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Ganglioside GM3 analogues as inhibitors for Staphylococcal Enterotoxin B and Endoglycoceramidase II from *Rhodococcus Sp.* Docking and ADME screening studies

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ABSTRACT

Modeled library of Ganglioside GM3 analogues with structural modification at different R positions were substituted to the original structure of GM3. Their molecular interactions and binding affinities with Staphylococcal Enterotoxin B (SEB) and Endoglycoceramidase II of *Rhodococcus Sp.* have been computationally studied using the docking-molecular mechanics based generalized Born/surface area (MM-GB/SA) solvation model. The absorption, distribution, metabolism and excretion (ADME) properties are considered for the final screening of these analogues. The proposed GM3 analogues are docked into the active site of Staphylococcal Enterotoxin B (SEB) and Endoglycoceramidase II of *Rhodococcus Sp.* These analogues qualify ADME properties and showed suitable drugable characters. The present work shed more light to modify the different R substituent in the GM3 scaffold to model and prepare synthetic analogues for drug development against the bacterial toxin Staphylococcal Enterotoxin B and Endoglycoceramide II from *Rhodococcus sp.*

INTRODUCTION

The availability of three dimensional structural details of proteins and receptors fuel the modern approaches for designing new leads for therapeutic targets. Docking simulation is an effective way to predict binding structure of a substrate in its receptor and it has been successfully proved in many applications. Rational drug design applies the combinations of the docking method with MD simulation, free energy binding calculation, comparative molecular field analysis (CoMFA) and comparative molecular similarity indices analysis (CoMSIA) which will helpful in revealing lots of insights on biological systems. There are many reports suggesting the calculation of free energy of binding (FEB) and enabled in different applications. [1] have successfully applied Monte Carlo and Linear Response Equation (LRE) on many systems to calculate binding affinities. [2] reported the molecular mechanics based Poisson-Boltzmann/surface area (MM-PBSA) solvation model and the method is incorporated in the predication of activity of 12 TIBO-like inhibitors [3].

The unacceptable absorption, distribution, metabolism and excretion (ADME) properties of new drug candidates accounts for the cause of failures in several clinical phase drug development [4] in the pharmaceutical industry that cost lot of loss. Thus, the investigation of the ADME properties of new chemical entities via *in vitro* approaches are now widely used in addition to computational (*in silico*) modelling as a tool to optimize selection of the most suitable candidates for drug development.

In the present work Ganglioside GM3 and 23 structural GM3 derivatives were used to study the binding modes and binding affinities to the receptor. The flexible docking (Glide) approach to predict the "preferable" binding structure of a ligand for the 2 bacterial toxins (Staphylococcal Enterotoxin B (SEB) and Endoglycoceramidase II of *Rhodococcus Sp.*) is used to study the association of the ligands with the receptor further. We used traditional, simple, fast and straightforward molecular mechanics methods to calculate ligand-receptor interaction energies (electrostatic energy (*Gele*), van der Waals energy (*GvdW*)), with a generalized-Born/surface area (GB/SA) solvation method for electrostatic part of solvation energy (*Gsolv*) [5-7] and solvent-accessible surface for the nonpolar part of solvation energy [8]. It benefits the calculation of relative binding affinity needed to evaluate the activity of large set of molecules in rational drug design. The final screening of the GM3 and its analogues for the probable absorption, distribution, metabolism and excretion (ADME) properties are calculated using the Qikprop program (Schrödinger, Inc.).

Hence, in the present work, we have set out to study the

binding mode of GM3 analogues using a combined approach of docking-MM-GB/SA and their final screening based on ADME properties, leading to successful drug development.

MATERIALS AND METHODS

Preparation of protein target structure

The cartesian co-ordinates for atoms of the bacterial toxins (Staphylococcal Enterotoxin B and Endoglycoceramidase II from *Rhodococcus Sp*.) were taken from the Protein Data Bank and further modified to be used for Glide docking (Schrödinger, Inc.). The complex were loaded into maestro window (Schrödinger, Inc.), the co-crystallized ligands were identified and removed from the structure and the protein preparation wizard (shipped by Schrödinger) was used to minimize the protein using by applying (Optimized Potential for Liquid Simulations) OPLS_2005 force field by application of the autoref.pl script. Non hydrogen atoms were subjected to progressively weaker restraints. Since the Glide uses the full OPLS-AA force field at an intermediate docking stage and is claimed to be more sensitive towards geometric details than other docking tools, the refinement procedure is necessary and it is recommended by Schrödinger software. The convergence criteria for minimizations were until the average root mean square deviation of the non-hydrogen atoms reached 0.1 Å.

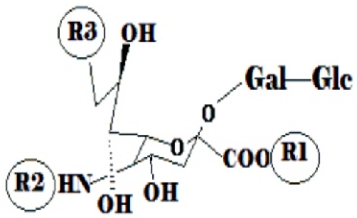
Preparation of compound libraries

The library of analogues was generated by modifying the respective functional groups (R) in the scaffold structure with sterically and conformationally allowed substituents. GM3 analogues are divided into 2 phases based on the substitutions at the R position. The GM3 analogues with single substituents and multiple substituents modeled in this study are compiled in Table 1 and Table 2. In the Single substitution, the C-9/C-5/C-1 positions respectively [9] have been modified, whereas in multiple substitutions, C-1/C-4/C-8/C-9 has been substituted [10-11]. The inhibitors were converted to .mae format (Maestro, Schrödinger Inc.) and optimized by means of the (Merck Molecular Force Field) MMFF94 force field using default setting.

