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Anthracycline Antibiotics

New Analogues, Methods of Delivery, and Mechanisms of Action

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Preface

DESPITE MORE THAN 20 YEARS OF EFFORT by the pharmaceutical industry and academia to develop new anticancer agents and to find a "better doxorubicin," doxorubicin remains a frontline chemotherapeutic agent in the treatment of human cancers, especially solid tumors such as carcinomas and sarcomas. The failure to find a clearly better doxorubicin has led in turn to the decline of interest in anthracyclines and to a reduced emphasis on such projects throughout the pharmaceutical industry. However, the clinical use of two other anthracycline antibiotics, epirubicin and idarubicin, has increased significantly in recent years, especially in Europe. Also, difficulties in developing biologicals as important chemotherapeutic drugs and the promising results of clinical studies of drugs such as Taxol (paclitaxel) have revived interest in anthracycline antibiotics and in more traditional approaches to drug development.

The development of new anthracyclines was hampered by several factors, especially a lack of knowledge of the exact mechanism of action. Early studies focused on the structure–activity relationship, and trials to establish the exact mechanism of action on a molecular level were not successful. Attempts to identify a mechanism of action for doxorubicin were complicated by the fact that anthracycline antibiotics exert their antitumor properties not through a single mechanism but more probably through an array of unrelated biochemical processes involving numerous cellular targets. Even such basic questions as why daunorubicin and doxorubicin differ in antitumor activity and how this relates to a single chemical change, i.e., the replacement of hydrogen at C-14 with hydroxyl, still remain unanswered. For years these questions have had enormous clinical and commercial significance because daunorubicin's use is limited to leukemia, whereas doxorubicin shows both antileukemic and solid tumor activity.

Development of new analogues was also influenced by self-imposed limitations based on initial structure–activity relationship studies by leading groups and the belief that water solubility was necessary for pharmaceutical formulation and development. Thus, in practice, water-insoluble, lipophilic compounds were neglected and insufficiently evaluated. This neglect was unfortunate because, despite the obvious problems of formulation, such analogues can offer desirable properties, such as a different spectrum of activity and reduced toxicity. Some of these properties are due to different intracellular, tissue, and organ distributions.

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In recent years, problems associated with the development of new anthracyclines and methods of their delivery have been addressed directly by many groups. New developments in other fields have led to the discovery of an important mechanism of action of anthracyclines by topoisomerases, and a more detailed picture of interaction of anthracyclines with DNA is emerging. Other new mechanisms of action have been proposed and are discussed in this book. Development of resistance is now perceived as one of the major obstacles to effective chemotherapy, and as such, it already significantly influences the design of new drugs and methods for evaluating them.

The main goal of the symposium on which this book is based was to bring together researchers who are involved in the direct design and synthesis of new drugs with researchers who are investigating biochemical processes and mechanisms of action. We hoped that such a meeting, by reviewing recent developments in related disciplines, would help to point out future directions in research and to facilitate close interdisciplinary efforts. The meeting was attended by chemists, medicinal chemists, biochemists, pharmacologists, and clinicians. We hope that this book will help to achieve the symposium's goals on a global scale and to stimulate further research and new discoveries in this area.

This book covers research relevant to the development of novel anthracyclines, such as the synthesis of promising new analogues, studies of mechanisms of action, and new approaches to improving properties for this class of compounds by using different drug-delivery and tumor-targeting systems. Although it occurred too late to be included in this volume, Bristol-Myers Squibb recently began clinical studies on a doxorubicin covalently bound to a chimeric (mouse–human) monoclonal antibody, BR96. The results of preclinical in vivo evaluation are promising (Trail, P. A. et al. *Science (Washington, D.C.)* **1993,** *261*, 212), and the success or failure of this study could influence this approach in years to come.

It is becoming apparent that to achieve success at the clinical stage, the development of new analogues should parallel and be closely connected to the development of methods to increase tumor targeting, to avoid organ-targeted toxicities, and to limit other undesired side effects. Because anthracyclines are highly flexible with regard to structural modifications, the physicochemical properties and potency of new analogues can be altered without sacrificing efficacy. In consequence, anthracyclines can be designed to be compatible with a specific delivery or targeting system, thus allowing one to take full advantage of a particular system.

