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Drug discovery for primary amebic meningoencephalitis: from screen to identification of leads

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Abstract

Introduction: *Naegleria fowleri* is responsible for primary amebic meningoencephalitis (PAM) which has a fatality rate of >97%. Because of the rarity of the disease, pharmaceutical companies do not pursue new drug discovery for PAM. Yet, it is possible that the infection is underreported and finding a better drug would have an impact on people suffering from this deadly infection.

Areas covered: This paper reports the efforts undertaken by different academic groups over the last 20 years to test different compounds against *N. fowleri*. The drug discovery research encompassed synthesis of new compounds, development and use of high-throughput screening methods and attempts to repurpose clinically developed or FDA-approved compounds for the treatment of PAM.

Expert opinion: In absence of economic investment to develop new drugs for PAM, repurposing the FDA-approved drugs has been the best strategy so far to identify new leads against *N. fowleri*. Increasing use of high-throughput phenotypic screening has the potential to accelerate the identification of new leads, either in monotherapy or in combination treatment. Since phase II clinical trial is not possible for PAM, it is critical to demonstrate *in vivo* efficacy of a clinically safe compound to translate the discovery from lab to the clinic.

Keywords

Naegleria fowleri ; primary amebic meningoencephalitis; drugs; leads; screen; high-throughput

1. Introduction

Naegleria spp. are free-living amebae that belong to the family Vahlkampfiidae and class Heterolobosea. Although these amebae are around for more than 100 years, a thermophilic ameboflagellate *N. fowleri* has attracted much attention because it causes

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a rapidly progressive and often fatal meningoencephalitis known as primary amebic meningoencephalitis (PAM). The infective form of *N. fowleri* is the trophozoite state, which is able to infect human by entering through the nasal cavity during swimming or other recreational water activities, and nasal cleansing. The trophozoite then attaches to the nasal mucosa, penetrates the olfactory neuroepithelium, migrates through the olfactory nerve, crosses the cribriform plate and reaches the olfactory bulb [1,2]. Once *N. fowleri* trophozoites reach brain parenchyma, they induce extensive inflammation, leading to the destruction of host nerves and the subsequent tissue damage in the central nervous system (CNS) [3]. The clinical symptoms can be divided into stage 1 and stage 2. Symptoms in stage 1 include severe frontal headache, nausea, vomiting, and a fever. Progression of these initial symptoms leads to a more severe stage 2 that includes stiff neck, altered mental status, seizures, cerebral edema, cerebellar herniation, and finally coma leading to death in approximately one week after the onset of symptoms [4]. These symptoms resemble the signs of bacterial or viral meningitis and delay the timely diagnosis of PAM.

In a recent study, a total of 381 global cases of PAM were identified from 1937 through 2018 and there were only seven reported survivors [5]. Although the infection is considered as rare and majority (156 cases) was reported from the United States, it is possible that the true number of cases worldwide is underestimated because of lack of surveillance or available diagnostic capacity in other countries [5,6]. New cases of PAM have arisen due to various factors such as ablution, and the way water is stored in developing countries [7]. Treatment history of seven confirmed survivors showed that all survivors received amphotericin B, either intravenous or both intravenous and intrathecal. In addition, majority received miconazole or fluconazole, azithromycin, rifampin, miltefosine and dexamethasone [5]. Only one survivor received amphotericin B alone, rest survivors were treated with a combination of drugs [5]. The current recommended treatment for PAM under the guidance of Centers for Disease Control and Prevention (CDC) is amphotericin B, azithromycin, fluconazole, rifampicin and miltefosine. Despite the use of a cocktail of drugs including amphotericin B, the mortality rate of PAM is more than 97% and only four patients survived so far in the US. In order to reduce PAM mortality, efforts were made to identify better drug leads for the treatment of PAM. Because of the rarity and fatality of the disease, no phase II clinical trials of safe and efficacious leads are feasible, leaving the efficacy assignment of one clinically safe compound over another to *in vitro* assays and animal tests.

