FARMASEUTTINEN AIKAKAUSKIRJA

DOSIS

VOL. 22 2 / 06

DOSIS Theme Issue "Protein Kinase Research"

SISÄLTÖ
Table of Contents

DOSIS

FARMASEUTTINEN AIKAKAUSKIRJA
VOL 22 N:O 2, 2006

Kustantaja
Suomen Farmasialiitto
Iso Roobertinkatu 7
00120 Helsinki
Puh. (09) 6962 2729

Julkaisija
Suomen Farmasialiitto/Proviisoriliitto

Päätoimittaja
Prof. Jouni Hirvonen
Helsingin yliopisto
Farmasian tiedekunta
PL 56
00014 Helsingin yliopisto

Toimitusneuvosto
Prof. Marja Airaksinen
FaT Jussi Holmalahti
FaT Nina Katajavuori
Dos. Anna Maria Nuutila
Dos. Kirsi-Marja Oksman-Caldentey
Dos. Jouko Savolainen
FaT Mikko Unkila
Prov. Harri Ovaskainen
FaT Markus Forsberg

Toimitus
Suomen Farmasialiitto
Toimitussihteeri Annemari Backman
Iso Roobertinkatu 7
00120 Helsinki

Ilmoitusmyynti
Anna-Liisa Virkki, T:mi Cancro
Puh. 0400 609 451
S-posti: anna-liisa.virkki@kolumbus.fi

Taitto
Jukka Korpela

Painopaikka
Forssan Kirjapaino Oy
Forssa

ISSN 0783-4233

PROTEIN KINASES AS DRUG TARGETS

In the present issue of Dosis, the reader will be introduced to protein kinases (PKs), particularly those being studied in the Pro-Kinase Research Consortium. During the Helsinki Drug Research 2006 congress, a broader view of PKs and, especially, PKs as drug targets, will be given by leading scientists in the field.

Protein kinases; localization and function in the cell

Protein kinases form the largest single family of enzymes, numbering over 500 and accounting for almost 2% of the proteins encoded by the human genome (Cohen, 2002a). PKs catalyse protein phosphorylation, an important mean by which cell functions are regulated (in concert with protein dephosphorylation catalysed by protein phosphatases). Phosphoproteins are the main effectors in intracellular signal transduction of chemical or hormonal stimuli leading, in most cases, to changes in gene-expression and sustained cellular responses (Cohen, 2002b).

The amino acid residues in the target (substrate) proteins that are phosphorylated by the PKs are serine, threonine and tyrosine. Therefore, PKs are classified as either serine/threonine (Ser/Thr) or tyrosine (TK) kinases, respectively. Protein kinases are expressed in various cellular compartments, i.e. on the cell surface (or the plasma membrane), the cytoplasm, as well as within intracellular organelles, including the nucleus.

Receptor tyrosine kinases (RTKs) are plasma membrane PKs that function as receptors for various extracellular mediators (hormones or other transmitters). This can lead to activation of an intracellular kinase-cascade causing activation of several other kinases in a concerted action and, finally, induce changes in cellular functions. An example of RTK is the insulin receptor, the activation of which causes the glucose transporter to be translocated from intracellular sites to the cell membrane, a prerequisite for glucose intake (Watson & Pessin, 2001).

Cytoplasmic PKs can be activated by intracellular second messengers which, in turn, are mostly generated by activation of G-protein coupled receptors (GPCRs) in the plasma membrane by extra-cellular mediators. Well-known examples for such processes are the activation of protein kinase A (PKA) by cyclic AMP (cAMP), and the activation of protein kinase C (PKC) by diacylglycerol (DAG). PKA can also be negatively regulated by a cell surface receptor via an inhibitory G-protein. In this case, the activity of PKA is decreased as a consequence of receptor activation. In addition to activation through GPCRs, the intracellular kinases can be activated by upstream PKs as well.

An interesting family of PKs is the cyclin-dependent protein kinases (CDKs) that include both protein activators and protein inhibitors. The CDKs is an important class of kinases because their physiological peptide modulators can serve as templates for development of peptidomimetic chemical drugs. The CDKs are of key importance in the control of cell cycle and also in the function of the nervous system (Knockaert et al., 2002).

Thus, PKs can be activated by extracellular and intracellular messengers, and they can be activated by other kinases. Their activation leads to modification of cellular responses and regulation of huge variety of physiological events such as function of ion channels, regulation cell cycle, regulation of nerve cell plasticity, to name a few.

Protein kinases as drug targets

Aberrant protein phosphorylation is involved in the mechanism of many disease processes, e.g. the development of cancer. PKs are also major players in the signalling of cytokines and other mediators of inflammation. Interestingly, in non-mammalian cells, such as in certain parasites (e.g. Leishmania), PKs may differ in structure from those of mammalian cells (Doerig et al. 2002), and are therefore most interesting targets for PK

inhibitors (for a comprehensive review see Pechan and Jaffe, in this issue). Thus, drugs affecting protein kinases (or more widely protein phosphorylation) may be of great importance in future treatment strategies of a large variety of diseases.

The cell surface kinases are attractive drug targets. The receptor tyrosine kinases have an extracellular ligand binding domain (regulatory domain) and intracellular protein kinase domain (catalytic domain) that are linked via the cell membrane perturbing domain. The intracellular protein kinase activity is increased when a specific ligand binds to the extra-cellular domain. In principle, it is possible to develop either small molecule drugs or biological drugs which would behave as agonists or antagonists in the ligand binding domain of such PKs (Bennasroune et al., 2004).

RET tyrosine kinase is a transmembrane TK activated by the endogenous activator Glial cell-line Derived Neurotrophic Factor (GDNF) (Airaksinen & Saarma, 2002). More precisely, GDNF binds to the co-receptor GFRalpha1 and the GDNF-GFRalpha complex activates the RET-TK. In the brain, GDNF can restore dopaminergic function of the brain nigro-striatal system that is degenerated in Parkinson's disease. Indeed, GDNF has shown promising effects in experimental models of Parkinson's disease (Eslamboli, 2005), but clinical trials have been controversial (Sherer et al., 2006). It is obvious that chemical agonists of this receptor would be of great interest, at least for experimental medicine. Another interesting example is Multiple endocrine neoplasia (MEN), a disease where RET TK is constitutively active due to a genetic abnormality. As a result of this unregulated RET-TK activity, MEN patients have multiple endocrine tumours. Hence, RET-TK is an obvious drug target that is also studied within the Pro-Kinase Research Consortium.

A major breakthrough in protein kinase clinical pharmacology was the discovery of imatinib (Glivec® trade name by Novartis), an inhibitor of the tyrosine kinases BCR-ABL, c-KIT, and the platelet-derived growth factor receptor (PDGF) TK. Imatinib exhibits high efficacy in the clinic in the treatment of tumours that possess constitutively active forms of c-ABL, c-KIT, or PDGF and, importantly, it turned out to be superior to the other available drug therapies. Among the tumours that can be treated successfully by imantinib are Philadelphia-chromosome-positive (Ph-positive) chronic myeloid leukemias (Abl-Bcl), gastrointestinal stromal tumours (c-KIT), and PDGF-positive leukaemias. Imatinib is well tolerated, but some tumour cells develop resistance to it (Klein et al., 2005). Imatinib also highlighted the importance of discriminating between the active and inactive conformations of tyrosine kinases when designing inhibitors. This drug binds to the ATP-binding site of the tyrosine kinase and thus its site of action is intracellular. Recently, allosteric inhibitors of the Abl-Bcl kinase have been discovered (Adrian et al., 2006). Many monoclonal antibodies directed to the extracellular ligand-binding domain of receptor tyrosine kinases have been raised for therapeutic use (Bennasroune et al., 2004). An example of these is trastuzumab (Herceptin®, Genentech). Carlo Gambacorti-Passerini and Leonardo Scapozza will summarize tyrosine kinases as drug target in more detail in this Dosis.

