



Antimicrobial activity of clioquinol and nitroxoline: a scoping review

Rachel Wykowski¹ · Alexandre Meneghello Fuentefria^{1,2} · Saulo Fernandes de Andrade^{1,3}

Received: 8 June 2022 / Accepted: 4 July 2022 / Published online: 30 July 2022
© The Author(s), under exclusive licence to Springer-Verlag GmbH Germany, part of Springer Nature 2022

Abstract

Clioquinol and nitroxoline, two drugs with numerous pharmacological properties fallen into disuse for many decades. The first was considered dangerous due to contraindications and the second mainly because was taken as ineffective, despite its known antibacterial activity. In the last decades, the advances in pharmaceutical chemistry, molecular biology, toxicology and genetics allowed to better understand the cellular action of these compounds, some toxicological issues and/or activity scopes. Thus, a new opportunity for these drugs to be considered as potential antimicrobial agents has arisen. This review contemplates the trajectory of clioquinol and nitroxoline from their emergence to the present day, emphasizing the new studies that indicate the possibility of reintroduction for specific cases.

Keywords Review · Clioquinol · Nitroxoline · Antimicrobial activity

Introduction

Microbial resistance has been a steadfast approach in the last 2 decades. The indiscriminate prescription of antimicrobials provided selective pressure to the environment enough to favor the emergence of resistant strains of fungi and bacteria (Mancuso et al. 2021). Thus, classic antimicrobials (micafungin, fluconazole, amphotericin B, doxycycline, sulfamethoxazole, and azithromycin) to which mankind has safely resorted to fighting infections have proven to be partially or completely ineffective, depending on the pathogen to be eliminated. Add to this fact the aggravating factor that, although they are substances approved by the Food and Drug Administration (FDA), such antimicrobials have always brought, in their use, adverse reactions (Lombardi

and Ouanounou 2020). This context may have worsened in the last 2 years with the pandemic of Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV2) infection. The scenario of collapsing health systems worldwide has aggravated the overuse of antimicrobials and combinations of antimicrobials, although these substances demanded clinical trials against the virus causing COVID-19. Several countries adopted this practice to provide a quick way out of the collapse of their health services, generated by the high demand for hospitalizations due to Severe Acute Respiratory Syndrome (SARS). However, such actions corroborated the worsening of nosocomial infections, since they increased the selective pressure on microorganisms in hospital environments (Segala et al. 2021).

Given this scenario, it is time to explore new molecules with antimicrobial activity, which offer safety in their use, not only in efficacy against pathogens but also in low adverse reactions to patients (Joaquim et al. 2021). In some cases, researchers have worked in different ways with drugs that were forgotten for decades by the pharmaceutical industry, such as clioquinol and nitroxoline (Fig. 1): the first, feared in the past due to a severe adverse reaction, even leaving clinical practice, since the mechanisms behind the compound's toxicity were unknown (Konagaya 2015). The second drug was underestimated concerning its antimicrobial activity due to incipient pharmacodynamic and pharmacokinetic knowledge at its discovery (Wijma et al. 2018). These compounds are underutilized, however, advances in chemistry, molecular

Communicated by Erko Stackebrandt.

✉ Saulo Fernandes de Andrade
saulo.fernandes@ufrgs.br

- ¹ Programa de Pós-Graduação Em Microbiologia Agrícola E Do Ambiente, Universidade Federal Do Rio Grande Do Sul, Porto Alegre, RS, Brazil
- ² Departamento de Análises, Faculdade de Farmácia, Universidade Federal Do Rio Grande Do Sul, Porto Alegre, RS, Brazil
- ³ Departamento de Produção de Matéria-Prima, Faculdade de Farmácia, Universidade Federal Do Rio Grande Do Sul, Porto Alegre, RS, Brazil

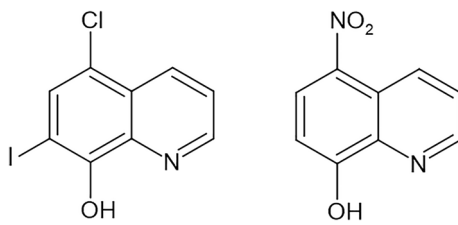


Fig. 1 Chemical structures of clioquinol (1) and nitroxoline (2)

biology, and genetics have allowed for better elucidation of the factors underlying the occurrence of drug-related undesirable effects, as well as the definition of a correct therapeutic window in which the drug exhibited satisfactory activity with reduced toxicity to the body (Kuru 2021).

Despite its versatile pharmacological properties (antiparasitic, antibacterial, antifungal, anti-inflammatory, attenuating neurodegenerative diseases, antineoplastic), for many decades, clioquinol raised fears about its clinical use due to a common serious adverse reaction resulting from its use mainly in Japan: the Subacute Myelo-Optic Neuropathy (SMON), discovered in Japan around the year 1958. Patients treated with clioquinol complained of nonspecific symptoms such as body aches, numbness, diarrhea, and abdominal pain, which soon culminated in paralysis of several body regions and blindness. However, all had three notorious symptoms in common: a green spot on the tongue, green urine, and green feces. After Japanese researchers reproduced SMON in dogs, which allowed the evaluation of brain damage and the identification of clioquinol in urine, the severe toxicity of clioquinol was widely reported in the scientific community, and the substance fell into disuse (Kuru 2021).

However, with the emergence of molecular biology and the advances in analytical methodologies in chemistry, this background took a turn in the mid-nineties by the Australian researcher Ashley Bush. Ashley discovered that the amyloid plaques formed in the brains of Alzheimer's disease patients could be caused by the accumulation and deposition of heavy metals in the brain, especially copper and zinc. Knowing the property of clioquinol as a chelator of metals, the group decided to employ the substance in a murine model of Alzheimer's disease, the result being the reduction of amyloid plaques in the brains of mice. In 1997, in Melbourne, the researcher began clinical trials with clioquinol against the disease, improving the cognitive ability of patients with Alzheimer's after 36 weeks of treatment. Since the dosage of clioquinol used in the trials was low and controlled, there were no adverse effects associated with the use of the drug. Finally, the fear regarding the therapeutic employment of clioquinol was beginning to dim in academia even though, due to the background, researchers understood the importance of the highly controlled use of the compound; thus,

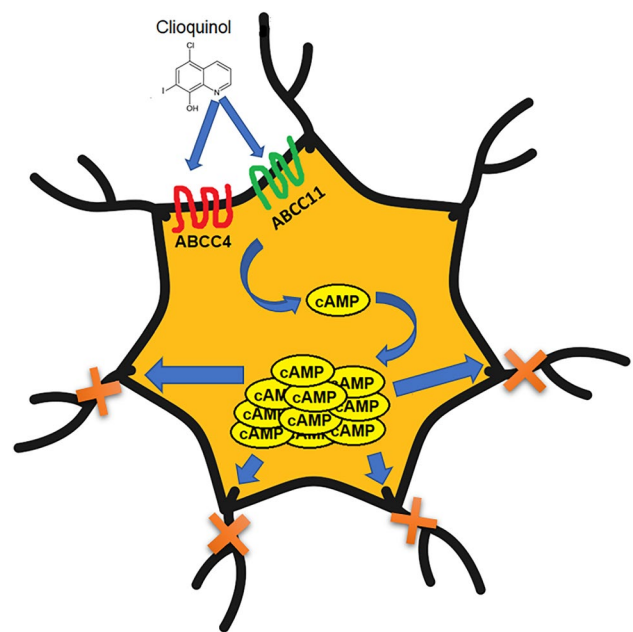


Fig. 2 Interaction of clioquinol with the polymorphous receptors ABCC4 and ABCC11 of the neuronal cell, which results in the accumulation of cAMP in the neuron and the consequent neuronal death by degeneration

more clinical trials emerged around the world employing clioquinol against Alzheimer's disease (Perez et al. 2019).

The advent of molecular biology, medicinal chemistry, and genetics favored studies that sought to unravel the factors and mechanisms behind Subacute Myelo-Optic Neuropathy. Thus, these studies suggested that the symptoms of SMON were more severe and could occur more frequently in: (a) patients who already had natural zinc deficiency; (b) anemic patients and patients with an increased tendency to anemia; (c) patients with natural vitamin B12 deficiency, considering the chelating property of clioquinol (You et al. 2020). Another theory behind the development of SMON would be polymorphisms in the neuronal receptors ABCC4 and ABCC14 (Fig. 2) that would occur mainly in the Japanese population, causing the deposition of cAMP in the neuronal cell and the consequent neuron death by apoptosis (Perez et al. 2019). Once there are genetic factors or particular conditions of the organism preponderant to an unwanted action for a specific drug, it is possible that this type of occurrence is not a common adverse reaction to the drug but is a contraindication. In some instances, contraindications can be identified and monitored through careful clinical evaluation of patients, just as the performance of blood tests that provide a drug profile in the patient's body (Shankar et al. 2021). Thus, by ascertaining the genetic disposition or deficiency of certain nutrients for the events associated with clioquinol, it would be possible to consider employment in the treatment of Alzheimer's, Parkinson's,

and Huntington's diseases after approval in clinical phases, as well as reintroduction as an antimicrobial, preserving patients susceptible to adverse effects (You et al. 2018; Pippi et al. 2019b; Mustazza et al. 2020; Tavares et al. 2020; Olalaye et al. 2021).

Nitroxoline is less controversial and less explored for its potential antimicrobial activities. The drug is known for its bacteriostatic activity against *Escherichia coli*, an important etiological agent causing urinary tract infections in hospitalized patients (Sobke et al. 2018). Nitroxoline was first employed in 1962 for the treatment and prophylaxis of acute and recurrent urinary tract infections caused by *E. coli* in both adult and pediatric patients. However, the incipient knowledge of pharmacology and pharmacotechnics back in the day made it challenging to define an adequate therapeutic window for the effective antibacterial action of the substance and understand its spectrum of action, causing the compound to be mistakenly used against resistant microorganisms. The corroboration between these factors led to the rapid abandonment of nitroxoline as an antimicrobial. However, the substance remained clinically active in Eastern Europe, in limited cities in Germany, where the resistance rate among *E. coli* strains remained low (Wijma et al. 2018). The decades that followed were of prescription abuse of antibiotics, especially of bactericides, promoting a great selective pressure in the environment, favoring the emergence of multidrug-resistant strains of bacteria, currently known by the term "ESKAPE": (a) E = *Enterococcus faecium*; (b) S = *Staphylococcus aureus*, *Stenotrophomonas maltophilia*; (c) K (or C) = *Klebsiella pneumoniae*, *Clostridioides difficile*; (d) A = *Acinetobacter baumannii*; (e) P = *Pseudomonas aeruginosa*; (f) E = Enterobacteriaceae—currently, primary targets of pharmaceutical companies in the search for new antibiotics, as well as in the search for new molecular targets (Giacomini et al. 2021). With antibiotic options increasingly scarce in the face of a new generation of resistant bacteria, it is possible to revisit and even reposition classes of compounds that were forgotten. After all, with the evolution of microorganisms comes the human evolution in terms of knowledge about how drugs interact with living organisms and how such compounds can be structurally improved to optimize their affinity and selectivity with the molecular targets on which they should act. Against this backdrop, work such as that of Cherdtrakulkiat et al. (2019), in which nitroxoline is tested for its antibacterial activity against bacteria of the dreaded genus Enterobacteriaceae; and the trial of Fuchs et al. (2019) that employed nitroxoline against *Neisseria gonorrhoeae*, have emerged. Some see the antimicrobial potential of nitroxoline even further, such as Laurie et al. (2018), who revealed the substance as a potent amebicide by testing it against the pathogen *Balamuthia mandrillaris*; and Zhang et al. (2020), who determined the antiviral activity of nitroxoline against Japanese encephalitis virus (JEV). In the

era of the search for new antibiotics, nitroxoline, a molecule belonging to a promising class of compounds, is expected to reveal its full antimicrobial potential as in-depth studies are performed to elucidate its scope of action and the cellular actions and molecular factors involved.

Against all the background mentioned, this review brings the main novelties for repositioning and expanding the scope of the drugs clioquinol and nitroxoline in terms of their antimicrobial activity, as evidenced by studies published in the literature over the last ten years, and advantages and limitations concerning each study listed.

Methods

Data sources

The literature review was based on a search of the PubMed, Embase, and Web of Science databases for papers dealing with in vitro, in vivo, ex vivo trials, case reports, retrospective studies, and observational studies reporting the antimicrobial efficacy of clioquinol and nitroxoline, dated between the years 2012 and 2021, employing the search filters described below.

Search filters

Pubmed

(Clioquinol[mh] OR Clioquinol[tw] OR 5-Chloro-7-iodo-8-quinolinol[tw] OR 5 Chloro 7 iodo 8 quinolinol[tw] OR Iodochloroxyquinoline[tw] OR Chloroiodoquine[tw] OR Iodochlorhydroxyquin[tw] OR nitroxolin*[tw] OR hydroxyquinoline moiety[tw]) AND (2012:2021[dp]) NOT (Neoplasms[mh] OR Alzheimer Disease[mh]).

Embase

'clioquinol'/exp OR 'nitroxoline'/exp OR (Clioquinol OR '5-Chloro-7-iodo-8-quinolinol' OR '5 Chloro 7 iodo 8 quinolinol' OR Iodochloroxyquinoline OR Chloroiodoquine OR Iodochlorhydroxyquin OR nitroxolin* OR 'hydroxyquinoline moiety'):ti,ab,kw NOT 'neoplasm'/exp OR 'Alzheimer disease'/exp OR (Neoplasm* OR cancer* OR Alzheimer):ti,ab,kw.

Web of science

TS=(Clioquinol OR "5-Chloro-7-iodo-8-quinolinol" OR "5 Chloro 7 iodo 8 quinolinol" OR Iodochloroxyquinoline OR Chloroiodoquine OR Iodochlorhydroxyquin OR nitroxolin* OR "hydroxyquinoline moiety") NOT TS=(Neoplasm* OR cancer* OR Alzheimer) AND PY=(2012–2020).

Inclusion criteria

The works included in the scoping review contemplated:

1. Papers dealing with *in vitro* antimicrobial activity for clioquinol or nitroxoline;
2. Retrospective analyses and observational studies dealing with the employment of the drugs, such as antimicrobials, in clinical practice;
3. Studies of antimicrobial action mechanism or microbial resistance mechanism for both drugs.

Exclusion criteria

The works excluded from the scoping review contemplated:

1. Reviews, book chapters;
2. Articles dealing primarily with derivatives of clioquinol or nitroxoline, not the substances themselves;
3. Articles dealing with antineoplastic, antiparkinsonian or anti-Alzheimer activity of the substances.

Selection of articles

The searches in PubMed, Embase, and Web of Science databases returned 255 articles. After applying the exclusion criteria and meeting the inclusion criteria, 44 articles remained and were included in the scoping review, according to the flowchart in Fig. 3.

