitors is correlated to their interactions with the active site of NA. In the BANA 108 complex, there are no extra interactions other than those of the carboxylate and the acetylamino groups. Its benzene ring is not constrained to the optimal binding position (a 10° rotation when compared to DANA). As a result, it is the weakest inhibitor of the three. In the BANA106 complex, the addition of a hydroxyl group at the C-4 position eliminated the rotation of the benzene ring and also resulted in an increase in the binding affinity of hydrogen bond to Asp151. The presence of an amino group in BANA106 does not introduce any additional interactions. The enhanced inhibitory activity of BANA105 must be due to the presence of the nitro group at the C-6 position, since it has the same OH interactions as BANA106. The contribution of the NO_2 group is not totally evident, but appears in part to result from a slight repositioning of this inhibitor. On the basis of the above three preliminary inhibitors and computational chemistry methods, a series of more potent inhibitors is being designed.

In the same year (1995), Singh [57] *et al.* utilized the benzene ring of 4-(*N*-acetylamino) benzoic acids as a cyclic template to substitute for the dihydropyran ring of Neu5Ac2en. And they synthesized several 3-(*N*-acylamino) derivatives (**24, 25, 26, 27**) as potential replacements for the glycerol side chain of Neu5Ac2en, and some were found to interact with the same binding subsite of NA. Greater significance was the observation that the 3-guanidinobenzoic acid derivative, the most potent benzoic acid inhibitor of influenza NA (IC_{50}=10μM), occupied the glycerol-binding subsite on NA as opposed to the guanidine-binding subsite. These benzoic acid derivatives thus provide a novel class of compounds that interact in a novel manner with the catalytic site of influenza NA.



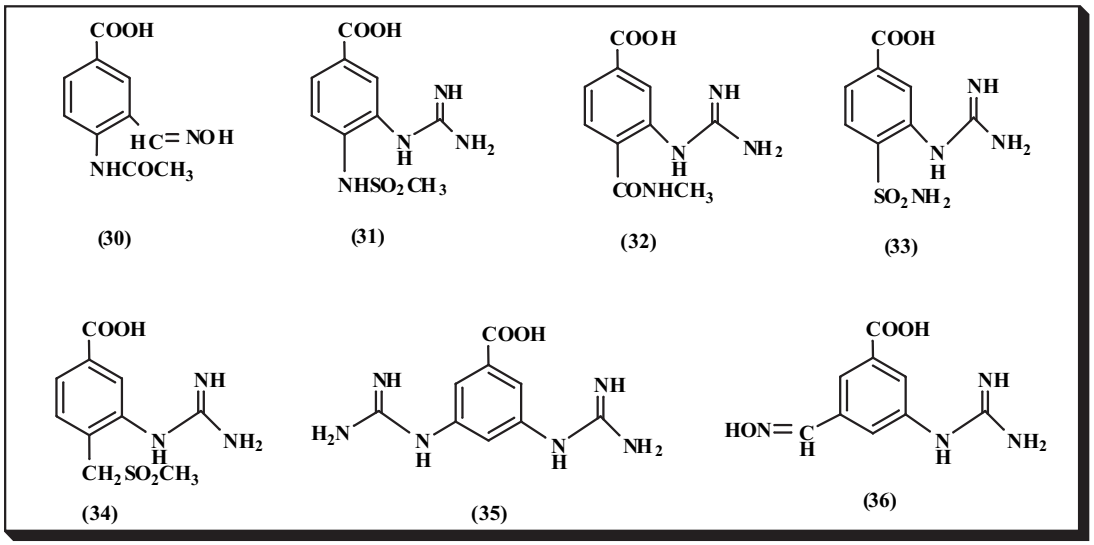
Soon later (1995), Williams [58] and his colleagues synthesized aromatic analogs of SA (**28 and 29**) as potential influenza NA inhibitors. Inspection of the X-ray structure of the NA-ZMV complex revealed that the half-chair conformation of the dihydropyran ring in ZMV is almost flat, and all equatorial C4,C5, and C6 substituents on the ring lay on the same flat plane. Therefore, in designing a new series of NA inhibitors, it is determined to mimic this planar arrangement of substituents by using a benzene ring as a replacement for the dihydropyran ring in ZMV. The benzene ring scaffold has advantages of non-chirality, chemical and metabolic stability and increased lipophilicity compared with the dihydropyran ring. These factors may be important for improving the deficient pharmacokinetic profiles observed for ZMV.

Compound BANA113 (**28**) exhibited good NA inhibitory activity (K_i=8μM, IC_{50}=20μM) and good cell culture inhibitory activity (EC_{50}=65μM). Interestingly, compound (**29**), with the glycerol side chain at the C5 position, did not show the NA inhibitory activity up to 100μM. As demonstrated by many X-ray crystallographic structures of NA-inhibitor complexes [59], the binding pocket of the influenza NA is very shallow and dictates that good inhibitors must meet stringent structural requirements. Clearly, modification of the dihydropyran ring in ZMV to a benzene ring does not allow correct orientation of the ring substituents in NA active site.



In 1997, Chand [60] and his coworkers synthesized a series of benzoic acid derivatives (**30~36**) and tested for their ability to inhibit influenza virus NA.

In order to prepare orally effective, potent, and selective viral NA inhibitors which could be easily and economically prepared, they observed cyclization in BANA113 (**28**), which resulted in the loss of NA inhibitory potency. By studying the interactions of (**28**) and other derivatives with the active site of NA, they designed analogs which contained different substituents and also imparted chemical stability to

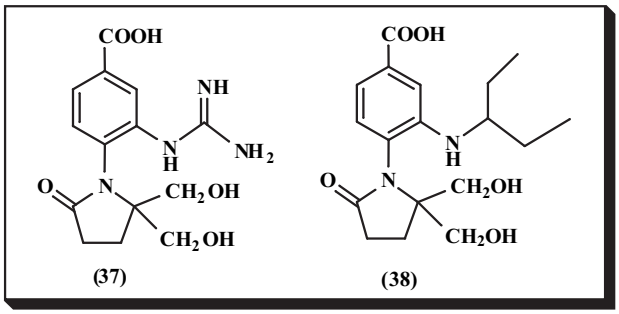


these planar molecules. They found that the planar benzene ring provided a scaffold to support substituents which would interact with the active site of NA. From the enzyme-inhibitor complex of (**28**), it can be seen that the interaction of the guanidine substituent is more favorable rather with the active site pocket where the glycerol substituent of OMV resides, than with the pocket where the guanidine substituent of OMV bonds. They found that interactions of individual substituents on the benzene ring with the active site could not be considered additive. Each combination of substituents had unique steric and electronic interactions with each other, which influenced the overall interaction of the compound with the active site. This made it difficult to predict a substituent arrangement for this series that would give an enhanced bonding to the active site.

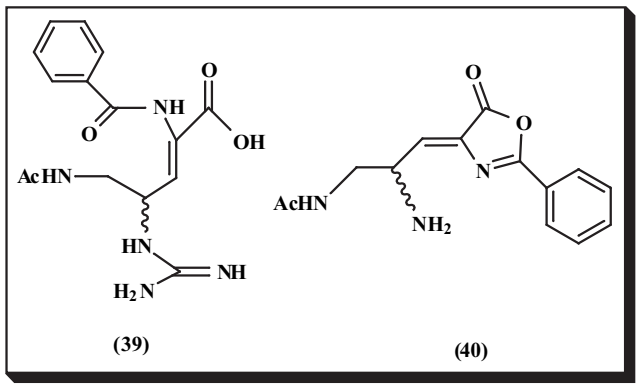
Two years later, in 1999, Atigadda [61] and colleagues designed several novel aromatic inhibitors (**37 and 38**) of NA on the basis of the lead compound BANA113 (**28**), which inhibited influenza virus A with a K_i of 2.5 μM. In their study, the N-acetyl group of BANA113 was replaced with a 2-pyrrolidinone ring, which was designed in part to offer opportunities for the introduction of spatially directed side chains that could interact with NA.

While the parent structure 1-(4-carboxy-2-guanidinopheny) pyrrolidin-2-one was only a modest inhibitor of NA, the introduction of a hydroxymethyl or *bis*(hydroxymethyl) substituent at the C-5' position of the 2-pyrrolidinone ring resulted in inhibitors with low micromole activity. Replacement of the guanidine in (**37**) with a hydrophobic 3-pentylamino group resulted in a large enhancement in binding to produce an inhibitor (**38**) with an IC_{50} of about 50nM against influenza virus A, although the inhibition of influenza virus B was 2000-fold less. The high selectivity of (**38**) for type A NA over type B NA is consistent with the higher energy cost; in type B NA, it is associated with the movement of Glu278 to form a new salt bridge.

In 2001, Mauldin [62] *et al.* reported the synthesis of (2*Z*,4*R*)-5-acetamido-2-benzamidopent-2-enoic acid (sodium salt) (**39**) and another compound (**40**). And the two compounds were assayed for activity against influenza virus NA by modification of a fluorescence assay as described by Woods *et al.* [63]. Compound (**39**) was found to give 33% inhibition of NA, whereas analog (**40**) was devoid of inhibition. These results indicated that moderate activity was preserved by preparing a ring-opened version of ZMV in conjunction with substituting oxygen for nitrogen at position 2.

