A review of the therapeutic properties of dithiocarbamates

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Abstract
The persistence of infectious diseases that continue to plague the world, as well as the formation of harmful substances within the human body, such as free radicals and reactive oxygen species (ROS) have sparked new research. Thus, the need for innovative approaches for developing new or modification of existing therapeutic agents. The design of biologically important metal complexes of dithiocarbamates (DTCs) has been made possible by recent advancements in innovative research. Dithiocarbamates are reduced thiuram disulfides with excellent complexing capabilities and have various applications. They are potent and work in tandem with the core metal ions of coordinating compounds to produce synergistic effects. Dithiocarbamates have many uses, including as antidotes for metal poisoning, cisplatin or carboplatin toxicity, and clinical trials for cancer, Lyme disease, human immunodeficiency virus and antibiotics. They exert anti-oxidant effect in cells. The understanding of the mechanisms of action of this therapeutic agent is important in drug repurposing. This review highlights the protective and therapeutic properties of dithiocarbamate compounds in biological systems.

Keywords
Dithiocarbamate, Biological properties, Antioxidant properties, Apoptosis, Reactive oxygen species
Introduction
Dithiocarbamates (DTCs) have lately reappeared as possible therapeutic agents because of their metal chelating properties and affinity for thiol groups (Kaul et al., 2021). Only a few studies have shown the growing DTCs’ importance in medicine and the promise for various medicinal applications. DTC moieties have been studied as antimicrobial agents, cancer therapies, and neurology and cardiology applications. New chemical compounds containing DTC moieties have also been developed and investigated for anti-cancer, neurological, and antimicrobial uses (Kaul et al., 2021).

Dithiocarbamates are biologically active chemical compounds with the -N(C=S)- moiety that is made by reacting amines or their corresponding derivatives with carbon disulfide in the presence of a base. They are a type of soft sulfur donor ligand (mono-anionic 1,1-dithiolate), capable of forming stable metal complexes (Adeyemi & Onwudie, 2020). These ligands are mono-anionic 1,1-dithiolate. DTC ligands have a significant metal binding potential because of the presence of two sulfur atoms in their structure that are available for complexation, which are effective in enzyme inhibition in biological systems (Adeyemi & Onwudie, 2020; Yeo et al., 2021). DTC ligands are lipophilic and can act as mono- or bi-dentate bridging ligands for metal ions (Hogarth, 2005). Their widespread use and applications are due to the relative simplicity of H+ replacement from the -SH group inside their molecules, as well as the potential for complex formation. DTCs can suppress bacterial growth in biological systems by modifying the metabolic processes of the organism. The existence of carbon–sulfur bonds has been attributed to most biological features exhibited by dithiocarbamate molecules. This helps with the synthesis of organic intermediates with interesting chemistry (Movassagh & Shokri, 2012). In research, their strong nucleophilic attributes and unique characteristics to perform a redox reaction have been credited with their application in enzyme catalysis, redox signaling and protein folding (Lal, 2014). They also demonstrate anticancer capabilities and the potential for use in treating various ailments, including viral infections and inflammation. Key proteins involved in apoptosis, degradation, oxidative stress, and transcription can be altered to help achieve these goals (Buac et al., 2012).

The antioxidant behavior of dithiocarbamate includes scavenging the superoxide radical and eliminating hydrogen peroxide (Mankhetkorn et al., 1994), peroxynitrite and the hydroxyl radical (Liu et al., 1996) and peroxyl radical, a product of lipid peroxidation (Zanocco et al., 1989). Excessive generation of free radicals causes damage to DNA, lipids, and proteins, which promote aging, cancer, and various brain and cardiac problems (Onwudie & Ekenia, 2017). Dithiocarbamate thiol radicals are formed when DTCs react with nitrogen species and reactive oxygen, and then dimerize to form thiuram disulfides (Zanocco et al., 1989), the oxidized form of DTCs. Thiuram disulfides, which are characterized by their intense oxidation of GSH and protein thiols, are responsible for much of the pro-oxidant effects of DTCs. (Nobel et al., 1995). The oxidation of diethylthiocarbamate and PDTC by copper (II) is an example of thiuram disulfide production that is metal-dependent (Burkitt et al., 1998).

In studies of DTC activity, the antioxidant properties of these compounds have been emphasized, whereas their pro-oxidant properties have received less attention.

