

Protein Kinase Inhibitors

(protein kinase / protein kinase inhibitor / cancer therapy)

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Abstract. Since protein kinases have been found to be implicated in many diseases, first of all malignancies, they are considered as promising therapeutic targets. Many protein kinase inhibitors have been designed by now. These molecules have a low molecular weight and most of them bind to protein kinases competing with ATP for the ATP-binding site. Some protein kinase inhibitors currently undergo clinical trials or have already been successfully introduced into treatment as exemplified by Bcr-Abl, c-kit and PDGFR inhibitor imatinib mesylate (Gleevec), flavopiridol and roscovitine, inhibitors of cyclin-dependent kinases, or erlotinib and gefitinib inhibiting EGFR. Discovery of these molecules seems to begin a new era in medicine, especially oncology. Targeting protein kinases represents a promising approach and gives us new hopes of effective non-invasive cancer treatment.

Introduction

Protein kinases (PKs) are indispensable for numerous processes in the cell. These enzymes catalyze phosphorylation of different cellular substrates. Phosphorylation in turn regulates various cellular functions. Normally, their activity is stringently regulated.

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Abbreviations: Abl – Abelson tyrosine kinase, CDK – cyclin-dependent kinase, CML – chronic myelogenous leukaemia, EGFR – epidermal growth factor receptor, ERK – extracellular signal-regulated kinase, FGFR – fibroblast growth factor receptor, Flavo – flavopiridol, Flt-3 – FMS-like tyrosine kinase-3, Hsp90 – heat-shock protein 90, JNKs – c-Jun N-terminal kinases, MAP – mitogen-activated protein, MAPK – mitogen-activated protein kinase, MKK – MAP kinase, PDGF – platelet-derived growth factor, PDGFR – platelet-derived growth factor receptor, PDK-1 – phosphoinositide-dependent kinase-1, PI3K – phosphatidylinositol 3-kinase, PK – protein kinase, PKC – protein kinase C, PKI – protein kinase inhibitor, RPTK – receptor protein tyrosine kinase, STAT – signal transducer and activator of transcription, TK – tyrosine kinase, VEGFR – vascular epidermal growth factor receptor.

However, under pathological conditions PKs can be deregulated, leading to alterations in the phosphorylation and resulting in uncontrolled cell division, inhibition of apoptosis, and other abnormalities and consequently to diseases (Shchemelinin et al., 2006).

Various cancers and other diseases are known to be caused or accompanied by deregulation of the phosphorylation. Inhibition of PKs has been shown to be a promising therapeutic strategy. Many PK inhibitors (PKIs) have been produced and tested in clinic by now. Inhibitors of Bcr-Abl, epidermal growth factor receptor (EGFR), HER2 and protein kinase C (PKC), all of which are deregulated in human malignancies, were among the first ones (Holyoak, 2001; Lydon and Druker, 2004). One of the most effective approaches is to produce small organic molecules targeting the specific tyrosine kinases in the signalling pathway in tumours that can easily penetrate into the tumour cells. A good example of such a molecule is imatinib (imatinib mesylate, Gleevec, STI571) targeting Bcr-Abl fusion kinase in chronic myelogenous leukaemia (CML) (Zhang et al., 2003). There is a very distinct association between Bcr-Abl and CML; therefore Bcr-Abl tyrosine kinase was chosen to be a model target to prove the potential clinical utility of a whole range of tyrosine kinase inhibitors (Holyoak, 2001; Lydon and Druker, 2004). PKs are also considered as potential targets for antiviral drugs. Use of drugs that target cellular proteins required for several viral functions makes it possible to evade drug resistance caused by virus mutation. Certain PKIs could be used against a variety of unrelated viruses including emerging new viral strains because even distantly related viruses commonly require the same cellular proteins (Schang, 2005).

Used in laboratory, PKIs may help to understand intra- and intercellular molecular interactions, elucidate the role of many units of the cellular biochemical network and clear up yet unknown details of different cell processes such as division, metabolism and death.

New data concerning PK inhibition keep emerging. They show us new possibilities for PKI use in clinic and investigation, but also their interferences with normal cellular functions and consequently possible clinical restrictions. A massive amount of information about PKIs and their targets has been collected by now. In this review we tried to survey the most investigated PKs and link them up to the most important PKIs.

Protein kinase inhibitors

Two basic strategies have been developed to inhibit PKs: small organic molecules – PKIs, and monoclonal antibodies.

Protein kinase inhibitors (PKIs) are chemically diverse, low-molecular-weight, less than 600 Da, hydrophobic heterocycles. While most PKIs compete with the ATP substrate, there also exists a group of the ATP non-competitive inhibitors, which have been described as a group of peptide inhibitors of protein kinases (Bogoyevitch et al., 2005). The cyclin-dependent kinase (CDK) inhibitor flavopiridol (Flavo) should be mentioned as an exception. It inhibits CDK9 either in a non-competitive manner or by binding to the ATP site (Schang, 2005).

Many tyrosine kinases (TKs) have been successfully inhibited by now. First of them were the receptor TKs: c-kit, insulin-like growth factor receptor, EGFR, vascular EGFR (VEGFR), fibroblast growth factor receptor (FGFR), platelet-derived growth factor receptor (PDGFR). The first targeted non-receptor TK was fusion kinase Bcr-Abl (Blagden and de Bono, 2002).

Several kinase inhibitors are currently approved for clinical use, e.g. imatinib, erlotinib, gefitinib (Kondapalli et al., 2005). More than 30 ATP-competitive inhibitors currently undergo clinical trials. All drugs designated as kinase inhibitors target the ATP-binding site (Cheetham, 2004). A large number of PK inhibitors have been used in the laboratory as well.

