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Review

The revival of dithiocarbamates: from pesticides to innovative medical treatments

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SUMMARY

Dithiocarbamates (DTCs) have been used for various applications, including as hardening agents in rubber manufacturing, as fungicide in agriculture, and as medications to treat alcohol misuse disorder. The multi-faceted effects of DTCs rely mainly on metal binding abilities and a high reactivity with thiol groups. Therefore, the list of potential applications is still increasing, exemplified by the US Food and Drug Administration approval of disulfiram (Antabuse) and its metabolite diethyldithiocarbamate in clinical trials against cancer, human immunodeficiency virus, and Lyme disease, as well as new DTC-related compounds that have been synthesized to target diseases with unmet therapeutic needs. In this review, we will discuss the latest progress of DTCs as anti-cancer agents and provide a summary of the mechanisms of action. We will explain the expansion of DTCs' activity in the fields of microbiology, neurology, cardiology, and ophthalmology, thereby providing evidence for the important role and therapeutic potential of DTCs as innovative medical treatments.

INTRODUCTION

Chemical structures containing dithiocarbamates (DTCs) as functional group began their life as catalysts in the rubber vulcanization process in the early 1880s. In 1943, the first DTC derivative, nabam, was patented as fungicide for agricultural use, followed by zineb, the first DTC to be coupled with a metal. The best known DTC derivative, diethyldithiocarbamate (DDC), is the active metabolite of the anti-alcoholic drug, disulfiram (DSF) (Bala et al., 2014). Notably, DTCs have recently resurfaced as potential drug therapies, owing to (1) their ability to chelate metals and (2) their affinity for thiol groups present in human and microbial enzymes (Sauna et al., 2005; Buac et al., 2012).

To date, a small number of articles have described the emerging importance of DTCs in medicine and the potential of DSF for various biomedical applications. DSF and DDC represent interesting candidate drug therapies, as they are inexpensive and have an excellent safety record (Viola-Rhenals et al., 2018). In the past 20 years, DSF and DDC have been extensively investigated as cancer treatments, as antimicrobial agents, and for their application in cardiology and neurology. In addition, new chemical structures with DTC moieties have been synthesized and tested for their anti-cancer, antiviral, ophthalmic, and neurological applications (i.e., for the treatment of Alzheimer disease) (Figure 1).

DITHIOCARBAMATES: NEW ALLIES IN THE FIGHT AGAINST CANCER

According to the World Health Organization (WHO), in 2018, one in six deaths was caused by cancer worldwide. Cancer is broadly defined as cells that display abnormal and uncontrolled growth. Treatment options include chemotherapeutic agents that are usually associated with toxic side effects and limited therapeutic activity due to the development of drug resistances in a significant number of patients (Gillet and Gottesman, 2010). Therefore, new anti-cancer drugs that display selectivity for cancer cells without the associated development of resistance are urgently required.

Current status of research on DTC in cancer

The anti-cancer activity of DSF was first observed when an alcoholic patient showed complete remission of metastatic breast cancer when being treated with the anti-alcoholic drug DSF in 1977 (Skrott and Cvek, 2012). According to clinicaltrials.gov, DSF's anti-cancer properties have seen these agents as the subject of 21 clinical trials with the focus on metastatic breast, prostate, and pancreatic cancer; glioblastoma;

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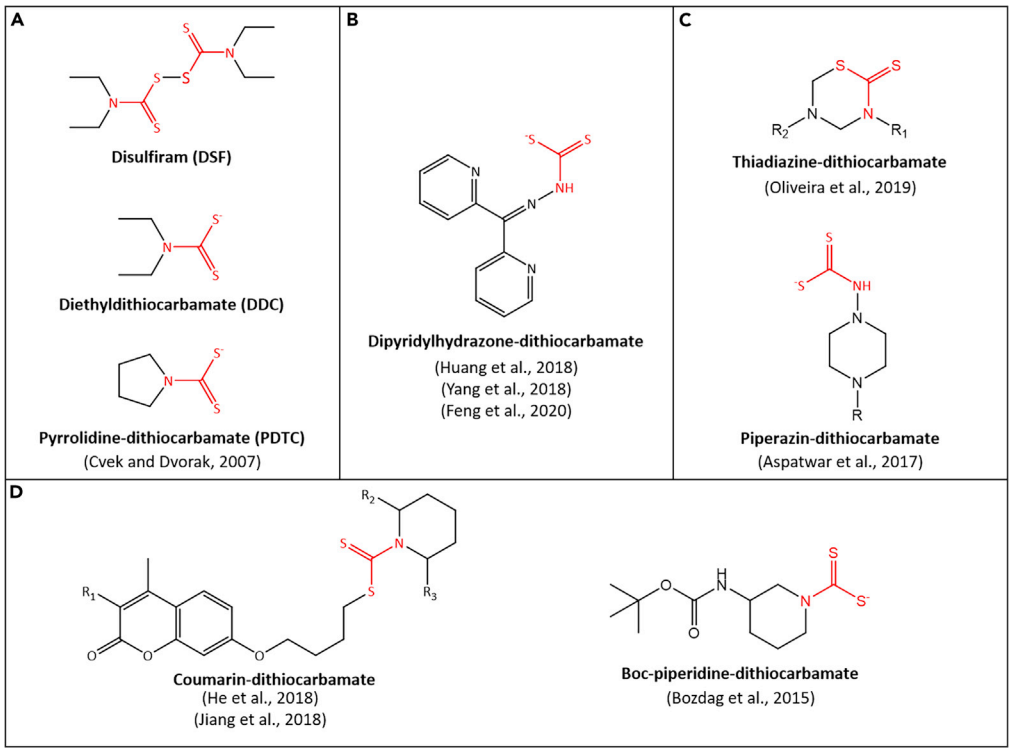

Figure 1. Selection of DTC derivatives with highlighted DTC moiety (red) according to their application

(A) Agents for alcohol misuse disorder and other fields, (B) anti-cancer agents, (C) antimicrobial agents, (D) neurological and ophthalmic application.

and melanoma. In these trials, DSF was investigated alone, with copper (Cu^{2+}) salts or as adjuvant to standard chemotherapy. Yang et al. (2019) reviewed the status of clinical trials involving treatment with DSF against cancer. Accordingly, this review will only provide an update on the most recent clinical studies and detail new treatment opportunities with other DTCs (Table 1).

Previously, the outcome of a phase IIb clinical trial indicated that combining DSF with cisplatin and vinorelbine prolonged progression-free and overall survival of patients diagnosed with phase IV non-small cell lung cancer (Nechushtan et al., 2015). Based on these results, the National Cancer Institute in Slovakia initiated a phase II study, evaluating the effectiveness of DSF-Cu with vinorelbine and cisplatin for the

Table 1. Clinical trials (from 2019 onward) involving DSF or DDC against cancer

Status	NCT	Date	DTC/DSF as	Disease	Study	Sponsor
Not yet recruiting	NCT04521335	2020/12–2025/12	DSF-Cu	Multiple myeloma	Phase I	University of Utah, United States
Recruiting	NCT04265274	2020/01–2023/01	DSF-Cu	Metastatic breast cancer	Phase II	National Cancer Institute, Slovakia
Recruiting	NCT03714555	2019/10–2021/07	DSF-Cu	Metastatic pancreatic cancer	Phase II	HonorHealth Research Institute, United States
Recruiting	NCT03950830	2019/05–2022/12	DSF	Germ cell tumors	Phase II	National Cancer Institute, Slovakia
Recruiting	NCT04234022	2020/04–2023/04	DDC-Zn	Multiple myeloma	Observational	The Royal Wolverhampton Hospitals NHS Trust, United Kingdom

treatment of metastatic breast cancer (NCT04265274). The inclusion of DSF-Cu was hypothesized to increase the objective response rate in patients with refractory metastatic breast cancer by overriding drug resistance in cancer cells and by inhibiting cancer stem cells through inactivation of aldehyde dehydrogenase (ALDH) (Nechushtan et al., 2015).

High ALDH levels have been observed in many cancer stem cells (Viola-Rhenals et al., 2018), including multiple relapsed germ cell tumors (Schmidtova et al., 2019) and metastatic pancreatic cancer cells (Kim et al., 2013). Accordingly, phase II clinical study patients are being recruited to investigate the objective response rate and patient survival when treated with DSF and cisplatin against multiple relapsed germ cell tumors (NCT03950830). Furthermore, treatment of metastatic pancreatic cancer with DSF-Cu plus gemcitabine alone, gemcitabine/nab-paclitaxel, and FOLFIRINOX is the focus of another phase II clinical study (NTC03714555) initiated in 2019. The objective of this study is to determine if the addition of DSF-Cu leads to a reduction of the tumor marker CA19-9.

Notably, DSF-Cu and DDC-Zinc (DDC-Zn) are the current focus of clinical trials to treat the hematological malignancy, multiple myeloma. The primary outcome of the phase I study using DSF-Cu is the evaluation of the dose-limiting toxicity rate of the DSF-Cu recommended phase 2 dose (NTC04521335). The observational study involving DDC-Zn aims to determine the *in vitro* efficacy and cytotoxicity of DDC-Zn alone or in combination with lenalidomide or pomalidomide against bone marrow samples from patients with multiple myeloma and those with acute leukemia (NCT04234022). The outcome of this study could lead to a first interventional trial comprising DDC as cancer treatment.

