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Analysis of two screens reveals a correlation between anti-amoebic 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 nitroxolin⁴ that showed promising results in the treatment of Balamuthia brain infection.⁵ Another one is miltefosine, a phospholipid group alkylphosphocholine used for leishmaniasis treatment⁶ and associated with recovery from *N.f.* encephalitis.⁷ Since there, CDC clinical care recommendation for *N.f.* infection include both molecules⁸ (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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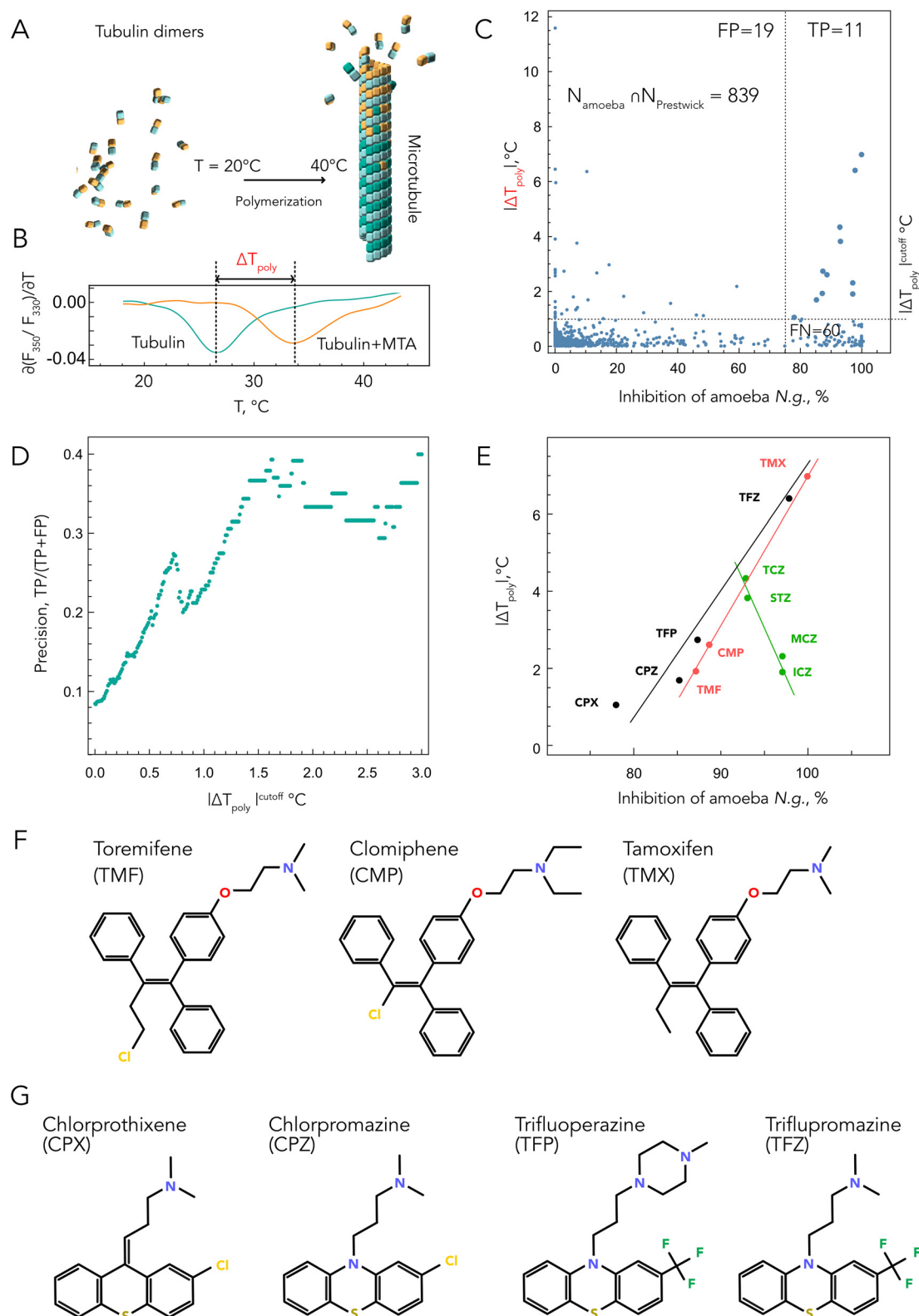


Fig. 1 (A) Schema of temperature-induced polymerization of tubulin dimers into microtubules; (B) changes in fluorescence signal upon tubulin heating allow to determine the temperature of tubulin polymerization in the absence (green line) and in the presence (orange line) of tested molecules and thus detect MTA by the shift of this temperature (ΔT_{poly}); (C) 2D distributions of absolute value of $|\Delta T_{\text{poly}}|$ obtained from nanoDSF screening¹⁰ and Inhibition of amoeba *N.g.*;⁹ (D) dependence of the precision of amoeba *N.g.* screening on $|\Delta T_{\text{poly}}|_{\text{cutoff}}, ^{\circ}\text{C}$; (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 antiamebic 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 antiameobic activity, ebelsen (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 microorganisms²² 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 alone²⁵ 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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