Many PK inhibitors cross-inhibit several PKs. An example could be imatinib, a Bcr-Abl, c-kit and PDGFR inhibitor, or sorafenib, known as a Raf, VEGFR, and PDGFR inhibitor. There are even suggestions that the therapeutic effectiveness of PKIs correlates with their ability to target additional protein kinases along with the main target (Wong et al., 2004).

The potency of a PKI is typically expressed as the IC_{50} value – concentration of the drug at which 50% of kinase activity is inhibited. Most kinase inhibitors are reversible, and their IC_{50} depends on the dissociation constant of the inhibitor and ATP concentration (Knight and Shokat, 2005).

As has been mentioned above, monoclonal antibodies have also been used to inhibit TKs, particularly EGFR (Bogoyevitch et al., 2005). They usually recognize the ligand-binding site and prevent interaction between the ligand and the receptor. Their binding results in growth inhibition or/and apoptosis (Zwick et al., 2002) and removal of the receptor by internalization (Bogoyevitch et al., 2005). We will not survey the group of monoclonal antibodies in this review.

Bcr-Abl, c-kit and PDGFR inhibitor imatinib mesylate

Imatinib mesylate (Gleevec, STI571) was initially designed for the inhibition of the Abl tyrosine kinase in

the Bcr-Abl oncoprotein (Sattler and Salgia, 2004) leading to clinical, hematological and molecular remissions in CML patients (Savage and Antman, 2002; Lydon and Druker, 2004). Imatinib is a small molecule ATP analogue. It competitively binds to the entire interlobal space of Bcr-Abl, where it is interposed between residues from both the N- and C-lobes, and inhibits this fusion tyrosine kinase. X-ray co-crystal structure of STI-571-Abl complex demonstrates that the drug targets an inactive conformation of Abl that is similar to autoinhibited crystal structures of PDGFR and c-kit (Mol et al., 2004). Imatinib binds to ATP binding domains of these PKs and thus cross-reacts with them (Buchdunger, 2000). Besides, imatinib inhibits Arg (Abl-related gene) product and probably some other enzymes. The drug is expected to be active against tumours where these PKs have been established to play a critical role in cancer pathogenesis (Lydon and Druker, 2002).

Krystal et al. (2000) have demonstrated that imatinib efficiently inhibits SCF-mediated kit activation at concentrations similar to those that inhibit both Bcr-Abl and the PDGFR. They have shown the inhibition of SCF-mediated small-cell-lung cancer cells, 70% of which express the kit receptor tyrosine kinase. It has been proved that imatinib inhibits the major signalling pathways of kit: PI3K/Akt and Ras/MAPK systems. However, only the inactive form of c-kit is inhibited by imatinib; the results of Mol and collaborators show that the active c-kit kinase with a phosphorylated juxtamembrane domain is resistant to STI-571 inhibition (Mol et al., 2003). Besides, only juxtamembrane domain mutation of kit has been shown to be inhibited by imatinib but not TK-II domain mutations.

Imatinib binds to PDGF as well. The studies of Buchdunger et al. (2000) demonstrated that the drug was a potent inhibitor of both PDGF subtypes.

Dewar et al. (2005) have shown that imatinib also affects macrophage colony-stimulating factor (M-CSF) receptor c-fms, despite previous supposition that the drug does not target this PK.

Because c-kit, PDGF and c-fms receptors are members of the type III receptor TK family, it could be assumed that other members of this family and closely related kinases might be inhibited by imatinib. The related receptor TKs Flt-3 (Bohmer et al., 2003), Kdr, Flt-1, Tek, and c-Met were evaluated for inhibition by imatinib but were not inhibited. This suggests that these TKs, although structurally related, have subtle differences in the structure of their ATP-binding domains. Many other kinases involved in intracellular signalling, e. g. Src or JAK kinases, are not inhibited by imatinib (Buchdunger et al., 2000).

Besides, it is hypothesized that in addition to its anti-tumour effects, imatinib might act indirectly on host cells outside of tumours. Borg and collaborators demonstrated this action of the drug on mouse tumour

models that were resistant to the anti-proliferative effects of imatinib *in vitro* but responded *in vivo* to long-term treatment with imatinib. The data indicated that imatinib stimulated dendritic cell (DC)-mediated natural killer (NK) cell activity via a direct action to kit expressed in DCs (Borg et al., 2004).

In this way, imatinib can be efficiently used in a variety of pathological conditions, first of all malignancies. Actually, it was initially designed for the inhibition of the Abl tyrosine kinase in the Bcr-Abl oncoprotein (Sattler and Salgia, 2004), and has been shown to induce clinical, haematological and molecular remissions in CML patients (Savage and Antman, 2002; Lydon and Druker, 2004). However, the sole Bcr-Abl inhibition has been found to be insufficient to eliminate all malignant cell populations. The results of Wong (2004) and collaborators suggest that the effectiveness of the drug in eliminating Ph⁺ CML populations may be due to its ability to suppress additional signalling pathways required for the survival of distinct leukaemic cell populations along with Bcr-Abl. Besides, several studies report the effective use of imatinib in polycythemia vera (Silver, 2005).

Zhang et al. (2003) have shown that imatinib can inhibit non-small-lung cancer cell growth in a dose-dependent manner. They revealed that A549 lung cancer cells express PDGFR- α , and proved that the inhibitory effect of imatinib on these cells is mediated through its inhibition. Besides, a synergistic effect of imatinib and cisplatin was shown, suggesting that imatinib can potentiate the effect of cisplatin on A549 cells. Nevertheless, the underlying mechanism of such synergism is unclear.