Furthermore, many newly synthesized compounds, based on DTCs, are being investigated for their anti-cancer activity in both *in vitro* and *in vivo* models. By linking DTC with other functional groups, compounds like diarylaminopyrimidine-DTC hybrids can inhibit a specific target, such as focal adhesion kinase (FAK). FAK is a regulator of cancer cell survival, proliferation, and angiogenesis and represents a good target for a chemotherapeutic drug owing to its multilateral affects. One diarylaminopyrimidine-DTC hybrid, synthesized by Su et al. (2019) to target FAK, showed selectivity toward tumor cells and had good anti-proliferative and anti-angiogenesis effects *in vitro*.

Additionally, some new active substances, based on the ability of DTCs to chelate metal ions, such as gold(III)-DTCs exhibited excellent *in vitro* and *in vivo* anti-tumor properties with reduced systemic toxicity (Nagy et al., 2012). In HER2-overexpressing cancer cells, the iron chelator dipyridylhydrazone-DTC exhibited anti-tumor activity by upregulating the tumor and metastasis suppressor gene NDRG1 (Yang et al., 2018). Furthermore, a newly synthesized DTC derivative, containing glucose and methionine, reduced cisplatin-induced nephrotoxicity by binding platin without compromising the therapeutic activity (Ge et al., 2019). In addition, a new platinum-containing compound, similar to cisplatin but based on a DTC moiety, showed *in vitro* and *in vivo* activity against a range of tumor cell lines without measurable nephrotoxicity (Nagy et al., 2012).

The well-known pyrrolidine dithiocarbamate (PDTC) has been investigated for anti-cancer effects against adult T cell leukemia/lymphoma. PDTC administration in mice models was shown to inhibit adult T cell leukemia/lymphoma cell growth and improved mouse survival (Nakamura et al., 2015).

Although both known and new DTCs are in different stages of development, an important question related to their mode of action remains: What mechanisms underlie the anti-cancer activities of DTCs, DSF, and their metal complexes?

Modes of action

Considerable progress as to the modes of action of DTC has been made by investigating different pathways, including their effects on the ubiquitin proteasome system (UPS), reactive oxygen species (ROS) generation, epigenetic factors, and cancer cell stemness (Figure 2). Many of these mechanisms have already been described in recent reviews (Viola-Rhenals et al., 2018; Yang et al., 2019; Jiao et al., 2016), with many of these articles focusing on the mechanisms of DSF and DDC with Cu^{2+}, but not other DTCs.

Inducing apoptosis through inhibition of UPS

The UPS is a crucial mechanism for the degradation of key signaling molecules such as cell cycle regulatory and apoptosis controlling proteins. Before their degradation, proteins are tagged with multiple ubiquitin

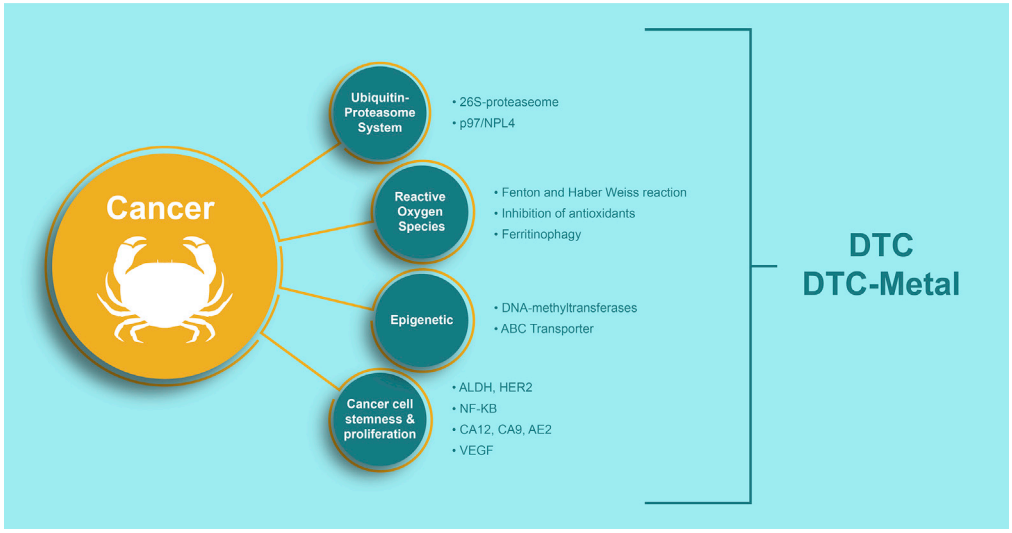

Figure 2. Different anti-cancer mechanisms of DTCs with and without metals

molecules, which are then recognized by the 26S-proteasome and consequently hydrolyzed (Cvek and Dvorak, 2008). Studies have shown that DTC copper complexes, such as DDC-Cu and PDTC-Cu, can inhibit proteasome activity in different cancer cells by interacting with two main processes within the UPS (Pang et al., 2007; Buac et al., 2012).

First, DTCs inhibit the 26S-proteasome by either binding to the 26S-proteasome or when complexed with Cu ions, by inhibiting the catalytic 20S-core (Yang et al., 2019; Viola-Rhenals et al., 2018). DDC-Cu and PDTC-Cu inhibit the 26S-proteasomes by interacting with the JAB1/MPN/Mov34 metalloenzyme domain, which is responsible for de-ubiquitination of proteins (Skrott and Cvek, 2012; Cvek, 2011). Inhibition of 26S-proteasome leads to the accumulation of ubiquitinated proteins and cytotoxic protein aggregates resulting in a disturbance of the protein homeostasis and leading to cell death (Cvek and Dvorak, 2008).

Second, Skrott et al. (2017) suggested that DDC-Cu interacts with the nuclear protein localization 4 (NPL4), a key component of p97 segregase. As a result, the process of protein ubiquitination is inhibited and protein aggregates are rapidly formed (Skrott et al., 2017). A recent paper (Pan et al., 2020) described that only cupric ions interact with NPL4, thereby immobilizing p97 segregase. DDC is then necessary to transport the cupric ions into the cells.

Reactive oxygen species

ROS are highly reactive molecules that, at low levels, are crucial for the regulation of normal physiological functions. An excess of cellular ROS activates its downstream c-Jun N-terminal kinases and p38 MAPK pathways, resulting in cell death (Yang et al., 2019).

DSF and DTCs, in combination with metal ions, can induce ROS production through the Fenton and Haber Weiss reaction, and also by inhibiting antioxidants such as superoxide dismutase and glutathione (Marikovsky et al., 2002; Skrott and Cvek, 2012). A recent article showed that a dipyridylhydrazone DTC induced ferritinophagy by mobilizing iron from ferritin and mediating the ROS/p53 pathway, resulting in the inhibition of gastric cancer cell line proliferation (Feng et al., 2020; Huang et al., 2018).

Epigenetic factors

Certain cancer types, including prostate cancer or glioma, show high expression of genes involved in DNA modifications. For instance, high levels of DNA-methyltransferases, which are responsible for DNA-methylation and leading to the silencing of genes, were observed in these cancer cell types. DSF is suggested to interfere with the catalytic center of DNA-methyltransferase 1, therefore inhibiting its activity. Exposure to DSF was found to lead to demethylation of hypermethylated genes and re-expression of silenced genes (Lin et al., 2011).

Another example is the overexpression of the O-6-methylguanine-DNA-methyltransferase gene in certain cancer cells, which encodes for the DNA-repair protein O-6-alkylguanine-DNA-alkyltransferase. This protein repairs methylations caused by the chemotherapeutic compound temozolomide and therefore diminishes its therapeutic effect. PDTC and other DTCs reduced the expression levels of O-6-methylguanine-DNA-methyltransferase in glioma cells (Tang et al., 2017).

Further epigenetic modifications within cancer cells leads to the overexpression of efflux pumps, such as the ATP-binding cassette (ABC) family of proteins. ABC transport proteins, such as P-glycoprotein, remove cytotoxic drugs from the cells, resulting in decreased intracellular accumulation of the drugs and thus lack of activity (Loo and Clarke, 2000). DSF binds to either the ATP-binding site or the drug substrate-binding sites of multiple ABC transporting proteins, potentially increasing the efficacy of other cancer drugs by inhibiting ABC activity or by blocking its maturation (Sauna et al., 2004).

Cancer cell stemness

Cancer stem cells are a subpopulation of cells within tumors, with the capability of differentiation and self-renewal, and are associated with metastasis, cancer recurrence, and therapy resistance. The importance of ALDH and HER-2 as marker for cancer stem cells and the inhibition through DSF was described in detail in recent reviews (Jiao et al., 2016; Yang et al., 2019).

The protein complex, nuclear factor-kappa B (NF-κB), is involved in multiple pathways necessary for cell survival and cancer cell stemness. NF-κB is a transcriptional regulator of proteins that inhibit apoptosis inhibitors, including XIAP, which are downregulated when exposed to DSF-Cu (Allensworth et al., 2015). DTCs inhibiting properties of NF-κB can, in part, be attributed to NF-κB being dependent on the UPS to split inhibitor-κB (I-κB) kinase and release the heterodimer p50:p65 for nuclear transcription (Cvek and Dvorak, 2008). Other hypotheses include the obstruction of I-κB kinase and therefore the prevention of the ubiquitination and the split of I-κB, along with the interaction of DTCs with various thiol groups present throughout the NF-κB pathway (Cvek and Dvorak, 2007).

