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Analysis of two screens reveals a correlation between antiamoebic and anti-tubulin activities of phenothiazine and triphenylethylene derivatives

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Naegleria fowleri (*N.f.*), commonly referred to as the "brain-eating amoeba", is a free-living amoeboflagellate excavate capable to cause primary amoebic meningoencephalitis (PAM)—a rapidly progressing and typically fatal brain infection. Current treatment options are limited, poorly effective, and highly toxic, underscoring the urgent need for novel therapeutics. In this study, we explore the potential of repurposing FDA-approved microtubule-targeting agents (MTAs) for anti-*N.f.* therapy. By performing a comparative analysis of two large-scale drug screens—one assessing anti-amoebic activity and the other evaluating effects on tubulin polymerization—we identify strong correlations between microtubule disruption and amoebic growth inhibition. Notably, we highlight three major drug families (triphenylethylene, phenothiazine, and miconazole derivatives) and describe how their anti-amoebic effects relate to their MTA activity. In particular, triphenylethylene and phenothiazine compounds demonstrate a high positive correlation between tubulin polymerization inhibition and *N.f.* suppression, suggesting a shared molecular mechanism. Furthermore, we identify potent MTAs such as ebselen and auranofin—both capable of crossing the blood–brain barrier—as promising candidates for repurposing. These findings demonstrate the value of MTA-based screening in anti-amoebic drug discovery and point toward new therapeutic avenues for treating this devastating disease.

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1. Introduction

Naegleria fowleri (*N.f.*), commonly known as the "brain-eating amoeba",[1] is a free-living amoeboflagellate excavate (amoeboid) and the most pathogenic species in *Percolozoa* phylum. It causes primary amoebic meningoencephalitis (PAM), a rare but almost always fatal brain infection. The protozoan is found in warm freshwater environments and may invade nasal epithelial cells of humans by entering through the nasal passages during water activities like swimming. After invading the brain *via* the olfactory nerves, *N. fowleri* rapidly proliferates, causing severe inflammation and death in over 97% of cases, often within two weeks.

Current treatment options are extremely limited. Until recently, the treatment choice was empirical and included triazole derivative fluconazole,[2] amphotericin B, rifampicin and azithromycin.[3] Amphotericin B, the most often used drug, shows poor efficacy and severe side effects, particularly nephrotoxicity. Moreover, its ability to cross the blood–brain barrier (BBB) is limited, requiring high doses that increase toxicity. Despite very intensive treatment, the survival rate is extremely low and does not exceed 5% in diagnosed cases.

Recently, two repurposed drugs showed promising results in the treatment of "brain-eating amoebae", both with not clearly described mechanisms of action. One is a very ancient oxyquinoline antibacterial drug nitroxolin[4] that showed promising results in the treatment of Balamuthia brain infection.[5] Another one is miltefosine, a phospholipid group alkylphosphocholine used for leishmaniasis treatment[6] and associated with recovery from *N.f.* encephalitis.[7] Since there, CDC clinical care recommendation for *N.f.* infection include both molecules[8] (consulted July, 01, 2025). Early diagnosis is critical for survival, but PAM is often misdiagnosed as bacterial or viral meningitis. Consequently, there is an urgent need for more effective and fast-acting drugs against *N.f.*

Developing new drugs against PAM is especially challenging due to the rarity of cases, limited knowledge of the pathogen, and the lack of suitable high-throughput screening (HTS) methods. Traditional *in vitro* assays for *N.f.* are slow, labor-intensive, and not adapted to modern drug discovery pipelines. To address these issues, new HTS *in vitro* assays using *Naegleria gruberi* (*N.g.*), a non-pathogenic but closely related species, as a model have been developed and validated.[9] In this study, authors focused on drug

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repositioning—the strategy of identifying new uses for existing drugs—to accelerate the discovery of anti-*Naegleria* agents. They screened a library of 1175 FDA-approved drugs and measured their inhibitory activity at a concentration of 10 μM. While the study yielded promising results and identified several candidates for drug repurposing, the exact molecular mechanisms of action likely differ among compounds with distinct chemical scaffolds. Therefore, further rational optimization of these drug candidates requires a better understanding of their specific molecular targets.

