molecules

MDPI

Article

Niclosamide Is Active In Vitro against Mycetoma Pathogens

Abdelhalim B. Mahmoud [1,2,3,†], Shereen Abd Algaffar [4,†], Wendy van de Sande [5], Sami Khalid [4], Marcel Kaiser [1,2] and Pascal Mäser [1,2,*]

1 Department of Medical Parasitology and Infection Biology, Swiss Tropical and Public Health Institute, 4051 Basel, Switzerland; halim.mahmoud@swisstph.ch (A.B.M.); marcel.kaiser@swisstph.ch (M.K.)
2 Faculty of Science, University of Basel, 4001 Basel, Switzerland
3 Faculty of Pharmacy, University of Khartoum, Khartoum 11111, Sudan
4 Faculty of Pharmacy, University of Science and Technology, Omdurman 14411, Sudan; phd_sh086@hotmail.com (S.A.A.); khalidseek@hotmail.com (S.K.)
5 Erasmus Medical Center, Department of Medical Microbiology and Infectious Diseases, 3000 Rotterdam, The Netherlands; w.vandesande@erasmusmc.nl
* Correspondence: pascal.maeser@swisstph.ch; Tel.: +41-61-284-8338
† These authors contributed equally to this work.

Abstract: Redox-active drugs are the mainstay of parasite chemotherapy. To assess their repurposing potential for eumycetoma, we have tested a set of nitroheterocycles and peroxides in vitro against two isolates of *Madurella mycetomatis*, the main causative agent of eumycetoma in Sudan. All the tested compounds were inactive except for niclosamide, which had minimal inhibitory concentrations of around 1 µg/mL. Further tests with niclosamide and niclosamide ethanolamine demonstrated in vitro activity not only against *M. mycetomatis* but also against *Actinomadura* spp., causative agents of actinomycetoma, with minimal inhibitory concentrations below 1 µg/mL. The experimental compound MMV665807, a related salicylanilide without a nitro group, was as active as niclosamide, indicating that the antimycetomal action of niclosamide is independent of its redox chemistry (which is in agreement with the complete lack of activity in all other nitroheterocyclic drugs tested). Based on these results, we propose to further evaluate the salicylanilides, niclosamide in particular, as drug repurposing candidates for mycetoma.

Keywords: mycetoma; *Madurella mycetomatis*; *Actinomadura*; drug repurposing; nitroimidazole; salicylanilide; niclosamide; MMV665807

Citation: Mahmoud, A.B.; Abd Algaffar, S.; van de Sande, W.; Khalid, S.; Kaiser, M.; Mäser, P. Niclosamide Is Active In Vitro against Mycetoma Pathogens. *Molecules* **2021**, *26*, 4005. https://doi.org/10.3390/molecules26134005

Academic Editor: Giuseppe Manfroni

Received: 30 May 2021
Accepted: 24 June 2021
Published: 30 June 2021

1. Introduction

First described from Madurai as Madura foot, the disease eumycetoma is endemic in the 'mycetoma belt' from India to the Middle East and across the Sahel [1,2]. The highest prevalence today is in Sudan [1,2]. Eumycetoma is a chronic subcutaneous mycosis that slowly spreads, starting from an initial lesion at the site of inoculation into the skin and deeper tissues, ultimately destroying muscles, tendons, and bones. The leg and foot are most often affected, likely due to inoculation via thorn pricks. While eumycetoma can be caused by various fungi of the orders Sordariales and Pleosporales [3], the majority of cases in Sudan are due to *Madurella mycetomatis* [2]. Eumycetoma is a debilitating, disfiguring, and stigmatizing disease. It is also an enigmatic disease in the light of the many open questions regarding its epidemiology, pathogenesis, and the biology of the causative agents [2,4].

In contrast to actinomycetoma, which is caused by filamentous bacteria and can be treated with antibiotics, there is no satisfactory treatment for eumycetoma. The current therapy consists of a combination of surgery and long-term chemotherapy with antifungal azoles, such as itraconazole [2]. However, the cure rates are low, and amputation of the affected limb may be the only measure to stop the flesh-eating fungus [5]. Given the urgent need for better drugs and the fact that eumycetoma is a neglected disease affecting

neglected patients, drug repurposing suggests itself as a fast and cost-effective way towards new antimycetomal agents [6,7].