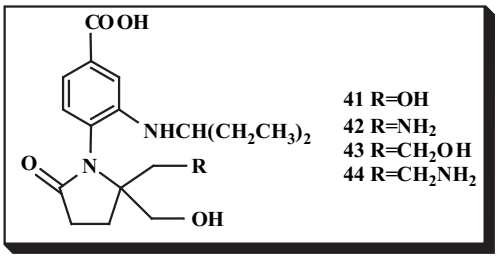


In 2003, Brouillette [64] *et al.* reported the benzoic acid 1-[4-carboxy-2-(3-pentylamino) phenyl]-5,5-*bis*(hydroxy-methyl)pyrrolidin-2-one (**41**), which was a potent inhibitor of avian influenza virus NA and, unlike other reported potent NA inhibitors, did not contain a basic aliphatic amine or guanidine nor a simple *N*-acetyl group. However, (**41**) was a poor inhibitor of influenza virus B. In their study, they further evaluated (**41**) as an inhibitor of human influenza virus A isolates, and it was effective against N2 NA but found to be 160-fold less active against N1 NA. They also

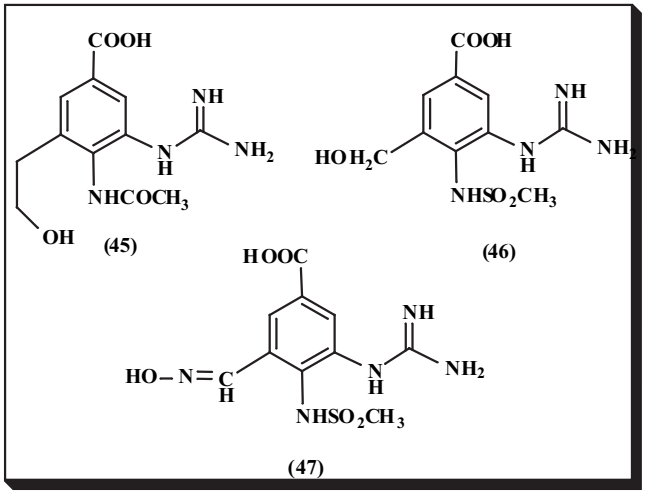


synthesized analogs of (**41**) involving moderate modifications of essential substituents on the pyrrolidinyl ring. Specifically, the aminomethyl (**42**), hydroxyethyl (**43**), and aminoethyl (**44**) analogs were prepared. Only the conservative change (**42**) resulted in continued effective inhibition of influenza virus A, in addition to a noteworthy increase in the activity of (**42**) for N1 NA. The effectiveness of (**42**) against influenza virus B was furthermore improved 10-fold relative to (**41**), but this activity remained 50-fold poorer than for type A NA.

The structure of the *bis*-(hydroxymethyl) compound (**41**) in complex with the N9 NA revealed that in one of the hydroxymethyl groups, hydrogen bonds to an ordered water molecule, whereas the other hydroxymethyl group replaces an ordered water molecule. The carboxylate makes salt bridges with Arg119, 294, and 372, and the pyrrolidinyl ring sits in the hydrophobic pocket created by Trp180 and Ile224. The 3-pentylamino moiety occupies the glycerol subsite of Neu5Ac2en, which is a hydrophobic pocket created by the rotation of Glu278 to form an intramolecular salt-bridge interaction with Arg226. Compound (**42**) exhibited potent inhibitory activity, comparable to (**41**), against N2 NA. However, compound (**41**) was also approximately 100-fold more active than (**40**) against N1 NA, revealing a broader spectrum activity against the two most important human strains of influenza A NA.



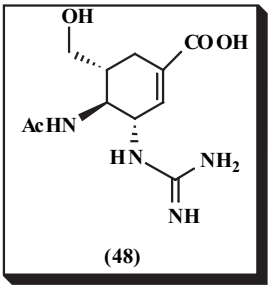
At the beginning of this year (2005), Chand [65] reported the synthesis and NA inhibitory activity of tetra-substituted benzoic acid derivatives (**45~47**). When compound (**45**) was prepared and docked with the crystals of NA, guanidine group did go to the glycerol pocket. The result of IC_{50} value (0.4mM) was disappointing, which can be explained by hydroxyethyl group not pointing to the same direction as glycerol in ZMV and also disturbed the binding affinity of other groups. When compound (**47**) (with carboxyl, oxime, guanidine and $NHSO_2CH_3$) was prepared and docked with the crystals of NA, the binding of the groups was the same as expected; guanidine group flipped back to guanidine pocket and oxime to glycerol pocket. However, the IC_{50} (0.08mM) was again not encouraging. Another compound (**46**) with COOH, $NHSO_2CH_3$ and CH_2OH and guanidine group was also prepared, which had very poor inhibitory activity.



3.3.3. Neuraminidase Inhibitors Containing Cyclohexene Scaffold

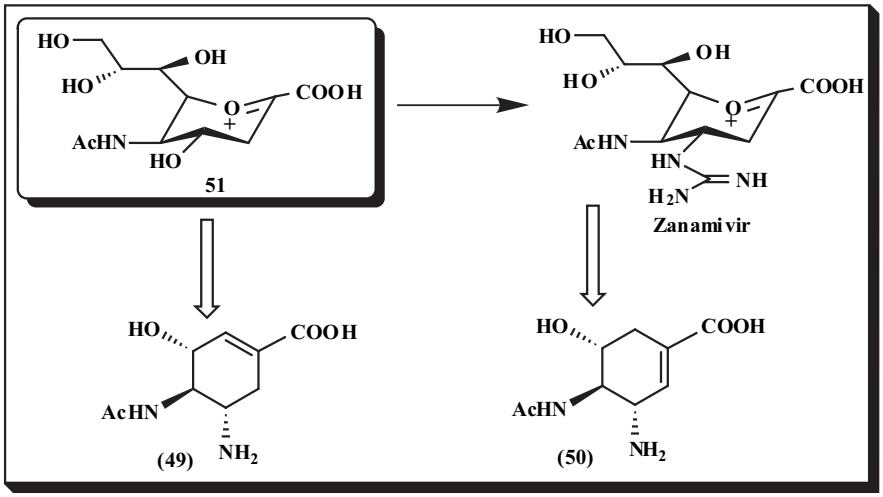
Because of the poor bioavailability of ZMV and the poor inhibitory activity of benzoic acid derivatives, several groups decided to investigate cyclohexene analogs.

In 1995, Chandler [66] reported the synthesis of truncated cyclohexene analogs (**48**) of 4-guanidino-Neu5Ac2en using Diel-Alder method. But the NA inhibitory activity was very poor.

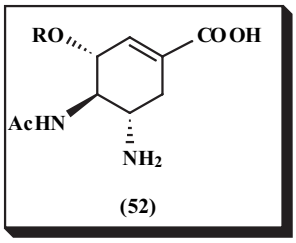


Two years later (1997), Kim [67] *et al.* reported the design, synthesis, and activity evaluation of the novel cyclohexenes (**49 and 50**) as transition-state-based inhibitors of influenza virus NA. They found that the double bond position in the cyclohexene analogs played an important role in NA inhibition as demonstrated by the antiviral activity of (**49**) (IC_{50}=6.3μM) vs (**50**) (IC_{50}>200μM).

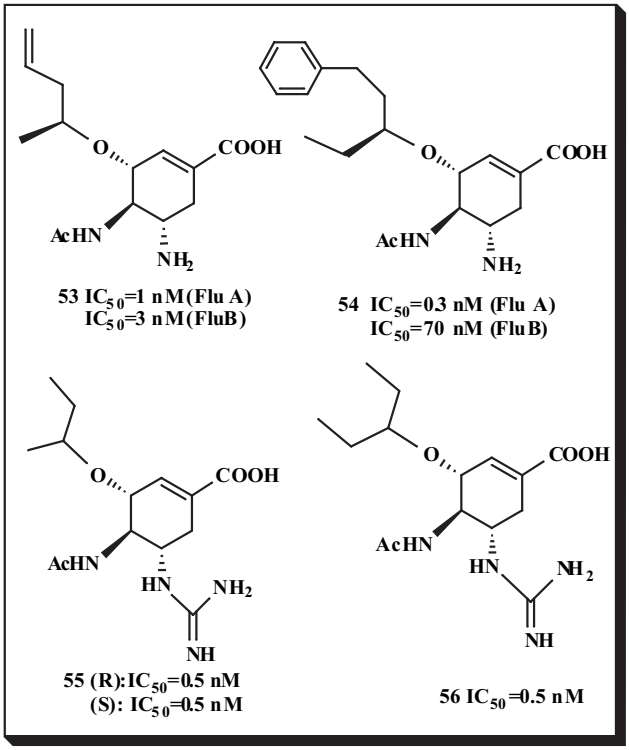
Transition-state mimics frequently are potent inhibitors of the catalyzing enzyme. The concept of structural similarity to the transition state has found wide application in drug design over the years. The multitude of enzyme-inhibitor interactions is governed by steric as well as electronic factors. In theory, compounds that closely resemble the transition-state structure should give high binding affinity toward the target enzyme [68]. Using intermediate (**51**) as a key transition-state mimic is a reasonable approximation in view of the X-ray crystallographic studies described above. Considering the flat oxonium cation of (**51**), as an isostere of the double bond, the cyclohexene scaffold was selected as a replacement for the oxonium ring of (**51**) which would keep the conformational changes to a minimum. In addition, the cyclohexene system was expected to be chemically versatile for the manipulation of side chains attached to the ring. And they also designed and synthesized the compounds (**49**) and (**50**) from shikimic acid. The inhibitory activity of (**49**) and (**50**) was directly determined in an NA enzyme assay. While (**49**) proved to be a potent NA inhibitor with an inhibitory concentration (IC_{50}) of 6.3μM, compound (**50**) did not exhibit inhibitory activity at concentrations up to 200μM. This result demonstrated that the double bond position in



the design of cyclohexene NA inhibitors plays an important role in inhibitory activity.



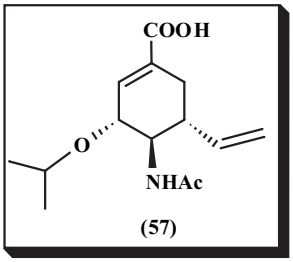
Kim synthesized a series of compound (**52**) from compound (**49**). In the cyclohexene structure (**52**), the C1 carboxylate, C4 acetamino, and C5 amino groups were kept constant, while the C3 aliphatic group was optimized for antiviral activity. They selected a general and efficient route to introduce various aliphatic side chains.



The NA inhibitory activity assay demonstrated that the length, size of branching and geometry of the alkyl groups in (**52**) profoundly influenced the NA inhibitory activity. In the design of enzyme inhibitors, consideration of hydrophobic interactions in the enzyme active site might lead to a new generation of structurally unique molecules.

