RESEARCH ARTICLE

In-silico Docking and Toxicity Analysis of *N-acetyl D- glucosamine* with Antimicrobial Proteins- A Novel Targeting against Antimicrobial Resistance

Pathakota Roja[1*], V. Santhosh Kumar[2]

[1]*School of Pharmaceutical Sciences, VELS Institute of Science Technology and Advanced Studies, Pallavaram, Chennai, India*

[2]*Department of Pharmacology, School of Pharmaceutical Sciences, VELS Institute of Science Technology and Advanced Studies, Pallavaram, Chennai, India*

Received: 10th January, 2023; Revised: 18th February, 2023; Accepted: 10th March, 2023; Available Online: 25th March, 2023

ABSTRACT

Nutraceuticals are popular health-promoting agents for various disease ailments such as food supplements, health promoters, etc. The rising antimicrobial resistance concerns are a serious challenge to researchers and need of the hour to be addressed by developing novel antimicrobial agents. One prospective nutraceutical that has been chosen as a candidate for development as an antibacterial agent is N-acetyl-D-glucosamine. GlcNAc is a monomer of chitin, a substance found in the cell walls of several fungi, mollusks, and cephalopod beaks. The present study aimed to evaluate NAG's antimicrobial potential by *in-silico* docking using Molegro virtual docker MVD 2013.6.0 as a novel approach. N-acetyl-D-glucosamine was tested against various targets like penicillin-binding protein (PDB3UDI) ligase (PDB2zdq), isomerase/isomerase inhibitor (PDB3TTZ), transferase (PDB2VEG), thymidylate kinase (PDB5UIV), dihydrofolate reductase (PDB3SRW), rifampicin-resistant, RNA polymerase (PDB6VVT) in different confirmations. Based on the docking scores obtained NAG was found to have potent activity against *Acinetobacter baumannii, Thermus thermophilus, Staphylococcus aureus, Streptococcus pneumoniae, Salmonella typhi, Mycobacterium smegmatis, Candida albicans* proving the therapeutic approach that can develop the NAG as antimicrobial agent. The toxicity analysis was performed using TEST software using different methods proving there no report of endotoxicity of GlcNAc molecule that tend to be promising for developing the GlcNAc as lead for antimicrobial resistance. The future lies in evaluating the *in-vivo* antimicrobial potential studies of NAG.

Keywords: GlcNAc, N-Acetyl -D-Glucosamine, Antimicrobial resistance, *In-silico* docking, Nutraceutical.

International Journal of Pharmaceutical Quality Assurance (2023); DOI: 10.25258/ijpqa.14.1.12

How to cite this article: Roja P, Kumar VS. *In-silico* Docking and Toxicity Analysis of *N-acetyl D- glucosamine* with Antimicrobial Proteins- A Novel Targeting against Antimicrobial Resistance. International Journal of Pharmaceutical Quality Assurance. 2023;14(1):66-75.

Source of support: Nil.

Conflict of interest: None

INTRODUCTION

The hazy concept of antibiotic resistance is the focus of global interest. The phrase "antimicrobial resistance" (AMR) refers to the spread of diseases caused by bacteria, fungi, viruses, algae, and other microbes as a result of poor hygiene or changes to the microbes brought on by the extended use of antibiotics.[1] The bacteria become resistant to ongoing medical therapy due to unanticipated genetic mutations or modifications.[2] Each person experiences a unique range of bacterial infections, with varying severity and complexity of illness.[3] Without the correct clinical intervention, this effect makes it impossible to control infection and increases sickness, which poses a severe risk to humanity. On November 17, 2021, the WHO identified antibiotic resistance as the tenth leading cause of severe deaths. A serious threat to humanity was presented by the global pandemic. Top organizations from all across the world attended the antimicrobial awareness week, which was conducted in November 2021 as a part of a stewardship program. The campaign highlighted the threat of disease spreading throughout the population as well as the inefficiency of the present medical treatments. The WHO Director-General formed the Strategic and Technical Advisory Group for Antimicrobial Resistance (STAG-AMR) to control policy, research, and the flow and monitoring of illnesses internationally in response to the need for a deeper understanding of the diseases. The strategic organization has vigorously enforced AMR's burden and response criteria while conducting a global surveillance campaign. Numerous public

**Author for Correspondence: natu9sea@gmail.com*

health systems and health security missions were created in reaction to societal crises. The WHO strategy committee has regulations in place to support research and development, keep an eye on voluntary norms, work together on risks and rewards, and examine neglected and preventable illnesses.[4] As a result, it is crucial to provide leads for microbiological targets. This is an intensely contentious area of research with significant implications for the public health system. Repurposing already-existing medications with great bioavailability and solubility may be a creative way to produce anti-AMR therapeutics in the face of increased antimicrobial leads.[5]

