SAS-8312 A NOVEL NEURAMINIDASE INHIBITOR OF CHAIN A OF INFLUENZA VIRUS B/Beijing/1/87

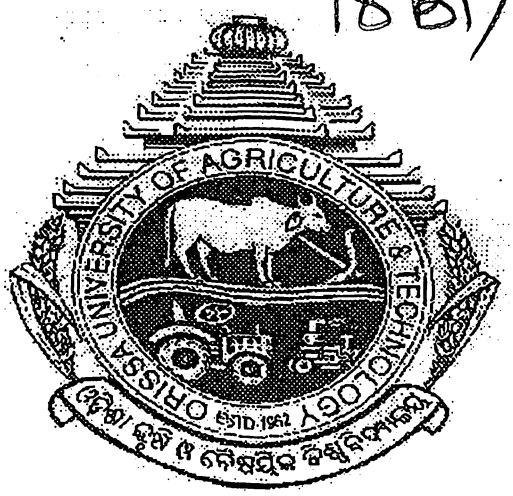
Project report submitted for partial fulfillment of Master degree in Bioinformatics under Dr. R. SARAVANAN, Chairman, Biomedical Engeneering Research Foundation, Salem, Tamilnadu.

TO

Orissa University Of Agriculture and Technology, Bhubaneswar, Orissa.

By

Ms. Sasmita Panda



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BIOMEDICAL ENGINEERING RESEARCH FOUNDATION

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This is to certify that **Ms. Sasmita Panda**, has carried out a major research work entitled "SAS-8312 a novel neuraminidase inhibitor of chain A of Influenza virus B/Beijing/1/87-Neuraminidase" as her project work towards the partial fulfillment of her M.Sc. Bioinformatics degree from, Orissa University of Agriculture and Technology, Bhubaneswar, Orissa,India.

The candidate executed the research work individually with diligence and endurance. It is further stated that this piece of research work has not formed the basis of award of any other diploma or degree. Due mention should be made for the research institute during the reproduction of this project work in any publication or future studies. The institute wishes her the best in all walks of her life.

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CERTIFICATE

This is to certify that the thesis entitled "*SAS-8312 A NOVEL NEURAMINIDASE INHIBITOR OF CHAIN A OF INFLUENZA VIRUS B/Beijing/1/87*" submitted by **Ms. Sasmita Panda,** Orissa University of Agriculture and Technology, Bhubaneswar, in the partial fulfillment of the requirements of the Degree of Masters of Science in Bioinformatics, has been approved /disapproved by students advisory committee after an oral examination of the same in collaboration with the external examiner.

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I here by declare that the dissertation entitled "**SAS -8312 a novel neuraminidase inhibitor of chain A of Influenza virus B/Beijing/1/87 Neuraminidase**" was submitted to the **Orissa University of Agriculture and Technology** in partial fulfillment of the requirements for the award of the degree of Master of Science Bioinformatics is a record of original research work done by me under the supervision and guidance of **Dr. R. Saravanan**, Chairman, Biomedical Engineering Research foundation, Salem. The dissertation has not formed the basis for the award of any Degree/Diploma/Associate ship/Fellowship or similar title to any candidate of any university.

Date: 04/10/2005

Place: Salem

Sasmita Panda

Signature of the candidate

Dedicated

to

my sister

For her unswerving love.

ACKNOWLEDGEMENT

I acknowledge with thanks to everyone who helped me for completing this project. I thank my Parents successfully for the support and encouragement they have forwarded to me.

This project has given me great joy in working and solving interesting problems. I owe the success of this project to all the people who have directly or indirectly helped me in this project. Right from the beginning of this project, our work was set with an attitude that this work is going to be of great importance to the scientific community because of its novel bioinformatics approach.

I would like to convey my heartfelt thanks to my guide, **Dr. R. Saravanan,** who has served as a constant source of inspiration and motivation which made me to complete this work as a successful one. And provided me with all the resources to complete this work with in time. No word would justify their genuine interest in the shaping of this project.

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ABSTRACT:

Neuraminidase is a surface glycoprotein of influenza viruses that cleaves terminal sialic acids from carbohydrates. It is critical for viral release from infected cells and facilitates viral spread in respiratory tract. The catalytic active site of neuraminidase is highly conserved in all type A and B influenza viruses, making it an excellent target for anti influenza drug design. Many regions of neuraminidase are likewise able to mutate (drift). Several drugs are invented to inhibit the action of this enzyme. We designed seven neuraminidase inhibitor drugs from which (SAS-8312) identified a novel neuraminidase inhibitor of A chain of influenza virus B/Beijing/1/87 neuraminidase.SAS-8312(5-(acetylamino)-3-(aminocarbonyl)-4 {[amino(imino)methyl]amino}-6-(1,2,3-trihydroxypropyl)tetrahydro-2*H*-pyran-2-carboxylic acid. It contains one –COOH, one –NHCOCH3, one –CONH2, one 1-methylguanidine and butane-1, 2, 3-triol groups. It is having molecular weight (377.35042). Energy minimization and docking with ArgusLab, the interaction of the designed inhibitors with the binding site of neuraminidase indicates that it is an extended conformation and makes excessive contact with viral enzyme. SAS-8312 binds to ARG222, GLU274, GLU275 and ARG291 of A chain of influenza virus B/Beijing/1/87 Neuraminidase inhibitor active site. So design of novel SAS-8312 is in order to optimize inhibitory activity.

1. INTRODUCTION:

Respiratory illnesses are responsible for more than half of the all acure illnesses each year in the U.S.A.

The Orthomyxo viruses (Influenza viruses) are a major determinant of morbidity and mortality caused by respiratory disease and outbreaks of infection sometimes occur in worldwide epidemic. The mutability and high frequency of genetic ressortment characteristic of orthomyxo viruses and resultant genetic changes in the viral surface glycoproteins make influenza viruses formidable challenges for control efforts.

Influenza viruses are of three types.

- Influenza A: Affects both humans and animals.
- Influenza B: Affects only humans.
- Influenza C: causes only mild illness.[1]

1.1

	Type A	TYPE B	TYPE C
Severity of illness	++++	++	+
Animal reservoir	Yes	No	No
Human pandemics	Yes	No	No
Human epidemics	Yes	Yes	No(sporadic)
Antigenic changes	Shift, drift	Drift	Drift
Segmented genome	Yes	Yes	Yes
Amantadine,rimantadine	Sensitive	No effect	No effect
Zanamivir(Relenza)	Sensitive	Sensitive	
Surface glycoprotein	2	2	(1)

Table 1: Comparison of influenza A, B, and C

Antigenic drift:

Minor antigenic changes are called antigenic drift and it is due to point mutations in the gene.

Antigenic shift:

Major antigenic changes are called antigenic shift. It reflects drastic changes in the sequence of viral surface protein due to mutation.

The most important viral surface proteins:

The viral envelope contains Hem agglutinin (HA) and Neuraminidase (NA) proteins.(1)

1.2 Neuraminidase (NA):

Viral surface protein contains nine antigenic subtypes (N1-N15).

- It playa a role in penetration of mucus layer that surround the target cell and in release of virus from the surface f the infected cell.
- It splits polysaccharides that are present on the surface and in mucus.
- Many regions of neuraminidase are likewise able to mutate (drift).
- Viral particle contains 100-250 copies of it.**(2)**

1.3 NEURAMINIDASE INHIBITORS:

Rationally designed NA inhibitors that block the viral life cycle. NA inhibitors effective against all influenza types.

1. Zanamivir:
2. Oseltamivir
3. Peramivir

N/mol of drug, which inhibits activity by 50%

	N1	N2	B
Zanamivir	0.5-2.5	0.9-5.6	1.0-7.9
Oseltamivir	0.3-1.0	0.2-0.8	1.7-18.3
Peramivir	0.2-1.4	0.5-0.9	0.6-11.0

Table 2: Inhibition of influenza A and B virus NAs by the NIs

1.4 SCIENTIFIC DEVELOPMENT OF THE ANTI-NA DRUGS:

Neuraminidase and its substrate sialic acid as a target for chemical inhibitors. The original neuraminic acid analogues were carefully investigated for anti-influenza effects on viral replication in cells. These drug transition state analogues were active at micro molar levels, reduced plaque size, caused virus to a mass at the budding stage, but disappointingly after intraperitoneal administration had no effect in models of viral infection in the lung of a mouse.

