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RESEARCH ARTICLE

In silico docking and adme-tox evaluation of annonacin: addressing contradictions in antimalarial evidence against pfdhodh and pfldh

Anindya Zerlina Tsaqif[1], Khodijah[1], Fahrizal Ryuzen Prayogi[2], Moh Mirza Nuryady[3], Kiky Martha Ariesaka[4*]

[1] Faculty of Medicine, State University of Malang, Malang, Indonesia
[2] Faculty of Mathematics and Science, State University of Malang, Malang, Indonesia
[3] Biology Education Department, Faculty of Teacher and Training Education, University of Muhammadiyah Malang, Malang, Indonesia
[4] Department of Parasitology, Faculty of Medicine, State University of Malang, Malang, Indonesia
[*]Correspondence: Kiky Martha Ariesaka; Address: Semarang street no. 5, Malang ; Email address: kiky.martha.fk@um.ac.id

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ABSTRACT

The rise of resistance to current antimalarial therapies highlights the urgent need for novel drug candidates. Annona muricata contains phenolic compounds, particularly annonaceous acetogenins, known for their bioactive properties and cytotoxicity. This study investigates the binding affinity of Annonacin to the malaria target proteins PfDHODH and PfLDH and assesses its toxicity and pharmacokinetic properties using in silico methods. Molecular docking was performed using Molegro Virtual Docker (MVD) version 6.0 to explore Annonacin's binding interactions with PfDHODH and PfLDH. Toxicity was evaluated using ProTox-II, focusing on cytotoxicity and LD_{50} values. Pharmacokinetic parameters, including gastrointestinal absorption and compliance with Lipinski's Rule of Five, were analysed using SwissADME. Docking analyses indicated stable complexes of Annonacin with both PfDHODH and PfLDH, suggesting strong inhibitory potential. Toxicity evaluation revealed significant cytotoxic effects, with an LD_{50} of 400 mg/kg, placing Annonacin in Toxicity Class IV, indicating risk upon ingestion. Pharmacokinetic assessments identified challenges, including low gastrointestinal absorption and a violation of Lipinski's rule due to Annonacin's high molecular weight. Annonacin demonstrates notable antimalarial potential; however, its development is limited by safety issues and pharmacokinetic constraints. Optimisation efforts are essential to enhance efficacy and safety, laying the groundwork for future research and development.

1. Introduction

Malaria remains one of the most devastating infectious diseases globally, accounting for over 600,000 deaths annually (Duguma *et al.,* 2022). Indonesia is among the nine Southeast Asian countries where malaria is endemic, reporting a troubling total of 443,530 cases in 2022. The eastern regions of Indonesia are particularly noted for their high malaria endemicity, and the government has pledged to eliminate malaria by 2030 (Philothra *et al.,* 2023). The disease is primarily caused by the protozoan parasite *Plasmodium falciparum,* which is transmitted through bites from *Anopheles mosquitoes* (Djihinto *et al.,* 2022). However, the efficacy of existing antimalarial drugs is increasingly compromised due to the rise of drug-resistant *P. falciparum* strains (Plowe, 2022). Resistance to artemisinin—the frontline treatment for malaria—has been documented in Southeast Asia and is spreading to other regions, presenting a significant challenge to

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malaria control efforts (Woodrow & White, 2017).

The escalating resistance to current treatments emphasises an urgent need for novel antimalarial agents. Previous studies have identified various bioactive compounds from different parts of the *Annona muricata* (soursop) plant, including leaves (K *et al.,* 2016; Nwonuma *et al.,* 2023), seeds, bark, and roots (Gavamukulya *et al.,* 2017), which exhibit antimalarial activity. However, findings have been inconsistent, with varying degrees of efficacy reported across specific compounds and experimental conditions. *A. muricata* contains natural phenolic compounds with antioxidant properties and bioactivity, and the annonaceous acetogenins, which exhibit cytotoxic potential (Rasyid *et al.,* 2023). Investigations into the plant's toxicology have revealed neurotoxic annonaceous acetogenins and benzyltetrahydro-isoquinoline alkaloids, providing new insights into its safety profile (Wahab *et al.,* 2018). Conversely, another study found that the aqueous leaf extract of *A. muricata* demonstrated significant dose-dependent antimalarial activity, inhibiting parasitemia by up to 85.61% without accompanying toxicity, while also extending the survival time of infected mice—suggesting its potential as a lead compound for malaria treatment (Somsak *et al.,* 2016). This contradiction highlights the necessity for further investigation into the potential of these compounds, particularly through in silico analyses, which could provide insights into their interactions with key molecular targets in malaria pathogenesis.

