Repurposing of Anticancer Drugs for the Treatment of Bacterial Infections

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Abstract

Despite the fact that bacterial infections are one of the leading causes of death worldwide and that mortality rates are increasing at alarming rates, no new antibiotics have been produced by the pharmaceutical industry in more than a decade. The situation is so dire that the World Health Organization warned that we may enter a "post-antibiotic era" within this century; accordingly, bacteria resistant against all known antibiotics are becoming common and already producing untreatable infections. Although several novel approaches to combat bacterial infections have been proposed, they have yet to be implemented in clinical practice. Hence, we propose that a more plausible and faster approach is the utilization of drugs originally developed for other purposes besides antimicrobial activity. Among these are some anticancer molecules proven effective *in vitro* for eliminating recalcitrant, multidrug tolerant bacteria; some of which also protect animals from infections and recently are undergoing clinical trials. In this review, we highlight the similarities between cancer cells/tumors and bacterial infections, and present evidence that supports the utilization of some anticancer drugs, including 5-fluorouracil (5-FU), gallium (Ga) compounds, and mitomycin C, as antibacterials. Each of these drugs has some promising properties such as broad activity (all three compounds), dual antibiotic and antivirulence properties (5-FU), efficacy against multidrug resistant strains (Ga), and the ability to kill metabolically dormant persister cells which cause chronic infections (mitomycin C).

1. INTRODUCTION

Due to the alarming global increase of antibiotic resistance observed in several important bacterial pathogens, the World Health Organization recently warned that we may enter a "post-antibiotic era" within this century and has proposed that urgent actions should be taken [1]. Nevertheless, the development of new antimicrobials is a slow process with a very low success rate due to several reasons, including that many compounds with acceptable antimicrobial activity *in vitro* often either fail to affect bacterial infections *in vivo*, present very high toxicity, or produce severe side effects to the host. Hence, an approach that will save enormous amounts of time and resources is the utilization of already available drugs that were originally developed and used for the treatment of non-infectious diseases, a process known as drug repurposing. This approach currently has remarkable attention in several fields, guided by the development of computational methods and systems biology approaches [2]. To date, several candidates for repurposing have been identified, including antivirals and fungicides with potent anticancer properties (e.g., nelfinavir and itraconazole) [3,4], pain killers for the treatment of opioid addiction (e.g., buprenorphine) [5] , drugs that reduce cholesterol levels, antifungal compounds, antihelminthic compounds, and anticancer drugs with antibacterial activities [6]. In this review we will discuss the remarkable antibacterial properties of diverse anticancer compounds as well as their potential future applications to treat bacterial infections.

Cancer cells and bacterial cells causing infections share several properties, such as high replication rates, virulence, modalities of spreading within the host, rapid development of resistance mechanisms against chemotherapeutical agents (e.g., active efflux and mutations of the drug target), and a tendency to become more aggressive during disease progression [7]. In addition, it has recently been hypothesized that cancer cells utilize cell-cell communication systems analogous to those of bacterial cells (quorum sensing or QS), that allow them to successfully coordinate their attacks against the host [8,9]. Utilization of these communication systems in bacterial populations allows for coordination of behaviors to better adapt to the environment, exhibiting properties that can be considered a mode of collective, primitive intelligence (e.g., learning, problem solving, and anticipation) [10]. QS in the case of cancer cells is only known to mediate the different steps of metastatic colonization [9], but coordination in a manner similar to bacterial populations could also in principle be utilized by cancer cells [11]. Relatedly, it is estimated that 65% to 80% of bacterial infections involve biofilm formation [12], which is mediated through QS. Microbial biofilms are recalcitrant structures that share many similarities with tumors, including the presence of oxygen and nutrient gradients across different spatial levels of the structure, which lead to a heterogeneity of metabolic activities among individual cells [13,14], and an increase in chemotherapy resistance, which allows the recalcitrance of either the infection [15] or the malignant cell mass [16,17].

In addition to population development modalities within a host, cancer cells and bacteria share numerous metabolic features and pathways that are present in nearly all forms of life. Therefore, it is not surprising that some anticancer drugs are also effective against bacterial infections, and vice versa [6]. In fact,

anthracyclines, which are among the most effective anticancer treatments available today and are used for the treatment of several types of cancer (e.g., leukemias, lymphomas, breast, bladder, and lung), were isolated from soil bacteria (*Streptomyces peucetius*) in a manner similar to the way antimicrobial compounds have been identified and were originally tested. Despite having good antibacterial activities, they also often exhibit high cytotoxicity, which precluded their utilization for treating internal bacterial infections. However, these compounds may be effective in treating chronic wound infections, where higher concentrations may be utilized with reduced concerns for cytotoxicity [18]; finding additional treatments for chronic wounds is of paramount importance since 1 to 2% of populations in developed countries will develop chronic skin wounds which cost $25B annually in the U.S. alone [19] Even given their cytotoxicity, in the 1960s many of these drugs were approved as anticancer compounds. Several other anticancer drugs such as antimetabolites methotrexate and 5-fluorouracil (5-FU), iron analogues like gallium, and the aziridine-containing compound mitomycin C also exhibit potent antibacterial properties with wide antibacterial spectra, and could eliminate multidrug resistant strains or even eradicate biofilm cells and metabolically dormant persister cells. Considering that the pharmaceutical industry has not produced a new class of antibiotic in over a decade, that several experimentally verified approaches to combat bacterial infections have yet to be implemented in clinical practice, and that therapeutically available options to treat resistant bacterial infections are becoming very scarce, the repurposing of already available drugs for the treatment of bacterial infections may be an effective and rapid method to combat multidrug resistant bacteria as modern medicine is on the verge of entering into the post-antibiotic era.

In this regard, we have consolidated information pertaining to several anticancer drugs with promising antibacterial activities (Table **1**). Most of them have been tested *in vitro* against various bacterial species, while some have been proven effective against infections in animal models, and a few are even undergoing clinical trials in humans to evaluate their antimicrobial activities. In this review we summarize the current findings for the most promising drugs to be repurposed and indicate the attractive features of anticancer drugs that may be suitable antibacterial compounds for rapid implementation as clinical treatments for bacterial infections.

1.1 Current Anticancer Chemotherapy

Cancer is characterized by uncontrolled cell proliferation which causes interference of normal organ function due to the growing number of tumor cells. It becomes more dangerous when it spreads to other organs after metastasis; in the case of leukemia, the high number of malignant blast cells prevents normal blood function. Since cancer pathogenicity is based on cell proliferation, anticancer drugs damage rapidly multiplying cells or interfere with cellular processes required for cell division and survival [17]. Cytotoxic drugs impair DNA metabolism or mitosis progression leading to apoptosis. This group of drugs includes alkylating agents that cross-link DNA, antimetabolites that inhibit enzymes of nucleotide biosynthetic pathways or that are incorporated into DNA, DNA intercalating agents that halt DNA replication and inhibit topoisomerases

causing DNA breaks, and drugs that interfere with cytoskeleton dynamics by preventing tubulin polymerization and/or depolymerization. These drugs however, also affect normal proliferating cells [17]. Higher selectivity is achieved by inhibiting processes to which certain cancer cells are highly dependent [20], such as using kinase inhibitors to block inappropriate signal transduction from intra- or inter-cellular signaling or using hormone modulators that inhibit hormone synthesis or block specific receptors. Other drugs like interleukin 2, Toll-like receptor agonists, or monoclonal antibodies (mAbs) can be used to stimulate the antitumor immune response. The high specificity of mAbs allows selective targeting of antigen-positive tumor cells for antibody-dependent complement-mediated destruction by cytotoxic T lymphocytes, for specific delivery of drugs or radioactive isotopes which are released after internalization by tumor cells, and for signaling impairment due to the binding of mAbs to the extracellular domains of some tyrosine kinase receptors. Other types of chemotherapeutic agents inhibit cellular processes like proteasome function, histone deacetylation, and DNA methylation or they limit cancer cells of specific nutrients, like L-asparagine in some leukemias.

Anticancer drugs are often used in combination therapies to overcome drug resistance and reduce toxicity [17]. However, the high heterogeneity of cancer cells, the complexity of tumor microenvironments, the many similarities between cancer and normal cells, and chemoresistance, limit the efficacy of anticancer chemotherapy and drive research of new drugs based on tumor biology [20]. The cytotoxic nature of anticancer chemotherapeutic agents reflects their potential to be used as antimicrobial drugs, as described below.

2. ANTICANCER DRUGS WITH PROMISING ANTIBACTERIAL ACTIVITIES

2.1 5-Fluorouracil an Antimetabolite with Antibacterial, Antibiofilm, and Antivirulence Properties

5-fluorouracil is a uracil analog with a fluorine atom at the C-5 position replacing hydrogen (Fig. **1**). It was originally developed in the 1950s as an antimetabolite after it was recognized that uracil metabolism was a potential target for chemotherapy because rat hepatomas incorporated uracil at higher rates than normal tissues [6]. 5-FU is converted intracellularly to several active metabolites including fluorodeoxyuridine monophosphate, fluorodeoxyuridine triphosphate, and fluorouridine triphosphate, which exert their effects in cancer cells primarily by incorporation into RNA. Also they inhibit the enzyme thymidylate synthase (TS) that catalyzes the conversion of deoxyuridine monophosphate to deoxythymidine monophosphate [21]. The inhibition of TS causes an imbalance of deoxynucleotides and increases levels of deoxyuridine triphosphate, promoting damage to DNA [21]. Currently, 5-FU is widely used to treat several kinds of cancers, being applied either systemically to treat colorectal, esophageal, stomach, anal, breast, pancreatic, head, and neck cancers or topically for skin cancers, actinic keratoses, and Bowen's disease [6,21]. Based on its wide effects against cancer cells, it is not surprising that 5-FU also has potent antibacterial effects. Indeed in 1986 Gieringer et al. [22] studied the interactions of four antineoplastic agents and five antimicrobials, finding out

