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Original Article

A MOLECULAR MODELLING APPROACH FOR STRUCTURE-BASED VIRTUAL SCREENING AND IDENTIFICATION OF NOVEL ISOXAZOLES AS POTENTIAL ANTIMICROBIAL AGENTS AGAINST *S. AUREUS*

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ABSTRACT

Objective: Universal use of antibacterial agents and swift development of resistance by the microorganisms pose a major threat to public health. Hence, there is a pressing need to develop novel antimicrobials. Isoxazole derivatives exhibiting versatile biological activities have been widely used as important scaffolds in the field of drug designing.

Methods: Twenty isoxazole derivatives were virtually screened by means of the molecular docking approach in order to identify potential antimicrobials against the most common disease-causing bacteria, *S. aureus*. *In silico* studies were done to detect the selectivity of the novel isoxazole derivatives for the selected bacterial protein targets using 'Glide'. *In silico* docking was carried out on few essential enzymes of *S. aureus*; Dihydrofolate reductase (DHFR), DNA gyrase, Dihydropteroate Synthetase (DHPS), Pyuvate kinase (PK). The compounds were subjected to energy minimization, followed by optimization and minimization of protein and generation of 3D grid at its active site. The ligands were subjected to molecular docking the Standard Precision and Extra Precision modes.

Results: Docking of the compounds with Pyruvate Kinase and dihydrofolate reductase are quite encouraging.2C (4-hydroxy) and 2D (4-hydroxy) analogues gavea G Score of-8.33 and-8.64 with DHFR and Pyruvate Kinase respectively. However, the dock scores for the other target proteins indicate that the scaffolds have not bound with those bacterial targets. Moreover, ADME studies indicate that the derivatives do not show any violations in the rules for the requirements of orally active drugs.

Conclusion: Study suggests that the derivatives 2C (4-hydroxy) and 2D(2-hydroxy) specifically bind to the active site of PK and DHFR. *In silico* ADME studies predicted the compounds to be "drug-like." Hence the hydroxy derivatives may be considered as leads for further structural modifications to arrive at potential anti-bacterial agents.

Keywords: Antimicrobial, Isoxazoles, Molecular docking, *S. aureus*, *In silico*, ADME

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INTRODUCTION

Staphylococcus aureus is one of the most common and destructive human pathogen [1]. Being a gram-positive bacterium, it is prevalent in milk and dairy products [2] and it is instrumental in causing numerous human infections, including bacteremia [3], hospital-acquired respiratory tract infections [4], infective endocarditis, skin and soft tissue infections, gastroenteritis, septic arthritis, osteomyelitis, prosthetic device infections, pulmonary infections, urinary tract infections and toxic shock syndrome [2]. Strains of *S. aureus* are becoming increasingly virulent and resistant to the existing antibiotics [3]. Management of *S. aureus* infections is becoming progressively challenging owing to the emergence of multi-drug resistant strains such as MRSA [5, 6].

Many synthetic compounds containing isoxazole nucleus possess various pharmacological activities such as antibacterial, antifungal, antiviral, antidepressant, anti-inflammatory, anti-ulcer, diuretic and antihypertensive activities [7]. With this standpoint, novel isoxazole derivatives were designed with the intent of discovering potent antibacterial agents which may be effective against *S. aureus*.

Virtual screening is recognized as an effective method for the discovery of hit compounds and help towards lead optimization in structure-based drug discovery. There are roughly 200 vital proteins in bacteria, yet very few bacterial targets have been exploited. Molecular docking studies helps to recognize prospective lead candidates and fewer compounds need to be experimentally screened. Besides recognizing small molecules which are likely to bind well to the protein target, docking studies also explain the binding interactions [8] of these compounds with target, thereby augmenting the knowledge for structural optimization. Docking of all the isoxazole derivatives were done at the active site of the specific proteins using "Glide" [9]. To reveal the type of interaction of the designed compounds with the bacterial targets, the compounds were docked onto the essential bacterial proteins [10]. The interactions of the isoxazole derivatives were studied at the active site of four essential proteins of *S. aureus*; Dihydrofolate reductase (PDB ID: 3SRW), Dihydropteroate synthetase (PDB ID: 1AD4), Pyuvate kinase (PDB ID: 3TO7) and DNA gyrase (PDB: 5BS3).

