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# A Decade of In Silico Drug Discovery for Naegleria fowleri: Progress, Challenges, and Future Directions

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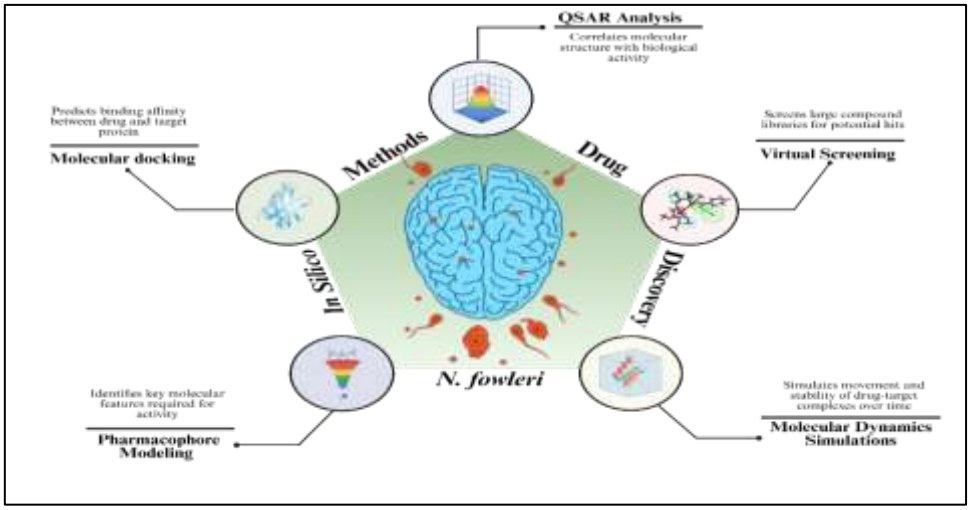
**Abstract**
The brain-eating amoeba, more formally known as *Naegleria fowleri* (*N. fowleri*), is the main cause of Primary Amoebic Meningoencephalitis (PAM), a rare albeit universally fatal infection. The rapid infectivity in conjunction with its guaranteed lethality alongside its limited treatment options should be the proverbial klaxon that signals for new therapeutic techniques. In recent years, *in silico* drug discovery, which includes computational techniques like molecular docking, molecular dynamics simulations, pharmacophore modelling, virtual screening, and QSAR analysis, has emerged as a powerful tool to identify potential treatments for PAM. Researchers have expended no small number of cognitive resources in fully maximising these methods to identify promising drug candidates, including both novel molecules and repurposed FDA-approved drugs. These efforts target essential proteins and metabolic pathways in *N. fowleri*, with structural bioinformatics helping to identify key drug targets such as proteases, kinases, and metabolic enzymes. The integration of AI and machine learning has further optimised the drug discovery process, enhancing prediction accuracy and compound optimisation. Additionally, diving into green chemistry with the goal of minimising toxicity and environmental impact. However, challenges remain. These include a shortfall of detailed protein structures, limited experimental validation, gaps between computational predictions, and clinical effectiveness. Progressing forward, researchers are encouraged to integrate multi-omics data, expand screening libraries, and improve predictive models to accelerate the discovery of eco-friendly and effective therapies. Combining bioinformatics, chemistry, and experimental biology is paramount for translating. In accordance with PRISMA 2020 guidelines, this study reviews *in silico* studies published between 2015 and 2025 using search engines like PubMed and Google Scholar. A multidisciplinary approach combining bioinformatics, chemistry, and experimental biology is paramount to translate computational leads into clinically viable treatments and experimental workflows for neglected protozoan diseases.

