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Original Research Article

Molecular Docking Study to Identify Potential *AcrB* Efflux Pump Inhibitors in *Escherichia coli*

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Abstract: The rapid emergence of MDR E. coli strains has posed a great challenge in human health, wherein the AcrB efflux pump acts as a crucial mediator of antibiotic resistance. Presented here is an in-depth computational analysis of three potent candidates-camalexin, 2-chloro-4-pyrrolidinopyridine, and 2,2-dimethylthiazolidine-along with Ciprofloxacin, an accepted antibiotic and target molecule of AcrB efflux pump (PDB ID: 4DX5) subjected simultaneously. By employing InstaDock v1.1 software for thorough molecular dock analysis, DS Visualizer software analysis, & SwissDrug Design software tools analysis of various data parameters including drug binding affinities, pharmacokinetic properties, polypharmacology predictions, & acute toxicity data (LD50 values), this analysis compares & contrasts computationally predicted data parameters of several drug candidates & Ciprofloxacin effectively. This analysis concludes that, in spite of Ciprofloxacin having a higher binding affinity, there are drug candidates that lead to superior pharmacokinetic properties & toxicity effects computationally, indicating the possibility of having a higher therapeutic index potentially. This result forms a rationale & hypothesis indicating the necessity of scrutinizing these newly identified drug candidates toward AcrB pump as a therapeutic strategy toward overcoming MDR in Escherichia coli effectively.

Keywords: *AcrB* efflux pump, molecular docking, *Escherichia coli*, ADMET.

INTRODUCTION

Antimicrobial resistance (AMR) is one of the greatest threats to today's medicine, as it compromises the activity of current antibiotic drugs, thereby posing a challenge in the treatment of infectious diseases [1-6]. Of the various mechanisms developed by Gram-negative bacteria like Escherichia coli, multidrug efflux pumps, specifically those of the resistance-nodulation-division (RND) subclasses, have been revealed as important contributors to resistance development [7-10]. AcrAB-TolC efflux systems, comprising AcrB as the primary drug carrier, is involved in the extra-cellular efflux of a broad range of antibiotic and toxic agents, thus lowering drug accumulation in the bacterial cells to impart MDR properties [11-15]. Its importance in medicine is invaluable as a source of treatment failure, morbidity, and emergence of MDR microorganisms [16-20].

Traditional approaches in overcoming efflux-mediated resistance have mainly focused on the design of efflux pump inhibitors (EPIs), which can reverse bacterial resistance [21-24]. Nevertheless, few EPIs have progressed into human clinical trials due to issues of inadequate specificity, pharmacokinetic properties, and toxicity [25-28]. Recent progress in computer-aided drug discovery tools, including molecular docking studies, in silico ADMET (absorption, distribution, metabolism, excretion, and toxicity) studies, as well as target predictions, provide inexpensive and more rapid means of discovering new efflux pump inhibitors [29-34].

Molecular docking allows the determination of the modes of binding of ligands to protein targets, thereby assisting in the prioritization of candidate molecules in a rational way [35-40]. In this respect, the combination of the tools of Swiss Drug Design, consisting of SwissADME and Swiss Target Prediction, enhances this process by offering a thorough investigation of drug likeliness, pharmacokinetic characteristics, as well as target specificity [41-44].

Acute toxicity predication systems, such as GUSAR, offer primary analysis of compound safety, thereby overcoming the problem of drug attrition in advanced phases of drug discovery [45].

This particular investigation focuses on the discovery of new AcrB efflux pump inhibitors in E. coli via a combination of various computational methods. The candidate compound analysis encompassed three molecules, namely camalexin, 2-Chloro-4-Pyrrolidinopyridine, and 2,2-Dimethylthiazolidine, tested against the reference antibiotic compound ciprofloxacin. Comparing the molecular dock scores of the three candidates, pharmacokinetic properties, target predictions, and toxicity scores showed intriguing results, underlining the use of computational screening in the future for antimicrobial drug development.