Dihydrofolate reductase (DHFR) catalyzes the reduction of dihydrofolate into tetrahydrofolate. It is required for the synthesis of thymidylate, purines and some amino acids, which are essential for growth and multiplication of cell. DHFR inhibitors are bactericidal. As the bacterial dihydrofolate reductase is different from the human enzyme, there is renewed interest in the development of new-generation bacterial DHFR inhibitors as effective antibacterial agents [11-14].

Dihydropteroate synthetase (DHPS) [15] is involved in the folate synthesis. It catalyzes the condensation of 6-hydroxymethyl-7,8-dihydropteridine pyrophosphate with p-aminobenzoic acid to form 7,8-dihydropteroate. It has two binding pockets: one which binds with dihydropterin pyrophosphate (DHPP) and the other which binds with p-amino benzoic acid (PABA). Although it is essential for bacteria, it is not expressed in most eukaryotes, including humans. This makes it a useful target which competes with the PABA precursor [15].

Pyruvate Kinase (PK) is a probable novel target for antibacterial activity. It catalyzes the last stage of glycolysis, which is the irreversible conversion of phosphoenolpyruvate to pyruvate with the concomitant phosphorylation of ADP to ATP. It plays a major role in regulation of glycolysis and its inhibition leads to the interruption of carbohydrate metabolism and energy depletion. Besides, the structure and protein sequence of bacterial pyruvate kinase is different from the human protein. Hence it is a potential target for antibacterial activity [16, 17].

DNA gyrase is an enzyme in the class of topoisomerases that relieves strain while double-stranded DNA is being unwound by helicase. This causes negative supercoiling of the DNA. The process occurs in prokaryotes (predominantly in bacteria), whose single circular DNA is cut by DNA gyrase and the two ends are twisted around each other to form supercoils. Bacterial DNA gyrase is the target of many antibiotics, including ciprofloxacin, nalidixic acid, etc [18-20]. Novel bacterial topoisomerase inhibitors (NBTIs) represent a new class of broad-spectrum antibacterial agents targeting bacterial gyrase [21].

The aim of the present study is to use *in silico* tools to identify novel chemical entities effective against *S. aureus*

MATERIALS AND METHODS

Molecular docking and scoring

Molecular modeling was done using GLIDE (Grid-based Ligand Docking with Energetics) running on an Intel® Core TM i3-2130 CPU@ 3.40 GHz processor using Linux professional workstation.

Ligand preparation

Twenty novel isoxazole derivatives were drawn in 2D and converted to 3D using the 3D sketcher of Maestro. The molecules were optimized and their energy minimized using LigPrep module. The execution was done with the graphical user interface of Maestro software package by means of the OPLS_2005 force field [22]. Optimization of bond lengths and bond angles as well as assignment of protonation states at biologically relevant pH were executed. Optimized conformations of the molecules were taken up for molecular docking studies.

Protein preparation

X-ray crystallographic structures of the four target proteins of *S. aureus* were downloaded from the Protein Data bank (extracted from the Brookhaven Protein Database http://www.rcsb.org/pdb) and used for docking studies. Structures of selected proteins were imported on the basis of the Resolution factor R. Dihydrofolate reductase complexed with novel 7-aryl-2,4-diaminoquinazoline-a monomer with Resolution value R 1.7Å (PDB entry code 3SRW); pyruvate kinase complexed with a natural bis-indole alkaloid-tetramer (Chains A,B,C,D) with R-value 2.3Å (PDB entry code 3T07); Bacterial topoisomerase complexed with tricyclic 1,5-naphthyridinone oxabicyclooctane-dimer (Chains Band D) with R 2.65Å (PDB entry code 5BS3); dihydropteroate synthetase complexed with OH-CH_2-pterin-pyrophosphate-dimer (Chains A,B) and Resolution 2.40 Å (PDB entry code 1AD4).

Protein preparation wizard tool of Maestro [23] was used to prepare the protein in order to fix common problems like protonation or missing disulphide bonds, side chains and loops. All unwanted water molecules beyond a certain distance were removed. Hydrogen bond optimization and restrained minimization was done. The optimized and minimized structure of the individual target protein was taken up for receptor grid generation.

Receptor grid generation

Optimized protein with co-crystallized ligand was engaged to generate a 3D grid (20 x 20 x 20 A°) at the active site of the target protein as per the standard protocol of glide manual [24]. Co-crystallized ligand molecule is detached and a 3D grid is introduced in its place. Receptor grid generation allows to define the position and size of the active site for ligand docking since center of the grid is located at the center of the co-crystallized ligand.

