INFORMATION TO USERS

This manuscript has been reproduced from the microfilm master. UMI films the text directly from the original or copy submitted. Thus, some thesis and dissertation copies are in typewriter face, while others may be from any type of computer printer.

The quality of this reproduction is dependent upon the quality of the copy submitted. Broken or indistinct print, colored or poor quality illustrations and photographs, print bleedthrough, substandard margins, and improper alignment can adversely affect reproduction.

In the unlikely event that the author did not send UMI a complete manuscript and there are missing pages, these will be noted. Also, if unauthorized copyright material had to be removed, a note will indicate the deletion.

Oversize materials (e.g., maps, drawings, charts) are reproduced by sectioning the original, beginning at the upper left-hand corner and continuing from left to right in equal sections with small overlaps. Each original is also photographed in one exposure and is included in reduced form at the back of the book.

Photographs included in the original manuscript have been reproduced xerographically in this copy. Higher quality 6” x 9” black and white photographic prints are available for any photographs or illustrations appearing in this copy for an additional charge. Contact UMI directly to order.
STRUCTURE-ACTIVITY RELATIONSHIPS FOR MITOMYCIN C AND MITOMYCIN A ANALOGUES

by

Kenneth Robert Kunz

Copyright © Kenneth Robert Kunz

A Dissertation Submitted to the Faculty of the DEPARTMENT OF PHARMACEUTICAL SCIENCES In Partial Fulfillment of the Requirements For the Degree of DOCTOR OF PHILOSOPHY In the Graduate College THE UNIVERSITY OF ARIZONA

1996
As members of the Final Examination Committee, we certify that we have read the dissertation prepared by Kenneth R. Kunz entitled STRUCTURE-ACTIVITY RELATIONSHIPS FOR MITOMYCIN C AND MITOMYCIN A DERIVATIVES and recommend that it be accepted as fulfilling the dissertation requirement for the Degree of Doctor of Philosophy.

William A. Remers
Arnold R. Martin
David S. Alberts

Final approval and acceptance of this dissertation is contingent upon the candidate's submission of the final copy of the dissertation to the Graduate College.

I hereby certify that I have read this dissertation prepared under my direction and recommend that it be accepted as fulfilling the dissertation requirement.

Dissertation Director William A. Remers
STATEMENT BY AUTHOR

This dissertation has been submitted in partial fulfillment of requirements for an advanced degree at the University of Arizona and is deposited in the University Library to be made available to borrowers under rules of the Library.

Brief quotations from this dissertation are allowable without special permission, provided that accurate acknowledgment of source is made. Requests for permission for extended quotation from or reproduction of this manuscript in whole or in part may be granted by the copyright holder.

SIGNED: [Signature]
ACKNOWLEDGMENTS

I would like to extend my sincere appreciation to the Medical Research Council of Canada for a three-year postdoctoral fellowship award which provided the funding for this project.

I would like to thank professors Barry Blackburn and Harold Hutton at the University of Winnipeg for giving me the courage and support to pursue a career in pharmaceutical sciences as it applies to cancer research.

I extend my respect, admiration and gratitude to Dr. William A. Remers, my research supervisor. Dr. Remers' valuable insight was instrumental in bringing this project to completion. Special thanks are due to Drs. David S. Alberts and Robert Dorr of the Arizona Cancer Center, for welcoming me into their laboratories and providing encouragement and support on a personal level.

My laboratory co-workers, Drs. Bhashyam Iyengar and Salah M. Sami, are deserving of an appreciation beyond words, having attained a level of family to me.
TABLE OF CONTENTS

LIST OF ILLUSTRATIONS ................................................................. 7
ABSTRACT .......................................................................................... 8

CHAPTER

INTRODUCTION .................................................................................. 9
Background ....................................................................................... 9
Explanation of the Problem and it’s Context ................................. 9
Review of the Literature ................................................................. 12
Structure of the Mitosanes .............................................................. 12
Mode of Action ............................................................................... 13
Evidence for Bifunctional Alkylation of DNA ............................... 16
Molecular Modeling Studies ............................................................ 18
Effect of DNA Structure on Cross-Linking ................................... 20
Biological Consequences of DNA Cross-Linking ......................... 25
QSAR for Mitomycins ...................................................................... 27
Explanation of the Dissertation Format ......................................... 30
Strategy of the Present Research .................................................... 30

PRESENT STUDY ............................................................................ 37
Quantitative Structure-Activity Relationships ............................... 37
QSAR for Mitomycin C subsets ....................................................... 40
QSAR for the Mitomycin A Derivatives .......................................... 42
DNA binding energy parameter .................................................... 44
Summary of Results and Conclusions ........................................... 45
Appendix: J. Med. Chem. Title Page ............................................... 48
Statement of Permission to use Copyright Material ........................ 49
J. Med. Chem. Reprint Article .................. 50
List of References .................................. 56
LIST OF ILLUSTRATIONS

Figures

1a. Structure and Numbering of the Mitosanes .......... 10
1b. X-ray Crystallographic Structure of Mitomycin A ...... 10
2. Pathway For Mitomycin C Alkylation of DNA .......... 15
3. Bifunctional Mitomycin C-2-deoxyguanosine Adduct ...... 17
4. Stereopair for Mitomycin C-DNA Adduct ............... 21
5. Preferred DNA Base Pair Sequence for Mitomycin Interstrand Cross-Linking ....................... 22
6. Intrastrand Cross-Linked Adduct of Mitomycin With 5′ GG DNA Sequence .......................... 23
7. Preparation of Mitomycin C and Mitomycin A Analogs .... 35
ABSTRACT
A set of 30 mitomycin C and mitomycin A analogues, including 5 new compounds, was screened against 3 different solid human tumor cell lines using the MTT tetrazolium dye assay. A statistically significant correlation among antitumor activity, quinone reduction potential ($E^\ddagger$) and the logarithm of the partition coefficient ($\log P$) was obtained, with the most easily reduced and the most lipophilic compounds being the most potent. When these analogues were separated into mitomycin C and mitomycin A subsets, the former gave a correlation only with $E^\ddagger$, whereas the latter (which differ little in their $E^\ddagger$ values) gave a correlation only with $\log P$. These correlations are in contrast to those made in the P388 leukemia assay in mice wherein the most active mitomycin C and mitomycin A analogues were the most hydrophilic ones. When the same compounds were tested against P388 leukemia cells in the MTT assay, the results were the same as those of the solid tumor assays. Thus, the substantial differences in relative potencies of mitomycins are related not to the kind of tumor cell, but to the type of assay performed, cell culture versus whole animal.
No correlation was found between antitumor potency in the cell culture systems and calculated relative DNA binding strengths, probably because the limiting factors in antitumor potency of mitomycins appear to be tumor cell uptake ($\log P$) and/or bioreductive activation ($E^\ddagger$).
INTRODUCTION

Background

The mitomycins are a potent family of antitumor antibiotics which were isolated from the culture broth of *Streptomyces caespi tosus* in the late 1950's.\(^1\)\(^2\) Although this class of alkaloid contains a number of remarkable and active structures,\(^3\) mitomycins A and C (Figure 1a) are two compounds which have received the widest attention in recent years. Of these, mitomycin C is thought to have the best clinical utility because of its broad spectrum of anticancer activity and lower, albeit significant toxicity profile.\(^4\) Consequently, it has received the most intensive investigation and is the best biologically characterized compound of the series.

An Explanation of the Problem and its Context

Although mitomycin C is a very effective antitumor agent, the clinical use of this drug has been limited because of a relatively narrow therapeutic index in comparison to other cancer drugs. The major organ systems susceptible to damage by therapeutic doses of mitomycin include all three cell lines of the bone marrow (white cells, red cells and platelets), and the epithelial lining of the gastrointestinal tract.
Figure 1a. Structure and numbering of the mitosanes.

Mitomycin A  \( \text{CH}_3\text{O} \)
7-Alkoxymitosane \( \text{RO} \)
Mitomycin C \( \text{H}_2\text{N} \)
7-Aminomitosane \( \text{RHN} \)

Figure 1b.

Toxic hematologic manifestations of mitomycin therapy include neutropenia, which may predispose to severe or fatal infections, anemia leading to profound weakness, and thrombocytopenia, which may cause hemorrhagic complications. The gastrointestinal side effects include mucositis, nausea, vomiting, anorexia and diarrhea, all of which may contribute to malnutrition of the patient.

Therefore, considerable interest exists with regard to developing derivatives as clinically effective as mitomycin C, but with less systemic toxicity. To facilitate the search for new, active and hopefully less toxic compounds, quantitative structure-activity relationships (QSAR) for the mitomycins must be developed. This involves the study of how slight variations in the chemical structure of the mitomycins will correlate with changes in their spectrum of biologic activity.

Although numerous attempts have been made to establish structure-activity relationships for this class of drug, the results, up to the present study, have been generally inconclusive. It was therefore my desire to reevaluate which physicochemical properties of the mitomycins were important in determining biologic activity, and how these might be varied in order to develop promising new drugs for the clinic.
REVIEW OF THE LITERATURE

Structure of the Mitosanes

Both mitomycin A and C contain the basic azirino(2',3':3,4)pyrrolo(1,2-a)indoloquinone nucleus with a carbamoyloxymethyl substituent at the C(9) position (Figure 1a). The trivial name 'Mitosane' has been assigned for this structure along with the numbering system indicated in Figure 1a. When the C(9a) methoxy group is lost with subsequent formation of a C(9) - C(9a) double bond, the structure is referred to as an aziridinomitosene (see Figure 2). Derivatives of mitomycins A and C with varying substituents at the C(7) position are referred to as 7-alkoxy and 7-aminomitosanes respectively. Because of the very potent antitumor activity but high toxicity of the mitosanes, medicinal chemists have prepared hundreds of semisynthetic analogues over the years with the hope of obtaining new clinical agents with improved therapeutic ratios.