Here, I describe the efforts undertaken by different groups over the last 20 years to develop and test different compounds against *N. fowleri*. The researchers employed a two-pronged approach: the first was to synthesize different compounds, either synthetic or natural product-derived, and test their activities against the pathogen; the other one was to test clinically developed or FDA-approved drugs to find safer and efficacious drugs for the treatment of PAM. The latter method can rapidly and cost-effectively identify drugs to treat this deadly disease. Both approaches were benefited from the development of a high-throughput phenotypic screen targeted against the trophozoite stage of *Naegleria*. Finally, I discuss the animal studies performed in a limited capacity to test the efficacy of some compounds, both in a monotherapy and in a drug combination study.

2. *In vitro* studies on drug discovery

Current drug development process in general utilizes two approaches for lead identification, one uses the screening of compound libraries *in vitro*, also known as whole-organism screening [8] and another employs the target-based drug development strategy [9]. The target-based approach can involve *in silico* methods, including the use of genomic and proteomic databases, minimizing the number of further *in vitro* screenings [9,10] (Figure 1A). This approach is very attractive from a theoretical point of view, but it has not been much successful in drug development in general or in antiparasitic drug development in particular [11].

The whole-organism screening approach has been the 'golden' standard for drug development and it is based on the use of *in vitro* assays for testing the novel compounds or clinically developed or FDA-approved drugs. It has notable advantages over the target-based approach like the consideration of both known and unknown targets, more physiological-like test conditions or higher number of variables that may be tested [8]. All antiparasitic drugs available on the market have been identified by using this method. The rational process behind this drug development approach starts with a high-dose testing of the library as first purge based on the efficacy of compounds, also known as primary screening, followed by the secondary screening which identifies the potency of the candidates via determination of their minimal inhibitory concentration, or inhibitory concentration 50 (IC_{50}) [12,13] (Figure 1B). Both steps should be supported by a proper monitoring system based on either qualitative methods, such as morphological assays, or quantitative methods, like colorimetry, luminometry or fluorescence [12,13]. The most promising compounds or leads are then tested in a suitable *in vivo* model, usually infected mice, for the future preclinical tests, including pharmacokinetic, pharmacodynamic, toxicology and efficacy studies.

A parallel work in drug development research is the target identification or the elucidation of mechanism of action. This is an inherently difficult aspect in the whole organism screening-based approach of drug development where different approaches can produce contradictory results. Nevertheless, genetic and biochemical methods can be performed *in vitro* to understand the mechanism of action [12,14]. In the past 20 years, drug discovery research for the treatment of PAM encompassed repurposing strategy and testing of novel compounds. In most cases, the methodology relied on phenotypic whole-organism screening and in some cases, a combination of phenotypic screening and target identification was employed.

2.1. Repurposing of antifungal drugs

Repurposing of antifungal drugs in the drug discovery of PAM has a historical context. Since antifungal amphotericin B was used in all confirmed survivors and it was administered to about three-quarters of PAM patients without having a universal effectiveness [5], researchers investigated the effect of other antifungal drugs in order to identify a more active and less toxic drug than amphotericin B. While *N. fowleri* trophozoites were found resistant to fluconazole, they displayed better sensitivity to ketoconazole with an IC_{50} ranging between 0.1 and 0.2 μM [15–19]. A systematic assessment of antifungal azole drugs, known as conazoles, identified newer azoles posaconazole and isavuconazole demonstrating

a nanomolar potency against *N. fowleri* [15]. This was independently confirmed by Colon *et al* [20] when they identified posaconazole as a promising hit from a screen of an FDA-approved compound library (Table 1).

A water-soluble polyene macrolide, corifungin, belonging to the same class as amphotericin B, was tested against *N. fowleri* trophozoites and the compound was found two-fold more potent than amphotericin B (Table 1). Although *in vitro* studies suggested that corifungin may have similar mechanism of action to that of amphotericin B in *N. fowleri*, the increased solubility of corifungin may have contributed to its better tolerability and pharmacokinetic distribution in animals [21].

