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Research Article

IN SILICO STUDIES OF N-(PHENYL SUBSTITUTED) 2-([PHENYL SUBSTITUTED) METHYLIDENE] AMINO)-N,4- DIPHENYL-6H-1,3-OXAZIN-6-AMINE DERIVATIVES AS POTENTIAL ANTIBACTERIAL AGENTS

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ABSTRACT

Resistance to bacteria is a growing threat to human health worldwide. The rate of discovery of new antibacterial is far outshined by the rate at which resistance is spreading. Therefore, there remains a pressing need for the development of new antibacterial drugs. Recent alarm estimates that deaths due to antimicrobial resistance may increase from 700,000 million lives annually by 2050. Glucosamine-6-phosphate (GlcN-6-P) synthase represents an interesting protein target because it plays an essential role in the protection of cell wall. The primary aim and objective of this study is to identify lead molecules as promising antibacterial agents by inhibiting Glucosamine-6-phosphate (GlcN-6-P) synthase enzyme. Autodock 4. 2, the effective tool for exploring the binding affinity of small molecule to enzyme target was used to study the interactions between the oxazine derivatives and the GlcN-6-P synthase binding site. The ligands were optimized for improving their efficacy and safety. Lead optimization was performed using Molinspiration server and the ligands were optimized for evaluating their oral bioavailability. With Glucosamine 6 phosphate synthase receptor, the binding energy was found to be best for 5 compounds SZ-3 (-5.27 kcal/mol), SZ-4 (-6.02 kcal/mol), SZ-5 (-5.35 kcal/mol), SZ-8 (-5.62kcal/mol), SZ-10 (-5.29 kcal/mol) when compared to the standard ligand, Ciprofloxacin (-5.09 kcal/mol) and were interacting well with the key residues TYR 304, GLU 438, LEU 484. Docking study strongly enhanced the activity of oxazine derivatives as new discovered hits.

Key words: Oxazines, Glucosamine-6-phosphate synthase, Virtual Screening, Autodock, Molecular docking, Antibacterial

INTRODUCTION

During the past years considerable evidence have also accumulated to demonstrate the efficacy of 1,3-oxazines including antibacterial [1,2,3,4], antitubercular [5,6,7], anticancer [8,9], anticoagulant [10,11] and anti-HIV activity [12,13]. Literature survey revealed that slight modification in the structure can cause wide variations in activity, which prompted us to undertake the synthesis of various Schiff bases of oxazine derivatives with the aim of having improved activity, lesser toxicity. Based on the findings of various literatures, we planned to synthesize substituted 1,3-oxazine compounds to get potent bioactive molecule.

To explain the promising activity of these derivatives as antimicrobial agents, this research includes the molecular docking of discovered hits within the binding pocket of L-Glutamine: D-fructose-6-phosphate amido transferase, known as glucosamine-6- phosphate synthase (GlcN-6-P) which is the effective target in antimicrobial chemotherapy [14,15].

MATERIALS AND METHODS

Glucosamine -6-phosphate synthase

Glucosamine -6-phosphate synthase enzyme catalyzes the first step in hexosamine biosynthesis, converting D-fructose 6-phosphate into D-glucosamine-6-phosphate (GlcN-6-P) using glutamine as the ammonia source and leading to the eventual formation of uridine-5-diphospho-N-acetyl-D- glucosamine, the important point of metabolic control in the biosynthesis of amino sugar containing macromolecules which is necessary for the cell wall assembly in bacteria and fungi [16,17]. Inhibition of this enzyme will restrain the cell membrane production and thereby significantly reduces the population of bacteria.

In silico studies

Computer docking technique plays an important role in the drug design and discovery, as well as in the mechanistic study by placing a molecule into the binding site of the target macromolecule and predicting the binding geometry and hydrogen bonds formed with the surrounding amino acids as well as the mode of the ligand to the receptor, which all contribute to the docking score.

