CHAPTER 11

THE CONTRIBUTION OF SYNTHETIC ORGANIC CHEMISTRY TO ANTICANCER DRUG DEVELOPMENT

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Summary

Synthetic organic chemistry has always played a vital role in anticancer drug development, but the nature of its major contributions has varied over time. In the 1950s, knowledge of DNA metabolism allowed the semirational development of nucleotide mimics and antagonists. From about 1960 to 1985, random screening (of natural products and synthetics) was dominant, driven primarily by the large screening program of the U.S. National Cancer Institute. This empirical approach had merit when there were few targets besides DNA to focus on, but it also had drawbacks. The nature of the screens facilitated selection of antiproliferative cytotoxins, and natural products identified were usually too complex to be modified. The main value of natural-product screening is now as lead discovery, since quite complex structures can now be synthesised economically due to improvements in organic synthesis. Better ways of defining molecular structure in terms of numeric parameters allowed the development of quantitative relationships between drug structure and biological activity. Organic chemistry has also contributed many concepts to the rational design of drug classes, such as tumor-activated prodrugs of cytotoxins, especially for methods of deactivating the toxins. Finally, anticancer drug development in the genomics era

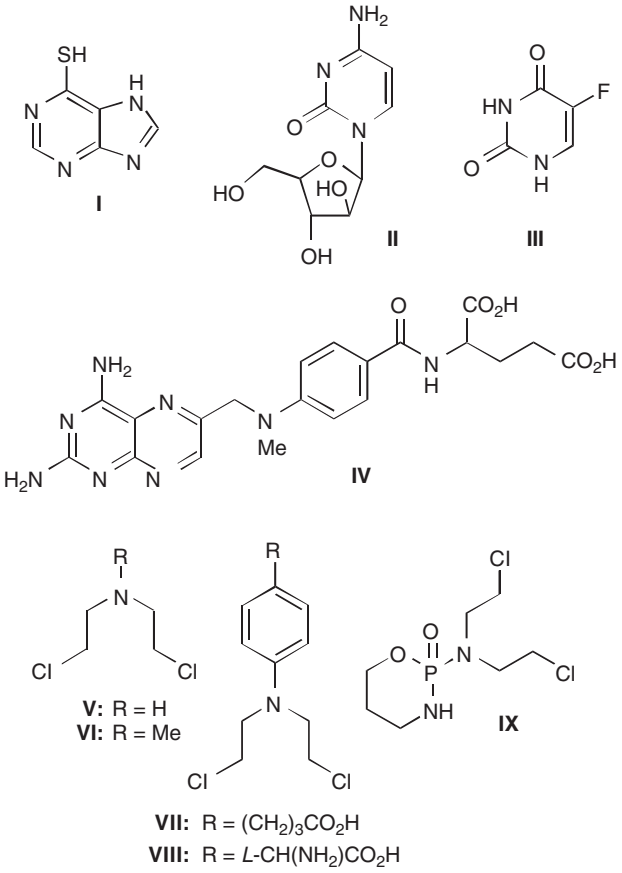
again focuses on the random screening of massive numbers of compounds, using automated high-throughput screening against pure enzymes. Organic chemistry has responded by the development of combinatorial methods of synthesis, enabling the simultaneous preparation of large numbers of compounds. Initially used to prepare polypeptides, this has been adapted to the preparation of a wide range of compounds, including complex natural products.

1. Introduction

Synthetic organic chemistry has always been a vital part of the highly integrated and multidisciplinary process of anticancer drug development. However, the nature of its major contributions has varied over time. Its relative importance in driving changes in the philosophy of anticancer drug development has also waxed and waned in comparison with other disciplines such as biochemistry, enzymology, and molecular biology. This chapter describes the particular contributions of synthetic organic chemistry to the philosophy of anticancer drug development, using particular drug classes as illustrations.

2. Early Rationality

In the earliest days of chemotherapy, knowledge of DNA metabolism allowed pioneers such as George Hitchings, Gertrude Elion, and Charles Heidelberger to develop nucleotide mimics and antagonists, largely by rational design (Elion, 1989). This led to such drugs as 6-mercaptopurine (I), cytosine arabinoside (II), 5-fluorouracil (III), and methotrexate (IV), which are still widely used in cytotoxic chemotherapy. At about the same time, Haddow, Ross and others were exploring the family of nitrogen mustards, sparked by the initial observation of depression of white cell count in people exposed to the war gas bis(2-chloroethyl)amine (mustard gas; V). This led to the development of mechlorethamine (VI) (Goodman *et al.,* 1946) and the less vesicant analogue chlorambucil (VII), melphalan (VIII), and the still widely used cyclophosphamide (IX) (Colvin, 1999). Chemistry played a leading intellectual role in the development of these drugs. In the case of the mustards, a good understanding was gained of the relationships between structure, reactivity, potency, and efficacy (at least in forming the primary DNA lesions) (Wilman and Connors, 1983).

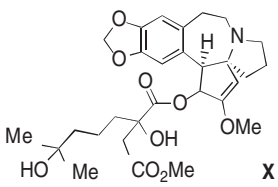


3. The Random Screening Era: Directly from Screen to Clinic

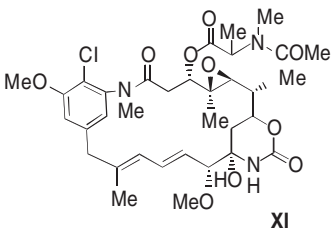
In the ensuing 25 years, from about 1960 to 1985, there was a great expansion of effort in seeking new anticancer drugs, with much of this expended in more or less random screening of compounds. These included compounds isolated from natural sources as well as synthetic compounds usually produced for some other purpose. An important impetus to this work was the large screening program of the National Cancer Institute (Zee-Cheng and Cheng, 1988), which during that time evaluated approximately 600,000 materials (both pure compounds and crude extracts from synthetic and natural sources). The primary screen comprised mouse leukemia cell lines, both in culture but primarily implanted intraperitoneally in mice. About 300 compounds from this screen proceeded to some level of evaluation in humans, and 42 were approved by the U.S. Food and Drug Administration as available medicines (Zee-Cheng and Cheng, 1988). However, a much smaller number went on to become useful drugs.

In a time when there were few targets other than DNA to focus on, such an empirical approach had merit, but also drawbacks. Apart from the fact that screens of this nature selected out antiproliferative cytotoxins, whose main therapeutic edge was based on cytokinetics (selectivity for cycling cells rather than cancer cells), it made little creative use of synthetic organic chemistry in the drug development process. While state-of-the-art structural chemistry was required to

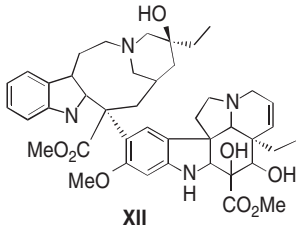
elucidate the structures of the many cytotoxic natural products revealed by these screens, the compounds were usually so complex that neither the compounds themselves or their analogues could be economically produced by synthesis. This is well illustrated by compounds such as homoharringtonine (X) (Zhou *et al.,* 1995), maytansine (XI) (Issell and Crook, 1978; Hamel, 1992), vincristine (XII) (Zhou and Rahmani, 1992), halichondrin B (XIII) (Hamel, 1992; Munro *et al.,* 1999), and esperamicin A_1 (XIV) (Golik *et al.,* 1987; Smith and Nicolaou, 1996). The structural complexity of these compounds severely limited the development of improved analogues and, in many cases, the production of sufficient quantities of drug to be viable. Thus chemistry could not be utilized to optimize the potencies of these compounds at their cellular target or to improve their pharmacokinetic properties. Hits from the primary screen progressed through a complex evaluation pathway to the clinic without chemical modification, and often without knowledge of their mechanism of action, with a heavy emphasis being placed on absolute potency.



X



XI



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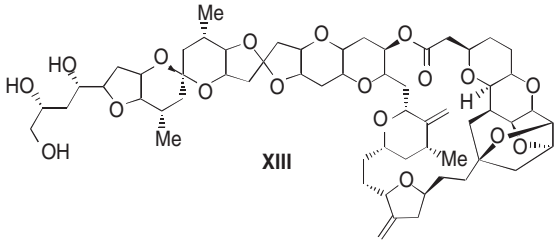
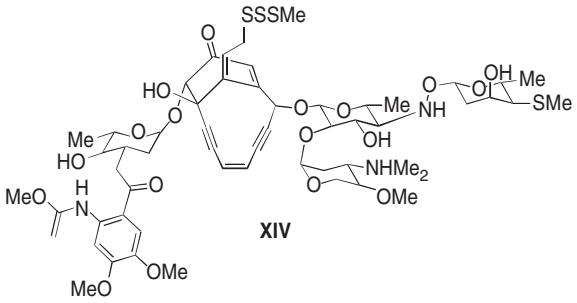


XIII



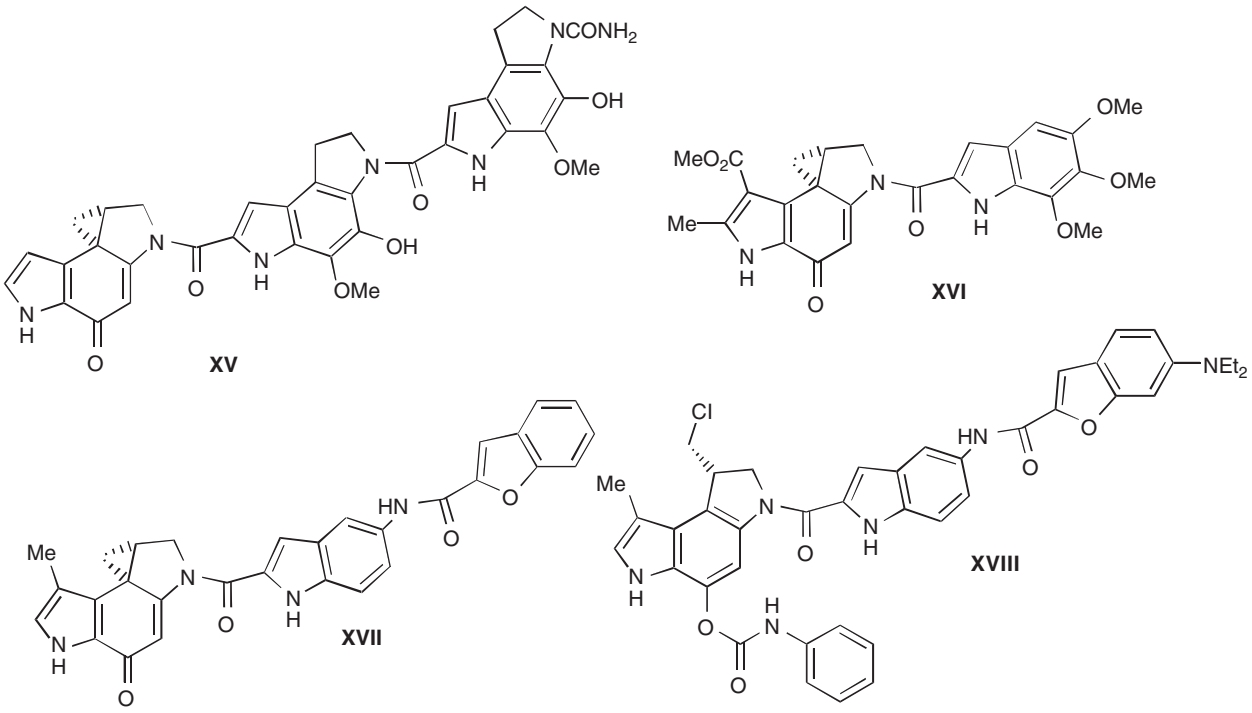
XIV

4. Organic Synthesis Catches Up: Development of Natural Product Leads

The "random screening" of natural products for their direct use as drugs was successful, introducing compounds such as anthracyclines (Hortobagyi, 1997), *Vinca* alkaloids (Zhou and Rahmani, 1992), epipodophyllotoxins (Damayanthi and Lown, 1998), and taxanes (Wall, 1998) into clinical use. However, its main value is now seen by many more in the discovery of new leads than in the direct procurement of clinical agents. This has come about partly because of a better understanding of the desirable physicochemical requirements of an anticancer drug, such as solubility, distributive properties, pharmacokinetics, and resistance to metabolism. There is no reason to expect that a natural product, evolved over millions of years to fulfill a particular function for its host, will have these properties optimized. This view has also been driven by the increasing power of modern synthetic organic chemistry. It is now much more likely that complex natural-product leads can be synthesized economically and therefore that analogues can be explored in an effort to optimize physicochemical properties. Two recent examples of the relatively rapid development of simpler clinical candidates from a complex natural-product lead are the cyclopropylindolines and the epothilones.