2.2. Use of nanotechnology

Since nanomaterials-based drug delivery systems may improve the pharmacokinetics and pharmacodynamics of cargo drugs, nanoparticles gained much attention in the drug discovery study. Silver, gold and iron oxide are considered as the most common metal carriers for nanoparticle-based drug delivery systems [22]. Both currently available drugs and natural products can be conjugated with nanoparticles to enhance the activity of the compounds. Silver nanoparticle conjugation was performed with amphotericin B and fluconazole and the conjugated nanoparticles were tested for their activity against *N. fowleri*. While silver nanoparticles conjugation enhanced amebicidal activity of amphotericin B (Table 1), conjugated fluconazole exhibited limited activity [22]. Future animal efficacy study is required to confirm if this increased amebicidal activity of conjugated amphotericin B *in vitro* translates to a better delivery of the drug in the animal model and improved efficacy *in vivo*.

2.3. Synthesis and testing of novel azoles and quinazolinones

Considering that azoles are antifungals and demonstrate wide range antimicrobial properties, six novel benzimidazole, indazole, and tetrazole derivatives were synthesized and tested against *N. fowleri*. One indazole and one tetrazole compound showed moderate activity at 50 μM concentration [23] (Table 1). Based on the documented antifungal activities of quinazolinones, 34 novel arylquinazolinones were also synthesized and tested for activities on *N. fowleri*. A relatively higher concentration of these compounds inhibited the growth of the trophozoites [24]. Further improvement of these compounds will be required to achieve increased potency.

2.4. Sterol biosynthesis inhibitors as new leads

Antifungal conazoles, identified as amebicidal agents against *N. fowleri*, are CYP51 inhibitors. CYP51 catalyzes removal of the 14-methyl group from the sterol tetracyclic core and CYP51 inhibitors deplete ergosterol pool and lead to accumulation of large amounts of sterol biosynthetic intermediates and non-physiological end-products[15,25]. Since ergosterol is one of the major sterols in the membrane of free-living amebae [15,25–27], disruption of isoprenoid and sterol biosynthesis by small-molecule inhibitors may be an effective intervention strategy against PAM. To perform ergosterol synthesis, *N. fowleri*, in addition to CYP51, encodes for sterol C24-methyltransferase (SMT), sterol Δ^8–Δ^7 isomerase (ERG2), protein farnesyltransferase (FT), HMG-CoA reductase. FDA-approved inhibitors of

SMT and ERG2, abafungin and tamoxifen, showed an IC_{50} of 3 and 6 μM [25]. In another study, eight experimentally active small molecules with moderate activity were identified through homology modeling of *N. fowleri* ERG2 [28]. While inhibitors of SMT, ERG2, and FT demonstrated an IC_{50} of low micromolar concentration against the European strain of *N. fowleri* [25,28,29], HMG-CoA reductase inhibitors fluvastatin [30,31] and pitavastatin [30] displayed a nanomolar potency against the same strain (Table 1). Cell biological studies also validated HMG-CoA reductase as a drug target of statins in *N. fowleri*. Although pitavastatin is considered blood-brain barrier permeable, future animal studies can confirm the *in vivo* efficacy and presence of the drug in *N. fowleri*-infected animals. In addition to these sterol biosynthesis inhibitors, two inhibitors of fatty acid oxidation, thioridazine and perhexiline, were found to inhibit 50% growth of *N. fowleri* at about 7 μM [32] (Table 1).

2.5. *Identification of leads from high-throughput screens* (HTS)

In the absence of a high-throughput screen, research on drug discovery for *N. fowleri* relied on low-throughput methods such as microscopy or staining, which were laborious and time consuming. With the availability of larger compound libraries, development of an HTS was a necessity that could accelerate the identification of new leads. The first HTS was developed in both 96- and 384-well microtiter plates with *N. gruberi*, a nonpathogenic relative of *N. fowleri* [21]. This screen was performed with an FDA-approved compound library and two kinase-targeted compound libraries. Five confirmed kinase-targeted hits were identified from this study and a known bioactive compound BAY-11-7085 showed strong inhibition against *N. gruberi* [21]. A follow-up study with *N. fowleri* identified a closely related compound BAY-11-7082 and another CNS-active compound ebselen as active against the pathogen [33].

