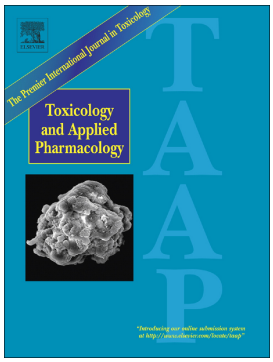

Upcycling the anthracyclines: New mechanisms of action, toxicology, and pharmacology

Claudine E. Bayles, Danielle E. Hale, Ali Konieczny, Veronica D. Anderson, Claire R. Richardson, Katelyn V. Brown, Jennifer T. Nguyen, Jacob Hecht, Nora Schwartz, Madan K. Kharel, Felix Amissah, Thomas C. Dowling, S. Eric Nybo

PII: S0041-008X(22)00507-5

DOI: https://doi.org/10.1016/j.taap.2022.116362

Reference: YTAAP 116362

To appear in: *Toxicology and Applied Pharmacology*

Received date: 25 August 2022

Revised date: 14 November 2022

Accepted date: 27 December 2022

Please cite this article as: C.E. Bayles, D.E. Hale, A. Konieczny, et al., Upcycling the anthracyclines: New mechanisms of action, toxicology, and pharmacology, *Toxicology and Applied Pharmacology* (2022), https://doi.org/10.1016/j.taap.2022.116362

Upcycling the anthracyclines: new mechanisms of action, toxicology, and pharmacology

Claudine E. Bayles [†,1], Danielle E. Hale [†,1], Ali Konieczny [†,1], Veronica D. Anderson [†,1], Claire R. Richardson [1], Katelyn V. Brown [1], Jennifer T. Nguyen [1], Jacob Hecht [1], Nora Schwartz [1], Madan K. Kharel [2], Felix Amissah [1], Thomas C. Dowling [1], S. Eric Nybo [1,*]

[1] Department of Pharmaceutical Sciences, College of Pharmacy, Ferris State University, Big Rapids, Michigan, USA

[2] Department of Pharmaceutical Sciences, School of Pharmacy and Health Professions, University of Maryland Eastern Shore, Princess Anne, Maryland, USA

Email: EricNybo@Ferris.edu

ABSTRACT

The anthracyclines are a family of natural products isolated from soil bacteria with over 2,000 chemical representatives. Since their discovery seventy years ago by Waksman and co-workers, anthracyclines have become one of the best-characterized anticancer chemotherapies in clinical use. The anthracyclines exhibit broad-spectrum antineoplastic activity for the treatment of a variety of solid and liquid tumors, however, their clinical use is limited by their dose-limiting cardiotoxicity. In this review article, we discuss the toxicity of the anthracyclines on several organ systems, including new insights into doxorubicin-induced cardiotoxicity. In addition, we discuss new medicinal chemistry developments in the biosynthesis of new anthracycline analogs and the synthesis of new anthracycline analogs with diminished cardiotoxicity. Lastly, we review new studies that describe the repurposing of the anthracyclines, or "upcycling" of the anthracyclines, as anti-infective agents, or drugs for niche indications. Altogether, the anthracyclines remain a mainstay in the clinic with a potential new "lease on life" due to deeper insight into the mechanism underlying their cardiotoxicity and new developments into potential new clinical indications for their use.

Keywords: Anthracycline, chemotherapy, toxicology, medicinal chemistry, biosynthesis

Introduction

Anthracycline natural products are a group of structurally diverse drugs used for the treatment of human cancers. The first anthracycline, rhodomycin A, was discovered by Nobel laureate Selman A. Waksman in 1951 from *Streptomyces griseus*. He previously discovered streptomycin in 1944 (Jones et al., 1944; Schatz et al., 2005; Shockman and Waksman, 1951). In 1964, daunomycin, also known as daunorubicin, was discovered by Arcamone et al. from *Streptomyces peucetius* isolated near the Castel del Monte in Italy (Arcamone et al., 1964). Doxorubicin, or 14-hydroxy-daunorubicin, also known as Adriamycin®, was isolated from a mutant strain of *S. peucetius* in 1969 (Arcamone et al., 1969).

During the next five decades, several other naturally occurring anthracycline analogs, such as nogalamycin, keyicin, aclacinomycin, or semi-synthetic analogs, including epirubicin, amrubicin, and idarubicin were discovered and developed (Hulst et al., 2022 (Figure 1). The anthracyclines exert their mechanism of action via intercalation into the double helix of DNA and binding to topoisomerase II (Minotti et al., 2004). Anthracyclines feature an anthraquinone pharmacophore, which undergoes redox chemistry to form reactive oxygen species (ROS) that can damage DNA and proteins (Gammella et al., 2014). This cytotoxic mechanism of action is responsible for broad-spectrum anticancer activity against leukemia, non-Hodgkin's and Hodgkin's lymphoma, ovarian cancer, thyroid cancer, breast cancer, and sarcoma (Weiss, 1992). However, the anthracyclines also exhibit dose-limiting cardiotoxicity that results in doxorubicin-induced congestive heart failure (Shan et al., 1996). This observation has motivated further drug development for less cardiotoxic anthracyclines.

Various microorganisms are developing drug resistance to frontline medications that are used to treat them, some of which include *Leishmania spp.,* HIV, SARS-CoV-2 virus, *Mycobacterium tuberculosis*, and methicillin-resistant *Staphylococcus aureus* (MRSA) (Bagre et al., 2022). Therefore, there is an urgent need to identify new anti-infective drugs or to repurpose approved medications to combat the emergence of drug-resistant pathogens. Alternatively, many orphan indications, such as Huntington's Disease, have few therapeutic options available (Frank, 2013). This necessitates the preclinical development of new classes of medications or "upcycled" medications from already-approved classes that could provide some unanticipated therapeutic benefit (Novack, 2021). Here, we define drug repurposing as "the investigation of medications that already have FDA-approved indications for therapeutic intervention in an entirely new indication" (S. Appleby and L. Cummings, n.d.). Drug repurposing offers significant time-savings and cost-savings over the development of new medications since the safety and efficacy profile of FDA-approved medications is already known (Spellicy and Hess, 2022). Presently, anthracyclines have been subject to several significant drug repurposing studies, which portends that the anthracyclines could find clinical utility outside the field of oncology. This literature review will investigate research concerning the toxicities of anthracyclines and new alternative uses for anthracyclines.

Anthracycline Toxicities

Anthracyclines are associated with several different toxicities including cardiotoxicity, mucositis, and various hematologic dyscrasias. Some of these toxicities are related to its mechanism of action, while cardiotoxicity is caused by the formation of toxic byproducts during anthracycline metabolism (Volkova and Russell, 2011) The anthracyclines work through

inhibition of topoisomerase II, which inhibits DNA replication and causes cell death. This cytotoxicity is what makes anthracyclines such powerful anticancer agents, but also results in damage to other tissues (Minotti et al., 2004) Other rapidly dividing cells like those found in the epithelium, erythrocytes, and leukocytes are also affected. The death of these cells can cause a wide array of adverse effects (Figure 1).

Liver toxicity

Doxorubicin can cause anthracycline-induced liver injury (AILI) during systemic administration (Figure 1) (Zhang et al., 2020). Liver injury is clinically assessed via serum transaminase levels that serve as an indicator of hepatocyte necrosis (Kagawa et al., n.d.). Zhang and coworkers investigated exosomal microRNAs that indicated liver toxicity in seven breast cancer patients receiving anthracycline therapy (Zhang et al., 2020). The authors discovered 30 differentially expressed microRNAs and 79 target genes associated with AILI. Genes related to AILI were characterized by the NOD-like receptor signaling pathway, the HIF-1 signaling pathway, and the FoxO signaling pathway. Interleukin-6 and superoxide dismutase-2 were found to be correlated with AILI and may mediate toxicity through activation of the immune response. Like the study by Kagawa et al., miR-1-3p seems to regulate most of the microRNAs discovered concerning AILI (Kagawa et al., n.d.).

Kidney toxicity

Anthracyclines have been found to cause acute kidney injury (AKI) in pediatric patients undergoing chemotherapy (Figure 1) (Bárdi et al., 2007). AKI is centered around damage to the proximal renal tubules, which is assessed clinically via serum creatinine, urinary N-acetyl-beta-D-glucosaminidase activity indices (NAGi), and microalbuminuria (MA). NAGi was especially found to be increased in children receiving doxorubicin, daunorubicin, idarubicin, or epirubicin treatments. Daunorubicin was especially found to contribute to decreasing proximal renal tubule function (Bárdi et al., 2007). The cardioprotective drug (dexrazoxane) was also found to provide significant benefits in protecting against AKI in patients treated with the drug as compared to patients who were not treated with dexrazoxane. Fortunately, damage to the proximal renal tubule is clinically mild and can be reversed in these patients, with only a minority of patients developing long-term tubulopathy.