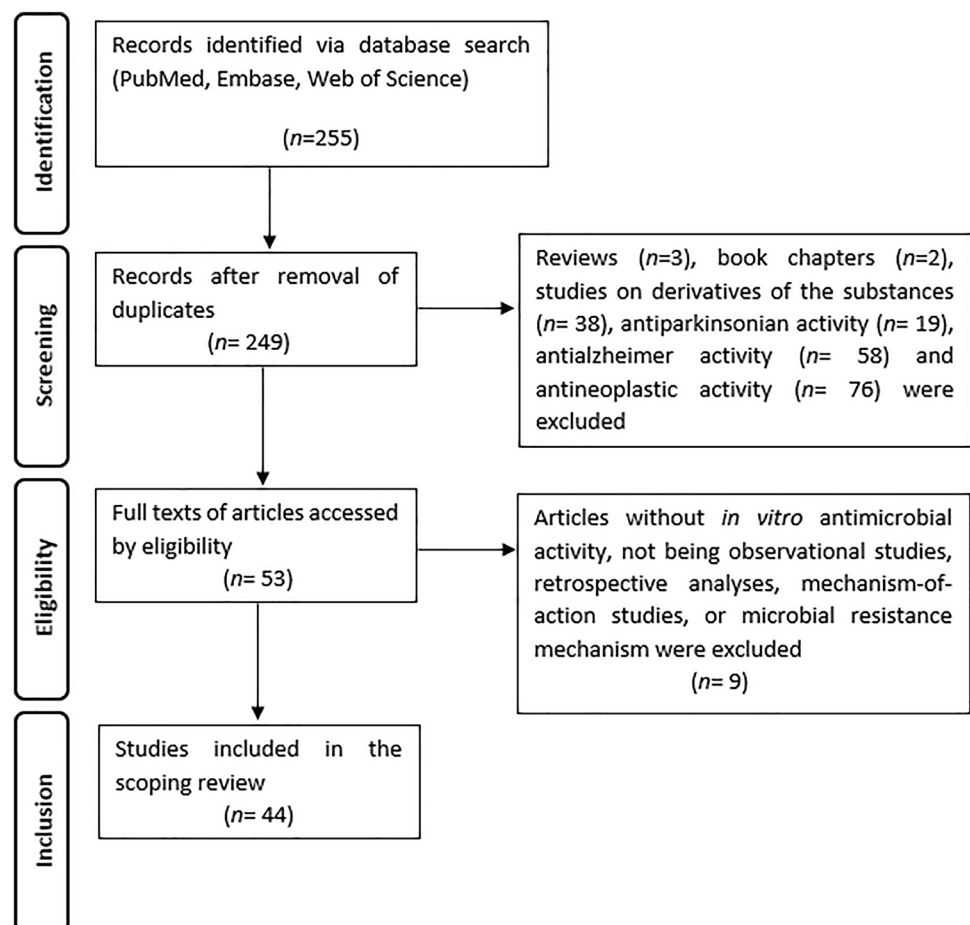
Results and discussion

Clioquinol

Antifungal activity

Among the versatile activities of clioquinol, the most reported in the literature is the antifungal activity, consisting of 14 articles from the previously mentioned databases. Such references encompass works against determined species of fungi, such as that of Alvarez and Sanhueza (2016) and Laskaris et al. (2016), and works that tested the activity of clioquinol against a wide variety of fungi, such as that of Leonardelli et al. (2019) and Pippi et al. (2019a).

Fig. 3 Flowchart illustrating the selection of articles, inclusion and exclusion criteria for the papers



Employing polymerase chain reaction (PCR) and broth microdilution, Alvarez and Sanhueza (2016) found optimal activity for clioquinol against *Scedosporium dehoogii*, an opportunistic soil-dwelling fungus, with variable MICs between 0.5 and 1 µg/mL. Employing similar techniques, Chaves et al. (2020) found MICs between 0.5 and 2 µg/mL for clioquinol against *Fusarium* species, which also denotes excellent activity against the fungus. In addition, the research team also found excellent antibiofilm activity for clioquinol (MAC = 5–10 µg/mL) against isolated *Fusarium* species. Also considering in vitro microdilution assays, Laskaris et al. (2016) combined broth microdilution with bioluminescence, finding optimal activity for clioquinol against *Aspergillus fumigatus* (MIC = 6 mg/L). Yousfi et al. (2020) studied the effect of clioquinol on a wide variety of fungal species (Table 1), which included *Aspergillus calidoustus*, *Aspergillus flavus*, and *Aspergillus niger*, obtaining a growth inhibition rate of 73%, 71%, and 75%, for each fungus, respectively. The growth inhibition range was 71–85% for the other fungal species. The Leonardelli et al. (2019) group, studying distinct species of Mucorales (Table 1), obtained intense antifungal activity (MIC = 4–8 µg/mL) for clioquinol against the fungal species addressed.

Studying different *Candida* species, Pippi et al. (2018) obtained excellent antifungal activity for clioquinol (PMIC₅₀ of 0.031–0.5 µg/mL) against the contemplated species of the fungus (Table 1). Regarding biofilm eradication, clioquinol proved to be most successful in eradicating biofilms caused by *C. glabrata* (100%), followed by *C. tropicalis* (99.95%) and *C. albicans* (97.92%). In subsequent work, the research group also revealed clioquinol as an antifungal capable of inhibiting pseudohyphae formation (in yeasts), and inhibiting hyphal growth in filamentous fungi (by altering cell permeability) (Pippi et al. 2019a). Clioquinol also protected *Drosophila* flies from *C. albicans* infection, making the survival curve of exposed animals equal to that of the uninfected control group (Pippi et al. 2019b). Still considering *Candida* species, Yan et al. (2018), combining PCR with MALDI TOF–MS, studied the effect of clioquinol on the *Candida albicans* cell cycle. The authors concluded that the substance exerts a fungistatic effect, inhibiting fungal cell growth in the G2/M phase of its cell cycle. The You et al. (2020) group addressed the little-explored heavy metal chelating ability of clioquinol and the influence of this property on the antifungal activity of the substance against the *C. albicans* cell wall as well as against biofilms formed by the fungus. In the study, clioquinol lysed the cell membrane of *C. albicans* at the concentration of 32 µg/mL and at the concentration of 5 µM, clioquinol chelated heavy metal ions (Table 1), inhibiting from the fungal cell, a condition reversible with the exogenous addition of the chelated ions.

Working with *Nannizzia gypsea*, *Microsporium canis*, and *Trichophyton* species, Costa et al. (2020) obtained intense

antifungal activity for clioquinol (MIC = 0.5–2 µg/mL) and intense fungicidal activity, evidenced by a 99.9% reduction in colony-forming units (CFUs). The group also achieved 90% cell viability for clioquinol against human leukocytes, indicating low toxicity to the organism. In a subsequent paper, the authors evaluated combinations of clioquinol with terbinafine and ciclopirox against ex vivo models of *Trichophyton rubrum* and *Microsporium canis* infection in pig hooves and dog fur. In the study, clioquinol associated with both terbinafine and ciclopirox was effective in removing 100% of the colony-forming units (CFUs) formed on pig hooves and being able to prevent biofilm formation on dog fur effectively. As for the ability to cause irritation, via the HET-CAM (chicken egg embryonic chorioallantoic membrane) method, the researchers obtained an irritation index for the antifungal combinations employed in the range of 2.83–3.41. These results classify such associations as non-irritating added to the fact that histopathology studies carried out on pigs' ears revealed no microscopic lesions resulting from the application of the antifungal combinations mentioned (Costa et al. 2021). Still dealing with *Trichophyton* species, Dunmade et al. (2012) brought up a case report of fungal rhinosinusitis by *T. mentagrophytes* in Nigerian patient of Yoruba ethnicity. In that case, clioquinol was associated with flumetasone, administered intra-antral after informed consent was obtained (weekly for six weeks), being able to completely eradicate the symptoms of the disease presented by the patient. It demonstrates the efficacy of the substance used in clinical practice, facing a confirmed case of infection in which the researchers could observe the evolution of the clinical picture and possible adverse effects resulting from treatment.

Antibacterial activity

Searches in the Pubmed, Embase and Web of Science databases returned 6 articles for the antibacterial activity of clioquinol, thus being the second most reported activity in the literature for the substance. In contrast to the studies on clioquinol as an antifungal agent, the researchers have studied the antibacterial activity of the substance in trials focused on one or a few species of bacteria, usually exploring mechanisms of bacterial resistance to clioquinol, such as the work of Blanco et al. (2018). These authors approached the effect of clioquinol on the expression of the *smeVWX* gene (encoding efflux pumps and resistance mechanisms) of *Stenotrophomonas maltophilia* (Table 2). The researchers concluded that the mechanism of bacterial resistance induction by clioquinol is selective. The compound is a potent inducer of resistance in the studied *S. maltophilia* strain PBT02. The methodology used by the authors was the Biolog Phenotype Microarrays technology. Similarly, the trial by Majumdar et al. (2013) studied the *rarA* gene

Table 1 Main findings pertinent to antifungal activity studies of clioquinol

References	Species	Methodology	Findings
Costa et al. (2021)	<i>Trichophyton rubrum</i> <i>Microsporum canis</i>	(a) The ability of antifungal combinations to promote irritation was evaluated via the HET-CAM method (embryonic chorioallantoic membrane from chicken eggs) (b) The histopathological evaluation was carried out in pig ears to observe possible tissue damage resulting from the application of antifungal associations (c) An ex vivo model of pig hooves infected with <i>T. rubrum</i> was prepared to evaluate the antifungal efficacy of the combinations of the three test compounds (among which, clioquinol) (d) Similarly, an ex vivo model of dog fur was prepared to determine the effectiveness of the combination of the tested substances in protecting the fur of veterinary origin from the formation of fungal biofilms (species previously mentioned) and removing them	(a) The irritation index for the combination of clioquinol (0.125 µg/mL) + terbinafine (0.03125 µg/mL) was 3.17; for the combination of clioquinol (0.125 µg/mL) + cyclopirox (0.5 µg/mL) was 2.83; and for the combination of clioquinol (0.125 µg/mL) + cyclopirox (0.250 µg/mL) + terbinafine (0.03125 µg/mL) was 3.41. Since all combinations exhibited irritation indices less than 5, all were classified as non-irritant according to the methodology employed (b) The histopathology studies on the pig's ear revealed no microscopic lesions resulting from the application of the antifungal combinations mentioned (c) The double combinations of antifungals were sufficient to remove 100% of the colony-forming units (CFUs) formed on the pig hooves
Chaves et al. (2020)	<i>Fusarium proliferatum</i> <i>Fusarium falciforme</i> <i>Fusarium incarnatum</i> <i>Fusarium keratoplasticum</i> <i>Fusarium oxysporum</i> <i>Fusarium solani</i>	(a) Clinical <i>Fusarium</i> isolates were obtained from patients with onychomycosis or fungal keratitis and identified by PCR (Polymerase Chain Reaction) (b) The susceptibility of the fungi (Minimum Inhibitory Concentration—MIC) to the antifungals clioquinol, voriconazole, natamycin, terbinafine and ciclopirox olamine was determined using the broth dilution method (c) Biofilm formation in conidia suspensions was evaluated by spectrophotometric method (d) The minimum antibiofilm concentration (MIC) and the minimum concentration for biofilm eradication (MBEC) were determined using the plate dilution method (e) The interaction between the antifungals used in the experiment against each species of fungus mentioned was investigated (f) The ability of the compounds to remove biofilms was evaluated using the checkerboard test (g) A hypoallergenicity test of the antifungals was performed against chicken embryonated eggs (h) Cytotoxicity study: lymphocytes and leukocytes from an 18-year-old volunteer without chronic use of medication (i) Toxicity prediction performed using the programs: pkCSM®, PreADMET®, Molinspiration Cheminformatics®, ChemIDPlus®, LAZAR Toxicity Predictions®	a) The work brought to light excellent fungal growth inhibition values (MIC = 0.5–2 µg/mL) and antibiofilm values (MAC = 5–10 µg/mL) for clioquinol against the different <i>Fusarium</i> species isolated b) Revealed the synergy between clioquinol and voriconazole and the safety of the association, evidenced in the hypoallergenicity test

Table 1 (continued)

References	Species	Methodology	Findings
Costa et al. (2020)	<i>Nannizzia gypsea</i> <i>Microsporium canis</i> <i>Trichophyton mentagrophytes</i> <i>Trichophyton rubrum</i>	(a) The study included 12 isolates of the fungi <i>N. gypsea</i> , <i>M. canis</i> , <i>T. mentagrophytes</i> , and <i>T. rubrum</i> (b) The minimum inhibitory concentrations (MICs) of the mentioned antifungals was determined by broth microdilution method (c) The interaction between the three tested substances was evaluated using the checkerboard method. The experiments were performed in triplicate and incubated at 32 °C for 5 days (d) Time-kill assay: using <i>T. rubrum</i> strain (TRU47), evidencing the synergistic effect for all tested antifungal combinations, compared to each compound individually (e) Sequentially, the fungal colonies formed were counted to ascertain the fungicidal effect of the combinations of substances (g) Cytotoxicity assay: antifungals against leukocytes from a volunteer older than 18 years without chronic medication use. The loss of leukocyte membrane integrity was verified with trypan blue coloration	a) The work reveals potent antifungal activity for clioquinol (MIC = 0.5–2 µg/mL) and strong fungicidal activity (evidenced by 99.9% reduction in CFU) b) The experiment also addressed the interaction of clioquinol with other antifungal drugs, which contributes to the future elucidation of other drug combinations c) The cytotoxicity test, both of clioquinol, alone and in association with other antifungal drugs, showed cell viability $\geq 90\%$, a strong indication of low toxicity to the organism, to be confirmed by future in vivo tests
You et al. (2020)	<i>Candida albicans</i>	(a) MIC ₅₀ and MIC ₁₀₀ were determined for clioquinol and other antifungals against a representative strain of <i>C. albicans</i> using the broth microdilution method (b) The minimum fungicidal concentration (MIC) for clioquinol was measured against <i>C. albicans</i> using the broth microdilution method and inoculation on Sabouraud Dextrose agar (c) The effect of clioquinol on biofilm formation was observed using the XTT reduction assay (d) The MICs of clioquinol and caspofungin (positive control) were measured in the presence and absence of sorbitol (0.8 M) by the broth microdilution method (e) The effect of clioquinol on the fungal cell membrane was studied using the propidium iodide influx assay (f) The ability of clioquinol to depolarize the fungal cell membrane and its interaction with ergosterol in the cell membrane was analyzed by the broth microdilution method (g) Finally, the influence of metal ions on the antifungal activity of clioquinol was evaluated	(a) Performance profile of clioquinol against the fungal cell wall and its performance against biofilms formed by <i>C. albicans</i> (b) Presented the clioquinol as capable of lysing the cell membrane of <i>C. albicans</i> at the concentration of 52 µg/mL (c) At a concentration of 5 µM, clioquinol proved capable of chelating Fe ³⁺ , Fe ²⁺ , Cu ²⁺ , Zn ²⁺ , Mg ²⁺ , and Ca ²⁺ ions, thus inhibiting fungal cell growth. However, this condition could be reversed by the exogenous addition of these ions

Table 1 (continued)