Types and structure of dithiocarbamates
Dithiocarbamates are divided into four groups:

a) Monoalkyldithiocarbamates e.g. ethyldithiocarbamate

b) Asymmetric (dialkyldithiocarbamate) e.g. ethylmethyldithiocarbamate

c) Symmetric (dialkyldithiocarbamate) e.g. diethyldithiocarbamate

d) Heterocyclic dithiocarbamates e.g. pyrrolidinedithiocarbamate

Free radicals, reactive oxygen species and oxidative stress
Molecules or molecular fragments with one or more unpaired electrons in atomic or molecular orbitals are known as free radicals (Halliwell & Gutteridge, 2006). The free radical usually has a lot of reactivity because of the unpaired electron(s). The most common type of radical species produced in living systems is radicals originating from oxygen (Miller et al., 1990). The electrical arrangement of molecular oxygen (dioxygen) is unusual, making it a radical.

When one electron is added to dioxygen, the superoxide anion radical (O2·−) is generated (Miller et al., 1990). Superoxide anion is the “primary” ROS, formed either using metabolic processes or after physical irradiation “activates” oxygen. It can then combine with other molecules to generate five “secondary” ROS, either directly or through enzyme or metal-catalyzed processes (Valko et al., 2005). The most prevalent ROS include superoxide anion, hydrogen peroxide, peroxyl radicals, and highly reactive hydroxyl (OH·) radicals.

Although, oxygen is required by cells, its metabolites such as the ROS are lethal to cells (de Lamirande & Gagnon, 1995). As a result of its toxicity to cells, ROS must continually be inactivated to keep only a small amount necessary to maintain normal
cell function. “Oxidative stress” (OS) is a condition associated with an increased rate of cellular damage induced by oxygen and oxygen-derived oxidants (Sikka et al., 1995). The imbalance between ROS and the antioxidant defense mechanism of the cell also causes oxidative stress (Sikka, 2001). Carcinogenesis, aging, infection, physical injury, acquired immunodeficiency syndrome, and toxin exposure have all been linked to ROS (Joyce, 1987). It’s also been hypothesized that oxidative stress plays a role in many disorders linked to reproductive dysfunction (Sharma & Agarwal, 1996).

**Dithiocarbamates in treatment and prevention of organ damage**

Pyrrolidine dithiocarbamate (PDTC) is an antioxidant and an inhibitor of NF-κB. In cells treated with IL-1, LPS, phorbol ester, and TNF-α, micromolar levels of PDTC inhibited the release of the inhibitory subunit IκB from the latent cytoplasmic form of NF-κB (Schreck et al., 1992). Hepatic fibrosis, triggered by hepatocyte damage results in the recruitment of inflammatory cells and the subsequent release of cytokines and growth factors (Muriel, 2007a; Muriel, 2007b) that are thought to link the inflammatory and reparative phases of fibrosis by activating hepatic stellate cells (HSC) (Parsons et al., 2007). HSC is triggered by oxidative stress and responds to IL-1β and TNF-α therapy, resulting in NF-κB nuclear translocation and IκBα breakdown. Activated HSCs are the primary source of extracellular matrix synthesis, and the expression of adhesion molecules and cytokines contributes to liver injury (Hellerbrand et al., 1998).

In experimental models of liver damage, activation of NF-κB has been linked to the pathophysiology of cell injury. In a rat model of thioacetamide-induced liver failure, Bruck et al. (2002) investigated whether PDTC could reduce hepatic damage. They discovered that giving thioacetamide-treated animals PDTC intraperitoneally reduced immediate liver damage and increased survival. Reduced oxidative stress, reduced hepatic hydroxyproline levels, inhibition of NF-κB, reduced spleen weight and fibrosis score, as well as inhibition of HSC activation, reduced collagen content, and tissue inhibitor of metalloproteinase-2 and collagen α1(I) gene expression in the liver of PDTC-treated rats, could all contribute to this effect.

Eren et al. (2010) reported that PDTC reduced biochemical and structural derangement of diabetic lungs. Another finding revealed that in the rat aorta, pyrrolidine dithiocarbamate inhibits the loss of contractile responsiveness caused by interleukin-1-mediated stimulation of inducible nitric oxide synthase (Schini-Kerth et al., 1994).

Borghesi et al. (2018) reported that in the kidney, PDTC inhibited diclefenac-induced morphological changes, oxidative stress, NF-κB activation, pro-inflammatory cytokine production and increased antioxidant defenses and anti-inflammatory cytokine (IL-10). Additionally, N-benzyl-D-glucamine dithiocarbamate (BGD) protects against renal toxicity in rats during repeated cisplatinadministration (Hidaka et al., 1995).