Glide-High Throughput Virtual Screening (HTVS)

Virtual screening (VS) is a computational technique used in drug discovery research. It involves the rapid *in silico* assessment of large libraries of chemical structures in order to identify those structures most likely to bind to a drug target; typically a protein receptor or enzyme [12, 13].Virtual screening has become an integral part of the drug discovery process. Related to the more general and long pursued concept of the database searching, the term "Virtual Screening" is relatively new. Walters *et al.,* 1988 [13] define virtual screening as "automatically evaluating very

Table1: Ganglioside GM3 analogues with single substituent modification at C-9/C-5/C-1 positions in its NeuAc

S.NO	GANGLIOSIDE GM3 DERIVATIVE	SUBSTITUENTS		
		R1	R2	R3
	GM3	H	CH3CO	HO
1.	9-Deoxy-NeuAc	H	CH3CO	H
2.	9-Amino-NeuAc	H	CH3CO	H2N
3.	9-Acetamido-NeuAc	H	CH3CO	CH3CO-NH
4.	9-N-Gly-NeuAc	H	CH3CO	H2NCH2C0-NH
5.	9-N-Succ-NeuAc	H	CH3CO	HOOC(CH2)2CONH
6.	9-iodo-NeuAc	H	CH3CO	I
7.	9-thio-NeuAc	H	CH3CO	HS
8.	9-ScH3-NeuAc	H	CH3CO	CH3S
9.	5-N-fluoroac-neu	H	FCH2CO	HO
10.	5-N-trifluoroac-Neu	H	CF3CO	HO
11.	5-N-Gly-Neu	H	H2NCH2CO	HO
12.	5-N-Succ-Neu	H	HOOC(CH2)2CO	HO
13.	NeuAc-Me-ester	H3C	CH3CO	HO
14.	NeuAc-Et-ester	H5C2	CH3CO	HO

libraries of compounds" using computer programs.

In a HTVS, each chemical compound available in the library is sequentially docked at every possible pose at the active site of the target molecule (often protein). Docked molecules with the lowest free energy of interactions are ranked against all docked molecules in the list and the best ranked could be considered as potential 'hits'. These sets of compounds can be subjected to experimental assays to determine if they are appropriate to select as lead for the optimization studies. HTVS is performed by importing 23 energy minimized GM3 analogues using Glide module of Schrodinger software. Primarily the protein is prepared using the protein preparation wizard.

Induced Fit: Fitting of GM3 analogues into the binding site of Bacterial Toxins using Glide Sp & Xp Algorithm

Induced fit is a novel method for fast and accurate prediction of ligand induced conformational changes in receptor active sites. The active site geometry of a protein complex depends heavily upon conformational changes induced by the bound ligand. However, resolving the crystallographic structure of a protein-ligand complex requires a substantial investment of time, and is frequently infeasible or impossible. Schrodinger's induced fit (IFD) protocol solves this problem by using glide and prime to exhaustively consider possible binding modes and the associated conformational changes within receptor active sites [14].

Induced fit is carried out for the top three scoring GM3 analogues to predict the ligand induced conformational changes in receptor active sites. The best 10 poses and corresponding scores have been evaluated using Glide in single precision mode (Glide SP) for the each modeled ligand. For each screened ligand, the pose with the lowest Glide SP score have been taken as the input for the Glide calculation in Extra Precision mode (Glide XP).

Table 2: Ganglioside GM3 analogues with multiple modifications at with C-1/C-4/C-8/C-9 positions in its NeuAc

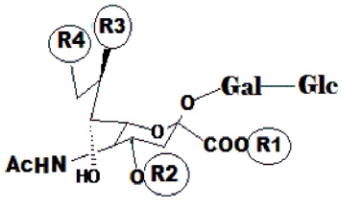
	GANGLIOSIDE GM3 DERIVATIVE	SUBSTITUENTS			
S.NO		R1	R2	R3	R4
	GM3	H	H	OH	OH
1.	methyl 5-N-acetyl Neuraminate	CH3	H	OH	OH
2.	Benzyl 2alpha-O-methyl-5-n-acetyl-8,9-O-isopropylidene Neuraminate	CH2Ph	H	OCH3	OCH3
3.	Benzyl 2alpha-O-methyl-4-O-Capriloyl-5-N-n-acetyl-8,9-O-isopropylidene Neuraminate	CH2Ph	CO2(CH2)6CH3	OCH3	OCH3
4.	Benzyl 2alpha-O-methyl-4-O-Capriloyl-5-N-n-acetyl- Neuraminate	CH2Ph	CO2(CH2)6CH3	OH	OCH3
5.	2alpha-O-methyl-4-O-Capriloyl-5-N-n-acetyl- Neuraminic acid	H	CO2(CH2)6CH3	OH	OCH3
6.	Benzyl 2alpha-O-methyl-4-O-(8-morpholin)-Capriloyl-5-N-n-acetyl-8,9-O Isopropylidene Neuraminate	CH2Ph	CO2(CH2)7O(CH2CH2)2NH	OCH3	OCH3
7.	Benzyl 2alpha-O-methyl-4-O-(8-morpholin) Capriloyl-5-N-n-acetyl-Neuraminate	CH2Ph	CO2(CH2)7O(CH2CH2)2NH	OH	OH
8.	2alpha-O-methyl-4-O-(8-morpholin)-Capriloyl-5-N-n-acetyl-Neuraminic acid	H	CO2(CH2)7O(CH2CH2)2NH	OH	OH
9.	5-N-Acetyl-9-amino -9-deoxy-Neuraminic acid	H	H	OH	NH2

Table 3 : Information for the crystal structures of the proteins used in the study.