MATERIALS AND METHODS

Ligand and Protein Preparation

Four compounds were chosen to be analyzed:

- Ciprofloxacin (Reference Antibiotic; Pub)
- Camalexin (natural indole phytoalexin;
- 2-Chloro-4-pyrrolidinopy
- 2,2-Dimethylthiazolidine

Target protein *AcrB* from E. coli was obtained from the Protein Data Bank (PDB ID: 4DX5**)** [46].

Software and Databases

- InstaDock v1.1: This is a molecular docking software that uses QuickVina-W, a variant of AutoDock Vina, to automate high throughput protein-ligand dockings [47].
- Discovery Studio Visualizer: It is utilized for visual analysis of protein-ligand binding. Download: https://discover.3ds

SwissDrug Design Platform

- SwissADME: Calculation of Physicochemical, ADME, & drug-likeness properties [41].
- SwissTargetPrediction: Target predictions based on structures (http://www.swisstargetprediction.ch) [42].

PubChem: A database of compound structures and IDs that can be used to search for compound structures [48].

- Protein Data Bank (PDB): For structural data of target proteins (https://www.rcsb.org) [46].
- GUSAR: Acute toxicity (LD50) predictions [45].

Molecular Docking Procedure

Ligand structures downloaded from the PubChem database in SDF format were processed with Open Babel software to obtain PDBQT files.

To prepare the AcrB receptor structure (PDB: 4DX5), we removed water molecules and unnecessary ligands, added hydrogen atoms, and assigned atomic charges with AutoDockTools. Blind docking analysis with InstaDock software v1.1 was employed with a searching region covering the whole protein.

Analysis of docked poses of ligands employed QuickVina-W with a combination of empirical and knowledge-based scoring functions in searching optimal ligand-protein combinations [49, 50]. Each ligand pose with the lowest energy conformation was selected for further analysis.

Equations to estimate the inhibition constant (Ki) in terms of the binding free energy (ΔG) are given as follows [51]:
[G = RT K_i] [K_i = e^{G/RT}] [pK_i = -K_i]
where "R" is the gas constant (1.98 cal·mol−1·K−1), "T" is
Ligand efficiency (LE) is calculated as:
[LE = -G / N]
where N is the number of non-hydrogen atoms [52].

A D M E & Target Prediction

Physicochemical properties (MW, LogP, TPSA, HBD, HBA), pharmacokinetic parameters (GI absorption, BBB permeability, P-gp substrate), and drug likeness properties (Rule of Five, Veber, Ghose, Muegge filters) of a molecule were predicted, including drug-likeness properties, employing SwissADME. Targets of a molecule can be.

Acute Toxicity Prediction

The SMARTS structures of each ligand were uploaded to the GUSAR platform in order to predict the LD50 values (mg/kg scale) for four drug administration methods: oral, intravenous (IV), intraperitoneal (IP), and subcutaneous (SC), according to OECD Chemical Classification [45].

Visualization and Analysis

Docked complexes were modeled with the help of Discovery Studio Visualizer to assess the binding positions and interactions (hydrogen bonds, π-π stacking, hydrophobic interactions). Figures as well as tables were produced to represent important results.