**Dithiocarbamates in sepsis treatment and apoptosis**

Sepsis is a severe systemic inflammation caused by dysregulated host response to pathologic infection (Singer et al., 2016), a report estimated its global incidence at 19 million people each year (Fleischmann et al., 2016). Free-oxygen radicals, lipid and protein oxidation have been implicated in sepsis pathogenesis (Goodee & Webster, 1993; Prauchner, 2017). Lipopolysaccharides activate nuclear factor kappa B (NF-κB), activation of NF-κB results in the expression of pro-inflammatory mediators and increased biosynthesis in sepsis, which has been linked to multiple organ injury (Ang et al., 2011). Pyrrolidine dithiocarbamate has been reported to be an effective medication for sepsis treatment (Gezmis et al., 2019; Liu et al., 1999).

Due to their pleiotropic effects on cells, dithiocarbamates may both prevent and trigger apoptosis (Ortenius et al., 1996). They prevent apoptosis induced by a range of stimuli in short-term incubations. This has been interpreted to mean that ROS plays a function in apoptosis (Wolfe et al., 1994). Others, on the other hand, believe that dithiocarbamate prevention of apoptosis is due to oxidation of key thiols rather than broad scavenging of oxygen radicals (Nobel et al., 1997a). Thus, disulfiram inhibits caspase-3, caspase-1 (whose sensitivity to disulfiram varies in vitro), and most likely additional members of the caspase family (Nobel et al., 1997a). Dithiocarbamate induces apoptosis via intracellular uptake of copper by triggering the formation of ROS and proteasome inhibition (Hogarth & Onwudie, 2021; Nobel et al., 1995). The precise molecular processes underlying their anticancer effect are unknown, although mechanistic investigations have revealed that they can operate as proteasome inhibitors (Milacic et al., 2006), DNA intercalators (Ronconi et al., 2006), nuclear factor kappa B (NF-κB) inhibitors, and inactivators of various metal-containing enzymes (Nobel et al., 1997b). In the presence of copper ions, disulfiram acts as an anticancer agent. With copper (Cu), it does not form a stable complex, but reacts rapidly. They are most likely transformed to thiram disulfides, which are powerful glutathione oxidants, using a copper-catalysed process (Burkitt et al., 1998). Park et al. (2003) has postulated a mechanism that involves the initial reduction of Cu(II) to Cu(I) and the creation of bitet-4 2+, the oxidized form of disulfiram, at the same time. The bitet-4 2+ molecule is unstable and decomposes catastrophically, resulting in the generation of 30 electrons per molecule and oxidative stress in cells (Cen et al., 2004). The significant cell death observed when exposed to disulfiram-copper combinations is most likely due to this (Chen et al., 2006).

Disulfiram and Cu(II) also induce cellular apoptosis in prostate and human breast cancer cells (Daniel et al., 2005; Daniel et al., 2007), suggesting that they could be used to treat resistant neuroblastoma in children (Hogarth & Onwudie, 2021). Based on IC50 values, Zhang et al. (2008) found that this combination suppressed the growth of BE (2/C cells (a human neuroblastoma cell line) and was more potent than cisplatin. These DTC salts reduce cancer cell migration and invasion by decreasing cell proliferation and inducing apoptosis and autophagy.
**Dithiocarbamates in the fight against microbial pathogens**

Antimicrobial resistance and the subsequent lack of effective antimicrobials to combat infectious diseases has remained a global health challenge. Understanding the mechanisms of resistance, which enables new diagnostic and therapeutic approaches, antimicrobial resistance drivers in the environment, will help in combating this threat. Pathogens have evolved defensive mechanisms, such as preventing drug entry or export, altering the drug target or generating enzymes that degrade or modify the antimicrobial. As a result, antimicrobial resistance could be thought of as a Darwinian competition from antimicrobial compounds originating from natural microorganisms (Holmes et al., 2016).

This challenge has resulted in fewer treatment options for patients and an increase in morbidity and mortality. As a result, we now have more serious infections that require comprehensive treatment, as well as lengthier illness causes that frequently necessitate protracted hospitalization. The costs of treating these illnesses have skyrocketed because of this. As a result, despite multiple discoveries and antimicrobial medicines already accessible for therapeutic use, continued hunt for novel drugs to combat rapidly evolving pathogens remains critical.