A. Cyclopropylindolines

In 1980, chemists at Upjohn determined the structure of the extraordinarily potent compound CC-1065 (XV) isolated from *Streptomcyes zelensis* (Martin *et al.,* 1980), and others later isolated related analogues (duocarmycins; e.g., XVI) from other *Streptomyces* species (Ichimura *et al.,* 1990). These compounds were shown to be very selective DNA alkylating agents, reacting only at adenine N3 sites in runs of adenines (Boger and Johnson, 1996). They demonstrated extraordinary cytotoxicity in cell culture (IC_{50} values down to 0.02 nM) and good antitumor activity in animal models at extremely low doses (Boger and Johnson, 1995, 1996). This high potency is thought to be due in part to their DNA-binding-induced activation, where a change in the conformation of the molecule as it binds to DNA disrupts its coplanar structure (Boger and Garbaccio, 1999). CC-1065 itself was shown to have chronic toxicity in animal models that prevented its further development (McGovren *et al.,* 1984). However, a large synthetic effort, devoted initially to the synthesis of CC-1065 itself (Kelly *et al.,* 1987; Boger and Coleman, 1988), allowed the preparation of other



analogues such as adozelesin (XVII) (Li *et al.,* 1991) and carzelesin (XVIII) (Mealy and Castaner, 1996) which did not have this side effect, and these synthetic compounds went on to clinical trial (Cristofanilli *et al.,* 1999; Pavildis *et al.,* 2000). Many other analogues of the original natural products have now been prepared, including some designed for use in prodrug approaches (Atwell *et al.,* 1998) (see Section 7), by a variety of routes.

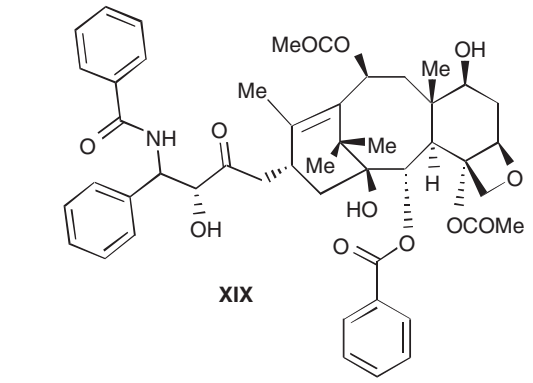
B. Microtubule-Stabilizing Agents

The microtubule stabilizing agent paclitaxel (XIX) is a major anticancer drug, originally isolated from the bark of the yew tree *Taxus baccata,* and is still produced from natural sources. More recently, other natural product microtubule-stabilizing agents have been reported, where organic synthesis has played a more important role. The cryptophycins are cyclic depsipeptide metabolites from cyanobacteria (Smith *et al.,* 1994), and their discovery sparked intensive synthetic studies (Liang *et al.,* 2000; Dhokte *et al.,* 1998), so that the first analogue to be tested clinically (cryptophycin-52; LY-355703) (XX) is a synthetic product (Panda *et al.,* 2000). Similarly, discovery (Bollag *et al.,* 1995; Gerth *et al.,* 1996) of the epothilones (e.g., epothilone A; XXI), metabolites from the myxobacterium *Sorangium cellulosum,* has sparked a similarly intensive synthetic effort (Harris and Danishefsky, 1999; Chappell *et al.,* 2000), allowing the development of synthetic analogues (e.g., desoxyepothilone B; XXII) with superior properties (Chou *et al.,* 1998). Comparison of structure–activity relationships for tubulin binding, together with binding models based on crystal structure data, have allowed postulation of a "common pharmacophore" shared by both paclitaxel and epothilone analogues (Giannakakou *et al.,* 2000; He *et al.,* 2000).

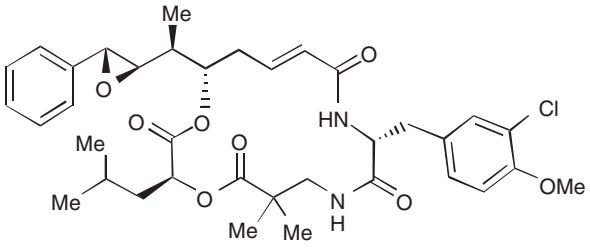
5. Development of Synthetic Compounds: Structure–Activity Relationships

Many cytotoxic synthetic compounds were also discovered during the random screening period. These were usually much less complex molecules than the natural products. There were also substantial advances in synthetic chemistry methodology, especially in mild and selective methods for carbon–carbon bond formation (Luh *et al.,* 2000) and in selective protecting groups. The range of analogues of these leads that could be prepared and tested was thus greatly expanded. Furthermore, advances in physical-organic chemistry led to a better understanding of how to define molecular structure in terms of a series of quantifiable parameters. Properties such as molecular charge distribution, energies of molecular orbitals, and both local (substituent) and global (whole-molecule) electronic, steric, and hydrophobic effects were accurately parameterized (Hansch and Leo, 1979).

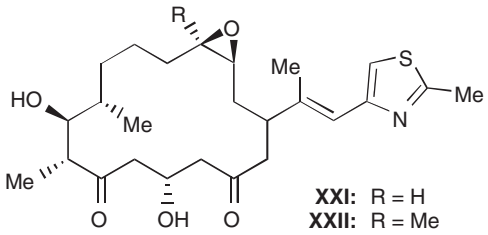
These advances provided the impetus for the development of quantitative structure–activity relationships (QSAR) for anticancer drugs, relating drug structure (defined in terms of the above parameters) to measures of biological activity such as cy-



XIX



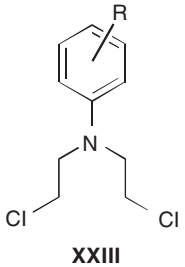
XX



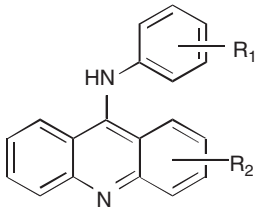
XXI: R = H
XXII: R = Me

totoxicity (Panthananickal *et al.,* 1979; Gupta, 1994), using the statistical methods originally developed by Hansch's group (Hansch *et al.,* 1996). For example, studies with aromatic nitrogen mustard alkylating agents (XXIII) quantified relationships between cytotoxicity and the electronic properties of substituents (R) (Panthananickal *et al.,* 1979; Palmer *et al.,* 1990) that were important in the design of hypoxia-selective drugs (Denny and Wilson, 1986) (see also Section 7). Similar QSAR studies with synthetic DNA-intercalating agents, especially the 9-anilinoacridines (XXIV), showed that their potency usually correlated positively with their strength of binding to DNA (Fink *et al.,* 1980; Denny *et al.,* 1982; Hartley *et al.,* 1988), resulting in a search for more tightly binding analogues. However, in this case the discovery that the antitumor activity of these compounds is due primarily to their ability to produce DNA strand breaks by interfering with the normal functioning of topoisomerase enzymes (Zwelling *et al.,* 1981; Drlica and Franko, 1988) showed that this relationship is not simple.

In addition to delineating structure–activity relationships for many individual classes of compounds, QSAR studies also provided more global information, including the likely



XXIII



XXIV

optimal lipophilicity for activity in particular cytotoxicity assays (Selassie *et al.,* 1986; Gupta, 1994). Approaches to minimize the effect of induced cellular resistance due to enhanced drug efflux were also suggested (Howbert *et al.,* 1990; Selassie *et al.,* 1990). Overall, the experience gained in this period in the application of QSAR ensured its acceptance as a standard tool in the development of anticancer drugs.

6. Immunotoxins: Synthetic Organic Chemistry Applied to Large Molecules

The first immunotoxins employed polypeptide cytotoxins or radioisotopes such as iodine-125 linked to monoclonal antibodies to improve delivery to tumor cells by antibody binding to tumor-associated antigens (Ghetie and Vitetta, 1994). Problems with this approach include difficulties in the consistent construction of suitable drug-antibody conjugates and the low proportion of toxin delivered (typically 0.001–0.01%), requiring very potent agents. The lack of toxin bystander effects to overcome the heterogeneity of clinical solid tumors (where many cells express little or no target antigen) was also a problem (Bodey *et al.,* 2000). However, this field is undergoing renewed interest, partly because of advances in organic chemistry that have provided more specific methods for stable linking of cytotoxins and antibodies (Carroll *et al.,* 1994). More efficient mechanisms of toxin cleavage in tumor tissue (DeFeo-Jones *et al.,* 2000) and very potent cytotoxins with good bystander effects, such as cyclopropylindoles (Charie *et al.,* 1995) and enediynes (Sievers *et al.,* 1999), have now been used.

7. Organic Synthesis in Rational Design: Tumor-Activated Prodrugs of Cytotoxins

This concept was devised to improve the specificity of the "classical" DNA-targeted systemic cytotoxins that essentially target cycling cell rather than tumor cell populations. Prodrugs can be defined as entities that are acted on in the body, either by chemical reactions or metabolism, resulting in formation of the desired, pharmacologically active species.

Tumor-activated prodrugs are systemic compounds that are activated selectively in tumor tissue by exploiting some unique physiologic, metabolic, or genetic difference between tumor and normal cells. The design of prodrug structures to meet the exacting set of criteria required draws heavily on concepts from synthetic organic chemistry. A modular approach to the design of such prodrugs has been suggested (Denny *et al.,* 1996), where a "trigger" unit is joined to a separate "effector" unit by a "linker," resulting in deactivation of the effector until metabolism of the trigger (Fig. 1). One advantage of this approach is that the properties of the units can be optimized independently for their particular role. One of the main challenges is to learn how to control and thus optimize both the distributive properties of the prodrug and the bystander effects of the activated effector, by modulating their chemical properties. Again, this requires novel chemical approaches.

A. The Trigger Unit

The primary function of the trigger unit is to determine selectivity by undergoing tumor-specific metabolism and (when the activation mechanism is extracellular) by containing functionality that excludes the prodrug from cells. The required properties of the trigger depend on the mechanism of selective activation that is chosen. The main phenomena exploited for selective activation of prodrugs in tumor tissue are discussed below.

1. Tumor Hypoxia

Tumor hypoxia is now recognized as a consistent and unique property of cells in solid tumors, as a result of a disorganized microvascular system in tumors (Vaupel *et al.,* 1991; Brizel *et al.,* 1994). Hypoxic cells in tumors limit response to radiation therapy (and some chemotherapy) and drive key aspects of tumor progression, such as angiogenesis and the selection of p53 mutations (Brown and Giaccia, 1998). Triggers for hypoxia-activated prodrugs are substrates for endogenous one-electron reductases such as cytochrome P450 reductase (Patterson *et al.,* 1998). The initial one-electron adducts formed by these compounds are rapidly back-oxidized by free oxygen in normal tissue to form the parent prodrug in a futile metabolism cycle. However, in hypoxic cells the one-electron adducts undergo further irreversible reduction or rearrangement to give cytotoxic species. Units used as triggers include nitroaromatics (e.g., in CB-1954; XXV) (Knox *et al.,* 1993), aliphatic *N*-oxides (e.g., in AQ4N; XXVI) (Patterson *et al.,* 2000), transition metal complexes (e.g., in SN-24771; XXVII) (Ware *et al.,* 1993), anthraquinones (e.g., in porfiromycin; XXVIII) (Haffty *et al.,* 1997), and aromatic di-*N*-oxides (e.g., in tirapazamine; XXIX) (Brown and Wang, 1998). Compounds using the latter two triggers, porfiromycin and tirapazamine, are in clinical trial as hypoxia-selective drugs, with tirapazamine showing particular promise (Denny and Wilson, 2000).