The studies presented in this book clearly indicate that the recent progress in studies of the mechanism of action, the development of new anthracyclines, and the use of drug delivery to increase tumor targeting will soon result in new, more selective chemotherapeutics.

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Chapter 1

Unresolved Structure–Activity Relationships in Anthracycline Analogue Development

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Continuing interest in quinone-altered anthracyclines, for example, can be explored by screening against a panel of 60 human tumor cell lines in culture. 5-Iminodaunorubicin gave a pattern of cytotoxic potencies that was distinct from the pattern produced by doxorubicin, daunorubicin, and other anthracyclines. This was consistent with accumulated evidence for an altered mechanism of action with the modified quinone. Previously, 5-iminodaunorubicin was active but not superior to the parent quinone when screened against leukemia P388 in the mouse. As a model quinone isostere synthesized in 7 steps, 1-hydroxy-7, 8-bis-(morpholinomethyl)phenazine-5,10-dioxide was cytotoxic across the 60-line panel. Without N-oxidation to the quinone isostere level, the phenazine was noncytotoxic.

It is ironic that the volume of anthracycline research has declined in recent years. The outlook for progress toward specific goals is better that ever, yet major objectives of anthracycline analogue development have yet to be attained. This is despite the voluminous clinical and experimental work done since the introduction of daunorubicin and doxorubicin in the 1960s. These molecules continue to offer an excellent point of departure for studies to develop better cancer drugs and explore antitumor mechanisms.

Needed Improvements

More than a thousand analogues have been obtained and tested, but anthracyclines with better activity and less toxicity are still needed (*1, 2*). It is precisely because of the attention given this series that the various properties needed in an improved analogue have been defined (*1-3*). Doxorubicin has been called the most active single agent against cancer because of its broad antitumor spectrum, but there are some important tumor types (e.g., colon, lung) that do not respond; an even broader spectrum is needed. Among responding tumors, the rate of response is often low--for doxorubicin as a single agent, sometimes 30%; even a doubling of this rate could give an enormous benefit. In many cases, initially responding tumors become resistant to anthracyclines over time, and this resistance often extends to chemically unrelated

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drugs (multidrug resistance); analogues effective in resistant tumors are widely sought. Clinicians have learned how to manage the toxic side effects--acute myelosuppression and chronic cardiotoxicity (*1, 2, 4*)--that were recognized early in the clinical application of daunorubicin and doxorubicin, but they still impose treatment limitations (on dose level and on the number of drug courses); less toxic analogues would provide significant therapeutic benefit.

There are basically two needs in an approach to these problems through analogue development: identification of significant alterations in the parent structure, and comparative testing of new compounds. Design of the structural changes can be based on observed structure-activity relationships together with considerations of synthetic accessibility. Equally productive may be to consider unresolved questions of structure-activity relationships in the results accumulated to date. Also, a survey of the analogues obtained so far will show what types of structure changes are underrepresented. For example, few changes have been made in the carbon skeleton of the aglycone. Some will be presented (*5*) at this symposium, but they are rare. This is surprising in view of the creative studies of aglycone total synthesis that have come from various groups of leading synthetic chemists (*6, 7*). Furthermore, there have been few changes of any kind at the quinone function. This is ironic because the quinone is an important site of mechanistic action in the anthracycline molecule. It could be useful to explore the effect on activity of various changes at the quinone.