PDB Code	Classification	Resolution (Å)	*R*-value	No. of residues
1SE3	Toxin	2.30	0.220	239
2OSX	Hydrolase	1.10	0.122	481

Glide docking and scoring function

Schrödinger, Inc. Impact version v50109 [15-17] is used for Docking calculations. It's Glide module executes grid-based ligand docking using energetic and favorable interactions between one or more typically small ligand molecules and a typically larger receptor molecule, usually a protein is analysed. Test calculations are recommended by Schrödinger with different scaling factors for the receptor and ligand atom through van der Waals radii, because steric repulsive interactions might otherwise be overemphasized, leading to rejection of overall correct binding modes of active compounds.

After fixing the initial configurations for protein and ligands are in correct form for docking, the receptor-grid files were generated using grid-receptor generation program. At the centroid of the active site, a grid box of size was generated and the sizes of ligands to be docked were selected from the workspace. "Single precision" and "xtra precision" Glide algorithm is used for docking the ligands into the active site. The ligand is first placed at the centre at various grid positions of a 1 Å grid and three Euler angles rotation is applied. The unfavourable binding modes are eliminated at this stage using crude score values and geometric filters. A grid-based force field evaluation, torsional & rigid-body movements of the ligand is calculated for refinement of docking solutions. The force field selected to be used is OPLS-AA. The final energy evaluation is done with Glide score (*GScore*) and a single best pose is generated as the output for a particular ligand.

$$GScore = a * vdW + b * Coul + Lipo + Hbond + Metal + BuryP + RotB + Site$$

where *vdW* is van der Waals energy; *Coul*, Coulomb energy; *Lipo*, lipophilic contact term; *HBond*, hydrogen-bonding term; *Metal*, metal-binding term; *BuryP*, penalty for buried polar groups; *RotB*, penalty for freezing rotatable bonds; *Site*, polar interactions in the active site; and the coefficients of *vdW* and *Coul* are: $a = 0.065, b = 0.130$.

The model energy score (*Emodel*) that combines Glide score, the nonbonded interaction energy and the excess internal energy of the generated ligand conformation dictates the selection of the best docked structure for each ligand. The Coulomb-van der Waals interaction-energy score (CvdW) contributes the nonbonded interaction energy in Glide computation. CvdW is formulated to avoid overlay rewarding charge-charge interactions at the expense of charge-dipole and dipole-dipole interactions. This CvdW score is designed to be more suitable for the comparative study of the binding affinities of different ligands than the "raw" Coulomb-van der Waals interaction energy.

MM and binding free energies

The Glide-XP docking output was considered for the computation of free energy of binding (FEB) of the ligands with the two bacterial toxins and only the best scoring pose for each ligand was taken into consideration. Implicit solvent (generalized Born) energy minimization is carried out for each ligand to determine the protein-ligand complex energy ($E_{lig\text{-}prot}$), the free protein energy (E_{prot}), and the free ligand energy (E_{lig}). Calculation of ligand-receptor interaction energies (G_{ele}, G_{vdW}, G_{solv}) is performed using traditional MM methods with a Gaussian smooth dielectric constant function method [18] for electrostatic part of solvation energy and solvent-accessible surface for the nonpolar part of solvation energy. The energy difference was calculated using the equation:

$$\Delta E^{o} = E_{complex} - E_{ligand} - E_{protein}$$

The complete effects of relaxation and solvations are incorporated in this mode.

ADME screening

The absorption, distribution, metabolism, and excretion (ADME) properties of the analogues are computed using the QikProp program [19]. This computation reveals both physically significant descriptors and pharmaceutically relevant properties. All the analogues were loaded into Qikprop after essential neutralization. Because in normal mode QikProp is unable to neutralize a structure and no properties will be generated. 44 properties were predicted for the selected 23 molecules when the program was processed in normal mode with the following principal descriptors and the physiochemical properties in a detailed analysis: predicted octanol/water partition coefficient (QPlogPo/w), predicted octanol/gas partition coefficient (QPlogPoct), predicted water/gas partition coefficient (QPlogPw), predicted polarizability in cubic angstroms (QPpolrz), % human oral absorption in intestine (QP%), predicted brain/blood partition coefficient (QPlogBB), predicted IC50 value for blockage of HERG(Human Ether-à-go-go Related Gene) K^{+} channels (log HERG), predicted skin permeability (QPlogKp), prediction of binding to human serum albumin (QPlogKhsa), predicted apparent Caco-2 cell permeability in nm/sec (QPPCaco) and predicted apparent (Madin-Darby Canine Kidney Cells)MDCK cell permeability in nm/sec (QPPMDCK). Caco-2 cells are a model for the gut-blood barrier whereas MDCK cells are considered to be a good mimic for the blood-brain barrier. Lipinski's rule of 5 (number of violations of Lipinski's rule of five) is applied to evaluate the acceptability of the analogues which is essential for rational drug design. Poor absorption or permeation are more likely when a ligand molecule violates Lipinski's rule of five i.e., has more than 5 hydrogen bond donors, the molecular weight is over 500, the log P is over 5 and the sum of N's and O's is over 10.

