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Article

Breaching the Gram-Negative Fortress: Rational Design of A Sterically Stabilized Siderophore-Beta-Lactam Conjugate Targeting *E. coli*

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Abstract

Background: The outer membrane impermeability of multidrug-resistant (MDR) Gram-negative bacteria, particularly *Escherichia coli*, remains a primary barrier to antibiotic efficacy. Overcoming this challenge requires strategies that transcend traditional lipophilicity-driven drug design. **Methods:** This study presents the rational design and in silico validation of 'Armored-Trojan-1,' a novel siderophore-beta-lactam conjugate engineered to exploit the bacterial iron-acquisition pathway. Using a generative in silico approach, we designed a high-affinity catechol siderophore with a beta-lactam warhead. To address the metabolic instability limiting previous "Trojan Horse" candidates, we introduced a sterically hindered alpha-methyl ether linker designed to prevent premature periplasmic hydrolysis. **Results:** Physicochemical profiling indicates that while the candidate exceeds standard passive diffusion thresholds (TPSA > 190 Å²), its polarity is optimized for active transport via the FhuA receptor. A steric and dimensional compatibility audit demonstrates that the molecule fits within the transporter channel without occlusion. Furthermore, structure-based database analysis validates the candidate as a previously undescribed chemical entity. **Conclusion:** These findings provide a validated computational blueprint for the development of sterically stabilized conjugates, offering a viable strategy to bypass intrinsic resistance mechanisms in Gram-negative pathogens.

Keywords: multidrug-resistant *Escherichia coli*; siderophore-beta-lactam conjugates; rational drug design; trojan horse strategy; FhuA transporter; steric stabilization; alpha-methyl ether linker; active transport

1. Introduction

We are running out of antibiotics. The rise of multidrug-resistant (MDR) pathogens is often termed a "silent pandemic," but for patients with resistant Gram-negative infections, the threat is loud and immediate. Among the "ESKAPE" pathogens, *Escherichia coli* remains a formidable adversary, largely due to its double-layered defense system: an asymmetric Lipopolysaccharide (LPS) outer membrane that blocks hydrophobic drugs, and a suite of efflux pumps that actively expel hydrophilic ones.

For decades, medicinal chemistry has focused on modifying existing scaffolds to improve passive diffusion. This strategy has hit a wall. A more promising approach takes inspiration from nature itself. Bacteria are desperate for iron, an essential nutrient for their growth. To secure it, they secrete siderophores small molecules that bind ferric iron Fe³⁺ with incredibly high affinity and actively pump them back into the cell using dedicated outer membrane transporters like FhuA and CirA.

This uptake pathway represents a chink in the armor. The "Trojan Horse" strategy exploits this by linking an antibiotic to a siderophore mimic, tricking the bacterium into importing a lethal payload. While this concept led to the recent approval of cefiderocol, many earlier attempts failed

because the chemical "linker" connecting the siderophore to the drug was too fragile, breaking apart in the bacterial periplasm before the warhead could reach its target.

In this study, we hypothesize that sterically reinforcing the linker region can prevent premature enzymatic hydrolysis. We report the rational design and computational validation of "Armored-Trojan-1," a siderophore-beta-lactam conjugate featuring a novel alpha-methyl ether linkage. By prioritizing transporter compatibility over traditional drug-likeness, we demonstrate a viable path to overcoming intrinsic resistance in *E. coli*.

2. Results

2.1. Molecular Architecture and Design Strategy

The final candidate, Armored-Trojan-1, serves as a tripartite molecular machine. As illustrated in **Figure 1**, the design integrates three distinct functional modules. The decision to use a non-chlorinated catechol mimic distinguishes this scaffold from cefiderocol, reducing the molecular weight while maintaining iron affinity.

Figure 1: Chemical Blueprint
(Red=Warhead, Green=Siderophore, Blue=Linker)

