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Molecular Modeling and Docking Studies of Neu5Ac2en analogues against Cholera toxin

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Abstract

Neu5Ac2en (2-deoxy-2, 3-didehydro-N-acetylneuraminic acid) analogues were modified in two different positions C-4 and C-9 were investigated using molecular modeling and molecular docking techniques. Cholera toxin is an protein complex made up of AB_5 subunits secreted by the pathogenic organism Vibrio cholerae. In present days these organism shows resistance towards antibiotics. In our present study, Cholera toxin 3D protein structure was optimized and minimized using maestro v9.2. Twelve synthetic Neu5Ac2en analogues were modeled using ACD/ChemSketch and optimized in LigPrep which is a tool in Schrödinger suite. Active site of cholera toxin protein was analyzed using PDBsum database. Molecular docking of Neu5Ac2en analogues into the active site of cholera toxin protein were carried out using Glide v5.7. All the 12 analogues of Neu5Ac2en show good binding affinity towards the cholera toxin with least docking (XPG) energy score and also these analogues have good pharmacological properties. Neu5Ac2en analogues blocks the binding site residues of cholera toxin directly through intermolecular hydrogen bonding.

Keywords: Molecular modeling, molecular docking, Neu5Ac2en analogues, Cholera toxin.

Introduction

Sialic acid or N-acetylneuraminic acid (Neu5Ac or NeuNAc) is the derivatives of neuraminic acid with 9 carbon monosaccharides found in the terminal components of glycoconjugates found in the cell surface of mammalian and blood cells of some non-mammalian and they involved in cellular and molecular interactions[1-3]. Sialic acid residues mask the bacterial cell surface from the host immune system and some of the bacterial pathogens are *Campylobacter jejuni*[4], *Escherichia coli*[5], *Haemophilus influenza*[6], *Haemophilus ducreyi*[7], *Neisseria gonorrhoeae*[8], *Neisseria meningitides*[9] and *Streptococcus agalactiae*[10]. Sialic acids are the natural ligands for both Hemagglutinin (HA) and Neuraminidase (NA)[11]. It is difficult to explain the general role of sialic acid because it involve directly or indirectly in various cellular events. Due to their negative charge Neu5Ac functions are divided into two groups. First, sialic acid act as the masking agent which protects recognition sites such as proteins, macromolecules and receptor molecules. Second, sialic acid is the ligand for large number of molecules such as hormones, lectins, antibodies, and inorganic cations[12].

2-deoxy-2,3-didehydro-D-N-acetylneuraminic acid (Neu5Ac2en) is the derivative of unsaturated sialic acid. Neu5Ac2en is a sialosyl cation transition-state analogue and it is a strong inhibitor core template. The structurally modified Neu5Ac2en analogues give more effective inhibitors[13]. Also Neu5Ac2en and N-acyl derivatives inhibit Newcastle disease virus (NDV), simian virus 5, and Sendai virus sialidases[14].

Cholera toxin (CT) is a protein complex produced by the bacterium *Vibrio cholera*[15]. CT is made up of six protein subunits one A subunit with enzymatic activity and five B subunit with receptor binding. *Vibrio cholera* sticks to the human small intestinal epithelium cells and cause diarrhea due to the production of cholera toxin. In 1970s ganglioside (G_{M1}) was recognized as the receptor for CT[16]. However, antibiotics are commonly administered as part of the treatment regimen. In present days, the organism becomes resistance towards the multiple antibiotics[17]. The active site of cholera toxin is found in the single B-subunit and also a single solvent mediated hydrogen bond from the amino acid residue Gly 33 to the neighboring subunit. The majority of the interaction between the receptor and the cholera toxin is due involvement of two terminal sugar of G_{M1} such as galactose and sialic acid[18]. The binding site of cholera toxin can be blocked by inhibition using sialic acid analogues[19].

In our present work, molecular modeling of Neu5Ac2en and its derivatives have substituted in two different positions C-4 and C-9. The Neu5Ac2en analogues were modeled using chemical drawing software chemsketch. Absorption, Distribution, Metabolism and Excretion (ADME) properties were done using QikProp to know the pharmacological activity of the compound as the drug molecule. The 3D crystal structure of cholera toxin is available in the protein data bank (PDB). Docking was done to study the binding mode of Neu5Ac2en analogues into the binding pocket of cholera toxin. Furthermore we describe the effective interaction between the Neu5Ac2en analogues and the target protein cholera toxin.