In our recent study we screened a library of 1520 FDA-approved drugs to evaluate their anti-tubulin activity.[10] Microtubule-targeting agents (MTAs) have been successfully used in anticancer, antifungal, and antiparasitic therapies, as they disrupt tubulin—a key component involved in essential cellular processes such as motility, division, and intracellular transport. Motivated by results of these two screenings, we performed a comparative analysis, aiming to identify MTAs with potential anti-*N.f.* activity.

2. Methods

Screening assay datasets were obtained from SI[9] or directly from the authors upon request.[10] Correlation between inhibition of *Neisseria gonorrhoeae* growth and the change in polymerization temperature (ΔT_{poly}) was analyzed in OriginPro (OriginLab).

3. Drug families with strong correlation of MTA and antiamoebic activities

We conducted a comparative analysis of two screenings from FDA-approved drug libraries. In the first study, the authors evaluated the efficacy of drugs against the amoeba *N.g.*,[9] while the second focused on identifying novel microtubule-targeting agents (MTAs) by assessing the impact of these drugs on tubulin polymerization (Fig. 1A), measured as the change in polymerization temperature, ΔT_{poly} (Fig. 1B).[10]

Out of the screened compounds, 839 drugs were common to both libraries. Our comparison revealed that approximately 15% of the hits identified in the MTA screening showed more than 40% inhibition of amoebic growth (Fig. 1C). This suggests that integrating nanoDSF-based MTA screening into anti-amoebic drug discovery could significantly enhance the accuracy of identifying true positive hits. Indeed, the true positive rate increased from 8% to 40% when incorporating MTA information (Fig. 1D). Even higher accuracy could potentially be achieved in future studies by using *N.f.*-derived tubulin directly in the screening. Indeed, only 85% of homology between *N.f.* and *Ovis aries* tubulin used in MTA screening. Notably, the overall correlation between the degree of *N.g.* growth inhibition and the ΔT_{poly} of tubulin (r = 0.53) was significantly stronger than the correlation observed with the cytotoxic effects on human U87MG glioblastoma cells.[10] This finding supports the hypothesis that the anti-amoebic activity of several identified compounds may, at least in part, be explained by their MTA activity.

Here, we focused exclusively on MTAs that inhibited *N.g.* growth by at least 80%. Among these highly active anti-amoebic compounds with strong microtubule-targeting activity, we identified three distinct structural clusters: (i) triphenylethylene derivatives, including tamoxifen (TMX), clomiphene (CMP), and toremifene (TMF) (Fig. 1F); (ii) imidazole derivatives such as tioconazole (TCZ), sertaconazole (STZ), miconazole (MCZ), and isoconazole (ICZ); and (iii) phenothiazine derivatives, including chlorpromazine (CPZ), trifluoperazine (TPZ), and triflupromazine (TFZ) (Fig. 1G).

Interestingly, the anti-amoebic activity of phenothiazine and triphenylethylene derivatives shows a strong positive correlation with their ability to inhibit tubulin polymerization (ρ = 0.96, p = 0.04). This allows us to hypothesize that the molecular mechanism underlying their anti-amoebic effect involves interference with tubulin polymerization. In contrast, miconazole derivatives exhibit a strong negative correlation between their anti-amoebic activity and their ability to inhibit tubulin polymerization. This suggests the involvement of an alternative mechanism, more harmful to *N.g.* growth, and implies that increased tubulin binding may actually reduce the effective concentration of the drug available to act through this primary mechanism. Indeed, miconazole derivatives are known to target an essential *N.f.* enzyme, sterol 14-demethylase (NfCYP51).[11]

Triphenylethylene derivatives primarily target estrogen receptors and are commonly used for ovulation induction and infertility treatment, with additional applications in cancer therapy.[12–14] Among them, TMX shows the strongest dual activity—both in inhibiting tubulin polymerization and in suppressing *N.g.* growth. Notably, TMX is known to cross the blood–brain barrier (BBB),[15] making it a particularly strong candidate for repurposing in PAM therapy. Recently, it has been proposed for such repurposing in combination with isavuconazole or epiminolanosterol due to its strong synergistic effects.[16] The recent discovery that TMX also disrupts tubulin polymerization[10] provides new insight into the molecular mechanisms underlying its anti-amoebic activity. Moreover, observed earlier antifungal[17] activity of TMX also could be explained by the MTA mechanism.