- Neu5Ac2en was a dehydrated neuraminidase acid derivative that mimicked the geometry of the transition state during the enzymatic reaction. Taking Neu5ac2en as the basic inhibitor a guanidinyl group was substituted for a hydroxyl carbon atom to make zanamivir (Relenza)
- A cyclohexene ring and replaced a polar glycerol with lipophilic side chains this is the drug. Oseltamivir (Tamiflu) The bioavailable drug is an ethyl ester that is converted into the active carboxylate by esterases in the liver.
- A cyclopentane derivative with a guanidinyl group and lipophilic chains this drug is RWJ-270201 (also known as BCX-1812 or peramivir). All three drugs (zanamivir, oseltamivir and peramivir) were shown to be powerful inhibitors of influenza A and B virus NAs. All nine influenza A NA subtypes are inhibited.

1.5 PHARMACOKINETICS AND CLINICAL SAFETY OF NIS:

Zanamivir and oseltamivir have proven to be safe drugs and have been administered around the world in hundreds of thousands of doses. Zanamivir has been exacerbation of reactive airways disease. Oseltamivir causes some upper gastrointestinal effects such as nausea and these effects are noted after the first dose and usually resolve after 24–48 h. Both zanamivir and oseltamivir deliver high levels of the active inhibitor in the respiratory tree of humans.[3]

2. REVIEW OF LITERATURE:

The complete coding sequence of neuraminidase gene of influenza virus was determined in 1918. The complete sequence was generated from A/Brevig Mission /1/18, with confirmatory sequencing carried out on A/south Carolina/1/18 and A/New york/1/18. The gene sequence was compared with N1 and N9 sub types. Phylogenetically, the 1918 neuraminidase gene appears to be intermediate between mammals and birds. (Ann H.Reid *et.al.*, 2000)

The three dimensional X-ray structure of a complex of the potent neuraminidase inhibitor 4-guanidino-Neu5Ac2en and influenza virus neuramindase (N9) has been obtained utilizing diffraction data 1.8Å resolution. The 4-guanidino-Neu5Ac2en group of the inhibitor is strongly conserved and is basis for all strains of influenza. Different solvent structure in the active site may vary in the affinities of inhibitors to different subtypes of influenza (J.N. VARGHESE *et.al.*, 1995)

Crystal structure of enzymatically active head of influenza B virus neuraminidase has been refined to a crystallographic R-factor of 14.8% at 2.2Å resolution and its complex with sialic acid refined at 2.8Å resolution. The overall fold of the molecule I similar to structure NA from influenza A. Two calcium binding site, one is located between active site and large surface of the antigenic loop. The calcium ion is octahedrally coordinated by 5 oxygen from the protein and one water molecule. Sequence comparison suggests that calcium site should occur in all influenza A and B virus neuraminidase. Soaking of sialic acid into the crystals has enabled the mode of binding of the reaction product in the putative active site of pocket to be revealed. (WP Burmeister *et.al*)

The sialidase (NA) inhibitor 4-guanidino-2, 4-dideoxy-2, 3-dehydro-Negative-acetylneuraminic acid (4-guanidino-Neu5Ac2en) has been examined to inhibit replication of influenza A and B at lower concentration. 4-guanidino-Neu5Ac2en was potent inhibitor of all influenza virus with 50% inhibitory concentration ranging from 0.00064 to 0.0079μM.No toxicity was observed at 10mM.. 4-guanidino-Neu5Ac2en ia new potent inhibitor of Influenza A and B virus sialidase activity and replication *in vitro* (JM Woods *et.al.*, 1993)

Sensitivity molecular docking GOLD is used to induce fit effects in influenza virus neuraminidase. The lowest energy structure was correctly docked (i.e., RMSD<1.5Å away from the crystal refine structure) in 84% of proteins and the most promiscuous protein (1mWe) was able to dock all 15 ligands. Overall, GOLD has shown itself to be an extremely good, robust docking program for this system (Birch L *et.al*, 2002)

An influenza B virus plasmid based rescue system was used to introduce site specific mutations, previously observed in NA inhibitor resistant virus into the NA protein of six recombinant viruses. Mutants were introduced into B/Beijing/1/87 virus neuraminidase protein to change residue E116 to glycine, alanine or aspartic acid. Mutant viruses displayed increased resistance to zanamivir, Oseltamivir and Peramivir, with certain viruses displaying cross-resistance to all three drugs. (Jackson d *et.al.*, 2005)

Zanamivir and Oseltamivir block influenza neuraminidase and prevent cleavage site of sialic acid residues. To minimize the development of resistant, the neuraminidase inhibitors represent a new and unique class of anti influenza

agents that can potentially reduce the morbidity associated with influenza. (MC Nicholl IR, *et.al.*, 2001)

The use of 3D structures of neuraminidase inhibitor complexes to derive QSARs to understand the mechanism of the inhibition and the discovery of new drugs. Crystal structures of NA inhibitor complexes were used alongside modeled complexes to derive QSARs model by COMperetive BINding Energy (COMBINE) analysis. The QSAR model provides guidelines for structural modification of current inhibitor and design of novel inhibitor in order to optimize inhibitory activity.(Ting Wang *et.al.*,2001)

Oseltamivir is an ethyl pro drug of Ro 64-0802, a selective inhibitor of influenza virus NA. Oral administration of oseltamivir delivers the active antiviral Ro 64-0802 to blood. Oseltamivir is a prodrug of oseltamivir carboxylate (Ro 64-0802, GS 4071) a potent and selective inhibitor of Na glyco protein essential for replication of influenza A and B virus (He G *et.al.*, 1999).

Structure based design has led to the synthesis of a novel analogue of GS-4071, an influenza neuraminidase inhibitor, in which the basic amino group has been replaced by hydrophobic vinyl group. An X-ray co crystal structure of the new inhibitor (K(I)=45nM) bound to the active site shows that the vinyl group occupies the same sub site as the amino group in GS-4071(Hanessian S,*et.al.*,2003)

Cyclopentane derivatives, designated as BCX-1812, BCX-1827, BCX-1898, and BCX-1923, were tested in parallel with oseltamivir carboxylate and zanamivir for the in vivo activity in mice infected with A/Turkey/Mas/76 X

A/Beijing/32/92 (H6N2) influenza virus. On comparison with oseltamivir carboxylate and zanamivir, these four cyclopentane derivatives have shown equal or better efficacies. The synthesis of two new compounds, BCX-1898 and BCX-1923, is also described. (Chand P *et.al.*, 2005)

A reverse genetics study of resistance to neuraminidase inhibitors in an influenza A/H1N1 virus. In NA inhibition assays, the His274Tyr mutant was resistant to oseltamivir (430-fold over wild-type) and BCX-1812 (50-fold) but was sensitive to zanamivir The Glu119Gln mutant expressed a low level of resistance to oseltamivir (nine-fold) and zanamivir (fourfold) in NA inhibition assay but was only marginally resistant to oseltamivir (fourfold) in PRA. The replication capacity of both mutants, in particular that of the His274Tyr virus, was impaired when compared with the wild-type virus in vitro. (Abed Y *et.al.*, 2004)

A total of 126 influenza B isolates were tested for their sensitivity to the neuraminidase (NA) inhibitor drugs zanamivir and oseltamivir carboxylate using a fluorescence-based enzyme assay. Sequence analysis of the haemagglutinin HA1 region and the neuraminidase gene of B/Perth/211/2001 revealed no amino acid changes in sites that have previously been reported to confer resistance to either of the NAI drugs. (Hurt AC *et.al.*,2004)

Experiments were run to determine the effect of oral gavage treatment with the cyclopentane influenza virus neuraminidase inhibitor peramivir (BCX-1812, RWJ-270201) in influenza A (H1N1) virus-infected mice that had their immune system suppressed by cyclophosphamide (CP) therapy or in severe combined immune deficient (SCID) mice These data indicate that peramivir

may have potential for treatment of influenza virus-infected immunosuppressed patients. (SidWell RW *et al.,* 2003).

