Journal of Medical Bacteriology

The Role of Artificial Intelligence in the Development of Efflux Pump Inhibitors

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ARTICLE INFO

Article type:
Review Article

Article history:

Received	08	Nov	2025
Revised	09	Dec	2025
Accepted	04	Jan	2026
Published	11	Feb	2026

Keywords:
Artificial intelligence, Antimicrobial resistance, Drug discovery, Efflux pumps, Inhibitors.

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ABSTRACT

Background: Antimicrobial resistance (AMR) mediated by efflux pumps constitutes a critical health problem, necessitating urgent strategies for the development of new efflux pump inhibitors (EPIs). In this regard, artificial intelligence (AI) seems to be an innovative strategy for accelerating discovery, optimization, and understanding of EPIs mechanisms of action.

Conclusion: This review summarizes recent advances regarding the role of AI in the development of new EPI, with emphasis on machine learning (ML) based inhibitor prediction, molecular dynamics (MD) for binding analysis, and quantitative structure-activity relationship modeling (QSAR). By regrouping data from recent studies, we discuss here the important role played by AI in the improvement of lead identification, inhibitor designs, and the study of the resistance mechanisms. Despite current limitations such as limited, fragmented data and structural complexity of efflux pumps, AI offers great promise to revolutionize EPI development. In order to effectively combat AMR, we address here some key approaches, applications, challenges, and future directions, demonstrating the urgent need for interdisciplinary collaboration.

- ***Please cite this paper as:*** Najoua E, Chaimaa K, Hanane AH. The Role of Artificial Intelligence in the Development of Efflux Pump Inhibitors. *J Med Bacteriol.* 2026; **14** (1): pp.62-72. DOI:

Introduction

Antimicrobial resistance (AMR) has been long a threatening public health problem in the 21st century. It is estimated to cause around 10 million deaths per year by 2050 (1). In fact, 2019 saw approximately 4.95 million deaths attributed to AMR, underscoring the immediate need for effective agents against this problem (2). The emergence of multidrug-resistant (MDR) bacteria, often termed "superbugs," compromises the efficacy of existing antibiotics, causing severe infections, high rates of mortality and expensive healthcare costs (3). The overexpression of efflux pumps, a sophisticated membrane protein system, making a substantial contribution to the emergence of AMR by actively expelling antimicrobials out of the cell, thus keeping the intracellular accumulation of the antibiotics below effective levels (4).

Efflux pumps, like the AcrAB-TolC system in *Escherichia coli*, confer resistance to wide spectrum classes of antibiotics, notably β-lactams, tetracyclines, and fluoroquinolones (4). For this, the development of novel EPIs presents a valuable strategy to restore antibiotic susceptibility by inhibiting the extrusion process, thereby increasing susceptibly of resistant strains to the treatment. In this regard, recent reports showed the ability of novel molecules acting as "molecular wedges" to inhibit efflux pumps such as AcrAB-TolC. This finding has successfully restored antibiotics efficacy in Gram-negative bacteria, opening a promising path for novel treatments (5).

The development of EPIs using conventional methods, like high-throughput screening and structure-based drug design is hampered by many limitations, including high costs, time consumption, efflux pumps structures complexity and efflux pumps wide variety of substrates (6). To circumvent this problem, artificial intelligence (AI) and machine learning (ML) have been recently used for drug discovery, using fast analysis of huge amounts of biological compounds, chemical datasets, predictive modeling, and molecular structures (7). In this context, a recent study has demonstrated the power of deep learning in halicin identification, a compound with EPI-like activity against MDR bacteria such as *Acinetobacter baumannii*, highlighting the immense potential of AI in antibiotic discovery (8). Thus, AI can streamline the development of new inhibitors, enhance binding affinity, and clarify the understanding of efflux-mediated resistance mechanisms. ML algorithms have also been used to screen molecules targeting efflux pumps such as AcrB and MexB for the identification of new inhibitors with higher binding properties (9). Molecular docking (MD) plays an important role in the improvement of the computational study of efflux pumps, as shown by discoveries fascinating their transport models using AI, which helps to identify potential compounds faster than using laborious assays and allows for a deeper insight into the structural characterizations of efflux pumps. Nevertheless, AI-based methods depend on high-quality databases to make reliable predictions of molecular interacting which is indispensable for EPI development (10). This review synthesizes recent advancements in AI-driven EPI development, exploring methodologies, key applications, current limitations and future perspectives, with a focus on their transformative potential in addressing the global AMR crisis.