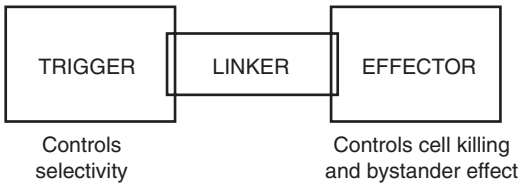


FIGURE 1 Modular design of hypoxia-activated prodrugs.

2. Lower Extracellular pH

Tumor cells in solid tumors consistently have lower extracellular pH levels than normal tissues because of the inefficient clearance of metabolic acids from chronically hypoxic cells (Tannock and Rotin, 1989). However this difference (0.6–0.8 pH unit) is small in chemical terms and has proved difficult to exploit. One interesting approach used releases a phosphoramide mustard from the sugar acetal (XXX) (Tietze *et al.,* 1989).

3. Therapeutic Radiation

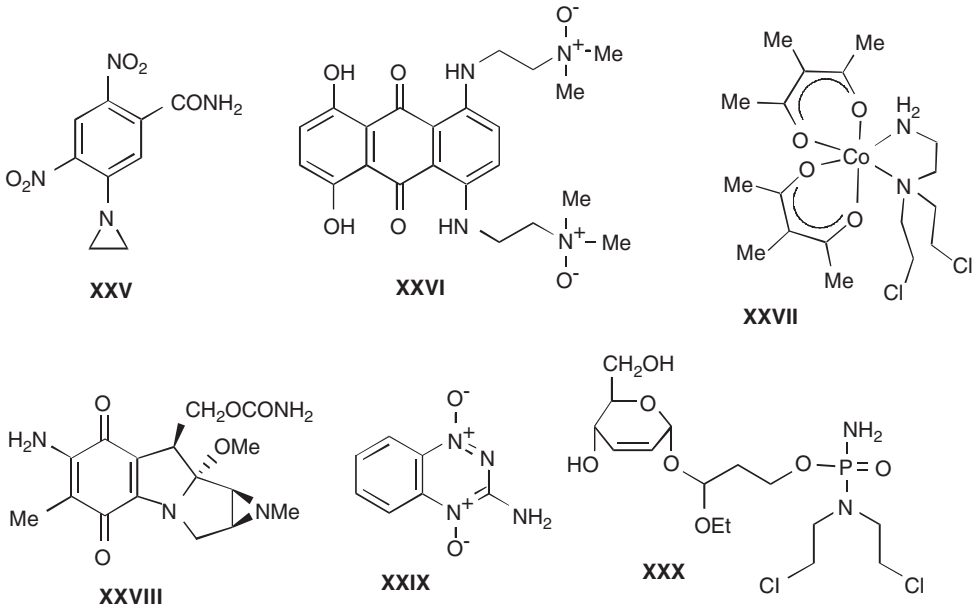
In principle, prodrugs can be activated by use of the reducing species produced from the radiolysis of water by ionizing radiation (Wilson *et al.,* 1998b). This obviates the need to rely on what may be variable levels of endogenous activating enzymes. However, a therapeutic dose of radiation (about 2 Gy) generates only a very small amount of reducing equivalents. This requires prodrugs with very efficient trigger units to capture these, substances capable of releasing/activating very potent effectors in an oxygen-inhibited process (Wilson *et al.,* 1998b). The design of such agents requires novel chemistry; for example, heterocyclic quaternary ammonium salts may be suitable triggers (Wilson *et al.,* 1998a).

4. Exogenous Enzymes; ADEPT and GDEPT Strategies

There are methodologies for either specifically locating (antibody-directed enzyme-prodrug therapy; ADEPT) or specifically generating (gene-directed enzyme-prodrug therapy; GDEPT) a nonhuman enzyme on tumor cells, and using this to selectively and catalytically activate a prodrug. In ADEPT, the enzyme is delivered as an antibody-enzyme conjugate that locates preferentially on tumor cells (Syrigos and Epenetos, 1999). In GDEPT, gene therapy methods are used to express the foreign enzyme preferentially in tumor cells (Denny and Wilson, 1998). In these approaches, the selected enzyme must efficiently and selectively metabolize the trigger unit.

B. The Linker Unit

This must deactivate the effector in the intact prodrug yet rapidly transmit an activating signal on metabolism of the trigger (Denny *et al.,* 1996). Several concepts for linkers have been explored, drawn from mechanistic organic chemistry:



1. Electron Release through an Aromatic System

Structure–activity relationship studies with aromatic nitrogen mustards have shown the high dependence of cytotoxicity on electron density at the mustard nitrogen (Panthananickal *et al.,* 1979; Palmer *et al.,* 1990). Thus, reduction of an electron-withdrawing nitro group to highly electron-donating hydroxylamine or amine groups on an aromatic system results in very large and instantaneous changes in electron redistribution, which can provide a large increase in the cytotoxicity of prepositioned groups. Thus, the simple 4-aminoaniline mustard (XXXI) is 17,000-fold more cytotoxic (CT_{10} clonogenic assay, UV4 cells) than its 4-nitro precursor (XXXII) (Palmer *et al.,* 1990).

2. Generation of Cationic Species

The concept of cation masking arose from knowledge about the chemical properties of tertiary amine *N*-oxides, which have much lower pK_a values (up to 5 pK_a units) than the amines themselves. This has led to the development of *N*-oxide prodrugs of DNA intercalating agents, which are much less toxic due to loss of the DNA binding ability normally conferred by the cationic tertiary amine (Wilson *et al.,* 1992). The hypoxia-selective prodrug AQ4N (XXVI) (Patterson *et al.,* 2000) is of this type.

3. Induced "Through-bond" Fragmentation

Charge-transfer reactions resulting in fragmentation of a molecule are well known in organic chemistry and have been adapted to the development of prodrugs. The best known examples are the 4-nitrobenzyl carbamates, wherein reduction to the corresponding 4-hydroxylamines is followed by fragmentation, because the increased electron release to the π system stabilizes the developing positive charge on the benzylic carbon (Hay *et al.,* 1999b) (Fig. 2). For example, 4-nitrobenzyl carbamate derivatives (XXXII, XXXIV) of mitomycin C (Mauger *et al.,* 1994) and amino-CBI (Hay *et al.,* 1999a) respectively are substrates for the nitroreductase from *E. coli* and show higher potencies in cell lines transfected with this enzyme. The kinetics of the fragmentation of the intermediate 4-hydroxylamines has been studied in a model system and can be modulated by additional substituents on the aromatic ring (Hay *et al.,* 1999b).

4. Induced "Through-space" Intramolecular Cyclization

Mechanisms related to the above but whereby the fragmentation is induced by "through-space" electronic interactions also have many parallels in organic chemistry, and allow electronic uncoupling of the trigger and effector units.

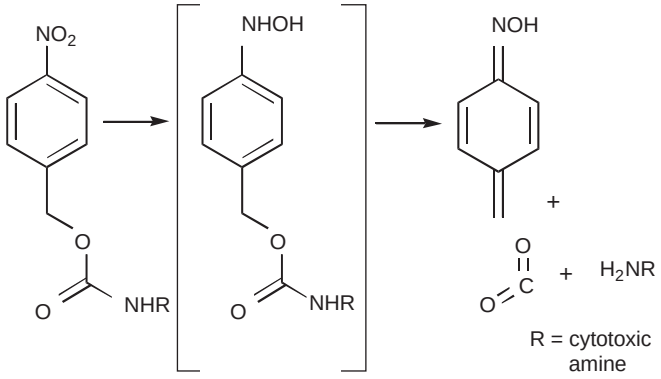


FIGURE 2 "Through-bond" fragmentation of reduced 4-nitrobenzyl carbamates.

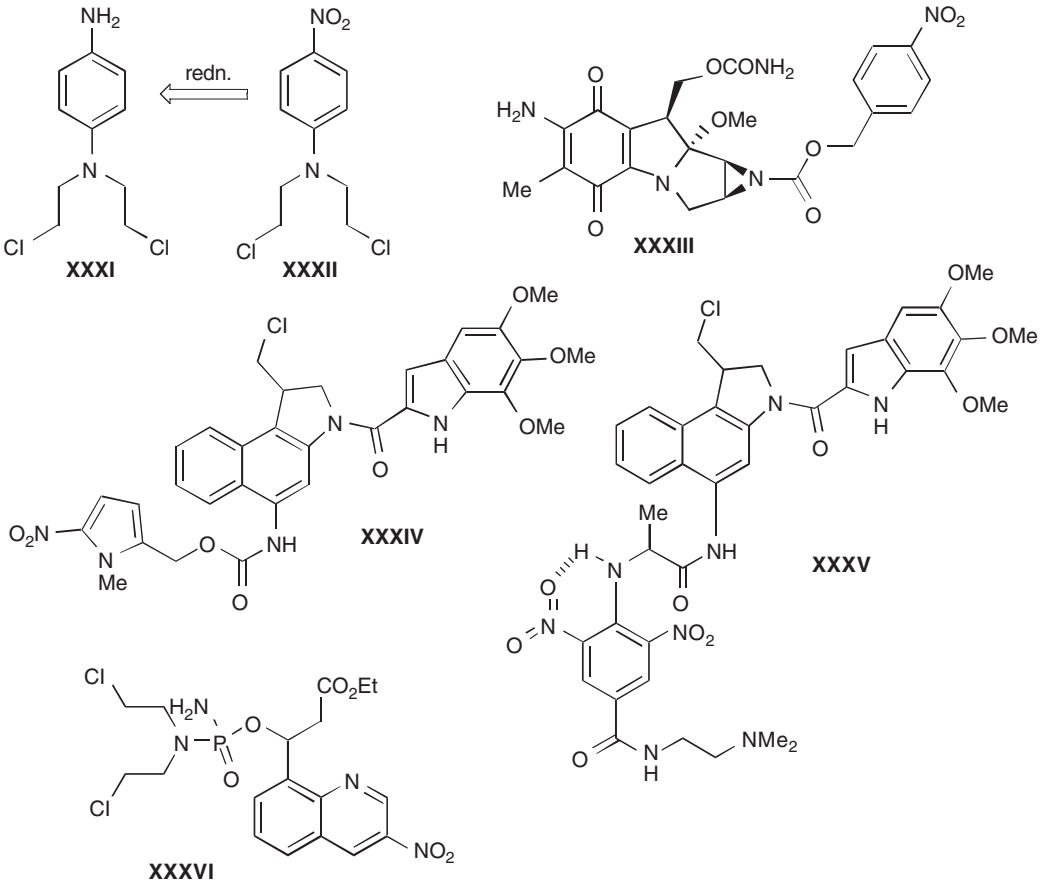
For example, the prodrug (XXXV) (Sykes *et al.,* 1999), with a *N*-(2,6-dinitrophenyl)amino trigger, is based on a concept used previously for the sequential cleavage of peptide bonds (Kirk and Cohen, 1969). H-bond "locking" by the second nitro group correctly positions the initial hydroxylamine or amine for fast cyclization/extrusion, resulting in facile cyclization even of very electron-deficient amines. Reduction of the nitroquinoline (XXXVI) was postulated to result in a related base-catalyzed through-space rearrangement, releasing a phosphoramide cytotoxin (Firestone *et al.,* 1991).