The cytoplasmic PKs are obvious drug targets as well. The CDKs control cell division by controlling cell cycle in its various phases. The regulation is complex and involves both activation and inhibition of CDKs. The CDK5 isozyme is an important regulator of nerve cell function. Several compounds affecting CDKs are in clinical trials for cancer and other diseases (Knockaert et al., 2002).

One of the classical PKs is the family of protein kinase C (PKC) isozymes. These kinases are cytoplasmic when inactive, but upon activation by DAG (with or without calcium), they are translocated to cell membranes (Parker & Murray-Rust, 2004). These PKs are essential players in signal transduction and may have significance in diseases such as cancer and diabetic vascular complications. It is possible to develop activators and inhibitors targeting the regulatory domain of PKCs (see Yli-Kauhaluoma et al. in this issue). Even so, the most promising compounds that have been developed so far target the ATP binding site of these enzymes (Goekjian & Jirousek, 2001). Also, the anchoring of the PKCε to RACK2 is a possible target for drug intervention (Hofmann, 2004). For more details, see Garczarczyk et al. in this issue. PKC isozymes as drug targets have been in the focus of research

for a quarter of century. A compound inhibiting the PKCβ isozyme had promising activity against diabetic complications. Several lines of evidence suggest a role for PKC in modulating agents for the treatment of leukaemia (see Wang et al. in this Dosis).

Drug binding sites on protein kinases

Regulatory domain of PKC isoenzymes. PKC-regulatory domain is unique to PKC and is not found in other PKs. The C1-domain of the regulatory domain of PKC is the binding site of DAG, the physiological activator of the enzyme. The C1-domain of various PKC-isoenzymes is an attractive drug-target, since the drugs would probably have specificity for PKCs over other kinases, and it would also be possible to discover PKC-isozyme-selective inhibitors. For details, see Yli-Kauhaluoma et al. in this issue.

Catalytic domain of various PKs. The catalytic domain of various PKs is highly conserved, especially the ATP binding site. On the other hand, the region surrounding the *sensu stricto* ATP binding site (the so-called "pharmacophore") is quite variable from one kinase to another, and can be exploited for the specific binding of ligands that will act as selective inhibitors competing with ATP (Noble et al., 2004). Indeed, most of the currently available PK-inhibitors, such as imatinib (Glivec®) and LY333531, a PKCβ-selective inhibitor, are targeted to the ATP binding site of the catalytic domain (see Goekjian in this issue)

Anchoring proteins. The receptors for activated C-kinase, RACK1 and RACK2, bind to PKCε and translocate it to the site of catalytic activity (Mackay & Mochly-Rosen, 2001). Several peptides inhibit the binding of PKCε to RACK1 or RACK2 (Hool, 2005). The PKCε-derived octa-peptide HDAPIGYD has been shown to be a PKCε agonist and prevents the heart from ischemic damage (Schechtman et al., 2004).

Pro-KinaseResearch Consortium

In the Pro-Kinase Research Consortium, much emphasis has been paid to the characterization of the role of selected PKs in signal transduction pathways and in various disease models, such as proliferation of tumour cells, functions of immune cells and vascular cells, the parasitic infection leishmaniasis, and also in the physiology of the central nervous system.

The Consortium is organized as five sub-projects, Work Packages (WP1-WP5). In WP1, several hundred extracts have been prepared from cyanobacterial and fungal strains, as well as from plants. Pure compounds from the extracts have been isolated and preliminary activity screening has been done. The WP2 is concentrating on virtual screening of known chemicals libraries and on developing new computer programs for virtual screening. Several hundred compounds targeted to various PKs have been synthesised in WP 3, in groups oriented to synthetic organic chemistry. They use modern methods of organic synthesis to prepare compounds that are based on natural product screening (WP1) or virtual screening of known chemicals (WP2). These synthesis products can be tested in various protein kinase binding and activity assays to find hit molecules. This provides essential feed-back information for the molecular modellers and synthetic chemists to refine the chemical structure of the found hits within the iterative process of drug discovery and development.

A number of the novel compounds that show biological activity in various assay systems utilising purified kinase enzyme preparations have been found. Several recombinant protein kinase domains have been expressed and used in the screening for ligand binding. Indeed, there are more than 150 different kinase activity assays available within the Consortium, thus the binding affinity and biological activity of potential protein kinase inhibitors/activators can be screened with the help of extensive collaboration. Rational drug design relies on three dimensional models of proteins and more specifically of the active site – the ligand binding site. In order to determine the crystal structure of the critical binding sites of different kinases, large quantities of various protein kinases have been produced in cell cultures. Structures of different kinase/inhibitor complexes and a novel protein kinase have been solved. (WP4)

In WP5, various model systems for a range of diseases have been utilised to test therapeutic potential of novel compounds of both natural and synthetic origin. The diseases include cancer (particularly leukaemia and also breast, glia and

gastric tumours), epilepsy, heart disease and post-stroke neurodegeneration, leishmaniasis and inflammatory disease. The protein kinases that have been the focus of the project include tyrosine kinases (receptor and cytoplasmic), Protein kinase C, MAP kinases and Cyclin Dependent Kinases.

A recent article by Vieth et al. (2005) summarised the status of protein kinase targeted drug development in the USA. At that time (June 2005), there were 76 different compounds that were active in clinical trials. Most of them were for oncological diseases. It is obvious that we need small molecule chemical substances that are rather selective to one or several kinases. Opposite to a major pharmacological principle, protein kinase inhibitors may not necessarily need to be specific to a single PK but, rather, could inhibit several "correct" kinases simultaneously. This is of course a major challenge to rational drug discovery and, indeed, there is an increasing need for better *in vitro* and *in vivo* models of diseases to test such compounds.

➤ **Raimo K. Tuominen, M.D.**
Coordinator of Pro-KinaseResearch Consortium
Division of Pharmacology and Toxicology,
Faculty of Pharmacy, P.O.Box 56,
FI-00014 University of Helsinki,
Helsinki, Finland

YHTEENVETO

PROTEIINIKINAASIT LÄÄKEVAIKUTUKSEN KOHDEMOLEKYYLEINÄ

Proteiinikinaasit (PK) ovat suurin yksittäinen entsyymiperhe, jossa on yli 500 proteiinia. Se kattaa lähes 2 % ihmisen genomin koodaamista proteiineista. PK:t katalysoivat proteiinien fosforylaatiota, joka on tärkeä vaihe kemiallisessa tai hormonaalisessa solunsisäisessä signaalinkujetuksessa johtaen muutoksiin geenien ilmentymisessä ja pitkäkestoisissa soluvasteissa. PK:t fosforyloivat kohdeproteiinin seriini-, treoniini- tai tyrosiini-aminohappotähteitä. Tämän vuoksi PKt luokitellaan joko seriini-/treoniinikinaaseiksi tai tyrosiinikinaaseiksi. Proteiinikinaaseja esiintyy joko solukalvoilla, sytoplasmassa tai solunsisäisissä organelleissa, esim. tumassa.

