Nitroimidazoles: Molecular Fireworks That Combat a Broad Spectrum of Infectious Diseases

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ABSTRACT: Infectious diseases claim millions of lives every year, but with the advent of drug resistance, therapeutic options to treat infections are inadequate. There is now an urgent need to develop new and effective treatments. Nitroimidazoles are a class of antimicrobial drugs that have remarkable broad spectrum activity against parasites, mycobacteria, and anaerobic Gram-positive and Gram-negative bacteria. While nitroimidazoles were discovered in the 1950s, there has been renewed interest in their therapeutic potential, particularly for the treatment of parasitic infections and tuberculosis. In this review, we summarize different classes of nitroimidazoles that have been described in the literature in the past five years, from approved drugs and clinical candidates to examples undergoing preclinical or early stage development. The relatively "nonspecific" mode of action and resistance mechanisms of nitroimidazoles are discussed, and contemporary strategies to facilitate nitroimidazole drug development are highlighted.
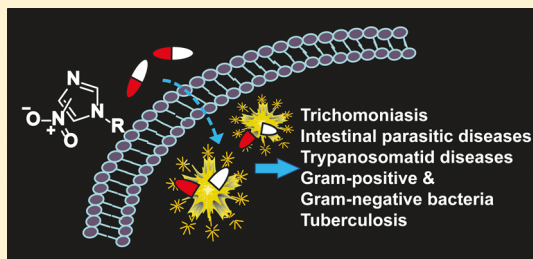
1. INTRODUCTION

Infectious diseases are a major health problem worldwide, killing more than nine million people every year.[1] Parasitic diseases and tuberculosis (TB) constitute a particularly significant burden to low- and middle-income countries as current treatments are inadequate. While the incidence rates in developed countries are comparatively lower, the threat to human health and economic burden of these diseases is still considerable. International travel has facilitated the distribution and transmission of infectious diseases, including those caused by drug-resistant microorganisms, making the fight against the diverse range of infectious agents a challenging global issue.

Neglected tropical diseases (NTD), in particular African sleeping sickness, Chagas disease, and leishmaniasis, are considered "infectious diseases of poverty" alongside TB, HIV, and malaria.[2] African sleeping sickness, or human African trypanosomiasis (HAT), is a major health concern threatening 70 million people in Africa.[3] Chagas disease (or American trypanosomiasis) affects people primarily in Latin America and causes a huge estimated annual economic loss of $7.2 billion globally.[4] Leishmaniasis is caused by over 20 species of the kinetoplastid protozoan *Leishmania* and kills 20000−30000 people yearly.[5] Diarrheal infections, which can be caused by bacteria, viruses, and parasites, are reported as the second highest cause of death among infants and children under 5 years old.[6] Parasitic diarrheal diseases including giardiasis and amebiasis are major causes of severe diarrhea. There are ∼280 million annual cases of giardiasis worldwide, with an estimated 1.2 million annual cases of giardiasis in the United States.[7,8] Amebiasis affects more than 50 million people each year worldwide, causing 40000−110000 deaths annually.[9] TB, an old millennium disease which was once epidemic in Europe and America in the 18th and 19th centuries,[10] has reappeared as the leading cause of death from infectious diseases.[11] There were an estimated 10.4 million new cases of TB in 2015, and 26% of these cases were within the African region.[11] Current treatment options for these infectious diseases are inadequate due to issues such as treatment-limiting toxicity, low efficacy, high cost, and emerging drug resistance.[12]

Despite the high burden of infectious diseases and continuous demand for new drugs, R&D funding in this field is disproportionate compared to funding for noncommunicable diseases in wealthier, developed countries. According to the Drugs for Neglected Diseases *initiative* (DND*i*), a mere "10% of R&D investment goes into 90% of global health needs".[13] Investment in neglected diseases fell by $193 million in 2013, and pharmaceutical companies contributed just 12% ($401 million) of the total global funding ($3219 million).[14] A poor return on investment, reflected by the low net present value (NPV) of antibiotics, has discouraged most major pharmaceutical companies from investing in the development of novel drug candidates to treat infectious diseases. NPV is usually risk adjusted to determine the viability of a drug development project, taking into account the projected costs, revenues, and probability of the drug being approved for market.[15] For instance, the risk adjusted NPV × $1,000,000 for an injectable antibiotic against Gram-positive bacteria is only 100 in contrast to 300 for an anticancer drug and 1150 for a musculoskeletal drug, making antibacterial drug development less attractive for pharmaceutical companies.[16] The chance for a drug against a neglected disease to be approved for market is 13-fold lower than for central nervous system disorders or cancers.[17] In 2012, Pfizer pulled out from its anti-infective research, while AstraZeneca shut down its TB research site in India in 2014 and spun out its antibiotic research group in 2015.[18,19]

Nitroimidazoles belong to a group of nitroheterocyclic compounds with broad spectrum activity against parasites, mycobacteria, and Gram-positive and Gram-negative bacteria.[20−22] The versatility of nitroimidazoles is further demonstrated by considering that infection from these pathogens occurs in many different sites of the body (Figure 1). Nitro groups in
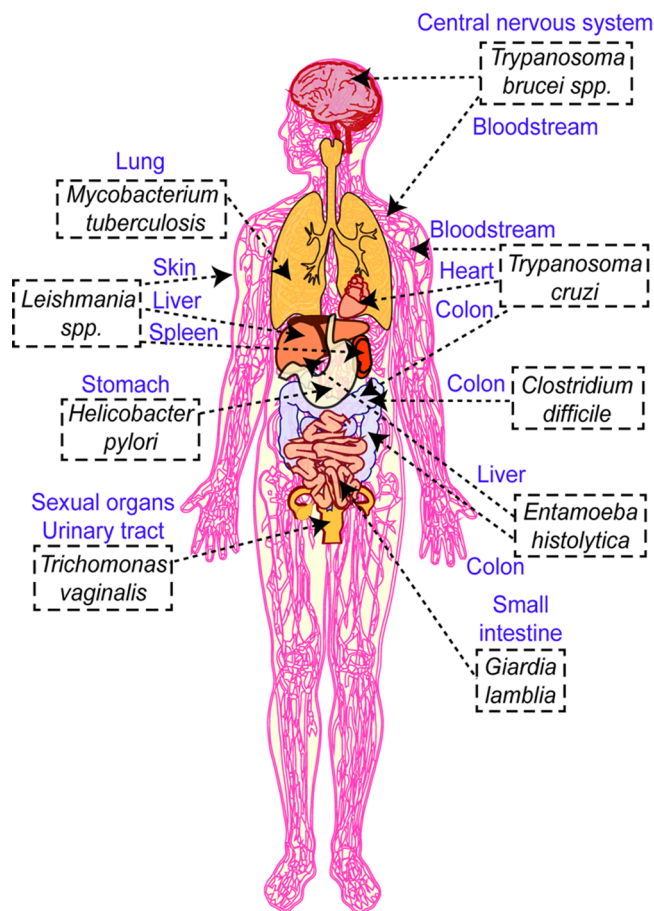

Figure 1. Nitroimidazoles can target a broad range of parasitic and bacterial pathogens that infect different sites within the human body.

compounds are often considered to be "undesirable" in drug discovery due to possible toxicity issues such as mutagenicity and hepatotoxicity.[23,24] However, the nitro group is critical for the mode of action and subsequent anti-infective activity of nitro-based antimicrobial drugs, including nitroimidazoles, nitrothiazoles, and nitrofurans. Nitroimidazoles, such as metronidazole (1) (Figure 2) are still considered the treatment of choice for anaerobic infections, clearly demonstrating that it is possible to select nitroimidazoles with an acceptable therapeutic index.[20,22] While concerns over potential toxicity has limited the number of nitroimidazoles under development in the past, there has recently been a renewed effort to develop nitroimidazoles against parasitic diseases and tuberculosis, largely driven by the lack of superior alternatives.

In this Perspective, we summarize the therapeutic values of different classes of nitroimidazoles, discuss the advantages and disadvantages of their complex and relatively "dirty" mode of action, and cover recent developments of the nitroimidazole class as agents to treat different parasitic and bacterial infections. Finally, a brief overview of current strategies to facilitate further nitroimidazole drug development targeting infectious diseases is

presented. This includes a discussion on the current focus of repurposing and repositioning both old and new nitroimidazoles as well as the open resources and platforms available to maximize the potential of nitroimidazoles in development.

2. HISTORY OF NITROIMIDAZOLE ANTIMICROBIAL AGENTS

The discovery of nitroimidazole antibiotics dates back to the early 1950s (Figure 2), when azomycin (2), a 2-nitroimidazole, was first isolated from a crude extract of *Streptomyces* bacteria.[25] Nitroimidazole 2 was shown to have potent activity against trichomoniasis, a sexually transmitted parasitic disease caused by *Trichomonas vaginalis*.[26] This finding inspired the researchers at Rhône-Poulenc to develop a series of derivatives of 2, but unfortunately all attempts to synthesize this 2-nitroimidazole were unsuccessful.[27] Rhône-Poulenc researchers shifted their attention to the synthesis of 5-nitroimidazole regioisomers, which fortuitously led to the identification of 1 with even greater activity than 2.[27]

The efficacy of 1 was not restricted to the treatment of trichomoniasis. The antibacterial activity of 1 was discovered serendipitously in 1962 when a patient with *T. vaginalis* and ulcerative gingivitis was successfully cured of both infections by 1.[28] In 1966, 1 was used to treat amoebic dysentery caused by *Entamoeba histolytica*, and in the 1970s, the treatment spectrum was expanded to include *Giardia lamblia*.[29] Since its discovery, 1 has demonstrated broad spectrum activity against both Gram-positive and Gram-negative anaerobes[22] and was included as part of a combination therapy against *Helicobacter pylori* to treat stomach ulcers.[30] In the mid-1990s, 1 demonstrated antitubercular activity against anaerobic, nonreplicating *Mycobacterium tuberculosis*.[31,32]

The synthesis of 2-nitroimidazoles was finally achieved 10 years after that of 1 (Figure 2).[33] 2-Nitroimidazoles with a variety of substituents at the 1- and 5-positions were the first class in this family that were shown to have antitubercular activity in the 1970s.[31,34] However, they possess reduction potentials approximately 150 mV higher than 5-nitroimidazoles and are therefore more readily reduced by mammalian cells, causing nonselective side effects.[31] This undesirable property means that interest in the nitroimidazole scaffold for anti-infectives has mainly been directed toward the 4- and 5-nitroimidazole regioisomers. However, 2-nitroimidazoles have found utility as radiosensitizing agents in cancer treatment,[35] and misonidazole (3) and pimonidazole (4) have been exploited in tumor hypoxia imaging for cancer management.[36] Benznidazole (5) is the only 2-nitroimidazole used as an anti-infective agent and has been prescribed as the first-line drug against *Trypanosoma cruzi* to treat Chagas disease since the 1960s.[37]

Around the same time as the discovery and development of the first members of the nitroimidazole class, the antitrypanosomal activity of nitrofurazone (6, Figure 3), a nitroaromatic compound belonging to the nitrofuran family, was recognized (recently reviewed by Patterson et al.).[24] Nitrofurans generally possess higher redox potential than nitroimidazoles and can be reduced by a wide range of enzymes.[38,39] Therefore, nitrofurans can also have issues due to mutagenicity and reduced selectivity between the target organism and mammalian cells. Nonetheless, nitrofurans such as nifurtimox (7) have been developed and are used as a second-line treatment of Chagas disease.[24]