RESULTS AND DISCUSSION

Computer Aided Structure Based Design of GM3 analogues as inhibitors for Staphylococcal Enterotoxin B and Endoglycoceramidase II from *Rhodococcus Sp.*

Two Protein Data Bank files (1SE3 and 2OSX) of high resolution crystal structures were used (Table 3) in the present docking study. The active site residues of Staphylococcal Enterotoxin B (1SE3) (Fig. 1) are Ile41, Ser52, Asp62, Asn63, Lys54, Asp108, Gln106 and the active site residues for the Endoglycoceramidase II from *Rhodococcus sp.* (2OSX) (Fig. 2) are Ser63, Ala155, Ile156, Lys66, Asp137, Gln233 and Trp178.

Staphylococcus Enterotoxin B

Molecular Docking of Staphylococcus Enterotoxin B and its GM3 analogues

High Throughput Virtual Screening was done for 23 GM3 analogues to find out the structures (ligands) most likely bind to the Staphylococcus Enterotoxin B (SEB). Among the 23 GM3 derivatives, 9-N-Gly-NeuAc (Table 1, analogue 4), NeuAc-Me-ester (Table 1, analogue 13), 5-N-Gly-Neu (Table 1, analogue 11), Ganglioside GM3, 9-ScH3-NeuAc (Table 1, analogue 2) are the ligands with best GLIDE score such as -5.2438, -4.8920, -4.6470, -4.5305 and -4.4735 respectively. The top 4 configurations were then subjected to SP and XP docking using GLIDE for further good results. The RMSD was calculated for each one in compare

Table 4: The docking results of Staphylococcus Enterotoxin B (1SE3) with GM3 analogues with its modifiedNeuAc using GLIDE-XP.

S.NO	TITLE	[a]GLIDE SCORE	[a]GLIDE ENERGY	[a]GLIDE EMODEL	FEB	[b]ΔE^o	[c]RMSD (Å)
1.	NeuAc-Et-ester (Phase 1, analogue 14)	-8.4518	-31.9123	-42.5209	-26.90	-18.34	0.30
2.	5-N-Succ-Neu (Phase 1, analogue 12)	-8.1510	-47.9314	-53.5102	-43.80	-35.25	0.65
3.	5-N-FluoroacNeu (Phase 1, analogue 9)	-8.0343	-53.0979	-64.7694	-31.85	-23.30	0.36
4.	9-N-Gly-NeuAc (Phase 1, analogue 4)	-7.8963	-50.0996	-62.5728	-8.55	0.0	0.5

[a]Glide Score, Energy and Emodel are expressed as kcal/mol; [b]$\Delta E^o = Ei - E$lowest; [c]RMSD between docked and crystallographic GM3.

Table 5: Inter molecular interactions between the ligand and the Staphylococcal Enterotoxin B.

S.NO	GM3 DERIVATIVE AND ITS MODIFIED NeuAc	POSE NO	LIGAND ATOM	PROTEIN		DISTANCE (Å)
				RESIDUE	ATOM	
	9-N-Gly-NeuAc (Phase 1, analogue 4)	7	O52	GLN:106	OE1	2.925
			N57	ASP:62	O	3.001
			O52	ASP:63	ND2	2.948
			N44	ASP:108	OD2	3.063
			O14	LYS:109	NZ	3.136
			O3	LYS:54	NZ	2.879
			O8	ASP:62	OD2	2.884
			O25	ASP:108	OD2	2.853
			O52	ASP:108	N	3.154
	5-N-fluoroAc-Neu (Phase 1, analogue 9)	1	O51	THR:99	N	3.410
			O43	ASN:63	OD1	2.768
			O25	ASN:108	OD2	2.914
			O8	ASP:62	OD1	2.454
			O14	ASP:62	OD2	3.192
			O51	ASP:108	OD1	2.604
			H71	ASP:108	O	2.895
	5-N-Succ-Neu (Phase 1, analogue 12)	2	O43	ASP:62	O	3.276
			O37	ASP:63	ND22	3.068
			O14	ASP:62	OD2	2.839
			O51	THR:99	N	3.290
			O51	ASP:108	OD1	2.850
			O8	ASP:62	OD1	2.786
			N44	ASP:108	OD2	3.153
			O34	ASP:108	OD2	2.841
			O37	GLN:106	OE1	2.561
	NeuAc-Et-ester (Phase 1, analogue 14)	1	O52	GLN:106	OE1	2.914
			O43	GLN:106	OE1	2.944
			O37	GLN:106	OE1	3.027
			O37	ASN:63	ND2	2.802
			O53	ASP:108	OD2	2.638
			O14	ASP:62	O	2.855
			O8	SER:52	O	3.126

Table 6: The docking results of Endoglycoceramidase II from Rhodococcus Sp. (2OSX) with GM3 analogues with its modified NeuAc using GLIDE-XP.