Screening Methods

Once certain structure changes have been selected, the question is what standardized screen to use for analogue comparison. Cancer screening has been the subject of recent reviews (*8-11*). For many years, mouse leukemia P388 provided a good standard test in the mouse. Potency was measured in terms of the required drug dose, and efficacy in terms of host survival time. Quantitative data were generated on many compounds, and if test parameters like tumor inoculum and dosing schedule were kept the same (as in the National Cancer Institute [NCI] screen), the compounds could be quantitatively compared. However, predictive value for clinical activity was at best only qualitative. Efficacy and potency in the mouse did not correlate directly with specific aspects of clinical activity like spectrum or rate of response. Development of the immune-suppressed nude mouse permitted testing against human tumor lines, but this has been an expensive animal model, and its predictiveness is still unsettled. For one thing, it is not clear how many human lines should be used to evaluate a given compound. And there are the unavoidable differences in pharmacology and pharmacokinetics between any animal model and man. Perhaps for these reasons (and others), high-volume antitumor screening has recently tended toward in vitro testing.

That may seem surprising, but methodology has been developed for the use of panels of human tumors in culture, which should give representative average values for cytotoxic potencies and may also give an indication of antitumor spectrum. The human tumor clonogenic assay (also called the Salmon stem-cell assay) tests for the inhibition of colony formation using fresh tumor explants (*11*). An encountered shortcoming was that many patient tumors could not be grown for the assay. The NCI has recently organized a screen using a panel of 60 established human tumor cell lines (*9*). It is called a "disease-oriented" screen because it is comprised of eight subpanels representing important tumor types found in human disease. The assay is based on the measurement of cell growth. Of course, the 60 lines in any test will vary in their responses. A working hypothesis of this new assay at NCI was that compounds of interest would show specificity (in terms of increased potency) for a particular subpanel. Another aspect of the assay is that the pattern of the 60 responses (apart from any subpanel specificity) may be characteristic of the compound, or the

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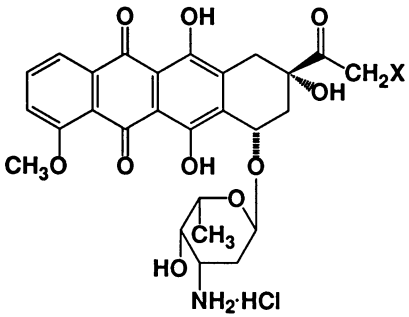
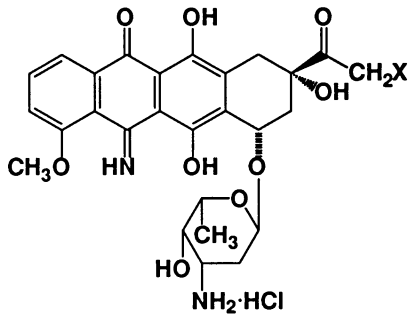
class of compound. Statistical methods have been developed for comparing the patterns and quantifying their degree of similarity (*8*). This in vitro assay, then, gives a type of result that is quite different from that obtained in the mouse. It may be of interest to compare results obtained from such different screens as mouse leukemia and the 60-cell-line panel on one drug candidate. A useful example should be 5-iminodaunorubicin .



X = H, daunorubicin
X = OH, doxorubicin



X = H, 5-iminodaunorubicin
X = OH, 5-iminodoxorubicin

Iminoquinones

The iminoquinones are among the relatively few quinone-altered anthracyclines. Iminoquinones have been prepared (*12, 13*) from both daunorubicin and doxorubicin, but most studies (*14-16*) have been carried out with 5-iminodaunorubicin because it is obtainable in a single step. 5-Iminodoxorubicin, on the other hand, requires five semisynthetic steps. 5-Iminodaunorubicin is a good example of a mechanistically altered and active analogue that was not selected for development because it failed to stand out in a mouse screen. Amination of the quinone causes suppression of the usual redox cycling and generation of oxygen radicals. Probably because of this, 5-iminodaunorubicin was found to be noncardiotoxic in rats and dogs, confirming a number of predictive tests. Less attention has been paid to the evident deletion of the reductive deglycosidation that is the normal metabolic process for anthracycline deactivation. No evidence for occurrence of this step could be identified when metabolism was studied in the rat (*17, 18*). The altered pharmacokinetics thereby predicted for 5-iminodaunorubicin were not considered when (at an earlier time) the screening regimens for this compound were chosen. Beginning with leukemia P388, 5-iminodaunorubicin was tested against various tumors in the mouse. It was active in 16 different regimens but was not superior to daunorubicin or doxorubicin. But if mouse activity parameters do not correlate directly with clinical activity factors, results from a quite different type of screen may be of equal interest.