Cardiotoxicity

One of the most significant limitations of anthracycline use is the risk of cardiotoxicity (Figure 1). Compared to the body of research surrounding anthracycline use in various cancers, relatively little is known about the mechanism behind cardiac myocyte damage and how to prevent it. To date, there is only one FDA-approved compound to prevent cardiotoxicity in anthracycline administration: the EDTA derivative dexrazoxane (Doroshow, 2012). The adverse cardiac effects of anthracycline use have also been found to be cumulative, both limiting the maximum dose and the overall length of treatment. Several different mechanisms to prevent cardiac damage have been offered.

One proposed mechanism is explored by Maeda et al. in a 2010 article. Amrubicin, an anthracycline compound approved for use in various lung cancers in Japan, has also been found to exhibit dose-dependent cardiotoxic effects. One of the active metabolites of amrubicin, amrubicinol, has been suggested to have up to 200 times the cytotoxicity of the parent drug (Maeda et al., 2010). The researchers in this study explored the effect of coadministration with a

second cancer treatment drug with a similar mechanism of action, irinotecan, and found that the metabolism of amrubicin to active metabolites could be manipulated. The article details the co-administration of irinotecan and amrubicin (abbreviated to CPT-11 and AMR in the manuscript, respectively) both at a concentration of 10 mg/kg in rats. Blood and bile samples were taken at set intervals, in addition to tissue samples from the kidneys, lungs, and other organs for analysis. Concentrations of amrubicin and the metabolite amrubicinol (abbreviated AMR-OH) were measured using HPLC. The amrubicinol plasma area under the curve (AUC) was found to be significantly decreased in the presence of irinotecan while concentrations of the parent compound were not significantly affected. The decrease in toxic metabolites could suggest that other compounds that inhibit specific metabolic pathways may allow for the same therapeutic anthracycline dose to have less overall toxicity.

More recently, a 2020 analysis by Cardinale et al. has reexamined the previous studies that led to the belief that cardiac damage from anthracyclines is irreversible (Cardinale et al., 2020). This article makes the argument that heart failure therapeutics at the time of those studies were limited and suggests that the recommended treatments be updated with recent advancements in mind. Many older studies focus on the treatment of patients with advanced heart failure, often occurring years after anthracycline treatment. Once developed, heart failure cannot be reversed, only treated. The treatment for anthracycline-induced cardiac damage should therefore be mainly focused first on prevention, and second on prompt measures to stop the progression of heart damage. Troponin levels can be used to assess the onset and severity of the damage, and pharmacologic treatments with heart failure medications such as angiotensin-converting enzyme (ACE) inhibitors or beta blockers can stop further progression and preserve cardiac function. The article compiles data from several other studies, all of which suggest that the effectiveness of this treatment is contingent on initial treatment. The idea that anthracycline cardiotoxicity is not necessarily permanent if caught and treated early is a crucial step towards making anthracycline treatment in other disease states feasible rather than just theoretical.

Anthracyclines have been historically thought to be cardiotoxic due to both the byproducts of their metabolism and their inhibition of topoisomerase II. Metabolism of anthracyclines was thought to cause the formation of free radicals that cause cardiomyocyte death (Minotti et al., 2004). The formation of free radicals was hypothesized to occur via the Fe^{2+}-mediated Fenton reaction (Gammella et al., 2014). Notably, Ichikawa et al. demonstrated that the accumulation of iron occurs after doxorubicin treatment in the mitochondria of isolated cardiomyocytes. (Ichikawa et al., 2014). To provide more support for this hypothesis, the authors overexpressed the *abcb8* gene product, which encodes a mitochondrial iron export protein, *in vitro* and *in vivo* mouse models. The result of *abcb8* overexpression was a decrease in mitochondrial iron concentration, a decrease in cellular ROS concentration, and protection against doxorubicin-induced cardiomyopathy. Furthermore, the authors demonstrated that co-administration of doxorubicin with dexrazoxane, a drug that can mitigate the effects of doxorubicin-induced cardiotoxicity, results in a decrease in mitochondrial iron levels and reverses doxorubicin-induced damage. Lastly, hearts from patients with doxorubicin-induced cardiomyopathy exhibited higher mitochondrial iron levels than hearts from patients with other cardiomyopathies or normal cardiac physiology. The mechanism of action for dexrazoxane was originally inferred to be an iron chelator. However, it may also play a role as a competitive inhibitor of topoisomerase II (see below) (Swain et al., 1997).

The formation of reactive oxygen species (ROS) occurs in the mitochondria of cardiomyocytes. Here, the anthracycline is reduced by NADH dehydrogenase, and a semiquinone radical is formed. This semiquinone radical then reacts with free oxygen which generates a superoxide radical. The second pathway of free radical formation may be due to the formation of anthracycline-iron complexes. These complexes can catalyze the transformation of hydrogen peroxide to toxic hydroxyl radicals (Gammella et al., 2014). The superoxide free radicals increase the permeability of the mitochondrial membrane by both directly binding to phospholipids and activating enzymes that facilitate the opening of the mitochondrial permeability transition pore. This causes cytochrome C to leak out of the mitochondria and the cell to undergo apoptosis. This is known as ferroptosis, or iron-dependent programmatic cell death (Javadov et al., 2022; Li et al., n.d.). However, considering recent discoveries, the iron-mediated formation of ROS may be a corollary pathology instead of the primary etiology of cardiotoxicity.

More recently, Zhang and coworkers identified the molecular basis for doxorubicin-induced cardiotoxicity as the result of its binding to topoisomerase-IIβ (Top2b) in cardiomyocytes (Zhang et al., 2012). Zhang et al. demonstrated that deletion of the *Top2b* gene, which encodes the Top2b isoenzyme, protected the heart cells from doxorubicin-induced DNA double-strand breaks. In turn, this prevented the subsequent mitochondrial dysfunction and formation of reactive oxygen species that are hallmarks of the cardiotoxicity pathology. More importantly, the deletion of *Top2b* prevented mice from progressing to anthracycline-induced progressive heart failure (Zhang et al., 2012). These results explain in large part the effectiveness of dexrazoxane (Zinecard®) in preventing doxorubicin-induced cardiotoxicity since it has been hypothesized to deplete expression of topoisomerase-IIα and -IIβ isoforms and may compete directly with doxorubicin for binding to topoisomerase-IIβ (Deng et al., 2014; Sobek and Boege, 2014).

Anthracycline toxicity presents itself as congestive heart failure. Patients being treated with anthracyclines may develop left ventricular dysfunction and have a reduced left ventricular ejection fraction (LVEF). Ventricular ejection fraction may also be lowered in patients who do not show any other symptoms of heart failure (Jeyaprakash et al., 2021). Cardiomyopathy may develop within months, or it may not show until many years after treatment. Pediatric patients often do not develop heart failure for many years after receiving the medication (Kremer et al., 2002). Anthracyclines exhibit dose-dependent toxicity in the heart which limits the maximum lifetime dose that a patient can receive. For instance, doxorubicin has a maximum dose of 450-550 mg/m^2 (Henriksen, 2018). A maximum lifetime cumulative dose (MLCD) of 550 mg/m^2 is associated with a 65% risk of developing heart failure, while an MLCD of 350 mg/m^2 has an 18% risk. Notably, the FDA approval of epirubicin (Ellence®) in 2006 resulted in a new anthracycline analog with significantly diminished cardiotoxicity (Hurteloup et al., 1983; Villani et al., 1983). Epirubicin, or 4'-epi-doxorubicin, is the 7-*O*-4'-*epi*-L-daunosamine (L-acosamine) derivative of doxorubicin (Figure 2). Epirubicin features an approximately 2-fold increase in the maximum lifetime cumulative dose of 900 mg/m^2 (Ryberg et al., 1998). A clinical trial including four hundred sixty-nine anthracycline naïve patients with metastatic breast cancer evaluated dose intensity, cumulative dose, and scheduling of epirubicin on the development of congestive heart failure (Ryberg et al., 1998). At a maximum cumulative dose of 900 mg/m^2, 34 (7.2%) patients developed congestive heart failure, whereas at a maximum cumulative dose of 1,000 mg/m^2 15% of patients developed congestive heart failure. This sparked interest in the development of glycodiversified anthracyclines with substitutions to the appended 7-*O*-glycoside

functionality to identify new lead drug candidates for the treatment of cancers with lessened cardiotoxicity.

Gastrointestinal toxicity

Anthracyclines cause gastrointestinal (GI) mucositis by killing the epithelial cells on the surfaces of the GI tract (Figure 1). Damage to these cells that make up the mucosal lining can cause oral ulcers, gastritis, nausea, and diarrhea. Both the inhibition of DNA synthesis and the formation of reactive oxygen species are believed to be what cause mucositis (Kwon, 2016). Oral mucositis can cause discomfort and may even delay chemotherapy treatment in severe cases. Oral mucositis can also make a patient more susceptible to bacterial and fungal infections in the mouth. Mucositis of the stomach and intestinal tract can cause nausea, diarrhea, and even malabsorption. These toxicities resolve with the discontinuation of anthracycline (Kwon, 2016).