that 5- FU inhibited the growth of *Staphylococcus aureus* and *Staphylococcus epidermidis*, with an MIC50 ≤0.8 μg/mL. In addition they demonstrated that 5-FU combined with β-lactams acts synergistically against Gram (−) bacteria [22]. Their activity against important Gram (+) bacterial pathogens was later confirmed when synergy of 5-FU with tobramycin was discovered against *S. aureus* [23]. Remarkably, in 2009 Ueda and coworkers discovered that the uracil biosynthetic pathway is required for robust biofilm formation and for the production of QS regulated virulence factors in *Pseudomonas aeruginosa*, and tested a battery of uracil analogues as possible biofilm inhibitors, finding that out of six uracil analogues only 5-FU was able to decrease biofilm formation. Accordingly, 5-FU inhibited the production of several QS-controlled virulence factors including the production of elastase, rhamnolipids, and pyocyanin, swarming motility, and the *Pseudomonas* quinolone signal autoinducer. It also decreased *P. aeruginosa* virulence against barley [24]. Later, Imperi and coworkers confirmed that 5-FU inhibits *P. aeruginosa* virulence, by demonstrating that the antifungal drug 5-fluorocytosine, when converted to 5-FU by bacterial enzymes, inhibits the production of pyoverdine, PrpL protease, and exotoxin A, and decreases *P. aeruginosa* pathogenicity in mouse lung infections [25]. Importantly, 5-FU is also generally effective at decreasing the virulence factor production of *P. aeruginosa* clinical strains [26]. Regarding the effects of 5-FU in other bacteria, it also decreases biofilm formation of the common lab strain *Escherichia coli* K-12, of the pathogenic enterohemorrhagic *E. coli* O157:H7 (EHEC) strain [27], and of *Staphylococcus epidermidis* [28]. It is worth noting that the roles of uracil or related compounds as bacterial signals for mediating pathogenicity are not yet fully described. However, in agreement with the findings of Thomas Wood´s group for *E. coli* and *P. aeruginosa* [24,27], it was recently discovered that in the gut of *Drosophila melanogaster*, pathogenic bacteria, but not commensal bacteria, produce uracil that promotes inflammation and binds a dual oxidase called DUOX, which catalyzes the production of reactive oxygen species. This uracil-induced immune response (i.e., uracil-based interkingdom signaling) is essential for the elimination of undesired bacteria and for intestinal cell repair [29]; however, whether a similar phenomenon exists in mammalian guts is yet unknown. The positive antibacterial and antivirulence properties of 5-FU prompted the implementation of large scale clinical trials in humans, testing the effect of 5-FU as an antibiofilm external coating of central venous catheters in critically ill patients. Remarkably, although 5-FU showed similar results to a mixture of chlorhexidine and silver sulfadiazine (positive control) for decreasing the percentage of bacterial colonization of the catheter surfaces, the catheters coated with 5-FU only allowed colonization by Gram (+) bacteria while abolishing colonization by Gram (-) bacteria and *Candida* species, in contrast to the positive control which allowed colonization by these organisms. Moreover, the treatment with 5-FU abolished the production of catheter-related bloodstream infections that occurred in 0.4% of the patients with chlorhexidine/silver sulfadiazine coated catheters; hence, the authors concluded that coating central venous catheters with 5-FU was a safe and effective alternative to prevent biofilm formation and to avoid the spreading of bacterial infections to the bloodstream [30]. Unfortunately, no further studies using 5-FU as antibacterial in clinical trials have been published, and this method has not yet been incorporated clinically to combat bacterial infections although Angiotech Pharmaceuticals received U.S. FDA approval to make commercial 5-FU coated catheters. This is an important

milestone as it represents the first potential commercial use of an anti-signaling compound as an antimicrobial.

2.2 Gallium a Non-Redox Iron Analogue with Wide Spectrum Antimicrobial Activity

Iron is an essential element for virtually all known life forms, including most pathogenic bacteria, since it is involved as a cofactor for several enzymes that mediate electron transport, DNA synthesis, the defense against toxic reactive oxygen species (ROS), and other metabolic processes [31]. The iron participation in all these processes is linked to its redox activity, and its importance for infections is highlighted by the fact that both mammalian hosts and pathogens have developed a series of strategies to increase access to iron sources, including the utilization of high affinity iron-binding proteins like transferrin, lactoferrin, and ferritin by hosts or siderophores, haemophores, and exoproteases (which cleave iron binding proteins, releasing iron) by bacterial pathogens [29]. Hence, targeting iron metabolism could be an effective strategy to combat bacterial infections, and one way to do this is by exploiting "Trojan horse" strategies which utilize the natural iron-uptake bacterial systems to internalize antimicrobial compounds [32]. Perhaps the best characterized Trojan horse compounds known to date are those containing the element gallium, a group IIIA metal with no known essential biological function which has remarkably similar physicochemical properties to Fe^{3+}, including its ionic radii, electronegativities, coordination geometries, and electron and ligand affinities [33]. Despite all of these similarities with iron, Ga^{3+} cannot participate in redox reactions; hence, Ga has the ability to disrupt iron-dependent enzymes. The utilization of gallium in the medical field began in the early 1950s when it was used as a radiotracer for the early detection of bone malignancies. Later in the 1970s, with the introduction of the radioisotope ^{67}Ga, diagnostic gallium scintigraphy became widespread. Most recently, ^{67}Ga scanning is used for the diagnosis and staging of lymphomas, and citrated $Ga(NO_3)_3$ was administered as intravenous injections for the treatment of hypercalcemia of malignancy for several years, although the commercial formulation Ganite® was discontinued in 2012. In addition, gallium nitrate (Fig. **1**) is clinically active against several kinds of cancer including hepatocellular carcinoma, bladder cancer, lymphoma, ovarian cancer, and multiple myeloma [33,34].

The use of gallium as an antimicrobial compound began in 1931, when it was successfully used to alleviate syphilis in rabbits and trypanosomiasis in mice [33]. Later, its antimicrobial activities were also demonstrated in several important pathogenic bacteria, including *Mycobacterium tuberculosis* grown in cultures or intracellularly inside macrophages [35]. Remarkably, gallium nitrate treatment prevents the death of *M. tuberculosis* infected mice, significantly decreasing the bacterial counts in their lungs [36]. Moreover, laboratory strains and several clinical strains of the recalcitrant bacteria *P. aeruginosa* are sensitive to gallium nitrate, and *in vivo* treatment increases the survival of mice with both acute and chronic infections [37]. Similarly, gallium nitrate is very effective at decreasing growth of the multidrug resistant bacteria *Acinetobacter baumannii* (including several clinical isolates) in both chemically-defined medium and human

serum, it protects *Galleria mellonella* larvae from lethal *A. baumannii* infections [38], and it prevents growth and accelerates clearance of *A. baumannii* in mouse lung infections [39]. Gallium nitrate also is effective to varying degrees against several other pathogenic bacteria such as *Francisella novicida*, *Francisella tularensis*, and *Burkholderia cepacia* [6,40].

Despite the potent antimicrobial effects of gallium nitrate, administration for treating hypercalcemia of malignancy requires continuous intravenous (IV) infusion at a dose of 100-200 mg/m^2 per day (usually for 5 days), which results in moderate nephrotoxicity and limits the range for its possible applications against bacterial infections. Nevertheless, there are ongoing clinical trials to test IV administration of $Ga(NO_3)_3$ in patients with cystic fibrosis to assess the pulmonary safety of the compound and its efficacy in improving lung function and decreasing *P. aeruginosa* counts. In addition, searching for novel gallium formulations with higher bioavailability and lower toxicity is an active research field. Among the most effective novel gallium formulations are: i) gallium maltolate *tris*(3-hydroxy-2-methyl-4*H*-pyran-4-onato) gallium (GaM), a low toxicity and highly bioavailable gallium formulation [41] effective for inhibiting the replication of hepatocellular carcinoma cell lines [42] and lymphoma cell lines with significantly lower concentrations than gallium nitrate, with the ability to block the proliferation of lymphoma cells resistant to gallium nitrate [43], and with antimicrobial activity against several bacterial pathogens (e.g., *P. aeruginosa* [6], methicillin-resistant *Staphylococcus aureus* [MRSA] [44], and *Mycobacterium avium* [45]); ii) gallium curcumin complex, which is effective for inhibiting the growth of breast cancer cell lines, bladder cancer cell lines, and prostate carcinoma cells, with antibacterial activity against *S. aureus* and *E. coli* [46]; and iii) gallium-thiosemicarbazone complexes, since the synthesis of complexes based in 2-acetylpyridine thiosemicarbazones, 3-aminopyridine-2-carboxaldehyde, thiosemicarbazone, 3-acetylpyridine, 2-acetylpyridine-R and other thiosemicarbazones, result in a numerous compounds with antimicrobial and antineoplastic activity, showing highly cytotoxic effects at nanomolar concentrations against malignant glioblastoma and breast cancer cells, with potent antimicrobial activities against *P. aeruginosa* and *Candida albicans* [47].

Another area of opportunity for repurposing gallium compounds as an antimicrobial is their potential utilization for the prophylaxis of burn patients and the treatment of infected burns. In 2009, DeLeon and coworkers demonstrated that doses of 25 mg/kg of body weight of GaM, administered subcutaneously to thermally injured mice, provided 100% survival in an otherwise lethal *P. aeruginosa* infection. The concentrations of GaM four times higher eradicated this bacterium from the wounds and prevented the bacterial spread to the liver and spleen. Additionally, GaM also prevented the systemic spread of bacteria from previous *P. aeruginosa* infected injuries and it was effective at decreasing the growth of two other recalcitrant bacterial species that commonly colonize wounds, *S. aureus* and *A. baumannii*, although in contrast with *P. aeruginosa*, the 100 mg/kg concentration was not enough to completely eradicate these

bacteria [51]. Nevertheless, this study was done with laboratory strains of bacteria, so the efficacy of GaM against infections in wounds from clinical strains remains unknown.