Figure 1 shows the 60 dose-response curves for 5-iminodaunorubicin (NSC 254681) in a single test from NCI's new disease-oriented screen. Cell growth (in percent relative to untreated control cells) is plotted against drug concentration (in molarity on a logarithmic scale). Activity of the drug across the panel can be compared at any selected level of growth: e.g., +50% = 50% growth inhibition; 0% = cytostasis; and -50% = 50% cell kill, or LC_{50}. The pattern of responses will be

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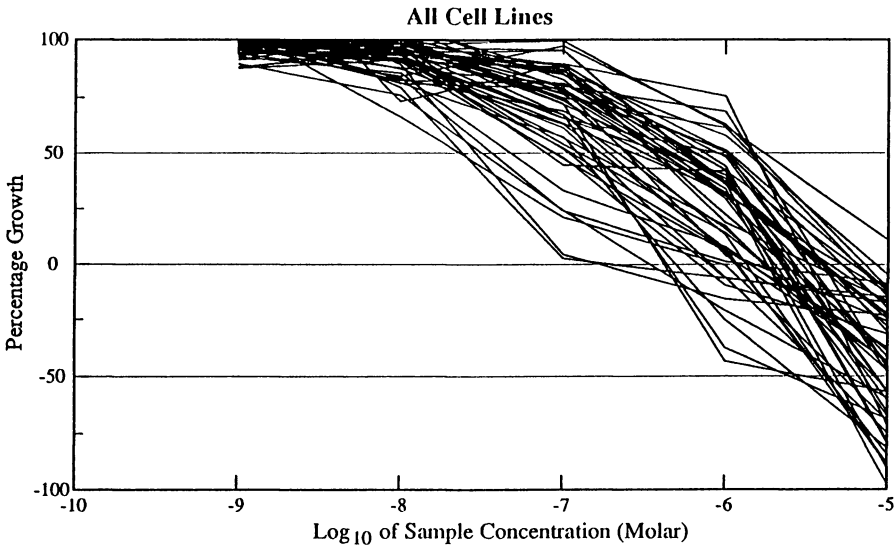


Figure 1. Dose-response curves for 5-iminodaunorubicin (NSC 254681; Exp ID 90009NS60) against 60 human tumor cell lines in disease-oriented NCI screen.

somewhat different at each level of growth. For development of cytotoxic agents, interest is primarily at the level of cell kill. In Figure 1, the highest concentration was 10^{-5} M, and many of the cell lines underwent 50% cell kill only above that level. In a test where the highest concentration was raised to 10^{-4} M, all but two of the lines (colon subpanel) underwent 50% cell kill and gave measured LC_{50} values. Results from this test are shown in Figure 2, in bar-graph form, with each log LC_{50} represented by a bar extending from the mid value (-4.74) either to the right (lower values, more sensitive lines) or left (higher values, more resistant lines). The cell lines in Figure 2 are grouped by subpanel but are not named. The purpose here is to show the distinctive overall pattern produced by 5-iminodaunorubicin in comparison with daunorubicin (NSC 82151; averaged from several dozen tests). Most anthracyclines--including doxorubicin--give a pattern resembling that shown for daunorubicin in Figure 2 (*19*). One of the few to give a pattern that is different is 5-iminodaunorubicin. The difference that is visually evident in Figure 2 was also observed in a preliminary use of the COMPARE pattern recognition program (*8*) being developed by NCI. Existence of the difference seems consistent with various results (*14*) on the alteration in biochemical mechanisms with the imino quinone. Perhaps screening results from this panel of cell lines can be used together with results from the mouse screen in choosing distinctive members of an analogue series for further study. It has always been easier to find active analogues than to choose among them.

Quinone Isosteres

Some years ago, we proposed to synthesize another type of quinone-altered analogue. These were isosteric structures (Figure 3) in which the quinone carbonyls were to be replaced by *N*-oxide functions (*20*).

