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Identifying Promising Novel Compounds Against Free-Living Amoebae: A Systematic Review of In vitro and in vivo Studies

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Abstract

The increasing global incidence of infections caused by free-living amoebae (FLA) and the lack of effective, safe, and approved treatments highlight the urgent need for novel amoebicidal compounds with pharmacological potential. Despite a growing body of literature on the anti-FLA properties of various compounds, comprehensive reviews summarizing this progress remain scarce. This study aimed to identify the most promising compounds tested in vitro and/or in vivo for anti-FLA activity. A systematic review was conducted, analyzing 108 studies published between 1986 and 2024, selected from an initial pool of 23,653 database results. A total of 537 compounds were evaluated for their in vitro anti-FLA activity, with those demonstrating amoebicidal activity ≥50% further analyzed to identify the most promising candidates based on anti-FLA potency and cytotoxicity profiles in non-target mammalian cells.

Image Attribution

Figure 1 Summary of the search and selection process for scientific articles included in this review.

Methodology

1 Objectives of the Review
This review was conducted to identify the most promising new chemical compounds (those not yet established as drugs) with potential pharmacological applications that have been tested in in vitro or in vivo studies against FLA. It aims to provide insights relevant to the field of antimicrobial drug development, particularly for the treatment of infections caused by FLA.

2 Additionally, this review seeks to identify compounds that, beyond their potential as therapeutic drugs, may also be suitable for clinical use as antiseptic agents or environmental disinfectants.

3 Furthermore, the review aims to highlight molecules that, although not yet fully optimized for clinical or environmental applications, may serve as valuable scaffolds or starting points for future chemical engineering efforts aimed at enhancing their biological activity, selectivity, or physicochemical properties, thus improving their suitability for therapeutic, prophylactic, or sanitation purposes.

4 The specific questions guiding this review are as follows:
- Which novel chemical compounds (those not yet established as drugs) have been tested in vitro and/or in vivo against FLA?
- Which tested compounds demonstrate promising performance in terms of amoebicidal activity, effective concentration, and mammalian toxicological profile?

Data collection

5 The planning, conduct, and reporting of this review were guided by the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA 2020) statement (Page et al. 2020). Primary studies were identified through a comprehensive search strategy applied across PubMed, EMBASE, ProQuest, and Web of Science, using the following search strategy "Acanthamoeba* OR Balamuthia OR Naegleria* OR Vermamoeba." Two authors validated the performance of the search strategy, and one author conducted the formal search for articles in the databases on June 11, 2024.

6 Inclusion Criteria: Studies were selected based on the following criteria: (1) full texts were accessible online, including those provided by authors upon request; (2) written in English, Portuguese, Spanish, or another language translatable with online tools; and (3) reporting in vitro or in vivo evaluation of a novel compound against FLA, specifically Acanthamoeba, Balamuthia, Naegleria, or Vermamoeba (Figure 1).

7 Exclusion Criteria: Studies were excluded if they were (1) non-primary research, (2) had unclear or unreliable methodology or data, (3) tested a complex mixture of chemical compounds, (4) did not report quantifiable amoebicidal effects, (5) tested approved

drugs or commercial disinfectants, or (6) if they presented only the chemical structures of the compounds without corresponding names, or if the names (whether common or IUPAC - International Union of Pure and Applied Chemistry) were unavailable or inaccessible. Studies involving pure compounds extracted from plants were also not included, as these have already been addressed in a specific review (Chaúque et al. 2025).

8 The references of the selected articles were evaluated in search of additional relevant studies that may not have been identified through the primary database search.