In this article, we pursued this strategy by testing a small set of redox-active parasiticides and antibiotics for their in vitro activity against *M. mycetomatis*. Redox-active molecules are the mainstay of current parasite chemotherapy [8]. The artemisinins for malaria, benznidazole and nifurtimox for Chagas' disease, fexinidazole for sleeping sickness, and metronidazole for intestinal protozoa: all of these are prodrugs that will produce cytotoxic radicals once they are activated by the parasite's metabolism. The activation occurs by chemical reduction, i.e., the acquisition of an electron [9]. Since saprophytic fungi dwell in hypoxic environments and may possess reducing agents of low redox potential, we speculated that *Madurella*, too, might be susceptible to prodrugs that are activated by electron transfer.

2. Results

A selection of redox-active drugs and experimental compounds was evaluated for their in vitro activity against two different isolates of *M. mycetomatis*, SO1 and CBS131320. It included six nitroimidazoles (fexinidazole, metronidazole, secnidazole, plus three experimental compounds [10,11]), three nitrofurans (nifurtimox, nifuroxacide, nitrofurantoine), a salicylanilide (niclosamide), and five peroxides (artemisinin, dihydroartemisinin, artesunate, artemether, and the experimental ozonide OZ78). Itraconazole was included as a reference. All molecules were tested in serial dilution against *M. mycetomatis* cultures [12]. The minimal inhibitory concentration (MIC) was defined as the lowest concentration that, after 7 days of incubation, had inhibited the growth by at least 80% compared to untreated cultures. Itraconazole had a MIC of 0.13 µg/mL and 0.25 µg/mL against SO1 and CBS131320, respectively (Table 1). The tested peroxides were inactive, which is in agreement with the reported lack of activity of artemisinin [13]. Moreover, all the nitroimidazoles and nitrofurans were inactive. The one notable exception was niclosamide (Figure 1), which had a MIC around 1 µg/mL (Table 1).

Table 1. Selected redox-active agents and their in vitro activity against *M. mycetomatis*: All assays were performed in triplicate. Values are minimal inhibitory concentrations (MIC) in µg/mL.

Compound	Class	Indication [1]	SO1 [2]	CBS131320 [2]
Niclosamide	Salicylanilide	Tapeworms	0.78	1.6
Secnidazole	Nitroimidazole	Bacterial vaginosis	>256	>256
Metronidazole	Nitroimidazole	Broad spectrum antibiotic	>256	>256
Fexinidazole	Nitroimidazole	Human African trypanosomiasis	>256	>256
RJ-164	Nitroimidazole	(Human African trypanosomiasis)	>256	>256
RJ-55	Nitroimidazole	(Human African trypanosomiasis)	>256	>256
Ro 15-6547	Nitroimidazole	(Human African trypanosomiasis)	>256	>256
Nifurtimox	Nitrofuran	Chagas' disease, HAT	>256	>256
Nifuroxazide	Nitrofuran	Colitis and diarrhea	>256	>256
Nitrofurantoine	Nitrofuran	Urinary tract infections	>256	>256
OZ 78	Peroxide	(Malaria, trematodes)	>256	>256
Artemisinin	Peroxide	Malaria	16	16
Dihydroartemisinin	Peroxide	Malaria	>256	>256
Artesunate	Peroxide	Malaria	>256	>256
Artemether	Peroxide	Malaria	64	64
Itraconazole	Triazole	Antifungal	0.13	0.25

[1] For experimental compounds, the envisaged indication is in parentheses; [2] SO1 and CBS131320 are two different isolates of *M. mycetomatis*.