Stereoselective total synthesis of racemic BCX-1812 (RWJ-270201) for the development of neuraminidase inhibitors as anti-influenza agents. . In addition, the size of the core ring can be varied depending on the size of the diene used for the preparation of the key cycloadduct 10 using an acylnitroso-based hetero-Diels-Alder reaction. Elaboration of 10 to methyl ester 14 followed by a precedented [3+2] dipolar cycloaddition gave bicyclic isoxazoline 17 in a regio- and stereoselective fashion. Incorporation of the peripheral guanidino group and subsequent deprotection provided the target molecule (MinenoT*etal.*,2003)

A point mutation in influenza B neuraminidase confers resistance to peramivir and loss of slow binding. Whereas the wild type (WT) virus was inhibited by peramivir with an EC(50) value of 0.10 microM, virus isolated at passages 3 and 15 displayed EC(50) values of 10 and >50 microM respectively. Passage 3 virus contained 3 hemagglutinin (HA) mutations, but no NA mutation. Passage 15 (P15R) virus contained an additional 3 HA mutations, plus the NA mutation His273Tyr. The mechanism of inhibition of WT and P15R NA displayed IC(50) values of 8.4+/-0.4 and 127+/-16 nM, respectively, for peramivir. . Peramivir inhibited the WT enzyme in a time-dependent fashion, with a K(i) value of 0.066+/-0.002nM the P15R enzyme did not display the property of slow binding and was inhibited competitively with a K(i) value of 4.69+/-0.44nM. Molecular modeling suggested that His273 was relatively distant from peramivir (>5A) in the NA active site, but that Tyr273 introduced a repulsive

interaction between the enzyme and inhibitor, which may have been responsible for peramivir resistance.(Baum EZ *et al.*,2003)

A-315675 is a novel influenza virus NA inhibitor that has potent enzyme activity and is highly active in cell culture against a variety of strains of influenza A and B viruses. To further assess the therapeutic potential of this compound, in vitro resistance studies have been conducted and a comparative assessment has been made relative to oseltamivir carboxylate Passage experiments with A-315675 identified a variant at passage 8 that was 60-fold less susceptible to the compound. Sequencing of the viral population identified an E119D mutation in the NA gene, but no mutations were observed in the hemagglutinin (HA) gene. However, by passage 10 (2.56 μM A-315675), two mutations (R233K, S339P) in the HA gene appeared in addition to the E119D mutation in the NA gene, resulting in a 310-fold-lower susceptibility to A-315675This P15 virus displayed 355-fold-lower susceptibility to A-315675 and >175-fold-lower susceptibility to zanamivir than did wild-type virus, but it retained a high degree of susceptibility to oseltamivir carboxylate). This suggests that cross-resistance between A-315675- and oseltamivir carboxylate-selected variants in vitro is minimal.(Akhteruzzaman Molla *et al.*,2002)

A new inhibitor under development by Biocryst Pharmaceuticals, BCX-1812, has both a guanidino group, as in zanamivir, and a bulky hydrophobic group, as in oseltamivir. The X-ray crystal structures of the complexes of BCX-1812 with the wild type and the two mutant neuraminidases were determined. The ligand is bound in an identical manner in each structure, with a rearrangement of the side chain of E276 from its ligand-free position. A structural explanation

of the mechanism of resistance of BCX-1812, relative to zanamivir and oseltamivir in particular, is provided.(Smith BJ *et al.*,2002)

The increasing use of influenza virus neuraminidase (NA) inhibitors (NIs) necessitates the development of reliable methods for assessing the NI susceptibility of clinical isolates We evaluated three NA inhibition assays against a panel of five clinical isolates each of influenza virus A/H1N1, A/H3N2, and B strains and four viruses with a defined resistance genotype (R292K, H274Y, R152K, and E119V). Four different operators repeated the assays several times in a blinded fashion with both zanamivir and oseltamivir carboxylate (GS4071) to determine intra- and interassay variations. Mixing experiments, whereby increasing fractions (0, 20, 40, 60, 80, and 100%) of NA from a known NI-resistant virus were mixed with the corresponding NI-sensitive parental NA, indicated that the resolution of IC_{50} values was clearer with the CL assay than with FA-2 for two of the resistant variants (R152K and E119V). The FA and CL methods were reliable for the detection of NI resistance(N. T. Wetherall,[1] *et al.*,2003)

A-315675 is a novel, pyrrolidine-based compound to inhibit A and B strain influenza virus neuraminidases in enzyme assays and influenza virus replication in cell culture. . A-315675 effectively inhibited influenza A N1, N2, and N9 and B strain neuraminidases with inhibitor constant (K(i)) values between 0.024 and 0.31 nM. These values were comparable to or lower than the K(i) values measured for oseltamivir carboxylate (GS4071), zanamivir, and BCX-1812, except for the N1 enzymes that were found to be the most sensitive to BCX-1812. A-315675 was found to be significantly more potent than oseltamivir carboxylate against the B strain isolates.(Kati WM,*et al.*,2002)

X-ray crystal structures of complexes of neuraminidase with known five- and six-membered ring inhibitors revealed that potent inhibition of the enzyme is determined by the relative positions of the interacting inhibitor substituents (carboxylate, glycerol, acetamido, hydroxyl) rather than by the absolute position of the central ring. scaffold for substituents (carboxylate, guanidino, acetamido, alkyl) that would interact with the four binding pockets of the neuraminidase active site at least as effectively as those of the established six-membered ring inhibitors such as DANA (2), zanamivir (3), and oseltamivir (4). A synthetic route to the identified candidate 50 was developed, which featured (3 + 2) cycloaddition of 2-ethylbutyronitrile oxide to methyl (1S,4R)-4[(tert-butoxycarbonyl)amino]cyclopent-2-ene-1-carboxylate (43). Two new neuraminidase inhibitors discovered in this work, 50 and 54, have IC(50) values vs neuraminidase from influenza A and B of <1 and <10 nM, respectively. The synthetic route used to prepare 50 and 54 was refined so that synthesis of pure active isomer 54, which has five chiral centers, required only seven steps from readily available intermediates. Further manipulation was required to prepare deoxy derivative 50. Because the activities of the two compounds are comparable and 54 [RWJ-270201 (BCX-1812)] is the easier to synthesize.(Chand P,*et al.*,2001)

Influenza A and B virus neuraminidases and a potent inhibitor of influenza A and B virus replication in cell culture The in vitro potency appears to be greater than either zanamivir or oseltamivir carboxylate based on the generally lower EC_{50} values seen using peramivir in studies run in parallel with each compound. Viruses with neuraminidase mutations are not necessarily all cross-resistant to peramivir, zanamivir and oseltamivir carboxylate oral treatment with peramivir significantly reduced nasal wash virus titres with no adverse effects(Donald F Smee *et al.*,2002)

Two known active influenza virus inhibitors, ribavirin and the novel cyclopentane influenza virus neuraminidase inhibitor (1S,2S,3R,4R)-3-[(1S)-(acetylamino)-2-ethylbutyl]-4-[(aminoiminomethyl)amino]-2-hydroxy-cyclopentanecarboxylic acid (RWJ-270201, BCX-1812) were studied. Mice were treated with ribavirin at 20 and 6.25 mg/kg/day combined with RWJ-270201 at 1, 0.32, or 0.1 mg/kg/day, or used alone. The combination of the two inhibitors produced additive to synergistic interactions in these mouse experiments with no enhancement of host toxicity. Treatment of influenza infections in the clinical setting may benefit by these two agents in combination (Donald F Smee *et al.*,2002)

Sialic acid analog 4-guanidino-Neu5Ac2en (GG167), an inhibitor of influenza virus neuraminidase. Four randomized, double-blind, placebo-controlled trials involving three prophylaxis limbs, two early treatment limbs, and one delayed treatment limb were conducted Intranasal GG167 was well tolerated for both prophylaxis and therapy. Direct respiratory administration of the selective neuraminidase inhibitor GG167 appears safe and effective for both prevention and early treatment of experimental influenza. Influenza virus neuraminidase is important for viral replication in humans.(F. G.Hayden *etal.*,1996)

