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Research Article

HOMOLOGY MODELING OF POLYMERASE AND CPS BIOSYNTHESIS PROTEINS IN CGSP14 STRAIN OF STREPTOCOCCUS PNEUMONIA AND ITS LIGAND IDENTIFICATION: AN INSILICO APPROACH

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ABSTRACT

Objective: Identifying specific ligands for polymerase and capsular polysaccharide biosynthesis proteins in CGSP14 Strain of streptococcus pneumonia using virtual screening approach along with the validation of ADMET descriptors.

Methods: The target sequences were retrieved from UniProt database,The homology modeling was performed by using Modeller 9v7 which is followed by verification using Ramachandran plot, ligands and their analogs were obtained from Pubchem database,docking with polymerase and capsular polysaccharide biosynthesis proteins and its respective analogs was performed by using Discovery studio.

Results: The docking results show that 6R,7R)-7-[[(2Z)-2-(2-amino-1,3-thiazol-4-yl)-2-methoxyiminoacetyl]amino]-3-[(2-methyl-6-oxido-5-oxo-1,2,4-triazin-3-yl)sulfanyl methyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate and (4S,4aR,5S,5aR,6R,12aR)-4(dimethylamino)-1,5,10,11,12a-pentahydroxy-6-methyl-3,12-dioxo-4a,5,5a,6-tetrahydro-4H-tetracene-2-carboxamide were the two analogs with maximum dock score when it binds with polymerase and capsular polysaccharide biosynthesis proteins respectively.

Conclusion: Our Insilco analysis illustrate that polymerase and capsular polysaccharide biosynthesis proteins have a greater potential to become drug target in CGSP14 Strain of streptococcus pneumonia and the above mentioned ligands having higher dock score may be considered as the potential lead molecules for drug discovery.

Keywords: Streptococcus pneumonia, CGSP14, polymerase , Capsular biosynthesis protein, Modeling and Docking,.

INTRODUCTION

Pneumonia is a lung inflammation which is caused by the infection with bacteria, viruses and other pathogens. *Streptococcus pneumoniae* is one of the most significant microbes which are responsible for causing bacterial diseases in humans [1, 2]. *S. pneumoniae* is the most common cause of bacterial decease in humans. The equilibrium shift from commensal bacterium to opportunistic pathogen occurs in the respiratory tract.

Pathogens exhibit a number of virulence factors to invade and colonize the host tissues. These virulence factors are displayed on the cell surface and include adhesins that mediates the attachment to host cells. Pneumolysin is an important toxin produced by all strains of *S. pneumonia* [3]. *S. pneumoniae* contain a polysaccharide capsule which acts as a virulence factor. At present more than 90 serotypes of distinct nature are known in *S. pneumonia* and these serotypes differ in virulence, prevalence and extent of drug resistance. The efficient degradation of glycoproteins in host is integral to pneumococcal virulence [4,5]. As part of its life cycle, pneumococcus exists as a commensal bacterium that inhabits and colonizes the nasopharynx [6,7].

It has been estimated that more than a million people die every year from pneumococcal infections in a global spectrum [8, 9]. General vaccination with the 7-valent pneumococcal conjugate vaccine was recommended in Germany during July 2006 for children greater than 2 years [10, 11]. In United States and elsewhere, resistance to a range of antibiotics is increasing among the clinical isolates of *S. Pneumoniae* [12-15].

The genome of *S. pneumoniae* CGSP14 virulent strain is of a chromosome with 2 million base pairs having 39.5% of GC content which codes 2206 proteins and 70 structural RNAs. In this present study we focus on two proteins namely polysaccharide polymerase (B2ILP9) and Capsular polysaccharide biosynthesis protein (B2ILP4) were modeled and potential inhibitors were screened.

