Antimicrobial Susceptibility Testing of Novel Anticancer Derivatives against Infectious Bacteria for the Potential Minimization of Nosocomial Infections

A Senior Honors Thesis

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By

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Abstract

Background: Nosocomial infections are an increasing problem in the United States with a reported 2 million American patients per year developing an infection as a result of their hospital stay (3). Newly synthesized derivatives of two classes of anti-cancer drugs known as anthracyclines and indolocarbazoles have recently been tested against leukemia K562 cells, colon cancer SW620 cells (15-19) and now *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Enterococcus faecalis* and *Streptococcus pyogenes*. ***Methods:*** Minimum Inhibitory Concentration (MIC) values were collected for 40 newly synthesized anticancer agents via microdilution antimicrobial susceptibility testing. ***Results:*** Thirteen anthracycline derivatives and three indolocarbazoles derivatives seemed to show an inhibitory effect to either one or all of the gram-positive organisms tested. The antimicrobial activities obtained in this study were then compared to the anticancer activities published in previous literature, resulting in the discovery of five anthracycline and one indolocarbazole derivatives which demonstrate good inhibition against both bacterial and cancer cell proliferation. ***Conclusion:*** The present study dealing with the effects of these drugs on prokaryotic system may help distinguish which drugs could be able to minimize a patient's chance of acquiring a bacterial infection while in use during chemotherapy. In addition, these studies could also provide more information in terms of the structure-activity relationship (SAR) between the drugs and their designated cellular targets.

Chapter 1: Introduction

Nosocomial infections have become a very significant problem in clinical settings all across the world. For example, in Intensive Care Unit (ICU) hospital settings (representing 8 % to 15% of hospital admissions), patients suffer from a substantially higher percentage of nosocomial infections, leading to increases in morbidity, mortality and financial cost (12). Some of these ICU patients may be cancer patients that are undergoing (or had previously undergone) chemotherapy. Due to the negative effects that chemotherapy drugs have on the immune system, patients undergoing chemotherapy have a higher chance of acquiring nosocomial infections as a result of their hospital stay (14). As a result, the importance of preventing infection in cancer patients undergoing chemotherapy is significant.

Anthracyclines, a class of drugs known to target DNA topoisomerase II in eukaryotes, are known to be some of the most effective drugs against a wide variety of solid tumors in human patients (2). Daunomycin and doxorubicin are two anthracyclines that have been widely used in clinical settings since the 1970s (13). Currently, the common clinical administration of daunomycin is daily intravenous injections of 30-60 mg/m^2 for 3-6 days. This dosage is not to exceed a cumulative-lifetime dose of 900 mg/m^2 in adults and 25 mg/kg in children (25). The mechanism of action for these drugs is believed to involve the drug's intercalation with DNA and stabilization of a topoisomerase II-DNA-drug ternary complex. Due to the growing demand for the introduction of new anthracycline derivatives, anthracyclines have been extensively researched in the hope of

finding more potent agents that overcome multidrug resistance and show reduced cardiotoxicity (4, 7). Recently, several novel anthracycline derivatives have been synthesized and tested against a variety of normal and drug resistant cancer cell lines (15-18). Some of these results have even discovered a reduction in cardiotoxicity with newly synthesized disaccharide derivatives, a major problem that most anthracycline monosaccharides such as Daunomycin cannot overcome (23, 24).

Indolocarbazoles are a newer class of anticancer drugs than the more well known anthracyclines used since the 1970s. Indolocarbazoles are most well known for their inhibitory role towards Topoisomerase I (21). Topoisomerase I is responsible for condensing and opening chromosomes during mitosis which allows for the replication and transcription of DNA (21). Indolocarbazoles work almost in the same way as anthracyclines by intercalating in between the DNA and stabilizing the DNA-Topoisomerase I-Drug complex (22). This stabilization prevents the Topoisomerase I enzyme from cleaving and religating the DNA strands, causing apoptosis. Another documented target of the indolocarbazoles is protein kinase C (22). The most famous of the indolocarbazoles is rebeccamycin and several derivatives have been synthesized in the hope of creating more potent Topoisomerase I inhibitors. Currently, rebeccamycin is clinically administered at 16-128 mg/m^2/week for several weeks, with a maximum tolerated dosage (MTD) of 572 mg/m^2 (26). This study measures the antimicrobial activity of seven new indolocarbazole derivatives that have recently been synthesized and tested against common cancer cell lines (19). In addition,

one of the most favorable qualities that these anticancer agents possess over the anthracycline family is reduced cardiotoxicity.

