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Research Article

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Ab initio and DFT investigation of C4 & C7 position of sialidase antiviral inhibitor

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ABSTRACT

DANA is the first sialidase antiviral inhibitor and its C4 position plays a vital role in inhibiting the neuraminidase virus. However, C7 position remains free with involving in any receptor interaction. Hence, various substitutes were introduced at the key functional site of DANA to design new sialidase inhibitor. The investigation reveals that at the C4 position amino, guandino and thiol group improves the binding affinity. Especially guandino at the C4 position enormously increases the binding affinity and acts as promising candidate for neuraminidase antiviral inhibitor. Meanwhile the investigation at C7 position discloses that the substituents such as amino, methoxy and methyl group increases the binding affinity and could act as potential antiviral neuraminidase inhibitor.

Keywords: Influenza, neuraminidase virus, antiviral inhibitor, DANA, binding affinity

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INTRODUCTION

Influenza, commonly known as flu affects major human population and causes mortality [1]. 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 [2]. Most of the influenza infections are spread by virus-laden respiratory droplets containing several microns in diameter that are expelled during coughing and sneezing. Despite significant knowledge of viral infectivity, current therapeutic measures could not control the viral disease. Vaccination has provided only a limited control because of the tendency of the virus to mutate and to escape from the immune system. So as the vaccines must be reformulated each year because of high antigenic drift neuraminidase virus

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. So it fails to contain the influenza virus B and also the clinical use of these agents is limited because of the rapid emergence of resistance [3]. The influenza virus neuraminidase inhibitors (NAIs) have currently been emerged as promising therapeutics for the treatment of influenza [4]. Two such inhibitors are Relenza (Zanamivir) and Tamiflu **2** (Oseltamivir) successfully contains the neuraminidase virus and further emphasize the importance of NA as a valid anti-influenza drug target [5]. The essential role of NA in influenza virus replication and the highly conserved enzyme active site in influenza viruses A and B, causes more attention of scientist to be focussed on the development of selective inhibitors of this enzyme. In recent past years, tremendous progress has been made in the discovering of this new class of anti-influenza agents [6]. Sialic acid is the active site of neuraminidase virus [7], which has four binding pocket and bound to various amino acid residues and it has been schematically shown in the figure-1. It is reported that the virus proliferates by cleavage of the bond between the carboxylic acid group of sialic acid and arginine amino acid residue [8]. The cleavage mechanism of sialic acid proceeds through the formation of sialosyl cation intermediate and hence, the

search for the neuraminidase inhibitors is focussed on the structure of cation intermediate [9]. The first such a sialidase antiviral inhibitor designed is DANA (Dehydrodeoxy-N-acetylneuraminic acid) bound to the active site [10]. Later on the replacement of hydroxyl group in DANA with amino group resulted in new inhibitor known as 4-amino-DANA. Binding affinity of 4-amino-DANA is effective than DANA due to replacement of C4 hydroxyl group [11].

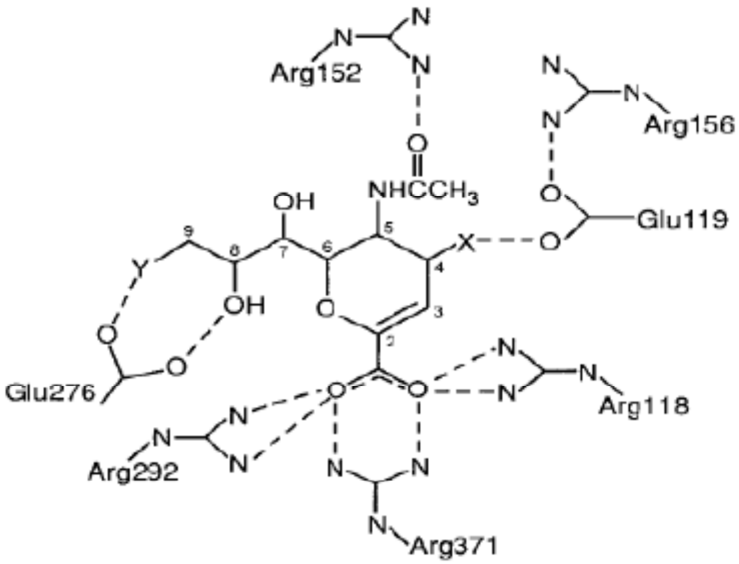

Figure-1, Schematic interactions of DANA inhibitor (X=OH, Y=OH)

In kinetics theory, the compounds that closely resemble the reaction intermediate should have a higher binding affinity towards the enzyme substrate [12]. As DANA mimics the structure of sialyl cationic intermediate; its further structural modification by various substituents will provide a new inhibitor with better binding affinity. Therefore various substituents were replaced at C4 position and validated the binding affinity of each inhibitor. The substituent which has more binding affinity toward viral target will become potential sialidase antiviral inhibitor. The C7 side chain hydroxyl group of DANA remains free without involving in receptor interaction and hence, its position with various groups is investigated to improve binding affinity and to design potent neuraminidase antiviral inhibitor.

