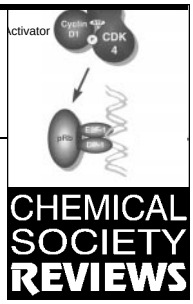
Cancer therapy: a move to the molecular level



F. Thomas Boyle and Gerard F. Costello*

Zeneca Pharmaceuticals, Mereside, Alderley Park, Macclesfield, UK SK10 4TG
E-mail: Gerard.Costello@Alderley.Zeneca.Com

The development of cancer therapeutics has traditionally been based on an empirical approach. As modern biological techniques have begun to open the way to understanding of key cellular processes at the individual protein level, it has become possible to take a more mechanistic approach to the discovery of antitumour agents. This review describes some of the areas in which target mechanisms have been identified and the drugs which have been developed or are currently being investigated. Some of the interventions are not aimed primarily at the tumour, but at the host systems.

1 Introduction

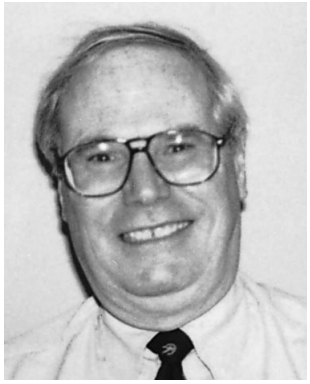
Cancer is not a single disease but a broad group characterised by uncontrolled proliferative growth and the spread of aberrant cells from their site of origin. At the simplest level, cancer cells may be regarded as having lost touch with their environment so that they are no longer responsive to the controlling signals and interactions which occur continuously in normal, healthy tissues. In general, cancer incidence increases with age and most of the major cancers occur in localised tissues.[1] This has led to them being described as solid tumours, *e.g.* lung, colon, prostate, to distinguish them from those such as the leukaemias (blood) and lymphomas. To some extent, this also helps to explain why surgery and radiotherapy are predominant in cancer treatment. Chemotherapy, though widely used, is still a relatively minor weapon in the fight against solid tumour disease.

Clinically, cancers have been categorised by the organ or structure in which they originated, *e.g.* breast, colon. This has tended to reinforce the treatment by clinical speciality whilst sometimes obscuring any commonality of disease mechanism across tissue tumour types. This review will attempt to illustrate the opportunity provided by the rapidly increasing understanding of such underlying molecular mechanisms in cancer, which has been made possible by the revolution in molecular and cell biology in recent years. To harness this insight most effectively, clinical testing and practice may have to accommodate corresponding changes in tumour classification and treatment.

2 Cytotoxics

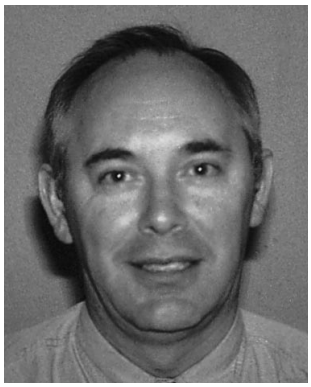
Current chemotherapy consists of cytotoxic (cell-killing) agents and anti-hormonal drugs, which reduce the proliferative drive to the tumour.[2] Many compounds with good tumour cell-killing activity have been discovered, but few have found clinical utility. This reflects a lack of discrimination between effects on tumour and normal tissue, the cell-cycle dependency of many cytotoxic drugs and their frequent susceptibility to induced drug resistance. Significant side-effects such as nausea, vomiting, diarrhoea, hair loss and serious infection are often encountered during chemotherapy, since healthy tissue in the gastrointestinal tract, hair follicle and bone marrow proliferates at least as rapidly as most tumours. Furthermore, quiescent (non-proliferating) tumour cells can remain largely unaffected by treatment and may subsequently begin to divide and grow. Clinical strategies have been developed to address such issues. These involve cycles of therapy to allow recovery of normal tissue in between and attack on those tumour cells which have grown out since the previous treatment. Unfortunately, this process may generate increased selection pressure for changes

Tom Boyle is a Zeneca Research Associate who was born in Cheshire in 1944. He obtained his BSc at the University of Salford before moving to ICI in 1964. After completing an industrially based MSc on ring opening reactions of pyridines he moved to the University of East Anglia to complete his PhD working with Richard A. Y. Jones and Alan Katritzky on the synthesis and tautomerism of N-oxides. He rejoined ICI at their Central Research Laboratories before moving to the Pharmaceutical Division in 1973 working on aspects of infection and animal health. The latter area took him to Australia in 1979 to work on the small molecule modulation of ruminant nutrition. On returning to the UK he focused his research interests in cancer and has been involved in the small molecule approaches to aromatase inhibition, cytotoxic therapies based on the inhibition of thymidylate synthase, antibody directed prodrug therapies (ADEPT) and inhibition of cell signalling and apoptopsis.



Tom Boyle

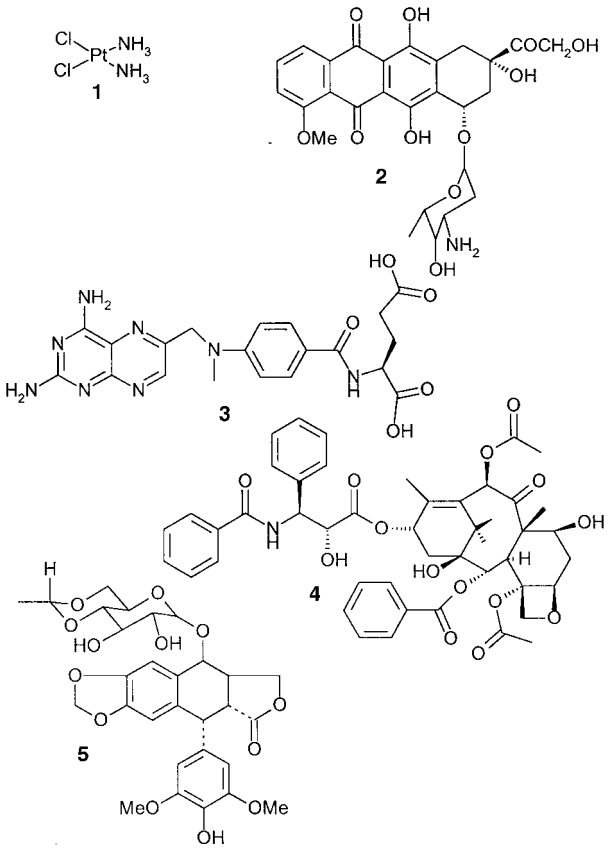
Gerard Costello was born in Scunthorpe, England in 1952 and obtained his BA (Chemistry) from the University of Oxford in 1975. He moved to Leeds to work for his PhD with Edwin Saxton on total synthesis of Aspidosperma alkaloids. After gaining his PhD in 1978, he spent two years working in the laboratory of Professor Albert Eschenmoser at ETH Zurich on a NATO postdoctoral fellowship before joining ICI Pharmaceuticals at Alderley Park as a medicinal chemist in 1981. He has also worked in chemical process development and as an International Product Development Manager with responsibility for cancer projects from 1987–1991. Since 1991 he has been Section/Project Manager in the Cancer Research Department of Zeneca (formerly ICI) Pharmaceuticals.



Gerard Costello

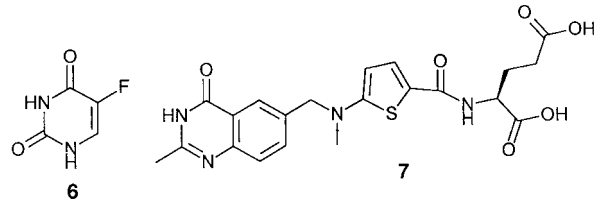
which induce drug resistance in what is, by definition, a genetically-labile cell population. In the clinic, early responses to therapy are often followed by disease progression or recurrence with reduced tumour susceptibility to the original or other drug treatment.

To a greater or lesser extent, this general profile applies to cytotoxic agents from a wide range of mechanistic classes, *e.g.* alkylating agents, DNA intercalators, antifolates, tubulin binders, topoisomerase inhibitors. This includes many of the best known and most widely-used anticancer drugs, such as cisplatin **1**, doxorubicin **2**, methotrexate **3**, paclitaxel **4** and etoposide **5**.



