BETA-LACTAMS AND THEIR POTENTIAL USE AS NOVEL ANTICANCER CHEMOTHERAPEUTICS DRUGS

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1. ABSTRACT

The discovery of natural and synthetic antibiotics is one of the most important medical breakthroughs in human history. Many diseases, such as bacterial meningitis, pneumonia, and septicemia, are now curable with the use of antibiotics. Antibiotics are efficacious, generally well tolerated in patients, and have a low toxicity level. It is for these reasons antibiotics remain an attractive target for drug discovery. Traditional beta-lactam antibiotics (e.g. penicillins, penems, cephalosporins) have a bicyclic ring structure that is conformationally rigid and functions to inhibit bacterial cell wall synthesis. In addition to the bactericidal action of antibiotics, it has been discovered that many antibiotics are capable of inhibiting tumor cell growth. There are currently many antitumor antibiotics approved for cancer therapy, which work to inhibit tumor cell growth by DNA intercalation. The use of beta-lactams as prodrugs has also met with success by aiding delivery of the chemotherapeutic directly to tumor sites. Recently, a novel class of N-thiolated monobactams, so termed because they possess a monocyclic ring instead of the bicyclic ring, has been found to induce apoptosis potently and specifically in many tumor cell lines but not in normal, non-transformed cell lines. Other beta-lactams, such as the polyaromatics, have been found to slow or inhibit tumor cell growth, and the 4-alkylidene beta-lactams are capable of inhibiting matrix metalloproteinases and leukocyte elastase activity. These data indicate that synthesis and evaluation of beta-lactams are a promising area for further development in anticancer research.

2. INTRODUCTION

Cancer is a heterogeneous disease and can be characterized as the growth of a malignant cell population that eventually leads to the interference of normal physiological functions. Anticancer drug research focuses on inhibition of tumor cell growth and induction of apoptosis in the malignant cell population. Apoptosis, or programmed cellular death, first described by Kerr et al. in 1972, is characterized by the ability of a cell to undergo a step-by-step self suicide program without affecting neighboring or adjoining cells (1). Activation of the apoptotic program in tumorigenic cells is essential for cancer prevention and treatment. A significant focus in anticancer drug discovery is to selectively induce tumor cell apoptosis with limited toxicity to normal cells. Tumor
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![Diagram of beta-lactams](image)

Figure 1. Structure of bicyclic (A) and monocyclic (B) beta-lactam families.

Bacteria from the genus *Penicillium* and Sir Alexander Fleming first coined the name “penicillin” in 1928. Fleming observed bacteriolysis in a broth contaminated with coagulase-negative staphylococci. He subsequently isolated the first antibiotic, which he called penicillin, from a fungus in a laboratory at St. Mary’s Hospital in London, England (4). It would be many years until the importance of this discovery was fully appreciated. Later studies at Oxford by Abraham, Florey and Chain resulted in the isolation of penicillin and subsequent drug trials (4). X-ray crystallography performed by Dorothy Hodgkin revealed that penicillin is a thiazolidine ring fused to a four membered beta-lactam ring (5; figure 1A). Later research focused on identifying several other antibiotics isolated from natural sources. Bacteria from the genus *Cephalosporium* also excrete beta-lactam containing compounds. Today the cephalosporins, and their derivatives, comprise a large portion of the antibiotic therapies used (6,7). Several other classes of bicyclic beta-lactams were found to also possess antibacterial properties, such as penams, carbapenems and clavulanic acids (figure 1A).

The beta-lactams are powerful and potent inhibitors of bacterial growth and many different moieties of bicyclic beta-lactams have been isolated or synthesized since the discovery of penicillin (8). In 1981, two independent groups from the Squibbs and Takeda laboratories isolated the first N-thiolated beta-lactams from natural sources (9,10). These beta-lactams were the first to have a N-sulfonic acid group attached directly to the nitrogen in the lactam ring. The term “monobactam” was coined for these lactams, which have a flexible monocyclic ring, lack the carboxylic acid moiety, yet still retain a high bactericidal property (figure 1B).

Of late, a new class of N-thiolated beta-lactams was found to inhibit *Staphylococcus aureus* and methicillin-resistant *S. aureus* (MRSA) growth (11-13). The novel lactams most active against MRSA have an N-methylthio-substitution. These compounds are unaffected by penicillinases, such as beta-lactamase, an enzyme produced by some bacteria that degrades beta-lactams (14).