PK:t ja proteiinifosforylaatio ovat tärkeässä roolissa monissa sairauksissa, esim. syövän kehittymisessä tai tulehdusreaktioissa. On havaittu myös, että parasiittien (esim. Leishmania) solujen PK:t poikkeavat rakenteeltaan nisäkässolujen vastaavista, ja ovat näin hyvin kiinnostava lääkevaikutuksen kohde. Proteiinikinaasien rakenteissa on useita potentiaalisia yhdisteiden sitoutumiskohtia, kuten PKC isoentsyymien säätelyosa ja katalyyttisen osan ATP:n sitoutumiskohta, joita tutkitaan lääkevaikutuksen kohteina. Myös ns. proteiinikinaaseihin ankkuroituvat proteiinit ovat kiivaan tutkimuksen kohteina.

EU:n rahoittama Pro-Kinase Research Consortium, jonka koordinaattorina Helsingin yliopiston farmasian tiedekunta (Prof. Raimo K. Tuominen) toimii, karakterisoi tiettyjen PK:ien roolia signaalin kuljetusreiteissä ja erilaisissa sairausmalleissa, kuten kasvainsolujen jakaantumisessa, immuuni- ja verisuonten solujen toiminnassa, leishmaniaasissa ja myös keskushermoston toiminnassa. Konsortion partnerit eristävät luonnonaineista ja syntetisoivat yhdisteitä, jotka voisivat toimia joko PK-inhibiittoreina tai aktivaattoreina. Pienimolekyylisten yhdisteiden, jotka olisivat selektiivisiä joko yhdelle tai useammalle proteiinikinaasille, kehittäminen on erittäin tärkeää. Lisäksi on olemassa yhä suurempi tarve paremmille *in vitro* ja *in vivo* -sairausmalleille, joissa voitaisiin näitä yhdisteitä testata. Tämä on suuri haaste lääkekehitykselle. Pro-Kinase Konsortio pyrkii ratkaisemaan näitä kysymyksiä. Hanke on viisivuotinen ja EU rahoittaa sitä 15 miljoonalla eurolla. Tämän käsilläolevan DOSIS-teemanumeron kirjoittajat ovat Pro-Kinase Konsortiumin partnereita.

➤ **Prof. Raimo K. Tuominen**

REFERENCES:

Adrian FJ, Ding Q, Sim T, Velentza A, Sloan C, Liu Y, Zhang G, Hur W, Ding S, Manley P, Mestan J, Fabbro D, Gray NS: Allosteric inhibitors of Bcr-abl-dependent cell proliferation. Nature Chem Biol 2: 95-102, 2006

Airaksinen MS, Saarma M: The GDNF family: signalling, biological functions and therapeutic value. Nature Rev Neurosci 3: 383-394, 2002

Bennasroune A, Gardin A, Aunis D, Cremel G, Hubert P: Tyrosine kinase receptors as attractive targets of cancer therapy. Crit Rev Oncol-Hematol 50: 23-38, 2004

Cohen P: Protein kinases – the major drug targets of the twenty-first century? Nature Rev Drug Discov 1: 309-315, 2002a

Cohen P: The origins of protein phosphorylation. Nature Cell Biol 4: E127-130, 2002b

Doerig C, Meijer L, Mottram JC: Protein kinases as drug targets in parasitic protozoa. Trends Parasitol 18: 366-371, 2002

Eslamboli A: Assessment of GDNF in primate models of Parkinson's disease: Comparison with human studies. Rev Neurosci 16: 303-310, 2005

Goekjian PG, Jirousek MR: Protein kinase C inhibitors as novel anticancer drugs. Exp Opin Invest Drugs 10: 2117-2140, 2001

Hofmann J: Protein kinase C isozymes as potential targets for anticancer therapy. Curr Cancer Drug Target 4: 125-146, 2004

Hool LC: Protein kinase C isozyme selective peptides - A current view of what they tell us about location and function of isozymes in the heart. Curr Pharm Design 11: 549-559, 2005

Klein S, McCormick F, Levitzki A: Killing time for cancer cells. Nat Rev Cancer 5: 573-580, 2005

Knockaert M, Greengard P, Meijer L: Pharmacological inhibitors of cyclin-dependent kinases. Trends Pharmacol Sci 23: 417-425, 2002

Mackay K, Mochly-Rosen D: Localization, anchoring, and functions of protein kinase C isozymes in the heart. J Mol Cell Cardiol 33: 1301-1307, 2001

Noble ME, Endicott JA, Johnson LN: Protein kinase inhibitors: insights into drug design from structure. Science 303: 1800-1805, 2004

Parker PJ, Murray-Rust J: PKC at a glance. J Cell Sci 117: 131-132, 2004

Schechtman D, Craske ML, Kheifets V, Meyer T, Schechtman J, Mochly-Rosen D: A critical intramolecular interaction for protein kinase Cepsilon translocation. J Biol Chem 279: 15831-15840, 2004

Sherer TB, Fiske BK, Svendsen CN, Lang AE, Langston JW: Crossroads in GDNF therapy for Parkinson's disease. Mov Disord 21: 136-141, 2006

Watson RT, Pessin JE: Subcellular compartmentalization and trafficking of the insulin-responsive glucose transporter, GLUT4. Exp Cell Res 271: 75-83, 2001

Vieth M, Sutherland JJ, Robertson DH, Campbell RM: Kinomics: characterizing the therapeutically validated kinase space. Drug Discov Today 10: 839-846, 2005

Protein Kinase C Isozymes – Targets for Potential Therapies

➤ **Dorota Garczarczyk**
➤ **Florian Rechfeld**
➤ **Georg Hechenberger**
➤ **Johann Hofmann**

Biocenter, Division of Medical Biochemistry
Innsbruck Medical University
Fritz-Pregl-Str. 3, A-6020 Innsbruck
Austria

Phone: +43-512-507-3505
Fax: +43-512-507-2872
E-mail: johann.hofmann@i-med.ac.at

INTRODUCTION

Protein kinase C (PKC) is a family of serine/threonine-specific protein kinases. The PKC isoenzymes can be classified into three groups: i) the conventional (cPKCs) α, βI, βII, and γ (require negatively charged phospholipids, diacylglycerol or phorbol ester, and calcium for optimal activation), ii) the novel (nPKCs) δ, ε, θ, η/L (mouse/human) and μ (require negatively charged phospholipids, diacylglycerol or phorbol ester, but no calcium), and iii) the atypical (aPKCs) λ/ι (mouse/human) and ζ (do not require calcium, diacylglycerol or phorbol ester, but only negatively charged phospholipids for optimal activity) (Nishizuka, 1995; Newton and Johnson, 1998). The PKC isoenzymes (**Figure 1**) are characterized by four conserved (C1 – C4) and five variable (V1 – V5) domains (Stabel and Parker, 1991; Azzi et al., 1992; Hug and Sarre, 1993; Stabel, 1994). The regulatory domain consists of the C1 and the C2 region. C1 contains the pseudo-substrate region that can inhibit the enzyme by binding to the catalytic site (C4). C1 also contains