For the most part, cytotoxic drugs have been developed empirically and their major locus of action identified in parallel or afterwards. Information gained from clinical study has been used to derive new approaches based on mechanistic considerations in addition to the more traditional compound screening methods. As an example, inhibition of transcription by targeting compounds to specific sequences in the minor groove of DNA is an area of much research activity which has been greatly assisted by advances in molecular structural techniques.[3] In another area, the powerful pairing of biosynthetic pathway elucidation (Fig. 1) and molecular modelling (Fig. 2) has led to a new class of antifolate agents which selectively inhibit thymidylate synthase (TS).

This enzyme is critical for the *de novo* biosynthesis of thymidine, the only nucleotide required exclusively for the synthesis of DNA rather than RNA. Inhibition of this enzyme is one of the actions of the widely-used agent 5-fluorouracil **6**. The recent introduction of the new TS-specific drug raltitrexed **7**, shown bound in the enzyme complex in Fig. 2, will allow a



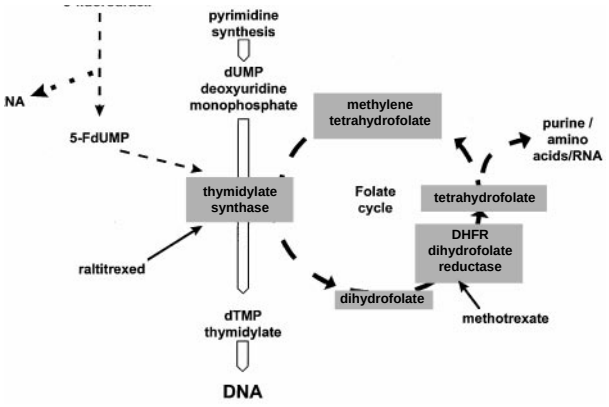


Fig. 1 Thymidine biosynthesis pathway

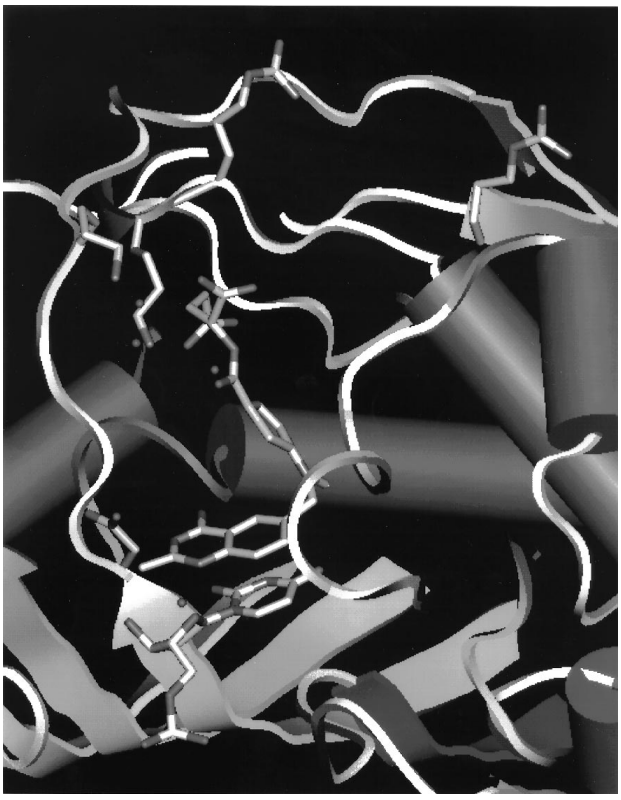


Fig. 2 Raltitrexed bound in the ternary complex of thymidylate synthase

realistic assessment of the clinical advantages arising from such mechanistic selectivity.[4]

Despite an improved basis for designing 'conventional' DNA-targeted cytotoxic agents, there must be a high risk that the inherent problems described above preclude any major therapeutic breakthrough with this category of drugs. At the same time, it should be recognised that incremental improvements in the treatment of solid tumour disease in particular remain highly desirable.

3 Antibody-targeting

In order to overcome the problem of normal tissue toxicity, efforts have been made to achieve direct targeting of tumour cells, usually by means of antibodies to tumour-specific antigens. This is the embodiment of the 'magic bullet' long sought after in cancer therapy. Despite much early promise, there have not been any real successes with antibody treatment of major solid tumours.[5] There are a number of problems which have been found in using antibody therapy:

(*i*) It is remarkably difficult to achieve tumour-specific antibodies and also to have high affinity.
(*ii*) Unlike the laboratory situation, clinical tumours do not have consistent expression of target antigen throughout their mass.
(*iii*) Antibodies are large molecules which do not penetrate solid tumours well.

As a result, only a very small amount of dosed antibody (much less than 1%) reaches the tumour and much of that localises to the vasculature. This means that systems using antibodies linked to radio-isotopes have the problem that the high doses given to achieve the desired effect at the tumour result in extended circulation times and most of the radiation being received by other tissues. Antibody–toxin and antibody–drug conjugates may suffer from their only being active against tumour cells bearing the relevant antigen and any instability in the chemical link to the antibody could result in undesirable systemic toxicity.

To date, there has been little success with antibodies alone. This could be a reflection of the majority of clinical studies being conducted with murine antibodies, which suffer from both immunogenicity and poor recruitment of effector mechanisms. Whilst human antibodies might be more effective, it is worth noting that T-cells are recruited in large numbers to some solid tumours apparently without the necessary activation to achieve cell killing. In fact, new immunological stimulation approaches are being investigated based on T-cell signalling targets and there is also renewed interest in cancer therapeutic vaccines.

3.1 ADEPT

One of the approaches most likely to overcome the shortcomings described above is antibody-directed enzyme prodrug therapy (ADEPT).[6,7] This is a two-phase therapy (Fig. 3) which uses an antibody–enzyme conjugate to achieve localisation to the tumour and follows up with a prodrug of low toxicity which is converted only by that enzyme to a very potent, short-acting drug. In this way, an amplification mechanism for targeted drug delivery is provided which can also achieve a 'bystander effect' and kill cells not bearing the antigen.

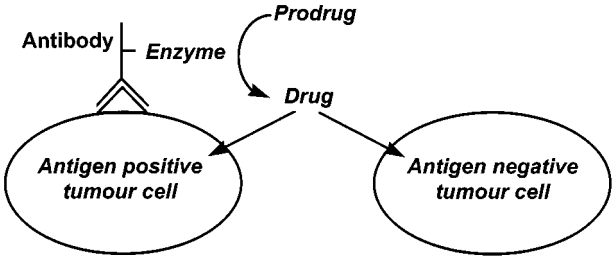


Fig. 3 ADEPT system

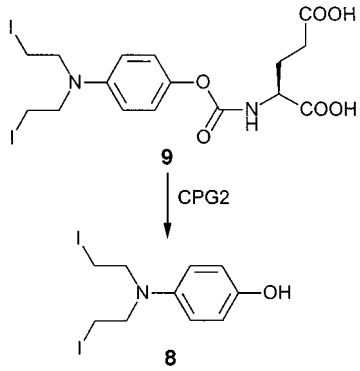
A system of this type is currently under clinical investigation. It uses a very selective antibody against carcinoembryonic antigen (CEA) and binds to most colon, gastric and non-small cell lung cancers as well as many breast and ovarian tumours. The antibody is murine and it is linked to a bacterial enzyme, carboxypeptidase G2 (CPG2). This means that the enzyme does not occur naturally in man and, by designing the prodrug appropriately, liberation of free drug away from the tumour is avoided. Careful consideration of the properties required for the prodrug–drug combination determined the design process. The drug had to:

(*i*) Be small enough to be readily-diffusible through the tumour mass.
(*ii*) Show high cytotoxic potency against both dividing and quiescent cells.
(*iii*) Have a rather short chemical half-life to avoid toxicity caused by escape of the drug from the tumour into the circulation.

In contrast, the prodrug had to:

(*i*) Show markedly less cytotoxicity than the drug, since it would be administered systemically.
(*ii*) Exhibit good enzyme kinetics as a substrate in order to allow rapid production of sufficient drug to have the desired effect.

