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Computational Strategies for the Rational Design of Tetrahydroisoquinoline-3-Carboxylic Acid Derivatives as Novel Antibacterial Agents

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Abstract

The rising incidence of antibiotic resistance is one of the most urgent issues in the treatment of bacterial diseases. Furthermore, certain bacterial strains have developed resistance to a number of medications. As a result, an effective treatment option for bacterial infections needs to have higher bioavailability, good therapeutic effects, and minimal side effects. In order to combat this threat, the current study employed computational techniques like docking and in silico ADMET analysis to develop a new Tetrahydroisoquinoline-3-carboxylic acid-based derivative. After a review of the literature, molecules were designed. All designed compounds underwent molecular docking, and ADMET analysis. Protox II and pkCSM toxicity estimates showed that these compounds were non-toxic and had negligible to no negative effects. Molecular docking studies carried out with THIQ-3CA derivatives (referred AS-1 -AS-5) docked against selected target proteins (**PDB ID: 4DUH**) of *DNA Gyrase enzyme* demonstrated ideal binding energies ranging from -8.7 to -7.8 kcal/mol. **AS-3 and AS-4** were identified to exhibit better binding affinity compared to the marketed drug ciprofloxacin, showing their potential as anti-bacterial medicines. Among all 5 compounds, **AS-3** showed the most promising ADME profiles as confirmed by pkCSM, demonstrating good pharmacokinetic properties. Moreover, **AS-5** has the least toxicity, with no AMES toxicity and a high maximum tolerated dose, making it the safest among the AS compounds. This research work will provide ample opportunities to explore medicinal and computational research areas. It will facilitate the development of novel antibacterial agents in future experimental studies. Numerous opportunities to investigate computational and medical research fields will arise from this research work. It will facilitate the development of novel antibacterial agents in future experimental studies.

Keywords: *DNA Gyrase enzyme*, Tetrahydroisoquinoline-3-carboxylic acid derivatives, docking studies, ADME, toxicity

1. INTRODUCTION

Microbial infections are associated with higher rates of morbidity and mortality [1]. Common bacterial resistance to current antibiotic treatments is quickly growing into a significant global public health issue. The occurrence of multi-drug resistant Gram-positive and Gram-negative bacteria is increasing and diseases caused by them are becoming problematic now-a-days [2], and multiple studies have been claimed to increase and improve the present antibacterial compounds. More than 70% of bacteria have been shown to be resistant to one or more of the antibiotics that are typically used to treat the infection [3]. Although the emergence of multidrug resistance in *Bacillus subtilis* is not a recent development, research on *B. subtilis* inhibition profiling is currently being conducted to find ways to counteract this resistance [4–7].

As a result, development of new antimicrobial drugs is a topic for current research that seeks to control resistant bacteria strains worldwide [8]. We may highlight Tetrahydroisoquinolines-3-carboxylic acid hydrazides as one of the chemical compounds with biological potential that could be new potential antibacterial agents [9].

Nitrogen heterocycles are essential components of many compounds with potential applications in medicine and act as basic building blocks for the discovery of new medications [10]. The chemical and biological significance of the isoquinoline skeleton cannot be overstated. Medications containing this skeleton, whether natural or synthetic, can be used for a variety of therapeutic purposes [11]. One of the most advantageous heterocyclic scaffolds is the tetrahydroisoquinoline moiety, which is widely found in a variety of plants, soils, and marine microbes. Tetrahydroisoquinolines have a wide range of applications in medicine, including analgesic, antibacterial, antifungal, anticancer, anti-inflammatory, anticonvulsant, antileukemic, anti-HIV, and antithrombotic [12].

Moreover, THIQ-containing isolated alkaloids from natural sources are found in large quantities in a number of pharmaceuticals. Based on the 1,2,3,4-tetrahydroisoquinoline molecule, the most well-known medicinal drugs are quinapril, noscapine, and praziquantel [13].

The vast diversity of biological importance of hydrazide-hydrazones is potentially due to azomethine group in their structure. Mainly antibacterial, antitubercular, antifungal, anti-inflammatory, and antioxidant activities are among their biological effects [14]. Their activity was occasionally significantly higher than that of the reference drugs (ampicillin, nitrofurantoin, cefuroxime, or ciprofloxacin) [15–18].