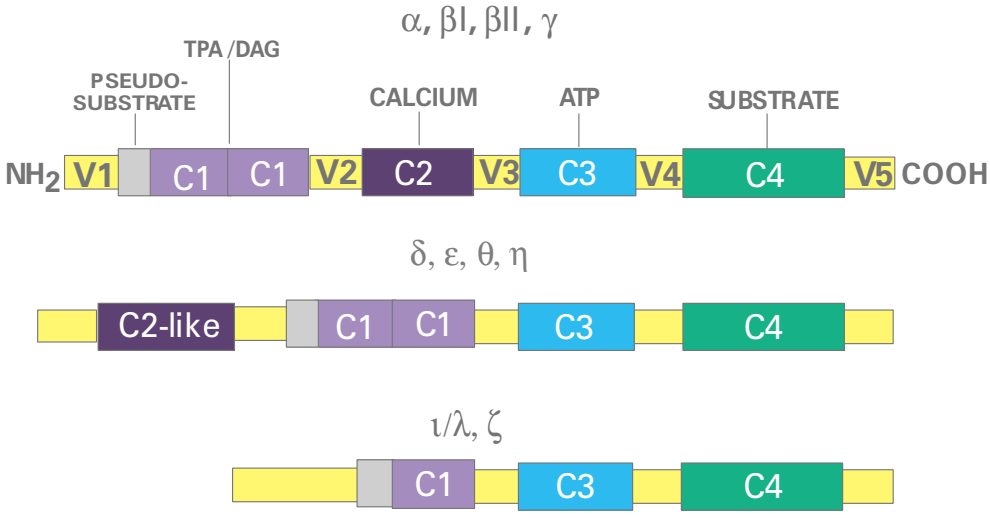


Figure 1. Structures of the PKC isozymes and their domains

tandemly repeated cysteine-rich regions to which diacylglycerol (DAG), phorbol esters and bryostatins can bind. cPKCs and nPKCs contain two zinc fingers in the phorbol ester binding site, aPKCs are characterized by a single zinc finger. C2 contains the calcium binding region present only in cPKCs but not in nPKCs and aPKCs. Between the C2 and the C3 region the so called hinge region is situated, which serves as cleavage site for calpain and trypsin during degradation. The C3 region is believed to be the ATP binding site and the C4 region the catalytic site.

Investigations into the expression of distinct PKC isoenzymes in various tissues revealed a highly variable tissue distribution. PKCα and PKCζ are ubiquitously expressed. Brain contains all isoenzymes, whereas others such as skin and skeletal muscle contain only a few (Blobe et al., 1994). Such a different pattern of expression suggests that the PKC isozymes play different roles in the tissue of expression.

PKC isoenzymes seem to play important roles in activation of signal transduction pathways leading to synaptic transmissions, the activation of ion fluxes, secretion, proliferation, cell cycle control, differentiation and tumor proliferation. PKC has become of major interest as target for therapeutic intervention in a range of different diseases (Nixon, 1997; Goekjian and Jirousek, 1999). PKC is the intracellular receptor for tumor promoting phorbol esters (Castagna et al., 1982; Niedel et al., 1983; Leach et al., 1983). Short term exposure of intact cells with phorbol esters such as 12-*O*-tetradecanoylphorbol-13-acetate (TPA) activates PKCs (by binding to the diacylglycerol-binding site), long term exposure down-regulates PKC activity. Phorbol esters are able to promote tumor formation. Therefore, it was presumed that activation of PKC by TPA induces tumors and inhibition may reduce carcinogenesis or inhibit tumor growth. However, investigations revealed that the situation is more complicated: For example, bryostatin, a PKC modulator with properties similar to those of TPA (Blumberg, 1991; Kennedy et al., 1992; Szallasi et al., 1994) does not induce tumor formation. PKC modulators such as miltefosine (hexadecylphosphocholine), bryostatin, safingol, midostaurin (PKC412, CGP41251) and UCN-01 (7-hydroxystaurosporine) are used in the clinic or are in clinical evaluation as antitumor agents. However, PKC is not the only target of these compounds. They also interfere with other targets (Hofmann, 2004).

The different PKC isozymes complicate the situation. Their exact functions are not known at present. In this review potential roles of the different PKC isozymes and possible effects of their modulation are discussed.

PKCα

PKCα has been implicated in a variety of cellular functions including proliferation, apoptosis, differentiation, motility, and inflammation. However, the responses induced by activation or overexpression of PKCα vary depending on the types, and sometimes conditions, of cells. For example, in some types of cells, PKCα is implicated in cell growth. In contrast, it may play a role in cell cycle arrest and differentiation in other types of cells. PKCα exhibits proapoptotic or antiapoptotic functions in different cell types (Nakashima, 2002).

Hearts of PKCα-deficient mice are hypercontractile, whereas those of transgenic mice overexpressing PKCα are hypocontractile. Adenoviral gene transfer of dominant-negative or wild-type PKCα into cardiac myocytes enhances or reduces contractility, respectively (Braz et al., 2004). PKCα transgenic mice exhibit severe intraepidermal neutrophilic inflammation, disruption of the epidermis and upper hair follicles when treated topically with TPA, conditions that characterize several human cutaneous diseases such as pustular psoriasis and acute generalized exanthematous pustulosis (Cattaison et al., 2003).

PKCα was found to be activated *in situ* in a significant number of human breast tumors (Ng et al., 1999). In contrast, down-regulation of PKCα in breast carcinomas was found by Kerfoot et al. (2004). PKCα overexpressing MCF-7 cells exhibited a significant reduction in estrogen receptor expression (Ways et al., 1995, Manni et al., 1996) and PKCα levels may especially be increased in breast cancer patients with low or negative estrogen receptor levels (Lahn et al., 2004). PKCα has been shown to activate telomerase in human breast cancer cells, which may represent an essential step in the maintenance of proliferation in human cancers (Li et al., 1998). On the other hand, increased

expression of PKCα led to cessation of growth, induction of differentiation in B16 melanoma cells, and to inhibition of proliferation in K562 cells (Gruber et al., 1992).

Cell lines derived from high-grade gliomas expressed higher levels of PKCα than did cell lines derived from low-grade gliomas. In glioblastoma-derived cell lines PKCα was mainly expressed in the cytosolic fraction, indicating an inactive state of the enzyme. The PKC modulator bryostatin 1 specifically down-regulated PKCα in glioblastoma-derived cell lines. However, this was not associated with significant growth inhibition illustrating that PKCα seems not to be essential for proliferation (Zellner et al., 1998). In models of melanoma, PKCα activation is typically associated with increased tumor cell proliferation, invasiveness and decreased differentiation (Lahn and Sundell, 2004).

Decreased PKCα seems to be of major importance for the development of colorectal cancers (Suga et al., 1998). Transfection of CaCo-2 cells with PKCα in the antisense orientation resulted in enhanced proliferation and decreased differentiation (Scaglione-Sewell et al., 1998). Human HT-29M6 colon cancer cells transfected with an activated form of PKCα showed decreased proliferation and increased invasion due to alterations in cell adhesion. When these cells were xenografted into athymic mice, higher expression of activated PKCα led to a reduction in tumor size (Batlle et al., 1998). PKCα was decreased in clear cell renal carcinoma versus normal tissue (Brenner et al., 2003).

Elevated levels of PKC were found in thyroid cancers if compared to normal thyroid tissue (Hagiwara et al., 1990; Hatada et al., 1992). In human thyroid cancers (Prevostel et al., 1995; Prevostel et al., 1997) and in pituitary cancers (Alvaro et al., 1993; Alvaro et al., 1997) point mutations in PKCα were detected. However, 11 human pituitary tumors showed a normal wild-type sequence of PKCα DNA (Schiemann et al., 1997). A single point mutation in D294 was found in a subpopulation of thyroid and pituitary tumors with more invasive phenotype, suggesting a role in tumor progression (Alvaro et al., 1993). A PKCα-D294G mutant was found to be unable to bind to cellular membranes tightly despite the fact that it translocates to the membrane as efficiently as the wild-type PKCα upon treatment of phorbol ester. The impaired membrane binding is associated with this mutant's inability to transduce several antitumorigenic signals. Thus, the PKCα-D294G seems to be a loss-of-function mutation. Therefore, developing selective activators instead of inhibitors of PKCα was suggested for the treatment of certain endocrine tumors (Zhu et al., 2005).