N-acetyl glucosamine (GlcNAc), an amine derivative of glucose, is produced by the secondary amide reaction between glucosamine and acetic acid. The polymer, impacted by N-acetyl muramic acid, is a component of bacterial cell walls.[6-8] It is also frequently seen in the exoskeletons of insects and other arthropods, the cell walls of fungi, and the extracellular matrix of human cells. The GlcNAc is a common treatment for autoimmune diseases and is well known for promoting the processes of animal cell protein glycosylation to promote cell signaling pathways.[9] Recent investigations have discovered a new function for GlcNAc, which was originally morphogenically suited to the human fungal infection *Candida albicans*. The pathogenic bacteria *E. coli* also undergoes morphogenesis in response to GlcNAc, promising in the production of antibiotics.[10] The discovery of GlcNAc as a lead for antimicrobial resistance is necessary due to the many roles of GlcNAc in cell signaling pathways for bacteria, fungi, and mammalian cells.[11] The major aim of the present study is to generate a novel medicinal chemical to combat antimicrobial resistance by *in-silico* docking GlcNAc to several targeted antimicrobial proteins using inexpensive molecular docking methods. What distinguishes the study is how GlcNAc forms as a lead for antibiotic resistance.

METHODOLOGY

The antimicrobial protein targets were identified from the literature, and the protein structure was available for RSDB database to be downloaded with PDB id. The GlcNAc structure was made available as PDB and other formats from drug bank. In order to perform the molecular docking, the protein and the ligand GlcNAc should be prepared individually and then docking is performed. The program Molegro Virtual Docker MVD 2013, version 6.0, was used to perform molecular docking. The docking studies included these three steps:

Preparation of Protein

The proteins were selected based on the literature and the PDB structure of the selected PDB ids was obtained from the RSDB database of proteins. The selected PDB ids of proteins along with the structure. For conducting the docking studies, the protein should be prepared initially by modifying the protein with the attachment of polar hydrogens, Kollman charges and removing the water molecules. Thus, the prepared protein is saved in PDBQT format for docking. The grid coordinates are also set to perform the docking. The test organisms or the antimicrobial drug targets include:

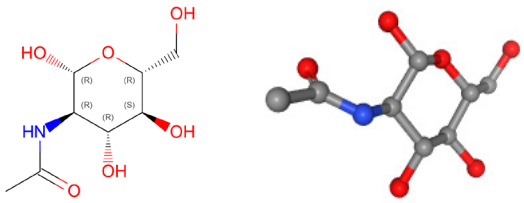


Figure 1: Structure of NAG along with 3D structure

Table 1: chemical details of structure

Formula	$C_8 H_{15} N O_6$
Molecular weight	221.21
IUPAC name	2-acetamido-2-deoxy-beta-D-glucose
Formal Charge	0
Atom Count	30
Chiral Atom Count	5
Bond Count	30
Aromatic Bond Count	0

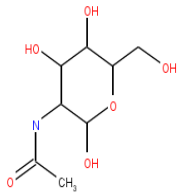


Figure 2: Structure of NAG molecule in the TEST Software module.

- *Staphylococcus aureus.*
- *Streptococcus pneumoniae.*
- *Bacillus cereus.*
- *Escherichia coli.*
- *Pseudomonas aeruginosa.*
- *Klebsiella pneumoniae.*
- *Salmonella typhi.*
- *Proteus vulgaris.*
- *Shigella flexineri.*
- *Candida albicans.*
- *Aspergillus niger.*

Preparation of Ligand

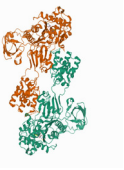
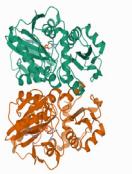
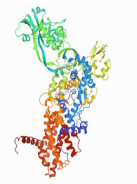
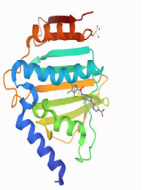
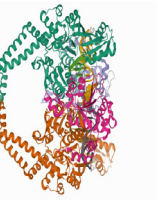
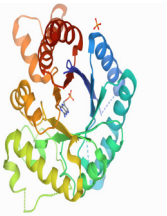
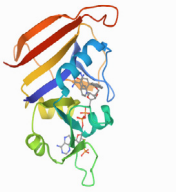
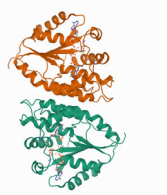
The GlcNAc/NAG structure was drawn using the Chem Draw. Further, the ligand was prepared by the inclusion of torsions and additional charges. The PDB format of the ligand was obtained from the drug bank which was enabled by converting into PDBQT format using the OPEN Babel software. Now the ligand GlcNAc is ready for docking.[12]

Details of Chemical Structure[13,14]