Targeting nosocomial infections requires identifying the bacteria that are known to cause such infections in hospital patients. In ICU settings, it has been discovered that the urinary tract, pneumonia and primary bloodstream are the most commonly infected areas, constituting 77% of all nosocomial infection sites (11). Of those commonly infected areas, coagulase-negative staphylococci constitute 36% of bloodstream infections while enterococci constitute 16% of bloodstream infections, 14% of urinary tract infections and 43% of cardiovascular infections. In the case of individual organisms, *S. aureus*, *P. aeruginosa* and *E. coli* are responsible for a combined 19% of bloodstream infections, 45% of pneumonia infections and 16% of urinary tract infections (11). This study focuses on inhibiting *E. coli*, *P. aeruginosa*, *S. aureus*, *E. faecalis* and *S. pyogenes* due to their obvious role in hospital acquired infections. With the known inhibitory potential of doxorubicin, daunomycin (6, 14) and rebeccamycin (21, 22) against gram positive organisms, this study shows similar inhibitory roles concerning newly synthesized anticancer agents against similar prokaryotes. By exploiting these bacterial-cancer inhibitory effects, this study has the potential to discover which of these new anticancer derivatives have the ability to inhibit common nosocomial bacteria for the possible prevention of infections in patients who are administered these drugs during cancer chemotherapy. Our aim is to test these anthracycline and indolocarbazole derivatives against infectious bacteria that have been known to play significant roles in

nosocomial infections. Since it has been reported that anticancer drugs may be antagonistic in the action of several antibiotics (6, 8, 9, 10) and that anthracycline and indolocarbazole agents have antibacterial activity (5, 6, 14), the significance of antibacterial testing with new anticancer derivatives may help find new therapeutic ways of treating cancer patients.

Chapter 2: Materials and Methods

Bacterial isolates. *E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853, *S. aureus* ATCC 29212, *E. faecalis* ATCC 29212 and *S. pyogenes* ATCC 19615 were the reference strains used in the antimicrobial susceptibility testing. In addition, Erythromycin, Ampicillin and Daunomycin were tested against all six strains and compared with literature values (1, 6) for quality control reasons.

Anticancer Derivatives. All anticancer derivatives were synthesized at The Ohio State University Biochemistry Department, Columbus, Ohio using procedures specified in previous literature (15-19). Stock solutions were prepared by dissolving 2.0 mg of each purified drug solid in 750 µl of DMSO. Erythromycin, Ampicillin and Daunomycin were obtained as reagent grade powders and stock solutions of each were prepared in the same fashion as the anthracycline derivatives. The resulting 2667 µg/ml stock solutions were stored in 4^{o} C refrigeration for no longer than 30 days. For experimentation, 25 µl of each stock solution was individually diluted in 975 µl Mueller-Hinton broth (MHB; Difco Laboratories, Detroit, Michigan) to afford a concentration of 66 µg/ml. The antimicrobial concentration range tested for the MIC determinations of each individual drug against each individual organism was 33 µg/ml to 0.125 µg/ml.

MIC determination. The minimum inhibitory concentrations (MICs) were determined via a microdilution method that involved sterile 24-well plates (Corning). *E. coli*, *P. aeruginosa*, *S. aureus* and *E. faecalis* were incubated on Tryptic soy agar (TSA;

Difco Laboratories, Detroit, Michigan) overnight and stored at 4° C for no longer than 40 days. TSA was made by mixing 10 grams of powder into 250 ml of distilled water. This solution was then brought to a boil over a hot plate and heated until all the powder had dissolved. This mixture was then placed into an autoclave for 20 minutes at 120° C removed afterwards until the solution cooled down to 50° C. Just before the TSA solution hardens it is transferred over to Petri dishes and incubated overnight to check for any contamination. *S. pyogenes* was incubated in TSA with 5% Defibrinated Horse blood overnight and stored at 4° C for no longer than 30 days. For making TSA with 5% Defibrinated Horse blood, the blood is added to the agar right before the TSA hardens and after it has cooled down to 50° C.

