

# THE DESIGN AND DEVELOPMENT OF ANTI-CANCER DRUGS

Cancer is one of the most widespread and feared diseases in the Western world today - feared largely because it is known to be difficult to cure. The main reason for this difficulty is that cancer results from the uncontrolled multiplication of subtly modified normal human cells. One of the main methods of modern cancer treatment is drug therapy (chemotherapy).

The majority of drugs used for the treatment of cancer today are cytotoxic (cell-killing) drugs that work by interfering in some way with the operation of the cell's DNA. Cytotoxic drugs have the potential to be very harmful to the body unless they are very specific to cancer cells - something difficult to achieve because the modifications that change a healthy cell into a cancerous one are very subtle. A major challenge is to design new drugs that will be more selective for cancer cells, and thus have lesser side effects. Initially the specificity of drugs was worked out simply by testing on animals, but now it is possible to use our knowledge of cancer cell biology to actively design drugs to be more specific. However, animal tests still need to be carried out at some point.

As with any pharmaceutical, new anticancer drugs are developed in a three-step process.

## **Step 1 - Initial discovery**

A wide range of compounds, both natural and synthetic, are tested in high-capacity screens to discover molecules with useful properties.

## **Step 2 - Molecular modification of a known compound**

A molecule that shows suitable properties is chemically altered to give it the best combination of properties to make the most effective anti-cancer drug.

## **Step 3 - Development into a useful pharmaceutical**

Because the above process is very time-consuming and expensive, the new discovery is usually patented at this time so that the discoverers can eventually recover some of these costs. The most effective route for synthesising the molecule is then worked out. A long process of advanced testing is then begun, ending up with tests on patients in specialised hospitals. If the results are favourable, the drug is then able to be released for use.

The process of drug development is very long and involved, with maybe only one in ten thousand of the molecules originally tested finally being clinically used. This article describes different types of drug interactions and discusses the development of the cytotoxic drug *asulacrine* from the less cytotoxic *amsacrine*.

## INTRODUCTION

Cancer is a major disease. About one in four people will get it in some form during their lifetime, and at the present time about one in five of all deaths are due to cancer. Currently there are three major ways of treating cancer: radiation therapy, surgery and cytotoxic drugs. All of these have significant limitations, but drugs offer the only approach to treat cases where the cancer has spread (metastasised) through the body. Other less well established

options include drugs that can stimulate the immune system to assist the body itself to fight the disease, and non-cytotoxic drugs that can prevent cancer cells from multiplying.

This article focuses on the development of drugs to combat cancer. Over the last fifty years about 500 000 natural and synthetic chemical compounds have been tested for anticancer activity, but only about 25 of these are in wide use today. This gives an indication of the difficulty of this problem. Currently drugs are available that significantly reduce the mortality rates for some cancers (e.g. leukemia and testicular and ovarian cancer), and give longer overall patient survival times. However, there is a long way to go before truly curative drugs are available for most cancers. The reason for this is simple: cancer cells are not foreign to the body but are simply subtly mutated forms of normal human cells, and it is very different to synthesise drugs that can tell the difference.

### **The origins of cancer**

Cancer is a term which describes a group of perhaps 120 different diseases which share some broad similarities. In these diseases, a single cell begins to divide uncontrollably forming a tumour, and eventually bits of this tumour break off and form new tumours (this is known as metastasis). Normal cells do not divide in this fashion, being kept under tight control by a number of different biological mechanisms that are still being explored. We do know that cell division is controlled by a relatively small group of enzymes. Some of these operate to form a communication network, relaying growth signals from the surface of the cell to its DNA and telling it when to begin dividing. Others work as a surveillance team, preventing a cell with damaged DNA from reproducing by first repairing the damage or by instructing it to die. However, sometimes the damage (mutation) occurs in the DNA that codes for these enzymes, so that they are themselves defective. Such a cell will divide uncontrollably, and produce daughter cells that do the same.

A human cell contains approximately 100 000 genes, of which about 50 are known as proto-oncogenes<sup>1</sup>. Many of these code for the enzymes that make up the communication and surveillance systems described above. If a cell accumulates critical mutations in five or six of these proto-oncogenes, the resulting multiple but subtle changes are likely to result in a fully malignant cell, capable of forming a tumour.

## **DESIGNING DRUGS TO COMBAT CANCER**

The drugs used to combat cancer belong to one of two broad categories. The first is cytotoxic (cell killing) drugs and the second is cytostatic (cell stabilising drugs). Both categories lead to a reduction in the size of the tumour because cancer cells (for various reasons) have such a high mortality rate that simply preventing them from dividing will lead to a reduction in the population.

### **Cytotoxic drugs**

Cytotoxic drugs work by interfering with DNA replication. Because cancer cells are rapidly dividing they are rapidly synthesizing new DNA - and if this is damaged the cell will die. There are three main groups of molecules that can be used to interfere with DNA replication:

- *antimetabolites*: molecules that appear to be nucleotides and so are incorporated into DNA, leading to non-functional DNA.