This approach was partly justified by modest antitumor activity observed for some simple phenazine-5,10-dioxides. Work toward these targets was initiated but not completed because of certain synthetic difficulties and delays. Intermediates that were synthesized are summarized in Scheme I. The linear tetracycles with two

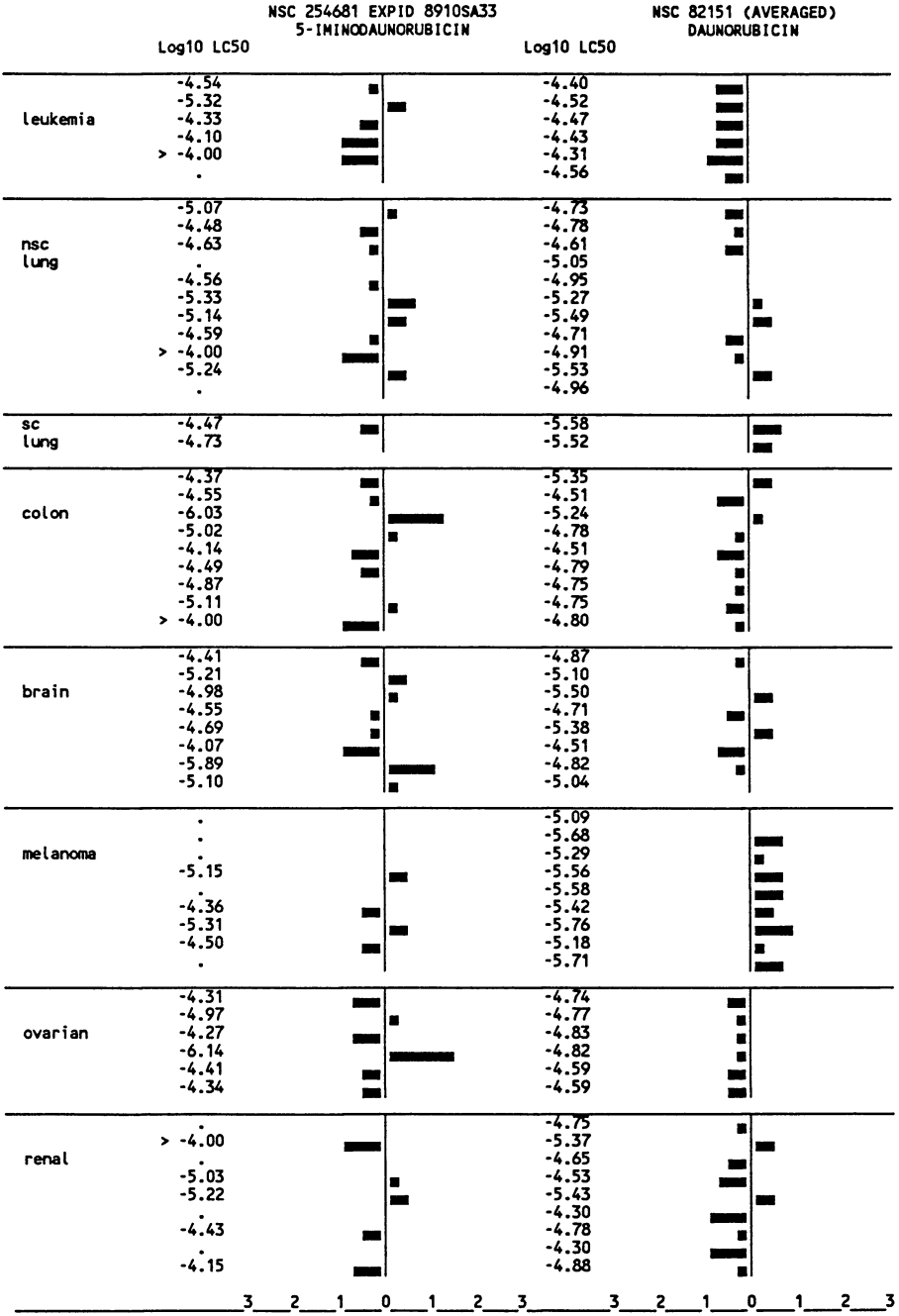
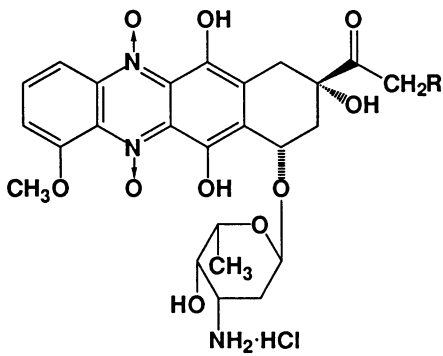
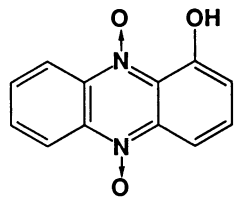