Serial twofold dilutions of the drugs in MHB were used for the testing of *E. coli*, *P. aeruginosa*, *S. aureus*, *E. faecalis*, and *S. pyogenes* (Figure 1 represents a 24-well plate containing with each well's individual contents. The first two rows of the plate are used for one drug while the other two are used for another drug. One plate tests two drugs against one organism). This two-fold dilution technique was done by first transferring 50 ml of the 2667µg/ml stock solutions into 925 ml of sterile MHB in a centrifuge tube. 500 µl of the resulting 132 µg/ml drug concentration is then mixed with a sterile 500 µl of MHB in well 2. 500 µl of the resulting dilution is then transferred to well 3, mixed, and the same is done all the way down to well 8, after which 500 µl is removed from well 8. This stepwise mixture and transfer creates a concentration gradient from 66 µg/ml-0.25 µg/ml spanning wells 2-10, respectively. After the addition of 500 µl of

inocula to wells 2-10 later in the procedure, the resulting concentration gradient spans 33 µg/ml-0.125 µg/ml (wells 2-10, respectively). Well 1 is used as a sterile control while well 12 is used as an inoculated control.

To achieve the necessary cell suspension density, each organism was individually incubated in 10 ml of MHB at 37^{o} C using an incubator shaker until a concentration of 2 X 10^{5} cells/ml was obtained (~100 minutes for each organism). This length of time was found by doing individual growth curves for each individual organism. The resulting inocula were transferred to their individual well plates containing the designated drug dilutions (500 µl of cell suspension was added to wells 2-10, 1000 µl was added to well 12). Lastly, the well plates were incubated for 20–24 hrs at 37^{o} C, after which the plates were observed macroscopically for growth. Tests were repeated at least two times for additional quality control. The technique was confirmed by testing Ampicillin and Erythromycin against the same reference strains used in this study and comparing it to literature MIC values (18).

Figure 1: A model 24-well plate containing divided into two halves.

1. 1000 µl of MHB (Sterile Control)	**2.** 500 µl of Inoculum and 500 µl of MHB	**3.** 500 µl of Inoculum and 500 µl of MHB	**4.** 500 µl of Inoculum and 500 µl of MHB	**5.** 500 µl of Inoculum and 500 µl of MHB	**6.** 500 µl of Inoculum and 500 µl of MHB
7. 500 µl of Inoculum and 500 µl of MHB	**8.** 500 µl of Inoculum and 500 µl of MHB	**9.** 500 µl of Inoculum and 500 µl of MHB	**10.** 500 µl of Inoculum and 500 µl of MHB	**11.** Blank	**12.** 1000 µl of Inoculum (Inoculated Control)
1. 1000 µl of MHB (Sterile Control)	**2.** 500 µl of Inoculum and 500 µl of MHB	**3.** 500 µl of Inoculum and 500 µl of MHB	**4.** 500 µl of Inoculum and 500 µl of MHB	**5.** 500 µl of Inoculum and 500 µl of MHB	**6.** 500 µl of Inoculum and 500 µl of MHB
7. 500 µl of Inoculum and 500 µl of MHB	**8.** 500 µl of Inoculum and 500 µl of MHB	**9.** 500 µl of Inoculum and 500 µl of MHB	**10.** 500 µl of Inoculum and 500 µl of MHB	**11.** Blank	**12.** 1000 µl of Inoculum (Inoculated Control)

Chapter 3: Results

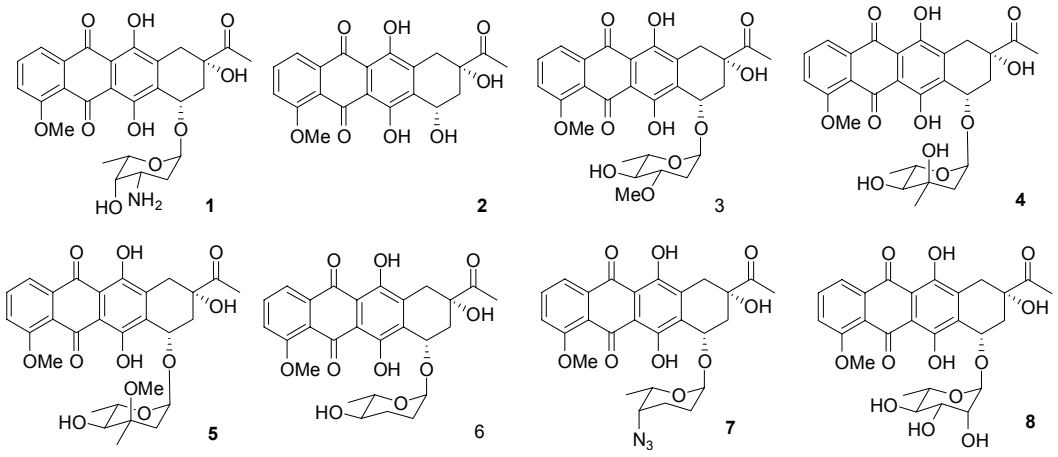

Figure 2. Drugs 1-8 represent monosaccharide anthracyclines with Drug 1 representing Daunorubicin (Daunomycin).

