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DESIGN, BINDING AFFINITY STUDIES AND IN-SILICO ADMET PREDICTIONS OF NOVEL ISOXAZOLES AS POTENTIAL ANTI-BACTERIALS

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ABSTRACT

Globally bacterial infections are on the rise owing to the present lifestyle and environmental conditions. Drug resistant strains have developed rapid development of resistance to the existing drugs and multidrug resistance to some combinational treatments. Presently, the world faces a post-antibiotic period, where typical bacterial infection and mild injuries might lead to death. As per the recent report (Center for Disease Control and Prevention), over 2 million individuals get affected by antibiotic-resistant bacteria in the United States. At least 23,000 people end their lives every year. Hence there is a dire need to develop novel, effective antibacterials.

Experimental Work: With the intent to discover potent novel anti-bacterials, we have designed a set of compounds containing the isoxazole nucleus by using software tools like Discovery studios, PyRx, PyMOL, SWISSPDB. ADMET studies were carried out by using SWISS ADMET and pkCSM. Molecular docking studies were carried out on the target proteins of both gram positive and gram-negative bacteria in order to assesses binding affinity for the proteins.

Result and Discussion: Designed scaffold was designed by Benzene Derivatives Tethered With 5(4-chloro-3-nitro phenyl-1-yl)isoxazole. All the derivatives were docked against the five proteins namely DNA Ligase (PDB ID: 3PN1), Topoisomerase (PDB ID: 3TTZ), Sterol demethylase (PDB ID: 5FSA), The compound JJC3F has shown best binding score against DNA ligase, sterol demethylase protein. Further, compound JJC3A has shown better binding affinity towards topoisomerase than the standard drugs.

Conclusion: Molecular Docking study indicates that isoxazole derivatives may be effective inhibitors for the different microbial proteins. Additionally, *in silico* ADMET studies predicts drug like features. Hence, these compounds may be considered as leads and further investigation of their analogues may help in development of novel drugs for the treatment of microbial diseases.

Key words: Drug resistant, ADMET, Benzene Derivatives, Molecular Docking

INTRODUCTION

Drug discovery is a method and time-thorough process which is aimed at developing new drug candidates. By using the aid of computational means in the pre-clinical phase of drug discovery. Computer-aided drug design (CADD) is defined as discover, develop, and analyse drug and active molecules with similar biochemical properties by using computational approaches. Some of them are Homology modelling; molecular docking, virtual screening (VS) or virtual high-throughput screening (vHTS), quantitative structure reactivity relationship (QSAR) and three-dimensional (3D) pharmacophore mapping generally, are the main constituents of CADD. Among these techniques, it seems that virtual screening is the major contributor to CADD and it has become somewhat a proven and well-appreciated computational method, which stands as a contemporary to the experimental high-throughput screening for hit identification and optimization, This computational method mainly based upon the improvements in computing algorithms, a considerable development of computers processing power and as well as in the vast knowledge of structural and physico-chemical properties of compounds in libraries and databases like pubchem etc., and the increased knowledge of the structural and functional properties of protein molecules. This computational method can be applied to screen for chemical compounds (natural and synthesized), peptides or proteins [1].

Molecular docking is used to find out the hit molecule from numerous compounds to a particular protein 3d structure is available. Lock and key, induced work and ensemble these are the three categories of docking; Docking methodology are following rigid ligand and rigid receptor docking; flexible ligand and rigid receptor docking; and flexible ligand and flexible receptor docking. Docking software's are available in-house and out-house for use [2].

The main aim of docking is to find the ligand which is fitting into the binding site of a receptor results like binding affinity, force-field, empirical etc., are useful to find out best conformation between ligand and receptor [2].

Microbial diseases are becoming global threat because of the effective decrease in the potential activity of anti-microbial therapies and also bacteria's like *Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumonia, Acinetobacter baumannii, Pseudomonas aeruginosa, Enterobacter* these families of organism becomes serious threat to the society because of change in the pathology of these organisms[3][4].

Microbial organisms are adapting to the condition and evolving their nature and acquiring resistance to the available anti-microbial drugs. So that there is need for developing novel anti-microbial agents [5].

Based on the need for novel microbial agents we designed a set of isoxazole contains compounds by using this computational software's.

Isoxazole is a five membered ring with two hetero atoms like oxygen and nitrogen which are present adjacent to each other, isoxazole are most widely used against insecticidal, antibacterial, antibiotic, antitumour, antifugal, antituerculosis, anticancer and ulcerogenic agents [6].