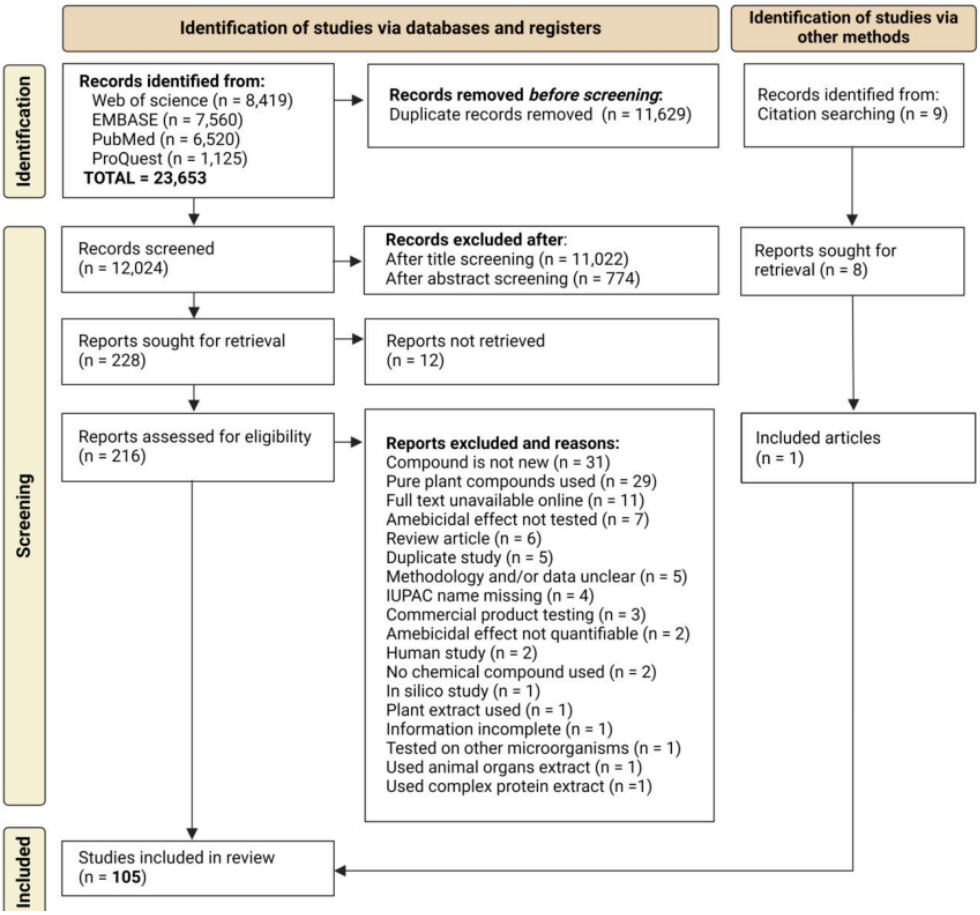

Figure 1 Summary of the search and selection process for scientific articles included in this review.

Screening stages

9 The screening of the identified records was carried out in three main stages: (1) Removal of duplicates - Duplicated titles were removed using semi-automated tools available in Mendeley Reference Manager. (2) Title screening - The titles were read and those clearly unrelated to the scope of the study were excluded. Titles that did not allow a clear judgment about their relevance were retained for further evaluation. (3) Abstract

screening - Abstracts were reviewed to identify and exclude studies whose content, based on the abstract, was evidently outside the scope of the review.

10 All screening stages were conducted independently by at least three authors. Discrepancies were resolved through consensus meetings. Studies selected by consensus were then included in the next steps of full-text retrieval and data extraction.

Data extraction quantification of compound efficacy

11 Data extraction was initially carried out by one author, followed by verification for accuracy by two or more independent authors. The extracted data from the articles included: reference, country (of all study authors), compound name, FLA (genus or species), amoebae form (cyst or trophozoite), density of FLA used in the tests, effective concentration (exhibiting the most relevant effect), exposure time, FLA mortality, concentration causing 50% FLA mortality (IC_50), highest concentration tested for toxicity in mammalian cells (CT-toxicity), cytotoxicity in mammalian cells, and the cell line used in the toxicity test.

12 For each compound identified in the included publications, biocidal efficacy data were extracted as reported by the original study authors. Whenever available, values for the MIC, IC*50, or the concentration corresponding to the maximum observed effect (E*max) were collected.

13 When multiple measurements were available, the value indicating the greatest efficacy against amoebae (according to the source article) was selected. These measurements were used as comparative indicators of the compounds' amoebicidal activity. No extrapolation or artificial standardization was applied to homogenize the data across studies, due to methodological heterogeneity among them. The compounds were then grouped according to the highest and lowest reported effects, aiming to provide a useful comparative reference for future studies investigating these molecules in greater depth.

Data analysis procedure

14 Before analysis, the compounds were alphabetically ordered and coded starting from "0" using the label encoder function of Scikit-learn. The compounds and their corresponding codes are presented in Table S1.

15 Following this, the compounds were ranked based on Effective Concentration and Mortality values. The ranking criteria considered the range of values (minimum and maximum) and the relative distance between each compound's Effective Concentration or Mortality values. Rankings started from '1,' with lower Effective Concentration values and higher Mortality values assigned a ranking of '1.'

16 An average ranking value was calculated for each compound based on its Effective Concentration and Mortality rankings. Compounds with lower average ranking values (indicating higher mortality rates at lower concentrations) were considered the most

effective. These average ranking values (rankAvg) were visualized using scatter plots (Figure S2).

17 Mean values and standard deviations (with 95% confidence intervals) were calculated for the variables: effective concentration, exposure time, FLA mortality, IC_50, CT-toxicity, and cytotoxicity. These calculations focused on compounds that exhibited mortality rates ≥ 50%. Mean values were further grouped and analyzed by compound, species, and amoebae form.

18 The primary data and analyzes are summarized in Table S2. All analyzes were conducted using Python 3 and GraphPad Prism 8.02 software.

19 Compounds mentioned more than once in the same or different graphs (Figure 4 - 31) for the same amoeba genus mean that they were tested in more than one species.

Protocol references

Raghavan and Rammohan 2024; Aykur et al. 2024; Hall et al 2024; Haston and Cope 2023; Schafer et al. 2015; Wang et al. 2020; Kot et al. 2021; Stetkevich et al. 2022; Haston et al. 2023; Aksozek et al. 2002; Chaúque et al. 2022; Chaúque et al. 2023; da Silva et al. 2024; Glotova et al. 2024; Pérez-Pérez et al. 2024; Mahdavi et al. 2024; Chaúque and Rott 2021; Ramírez-Flores et al. 2023; Dos Santos et al. 2023; Liu et al. 2024; Ali et al. 2024; Choi et al. 2024; Spottiswoode et al. 2024; Lemke et al. 2010; Aqeel et al. 2012; Tiewcharoen et al. 2014; Rabablert et al. 2015; Wehelie et al. 2022; Akbar et al. 2022a; Siddiqui et al. 2022c; Lemke et al. 2010; Aqeel et al. 2012; Tiewcharoen et al. 2014; Rabablert et al. 2015; Wehelie et al. 2022; Akbar et al. 2022a; Siddiqui et al. 2022c