The pathogenesis of malaria involves several key proteins essential for the survival and proliferation of *P. falciparum* within the human host. Notably, Plasmodium falciparum Dihydroorotate Dehydrogenase (PfDHODH) and *Plasmodium falciparum* Lactate Dehydrogenase (PfLDH) are crucial in this process (Heikal *et al.,* 2023; Owoloye, 2020). PfDHODH plays a vital role in the de novo pyrimidine biosynthesis pathway, which is essential for DNA replication and cell proliferation in the parasite. Inhibiting this enzyme disrupts nucleotide synthesis, effectively halting parasite growth (Hartuti *et al.,* 2021). Meanwhile, PfLDH is critical in the glycolytic pathway, serving as the primary energy source (ATP) for *P. falciparum* in the anaerobic environment of red blood cells. Inhibition of PfLDH can deprive the parasite of energy, leading to its demise (Akinnusi *et al.,* 2023).

Given the pivotal roles of PfDHODH and PfLDH in *P. falciparum* survival, targeting these enzymes represents a promising strategy for developing new antimalarial drugs. This study aims to leverage in silico methods to explore the interactions between bioactive compounds derived from *A. muricata* and these crucial proteins. By identifying potential inhibitors through molecular docking studies, this research seeks not only to contribute to the discovery of new, effective antimalarial agents that can overcome existing drug resistance but also to clarify the inconsistencies observed in previous studies by elucidating how these compounds interact at the molecular level. Ultimately, this could validate *A. muricata* as a viable source for developing new antimalarial drugs, supporting global efforts toward malaria elimination.

2. Materials and Methods

Ligand Structure Preparation

Information regarding the IUPAC names of compounds derived from *Annona muricata* was sourced from the PubChem database (https://pubchem.ncbi.nlm.nih.gov). The ligand structures were initially constructed in 2D format using ChemBioDraw Ultra 16. These 2D structures were then converted into 3D models utilising the ChemBio3D Ultra 16 software. Subsequently, energy minimisation of the generated 3D models was performed using the Merck Molecular Force Field 94 (MMFF94) method in ChemDraw to optimise the ligand conformations.

Protein Structure Preparation

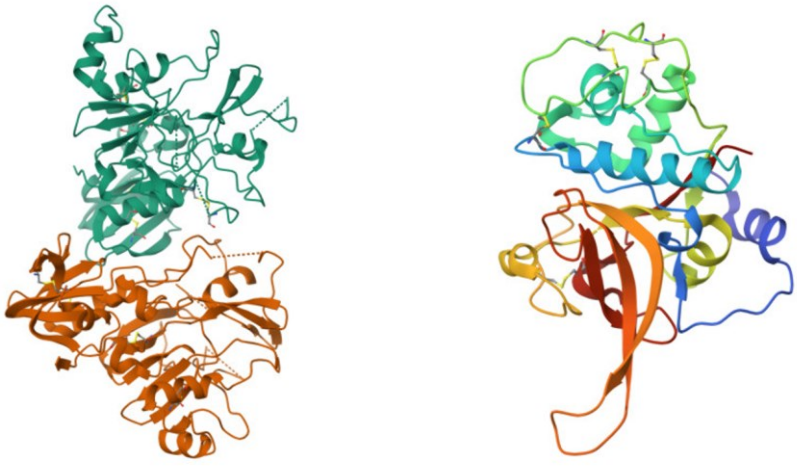
Two proteins, PfDHODH (PDB ID: 1Z40) and PfLDH (PDB ID: 2GHU), were selected for the analysis. The structural data for these proteins in PDB format were retrieved from the RCSB Macromolecular Structure Database (https://www.rcsb.org). The specific proteins obtained were PfDHODH (PDB ID: 1Z40) and PfLDH (PDB ID: 2GHU). A schematic representation of the crystal structures of both proteins is illustrated in Figure 1.