C. The Effector Unit

This requires a chemistry that allows it to be substantially deactivated in the prodrug form, but in the active form to be a potent cytotoxin capable of killing cells under a variety of pH and oxygen levels, and at all phases of the cell cycle. It is also required to have a substantial bystander effect (the ability to diffuse some distance from the cell where it is generated to kill surrounding tumor cells). This allows for the fact that only a small proportion of cells in a tumor may be capable of activating a particular prodrug. The most widely employed effectors have been DNA-alkylating agents, which have a well-defined chemistry and fulfill many of the above criteria (Denny and Wilson, 1993).

8. Early Genomics: Inhibitors of Transmembrane Tyrosine Kinases

During the 1990s, the overexpressed or mutated protein products of many oncogenes were identified and characterized, in work that significantly preceded the Human Genome Project. Many of these proteins were enzymes in the growth signal transduction pathways in cells, and particularly the transmembrane tyrosine kinases that initiate these pathways (Sedlacek, 2000). The development of specific small-molecule inhibitors of the intracellular kinase domains of these enzymes has been one of the major anticancer success stories of the second half of the 1990s and represents a major move away from drugs targeted at DNA function (Gibbs, 2000). The rapid development of this new class of agents has been achieved by mass screening in high-throughput isolated enzyme assays for initial leads, followed by optimization of these leads in the same assays. Analogues were prepared by a combination of classical one-at-a-time (singleton) synthesis and combinatorial



synthesis methods (see Section 9), with the latter becoming increasingly important. Development has also been greatly assisted by the availability of crystal structures (or derived computer models) of enzyme–inhibitor complexes.

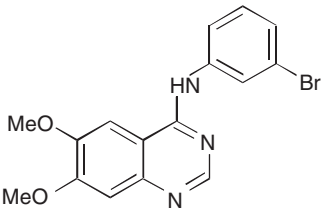
An illustrative example of these points is the development of inhibitors of the epidermal growth factor receptor, a member of the *erbB* family of transmembrane tyrosine kinases (Daly, 1999). Initial leads with moderate potencies in the enzyme assay were rapidly developed into extraordinarily potent and selective inhibitors (e.g., PD-153035; XXXVII; IC_{50} 0.029 nM) (Fry *et al.,* 1994; Bridges *et al.,* 1996). Work in several laboratories led to the development of the clinical agents Iressa (XXXVIII) (Ciardello *et al.,* 2000) and CP-358774 (XXXIX) (Moyer *et al.,* 1997). Later studies of the enzyme binding mode of these compounds led to the development of the irreversible inhibitor CI-1033 (XL) (Smaill *et al.,* 2000; Bridges, 1999), by appropriate positioning of a weak alkylating agent to pick up a unique cysteine residue (cys773) in the enzyme binding site. Another drug resulting from this new approach now in clinical trial is STI-571 (XLI) targeted at *c-abl* kinase (Schindler *et al.,* 2000). Also in development are compounds targeted against later enzymes in these pathways (MEK, ERK) (Gould and Stephano, 2000), and against the cyclin kinases that control the cell cycle checkpoints (Senderowicz and Sausville, 2000).

This work has shown that medicinal chemistry can be used to rapidly refine leads, achieving large increases in potency in isolated enzyme assays. However, it must be remembered that these screens do not provide information on the effectiveness of the compounds in living hosts. Thus, a constant emphasis on "drug-like" structures and early pharmacokinetic screening of representative analogues is important.

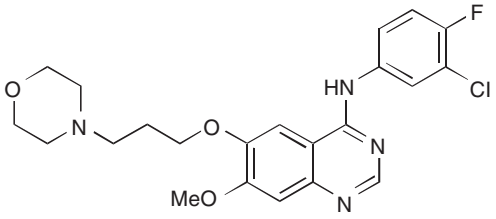
9. The Genomics/Proteomics Era: Combinatorial Chemistry

The imminent completion of the Human Genome Project (identification of all human genes) and the subsequent delineation of their function is expected to lead to the addition of a plethora of potential new enzyme targets for anticancer drugs, in addition to those uncovered recently (see Section 8). Rapid exploitation of this multiplicity of targets will require greater use of the various combinatorial methods of drug synthesis that have been developed over the last few years.

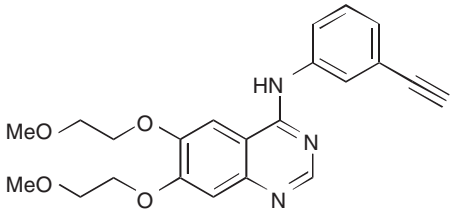
Combinatorial synthesis allows the rapid preparation of large numbers (libraries) of related compounds. The concept arose originally from the Merrifield solid-phase synthesis of peptides, and most of the first libraries were of polypeptides or oligonucleotides. However, more recently the technique



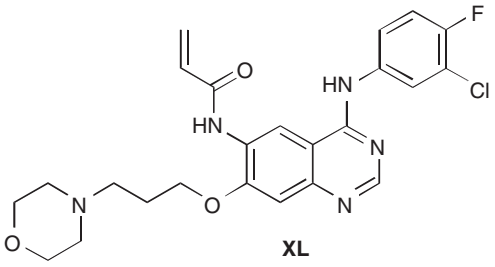
XXXVII



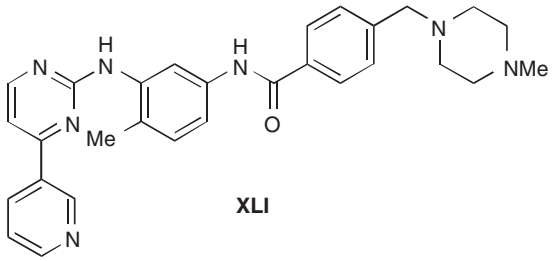
XXXVIII



XXXIX



XL



XLI

has expanded to cover many other types of small molecules, particularly heterocycles (Nefzi *et al.,* 1997). In conjunction with high-throughput screening, usually against purified enzyme targets, combinatorial synthesis has greatly influenced the way in which drugs are discovered and developed. Over the period from 1992 to 1999, the synthesis of 975 distinct libraries of compounds was reported in the literature (Dolle, 2000). Of the 240 for which biological data were reported, only a small proportion (less than 14%) were of cytotoxic anticancer drugs, but this proportion will presumably increase as enzyme inhibitors become a major class of anticancer drugs (see Section 8).

The basic idea behind combinatorial synthesis is to rapidly generate libraries of compound with chemical diversity. Two main types of libraries can be distinguished: discovery libraries and optimization libraries. Discovery libraries are large (arbitrarily defined as more than 5000 members) (Dolle, 2000) and not prepared against any preconceived target. The main aims in such libraries, which are used for primary screening for hits against new targets, is that members should be "drug-like" and as chemically diverse as possible. Optimization libraries are smaller, and the main aims are to improve the "drug-like" properties (potency, selectivity, solubility, pharmacokinetics, etc.) of some lead molecule.

While definition of drug-like character is difficult, the simple "rule-of-five" developed by Lipinski *et al.* (1997) is easy to apply as a rough guideline. This states that the lipophilicity (computed by the program CLOGP) should be less than 5, the molecular weight should be less than 500, the number of H-bond donor groups should be less than 5, and the number of H-bond acceptor groups should be less than 10. More sophisticated computer models that compare structure with data parameters constructed from existing drugs are also available (Wang and Ramnarayan, 1999; Frimurer *et al.,* 2000). True diversity in these larger sets is also hard to achieve; while computer programs to maximize this have been devised (Koehler and Villar, 2000), library diversity is often compromised by the availability of starting materials and the scope of the reactions employed.

A. Methods of Combinatorial Chemistry

Combinatorial chemistry is now a huge subject, and much has been written about the various concepts for library generation, as well as their strengths and weaknesses (Thompson and Ellman, 1996; Lam, 1997; Nefzi *et al.,* 1997; Pirrung, 1997). Most of the focus has been on "solid-phase" synthesis, since the concept arose originally from the Merrifield resin-attached approach to the synthesis of peptides. In this, temporary covalent attachment of the first building block to a resin allowed the stepwise construction of long polypeptides in an automatable fashion, with excess reagents being washed away after each step. Cleavage of the initial resin-building block bond allowed easy isolation of a pure, homogeneous product (Fig. 3).

The shift to true combinatorial synthesis (initially of peptides but later of other molecules) came with the "split-and-mix" concept, which allowed the preparation of very large libraries (Furka *et al.,* 1991; Lam *et al.,* 1991). In this method (Fig. 4), the first component is attached to small (100 μm) resin beads by a cleavable linkage, and the beads are divided into n equal portions. Each portion is then reacted with one of n different second components. After the reaction the individual portions are then remixed and redivided for a further round of treatment. After x such split-and-mix reaction cycles the beads will contain n^x different compounds, but each bead will contain only one pure compound (the "one-bead-one-compound" concept; Lam *et al.,* 1991). While peptide libraries built in this way are usually linear in nature, there is more scope with small-molecule libraries, where branched and scaffolded libraries can be constructed (Lam, 1997).

There are many methods for biological screening of the products from combinatorial libraries. For products designed to bind to enzyme receptors, enzyme-linked colorimetric as-

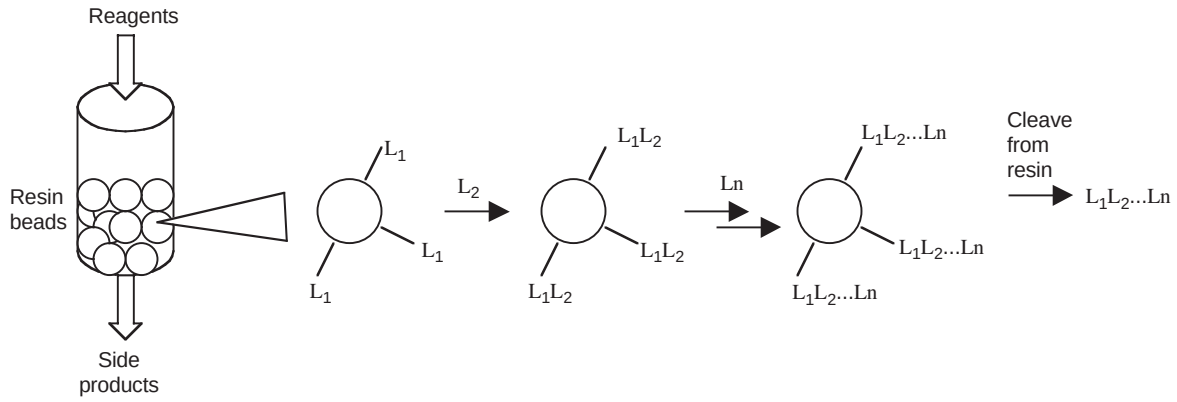


FIGURE 3 Concept of automated solid-phase synthesis.