The role of efflux pumps in antimicrobial resistance

Efflux pumps are sophisticated membrane proteins existing in both Gram-positive and Gram-negative bacteria (11). They represent a major source of resistance by actively extruding a wide range of antimicrobials and other toxic substances out of bacterial cells (11). According to their structure and mechanisms of action, these transporters are classified into five families, including the Resistance-Nodulation-Division

(RND), Major Facilitator Superfamily (MFS), ATP-Binding Cassette (ABC), Small Multidrug Resistance (SMR), and Multidrug and Toxic Compound Extrusion (MATE) (12).

Among these transporters, RND-type efflux pumps like the AcrAB-TolC system are particularly noteworthy owing to their broad-spectrum specificity and high efficiency in expelling antibiotics and other compounds (13). They are commonly found in Gram-negative bacteria like *E. coli*, *Pseudomonas aeruginosa*, and *A. baumannii* (9), and they are formed by three domains: AcrB (an inner membrane transporter), AcrA (a periplasmic adaptor protein) and TolC (an outer membrane channel) (14). These proteins form together a tripartite complex fixed on the bacterial cell envelope, which actively extrude antibiotics to the extracellular environment (15). Thus, these transporters contribute to MDR by reducing the accumulation of antimicrobials or by working in synergy with other resistance mechanisms, like enzymatic degradation or target modification (11-12). For instance, in *P. aeruginosa*, the MexAB-OprM transporter pumps out β-lactams and fluoroquinolones, while the NorA efflux pump of the MFS family confers resistance to quinolones and many other drugs in *Staphylococcus aureus* (16). In the clinic, efflux-mediated resistance poses a significant challenge, especially in hospital environment where MDR species cause severe nosocomial infections (17). EPIs are intended to inhibit these pumps by preventing the extrusion of antibiotics and restoring their efficacy (12). Nevertheless, developing novel EPIs comes with considerable hurdles due to the structural complexity of efflux transporters, of their capacity of recognition of a wide variety of substrates, and bacteria's potential to upregulate pump expression or develop resistance to EPIs themselves, which pose significant challenges to their development (12).

Traditional EPIs discovery approaches, including high-throughput screening of chemical libraries and crystallographic analysis search for compounds that bind at efflux pump active sites. However, they are time-consuming and often lead to limited success due to the fact that pump structures are dynamic (18). In recent years, AI-driven approaches have emerged as innovative solutions, including ML, MD simulations, and quantitative structure-activity relationship (QSAR) modeling (10). These methods enable rapid screening of huge chemical libraries, predicting inhibitor-pump interactions, and contributing to the optimization of lead compounds (10). Hence, by integrating computational and experimental data, AI can significantly accelerate the development of EPIs, providing a crucial tool to fight the global AMR crisis.

Introduction to artificial intelligence in efflux pump inhibitor development

Machine learning for efflux pump inhibitor prediction

Supervised ML algorithms have achieved significant success in EPIs prediction by analyzing large biological and chemical datasets (9). However, the structural complexity and broad-spectrum drug recognition of these transporters present a critical hurdle, emphasizing the crucial need for new EPIs to restore antibiotic efficiency (19). ML facilitates the development of EPIs by analyzing large datasets to identify potential inhibitors, predict their interaction mechanisms with efflux pumps and optimize their chemical properties. This significantly facilitates the discovery process and provides a viable way for getting the challenges of efflux pump inhibition (9). In fact, recent studies in neural networks have introduced hybrid representations coupling convolutional neural networks with molecular descriptors. Such progress has been seen to offer increased flexibility and improve predictive capability on structurally diverse molecules, including EPIs. These ML approaches, supported

by improved computational power, are now enabling both predictive and generative models for novel antibacterials discovery (20). In this regard, deep learning has been recently used for drug discovery by identifying halicin as a novel antibiotic that exhibits an EPI-like activity. This compound was able to disrupt resistance mechanisms in bacteria such as A. baumannii and *E. coli*. The deep neural network used in this study, trained on a large chemical library of more than 6,000 compounds and powerfully proved the ability of AI's algorithms to discover novel molecules characterized with a broad-spectrum antimicrobial activity, including efflux-mediated resistance (8).