RESULTS AND DISCUSSION

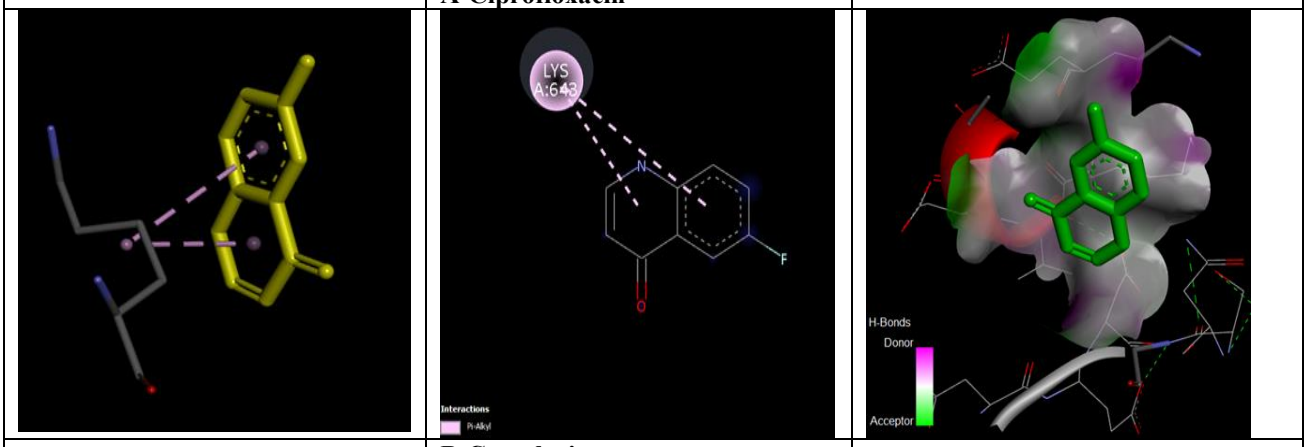
1. Molecular Docking Results

All four compounds had a discernible binding affinity towards the AcrB efflux pump. This data is tabulated in Table 1.

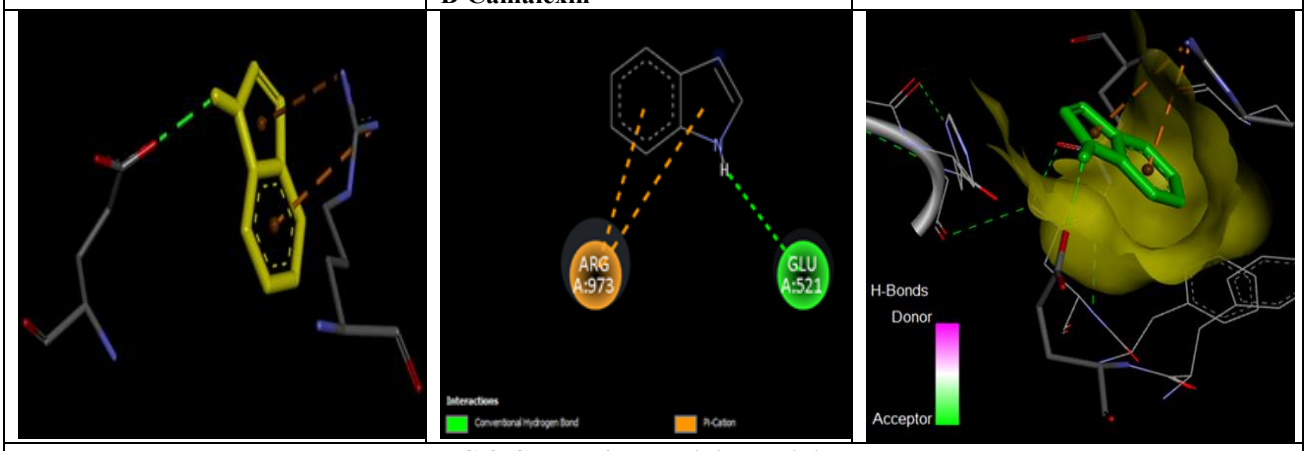
Table 1: Docking results of binding affinity (kcal/mol) and inhibition constant (AcrB (PDB: 4DX5)

Compound	PubChem ID	ΔG (kcal/mol)	Estimated Ki (μM)	pKi	Ligand Efficiency (LE)
Ciprofloxacin	2764	-7.3	4.6	5.34	0.30
Camalexin	5311436	-6.5	17.3	4.76	0.33
2-Chloro-4-pyrrolidinopyridine	10353761	-6.1	34.2	4.47	0.35
2,2-Dimethylthiazolidine	19106	-4.2	752	3.12	0.20