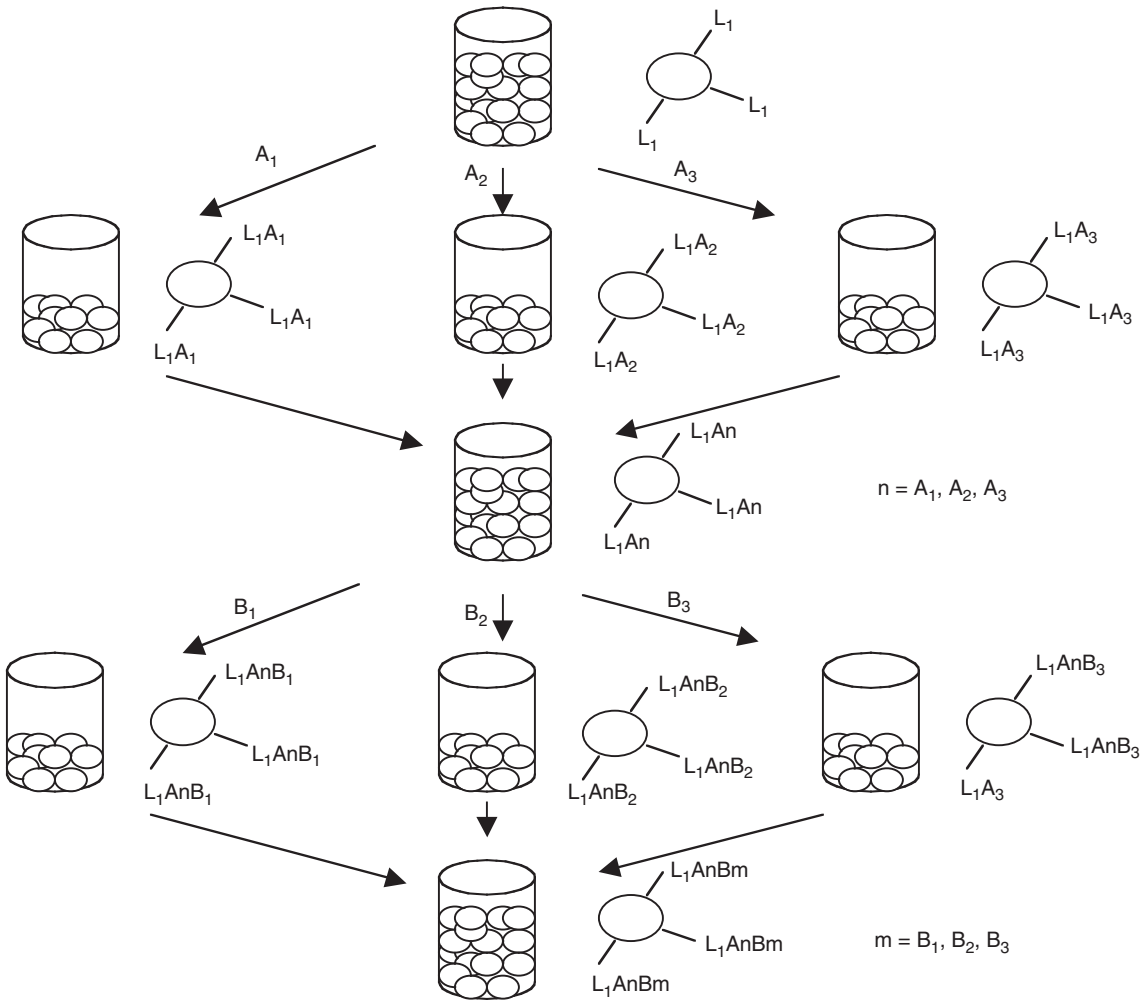


FIGURE 4 Concept of "split and mix, one-bead-one-compound," solid-phase synthesis.

says can be used to pick out individual "active" beads, where the synthesized compounds are still attached to the beads (Lam *et al.,* 1991). An ingenious approach (Salmon *et al.,* 1996) for screening using cellular cytotoxicity assays (of particular value for anticancer drugs) is to attach the compounds to the beads using two different linkers that release the compounds at different pHs. The beads are plated with tumor cells in soft agarose, and one of the linkers is cleaved at the resulting neutral pH, so that any active compounds released cause a zone of lysis around the bead. These beads can then be recovered, and the remaining compound still attached by the second linker can be cleaved and analyzed to determine its structure.

Many other methods of separately "tagging" beads to record their history and thus allow "deconvolution" of the library, allowing determination of the structure of the particular compound attached to a particular bead, have been reported (Lam *et al.,* 1997; Fitch *et al.,* 1999). These include the use of polynucleotides (Needels *et al.,* 1993) and small polypeptides (Kerr *et al.,* 1993), for which there are routine and sensitive detection methods. Tags more compatible with organic reagents include halocarbons (determined by gas chromatography after silylation) (Burbaum *et al.,* 1995) and alkylamines (recovered by hydrolysis, dansylated and analyzed by high-performance liquid chromatography) (Ni *et al.,* 1996). More recently, radiofrequency transmitting chips have been used to encode the chemical reaction history of each bead (Moran *et al.,* 1995). These have the advantage of nondestructive readout but are expensive. Smaller optimization libraries are often produced by solution-phase parallel synthesis, avoiding the deconvolution problem altogether (Schrieber, 2000).

B. Application of Combinatorial Chemistry to Anticancer Drugs

As for many applications, a good proportion of anticancer agent combinatorial libraries are on-bead libraries of peptides

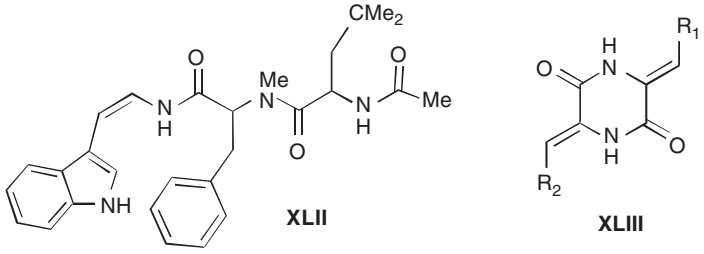
or peptidomimetics. Recent examples include synthetic epitopes for tumor-specific cytotoxic T lymphocytes (Linneman *et al.,* 1998), mimics of the tumor-associated antigen sialyl-Lewis A (Insug *et al.,* 1999), *O*-phosphotyrosyl surrogate-containing p56lck SH2-domain-binding peptides (Broadbridge and Sharma, 1999), peptide aptamers designed for E2F's DNA binding and dimerization domains (Fabrizio *et al.,* 1999), and peptidomimetic ligands for the pp60c-src substrate binding site (Orfi *et al.,* 1999; Maly *et al.,* 2000) and for farnesyl-protein transferase (Wallace *et al.,* 1996).

Smaller libraries of relatively simple nonpeptide compounds have largely been prepared by classical solution-phase parallel synthesis methods. Recent examples include analogues of aspergillamide (XLII) (Beck and Hess, 2000), piperazine-2,5-diones (XLIII) (Loughlin *et al.,* 2000), 2,5-diphenyloxazole-based inhibitors of Cdc25 phosphatase (XLIV) (Ducruet *et al.,* 2000), disulfide inhibitors of the thioredoxin redox system (XLV) (Kirkpatrick *et al.,* 1999), and 2,6,9-trisubstituted purines as CDK inhibitors (Chang *et al.,* 1999). Libraries of paclitaxel C7 esters (Bhat *et al.,* 1998) and benzodiazepines (as c-Src inhibitors) (Ramdas *et al.,* 1999) prepared by this method have also been reported. The technique has also been extended to the synthesis of tetraphenylporphyrins as potential photodynamic anticancer drugs (Berlin *et al.,* 1998).

Libraries of more complex molecules, requiring a greater number of steps to be performed, have been constructed using solid-phase synthesis. A series of 7α-alkylamide estradiols (e.g., XLVI) were prepared as estrogen receptor antagonists derivatives on an aminomethyl resin via a photolabile *o*-nitrobenzyl linker (Tremblay *et al.,* 1999). Finally, the total syntheses by Nicolaou's laboratory of libraries of analogues of the natural-product microtubule-stabilizing agents epothilone (XXI) (Nicolaou *et al.,* 1997a, 1997b) and sarcodictyin (XLVII) (Nicolaou *et al.,* 1998) by solid-phase parallel synthesis, and the recent report by Hecht's group (Leitheiser *et al.,* 2000) of the solid-phase synthesis of two deglycobleomycin analogues, indicate the combined power of this technique and modern organic synthesis to rapidly optimize the activity of very complex molecules.

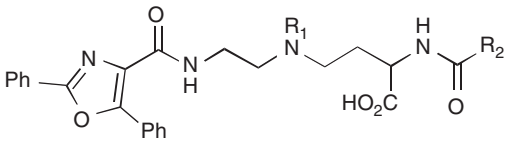
10. Conclusion

The role of synthetic organic chemistry in the development of anticancer drugs has changed over time as the power of the synthetic techniques has increased and as it has adapted to other changes in drug development. In the random screening era, particularly for natural products, the role of organic chemistry was primarily isolation and structure determination. A great deal of work on the total synthesis of such natural products was carried out, and this greatly helped to develop the power of modern organic synthesis. However, these syntheses were not generally economically competitive with purification from natural sources. The availability of analogues to allow

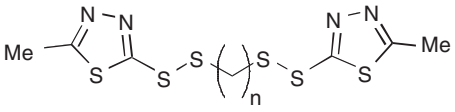


XLII

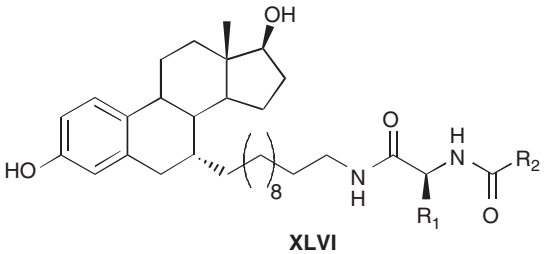
XLIII



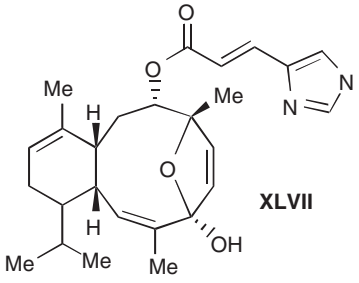
XLIV



XLV



XLVI



XLVII

the development of structure–activity relationships was also limited. In contrast, the easier preparation of analogues of the synthetic compounds discovered during this same era allowed the exploration of detailed structure–activity relationship studies, and these served to develop the tools of compound paramerization, molecular diversity, and QSAR modeling that have become standard tools of drug design.

More recently, the increasing power of synthetic organic chemistry has resulted in economic total syntheses of complex natural products, making these (and their possibly improved analogues) available in sufficient quantities to allow their clinical development. Natural products will thus increasingly serve as ideas for the development of anticancer drugs, rather than the drugs themselves. In addition, organic chemistry continues to provide novel concepts for the development of new types of anticancer drugs; a good example is the tumor-activated prodrugs.

The field of organic chemistry has also responded to the molecular biology revolution, which is in the process of identifying many new enzymes as potential targets for anticancer drugs, by the development of combinatorial methods for the simultaneous or parallel preparation of large numbers of compounds to accelerate drug discovery. In the process it is also developing the discrimination tools that will hopefully allow the preparation of compound libraries of diverse but "drug-like" structures. Recent work suggests that even very complex natural products and their analogues (epothilones, bleomycins) can be prepared by combinatorial methods. This will allow the development of much more detailed structure–activity relationships in order to optimize the anticancer activities of these compounds.

References

Atwell, G. J., Tercel, M., Boyd, M., Wilson, W. R., and Denny, W.A. (1998). Synthesis and cytotoxicity of 5-amino-1-(chloromethyl)-3-[(5,6,7-trimethoxyindol-2-yl)carbonyl]-1,2-dihydro-3*H*-benz[*e*]indole (amino-*seco*-CBI-TMI) and related 5-alkylamino analogues: new DNA minor groove alkylating agents. *J. Org. Chem.* **63,** 9414–9420.

Beck, B., and Hess, S. (2000). One-pot synthesis and biological evaluation of aspergillamides. *Bioorg. Med. Chem. Lett.* **10,** 1701–1705.

Berlin, K., Jain, R. K., and Richert, C. (1998). Are porphyrin mixtures favorable photodynamic anticancer drugs? A model study with combinatorial libraries of tetraphenylporphyrins. *Biotechnol. Bioeng.* **61,** 107–118.

Bhat, L., Liu, Y., Victory, S. F., Himes, R. H., and Georg, G. I. (1998). Synthesis and evaluation of paclitaxel C7 derivatives: solution phase synthesis of combinatorial libraries. *Bioorg. Med. Chem. Lett.* **8,** 3181–3186.

