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PRINCIPAL INVESTIGATOR: Q. Ping Dou, Ph.D.

CONTRACTING ORGANIZATION: Wayne State University
Detroit, MI 48202-3622

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Examination of Potential Anti-Tumor Activity of N-Thiolated B-Lactam Antibiotics in Nude Mice Bearing Human Breast Tumors

Q. Ping Dou, Ph.D.

Wayne State University
Detroit, MI 48202-3622

Activation of the cellular apoptotic program is a current strategy for the prevention and treatment of human cancer including breast cancer. Because of the ease of synthesis and structural manipulation, small molecules with apoptosis-inducing ability have great potential to be developed into chemotherapeutic drugs. The beta-lactam antibiotics have for the past 60 years played an essential role in treating bacterial infections without causing toxic side effects in the host. We hypothesized that active N-thiolated b-lactams can damage DNA and induce apoptosis in human breast cancer cells in nude mice. In this summary report, we have first evaluated potencies of several novel synthetic beta-lactams to inhibit proliferation and induce apoptosis in human cancer cells. We then determined whether one of these b-lactams, HY14, could inhibit breast tumor growth in vivo. We have found that HY14 inhibited growth of implanted MDA-MB-231 breast tumors in a concentration-dependent manner, associated with its DNA-damaging activity. We are currently testing more novel beta-lactams in nude mice bearing human breast tumors. Our studies have provided strong support for proof-of-concept of the potential use of these N-thiolated beta-lactams in breast cancer prevention and treatment.
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INTRODUCTION

Activation of the cellular apoptotic program is a current strategy for the prevention and treatment of human cancer including breast cancer (1). Because of the ease of synthesis and structural manipulation, small molecules with apoptosis-inducing ability have great potential to be developed into chemotherapeutic drugs (2). One particularly important class of small molecule drugs, the beta-lactam antibiotics, have for the past 60 years played an essential role in treating bacterial infections without causing toxic side effects in the host (3, 4). Recently we uncovered new members of this family of drugs, termed N-thiolated beta-lactams, which are highly effective at inhibiting bacterial growth in drug-resistant strains of Staphylococcus aureus (5, 6). Their mode of action appears to differ from that of traditional beta-lactam antibiotics. Most innovatively, we have discovered and characterized for the first time the anti-proliferative and apoptosis-inducing properties of N-thiolated beta-lactam antibiotics against human tumor cells (7). However, whether N-thiolated beta-lactam antibiotics have actual anti-breast cancer effects in vivo remains unknown.

BODY

For details, please see the included APPENDICES. Please also see below KEY RESEARCH ACCOMPLISHMENTS.

Structure-activity relationships of N-methylthiolated beta-lactam antibiotics with C₃ substitutions and their selective induction of apoptosis in human cancer cells (8 and see Appendices). The development of novel anti-cancer drugs that induce apoptosis has long been a focus of drug discovery. Beta-lactam antibiotics have been used for over 60 years to fight bacterial infectious diseases with little or no side effects observed. Recently a new class of N-methylthiolated beta-lactams has been discovered that have potent activity against methicillin resistant Staphylococcus aureus. Most recently, we determined the potential effects of these N-thiolated beta-lactams on tumorigenic cell growth and found that they are apoptosis-inducers in human cancer cell lines. In the current study, we further determined the effects of the substitution of the O-methyl moiety on C₃ and stereochemistry of the beta-lactams on the anti-proliferative and apoptosis-inducing abilities. We have found that Lactam 18, in which C₃ is substituted with an acrylate ester group, is a very effective proliferation inhibitor against human premalignant and malignant breast, leukemic, and simian virus 40-transformed fibroblast cells. Generally speaking, increasing the size of the moiety on C₃ decreases its anti-proliferation potency, possibly indicating steric hindrance with the cellular target or decreased permeability through the cell membrane. We also found that the stereochemistry of the beta-lactams plays an important role in their potency. The 3S,4R isomers are more effective than their enantiomers (3R,4S), suggesting that 3S,4R configuration is more favorable for target interaction.

Beta-lactams and their potential use as novel anticancer chemotherapeutics drugs (9 and see Appendices). 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 sepsisemia, 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.


**Cell death-inducing activities of novel beta-lactams.** In order to discover more potent β-lactams against cancer, we have tested numerous of β-lactams that were synthesized by our chemistry collaborators. There are 8 compounds in HY group (HY14 to HY21) and 24 compounds in JG group (JG1 to JG 24). Chemical structures of some of the compounds are shown in Fig. 1. Our results indicate that in JG group, JG19 and JG5 were most potent cell death inducers when tested in human leukemia HL60 and Raji cell lines (Fig. 2A, B) and that in HY group, the order of potency to induce HL60 cell death was: HY20 > HY18 > HY16 = HY15 > HY14 > HY19 > HY17 (Fig. 2C). Beta-lactam L-1 was used as a comparison (Fig. 2). The results from Western blot analysis also showed that JG19 and JG20 could induce PARP cleavage, a cellular apoptotic marker (Fig. 3).

**Beta-lactams could effectively inhibit proliferation and induce apoptosis in human breast cancer cells.** Previously we reported that L-1 has the great potency to induce apoptosis in cancer cells showed by MTT assay and PARP cleavage. In current experiment we screened more beta-lactams (Fig. 4) in order to discover more potent analogs. Human breast cancer MCF-7 cells were treated with each of the indicated beta-lactams at 1, 25 or 50 µM or DMSO (as solvent control) for 24 h, followed by performance of an MTT assay, which measures the status of cell viability and, thus, cell proliferation. After treatment with 50 µM of L-47, cellular viability of MCF-7 was decreased by 73%. Compared with 63% inhibition by 50 µM of L-1, L-47 was the most potent one in the tested beta-lactams (Fig. 5A).

We then treated another human breast cancer cell line MDA-MB-231 with 50 µM of L-1, L-30, L-47 or L-53 for different time points, followed by preparation of cell lysates and measurement of PARP cleavage, a cellular apoptotic marker, by Western blotting. The results showed that among the tested lactams, L-47 had the greatest potency to induce PARP cleavage within 8 h of treatment (Fig. 5B).
Beta-lactam HY14, an analog of L-47, significantly inhibits the growth of breast cancer xenografts, associated with its DNA damaging activity in vivo. The data described above clearly demonstrate that β-lactams are apoptosis inducer in cultured leukemia and breast cancer cells. Our experimental results also showed that HY14 was more potent than L-1 in inducing cell death in cancer cells (Fig. 2C). Since we have a large quantity of HY14, we then examined anti-tumor activity of HY14 in vivo. We implanted MDA-MB-231 cells s.c. in nude mice. When the tumors became ~200 mm$^3$, the mice were i.p. treated with either vehicle control or HY14 at 0.3 or 3.0 mg/kg/day. The inhibition (up to 53%) of tumor growth by 3.0 mg/kg/day treatment of HY14 was observed after 30 days injection (Fig. 6A) but only 13% inhibition of tumor growth was showed by 0.3 mg/kg/day treatment, indicating that HY has anti-tumor activity which is dose-dependent (Fig. 6). The immunohistochemistry results showed that apoptosis-specific TUNEL positivity was found mainly in MDA-MB-231 tumors treated with HY14 at 3.0 mg/kg/day, less in those treated with 0.3 mg/kg/day of HY14, but none in vehicle-treated tumor (Fig. 6B).

Our future studies will focus on examining more novel β-lactams and determining whether their in vivo apoptosis-inducing abilities are related to their anti-tumor activities using nude mice bearing human breast tumors. These studies should provide strong support for proof-of-concept of the potential use of these N-thiolated beta-lactams in breast cancer prevention and treatment.
Figure 1. Chemical structure of related beta-lactams
Figure 2. Beta-lactams could induce cell death in human leukemia cells.

Trypan blue assays: human leukemia HL60 (A, B) and Jurkat cell line (C) were treated with 50 or 100 µM of each beta-lactam for 24 hrs, and then non-viable cells were determined by Trypan Blue. L1 was used as a positive control.
Figure 3. **JG19 and JG20 could induce apoptosis-associated PARP cleavage.**

Raji cells were treated with 50 uM JG19 or JG20 for 4, 8, or 20 hours before being harvested. The results show that both JG19 and JG20 induce PARP cleavage, indicative of apoptosis.

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Figure 4. **Chemical structures of more beta-lactams**

- **Lactam 1**
- **Lactam 30**
- **Lactam 44**
- **Lactam 45**
- **Lactam 47**
- **Lactam 50**
- **Lactam 51**
- **Lactam 52**
- **Lactam 53**
Figure 5. β-lactams could effectively inhibit proliferation and induce apoptosis in human breast cancer cells.

A. MCF-7 cells were treated with either 1, 25 or 50 µM of indicated lactams or DMSO as solvent control (Ctrl) for 24 h, followed by performance of an MTT assay.

B. MDA-MB-231 cells were treated with 50 µM of indicated β-lactams for different time points, followed by preparation of cell lysates and measurement of PARP cleavage by Western blot.
Figure 6. β-lactam HY-14 could inhibit the tumor growth in nude mice implanted by human breast cancer MDA-MB-231 cells.

Female athymic nude mice (NCRNU-M) were xenografted by injection of 6 X 10⁶ MDA-MB-231 cells. 15 days after the injection, the mice were divided into three groups: solvent control, low dose (0.3 mg/kg) and high dose (3 mg/kg) treatment with β-lactam HY-14 by subcutaneous injection daily. Tumor size was measured every 5 days and tumor volume (V) was determined by the equation: 

$$V = L \times W^2 \times 0.5$$

where L is the length and W is the width of a tumor. Tumor volume was calculated and expressed as cubic millimeters (A). TUNEL (Terminal deoxynucleotidyl Transferase Biotin-dUTP Nick End Labeling) analysis of tumor tissues from three different groups was performed. Nuclei stained in dark brown indicate TUNEL positive (B).
KEY RESEARCH ACCOMPLISHMENTS

- Published 3 articles and 1 abstract
- Gave 5 scientific presentations
- Trained and graduated two Ph.D. students
- Partially supported several personnel (Deborah Kuhn, Ph.D., Kenyon Daniel, Ph.D., Di Chen, Ph.D., Shirley Orlu, B.S.)

REPORTABLE OUTCOMES

Provide a list of reportable outcomes that have resulted from this research to include:

**Manuscript Publication (see Appendices):**


**Abstracts (see Appendices):**


**Presentations:**

Minic V, Kuhn D, Daniel K, Chen D, Landis K, Turos E, Dou QP. Potential Use of β-Lactam antibiotics in Cancer Prevention and Therapy. Poster presentation. Cancer Biology Program, Karmanos Cancer Institute and Wayne State University, Detroit, MI, September 8, 2004

Dou QP. Invited Speaker. Synthetic beta-lactam antibiotics and a selective breast cancer cell apoptosis inducer: Significance in breast cancer prevention and treatment. The Breast Cancer Group, Karmanos Cancer Institute, Detroit, MI, May 6, 2004
**Dou QP. Invited Speaker. Discovery of Novel Small Molecules: Rational Design, Structure-Activity Relationships, Cellular Targets, and Potential Uses for Cancer Treatment and Prevention. The Developmental Therapeutics Group, Karmanos Cancer Institute, Detroit, MI, September 8, 2004**

Minic V, Kuhn D, Daniel K, Chen D, Landis K, Turos E, **Dou QP. Potential Use of β-Lactam antibiotics in Cancer Prevention and Therapy. Poster presentation. Graduate student recruitment for Cancer Biology Program, Karmanos Cancer Institute and Wayne State University, April 2, 2005**


**Patents and licenses applied for and/or issued:**
None

**Degrees obtained that are supported by this award:**
Kenyon Daniel, Ph.D., graduated from Department of Biochemistry & Molecular Biology, University of South Florida College of Medicine on April 5, 2004. Dissertation Title: “Strategies for Cancer Therapy Through Regulation of Apoptotic Proteases” (Advisor: Q. Ping Dou). Currently working as a postdoctoral fellow in my laboratory at Karmanos Cancer Institute, Wayne State University

Deborah Kuhn, Ph.D., graduated from Cancer Biology Program, University of South Florida College of Medicine on November 7, 2004. Dissertation Title: “Novel Approaches to Targeting Tumor Cell Apoptotic Signaling Pathways” (Advisor: Q. Ping Dou). Currently working as a postdoctoral fellow in University of North Carolina.

**Development of cell lines, tissue or serum repositories; infomatics such as databases and animal models, etc:**
None.

**Funding applied for based on work supported by this award:**
Alliance For Cancer Gene Therapy. Dual-agent nanoparticles to overcome drug resistance. 5% Effort (Co-PI: Q. Ping Dou; PI: Jayanth Panyam). 07/01/05-06/30/08. Total Direct Costs: $449,991; Total Indirect Costs: $44,999
The Michigan Technology Tri-Corridor Fund, Fiscal Year 2005 Competition. DUAL-AGENT NANOPARTICLES TO OVERCOME DRUG RESISTANCE. 5% Effort (Co-PI: Q. Ping Dou; PI: Jayanth Panyam). 07/01/05-06/30/07. Total Direct Costs: $85,320 (to Dou Lab)

NIH R21. Dual-agent nanoparticles to overcome drug resistance. 5% Effort (Co-PI: Q. Ping Dou; PI: Jayanth Panyam). 07/01/05-06/30/07. Total Direct Costs: $250,000; Total Indirect Costs: $124,950

Wayne State University Stimulus responsive Nanosystems Proposal. Stimulus Controlled Nanosystems for Cancer Imaging and Treatment. (Co-I: Q. Ping Dou; PI: Stephanie L. Brock). Total Direct Costs (Dou Lab): $40,000

Susan Komen Foundation. Nanoparticle-mediated combination photodynamic and chemotherapy to overcome refractory tumors. 5% Effort (Co-PI: Q. Ping Dou; PI: Jayanth Panyam). 05/01/06-04/30/08. Total Direct Costs: $200,000

Employment or research opportunities applied for and/or received based on experience/training supported by this award:

Deborah Kuhn, Ph.D.
Kenyon Daniel, Ph.D.
Huanjie Yang, Ph.D.
Haiyan Pang, Ph.D.
Di Chen, Ph.D.
Shirley Orlu, B.S.
Cindy (Qiuzhi) Cui, Technician

CONCLUSIONS

We have determined whether one of novel beta-lactams, HY14, can inhibit tumor growth in vivo. We have found that HY14 inhibited growth of implanted MDA-MB-231 breast tumors in a concentration-dependent manner, associated with its DNA-damaging activity. We are currently testing more novel beta-lactams in nude mice bearing human breast tumors. Our studies have provided strong support for proof-of-concept of the potential use of these N-thiolated beta-lactams in breast cancer prevention and treatment.
REFERENCES

APPENDICES


Curriculum vitae.
Novel \(N\)-thiolated \(\beta\)-lactam antibiotics selectively induce apoptosis in human tumor and transformed, but not normal or nontransformed, cells

Aslamuzzaman Kazi\textsuperscript{a}, Randy Hill\textsuperscript{a}, Timothy E. Long\textsuperscript{b}, Deborah J. Kuhn\textsuperscript{a}, Edward Turosa\textsuperscript{a,b}, Q. Ping Dou\textsuperscript{a,*}

\textsuperscript{a}Drug Discovery Program, H. Lee Moffitt Cancer Center and Research Institute, Departments of Interdisciplinary Oncology and Biochemistry & Molecular Biology, College of Medicine, University of South Florida, Tampa, FL 33612, USA

\textsuperscript{b}Department of Chemistry, College of Arts and Sciences, University of South Florida, Tampa, FL 33612, USA

Received 13 May 2003; accepted 4 September 2003

Abstract

Historically, it has been shown that the \(\beta\)-lactam antibiotics play an essential role in treating bacterial infections while demonstrating selectivity for prokaryotic cells. We recently reported that certain \(N\)-methylthio-substituted \(\beta\)-lactam antibiotics had DNA-damaging and apoptosis-inducing activities in various tumor cells. However, whether these compounds affect human normal or nontransformed cells was unknown. In the current study, we first show that a lead compound (lactam 1) selectively induces apoptosis in human leukemic Jurkat T, but not in the nontransformed, immortalized human natural killer (NK) cells. Additionally, we screened a library of other \(N\)-methylthiolated \(\beta\)-lactams to determine their structure–activity relationships (SARs), and found lactam 12 to have the highest apoptosis-inducing activity against human leukemic Jurkat T cells, associated with increased DNA-damaging potency. Furthermore, we demonstrate that lactam 12, as well as lactam 1, potently inhibits colony formation of human prostate cancer cells. We also show that lactam 12 induces apoptosis in human breast, prostate, and head-and-neck cancer cells. Finally, lactam 12 induces apoptosis selectively in Jurkat T and simian virus 40-transformed, but not in nontransformed NK and parental normal fibroblast, cells. Our results suggest that there is potential for developing this class of \(\beta\)-lactams into novel anticancer agents.

Keywords: \(N\)-thiolated \(\beta\)-lactam; Antibiotics; DNA damage; Apoptosis; Anticancer drugs

1. Introduction

Apoptosis, or programmed cell death, is a highly regulated process important in embryonic and immune system development and tissue homeostasis [1,2]. Perturbation of this pathway can lead to autoimmunity, acquired immune deficiency syndrome, neurodegenerative disorders, and cancer [3,4]. Initiation, commitment, and execution are

the three fundamental steps of apoptosis [5]. Several apoptotic stimuli, such as death receptor-binding ligands, signal to activate the initiator caspases (caspases-2, -8, -9, -10), which in turn activates downstream effector caspases (caspases-3, -6, -7). The effector caspases can also be activated through the release of key mitochondrial proteins, such as cytochrome \textit{c}, cell death inducer second mitochondria-derived activator of caspases (Smac), and apoptosis initiating factor [6]. It is generally believed that proteolytic cleavage of a variety of intracellular substrates, including poly(ADP-ribose) polymerase (PARP) [7,8] and the retinoblastoma protein (RB) [9–11], by effector caspases leads to apoptosis.