Molecular Docking

Molecular docking was performed using Molegro Virtual Docker (MVD) software. The prepared ligand compounds and the protein structures of PfDHODH and PfLDH were introduced into the software for docking analysis. Prior to docking, water molecules (H2O) present in the protein structures were removed to prevent interference during the docking process, ensuring that only the ligands interacted with the target proteins. The docking procedure involved cavity detection, validation of the docking method, and subsequent docking of the candidate compounds with the target receptors.

Prediction of Compound Toxicity

To evaluate the toxicity properties of the compounds, toxicity prediction was performed using



(a) (b)

Figure 1. Crystal structure of (a) PfDHOH and (b) PfDLH protein

Table 1. Validation Results of the Docking Method

Protein	Ligand	RMSD (Å)	Rerank Score (kcal/mol)
PfDHODH	Annonacin	0.0	-5.9
PfLDH	Annonacin	0.0	-6.0

Table 2. Molecular Docking Results and Amino Acid Interactions

Compound	Binding Affinity	Protein Target	Residual Amino Acid Interactions
Annonacin	-5.9 kcal/mol	PfDHODH	Hydrogen bonds: Asn 258, Gln 349, Ser 347 Van der Waals: Gln 352, Tyr 353, Glu 365, Asn 257, Glu 256, Pro 350, Asn 327, Arg 128, Asn 413, Lys 351, Tyr 397, Gly 396, Glu 343, Glu 336, Ala 346, Ser 345 Alkyl Pi-Alkyl: Lys 339, Leu 340, Lys 395, Pro 330, Phe 329
	-6.0 kcal/mol	PfLDH	Hydrogen bonds: Gln 36, Asn 16, Phe 17 Van der Waals: His 174, Cys 42, Trp 206, Asp 35, Lys 37, Asn 38, Trp 210, Glu 222, Asp 18, Glp 209, Ala 20, Tyr 198 Alkyl Pi-Alkyl: His 19, Ala 157, Lys 196

the ProTox-II web platform (https://tox-new.charite.de/protox_II/). The canonical SMILES representations of the compounds were employed to process and assess their toxicity profiles.

Pharmacokinetic analysis

Pharmacokinetic analysis was conducted using the SwissADME tool, available at (http://www.swissadme.ch/). The canonical SMILES of the active compounds were copied and input into the SwissADME input field. The analysis of pharmacokinetic parameters and bioavailability profiles was initiated by clicking the run button, allowing for a comprehensive evaluation.

3. Results

Molecular Docking

To validate the docking method, the native ligand was re-docked with the target proteins PfDHODH (PDB ID: 1Z40) and PfLDH (PDB ID: 2GHU). The validation process relied on the RMSD (Root Mean Square Deviation), which quantifies changes in ligand interactions with the protein's crystal structure before and after docking. A docking method is deemed valid if the RMSD value (Table 1) indicates accurate predictions. Lower RMSD values suggest more precise ligand positioning.

Docking Outcomes and Amino Acid Interactions

The docking analysis of PfDHODH (PDB ID: 1Z40) and PfLDH (PDB ID: 2GHU) with Annonacin revealed notable binding affinities of -5.9 kcal/mol and -6.0 kcal/mol, respectively (Table 1). These results indicate that Annonacin forms stable interactions with both proteins, underscoring its potential as an inhibitor.