Bone marrow suppression

Anthracyclines inhibit the replication of hematopoietic stem cells through the inhibition of DNA synthesis (Figure 1). This can cause predictable neutropenia and can also cause thrombocytopenia. Anthracycline-induced neutropenia usually occurs between 10 and 14 days after administration of the drug (Moore, 2016). Neutropenia increases a patient's risk of developing systemic bacterial infections that can lead to sepsis and death. Patients may need to be treated with colony-stimulating factors to support neutrophil formation. The neutrophil count typically returns to normal within 4 weeks after discontinuation (Moore, 2016).

Anthracyclines may also cause thrombocytopenia through both bone marrow suppression, and by being directly toxic to mature platelets. *In vitro* studies have shown that the generation of reactive oxygen species leads to apoptosis and cell lysis of platelets (Kim et al., 2009). Typically, thrombocytopenia is seen within 7 to 10 days after administration of chemotherapy and can return to normal within 28 to 35 days after discontinuation of treatment (Kuter, 2015). Thrombocytopenia can increase the patient's risk for bleeding and the patient may require treatment with transfusions.

Molecular basis for anthracycline toxicity

Recently, several new reports from the Prof. Sjaak van Neefjes group at Leiden University in the Netherlands have illuminated important structure-activity relationships that dissect the beneficial antineoplastic activity of the anthracyclines from the cardiotoxic activity. In these studies, the group has synthesized several new anthracycline analogs featuring *N,N*-dimethyl-aminosugars that exhibit beneficial histone eviction activity while minimizing topoisomerase-IIβ inhibition and cardiotoxicity. Van der Zanden et al. described the various anticancer activities and adverse effects of doxorubicin, including the inhibition or poisoning of topoisomerase-II, which ultimately prevents topoisomerase II from re-ligating broken DNA (van der Zanden et al., 2021). By studying the adverse effects of anthracyclines, new strategies and techniques were developed to decrease these effects. For example, novel strategies for tumor-specific drug delivery systems such as nanoparticle-encapsulated liposomal doxorubicin (LD) and pegylated LD (PLD) doxorubicin have been shown to reduce anthracycline-induced toxicity. Based on the current understanding of anthracycline activity and effects, the authors asserted that the development of new anthracyclines should focus on reducing DNA-damaging activity from chromatin-damaging activity (van der Zanden et al., 2021).

Qiao et. al. showed that anthracycline-induced cardiotoxicity requires a combination of DNA damage and chromatin damage (Qiao et al., 2020). Anthracyclines that have either cellular activity alone (aclarubicin and etoposide) exhibit robust anticancer activity but fail to induce cardiotoxicity in mice and human cardiac microtissues. The cardiotoxicity of doxorubicin can be prevented by chemically separating these two activities. Variants that only induce histone eviction maintain similar anticancer potency, challenging the concept that anthracyclines work primarily by inducing DNA damage and implying that chromatin damage also constitutes a major cytotoxic mechanism of anthracyclines. Therefore, anthracycline variants targeting primarily chromatin damage may be beneficial in the prolonged treatment of cancer patients (Qiao et al., 2020).

Wander et. al. demonstrated some key differences in the mechanisms of doxorubicin and aclarubicin (Wander et al., 2020). Doxorubicin's mechanisms of action include inducing double-stranded DNA breaks by poisoning topoisomerase IIA and a newly discovered secondary mechanism, histone eviction from chromatin. Aclarubicin's mechanism of action only involves histone eviction. Aclarubicin is less cardiotoxic and equally effective as doxorubicin. This suggests that the combination of DNA damage and histone eviction displayed by doxorubicin could be to blame for severe cardiotoxicity and secondary tumor formation. Generation and testing of doxorubicin and aclarubicin hybrid structures differing in the tetracyclic aglycon, sugar moiety, and *N*-alkylation pattern lead to the conclusion that structures with *N, N*-dimethylation of the amino sugar induce little to no DNA breakage, yet remain cytotoxic. This indicates that to generate more potent, less cardiotoxic anthracycline derivatives, it is imperative to consider the addition of an *N, N*-dimethylated sugar to the aglycon anthracycline structures.

Wander et al. further investigated the possible structural basis for the differing mechanisms of action of doxorubicin and aclarubicin (Wander et al., 2021). The structures of both anthracyclines are similar, sharing an anthraquinone core and a basic aminosugar, but differ in the oxidation patterns on the aglycon, the number of sugars attached to the aglycon, and the absence or presence of *N, N*-dimethylation on the amino sugar. A study of a small library of doxorubicin/aclarubicin hybrid molecules revealed that the oxidation pattern on the aglycon did not have a significant effect on cytotoxicity, histone eviction, or topoisomerase II inhibition. The number of sugars attached to the aglycon also did not have a significant effect on any parameter. However, *N, N*-dimethylation of the amino sugar led to improved histone eviction and cytotoxicity of the compound compared to an anthracycline with an unmethylated primary amine. This study further reinforced the conclusion that to synthesize more potent anthracycline analogs with minimal cardiotoxicity, it would be prudent to consider adding an *N, N*-dimethylated sugar, like that seen in aclarubicin, appended to the aglycon rather than appending a sugar with a primary amine like that seen in doxorubicin (Wander et al., 2020).

Biosynthetic studies on nogalamycin-type anthracyclines and "upcycling" for new indications

Anthracyclines are a group of naturally occurring antibiotics that are derived from *Streptomyces* bacteria. As natural products, anthracyclines have attracted considerable interest over the last three decades concerning the elucidation of their biosynthesis and thorough investigation of their biosynthetic enzymes. Biosynthetic studies reveal the elaborate

biochemistry of the anthracycline pathway enzymes, but biosynthetic studies can also result in the generation of new anthracycline analogs through a process known as “combinatorial biosynthesis” (Weissman and Leadlay, 2005). Combinatorial biosynthesis has been defined as “the application of genetic engineering to modify biosynthetic pathways to natural products to produce new and altered structures using nature’s biosynthetic machinery” (Floss, 2006).

For example, Madduri et al. engineered *Streptomyces peucetius* to produce the clinically advantageous analog epirubicin instead of the more cardiotoxic natural metabolite doxorubicin (Madduri et al., 1998) (Figure 2). To accomplish this, Madduri and coworkers knocked out the endogenous gene *dnrV* from *Streptomyces peucetius* is responsible for the 4’-ketoreduction step to furnish TDP-L-daunosamine, which resulted in the abrogation of the biosynthesis of doxorubicin. The authors then successfully modified the biosynthetic pathway via heterologous expression of 4’-ketoreductase genes *aveBIV* or *eryBIV* from the avermectin and erythromycin pathways, respectively, redirecting metabolic flux towards the biosynthesis of epirubicin (Madduri et al., 1998).

While many biosynthetic studies into the anthracyclines are motivated by the development of new anticancer drugs, other recent studies have tested the possibility of using nogalamycin and keyicin for Huntington’s disease, malaria, and tuberculosis, respectively (Figure 2). Keyicin is an antibiotic that is produced from the co-culturing of two bacteria, *Rhodococcus* and *Micromonospora.* Adnani and coworkers initially hypothesized that direct contact between the microbial cells was a prerequisite to eliciting the production of keyicin (Adnani et al., 2017). The authors devised an ingenious system in which a membrane separated two bacterial cultures but allowed for the diffusion of small molecules/metabolites across the membrane (Adnani et al., 2017). This experiment suggested that a diffusible chemical signal must be transported from one bacterial species to another across the membrane to induce expression of the keyicin cluster.

In another study conducted by Acharya and coworkers, they discovered that a small molecule signal from *Rhodococcus* stimulates *Micromonospor*a to produce Keyicin (Acharya et al., 2019). They used genomics, transcriptomics, and metabolomics methods to show how *Rhodococcus* and *Micromonospora* interactions produce keyicin. The study showed that co-culturing these two bacteria activates silent biosynthetic gene clusters. Importantly, the supernatant from *Rhodococcus* fermentations could stimulate *Micromonospora* to produce keyicin (Acharya et al., 2019). Furthermore, this experiment also demonstrated that *Micromonospora* responds to a small molecular signal from *Rhodococcus*, which describes a collaborative evolution between the two bacteria. Interestingly, keyicin exhibited antibacterial activity against gram-positive bacteria, including *Mycobacterium spp.,* methicillin-sensitive *Staphylococcus aureus*, and *Rhodococcus spp.* (Adnani et al., 2017). This is especially important since *Mycobacterium tuberculosis* is the etiologic agent for tuberculosis, a contagious respiratory infection that is characterized by the emergence of multidrug-resistant (MDR) and extensively drug-resistant (XDR) tuberculosis strains (Seung et al., 2015). Furthermore, methicillin-resistant *Staphylococcus aureus* (MRSA) is another gram-positive multidrug-resistant bacterium for which new antibiotics are urgently needed (Hassoun et al., 2017). Keyicin could be a promising new antibacterial lead compound, especially since its mechanism of action, unlike other anthracyclines, does not appear to involve the induction of double-stranded DNA breaks (Adnani et al., 2017).

