TIMELINE

Protein kinases — the major drug targets of the twenty-first century?

Philip Cohen

Protein phosphorylation regulates most aspects of cell life, whereas abnormal phosphorylation is a cause or consequence of disease. A growing interest in developing orally active protein-kinase inhibitors has recently culminated in the approval of the first of these drugs for clinical use. Protein kinases have now become the second most important group of drug targets, after G-protein-coupled receptors. Here, I give a personal view of some of the most important advances that have shaped this field.

Protein phosphorylation was originally identified as a regulatory mechanism for the control of glycogen metabolism, and it took many more years before its general significance came to be appreciated[1]. Two observations that were made more than 20 years ago, and did much to stimulate awareness of the importance of protein phosphorylation, also provided the first connections between abnormal protein phosphorylation and disease (see TIMELINE). In 1978, Ray Erikson found that the transforming factor of the Rous sarcoma virus (v-Src) was a protein kinase[2], and — during a short visit to Yasutomi Nishizuka's laboratory in 1981 — Monique Castagna discovered that tumour-promoting phorbol esters were potent activators of protein kinase C (PKC)[3] that probably mimicked activation by the second messenger diacylglycerol[4].

The first protein-kinase inhibitors

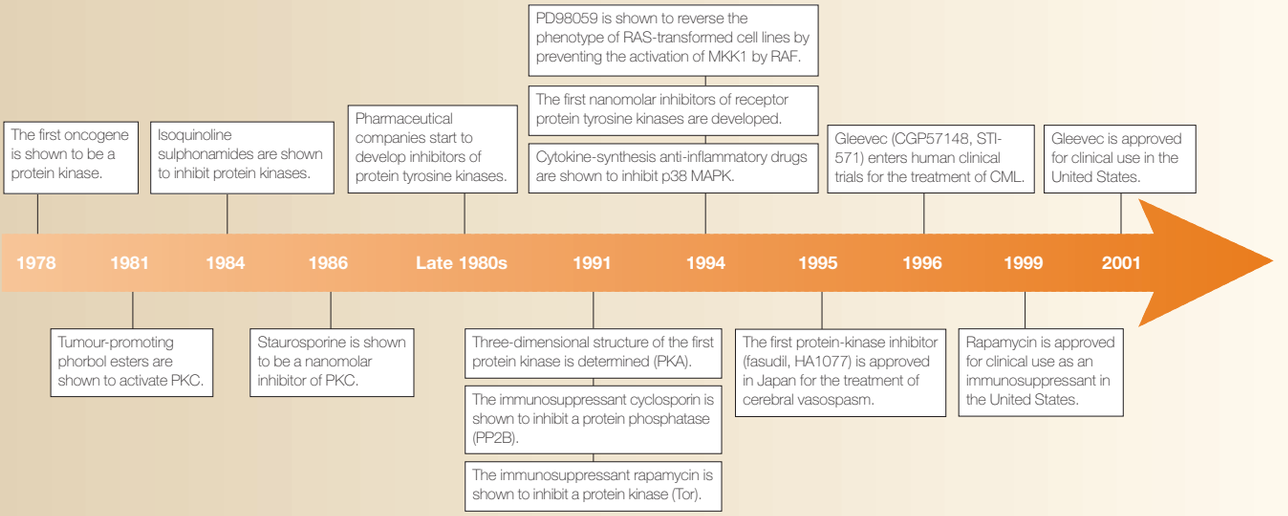
The first protein-kinase inhibitors were developed in the early 1980s by Hiroyoshi Hidaka. Naphthalene sulphonamides, such as *N*-(6-amino-hexyl)-5-chloro-1-naphthalenesulphonamide (W7), which had already been developed as antagonists of the calcium-binding protein calmodulin, were also found (at higher concentrations) to inhibit several protein kinases. However, when the naphthalene ring was replaced by isoquinoline, Hidaka observed that the derivatives were no longer calmodulin antagonists, but retained the ability to inhibit protein kinases. One of these compounds — termed H8 (FIG. 1) — was a much stronger inhibitor of cyclic-AMP- and cGMP-dependent protein kinases than four other protein kinases tested, whereas another (H7) inhibited cyclic-nucleotide-dependent protein kinases and PKC with similar potency. These compounds were ATP competitive and, importantly, were cell permeable, which indicated their potential use *in vivo*[5].