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<sup>1</sup>Protooncogenes are genes that, if mutated, predispose the cell to becoming a cancer cell.

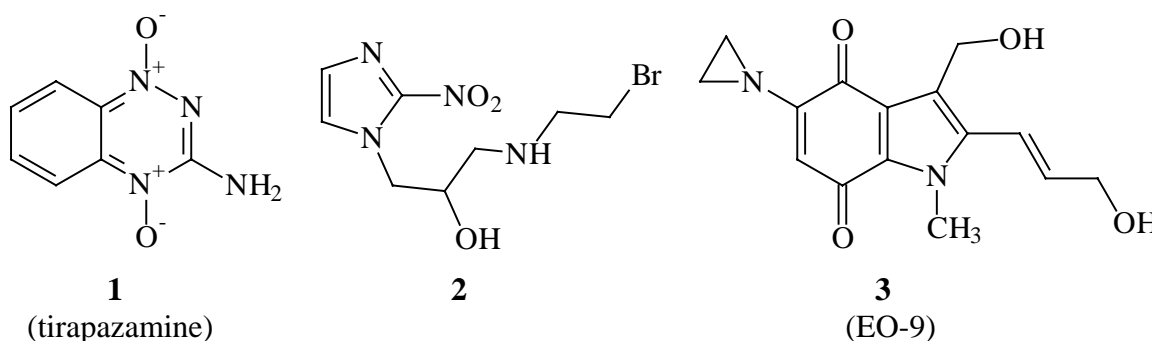
- *alkylating agents*: molecules that permanently attach to the DNA, distorting its shape. Unfortunately these also attach to many other molecules in cells.
- *DNA-binding agents*: molecules that attach to the DNA chain, break it, disengage from the chain and then attach to another chain to repeat the process. These usually function in conjunction with an enzyme.

DNA-binding agents are currently the most effective drugs used, but usually a patient is given a combination of drugs from several of these groups to take advantage of the different ways in which they work.

None of these drugs are 'cancer cell-specific' — they are all simply 'quickly dividing cell-specific'. This is the explanation of the side effects associated with chemotherapy (nausea, immunosuppression, ulceration and hair loss): the drugs not only attack cancer cells but also any other quickly dividing cells such as those in bone marrow or the gut. For this reason, work is currently being done into targeting drugs more specifically for cancer cells by giving the patient a precursor of the drug (a 'prodrug') which is only activated in the cancerous tumour. Two possible methods for doing this are outlined below.

#### *Prodrug activation by hypoxic cancer cells*

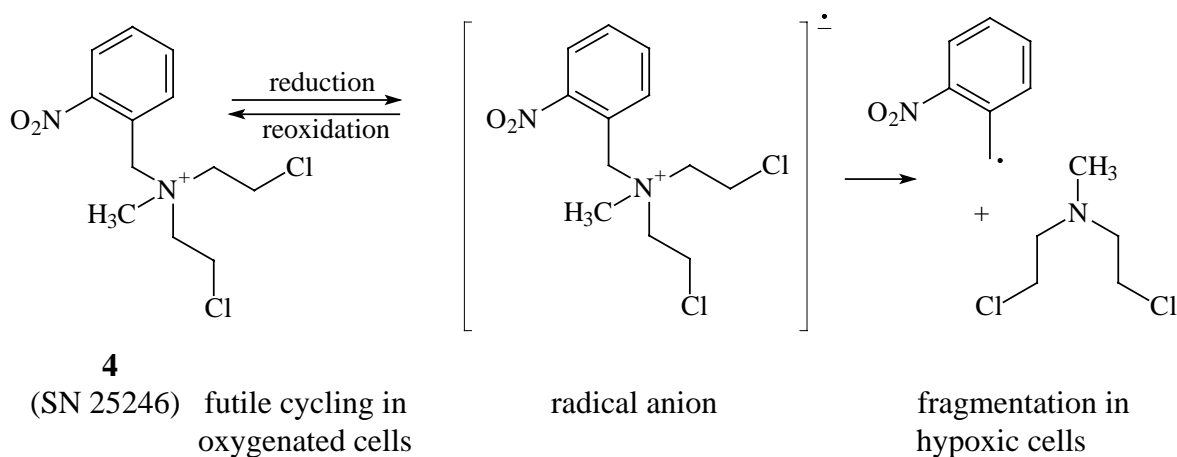
It has been noticed that cells in solid tumours have chronic hypoxia (oxygen deprivation) and that this property is unique to these cells. In the body, the oxygen needed by cells for respiration is carried over long distances in the bloodstream complexed with hemoglobin, but has to get from the bloodstream to cells by diffusion. Because it is consumed by living cells, oxygen concentration decreases with distance from the nearest blood vessel, and at about 150  $\mu\text{m}$  falls essentially to zero. In normal tissue the blood vessel network is so well-developed that all cells are well supplied with oxygen, but virtually all solid tumours larger than about 1mm in diameter possess a proportion (usually a few percent) of chronically hypoxic cells. Much work has recently gone into the concept of developing drugs that are activated only in hypoxic cells, thus leaving healthy cells intact. Molecules designed as hypoxia-activated prodrugs have a variety of different chemical structures, and three examples (compounds **1** - **3**) are shown below. Two of these (tirapazamine and EO-9) are currently in clinical trials.