MATERIAL & METHODS

The sequences of the polysaccharide polymerase (B2ILP9) and Capsular polysaccharide biosynthesis protein (B2ILP4) were obtained from UniProtKB. Since these proteins do not have a structure, homology model building was performed using Modeler9v7 [16-18]. The template structure was obtained from protein data bank (PDB Id: 3N7K: A and 3NEP: X). The modeled structures were validated using SAVS, an online server [19].The binding sites of the protein molecules were predicted by CASTp server. Further, on the basis of high throughput method lead molecules having more affinity with the target proteins were obtained from DrugPort database. Then the structurally similar compounds were obtained using PubChem database. Finally a datset was created for potential ligands inhibiting the target proteins from the *Streptococcus pneumonia* CGSP14 strain using vegaZZ software. Accelrys Discovery Studio 2.0 was used to analyze specific protein-ligand docked complexes and finally toxicity of the ligand molecules were analysed using ADMET descriptors.

RESULTS

Homology modeling

Homology modeling was performed for polysaccharide polymerase (B2ILP9) and Capsular polysaccharide biosynthesis protein (B2ILP4) using the respective templates structures (PDB Ids: 3N7K: A, 3NEP: X). The modeled proteins were validated through SAVS and the validation results are shown in **Table 1**. From the table it is found that more than 80% residues in target proteins are present in the allowed region of Ramachandran Plot. The final structures of modeled protein are shown in **Figure 1**.

Ligand search

Ligands for polysaccharide polymerase (B2ILP9) and Capsular polysaccharide biosynthesis protein (B2ILP4) were retrieved from Drug Port sharing more than 40% identity with related protein

sequence for which a drug which exists already. The analogs for those ligands were obtained from Pubchem and for each protein best analogs were chosen from the hit. The docking was performed with those analogs using Discovery studio software. Dock score was calculated for all the analogs with their respective proteins and the affinity was more when the binding energy is less.

Fig. 1: Homology Modeled Structures of polysaccharide polymerase (B2ILP9) and Capsular polysaccharide biosynthesis protein (B2ILP4).

Capsular polysaccharide biosynthesis protein (B2ILP4)

Amitriptyline, ceftriaxone and Fluoxetine were found to be the best ligand for the protein Capsular polysaccharide biosynthesis protein (B2ILP4) which had an identity of 90,95 and 96 percent with target protein and (3Z)-3-(3-hydroxy-5,6-dihydrodibenzo[1,2-a:1',3'-e][7]annulen-11-ylidene)-N,N-dimethylpropan-1-amine oxide, (6R,7R)-7-[[(2Z)-2-(2-amino-1,3-thiazol-4-yl)-2-methoxyiminoacetyl]amino]-3- [(2-methyl-6-oxido-5-oxo-1,2,4-triazin-3-yl)sulfanylmethyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate and N-methyl-N-[3-phenyl-3-[4-(trifluoromethyl) phenoxy] propyl] acetamide were the best analogs with the dock score of 60.909, 78.531 and 43.645 respectively as the descriptions are mentioned in **Table 2**.The best analogs of Amitriptyline, ceftriaxone and Fluoxetine had a non bonding interaction with the Capsular polysaccharide biosynthesis protein (B2ILP4) with a distance of 1.40527, 1.59782 and 2.29248 respectively as shown in **Table 3** and the interactions between the Capsular polysaccharide biosynthesis protein (B2ILP4) and analogs of selected ligands are illustrated in **Figure 2**.

Table 1: **Percentage of residues in the modeled structure present in allowed region of Ramachandran plot as predicted by SAVS with its similarity and template description.**

Target Protein	Sequence length	Template	Length	Similarity (%)	Ramachandran Plot (%)
Capsular polysaccharide biosynthesis protein Cps14C (B2ILP4)	230	3NEP(X)	305	37.3%	89.6
Polysaccharide polymerase(B2ILP9)	390	3N7K(A)	422	30.8%	84.7

Table 2: **Docking Analysis of Capsular polysaccharide biosynthesis protein (B2ILP4) with respect to ligands with its analogs.**