Although these and many other isoquinolinesulphonamides that were developed subsequently are of relatively low potency and inhibit several protein kinases, one of these compounds (HA1077, also known as AT877 or fasudil hydrochloride; TABLE 1; FIG. 2) progressed to human clinical trials in the early 1990s. It was approved in Japan in 1995 for the treatment of cerebral vasospasm after surgery for subarachnoid haemorrhage, and associated cerebral ischaemic symptoms. HA1077 had no marked side effects over a two-week period when given by intravenous injection (see, for example, REFS 6,7). At micromolar concentrations, HA1077 inhibits several protein kinases, such as the RHO-dependent protein kinase ROCK[8], but it is unclear whether its clinical efficacy results from inhibition of this or other protein kinases, or whether it is due to a non-kinase effect. ROCK can constrict blood vessels by inhibiting smooth-muscle myosin phosphatase[9], and Y27632 — a more specific ROCK inhibitor — might exert its anti-hypertensive effects in this way[10].

It was the discovery that staurosporine (FIG. 1), an antifungal agent that is produced by bacteria of the genus *Streptomyces*, was a nanomolar inhibitor of PKC[11] that really made the pharmaceutical industry sit up and take notice. This discovery led several companies to make and test many derivatives of this bisindolyl maleimide. Two such compounds, which were developed by Roche (Ro31-8220)[12] and Goedke (Go6850; also called GF109203X)[13], were used subsequently in cell-based assays by hundreds of laboratories to invoke a myriad of roles for PKC. However, these and several other bisindolyl maleimides were later shown to lack specificity, and inhibited several other protein kinases *in vitro*[8,14].

Nevertheless, several bisindolyl maleimides have progressed to human clinical trials, although it is unclear whether their efficacy stems from the inhibition of PKC, another protein kinase or even the combined inhibition of several protein kinases. The compound 7-hydroxystaurosporine (UCN-01; TABLE 1; FIG. 2) also blocks cell-cycle progression and might act by inhibiting the cell-cycle checkpoint-control kinase CHK1 (REF. 15). *N*-benzoyl staurosporine (PKC412), which is being developed by Novartis, has entered human clinical trials for the treatment of advanced cancers (TABLE 1). It also inhibits retinal neovascularization and laser-induced choroidal neovascularization in mouse models, and is now in Phase I clinical trials for the treatment of ischaemic retinopathy[16]. Another bisindolyl maleimide, LY333531, which inhibits the PKC-β isoform more potently than other PKC isoforms, is reported to normalize the elevated levels of

Timeline | **Key events in the development of protein-kinase inhibitors**



CML, chronic myelogenous leukaemia; EGF, epidermal growth factor; MAPK, mitogen-activated protein kinase; MKK1, mitogen-activated protein kinase kinase 1; PKA, protein kinase A; PKC, protein kinase C; Tor, target of rapamycin.

PKC activity in the retina and kidneys of diabetic rats[17]. It is in Phase III clinical trials for the treatment of diabetic microvascular disease (TABLE 1). The staurosporine analogue CEP-1347 (FIG. 2) is reported to prevent the activation of the c-JUN amino-terminal kinase (JNK) by inhibiting the mixed-lineage kinase (MLK), which is an 'upstream' component of this signalling pathway[18]. It has entered human clinical trials for the prevention of neuronal apoptosis and neurodegeneration.

Immunosuppressant drugs

By the end of the 1980s, no protein-kinase inhibitors had entered human clinical trials. Moreover, virtually all of the protein-kinase inhibitors that had been developed were ATP competitive, and the difficulties that were involved in developing compounds with sufficient potency to compete with the ATP concentrations that are present in the intracellular millieu (2–10 mM) were becoming apparent. Furthermore, with the determination of the first three-dimensional structure of a protein kinase (protein kinase A; PKA)[19], it had become apparent that the residues that were involved in binding ATP were conserved from kinase to kinase. A myth therefore began to permeate the field that it was 'impossible' to develop protein-kinase inhibitors with the requisite potency and specificity.

A turning point came in 1991, when Stuart Schreiber identified cellular targets for cyclosporin (FIG. 1) and FK506. Cyclosporin A, which is a naturally occurring secondary metabolite that is produced by *Tolypocladium inflatum* (a fungus isolated from a Norwegian soil sample), was approved for clinical use in 1983, and has revolutionized organ transplantation by its ability to prevent graft rejection. However, the mechanism by which it inhibited T-cell activation remained unknown for many years. Schreiber and his colleagues showed that the complex that is formed between cyclosporin and cyclophilin (its intracellular receptor protein) was a potent and specific inhibitor of calcineurin[20], a Ca^{2+}–calmodulin-dependent protein phosphatase that had been identified ten years previously in a completely different context[21]. FK506 is produced by *Streptomyces tsukubaensis*, a soil bacterium that originates from the north of Japan. The complex that is formed between FK506 and the FK-binding protein (FKBP) also inhibits calcineurin, which indicates a similar mechanism of action to that of cyclosporin[20]. The inhibition of calcineurin prevents the dephosphorylation of transcription factors of the NFAT (nuclear factor of activated T cells) family in T cells, which inhibits their entry into the nucleus. This stops the production of interleukin-2 (IL-2), and hence T-cell proliferation. So, an important drug that is already in clinical use exerted its effects by modulating the phosphorylation of one or more intracellular proteins.