For nearly 60 years \(\beta\)-lactam compounds have been used in the treatment of bacterial infections [12]. Following the initial introduction of penicillin, a variety of other classes of \(\beta\)-lactam antibiotics were subsequently identified and used clinically, including cefalosporins, penems,
carbapenems, nocardicins, and monobactams [13]. The bacterial targets of these antibiotics are membrane-bound transpeptidases referred to as the penicillin-binding proteins, which are responsible for creating crosslinks within the bacterial cell wall [13]. By disrupting these cross-linking proteins, the β-lactams induce structural deformities within the cell wall, which cause the bacteria to lyse. Recently, a novel class of N-thiolated β-lactams has been shown to inhibit Staphylococcus aureus and methicillin-resistant S. aureus growth [14–16].

Previously we showed that N-thiolated β-lactams, such as β-lactam 1, induced DNA damage, inhibited DNA replication, and induced tumor cell apoptosis in a time- and concentration-dependent manner [17]. Our current study shows, for the first time, that the N-thiolated β-lactam 1 can preferentially induce apoptosis in leukemic Jurkat T cells, but not nontransformed, immortalized human NK cells. Additionally, we also show that lactam 12, an analog of lactam 1, has enhanced apoptosis-inducing activity in Jurkat T cells compared to lactam 1. Furthermore, this study reveals that lactam 12 can induce apoptosis in other human solid tumor cell lines such as breast, prostate, and head and neck. Lactam 12 also induces apoptosis selectively in Jurkat T, but not human NK, cells, and in simian virus 40 (SV40)-transformed human fibroblasts (VA-13), but not in their parental counterpart (WI-38). Both lactams 1 and 12 are able to activate caspase-3 in human prostate cancer cells and inhibit colony formation of these cells in soft agar. These data indicate that further study of N-thiolated β-lactams in the treatment of cancer is warranted.

2. Materials and methods

2.1. Materials

Fetal bovine serum (Tissue Culture Biologicals), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), dimethyl sulfoxide (DMSO), and trypsin blue were purchased from Sigma-Aldrich. RPMI 1640, Dulbecco’s modified Eagle’s medium (DMEM), MEM nonessential amino acids solution, MEM sodium pyruvate solution, penicillin, and streptomycin were purchased from Invitrogen. Fluorogenic peptide substrate Ac-DEVD-AMC (the specific caspase-3/-7 substrate) was obtained from Calbiochem. Polyclonal antibody to human PARP was obtained from Roche Molecular Biochemicals. The APO-DIRECT kit for terminal deoxynucleotidyl transferase-mediated UTP nick-end labeling (TUNEL) staining was purchased from BD Pharmingen.

2.2. Synthesis of β-lactams

The β-lactam analogs (Fig. 1A) were prepared as racemates (with cis stereochemistry) using a procedure described previously [14,15].

2.3. Cell culture, protein extraction, and Western blot assay

Human Jurkat T cells and human prostate cancer LNCaP cells were cultured in RPMI 1640 medium, supplemented with 10% fetal bovine serum, 100 U/mL penicillin, and 100 µg/mL streptomycin. Human YT cells were cultured in RPMI 1640 medium supplemented with 1 mM MEM sodium pyruvate solution, 0.1 mM MEM nonessential amino acids solution, 0.1% fetal bovine serum, 100 U/mL penicillin, and 100 µg/mL streptomycin. Human breast cancer MCF-7 cells, head-and-neck cancer PCI-13 cells, prostate cancer DU-145 cells, normal (WI-38) and SV-40 transformed (VA-13) human fibroblasts cells were grown in DMEM containing 10% fetal calf serum, 100 U/mL penicillin, and 100 µg/mL streptomycin. All cell lines were maintained at 37°C in a humidified incubator with an atmosphere of 5% CO2.

A whole-cell extract was prepared as described previously [18]. Briefly, cells were harvested, washed with PBS and homogenized in a lysis buffer (50 mM Tris–HCl, pH 8.0, 5 mM EDTA, 150 mM NaCl, 0.5% NP-40, 0.5 mM phenylmethylsulfonyl fluoride, and 0.5 mM dithiothreitol) for 30 min at 4°C. Afterwards, the lysates were centrifuged
at 12,000 g for 15 min at 4°C and the supernatants collected as whole-cell extracts. Equal amounts of protein extract (60 μg) were resolved by SDS-polyacrylamide gel electrophoresis and transferred to a nitrocellulose membrane (Schleicher & Schuell) using a Semi-Dry Transfer System (Bio-Rad). The enhanced chemiluminescence Western blot analysis was then performed using specific antibodies to the proteins of interest.

2.4. Trypan blue assay

The trypan blue dye exclusion assay was performed by mixing 20 μL of cell suspension with 20 μL of 0.4% trypan blue dye before injecting into a hemocytometer and counting. The number of cells that absorbed the dye and those that exclude the dye were counted, from which the percentage of nonviable cell number to total cell number was calculated.

2.5. Morphological assessment of apoptosis

To assess morphological changes of cells, 50 μL of treated or untreated cell suspension were transferred to a glass slide at the indicated time points. The slides were observed under a phase-contrast microscope (Leica) and photographs were taken (100×). Apoptotic cells were identified by their distinct morphological changes.

2.6. TUNEL assay

Terminal deoxynucleotidyl transferase-mediated nick-end labeling (TUNEL) was used to determine the extent of DNA strand breaks [19]. The assay was performed following manufacturer’s instruction using the APO-Direct kit. In brief, the harvested cells were fixed in 1% paraformaldehyde for 15 min on ice, washed with PBS, and then fixed again in 70% ethanol at −20°C overnight. The cells were then incubated in DNA labeling solution (containing terminal deoxynucleotidyl transferase (TdT) enzyme, fluorescein-conjugated dUTP and reaction buffer) for 90 min at 37°C. After removing the DNA labeling solution by rinsing cells with Rinsing Buffer, the cells were incubated with the propidium iodide/RNase A solution, incubated for 30 min at room temperature in the dark, and then analyzed by flow cytometry within 3 hr of staining.

2.7. Caspase-3/-7 activity assay

To measure cell-free caspase-3/-7 activity, whole-cell extracts (20–30 μg) from untreated or treated LNCAp, MCF-7, PCI-13, DU-145, VA-13, and WI-38 cells were incubated with 20 μM of the fluorogenic substrate caspase-3/-7 (Ac-DEVD-AMC) for 30 min at 37°C in 100 μL of assay buffer (50 mM Tris, pH 8.0). Measurement of the hydrolyzed AMC groups was performed on a VersaFluor™ Fluorometer (Bio-Rad) as described previously [20].

2.8. Soft agar assay

The soft agar assay was performed as described previously [21] with a few modifications. In brief, in a 6-well plate, a bottom feeder layer (0.6% agar) was prepared with DMEM media containing 10% fetal bovine serum, 100 U/mL penicillin, and 100 μg/mL streptomycin. A top layer (0.3% agar) was prepared with DMEM and the same media as described above but containing 2 × 10⁴ prostate cancer LNCaP cells and 50 μM of lactam 1 or 12, or equal volume of solvent (DMSO) as a control. Plates were incubated at 37°C in 5% CO₂ in a humidified incubator for 3 weeks. MTT (1 mg/mL) was added to each well and incubated overnight to allow complete formation of purple formazan crystals. The plates were then scanned and photographed, and the number of colonies was quantified by Quantity one v. 4.0.3 software (Bio-Rad).

2.9. Nuclear staining

To assay nuclear morphology, the detached or remaining attached cells were washed with PBS, fixed with 70% ethanol for 1 hr, and stained with Hoechst 33342 (1 mM) for 30 min. The nuclear morphology was visualized by fluorescence microscopy (40×; Leitz) [18].

3. Results

3.1. Screening for more apoptotically active analogs of lactam 1

Lactam 1 contains a chloro (−Cl) group in the ortho position on the benzene ring (Fig. 1A). To examine whether deletion or substitution of the Cl group could affect its cell death-inducing ability, other halogen and nonhalogen analogs of lactam 1 were synthesized (Fig. 1A). These compounds were then tested in the trypan blue dye exclusion assay, using lactam 1 as a comparison (Fig. 1B). Jurkat T cells were treated with each of these compounds at 50 μM for 24 hr, followed by measurement of loss of cell membrane permeability, indicative of a late apoptotic stage (Fig. 1B) [22,23]. As a control, lactam 1 induced ~52% cell death (Fig. 1B). Interestingly, removal of the Cl group from the benzene ring significantly decreased the cell death-inducing activity to ~25% (lactam 8; Fig. 1B). Furthermore, replacement of the Cl group with a smaller halogen atom (−F; lactam 9) also decreased the death-inducing activity (to ~35%), while analogs with a larger halogen group (−Br and −I; lactams 10 and 11, respectively; Fig. 1A) increased the cell death rates to 55 and 60% (Fig. 1B). These data suggest that the size of the group in the ortho position on the benzene ring is important for the compound’s cell death-inducing activity. Indeed, the analog with −NO₂ substitution, lactam 12 (Fig. 1A), exhibited the strongest effect with a total of
~94% cell death (Fig. 1B). Therefore, the order of potency of the tested compounds was: $X = H < F < Cl < Br < I < NO_2$.

3.2. Lactam 1 induces apoptosis preferentially in leukemic Jurkat T over nontransformed, immortalized NK cells

Previously, we reported that β-lactam analogs, such as lactam 1 (Fig. 1A) [17], were able to induce tumor cell apoptosis. However, whether lactam 1 affects normal or nontransformed cells was unknown. To determine whether lactam 1 was able to induce apoptosis preferentially in tumor/transformed vs. normal/nontransformed cells, we treated human leukemic Jurkat T cells and immortalized, nontransformed NK cells (YT line) [24] with lactam 1 in both concentration- and time-dependent experiments. Treatment of Jurkat T cells with 10 μM of lactam 1 for 24 hr induced apoptosis-specific PARP cleavage fragment p85 (Fig. 2A), whose levels were further increased when 25 μM of lactam 1 was used (Fig. 2A). After treatment with 50 μM of lactam 1, PARP degradation was further increased, as evidenced by a significant decrease in expression of intact PARP protein (Fig. 2A). In contrast, no PARP cleavage was detectable in the YT cells after treatment with lactam 1 at even 50 μM (Fig. 2A).

In the kinetic experiment, both Jurkat T and YT cells were treated with 30 μM of lactam 1 for 3, 6, or 24 hr. PARP cleavage was detected in Jurkat T cells first at 3 hr, which was then increased at 6 hr (although the levels of PARP/p85 fragments at 24 hr were decreased in this Western blotting; Fig. 2B). Importantly, no PARP cleavage was observed in YT cells in the same kinetic experiment (Fig. 2B). To confirm the tumor cell-selective killing activity of lactam 1, a trypan blue dye exclusion assay was performed in the same kinetic experiment. After 24 hr, there was 42% cell death in the Jurkat T cells compared to 9% in YT cells (Fig. 2C). Furthermore, by using phase-contrast microscopy, more cell death was observed in Jurkat T cells than YT cells (Fig. 2D). These data support the conclusion that lactam 1 could induce apoptotic cell death selectively in tumor over nontransformed cells.

3.3. Lactam 12 has enhanced apoptosis-inducing activity specific to Jurkat T, but not normal YT cells

To determine if lactam 12 is capable of inducing apoptosis at lower concentrations than lactam 1, a dose–response experiment was performed with both compounds. Jurkat T cells were treated with lactam 12 at 2, 10, 25, and 50 μM for 24 hr, using 50 μM of lactam 1 as a comparison. Again, treatment with lactam 1 caused ~50% cell death, measured by trypan blue exclusion assay (Fig. 3A). Under the same experimental conditions, lactam 12 induced cell death in a concentration-dependent manner: 25% at 10 μM, 45% at 25 μM, and 80–90% at 50 μM (Fig. 3A). Therefore, lactam 12 is ~2-fold more potent than lactam 1. This conclusion was further supported by PARP cleavage

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Fig. 2. Selective induction of apoptosis by lactam 1 in leukemic Jurkat T over immortalized/nontransformed NK cells. Jurkat T and NK (YT) cells were treated with 10, 25, and 50 μM of lactam 1 for 24 hr (A) or with 30 μM of lactam 1 for indicated hours (B–D). (A and B) Measurement of PARP cleavage in Western blot assay. The intact PARP (116 kDa) and a PARP cleavage fragment (p85) are shown. (C) Trypan blue dye exclusion assay. The numbers given are percentages of nonviable cells to total cells. Standard deviations are shown with error bars from a mean of at least three different experiments. (D) Morphological changes of Jurkat T and YT cells after treatment. Photographs under a phase-contrast microscope (100×).
assay using lysates prepared after 12-hr treatment (Fig. 3B). Cleavage of PARP occurred in lactam 12-treated cells in a dose-dependent manner with the highest level of PARP cleavage observed at 50 μM (Fig. 3B). The levels of PARP cleavage induced by 50 μM of lactam 1 were equivalent to ~50% of that by 50 μM of lactam 12 (Fig. 3B).

In the same experiment, when immortalized, nontransformed NK cells were treated with lactam 12 (using lactam 1 as a control), neither cell death (Fig. 3C) nor PARP cleavage (Fig. 3D) were observed. Therefore, like lactam 1, lactam 12 also induces apoptotic cell death preferentially in tumor over nontransformed cells.

To further compare the potency of lactams 1 and 12, Jurkat T cells were treated with 25 μM of lactam 12 vs. 50 μM of lactam 1 for 3, 6, 12, and 24 hr, followed by determination of trypan blue incorporation and PARP cleavage. After 3 hr, lactam 12 at 25 μM caused 15% vs. 11% cell death with lactam 1 at 50 μM (Fig. 4A). Similarly, at 6 hr, 24% of trypan blue-positive cells were found after 25 μM lactam 12 treatment, while only 20% observed in 50 μM lactam 1-treated cells (Fig. 4A). Only at later time points (12 and 24 hr), lactam 1 at 50 μM was slightly more potent than lactam 12 at 25 μM (Fig. 4A). Similar levels of cleaved PARP were observed in Jurkat T cells treated with either 25 μM of lactam 12 or 50 μM of lactam 1 at each time point (Fig. 4B). Therefore, lactam 12 is able to induce similar amounts of apoptosis in Jurkat T cells at a concentration half of that of lactam 1.

Furthermore, we examined levels of sub-G1 populations, as a measurement of cells with DNA fragmentation [18], in Jurkat T cells treated with lactam 12 or 1. Treatment with 50 μM of lactam 12 increased the sub-G1 populations by 34 and 57%, respectively, at 12 and 24 hr (Fig. 5A). In comparison, 50 μM of lactam 1 treatment for 12 and 24 hr induced sub-G1 populations by 10 and 16%, respectively [17], confirming the greater potency of lactam 12.

3.4. Lactam 12 is able to induce DNA damage in Jurkat T cells

We have previously shown that lactam 1 induces damage to DNA, leading to the inhibition of DNA replication and...
subsequent induction of apoptosis [17]. To determine whether lactam 12 is also capable of damaging tumor cell DNA, Jurkat T cells were treated with 50 \( \mu M \) of lactam 12, followed by performance of TUNEL assay, which detects DNA strand breaks [17]. A significant population (\( \geq 70\% \)) of the cells exhibited DNA strand breaks after 3 hr of incubation with lactam 12 (Fig. 5B). A total of 82–90% of the cells were TUNEL-positive after 12–24-hr treatment with lactam 12 (Fig. 5B). In this experiment, 66% of TUNEL-positive cells were observed after treatment with 50 \( \mu M \) of lactam 1 for 24 hr (data not shown). Thus, the increased DNA-damaging capability of lactam 12 is most likely responsible for its enhanced cell death-inducing activity (Figs. 1–4).

3.5. Lactams 1 and 12 induce apoptosis and inhibit colony formation in human prostate cancer cells

So far, we demonstrated that lactam 12 is also capable of damaging tumor cell DNA, Jurkat T cells were treated with 50 \( \mu M \) of lactam 12, followed by performance of TUNEL assay, which detects DNA strand breaks [17]. A significant population (\( \sim 70\% \)) of the cells exhibited DNA strand breaks after 3 hr of incubation with lactam 12 (Fig. 5B). A total of 82–90% of the cells were TUNEL-positive after 12–24-hr treatment with lactam 12 (Fig. 5B). In this experiment, 66% of TUNEL-positive cells were observed after treatment with 50 \( \mu M \) of lactam 1 for 24 hr (data not shown). Thus, the increased DNA-damaging capability of lactam 12 is most likely responsible for its enhanced cell death-inducing activity (Figs. 1–4).

In a previous study, we showed that lactam 1 induced apoptosis in several solid tumor cell lines [17]. In this study we also investigated the effects of lactam 12 on several solid tumor cell lines including human breast (MCF-7), head-and-neck (PCI-13), and prostate (DU-145) cancer cells. Furthermore, we wanted to investigate whether lactam 12-induced cell death was selective in transformed (VA-13) over the normal (WI-38) human fibroblasts. We treated these cell lines with 50 \( \mu M \) lactam 12 or an equal percentage of DMSO, followed by separation of the attached and detached cell populations. Both attached and detached cell populations were then used for detection of apoptotic nuclear condensation. We found that after a 48-hr treatment with lactam 12, \( \sim 60\% \) of MCF-7 and PCI-13 cells and \( \sim 50\% \) of DU-145 and VA-13 cells became detached. However, no detachment was observed in WI-38 cells after treatment with lactam 12. Little or no detachment was observed in all the cell lines treated with DMSO. All the detached tumor or transformed cells exhibited typical apoptotic nuclear condensation.
and fragmentation (Fig. 7A). In addition, apoptosis-specific nuclear condensation was also observed in the remaining attached solid tumor (MCF-7, PCI-13, and DU-145) and transformed (VA-13), but not the normal (WI-38), cells (Fig. 7A). These results strongly suggest that lactam 12 induces apoptosis that lead to detachment preferentially in the tumor and transformed cells.

To confirm lactam 12-mediated apoptotic cell death, in the same experiment, aliquots of both detached and attached cells of each line were combined and used for whole-cell extract preparation. This was followed by measurement of cell-free caspase-3/-7 activity. Consistent with the apoptotic nuclear changes (Fig. 7A), treatment of MCF-7, PCI-13, DU-145, and VA-13 cells with lactam 12 also increased levels of caspase-3/-7 activity by 11.0-, 10.2-, 5.2-, and 5.3-fold, respectively, over the control DMSO-treated cells (Fig. 7B). In addition, accompanying the lack of the detachment in normal WI-38 cells treated with lactam 12 (Fig. 7A), there was little or no induction of caspase-3/-7 activity observed in these cells (Fig. 7B). Taken together, these data further support the conclusion that lactam 12 is able to induce apoptotic cell death preferentially in tumor and transformed over the normal cells.