In this system, the preferred drug **8** is an aromatic 'mustard' compound. The advantages of this class of alkylating agent are that they are not cell-cycle-specific, so are also effective against quiescent cells, and they tend to be less susceptible to induced resistance than most anti-cancer agents. From a medicinal chemistry viewpoint, there is also a reasonable basis of understanding of how to modulate the cytotoxic potency of such agents. A thorough investigation of the interactions between the various component parts of the prodrug **9** was necessary before an optimal system was achieved.



Obviously, this antibody–enzyme conjugate will almost certainly be immunogenic in man. This may well restrict the number of doses which can be given to cancer patients. Research is continuing to examine the feasibility of producing humanised systems with much reduced potential for immunogenicity which could allow more treatment cycles to be undertaken.

4 Anti-hormonal agents

Perhaps the first example of an area of medical treatment for cancer to benefit from a detailed understanding of biochemical mechanism is that of anti-hormonal therapy. It is interesting to note that the approach derives from surgical discoveries over the last century. Removal of the ovaries or testes was shown to give clinical responses in a significant proportion of breast and prostate cancer patients respectively. Responsive tumours were found to be dependent on the relevant sex hormone, oestrogen or testosterone, for their growth.[8] Extensive research over many years into the biosynthesis and action of the sex hormones then provided the basis for targeting interventions and drug design (Fig. 4).

4.1 Anti-oestrogens

Even with extensive background knowledge, the first and still the most successful anti-hormonal drug is tamoxifen **10**, which was discovered in a programme originally aimed at anti-fertility treatment.

To some extent, this reflected the concern that medical treatment would not be able to match the efficacy of surgery in cancer. It also resulted from the apparent paradox that this oestrogen receptor antagonist does not cause direct killing of breast cancer cells, yet can achieve good clinical anti-tumour effects.[9,10] Additional complexity was provided by the fact that tamoxifen acted as a full antagonist in some tissues and as a partial agonist in others, even within the same species. A pure oestrogen receptor antagonist, ICI182780 **11**, was subsequently discovered and is now in late-stage clinical trial.

4.2 Aromatase inhibition

As indicated in Fig. 4, there are other points in the oestrogen biosynthetic pathway which offer potential for breast cancer

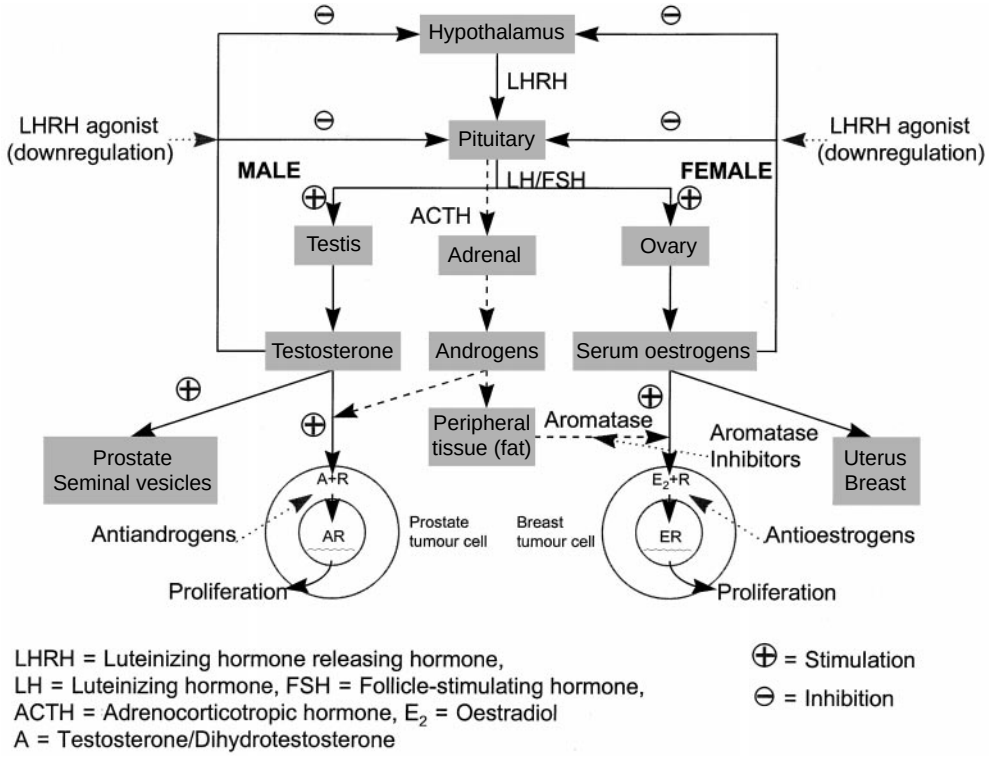
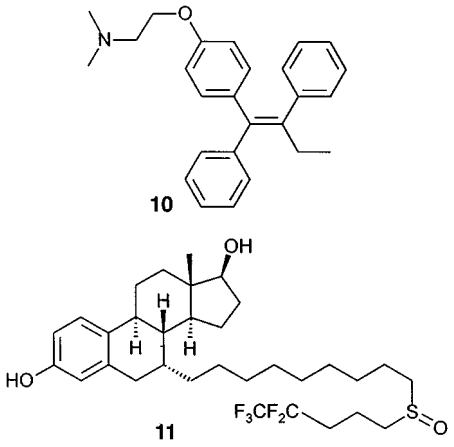
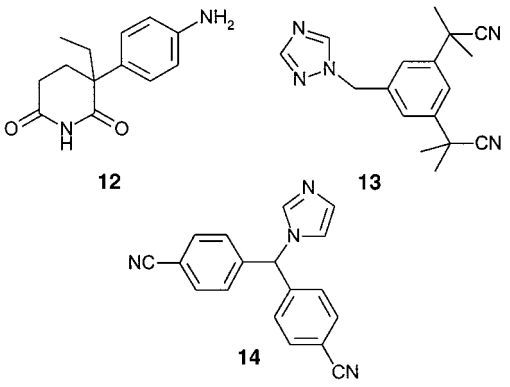


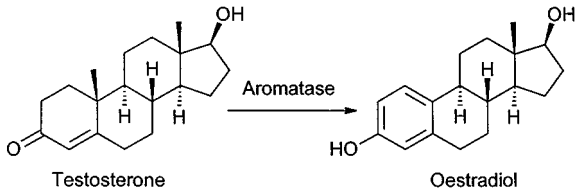
Fig. 4 Hypothalamic/pituitary axis and sex hormone action



treatment interventions. Inhibition of the enzyme steroid aromatase, which effects the conversion of androgens to oestrogens (Scheme 1), has been a target of much recent research.[11] Once again, the interest was stimulated by clinical observation, this time with the drug aminoglutethimide **12**. Originally developed as an anti-convulsant, this compound had been found to be a non-selective inhibitor of steroid biosynthesis. In particular, it was shown to be an effective inhibitor of cytochrome P-450 enzymes, many of which (including aromatase) are involved in the steroid pathways. Whilst interesting from a mechanistic viewpoint, the more important finding clinically was that the drug lowered circulating oestrogen levels by around 50% in post-menopausal women and the responses seen in breast cancer patients broadly reflected the reduction in hormone levels.

Efforts were then focused not only on evaluating structures with known cytochrome P-450 inhibitory potential (especially from the anti-fungal area), but also on using molecular modelling based on homology with X-ray structures of bacterial enzymes to build in the selectivity required to avoid the severe side-effects seen with aminoglutethimide.[12,13] This has proved a very successful approach and a number of compounds, generally classified as 'azoles', have been evaluated clinically. Two recently introduced drugs from this class, anastrozole **13** and letrazole **14** appear to deliver the improvements in side-effects and clinical efficacy being sought. The increased efficacy results from a more profound lowering of circulating oestrogen levels.