Remarkably, although the utilization of gallium as an antibacterial compound is not yet implemented in the clinic, the possible ways in which pathogenic bacteria such as *P. aeruginosa* can adapt to it and acquire resistance are beginning to be elucidated. It was found that gallium can enter the cell through the secondary iron transporters HitAB and by the secondary siderophore pyochelin; accordingly, mutants with defects in these systems had 2 to 4 fold higher MIC50 against gallium than the parental strains [48] . In addition, the overexpression or external addition of the main siderophore pyoverdine increases gallium resistance, likely by sequestering gallium outside the cell and avoiding its internalization [37]. Moreover, *P. aeruginosa* clinical isolates that produce higher pyoverdine levels are more resistant to gallium than low pyoverdine producers when cultured in complement-free human serum, since pyoverdine allows iron sequestration from iron serum binding proteins like transferrin. Similarly, those clinical isolates with high exoprotease production were less susceptible to gallium, since the hydrolysis of iron containing proteins releases iron that can be used by the bacteria [49]. These abilities of high proteolysis and high pyoverdine production by clinical isolates in combination with the relatively high gallium concentration required to inhibit the growth in human serum is a potential limitation to the utilization of gallium for the treatment of *P. aeruginosa* bloodstream infections [49]. Interestingly, the overproduction or addition of the phenazine pyocyanin to cultures of the PA14 laboratory strain confer protection against gallium [48], but whether this is also the case for clinical isolates remains to be elucidated. Since the production of pyoverdine, pyocyanin, and exoproteases are positively regulated by QS, a more effective gallium treatment for combating *P. aeruginosa* infections may be in combination with a QS inhibitor. In agreement, the simultaneous administration of the quorum quenching compound C-30 with gallium nitrate increased the gallium growth inhibitory effect *in vitro* [50]. Another potential drawback for gallium utilization as an antimicrobial is the fact that for *P. aeruginosa*, at least *in vitro*, the utilization of sub-lethal gallium concentrations promotes the expression of some virulence factors, likely by inducing an iron starvation response [50]; however, the effect of sub-lethal gallium concentrations on the *in vivo* virulence of bacteria remains unexplored.

2.3 Mitomycin C Exhibit Potent Antibacterial and Antipersistent Activities

Many anticancer compounds are DNA-alkylating compounds, which form DNA cross-links to disrupt replication and transcription. This mechanism is most effective against rapidly growing tumor cells, in which DNA lesions are less likely to be repaired before the replication cycle is disrupted. Mitomycin C (Fig. **1**) is one such alkylating agent that is FDA-approved [52] as a chemotherapeutic agent for numerous cancer treatments (e.g., bladder, gastric, and pancreatic) [53]. Mitomycin C is an amphipathic molecule that freely diffuses through cellular membranes [54], and forms a spontaneously-reacting quinone methide structure [55] upon reduction from its nascent form. In its reduced form, mitomycin C reacts with two adjacent guanine residues in 5'-CG sequences, forming an interstrand DNA crosslink [56]. This dependence on reductive

activation is a key factor in the high cytotoxic activity of mitomycin C against tumor cells in comparison to non-tumor cells because the tumor microenvironment is typically hypoxic and low pH, which both lead to increased activity of mitomycin C [57]. Consequently, the bacterial cytoplasm is a reducing environment [58], which means that bacteria are also susceptible to the active form of mitomycin C.

The antibacterial activity of mitomycin C has long been established [58]; however, clinical use against bacterial infections has largely been foregone likely due to cytotoxic side effects and the advent of effective, lower toxicity alternative antibiotics. Due to the recent decline in novel antibiotic discovery, persister cells have become a focus of recent research in an effort to find methods of improving the efficacy of current antibiotics for treating infections. Persister cells are a metabolically dormant [59-61] subset of bacterial populations across all species [61, 62], and as a result of this inactivity, these cells are highly tolerant against all traditional classes of antibiotics, which primarily target actively growing cells. These persisters arise both stochastically [63] and through environmental influence [60, 64-68]; however, this multidrug tolerance is not a heritable trait [69]. Persister cells contribute to the recalcitrance of numerous infections, particularly when biofilms are present to harbor the persisters between rounds of antibiotic treatments [64]. Therefore, persister cells have become an important target in development of novel antibacterial treatments.

In response to the need for persister treatments, it was recently demonstrated through two independent studies that mitomycin C treatment is a highly effective method for eradication of these persister cells [70, 71] Passive transport and spontaneous activity make mitomycin C a particularly simple, but clever method of killing persisters, which tolerate traditional antibiotics due to lack of active transport and metabolic inactivity [70]. Kwan et al. highlighted the bactericidal activity of mitomycin C against both commensal and pathogenic species of *E. coli*, *S. aureus*, and *P. aeruginosa* under numerous different states of growth, including highly tolerant persister cells [70]. In several of these cases, mitomycin C was so potent that all the treated cells were eradicated [70]. Additionally, mitomycin C efficacy in an animal model was demonstrated through improved survival of the nematode *Caenorhabditis elegans* when infected with EHEC [70]. Critically, they also demonstrated that mitomycin C was effective in an *in vitro* wound model against *S. aureus* and *P. aeruginosa*, where higher concentrations of antimicrobials are tolerated [70]. Sharma et al. demonstrated that mitomycin C is also highly effective against persisters, as well as growing cells of *Borrelia burgdorferi*, the causative microbe of Lyme disease [71]. Therefore, mitomycin C has highly potent antibacterial and antipersister activities, and is an appropriate candidate as an anticancer drug that should be repurposed for treatment of clinical infections.

Figure 1

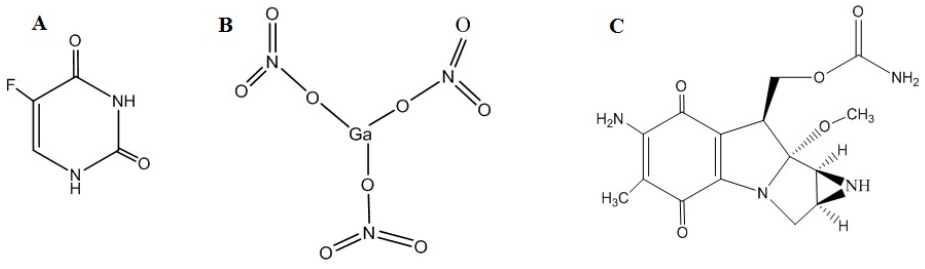

Figure 1) Structures of**:** A) 5-fluorouracil, B) gallium nitrate and C) mitomycin C.

3. OTHER ANTICANCER DRUGS WITH ANTIBACTERIAL ACTIVITIES

Historically, many anticancer drugs were isolated from natural products such as the microbial metabolite pool [70]. For this reason, we hypothesized that many anticancer drugs (in addition to 5-FU, gallium, and mitomycin C) possess antibacterial activities and are candidates for drug repurposing. We reviewed more than 200 currently used (and mostly FDA-approved) anticancer drugs listed on the National Cancer Institute (http://www.cancer.gov/about-cancer/treatment/drugs; updated on 1 June 2015), summarized all drugs with antibacterial activities in Table **1**, and briefly discussed their potentials as antibacterial drugs.

3.1 Alkylating Agents and DNA Crosslinkers

DNA has been the main target for the development of anticancer drugs owing to the rapid proliferation of cancer cells. Specifically, these DNA-targeting compounds (commonly known as alkylating agents and DNA crosslinkers) inhibit DNA replication by attaching alkyl groups to DNA bases and forming inter- or intramolecular cross-bridges. Examples of alkylating agents and DNA crosslinkers include mitomycin C, nitrogen mustard derivatives (chlorambucil, mechlorethamine), and nitrosoureas (carmustine, lomustine, streptozotozin). Of all the anticancer drugs with antibacterial properties reviewed, as many as nine compounds belong to this class of chemicals (Table **1**, Fig. **2**), which suggests that interruption of DNA synthesis is a general and effective strategy to eliminate bacterial cells. Although the alkylating agents and DNA crosslinkers in Table **1** show moderate bactericidal and/or bacteriostatic effects against planktonic cells, their effective concentrations against biofilm, persister, and wound cells must be further elucidated, as in the case for mitomycin C.

Busulfan is a 1,4-butanediol dimethanesulfonate (Fig. **2**), and its two labile methanesulfonate groups are hydrolyzed releasing butyl carbonium ions which alkylate DNA forming intrastrand cross-links at 5′-GA-3′ and, to a lesser extent, 5′-GG-3’ sites, as well as mono-alkylation through a S_N2 reaction in which the nucleophilic guanine N7 attacks the carbon adjacent to the mesylate leaving group. This DNA damage may contribute to the antibacterial activity against *S. aureus*, *Enterococcus faecium*, and *P. aeruginosa* [73].

Carmustine (1,3-bis(2-chloroethyl)-1-nitrosourea, Fig. **2**), like other nitrosoureas, spontaneously forms 2-chloroethyl carbonium ions which alkylate DNA nitrogen bases. Displacement of the reactive chlorine atom leads to interstrand or intrastrand cross-links. The spontaneous degradation of carmustine also produces isocyanates that carbamoylate lysine residues of proteins inactivating DNA repairing enzymes [74]. This double effect on DNA and proteins must contribute to the genotoxic effect against *E. coli* cells [75].

Chlorambucil is an aromatic nitrogen mustard whose biological activity depends on the two 2-chloroethyl groups bound to the aminophenyl butyric acid (Fig. **2**). The presence of the aromatic group results in higher

selectivity, stability, and lower toxicity than mechlorethamine [74,76]. The reactivity of the 2-chloroethyl carbonium ions is similar to those derived from carmustine resulting in DNA alkylation. However, the DNA-damaging activity in *E. coli* is dependent on polymyxin B nonapeptide permeabilization [77].

Diaziquone is a synthetic aziridinylbenzoquinone with two carbethoxyamino groups and two alkylating aziridine rings (Fig. **2**). This drug needs metabolic reduction by the two-electron quinone reductase DT-diaphorase to trigger the aziridine DNA crosslinking process. This enzyme is also present in *E. coli* which may be the reason for the bacteriostatic activity [77].

Lomustine is a nitrosourea that bears a 2-chloroethyl group and a cyclohexylamine moiety (Fig. **2**). Activation of lomustine in the liver releases 2-chloroethyl carbonium ions which react mainly with the O6 position of guanine. Activation also produces isocyanates that carbamoylate lysine residues damaging cellular proteins [78]. This drug showed moderate bactericidal activity against *E. coli* and *S. aureus* [79].