4. Discussion

Developing novel anticancer drugs that induce apoptosis in tumor cells has long been a goal of cancer drug discovery research. Many of the drugs in current use focus on targeting dysregulated cell cycle and apoptosis programs in cancer cells [25]. We previously have shown that N-thiolated β-lactams cause DNA damage in tumor cells that leads to induction of apoptosis through p38 activation, cytochrome c release, and caspase activation [17]. Here we show that lactam 1 selectively induces apoptosis in human leukemic Jurkat T cells, but not nontransformed, immortalized human NK cells (Fig. 2). Furthermore, lactam 1 is capable of inducing Jurkat cell apoptosis at concentrations as low as 10 μM after 24-hr treatment (Fig. 2A).

Often, addition/substitution of groups on a molecule leads to development of more potent drugs. In order to determine whether structural changes to lactam 1 could produce a more potent tumor cell death inducer, analogs of lactam 1 were synthesized (Fig. 1A). Substitutions of the –Cl group with other halogens of higher atomic mass (–Br, –I) did increase the efficacy of the compound. In contrast, substitution with a lower atomic mass halogen (–F) or a hydrogen (H) atom had a concomitant decrease in cell death induction (Fig. 1). Lactam 12, containing an –NO2 substituent, proved to be a highly active compound and induced 93–100% of cell death at 50 μM vs. 52% of cell death by lactam 1 at the same concentration (Fig. 1B).

Furthermore, lactam 12 was superior to lactam 1 at inducing apoptosis in human Jurkat T cells because lactam 12 can induce the same amount of PARP cleavage at a lower concentration than lactam 1 (Figs. 3 and 4). Additionally, lactam 12 at 25 μM was able to exert its cell death-inducing effect at as early as 3 hr (Fig. 4A and B). We also found that lactam 12 had greater potency than lactam 1 when used in human prostate cancer cells to activating caspase-3/-7 and inhibiting colony formation (Fig. 6). Similar to our previous results with lactam 1 [17], we found that lactam 12 induces apoptosis in several solid tumor cell lines (e.g. MCF-7, PCI-13, DU-145) in a caspase-dependent manner (Fig. 7). Due to lack of caspase-3 in MCF-7 cells, it was believed that lactam 12-mediated MCF-7 cell death was associated with caspase-7 activity (Fig. 7). Additionally, like lactam 1, lactam 12 was also able to selectively induce apoptosis in human leukemic Jurkat T cells over nontransformed,
Fig. 7. Lactam 12 induces apoptosis and caspase activation in different solid tumor cell lines and SV40-transformed but not normal cells. (A) Nuclear staining assay. MCF-7, PCI-13, DU-145, VA-13, and WI-38 cells were treated with 50 μM lactam 12 or DMSO for 48 hr, followed by collection of both detached and attached cell populations. After lactam 12 treatment, ~60% of MCF-7 and PCI-13 cells and ~50% of DU-145 and VA-13 cells became detached, whereas <5% were detached from each of these cell lines after DMSO treatment. No detachment was found in WI-38 cells after each treatment. Both detached and attached cell populations were stained with the nuclear staining dye Hoechst 33342. Each sample was then analyzed by fluorescence microscopy for nuclear morphology. (B) Cell-free caspase-3/-7 activity assay. Aliquots of the above detached and attached cells of each line were combined for whole-cell extraction. Cell-free caspase-3/-7 activity was then determined by incubating each whole-cell extract with caspase-3/-7 substrate and measuring free AMCs. Error bars denote standard deviations. Similar results were obtained in three independent experiments.
immortalized human NK cells (Fig. 3). Also, lactam 12 was able to selectively induce apoptotic cell death in simian virus 40-transformed, but not the parental normal, human fibroblasts (Fig. 7). The molecular mechanism for the enhanced activity in lactam 12 remains unknown. One interpretation is that the presence of –NO2 group in this drug increases its binding to the cellular target(s). Alternatively, this drug might have increased uptake rates by the cells.

The mechanism of action of many chemotherapeutic drugs is through DNA damage and then subsequent apoptosis induction in tumor cells [26]. As mentioned above, we have recently shown that lactam 1 is capable of inducing apoptosis after DNA damage [17]. In the present study, we show, by TUNEL assay, that lactam 12 also causes DNA damage in ~70% of cells just after 3-hr treatment (Fig. 5B). At this time, there was only 2% cell death (Fig. 5A), suggesting that the DNA damage occurs much earlier than apoptotic cell death. However, apoptotic cells increased at later time points with increased TUNEL-positive cells (Fig. 5A and B). This result is consistent with our previous study [17] and several other studies that have shown that several traditional chemotherapeutic drugs or DNA-damaging agents cause DNA strand breaks that trigger apoptotic cell death [27,28].

Malignant transformation of a cell can lead to tumor formation and metastasis. The desired effect of any anticancer drug is to inhibit tumor growth and formation in situ. Soft agar colony forming assay is an assay that has been developed to mimic tumor cellular growth in tissue. We hypothesized that the N-thiolated β-lactams that induce cell death should be able to inhibit colony formation in soft agar assay. Indeed, when LNCaP prostate cancer cells were cultured in the presence of lactam 1 or 12, 91 and 100% inhibition of colony formation was observed, respectively, as compared to the solvent control (Fig. 6).

Based on our previous [17] and current studies, we propose that these N-thiolated β-lactams act by inducing DNA damage that leads to apoptosis preferentially in cancer and transformed over normal/nontransformed cells. Although it appears that the N-methylthio moiety is necessary for the cell death-inducing activity [17], addition of a larger group in the ortho position on the phenyl ring can also increase the effectiveness of the compound (Fig. 1). Our results strongly suggest the potential for developing this class of β-lactams into novel anticancer agents. Immediate future studies focusing on determining the molecular targets and chemical action of the N-thiolated β-lactams would help rational development of these compounds.

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References


STRUCTURE-ACTIVITY RELATIONSHIPS OF N-METHYLTHIOLATED BETA-LACTAM ANTIBIOTICS WITH C3 SUBSTITUTIONS AND THEIR SELECTIVE INDUCTION OF APOPTOSIS IN HUMAN CANCER CELLS

Deborah J. Kuhn 1, Yang Wang 2, Vesna Minic 1, Cristina Coates 2, G. Suresh Kumar Reddy 2, Kenyon G. Daniel 1, Jeung-Yeop Shim 2, Di Chen 1, Kristin R. Landis-Piwowar 1, Fred R. Miller 3, Edward Turos 2 and Q. Ping Dou 1

1 The Prevention and 3 Breast Cancer Programs, Barbara Ann Karmanos Cancer Institute, and Department of Pathology, School of Medicine, Wayne State University, Detroit, Michigan, USA, 2 Department of Chemistry, College of Arts and Sciences, University of South Florida, Tampa, Florida, USA.

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1. ABSTRACT

The development of novel anti-cancer drugs that induce apoptosis has long been a focus of drug discovery. Beta-lactam antibiotics have been used for over 60 years to fight bacterial infectious diseases with little or no side effects observed. Recently, a new class of N-methylthiolated beta-lactams has been discovered that have potent activity against methicillin resistant Staphylococcus aureus. Most recently, we determined the potential effects of these N-thiolated beta-lactams on tumorigenic cell growth and found that they are apoptosis-inducers in human cancer cell lines. In the current study, we further determined the effects of the substitution of the O-methyl moiety on C3 and stereochemistry of the beta-lactams on the anti-proliferative and apoptosis-inducing abilities. We have found that lactam 18, in which C3 is substituted with an acrylate ester group, is a very effective proliferation inhibitor against human premalignant and malignant breast, leukemic, and simian virus 40-transformed fibroblast cells. Generally speaking, increasing the size of the moiety on C3 decreases its anti-proliferation potency, possibly indicating steric hindrance with the cellular target or decreased permeability through the cell membrane. We also found that the stereochemistry of the beta-lactams plays an important role in their potency. The 3S,4R isomers are more effective than their enantiomers (3R,4S), suggesting that 3S,4R configuration is more favorable for target interaction.

2. INTRODUCTION

Selectively targeting tumorigenic cells versus normal cells is a primary goal in anti-cancer drug discovery. Small molecules with apoptosis-inducing ability have great potential to be developed into novel chemotherapeutic drugs because of the ease of synthesis and structural manipulation (1-3). Initiation, commitment, and execution are the three fundamental steps of apoptosis (4). Several apoptotic stimuli, such as irreparable DNA damage, signal to activate the initiator caspases (e.g. caspases-8/10), which in turn activate downstream effector caspases (e.g. caspases-3/7). The effector caspases can also be activated through the release of mitochondrial proteins, such as cytochrome c (5). It is generally believed that proteolytic cleavage of a variety of intracellular substrates by effector caspases leads to apoptosis (6-9).

One particularly important class of small molecule drugs, the beta-lactam antibiotics, have played an essential role in treating bacterial infections without causing toxic side effects in the host for the past 60 years. Sir Alexander Fleming first coined
the name “penicillin” in 1928 after his discovery that molds from the *Penicillium* genus secrete powerful antimicrobial compounds, called beta-lactams (10). X-ray crystallography revealed that penicillin is a thiazolidine ring fused to a four-membered beta-lactam ring (11). 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 (12). There are several classes of bicyclic beta-lactams that possess antibacterial properties, including the penams, penems, carbapenems, cephalosporins, and clavulanic acids (10).

A novel class of beta-lactams was discovered by the Squibbs and Takeda laboratories in 1981, which have an N-sulfonic acid group attached directly to the nitrogen in the lactam ring (13, 14). The term “monobactam” was coined for these lactams, which have a flexible monocyclic ring and lack the carboxylic acid moiety, yet still retain a high bactericidal potency. Recently, a structurally related family of N-thiolated compounds, termed *N*-methylthio beta-lactams, was found to inhibit growth of *Staphylococcus* and *methicillin-resistant S. aureus* (MRSA) (15-17). Additionally, we have shown that these *N*-methylthio beta-lactams possess potent anti-proliferative properties, and are capable of inducing DNA strand breakage, inhibiting DNA replication, and inducing apoptosis in a time- and concentration-dependent manner when tested in several human cancer, but not normal cell lines (18, 19).

In this study, we screened several additional *N*-thiolated beta-lactams with substitutions made to the *O*-methyl moiety of carbon 3 (C3) for their structure-activity relationships and found that increasing the size of the C3 substitution results in decreased anti-proliferative activity in human breast cancer cells. Additionally, increasing the size of the C3 substituent may interfere with cellular uptake. We identified one particularly active lactam (lactam 18), which possesses an acrylate ester moiety off of C3, for further study. Lactam 18 induces caspase-3 activation and apoptosis, associated with increased Hsp70 protein expression and p38 phosphorylation. We have also found that the stereochemistry plays an important role in the activities of *N*-thiolated beta-lactam antibiotics, including anti-proliferation, S/G2/M cell cycle arrest, and apoptosis induction. The 3S,4R-configured [(+)]-isomers of lactam 18 and another lactam 19 are more potent than their 3R,4S-configured isomers or the racemic mixtures. Furthermore, these (+)-lactams are more efficacious than racemic lactam 1, which was identified from our previous studies (18). These effects of beta-lactams were found mainly in cultured human cancer and transformed cells, but not in normal/non-transformed cells. These data indicate that further study of *N*-thiolated beta-lactams in the treatment of cancers is warranted.

**MATERIALS AND METHODS**

3.1. Reagents

Fetal Bovine Serum was purchased from Tissue Culture Biologicals (Tulare, CA). Mixture of penicillin-streptomycin-L-glutamine, RPMI, Dulbecco’s modified Eagle’s medium (DMEM), DMEM/F12 (1:1) medium, horse serum, MEM non-essential amino acids solution, MEM sodium pyruvate solution, penicillin, and streptomycin were purchased from Invitrogen (Carlsbad, CA). (4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), dimethylsulfoxide (DMSO), epidermal growth factor (EGF), sodium bicarbonate, hydrocortisone, cholera enterotoxin, bovine insulin, propidium iodide and trypan blue were purchased from Sigma-Aldrich (St. Louis, MO). Polyclonal antibodies to actin, monoclonal antibodies to HSP70 and p-p38, and anti-goat and anti-mouse IgG-horseradish peroxidase were obtained from Santa Cruz Biotechnologies (Santa Cruz, CA). CaspACE FITC-VAD-FMK marker was purchased from Promega (Madison, WI). Fluorogenic peptide substrate Ac-DEV-DAMC (for caspase-3/-7 activities) was obtained from Calbiochem (San Diego, CA).

3.2. Synthesis of beta-lactams

The beta-lactam analogs (Figure 1) were prepared as racemates and enantiomers (with *cis* stereochemistry) using a procedure described previously (15, 16).

3.3. Cell culture, protein extraction, and Western blot assay

Human leukemia Jurkat T cells and natural killer YT cells were cultured in RPMI 1640 medium, supplemented with 10% fetal bovine serum, 100 units/ml penicillin, and 100 µg/ml streptomycin. Further supplementation with 1 mM MEM sodium pyruvate solution, 0.1 mM MEM non-essential amino acids solution was added to YT cells. Human breast cancer MCF-7 cells, normal (WI-38) and SV-40 transformed (VA-13) human fibroblasts cells were grown in Dulbecco’s modified Eagle’s medium containing 10% fetal calf serum, penicillin, and streptomycin. Premalignant MCF10AT1Kcl.2 transformed human breast cells were cultured in DMEM/F12 (1:1) supplemented with 10 µg/ml bovine insulin, 100ng/ml cholaer enterotoxin, 20 ng/ml epidermal growth factor, 500 ng/ml hydrocortisone, 29 mM sodium bicarbonate, 5% horse serum, 100 units/ml penicillin, and 100 µg/ml streptomycin. All cell lines were maintained at 37°C in a humidified incubator with an atmosphere of 5% CO₂. A whole-cell extract was prepared and Western blotting was performed as described previously (18).

3.4. Cellular proliferation assay

The MTT assay was used to determine the effects of beta-lactams on overall proliferation of tumor cells. Cells were plated in a 96-well plate and grown to 70-80% confluence, followed by addition of each compound at an indicated concentration for 24 h. MTT (1 mg/ml) in PBS was then added to wells and incubated at 37°C for 4 hours to allow for complete cleavage of the tetrazolium salt by metabolically active cells. Next, MTT was removed and 100 µl of DMSO was added, followed by
SAR of N-methylthiolated beta-lactams

colorimetric analysis using a multilabel plate reader at 560 nm (Victor3; Perkin Elmer). Absorbance values plotted are the mean from triplicate experiments.

3.5. Trypan blue assay

The trypan blue dye exclusion assay was performed by mixing 20 µl of cell suspension with 20 µl of 0.4% trypan blue dye before injecting into a hemocytometer and counting. The number of cells that absorbed the dye and those that exclude the dye were counted, from which the percentage of nonviable cell number to total cell number was calculated.

3.6. Cell cycle assay

Cell cycle analysis based on DNA content was performed as follows. Cells were harvested, counted, and washed twice with PBS. Cells (5 × 10^6) were then suspended in 0.5 ml of PBS, pipetted, and fixed in 5 ml of 70% ethanol for at least 2 h at -20°C. Cells were centrifuged, resuspended in 1 ml of propidium iodide staining solution (50 µg propidium iodide, 1 mg RNase A, and 1 mg of glucose per ml of PBS) and incubated at room temperature for 30 min before flow cytometry analysis. The cell cycle distribution is shown as the percentage of cells containing G1, S, G2, and M DNA judged by propidium iodide staining.

3.7. Caspase-3 activity assay

To measure cell-free caspase-3 activity, whole cell extracts (30 µg) from untreated or treated cells were incubated with 20 µM of the fluorogenic substrate caspase-3/-7 (Ac-DEVD-AMC) for 30 min at 37°C in 100 µl of assay buffer (50 mM Tris, pH 8.0). Measurement of the hydrolyzed AMC groups was performed on a Victor3 Multilabel plate readerTM (Perkin Elmer) as described previously [18].

3.8. Immunostaining of apoptotic cells

Immunostaining of apoptotic cells was performed by addition of the FITC-VAD-FMK marker and visualized on an Axiovert 25 microscope (Zeiss; Thornwood, NY). Briefly, cells were grown to ~80% confluency in 60 mm dishes, and then treated under conditions described in the figure legends. Detection of caspase activity was determined according to the manufacturer’s protocol with a few modifications. Briefly, total cell population was collected and incubated with a 10 µM FITC-VAD-FMK for 20 min in the dark. Cells were then centrifuged at 300 xg for 3 minutes, washed 3X in PBS, and then resuspended in 50 µl PBS. Cell suspension was then transferred to glass slides in the presence of Vector Shield mounting medium with DAPI. Images were captured using AxioVision 4.1 and adjusted using Adobe Photoshop 6.0 software. Apoptotic cells were quantified by counting the number of apoptotic cells over the total number of cells in the same field.

3.9. Nuclear staining

After each drug treatment, both detached and attached populations of VA-13 and WI-38 lines were stained with Hoechst 33342 to assess apoptosis. Briefly, cells were washed 2X in PBS, fixed for 1 h with 70% ethanol at 4°C, washed 3X in PBS, and stained with 50 µM Hoechst 30 for 30 min in the dark at room temperature. Detached cells were plated on a slide and attached cells were visualized on the culture plate with a fluorescent microscope at 10X or 40X resolution (Zeiss, Thornwood, NY). Images were obtained using an AxioVision 4.1 and adjusted using Adobe Photoshop 6.0.