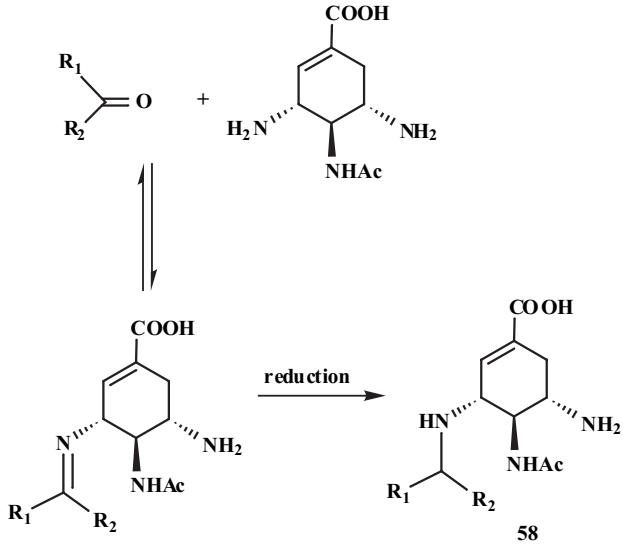
In 1998, Kim [69] *et al.* synthesized another series of influenza NA inhibitors (**53~56**) with the cyclohexene scaffold containing lipophilic side chains. The compound (**53**) had a good NA inhibitory activity *in vitro*. The (*S*)-isomer of (**54**) was more potent than the (*R*)-isomer for influenza virus A, but their influenza virus B inhibitory activity was comparable. The X-ray crystal structures of (**54**) bound to NA revealed that a portion of the phenyl ring is exposed to water. They also prepared a series of guanidine analogs (**55**) and (**56**), and their NA inhibitory activities were compared with those of corresponding amino analogs. The different degrees of the binding enhancement observed in the guanidine series might suggest that the hydrophobic interactions of the C3-alkyl groups influenced the hydrogen bond interaction of the guanidine groups with the amino acid residues of the NA active site.

In 2002, Hanessian [70] synthesized a novel analog (**57**) of OMV, which the basic amino group had replaced by a hydrophobic group. The X-ray co-crystal structure of the new inhibitor bound to the NA active site showed that the vinyl group occupied the same subsite as the amino group in OMV. Molecular modeling studies indicated that six-membered analogs of OMV could be designed with hydrophobic olefinic substituents which could replace the primary amino group. With this premise in mind, compound (**57**) was designed and synthesized. The 2-propyl ether substituent of (**57**) is likely to be suboptimal for



interaction within subsites of NA and should be more properly compared to the corresponding analog of OMV. The replacement of the amino group in OMV by a vinyl group leads to an inhibitor of comparable activity to the parent series.

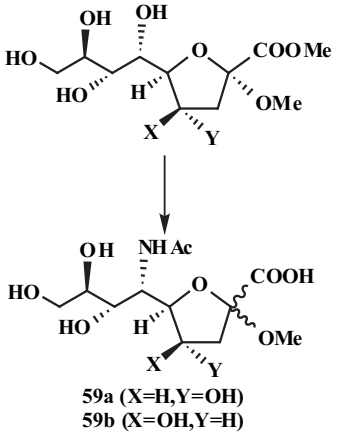
In 2003, Hochgurtel [71] and colleagues discovered a new and potent inhibitor (**58**) of NA in dynamic combinatorial libraries based on ketones and amines as building blocks, and they are the first who reported the use of ketones as building blocks of dynamic libraries. They used diamine which is structurally similar to some known NA inhibitors [72] as the scaffold for the dynamic libraries. The NA is a dynamic combinatorial chemistry (DCC) target. Equilibration of the scaffold with a mixture of ketones was expected to produce a set of imines (**Scheme 2**)**.** The imines were then reduced to the secondary amines of general structure (**58**).



Scheme 2.

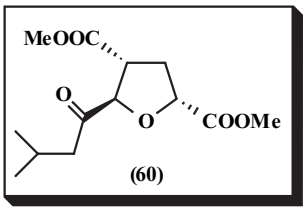
3.3.3. Neuraminidase Inhibitors Containing Furan Scaffold

In 1992, Yamamoto [73] *et al.* reported the synthesis of isomers of SA with 6-acetylamino group (**59a and 59b**) through aldol condensation of D-glucose with oxalacetic acid (Scheme **3**). These compounds inhibit NA of influenza viruses A, A_2, and B remarkably.

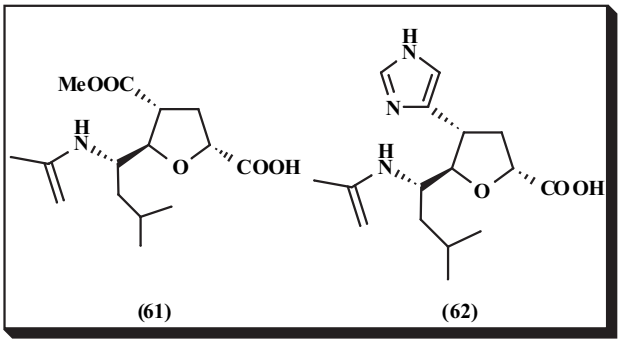


Scheme 3.

In 2001, Wang [74] reported the synthesis of (±)-(2*R,*3*,*5*R*)-tetrahydrofuran-3,5-dicarboxylic acid dimethyl ester (**60**). It is a key intermediate in the synthesis of NA inhibitors containing furan scaffold.



At the beginning of this year (2005), Wang [75] and coworkers synthesized (±)-(2*R*,3*R*,5*R*)-[2-(10-*S*-Acetamido-30-methyl) butyl-3-methoxycarbonyl]tetrahydrofuran-5-carboxylic acid (**61**) and (±)-(2*R*,3*R*,5*R*)-[2-(10-*S*-acetamido-30-methyl)butyl-3-(4-imidazolyl)] tetrahydrofuran 5-carboxylic acid (**62**) as inhibitors of influenza NA. Both the compounds (**60**) and (**61**) inhibited influenza virus A with an IC_{50} of about 0.5μM and influenza virus B with an IC_{50} of 1.0μM. However, these compounds have reduced inhibitory potency in comparison to the corresponding pyrrolidine analogs.



3.3.4. Neuraminidase Inhibitors Containing Cyclopentane Scaffold

The crystal structure of compound (**59**) complexed with N9 NA and its activity against influenza NA suggested that a cyclopentane ring might be a suitable scaffold for a novel NA inhibitor.

In 1993, Von Itzstein [76] designed the compound (**63**) to exploit the charged nature of the fourth pocket. It was initially used for modeling cyclopentane NA inhibitors. This compound was synthesized as a racemic mixture, and its crystal structure bound to N9 NA revealed that the guanidino group occupied the fourth binding pocket replacing the existing water molecule, and it was involved in charge-based interactions with residues Asp151, Glu119, and Glu227. This mode of binding for the guanidino group was analogous to the one observed in the crystal structure of ZMV with influenza A NA.

In 2000, Babu [77] *et al.* analyzed the crystal structures of influenza virus A NA complexed with DANA (**7**) (Fig **4**). They divided the active site of NA into four binding pockets: ¢Ÿ an acid pocket where the carboxylic acid of DANA has hydrogen bond interactions with a triad of Arg residues (118, 292, and 371); ¢⁄ an acetamido binding pocket that has a hydrophobic patch formed by a Trp178 and Ile222 and a buried water molecule; ¢€ the glycerol binding pocket; ¢‹ the fourth pocket into which the C4-hydroxyl of

DANA is positioned and a water molecule surrounded by negatively charged residues characterizes the fourth pocket.

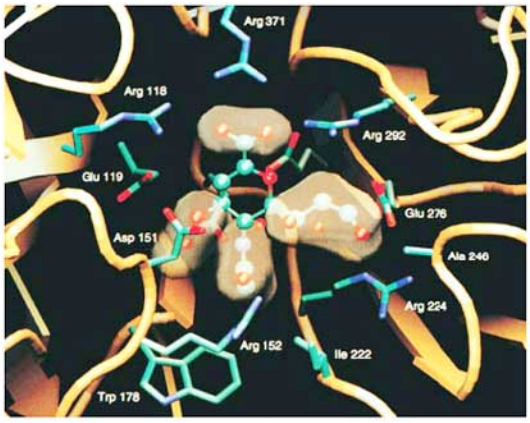
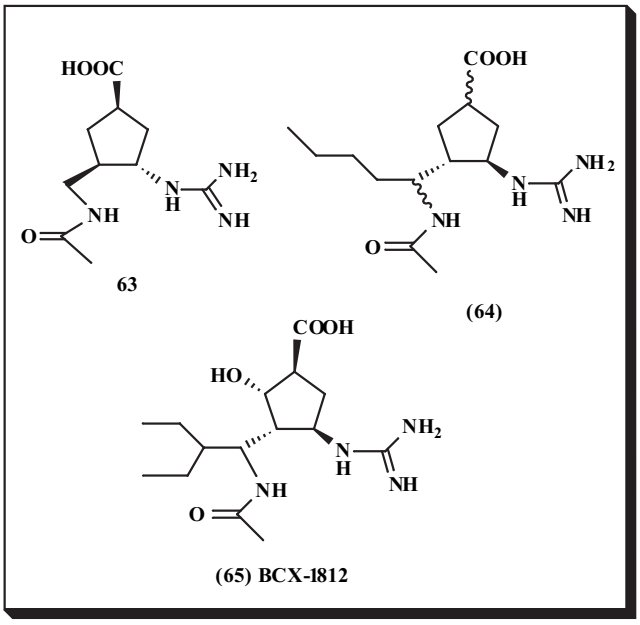


Fig. (4). Binding of DANA (7) to NA N9 showed the four binding pockets in active site (reference 77).

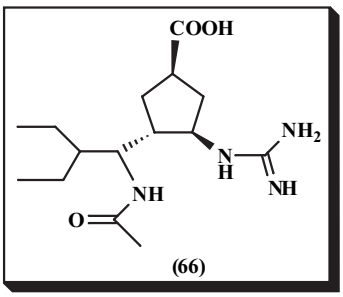
On the basis of the crystal structure of compound (**63**) with NA, structure (**64**) was designed. The absolute configuration at C4 having the guanidino group is *R* in (**64**) and *S* in (**63**) as observed from the crystal structures of the complexes with NA. As a consequence of these differences in the stereochemistry, the guanidino group of (**64**) is oriented differently into the fourth pocket compared to the guanidino group of (**63**) and ZMV. Later, structure of (**65**) (BCX-1812) was designed by taking advantage of both the hydrophobic pockets in the active site. Again, this compound was synthesized as a racemic mixture, and the crystal structure showed that the active isomer was the same as that of (**64**), and there was a similar altered orientation of the guanidino group in the fourth pocket compared to the guanidino group in ZMV. The significance of these differences in the orientation of the guanidino group in the fourth pocket of the NA active site is emphasized by the observation that (**65**) retains its inhibitory activity against the ZMV-resistant Glu119 Gly variant of influenza A NA. Due to *in vitro* and *in vivo* activity of (**65**) against influenza A and B, further studies with (**65**) in humans are warranted,



and it is currently in human clinical trials for the management of influenza.