### 3. CURRENT ANTIBIOTIC MODALITIES IN CANCER TREATMENT

The tetracyclines are antibiotics that have been used for the treatment of infections for decades (15). This family of compounds includes: tetracycline, doxycycline, and minocycline. Although these compounds are known for their effects on mitochondria, their ability to inhibit matrix metalloproteinase (MMP) enzymes required for angiogenesis, may prove more beneficial (15). Col-3, a modified tetracycline, is now in clinical trials. Doxycycline has been shown to reduce tumor burden in mouse models and osteolytic bone metastasis as a result from breast cancer (15). These results have enabled doxycycline to enter clinical trials.

**Rapamycin** (RAPA) is a microbial macrolide from *Streptomyces hygroscopicus* and is used as an immunosuppressive to prevent organ rejection (16). It can also induce anti-proliferative effects by inhibiting cyclin-dependent kinases and inhibit retinoblastoma protein phosphorylation leading to cell cycle arrest (17-19). RAPA has been shown to have growth-suppressive effects in a broad range of cancers (20). For instance, Nepomuceno et al., have shown that RAPA is capable of preventing the growth of Epstein Barr virus positive (EBV+) B-cell lymphomas. Severe Combined Immune Deficient (SCID) mice treated with 1.5 mg/kg/day RAPA remained tumor free for up to six weeks after injection with peripheral blood from liver transplant patients, while mice without RAPA treatment developed tumors within three weeks (21). Their results suggest that rapamycin may be able to control other EBV-related cancers and those associated with organ transplant.

The novel histone deacetylase inhibitor, FK228, has been recently isolated from *Chromobacterium violaceum* (22). This antibiotic is a bicyclic peptide with a non-cysteine disulfide bridge that has been found to reverse H-ras transformed NIH-3T3 cells (22). The activity of FK228 against tumor cells is in the ng/mL range while effects against normal cells are not seen below concentrations of 1 µg/mL (23). The action of FK228 indicates that it is more effective against large tumors with an established blood supply over small tumors that do not yet require a capillary network (23). Specifically, FK228 down-regulates mRNA levels of vasoendothelial growth factor, a principle component of the angiogenesis pathway. This evidence strongly supports FK228 as a potential candidate for cancer chemotherapy.

**Lavendamycin**, which possesses a quinoline-5,8-dione core, is an antibiotic derived from *Streptomyces*...
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lavendulae and isolated in 1981 (24). While the native compound did not pass clinical trials due to poor solubility and general toxicity, newly designed analogs show promise as potential chemotherapeutic drugs (25). The analogs of this compound possess a modified ring structure of the native compound to increase solubility and selectivity for p53-deficient cells (25). When lung carcinoma A549 cells were treated with the analog MB-97, the cells accumulated and activated p53 (25). These results suggest that this compound acts as a strong DNA damaging agent, a property it shares with other antibiotic/antitumor compounds like streptonigrin and the anthracyclines (25). Parental compounds of the MB-97 lavendamycin analog showed in vivo toxicity in the range of 0.4 mg/kg, whereas the new analog did not display toxicity until a treatment of 400 mg/kg was reached. Administration of another lavendamycin analog, MB-51, at doses of 300 mg/kg to mice bearing tumors resulted in an 80% reduction of tumor mass (25). These results strongly suggest that further examination of lavendamycin analogs as chemotherapeutic agents is necessary.

Discovered in 1966, bleomycin (derived from Streptomyces verticillus) is a well-studied antibiotic/antitumor agent (26). This compound is a principle treatment for testicular cancer and demonstrates reduced myelotoxicity (27). The most well known mode of action for this compound is its oxygen-dependent degradation of DNA (27). Unfortunately, bleomycin possesses potential fatal pulmonary toxicity (27). A recent 2003 clinical trial examined the effects of bleomycin treatment with mitomycin C as a follow up treatment to postoperative irradiation for patients with advanced head and neck cancer (28). This combined therapy improved survival and the toxic effects (primarily mucositis) were considered within acceptable limits (28).