4. RESULTS

4.1. Structure-activity relationship analysis of N-thiolated beta-lactams

We have previously shown that lactam 1, which contains an O-methyl moiety at carbon-3 (C3) of the beta-lactam ring, induces apoptosis in a relatively selective manner in tumor and transformed cells, but not normal or non-transformed cell lines (18). More than 35 N-thiolated beta-lactam analogs were then screened using an MTT assay in breast cancer MCF-7 cells to assess their anti-proliferative potency compared to lactam 1. Several of these analogs with substitutions to the O-methyl group at C3 (Figure 1) were then selected for further structure-activity relationship (SAR) studies (Figure 2). MCF-7 cells were treated with 50 µM of selected lactams for 24 h, followed by MTT assay. Lactam 18, which possesses an ester moiety at C3, is twice as potent as lactam 1 (Figure 2). Additionally, it was found that as the C3 group increased in size, the effectiveness to inhibit proliferation decreased. For instance, increasing the size of the halogen, from –Cl to –I (lactam 13 versus lactam 14), lead to a 2-fold decrease in potency (Figure 2). A similar observation was made in comparing the bioactivities of C3-sulfonated lactams 16 and 17. Mesyl lactam 16 inhibited 57% of MCF-7 cell growth, while dansyl lactam 17 has much less effect on inhibiting proliferation (Figure 2). A possible explanation may be that lactam 17 with the large dansyl group is incapable of crossing the cell membrane. Although lactam 15 with an N3 group at C3 is less potent than lactam 14 with 1 at C3, as predicted, it is yet unclear why lactam 15 is less potent than mesyl lactam 16 (Figure 2). The order of potency is determined as follows: Lactam 18 > 1 > 16 ≥ 13 > 14 > 17 > 15.

4.2. Lactam 18 is more potent than lactam 1 at inducing apoptosis associated with Hsp70 expression and p38 phosphorylation

We decided to focus on lactam 18 due to its increased anti-proliferative potency over lactam 1 (Figure 2). To determine whether lactam 18 is capable of inducing apoptosis, we treated leukemia Jurkat T cells with 20 µM lactam 18 for 24 h, using lactam 1 as a control. A fluorescent marker specific for activated caspases was then added to the cells and then visualized by fluorescence microscopy (Figure 3A). Lactam 18 displayed greater apoptosis-inducing activity than lactam 1 (Figure 3A).
Microarray analysis showed up-regulation of many genes by lactam 1 treatment, including HSP70 (manuscript in preparation). To confirm this finding, Jurkat T cells were treated with lactam 1 and lactam 18 for 16 h at either 25 or 50 µM. It was found that Jurkat T cells had increased Hsp70 protein expression after treatment of both lactams; lactam 18 induced a greater amount of Hsp70 expression at 25 µM than lactam 1 at the same concentration (Figure 3B). This is consistent with the idea that the beta-lactams induce a stress response in the cell, most likely due to their disruption of DNA synthesis (18).

We have previously reported that p38 MAP kinase activation is associated with β-lactam-induced apoptosis (18). Activation of p38 MAP kinase can trigger apoptosis following multiple stimuli, such as DNA damage (21, 22). Western blot for phosphorylated (activated) pp38 reveals that treatment with 25 µM lactam 18 leads to an increase in pp38 levels (9.0-fold), compared to that of 25 µM lactam 1 (2.6-fold) (Figure 3B). Actin was used as a loading control.

4.3. (+)-Lactam 19 inhibits cellular proliferation more effectively than (-)-lactam 19

To determine if the stereochemistry has any bearing on the potency of β-lactams, lactams 18 and 19 were synthesized in enantiomerically pure forms (Figure 1). Premalignant MCF-10AT1Kcl.2 breast cancer cells were treated with (+)-lactam 19 (3S,4R-configuration), (-)-lactam 19 (3R,4S-configuration), or racemic lactam 1 (as a control) for 24 h at indicated doses (Figure 4A). All of these lactams inhibited proliferation in a dose-dependent manner, with (+)-lactam 19 at 50 µM inhibiting 85% cell growth, (-)-lactam 19 inhibiting 56% and lactam 1 inhibiting 49% at the same concentration (Figure 4A). These results are similar to that from another experiment using malignant MCF-7 breast cancer cells (data not shown). Trypan blue incorporation also shows increased tumor cell killing with (+)-lactam 19 to that of (-)-lactam 19, 42% versus 26%, respectively, at 25 µM (Figure 4B).

4.4. β-Lactams 18 and 19 induce tumor cell-selective apoptosis

We have previously shown that lactam 1 preferentially induces apoptosis in human cancer cells over normal, non-transformed cells lines (19). To determine if lactam 18 possessed a similar tumor cell-specific activity, human leukemic Jurkat T cells and immortalized, non-transformed natural killer cells (YT) were treated with lactam 1 and lactam 18, and the effects were determined. After 16 h treatment, it was found that lactam 18-treated Jurkat cells had a 19-fold increase in caspase-3 activity at 25 µM, compared to 9-fold induced by lactam 1 at the same concentration (Figure 5A). Both lactam 18 and lactam 1 had little or no apoptosis-inducing effects on the immortalized, non-transformed YT cells (Figure 5A).

To assess whether the isomers of lactam 19 (Figure 1) also have explicit activity against cancer cells, we treated Jurkat T cells and YT cells with (+)-lactam 19, (-)-lactam 19 and racemic lactam 1 (as a control), and determined the effects on apoptotic cell death. We found that only the Jurkat, but not YT, cells exhibited high levels of caspase-3 activity when treated with these beta-lactams (Figure 5B). Additionally, (+)-lactam 19 at 25 µM induced a 7.4-fold increase in caspase-3 activity compared to 5.5-fold increase by (-)-lactam 19 and 3.1-fold increase by lactam 1 at 25 µM (Figure 5B). Therefore, the 3S,4R-configured isomer, (+)-lactam 19, is more potent than (-)-Lactam 19 and racemic lactam 1.

To further confirm the effect of stereoselectivity on the apoptosis-inducing effects of β-lactams, we synthesized an isomeric pair of lactam 18, 3S,4R-isomer [(+)-lactam 18] and 3R,4S-isomer [(-)-lactam 18] (Figure 1). Jurkat and YT cells were then treated with 50 µM (+)-lactam 18, (-)-lactam 18, racemic lactam 18 or lactam 1 for 24 h, followed by trypan blue dye exclusion assay (Figure 6A). (+)-lactam 18 induced much higher amount of cell death than its isomer (-)-lactam 18, 98% vs. 58%, respectively (Figure 6A). Interestingly, the racemic lactam 18 was almost equally potent to that of (+)-lactam 18 (Figure 6A). All lactam 18 compounds initiated more cell death than lactam 1 (Figure 6A). Again, it was found that normal, non-transformed YT cells did not undergo cell death after treatment with any of these beta-lactams (Figure 6A).

Another experiment using SV-40 transformed (VA-13) and normal (WI-38) human fibroblasts demonstrates again that (+)-lactam 18 is the more active isomer. A nuclear stain of VA-13 and WI-38 cell lines treated with 50 µM of each beta-lactam for 24 h reveals that there is a high degree of detachment and DNA condensation, characteristics indicative of apoptosis, in cells treated with (+)-lactam 18, racemic lactam 18 and lactam 1 (Figure 6B). (-)-lactam 18, on the other hand, showed decreased activity when compared with (+)-lactam 18 and racemic lactam 18. There was a very minor amount of cellular detachment observed in the normal WI-38 fibroblasts treated with (+)-lactam 18 (data not shown), supporting that these beta-lactams selectively kill transformed VA-13 cells (Figure 6B).

4.5. (+)-Lactam 18 induces S/G2/M cell cycle arrest

We have previously shown that beta-lactams decrease G1 population, associated with DNA damage (18). To further investigate the cause of apoptosis after N-methylthio beta-lactam treatment, analysis of cell cycle changes were performed on an exponentially growing cell population (Table 1). As a control, lactam 1 was found to decrease G1 phase DNA content by 6% after 6 h incubation (Table 1). Racemic lactam 18 had a very similar effect on cell cycle as racemic lactam 1 (Table 1). However, when cells were treated with (+)-lactam 18, there was a 15% decrease in G1, demonstrating that the 3S,4R configuration has increased growth-inhibitory activity (Table 1).
5. DISCUSSION

Currently, many anticancer therapies, from radiation treatment to chemotherapeutic agents, are very toxic. Therefore, drug discovery for anticancer therapy is as concerned with selectivity of normal versus cancer tissues, as it is the potency of the therapy itself. Antibiotic therapies have typically used the unique molecular targets of microbes in order to avoid toxicity to the patient during treatment. Recently we have shown that some of these compounds possess anti-proliferation activity in human tumor cells (18). Thus, these compounds that are already known to be essentially non-toxic to humans may be anticancer agents as well. Of particular interest are the N-thiolated beta-lactams, which we have previously found to be potent against MRSA (17). Additionally, we found these compounds act as potential anticancer agents through S-phase arrest, DNA damage, and apoptosis induction (18). These compounds are also able to selectively induce apoptosis in cancerous over normal cells (19).

This novel class of N-thiolated beta-lactams possesses potent anti-cancer activity, which is directly related to the nature of the substituents on each of the four ring sites. We have previously reported on the effects of additions/substitutions to the N-thio group and aryl ring (18, 19). The work reported here is a further characterization of the SAR between the various substitution groups on beta-lactam ring. At the core of the beta-lactam molecule is a four-membered ring that is substituted at each position (Figure 1). Each of the positions, we have previously published, plays a role in the potency of the compound. Position 1 is the N-methylthio position and changes at this position that either eliminate the methylthio moiety or lengthen the chain result in decreased potency (18). Position 2 is substituted with a benzene ring and changes here also effect potency (19). Physical position on the ring with regards to ortho-, para-, or meta substitution as well as the nature of the substituent affected the potency dramatically [for details see (19)]. Position 4, which is substituted with a double-bonded oxygen, is the “backbone” of the beta-lactam and therefore cannot be changed without losing the general beta-lactam framework (10, 23). The work presented here examines the SAR at position 3 and completes the survey of each position of the four-membered ring.

We determined that the size and polarity of the group at C3 is important for these lactam’s activity. As these C3 substituents increase in size or in polarity, the efficacy of the compound seems to drop. For example, replacing the chloro (Cl) moiety of lactam 13 for azido (N3), lactam 15, decreases the anti-proliferative activity from 75 to 5% respectively (Figure 2). However, a simple single atom change in the same period (Cl to I; lactam 13 vs. lactam 14) seems to result in only a partial increase in potency. Similarly, the potencies of C3-sulfonated compounds 16 and 17 can be directly attributed to their C3 substitutions: lactam 17 with its large, polar dansyl moiety has significantly diminished activity compared to its smaller, less polar mesyl analog, lactam 16 (Figure 2). Likewise, lactam 16 seems to be similar in or slightly less potent than lactam 14, indicating that the size of the substituent may be slightly more significant than the overall charge (Figure 2). Lactam 18 displays very potent activity with its acrylate ester off C3. This may indicate that these substitutions may affect the capability of these compounds to cross the cell membrane.

Of primary importance in anticancer drug research is that the compound being investigated demonstrate selectivity between normal cells and tumor cells. Cytotoxic agents are less desirable than those compounds that can differentially activate apoptosis in cancer cells vs. tumor cells. Previously we have reported that lactam 1-induced apoptosis is caspase-dependent and associated with cytochrome c release (18). Here we show that lactam 18-induced apoptosis is also caspase-dependent (Figure 5A). However, the efficacy to induce apoptosis by lactam 18 is much improved over lactam 1 (Figure 3) and that the apoptosis induced by lactam 18 is tumor cell-specific (Figure 5A). Beta-lactams cause DNA strand breakage and subsequent cell cycle arrest (18). Our microarray studies show a 3.5-fold increase in HSP70 expression in Jurkat T cells treated with lactam 1 (data not shown). Increased expression of Hsp70 protein (Figure 3B) indicates that treatment with lactam 18 induces a stress response in leukemic Jurkat T cells. Another important molecular event in lactam-induced apoptosis is the increase in p38 phosphorylation. Abrogation of pp38 activity with a specific inhibitor (PD169316) leads to tumor cell survival (18). Not only is lactam 18 capable of inducing p-38 activation, it is capable of inducing a greater amount of pp38 levels at 25 μM compared to 50 μM of lactam 1 (Figure 3B).

Stereochemistry can play an important role in the efficacy of a particular compound. Often only one of the isomers displays a significant selectivity for the molecular target while the other can cause adverse side effects (24-26). Here we find that two 3S,4R-configured beta-lactam compounds, (+)-lactam 18 and (+)-lactam 19, do have a higher potency than their 3R,4S enantiomers or a racemic mixture. Specifically, (+)-lactam 19 has greater anti-proliferation and cell death-inducing activities than both (-)-lactam 19 and the racemic lactam 1 (Figure 4). (+)-Lactam 19 triggers an equivalent amount of caspase-3 activation at half the concentration of lactam 1 and this activity again is tumor cell-selective (Figure 5). Additionally, another isomer, (+)-lactam 18, displays a similar potency to (+)-lactam 19 while still retaining the tumor cell-selectivity (Figures 5B and 6). A vast amount of anti-cancer research is ongoing to develop apoptosis-inducing drugs. While the molecular targets and chemical actions of N-thiolated beta-lactams are not fully characterized, we believe that the compounds possess great potential for chemotherapeutic drug development. These antibiotics compounds are predicted to have little to no effect on normal cells, supported by our results. Thus, the anti-tumor potential and expected lack of toxicity of these beta-lactams makes them excellent candidates for anticancer drug development. Our ongoing studies focus on identifying the molecular interactions of beta-lactams in human cancer cells and their anti-tumor activities in vivo.
6. ACKNOWLEDGMENTS

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7. REFERENCES


SAR of N-methylthiolated beta-lactams


**Key Words:** beta-lactams, cancer, apoptosis, antibiotics, structure-activity relationship

**Send correspondence to:** Dr Q. Ping Dou, Karmanos Cancer Institute, 640.01 HWCRC, 4100 John R, Detroit, Michigan, 48201. Tel: 313-966-0641, Fax 313-993-0193; Alt. Fax 313-966-7368; E-mail: doup@karmanos.org

**Figure 1.** Chemical structures of N-methylthiolated beta-lactams.

**Figure 2.** Structure-activity relationship (SAR) analysis of N-thiolated beta-lactams. MCF-7 cells were plated in a 96-well plate and grown to 70-80% confluency followed by addition of 50 µM beta-lactam for 24 h. Cells were then incubated with 1 mg/ml MTT for 3 h and proliferation rates were determined using a multi-label plate reader (Victor3, Perkin Elmer; ±SD).

**Figure 3.** Lactam 18 induces caspase activity associated with Hsp70 expression and p38 phosphorylation. A. Jurkat T cells were treated with 20 µM lactam 1 or lactam 18 for 24 h. Following the treatment, the cells were then incubated with a FITC-conjugated marker that binds to activated caspasases. Cell suspension was then transferred to glass slides in the presence of Vector Shield mounting medium with DAPI. Images were captured using AxioVision 4.1 and adjusted using Adobe Photoshop 6.0 software. B, Jurkat cells treated with 25 or 50 µM of lactam 1 or lactam 18 for 16 h, followed by Western blot analysis using specific antibodies to HSP70, p-p38, and Actin. Data shown are representative from three independent experiments.

**Figure 4.** (+)-Lactam 19 effects proliferation and cell death in a dose-dependent manner. A, MCF10AT1Kcl.2 cells were plated in a 96-well plate and grown to 70-80% confluency followed by addition of 50 µM of indicated beta-lactams for 24 h. Cells were then incubated with 1 mg/ml MTT for 4 h and proliferation rates were determined using a multi-label plate reader (Victor3, Perkin Elmer; ±SD). B, Jurkats T cells treated with lactam 1, (+)-lactam 19, (-)-lactam 19 at indicated doses and assayed for cell death by trypan blue incorporation (±SD).

**Figure 5.** beta-Lactams induce apoptosis in a tumor cell-specific manner. A, Jurkat T and YT cells were treated with lactam 1 and lactam 18 at indicated concentration for 16 h, followed by measurement of cell-free caspase-3 activity by incubating whole cell extracts with caspase-3 substrate and measuring free AMCs. B, (+)-lactam 19 is 2-fold more potent that lactam 1 at inducing apoptosis in a tumor cell specific manner. Jurkat T and YT cells were treated for 24 h with 25 and 50 µM of lactam 1 versus 25
μM of (+)-lactam 19 and (-)-lactam 19. Cell-free caspase-3 activity was then determined by incubating whole cell extracts with caspase-3 substrate and measuring free AMCs.

**Figure 6.** (+)-Lactam 18 induce apoptosis selectively in tumorigenic cells. A, Leukemic Jurkat T and non-transformed YT cells were treated with lactam 1 or isomers of lactam 18 at 50 μM for 24 h. Cell death is given as a percent of dead cells over total cell population (±SD). B, Both detached and attached VA-13 and WI-38 fibroblast cell populations were collected and stained with the nuclear staining dye Hoechst 33342. Each sample was then analyzed by fluorescence microscopy for nuclear morphology.

**Table 1.** Cell cycle analysis of asynchronous Jurkat T cells treated with beta-lactams at 50 μM for 6 h. 1

<table>
<thead>
<tr>
<th></th>
<th>No treat</th>
<th>Lactam 1</th>
<th>Lactam 18</th>
<th>(+)-Lactam 18</th>
</tr>
</thead>
<tbody>
<tr>
<td>% G0/G1</td>
<td>42</td>
<td>36</td>
<td>36</td>
<td>27</td>
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<td>% S</td>
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<tr>
<td>% G2/M</td>
<td>16</td>
<td>26</td>
<td>23</td>
<td>27</td>
</tr>
<tr>
<td>% G0/G1 Δ2</td>
<td></td>
<td>-6</td>
<td>-6</td>
<td>-15</td>
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1The cell cycle distribution was measured as the percentage of cells that contain G1, S, G2, and M DNA (G1/S/G2/M = 100%).
2The percent change from control cells (no treat) is shown as % Δ.
Figure 1
Figure 3
Figure 4
Figure 5
Figure 6
BETA-LACTAMS AND THEIR POTENTIAL USE AS NOVEL ANTICANCER CHEMOTHERAPEUTIC DRUGS

Deborah Kuhn 1, Cristina Coates 2, Kenyon Daniel 1, Di Chen 1, Mohammad Bhuiyan 1, Aslamuzzaman Kazi 1, Edward Turos 2 and Q. Ping Dou 1

1 The Prevention Program, Barbara Ann Karmanos Cancer Institute, and Department of Pathology, School of Medicine, Wayne State University, Detroit, Michigan, USA, 2 Department of Chemistry, College of Arts and Sciences, University of South Florida, Tampa, Florida, USA

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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 sepsis, 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 cells often have multiple alterations in their apoptotic machinery and/or signaling pathways that lead to increased levels of
growth and proliferation. The absence of a tumor suppressor protein (such as p53) or the activation of an oncogenic protein (such as Bcl-2) can inhibit tumor cell apoptosis (2,3). Therefore, overriding these mutations can lead to stimulation of the apoptotic signaling pathway and cell death in tumor cells.