Closer examination of the structure of the mitosanes reveals the presence of three key functional groups which act collectively to produce an efficient bifunctional alkylating agent (Figure 1a). These groups include a quinone, a carbamate side chain, and a highly strained aziridine ring. The aziridine and the carbamate substituents are essential for
good antitumor activity since they are individually capable of alkylation and together can significantly damage DNA through covalent cross-linking. The quinone ring, while controlling the reactivity of the aziridine and carbamate groups, is believed to undergo redox cycling to generate reactive superoxide and hydroxyl radicals that cause DNA strand scission.

In the quinone oxidation state, the mitosanes are relatively inactive alkylating agents. This is because the powerful electron-withdrawing effect of the quinone ring delocalizes the electron density of the indole and aziridine nitrogens by overlap of their p orbitals. Consequently, the molecule is held in an inactive, bent shape (Figure 1b). However, upon reduction of the quinone to the corresponding hydroquinone (Figure 2), electron density in the pyrrolo(1,2a)indole system is greatly enhanced. This leads to structural changes and flattening of the molecule which renders the aziridine and carbamate moieties highly reactive. When the reduction and subsequent trapping of nucleophiles is mediated by enzymes in a biological system, the process is referred to as "bioreductive alkylation."

Mode of Action

Iyer and Szybalski were the first to postulate that after
mitomycin is reduced, the C(1) and C(10) positions may be capable of covalently cross-linking DNA. The mechanism was later refined by Moore, who suggested that displacement of the aziridine and carbamate functionalities occurred by $S_{N1}$ type steps to generate reactive intermediates which could then alkylate DNA (Figure 2).

Although the mitosane quinone ring can be reduced in either a one- or two-electron process, reduction by biological systems such as xanthine oxidase or NADPH-cytochrome C P-450 reductase have been shown to involve an initial one-electron transfer. This process generates a reactive semiquinone radical intermediate which is thought to disproportionate to yield a hydroquinone (Figure 2) and mitosane. Assisted by resonance from the pyrrolo(1,2a)indole system, the hydroquinone can lose a mole of methanol from the C(9a) position to form the corresponding aziridinomitosene. A series of prototropic shifts with subsequent aziridine ring opening is thought to generate a reactive quinone methide. The latter species can undergo nucleophilic attack at C(1) by DNA to give a monofunctional adduct. Loss of the C(10) carbamate with generation of an iminium ion intermediate can provide a second site for DNA alkylation. When this occurs with a nucleophilic group on the complementary DNA strand, a bifunctional, cross-linked adduct is formed (Figure 2). Once covalently bound, the hydroquinone can be reoxidized to the semiquinone or
Figure 2: Proposed pathway for mitomycin C alkylation of DNA.
Evidence for Bifunctional Alkylation of DNA

The first experiments to characterize the alkylation products of mitomycin C with DNA revealed that the adducts were predominantly of a monofunctional nature. These studies involved either enzymatic reduction or catalytic hydrogenation of mitomycin in the presence of calf thymus DNA. After hydrolysis of the alkylated DNA, the major product (95%) was found to be mitomycin covalently linked through its C(1) position to the exocyclic 2-amino group of guanine, affording a monofunctional adduct. This result was somewhat perplexing, as previous studies had revealed altered macromolecular properties of the mitomycin-treated DNA which were strongly suggestive of covalent cross-linking.

In an attempt to isolate and characterize a bifunctional, cross-linked adduct, Tomasz and co-workers tried a new approach by reducing the mitomycin with sodium dithionite (Na$_2$S$_2$O$_4$) in the presence DNA isolated from the microorganism Micrococcus luteus. The investigators chose M. luteus DNA because of this bacterium's high deoxyguanylate-cytidylate content, which accounts for approximately 72% of the base pairs. The bound DNA was hydrolyzed with a mixture of nucleases and the major product (70%) was characterized by $^1$H-
Figure 3. Bifunctional mitomycin C-2-deoxyguanosine adduct.
NMR, FT-IR, FAB mass spectroscopy, UV and circular dichroism spectroscopy. The product was a bisadduct covalently linked through the predicted C(1) and C(10) positions of mitomycin and the exocyclic 2-amino groups of two deoxyguanosine residues (Figure 3). This structure has since been verified by a number of studies, including 2-D homo- and heteronuclear NMR, in combination with energy minimized computational analysis. The bifunctional adduct provided final evidence that mitomycin could cross-link DNA and confirmed the original hypothesis of Iyer and Szybalski.

Molecular Modeling Studies

Before the bifunctional mitomycin C-2-deoxyguanosine adduct was identified, molecular mechanics simulations were used in an attempt to predict the mode of binding of mitomycins to double stranded DNA. In the first study, interactions between reduced mitomycin and the decanucleotide GC10 were examined. The model predicted low-energy, noncovalent complexes with the drug which suitably oriented the C(1) and C(10) atoms to form covalent cross-linkages with either the O(6) atoms of guanines in the major groove or the N(2) atoms of guanines in the minor groove. The energy calculations of this study seemed to suggest that binding and subsequent cross-linking of mitomycin in the major groove of DNA might be favored over the minor groove.
However, when the mitomycin C-2-deoxyguanosine bisadduct (Figure 3) was isolated by Tomasz, the molecular mechanics simulations were reexamined with the emphasis on mitomycin and its analogs alkylating DNA at the 2-amino groups of adjacent guanines in the minor groove.\textsuperscript{28,29} In one study, Remers used the model to demonstrate the excellent fits and strong binding affinities of four highly potent mitomycins with duplex DNA.\textsuperscript{28} As an additional and unexpected surprise, this work revealed an "apparent" qualitative correlation between the predicted binding energies of the drugs and their biological activity against P388 leukemia in mice. Although the authors stressed that there were too few compounds to substantiate a firm correlation, they suggested that the computer predicted DNA binding energies of mitomycin analogues may provide a valuable new parameter to help guide the preparation of more active analogues. They recommended that a more extensive investigation be undertaken to compare antitumor activities with the new DNA binding energies in addition to other well known drug parameters such as lipophilicity and quinone reduction potential.

Interestingly, Remers also used molecular modeling studies to predict that a specific DNA nucleotide sequence might favorably influence mitomycin-DNA binding.\textsuperscript{28} The energy calculations seemed to suggest that there was one best fit for mitomycins in the minor groove of DNA and that the most
favored sequence might be 5'CGT segment (Figures 4, 5). This theory was based on the observation that once C(1) of mitomycin had alkylated the first guanine (5'CGT), its carbamate carbonyl was close enough to form a strong hydrogen bond with the amino group of the adjacent guanine on the complementary strand (Figure 4). The required adjacent thymine gave further stabilization to the complex by providing a hydrogen bond between its carbonyl oxygen and the protonated amine at C(2) of mitomycin (Figure 4). Recent experiments concerning mitomycin-DNA sequence specificity have verified these predictions and are detailed in the following section.\textsuperscript{24,30,31}

**Effect of Local DNA Structure On Mitomycin Cross-Linking**

Once it had been established that mitomycin could cross-link adjacent deoxyguanosines of duplex DNA, investigators thought to determine what role the surrounding DNA structure might play on the bonding process.

Experiments using synthetic DNA oligomers have clearly shown that mitomycin recognizes and alkylates specific base pair sequences.\textsuperscript{24,30,31} The drug predominantly causes intermolecular cross-links with 5'CG sequences (Figure 5) and has no affinity whatever for 5'GC sequences. Further, runs of alternating 5'(CG)\textsubscript{n} sequences are even more conducive to the cross-linking
Figure 4. Stereo pair for the C(1) monolinked complex of mitomycin C in the minor groove of duplex DNA. Taken from *J. Med. Chem.* 1988, 31, 1616.
Figure 5.

Preferred base pair sequence for mitomycin interstrand cross-linking of DNA.
Intrastrand cross-linked adduct of mitomycin with 5' GG DNA sequence.
Recently, Tomasz and co-workers reported the isolation and structure of an intrastrand cross-linked adduct of mitomycin C with 5'GG sequences (Figure 6). The latter product however, is approximately 3.6-fold less favored than the 5'CG interstrand cross-linked adduct.32

The mitomycin-DNA sequence specificity concept was further developed to show that when the 5'CG guanine was followed by a pyrimidine on its 3' side, such as in the nucleotide sequence 5'CGThymine (Figure 5), the locus was 2-fold more susceptible to cross-linking.24 This is in agreement with the predictions made earlier by Remers and associates using molecular modeling studies.28 To explain this sequence specificity, Tomasz and co-workers reasoned that the negative dipole of the thymine O(2) enhances the nucleophilicity of the neighboring guanine amino group,24 presumably facilitating the latter's alkylation by C(1) of mitomycin.

Li and Kohn 31 also recognized the importance of the 5'CGPyrimidine sequences, and were the first investigators to introduce the concept that 5'GG pairs also appeared to be alkylated. In addition, they presented a hypothesis for the 5'CG specificity. The theory requires the formation of a key hydrogen bond between the C(8) hydroxyl group of the mitomycin hydroquinone and the N(3) of the guanine on the complementary (3'GC) strand, prior to the C(1) alkylation event. Teng and
co-workers also observed the absolute requirement for 5'CG sequences, and used molecular modeling to explain the phenomenon in terms of the minor groove geometry of the exocyclic guanine amino groups. In the less favored 5'GC sequence, they showed that this distance is substantially greater than that required for bifunctional covalent bond formation.

A number of regulatory proteins (histones, topoisomerases, etc.) are known to associate with DNA and induce significant bends and distortions upon the double helix. Such interactions might be expected to effect the way in which mitomycins interact with DNA. To show this, Cera and Crothers complexed the transcription regulating CAP protein with Escherichia coli DNA and found that the conformation of the minor groove was significantly altered. Widening of the minor groove reduced mitomycin cross-linking efficiency by 25%, while narrowing decreased it 4-fold. The authors reasoned that changes in the distance between the two exocyclic guanine amino groups accounted for these variations. They concluded that local DNA structure plays a critical role in the efficiency of the mitomycin cross-linking process.

