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Quantum Chemical Investigation of C12 and C6 Position of Oseltamivir Sialidase Antiviral Inhibitor

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ABSTRACT

The ab initio and DFT investigation of C12 & C6 position of oseltamivir sialidase inhibitor reveals that the absence of pyranose oxygen ring in the inhibitor structure drastically increases binding affinity of the inhibitor in relation to the pyranose based inhibitors. The investigation further reveals that the methyl and ethyl group at the C12 position have substantial binding affinity due to their inherent hyperconjugative and charge transfer effects between C4 and C13 bond. The analysis at C6 position of oseltamivir inhibitor discloses that the methyl amine group increases the binding affinity due to their strong hydrogen bonding tendency with the vicinity receptors. Hence, the investigation validates that the 12-methyl-oseltamivir, 12-ethyl-oseltamivir and 6-methylamine-oseltamivir inhibitor become the potential candidate for the development effective sialidase antiviral inhibitors.

INTRODUCTION

Influenza virus is a RNA virus which contains two surface proteins namely hemaglutinnin (HA) and neuraminidase (Chak *et al*., 2007). Among the two proteins, hemagglutinin is the prime target of vaccines and neuraminidase is the main target of anti viral drugs. Vaccines against influenza virus are frequently inactive due to the rapid emergence of mutant viral antigens (Gerdon *et al*., 2005) and hence, it creates an inevitable need of antiviral drugs. NA is an enzyme protein, the influenza virus cleaves the alpla ketosidic linkage of the neuraminidase active site (sialic acid) and the adjacent arginine amino residue of the host cell receptor (Jarreau *et al*., 1992) The cleavage process proliferate the viral infection to the other cells and thus continues its lifecycle. Inhibiting the activity of neuraminidase will trap the virus particle inside the infected host cell. The binding site of NA is smaller and has a highly selective binding pocket and hence, this site is an ideal target for design of antiviral drugs against a broad range of influenza virus (Vincent *et al*., 2003). The first two antiviral drugs amantadine and rimantadine which block the M2 protein ion channel function will be effective in blocking only the influenza A type virus (Hayden *et al*., 1992) and fails to contain the influenza B type virus, moreover resistant mutants are rapidly generated for the above compounds. Therefore alternative treatment to contain the influenza virus focused towards the development of sialidase antiviral inhibitors. Meanwhile, antiviral inhibitor treatments for influenza virus (neuraminidase inhibitors) have been emerged as potential therapeutics to treat the influenza. The two such compounds developed are zanamivir (Macdonald *et al*., 2005) and oseltamivir (Harrington *et al*., 1996) successfully contains the rapid generation of mutants of influenza A & B. The developments of zanamivir, oseltamivir, and BCX (Chand *et al*., 2004) antiviral inhibitors are purley based on the transition structure of neuraminidase with its natural substrate sialic acid. Sialic acid is the active site of neuraminidase and it has the five binding pocket; each of pocket bound to the adjacent amino acid residues, its structure is shown in the figure-1.

Natural substrate sialic acid cleaves from the neuraminidase protein by enzyme catalytic process and proliferates the viral infection due to the low binding affinity with its receptor protein residues. The process of cleavage of sialic acid from the adjacent arginine amino acid residue proceeds through the formation of sialyl cation intermediate (Colmann *et al*., 1994) therefore the search for neuraminidase inhibitor is focussed on the structure of cation intermediate.

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The first developed sialcidase inhibitor is DANA (Alexander *et al.,* 2000). The DANA is the dehydrated derivative of the sialic acid; which resembles the transition state geometry of the sialyl cation intermediate formed during the sialidase catalytic process. The replacement of hydroxyl group in DANA with amino group resulted in the 4-amino-DANA; which is more potent than DANA. All the inhibitors DANA, 4-amino-DANA, 4-guanidino-DANA contains oxygen atom in the ring and thereby it suffers in conformation flexibility during binding with receptors. Tamiflu or oseltamivir (Lew *et al*., 2000) is the first inhibitor developed with carbocyclic ring which does not have oxygen atom in the ring with the high degree of stability. Moreover this non-pyranose inhibitor reduces the polarity of the compound and increases the bio-availability of the compound. Hence, this carbocyclic inhibitor established a new era in anti influenza drugs. It appears that C7 and C12 position of tamiflu remains free without involving in any interaction with its receptor amino acid residues. Hence, the investigation of C7 and C12 position of oseltamivir with various substituent could improve its potency and will acts as potential antiviral inhibitor. Besides, recent ab initio investigation (Krishnan *et al*., 2015) of DANA based sialidase antiviral inhibitors reveals that substituent at certain position drastically increases the binding affinity of the inhibitor and becomes the new antiviral drugs.