Currently, the beta-lactams are the most exploited family of antibiotics used for the treatment of bacterial infections (figure 1A). Beta-lactams are secreted by molds from the Penicillium genus and Sir Alexander Fleming first coined the name “penicillin” in 1928. Fleming observed bacteriolysis in a broth contaminated with Penicillium at St. Mary’s Hospital in London, England (4). It would be many years until the import 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 cephalosporin antibiotics, 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 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 infection 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 metalloproteinases, the 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 vasodeothenial 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 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 streptogin 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.
**N-thiolated beta-lactams as anticancer drugs**

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 unconjugated enzyme must be readily cleared from the blood-stream 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, chlorambucil, 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 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 unconjugated enzyme must be readily cleared from the blood-stream before the prodrug is administered. The following section will discuss antibody-enzyme-prodrug systems that have undergone evaluation to treat various types of cancers.

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 and CCM) were significantly less toxic than PDM.

The prodrug C-Mel, a cephalosporin carbonate derivative of melphalan, was also shown to have antitumor activity (50). C-Mel was activated in an immunologically specific manner by the L49-sFv-βL conjugate. In vitro cytotoxicity assays using 3677 human melanoma cells treated with the C-Mel prodrug in conjunction with L49-sFv-βL 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-βL 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 antimetabolites has been implicated due to the nucleoside transporters (NT), which mediate uptake of nucleic acids into dividing cells (57). 5-Fluorouracil is an anticancer drug 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-ECIIβ-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 xenografts (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 chemotherapies. 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-labeled cephalosporin conjugated to Tositumomab, a mAb specific for the CD20 antigen via a synthetic linker. Upon administration of the beta-lactamase 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
N-thiolated beta-lactams as anticancer drugs

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-methyl-thio 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 shown 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-methyl-thio 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 > 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 mitochondria-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-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 apoptosis signaling cascade after gamma-lactam treatment (74). Lactacytin, a gamma-lactam possessing a thio ester moiety and originally isolated from actinomycetes (75), has been found to be a potent inhibitor of chymotryptic- and tryptic-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), IκB-α (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).
N-thiolated beta-lactams as anticancer drugs

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 [3H] thymidine incorporation assay. DNA replication was inhibited in a time- and concentration-dependent manner with a half-maximal inhibition (IC50) of [3H] thymidine in Jurkat cells at 32 µM 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 genomic DNA. After just 1 h of treatment with, over half of the cell population contained DNA strand breaks and after 4 h 98 % of the cells showed DNA strand breakage (37). In a similar experiment it was found that Lactam 12 induced greater DNA damage that Lactam 1 (by 27%) after a 24 h period (37).

It was also determined that p38 MAP kinase activation is required for beta-lactam induced apoptosis (37). Activation of p38 MAP kinase can trigger apoptosis following multiple stimuli, such as DNA damage (85, 86). Protein levels of phosphorylated p38 increased significantly with Lactam treatment and cotreatment with the p38 inhibitor (PD169316) inhibited PARP cleavage and activation of caspase-3, -8, and -9 (37). Additional experiments revealed that p38 activation occurs upstream of caspase activation and that p38 activity was necessary for caspase-mediated cell death in beta-lactam treatment. Conversely, DNA strand breaks were still observed after cotreatment with PD169316 and Lactam 1, indicating that N-thiolated beta-lactams induced DNA damage leading to p38 activation, followed by caspase activation and subsequent apoptotic cell death.

6.1.4. Preferential Tumor Cell Killing
Many currently used chemotherapeutic drugs for cancer intercalate with cellular DNA, thus making it impossible for the cell to function which leads to subsequent apoptotic death. Unfortunately, these drugs are not “tumor-specific” and they will intercalate with any rapidly dividing cell, such as the epithelial cells lining the gastrointestinal tract, which can lead to nausea and vomiting. Tumor cell specific therapies are those that solely target tumor cell characteristics exclusively. For instance, Gleevec (STI571) is an ATP inhibitor that targets growth and proliferative signaling pathways stimulated by the Bcr-Abl oncoprotein in chronic myelogenous leukemia (87).

To determine if Lactam 1 possessed a tumor cell-specific activity human leukemic Jurkat T cells and immortalized, non-transformed natural killer cells (YT cells) were treated with Lactam 1 and the effects were determined. It was found that only the Jurkat, but not YT, cells showed apoptosis-specific PARP cleavage and decreased cell viability in both time- and concentration-dependent manner (38). Additionally, treatment with Lactam 12, which substitutes the –Cl moiety for a –NO2 on the benzene ring, was found to potently and specifically induce apoptosis in only the Jurkat T cells while not affecting the non-transformed YT cells. Both Lactam 1 and Lactam 12 inhibited colony formation, indicative of cellular transformation, of prostate cancer LNCaP cells as observed in a soft agar assay. These lactams were also able to induce TUNEL-positive cells as well as caspase-3/-7 activity and apoptotic nuclei in a number of transformed tumor cell line types, but not in non-transformed cell lines (38). For example, Lactam 12 treatment induced morphological changes and caspase-3 activity exclusively in SV-40 transformed human fibroblasts (VA-13) but not in normal non-transformed fibroblasts (WI-38) (38). This is consistent with the idea that beta-lactams could be developed into tumor-specific drugs.

6.2. 4-Alkylidene-beta-Lactams
The matrix metalloproteinases (MMPs) are a class of mammalian proteases that can, among other functions, degrade the extracellular matrix (88). Angiogenesis, the formation of new blood vessels, requires the activity of the MMPs to digest the basement membrane. The MMPs play a pivotal role in cancer progression by allowing neovascularization, which is essential for tumor growth, invasion, and metastasis (89). MMPs can be constitutively activated in cancer cells, but not in normal cells (90). Thus, targeting MMP expression and activity is a unique approach in the field of cancer research.

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 IC50 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 (IC50=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 IC50s 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
**N-thiolated beta-lactams as anticancer drugs**

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 \(7 \pm 2\) days in mice injected with HT-29 cells. Additionally, many of the mice injected with 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 almost 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 cells 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.

**8. ACKNOWLEDGMENTS**

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Send correspondence to: Q. Ping Dou, Ph.D., The Prevention Program, Barbara Ann Karmanos Cancer Institute, and Department of Pathology, School of Medicine, Wayne State University, 4100 John R Road, Detroit, MI 48201, Tel: 313-966-0641, Fax: 313-993-0193, E-mail: doup@karmanos.org

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

Figure 2. Diagramatic presentation of cephalosporin prodrug reaction. Beta-lactamase induces hydrolytic cleavage of the beta-lactam ring, which causes a secondary reaction leading to the release of the anticancer drug in the 3’position.

Figure 3. Structure of N-thiolated monobactams.

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1. Title: Change ‘CHEMOTHERAPIUTICS’ to ‘CHEMOTHERAPEUTICS’
2. Section 3, p. 3, paragraph 1: Change last sentence to, “This combined therapy improved survivability and the toxic effects (primarily mucositis) were considered within acceptable limits.”
3. Section 4, p. 3, paragraph 1: Change last sentence from “meticillin-resistance” to “methicillin-resistant”.
4. Section 4, p. 3, paragraph 2, line 8: Sentence should read, “Cainelli et al., describe that 4-alkyliden-beta-lactams…”
5. Section 6.1.3, p. 7, paragraph 1, lines 3-4: Sentence should read, “…cells to undergo DNA replication as was found from a [3H] thymidine…”
6. Section 6.2, p. 7, paragraph 2, line 3: Change “…hydroxy groups (Compound 1 and 8)…” to hydroxy groups (Compounds 1 and 8)…”
RELATIONSHIPS OF STRUCTURES OF N-METHYLTHIOLATED BETA-LACTAM ANTIBIOTICS TO THEIR APOPTOSIS-INDUCING ACTIVITY IN HUMAN BREAST CANCER CELLS

Deborah Kuhn, Yang Wang, Vesna Minic, Cristina Coates, G. Suresh Kumar Reddy, Kenyon, G. Daniel, Jeung-Yeop Shim, Di Chen, Kristin R. Landis-Piwowar, Edward Turos, And Q. Ping Dou, Ph.D.
The Prevention Program, Barbara Ann Karmanos Cancer Institute, and Department of Pathology, School of Medicine, Wayne State University, Detroit, MI, and Department of Chemistry, College of Arts and Sciences, University of South Florida, Tampa, FL.
doup@karmanos.org
Activation of the cellular apoptotic program is a current strategy for the prevention and treatment of human cancer including breast cancer. The beta-lactam antibiotics have played an essential role in treating bacterial infections without causing toxic side effects for the past 60 years. We hypothesize that active N-thiolated beta-lactams can target a tumor-specific protein(s) and selectively induce apoptosis in human breast cancer but not normal cells.

To test this hypothesis, we designed and synthesized numerous new N-thiolated beta-lactams analogs, evaluated potencies of these synthetic beta-lactams to inhibit proliferation and induce apoptosis in human breast cancer cells, and investigated whether these beta-lactams can induce apoptosis selectively in breast tumor vs. normal cells and the involved molecular mechanisms.

We tested a library of lactam compounds and found that many of them are able to inhibit proliferation and induce apoptosis in human breast cancer cells in a time- and concentration-dependent manner. These active beta-lactams are capable of inducing DNA strand breakage, inhibiting DNA replication, and inducing p38 MAP kinase activation. Consistent with the idea that these beta-lactam antibiotics are potent anti-cancer agents, the active analogs are also capable of inhibiting colony formation potential of breast cancer cells. Furthermore, the active beta-lactams have much reduced effects on human normal or non-transformed cells. Our findings also yielded several important structure-activity relationships (SARs). The N-methylthio group is necessary for the apoptosis-inducing activity. Also observed is the inverse relationship between the number of carbon atoms off the N-thio group and apoptotic activity. Substitutions to C3 reveal that as the substituents increase in size or in polarity, the efficacy of the compound decreases. Therefore, the overall size of the beta-lactam is important, possibly indicating steric hindrance with the cellular target or permeability to the cell membrane. We also found that the stereochemistry of the beta-lactams play an important role in their potency. The 3R,4S enantiomers are more efficacious than the 3S,4R isomers, which may indicate a more favorable configuration for target interaction.

Our future studies will focus on the biochemical target of N-thiolated beta-lactams, whether the N-thiolated beta-lactams can induce apoptosis selectively in breast tumor vs. normal breast cells, and whether the in vivo apoptosis-inducing ability of the N-thiolated beta-lactams is related to their cancer-preventive and anti-tumor activities using nude mice bearing human breast tumors. These studies should provide strong support for proof-of-concept of the potential use of these N-thiolated beta-lactams in breast cancer prevention and treatment.

The U.S. Army Medical Research and Materiel Command under DAMD17-03-1-0175 and W81XWH-04-0688 supported this work.
Q. Ping Dou, Ph.D.

CURRICULUM VITAE

Q. Ping Dou, Ph.D.

Date of Preparation: March 23, 2006

Signature: ____________________

OFFICE ADDRESS

The Prevention Program
Barbara Ann Karmanos Cancer Institute, and
Department of Pathology
Wayne State University School of Medicine
640.1 Hudson-Webber Cancer Research Center
4100 John R Road
Detroit, MI 48201-2013
Telephone: 313-576-8301 (Office)
313-576-8264/-8247/-8248/-8249/-8250/-9397 (Lab)
313-576-8299 (Adm. Assistant)
Fax: 313-576-8307 (Office)
313-576-8306 (Adm. Assistant)
E-mail: doup@karmanos.org

EDUCATION:
B.S. in Chemistry, Shandong University, Jinan, Shandong, People’s Republic of China, 1981
Ph.D. in Chemistry, Rutgers University, Piscataway, NJ (Mentor: Kuang Yu Chen), 1988

TRAINING:
Postdoctoral Research Fellow in Molecular Biology and Pharmacology, Dana-Farber Cancer Institute, Harvard Medical School, Boston, MA (Mentor: Arthur B. Pardee), 1988-1992

FACULTY APPOINTMENTS:
Instructor, Department of Medicine, Harvard Medical School, at Dana-Farber Cancer Institute and Beth Israel Hospital, Boston, MA, 1992-1993
Assistant Professor, Department of Pharmacology, University of Pittsburgh School of Medicine, Pittsburgh, PA, 1993-1998
Assistant Professor, Biochemistry and Molecular Genetics Graduate Training Program, Interdisciplinary Biomedical Graduate Program, University of Pittsburgh School of Medicine, Pittsburgh, PA, 1997-1998
Member, Experimental Therapeutic Program, University of Pittsburgh Cancer Institute, Pittsburgh, PA, 1993-1998
Q. Ping Dou, Ph.D.

Member in Residence, Drug Discovery Program, H. Lee Moffitt Cancer Center & Research Institute, Tampa, Florida, 1998-2003
Associate Professor, Department of Biochemistry and Molecular Biology, University of South Florida College of Medicine, Tampa, Florida, 1998-2003
Associate Professor, Department of Interdisciplinary Oncology, University of South Florida College of Medicine, Tampa, Florida, 2000-2003
Member, the Institute for Biomolecular Science, University of South Florida, Tampa, Florida, 1998-2003
Assistant Program Leader and Scientific Member, Population Studies and Prevention Program, Barbara Ann Karmanos Cancer Institute, Detroit, MI, 2003-present
Leader and Scientific Member, Prevention Program, Barbara Ann Karmanos Cancer Institute, Detroit, MI, 2003-present
Full Professor (with Tenure), Department of Pathology, Wayne State University School of Medicine, Detroit, MI, 2003-present
Full Professor, Cancer Biology Program, Barbara Ann Karmanos Cancer Institute, Wayne State University School of Medicine, Detroit, MI, 2003-present
Full Member, Gene Regulation and Genetics Research Program, Institute of Environmental Health Sciences, Wayne State University, Detroit, MI, 2004-present
Member, Scientific Leadership Council (SLC), Barbara Ann Karmanos Cancer Institute, Detroit, MI, 2004-present
Member, the NanoSciences Institute, Wayne State University, Detroit, MI, 2005-present
Visiting Professor in the Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, Beijing, China (April 19-20, 2006)
Visiting Professor in the Institution of Environmental Sciences, Shandong University, Jinan, Shandong, China, 2006-

MAJOR PROFESSIONAL SOCIETIES:
American Association for Cancer Research, Inc.
American Association for the Advancement of Science
American Society for Biochemistry and Molecular Biology
American Society for Pharmacology and Experimental Therapeutics
Society of Chinese Bioscientists in America
International Society for Study of Comparative Oncology, Inc.
American Chemistry Society

HONORS AND AWARDS:
Summer Research Prize in recognition of outstanding accomplishments in research. Rutgers University, 1988
Biochemical Research Support Grant Award. Dana-Farber Cancer Institute, 1991
Barr Program Small Grant Award. Dana-Farber Cancer Institute, 1992
Co-Discussion Leader, University of Pittsburgh Cancer Institute Scientific Retreat, 1995
NIH Director James A. Shannon Award, 1 R55 AG/OD13300-01, 1995-1997
NIH FIRST Award, R29 AG13300-05, 1996-2001
A Predoctoral Trainingship in Breast Cancer Biology and Therapy from the United States Army Medical Research, Development, Acquisitions, and Logistics Command (to Cheryl L. Fattman), 1997-1999

58
Q. Ping Dou, Ph.D.

The Best Poster Presentation (An B et al.), Scientific Retreat, Department of Pharmacology, University of Pittsburgh School of Medicine, 1997

Chairman for Session of Clinical Oncology/Apoptosis. 2nd World Congress on Advances in Oncology, Athens, Greece, 16-18 October, 1997

Award for the Best Abstract, 2nd World Congress on Advances in Oncology, Athens, Greece, 16-18 October, 1997

Cheryl L. Fattman, Ph.D. Graduation with Honor from University of Pittsburgh (mentor: Q. Ping Dou), 1999

Moffitt's Cancer Center Director’s Award (for the article published by Li B and Dou QP in Proc. Natl. Acad. Sci. USA, 2000; 97: 3850-3855). Moffitt Cancer Center & Research Institute, 2000

An AACR-AFLAF Scholar-in Training Award ($400 to Aslam Kazi mentor: QP Dou), for a selected poster (Abstract #788, Kazi A, Smith DM, Zhong Q and Dou QP. Down Regulation of Bcl-XL Protein Expression by Green Tea Polyphenols Is Associated with Prostate Cancer Cell Apoptosis. AACR-NCI-EORTC. Molecular Targets and Cancer therapeutics, Miami Beach, FL, October 29-November 2, 2001

Abstract was chosen as one of the selected few for News Briefing (Abstract #788, Kazi A, Smith DM, Zhong Q and Dou QP. Down Regulation of Bcl-XL Protein Expression by Green Tea Polyphenols Is Associated with Prostate Cancer Cell Apoptosis. AACR-NCI-EORTC. Molecular Targets and Cancer therapeutics, Miami Beach, FL, October 29-November 2, 2001

Nominee of Team Award (Cancer Control Program), 2002

Kenyon G. Daniel, Ph.D. a Winner of the 2004 Outstanding Dissertation Award from University of South Florida (Major Professor: Q. Ping Dou), 2004

Invited Visiting Professor in the Department of Urology at the University of California San Francisco and San Francisco VA Medical Center (April 28, 2005), with seminar presentation, "Searching for novel polyphenol proteasome inhibitors for cancer prevention and treatment"

Training Grant (Kristin Landis; Mentor: Q. Ping Dou). Wayne State University, 2005-2007

First Place, Oral Presentation (Krisitn Landis; Mentor: Q. Ping Dou). Wayne State University School of Medicine, Graduate Student Research Day, September 22, 2005. “Molecular Studies for the Regulation of Green Tea Polyphenol Biological Function by the Polymorphic Gene Product Catechol-O-Methyltransferase”.