Bodey, B., Bodey, B., Jr., Siegel, S. E., and Kaiser, H. E. (2000). Genetically engineered monoclonal antibodies for direct anti-neoplastic treatment and cancer cell specific delivery of chemotherapeutic agents. *Curr. Pharm. Des.* **6,** 261–276.

Boger, D. L., and Coleman, R. S. (1988). Total synthesis of (+)-CC-1065 and ent-(-)-CC-1065. *J. Am. Chem. Soc.* **110,** 1321–1323.

Boger, D. L., and Garbaccio, R. M. (1999). Shape-dependent catalysis: insights into the source of catalysis for the CC-1065 and duocarmycin DNA alkylation reaction. *Acc. Chem. Res.* **32,** 1043–1052.

Boger, D. L., and Johnson, D. S. (1995). CC-1065 and the duocarmycins: unraveling the keys to a new class of naturally derived DNA alkylating agents. *Proc. Natl. Acad. Sci. USA* **92,** 3642–3649.

Boger, D. L., and Johnson, D. S. (1996). CC-1065 and the duocarmycins: understanding their biological function through mechanistic studies. *Angew. Chem., Int. Ed. Engl.* **35,** 1438–1474.

Bollag, D. M., McQueney, P. A., Zhu, J., Hensens, O., Koupal, L., Liesch, J., Goetz, M., Lazarides, E., and Woods, C. M. (1995). Epothilones, a new class of microtubule-stabilizing agents with a taxol-like mechanism of action. *Cancer Res.* **55,** 2325–2333.

Bridges, A. J. (1999). The rationale and strategy used to develop a series of highly potent, irreversible, inhibitors of the epidermal growth factor receptor family of tyrosine kinases. *Curr. Med. Chem.* **6,** 825–843.

Bridges, A. J., Zhou, H., Cody, D. R., Rewcastle, G. W., McMichael, A., Showalter, H. D. H., Fry, D. W., Kraker, A. J., and Denny, W. A. (1996). Tyrosine kinase inhibitors. 8. An unusually steep structure-activity relationship for analogues of 4-(3-bromoanilino)-6,7-dimethoxyquinazoline (PD 153035), a potent inhibitor of the epidermal growth factor receptor. *J. Med. Chem.* **39,** 267–276.

Brizel, D. M., Rosner, G. L., Harrelson, J., Prosnitz, L. R., and Dewhirst, M. W. (1994). Pretreatment oxygenation profiles of human soft tissue sarcomas. *Int. J. Radiat. Oncol. Biol. Phys.* **30,** 635–642.

Broadbridge, R. J., and Sharma, P. P. (1999). p56lck SH2 domain binding motifs from bead binding screening of peptide libraries containing phosphotyrosine surrogates. *Lett. Pept. Sci.* **6,** 335–341.

Brown, J. M., and Giaccia, A. J. (1998). The unique physiology of solid tumors: opportunities (and problems) for cancer chemotherapy. *Cancer Res.* **58,** 1405–1416.

Brown, J. M., and Wang, L. H. (1998). Tirapazamine: laboratory data relevant to clinical activity. *Anticancer Drug Des.* **13,** 529–539.

Burbaum, J. J., Ohlmeyer, M. H. J., Reader, J. C., Henderson, I., Dillard, L. W., Li, G., Randle, T. L., Sigal, N. H., Chelsky, D., and Baldwin, J. J. (1995). A paradigm for drug discovery employing encoded combinatorial libraries. *Proc. Natl. Acad. Sci. USA* **92,** 6027–6031.

Carroll, S. F., Bernhard, S. L., Goff, D.A., Bauer, R. J., Leach, W., and Kung, A. H. C. (1994). Enhanced stability in vitro and in vivo of immunoconjugates prepared with 5-methyl-2-iminothiolane. *Bioconjugate Chem.* **5,** 248–256.

Chang, Y-T., Gray, N. S., Rosania, G. R., Sutherlin, D. P., Kwon, S., Norman, T. C., Sarohia, R., Leost, M., Meijer, L., and Schultz, P. G. (1999). Synthesis and application of functionally diverse 2,6,9-trisubstituted purine libraries as CDK inhibitors. *Chem. Biol.* **6,** 361–375.

Chappell, M. D., Stachel, S. J., Lee, C. B., and Danishefsky, S. J. (2000). A. En route to a plant scale synthesis of the promising antitumor agent 12,13-desoxyepothilone B. *Org. Lett.* **2,** 1633–1636.

Charie, R. V. J., Jackel, K. A., Bourret, L. A., Derr, S. M., Tadayoni, B. M., Mattocks, K. M., Shah, S. A., Liu, C. N., Blattler, W. A., and Goldmacher, V. S. (1995). Enhancement of the selectivity and antitumor efficacy of a CC-1065 analogue through immunoconjugate formation. *Cancer Res.* **55,** 4079–4084.

Chou, T. C., Zhang, X. G., Balog, A., Su, D. S., Meng, D., Savin, K., Bertino, J. R., and Danishefsky, S. J. (1998). Desoxyepothilone B: an efficacious microtubule-targeted antitumor agent with a promising in vivo profile relative to epothilone B. *Proc. Natl. Acad. Sci. USA* **95,** 9642–9647.

Ciardiello, F., Caputo, R., Bianco, R., Damiano, V., Pomatico, G., De Placido, S., Bianco, A. R., and Tortora, G. (2000). Antitumor effect and potentiation of cytotoxic drugs activity in human cancer cells by ZD-1839 (Iressa), an epidermal growth factor receptor-selective tyrosine kinase inhibitor. *Clin. Cancer Res.* **6,** 2053–2063.

Colvin, O. M. (1999). An overview of cyclophosphamide development and clinical applications. *Curr. Pharm. Des.* **5,** 555–560.

Cristofanilli, M., Bryan, W. J., Miller, L. L., Chang, A. Y-C, Gradishar, W. J., Kufe, D. W., and Hortobagyi, G. N. (1998). Phase II study of adozelesin in untreated metastatic breast cancer. *Anticancer Drugs* **9,** 779–782.

Daly, R. J. (1999). Take your partners, please—signal diversification by the erbB family of receptor tyrosine kinases. *Growth Factors* **16,** 255–263.

Damayanthi, Y., and Lown, J. W. (1998). Podophyllotoxins: current status and recent developments. *Curr. Med. Chem.* **5,** 205–252.

DeFeo-Jones, D., Garsky, V. M., Wong, B. K., Feng, D-M., Bolyar, T., and Haskel, K. (2000). A peptide-doxorubicin 'prodrug' activated by prostate-specific antigen selectively kills prostate tumor cells positive for prostate-specific antigen in vivo. *Nat. Med.* **6,**1248–1252.

Denny, W. A., Atwell, G. J., Cain, B. F., Leo, A., Panthananickal, A., and Hansch, C. (1982). Potential Antitumor Agents. Part 36. Quantitative relationships between antitumor potency, toxicity and structure for the general class of 9-anilinoacridine antitumor agents. *J. Med. Chem.* **25,** 276–316.

Denny. W. A., Wilson. W. R., and Hay, M. P. (1996). Recent developments in the design of bioreductive drugs. *Br. J. Cancer* **74** (Suppl. 17), 32–38.

Denny, W. A., and Wilson, W. R. (1986). Considerations for the design of nitrophenyl mustards as drugs selectively toxic for hypoxic mammalian cells. *J. Med. Chem.,* **29,** 879–887.

Denny, W. A., and Wilson, W. R. (1993). Bioreducible mustards: a paradigm for hypoxia-selective prodrugs of diffusible cytotoxins (HPDCs). *Cancer Metab. Rev.* **12,** 135–151.

Denny, W. A., and Wilson, W. R. (1998). The design of selectively-activated anti-cancer prodrugs for use in antibody-directed and gene-directed enzyme-prodrug therapies. *J. Pharm. Pharmacol.* **50,** 387–394.

Denny, W. A., and Wilson, W. R. (2000). Tirapazamine: a bioreductive anticancer drug which exploits tumor hypoxia. *Exp. Opin. Inv. Drugs,* **9,** 2889–2901.

Dhokte, U. P., Khau, V. V., Hutchison, D. R., and Martinelli, M. J. (1998). A novel approach for total synthesis of cryptophycins via asymmetric crotylboration protocol. *Tetrahedron Lett.* **39,** 8771–8774.

Dolle, R. E. (2000). Comprehensive survey of combinatorial library synthesis: 1999. *J. Comb. Chem.* **2,** 383–433.

Drlica, K., and Franko, R. J. (1988). Inhibitors of DNA topoisomerases. *Biochemistry* **27,** 2253–2259.

Ducruet, A. P., Rice, R. L., Tamura, K., Yokokawa, F., Yokokawa, S., Wipf, P., and Lazo, J..S. (2000). Identification of new dual specificity phosphatase inhibitors in a targeted small molecule array. *Bioorg. Med. Chem.* **8,** 1451–1466.

Elion, G. B. (1989). Nobel Lecture. The purine path to chemotherapy. *Bioscience Rep.* **9,** 509–529.

Fabrizio, E., Le Cam, L., Polanowska, J., Kaczorek, M., Lamb, N., Brent, R., and Sardet, C. (1999). Inhibition of mammalian cell proliferation by genetically selected peptide aptamers that functionally antagonize E2F activity. *Oncogene* **18,** 4357–4363.

Fink, S. I., Leo, A., Yamakawa, M., Hansch, C., and Quinn, F. R. (1980). The quantitative structure-selectivity relationship of anthracycline antitumor activity and cardiac toxicity. *Farmaco* **35,** 965–979.

Firestone, A., Mulcahy, R. T., and Borch, R.F. (1991). Nitroheterocycle reduction as a paradigm for intramolecular catalysis of drug delivery to hypoxic cells. *J. Med. Chem.* **31,** 2933–2935.

Fitch, W. I , Baer, T. A., Chen, W., Holden, F., Holmes, C. P., Maclean, D., Shah, N., Sullivan, E., Tang, M., and Waybourn, P. (1999). Improved methods for encoding and decoding dialkylamine-encoded combinatorial libraries. *J. Comb. Chem.* **1,** 188–194.

Frimurer, T. M., Bywater, R., Nrum, L., Lauritsen, L. N., and Brunak, S. (2000). Improving the odds in discriminating "drug-like" from "non drug-like" compounds. *J. Chem. Inf. Comput. Sci.* **40,** 1315–1324.

Fry, D. W., Kraker, A. J., McMichael, A., Ambroso, L. A., Nelson, J. M., Leopold, W. R., Connors, R. W., and Bridges, A.J. (1994). A specific inhibitor of the epidermal growth factor receptor tyrosine kinase. *Science* **265,** 1093–1095.

Furka, A., Sebestyen, F., Asgedom, M., and Dibo, G. (1991). General method for rapid synthesis of multicomponent peptide mixtures. *Int. J. Pept. Protein Res.* **37,** 487–493.

Gerth, K., Bedorf, N., Hoefle, G., Irschik, H., and Reichenbach, H. (1996). Antibiotics from gliding bacteria. 74. Epothilons A and B: antifungal and cytotoxic compounds from *Sorangium cellulosum* (Myxobacteria): production, physico-chemical and biological properties. *J. Antibiot.* **49,** 560–563.

Ghetie, V., and Vitetta, E. (1994). Immunotoxins in the therapy of cancer: from bench to clinic. *Pharmacol. Ther.* **63,** 209–234.

Giannakakou, P., Gussio, R., Nogales, E., Downing, K. H., Zaharevitz, D., Bollbuck, B., Poy, G., Sackett, D., Nicolaou, K. C., and Fojo, T. (2000). A common pharmacophore for epothilone and taxanes: molecular basis for drug resistance conferred by tubulin mutations in human cancer cells. *Proc. Natl. Acad. Sci. USA* **97,** 2904–2909.

Gibbs, J. B. (2000). Mechanism-based target identification and drug discovery in cancer research. *Science* **287,** 1969–1973.