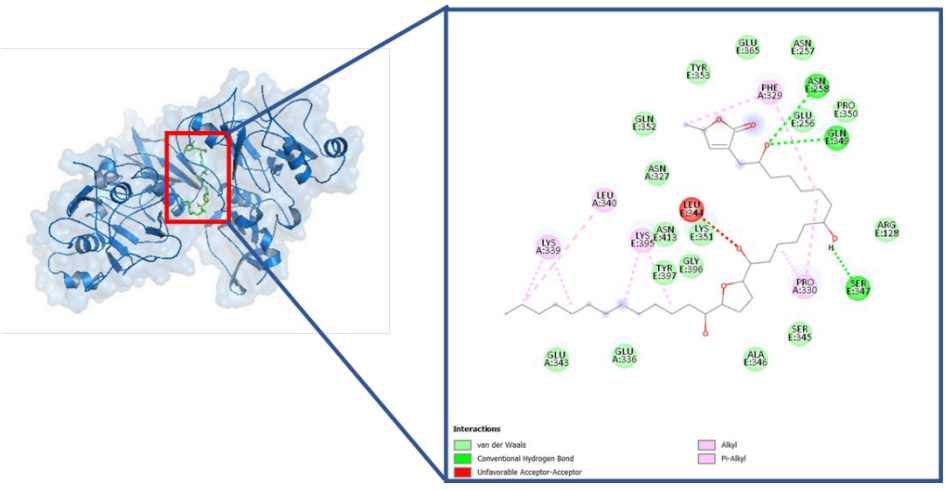


Figure 2. 3D visualization of the interaction between Annonacin and PfDHODH (1Z40)

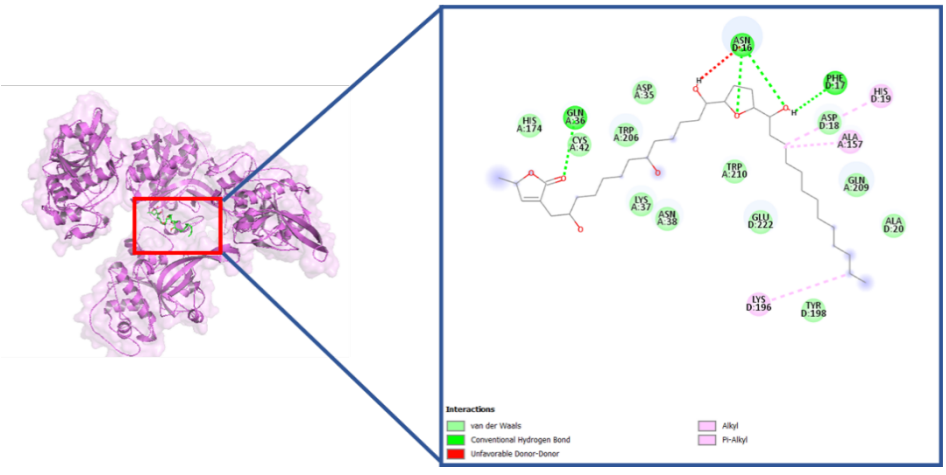


Figure 3. 3D visualisation of the interaction between annonacin and PfLDH (2GHU)

Table 3. Toxicity analysis summary

Compound	LD_{50}	Toxicity Class	Hepatotoxicity	Carcinogenicity	Mutagenicity	Cytotoxicity
Annonacin	400 mg/kg	Class IV	Inactive	Inactive	Inactive	Active

For PfDHODH, the binding affinity was -5.9 kcal/mol. This interaction is stabilised by multiple hydrogen bonds formed with key residues, including Asn 258, Gln 349, and Ser 347 (Figure 2). Additionally, several van der Waals interactions enhance the stability of the ligand-protein complex. Alkyl and Pi-Alkyl interactions with residues such as Lys 339 and Leu 340 further contribute to the overall stability (Table 2).

In the case of PfLDH, Annonacin exhibited a slightly stronger binding affinity of -6.0 kcal/mol. Key interactions include hydrogen bonds with Gln 36, Asn 16, and Phe 17, while van der Waals interactions with multiple amino acids, including His 174 and Trp 210, provide additional stabilisation (Figure 3). Furthermore, Alkyl and Pi-Alkyl interactions with residues like His 19 and Lys 196 also play significant roles in maintaining binding stability (Table 3).

Toxicity Prediction

Toxicity prediction for Annonacin was conducted using the ProTox-II tool, which revealed an LD_{50} of 400 mg/kg. According to the Globally Harmonised System (GHS) classification, substances with an LD_{50} range of 300 to 2000 mg/kg are categorised under Toxicity Class IV. This classification indicates that Annonacin is harmful if swallowed (Table 3). While it is less hazardous than substances classified in Classes I through III, it still presents a considerable risk if

Table 4. Pharmacokinetic properties of annonacin

Parameter	Annonacin
Molecular weight (g/mol)	596.88 g/mol
Hydrogen bond donors	4
Hydrogen bond acceptors	7
Rotatable bonds	26
Total polar surface area	116.45 Å²
Log P (iLOGP)	6.79
Log S (ESOL)	-7.13
GI Absorption	Low
Lipinski's (Ro5)	Yes, 1 violation
Bioavailability score	0.55

ingested in large quantities.