References	Species	Methodology	Findings
Yousfi et al. (2020)	<i>Aspergillus calidoustus</i> <i>Aspergillus flavus</i> <i>Aspergillus niger</i> <i>Fusarium oxysporum</i> <i>Fusarium solani</i> <i>Rhizopus oryzae</i> <i>Lomentospora prolificans</i> <i>Lichtheimia corymbifera</i>	(a) The Prestwick Chemical Library, located in France, was searched to locate molecules in its database capable of inhibiting the growth of the selected fungi (b) Tests documented in the library were performed in dimethylsulfoxide (DMSO) at a concentration of 10 mM for each compound to obtain, via serial dilutions, a final concentration of 10 μ M (0.5%) of each substance tested in DMSO (c) The fungal inoculum for the tests documented were all prepared in RPMI-1640 medium and were derived from clinical isolates from several French hospitals (d) The determination of the fungal growth inhibition rate for each compound studied against each fungus was performed by optical densitometry, according to the CLSI protocol M38-A	(a) In this work, clioquinol exhibited a fungal growth inhibition range of 71–85%, against the variety of fungi it was tested on
Leonardelli et al. (2019)	<i>Rhizopus microsporus</i> <i>Rhizopus oryzae</i> <i>Syncephalastrum racemosum</i> <i>Mucor circinelloides</i> <i>Lichtheimia corymbifera</i> <i>Cunninghamella bertholletiae</i>	(a) Twenty-five isolates were tested; among them, 18 of <i>R. microsporus</i> , 2 of <i>R. oryzae</i> , 1 of <i>M. circinelloides</i> , 1 of <i>L. corymbifera</i> , 1 of <i>C. bertholletiae</i> (b) For quality control of antifungal susceptibility, the ATCC 22,019 strain of <i>C. parapsilosis</i> was used (c) <i>Mucorales</i> isolates were identified by internal transcribed spacer sequencing (d) The concentration range of the antifungals—amphotericin B (AMB), posaconazole (POSA)—as well as the MICs of each Zn chelating compound—clioquinol (CLIO), 1,10-phenanthroline (PHEN), N,N,N',N'-tetrakis(2-pyridylmethyl) ethane-1,2-diamine (TPEN)—were determined according to CLSI guide M38 (e) Interactions between the antifungals and Zn chelating compounds were studied by calculating the fractional inhibitory concentration index (FICI) using modified CLSI protocols for the microdilution and checkerboard methods (f) The concentration ranges tested on checkerboard plates were 0.06 to 4 μ g/mL for AMB, 0.12 to 8 μ g/mL for POS, and 0.03 to 16 μ g/mL for CLIO, PHEN, and TPEN	(a) Broad action profile of the compounds studied (b) Brings the little-explored chelating property of heavy metals as a promising mechanism of antifungal action (c) Clioquinol showed good MIC values (4–8 μ g/mL) against most fungal strains tested (d) Promising synergistic interaction between posaconazole and clioquinol (24% synergy), especially against <i>R. microsporus</i>

Table 1 (continued)

References	Species	Methodology	Findings
Pippi et al. (2019a)	<i>Candida albicans</i> <i>Candida glabrata</i> <i>Candida krusei</i> <i>Candida parapsilosis</i> <i>Candida tropicalis</i> <i>Microsporium canis</i> <i>Microsporium gypseum</i> <i>Trichophyllum mentagrophytes</i> <i>Trichophyllum rubrum</i>	(a) The MICs of clioquinol and its two derivatives (8-hydroxy-5-quinolinesulfonic acid and 8-hydroxy-7-iodo-5-quinolinesulfonic acid) were determined against <i>Candida</i> spp. and dermatophyte fungi using the microdilution method (b) The effect of clioquinol and its cited derivatives on fungal cell wall integrity was evaluated using the sorbitol protection assay (c) The leakage of intracellular contents was measured for a representative strain of each fungal species studied by spectrophotometric method (d) The capacity of clioquinol and its derivatives to complex with ergosterol of the fungal cell membrane was evaluated, measuring the MICs for each substance in the presence and absence of ergosterol (e) Morphological changes in <i>T. mentagrophytes</i> and <i>M. canis</i> cells were examined by scanning electron microscopy. The growth of fungi was exposed to concentrations of clioquinol and other derivatives defined by the broth dilution method	(a) The work evidences the action of the studied substances on the fungal cell as a whole, considering the activity on the fungal cell membrane and the capacity of these compounds to lyse such cells. Detected changes in morphology caused in fungal cells by the studied compounds (b) Presented clioquinol as an antifungal able to damage the cell wall of fungi, acting to inhibit the formation of pseudohyphae (in yeasts), and inhibiting the growth of hyphae in filamentous fungi (by altering cell permeability)
Pippi et al. (2019b)	<i>Candida albicans</i>	(a) The study determined the ability of clioquinol and its derivatives (8-hydroxy-5-quinolinesulfonic acid and 8-hydroxy-7-iodo-5-quinolinesulfonic acid) to protect Toll-deficient (genetically immunosuppressed) <i>Drosophila</i> flies against <i>C. albicans</i> infection by plotting survival curves using Kaplan–Meier analysis (b) The amount of <i>Candida</i> cells in the tissues of the flies after 7 days of infection was measured using the Kruskal–Wallis test (c) A simulation of the pharmacokinetic and pharmacodynamic profiles of clioquinol in flies was performed using a two-compartment model. This model was previously adapted from human studies in the literature, from which three oral dose regimes were simulated. The analyses of this step were performed with the software Scientist v3 (d) Using embryonated chicken eggs, we performed a study of lethal dose and maximum sublethal dose in embryos for clioquinol and its two derivatives, observing the arterial pulsation, beak opening frequency, and movements of embryos for two minutes	(a) Pharmacokinetic, pharmacodynamic and toxicological parameters for the analyzed substances against <i>Candida albicans</i> (b) Regarding the ability to protect <i>Drosophila</i> flies from <i>C. albicans</i> infection, clioquinol proved to protect the flies completely since the survival curve of the exposed animals matched that of the non-infected control group (c) Clioquinol proved non-lethal to the chicken embryo at a 1 mg/mL concentration, a dose also contemplated for the second derivative studied

Table 1 (continued)

References	Species	Methodology	Findings
Pippi et al. (2018)	<i>Candida albicans</i> <i>Candida glabrata</i> <i>Candida krusei</i> <i>Candida parapsilosis</i> <i>Candida tropicalis</i>	(a) The minimum planktonic cell inhibitory concentrations (PMICs), for clioquinol and two derivatives (8-hydroxy-5-quinolinesulfonic acid and 8-hydroxy-7-iodo-5-quinolinesulfonic acid), were determined against <i>Candida</i> planktonic cells (b) The percentage of inhibition of <i>Candida</i> biofilm formation (BFI) in microplates containing clioquinol and other derivatives was determined (c) <i>Candida</i> cells were cultured in microplates to allow biofilm formation. The fungi were then exposed to clioquinol as well as its other derivatives (d) The effect of clioquinol, as well as its derivatives on the resulting biofilms, was estimated using the XTT [2,3-bis(2-methoxy-4-nitro-5-sulfonyl)-2H-tetrazolium]] reduction assay (e) The ability of clioquinol and its derivatives to remove biofilms formed by <i>Candida</i> cells grown in microplates was evaluated (g) IUDs (Intrauterine Devices) adopted by the Brazilian Unified Health System (UHS) were exposed to clioquinol and fluconazole for 8 h at 35 °C (similar to the conditions of action of a vaginal cream) to investigate the ability of antifungal agents to remove biofilms formed on these devices	(a) Action profile of clioquinol and two other derivatives against several <i>Candida</i> species in the distinct life forms of the fungus: planktonic cells and biofilms (b) Regarding the action against <i>Candida</i> planktonic cells, clioquinol showed excellent activity, as evidenced by PMIC ₅₀ values of 0.031–0.5 µg/mL and PMIC ₉₀ of 0.063–1 µg/mL (c) Considering the percentage of biofilm eradication, clioquinol performed best against <i>C. glabrata</i> (100%), followed by <i>C. tropicalis</i> (99.95%) and <i>C. albicans</i> (97.92%) (d) Against <i>Candida</i> sessile cells, clioquinol proved to be more active against <i>C. glabrata</i> and <i>C. parapsilosis</i> , evidenced by SMIC ₅₀ (minimal inhibitory concentration of sessile cells) values of 4 µg/ml and SMIC ₉₀ of 32 µg/ml, for both species
Yan et al. (2018)	<i>Candida albicans</i> <i>Saccharomyces cerevisiae</i>	a) The effect of clioquinol on the growing cells of <i>C. albicans</i> and <i>S. cerevisiae</i> was tested, plotting the growth curve for both species b) The influence of clioquinol on the cell cycle of <i>S. cerevisiae</i> was evaluated using a combination of methods involving Fluorescence-Activated Cell Separator (FACS), PCR, and gel electrophoresis c) The ability of clioquinol to alter differential protein expression by <i>S. cerevisiae</i> cells was determined using MALDI TOF-MS technology by comparing the protein profile of yeast exposed to the substance with that displayed for untreated yeast cells (control) d) Finally, it was evaluated the effect of clioquinol on TDH3 gene expression in <i>S. cerevisiae</i> cells, in which mRNA from the yeast cells was analyzed using RT-PCR (Real-Time Polymerase Chain Reaction)	a) Acting of clioquinol on the cell cycle of the yeast studied, which strongly contributes to the consolidation of knowledge about the mechanism of action of clioquinol on fungal cells b) Revealed clioquinol as a fungistatic agent, inhibiting fungal cell growth in the G2/M phase of its cell cycle

Table 1 (continued)

References	Species	Methodology	Findings
You et al. (2018)	<i>Candida albicans</i> <i>Candida tropicalis</i> <i>Candida krusei</i> <i>Candida guilliermondii</i> <i>Malassezia furfur</i> <i>Malassezia globosa</i> <i>Malassezia sympodialis</i> <i>Trichophyllum rubrum</i> <i>Trichophyllum interdigitale</i> <i>Microsporium canis</i> <i>Sporothrix globosa</i> <i>Fusarium solani</i> <i>Aspergillus terreus</i> <i>Trichoderma harzianum</i>	a) The activity of 3% clioquinol and other antifungal agents (terbinafine, ketoconazole, bifonazole, triamcinolone acetate, econazole, natifine, fluconazole) was determined against the different species of fungus previously mentioned, by performing agar diffusion disk, microdilution and broth dilution for each species of fungus studied b) The antibacterial activity of clioquinol at 3% was determined, as well as for the other antifungals tested in the experiment. The techniques employed in that analysis were the same for the antifungal activity analyses, whose parameters were adapted for bacterial assays. The compounds in question were then tested against representative strains of bacteria: <i>Staphylococcus aureus</i> , <i>S. epidermidis</i> , <i>Escherichia coli</i> , <i>Propionibacterium acnes</i>	a) A broad notion of the spectrum of action of clioquinol and the other antifungals discussed in the study b) Clioquinol, in the 3% concentration, showed itself capable of inhibiting the growth of most of the fungi against which it was tested. For the species, <i>C. tropicalis</i> and <i>C. guilliermondii</i> , the antifungal activity of clioquinol proved to be superior to all the other antifungals analyzed in pharmaceutical formulations
Alvarez and Sanhueza (2016)	<i>Scedosporium dehoogii</i>	a) Fungal structures were obtained from 84 soil samples collected from 14 different regions of Chile; b) DNA was extracted from the isolated fungal colonies with the E.Z.N.A.® Fungal DNA mini kit; PCR with Primer Thermal Cycler (Technique, UK) primers Cal 1 and Cal2 for the calmodulin region and primers Tub2F and Tub2R for the β -tubulin gene from genomic sequences characteristic of <i>S. dehoogii</i> c) Phylogenetic analysis was performed, using the Maximum Parsimony (MP) method d) A susceptibility test to antifungal agents was carried out by microdilution of filamentous fungi protocol in the CLSI (Institute for Clinical and Laboratory Standards) document M38-A2	a) Extensive genetic variety of <i>S. dehoogii</i> strains in the test for in vitro susceptibility to antifungal agents, in which clioquinol showed MIC values ($\mu\text{g/mL}$) between 0.5–1. Therefore, the compound showed potency similar to that of azole drugs (0.12–2 $\mu\text{g/mL}$) and higher than that of echinocandins (0.12–4 $\mu\text{g/mL}$)

Table 1 (continued)

References	Species	Methodology	Findings
Laskaris et al. (2016)	<i>Aspergillus fumigatus</i>	<p>a) Three successive dilutions of clioquinol solution to obtain the final concentration of 0.5 mM clioquinol in 10% DMSO</p> <p>b) Conidia of a genetically modified strain (strain 2/7/1) of <i>Aspergillus fumigatus</i>, containing <i>Photinus pyralis</i> luciferase enzyme gene, were incubated for 7 days in 2% malt agar and later recovered by vortexing in 0.01% aqueous Tween 20 solution</p> <p>c) Homogeneous conidia suspension obtained by filtration of the recovered conidia on a 40 m pore membrane</p> <p>d) Seeding of 5×10^4 conidia in a 24-well plate</p> <p>e) Susceptibility testing on <i>A. fumigatus</i> strain 2/7/1 to clioquinol and other chelators (EDTA, DEDTC, DTPA, EDDA, TPEN, and phenanthroline) by bioluminescence detection via IVIS 100 system</p> <p>f) Murine model of invasive pulmonary aspergillosis, monitored by bioluminescence via IVIS 100 system in 165 mice</p> <p>g) The experiments performed on the mice were divided between:</p> <p>g.a) Extraction and homogenization of lungs from dead mice for determination of IL-6 concentration (an inflammatory mediator);</p> <p>g.b) Intraperitoneal administration of clioquinol at 15 mg/Kg/day and 30 mg/Kg/day and solutions prepared with the other chelating agents used in the study</p>	<p>a) Comprehensive profiling of clioquinol and other chelating compounds, in vivo, employing the substances against a murine model of aspergillosis</p> <p>b) Exploration of the heavy metal chelating property of clioquinol (especially Zn) as part of its mechanism of antifungal action, which is still poorly addressed by articles on antimicrobials</p> <p>c) Optimal activity for clioquinol evidenced by MIC-0 and MIC-2 values equal to 6 mg/L (in this article, MIC-0 is the lowest value required for 95% reduction in hypha bioluminescence, while MIC-2, the lowest value for 50% reduction in hypha bioluminescence) d) Clioquinol decreased or did not significantly alter mouse survival at doses evaluated, and clioquinol was abandoned in this paper</p>
Dunmade et al. (2012)	<i>Trichophyton mentagrophytes</i>	<p>a) A 30-year-old patient presenting with coryza undergoes bilateral nasal polypectomy and inferior meatal antrostomy. The physicians find a pale, shiny mass and grayish materials in both the patient's nasal and antral cavities</p> <p>c) An aspirate is taken from the patient's nasal and antral cavities, so the material is subjected to histopathological studies, which reveal <i>T. mentagrophytes</i> infection</p> <p>d) The individual undergoes conventional treatment in the region with 250 mg terbinafine daily for two weeks, with no remission of symptoms</p> <p>e) The patient is then submitted to experimental treatment with a combination of flumethasone and clioquinol, administered intra-antral weekly</p> <p>f) The patient presented complete remission of the symptoms</p>	<p>a) Intra-antral use of clioquinol in clinical practice against fungal rhinosinusitis, a public health problem in Nigeria</p> <p>b) The efficacy of clioquinol in this case was presented</p>

Table 2 Main findings pertinent to antibacterial activity studies of clioquinol.