The anthracycline class of antibiotics include doxorubicin, daunorubicin, idarubicin, and epirubicin. Doxorubicin (DOX) and daunorubicin (DNR) have been used for over 30 years to treat a variety of solid and hematological tumors. DOX and DNR work by intercalation into DNA and inhibition of Topoisomerase II via binding to the Topo II/DNA ternary complex to promote its stabilization (29,30). Unfortunately, DOX and DNR also have high toxicity due to their mechanism of action, production of reactive oxygen species (ROS), which leads to toxicity of the cardiomyocytes and subsequent chronic and acute cardiomyopathies (31-33). Improvements in DOX and DNR structures lead to the development of idarubicin and epirubicin. Although these two analogs do have decreased toxicity and improved activity, there still is a significant risk to patients using these chemotherapeutic drugs (34,35).

Antibiotics are an intriguing class of compounds, not only for their ability to control bacterial infection but also for their capability to function as chemotherapeutic agents in cancer. There are an unlimited number of compounds bacteria can create. Based on the properties of existing antibiotics, studies into active analogs or novel synthetic compounds continue. Typically, antibiotics have reduced or no toxicity though there are exceptions, e.g. high doses can result in toxicity. Furthermore, these compounds can serve the dual roles of treating cancer or fighting potential infection during chemotherapy as an adjunct treatment. One of the more recent antibiotics to enter into the class of antibiotic/antitumor compounds is the class of beta-lactams.

4. TRADITIONAL ROLES OF BETA-LACTAMS

The family of beta-lactams, so named because they all contain a beta-lactam ring, have been used for many years to treat bacterial infections. Traditional beta-lactam antibiotics, such as the penicillins and cephalosporins, contain, in addition to the beta-lactam ring, a carboxyl group in close proximity to the lactam nitrogen, which is required for antimicrobial activity. These antibiotics act as bactericidal agents by serving as a substrate for peptidoglycan transpeptidase, the enzyme responsible for crosslinking the N-acetylglucuronic acid (NAG) and N-acetylmuramic acid (NAM) moieties in the peptidoglycan layer surrounding the periplasmic space and membrane of bacteria. The transpeptidase enzyme is acylated by the beta-lactam, which results in a weakened cell wall and osmotic lysis of the bacteria. Since the commercial availability of penicillin in 1940, many other beta-lactam antibiotics with medicinal effects have been isolated and synthesized. Many thought that the war on infectious diseases was over after the discovery of penicillin as we have used antibiotics to treat and cure many diseases that were once fatal, namely tuberculosis, bacterial meningitis, and pneumonia. Unfortunately, bacteria developed resistance by producing enzymes to hydrolyze the beta-lactam moiety rendering the antibiotic inactive (36). This resistance has spawned a renewed interest in identifying and synthesizing new active antibiotics to treat resistant strains, derived mainly through resistant genes located in plasmids. Bacterial infections caused by methicillin-resistant Staphylococcus aureus (MRSA) are currently susceptible to only one current antibiotic (vancomycin).

Antibiotics are substances created by microorganisms or synthetically designed to kill or inhibit the growth of bacteria. These compounds are the staple of treatment for bacterial infection. In addition, there are numerous antitumor antibiotics that are currently used to treat cancer, such as the anthracyclines, bleomycin, mitomycin C, daunomycin, and mithramycin. The major mechanism of action for these antitumor antibiotics is DNA intercalation or inhibition of DNA synthesis. Beta-lactam antibiotics are traditionally used only for bacterial infections, however, several novel classes of beta-lactams have been shown to possess anticancer properties as well. We have found that a class of beta-lactams, the N-thiolated beta-lactams, induce tumor cell apoptosis by introducing DNA damage in a potent, and more importantly, a tumor cell-specific manner with little or no effect on normal cells (37, 38). Cainelli et al., describe that 4-alkylidene-beta-lactams inhibit matrix metalloproteinases-2, and -9 (MMP), essential for the tumor induced neovascularization (39). Banik et al., also show that beta-lactams with polyaromatic
substituents induce tumor cell death in a variety of cancer cell lines, such as ovarian, prostate, breast, colon, and leukemic *in vitro* and demonstrated inhibition of tumor cell growth in mice (40) (see Section 6 for details).

5. BETA-LACTAMS AS PRODRUGS FOR ANTICANCER CHEMOTHERAPIES

5.1. Background

The most widely applied beta-lactams for prodrug based cancer chemotherapy have been the cephalosporins. The cephalosporins were chosen as prodrug candidates because of their inherent reactivity when hydrolyzed by beta-lactamase enzymes. Hydrolytic cleavage of the beta-lactam ring causes a secondary reaction that triggers the expulsion of the 3’-substituent (figure 2). The cytotoxic component can be attached to this position and then released when the cephalosporin beta-lactam ring is hydrolyzed by the enzyme.