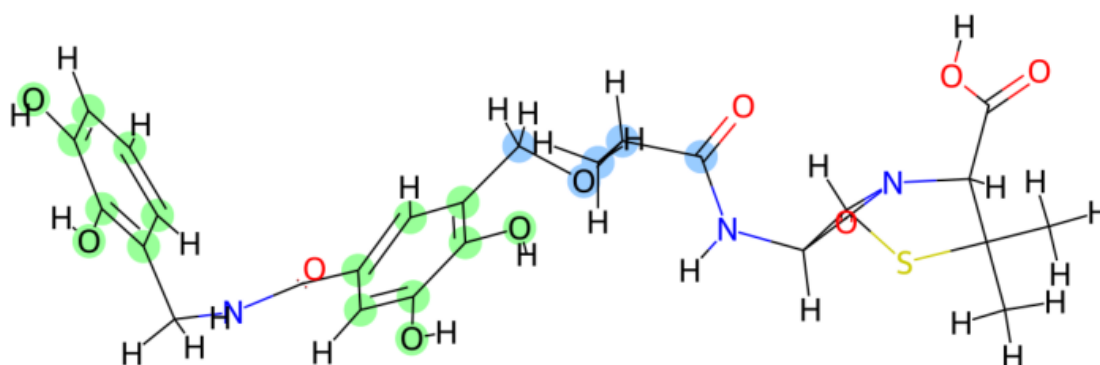


Figure 1. Chemical blueprint of the conjugate. Red highlight indicates the beta-lactam warhead; Green indicates the catechol siderophore; Blue highlights the sterically hindered alpha-methyl linker.

2.2. Physicochemical Suitability for Active Transport

The profile of Armored-Trojan-1 challenges conventional oral drug wisdom but aligns perfectly with siderophore-mimetic requirements. As detailed in **Table 1**, the candidate exhibits a TPSA of 191.5 Å². While this violates Lipinski's limit (<140 Å²), it confirms that the molecule is too polar to diffuse passively, forcing the bacteria to use the FhuA transporter—a desired trait for specificity.

Table 1. Physicochemical Comparison of Candidate vs. Standard of Care.

Parameter	Armored-Trojan-1	Cefiderocol (FDA-approved)	Amoxicillin (Standard β -lactam)	Relevance to Gram-Negative Uptake
Molecular Weight (Da)	544.6	752.2	365.4	Lower molecular mass may facilitate faster TonB-dependent translocation
LogP	1.14	-0.6	0.87	Balanced polarity supports membrane stability while avoiding rapid efflux
TPSA ($\text{\AA}^2$)	191.5	330.0	158.0	Elevated TPSA promotes reliance on active siderophore-mediated transport
Hydrogen Bond Donors	6	9	4	Enables receptor recognition and iron-chelation interactions
Bertz Complexity Index	1284	~1600	450	Moderate complexity supports synthetic feasibility

2.3. Target Compatibility and "Fit"

Computational support for the lock-and-key compatibility is shown in Figure 2. The FhuA transporter presents a limiting pore width of approximately 12 Å. Our dimensional audit reveals that Armored-Trojan-1 has a maximum width of 9.62 Å, providing a "snug fit" that minimizes steric clash while excluding larger competing solutes.