Regardless of their structure, hypoxia-activated prodrugs function by the inactive form of the drug being converted into the active drug by a mechanism that can only occur in hypoxic (and therefore cancer) cells. The initial prodrug must be nontoxic and able to diffuse to the hypoxic cells in a tumour, and is designed so that it can easily be reduced (i.e. pick up an electron from cellular enzymes). While this reduction will happen in all cells, in oxygenated

cells it will be immediately reversed to regenerate the parent prodrug. However, in hypoxic cells this step is unable to be reversed, allowing the reduced drugs to react further, becoming cytotoxic.

A specific example of a class of hypoxia-activated prodrugs being developed in the Cancer Research Laboratory in Auckland, and the mechanism of their function, is given in **Figure 1**. This prodrug (**4**), designated SN (screen number) 25246, contains the  $N(\text{CH}_2\text{CH}_2\text{Cl})_2$  group known as a mustard group. This is usually a very cytotoxic alkylating agent, able to permanently attach to the DNA, distorting its shape and preventing it from separating. In the prodrug form this mustard is deactivated by the positive charge on the nitrogen (the reactivity and toxicity of mustards depends on their having an electron-rich nitrogen). This prodrug is rapidly reduced by enzymes present in all cells, resulting in an intermediate radical anion (**Figure 1**). In normal, oxygenated cells this radical anion, once formed, is rapidly re-oxidised by the free oxygen present to reform the non-toxic prodrug. However, in the absence of oxygen the radical anion eventually fragments, releasing the mechlorethamine mustard. This is a cytotoxic drug that has been used in patients, but resulted in side effects. Such side effects will be lessened if the drug is released only in hypoxic (i.e. cancer) cells.



**Figure 1**

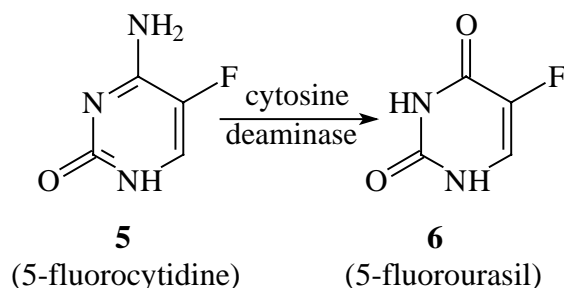
#### *Gene-Directed Enzyme-Prodrug Therapy (GDEPT)*

Alternatively, the prodrug can be activated by an enzyme that has been produced in only the cancer cells. A gene coding for a non-human enzyme is integrated into a retrovirus<sup>2</sup>, and the engineered retrovirus is used to infect cancer cells. Using a retrovirus makes the therapy dividing cell-specific, but still not cancer-cell specific. However, the treatment can be made cancer cell-specific by using a gene that is only coded for if activating proteins that are much more abundant in cancer cells than in healthy cells are used. The enzyme is then produced in these cells, meaning that the prodrug is activated only in cancer cells.

One disease for which this therapy could be useful is colon cancer. Compound **6** (5-fluorourasil) is currently widely used in the treatment of colon tumours, but has serious side effects. Cells in colon tumours over-express the gene coding for a particular transcriptional protein. A potential colon cancer therapy could involve inserting a gene coding for the

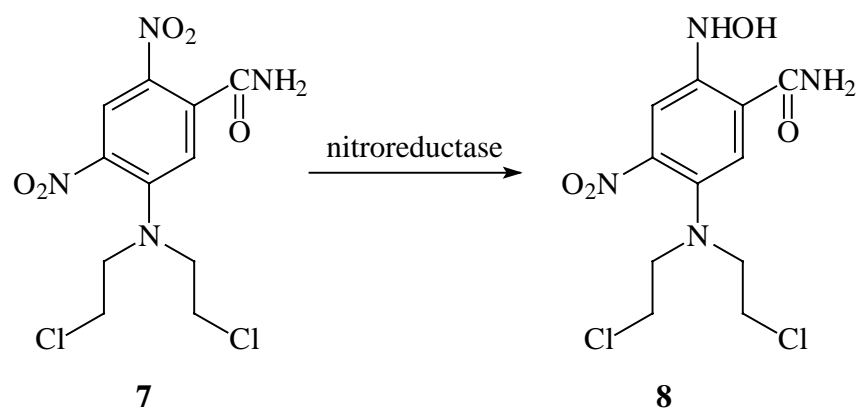
<sup>2</sup>A retrovirus is a virus that is able to incorporate its DNA into that of dividing cells.

bacterial enzyme cytosine deaminase into rapidly dividing cells. This gene is only transcribed in cells with a high level of the protein which is over-expressed in colon cancer cells, so cytosine deaminase is only produced in these cells. The patient is then given the 5-fluorourasil prodrug 5-fluorocytosine (**5**), which is only converted by cytosine aminase to the cytotoxic 5-fluorourasil in tumourous cells (**Figure 2**).