Material and Methods

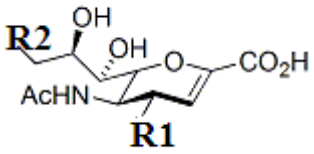
Molecular Modeling of Neu5Ac2en analogues and their modifications in two different positions C-4 and C-9 were collected from the literature shown in (table-1). The Neu5Ac2en derivatives are designed as described by Suzuki, *et al*.,[20].

Molecular Docking: Ligand preparation: The two dimensional chemical structures of the molecule are converted into three dimensional structures using a tool LigPrep in Schrödinger suite[21]. LigPrep produces different conformation of structure from each input with different ring conformations, ionization states, tautomers and stereochemistries. Ligands are minimized based upon the force field OPLS (Optimized Potentials for Liquid Simulations)[22]. The pharmacological properties of the ligands were calculated using QikProp software. In our study all the 12 compounds are biological active compounds.

Protein preparation: The 3D crystal structure of cholera toxin protein PDB id is 3CHB was downloaded from the protein data bank (PDB). The structural features and active site residues of the choler toxin protein 3CHB were analyzed using PDBsum database. Protein preparation wizard Maestro v9.2 is used to fix the atomic representations of protein and its optimization[23]. Minimization was carried out using molecular mechanics force field OPLS[22]. A receptor grid was generated in the binding pocket of cholera toxin protein using Glide v5.7[24].

Glide Docking: Two molecules bound to each other to form a stable complex to predict the preferred orientation of the both molecule is called docking[25]. Docking was carried out using the software Glide v5.7[24]. In the grid box of protein the prepared and optimized ligands were flexibly docked using Monte carlo based simulation algorithm (MCSA) based minimization. In our study two subsequent docking procedure were used they are standard precision (SP) and extra precision (XP)[26]. 32 poses were generated for each ligand during XP docking the conformation of each ligand with best pose was retaining after post docking. The molecules were ranked based on XPG score with least binding energy and glide score.

Table-1
Neu5Ac2en Analogues and their derivatives

Neu5Ac2en derivatives	**Substituent**	
 **Neu5Ac2en**	**R1=OH**	**R2=OH**
4-O-amidinomethyl-Neu5Ac2en	$OCH_2C(=NH)NH_2$	OH
4-O-carbamoylmethyl-Neu5Ac2en	$OCH_2C(=O)NH_2$	OH
4-O-thiocarbamoylmethyl-Neu5Ac2en	$OCH_2C(=S)NH_2$	OH
4-O-cyanomethyl-Neu5Ac2en	OCH_2CN	OH
9-acetamido-4-O-amidinomethyl-Neu5Ac2en	$OCH_2C(=NH)NH_2$	NHAc
9-acetamido-4-O-carbamoylmethyl-Neu5Ac2en	$OCH_2C(=O)NH_2$	NHAc
9-acetamido-4-O-thiocarbamoylmethyl-Neu5Ac2en	$OCH_2C(=S)NH_2$	NHAc
9-acetamido-4-O-cyanomethyl-Neu5Ac2en	OCH_2CN	NHAc
9-azido-4-O-amidinomethyl-Neu5Ac2en	$OCH_2C(=NH)NH_2$	N_3
9-azido-4-O-carbamoylmethyl-Neu5Ac2en	$OCH_2C(=O)NH_2$	N_3
9-azido-4-O-methoxy-iminomethyl- Neu5Ac2en	$OCH_2C(=NH)OCH_3$	N_3

Results and Discussion

Structural analysis of 3CHB: The three dimensional structure of cholera toxin protein 3CHB in complex with GAL-NGA-GAL-GLU-SIA were downloaded from PDB. The protein consists of 103 residues and comprises of 2 helices and 6 strands. The residues such as GLU11, TYR12, HIS13, LYS34, GLU51, GLN56, HIS57, ILE58, GLN61, TRP88, ASN90 and LYS91 were localized to be in van der Waal contact with GAL-NGA-GAL-GLU-SIA constitute as the active site residues. 3CHB residues GLU11, TYR12, HIS13, GLY33, LYS34, GLU51, GLN56, HIE57, ILE58, GLN61, TRP88, ASN90 and LYS91 were involved in hydrophobic interactions with GAL-NGA-GAL-GLU-SIA (figure 1). The above analysis were done using PDBsum database[27].