Scheme 1 Action of steroid aromatase

4.3 LHRH agonists

Luteinizing hormone releasing hormone (LHRH) agonists form a third class of hormonal therapy for breast cancer, albeit only in pre-menopausal women. This limitation arises from their inhibitory effect on luteinizing hormone (LH) release from the

pituitary (see Fig. 4) and consequent suppression of ovarian stimulation for oestrogen production. Whilst there are LHRH antagonists in clinical study, the ability of the agonists to mimic successfully the natural inhibitory feedback effect of oestrogen at the pituitary by inducing LHRH receptor downregulation provides a rare superiority over direct blockade. The agonists are all close analogues of LHRH **15**, but their potency has had to be even greater than that of the natural decapeptide hormone itself to achieve the downregulating effect.[15] The structures of the two major drugs, goserelin **16** and leuprorelin **17**, are aligned by amino acid sequence for comparison.

1 2 3 4 5 6 7 8 9 10

Glu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH_2 **15**

Glu-His-Trp-Ser-Tyr-D-Ser(tBu)-Leu-Arg-Pro-Azgly-NH_2 **16**

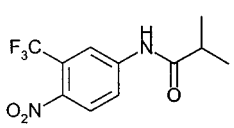
Glu-His-Trp-Ser-Tyr-D-Leu-Leu-Arg-Pro-NHEt **17**

To realise the full clinical benefit of their biological action required the development of sustained-release formulations of one month duration. Novel technology was needed to produce the bio-degradable carriers which allowed the usual problems of rapid metabolic cleavage of peptides to be overcome. The basis of these formulations was a lactide–glycolide co-polymer. Very small quantities of these peptide agents (3.6 and 10.8 mg in the case of goserelin for formulations of one month and three month duration respectively) as injectable depot preparations are sufficient to suppress serum oestrogen levels into the menopausal range and maintain that suppression throughout these extended periods.

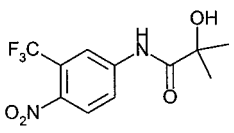
Since the LHRH agonists act at the pituitary (Fig. 4), they also have an inhibitory effect in men on hormonal drive to the testes, resulting in a reduction of serum testosterone to levels comparable with those achieved by surgical castration. By matching the effects of this standard treatment LHRH agonists have also become the first acceptable medical therapy for prostate cancer, the second largest cause of cancer deaths in men.

4.4 Anti-androgens

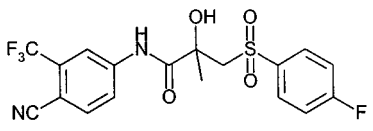
Whilst either surgical castration or treatment with LHRH agonists ablates testicular androgen production, a secondary source is provided by the adrenals (Fig. 4). To achieve what is referred to as 'total androgen blockade', androgen receptor antagonists **14** have been introduced in combination with either surgery or LHRH agonist treatment. The prototypic anti-androgen is the non-steroidal compound, flutamide **18**. Its biological activity however, derives mainly from a metabolite, hydroxyflutamide **19**, which is a much more potent androgen receptor antagonist. Subsequently, bicalutamide **20** was developed from consideration of the hydroxyflutamide structure and this drug is active in its own right and appears very well tolerated.



18



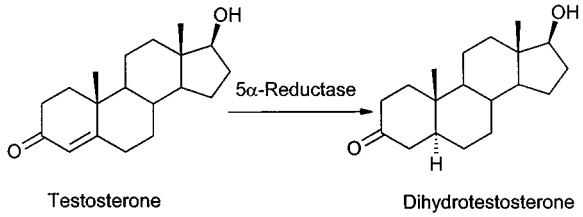
19



20

As yet, no anti-androgen has gained use as a single therapy in prostate cancer, though trials are taking place both in advanced and in early-stage disease. This forms a marked contrast with tamoxifen in breast cancer, where oestrogen receptor blockade was the first successful approach.

Another potential target for intervention in the androgen biosynthetic pathway is the enzyme 5α-reductase, which converts testosterone to the much more potent androgen dihydrotestosterone (Scheme 2). Although 5α-reductase inhibitors have been developed for the treatment of benign prostatic hypertrophy (BPH),[16] initial studies against the more demanding target of prostate cancer have not been as encouraging.



Scheme 2 Action of 5α-reductase

4.5 Anti-hormonal profile

Common features across the whole of anti-hormonal therapy are the need for continuous dosing of the agent over extended time periods (sometimes indefinitely) and the consequent requirement for a much better tolerability profile than, for example, cytotoxic therapy. These factors relate to the lack of direct cell-killing ability associated with this general therapeutic class. At the same time, the improved tolerability is often associated with better quality of life for the patient and increased compliance with therapy. Although differing mechanistically from cytotoxics, anti-hormonal agents are also subject to the development of resistance by the tumour. The timescale tends to be much longer than for cytotoxic therapy, but resistance may emerge and in certain cases it has been known for the drug to become stimulatory for tumour growth.

5 Signal transduction inhibition

Despite the great importance and value of anti-hormonal therapy, perhaps its most important deficiency is that it is essentially limited to use in breast and prostate cancer. However, the clinical profile shown by these agents opened up the prospect of being able to develop similar drugs for other tumours which were not hormonally-responsive.[17]

Without good clinical precedent to direct research towards a particular approach, it was unclear for a very long time how to make progress towards this new goal. In this case, the insight came from the laboratory and the rapid advances being made in the understanding of the genetic processes underlying cancer pathogenesis. Over the last two decades, there has been a fundamental change in the approach to cancer research with recognition of the primacy of molecular mechanism. The first wave of drug candidates derived from this change is just coming through into the clinic.

Amongst the first systems to have been investigated successfully are the growth factor signalling pathways (Fig. 5). Even as their detail and complexity have been emerging, the potential for therapeutic intervention at several different levels between the cell membrane and the nucleus has become evident and a variety of molecular biological and 'small molecule' tools applied to validating genes as targets.

5.1 Growth factor receptor antagonism

Antagonism of growth factors at their cell surface receptors was an early approach which helped to open up the area for further investigation.[18] Growth factor receptor blockade can be achieved in a number of cases, but its impact has generally not been sufficient to affect the proliferation of representative

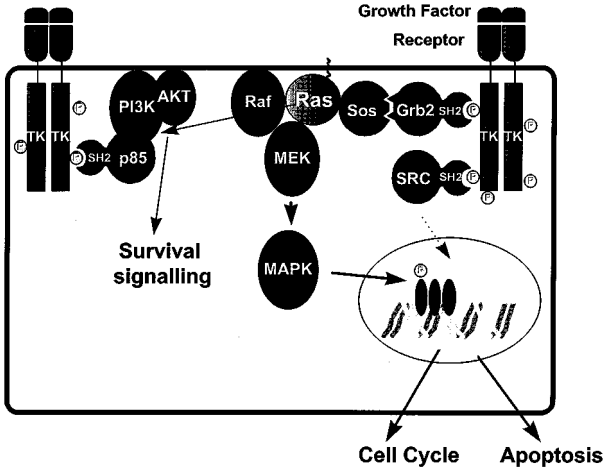
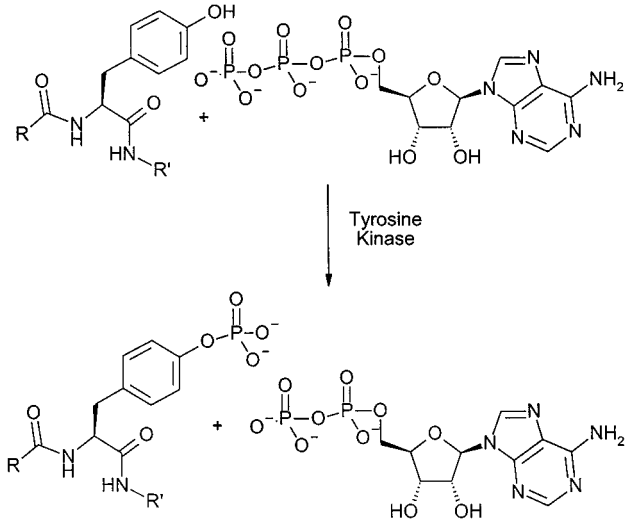


Fig. 5 Growth factor signalling pathways

tumour cells. This has been interpreted as a consequence of the inherent 'redundancy' within cell signalling pathways. This limits the extent to which proliferation, differentiation or survival signals depend on the binding of an individual ligand to a single receptor type. Best effects have been claimed for less-selective agents, which tends to support the view that selective growth factor antagonists will not be useful 'stand-alone' anti-cancer drugs.