**Biological Consequences of Mitomycin-DNA Cross-linking**

At least two different processes are thought to be responsible
for the extreme cytotoxicity of the mitomycins: alkylation of DNA and the generation of chemically reactive superoxide and hydroxyl free radicals.³

Alkylation with cross-linking of nucleic acids is thought to have serious consequences on cellular metabolism. The loss of DNA integrity and template function essentially prevents DNA replication and cell division.³ Transcription is also inhibited, and the resulting loss of RNA and subsequent protein synthesis contributes to cell death.³⁴ Moreover, other macromolecules such as polyribonucleotides,³⁵ proteins and polysaccharides are also believed to be alkylated, although to a lesser extent than DNA.³⁶ These processes are thought to be enhanced by the greater reducing power and lower pH of tumor cells, which may be relatively hypoxic and acidotic secondary to accelerated glycolysis.³

Once the mitomycin hydroquinone is covalently bound to DNA, it can react with oxygen in the presence of metal ions to generate the semiquinone radical and a superoxide radical.³⁹ The superoxide radical is believed to disproportionate to hydrogen peroxide and oxygen and further react with the peroxide to give hydroxyl radicals.⁹ If these reactive species are liberated in the vicinity of DNA, they can lead to DNA strand scission. This cascade has been shown to be inhibited by catalase, superoxide dismutase, radical scavengers and
sequestering agents.\textsuperscript{37}

\textbf{Structure-Activity Relationships For Mitomycins}

The clinical importance of mitomycin C as an anticancer drug has stimulated medicinal chemists to prepare many hundreds of derivatives.\textsuperscript{3,6} Because of the extreme bone marrow toxicity of the drug, the goal of this research has been to develop new compounds as active as mitomycin C but which cause less myelosuppression.\textsuperscript{38} This work has resulted in the accumulation of an extensive data base of antitumor activity, predominantly against the \textit{in vivo} P388 murine leukemia model. In this test system, leukemia cells are inoculated into the peritoneal cavity of mice, followed by varying concentrations of the mitomycin analogue to be studied. The antitumor activity of a particular compound is determined by the average length of time a group of treated animals survives beyond the average life span of the untreated controls.

There have been several attempts to develop structure-activity relationships for the mitomycins using the accumulated \textit{in vivo} biological data.\textsuperscript{39-44,46-49} As basic requirements, the quinone, carbamate, and aziridine substituents have all been shown to be essential for good antitumor activity. Furthermore, reduction of the quinone ring to the corresponding hydroquinone is necessary before the drug can cross-link
Prior to the present study however, most attempts to correlate mitomycin structure with biological activity had generally yielded only rough qualitative trends. For example, one of the first studies suggested that the quinone ring reduction potential ($E_{1/2}$) might be an important determinant in the activity of a particular compound.\textsuperscript{40} Lowering of the quinone ring reduction potential was believed to facilitate cellular bioactivation of the molecule, thus conferring greater potency on the analogue. This concept has been supported by a number of recent studies.\textsuperscript{39,46,49} However, although compounds with low reduction potentials were generally found to be more active, no quantitative correlations between $E_{1/2}$ and cytotoxicity had been established prior to the present work.

On the other hand, some studies have indicated a rough trend between antileukemic activity and the hydrophilicity of a compound, with the most water soluble derivatives showing the greatest efficacy.\textsuperscript{47,48} Concerning this latter phenomenon, there has been one statistically significant correlation ($R^2=0.85$) reported involving lipophilicity ($\log P$) and antitumor activity.\textsuperscript{42} This study showed that for a set of $N^7$-phenyl substituted mitomycin C derivatives tested against P388 murine leukemia \textit{in vivo}, the most active analogues were the most hydrophilic ones. In contrast to this discovery, when a large
series of mitomycin A analogues were screened against the same tumor model using the same parameter, log P, no statistically significant correlation could be obtained.\textsuperscript{43,44} This result created confusion concerning the value of log P in determining the antitumor activity of a particular compound. However, it has subsequently been shown that the log P values used in the mitomycin A experiment were inaccurate and that such a correlation does indeed exist.\textsuperscript{50}

In summary, despite the extensive amount of work that had been done to relate the physicochemical properties of the mitomycins with their antitumor activities, the correlations had been either too weak or outright conflicting. Until the present study, QSAR for these interesting and important compounds had remained largely undefined.
EXPLANATION OF THE DISSERTATION FORMAT

Strategy of the Present Research

As outlined in the *Journal of Medicinal Chemistry* article that follows, my purpose in conducting this project was to search for mitomycin derivatives of greater clinical utility by means of establishing quantitative structure-activity relationships (QSAR) for this class of drug.

To begin with, I desired a more convenient and reproducible biological system for testing new drugs than the *in vivo* P388 murine leukemia model. The latter assay caused problems because of considerable costs, length of time required to generate biologic activity, and variation in survival times of mice from experiment to experiment.

To screen our compounds, I decided to use an *in vitro* panel of cultured tumor cell lines, which is a method used by the National Cancer Institute (NCI).\(^1\) Referred to as the MTT assay,\(^2\) this method can give rapid, precise, and reproducible biologic activity values for compounds, and this data can subsequently be used in the development of quantitative structure-activity relationships.

The MTT assay is a colorimetric study of the mitochondrial
respiratory chain enzymes of living, metabolically active human tumor cells. The respiratory enzymes cleave the colorless substrate MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) to a highly colored formazan dye. This dye can be rapidly and quantitatively read on a scanning multiwell spectrophotometer with the absorbance proportional to the number of living cells at completion of the assay. The biologic activity of a compound is then expressed as an IC₅₀ value (nanomolar for the mitomycins), which is the concentration of a drug that will inhibit growth of the tumor cells by 50%. The length of time required to generate biological data for a drug is only seven days, and multiple compounds can be screened at several different concentrations in a single experiment.

In order for me to use this screening method, it was necessary to become familiar with all aspects of tissue culture technique. I selected three different human tumor cell lines and one mouse leukemia cell line to screen my compounds against. The human tumor cell types included breast, colon and ovarian cancer cells. Three different human tissue types as well as one mouse cell line were decided upon because I wanted to see if the mitomycins showed selective activity against any particular type of cancer cell, or whether they killed all varieties of cancer cells in a roughly equal pattern.
In addition to a new screening assay, I thought to investigate a new physicochemical parameter: the computer predicted binding energies of mitomycin analogues to DNA, as recently developed by Kollman, Remers and co-workers.\textsuperscript{26-29} The introduction of the computer generated mitomycin-DNA binding energies provided an encouraging new parameter to be used in the development of mitomycin QSAR. This was because of the previously unsuccessful attempts using more standard parameters such as the quinone ring reduction potential ($E_{1/2}$) and the lipophilicity (log P) of the mitomycin derivatives. Moreover, the preliminary molecular modeling work done by Remers et al. had suggested that there may be a correlation between the predicted mitomycin-DNA binding energies and the antitumor activity against P388 leukemia in mice.\textsuperscript{28} The authors of this investigation suggested that it might be worthwhile to include the DNA binding energies as a new parameter, along with other physicochemical properties such as log P and $E_{1/2}$ in a multiple linear regression study to generate mitomycin QSAR.\textsuperscript{27,28}

In order for me to use the new binding energy parameter, it was necessary to become familiar with the molecular mechanics program of AMBER\textsuperscript{55} as a means of calculating the DNA binding energies for both known and new, as yet unprepared compounds. This began with constructing the desired mitomycin C(7) substituent using CHEMLAB II, docking the new substituent at
the C(7) position of the mitosene-DNA complex using MIDAS\textsuperscript{56}, then and bringing the resulting structures to minimum energy conformation using the AMBER program.

For example, one compound that I designed using this method was N\textsuperscript{7}-(4-hydroxy-3-nitrophenyl) mitomycin C (see compound no. 16, table II, p.56). As a result of the favorable energy interactions of the new 4-hydroxy-3-nitrophenyl substituent, the calculated DNA binding energy of this compound was much higher than that of the parent compound mitomycin C, -137.7 kcal/mol as compared to -115.3 kcal/mol, respectively. Therefore the new compound might be expected to have greater biologic activity than the parent compound, and it was synthesized and subsequently screened against the cancer cell lines for evaluation.

Besides the use of a new screening assay, and the introduction of the novel mitomycin-DNA binding energy parameter, we had access to more accurate Log P estimations for the mitomycins than used in previous QSAR studies. This was because of the availability of a new computer program called "Leo’s CLOGP", which, with reasonable accuracy, can estimate the partition coefficient of a compound of potential interest.\textsuperscript{54} As outlined in the work to follow, these more accurate lipophilicity values, which were calculated by Dr. William Remers, were absolutely essential to our successful development of
structure-activity relationships for the mitomycins.

Finally, in our new attempt at developing mitomycin QSAR, I chose to prepare and screen a carefully selected series of C(7)-substituted alkoxy- and aminomitosanes (Figure 1a). The C(7) position of the quinone ring, besides being very susceptible to substitution reactions, is an important position because it can influence a number of physicochemical properties of the molecule. These include the quinone ring reduction potential (E_{1/2}), the lipophilicity (log P) and, theoretically, the DNA binding energy (Kcal/mole) of a particular compound.\textsuperscript{28,39} The functional groups selected for substitution at this position of the quinone ring were chosen to provide wide ranges of each of these physicochemical parameters (table II, p.56).