Hence, the present study focuses on designing of novel isoxazole derivatives, predicting their interactions with the selected target proteins and determining the ADMET properties of potent molecules using recent computational methods.

METHOD AND METHODOLOGY

Selection of target protein and ligand

In this study, three protein targets were selected namely DNA ligase, Topoisomerase, 14-α Sterol demethylase protein and their X-Ray Diffraction structures were taken from RCSB Protein Data Bank in PDB ID: 3PN1[6], 3TTZ[7], in PDB format respectively.

Preparation of protein

Preparation of protein was done on swisspdb after downloading the protein from protein data bank in pdb format and it was opened in swisspdb. It will automatically arrange all the missing amino acids and remove water molecule and add hydrogen bonds to it and save it in pdb format.

Preparation of ligands

Designed ten ligands structure were drawn by using chemsktech and saved in SDF format. All the ligands were then converted to cluster file in SDF format using Discovery Studio 2021 Client. Standard drugs Ciprofloxacin, Moxifloxacin were selected for docking on the same targets in order for comparison of docking scores. The structures of the standards were downloaded from Pubchem and saved in 3D conformer as SDF format.

Assigning grid box to define binding site

The structure of protein was loaded in PyRx software where the Kollmann and Gasteiger charges were assigned. The proteins were then converted to PDBQT file format. Then the ligands were loaded into PyRx, energy minimised and converted to PDBQT file format. Finally specific protein and ligands were selected for docking and grid box was assigned in the protein structure.

Molecular docking process

Molecular docking was performed in PyRx software after assigning grid dimensions. Docking of the ligands at the active site of the respective proteins was carried out. The docking scores and binding energy analysis were downloaded in the CVS format

Visualisation of docking poses

The ligand which gave best score compared to standard drugs against the proteins was chosen it will be opened along with protein in pymol for envisioning the 3D interaction. The multiple files will be compressed into a single file and its docking interactions were visualised in 2D conformation using Discovery Studio 2021 Client.

ADMET studies using pkCSM [8]

Physic-chemical properties	**compounds**	
	JJC3A	**JJC3F**
Descriptor	**Value**	**Value**
Molecular Weight	318.716	337.162
LogP	3.5708	4.5186
Rotatable Bonds	3	3
Acceptors	5	4
Donors	2	1
Surface Area	130.256	135.765

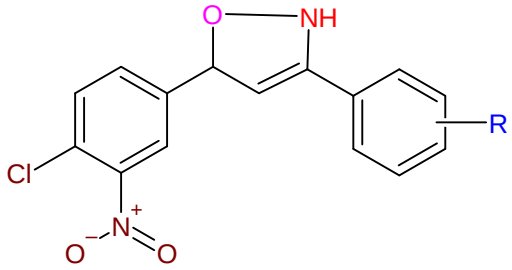
RESULTS AND DISCUSSION

Designed compound shows better binding affinity and also all the designed compounds are following Lipinski rule of five which means the designed may show potent activity towards selected targets docking results revealed that Substitution of OH group at 2nd position show

better activity against DNA gyrase, moreover compound JJC3A shows better activity against topoisomerase

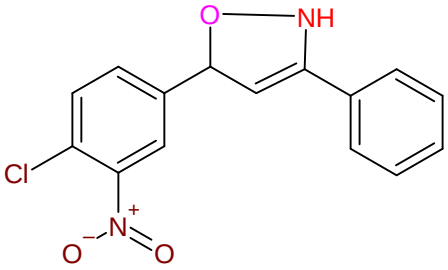
Scaffold Design [9]

The structure of scaffold is given in figure. Total Ten derivatives of the designed scaffold were prepared using ChemDraw 20.0 and their structures are given in figure.

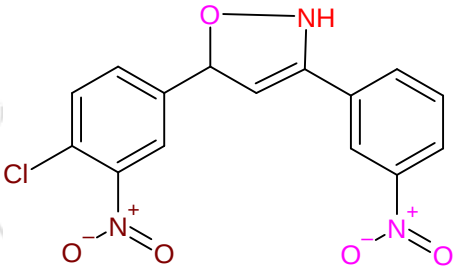


JJC3A-J

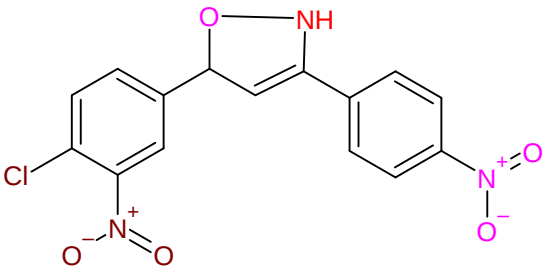
Figure:1 Benzene Derivatives Tethered With 5(4-chloro-3-nitro phenyl-1-yl)isoxazole