The orally administered neuraminidase (NA) inhibitor RWJ-270201 was tested in parallel with zanamivir and oseltamivir against a panel of avian influenza viruses for inhibition of NA activity and replication in tissue culture. The agents were then tested for protection of mice against lethal H5N1 and H9N2 virus infection. RWJ-270201 inhibited the replication of avian influenza viruses of both Eurasian and American lineages in MDCK cells (50% effective concentration, 0.5 to 11.8 μM) RWJ-270201 is at least as effective as either

zanamivir or oseltamivir against avian influenza viruses and may be of potential clinical use for treatment of emerging influenza viruses that may be transmitted from birds to humans. (Elena A *et al.*,2001)

The cyclopentane influenza virus neuraminidase inhibitor RWJ-270201 was evaluated against influenza A/NWS/33 (H1N1), A/Shangdong/09/93 (H3N2), A/Victoria/3/75 (H3N2), and B/Hong Kong/05/72 virus infections in mice. RWJ-270201 was inhibitory to all infections using doses as low as 1 mg/kg/day. Maximum virus titer inhibition was seen on day 1, with RWJ-270201 exhibiting the greater inhibitory effect, a titer reduction of >104 cell culture 50% infective doses (CCID50)/g. By day 8, the lung virus titers in mice treated with RWJ-270201 had declined to 101.2 CCID50/g, whereas titers from oseltamivir-treated animals were >103 CCID50/g. RWJ-270201 was nontoxic at doses as high as 1,000 mg/kg/day. These data indicate potential for the oral use of RWJ-270201 in the treatment of influenza virus infections in humans.(Robert W *et al.*,2001)

Two hundred and forty-five human influenza A and B viruses isolated in Australia between 1996 and 2003 were tested for their sensitivity to the NA inhibitor drugs, zanamivir and oseltamivir using a fluorescence-based neuraminidase inhibition assay. Based on mean IC_{50} values, influenza A viruses (with neuraminidase subtypes N1 and N2) were more sensitive to both the NA inhibitors than were influenza B strains. Influenza A viruses with a N1 subtype and influenza B strains both demonstrated a greater sensitivity to zanamivir than to oseltamivir carboxylate, whereas influenza A strains with a N2 subtype were more susceptible to oseltamivir carboxylate. A comparison of IC_{50} values for viruses isolated before and after the release of the NA inhibitors in Australia, found there was no significant difference in the sensitivity of strains

to either neuraminidase inhibitor and none of the isolates tested showed clinically significant resistance.(*Aeron C Hurtlet al.,2003*)

Neuraminidase (NA) is one of the most important targets to screen the drugs of anti-influenza virus A and B. After virtual screening approaches were applied to a compound database which possesses more than 10000 compound structures, 160 compounds were selected for bioactivity assay, then a High Throughput Screening (HTS) model established for influenza virus NA inhibitors was applied to detect these compounds. Finally, three compounds among them displayed higher inhibitory activities, the range of their IC50 was from 0.1 ¦Ìmol/L to 3¦Ìmol/L. Their structural scaffolds are novel and different from those of NA inhibitors approved for influenza treatment, and will be useful for the design and research of new NA inhibitors. The resuit indicated that the combination of virtual screening with HTS was very significant to drug screening and drug discovery. (LIU Ailin et al.,2005)

A new inhibitor under development by Biocryst Pharmaceuticals, BCX-1812, has both a guanidino group, as in zanamivir, and a bulky hydrophobic group, as in oseltamivir. Using influenza A/NWS/Tern/Australia/G70C/75 (H1N9), neuraminidase variants E119G and R292K have previously been selected by different inhibitors. The sensitivity of these variants to BCX-1812 has now been measured and found in both cases to be intermediate between those of zanamivir and oseltamivir. In addition, the X-ray crystal structures of the complexes of BCX-1812 with the wild type and the two mutant neuraminidases were determined. The ligand is bound in an identical manner in each structure, with a rearrangement of the side chain of E276 from its ligand-free position. (Smith BJ)

Forty-two influenza A and 23 influenza B isolates collected from untreated subjects during the 1999-2000 influenza season in Canada were tested for their susceptibility to three neuraminidase inhibitors (zanamivir, oseltamivir carboxylate and RWJ-270201 or BCX-1812) using a chemiluminescent neuraminidase assay. Influenza B isolates were less susceptible than A viruses to all tested drugs. RWJ-270201 was the most potent drug against both influenza A(H3N2) (mean IC(50): 0.60 nM) and B (mean IC(50): 0.87 nM) viruses. Oseltamivir carboxylate was more active than zanamivir for influenza A(H3N2) isolates (mean IC(50): 0.73 vs. 2.09 nM) whereas it was less potent against B viruses (mean IC(50): 11.53 vs. 4.15 nM).(Boivin G)

Oseltamivir phosphate (Tamiflu, Ro 64-0796) is the first orally administered neuraminidase (NA) inhibitor approved for use in treatment and prevention of influenza virus infection in man. Oseltamivir phosphate is the pro-drug of the active metabolite oseltamivir carboxylate (Ro 64-0802). . The predominant mutation seen is the substitution of arginine for lysine at position 292 of the viral NA. The infectivity and replicative abilities of R292K mutant virus were reduced by at least 2 logs in a mouse model of influenza infection and by 2 and 4 logs, respectively, in the ferret model. Pathogenicity of R292K influenza virus A/Sydney/5/97 was reduced in ferrets as measured by inflammatory and febrile responses at least in parallel to the decrease in replicative ability. The data indicate that the R292K NA mutation compromises viral fitness such that virus carrying this mutation is unlikely to be of significant clinical consequence in man. (Carr J)

They report molecular dynamics calculations of neuraminidase in complex with an inhibitor, 4-amino-2-deoxy-2,3-didehydro-*N*-acetylneuraminic acid (N-DANA), with subsequent free energy analysis of binding by using a combined

molecular mechanics/continuum solvent model approach. Computational analysis indicates a major van der Waals component to the inhibitor-neuraminidase binding free energy. Based on the N-DANA/neuraminidase molecular dynamics trajectory, a perturbation methodology was used to predict the binding affinity of related neuraminidase inhibitors by using a force field/Poisson-Boltzmann potential. Mutation of the key ligand four-substituent to a hydrogen atom indicates no favorable binding free energy contribution of a hydroxyl group; conversely, cationic substituents form favorable electrostatic interactions with neuraminidase (Pascal Bonnet and Richard A *et al.*,2004)

The tolerability and pharmacokinetics of Ro 64-0802, a potent, selective inhibitor of influenza neuraminidase, and its oral prodrug oseltamivir were investigated in three double-blind, placebo-controlled studies. Measurable plasma concentrations of the active metabolite appeared rapidly in plasma and were significantly higher and longer lasting than those of oseltamivir. Pharmacokinetics of both compounds were linear.(JW Massarella *et al.*,2000)

The target for the drug is the active site of neuraminidase, which is a pocket that has been totally conserved in both Type A and B influenza in all known subtypes of influenza (animal and human). Mutations in residues that surround this conserved pocket allow the virus to escape binding to circulating antibodies that recognise the molecular surface around the active site of the wild-type virus. High-affinity neuraminidase inhibitors have been designed that interact only with the conserved active site residues. The design of these sialic acid analogues was based on the crystal structure of influenza virus neuraminidase and its complex with N-acetyl neuraminic acid (sialic acid) and 2-deoxy-2,3-dehydro-N-acetyl neuraminic acid. These novel inhibitors are highly specific for influenza neuraminidase, and have been shown to inhibit

influenza virus replication in both cell culture and animal models. The development of drugs against a rapidly mutating organism like influenza has to address to the possibility of emerging drug resistance. This is examined in the light of drug resistant mutants selected after in vitro passaging of virus in the presence of neuraminidase inhibitors. (Joseph N. Varghese *et al.,* 1999)

An influenza virus neuraminidase inhibitor, RWJ-270201 (BCX-1812), a novel cyclopentane derivative discovered through structure-based drug design. RWJ-270201 was more effective in inhibiting A/H1N1 and B strain neuraminidases than oseltamivir carboxylate. When oseltamivir carboxylate or RWJ-270201 binds to the active site of the neuraminidase, the 3-pentyl hydrophobic group occupies a region formed by a reorientation of the side chain of Glu 276. In the case of influenza B neuraminidase, it has been reported that the rearrangement of Glu 276 is energetically less favorable although both oseltamivir carboxylate and RWJ-270201 have hydrophobic groups. RWJ-270201 demonstrates better potency on influenza B neuraminidase than oseltamivir carboxylate (S. Bantia *et al.,* 2001)

3. MATERIALS:

Receptor:

Influenza virus B/Beijing/1/87 Neuraminidase complexed with Zanamivir having PDB ID 1A4G was taken[37]. Residues of both chain A and B are 76-465. By the help of Swiss Pdb viewer chain A was taken.