X-ray crystal structures of complexes of NA with known five- and six-membered ring inhibitors revealed that potent inhibition of the NA is determined by the relative positions of the substituents (carboxylate, glycerol, acetamido, hydroxyl) rather than by the absolute position of the central ring. This theory leads to the design of potential NA inhibitors in which the cyclopentane ring served as a scaffold for substituents (carboxylate, guanidino, acetamino, alkyl) that would interact with the four binding pockets of the NA active site at least as effectively as those of the established six-membered ring inhibitors such as DANA (**7**), ZMV (**1**), and OMV (**2**).

In 2001, Chand [78] and colleagues developed a synthetic route to obtain candidate (**66**), and they discovered two NA inhibitors (**66**) and (**65**), which had IC_{50} values against NA from influenza viruses A and B of <1 and <10nM, respectively. These IC_{50} values were comparable to be superior to those for ZMV and OMV. Compounds (**65**) [RWJ-270201 (BCX-1812)] and (**66**) proved to be very similar in biological activity, but compound (**65**) required fewer steps for the synthesis. This compound became the agent for further biological evaluation and is now in phase III clinical trials.

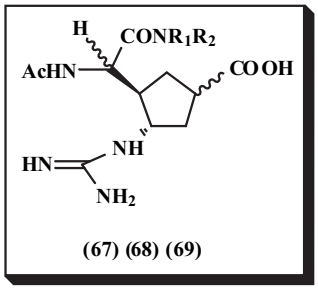


RWJ-270201 (BCX-1812) [79]: RWJ-270201 is a novel NA inhibitor that was designed by a rational, structures-based approach with the objectives of achieving high selectivity for influenza NA inhibition and broad-ranging activity against influenza virus A and B strains with oral activity. Preclinical studies have demonstrated the selectivity and potency of RWJ-270201 against an array of clinically significant influenza strains, including recent clinical isolates and avian strains with potential pandemic implications. In general, the activity of RWJ-270201 *in vitro* and in mice was comparable to be better than that of ZMV and OMV.

As a result of the encouraging preclinical data, clinical trails were initiated in order to determine the tolerability and efficacy of RWJ-270201. Phase II clinical studies have provided further evidence that RWJ-270201 is well tolerated and has antiviral activity when administered orally and once daily. These studies support the continued development of RWJ-270201 in phase III trails; such trails are currently underway. A favorable outcome of these trails could lead to the availability of a new weapon for combating the significant morbidity and mortality of annual epidemics and unpredictable pandemics of influenza.

Last year (2004), Chand [80] and coworkers synthesized a series of multisubstituted cyclopentane amide derivatives. Amides prepared were 14 compounds (**67a~n**) of N-

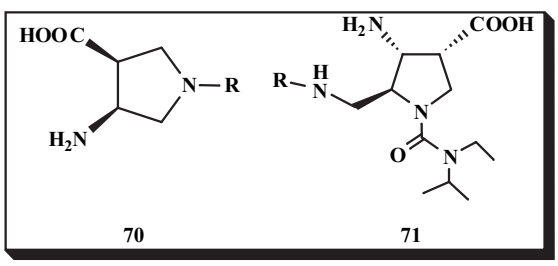
substituted alkyl or aralkyl types from primary amines, 13 compounds (**68a~m**) of the N,N-disubstituted alkyl, aralkyl, or substituted-alkyl type from secondary amines, and 12 compounds (**69a~l**) from cycloaliphatic or substituted cycloaliphatic secondary amines. These compounds bearing two chiral centers at position-1 in the ring and position-1' in the side chain attached at position 3, were tested for their ability to inhibit viruses A and B NA. The 1-ethylpropylamide, diethylamide, dipropylamide, and 4-morpholinylamide showed very good inhibitory activity (IC_{50} =0.015-0.080 μM) vs the NA A, but modest activity (IC_{50} =3.0-9.2 μM) *vs* the NA B. Since the parent amides bear two chiral centers (C-1 and C-1'), three of the better inhibitors were tested at higher levels of diastereomeric purity. The diastereomers corresponding to the active forms of the 1-(ethyl) propylamine, the diethylamide, and the dipropylamide, and the diastereomer of the diethylamide representing the active form both at C-1' and C-1 were isolated or synthesized from precursors that were isolated as diastereomers. These diastereomers showed some improvement in NA inhibition over the parent diastereomeric mixtures. 1-carboxy-1-hydroxy derivatives which are the best active compounds, the diethylamide and the dipropylamine, were also prepared. These compounds were not as active as the compounds without the 1-hydroxy group. The C-1' active isomer of the diethylamide from the 1-carboxy series was tested in influenza-infected mice by oral and intranasal administration and was found to be very effective only intranasally in preventing weight loss at doses as low as 0.1 (mg/kg)/day.



(67) (68) (69)

3.3.5 Neuraminidase Inhibitors Containing Pyrrolidine Scaffold

In 2001, starting with some pharmacophore structures discussed above and using available structural information of the NA active site as the guide, Wang [81] and colleagues synthesized analogs of tri- and tetrasubstituted pyrrolidines (**70**) and (**71**) by means of high-throughput parallel synthesis in solid or solution phase for expeditious SAR. Their effort in this area started with the identification of c*is-N*-Boc-3-aminopyrrolidine 4-carboxylic acid (**72**) as a modestly active NA inhibitor (IC_{50}=50 μM against NA A/Tokyo). On the basis of the literature data [82], they derived an "airplane" model of the NA active site as illustrated in (Fig. **5**), to summarize the basic structural requirements of a potent NA inhibitor.



70 71

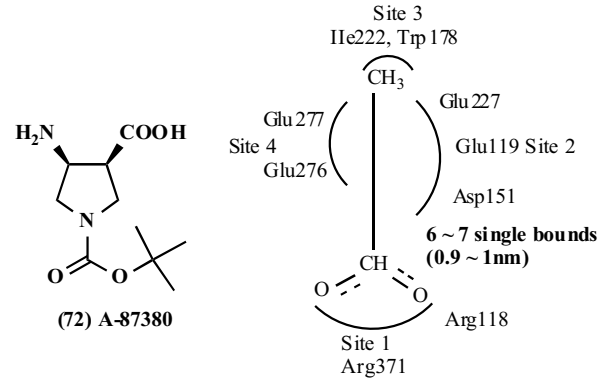
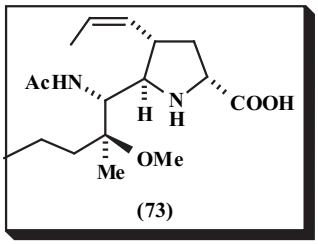


Fig. (5). NA active site (reference 82).

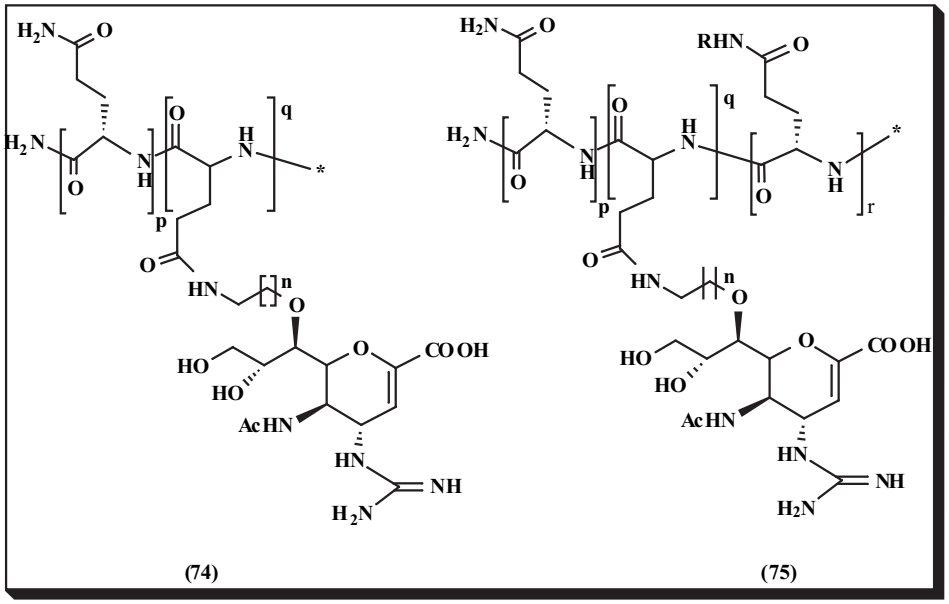
The active site of NA has four main well-conserved binding sites. The positively charged **site 1** consists of Arg118, Arg292, and Arg371 residues and interacts with the carboxylate of SA or GG167 via charge-charge interaction and hydrogen bonding. The negatively charged **site 2** consists of Glu119, Glu227, and Asp151 and interacts with the guanidino group of GG167. The small hydrophobic pocket consisting of Ile222 and Trp178 residues (**site 3**) accommodates the acetyl group of SA or GG167, and **site 4**, consisting of Glu276 and Glu277 residues, binds to the triglycerol side chain of SA or GG167. In the two-dimensional sense, **sites 1** and **3** are situated at the head and tail of the "airplane" respectively, separated by 9-10 Å or 6-7 single bond lengths, while **sites 2** and **4** are situated at two wings of the "airplane". In the three-dimensional sense, these binding pockets are clearly set off from the plane defined by the ring of the cyclic nucleus since aromatic (planar) mimics of GG167 are poor inhibitors of NA. Literature data indicated that occupation of all four pockets is necessary for potent inhibition.

In 2002, Hanessian [83] *et al.* reported a concise, stereocontrolled, and practical synthesis of a NA inhibitor (**73**) (A-315675) consisting of a highly functionalized *D*-proline scaffold. They described a highly stereocontrolled total synthesis of a novel NA inhibitor (**73**) in a 12.8% overall yield covering 22 steps from D-serine.