References	Species	Methodology	Findings
Chan et al. (2020)	<i>Pseudomonas aeruginosa</i> <i>Acinetobacter baumannii</i>	a) The ability of clioquinol and the other antimicrobials to form iron complexes (by adding FeCl ₃) was evaluated. These iron complexes are visualized by the intensity of the red coloration of the reaction medium, whose absorbance was measured by spectroscopy b) The ability of clioquinol and other antimicrobials to synergize with thioestreptone against <i>P. aeruginosa</i> and <i>A. baumannii</i> inoculums, obtained from clinical isolates, was verified by a 3D checkerboard test. The same test was performed against a hyper-resistant mutant strain of <i>P. aeruginosa</i>	a) The paper proposed optimizing the antimicrobial profile of a natural compound (thioestreptone) by combining it with other molecules of proven efficacy and safety b) At 1 µg/mL concentration, clioquinol showed strong synergy with thioestreptone, effectively inhibiting the growth of <i>A. baumannii</i>
Magallon et al. (2019)	<i>Acinetobacter baumannii</i>	a) The enzymatic acetylation capacity of the <i>A. baumannii</i> strains was tested using the phosphocellulose paper binding assay, with Acetyl Coenzyme A and Amikacin as the substrate for the reactions b) Time-kill and growth inhibition assay were performed for clioquinol against the three strains of <i>A. baumannii</i> , at indicated concentrations of amikacin, ionophore and zinc c) Using the LIVE/DEAD Kit to determine Cell Viability/Cytotoxicity, cytotoxicity assays were carried out for the evaluated molecules (in serial dilutions in DMSO) against <i>A. baumannii</i>	a) Shows the capacity of clioquinol and pyrrithione to complex with ions such as Zn ⁺² and Cu ⁺² , considering this property as part of the mechanism of action of the substances b) According to the results of the Time-Kill test and cytotoxicity curves for clioquinol against the three tested strains of <i>A. baumannii</i> , the compound showed to be excellent candidate as antimicrobial adjuvant to reduce amikacin resistance
Blanco et al. (2018)	<i>Stenotrophomonas maltophilia</i>	a) <i>S. maltophilia</i> strains PBT02, PBT03, and PBT10, which are reporter strains (encoding different efflux pumps and resistance mechanisms of the bacterium), were used b) Combining the use of Biolog Phenotype Microarrays technology with RT-PCR, clioquinol (and other antimicrobials) was evaluated to induce the expression of the <i>smeVWX</i> and <i>smeYZ</i> genes consequently, of efflux pumps	a) The work revealed that the mechanism of bacterial resistance induction by clioquinol is selective. The compound was a strong resistance inducer of the studied <i>S. maltophilia</i> strain PBT02
Salah and Faergemann (2015)	<i>Staphylococcus aureus</i> <i>Streptococcus A</i> <i>Streptococcus B</i> <i>Streptococcus C</i> <i>Streptococcus G</i>	a) A retrospective comparative analysis was performed on patients diagnosed with atopic dermatitis and impetigo at the Department of Dermatology, Sahlgrenska University Hospital. They were positive for <i>S. aureus</i> and <i>Streptococcus</i> ssp. skin swabs from 2005 to 2011 b) These patients were divided into 4 groups, which were compared in terms of age, sex, diagnosis, and choice of antimicrobial treatment	a) Use of clioquinol in clinical practice, in treating atopic dermatitis and impetigo (in association with betamethasone) in the Betnovat® topical formulation, which proved to be the main choice of topical treatment for these pathologies b) According to the study, the synergistic effect of the anti-inflammatory compound and the antimicrobial compound makes the Betnovat® formulation the preferable choice in treating infected dermatoses

Table 2 (continued)

Antibacterial clioquinol			
References	Species	Methodology	Findings
Majumdar et al. (2013)	<i>Klebsiella pneumoniae</i>	<p>a) The assays were performed with several strains of <i>K. pneumoniae</i>, including: a deleterious mutant for the <i>raraA</i> gene and the other naturally containing the <i>raraA</i> gene</p> <p>b) Growth curves were performed for strains of <i>K. pneumoniae</i> in the presence of 1% and 5% of Sodium Dodecyl Sulfate (SDS), and in the presence of 60 µg/ml of clioquinol</p> <p>c) The Biolog system was used for phenotypic monitoring of the growth of the two studied strains of <i>K. pneumoniae</i>, in presence of clioquinol</p>	<p>a) Elucidated the role of the <i>raraA</i> gene, present in <i>K. pneumoniae</i>, which encodes numerous resistance mechanisms to multiple antimicrobial drugs</p> <p>b) Presented the possible mechanism by which <i>raraA</i> confers clioquinol resistance to carrier bacteria: increased expression of nitric oxide synthase, since NO acts as an endogenous attenuator of the pharmacological action of clioquinol</p>
Loock (2012)	<i>Proteus</i> ssp. <i>Pseudomonas aeruginosa</i> <i>Staphylococcus aureus</i>	<p>a) The trial consisted of a single-blind randomized controlled trial, following the CONSORT group guidelines (http://www.consort-statement.org)</p> <p>b) Patients over 6 years of age with otorrheal symptoms diagnosed with chronic otitis media at the Tygerberg Hospital Otolaryngology Clinic (South Africa) were included in the study. These were divided into 3 groups</p> <p>c) Randomization was generated via a computer program (Randomisation.com), through which the pharmacist was instructed to dispense numbered envelopes (containing the treatment) according to the randomized sequence generated by the computer. Following this sequence, the nurses administered the treatment contained in each envelope to the study participants</p> <p>d) The treatments evaluated included 1% acetic acid, boric acid (50 g), ciprofloxacin hydrochloride eardrops 3 mg/mL, Quadriderm® (ointment composed of betamethasone valerate, gentamicin sulfate, tolnaftate, and clioquinol)</p>	<p>a) Even though Quadriderm® is considered the second line of treatment for patients with chronic otitis media, it proved to be the most effective among the treatment options tested, being able to provide relief from the symptoms presented by patients as early as the first application in the external auditory canal</p>

in *Klebsiella pneumoniae*, encoding numerous resistance mechanisms to multiple antimicrobial compounds. The experiment suggested that the *rarA* gene confers resistance to clioquinol to carrier bacteria by increasing nitric oxide synthase expression since NO acts as an endogenous attenuator of the pharmacological action of clioquinol.

Considering in vitro assays, Chan et al. (2020) evaluated the efficacy of the association of clioquinol with thiostrepton, relying on the heavy metal chelating capacity of the first compound to optimize the antibacterial activity of the second compound. According to the researchers, clioquinol, at a concentration of 1 µg/ml, together with thiostrepton, effectively inhibited the growth of *A. baumannii*. In this same line of study, there is Magallon et al. (2019) also emphasize the ability of clioquinol as well as pyrithione to complex with ions such as Zn⁺² and Cu⁺² as part of its antibacterial activity, reducing the levels of resistance in strains of *Acinetobacter baumannii*.

In retrospective analyses, Salah and Faergemann (2015) conducted a study with medical records of patients diagnosed with atopic dermatitis and impetigo at the Department of Dermatology, Sahlgrenska University Hospital. In these studies, clioquinol was used directly in clinical practice to treat these pathologies (in association with betamethasone in Betnovat® topical formulation). According to the authors, the synergistic effect of the anti-inflammatory compound and the antimicrobial compound guarantees the Betnovat® association's success in treating atopic dermatitis and impetigo. Finally, there is a single-blind randomized controlled trial by Loock (2012). The study has included patients over 6 years of age, presenting with otorrhea symptoms, diagnosed with Chronic Otitis Media at the Otolaryngology Clinic of Tygerberg Hospital in South Africa. In this study, Quadri-derm® (betamethasone, gentamicin, tolfanate and clioquinol) was considered a second-line treatment for patients with chronic otitis media. However, it proved to be the most effective of the treatment options tested, providing relief from symptoms in the first application to the external auditory canal.

Antiparasitic activity

The antiparasitic activity of clioquinol returned a total of six articles from the searches in the previously cited databases, being, therefore, as reported as the antibacterial activity of the drug.

Among the studies dealing with the antiparasitic activity of clioquinol, the one by Jong et al. (2014) stands out, which brings the use of the substance against *Dientamoeba fragilis* (Table 3), a protozoan estimated to be responsible for most cases of chronic or recurrent abdominal pain in schoolchildren in the U.S. and Europe (Jong et al. 2014). In contrast, there are the studies by Souza et al. (2019) and Tavares et al.

(2018), add fundamental data about the antiparasitic activity of clioquinol against different species of *Leishmania*, a known etiological agent of a neglected tropical disease of extreme impact in Latin America, either by the severity of the symptoms caused in affected patients, as well as by the number of vertebrate reservoirs that the etiological agent has (Moya-Salazar et al. 2021).

The study by Jong et al. (2014) consisted of a retrospective analysis, reviewing medical records of patients admitted to two different hospitals in the Netherlands; one group, consisting of patients admitted to the Hospital Jeroen Bosch, was diagnosed with chronic abdominal pain; another group is a control group consisting of patients admitted to the Psychiatric Hospital of Herlaarhof. Clioquinol improved symptoms in 62.5% of the treated patients that presented chronic abdominal pain caused by *D. fragilis*, and for 75% of these patients, no *D. fragilis* was detected after clioquinol treatment. Although these results are intriguing, the employment of clioquinol in the face of parasitosis should still be considered with caution, given the adverse effects already known for the substance (HUNSEL; NIEUWKOOP; STRICKER, 2017). Another retrospective study was the Schierenberg et al. (2019) group, which analyzed medical records of patients admitted to various health services with the diagnosis of gastroenteritis. 8.8% of the cases of gastroenteritis were treated with antimicrobials and clioquinol was used in 5% of the treatments. Although clioquinol is no longer available for systemic use, it is still used in some countries, such as the Netherlands, as a manipulated drug in the treatment of amoebiasis and *Dientamoeba fragilis* infections following strict recommendations to prevent the deposition of clioquinol in patients' bodies (Hunsel et al. 2017).

Souza et al. (2019) ascertained the antiparasitic activity of clioquinol against *Leishmania amazonensis* and *Leishmania infantum*, finding excellent in vitro antileishmanial activities (EC₅₀) (Table 3) against promastigote forms of *L. amazonensis* (7.90 ± 0.65 µM) and *L. infantum* (4.45 ± 0.98 µM), as well as against axenic amastigote forms of the parasites (2.27 ± 0.44 µM, for *L. amazonensis*; 3.65 ± 0.25 µM, for *L. infantum*). The researchers also determined a high selectivity index for both promastigote (77.63) and axenic amastigote (270.16) forms of *L. amazonensis*, as well as for promastigote (137.81) and axenic amastigote (168.0) forms of *L. infantum*. In a similar approach, Tavares et al. (2018) also found strong in vitro antileishmanial activities (EC₅₀) against promastigote forms of *L. amazonensis* (2.55 ± 0.25 µg/mL) and *L. infantum* (1.44 ± 0.35 µg/mL), as well as against axenic amastigote forms of the parasites (1.88 ± 0.13 µg/mL, for *L. amazonensis*; 0.98 ± 0.17 µg/mL, for *L. infantum*). The group further found high selectivity for clioquinol for both promastigote (99.9) and axenic amastigote (135.6) forms of *L. amazonensis*, as well as for promastigote (177.1) and axenic amastigote (260.1) forms of *L. infantum*. Unlike

Table 3 Main findings pertinent to antiparasitic activity studies of clioquinol

References	Species	Methodology	Findings
Tavares et al. (2020)	<i>Leishmania infantum</i>	<p>a) BALB/c female mice ($n = 60$; 12 per group), infected with promastigotes of <i>L. infantum</i> by subcutaneous injection, were divided into five groups: group 1 (control)—received saline solution subcutaneously; group 2—received empty Pluronic F127 mycelia; group 3—received miltefosine; group 4—received clioquinol; finally, group 5—received clioquinol incorporated into the Pluronic F127 mycelial system</p> <p>b) Half of the animals were euthanized 15 days after treatment to evaluate parasitological and immunological parameters</p> <p>c) Parasitism was investigated in the spleen, liver, and bone marrow of the animals belonging to the five groups by the dilution method</p> <p>d) The parasite load in the spleen of the animals was evaluated by RT-PCR</p> <p>e) The cytokine profile exhibited by CD4 + and CD8 + T cells of the animals belonging to the different groups was determined by flow cytometry</p> <p>f) The IgG1 and IgG2a anti-<i>L. infantum</i> produced by the animals was quantified by ELISA to determine the animals' humoral response</p>	<p>a) Clioquinol exhibited more significant antileishmanial activity, evidenced by the greater amounts of IL-12 and IFN-γ produced by the treated animals relative to control animals and those treated with empty mycelia alone</p> <p>b) Incorporated into the Pluronic F127 micellar system, clioquinol had the highest antileishmanial activity superior to all other treatments administered</p> <p>c) The amounts of nitrite produced by the animals were also higher in the groups treated with clioquinol and with the compound incorporated into the micellar system relative to the other treatments administered in the experiment</p>
Nunes et al. (2019)	<i>Plasmodium falciparum</i>	<p>a) Molecular targets were selected, whose three-dimensional structures were obtained from the PDB database, using the keyword "<i>Plasmodium falciparum</i>"</p> <p>b) Using the TDR Targets platform, the researchers have determined the ability of small compounds to bind with high affinity to the molecular targets of <i>P. falciparum</i> previously selected</p> <p>c) Data from the BraMMT database was evaluated against antimalarial compounds approved by the World Health Organization (WHO). With the help of the OCTOPUS engine software, the prediction of the correct molecular targets for the compounds was performed</p>	<p>a) Presented clioquinol as a highly active and selective compound against <i>P. falciparum</i> in vitro, with an IC₅₀ (Inhibitory Concentration for 50% of Cells) of 0.56 μM and a high Selectivity Index (SI) of 178.6</p> <p>b) A value of -19.41 kcal/mol suggests that clioquinol is also a potential protease inhibitor in <i>P. falciparum</i></p>

Table 3 (continued)

References	Species	Methodology	Findings
Souza et al. (2019)	<i>Leishmania amazonensis</i> <i>Leishmania infantum</i>	<p>a) The Effective Concentration for 50% of the Maximum Effect (EC₅₀) for clioquinol and amphotericin B against promastigotes and axenic amastigotes of <i>L. amazonensis</i> and <i>L. infantum</i> was determined via the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] method</p> <p>b) The cytotoxicity of clioquinol and amphotericin B was measured against murine macrophages and human erythrocytes, determining the Cytotoxic Concentration 50% (CC₅₀), as well as the Inhibitory Concentration on 50% of Red Blood Cells (RBC₅₀), for each one of the substances</p> <p>c) The efficacy of the studied compounds was evaluated against macrophages infected with <i>L. amazonensis</i> and <i>L. infantum</i>, counting the number of amastigotes per infected cell by optical microscopy</p> <p>d) The efficacy of clioquinol and amphotericin B was studied in mice subcutaneously infected with promastigote forms of <i>L. amazonensis</i></p> <p>e) The immune response of both the test substance-treated animals and the positive control group (subcutaneous saline injection) was investigated by determining the amount of anti-leishmania IgG1 and IgG2a in spleen cells of both groups of animals</p> <p>f) The organic toxicity of the test substances was evaluated by monitoring the renal and hepatic functions of the treated animals and the positive control group</p>	<p>a) Clioquinol exhibited excellent in vitro antileishmanial activities (EC₅₀) against promastigote forms of <i>L. amazonensis</i> (7.90 ± 0.65 μM) and <i>L. infantum</i> (4.45 ± 0.98 μM), as well as against axenic amastigote forms of the parasites (2.27 ± 0.44 μM, for <i>L. amazonensis</i>; 3.65 ± 0.25 μM, for <i>L. infantum</i>)</p> <p>b) Clioquinol also exhibited low toxicity against murine macrophages (CC₅₀ 613.27 ± 20.53 μM) and human erythrocytes (RBC₅₀ 1409.85 ± 64.35 μM)</p> <p>c) Clioquinol also showed high selectivity index for both promastigote (77.63) and axenic amastigote (270.16) forms of <i>L. amazonensis</i>, as well as for the promastigote (137.81) and axenic amastigote (168.0) forms of <i>L. infantum</i></p>
Schierenberg et al. (2019)	<i>Blastocystis hominis</i> <i>Cryptosporidium</i> spp. <i>Dientamoeba fragilis</i> <i>Entamoeba histolytica/dispar</i> <i>Giardia</i> spp.	<p>a) The study comprised a retrospective analysis of patient data seen by general practitioners in various primary health care settings via the Julius General Practitioner Network (JGPN), an electronic medical record system adopted in the Netherlands</p> <p>b) The researchers searched medical records of patients diagnosed with gastroenteritis from 2013 to 2014</p> <p>c) Information was collected for each patient, such as age, gender, number of medical visits per episode, comorbidities, immunosuppressive disorders, or occurrence of immunosuppressive therapy</p> <p>d) Finally, the type of therapy administered to each patient diagnosed with gastroenteritis was ascertained in cases which a drug was used</p>	<p>a) Therapeutic interventions employed in clinical practice and their rate of effectiveness</p> <p>b) In 5% of cases of gastroenteritis treated with the use of antimicrobials, clioquinol was used</p>