Another HTS was specifically developed for *N. fowleri* and this was utilized to screen novel 150 amidino derivatives. Testing of these compounds belonging to multiple structural classes identified both mono- and diamidino derivatives of nanomolar potency [34] (Table 1). This HTS was later applied to conduct phenotypic screens of a collection of 1134 FDA-approved drugs and a collection of 400 drug and probe-like molecules in the Medicines for Malaria Venture (MMV) Pathogen Box [20]. Screening of an FDA-approved compound library identified three new anti-amebic veterinary antibiotics, valnemulin, retapamulin, and tilmicosin, with nanomolar potency and validated previously reported anti-amebic azithromycin, clarithromycin, erythromycin, roxithromycin, itraconazole, ketoconazole, and posaconazole [15,20,35,36] (Table 1). Testing of compounds in MMV Pathogen Box identified six known antiparasitic compounds that exhibited low micromolar IC_{50} against *N. fowleri* [20]. Another library, consisting of 400 compounds that were either FDA-approved or in various stages of drug development, was available through MMV and was known as MMV Pandemic Response Box. This library was also screened against *N. fowleri* trophozoites and seven compounds - luliconazole, ravuconazole, CRS-3123, fludarabine, panobinostat, erythromycin, and terbinafine – achieved nanomolar potency [37] (Table 1). Luliconazole and ravuconazole add to the armamentarium of antifungal drugs as promising leads for the treatment of PAM.

A subset of compounds available in a high-value Repurposing, Focused Rescue and Accelerated MEdicinal chemistry (ReFRAME) library also identified FDA-approved panobinostat displaying a nanomolar potency. In addition, FDA orphan drug lestaurtinib was also found highly potent against *N. fowleri* [38]. Another FDA-approved drug auranofin was identified as a priority anti-*N. fowleri* compound in this study (Table 1). Auranofin, a broad-spectrum anti-parasitic agent, was shown to be active against the US strains [39], as well as against the strains of various genotypes originated from different geographic regions [40]. Auranofin is a known inhibitor of selenoprotein synthesis [41] and thioredoxin reductase function [42–46], which is involved in protecting cells from damage caused by oxidative stress. Consistent with the targeting of redox enzymes, exposure to auranofin led to accumulation of reactive oxygen species in *N. fowleri* trophozoites [40].

2.6. Natural product-derived compounds as inhibitors of N. fowleri

Despite the role of natural products in the development of drugs for over a century, research on identifying new natural product-based anti-*N. fowleri* agents is limited. Both plants and microbial secondary metabolites are sources of novel compounds that display antiparasitic activity [47–49]. Isoflavans, 3*S*(+)-7-methoxymanuifolin K and manuifolin K, were isolated from methanolic extracts of *Dalea aurea* (Fabaceae) and tested against *N. fowleri* trophozoites. At a concentration of 30 μM, the compounds exhibited growth inhibition of trophozoites comparable to that of amphotericin B [50]. Lignans isolated from creosote bush *Larrea tridentata* showed moderate amebicidal activity against *N. fowleri* [51]. Another plant secondary metabolite, cinnamic acid (*trans*-3-phenylacrylic acid), was tested for anti-*N. fowleri* activity. Cinnamic acid alone and gold nanoparticles conjugated with cinnamic acid demonstrated significant growth inhibition of trophozoites at 2.5 μM [52]. Curcumin, a polyphenol isolated from *Curcuma longa*, has a longstanding use as an antimicrobial and anti-inflammatory agent [53]. When tested against *N. fowleri*, curcumin and gold nanoparticle-conjugated compound showed amebicidal activity [54] (Table 1). Secondary metabolites representing a class of natural products pharmacophore can be further optimized to develop more potent antiamebic compounds.