**Keywords**: *Naegleria fowleri*; primary amoebic meningoencephalitis (PAM); *in silico* drug discovery; molecular docking; virtual screening; AI learning



## Introduction

The free-living thermophilic protozoan *Naegleria fowleri*, also known as the "brain-eating amoeba," is found primarily in warm freshwater habitats such as lakes, rivers, hot springs, and swimming pools with insufficient chlorination.[1] There are three different stages of *N. fowleri*: the trophozoite, which is the active feeding stage; the flagellate, which has flagella for movement; and the cyst, which is a resilient and dormant form that can withstand adversity.[2] Primary Amoebic Meningoencephalitis (PAM) is an uncommon but deadly infection that develops when contaminated water enters the body through the nasal passages and then invades the central nervous system (CNS) via the olfactory nerve. Once established in the brain, it elicits a rapidly progressive and almost universally fatal infection.

According to the epidemiological data, despite PAM's extreme rarity, the death rate exceeds 97%, with only a small number of survivors reported globally. In the US, for instance, fewer than 10 instances are recorded each year, and only four of the estimated 164 confirmed cases survived between 1962 and 2023. Approximately 98% of those affected die within one to two weeks of their first symptoms, making the death rate even more concerning on a global scale.[3] Despite improvements in our knowledge of this deadly organism, clinical results are still dismal for a number of reasons, such as delayed diagnosis, the disease's quick progression, and the ineffectiveness of the available treatments.[4] This underscores the critical need for increased awareness and preventive measures in environments where *N. fowleri* is prevalent.

Current therapeutic options for PAM are critically limited and face numerous challenges that impede effective intervention. Presently, amphotericin B, the mainstay of treatment, is typically combined with regimens like miltefosine and rifampin. Even with this combination, a startlingly high percentage of PAM cases still end in death since these drugs are extremely toxic and cause electrolyte imbalances and nephrotoxicity.[5] Moreover, these medications' poor penetration of the blood-brain barrier (BBB), a protective membrane that restricts the entry of chemicals into the brain, further reduces their efficacy.[4] The medications' general toxicity and inconsistent effectiveness against the amoeba, along with their limited penetration, present major obstacles to effective treatment outcomes.

Apart from the poor efficacy of medications, the rapid clinical course of PAM and the difficulties in detecting it exacerbate the dire prognosis. Traditional drug discovery procedures are also often time-consuming and costly, making it challenging to develop effective treatments. These limitations underscore the pressing necessity to link the large medical need for efficient PAM therapies with the creation of novel, secure, and potent therapeutic alternatives.

In recent years, *in silico* drug discovery, which includes computational techniques like molecular docking, molecular dynamics simulations, pharmacophore modelling, virtual screening, and QSAR analysis, has emerged as a powerful tool to identify potential treatments for PAM, an area that contributes to more extensive current developments in treatments for severe illnesses based on natural chemicals and nanoparticles.[1], [2], [6] These approaches not only speed up the search for new compounds but also make it easier to repurpose existing drugs by assessing how well FDA-approved compounds work against essential *N. fowleri* targets.[7], [8] Developments in machine learning and artificial intelligence have also increased the prediction capacity of computational models, improving toxicity profiling and lead optimization. [5] The use of *in silico* techniques reduces the need for lengthy laboratory experiments, which lessens the impact on the environment, chemical waste, and resource consumption. By encouraging more environmentally friendly and sustainable methods of drug development, this approach supports Sustainable Development Goal 12 of the UN, which is centered on responsible production and consumption. Furthermore, computational methods provide quick iterations and economical compound screening, which supports efficient and ecologically friendly development procedures.

The purpose of this systematic review is to provide an overview of the development of computational drug discovery for *N. fowleri* and PAM from 2015 to 2025, emphasizing important targets, approaches, and promising candidates. It also mentions present constraints along with examining potential future paths like multi-omics integration, larger screening libraries, and environmentally friendly medication creation. This study ultimately emphasizes a multidisciplinary approach by combining bioinformatics, chemistry, and experimental biology to translate computational leads into clinically viable treatments.