Treatment of mice bearing U-87 (Yazaki et al., 1996), T-24 bladder carcinoma, A549 lung carcinoma or Colo 205 colon carcinoma xenografts with antisense phosphorothioate oligonucleotides directed against PKCα (ISIS 3521, LY900003, aprinocarsen) resulted in suppression of tumor growth (Dean et al., 1996). Recent phase I and phase II trials in patients with colorectal adenocarcinomas (Cripps et al., 2002), breast and ovarian cancers (Lahn et al., 2004), NSCLC (Vansteenkiste et al., 2005), high grade lymphomas (Advani et al., 2005) and hematologic malignancies (Lahn et al., 2006) did not show promising results.

PKCβ

PKCβI and PKCβII are generated by alternative splicing from a single gene (Kawakami et al., 2002). Elevated expression of PKCβII seems to be an important early event in colon cancer development (Gokmen-Polar 2001). PKCβ knockout mice showed that this gene is important for B cell development and activation (Leitges et al., 1996). A dramatic increase in the angiogenic response to oxygen-induced retinal ischemia in transgenic mice overexpressing PKCβII and a significant decrease in retinal neovascularization in PKCβ null mice was observed (Suzuma et al., 2002). Hyperglycemia leads to PKCβI and II activation, which experimentally has been shown to contribute to the development and progression of diabetic microvascular complications (retinopathy, nephropathy and neuropathy). Animal and human studies using ruboxistaurin mesylate (LY333531), a novel, selective inhibitor of PKCβ, have shown a delay in the progression and, in some cases, reversal of diabetic retinopathy, nephropathy and neuropathy (Joy et al., 2005). The compound enhanced the antitumor activity of fractionated radiation, carboplatin, gemcitabine, paclitaxel, BCNU and 5-FU. The ruboxistaurin analogue, LY379196, suppressed

the growth of neuroblastoma cells and enhanced the antiproliferative activity of taxol and vincristine *in vitro*. The analogue LY317615 enhanced the antitumor activity of 5-FU and BCNU in tumor bearing mice (Teicher et al., 1999).

PKCγ

PKCγ is restricted to the brain and spinal cord (Hug and Sarre and 1993). In normal animals, peripheral nerve injury produces a persistent, neuropathic pain state. Mice that lack PKCγ displayed normal responses to acute pain stimuli, but they almost completely failed to develop a neuropathic pain syndrome after partial sciatic nerve section, and the neurochemical changes that occurred in the spinal cord after nerve injury were blunted (Malmberg et al., 1997). In mice lacking PKCγ, impaired motor coordination, but intact eyeblink conditioning, a form of component movement learning (Chen et al., 1995), spatial learning deficits, impaired hippocampal long-term potential (Abeliovich et al., 1993a, 1993b; Ramakers et al., 1999), and increased ethanol consumption and behavioural impulsivity (Bowers and Wehner, 2001), were observed. An analysis of PKCγ knockout mice supports the notion that the loss of the PKCγ gene may protect the functional μ-opioid receptors from degradation in the spinal cord and produce antinociception (Narita et al., 2001). Thus, PKCγ might be a potential target for prevention and therapy of persistent pain or alcohol problems. PKCγ-activating or inhibiting compounds are not available at present.

PKCδ

PKCδ is expressed ubiquitously among tissues. It is activated, in addition to by phospholipids, by caspase 3-cleavage (Emoto et al., 1995; Ghayur et al., 1996) or tyrosine-phosphorylation (Kikkawa et al., 2002). In contrast to other PKC isozymes, PKCδ is involved in tumor suppression and induction of apoptosis (Perletti et al., 1999; Gschwendt, 1999; Kikkawa et al., 2002). Transgenic mice overexpressing PKCδ in the epidermis were found to be resistant to skin tumor promotion by TPA (Reddig et al., 1999). PKCδ knock-out mice, due to reduced apoptosis, exhibited an increased smooth muscle cell accumulation (Leitges et al., 2001) and increased proliferation of B cells leading to autoimmune disease (Mathis and King, 2002; Miyamoto et al., 2002; Mecklenbrauker et al., 2004).

Rottlerin was reported to be an inhibitor of PKCδ (Gschwendt et al., 1994). However, the compound also inhibits other kinases. It seems to be a mitochondrial uncoupler and may inhibit PKCδ indirectly (Kikkawa et al., 2002). Bistratene A, an activator of PKCδ, induced translocation of PKCδ to the nucleus and induced growth arrest in G2/M in HL-60 cells (Griffiths et al., 1996). Therefore, activation of this isozyme seems to increase antitumor activity and activation of apoptosis. An activation of PKCδ seems to be responsible for the antitumor activity of the adriamycin-derivative *N*-benzyladriamycin-14-valerate (AD 198) (Lothstein et al., 2001). The anthracyclins adriamycin and daunorubicin are essential components of first-line chemotherapy in the treatment of solid and hematopoietic tumors. The compounds intercalate into DNA and inhibit topoisomerase II religation. Their efficacies are impeded by serious side effects (cardio-toxicity). To obtain derivatives lacking these disadvantages, compounds with alkylesters at C-14 have been synthesized, among them AD 198 (N-benzyladriamycin-14-valerate) and valrubicin (*N*-trifluoroacetyladriamycin-14-valerate). These alterations resulted in an increase in lipophilicity and rapid cellular penetration. Both compounds are localized in the perinuclear region and showed poor DNA-intercalation and interference with topoisomerase II. AD 198 shows a spatial homology with TPA and DAG and binds to the C1 region of PKC. In intact cells, AD 198 induced the translocation of PKCα and PKCδ in murine NIH3T3 cells (Roaten et al., 2001). The compound seems to induce apoptosis by activation and cleavage of PKCδ, but may also interact with other C1-containing proteins such as chimaerins, Ras-GRP or PKD.

PKCε

PKCε is expressed in many tissues but abundantly in neuronal, hormonal and immune cells. In neuronal cells, PKCε seems to be important for proliferation and differentiation (Hundle et al., 1995; Fagerstrom et al., 1996), alcohol sensitivity (Choi et al., 2002), nociceptor

function (Cesare et al., 1999; Dina et al., 2000) and cell death and survival (Jung et al., 2004). Overexpression of PKCε in NIH3T3 cells and Rat 6 embryo fibroblasts caused increased growth rates, growth to higher cell densities in monolyer, growth in soft agar and tumor formation in nude mice (Mischak et al., 1993; Cacace et al., 1993), indicating that it is an oncogene (Perletti et al., 1996).

Mice overexpressing PKCε in the skin exhibited reduced papillomas but enhanced carcinomas (Jansen et al., 2001; Li et al., 2005). A modest increase in overexpression of PKCε in transgenic mice exhibited protection against myocardial ischemia (Ping et al., 2002), whereas a marked increase exhibited cardiac hypertrophy and failure (Takeishi et al., 2000). Targeted disruption of the PKCε gene blocked cardioprotection caused by ischemic preconditioning (Gray et al., 2004) and led to decreased hypoxic pulmonary vasoconstriction (Littler et al., 2003). Compared to wild-type mice, PKCε knock-out mice also exhibited reduced macrophage activation (Castrillo et al., 2001; Aksoy et al 2004), reduced stress hormones, decreased anxiety-like behaviour (Hodge et al., 2002), decreased hyperalgesia (Khasar et al., 1999), less alcohol consumption, and greater acute sensitivity to alcohol (Choi et al., 2002, Newton and Messing, 2006).