**Figure 2 - Enzymatic conversion of prodrug to active drug in GDEPT**

If the foreign enzyme can be generated selectively in this way, the non-toxic prodrug will then only be converted to the cytotoxic drug in the target cells. In addition, because the active drug is released catalytically by the enzyme, and can be designed to diffuse short distances from where it is released, only a few percent of the target cells need to express the enzyme for the tumour to be completely destroyed. Many combinations of enzymes and prodrugs are currently under study for GDEPT. A project in the Cancer Research Laboratory in Auckland is concerned with the development of prodrugs able to be activated specifically by a bacterial nitroreductase enzyme to generate cytotoxic nitrogen mustards. The dinitrophenyl-carboxamide mustard (**7**) has been shown to be a suitable prodrug. It is essentially non-toxic because the three electron-withdrawing groups on the molecule remove electrons from the mustard nitrogen. It is activated by the bacterial nitroreductase (but not by cellular enzymes), *via* reduction of the 2-nitro group to give **8**, which is much more reactive (**Figure 3**).



**Figure 3 - Activation of a nitrogen mustard by nitroreductase**

### Cytostatic drugs

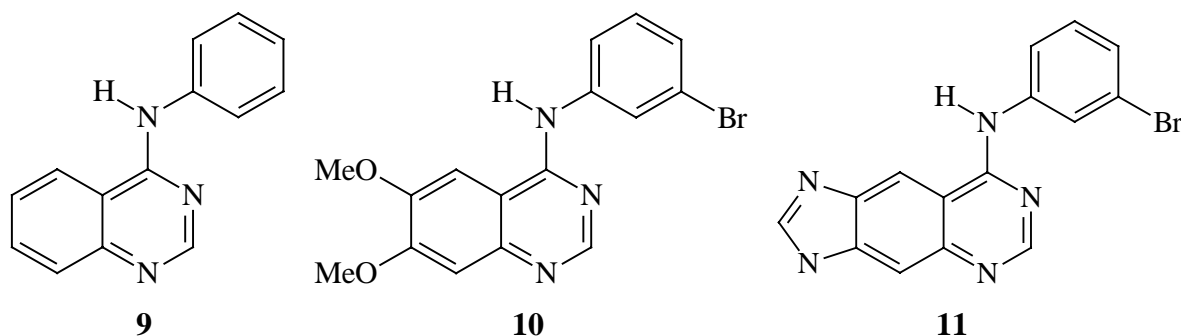
While the prodrug approach is being developed to try and improve the specificity of the common "cytotoxic" anticancer drugs, another new approach is to use "cytostatic" drugs. Many of these specifically target the altered biochemical pathways that enable cancer cells to reproduce quickly. These drugs are designed to deactivate the altered enzymes that result from changes in the oncogene involved. These drugs are not designed to kill the cell involved, but simply to prevent it from reproducing. However, because cancer cells have

very high death rates, due largely to the multiple mutations they possess, simply preventing reproduction is expected to lead to a reduction in tumour size. These drugs are theoretically 'cancer cell-specific' in that they target processes occurring only in cancer cells.

One of the targets for these drugs are the enzymes making up the communication networks by which growth signals instructing a cell to divide are transmitted from the outside of a cell to the nucleus. The growth signal pathways begin at the cell surface, with receptor enzymes that protrude through the cell membrane. Polypeptide growth factors bind (attach) to these, and this binding induces the receptor enzyme to change shape. This shape change turns on the enzyme, allowing it to phosphorylate (add a phosphate group to) tyrosine amino acids in other enzymes (ligands) that associate with its internal sections. This tyrosine phosphorylation activates the ligand, which then transmits the growth message to other enzymes in the pathway.

One of the most important of these receptor enzymes is called the epidermal growth factor receptor (EGFR). This does not usually occur in normal cells, but is present on the surface of a large proportion of human tumour cells. EGFR has a binding site for the ligand that it phosphorylates (to pass on the signal), and one for the cofactor ATP (which donates the phosphate). The role of the EGFR enzymes is really to bring these two components together. There is a great deal of interest in drugs that can selectively block the tyrosine kinase activity of EGFR (but not of similar, useful enzymes such as the insulin receptor). In theory this could be achieved by drugs which bind either at the ATP site, or at the ligand site.