In comparison to other anthracyclines, which exert their anticancer mechanism of action via inhibition of topoisomerase II, nogalamycin is structurally related to keyicin and is a topoisomerase I poison (Figure 2) (Sim et al., 2000). One promising new "upcycled" indication for nogalamycin could be a potential therapy for Huntington's disease. Huntington's Disease is a progressive brain disorder caused by a defective huntingtin (*htt)* gene on chromosome four that has an onset between the ages of 30 and 50 (Roos, 2010). Huntington's Disease is characterized by gradual, irreversible degradation of all psychomotor processes. Although there are drugs that can help with the symptoms of Huntington's disease, there are currently no drugs that can treat this disease. A study conducted by Lee and coworkers compared B6CBA-R6/2 (CAG 120 +/- 5) mice (i.e. transgenic mice that display a neurological phenotype that closely resembles Huntington's Disease) that were treated with nogalamycin at five weeks of age and those that were not (Lee et al., 2017). The front and hindleg movements were carefully documented to assess smooth motor control. Nogalamycin decreased H3K9me3 methylation and decreased transcription of *Setdb1/Eset*, which the authors hypothesized were genes implicated in Huntington's Disease progression. Nogalamycin was also found to improve behavioral irregularities and slow neuropathological progression, effectively extending the lifespan of the B6CBA-R6/2 mice. Overall, this study demonstrated that the role of nogalamycin lies in remodeling heterochromatin structure and alteration in histone modification, which could play significant roles in slowing the effects of Huntington's Disease.

Additionally, nogalamycin has been identified as a target compound for the treatment of malaria. Malaria is a parasitic disease caused by the genus *Plasmodium. Plasmodium* species that naturally infect humans and cause malaria in most parts of the world are *P. falciparum*, *P. vivax*, *P. malariae*, and *P. ovale. Of these, P. falciparum* malaria is the most prevalent and results in the most severe infection. *P. falciparum* is transmitted via *Anopheles* spp. mosquitoes (White, 2004). Malaria is thought to be the most prevalent and pernicious infectious disease in human history, with Whitfield estimating that malaria has killed half of all humans who have ever lived (Whitfield, 2002). Malaria has become resistant to the normal treatment regimen of chloroquine, sulfadoxine, and pyrimethamine (White, 2004). Tarique and colleagues hypothesized that PfMLH and PfUrD are key components in the survival of *Plasmodium falciparum*, the parasite that causes malaria (Tarique et al., 2017). The study showed that nogalamycin decreases the activity of ATPase of PfMLH by inhibiting its binding with the DNA. PfMLH is needed for the unwinding properties of PfUrD, the DNA helicase. Nogalamycin demonstrated inhibition of parasitic growth by 60% - 70% over 72 hours. This study showed nogalamycin's potential for development as an antiparasitic, due to its unique mechanism of action targeting proteins involved in DNA replication.

As a result, nogalamycin and keyicin have been identified as promising repurposed drug candidates for Huntington's, malaria, *Staphylococcus aureus* infection, and tuberculosis. Huntington's Disease is a sudden, irreversible neurodegenerative disorder, whereas malaria, tuberculosis, and methicillin-resistant *Staphylococcus aureus* infection are multidrug-resistant infections that require the development of new anti-infective drugs. Nogalamycin and keyicin are two promising anthracycline candidates that could fill each of these distinctive therapeutic niche areas and are worthy of further investigation.

Anthracyclines as antiviral and antiparasitic drugs

A study by Chandra et al. investigated potential inhibitors of SARS-COV-2 endoribonuclease (EndoU) using high-throughput screening of FDA-approved medications to

identify drug candidates that could be repurposed for the treatment of SARS-CoV-2 (Chandra et al., 2021). NSP15, an Indo viral RNA uridylate-specific endoribonuclease (NendoU), is a protein found in COVID-19 which plays a crucial role in the evasion of host cell defense mechanisms. This study used molecular dynamic simulations to see what medications would target the EndoU on COVID-19. The top 5 molecules that could bind with EndoU in the simulation study were dihydroergotamine, glisoxepide, idarubicin, ergotamine, and tasosartan. It was found that the complexes of EndoU with idarubicin, glisoxepide, and tasosartan had fewer motions during the ligand binding, indicating that they had stabilizing effects on the enzyme. Glisoxepide and idarubicin had binding energies of -141 kJ/mol and -134 kJ/mol respectively as well as hydrogen bonding interactions of 8 and 7. EndoU is a key factor in the mechanism for host invasion of COVID-19 and is a good target for drug therapies. Glisoxepide and idarubicin showed a good affinity for EndoU, but glisoxepide was more potent with an IC_{50} being 9.3 micromolar compared to idarubicin's IC_{50} being 30 micromolar. This study warrants further testing of these medications *in vitro* and *in vivo* for their efficacy against the COVID-19 virus (Chandra et al., 2021). This study, along with previous studies discussed, shows the importance of utilizing in silico methods to find alternative uses for medications, specifically focusing on anthracyclines. Finding alternative uses for existing medications could speed up the approval process as efficacy and safety have already been shown for other disease states. *In vitro* and *in vivo* studies would help determine the true efficacy and safety of the use of anthracyclines in disease states other than cancer.

HIV treatments have come a long way since the discovery of the virus in the early 80s, allowing patients on the right combination of drugs to reach an undetectable viral load in the blood, minimizing transmissibility and virtually eliminating AIDS. That, however, is completely contingent on the patient being able to afford all the drugs in their combination treatment, which can be especially difficult in poor populations, where infection rates can be the highest. A cure has yet to be found, but several mechanisms and drug candidates have been proposed, including a study by Shishido et al. in 2012 (Shishido et al., 2012). A potential cure for HIV infection would require the elimination of the reservoir of infected T-cells. HIV-infected T cells cannot be distinguished from non-infected cells via cell surface markers, so a different method must be utilized. Reactivating the latent infection in T-cells forces the HIV to reproduce, killing the cell, and bringing the virus into the blood to where it can then be eliminated. Most therapeutic research in the past for this mechanism of treatment has investigated monotherapies, which have not shown much efficacy to date. A drug combination would both have to lower the threshold for reactivation of the infection and then activate the reinfection. Aclacinomycin (aclarubicin) and dactinomycin were identified by a high-throughput drug screening assay as compounds that could achieve the synergistic effect desired to force the infected T-cell population from the latent to the lytic phase. The study suggests that the mechanism behind this reactivation is related to the cell-differentiating properties of both drugs rather than the DNA intercalation mechanism used in cancer treatment. This study utilized flow cytometry to detect GFP-tagged viral sequences in the cell cultures to quantitively determine the level of HIV transcription, which directly correlates to the rate of reactivation of viral infection. Interestingly, the data from this study also suggests that the combination of aclarubicin and dactinomycin does not increase infection rates of healthy cells after reactivation. Cell viability was preserved in the concentrations studied. The study concludes with a recommendation that the next logical step would be to screen for other activating agents that could work in conjunction with identified priming agents.

A case report from Gow et al. followed a 60-year-old man with HIV who also had visceral leishmaniasis (VL) co-infection that became resistant to other anti-leishmania treatments. The man was diagnosed with HIV in 1985 and was started on Zidovudine monotherapy, then was stepped up to a 3-drug antiretroviral therapy in 1996 (Gow et al., 2015). In March of 1997, the man was diagnosed with VL and treated for over 8 weeks with improvement however he had a VL relapse in July of 1997 and continued to have the infection for the next 15 years despite treatment. VL commonly happens in HIV patients that live in areas where the species is common. Treatment of the infection is important because co-infection with HIV can lead to death. Doxorubicin is an anthracycline that has been shown to have potent activity against *Leishmania donovani*, a type of VL, in both *in vitro* studies and animal models (Kansal et al., 2012; Sett et al., 1992). For his treatment, the clinicians decided to use liposomal daunorubicin to treat the infection because of personal and institutional experience in the use of the agent for the treatment of Kaposi's sarcoma in patients with HIV infection. After 6 doses of the medication over three months, there was no evidence of response to the treatment. The clinicians speculated the treatment did not work because inadequate concentrations of daunorubicin were used, in addition to it being less potent than doxorubicin. Their patient also had a different strain of VL, *L. infantum*, that had not been studied previously. They conclude that the previous treatments to treat the infection may have caused it to become resistant to medications (Gow et al., 2015). Although the above study does not demonstrate that daunorubicin was an effective treatment, the study was done on one person with a drug-resistant strain of bacteria. While it can be important to look at individual case situations, clinical trials across various groups of people should be performed before discrediting a possible treatment option. It must also be noted that this study involved a patient that had already developed drug resistance and that daunorubicin was being used as a last resort to see if the patient could get better.

The studies above demonstrate the importance of finding alternative uses for anthracyclines. High throughput screening has helped identify approved FDA drugs that could be used in the treatment of COVID-19, leishmania, and HIV. While the results described are promising for repurposing the anthracyclines, further drug development *in vitro* and *in vivo* will are required to advance anthracyclines as alternative therapies for antiviral and antiparasitic disease states. Since antiretroviral treatments are often continued for life, a less toxic anthracycline would have to be developed for this purpose to avoid induction of heart failure.