Docking Method

The preparation of proteins and ligand was done to ensure the molecules' least energy conformation. Using the software Molegro virtual Docker MVD 2013, Version 6.0. the docking

Table 2: crystal structures of the antimicrobial proteins

S. no	*PDB Id (Classification)*	*Name of the protein*	*Organism*	*Crystal structure*
1	3UDI (Penicillin-binding protein/ Antibiotic)	A combination of Acinetobacter baumannii PBP1a and penicillin	*Acinetobacter baumannii*	
2	2ZDQ (Ligase)	D-Alanine:D-Alanine Ligase from Thermusthermophius HB8 with ATP and D-Alanine:D-Alanine	*Thermust hermophilus*	
3	1JZQ (Ligase)	Complex of soleucyl-tRNA synthetase and soleucyl-adenylate analogue	*Thermus thermophilus*	
4	3TTZ (Isomerase)	Inhibitor of topoisomerase ATPase	*Staphylococcus aureus*	
5	3RAE(Isomerase/DNA, antibiotic)	Quinolone (Levofloxacin)-DNA cleavage complex of S. pneumonia type IV topoisomerase	*Streptococcus pneumonia*	
6	2VEG(Isomerase)	Streptococcus pneumoniae dihydropteroate synthase: combination with 6-hydroxymethyl-7,8-dihydropterin monophosphate	*Streptococcus pneumoniae*	
7	3SRW(Oxidoreductase/ Oxidoreductase Inhibitor)	Complexes of new 7-aryl-2,4-diaminoquinazolines with Dihydrofolate Reductase	*Staphylococcus aureus*	
8	5UIV(Transferase)	Thymidylate Kinase	*Candida albicans*	

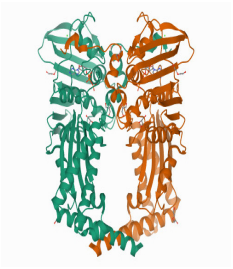
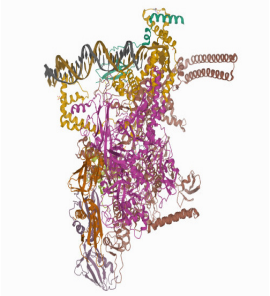
9	6J90(Isomerase)	Salmonella Typhi ATP-Gyrase B N-Terminal Domain Complex at 2.2A Resolution	*Salmonella enterica subsp. entericaserovar typhi*	
10	6VVT(Transcription,Transferase/ Dna/Antibiotic)	*Mycobacterium smegmatis* transcription initiation complex containing the antibiotic Sorangicin and RNA polymerase that is resistant to rifampin	*Mycolicibacterium smegmatis MC2 155,*	

Table 3: The TEST software module's method for predicting toxicity

Method	*Description*
Hierarchical method	The weighted average of the predictions from various cluster models is used to assess the toxicity for a certain query compound.
FDA method	A new model fitted to the chemicals most similar to the test compound is used to anticipate the behavior of each test chemical. Every model is created during runtime.
Single model method	A multi-linear regression model fitted to the training set is used to make predictions (using molecular descriptors as independent variables).
Group contribution method	A multi-linear regression model fitted to the training set is used to make predictions (using molecular fragment counts as independent variables).
Nearest neighbor method	The projected toxicity is calculated by averaging the three compounds in the training set that are most comparable to the test chemical.
Consensus method	By averaging the anticipated toxicities from the aforementioned QSAR approaches, the predicted toxicity is estimated (provided the predictions are within the respective applicability domains)
Random forest method	A decision tree analysis is used to evaluate the anticipated toxicity (using molecular descriptors as decision variables). Only the developmental toxicity endpoint is presently accessible for the random forest approach.

site was posed in several ways to determine the ideal binding posture for the investigation. The best confirmation position with a maximum number of binding sites was confirmed and evaluated with binding energies and structure as listed in Table 2.

Toxicity Analysis

Utilizing a variety of methodologies, including the hierarchical method, FDA method, single model method, group contribution method, nearest neighbour method, consensus method, and random forest method, the toxicity study of the GlcNAc/NAG molecule was carried out using the TEST software program. Each of the methods provided above has a benefit for calculating the best prediction, external validity, clustering with and without software, and rapid toxicity estimations, in that order (Table 3). The descriptors for the toxicity estimates include the following parameters determination:

- E-state counts, as well as values
- Topological and constitutional descriptors
- Walk and path counts
- Connectivity and content of the information
- 2D autocorrelation and the eigenvalue for Burden
- Molecular characteristics (such as the octanol-water partition coefficient)
- Hydrogen bond acceptor/donor numbers and kappa
- Molecular distance edge, and molecular fragment counts.

Initially, the structure was drawn in the software (Figure 1) and further different method of prediction was used to estimate the toxicity profile of the drugs. The different methods used to elucidate the toxicity profile are listed below in Table 2.