RESULTS AND DISCUSSION

Sialic acid is the active site of neuraminidase virus and its cleavage with arginine amino acid residue proliferates the infection. The investigation of mechanistic pathway of sialidase catalysis signifies that the cleavage proceeds via the formation of sialosyl cation intermediate. The structure of cation intermediate has the half chair configuration and the DANA compound too possess the structure of transition state cation intermediate and it is shown in the figure-2. The C4 position of DANA is the key position and it imparts the binding affinity to the compound and hence its position is investigated with various substituents to design the potent inhibitor[13]. The DANA inhibitor with hydroxyl group at the C4 position yields binding affinity of –105.4 kcal/mol. The substitution of amino group at the C4 position increases the binding affinity to 112.6 kcal/mol by forming strong hydrogen bonds with glutamic acid and thus acts as effective neuraminidase inhibitor. Meanwhile the substitution of amino group decreases the polarization between C2–O16 and accounts for effective inhibition. The substitution of fluorine at the C4 position increases the binding affinity to 107.9 kcal/mol. The electronegative fluorine binds better than the parent hydroxyl group and therefore it acts as a better inhibitor than DANA. The next electronegative substituent chlorine at C4 position scores binding affinity of 106.3kcal/mol; but it is lower than the corresponding fluorine. Despite it acts as a better inhibitor than the parent DANA compound.

Introduction of methoxy at the C4 position attains binding affinity of 109.51 kcal/mol, the oxygen atom of the methoxy group binds strongly with the hydrogen atom of glutammic acid by hydrogen bond. The strong binding affinity of 4-methoxy-DANA makes it as a better inhibitor than the fluorine and chlorine counterpart due to strong hydrogen bonding with gluttamic residue. Thiol group at the C4 position yields binding affinity of 105.61 kcal/mol, which is same as parent DANA. Hence the study found that the thiol group and hydroxyl group at the C4 position fails to make strong hydrogen bond with glutammic amino acid residue and hence both acts as a moderate neuraminidase inhibitors. Introduction of methyl group at the C4 position increases binding energy to 109.46 kcal/mol. Table I clearly shows that the methyl candidate is the next promising substituent to the amino group in

offering best binding affinity. It is attributed to the electron rich methyl group binds electronegative oxygen of the glutammic acid. Tri-fluoro carbon at the C4 position yields better binding affinity of 110.32 kcal/mol and thus it shares same platform in binding affinity amino group. It is apparent that the C4 investigation with various substituent reveals that the substituent which has multiple atoms alike CF_3 and CH_3 offers better binding affinity due to their tendency to form hydrogen bond with glutammic acid residue. The effect of substituent at the C4 position has also been investigated in solvent phase as well. It appears that the parent hydroxyl offers better binding affinity of 9.9 kcal/mol. It is quite unusual to signify that the thiol in the solvent phase exhibits strong binding affinity of 15.37 kcal/mol than the methyl, fluorine and methoxy group due to strong bonding in solvent phase. Amino group at the solvent phase also offers better binding affinity than all the seven substituents.

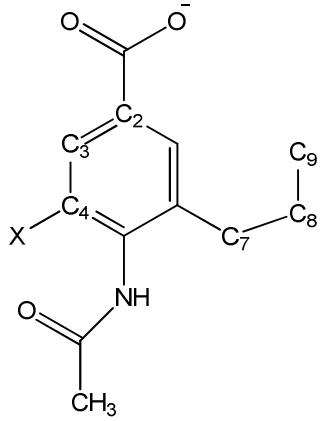

Figure 2, Structure of 1-deoxy-2,3-didehydro-N-acetylneuraminic acid (DANA). X- Substituents.