A second-generation of 5-nitroimidazoles, including tinidazole (8), ornidazole (9), and secnidazole (10), were developed in the 1960s and 1970s. These compounds displayed similar broad
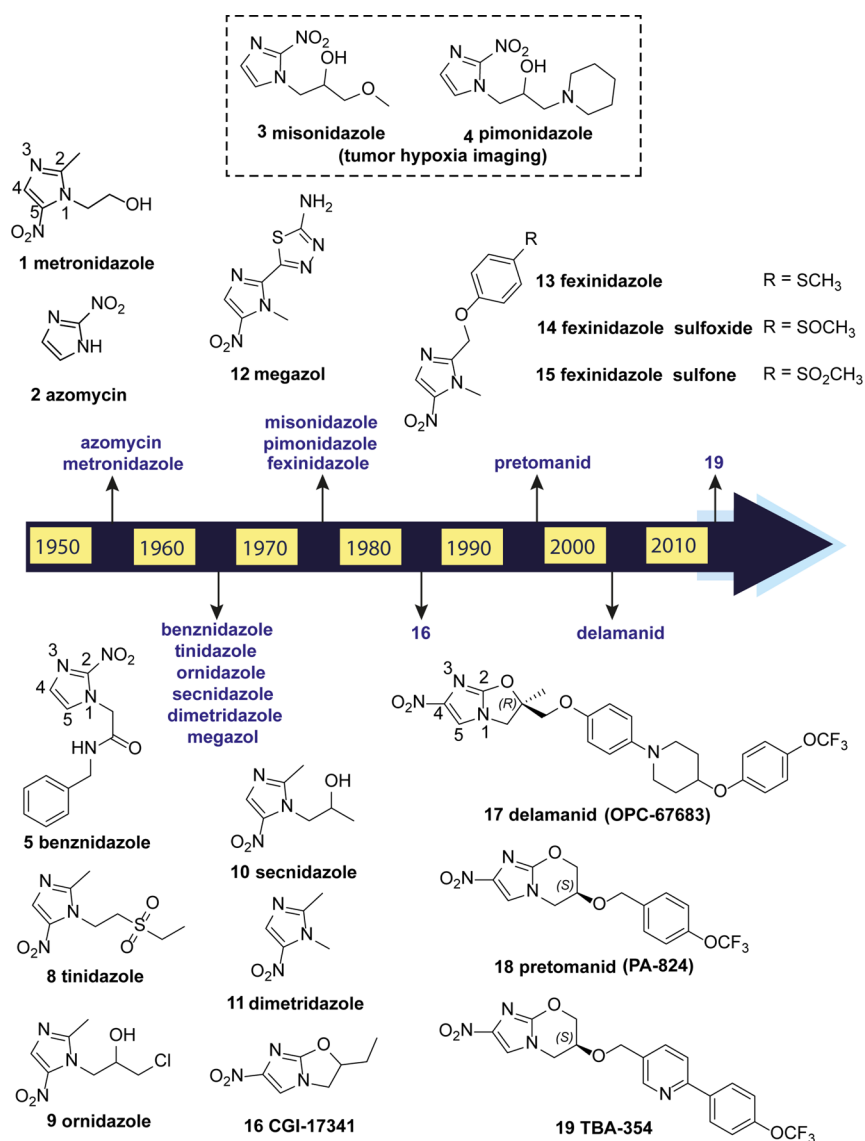
Figure 2. Timeline for the discovery of nitroimidazole anti-infective agents and examples of 2-nitroimidazoles (misonidazole **3** and pimonidazole **4**) that are used for tumor hypoxia imaging. The imidazole ring atoms are numbered for metronidazole **1** (5-nitroimidazole), benznidazole **5** (2-nitroimidazole), and delamanid **17** (4-nitroimidazole) to highlight the position of the nitro group relative to the other substituents.
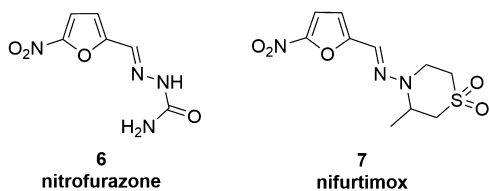

Figure 3. Structures of nitrofurans: nitrofurazone (**6**) and nifurtimox (**7**).

spectrum activity but better efficacy than **1**.[40−42] Dimetridazole (**11**), an analogue of **1** containing a methyl group rather than a hydroxyethyl group at the 1-position, was also discovered to have antiprotozoal activity. It was mainly used in food production livestock to treat parasitic infections such as histomoniasis and trichomoniasis but was banned by European Commission due to its potential carcinogenicity in humans.[43] Around the same time, additional nitroimidazoles with promising activity against trypanosomes were discovered. These nitroimidazoles included megazol (**12**), fexinidazole (**13**), and in vivo metabolites of

fexinidazole: fexinidazole sulfoxide (**14**) and fexinidazole sulfone (**15**).[20,44,45] Nitroimidazole **12** demonstrated promising activity against *Trypanosoma brucei* and *Trypanosoma cruzi* and showed mild activity against *Leishmania donovani*.[44,46] Unfortunately, further development of **12** was discontinued due to mutagenic and genotoxic effects observed in vitro and in vivo.[47] In comparison, **13** was discovered from **12**, and it maintains the 1-methyl-5-nitroimidazole core structure of **12**. An additional study has shown that **13** is rapidly oxidized in vivo, and that the metabolites (**14** and **15**) were active against *L. donovani* amastigotes grown in macrophages.[45]

The first bicyclic nitroimidazole reported to display in vitro and in vivo antitubercular activity was CGI-17341 (**16**), which was discovered by Hindustan Ciba-Geigy in 1989 (Figure 2).[48] For the purpose of this review, the numbering of the ring positions of the bicyclic nitroimidazoles will be kept consistent with the monocyclic nitroimidazoles rather than the chemical nomenclature of the bicyclic systems (i.e., the nitro group of **16** is at the equivalent 4-nitro position of respective monocyclic nitroimidazoles, compared to the 6-position of the chemical
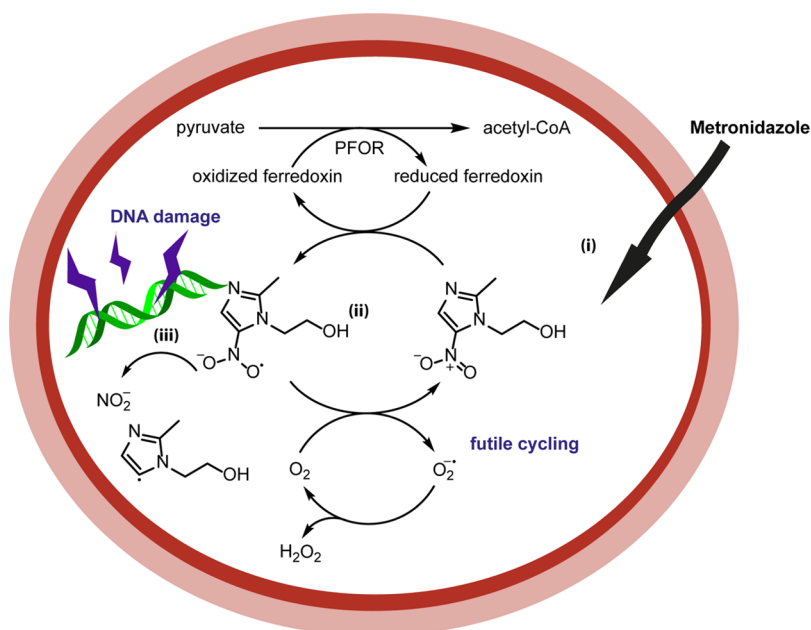
Figure 4. Mechanism of metronidazole (**1**) that involves bioreduction of the nitro group by ferredoxin to produce reactive radical species which can cause DNA damage and leads to cell death.[27,57]

nomenclature). While **16** was found to be active against drug susceptible TB and multidrug-resistant strains,[49] further development was discontinued due to mutagenicity observed in Ames testing.[48] Nonetheless, the promising antitubercular activity of this scaffold later encouraged optimization and development of related compounds. After more than 10 years, two new bicyclic nitroimidazoles with no evidence of mutagenicity were developed, delamanid **17** (OPC-67683) and pretomanid **18** (PA-824).[21,50]

Discovered by Otsuka Pharmaceuticals from a series of nitroimidazooxazoles, **17** was prepared by modifying the 2-position of the side chain with an oxygen containing linker.[51] It was granted conditional approval in 2014 by the European Medicines Agency (EMA) for treatment of pulmonary multidrug-resistant TB in adult patients.[52] Nitroimidazooxazine **18**, on the other hand, was identified by PathoGenesis from more than 300 analogues of **16**.[21] It is currently in phase II/III clinical trials for the treatment of TB.[53] To further improve the potency of **18**, a second-generation nitroimidazooxazine, TBA-354 (**19**), was recently developed by the Global Alliance for TB Drug Development and the University of Auckland.[54] It was advanced to a phase I clinical trial, but the study was halted in January 2016 due to observed neurotoxicity in healthy volunteers.[53,55]

3. MODE OF ACTION

The broad spectrum activity of nitroimidazoles across a variety of species can be explained by their mode of action. Nitroimidazoles are prodrugs that require bioactivation of the nitro group in order to exert an antimicrobial effect. While the nitro group is crucial for the antimicrobial activity, changes to the imidazole substituents leads to different spectra of activity and pharmacokinetic properties.[56] Although the mode of action involving bioactivation makes it challenging to elucidate the exact molecular mechanisms of nitroimidazoles, the mechanism is generally understood to involve the following steps: (i) molecules enter the cells through passive diffusion, (ii) the nitro group is reduced to reactive radical species, and (iii) the radicals react with cellular components such as DNA or protein

(Figure 4).[27,57] This process mimics the action of fireworks, which are innocuous until activated by an ignition source, at which point they explode spectacularly from the triggered redox reactions. The nitroimidazole reduction products formed in a cell depend on the redox potential of the compound and the number of electrons involved.[58] Under anaerobic conditions, the redox potential of the electron-transport system in microbes is sufficiently negative to reduce the nitro group. However, when oxygen is present, the cytotoxic nitro radical anion is rapidly reoxidized to its parent drug in a process resembling futile cycling, and the bactericidal effects are diminished.[24,27]

The general mechanism of action of metronidazole (**1**), one of the most prescribed antibiotics of this class,[59] has been studied for decades and is relatively well understood. After entering the cell, **1** is converted to a short-lived, reactive nitro radical anion, which then decomposes to form a nitrite anion and an imidazole radical (Figure 4).[27] These toxic radical species can inhibit DNA synthesis and cause DNA damage by oxidation, leading to DNA degradation and cell death.[22] However, recent studies have contributed to an improved understanding of the mechanism of action of **1** in different target organisms. Electron donors involved in this process vary across different species.[22] In anaerobic and microaerophilic protozoa, such as *T. vaginalis*, *E. histolytica*, and *G. lamblia*, ferredoxin, which receives electrons from pyruvate:ferredoxin oxidoreductase (PFOR), has been suggested to activate **1**.[60,61] The reduction creates a concentration gradient that favors further intracellular uptake of the drug.[27] However, PFOR may not be the only activator of nitroimidazoles in these pathogens (recently reviewed by Ansell et al.).[62]

Thioredoxin reductase (TrxR) and nitroreductase 1 (NTR1) have also been implicated in the activation of 5-nitroimidazoles.[63] Leitsch et al. reported that **1** and other nitroimidazoles are metabolized differently in *G. lamblia* compared to *T. vaginalis* and *E. histolytica*.[64−66] It was found that the 2-nitroimidazole **2** and 5-nitroimidazole ronidazole (**20**) (Figure 5) did not bind to any *G. lamblia* proteins including TrxR, although these compounds formed adducts with proteins in *T. vaginalis* and *E. histolytica*.[64]
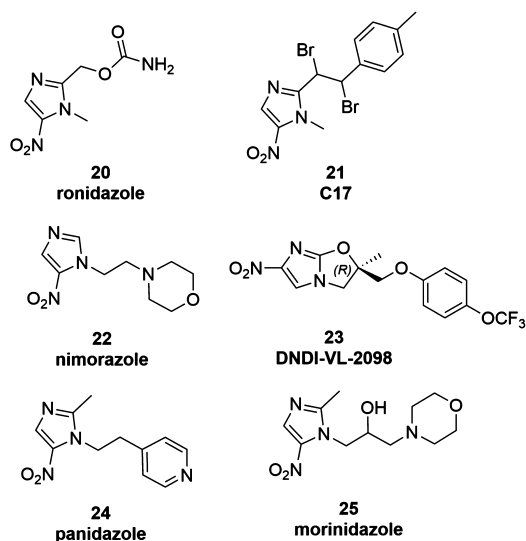
Figure 5. Chemical structures of other 4- and 5-nitroimidazoles with antimicrobial activity.