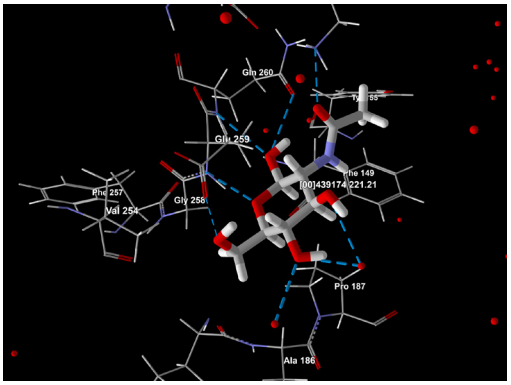
Thus the software is incorporated with different modules which were used to analyze the structure-based complexity and its nature that governs the internal toxicity of the compound NAG.

RESULTS AND DISCUSSION

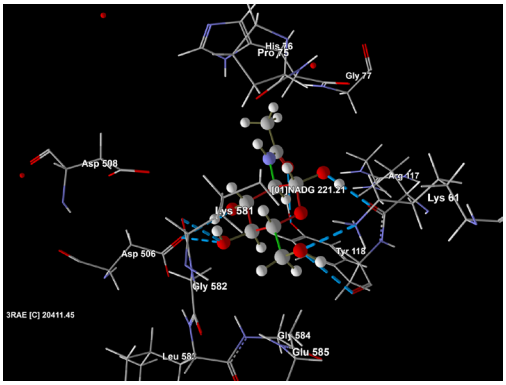
The docking analysis was done using the software Molegro virtual Docker MVD 2013 version 6.0. The docking scores were generated for individual proteins and the binding sites with the best confirmation were analyzed. The hydrogen binding efficiency was also determined.

Toxicity Analysis of NAG Ligand using TEST (Version 4.1)

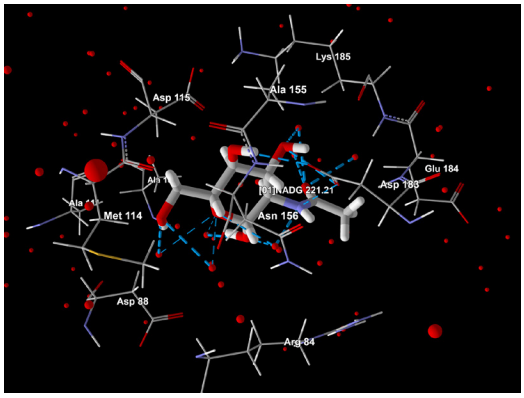
The toxicity profile of the NAG structure was investigated using the TEST (toxicity estimation software program)



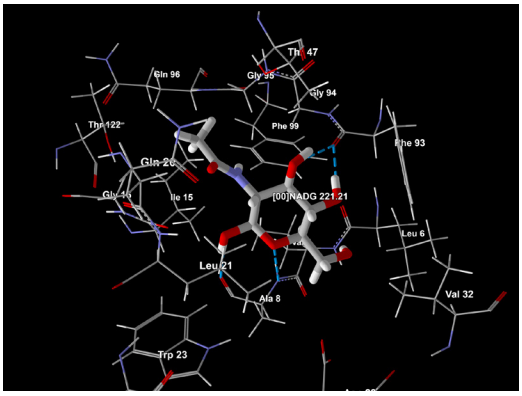
A) 3UDI (Penicillin-binding protein)



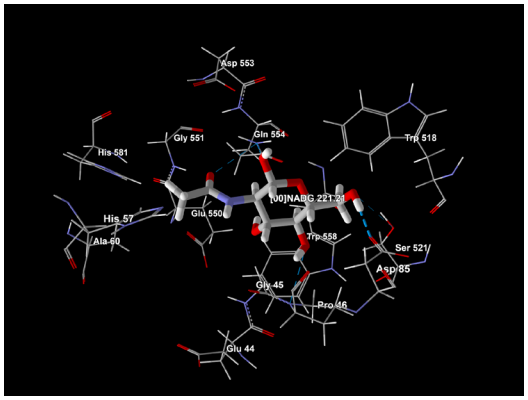
E) 3RAE (Isomerase/DNA)



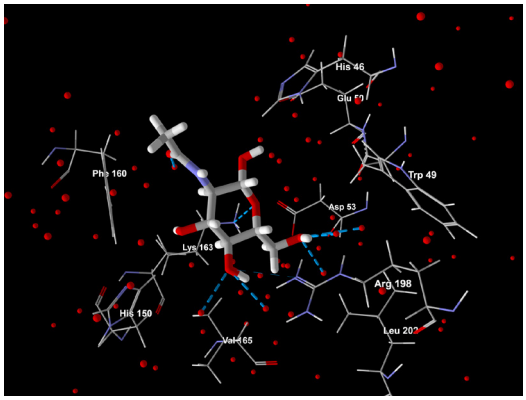
B) 2ZDQ (Ligase)