Table 1. MIC and IC_{50} values for daunomycin and seven monosaccharide derivatives

Monosacch-aride Cmpds. Series 1	Antimicrobial MIC (μg/mL)			Anti-cancer[a] IC_{50} (nM)
	S. aureus	E. faecalis	S. pyogenes	
1	4	4	0.25	15.6
2	> 33	> 33	> 33	> 2000
3	33	33	33	104
4	> 33	> 33	> 33	> 1000
5	> 33	> 33	> 33	> 1000
6	> 33	> 33	> 33	350
7	> 33	> 33	> 33	> 1000
8	> 33	> 33	2	265

a. Cytotoxicity of compounds 1–8 on colon cancer SW620 cells by MTS assay (cell survival is compared to control group without treatment of any drugs.)

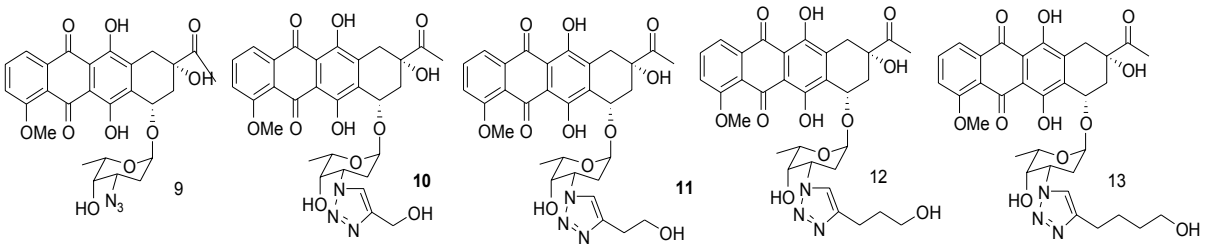

Figure 3. Drug 9 represents the monosaccharide ADNR (One substituent derivative of Daunomycin). Drugs 10-13 represent monosaccharide derivatives with an attached triazole group at the 3' carbon on the primary sugar bound to the anthracycline conjugated ring system.

Table 2. MIC and IC_{50} values for daunomycin and five monosaccharide derivatives

Monosacch-aride Cmpds. Series 2	Antimicrobial MIC (μg/mL)			Anti-cancer[a] IC_{50} (nM)
	S. aureus	E. faecalis	S. pyogenes	
1	4	4	0.25	15.6
9	> 33	> 33	> 33	75
10	> 33	> 33	2	~1000
11	> 33	> 33	4	~ 700
12	> 33	> 33	4	~ 400
13	> 33	> 33	4	~ 400

a. Cytotoxicity of compounds 1, 9–13 on leukemia K562 cells by MTS assay (cell survival is compared to control group without treatment of any drugs.)

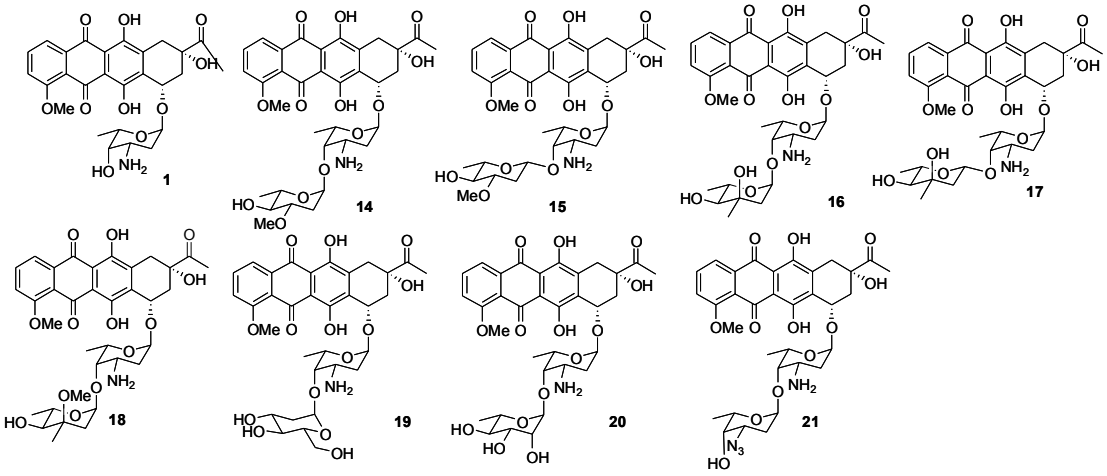

Figure 4. Drug 1 represents Daunomycin while Drugs 14-21 represent dissacharide derivatives with a common amine group attached to the 3' position of the primary sugar bound to the anthracycline conjugated ring system.