Anthracyclines as novel treatments for bacterial infections

Myriad studies have focused on alternative uses for anthracyclines, including their potential use as antimicrobial agents. A study by Gumbo et al. investigated the repurposing of medications for the treatment of *Mycobacterium abscessus* (Gumbo et al., 2020). They were able to identify 16 drug candidates to determine their MIC and efficacy in treating *Mycobacterium abscessus*. Daunorubicin was one of the 16 identified, but the researchers did not determine the MIC. The authors determined that daunorubicin exhibited a bactericidal dose-response relationship, and the drug effectively killed *Mycobacterium abscessus*. However, the MIC of daunorubicin for *M. abscessus* is yet to be established (Gumbo et al., 2020).

A study by Feng et al. looked at how well daunorubicin, daptomycin, and mitomycin C were able to eradicate biofilm structures of *Borrelia burgdorferi* as monotherapy and in combination with doxycycline and cefuroxime (Feng et al., 2016). *B. burgdorferi* can cause

Lyme disease, and even after treatment, some patients still experience symptoms, which are thought to be caused by persister microorganisms that develop resistance. The persisters have an altered RNA profile which allows them to tolerate treatment by current regimens. The study revealed daunomycin had the highest activity against the stationary phase cultures at all concentrations they tested (5, 10, 15, and 20 μM). When used in combination with doxycycline and cefuroxime, daunomycin had the best anti-persister activity, with only 12% of the remaining cells viable. To validate the results, they washed the cultures to remove the drug and incubated them in a fresh medium for 7 and 21 days. Daptomycin and daunorubicin treatments did not show any regrowth of bacteria at both 7 and 21 days. Their study showed daunorubicin and daptomycin have significant activity against *B. burgdorferi* by themselves and in combination with doxycycline and cefuroxime (Feng et al., 2016)). Future studies using rodent models could provide further insights into the potential application of these combinations.

She et al. specifically examined the "upcycled" use of idarubicin as an antibacterial agent against methicillin-resistant *Staphylococcus aureus* (MRSA) *in vitro* and *in vivo* (She et al., 2020). The researchers performed an antimicrobial susceptibility test along with various assays including disc diffusion, bactericidal pharmacokinetics, persister killing, biofilm inhibition, biofilm eradication, and enzyme inhibition. When compared to vancomycin in the biofilm inhibition assay, idarubicin had stronger inhibitory effects at a concentration of 2 ug/mL. Idarubicin produced a synergistic effect on growth inhibition when combined with fosfomycin. Sub-MIC levels of idarubicin when combined with 2-4 ug/mL fosfomycin could eliminate almost 100% of live bacteria within 6 to 8 hours. This highlights the potential application of idarubicin against MRSA by itself and in combination with fosfomycin (She et al., 2020). It is also noteworthy that the study of the synergistic activity that combination therapies exert on pathogens is severely under-investigated. Potential future studies could include the study of the fosfomycin/idarubicin combination in animal models. Alternatively, other anthracyclines, such as daunorubicin or nogalamycin, could be investigated for synergistic activities with doxycycline and cefuroxime to see if lower doses could still exhibit bactericidal activity. Achieving lower bactericidal dosages in an animal model remains one key objective. However, such a study would also have to account for potential drug-drug interactions between the medications.

Trombetta, and coworkers developed a high throughput screening (HTS) assay utilizing adenylate kinase to identify drug candidates useful for treating *Staphylococcus aureus* infections (Trombetta et al., 2018). First, the authors defined "small colony variants" as a unique morphological phenotype of *S. aureus* exemplified by abnormal growth patterns and drug resistance. The adenylate kinase assay identified medications that showed activity against the small colony variants by looking for an increase in adenylate kinase signal signifying bactericidal activity. In the adenylate kinase high throughput screen, four medications exhibited antibacterial activity against small colony variant *S. aureus*, which included daunorubicin, ketoconazole, sitafloxacin, and rifapentine. The researchers performed various tests to see how efficacious daunorubicin was in killing *S. aureus*, including antimicrobial susceptibility testing, biofilm susceptibility testing, and cytotoxicity testing. Daunorubicin showed significant potency against the bacteria but also showed cytotoxicity when used in mammalian serum (Trombetta et al., 2018).

Balasubramani, et al. developed an *in-silico* ligand binding model to identify potential drugs to treat *Mycobacterium tuberculosis* (Balasubramani et al., 2020). They docked the structures of 13,450 drug compounds in a protein model of *M. tuberculosis* DNA gyrase B

(GyrB) to determine drug fit. Doxorubicin and idarubicin were both identified as possible inhibitors of GyrB. Next, the authors subjected the anthracyclines to a DNA supercoiling assay using to analyze enzyme inhibition to assess the degree of enzyme inhibition against the control drug novobiocin. The authors also incorporated an ATPase binding assay into the study. Doxorubicin and daunorubicin exhibit affinity for GyrB and activity against *M. tuberculosis*, however epirubicin and echinacoside evinced greater activity (Balasubramani et al., 2020). In another report, Gajadeera and coworkers investigated the role of idarubicin and daunorubicin in inhibiting *M. tuberculosis* DnaG primase in a novel primase-pyrophosphatase assay (Gajadeera et al., 2015). Idarubicin and daunorubicin exhibited low-μM inhibition of DnaG and *M. tuberculosis*, further reinforcing the potential for anthracyclines to be developed as new antibacterial agents for the treatment of MDR or XDR.

Conclusions

The anthracyclines have been clinically used for nearly seven decades for the treatment of human cancers and are inhibited by dose-limiting adverse drug reactions. However, the anthracyclines may be on the verge of a significant "upcycling" via their repurposing for other indications. New studies into the structure-activity-relationships (SARs) of anthracycline cardiotoxicity are promising for the development of more efficacious and less toxic alternatives. The emergence of multidrug-resistant bacterial pathogens, such as *Mycobacterium tuberculosis*, *Borellia burgdorferi*, and *Staphylococcus aureus* pose significant challenges that could be addressed via the development of low-dose anthracycline anti-infective drugs. The identification of new drug targets in *M. tuberculosis*, such as DNA gyrase GyrB and DNA primase DnaG, provides new strategies for drugging this pathogen. Parasitic infections, such as leishmaniasis, and viral infections, such as HIV and SARS-CoV-2, serve as additional examples of the disease burden of communicable diseases. In addition, orphan indications such as Huntington's Disease have a significant window of opportunity for the development of new therapies. Altogether, the anthracyclines are privileged molecular scaffolds that could serve as the basis for entirely new pharmacological interventions.

Acknowledgments

Research reported in this publication was supported by the National Cancer Institute of the National Institutes of Health under award number R15CA252830 (S.E.N.) and supported in part by the National Science Foundation under Grant No. ENG-2015951 (S.E.N.).

Figures

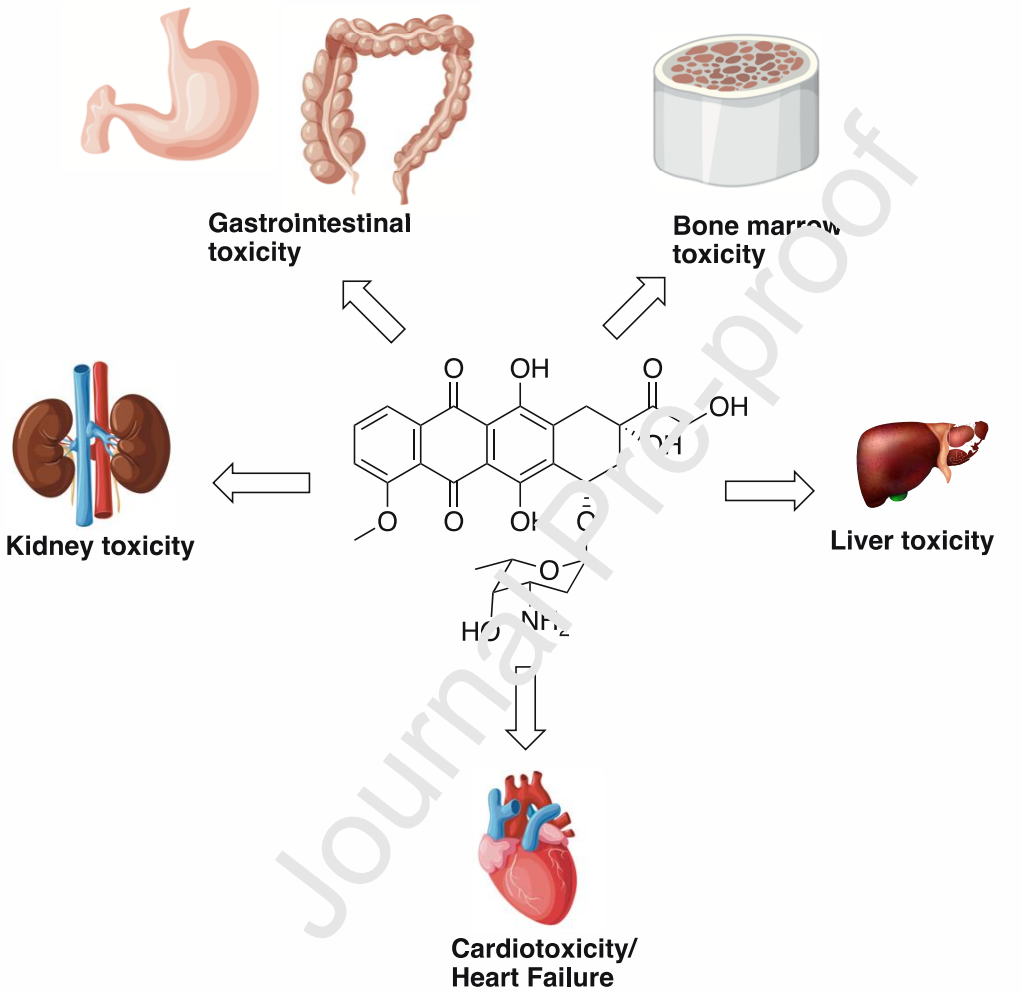

Figure 1 Organ toxicities caused by systemic administration of doxorubicin.