PKCε inhibitors might be potential drugs against certain tumors, anxiety, alcoholism, cardiac hypertrophy or pain. PKCε activators might be beneficial for prevention of myocardial ischemia or activation of macrophages.

Receptors for activated C kinases (RACKs) are proteins interacting with PKCs. RACK2 interacts with activated PKCε (Csukai et al, 1997). A short peptide (EAVSLKPT), corresponding to the amino acids 14 – 21 of the V1 region of PKCε, interferes with the PKCε/RACK2 interaction and selectively inhibits the translocation of PKCε by binding to RACK2 (Johnson et al., 1996). It has been shown that this peptide inhibits protection from hypoxia-induced cell death of cardiac myocytes (Gray et al., 1997).

The peptide HDAPIGYD corresponding to a part of the RACK sequence that binds to PKCε, selectively translocates PKCε from cytosolic to particulate subcellular fractions, a hallmark of PKC activation (Dorn et al., 1999; Mackay and Mochly-Rosen, 2001). Activation of endogenous PKCε in hearts of diabetic mice promoted the survival phenotype and inhibited the negative inotropic properties of chronic hyperglycemia (Malhotra et al., 2005). Combined treatment of the PKCε activator peptide and a PKCδ inhibitor reduced ischemia-reperfusion injury and decreased the resulting graft coronary artery disease induced by prolonged ischemia (Tanaka et al., 2005).

Recently the group of O'Brian described that the active-site cavity of human PKCε harbors a redox-sensitive cysteine residue, (Cys452), which inactivates the kinase when modified by thiol-disulfide exchange with small disulfides, such as cystamine, cystine and disulfiram or *N*-ethyl-maleimide (O'Brian et al., 2006). Cys452 is only conserved in 11 out of the approximately 500 human protein kinases.

PKCη

PKCη is strongly expressed in skin and lung but only slightly in spleen and brain. It is highly expressed in epithelial tissues, especially in squamous epithelia. PKCη is unique in that it is specifically activated by cholesterol sulfate and sulfatide, sulfated metabolites of cholesterol and cerebroside, respectively. PKCη overexpression induces G1 arrest and differentiation in keratinocytes. PKCη-induced differentiation is accompanied by the transcriptional activation of transglutaminase I, a key enzyme in squamous differentiation, and involucrin, a precursor of cornified envelopes. In keratinocytes, PKCη associates with the cyclin E/cdk2/p21 complex and inhibits the cdk2-kinase activity, leading to G1 arrest. Cholesterol sulfate inhibits the promotional phase of skin carcinogenesis. Moreover, PKCη-knock-out mice showed a much higher sensitivity to carcinogenesis, suggesting that PKCη is negatively involved in tumor promotion through stimulation of keratinocyte differentiation. In addition to epithelial cells, recent studies revealed that PKCη acts as a key regulator in early B-cell development. Although the functions of PKCη in other cell types are not yet fully elucidated, available evidence indicates that this particular isoform plays a crucial role in the signaling of cell differentiation in a cell-type-specific manner (Kashiwagi et al., 2002).

In PKCη-deficient mice wound healing on the

dorsal skin, particularly reepithelialization, was significantly delayed. These mice exhibited increased susceptibility to tumor formation in two-stage skin carcinogenesis by single application of 7,12-dimethylbenz(a)anthracene for tumor initiation and repeated applications of TPA for tumor promotion (Chida et al., 2003). A clear correlation of PKC expression with tumor progression in clear cell renal cell carcinoma was observed (Brenner et al., 2003). Compounds selectively modulating PKCη are not known at present.

PKCθ

PKCθ demonstrated a relatively restricted expression pattern, i.e. in skeletal muscle, lymphoid organs and hematopoietic cell lines, in particular T cells (Baier, 2003). T cell receptor-induced activation is blocked in T cells from two independent PKCθ knockout mouse lines. PKCθ is essential for the activation of mature T cells (Sun et al., 2000). Therefore, PKCθ appears to be a prime target for novel anti-inflammatory or immunosuppressive therapies.

PKCθ is necessary for induction of the interleukin-2 gene because the transcription factors AP-1 and NF-kappaB, which are essential for interleukin-2 gene promoter activation, are main targets of PKCθ. Therefore, given the essential role of PKCθ in interleukin-2 production, interleukin-2 gene regulation by PKCθ could also be of therapeutic interest. Recent studies revealed that PKCθ provides an important survival signal that protects leukemic T cells from Fas- or UV-induced apoptosis. These findings and the constitutive localization of PKCθ in the membrane of some leukemic T cells suggests that it plays a role in leukemic T cell survival and/or proliferation, and that selective PKCθ-inhibitory strategies may facilitate elimination of malignant T cells (Villalba and Altman, 2002). Duensing et al. (2004) showed that PKCθ is a diagnostic marker for gastro-intestinal stromal tumors.

PKCι

The human PKCι and its mouse ortholog PKCλ exhibit an amino acid identity of 98%. This isozyme is involved in cell polarization (Suzuki et al., 2001), fundamental for development and tissue functions. PKCλ knock-out mice die at an early embryonic stage (Suzuki et al., 2003). PKCι was found to be overexpressed in the vast majority of primary human non-small cell lung cancer (NSCLC) cells and in primary NSCLC tumors. Elevated PKCι expression levels in tumors predict poor survival. Therefore, PKCι expression profiling might be useful in identifying early-stage NSCLC patients at elevated risk of relapse. Disruption of PKCι signaling with a dominant negative PKCι blocks the transformed growth of human NSCLC cells harboring PKCι gene amplification. Thus, PKCι seems to be an attractive target for development of novel, mechanism-based therapeutics to treat NSCLC (Regala et al., 2005). The PKCι-RAC1-PAK-MEK-ERK pathway seems to be responsible for cell transformation. In this signal transduction, a complex of PKCι-RAC1-PAR6 is involved. Aurothioglucose, a gold compound used clinically to treat rheumatoid arthritis, and the related compound aurothiomalate, were identified as inhibitors of PKCι-PAR6 interactions. Aurothio-glucose inhibited the tumor growth of NSCLC A549 cells in nude mice (Stallings-Mann et al., 2006).

PKCζ

PKCζ is activated by phosphatidylinositol-3,4,5-trisphosphate (PIP_3). This PKC isozyme associates with other signaling and scaffold proteins and is involved in the mitogen-activated protein kinase, ribosomal S6 protein kinase and NFkB signaling (Hirai and Chida, 2003). The prostate androgen response-4 (Par-4) protein has been shown to inhibit and the lambda-interacting protein (LIP) to activate PKCζ. The level of Par-4 correlated with growth inhibition and apoptosis (Moscat and Diaz-Meco, 2000). In PKCζ knock-out mice the activation and survival of isolated purified cultures B cells are severely impaired (Martin et al., 2002).

PERSPECTIVES

The PKCα-specific antisense oligonucleotide aprinocarsen (ISIS 3521) did not show promising results as an antitumor agent. The PKCβ-specific inhibitor ruboxistaurin mesylate (LY333531) is in

phase III clinical trials. It has shown a delay in the progression of diabetic retinopathy, nephropathy and neuropathy. The experimental antitumor drug AD 198 is believed to exert its antitumor activity by activation of PKCδ. For the other PKC isozymes, no isozyme-specific small-molecule inhibitors or activators are available so far. Such modulators would be important for the elucidation of the exact functions of PKC isozymes. With such compounds also investigations into their possible therapeutic potential could be performed.