Cholera toxin- Neu5Ac2en analogues docking complex: Cholera toxin-Neu5Ac2en analogues docking complex was shown in (table-2 and 3). The good interaction between these analogues is due to various interactions such as hydrogen bond, hydrophobic, hydrophilic, electrostatic and steric interactions. Neu5Ac2en analogues such as Neu5Ac2en show least glide score of -8.08 and glide energy of -39.92 Kcal/mol with seven intermolecular hydrogen bond, 4-O-carbamoylmethyl-Neu5Ac2en -7.46 and -40.40 Kcal/mol with four intermolecular hydrogen bond, 4-O-cyanomethyl-Neu5Ac2en -7.37 and -43.97 Kcal/mol with seven intermolecular hydrogen bond, 4-O-thiocarbamoylmethyl-Neu5Ac2en -7.35 and -41.25 Kcal/mol with four intermolecular hydrogen bond, 9-azido-4-O-carbamoylmethyl-Neu5Ac2en -7.15 and -45.88 Kcal/mol with seven intermolecular hydrogen bond, 9-acetamido-4-O-cyanomethyl-Neu5Ac2en -7.09 and -41.01 with seven intermolecular hydrogen bond, 4-O-amidinomethyl-Neu5Ac2en -6.84 and -37.99 Kcal/mol with seven intermolecular hydrogen bond, 9-acetamido-4-O-carbamoylmethyl-Neu5Ac2en -6.75 and -36.36 Kcal/mol with five intermolecular hydrogen bond, 9-acetamido-4-O-amidinomethyl- Neu5Ac2en -6.71 and -38.41 with five intermolecular hydrogen bond, 9-azido-4-O-amidinomethyl-Neu5Ac2en -6.53 and -39.78 Kcal/mol with four intermolecular hydrogen bond, 9-acetamido-4-O-thiocarbamoylmethyl-Neu5Ac2en -6.52 and -37.11 Kcal/mol with seven intermolecular hydrogen bond and 9-azido-4-O-methoxy-iminomethyl-Neu5Ac2en -6.18 and -46.29 Kcal/mol with five intermolecular hydrogen bond.

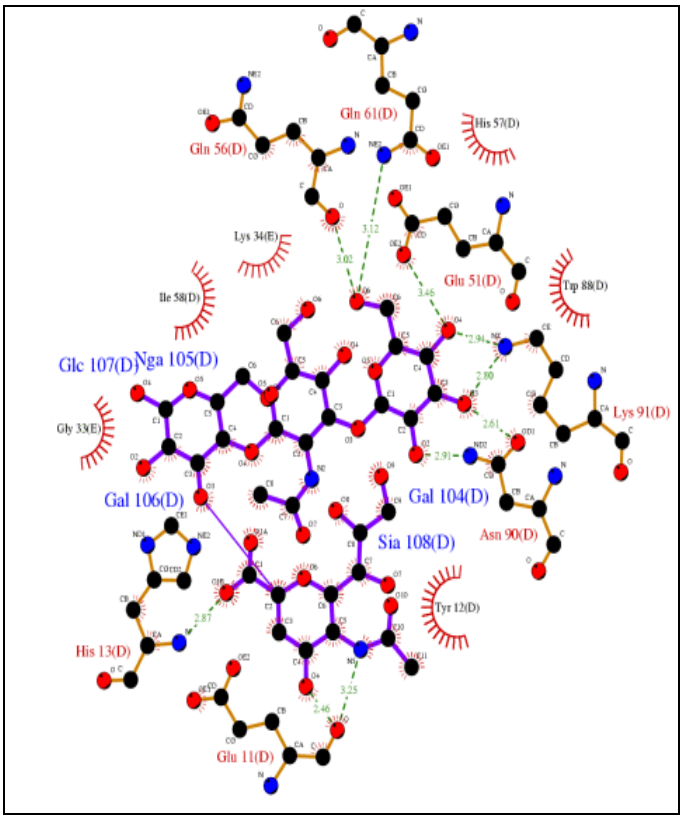


Figure-1
Graphical representation of interaction of ligands GAL-NGA-GAL-GLU-SIA with active site of 3CHB

Table-2
Glide docking score and glide energy of cholera toxin-Neu5Ac2en analogues