Table 3. MIC and IC_{50} values for daunomycin and eight disaccharide derivatives

Disacch-aride Cmpds. Series 3	Antimicrobial MIC (μg/mL)			Anti-cancer[a] IC_{50} (nM)
	S. aureus	E. faecalis	S. pyogenes	
1	4	4	0.25	15.6
14	4	8	4	39.5
15	8	8	1	45.8
16	4	4	2	44.3
17	> 33	> 33	> 33	1531.6
18	4	8	2	21.0
19	> 33	> 33	> 33	1378.3
20	> 33	> 33	8	>4000
21	4	4	2	31.3

a. Cytotoxicity of compounds 1, 14–21 on leukemia K562 cells by MTS assay (cell survival is compared to control group without treatment of any drugs).

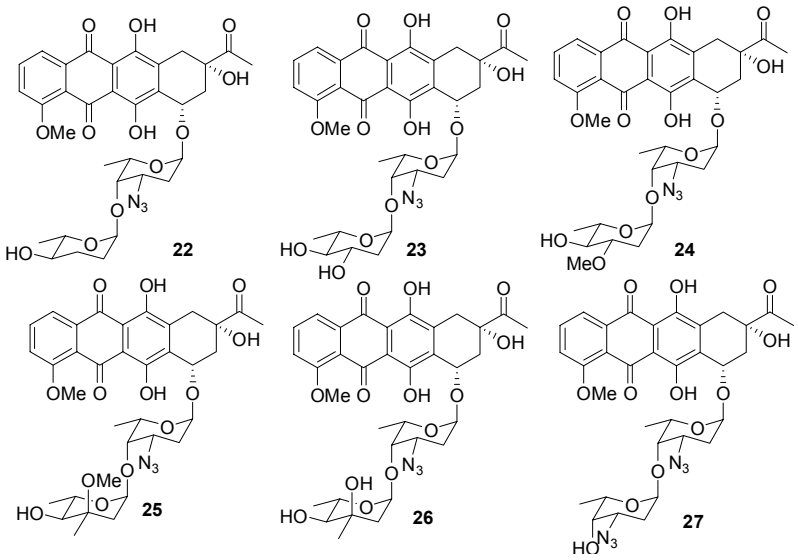

Figure 5. Drugs 22-27 represent dissacharide anthracycline derivatives containing an azido group attached to the 3' carbon on the primary sugar directly bound to the anthracycline conjugated ring system.

Table 4. MIC and IC_{50} values for daunomycin and six disaccharide derivatives

Disaccharide Cmpds. Series 4	Antimicrobial MIC (μg/mL)			Anti-cancer[a] IC_{50} (nM)
	S. aureus	E. faecalis	S. pyogenes	
1	4	4	0.25	15.6
22	> 33	> 33	> 33	790
23	> 33	> 33	4	290
24	> 33	> 33	> 33	280
25	> 33	> 33	> 33	220
26	> 33	> 33	> 33	450
27	> 33	> 33	> 33	1132

a. Cytotoxicity of compounds 1, 22–27 on leukemia K562 cells by MTS assay (cell survival is compared to control group without treatment of any drugs).