(73)

In the same year (2002), DeGoey [84] reported a stereoselective synthesis of A-315675 (**73**) that utilizes pyrrolinone as the key intermediate. Kati [85] evaluated A-315675 for its ability to inhibit influenza viruses NA in cell culture. They found that A-315675 effectively inhibited influenza virus A N1, N2, and N9 and B NA with constant (K_i) values between 0.024 and 0.31nM. These values were



(74) (75)

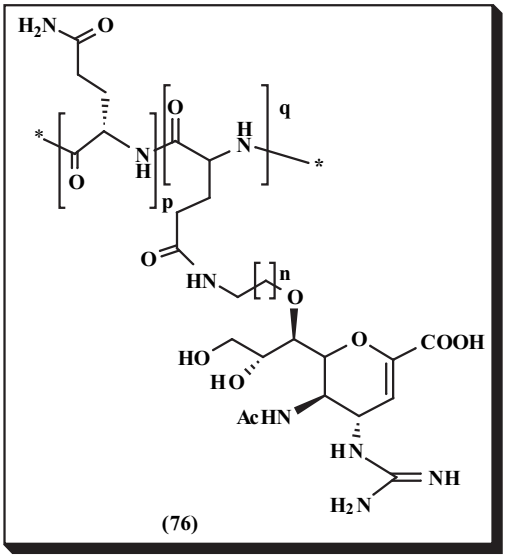
comparable to or lower than the K_i values measured for ZMV, OMV, and BCX-1812.

3.3.6. Polyvalent Neuraminidase Inhibitors

As there are about 50 tetrameric NA units on the surface of each influenza virion, several research groups became interested in exploring the antiviral properties of multimeric ZMV derivatives. They found that polyvalent NA inhibitors showed high *in vitro* anti-influenza activity.

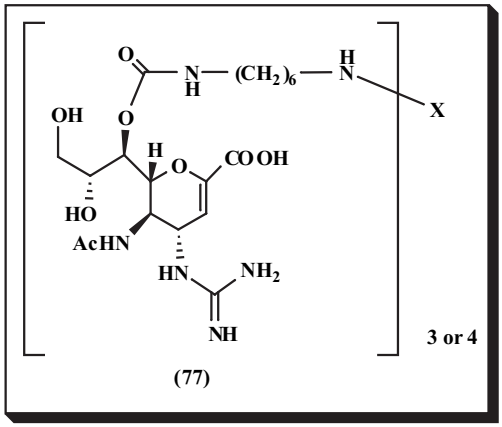
In 2002, Honda [86] and colleagues synthesized polyvalent NA inhibitors bearing 4-guanidino-Neu5Ac2en analogs on the polyglutamic acid backbone, via a spacer of alkyl ether at the C7 position. These multivalent conjugates (**74**) and (**75**) showed enhancement of antiviral activity against influenza virus A and more potent efficacy *in vivo* compared to a monomeric NA inhibitor.

In 2003, Masuda [87] *et al.* described polyvalent NA inhibitors (**76**) bearing 4-guanidino-Neu5Ac2en derivatives on a poly-L-glutamine backbone. In order to obtain a longer retention time of ZMV in bronchi and lungs, they focused on supermolecules bearing 4-guanidino-Neu5Ac2en derivatives bound at their C7 position through noncleavable alkyl ether linkages. They further synthesized poly-*L*-glutamine bearing 7-*O*-alkyl-4-guanidino-Neu5Ac2en derivatives linked by amide bonds (**76**), which showed enhanced antiviral activity against influenza virus A and more potent efficacy *in vivo*. A much greater efficacy against influenza A in the mice model by intranasal administration than ZMV was observed.



(76)

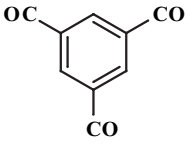
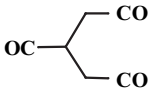
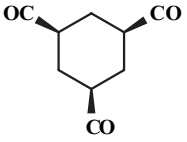
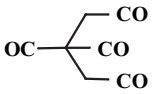
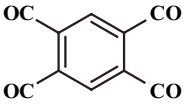
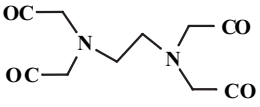
In 2004, Watsom [88] and colleagues synthesized a set of trimeric and tetrameric derivatives (**78~83**) of the influenza virus NA inhibitor ZMV by coupling a common monomeric ZMV derivative (**77**) onto various multimeric carboxylic acid core groups. All these multimeric compounds are significantly more effective than ZMV and also show outstanding long-lasting protective activity when tested in mouse influenza infectivity experiments. The structures and anti-influenza activity of (**76~81**) are shown in (Table **2**).



(77)

They also found that trimeric and tetrameric conjugates of ZMV showed remarkable potent antiviral activity and that it was necessary to use complex and high molecular weight polymeric conjugates to achieve long-lasting *in vivo* antiviral activity.

Table 2. Structure and Anti-Influenza Activity of (78~83) (Reference 88)

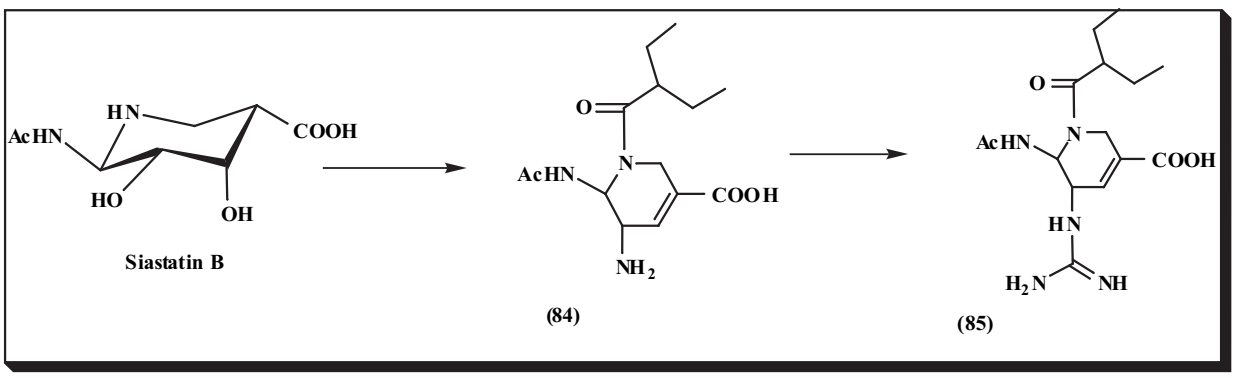
Compd	X	EC_{50}(ng/mL) (influenza A)	EC_{50}(ng/mL) (influenza B)
ZMV	-	32.5	12.2
Monomer	-	438	213
78		0.82	1.33
79		0.4	0.81
80		0.3	0.438
81		0.159	2.475
82		0.53	0.95
83		1.07	2.84

3.3.7. Other Neuraminidase Inhibitors

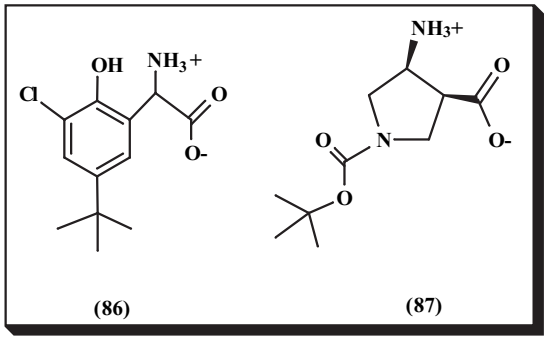
In 2000, Shitara [89] *et al.* synthesized 6-acetamido-5-guanidino-3,4-dehydro-*N*-(2-ethylbutyryl)-3-piperidinecarboxylic acids (**84**) and (**85**) starting form natural siastatin B (Scheme **4**), which was a bacterial NA inhibitor isolated from *Streptomyces* culture in a stereospecific fashion. A preliminary biological evaluation was carried out by using compounds (**84**) and (**85**). Unfortunately, the two compounds were found inactive to enhance the inhibitory activities of NA on influenza virus A/PR/8/34 (H1N1), A/Japan/307/56 (H2N2), A/Aichi/2/68 (H3N2), and B/Yamagata/16/88.

In 2001, Kati [90] and coworkers hypothesized that compounds which contain positively charged amino groups in an appropriate position to interact with the Asp152 or Tyr406 side chains, might be bound tightly by NA. They tested approximately 300 α- and β-amino acids as potential inhibitors of the NA catalytic domain from A/N2/Tokyo3/67 influenza virus. Although several amino acid inhibitors were identified during this test, the two most potent compounds were a phenylglycine (**86**) and a pyrrolidine (**87**).

They used enzyme mechanistic information to form a hypothesis about specific interaction which might be important for the tight binding of ligands to the active site of influenza virus NA. They demonstrated that interactions with Asp152 and Tyr406 and Glu120 were important contributors to the binding affinity of these compounds. Residues Asp152 and Tyr406 are particularly attractive for



targeted inhibitor design, because they are strictly conserved, and lab-generated mutant enzymes at these positions exhibit poor enzymatic activity. Thus, drug resistance may be less likely to develop for compounds which interact with these residues.



(86) **(87)**

Last year (2004), Harrington [91] *et al.* reported the research and development of second-generation compounds of OMV phosphate, a prodrug for a NA inhibitor. The overall yield from shikimic acid to OMV phosphate has been increased from 27 to 29% or 35-38%.

Since benzene derivatives were not better NA inhibitors, Chand [92] and coworkers explored the pyridine ring system this year. They replaced benzene ring by pyridine ring and synthesized di-, tri-, and tetra-substituted pyridine derivatives (**88~95**).

Replacement of benzene ring with pyridine ring gave compound (**92**) (IC_{50}=0.006mM) as the most potent compound in tri-substituted series. Addition of a fourth group in compound (**94**) did increase the potency slightly (IC_{50}=0.004mM) but not significant. The crystal structure of compound (**94**) showed that the affinity of guanidine group to glycerol pocket was much more than the guanidine pocket itself. The interaction of pyridine nitrogen in the active site was not of relevance for the activity.