Because of this, the current study focuses on designing novel derivatives of Tetrahyroisoquinoline-3-carboxylic acid and investigating these derivatives against the DNA Gyrase enzyme in silico. The purpose of the study is to screen the hit compounds for pharmacokinetic and toxicological profiles and to analyze the binding affinities of the newly design derivatives against the active pockets of the DNA Gyrase enzyme.

2. MATERIALS AND METHODS

2.1 Protein Preparation

Using the accession code 4DUH, the target protein structure was obtained from the Protein Data Bank (PDB). The protein structure was then transformed with AutoDock Vina version 1.5.6 into PDBQT format. The protein was then placed into AutoDock in order to extract the protein and ligand independently. This software was used to evaluate the ligands' binding affinities and choose the optimal lead molecule for antibacterial drugs [20].

2.2 Molecular Docking Program

By locating its binding site, the chosen protein 4DUH has been prepared. The ligand (RL1301) was first removed in order to validate the protein, and the altered structure was stored in PDB format. The protein in PDB format was then loaded into AutoDock Vina for docking studies after water and other redundant structures were removed, missing atoms were fixed, polar hydrogen atoms were added, and Kollman charges were applied. To develop the ligand, it was extracted from the protein, polar hydrogen atoms were added, the root was found, torsions were chosen (which were left flexible to explore various ligand conformations), and the ligand was eventually converted into a PDBQT file extension [20–22]. Finally, the ligand was created as the center of a grid box. Below is the configuration for docking:

center_x = 3.024

center_y = 2.008

center_z = 36.902

size_x = 40

size_y = 40

size_z = 40

3. 2-D Interaction Studies

Using the Discovery Studio molecular docking was performed, where the prepared protein and ligands were subjected to multiple docking runs. This procedure located the protein's binding site and evaluated the ligand-protein interaction patterns and binding affinities. [23, 24].

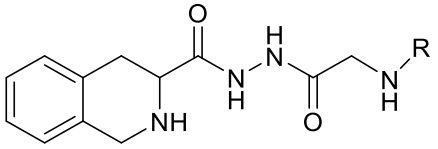
4. ADME and Toxicity Prediction

Using the pkCSM online platform, ADME (Absorption, Distribution, Metabolism, and Excretion) predictions were used to assess the pharmacokinetic features of each of these hit substances. ChemDraw 2D was initially used to convert the compounds to their corresponding SMILES IDs, which were subsequently input into the pkCSM portal. This study offered a thorough assessment of the ligands' potential as therapeutic candidates by shedding light on their drug-likeness, solubility, permeability, and metabolic stability [25]. Furthermore, the compounds underwent toxicity prediction with the use of web portals, namely protox-II and pkCSM [26-27].

5 Results and Discussion

AutoDock Vina 1.5.6 software was then used to perform molecular docking in order for assessing the binding affinity in the active site of the DNA Gyrase enzyme protein (PDB ID: 4DUH).. After the compounds were screened, their kcal/mol free binding energies were calculated. The compounds' binding affinities ranged from -8.7 to -7.8 kcal/mol, according to the molecular docking studies.

Table 1: Docking Scores of designed compounds in comparison to standard drug ciprofloxacin.

Docking Score			
			
S.No	**Compound**	**R**	**Binding Affinity (kcal/mol)**
1	**AS-1**	$-C_6H_4NO_2$	-7.8
2	**AS-2**	$-C_6H_3ClNO_2$	-8.0
3	**AS-3**	$-C_6H_4Br$	-8.7
4	**AS-4**	$-C_6H_3(CH_3)_2$	-8.5
5	**AS-5**	$-C_6H_4CH_3NH_2$	-8.1