S.NO	TITLE	[a]GLIDE SCORE	[a]GLIDE ENERGY	[a]GLIDE EMODEL	FEB	[b]ΔE°	[c]RMSD (Å)
1.	Benzyl 2α-O-methyl-5-n-acetyl-8,9-O-isopropylidene Neuraminate (Phase 2,analogue 2)	-11.6826	-73.0531	-110.3517	-103.04	-47.64	0.32
2.	9-N-Gly-NeuAc (Phase 1, analogue 4)	-12.7926	-64.2530	-90.9000	-55.74	-0.34	0.5
3.	9-N-Succ-NeuAc (Phase 1, analogue 5)	-12.6862	-77.8319	-108.3060	-121.19	-65.79	0.56
4.	9-thio-NeuAc (Phase 1, analogue 7)	-12.9526	-55.8415	-90.1640	-55.40	0.0	0.54

[a]Glide Score, Energy and Emodel are expressed as kcal/mol, [b]$\Delta E^{o} = Ei - E$lowest; [c]RMSD between docked and crystallographic GM3.

Table 7: Inter molecular interactions of the ligand and the Endoglycoceramidase II from *Rhodococcus Sp.*

.NO	GM3 DERIVATIVE WITH ITS MODIFIED NeuAc	POSE NO	LIGAND ATOM	PROTEIN		DISTANCE
				RESIDUE	ATOM	(Å)
	Benzyl-2α-O-methyl-5-n-acetyl-8,9-O-isopropylidene Neuraminate (Phase 2, analogue 2)	12	O43	ILE:156	O	2.774
			O37	ALA:155	O	2.937
			O43	LYS:66	HZ3	2.881
			O8	GLU:233	OE1	2.738
			O37	LYS:66	HZ2	3.130
			O13	GLU:233	OE2	2.832
	9-N-Gly-NeuAc (Phase 1, analogue 4)	4	O 13	GLU:233	OE2	2.685
			O34	TRP:382	NE1	3.024
			O37	LYS:66	NZ	3.028
			O43	LYS:66	NZ	2.860
			O43	ILE:156	O	2.837
			O37	ALA:155	O	2.996
			N57	ASP:355	OD2	2.765
	9-N-Succ-NeuAc (Phase 1, analogue 5)	4	O37	ALA:155	O	3.063
			O52	GLU:179	OE2	2.880
			O13	GLU:233	OE2	3.048
			O8	GLU:233	OE1	2.604
			O43	ILE:156	O	2.853
			O43	LYS:66	NZ	2.863
			O60	LEU:310	O	3.138
			O46	TRP:389	NE1	2.846
	9-thio-NeuAc (Phase 1, analogue 7)	2	O37	ALA: 155	O	2..947
			037	LYS:66	NZ	3.114
			O14	LYS:66	NZ	3.258
			O43	ILE:156	O	2.797
			O24	TRP:382	NE1	2.915
			O8	ASP:137	O	2.590
			O43	LYS:66	NZ	2.874
			O52	SER:63	O	2.909

Table 8: Screening of ADME properties for GM3 analogues with its modified NeuAc using Qikprop simulation

GM3 Analogues with its modified NeuAc	Mol.wt	QlogS	QPlog po/w	Log HERG	Dipole	SASA	RULE OF 5
Reference Range	130-175	-6.5-0.5	-2.0-6.5	< -5	1-12.5	300-1000	Max 4
9-deoxy-NeuAc	617.557	0.153	-5.297	-1.484	7.722	816.6	3
9-Amino-NeuAc	632.572	0.991	-8.568	-2.462	14.07	854.1	3
9-Acetamido-NeuAc	674.609	1.164	-6.71	-0.793	7.859	936.1	3
9-N-Gly-NeuAc	688.636	0.438	-8.826	-3.382	7.747	1007	3
9-N-Succ-NeuAc	732.645	1.4	-7.062	-0.707	8.51	1003	3
9-iodo-NeuAc	743.454	-0.424	-4.795	-1.586	12.54	849.3	3
9-thio-NeuAc	649.617	0.423	-5.236	-1.232	10.32	809.4	3
9-ScH3-NeuAc	663.644	0.245	-4.979	-2.156	2.562	915.4	3
5-N-fluoroac-neu	651.547	0.848	-5.943	-1.456	6.601	825.5	3
5-N-trifluoroac-Neu	687.528	-0.08	-5.31	-1.696	5.181	861.2	3
5-N-Gly-Neu	648.571	1.624	-9.243	-2.357	7.248	850.6	3
5-N-Succ-Neu	691.593	0.646	-6.349	0.304	4.427	865.2	3
NeuAc-Me-ester	647.583	0.951	-6.335	-3.496	3.293	860.6	3
NeuAc-Et-ester	661.61	0.322	-5.886	-3.593	4.572	889.2	3
methyl 5-N-acetyl Neuraminate	647.583	0.74	-6.574	-3.841	6.578	897.9	3
Benzyl 2α-O-methyl-5-n-acetyl-8,9-O-isopropylidene Neuraminate	751.734	-0.206	-3.758	-4.666	16.73	1000	3
Benzyl 2α-O-methyl-4-O-Capriloyl-5-N-n-acetyl-8,9-O-isopropylidene Neuraminate	877.932	0.219	-1.475	-4.136	4.403	1098	3
Benzyl 2α-O-methyl-4-O-Capriloyl-5-N-n-acetyl- Neuraminate	849.879	-1.232	-2.471	-5.079	6.579	1151	3
2α-O-methyl-4-O-Capriloyl-5-N-n-acetyl- Neuraminic acid	759.754	-0.508	-4.13	-2.604	2.329	1076	3
Benzyl 2α-O-methyl-4-O-(8-morpholin)-Capriloyl-5-N-n-acetyl-8,9-O Isopropylidene Neuraminate	963.038	0.379	-1.706	-5.7	7.689	1292	3
Benzyl 2α-O-methyl-4-O-(8-morpholin) Capriloyl-5-N-n-acetyl-Neuraminate	934.984	0.9	-3.322	-5.329	12.01	1206	3
2α-O-methyl-4-O-(8-morpholin)-Capriloyl-5-N-n-acetyl- Neuraminic acid	844.86	-0.394	-6.352	-3.862	14.15	1216	3
5-N-Acetyl-9-amino -9-deoxy-Neuraminic acid	632.572	1.032	-8.568	-2.403	13.82	850.1	3