Because most enzymes have sites of similar structure, where the ATP binds, it was felt until recently that drugs that worked by binding at this site would not be very selective. However, recent work in the Cancer Research Laboratory (in conjunction with the Parke-Davis Company) has discovered a novel class of drugs, the anilinoquinazolines, which inhibit EGFR by binding at the ATP site. The lead molecule, 4-anilinoquinazoline (**9**), was itself a quite potent inhibitor, but it was found that changes at three positions on the molecule increased the activity about a million-fold. Thus the related molecule (**10**) can inhibit the enzyme at a concentration of 25 pM (0.000025 M). The related tricyclic molecule **11** is even more potent, inhibiting at a concentration of 8 pM, i.e. about 2 mg dissolved in an Olympic-sized swimming pool. More detailed studies with **10** also show that it is a very selective drug. It inhibits the EGFR enzyme about a million-fold more effectively than it inhibits related tyrosine kinase enzymes. This work has shown for the first time that the extremely potent and selective capacity of the EGFR enzyme can be developed.



## THE DEVELOPMENT OF THE CYTOTOXIC DRUG ASULACRINE AN EXAMPLE OF PHARMACEUTICAL DEVELOPMENT

The development of a new pharmaceutical is a complex process, but can be broken down to three main steps:

- Discovery of a new potentially useful molecule.
- Appropriate molecular modification to produce a molecule with the best combination of properties.
- Development of this molecule into a safe and affordable drug.

This process is outlined below for the anticancer drug CI-921 (asulacrine), which was discovered and mainly developed by the Cancer Research Laboratory (CRL) in Auckland. The CRL was established in 1956 for the discovery and development of drugs useful for the treatment of cancer. The drug asulacrine was developed using funding from the Auckland Division of the Cancer Society of New Zealand, the Health Research Council of New Zealand and Warner-Lambert/Parke-Davis (an American pharmaceutical company).

### Step 1 - Initial Discovery

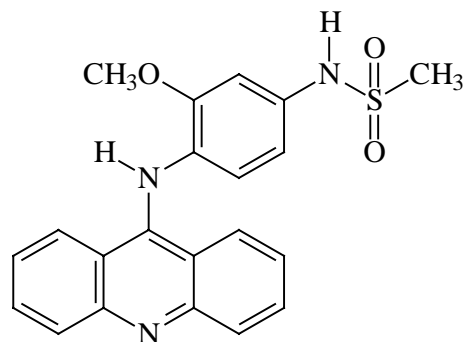
The first phase, the discovery of new classes of active compounds, is especially difficult in the case of anticancer drugs because (at least until recently) there were few identifiable targets to aim for. For this reason, many of the drugs used today were discovered from the random testing of compounds isolated from natural sources or made for other purposes. Not surprisingly, such an approach gives a very poor return, with less than one compound in ten thousand proving even slightly useful. The early tests for usefulness used human or animal tumour cells grown in culture. These measure the cell killing ability (cytotoxicity) of a molecule.

However, to measure how specific a given drug is for cancer cells it is necessary to see whether it will work against tumours in an animal model (usually mice). Such tests are expensive to carry out and can sometimes be misleading, since human and mouse tumours are quite different. By contrast, antibacterial drugs can be evaluated in a test tube against the very same bacterium which they are designed to kill in humans. However, limited animal testing is necessary to ensure that new drugs are useful and reasonably safe before being used in human patients.

Work in the CRL in the 1970s on drugs that bound reversibly to DNA resulted in the class of molecules known as the acridinylaminomethanesulfonanilides (AMSA compounds). One member of this class (amsacrine, **12**), after receiving advanced testing both in the CRL and by the American National Cancer Institute, was approved for human trials in 1978. After two years of clinical testing in America, Europe and Australasia (including in Auckland), amsacrine was approved for hospital use. It is particularly useful against leukemias, and is now one of the drugs of choice throughout the world for the treatment of this disease. In 1984 it became the first pharmaceutical developed in New Zealand to be registered for use in this country. Amsacrine is produced and marketed by the Warner-Lambert/Parke-Davis pharmaceutical company, with which the CRL has a close association.

Amsacrine itself is useful (in combination with other drugs) in the treatment of leukemia, but has little activity against the more common solid tumours. However, it is a novel molecule, working by causing DNA breaks in conjunction with an enzyme called topoisomerase II. It was thought that by changing its structure in certain ways a new and better drug might be

developed. If the drug properties important for activity could be determined, other molecules of this general structure but which were active against a wider range of tumours might be found.