Imatinib proved to be effective in GISTs interfering with oncogenic kit signalling mechanisms found in these tumours (Duensing et al., 2004a). The results of Nakatani et al. (2005) suggest that tyrosine-phosphorylated c-kit can bind to Hsp90, which is supposed to be necessary and sufficient for activation of c-kit in GIST-T1 cells. The c-kit treated with imatinib could not bind to Hsp90 and tyrosine phosphorylation of the c-kit did not occur in GIST-T1 cells. Furthermore, the authors investigated the difference between activated c-kit and dephosphorylated c-kit. In their study, activated c-kit molecules showed cell-surface clustering, which was inhibited by imatinib.

Imatinib does not penetrate across the blood-brain barrier. Wolff and collaborators have revealed in their study on murine Bcr-Abl retroviral transduction and transplantation model of CML that the spinal fluid concentration of imatinib was 155-fold lower than in plasma. Mice in their experiments developed progressive neurological deficits after 2 to 4 months of imatinib mesylate therapy because of central nervous system leukaemia. The authors proved that neurologic findings were not a direct neurotoxic effect of the long-term imatinib treatment (Wolff et al., 2003). Despite that,

imatinib and probably other TK inhibitors represent a prospective investigation subject when delivered intrathecally. Several studies show possibilities of imatinib application in certain CNS diseases. There are data that imatinib can be effective in treatment of malignant gliomas. Kilic et al. demonstrated that imatinib mesylate significantly inhibited growth of U343 and U87 glioblastoma cell lines *in vitro* and *in vivo* in heterotopic malignant glioma models, because of its potency to inhibit PDGFR- α and β (Kilic et al., 2000).

Netzer et al. (2003) determined that imatinib and inhibitor 2 (6-(2,6-dichlorophenyl)-8-methyl-2-(3-methylsulfanylphenyl-amino)-8H-pyrido[2,3-d]pyrimidin-7-one) inhibit β -amyloid production. β -amyloid (A β) is the metabolite of the amyloid precursor protein and is believed to be a major pathological effector of Alzheimer's disease. The authors investigated the possibility of using inhibitors of the ATP activity to affect A β production. It was revealed that imatinib suppressed A β production, though precise mechanisms of this action have not been cleared.

Alternative Abl kinase inhibitors

Imatinib binds to the inactive conformations of PKs. They are very distinct among different kinases, which explains the selectivity of imatinib. Besides, the inactive conformation may be altered by a mutation, which may lead to resistance. This appears to be the case in mutations that affect the activation loop. Some other TK inhibitors distinct from STI571 are capable of binding to both active and inactive conformations of kinases, implying their greater therapeutic effectiveness (Deininger, 2004). La Rosee and collaborators (La Rosee et al., 2002) showed that one of such compounds, PD180970, a dual-specific Abl and Src kinase inhibitor, binds to all of the common mutants of these kinases, with the exception of T315I. This compound was not suitable to be developed into a drug. However, it proved that alternative inhibitors is a promising strategy. Several such molecules are in development. The main problem here may be posed by a lower specificity of such compounds. If they are less specific, they may have more side effects (Deininger, 2004).

Another compound, AMN107, has been developed. It is 10 to 30 times more potent than imatinib against Bcr-Abl and has a similar activity against some other kinases. This molecule also inhibits the kinase activity of most Bcr-Abl mutants but, like PD180970, does not bind T315I. The significant inhibition of proliferation of cells transfected with Bcr-Abl mutants has been shown (Weisberg et al., 2005).

BMS-354825 is another alternative Bcr-Abl inhibitor. O'Hare et al. compared imatinib with AMN107 and BMS-354825 in their study. The results suggested that both inhibitors were more potent than

STI571 against all CML cell lines and targeted Abl kinase mutants except T3151 (O'Hare et al., 2005).

Dasatinib (BMS-354825) is an ATP-competitive, dual-specific Src and Abl-kinase inhibitor. Dasatinib is structurally not related to imatinib. It is 100- to 300-fold more potent as a Bcr-Abl inhibitor. Unlike imatinib, dasatinib binds to both the inactive and active configurations of Bcr-Abl (Lombardo et al., 2004). In addition, dasatinib combined with imatinib has an additive or synergistic inhibitory effect on the growth of Bcr-Abl-expressing cells. The potency of this combination has been shown on the Ba/F3 cells expressing wild-type Bcr-Abl (Cortes and Kantarjian, 2005).

A different approach to TK inhibition is to inhibit not the ATP binding, but the substrate binding to Bcr-Abl (Cortes and Kantarjian, 2005). Compounds of this group are described in the section „ATP non-competitive protein kinase inhibitors“.

Alternative kit and PDGF inhibitors

Indolinone TK inhibitors have been found to inhibit mutant kit and the SCF-dependent growth of small-cell-lung cancer (Sattler and Salgia, 2004). It has been established that imatinib mesylate only inhibits the juxtamembrane (JM) domain mutation of kit but does not target TK-II domain mutations, while indolinone derivatives (most recent are SU5416 and SU6597) inhibit both JM and TK-II domain mutations. These compounds are effective in inhibiting kit activation and kit-mediated viability in acute myeloid leukemia blasts (Sattler and Salgia, 2004). There is evidence that some indolinone derivatives effectively inhibit TK-II domain mutations in mast and germ cell neoplasms. Additionally, SU5416 inhibits VEGFR along with Flt-3 and c-kit (Krause and Van Etten, 2005).