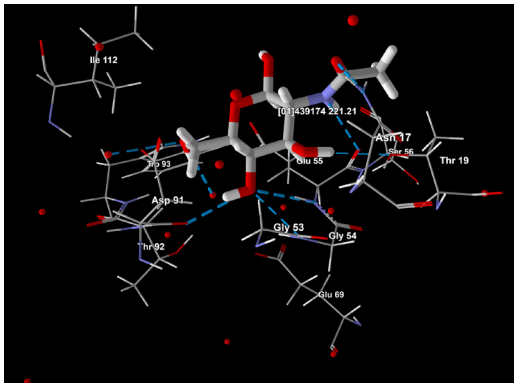
F) 3SRW (Oxidoreductase Inhibitor)



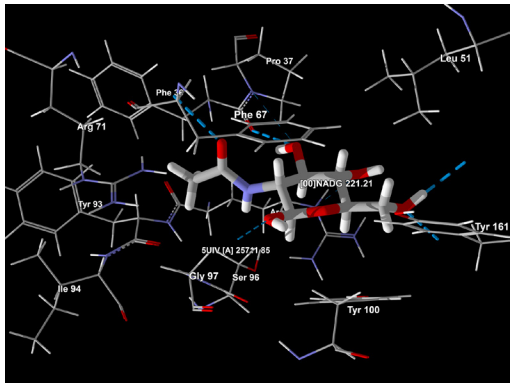
C) 1JZQ(Ligase)



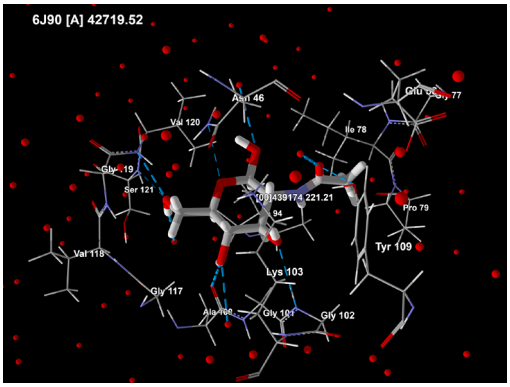
G) 3TTZ (Isomerase)



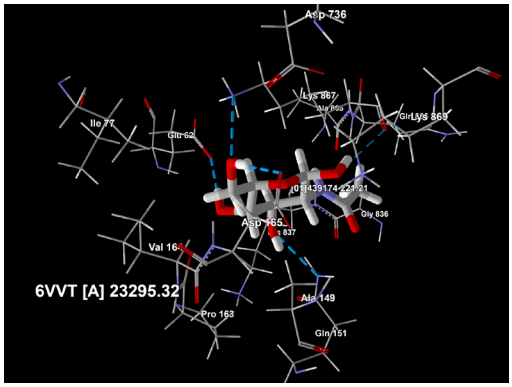
D) 2VEG(Isomerase)



H) 5UIV (Transferase)



I) 6J90 (Isomerase)



J) 6VVT (Transferase)

Figure 3: Docking analysis of nag with different microbial proteins

Table 4: Hydrogen bonding distance and bonding energies of proteins with NAG

S. no	*PDB Id (Classification)*	*Organism*	*Mol dock score*	*Aminoacid binding residues at site*	*H Bond energy*
1	3UDI(Penicillin binding protein/Antibiotic)	Acinetobacter baumannii	-65.9677	Asp, Gln, Glu, Gly, Luys, Phe, Val, Ala, Trp	-11.6492
2	2ZDQ (Ligase)	*Thermusthermophilus*	-65.5238	Ala, Asp, Arg, Asn, Glu, Lys, Met	-4.99971
3	1JZQ(Ligase)	*Thermus thermophilus*	-53.2385	Ala, Asp, Glu, Gln, Gly, His, Pro	-6.46437
4	3TTZ(Isomerase)	*Staphylococcus aureus*	-57.0446	His, Lys, Phe, Val, Arg, Asp, Glu, His	-5.02427
5	3RAE(Isomerase/DNA, antibiotic)	*Streptococcus pneumonia*	-69.5915	Gly, His, Pro, Arg, Lys, Tyr,Asp, Glu, Gly	-9.44296
6	2VEG(Isomerase)	*Streptococcus pneumoniae*	-38.7226	Asn, Asp, Glu, Gly, lle, Ser	-7.41369
7	3SRW(Oxidoreductase/ Oxidoreductase Inhibitor)	*Staphylococcus aureus*	-78.708	Ala, Asp, Gln, Gly, Ile, Leu, Phe	-9.73845
8	6VVT(Transcription, Transferase/DNA/Antibiotic)	*Mycobacterium smegmatis*	-67.3871	Ala, Asp, Gln, Glu, Ile, Pro, Val, Lys	-11.3837
9	6J9O(Isomerase)	*Salmonella Typhi*	-84.4374	Ala, Asn, Glu, Gly, Ile, Lys, Pro	-7.74649
10	5UIV(Transferase)	C. albicans	-67.1229	Arg, Gly, Ile, Leu, Phe, Pro, Ser, Tyr	-7.68865