Based on these views, an effort was made to design and develop new antibacterial agents by computational methods. The primary aim and objective of this study is to identify lead molecules as promising antibacterial agents by inhibiting Glucosamine-6-phosphate (GlcN-6-P) synthase enzyme.

Autodock 4. 2, the effective tool for exploring the binding affinity of small molecule to enzyme target was used to study the interactions between the oxazine derivatives and the Glc N-6-P synthase binding site. D-glucosamine-6-phosphate synthase (GlcN-6-P) enzyme inhibition will restrain the production of cell membrane and decreases the population of bacteria. The PDB file format of enzyme as receptor was obtained from the RCSB

Protein Data Bank (PDB code 1MOQ) and used as a rigid molecule [18].

Selection of target

The enzyme is commonly known by the trivial name of glucosamine-6-phosphate synthase, which is characterized as a new antimicrobial target. This enzyme carries out transferase reaction which involves the transfer of ammonia group from L-glutamine to fructose-6-phosphate (Fru-6-P), which is followed by isomerization of fructosamine-6-phosphate to glucosamine-6-phosphate.This reaction is involved in the formation of uridinediphosphate N-acetylglucosamine (UDP-GlcNAc), a product that is present in all class of organisms, but in case of fungi and bacteria it is solely used to build macromolecules related with cell wall assembly, *e.g.* chitins and mannoproteins in case of fungi and peptidoglycan in bacteria. Hence, the target glucosamine-6-phosphate synthase shown in Figure 1 was selected for performing the *in-silico* studies in the present study.

Virtual screening

Virtual screening was done by using iGEMDOCKv.2[19].

A small molecule library consisting of 15 compounds were constructed. Molecules were taken from ZINC, a free database of 13 million commercially available compounds in ready –to – dock, 3D formats for virtual screening. The protein with the accession code 1MOQ was selected from the RCSB protein data bank. The protein was refined using BIOVIA discovery studio visualizer. The protein was uploaded in the iGEMDOCKv.2 & the binding sites were chosen. Similarly, ligands were uploaded in the iGEMDOCKv.2. Start virtual screening module was clicked & the fitness value was saved. Among the 15 compounds of Glucosamine 6 phosphate synthase inhibitors screened, OXAZINES were found to be the lead. The snap shots of ligands binding are shown in Figure 2.

On analyzing the results, OXAZINE nucleus was found to have good fitness value. Hence from literature and virtual screening technique performed in iGEMDOCKv2, **OXAZINE** was taken as a lead in the present study. The fitness values are table in Table 1.

Lead Optimization

Lipinski's Rule of Five can be applied to evaluate drug likeness or determine if a chemical compound with a certain pharmacological or biological activity has properties that would make it a likely orally active drug in humans[20]. Lipinski's rule says that, in general, an orally active drug has not more than one violation of the following criteria:

The structures of the target compounds are tabulated in Table 2. The results of lead optimization are tabulated in Table 3.

Molecular Docking

The PDB file format of enzyme as receptor was obtained from the RCSB Protein Data Bank (PDB code 1MOQ) and used as a rigid molecule. Docking studies was performed with Autodock 4.2 version[21]. Steps involved in docking involves Enzyme Refinement, Protein structure refinement, Ligand File Format Conversion and docking.

The docking results of **Glucosamine 6 phosphate synthase (1MOQ.pdb)** with the ligands **SZ (1-10)** is reported below. The binding sites and the active sites are being presented in the snap shots as in Figure 3 and the binding energy was found to be more or less equivalent or lower when compared to the standard. The interaction profile of the compounds is tabulated in Table 4.

RESULTS AND DISCUSSION

During the past years considerable evidence have also accumulated to demonstrate the efficacy of 1,3-oxazines including antibacterial, antitubercular, anticancer, anticoagulant and anti-HIV activity. Literature survey revealed that slight modification in the structure can result in qualitative as well as quantitative changes in the activity, which prompted us to undertake the synthesis of various Schiff bases of oxazine derivatives with the aim of having improved activity, lesser toxicity. The review of literature reveals also prompted us to synthesize substituted 1,3-oxazine compounds to get potent bioactive molecule.