Mechlorethamine was formerly used as a war gas. It is a nitrogen mustard that bears two beta-haloalkyl groups bound to a nitrogen atom (Fig. **2**) and it spontaneously produces a reactive azidirinc cation which reacts with the N7 position of the imidazole ring of guanine. Each chlorine atom can undergo an intramolecular nucleophilic substitution which leads to interstrand crosslinks between guanines. This type of DNA damage may be the reason for the bacteriostatic and bactericidal activities of this drug against several bacterial species [80].

Streptozotocin is an amino sugar antibiotic produced by *Stretomyces achromogenes*. It is the most prominent diabetogenic chemical agent in diabetes research due to its specific cytotoxicity in pancreatic beta-cells. It bears a D-glucosamine bound to the amide group of N-methyl-N-nitroso carbamic acid (Fig. **2**). It has been reported that this drug inhibited growth of Gram (+) and Gram (-) bacteria [81].

ThioTEPA is a organophosphorus alkylating agent derived from N,N',N''-triethylenephosphoramide (TEPA) (Fig. **2**) that releases highly reactive ethylenimine groups. These groups react with nucleophilic groups like the N7 position of the imidazole ring of guanine [82]. This drug has shown genotoxic activity in *E. coli* [75].

Figure 2

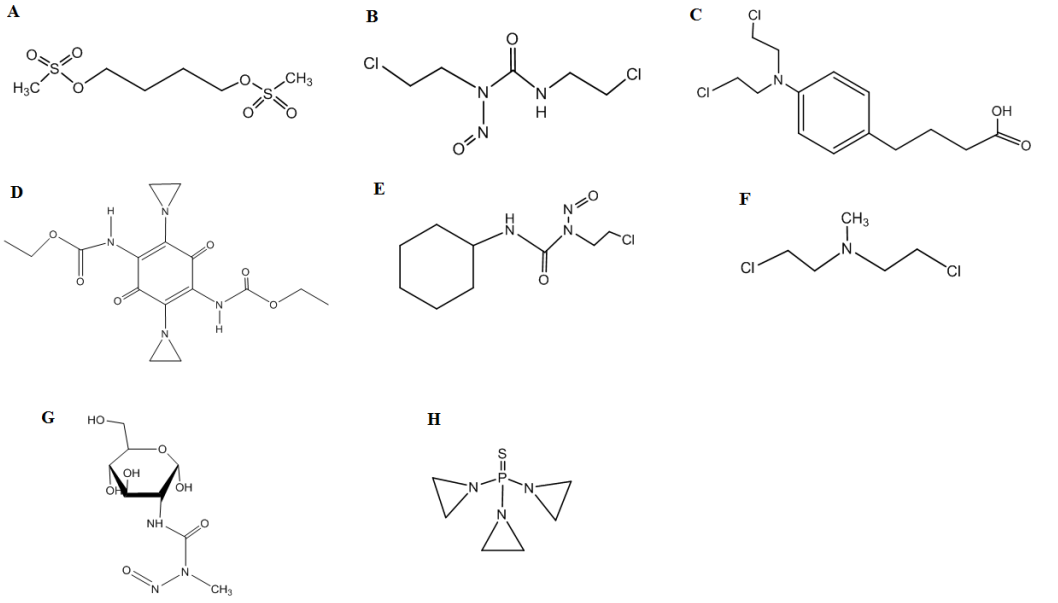

Figure 2) Structures of the Alkylating Agents and DNA Crosslinkers with antibacterial activities: **A)** busulfam, B) carmustine, C) chlorambucil, D) diaziquone, E) lomustine, F) mechloretamine, G) streptozotocin and H) thioTEPA

3.2 Antimetabolites

Another strategy to disrupt DNA synthesis is to poison the nucleotide pools, and this class of drugs is referred as antimetabolites. They include folic acid antagonists (methotrexate), purine analogs (mercaptopurine and thioguanine), and pyrimidine analogs (5-FU, gemcitabine, and azacitidine). Similar to 5-FU, the antimetabolites in Table **1** show promising bactericidal and/or bacteriostatic properties. In particular, mercaptopurine inhibits the growth of *Mycobacterium paratuberculosis* (a slow-growing strain) in a dose-dependent manner [83]. However, in contrast to 5-FU, their clinical efficacies as antibacterial drugs have yet to be evaluated.

The chemical structures of the antimetabolites in Table **1** are shown in Figure **3.** In cancer cells, most of these compounds have to be enzymatically activated by purine and pyrimidine metabolism to produce fraudulent nucleotides that deregulate the feedback controls of nucleotide biosynthetic pathways leading to a limitation of precursors for DNA and RNA synthesis. Some of them can also be incorporated into nucleic acids where they halt critical processes like DNA replication, transcription, or translation leading to cell death. These cytotoxic mechanisms may be similar in bacterial cells. Moreover, DNA and RNA are important components of biofilm architecture; thus, inhibition of nucleotide production may also impair biofilm formation as with *Streptococcus pneumoniae* [84].

Azacitidine is a 1,3,5-triazine ribonucleoside (Fig. **3**), its structure contains a 1,3,5-triazine ring instead of the normal 1,3-diazine pyrimidine ring of cytidine. Due to the presence of the hydroxyl group at position 2', it is mainly incorporated into RNA, and to a lesser extent into DNA. This drug showed significant antibacterial activity and reduced biofilm formation of *S. pneumoniae* [84].

Gemcitabine is a deoxycytidine analog, a pyrimidine 2'-deoxyribonucleoside which consists of a typical pyrimidine ring linked to a ribose which lacks a hydroxyl group at position 2'; instead, gemcitabine has two fluorine atoms (Fig. **3**). Gemcitabine was active against *Listeria*, *Bacillus*, *Enterococcus*, *Staphylococcus*, and *Streptococcus* species [85,86].

Thioguanine is a guanine analog and that bears a thioketone at position 6 instead of the ketone found in guanine (Fig. **3**). Similarly, mercaptopurine also bears a thioketone at position 6 (Fig. **3**). These are prodrugs and all the enzymes involved in their activation are present in human and bacterial cells. It seems likely that impairment of purine metabolism may be related to the potent bactericidal and bacteriostatic effects of these drugs [83,87,88].

Methotrexate is a folate analog with antineoplastic and immunosuppressant properties. It is a heterocyclic compound based on the 4-[(pteridin-6-ylmethyl)amino]benzoic acid skeleton conjugated with L-glutamate, methotrexate has the 2,4-diaminopteridin instead of the 2-amino-4-oxo-1H-pteridin moiety of folate (Fig. **3**). Because of this difference, it inhibits dihydrofolate reductase (DHFR), thereby limiting production of tetrahydrofolate which is necessary for thymidylate (dTMP) synthesis, an essential component of DNA. Several bacterial DHFRs are also inhibited by methotrexate which may be related to its bactericidal and bacteriostatic activities [89]. However, the immunosuppressant activity of this drug must be considered for repurposing efforts as an antibacterial compound.

Figure 3

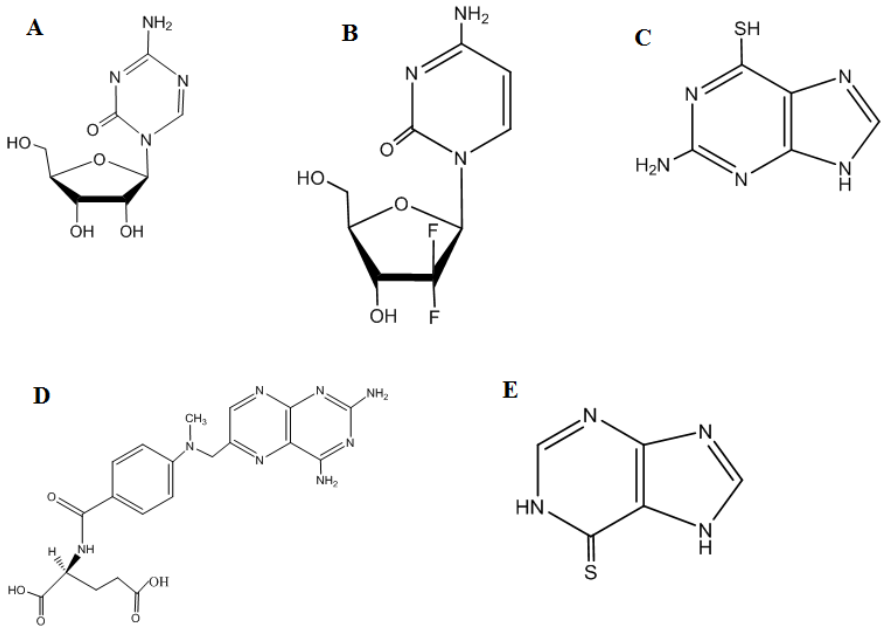

Figure 3) Structures of the antimetabolites with antibacterial activities: A) azacitidine, B) gemcitabine, C) thioguanine and D) methotrexate, E) mercaptopurine

3.3 Hormonal Modulators

Standard treatment for breast cancer includes hormonal therapy agents such as those that selectively bind estrogen receptors called selective estrogen-receptor modulators (SERMs). Depending on the tissue location, SERMs act as either agonists or antagonists, and thus allow stimulation or repression of estrogen-like actions in the local environment. Raloxifene, toremifene, and tamoxifen are three such compounds that show

significant antibacterial properties (Table **1**, Fig. **4**). Further tests should be conducted to investigate their uptake mechanism in bacteria (note that these hormonal modulators are fairly large and hydrophobic) and their roles in reducing pyocyanin production in *P. aeruginosa* or biofilm formation in *S. aureus* [90,91].

Raloxifene is benzothiophene derivative with a 4-hydroxyphenyl group at position 2 and, at position 3, it bears a phenyl methanone group bound to a piperidine moiety (Fig. **4**). Raloxifene is a SERM with estrogen antagonistic effects on the mammary gland. This drug strongly attenuates *P. aeruginosa* virulence in a *C. elegans* model of infection [90].