Of the thirty compounds chosen for use in this study, twenty-five were previously reported. However, it was necessary for me to synthesize, purify and confirm the structures of many of these again, prior to testing their biologic activity in the MTT assay. Five of the compounds were new, and were prepared according to the method of Iyengar et al.\textsuperscript{39}

In general, the synthesis of either a mitomycin A or C derivative began with the commercially available mitomycin C as a starting material (figure 7). The C(7) amino group is
Figure 7. Preparation of mitomycin C and mitomycin A derivatives.
converted to a hydroxyl group by hydrolysis with dilute sodium hydroxide, then careful adjustment to pH 4 with sulfuric acid. The C(7) hydroxyl group is then alkylated with diazomethane to produce mitomycin A, which can then be converted to the desired mitomycin A or C derivative (figure 7). The conversion to mitomycin A or C derivatives is facile because the C(7) methoxide, which is attached to a conjugated carbonyl system in the quinone ring, is a very good leaving group, and can readily be displaced by attack from a good nucleophile.

In the case of mitomycin C analogs, a methanolic solution of mitomycin A is stirred with an excess of the desired amine, which displaces the C(7) methoxy group in a three step addition-elimination reaction. Strong nucleophiles react quickly, but poorly nucleophilic amines, such as those containing an aromatic substituent, require a longer time to displace the C(7) methoxy group.

To prepare mitomycin A derivatives, mitomycin A can be dissolved in the alcohol corresponding to the desired new alkoxide. Addition of a small amount of potassium hydroxide will generate the desired alkoxide from the solvent, which can displace the C(7) methoxy group to afford the required C(7)-alkoxymitosane (figure 7).
PRESENT STUDY

The detailed methods, results, and conclusions of this study are presented in the *Journal of Medicinal Chemistry* reprint appended to this dissertation. The following is a summary of the most important findings in this publication.

**Quantitative Structure-Activity Relationships**

Quantitative structure-activity relationships were successfully established for a set of thirty mitomycin antitumor antibiotics. Nineteen mitomycin C derivatives, five of which were new, and eleven mitomycin A derivatives were prepared and screened against a panel of four cultured tumor cell lines using the MTT tetrazolium dye assay. Three of the cultured cell lines were solid human tumors and consisted of WiDr colon, MCF-7 breast and 2780 ovarian cancer. The remaining tumor type was the P388 murine leukemia cell line.

Three mitomycin physicochemical properties were investigated. These included the logarithm of the partition coefficient (log P), the quinone ring reduction potential ($E_{1/2}$), and the net binding energies of each analog to the DNA segment GC10 (kcal/mol). These properties were selected according to the sequents of events which could occur as mitomycin attacks a tumor cell. Initially, the partition coefficient (log P) may
be important in determining whether an analog will be able to enter the cell by crossing the plasma membrane. The quinone ring reduction potential (E_{1/2}) may then determine the degree of difficulty with which intracellular enzymes can bioactivate the drug. Finally, the net binding enthalpy (kcal/mol) of the analog could determine the relative energy with which it will alkylate DNA.

In terms of antitumor activity, all three physicochemical properties would be essential for a mitomycin analog to function effectively. However, for quantitative structure-activity relationships, each one of these three properties may not turn out to be statistically significant. As an example, an analog might easily penetrate the cell membrane and have a high DNA binding energy, but if its reduction potential is too high and bioactivation is difficult, it could turn out that E_{1/2} will be the only significant QSAR parameter.

The physicochemical properties and the antitumor activities of the mitomycins screened against the human tumor cell lines are contained in table II, p.56. The antitumor potencies are expressed as IC_{50} (nM) values for each of the three cell lines. The three IC_{50} values for each analog were then averaged, and the logarithm of the reciprocal, log(1/C), was obtained for use as the dependent variable in multiple linear regression analysis. The independent variables consisted of Log P, E_{1/2},
and the net DNA binding energies in kcal/mol. The IC50 (nM) and log (1/C) values for the same compounds screened against the P388 mouse leukemia cell line are listed in table IV, p.57.

A preliminary inspection of the IC50 values in tables II and IV will reveal that a particular mitomycin analog has roughly equal potency against all four cell lines, suggesting that none of the derivatives is selectively toxic to any particular tumor type, whether mouse or human in origin.

When multiple linear regression analysis was performed with all thirty compounds tested against the human tumor cells, a statistically significant correlation among antitumor activity, partition coefficient and reduction potential was obtained, affording the regression equation:

\[
\log (1/C) = 10.1 + 6.59E_{1/2} + 0.35\log P
\]

\[N=30, R=0.83, R^2=0.69, S=0.46, F=30.3, \text{sig} \ F=0.000\]

This equation relates that the most easily reduced and the most lipophilic compounds were the most potent. More specifically, the quinone ring reduction potential \(E_{1/2}\) accounts for 48% of the variance in the biological data, and the lipophilicity \(\log P\) accounts for another 21%.
When the same compounds were tested against the P388 murine leukemia cell line, the results were essentially the same. The regression equation was:

\[ \log \left( \frac{1}{C} \right) = 10.84 + 8.63E_{1/2} + 0.32 \log P \]

\[ N=27, \ R=0.87, \ R^2=0.76, \ S=0.46, \ F=36.2, \ \text{sig} \ F=0.0000 \]

As with the human tumor cells, the most easily reduced and most lipophilic compounds had the highest activity, and these qualities collectively accounted for about 76% of the variance in the biological data.

**QSAR for Mitomycin C Subsets**

Next, the compounds were divided into subsets consisting of mitomycin C (compounds 1-19, p.56) and mitomycin A derivatives (compounds 20-30, p.56). Using data from the human tumor cells, the mitomycin C derivatives were subjected to regression analysis with \( E_{1/2} \) and \( \log P \) as the independent variables. The only statistically significant parameter for the mitomycin C analogs was the quinone ring reduction potential, giving the regression equation:

\[ \log(1/C) = 11.5 + 9.45E_{1/2} \]

\[ N=19, \ R=0.73, \ R^2=0.53, \ S=0.48, \ F=19.4, \ \text{sig} \ F=0.0004 \]
The relationship between reduction potential and potency became even more apparent when the mitomycin C analogs were further subdivided into the monosubstituted N'-phenylmitosanes (compounds 7-14). In this instance, the sigma values of the phenyl ring substituents, which essentially determine the $E_{1/2}$ for a particular compound, gave the following equation:

$$\log(1/C) = 8.46 + 0.92\text{sigma}$$

$N=8$, $R=0.89$, $R^2=0.80$, $S=0.22$, $F=23.4$, $\text{sig } F=0.0029$

As anticipated, phenyl substituents with the greatest electron-withdrawing ability afforded the most potent compounds.

When the mitomycin C and the N'-phenylmitomycin C subsets were tested against the P388 murine cell line, the correlations paralleled those of the human tumor cells, with the only significant parameters being the $E1/2$ and sigma values, respectively (see equations on p. 2284).

The fact that the reduction potential was the only statistically significant parameter for mitomycin C analogs in the cell culture system was surprising to us. This was because a previous study by Sami et al.\textsuperscript{42} showed that for a set of N'-phenylmitomycin C derivatives tested against P388 murine leukemia \textit{in vivo}, the only statistically significant
correlation obtained was between the antileukemic activity and the lipophilicity. In that study, the most hydrophilic compounds, as determined by the hydrophobic fragment constants of the phenyl ring substituents, had the greatest biologic activity, $R^2=0.85$.

The very different results obtained when the same compounds are screened in the cultured tumor cells versus the live animal model are concerning because of the potential dilemma encountered when new agents are to be selected for use in clinical trials. Evidently, the biology of the cultured tumor cells and live animal tumor models is substantially different.

**QSAR for the Mitomycin A Derivatives**

Regression analysis was also performed independently on data from the mitomycin A analogs when screened against the tumor cells. It must be emphasized that the reduction potentials for the mitomycin A compounds (no.s 20-30, table II, p.56) remain essentially constant because they are all C(7)-alkoxymitosanes. A statistically significant correlation between the partition coefficient and antitumor activity against the human tumor cells was obtained:

$$\log(1/C) = 8.58 + 0.44 \log P$$
The equation reveals that the most lipophilic mitomycin A analogs had the greatest activity. The same correlation was obtained when the mitomycin A subset was tested against the P388 murine leukemia cells (p.57). Again, the most lipophilic derivatives were the most biologically active.

In two earlier studies from our laboratory, it was reported that there was no statistically significant correlation between the lipophilicity of mitomycin A analogs and biologic activity in the P388 mouse leukemia assay.\textsuperscript{43,44} For the present study, we had access to more accurate log P estimations,\textsuperscript{54} and these were used to reevaluate the biologic activity generated from the previous in vivo P388 mouse leukemia work (table III, p.57). This time, a statistically significant correlation was obtained which indicated that the least lipophilic compounds had the best activity:

\[
\log(1/C) = 7.07 - 0.56 \log P
\]

\(N=11, R=0.84, R^2=0.71, S=0.50, F=22.0, \text{sig } F= 0.0011\)

Thus, the results from the live animal model are the opposite from those obtained from the cultured tumor cells. As in the case of the mitomycin C derivatives above, this could present a dilemma if agents are to be chosen for further study in
DNA Binding Energy Parameter

Due to the extensive time and computer costs required, the net DNA binding energies to GC10 were calculated for only fourteen of the compounds in table II, (no.s 1-6, 12, 13 16, 17, 20, 25, 27, and 28). Ten of these were mitomycin C derivatives and four were mitomycin A derivatives. When this set was tested against the human tumor cells and the results subjected to multiple linear regression analysis with Log P, $E_{1/2}$, and the net DNA binding energies as the independent variables, no statistically significant correlation was obtained with any variable. Although there is no obvious relationship between DNA binding energies and antitumor activity, this is not surprising in retrospect. The binding energies for all of the compounds are high enough to suggest that once reduced and inside the cell, a compound's ability to alkylate DNA is not a limiting factor to its cytotoxicity.
Summary of Results and Conclusions

Statistically significant correlations were obtained among the antitumor activity, lipophilicity \( (\log P) \), and quinone reduction potential \( (E_{1/2}) \) for a series of mitomycin C and mitomycin A analogs when screened against four cultured tumor cell lines. Mitomycin C derivatives with the lowest reduction potential were found to the most active, while mitomycin A derivatives with the highest lipophilicity had the best activity. No correlation between biologic activity and the calculated DNA binding energies was apparent.