Molecular docking is a computer-assisted drug design (CADD) method used to predict the favorable orientation of a ligand (viz. drug) to a target (viz. receptor) when bound to each other to form a stable complex. By understanding of the favored orientation in turn can be used to find out the strength of binding affinity between ligand and target site, e.g., by docking score. Moreover, docking study can be used to find out type of interactions between ligand and receptor viz. hydrogen bonding and hydrophobic interactions. Hence, molecular docking can be considered as first-line technique for a pharmaceutical lead discovery.

To explain the promising activity of these derivatives as antimicrobial agents, this research includes the molecular docking of discovered hits within the binding pocket of L-Glutamine: D-fructose-6-phosphate amido transferase, known under the trivial name of glucosamine-6- phosphate synthase (GlcN-6-P) which represents the effective target in antimicrobial chemotherapy.

This enzyme catalyzes the first step in hexosamine biosynthesis, converting D-fructose 6- phosphate (Fru-6-P) into D-glucosamine-6-phosphate (GlcN-6-P) using glutamine as the ammonia source and leading to the eventual formation of uridine-5-diphospho-N-acetyl-D- glucosamine (UDP GlcNAc), the important point of metabolic control in the biosynthesis of amino sugar containing macromolecules which is necessary for the cell wall assembly in bacteria and fungi. Inhibition of this enzyme will restrain the production cell membrane and significantly decrease population of bacteria.

Autodock4. 2, the effective tool for exploring the binding affinity of small molecule to enzyme target was used to study the interactions between the oxazine derivatives and the Glc N-6-P synthase binding site.

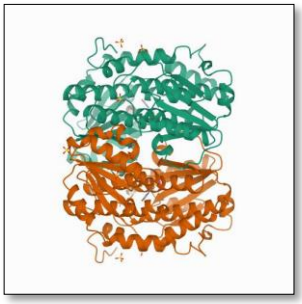


Figure 1: Glucosamine-6-phosphate synthase (GlcN-6-P) enzyme (PDB ID:1MOQ)

Virtual screening was done by using iGEMDOCK v. 2 where oxazines were obtained as the lead from a library of fifteen hits and focused as D-glucosamine-6-phosphate synthase (GlcN-6-P) enzyme inhibitors. The small molecular library was constructed from ZINC database. Virtual screening revealed that oxazine scaffold can act as promising D-glucosamine-6-phosphate synthase (GlcN-6-P) enzyme inhibitors.

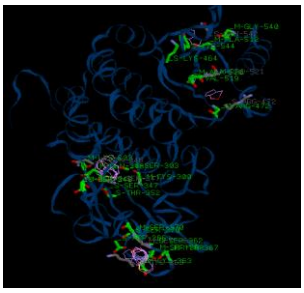


Figure 2: Binding of ligands with 1MOQ.pdb

Table 1: Fitness values		
S.No	**Compound code**	**Fitness value**
1	**OX-1**	**-61.96**
2	**OX-2**	**-54.57**
3	**OX-3**	**-59.25**
4	**OX-4**	**-63.37**
5	**OX-5**	**-62.44**
6	TZ-1	-44.75
7	TZ-2	-43.14
8	TZ-3	-38.26
9	TZ-4	-46.02
10	TZ-5	-50.52
11	PY-1	-47.99
12	PY-2	-45.71
13	PY-3	-37.77
14	PY-4	-42.27
15	PY-5	-47.26