ACKNOWLEDGEMENTS

Work concerning these areas is supported be grants P15039 and P16477 from the Austrian Science Fund, and 503467 Pro-KinaseResearch from the EC.

REFERENCES

Abeliovich A, Chen C, Goda Y, Silva AJ, Stevens CF, Tonegawa S: Modified hippocampal long-term potentiation in PKC gamma-mutant mice. Cell 75: 1253-1262, 1993a

Abeliovich A, Paylor R, Chen C, Kim JJ, Wehner JM, Tonegawa S: PKC gamma mutant mice exhibit mild deficits in spatial and contextual learning. Cell 75: 1263-1271, 1993b

Advani R, Lum BL, Fisher GA, Halsey J, Geary RS, Holmlund JT, Kwoh TJ, Dorr FA, Sikic BI: A phase I trial of aprinocarsen (ISIS 3521/LY900003), an antisense inhibitor of protein kinase C-alpha administered as a 24-hour weekly infusion schedule in patients with advanced cancer. Invest New Drugs 23: 467-77, 2005

Aksoy E, Goldman M, Willems F: Protein kinase C epsilon: a new target to control inflammation and immune-mediated disorders. Int J Biochem Cell Biol 36: 183-188, 2004

Alvaro V, Levy L, Dubray C, Roche A, Peillon F, Querat B, Joubert D: Invasive human pituitary tumors express a point-mutated alpha-protein kinase C. J Clin Endocrinol Metab 77: 1125-1129, 1993

Alvaro V, Prevostel C, Joubert D, Slosberg E, Weinstein IB: Ectopic expression of a mutant form of PKCalpha originally found in human tumors: aberrant subcellular translocation and effects on growth control. Oncogene 14: 677-685, 1997

Azzi A, Boscoboinik D, Hensey C: The protein kinase C and protein kinase C related gene families. Eur J Biochem 208: 547-557, 1992

Baier G: The PKC gene module: molecular biosystematics to resolve its T cell functions. Immunol Rev 92: 64-79, 2003

Batlle E, Verdu J, Dominguez D, del Mont Llosas M, Diaz V, Loukili N, Paciucci R, Alameda F, de Herreros AG: Protein kinase C-alpha activity inversely modulates invasion and growth of intestinal cells. J Biol Chem 273: 15091-15098, 1998

Blobe GC, Obeid LM, Hannun YA: Regulation of protein kinase C and role in cancer biology. Cancer Metastasis Rev 13: 411-431, 1994

Blumberg PM: Complexities of the protein kinase C pathway. Mol Carcinog 4: 339-344, 1991

Bowers BJ and Wehner JM: Ethanol consumption and behavioural impulsivity are increased in protein kinase Cg null mutant mice. J Neurosci 21, RC180, 2001

Braz JC, Gregory K, Pathak A, Zhao W, Sahin B, Klevitsky R, Kimball TF, Lorenz JN, Nairn AC, Liggett SB, Bodi I, Wang S, Schwartz A, Lakatta EG, DePaoli-Roach AA, Robbins J, Hewett TE, Bibb JA, Westfall MV, Kranias EG, Molkentin JD: PKC-alpha regulates cardiac contractility and propensity toward heart failure. Nature Med 10: 239-240, 2004

Brenner W, Farber G, Herget T, Wiesner C, Hengstler JG, Thuroff JW: Protein kinase C eta is associated with progression of renal cell carcinoma (RCC). Anticancer Res 23: 4001-4006, 2003

Cacace A M, Guadagno SN, Krauss RS, Fabbro D, Weinstein, IB: The epsilon isoform of protein kinase C is an oncogene when overexpressed in rat fibroblasts. Oncogene 8: 2095-2104, 1993

Castagna M, Takai Y, Kaibuchi K, Sano K, Kikkawa U, Nishizuka Y: Direct activation of calcium-activated, phospholipid-dependent protein kinase by tumor-promoting phorbol esters. J Biol Chem 257: 7847-7851, 1982

Castrillo A, Pennington DJ, Otto F, Parker PJ, Owen MJ, Bosca L: Protein kinase Cepsilon is required for macrophage activation and defense against bacterial infection. J Exp Med 194: 1231-1242, 2001

Cataisson C, Joseloff E, Murillas R, Wang A, Atwell C, Torgerson S, Gerdes M, Subleski J, Gao JL, Murphy PM, Wiltrout RH, Vinson C, Yuspa SH: Activation of cutaneous protein kinase C alpha induces keratinocyte apoptosis and intraepidermal inflammation by independent signaling pathways. J Immunol 171: 2703-13, 2003

Cesare P, Dekker LV, Sardini A, Parker PJ, McNaughton PA: Specific involvement of PKC-epsilon in sensitization of the neuronal response to painful heat. Neuron 23: 617-624, 1999

Chen C, Kano M, Abeliovich A, Chen L, Bao S, Kim JJ, Hashimoto K, Thompson RF, Tonegawa S: Impaired motor coordination correlates with persistent multiple climbing fiber innervation in PKC gamma mutant mice. Cell 83: 1233-1242, 1995

Chida K, Hara T, Hirai T, Konishi C, Nakamura K, Nakao K, Aiba A, Katsuki M, Kuroki T: Disruption of protein kinase Ceta results in impairment of wound healing and enhancement of tumor formation in mouse skin carcinogenesis. Cancer Res 63: 2404-2408, 2003

Choi DS, Wang D, Dadgar J, Chang WS, Messing RO: Conditional rescue of protein kinase C epsilon regulates ethanol preference and hypnotic sensitivity in adult mice. J Neurosci 22: 9905-9911, 2002

Cripps MC, Figueredo AT, Oza AM, Taylor MJ, Fields AL, Holmlund JT, McIntosh LW, Geary RS, Eisenhauer EA: Phase II randomized study of ISIS 3521 and ISIS 5132 in patients with locally advanced or metastatic colorectal cancer: a National Cancer Institute of Canada Clinical Trials Group Study. Clin Cancer Res 8: 2188-2192, 2002

Csukai M, Chen CH, De Matteis MA, Mochly-Rosen D: The coatomer protein beta'-COP, a selective binding protein (RACK) for protein kinase Cepsilon. J Biol Chem 272: 29200-29206, 1997

Dean N, McKay R, Miraglia L, Howard R, Cooper S, Giddings J, Nicklin P, Meister L, Ziel R, Geiger T, Muller M, Fabbro D: Inhibition of growth of human tumor cell lines in nude mice by an antisense oligonucleotide inhibitor of protein kinase C-α expression. Cancer Res 56: 3499-3507, 1996

Dina OA, Barletta J, Chen X, Mutero A, Martin A, Messing RO, Levine JD: Key role for the epsilon isoform of protein kinase C in painful alcoholic neuropathy in the rat. J Neurosci 20: 8614-8619, 2000

Dorn GW 2nd, Souroujon MC, Liron T, Chen CH, Gray MO, Zhou HZ, Csukai M, Wu G, Lorenz JN, Mochly-Rosen D: Sustained in vivo cardiac protection by a rationally designed peptide that causes epsilon protein kinase C translocation. Proc Natl Acad Sci USA 96: 12798-12803, 1999

Duensing A, Joseph NE, Medeiros F, Smith F, Hornick JL, Heinrich MC, Corless CL, Demetri GD, Fletcher CD, Fletcher JA: Protein Kinase C theta (PKCtheta) expression and constitutive activation in gastrointestinal stromal tumors (GISTs). Cancer Res 64: 5127-5131, 2004