6	Co-Crystallized Ligand	-	-7.4
7	Ciprofloxacin	-	-8.2

Table 2: Types of Interactions for Compounds

S. No.	Compound	Binding Affinity (kcal/mol)	Hydrogen Bond Interactions	Carbon Hydrogen Bond Interactions	π-σ Interactions	π-alkyl Interactions	Pi-Cation	Halogen (Fluorine) Interaction	Amide-Pi Stacked	Pi-sulphur Interactions
1.	AS-1	**-7.8**	VAL120 HIS99 LYS103 ASP49	GLY117	ILE48	LYS103	-	-	-	
2.	AS-2	**-8.0**	ARG136 ARG76	HIS83	-	ALA90 PRO79 ILE78	-	-	GLY77	
3.	AS-3	**-8.7**	VAL120 ASN46	GLY119 GLY101	-	ILE78 LYS103	GLY117 GLU50	-	ASN46	
4.	AS-4	**-8.5**	GLY117 PHE104 LYS103	--	--	ILE78	GLU50 GLY117 LYS103	-		
5.	AS-5	**-8.1**	GLY101 LYS103	--	ASN46 ILE78	PRO79 ALA90	--	--	VAL120	
6.	Co-Crystallized Ligand	**-7.4**	ALA100 ASP45 ASP49	PRO79	ILE94	LYS103	LYS103 GLY117	--	--	PHE104
7.	Ciprofloxacin	**-8.2**	GLY77 ALA100	ILE94 GLY119 PRO79	--	ILE78 VAL120 ILE94 LYS103	- LYS103 GLU50	GLY101 ALA100	-	

Furthermore, compounds with either strong or equipotent activity in relation to the ligand and the reference medication ciprofloxacin (binding affinity = - 8.2 kcal/mol) were found by analysis. With a docking score of -8.7 kcal/mol, compound AS-3 demonstrated the most favorable results, suggesting a substantial binding affinity to the target protein. (Reference Table 1). To determine how these five chemicals interacted with the protein's amino acids (given in Table 2), a thorough interaction study was conducted. The results, which are shown in Table 2, provide further information about the binding mechanisms of each chemical by highlighting several interaction types such as π-π interactions, π-alkyl interactions, hydrogen bonds (H-bonds), and π-cation interactions.

As depicted in Figs. (**1**), the interaction analysis of **AS-3** with the highest binding affinity (-8.7 kcal/mol), reveals that the compound exhibits significant hydrogen bond interactions with VAL120 and ASN46, carbon hydrogen bonding with GLY119 and GLY101 and π-alkyl interactions with GLU50 and GLY117. A special type of interaction, namely amide-pi Sacked and pi cation was also found between enzyme protein and compound **AS-3** involving the amino acid ASN46. and GLY117, GLU 50 respectively.

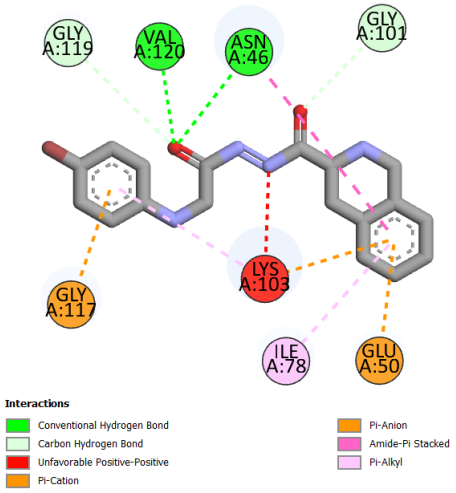


Figure-1 Interaction of AS-3 & DNA gyrase protein (4DUH)

The interactions of compounds **AS-4 are** presented in Figs. (**2**), compound was found to have **three** H-bond interaction with GLY117, PHE104 and LYS103, and also showed a π-alkyl interaction with ILE78. Furthermore, this compound engaged in π-alkyl interactions with residues such as PHE764, LEU, and MET895.

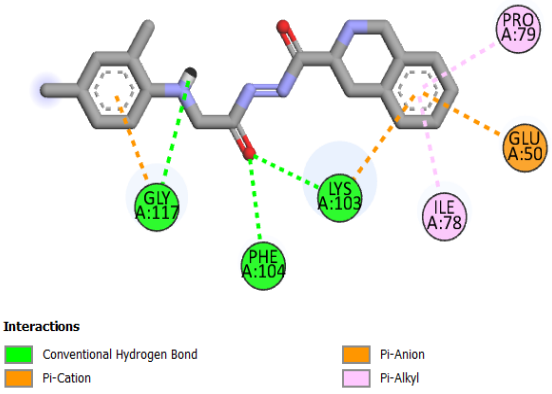


Figure-2. Interaction of AS-4 & DNA gyrase protein (4DUH)

AS-5 (Fig-3) also demonstrate the H-bond interaction with residue GLY101 and LYS103, π-sigma interactions with residues ASN46 and ILE78. Along with that this compound also show a π-alkyl interaction with PRO79 and ALA90. Furthermore, this compound engaged in amide π-sacked interactions with residues such as VAL120.