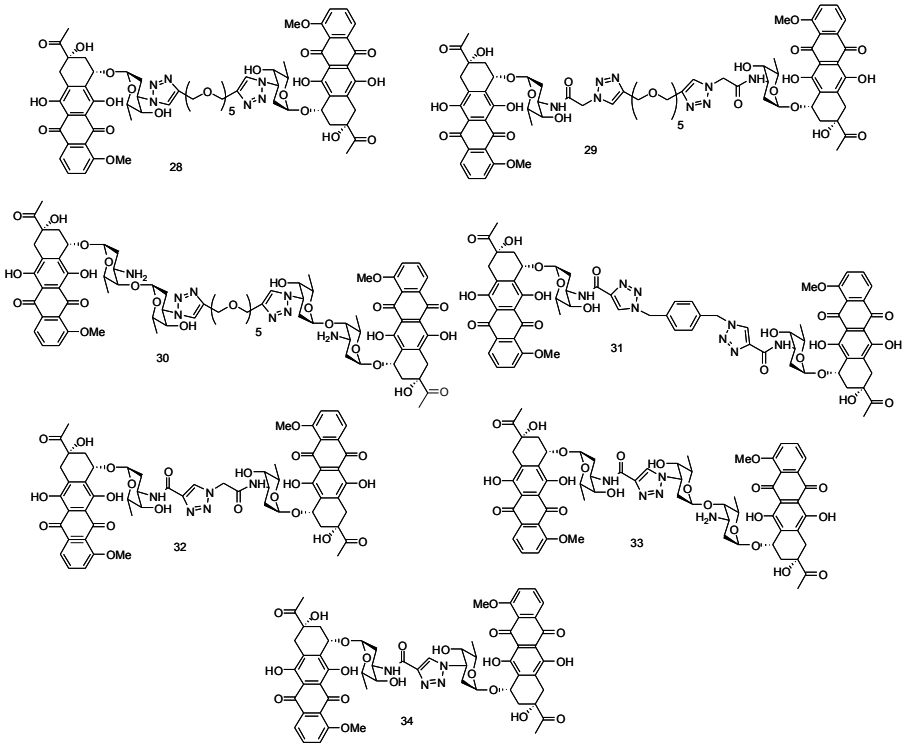

Figure 6. Drugs 28-34 represent various anthracycline dimmer derivatives linked together via ether, amide and sugar groups.

Table 5. MIC and IC_{50} values for daunomycin and seven dimer derivatives

Dimer Cmpds. Series 5	Antimicrobial MIC (μg/mL)			Anti-cancer[a] IC_{50} (μM)
	S. aureus	E. faecalis	S. pyogenes	
1	4	4	0.25	15.6
28	> 33	> 33	> 33	> 5
29	> 33	> 33	> 33	4
30	> 33	> 33	> 33	4
31	> 33	> 33	> 33	N.A.[b]
32	> 33	> 33	> 33	N.A.
33	> 33	> 33	> 33	N.A.
34	> 33	> 33	> 33	> 5

a. Cytotoxicity of compounds 1, 28–34 on leukemia K562 cells by MTS assay (cell survival is compared to control group without treatment of any drugs). B. N.A., not active

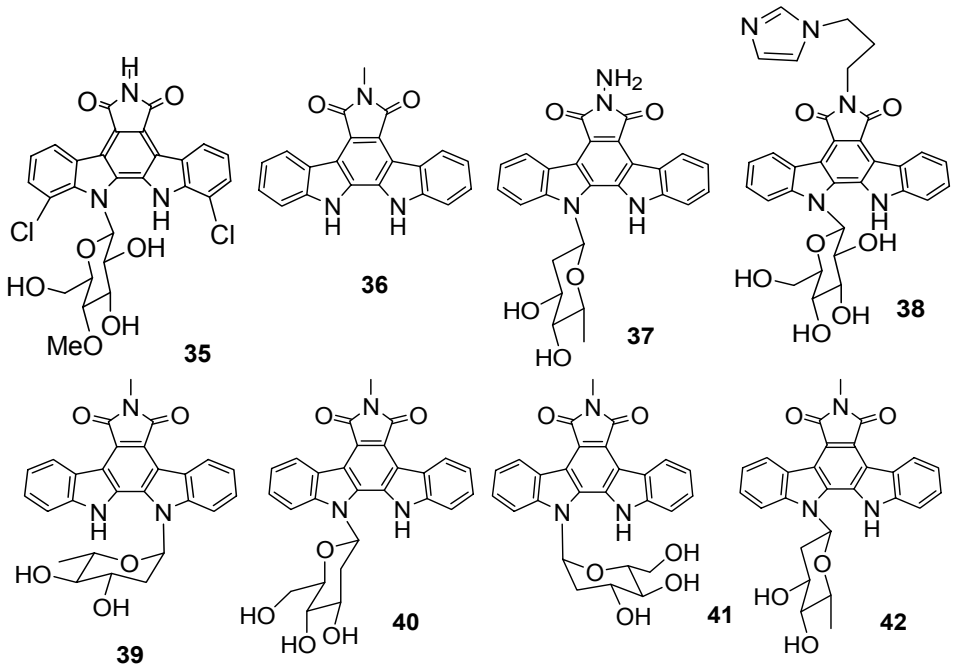

Figure 7. Drug 35 represents Rebeccamycin while Drugs 36-42 represent monosaccharide indolocarbazole derivatives.