In the cultured tumor cell lines, it might be inferred that the quinone ring reduction potential is the overall most important parameter in determining biologic activity. For example, the set of mitomycin C analogs studied here had wide variations in both their reduction potentials and their lipophilicities. Cell penetration however, was not a limiting factor to a particular compound's activity because only the ease of reduction was of statistical significance. In the case of the mitomycin A derivatives, the reduction potentials were nearly all the same, making this parameter essentially a constant and removing it from statistical significance. Therefore, the lipophilicity was allowed to emerge secondarily as the dominant factor in determining activity for a compound. As mentioned earlier, the DNA binding energy parameter was not
statistically significant in this study probably because the energies are so favorably high for all the compounds that their ability to alkylate DNA was not a limiting factor to their cytotoxicity.

In contrast to the QSAR results from cell culture assays, only the lipophilicity was important in the P388 leukemia assay in mice, where the most hydrophilic compounds had the greatest activity. In the in vivo P388 murine leukemia model, the tumor cells are suspended in an aqueous environment confined to the abdominal cavity. It could be that when the drugs are administered intraperitoneally, the most lipophilic will disappear into the tissues while the most hydrophilic remain in the peritoneal fluid. If this were true, then the most hydrophilic compounds would gain longer access to the tumor cells and would ultimately have the best activity in this system.

The surprising differences in mitomycin QSAR between cultured tumor cells and the in vivo P388 leukemia model in mice pose interesting questions in terms of drug discovery programs. For example, if a number of compounds are tested in cell culture systems, and the most active are selected for further study in animal models, it could happen that the best compounds would be overlooked. Investigators therefore must be aware of the inherent differences in the biology of the
screening systems in order to develop effective drug development programs.
APPENDIX

JOURNAL OF MEDICINAL CHEMISTRY®
Registered in U.S. Patent and Trademark Office
© Copyright 1991 by the American Chemical Society

Volume 34, Number 7
July 1991

PERSPECTIVE

1935 Design of Enzyme Inhibitors Using Iterative Protein Crystallographic Analysis
Krzysztof A. Aspeli, Russell J. Basureau, Charlette A. Bartlett, Carol L. J. Becht, Stephan T. Freer,
Mary Ann F. Fohy, Michael R. Gehring, Steve M. Herrmann, Elesser F. Hewland, Cheryl A. James,
Teresa L. Janes, Chen-Chen Kan, Vitali Kastrukov, Kathleen K. Lewis, Gifford P. Marra,
David A. Mathews, Christopher Meyer, Ellen W. Moesch, Catharina A. Morrow, Stuart J. Oatley,
Richard C. Ogden, Maryvale Rami Reddy, Siegfried H. Reigh, Warren S. Schecter, Ward W. Smith,
Michael D. Varney, J. Ernest Villarreal, Robert W. Ward, Stephanie Webber, Stephen E. Webber,
Katharine M. Welch, and Jennifer White

ARTICLES

1935 Rasin Inhibitors Containing Esters at the P, Position. Oral Activity in a Derivative of Methyl Aminomalonic Esters
Joseph T. Ramos, Richard J. Himmelstein, John C. Rodot, James S. Kalleshrens, Ilia Bivane,
Richard W. Skoar, Sean T. Brownian, Timothy R. Hurley, Elizabeth Lussey, Christian C. Humbert,
Ronald E. Welsch, Stephen Elamunio, Michael J. Rynse, David G. Taylor, Jr., Stephen C. Glesa,
Barbara M. Michelenow, Brian E. Kunkin, Daniel T. Reimer, and Michael D. Taylor*

1944 Structural Studies on Tambelast
Carl A. Toone, Carl H. Schwaib, Neil S. Ringham, Peter A. Lambert, Philip R. Lowe, and Ving J. Lee

1947 Additional Nucleotide Derivatives of Milliconce. Synthesis and Activity against Parental and Multidrug
Rapidant li210 Leukoma
Bhaskar S. Iyengar, Robert T. Dott, and William A. Remmers*

1951 Synthetic Approaches to the Guanacine and Xanthines Analogue
3-Amino-3-D-ribofuransopyrimidoino[3,4-e][1,3]oxan-7-one and 3-3-D-ribofuransopyrazinono[3,4-e][1,3]oxan-7,7-dione
and Studies of Their Antimotor Potential
Raiming Zou, Vladimir G. Bayling, Michael P. Grewink, Linda L. Wering, and Leroy B. Townsend*

1952 Synthesis and Biological Evaluation of Quainacrin Derivatives: Thiophenyl-Substituted Guanines and Hydroxyguanine
Hiromasa Saito, Tadashi Hijata, Masaji Inaka, Katsuhisa Fumitsuka, Tadashi Ashizawa, Makoto Marima, and Akira Sato*

1952 Persistent Binding of Fatty Acyl Derivatives of Naltrexone to Opioid Receptors
P. S. Portoghese, A. Garson-Aburze, and D. L. Larsen

1959 Lipopeptides Containing 2-(Palmitoylamino)-6,7-bis(palmitoyloxy)heptanoic Acid: Synthesis, Stereo-specific Stimulation of L-Lymphocytes and Macrophages, and Adherence in Vivo and in Vitro
Jörg Mutzger, Gisbert Jung, Wolfgang G. Becker, Petra Hoffmann, Marianne Strecker,
Albrecht Lieberknecht, and Ulrich Schmidt

Reday C. Sokur,* Randall J. Gallekauk, David E. Straight, Martin Griesen, Donald Z. Glesa,
Peter Goodwin, Patricia A. McNiff, Austin F. J. Flitt, F. Michael Mungan, Thank H. Glesa, and
Vihanet A. Pelleck
DATE: January 2, 1996

MEMORANDUM

TO: Kenneth R. Kunz, M.D., University of Arizona
   Health Sciences Center
   Department of Pharmacy Practice
   Tucson, Arizona 85721

FROM: C. Arleen Courtney
      Copyright Assistant
      (202) 872-4368

RE: Your letter dated October 17, 1995

Thank you for your recent letter, regarding your request for permission to include your paper(s), per your attached letter, in your thesis. Please note the following:

* If your paper has already been published by ACS, I would be happy to grant you this permission royalty free provided that you print the required ACS copyright credit line on the first page of your article: "Reprinted with permission from FULL REFERENCE CITATION. Copyright YEAR American Chemical Society."

* If you plan to submit your thesis to UMI, please inform them that permission to include your already published ACS article as part of your thesis is granted for paper and microform copies only; the ACS copyright notice (see above) must appear on the first page of the ACS article.

* If your paper has not already been published by ACS, you may include it in your thesis provided that you print the following ACS copyright credit line on the first page of your article: "Reprinted with permission from JOURNAL NAME, in press. Unpublished work copyright CURRENT YEAR American Chemical Society." You may NOT include the ACS paper in the version that you submit to UMI until ACS has published your paper.

* Other:

Thank you for writing. If you have any questions, please call me at 202/872-4368.
Structure–Activity Relationships for Mitomycin C and Mitomycin A Analogues

Kenneth R. Kunz,1 Bhashyam S. Iyengar,1 Robert T. Dorr,2 David S. Albert, and William A. Remers1

Department of Pharmaceutical Sciences and Cancer Center, University of Arizona, Tucson, Arizona 85721.

Received August 31, 1990

A set of 30 mitomycin C and mitomycin A analogues, including five new compounds, was screened against three different solid human tumor cell lines using the MTT tetrazolium dye assay. A statistically significant correlation among antitumor activity, quinone reduction potential (E1/2), and the logarithm of the partition coefficient (log P) was obtained, with the most easily reduced and the most lipophilic compounds being the most potent. When these analogues were separated into mitomycin C and mitomycin A subsets, the former gave a correlation only with E1/2, whereas the latter (which differ little in their E1/2 values) gave a correlation only with log P. These correlations are in contrast to those made in the P388 leukemia assay in mice wherein the most active mitomycin C and mitomycin A analogues were the most hydrophilic ones. When the same compounds were tested against P388 leukemia cells in the MTT assay, the results were the same as those of the solid tumor assay. Thus, the substantial differences in relative potencies of mitomycins appear to the kind of tumor cell, but not to the type of assay performed, cell culture versus whole animal. No correlation was found between antitumor potency in the cell culture systems and calculated relative DNA binding strengths, probably because the limiting factors in antitumor potency of mitomycins appear to be tumor cell uptake (log P) and/or bioreductive activation (E1/2).