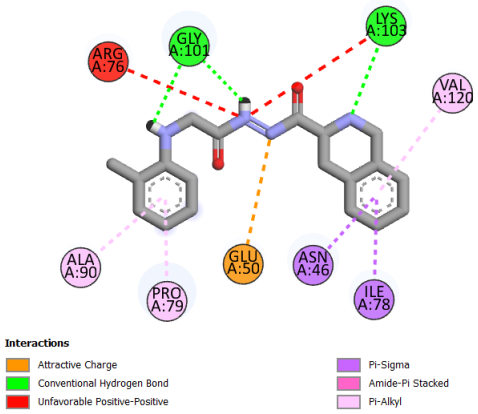


Figure-3. Interaction of AS-5 & DNA gyrase protein (4DUH)

Figures (4 and 5) depict the interactions of compounds AS-1 and AS-2, respectively. It was discovered that both compounds exhibited hydrogen bond interactions; AS-1 interacted with residues VAL120, HIS99, LYS103, and ASP49, while AS-2 interacted with ARG136 and ARG76 in the DNA gyrase enzyme's active site. Other than that, C-H interactions were shown by both compounds with ILE48 and HIS83, respectively. Whereas AS-2 has three π-alkyl interactions with ALA90, PRO79, and ILE78, AS-1 only exhibits one π-alkyl contact with LYS103.

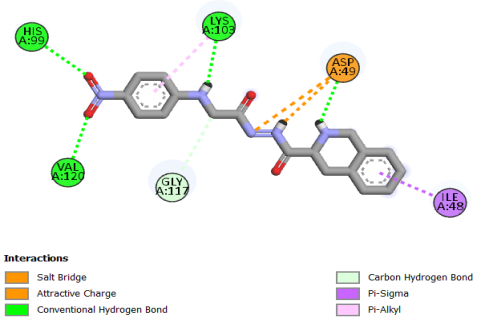


Figure-4. Interaction of AS-1 & DNA gyrase protein (4DUH)

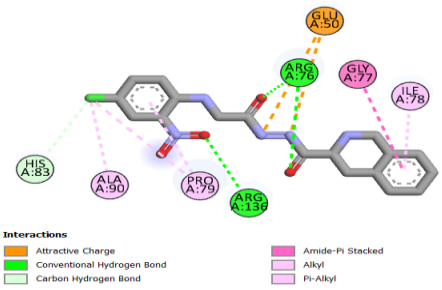


Figure-5. Interaction of AS-2 & DNA gyrase protein (4DUH)

The co-crystallized ligand is presented in **Fig. (6)** have H-bond interaction with ALA100, ASP45 and ASP49, C-H Interaction with PRO79, also have one π-alkyl and π -sigma interactions with ILE94 and LYS103 respectively. Along with this it displayed pi-cation interaction with LYS103 and GLY117, and have one Pi-sulphur interaction with PHE104.

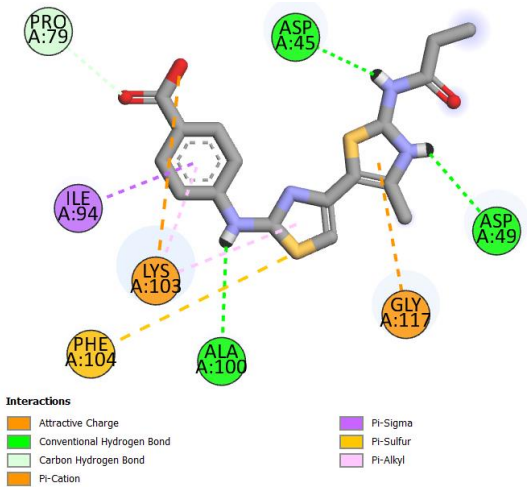


Figure-6. Interaction of Ligand & DNA gyrase protein (4DUH)