Fig. 1: Structure of sialic with Five Binding Pockets.

COMPUTATIONAL METHOD

All the oseltamivir compounds are invariably optimized using the HF/6-31G(d) level of theory. The oseltamivir compounds and its substituted compounds are optimized in anionic form using the above level of theory. Single point energy calculation of all the optimized oseltamivir compounds were carried out using B3LYP/6-31G(d) level of theory. Single point energy calculations of all the compounds were performed in both gas phase and solvent phase. CPCM model [Conductor like polarized continuum model] is employed for the solvent energy calculation and water is the only solvent used for solvation models. Likewise the receptor model compound guanidino cation compound is optimized using the HF/6-31G(d) level of theory and single point energy calculation is performed using B3LYP/6-31G(d) level of theory in both gas phase and solvent phase (CPCM solvent model). The binding affinity of oseltamivir and its substituted compounds were estimated by using the following equation,

$$\Delta E_{(Bind)} = E_{g(Substrate\text{-}Ligand\ Complex)} - (E_{(substrate)} + E_{(Ligand)g})$$

RESULTS AND DISCUSSION

Oseltamivir on interaction with methyl guanidino provides ligand binding energy of 122.2 kca/mol. Methyl guanidino is used as model compound to replace the amino acid residue (argnine) as a receptor binding with the oseltamivir. The structure of oseltamivir is shown in fugure 2. But the pyranose derivative yields binding energy of 109.65 kcal/mol. So it is apparent that the non-pyranose derivative acts as the powerful inhibitors. Substitution of methoxy group at the C12 position provides the binding affinity of 121.56 kcal/mol; so it shares same platform with with oseltamivir inhibitor. The introduction of ethoxy group at the C12 position does not produce significant effect on the binding affinity due to the highly polar nature. It provides the binding energy of 121.66 kcal/mol. The polar nature of methoxy and ethoxy group decreases the negative inductive effect between conjugative carbon C4=C13 bond and thus have little effect on binding affinity.

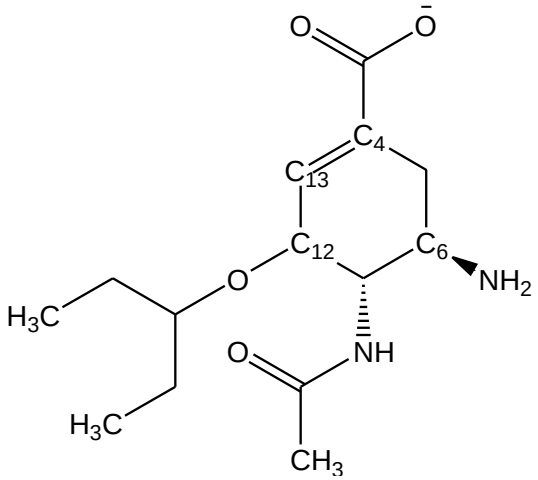

Fig. 2: Structure of Oseltamivir anion.

The introduction of methyl group at the C12 position of oseltamivir increases the binding affinity to 120.04 kcal/mol and it is higher than the parent oseltamivir inhibitor. Increase in binding affinity is attributed to the fact the presence of methyl group promotes the hyperconjugative effect and pi electron delocalization between C13 and C4 carbon atoms. In addition to the hyperconjugative effect, the absence of polar group also accounts for the higher binding energy of 12-methyl oseltamivir inhibitor.

Table 1 : Effect of C12 substituents on the binding affinity of oseltamivir.