Furthermore, carbonic anhydrase (CA) 12 and its associated transporter anion exchanger (AE) 2 can also play an important role in invasion and cell migration in both lung cancer and breast cancer cell lines. An upregulation of AE2 has been observed in various cancer cell types, in which DSF reduced the surface expression of both CA12 and AE2 (Hwang et al., 2019). Additionally, CA9 and CA12 are overexpressed in hypoxic tumor cells and were inhibited by various DTC derivates. Especially CA9 was still inhibited despite a high structural diversity of the DTC derives, including aliphatic, aromatic, aralkyl, and hetaryl moieties (Carta et al., 2012a). Last, the vascular endothelial growth factor (VEGF) plays a key role in angiogenesis and therefore in abnormal vessel growth at the tumor site, thereby contributing to the progression of solid malignancies. DSF-Cu showed improved inhibition of endothelial cell proliferation, migration, invasion, adhesion, and tube formation by inhibiting the EGFR/Src/VEGF pathway (Li et al., 2015).

Through various mechanisms, DSF and DTCs inhibit angiogenesis and proliferation, overcome apoptotic signals, and induce apoptosis in cancer cells. However, these targets are not only relevant in tumor therapy but also play an important role in the virulence of microbial diseases.

Extension of dithiocarbamates' activities to the microbial world

The WHO predicts that 10 million people will die from antimicrobial-resistant infections by the year 2050. This is partly caused by the rise of microbial drug resistances, as well as by the reduced pace in the development of new antimicrobials (Thakare et al., 2019). DSF and DTCs were investigated as antimicrobial candidates and showed activity against viruses, bacteria, fungi, and parasites, in some cases leading to clinical trials, as described in Table 2.

Antiviral properties

Coronavirus

Considering that three coronavirus outbreaks have occurred in the past 20 years, including the current 2019 novel coronavirus pandemic, there is an urgent need for new antiviral therapies. It has been previously demonstrated that DSF inhibits main protease and papain-like protease, which play an important role in viral replication of Middle East respiratory syndrome (MERS) coronavirus and severe acute respiratory

Table 2. Clinical trials involving DSF against viruses and bacteria

Status	NCT	Date	DTC/DSF as	Disease	Study	Sponsor
Not yet recruiting	NCT04485130	2020/09–2021/09	DSF	COVID-19	Phase II	University of California, United States
Terminated	NCT03198559	2017/08–2019/04	DSF	HIV	Phase I/II	The Peter Doherty Institute for Infection and Immunity, Australia
Completed	NCT01944371	2013/09–2014/05	DSF	HIV	Phase I/II	University of California, United States
Completed	NCT01286259	2011/01–2014/05	DSF	HIV-1	Not applicable	University of California, United States
Recruiting	NCT03891667	2019/07–2021/03	DSF	Lyme disease	Phase I/II	Research Foundation for Mental Hygiene Inc., United States

syndrome (SARS) coronavirus (Lin et al., 2018). DSF and DDC-Cu have been shown to inactivate crucial viral proteins of the 2019 novel coronavirus such as thio-protease and RNA replicase, by oxidizing the thiol/thiolate to disulfides (Xu, L., Tong, J., Wu, Y., Zhao, S. and Lin, B.-L. 2020. *Targeted oxidation strategy (TOS) for potential inhibition of coronaviruses by disulfiram — a 70-year old anti-alcoholism drug*. ChemRxiv, under review). DSF is now being investigated in the clinical trial DISCO (NCT04485130) to assess adverse events and changes in the COVID-19 symptom severity score.

Human immunodeficiency virus

DSF and its metabolite DDC also display antiviral activities against human immunodeficiency virus (HIV)-1 and have been used in clinical trials (clinicaltrials.gov). The progression of HIV-1 can be controlled with combination anti-retroviral therapy. However, to date no cure has been identified owing to the persistence of replication-competent, latent provirus in long-lived resting T cells. A recent approach, termed the "shock and kill strategy," aims to reactivate the virus reservoirs and destroy latently infected cells. In combination with anti-retroviral therapy, this approach could lead to HIV-1 cure (Spivak and Planelles, 2018). The possible mechanism of the viral reactivation properties of DSF has been attributed to the stimulation of the protein kinase B signaling pathway. The protein kinase B pathway initiates NF-κB-dependent proviral transcription, and consequently, reactivation of latent HIV-1 expression (Doyon et al., 2013; Spivak and Planelles, 2018).

Clinical studies performed in 2011 (NCT01286259) and 2013 (NCT01944371) showed that treatment with DSF resulted in a dose-dependent increase of cell-associated unspliced HIV-RNA and plasma HIV-RNA (Spivak et al., 2013; Elliott et al., 2015; Lee et al., 2019). However, a reduction of the size of the latent reservoir could not be achieved. It was postulated that this was the result of a lack of potency of DSF, the challenges of measuring the size of the reservoir, and the presence of additional viral reservoirs, such as in the central nervous system (Knights, 2017). Another study (NCT03198559) investigating the changes in HIV-RNA levels after treatment with DSF and another latency-reversing agent, vorinostat, had to be terminated due to an increased risk for patients.

Nevertheless, these studies suggest that DSF is a promising treatment option to reactivate latent virus, but methods to determine the size of the latent reservoir need to be optimized and the benefit of combination therapy with anti-retroviral compounds requires further investigation.

Other viruses

Other DTCs, such as PDTC, were investigated against multiple viruses in numerous *in vitro* and *in vivo* infection models, as described in Table 3.

The antiviral activity of PDTC is related to the inhibition of NF-κB. Similar to its role in anti-cancer activity, virus-induced NF-κB activation leads to inhibition of apoptosis and enhanced survival of the host cells. In addition, NF-κB is important in the regulation of the innate and adaptive immune system, giving the virus more time to replicate (Santoro et al., 2003).

Table 3. PDTC antiviral effects against a range of viruses

Virus	Therapy with	Model	Outcome	References
Influenza A	PDTC	Infected mice	Increased the survival rate Reduced influenza A virus-induced weight loss Reduced viral replication in lung tissue in a dose- and time-dependant manner	(Wiesener et al., 2011)
Dengue virus 2	Gefitinib + PDTC	*In vitro*	Decreased virus replication Decreased antiviral cytokine production	(Duran et al., 2017)
Rhinovirus	PDTC	*In vitro*	Reduced virus multiplication	(Nirwan and Kakkar, 2019; Krenn et al., 2005)
Enterovirus 71	PDTC	*In vitro*	Repressed viral replication Reduced protein production	(Lin et al., 2015)
Coxsackievirus B3	PDTC-Cu PDTC-Zn	*In vitro*	Repressed viral replication Reduced protein production	(Si et al., 2005)
Herpes simplex	PDTC-Zn	*In vitro*	Suppressed the expression of herpes simplex virus genes Suppressed the production of viral progeny	(Qiu et al., 2013)

The antiviral activity of PDTC is not only dependent on the inhibition of NF-κB, especially when complexed with metals. For example, PDTC-Zn disturbed the RNA-polymerase enzyme function in rhinovirus by increasing the cellular concentrations of zinc (Zn) ions (Nirwan and Kakkar, 2019; Krenn et al., 2005). In addition, the antiviral activity of PDTC was also linked to the UPS, with PDTC-mediated inhibition of ubiquitination, leading to repressed viral replication and protein production of enterovirus 71 *in vitro* without leading to apoptosis of the cells (Lin et al., 2015). Similar results have already been described for coxsackievirus B3 when exposed to PDTC-Cu and PDTC-Zn (Si et al., 2005) and for herpes simplex virus 1 and 2 when exposed to PDTC-Zn (Qiu et al., 2013).

These results suggest that PDTC, in combination with metals, has antiviral properties via inhibition of NF-κB and UPS in a broad range of viral pathogens.

Although DTCs and DSF are expected to play an important role in the fight against viruses, their antimicrobial activity has been studied in other infectious diseases, such as bacterial infections.

Antibacterial properties

Bacterial infections can affect multiple human organ systems, including the mouth and teeth, the skin, and the lungs (Figure 3). Some of these infections are caused by pathogens on the WHO's global priority list of antibiotic-resistant bacteria, such as *Mycobacterium tuberculosis* and *Staphylococcus aureus*, for which there is an urgent need for research and development of new treatments.