In contrast to triphenylethylene derivatives, the anti-tubulin activity of some phenothiazine derivatives—antipsychotic drugs that target dopamine receptors—has been known or suspected for a long time[18,19] and for others, it has been reported recently.[10] The same is true for their anti-amoeba activity.[20,21] However, only the two recent screenings with quantitative readouts have revealed a high correlation between these two activities, strongly suggesting that the microtubule-targeting mechanism mostly contributes to the anti-amoebic effects of phenothiazine derivatives.

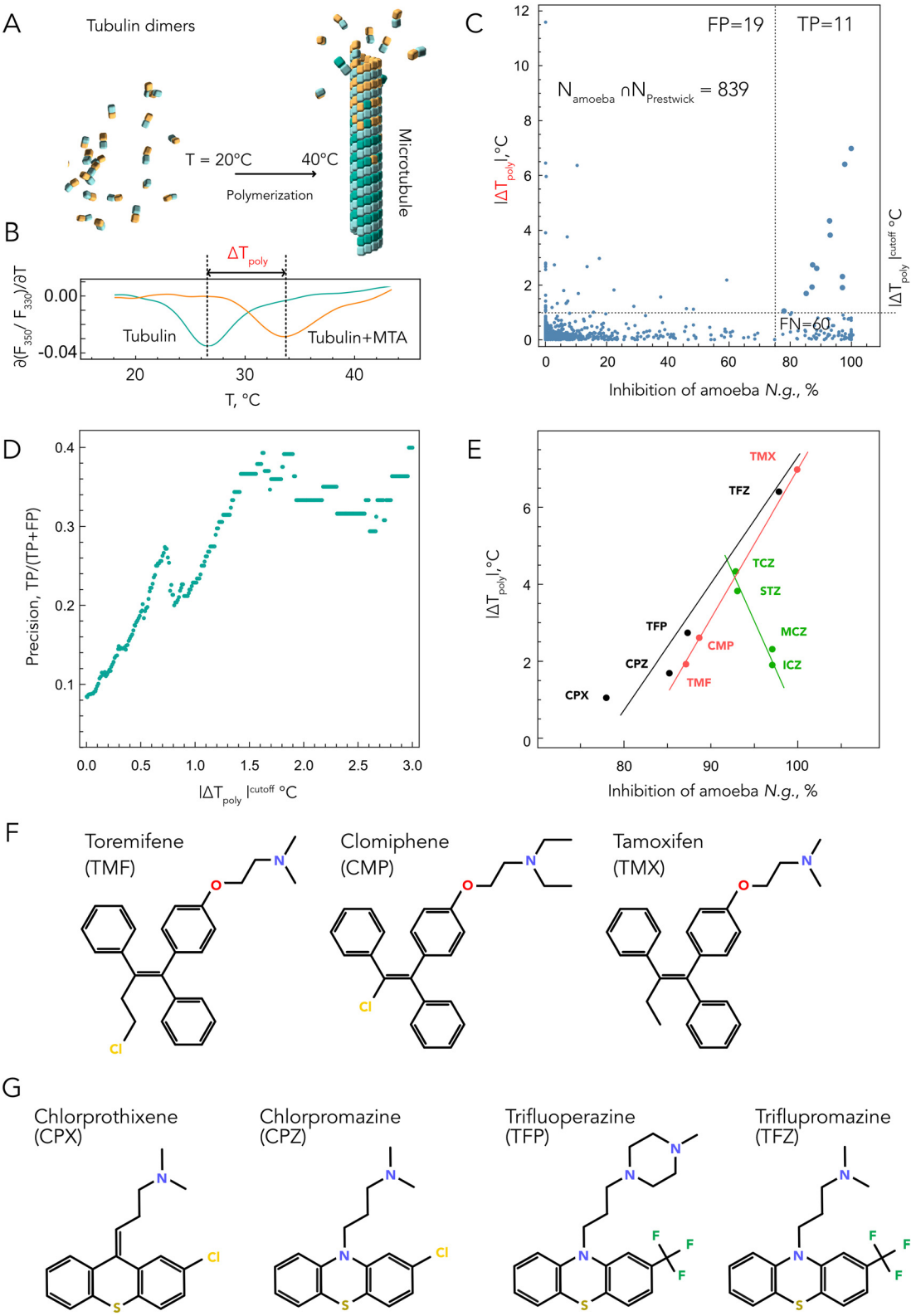

Fig. 1 (A) Schema of temperature-induced polymerization of tubulin dimers into microtubules; (B) changes in fluorescence signal upon tubulin heating allow to determine the temperature of tubulin polymerization in the absence (green line) and in the presence (orange line) of tested molecules and thus detect MTA by the shift of this temperature (ΔT_{poly}); (C) 2D distributions of absolute value of $|\Delta T_{poly}|$ obtained from nanoDSF screening[10] and Inhibition of amoeba *N.g.*;[9] (D) dependance of the precision of amoeba *N.g.* screening on $|\Delta T_{poly}|^{cutoff}$; (E) correlation of MAT and antiamoebal effects of three drug families triphenylethylene, phenothiazine, and miconazole derivatives shown in red, black and blue respectively. Triphenylethylene (F) and phenothiazine (G) derivatives with MTA and anti-*N.g.* activities.

4. Strong MTAs with antiamoebic activity

In recently published anti-tubulin screening, approximately 20 compounds were identified that completely inhibit tubulin polymerization.[10] As a result, they lack measurable ΔT_{poly} values and were excluded from the correlation analysis. Nevertheless, several of these compounds have previously reported antiamoebic activity. Unfortunately, due to the absence of structurally similar drugs with available ΔT_{poly} values, it is not possible to establish a correlation between their anti-tubulin and antiamoebic activities. However, this potential link remains worth investigating, particularly for antiamoebic compounds with unknown molecular mechanisms of toxicity.

Among strong MTAs with antiamoebic activity, ebselen (EBS) and auranofin (AUF)—selenium- and gold-containing drugs, respectively—stand out as promising candidates for repurposing in anti-*N.f.* therapy, given their ability to cross the blood–brain barrier. EBS, known for its anti-inflammatory, antioxidant, and cytoprotective effects, has already been proposed for repurposing against infections caused by multidrug-resistant microorganisms[22] and PAM.