Rapamycin (FIG. 1) is produced by *Streptomyces hygroscopicus*, a soil bacterium that originates from Easter Island (Rapa Nui is the native name for Easter Island) and was identified more than 30 years ago. It was originally purified as an antifungal agent, but was initially discarded because of its undesirable immunosuppressive side effects[22]. It is only more recently that the potential of rapamycin as an immunosuppressant was explored, and it was approved for clinical use in 1999. Since that time, rapamycin has rapidly become the immunosuppressant of choice to prevent rejection after kidney transplantation, because its side effects are less severe than those of cyclosporin. It also prevents the rejection of pancreatic islets, the transplantation of which is a potential way to treat insulin-dependent diabetes mellitus (IDDM) without the need for daily injections of insulin.

The anticancer properties of rapamycin, which was the first cytostatic agent to be identified, were first noted in the mid-1970s, and the compound was sent to the National Cancer Institute (NCI) in the United States for testing by Suren Sehgal, a scientist at Ayerst Research Laboratories in Montreal. The impressive activity of rapamycin against solid tumours, when used in combination with chemotherapy, led the NCI to designate it a 'priority drug'. However, when Ayerst closed down the Montreal laboratories in 1982, the rapamycin programme was abandoned. It was only when the two subsidiaries of American Home Products — Wyeth and Ayerst — merged in 1988 that Sehgal managed to get the project resurrected. CCI779, a close analogue of rapamycin that has improved pharmacological properties, has shown activity against a wide range of cancers[23], and Phase II trials are underway. Another rapamycin analogue, RAD001, which is being developed by Novartis, has also entered human clinical trials (TABLE 1).

Remarkably, the intracellular receptor for rapamycin is also FKBP, but in contrast to the FK506–FKBP complex, rapamycin–FKBP does not inhibit calcineurin. Michael Hall identified the molecular target of rapamycin–FKBP in yeast as being a protein kinase, which he termed the 'target of rapamycin' (Tor)[24]. The mammalian homologue, which is called mTOR, or the FKBP–rapamycin-associated protein (FRAP), was identified subsequently. Rapamycin was the first drug to be approved for clinical use that seems to inhibit one protein kinase specifically[8].

mTOR is involved in a phosphatidylinositol (PtdIns)-3-kinase-dependent signalling pathway that has a crucial role in cell-cycle progression from G1-to-S phase, as well as in IL-2-stimulated T-cell proliferation; these are activities that underlie its anticancer and immunosuppressive properties. Consistent with its requirement for the G1-to-S transition, the cytostatic effects of CCI779 are most marked in tumours in which the level of PtdIns-3,4,5-trisphosphate (the product of the class 1 PtdIns 3-kinases) is elevated, such as occurs in tumours that are deficient in the phosphatase-and-tensin homologue (PTEN), which is the phosphatase that converts PtdIns(3,4,5)P_3 to PtdIns(4,5)P_2 (REFS 25,26).

CSAIDs

During bacterial infection, lipopolysaccharide (LPS) — a component of the cell wall of gram-negative bacteria — triggers cells of the immune system to produce proinflammatory cytokines, such as tumour-necrosis factor (TNF) and IL-1, which are released into the circulation, where they help to mount the immune responses that fight — and eventually kill — the invading bacteria. However, this defence mechanism is a double-edged sword, because the uncontrolled production of proinflammatory cytokines can be a cause of chronic inflammatory diseases, such as rheumatoid arthritis, inflammatory bowel disease and septic shock. For this reason, many companies have sought to develop compounds that suppress TNF production.

More than 20 years ago, SmithKline and French developed a new class of pyridinyl imidazoles — exemplified by SKF86002 — that showed efficacy in animal models of chronic inflammatory disease. They were therefore called cytokine-synthesis anti-inflammatory drugs (CSAIDs; see REF. 27 for a review of early work in this area).