to the co-crystallized GM3. The RMSD value for SEB was found between 0.3 Å to 0.6Å. The ranking of ligands was done based on the GLIDE score (Table 4). All the 23 ligands have accepted poses with the receptors. The docking score (*GScore*) using GLIDE varies from −7.89 (9-N-Gly-NeuAc) to a minimum of −8.45 (NeuAc-Et-ester) in Staphylococcal Enterotoxin B. Based on *GScore* it is revealed that the analogues of GM3 prepared in this study could be the potential inhibitors for second generation drug development. The GM3 analogues with modified NeuAc such as 9-N-Gly-NeuAc, 5-N-fluoroAc-Neu, 5-N-Succ-Neu and NeuAc-Et-ester at the active site of Staphylococcus Enterotoxin B (SEB) with their molecular interactions are displayed in the Fig. 3-6 and in Table 5.

The ligplot interaction study shows that the reference PDB ligand for the protein Staphylococcus Enterotoxin B (1SE3) shows a single hydrogen bond interaction with Gln106 amino acid residue, Whereas, the present case shows more hydrogen bond interactions for the GM3 analogues with 9-N-Gly-NeuAc . It forms 9 hydrogen bonds with Gln106, Asp62, Asp63, Asp108 and Lys54. And the GM3 analogue with 5-N-Succ-Neu shows interaction with Asp62, Asp63, Thr99, Asp108, Gln106. And GM3 analogue with NeuAc-Et-ester shows 7 hydrogen bond interactions with Gln106, Asn63, Asp108, Asp62 and Ser52. Therefore it is prominent that our synthetic GM3 analogue could inhibit Staphylococcal Enterotoxin B well better than the reference ligand.

Endoglycoceramidase II from *Rhodococcus Sp.*

Modeling of GM3 analogues with Endoglycoceramidase II from *Rhodococcus Sp.* Complexes - HTVS

High Throughput Virtual Screening was done for 23 GM3 analogues to find out the structures (ligands) most likely bind to the Endoglycoceramidase II from *Rhodococcu*s Sp. Among the 23 GM3 derivatives having Benzyl2α-O-methyl-5-n-acetyl-8,9-O-isopropylidene Neuraminate (Table 2, Analogue 2), 9-thio-NeuAc (Table 1, analogue 7), 5-N-Succ-Neu (Table 1, analogue 12), 9-N-Gly-NeuAc (Table 1, analogue 4), 5-N-Gly-Neu (Table 1, analogue 11), 5-N-fluoroAc-Neu (Table 1, analogue 9), methyl 5-N-acetyl Neuraminate (Table 2,analogue 1), 9-N-Succ-NeuAc (Table 1, analogue 5), Gangliosides GM3 and Benzyl 2α-O-methyl-4-O-Capriloyl-5-N-acetyl- Neuraminate (Table 2, analogue 5) are the ligands with best score respectively. The original crystal structures of Endoglycoceramidase II were used to validate the GLIDE-XP docking. The top 4 configurations after docking were taken into consideration to validate the result. The RMSD was calculated for each one in compare to the co-crystallized GM3. The RMSD value for Endoglycoceramidase II from *Rhodococcus Sp.* (2OSX) was found between 0.32Å to 0.56 Å (Table 6). The GM3 analogues with Benzyl 2α-O-methyl-5-n-acetyl-8,9-O-isopropylidene Neuraminate, 9-N-Gly-NeuAc, 9-N-Succ-NeuAc and 9-thio-NeuAc at the active site of Endoglycoceramidase II from *Rhodococcus Sp.* with their molecular interactions are displayed in the Fig. 7-10 and in Table 7.

The GM3 analogues (ligands) are superimposed at the active site of Staphylococcal Enterotoxin B and Endoglycoceramidase II from *Rhodococcus Sp.* respectively in the Fig. 11 and 12.

ADME screening

We have analyzed 23 physically significant descriptors and pharmaceutically relevant properties of GM3 and its analogues, among which were molecular weight, polarizability (Å), log P

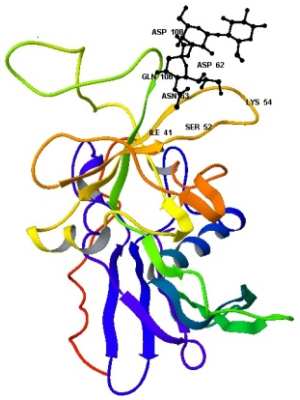


Fig. 1: Staphylococcal Enterotoxin B complexed with GM3 Trisaccharide.