Table 3 (continued)

References	Species	Methodology	Findings
Tavares et al. (2018)	<i>Leishmania amazonensis</i> <i>Leishmania infantum</i>	<p>a) Representative strains of <i>Leishmania amazonensis</i> and <i>Leishmania infantum</i> were grown in Schneider's medium and subsequently identified by light microscopy via Giemsa staining</p> <p>b) In vitro growth inhibition of both parasite species was assessed in the presence of clioquinol (0–20.0 µg/mL) for both promastigotes and axenic amastigotes via the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] method</p> <p>c) The cytotoxicity of clioquinol and amphotericin B was measured against murine macrophages and human erythrocytes, determining the Cytotoxic Concentration on 50% (CC₅₀), as well as the RBC₅₀ (Inhibitory Concentration on 50% of Red Blood Cells), for each one of the substances</p> <p>d) The inhibition of infection in murine macrophages was evaluated using promastigotes of <i>L. amazonensis</i> pre-incubated with clioquinol and amphotericin B</p> <p>e) The effect of the substances tested was observed on morphology and cell volume and in the mitochondrial function of promastigote cells of <i>L. amazonensis</i></p> <p>f) The in vivo toxicity of clioquinol was studied in BALB/c rats, monitoring the cardiac, hepatic, and renal functions of the animals</p>	<p>a) Clioquinol exhibited excellent in vitro antileishmanial activities (EC₅₀) against promastigote forms of <i>L. amazonensis</i> (2.55 ± 0.25 µg/mL) and <i>L. infantum</i> (1.44 ± 0.55 µg/mL), as well as against axenic amastigote forms of the parasites (1.88 ± 0.13 µg/mL, for <i>L. amazonensis</i>; 0.98 ± 0.17 µg/mL, for <i>L. infantum</i>)</p> <p>b) Clioquinol also exhibited low toxicity against murine macrophages (CC₅₀ = 254.90 ± 22.60 µg/mL) and human erythrocytes (RBC₅₀ = 488.90 ± 19.50 µg/mL), as well as high selectivity index for both promastigote (99.9) and axenic amastigote (135.6) forms of <i>L. amazonensis</i>, as well as for promastigote (177.1) and axenic amastigote (260.1) forms of <i>L. infantum</i></p> <p>c) The percentages of infection of murine macrophages by promastigotes of <i>L. amazonensis</i> and <i>L. infantum</i>, pretreated with clioquinol, were 16.5% ± 3.5% and 19.8% ± 1.9%, respectively</p> <p>d) At a subcutaneous dosage of 50 mg/kg/day for 10 days, clioquinol caused no significant alteration in the cardiac, hepatic, and renal functions of the BALBc mice tested</p>
Jong et al. (2014)	<i>Dientamoeba fragilis</i>	<p>a) The work consisted of a retrospective analysis study reviewing medical records of patients admitted to two different hospitals in the Netherlands</p> <p>b) From April 2011 to April 2013, 132 patients with chronic abdominal pain, aged 8 to 18 years, admitted to Jeroen Bosch Hospital, were analyzed</p> <p>c) The control group included 77 patients aged 8–18 years admitted to the Herlaarhof Psychiatric Hospital</p> <p>d) In the <i>D. fragilis</i> positive group presenting chronic abdominal pain, the ability of metronidazole or clioquinol to eradicate the parasite infection (evaluated by PCR of the patients' fecal samples) was determined</p>	<p>a) Clioquinol was able to improve symptoms in 62.5% of treated patients presenting <i>D. fragilis</i> and chronic abdominal pain and completely eradicate <i>D. fragilis</i> infection (75% of patients) in these patients</p>

the previous study, in this work, the authors have determined the percentage of infection of murine macrophages by promastigotes pretreated with clioquinol, which ranged from $16.5\% \pm 3.5\%$ for *L. amazonensis*, $19.8\% \pm 1.9\%$ for *L. infantum*. In a subsequent study, Tavares et al. (2020) sought to determine the optimization of the antiparasitic activity of clioquinol by including the substance in a Pluronic F127 micellar system. In this study, the group showed higher production of IL-12 and IFN- γ in animals infected with *L. infantum*, which were treated with clioquinol, than control animals (which received saline solution) and animals that received the empty mycelia. Clioquinol had the highest antileishmanial activity, superior to all other treatments administered. With clioquinol incorporated into the micellar system Pluronic F127, the amount of nitrite produced by the animals given this form of treatment was greater relative to the nitrite production by the animals given the other forms of treatment.

Antiviral activity

In a SARS-CoV-2 pandemic scenario, since the year 2020, the race for substances capable of containing the advance of the virus has become more intense, Olaleye et al. (2021) have dared to venture into a little-explored property for clioquinol: the antiviral one. The authors tested clioquinol and other analogs against SARS-CoV-2 infection in Vero E6 cells from African green monkeys (Table 4) by combining luminescence with high-performance screening (HTS). The researchers found potent antiviral activity for clioquinol, evidenced by IC_{50} of $12.62 \mu\text{M}$, in high-throughput cell screening against SARS-CoV-2. The substance effectively inhibited the exopeptidase activity of the human ACE2 receptor protein, evidenced by an IC_{50} value of $5.36 \mu\text{M}$. The group also observed the effect of clioquinol and analogous compounds on the interaction of the human ACE2 receptor protein with the SARS-CoV-2 viral receptor protein Spike using a modified ELISA assay. Not a definitive cure, nor a "miracle cure", but indeed an excellent substance to be investigated in in vivo models for the treatment of SARS-CoV-2 infections (Olaleye et al. 2021).

Antiprionic activity?

Prion diseases include a group of fatal neurodegenerative diseases affecting humans and animals. These illnesses are caused by infectious protein particles resistant to inactivation by many procedures that modify nucleic acids, particles known as "prions" (Mustazza et al. 2020). The group Mustazza et al. (2020) brought to light a series of data concerning small molecules with antiprionic activity, compounds capable of increasing the survival rate of animal models infected

Table 4 Main findings pertinent to antiviral activity studies of clioquinol

References	Species	Methodology	Findings
Olaleye et al. (2021)	SARS-CoV-2	<p>a) The efficacy of clioquinol and analogs against SARS-CoV-2 infection in Vero E6 cells from African green monkeys was assessed by combining luminescence with high-throughput screening (HTS)</p> <p>b) The cytotoxic effect of clioquinol and its derivatives on Vero E6 cells was evaluated using Cell Titer-Glo Luminescent Viability Assay</p> <p>c) The effect of clioquinol and derivatives on the exopeptidase activity of ACE2 protein was determined using an adapted fluorometric assay</p> <p>d) The effect of clioquinol and analogous compounds on the interaction of the human ACE2 receptor protein with the SARS-CoV-2 viral receptor protein Spike was observed using a modified ELISA assay</p>	<p>a) Clioquinol exhibited potent antiviral activity, evidenced by IC_{50} of $12.62 \mu\text{M}$, in the high-performance cell screen against SARS-CoV-2</p> <p>b) In the luminescent cell viability assay, clioquinol showed $CC_{50} > 30 \mu\text{M}$ and minimal cell viability of 61.83%, denoting low cytotoxicity</p> <p>c) Clioquinol was also shown to effectively inhibit the exopeptidase activity of the human ACE2 receptor protein, evidenced by the $IC_{50} = 5.36 \mu\text{M}$</p>

with prions. In one of these works, clioquinol, allied to its Cu/Zn chelating capacity, extended the survival time of animals infected with prions by 60% (Mustazza et al. 2020, p. 5453, apud Ponti et al. 2004, p. 307). This result is because prion proteins play an essential role in copper metabolism, which is also related to superoxide dismutase activity. Clioquinol showed good potential antipryonic activity in this study. The authors hope that this study will stimulate more in vitro trials with new molecules with antipryonic activity. The perspective is that more studies for antipryonic activity of the substance and its derivatives will emerge in the future as knowledge about molecular targets (more precisely, genes encoding potential prion proteins) grows (Mustazza et al. 2020).

Toxicological data for clioquinol

From the mid 1958s through the 1970s, Subacute Myelo-optic Neuropathy (SMON) afflicted many Japanese patients. This disease began with abdominal pain and diarrhoea and ended with degeneration of the peripheral nerves, leading to severe impairment of the patients' vision, communication, and gait. Pesticide poisoning, metabolic disorders, vitamin deficiencies, bacterial and viral infections, many were the hypotheses that puzzled doctors. However, researchers have found that only patients taking clioquinol developed the disease. Later, they have the ability of clioquinol to chelate heavy metals such as Fe(III) as one of the possible causes behind the onset of SMON (Perez et al. 2019). The pathophysiology of SMON is not entirely elucidated (even after 4 decades of discontinuing clioquinol in Japan). However, clinicians observed that SMON symptoms were more severe and occurred more frequently in patients with natural zinc deficiency or natural vitamin B12 deficiency. Suppose there are physiological and genetic conditions for predisposition to the unwanted effects of clioquinol. In that case, patients could use clioquinol with great caution by monitoring serum metal ion levels and concomitant administration with antioxidants, as long as there is an indication from the health surveillance agencies in this sense (Leuci et al. 2020).

The study by Kawamura et al. (2014) sought to unravel the molecular mechanism behind the neurotoxicity of clioquinol. For this, the researchers have employed the human cell line SH-SY5Y (neuroblastoma). The most important findings obtained by the authors were: (a) The induction of apoptotic cell death, as well as the intracellular increase of reactive oxygen species (ROS) promoted by clioquinol; (b) Inhibition of superoxide-dismutase-1 (SOD1) enzymatic activity. However, in a feedback mechanism, exogenous SOD1 was shown to reduce ROS production and optimize the viability of clioquinol-treated cells (Kawamura et al. 2014). In a similar approach, Kuru (2021) emphasized the similarities between SMON and copper deficiency

myelopathy, pointing out that copper is the primary cofactor of the enzyme superoxide-dismutase-1 (SOD1) and that clioquinol, by chelating Cu, would promote intracellular Zn deposition in neuronal cells. Added to this factor, the author raises the possibility of polymorphism of the enzyme NADH quinone oxidoreductase (NQO1) among the Japanese population, which, although not fully elucidated, would explain why SMON occurred virtually only in Japan (Kuru 2021). Fukui et al. (2015) observed that at 1 μM concentration, clioquinol reduced acetylated histone levels and a histone deacetylase inhibitor was able to prevent the neuronal cell damage caused by clioquinol (Fukui et al. 2015).

In a retrospective analysis of data from the Dutch Poison Information Center (DPCI), Van Velzen and De Vries (2017) revealed that the center received 51 cases between 2011 and 2015 dealing with intoxication related to oral exposure to clioquinol. However, the researchers ascertained that 50 of these patients had become intoxicated with the substance due to medication administration errors. The majority of these patients ($n=25$) belonged to the age group of 0–4 years, followed by patients aged 5–12 years ($n=17$) and adults ($n=8$). Regarding the origin of medication errors, the researchers have found that caregivers of the patient caused 32 cases, the patients themselves caused 7 cases, physicians caused 6 cases, pharmacists caused 4 cases, and a physician or a pharmacist may have caused 1 case. The study postulated that the errors caused by the patients or caretakers were due to the administration of overdoses, in some cases about ten times higher than the recommended dosage.

Of the 51 patients seen by the DPCI, 7 patients showed neurotoxicity (dizziness, dysarthria). In contrast, the errors caused by physicians or pharmacists originated from errors in the prescribed dose or in reading the label. Finally, the paper concludes that many of the cases of intoxication with clioquinol stem from overdoses and that to prevent serious adverse effects, such as neurotoxicity, the health professionals need to make a thoughtful work in instructing patients and caregivers about the correct way to take the drug, as well as an effective role of pharmacists to avoid prescription errors (Van Velzen and De Vries 2017).

Nitroxoline

Antifungal activity

Candida auris is an emerging species that is rapidly spreading worldwide, causing many nosocomial infections and bringing a laborious dilemma to physicians regarding resistance to multiple antifungal drugs. Within this context, Fuchs et al. (2021) performed susceptibility testing of 35 fungal isolates of *C. auris* to nitroxoline and 4 other antifungals (fluconazole, voriconazole, amphotericin B, and anidulafungin) employed for comparison (Table 5). In the study,

the authors found MICs for nitroxoline in the range of 0.125 to 1 mg/L, which denotes the excellent antifungal activity of nitroxoline against the *C. auris* strains employed resistance to several antifungal drugs.

Another study to address the antifungal activity of nitroxoline was that of Cherdtrakulkiat et al. (2016), in which the MIC of nitroxoline was determined against *Saccharomyces cerevisiae* and *Candida albicans*, obtaining a value of 42.07 μM for both fungal species (an indication of moderate antifungal activity). A comparison of the results obtained by Fuchs et al. (2021) with those displayed by Cherdtrakulkiat et al. (2016) allows us to infer that the antifungal activity of nitroxoline is species-dependent. In addition, the fact that nitroxoline is an FDA (Food and Drugs Administration) approved drug will facilitate future clinical trials using the substance for the management of urinary tract infection or colonization not only by *C. auris*, but by other fungal species (Fuchs et al. 2021).

Antibacterial activity

Unlike clioquinol, the activity best reported in the literature for nitroxoline was antibacterial activity, consisting of 14 articles from Pubmed, Embase, and Web of Science databases. However, similar to what occurred for the antifungal activity of clioquinol, the articles dealing with the antibacterial activity of nitroxoline are divided into articles

focused on one or a few microorganisms, such as the one by Ancuta et al. (2016), the one by Fuchs et al. (2019) and that of Kresken and Körber-Irrgang (2014), as well as articles investigating the spectrum of action of the drug against several microorganisms, such as that of Cherdtrakulkiat et al. (2016) and Kudera et al. (2020).