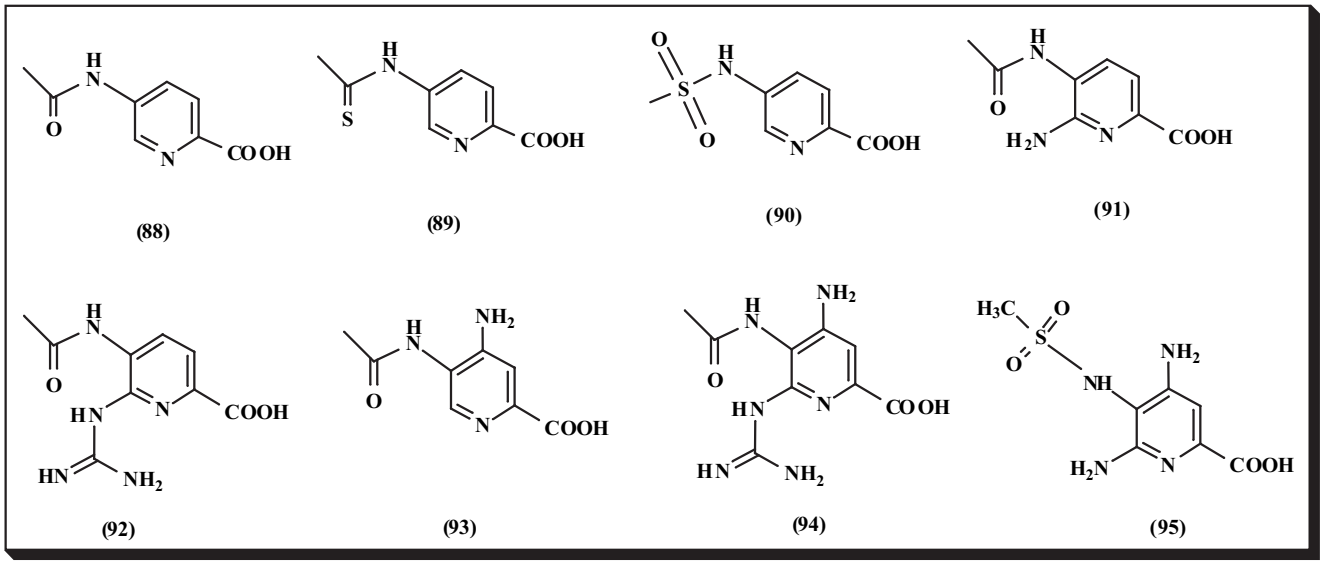
In 2005, Macdonald [93] and colleagues reported the synthesis, antiviral and pharmacokinetic properties of ZMV dimmers (**96**) and (**97**). The two compounds are highly potent NA inhibitors being investigated as potential second generation inhaled therapies both for the treatment of influenza and for prophylactic use. They show outstanding activity in a one-week mouse influenza prophylaxis assay, and compared with ZMV, high concentrations of (**96**) and (**97**) are found in rat lung tissue after one week. In plaque reduction assays, (**96**) and (**97**) show outstanding potency against a panel of nine flu A and B virus strains. Consistent with its shorter and more rigid linking group, dimmer (**96**) has been successfully crystallized.

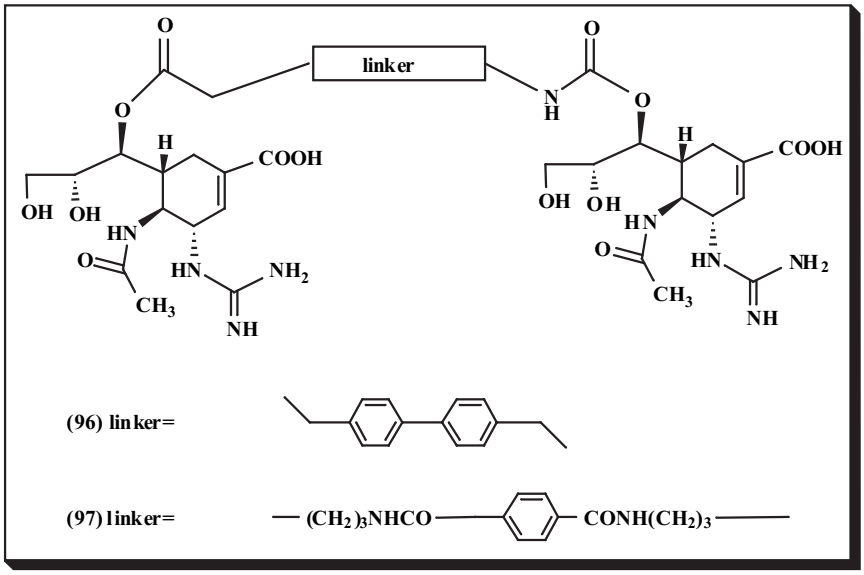
The potency of (**96**) and (**97**) in the described biological assays and their ability to have a prolonged antiviral effect can be explained in terms of their polarity, molecular weight and dimeric structure which leads to a multivalent binding effect [94]. The properties of dimmers (**96**) and (**97**) are commensurate with a superior treatment for influenza virus A or B and long-acting prophylaxis, and the compounds are potential candidates for further development. They are likely to be effective against strains of NA that may yet develop.

4. RESISTANCE OF INFLUENZA VIRUSES TO NA INHIBITORS [95]

It has been presumed, given the quasi-species nature of influenza virus, that the virus was resistant to the NIs even before the drugs were discovered. However, up to date, no such viruses have been uncovered. Rather, quite extensive laboratory passage of influenza viruses A and B in cell culture is required to select an NI-resistant mutant. Some mutants have also been recovered from patients. However, more significant is the observation that the drug-resistant mutants are somewhat comprised in virulence.

Last year, Gubareva [96] reported the molecular mechanisms of influenza virus resistance to NA inhibitors (NAIs). *In vitro* studies demonstrate that both NA and HA influence virus susceptibility to NAIs. Drug resistance conferred due to changes in the NA could be monitored in the NA inhibition assays. ZMV-selected viruses acquired the NA substitutions at residues 119 and 292; OMV-selected- at 274 and 292; Peramivir (BCX-1812)-selected- at 292; and A-315675-selected-at 119. The NA binding efficiency and the susceptibility to NAIs are affected by the amino acids





forming the NA receptor-binding site, the location and number of oligosaccharide chains, and structure of the SA-containing cellular receptors. Emergence of the viruses with the NIs-induced substitutions in the NA is uncommon in drug-treated humans; however, a compromised state of the immune system promotes emergence of drug resistance. *In vivo*, the ZMV-selected mutant contained substitutions at 152 (B/NA), the OMV-selected mutants at residues 119 (A/N2), 198(B/NA), 274(A/N1), and 292(A/N2). Substitutions in the NA were often accompanied by impairment of virus infectivity and virulence in animal models.

The recent studies demonstrate that emergence of the NIs-resistant viruses with the altered NA, as a result of the drug treatment is uncommon in the immunocompetent people as it was predicted based on the design of these drugs [97].

In 2002, Smith [98] *et al.* carried out structural studies of the resistance of influenza virus NA to inhibitors. They have proposed that a reduced energy is required for reorientation of the resistance of the R292K variant toward compounds such as BCX-1812 with a large hydrophobic group. The large difference in sensitivity of the N2 and N9 R292K mutants toward BCX-1812 suggests a reduced energy requirement for the reorganization of the active site in the N2 variant compared to N9. The reorganization of E276 in the NA from influenza virus B has been shown from molecular dynamics simulations to occur less frequently than in NA from influenza A. The difference in the energy used accounts for the selectivity of inhibitors that require this reorientation for binding. However, both in N2 and N9 backgrounds, the R292K variant shows similarly high resistance to OMV. The energy penalty for the conformational change in E276 is presumably not the only determinant of the resistance of R292K to OMV.

The only case of a drug-resistant variant in human reported to date (February 1999) involves the compassionate use of ZMV in an immuno-compromised child infected with influenza virus B. A variant virus with a mutation in the NA of R152K was isolated after a prolonged (14 days) treatment with the drug [99]. Arg152 interacts with the carbonyl of the acetamido side chain of substrate and inhibitors, and the mutation to Lys affects enzyme activity and inhibitor binding. The more relevant test of susceptibility to drug-resistance will come from future studies of resistant variants arising during the acute exposure of virus to drug in the course of normal treatment of influenza.

5. CONCLUSION AND PERSPECTIVE

The two influenza virus NA inhibitors-ZMV and OMV have been approved by the Food and Drug Administration (FDA) for the treatment and prophylaxis of influenza. Compared with other anti-influenza agents, the NA inhibitors are better tolerated and more effective against all influenza types. There has been little evidence of the emergence of viral resistance. NA inhibitors provided an important new therapeutic weapon for the prevention of influenza infection.

The benefits of using a rational drug design (RDD) strategy are exemplified in the development of ZMV as a potent anti-influenza agent. The recent discoveries with respect to structurally modified analogs of ZMV result in a series of potent NA inhibitors. However, both the two NA inhibitors have disadvantages. Due to the poor oral bioavailability of ZMV, it must be administrated by inhalation. OMV is an ester prodrug to be converted to the active free carboxyl form upon administration. The most common side effects reported with ZMV included headache and diarrhea, whereas OMV caused nausea and vomiting. So, the NA inhibitors with novel structure need to be discovered.

The success of these SBDD studies with influenza virus NA improves current knowledge on influenza virus pathogenesis, innate anti-influenza immunity, and facilitates the development of novel approaches to prophylaxis and treatment of influenza. The crystallographic studies, molecular modeling and computational chemistry analysis, understanding of the enzyme mechanism and synthetic chemistry may provide encouragement for future studies of NA inhibitors as antiviral agents. These antiviral agents will translate economic benefits in preventing disease or

shortening the duration of the clinical signs of influenza in the 'at risk' groups and working community.

The focus of the research is to identify novel, highly potent, effective orally, and selective NA inhibitors, which could serve as lead structures for a program to develop novel anti-influenza agents.