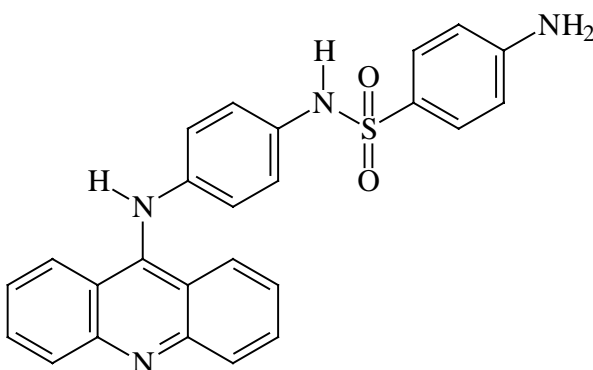


**12**

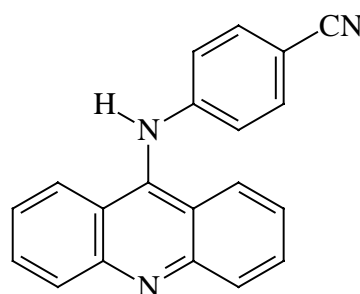
Amsacrine

### Step 2 - Molecular modification of a known compound

It was known that the biological effects of the AMSA molecules was greatly affected by substituents placed on the parent structure. For example, the two compounds **13** and **14** differ in their biological effectiveness (potency) by more than 50 000-fold.



**13**



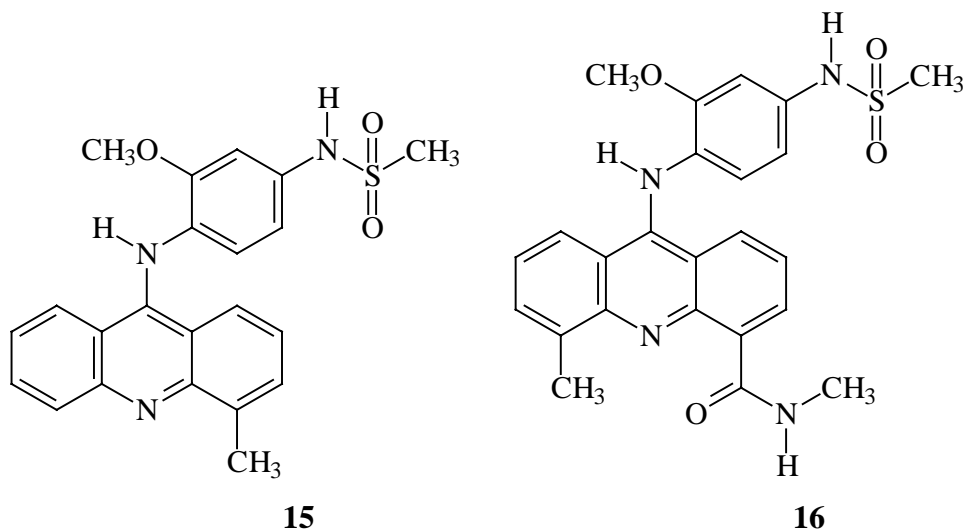
**14**

The goal of the drug development work, begun in the early 1980s, was to obtain analogues that retained potency and effectiveness against leukemia, but also had a wider range of activity against solid tumours. The next step was to make compounds of a similar structure to amsacrine, and test them for the physical and biological properties. Their ability to bind to DNA, their ability to kill leukemia and lung cells in culture, their effects on leukemia and lung cancer cells in culture and their effects on leukemias and lung tumours in mice were evaluated. The lung tumour test was considered more relevant for selecting compounds with possible activity against human solid tumours. Most of the drugs already in clinical use against solid tumours had some activity in this mouse lung tumour, but amsacrine (not effective against human lung cancer) did not.

How were the target molecules to be selected? Modifications could not be usefully made randomly: the science of organic chemistry is too vast for that to be practical. Even if only 20 different substituents were used, placed two at a time at available positions of the amsacrine structure, more than  $10^9$  different possibilities would result. Instead, the molecule has to be looked at as an entity, and the effect that an attached substituent will have must be predicted as far as possible. For example, if the 4-methyl analogue is made (**15**), the added



methyl group will alter the overall solubility of the molecule and the base strength of the acridine nitrogen. Its steric bulk will affect the ability of the molecule to bind to DNA, and it may provide a new site for metabolic breakdown. All of these factors, and others, will influence the biological activity of **15** in different ways, but we will see only the overall result. Rational design requires an appreciation of the molecular properties that might improve biological activity, and a knowledge of how the addition of substituents to a molecule will contribute to all these properties. This is the science of molecular design.



Previous work had shown that high antitumour activity was generally associated with strong binding to DNA, and also with slow binding (where the drug attaches to one particular site for a long time, rather than coming on and off very quickly). It was also thought that the drug would be able to diffuse throughout the body better if it were only weakly basic, because this would mean that more of the drug was in the neutral (uncharged) form. Finally, for ease of administration a drug somewhat more water-soluble than amsacrine would be very desirable. Modifications to the acridine portion of amsacrine were sought that would achieve all these effects, and in so doing enhance its antitumour activity and usefulness.