Table 2: Structure of target compounds

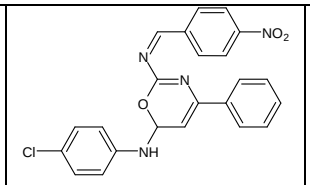
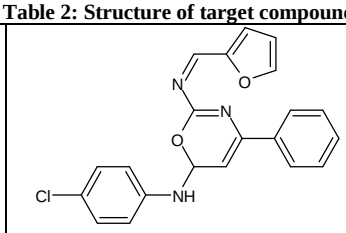
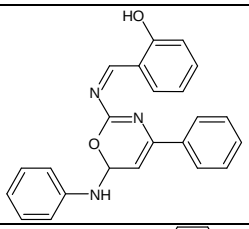
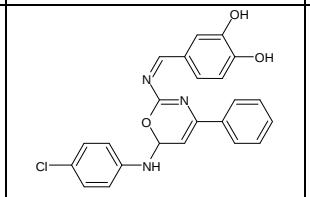
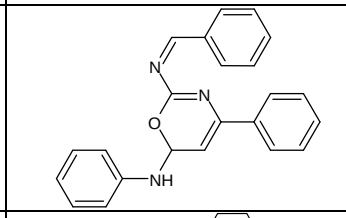
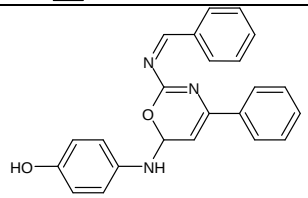
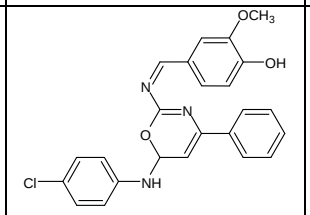
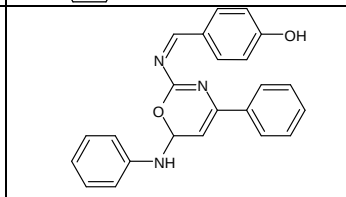
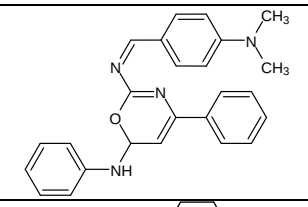
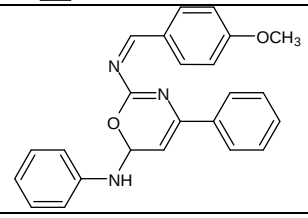
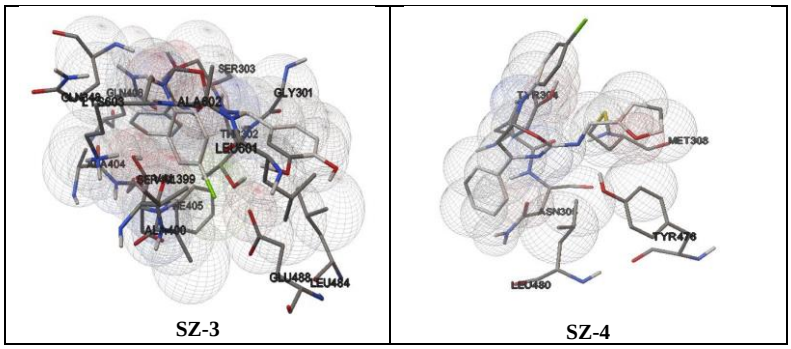
SZ-1		**SZ-4**		**SZ-7**	
SZ-2		**SZ-5**		**SZ-8**	
SZ-3		**SZ-6**		**SZ-9**	
				SZ-10	

Table 3: Drug likeness score of ligands									
S.No	**Compound Code**	**Log P**	**TPSA**	**No. of ATOMS**	**Molecular Weight**	**No. of ON**	**No. of OHNH**	**No. of violations**	**No. of rotational bonds**
1	SZ-1	5.47	91.81	31	432.87	7	1	1	6
2	SZ-2	4.54	86.44	30	419.87	6	3	0	5
3	SZ-3	4.85	75.45	31	433.89	6	2	0	6
4	SZ-4	4.76	59.13	27	377.83	5	1	0	5
5	SZ-5	4.83	45.99	27	353.43	4	1	0	5
6	SZ-6	4.35	66.22	28	369.42	5	2	0	5
7	SZ-7	4.77	66.22	28	369.42	5	2	0	5
8	SZ-8	4.35	66.22	28	369.42	5	2	0	5
9	SZ-9	4.93	49.23	30	396.49	5	1	0	6
10	SZ-10	4.89	55.22	29	383.45	5	1	0	6

Lead optimization was performed using Molinspiration server and the ligands were optimized for evaluating their oral bioavailability. The ligands were optimized for improving their efficacy and safety. The results of the study revealed that all the ten derivatives possess good drug likeness score.