Another class of kit inhibitors includes quinoxalines, including AGL2043, that have also been found to inhibit Flt-3 and PDGFR TKs (Sattler and Salgia, 2004). As an alternative PDGFR inhibitor should be mentioned PTK787/ZK222584 as well, which is also known to inhibit VEGFR (Kesari et al., 2005).

CDK inhibitors

CDK inhibitors are a heterogeneous group of compounds that are able to inhibit CDKs involved in the cell cycle (CDK1, CDK2, CDK3, CDK4, CDK6, and CDK7), transcription (CDK7, CDK8 and CDK9), or neuronal functions (CDK5 and CDK11). CDK inhibitors are chemically diverse, low-molecular-weight (< 600 Da) flat, hydrophobic heterocycles. CDK inhibitors compete with the ATP like most PKIs. No CDK inhibitor has been shown to compete with the target proteins of CDKs. As an exception, Flavo inhibits CDK9 in both a non-competitive and ATP-competitive manner (Schang, 2005).

The first CDK inhibitors were the natural products flavopiridol, butyrolactone, the paullones, indirubin and staurosporine with its 7-hydroxy-derivative UCN-01. Later, purine and pyrimidine analogues were produced: olomoucine, R-roscovitine, CGP74514A, BMS-387032, purvalanol B, PD0183812 and other chemical derivatives including the sulphonamide E7070. Low selectivity of these compounds was demonstrated. They inhibited not only CDKs but also other kinases, including the MAP kinases Erk1 and Erk2 (Vesely, 1994).

Flavopiridol (Flavo) is one of the best characterized CDK inhibitors. It is a semi-synthetic flavonoid derived from the natural alkaloid, rohitukine, originally isolated from leaves and stems of *Amoora rohituka* (Schang, 2005). Flavo was initially developed as an inhibitor of EGFR and protein kinase A (Sattler and Salgia, 2004). However, the compound was found to inhibit cell replication CDKs at far lower concentrations than those required for EGFR or protein kinase A inhibition. Then it was revealed that at nanomolar concentrations it inhibits CDK1, 2, 4, 6, 9 and likely 6 (Schang, 2005; Senderowicz and Sausville, 2000). Although Flavo can also bind to the ATP binding pocket of CDK9, it is able to inhibit this kinase non-competitively. Flavo inhibits several other enzymes, such as GSK-3b kinase, PKC, glycogen phosphorylase (a and b forms), binds to cytosolic aldehyde dehydrogenase and duplex DNA and stimulates the ATPase activity of multidrug resistance associated protein 1 (MRP1) (Schang, 2005).

Flavo induces cell cycle arrest in G1 *in vivo* and *in vitro*, perhaps by inhibiting CDK1 and 2. It is cytotoxic to cells synthesizing DNA and causes apoptosis, probably by inhibiting CDK1, 2 or 9 (Carlson, 1999). The compound also inhibits *in vitro* transcription by RNA polymerase II and at higher concentrations it inhibits expression of 5.6% of genes in cultured cells (284 of 5032 genes). These inhibitory effects result from inhibition of CDK9 and resemble the effects of global transcriptional inhibitors, such as actinomycin D and DRB (Carlson, 1999, Schang, 2005).

Another well-characterized CDK inhibitor of the first generation is UCN-01 (7-hydroxystaurosporine). UCN-01 is an alkaloid from *Streptomyces* bacteria, derived from staurosporine. Initially discovered to target CDK1 and CDK2, it is now known to have pan-CDK inhibitory activity, as well as promoting p53-independent apoptosis by targeting Chk1 and Chk2 (Wang et al., 1996). The drug may inhibit Akt signalling via PDK1 inhibition. In *in vitro* assays UCN-01 causes cell cycle arrest and apoptosis (Akiyama et al., 1997).

The search for specific pharmacological CDK inhibitors resulted in the discovery of 6-aminopurines, semi-specific but not very potent CDK inhibitors. The next substantial stride was the discovery of other purine-related CDK inhibitors. Compounds containing a purine-like ring (purine-type CDK inhibitor) include

roscovitine, olomoucine, the purvalanols and related compounds. Purine-type CDK inhibitors preferentially inhibit CDK1, 2, 5 and 7, but not CDK4, 6, or 8 (Schang, 2005). They also affect ERK1, ERK2 and DYRK1a at concentrations approximately 50- to 1,000-fold higher than those that inhibit CDKs. Olomoucine (Olo) was the first specific and relatively potent CDK inhibitor discovered. More potent but equally specific roscovitine (Rosco) was then discovered (Schang, 2005).

R-roscovitine (CYC202) is an orally bioavailable purine analogue that competes for the ATP-binding site of CDK2/cyclin E, CDK4/cyclin D1, CDK7/cyclin H, CDK9/cyclin T1 (McClue et al., 2002). Rosco inhibits MDM2 expression and thus blocks p53 degradation. Studies in the Lovo colorectal carcinoma cell line showed that roscovitine induced cell death in all stages of the cell cycle. In xenograft studies, roscovitine administered orally or intraperitoneally induced tumour growth delay (McClue et al., 2002). The anti-tumour efficacy of roscovitine has been tested in a panel of 77 human tumour xenografts in order to find out which tumour types are sensitive to Rosco. A dose-dependent anti-tumour activity of CYC202 has been detected. CYC202 was most active in inhibiting the proliferation of colon, non-small-cell lung, breast and prostate human cancer xenografts (Blagden and de Bono, 2002).

E7070 is another representative of the second generation. E7070 induces G1/S cell cycle arrest at low nanomolar concentrations. It inhibits CDK2 and cyclin E, down regulates cyclin H and at higher concentrations upregulates p53 and p21, inducing apoptosis (Ozawa et al., 2001). E7070 shows efficiency *in vivo*. It has a broad spectrum of anti-tumour activity with a wide range of IC₅₀ values in 42 different cell lines (Blagden and de Bono, 2002).