Table 6. MIC and IC_{50} values for rebeccamycin and seven rebeccamycin derivatives

Rebeccamycin Derivatives	Antimicrobial MIC (μg/mL)			Anticancer[a] IC_{50} (μM)
	S. aureus	E. faecalis	S. pyogenes	
35	NA[b]	NA	NA	4
36	> 33	> 33	> 33	> 100
37	> 33	> 33	> 33	> 100
38	> 33	> 33	> 33	2
39	> 33	> 33	> 33	34
40	1	2	0.5	13
41	8	> 33	4	32
42	8	> 33	4	24

a. Cytotoxicity of compounds 1, 28–34 on leukemia K562 cells by MTS assay (cell survival is compared to control group without treatment of any drugs). b. N.A., not active

Chapter 4: Discussion

Ampicillin, Erythromycin and Daunomycin (drug **1**) all demonstrated antibacterial activity. Ampicillin was found to inhibit *S. aureus* at 0.25 μg/ml, *E. faecalis* at 1 μg/ml and *S. pyogenes* at less then 0.125 μg/ml. Erythromycin inhibited *S. aureus* and *E. faecalis* at 0.5 μg/ml, *S. pyogenes* at less then 0.125 μg/ml and *E. coli* at 4 μg/ml.

Figures 2-7 represent all the anthracycline drugs tested against the three gram-positive bacteria in the study. Figure 6 represents the seven indolocarbazole derivatives tested, with drug **35** representing the original rebeccamycin (a drug that was not tested in our study). It was observed that none of the drug derivatives (including daunomycin) tested worked on gram-negative organisms (*E. coli* and *P. aeruginosa*). The values in Tables 1-6 correspond to Figs. 2-7, respectively. Tables 1-6 contain each derivative's IC_{50} anti-cancer concentrations (15-19) as well as the MICs against *S. aureus*, *E. faecalis* and *S. pyogenes*.

This study shows some striking similarities amongst the drugs that work against cancer cells as well as bacterial cells. In the case of the original daunomycin, the drug demonstrated an IC_{50} concentration of 33.4 nM in colon cancer SW620 cells as well as 15.6 nM in leukemia K562 cells. In addition, daunomycin demonstrated antibacterial MIC values of 4 μg/ml in both *S. aureus* and *E. faecalis* as well as 0.25 μg/ml in *S. pyogenes*. When dealing with the newly synthesized anthracycline derivatives in this study, Table 3 shows several similarities that, when compared with Table 4, demonstrate

the importance of the amino group on the 3' carbon of the sugar directly attached to the anthracycline conjugated ring system. This discovery is also evident when comparing daunomycin (drug **1**) with drug **9**, in which daunomycin, containing an amine group on the 3' carbon, shows bacterial inhibition while drug **9** with the azido group at the same position fails to inhibit bacteria at a concentration below 33 μg/ml. In the comparison between the antibacterial and anticancer effects of the drugs in Table 3, drugs **14, 15, 16, 18** and **21** seem to demonstrate the greatest clinical potential. Drug **18** demonstrated strong anticancer activity (21.0 nM in leukemia K562 cells) as well as fairly strong antibacterial activity (4 μg/ml in *S. aureus*, 8 μg/ml in *E. faecalis* and 2 μg/ml in *S. pyogenes*). When comparing drug **18** to drugs **16** and **17**, the linkage between the two carbohydrates seems to be more important for the anticancer and antibacterial activity than the change on the 3' carbon of the second carbohydrate. This is more evident when comparing the activities of drug **16** to drug **17**. Drug **16** contains an alpha linkage between the disaccharides and showed better anticancer and antibacterial inhibition than drug **17**, which contains a beta linkage. Drug **21** demonstrated a slightly higher IC_{50} concentration against leukemia K562 cells (31.3 nM) when compared with Drug **18**, as well as a slightly lower MIC value against *E. faecalis* (4 μg/ml). Drugs **15** and **16** compared fairly well with each other in terms of anticancer activity (45.8 nM and 44.3 nM against leukemia K562 cells, respectively) but drug **16** seemed to have a lower bacterial inhibitory concentration when measured against *S. aureus* (4 μg/ml) and *E. faecalis* (4 μg/ml), while drug **15** inhibited *S. pyogenes* slightly better than drug **16** (1

μg/ml in drug **15** and 2 μg/ml in drug **16**). The last of the anticancer/antibacterial agents, Drug **14**, showed fairly good anticancer activity (39.5 nM) as well as fairly good antibacterial activity, inhibiting *S. aureus* at 4 μg/ml, *E. faecalis* at 8 μg/ml and S. *pyogenes* at 4 μg/ml. However, when drugs **14** and **15** are compared to each other, the linkage between their disaccharides does not seem to be an important factor in bacterial or cancer inhibition.