## Methods and Methodology

### A. Literature Search Strategy

A comprehensive and methodical search for relevant literature was conducted utilizing major academic databases such as Web of Science, PubMed, Scopus, and Google Scholar, covering the duration from 2015 to 2025. The objective was to find a large number of pertinent papers about computational drug development that targeted *N. fowleri*. Boolean operators were deliberately used to optimize coverage, and a range of targeted keywords were used to increase the search's comprehensiveness. These include "*Naegleria fowleri*," "*in silico* drug discovery," "molecular docking," "molecular dynamics simulations," "pharmacophore modeling," "Quantitative Structure-Activity Relationship (QSAR) analysis," "AI-based drug screening," and "virtual screening techniques." Additionally, the reference lists of chosen papers were further examined in order to find other pertinent works.

### B. Data Selection

The resulting publications are then filtered for legitimate selection, excluding non-peer-reviewed and irrelevant papers related to the topic of free-living amoeba. Following another screening for eligibility, the chosen articles were eliminated for high quality if they did not contain *N. fowleri* and *in silico* results, had inaccessible protocols, or several other reasons. From each chosen paper, certain details were taken out, such as the computational methods applied, the target proteins or pathways examined, and the types of chemicals assessed. All selected papers were exported into Zotero and duplicated records were removed automatically.

### C. Data Review

To improve the process's objectivity, the papers were evaluated separately by a number of reviewers. In order to reduce the possibility of bias, disagreements were settled by debate and agreement. A strong dataset was created by this methodical approach, which also offered insightful information about how well *in silico* drugs may suppress *N. fowleri* vitality and help in the future therapeutic advancements.