[23] Similarly, AUF, also an anti-inflammatory agent, has demonstrated *in vitro* antifungal and antibiofilm activities,[24] which were recently linked to its MTA activity [manuscript under consideration]. In anti-*N.g.* screening, AUF exhibited strong inhibitory activity (94%).[9] It has previously been proposed for repurposing in PAM treatment, either alone[25] or in combination with amphotericin B.[26] While its therapeutic effect has primarily been attributed to the inhibition of antioxidant pathways,[26] emerging evidence suggests that its MTA activity should also be taken into account.

Finally, two compounds newly identified as strong MTAs —bithionol (BTN) and nifedipine (NFD)—have not previously been reported for anti-*N. fowleri* activity but demonstrated toxicity against other protists. BTN showed significant activity against *Neoparamoeba* spp., a genus of Amoebozoa known to cause amoebic gill disease (AGD) in fish,[27] while NFD exhibited notable antiproliferative effects against *Acanthamoeba castellanii* trophozoites, another Amoebozoa that can cause granulomatous amoebic encephalitis (GAE) upon CNS infection.[28]

5. Summary

In summary, the comparative analysis of the two screenings demonstrated that the cytotoxic effects of certain antiamoebic drugs may be directly associated with their anti-microtubule activity. Notably, phenothiazine and triphenylethylene derivatives exhibited a strong correlation between their antiamoebic efficacy and anti-tubulin effects. Given that compounds from both classes are known to cross the blood–brain barrier, they represent promising candidates for repurposing in the treatment of primary amoebic meningoencephalitis and other amoebic brain infections.

Conflicts of interest

Authors declare no competing interests.

Data availability

Data supporting this study are available from published sources. Inhibition percentages for FDA-approved drugs against *Naegleria gruberi* were extracted from Table S1 of Martín-Escolano *et al.*, "Repurposing *in vitro* approaches for screening anti-parasitic drugs against the brain-eating amoeba *Naegleria fowleri*"[9] (SI file: **https://ars.els-cdn.com/content/image/1-s2.0-S221132072100052X-mmc1.docx**). Changes in tubulin polymerization temperature (ΔT_{poly}) in the presence of FDA-approved drugs from the Prestwick Chemical Library were provided by the authors of Baksheeva *et al.*, "NanoDSF Screening for Anti-tubulin Agents Uncovers New Structure–Activity Insights",[10] upon request to the corresponding authors. No new experimental data were generated for this work.

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