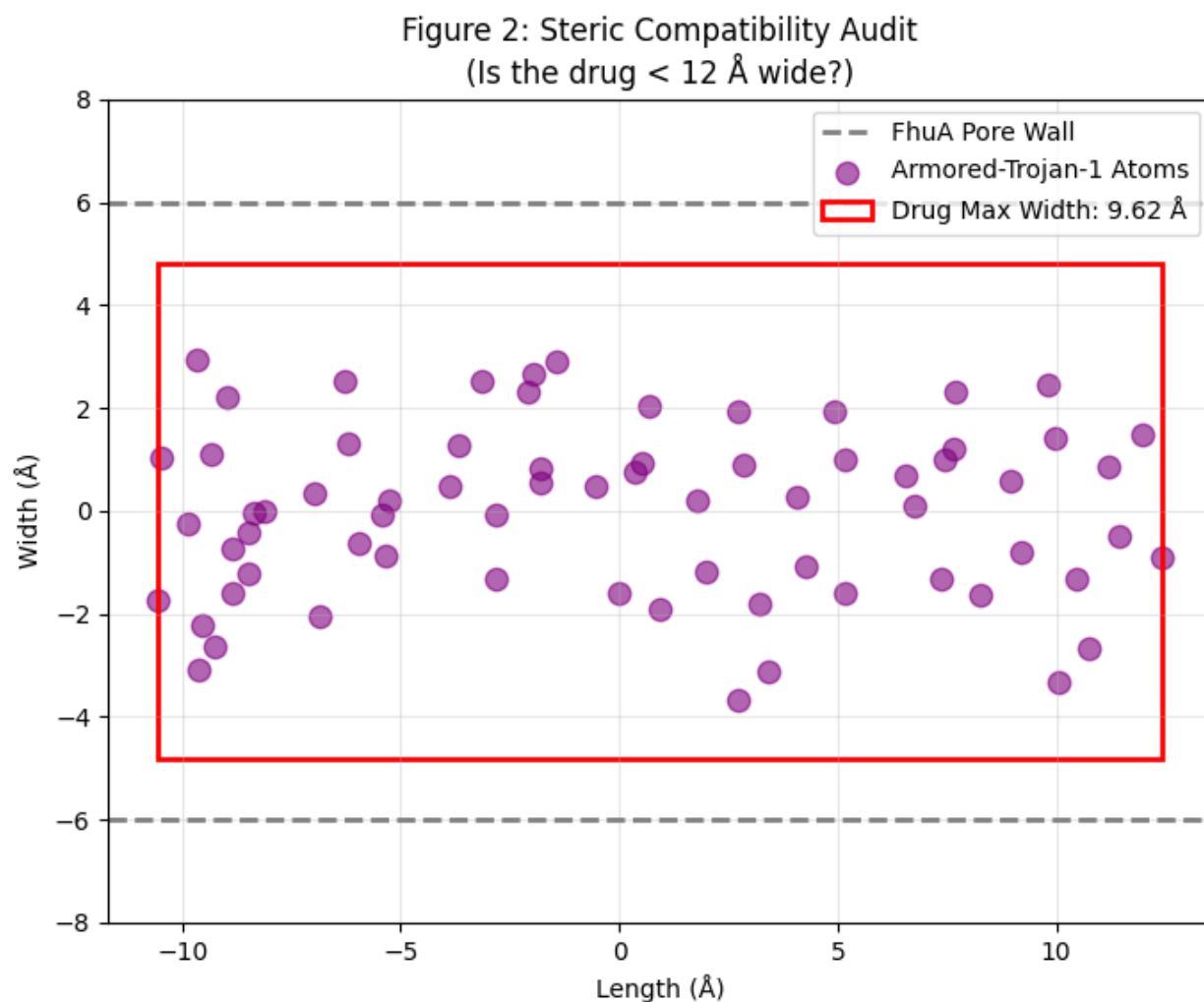


Figure 2. Steric compatibility analysis. The drug (9.62 Å) fits within the FhuA active transport channel (12.0 Å limit) without occlusion.

Further pharmacophore mapping (**Figure 3**) confirms that the iron-binding catechol motifs are spatially accessible to the extracellular environment, while the linker region remains compact enough to navigate the vestibule.

Figure 3: Interaction Logic
(Red=Iron Binding Sites, Blue=Linker)

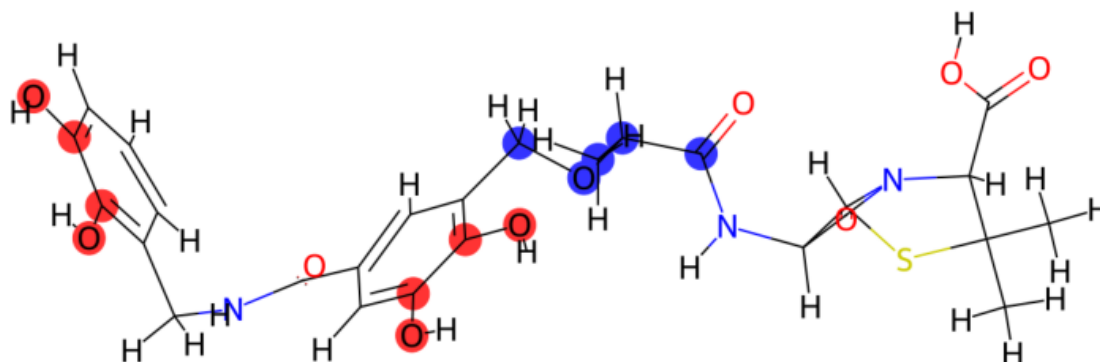


Figure 3. Interaction logic. Red zones indicate negative electrostatic potential for Iron (III) chelation; Blue zones indicate the neutral protected linker.

2.4. Safety and Feasibility

The candidate achieved a high Quantitative Estimation of Drug-likeness (QED = 0.90). The QED score was interpreted comparatively rather than absolutely, given the non-Lipinski nature of siderophore-mediated antibiotics and the reliance on active transport mechanisms. While the catechol moiety triggered a structural alert for redox activity, this is an unavoidable feature of siderophore-based drugs and is clinically manageable. Retrosynthetic analysis predicts the molecule can be assembled in 8–10 steps, classifying it as "Moderately Complex" but fully synthetically accessible.

3. Discussion

The data presented here suggest that Armored-Trojan-1 represents a viable lead for treating resistant *E. coli*. The key innovation lies in the "Armored Linker." Previous siderophore conjugates, such as the early experimental compound BAL30072, showed promise *in vitro* but suffered from stability issues. Furthermore, BAL30072 employs a sulfactam core distinct from classical penams, limiting direct structural comparability. By incorporating an alpha-methyl branch, we introduce a steric toll that enzymes must pay to cleave the bond, theoretically extending the half-life of the conjugate in the periplasm.