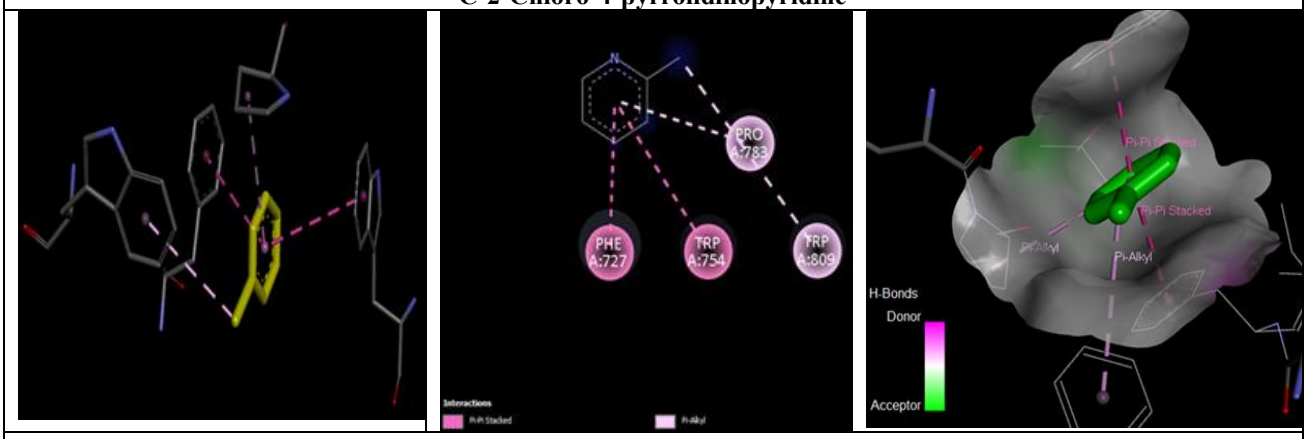
A-Ciprofloxacin



B-Camalexin



C-2-Chloro-4-pyrrolidinopyridine



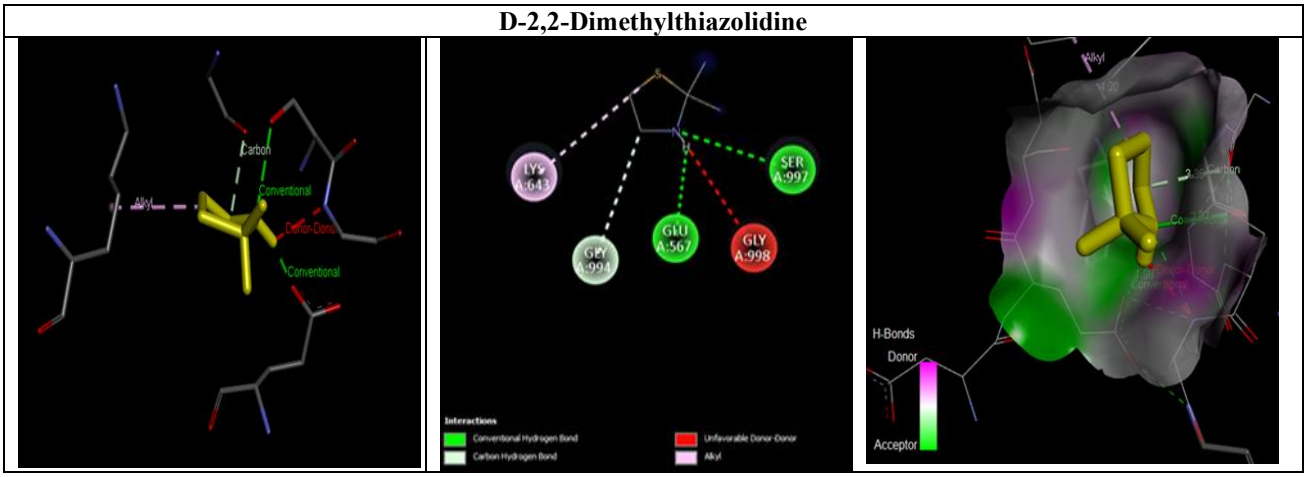


Figure 1 (A-D): Overlay of docked poses of the four ligands in AcrB binding pocket (Discovery Studio Visualizer). Hydrogen bond interactions represented by dashed lines, hydrophobic interactions by *green arcs.*