The 2-aminothiazole, BMS-387032 is a potent, selective ATP-competitive small molecule inhibitor of the CDK2/cyclin E complex. BMS-387032 shows a lower potency against CDK1 and CDK4 and some other non-CDK kinases. The X-ray crystal structure of the BMS-387032-CDK2 complex has been described. It discovers the mechanisms by which this compound interacts with the CDK2 ATP-binding site (Misra et al., 2004). *In vitro*, the aminothiazoles induce cell cycle arrest in the A2780 ovarian carcinoma cell line, inhibiting CDK2 phosphorylation and the phosphorylation of downstream targets of CDK2 including the retinoblastoma protein, histone H1 and DNA polymerase- α (Blagden and de Bono, 2002).

CDKs represent promising cellular targets for antiviral drugs (Schang, 2002). They are apparently well tolerated, which has been shown in animal experiments and human clinical trials against cancer. Therefore, they may soon be tested as clinical antivirals (Schang, 2005).

The smallest DNA viruses require cell progression into S-phase and CDK2 activity for virus replication. Most of these viruses activate CDK2 and stimulate

expression of cellular DNA replication proteins, thus forcing cells to enter S-phase. Several viruses that replicate in both cycling and non-cycling cells also require CDK activities. These viruses include herpes simplex virus types 1 and 2 (HSV-1 and HSV-2) (Schang et al., 2000), hepatitis B virus (HBV), and HIV (Schang, 2004). Replication of human T lymphotropic virus (HTLV), Kaposi's sarcoma-associated herpesvirus (KSHV), human cytomegalovirus (HCMV), varicella-zoster virus (VZV), EBV, adeno- and other viruses require CDK activities. It should be expected that viral replication functions of these viruses may be efficiently inhibited by CDK inhibitors (Schang 2004, 2005).

Many other non-purine related CDK inhibitors have been designed, including other flavonoids, paullones, indirubines and aloisines. The development of novel CDK inhibitors still continues and new compounds are continuously added to this group (Schang, 2005).

EGFR inhibitors

EGFR inhibitors can have single, dual, or pan-EGFR receptor specificity (Arteaga, 2003). Examples are erlotinib and gefitinib, both c-ERBB1 (EGFR)-specific reversible TK inhibitors, and GW572016 or PKI166, dual TK inhibitors (Raizer, 2005).

Like other TK inhibitors, EGFR inhibitors target the intracellular domain of the receptor TK competing with ATP for the intracellular catalytic site of EGFR and thus block its downstream signalling. Therefore, they inhibit tyrosine autophosphorylation, resulting in a blockade of EGFR signal transduction pathways. Preclinical studies have described the blockade of TK activity, proapoptotic effects, and inhibition of cell proliferation and angiogenesis as the result of EGFR inhibition (Vallbohmer and Lenz, 2005).

The activity of the two main reversible EGFR-TK inhibitors erlotinib and gefitinib (ZD1839, Iressa), orally administered small-molecule TK inhibitors, has already been investigated in clinic (Vallbohmer and Lenz, 2005).

Laboratory studies have shown that erlotinib reduces EGFR autophosphorylation in tumour cells, inhibits EGFR-dependent cell proliferation, and blocks cell-cycle progression at G1-phase (Vallbohmer and Lenz, 2005).

Gefitinib has been described as a drug reducing the cell proliferation and inducing cell-cycle arrest. Increased apoptosis and anti-angiogenic effect are described as a result of gefitinib activity (Kesari et al., 2005, Vallbohmer et al., 2005). Gefitinib has demonstrated anti-tumour activity in preclinical studies (Barlesi et al., 2005). However, there are some mechanisms of gefitinib resistance e. g. in recurrent non-small-lung cancer that were found by Kwak et al. (2005). These mechanisms can be circumvented by irreversible TK inhibitors. The findings of the authors suggest that one of

these, HKI-272, may prove highly effective in the treatment of EGFR-mutant NSCLCs, including tumours resistant to gefitinib or erlotinib (Kwak et al., 2005).

Other compounds have been shown to be promising EGFR inhibitors: PKI166, PD153035, canertinib, GW572016 and CI-1033. GW572016 (lapatinib) is a selective inhibitor of both EGFR and HER-2 TKs, instead of. It has shown the most notable activity in advanced or metastatic breast cancer. PKI166-A is a dual EGFR-ErbB TK inhibitor. It inhibits signalling through the ligand-activated EGFR in cells (Traxler et al., 2001). PD153035 has been shown to irreversibly inhibit proliferation in cell lines with a high proportion of EGFRs. PD168393, an irreversible EGFR inhibitor, was reported to have anti-tumour activity in A431 epidermoid xenografts (Kondapalli et al., 2005).

CI-1033 (canertinib) is an orally available 3-chloro, 4-fluoro, 4-anilinoquinazoline. It has the two distinctions: irreversible binding to the TK active site and pan-erbB specificity. It tightly binds to all four members of the EGFR family and provides a prolonged suppression of EGFR-mediated cell signalling. Since CI-1033 possesses an irreversible effect, synthesis of new receptors is required to re-establish signal transduction through this growth-promoting pathway (Dewji, 2004). CI-1033 currently undergoes clinical trials.

JAK inhibitors

Among the Janus kinase (JAK) family members the main achievements have been made in inhibition of JAK3 and JAK2.