1: M54967. Reports Influenza B/Beiji...[gi:325211] Links

```
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  AUTHORS   Burmeister,W.P., Daniels,R.S., Dayan,S., Gagnon,J.,
Cusack,S. and
            Ruigrok,R.W.
  TITLE     Sequence and crystallization of influenza virus
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            neuraminidase
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LAHSGVMVSMKEPGWYSFGFEIKDKKCDVPCIGIEMVHDGGKKTWHSAATAIYCLMGS

GQLLWDTVTGVDMAL". [37]

4. METHODS:

4.1 Structure visualization by Swiss-PDB Viewer 3.7:

Swiss-PdbViewer is tightly linked to SWISS-MODEL, an automated homology modeling server developed within the Swiss Institute of Bioinformatics (SIB) in collaboration between GlaxoSmithKline R&D and the Structural Bioinformatics Group at the Biozentrum in Basel. Swiss-PdbViewer is an application that provides a user friendly interface allowing to analyze several proteins at the same time. The proteins can be superimposed in order to deduce structural alignments and compare their active sites or any other relevant parts. Amino acid mutations, H-bonds, angles and distances between atoms are easy to obtain thanks to the intuitive graphic and menu interface.

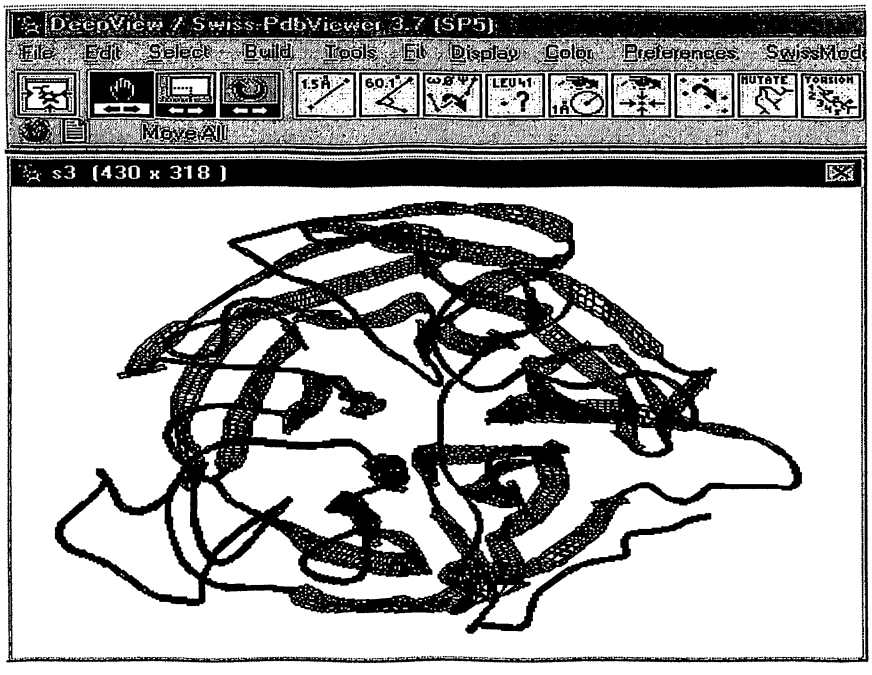

Fig-1 3D structure visualization of chain A of Influenza virus B/Beijing/1/87 Neuraminidase

This tool is used for visualization of receptor i.e. chain A of Influenza Virus B/Beijing/1/87 Neuraminidase. After docking of designed ligand with neuraminidase bond length, deviaton in ω, Φ and Ψ of bonded amino acids, torsion angles, Ramachandran plot and RMSD are calculated by this tool. [38]

4.2 DRAWING CHEMICAL STRUCTURE:

ChemSketch 8.0:

ChemSketch, from ACD Labs is used to draw chemical structures, and to view them as three dimensional (3D) models. A new feature of ChemSketch, starting with v 5, is the ability to name simple organic molecules. With the help of this tool 8 ligand molecules were drawn and named. Empirical formula and molecular weight was calculated.

Name of compound	Name	Empirical formula	Molecular weight
4-(acetylamino)-5-amino-3-(2-amino-2oxoethoxy)cyclohex-1-ene-1-carboxylic acid	SAS-8112	C11H17N3O5	271.26978
6-(acetylamino)-5-amino-3-(1,2,2-trihydroxyethyl)cyclohex-2-ene-1-carboxylic acid	SAS-8212	C11H18N2O6	274.27042
5-(acetylamino)3(aminocarbonyl)-4-{[amino(imino)methyl]amino}-6-(1,2,3trihydroxypropyl)tetrahydro-2*H*-pyran-2-carboxylic acid	SAS-8312	C13H23N5O8	377.35042
6-(acetylamino)-3-(amino carbonyl)-2-(dihydroxymethyl)-3,4-dihydro-2*H*-pyran-5-carboxylic acid	SAS-8412	C10H14N2O7	274.22736
N-{4-(dihydroxymethyl)-5-[(2-oxopropyl)amino]tetrahydrofuran-3-yl}acetamide	SAS-8512	C10H18N2O5	246.26032

Table 4. Nomenclature of five drawn molecules.

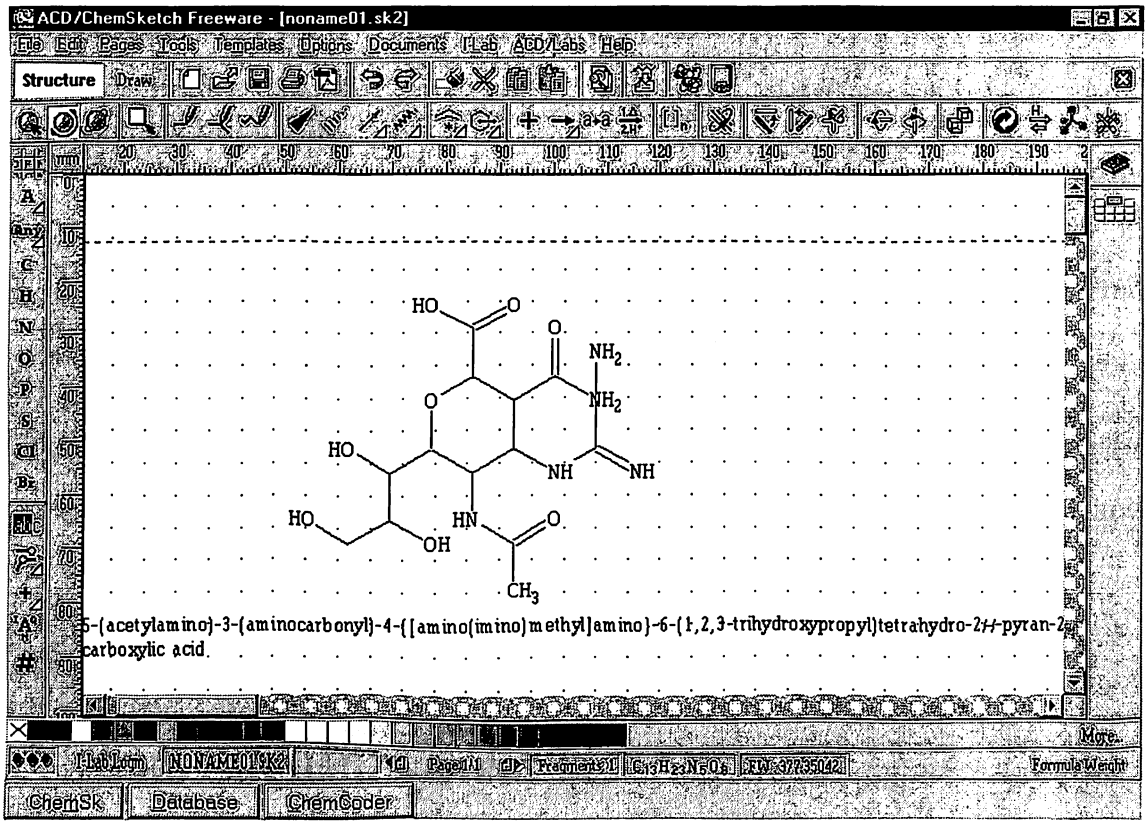

Fig-2 Chemical structure of SAS-8312.