Table 5: The binding efficiency of ligand nag with different proteins (PDBID) with moldock score and H –BOND

1JZQ				
NAME	*Ligand*	*MolDock Score*	*Rerank Score*	*H-bond Binding Energy*
[00] NADG	NADG	-69.4116	-63.1683	-5.82859
[02] NADG	NADG	-63.1102	-57.5407	-4.31518
[03] NADG	NADG	-60.3103	-53.2385	-6.46437
[01] NADG	NADG	-58.1666	-46.4609	-4.73254
2VEG				
[00] NADG	NADG	-74.9008	-66.616	-12.1046
[02] NADG	NADG	-71.6546	-38.7226	-7.41369
[03] NADG	NADG	-67.7176	-59.7266	-6.83042
[01] NADG	NADG	-66.5428	-59.93	-13.3769
2ZDQ				
[01] NADG	NADG	-75.5765	-72.7458	-4.79937

[00] NADG	NADG	-73.8941	-43.8926	-2.73852
[04] NADG	NADG	-63.7518	-63.6603	-0.0807009
[03] NADG	NADG	-62.9705	-65.5238	-4.99971
[02] NADG	NADG	-62.4561	-37.8488	-4.4402
3RAE				
[01] NADG	NADG	-83.126	-71.567	-10.7726
[00] NADG	NADG	-81.3009	-72.5841	-8.47383
[03] NADG	NADG	-79.1108	-69.5915	-9.44296
[04] NADG	NADG	-74.2735	-66.8746	-6.87056
[02] NADG	NADG	-74.0047	-42.2343	-7.73639
3SRW				
Name	Ligand	MolDock Score	Rerank Score	H-Bond
[00] NADG	NADG	-85.9289	-78.708	-9.73845
[03] NADG	NADG	-74.4101	-67.7278	-3.36243
[01] NADG	NADG	-73.2785	-68.9613	-8.03745
[02] NADG	NADG	-72.9516	-67.7215	-4.38102
3TTZ				
[01] NADG	NADG	-62.0585	-61.031	-2.26559
[00] NADG	NADG	-61.5733	-57.0446	-5.02427
[02] NADG	NADG	-55.5492	-54.6229	-1.69513
[03] NADG	NADG	-53.4724	-51.9379	-2.16831
3UDI				
[01] NADG	NADG	-83.911	-76.7022	-8.89208
[00] NADG	NADG	-74.0033	-65.9677	-11.6492
[02] NADG	NADG	-70.6986	-63.786	-9.25248
[03] NADG	NADG	-68.8653	-62.7365	-2.28372
[04] NADG	NADG	-63.8442	-58.1977	-6.05051
5UIV				
[00] NADG	NADG	-95.7302	-26.9556	-2.90047
[01] NADG	NADG	-82.1316	-57.0312	-1.87091
[03] NADG	NADG	-71.9778	-67.1373	-0.088009
[04] NADG	NADG	-67.9507	35.3705	-1.5411
[02] NADG	NADG	-67.8876	-67.1229	-7.68865
6J9O				
Name	Ligand	MolDock Score	Rerank Score	HBond
[00] NADG	NADG	-91.7445	-84.4374	-7.74649
[01] NADG	NADG	-85.9291	-77.7984	-5.82844
[03] NADG	NADG	-85.2492	-77.4234	-6.79898
[04] NADG	NADG	-85.0877	-78.937	-2.13049
[02] NADG	NADG	-84.7947	-77.4842	-5.92637
6VVT				
[00] NADG	NADG	-75.5239	-67.3871	-11.3837
[01] NADG	NADG	-73.7375	-62.8148	-10.1347
[03] NADG	NADG	-70.2099	-62.7439	-9.30764
[04] NADG	NADG	-61.2029	-39.2977	-8.03005

Table 6 (a): Prediction results

S. No.	*Endpoint*	*Experimental Value*	*Predicted Value*
1	Oral rat LD_{50} –Log10 (mol/kg)	N/A	1.74
2	Oral rat LD_{50} mg/kg	N/A	410.03

Table 6 (b): Nearest neighbors from the training set

S. No.	*CAS*	*Experimental value-log10 (mol/kg)*	*Similarity coefficient*
1	C8H14N06_1637338255376 (Test chemical)	N/A	N/A
2	35849-41-3	2.68	0.60
3	107187-05-3	2.52	0.57
4	2540-82-1	3.01	0.54

(version 4.1). The projected oral rat for LD_{50} determination was conducted out using several TEST software modules. The structure's anticipated toxicity levels after 96 hours may indicate that there is no discernible difference in the LD_{50} levels, indicating that the molecular structure is not poisonous.