In the late 1980s, John Lee and colleagues found that SKF86002 suppressed the LPS-induced production of IL-1 and TNF in human monocytes[28]. Subsequently, more

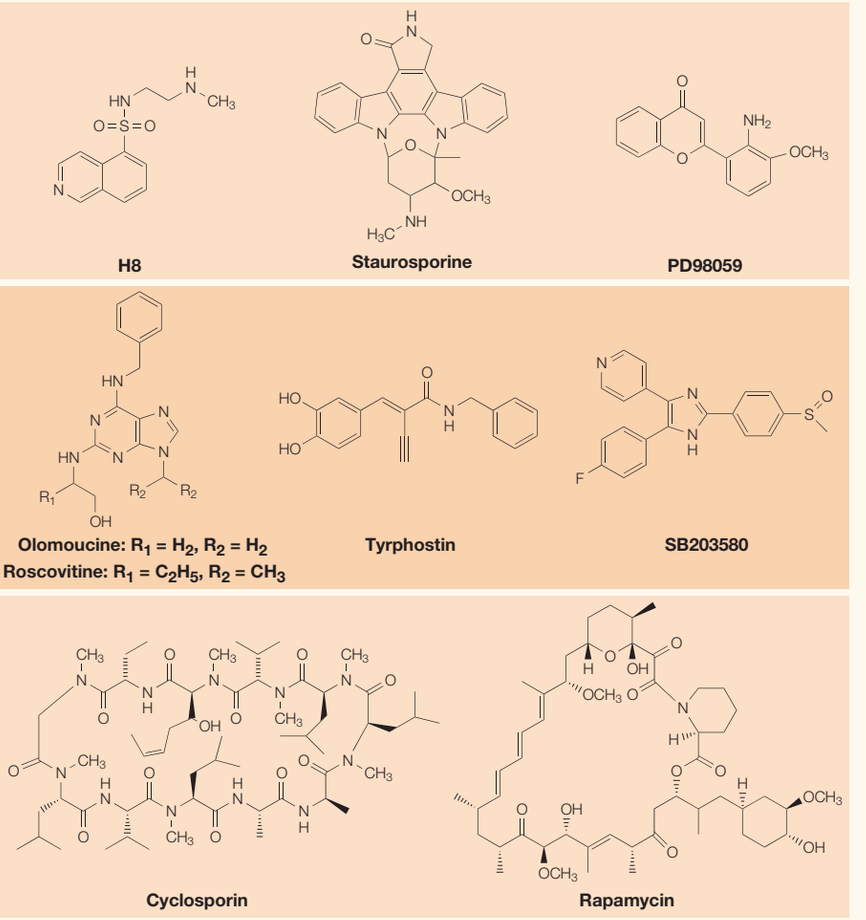


Figure 1 | **Chemical structures of some compounds that have been important for stimulating interest in the development of protein-kinase inhibitors.**

potent pyridinyl-imidazole inhibitors of TNF production, such as SB202190, were shown to prevent translation of the messenger RNA that encodes TNF. A protein that bound these pyridinyl imidazoles specifically was purified from the cell extracts, which led to its identification as a mitogen-activated protein kinase (MAPK) family member that was termed the CSAID-binding protein (CSBP)[29]. This protein kinase was identified independently by several other laboratories[30–32], and is now usually called p38 MAPK. At least three inhibitors of p38 MAPK have now entered human clinical trials for the treatment of rheumatoid arthritis or psoriasis (TABLE 1), and several other companies have potent inhibitors of this enzyme at a preclinical stage.

Although SB203580 (FIG. 1) and the closely related SB202190 are ATP competitive inhibitors, they are relatively specific for p38 MAPK *in vitro* and do not inhibit many other protein kinases that have been tested[8,33]. The basis for this high degree of specificity was shown by the determination of the three-dimensional structure of p38 MAPK in complex with closely related compounds. These studies showed that only a portion of the drug interacts with the residues that bind ATP, whereas the 4-fluorophenyl moiety binds in a small hydrophobic groove that is adjacent to the ATP-binding site[34] (FIG. 3). This groove is created by the presence of a small side chain (threonine) at position 106, and its mutation to a bulky hydrophobic residue, which is found at this position in most other protein kinases, makes p38 MAPK insensitive to SB203580 (REFS 35,36). The finding that specificity is conferred largely by interaction with residues that lie near, but out with, the ATP-binding pocket also explains the high degree of specificity of several other ATP competitive inhibitors of different protein kinases that were developed subsequently. Examples include SU5402, an inhibitor of the fibroblast-growth-factor receptor (FGFR) tyrosine kinase[37], and two cyclin-dependent protein-kinase inhibitors — roscovitine (REF. 38; FIG. 1) and purvalanol[39] (FIG. 3).