Furthermore, the effects of different nitroimidazoles on intracellular thiol levels in *G. lamblia* also differed from the other two microaerophiles.[65,66] In *H. pylori*, the reduction of **1** involves a separate mechanism which includes a two-electron transfer step using an oxygen-insensitive NADPH nitroreductase (encoded by the *rdxA* gene).[67]

Modulation of nitroreductase levels has been shown to directly affect the sensitivity of trypanosomatids to nitro compounds in vitro. A 2-nitroimidazole drug **5** that is currently used for treatment of *T. cruzi* utilizes an NADH-dependent trypanosomal type I nitroreductase for bioactivation.[68] The reductive pathway proceeds through two-electron transfer, forming a nitroso intermediate before conversion to a hydroxylamine.[68] This hydroxylamine is then transformed to a more stable dihydro–dihydroxyimidazole product through nitrenium and hydroxyl intermediates.[68] The dihydro–dihydroxy product breaks down to release a cytotoxic glyoxal and a substituted guanidine product, as shown in Figure 6.[24] Glyoxal can form guanosine–glyoxal adducts, leading to DNA cross-links and mutations.[68] However, as the release of glyoxal is inefficient, it is suggested that the antitrypanosomal activity of **5** probably relies on the combined effects of several metabolites including 4,5-dihydro-4,5-dihydroxyimidazole as the major metabolite.[68,69] A 5-nitroimidazole anti-HAT clinical candidate, **13**, that was also potent against visceral leishmaniasis (VL), utilizes the same type I nitro-

reductase enzyme for activation in both *T. brucei* and *L. donovani*.[70]

Like other nitroimidazole drugs, the bicyclic 4-nitroimidazole analogues delamanid (**17**) and pretomanid (**18**) are pro-drugs that require bioactivation of the nitro group. Unlike **1**, which is only active against anaerobic, nonreplicating *M. tuberculosis*, these compounds have activity against both replicating and hypoxic nonreplicating mycobacteria. Under aerobic growth conditions, the bicyclic 4-nitroimidazoles were found to inhibit mycolic acid synthesis, a key reaction for cell wall formation.[21,71] When *M. tuberculosis* is in a nonreplicating growth stage, such as when cells are exposed to hypoxia, the cell wall is not actively synthesized.[71] Instead, the bicyclic nitroimidazoles such as **18** induce respiratory poisoning via nitric oxide release, contributing to the compound's bactericidal activity.[72]

Characterization of in vitro generated pretomanid-resistant mutants showed that both deazaflavin-dependent nitroreductase (Ddn) and F_{420}-dependent glucose-6-phosphate dehydrogenase (FGD1) were essential for the activity of **18**.[73] However, only Ddn was found to directly reduce **18**, producing three primary metabolites and reactive nitrogen species.[74,75] Formation of the major *des*-nitro metabolite correlated with the rate of release of nitric oxide.[76] Nitric oxide reacts with respiratory chain cytochromes and cytochrome c oxidases, which disrupts regular electron flow and ATP homeostasis.[76] This results in cell death of nonreplicating *M. tuberculosis* under anaerobic conditions.[76] Although nitric oxide was also released in the presence of oxygen, it was insufficient to kill the replicating cells.[76] While second-generation bicyclic nitroimidazoles were found to be activated exclusively by Ddn, activation of the original parent molecule **16** has been found to depend on a wider range of F_{420}-dependent reductases including Rv1261c, Rv1558, and Rv3178.[74]

The mode of action of bicyclic nitroimidazoles in *Leishmania* is different from the mode of action discussed above for *M. tuberculosis*. It has recently been discovered that bicyclic nitroimidazoles are activated by a novel nitroreductase (NTR2) in *Leishmania*.[77] In contrast, monocyclic nitroimidazoles such as fexinidazole metabolite **15** were activated by the previously identified type I nitroreductase.[77] This clearly demonstrates the diverse mode of action of nitroimidazoles, both within an organism and also between different target organisms. It also highlights the difficulty in optimizing the potency of nitroimidazole drugs when the mode of action can be varied and often poorly defined.
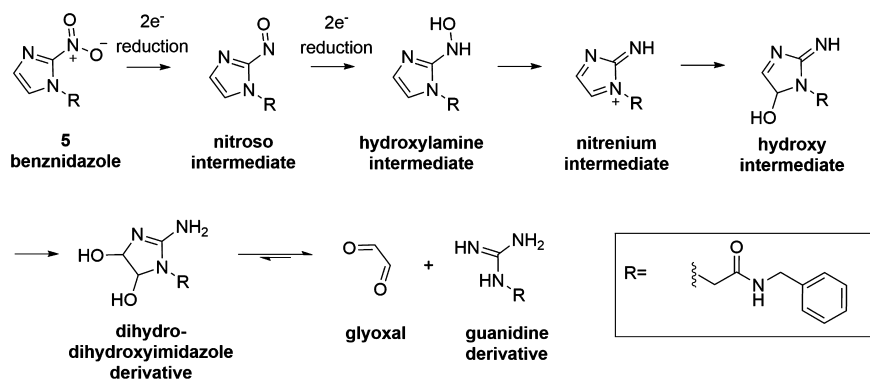

Figure 6. Mechanism of benznidazole (**5**) in *T. cruzi*, which involves bioactivation with type I nitroreductase.[24,68]

4. RESISTANCE MECHANISMS

Given that the mode of action of nitroimidazole drugs depends on the bioactivation of their nitro group by the target cell, resistance can occur when there is decreased expression/activity of the reductive enzymes.[78] These resistance mechanisms are different depending on the organism in question, but they share a similar basis. For example, reduced levels of PFOR have been linked to nitroimidazole resistance even though some in vitro selected strains deficient in PFOR only showed low levels of resistance to metronidazole (**1**).[61] The loss of PFOR activity was reported by Kulda et al. and Brown et al. in anaerobic induced metronidazole-resistant *T. vaginalis*.[79,80] In contrast, Land et al. showed that a ferredoxin knockout mutant of *T. vaginalis* with 95% reduced activity of PFOR remained sensitive to metronidazole.[81] In some **1**-resistant strains of *G. lamblia*, PFOR is downregulated up to 5-fold, which is consistent with reduced activation of the drug in other anaerobes.[61] A recent report describes a second-generation 5-nitroimidazole, C17 (2-[1,2-dibromo-2-(4-methylphenyl)ethyl]-1-methyl-5-nitro-1*H*-imidazole, **21**) that displays a high level of cross-resistance to **1** in metronidazole-resistant *G. lamblia*.[82] Resistant strains against **21** were found to reduce **1** at a similar level (60−80%) as metronidazole-sensitive isolates, indicating that functional PFOR was still expressed in these resistant strains.[83] Therefore, it is likely that resistance to 5-nitroimidazoles including **1** in *G. lamblia* involves more than one mechanism distinct from the activity of PFOR/ferredoxin.[83] In metronidazole-resistant strains of *H. pylori*, mutations in an *rdxA* gene that encodes an NADPH nitroreductase were found to reduce the level of PFOR indirectly, although the mechanism of this reaction remains unknown.[84]

Altered levels of nitroreductase expression are also responsible for resistance to nitroimidazoles. In laboratory-generated metronidazole-resistant *G. lamblia* cell lines, the expression of nitroreductase I (GlNR1) involved in the activation of **1** was found to be downregulated.[85] On the other hand, GlNR2, which can reduce the nitro group of the drug to a nontoxic amine group, was shown to be upregulated.[86] Ansell et al. have recently utilized RNA sequencing to study the differences in transcription products of three metronidazole-resistant *Giardia* lines, 106-21D$_{10}$, 713-M3, and WB-M3.[87] Both passive resistance mechanisms, as a result of reduced activation of **1**, and active resistance mechanisms, such as reduction of **1** to inactive products, were associated with metronidazole resistance.[87] However, differences were noted between 106-21D$_{10}$ and the 713-M3 and WB-M3 strains, supporting the hypothesis that varied mechanisms can underpin similar resistance phenotypes.[87] A recent study with *L. donovani* showed that loss of a single chromosomal copy of the nitroreductase gene resulted in weak resistance to the active fexinidazole metabolite **15**.[70] A similar effect was also observed in benznidazole-resistant *T. cruzi* laboratory strains, where loss of a single copy of the gene encoding a mitochondrial nitroreductase (TcNTR) resulted in abolished enzyme activity.[88]

Consistent with the different mode of action of bicyclic nitroimidazoles in *M. tuberculosis*, distinct resistance mechanisms to bicyclic nitroimidazoles have been observed. In laboratory-generated pretomanid-resistant *M. tuberculosis*, 83% of the mutants had a single mutation in one of the genes involved either in the activation of **18** (*ddn* and *fgd1*) or the F$_{420}$ biosynthetic pathway (*fbiA*, *fbiB*, and *fbiC*).[73] Of the five target genes, *ddn* exhibited the highest mutation frequency.[73] Crystal structures (PDBs 3R5R and 3B4Y) showed that the identified mutations were located within the catalytic domain of Ddn and FGD1, which is essential for prodrug activation.[73] Likewise, *M. tuberculosis* resistance to **17** was suggested to occur by similar mechanisms.[89] In clinical isolates of delamanid-resistant *M. tuberculosis*, reduced conversion of the prodrug to the *des*-nitro product was observed.[89]

Understanding the mechanism of nitroimidazole resistance is key for the development of new effective therapeutic agents and for the improved management of diseases. In certain cases, nitroimidazole-resistant microbes can be treated by other nitroimidazoles,[90] but some microbial strains have gained cross-resistance to multiple drugs from the same class.[91] Exposure to subtherapeutic concentrations among patients, especially from low- and middle-income countries, may lead to a rapid increase in resistance. Therefore, an optimized dosage to achieve adequate drug concentration at the site of infection is important. Combination therapy using partner drugs with distinct modes of action is another useful approach and has been successful in minimizing drug-resistant tuberculosis and malarial infections.[92] This strategy is actively being pursued in new tuberculosis treatment regimes. For example, the combination of **18** with moxifloxacin and pyrazinamide is currently in phase III clinical trials to treat drug-sensitive and multidrug-resistant TB (MDR-TB) (ClinicalTrials.gov Identifier: NCT02342886).[93]

5. NITROIMIDAZOLES: ANTIPARASITIC AND ANTIBACTERIAL AGENTS

There is an urgent need to develop new and more effective drugs to combat various neglected infectious diseases. The following section reviews the different types of parasitic and bacterial infections typically treated with nitroimidazole therapeutics, including discussion of current nitroimidazole treatment options with a focus on recent preclinical and clinical developments of nitroimidazoles.