JAK3 inhibition blocks several cytokine signals in NK cells and in T and B lymphocytes. It can provoke immunosuppression by altering the expansion and function of these cells. Therefore, targeting JAK3 may theoretically be used for immune suppression where it is needed, e. g. on cells actively participating in transplant rejection without affecting any cells outside of these cell populations (Borie et al., 2004). The lack of information about the three-dimensional structure of JAKs seriously complicates the design of putative specific JAK3 inhibitors (Misra et al., 2004). Nevertheless, several compounds able to inhibit JAK3 exist.

The development of a new JAK3 inhibitor named CP-690 550 has been reported by Changelian. JAK3 inhibition with CP-690 550 induced a substantial inhibition of *in vitro* cellular allo-immune responses. CP-690 550 potently inhibited mixed leukocyte reactions in mice, monkeys or humans (Changelian et al., 2003).

PNU156804 is an antibiotic of the undecylprodigiosin family, which was also reported to block JAK3 autophosphorylation, the IL 2-mediated tyrosine phosphorylation of JAK3 and STAT5 and IL 2-induced T-cell proliferation. The effect was restricted to activated cells and no effect was found when PNU156804 was

added to growing Jurkat cells that did not express JAK3 (Borie et al., 2004).

AG-490 is a membrane-soluble JAK 2 and 3 inhibitor. First it was shown to inhibit JAK2 activity and was successfully used to control abnormal constitutive JAK2 activation in human cells obtained from patients with acute lymphoblastic leukaemia (Meydan et al., 1996). The subsequent evaluation of AG-490 revealed the inhibition of IL-2-mediated growth of mitogen-activated human T cells. Hence, an inhibitory activity of AG-490 against the JAK3-STAT5 pathway may be presumed (Kirken et al., 1999). In a rodent model of allotransplantation AG-490 showed a dose-related, but only modest prolongation of rat heterotopic heart allograft survival (Behbod et al., 2001).

Other known JAK inhibitors are WHI-P131 and WHI-P154. They represent prospective PKIs, although proved to be 1,000–10,000 times less potent than CP-690 550 (Changelian et al., 2003).

Some other compounds, such as A77 1726 (an immunosuppressive metabolite of leflunomide), have been reported to affect signal transduction via the JAK-STAT pathway, yet not specifically and at high supra-micromolar concentrations (Elder et al., 1997).

ATP non-competitive proteine kinase inhibitors

ATP competitive PKIs have a drawback – they must compete with high intracellular ATP concentrations. Moreover, to be specific these inhibitors must discriminate between the ATP-binding sites resembling in multiple human proteins that also utilize ATP, including other PKs. Therefore, it may be beneficial to target sites on protein kinases other than the ATP-binding site distinct in different PKs (Bogoyevitch et al., 2005).

Kamath et al. (2003) designed a series of pseudosubstrate-based peptide inhibitors specific to the enzyme-substrate interaction site of the non-receptor PTK p60c-Src, which is involved in proliferation and mitosis, and whose deregulation may lead to tumorigenesis. The authors produced cysteine-containing hexa- and heptamers, which proved to potently inhibit p60c-Src kinase activity.

Pero et al. (2004) identified peptide EC-1, which was able to bind to the extracellular domain of ErbB2, leading to inhibition of ErbB2 autophosphorylation. EC-1 was found to inhibit the proliferation of ErbB2-overexpressing breast cancer cells.

The domains of interactive partners have been used in order to inhibit c-Jun NH2-terminal kinase (JNK). One of such partners is the scaffold protein JIP. It has been shown that overexpression of JIP protein itself can inhibit JNK. TI JIP is a potent inhibitory peptide that resembles the kinase interaction motif of JIP (Barr et al., 2004).

Bonny et al. (2001) produced cell-permeable peptide inhibitors of JNK by linking the 20-amino acid inhibitory domain of JIP-1 to a 10-amino-acid HIV-TA sequence, which can rapidly penetrate inside cells. This peptide was introduced into pancreatic β TC-3 cells and blocked JNK-mediated activation of c-Jun.

Some ATP non-competitive PKIs function by targeting Src homology-2 (SH2) domains. These domains recognize and bind to proteins containing a phosphorylated tyrosine (Mendoza et al., 2005). Peptidomimetic compounds were produced to imitate sequences of target proteins bearing a phosphorylated tyrosine. However, phosphotyrosine groups happened to be very susceptible to chemical and enzymatic degradation, which might pose a problem in regard to practical use (Mendoza et al., 2005). Burke et al. sought for phosphotyrosine analogues that had high stability. This has led to the discovery of *c*-phosphonomethylphenylalanine, a phosphotyrosine mimetic that is phosphatase-resistant and has very similar biological properties to phosphotyrosine (Burke et al., 2001).

Tyrphostins are small synthetic molecules. They have been shown to specifically inhibit PKs by interfering not only with the binding of ATP, but also of ligands. AG957 is a tyrphostin that inhibits Bcr-Abl and other TKs in an ATP non-competitive manner. Adaphostin is an ester of AG957 that shows greater *in vitro* potency than AG957 (Gumireddy et al., 2005). Adaphostin has been shown to induce apoptosis in imatinib-resistant cell lines and act synergistically with imatinib. Another small-molecule kinase inhibitor unrelated to ATP is ON012380. It has demonstrated significant inhibition of all mutants of Bcr-Abl, including imatinib-resistant ones, resulting in apoptosis of leukaemic cells, and regression of leukaemias in mice (Gumireddy et al., 2005).

The peptide F56, which specifically binds to VEGF, is able to nearly abolish VEGF binding to its receptor, Flk-1, *in vitro*. *In vivo*, this peptide is able to inhibit tumour growth and metastases (Mendoza et al., 2005).