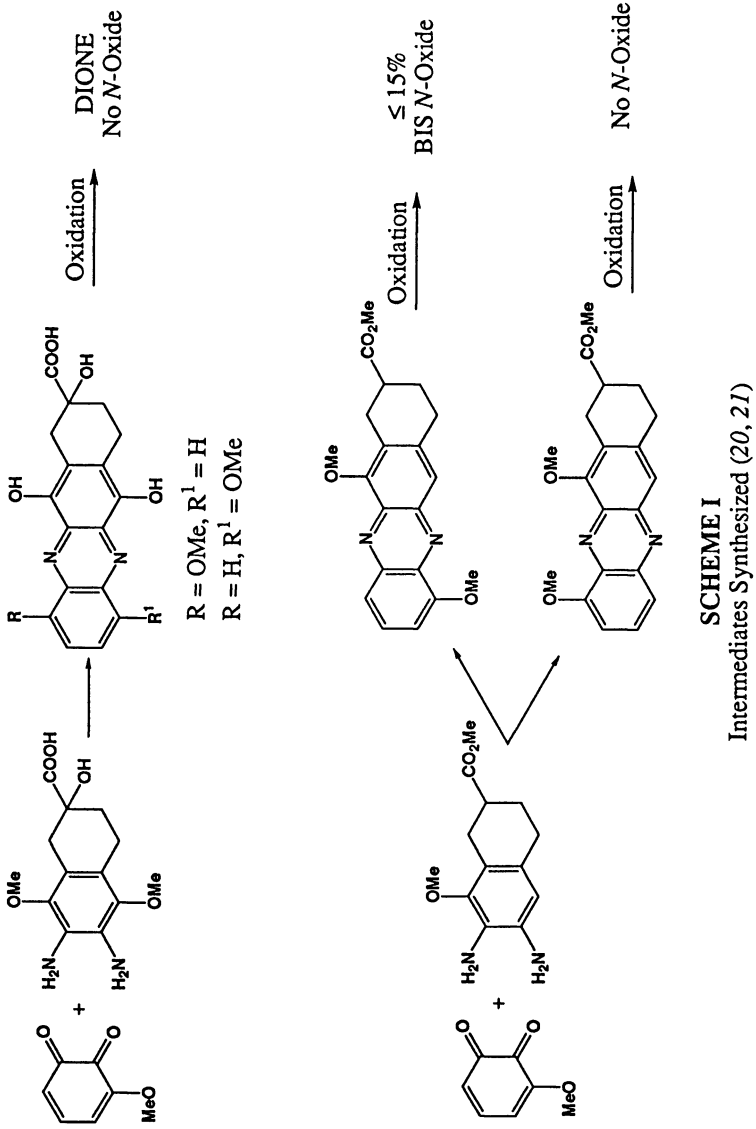
Figure 2. Comparison of 5-iminodaunorubicin and daunorubicin in the NCI screen. Cell lines grouped by subpanel. Responses expressed as bargraphs giving a pattern for each compound.





Borderline antitumor activity

Figure 3. Proposed quinone isosteres and a known phenazine dioxide (*20*)



SCHEME I
Intermediates Synthesized (*20*, *21*)