5.1. Trichomoniasis. *T. vaginalis* is a sexually transmitted anaerobic, flagellated protozoan that can cause trichomoniasis in both men and women. Nitroimidazoles, including metronidazole (**1**) and tinidazole (**8**), are the only class of antimicrobial drugs approved to treat trichomoniasis in the United States.[94] Nitroimidazole **1** is more widely used, inexpensive, and generally well-tolerated. In the presence of oxygen, **1** exhibits a higher (less effective) minimum lethal concentration (MLC) as oxygen prevents the reduced nitro group from causing cellular damage.[95] Depending on the assay system, most strains of *T. vaginalis* that are clinically susceptible to **1** display aerobic MLCs in the range of 0.5−32 µg/mL,[96] while aerobic MLCs on clinically resistant isolates increase to 25 to >200 µg/mL.[97] Under anaerobic conditions, the MLC of susceptible isolates was 0.5−1 µg/mL, while a clinically resistant isolate BRIS/92/STDL/B7268 was 4.25 µg/mL.[97]

Other factors complicating adequate management of trichomoniasis infections also contribute to treatment failure. Reinfection from untreated/infected baseline sexual partners is not uncommon, as the majority of women (85%) and men (77%) with trichomoniasis are asymptomatic (reviewed by Kissinger).[98] Therefore, concurrent treatment of the sexual partner(s) of patients is critical. One option to treat sexual partners is called expedited partner therapy, in which medications can be provided to patients to give to their partners without the physician examining the partners.[98] According to Centers for Disease Control and Prevention guidelines, the recommended oral dose regimen of **1** is a single 2000 mg dose, or

alternatively, 500 mg twice a day for 7 days.[94] Intravenous administration of **1** results in similar cure rates but with fewer side effects than oral regimens.[99] However, this route of administration is not commonly used as it is less practical compared to oral dosing.[99] An intravaginal gel of **1** is also available but is not as favored due to the slightly lower cure rate compared to its oral form.[100]

Treatment with **8** is recommended when **1** is not tolerated or ineffective. The ethylsulfonylethyl side chain of **8** promotes a longer half-life (12−14 h)[101] and better tissue distribution than **1**. Nitroimidazole **8** is delivered more efficiently to the infected area as the concentration of drug found in vaginal secretions is comparable to the serum level.[99] In various *T. vaginalis* strains, **8** demonstrated lower MLCs than **1**. From an evaluation of 104 clinical isolates derived from patients where treatment with **1** failed, **8** showed lower mean aerobic MLC at 1015 μM compared to **1** at 2618 μM.[102] Although **8** may be used to treat metronidazole-resistant trichomoniasis, the development of cross-resistance is a concern.[99] Other 5-nitroimidazoles have also been investigated for the treatment of *T. vaginalis*. The elimination half-lives of most of the analogues, such as **9** and **10**, are longer than that of **1**. Nimorazole (**22**) is an exception, as it is rapidly metabolized to two major metabolites (the *N*-oxide and 3-oxomorpholine). However, **22** is still effective as the metabolites have potent activity and are indeed more active than the metabolites of **1**.[99,103]

5.2. Intestinal Parasitic Diseases. *G. lamblia* and *E. histolytica* are two of the most common diarrhea-associated intestinal protozoan parasites, causing giardiasis and amebiasis, respectively. Despite the recognition of these diseases for many decades, only a handful of treatment regimens are available and some of them have adverse effects and issues with resistance.[61,104] Metronidazole (**1**) is the most common drug for treatment of giardiasis worldwide. The clinical efficacy of **1** when dosed at 250 mg two or three times daily for 5−10 days in adult and pediatric patients was reported to be superior (median efficacy at 88%) to a single dose at 2000 or 2400 mg/dose (median efficacy at 48%).[104]

Other nitroimidazoles that are used to treat *G. lamblia* include **8**, **9**, and **10**, and they are suitable for once-daily dose administration.[105] In many countries excluding the United States, **8** is an approved front-line drug for the treatment of giardiasis. When given as a single 2 g dose in adults, **8** had a very high median efficacy at 92% and reduced clinical adverse effects compared to **1** in a single-dose regimen.[104] When given as a single dose, **9** had a slightly better efficacy compared to **8**, with a cure rate of 92−100% in adults.[104] In children, a single-dose (40 mg/kg) administration of **9** showed comparable efficacy to a seven-day course of **1** with enhanced patient compliance.[106] With an even longer half-life of 17−29 h, **10** was also given as a single dose of 2 g in adults, or 30 mg/kg in children. This treatment resulted in 80−100% parasitological cure rate among patients, comparable to that of the multiple dosage regimens of **1** or **8**.[107]

Like giardiasis, the major drug of choice against amebiasis is still **1**. Different minimum inhibitory concentrations (MIC) for clinical isolates and reference strains of *E. histolytica* have been reported in the past, and indiscriminate use of nitroimidazole drugs may over time increase the therapeutic concentration required for most of the antiamebic agents.[108] The MIC of **1** in laboratory-passaged strains as reported by Upcroft was 12.5−25 μM.[109] Adagu et al. showed that the mean IC$_{50}$ value of **1** was 18.5 μM for the susceptible isolates of *E. histolytica*.[110] Higher IC$_{50}$ values for clinical isolates compared to a reference strain

(HM1:IMSS) was also reported by Bansal et al.[108] Because **8** has similar efficacy to **1** against intestinal amebiasis and amebic liver abscess, it remains an alternative treatment options in countries that have approved this agent.[27] In patients with invasive amebiasis, **1** is usually prescribed for the first 5 or 10 days to eradicate tissue trophozoites.[27] As both **1** and **8** fail to reach high concentrations within the lumen of intestines and are less effective against the cyst form,[111] subsequent treatment with nonorally absorbed luminal amebicides (such as paromomycin and iodoquinol) to eliminate any surviving amoeba in the colon is often recommended.[112]

5.3. Kinetoplastids. Kinetoplastids are a group of flagellated protozoans that cause HAT, Chagas disease, and leishmaniasis. The DND*i* has recently established the target product profiles (TPPs) for these three trypanosomatid diseases.[113] The ideal TPPs focus on new regimens that are active against all strains/subspecies, with good oral bioavailability, short treatment durations, improved safety to efficacy ratios, and low cost.[114−116]

5.3.1. Human African Trypanosomiasis (HAT). HAT is caused by two subspecies: *Trypanosoma brucei gambiense* (responsible for over 98% of reported cases) and *Trypanosoma brucei rhodesiense*. Patients are asymptomatic during the early stage of the disease, and thus many infections are undiagnosed. However, during late stage disease, the parasites cross the blood−brain barrier and infect the central nervous system, causing serious neurological issues including paralysis, disturbance of sleep cycle, confusion, and progressive mental deterioration.[117] Current treatment of HAT depends on the type of infection and the disease stage. Pentamidine and suramin are the drugs of choice for stage 1 HAT but are ineffective at treating stage 2 HAT. Melarsoprol is the only available drug effective against *T. brucei rhodesiense* in stage 2, whereas eflornithine and nifurtimox/eflornithine combination therapy (NECT) are used for treating *T. brucei gambiense* stage 2 infection.[117,118]

Nitroimidazole **13** represents the first new clinical candidate against trypanosomatid diseases in 30 years. This antimicrobial was in preclinical development in the 1970s−1980s for its broad spectrum antimicrobial activity. Further development of **13** was not pursued at that time, partly due to the concerns over the mutagenicity of nitroaromatic compounds.[24] It was later "rediscovered" by DND*i* from ∼700 nitroheterocyclic compounds in a search for potential drug candidates for treatment of stage 2 HAT.[20]

Nitroimidazole **13** and its metabolites (**14** and **15**) have comparable activities against *T. brucei* laboratory strains and clinical isolates, with IC$_{50}$ in the range of 0.2−0.9 μg/mL.[119] Oral administration of **13** was effective against acute and chronic diseases in mice at 100 mg/kg/day for 4 days and 200 mg/kg/day for 5 days, respectively.[20] Pharmacokinetic data in mice, rats, and dogs indicated that the metabolites **14** and **15** likely accounted for the in vivo efficacy.[20] When tested in an Ames assay, **13** showed reduced mutagenicity activity in nitroreductase-deficient *Salmonella* strains compared to the standard strains.[20] This indicates that the mutations were mostly induced by bacterial nitroreductase enzymes.[20] Genotoxicity in mammalian cells was not observed for **13**, and it is currently in phase II/III clinical development to treat HAT.[120] However, cross-resistance between **13** and its metabolites **14** and **15** to **7** in *T. brucei*, due to their common mode of action involving NTR activation, is of concern.[121,122]

5.3.2. Chagas Disease. The causative agent for Chagas disease is *T. cruzi*, an insect-transmitted protozoan parasite. The

initial acute stage of Chagas disease is relatively mild and can be partially cleared by the body's immune system.[123] The chronic stage can be asymptomatic for years or develop to symptomatic chronic complications such as cardiomyopathy (enlarged heart), heart failure, and megacolon that can lead to death.[118] The benchmark of clinical efficacy for Chagas disease is **5**, which is the front-line 2-nitroimidazole drug against *T. cruzi* in acute stage disease.[124] Treatment with **5** resulted in a long-term cure rate of 70%.[24] It is administered orally at 5−10 mg/kg daily for 30−60 days for both adults and children.[125] However, its efficacy in chronic stage disease remains controversial. A study in 2002 demonstrated that the treatment of patients with chronic *T. cruzi* infection resulted in a mere 8% cure rate compared to 76% in patients with acute disease.[126] In murine models, a recent study using a highly sensitive bioluminescence imaging method found that chronic infections could be cured with **5** at 100 mg/kg daily dose within 5 days compared to acute stage infections, which required 20 days to achieve a similar curative outcome.[123] Another front-line drug for Chagas disease is the nitrofuran **7**, which was found to be trypanocidal against both circulating trypomastigotes and amastigotes.[118] However, both **5** and **7** produce side effects that increase with the age of patients.[126,127]

Considering the potential of **13** as a treatment for HAT, **13** was also selected for investigation for treatment of Chagas disease. Studies have shown that **13** is effective at curing both acute and chronic forms of *T. cruzi* infections, including benznidazole-resistant strains.[90] In chronic infections with benznidazole-resistant VL-10 strains, **13** was able to reduce myocarditis in all infected mice. At higher doses, **13** was superior to **5** in the prevention of cardiac inflammation.[90] Interestingly, fexinidazole metabolites (**14** and **15**) achieved a higher cure rate than **5** or **13** itself in treating acute stage *T. cruzi* infection in mouse models.[128] An initial phase II study of **13** was initiated in 2014 but was interrupted due to safety concerns with high doses administered for more than 14 days. However, the acceptable safety and tolerability of **13** at lower doses and shorter treatment duration have warranted further investigation and a new phase IIa clinical trial is planned in 2017.[129]

5.3.3. Leishmaniasis. There are different forms of leishmaniasis in humans, with visceral leishmaniasis (VL) and cutaneous leishmaniasis (CL) being the most common. VL is normally caused by *L. donovani* and *L. infantum*. VL is life threatening and affects internal organs including the spleen, liver, and bone marrow.[130] CL is caused by over 15 species of *Leishmania* and is associated with skin sores.[118] One of the available treatment options for leishmaniasis, miltefosine, is teratogenic and expensive, and resistance to miltefosine is developing rapidly.[45] Another front-line drug, amphotericin B, requires intravenous infusion and is unstable at high temperatures.[118] In the search for new drugs to treat leishmaniasis, **13** was also assessed for its therapeutic potential against VL.[45] In a mouse model of *Leishmania* infection, the administration of **13** in a once-daily 200 mg/kg regimen for 5 days resulted in 98% suppression of infection, which was comparable with the potency of the front-line drug miltefosine.[45] Both fexinidazole metabolites **14** and **15** were active in vitro against intracellular *L. donovani* amastigotes in peritoneal mouse macrophages, but the parent compound was found to be inactive.[45] This suggests that **14** and **15**, not **13** itself, likely contributed to the therapeutic effect. The parent drug, **13**, was advanced into a phase II proof-of-concept study in Sudan, but the trial was terminated in May 2014 as it failed to demonstrate conclusive efficacy in the majority of patients.[131] DND*i* has recently completed the assessment of the combination

of **13** and miltefosine for the treatment of VL patients in eastern Africa.[131]

Nitroimidazole **17**, which was granted conditional approval for the treatment of MDR-TB, has also been found to be effective against various *Leishmania* strains in laboratory studies. It was found that **17** (EC_{50} = 0.087 μM) surpassed the activity of the standard drug miltefosine (EC_{50} = 3.3 μM) and active fexinidazole metabolite (**15**) (EC_{50} = 5.3 μM) when assessed against intracellular *L. donovani* amastigotes.[132] The *S*-enantiomer of **17** was less potent, which was similar to its enantiomeric specificity in *M. tuberculosis*.[51] Nitroimidazole **17** also demonstrated favorable activity in vivo, with ED_{50} and ED_{90} of 7.3 and 21.5 mg/kg, respectively, in twice daily dosing in BALB/c mice that were infected with ex vivo *L. donovani* amastigotes.[132]