Molecular docking

GLIDE docking and scoring methods were used to identify the binding interactions at the active site of the selected proteins. Glide was run on flexible docking mode where the protein is rigid and the ligand is flexible. By design, this generates various conformations for each ligand. The ligand poses generated by Glide passes through a series of hierarchical filters that assess its interaction with the receptor [23]. Ligand poses that pass through these filters are subjected to evaluation and energy minimization. The poses are then scored using the Glidescore (GScore). Glide uses Emodel scoring function to select between protein-ligand complexes of a particular ligand and GScore function to rank-order compounds so as to separate compounds that bind strongly (actives) from those that do not (inactives). The Emodel scoring function is primarily defined by the protein-ligand coulomb-vdW energy. GScore [24] is an empirical scoring function designed to maximize the separation of compounds with strong binding affinity with little to no binding ability [24-26].

$$\text{GScore} = 0.05*\text{vdW}+0.15*\text{Coul}+\text{Lipo}+\text{Hbond}+\text{Metal}+\text{Rewards}+\text{RotB}+\text{Site}$$

(VdW=Van der Waals energy, Coul= Coulonb energy; Lipo=lipophilic 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)

It accounts for the physics of the binding process comprising lipophilic-lipophilic term, hydrogen bond terms, a rotatable bond penalty, and contributions from protein-ligand coulomb-vdW energies [24-26]. It also comprises terms to account for hydrophobic enclosure, which is the displacement of water molecules by a ligand from areas with proximal lipophilic protein atoms [27].

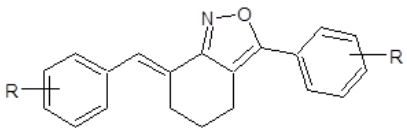
In silico prediction of pharmacokinetic properties

The properties that differentiate drugs from other chemicals can be considered as drug-like properties. A set of ADME-related properties (molecular descriptors) were calculated by using the Swiss ADME online server (http://www.swissadme.ch). It allows to compute appropriate physicochemical descriptors and to predict ADME parameters and pharmacokinetic properties [28]. It analyses the drug-likeness of compounds by applying Lipinski's rule of five [29] together with the Ghose, Veber, Egan and Muegge methods [30-33]. The predicted properties include molecular weight, hydrogen bond donors and acceptors, number of rotatable bonds, $LogP_{o/w}$, number of metabolic reactions, Solubility, BBB penetration, GI absorption, etc.

RESULTS

Molecular docking studies of the title compounds (fig. 1) with four potential targets of *Staphylococcus aureus* was implemented. Glide combines a powerful sampling protocol with a custom scoring function which is designed to identify ligand poses. Individual poses were studied to recognize the interactions at the active site of the respective protein, and the ligands were evaluated in terms of Glide score and Emodel. The docked poses were ranked in accordance to their GScores. The ranking of the ligands was also on the basis of their binding energy with the enzyme. If the binding energy is less, it is more active.

Although docking simulations were executed in both the standard precision (SP) and extra precision (XP) modes of the Glide module, the XP results are discussed. The benefit of XP mode is that it discards false positives and provides a superior association between good scores and good poses. Moreover, it contains extra terms and more stringent filters over the SP scoring function and provides a more comprehensive treatment of some of the SP terms like scoring of H-bonds, detection of buried polar groups, etc.



Comp code	R
2A	H
2B	Furfuryl
2C	4-hydroxy
2D	2-hydroxy
2E	4-methyl
2F	4-ethyl
2G	4-nitro
2H	3-nitro
2I	4-dimethyl amino
2J	4-chloro
2K	3-chloro
2L	2,4-dichloro
2M	2,6-dichloro
2N	3-methoxy
2O	4-methoxy
2P	3,4-dimethoxy
2Q	3,4,5-trimethoxy
2R	2-nitro
2S	3-methoxy-4-hydroxy
2T	3-ethoxy-4-hydroxy