Awardee (Kristin Landis; Mentor: Q. Ping Dou) for the Honors Recognition Program for Wayne State University School of Medicine, Wayne State University, 2005

Invited Visiting Professor in the Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, Beijing, China (April 19-20, 2006), with seminar presentation, "Cancer Prevention and the Role of Environmental Factors"

Invited Visiting Professor in the Institution of Environmental Sciences, Shandong University, Jinan, Shandong, China (April 24-25, 2006), with seminar presentations, "Nutrient-Gene-Environment Interactions and Molecular Prevention of Cancer" and "Discovery of Novel Small Molecules from Nature and Laboratories for Cancer Therapies"

SERVICE:

Professional Consultation
Tours for University of Pittsburgh Cancer Institute
Q. Ping Dou, Ph.D.

Tours for Drug Discovery Program Moffitt Cancer Center & Research Institute
Advisor for Project LINK (Leaders In New Knowledge) Students
Advisor for Moffitt Summer Interns
Advisor for Undergraduate Student Honor’s Thesis Research
Member, the Scientific Advisory Board, Torrey Pharmaceuticals, LLC, San Diego, CA, 2000-present
Graduate student recruitment faculty for Cancer Biology Program Karmanos Cancer Institute and Wayne State University, April 3, 2004, April 2, 2005
Presentation to Cancer Biology Program Candidates, Karmanos Cancer Institute and Wayne State University, March 25, 2006 (Kristin Landis and Vesna Minic from Dr. Dou’s laboratory)
Preferred Consultant, MEDACorp, Boston, Massachusetts, 2004-present
Speaker (Kristin Landis from the laboratory of Q. Ping Dou), "Friends Raising Funds" program, March 5th, 2006, the Powerhouse Gym, West Bloomfield, MI.

Journal/Editorial Activity

Editorial Board Memberships
Invited member of the Editorial Board of the Oncology Reports, 1996-present
Invited member of the Editorial Board of Frontiers In Bioscience, 1997-present
Invited member of the Editorial Board of LifeXY (Currently International Archives of Biosciences), 2001-present
Invited panel evaluator of Current Drugs, 2001-present
Invited member of The Science Advisory Board, 2002-present
Invited managing editor of Frontiers In Bioscience, 2003-present
Invited managing editor of Frontiers In Bioscience for a special issue of “Potential Molecular Targets for Chemoprevention”, 2004-present

Reviewer for Journal Manuscripts
Proceeding of National Academy of Sciences USA
FASEB
Oncogene
Chemistry & Biology
Cancer Research
Clinical Cancer Research
Molecular Cancer Therapeutics
Cell Death & differentiation
Molecular Pharmacology
Journal of Pharmacology & Experimental Therapeutics
Drug Discovery Today
Microbes and Infection
Leukemia
Cancer Letters
FEBS Letters
Carcinogenesis
International J. Oncology
Breast Cancer Research and Treatment
J. Cell. Biochemistry
Biochemical Pharmacology
European Journal of Medicinal Chemistry
European Journal of Cancer
Arch Biochem Biophys
Journal of Pharmacy and Pharmacology
Head & Neck
Apoptosis
Journal of Agriculture and Food Chemistry
Obesity Research
Molecular Nutrition and Food Research
Endocrine
Natural Immunity
Molecular Biology Reports
Gene Therapy
Cellular and Molecular Life Sciences
Expert Opinion on Investigational Drugs
Expert Review of Anticancer Therapy
Expert Review of Proteomics
Evidence-Based Integrative Medicine
Life XY (Currently International Archives of Biosciences)
The Pittsburgh Undergraduate Review

Reviewer for Grant Applications
Competitive Medical Research Fund (Ad Hoc Reviewer), University of Pittsburgh School of Medicine, 1996
Competitive Medical Research Fund, University of Pittsburgh School of Medicine, 1997
Central Research Development Fund, University of Pittsburgh, 1997
National Science Foundation, 1998
Merit Review Subcommittee for Oncology, the Veterans Affairs Medical Research Programs, US Department of Veterans Affairs, 1999-2005
Member, Cancer Study Section, The Tobacco-Related Disease Research Program, University of California, San Francisco, CA, 2004-present
Invited Reviewer, Biotechnology and Biological Sciences Research Council Research Grant, United Kingdom, 2004, 2006
Invited Reviewer, the Strategic Research Initiative Awards, Barbara Ann Karmanos Cancer Institute, Wayne State University School of Medicine, Detroit, MI, 2005
Invited Reviewer, the Seed Money Grants, Barbara Ann Karmanos Cancer Institute, Wayne State University School of Medicine, Detroit, MI, 2006
Invited Reviewer, the European Commission's Research Program on "Combating Cancer" - Apoptosis, Brussels, Belgium, 2006
Other Professional Related Service

Faculty reviewer for applications to University of Pittsburgh and University of Pittsburgh Cancer Institute
Faculty reviewer for applications to Moffitt Cancer Center & Research Institute and University of South Florida
Faculty reviewer for applications to Karmanos Cancer Institute and Wayne State University (2003-)
Faculty reviewer for Deputy Director/Associate Center Director Candidates to Karmanos Cancer Institute and Wayne State University (2004)
Faculty reviewer for Lambert – Webber Endowed Chair, Division Chief, Hematology and Oncology, Department of Internal Medicine, Wayne State University, Program Leader, Developmental Therapeutics, Karmanos Cancer Institute (2005)

National and International Boards and Committees

Ad Hoc Reviewer, National Science Foundation, 1998
Ad Hoc Reviewer, Merit Review Subcommittee for Oncology, the Veterans Affairs Medical Research Programs, US Department of Veterans Affairs, 1999-2001
Member, Department of Veterans Affairs (VA) Medical Research Service Merit Review Subcommittee for Oncology, 2001-2005
Member, the Scientific Advisory Board, Torrey Pharmaceuticals, LLC, San Diego, CA, 2000-present
Council Member, Gerson Lehrman Group's Council of Healthcare Advisors, New York, NY, 2004-present
Member, Cancer Study Section, The Tobacco-Related Disease Research Program, University of California, San Francisco, CA, 2004-present
Invited Reviewer, Biotechnology and Biological Sciences Research Council Research Grant, United Kingdom, 2004, 2006
Preferred Consultant, MEDACorp, Boston, Massachusetts, 2004-present
Judge, Cell Biology & Cell Signaling Section, 2nd Annual Research Symposium, Henry Ford Health System Academic Affairs, Detroit, MI, April 15, 2005
Invited Reviewer, the European Commission's Research Program on "Combating Cancer" - Apoptosis, Brussels, Belgium, 2006

State and Local Boards and Committees

Department of Pharmacology, University of Pittsburgh School of Medicine
Comprehensive Examination Committee, Department of Pharmacology, University of Pittsburgh School of Medicine, 1993-1998
Committee of Graduate Studies, Department of Pharmacology, University of Pittsburgh School of Medicine, 1994-1998
Chairman of Graduate Evaluations, Department of Pharmacology, University of Pittsburgh School of Medicine, 1994-1998
NIH Predoctoral Training Grant Selection Committee, 1995
Director of Departmental Seminar Program, 1997

University of Pittsburgh School of Medicine
Competitive Medical Research Fund Review Committee (Ad Hoc Reviewer), University of Pittsburgh School of Medicine, 1996-1997
The Graduate Progress Evaluation Committee, University of Pittsburgh School of Medicine, 1997
Central Research Development Fund, University of Pittsburgh, 1997

**University of South Florida and Moffitt Cancer Center & Research Institute**
- Member, Search Committee for Assistant Professor position in Molecular, Epidemiology Program of Cancer Control Division at Moffitt Cancer Center & Research Institute, 2001
- Member, Rb Club, Moffitt Cancer Center & Research Institute, 2001-2002
- Member, The Summer Intern Program Committee at Moffitt Cancer Center & Research Institute, 2002
- Member, Preliminary Data Club, Moffitt Cancer Center & Research Institute, 2002-2003
- Member, Proteomics Task Force Committee, Moffitt Cancer Center & Research Institute, 2002
- Member, Search Committee for Structural Biology Faculty position in Drug Discovery Program at Moffitt Cancer Center & Research Institute, 2003
- Member, Search Committee for the Cancer Prevention Faculty position in Molecular Epidemiology Program of Cancer Control Division at Moffitt Cancer Center & Research Institute, 2003
- Member, Search Committee for Chemistry Faculty position in Drug Discovery Program at Moffitt Cancer Center & Research Institute, 2003
- Member, SPARK (Summer Program for the Advancement of Research Knowledge) Selection Committee at Moffitt Cancer Center & Research Institute, 2003

**Wayne State University and Karmanos Cancer Institute**
- Graduate student recruitment faculty for Cancer Biology Program Karmanos Cancer Institute and Wayne State University, April 3, 2004
- Graduate student recruitment faculty for Cancer Biology Program Karmanos Cancer Institute and Wayne State University, April 2, 2005
- Invited Reviewer, the Strategic Research Initiative Awards, Barbara Ann Karmanos Cancer Institute, Wayne State University School of Medicine, Detroit, MI, 2005
- Invited Judge, the 2nd Annual Research Symposium, Henry Ford Health System, Detroit, MI, 2005
- Invited Reviewer, the Seed Grants, Barbara Ann Karmanos Cancer Institute, Wayne State University School of Medicine, Detroit, MI, 2006
- Co-Director and Member of a P01 Group, “AR-mediated cell cycle-dependent anti-apoptotic events in prostate cancer”, Wayne State University and Henry Ford Health System, Detroit, MI, 2004-present
- Speaker (Kristin Landis from the laboratory of Q. Ping Dou), "Friends Raising Funds" program, March 5th, 2006, the Powerhouse Gym, West Bloomfield, MI.
- Member, HFHS/WSU Prostate Journal Club, 2003-
- Co-Director and Member of a P01 Group, “AR-mediated cell cycle-dependent anti-apoptotic events in prostate cancer”, Wayne State University and Henry Ford Health System, Detroit, MI, 2004-present
- Dr. Dou has the following responsibilities:
  1. Assist the Program Leader in the entire Population Studies and Prevention program and provide leadership in the Prevention sub-program, particularly with respect to basic science research, molecular targeting and chemoprevention;
  2. Participate in Population Studies and Prevention leadership meetings;
  3. Participate in updating and maintaining strategic planning for the Prevention sub-program;
Q. Ping Dou, Ph.D.

(4) Facilitate inter- and intra-programmatic interactions between the Prevention faculty members and members of Population Studies, Communication & Behavioral Oncology, and other cancer center programs;
(5) Organize and lead the monthly Prevention group meetings;
(6) Recruit new members into the Prevention sub-program and mentor junior faculty;
(7) Advocate for shared facilities that meet the needs of the Prevention members;
(8) Develop the Prevention sub-program into an independent program in the next five years.

**TEACHING:**

**Years at Wayne State University:** Since August 1, 2003

**Years at Other Universities:**
- Harvard Medical School, 1 year
- University of Pittsburgh, 5 years
- University of South Florida, 5 years

**Courses Taught at Wayne State University**
- 2003- CB 7250: CANCER CONTROL. 3 credits. 20 students
- 2004 CB 7230: BREAST CANCER. 2 credits. 10-12 students (December 8, 2004, 10:00 AM- 12:00 PM, 1140 Scott Hall).

**Courses Taught at University of South Florida**
- 1999-2001 BCH 6411: Molecular Biology. Lecture. 3 credits. 25-30 students
- 2001- Cancer Biology I Course Lecture. 3 credits. ~10 students

**Courses Taught at University of Pittsburgh**
- 1993-1998 MS MIC 2355: Advanced Molecular Genetics. Lecture and Paper Discussion. 3 credits. 8-16 students
- 1993-1998 PHL 3510: Receptors and Signal Transduction. Lecture and Paper discussion. 3 credits. 10-15 students
- 1993-1998 2563: Cancer Pharmacology. Lecture. 3 credits. ~5 students
- 1993-1998 Medical Student Program: Problem-Based Learning Sessions. 8-10 students
- 1995 Medical Student Program: Pharmacology Conference. ~20 students
- 1997 Medical Student Program: Neoplasia and Neolastic Disease. 16 students
- 1996-97 The Pennsylvania Governor’s School Program. 6-8 students
- 1997 Foundations of Biomedical Science. Small group conference. 3 credits. ~8 students

**Undergraduate and Graduate/Medical Student Supervision**
- 1994 Chen Yu, Harvard University, ASPET undergraduate
- 1995 Peggy Lin, Penn State-Jefferson
- 1995 Bill Wang, California University of PA
- 1995 Vivian Lui, Department of Pharmacology, University of Pittsburgh School of Medicine, one lab rotation
- 1996 Toni A. Termin, Saint Vincent College, ASPET undergraduate
Q. Ping Dou, Ph.D.

1996  Kirk E. Dineley, Department of Pharmacology, University of Pittsburgh School of Medicine, two lab rotations

1996-1999 Cheryl Fattman, Department of Pharmacology, University of Pittsburgh School of Medicine. Ph.D., Dissertation Title: “Molecular mechanisms for apoptosis-associated the retinoblastoma protein (RB) internal cleavage”. Graduation with Honor (Mentor: Q. Ping Dou). Currently working as a postdoctoral fellow in Department of Pathology, University of Pittsburgh School of Medicine

1996-1999  Cheryl Fattman, Department of Pharmacology, University of Pittsburgh School of Medicine. Ph.D., Dissertation Title: “Molecular mechanisms for apoptosis-associated the retinoblastoma protein (RB) internal cleavage”. Graduation with Honor (Mentor: Q. Ping Dou). Currently working as a postdoctoral fellow in Department of Pathology, University of Pittsburgh School of Medicine

1997  Lachelle Sussman, University of New York at Buffalo, ASPET Undergraduate, University of Pittsburgh School of Medicine, one lab rotation

1997  Kristin S Morrow, Department of Biology University of South Florida, master graduate student

1998-  Yaser S. Bassel, University of South Florida College of Medicine, medical student

1998-  Jason A. Evangelista, University of South Florida College of Medicine, medical student

1998-  Joseph J. Kavanagh, University of South Florida College of Medicine, medical student

1998-  Alexander Paloma, University of South Florida College of Medicine, medical student

1998-  Gregory A. Russell, University of South Florida College of Medicine, medical student

1999-2002  David M. Smith, Department of Biochemistry & Molecular Biology, University of South Florida College of Medicine, one lab rotation and Ph.D. student in my lab (graduated in 2002). Dissertation Title: “Mechanistic Studies on Tumor Cell Cycle Disruption and Apoptosis by Green Tea Polyphenols and N-Thiolated beta-Lactams”. Currently working as a postdoctoral fellow in Dr. Fred Goldberg’s laboratory at Harvard Medical School

1999  Lisa Smith, Department of Biology University of South Florida, undergraduate student (currently a graduate student in University of North Carolina)

1999  Jessica Hu, Harvard University, undergraduate student

1999  Daniel Lorch, University of Florida, undergraduate student

1999  Sun Hee Rim, Hillsborough High School, student

1999  Alvin Jones, Land O’Lakes High School, student

1999  Kristie Main, Department of Biochemistry & Molecular Biology, University of South Florida College of Medicine, one lab rotation

1999-2000  Kenyon Daniel, Department of Biology University of South Florida, undergraduate student. Research for Honor’s Thesis

2000  Lisa Smith, Moffitt Summer Intern, Department of Biology University of South Florida, undergraduate student (current graduate student at University of North Carolina)

2000-2002  Marie Bosley, Project LINK (Leaders In New Knowledge) Student and a Moffitt Summer Intern, Department of Biology University of South Florida, undergraduate student

2000  John (Chilu) Chen, Department of Biochemistry & Molecular Biology, University of South Florida College of Medicine, one lab rotation

2001  Jonathan S. Anderson, Moffitt Summer Intern, Zoology, University of Florida, undergraduate student
2001  
Kyleen Charlton, Moffitt Summer Intern, Boston College, undergraduate student

2001-2002  
Priyanka Kamath, Volunteer, Hillsborough High School, high school student

2000-2004  
Kenyon Daniel, Department of Biochemistry & Molecular Biology, University of South Florida College of Medicine, one lab rotation and Ph.D. student in my lab (graduated in 2004). Dissertation Title: “Strategies for Cancer Therapy Through Regulation of Apoptotic Proteases”. Currently working as a postdoctoral fellow in my laboratory at Karmanos Cancer Institute, Wayne State University

2001-2004  
Deborah Kuhn, Cancer Biology Ph.D. Program (IOP), University of South Florida College of Medicine, one lab rotation and Ph.D. student in my lab (graduated in November of 2004). Dissertation Title: “Novel Approaches to Targeting Tumor Cell Apoptotic Signaling Pathways”. Currently working as a postdoctoral fellow in University of North Carolina.