In 1987, Taylor et al. (1987) were the first group investigating the antibacterial properties of DDC against methicillin-resistant *Staphylococcus aureus* (MRSA). Subsequent to these studies, Phillips et al. (1991) investigated the antibacterial properties of DSF against a range of Gam-positive and Gram-negative bacteria. They concluded that DSF inhibits the growth of Gram-positive bacteria, such as MRSA, but not Enterobacteriaceae or *Pseudomonas aeruginosa*. Recent studies confirmed inhibition of growth in Gram-positive bacteria through DSF, especially in MRSA (Frazier et al., 2019; Long, 2017; Sheppard et al., 2018). However, DDC alone did not demonstrate significant inhibition of growth against Gram-positive bacteria (Frazier et al., 2019). PDTC showed some antibacterial activity against pathogens causing

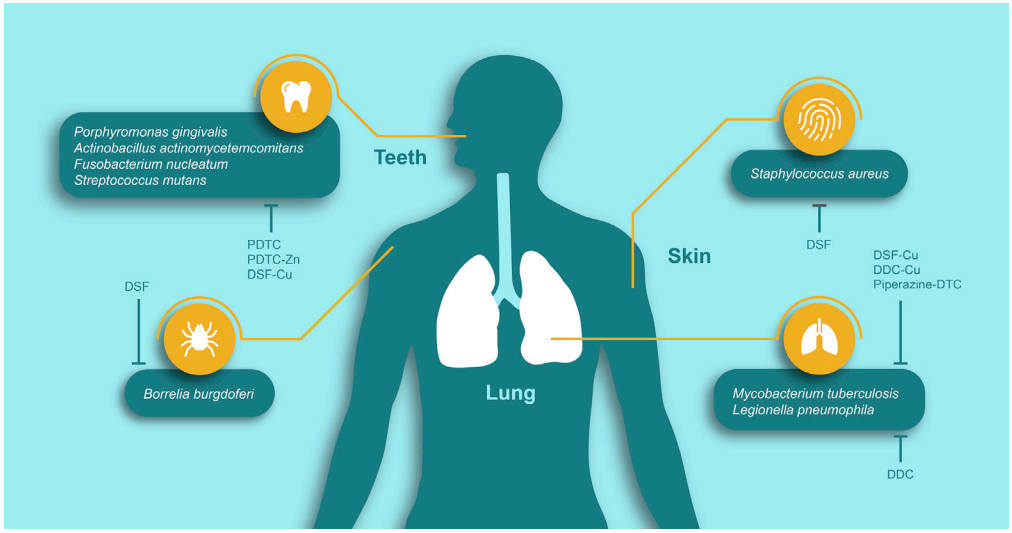

Figure 3. DTCs' activity against bacteria

periodontitis, such as *Porphyromonas gingivalis* and *Actinobacillus actinomycetemcomitans*; medium sensitivity against *S. aureus;* and only low sensitivity against *Escherichia coli* (Kang et al., 2008).

Both DDC and PDTC were highly active against growing and nongrowing persister *Mycobacterium tuberculosis* and enhanced the activity of established tuberculosis drugs (Byrne et al., 2007). However, Dalecki et al. (2015) demonstrated that the antibacterial properties of DSF and DDC in *M. tuberculosis* are strictly Cu ion dependent. In contrast, other DTCs neither benefited nor required Cu ions for their antibacterial activity. The addition of Cu ions reduced the antimicrobial activity of PDTC against *Fusobacterium nucleatum* and *Porphyromonas gingivalis,* whereas it was enhanced by the addition of Zn ions (Choi et al., 2010). Moreover, the antibacterial activity of synthesized potassium morpholine dithiocarbamate against Gram-positive and Gram-negative bacteria was higher than the antibacterial activity of this compound complexed to nickel or copper ions (Balakrishnan et al., 2019).

To date, only a small number of articles have described the antibacterial properties of DDC or DSF combined with Cu^{2+}. In addition, little research has been done to investigate the activity of DTCs against biofilms. Biofilms are clusters of bacteria embedded in a protective matrix (Costerton et al., 1999). Only Saputo et al. (2018) highlighted that DSF and Cu^{2+} reached synergistic effects against biofilms and planktonic *Streptococcus mutans*.

Based on the heterogeneity between different bacterial species, from the membrane constitution of Gram-positive and Gram-negative bacteria to the expression of different proteins within a species, determining the mode of action of DSF and DTCs with and without copper is challenging. The first hypothesis for DSF was based on the metabolization to DDC, which chelates vital metals and inhibits enzymes (Phillips et al., 1991). Later, it was suggested that the complex DDC-Cu acts like a "Trojan horse," helping copper to enter the bacteria, overcoming the bacterial resistance mechanisms, and inhibiting targets that usually were not accessible to free Cu ions (Dalecki et al., 2015).

Despite the species specificity of copper resistance mechanisms, some similarities are found within all bacteria. The copper homeostasis consists of three main components: a copper-exporting ATPase, a copper chaperone, and a copper-responsive regulator. These components differ within species and are particularly expressed in Gram-negative bacteria (Solioz, 2018). Based on our experimental results (L. Kaul, A. Zannettino, R. Süss, K. Richter (2021). *The combination of diethyldithiocarbamate and copper ions presents in vitro activity against staphylococci biofilms*. Manuscript in preparation) we suggest that in Gram-positive bacteria DDC-Cu inhibits at least one of these components, leading to accumulation of free toxic Cu ions. In addition, the presence of glutathione in Gram-negative bacteria can lead to thiol-disulfide exchange between glutathione and DTCs and consequently reduce the antibacterial activity (Long, 2017; Sheppard et al., 2018).

Interestingly, recent studies have revealed antibacterial activity of these compounds against certain Gram-negative bacteria. Following the high *in vitro* activity of DSF against the tick-transmitted *Borrelia burgdorferi*, the cases of three patients taking DSF daily for the symptoms of chronic relapsing neurological Lyme disease and relapsing babesiosis were reported. All patients were able to discontinue antimicrobial treatment and remained clinically well for 6 to 23 months (Liegner, 2019). A pilot study (NCT03891667) is currently recruiting patients with persistent symptoms before antibiotic treatment for Lyme disease to investigate the side effects, tolerability, and effectiveness of DSF (Younger and Murphy, 2020).

Furthermore, DDC inhibited the carbonic anhydrase (CA) in *Legionella pneumophila* (Nishimori et al., 2014). DTCs displayed selectivity for the inhibition of bacterial CA over the host enzyme (Capasso and Supuran, 2017) with some derivatives demonstrating high inhibition of *M. tuberculosis* CA (Maresca et al., 2013). Aspatwar et al. (2020) determined a DTC derivative with high *in vitro* activity against *M. tuberculosis* CA and minimal *in vivo* toxicity, making it a promising candidate for preclinical assays. Previously, the same group investigated a piperazine-DTC induced growth inhibition of *M. tuberculosis* CA and *M.* marinum *in vitro*. In addition, the compound showed growth inhibition of *M. marinum in vivo* and low toxicity in a zebrafish larvae model (Aspatwar et al., 2017).

Considering that CAs are metalloenzymes playing a crucial role in most living organisms and that DTCs interact with these enzymes in cancer and bacterial cells, a possible application for DTCs might also be found in fungi and parasites.

Antifungal properties

A series of DTC analogs selected by Vullo et al. (2017) were tested against a subtype of CA from *Malassezia globosa*. This fungus is considered to be one of the causes for dandruff, a condition affecting more than 50% of the population worldwide. Compared with the clinically used acetazolamide, all DTC derivates were more potent inhibitors of the *M. globosa* CA. An aliphatic DTC with an alcohol moiety and a quinuclidine-3-DTC showed the highest *M. globosa* CA inhibition (Vullo et al., 2017). A similar screening was previously performed for DTC analogs against a CA subtype from different *Candida* species. Dimethyl- and diethyldithiocarbamate were the weakest inhibitors, whereas other derivatives with aryl, arylalkyl-, heterocyclic, and aliphatic moieties led to potent fungal CA inhibition (Monti et al., 2012). The biochemical activities of other vital enzymes, such as ALDH and urease were reduced by DSF, thereby inhibiting the growth of *Pythium insidiosum*, the oomycete microorganism causing the life-threatening infection pythiosis (Krajaejun et al., 2019).

Furthermore, DSF showed fungicidal activity against yeast isolates, *Aspergillus* isolates and *Candida* isolates (Khan et al., 2007). Similar to cancer cells, *Candida* spp. can overexpress ABC transporters, such as Cdr1p supporting efflux of drugs and contributing toward the development of resistance. DSF inhibited Cdr1p and increased the sensitivity of Cdr1p expressing *S. cerevisiae* to different antifungal drugs (Shukla et al., 2004). However, neither synergy nor enhancement could be observed when DSF and fluconazole were tested against resistant *Candida albicans* and other *Candida* isolates (Khan et al., 2007).

Interestingly, DDC showed high antifungal activity against several *C. albicans* and *Candida tropicalis* biofilms (Harrison et al., 2007). The combination of DDC with amphotericin B resulted in a reduced biofilm viability when compared with the compounds alone, as DDC inhibits the *C. albicans* biofilm persistence mechanism superoxide dismutase (De Brucker et al., 2013).

Antiparasitic properties

Superoxide dismutase inhibition by DDC also enhanced *Leishmania braziliensis* killing by macrophages *in vitro* through an increase of superoxide anion release while decreasing lesion size and parasite load in *vivo* (Khouri et al., 2010). Similar results were observed when DDC was incorporated in bacterial cellulose, making it a promising topical formulation for chemotherapy of cutaneous leishmaniasis (Celes et al., 2016). Likewise, DTC pesticides maneb, zineb, and propineb were investigated for their capacity to inhibit CA activity in *Leishmania* species. Mammalian cells were unaffected by the three compounds, whereas exposure to the external and motile form of *Leishmania major* led to apoptotic and necrotic death of the parasite, as well as the reduction of the intracellular parasite burden (Pal et al., 2015).

Another organism relying on antioxidants is the parasite causing malaria. In chloroquine-resistant *Plasmodium berghei*, higher levels of glutathione and glutathione-S-transferase were observed. The mechanism

of chloroquine is based on the inhibition of the crucial biomineralization from hemoglobin to hemozoin. As a result, toxic ferriprotoporphyrin IX accumulates in the membrane fraction of infected cells and binds to membrane proteins. However, free ferriprotoporphyrin IX can be degraded by glutathione (Ginsburg et al., 1998). Disulfiram interacts with GSH and decreases the reduced/oxidized GSH ratio in erythrocytes of uninfected mice. DSF substantially potentiated the antimalarial activity of chloroquine and amodiaquine and prolonged the survival of mice infected with chloroquine-resistant *Plasmodium* strains (Deharo et al., 2003).