The antileishmanial potential of **18** and its *R*-enantiomer analogue was also investigated by Patterson et al.[133] In contrast to the poor activity (MIC > 100 μM) shown by (*R*)-PA-824 in *M. tuberculosis*, this enantiomer was potent against *L. donovani* growth, with EC_{50} of 0.16 and 0.93 μM against promastigotes and intracellular amastigotes in macrophages, respectively. It was 5-fold more active than (*S*)-**18** in vitro and showed almost complete clearance of infection in a mouse model of VL at 100 mg/kg, twice daily oral dosing for 5 days.[133] The combination of (*R*)-PA-824 and **15** demonstrated additive effects against *L. donovani* promastigotes, suggesting some future potential as a combination therapy for VL.[133] Interestingly, **17** and (*R*)-PA-824 were activated differently from the monocyclic nitroimidazole **13** in *L. donovani*, with the latter known to be catalyzed by NADH-dependent type I nitroreductase.[132,133]

DND*i* recently identified DNDI-VL-2098 (**23**, Figure 5) as a preclinical candidate for the oral treatment of VL, selected from a library of nitroimidazole compounds originally synthesized to assess the antimycobacterial activity of the class.[134] Nitroimidazole **23** is the *R*-enantiomer of a nitroimidazooxazole with the same bicyclic core structure found in **17**. It displayed potent submicromolar in vitro activity in a macrophage amastigote model against various *L. donovani* strains, including resistant and clinical strains.[135] In a HU3/BALB/c mouse model, **23** also demonstrated excellent in vivo activity, with a 90% effective dose (ED_{90}) at 3.7 mg/kg.[134] The *S*-enantiomer was much less active and failed to reach the ED_{90} when administered at 12.5 mg/kg.[134] In hamsters, **23** induced host immunity via activation of host-protective Th1-dominated immune responses, providing long-term activity in the chronic models.[134] Unfortunately, **23** was recently linked to testicular toxicity in animal models and it was concluded that **23** lacked an adequate safety margin for further progression.[136]

Fortunately, two lead molecules DNDI-8219 (structure not disclosed) and DNDI-0690 (structure not disclosed) from the nitroimidazooxazine subclass were identified as back up candidates for further investigation.[137] These compounds displayed promising in vivo efficacy, better solubility, and lower potential for cardiotoxic effects.[137] Importantly, a 14-day toxicity evaluation supported further progression of DNDI-0690 as a preclinical candidate for treatment of VL and potentially also for CL.[137] This study demonstrates the value of mining compound libraries that were prepared for treatment of one disease (in this case TB), as these may be repositioned to identify compounds with desirable activity, ADMET, and PK/PD characteristics that better suit the required TPPs of other organisms.

5.4. Gram-Positive and Gram-Negative Bacteria.
Antimicrobial drug resistance is a major problem to human health and will continually fuel the need to develop new antibiotics. It is not only acquired resistance but intrinsic resistance that poses a great challenge to the development of new antibiotics. Compared to Gram-positive bacteria, Gram-negative organisms are more resistant to chemotherapeutic agents as they have a largely impermeable outer cell wall that excludes most antibiotics.[138] Therefore, identifying antimicrobial agents that, in particular, have activity against Gram-negative bacteria, is extremely important. Nitroimidazoles satisfy this requirement as they are active against both Gram-positive and Gram-negative bacteria, although they generally only display activity against anaerobic and microaerophilic bacterial species.

Metronidazole (**1**) is potent against a range of Gram-positive and Gram-negative bacteria. This antimicrobial agent is especially important for the treatment of anaerobic infections caused by *Bacteroides fragilis*, *Clostridium difficile*, and *Fusobacterium nucleatum*.[22] For the treatment of mixed aerobic and anaerobic infections, **1** can be used in combination with other antibacterial agents that are effective against aerobic bacteria.[22] For example, a combination of ciprofloxacin and **1** showed favorable efficacy compared to *β*-lactam agents alone in intra-abdominal infections.[139] *B. fragilis* is a Gram-negative obligate anaerobic, nonspore forming bacterium that infects the human gut. Comparative studies showed that most 5-nitroimidazoles, including **8**, **9**, **10**, and panidazole (**24**), had similar or better activity than **1** against *B. fragilis* ATCC 23745 strain, with MIC ranging between 0.8–3.7 μM.[140]

Bacterial vaginosis (BV) is one of the most common vaginal infections in women of reproductive age that is caused by an overgrowth of Gram-positive or Gram-negative anaerobes (such as *Gardnerella vaginalis*, *Prevotella* spp., and *Mobiluncus* spp.) in the vaginal flora.[141] Current treatment of BV relies on **1**, which can be given orally (recommended treatment regimen at 500 mg, twice a day for 7 days) or in gel form.[141] Oral treatment with **1** for 7 days has a cure rate of 80–90% after 1 month but suffers from high recurrence rates when assessed 12 months following treatment.[142] Another first-line therapy is clindamycin, with equivalent efficacy as **1** in the short term.[142] Recently, Symbiomix instigated clinical trials toward the approval of SYM-1219, a granule formulation of **10**, as a single-dose treatment for BV in the USA. The half-life of **10** is superior when compared to **1**, and **10** is known to be active against many anaerobic bacteria associated with BV.[143] It has completed a phase III, randomized, double-blind, placebo-controlled study using a single, oral dose in 189 patients infected with BV.[144] It is anticipated to be the first single-dose oral regimen approved in the USA for the treatment of BV, with better patient adherence than the current therapy.[144]

Morinidazole (**25**), a third-generation 5-nitroimidazole, was approved in 2014 by the China Food and Drug Administration (CFDA) for treatment of bacterial infections such as pelvic inflammatory disease (PID). PID can be caused by a variety of organisms including the sexually transmitted Gram-negative bacteria *Neisseria gonorrheae* and *Chlamydia trachomatis* as well as *Mycoplasma genitalium* and other anaerobes that comprise vaginal flora.[145] Nitroimidazole **25** is used in the treatment regimen to improve anaerobic bacteria coverage and was less toxic than **1** and **9** in a mouse model of infection.[146]

C. difficile is a Gram-positive, spore forming, anaerobic bacterium that causes infection in the gastrointestinal tract, usually through the fecal–oral route.[147] As the first-line therapy, **1** is used to treat mild *C. difficile* infections (CDIs), while oral

vancomycin and fidaxomicin are alternative antibiotics, more commonly used to treat moderate to severe infections.[148] In cases of ileus or toxic megacolon, combination therapy of intravenous **1** and enteral vancomycin may be used.[147] Oral **1** has very high bioavailability, with a low concentration of **1** excreted in the formed stools (1.2 μg/g) compared to watery stools (9.3 μg/g) after treatment.[149] The reduced concentration of **1** in the colon, due to its high systemic uptake, is thought to contribute to lower efficacy against severe CDI and may potentially increase the generation of resistance.[148] The MIC$_{90}$ of **1** against *C. difficile* is in the range of 0.20–2.0 μg/mL, with resistant strains displaying MICs of 8–64 μg/mL.[150] Although **8** is not an approved drug for CDI, it possessed superior activity compared to **1** against resistant strains, while **1** was more active against susceptible strains.[151]

Another disadvantage of using **1** to treat CDI is its broad spectrum activity against Gram-positive and Gram-negative bacteria, which promotes dysbiosis of the microbiota and leaves patients vulnerable to recurrent infections. Development of antibiotics that are more selective for *C. difficile*, that are available at high concentrations in the colon, and which inhibit spores, are major focus areas for new treatments against CDI, as are the development of nonantibiotic microbiota based therapeutics (reviewed by Jarrad et al.).[148]

H. pylori is a Gram-negative, microaerophillic bacterium that can cause gastritis, peptic ulcer disease, and gastric cancer. Different treatment regimens have been used to treat *H. pylori* infections, which generally require two or more antibiotics. Standard triple therapy is composed of a proton pump inhibitor (such as omeprazole), clarithromycin, and amoxicillin, but its effectiveness is compromised by the increasing resistance to this treatment.[152] Nitroimidazole **1** is used as part of multidrug regimens in empiric therapy. The combination of **1**, omeprazole, and clarithromycin was found to be effective, with a cure rate of 88%.[153] Other triple/quadruple therapies drug cocktails including **1** have also been developed to treat *H. pylori* infections.

Resistance to **1** can be overcome by adding a proton pump inhibitor to the therapy (if one is not already present) or by increasing either the dose or the treatment duration.[152,154] A quadruple therapy containing high dose of **1** with tetracycline, bismuth subsalicylate, and omeprazole for 14 days achieved a high cure rate of 92% in patients with metronidazole-resistant *H. pylori*.[155] However, the optimal dose and duration of **1** for different levels of resistance is still unknown and yet to be determined.[152] MICs of **1** against susceptible and resistant *H. pylori* strains range from 0.25 to 2 μg/mL and >8 μg/mL, respectively.[156] As an alternative to **1**, **8** was shown to eradicate *H. pylori* more effectively than **1**.[157] In a randomized, blinded study, a regimen based on **8** achieved a high eradication rate of >90% in patients with metronidazole susceptible strains of *H. pylori*, but these regimes were not effective against strains with high levels of resistance to **1** (MIC = ≥256 μg/mL).[158]

5.5. Tuberculosis. One major hurdle to the successful treatment of TB infections is the length and complexity of the current available treatment regimens, which leads to poor patient compliance and the emergence of resistant strains. Therefore, the desired TPPs of new TB drugs focus on shortening the treatment duration, reducing the dosing frequency, and having the capacity to treat drug-resistant TB.[159] Nitroimidazoles can potentially shorten the treatment duration, as the mode of action to produce nitric oxide and other reactive intermediates can target the hypoxic, dormant stage of mycobacteria within granulomas.[72] 2-Nitroimidazoles were the first in this class reported to have
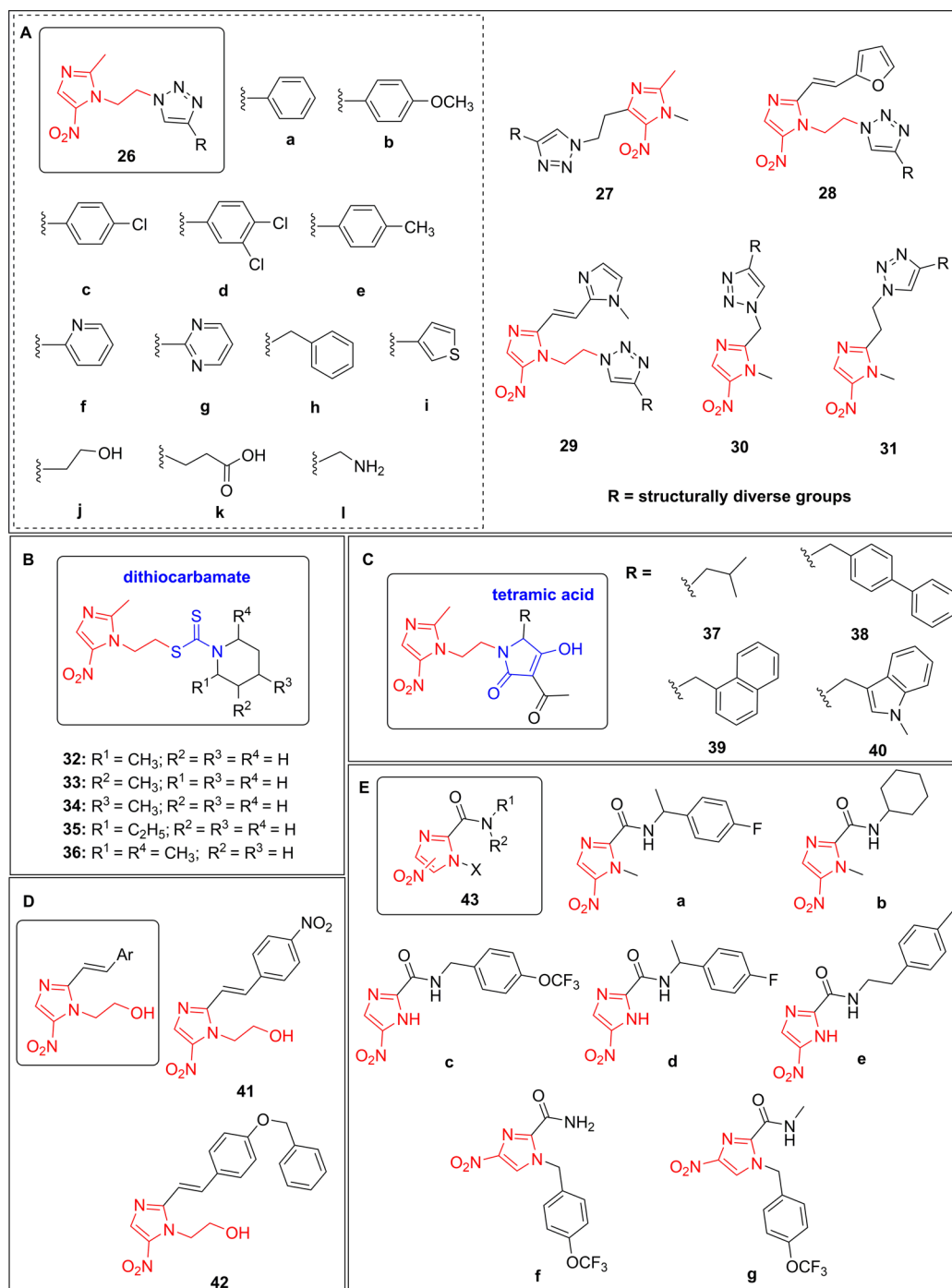
Figure 7. Recent development of 5-nitroimidazoles: (A) metronidazole-triazole analogues (**26−31**),[175,177] (B) metronidazole-thiocarbamate analogues (**32−36**),[180] (C) metronidazole hybrids with tetramic acid substituents (**37−40**),[181,182] (D) 2-styryl 5-nitroimidazole derivatives (**41,42**),[183] and (*E*)5-, 4(5)- and 4-nitroimidazole carboxamides (**43a−g**).[184]