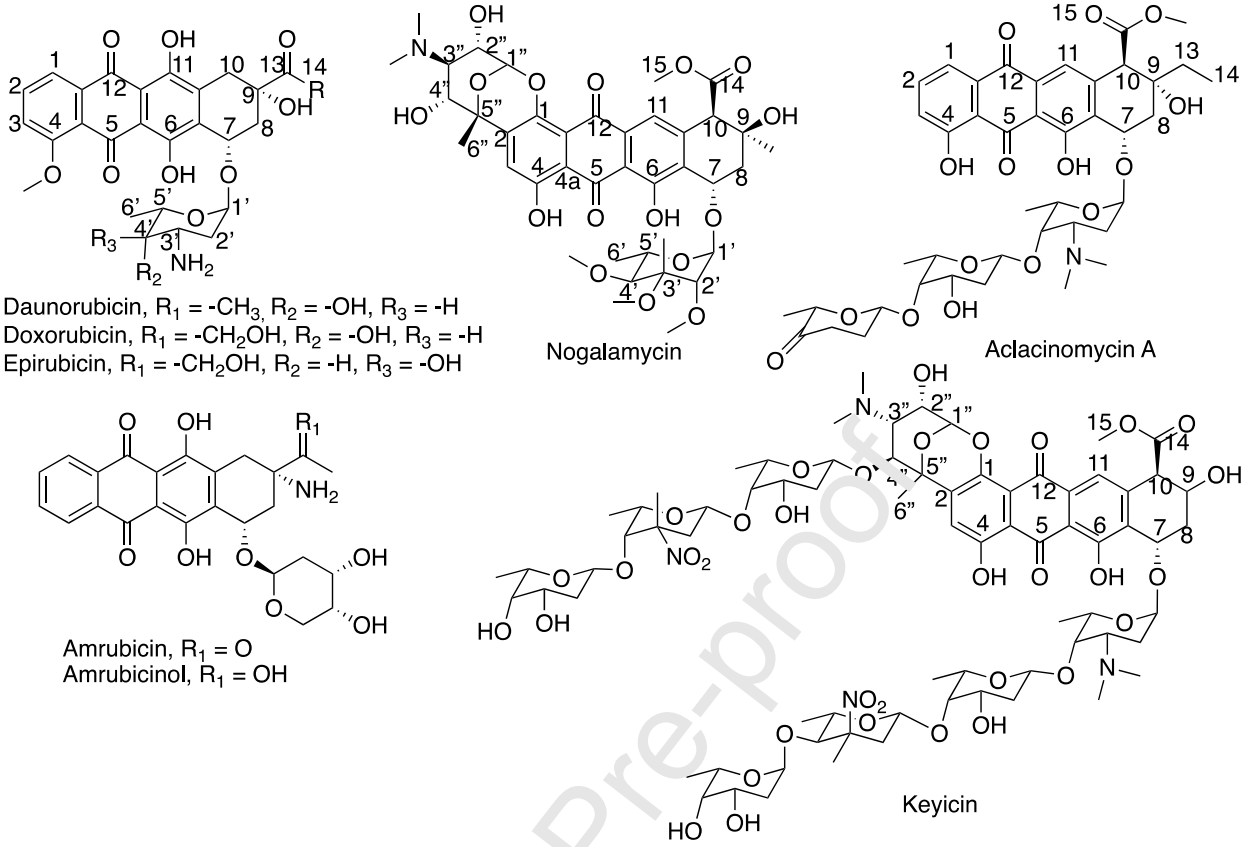

Figure 2 Structures of anthracyclines discussed in this manuscript.

Table 1 Summary of new "upcycled" indications for anthracyclines.

Drug	Indication	References
Aclacinomycin	Antiviral, Human immunodeficiency virus	(Shishido et al., 2012)
Daunorubicin/doxorubicin	Visceral Leishmaniasis	(Gow et al., 2015; Kansal et al., 2012; Sett et al., 1992)
Daunorubicin	Antituberculosis, *M. abscessus*	(Gajadeera et al., 2015; Gumbo et al., 2020)
Daunorubicin	Antibacterial, MRSA	(Trombetta et al., 2018)
Daunorubicin	Lyme disease, *B. burgdorferi*	(Feng et al., 2016, 2015)
Doxorubicin	Antituberculosis, *M. tuberculosis*	(Balasubramani et al., 2020)
Idarubicin	Antituberculosis, *M. tuberculosis*	(Balasubramani et al., 2020; Gajadeera et al., 2015)
Idarubicin	Antibacterial, MRSA	(She et al., 2020)
Idarubicin	SARS-CoV-2	Chandra et al., 2021)
Keyicin	Antibacterial, MRSA, *M. tuberculosis*	(Adhani et al., 2017)
Nogalamycin	Huntington's Disease	(Lee et al., 2017; Roos, 2010; Sim et al., 2000)
Nogalamycin	Malaria, *P. falciparum*	(Tarique et al., 2017; White, 2004; Whitfield, 2002)

References

Acharya, D., Miller, I., Cui, Y., Braun, D.R., Berres, M.E., Styles, M.J., Li, L., Kwan, J., Rajski, S.R., Blackwell, H.E., Bugni, T.S., 2019. Omics Technologies to Understand Activation of a Biosynthetic Gene Cluster in Micromonospora sp. WMMB235: Deciphering Keyicin Biosynthesis. ACS Chem Biol 14, 1260–1270. https://doi.org/10.1021/acschembio.9b00223

Adnani, N., Chevrette, M.G., Adibhatla, S.N., Zhang, F., Yu, Q., Braun, D.R., Nelson, J., Simpkins, S.W., McDonald, B.R., Myers, C.L., Piotrowski, J.S., Thompson, C.J., Currie, C.R., Li, L., Rajski, S.R., Bugni, T.S., 2017. Coculture of Marine Invertebrate-Associated Bacteria and Interdisciplinary Technologies Enable Biosynthesis and Discovery of a New Antibiotic, Keyicin. ACS Chem Biol acschembio.7b00688. https://doi.org/10.1021/acschembio.7b00688

Arcamone, F., Franceschi, G., Orezzi, P., Cassinelli, G., Barbieri, Wanda., Mondelli, Rosanna., 1964. Daunomycin. I. The Structure of Daunomycinone. J Am Chem Soc 86, 5334–5335. https://doi.org/10.1021/ja01077a059

Arcamone, F., Franceschi, G., Penco, S., Selva, a, 1969. Adriamycin (14-hydroxydaunomycin), a novel antitumor antibiotic. Tetrahedron Lett 40, 1007–1010. https://doi.org/10.1016/S0040-4039(01)97723-8

Bagre, A., Patel, P.R., Jain, K., Naqvi, S., 2022. Emerging concerns of infectious diseases and drug delivery challenges. Nanotheranostics for Treatment and Diagnosis of Infectious Diseases 1–23. https://doi.org/10.1016/B978-0-323-91201-3.00013-X

Balasubramani, G.L., Rajput, R., Gupta, M., Dahiya, P., Thakur, J.K., Bhatnagar, R., Grover, A., 2020. Structure-based drug repurposing to inhibit the DNA gyrase of Mycobacterium tuberculosis. Biochemical Journal 477, 4167–4190. https://doi.org/10.1042/BCJ20200462

Bárdi, E., Bobok, I., Oláh, A. v., Kappelmayer, J., Kiss, C., 2007. Anthracycline antibiotics induce acute renal tubular toxicity in children with cancer. Pathol Oncol Res 13, 249–253. https://doi.org/10.1007/BF02893506

Cardinale, D., Iacopo, F., Cipolla, C.M., 2020. Cardiotoxicity of Anthracyclines. Front Cardiovasc Med 7, 26. https://doi.org/10.3389/FCVM.2020.00026

Chandra, A., Gurjar, V., Qamar, I., Singh, N., 2021. Identification of potential inhibitors of SARS-COV-2 endoribonuclease (EndoU) from FDA approved drugs: a drug repurposing approach to find therapeutics for COVID-19. J Biomol Struct Dyn 39, 4201–4211. https://doi.org/10.1080/07391102.2020.1775127