Tamoxifen is a stilbene derivative bearing, at position 8, a phenoxy group bound to a dimethylethanamine moiety, and at position 7, an enyl group (Fig. **4**). Toremifene has a similar structure but bears a chlorine atom attached to the enyl group (Fig. **4**). These drugs are also SERMs with anti-estrogen activity in the mammary tissue. Both drugs were active as antimicrobials: tamoxifen was active against *E. faecium*, *A. baumannii*, *S. aureus*, *E. coli* and *P. aeruginosa* [92,93] whereas toremifene inhibited biofilm formation by *S. aureus in vivo* and *in vitro* [91].

Figure 4

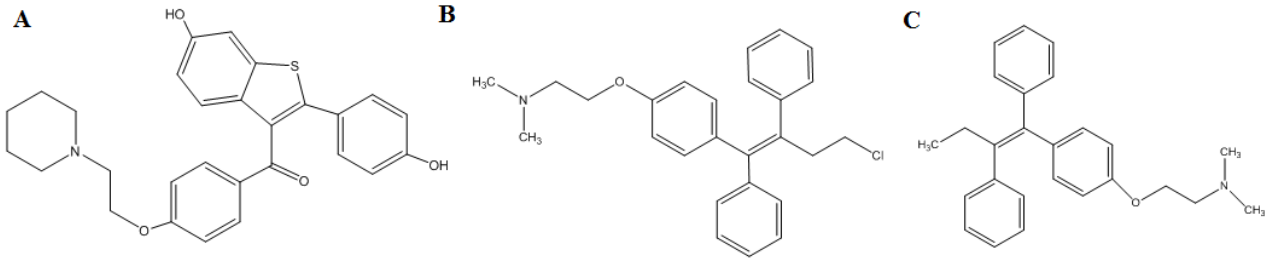

Figure 4) Structures of the hormone modulators with antibacterial activities: A) raloxifene, B) toremifene and C) tamoxifen

3.4 Radiomimetic Compounds

Bleomycin and streptonigrin are two radiomimetic compounds that showed antibacterial properties (Fig. **5**). The effects of radiomimetic compounds on cells are broad and poorly understood, as they generate free radicals and activate iron-dependent Fenton reactions to damage DNA and proteins. Therefore, radiomimetic compounds are unlikely to be suitable candidates for drug repurposing.

Bleomycin A2 is a complex glycopeptide antibiotic from *Streptomyces verticillus*, it contains a carbohydrate component attached to a hybrid peptide component (Fig. **5**). In the presence of iron and oxygen, it produces

superoxide and hydroxyl radicals that damage DNA and other cellular components. It showed antibacterial activity against several bacteria [94].

Streptonigrin is an aminoquinone antibiotic from *Streptomyces flocculus* (Fig. **5**), its cytotoxic activity is related to topoisomerase II inhibition and production of free radicals which damage cellular components. It showed antibacterial activity in *E. coli* [95].

Figure 5

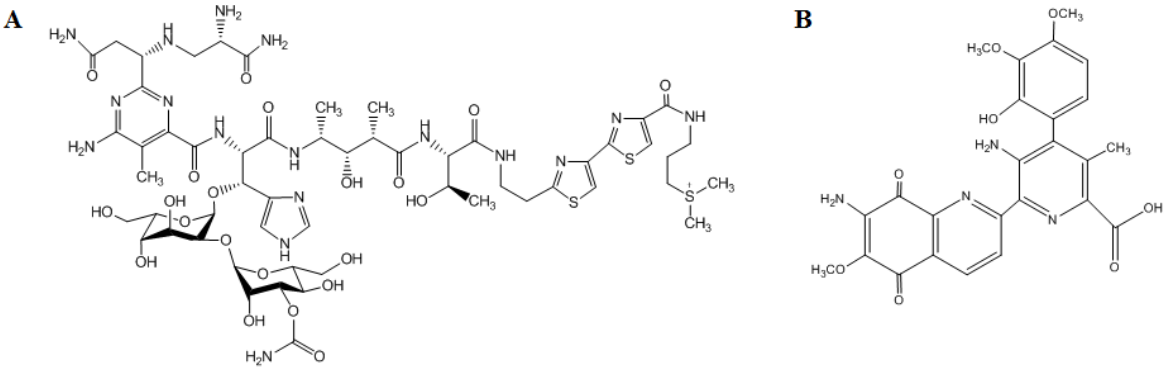

Figure 5) Structures of the radiomimetic compounds with antibacterial activities A) bleomycin and B) streptonigrin

3.5 Tyrosine Protein Kinase Inhibitors (TKI)/Signal Transduction Inhibitors

Gefitinib, imatinib, and ibrutinib target host cell proteins relevant to the infectious processes of intracellular pathogens like *M. tuberculosis*, *Mycobacterium smegmatis*, *Mycobacterium marinum*, or *Shigella flexneri* (Table **1**, Fig. **6**) [96-98]. Thalidomide also modulates host cell proteins to stimulate an immune response against *E. coli* and *P. aeruginosa* [99,100]. Although these drugs do not show direct antibacterial activity, repurposing strategies may improve host cell capacity to fight infections. It has been shown that tyrosine kinases mediate entry and intracellular survival of some *Mycobacterium* spp. Remarkably, imatinib showed synergistic effects when combined with current first-line anti-tuberculosis drugs like rifampicin and rifabutin [97], and inhibition of the Abelson Tyrosine Kinase (Abl) with imatinib increased lysosomal acidification, facilitating *M. tuberculosis* cell destruction by human macrophages [101]. Whereas inhibition of the epidermal growth factor receptor (EGFR) tyrosine kinase with gefitinib has been proposed to increase autophagy resulting in enhanced *M. tuberculosis* cell elimination [96].

Gefitinib is a quinazolinamine with the heterocyclic aromatic quianazoline moiety substituted by one 3-chloro-4-fluorophenylamino group at position 4, one methoxy group at position 7, and a 3-morpholin-4-yl-propoxy group at position 6 (Fig. **6**). Gefitinib inhibits the epidermal growth factor receptor (EGFR) tyrosine kinase by binding to the ATP-binding site of the enzyme, thereby reducing the activation of downstream

signaling cascades that promote cell proliferation in some cancer cells and that, in macrophages infected with *M. tuberculosis*, contribute to bacterial infection [98].

Ibrutinib [1-[(3R)-3-[4-amino-3-(4-phenoxyphenyl)pyrazolo[3,4-d]pyrimidin-1-yl]piperidin-1-yl]prop-2-en-1-one, Fig. **6**] is an irreversible inhibitor of Bruton's tyrosine kinase (BTK) because it forms a covalent bond with the cysteine-481 residue in the active site of the kinase. Ibrutinib did not show direct antibacterial activity; however, targeting host cell molecules, it reduced spread of infection by *S. flexneri* in intestinal cell lines [96].

Imatinib is a n-phenylbenzamide bearing methylpiperazine, pyrimidine, and pyridine moieties (Fig. **6**). It specifically inhibits the Bcr-Abl tyrosine kinase, a deregulated kinase created by the Philadelphia chromosome abnormality in some leukemias. It binds close to the ATP binding site locking the kinase in an inhibited conformation. Like gefitinib and ibrutinib, imatinib also shows antibacterial activity only in the context of host cell infection, reducing bacterial burden in macrophages infected with *M. tuberculosis* [97].

Thalidomide is a dioxopiperidinyl isoindolone (Fig. **6**) that inhibits production of two important extracellular signaling proteins in mammalian cells: the tumor necrosis factor-alpha and the vascular endothelial growth factor. This drug shows antimicrobial activity against *E. coli* and *P. aeruginosa* during experimental sepsis; this effect was attributed to immunomodulatory effects on the host [99,100].

Figure 6

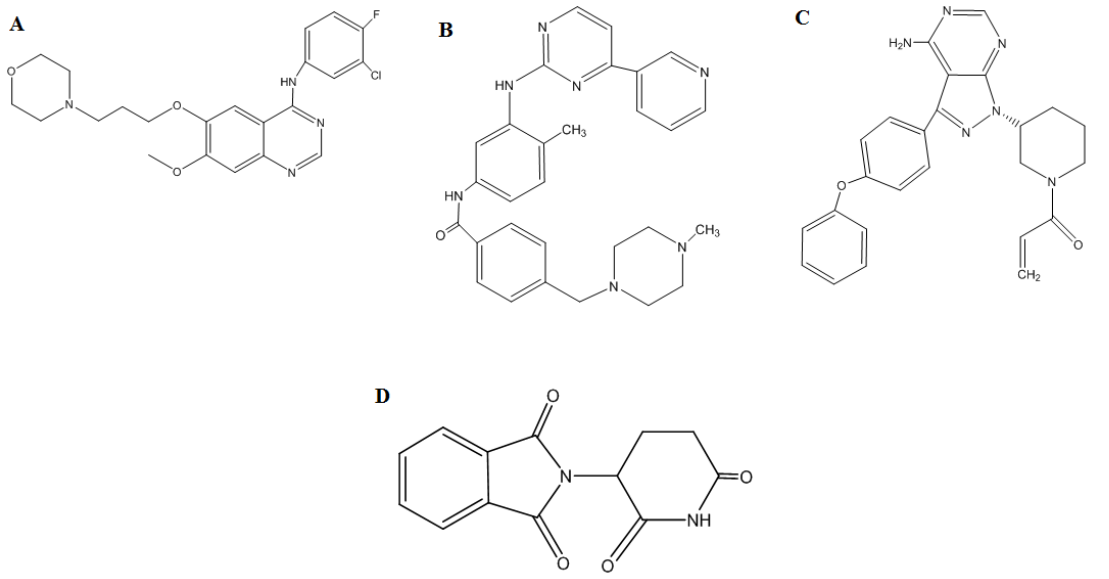

Figure 6) Structures of the tyrosine protein kinase inhibitors (TKI)/signal transduction inhibitors with antibacterial activities: A) gefitinib, B) imatinib, C) ibrutinib and D) thalidomide

3.6 Topoisomerase Inhibitors

Daunorubicin, doxorubicin, epirubicin, and idarubicin are anthracyclines with antibacterial activity. The affinity for the DNA double strand may explain the inhibitory effect of these anthracyclines on the essential Mycobacterial enzyme DnaG (Table **1**) [102]; this potential therapeutic target is a DNA-dependent RNA polymerase that synthesizes short oligonucleotides during DNA replication. Anthracyclines are natural or synthetic compounds with a skeleton of 7,8,9,10-tetrahydro-tetracene-5,12-quinone with one to four saccharide moieties attached by glycosidic linkage usually to the ring D of the aglycone (Fig. **7**) [103]. The saccharide moiety of daunorubicin, doxorubicin, epirubicin, and idarubicin is 4-amino-5-hydroxy-6-methyloxan; these drugs bear different groups attached to the polyhydroxylated quinones whereas epirubicin is the 4'-epi-isomer of doxorubicin (Fig. **7**). Their planar hydrophobic structure facilitates the intercalation between adjacent base pairs in the DNA-topoisomerase complex turning topoisomerases into DNA-damaging agents. Topoisomerases are enzymes that release the tension of the DNA double helix during DNA replication and transcription; to this end, topoisomerases break one or two strands and after untangling the double helix, they religate the broken strands. Besides inhibiting topoisomerase activity, anthracyclines promote DNA breaks by increasing the rate of DNA cleavage or preventing ligation [103]. The bacterial topoisomerase inhibitors fluoroquinolones have been used as antibacterial drugs in the clinic; however, resistance can arise [104]. The development of new bacterial topoisomerase poisons may take advantage of the experience and knowledge of anthracycline synthesis and their clinical use. Although the promising antibacterial activity of topoisomerase inhibitors used in anti-cancer chemotherapy (Table **1**) [102,105-107] stimulates repurposing strategies, cardiotoxicity of anthracyclines must be carefully considered.