Using the broth microdilution method, Abouelhasan et al. (2017) measured the minimum inhibitory concentrations (MICs) against different bacterial species (Table 6) and, using the Calgary device, subsequently measured the minimum concentration for biofilm eradication (MBEC). The authors found a broad-spectrum antibacterial activity for nitroxoline, with MICs of 4.69–6.25 μM for *A. baumannii*, 12.5 μM for *E. coli*, 9.38–25 μM for *S. aureus*, 18.8 μM for *S. epidermidis*. As for biofilm eradication, nitroxoline was more active against *A. baumannii* (MBEC = 46.9–62.5 μM) biofilms and *E. coli* (MBEC = 62.5 μM) than for biofilms formed by the other bacterial species. In a similar study, Cherdtrakulkiat et al. (2016) obtained MICs of 42.07 μM for *S. aureus* and *S. epidermidis*, 21.03 μM for *Escherichia coli*, and 84.14 μM for *Pseudomonas aeruginosa* as well as *Klebsiella pneumoniae*. In a similar approach, Cherdtrakulkiat et al. (2019) investigated the effect of metal ions on the antimicrobial activity of nitroxoline. The authors concluded that Cu^{2+} and Fe^{3+} caused reduced activity, while Ca^{2+} , Mg^{2+} , and Mn^{2+} exerted no effect on the antibacterial activity of the compound. In addition, the group found an

Table 5 Main findings pertinent to the antifungal activity studies of nitroxoline

Antifungal nitroxoline			
References	Species	Methodology	Findings
Fuchs et al. (2021)	<i>Candida auris</i>	a) The susceptibility of 35 clinical isolates of <i>C. auris</i> to nitroxoline, fluconazole, voriconazole, amphotericin B, and anidulafungin was determined via the microdilution method recommended by EUCAST b) Similarly, the susceptibility of <i>C. auris</i> isolates to nitroxoline and four other antifungals were determined via the disk-diffusion method	(a) Cepa-dependent antifungal activity for nitroxoline b) Nitroxoline showed good antifungal activity against different isolates of <i>C. auris</i> (MIC = 0.125–1 mg/L)
Cherdtrakulkiat et al. (2016)	<i>Candida albicans</i> <i>Saccharomyces cerevisiae</i>	a) The antimicrobial activity of nitroxoline and eight derivatives of 8-hydroxyquinoline was determined against the previously mentioned microorganisms using the agar dilution method b) The 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay was performed to determine the antioxidant activity of nitroxoline and other compounds tested c) A cytotoxicity assay was carried out for nitroxoline and other studied substances using the MTT reduction method (3–4,5-dimethyl-thiazol-2-yl-2,5-diphenyltetrazolium bromide)	a) Nitroxoline also showed good antifungal activity, evidenced by the MIC value of 42.07 μM for <i>C. albicans</i> and <i>S. cerevisiae</i>

Table 6 Main findings pertinent to the antibacterial activity studies of nitroxoline

References	Species	Methodology	Findings
Kudera et al. (2020)	<i>Bacillus cereus</i>	<p>a) The MICs of nitroxoline and the other test compounds were evaluated against representative strains of the bacteria previously mentioned by the broth microdilution method</p> <p>b) A cytotoxicity assay was carried out to analyze the antiproliferative activity of nitroxoline and the other substances studied against normal and cancerous human intestinal cell lines, using the 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) method</p> <p>c) The selective toxicity calculation was performed to determine which substances would be selective against diarrheagenic and probiotic bacteria strains</p> <p>d) Finally, the correlation between the tested compounds' biological activities and their chemical structures was ascertained via the Principal Component Analysis (PCA) method</p>	<p>(a) Nitroxoline exhibited moderate to intense inhibitory activity against all diarrheagenic bacteria (\bar{x}-MIC = 12 ± 10 $\mu\text{g/mL}$). The compound also exhibited intense inhibitory activity against <i>B. cereus</i>, <i>Clostridium</i> species, <i>E. coli</i>, and <i>S. flexneri</i> (MICs = 2–4 $\mu\text{g/mL}$)</p>
	<i>Clostridium difficile</i>		
	<i>Clostridium perfringens</i>		
	<i>Enterococcus faecalis</i>		
	<i>Salmonella Enteritidis</i>		
	<i>Salmonella Typhimurium</i>		
	<i>Vibrio parahaemolyticus</i>		
	<i>Yersinia enterocolitica</i>		
	<i>Bacteroides fragilis</i>		
	<i>Bifidobacterium adolescentis</i>		
	<i>Bifidobacterium animalis</i> spp. <i>lactis</i>		
	<i>Bifidobacterium bifidum</i>		
	<i>Bifidobacterium breve</i>		
	<i>Bifidobacterium longum</i>		
Principe et al. (2020)	<i>Lactobacillus casei</i>	<p>a) MICs were determined for nitroxoline and other zinc chelators evaluated, against the bacterial species mentioned, using the broth microdilution method</p> <p>b) The interaction between meropenem and the tested zinc chelators (among them nitroxoline) was evaluated using the double qualitative diffusion-disc method</p> <p>c) Positive interactions, indicated by the diffusion-disc method, were investigated in detail via the checkerboard assay</p> <p>d) A time-kill assay was performed in Cation-Adjusted Müller-Hinton Broth (CAMHB)</p> <p>e) <i>Galleria mellonella</i> larvae were inoculated by injecting bacterial suspensions with an insulin syringe in the left pro-leg region</p> <p>f) Similarly, the toxicity of the test compounds against <i>G. mellonella</i> larvae was determined by injecting specific concentrations of the substances into the animals</p> <p>g) Finally, the ability of the zinc chelating substances to eradicate bacterial infections induced in <i>G. mellonella</i> larvae was evaluated, determining the viability of the larvae 120 h after the experiment</p>	<p>a) Nitroxoline showed MIC values from 1 to 4 mg/L for all bacterial species, except for <i>K. pneumoniae</i>, against which the disk-diffusion test showed inactivity</p> <p>b) Inoculated in <i>G. mellonella</i> larvae at 128 mg/L, nitroxoline did not increase hemocyte density compared to the larval control</p> <p>c) Nitroxoline showed intrinsic antibacterial activity in vivo, increasing larval survival time (except when infected with <i>K. pneumoniae</i>) beyond 120 h</p>
	<i>Lactobacillus reuteri</i>		
	<i>Lactobacillus rhamnosus</i>		
	<i>Klebsiella pneumoniae</i>		
	<i>Stenotrophomonas maltophilia</i>		
	<i>Chryseobacterium indologenes</i>		
	<i>Elizabethkingia meningoseptica</i>		

Table 6 (continued)

References	Species	Methodology	Findings
Cherdtrakulkiat et al. (2019)	<i>Escherichia coli</i> <i>Klebsiella pneumoniae</i> <i>Providencia rettgeri</i>	a) The antimicrobial activity of nitroxoline and two analogs of 8-hydroxyquinoline was determined against 56 isolates of the mentioned bacterial species by the microdilution method, recommended by the Clinical Laboratory Standards Institute (CLSI) b) By the same method, the minimum bactericidal concentration (MBC) was determined for nitroxoline and 8-hydroxyquinoline derivatives against representative strains of the mentioned bacterial species c) Also, by the microdilution method recommended by the CLSI, the activity of nitroxoline and the other compounds evaluated was determined in the presence of metal ions (Ca^{2+} , Mg^{2+} , Mn^{2+} , Zn^{2+} , Cu^{2+} , and Fe^{3+})	a) Nitroxoline, among all the compounds tested, was the one that presented the highest antibacterial activity against the species of bacteria in the experiment, presenting MICs in the range of 21.03–84.14 μM
Fuchs et al. (2019)	<i>Neisseria gonorrhoeae</i>	a) Clinical isolates of <i>N. gonorrhoeae</i> were collected between 2015 and 2018 from two German medical centers, Cologne and Bonn. Among these isolates, those with high MICs for penicillin (MIC 0.125 mg/L) were selected, resulting in 27 selected isolates b) The MICs for penicillin and cefotaxime against clinical isolates were determined by agar diffusion gradient (chocolate agar). The susceptibility of <i>N. gonorrhoeae</i> isolates to antimicrobials was interpreted based on EUCAST clinical cut-off points c) The activity of nitroxoline against <i>N. gonorrhoeae</i> was determined by agar dilution and agar disk-diffusion methods	a) The MICs of nitroxoline against different <i>N. gonorrhoeae</i> strains ranged from 0.125–4 mg/L
Fuchs and Hamprecht (2019)	<i>Klebsiella pneumoniae</i> <i>Escherichia coli</i> <i>Enterobacter cloacae</i> <i>Citrobacter freundii</i> <i>Proteus mirabilis</i> <i>Klebsiella oxytoca</i> <i>Klebsiella aerogenes</i> <i>Raoultella ornithinolytica</i>	a) The MICs of meropenem, imipenem, and ertapenem were determined against the addressed bacterial species by agar diffusion gradient using MIC test strips b) Finally, the MIC of nitroxoline against representative strains of the bacteria mentioned was determined by agar dilution method	a) Because the work involves several species of bacteria, it provides a broad notion of the spectrum of action of the substance tested c) Nitroxoline presented MIC values of 1–32 mg/L against representative strains of the bacterial species tested, which denotes optimal broad-spectrum antibacterial activity
Li et al. (2019)	<i>Bartonella henselae</i>	a) The growth curve of <i>B. henselae</i> was determined in a consistent stationary phase of modified Schneider medium, using the SYBR Green method b) The drug exposure assay was performed by resuspending the <i>B. henselae</i> stationary phase (5 days after the viability assay) in Eppendorf and recovering viable cells by centrifugation. Afterward, the resulting cells were seeded on blood agar plates for counting	a) After exposure of <i>B. henselae</i> stationary phase to nitroxoline, 6% viable cells remained b) The MIC in the range 0.31–0.63 $\mu\text{g/mL}$ confirms that nitroxoline has high potency against <i>B. henselae</i>

Table 6 (continued)

References	Species	Methodology	Findings
Valentine-King et al. (2019)	<i>Ureaplasma parvum</i> <i>Ureaplasma urealyticum</i> <i>Mycoplasma hominis</i> <i>Mycoplasma pneumoniae</i> <i>Mycoplasma genitalium</i>	a) The MIC for nitroxoline and the other test compounds were determined against the bacterial species previously mentioned, using the broth microdilution method and the agar dilution method b) Similarly, the minimum bactericidal concentration (MBC) was evaluated for nitroxoline and other test compounds against the bacterial species employed, using culture tubes	a) Nitroxoline proved to be more efficient against species of <i>Ureaplasma</i> spp., against which it presented MIC ₅₀ = 3.13 µM and MIC ₉₀ = 6.25 µM. Considering <i>Mycoplasma</i> species, nitroxoline presented MIC values of 12.5–50 µM b) Nitroxoline showed bactericidal effect against <i>U. parvum</i> , while it showed bacteriostatic effect for <i>U. urealyticum</i>
Hof and Juretschke (2019)	<i>Klebsiella pneumoniae</i> <i>Proteus mirabilis</i>	a) Case report: a 68-year-old patient with multi-morbidities contracted rectal colonization by multidrug-resistant bacteria b) After a coughing fit, the patient developed difficulty swallowing and was admitted to the hospital's pulmonary department. On X-ray imaging, however, there were no signs of aspiration pneumonia c) Clinical evaluation provided evidence of symptomatic urinary tract infection, supported by signs of urine turbidity, proteinuria, and massive leukocytosis triggered by an indwelling catheter d) Microbiological examination of midstream urine revealed the presence of two bacteria: <i>Klebsiella pneumoniae</i> and <i>Proteus mirabilis</i> . Susceptibility to both microorganisms was determined by the disc-diffusion test	a) Performance of the evaluated substances (among which nitroxoline) directly in clinical practice, in their pharmacological parameters b) The antibiogram showed that nitroxoline was the only substance active against both pathogens
Sobke et al. (2018)	<i>Klebsiella pneumoniae</i> <i>Enterococcus faecium</i> <i>Staphylococcus aureus</i> <i>Escherichia coli</i> <i>Proteus mirabilis</i> <i>Enterococcus faecalis</i>	a) The susceptibility test to nitroxoline (and other studied compounds) was performed against representative strains of the mentioned bacterial species, combining the VITEK®2 system methodology with double disk diffusion b) The susceptibility of the bacterial species to nitroxoline and nitrofurantoin was also tested utilizing disk diffusion c) The MIC for nitroxoline and nitrofurantoin was determined against the mentioned bacterial species by broth microdilution method, as recommended by EUCAST d) Also the researchers evaluated the minimum bactericidal concentration (MBC) for nitrofurantoin and nitroxoline against <i>E. coli</i> , <i>P. mirabilis</i> , and <i>E. faecalis</i> e) Time-kill test was performed for nitrofurantoin and nitroxoline against <i>E. coli</i> , <i>P. mirabilis</i> , and <i>E. faecalis</i> , using concentrations of 10, 50, and 200 mg/L of the compounds for each bacterial species	a) Among susceptible strains of bacteria, nitroxoline presented MICs in the range of ≤ 16 mg/L, while this value varied from 16–64 mg/L for resistant strains b) In the time-kill assay, nitroxoline reduced the number of viable cells of representative strains of the different bacterial species by magnitude of ≤ 2 log at a concentration of 10 mg/L c) In artificial urine, the MICs of nitroxoline varied from 0.25–2 mg/L in pH ranges from 5.5 to 7.5 against <i>E. coli</i>

Table 6 (continued)

References	Species	Methodology	Findings
Abouelhassan et al. (2017)	<i>Acinetobacter baumannii</i> <i>Pseudomonas aeruginosa</i> <i>Staphylococcus aureus</i> <i>Staphylococcus epidermidis</i> <i>Enterococcus faecium</i> <i>Escherichia coli</i>	a) The minimum inhibitory concentration (MIC) for nitroxoline was determined against the different strains of pathogenic bacteria studied using the broth microdilution method b) Using the Calgary device, the minimum concentration for biofilm eradication (MBEC) of nitroxoline was measured against the previously mentioned bacterial species c) The capacity of nitroxoline to remove biofilms of the different bacterial species studied, formed on fragments of pigskin (ex vivo model), was investigated d) MRSA-2 stationary cell death kinetics assay was performed for nitroxoline, against the bacterial species mentioned before e) The minimum biofilm inhibitory concentration (MBIC) for nitroxoline was measured against the different bacterial species	a) Nitroxoline demonstrated broad-spectrum antibacterial activity, showing MICs of 4.69–6.25 μM for <i>A. baumannii</i> , 12.5 μM for <i>E. coli</i> , 9.38–25 μM for <i>S. aureus</i> , 18.8 μM for <i>S. epidermidis</i> b) Regarding biofilm eradication, nitroxoline was more active against biofilms of <i>A. baumannii</i> (MBEC = 46.9–62.5 μM) and <i>E. coli</i> (MBEC = 62.5 μM) than for biofilms formed by the other bacterial species c) In an ex vivo model of wound infection by bacterial biofilms (with pigskin fragments), nitroxoline reduced the viability of biofilms formed by <i>A. baumannii</i> , <i>E. coli</i> , <i>S. aureus</i> , and <i>S. epidermidis</i> , with a 99% kill rate of viable biofilm cells
Cherdrakulkiat et al. (2016)	<i>Escherichia coli</i> <i>Klebsiella pneumoniae</i> <i>Serratia marcescens</i> <i>Salmonella Typhimurium</i> <i>Salmonella Choleraesuis</i> <i>Salmonella Enteritidis</i> <i>Shigella dysenteriae</i> <i>Morganella morganii</i> <i>Citrobacter freundii</i> <i>Plesiomonas shigelloides</i> <i>Aeromonas hydrophila</i> <i>Pseudomonas aeruginosa</i> <i>Pseudomonas stutzeri</i> <i>Shewanella putrefaciens</i> <i>Achromobacter xylosoxidans</i> <i>Staphylococcus aureus</i> <i>Staphylococcus epidermidis</i> <i>Micrococcus luteus</i> <i>Enterococcus faecalis</i> <i>Corynebacterium diphtheriae</i> <i>Bacillus subtilis</i> <i>Listeria monocytogenes</i> <i>Bacillus cereus</i>	a) The antimicrobial activity of nitroxoline and eight derivatives of 8-hydroxyquinoline was determined against the previously mentioned microorganisms using the agar dilution method b) The 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay was performed to determine the antioxidant activity of nitroxoline and other compounds tested c) A cytotoxicity assay was carried out for nitroxoline and other studied substances using the MTT reduction method (3–4,5-dimethyl-thiazol-2-yl-2,5-diphenyltetrazolium bromide)	a) Nitroxoline exhibited promising antimicrobial activity, evidenced by MIC values between 5.26–84.14 μM , these values being dependent on the species and strain of microorganism tested b) Nitroxoline exhibited low cytotoxicity, which is reflected in the value of CC_{50} = 88.14 \pm 2.11 μM