Molecular dynamics and docking simulations for efflux pump inhibitors

Molecular dynamics simulations and MD studies provide an important overview regarding the binding interactions between bacterial efflux pumps and EPIs. In this regard, a recent study focused on *S. aureus* NorA efflux pump used supervised molecular dynamics (SuMD) and molecular docking in order to study the dynamic interaction pattern between this pump and its substrates including PQQ16P, a potent NorA inhibitor (21). Hence the use of SuMD contributed to the reconstruction of the molecular recognition pathway of PQQ16P from the first ligand-free state to the formation of the final protein-ligand complex. The investigation results showed that after only 8 ns the EPI reached its binding site. Regarding MD stimulations, binding sites identified by SuMD were commonly shared between the most potent studied EPIs. Indeed, 13 of the very active 2-phenylquinoline analogs, characterized with 95% inhibition of EtBr efflux, and exhibited the same predicted inhibitor-binding conformation in NorA (21). Hence, SuMD and MD algorithms might constitute a valuable option to explore the ligand binding region in structure based virtual screening for potent EPIs.

Introduction to QSAR modeling for efflux pumps inhibitors prediction

QSAR modeling is central to the prediction of EPIs efficacy. This computational method develops correlations between molecule's structure and its biological activity, a step of considerable importance in the search for new therapeutics against MDR bacteria, especially those targeting efflux pumps such as NorA in S. aureus.

QSAR models and flavonoid derivatives

Predictive capacity of QSAR models in studying prenylated isoflavonoids as NorA EPI in *S. aureus* was performed previously (22). In this study, authors optimized models with closely related hydrophobicity, molecular size, and partial charge descriptors for inhibitory activity detection using the Molecular Operating Environment (MOE) software. Strong correlation ($r \geq 0.95$, $p < 0.05$) were observed between some calculated molecular descriptor properties and the ability of prenylated isoflavonoids like neobavaisoflavone, glabrene, glyceollin I, and glyceollin III to potentiate ciprofloxacin effect by reducing its minimum inhibitory concentration (MIC) by fourfold in fluoroquinolone-resistant *S. aureus* (Figure 1). Hydrophobic surface area and volume descriptors (41%) seemed to be the important factors of this correlation, followed by hydrophobic/hydrophilic balance (24%), partial charge-related descriptors (12%), and molecular size (12%). These findings suggest that hydrophobicity facilitates interactions with bacterial membranes and facilitates π–π stacking with phenylalanine residues within NorA's binding pocket, while hydroxyl groups ensure a balance for effective binding. The results align with prior QSAR studies on anti-MRSA activity, indicating shared molecular features for antibacterial and EPI activities, though not all potent EPIs exhibited strong antibacterial effects. This study emphasizes the need for further research, including compound libraries of larger

sizes and experimental protein–ligand binding assays, to better fine-tune like associations and final verification of the proposed binding mechanisms (22).

QSAR and virtual screening for novel efflux pumps inhibitors

In other to discover new NorA EPI derived from natural products, a report made by Thai et al. (2015) employed models based on modeling, virtual screening, and MD. The developed QSAR model using 47 diverse compounds from existing literature validated with an external set of 15 compounds, exhibited strong predictive power evidenced by robust statistical metrics (n = 45, Q^2 = 0.80, RMSE = 0.20 for the full dataset; n = 15, R^2 = 0.60, |res|max = 0.75, |res|min = 0.02 for the test set) (23). Based on this validated model, the screening of huge library of 182 flavonoids and a traditional Chinese medicine (TCM) database was performed, leading to the identification of 33 lead compounds that nor only showed high predicted (pIC50 values) but also adhered to Lipinski's Rule of Five (23). Docking studies were further performed in order to evaluate the effect of these compounds on a NorA homology model (due to the lack of NorA crystal structure). The goal was to target the central channel and Walker B active sites. Results elucidated the NorA EPI activity of these compounds, highlighting the important role of QSAR-driven models in the process of discovery of novel EPI (23).