Ligands	Analogues	PLP1	PLP2	Jain	-PMF	Dock score
Amitriptyline	3-(5,6-dihydrodibenzo[2,1-b:2',1'-f][7] annulen-11-ylidene)-N,N dimethylpropan-1-amine.	44.63	42.52	2.04	67.4	42.161
	(11Z)-11-[3-(dimethylamino) propylidene]-5,6-dihydrodibenzo[2,1-b:3',1'-f][7]annulen-3-ol.	50.47	51.17	1.48	62.3	44.692
	(3Z)-3-(3-hydroxy-5,6-dihydrodibenzo[1,2-a:1',3'-e][7]annulen-11-ylidene)-N,N-dimethylpropan-1-amine oxide	71.37	69.23	2.95	87.12	60.909
Ceftriaxone	(6R,7R)-7-[[(2Z)-2-(2-amino-1,3-thiazol-4-yl)-2-methoxyiminoacetyl]amino]-3- [(2-methyl-6-oxido-5-oxo-1,2,4-triazin-3-yl)sulfanylmethyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate.	70.65	58.32	3.54	76.99	78.531
	(6R,7R)-7-[[(2Z)-2-(2-amino-1,3-thiazol-4-yl)-2methoxyiminoacetyl]amino]-3-[(2methyl-5,6-dioxo-1H-1,2,4-triazin-3-yl)sulfanylmethyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.	63.71	56.24	-1.08	103.7	57.568
	7-[[(2Z)-2-(2-amino-1,3-thiazol-4-yl)-2-methoxyiminoacetyl]amino]-3-[(2-methyl-5,6 -dioxo-1H-1,2,4-triazin-3-yl)sulfanylmethyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.	35.11	31.31	-0.77	69.9	68.852
Fluoxetine	N-methyl-3-phenyl-3-[4-(trifluoromethyl)phenoxy]propan-1-amine.	49.17	48.27	-0.18	61.81	39.759
	1-dideuterio-N-methyl-3-phenyl-3-[4-(trifluoromethyl)phenoxy]propan-1-amine.	.....	---	-----		-----
	N-methyl-N-[3-phenyl-3-[4-(trifluoromethyl)phenoxy]propyl]acetamide	47.54	45.58	0.53	52.47	43.645

Table 3: Interaction between Capsular polysaccharide biosynthesis protein (B2ILP4) and its ligands analogs.

Ligand	Analog	Receptor		Ligand	Distance
		Amino acid	Atom	Atom	
Amitriptyline	CID2160	ARG178	HH	N1	2.23
	CID6428581	GLN4	HE21	O1	2.49003
	CID6428582	ARG178	HH11	O1	1.40527
Ceftriaxone	CID46173365	LYS113	HZ1	O9	1.59782
	CID5479530	ARG143	HH11	O7	1.94842
	CID5896995	LYS64	H23	O5	2.3212

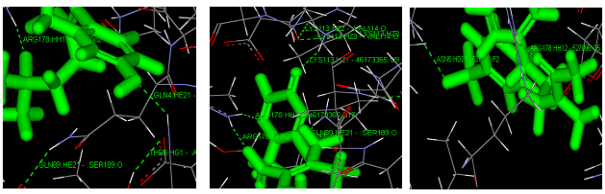
	CID3368	ARG178	HH12	F2	2.02082
Fluoxetine	CID6914091	---	----	----	-----
	CID527096	ASN5	HD21	F2	2.29248

Table 4: Docking Analysis of polysaccharide polymerase (B2ILP9) with respect to ligands with its analogs.