Additionally, Fig. (7) showed the interactions of the standard drug ciprofloxacin. Two H-bond interactions between ciprofloxacin and distinct amino acid residues, GLY77 and ALA100, were observed. Apart from that, it displayed, C-H interaction with ILE94. GLY119 and PRO79, several π-alkyl interactions with ILE78, VAL120, ILE94, and LYS103, and pi-cation interaction with LYS103 and GLU50. Notably, it showed Halogen interaction with GLY101 and ALA100. The interaction patterns showed that several residues were common between Ciprofloxacin and the designed ligands.

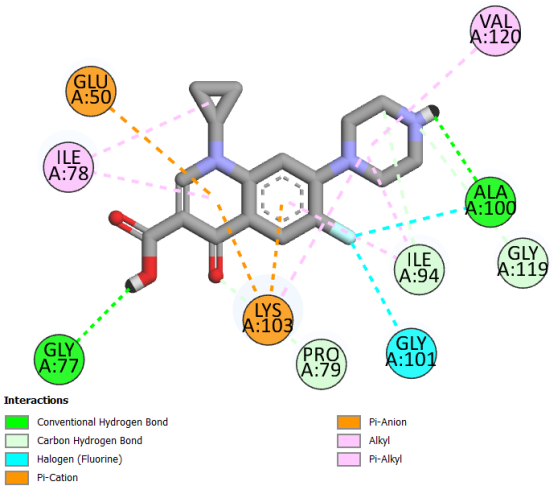


Figure-7. Interaction of Ciprofloxacin & DNA gyrase protein (4DUH)

Based on the interaction patterns that the majority of ligands and ciprofloxacin displayed with VAL120 and LYS103, it can be deduced that the designed ligands display interactions that are comparable to those of the standard drug ciprofloxacin, which makes them promising compounds for the treatment of bacterial infection.

5.1 ADME Analysis

ADME analysis was carried out to evaluate their potential even further, since these physicochemical characteristics are critical in defining a compound's outcome. Table 3 displays the physicochemical parameters, such as molecular weight, Log P values and water solubility, for the hit compounds. A range of molecular weights, from 338.411 to 639.381 g/mol, is revealed by the ADME analysis of five new substances (AS-1 to AS-5) and a reference medication (ciprofloxacin), with AS-1 having the highest value. The range of lipophilicity (LogP) is 0.8686 (AS-1) to 1.7229 (AS-3), suggesting a range of hydrophobicity that might affect the permeability and solubility of membranes. Each compound exhibits intestinal absorption ranging from 38.87% (AS-5) to 97.31% (Ciprofloxacin), and moderate to high water solubility (log mol/L between -3.249 and -3.338). With distribution values (VDss) close to neutral, they are all P-glycoprotein (P-gp) substrates with restricted tissue distribution. Permeability of the blood-brain barrier (BBB) ranges from -1.347 to -0.534, and the CNS is likewise limited in permeability for all substances (log PS values < -2.5). With the exception of ciprofloxacin, all substances are substrates of CYP2D6 and CYP3A4. The compounds' total clearance values differ, with AS-1 exhibiting the maximum clearance (0.874 log ml/min/kg).

Table 3. Drug likeness property of hit compounds using pkCSM.

S. No	Code	Molecular Weight (g/mol)	LogP	Water Solubility (log mol/L)	Intestinal Absorption (human %)	P-gp Substrate	VDss (human, log L/kg)	BBB Permeability (log BB)	CNS Permeability (log PS)	CYP2D6 Substrate	CYP3A4 Substrate	Total Clearance (log ml/min/kg)
1.	**AS-1**	639.381	0.8686	-3.307	68.591	Yes	-0.068	-1.169	-2.946	Yes	Yes	0.874
2.	**AS-2**	403.026	1.522	-3.317	69.729	Yes	-0.016	-1.347	-2.818	Yes	Yes	0.77
3.	**AS-3**	403.28	1.7229	-3.338	94.816	Yes	-0.024	-1.153	-2.592	Yes	Yes	1.131
4.	**AS-4**	352.438	1.57724	-3.292	96.139	Yes	0.046	-0.99	-2.581	Yes	Yes	1.112
5.	**AS-5**	338.411	1.26882	-3.249	38.872	Yes	0.027	-0.967	-2.649	Yes	Yes	1.154
6.	**Ciprofloxacin**	331.347	1.5833	-3.172	97.306	Yes	0.042	-0.534	-3.03	Yes	No	0.545