antitubercular activity in the 1970s. However, their development was not pursued as they were less selective and more readily reduced by mammalian enzymes compared to the 5-nitro regioisomers.[31] The 5-nitroimidazole metronidazole (**1**) was found to be active against nonreplicating, hypoxic *M. tuberculosis* in vitro, but no activity was observed under aerobic conditions.[160] The efficacy of **1** in animal models has been linked to the presence of hypoxic lesions. For instance, **1** showed good efficacy in *M. tuberculosis*-infected guinea pigs and rabbits with developed necrotic lesions but lacked activity in mice models of *M. tuberculosis* infections where hypoxic lesions did not devel-

op.[161,162] A randomized, double-blind, placebo-controlled phase II study has shown that **1** may increase early sputum smear and culture conversion, but it was unfortunately too neurotoxic for longer term use.[161]

Bicyclic nitroimidazoles such as **17** and **18** have demonstrated potent activity against both actively growing and nonreplicating mycobacteria.[31] Nitroimidazole **17** has a potent MIC range of 0.006−0.024 μg/mL against standard and clinical isolate strains[50] and rapidly eradicated bacilli in mouse models.[51] It was not significantly metabolized by liver microsomes after 2 h and showed no inductive or inhibitory effect on cytochrome

P450 enzymes when tested at concentrations up to 100 μM.[50] In *Salmonella typhimurium*, no metabolism of **17** was observed,[163] which might explain its lack of mutagenicity in the Ames test.[50] In addition, **17** was not genotoxic in either in vitro or in vivo studies. When incubated with human NQO1 and NQO2 nitroreductase enzymes for up to 24 h, **17** was not reduced.[163] Neither cross-resistance nor antagonistic effects were observed against the first-line TB drugs such as isoniazid, rifampin, and ethambutol.[50] In macrophage-derived THP-1 cells, the intracellular activity of **17** at 0.1 μg/mL was similar to the effect of rifampin at 3 μg/mL and was superior to isoniazid.[50]

In a phase IIa clinical trial, **17** demonstrated measurable activity as a monotherapy in drug-susceptible TB patients.[164] However, **17** has poor absorption at higher doses which contributes to a requirement for twice daily dosing. In a two-month study that evaluated bacterial colony forming units (CFU) in cultured sputum samples from MDR-TB patients, the combination of **17** with an optimized background regimen (OBR) increased sputum culture conversion by approximately 50%.[165] It was granted conditional approval in the European Union, Japan, and Korea for the treatment of MDR-TB in 2014.[166] A phase III clinical trial involving 6 months of treatment with **17** plus OBR in MDR-TB patients, including those with HIV coinfection, has just been completed, with results expected in 2018 (ClinicalTrials.gov Identifier: NCT01424670).[53,167]

Similar to **17**, **18** is active against both replicating and nonreplicating *M. tuberculosis*, which is considered important for shortening the duration of TB treatment. The MIC values of **18** ranged from 0.015 to 0.25 μg/mL against a panel of susceptible and rifampin monoresistant clinical isolates.[21] No cross-resistance of **18** with other antitubercular drugs has been reported so far, highlighting its potential for treating multidrug-resistant strains.

The efficacy of **18** in in vivo animal infection models was promising. In mice, daily doses of **18** at 25, 50, and 100 mg/kg for 10 days showed comparable activity to isoniazid at 25 mg/kg in reducing mycobacterial burden in spleen and lung tissues.[21] A similar effect was observed in a *M. tuberculosis* aerosol challenged guinea pig model, where **18** was given orally at 40 mg/kg once daily for 30 days.[21] A combination of **18** with bedaquiline and sutezolid (currently in phase IIa development) showed greater sterilizing activity than the first-line regimen of rifampin, isoniazid, and pyrazinamide in a murine model of TB.[168] Another combination in clinical development, pretomanid−moxifloxacin−pyrazinamide, was evaluated in an early bactericidal activity study that measured the fall in CFU of *M. tuberculosis* in sputum samples of patients with smear-microscopy-positive pulmonary TB over 14 days of treatment.[169] This combined therapy was well tolerated and demonstrated comparable activity to the standard therapy of isoniazid, rifampin, pyrazinamide, and ethambutol.[169] A more recent study also showed that this combination had superior bactericidal activity than the standard regimen in the first 8 weeks of treatment.[93,170]

6. RECENT NITROIMIDAZOLE MEDICINAL CHEMISTRY CAMPAIGNS

Given the success of metronidazole (**1**) treatment of various anaerobic bacterial and protozoal infections and the advancement of delamanid (**17**) and pretomanid (**18**) in TB clinical development, there is now a renewed interest in developing new nitroimidazole analogues to meet the challenge of emerging and evolving infectious diseases. The following section is limited to recent medicinal chemistry studies, published from 2011 to 2016, that describe 5- and 4-nitroimidazoles. This is because there have been no other 2-nitroimidazoles in the drug development pipeline for infectious diseases since the discovery of **5** as the front-line drug to treat Chagas disease. Modifications of the 2-nitroimidazole scaffold for the past five years have instead focused on hypoxia imaging and radiosensitizing applications in cancer therapy.[171,172] Various chemical classes of nitrotriazole-based compounds with antitrypanosomal activity were synthesized based on **5**, but these do not contain the core nitroimidazole structure.[173,174]

6.1. 5-Nitroimidazole Analogues. Nitroimidazole **1** has an imidazole core structure, with a nitro group at the 5-position which is essential for its antimicrobial activity and a hydroxyethyl side chain at the 1-position which is commonly used for modifications to improve biological efficacy. The 2- and 4-positions also have potential to be modified. Metronidazole−triazole conjugates have recently received attention from several research groups. A large library of structurally diversified 5-nitroimidazoles (>650 compounds, **26**−**31**, Figure 7A) was prepared utilizing a modular synthesis through the copper(I)-catalyzed azide alkyne cycloaddition (CuAAC, or "click reaction").[175] Crude reaction mixtures (≥80% purity by LC-MS) were tested, and many of these metronidazole−triazole compounds showed improved activity against a range of microbes, including *G. lamblia*, *T. vaginalis*, *H. pylori*, *C. difficile*, and *B. fragilis*. Remarkably, most of these compounds were even active against metronidazole-resistant strains of *T. vaginalis* and *G. lamblia*, and a number of derivatives displayed efficacy in a mouse model of giardiasis.[175] Several compounds were tested in micronucleus assay using mammalian CHO cells, and no acute genotoxicity was observed.[175]

Negi et al. extended the study of metronidazole−triazole analogues (**26**) to *E. histolytica*.[176] Compounds derived from an in-house database were docked against *E. histolytica* thioredoxin reductase, and the top 10 hybrids with the best docking scores were assessed for their activity against HM1:IMSS strains. Inhibition of *E. histolytica* growth in vitro corroborated the initial docking predictions. Most of the potent compounds were found to interact with the arginine residues in the reductase binding pocket through their nitroimidazole ring head groups.[176]

Jarrad et al. also reported the synthesis of a library of structurally related metronidazole−triazole analogues (**26**).[177] Several compounds were identified with excellent broad spectrum activity targeting *C. difficile*, *E. histolytica*, and *G. lamblia*, but no activity was observed against the facultative anaerobic bacteria tested. Hydrophobic derivatives with phenyl (**26a−e**) or benzyl (**26h**) substituents as well as heterocyclic R groups such as pyridine **26f** and thiophene **26i** favored broad spectrum anaerobic activity in contrast to polar analogues (e.g., **26g**, **26j**, **26k**, and **26l**).[177] These metronidazole−triazoles showed cross-resistance against metronidazole-resistant strains of *C. difficile*, *H. pylori*, and *G. lamblia*. However, for the most potent derivatives, several compounds were active in a therapeutically relevant range for treatment of *G. lamblia*.[177]