Considering accumulated experience about how these drugs interacted with DNA, modifications concentrated on 3-, 4- and 5-substitution patterns. A large number (upward of 200) compounds were made and evaluated for both the above desired physical properties and for antitumour activity in both the leukemia and lung tumour models. Several analogues showed excellent activity against lung tumours, and three of these were sent for additional testing in overseas laboratories. On the basis of all the results, the 4-methyl-5-methylcarboxamide (**16**, CI-921, asulacrine) was chosen for clinical trial.

A comparison of a number of important physical properties of asulacrine (**16**) with those of the parent amsacrine (**12**) shows that these modifications have largely achieved the specific goals (**Table 1**). Placing two substituents, one of them the electron-withdrawing carboxamide, adjacent to the acridine nitrogen has lowered the basicity by over one pK unit. At the normal body pH of 7.2 this means that 69% of the asulacrine is in the diffusible neutral form compared with only 12% of the amsacrine. At the same time, DNA binding has increased 16-fold, due mainly to the carboxamide (placed on the molecule so that it can make specific binding contact in the minor groove of the DNA). The average residence time of the molecule on the DNA has also been increased 20-fold, and the water solubility is improved.

However, these changes have not significantly affected the oxidizability of the aniline portion of the molecule, as shown by the similar oxidation potentials.

**Table 1 - A comparison of the physicochemical properties of amsacrine and asulacrine**

	amsacrine	asulacrine
Solubility of hydrochloride salt in water / mg mL <sup>-1</sup>	0.12	0.71
Strength of DNA binding / log K	5.11	0.32
Average residence time at a DNA site / milliseconds	5.5	108
% ionised (i.e. protonated) at pH 7.2	88	33
Oxidation potential / V (c.f. SCE)	0.282	0.243
Basicity of acridine nitrogen / pK <sub>a</sub> )	7.93	6.90

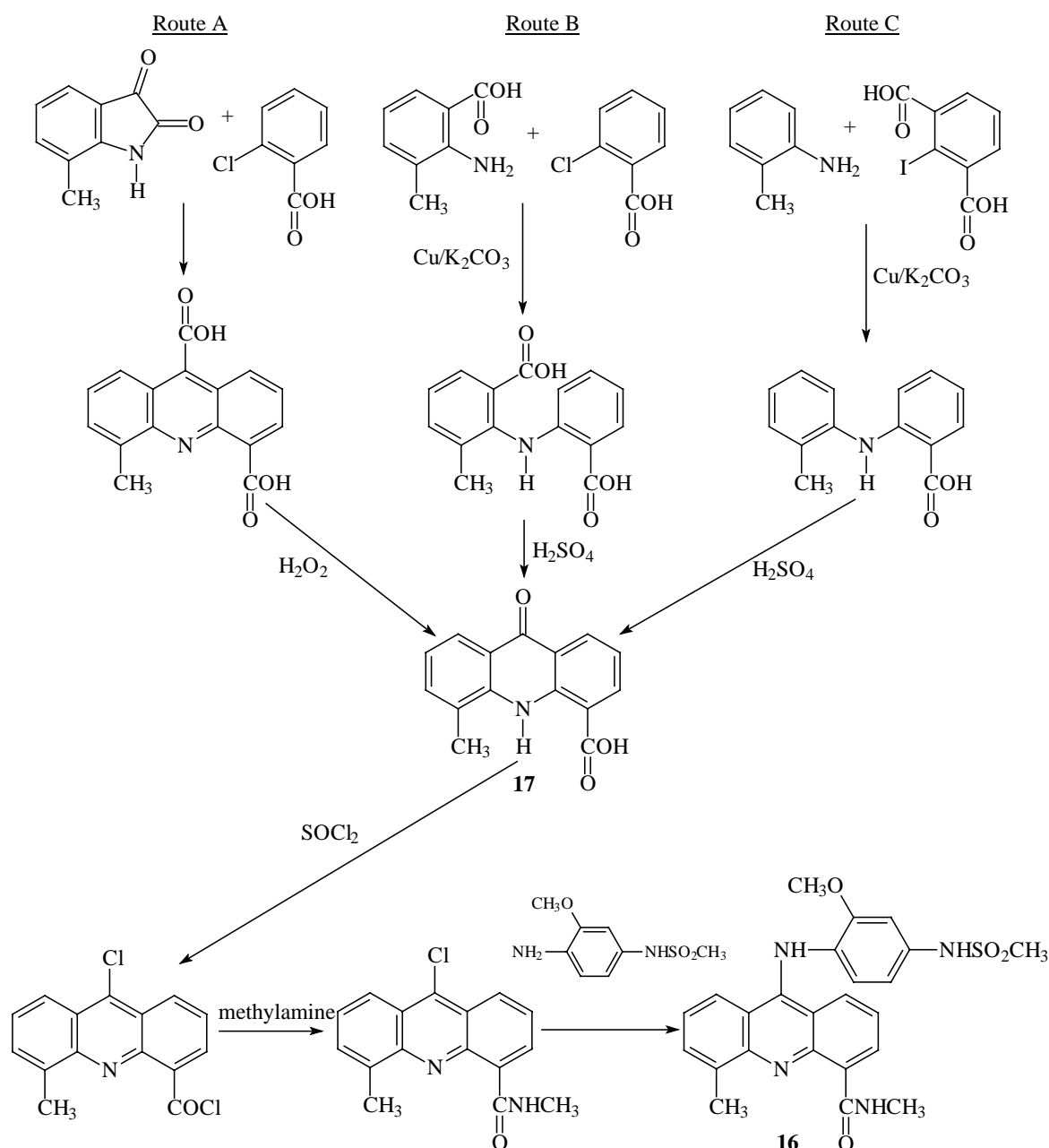
It is reasonable to suppose that these alterations in physical properties have contributed to the greatly improved experimental antitumour activity of asulacrine compared to amsacrine (Table 2). While the potency of both drugs is similar, asulacrine shows a significant proportion of complete cure in animals bearing either leukemia or lung tumours, whereas amsacrine under the same conditions achieves no cures.