In this study, Auto Dock 4.2 package software was used to investigate the affinity of synthesized compounds to the binding pocket of GlcN-6-P synthase. The PDB file format of enzyme as receptor was obtained from the RCSB Protein Data Bank (PDB code 1 MOQ) and used as a rigid molecule. In this study, Auto dock 4.2 was used to evaluate the binding energy of ligands inside the known 3D structure of target enzyme.



SZ-3 **SZ-4**

Figure 3: Docking poses of ligands with 1MOQ.pdb

Table 4: Binding energy of docked compounds

S.No	Compound Code	Binding energy Kcal/mol	Interacting amino acids
1.	SZ-1	-4.17	Ala 496, Arg 311, Leu 480, Met 308, Tyr 304, Tyr 476
2.	SZ-2	-5.17	Ala 483, Ala 496, Asn 305, Glu 495, Gly 301, Leu 480, Leu 484, Tyr 304
3.	SZ-3	-5.27	Ala 400, Ala 404, Ala 602, Gln 349, Gln 408, Glu 488, Gly 301, Leu 601, Ser 303
4.	SZ-4	-6.02	Asn 306, Leu 480, Met 308, Tyr 304, Tyr 476
5.	SZ-5	-5.35	Ala 496, Asn 305, Glu 438, Leu 484, Tyr 304
6.	SZ-6	-5.05	Ala 483, Ala 496, Asn 305, Leu 480, Leu 484, Lys 487, Met 308, Tyr 304, Tyr 476
7.	SZ-7	-4.86	Ala 483, Ala 496, Ile 326, Gly 301, Leu 480, Leu 484, Lys 487, Tyr 304
8.	SZ-8	-5.62	Asn 305, Ile 326, Gly 301, Leu 480, Leu 484, Tyr 304
9.	SZ-9	-5.19	Ala 602, Asn 305, Gly 301, Leu 480, Leu 488, Leu 601, Tyr 304, Val 399
10.	SZ-10	-5.29	Asn 305, Gly 301, Leu 480, Leu 484, Lys 487, Tyr 304, Tyr 476
11.	Ciprofloxacin	-5.09	Tyr 304, Glu 438, Leu 484

With Glucosamine 6 phosphate synthase receptor, the binding energy was found to be best for 5 compounds SZ-3 (-5.27 kcal/mol), SZ-4 (-6.02 kcal/mol), SZ-5 (-5.35 kcal/mol), SZ-8 (-5.62kcal/mol), SZ-10 (-5.29 kcal/mol) when compared to the standard ligand, Ciprofloxacin (-5.09 kcal/mol) and were interacting well with the key residues TYR 304, GLU 438, LEU 484.

CONCLUSION

New oxazine derivatives were designed and synthesized. Molecular docking studies were carried out to know the interaction of synthesized compounds against active site of glucosamine-6-phosphate synthase (GlcN-6-P) which is the target for antibacterial agents. GlcN-6-P synthase is considered as primary target because it is essential building block of bacteria which involves in biosynthesis of cell wall in most of bacteria and fungi. Inhibition of this enzyme will restrain the production cell membrane and significantly decrease population of bacteria.

To explain the activity of new derivatives, we have explored the binding affinity of the compounds against glucosamine-6-phosphatesynthase, the target enzyme for the antibacterial activity. Docking study strongly enhanced the activity of these compounds as new discovered hits. This computational prediction is in a very good agreement which proves the significant impact of this molecular docking study. The novel oxazine derivatives were identified as promising antibacterial agents.

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