Cephalosporins have also been used in the selective targeting of anticancer compounds to tumor cells using Antibody Directed Enzyme Prodrug Therapy (ADEPT) and this method has received much attention in recent years (41,42). ADEPT is a drug delivery strategy that employs an enzyme covalently attached to a monoclonal antibody that is specific for a tumor cell antigen. This strategy allows for the delivery of the cytotoxic agent masked as a prodrug to specifically target tumor cells. To achieve site-specific generation of the cytotoxic agent, the monoclonal antibody-enzyme immunoconjugate (mAb-enz) is given to the patient first. This allows for prelocalization of the mAb-enz on the targeted tumor cell surface. The prodrug, which is the substrate of the enzyme, is then administered leading to targeted release of the drug.

The benefits of this method are many compared to administering the prodrug or parent drug alone (43-46). The catalytic nature of the enzyme will allow for the conversion of a stoichiometric excess of prodrug substrate. In addition, lower doses of the antibody-enzyme conjugate and higher doses of the prodrug can be administered, which reduces the toxicity. The surrounding tumor cells not bearing the target epitope can also be effectively dosed with the cytotoxic drug. This can lead to lower side effects due to tumor-specific localization of the parent drug. Furthermore, beta-lactamase enzymes are not endogenous to humans, making them highly exploitable for ADEPT and therefore eliminating premature cleavage of the prodrug by other naturally occurring enzymes. In order for the monoclonal antibody-enzyme immunoconjugate system to be successful, there are several criteria required for therapeutic efficacy. First, the mAb-enzyme conjugate should not elicit an immune response and have a high tumor/blood ratio. Secondly, the antibody portion of the immunoconjugate must selectively target and have a high binding affinity for the tumor cell antigen. Lastly, the unbound conjugate must be readily cleared from the bloodstream before the prodrug is administered. The following section will discuss antibody-enzyme-prodrug systems that have undergone evaluation to treat various types of cancers.

5.2. Nitrogen Mustards

The alkylating agents or nitrogen mustards include cyclophosphamides, chloroambucil, and melphalan, and function by replacing an alkyl group for a hydrogen atom, leading to the formation of DNA adducts, or abnormal base pairing and cross linking of DNA. Cyclophosphamide and ifosfamide exist as prodrugs and are activated by hepatic enzymes into active species (47). While the nitrogen mustards are efficacious, they are extremely cytotoxic which results in unwanted side effects. For example, 7-(phenylacetamido) cephalosporin mustard (CM) prodrug was at least 50 times less toxic than phenylenediamine mustard (PDM) toward H2981 human adenocarcinoma cell line *in vitro* (48). The monoclonal antibody-enzyme conjugate L6-BCβL was shown to activate CM in an immunologically specific manner, which resulted in a level of cytotoxicity comparable to PDM (48). *In vivo* studies indicated that CM was less toxic to nude mice than PDM, however treatment was hampered due to severe tail necrosis following intravenous injection (49).

A modified analog of CM, 7-(4-carboxybutamido) cephalosporin mustard (CCM), showed higher activity *in vitro* when administered with L6-ECβL. CCM was found to be less cytotoxic than PDM on H2981 cells, with IC50 values of 25-45 µg and 1.5 µg, respectively. When the monoclonal antibody-enzyme conjugate L6-ECβL was administered 96 h prior to CCM, there was observed significant antitumor activity *in vivo* (49). The *in vivo* experiments concluded that administration of CCM in nude mice was less toxic than CM, and both prodrugs (CM
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and CCM) were significantly less toxic than PDM.

The prodrug C-Mel, a cephalosporin carbamate derivative of melphalan, was also shown to have antitumor activity (50). C-Mel was activated in an immunologically specific manner by the L49-sFv-beta-lactamase conjugate. In vitro cytotoxicity assays using 3677 human melanoma cells treated with the C-Mel prodrug in conjunction with L49-sFv-beta-lactamase showed that c-Mel was 40 times less toxic than melphalan alone, IC₅₀ = 53µg and 1.3 µg, respectively. In vivo studies demonstrated that nude mice with growing tumors treated with L49-sFv-beta-lactamase and C-Mel at 150 mg/kg/injection underwent complete regressions, and 3 out of 5 mice were eventually cured (50).