This structure-activity relationship amongst bacteria and cancer cell lines is also evident when looking at Table 6 and the indolocarbazole derivatives. Beginning with drug **36**; just as the aglycan version of daunomycin was unable to inhibit either cancer or bacterial cell growth, the same is true with the aglycan indolocarbazole derivative. When comparing drugs **37** and **42**, Table 6 quickly points out the significance of the imide nitrogen at the top of the indolocarbazole substituted ring system. The comparison shows that an amino substituent attached to the imide nitrogen, as opposed to a methyl or hydrogen substituent, seems to decrease biological activity. However, in terms of cancer cell growth inhibition, drug **38** demonstrates very strong cancer cell inhibitions (2 μM); the opposite is observed when this same drug was tested against bacteria (> 33 μg/ml).

It is interesting to note that previous literature on the use of indolocarbazoles derivatives and their biological activity has noted that the two chlorine atoms on rebeccamycin actually prevents the drug from intercalating with the DNA (20). In addition, the same study mentions that the sugar portion of indolocarbazoles is extremely

important in the inhibition of Topoisomerase I. This was found to be true in the study that our lab has conducted when comparing the activities of the aglycan indolocarbazole derivative **36** with those containing carbohydrate groups (**37**-**42)**. However, the literature also points out that indolocarbazoles with a beta-glycosidic linkage seem to be the only potent Topoisomerase I inhibitors (20). In some cases, our study agrees with these results when comparing the activity of the beta linked drug **42** and the alpha linked drug **39**. On the other hand, our study with bacteria and Dr. Sun's study with the cancer shows at least some inhibitory activity regardless of linkage (as seen with drugs **40** and **41**).

The potential of these new anticancer drug derivatives to protect a patient from obtaining a nosocomial infection mainly depends on the dosage. After examining which of these drugs show the most promise to accomplish this task, drugs **14**, **15**, **16**, **18** and **21** in particular are disaccharide anthracyclines, proven to have less cardiotoxicity than the monosaccharide Daunomycin (23). For a general estimation of what concentration of an antimicrobial agent will be needed to elicit an antibiotic effect on the body, the MIC value obtained from in vitro studies is first multiplied by 2-4 times. The resulting concentration is further multiplied by approximately 70% of a patient's body weight in volume (~40 Liters). When applying these calculations to the anthracyclines **14**, **15**, **16**, **18** and **21**, with an average MIC value of 4 μg/ml, a cumulative dosage of 460 mg would be necessary to inhibit bacterial growth in body fluids. Since these drugs exhibit lower cardiotoxicity, they may be used at higher cumulative concentrations than daunomycin

(90-360 mg/m^2). In the case of the indolocarbazole derivative **40**, with an average MIC value to 1 μg/ml, general estimates indicate that a cumulative concentration of 120 mg would be needed to inhibit bacterial growth. This estimate in particular seems very attractive when noticing that rebeccamycin is used at 16-128 mg/m^2/week for several weeks, with a maximum tolerated dosage (MTD) of 572 mg/m^2. However, since several clinical studies are normally done to discover the ideal dosage for antibiotics, this rough estimate should be looked at only as being preliminary.

In particular, *S. pyogenes* was observed to be more sensitive to most of the drugs tested. In the case of drugs **8**, **10-13**, **20** and **23**, *S. pyogenes* was the only one inhibited in the study. Generally, most of the drugs that contain a general inhibitory effect against all three gram-positive organisms also show anticancer activity as well. Due to the limited understanding of how anthracyclines and indolocarbazoles work in mammalian and bacterial systems, a structural reasoning for the similarities in activity can only be created through the testing of more drugs against both classes of organisms. Nevertheless, several drugs in this study (particularly drugs **14, 15, 16, 18, 21** and **40**) may prove to be studied further in the hopes of achieving more pharmaceutical agents that minimize nosocomial infections as well as inhibit cancer cell proliferation.

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