5.2 Receptor tyrosine kinase inhibition

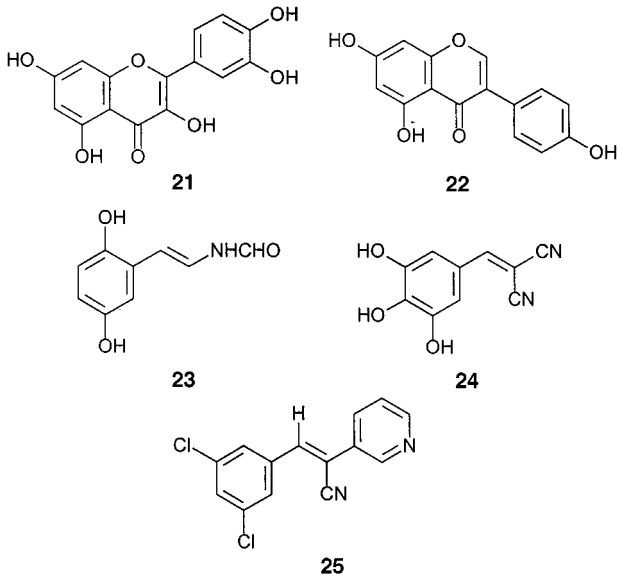
As shown in Fig. 5, the next stage in the signalling cascade involves the phosphorylation of protein tyrosine moieties by the cytoplasmic domains of growth factor transmembrane receptors. The enzymic addition of phosphate groups is carried out by kinases. Protein tyrosine kinase activity (Scheme 3) is associated with many of the growth factor receptors and also with oncogenic, non-receptor proteins such as src. A number of tyrosine kinases are overexpressed and/or show increased activity in human tumours. Whilst this is not proof of a causal relationship, it provides some evidence for the contribution of tyrosine kinase activity to cancer growth.



Scheme 3 Protein tyrosine kinase activity

For these reasons, inhibition of tyrosine kinases was seen as an attractive and chemically feasible opportunity. The main issue around these targets was one of selectivity. In this case, selectivity refers to tumour *versus* normal cells and also across the different classes of tyrosine kinase. A combination of high-throughput screening and structure-based design approaches was used to derive the first compounds which served as pharmacological tools to demonstrate that the principle of intervening at this level in signal transduction pathways was valid.

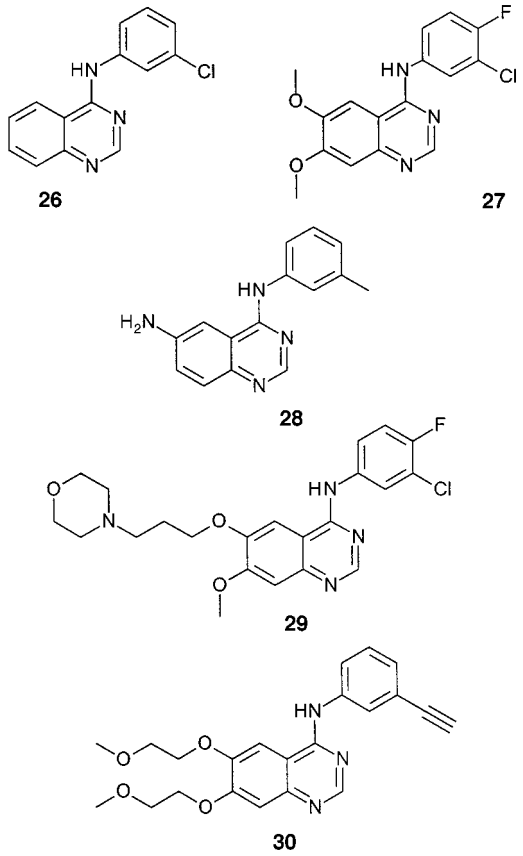
Flavonoid natural products like quercetin **21** and genistein **22** were not generally selective and had other actions such as topoisomerase inhibition, but they showed that compounds competitive with ATP could at least discriminate between tyrosine and serine/threonine kinases. Another natural product, erbstatin **23**, engendered great interest because of its simple structure. Many analogues of the tyrphostin type,[19] represented by **24** and **25**, were made and provided yet more support for the approach because they also showed a degree of discrimination between individual tyrosine kinases and some had anti-tumour activity in animal models.[20]



The epidermal growth factor (EGF) receptor was one of the first targets for drug discovery because of its known over-expression in human tumours such as non-small cell lung cancer (NSCLC) and head and neck cancer. Using the known sequence of the EGF receptor tyrosine kinase catalytic domain alongside X-ray crystal structures of cyclic adenosine monophosphate (cAMP)-dependent protein kinase, a homology model could be built.

Structural types identified by screening against the human enzyme derived from the membranes of A431 tumour cells were assessed against this model. A number of different compound classes showing reasonably selective inhibition of EGF receptor tyrosine kinase activity has been identified. Perhaps the most significant of these to date is the anilinoquinazoline class. These agents have also been shown to be competitive with ATP and have exhibited a high degree of selectivity.

The prototypic structure, represented by **26**, was identified by targeted screening and was enhanced to produce compounds like **27**, which is an extremely potent inhibitor and very selective for the EGF receptor. However, the physical properties of these agents were not optimal for *in vivo* activity in animal tumour models. The breakthrough in this area came with the discovery of *in vivo* activity with the 6-aminoquinazoline compound **28**. Although very much less potent against the enzyme than many of the compounds referred to above, it had a better pharmacokinetic profile in animals. This was particularly important because these inhibitors had the desired profile incorporating a separation of anti-proliferative and cell-killing actions. In the absence of direct cell-killing, it appears essential to have significant levels of compound in the blood at all times in order to see reproducible effects in the tumour models.



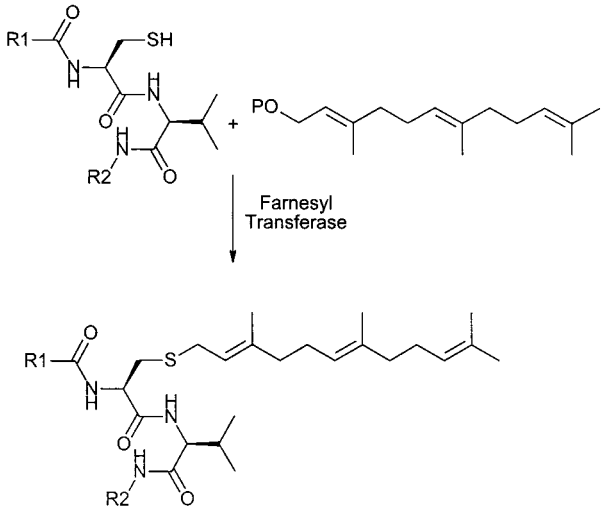
Optimisation based around pharmacokinetic properties resulted in the compound ZD1839 **29** which is currently undergoing clinical trials.[21] Another compound **30** with very close structural similarity to **27**, has also shown good *in vivo* activity. These agents and a small number of compounds directed against other tyrosine kinase targets should provide the first clinical test of whether intervention at this point in the signalling cascade can produce useful anti-tumour efficacy. Whilst aberrant signalling is a feature of many tumour cells, the same pathways are essential for maintenance of some normal tissues. It is hoped that a balance similar to that seen in the animal models will be found in the clinic between activity against the poorly structured tumour and toxicity to the patient.