Buerger et al. targeted the intracellular domain of PKs by importing inhibitors into the cell by means of protein transduction. They produced a peptide able to bind to the kinase domain of EGFR and fused the peptide sequence with the bacterial protein thioredoxin. Such type of fusion proteins is called aptamers. The aptamers were produced in bacteria and were imported into the cell by means of protein transduction, a process in which extracellular proteins are unfolded, transported through the cell membrane, and refolded in the cell with conserved activity (Buerger et al., 2003).

Other PK inhibitors

VEGFR. SU5416 (semaxanib) inhibits vascular endothelial growth factor receptors (VEGFR) in addition to Flt-3 and c-kit (Krause and Van Etten, 2005).

SU5416 was the first specific VEGFR inhibitor used in clinic in malignant gliomas (Kesari et al., 2005). Semaxanib is particularly potent in combination with STI571/Gleevec. This combination has been found to be most effective in reducing tumour growth, even in the late stages, where treatment with sole SU5416 is inefficient (Saharinen and Alitalo, 2003).

PTK787/ZK222584 potentially inhibits recombinant VEGFR kinases and is the most selective VEGFR kinase inhibitor described. PTK787 readily penetrates into cells and inhibits the autophosphorylation of VEGFR-2 (Wood et al., 2000). It possesses good functional activity in cellular systems, inhibiting VEGF-mediated cell proliferation, cell survival and cell migration. Besides, PTK787 has been shown to inhibit PDGFR (Kesari et al., 2005).

MAPK. CEP-1347 (KT7515) is an indolocarbazole, a derivative of the natural product K252a isolated from the culture broth of K252 strain of *Nocardiosis* sp. bacteria (Wang et al., 2004). CEP1347 was the first efficient compound targeting the JNK signalling pathway, which inhibits mixed lineage kinases (MLKs), a group of activating MEKKs (Maroney et al., 1998, Wang et al., 2004). The compound inhibits neuronal death in *in vitro* and *in vivo* models. It was safe and well tolerated in a short-term human trial. CEP-1347 and related MLK inhibitors offer prospective compounds for treatment of neurodegenerative disorders such as Alzheimer's and Parkinson's diseases (Parkinson Study Group, 2004).

The drug SB203580 inhibits p38 MAPK by interacting not only with the ATP-binding pocket, but also with a small hydrophobic groove next to it (Tong et al., 1997).

BIRB 796, a p38 inhibitor, induces a conformational change after binding that renders the ATP-binding pocket unable to subsequently bind ATP (Regan et al., 2003).

FGFR. SU5402 interacts not only with the ATP-binding pocket of the FGFR, but also with another binding pocket next to it. The mechanism is analogous to the p38 inhibitor SB203580 (Mohammadi et al., 1997).

Aurora. There are three chemical Aurora inhibitors currently in development: ZM447439 (Ditchfield et al., 2004), Hesperadin (Hauf et al., 2003) and VX-680 (Harrington et al., 2004). Like many other PKIs they act by blocking the ATP-binding pocket. There is a high degree of homology between the three Aurora kinases (Brown et al., 2004), and although ZM447439 and hesperadin were designed to inhibit Aurora B, they have been shown to inhibit Aurora A as well, yet with a lower potency. It is likely that these agents also inhibit Aurora C. The agent VX-680 targets Aurora A, B and C, and also Flt-3 (Harrington et al., 2004), an unrelated TK involved in the progression of myeloid and some lymphoid leukaemias. The three drugs have been shown to inhibit cell division, but not cell cycle progression. Treated cells become polyploid and, depending on the p53 status, this leads to arrest in G1 or apoptosis (Keen

Table 1. Protein kinases involved in pathogenesis of cancer and other diseases and their inhibitors