The effectiveness of mitomycin C (1) as a clinical antitumor agent has stimulated the preparation and evaluation of hundreds of analogues.1 This research has resulted in a substantial data base of antitumor activity, especially against P388 leukemia in mice. A number of attempts have been made to correlate this activity with structural features and physical properties of the mitomycins.2-7 Among the structural features, the aziridine ring and the carbamate substituent are especially important for good activity. Mitomycins are more potent than the corresponding mitomycin C analogues, probably because the indoloquinone chromophores of the latter are more difficult to reduce than the benzoquinone chromophores of the former.2 Reduction is essential to bioactivation of both mitomycins and mitomones as bifunctional alkylating agents. Accordingly, attempts to correlate the antitumor activity of mitomycines with physical properties such as partition coefficient, quinone reduction potential, and substituent size have been generally disappointing. Early studies on mitomycines, wherein substituents varied at a number of positions on the molecule, suggested an inverse correlation between antitumor activity and quinone reduction potential6 or the presumably related redox-electrode power of quinone-ring substituents.4 Subsequent studies on mitomycin C analogues, with substituents varied only on the quinone-ring amino group (N') revealed no statistically significant correlations for sets of compounds consisting of substituted methylamines,6 substituted ethylamines,10 or secondary amines.11 It was possible to obtain a correlation (R² = 0.85) between partition coefficient and antitumor potency (minimum effective dose (MDE) against P388 leukemia in mice) within a set of N'-phenyl analogues.5 Correlations of antitumor potency with quinone reduction potential (E1/2), which are related not against σ values for substituents on the phenyl group, or substituent size were statistically insignificant. More recently, two different sets of mitomycin C analogues did not give statistically significant correlations between partition coefficients and MED values against P388 murine leukemia.6,7

Despite the generally unsatisfactory previous results on mitomycin QSAR, we thought that it might be possible to obtain better correlations between antitumor activity and physical properties if a more reproducible test system and more accurate estimations of physical properties, including net binding enthalpies, were used. In particular, the P388 leukemia assay in mice caused problems because of variations in survival times from run to run. Consequently, we decided to use in vitro assays, which could give precise and reproducible IC50 values for QSAR. Three different human tumor cell lines, WI-38 normal,12 2780 ovarian,13 and MCF-7 breast cancer,14 were chosen for this purpose. The physical properties investigated were log P, E1/2, and the net binding enthalpies of drugs to the DNA segment, GC10, as determined by molecular mechanics calculations. These properties follow from the sequence of events that occur in mitomycin antitumor activity. Thus, log P may be related to the partitioning of mitomycines into tumor cell membranes. E1/2 is related to their bioreductive activation, and net binding enthalpy is related to their ability to alkylate DNA in the nucleus once they reach it. Effects of substituent size should be accounted for in the net binding calculations, although they were found previously to be insignificant in the set of N'-phenyl derivatives.6 Although each of these three effects, cell penetration, bioactivation, and DNA binding, is essential for antitumor activity, they might not all turn out to be statistically significant in the QSAR. For example, if cell penetration

1991 American Chemical Society
Table I. Preparation and Properties of New Mitomycin C Analogues

<table>
<thead>
<tr>
<th>Yield</th>
<th>Solvents for Silica Gel Chromatography</th>
<th>¹H NMR for the 7-substituent*</th>
</tr>
</thead>
<tbody>
<tr>
<td>10%</td>
<td>CHCl₃/MeOH/91:1, 9:2, then CHCl₃/MeOH</td>
<td>129-131, 7.04 (d, 2H, 7.50 (d, 2H), S.06 (br a. 1)</td>
</tr>
<tr>
<td>11%</td>
<td>CHCl₃/MeOH/91:1, 125-128, 7.10 (d, 2H, 7.65 (d, 2H), 8.00 (br a. 1)</td>
<td></td>
</tr>
<tr>
<td>15%</td>
<td>CHCl₃/MeOH/91:1, 123-128, 7.10 (d, 2H, 7.65 (d, 2H), 8.00 (br a. 1)</td>
<td></td>
</tr>
<tr>
<td>16%</td>
<td>CHCl₃/MeOH/91:1, 123-128, 7.10 (d, 2H, 7.65 (d, 2H), 8.00 (br a. 1)</td>
<td></td>
</tr>
<tr>
<td>13%</td>
<td>CHCl₃/MeOH/91:1, 123-128, 7.10 (d, 2H, 7.65 (d, 2H), 8.00 (br a. 1)</td>
<td></td>
</tr>
<tr>
<td>17%</td>
<td>CHCl₃/MeOH/91:1, 123-128, 7.10 (d, 2H, 7.65 (d, 2H), 8.00 (br a. 1)</td>
<td></td>
</tr>
</tbody>
</table>

*Analytical results were within ±0.40% of the theoretical value for C, H, N, and Br (101), unless stated otherwise. *The solvent was acetone-d₆ unless specified otherwise and TMS was the internal standard. *The solvent was CDCl₃. *The solvent was CD$_2$OD. *N: calcd 11.44, found 10.95. C: calcd 44.77, found 47.72. *N: calcd 13.90, found 12.29. *N: calcd 14.30, found 13.73.

![Figure 1. Mitomycin C analogues: log (1/C) vs $E_{1/2}$ Data from Table I.](image)

Figure 1. Mitomycin C analogues: log (1/C) vs $E_{1/2}$. Data from Table I.

is difficult, but bioactivation and DNA binding are easy once the mitomycin enters the cell. It is possible that log P will be the only significant factor. In addition to a new test system, better log P estimations were possible than we had in previous QSAR studies because of the availability of Leo's CLOP computer program. A set of 30 different mitomycin C and mitomycin A analogues was chosen for this study. Those already on hand were checked for purity by TLC and repurified by preparative TLC if necessary. Known compounds not available were prepared as described previously. Compounds 10, 11, 15, 16, and 17 were new. Among them 16 and 17 were "rationally" designed to have enhanced DNA binding. As indicated in Table II, 16 and 17 have significant calculated net binding energies. Compounds 11 and 15 were based on $N^\alpha$-4-(2-indolyl) analogues 12, after it showed unexpectedly high activity in the human tumor cell assay. Initially, we wished to prepare the diiodophenol derivative by reduction of 2,6-diiodo-4-nitrophenol. However, this compound lost one of the iodine atoms in the process.

Chemistry

The five new analogues 10, 11, 15, 16, and 17 were prepared by treating mitomycin A (20) in methanol with the appropriate amines. Triethylamine in water was used to promote the slow reaction of 16. The yields and physical properties of the new analogues in Table I. 4-Amino-2-iodophenol was prepared by sodium dithionite reduction of 2,6-diiodo-4-nitrophenol. It has been reported, but not characterized. Appropriate data are given in the Experimental Section.

Antitumor Activity

Antitumor potencies (IC₅₀) of the mitomycin analogues against the three human tumor cell lines were determined by the MTT assay, as described in the Experimental Section. The results are listed in Table II, which also gives the values for log P, $E_{1/2}$, and net binding energy to be used in QSAR. It can be seen that potencies of a particular drug against all three tumors are roughly comparable, suggesting that mitomycins are not cell-line selective in their toxicity. There are some exceptions to this generalization, but the variations in activity for a compound against the three tumors are usually much less than the variations among all compounds for a particular tumor. Among the more apparent relationships in Table II are the superior in vitro antitumor potencies for lipophilic mitomycin A analogues and certain $N^\alpha$-aryls derivatives of mitomycin C. Only 14 of the compounds in Table II were characterized by cal-

(15) CLOP is a tool developed by R. Kinnamon at the U.S. Environmental Protection Agency.

was attempted with the monosubstituted N'-aryl-
mitomycin C derivatives in Table II (compounds 7-14),
using e and s values of substituents as the independent
variables. The result gave a statistically significant cor-
correlation between the e values, with the most electron-
withdrawing substituents conferring the greatest potency
(lowest IC50). The regression equation was log (1/C) = 6.46
+ 0.92e; N = 8, R = 0.89, R² = 0.80, S = 0.22, F = 23.4,
sig F = 0.0029. 17 There was no statistically significant
correlation with s. The difference between the correlation
obtained from the P388 leukemia assay in mice and the
assays with cultured human tumor cells is surprising. It
suggests that the two test systems are substantially unlike
in terms of sensitivity to different mitomycin analogues.
Of course, the mouse system is much more complex than
the cell cultures, but even so log P still accounted for 85%
of the variance.

Statistical analysis was performed on the mitomycin A
analogues 20-30 with log (1/C) values from the human
tumor cell assays (Table II) and log P as the independent
variable. These compounds have little variation in E80
because they all are 7-alkoxymitosanes. A statistically
significant correlation was obtained: N = 11, R = 0.90, R²

Figure 2. Mitomycin A analogues: log (1/C) vs log P. Data from
Table I

= 0.64; S = 0.46, F = 15.9, sig F = 0.0032. The equation
was log (1/C) = 6.88 + 0.44 log P,17 indicating that the
most lipophilic compounds have the greatest potencies.
These data are plotted in Figure 2. Most of the points
lie close to the least-squares line, but mitomycin A (30)
is substantially more potent than predicted and (hydroxy-
ethoxy)ethoxy analogue 27 is substantially less potent
than predicted. The reasons for these deviations are not
obvious. In two earlier publications, we noted that mito-
mycin A analogues did not give statistically significant
correlations with log P in the P388 leukemia mouse assay.15

(17) In this calculation, the number of compounds is small for
multiparameter analysis; however, because only one variable
is significant, the equation reduces to that of a straight line.
The number of points is adequate for this equation.
Nevertheless, we examined the earlier antitumor potencies in a correlation with a better estimate of \( p \) values of compounds 20–30 as listed in Table III. This time, a statistically significant correlation was obtained: \( N = 27 \), \( R = 0.84 \), \( R^2 = 0.71 \), \( S = 0.50 \), \( F = 22.0 \), \( \text{sig } F = 0.0011 \). The equation was \( \log (1/C) = 7.07 - 0.56 \log P \), indicating that the least lipophilic compounds have the highest potency. This result is the opposite of that obtained in the human tumor cells.