3. Discussion

In spite of a relatively small number of researchers working in the field of PAM, significant progress has been made in the last 20 years to identify new leads for the treatment of PAM. With the advent of HTS, PAM drug discovery has gained further momentum in the past decade. The bioluminescence-based microtiter plate assay brought additional advantage of evaluating the rate of action of active compounds against *N. fowleri*. Since PAM has a rapid clinical course, it is important to identify a drug that is fast-acting. When tested with the high-throughput cell viability assay, currently used anti-*N. fowleri* drugs miltefosine, amphotericin B, azithromycin, and fluconazole demonstrated delayed onset of action [20]. Newly identified leads posaconazole, terbinafine, panobinostat, lestaurtinib, auranofin, and pitavastatin, on the other hand, were fast-acting against *N. fowleri* trophozoites [15,20,29,30,38]. Identification of these rapidly acting drugs has the potential to increase survival rates in PAM.

Since drug susceptibility varies considerably among strains of different genotypes of *N. fowleri* [55], it is important to test the effect of drugs against strains of different genotypes and identify leads that are almost equally active on multiple strains. Although some of the earlier studies tested the activity of compounds against a limited number of strains [18,19,56], the microtiter plate-based assay provides an opportunity to test the active leads on diverse strains in a relatively shorter time period. Some of the new leads that were tested on multiple strains and exhibited equal activity against different genotypes are posaconazole [20], auranofin [40], and pitavastatin [30].

Except one, all confirmed survivors of PAM were treated with a combination of drugs [5]. Among the drugs used in the survivor, only miconazole and azithromycin were reported to have an additive or synergistic effect with amphotericin B [57,58]. In addition to identification of leads in monotherapy, researchers are now increasingly investigating the effect of a combination of different compounds on *N. fowleri*. The phenotypic screening method available in a 96-well plate has accelerated this exploration. While combinations between new lead posaconazole and currently used drugs miltefosine, amphotericin B, or azithromycin showed additivity [20], combination of isavuconazole and tamoxifen elicited synergism *in vitro* [25]. Combination of two sterol biosynthesis inhibitors, lonafarnib and pitavastatin also induced synergistic activity in the culture [29]. When auranofin was paired with amphotericin B, the combination results reduced the concentration of amphotericin B by an order of magnitude [40]. The combination experiments not only provide the opportunity to increase the effect of individual compounds at a lower dose, it may also allow to minimize the dose-limiting adverse effects associated with any compound.

Most of the studies reported above relied on *in vitro* experiments and lacked *in vivo* proof-of-concept validation. Since 2003, a few studies extended the *in vitro* identification of potent anti-*Naegleria* compounds to a further investigation in a monotherapy or in a combination therapy in the animal model of PAM. In a monotherapy, intraperitoneal injections for 5 days once daily of ketoconazole at 25 mg/kg (Table 1), antibiotic minocycline at 50 mg/kg, and antipsychotic trifluoperazine at 2.5 mg/kg provided only 30%, 10%, and 20% protection from PAM [16]. In a combination therapy, two pristinamycin derivatives quinupristin and dalfopristin protected 50% of mice at an i.p. dose of 150 mg/kg q.d. for 5 days [16].

Earlier finding of *in vitro* activity of azithromycin alone and synergistic activity of amphotericin B and azithromycin led to a further testing of these drugs *in vivo*. When treated with i.p. injections of 75 mg/kg azithromycin alone q.d. for 5 days and a combination of 2.5 mg/kg amphotericin B and 25 mg/kg azithromycin q.d. for 5 days, both monotherapy and the drug combination provided 100% protection to *N. fowleri*-infected mice [35,58] (Table 1).

Based on the *in vitro* activities of antibacterial rokitamycin and roxithromycin [20,36], and antipsychotic chlorpromazine [59], *in vivo* efficacy studies were designed. 20 mg/kg of rokitamycin, roxithromycin and chlorpromazine administered i.p. three times at four-day intervals protected 80%, 25%, and 75% of mice, respectively [36,60] (Table 1).

Finally, corifungin and posaconazole, identified from HTS, were investigated for efficacy in a PAM murine model. While i.p. administration of corifungin at 9 mg/kg q.d. for 10 days caused 100% survival of *N. fowleri*-infected mice, intravenous administration of posaconazole at 20 mg/kg q.d. for 3 days caused 33% survival of infected mice. Increased survival (53%) was observed when posaconazole at 20 mg/kg i.v. was combined with 25 mg/kg of azithromycin i.p. for 3 days [20] (Table 1).