Neu5Ac2en derivative	Glide energy (Kcal/mol)	Glide score
Neu5Ac2en	-39.92	-8.08
4-O-carbamoylmethyl-Neu5Ac2en	-40.40	-7.46
4-O-cyanomethyl-Neu5Ac2en	-43.97	-7.37
4-O-thiocarbamoylmethyl-Neu5Ac2en	-41.25	-7.35
9-azido-4-O-carbamoylmethyl-Neu5Ac2en	-45.88	-7.15
9-acetamido-4-O-cyanomethyl-Neu5Ac2en	-41.01	-7.09
4-O-amidinomethyl-Neu5Ac2en	-37.99	-6.84
9-acetamido-4-O-carbamoylmethyl-Neu5Ac2en	-36.36	-6.75
9-acetamido-4-O-amidinomethyl-Neu5Ac2en	-38.41	-6.71
9-azido-4-O-amidinomethyl-Neu5Ac2en	-39.78	-6.53
9-acetamido-4-O-thiocarbamoylmethyl-Neu5Ac2en	-37.11	-6.52
9-azido-4-O-methoxy-iminomethyl- Neu5Ac2en	-46.29	-6.18

Table-3
Intermolecular hydrogen bond interaction between the cholera toxin-Neu5Ac2en analogues

Neu5Ac2en derivative	Ligand atom	Protein		Distance (Å)
		Residue	Atom	
Neu5Ac2en	O38	LYS:91	HZ1	1.774
	H33	GLN:56	O	1.684
	H37	GLN:56	O	2.121
	O	GLN:61	HE22	2.477
	O	ASN:90	HD22	1.817
	H30	HIE:13	O	1.859
	H32	HIE:13	O	1.914
4-O-carbamoylmethyl-Neu5Ac2en	H35	ASN:90	OD1	1.976
	O19	TRP:88	HE1	1.972
	O23	HIE:13	H	1.993
	H34	GLU:51	OE1	1.732
4-O-cyanomethyl-Neu5Ac2en	H33	GLU:51	OE1	2.023
	O	LYS:91	HZ1	1.860
	O42	ASN:90	HD22	1.561
	H34	GLN:56	O	2.376
	H35	GLN:56	O	2.096
	O15	GLN:61	HE22	2.431
	O19	TRP:88	HE1	2.187
4-O-thiocarbamoylmethyl-Neu5Ac2en	O19	GLN:61	HE21	1.877
	H34	ILE:96	O	2.016
	H36	ILE:96	O	1.837
	H37	ALA:97	O	1.625
9-azido-4-O-carbamoylmethyl-Neu5Ac2en	N25	HIE:13	HE2	2.055
	H36	HIE:13	O	2.034
	O	ASN:90	HD22	2.207
	O	ASN:90	HD22	2.248
	O46	LYS:91	HZ1	1.696
	H45	GLN:56	O	1.974
	H44	GLN:61	OE1	1.997
9-acetamido-4-O-cyanomethyl-Neu5Ac2en	H39	GLN:56	O	2.027
	O14	TRP:88	HE1	2.397
	O	ASN:90	HD22	2.041
	O48	LYS:91	HZ2	1.916
	O15	HIE:13	H	2.045
	H38	HIE:13	O	2.064
	H36	HIE:13	O	2.031
4-O-amidinomethyl-Neu5Ac2en	O46	LYS:91	HZ1	1.788
	H42	GLN:56	O	1.910
	O	ASN:90	HD22	1.818
	H35	HIE:13	O	2.473
	H36	HIE:13	O	1.812
	O15	TRP:88	HE1	2.166
	O13	HIE:13	H	1.832
9-acetamido-4-O-carbamoylmethyl-Neu5Ac2en	H39	GLN:61	OE1	1.939
	H38	GLN:56	O	1.784
	O51	LYS:91	HZ1	1.952
	O	ASN:90	HD22	1.877
	O26	HIE:13	H	2.044
9-acetamido-4-O-amidinomethyl-Neu5Ac2en	O19	GLN:61	HE21	2.097
	H51	GLU:11	OE2	1.875
	H49	GLU:11	OE2	1.980
	H39	ALA:97	O	2.164
	H37	ALA:97	O	1.981