Fig. 1: General structure of isoxazole derivatives with substitutions

Table 1: Molecular docking results of isoxazoles with pyruvate kinase

Compound code	R	SP mode		XP mode		
		G Score	Emodel	G Score	Emodel	Interactions at active site
2D	2-hydroxy	-8.236	-64.696	-8.64	-54.523	H-bond with Ala 358
Ciprofloxacin		-8.143	-59.086	-6.511	-53.47	Hie 365, Ser 362, Thr 366
Amoxacillin		-5.863	-54.217	-5.935	-50.319	Lys341, Asn 369, His 365
Trimethoprim		-6.391	-48.765	-5.947	-43.595	Solvent exposure

SP: Standard Precision XP: Extra Precision Ala: Alanine Thr: Threonine

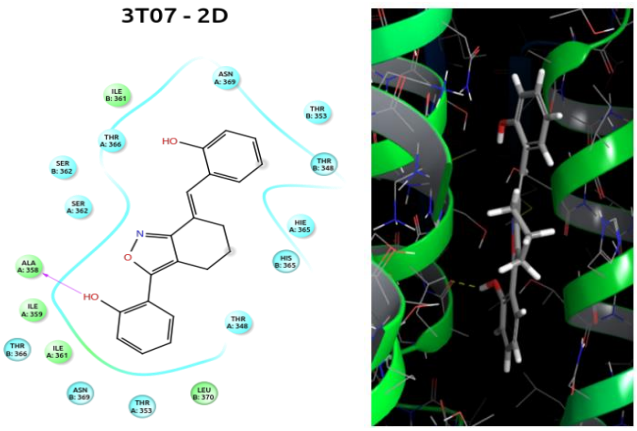


Fig. 2: XP docked pose of compound 2D with pyruvate kinase (PDB ID: 3T07)

Table 2: Molecular docking results of isoxazoles with DHFR of *S. aureus*

Comp code	R	SP mode		XP mode		
		G Score	E model	G Score	E model	Interactions at active site
2C	4-hydroxy	-8.701	-65.495	-8.33	-60.897	H-bonds with Thr 122 and Leu 29
Ciprofloxacin		-9.095	-74.145	-8.607	-64.65	Asp28
Amoxacillin		-8.266	-80.979	-7.937	-66.441	Asp28
Trimethoprim		-8.889	-72.433	-9.559	-60.018	Leu 6, Phe 93, Asp 28

SP: Standard Precision Thr: Threonine, XP: Extra Precision Leu: Leucine, DHFR: Dihydrofolate reductase

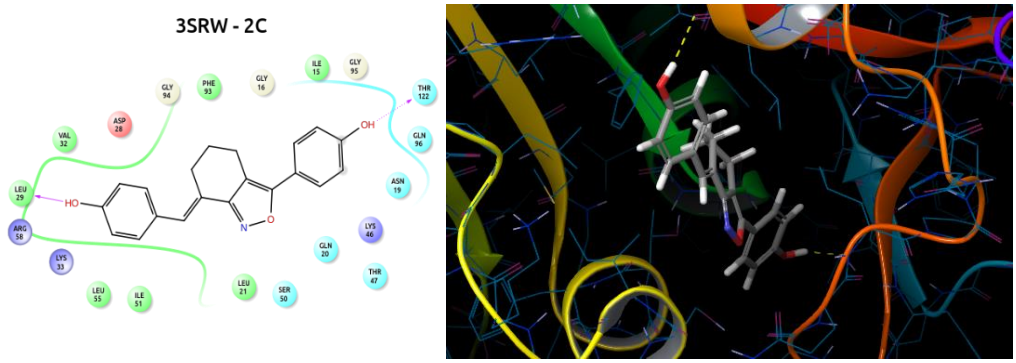


Fig. 3: XP docked pose of compound 2C with DHFR (PDB ID: 3SRW)

Table 3: Molecular docking results of isoxazoles with DHPS of *S. aureus*

Compound code	R	SP mode		XP mode		
		G Score	E model	G Score	E model	Interactions at active site
2N	3-methoxy	-4.091	-40.105	-3.523	-34.213	H-bonds-Hie 55, Asn 103 and Ash 84, pi-pi stacking-Arg 239, Hie 55
Ciprofloxacin		-4.997	-52.864	-4.623	-43.792	Arg 239, Glu 56
Amoxacillin		-4.631	-49.15	-6.043	-51.152	Asn 11, Hie 55, Arg 52, Glu 56
Trimethoprim		-4.363	-37.742	-4.123	-40.111	Val 12, Arg 52, Val 49