Golik, J., Dubay, G., Groenewold, G., Kawaguchi, H., Konishi, M., Krishnan, B., Ohkuma, H., Saitoh, K., and Doyle, T. W. (1987). Esperamicins, a novel class of potent antitumor antibiotics. 3. Structures of esperamicins A1, A2, and A1b. *J. Am. Chem. Soc.* **109,** 3462–3464.

Goodman, L. S., Wintrobe, M. M., Damesheck, W., Goodman, M. J., Gilman, A., and McLennan, M. T. (1946). Nitrogen mustard therapy. Use of methyl-bis(β-chloroethyl)amine hydrochloride and tris-(β-chloroethyl)-amine hydrochloride for Hodgkin's disease, lymphosarcoma, leukemia and certain allied and miscellaneous disorders. *J. Am. Med. Assoc.* **132,** 126–132.

Gould, M. C., and Stephano, J.L. (2000). Inactivation of Ca2+ action potential channels by the mek inhibitor PD 98059. *Exp. Cell Res.* **260,** 175–179.

Gupta, S. P. (1994). Quantitative structure-activity relationship studies on anticancer drugs. *Chem. Rev.* **94,** 1507–1551.

Haffty, B. G., Son, Y. H., Wilson, L. D., Papac, R., Fischer, D., Rockwell, S., Sartorelli, A. C., Ross, D., Sasaki, C. T., and Fischer, J. J. (1997). Bioreductive alkylating agent porfiromycin in combination with radiation therapy for the management of squamous cell carcinoma of the head and neck. *Radiat. Oncol. Inv.* **5,** 235–245.

Hamel, E. (1992). Natural products which interact with tubulin in the vinca domain: maytansine, rhizoxin, phomopsin A, dolastatins 10 and 15 and halichondrin B. *Pharmacol. Ther.* **55,** 31–51.

Hansch, C., Hoekman, D., and Gao, H. (1996). Comparative QSAR: toward a deeper understanding of chemicobiological interactions. *Chem. Rev.* **96,** 1045–1075.

Hansch, C., and Leo, A. J. (1979). "Substituent Constants for Correlation Analysis in Chemistry and Biology." Wiley-Interscience, New York, NY.

Harris, C. R., and Danishefsky, S. J. (1999). Complex target-oriented synthesis in the drug discovery process: a case history in the dEpoB series. *J. Org. Chem.* **64,** 8434–8456.

Hartley, J. A., Reszko, K., Zuo, E. T., Wilson, W. D., Morgan, A. R., and Lown, J. W. (1988). Characteristics of the interaction of anthrapyrazole anticancer agents with deoxyribonucleic acids; structural requirements for DNA binding, intercalation and photosensitisation. *Mol. Pharmacol.* **33,** 265–271.

Hay, M. P., Sykes, B. M., Denny, W. A., and Wilson, W. R. (1999a). A 2-nitroimidazole carbamate prodrug of 5-amino-1-(chloromethyl)-3-[(5,6,7-trimethoxyindol-2-yl)carbonyl]-1,2-dihydro-3H-benz[e]indole (amino-*seco*-CBI-TMI) for use with ADEPT and GDEPT. *Bioorg. Med. Chem. Lett.* **15,** 2237–2242.

Hay, M. P., Sykes, B. M., O'Connor, C. J., and Denny, W. A. (1999b). Substituent effects on the kinetics of reductively-initiated fragmentation of nitrobenzyl carbamates designed as triggers for bioreductive drugs. *J. Chem. Soc. Perkin Trans.* **I,** 2759–2770.

He, L., Jagtap, P. G., Kingston, D. G. I., Shen, H-J., Orr, G. A., and Horwitz, S. B. (2000). A common pharmacophore for taxol and the epothilones based on the biological activity of a taxane molecule lacking a C-13 side chain. *Biochemistry* **39,** 3972–3978.

Hortobagyi, G. N. (1997). Anthracyclines in the treatment of cancer. An overview. *Drugs* **54** (Suppl 4), 1–7.

Howbert, J. J., Grossman, C. S., Crowell, T. A., Rieder, B. J., Harper, R. W., Kramer, K. E., Tao, E. V., Aikins, J., and Poore, G. A. (1990). Novel agents effective against solid tumors: the diarylsulfonylureas. Synthesis, activities, and analysis of quantitative structure-activity relationships. *J. Med. Chem.* **33,** 2393–2407.

Ichimura, M., Ogawa, T., Takahashi, K., Kobayashi, E., Kawamoto, I., Yasuzawa, T., Takahashi, I., and Nakano, H. (1990). Duocarmycin SA, a new antitumor antibiotic from Streptomyces sp. *J. Antibiot.* **43,** 1037–1038.

Insug, O., Kieber-Emmons, T., Otvos, L., and Blaszczyk-Thurin, M. (1999). Peptides mimicking sialyl-Lewis A isolated from a random peptide library and peptide array. *Ann. NY. Acad. Sci.* **886,** 276–279.

Issell, B. F., and Crooke, S. T. (1978), Maytansine. *Cancer Treat. Rev.* **5,** 199–207.

Kelly, R. C., Gebhard, I., Wicnienski, N., Aristoff, P. A., Johnson, P. D., and Martin, D. G. (1987). Coupling of cyclopropapyrroloindole (CPI) derivatives. The preparation of CC-1065, ent-CC-1065, and analogs. *J. Am. Chem. Soc.* **109,** 6837–6838.

Kerr, J. M., Banville, S. C., and Zuckermann, R. N. J. (1993). Encoded combinatorial peptide libraries containing non-natural amino acids. *J. Am. Chem. Soc.* **115,** 2529–2531.

Kirk, K. L., and Cohen, L. A. (1969). Participation of the anilino group in peptide bond cleavage. The use of *t*-butyl 3,5-dinitro-2-fluorocarbanilate as a peptide reagent. *J. Org. Chem.* **34,** 395–397.

Kirkpatrick, D. L., Watson, S., Kunkel, M., Fletcher, S., Ulhaq, S., and Powis, G. (1999). Parallel syntheses of disulfide inhibitors of the thioredoxin redox system as potential antitumor agents. *Anticancer Drug Des.* **14,** 421–432.

Knox, R. J., Friedlos, F., and Boland, M. P. (1993). The bioactivation of CB 1954 and its use as a prodrug in antibody-directed enzyme prodrug therapy (ADEPT). *Cancer Metab. Rev.* **12,** 195–212.

Koehler, R. T., and Villar, H. O. (2000). Design of screening libraries biased for pharmaceutical discovery. *J. Comput. Chem.* **21,** 1145–1152.

Lam, K. S., Salmon, S., Hersh, E., Hruby, V., Kazmierski, W., and Knapp, R. (1991). A new type of synthetic peptide library for identifying ligand-binding activity. *Nature* **354,** 82–84.

Lam, K. S. (1997). Application of combinatorial library methods in cancer research and drug discovery. *Anticancer Drug Des.* **12,** 145–167.

Lam, K. S., Lebl, M., and Krchák, V. (1997). The "One-Bead-One-Compound" combinatorial library method. *Chem. Rev.* **97,** 411–448.

Leitheiser, C. J., Rishel, M. J., Wu, X., and Hecht, S. M. (2000). Solid-phase synthesis of bleomycin group antibiotics. Elaboration of deglycobleomycin A5. *Org. Lett.* **2,** 3397–3399.

Li, L. H., Kelly, R. C., Warpehoski, M. A., McGovern, J. P., Gebhard, I., and DeKoning, T. F. (1991). Adozelesin, a selected lead among cyclopropylpyrroloindole analogs of the DNA-binding antibiotic, CC-1065. *Invest. New Drugs* **9,** 137–148.

Liang, J., Moher, E. D., Moore, R. E., and Hoard, D. W. (2000). Synthesis of Cryptophycin 52 using the Sharpless asymmetric dihydroxylation: diol to epoxide transformation optimized for a base-sensitive substrate. *J. Org. Chem.* **65,** 3143–3147.

Linnemann, T., Brock, C., Sparbier, K., Muche, M., Mielke, A., Lukowsky, A., Sterry, W., Kaltoft, K., Wiesmuller, K. H., and Walden, P. (1998). Identification of epitopes for CTCL-specific cytotoxic T lymphocytes. *Adv. Exp. Med. Biol.* **451,** 231–235.

Lipinski, C. A., Lombardo, F., Dominy, B. W., and Feeney, P. J. (1997). Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Adv. Drug Delivery Rev.* **23,** 3–25.

Loughlin, W. A., Marshall, R. L., Carreiro, A., and Elson, K. E. (2000). Solution-phase combinatorial synthesis and evaluation of piperazine-2,5-dione derivatives. *Bioorg. Med. Chem. Lett.* **10,** 91–94.

Luh, T. Y., Leung, M. K., and Wong, K. T. (2000). Transition metal-catalyzed activation of aliphatic C-X bonds in carbon-carbon bond formation. *Chem. Rev.* **100,** 3187–3204.

Maly, D. J., Choong, I. C., and Ellman, J. A. (2000). Combinatorial target-guided ligand assembly: Identification of potent subtype-selective c-Src inhibitors. *Proc. Natl. Acad. Sci. USA* **97,** 2419–2424.

Martin, D. G., Chidester, C. G., Duchamp, D. J., and Mizsak, S.A. (1980). Structure of CC-1065 (NSC-298223), a new antitumor antibiotic. *J. Antibiot.* **33,** 902–903.

Mauger, A. B., Burke, P. J., Somani, H. H., Friedlos, F., and Knox, R. J. (1994). Self-immolative prodrugs: candidates for antibody-directed enzyme prodrug therapy in conjunction with a nitroreductase enzyme. *J. Med. Chem.* **37,** 3452–3458.

McGovren, J. P., Clarke, G. L., Pratt, E. A., and DeKoning, T.F. (1984). Preliminary toxicity studies with the DNA-binding antibiotic, CC-1065. *J. Antibiot.* **37,** 63–70.

Mealy, N., and Castaner, J. (1996). Carzelesin. U-80244. NSC-619029. Antineoplastic. Alkylating agent. *Drugs Future* **21,** 245–248.

Moran, E. J., Sarshar, S., Cargill, J. F., Shahbaz, M. M., Lio, A., Mjalli, A. M. M., and Armstrong, R. W. (1995). Radio frequency tag encoded combinatorial library method for the discovery of tripeptide-substituted cinnamic acid inhibitors of the protein tyrosine phosphatase PTP1B. *J. Am. Chem. Soc.* **117,** 10787–10788.

Moyer, J. D., Barbacci, E .G., Iwata, K. K., Arnold, L., Boman, B., Cunningham, A., Diorio, C., Doty, J., Morin, M. J., Moyer, M. P., Neveu, M., Pollack, V. A., Pustilnik, L. R., Reynolds, M. M., Sloan, D., Theleman, A., and Miller P. (1997). Induction of apoptosis and cell cycle arrest by CP-358774, an inhibitor of epidermal growth factor receptor tyrosine kinase. *Cancer Res.* **57,** 4838–4848.

Munro, M. H., Blunt, J. W., Dumdei, E. J., Hickford, S. J., Lill, R. E., Li, S., Battershill, C. N., and Duckworth, A. R. (1999). The discovery and development of marine compounds with pharmaceutical potential. *J. Biotech.* **70,** 15–25.

Needels, M. C., Jones, D. G., Tate, E. H., Heinkel, G. L., Kochersperger, L. M., Dower, W. J., Barrett, R. W., and Gallop, M. A. (1993). Generation and screening of an oligonucleotide-encoded synthetic peptide library. *Proc. Natl. Acad. Sci. USA* **90,** 10700–10704.

Nefzi, A., Ostresh, J. M., and Houghten, R. A. (1997). The current status of heterocyclic combinatorial libraries. *Chem. Rev.* **97,** 449–472.