Applications of artificial intelligence in efflux pump inhibitor development

AI is transforming the identification of EPIs, which are very important in the fight against antibiotic-resistant bacteria through re-sensitization of conventional antibiotics. Efflux pumps, such as AcrAB-TolC in E. coli and NorA in S. aureus, have been considered to be prime drives of the globalization of MDR due to their active efflux of antibiotics from bacterial cells (6). To overcome this challenge, AI-driven approaches are accelerating EPI discovery and optimization, providing innovative solutions to address this global health crisis.

Acceleratiion of efflux pumps inhibitors lead identification

AI has significantly enhanced the screening of large chemical libraries for EPIs discovery. Indeed, ML models, such as deep neural networks and support vector machines, are leaders of this growth. These models examine the molecular structures of compounds and estimate their direct effect of efflux pumps. Similarly, research conducted by Liu and his collaborators (2023) screened around 7,500 molecules using a deep molecular property neural network (D-MPNN). Among 480 identified active compounds, abaucin showed a strong activity against A. baumannii (24). Similarly, an interpretable substructure-based method was performed using a graph neural network (25). This model evaluated 39,312 compounds and made predictions on antibiotic activity and cytotoxicity for over 12,076,365 compounds. This discovery has resulted of novel classes of antibiotics with low toxicity and potent antibiotic activity (25). Indeed, among 283 tested compounds, one identified class presented an important against methicillin-resistant *S. aureus* MRSA and vancomycin-resistant enterococci, with decreased resistance development, with promising results in an infection mouse model (25). This study underscores the important role of ML in antibiotics discovery to both predict drug's efficacy and offer insights into key structures for targeted antibiotics.

AI also enhances inhibitor discovery by integrating virtual screening with MD simulations, a powerful combination that greatly improves discovery of new effective compounds (26). In fact, Phan et al. (2023) employed this approach to identify new AcrAB-TolC inhibitors from

DrugBank and ZINC15 databases. Authors performed a virtual screening of twenty inhibitor candidates of this system using 3D pharmacophore and two 2D-QSAR models on the AcrB protein (PDB 4DX7) based on a dataset of 170 compounds with varied structures obtained from 28 research journals. Results from these investigations demonstrated that three components notably DB09233, DB02581, and DB15224 exhibited an important EPI activity with ΔGbind of -42.30 ± 4.58, -40.76 ± 7.30 and -31.06 ± 7.63 kcal.mol-1 (27). Thus, AI enabled the screening of compounds based on predicted binding potential to targets like AcrAB-TolC as well as their desirable drug-like characteristics. These AI-driven screening methods significantly accelerate the discovery of novel molecular scaffolds and reduce the time and cost associated with traditional high-throughput screening technologies (28).

Optimization of efflux pumps inhibitors designs

Artificial intelligence is major contributor to EPIs development through important enhancement of their design and characteristics. Molecular modifications can also be predicted by AI algorithms in order to enhance properties such as binding affinity and pharmacokinetics (29). For example, halicin identification has been performed using deep learning models, this compound exhibits EPI-like properties against a wide variety of MDR pathogens, notably A. baumannii and *E. coli*. The model based on a large chemical library, predicted molecular features capable that could disrupt resistance mechanisms mediated by efflux pumps, demonstration the important role played by AI in EPI screening (10).

In the same context, Vamathevan et al. (2019) demonstrated the ability of AI models to screen novel EPIs by combining molecular details with clever predictive algorithms (10). In fact, recent investigations showed the important role of AI in EPI development design, by studying Absorption, Distribution, Metabolism, Excretion, Toxicity (ADMET) profiles (31). This capability allows for the rational design of EPIs that are not only potent but also possess improved pharmacokinetic properties, addressing key challenges such as poor membrane permeability and toxicity that often hinder drug development (30).