DTCs also showed activity in Chagas disease caused by *Trypanosoma cruzi* and in sleeping sickness caused by *Trypanosoma brucei*. Synthesized compounds with a DTC moiety showed antiparasitic activity based on the chelation of metals in the active center of enzymes responsible for the oxidation metabolism of protozoa and therefore disturbing important functions in the parasite's biology. Additional compounds were synthesized by linking DTC with thiadiazine, which are known to inhibit vital parasitic cysteine proteases (Oliveira et al., 2019).

Although new DTC derivatives are being investigated for their antimicrobial activity, DSF and its metabolite DDC appear to be the key candidates. These two compounds inhibit crucial cellular mechanisms and are predicted to play an important role in the battle against cancer, viruses, bacteria, fungi, and parasites.

From head to heart

Initially, DSF was used for the treatment of chronic alcoholism, but the research focus also expanded toward the treatment of cocaine addiction among alcohol users (Suh et al., 2006). Cocaine use leads to increased monoamine levels in the brain, by inhibiting monoamine transporters. It was suggested that the increased synaptic dopamine levels and the norepinephrine levels were responsible for the addictive effect. DDC inhibits the enzyme dopamine b-hydroxylase, which catalyzes the conversion from dopamine to norepinephrine. Therefore, dopamine levels rise during DDC treatment, whereas norepinephrine levels drop (De Sousa, 2019).

The efficacy of DSF for the treatment of cocaine addiction has been attributed to a decrease of the reward mechanisms and an increase in cocaine aversion due to more side effects. In addition, DSF leads to higher basal dopamine levels, thus restoring the reward function of hypodopaminergic addicts and preventing stress-induced reinstatement of cocaine seeking (Devoto et al., 2012). In a study performed by Kampangkaew et al. (2019) patients with genetically higher levels of dopamine transporter, correlating with a lower basal level of dopamine in the synapse, experienced better treatment outcomes with DSF than those with lower dopamine transporter levels (Kampangkaew et al., 2019). In addition, a systematic review performed in 2010 described a statistically significant difference in favor of DSF for the treatment of cocaine dependence when compared with no pharmaceutical treatment. However, they concluded that there was insufficient evidence for the treatment of cocaine abuse with DSF (Pani et al., 2010).

Moreover, DTC pesticides also cause changes in dopamine levels. Maneb triggers dopaminergic cell loss and has been associated with neurotoxic effects leading to an increased risk of developing Parkinson disease (Roede et al., 2011). In contrast, other DTCs have been investigated as treatment options for diseases associated with neuroinflammation, as many have low molecular weight and good blood-brain barrier permeability.

Neuroinflammation is considered a critical risk for the development of neurodegenerative diseases, such as Alzheimer disease and psychiatric illnesses. Alzheimer disease is an irreversible, progressive neurodegenerative disorder that affects approximately 50 million people worldwide. Both the deficit of acetylcholine in the brain region and the aggregation of amyloid-β to plaques can lead to cognitive impairment and dementia (He et al., 2018; Jiang et al., 2018).

It was demonstrated that coumarins inhibit acetylcholinesterase by binding to the peripheral anionic site of the enzyme and display potent monoamine oxidase B inhibitory activity. The DTC moiety interacts with the catalytic anionic site of acetylcholinesterase. To this end, a coumarin-dithiocarbamate hybrid synthesized by He et al. (2018), displayed promising inhibitory activity against monoamine oxidase B and inhibition of acetylcholinesterase through dual binding. Another coumarin-dithiocarbamate hybrid synthesized by Jiang et al. (2018) exhibited potent inhibition toward acetylcholinesterase and self-induced β-amyloid

aggregation. Both compounds demonstrated good ability to penetrate the blood-brain barrier, and no acute toxicity could be observed *in vitro* and *in vivo* (He et al., 2018; Jiang et al., 2018).

Similar to coumarins, several phthalimide analogs were reported to exhibit high inhibitory activity against acetylcholinesterase. Synthesized phthalimide-dithiocarbamate hybrids showed potent anti-acetylcholinesterase and anti-butyrylcholinesterase activity, as well as good blood-brain barrier penetration (Asadi et al., 2019). Through innovative multitargeting, hybrids based on a DTC moiety are promising compounds in the research against Alzheimer disease.

In addition, activation of NF-κB pathways contributes to long-term neuroinflammation and epilepsy. Studies focusing on treatment with the NF-κB inhibitor PDTC showed improved memory function in different models of neuroinflammation and cognitive disorder (Kan et al., 2016; Li et al., 2012; Zhang et al., 2014). Lv et al. (2014) demonstrated that pre-treatment with PDTC prevented neuroinflammation through inhibition of the chemokine MCP-1 and attenuated microglial activation in a status epilepticus rat model. Although PDTC enhanced the susceptibility to seizures, it reduced their frequency and their severity, making it a promising agent for the treatment of status epilepticus (Lv et al., 2014).

Nevertheless, the anti-inflammatory activity of PDTC, through inhibition of NF-κB, is not restricted to neuroinflammation. Indeed, PDTC reduced collagen deposition in non-infarcted areas and improved sympathetic nerve hyperinnervation in rat hearts, resulting in decrease of ventricular arrhythmia incidences and improved myocardial remodeling after myocardial infarct (Wang et al., 2019; Jin et al., 2016). Treatment with PDTC also attenuated pulmonary arterial hypertension and protected pulmonary endothelium in rat models by preventing elevated lipid peroxidation and reducing collagen deposits (Yavuz et al., 2013). Furthermore, PDTC-induced NF-κB inhibition led to reduced renal angiotensin 1 receptor expression and its mediated vasoconstriction, improved oxidative stress, attenuated proinflammatory cytokines, and upregulated anti-inflammatory cytokines in hypertensive transgenic rats (Luo et al., 2015).

Finally, DTCs reduce the intraocular pressure by inhibiting CA2 and CA12, both enzymes playing an important role in the development of glaucoma. First analyses of DTCs with hydrogen, alkyl, aryl, aralkyl, hetaryl, and cyclic moieties by Carta et al. (2012a) resulted in two selected compounds with excellent human CA2 and CA12 inhibitory properties, which lowered the intraocular pressure over 4–8 h in a rabbit model. The potent inhibitory activity of certain DTCs against CA2 was suggested by analyzing the crystallographic structure of CA2. The thiol groups of DTCs bind the zinc in the active site and are further stabilized in the active site pocket through multiple interactions of the tail groups, such as hydrophobic interactions and hydrogen bonds with different amino acids (Bozdag et al., 2015; Carta et al., 2012b). In 2015, another screening of multiple DTCs revealed a superior candidate, as the boc-piperidine-DTC selectively inhibited CA2 and CA12 over the off-target isoform CA1. This DTC analog was also investigated in an *in vivo* model and reduced the intraocular pressure significantly compared with the standard of care dorzolamide (Bozdag et al., 2015). Similar results were also observed with xanthate and trithiocarbamate analogs (Carta et al., 2013).

CONCLUSION

In conclusion, the mechanisms of action of DTCs and DSF remain unclear and require further investigation. However, recent studies have shed light on the interactions of DTCs with enzymes, intracellular metal concentrations, and oxidative processes, which represent important targets in various disease states. DSF, DDC, PDTC, and many newly synthesized DTC derivatives have displayed encouraging results in *in vitro* experiments, often leading to further *in vivo* assays. In addition, DSF and DDC are currently being investigated in multiple clinical trials for applications in cancer, infectious diseases, and substance abuse. An exciting path lies ahead unraveling the potential and pitfalls of DTCs as innovative medical treatments.

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AUTHOR CONTRIBUTIONS

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DECLARATION OF INTERESTS

K.R. has filed an International Patent Application (PCT/AU2020/050661) on DDC-Cu as a combination treatment for microorganisms.

REFERENCES

Allensworth, J.L., Evans, M.K., Bertucci, F., Aldrich, A.J., Festa, R.A., Finetti, P., Ueno, N.T., Safi, R., Mcdonnell, D.P., Thiele, D.J., et al. (2015). Disulfiram (DSF) acts as a copper ionophore to induce copper-dependent oxidative stress and mediate anti-tumor efficacy in inflammatory breast cancer. Mol. Oncol. *9*, 1155–1168.

Asadi, M., Ebrahimi, M., Mohammadi-Khanaposhtani, M., Azizian, H., Sepehri, S., Nadri, H., Biglar, M., Amanlou, M., Larijani, B., Mirzazadeh, R., et al. (2019). Design, synthesis, molecular docking, and cholinesterase inhibitory potential of phthalimide-dithiocarbamate hybrids as new agents for treatment of alzheimer's disease. Chem. Biodivers *16*, e1900370.

Aspatwar, A., Hammaren, M., Koskinen, S., Luukinen, B., Barker, H., Carta, F., Supuran, C.T., Parikka, M., and Parkkila, S. (2017). Beta-CA-specific inhibitor dithiocarbamate Fc14-584B: a novel antimycobacterial agent with potential to treat drug-resistant tuberculosis. J. Enzyme Inhib. Med. Chem. *32*, 832–840.