In summary, identification of rapidly acting potent compounds, which are active on diverse strains, either alone or in combination with currently used drugs, and validated in the animal model of PAM, may translate the discovery from bench to bedside.

4. Expert opinion

Despite the fact that precious lives are lost from PAM, economic incentives to invest in development of anti-PAM drugs by the pharmaceutical industry are lacking. In the absence of investment in development of anti-PAM drugs by the pharmaceutical industry, drug discovery for PAM largely relies on academic research centers. Because of the rarity of the disease, research on drug discovery for this fatal infection faces continuous difficulty in acquiring fund from different sources. In this situation, it is very important to use the available drug discovery resources wisely, especially the compound libraries available from different sources should be utilized in a nonredundant manner. That would help the community to avail the data in a relatively timely fashion without draining resources available to different researchers.

The conventional route of drug development is a long and expensive process and involves about $2.6 billion to bring a drug to the market [61]. The use of clinically developed compounds can reduce the development time and allows to cost-effectively repurpose these drugs for the treatment of PAM. Considering the rarity of the disease and more than a quarter of all annotated drugs are associated with off-label uses [62,63], PAM drug discovery strategies are continuously exploring the repurposing opportunities. Repurposing may employ both phenotypic screening and target-based approaches. Despite the availability of molecular biology and genomic tools, a handful of druggable targets (pore forming protein, cysteine protease, CYP51, 24-SMT, ERG2) were identified in *N. fowleri* [15,25,28,64–66]. The development of robust assay based on phenotypic screening led to the reliance on this screen to identify new leads. Earlier analysis of first-in-class small-molecule drugs approved by the FDA between 1999 and 2008 showed that the phenotypic screening contributed more to the discovery of new leads than that of target-based approaches, demonstrating the utility of the phenotypic screening strategy [67]. A mechanism-based discovery of new anti-*Naegleria* compounds can be explored in conjunction with the phenotypic screening to identify target-based inhibitors.

The paucity of blood-brain barrier permeable anti-PAM drugs currently used in the treatment may be one of the factors for not providing uniform protection, but whether blood-brain barrier permeability of a compound is an absolute requirement for successful treatment may require further research. Identification of a blood-brain barrier permeable compound that is highly potent against *N. fowleri* may contribute to the success in the proof-of-concept

study. In case of challenges associated with blood-brain barrier permeability of a compound, intranasal delivery can deliver the drug directly to the inoculation sites of amebae [68] and may result in an improved outcome.

It is unlikely that we can depend on a single “magic bullet” for the treatment of PAM. Researchers will increasingly employ combination strategy, both *in vitro* and *in vivo*.

Considering the high mortality of PAM, a phase II clinical trial for this infection is not possible and it is therefore critical to demonstrate *in vivo* efficacy in order to obtain a faster track to the clinic. The rarity of this disease also brings opportunities in the form of FDA orphan drug designation, rare pediatric disease designation and expanded access under a treatment protocol or Investigational New Drug (IND) program.

Future efforts should include (1) increased international cooperation to accelerate the research on drug discovery, (2) avail collections of approved and investigational products through different public and private sector initiatives, (3) involvement in the programs that support drug repurposing, and (4) enhance partnerships between academic institutions, industry, patient advocacy groups, different foundations and government regulatory agencies.

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Article highlights

- *Naegleria fowleri* is a free-living ameba that causes a brain infection called primary amebic meningoencephalitis.
- Current treatment relies on amphotericin B, an azole drug, azithromycin, rifampin, and miltefosine, but the combination treatment does not provide uniform protection.
- High-throughput screens identified FDA-approved drugs including sterol biosynthesis inhibitors as new leads for the treatment of PAM.
- Natural product-derived compounds and newly synthesized azoles demonstrated activity against *N. fowleri*. Nanoparticle conjugation enhanced the activity of compounds against *N. fowleri*.
- Drug combination experiments and testing of compounds on diverse strains have the potential to bring better outcome to patients.
- In absence of a phase II clinical trial, demonstration of *in vivo* efficacy of compounds is critical to obtain approval for future clinical use.