5.3. Methotrexate

Methotrexate (MTX) is a folic acid analog, or antimetabolite, which was first developed and used clinically in the 1940’s (51). MTX interacts with dihydrofolate reductase (DHFR), an enzyme critical in folate metabolism (52). Unfortunately, drug resistance can often occur due to increased endocytosis of the antifolate by multidrug resistance protein (MDRPs) pumps and folate transporters (53-56). The prodrug of MTX is a potent cytotoxic agent and antimetabolite developed for ADEPT therapy. MTX was found to be a good substrate of beta-lactamase but showed identical cytotoxicity to that of the parent drug alone (45). No further evaluations have been conducted.

5.4. 5-Fluorouracil

Another group of antimetabolites include the pyrimidine analogs, such as 5-fluorouracil and gemcitabine, and the purine analogs, such as 6-mercaptopurine and 6-thioguanine. These analogs substitute for nucleic acid bases in both DNA and RNA synthesis, but drug resistance to these antimonials has been implicated due to the nucleoside transporters (NT), which mediate uptake of nucleic acids into dividing cells (57). 5-Fluorouracil is an antitumor agent often used in the treatment of colon cancer. The prodrug of 5-fluorouracil was shown to be cleaved by beta-lactamase, however, the in vitro cytotoxicity was found to be the same as that of the parent drug alone (58).

5.5. Vinca Alkaloids

The vinca alkaloids are potent anticancer drugs derived from natural sources that are used to treat the acute leukemias, lymphomas, and some solid tumors. Two cephalosporin-vinca alkaloid prodrugs were found to have a 5-fold less cytotoxicity to LS174T colon adenocarcinoma cells than the parent drug LY233425 and LY266070 (59,60). LY233425-cephalosporin prodrug was found to be equipotent when administered with the F(ab’)-beta-lactamase conjugate with the parent drug (59). In vivo studies with mouse models of human colorectal carcinoma tumors demonstrated long term regressions when LY266070 cephalosporin prodrug was administered with mAb-beta-lactamase (60).

5.6. Doxorubicin

Cephalosporin-doxorubicin prodrugs have been developed that show promising anticancer properties when used in conjunction with mAb-beta-lactamase conjugates. In vitro studies of C-Dox on H2981 lung adenocarcinoma cells revealed that the prodrug was less toxic than doxorubicin alone. The prodrug was also immunospecifically activated by the L6-ECl-beta-lactamase conjugate to release doxorubicin in vitro (61). Additionally, another study showed that a different mAb-beta-lactamase/cephalosporin-doxorubicin prodrug system effectively delivered doxorubicin to a series of MCF7 breast carcinoma, OVCAR3 ovarian carcinoma, and T380 and LS174T colon tumor xenographs (62). The maximum tolerated dose of the prodrug was equivalent to that of the free drug when compared to the degree of tumor suppression, however tumors did not regress. A polymer prodrug of cephalosporin-doxorubicin has been developed and was shown to increase the survival rate and decrease the tumor growth rate of mice when treated in conjunction with a polymer bound beta-lactamase enzyme (63). The combination of polymer-prodrug and polymer-enzyme was non-toxic with the doses used in the study.

5.7. Mitomycin C

Two cephalosporin prodrugs of mitomycin C were evaluated against H2987 lung adenocarcinoma and clone 62 melanoma cell lines (64). In vitro studies showed that one of the prodrugs (prodrug 1) had comparable cytotoxicity to the parent drug, whereas another prodrug (prodrug 3) was 40- and 10-fold less toxic toward H2987 and clone 62 melanoma cells. Prodrug 3 also was immunospecifically activated by L6-F(ab’)-beta-lactamase and 96.5-F(ab’)-beta-lactamase conjugates that are selective toward H2987 and clone 62 cells, respectively (64).

5.8. Paclitaxel

Anticancer drugs derived from natural sources comprise a large body of the drugs currently approved for chemotherapy. Treatments with paclitaxol (Taxol), derived from Taxus brevifolia (Pacific yew tree) and a semisynthetic analog, docetaxol, target rapidly dividing cancer cells by increasing microtubule polymerization, thereby inhibiting anaphase during cell cycle (65,66).