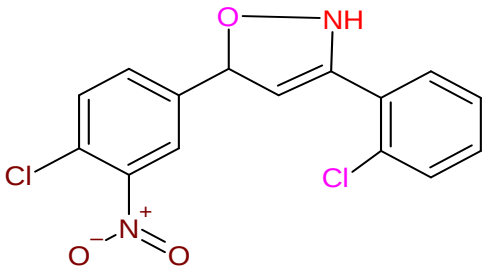
JJC3A



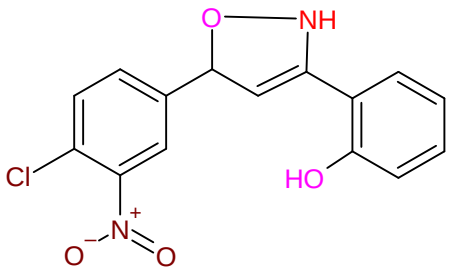
JJC3B



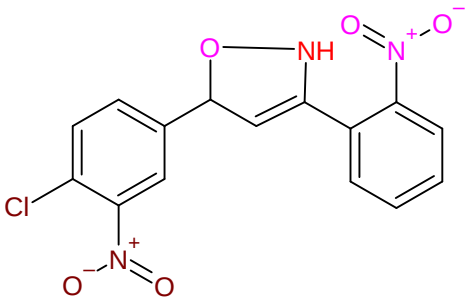
JJC3C



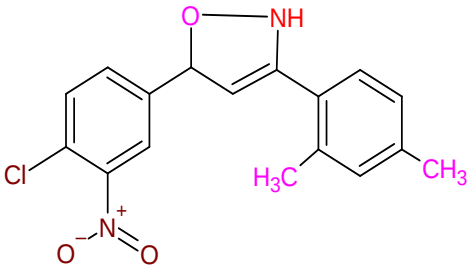
JJC3D



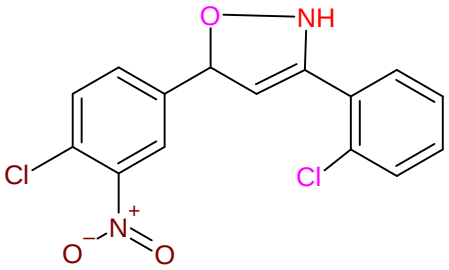
JJC3E



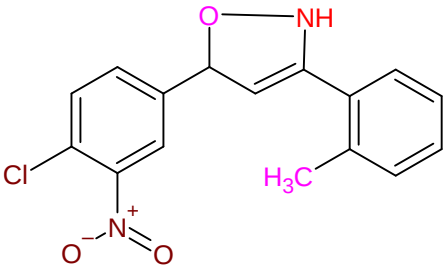
JJC3F



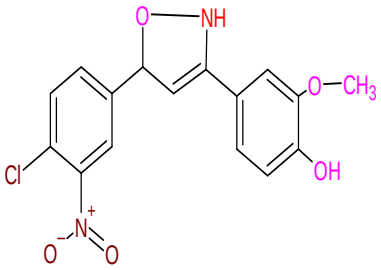
JJC3G



JJC3H



JJC3I



JJC3J

Molecular Docking Studies

Molecular docking is the computational method of drug discovery which is use for prediction of interaction between the ligand and protein The interaction energy includes van der Waals energy, electrostatic energy, as well as intermolecular hydrogen bonding were calculated for each minimized complex. The residues thus predicted are energetically important for ligand binding inside the binding site via hydrophobic, hydrogen bond interactions in almost all complexes.

Table 1: Docking Scores of ligands against selected target proteins

S.NO	LIGAND	DOCKING SCORE	
		DNA Ligase (3PN1)	Topoisomerase (3TTZ)
1.	JJC3A	9.2	-9.3
2.	JJC3B	9.6	-8
3.	JJC3C	9.0	-86
4.	JJC3D	8.8	-8.8
5.	JJC3E	8.7	-9
6.	JJC3F	10.1	-8.1
7.	JJC3G	9.9	-8.8
8.	JJC3H	9.5	-8.6
9.	JJC3I	9.6	-8.7
10.	JJC3J	9.9	-8.6
S1	Ciprofloxacin	-8.9	-7.6
S2.	Moxifloxacin	-9.9	-7.1

All the ligands were docked against the selected proteins. Among them compound JJC3F has shown best binding score against DNA ligase. Further, compound JJC3A has shown better binding affinity towards topoisomerase than the standard drugs. The 2D interactions of potent molecules with the target proteins are shown in below figures 2-4.