According to the World Health Organization, antimicrobial-resistant illnesses are expected to result in the death of 10 million people by 2050. This is due in part to an increase in microbial drug resistance, as well as a slower rate of discovery of new antimicrobials (Kaul et al., 2021). Thus, there is an obvious need to discover new and effective antibiotics. A practical strategy is to focus on metallodrugs, which provide novel drug discovery prospects due to their increased potency and different modes of action (Yeo et al., 2021). DTCs have been studied as antibacterial possibilities, with activity against viruses, bacteria, fungi, and parasites, leading to clinical trials in some cases. With the recognized therapeutic usage and promise of DTC derivatives and a growing understanding of the role of metal-based medications, it seems only logical that DTC derivatives be investigated as possible antimicrobial agents. Interference with cell wall (by affecting cell permeability), metabolic interference with cellular enzymes, cellular damage owing to protein denaturing, and disruption of normal cell processes as a result of hydrogen bonding with active cellular constituents through the azomethine group (Adeyemi & Onwudiwe, 2018). The permeability of these compounds through the cell membrane/wall of either Gram-positive or Gram-negative organisms was considered to guide these activities. Gram-negative bacteria pose a greater challenge because of the complex outer-lipid membrane, which is made of lipopolysaccharide. This outer membrane contributes to their antigenic specificity and makes them less penetrable than Gram-positive bacteria with simpler cell membrane (Jabbar et al., 2012).

Several metal–dithiocarbamate complexes have shown moderate-to-high antibacterial activity against Gram-negative and Gram-positive bacterial pathogens on the World Health Organization’s global priority list of antibiotic-resistant species. Disulfiram (DSF), a DTC derivative, inhibits Gram-positive bacterial growth in recent research, especially methicillin-resistant Staphylococcus aureus (MRSA) (Frazier et al., 2019; Sheppard et al., 2018). Diethylthiocarbamate (DTC), the bestknown DTC derivative, on the other hand, did not show any substantial inhibition of Gram-positive bacterial growth when used alone (Frazier et al., 2019). Periodontitis-causing organisms such as Actinobacillus actinomycetemcomitans and Porphyromonas gingivalis were inhibited by PDTC, which had a medium action against S. aureus and low sensitivity to Escherichia coli (Kang et al., 2008).

DTC derivatives (PDTC and DDC) have been reported to be highly effective against both growing and non-growing Mycobacterium tuberculosis persisters. These derivatives improved the efficacy of existing tuberculosis drugs (Byrne et al., 2007; Dalecki et al. (2015) on the other hand, reported that the bactericidal activities of DSF and DDC in M. tuberculosis are solely dependent on Cu⁺. This indicates a synergistic antibacterial activity of DSF and Cu⁺. This allows the complex to penetrate M. tuberculosis’s cellular defenses and its drug resistance machinery. As a result, M. tuberculosis is vulnerable to chemical attacks, and copper-interacting compounds are identified as a unique family of bacterial inhibitors. Liegnier (2019) presented a case of three patients, who were treated with DSF for Lyme disease and relapsing babesiosis caused by Borrelia burgdorferi. Patients who previously required intense open-ended antibiotic therapy for Lyme disease were able to quit treatment after completing a finite course of treatment alone with DSF and were clinically healthy for periods of observation ranging from 6–23 months (Liegnier, 2019).

The metal chelation properties of DTC complexes depend on the polarity of the metal. Due to partial positive charge sharing with donor groups and the potential for n-electron delocalization over the entire chelate ring, the polarity of the metal is greatly reduced during complexation (Adeyemi & Onwudiwe, 2020; Manoussakis et al., 1987). In this process, the complexes’ permeability triumphs over the bacteria’s lipophilic membrane, through chelation with DTC ligands, this mechanism of action was thought to block biological activities within the organism by suppressing physiologically important metals like Zn and Cu (Manoussakis et al., 1987; Vuksanović et al., 2013).

Another mechanism is thought to be exploited by DTCs is the formation of a hydrogen bond with the active centers of the bacterium cell constituents via the -N=C(S)SH group, which disrupts normal cell processes (Manoussakis et al., 1987). A well-known DTC derivative, potassium-3-dithiocarboxy-3-aza-5-aminopentanolate, has been postulated to react with metalloenzymes in bacteria, killing them (Vuksanović et al., 2013).

In a study by Khan et al. (2007), the antifungal activities of DSF against Aspergillus, Candida and yeast isolates that cause life-threatening infections in immunocompromised patients were reported. Similarly, a series of DTCs were investigated against β-class carbonic anhydrase from Malassezia globosa, a fungal pathogen causing dandruff (Vullo et al., 2017).
Compared to the typical sulfonamide medication acetazolamide, several DTCs were found to be more effective in suppressing *M. globosa*. These studies show the antifungal properties of DTCs and its derivatives in combating fungal infections.