Aspatwar, A., Hammaren, M., Parikka, M., Parkkila, S., Carta, F., Bozdag, M., Vullo, D., and Supuran, C.T. (2020). In vitro inhibition of Mycobacterium tuberculosis beta-carbonic anhydrase 3 with Mono- and dithiocarbamates and evaluation of their toxicity using zebrafish developing embryos. J. Enzyme Inhib. Med. Chem. *35*, 65–71.

Bala, V., Gupta, G., and Sharma, V.L. (2014). Chemical and medicinal versatility of dithiocarbamates: an overview. Mini Rev. Med. Chem. *14*, 1021–1032.

Balakrishnan, S., Duraisamy, S., Kasi, M., Kandasamy, S., Sarkar, R., and Kumarasamy, A. (2019). Syntheses, physicochemical characterization, antibacterial studies on potassium morpholine dithiocarbamate nickel (II), copper (II) metal complexes and their ligands. Heliyon *5*, e01687.

Bozdag, M., Carta, F., Vullo, D., Akdemir, A., Isik, S., Lanzi, C., Scozzafava, A., Masini, E., and Supuran, C.T. (2015). Synthesis of a new series of dithiocarbamates with effective human carbonic anhydrase inhibitory activity and antiglaucoma action. Bioorg. Med. Chem. *23*, 2368–2376.

Buac, D., Schmitt, S., Ventro, G., Kona, F.R., and Dou, Q.P. (2012). Dithiocarbamate-based coordination compounds as potent proteasome inhibitors in human cancer cells. Mini Rev. Med. Chem. *12*, 1193–1201.

Byrne, S.T., Gu, P., Zhou, J., Denkin, S.M., Chong, C., Sullivan, D., Liu, J.O., and Zhang, Y. (2007). Pyrrolidine dithiocarbamate and diethyldithiocarbamate are active against growing and nongrowing persister Mycobacterium Tuberculosis. Antimicrob. Agents Chemother. *51*, 4495–4497.

Capasso, C., and Supuran, C.T. (2017). Inhibition of bacterial carbonic anhydrases as a novel approach to escape drug resistance. Curr. Top. Med. Chem. *17*, 1237–1248.

Carta, F., Aggarwal, M., Maresca, A., Scozzafava, A., Mckenna, R., Masini, E., and Supuran, C.T. (2012a). Dithiocarbamates strongly inhibit carbonic anhydrases and show antiglaucoma action in vivo. J. Med. Chem. *55*, 1721–1730.

Carta, F., Aggarwal, M., Maresca, A., Scozzafava, A., Mckenna, R., and Supuran, C.T. (2012b). Dithiocarbamates: a new class of carbonic anhydrase inhibitors. Crystallographic and kinetic investigations. Chem. Commun. (Camb) *48*, 1868–1870.

Carta, F., Akdemir, A., Scozzafava, A., Masini, E., and Supuran, C.T. (2013). Xanthates and trithiocarbonates strongly inhibit carbonic anhydrases and show antiglaucoma effects in vivo. J. Med. Chem. *56*, 4691–4700.

Celes, F.S., Trovatti, E., Khouri, R., Van Weyenbergh, J., Ribeiro, S.J., Borges, V.M., Barud, H.S., and De Oliveira, C.I. (2016). DETC-based bacterial cellulose bio-curatives for topical treatment of cutaneous leishmaniasis. Sci. Rep. *6*, 38330.

Choi, E., Lee, H., Kang, M., Kim, B., Lim, H., Kim, S., and Kang, I. (2010). Potentiation of bacterial killing activity of zinc chloride by pyrrolidine dithiocarbamate. J. Microbiol. *48*, 40–43.

Costerton, J.W., Stewart, P.S., and Greenberg, E.P. (1999). Bacterial biofilms: a common cause of persistent infections. Science *284*, 1318–1322.

Cvek, B. (2011). Targeting malignancies with disulfiram (Antabuse): multidrug resistance, angiogenesis, and proteasome. Curr. Cancer Drug Targets *11*, 332–337.

Cvek, B., and Dvorak, Z. (2007). Targeting of nuclear factor-kappaB and proteasome by dithiocarbamate complexes with metals. Curr. Pharm. Des. *13*, 3155–3167.

Cvek, B., and Dvorak, Z. (2008). The value of proteasome inhibition in cancer. Can the old drug, disulfiram, have a bright new future as a novel proteasome inhibitor? Drug Discov. Today *13*, 716–722.

Dalecki, A.G., Haeili, M., Shah, S., Speer, A., Niederweis, M., Kutsch, O., and Wolschendorf, F. (2015). Disulfiram and copper ions kill Mycobacterium Tuberculosis in a synergistic manner. Antimicrob. Agents Chemother. *59*, 4835–4844.

De Brucker, K., Bink, A., Meert, E., Cammue, B.P., and Thevissen, K. (2013). Potentiation of antibiofilm activity of amphotericin B by superoxide dismutase inhibition. Oxid Med. Cell Longev. *2013*, 704654.

De Sousa, A. (2019). Disulfiram in the management of cocaine dependence and other psychiatric disorders. In Disulfiram (Springer Singapore), pp. 31–42.

Deharo, E., Barkan, D., Krugliak, M., Golenser, J., and Ginsburg, H. (2003). Potentiation of the antimalarial action of chloroquine in rodent malaria by drugs known to reduce cellular glutathione levels. Biochem. Pharmacol. *66*, 809–817.

Devoto, P., Flore, G., Saba, P., Cadeddu, R., and Gessa, G.L. (2012). Disulfiram stimulates dopamine release from noradrenergic terminals and potentiates cocaine-induced dopamine release in the prefrontal cortex. Psychopharmacology *219*, 1153–1164.

Doyon, G., Zerbato, J., Mellors, J.W., and Sluis-Cremer, N. (2013). Disulfiram reactivates latent HIV-1 expression through depletion of the phosphatase and tensin homolog. Aids *27*, F7–F11.

Duran, A., Valero, N., Mosquera, J., Fuenmayor, E., and Alvarez-Mon, M. (2017). Gefitinib and pyrrolidine dithiocarbamate decrease viral replication and cytokine production in dengue virus infected human monocyte cultures. Life Sci. *191*, 180–185.

Elliott, J.H., Mcmahon, J.H., Chang, C.C., Lee, S.A., Hartogensis, W., Bumpus, N., Savic, R., Roney, J., Hoh, R., Solomon, A., et al. (2015). Short-term administration of disulfiram for reversal of latent HIV infection: a phase 2 dose-escalation study. Lancet HIV *2*, e520–e529.

Feng, J., Li, C., Xu, R., Li, Y., Hou, Q., Feng, R., Wang, S., Zhang, L., and Li, C. (2020). DpdtC-induced EMT inhibition in MGC-803 cells was partly through ferritinophagy-mediated ROS/p53 pathway. Oxid Med. Cell Longev. *2020*, 9762390.

Frazier, K.R., Moore, J.A., and Long, T.E. (2019). Antibacterial activity of disulfiram and its metabolites. J. Appl. Microbiol. *126*, 79–86.

Ge, Y., Zheng, N., Chen, X., Zhu, J., Sun, W., Olson, J.R., Aga, D.S., Hu, W., Tang, X., and Ren, X. (2019). GMDTC chelating agent attenuates cisplatin-induced systemic toxicity without affecting antitumor efficacy. Chem. Res. Toxicol. *32*, 1572–1582.

Gillet, J.P., and Gottesman, M.M. (2010). Mechanisms of multidrug resistance in cancer. In Multi-Drug Resistance in Cancer. Methods in Molecular Biology (Methods and Protocols), J. Zhou, ed. (Humana Press), pp. 47–76.

Ginsburg, H., Famin, O., Zhang, J., and Krugliak, M. (1998). Inhibition of glutathione-dependent degradation of heme by chloroquine and amodiaquine as a possible basis for their antimalarial mode of action. Biochem. Pharmacol. *56*, 1305–1313.

Harrison, J.J., Turner, R.J., and Ceri, H. (2007). A subpopulation of Candida albicans and Candida tropicalis biofilm cells are highly tolerant to chelating agents. FEMS Microbiol. Lett. *272*, 172–181.

He, Q., Liu, J., Lan, J.S., Ding, J., Sun, Y., Fang, Y., Jiang, N., Yang, Z., Sun, L., Jin, Y., et al. (2018). Coumarin-dithiocarbamate hybrids as novel multitarget AChE and MAO-B inhibitors against Alzheimer's disease: design, synthesis and biological evaluation. Bioorg. Chem. *81*, 512–528.

Huang, T., Sun, Y., Li, Y., Wang, T., Fu, Y., Li, C., and Li, C. (2018). Growth inhibition of a novel iron chelator, DpdtC, against hepatoma carcinoma cell lines partly attributed to ferritinophagy-mediated lysosomal ROS generation. Oxid Med. Cell Longev. *2018*, 4928703.

Hwang, S., Shin, D.M., and Hong, J.H. (2019). Drug repurposing as an antitumor agent: disulfiram-mediated carbonic anhydrase 12 and anion exchanger 2 modulation to inhibit cancer cell migration. Molecules *24*, 3409.

Jiang, N., Huang, Q., Liu, J., Liang, N., Li, Q., Li, Q., and Xie, S.S. (2018). Design, synthesis and biological evaluation of new coumarin-dithiocarbamate hybrids as multifunctional agents for the treatment of Alzheimer's disease. Eur. J. Med. Chem. *146*, 287–298.