Neu5Ac2en derivative	Ligand atom	Protein		Distance (Å)
		Residue	Atom	
9-azido-4-O-amidinomethyl-Neu5Ac2en	O14	TRP:88	HE1	2.008
	H37	HIE:13	O	2.022
	O	ASN:90	HD22	1.691
	O47	LYS:91	HZ1	1.704
9-acetamido-4-O-thiocarbamoylmethyl-Neu5Ac2en	O22	GLN:61	HE22	2.270
	H39	GLN:56	O	1.978
	H38	GLN:56	O	1.878
	O	LYS:91	HZ1	1.771
	O51	ASN:90	HD22	1.893
	H49	ASN:90	OD1	2.042
	H50	HIE:13	O	2.069
9-azido-4-O-methoxy-iminomethyl- Neu5Ac2en	O18	GLN:61	HE21	2.033
	N25	LYS:91	HZ2	1.942
	H37	GLN:56	O	1.864
	H45	GLU:11	O	1.924
	O49	HIE:13	H	1.986

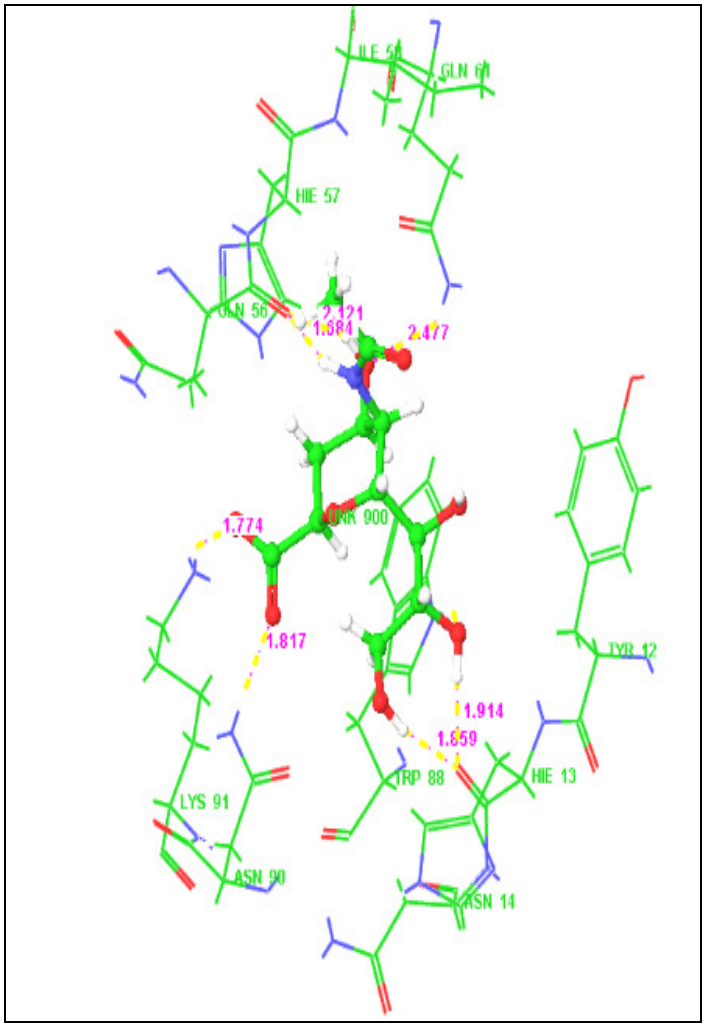


Figure-2
Neu5Ac2en docked in cholera toxin binding pocket

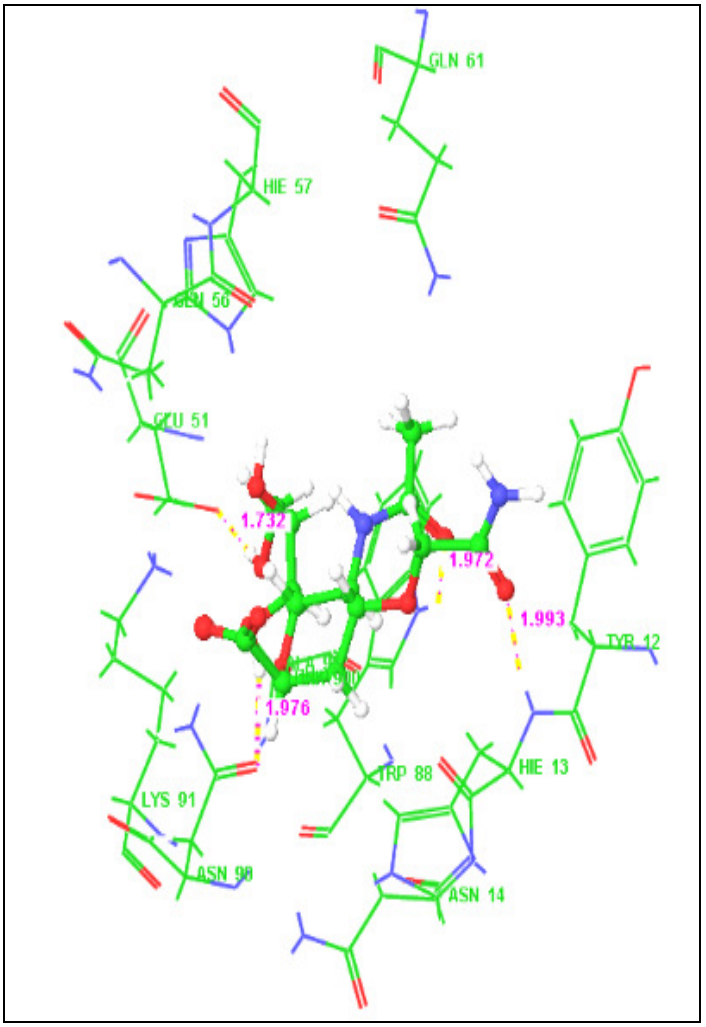


Figure-3
4-O-carbamoylmethyl- Neu5Ac2en docked in cholera toxin binding pocket

Table-4
QikProp Pharmacopore Prediction

Ligands Name	Molecular Weight	H-bond donors	H-bond acceptors	QPlogPo/w
Neu5Ac2en	293.273	6	13	-2.452
4-O-carbamoylmethyl-Neu5Ac2en	350.325	7	15	-3.652
4-O-cyanomethyl-Neu5Ac2en	332.310	5	14	-2.651
4-O-thiocarbamoylmethyl-Neu5Ac2en	366.385	7	15	-2.224
9-azido-4-O-carbamoylmethyl-Neu5Ac2en	375.338	6	16	-3.980
9-acetamido-4-O-cyanomethyl-Neu5Ac2en	373.362	5	15	-2.831
4-O-amidinomethyl-Neu5Ac2en	349.340	8	14	-2.792
9-acetamido-4-O-carbamoylmethyl-Neu5Ac2en	391.377	7	16	-3.952
9-acetamido-4-O-amidinomethyl-Neu5Ac2en	390.392	8	15	-3.187
9-azido-4-O-amidinomethyl-Neu5Ac2en	374.353	7	15	-3.229