SAS-8312(5-(acetylamino)-3-(aminocarbonyl)-4-{[amino(imino)methyl]amino}-6-(1,2,3-trihydroxypropyl)tetrahydro-2*H*-pyran-2-carboxylic acid).It contains one –COOH, one –NHCOCH3, one –CONH2, one 1-methylguanidine and butane-1,2,3-triol groups. And having molecular weight 377.35042 Dalton.[40]

4.3 VISUALIZATION OF 3D STRUCTURE:

CS Chem3D Pro 5.0:

CS chem.3D is an application specifically designed to aid scientists in modeling chemicals. It combines powerful building analysis and computational tools with a friendly graphical interface.

Chem3D provides computational tools based on molecular mechanics for optimizing models, conformational searching, molecular dynamics and calculating single point energies for molecules.

2D structure of the ligand is visualized with this tool.

4.4 ENERGY MINIMIZATION

CS MOPAC

It is an implementation of MOPAC, the well known semi-empirical modeling application that take advantage of the easy-to-use interface of Chem3D. CS MOPAC includes MOPAC97 supported by Fugitsu Corporation and MOPAC's author Dr. James Stewart.

Single Point energy of drawn molecule is calculated. Energy minimization of the molecule was done with 0.100 RMS Gradient. After energy minimization the most stable structure was taken as ligand.

Name	Empirical formula	Energy minimization
SAS-8112	C11H17N3O5	0.09395 kacl/mol
SAS-8212	C11H18N2O6	0.07512 kacl/mol
SAS-8312	C13H23N5O8	0.08327 kcal/mol
SAS-8412	C10H14N2O7	0.08785 kacl/mol
SAS-8512	C10H18N2O5	0.12996 kacl/mol

Table 5. Energy minimization of compounds with CS-MOPAC

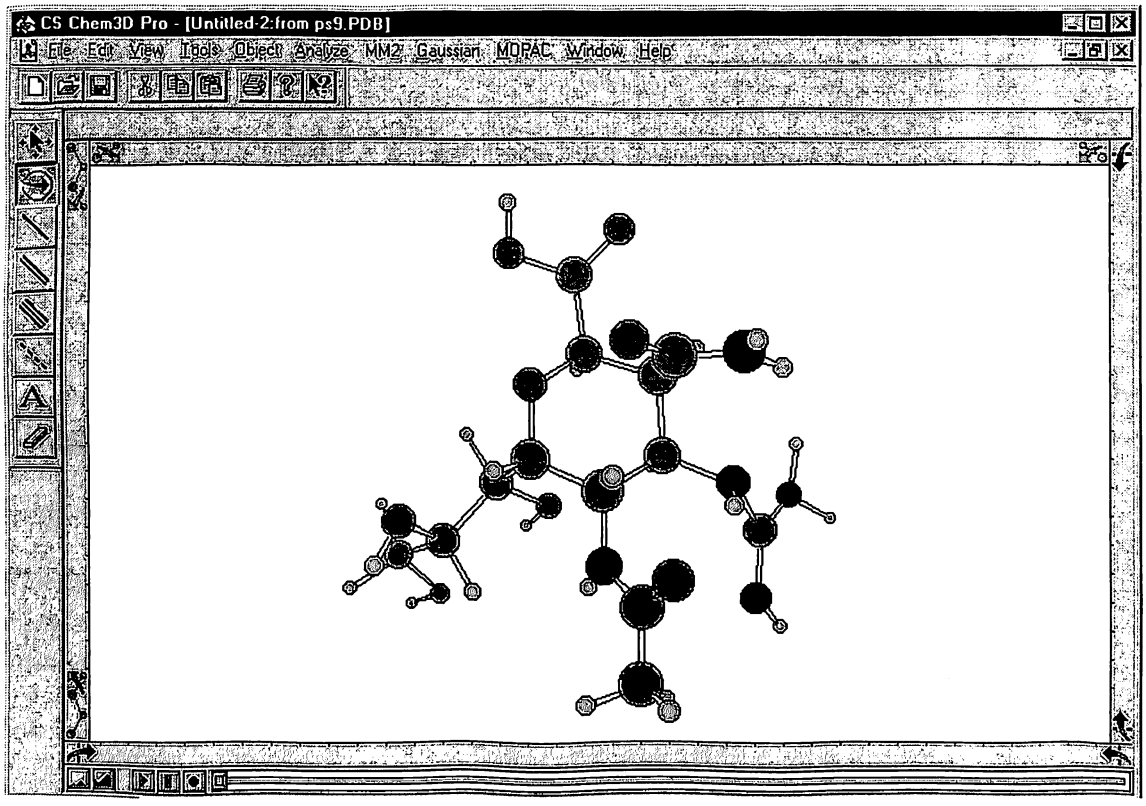

Fig-3. Single point energy minimization of SAS-8312

After single point energy minimization with CS-MOPAC, the energy of SAS-8312 is minimized to minimum RMS gradient 0.100 and using theory AM1 method and wave function closed shell (restricted).

4.5 DETERMINATION OF PROPERTIES OF LIGAND:

PreADME molecular descriptor:

This program can calculate a number of molecular descriptors of chemical compounds.

Functional Groups:

No. amino groups primary:	1
No. alcohol groups primary :	1
Monocyclic compounds carbocycles:	1
No. amino groups secondary:	1
No. amide groups:	2
No. alcohol groups secondary:	2

Constitutional Other constitutinal descriptors

Molecular weight:	377.353400
Molecular formula:	C13H23N5O8
No. Total atoms:	49
Fraction of Rotatable bonds:	0.261239
No. Rings:	1
No. of single bonds:	45
No. Rotatable bonds:	7

No. Rigid bonds:	42
No. H-bond acceptors:	8
No. positive charged groups:	1
No. negative charged groups:	1
No. positive chargable groups:	3
No. negative chargable groups:	2
Ratio donors to acceptor:	1.000000

Electrostatic descriptors only

Max positive charge:	0.807416
Max negative charge:	1.280054
Local dipole index:	0.426319

Physicochemical descriptors

SKlogP value:	8.362920
Water solubilityl:	9.65291e+06
Vapor pressure:	2.18038e-23
Buffer solubility:	5.10668e+06