A different type of topoisomerase inhibitor with antibacterial activity is etoposide. This is a semi-synthetic derivative of podophyllotoxin, a tetralin lignan in which the benzene moiety of the tetralin skeleton is fused to a 1,3-dioxolane and the cyclohexane is fused to a butyrolactone (pyrrolidin-2-one) (Fig. **7**). This drug was an efficient antibacterial agent against *Kocuria rhizophila* but did not affect growth of *E. coli*, *P. aeruginosa*, or *Klebsiella pneumoniae* [108]; this illustrates the bacterial heterogeneity in topoisomerases and DNA repair mechanisms.

Figure 7

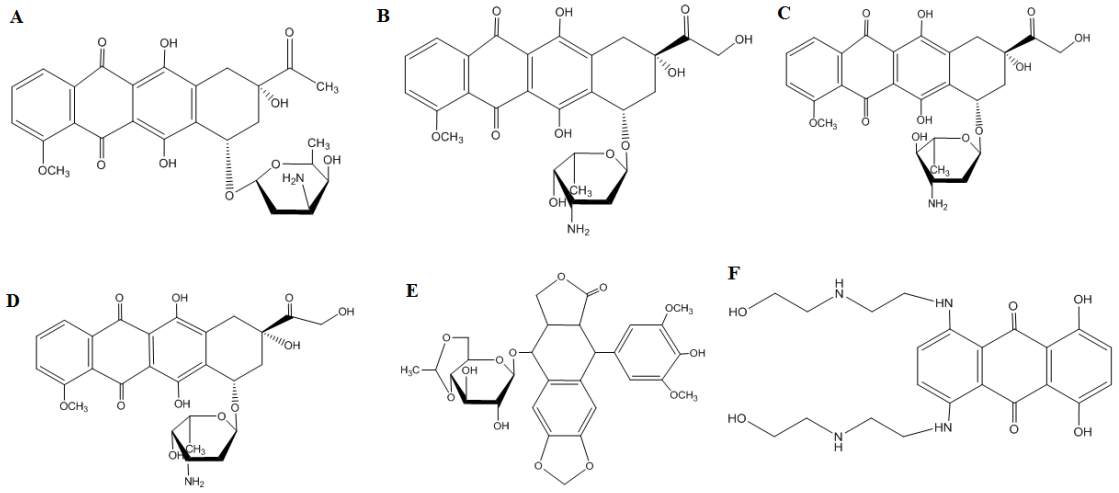

Figure 7) Structures of the topoisomerase inhibitors with antibacterial activities: A) daunorubicin, B) doxorubicin, C) epirubicin, D) idarubicin,E) etoposide, and F) mitoxantrone

3.7 Miscellaneous

Aminolevulinic acid [5-amino-4-oxopentanoic acid, Fig. **8**] is converted into protoporphyrin IX (PpIX) by enzymes of the heme biosynthetic pathway. PpIX is a photosensitizer, when exposed to light of appropriate energy in the presence of oxygen; it produces singlet oxygen which in turn produces superoxide and hydroxyl radicals damaging cellular components. As the protoporphyrin IX producing pathway is highly conserved, aminolevulinic acid shows antibacterial activity upon light exposure against *S. aureus*, *S. epidermidis* and *P. aeruginosa* [109-111].

The drug Mesna (2-sulfanylethanesulfonate) (Fig. **8**), an adjuvant in cancer chemotherapy used for the detoxification of the urotoxic derivatives of ifosfamide and cyclophosphamide, also shows bacteriostatic activity against *P. aeruginosa* [112].

Tirapazamine is an aziridinylquinone it is activated under hypoxic conditions to form a free radical at position 1, where the oxido group is attached (Fig. **8**), which damages DNA [113]. Tirapazamine is very active against *E. coli*, *S. aureus* and *Clostridium difficile* under hypoxic conditions [114].

Zoledronic acid is a bisphosphonate bearing an imidazole ring (Fig. **8**). It reduces bone resorption and turnover in osteoclasts. It promotes host defense against *Chlamydia pneumoniae* infections working as an immune regulator drug [115].

Figure 8

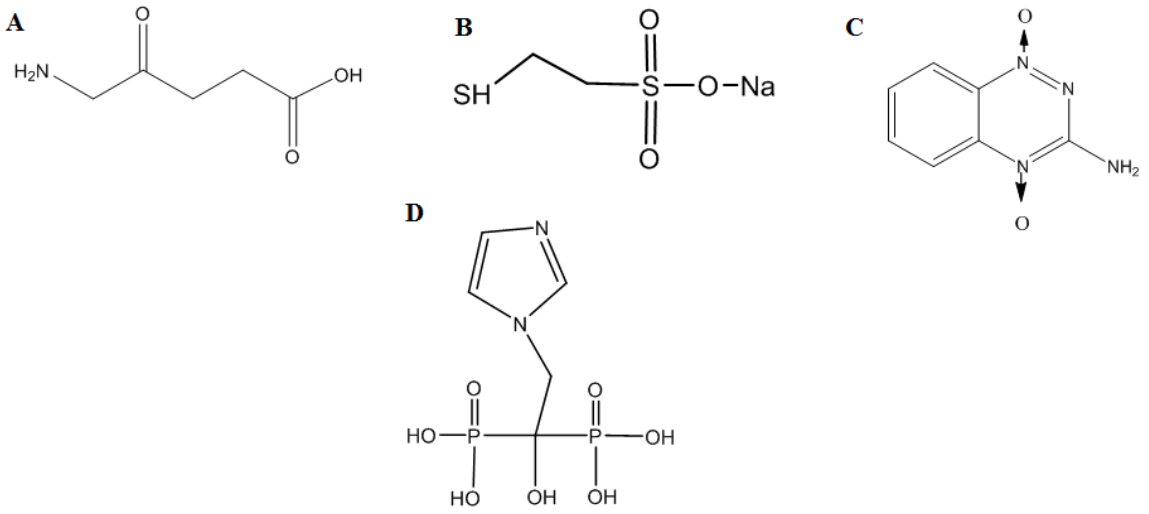

Figure 8) Structures of the miscellaneous anticancer compounds with antibacterial activities: A) aminolevulinic acid, B) mesna, C) tirapazamine and D) zoledronic acid.

4. DISCUSSION

In this review, we present a compilation of anticancer compounds with known antimicrobial activities, focusing on the properties of three of them, the antimetabolite 5-fluorouracil, the non-redox iron analogue gallium nitrate, and the potent DNA cross-linker mitomycin C. These three compounds present remarkable antibacterial properties, including broad spectrum activity, as well as the ability to kill biofilm cells, and for mitomycin C, the ability to kill persister cells; their respective applications for combating bacterial infections have been appropriately indicated [6,40,70,116]. 5-FU clinical trials in humans for antimicrobial activity were successful, demonstrating 5-FU is an effective coating agent to prevent the bacterial colonization of central venous catheters with even better effects than the positive control, while gallium nitrate clinical trials for efficacy in cystic fibrosis patients infected with *P. aeruginosa* are currently underway. In the case of mitomycin C, no clinical trials for antimicrobial efficacy have been done yet, likely because the strong potential for clinical efficacy against bacterial infections was very recently discovered [70,71].