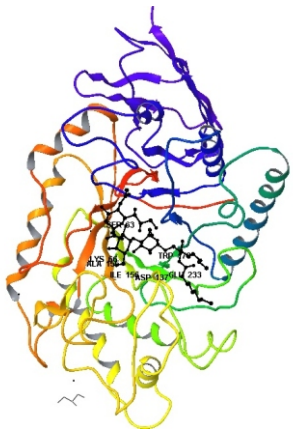


Fig. 2: Endo-glycoceramidase II from *Rhodococcus sp.*: Ganglioside GM3 Complex.

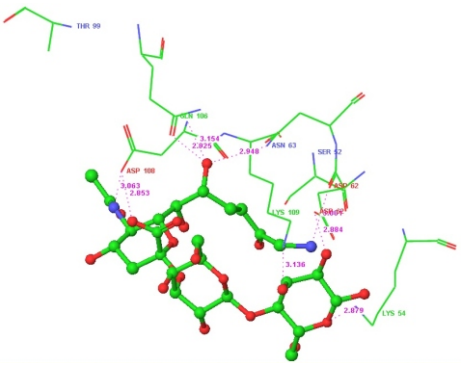


Fig. 3: View of binding mode of GM3 analogue with 9-N-Gly-NeuAc - Staphylococcal Enterotoxin B. complex.

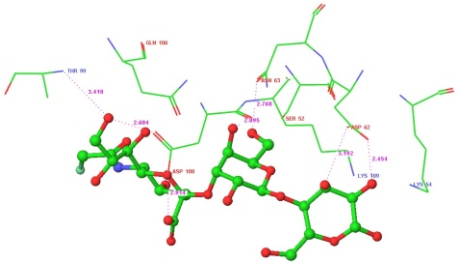


Fig. 4: View of binding mode of GM3 analogue with 5-N-fluoroac-neuAc - Staphylococcal Enterotoxin B complex

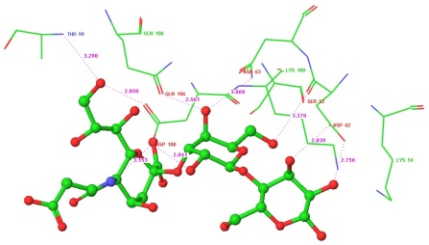


Fig. 5: View of binding mode of GM3 analogue with 5-N-Succ-NeuAc - Staphylococcal Enterotoxin B complex.

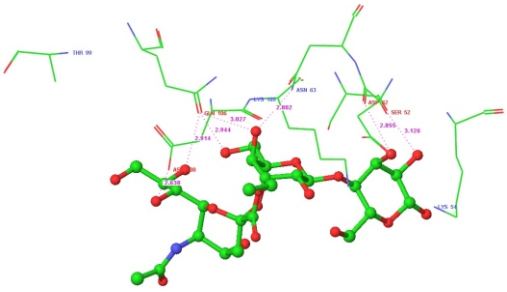


Fig. 6: View of binding mode of GM3 analogue with NeuAc-Et-ester - Staphylococcal Enterotoxin B complex.

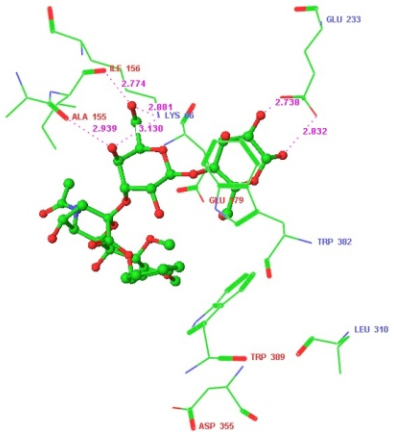


Fig. 7: View of binding mode of GM3 analogue with Benzyl 2α-O-methyl-5-n-acetyl-8,9-O-isopropylidene Neuraminate Endoglycoceramidase II complex

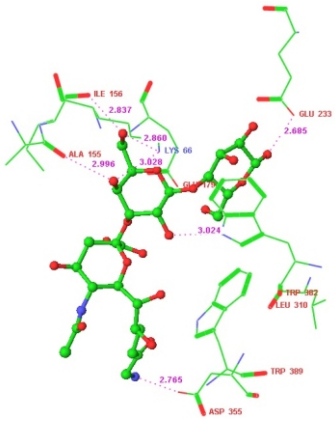


Fig. 8: View of binding mode of GM3 analogue with 9-N-Gly-NeuAc - Endoglycoceramidase II complex.

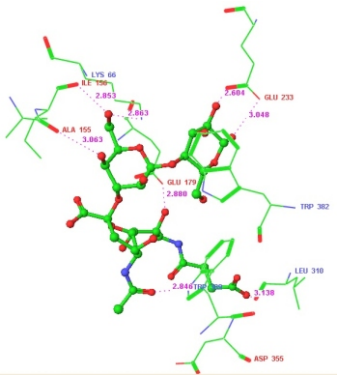


Fig. 9: View of binding mode of GM3 analogue with 9-N-Succ-NeuAc - Endoglycoceramidase II complex.