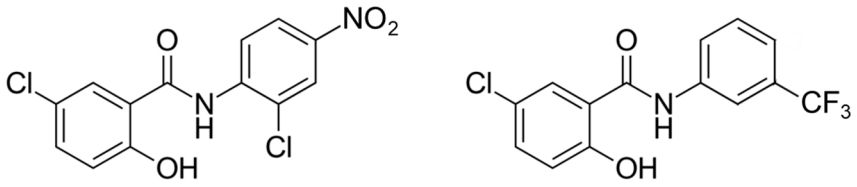

Figure 1. Chemical structure of niclosamide and MMV665807: Both compounds are salicylanilides, i.e., amides of salicylic acid and aniline.

This interesting finding was followed up by testing the three compounds niclosamide, niclosamide-ethanolamine (NEN), and MMV665807 against the two isolates of *M. mycetomatis* and two species of *Actinomadura*, *A. madurae* and *A. syzygii*, causative agents of actinomycetoma. NEN is the ethanolamine salt of niclosamide [14]. MMV665807 (Figure 1) is a salicylanilide from the Medicines for Malaria Venture's malaria box [15] that has shown antibacterial [16], antiprotozoal [17,18], and anticestodal [19,20] activity. All three compounds exhibited good activity against *Madurella* as well as *Actinomadura*, with MIC values somewhat higher than the reference drug itraconazole for *M. mycetomatis* and considerably lower than the reference drug cotrimoxazole for *Actinomadura* (Table 2).

Table 2. In vitro activity of niclosamide and related compounds against causative agents of mycetoma: All assays were performed in triplicate. Values are MIC in μg/mL.

Compound	SO1 [1]	CBS131320 [1]	SAK-A05 [2]	SAK-A08 [2]
Niclosamide	0.78	1.6	0.39	0.39
Niclosamide-ethanolamine	0.78	1.6	0.19	0.39
MMV665807	1.6	1.6	0.39	0.39
Itraconazole	0.13	0.25	n.d.	n.d.
Cotrimoxazole	n.d.	n.d.	20	10

[1] SO1 and CBS131320 are two different isolates of *M. mycetomatis*.[2] SAK-A05 and SAK-A08 are two species of *Actinomadura* (*A. madurae* and *A. syzygii*, respectively).

3. Discussion

In this small drug repurposing study, we have evaluated redox-active parasiticides and antibiotics against *M. mycetomatis* based on the rationale that the metabolism of the fungus would be able to reduce, and thereby activate, the prodrugs. This hypothesis turned out to be wrong, as all tested molecules were inactive—except for niclosamide. Given the lack of activity of the tested nitrofurans and nitroimidazoles, the observed activity of niclosamide is likely not due to its nitro group, but rather due to the salicylanilide moiety. This is supported by the good activity of MMV665807 against *M. mycetomatis*, a salicylanilide that lacks a nitro group (Figure 1). Thus, the discovery of niclosamide as a hit for mycetoma pathogens was serendipitous. The nitro group of niclosamide was found to be dispensable also for its inhibitory action on Wnt signaling, an activity that has raised interest in niclosamide as an anticancer agent [21]; another such activity is mitochondrial uncoupling [22,23].

Niclosamide is an old drug of many uses [24,25]. It was developed by Bayer (Bayer 2353) in the 1950s as a molluscicide for schistosomiasis control. Since 1982, when it was approved by the FDA for human use, its primary indication has been as a broad-spectrum anthelmintic for tapeworms (*Taenia* spp., *Diphyllobothrium latum*) and intestinal fluke (*Fasciolopsis buski*) [26]. Niclosamide was shown to have promising activity against bacteria [27,28], fungi [29], and even coronavirus [30,31]. What restricted its use to intestinal pathogens was the poor oral bioavailability, i.e., the fact that niclosamide is not significantly absorbed from the gastrointestinal tract [32,33]. Different carriers or formulations have been employed to overcome this issue, for example, [34–36]. The ethanolamine salt of

niclosamide (NEN, also called niclosamide olamine or clonitralide) has a better water-solubility and bioavailability [33], and it is being considered for different (re)purposes [22, 37–40]. In our in vitro assays, NEN was as active as niclosamide against *M. mycetomatis* and *Actinomadura* spp.