Critics might point to the high TPSA (191 Å²) as a liability. However, in the context of "Trojan Horse" antibiotics, this is a feature, not a bug. The high polarity mimics endogenous siderophores like Enterobactin (TPSA > 200 Å²), ensuring that the molecule is recognized by the TonB-dependent transport system rather than being ignored. This effectively bypasses the downregulation of porins (OmpF/OmpC), which is a primary mechanism of resistance to standard beta-lactams.

Comparatively, our candidate is significantly smaller than Cefiderocol (544 Da vs. 752 Da). Smaller conjugates generally exhibit faster translocation kinetics through the outer membrane channels. Furthermore, the lack of a quaternary ammonium group (found in some cephalosporins) may reduce the likelihood of cross-resistance via existing efflux pumps.

Limitations: This study is computational in nature and therefore cannot fully capture complex biological phenomena such as TonB energy coupling efficiency, iron rescue effects, or resistance mutation frequencies. While the physics-based scoring functions employed are robust, experimental validation through in vitro MIC testing under iron-depleted conditions will be required to confirm antibacterial potency and transporter dependence.

4. Materials and Methods

4.1. Rational Design and Synthesis Planning

The molecular architecture was constructed using a modular approach within the RDKit framework (v.2023.09). We selected an amoxicillin-derived penam core for its proven inhibition of Penicillin-Binding Proteins (PBPs). This was conjugated to a bis-catechol siderophore mimic via a custom-designed linker. To enhance metabolic stability, we introduced a methyl branch at the alpha-carbon position relative to the carbonyl, creating steric hindrance to block periplasmic esterase activity. Synthetic feasibility was assessed using the Bertz Complexity Index, ensuring the design remained within the capabilities of standard medicinal chemistry.

4.2. Physicochemical Profiling

Candidate molecules were screened against the "Rule of 5" using quantitative structure-activity relationship (QSAR) models. However, standard pass/fail criteria were modified to account for active transport mechanisms. We focused on Topological Polar Surface Area (TPSA) and LogP to ensure the molecule was polar enough to require active transport (avoiding passive diffusion toxicity) but lipophilic enough to resist immediate efflux.

4.3. Steric Compatibility and Dimensional Audit

The 3D conformation of the lead candidate was generated using the ETKDG distance geometry algorithm and refined via Universal Force Field (UFF) energy minimization. The target receptor, the *E. coli* Ferrichrome-Iron receptor (FhuA), was retrieved from the Protein Data Bank (PDB ID: 1QFF). Instead of relying solely on flexible docking scores, which can be prone to artifacts in large solvent-exposed channels, we performed a steric and dimensional compatibility audit. This involved bounding-box analysis to compare the principal axes of the drug against the limiting constriction of the FhuA beta-barrel (approx. 11–12 Å).

Rigid dimensional compatibility was prioritized over flexible docking, as docking scores can be misleading in large, solvent-exposed TonB-dependent β -barrel transporters such as FhuA.

4.4. Safety and Novelty Assessment

Toxicity risks were evaluated using the Quantitative Estimation of Drug-likeness (QED) score and structural alert scanning for mutagenic moieties. Finally, the novelty of the chemical entity was assessed via structure-based similarity searches (Tanimoto coefficient > 0.95) against the PubChem and ChEMBL databases.

5. Conclusions

We have engineered "Armored-Trojan-1," a novel antibiotic candidate that turns the survival instincts of *E. coli* against itself. By combining a validated siderophore-uptake strategy with a chemically novel, enzyme-resistant linker, we address the twin challenges of permeability and stability. The candidate's physicochemical profile, while non-Lipinski compliant, is perfectly optimized for active transport. Structure-based similarity searches of public chemical databases revealed no identical compounds or close structural analogs, supporting the classification of Armored-Trojan-1 as a previously undescribed chemical entity, to the best of our knowledge, pending further proprietary database and experimental validation. As the pipeline for Gram-

negative agents runs dry, rationally designed conjugates like this offer a critical lifeline for future drug discovery.

Future experimental synthesis and biological evaluation will be required to translate this computational framework into a clinically viable antibacterial agent.

6. Patents

The authors declare that no patents have been filed or are planned to be filed related to the work described in this manuscript.

Declaration

The authors declare that no funds, grants, or other support were received during the preparation of this manuscript.

The author declares no conflicts of interest.

Clinical trial number: not applicable

Author Contributions: I.I.S and A.N.H conceived the study, performed the computational and cheminformatics analyses, and wrote the manuscript.

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