Table 7 (a): Prediction results

S. No.	*Endpoint*	*Experimental value*	*Predicted Value*
1	Oral rat LD_{50} –Log10 (mol/kg)	N/A	2.24
2	Oral rat LD_{50} mg/kg	N/A	1278.84

Table 7 (b): Individual Predictions

S. No.	*Method*	*Predicted value-log10 (mol/kg)*	*Test chemical*
1	Hierarchical clustering	N/A	$C_8H_{14}N_{6\_}$ 1637338255376
2	FDA	1.75	
3	Nearest neighbor	2.74	

Predicted toxicity analysis using different modules of TEST software:

- Predicted Oral rat LD_{50} for C8H14N06_1637338255376 for Nearest Neighbor Method

Table 6 (a and b).

Table 8 (a): Prediction results

S. No.	*Endpoint*	*Experimental value*	*Predicted value*	*Prediction interval*
1	Fathead minnow LC_{50} (96 hours)–Log10 (mol/kg)	N/A	2.43	$1.97 \leq Tox \leq 2.90$
2	Fathead minnow LC_{50} (96 hours) mg/kg	N/A	823.76	$281.73 \leq Tox \leq 2408.63$

Table 8 (b): Cluster model predictions and statistics

S. No.	*Cluster model*	*Test chemical descriptor values*	*Prediction interval-log10 (mol/kg)*	R^2	Q^2	*# Chemicals*	*Test chemical*
1	1296	Descriptors	4.44 ± 0.61	0.828	0.757	74	$C_8H_{14}N_{06\_}$ 1637338255376
2	1305	Descriptors	0.44 ± 1.00	0.841	0.797	143	
3	1308	Descriptors	1.81 ± 0.92	0.848	0.811	187	
4	1314	Descriptors	0.73 ± 1.17	0.750	0.704	477	
5	1315	Descriptors	1.82 ± 1.33	0.716	0.689	563	
6	1316	Descriptors	1.16 ± 1.27	0.758	0.734	549	

Table 9: Predicted Oral rat LD_{50} for $C_8H_{14}N_{06_}$1637338255376 from FDA method

S. No.	*Endpoint*	*Experimental value*	*Predicted value*	*Prediction interval*
1	Oral rat LD_{50} –Log10 (mol/kg)	N/A	1.75	$1.26 \leq Tox \leq 2.23$
2	Oral rat LD_{50} mg/kg	N/A	3988.59	$1311.39 \leq Tox \leq 12131.26$

Table 10: Cluster Model Predictions and Statistics

S. No.	*Cluster model*	*Test chemical descriptor values*	*Prediction interval-log10 (mol/kg)*	R^2	Q^2	*# Chemicals*	*Test chemical*
1	FDA Model	Descriptors	1.75 ± 0.48	0.920	0.846	30	$C_8H_{14}N0_{6_}$1637338255376

Table 11 (a): Prediction results

S. No.	*Endpoint*	*Experimental value*	*Predicted value*
1	Fathead minnow LC_{50} (96 hours)–Log10 (mol/kg)	N/A	1.35
2	Fathead minnow LC_{50} (96 hours) mg/kg	N/A	10016.77

Table 11 (b): Individual predictions

S. No.	*Model*	*Prediction value- Log10(mol/L)*	*Test chemical*
1	Hierarchical clustering method	2.43	
2	Single model	1.16	
3	Group contribution	0.23	$C_8H_{14}N_6$_1637338255376
4	FDA model	1.57	
5	Nearest neighbor method	N/A	

- Predicted Oral rat LD_{50} for $C_8H_{14}N_6$_1637338255376 from Consensus method

Table 7 (a and b).

- Predicted Fathead minnow LC_{50} (96 hours) for C8$H_{14}N_6$_1637338255376 from Hierarchical clustering method

Table 8 (a and b).

- Predicted Oral rat LD_{50} for $C_8H_{14}N_{06}$_1637338255376 from FDA method.

Table 9.

- Cluster Model Predictions and Statistics

Table 10.

- Predicted fathead minnow LC_{50} (96 hours) for C_8H_{14}N06_1637338255376 from consensus method

Table 11 (a and b).