Furthermore, the toxicity analysis indicated that Annonacin is predicted to be non-carcinogenic, non-mutagenic, and non-hepatotoxic. However, it exhibits cytotoxic properties, suggesting caution is warranted. These findings imply that although Annonacin shows promise as a drug candidate, careful consideration of its dosage and formulation is essential to mitigate potential adverse effects. Ensuring an appropriate risk-benefit balance will be critical in advancing its development for therapeutic use.

Pharmacokinetic Analysis

The pharmacokinetic profile of Annonacin was evaluated using the SwissADME tool. The analysis indicated that Annonacin exhibits low gastrointestinal absorption and violates one of Lipinski's Rules of Five due to its molecular weight. Despite these challenges, the compound achieved a moderate bioavailability score of 0.55, suggesting that it can be absorbed to some extent when administered (Table 4).

However, Annonacin's pharmacokinetic profile, characterised by low gastrointestinal absorption and the violation of one of Lipinski's rules, presents significant hurdles for its use as an orally administered drug. The bioavailability radar further illustrated that Annonacin aligns with the optimal range for oral bioavailability in some parameters but not all (Figure 4).

Given the moderate bioavailability score coupled with low solubility, it is evident that formulation optimisation will be necessary to enhance Annonacin's pharmacokinetic properties. Improvements in its formulation could potentially increase the drug's absorption and overall efficacy in therapeutic settings.

4. Disscussion

This study aimed to investigate the potential of Annonacin, a bioactive compound derived from A. muricata (Wijaya *et al.,* 2021), as a candidate for antimalarial drug development. Molecular docking techniques were employed to assess the binding affinity

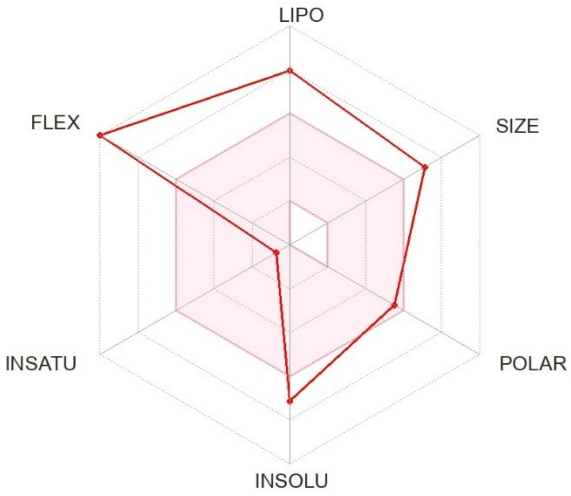


Figure 4. Bioavailability radar

of Annonacin with two critical malaria proteins: PfDHODH (PDB ID: 1Z40) and PfLDH (PDB ID: 2GHU). Additionally, the toxicity and pharmacokinetic properties of Annonacin were analysed to evaluate its viability as a therapeutic agent.

The molecular docking analysis revealed that Annonacin establishes multiple significant interactions with both PfDHODH and PfLDH. These interactions include hydrogen bonds, van der Waals forces, and Alkyl-Pi-Alkyl interactions, all of which are essential for securing the ligand within the enzymes' binding pockets (Niranjan *et al.,* 2021). Hydrogen bonds are particularly critical, as they provide strong and specific interactions that anchor Annonacin in the active sites of the enzymes. This anchoring is vital for maintaining the ligand's position, thereby enhancing its potential to effectively inhibit enzymatic function (Lin & MacKerell, 2017).

While van der Waals interactions are comparatively weaker, they play a crucial role in stabilising the ligand-protein complex by ensuring close contact between Annonacin and the surrounding amino acids in the binding pocket. These interactions maximise the likelihood of successful enzyme inhibition by keeping the ligand in proximity to the active site (Bitencourt-Ferreira *et al.,* 2019). Furthermore, Alkyl and Pi-Alkyl interactions reinforce the stability of Annonacin within the hydrophobic regions of the binding pocket. Such hydrophobic interactions are particularly significant in environments where water molecules are typically excluded, as in the enzyme's active site, thereby enhancing the ligand-protein complex's stability and resistance to dissociation (Elkahoui *et al.,* 2024).