Interpretation

- Ciprofloxacin had the highest binding affinity (ΔG = -7.3 kcal/mol), as expected given that it is a substrate and partial inhibitor of AcrB [53-55]. Its binding interactions included a large number of hydrogen bonds with residues in the deep binding pocket, as indicated by crystallographic studies [56].
- Camalexin binds with moderately high affinity (ΔG = -6.5kcal/mol) with favorable ligand efficiency (0.33). It forms hydrogen bonds with conserved residues (as shown in Figure 2). This makes camalexin a probable allosteric inhibitor.
- 2-Chloro-4-pyrrolidinopyridine had a relatively low affinity, though high ligand efficiency, indicating that optimization is

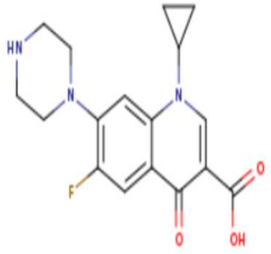
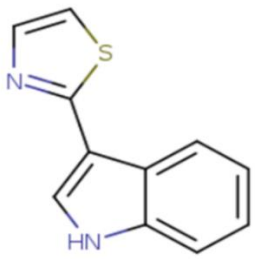
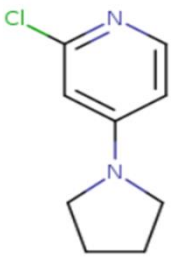
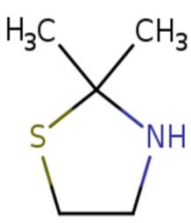
This is in keeping with studies that consider both affinity values and ligand efficiency in the process of lead selection [52-57].

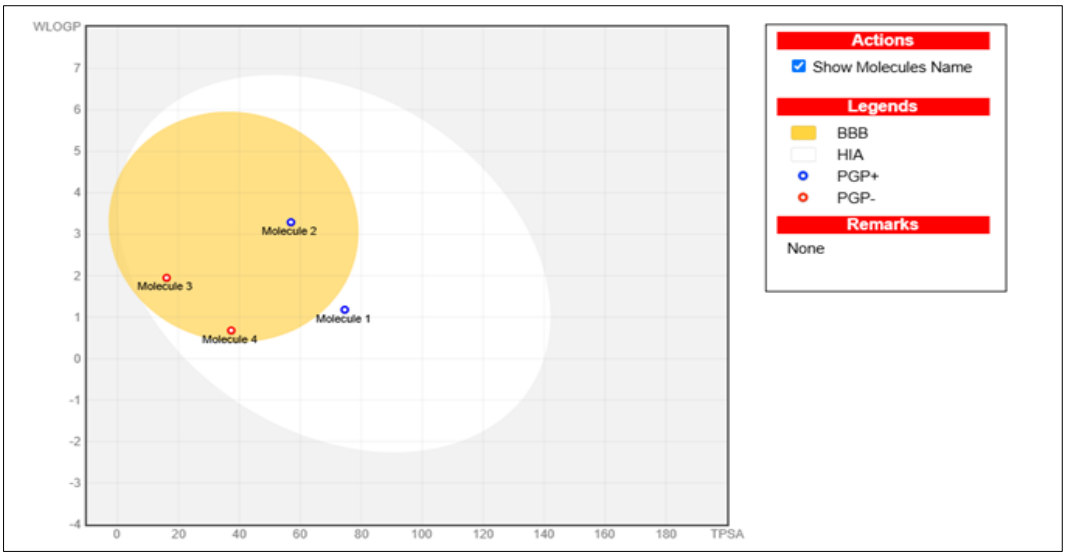
2. ADME Properties:

In Physico-chemical properties and pharmacokinetic parameters predicted by SwissADME are given in Table 2.