DHPS: Dihydropteroate synthetase

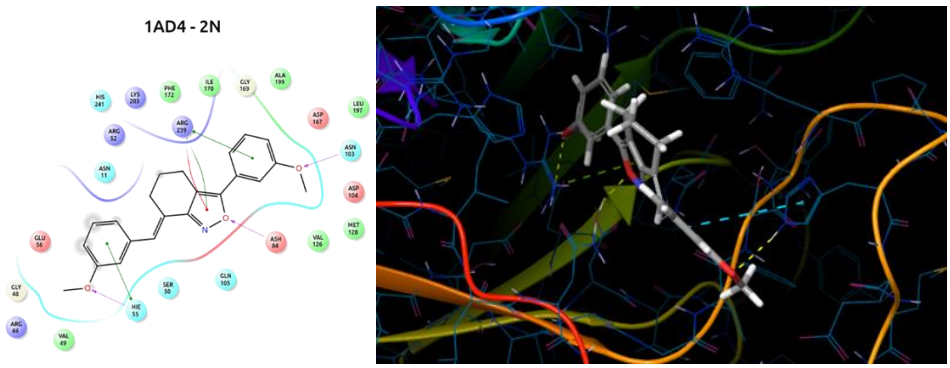


Fig. 4: XP docked pose of compound 2N with DHPS (PDB ID: 1AD4)

Table 4: Molecular docking results of isoxazoles with DNA gyrase of *S. aureus*

Comp code	R	SP mode		XP mode		
		G Score	E model	G Score	E model	Interactions at active site
2C	4-hydroxy	-7.424	-59.979	-6.797	-58.365	H-bond with Met 1121, Pi-pi stacking with residue G
Ciprofloxacin		-4.908	-46.616	-6.380	-57.246	Arg 1122
Amoxacillin		-6.994	-77.646	-5.993	-62.359	Ala 1068, Asp 1083
Trimethoprim		-5.218	-51.418	-5.220	-40.249	Asp 1083

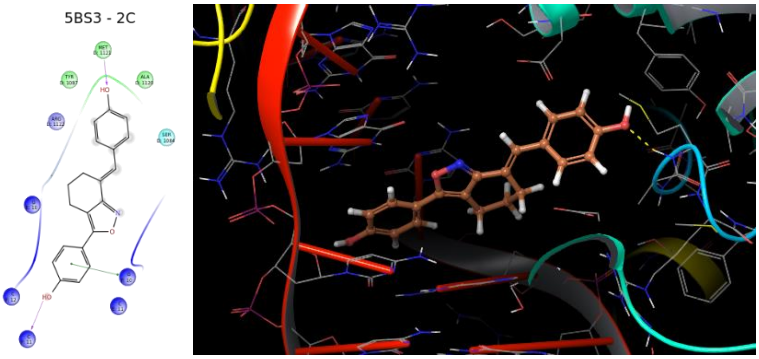


Fig. 5: XP Docked pose of compound 2C with DNA Gyrase (PDB ID: 5BS3)

Table 5: Predicted ADME properties of isoxazole derivatives with the top glide scores

Title	MW	#rotor (NRB)	HBD	HBA	log Po/w	# metab	GI absorption	Ro5
2A	287.36	2	0	2	4.80	2	High	0
2C	319.359	2	2	3	4.05	4	High	0
2D	319.359	2	2	4	4.05	4	High	0
2N	347.413	4	0	4	5.733	4	High	0

MW: Molecular weight, #rotor (NRB): Number of non-hindered rotational bonds, HBD: Hydrogen bond donor, HBA: Hydrogen bond acceptor, Log Po/w: Predicted octanol/water partition coefficient, #metab: Number of metabolites, GI Absorption: Predicted human oral abs option, Ro5: Number of violations from Lipinski rule of five

DISCUSSION

Docking of the title compounds with pyruvate kinase

XP docking mode has presented significant results with Pyruvate Kinase. Docking results graded Compound 2D (2-hydroxy derivative) with GScore-8.64 and Emodel-54.523 with the highest score (table 1). The hydroxyl group of the ligand formed H-bond with ALA 358 at the active site (Fig.2). The docked pose indicates that the ligand has fit well into the protein pocket. Most of the other derivatives have revealed good docking scores with H-bonding interactions at the active site of the enzyme. The dock scores have been compared with the standard drugs and it was seen that the scaffolds exhibited better score than the standard drugs.