The antiviral properties of DTC and its derivatives have also been reported in the literature. Several studies and DTC drugs are in preclinical and clinical stages against coronaviruses and human immunodeficiency virus (Kaul et al., 2021). However, certain DTCs have been reported to be effective against other viruses. The antiviral activities of PDTC against influenza A (Wiesener et al., 2011), enterovirus 71 (Lin et al., 2015), herpes simplex 1 and 2 (Qiu et al., 2013), and dengue virus 2 (Duran et al., 2017) have been reported.

The blockage of influenza virus-induced apoptosis is responsible for PDTC’s antiviral action against influenza virus (Uchide et al., 2002). In primary cultured choriocarcinoma cells generated from human fetal membranes, influenza virus increased ROS generation and apoptotic fragmentation of DNA as genetic material. The anti-influenza properties of PDTC were demonstrated by inhibition of induction of DNA fragmentation, ROS overproduction and the release of influenza particles from infected cells. Furthermore, PDTC suppressed the synthesis of complementary (cRNA and mRNA) RNAs, viral (vRNA), and influenza virus hemagglutinin up to 6 hours after infection, as well as delaying and deceasing hemagglutinin protein synthesis. PDTC did not affect apoptosis or influenza virus formation, but did inhibit ROS overproduction, suggesting that PDTC inhibited apoptosis by decreasing viral macromolecule synthesis rather than through its antioxidant impact (Uchide et al., 2002).

The mechanism of PDTC on influenza virus gene replication and transcription is by chelating divalent metal ions and rapid recruitment of copper and zinc ions into cells from the extracellular medium (Kim et al., 1999). The activity of viral RNA-dependent RNA polymerase is inhibited by zinc or copper ions. Bathocuproine–copper or bathocuproine–zinc complexes have a higher inhibitory impact than bathocuproine alone (Oxford & Perrin, 1974). Thujaplicin–copper complex, a metal chelator, prevents influenza virus multiplication (Miyamoto et al., 1998). As a result, it’s possible that PDTC suppresses viral gene replication and transcription by increasing intracellular copper and zinc ions, or intracellular PDTC–copper and PDTC–zinc complexes, by inhibiting RNA-dependent RNA polymerase activity. If PDTC worked just as a replicative enzyme inhibitor, the viral RNA production that PDTC halted would not resume in its presence; also, earlier exposure of cells to PDTC would not amplify its action (Takizawa et al., 1993).

**Limitations of dithiocarbamates as therapeutic agents**

Dithiocarbamate and its metal complexes have been shown in numerous studies to be effective biological agents, particularly at the cellular level. However, most of these complexes are not clinically resolved. A few of the metallo-complexes have been linked to negative consequences on biological systems. These are frequently related to either the metal’s toxicity or the instability of the ligand moiety (Adyemey & Onwudiwe, 2018). Metal complexation, on the other hand, is hypothesized to have a modulating effect on the metal ion’s toxicity while also allowing the ligand to become more stable, thus, reducing its availability for additional side reactions. Adokoh (2020) reported that these complexes have low stability in biological systems under physiological conditions as therapeutic agents. This property continues to be a major stumbling block in the development of this type of drug. Another challenge associated with the use of DTC complexes, particularly gold complexes, is the likelihood of oxidation state shift (Adokoh, 2020). Because most gold (III) compounds are largely unstable under physiological conditions and are frequently transformed into more thermodynamically stable gold (I) complexes, part of this compound’s utility has been limited. Despite numerous publications suggesting that these gold (I) complexes have a more promising potential than most currently used anticancer medicines, the instability of gold (III) complex oxidation impedes the development of innovative therapeutic agents (Adokoh, 2020).

**Conclusions**

There has been limited information about the protective and therapeutic properties of dithiocarbamate compounds and the mechanisms of action is yet unknown. Recent research, on the other hand, has revealed information about DTC interactions with enzymes, intracellular metal concentrations, and oxidative processes, all of which are important targets in many diseases. This review gives comprehensive information about the protective effect of DTC compounds on ameliorating damage done to various organs and its use against microbial pathogens. In vitro tests on DTC and its derivatives have yielded promising results, prompting additional in vivo testing. The utilization of these complexes in drug design could have a tremendous impact on human health and provides alternative to currently available drugs that is both cheaper and more effective. Because of their biological potentials, DTC complexes could be effective in the fight against antibiotic resistance. However, understanding their precise mechanism of action and side effects requires a significant amount of effort. Regardless of the limitations, these complexes can provide a platform for the development of novel therapeutic drugs, hence fresh approaches as well as detailed studies are required to overcome their drawback. Uncovering the possibilities and drawbacks of DTCs as revolutionary medical treatments is an interesting journey ahead.

**Data availability**

**Underlying data**

There are no data associated with this article.
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