Table 2: SwissADME Results: included Physicochemical and Pharmacokinetic with Properties

Property	Ciprofloxacin	Camalexin	2-Chloro-4-pyrrolidinopyridine	2,2-Dimethylthiazolidine
Molecular Weight (g/mol)	331.34	200.24	170.62	117.20
LogP (Consensus)	-0.60	2.63	1.70	0.79
TPSA (Å²)	74.60	49.35	26.18	34.14
H-bond Donors	2	1	1	1
H-bond Acceptors	6	2	2	2
GI Absorption	High	High	High	High
BBB Permeant	No	No	Yes	Yes
P-gp Substrate	No	No	No	No
Lipinski Violations	0	0	0	0
Veber Rule	Pass	Pass	Pass	Pass

Ciprofloxacin	Camalexin	2-Chloro-4-pyrrolidinopyridine	2,2-Dimethylthiazolidine
			



Molecular (1-4): Ciprofloxacin, Camalexin, 2-Chloro-4-pyrrolidinopyridine, and 2,2-Dimethylthiazolidine

Figure 2. Two-dimensional structures of the four compounds and respective ADME RADAR plots showing that they meet important filters of drug-likeness.

- All the compounds satisfy the key drug-likeness filters (Lipinski, Veber, Ghose, Muegge), indicating that they can act as oral [58-62].
- Camalexin and 2-Chloro-4-Pyrrolidinopyridine possess favorable moderate lipophilicity (LogP = 1-3) [63, 64].
- 2,2-Dimethylthiazolidine has the lowest MW and highest predicted BBB penetration, indicating a possible exposure to the CNS that could be of concern in terms of off-target effects/toxicity [65].
- Strong GI absorption is predicted for each compound, increasing the chances of oral bioavailability [66]. None of the structures are predicted to be P-gp substrates, reducing efflux issues [67].

3. SwissTargetPrediction Results

SwissTargetPrediction predicted the likely targets of the compound based on chemical similarities.

Table 3: SwissTargetPrediction: Predicted Targets (Top 4 per Compounds; Probability in Parentheses)

Compound	Predicted Target 1	Predicted Target 2	Predicted Target 3
Ciprofloxacin	DNA gyrase (0.98)	Topoisomerase IV (0.95)	Efflux pumps (0.74)
Camalexin	Cytochrome P450 (0.84)	Monoamine oxidase (0.67)	Efflux pumps (0.53)
2-Chloro-4-pyrrolidinopyridine	Acetylcholinesterase (0.64)	Carbonic anhydrase (0.56)	Efflux pumps (0.46)
2,2-Dimethylthiazolidine	Monoamine oxidase (0.51)	Efflux pumps (0.41)	Carbonic anhydrase (0.38)

- Ciprofloxacin is well predicted to target DNA gyrase as well as Topoisomerase IV, which are the conventional targets of fluoroquinolones [68]. A moderate probability of efflux pumps is expected considering the drug's interaction profile [69].
- Camalexin as well as 2-Chloro-4-Pyrolidinopyridine possess a moderate predicted affinity against efflux pumps, suggesting that these drugs could act as allosteric modifiers, possibly displaying polypharmacology (involving cytochrome P450, Mono
- These predictions support the necessity of target validation in vitro, but having AcrB or efflux proteins as one of the predicted targets is promising [70, 72].

4. Acute Toxicity (LD50)

GUSAR predictions of acute toxicity (LD50, mg/kg) values over four routes of administration are given in Table 4.