The cumulative effect of these interactions is an increased residence time of Annonacin within

the enzyme's active site, reducing the likelihood of dissociation before exerting its inhibitory effects. This stability is essential for enhancing the effectiveness of enzyme inhibition, which is crucial for disrupting the biological pathways that the parasite relies upon for survival (Matthews *et al.,* 2020).

Furthermore, the docking results demonstrated notable binding affinities, with binding energies of -5.9 kcal/mol for PfDHODH and -6.0 kcal/mol for PfLDH. These values indicate that Annonacin forms stable complexes with both proteins, reinforcing its potential efficacy as an antimalarial compound. Given these findings, Annonacin emerges as a promising candidate for further development as an antimalarial agent, warranting additional studies to optimise its pharmacokinetic properties and therapeutic potential.

The docking results indicate that Annonacin effectively mimics the binding characteristics of the native ligands of PfDHODH and PfLDH, which are essential enzymes in the survival of the P. falciparum parasite (Hartuti *et al.,* 2021; Heikal *et al.,* 2023). For PfDHODH, Annonacin forms hydrogen bonds with critical residues, including Asn 258, Gln 349, and Ser 347. These interactions are vital for stabilising ligand binding within the enzyme's active site. The formation of these hydrogen bonds implies that Annonacin can competitively inhibit PfDHODH by occupying the same binding site as its native ligand. This competitive inhibition blocks the enzyme's role in the pyrimidine biosynthesis pathway, a process crucial for DNA replication and cell proliferation in the parasite (Owoloye, 2020).

Similarly, in PfLDH, Annonacin interacts with key residues, including Gln 36, Asn 16, and Phe 17. These residues are instrumental in the enzyme's activity, particularly within the glycolytic pathway, which serves as the primary source of ATP production for the parasite. By establishing stable interactions with these vital residues, Annonacin has the potential to disrupt the glycolytic process, effectively depriving the parasite of the energy necessary for survival and proliferation (Owoloye, 2020). Annonacin's ability to mimic the binding characteristics of native ligands in both enzymes suggests it could act as a potent inhibitor, targeting critical metabolic pathways essential to the malaria parasite and ultimately leading to its demise.

This dual mechanism of action, in which Annonacin inhibits both pyrimidine biosynthesis via PfDHODH and glycolysis via PfLDH, enhances its potential as a broad-spectrum antimalarial agent. By simultaneously targeting multiple crucial pathways, Annonacin increases the likelihood of overcoming drug resistance, which often develops when a single pathway is targeted for treatment (Shibeshi *et al.,* 2020). Thus, these docking results not only underscore the potential efficacy of Annonacin but also highlight its versatility in disrupting various essential functions within the malaria parasite.

In drug discovery, toxicity evaluation is paramount, as it determines a compound's safety and viability as a potential therapeutic agent. A favourable balance between efficacy and toxicity is essential; the compound must be potent enough to achieve its therapeutic effect while ensuring safety for human consumption. Therefore, the identification and evaluation of toxicity during the early stages of drug development are crucial to prevent promising candidates from posing significant risks to patients. Compounds associated with high toxicity are often excluded from further development or require substantial modifications to mitigate harmful effects (Tran *et al.,* 2023). Careful assessment of Annonacin's toxicity and its pharmacokinetic profile will be essential to determine its suitability as a safe and effective antimalarial therapy.

The toxicity analysis of Annonacin revealed that although the compound is non-carcinogenic, non-mutagenic, and non-hepatotoxic, it exhibits cytotoxic properties. While Annonacin does not pose a risk for cancer, genetic mutations, or liver damage, its cytotoxicity raises concerns regarding its safety profile. The compound's LD_{50} value, indicative of acute toxicity, was determined to be 400 mg/kg, placing Annonacin in Toxicity Class IV. This classification suggests that Annonacin could be harmful if ingested in large amounts, presenting a significant challenge for its development as an oral drug (Bhat & Chatterjee, 2021). Although it is less toxic than compounds in higher classes, the risk of harm at doses encountered during treatment could limit its therapeutic window—the range between an effective dose and a toxic dose—making safe administration more difficult.