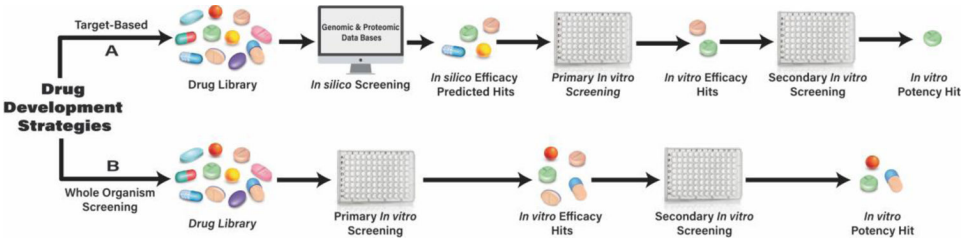

Figure 1.

Drug development strategies. (A) The target-based strategy starts with a drug library screening *in silico* against certain target using genomic and proteomic information. It reduces the number of primary and secondary *in vitro* screenings required to determine the compound efficacy and potency. (B) The whole-organism screening strategy starts directly with the primary *in vitro* screening of the whole drug library to determine the compound efficacy, followed by the secondary *in vitro* screening to determine the potency of the leads.

Table 1.

Summary of the leads identified from *in vitro* and *in vivo* studies performed in the last 20 years against *N. fowleri*.

Compound classes	Compound names	*In vivo* efficacy	References
CYP51 inhibitors and other antifungals	Ketoconazole	30% protection at 25 mg/kg q.d. for 5 days	[15–19]
	Posaconazole	33% protection at 20 mg/kg q.d. for 3 days 53% protection at 20 mg/kg posaconazole + 25 mg/kg azithromycin q.d. for 3 days	[15,20]
	Isavuconazole		[15,20]
	Luliconazole		[37]
	Ravuconazole		[37]
	Terbinafine		[37]
	Corifungin	100% protection at 9 mg/kg q.d. for 10 days	[21]
	Silver nanoparticles conjugated amphotericin B		[22]
Novel azoles	(*E*)-2-(6,6-Dimethyl-1-phenyl-1,5,6,7-tetrahydro-4*H*-indazol-4-4ylidene)hydrazine-1-carbothioamide		[23]
	5-(3-Chlorobenzyl)-1*H*-tetrazole		[23]
C24-methyltransferase (SMT) inhibitor	Abafungin		[25]
Sterol Δ^8–Δ^7 isomerase (ERG2) inhibitor	Tamoxifen		[25]
Protein farnesyltransferase (FT) inhibitor	Lonafarnib		[29]
HMG-CoA reductase inhibitors (statins)	Pitavastatin		[30]
	Fluvastatin		[30,31]
Fatty acid oxidation inhibitors	Thioridazine		[32]
	Perhexiline		[32]
Bis-benzimidazole	Mono- and diamidino derivatives		[34]
Antibiotics	Valnemulin		[20]
	Retapamulin		[20]
	Tilmicosin		[20]
	Roxithromycin	25% protection at 20 mg/kg three times at four-day intervals	[20,36]
	Rokitamycin	80% protection at 20 mg/kg three times at four-day intervals	[36]
	Azithromycin	100% protection at 75 mg/kg q.d. for 5 days 100% protection at 25 mg/kg azithromycin + 2.5 mg/kg amphotericin B q.d. for 5 days	[35,58]

Compound classes	Compound names	*In vivo* efficacy	References
Anticancer	Fludarabine		[37]
	Panobinostat		[37]
	Lestaurtinib		[38]
Thioredoxin reductase inhibitor	Auranofin		[39]
Natural product	3*S*(+)-7-methoxymanuifolin K from *Dalea aurea*		[50]
	Manuifolin K from *D. aurea*		[50]
	Lignans from *Larrea tridentata*		[51]
	Cinnamic acid		[52]
	Curcumin		[54]

q.d., once a day; HMG-CoA, 3-hydroxy-3-methylglutaryl coenzyme A