5.3 Ras inhibition

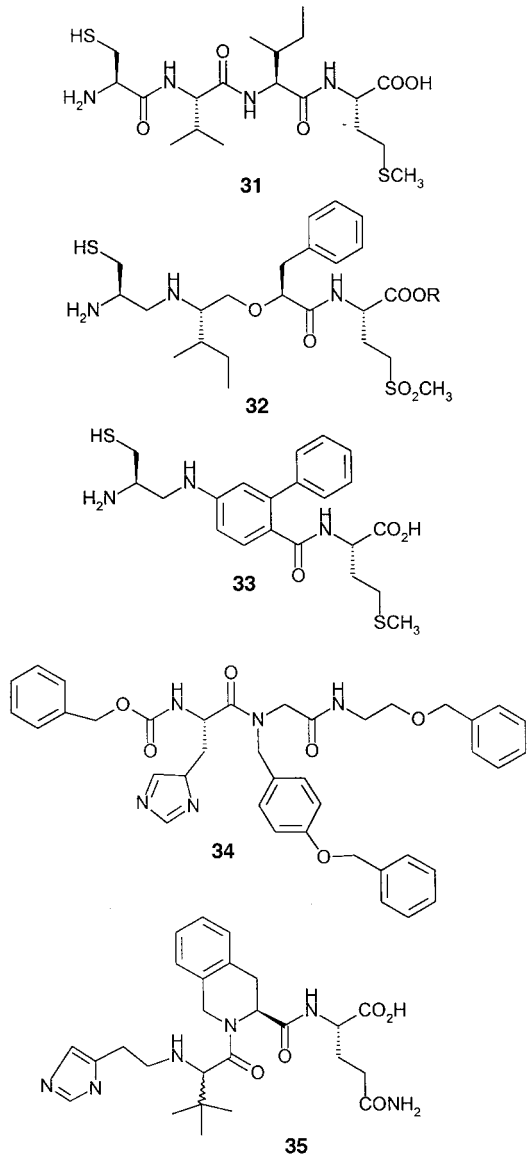
Another key signalling system is the ras pathway (Fig. 5). Most interest in modifying the action of the ras oncogene has been focused on inhibition of the farnesyl transferase enzyme. In order to exert its functional effects, ras has to be docked into the cell membrane. The cytosolic protein has to be modified at the C-terminus by addition of a lipophilic 'tail' which then anchors it into the cell membrane. A key step in this process is the addition of a farnesyl group to a cysteine thiol, which is catalysed by farnesyl transferase (Scheme 4).[22] Each of the different ras forms, mutated or normal, has a C–A–A–X terminal peptide sequence which confers farnesyl transferase substrate activity. A range of structures has been identified, usually based around the tetrapeptide motif **31**,[23] amongst which there are very potent enzyme inhibitors **32**–**35**. As yet, none of these compounds is known to have progressed to the clinic.

5.4 Other signalling pathway interventions

SH2 (src-homology) domain containing adapter proteins determine which of the associated proteins will interact with tyrosine phosphorylation sites on receptors to propagate the signal (Fig. 5).



Scheme 4 Action of ras farnesyl transferase



Structural studies on various SH2-containing proteins have provided some insights into how they perform their adapter

role.[24] The N- and C-terminal SH2 domains of the p85 sub-unit of phosphatidyl inositol (PI) 3 kinase have provided excellent examples of protein structure determination by NMR methods. However, the fact that the key interactions in this case are between two proteins, at least one of which is phosphorylated, has made it more difficult to find good chemical starting points from normal compound library screening. Even peptide-based medicinal chemistry approaches do not appear to have made much progress against this category of target and the first real breakthrough is still awaited.

There has been a great deal of work done around the protein kinase C (PKC) family which has produced some interesting compounds, though the problem of selectivity between isoforms of this important serine/threonine kinase has not been overcome. Another family of signalling proteins exciting great interest is the mitogen-activated protein (MAP) kinases, which are involved in the linkage of tyrosine phosphorylation signals to serine/threonine phosphorylation signals. They are important enzymes in growth modulation signalling and have become leading drug discovery targets. When assessing intervention options closer to the cell nucleus like these, there may be increased concern that the balance being sought between efficacy and toxicity will be shifted towards a profile more closely resembling that of the cytotoxic agents.

6 Cell cycle modulation

The concern about increased toxicity is even greater when considering intervention at the level of the cell cycle. The nuclear process of replication and division involves a number of phases (Fig. 6).

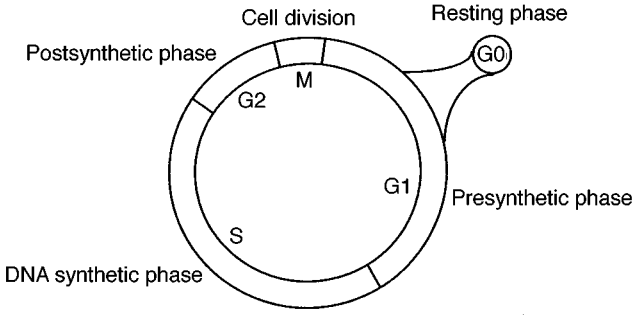


Fig. 6 The cell cycle

Rapid growth in understanding of the basic machinery has been accompanied by insight into how mitogenic and inhibitory pathways couple to the cell cycle and how it is deregulated in cancer.[25] Entry into the cell cycle is controlled by a balance of activating factors such as mitogen and oncogene signals and inhibitory elements such as transforming growth factor (TGF)β and tumour suppression genes.

6.1 Cyclin-dependent kinase inhibition

One of the prime targets is cyclin-dependent kinase (CDK)4, which acts at the G1/S interface. The response to DNA damage in normal cells is to arrest the cell at this starting point of its cycle. CDK4 activity is known to be increased in a wide variety of solid tumours and this may be associated with overexpression of cyclin D1, TGF-β signalling defects and reduction or loss of the tumour suppressors p16, p21 and p53 (Fig. 7).

Inhibition of CDK4 should block entry into the cell cycle but the degree of selectivity for tumour over normal cells has not yet been established. A broad spectrum CDK inhibitor, flavopiridol **36**, is currently under clinical investigation[26] and appears to be showing a cytotoxic profile in line with expectation.

X-Ray structural studies[27] with CDK2 have been successful which provides extra information for design not only of

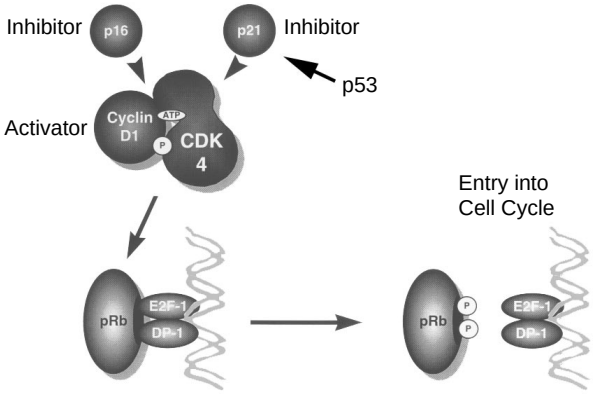
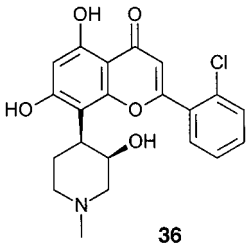


Fig. 7 Pathway to cell cycle entry



36

inhibitors of that enzyme but also of other CDK enzymes by homology modelling.

7 Apoptosis

All of the approaches described in Sections 4–6 have as their main aim an anti-proliferative effect without direct cell-killing. There are also options for cell death approaches[28] which can exploit the differences between tumour and normal cells and so avoid the drawbacks of the 'conventional cytotoxics'. The most important area for consideration involves the process of apoptosis or programmed cell death. Apoptosis is an important and widespread biological process which seems to be complementary to mitosis (cell-division) in the regulation of cell populations. It plays a critical role in development and is often a result of tissue damage. Disruption or inhibition of apoptosis is frequently seen as a major component of malignancy. Induction of apoptosis either by blockade of survival signals or activation of programmed cell death signals is attractive because it is a process which does not occur randomly in all cells of a tissue. Some precedent for efficacy is provided by the fact that many of the known cytotoxic agents induce apoptosis, albeit in a non-specific manner.

Progress in understanding the range of mechanisms involved in apoptosis has occurred at a remarkable rate, which reflects it being currently one of the most intensively studied biological areas. Once a cell is stimulated to enter the cell cycle, signals at certain stages direct it either to complete the cycle or to undergo apoptosis (Fig. 8).