At this point in the study, it seemed important to establish whether the surprising differences in mitomycin QSAR between human solid tumor cells in culture and P388 leukemia in mice are caused by the nature of the tumors (solid versus hematological) or the test systems (cell culture versus animal). Consequently, we tested the mitomycin A analogues 20–30 and most of the mitomycin C analogues (1–14, 18, and 19) against P388 leukemia cells in the MTT assay. The results were closely parallel to those obtained with the human solid tumor cells, as shown in Table IV. Thus, for all analogues the \( \log (1/C) \) value for the test system was determined. For example, \( N = 27 \), \( R = 0.87 \), \( R^2 = 0.76 \), \( S = 0.46 \), \( F = 36.2 \), \( \text{sig } F = 0.0000 \). Separation into the subsets gave \( \log (1/C) = 13.3 + 14.45 \), for the mitomycin C analogues (\( N = 16 \), \( R = 0.91 \), \( R^2 = 0.83 \), \( S = 0.36 \), \( F = 63.7 \), \( \text{sig } F = 0.0000 \)) and \( \log (1/C) = 8.8 + 0.32 \log P \), for the mitomycin A analogues (\( N = 11 \), \( R = 0.62 \), \( R^2 = 0.39 \), \( S = 0.53 \), \( F = 6.52 \), \( \text{sig } F = 0.04 \)) for the mitomycin A analogues.

Application of the \( s \), \( r \) analysis to the \( N^2 \)-phenyl-substituted mitomycin C derivatives gave \( \log (1/C) = 8.50 + 1.07e \). \( N = 8 \), \( R = 0.96 \), \( R^2 = 0.91 \), \( S = 0.15 \), \( F = 64.4 \), \( \text{sig } F = 0.002 \).

Those compounds for which net binding energies to GC10 had been calculated (1–6, 12, 13, 16, 17, 20, 25, 27, and 28) in Table III were subjected to multiple linear regression analysis with \( p, E_{1/2} \), and net binding energy as the independent variables. The results showed that there was no statistically significant correlation (95% confidence limit) with any variable.

**Summary and Conclusions**

In contrast to earlier studies, statistically significant correlations were made between antitumor potencies and physicochemical properties for a variety of mitomycin C and mitomycin A analogues. The property that dominated the correlation depended on the type of mitomycin analogue (C or A) and the tumor system. Thus, the potencies of mitomycin A analogues, which are readily susceptible to bioreductive activation, were determined by \( p \). This suggests that uptake by the tumor cells probably was the controlling event and that there is a striking difference between partitioning of the drug in cultured cells and in mice. The determining factor for potencies of the less readily reduced mitomycin C analogues also depended on the assay system. Against tumor cells in culture, the quinone reduction potential was determining, whereas \( p \) was determining against leukemia cells in mice. The effects of the test system on QSAR should be studied further using other antitumor drugs and other test systems, such as human solid tumor xenografts in mice.

A possible explanation for these results may lie in the inherent differences of the test systems. In the P388 murine leukemia model, the tumor cells are suspended in ascites, which is an aqueous environment confined to the abdominal cavity. Considering that the drugs are administered intraperitoneally, the more hydrophilic mitomycins would have a tendency to remain in this compartment longer, whereas the concentrations of the lipophilic derivatives may fall rapidly as these analogues penetrate, redistribute, and are subsequently metabolized by the surrounding solid tissues. This supposition implies that bioavailability of the mitomycins may supersede both the \( E_{1/2} \) and \( p \) in determining the activity of a particular compound. In the MTT assay system where drug exposure parameters do not vary, the ease of reduction appears to emerge as the dominant factor. However, when both the bioavailability and \( E_{1/2} \) are held essentially constant, as for the MMA analogues in culture, the relative lipophilicity of the cell penetration that determines activity. As previously mentioned, the calculated DNA binding energies of the mitomycins are favorable enough to suggest that once a compound enters a cell, its ability to alkylate DNA is not a limiting factor to its cytotoxicity.

Our observation that the QSAR of the mitomycins appears to vary with the tumor system is consistent with the

---

*Table III. Activity of Mitomycin A Analogues against P388 Leukemia in Mice*

<table>
<thead>
<tr>
<th>no.</th>
<th>MED (mol/L)</th>
<th>log (1/C)</th>
<th>log P</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.14</td>
<td>6.85</td>
<td>0.29</td>
</tr>
<tr>
<td>2</td>
<td>0.13</td>
<td>6.89</td>
<td>0.23</td>
</tr>
<tr>
<td>3</td>
<td>1.03</td>
<td>5.97</td>
<td>1.33</td>
</tr>
<tr>
<td>4</td>
<td>0.99</td>
<td>8.00</td>
<td>2.47</td>
</tr>
<tr>
<td>5</td>
<td>0.24</td>
<td>6.87</td>
<td>1.34</td>
</tr>
<tr>
<td>6</td>
<td>0.017</td>
<td>7.78</td>
<td>-1.10</td>
</tr>
<tr>
<td>7</td>
<td>3.5</td>
<td>5.45</td>
<td>1.74</td>
</tr>
<tr>
<td>8</td>
<td>0.30</td>
<td>7.53</td>
<td>-1.08</td>
</tr>
<tr>
<td>9</td>
<td>0.11</td>
<td>6.84</td>
<td>-0.46</td>
</tr>
<tr>
<td>10</td>
<td>0.47</td>
<td>5.17</td>
<td>2.38</td>
</tr>
<tr>
<td>11</td>
<td>0.008</td>
<td>7.59</td>
<td>0.35</td>
</tr>
</tbody>
</table>


---

*Table IV. Activity of Mitomycin Analogues against P388 Leukemia Cells in Culture*

<table>
<thead>
<tr>
<th>no.</th>
<th>IC50 (nm)</th>
<th>log (1/C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>50.8</td>
<td>7.29</td>
</tr>
<tr>
<td>2</td>
<td>499</td>
<td>6.34</td>
</tr>
<tr>
<td>3</td>
<td>9.7</td>
<td>8.04</td>
</tr>
<tr>
<td>4</td>
<td>34.6</td>
<td>7.68</td>
</tr>
<tr>
<td>5</td>
<td>28.6</td>
<td>7.58</td>
</tr>
<tr>
<td>6</td>
<td>32.5</td>
<td>7.27</td>
</tr>
<tr>
<td>7</td>
<td>13.1</td>
<td>8.77</td>
</tr>
<tr>
<td>8</td>
<td>18.8</td>
<td>7.73</td>
</tr>
<tr>
<td>9</td>
<td>3.03</td>
<td>8.52</td>
</tr>
<tr>
<td>10</td>
<td>1.02</td>
<td>9.72</td>
</tr>
<tr>
<td>11</td>
<td>1.42</td>
<td>8.85</td>
</tr>
<tr>
<td>12</td>
<td>1.62</td>
<td>8.79</td>
</tr>
<tr>
<td>13</td>
<td>3.56</td>
<td>8.33</td>
</tr>
<tr>
<td>14</td>
<td>0.44</td>
<td>9.35</td>
</tr>
<tr>
<td>15</td>
<td>0.50</td>
<td>9.30</td>
</tr>
<tr>
<td>16</td>
<td>0.49</td>
<td>9.30</td>
</tr>
<tr>
<td>17</td>
<td>1.55</td>
<td>7.81</td>
</tr>
</tbody>
</table>

*Activities determined by the MTT assay according to the procedure of Mosmann, T. J. Immunol. Meth. 1983, 65, 55.
extensive evidence recently reviewed by Phillips and co-workers that additional factors, besides the inherent chemosensitivity of tumor cells, may significantly influence the outcome of in vivo chemotherapy. Thus, in order to extract the maximum therapeutic advantage of a drug or class of drugs, it may be important to consider their persistent physicochemical properties as they relate to both the in vivo pharmacokinetics and the biology of the tumor system. For example, selection of a very hydrophilic mitomycin for the intracavitary therapy of gynecologic tumor systems such as malignant ovarian disease and pleural effusions may maximize drug bioavailability thereby enhancing the curative potential of the tumor. A recent study involving the use of a lipophilic derivative of peritoneal mitomycin C (10 mg/m² every 4 weeks) for persistent recurrent peritoneal carcinomatosis secondary to ovarian cancer revealed an objective tumor response rate of 86%. The toxicities included mild myelosuppression (suggesting that a fraction of the dose was absorbed) and abdominal discomfort that was dose limiting after approximately four courses. The choice of a more hydrophilic derivative may allow dose reductions that may ameliorate these side effects to some degree. On the other hand, selection of a lipophilic derivative with a low reduction potential for the continuous regional infusion of localized neoplasia may enhance tissue extraction of an active analogue while potentially minimizing the systemic toxicities. It is therefore unlikely that any single mitomycin derivative would be sufficient to treat all varieties of neoplasia.

It is becoming apparent that the appropriate choice of test systems in a stepwise protocol for selecting antitumor drugs is extremely important. Furthermore, the test systems should closely resemble the disease to be treated. For example, if a protocol for selecting mitomycin A analogues were based on optimizing activity against cultured human tumor cell lines (e.g., P388 leukemia cells) and/or P388 leukemia cells, and then confirming activities of the “best” analogues in the P388 mouse leukemia system, it would be found that they were poor choices and, possibly, the most active compounds would have been rejected prematurely. Of course, P388 mouse leukemia does not need to be the secondary screen, but the concept of compatibility between in vitro and in vivo screens still is critical. It appears that if laboratories have drug development protocols based on cell culture assays followed by “confirmation” in mouse systems, and they will need to carefully validate and compare these assays.

Experimental Section

Melting points were recorded on a Mel-Temp melting point apparatus and are uncorrected. 1H NMR spectra were recorded on a JEOL FX-90Q (90 MHz) spectrometer using tetramethylsilane as the standard. Elemental analyses were performed by Desert Analytics, Tucson, AZ. Analytical results were within ±0.4% of theoretical values unless specified otherwise.

Preparation of New Mitomycin C Analogues (General Method). A solution of mitomycin A (23-52 mg, 0.07-0.15 mmol) in anhydrous methanol (3-10 mL) was stirred with about 1.25-2.25 equiv of amine at room temperature. For the preparation of 18 this ratio was 6:7. Progress of the reaction was followed by thin-layer chromatography on silica gel with CHCl₃-MeOH (9:1 v/v) as solvent. The preparation of 16 was conducted in 1-tritylmethylamine (1:2 v/v) because it was too slow to monitor. When the conversion was complete, the mixture was filtered, the filtrate was concentrated under reduced pressure, and the crude product was purified by preparative thin-layer chromatography using precoated silica gel plates (20 × 20 cm and 2-mm thickness) and the solvent system specified in Table I. The appropriate bands were scraped from the plates and extracted with solvents given in Table I. This table also gives the yields and properties of the products.