Docking of the title compounds with DHFR

Results of the docking studies with DHFR are quite significant. 2C (4-hydroxy derivative) is ranked with highest GScore-8.33 and Emodel-60.897 (table 2). The two hydroxyl groups of 2c have formed H-bonds with LEU 29 and THR 122 (fig. 3). Surprisingly, only the hydroxyl and nitro-substituted derivatives have displayed binding interactions with the amino acids. Moreover, some of the derivatives have not given any score with the protein, demonstrating that the active poses of the compounds were not identified. However, GScore of 2C was lesser when compared to the standard DHFR inhibitor, trimethoprim (-9.559). Moreover, the interactions were with different amino acids at the active site of the protein.

Docking the title compounds with DHPS

The title compounds have not exhibited significant results with the DHPS. GScores ranged from 0.536 to 3.523 (table 3). Most of the compounds have formed pi-pi stacking interactions with HIE 55 and ARG 239 at the active site of DHPS (fig. 4) and compound 2N has exhibited the highest score among the derivatives. However, poor GScores indicated that the compounds have not docked well into the protein pocket. Hence the scaffolds are not expected to bind with the DHPS protein, although they interact with many amino acids at the active site.

Docking of the title compounds with DNA gyrase

Docking of the ligands with DNA gyrase have revealed reasonable glide scores. Compound 2C (4-hydroxy derivative) with GScore-6.797 and Emodel-58.365 presented the highest score (table 4). Docked pose indicates that ligand has formed H-bond with MET 1121 and C residue (of chain F). Furthermore, ligand has displayed pi-pi stacking interactions with residue G of chain F (fig. 5). Interestingly, only the hydroxy derivatives have formed interactions with amino acids at the active site. Most of the other derivatives have only formed pi-pi interactions with the residues of the protein DNA gyrase. The GScore of the 4-hydroxy derivative (2C) is comparable with the standard DNA gyrase inhibitor, Ciprofloxacin with a Gscore-6.380. However, ciprofloxacin bids with ARG 1122 at the active site, while 2C binds with MET 1121.

Binding score of the potent molecules at the active site of the proteins were comparable with the docking scores of the compounds discussed in similar articles [34, 35]. Hence, they may be considered as potential leads.

Drug-likeness assessment

Pharmacokinetic properties of the compounds were analyzed using the Swiss ADME. Results for the ADME predictions are given in table 5. Molecular weight of all the compounds were below 500. Some compounds presented slightly high logP values. The number of HBA (Hydrogen bond acceptors) ranged from 1-6, while the number of HBD (Hydrogen bond donors) were form 0-2. Most of the compounds have no violations from Lipinski's rule of 5 (ro5). However, some compounds show one violation, which fall within the "Lipinski region of interest," and hence they are "drug-like". Most of the compounds do not have any violations based on the Ghose and Veber rules for Drug-likeness assessment. Compounds 2C (4-hydroxy derivative) and 2D (4-hydroxy derivative), which displayed the maximum predicted binding affinity for the targets, did not exhibit any violations from the Lipinski ro5, Veber rules, or Ghose rules for drug-likeness.

Compliance with the rules indicate that the compounds own properties that make them 'drug-like.'

CONCLUSION

In silico molecular docking has been executed for all the twenty designed isoxazole derivatives on four antibacterial target enzymes of *S. aureus*. The molecules were ranked according to the results of the docking simulations i.e. by their docking score (GScore) and their binding energy (Emodel). Generally, a lower Glide score indicates good affinity for the receptor. If the binding energy is less, the ligand is more stable in the active site of the receptor. Docking studies identified compounds 2C (4-hydroxy derivative) and 2D (2-hydroxy derivative) with good binding interactions at the active site of Dihydrofolate reductase and Pyruvate Kinase of *S. aureus*. *In silico* predictions suggest that title compounds may be considered as lead molecules showing selectivity in inhibiting Pyruvate Kinase of *S. aureus*. Additionally, *in silico* pharmacokinetic studies confirmed the drug-likeness of the compounds; with these encouraging results, the compounds may be considered as leads and can be further explored for structural modifications and detailed microbiological investigations to obtain promising antibacterials 'effective against *S. aureus*.

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AUTHORS CONTRIBUTIONS

Judy Jays performed the experimental work and penned the manuscript. Saravanan J helped in drafting and revising the manuscript. Both authors approved the final manuscript.

CONFLICT OF INTERESTS

Declared none

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