A prodrug of paclitaxel has been shown to be immunospecifically activated by the fusion protein L49-sFv-beta-lactamase (67). In vitro cytotoxicity assays performed on 3677 melanoma cells expressing the melanotransferrin (p97) antigen revealed that the prodrug was 12 to 30 times less cytotoxic than the parent drug (67).

5.9. Radioimmunoconjugates

Radioimmunotherapy is a method to deliver a radioisotope to a specific target area namely tumor cells. One disadvantage of method is that it can lead to dose-limiting toxicities through radiation exposure to non-targeted organs. A recent study has shown that a radioimmunoconjugate containing a cleavable linker can release the radioisotope upon administering an enzyme thus lowering systemic radiation exposure (68). This approach utilized a ¹³¹I-labled cephalosporin conjugated to Tositumomab, a mAb specific for the CD20 antigen via a synthetic linker. Upon administration of the beta-lactamase
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enzyme, the radiolabel would be released causing rapid clearance from the blood and normal organs. In vivo studies of mouse models with human Ramos B lymphoma tumor xenografts revealed no decrease of the injected dose after 1 h of beta-lactamase treatment (68). However, after 4 h there was a noticeable decrease in the radioactive content from the tumor as well as blood, liver, lung and marrow demonstrating that there was rapid clearance of the radiolabel after injection of the radioimmunoconjugate and beta-lactamase enzyme (68). In addition, there was an enhanced tumor to blood % injected dose ratio at the beginning time points of the study.

The ADEPT system allows for the use of agents that, when given systemically, are too toxic for use in the clinic. The diversity of cancer drugs that are utilized is well demonstrated. Additionally, many studies have shown that the active drug is generated at the tumor site and at concentrations that could not be used in normal systemic administration of the parent drug. Further advancements to improve the efficacy of mAb-enzyme/prodrug therapies have resulted in modifying the mAb-beta-lactamase conjugate. Recombinant formed monoclonal antibody-beta-lactamase conjugates showed improved anticancer therapeutic activities compared to the synthetically formed conjugates (69).

6. POTENTIAL USE OF BETA-LACTAMS AS ANTICANCER DRUGS

6.1. N-Thiolated beta-Lactams

6.1.1. Structure-Activity Relationships

Currently, there are many types of antibiotics (e.g. anthracyclines, bleomycin) that have been used to treat cancer. However, research into the possibility of utilizing beta-lactam antibiotics as potential anticancer medications has been relatively non-existent. Our most recent studies suggest that beta-lactams could play a role as anticancer drugs (37,38). In the following sections, we will discuss the different N-methylthio beta-lactams and their effects on cancer cells as well as some insights into possible mechanisms of action. We have demonstrated that N-thiolated beta-lactams have tumor cell-killing ability through induction of DNA-damage and subsequent apoptosis (37). Synthesis of other beta-lactams have aided in identifying important structure-activity-relationships (SARs). Additionally, we have also showed that N-thiolated beta-lactams have the ability to preferentially induce apoptosis in tumor cells, but not in normal or non-transformed cell (38).

Perhaps one of the most important findings with beta-lactams was that they did not need to possess a bicyclic ring with its rigid conformity to be bactericidal (9,10). This allows for a broader range of synthetic analogs to be made that possess antibacterial or antitumor activity. We have screened a large number of synthetic beta-lactams for their ability to promote tumor cell death (37,38). Of the compounds screened, we chose a lead compound, Lactam 1, to be the basis for additional synthesis of analogs (figure 3). Our findings yielded several important SARs. Lactam 1 was shown to induce apoptosis in a variety of tumor cell lines, namely, breast (MDA-MB-231, MCF-7), prostate (PC-3, DU-145), head-and-neck (PCI-13), SV-40 transformed lung cells (VA-13) and leukemic (Jurkat T) cell lines (37, 38).