The finding that a drug like niclosamide, which is on the WHO's list of Essential Medicines, exhibited in vitro activity against both *Madurella mycetomatis* and *Actinomadura* spp. in the same range as the reference compounds, warrants the testing of further salicylanilides against these pathogens and the consideration of niclosamide as a repurposing candidate for mycetoma.

4. Materials and Methods

4.1. Chemicals

MMV665807 (Princeton Bio Molecular Research) and niclosamide-ethanolamine (2A Biotech) were received from Britta Lundström-Stadelmann (University of Bern, Switzerland), the five peroxides from Jonathan Vennerstrom (University of Nebraska, USA). All other test compounds were obtained from DNDi (Geneva, Switzerland). Ro 15-6547 is 4′[(1-methyl-2-nitroimidazole-5-yl)methoxy]-1-pyrrolidine-acetanilide, and RJ-55 and RJ-164 are 1-aryl-4-nitroimidazoles; the three nitroimidazoles were developed for African trypanosomiasis [10,11]. OZ78 is an ozonide related to arterolane [41,42] that has mainly been studied for its activity against trematodes [43–45]. All compounds were dissolved in DMSO.

4.2. Strains

Madurella mycetomatis isolate SO1 was originally isolated from a Somalian patient and CBS131320 from a Sudanese patient. The two PCR-identified strains were obtained from the mycetoma collection of the Erasmus Medical Centre, Rotterdam, The Netherlands. The mycelia were grown at 37 °C in RPMI 1640 medium supplemented with 0.35 g/L L-glutamine and 1.98 mM 4-morpholinepropane sulfonic acid. *Actinomadura madurae* SAK-A05 and *Actinomadura syzygii* SAK-A08 were originally isolated from Sudanese patients and grown in the pharmaceutical research laboratory, University of Science and Technology repository (Omdurman, Sudan). The bacterial suspensions were prepared in Mueller Hinton II broth (CAMHB) media and propagated at 35 °C.

4.3. In Vitro Drug Efficacy Testing

M. mycetomatis mycelia in RPMI 1640 medium were sonicated for 10 s (QSONICA Q55) and centrifuged at 2600× *g* for 5 min. The mycelia were washed and resuspended in fresh RPMI 1640 medium to obtain a fungal suspension of 68% to 72% transmission at 660 nm (UV/Vis Spectrophotometer 6305, Jenway, UK). A 1:2 serial drug dilution covering a range from 256 µg/mL to 0.063 µg/mL of test compound was prepared in a round-bottom 96-well microtiter plate (Corning, CLS379). Itraconazole was used as the positive control at a range from 1 µg/mL to 0.03 µg/mL. Exactly 100 µL of adjusted fungal suspension was added to each well along with 1 µL of the drug. Next, 1 µL of resazurin was added to a final concentration of 0.15 mg/mL [12]. The plates were sealed and incubated at 37 °C for 7 days. All assays were performed in triplicate. For further details see [12].

Actinomadura suspensions were adjusted to absorbance of 0.08–0.1 at 625 nm. A 1:2 serial drug dilution covering a range from 256 µg/mL to 0.063 µg/mL was prepared in a 96-well microtiter plate. Co-trimoxazole (fixed combination of 1 part trimethoprim and 5 parts sulfamethoxazole) was employed as the positive control at a starting concentration of 80 µg/mL. Next, 100 µL of adjusted bacterial suspension were added to each well along with 1 µL of the drug. Precisely 1 µL of resazurin was added to a final concentration of 0.15 mg/mL. The plates were incubated at 35 °C for 5 days. All assays were performed in triplicate.

The assay plates were inspected for visual and spectrometric endpoints. Absorbance was measured at 600 nm (Thermo Scientific Multiskan Spectrum, Thermo Fisher Scien-tific,

Vantaa, Finland). The minimal inhibitory concentration (MIC) was defined as the lowest concentration of test compound that produced ≥80% growth inhibition as compared to the untreated cultures (negative control).