Table – I Effects of C4 substituent on DANA binding affinity

Substituent	Ligand Binding Energy HF\6-31g (kcal/mo)	Ligand Binding Energy B3LYP\6-31G(d) (kcal/mol)	r(C2=C3) (Å)	r(C2–O16) (Å)
Hydroxyl	105.61	105.20	1.321	1.382
Amino	107.95	112,62	1.324	1.390
Methoxy	106.91	110.19	1.324	1.389
Fluorine	102.81	107.92	1.321	1.388
Methyl	106.18	109.46	1.324	1.384
Chlorine	102.96	106.30	1.321	1.382
Thiol	101.76	105.61	1.323	1.382
CF_3	104.54	110.32	1.321	1.383

Effect of substituent at the C7 position

It is apparent from discussion that the DANA compound with C4 replacement of amino group drastically increases the binding affinity and in the same line C7 position also examined and its structure is shown in figure-3. It appears that the C7 position of DANA remains free without involving in any interactions and it is shown in figure-1[14] and hence, various substituents can be introduced to evaluate the binding affinity at this position. The substituent which score high binding affinity can be used to design new antiviral inhibitor based on pyranose cyclohexane ring series. The 7-amino-DANA provides binding affinity of 110.6 kcal/mol with its receptor and its affinity is almost same as the parent compound. The substitution of methoxy position at the C7 position decreases the binding affinity to 109.5 kcal/mol; lower than the parent compound. The methoxy group might get polarized and fails to attain high binding affinity. Fluorine at the C7 position does not significantly increase the binding and it provides 109.5 kcal/mol.

The electronegative fluorine could form intramolecular hydrogen bond with the vicinyl hydroxyl groups and as a result attains low binding affinity. Substitution of methyl group at the C7 position shares same domain with fluorine and methoxy substituents of 110.6 kcal/mol and increases the binding affinity than the parent hydroxyl group at the C7 position.

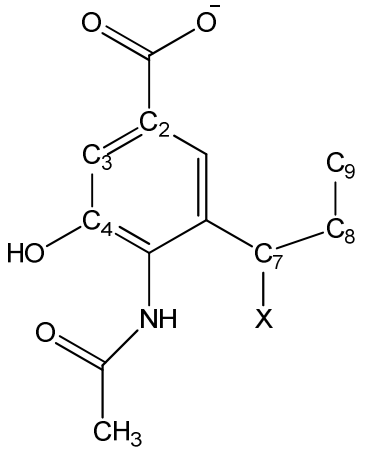

Figure 3, Structure of 1-deoxy-2,3-didehydro-N-acetylneuraminic acid (DANA). X- Substituents.

Table – II Role of C7 substituents on the biinding affinity in gas phase

Substituent	Ligand Binding Energy HF\6-31g (kcal/mol)	Ligand Binding Energy B3LYP\6-31G(d) (kcal/mol)	r(C2=C3) (Å)	r(C2–O16) (Å)
Hydroxyl	105.61	105.20	1.319	1.382
Amino	107.69	109.65	1.319	1.380
Methoxy	106.34	109.51	1.319	1.381
Fluorine	106.34	109.42	1.319	1.380
Chlorine	87.60	96.31	1.490	1.382
Methyl	107.86	110.66	1.319	1.382
Thiol	103.40	108.56	1.319	1.379
Guandino	113.68	115.42	1.322	1.382
CF_3	104.39	108.12	1.318	1.381

Introduction of chlorine at the C7 position drastically decreases the binding affinity due to the polarization effect and poor bonding with protein residues. The thiol group at the C7 position significantly increases the binding affinity due to strong hydrogen bonding tendency of thiol with the amino acid residues. The tri-fluoro carbon at the C7 position offers same binding affinity of 108.12 kca/mol as thiol group. Introduction of tri-fluorocarbon at the C7 position alters the pyranose ring structure by elongating C2–O16 bond; thus it offers moderate binding affinity. Finally, the substitution of guandino at the C7 hydroxyl group enormously increases the binding affinity to 115.42 kcal/mol. Basically guandino has two amino groups in its structure and these groups polarize strongly and made strong hydrogen bond with the arginine amino acid resides. This strong electrostatic hydrogen bonding stabilizes the cyclic pyranose ring by attaining the perfect half planar configuration. Substitution of guandino increases the delocalization of electron by decreasing the polarization between C2–O16 bond. It is apparent from table II that the introduction of guandino group at C7 position decreases the C–O bond distance from 1.382Å to 1.322Å, In a nutshell the effect of C7 substituents survey reveals that the methyl group and guandino group acts as a potential anti-viral agents.