Ligands	Analogues	PLP1	PLP2	Jain	-PMF	Dock score
Imipramine	3-(5,6-dihydrobenzo[b][1]benzazepin-11-yl)-N,N-dimethylpropan-1-amine.	38.69	38.14	0.39	60.23	34.609
	N,N-dimethyl-3-(11-oxido-5,6-dihydrobenzo[b][1]benzazepin-11-ium-11-yl)propan-1-amine.	36.47	36.07	0.53	35.08	48.628
	3-(5,6-dihydrobenzo[b][1]benzazepin-11-yl)-N,N-dimethylpropan-1-amine oxide.	35.9	38.15	0.52	24.71	52.162
Doxycycline	(4S,4aR,5S,5aR,6R,12aR)-4(dimethylamino)-1,5,10,11,12a-pentahydroxy-6-methyl-3,12-dioxo-4a,5,5a,6-tetrahydro-4H-tetracene-2-carboxamide.	8.08	10.23	-3.15	21.74	57.068
	(4S,5S,6R,12aR)-4-[bis(trideuteriomethyl) amino]-1,5,10,11,12a-pentahydroxy-6 methyl-3,12-dioxo-4a,5,5a,6-tetrahydro -4H-tetracene-2-carboxamide.	---	---	---	---	---
	(4S,4aR,5S,5aR,6R,12aS)-4(dimethylamino) -3,5,10,11,12a-pentahydroxy-6-methyl-1,12-dioxo-4a,5,5a,6-tetrahydro-4H-tetracene-2-carboxamide.	34.25	32.59	-1.15	88.05	40.196
Amiodarone	(2-butyl-1-benzofuran-3-yl)-[4-[2-(diethylamino)ethoxy]-3,5-diiodophenyl]methanone.	49.34	47.54	-2.05	66.67	37.082
	2-[4-[(2-butyl-1-benzofuran-3-yl)methyl]-2,6-diiodophenoxy]-N,N-diethylethanamine.	37.47	35.03	-2.16	68.92	37.318
	(2-butyl-1-benzofuran-3-yl)-[3,5-diiodo-4-[1,1,2,2-tetradeuterio-2 (diethylamino) ethoxy] phenyl] methanone.	---	----	---	-----	----

Table 5: Interaction between polysaccharide polymerase (B2ILP9) and its ligands analogs.

Drug	Ligand	Receptor		Ligand	Distance
		Amino acid	Atom	Atom	
	CID3696	ARG144	HH12	N2	2.40649
Imipramine	CID75155	ARG144	HH12	O1	1.92517
	CID65589	ARG161	HE	O1	2.01325
	CID54671203	ARG265	HH22	O8	2.39112
Doxycycline	CID54727554	---	---	---	---
	CID54706023	ARG144	HH12	O7	1.9984
	CID2175	ARG144	HH12	O5	2.05672
Amiodarone	CID164007	ARG189	HH21	O3	2.44359
	CID45038159	---	---	---	---



(a) (b) (c)

a. (3Z)-3-(3-hydroxy-5,6-dihydrodibenzo[1,2-a:1',3'-e][7]annulen-11-ylidene)-N,N-dimethylpropan-1-amine oxide
b. (6R,7R)-7-[[(2Z)-2-(2-amino-1,3-thiazol-4-yl)-2-methoxyiminoacetyl]amino]-3- [(2-methyl-6-oxido-5-oxo-1,2,4-triazin-3-yl)sulfanylmethyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate.
c. N-methyl-N-[3-phenyl-3-[4-(trifluoromethyl)phenoxy]propyl]acetamide

Fig. 2: Docking results of best analogs with Capsular polysaccharide biosynthesis protein (B2ILP4)

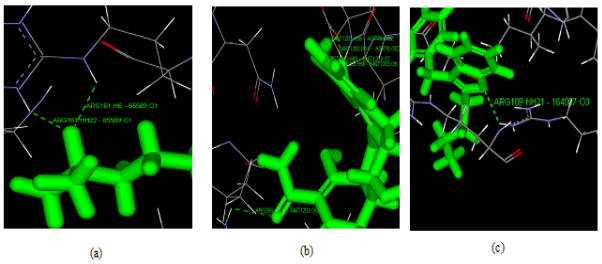
Polysaccharide polymerase (B2ILP9)

Imipramine, Doxycycline and Amiodarone were found to be the best ligands for the Polysaccharide polymerase (B2ILP9) and 3-(5,6-dihydrobenzo[b][1]benzazepin-11-yl)-N,N-dimethylpropan-1-amine oxide, (4S,4aR,5S,5aR,6R,12aR)-4(dimethylamino)-1,5,10,11,12a-pentahydroxy-6-methyl-3,12-dioxo-4a,5,5a,6-tetrahydro-4H-tetracene-2-carboxamide and 2-[4-[(2-butyl-1-benzofuran-3-yl)methyl]-2,6-diiodophenoxy]-N,N-diethylethanamine were identified as the best analog with the dock score of 52.162, 57.068 and 37.318 respectively as illustrated in **Table 4**.