Jiao, Y., Hannafon, B.N., and Ding, W.Q. (2016). Disulfiram's anticancer activity: evidence and mechanisms. Anticancer Agents Med. Chem. *16*, 1378–1384.

Jin, J.-L., Lv, R.-G., Guo, J., Liu, X.-H., Liang, Y.-W., Wei, J.-R., and Wang, L. (2016). Improvement of left ventricular remodelling by inhibition of NF-κB in a rat model of myocardial infarction. Heart Lung Circ. *25*, 1007–1012.

Kampangkaew, J.P., Spellicy, C.J., Nielsen, E.M., Harding, M.J., Ye, A., Hamon, S.C., Kosten, T.R., and Nielsen, D.A. (2019). Pharmacogenetic role of dopamine transporter (SLC6A3) variation on response to disulfiram treatment for cocaine addiction. Am. J. Addict. *28*, 311–317.

Kan, M.H., Yang, T., Fu, H.Q., Fan, L., Wu, Y., Terrando, N., and Wang, T.-L. (2016). Pyrrolidine dithiocarbamate prevents neuroinflammation and cognitive dysfunction after endotoxemia in rats. Front. Aging Neurosci. *8*, 175.

Kang, M., Choi, E., Choi, D., Ryu, S., Lee, H., Kang, H., Koh, J., Kim, O., Hwang, Y., Yoon, S., et al. (2008). Antibacterial activity of pyrrolidine dithiocarbamate. FEMS Microbiol. Lett. *280*, 250–254.

Khan, S., Singhal, S., Mathur, T., Upadhyay, D.J., and Rattan, A. (2007). Antifungal potential of disulfiram. Jpn. J. Med. Mycol. *48*, 109–113.

Khouri, R., Novais, F., Santana, G., De Oliveira, C.I., Vannier Dos Santos, M.A., Barral, A., Barral-Netto, M., and Van Weyenbergh, J. (2010). DETC induces leishmania parasite killing in human in vitro and murine in vivo models: a promising therapeutic alternative in leishmaniasis. PLoS One *5*, e14394.

Kim, S.K., Kim, H., Lee, D.-H., Kim, T.-S., Kim, T., Chung, C., Koh, G.Y., Kim, H., and Lim, D.-S. (2013). Reversing the intractable nature of pancreatic cancer by selectively targeting ALDH-high, therapy-resistant cancer cells. PLoS One *8*, e78130.

Knights, H.D.J. (2017). A critical review of the evidence concerning the HIV latency reversing effect of disulfiram, the possible explanations for its inability to reduce the size of the latent reservoir in vivo, and the caveats associated with its use in practice. AIDS Res. Treat. *2017*, 8239428.

Krajaejun, T., Lohnoo, T., Yingyong, W., Rujirawat, T., Kumsang, Y., Jongkhajornpong, P., Theerawatanasirikul, S., Kittichotirat, W., Reamtong, O., and Yolanda, H. (2019). The repurposed drug disulfiram inhibits urease and aldehyde dehydrogenase and prevents in vitro growth of the oomycete pythium insidiosum. Antimicrob. Agents Chemother. *63*, e00609–e00619.

Krenn, B.M., Holzer, B., Gaudernak, E., Triendl, A., Van Kuppeveld, F.J., and Seipelt, J. (2005). Inhibition of polyprotein processing and RNA replication of human rhinovirus by pyrrolidine dithiocarbamate involves metal ions. J. Virol. *79*, 13892–13899.

Lee, S.A., Elliott, J.H., Mcmahon, J., Hartogenesis, W., Bumpus, N.N., Lifson, J.D., Gorelick, R.J., Bacchetti, P., Deeks, S.G., Lewin, S.R., et al. (2019). Population pharmacokinetics and pharmacodynamics of disulfiram on inducing latent HIV-1 transcription in a phase IIb trial. Clin. Pharmacol. Ther. *105*, 692–702.

Li, J., Sheng, W., Feng, C., and Zuo, Z. (2012). Pyrrolidine dithiocarbamate attenuates brain Abeta increase and improves long-term neurological outcome in rats after transient focal brain ischemia. Neurobiol. Dis. *45*, 564–572.

Li, Y., Fu, S.Y., Wang, L.H., Wang, F.Y., Wang, N.N., Cao, Q., Wang, Y.T., Yang, J.Y., and Wu, C.F. (2015). Copper improves the anti-angiogenic activity of disulfiram through the EGFR/Src/VEGF pathway in gliomas. Cancer Lett. *369*, 86–96.

Liegner, K.B. (2019). Disulfiram (tetraethylthiuram disulfide) in the treatment of lyme disease and babesiosis: report of experience in three cases. Antibiotics *8*, 72.

Lin, J., Haffner, M.C., Zhang, Y., Lee, B.H., Brennen, W.N., Britton, J., Kachhap, S.K., Shim, J.S., Liu, J.O., Nelson, W.G., et al. (2011). Disulfiram is a DNA demethylating agent and inhibits prostate cancer cell growth. Prostate *71*, 333–343.

Lin, L., Qin, Y., Wu, H., Chen, Y., Wu, S., Si, X., Wang, H., Wang, T., Zhong, X., Zhai, X., et al. (2015). Pyrrolidine dithiocarbamate inhibits enterovirus 71 replication by down-regulating ubiquitin-proteasome system. Virus Res. *195*, 207–216.

Lin, M.H., Moses, D.C., Hsieh, C.H., Cheng, S.C., Chen, Y.H., Sun, C.Y., and Chou, C.Y. (2018). Disulfiram can inhibit MERS and SARS coronavirus papain-like proteases via different modes. Antivir. Res. *150*, 155–163.

Long, T.E. (2017). Repurposing thiram and disulfiram as antibacterial agents for multidrug-resistant Staphylococcus aureus infections. Antimicrob. Agents Chemother. *61*, e00898–17.

Loo, T.W., and Clarke, D.M. (2000). Blockage of drug resistance in vitro by disulfiram, a drug used to treat alcoholism. J. Natl. Cancer Inst. *92*, 898–902.

Luo, H., Wang, X., Wang, J., Chen, C., Wang, N., Xu, Z., Chen, S., and Zeng, C. (2015). Chronic NF-κB blockade improves renal angiotensin II type 1 receptor functions and reduces blood pressure in Zucker diabetic rats. Cardiovasc. Diabetol. *14*, 76.

Lv, R., Xu, X., Luo, Z., Shen, N., Wang, F., and Zhao, Y. (2014). Pyrrolidine dithiocarbamate (PDTC) inhibits the overexpression of MCP-1 and attenuates microglial activation in the hippocampus of a pilocarpine-induced status epilepticus rat model. Exp. Ther. Med. *7*, 39–45.

Maresca, A., Carta, F., Vullo, D., and Supuran, C.T. (2013). Dithiocarbamates strongly inhibit the beta-class carbonic anhydrases from Mycobacterium tuberculosis. J. Enzyme Inhib. Med. Chem. *28*, 407–411.

Marikovsky, M., Nevo, N., Vadai, E., and Harris-Cerruti, C. (2002). Cu/Zn superoxide dismutase plays a role in angiogenesis. Int. J. Cancer *97*, 34–41.

Monti, S.M., Maresca, A., Viparelli, F., Carta, F., De Simone, G., Muhlschlegel, F.A., Scozzafava, A., and Supuran, C.T. (2012). Dithiocarbamates are strong inhibitors of the beta-class fungal carbonic anhydrases from Cryptococcus neoformans, Candida albicans and Candida glabrata. Bioorg. Med. Chem. Lett. *22*, 859–862.

Nagy, E.M., Ronconi, L., Nardon, C., and Fregona, D. (2012). Noble metal-dithiocarbamates precious allies in the fight against cancer. Mini Rev. Med. Chem. *12*, 1216–1229.

Nakamura, D., Yoshimitsu, M., Kuroki, A., Hachiman, M., Kamada, Y., Ezinne, C.C., Arai, A., Inoue, H., Hamada, H., Hayashida, M., et al. (2015). A new ATL xenograft model and evaluation of pyrrolidine dithiocarbamate as a potential ATL therapeutic agent. Exp. Hematol. *43*, 944–950.

Nechushtan, H., Hamamreh, Y., Nidal, S., Gotfried, M., Baron, A., Shalev, Y.I., Nisman, B., Peretz, T., and Peylan-Ramu, N. (2015). A phase IIb trial assessing the addition of disulfiram to chemotherapy for the treatment of metastatic

non-small cell lung cancer. Oncologist *20*, 366–367.

Nirwan, S., and Kakkar, R. (2019). Rhinovirus RNA polymerase: structure, function, and inhibitors. In Viral Polymerases, S.P. Gupta, ed. (Academic Press), pp. 301–331.

Nishimori, I., Vullo, D., Minakuchi, T., Scozzafava, A., Osman, S.M., Alothman, Z., Capasso, C., and Supuran, C.T. (2014). Anion inhibition studies of two new β-carbonic anhydrases from the bacterial pathogen Legionella pneumophila. Bioorg. Med. Chem. Lett. *24*, 1127–1132.

Oliveira, J.W.D.F., Rocha, H.a.O., De Medeiros, W.M.T.Q., and Silva, M.S. (2019). Application of dithiocarbamates as potential new antitrypanosomatids-drugs: approach chemistry, functional and biological. Molecules *24*, 2806.

Pal, D.S., Mondal, D.K., and Datta, R. (2015). Identification of metal dithiocarbamates as a novel class of antileishmanial agents. Antimicrob. Agents Chemother. *59*, 2144–2152.