Author Contributions: Conceptualization, A.B.M., M.K., S.K. and P.M.; methodology, S.A.A., W.v.d.S., S.K.; validation, S.A.A. and A.B.M.; formal analysis, S.A.A. and A.B.M.; investigation, S.A.A. and A.B.M.; resources, M.K., S.K., W.v.d.S.; data curation, S.A.A. and A.B.M.; writing—original draft preparation, P.M.; writing—review and editing, A.B.M. and S.A.A.; visualization, P.M.; supervision, M.K., S.K. and P.M.; project ad-ministration, P.M.; funding acquisition, A.B.M. and P.M. All authors have read and agreed to the published version of the manuscript.

Funding: We gratefully acknowledge financial support by the Emilia Guggenheim-Schnurr Foundation (www.ngib.ch/stiftung-egs/) to A.B.M. The funder had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: All data are contained within the article.

Acknowledgments: We thank DNDi for the nitroheterocyclic drugs, Jonathan Vennerstrom for the peroxides, and Britta Lundström-Stadelmann and Reto Rufener for MMV665807 and helpful advice. We are most grateful to Marcel Tanner and Suad Sulaiman for their continuous support.

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

Sample Availability: For samples of the compounds please contact the corresponding author, who will link you up with the compound providers (see Acknowledgments above).

References

1. Nenoff, P.; van de Sande, W.W.; Fahal, A.H.; Reinel, D.; Schofer, H. Eumycetoma and actinomycetoma–an update on causative agents, epidemiology, pathogenesis, diagnostics and therapy. *J. Eur. Acad. Dermatol. Venereol.* **2015**, *29*, 1873–1883. [CrossRef] [PubMed]
2. Zijlstra, E.E.; van de Sande, W.W.J.; Welsh, O.; Mahgoub, E.S.; Goodfellow, M.; Fahal, A.H. Mycetoma: A unique neglected tropical disease. *Lancet Infect. Dis.* **2016**, *16*, 100–112. [CrossRef]
3. Ahmed, S.A.; van de Sande, W.W.; Stevens, D.A.; Fahal, A.; van Diepeningen, A.D.; Menken, S.B.; de Hoog, G.S. Revision of agents of black-grain eumycetoma in the order Pleosporales. *Persoonia* **2014**, *33*, 141–154. [CrossRef] [PubMed]
4. van de Sande, W.; Fahal, A.; Ahmed, S.A.; Serrano, J.A.; Bonifaz, A.; Zijlstra, E. eumycetoma working, g. Closing the mycetoma knowledge gap. *Med. Mycol.* **2018**, *56*, 153–164. [CrossRef] [PubMed]
5. Suleiman, S.H.; Wadaella, E.S.; Fahal, A.H. The Surgical Treatment of Mycetoma. *PLoS Negl. Trop. Dis.* **2016**, *10*, e0004690. [CrossRef]
6. Ferreira, L.G.; Andricopulo, A.D. Drug repositioning approaches to parasitic diseases: A medicinal chemistry perspective. *Drug Discov. Today* **2016**, *21*, 1699–1710. [CrossRef]
7. Lim, W.; Melse, Y.; Konings, M.; Phat Duong, H.; Eadie, K.; Laleu, B.; Perry, B.; Todd, M.H.; Ioset, J.R.; van de Sande, W.W.J. Addressing the most neglected diseases through an open research model: The discovery of fenarimols as novel drug candidates for eumycetoma. *PLoS Negl. Trop. Dis.* **2018**, *12*, e0006437. [CrossRef]
8. Pal, C.; Bandyopadhyay, U. Redox-active antiparasitic drugs. *Antioxid. Redox Signal.* **2012**, *17*, 555–582. [CrossRef]
9. Mäser, P. Cherchez l'Electron. *Mol. Microbiol.* **2017**, *106*, 183–185. [CrossRef]
10. Richle, R.; Hofheinz, W. Chemotherapeutische Wirksamkeit von 2 neuen 2-Nitroimidazolderivaten gegen Trypanosoma brucei rhodesiense bei der experimentellen Schlafkrankheit von Maus und Kaninchen. *Mitt. Österr. Ges. Tropenmed. Parasitol.* **1983**, *5*, 143–149.
11. Trunz, B.B.; Jedrysiak, R.; Tweats, D.; Brun, R.; Kaiser, M.; Suwinski, J.; Torreele, E. 1-Aryl-4-nitro-1H-imidazoles, a new promising series for the treatment of human African trypanosomiasis. *Eur. J. Med. Chem.* **2011**, *46*, 1524–1535. [CrossRef]
12. Abd Algaffar, S.O.; Verbon, A.; van de Sande, W.W.J.; Khalid, S.A. Development and Validation of an In Vitro Resazurin-Based Susceptibility Assay against Madurella mycetomatis. *Antimicrob. Agents Chemother.* **2021**, *65*. [CrossRef] [PubMed]
13. van de Sande, W.W.; Fahal, A.H.; Riley, T.V.; Verbrugh, H.; van Belkum, A. In vitro susceptibility of Madurella mycetomatis, prime agent of Madura foot, to tea tree oil and artemisinin. *J. Antimicrob. Chemother.* **2007**, *59*, 553–555. [CrossRef] [PubMed]
14. Hecht, G.; Gloxhuber, C. Tolerance to 2′, 5-dichloro-4-nitrosalicylanilide ethanolamine salt. *Z. Tropenmed. Parasit.* **1962**, *13*, 1–8.