Table 1 | **Some small-molecule inhibitors of protein kinases that are undergoing human clinical trials**

Kinase targeted	Inhibitor	Company	Disease	Status
Tyrosine kinases				
ABL (KITR, PDGFR)	Gleevec (Glivec, STI-571)	Novartis	Cancer	FDA approval, May 2001
EGFR	ZD1839 (Iressa) OSI774	AstraZeneca OSI Pharmaceuticals/Roche/Genentech	Cancer Cancer	Phase III Phase III
EGFR, ERB2R	CI1033 EKB569 GW2016 PKI166	Pfizer Wyeth-Ayerst GlaxoSmithKline Novartis	Cancer Cancer Cancer Cancer	Phase I Phase I Phase I Phase I
VEGFR (PDGFR, FGFR)	SU6668	Pharmacia Corporation	Cancer	Phase I
VEGFR	PTK787/ZK222584	Novartis/Schering-Plough	Cancer	Phase II
VEGFR (EGFR)	ZD6474	AstraZeneca	Cancer	Phase I
NGFR	CEP2583	Cephalon	Cancer	Phase II
Serine/threonine kinases				
PKC, KITR, PDGFR?	PKC412	Novartis	Cancer, retinopathy	Phase I
CDKs?	Flavopiridol	Aventis	Cancer	Phase II
CDK2	CYC202	Cyclacel	Cancer	Phase I
MKK1	PD184352	Pfizer	Cancer	Phase I
RAF?	BAY43-906	Onyx Pharmaceuticals/Bayer	Cancer	Phase I
CHK1, PKC, others?	UCN-01	Kyowa Hakko	Cancer	Phase I
mTOR	CCI779 RAD001 Rapamycin (Sirolimus)	Wyeth-Ayerst Novartis Wyeth-Ayerst	Cancer Cancer Immunosuppression	Phase II Phase I FDA approval, 1999
ROCK?	HA1077 (AT877, fasudil)	Asahi Chemical Industry	Cerebral vasospasm	Approved in 1995 (Japan)
PKCβ	LY333531	Eli Lilly	Diabetic retinopathy	Phase III
p38/SAPK2a	SB281832 BIRB0796 Ro320-1195	GlaxoSmithKline Boehringer Ingelheim Roche	Rheumatoid arthritis Rheumatoid arthritis Rheumatoid arthritis	Phase I Phase II Phase I
MLK	CEP-1347	Cephalon	Neurodegeneration	Phase I

ABL, Abelson tyrosine kinase; CDK, cyclin-dependent kinase; CHK1, checkpoint kinase 1; EGFR, epidermal-growth-factor receptor; ERB2R, ERB2 receptor; FDA, US Food and Drug Administration; FGFR, fibroblast-growth-factor receptor; KITR, c-KIT receptor; MKK1, mitogen-activated protein kinase kinase 1; MLK, mixed-lineage protein kinase; mTOR, target of rapamycin (mammalian); NGFR, nerve-growth-factor receptor; p38, p38, mitogen-activated protein kinase; PDGFR, platelet-derived-growth-factor receptor; PKC, protein kinase C; ROCK, RHO-dependent protein kinase; SAPK, stress-activated protein kinase; VEGFR, vascular-endothelial-growth-factor receptor.

Anticancer agents

Cyclosporin and rapamycin were the first compounds to be approved as drugs that exert their effects by inhibiting a particular protein phosphatase or protein kinase. However, these compounds — as well as the CSAIDs that inhibit p38 MAPK — were not developed as a result of this knowledge, and their clinical efficacy was known before their mechanism of action was elucidated. It is in the field of cancer in which much of the effort to develop drugs that target specific protein kinases has been concentrated. At present, some of the most promising drugs in development as anticancer agents are inhibitors of protein tyrosine kinases. Progress in this area has been greatly influenced by seminal studies that were carried out 10–15 years ago, which identified important compound classes, such as quinazolines and tyrphostins, that potently inhibit protein tyrosine kinases (reviewed in REFS 40–42).

Growth factors, such as epidermal growth factor (EGF), activate the classical RAS-dependent MAPK cascade, which is required for the proliferation of some cells and the differentiation of others. However, the uncontrolled activation of this pathway is now known to cause cancer. This can occur as a result of the overexpression of particular growth-factor-receptor tyrosine kinases or their mutation to constitutively active forms. For example, the EGF receptor (EGFR) is overexpressed in many cancers of epithelial origin, such as lung and breast cancers. The mutation of RAS itself to constitutively active, oncogenic forms occurs in 25% of human cancers, whereas the protein kinase RAF was discovered to be a viral oncogene. There has therefore been considerable interest in the development of drugs to treat different cancers that target particular protein kinases in this pathway.