Sl No.	C12 substituents of oseltamivir	Ligand Binding Energy HF/6-31G(d) (kcal/mol)	Ligand Binding Energy B3LYP/6-31G(d) (kcal/mol)	r(C4=C12) (Å)
1	Oseltamivir	118.34	122.21	1.326
2	Methoxy	117.27	121.56	1.327
3	Ethoxy	117.34	121.66	1.327
4	Methyl	119.91	124.03	1.327
5	Ethyl	119.87	124.12	1.326
6	Chlorine	110.9	117.72	1.326
7	Fluorine	116.67	121.21	1.326
8	Thiol	115.38	119.32	1.326
9	Guanidino	115.67	118.38	1.327
10	Tri fluoro carbon	115.53	120.81	1.324

Substitution of ethyl group at the C12 position increases the binding affinity to 124.03 kcal/mol. However, in relative to the methyl group; it produces the same effect in binding affinity and which implies that the lengthening of alkyl group does not increase the binding affinity. It is apparent from table 1 that the introduction of chlorine and fluorine at the C12 position decreases the binding affinity to 117.72 and 121.20 kcal/mol. It appears that both the halogens have failed to form strong hydrogen bonds with receptor and thus attains moderate binding affinity. Substitution of thiol at the C12 does not produce the required effect and it attains the binding energy of 119.32 kcal/mol.

Likewise the substitution of guanidino and tri fluoro carbon decreases the binding affinity of oseltamivir due to the lack of delocalization of π electrons between C2=C14 bond. It is clear from table 1 that the substituents like tri fluoro cadrbon, guanidino and thiol at the C12 position decreases the electron density on C2 atom and decreases the binding affinity. Summary of table 1 predicts that the alkyl groups like ethyl and methyl increases the binding affinity of oseltamivir and become the potent antiviral inhibitor.

SOLVATED LIGAND BINDING ENERGY

The study of binding affinity of tamiflu in solvent phase in inevitable because its interaction with protein and amino acids are involved in the solvent phase. Parent oseltamivir provides provides solvated binding energy of 20.82 kcal/mol. Substitution of methoxy group at the C12 position decreases the solvated binding energy to 14.03 kcal/mol.

The hydrophobic methoxy group fails to bind effectively with receptor and as a result it attains low binding affinity. Introduction of ethoxy group at the C12 position also decreases the binding affinity, it is due to the polarisation of the ethoxy group at the C12 position. Likewise methyl group at C12 position decreases the binding affinity to 13.84 kcal/mol due to its bulkiness and hydrophobic nature. Substitution of ethyl at C12 position decreases the binding affinity to 14.54 kcal/mol. It is clear from table 2 that alkyl group could not increase the binding affinity in solvent due its bulky hydrophobic behaviour. However, alkyl group increases the binding affinity in gas phase and becomes the potential candidate for the antiviral inhibitor. Substitution of chlorine in the C12 position decreases the binding affinity and yields binding energy of 117.72 kcal/mol. Likewise fluorine at C12 position of oseltamivir has no effect on binding affinity and attains binding energy of 121.2 kcal/mol

Table 2: Solvated Binding energy of C12 substituents .

Sl No.	C12 substituents of oseltamivir	Ligand Binding Energy HF/6-31G(d) (kcal/mol)	Ligand Binding Energy B3LYP/6-31G(d) (kcal/mol)	Mulliken Charge on C13 carbon
1	Oseltamivir	3.59	20.83	-0.107
2	Methoxy	6.66	14.81	-0.1
3	Ethoxy	6.85	15.07	-0.101
4	Methyl	5.11	13.84	-0.112
5	Ethyl	5.36	14.54	-0.112
6	Chlorine	0.63	12.85	-0.093
7	Fluorine	5.86	14.12	-0.093
8	Thiol	5.38	13.69	-0.1
9	Guanidino	11.19	16.66	-0.096
10	Tri fluoro carbon	5.18	14.09	-0.093

It is clear from table 2 that halogens at the C12 position disperses the electron density at the C2 carbon and causes low binding affinity and hence its effect remains futile. Substitution of sulphur based thiol at the C12 position also decreases the binding affinity and attains the binding energy of 119.32 kcal/mol. Substitution of thiol decreases the binding affinity due to hydrophobic nature. Substitution of guanidino at the C12 position decreases the binding affinity to 118.38 kcal/mol.