9-acetamido-4-O-thiocarbamoylmethyl-Neu5Ac2en	407.438	7	15	-2.388
9-azido-4-O-methoxy-iminomethyl-Neu5Ac2en	389.364	5	15	-2.225

QikProp pharmacokinetic prediction: QikProp is used to predict the molecular properties of the known drug. Pharmacokinetic properties like molecular weight, hydrogen bond donor, hydrogen bond acceptor, oral absorption, QPlogPo/w are calculated based on Lipinski's rule.

Conclusion

Docking study revealed that the Neu5Ac2en analogues with substitution in two different position C-4 and C-9 have good interaction in the binding site of cholera toxin protein. Among the 12 analogues 6 shows least glide (docking) score for the compounds Neu5Ac2en, 4-O-carbamoylmethyl-Neu5Ac2en, 4-O-cyanomethyl-Neu5Ac2en, 4-O-thiocarbamoylmethyl-Neu5Ac2en, 9-azido-4-O-carbamoylmethyl-Neu5Ac2en, 9-acetamido-4-O-cyanomethyl-Neu5Ac2en such as -8.08, -7.46, -7.37, -7.35, -7.15 and -7.09 respectively and minimum glide energy such as -39.92 Kcal/mol, -40.40 Kcal/mol, -43,97 Kcal/mol, -41.25 Kcal/mol, -41.01 Kcal/mol and -41.01 Kcal/mol respectively. Neu5Ac2en analogues blocked the active site residues LYS:91, GLN:56, GLN:61, ASN:90, HIE:13, ALA:97, GLU:11, TRP:88 and ILE:96 directly through intermolecular hydrogen bonding. Neu5Ac2en analogues have better pharmaceutical properties thus it could be used as futuristic drug for cholera.

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