Other important SARs were observed also when cells were treated with the beta-lactams in cellular toxicity assays. Foremost, is the necessity of the N-methyl-thio group that when absent abolishes the apoptosis-inducing activity. Also observed was the inverse relationship between the number of carbon atoms off the N-thio group. Increasing the number of carbons from one to two decreased the amount of apoptosis observed by ~50% (Lactam 1 vs. Lactam 3). A four carbon chain off the N-thio group further decreased apoptosis-inducing activity by ~65%, and substitution of the N-methylthio group with a N-benzylthio group lead to ~70% decrease in apoptosis. Observations about the position of the chloro group off the phenyl ring also provided key SAR information. Isomers with the –Cl group in the meta or para position revealed that Lactams-5 and –6, while still capable of inducing apoptosis, were less potent than Lactam 1 (37). To determine if deletion or substitution of the ortho –Cl on the phenyl ring would increase or decrease activity, several analogs of Lactam 1 were synthesized with substituted halogen or non-halogen groups for the –Cl (figure 3). It was found that increasing the size of the group in the ortho position correlated with increased cell death (38). In fact, elimination of the ortho substituted group resulted in the least amount of activity, while substitution with a nitro group lead to the greatest amount of activity. The –NO2 substituted analog, Lactam 12, exhibited the strongest effect and consistently induced apoptosis comparable with Lactam 1, but at half the concentration (38).

According to these results, several key features should be retained for future design and synthesis of beta-lactams with antitumor properties: 1) the N-methyl-thio group must remain intact without additional carbon chains, 2) although meta and para substitutions on the phenyl ring still preserve their apoptotic producing abilities, substitutions in the ortho position are most potent, and lastly 3) the larger groups in the ortho position correlates with enhanced apoptotic-inducing activity (37,38).

6.1.2. Apoptosis Induction

A number of synthetic N-methylthio-beta-lactam compounds have been found to induce apoptosis in a number of tumor cell lines, such as the human leukemia Jurkat T cells, breast cancer (MCF-7, MDA-MB-231), prostate (PC-3, DU-145), and head-and-neck (PCI-13) cells (37,38). Several of these compounds (figure 3) caused induction of caspase-3/-7 activity, effector caspases whose activation is indicative of the apoptosis (70). The potency of these beta-lactams is as follows, Lactam 12 > 10 > 11 > 1 > 6 ≥ 5 > 3 > 4 > 9 > 8 > 7 > 2 (37,38). A nuclear stain to determine the morphological changes of apoptotic nuclei showed that Lactam 12 induced cellular detachment of 50-60 % of total cell population (38). Additionally, beta-lactam treatment instigated cleavage of poly(ADP-ribose) polymerase (PARP) from its 116 kDa full length form to the 85 kDa fragment (37) which occurred in conjunction
with caspase-3 activity, the caspase shown to directly cleave PARP (71). Caspase-8, an initiator caspase capable of mitochondrion-dependent and -independent apoptosis initiation (72), was also found to become active after beta-lactam treatment (37). Cytochrome 
\(c\) is a mitochondrial protein that is released during apoptosis when the membrane potential of the mitochondria is compromised and combines with several other proteins (dATP, Apaf-1, caspase-9) to form the apoptosome, which is capable of activating caspase-3 (73). The N-
\(\text{ methylthio} \) beta-lactams are also able to cause cytochrome 
\(c\) release from the mitochondria, prior to activation of caspase-3, in time- and concentration-dependent manners (37). These data confirm that beta-lactams can indeed cause apoptosis in tumor cells.

There are other lactam compounds that can also induce apoptosis. Watabe et al. found that gamma-lactams, which contain a five-membered ring, are capable of inducing apoptosis in HL-60 cells (74). MT-21, a synthetic gamma-lactam, activates caspase-9 followed by the subsequent activation of caspase-3. Unlike our findings with beta-lactams, caspase-8 was not found to participate in the apoptotic signaling cascade after gamma-lactam treatment (74). Lactacystin, a gamma-lactam possessing a thioester moiety and originally isolated from actinomycetes (75), has been found to be a potent inhibitor of chymotryptic- and trypptic-like catalytic activities of the proteasome through covalent bonding to the N-terminal threonine of the beta-subunits (76). Proteasome inhibition leads to an accumulation of p27 (77), I\(\kappa\)B-\(\alpha\) (78), and Bax (79), which can cause G1 cell cycle arrest and apoptosis (80, 81). It is for these reasons many believe that proteasome inhibitors are good candidates for anticancer chemotherapeutic drugs (82-84).