Preparation of 4-Amino-2-imidazolone. To a yellow suspension of 2.6-diido-4-nitrophenol (1.26 g) in 25 mL of 25% NaOH was added sodium dithionite (1.8 g), and the mixture was heated with stirring at 75 °C until it turned white. It was filtered and the solids were washed with deionized water and dried in air to give 0.2 g (30%) of 4-amino-2-imidazolone as white solid with mp 157-158 °C. 1H NMR (DMSO-d₆ + D₂O + TMS) δ 7.02 (d, 1, J = 2.5 Hz), 6.65 (dd, 1, J = 13.5 Hz, 6.55 (dd, 1, J = 2.5, 12.5 Hz); 13C NMR (DMSO-d₆, decoupled) δ 148 (C1), 142 (C4), 124 (C3), 116.2 (C5), 115.5 (C6). Anal. (C₉H₇N₂O₂) C; H; N: calcd 53.86, found 54.83.

Antitumor Assays. The MTT tetrazolium dye reduction assay was used to determine antitumor activities of the mitomycins in three adherent human tumor cell lines: WI-DR colon (R. Wallace, American Cyanamid Co., Pearl River, NY), 2780 ovarian (R. Gosl, Fox Chase Cancer Center, Philadelphia, PA), and MCF-7 breast (American Type Culture Collection, Rockville, MD). These compounds were further screened against P388 murine leukemia cells (American Type Culture Collection, CCL-46, Rockville, MD).

The human tumor cells were grown in RPMI-1640 culture medium (Grand Island Biologicals, NY) supplemented with 5% (v/v) fetal bovine serum, L-glutamine 292 µg/mL, and 1% (v/v) each of penicillin G (100 µg/mL) and streptomycin (100 µg/mL). For the antitumor assays, single cell suspensions of WI-DR colon, 2780 ovarian, MCF-7 breast, and P388 leukemia cells were plated at concentrations of 300, 750, 2000, and 3000 cells per well, respectively, onto 96-well microtiter plates (Costar, Cambridge, MA). On day two (day one for P388), drugs dissolved initially in DMSO (J. T. Baker, analytical grade) and then diluted serially with phosphate buffered saline (pH 7.4) were added at concentrations of 10⁻⁶ to 10⁻¹ ng/mL in half-log gradations. Final concentrations of DMSO did not exceed 0.1%. The plates were incubated at 37 °C with 5% CO₂, 95% air, and 100% relative humidity for 6 days.

After the 6-days exposure period, 50 µL of a 2 mg/mL MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium brosme, Sigma Chemical, St. Louis, MO) dye solution was added to each of the wells and the plates were incubated an additional 4 h. The blue dye forms a colored formazan product upon the action of mitochondrial reductases in viable cells. The medium was then aspirated and the formazan product was solubilized by DMSO (100 µL/well). The intensity of the color, which is proportional to viable cell numbers, was measured at an excitation of 570 nm on an automated microculture plate reader (Bioscan 1000, Beckman Instruments, Fullerton, CA). Test results were calibrated in percent control absorbance from unexposed tumor cell cultures. Each drug concentration was tested in 6 wells and each mitomycin assay was conducted at least twice. The IC₅₀ values were then averaged. These results are given in Table II.

Solutions of mitomycins in DMSO could be stored at −5 °C without any diminution in potency; however, certain compounds lost potency when their aqueous solutions were frozen and thawed.

Statistical Analysis. Multiple linear regression analysis was performed using SPSS-X releases 3.1 for VAX/VMS. The new assay program was used with stepwise entry of variables and an entry criterion of P<0.05 (95% confidence limit).

The octanol-water partition coefficients were calculated with the aid of Leo's LOGP program on a microcomputer, using the experimentally determined log P values for mitomycins A and C as starting points. For example, in the calculation of log P for compound 2, the value for 1-propanol minus the contribution made by one hydrogen on C-3, was determined by log P to be 0.594 - 0.227 = 0.367. The experimentally determined log P for mitomycin C was corrected for removal of one hydrogen from the 7-NH₂ group to give −0.380 - (−0.030) = −0.350.

(22) SPSS Inc. Suite 3300, 444 N. Michigan Ave., Chicago, IL 60611.
of the corrected 1-propanol and mitomycin C fragments then gave a net ICP of 0.282 for 2. The contribution of one hydrogen to the 7-NH<sub>2</sub> group was determined by a calculation for analogs using the CLEOF. For the substituted N<sup>2</sup>-phenylaminoxytocin C analogues, the experimentally determined ICP for N<sup>2</sup>-phenylaminocytosin C (7) was used and s values for the substituents were taken from the literature. The R<sub>1</sub>, s values were previously published, except for those of 18, 11, 15, 16, and 17, which were unknown. For the first four of these compounds, E<sub>1/2</sub> was calculated using an equation based on the linear relationship between E<sub>1/2</sub> and s values for substituents on the benzene ring of N<sup>2</sup>-arylaminoxytocin C analogues previously published. This equation, derived by statistical analysis of the earlier data using SPSSX, was E<sub>1/2</sub> = –0.34 + 0.072s. In the calculations for s in compounds with substituted N<sup>2</sup>-phenyl groups, e<sub>1</sub> and e<sub>2</sub> values for each substituent are taken from the literature and it is assumed that they can be added together to obtain a combined s for all substituents. Using compound 15 as an example, E<sub>1/2</sub> = –0.34 + 0.072(e<sub>1</sub> + e<sub>2</sub>) = –0.34 + 0.072(–0.37 + 0.35) = –1.34 V. Following this procedure, the E<sub>1/2</sub> value for 16 is –0.32 V. The E<sub>1/2</sub> for N<sup>2</sup>-indolyl derivative 17 was estimated to be –0.36 V by comparing the nearly equal calculated electron densities (partial atomic charges) on 5-aminoisoquinoline (–0.436) and 4-aminoquinoline (–0.474), as determined by quantum mechanics using GAUSSIAN-90 UCP.6 Molar Molecular Modeling. New mitomycin analogues were obtained by displaying the mutant forms of mitomycin C and mitomycin A with no substituent on C1, which has been previously subjected to energy refinement with the molecular mechanics program of AMBER and docking the new substituent groups onto them using MDGRAF.24 These substituent groups were constructed using CHIMERIC II, and their partial atomic charges (ESP) were calculated with GAUSSIAN-90 UCP.25 The resulting structures were then brought to minimum energy conformations using AMBER.25 Parameters previously outlined were used for the mitomycin part of the structure. Parameters for the new substituents were based on those already in AMBER.25 For example, the indole substituent and the p-hydroxyphenyl substituent were taken from tryptophan and tyrosine, respectively. The united atom force field of AMBER 8.0 was used and all structures were refined until the root mean square gradient was less than 0.1 kcal/A. A distance-dependent dielectric constant was used, and all hydrogen pairs were included in the calculations. These same parameters and conditions were used for the decametocytosine duplexes and their covariant complexes with the mitomycins described below.

The decametocytosine diGGCCCCCCG, (referred to henceforth as GC10) was generated in the B form with Asof's geometry27 and brought to a minimum-energy conformation. Mitomycin A and mitomycin C were docked onto it near the 2-amino group of the fifth guanine residue in the first strand using MDGRAF. The coordinates were captured, and the structures of the resulting complexes, made covariant by defining a 1.47 A bond between C1 of the mitomycin and N2 of the guanine, were subjected to energy refinement using AMBER. Models for other covariantly bound mitomycin analogues were derived from those of mitomycin A and mitomycin C by docking the new substituent onto the mitomycin A 7-CH<sub>3</sub>O or mitomycin C 7-NH<sub>2</sub>, removing hydrogens, and carrying out energy minimization on the resulting structures. Using the ANALYSIS mode of AMBER, the energies for interactions between the mitomycin analogues and GC10, as well as the internal energies of each, were calculated. Distortion energies in the GC10, resulting from induced fits with the mitomycins, were calculated by subtracting energies of GC10 minimized alone from those of GC10 in the covariant complex. In the same manner, distortion energies in the mitomycins were obtained by subtracting energies of the mitomycin minimized alone from those in the covariant complex. Net binding energies, which are used in the comparison of relative binding strengths of mitomycins to GC10, were obtained by adding the calculated intermolecular binding energies (electrostatic + van der Waals) and the two distortion energies. These data are given in Table II.

Concerning the helix distortion energies, their absolute values cannot be used to compare the distortion in a complexed polynucleotide relative to uncomplexed polynucleotide because they are chemically different molecules. It is, however, meaningful to compare the distortion energies among various complexes based on the same drug and polynucleotide and to draw inferences on their relative stabilities. The dominant components in the relative distortions are van der Waals and electrostatic interactions. Bond length and bond angle contributions make little difference. Previous publications discuss the scope of application of AMBER to drug-macromolecule complexes and the validity of parameters in its force fields.

Acknowledgment. We gratefully acknowledge the Medical Research Council of Canada for a fellowship to K.R.K. Dr. S. M. Sama (University of Arizona) generously provided some of the mitomycins used in this project. The CLEOF program was made available by Dr. S. Yalkowsky (University of Arizona). AMBER was a gift of Professor Peter A. Kollman (University of California, San Francisco).

References:

REFERENCES


(32) Bizanek, R.; McGuinness, B.F.; Nakanishi, K.; Tomasz, M.


(54) CLOGP in PCGEMS was provided by R. Kinnerson at the U.S. Environmental Protection Agency.