SOLVENT PHASE BINDING ENERGY AT C4 POSITION

The parent hydroxyl group at the C4 position yields ligand binding energy of 9.99 kcal/mol in solvent phase. It is obvious from table III that the replacement of hydroxyl group by amino group at the C4 position drastically increases the solvated ligand binding energy from 9.99 kcal/mol to 18.03 kcal/mol. It appears that the two hydrogen atoms can easily bind the oxygen atom of glutammic acid and increases the binding affinity. The thiol group at the C4 position scores effective binding energy of 15.37 kcal/mol, next to the amino group. The binding energy of thiol in solvent phase is relatively better to gas phase. The hydrophillic nature of thiol group provides superior binding affinity in solvent phase. Fluorine attains binding affinity of 14.24 kcal/mol in the solvent phase. The electronegative fluorine may bind the glutammic acid presumably by the presence of bridged water molecule; which in turns scores strong binding energy of fluorine. Chlorine and methyl group attains poor binding affinity of 106 & 109 kcal/mol in solvent phase, due to their hydrophobic character. Investigation of solvated binding energy reveals that the bulky group at C7 position attains low binding affinity.

Table III Effect of C4 substituents on the binding affinity of DANA in solvent phase

C4 Substituents of DANA	Solvated Ligand Binding Energy HF\6-31g (kcal/mol)	Solvated Ligand Binding Energy B3LYP\6-31G(d) (kcal/mol)	Charges on the carbon (C2)	Charges on the oxygen (O16)
Hydroxyl	8.22	9.99	0.310	-0.789
Fluorine	9.25	14.24	0.346	-0.787
Chlorine	5.59	13.15	0.344	-0.782
Methyl	7.02	13.76	0.314	-0.788
Methoxy	5.86	12.76	0.358	-0.781
Amino	6.33	18.03	0.313	-0.789
CF3	3.78	12.68	0.350	-0.781
Thiol	6.57	15.37	0.315	-0.783

SOLVENT PHASE BINDING ENERGY AT C7 POSITION

Introduction of amino group at the C7 position yields binding energy of 18.06 kcal/mol. The protonated amino group in solvent phase easily binds the arginine amino acid residue and attains higher binding affinity. The results in table IV clearly shows that the 7-amino-DANA acts as potential antiviral neuramindase inhibitor. Next to the amino group, guandino group scores high solvated binding energy of 17.57 kcal/mol. The two amino groups in guandino offers better binding affinity with the arginine residue. It is clear from table III that the amino, methoxy, methyl, fluorine and thiol groups almost have same binding energy of 12-13 kcal/mol due to poor ionic and polar effects. Investigation of C7 substituent in the solvent phase discloses that the amino group and guandino group bound strongly with the arginine residue and thus acts a potential antiviral inhibitors. The rest of bulky substituents like methyl, chlorine and fluorine experience low binding affinity at the C7 position due to the lack of hydrophilic and hydrogen bonding character.

Table IV Effect of C7 substituents on the binding affinity of DANA (solvent phase)

Substituents	Solvated Ligand Binding Energy HF\6-31g (kcal/mol)	Solvated Ligand Binding Energy B3LYP\6-31G(d) (kcal/mol)	Charges on the carbon C2	Charges on the oxygen O16
Hydroxyl	8.22	9.99	0.310	-0.789
Amino	6.10	18.64	-0.803	-0.803
Methoxy	5.85	12.80	-0.797	-0.797
Fluorine	6.26	12.99	-0.805	-0.805
Methyl	6.02	12.87	-0.796	-0.796
Chlorine	10.48	1.75	-0.786	-0.786
Thiol	3.60	12.71	-0.795	-0.795
CF_3	1.50	13.61	-0.791	-0.791
Guandino	11.94	17.57	-0.798	-0.798

CONCLUSION

Ab initio and DFT investigation of C4 & C7 sialidase inhibitors reveals that the amino, thiol and fluorine group at the C4 position offers better binding affinity in the gas phase. It is significant to mark that the above stated groups have strong tendency to form hydrogen bonding network with the amino acid residues and could act as potentical antiviral inhibitor candidates. Meanwhile amino and guandino group at the C7 position attains higher binding affinity at the C7 position. Both the amino and guandino group contain two hydrogen atoms to bind the oxygen atom of the carboxyling group of the glutammic acid residue. Furthermore the investigation of solvated binding energy of various substituents reveals that the thiol group and amino group have excellent binding affinity at the C4 position due to the hydrophilic character. However at the C7 position guandino and amino group socres high solvated binding energy due to high polar amine groups. Hence, the ab initio and DFT investigation concludes that the amino, guandino and thiol group at the C4 and C7 position acts as a potential antiviral neuraminidase inhibitors. These findings will provide greater impetus to contain the formidable neuraminidase virus.

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