In order to advance the repurposing of anticancer drugs as antimicrobials, it is necessary to further investigate the potency and limitations of such compounds in both animal models and clinical trials. For example, clinical trials with a larger patient sample size are necessary for testing the effects of catheter coating with 5-FU, and animal models should be implemented to evaluate the effects of 5-FU as a prophylactic for prevention of external infections as well as an adjuvant against biofilms in wounds. The use of 5-FU as an epidermal

prophylactic is a straightforward study because 5-FU is administered topically to treat skin cancers, and models of skin infections [117,118] and thermally induced wounds [51] are already established in mice. In the case of gallium nitrate, current clinical trials to test efficacy in cystic fibrosis patients involves intravenous administration; hence, the local concentration of gallium that reaches bacteria in the lungs is severely limited and may be below therapeutic levels. Therefore, we propose that an intranasally administered regimen should be tested. Additionally, since gallium is administered intravenously, its activity against bacteremia and bloodstream infections should be determined in mouse models to characterize *in vivo* potency, since *in vitro* studies with *A. baumannii* [38] and *P. aeruginosa* [49] showed increased gallium resistance of clinical strains cultured in human serum. Additionally, potential applications for gallium against epidermal and wound infections should be explored on the premise that GaM effectively protects thermally induced burns against bacterial colonization [51]. Mitomycin C kills biofilm and persister cells, so its potential applications for treating chronic infections are attractive. It was demonstrated that mitomycin C improves survival of the nematode *C. elegans* against an EHEC infection, so a mammalian infection model is logically the next step. Similarly, mitomycin C efficacy was demonstrated *in vitro* against *B. burgdorferi*, the causative agent of Lyme disease, so it is pertinent to test whether this activity is maintained in a Lyme disease infection model, which is available in both mice and monkeys [119]. In addition, the discovery of the antipersister activity of mitomycin C serves as a paradigm in the search for other anticancer drugs with activity against these dormant persisters. Mitomycin C is effective against persisters due a series of drug mechanics that are independent of cell metabolism. This mechanism includes uptake via passive diffusion [52], followed by the reduction of its quinone functional group [54] within the naturally reducing environment of the cytoplasm [53], which triggers a series of spontaneous transformations, ending in the opening of the aziridine ring to produce an unstable quinone methide that alkylates DNA [53]. Hence, based on this mechanistic background, we speculate that other anticancer compounds containing an activated aziridine ring should have activity against persister cells. As such, we propose that thioTEPA and diaziquone (Table **1**) should be investigated for antibacterial activity. Since thioTEPA enters cells through passive diffusion [82,120] and contains three activated aziridine rings (Fig. **2**), which spontaneously react with DNA [121] via a ring solvolysis reaction [83]. In agreement, thioTEPA displays a non-specified level of lethality against *E. coli* [75]; however, glutathione may serve as a resistance mechanism against alkylation [82]. Similarly, diaziquone (Fig. **2**) should enter cells through passive diffusion due to the presence of two carbethoxyamino groups which facilitate transport across the blood-brain barrier [75]. Diaziquone contains a quinone moiety and two alkylating aziridine rings [78], similar to mitomycin C with a quinone moiety and an aziridine ring [54]. Diaziquone is reductively activated by NADH and NADPH to initiate the aziridine DNA crosslinking mechanism [78]. Additionally, *in vivo* activity of diaziquone has been demonstrated in *E. coli* K-12 via lack of growth [122]. Relatedly, despite lacking an aziridine ring, tirapazamine (Fig. **8**) is also considered an aziridinylquinone [113] and it has antimicrobial activity [114]. The bactericidal activities of mitomycin C and tirapazamine suggest the importance of the aziridinylquinone structure for killing bacteria. Apaziquone and triaziquone are two other aziridinylquinones, so we anticipate that these compounds should also be active against persisters.

If these compounds are proven effective against persister cells, this would suggest the importance of investigating anticancer drugs with the aziridinylquinone structure as candidates to repurpose for clinical use against bacterial infections.

Table **1** summarizes several additional anticancer compounds with antibacterial effects including diverse alkylating agents, DNA crosslinkers, antimetabolites, immune modulators, signal transduction inhibitors, and radiomimetic compounds, demonstrating the large variety of antibacterial mechanisms of action. Moreover, since natural products are the source of most antibiotics and since the use of natural compounds for the treatment of cancer has been extensively studied [123-125] (approximately 70% of anticancer compounds are based on natural products or derived from their structural scaffolding [126]), it is expected that several new natural compounds will be derived from plants with both remarkable anticancer properties and antimicrobial activities. Accordingly, the compound berberine is an alkaloid with potent apoptotic properties that interferes with tumor progression and metastasis [127], and also shows antibacterial activity against *Streptococcus agalactiae* (MIC 78 μg/mL)[128]. Curcumin is a polyphenolic compound with the potential to release electrons facilitating its scavenging activity; this property may be the main responsible for its anticancer effect [125]. Curcumin exerts cytotoxic effects on cancer stem cells and has antitumor activity [129,130]; concomitantly, several studies have reported the broad-spectrum antibacterial activity (bactericidal, antivirulence, and antibiofilm effects) of curcumin across diverse bacterial pathogens including *E. coli*, *Helicobacter pylori, P. aeruginosa,* MRSA strains, and periodontopathic bacteria [131,132]. Genistein is an isoflavonoid effective against several tumor types such as breast, prostate, colon, gastric, and ovarian cancer [125]. This metabolite also shows antibacterial activity against different bacterial species such as *E. coli*, *P. aeruginosa*, *Proteus mirabilis*, *K. pneumoniae*, *A. baumannii*, *S. aureus*, *Enterococcus faecalis* and *Bacillus subtilis* [133]. Resveratrol is a phytoalelexin that suppresses cell proliferation, induces apoptosis and suppresses metastasis and invasion in different cell lines [134]. This compound exhibits antibacterial properties against *Propionibacterium acnes*, *Haemophilus ducreyi*, *Arcobacter butzleri*, *Arcobacter cryaerophilus*, and *E. coli.* In addition, resveratrol and related compounds inhibit *S. aureus* virulence [135]. Recently, it was shown that reactive oxygen species production and the suppression of FtsZ expression and Z-ring formation is correlated with its antibacterial activity [136,137]. Hence, multiple plant metabolites with diverse action mechanisms exhibit both anticancer and antibacterial properties.

The evidence presented above suggests that a plausible alternative to combat bacterial infections, including those by multidrug resistant pathogens, is to repurpose anti-cancer drugs and highlights the potential of those drugs with better characterized antimicrobial properties, such as gallium compounds, 5-FU, and the unique properties of mitomycin C regarding persister elimination., We hope this work will encourage further basic research that could help us to improve our current knowledge about the antibacterial properties of some of these drugs, to better characterize their molecular mechanisms in microorganisms (since they may be not always the same than in cancer cells) to discover new anticancer compounds with promising antibacterial

potential, to search for possible synergistic interactions between anticancer compounds, traditional antibiotics and/or antivirulence compounds and to ultimately promote their utilization as antibacterials in the clinic, which may decrease the high morbidity and mortality associated to currently untreatable recalcitrant bacterial infections.

5. ACKNOWLEDGMENTS

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Table 1. Anticancer Drugs That Possess Antibacterial Activities.

Drug	Mode of action	Currently used to treat	Antibacterial property	Reference(s)
Alkylating Agents and DNA Crosslinkers				
Busulfan	Alkylates DNA to form crosslinks interfering with DNA replication and transcription.	Chronic myelogenous leukemia	Showed both bacteriostatic and/or bactericidal activity against *S. aureus*, *E. faecium*, and *P. aeruginosa* at 0.5 mg/ml.	[73]
Carmustine	Alkylates DNA and RNA to form crosslinks inhibiting DNA replication and transcription	Brain tumors, Hodgkin's and non-Hodgkin's lymphomas Multiple myeloma	Genotoxic in *E. coli* (concentration not specified in the original article)	[75]
Chlorambucil	Nitrogen mustard that alkylates DNA to form crosslinks thereby inhibiting DNA replication and transcription. It also induces mispairing of the nucleotides leading to mutations.	Chronic lymphocytic leukemia, Hodgkin's and non-Hodgkin's lymphomas	Cannot permeabilize *E. coli* outer membrane alone, but can be permeabilized with polymyxin B nonapeptide. *E. coli* growth is reduced with 1 mM chlorambucil and 10 μg/ml polymyxin B nonapeptide.	[77]
Diaziquone	Alkylates DNA to form crosslinks	Broad activity	Showed bacteriostatic activity in *E. coli*.	[78]
Lomustine	Alkylates DNA to form crosslinks, this DNA damage interferes with DNA synthesis and transcription.	Brain tumors, Hodgkin's lymphoma	Showed moderate bactericidal activity against *E. coli* and *S. aureus* at 500 μM/ml.	[79]
Mechlorethamine	Nitrogen mustard that alkylates DNA to form crosslinks, this DNA damage interferes with DNA synthesis and transcription. It also induces mispairing of the nucleotides leading to mutations.	Bronchogenic carcinoma, chronic lymphocytic leukemia, chronic myelogenous leukemia, Hodgkin's and non-Hodgkin's lymphomas, malignant pleural, pericardial, and peritoneal effusions, mycosis fungoides	Showed both bacteriostatic and bactericidal activity against numerous bacterial species (MIC 125 – 1000 μg/ml).	[80]

Mitomycin C	Potent DNA alkylating agent, it causes crosslinks and interferes with DNA replication and transcription.	Upper gastro-intestinal cancers, anal and breast cancers, superficial bladder tumors.	Able to kill *E. coli, S. aureus* and *P. aeruginosa* planktonic (MIC 50 0.2-15 μg/ml) and biofilm cells as well as persister cells. Killed *B.burgdorferi* stationary phase cells and persisters (MIC 0.2 μg/ml).	[70,71, 138]
Streptozotocin	Lipophilic compound, alkylates DNA to form crosslinks	Metastatic cancer in pancreatic islet cells	Inhibited growth of Gram (+) and Gram (-) bacteria.	[81]
ThioTEPA	Alkylates DNA to form crosslinks interfering with DNA replication and transcription.	Bladder, breast, and ovarian cancers, malignant pleural, pericardial, and peritoneal effusions	Genotoxic in *E. coli* (concentration not specified in the original article)	[75]
Antimetabolites				
Azacitidine	Cytosine analog that gets incorporated into DNA/RNA to disrupt transcription and translation.	Myelodysplastic syndromes	Showed minor bacteriostatic activity against *S.pneumoniae*, but showed significant inhibition of biofilm formation at <500 μM.	[84]
5-Fluorouracil	Irreversible inhibitor of thymidylate synthase which results in the inhibition of thymidylate production from uracil, thereby inhibiting DNA synthesis. It can also be incorporated into RNA interfering with RNA processing and translation.	Superficial basal cell carcinomas, colon, esophageal, gastric, rectum, breast, biliary tract, stomach, head and neck, cervical, pancreas, renal cell, and carcinoid cancers	Growth and biofilm inhibition of Gram (+) and (−) bacterial species including *E. coli* (10 μM), *P. aeruginosa* and *S. epidermidis* (MIC 0.5-1 μg/ml).Repression of QS and virulence *in P. aeruginosa* (10 μM). Successful in clinical trials in humans, as external coating of central venous catheters that inhibited colonization by microbes and prevented bloodstream infections.	[22-28]
Gemcitabine	Cytidine analog that inhibits deoxycytidylate deaminase and ribonucleotide reductase leading to	Breast, ovarian, and pancreatic cancers, non-small cell lung	Very active against *Listeria*, *Bacillus*, *Enterococcus*,	[85,86]