Overexpression of the proto-oncogene bcl-2 seems to limit the effects of chemotherapy and radiation treatment.[29] It appears to function as a negative regulator of apoptosis and much effort is being made to discover agents capable of blocking its action. Similarly, loss of p53 activity by mutation of that tumour suppressor gene causes resistance to apoptosis induction. Both of these drug targets involve protein–protein interactions and initial approaches have consequently been built mainly around peptides to provide validation tools.

An alternative approach is to reduce the enhanced survival signalling which may occur in tumour cells. There are targets of this type, such as insulin-like growth factor (IGF)-1 receptor and focal adhesion kinase (FAK) activity, which seem more

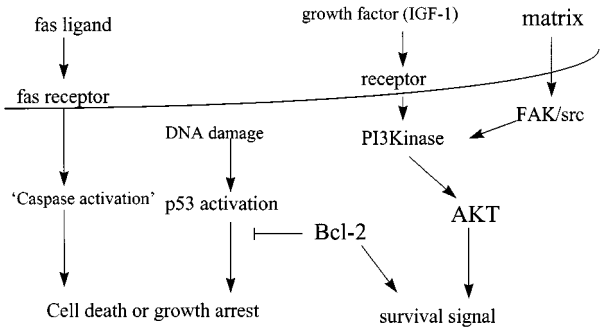


Fig. 8 Cell death and survival signalling pathways

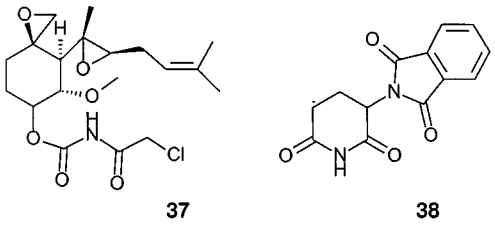
akin to the anti-proliferative signalling targets. Intervention using more precedented medicinal chemistry approaches and low molecular-weight compounds seems more feasible in such systems.

8 Angiogenesis inhibition

In addition to the majority of the treatments aimed solely at the tumour cell, there are also therapeutic approaches targeted at the host or host–tumour interaction. The two major areas here are angiogenesis (generation of new blood levels from existing vasculature)[30] and invasion[31] (Fig. 9).

Tumours require a blood supply in order to grow. Since angiogenesis in adults is normally a transient local process controlled by a balance of angiogenic and angiostatic factors, tumours have to subvert this to achieve sustained blood vessel formation. In many cases, this leads to tumour blood vessels being structurally abnormal and potentially usefully different from the rest of the vascular system. An anti-angiogenic agent should, in principle, be useful in all solid tumour disease to produce at least growth stasis.

There are a number of biopharmaceutical approaches directed against angiogenesis, including antibodies and angiostatic factors, which have yet to be fully tested in the clinic. Some low molecular-weight compounds are also known to be anti-angiogenic, including the natural product-derived TNP470 **37**[32,33] and thalidomide **38**. It is likely that the anti-angiogenic actions of the latter compound contribute to its well-known production of birth defects. Angiogenesis is a multiple-step process involving activation of endothelial cells, synthesis and release of degradative enzymes, migration and proliferation of the cells and then organisation and differentiation to form the new structure. Consequently, it is not always possible to determine which steps are affected by a given compound. As the process has been studied in greater depth, specific opportunities for therapeutic intervention have emerged.



8.1 VEGF receptor tyrosine kinase inhibition

Amongst the mechanisms involved, the vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF) receptor tyrosine kinases represent targets which look amenable to drug discovery efforts. Of the two, the VEGF receptor would seem to offer the best chance for selectivity since it is found predominantly on the vascular endothelium and most research has focused on this target.[34] VEGF is also known as vascular permeability factor and it is suggested that it facilitates tumour progression by stimulating angiogenesis and increasing vascular permeability. Tumour cells are known to make and secrete VEGF, which then acts locally on endothelial cells. This should not only provide some tumour selectivity, but also may help to avoid the sort of drug resistance mechanisms utilised by tumours because endothelial cells are not similarly genetically unstable.

There are two forms of the VEGF receptor, KDR and Flt, against which compounds have been tested for their ability to inhibit tyrosine kinase activity. No strong evidence exists for one form being significantly more important than the other for the angiogenic process and most inhibitors seem to have at least some activity against both enzymes. Clinical assessment of drug candidates with different profiles against the receptor forms should be available soon as it is known that compounds have entered pre-clinical development.

9 Anti-invasion approaches

Invasion is another complex process which occurs in both normal and disease situations. With regard to tumours, the definition of malignancy has always been made pathologically in terms of whether growth has been accompanied by invasion into other tissue. The three major components of tumour

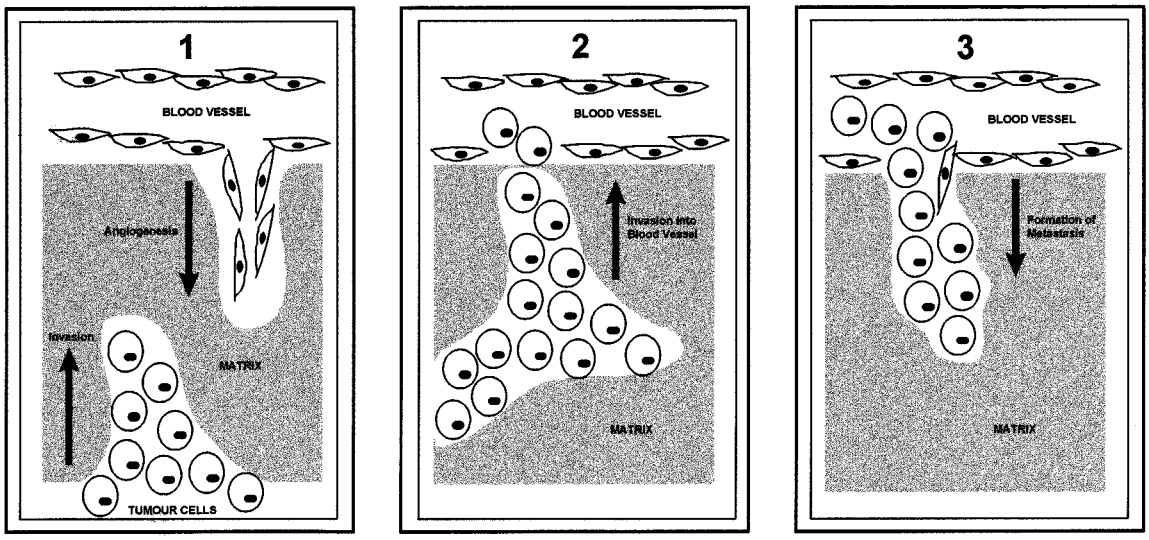


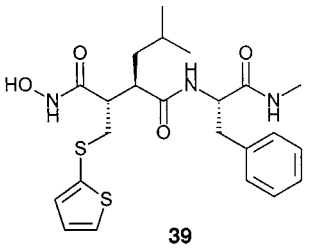
Fig. 9 Angiogenesis and invasion

invasion are tissue degradation, adhesion and migration. Contributory mechanism targets have been identified in all three areas and there is overlap in some cases with angiogenesis approaches.

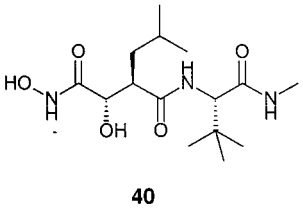
9.1 MMP inhibition

Most research has been carried out in the area of tissue degradation, which is also of great relevance in diseases such as arthritis. Inhibition of matrix metalloproteinases (MMPs) has been by far the most investigated of the approaches.[35] These enzymes constitute a family of zinc and calcium-dependent endoproteinases which is capable physiologically of breaking down all of the protein components in the extracellular matrix. Normal tissue remodelling involving MMPs occurs in processes such as wound healing and connective tissue maintenance, but the same processes are important in tumour invasion and the enzymes have been found in a range of solid tumour types. The three major MMP classes are collagenases, stromelysins and gelatinases. On the basis of tumour association and their ability to degrade basement membrane, gelatinases are claimed to be the preferred target in cancer. It seems likely that, as with some of the areas described above, clinical testing of compounds with differing profiles against the MMP classes will determine which are most relevant.