15. Spangenberg, T.; Burrows, J.N.; Kowalczyk, P.; McDonald, S.; Wells, T.N.; Willis, P. The open access malaria box: A drug discovery catalyst for neglected diseases. *PLoS ONE* **2013**, *8*, e62906. [CrossRef]
16. Zapotoczna, M.; Boksmati, N.; Donohue, S.; Bahtiar, B.; Boland, A.; Somali, H.A.; Cox, A.; Humphreys, H.; O'Gara, J.P.; Brennan, M.; et al. Novel anti-staphylococcal and anti-biofilm properties of two anti-malarial compounds: MMV665953 {1-(3-chloro-4-fluorophenyl)-3-(3,4-dichlorophenyl)urea} and MMV665807 {5-chloro-2-hydroxy-N-[3-(trifluoromethyl)phenyl]benzamide}. *J. Med. Microbiol.* **2017**, *66*, 377–387. [CrossRef]
17. Aleman, A.; Guerra, T.; Maikis, T.J.; Milholland, M.T.; Castro-Arellano, I.; Forstner, M.R.; Hahn, D. The Prevalence of Trypanosoma cruzi, the Causal Agent of Chagas Disease, in Texas Rodent Populations. *Ecohealth* **2017**, *14*, 130–143. [CrossRef]
18. Muller, J.; Winzer, P.A.; Samby, K.; Hemphill, A. In Vitro Activities of MMV Malaria Box Compounds against the Apicomplexan Parasite Neospora caninum, the Causative Agent of Neosporosis in Animals. *Molecules* **2020**, *25*, 1460. [CrossRef]
19. Ritler, D.; Rufener, R.; Sager, H.; Bouvier, J.; Hemphill, A.; Lundstrom-Stadelmann, B. Development of a movement-based in vitro screening assay for the identification of new anti-cestodal compounds. *PLoS Negl. Trop. Dis.* **2017**, *11*, e0005618. [CrossRef] [PubMed]
20. Stadelmann, B.; Rufener, R.; Aeschbacher, D.; Spiliotis, M.; Gottstein, B.; Hemphill, A. Screening of the Open Source Malaria Box Reveals an Early Lead Compound for the Treatment of Alveolar Echinococcosis. *PLoS Negl. Trop. Dis.* **2016**, *10*, e0004535. [CrossRef]
21. Mook, R.A.; Jr Wang, J.; Ren, X.R.; Chen, M.; Spasojevic, I.; Barak, L.S.; Lyerly, H.K.; Chen, W. Structure-activity studies of Wnt/beta-catenin inhibition in the Niclosamide chemotype: Identification of derivatives with improved drug exposure. *Bioorg. Med. Chem.* **2015**, *23*, 5829–5838. [CrossRef]
22. Alasadi, A.; Chen, M.; Swapna, G.V.T.; Tao, H.; Guo, J.; Collantes, J.; Fadhil, N.; Montelione, G.T.; Jin, S. Effect of mitochondrial uncouplers niclosamide ethanolamine (NEN) and oxyclozanide on hepatic metastasis of colon cancer. *Cell Death Dis.* **2018**, *9*, 215. [CrossRef]
23. Kumar, R.; Coronel, L.; Somalanka, B.; Raju, A.; Aning, O.A.; An, O.; Ho, Y.S.; Chen, S.; Mak, S.Y.; Hor, P.Y.; et al. Mitochondrial uncoupling reveals a novel therapeutic opportunity for p53-defective cancers. *Nat. Commun.* **2018**, *9*, 3931. [CrossRef]
24. Chen, W.; Mook, R.A.; Premont, R.T., Jr.; Wang, J. Niclosamide: Beyond an antihelminthic drug. *Cell Signal* **2018**, *41*, 89–96. [CrossRef]
25. Kadri, H.; Lambourne, O.A.; Mehellou, Y. Niclosamide, a Drug with Many (Re)purposes. *ChemMedChem* **2018**, *13*, 1088–1091. [CrossRef]
26. NIH National Center for Advancing Translational Sciences, Inxight: Drugs. Niclosamide. Available online: https://drugs.ncats.io/drug/8KK8CQ2K8G (accessed on 20 May 2021).