ABBREVIATIONS

NA	=	Neuraminidase
HA	=	Haemagglutinin
NAIs	=	Neuraminidase inhibitors
SA	=	Sialic Acid
ZMV	=	Zanamivir
OMV	=	Oseltamivir
DANA	=	Dehydrodeoxy-N-acetylneuraminic acid
DCC	=	Dynamic Combinatorial Chemistry
SAR	=	Structure Activity Relationshi
SBDD	=	Structure-Based Drug Design
RDD	=	Rational Drug Design
FDA	=	Food and Drug Administration

REFERENCES

[1] Nicholson, K.G. *Epidemiol. Infect.* **1996,***116,* 51.
[2] Naem, S. *J. Biomed. Biotechnol.* **2001**, *1*(2), 89.
[3] Bridges, C.B; Fukuda, K; Uyeki, T.M; Cox, N.J. *MMWR Reconm. Rep.* **2002**, *51*(RR-3), 1.
[4] Alexander, DJ. *Vet. Microbiol.* **2000**, *74*(1-2)*,* 3.
[5] Landman, W.J.; Schrier, C.C.; *Tijdschr. Diergeneeskd.* **2004,** *129*(23), 782.
[6] Heins, J.R.; Plamp, J. *S. D. J. Med.* **2004**, *57*(12), 529.
[7] Oxford, J.S.; Novelli, P.; Sefton, A.; Lambkin, R. *Antivir. Chem. Chemother.* **2002**, *13,* 205.
[8] Dreitlein, W.B.; Maratos, J.; Brocavich, J. *Clin. Ther.* **2001**, *23(3),* 327.
[9] Diane, Y.; Cynthia, F.; Karen, B. *Phil. Trans. R. Soc. Lond. B* **2001,** *356,* 1905.
[10] Kati, W.M.; Montgomery, D.; Carrick, R.; Gubareva, L.; Maring, C.; Mcdaniel, K.; Steffy, K.; Molla, A.; Hayden, F.; Kempf, D.; Kohlbrenner, W. *Antimicrob. Agents Chemother* **2002**, *46(4),* 1014.
[11] Frederick, G. H. *Int. Congr. Ser.* **2001**, *1219,* 797.
[12] Whittington, A.; Bethell, R. *Curr. Opin. Thera. Patents* **1995,** *5,*793.
[13] Paulson, J.C. In *The Receptors*, Academic Press: Orlando, FL, **1985,** *2*, 131.
[14] http://www-micro.msb.le.ac.uk/3035/Orthomyxoviruses.html
[15] Winn, W.C.; Westenfeld, F.W. *New Eng. J. Med.* **1995,** *333*, 912.
[16] Pinto, L.H.; Holsinger, L.J.; Lamb, R.A. *Cell* **1992,** *69,* 517.
[17] Laver, W.G.; Valentine, R.C. *Virology* **1969,** *38*, 105.
[18] Varghese, J.N.; Colman, P.M. *J. Molec. Biol.* **1991,** *221*, 473.
[19] Colman, P.M.; Varghese, J.N.; Laver, W.G... *Nature* **1983,** *303,* 41.
[20] Varghese, J.N.; Colman, P.M. *J. Molec. Biol.* **1991,** *221*, 473.
[21] Schauer, R. *Cell Biology Monographs* **1982,** *10*, 53.
[22] Liu, C.G.; Eichelberger, M.C.; Compans, R.W.; Air, G.M. *J. Virol* **1995,** *69*, 1099.
[23] Herrler, G., Hausmann, J.; Klenk, H.D. *Plenum Press, New York* **1995,** *3,* 315.
[24] Wagner. R; Matrosovich, M.; Klwnk, H.D. *Rev. Med. Virol.* **2002**, *12,* 159.
[25] Chong, A.K.J.; Pegg, M.S.; Taylor, N.R.; von Itzstein, M. *Eur. J. Biochem.* **1992,***207,* 335.
[26] Chong, A.K.J.; Pegg, M.S.; Taylor, N.R.; von Itzstein, M. *Eur. J. Biochem.* **1992,***207,* 335.
[27] Edmond, J.D.; Johnston, R.G.; Kidd, D.; Rylance, H.J.; Sommerville, R.G. *Br. J. Pharmac. Chemother.* **1966,** *27,* 415.
[28] Air, G.M.; Ghate, A.A.; Stray, S.J. *Adv. Virus Res.* **1999**, *54,* 375.
[29] Blix, G.; Svennerholm, L.; Werner, I. *Acta Chem. Scand.* **1952**, *6,* 358.
[30] Holzer, C. T.; von Itzstein, M.; Jin, B.; Pegg, M. S.; Stewart, W. P.; Wu, W. Y. *Glycoconjugate J.* **1993**, *10,* 40.
[31] Rosenberg, A.; Schengrund, C.L., Eds. *Plenum Press: New York,* **1976**.
[32] Krizanova, O.; Rathova, V. S. *Curr Top Microbiol. Immunol.* **1969**, *47,* 125.
[33] Edmond, J.D.; Johnston, R.G.; Kidd, D.; Rylance, H.J.; Sommerville, R.G. *Bri J Pharmacol. Chemother.* **1966**, *27,* 415.
[34] Meindl, P.; Tuppy, H. *Monatsh. Chem.* **1969**, *100,* 1295.
[35] Varghese, J.N.; Laver, W.G.; Colman, P.M. *Nature* **1983**, *303,* 35.
[36] Vincent, S.; Kent D.; Stewart, C.J.; Maring, S.M.; Vincent G.; Yu-gui G.; Gary W.; Yuanwei C.; Minghua S.; Chen Z.; April L.; Kennedy, D.L.; Madigan, Y.X.; Ayda S.; Warren K.; Graeme L.; Thomas S.; Hing L.S.; Jonathan, G.; Dale K. *Biochemistry* **2003**, *42*, 718.
[37] Bossart, W.P.; Carson, M.; Babu, Y.S.; Smith, C.D.; Laver, W.G.; Air, G.M. *J. Mol. Biol.* **1993**, *232,* 1069.
[38] Smith, P.W.; Sollis, S.L.; Howes, P.D.; Cherry, P.C.; Cobley, K.N.; Taylor, H.; Whittington, A.R.; Scicinski, J.; Bethell, R.C.; Taylor, N.; Skarzynski, T.; Cleasby, A.; Singh, O.; Wonacott, A.; Varghese, J.; Colman, P. *Bioorg. Med. Chem. Lett.* **1996**, *6,* 2931.
[39] Palese, P.; Schulman, J.L. *Edited by J.S. Oxford. CRC Press,* **1977**,*19,* 189.
[40] Colman, P.M.; Varghese, J.N.; Laver, W.G. *Nature* **1983**, *303,* 41.
[41] Meindl, P.G.; Bodo, P.; Palese, J.S.; Tuppy, H. *Virology* **1974**, *58*, 457.
[42] McNicholl, I.R.; McNicholl, J.J. *Ann. Pharmacother.* **2001**, *35(1),* 57.
[43] Lew, W.; Chen, X.; Kim, C.U. *Curr Med Chem.* **2000**, *7*(6), 663.
[44] Miler, C.A.; Wang, P.; Flasner, M. *Biochem. Biophys. Res. Commun.* **1978**, *83,* 1479.
[45] Silvana, C.; von Itzstein, M. *Tetrahedron Lett.* **1995**, *36*(30), 5405.
[46] Scheigetz, J.; Zamboni, R.; Bernstein, M.A.; Roy B. *Organic Preparations and Procedures International* **1995**, *27*(6), 637.
[47] Smith, P.W.; Sollis, S.L.; Howes, P.D.; Cherry, P.C.; Starkey, I.D.; Cobley, K.N.; Weston, H.; Scicinski, J.; Merritt, A.; Whittington, A.; Wyatt, P.; Taylor, N.; Green, D.; Bethell, R.; Madar, S.; Fenton, R.J; Morley, P.J,; Patemanr, T.; Beresfordr, A. *J. Med. Chem.* **1998**, *41,* 787.
[48] Smith, P.W.; Robinson, J.E.; Evans, D.N.; Sollis, S.L.; Howes, P.D.; Trivedi, N.; Bethell, R.C. *Bioorg. Med. Chem. Lett.* **1999**, *9,* 601-604.
[49] Taylor, N.R.; Cleasby, A.; Singh, O.; Skarzynski, T.; Wonacott, A.J.; Smith, P.W.; Sollis, S.L.; Howes, P.D.; Cherry, P.C.; Bethell, R.; Colman, P.; Varghese, J. *J. Med. Chem.* **1998**, *41,* 798.
[50] Andrews, D.M.; Cherry, P.C.; Humber, D.C.; Jones, P.S.; Keeling, S.P.; Martin, P.F.; Shaw, C.D.; Swanson, S. *Eur. J. Med. Chem.* **1999**, *34,* 563.
[51] Wyatt, P.G.; Coomber, B.A.; Evans, D.N.; Jack, T.I.; Fulton, H.E.; Wonacott, A.J.; Colman, P.; Varghese, J. *Bioorg. Med. Chem. Lett.* **2001**, *11,* 669.
[52] Smith, P.W.; Sollis, S.L.; Howes, P.D.; Cherry, P.C.; Starkey, I.D.; Cobley, K.N.; Weston, H.; Scicinski, J.; Merritt, A.; Whittington, A.; Wyatt, P.; Taylor, N.; Green, D.; Bethell, R.; Madar, S.; Fenton, R.J.; Morley, P.J.; Patemanr, T.; Beresfordr, A. *J. Med. Chem*. **1998**, *41,* 787.
[53] Honda, T.; Masuda, T.; Yoshida, S.; Arai, M.; Kobayashi, Y.; Yamashita, M. *Bioorg. Med. Chem. Lett.* **2002**, *12,* 1921.
[54] Honda, T.; Masuda, T.; Yoshida, S.; Arai, M.; Kobayashi, Y.; Yamashita, M. *Bioorg. Med. Chem. Lett.* **2002**, *12,* 1921.
[55] Masuda, T.; Shibuya, S.; Arai, M.; Yoshida, S.; Tomozawa, T.; Ohno, A.; Yamashita, M.; Honda, T. *Bioorg. Med. Chem. Lett.* **2003**, *13,* 669.
[56] Jedrzejas, M.J.; Singh, S.; Brouillette, W.J.; Laver, W.G.; Air, G.M.; Luo, M. *Biochemistry* **1995**, *34,* 3144.
[57] Singh, S.; Jedrzejas, M.J.; Air, G.M.; Luo, M.; Laver, W.G.; Brouillette, W. *J. Med. Chem.* **1995**, *38,* 3217.
[58] Williams, M.; Bischofberger, N.; Swaminathan, S.; Kim, C.U.. *Bioorg Med Chem. Lett.* **1995**, *5(19),* 2251.

[59] von Itzstein M.; Wu, W.Y.; Kok, G.B.; Pegg, M.S.; Dyason, J.C.; Jin, B.; Phan, T.V.; Smythe, M.L.; White, H.F.; Oliver, S.W.; Colman, P.M.; Varghese, J.N.; Ryan, D.M.; Wood, J.M.; Bethell, R.C.; Hotman, V.J.; Cameron, J.M.; Penn, C.R. *Nature* **1993**, *363,* 418.