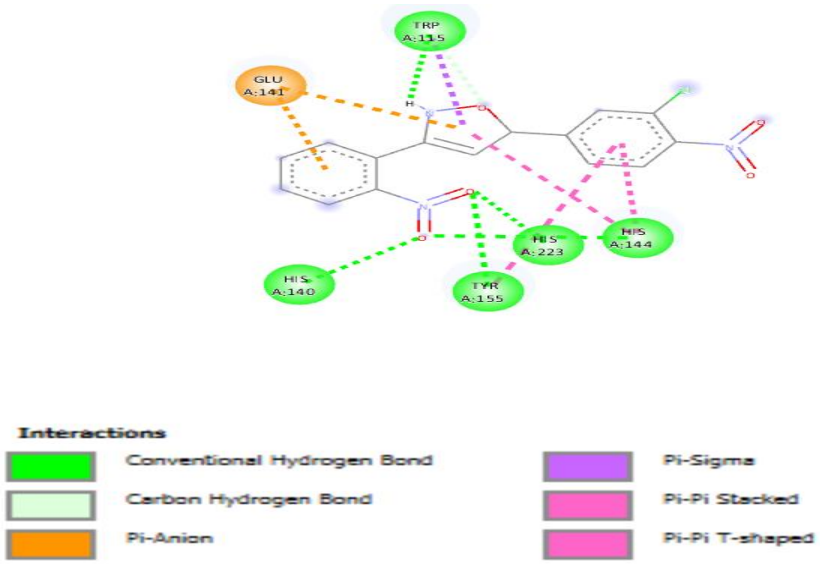


Figure 2: 2D interaction of compound JJC3F with DNA Ligase

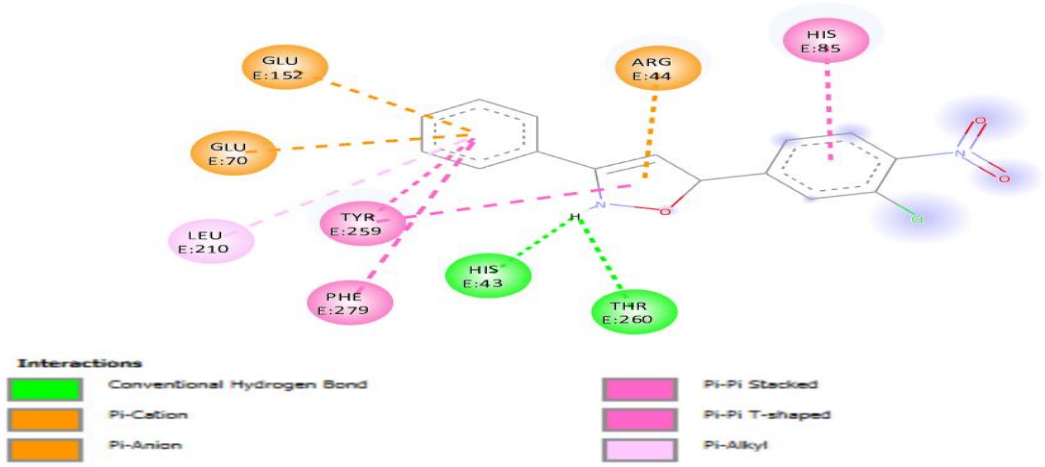


Figure 4: 2D interaction of compound JJC3A with Topoisomerase

CONCLUSION

Molecular Docking study indicates that Benzene Derivatives Tethered with 5(4-chloro-3-nitro phenyl-1-yl) isoxazole may be effective inhibitors for selected bacterial proteins. Additionally, *in silico* ADMET studies predicts drug like features. Therefore, these compounds can be considered as leads for further investigation in development of novel, effective antibacterials.

Data Availability

The data used to support the findings of this study are available with the corresponding author upon request.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

Authors' Contributions

The study protocol was designed by Judy Jays and coordinated the overall project. Molecular docking and ADMET studies were performed by G. Chaitanya Sai along with Burhanuddin Madriwala

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