2002  
Naveen Kumar, Moffitt Summer Intern, New York University, undergraduate student

2002  
Randy Hill, Moffitt Summer Intern, University of Wisconsin, undergraduate student (currently a graduate student in University of Wisconsin)

2002  
Seth Pross, Moffitt Summer Intern, Hillsborough High School, high school student (currently a graduate student in University of Pennsylvania)

2002-2003  
Audrey Burns, Department of Biochemistry & Molecular Biology, University of South Florida College of Medicine, one lab rotation and Ph.D. student in my lab

2002-2003  
Sam Falsetti, Department of Biochemistry & Molecular Biology, University of South Florida College of Medicine, one lab rotation and Ph.D. student in my lab

2002  
Jennelle McQuown, Cancer Biology Ph.D. Program (IOP), University of South Florida College of Medicine, one lab rotation

2002-2003  
Daniel Urbizu, Project LINK (Leaders In New Knowledge) Student, Department of Biology University of South Florida, undergraduate student

2003-  
Thomas Lendrihas, Department of Biochemistry & Molecular Biology, University of South Florida College of Medicine, one lab rotation

2003  
Seth Pross, Moffitt Summer Intern, University of Pennsylvania, undergraduate student

2003  
Shuojing Song, Moffitt Summer Intern, C. Leon King High School, high school student (currently an undergraduate of MIT)

2003  
R. Hope Harbach, Summer Student, Department of Chemistry, Eckerd College, undergraduate student

2003  
Daniel Urbizu, Summer/Project LINK (Leaders In New Knowledge) Student, Department of Biology University of South Florida, undergraduate student

2003  
Marina Si Chen, Summer Research Volunteer, King High School, high school student

2003  
Sydnor M. Richkind, Summer Research Volunteer, Hillsborough High School, high school student (currently a graduate student in University of Florida)

2004-present  
Kristin Landis, Cancer Biology Program, Wayne State University, one lab rotation and Ph.D. student in my lab

2004  
Marina Si Chen, Summer Research Hourly Technician, Emory University, undergraduate student
Q. Ping Dou, Ph.D.

2005-present Vesna Minic, Cancer Biology Program, Wayne State University, one lab rotation and Ph.D. student in my lab

2005- Joan McCallum, Cancer Biology Program, Wayne State University, one lab rotation in my lab
2006- Mike Frezza, Cancer Biology Program, Wayne State University, one lab rotation in my lab
2006- Benchamart Moolmuang, Cancer Biology Program, Wayne State University, one lab rotation in my lab
2006- Andy Yang, Summer Research Student, Webster Thomas High School, New York

Theses/ Dissertation or Comprehensive Examination Committees

Ph.D. Dissertation Committees
1995 Kirti G. Goyal, Molecular Genetics and Biochemistry, University of Pittsburgh School of Medicine (Advisor: Dr. Leaf Huang)
1997 Jie-Gen Jiang, Pathology, University of Pittsburgh School of Medicine (Advisor: Dr. Reza Zarnegar), graduated in 12/97
1997-1998 Marni Brisson, Pharmacology, University of Pittsburgh School of Medicine (Advisor: Dr. Leaf Huang)
1997-1998 Donald Schwartz, Pharmacology, University of Pittsburgh School of Medicine (Advisor: Dr. John Lazo)
1996-1998 Robert Rice, Pharmacology, University of Pittsburgh School of Medicine (Advisor: Dr. John Lazo)
1996-1999 Cheryl Fattman, Pharmacology, University of Pittsburgh School of Medicine (Advisor: Dr. Q. Ping Dou)
1999-2002 David M. Smith, Department of Biochemistry & Molecular Biology, University of South Florida College of Medicine (graduated; Ph.D. Advisor: Dr. Q. Ping Dou)
2000-2004 Kenyon Daniel, Department of Biochemistry & Molecular Biology, University of South Florida College of Medicine (Graduated; Ph.D. Advisor: Dr. Q. Ping Dou)
2001-2005 Deborah Kuhn, Cancer Biology Ph.D. Program (IOP), University of South Florida College of Arts and Sciences (Advisor: Dr. Q. Ping Dou). Graduated in November, 2004.
2002-2003 Audrey Burns, Department of Biochemistry & Molecular Biology, University of South Florida College of Medicine (Advisor: Dr. Q. Ping Dou)
2002-2003 Sam Falsetti, Department of Biochemistry & Molecular Biology, University of South Florida College of Medicine (Advisor: Dr. Q. Ping Dou)
2003 Bonnie Goodwin, Department of Biochemistry & Molecular Biology, University of South Florida College of Medicine (Advisor: Dr. Duane Eichler)
2004 Kristin Landis, Cancer Biology Program, Wayne State University (Advisor: Dr. Q. Ping Dou)
2005 Vesna Minic, Cancer Biology Program, Wayne State University (Advisor: Dr. Q. Ping Dou)

Comprehensive Examination Committees
1994 Xiang Gao, Pharmacology, University of Pittsburgh School of Medicine
1995 Jeff Phillips, Pharmacology, University of Pittsburgh School of Medicine
1995  Chialin Cheng, Pharmacology, University of Pittsburgh School of Medicine
1995  Cheryl Fattman, Pharmacology, University of Pittsburgh School of Medicine
1996  Marni Brisson, Pharmacology, University of Pittsburgh School of Medicine
2003  Deborah Kuhn, Cancer Biology Ph.D. Program, University of South Florida College of Arts and Sciences

Research Associates and Others
2005-present  Haiyan Pang, Ph.D., Research Associate
2005-present  Huanjie Yang, Ph.D., Post-Doctoral Fellow
2004-present  Cindy (Qiuzhi) Cui, Technician
2004-present  Marcianna Norris, Administrative Assistant (Supervisor: Dr. Q. Ping Dou)
2003-present  Di Chen, Ph.D., Research Associate
2005-present  Jaiwei Ren, Technician

2005  Alejandro Diez, M.D., Physician Intern
2004-2005  Shirley Adanta Orlu, Research Assistant
2005-2005  Yezhou Sun, Student Assistant
2004-2005  Kenyon Daniel, Ph.D., Post-Doctoral Fellow
2003-2004  MaryAnn Sparkman, Administrative Assistant (Supervisor: Dr. Q. Ping Dou)
2003-2004  Mohammad Bhuiyan, Ph.D., Research Associate
2000-2003  Aslamuzzaman Kazi, Ph.D., Research Associate
2002  Robin Shear, Research Volunteer
2000-2002  Sherry Zhong, Research Assistant
2001-2002  Puja Gupta, Research Volunteer
2000-2001  Hongwei Wang, Research Assistant
2000-2001  Kenyon Daniel, Research Assistant
1998-2000  Sangkil Nam, Ph.D., Research Associate
2000  Gen Wang, Ph.D., Research Associate
1999-2000  Xiaoxia Zhang, M.S., Research Assistant
1998-2000  Gui Gao, Ph.D., Research Associate
1998-2000  Benyi Li, M.D., Research Associate
1998-1999  Roland Cooper, Ph.D., Research Associate
1998  Jieliu Tang, Ph.D., Research Associate
1994-1998  Bing An, Research Associate
1996-1998  Terence F. McGuire, Ph.D., Instructor
1997-1998  Yibing Peng, M.S., Research Assistant
1995-1996  Jia-Rui Jin, Visiting Scholar
1995  Leilei Zhang, Visiting Scholar

GRANT SUPPORT:

Completed support

American Cancer Society Institutional Research Grant. Cyclins, transcription and defective growth control in cancer. Principal Investigator: Qing Ping Dou. 10/01/93-06/30/95. Total Direct Costs: $10,000

Agreement with Beth Israel Hospital. Molecular Biology of Aging. Principal Investigator: Jeanne Y. Wei. 1994. Total Direct Costs: $10,000
Q. Ping Dou, Ph.D.

NIH R01. Molecular Biology of G1/S Regulation in Murine Cells. 07/01/93-06/30/96. Subcontract Total Direct Costs: $82,040; Total Indirect Costs: $38,239 (Principal Investigator: Arthur B. Pardee)

NIH Shannon Award. Functions of RB-protease(s) in apoptosis. Principal Investigator: Qing Ping Dou. 09/15/95-08/31/97 (replaced by R29 on 04/14/96). Total Direct Costs: $80,000; Total Indirect Costs: $20,000

UPCI Breast Cancer Pilot Grant. Induction of p53-independent apoptosis and treatment of human breast cancer. Principal Investigator: Qing Ping Dou. 03/15/96-09/30/97. Total Direct Costs: $20,000; Total Indirect Costs: $10,200

NIH R29. Functions of RB-protease(s) in apoptosis. Principal Investigator: Q. Ping Dou (50%). 04/15/96-02/28/01. Total Direct Costs: $349,996; Total Indirect Costs: $178,298

NIH R01. Growth Inhibition by IL-2 of IL2R+ oral carcinomas. Principal Investigator: Q. Ping Dou (10%). 04/01/98-03/31/01. Total Direct Costs: $105,270; Total Indirect Costs: $44,740 (a subcontract from University of Pittsburgh)

Department of the Army Advanced Cancer Detection Center Research Grant (Moffitt). Significance of Bax-Dependent Apoptosis in Prevention and Detection of Human Prostate and Lung Cancer. Principal Investigator: Q. Ping Dou. 10/01/00-9/30/01. Total Direct Costs: $114,773

Administrative Supplement from Moffitt Cancer control. Co-Principal Investigator: Q. Ping Dou. $4,285

Administrative Supplement from Moffitt Foundation. Co-Principal Investigator: Q. Ping Dou. $2,300

Agreement from University of North Taxes. Co-Principal Investigator: Q. Ping Dou. $3,000

NIH R03. Tea Targeting Proteasome: A Role in Cancer Prevention. Principal Investigator: Q. Ping Dou (10%). 07/01/01-06/30/03. Total Direct Costs: $100,000; Total Indirect Costs: $45,000

Supplement for Correlative Studies Related to estrogen Receptor Negative (ER-negative) Breast Cancer (Moffitt CCOP Research Base). PI: Krischer; Co-Investigator: Q. Ping Dou. $36,040

U10 CA81920. A Clinical Trial of the Action of Isoflavones in Breast Neoplasia: Administration Prior to Mastectomy or Lumpectomy - A Pilot Study. PI: Krischer; Co-Investigator: Q. Ping Dou. 12/01/01-11/30/03

U10 CA81920. The Specific Role of Isoflavones in reducing Prostate Cancer Risks. PI: Krischer; Co-Investigator: Q. Ping Dou. 12/01/01-11/30/03
U10 CA81920. A Randomized Pilot Clinical Trial of the Action of Isoflavones and Lycopene in Localized Prostate Cancer: Administration Prior to radical Prostatectomy. PI: Krischer; Co-Investigator: Q. Ping Dou. 12/01/01-11/30/03

**Approved but not funded**

American Cancer Society. Induction of an RB-associated phosphatase and cancer cell apoptosis *(Score: the second decile).* Principal Investigator: Q. Ping Dou. 01/01/97-12/31/99. Total Direct Costs: $252,151; Total Indirect Costs: $63,038

American Institute for Cancer Research. Tea polyphenols target proteasome-mediated Bax degradation pathway: Significance in prostate cancer prevention and treatment *(Score: 2.92).* Principal Investigator: Q. Ping Dou. 02/01/00-01/31/03. Total Direct Costs: $150,000; Total Indirect Costs: $15,000

**Present support**


NIH R01. N-Thiolated β-Lactams. Co-Principal Investigator: Q. Ping Dou (5%) (PI: Ed Turos). 03/01/02-02/28/07. Total Direct Costs (to Dou lab): $200,000; Total Indirect Costs: $90,000

DOD Breast Cancer Research Program-Concept Award. Examination of potential anti-tumor activity of N-thiolated β-lactam antibiotics in nude mice bearing human breast tumors. 5% Effort (PI: Q. Ping Dou). 10/01/04-09/30/06. Total Direct Costs: $75,000; Total Indirect Costs: $38,250

NIH R03. The Proteasome as Molecular Target of Grape Polyphenols. 5% Effort (PI: Q. Ping Dou). 12/01/04-11/30/06. Total Direct Costs: $100,000; Total Indirect Costs: $51,000

NIEHS P50 ES012395. Center for Urban African American Health. 3.0% Effort (Co-I: Q. Ping Dou; PI: John Flack). 06/01/05-05/31/07.

T32-CA09531-19 NIH Training Grant. “Training Program in the Biology of Cancer" (Ms. Kristin R. Landis-Piwowar; Mentor: Q. Ping Dou) 09/01/05-8/31/07.

NCI/NIH the Cancer Center Support Grant (PI: Ruckdeschel). Population Studies & Prevention Program (Program PI: Schwartz; Co-I: Q. Ping Dou, 5%) 10/01/05-9/31/10.

CellQuest, Inc. CellQuest, A Musaceas Plant Extract: Implications in cancer prevention. Principal Investigator: Q. Ping Dou. 10/01/02-09/30/06. Total Direct Costs: $375,000 (Approved)

Karmanos Cancer Institute Startup funds. Principal Investigator: Q. Ping Dou. 08/01/03 -.
Karmanos Cancer Institute Indirect Account. Principal Investigator: Q. Ping Dou. 08/01/03 -.

Pending support

NIH R01. Elucidation of molecular mechanisms for the ubiquitin-proteasome pathway in malignant pleural mesothelioma (Co-I: Q. Ping Dou; PI: Anil Wali). 10/01/05-9/30/10. Total Direct Costs: $1,250,000; Total Indirect Costs: $586,500

NIH R21. Molecular Study on Novel NCI Potential Anti-tumor Drugs. 15% Effort (PI: Q. Ping Dou). 04/01/05-03/31/07. Total Direct Costs: $200,000; Total Indirect Costs: $102,000

NIH R03. Targeting tumor endogenous copper with clioquinol. 5% Effort (PI: Q. Ping Dou). 07/01/05-06/30/07. Total Direct Costs: $100,000; Total Indirect Costs: $51,000

NIH R21. Dual-agent nanoparticles to overcome drug resistance. 5% Effort (Co-PI: Q. Ping Dou; PI: Jayanth Panyam). 07/01/05-06/30/07. Total Direct Costs: $250,000; Total Indirect Costs: $124,950

Alliance For Cancer Gene Therapy. Dual-agent nanoparticles to overcome drug resistance. 5% Effort (Co-PI: Q. Ping Dou; PI: Jayanth Panyam). 07/01/05-06/30/08. Total Direct Costs: $449,991; Total Indirect Costs: $44,999

DOD Breast Cancer Research Program-Concept Award. Determination of potential anti-cancer activity of synthetic acetylated EGCG analog prodrugs in nude mice bearing human breast tumor xenografts. 5% Effort (PI: Q. Ping Dou). 07/01/05-06/30/06. Total Direct Costs: $75,000; Total Indirect Costs: $38,250

DOD Breast Cancer Research Program-Concept Award. Synchronized Gene Silencing and Drug Delivery to Overcome Drug Resistance in Breast Cancer. 5% Effort (Co-PI: Q. Ping Dou; PI: Jayanth Panyam). 07/01/05-06/30/06. Total Direct Costs: $75,000; Total Indirect Costs: $38,250

NIH R01. Synthesis and evaluation of prodrugs of green tea polyphenol EGCG analogs. 20% Effort (PI: Q. Ping Dou). 12/01/05-11/30/10. Total Direct Costs: $1,250,000; Total Indirect Costs: $380,460

DOD Prostate Cancer Research Program-Idea Development Award. MOLECULAR TARGETS OF NOVEL NCI POTENTIAL ANTICANCER DRUGS IN HUMAN PROSTATE CANCER CELLS. 20% Effort (PI: Q. Ping Dou). 10/01/05-09/30/08. Total Direct Costs: $375,000; Total Indirect Costs: $191,250

The Michigan Technology Tri-Corridor Fund, Fiscal Year 2005 Competition. DUAL-AGENT NANOPARTICLES TO OVERCOME DRUG RESISTANCE. 5% Effort (Co-PI: Q. Ping Dou; PI: Jayanth Panyam). 07/01/05-06/30/07. Total Direct Costs: $85,320 (to Dou Lab)
Q. Ping Dou, Ph.D.

DOD Breast Cancer Research Program/IDEA Award. The potential use of the anti-alcoholism drug disulfiram in breast cancer prevention and treatment. 15% Effort (PI: Q. Ping Dou). 01/01/06-12/31/08. Total Direct Costs: $300,000; Total Indirect Costs: $146,403

NIH R01. Roles of polymorphic COMT, tea polyphenols and proteasome in cancer prevention. 20% Effort (PI: Q. Ping Dou). 04/01/06-03/31/11. Total Direct Costs: $1,250,000; Total Indirect Costs: $421,137 (received a Priority Score "150" and a Percentile "6.0")

DOD Prostate Cancer Research Program/Physician Research Training Award. Novel organic copper complex PDC-Cu for molecular therapy of prostate cancer facilitated by PET imaging (PI: Fangyu Peng; Mentor: Q. Ping Dou). 10/01/05-09/30/10. Total Direct Costs: $634,445; Total Indirect Costs: $50,756

Wayne State University Stimuli_responsive Nanosystems Proposal. Stimulus Controlled Nanosystems for Cancer Imaging and Treatment. (Co-I: Q. Ping Dou; PI: Stephanie L. Brock). Total Direct Costs (Dou Lab): $40,000

Susan Komen Foundation. Nanoparticle-mediated combination photodynamic and chemotherapy to overcome refractory tumors. 5% Effort (Co-PI: Q. Ping Dou; PI: Jayanth Panyam). 05/01/06-04/30/08. Total Direct Costs: $200,000

Wilson Foundation. Targeting tumor endogenous copper with the antibiotic clioquinol: A novel approach for cancer-specific killing with no or low toxicity. 15% Effort (PI: Q. Ping Dou). 01/01/06-12/31/07. Total Direct Costs: $200,000

VA Merit. Ubiquitin Protease System in Malignant Pleural Mesothelioma. 10% Effort. (Co-I: Q. Ping Dou; PI: Anil Wali). Total Direct Costs: $795,600

NIH R01. Copper as a novel target for determining fate of AR and prostate cancer cells. 20% Effort (PI: Q. Ping Dou). 07/01/06-06/30/11. Total Direct Costs: $1,250,000; Total Indirect Costs: $628,693

NIH R21 (resubmission). Dual-agent nanoparticles to overcome drug resistance. 5% Effort (Co-PI: Q. Ping Dou; PI: Jayanth Panyam). 07/01/06-06/30/08. Total Direct Costs: $250,000; Total Indirect Costs: $126,250

NIH R01. The Chinese Thunder of God Vine: Active Components & Biological Targets in Cancer. 20% Effort (PI: Q. Ping Dou). 12/01/06-11/30/11. Total Direct Costs: $1,250,000; Total Indirect Costs: $568,125


DOD Breast Cancer Research Program-Concept Award. Chemosensitization of human breast cancer cells by an active compound purified from the Chinese medicine Thunder
of God vine. 5% Effort (PI: Q. Ping Dou). 07/01/06-06/30/07. Total Direct Costs: $75,000; Total Indirect Costs: $37,875

NIH R01. Maspin in Hormone Refractory Prostate Cancer Intervention (Co-I: Q. Ping Dou, 5%; PI: Shijie Sheng). 12/01/06-11/30/11. Total Direct Costs: $1,250,000; Total Indirect Costs: $568,125

NIH R01 (resubmission). Elucidation of molecular mechanisms for the ubiquitin-proteasome pathway in malignant pleural mesothelioma (Co-I: Q. Ping Dou; PI: Anil Wali). 12/01/06-11/30/11. Total Direct Costs: $1,250,000; Total Indirect Costs: $586,500

VA Merit (resubmission). Ubiquitin Protease System in Malignant Pleural Mesothelioma. 10% Effort. (Co-I: Q. Ping Dou; PI: Anil Wali). Total Direct Costs: $795,600

MICHIGAN ECONOMIC DEVELOPMENT CORPORATION (MEDC). Development of natural pharmaceuticals to protect against low-intensity radiation exposure. 5% Effort (Co-PI: Q. Ping Dou; PI: Michael C Joiner). 10/01/06-09/30/09. Total Direct Costs: $1,026,000; Total Indirect Costs: $153,900


PUBLICATIONS:

Original Observations in Referred Journals

1. Chen KY and Dou QP. NAD+ stimulated the spermidine-dependent hypusine formation on the 18,000-dalton protein in cytosolic lysates derived from NB-15 mouse neuroblastoma cells. FEBS Letters, 1988; 229: 325-328


20. Fattman CL, An B and **Dou QP**. Characterization of interior cleavage of retinoblastoma protein in apoptosis. J. Cell. Biochem. (A figure was selected as the cover of the journal), 1997; 67: 399-408


30. Sun J, Nam S, Lee C-S, Li B, Coppola D, Hamilton AD, **Dou QP** (co-corresponding author) and Sebti SM. CEP1612, a dipeptidyl proteasome inhibitor, induces p21^WAF1 and p27^KIP1 expression and apoptosis and inhibits the growth of the human lung adenocarcinoma A-549 in nude mice. Cancer Res. (Advances in Brief), 2001; 61: 1280-1284

31. Fattman CL, Delach S, **Dou QP** and Johnson DE. Sequential two-step cleavage of the retinoblastoma protein by caspase-3 during etoposide-induced apoptosis. Oncogene, 2001; 20: 2918-26


44. Chen MS, Chen D and Dou QP. Inhibition of the proteasome activity by various fruits and vegetables is associated with cancer cell death. IN VIVO, 2004; 18: 73-80.