	inhibition of DNA synthesis. When incorporated into DNA, it causes termination of DNA synthesis.	cancer	*Staphylococcus*, and *Streptococcus* species (MIC 0.001–1.0 μM).	
Mercaptopurine	This compound and its cellular derivatives inhibit several steps of the purine nucleotide synthesis	Acute lymphoblastic leukemia	Showed both bacteriostatic (1-4 μg/ml) and bactericidal (8-32 μg/ml) activity against different strains of *M. paratuberculosis*.	[83]
Methotrexate	Competitive inhibitor of dihydrofolate reductase, thereby impairing purine and pyrimidine synthesis.-	Acute lymphoblastic leukemia, breast, head and neck, and lung cancers, gestational trophoblastic disease, mycosis fungoides, non-Hodgkin's lymphoma, osteosarcoma	Active against *S. aureus* (MIC 10 μg/L). Showed bacteriostatic activity against *S. aureus* and *P. aeruginosa* at 9 mg/ml	[89]
Thioguanine	Sequential blockade of the utilization and synthesis of the purine nucleotides	Acute non-lymphocytic leukemia	Bactericidal effect against *S. aureus* isolated from non-leukemic children (MIC 16 μg/ml). Showed bacteriostatic activity against *E. coli* and *Salmonella typhimurium* (0.25 μM).	[87,88]
Hormonal Modulators				
Raloxifene	Induces the effect of estrogen by binding estrogen receptors on bone and cholesterol metabolism. It also works as estrogen antagonist on mammary gland, which results in a blockage or change in the expression of estrogen dependent genes.	Reduces breast cancer risk in post-menopausal women	Reduced pyocyanin production in *P. aeruginosa*. Raloxifene (100 mg/L) also promoted the survival of *C. elegans* infected with *P. aeruginosa* to 75-95%.	[90]
Toremifene	Binds estrogen receptors to block estrogen binding on mammary gland tumors.	Breast cancer	Inhibited *in vivo* biofilm formation by *S. aureus* at rat subcutaneous catheter by 57%. *In vitro* BIC-2 for *S. aureus* was 3.5 μM.	[91]
Tamoxifen	Selectively binds to estrogen receptors, inducing a conformational change in the receptor which eventually results	Metastatic breast cancer in women and ductal carcinoma in men (*in situ*)	Exhibited MICs of 8 μg/ml against *E. faecium* and *A. baumannii*. Also showed bactericidal effect	[92, 93, 139]

	in a blockage or change in the expression of estrogen dependent genes on mammary gland tumors.		against MRSA, *E. coli* K1, and *P. aeruginosa* PAO1.	
Radiomimetic Compounds				
Bleomycin	Radiomimetic compound that promotes DNA damage thereby inhibiting DNA synthesis.	Hodgkin's and non-Hodgkin's lymphomas, testicular, ovarian, and cervical cancers	At 1 mM Inhibited growth of several Gram (+) and Gram (-) bacteria including: *S. epidermidis*, *K. pneumoniae and E. coli*	[94]
Streptonigrin	Damages cells using free radicals and iron-activated Fenton reactions	Used until 1971 to treat lymphoma and solid tumors	Showed bactericidal activity in *E. coli*.	[95]
Signal Transduction Inhibitors				
Gefitinib	Inhibits the epidermal growth factor receptor (EGFR) tyrosine kinase, thereby inhibiting the Ras signal transduction cascade.	Non-small cell lung cancer	Computationally predicted to be an antibacterial agent. Showed antibacterial activity exclusively in the context of macrophage infection by *M. tuberculosis*. At 10 μM reduced 50% bacterial proliferation in cultured macrophages and at 100 mg/kg/day reduced 36% CFU in mouse lungs.	[98,140]
Ibrutinib	Inhibits the BTK tyrosine kinase, thereby inhibiting signaling through the B-cell receptors and reducing malignant B-cell proliferation and survival.	Mantle cell lymphoma and chronic lymphocytic leukemia.	At 1 μM impaired spread of infection by reducing actin-based motility of *S. flexneri* during infection of intestinal cell lines.	[96]
Imatinib	Inhibits the Bcr-Abl tyrosine kinase created by the Philadelphia chromosome abnormality, thereby inhibiting several signaling pathways which reduce proliferation and induce apoptosis in Bcr-Abl positive cells.	Philadelphia chromosome positive chronic myeloid leukemia, acute lymphoblastic leukemia, chronic eosinophilic leukemia, dermatofibrosarcoma protuberans, and malignant gastrointestinal stromal tumors	Showed antibacterial activity exclusively in the context of macrophage infection by *M. tuberculosis*: at 10 μM, imatinib reduced bacterial CFU/mL in macrophages lysates by 6 fold and at 66.7 mg/kg/day, imatinib caused a 185-fold reduction in the bacterial	[97]

			burden in mouse lungs. At 100 mg/kg/day, imatinib reduced the *M. marinum* burden in mouse liver, lung, tail, and spleen by 13-64 fold.	
Thalidomide	VEGF inhibitor displaying anti-angiogenic activity. It also inhibits biosynthesis of tumor necrosis factor alpha (TNF-α).	Multiple myeloma	Active against clinical isolates of *E. coli* (MIC 712.5 mg/L). Prevented the evolution of sepsis with *E. coli*. Prolonged survival in experimental sepsis by multidrug resistant *P. aeruginosa* attributed to immunomodulatory effects.	[99,100]
Topoisomerase Inhibitors				
Daunorubicin	DNA intercalating agent. Inhibits topoisomerase II activity and cause DNA breaks.	Acute non-lymphocytic leukemia (myelogenous, monocytic, erythroid) of adults and acute lymphocytic leukemia of children and adults	Very active against *M. smegmatis* (MIC 0.5 μM), active against *M. tuberculosis* (MIC 1.25 μM). Antibacterial activity against *B. subtilis* and the protoplast type L-forms of *E. coli* B, and *P. mirabilis* (MIC 125 μg/ml)	[102,105]
Doxorubicin	Inhibits topoisomerase II via intercalation into DNA to ultimately block DNA replication. Also generates free radicals that damage DNA and cells.	Acute lymphoblastic leukemia, acute myeloid leukemia, Hodgkin's and non-Hodgkin's lymphomas, neuroblastomas, breast, gastric, and ovarian cancers, small cell lung cancer, soft tissue and bone sarcomas, thyroid cancer, transitional cell bladder cancer, Wilms tumor	Retarded the growth of *S. aureus* (1 μg/ml), *S. epidermis* (1 μg/ml), and *Streptococcus sanguis* (10 μg/ml) in broth cultures. Active against *M. smegmatis* (MIC 8 μM) and *M. tuberculosis* (MIC 5 μM).	[102,106]
Epirubicin	Inhibits topoisomerase II via intercalation into DNA, to ultimately block DNA replication.	Breast cancer	Active against *Mycobacterium chelonae* (MIC 7.25 mg/L) and	[107]

	Also generates free radicals that damage DNA and cells.		*Mycobacterium abscessus* (MIC >7.25 mg/L).	
Etoposide	Causes breakage of DNA strands by forming a ternary complex with DNA and topoisomerase II.	Small cell lung cancer, testicular cancer, lymphoma, non-lymphocytic leukemia, and glioblastoma multiforme	Very active against *K. rhizophila* (MIC 6.2 mg/L, formerly known as *Sarcina lutea*), *S. faecalis* (50 mg/L), and *B. subtilis* (50 mg/L); moderately active against *S. aureus* (12.5–16 mg/L). Not active against *E. coli*, *P. aeruginosa*, and *K. pneumoniae*.	[108]
Idarubicin	DNA intercalating agent. Inhibits topoisomerase II activity and cause DNA breaks.	Acute myeloid leukemia	Very active against *M. smegmatis* (MIC 0.6 μM), active against *M. tuberculosis* (MIC 80 μM).	[102]
Miscellaneous				
Aminolevulinic acid	Promotes the accumulation of protoporphyrin IX, and after irradiation with visible light promotes the synthesis of ROS and induces apoptosis.	Visualize bladder cancer and photodynamic therapy to treat superficial basal cell carcinoma (BCC) and Bowen's disease	Able to inhibit biofilm formation of *S. aureus* and *S.epidermidis* (40 mM and light exposure) alone or in combination with gentamicin. Treatment of *S. aureus* and *P.aeruginosa* with 10 mM aminolevulinic acid and 90 min light activation (635 nm) eradicated cells.	[109-112]
Gallium nitrate	Non-redox iron analog that interferes with iron metabolism.	Cancer related hypercalcemia	Growth inhibition of several recalcitrant bacterial pathogens (including clinical isolates with multidrug resistance such as *P. aeruginosa* (5 μM), *A.baumanii* (10 μM) and *M. tuberculosis* (10 μM). Effective in animal models to treat acute and	[33-40]

			chronic mouse infections of *P. aeruginosa* and *A. baumannii* infections in caterpillars (1.2 mmol/kg) and mice (25 mg/kg).	
Mesna	Mucolytic agent that acts as an antioxidant. It reacts with the urotoxic derivatives of ifosfamide and cyclophosphamide resulting in detoxification.	Hemorrhagic cystitis	At 1% showed bacteriostatic activity against *P. aeruginosa*.	[141]
Tirapazamine	Activated to become a toxic radical under hypoxic conditions, such conditions are common in solid tumors.	Experimental cancer drug, currently in clinical trials for several types of solid tumors.	Showed antibacterial activity against *E. coli* (MIC 10 μM aerobic and 0.37 μM anaerobic, against *S. aureus* (MIC 2 μM anaerobic) and *C. difficile* (MIC 42 nM anaerobic conditions).	[114]
Zoledronic acid	Slows down bone resorption, allowing the bone-forming cells time to rebuild normal bone.	Hypercalcemia of malignancy. Prevents skeletal fractures in patients with multiple myeloma and prostate cancer. Inhibit bone metastasis in patients with breast cancer.	At 10 μM promoted host defense against *C. pneumoniae* infection.	[115]

MIC, minimal inhibitory concentration; BIC-2, biofilm inhibitory concentration that inhibits biofilm formation 2-fold.

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