Table 6 (continued)

References	Species	Methodology	Findings
Ancuta et al. (2016)	<i>Staphylococcus aureus</i>	<p>a) Ceramic disks were prepared and then characterized using air and water weight method</p> <p>b) The ceramic disks were impregnated with nitroxoline and silver ions</p> <p>c) The ceramic discs were incubated with <i>Staphylococcus aureus</i> cultures at 37 °C for 24 h to evaluate the impregnated compounds' antibacterial activity by measuring the resulting halo of inhibition (disc diffusion method)</p>	<p>a) The nitroxoline-impregnated discs caused a halo of inhibition > 30 mm, an effect that remained even 24 h after removal of the discs from the Petri dish containing <i>S. aureus</i>, demonstrating strong antimicrobial activity</p>
Kresken and Körber-Irrgang (2014)	<i>Escherichia coli</i>	<p>a) The in vitro activity of nitroxoline was determined against 499 <i>E. coli</i> isolates from urine samples from different German laboratories</p> <p>b) MALDI Biotyper (for species confirmation) and susceptibility testing were performed at a central laboratory (Antiinfectives Intelligence)</p> <p>c) MICs for nitroxoline and other tested compounds were determined by the broth microdilution method as recommended by the International Organization for Standardization (ISO)</p>	<p>a) Nitroxoline showed a constant activity profile among the different isolated strains of <i>E. coli</i>, evidenced by MIC₅₀ = 2 mg/L and MIC₉₀ = 4 mg/L</p>
Wagenlehner et al. (2014)	<i>Escherichia coli</i> <i>Klebsiella pneumoniae</i> <i>Proteus mirabilis</i> <i>Staphylococcus saprophyticus</i>	<p>a) Six volunteers received 250 mg single dose nitroxoline, or 200 mg trimethoprim, in order to have their urinary inhibitory titers (UITs) and their urinary bactericidal titers (UBT) determined. Three healthy volunteers received 250 mg of nitroxoline three times a day to determine their urinary bactericidal kinetics (UBK)</p> <p>b) The MICs of nitroxoline and trimethoprim were determined by the broth microdilution method against representative strains of the aforementioned bacterial species</p> <p>c) The UIT and UBT of the volunteers were determined via the microdilution method. UBK was determined by bacterial counting at different times</p> <p>d) Finally, urinary concentrations and analyses of metabolites were performed by liquid chromatography and mass spectrometry</p>	<p>a) Nitroxoline presented MIC value between 2 and 8 mg/L against representative strains of the different species of bacteria mentioned, which denotes high antibacterial activity</p> <p>b) The authors mentioned no serious adverse events related to the drugs administered to the study volunteers</p>

MIC₅₀ value of 42.07 µM for nitroxoline against a multi-drug-resistant strain of *E. coli*. In a paper that determined MICs of nitroxoline against carbapenemase-producing bacteria, Fuchs and Hamprecht (2019) obtained MIC values between 2 and 8 mg/L for *E. coli*, between 2 and 32 mg/L for *K. pneumoniae*, and between 8 and 16 mg/L for *P. mirabilis*. For the other bacterial species employed in the study (Table 6), the MIC ranged between 1 and 32 mg/L. Wagenlehner et al. (2014) conducted a comparative study involving volunteers who received nitroxoline or trimethoprim in order to have their urinary inhibitory titers (UITs) and their urinary bactericidal titers (UBT) determined (Table 6). In the study, nitroxoline showed MIC values of 2–4 mg/L for *E. coli*, 4 mg/L for *K. pneumoniae*, and 8 mg/L for *P. mirabilis* and *S. saprophyticus*. Kresken and Körber-Irrgang (2014) determined the in vitro activity of nitroxoline against 499 *E. coli* isolates from urine samples from different German laboratories. In the study, nitroxoline showed a constant activity profile among the isolated *E. coli* strains, evidenced by MIC₅₀ = 2 mg/L and MIC₉₀ = 4 mg/L.

Kudera et al. (2020) evaluated the MICs of nitroxoline against representative strains of 17 bacterial species (Table 6) via the broth microdilution method. The authors obtained vital bacterial growth inhibitory activities (MICs = 2–4 µg/mL) against *B. cereus*, *Clostridium* spp., *E. coli*, and *S. flexneri*. Principe et al. (2020) determined the MIC for nitroxoline against the bacterial species mentioned in Table 6 using the broth microdilution method. In addition, the researchers tested the substance against *Galleria mellonella* larvae to assess the compound's toxicity in the animal model. As a result, the group obtained MIC values of 1–4 mg/L for all bacterial species, except for *K. pneumoniae*, against which the disk-diffusion test showed inactivity. Inoculated in *G. mellonella* larvae at 128 mg/L, nitroxoline did not increase hemocyte density compared to the larval control. Furthermore, nitroxoline showed intrinsic antibacterial activity in vivo, increasing larval survival time (except when infected with *K. pneumoniae*) beyond 120 h. Sobke et al. (2018) performed nitroxoline susceptibility testing against representative strains of the bacterial species mentioned in Table 6, combining the VITEK®2 system methodology with double disc-diffusion. Among strains of the bacteria studied, nitroxoline presented MICs in 0.25–32 mg/L for *E. coli*, 1–32 mg/L for *K. pneumoniae*, 1–16 mg/L for *S. aureus*, and 2–64 mg/L for *E. faecalis*. In a case report, Hof and Juretschke (2019) tested by disc diffusion the susceptibility of *Klebsiella pneumoniae* and *Proteus mirabilis*, isolated from a 68-year-old patient who had contracted urinary tract infection by multidrug-resistant bacteria. In the paper, the antibiogram showed that nitroxoline was the only active substance against both pathogens. Valentine-King et al. (2019) determined the MIC for nitroxoline and other test compounds against the bacterial species

listed in Table 6, employing the broth microdilution method and the agar dilution method. Nitroxoline exhibited greater effectiveness against *Ureaplasma* spp. species, against which it presented MIC₅₀ = 3.13 µM and MIC₉₀ = 6.25 µM. Considering *Mycoplasma* species, nitroxoline presented MIC values of 12.5–50 µM. In addition, nitroxoline demonstrated a bactericidal effect against *U. parvum*, while it demonstrated a bacteriostatic effect for *U. urealyticum* (Valentine-King et al., 2019).

Ancuta et al. (2016) worked with *Staphylococcus aureus* employing nitroxoline-impregnated ceramic discs against the microorganism (disc diffusion method). Thus, the nitroxoline-impregnated discs caused a halo of inhibition > 30 mm, which remained 24 h after the discs were removed from the Petri dish containing *S. aureus*, demonstrating intense antimicrobial activity.

In a trial with *Neisseria gonorrhoeae*, Fuchs et al. (2019) collected clinical isolates between 2015 and 2018 from two German medical centers, Cologne and Bonn. Among these isolates, the researchers have selected those with high penicillin resistance. As a result, the authors obtained high antimicrobial activity for nitroxoline against *N. gonorrhoeae*, of which the MIC values = 0.125–4 mg/L evidences.

Li et al. (2019), working with *Bartonella henselae* obtained excellent antibacterial activity for nitroxoline against the pathogen, with MIC in the range of 0.31–0.63 µg/mL. In a cell viability assay, after exposure to nitroxoline, only 6% viable *B. henselae* cells remained, confirmed by the authors using PCR.

Antiparasitic activity

Working with *Balamuthia mandrillaris*, a free-living pathogen that occasionally causes fatal infection in the central nervous system, Laurie et al. (2018) performed a scan of clinically approved compounds (a total of 2177, among these, nitroxoline) against trophozoites of the parasite (Table 7). In the study, nitroxoline showed inhibitory concentration IC₅₀ = 4.77 µM against suspensions of *B. mandrillaris* trophozoites, denoting good anti-amebic activity. Furthermore, samples of brain tissue, previously collected from a patient (with the proper authorization and supervision of the institutional ethical regulations), were infected and nitroxoline prevented destruction of the brain tissue caused by *B. mandrillaris*.

Granulomatous amebic encephalitis (GEA), caused by *Balamuthia mandrillaris*, is a rare fatal pathology that does not have an established treatment. It is common for patients to receive extremely aggressive experimental treatments, hence the importance, highlighted by the authors, of the search for new anti-amebic compounds with high efficacy and low side effects (Laurie et al. 2018).

Table 7 Main findings pertinent to the antiparasitic activity studies of nitroxoline

References	Species	Methodology	Findings
Laurie et al. (2018)	<i>Balamuthia mandrillaris</i>	<p>a) Clinically approved compounds (a total of 2,177, among them nitroxoline) were scanned against <i>B. mandrillaris</i> trophozoites</p> <p>b) The percentage inhibition of such compounds against <i>B. mandrillaris</i> was calculated by CellTiter-Glo R luminescence assay</p> <p>c) A secondary scan was performed to find hit compounds whose dose-response was determined against <i>B. mandrillaris</i> trophozoites, human fibroblast HFF-1, and human neuroglioma H4 cells, also by CellTiter-Glo R luminescence</p> <p>d) A dose-response assay was performed, with 90 compounds (including nitroxoline) against suspensions of <i>B. mandrillaris</i> trophozoites and human cells of HFF-1 (fibroblast), H4 (glial), U87 (glial), HEK-293 T (kidney), and Hep-G2 (liver) lineages, in 96-well plates</p> <p>e) Recrudescence assays of <i>B. mandrillaris</i> were carried out using the minimum amoebicidal concentration of trophozoites method, specially adapted for this study</p> <p>f) Brain tissue was infected with <i>B. mandrillaris</i> to investigate the efficacy of nitroxoline in an infection model</p>	<p>a) Nitroxoline showed inhibitory concentration $IC_{50} = 4.77 \mu\text{M}$ against suspensions of <i>B. mandrillaris</i> trophozoites, thus having good anti-amebic activity</p> <p>b) In the ex vivo model, nitroxoline prevented destruction of the brain tissue caused by <i>B. mandrillaris</i></p>

Antiviral activity

Within the little-explored antiviral activity, Zhang et al. (2020) worked with the Japanese encephalitis virus (JEV); the researchers have encoded an eGFP reporter gene containing a sequence encoding a fluorescent protein. As a result, the authors found potent antiviral activity for nitroxoline (Table 8), against Japanese encephalitis virus (JEV), denoted by $EC_{50} = 2.482 \mu\text{M}$.

Japanese encephalitis virus (JEV) is a mosquito-borne virus that belongs to the genus *Flavivirus*, the same as other important human pathogens such as yellow fever virus, West Nile virus and Dengue virus. The disease has no clinically approved treatment, and the vaccine is the only form of prevention, hence the importance, highlighted by the authors, of identifying compounds approved by the FDA, which have activity against the virus (Zhang et al. 2020).

Clioquinol and nitroxoline: a comparison.

Although antifungal activity is the most reported in the literature for clioquinol, as is the antibacterial activity for nitroxoline, care must be taken not to confuse popular action with preponderant action, or to claim that one drug is more active as an antifungal while another, as an antibacterial. After all, Olaleye et al. (2021) and Tavares et al. (2020) found such good antiviral and antiparasitic activity for clioquinol, just as Costa et al. (2021) and Pippi et al. (2018) reported excellent antifungal activity for the drug. For now, antiviral activity is not as explored for clioquinol as the antifungal activity. Similarly, for nitroxoline, Zhang et al. (2020) and Laurie et al. (2018) proved that the drug has as good antiviral and antiparasitic action as Cherdtrakulkiat et al. (2016) and Kudera et al. (2020) proved its known antibacterial activity. Dealing with the antiviral activity of nitroxoline, the result found by Zhang et al. (2020) ($EC_{50} = 2.482 \mu\text{M}$) against Japanese encephalitis virus (JEV) was promising as the result found by Olaleye et al. (2021) for antiviral activity of clioquinol ($IC_{50} = 12.62 \mu\text{M}$) against SARS-CoV-2. Fuchs et al. (2021) found excellent antifungal activity for nitroxoline against *Candida auris* isolates, with MICs (Minimum Inhibitory Concentrations) between 0.125 and 1 mg/L, a crucial step in the, so far, new possible use for the substance.

Conclusions

The present review brought to light the broad-spectrum antimicrobial activity for clioquinol and nitroxoline and the broadening of the scope of both drugs as antiparasitic and antiviral, in addition to their antibiofilm action. By organizing the information from each reference in the form of

Table 8 Main findings pertinent to the antiviral activity studies of nitroxoline

NITROXOLINE ANTIVIRAL	
References	Species
Zhang et al. (2020)	Japanese Encephalitis Virus (JEV)
	Methodology
	Findings

(a) The researchers built a Japanese encephalitis virus (JEV) encoding an eGFP reporter gene containing a fluorescent protein-coding sequence

(b) The stability of the eGFP-JEV produced was tested against C6/36 *Aedes albopictus* cells

(c) Assays evaluating antiviral activity were performed in eGFP-JEV gene-infected Huh7 cells with the test compound NITD008 and other substances (including nitroxoline).

(a) Nitroxoline exhibited high antiviral activity against Japanese encephalitis virus (JEV), denoted by $EC_{50} = 2.482 \mu\text{M}$

tables, the work constitutes a concise guide to orient future studies of researchers in the respective academic area, assisting in the decision about which substance and with which species of microorganism to work. In addition to these factors, the comparison between the findings of the tabulated studies allows the conclusion that both drugs have vast latent antimicrobial potential. However, to properly understand and exploit this potential, more work is needed to explore the less addressed activities for clioquinol and nitroxoline. A more detailed study is also needed on the mechanism of action of the drugs, which still lacks proper elucidation, despite both drugs being old.

As a perspective for clioquinol and nitroxoline, there is the future return of these drugs to clinical practice, within a cautious and controlled use, respecting the recommendations proposed by the health agencies in force in each country.

Author contributions Literature search, papers' selection, drafting and artwork was performed by Rachel Wykowski. Conception, revision, design and supervision was performed by Saulo Fernandes de Andrade and Alexandre Meneghello Fuentefria.

Funding This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior -Brasil (CAPES) - Finance Code 001.

Declarations

Competing interests The authors declare no competing interests.