The hybridization of **1** with other active ligands is a promising approach to form chimeric compound classes with improved biological activity, although with the caveat that formation of chimeric compounds may be detrimental to drug-like properties such as oral availability and toxicity. To increase trichomonacidal activity and to block the metabolism of the 1-hydroxyethyl side chain of **1** to 1-acetic acid, Kumar et al. synthesized a library of new piperidine dithiocarbamate hybrids by substituting the
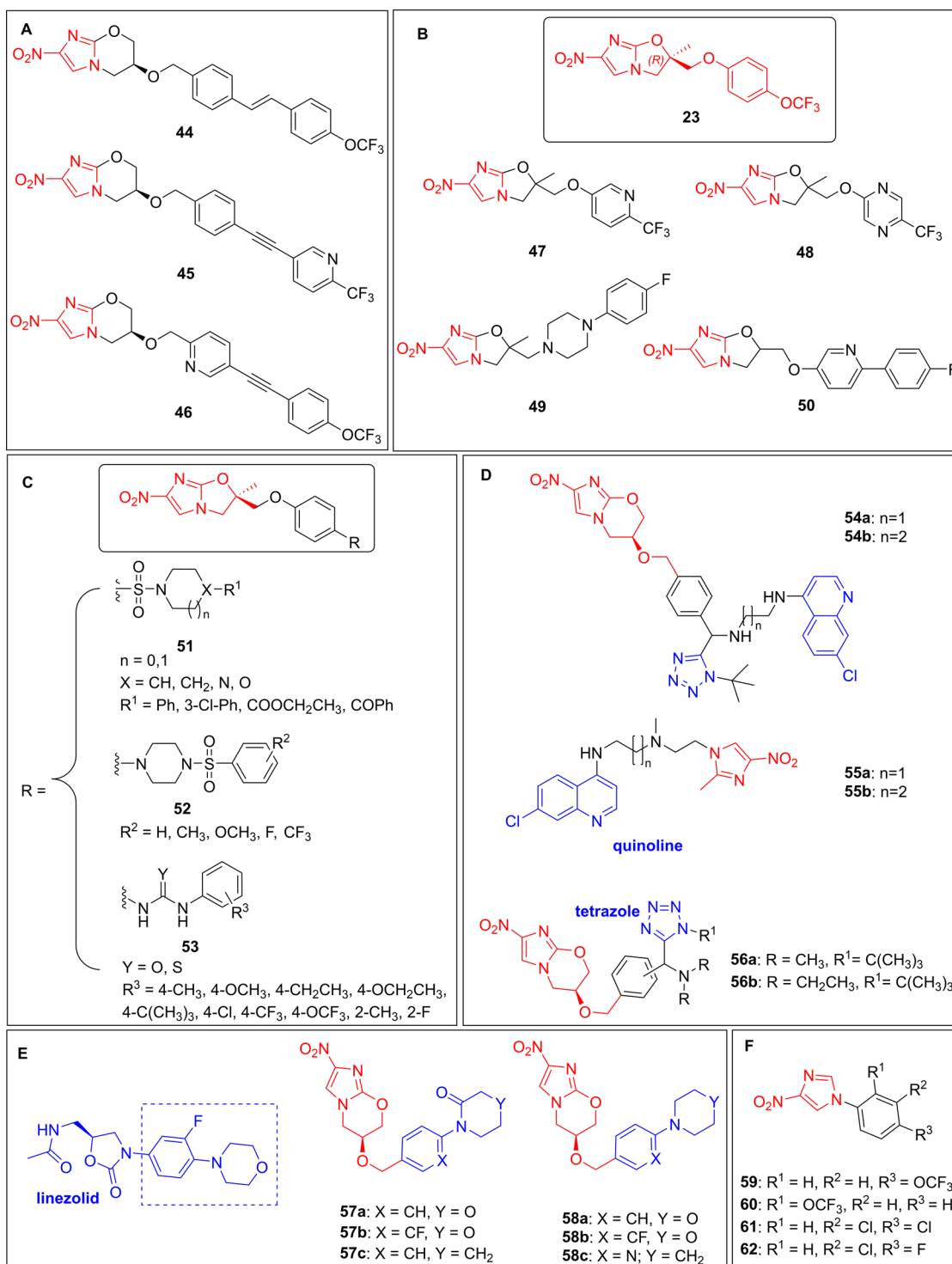
Figure 8. New 4-nitroimidazole analogues: (A) pretomanid derivatives with extended side chains (**44**–**46**),[186] (B) backup series for **23** (**47**–**50**),[187] (C) delamanid analogues with polar functionalities such as sulfonyl and thiouridyl groups (**51**–**53**),[188] (D) pretomanid conjugated with tetrazole and/or quinoline rings (**54**–**56**),[189] (E) pretomanid–oxazolidinone hybrids (**57**–**58**),[190] and (F) monocyclic 1-aryl-4-nitro-1*H*-imidazoles (**59**–**62**).[191]

hydroxyl group with various dithiocarbamates, which are well documented for their antimicrobial activity.[178,179] Using 3D-QSAR analysis, they subsequently designed another five compounds (**32**–**36**, Figure 7B) that surpassed the antitrichomonas activity of **1**. Compounds **32**–**36** showed a minimum 3-fold and 10-fold improvement in activity compared to **1** against metronidazole-susceptible and -resistant strains of *T. vaginalis*, respectively.[180]

In an attempt to improve the localization of nitroimidazoles to the GI tract, Cherian et al. prepared metronidazole derivatives containing a tetramic acid moiety.[181] The tetramic acid moiety exists abundantly in natural products, shows narrow-spectrum activity against Gram-positive bacteria (such as *Clostridium difficile*, *Staphylococcus aureus*, and *Bacillus anthracis*), and has poor permeability.[182] These hybrids (**37**–**40**, Figure 7C) retained similar activity to **1** against *C. difficile* but were absorbed

at a lower rate and retained in the GI tract, a useful property to deliver drugs for treatment of microbial infections that are confined to the gastrointestinal tract.[181]

Derivatives based on 2-styryl 5-nitroimidazole (Figure 7D) were reported and screened for antibacterial activity by Duan et al.[183] These compounds, potential FabH inhibitors, were tested against two Gram-positive (*Bacillus subtilis* and *Bacillus thuringiensis*) and two Gram-negative (*Escherichia coli* and *Pseudomonas aeruginosa*) bacterial strains.[183] Compounds **41** and **42** showed the most potent FabH inhibition activities (IC_{50} = 2.1, 3.4 μM) and were active against both Gram-positive (IC_{50} = 6.3−13.9 μg/mL) and Gram-negative (IC_{50} = 14.5−36.4 μg/mL) bacteria.[183]

Modification of the 2-position of the imidazole ring was also recently explored by Jarrad et al. in a study that reinvestigated nitroimidazole carboxamides for therapeutic potential against a range of parasites.[184] This study expanded upon a patent filed over 40 years ago by Merck & Co. Inc. in 1973 that disclosed the efficacy of several 1-methyl 5-nitroimidazole 2-carboxamides in trichomoniasis and histomoniasis animal models,[185] but further studies were never reported. A library of 32 novel nitroimidazole carboxamides (Figure 7E) were synthesized and assessed for inhibitory activity against *G. lamblia*, *E. histolytica*, *T. vaginalis*, and *C. difficile*.[184] Several of the new compounds (**43a**, **43c**, and **43e**) were active against metronidazole-sensitive and -resistant *G. lamblia* strains (EC_{50} = 0.1−2.9 μM), while others (**43b** and **43d**) showed improved activity against *E. histolytica* (EC_{50} = 1.7−3.7 μM). 4-Nitroimidazole carboxamides such as **43f** and **43g** were also synthesized. In general, while the 4-nitro series displayed potent activity toward *G. lamblia* (the majority of compounds EC_{50} = 2.7−8.4 μM, cf. **1** EC_{50} = 6.1 μM), only minimal activity was observed against *E. histolytica* (EC_{50} = 10 to >50 μM) and *C. difficile* (MIC = 16 to >64 μg/mL).[184] Importantly, several compounds examined for metabolic stability were stable after 2 h incubation with human liver microsomes.[184]

6.2. 4-Nitroimidazole Analogues. The recent focus on bicyclic 4-nitroimidazoles against TB has encouraged medicinal chemists to seek new generations with improved biological profiles. The limited solubility of **17** and **18** has provided opportunities to improve their physiochemical properties. Palmer et al. reported the antitubercular activity and pharmacokinetic data of 80 new extended side chain analogues of **18**, with a variety of linker functionalities between two aryl rings (Figure 8A).[186] Most of the lipophilic and highly polar functionalities were well tolerated in vitro (except for sulfonamide or aliphatic NH moieties), while aqueous solubility was improved when hydrophilic linkers were introduced. Three derivatives (**44**−**46**) surpassed the efficacy of **18** in a mouse model of acute tuberculosis, and compound **46** was about 24-fold more potent than **17** in the stringent chronic model.[186] Ames testing against *Salmonella* strains showed that compound **46** was not mutagenic, which is an important property to justify further development from a safety−efficacy perspective.[186]

One backup series (**47**−**50**, Figure 8B) to the preclinical lead nitroimidazooxazole **23** focused on improving the physiochemical/pharmacological properties as well as obtaining a deeper understanding of the SAR.[187] The replacement of phenyl rings by pyridine **47** and pyrazine **48** or the use of arylpiperazine **49** as a biaryl moiety at the right-hand terminus were able to improve the solubility by 10−20-fold and 6-fold, respectively. In a mouse model of acute *L. donovani* infection, arylpyridine **50** demonstrated superior efficacy to the preclinical lead, with the *S* enantiomer showing a slightly preferred profile.[187] Polar

functionalities, including sulfonyl, uridyl, and thiouridyl groups, were also utilized by Yempalla et al. to synthesize three series of nitrodihydroimidazooxazoles (**51**−**53**, Figure 8C) with enhanced aqueous solubility compared to **17**.[188]

4-Nitroimidazole and nitroimidazooxazine analogues with tetrazole and quinoline hybrid structures (Figure 8D) were reported by Tukulula et al. in 2013 as antimalarial and antitubercular candidates.[189] Pretomanid−chloroquinoline hybrids **54a** and **54b** as well as methylnitroimidazole−chloroquinoline hybrid **55b** showed superior antiplasmodial activity (IC_{50} = 0.094−0.164 μM) compared to the standard chloroquine (IC_{50} = 0.213 μM) and primaquine (IC_{50} = 0.643 μM) antimalarial treatments against the K1 strain of *Plasmodium falciparum*. For antimycobacterial activity, most of the tetrazole series (such as **56a** and **56b**, **54a**, and **54b**) showed better activity (MIC of 0.313−0.625 μM) against *Mtb* H37R_V strains compared to the rest.[189]

To develop improved antitubercular drugs, hybrids of **18** and oxazolidinone (Figure 8E) were synthesized by Rakesh et al. to utilize the excellent physiochemical properties and promising antimycobacterial activity of linezolid.[190] The (4-trifluoromethoxy)benzyl side chain of **18** was replaced by the outer ring elements of linezolid analogues without modification of the nitroimidazole core. Although the physiochemical properties were improved and the in vitro antitubercular activity was maintained, these new hybrids (**57** and **58**) lost efficacy in in vivo models of infection.[190]

Trunz et al. have reported the synthesis of monocyclic 4-nitroimidazoles (Figure 8F) with potential antitrypanosomal activity.[191] Two of the most potent compounds, **59** and **61** (IC_{50} = 0.10−0.16 μM), showed comparable activity to **12** against *T. b. rhodesiense* and were at least 16-fold more active than **13**.[191] Compounds **59**−**62** showed mutagenicity when assessed in standard *Salmonella*, but this was reduced or abolished when nitroreductase-deficient strains were employed.[191] The compounds were nongenotoxic when tested in human lymphocytes, making this monocyclic scaffold a possible lead for the treatment of HAT.

To summarize, recent medicinal chemistry efforts have focused on a number of different approaches to reinvigorate and obtain new value from the "old" nitroimidazole scaffold. The hydroxyl side chain of **1** has proven to be a robust site for modification. The hydroxyl group has been replaced with a triazole linker,[175−177] dithiocarbamates,[180] and tetramic acids,[181] with goals ranging from improving the activity to reducing the oral absorption in order to increase the concentration in the gastrointestinal tract and better target microorganisms that reside there. Reinitiating studies on "forgotten" nitroimidazoles, such as the nitroimidazole carboxamide scaffold, has also been a means to identify new active compounds.[184] Significant efforts have been directed toward bicyclic 4-nitroimidazoles to not only improve their antitubercular activity and compound properties but also to reposition these toward other organisms including *L. donovani* and *P. falciparum*. Some nitroimidazoles, including **12** and **16**, are associated with mutagenicity and genotoxicity which have precluded their further development, so developing preclinical candidates that are not mutagenic remains an important priority.

7. CURRENT STRATEGIES TO FACILITATE DRUG DEVELOPMENT

7.1. Drug Repurposing. Nobel laureate Sir James Black once stated that "The most fruitful basis for the discovery of a

new drug is to start with an old drug".[192] Drug repurposing or repositioning is an attractive strategy to find new treatments based on existing drugs or previously failed clinical candidates. These strategies can save significant time and money compared with the development of a new chemical class because pharmacology and toxicity data are already known in humans. As cited from a recent review article by Njoroge et al., drug repurposing refers to the situation where "an approved drug in one disease area is found to be active in another disease", whereas drug repositioning involves "the uses of a drug active in one disease as a template for the synthesis of derivatives active in another disease".[193] Another term, drug rescue, refers to "developing new uses of a drug that failed to progress through clinical studies or which was removed from the market".[193] These approaches have been applied to nitroimidazoles and have significant appeal for the drug discovery process of neglected diseases where resources are limited and the financial return is poor.

As outlined in earlier sections of this manuscript, these strategies have been pursued for both **13** and the nitroimidazo-oxazoles/oxazines. As an "old" nitroimidazole, **13** was "rescued" and rediscovered for its antitrypanosomal activities. It is currently in clinical development and will be the first oral drug for phase I and II HAT if successful. Nitroimidazole **13** has also been used to treat other trypanosomatid-related diseases such as Chagas and leishmaniasis. A second great example of drug repurposing and repositioning includes the investigation of **17** and development of DNDI-0690 for treatment of VL. Unlike **17**, which is a TB clinical candidate, DNDI-0690 had not previously entered preclinical development, slowing down its progression into VL clinical trials. However, the knowledge of how **17** behaves in the clinic is still of some value for the advancement of DNDI-0690 as a potential new treatment for VL.