27. Rajamuthiah, R.; Fuchs, B.B.; Conery, A.L.; Kim, W.; Jayamani, E.; Kwon, B.; Ausubel, F.M.; Mylonakis, E. Repurposing salicylanilide anthelmintic drugs to combat drug resistant Staphylococcus aureus. *PLoS ONE* **2015**, *10*, e0124595. [CrossRef]
28. Imperi, F.; Massai, F.; Ramachandran Pillai, C.; Longo, F.; Zennaro, E.; Rampioni, G.; Visca, P.; Leoni, L. New life for an old drug: The anthelmintic drug niclosamide inhibits Pseudomonas aeruginosa quorum sensing. *Antimicrob. Agents Chemother.* **2013**, *57*, 996–1005. [CrossRef]
29. Garcia, C.; Burgain, A.; Chaillot, J.; Pic, E.; Khemiri, I.; Sellam, A. A phenotypic small-molecule screen identifies halogenated salicylanilides as inhibitors of fungal morphogenesis, biofilm formation and host cell invasion. *Sci. Rep.* **2018**, *8*, 11559. [CrossRef]
30. Jeon, S.; Ko, M.; Lee, J.; Choi, I.; Byun, S.Y.; Park, S.; Shum, D.; Kim, S. Identification of Antiviral Drug Candidates against SARS-CoV-2 from FDA-Approved Drugs. *Antimicrob. Agents Chemother.* **2020**, *64*. [CrossRef] [PubMed]
31. Wu, C.J.; Jan, J.T.; Chen, C.M.; Hsieh, H.P.; Hwang, D.R.; Liu, H.W.; Liu, C.Y.; Huang, H.W.; Chen, S.C.; Hong, C.F.; et al. Inhibition of severe acute respiratory syndrome coronavirus replication by niclosamide. *Antimicrob. Agents Chemother.* **2004**, *48*, 2693–2696. [CrossRef]
32. Barbosa, E.J.; Lobenberg, R.; de Araujo, G.L.B.; Bou-Chacra, N.A. Niclosamide repositioning for treating cancer: Challenges and nano-based drug delivery opportunities. *Eur. J. Pharm. Biopharm.* **2019**, *141*, 58–69. [CrossRef]
33. Schultz, D.P.; Harman, P.D. Uptake, distribution, and elimination of the lampricide 2',5-dichloro-4'-nitro[14C]salicylanilide (Bayer 2353) and its 2-aminoethanol salt (Bayer 73) by largemouth bass. *J. Agric. Food Chem.* **1978**, *26*, 1226–1230. [CrossRef]
34. Guo, J.; Tao, H.; Alasadi, A.; Huang, Q.; Jin, S. Niclosamide piperazine prevents high-fat diet-induced obesity and diabetic symptoms in mice. *Eat. Weight Disord.* **2019**, *24*, 91–96. [CrossRef]
35. Rehman, M.U.; Khan, M.A.; Khan, W.S.; Shafique, M.; Khan, M. Fabrication of Niclosamide loaded solid lipid nanoparticles: In vitro characterization and comparative in vivo evaluation. *Artif. Cells Nanomed. Biotechnol.* **2018**, *46*, 1926–1934.
36. Zhang, X.; Zhang, Y.; Zhang, T.; Zhang, J.; Wu, B. Significantly enhanced bioavailability of niclosamide through submicron lipid emulsions with or without PEG-lipid: A comparative study. *J. Microencapsul.* **2015**, *32*, 496–502. [CrossRef] [PubMed]
37. Han, P.; Zhan, H.; Shao, M.; Wang, W.; Song, G.; Yu, X.; Zhang, C.; Ge, N.; Yi, T.; Li, S.; et al. Niclosamide ethanolamine improves kidney injury in db/db mice. *Diabetes Res. Clin. Pract.* **2018**, *144*, 25–33. [CrossRef]
38. Li, S.L.; Yan, J.; Zhang, Y.Q.; Zhen, C.L.; Liu, M.Y.; Jin, J.; Gao, J.L.; Xiao, X.L.; Shen, X.; Tai, Y.; et al. Niclosamide ethanolamine inhibits artery constriction. *Pharmacol. Res.* **2017**, *115*, 78–86. [CrossRef]
39. Park, J.S.; Lee, Y.S.; Lee, D.H.; Bae, S.H. Repositioning of niclosamide ethanolamine (NEN), an anthelmintic drug, for the treatment of lipotoxicity. *Free Radic Biol. Med.* **2019**, *137*, 143–157. [CrossRef]