Initial medicinal chemistry interest has been centred around broad-spectrum peptidic structures bearing a zinc-binding ligand, often a hydroxamic acid. Whilst this has resulted in extremely potent compounds being discovered and batimistat **39** and marimistat **40** being taken into the clinic, there are a



39



40

number of problems with inhibitors of this type. In particular, they often have very poor aqueous solubility which can lead to difficult formulation and contribute to poor bioavailability and pharmacokinetics. Adverse effects such as joint pain have also been seen in the clinic and these findings cannot be attributed with any certainty to the general approach given the lack of enzyme selectivity with these compounds.

The more recent availability of X-ray and NMR structures of collagenase and stromelysin combined with high-throughput screening should help to provide additional start points to those derived so far from rational design based on substrate cleavage sites. Modification of physical properties, particularly by introduction of non-peptide structures and replacement of the widely used zinc ligands, to improve pharmacokinetics and metabolism remains the goal of second generation MMP inhibitors in cancer.

It is still much too early to say whether an anti-invasive agent will be sufficiently effective as a single agent in cancer. Although it is generally true that cancer treatments will involve multiple drug therapy, there is a greater expectation that anti-invasives, and anti-angiogenic agents, will be used in combination with drugs targeting the tumour cell exclusively.

10 Conclusion

The breadth of cancer therapeutic research means that only a limited, illustrative coverage of a few key areas has been attempted. For example antisense oligonucleotide[35,37] and gene therapy[38] approaches to cancer have not been considered. In both cases, the technologies and therapeutics differ sufficiently from previous pharmaceutical systems to require fuller explanation. Furthermore, whilst clinical studies are being conducted with examples of both types, their prospects in solid tumour disease are probably confined to proof of principle in this phase. Similarly, understanding of differentiation mechanisms is still at an early stage, despite the interesting activities of retinoid compounds,[39] and approaches to restoration of normal morphology and function to tumour cells are not sufficiently advanced for inclusion.

Nevertheless, the general message for cancer therapy is that a new era has begun. It started with the development of the techniques of molecular biology which allowed identification and investigation of individual components in key cell systems. This not only provided the basis for elucidating molecular mechanisms, but also allowed the production of individual proteins or their relevant domains (often as the human version) for structural study and use in compound screening. Now that targets of particular relevance to tumours can be more readily identified, drug discovery research has started to operate at the molecular level. The final phase requires that the clinical approach builds on this process and ensures that the developing speciality of molecular medicine becomes established in cancer.

11 Acknowledgements

We thank Stephen Green, Phillip Hedge and Donald Ogilvie for providing diagrams to us and Andrea Torkington for preparation of this manuscript.

12 References

1 *Cancer Facts and Figures*, American Cancer Society, Atlanta, 1997, pp. 1–17.
2 B. Chabner, *Cancer Principles and Practice in Oncology*, ed. V. T. DeVita, S. Hellman, S. A. Rosenberg and J. B. Lippincott, Philadelphia, 1993, pp. 325–417.
3 J. O. Trent, G. R. Clarke, A. Kamur, W. Wilson, D. W. Boykin, J. E. Hall, R. R. Tidwell, B. L. Blackburn and S. Neidle, *J. Med. Chem.*, 1996, **39**, 4554.
4 A. L. Jackman and A. H. Calvert, *Ann. Oncol.*, 1995, **6**, 871.
5 D. C. Blakey, *Acta Oncologica*, 1992, **31**, 91.
6 K. D. Bagshawe, *Mol. Med. Today*, 1995 **1**, 424.
7 R. Melton, R. Knox and T. A. Connors, *Drugs of the Future*, 1996, **21**, 167.
8 A. Howell, R. B. Clarke and E. Anderson, *Endocrine-Related Cancer*, 1997, **4**, 371.
9 T. J. Powles, *Semin. Oncol.*, 1997, **24**, Suppl. 1: S1-48-S1-54.
10 W. J. Gradishar and V. C. Jordan, *J. Clin. Oncol.*, 1997, **15**, 840.
11 P. E. Goss and K. M. E. H. Gwyn, *J. Clin. Oncol.*, 1994, **12**, 2460.
12 Y.-H. Kao, L. L. Cam, C. Laughton, D. Zhou and S. Chen, *Cancer Res.*, 1996, **56**, 3451.
13 S. Graham-Lorence, B. Amarneh, R. H. White, J. A. Peterson and E. R. Simpson, *Protein Sci.*, 1995, **4**, 1065.
14 W. R. Fair, M. S. Cookson, N. Stroumbakis, D. Cohen, A. G. Aprikian, Y. Wan, P. Russo, S. M. Soloway and J. Sogani, *Urology*, 1997, **43** (3A) Suppl, 46.
15 B. J. A. Furr, G. R. P. Blackledge and I. D. Cockshott, *Hormone Dependent Cancer*, ed. J. R. Pasqualini and B. S. Katzenellenbogen, Dekker, New York, 1996, pp. 397–424.
16 J. L. Tenover, G. A. Pagano, A. S. Morton, C. L. Liss and C. A. Bymes, *Clin. Ther.*, 1997, **19**, 243.
17 G. Powis, *Curr. Opin. Oncol.*, 1995, 554.
18 J. B. Trepel, J. D. Moyser and F. Cuttita, *Biochem. Biophys. Res. Commun.*, 1988, **156**, 1383.
19 A. Gazit, N. Osherov, C. Giton and A. Lavitzki, *J. Med. Chem.*, 1996, **39,** 4905.
20 N. M. Law and N. B. Lydon, *Emerging Drugs*, 1996, 241.
21 J. Woodburn, A. J. Barker, K. H. Gibson, S. E. Ashton, A. E. Wakeling, B. J. Curry, L. Scarlett and L. R. Henthorn, *J. Immunotherapy*, 1997, **20**, 408.
22 M. Yongqi, C. Omer and R. A. Gibbs, *J. Am. Chem. Soc.*, 1996, **118**, 1817.
23 S. Graham and T. M. Williams, *Exp. Opin. Ther. Patents*, 1996, **6**, 1295.
24 T. Pawson and J. D. Scott, *Science*, 1997, **278**, 2075.

25 L. Meijer, S. Guidet and H. Y. L. Tung, *Progress in Cell Cycle Research*, Plenum Press, New York, 1996, vol. 1, 373.
26 H. H. Sedlacek, J. Czech, R. Nqaik, G. Kaur, W. Worland, M. Losiewicz, B. Parker, B. Carlson, A. Smith, A. Senderowicz and E. Sausville, *Int. J. Oncol.*, 1996, 1143.
27 W. F. Jr. De Azevedo, H.-J. Mueller-Dieckmann, U. S. Gahmen, P. J. Worland, E. Sausville and S.-H. Kim, *Proc. Natl. Acad. Sci. USA*, 1996, **93**, 2735.
28 E. White, *Genes and Development*, 1995, 1.
29 J. C. Reed, *Nature*, 1997, **387**, 773.
30 W. Risau, *Nature*, 1997, **386**, 671.
31 J. Folkman, *New Eng. J. Med.*, 1995, **333**, 1757.
32 D. Ingber, K. Takeshi, S. Shoji, T. Kanamura, H. Brem and J. Folkman, *Nature*, 1990, **340**, 555.
33 M. Skobe, P. Rockwell, N. Goldstein, S. Vosseler and N. Fusenig, *Nature Med.*, 1997, **3**, 1222.
34 L. Liotta, *Cancer Res.*, 1986, **46**, 1.
35 H. S. Rammusen and G. M. Hockel, *Pharm News*, 1997, **4**, 11.
36 C. A. Stein, *Cancer Principles and Practice in Oncology*, ed. V. T. DeVita, S. Hellman, S. A. Rosenberg and J. B. Lippincott, Philadelphia, 1993, pp. 2646–2648.
37 C. Helene, *New Approaches in Cancer Pharmacology: Drug Design and Development*, ed. P. Workman, Springer-Verlag, Heidelberg, 1992, vol. 1, pp. 13–24.
38 M. Singh, V. Parikh and A. Sharma, *Drugs of the Future*, 1997, **22**, 995.
39 L. M. De Luca, *FASEB J.*, 1993, 2924.

Received 23rd January 1998
Accepted 23rd February 1998