Table 4: In Silico Acute-Toxicity Prediction (LD50, mg/kg)

Compound	Oral LD50	IV LD50	IP LD50	SC LD50	OECD Class
Ciprofloxacin	5000	350	1700	2100	5 (low)
Camalexin	7200	520	2100	2400	5 (low)
2-Chloro-4-pyrrolidinopyridine	3800	270	1100	1300	4 (moderate)
2,2-Dimethylthiazolidine	12000	980	3200	3200	5 (low)

- All compounds, except for 2-Chloro-4-Pyrrolidinopyridine, belong to OECD Class 5 (low toxicity), since they have high LD
- 2,2-Dimethylthiazolidine has the highest predicted LD50 value, showing a high safety margin in acute toxicity
- 2-Chloro-4-pyrrolidinopyridine is a Class 4 compound (moderate toxicity
- These results agree with the literature data on early toxicity de-risking, where high LD50 values are linked to a reduced likelihood of acute adverse effects in vivo [73-75].

5. Comparative Efficacy and Safety Profiles

Docking vs. ADMET and Toxicity Correlation

- The most promising compound, ciprofloxacin, retains the highest binding affinity and strong ADMET profile but as a known antibiotic is prone to AcrBmediated efflux which limits its long-term use against MDR E. coli [76, 77].
- Camalexin is the most promising candidate due to its moderately high affinity, excellent ligand efficiency, excellent ADME profile, and low predicted toxicity; its moderate efflux pump target probability and natural product status further support its development as an EPI [78-80].
- 2-Chloro-4-pyrrolidinopyridine and 2,2-Dimethylthiazolidine exhibit lower docking scores, but the latter outperforms them in terms of safety (highest LD50) and blood-brain barrier permeability, pointing to its possible repurposing for CNS infections or as a scaffold for further optimization [81].

Limitations and Future Directions

While powerful, the computed docking scores only provide a useful proxy for binding potential and do not take into consideration dynamic conformational changes of AcrB or effects due to the presence of the bacterial membrane itself [82-85]. While robust, ADMET prediction requires in vitro and in vivo validation for confirmation of bioavailability and metabolic stability [86-88]. Off-targets will also have to be assayed stringently, particularly those compounds predicted to exhibit polypharmacology [89, 90]. However, the in silico workflow presented herein is consistent with the current state of the art for virtual screening protocols and represents a powerful approach toward early-stage discovery of antimicrobials [91-95].

CONCLUSIONS

This in silico study demonstrates that some of the candidate compounds, predominantly Camalexin, possess excellent AcrB efflux pump inhibition potential through a competitive binding affinity with an improved pharmacological profile as compared to Ciprofloxacin. Integration of molecular docking, ADME evaluation, target prediction, and acute toxicity modeling provides a comprehensive framework during early-stage drug discovery process against MDR E. coli. Camalexin, specifically, deserves further preclinical investigation as an efflux pump inhibitor. Despite being a strong binder, Ciprofloxacin is prone to efflux and has developed resistance mechanisms that preclude its independent clinical use. The candidate molecules identified herein offer the possibility of restoring antibiotic efficacy and overcoming MDR via efflux inhibition.

Recommendations

- The outcome of these predictions needs experimental validation through in vitro efflux inhibition assays and whole-cell susceptibility studies in E. coli strains expressing AcrB.
- Optimization of Lead Molecules: In this respect, the SAR studies and medicinal chemistry optimization should focus on improved AcrB affinity and specificity, particularly against Camalexin and 2,2-Dimethylthiazolidine.
- Pharmacokinetics and Safety: Confirmation of the predicted oral bioavailability and safety margins by in vivo pharmacokinetic and toxicity profiling is recommended.
- Combination Therapy: Evaluate the effectiveness of candidate EPIs in combination with antibiotics against MDR E. coli using relevant infection models.

Research Ethics Statement

In this study, all computational analyses were performed using open-source or licensed software, namely InstaDock, SwissDrug Design, and GUSAR, in strict accordance with their terms of use as determined by the cited programs. No human or animal subjects were used in this study, and all chemical structures and protein targets used were from publicly available databases, such as PubChem and PDB, respectively; hence, data privacy and integrity are guaranteed. Consequently, the research was conducted in full adherence to open science principles for transparency, reproducibility, and responsible data sharing. No conflict of interest or external funding influenced either the study design, execution, or interpretation of the results.

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