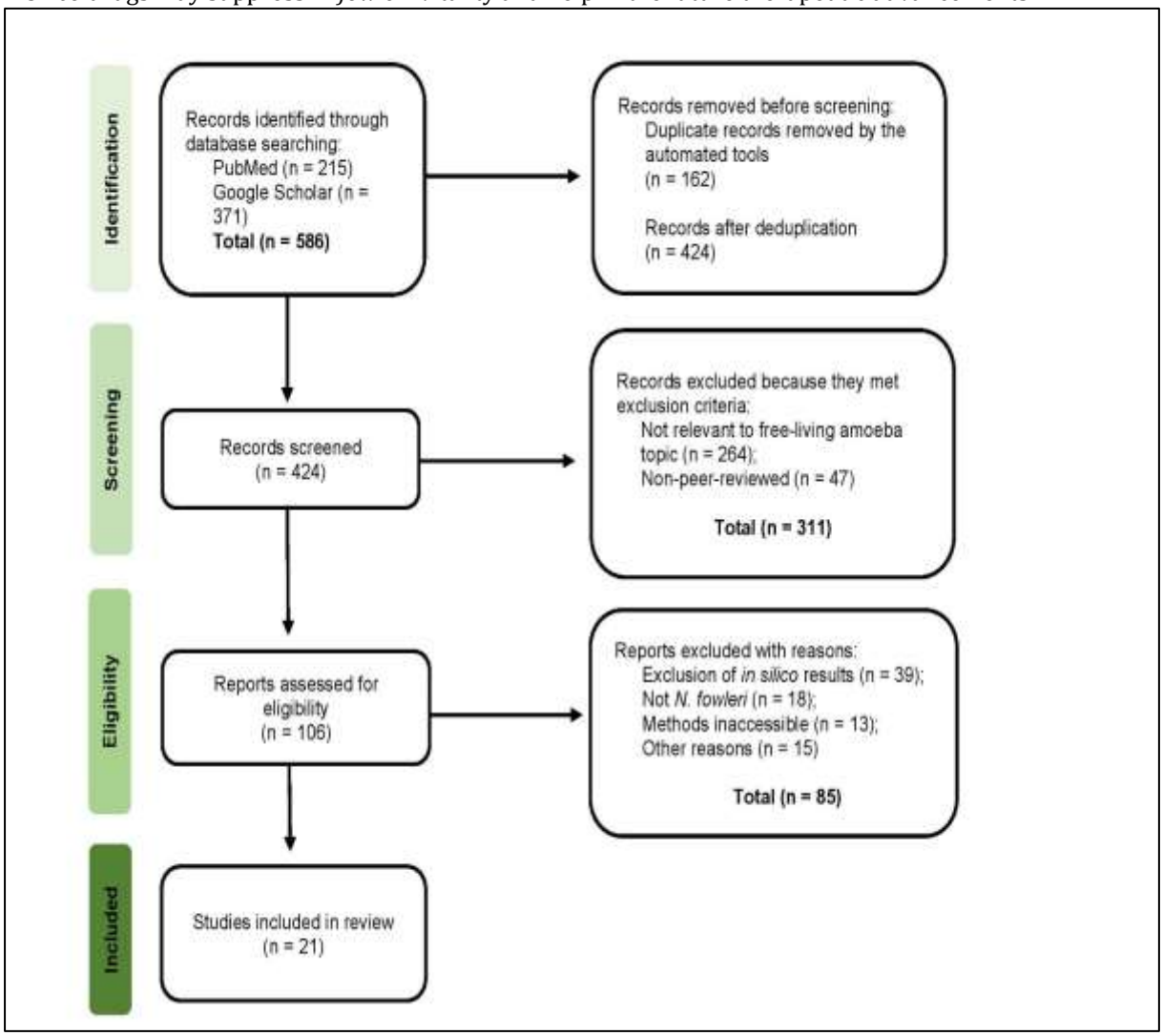



**Fig. 1: Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) flow chart outlining the methodology and the number of citations (n) obtained at each stage for this review.**

## Results

A series of experimental and computational investigations conducted between 2015 and 2025 have systematically advanced the knowledge and targeting of *N. fowleri* by using a variety of molecular targets and *in silico* techniques. [9], [10] Applying techniques like *in silico* microRNA identification, docking, and X-ray crystallography, early studies focused on regulatory miRNA identification, heat shock proteins, and glucokinase in order to provide fundamental structural insights for drug design [11,12]. Subsequent research included long-read genome assembly, enzymology, and homology modelling, which enhanced target validation and guided the development of inhibitors with a focus on low toxicity profiles. [12,13] The integration of chemoinformatics, virtual screening, and reverse vaccinology enabled the prioritization of natural product inhibitors and multi-epitope vaccine candidates. [14–17] To improve ligand binding accuracy and target specificity [18,19], more recent methods have made use of metabolomics-guided

validation. [17,18,20] Numerous flavonoids and phytochemicals showed encouraging inhibitory activity against important enzymes, such as CYP51 and SAHH, which was corroborated by thorough structure-activity relationship investigations and molecular dynamics simulations. [21–24] **Table 1** presents a comprehensive review of the important discoveries and evolving computational tools that support ongoing efforts to develop preventive and therapeutic approaches against *N. fowleri*.

**Table 1: Summary of *in silico* techniques used for drug target identification and validation in *N. fowleri* between 2015 and 2025.**