Similarly, prenylated isoflavonoids like neobavaisoflavone and glabrene isolated from plants belonging to Fabaceae family, were identified as potent NorA EPIs in S. aureus (22). This investigation confirmed that the combination of these compounds with fluoroquinolones can increase their antibacterial effect by eight times. This positive effect was linked to molecular characteristics like hydrophobic surface area and hydrophilic/hydrophobic balance. The combination of these compounds with fluoroquinolones made QSAR predictions on enhanced activity possible; thus, building on previous work and targeting even more improved compounds (22).

Role of artificial intelligence in the resistance mechanisms understanding

Microbial resistance mechanisms have been strongly studied owing to AI models, primarily by providing detailed studies regarding structural and dynamic properties of efflux pumps. Indeed, MD models have been employed in order to study AcrB efflux pump in *E. coli* (15). These simulations helped defining key substrate-binding sites and conformational changes that promote the bacterial resistance by extruding multiple drugs out of the cell. This provides vital insights into the molecular interactions that control pump function, ultimately making it easier to design targeted inhibitors (15).

In a similar way, Wang and collaborators (2023) conduced molecular docking and MD simulations to study the binding mechanisms of EPIs to the AcrB pump in *E. coli*. Their investigation showed important inhibition mechanisms (32). Furthermore, new resistance mechanisms discovery can be dramatically improved by the

usage of AI tools. This is exemplified by a recent study showcasing the part efflux pumps play in colistin resistance in *Klebsiella pneumoniae*, by analyzing the effect of proton motive force-dependent pumps using AI (33). Results suggest that by interfering with the efflux pumps functions inhibitors like carbonyl cyanide m-chlorophenyl hydrazone (CCCP) may be able to reverse resistance. Hence, by combining AI tools with structural biology and bioinformatics, scientists can now possess a better understanding of efflux pump dynamics, allowing them to discover news effective pathways for combating AMR.

Challenges in AI-driven efflux pump inhibitor development

ML and MD tools offer innovative strategies to combat MDR. Using these approaches, the efficacy of existing antimicrobial drugs could be restored by accelerating the development, of promising compounds, and the discovery of novel EPIs (34). Despite this potential, many hurdles circumvent the progress in this area, impacting the successful clinical application of AI-predicted EPIs.

Data quality and availability

AI training models require high-quality open access dataset libraries with validated EPI activities. In fact, computational model's effectiveness in the discovery of EPI is directly Complexity of efflux pumps

In bacteria, the structural and functional diversity of efflux pumps makes the discovery of broad-spectrum efflux pump inhibitors (EPIs) a significant challenge. As previously cited, efflux pumps are grouped into several superfamilies characterized by special mechanisms for transporting and recognizing substrate (36). In fact, RND pumps, like AcrB in *E. coli* and MexB in *P. aeruginosa*, are polyspecific, extruding many different, unrelated compounds through complex binding pockets that include hydrophobic traps and distal binding sites (36). Hence, universal inhibitors designs can disrupt several types of pumps in both Gram-negative and Gram-positive bacteria.

For example, the AcrAB-TolC pump in *Enterobacteriaceae* confers resistance to antibiotics like tetracyclines and fluoroquinolones, while the MexAB-OprM in *P. aeruginosa* additionally targets substrates like macrolides (37).

linked to the rich comprehensive data, including molecular structures, binding affinities, and biological activities (35). However, the efficiency AI algorithms are limited with many drawbacks, notably the insufficiency of standardized and large-scale datasets which reduce the predictive performance and limits EPI optimizations using ML models. Thus, collaboration between scientists working in different fields could be a valuable strategy to developpe unfragmented open access databases libraries by regrouping chemical, biological, and structural information to strengthen AI-based model training destinated to EPI development. Recent initiatives, such as the Antimicrobial Resistance-focused Pharmaceutical Developing Research (APDR) program, aim to address this by generating publishable datasets through undergraduate research (10).