Several antibodies that bind to the extracellular domain of the EGFR are undergoing clinical trials, including C225 (also called Cetuximab), which was developed by Imclone Systems and is in Phase III clinical trials for the treatment of several cancers. C225 has to be administered intravenously; however, several promising orally active drugs that are potent and relatively specific inhibitors of the EGFR tyrosine kinase are now well advanced in human clinical trials (TABLE 1). The AstraZeneca compound ZD1839, which is now called Iressa, has shown marked efficacy against several cancers in human clinical trials (reviewed in REF. 43), and will probably be approved for clinical use in 2002 (FIG. 2). Another compound, OSI774, is also in Phase III clinical trials[43].

Another promising approach to the development of anticancer drugs with broad efficacy is based on Judah Folkman's 25-year-old idea of destroying the blood supply to a tumour. Angiogenesis depends on growth factors, such as platelet-derived growth factor (PDGF), FGF and vascular endothelial growth factor (VEGF). Antibodies have been raised that bind to the extracellular domain of the VEGF receptor, and one that has been developed by Genentech is now in Phase III clinical trials as an anti-angiogenic agent. However, several promising orally active inhibitors of the VEGF receptor (VEGFR) protein tyrosine kinase — including SU6668, PTK787 and ZD6474 (TABLE 1) — are undergoing human clinical trials (reviewed in REF. 43) .

Cyclin-dependent protein kinases (CDKs) have essential roles in cell proliferation, which has stimulated considerable interest in the development of inhibitors of these enzymes to suppress tumour growth. Two CDK inhibitors that are undergoing human clinical trials are shown in TABLE 1, the first of which was Flavopiridol[44]. More potent and specific CDK inhibitors have been developed by many other companies, but whether they have entered human clinical trials has not yet been disclosed.

Interestingly, certain indirubin dyes, which are the active ingredients in an ancient Chinese herbal remedy that has been used for centuries to treat diseases such as cancer, are potent CDK inhibitors[45]. So, without anyone realising it, CDK inhibitors might actually have been in clinical use for rather a long time!

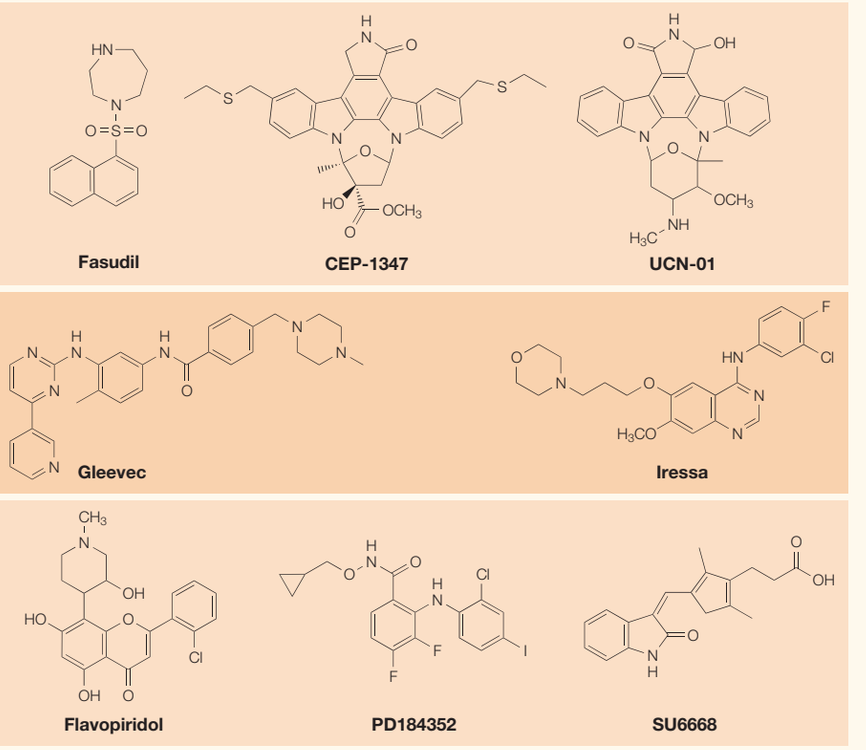


Figure 2 | **Chemical structures of some small-molecule inhibitors of protein kinases that have been approved or are undergoing human clinical trials.** Further information is given in the TIMELINE.