| Year | Target | *In silico* methods | Key Findings | Ref |
|---|---|---|---|---|
| **2015** | Regulatory microRNAs | In-silico miRNA discovery | Predicted *N. fowleri* miRNAs suggest novel regulatory/druggable axes. | [25] |
| **2017** | Heat shock protein 70 (Hsp70) | Docking (phytochemical panel) | Early ligandability signals for Hsp70 in *N. fowleri*. | [26] |
| **2019** | Sterol Δ8–Δ7 isomerase (ERG2) | Homology modeling; docking; phenotypic triage | Docking triage prioritized 4 selective, low-toxicity amoebicidal hits. | [27] |
| | Glucokinase (NfGlck/NfHK) | Target validation; enzymology | Validated glycolytic node; structure informs inhibitor design. | [11] |
| | | X-ray crystallography (PDB 6DA0) | Crystal structure published to support future docking and design | |
| | Genomics resource | Long-read assembly (ONT) | Improved assembly quality supports target mining and annotation | [12] |
| **2020** | Protein farnesyltransferase axis | Cheminformatics | Prenylation inhibitors highlighted as potential anti-amoebic avenue. | [28] |
| **2021** | Structural resource (SSGCID) | Structural genomics; crystallography | 19 unique *N. fowleri* protein structures deposited to enable SBDD. | [13] |
| **2022** | Sterol 24-C methyltransferase (SMT) | Virtual screening (alkaloids/terpenoids); ADMET; MD | Natural products prioritized as SMT inhibitors with stable MD. | [14] |
| **2023** | Lanosterol 14α-demethylase (CYP51) | Cheminformatics; docking; lead optimization | Miconazole-like scaffold produced multiple sub-10 μM inhibitors. | [21] |
| | Multi-epitope vaccine (NaeVac) | Reverse vaccinology; docking (TLRs); MD | Stable TLR binding and favorable immunoinformatics indices. | [15] |
| | CYP51 (Indonesian medicinal plants) | QSAR → docking → MD; ADMET filters | Six phytochemicals passed ADMET and showed stable 1–3 Å RMSF trajectories. | [16] |
| | Glucokinase (NfGlck) | Structure-guided KTGS + computational analysis | KTGS hits informed by structural analysis; platform for future design. | [18] |
| | Karachi NF001 strain proteins | Homology modeling; active-site mapping; docking; MD | Stability supported for top ligands against selected NF001 proteins. | [2] |
| **2024** | Enolase (NfENO) | Docking; metabolomics-guided validation | HEX docks to NfENO and shows potent whole-cell activity. | [19] |
| | Vaccine candidate peptides | Immunoinformatic | Diagnostic/vaccine epitopes proposed with high predicted antigenicity. | [17] |
| | In-silico vaccine design | | High-scoring multi-epitope construct proposed for *N. fowleri* | |
| | CYP51 (flavonoids) | Docking against NfCYP51 | Several flavonoids scored favorably vs. voriconazole benchmark. | [22] |
| **2025** | Tubulin (colchicine site) | Homology modeling; docking | Colchicine/thiocolchicine rank highly but host–pathogen selectivity is a concern. | [7] |

| | | | | |
|---|---|---|---|---|
| | S-adenosyl-L-homocysteine hydrolase (SAHH) | Docking; MD (Asteraceae library) | Several phytochemicals predicted to stably bind NfSAHH active site. | [23] |
| | SAHH | Structure-based screen; docking; MD (cotton metabolites) | Heliocide-like candidates prioritized for experimental follow-up. | [24] |
| | CYP51 (iridoids) | Docking; MD; MM/PBSA; DFT | Two iridoids out-scored voriconazole and remained stable in MD. | [22] |
| | Serine Carboxypeptidase, Rab GTPase (malabaricones) | Docking; MD; MM/PBSA–GBSA; ADMET | With the highest amoebicidal efficacy, malabaricone B had the strongest binding and stability; all other drugs exhibited low toxicity and good absorption. | [29] |