References

- Abouelhassan Y, Yang Q, Yousaf H et al (2017) Nitroxoline: a broad-spectrum biofilm-eradicating agent against pathogenic bacteria. *Int J Antimicrob Agents* 49:247–251. <https://doi.org/10.1016/j.ijantimicag.2016.10.017>
- Alvarez E, Sanhueza C (2016) *Scedosporium dehoogii*, un nuevo reporte de Chile: filogenia y perfiles de sensibilidad a antifúngicos clásicos y a nuevas moléculas con potencial antifúngico. *Rev Iberoam Micol* 33:224–229. <https://doi.org/10.1016/j.riam.2016.03.007>
- Ancuta D, Gorea M, Avram A (2016) Antimicrobial activity of ceramic disks loaded with silver ions and nitroxoline. *Bacteriology and Bacterial Zoonoses*. <https://www.researchgate.net/publication/305618803>. Accessed 06 July 2020
- Blanco P, Corona F, Martínez JL (2018) Biolog phenotype microarray is a tool for the identification of multidrug resistance efflux pump inducers. *Antimicrob Agents Chemother* 62(11):e01263-e1318
- Chan DCK, Guo I, Burrows LL (2020) Forging new antibiotic combinations under iron-limiting conditions. *Antimicrob Agents Chemother* 64(3):e01909-e1919. <https://doi.org/10.1128/AAC.01909-19>
- Chaves MA, Ferreira do Amaral T, Monteiro da Silva Rodrigues Coutinho N, et al (2020) Synergistic association of clioquinol with antifungal drugs against biofilm forms of clinical *Fusarium* isolates. *Mycoses* 63:1069–1082. <https://doi.org/10.1111/myc.13142>

- Cherdtrakulkiat R, Boonpangrak S, Sinthupoom N et al (2016) Derivatives (halogen, nitro and amino) of 8-hydroxyquinoline with highly potent antimicrobial and antioxidant activities. *Biochem Biophys Reports* 6:135–141. <https://doi.org/10.1016/j.bbrep.2016.03.014>
- Cherdtrakulkiat R, Lawung R, Nabu S et al (2019) Nitroxoline: A potent antimicrobial agent against multidrug resistant enterobacteriaceae. *EXCLI J* 18:445–453. <https://doi.org/10.17179/excli2019-1378>
- Costa B, Pippi B, Andrzejewski Kaminski TF et al (2020) *In vitro* antidermatophytic synergism of double and triple combination of clioquinol with ciclopirox and terbinafine. *Mycoses* 63:993–1001. <https://doi.org/10.1111/myc.13127>
- Costa B, Pippi B, Berlitz SJ et al (2021) Evaluation of activity and toxicity of combining clioquinol with ciclopirox and terbinafine in alternative models of dermatophytosis. *Mycoses* 64:727–733. <https://doi.org/10.1111/myc.13276>
- de Tavares G, SV, Mendonça DVC, Lage DP, et al (2018) Antileishmanial activity, cytotoxicity and mechanism of action of clioquinol against *Leishmania infantum* and *Leishmania amazonensis* species. *Basic Clin Pharmacol Toxicol* 123:236–246. <https://doi.org/10.1111/bcpt.12990>
- Dunmade AD, Afolabi OA, Alabi BS et al (2012) Intra-antral application of an anti-fungal agent for recurrent maxillary fungal rhinosinusitis: a case report. *J Med Case Rep*. <https://doi.org/10.1186/1752-1947-6-245>
- Fuchs F, Hamprecht A (2019) Susceptibility of carbapenemase-producing Enterobacterales (CPE) to nitroxoline. *J Antimicrob Chemother* 74:2934–2937. <https://doi.org/10.1093/jac/dkz275>
- Fuchs F, Wille J, Hamprecht A et al (2019) *In vitro* activity of mecillinam and nitroxoline against *Neisseria gonorrhoeae* - re-purposing old antibiotics in the multi-drug resistance era. *J Med Microbiol* 68:991–995. <https://doi.org/10.1099/jmm.0.001014>
- Fuchs F, Hof H, Hofmann S et al (2021) Antifungal activity of nitroxoline against *Candida auris* isolates. *Clin Microbiol Infect* 27:1697.e7-1697.e10. <https://doi.org/10.1016/j.cmi.2021.06.035>
- Fukui T, Asakura K, Hikichi C et al (2015) Histone deacetylase inhibitor attenuates neurotoxicity of clioquinol in PC12 cells. *Toxicology* 331:112–118. <https://doi.org/10.1016/j.tox.2015.01.013>
- Giacomini E, Perrone V, Alessandrini D et al (2021) Evidence of antibiotic resistance from population-based studies: a narrative review. *Infect Drug Resist* 14:849–858. <https://doi.org/10.2147/IDR.S289741>
- Hof H, Juretschke C (2019) Nitroxoline: an option for the treatment of urinary tract infection with multi-resistant uropathogenic bacteria. *Infection* 47:493–495. <https://doi.org/10.1007/s15010-018-1253-y>
- Joaquim AR, Reginatto P, Lopes MS et al (2021) New 8-hydroxyquinoline derivatives highlight the potential of this class for treatment of fungal infections. *New J Chem* 45:18158–18170. <https://doi.org/10.1039/d0nj06188c>
- Jong MJ, Korterink JJ, Benninga MA et al (2014) *Dientamoeba fragilis* and chronic abdominal pain in children: a case-control study. *Arch Dis Child Educ Pract Ed* 99:1109–1113. <https://doi.org/10.1136/archdischild-2014-305942>
- Kawamura K, Kuroda Y, Sogo M et al (2014) Superoxide dismutase as a target of clioquinol-induced neurotoxicity. *Biochem Biophys Res Commun* 452:181–185. <https://doi.org/10.1016/j.bbrc.2014.04.067>
- Konagaya M (2015) SMON: toxicity of clioquinol and the *status quo*. *Brain Nerve* 67(1):49–62. <https://doi.org/10.11477/mf.1416200086>
- Kresken M, Körber-irrgang B (2014) *In vitro* activity of nitroxoline against *Escherichia coli* urine isolates from outpatient departments in Germany. *Antimicrob Agents Chemother* 58:7019–7020. <https://doi.org/10.1128/AAC.03946-14>
- Kudera T, Doslak I, Salmonova H et al (2020) *In vitro* selective growth-inhibitory activities of phytochemicals, synthetic phytochemical analogs, and antibiotics against diarrheagenic / probiotic bacteria and cancer / normal intestinal cells. *Pharmaceuticals* 13(9):1–17
- Kuru S (2021) Fifty years after the identification of the cause of SMON. *Clin Neurol* 61:109–114. <https://doi.org/10.5692/clinicalneurol.cn-001500>
- Laskaris P, Atrouni A, Calera JA et al (2016) Administration of zinc chelators improves survival of mice infected with *Aspergillus fumigatus* both in monotherapy and in combination with caspofungin. *Antimicrob Agents Chemother* 60:5631–5639. <https://doi.org/10.1128/AAC.00324-16>
- Laurie MT, White CV, Retalack H et al (2018) Functional assessment of 2,177 U.S. and international drugs identifies the quinoline nitroxoline as a potent amoebicidal agent against the pathogen *Balamuthia mandrillaris*. *mBio* 9(5):e02051-18. <https://doi.org/10.1128/mBio>
- Leonardelli F, Macedo D, Dudiuk C et al (2019) *In vitro* activity of combinations of zinc chelators with amphotericin B and posaconazole against six mucorales species. *Antimicrob Agents Chemother*. <https://doi.org/10.1128/AAC.00266-19>
- Leuci R, Brunetti L, Laghezza A et al (2020) Importance of biometals as targets in medicinal chemistry: an overview about the role of zinc (II) chelating agents. *Appl Sci* 10(12):4118. <https://doi.org/10.3390/app10124118>
- Li T, Feng J, Xiao S et al (2019) Identification of FDA-approved drugs with activity against stationary phase *Bartonella henselae*. *Antibiotics*. <https://doi.org/10.3390/antibiotics8020050>
- Lombardi A, Ouanounou A (2020) Fungal infections in dentistry: clinical presentations, diagnosis, and treatment alternatives. *Oral Surg. Oral Med Oral Radiol* 130:533–546
- Loock JW (2012) A randomised controlled trial of active chronic otitis media comparing courses of eardrops versus one-off topical treatments suitable for primary, secondary and tertiary health-care settings. *Clin Otolaryngol* 37(4):261–270. <https://doi.org/10.1111/j.1749-4486.2012.02532.x>
- Magallon J, Chiem K, Tran T et al (2019) Restoration of susceptibility to amikacin by 8- hydroxyquinoline analogs complexed to zinc. *PLoS ONE* 14(5):e0217602. <https://doi.org/10.1371/journal.pone.0217602>
- Majumdar S, Veleba M, Finn S et al (2013) Elucidating the regulon of multidrug resistance regulator *rarA* in *Klebsiella pneumoniae*. *Antimicrob Agents Chemother* 57:1603–1609. <https://doi.org/10.1128/AAC.01998-12>
- Mancuso G, Midiri A, Gerace E, Biondo C (2021) Bacterial antibiotic resistance: the most critical pathogens. *Pathogens* 10(10):1310. <https://doi.org/10.3390/pathogens10101310>
- Moya-Salazar J, Pasco IA, Cañari B, Contreras-Pulache H (2021) Cutaneous leishmaniasis associated with the level of poverty of the andean rural population: a five-year single-center study. *Electron J Gen Med* 18(6):335. <https://doi.org/10.29333/ejgm/11335>
- Mustazza C, Sbriccoli M, Minosi P, Raggi C (2020) Small molecules with anti-prion activity. *Curr Med Chem* 27:5446–5479. <https://doi.org/10.2174/0929867326666190927121744>
- Nunes RR, Da Fonseca AL, Pinto ACDS et al (2019) Brazilian malaria molecular targets (BraMMT): selected receptors for virtual high-throughput screening experiments. *Mem Inst Oswaldo Cruz*. <https://doi.org/10.1590/0074-02760180465>
- Olaleye OA, Kaur M, Onyenaka C, Adebusuyi T (2021) Discovery of clioquinol and analogues as novel inhibitors of severe acute respiratory syndrome coronavirus 2 infection, ACE2 and ACE2—spike protein interaction *in vitro*. *Heliyon* 7(3):e06426. <https://doi.org/10.1016/j.heliyon.2021.e06426>

- Perez DR, Sklar LA, Chigaev A (2019) Clioquinol: To harm or heal. *Pharmacol Ther* 199:155–163. <https://doi.org/10.1016/j.pharmthera.2019.03.009>
- Pippi B, da Machado G, RM, Bergamo VZ, et al (2018) Clioquinol is a promising preventive morphological switching compound in the treatment of *Candida* infections linked to the use of intrauterine devices. *J Med Microbiol* 67:1655–1663. <https://doi.org/10.1099/jmm.0.000850>
- Pippi B, Lopes W, Reginatto P et al (2019a) New insights into the mechanism of antifungal action of 8-hydroxyquinolines. *Saudi Pharm J* 27:41–48. <https://doi.org/10.1016/j.jsps.2018.07.017>
- Pippi B, Merkel S, Staudt KJ et al (2019b) Oral clioquinol is effective in the treatment of a fly model of *Candida* systemic infection. *Mycoses* 62:475–481. <https://doi.org/10.1111/myc.12888>
- Principe L, Vecchio G, Sheehan G et al (2020) Zinc chelators as carbapenem adjuvants for metallo- β -lactamase-producing bacteria: in vitro and in vivo evaluation. *Microb Drug Resist* 26:1133–1143. <https://doi.org/10.1089/mdr.2020.0037>
- Salah LA, Faergemann J (2015) A retrospective analysis of skin bacterial colonisation, susceptibility and resistance in atopic dermatitis and impetigo patients. *Acta Derm Venereol* 95:532–535. <https://doi.org/10.2340/00015555-1996>
- Schierenberg A, Buijning-Verhagen PCJ, Van Delft S et al (2019) Antibiotic treatment of gastroenteritis in primary care. *J Antimicrob Chemother* 74:207–213. <https://doi.org/10.1093/jac/dky385>
- Segala FV, Bavaro DF, Di Gennaro F et al (2021) Impact of sars-cov-2 epidemic on antimicrobial resistance: a literature review. *Viruses* 13(11):2110. <https://doi.org/10.3390/v13112110>
- Shankar PR, Palaian S, Gulam SM (2021) Hydroxychloroquine and chloroquine in prophylaxis and treatment of COVID-19: what is known? *J Pharm Bioallied Sci* 13(1):4–10. https://doi.org/10.4103/jpbs.JPBS_404_20
- Sobke A, Makarewicz O, Baier M et al (2018) Empirical treatment of lower urinary tract infections in the face of spreading multidrug resistance: in vitro study on the effectiveness of nitroxoline. *Int J Antimicrob Agents* 51:213–220. <https://doi.org/10.1016/j.ijantimicag.2017.10.010>
- Sousa JKT, Antinarelli LMR, Mendonça DVC et al (2019) A chloroquinoline derivate presents effective *in vitro* and *in vivo* antileishmanial activity against *Leishmania* species that cause tegumentary and visceral leishmaniasis. *Parasitol Int* 73:101966. <https://doi.org/10.1016/j.parint.2019.101966>
- Tavares GSV, Mendonça DVC, Pereira IAG et al (2020) A clioquinol-containing Pluronic®F127 polymeric micelle system is effective in the treatment of visceral leishmaniasis in a murine model. *Parasite* 27:29. <https://doi.org/10.1051/parasite/2020027>
- Valentine-King MA, Cisneros K, James MO et al (2019) Turning the tide against antibiotic resistance by evaluating novel, halogenated phenazine, quinoline, and NH125 compounds against ureaplasma species clinical isolates and mycoplasma type strains. *Antimicrob Agents Chemother* 63(3):e02265-18. <https://doi.org/10.1128/AAC.02265-18>
- Van Hunsel F (2017) Clioquinol use for *Dientamoeba fragilis* infections is questionable. *Ned Tijdschr Geneesk* 161:D1477
- Van Velzen AG, de Vries I (2017) Nearly all cases of clioquinol intoxication result from medication errors. 37th International Congress of the EAPCCT 16–19. *Clin Toxicol* 55(5):462
- Wagenlehner FME, Münch F, Pilatz A et al (2014) Urinary concentrations and antibacterial activities of nitroxoline at 250 milligrams versus trimethoprim at 200 milligrams against uropathogens in healthy volunteers. *Antimicrob Agents Chemother* 58:713–721. <https://doi.org/10.1128/AAC.02147-13>
- Wijma RA, Huttner A, Koch BCP et al (2018) Review of the pharmacokinetic properties of nitrofurantoin and nitroxoline. *J Antimicrob Chemother* 73:2916–2926. <https://doi.org/10.1093/jac/dky255>
- Yan C, Wang S, Wang J et al (2018) Clioquinol induces G2/M cell cycle arrest through the up-regulation of TDH3 in *Saccharomyces cerevisiae*. *Microbiol Res* 214:1–7. <https://doi.org/10.1016/j.micres.2018.05.006>
- You Z, Ran X, Dai Y, Ran Y (2018) Clioquinol, an alternative antimicrobial agent against common pathogenic microbe. *J Mycol Med* 28:492–501. <https://doi.org/10.1016/j.mycmed.2018.03.007>
- You Z, Zhang C, Ran Y (2020) The effects of clioquinol in morphogenesis, cell membrane and ion homeostasis in *Candida albicans*. *BMC Microbiol* 20(1):165. <https://doi.org/10.1186/s12866-020-01850-3>
- Yousfi H, Ranque S, Cassagne C et al (2020) Identification of repositionable drugs with novel antimycotic activity by screening the Prestwick Chemical Library against emerging invasive moulds. *J Glob Antimicrob Resist* 21:314–317. <https://doi.org/10.1016/j.jgar.2020.01.002>
- Zhang Z, Zhang H, Li X et al (2020) Generation and characterization of Japanese encephalitis virus expressing GFP reporter gene for high throughput drug screening. *Antiviral Res* 182:104884. <https://doi.org/10.1016/j.antiviral.2020.104884>

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Springer Nature or its licensor holds exclusive rights to this article under a publishing agreement with the author(s) or other rightsholder(s); author self-archiving of the accepted manuscript version of this article is solely governed by the terms of such publishing agreement and applicable law.