40. Tao, H.; Zhang, Y.; Zeng, X.; Shulman, G.I.; Jin, S. Niclosamide ethanolamine-induced mild mitochondrial uncoupling improves diabetic symptoms in mice. *Nat. Med.* **2014**, *20*, 1263–1269. [CrossRef]
41. Lanteri, C.A.; Chaorattanakawee, S.; Lon, C.; Saunders, D.L.; Rutvisuttinunt, W.; Yingyuen, K.; Bathurst, I.; Ding, X.C.; Tyner, S.D. Ex vivo activity of endoperoxide antimalarials, including artemisone and arterolane, against multidrug-resistant Plasmodium falciparum isolates from Cambodia. *Antimicrob. Agents Chemother.* **2014**, *58*, 5831–5840. [CrossRef]
42. Vennerstrom, J.L.; Arbe-Barnes, S.; Brun, R.; Charman, S.A.; Chiu, F.C.; Chollet, J.; Dong, Y.; Dorn, A.; Hunziker, D.; Matile, H.; et al. Identification of an antimalarial synthetic trioxolane drug development candidate. *Nature* **2004**, *430*, 900–904. [CrossRef] [PubMed]
43. Keiser, J.; Utzinger, J.; Tanner, M.; Dong, Y.; Vennerstrom, J.L. The synthetic peroxide OZ78 is effective against Echinostoma caproni and Fasciola hepatica. *J. Antimicrob. Chemother.* **2006**, *58*, 1193–1197. [CrossRef]
44. Keiser, J.; Xiao, S.H.; Dong, Y.; Utzinger, J.; Vennerstrom, J.L. Clonorchicidal properties of the synthetic trioxolane OZ78. *J. Parasitol.* **2007**, *93*, 1208–1213. [CrossRef] [PubMed]
45. Xiao, S.H.; Keiser, J.; Chollet, J.; Utzinger, J.; Dong, Y.; Endriss, Y.; Vennerstrom, J.L.; Tanner, M. In vitro and in vivo activities of synthetic trioxolanes against major human schistosome species. *Antimicrob. Agents Chemother.* **2007**, *51*, 1440–1445. [CrossRef] [PubMed]