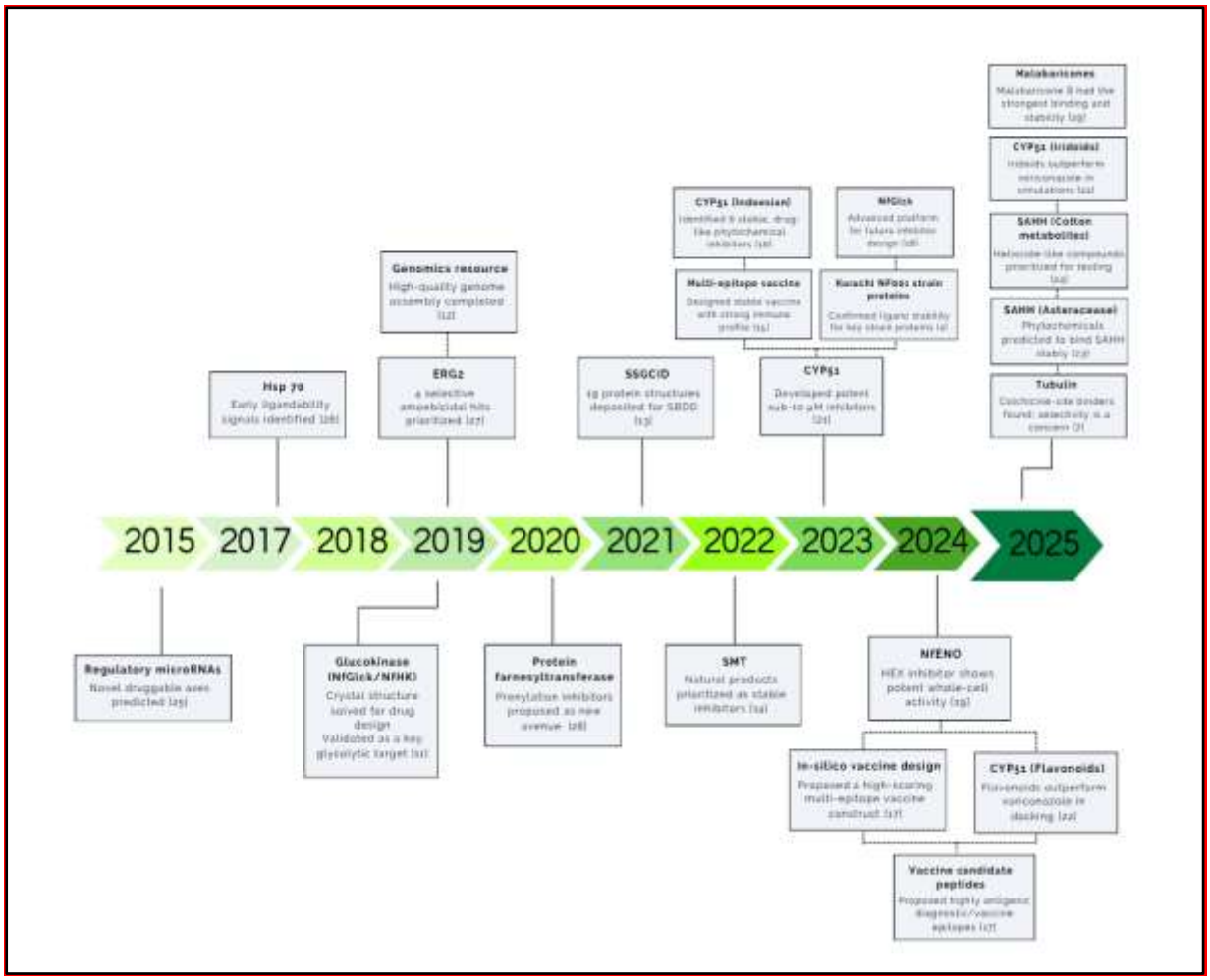



**Fig. 2: A decade of key milestones in *Naegleria fowleri* drug discovery. This timeline illustrates the progression of major targets, resources, and findings from 2015 to 2025.**

highlighting the shift from foundational genomics and structural work to advanced inhibitor design and vaccine development. Key achievements are plotted above the year of their publication, with citation numbers in brackets.

## Discussion

In warm freshwater habitats like lakes, rivers, and hot springs, *N. folweri* often flourishes and presents major health hazards, particularly in places where recreational water activities are common. [30], [9] According to recent studies, rising global temperatures and changing climatic patterns are contributing to the increased persistence of *N. fowleri* in the environment, as well as its spread into new geographic regions. [31] Higher water temperatures increase the amount of time that this thermophilic organism can exist in these habitats, increasing the danger of exposure to humans. [9] This is especially alarming as temperate