CONCLUSION

N-acetyl glucosamine/NAG is active pharmacophore identified with amine bonded to glucose and an active representative in the treatment of various autoimmune diseases. The challenging quest by the microbes in terms of antimicrobial resistance poses a threat to conventional antimicrobial therapy in the long run. Hence the surge and urgency created by the AMR govern the search of new leads which are readily available, cheap and non-toxic. Using *in-silico* docking analysis by Molegro Virtual Docker version 6.0 against several antimicrobial proteins that were retrieved as PDB structures from the RSDB database, an attempt was made to report the activity of NAG as an antibacterial agent. Further, the toxicity analysis using the software tool TEST was performed, which could validate the compound NAG has less internal toxicity. Thus, the pharmacophore molecule NAG is identified as a best-fit inducer model to the selected broad range of antimicrobial proteins. The need of further formulation development or a modification in the NAG drug development with an *in-vivo* clinical evaluation establishes a safety protocol for the usage of the drug in a large group of population and effectively can establish an *in-silico* and *in-vivo* correlation.

ACKNOWLEDGMENT

The authors are thankful to VISTAS institute for the support and encouragement throughout the study.

REFERENCES

1. Basu S, Garg S. Antibiotic prescribing behavior among physicians: ethical challenges in resource-poor settings. Journal of medical ethics and history of medicine. 2018;11.
2. WHO Global action plan on Antimicrobial Resistance. WHO (World Health Organization), January 2016, ISBN: 9789241509763
3. Tomczyk S, Taylor A, Brown A, de Kraker MEA, El-Saed A, Alshamrani M, Hendriksen RS, Jacob M, Löfmark S, Perovic O, Shetty N, Sievert D, Smith R, Stelling J, Thakur S, Vietor AC, Eckmanns T; WHO AMR Surveillance and Quality Assessment Collaborating Centres Network. Impact of the COVID-19 pandemic on the surveillance, prevention and control of antimicrobial resistance: a global survey. Journal of Antimicrobial Chemotherapeutics. 2021 Oct 11;76(11):3045-3058. doi: 10.1093/jac/dkab300.
4. Health-topics on antimicrobial-resistance, WHO, November 17, 2021
5. World Health Organization, Global Antimicrobial Resistance and Use Surveillance System (GLASS) Report: 2021
6. Brandt AU, Sy M, Bellmann-Strobl J, et al. Association of a Marker of N-Acetylglucosamine With Progressive Multiple Sclerosis and Neurodegeneration. JAMA Neurology. 2021;78(7):842–852. doi:10.1001/jamaneurol.2021.1116
7. Emadi A, Chua JV, Talwani R, Bentzen SM, Baddley J. Safety and Efficacy of Imatinib for Hospitalized Adults with COVID19: A structured summary of a study protocol for a randomised controlled trial, Trials. 2020 Oct 28;21(1):897. doi: 10.1186/s13063-020-04819-9
8. Aflaki M, Flannery A, Ferreira JP, Cheng MP, Kronfli N, Marelli A, Zannad F, Brophy J, Afillalo J, Huynh T, Giannetti N, Bessissow A, Ezekowitz JA, Lopes RD, Ambrosy AP, Craig M, Sharma A. Management of Renin-Angiotensin-Aldosterone System blockade in patients admitted to hospital with confirmed coronavirus disease (COVID-19) infection (The McGill RAASCOVID- 19): A structured summary of a study protocol for a randomized controlled trial. Trials. 2021 Feb 5;22(1):115. doi: 10.1186/s13063-021-05080-4.
9. Ong Q, Han W, Yang X. O-GlcNAc as an Integrator of Signaling Pathways. Front Endocrinology (Lausanne). 2018, 9:599. doi: 10.3389/fendo.2018.00599
10. Yang X, Qian K. Protein O-GlcNAcylation: emerging mechanisms and functions. Nature Reviews Molecular Cell Biology. 2017, 18:452–65. doi: 10.1038/nrm.2017.22 11.
11. Ryan P, Xu M, Davey AK, Danon JJ, Mellick GD, Kassiou M, et al. O-GlcNAc Modification Protects against Protein Misfolding and Aggregation in Neurodegenerative Disease. ACS Chemical Neuroscience. 2019, 10:2209–21. doi: 10.1021/acschemneuro.9b00143.
12. Bethesda (MD): National Library of Medicine (US), National Center for Biotechnology Information; 2004. PubChem Compound Summary for CID 439174, N-Acetyl-D-Glucosamine; [cited 2021 July 8].from: https://pubchem.ncbi.nlm.nih.gov/compound/N-Acetyl-D-Glucosamine

13. The Protein Data Bank https://www.rcsb.org.in
14. Chen JK, Shen CR, Liu CL. N-acetylglucosamine: production and applications. Marine drugs. 2010 Sep 15;8(9):2493-516. https://doi.org/10.3390/md8092493
15. Okamoto M, Kouwaki T, Fukushima Y, Oshiumi H. Regulation of RIG-I activation by K63-linked polyubiquitination. Frontiers in immunology. 2018 Jan 5;8:1942. doi: 10.3389/fimmu.2017.01942