Pan, M., Zheng, Q., Yu, Y., Ai, H.-S., Xie, Y., Zeng, X., Wang, C., Liu, L., and Zhao, M. (2020). Seesaw conformations of Npl4 in the human p97 complex and the inhibitory mechanism of a disulfiram derivative. bioRxiv.

Pang, H., Chen, D., Cui, Q.C., and Dou, Q.P. (2007). Sodium diethyldithiocarbamate, an AIDS progression inhibitor and a copper-binding compound, has proteasome-inhibitory and apoptosis-inducing activities in cancer cells. Int. J. Mol. Med. *19*, 809–816.

Pani, P.P., Trogu, E., Vacca, R., Amato, L., Vecchi, S., and Davoli, M. (2010). Disulfiram for the treatment of cocaine dependence. Cochrane Database Syst. Rev. Cd007024.

Phillips, M., G M, Nedunchezian, D., Lukrec, A., and Howard, R.G. (1991). Disulfiram inhibits the in vitro growth of methicillin-Resistant Staphylococcus aureus. Antimicrob. Agents Chemother. *35*, 785–787.

Qiu, M., Chen, Y., Cheng, L., Chu, Y., Song, H.-Y., and Wu, Z.-W. (2013). Pyrrolidine dithiocarbamate inhibits herpes simplex virus 1 and 2 replication, and its activity may be mediated through dysregulation of the ubiquitin-proteasome system. J. Virol. *87*, 8675–8686.

Roede, J.R., Hansen, J.M., Go, Y.-M., and Jones, D.P. (2011). Maneb and paraquat-mediated neurotoxicity: involvement of peroxiredoxin/thioredoxin system. Toxicol. Sci. *121*, 368–375.

Santoro, M.G., Rossi, A., and Amici, C. (2003). NF-kappaB and virus infection: who controls whom. EMBO J. *22*, 2552–2560.

Saputo, S., Faustoferri, R.C., and Quivey, R.G., Jr. (2018). A drug repositioning approach reveals that Streptococcus mutans is susceptible to a diverse range of established antimicrobials and nonantibiotics. Antimicrob. Agents Chemother. *62*, e01674–17.

Sauna, Z.E., Peng, X.H., Nandigama, K., Tekle, S., and Ambudkar, S.V. (2004). The molecular basis of the action of disulfiram as a modulator of the multidrug resistance-linked ATP binding cassette transporters MDR1 (ABCB1) and MRP1 (ABCC1). Mol. Pharmacol. *65*, 675–684.

Sauna, Z.E., Shukla, S., and Ambudkar, S.V. (2005). Disulfiram, an old drug with new potential therapeutic uses for human cancers and fungal infections. Mol. Biosyst. *1*, 127–134.

Schmidtova, S., Kalavska, K., Gercakova, K., Cierna, Z., Miklikova, S., Smolkova, B., Buocikova, V., Miskovska, V., Durinikova, E., Burikova, M., et al. (2019). Disulfiram overcomes cisplatin resistance in human embryonal carcinoma cells. Cancers *11*, 1224.

Sheppard, J.G., Frazier, K.R., Saralkar, P., Hossain, M.F., Geldenhuys, W.J., and Long, T.E. (2018). Disulfiram-based disulfides as narrow-spectrum antibacterial agents. Bioorg. Med. Chem. Lett. *28*, 1298–1302.

Shukla, S., Sauna, Z.E., Prasad, R., and Ambudkar, S.V. (2004). Disulfiram is a potent modulator of multidrug transporter Cdr1p of Candida albicans. Biochem. Biophysical Res. Commun. *322*, 520–525.

Si, X., Mcmanus, B.M., Zhang, J., Yuan, J., Cheung, C., Esfandiarei, M., Suarez, A., Morgan, A., and Luo, H. (2005). Pyrrolidine dithiocarbamate reduces coxsackievirus B3 replication through inhibition of the ubiquitin-proteasome pathway. J. Virol. *79*, 8014–8023.

Skrott, Z., and Cvek, B. (2012). Diethyldithiocarbamate complex with copper: the mechanism of action in cancer cells. Mini Rev. Med. Chem. *12*, 1184–1192.

Skrott, Z., Mistrik, M., Andersen, K.K., Friis, S., Majera, D., Gursky, J., Ozdian, T., Bartkova, J., Turi, Z., Moudry, P., et al. (2017). Alcohol-abuse drug disulfiram targets cancer via p97 segregase adaptor NPL4. Nature *552*, 194–199.

Solioz, M. (2018). Copper homeostasis in gram-positive bacteria. In Copper and Bacteria (SpringerBriefs in Biometals), pp. 21–48.

Spivak, A.M., Andrade, A., Eisele, E., Hoh, R., Bacchetti, P., Bumpus, N.N., Emad, F., Buckheit, R.I., Mccance-Katz, E.F., Lai, J., et al. (2013). A pilot study assessing the safety and latency-reversing activity of disulfiram in HIV-1–infected adults on antiretroviral therapy. Clin. Infect. Dis. *58*, 883–890.

Spivak, A.M., and Planelles, V. (2018). Novel latency reversal agents for HIV-1 cure. Annu. Rev. Med. *69*, 421–436.

Su, Y., Li, R., Ning, X., Lin, Z., Zhao, X., Zhou, J., Liu, J., Jin, Y., and Yin, Y. (2019). Discovery of 2,4-diarylaminopyrimidine derivatives bearing dithiocarbamate moiety as novel FAK inhibitors with antitumor and anti-angiogenesis activities. Eur. J. Med. Chem. *177*, 32–46.

Suh, J.J., Pettinati, H.M., Kampman, K.M., and O'brien, C.P. (2006). The status of disulfiram: a half of a century later. J. Clin. Psychopharmacol. *26*, 290–302.

Tang, J.H., Huang, G.H., Mou, K.J., Zhang, E.E., Li, N., Du, L., Zhu, X.P., Chen, L., Yang, H., Zhang, K.B., et al. (2017). Pyrrolidine dithiocarbamate sensitizes U251 brain glioma cells to temozolomide via downregulation of MGMT and BCL-XL. Oncol. Lett. *14*, 5135–5144.

Taylor, E.H., Walker, E., Bartelt, M., Day, S., and Pappas, A. (1987). In vitro antimicrobial activity of diethyldithiocarbamate and dimethyldithiocarbamate against methicillin-resistant Staphylococcus. Ann. Clin. Lab. Sci. *17*, 171–177.

Thakare, R., Shukla, M., Kaul, G., Dasgupta, A., and Chopra, S. (2019). Repurposing Disulfiram for treatment of Staphylococcus aureus infections. Int. J. Antimicrob. Agents *53*, 709–715.

Viola-Rhenals, M., Patel, K.R., Jaimes-Santamaria, L., Wu, G., Liu, J., and Dou, Q.P. (2018). Recent advances in antabuse (disulfiram): the importance of its metal-binding ability to its anticancer activity. Curr. Med. Chem. *25*, 506–524.

Vullo, D., Del Prete, S., Nocentini, A., Osman, S.M., Alothman, Z., Capasso, C., Bozdag, M., Carta, F., Gratteri, P., and Supuran, C.T. (2017). Dithiocarbamates effectively inhibit the beta-carbonic anhydrase from the dandruff-producing fungus Malassezia globosa. Bioorg. Med. Chem. *25*, 1260–1265.

Wang, Y., Tan, J., Yin, J., Hu, H., Shi, Y., Wang, Y., Xue, M., Li, X., Liu, J., Li, Y., et al. (2019). Targeting blockade of nuclear factor-κB in the hypothalamus paraventricular nucleus to prevent cardiac sympathetic hyperinnervation post myocardial infarction. Neurosci. Lett. *707*, 134319.

Wiesener, N., Zimmer, C., Jarasch-Althof, N., Wutzler, P., and Henke, A. (2011). Therapy of experimental influenza virus infection with pyrrolidine dithiocarbamate. Med. Microbiol. Immunol. *200*, 115–126.

Yang, Q., Yao, Y., Li, K., Jiao, L., Zhu, J., Ni, C., Li, M., Dou, Q.P., and Yang, H. (2019). An updated review of disulfiram: molecular targets and strategies for cancer treatment. Curr. Pharm. Des. *25*, 3248–3256.

Yang, Y., Liu, Y., Guo, R., Fu, Y., Zhang, Z., Zhang, P., Zhou, P., Wang, T., Huang, T., Li, X., et al. (2018). The novel dithiocarbamate, DpdtC suppresses HER2-overexpressed cancer cells by up-regulating NDRG1 via inactivation of HER2-ERK 1/2 signaling. Sci. Rep. *8*, 3398.

Yavuz, T., Uzun, O., Macit, A., Comunoglu, C., Yavuz, O., Silan, C., Yuksel, H., and Yildirim, H.A. (2013). Pyrrolidine dithiocarbamate attenuates the development of monocrotaline-induced pulmonary arterial hypertension. Pathol. Res. Pract. *209*, 302–308.

Younger, D., and Murphy, B. (2020). Antabuse for lyme disease: the way forward. World J. Neurosci. *10*, 1–7.

Zhang, J., Jiang, W., and Zuo, Z. (2014). Pyrrolidine dithiocarbamate attenuates surgery-induced neuroinflammation and cognitive dysfunction possibly via inhibition of nuclear factor κB. Neuroscience *261*, 1–10.