Solvation Free Energy:	39.700000**

4.6 DOCKING:

ArgusLab 4.0.1:

It is a Computational Chemistry Software for molecular modeling, graphics, and drug design program. It uses new drug docking code, Contains both the GADock and ArgusDock docking engines and the AScore scoring function with a preliminary set of parameters.

- The structure of both receptor i.e. neuraminidase and designed ligand are opened in Brookhaven Protein Databank format.
- 274GLU amino acid of chain A of receptor was selected for making the binding site group.
- One ligand group is created from the residue option of the ligand.
- It creates a dock setting dialog box. This binding site bounding box is adjusted to 25.50, 20.75, 15.25 in X-, Y- & Z- axes respectively.
- 0.4 Å grid resolution was taken. Argusdock from Docking Engine, dock from Calculation Type and flexible from Ligand options were selected. Then start button was selected for docking.

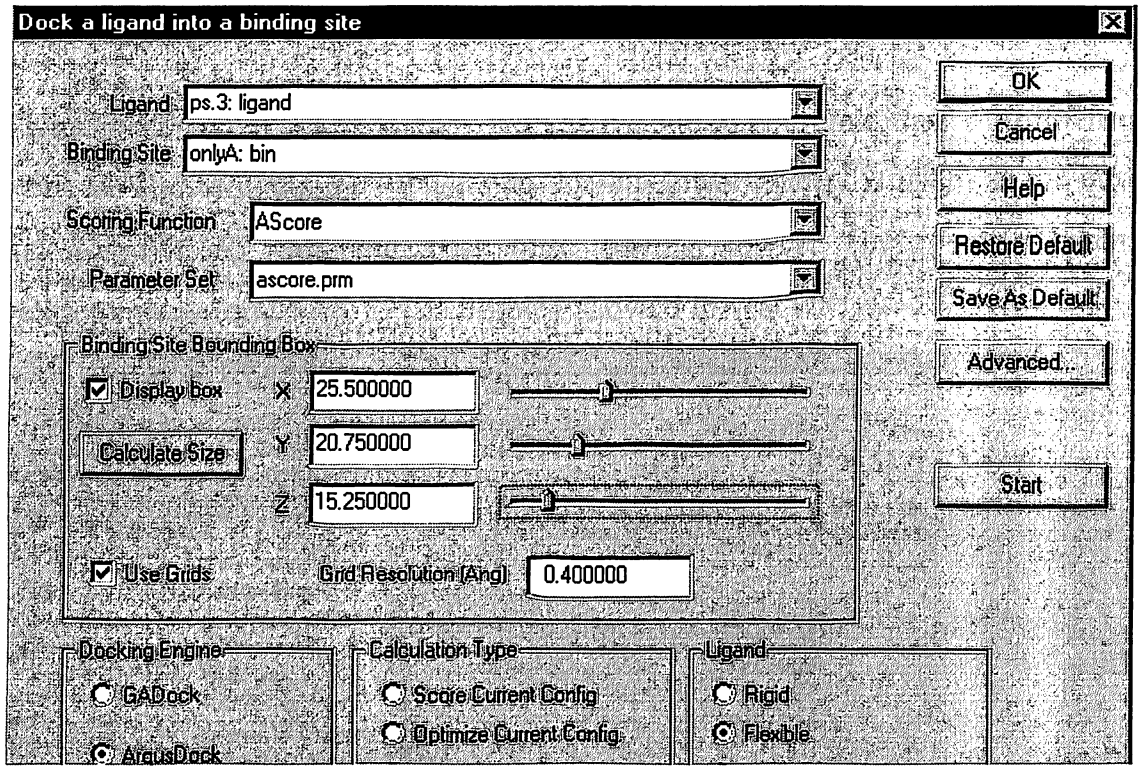

Fig-4 parameters required for docking of SAS-8312 with binding sites.

5. RESULT:

Eight ligands are allowed to dock with active site of neuraminidase of Influenza virus B. The receptor-ligand complex having lower energy is taken as the best hit. Out of 53 hits the best pose having lowest energy was taken. Neuraminidase and SAS-8312 complex showed lowest energy -8.61Kcal/mol.

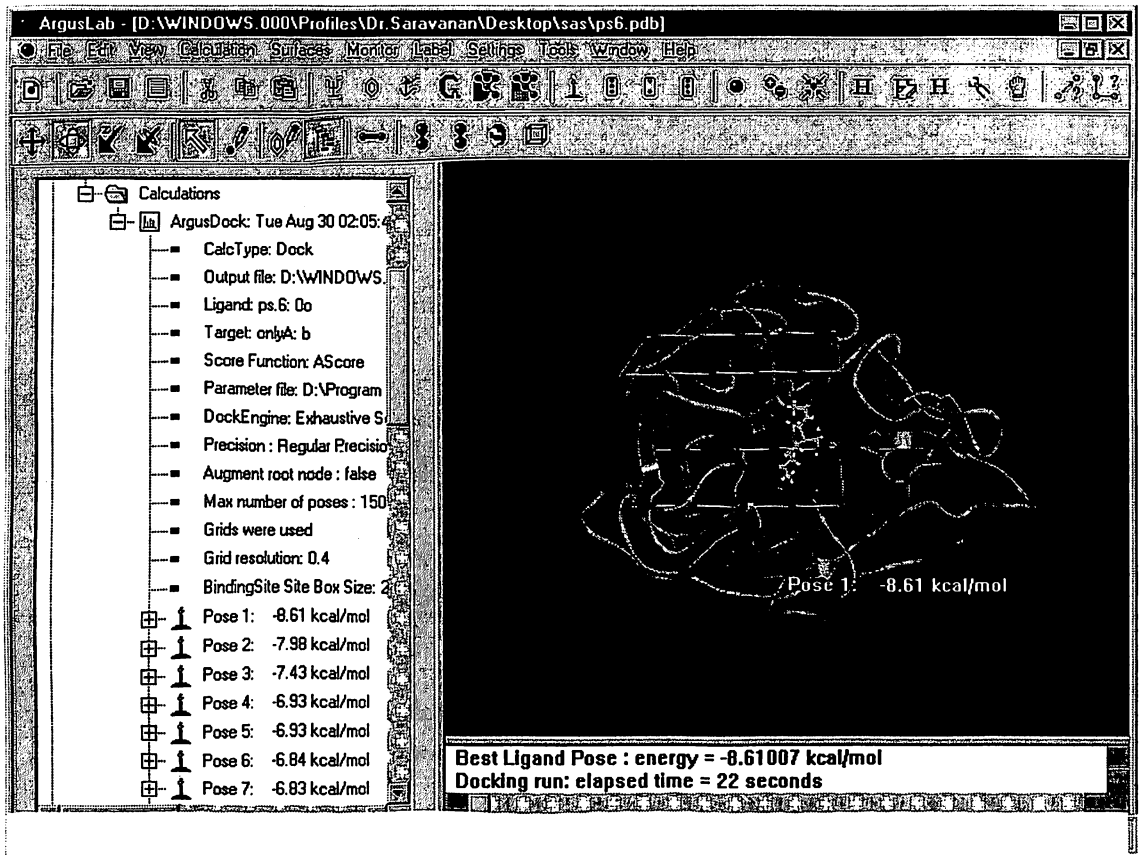

Fig-5 Docking of SAS-8312 to chain A of Influenza Virus B/Beijing/1/87 Neuraminidase

Name	Empirical. Formula	Energy
SAS-8112	C11H17N3O5	-6.30kcal/mol
SAS-8212	C11H18N2O6	-8.40kcal/mol
SAS-8312	C13H23N5O8	-8.61kcal/mol
SAS-8412	C10H14N2O7	-6.27kcal/mol
SAS-8512	C10H18N2O5	-7.12kcal/mol

Table6 Docking result of five ligands.

The complex is visualized with Swiss-pdb Viewer tool. ARG222, GLU274, GLU275 and ARG291 of chain A Influenza Virus B/Beijing/1/87 Neuraminidase active site is bound with SAS-8312.

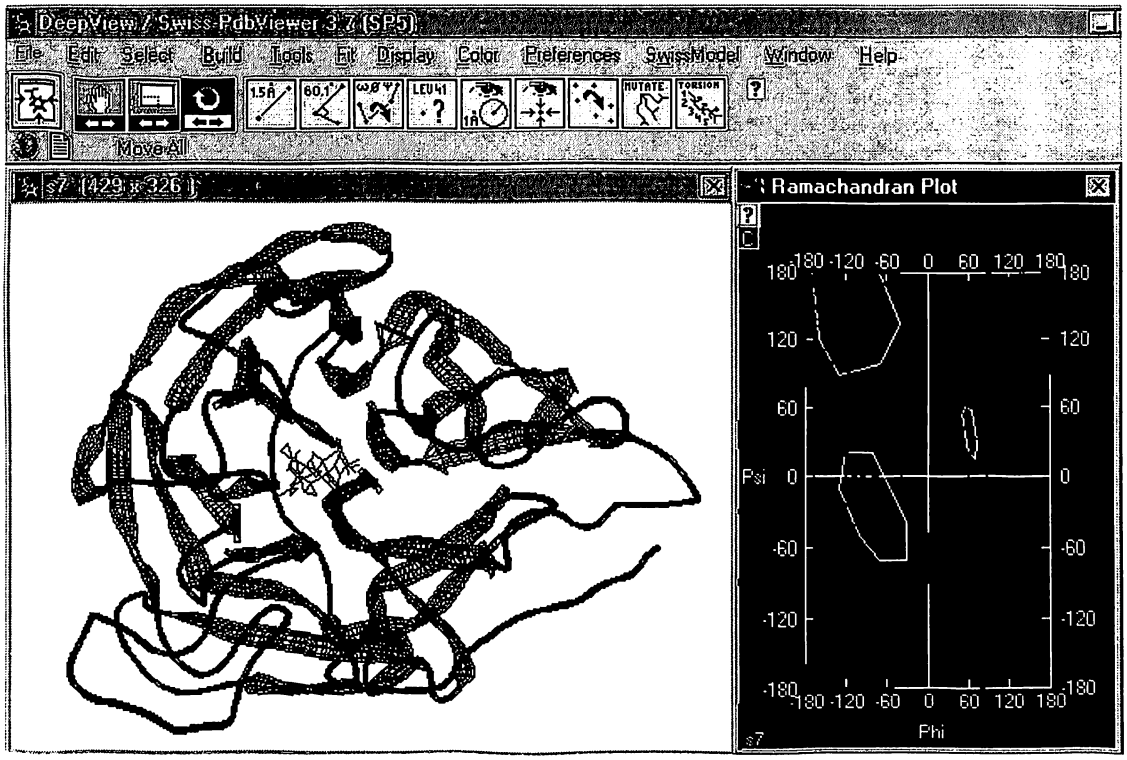

Fig-6 Visualization of SAS-8312 complexed with chain A of neuraminidase and Ramachandran plot.

A graph called Ramachandran plot describes the allowed combinations of phi versus psi for this complex structure.RMS deviation is calculated by Swiss-pdb

viewer (>1.5 Å). No changes found in ω, Φ, Ψ angles of neuraminidase active site.

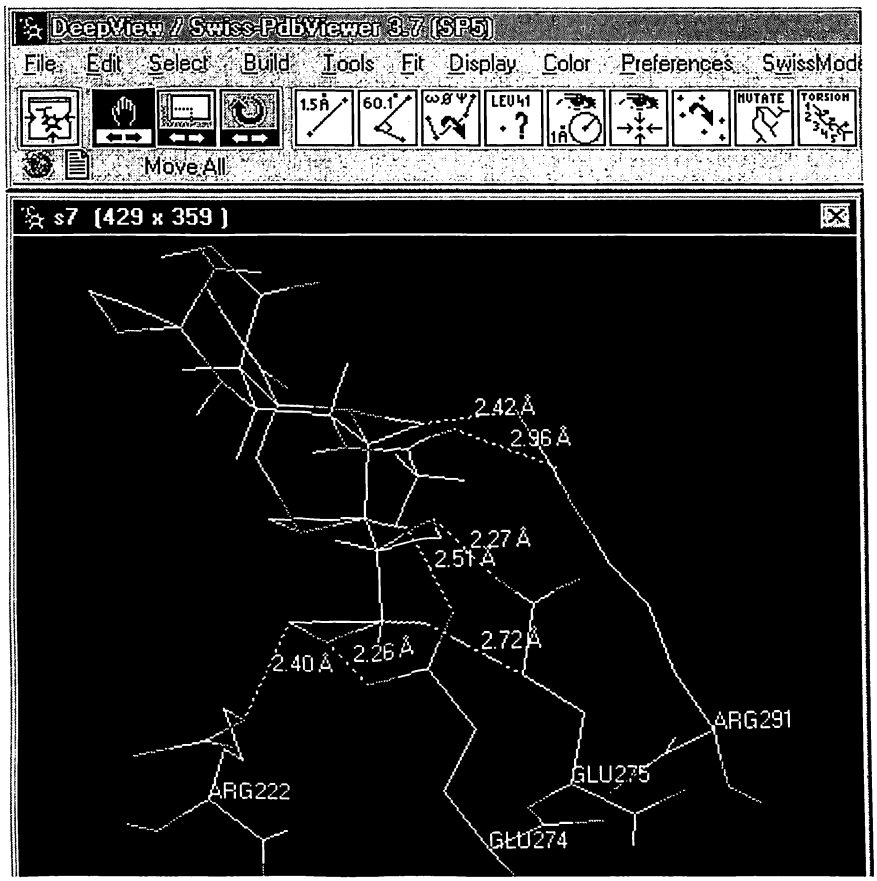

Fig-7. Binding of SAS-8312 with active site residues.

Name	Name of residues	No. of bonds
SAS8112	GLU274,GLU275	3
SAS8312	ARG222,GLU274,GLU275,ARG291	4
SAS8312	ARG222,GLU274,GLU275,ARG291	7
SAS8412	ARG222,GLU274,ARG291	3
SAS8512	ARG291,GLU274	3

Table – 7: Name of residues with number of bonds

It was found out that SAS-8312 was bonded by hydrogen and vander waals force of attraction with four amino acid residues i.e., ARG222, GLU274, GLU275 and ARG 291 with bond length 2.40Å, 2.26Å, 2.99Å, 2.27Å, 2.46 Å, 2.96 Å and 2.42 Å of chain A of Influenza virus B/Beijing/1/87 Neuraminidase.