Gleevec

A landmark event occurred in May 2001 when Gleevec — the first important drug to be developed by targeting a protein kinase specifically (the Abelson tyrosine kinase, ABL) — was approved for clinical use. ABL becomes fused to the breakpoint cluster region (BCR) protein as a result of a chromosome rearrangement in nearly all cases of chronic myelogenous leukaemia (CML). This creates a kinase with enhanced activity that might cause CML by eliciting uncontrolled activation of the MAPK cascade.

A programme to develop an inhibitor of ABL was initiated by Nick Lydon at Novartis in 1986. However, it was only when baculovirus expression systems for the large-scale production of several protein tyrosine kinases were developed by Tom Roberts and Nick Lydon in the late 1980s that the programme really started to take off. The compound that subsequently entered human clinical trials (CGP57148B; later called STI-571 and then Gleevec/Glivec) was discovered around 1992 (FIG. 2)[46], but its development was not initially given a high priority because of the relatively low incidence of CML. Support for the development of CGP57148B came as a result of a pivotal study that was carried out by Brian Druker, which profiled this compound in *ex vivo* colony-forming assays using cells from CML and control cells[47]. After the initiation of a clinical trial, spectacular efficacy and minimal side effects were shown[48]. This led to its extremely rapid approval for clinical use, and has created great excitement in the pharmaceutical industry (reviewed in REFS 43,49).

Although Gleevec is an ATP competitive inhibitor, the determination of the three-dimensional structure of ABL in complex with Gleevec showed that the drug extends much further into the catalytic site[50]. In particular, it straddles the highly conserved amino-terminal region of the 'activation loop' that controls the catalytic activity of many protein kinases by switching them from an inactive dephosphorylated form to an active phosphorylated state. Intriguingly, the binding of Gleevec induces a structural transition that causes the kinase to adopt the inactive conformation. The compound BIRB0796 (TABLE 1) has recently been shown to interact with p38 MAPK in an analogous way (S. Jakes, personal communication).

Although Gleevec is a relatively specific inhibitor of ABL, it also inhibits the c-KIT and PDGF-receptor tyrosine kinases with similar potency[51]. It has been undergoing clinical trials for the treatment of gastrointestinal stromal tumours and other cancers in which c-KIT or PDGF-receptor signalling is deregulated[52], and was recently approved for the treatment of gastrointestinal stromal tumours.

Preventing kinase activation

Alan Saltiel and his colleagues at Parke-Davis developed the compound PD98059 (FIG. 1) during a screen to identify inhibitors of a 'constitutively' active mutant of MAPK kinase-1 (MKK1), which can be produced by mutating the residues that are phosphorylated by RAF to glutamic acid (MKK1-EE)[53]. However, we were surprised to find that PD98059 does not inhibit MKK1 that had been activated by RAF. Because MKK1-EE has < 1% of the activity of phosphorylated MKK1, this indicates that the screen selected for compounds that bind much more strongly to the inactive form of MKK1.

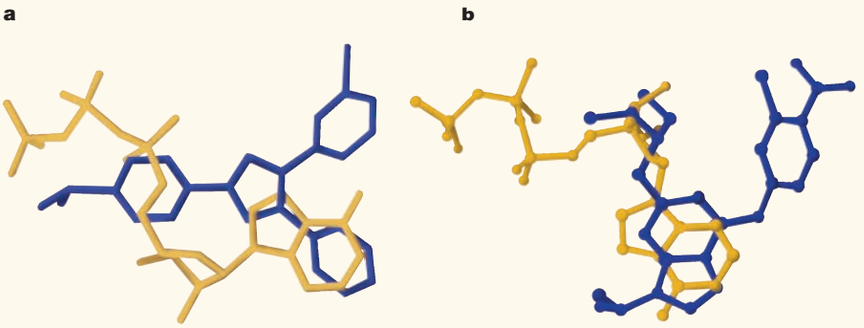


Figure 3 | **Basis for the specificity of two ATP-competitive protein-kinase inhibitors. a** | Comparison of the binding of SB203580 (blue) and ATP (yellow) to p38 MAPK[34]. **b** | Comparison of the binding of purvalanol (blue) and ATP (yellow) to CDK2[39]. Drug specificity is largely determined by the part of the molecule that does not interact with residues that bind ATP, but which occupies a hydrophobic binding pocket near the ATP-binding site. CDK2, cyclin-dependent kinase 2; p38 MAPK, p38 mitogen-activated protein kinase.