5.2 Toxicity Analysis

Protox-II and pkCSM, which are accessible online at https://tox-new.charit e.de/protox_II/ and https://biosig.lab.uq.edu.au/pkcsm//, respectively, were used to predict the toxicity of these chemicals. The highest tolerated dose (HTD), oral rat acute toxicity (LD50), AMES toxicity, hERG I and II inhibitor, organ toxicity (particularly hepatotoxicity), skin sensitization, T. pyriformis toxicity, and minnow toxicity are all included in Table 4's toxicity predictions for these three drugs. With the exception of AS-1 and AS-5, all drugs exhibit active neurotoxicity but lack hepatotoxicity. In general, nephrotoxicity is inert; however, AS-2 and Ciprofloxacin exhibit efficacy. With the exception of AS-1, most chemicals are respiratoryly poisonous. T. pyriformis toxicity varies from 0.291 to 0.318 log µg/L, and none of the chemicals show signs of skin sensitization. Each of the compounds are AMES hazardous, except AS-5, which is non-AMES toxic like Ciprofloxacin. All substances, with the exception of ciprofloxacin, inhibit hERG II but not hERG I. Minnow toxicity values range from 1.129 log mM for ciprofloxacin to 4.152 log mM for AS-1.

Table 4. Predicted toxicity for hits compounds.

S. No	Code	AMES Toxicity	Max Tolerated Dose (log mg/kg/day)	hERG I Inhibitor	hERG II Inhibitor	ORAT (LD50, mol/kg)	ORCT (LOAEL)	Hepato-toxicity	Neuro-toxicity	Nephro-toxicity	Respiratory toxicity	Cardio Toxicity	Skin Sensitisation	T. Pyriformis Toxicity (log µg/L)	Minnow Toxicity (log mM)
1.	AS-1	Yes	-0.079	No	Yes	2.47	2.686	Inactive	Inactive	Inactive	Active	Inactive	No	0.294	4.152
2.	AS-2	Yes	-0.097	No	Yes	2.407	2.54	Inactive	Active	Active	Active	Inactive	No	0.291	3.569
3.	AS-3	Yes	-0.03	No	Yes	2.549	2.109	Inactive	Active	Inactive	Active	Inactive	No	0.318	2.359
4.	AS-4	Yes	-0.045	No	Yes	2.565	1.951	Inactive	Active	Inactive	Active	Inactive	No	0.316	2.88
5.	AS-5	No	-0.035	No	Yes	2.504	2.042	Inactive	Active	Inactive	Active	Inactive	No	0.315	2.917
6.	Ciprofloxacin	No	0.274	No	No	2.2	0.994	Inactive	Active	Active	Active	Inactive	No	0.295	1.129

CONCLUSION

Our goal in this work was to use computational techniques to create and find effective antibacterial chemicals. Novel compounds were first created by a survey of the literature. These compounds were then docked using AutoDock Vina 1.5.6. The docking analysis revealed that two compounds, AS-3 and AS-4, had better binding affinities than the commercial medication ciprofloxacin, suggesting that they may be used as antibacterial agents. As validated by pkCSM, AS-3 showed encouraging ADME profiles among these drugs, suggesting good pharmacokinetic qualities. Furthermore, AS-5 is the least hazardous of the AS compounds due to its lack of hERG I inhibition, high maximum tolerated dose, and lack of AMES toxicity.

This work introduces new opportunities to investigate computational and therapeutic studies to create new antibacterial agents. In addition, further research in the synthesis and biological assessment of

these strong leads might yield new and less toxic DNA gyrase inhibitors for the treatment of bacterial infections.

COMPETING INTERESTS

The authors declare no conflict of interest, financial or otherwise.

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NA

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