56 Lu M, **Dou QP**, Kitson RP, Smith DM, and Goldfarb RH. Differential effects of proteasome inhibitors on cell cycle and apoptotic pathways in human YT and Jurkat cell. J Cell Biochem; Published Online: 19 Sep 2005


59 Daniel KG, Chen D, Orlu S, Cui QC, Miller FR, and **Dou QP**. Clioquinol and pyrrolidine dithiocarbamate complex with copper to form proteasome inhibitors and apoptosis inducers in human breast cancer cells. Breast Cancer Res, 2005; 7:R897-R908


61 Yang HJ, Chen D, Cui QC, Yuan X, and **Dou QP**. Celastrol, a triterpene extracted from the Chinese Thunder of God Vine, is a potent proteasome inhibitor and suppresses human prostate cancer growth in nude mice (Cancer Res., Accepted).

**Invited Review Articles and Book Chapters:**


3. **Dou QP** and Pardee AB. Transcriptional activation of thymidine kinase, a marker for cell cycle control. Progress in Nuclear Acid Research and Molecular Biology, 1996; 53: 197-217


5. **Dou QP**. Putative roles of retinoblastoma protein in apoptosis. Apoptosis, 1997; 2: 5-18


23. Kuhn DJ and Dou QP. The Role of Interleukin-2 Receptor Alpha in Cancer. Frontiers in Bioscience, 2005; 10: 1462-1474

26. Daniel KG, Chen D, Yan B and **Dou QP**. Copper-binding compounds as proteasome inhibitors and apoptosis inducers in human cancer. Frontiers in Bioscience, 2006; in press

**Patents:**

1. “Multicatalytic Protease (Proteasome) Inhibitors for Use as Anti-Tumor Agents”, filed in on 12/16/97 (US, Ser. No. 60/069,804) and (International, WO 99/30707)
5. “Novel Beta-Lactams as Potential Anticancer Agents”, filed on 4/18/2001 (USF Reference No.: 01A032)
6. “Chemical synthesis and biological activities of the polyphenols GCG (gallocatechin-gallate) and EGCG (epigallocatechin-gallate)”, filed on 8/29/2002
7. “Organic Copper Compounds as Potent Proteasome Inhibitors and Potential Anticancer Agents”, filed on 4/17/2002 (USF Reference No.: 02A033)
8. “Computational Docking Model Development of Tea Polyphenol Proteasome Inhibitors: Applications to Rational Drug Design”, filed on 12/18/2002 (USF Reference No.: 03A003)
11. Peracyloxyl Protected (-)-Epigallocatechin Gallate Derivatives and their Prodrugs as Proteasome Inhibitors and Cancer Cell Apoptosis Inducers” (filled on February 4, 2005) (with the USPTO; patent number to be assigned).

**ABSTRACTS FOR POSTER PRESENTATION IN THE LAST FIVE YEARS:**

Fattman CL and **Dou QP**. Distinct ICE-like proteases mediate cleavage of retinoblastoma protein and poly(ADP-ribose) polymerase during apoptosis. AACR 88th annual Meeting, San Diego, California, April 12-16, 1997.


Fattman CL, An B, Zhang LL, Dineley K and **Dou QP**. Dephosphorylation and cleavage of the retinoblastoma protein during p53-dependent and -independent apoptosis of

**Dou QP.** Cell cycle checkpoint proteins as apoptosis therapeutic targets. NATO Advanced Research Workshop at H. Lee Moffitt Cancer Center and Research Institute University of South Florida, Protein-Protein and Protein-Lipid Interactions in Signal Transduction: Use of Small Synthetic Molecules as probes and Therapeutic Agents, Clearwater Beach, Florida, December 5-9, 1997


**Dou QP** and Li B. Bax degradation by the ubiquitin/proteasome-dependent pathway: involvement in tumor survival and progression. AACR 91st Annual Meeting, San Francisco, CA, April 1-5, 2000

**Dou QP,** Gao G and Zhang X. Insulin/IGF-I receptor-mediated signal transduction pathway regulates G1 phase-dependent Bcl-2 expression and tumor chemoresistance. AACR 91st Annual Meeting, San Francisco, CA, April 1-5, 2000


**Gao G** and **Dou QP.** G1 phase-dependent Bcl-2 expression correlates with chemoresistance of human cancer cells. 29th Annual Scientific Meeting of the International Society for Experimental Hematology, Tampa, FL, July 8-11, 2000

**Smith DM** and **Dou QP.** Green tea targets human tumor cell DNA synthesis and consequently induces apoptosis. Poster presentation. New Molecular Targets for Cancer Therapy, St. Peters burg Beach, FL, October 14-17, 2000

**Smith DM,** Nam S and **Dou QP.** Studies on tumor related targets of green tea polyphenols. Poster presentation. 2nd International Conference. Mechanisms of Cell Death and Disease: Advances in Therapeutic Intervention, North Falmouth, MA, June 2-6, 2001

**Daniel KG,** Zhong Q, Gupta P and **Dou QP.** Etoposide induces activation of calpain in early stages of apoptosis. Poster presentation. 22nd Annual South East Pharmacology Society Meeting (SEPS), Drug Development Symposium (from Bench to Bedside), Clearwater, FL, October 4-6, 2001

**Kazi A,** Smith DM, Zhong Q and **Dou QP.** Down Regulation of Bcl-XL Protein Expression by Green Tea Polyphenols Is Associated with Prostate Cancer Cell Apoptosis. Poster presentation. AACR-NCI-EORTC. Molecular Targets and Cancer therapeutics, Miami Beach, FL, October 29-November 2, 2001


Nam S, Dalton WS, Trotti AM, **Dou QP** and Calvin DP. Cell adhesion to fibronectin (FN) through β1 integrins results in cell adhesion mediated ionizing radiation resistance (CAM-RR) in human LNCaP prostate cancer cells: the potential involvement of proteasome chymotrypsin-like activity. Poster presentation. AACR 93rd Annual Meeting, San Francisco, CA, April 6-10, 2002

Nam S, Dalton WS, Q. Ping Dou, Jove R and Calvin DP. Proteasome chymotrypsin-like activity (PCA) is implicated in LNCaP prostate cancer cell adhesion mediated ionizing radiation (IR) resistance (CAM-RR). Poster presentation. Molecular Targets for Cancer Therapy, St. Pete Beach, FL, October 11-15, 2002


Lu M, Q. Ping Dou, Kitson RP, Smith DM, and Goldfarb RH. Differential Effects of Proteasome Inhibitors on Cell Cycle Progression and Molecular Modulation in Human Natural Killer Cells and T Lymphocytes. AAI, 2003


Minic V, Kuhn D, Daniel K, Chen D, Landis K, Turos E, Q. Ping Dou. Potential Use of beta-Lactam antibiotics in Cancer Prevention and Therapy. Poster presentation. Cancer Biology Program, Karmanos Cancer Institute and Wayne State University, Detroit, MI, September 8, 2004


Minic V, Kuhn D, Daniel K, Chen D, Landis K, Turos E, Q. Ping Dou. Potential Use of beta-Lactam antibiotics in Cancer Prevention and Therapy. Poster presentation. Graduate student recruitment for Cancer Biology Program, Karmanos Cancer Institute and Wayne State University, April 2, 2005

Di Chen, Marina Chen, Kenyon G. Daniel, Deborah J. Kuhn, Kristin Landis, and Q. Ping Dou. Dietary Flavonoids as Proteasome Inhibitors and Apoptosis Inducers in Human Leukemia Cells and Their Structure-activity Relationships. Poster presentation. Graduate student recruitment for Cancer Biology Program, Karmanos Cancer Institute and Wayne State University, April 2, 2005

Kristin R. Landis-Piwowar, Deborah J. Kuhn, Sheng Biao Wan, Di Chen, Tak Hang Chan, and Q. Ping Dou. Evaluation of Proteasome-Inhibitory and Apoptosis-inducing
Potencies of Novel (-)-EGCG Analogs and their Prodrugs. Poster presentation. Graduate student recruitment for Cancer Biology Program, Karmanos Cancer Institute and Wayne State University, April 2, 2005

Di Chen, Kenyon G. Daniel, Marina S. Chen, Deborah J. Kuhn, Kristin R. Landis Piwowar, Wai Har Lam, Larry M. C. Chow, Tak Hang Chan and Q. Ping Dou. Dietary and synthetic polyphenols as proteasome inhibitors and apoptosis inducers in human cancer cells. 5th Annual Symposium, Michigan Prostate Research Colloquium, Basic and Clinical Advances in Prostate Cancer Research, Wayne State University School of Medicine, Detroit, MI 48201, April 23, 2005


INVITED ORAL PRESENTATIONS (IN THE LAST FIVE YEARS):

Dou QP. Apoptosis control and cancer. Department of Pharmacology, University of Pittsburgh School of Medicine, February 7, 1997


Dou QP. RB and apoptosis. University of Pittsburgh Cancer Institute, Molecular Oncology Seminar Series, April 16, 1997

Dou QP. Activation of apoptotic death program in human cancer. H. Lee Moffitt Cancer Center & Research Institute at the University of South Florida, April 28, 1997


Dou QP. Apoptosis regulation in breast cancer. Second Annual Pittsburgh Minisymposium on Basic and Translational Research in Breast Cancer, Center for Environmental and Occupational Health and Toxicology, University of Pittsburgh, August 16, 1997

Dou QP. Invited speaker. RB and apoptosis control. Center for Clinical Pharmacology, University of Pittsburgh School of Medicine, September 25, 1997

Dou QP. Invited Speaker. Targeting the Apoptotic Signaling Pathway in Human Cancer. Departments of Biochemistry & Molecular Biology and Microbiology & Immunology, University of North Texas Health Science Center at Fort Worth, September 29, 1997

Dou QP. Invited Speaker and Session Chairman. Putative roles of retinoblastoma protein in apoptosis. 2nd World Congress on Advances in Oncology, Athens, Greece, 16-18 October, 1997

Dou QP. Invited Speaker. Cell cycle checkpoint proteins as apoptosis therapeutic targets. NATO Advanced Research Workshop at H. Lee Moffitt Cancer Center and Research Institute, University of South Florida, Protein-Protein and Protein-Lipid Interactions in Signal Transduction: Use of Small Synthetic Molecules as probes and Therapeutic Agents, Clearwater Beach, Florida, December 5-9, 1997

Dou QP. Targeting ubiquitin/proteasom-mediated protein degradation pathway in human cancers. Research Progress Seminar Series at H. Lee Moffitt Cancer Center and Research Institute and University of South Florida, Tampa, Florida, October 29, 1998
Dou QP. Invited Speaker. Targeting ubiquitin/proteasom-mediated protein degradation pathway in human cancers. Department of Pharmacology and Therapeutics, University of South Florida College of Medicine, Tampa, Florida, February 17, 1999

Dou QP. Invited Speaker. Bax degradation by the proteasome: a survival mechanism used by human cancer cells. Department of Biochemistry & Molecular Biology, University of South Florida College of Medicine, Tampa, Florida, October 15, 1999

Gao G and Dou QP. G1 phase-dependent Bcl-2 expression correlates with chemoresistance of human cancer cells. Oral presentation. 29th Annual Scientific Meeting of the International Society for Experimental Hematology, Tampa, FL, July 8-11, 2000

Smith DM and Dou QP. Green tea targets human tumor cell DNA synthesis and consequently induces apoptosis. Poster presentation. New Molecular Targets for Cancer Therapy, St. Petersburg Beach, FL, October 14-17, 2000

Dou QP. Invited Speaker. Proteasome inhibitors as novel anticancer drugs. Cancer Research and Biotechnology in the I-4 Corridor, Moffitt Cancer Center & Research Institute, Tampa, Florida, August 21, 2000

Dou QP. Invited Speaker. Therapeutic potential of proteasome inhibitors in cancer prevention and treatment. Moffitt Cancer Center Research Retreat, Saddlebrook Resort, FL, May 19, 2001

Smith DM and Dou QP. Drug Discovery: Hunting for Cancer-Specific Molecular Targets - from Natural to Synthetic Compounds. Drug Discovery and Developmental Therapeutics Seminar, Moffitt Cancer Center & Research Institute, June 21, 2001

Dou QP. Invited Speaker. Proteasome inhibitors. New drugs in hematologic malignancies, Institute Of Hematology and Medical Oncology, “Seragnoli”, University of Bologna, Bologna, Italy, November 12-14, 2001

Dou QP. Invited Speaker. Proteasome: a novel target for cancer prevention and treatment as well as anti-angiogenic therapy. Moffitt Grand Rounds, Moffitt Cancer Center & Research Institute, Tampa, FL, November 15, 2002

Dou QP. Invited Speaker. Identification of A Novel Molecular target for Anti-Copper and Anti-Angiogenic Therapies. Attenuon, L.L.C., San Diego, CA, November 25, 2002

Dou QP. Invited Speaker. Natural Proteasome Inhibitors and Chemoprevention. Karmanos Cancer Institute at Wayne State University, Detroit, MI, February 6, 2003


Dou QP. Invited Speaker. TBN. Department of Pathology, Wayne State University, Detroit, MI, June 25, 2003 (rescheduled)

Daniel KG and Dou QP. Organic-copper complexes as a new class of proteasome inhibitors: the potential of converting a pro-angiogenic factor to a cancer cell-specific killer. Drug Discovery and Developmental Therapeutics Seminar, Moffitt Cancer Center & Research Institute, May 29, 2003


Dou QP. Invited Speaker. The proteasome: a novel molecular target for cancer prevention and treatment. The Protease Group, Karmanos Cancer Institute, Detroit, MI, September 2, 2003

Dou QP. Invited Speaker. Proteasome inhibitors and chemoprevention. Great lakes chemoprevention retreat, Maumee Bay Resort, Ohio, September 13, 2003

Dou QP. Invited Speaker. Green tea and cancer prevention. Presentation to Cancer Biology Candidate Students, Karmanos Cancer Institute, Detroit, MI, April 3, 2004

Dou QP. Invited Speaker. Synthetic beta-lactam antibiotics and a selective breast cancer cell apoptosis inducer: Significance in breast cancer prevention and treatment. The Breast Cancer Group, Karmanos Cancer Institute, Detroit, MI, May 6, 2004

Dou QP. Invited Speaker. Tea Polyphenols. Karmanos Cancer Institute Research Retreat, Detroit, MI, August 27, 2004

Dou QP. Invited Speaker. Discovery of Novel Small Molecules: Rational Design, Structure-Activity Relationships, Cellular Targets, and Potential Uses for Cancer Treatment and Prevention. The Developmental Therapeutics Group, Karmanos Cancer Institute, Detroit, MI, September 8, 2004

Dou QP. Invited Speaker. Proteasome Inhibitors: Killing via Tumor-Specific Signaling. Basic and Translational Aspects of Cancer Cell Signaling Research Retreat, Karmanos Cancer Institute, Detroit, MI, January 14, 2005

Dou QP. Invited Speaker. Searching for Natural and Pharmacological Proteasome Inhibitors for Cancer Prevention and Treatment. Department of Pharmaceutical Sciences, Eugene Applebaum College of Pharmacy and Health Sciences, Wayne State University, Detroit, MI, April 13, 2005

Dou QP. Invited Speaker. Searching for Novel Polyphenol Proteasome Inhibitors for Cancer Prevention and Treatment. Department of Urology at the University of California San Francisco and San Francisco VA Medical Center, San Francisco, CA, April 28, 2005

Dou QP. Invited Speaker. Roles of polymorphic catechol-O-methyltransferase gene, tea polyphenols and proteasome in cancer prevention. Population Studies and Prevention Joint Meeting, Karmanos Cancer Institute, Detroit, MI, June 14, 2005

Dou QP. Invited Speaker. Tea polyphenols, Proteasome and Polymorphic Catechol-O-Methyltransferase: Use in Cancer Molecular Diagnosis, Prevention and Treatment. Department of Chemistry at McGill University and American Diagnostica Inc., Montreal, Quebec, Canada, August 22, 2005


Dou QP. Invited Speaker. Copper as a novel target for determining fate of AR and prostate cancer cells. Karmanos Cancer Institute Research Retreat, Detroit, MI, October 7, 2005

Dou QP. Searching for natural proteasome inhibitors for cancer prevention and anticancer drug discovery. Department of Pathology Retreat, Wayne State University School of Medicine, Detroit, MI, August 27, 2005 (canceled)


Dou QP. Invited Speaker. Cancer Prevention and the Role of Environmental Factors. The Eco-Environmental Sciences, Chinese Academy of Sciences, Beijing, China, April 19-20, 2006
Dou QP. Invited Speaker. Nutrient-Gene-Environment Interactions and Molecular Prevention of Cancer. The Institution of Environmental Sciences, Shandong University, Jinan, Shandong, China, April 24, 2006

Dou QP. Invited Speaker. Discovery of Novel Small Molecules from Nature and Laboratories for Cancer Therapies. The Institution of Environmental Sciences, Shandong University, Jinan, Shandong, China, April 25, 2006