Deng, S., Yan, T., Jendrny, C., Nemecek, A., Vincetic, M., Gödtel-Armbrust, U., Wojnowski, L., 2014. Dexrazoxane may prevent doxorubicin-induced DNA damage via depleting both Topoisomerase II isoforms. https://doi.org/10.1186/1471-2407-14-842

Doroshow, J.H., 2012. Dexrazoxane for the Prevention of Cardiac Toxicity and Treatment of Extravasation Injury from the Anthracycline Antibiotics. Curr Pharm Biotechnol 13, 1949. https://doi.org/10.2174/138920112802273245

Feng, J., Shi, W., Zhang, S., Zhang, Y., 2015. Identification of new compounds with high activity against stationary phase Borrelia burgdorferi from the NCI compound collection. Emerg Microbes Infect 4, e31. https://doi.org/10.1038/emi.2015.31

Feng, J., Weitner, M., Shi, W., Zhang, S., Zhang, Y., 2016. Eradication of Biofilm-Like Microcolony Structures of Borrelia burgdorferi by Daunomycin and Daptomycin but not Mitomycin C in Combination with Doxycycline and Cefuroxime. Front Microbiol 7. https://doi.org/10.3389/FMICB.2016.00062

Floss, H.G., 2006. Combinatorial biosynthesis-Potential and problems. J Biotechnol 124, 242–257. https://doi.org/10.1016/J.JBIOTEC.2005.12.001

Frank, S., 2013. Treatment of Huntington's Disease. Neurotherapeutics 2013 11:1 11, 153–160. https://doi.org/10.1007/S13311-013-0244-Z

Gajadeera, C., Willby, M.J., Green, K.D., Shaul, P., Fridman, M., Garneau-Tsodikova, S., Posey, J.E., Tsodikov, O. v., 2015. Antimycobacterial activity of DNA intercalator inhibitors of Mycobacterium tuberculosis primase DnaG. J Antibiot (Tokyo) 68, 153. https://doi.org/10.1038/JA.2014.131

Gammella, E., Maccarinelli, F., Buratti, P., Recalcati, S., Cairo, G., Arosio, P., 2014. The role of iron in anthracycline cardiotoxicity. https://doi.org/10.3389/fphar.2014.00025

Gow, N.J., Davidson, R.N., Ticehurst, R., Burns, A., Thomas, M.G., 2015. Case Report: No Response to Liposomal Daunorubicin in a Patient with Drug-Resistant HIV-Associated Visceral Leishmaniasis. PLoS Negl Trop Dis 9, e0003983. https://doi.org/10.1371/JOURNAL.PNTD.0003983

Gumbo, T., Cirrincione, K., Srivastava, S., 2020. Repurposing drugs for treatment of Mycobacterium abscessus: a view to a kill. J Antimicrob Chemother 75, 1212–1217. https://doi.org/10.1093/JAC/DKZ523

Hassoun, A., Linden, P.K., Friedman, B., 2017. Incidence, prevalence, and management of MRSA bacteremia across patient populations—a review of recent developments in MRSA management and treatment. Crit Care 21, 211. https://doi.org/10.1186/S13054-017-1801-3

Henriksen, P.A., 2018. Anthracycline cardiotoxicity: an update on mechanisms, monitoring and prevention. Heart 104, 971–977. https://doi.org/10.1136/HEARTJNL-2017-312103

Hulst, M.B., Grocholski, T., Neefjes, J.J.C., Wezel, G.P. van, Metsä-Ketelä, M., 2022. Anthracyclines: biosynthesis, engineering and clinical applications. Nat Prod Rep. https://doi.org/10.1039/D1NP00059D

Hurteloup, P., Cappelaere, P., Armand, J.P., Mathé, G., 1983. Phase II clinical evaluation of 4'-epi-doxorubicin. Cancer Treat Rep 67, 337–41.

Ichikawa, Y., Ghanefar, M., Bayeva, M., Wu, R., Khechaduri, A., Naga Prasad, S. v., Mutharasan, R.K., Jairaj Naik, T., Ardehali, H., 2014. Cardiotoxicity of doxorubicin is mediated through mitochondrial iron accumulation. J Clin Invest 124, 617. https://doi.org/10.1172/JCI72931

Javadov, S., Rico Hitoshi Kurose, P., Sun, S., Xing, Y., Zhang, G., Yuan, C., Su, X., Zhang, J., Gokulnath, P., Vulugundam, G., Li, G., Yang, X., An, N., Liu, C., Sun, W., Chen, H., Wu, M., 2022. Relevance of

Ferroptosis to Cardiotoxicity Caused by Anthracyclines: Mechanisms to Target Treatments. https://doi.org/10.3389/fcvm.2022.896792

Jeyaprakash, P., Sangha, S., Ellenberger, K., Sivapathan, S., Pathan, F., Negishi, K., 2021. Cardiotoxic effect of modern anthracycline dosing on left ventricular ejection fraction: A systematic review and meta-analysis of placebo arms from randomized controlled trials. J Am Heart Assoc 10, 18802. https://doi.org/10.1161/JAHA.120.018802/FORMAT/EPUB

Jones, D., Metzger, H.J., Schatz, A., Waksman, S.A., 1944. CONTROL OF GRAM-NEGATIVE BACTERIA IN EXPERIMENTAL ANIMALS BY STREPTOMYCIN. Science 100, 103–105. https://doi.org/10.1126/SCIENCE.100.2588.103

Kagawa, T., Shirai, Y., Oda, S., Yokoi, T., n.d. Identification of Specific MicroRNA Biomarkers in Early Stages of Hepatocellular Injury, Cholestasis, and Steatosis in Rats. https://doi.org/10.1093/toxsci/kfy200

Kansal, S., Tandon, R., Dwivedi, P., Misra, P., Verma, P.R.P., Dube, A., Mishra, P.R., 2012. Development of nanocapsules bearing doxorubicin for macrophage targeting through the phosphatidylserine ligand: a system for intervention in visceral leishmaniasis. Journal of Antimicrobial Chemotherapy 67, 2650–2660. https://doi.org/10.1093/JAC/DKS286

Kim, E.J., Lim, K.M., Kim, K.Y., Bae, O.N., Noh, J.Y., Chung, S.M., Shin, S., Yun, Y.P., Chung, J.H., 2009. Doxorubicin-induced platelet cytotoxicity: a new contributory factor for doxorubicin-mediated thrombocytopenia. Journal of Thrombosis and Haemostasis 7, 1172–1183. https://doi.org/10.1111/J.1538-7836.2009.03477.X

Kremer, L.C.M., van der Pal, H.J.H., Offringa, M., van Dalen, E.C., Voûte, P.A., 2002. Frequency and risk factors of subclinical cardiotoxicity after anthracycline therapy in children: a systematic review. Annals of Oncology 13, 819–829. https://doi.org/10.1093/annonc/mdf167

Kuter, D.J., 2015. Managing thrombocytopenia associated with cancer chemotherapy. Oncology 29, 282–282.

Kwon, Y., 2016. OncoTargets and Therapy Dovepress Mechanism-based management for mucositis: option for treating side effects without compromising the efficacy of cancer therapy. https://doi.org/10.2147/OTT.S96899

Lee, J., Hwang, Y.J., Kim, Y., Lee, M.Y., Hyeon, S.J., Lee, S., Kim, D.H., Jang, S.J., Im, H., Min, S.J., Choo, H., Pae, A.N., Kim, D.J., Cho, K.S., Kowall, N.W., Ryu, H., 2017. Remodeling of heterochromatin structure slows neuropathological progression and prolongs survival in an animal model of Huntington's disease. Acta Neuropathol 134, 729–748. https://doi.org/10.1007/S00401-017-1732-8/FIGURES/8

Li, J., Cao, F., Yin, H.-L., Huang, Z.-J., Lin, Z.-T., Mao, N., Sun, B., Wang, G., n.d. Ferroptosis: past, present and future. https://doi.org/10.1038/s41419-020-2298-2

Madduri, K., Kennedy, J., Rivola, G., Inventi-Solari, A., Filippini, S., Zanuso, G., Colombo, A.L., Gewain, K.M., Occi, J.L., MacNeil, D.J., Hutchinson, C.R., 1998. Production of the antitumor drug epirubicin

(4'-epidoxorubicin) and its precursor by a genetically engineered strain of Streptomyces peucetius. Nat Biotechnol 16, 69–74. https://doi.org/10.1038/nbt0198-69

Maeda, Y., Hamada, A., Sanematsu, E., Sasaki, J.I., Yokoo, K., Hira, A., Saito, H., 2010. Co-administration of irinotecan decreases the plasma concentration of an active metabolite of amrubicin, amrubicinol in rats. Cancer Chemother Pharmacol 65, 953–959. https://doi.org/10.1007/S00280-009-1102-X/FIGURES/5

Minotti, G., Menna, P., Salvatorelli, E., Cairo, G., Gianni, L., 2004. Anthracyclines: Molecular Advances and Pharmacologic Developments in Antitumor Activity and Cardiotoxicity. Pharmacol Rev 56, 185–229. https://doi.org/10.1124/pr.56.2.6

Moore, D.C., 2016. Drug-Induced Neutropenia A Focus on Rituximab-Induced Late-Onset Neutropenia PHARMACOVIGILANCE FORUM.