The best analogs of Imipramine, Doxycycline and Amiodarone had a non bonding interaction with the polysaccharide polymerase (B2ILP9) protein with a distance of 2.01325, 2.39112and 2.44359 respectively as shown in **Table 5** and the interactions between the polysaccharide polymerase (B2ILP9) and analogs of selected ligands are illustrated in **Figure 3**.

ADMET properties for the analogs of ligands having better dock score and maximum interaction with the active site residues were analyzed. The plot of polar surface area (PSA) vs logP is shown in **Figure 4**. Based on our analysis, it has been found that the analogs

which had maximum dock score have proper lopP, Absorption and Blood Brain Barrier values.



(a) (b) (c)

a. 3-(5,6-dihydrobenzo[b][1]benzazepin-11-yl)-N,N-dimethylpropan-1-amine oxide.
b. (4S,4aR,5S,5aR,6R,12aR)-4(dimethylamino)-1,5,10,11,12a-pentahydroxy-6-methyl-3,12-dioxo-4a,5,5a,6-tetrahydro-4H-tetracene-2-carboxamide.
c. 2-[4-[(2-butyl-1-benzofuran-3-yl)methyl]-2,6-diiodophenoxy]-N,N-diethylethanamine.

Fig. 3: Docking results of best analogs with polysaccharide polymerase (B2ILP9).

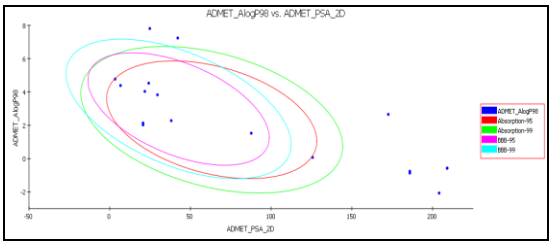


Fig.4: ADMET plot of the analog compounds.

DISCUSSION

There had been a lot of research on CGSP 14 and many other strains of *Streptococcus pneumoniae*, though there is a lot of worldwide mortality due to pneumonia. So we have concentrated on a virulent strain CGSP 14 for the analysis. We found that there are 2206 proteins coded by this strain in which many proteins are not crystallized and our objective is to identify the efficient drug target in these proteins. As we were analyzing these proteins, we found that the proteins polysaccharide polymerase (B2ILP9) and Capsular polysaccharide biosynthesis protein (B2ILP4) may act as drug target and hence we performed homology modeling to understand the role of these proteins in disease pathway on the basis of 3D structure. Based on the docking studies we came to a conclusion that the analog molecules having higher dock score can be considered as leads and the receptors as their targets.

CONCLUSION

Based on docking studies it has been concluded that 6R,7R)-7-[[(2Z)-2-(2-amino-1,3-thiazol-4-yl)-2-methoxyiminoacetyl]amino]-3-[(2-methyl-6-oxido-5-oxo-1,2,4-triazin-3-yl)sulfanyl methyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate and (4S,4aR, 5S,5aR,6R,12aR)-4(dimethylamino)-1,5,10,11,12a-pentahydroxy-6-methyl-3,12-dioxo-4a,5,5a,6-tetrahydro-4H-tetracene-2-carboxamide are the two analogs with maximum dock score when it binds with B2ILP4 and B2ILP9 respectively. ADMET descriptors were also analyzed for the drug candidates and these proteins have a greater potential to become drug targets and the above mentioned ligands having higher dock score may be considered as the potential lead molecules for drug discovery.

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