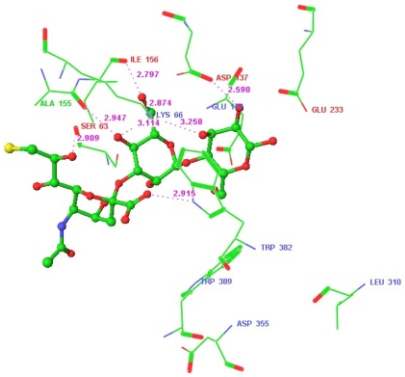


Fig. 10: View of binding mode of GM3 analogue with 9-thio-NeuAc - Endoglycoceramidase II complex.

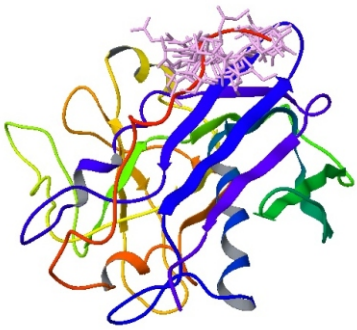


Fig. 11: GM3 analogues (Superimposed) at the binding site of Staphylococcal Enterotoxin B

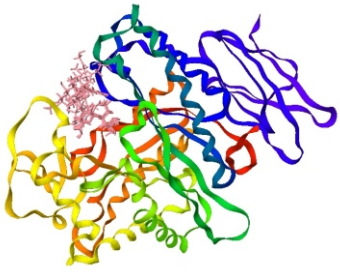


Fig. 12: GM3 analogues (Superimposed) at the binding site of Endoglycoceramidase II from *Rhodococcus Sp*.

(octanol/gas), log P (water/gas), log P (octanol/water), log BB (brain/blood), log P MDCK, log Kp (skin permeability), log Khsa (serum protein binding), solvent accessible surface area (SASA) etc. and their screening in accordance to Lipinski's rule of 5. For the log P (octanol/water), QP%, and log HERG if the value for a utilized descriptor exceeded the range for the experimental training set, it was flagged. In this study, out of 23 ligands, most of the structures showed significant values for the properties analyzed and showed drug like characteristic's based on Lipinski's rule of 5 (Table. 8).

CONCLUSION

The ganglioside GM3 analogue compounds were evaluated using docking-MM-GB/SA approach. The structurally homologous inhibitors dock in a very similar position and orientation to parent Ganglioside GM3, which suggests that the homologous inhibitors show similar binding patterns and interaction modes in the SEB and Endoglycoceramidase II. Furthermore, the most potent inhibitor should have the interaction with the highest affinity with the two toxins. The docking structures of all compounds showed that they bind in very similar pattern into the active site of Staphylococcal Enterotoxin B and Endoglycoceramidase II from *Rhodococcus Sp*. This concludes that the structural modification implemented in this study is significantly related to their activity. Also this proved the reasonability and reliability of the docking results. It can be seen that substitution of functional groups at position R1, R2, R3 and R4 leads to increase in binding affinity of modified analogues even more intense than that of co-crystallized ligand. Further ADME screening provided a detailed analysis for the final selection of the potential candidates from the dataset of compounds.

Docking studies revealed the mode of binding of GM3 analogues into the binding pocket of Staphylococcus Enterotoxin B. GM3 analogues with best docking score and interaction with Staphylococcus Enterotoxin B obtained from High Throughput Virtual Screening are GM3 analogues having 9-N-Gly-NeuAc, NeuAc-Me-ester, 5-N-Gly-Neu, 9-Amino-NeuAc, 5-N-trifluoroAc-Neu, NeuAc-Et-ester, 5-N-fluoroAc-Neu, N-Glycolyl Neuraminic Acid, 5-N-Succ-Neu. Best Docking scores are obtained by XP algorithm for GM3 analogues having 9-N-Gly-NeuAc, 5-N-fluoroAc-Neu, 5-N-fluoroAc-Neu and NeuAc-Et-ester.

Docking studies for binding of GM3 analogues into the binding pocket of Endoglycoceramidase II from *Rhodococcus Sp.* reveals High Throughput Virtual Screening compounds are GM3 analogues having Benzyl2α-O-methyl-5-n-acetyl-8,9-O-isopropylidene Neuraminate, 9-thio-NeuAc, 5-N-Succ-Neu,9-N-Gly-NeuAc,5-N-Gly-Neu, 5-N-fluoroAc-Neu, methyl 5-N-acetyl Neuraminate, 9-N-Succ-NeuAc, Gangliosides GM3 and Benzyl 2α-O-methyl-4-O-Capriloyl-5-N-acetyl-Neuraminate. The docking are done for GM3 analogues having Benzyl 2α -O-methyl-5-n-acetyl-8,9-O-isopropylidene Neuraminate, 9-N-Gly-NeuAc, 9-N-Succ-NeuAc and 9-thio-NeuAc.

Based on the overall analysis we can conclude that the GM3 analogues having 9-N-Gly-NeuAc, 5-N-fluoroac-Neu, 5-N-Succ-Neu and NeuAc-Et-ester are the most potent analogues for Staphylococcal Enterotoxin B and analogues Benzyl-2α-O-methyl-5-N-acetyl-8,9-O-Isopropylidene Neuraminate, 9-N-Gly-NeuAc, 9-N-Succ-NeuAc and 9-thio-NeuAc are the most potent against Endoglycoceramidase II from *Rhodococcus Sp*., which could be used for further study of drug development. The combination of docking-MM-GB/SA method and the ADME properties based final screening in the present work has the potential to predict the binding affinity of a large set of ligands to a receptor as well as their suitability as potential drug candidates for modern drug discovery process.

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