Novack, G.D., 2021. Repurposing medications. Ocul Surf 19, 336. https://doi.org/10.1016/J.JTOS.2020.11.012

Qiao, X., van der Zanden, S.Y., Wander, D.P.A., Borràs, D.M., Song, J.Y., Li, X., Duikeren, S. van, Gils, N. van, Rutten, A., Herwaarden, T. van, Tellingen, O. van, Giacomelli, E., Bellin, M., Orlova, V., Tertoolen, L.G.J., Gerhardt, S., Akkermans, J.J., Bakker, J.M., Zuur, C.L., Pang, B., Smits, A.M., Mummery, C.L., Smit, L., Arens, R., Li, J., Overkleeft, H.S., Neefj, J., 2020. Uncoupling DNA damage from chromatin damage to detoxify doxorubicin. Proc Natl Acad Sci U S A 117, 15182–15192. https://doi.org/10.1073/PNAS.1922072117

Roos, R.A.C., 2010. Huntington's disease: a clinical review. Orphanet J Rare Dis 5, 40. https://doi.org/10.1186/1750-1172-5-40

Ryberg, M., Nielsen, D., Skovsgaard, T., Hansen, J., Jensen, B.V., Dombernowsky, P., 1998. Epirubicin cardiotoxicity: an analysis of 469 patients with metastatic breast cancer. J Clin Oncol 16, 3502–3508. https://doi.org/10.1200/JCO.1998.16.11.3502

S. Appleby, B., L. Cummings, J., d. Discovering New Treatments for Alzheimer's Disease by Repurposing Approved Medications.

Schatz, A., Bugie, E., Waksman, S.A., 2005. Streptomycin, a substance exhibiting antibiotic activity against gram-positive and gram-negative bacteria. 1944. Clin Orthop Relat Res 437, 3–6. https://doi.org/10.1097/01.BLO.0000175887.98112.FE

Sett, R., Basu, N., Ghosh, A.K., Das, P.K., 1992. Potential of doxorubicin as an antileishmanial agent. Journal of Parasitology 78, 350–354. https://doi.org/10.2307/3283487

Seung, K.J., Keshavjee, S., Rich, M.L., 2015. Multidrug-Resistant Tuberculosis and Extensively Drug-Resistant Tuberculosis. Cold Spring Harb Perspect Med 5. https://doi.org/10.1101/CSHPERSPECT.A017863

Shan, K., Lincoff, A.M., Young, J.B., 1996. Anthracycline-induced cardiotoxicity. Ann Intern Med. https://doi.org/10.1059/0003-4819-125-1-199607010-00008

She, P., Li, S., Zhou, L., Luo, Z., Liao, J., Xu, L., Zeng, X., Chen, T., Liu, Y., Wu, Y., 2020. Insights into idarubicin antimicrobial activity against methicillin-resistant Staphylococcus aureus. Virulence 11, 636. https://doi.org/10.1080/21505594.2020.1770493

Shishido, T., Wolschendorf, F., Duverger, A., Wagner, F., Kappes, J., Jones, J., Kutsch, O., 2012. Selected Drugs with Reported Secondary Cell-Differentiating Capacity Prime Latent HIV-1 Infection for Reactivation. J Virol 86, 9055. https://doi.org/10.1128/JVI.00793-12

Shockman, G., Waksman, S.A., 1951. Rhodomycin--an antibiotic produced by a red-pigmented mutant of Streptomyces griseus - PubMed. Antibiot Chemother (1971) 1, 68–75.

Sim, S.P., Pilch, D.S., Liu, L.F., 2000. Site-specific topoisomerase I-mediated DNA cleavage induced by nogalamycin: A potential role of ligand-induced DNA bending at a distal site. Biochemistry 39, 9928–9934. https://doi.org/10.1021/bi000906h

Sobek, S., Boege, F., 2014. DNA topoisomerases in mtDNA maintenance and ageing. Exp Gerontol 56, 135–141. https://doi.org/10.1016/J.EXGER.2014.01.009

Spellicy, S.E., Hess, D.C., 2022. Recycled Translation: Repurposing Drugs for Stroke. Translational Stroke Research 2022 13:6 13, 866–880. https://doi.org/10.1007/S12975-022-01000-Z

Swain, S.M., Whaley, F.S., Gerber, M.C., Weisberg, S., York, M., Spicer, D., Jones, S.E., Wadler, S., Desai, A., Vogel, C., Speyer, J., Mittelman, A., Reddy, S., Hendergrass, K., Velez-Garcia, E., Ewer, M.S., Bianchine, J.R., Gams, R.A., 1997. Cardioprotection with dexrazoxane for doxorubicin-containing therapy in advanced breast cancer. Journal of Clinical Oncology 15, 1318–1332. https://doi.org/10.1200/JCO.1997.15.4.1318

Tarique, M., Chauhan, M., Tuteja, R., 2017. ATPase activity of Plasmodium falciparum MLH is inhibited by DNA-interacting ligands and dsRNAs of MLH along with UvrD curtail malaria parasite growth. Protoplasma 254, 1295–1305. https://doi.org/10.1007/S00709-016-1021-8

Trombetta, R.P., Dunman, P.M., Schwarz, E.M., Kates, S.L., Awad, H.A., 2018. A High-Throughput Screening Approach To Repurpose FDA-Approved Drugs for Bactericidal Applications against Staphylococcus aureus Small-Colony Variants. mSphere 3, 422–440. https://doi.org/10.1128/MSPHERE.00422-18

van der Zanden, S.Y., Qiao, X., Neefjes, J., 2021. New insights into the activities and toxicities of the old anticancer drug doxorubicin. FEBS J 288, 6095–6111. https://doi.org/10.1111/FEBS.15583

Villani, F., Comazzi, R., Lacaita, G., Genitoni, V., Guindani, A., Martini, A., 1983. Preliminary echocardiographic and polygraphic evaluation of cardiac toxicity of 4'-epi-doxorubicin. Int J Clin Pharmacol 21, 203–8.

Volkova, M., Russell, R., 2011. Anthracycline Cardiotoxicity: Prevalence, Pathogenesis and Treatment. Curr Cardiol Rev 7, 214–220.

Wander, D.P.A., van der Zanden, S.Y., van der Marel, G.A., Overkleeft, H.S., Neefjes, J., Codée, J.D.C., 2020. Doxorubicin and Aclarubicin: Shuffling Anthracycline Glycans for Improved Anticancer Agents. J Med Chem 63, 12814–12829. https://doi.org/10.1021/ACS.JMEDCHEM.0C01191/SUPPL_FILE/JM0C01191_SI_001.CSV

Wander, D.P.A., van der Zanden, S.Y., Vriends, M.B.L., van Veen, B.C., Vlaming, J.G.C., Bruyning, T., Hansen, T., van der Marel, G.A., Overkleeft, H.S., Neefjes, J.J.C., Codée, J.D.C., 2021. Synthetic (N, N-Dimethyl)doxorubicin Glycosyl Diastereomers to Dissect Modes of Action of Anthracycline Anticancer Drugs. Journal of Organic Chemistry 86, 5757–5770. https://doi.org/10.1021/ACS.JOC.1C00220/ASSET/IMAGES/LARGE/JO1C00220_0005.JPEG

Weiss, R.B., 1992. The anthracyclines: will we ever find a better doxorubicin? Semin Oncol 19, 670–686. https://doi.org/10.5555/uri:pii:009377549290036Z

Weissman, K.J., Leadlay, P.F., 2005. Combinatorial biosynthesis of reduced polyketides. Nature Reviews Microbiology 2005 3:12 3, 925–936. https://doi.org/10.1038/nrmicro1287

White, N.J., 2004. Antimalarial drug resistance. Journal of Clinical Investigation 113, 1084. https://doi.org/10.1172/JCI21682

Whitfield, J., 2002. Portrait of a serial killer. Nature. https://doi.org/10.1038/NEWS021001-6

Zhang, S., Liu, X., Bawa-Khalfe, T., Lu, L.S., Lyu, Y.L., Liu, L.F., Yeh, E.T.H., 2012. Identification of the molecular basis of doxorubicin-induced cardiotoxicity. Nature Medicine 2012 18:11 18, 1639–1642. https://doi.org/10.1038/nm.2919

Zhang, Y., Wang, D., Shen, D., Luo, Y., Che, Y.Q., 2020. Identification of exosomal miRNAs associated with the anthracycline-induced liver injury in postoperative breast cancer patients by small RNA sequencing. PeerJ 2020, e9021. https://doi.org/10.7717/PEERJ.9021/SUPP-4

Declaration of interests

☐The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☒The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Stephen Eric Nybo reports financial support was provided by National Science Foundation.
Stephen Eric Nybo reports financial support was provided by National Institutes of Health.

Highlights

- A brief review of new mechanisms underpinning anthracycline toxicity
- Updates on anthracycline structure-activity-relationships (SAR) to dissect the cardiotoxic activity from the antiproliferative mechanism of action.
- Discussion about new indications for anthracyclines as anti-infectives