locations that were previously thought to be at low risk for *N. fowleri* infections may potentially be more vulnerable to outbreaks. [9], [32] The ramifications of these discoveries have prompted more thorough research into key molecular targets, directing the creation of preventative and therapeutic approaches. [10] One of the most important therapeutic approaches for treating *N. fowleri* is sterol biosynthesis. Lanosterol 14α-demethylase, also known as CYP51, and sterol Δ8−Δ7 isomerase, also known as ERG2, are two of the most promising targets in this biosynthesis process. [10], [14]. Shi et al. [27]'s research used a multimodal strategy that includes phenotypic triage, molecular docking, and homology modeling. Four scaffolds formed from phytochemicals were identified as having low projected toxicity in mammalian cells and selective efficacy against amoebae as a result of this meticulous research. This innovative study offers strong computational support for the possibility of using *N. fowleri*'s sterol enzymes for medicinal purposes. Using molecular dynamics simulations, ADMET analysis, and virtual screening of alkaloids and terpenoids, Abraham et al. [14] have more recently prioritized natural product inhibitors of SMT with attractive stability profiles. These *N. fowleri*-specific results are consistent with a larger eukaryotic precedent: azole antifungals like fluconazole and posaconazole have been shown to block lanosterol 14α-demethylase (CYP51) in fungi [28], and both azole and non-azole CYP51 inhibitors have shown *in vivo* efficacy in protozoa like Trypanosoma cruzi and Leishmania donovani [13]. But in all of these systems, cross-reactivity against human sterol enzymes and resistance resulting from efflux pumps and target-site mutations continue to be major challenges [21]. Experimental research has demonstrated that natural terpenes can effectively reduce the cytopathogenicity of N. fowleri, further supporting the potential of terpenes as anti-amoebic drugs. [33]

Glucokinase (NfGlck) and enolase (NfENO) are tractable targets for glycolysis, which has become a major metabolic weakness in Naegleria fowleri [9], [18], [20]. The first high-resolution understanding of the amoeba's glycolytic machinery was given by the enzymatic and structural characterisation of NfGlck in 2019, which also confirmed glucokinase as a crucial node for parasite survival and laid the groundwork for the development of inhibitors [11]. Building on this, Kassu et al. [18] showed how structural insights might lead to logical optimization of glycolytic inhibitors by using structure-guided kinetic target-guided synthesis (KTGS) and computational analysis to find small-molecule hits. This focus was recently expanded to enolase by Milanes and colleagues [19], who used docking and metabolomics-guided validation to demonstrate that several inhibitors not only exhibited strong in silico binding but also disrupted parasite metabolism in phenotypic assays. This is one of the most lucid examples of computational predictions being translated into experimental activity in *N. fowleri*. In Leishmania, enolase also serves as a surface antigen, indicating dual potential as a drug and vaccine target [15], while in malaria, enolase inhibition decreases parasite viability but raises concerns about erythrocyte toxicity [21]. These findings are consistent with more general lessons learned from other parasites. All of these investigations point to glycolytic enzymes as potential therapeutic targets for *N. fowleri*, but they also stress the necessity of methods that optimize parasite selectivity in order to reduce the danger of host damage.

In *N. fowleri*, tubulin is a potential therapeutic target since *in silico* studies indicate that tubulin binds well to colchicine-site ligands [13]. Similar methods have shown that microtubule-disrupting substances like benzimidazoles can hinder the growth of parasites in other protozoa, such as Leishmania and Trypanosoma [16]. However, because inhibitors frequently involve large cytotoxicity risks, the great degree of tubulin conservation between parasites and hosts presents a constant difficulty. Comparative binding-site mapping of tubulin isotypes can reveal small structural changes that could be used to improve parasite selectivity, according to insights from comparable kinetoplastid systems [18]. Similar structure-guided approaches will be essential for designing tubulin-targeted inhibitors for *N. fowleri* that optimize antiparasitic efficacy while reducing host toxicity.