Drug repurposing initiatives can also promote partnerships between academics, not-for-profit organizations, and industry. For example, DND*i*, the organization developing **13** and **17/18** derivatives, has a large drug portfolio for NTD through a wide range of collaborators, with a major focus on HAT, Chagas disease, and leishmaniasis. All of the R&D activities are outsourced to minimize the cost, while funding of the projects arises from a range of contributing public/private partners. This is an important aspect for neglected disease drug discovery that attracts little interest from the big pharmaceutical players.

7.2. Open Resources and Platforms. Open databases established by academic and not-for-profit initiatives assist drug development in the field of rare and neglected diseases and can be a source of inspiration to identify nitroimidazoles with potential for further development. Compound specific databases such as PubChem, ChEMBL, ChemSpider, and IDMap provide chemical structural information and some associated biological activity, whereas DrugBank, Therapeutic Target Database, and SuperTarget provide drug profiles and information on target protein or diseases.[194] PubChem is organized into Substance, Compound, and BioAssay categories that provide information on chemical structure similarity and bioactivity data.[195] This data is an important resource to assist in the repositioning and repurposing of drugs and, in particular, of nitroimidazoles, because nitroimidazoles are likely to have broad spectrum activity against a range of microorganisms. For example, a search using the keyword "nitroimidazole[All Fields]" (April 12, 2017) identified 2697 molecules in the PubChem Compound database, with 1028 records of activity and binding data in the PubChem BioAssay database. However, nitroimidazoles are almost never

profiled against the breadth of microorganisms that they potentially can target, often due to limitations with microbiological screening available to the investigating groups. Therefore, even previously reported nitroimidazoles often have untapped potential that could be enabled by broader spectra of action profiling.

Screening platforms play an important role in accelerating the profiling of nitroimidazoles against the vast number of different target organisms. For example, the Johns Hopkins Clinical Compound Screening Initiative (JHCCSI) was launched in 2002 to support collaborative drug screening. Collections of existing drugs were screened at Johns Hopkins and by global collaborators against a variety of different diseases, including malaria and NTD.[194,196] The NIH, particularly the National Institute of Allergy and Infectious Diseases (NIAID), has established a program to screen compounds against *M. tuberculosis* from both academic and commercial sources. Compounds with good in vitro activity and acceptable selectivity index are progressed for further evaluation against intracellular *M. tuberculosis* in macrophage infection assays and for efficacy in in vivo models.[197] The Center for Discovery and Innovation in Parasitic Diseases (CDIPD) is an interdisciplinary research center that studies the basic biology and biochemistry of a range of parasites causing NTD. CDIPD also collaborates with academic researchers as well as nonprofit and industry partners in translational research to develop new leads into clinical candidates.[198]

One other example of an open collaborative initiative is the Community for Open Antimicrobial Drug Discovery (CO-ADD), which is funded by the Wellcome Trust and The University of Queensland. CO-ADD provides free screening of compounds from all over the world against five key ESKAPE bacterial pathogens and two fungi (*Candida albicans* and *Cryptococcus neoformans*).[199,200] While nitroimidazoles do not typically display activity against this panel, this initiative represents an important model to facilitate broad profiling of potential antimicrobials and is also useful as a counterscreen to better understand the spectrum of action of molecules of interest. The concentrated resources, expertise, and networks found at the above centers can play an important role in progressing new nitroimidazole drug development.

For researchers to develop active compounds from hits to leads, and ideally to clinical candidates, it is important to have a desirable TPP to guide medicinal chemistry optimization in a focused manner. To assist researchers in this area, in 2015, experts from the Japanese Global Health Innovative Technology (GHIT) Fund, Medicines for Malaria Venture (MMV), DND*i*, and TB Alliance, together with the representatives from the Bill & Melinda Gates Foundation, published disease-specific criteria for hits and leads in drug discovery for infectious diseases prevalent in the developing world.[124]

Recently there has been a discussion of different "open innovation" approaches in drug discovery.[201,202] This involves open sharing of data as well as ideas and workflows without barriers. These different approaches could be applied to nitroimidazole projects that rely on contributions from a wide variety of sources. For example, the Open Source Malaria project which commenced in 2011 is a transparent, online platform that allows scientists to share their research in antimalarial drug discovery. It uses an online electronic lab notebook system that records all the results and experimental procedures from around the world, with other scientists being free to access the data and compare their own results.[203] Further expansion of this approach

to different nitroimidazoles targeting a range of organisms in different ways, may prove beneficial. CO-ADD is also an example of an "open innovation" approach, as all data, including compound structures, will eventually be made publically accessible in an online database.

Another example of an open collaborative structure to enable research in NTD has been spear-headed by Michael Pollastri at North Eastern University. The NTD Data Sharing project is designed to facilitate sharing of information between researchers in NTD and to facilitate collaboration.[204] The NTD Data Sharing project is open to join, but the information shared among participants is "protected" (or closed) within a user login database, the Collaborative Drug Discovery Vault.[205] This model not only appeals to researchers who want to share data in a more timely fashion than publication and conference presentations but who also desire the security of a regulated community.[206] Unlike the Open Source Malaria project, which is a coordinated effort to identify new drugs, the NTD Data Sharing project does not seek to coordinate researchers' projects, although projects are discussed in consortium meetings to facilitate advancement of projects in a strategic fashion.

While resources that are available to individual groups often limits progression of compounds, the open innovation concepts above aim to make tools and capacity more widely available to assist with driving a project forward when it would otherwise hit a stalling point. This is particularly useful for the development of nitroimidazoles, as they are known for their complex mode of action and so require extensive biological profiling to sufficiently understand both the spectra and mechanisms of action. Ultimately, open innovation concepts promote a thorough assessment of the potential of nitroimidazoles for therapeutic development against a range of different potential target pathogens.

8. CONCLUSION AND FUTURE PERSPECTIVES

Infectious diseases are no longer a "problem for the poor" but have become a global health threat. As microbes rapidly gain resistance to current drugs, scientists are racing against time to find new treatment options before the antibiotic pipeline dries up. Many nitroimidazole drugs have been approved for the treatment of infectious diseases, but interest in this class waned several decades ago due to concerns over the potential toxicity of the nitro group. However, in the past decade there has been a resurgence of investigations into nitroimidazoles as a source of potential new treatments for infectious diseases. For example, **13** and **18** are in clinical development, and **17** has been granted conditional approval for treatment of MDR-TB. "Old" and abandoned nitroimidazoles have been rediscovered through drug repurposing or repositioning programs. This new avenue accelerates the otherwise lengthy process of drug discovery compared to the traditional de novo strategy, as human pharmacokinetic and safety data are often already known. Combination therapy may also be an effective strategy to increase the success rate of drug repurposing by utilizing the additive or synergistic effects of the combined drugs, with the multiple targeting mechanisms potentially able to overcome existing resistance or delay the induction of new resistance. However, the use of multiple drugs increases the risk of drug–drug interactions and other side effects, which must be characterized to prevent any toxicity or adverse effects.

Significant research efforts have focused on extensive SAR studies around new nitroimidazoles in order to develop new drugs. Alternatively, existing nitroimidazole drugs are being combined with other pharmacophores to develop hybrid molecules that can act as "double-edged swords". It is believed that these molecules will exhibit superior activity with dual action that can overcome resistance. While it is hoped that these can be advanced to clinical development in the near future, hybrid molecules must still progress through traditional preclinical toxicity studies, losing the advantage of repurposed entities.

An important factor for nitroimidazoles in drug development is the bioavailability and pharmacokinetics of these compounds relative to the disease target. The two newly emerging TB drug candidates, **17** and **18** suffer from solubility issues and require specialized formulation. Therefore, further modification is required to improve the oral bioavailability of these compounds.

There is a promising future in the development and expansion of the nitroimidazole family to address unmet need in the field of neglected infectious diseases. Recent research has led to an improved knowledge of how the nitroimidazole compound class works, providing insight not only into the multimodal mechanisms within specific pathogens but also variations between different organisms. Alternative models of open research and screening platforms may facilitate further development of the nitroimidazole class to maximize the use, benefits, and advantages that this scaffold offers. Future development of nitroimidazoles will rely on a strong research partnership and sustained commitment between private and public sectors to translate fundamental academic research into clinical interventions.

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Notes
The authors declare no competing financial interest.

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Angie M. Jarrad has expertise spanning medicinal chemistry, organic synthesis, and microbiology, with six years of experience in a broad range of antimicrobial drug development projects. She received her Ph.D. from the University of Queensland in 2016 for the development of novel structural classes derived from existing vancomycin and metronidazole-based therapies targeting gut pathogens, in particular *Clostridium difficile*, *Giardia lamblia*, and *Entamoeba histolytica*. Throughout her Ph.D.

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Matthew A. Cooper completed his Ph.D. in Australia (1995) and then spent 13 years in the UK at the University of Cambridge, start-ups, and biotechnology companies. He returned to Australia (2009) as an NHMRC Australia Fellow. He has consulted with Private Equity Investment, Pharmaceutical, Biotechnology, and Diagnostics companies and was Managing Director of Cambridge Medical Innovations (part of Alere Inc.), then CSO of Akubio Ltd. He is an inventor and driver of a several antibiotic drug discovery programs with preclinical lead compounds. Professor Cooper currently holds a fractional Professorial Research Fellow appointment at the University of Queensland with his remaining time as CEO of Inflazome Ltd. (Dublin, Ireland), a company developing drugs to address clinical unmet needs in inflammatory disease by targeting the inflammasome.

Mark A. T. Blaskovich is an experienced medicinal chemist with a career spanning three biotechnology companies in the USA and Australia, most recently as COO of Mimetica, where he was responsible for developing a first-in-class melanocortin-5 receptor antagonist that completed phase 2 clinical testing for the treatment of acne in 2016. He received his Ph.D. in chemistry from the University of Waterloo (1993) and since 2010 has been applying his drug discovery skills to the development of new antibiotics at The University of Queensland. He has coauthored more than 50 research publications, is author of *The Handbook on Syntheses of Amino Acids*, and is an inventor on 10 patent families containing over 50 granted patents. Dr. Blaskovich helped found the Community for Open Antimicrobial Drug Discovery and has been featured in media articles on the rise of antimicrobial resistance.

ABBREVIATIONS USED

BV, bacterial vaginosis; CDI, *Clostridium difficile* infection; CDIPD, Center for Discovery and Innovation in Parasitic Diseases; CFDA, China Food and Drug Administration; CFU, colony forming units; CL, cutaneous leishmaniasis; CO-ADD, Community for Open Antimicrobial Drug Discovery; Ddn, deazaflavin-dependent nitroreductase; DNDi, Drugs for Neglected Diseases *initiative*; EMA, European Medicines Agency; ESKAPE, *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* species; FabH, β-ketoacyl–acyl carrier protein synthase III; FDA, Food and Drug Administration; FGD1, F_{420}-dependent glucose-6-phosphate dehydrogenase; GHIT, Global Health Innovative Technology; GlNR, *G. lamblia* nitroreductase; HAT, human African trypanosomiasis; JHCCSI, Johns Hopkins Clinical Compound Screening Initiative; MLC, minimum lethal concentration; MMV, Medicines for Malaria Venture; mV, millivolts; NCATS, National Center for Advancing Translational Sciences; NECT, nifurtimox/eflornithine combination therapy; NIAID, National Institute of Allergy and Infectious Diseases; NPV, net present value; NTR, nitroreductase; NTD, neglected tropical diseases; OBR, optimized background regimen; PFOR, pyruvate:ferredoxin oxidoreductase; TcNTR, *T. cruzi* nitroreductase; TPP, target product profile; TrxR, thioredoxin reductase; VL, visceral leishmaniasis

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