[60] Chand, P.; Babu, Y.S.; Bantia, S.; Chu, N.M.; Cole, L.B.; Kotian, P.L.; Laver, W.G.; Montgomery, J.A.; Pathak, V.P.; Petty, S.L.; Shrout, D.P.; Walsh, D.A.; Walsh, G.M. *J. Med. Chem.* **1997**, *40 (25),* 4030.

[61] Atigadda, V.R.; Brouillette, W.J.; Duarte, F.; Ali, S.M.; Babu, Y.S.; Bantia, S.; Chand, P.; Chu, N.M.; Montgaomery, J.A.; Walsh, D.A.; Sudbeck, E.A.; Finley, J.; Luo, M.; Air, G.M.; Laver, G.W. *J. Med. Chem.* **1999**, *42(13),* 2332.

[62] Mauldin, S.C.; Hoornback, W.J.; Munroe, J.E. *J. Chem. Soc. Perkin Trans. 1* **2001,***15,* 1554.

[63] Woods, J.M.; Bethell, R.C.; Coates, J.A.; Healy, N.; Hiscox, S.A.; Pearson, B.A.; Ryan, D.M.; Ticehurst, J.; Tilling, J.; Walcott, S.M.; Penn, C.R. *Antimicrob. Agents Chemother.* **1993**, *37,* 1473.

[64] Brouillette, W.J.; Bajpai, S.N.; Ali, S.M.; Velu, S.E.; Atigadda, V.R.; Lommer, B.S.; Finley, J.B.; Luo, M.; Air, G.M. *Bioorg. Med. Chem.* **2003**, *11,* 2739.

[65] Chand, P.; Kotian, P.L.; Morris, P.E.; Bantia, S.; Walsh, D.A.; Babu, Y.S. *Bioorg Med. Chem.* **2005**, *13,* 2665.

[66] Chandler, M.; Cooper, W.J.; Lamont, R.B.; Scicinski, J.J.; Smart, J.E.; Storer, R.; Weir, N.G.; Wilson, R.D.; Wyatt, P.G. *J. Chem. Soc. Perkin Trans. 1.* **1995**, *9,* 1189.

[67] Kim, C.U.; Lew, W.; Williams, M.A.; Liu, H.T.; Zhang, L.J.; Swaminathan, S.; Bischofberger, N.; Chen, M.S.; Mendel, D.B.; Tai, C.Y.; Laver, W.G.; Stevens, R.C. *J Am. Chem. Soc.* **1997**, *119*(4), 681.

[68] Bartlett, P. A. *Stud. Org. Chem.* **1985**, *20,* 439.

[69] Kim, C.U.; Lew, W.; Williams, M.A.; Wu, H.W.; Zhang, L.J.; Chen, X.W.; Escarpe, P.A.; Mendel, D.B.; Laver, W.G.; Stevens, R.C. *J. Med. Chem.* **1998**, *41*(14), 2451.

[70] Hanessian, S.; Wang, J.C.; Montgomery, D.; Stoll, V.; Stewart, K.D.; Kati, W.; Maring, C.; Kempf, D.; Hutchins, C.; Laver, W.G. *Bioorg. Med. Chem. Lett.* **2002**, *12,* 3425.

[71] Hochgurtel, M.; Biesinger, R.; Kroth, H.; Piecha, D.; Hofman, M.W.; Krause, S.; Schaaf, O.; Nicolau, C.; Eliseev, A.V. *J. Med. Chem.* **2003**, *46*(3), 356.

[72] Lew, W.; Chen, X.W.; Kim, C.U. *Curr. Med. Chem.* **2000**, *7,* 663.

[73] Yamamoto, T.; Kumazawa, H.; Inami, K.; Teshima, T.; Shiba, T. *Tetrahedron Lett.* **1992**, *33*(39), 5791.

[74] Wang, G.T.; Wang, S.; Chen, Y.W.; Genties, R.; Sowin, T. *J. Org. Chem.* **2001**, *66*(6), 2052.

[75] Wang, G.T.; Wang, S.; Gentles, R.; Sowin, T.; Maring, C.J.; Kempf, D.J.; Kati, W.M.; Stoll, V.; Stewart, K.D.; Laver, G. *Bioorg. Med. Chem. Lett.* **2005**, *15,* 125.

[76] Von Itzstein, M.; Wu, W.Y.; Kok, G.B.; Pegg, M.S.; Dyason, J.C.; Phan, T.V.; Smythe, M.L.; White, H.F.; Oliver, S.W.; Colman, P.M.; Varghese, J.N.; Ryan, D.M.; Woods, J.M.; Bethell, R.C.; Hotham, V.J.; Cameron, J.M.; Penn, C.R. *Nature* **1993**, *363,* 418.

[77] Babu, Y.S.; Chand, P.; Bantia, S.; Kotian, P.; Dehghani, A.; El-Kattan, Y.; Lin, T.H.; Hutchison, T.L.; Elliott, A.J.; Parker, C.D.; Ananth, S.L.; Horn, L.L.; Laver, G.M.; Montgomery, J.A. *J. Med. Chem.* **2000**, *43*(19), 3482.

[78] Chand, P.; Kotian, P.L.; Dehghani, A.; El-Kattan, Y.; Lin, T.H.; Hutchison, T.L.; Babu, Y.S.; Bantia, S.; Elliiott, A.J.; Montgomery, J.A. *J. Med. Chem.* **2001**, *44*(25), 4378.

[79] Young, D.; Fowler, C.; Bush, K. *Phil. Trans. R. Soc. Land. B* **2001**, *356,* 1905.

[80] Chand, P.; Babu, Y.S.; Bantia, S.; Rowland, S.; Dehghani, A.; Kotian, P.L.; Hutchison, T.L.; Ali, S.; Brouillette, W.; El-Kattan, Y.; Lin, T.H. *J. Med. Chem.* **2004**, *47*(8), 1919.

[81] Wang, G.T.;, Chen, Y.W.; Wang, S.; Gentles, R.; Sowin, T.; Kati, W.; Muchmore, S.; Giranda, V.; Stewart, K.; Sham, H.; Kempf, D.; Laver, W.G. *J. Med. Chem.* **2001**, *44*(8), 1192.

[82] Grennel, S.J.; Garman, E.F.; Phiilppon, C.; Vasells, A.; Laver, W.G.; Vimr, E.R.; Talor, G.L. *J. Mol. Biol.* **1996**, *259,* 262.

[83] Hanessian, S.; Bayrakdarian, M.; Luo, X.L. *J. Am. Chem. Soc.* **2002**, *124*(17), 4716.

[84] Degety, D.A.; Chen, H.J.; Flost, W.J.; Grampovnik, D.J.; Yeung, C.M.; Kiein, L.L.; Kempf, D.J. *J. Org. Chem.* **2002**, *67*(16), 5445.

[85] Kati, W.M.; Montgomery, D.; Carrick, R.; Gubareva, L.; Maring, C.; McDaniel, K.; Steffy, K.; Molla, A.; Hayden, F.; Kempf, D.; Kohlbrenner, W. *Antimicrob. Agents Chemother.* **2002**, *46*(4), 1014.

[86] Honda, T.; Yoshida, S.; Arai, M.; Masuda, T.; Yamashita, M. *Bioorg. Med. Chem. Lett.* **2002**, *12,* 1929.

[87] Masuda, T.; Yoshida, S.; Arai, M.; Kaneko, S.; Yamashita, M.; Honda, T. *Chem. Pharm. Bull.* **2003**, *51*(12)*,* 1386.

[88] Watson, K.G.; Cameron, R.; Fenton, R.J.; Gower, D.; Hamilton, S.; Jin, B.; Krippner, G.Y.; Luttick, A.; McConnell, D.; MacDonald, Simon J.F.; Mason, A.M.; Nguyen, V.; Tucker, S.P.; Wu, W.Y. *Bioorg Med. Chem. Lett.* **2004**, *14,* 1589.

[89] Shitara, E.; Nishimura, Y.; Nerome, K.; Hiramoto, Y.; Takeuchi, T. *Org. Lett.* **2000**, *2*(24), 3837.

[90] Kati, W.M.; Montgomery, D.; Maring, C.; Stoll, V.S.; Giranda, V.; Chen, X.Q.; Laver, W.G.; Kohlbrenner, W.; Norbeck, D.W. *Antimicrob. Agents Chemother.* **2001**, *45*(9), 2563.

[91] Harrington, P.J.; Brown, J.D.; Foderaro, T.; Hughes, R.C. *Organic Process Research & Development* **2004**, *8*(1), 86.

[92] Chand, P.; Kotian, P.L.; Morris, P.E.; Bantia, S.; Walsh, D.A.; Babu, Y.S. *Bioorg. Med. Chem.* **2005**, *13,* 2665.

[93] Macdonald, S.J.F.; Cameron, R.; Demaine, D.A.; Fenton, R.J.; Foster, G.; Gower, D.; Hamblin J.N.; Hamilton, S.; Hart, G.J.; Hill, A.P.; Inglis, G.A.; Jin, B.; Jones, H.T.; McConnel, D.B.; McKimm-Breschkin, J.; Mills, O.G.; Van, N.; Owens, I.J.; Parry, N.; Shanahan, S.E.; Smith, D.; Watson, K.G.; Wu, W.Y.; Tucker, S.P. *J. Med. Chem.* **2005***, 48*(8), 2964.

[94] Macdonald, S.J.F.; Watson, K.G.; Cameron, R.; Chalmers, D.K.; Demaine, D.A.; Fenton, R.J.; Gower, D.; Hamblin, J.N.; Hamilton, S.; Hart, G.J.; Inglis, G.A.; Jin, B.; Jones, H.T.; McConnell, D.B.; Mason, A.M.; Nguyen, V.; Owens, I.J.; Parry, N.; Reece, P.A.; Shanahan, S.E.; Smith, D.; Wu, W.Y.; Tucker, S.P. *Antimicrob. Agents Chemother.* **2004***, 48,* 4542.

[95] McKimm-Breschkin J.L. *Antiviral Res.* **2000**, *47,* 1.

[96] Gubareva, L.V. *Virus Res.* **2004**, *103,* 199.

[97] Colman, P.M. *Protein Sci.* **1994***, 3,* 1687.

[98] Smith, B.J.; McKimm-Breshkin, J.L.; McDonald, M.; Femley, R.T.; Varghese, J.N.; Colman, P.M. *J. Med. Chem.* **2002**, *45*(11), 2207.

[99] Colman, P.M. *Vaccine* **2002***, 20*, S55.

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