Computational methods like end-point molecular mechanics, alchemical methods and ensemble methods utilizing physics-based calculation allows not only for high-throughput chemoinformatics approaches [34] but also considerably lowers the costs of resources for the discovery and development of antiparasitic and antiamoebic drugs, allowing for a more sustainable and environmental-friendly pipeline [35]. And with the advancement of machine learning and deep learning algorithms, data driven approaches have improved the accuracy for drug discovery [36]. Model-based ADMET models have further contributed, allowing researchers to further refine and improve leads for more desirable properties and diminish toxicophores in drug leads [37].

Protozoan parasites undergo fatal metabolic collapse when S-adenosylhomocysteine hydrolase (SAHH) is inhibited. SAHH is necessary for maintaining methylation homeostasis. This vulnerability has been confirmed in Trypanosoma brucei and Leishmania major [20]. According to computational studies, phytochemicals in *N. fowleri* have a promising affinity for binding SAHH [23]. If parasite-selective scaffolds are not prioritized, host off-target effects are likely to occur. To reduce host toxicity while maintaining antiparasitic action, comparative and structure-guided modeling techniques will be crucial.

Beyond enzymatic targets, epitope-based vaccine design offers a complementary approach for *N. fowleri*. As *in silico* designs have advanced to experimental validation in leishmaniasis and malaria, computationally predicted multi-epitope structures [15,17] represent effective approaches [19]. Wet-lab immunology and

bioinformatic pipeline integration allows for the logical prioritizing of antigenic areas, minimizing experimental burden and directing the creation of vaccines that can elicit protective immune responses.
Collectively, these studies expose several structural and metabolic weaknesses in N. fowleri, such as tubulin, SAHH, glycolysis, and sterol biosynthesis, while also emphasizing the potential of immunoinformatics in vaccine development. For all targets, striking a balance between potency and host selectivity is still crucial, and turning these discoveries into successful treatments will require combining computational predictions with experimental validation.

## Conclusion

This systematic review focuses on ten years of notable progress in the use of *in silico* drug discovery approaches aimed at combating *N. fowleri*. After starting with fundamental research on target prediction and structural analysis, the area of study has advanced to incorporate multi-epitope vaccine design and AI-driven predictions. Finding and confirming a number of important therapeutic targets has been made possible in large part by these computational techniques. Key vulnerabilities in the biology of *N. fowleri* have been meticulously characterized, including enzymes involved in the sterol biosynthesis pathway (CYP51, ERG2, SMT), central glycolytic enzymes (glucokinase, enolase), and essential proteins like tubulin and SAHH. The effective translation of computational predictions into actual results, as evidenced by the identification of compounds with strong whole-cell action and sub-μM inhibitors, highlights the growing dependability and efficiency of in silico methods. Furthermore, the field now includes the complex *in silico* design of multi-epitope vaccine candidates, which offers a promising preventive approach, in addition to small drugs.
However, there are still many obstacles to overcome. Significant barriers to therapeutic application include the absence of amoeba-specific, high-resolution protein structures, the necessity of parasite selectivity to prevent host toxicity, and the indispensable need for reliable experimental and phenotypic confirmation. Even while the use of potent technologies like AlphaFold is revolutionary, knowing the structure is only the beginning. Identifying key residues contributing to the target's activity is crucial to precisely target a binding site; physical and ML-based models need to be carefully improved to guarantee precise drug-binding predictions.
Future anti-*N. fowleri* drug discovery relies on expanding screening libraries to include a variety of natural product repositories, repurposing drug databases, and further integrating multi-omics data. Enhancing predictions about toxicity, efficacy, and blood-brain barrier permeability will also require ongoing improvement of AI and machine learning models. In the end, combating this underappreciated tropical illness will require persistent, interdisciplinary cooperation between experimental biologists, medicinal chemists, and computer scientists to close the gap between promising in silico leads and life-saving treatments. In addition to illuminating the way forward, the foundational work conducted between 2015 and 2025 changed the outlook for PAM from one of probable death to one of genuine hope.

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