PK	Main deregulation mechanisms supposed	Diseases suspected to involve PK	Inhibitor	Reference
Bcr-Abl	Reciprocal recombination occurs between <i>bcr</i> and <i>abl</i> genes. Because of an increased tyrosine kinase activity, Bcr-Abl causes cell growth and differentiation and reduces apoptosis.	CML	imatinib mesylate (Gleevec, ST1571), BMS-354825 (dasatinib), VX-680	Daley et al., 1992 Deininger et al., 2000 Holyoak, 2001
c-kit	1. Gain-of-function mutations leading to the permanent activation of c-kit signalling in the absence of binding of SCF, which leads to uncontrolled cell proliferation and resistance to apoptosis 2. ligand-mediated activation of kit	GISTs, lung cancers, Merkel cell carcinoma, Kaposi's sarcoma, germ cell tumours, mast cell tumours, melanoma, testicular and gynaecological cancers, neuroblastoma	imatinib SU5416 PKC412 MLN518	Sattler and Salgia, 2004 Verweij, 2004
PDGFR	Activating mutations resulting in uncontrolled cell proliferation and maintenance of tumour blood vessels	myeloproliferative disorders, gliomas, carcinomas, melanomas, sarcomas, GIST, breast and lung cancers, ovarian tumours	imatinib PKC412 SU11248 MLN518 PTK787 sorafenib	Buchdunger et al., 2000
CDKs	Cell cycle deregulation	various types of sarcomas, colorectal and lung cancers	flavopiridol roscovitine purvalanol B olomoucine UCN-01 E7070 BMS-387032 purvalanol A (P 4484) kenpauillone (K 3888) alsterpauillone (A 4847) indirubins staurosporine (S 4400)	Blagden and de Bono, 2005 Schang, 2005
MAPKs	Superfluous endothelial cell activation, T-effector cell differentiation and proliferation of vascular smooth muscle cells	diabetes, atherosclerosis, stroke, Parkinson's disease, Alzheimer's disease, arthritis, asthma	roscovitine, olomoucine, purvalanols, PD98059	Waetzig and Herdegen, 2005 Chang et al., 2003 Coffey et al., 2002
EGFR	Overexpression, point mutations in the kinase domain or both lead to different cellular processes involved in carcinogenesis such as cell proliferation, inhibition of apoptosis, angiogenesis, cell motility, and metastasis.	colorectal cancer, non-small-cell lung cancer, glioblastoma multiforme, different types of solid tumours	erlotinib, gefitinib (Iressa), PK1166, PD153035, canertinib	Mendoza et al., 2005 Vallbohmer and Lenz, 2005 Arteaga, 2003
JAKs	1. Deficiency of JAK3 2. A clonal somatic mutation in the pseudo-kinase domain JAK2 3. Aberrant activity of the JAK-Src kinase duet	1. SCID 2. polycythemia vera 3. haemopoietic abnormalities including leukaemia	CP-690 550 AG-490 WHI-P131 WHI-P154 A77 1726	Changelian et al., 2003 Borie et al., 1997 Kralovics et al., 2005
ROCK	1. Contribution to inhibition of apoptosis in tumour cells 2. Involvement of the ROCK pathways in motility and invasion of tumour cells	glioma, NSCLCs, cardiovascular disorders	Y27632 Y-30141	Rattan et al., 2006
PKC	1. Anti-apoptotic signalling 2. Promotion of the expression of cell surface receptors including the EGF receptor	GISTs, breast cancer, different malignancies	LY317615 PKC412	Lu et al., 2004 Duensing et al., 2004a
Src	Deregulation of multiple oncogenic pathways including PDGFR, VEGFR, and others	myeloproliferative disorders, gliomas, carcinomas, melanomas and other malignancies	dasatinib	Lombardo et al., 2004
Plk/ Plk-1	Deregulation of cell cycle progression	head and neck cancer, ovarian cancer, endometrial cancer, prostate cancer, NSCLC, glioma, breast cancer, melanoma, colorectal cancer	scytonemin	Eckerdt et al., 2005 McInnes et al. 2005
Aurora A Aurora B	1. Overexpression of both Aurora A and B 2. Gene amplification of Aurora A	1. breast and colon cancers 2. bladder, gastric and colorectal cancers	ZM447439, hesperadin, VX-680	Ditchfield et al., 2004 Hauf et al., 2003 Harrington et al., 2004
Flt-3	Mutations leading to constitutive activation of Flt-3, which enhance cell proliferation, differentiation, and survival	various haematologic malignancies incl. acute myeloid leukaemia	AG1295 AG1296 MLN518 SU5416 PKC412 CEP-701 SU11248 Ki23819	Krause and Van Etten, 2005
VEGFR	Ligand-mediated activation of VEGFR by VEGF secreted by tumour cells, which provides the tumour vascularization	breast cancer amyotrophic lateral sclerosis	PKC412, SU11248, PTK787, Gleevec +SU5416, sorafenib	Saharinen and Alitalo, 2003
Bub1, BubR1, Mps1	Mutation followed by spindle assembly checkpoint and cytokinesis deregulation	colorectal cancer	not available	Lew and Burke, 2003 Tighe et al., 2001

and Taylor, 2004). The preclinical data indicate that both hesperadin and ZM447439 selectively target different tumour cells *in vitro*, indicating that Aurora kinase inhibitors may be useful in cancer treatment.

BRAF. BAY 43-9006 is a small-molecule inhibitor of wild-type and mutant versions of BRAF, which has been shown to be important in pathogenesis of melanoma along with c-kit and PDGFR- α and β (Kondapalli et al., 2005).

Src. Src kinase modulates signal transduction through multiple oncogenic pathways including PDGFR, VEGFR, and may play a role in the development and progression of many tumours. This is why its inhibition is attractive. Dasatinib is an ATP-competitive, dual-specific Src and Abl-kinase inhibitor. It has been demonstrated to efficiently inhibit Src (Lombardo et al., 2004).

Rhe. Griffinn et al. (2003) revealed a genetic rearrangement in the eosinophilic cell line EOL-1 that resulted in the expression of a fusion protein comprising an N-terminal region encoded by a gene of unknown function (GenBank accession number NM_030917) and a C-terminal region originating from the intracellular domain of the PDGFR- α . They reported that STI571 inhibited this fusion kinase found in patients with idiopathic hypereosinophilic syndrome. It was the fusion kinase that was targeted by STI571 in idiopathic hypereosinophilic syndrome, the authors suggested.

PKC and PDK-1 inhibitors. LY317615 ATP competitively disrupts the intrinsic phosphotransferase activity of conventional and novel protein kinase C isoforms. This molecule selectively inhibits the PKC β isoform (PKC- β 2) (Kesari et al., 2005).

There are PKC and PDK-1 inhibitors chemically related to the bisindolyl maleimide group. Their protein data bank codes are 1OKY, 1OKZ, 1UU3, 1UU7, 1UU8, 1UU9 and 1UVR. The crystal structures of these compounds have been determined (Komander et al., 2004). The bisindolyl maleimide PKC and PDK-1 inhibitors may become a starting point for the discovery of clinically useful drugs (Cheatham, 2004).

ROCK. Fasudil and Y-27632 were the first small-molecule ROCK inhibitors discovered (Breitenlechner et al., 2003). They were shown to be moderate inhibitors of ROCK. However, their optimization led to production of very potent compounds, e. g. Y-30141 (Mueller et al., 2005).

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