**Table 2 - A comparison of anticancer activity of amsacrine and asulacrine in mice**

	Leukemia		Lung tumour	
	amsacrine	asulacrine	amsacrine	asulacrine
Dose of drug for best effect / mg kg <sup>-1</sup> )	13.3	20	13.3	20
Average % of animals cured	0	50	0	82

### Step 3 - Development to a useable pharmaceutical

The above improved physical properties and biological activity of asulacrine compared to amsacrine resulted in the drug being selected as a second-generation analogue of amsacrine for trial in human patients. This meant very extensive toxicology studies, and the production of large quantities of the drug. Such work is very time-consuming and expensive, and mostly beyond the scope of the CRL. A commercial partner was therefore needed. However, before a commercial company would agree to spending the many millions of dollars necessary to develop the drug for human trials, it was necessary to obtain a patent on it. A patent is granted if, in the view of the examiners, an invention is novel, not obvious from existing knowledge, and useful. Once a patent is granted in a particular country only the organisation to which the patent is assigned can make or market the product for a period of 17 years. In the case of asulacrine, patents were successfully obtained in a number of countries, and further development was then undertaken by the Warner-Lambert/Parke-Davis Company.



**Figure 4 - Synthetic routes to asulacrine**

While the CRL did not have the resources to carry out the commercial-scale synthesis of asulacrine, it did develop the synthetic route eventually used. Several different routes were evaluated, seeking the most efficient and cost-effective one. In large-scale synthesis, costs are dominated by the price of starting materials and the length and overall yield of the process. Ideal routes are of a few steps, using cheap starting materials and reagents that give high yields of products in pure form without chromatography. It was decided that the simplest synthesis of asulacrine (16) was from 5-methylacridone-4-carboxylic acid (17). Three routes for the synthesis of this were explored (Figure 4). Route A had already been reported but was found to be impractical for large-scale operations because it used dangerous reagents and had quite low yields. Route B gives a quantitative yield, as does Route C, but it is Route B that was selected for commercial use. The intermediate (17) is treated with thionyl chloride, cold methylamine and finally N-(4-amino-4-

methoxyphenyl)methanesulfonamide to give the water soluble isethionate salt of asulacrine (**16**) in very high yield.

Initial clinical trials of asulacrine were carried out in Auckland and the UK in the mid- to late 1980s. These suggested the drug does have some activity in certain types of lung and breast cancers. Further trials are currently in progress in the UK, controlled by the UK Cancer Research Campaign and Sparta Pharmaceuticals.

## CONCLUSIONS

The purpose of this article is to suggest that, although the design of drugs active against cancer is one of the most difficult pharmaceutical problems, much can be achieved by the intelligent application of chemical principles. Recent developments have led to drugs with novel action that are highly specific to cancer cells. The specific example of asulacrine, developed initially in New Zealand, illustrates the type of process that these drugs need to go through before they can be used clinically. Even the initial stage of drug development is a multi-disciplinary effort by chemists, biologists and biochemists. The further development of promising drugs such as asulacrine into useful pharmaceuticals requires the work of many more people in very different fields including patent lawyers, physicians, chemical engineers and accountants.

Article written by Bill Denny (Cancer Research Laboratory) and Heather Wansbrough and based on:

- Denny, William A.; *New Directions in Cancer Chemotherapy*; Chemistry in New Zealand/July 1995
- Denny, William A.; *The Design and Development of Anticancer Drugs*; Chemical Processes in New Zealand, edition one, volume two