Guanidino substituent decreases the electrostatic and polarization charge transfer in the solvation medium and thus decreases the binding affinity. Trifluoro carbon at the C12 position have little effect on the binding affinity and attains the binding energy of 120.8 kcal/mol. Tri fluoro carbon at C12 position increases the charge transfer between C12=C3 bond by decreasing the bond length from 1.326Å to 1.324Å. Although it has effective charge transfer between C12 and C3 carbon; the hydrophobic nature of fluorine causes low binding affinity.

EFFECT OF C6 SUBSTITUENT ON OSELTAMIVIR

The di-methoxy group present at the C12 position of oseltamivir kept constant and the amino group present at the C6 position replaced by various substituents to evaluate the binding affinity of C6 modified oseltamivir and reported results in table 3. Introduction of guanidino at the C6 position provides binding energy of 121.35 kcal/mol and thereby it shares same platform with amino group. The presence of two amino groups in the guanidino substituent does not make any significant change in binding affinity. Substitution of methyl group at C6 position of oseltamivir yields binding energy of 121.15 kcal/mol. It appears that the methyl group at C6 also has the same effect as amino group.

Table 3: Effect of C6 Substituents on the Binding Affinity of oseltamivir Compound.

Serial No.	Substituents	Binding energy HF/6-31G(d) Kcal/mol	Binding energy RB3LP/6-31G (d) Kcal/mol	Electronic Charge density on C4 carbon	Dipole moment (Debyes)
1	Oseltamivir	118.34	122.21	-0.107	9.07
2	Guanidino	117.73	121.35	-0.119	11.61
3	Methyl	116.82	121.15	-0.092	10.7
4	Fluorine	114.55	119.45	-0.12	11.39
5	Methyl amine	117.06	121.36	-0.092	10.61
6	Thiol	115.07	118.95	-0.091	10.96

It is apparent from table 3 that the methyl group increases the electron charge density on C2 by hyperconjugative effect and thus increases the binding affinity even though its bulkiness. Introduction of fluorine at the C6 position provides the binding affinity of 119.45 kcal/mol. It exhibits only a minor decrease in binding affinity in relative to the oseltamivir due to its negative inductive effect. The introduction of methyl amine at the C6 position attains the binding energy of 121.36 kcal/mol. It is clear from table 3 that the methyl amine increases the binding affinity by conjugative inductive effect and provides better binding affinity. The presence of amine group in methyl amine offers greater hydrogen bonding ability with the neighboring receptors and increases the binding affinity. Introduction of thiol at the C6 position decreases the binding affinity due to lack of hydrogen bonding with the receptors. The substitution of thiol at the C6 position poloarizes the oseltamivir molecule and decreases the binding affinity. In a nutshell the investigation reveals that among the five substituents only methylamine at C6 position increases the binding affinity and therefore it could acts a potential anti viral sialidase inhibitor in the future antiviral drug era.

CONCLUSION

Ab initio and DFT investigation of C12 position of sialidase oseltamivir inhibitor reveals thatthe methyl and ethyl group have attained higher binding affinity due to their hyper conjugative effects and charge transfer effects. 12-methyl oseltamivir and 12-ethyl oseltamivir could become the potential candidate for the development of potent sialidase antiviral inhibitors. Apart from the alkyl group, the alkoxy groups such as methoxy and ethoxy group also shows better binding affinity than the parent oseltamivir compound and further investigation on this compounds might produce promising results to produce the more potent sialidase inhibitors. The analysis of binding affinity of C12 substituents of oseltamivir compound in solvent phase indicates that the parent oseltamivir attains better binding affinity that all other substituents due to the lack of polarization effect and hydrogen bonding affinity with the neighboring receptors. The theoretical investigation of C6 position of oseltamivir inhibitor discloses that among all the five substituent, the methyl group increases the binding affinity. The presence of methyl group at the C6 position increases the charge transfer between C4=C13 by hyperconjugative effect and consequently increases the binding affinity. Hence the investigation concludes that the 6-methyl oseltamivir and 12-methyl oseltamivir compounds have shown better binding affinity and thus it paves way for development and design of potential sialidase antiviral inhibitor.

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