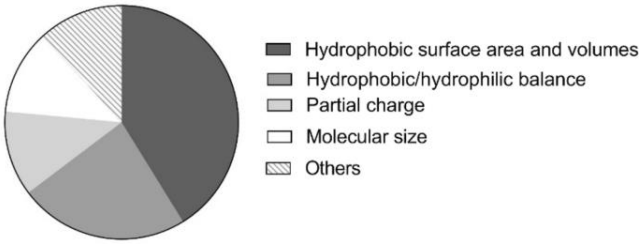

Figure 1. Molecular properties of prenylated isoflavonoids highly correlated ($r > 0.95$, $p < 0.05$) with EPI activities (fourfold reduction of ciprofloxacin MIC (22).

Given this complexity, AI-driven approaches for EPI discovery must consider these variations by incorporating multi-target modeling and species-specific structural data, which significantly increases computational complexity and requires advanced algorithms like fully connected graph neural networks (FCGNN) to predict inhibitor interactions across diverse pump architectures (38).

Validation requirements

AI-predicted EPI candidates require extensive in vitro and in vivo validations to confirm their efficacy, safety, and clinical viability, a process that is both resource-intensive and time-consuming. As showen by Duffey et al. (2024), rigorous experimental testing is required for computation predictions validation, this testing should include assays for pump inhibition, pharmacokinetic properties and antibiotic potentiation (e.g., ADMET) (39). Despite advances in AI-driven drug screening, no EPI has been approved by the U.S. Food and Drug Administration (FDA) largely due to challenges in translating computational hits into clinically effective compounds. For example, early EPI candidates like PAβN showed promising activity in vitro but failed clinical trials because of toxicity and poor pharmacokinetics (39, 31). In addition, resistance mechanisms and EPIs activitie's evaluation against numerous bacterial strains is another aspect of validation calling for high-throughput experimental platforms and standardized protocols. The intricacy of these validation procedures emphasizes the necessity of integrating AI-experimental pipelines to speed up in silico predictions into clinical candidates (41).

Resistance to efflux pumps inhibitors

Combating MDR is significantly influenced by bacterial resistance to EPIs, especially in Gram-negative bacteria, including *P. aeruginosa* and *Enterobacteriaceae*. Resistance mechanisms include overexpression of efflux pumps (e.g., MexCD-OprJ, AcrAB-TolC), horizontal transfer of resistance genes via plasmids or integrons and mutations in pump or regulatory genes (e.g., mexR, nfxB) which reduce the binding affinity of the inhibitor, (37). In this regard, a recent investigation made by Schuster and his collaborators (2024) showed genes mutations coding for bacterial efflux pumps (e.g., in AcrB's distal binding pocket) can distinctly influence EPIs potency. requiring sophisticated AI tools to optimize novel EPI capable of handling these adaptations (42).

Conclusion

The discovery and optimization of EPIs was revolutionized by AI-innovative approaches to fight against the urgent challenge of antimicrobial resistance. Using the power of ML, researchers can discover vast chemical libraries to find new EPIs. Meanwhile MD simulations make clear the mechanisms of action of efflux pumps, Therefore, designing inhibitors to fit all these aspects perfectly. In parallel, QSAR modeling adjusts such compounds so they can combat resistance. such as limited data, the complexity of pump structures, demanding validation processes and changing bacterial resistance, AI improves the discovery and complements experimental validation. Thus, interdisciplinary collaborations between microbiologists, clinicians and computational experts, the in-silico EPI models could be transformed into real-world therapies. Through advocating open-access data sharing and standardized experimental protocols, as well as in depth AI strategies of new breed medications, the scientific community can develop fresh antimicrobials that will provide lasting and truly global health protection.

Acknowledgements

The authors declare that there are no acknowledgements.

Funding Information

This work was not supported by any specific funding.

Ethics approval and consent to participate

Not applicable.

Conflict of interest

Elhidar Najoua, Katif Chaimaa and Ait Hammou Hanane declare that there are no conflicts of interest.

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