The cytotoxic effects of Annonacin necessitate careful management, as they can cause unintended damage to healthy cells, potentially leading to side effects or toxicity in patients (Zhang, 2018). Addressing this issue during the drug development process is essential, either through stringent dosage control to minimise exposure to toxic levels or by making structural modifications to reduce inherent cytotoxicity while preserving therapeutic efficacy (Sun *et al.,* 2022). Failure to manage these risks adequately could render Annonacin unsuitable as a treatment option by causing adverse effects that outweigh its potential benefits.

From a pharmacokinetic perspective, Annonacin presents several challenges that could impact its effectiveness as an oral drug. A primary concern is its

low gastrointestinal absorption, indicating that only a fraction of the orally administered dose reaches systemic circulation. This limitation significantly reduces the compound's bioavailability, implying that less of the active drug will be available to exert therapeutic effects. Low bioavailability often necessitates higher doses to achieve the desired effect, which is typically constrained by toxicity concerns, as elevated doses increase the risk of adverse effects (Vinarov *et al.,* 2021).

Additionally, Annonacin violates one of Lipinski's Rules of Five, which are guidelines for assessing the drug-likeness of compounds based on molecular properties, including molecular weight, lipophilicity, and hydrogen bond donors and acceptors (Ivanovic *et al.,* 2020). This violation, attributed to Annonacin's relatively high molecular weight, could further limit its oral bioavailability. High molecular weight often correlates with poor absorption and permeability, making it more challenging for the drug to cross biological membranes and reach target sites within the body. Poor absorption and low permeability can lead to insufficient drug levels in the bloodstream, complicating its therapeutic use (Yang *et al.,* 2017).

Despite these challenges, Annonacin's bioavailability score of 0.55 indicates some potential for systemic absorption, although it falls on the lower end of the range for orally active drugs. While Annonacin may reach systemic circulation to an extent, its pharmacokinetic properties demand significant optimization for it to become a viable candidate for oral administration. Strategies for optimisation might involve structural modifications aimed at reducing molecular weight, increasing lipophilicity, or enhancing membrane permeability. Such changes could improve the compound's absorption profile, ensuring more consistent and effective delivery to its target site.

The bioavailability radar tool, used to assess a compound's suitability as an oral drug, supports this assessment by demonstrating that Annonacin falls within the optimal range for certain pharmacokinetic parameters, while also highlighting areas for improvement. Specifically, the radar indicates suboptimal solubility and permeability, critical factors for efficient drug absorption and therapeutic concentration in the body (Liu *et al.,* 2024). Enhancing these properties could significantly improve Annonacin's drug-like characteristics, making it a more feasible candidate for development.

However, it is essential to recognise that even if pharmacokinetic improvements are achieved, they may not suffice to address the challenges posed by Annonacin's toxicity profile. The compound's cytotoxicity, alongside a narrow therapeutic window—where the difference between effective and toxic doses is minimal—introduces significant risks. Without effectively addressing these issues, pharmacokinetic enhancements may not ensure the compound's safety and efficacy as an antimalarial drug. Therefore, efforts to improve Annonacin's pharmacokinetics must be coupled with strategies to mitigate cytotoxic effects and widen its therapeutic window. This may involve designing analogues with reduced toxicity or developing targeted delivery systems that direct the drug to the site of infection while minimising exposure to healthy tissues.

Overall, while Annonacin shows considerable promise as an antimalarial agent, its development is fraught with challenges that demand careful consideration. The compound's pharmacokinetic limitations, combined with toxicity concerns, highlight the need for a multifaceted approach in optimisation efforts. Balancing efficacy with safety will be crucial in determining whether Annonacin can be successfully developed into a safe and effective treatment for malaria.

5. Conclusions

Annonacin shows promise as an antimalarial agent, but its development is hindered by low gastrointestinal absorption, high molecular weight, and cytotoxicity, which limit its effectiveness and safety as an oral drug. Addressing these challenges requires optimising its pharmacokinetics and reducing its toxicity.

Conflict of interest

All authors have no conflict of interest in this article.

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