This led us to discover that PD98059 prevents the activation of MKK1 by RAF[54]. So, PD98059 is not a kinase inhibitor, but binds to the inactive, dephosphorylated form of MKK1, preventing its activation by RAF both *in vitro* and in cell-based assays. The more potent compounds U0126 (REF. 55) and PD184352 (REF. 56; TABLE 1; FIG. 2), which were developed subsequently, also seem to prevent the activation of MKK1 much more potently than they inhibit MKK1 activity[8].

PD98059 is one of the few synthetic protein-kinase inhibitors that is not ATP competitive. The way in which it was identified is therefore instructive, and raises the possibility that completely different compounds might be obtained by screening with either high- or low-activity forms of particular protein kinases. PD98059 reverses the phenotype of RAS-transformed cell lines[53], and PD184352 was reported to inhibit the growth of human colon tumours that were implanted into mice without causing obvious adverse side effects over several months of treatment[56]. For this reason, PD184352 has now entered Phase I clinical trials. The absence of side effects that have been reported so far has come as a surprise to the cell-signalling community, because the classical MAPK cascade has been implicated in so many cellular processes. However, perhaps its essential roles in proliferation and differentiation are required only during embryogenesis, and the pathway might be far less crucial for normal function in adults than previously supposed.

The future

Three protein-kinase inhibitors (fasudil, rapamycin and Gleevec) and one protein-phosphatase inhibitor (cyclosporin) have so far been approved for clinical use, and a further 23 protein-kinase inhibitors are known to be undergoing human clinical trials (TABLE 1). However, many others have entered clinical trials without their structures being disclosed, and a great many more are still at the preclinical stage. As a result, protein kinases are already the second largest group of drug targets after G-protein-coupled receptors, and they account for 20–30% of the drug discovery programmes of many companies. There is clearly no shortage of potential targets, as protein kinases comprise the largest enzyme family, with ~500 being encoded by the human genome.

In the field of cancer, in which much of the effort so far has been directed, protein-kinase inhibitors are proving to be well tolerated compared with conventional chemotherapeutic treatments. This is encouraging, because several protein-kinase inhibitors that have entered human clinical trials are not very specific. This has become obvious only since the introduction of much larger panels of protein kinases for profiling specificity[8]. More extensive use of even larger panels will aid the future development of more specific protein-kinase inhibitors, and also allow these enzymes to be reclassified according to the similarities of their ATP-binding sites rather than their amino-acid sequences.

It will become more important to show that a drug is exerting its effect through inhibition of a particular protein kinase, by showing that the effect of the drug disappears when a drug-resistant mutant is over-expressed or replaces the wild-type enzyme. However, this has been shown in only a few cases (for example, REFS 57,58). The mutation of a single amino acid can make a kinase drug resistant[57,58], and mutations in ABL that make it resistant to Gleevec are the cause of relapse in patients who have the advanced stage of CML[59]. However, although such resistance presents new therapeutic challenges, resistance to Gleevec has been seen only in the most advanced forms of CML, in which extensive genomic instability occurs, which leads to many additional genetic changes.

Most protein-kinase inhibitors that are identified by high-throughput screening are ATP competitive and, although inhibitors of high specificity can clearly be obtained by targeting the ATP-binding site, the development of sufficient potency to compete with the ATP concentrations that are present *in vivo* is still a problem, especially for the many protein kinases that have a Michaelis–Menten constant (K_m) for ATP of 10 μM or less. Indeed, it might be no coincidence that a plethora of potent inhibitors that target the p38 MAPK have been developed, because its K_m for ATP is above 0.1 mM. It is surprising that virtually no compounds that compete for binding with the protein substrate have been identified. Unlike ATP competitive inhibitors, such compounds might have the potential to prevent a protein kinase from phosphorylating some substrates but not others[60], and their development is clearly an important challenge for the future. A much greater emphasis on developing compounds that bind preferentially to the inactive forms of protein kinases, or which prevent one protein kinase from activating another, might well pay dividends. Smarter ways to screen for — or design — inhibitors of the activity and activation of particular protein kinases on the basis of a detailed understanding of their catalytic and regulatory properties is likely to become more and more important.

Finally, it should be mentioned that protein-kinase inhibitors are important not just for the treatment of disease, but also as reagents to help us understand more about the physiological roles of protein kinases. The number of papers that have been referenced in this article that have been cited more than 1,000 times (for example, REFS 5,11,13, 28,53,54) is an indication of the acute need for such compounds by the cell-signalling community. Many specific protein-kinase inhibitors that cannot be used as drugs for reasons of toxicity, pharmacology or solubility, such as PD98059 and SB203580, could be extremely useful research reagents. Pharmaceutical companies have much to gain from the discoveries that will be made by exploiting such compounds, and it is to be hoped that more will be released for general use in the future.

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