Ni, Z-J., Maclean, D., Holmes, C. P., Murphy, M. M., Ruhland, B., Jacobs, J. W., Gordon, E. M., and Gallop, M. A. (1996). Versatile approach to encoding combinatorial organic syntheses using chemically robust secondary amine tags. *J. Med. Chem.* **39,** 1601–1608.

Nicolaou, K. C., Vourloumis, D., Li, T., Pastor, J., Winssinger, N., He, Y., Ninkovic, S., Sarabia, F., Vallberg, H., Roschangar, F., King, N. P., Finlay, M. R. V., Giannakakou, P., Verdier-Pinard, P., and Hamel, E. (1997a). Designed epothilones: combinatorial synthesis, tubulin assembly properties, and cytotoxic action against taxol-resistant tumor cells. *Angew. Chem., Int. Ed. Engl.* **36,** 2097–2103.

Nicolaou, K. C., Winssinger, N., Pastor, J., Ninkovic, S., Sarabia, F., He, Y., Vourloumis, D., Yang, Z., Li, T., Giannakakou, P., and Hamel, E. (1997b). Synthesis of epothilones A and B in solid and solution phase. *Nature* **387,** 268–272.

Nicolaou, K. C., Winssinger, N., Vourloumis, D., Ohshima, T., Kim, S., Pfefferkorn, J., Xu, J-Y., and Li, T. (1998). Solid and solution phase synthesis and biological evaluation of combinatorial sarcodictyin libraries. *J. Am. Chem. Soc.* **120,** 10814–10826.

Orfi, L., Waczek, F., Kovesdi, I., Meszaros, G., Idei, M., Horvath, A., Hollosy, F., Mak, M., Szegedi, Z., Szende, B., and Keri, G. (1999). New antitumor leads from a peptidomimetic library. *Lett. Pept. Sci.* **6,** 325–333.

Palmer, B. D., Wilson, W. R., Pullen, S. M., and Denny, W. A. (1990). Hypoxia-selective antitumor agents. 3. Relationships between structure and cytotoxicity against cultured tumor cells for substituted N,N-bis(2-chloroethyl)anilines. *J. Med. Chem.* **33,** 112–121.

Panda, D., Ananthnarayan, V., Larson, G., Shih, C., Jordan, M. A., and Wilson, L. (2000). Interaction of the antitumor compound cryptophycin-52 with tubulin. *Biochemistry* **39,** 14121–14127.

Panthananickal, A., Hansch, C., and Leo, A. (1979). Structure-activity relationship of aniline mustards acting against B-16 melanoma in mice. *J. Med. Chem.* **22,** 1267–1269.

Patterson, A. V., Saunders, M. P., Chinje, E. C., Patterson, L. H., and Stratford, I. J. (1998). Enzymology of tirapazamine metabolism: a review. *Anticancer Drug Des.* **13,** 541–573.

Patterson, L. H., McKeown, S. R., Ruparelia, K., Double, J. A., Bibby, M. C., Cole, S., and Stratford I. J. (2000). Enhancement of chemotherapy and radiotherapy of murine tumors by AQ4N, a bioreductively activated anti-tumor agent. *Br. J. Cancer* **82,** 1984–1990.

Pavlidis, N., Aamdal, S., Awada, A., Calvert, H., Fumoleau, P., Sorio, R., Punt, C., Verweij, J., van Oosterom, A., Morant, R., Wanders, J., and Hanauske A-R. (2000). Carzelesin phase II study in advanced breast, ovarian, colorectal, gastric, head and neck cancer, non-Hodgkin's lymphoma and malignant melanoma: a study of the EORTC early clinical studies group (ECSG). *Cancer Chemother. Pharmacol.* **46,** 167–171.

Pirrung, M. C. (1997). Spatially addressable combinatorial libraries. *Chem. Rev.* **97,** 473–488.

Ramdas, L., Bunnin, B. A., Plunkett, M. J., Sun, G., Ellman, J., Gallick, G., and Budde R. J. A. (1999). Benzodiazepine compounds as inhibitors of the Src protein tyrosine kinase: screening of a combinatorial library of 1,4-benzodiazepines. *Arch. Biochem. Biophys.* **368,** 394–400.

Salmon, S. E., Liu-Steven, R. H., Zhao, Y., Lebl, M., Krchnak, V., Wertman, K., Sepetov, N., and Lam, K. S. (1996). High-volume cellular screening for anticancer agents with combinatorial chemical libraries: a new methodology. *Mol. Diversity* **2,** 57–63.

Schindler, T., Bornmann, W., Pellicena, P., Miller, W.T., Clarkson, B., and Kuriyan, J. (2000). Structural mechanism for STI-571 inhibition of abelson tyrosine kinase. *Science* **289,** 1938–1942.

Schrieber, S. L. (2000). Target-oriented and diversity-oriented organic synthesis in drug discovery. *Science* **287,** 1964–1969.

Sedlacek, H .H. (2000). Kinase inhibitors in cancer therapy: a look ahead. *Drugs* **59,** 435–476.

Selassie, C. D., Strong, C. D., Hansch, C., Delcamp, T. J., Freisheim, J.H., and Khwaja, T. A. (1986). Comparison of triazines as inhibitors of L1210 dihydrofolate reductase and of L1210 cells sensitive and resistant to methotrexate. *Cancer Res.* **46,** 744–756.

Selassie, C. D., Hansch, C., and Khwaja T. A. (1990). Structure-activity relationships of antineoplastic agents in multidrug resistance. *J. Med. Chem.* **33,** 1914–1919.

Senderowicz, A. M., and Sausville E. A. (2000). Preclinical and clinical development of cyclin-dependent kinase modulators. *J. Natl. Cancer Inst.* **92,** 376–387.

Sievers, E. L., Appelbaum, F. R., Spielberger, R. T., Forman, S. J., Flowers, D., Smith, F. O., Shannon-Dorcy, K., Berger, M. S., and Bernstein I. D. (1999). Selective ablation of acute myeloid leukemia using antibody-targeted chemotherapy: A phase I study of an anti-CD33 calicheamicin immunoconjugate. *Blood* **93,** 3678–3684.

Smaill, J. B., Rewcastle, G. W., Bridges, A. J., Zhou, H., Showalter, H. D. H., Fry, D. W., Nelson, J. M., Sherwood, V., Elliott, W. L., Vincent, P. W., DeJohn, D., Loo, J. A., Gries, K. D., Chan, O. H., Reyner, E. L., Lipka, E., and Denny, W. A. (2000). Tyrosine kinase inhibitors. 17. Irreversible inhibitors of the epidermal growth factor receptor: 4-(phenylamino)quinazoline- and 4-(phenylamino)pyrido[3,2-*d*]pyrimidine-6-acrylamides bearing additional solubilizing functions. *J. Med. Chem.* **43,** 1380–1397.

Smith, A. L., and Nicolaou, K. C. (1996). The enediyne antibiotics. *J. Med. Chem.* **39,** 2103–2017.

Smith, C. D., Zhang, X., Mooberry, S. L., Patterson, G. M. L., and Moore, R. E. (1994). Cryptophycin: a new antimicrotubule agent active against drug-resistant cells. *Cancer Res.* **54,** 3779–3784.

Sykes, B. M., Atwell, G. J., Hogg, A., Wilson, W. R., O'Connor, C. J., and Denny, W. A. (1999). N-Substituted 2-(2,6-dinitrophenylamino)-propanamides: novel prodrugs that release a primary amine via nitroreduction and intramolecular cyclization. *J. Med. Chem.* **42,** 346–355.

Syrigos, K. N., and Epenetos, A. A. (1999). Antibody directed enzyme prodrug therapy (ADEPT): a review of the experimental and clinical considerations. *Anticancer Res.* **19,** 605–613.

Tannock, I. F., and Rotin. D. (1989). Acid pH in tumors and its potential for therapeutic exploitation. *Cancer Res.* **49,** 4373–4384.

Thompson, L. A., and Ellman, J. A. (1996). Synthesis and application of small molecule libraries. *Chem. Rev.* **96,** 555–600.

Tietze, L. F., Newman, M., Mollers, T., Fischer, R., Glusenkamp, K-H., Rajewsky, M., and Jahde, E. (1989). Proton-mediated liberation of aldophosphamide from a non-toxic prodrug: a strategy for tumor-selective activation of cytocidal drugs. *Cancer Res.* **49,** 4179–4184.

Tremblay, M. R., Simard, J., and Poirier, D. (1999). Parallel solid-phase synthesis of a model library of 7α-alkylamide estradiol derivatives as potential estrogen receptor antagonists. *Bioorg. Med. Chem. Lett.* **9,** 2827–2832.

Vaupel, P., Schlenger, K., Knoop, C., and Hockel, M. (1991). Oxygenation of human tumors: evaluation of tissue oxygen distribution in breast cancers by computerized O2 tension measurements. *Cancer Res.* **51,** 3316–3322.

Wall, M. E. (1998). Camptothecin and taxol: discovery to clinic. *Med. Res. Rev.,* **18,** 299–314.

Wallace, A., Koblan, K., Hamilton, K., Marquis-Omer, D. J., Miller, P. J., Mosser, S. D., Omer, C. A., Schaber, M. D., Cortese, R., Oliff, A., Gibbs, J. B., and Pessi A. (1996). Selection of potent inhibitors of farnesyl-protein transferase from a synthetic tetrapeptide combinatorial library. *J. Biol. Chem.* **271,** 31306–31311.

Wang, J., and Ramnarayan, K. (1999). Toward designing drug-like libraries: a novel computational approach for prediction of drug feasibility of compounds. *J. Comb. Chem.* **1,** 524–533.

Ware, D. C., Palmer, B. D., Wilson, W. R., and Denny, W. A. (1993). Hypoxia-selective antitumor agents. 7. Metal complexes of aliphatic mustards as a new class of hypoxia-selective cytotoxins. Synthesis and evaluation of cobalt(III) complexes of bidentate mustards. *J. Med. Chem.* **36,** 1839–1846.

Wilman, D. E. V., and Connors, T. A. (1983). Molecular structure and antitumour activity of alkylating agents. *In* "Molecular Aspects of Anticancer Drug Action" (S. Neidle and M. J. Waring, eds.), pp. 234–282, MacMillan, London.

Wilson, W. R., Ferry, D. M., Tercel, M., Anderson, R. F., and Denny, W. A. (1998a). Reduction of nitroarylmethyl quaternary ammonium prodrugs of mechlorethamine by radiation. *Radiat. Res.* **149,** 237–245.

Wilson, W. R., Tercel, M., Anderson, R. F., and Denny, W. A. (1998b). Radiation-activated prodrugs as hypoxia-selective cytotoxins: model studies with nitroarylmethyl quaternary salts. *Anticancer Drug Des.* **13,** 663–685.

Wilson, W. R., van Zijl, P., and Denny, W. A. (1992). Bis-bioreductive agents as hypoxia-selective cytotoxins: N-oxides of nitracrine. *Int. J. Radiat. Oncol. Biol. Phys.* **22,** 693–697.

Zee-Cheng, R. K-Y., and Cheng, C. C. (1988). Screening and evaluation of anticancer agents. *Methods Find. Exp. Clin. Pharmacol.* **10,** 67–101.

Zhou, D. C., Zittoun, R., and Marie, J. P. (1995). Homoharringtonine: an effective new natural product in cancer chemotherapy. *Bull. Cancer* **82,** 987–995.

Zhou, X. J., and Rahmani, R. (1992). Preclinical and clinical pharmacology of vinca alkaloids. *Drugs* **44** (Suppl. 4), 1–16.

Zwelling, L. A., Michaels, S., Erickson, L. C., Ungerleider, R. S., Nichols, M., and Kohn, K. W. (1981). Protein-associated deoxyribonucleic acid strand breaks in L1210 cells treated with the deoxyribonucleic acid intercalating agents 4′-(9-acridinylamino)methanesulfon-*m*-anisidide and Adriamycin. *Biochemistry* **20,** 6553–6563.