6. DISCUSSION:

Neuraminidase (NA) is one of the most important targets to screen the drugs of anti-influenza virus A and B. Zanamivir and Oseltamivir are two drugs for inhibiting neuraminidase. Sensitivity molecular docking GOLD is used to induce fit effects in influenza virus neuraminidase. GOLD is used for designing a neuraminidase inhibitor.Rational drug designing has led on synthesis of new drugs. But these are showing side effects because of low dissociation constants of drugs and IC50 value.

Out of which five molecule SAS-8312(5-(acetylamino)-3-(aminocarbonyl)-4-{[amino(imino)methyl]amino}-6-(1,2,3-trihydroxypropyl) tetrahydro-2*H*-pyran-2-carboxylic acid)

It contains one –COOH, one –NHCOCH3, one –CONH2, one 1-methylguanidine and butane-1,2,3-triol group and RMSD value>1.5Å.In this report we designed SAS-8312 molecule having molecular formula (C13H23N508) and molecular weight 377.35042 Dalton. It is bound with four residues i.e., ARG222, GLU274, GLU275 and ARG291 with bond length 2.40Å, 2.26Å, 2.99Å, 2.27Å, 2.46 Å, 2.96 Å and 2.42 Å. respectively.

Rationally designed NA inhibitors that block the viral life cycle. NA inhibitors effective against all influenza types. Zanamivir, oseltamivir and peramivir have proven to be safe drugs but they are showing side effects. So novel drug discovery is needed to reduce the side effects.

7.SUMMARY:

Neuraminidase is a glycoprotein present on the surface of the influenza virus. It is also called as sialidase, which cleaves sialic acid from glycoconjugates. It facilitates release of virus particles from the infected cell surfaces during the budding process. A chain of influenza virus B/Beijing/1/87 neuraminidase consists of 76-465 residues.

Compared with other anti-influenza agents the NA inhibitors are well tolerated and effective against all influenza subtypes. NA inhibitors provide an important new therapeutic weapon for the management of influenza infection.

In this report we discussed many tools. Chemsketch8.0 is a user friendly tool for drawing chemical structure of compounds. CS chem3D Pro 5.0 is used for viewing D structure of the drawn chemical compound and CS-MOPAC is a tool for minimizing the energy to 0.100 RMS gradients. After that Arguslab4.0.1 is used for docking of ligand and protein. It gives low energy value -8.61 kcal/mol. The best pose was taken which is more stable. Then our complex structure is visualized with Swiss Pdb viewer 3.7 tool. Ramachandran plot, RMS value and bond lengths are calculated by this tool.

It is concluded that SAS-8312 binds with four residues of with seven bonds i.e., four hydrogen and three van der Waals bonds. Hence, SAS-8312 is a novel neuraminidase inhibitor of chain A of influenza virus B/Beijing/1/87 neuraminidase

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