6.1.3. DNA-Damage and Signal Transduction Pathways

To further investigate the cause of apoptosis after N-methylthio beta-lactam treatment, analysis of cell cycle changes were performed. Lactam 1 was found to increase S-phase DNA content and initiate a concomitant decrease in G1 phase DNA. This S-phase cell cycle arrest was found to be due to an inability for treated cells to undergo DNA replication as was found from a [\(\text{3H}\)] thymidine incorporation assay. DNA replication was inhibited in a time- and concentration-dependent manner with a half-maximal inhibition (IC50) of [\(\text{3H}\)] thymidine in Jurkat cells at 32 \(\mu\)M with Lactam 1 treatment (37). A terminal deoxynucleotidyl transferase-mediated nick-end labeling (TUNEL) assay, which detects DNA strand breaks, then provided additional data to support the hypothesis that the DNA replication inhibition was due to damage of the
A class of beta-lactams, the 4-alkylidene-azetidin-2-ones, has been identified that exhibit inhibitory activity to both MMP-2 and MMP-9 as well as leukocyte elastase (LE) (39). LE can activate MMP-2 and MMP-9, and inactivate their tissue inhibitor (91). Compounds with protected hydroxy groups (Compounds 1 and 8) were found to inhibit LE. Compound 8 in particular showed an IC$_{50}$ of 9 µM to LE activity. The green tea polyphenol (−)-epigallocatechin-3-gallate (EGCG), a known LE inhibitor, was used as a comparison and was found to be ~22-fold more active (IC$_{50}=0.4$ µM) than Compound 8 (39). When the hydroxyl group was unprotected, or removed all together, the beta-lactam lost its potent activity against LE, but gained considerable activity against MMP-2 and MMP-9. Two compounds in particular (Compounds 2 and 18) showed the greatest inhibitory activity on MMP-2 with IC$_{50}$s of 85 µM and 60 µM, respectively (39). This is a promising area of drug research because inhibition of angiogenesis not only inhibits tumor growth, but also prevents invasion and metastasis via the circulatory system.

6.3. Polyaromatic Beta-Lactams

In 2001, Banik et al. described polyaromatic imine beta-lactams with biological activity against cancer cells (40). Several synthesized compounds were tested in vitro for their cytotoxicity on nine cancer cell lines (40). Compounds with phenanthrene and chrysene substituents had the most activity in vitro, as measured by MTT assay. The maximal activity concentrations ranged from 2.5 to 40.6 µM, some well within the therapeutic range. Conversely, beta-lactams with naphthalene, anthracene, and pyrene substituents showed virtually no cytotoxicity (40).

A series of in vivo assays using athymic nude (nu/nu) mice was performed with the active beta-lactams mentioned above. Mice were injected with K-562 leukemia, HT-29 colon, or SKOV-3 ovarian cancer cells. A variety of treatment times and regimens were tested (40). Mice given polyaromatic beta-lactam treatments (Compound 17a) at 60 mg/kg showed negligible toxicity compared to the control mice that were given cisplatin and Adriamycin. The polyaromatic induced only a slight weight loss (3.52 g), which was quickly recovered after discontinuation of treatment (40). It was also found that treatment with the beta-lactam compound delayed the onset of tumor formation by 7 ± 2 days in mice injected with HT-29 cells. Additionally, many of the mice injected with
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SKOV-3 cells did not form any tumors at all (40).

7. CONCLUSIONS AND PERSPECTIVES

Cancer is lethal to 42% of those diagnosed. With millions of new patients each year, the initiative to develop suitable chemotherapeutic agents is a driving focus of medical research. However, the currently available chemotherapeutic agents are incapable of selectively targeting cancer cells from normal cells leading to treatments that are as hazardous as the disease itself. Beta-lactams are compounds that have been used for many years to combat microbial infections. Therefore, it is already known that these compounds possess minimal effects on non-bacterial cells; this trait is desirable of chemotherapeutic agents. Recently, the potential of beta-lactams as anticancer agents has come to light. Beta-lactams can be used as pro-drugs that are capable of specifically targeting tumor cells. Likewise, the N-methylthiolated beta-lactams are capable of inducing apoptosis in a wide array of tumor cell types, with little effect on normal cells. Thus making these compounds, and other beta-lactams (eg. 4-alkylidene and polyaromatics), attractive targets for structure-activity relationship studies and analog synthesis. While further study on this class of compounds in animal models should be performed to completely assess their toxicity, selectivity, and efficacy in vivo, the profiles reported here show an optimistic future for expanding the role of these compounds from simple antibiotics to anticancer therapeutics.

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