Emoto Y, Manome Y, Meinhardt G, Kisaki H, Kharbanda S, Robertson M, Ghayur T, Wong WW, Kamen R, Weichselbaum R, Kufe D: Proteolytic activation of protein kinase C delta by an ICE like protease in apoptotic cells. EMBO J 14: 6148-6156, 1995

Fagerstrom S, Pahlman S, Gestblom C, Nanberg E: Protein kinase C-epsilon is implicated in neurite outgrowth in differentiating human neuroblastoma cells. Cell Growth Differ 7: 775-785, 1996

Ghayur T, Hugunin M, Talanian RV, Ratnofsky S, Quinlan C, Emoto Y, Pandey P, Datta R, Huang Y, Kharbanda S, Allen H, Kamen R, Wong W and Kufe D: Proteolytic activation of protein kinase C delta by an ICE/CED 3 like protease induces characteristics of apoptosis. J Exp Med 184: 2399-2404, 1996

Goekjian PG, Jirousek MR: Protein kinase C in the treatment of disease: signal transduction pathways, inhibitors, and agents in development. Curr Med Chem 6: 877-903, 1999

Gokmen-Polar Y, Murray NR, Velasco MA, Gatalica Z, Fields AP: Elevated protein kinase C betall is an early promotive event in colon carcinogenesis. Cancer Res 61: 1375-81, 2001

Gray MO, Karliner JS, Mochly-Rosen D: A selective epsilon-protein kinase C antagonist inhibits protection of cardiac myocytes from hypoxia-induced cell death. J Biol Chem 272: 30945-30951, 1997

Gray MO, Zhou HZ, Schafhalter-Zoppoth I, Zhu P, Mochly-Rosen D, Messing RO: Preservation of baseline hemodynamic function and loss of inducible cardioprotection in adult mice lacking protein kinase C epsilon. J Biol Chem 279: 35963-35604, 2004

Griffiths G, Garrone B, Deacon E, Owen P, Pongracz J, Mead G, Bradwell A, Watters D, Lord J: The polyether bistratene A activates protein kinase C-delta and induces growth arrest in HL60 cells. Biochem Biophys Res Commun 222: 802-808, 1996

Gruber JR, Ohno S, and Niles RM: Increased expression of protein kinase C alpha plays a key role in retinoic acid-induced melanoma differentiation. J Biol Chem 267: 13356-13360, 1992

Gschwendt M, Muller, HJ, Kielbassa K, Zang R, Kittstein W, Rincke G Marks F: Rottlerin, a novel protein kinase inhibitor. Biochem Biophys Res Commun 199: 93-98, 1994

Gschwendt M: Protein kinase Cδ. Eur J Biochem 259: 555-564, 1999

Hagiwara M, Hachiya T, Watanabe M, Usuda N, Iida F, Tamai K, Hidaka H: Assessment of protein kinase C isoenzymes by immunoassay and overexpression of type II in thyroid adenocarcinoma. Cancer Res 50: 5515-5519, 1990

Hatada S, Sakanoue Y, Kusunoki M, Kobayashi A, Utsunomiya J: Protein kinase C activity in human thyroid carcinoma and adenoma. Cancer 70: 2918-2922, 1992

Hirai T and Chida K: Protein kinase Czeta (PKCzeta): activation mechanisms and cellular functions. J Biochem 133: 1-7, 2003

Hodge CW, Raber J, McMahon T, Walter H, Sanchez-Perez AM, Olive MF, Mehmert K, Morrow AL, Messing RO: Decreased anxiety-like behavior, reduced stress hormones, and neurosteroid supersensitivity in mice lacking protein kinase Cepsilon. J Clin Invest 110: 1003-1010, 2002

Hofmann J: Protein kinase C isozymes as potential targets for anticancer therapy. Curr Cancer Drug Targets 4: 125-146, 2004

Hug H and Sarre TF: Protein kinase C isoenzymes: divergence in signal transduction? Biochem J 291: 329-343, 1993

Hundle B, McMahon T, Dadgar J, Messing RO: Overexpression of epsilon-protein kinase C enhances

nerve growth factor-induced phosphorylation of mitogen-activated protein kinases and neurite outgrowth. J Biol Chem 270: 30134-30140, 1995

Jansen AP, Verwiebe EG, Dreckschmidt NE, Wheeler DL, Oberley TD, Verma AK: Protein kinase C-epsilon transgenic mice: a unique model for metastatic squamous cell carcinoma. Cancer Res 61: 808-812, 2001

Johnson JA, Gray MO, Chen CH, Mochly-Rosen D: A protein kinase C translocation inhibitor as an isozyme-selective antagonist of cardiac function. J Biol Chem 271: 24962-24966, 1996

Joy SV, Scates AC, Bearelly S, Dar M, Taulien CA, Goebel JA, Cooney MJ: Ruboxistaurin, a protein kinase C beta inhibitor, as an emerging treatment for diabetes microvascular complications. Ann Pharmacother 39: 1693-1699, 2005

Jung YS, Ryu BR, Lee BK, Mook-Jung I, Kim SU, Lee SH, Baik EJ, Moon CH: Role for PKC-epsilon in neuronal death induced by oxidative stress. Biochem Biophys Res Commun 32: 789-794, 2004

Kawakami T, Kawakami Y, Kitaura J: Protein kinase C beta (PKC beta): normal functions and diseases. J Biochem 132: 677-682, 2002

Kashiwagi M, Ohba M, Chida K, Kuroki T: Protein kinase C eta (PKC eta): its involvement in keratinocyte differentiation. J Biochem 132: 853-857, 2002

Kennedy MJ, Prestigiacomo LJ, Tyler G, May WS, Davidson NE: Differential effects of bryostatin 1 and phorbol ester on human breast cancer cell lines. Cancer Res 52: 1278-1283, 1992

Kerfoot C, Huang W, Rotenberg SA: Immuno-histochemical analysis of advanced human breast carcinomas reveals downregulation of protein kinase C alpha. J Histochem Cytochem 52: 419-422, 2004

Khasar SG, Lin YH, Martin A, Dadgar J, McMahon T, Wang D, Hundle B, Aley KO, Isenberg W, McCarter G, Green PG, Hodge CW, Levine JD, Messing RO: A novel nociceptor signaling pathway revealed in protein kinase C epsilon mutant mice. Neuron 24: 253-260, 1999

Kikkawa U, Matsuzaki H, Yamamoto T: Protein kinase Cδ(PKCδ): activation mechanisms and functions. J Biochem 132: 831-839, 2002

Lahn M, Kohler G, Sundell K, Su C, Li S, Paterson BM, Bumol TF: Protein kinase C alpha expression in breast and ovarian cancer. Oncology 67: 1-10, 2004

Lahn MM and Sundell KL: The role of protein kinase C-alpha (PKC-alpha) in melanoma. Melanoma Res 14: 85-89, 2004

Lahn M, Sundell K, Köhler G: The role of protein kinase C-alpha in hematoligical malignancies. Acta Haematol 115: 1-8, 2006

Leach KL, James ML, Blumberg PM: Characterization of a specific phorbol ester aporeceptor in moujse brain cytosol. Proc Natl Acad Sci USA 80: 4208-4212, 1983

Leitges M, Schmedt C, Guinamard R, Davoust J, Schaal S, Stabel S, Tarakhovsky A: Immunodeficiency in protein kinase cbeta-deficient mice. Science 273: 788-791, 1996

Leitges M, Mayr M, Braun U, Mayr U, Li C, Pfister G, Ghaffari-Tabrizi N, Baier G, Hu Y, Xu Q: Exacerbated vein graft arteriosclerosis in protein kinase Cdelta-null mice. J Clin Invest 108: 1505-1512, 2001

Li H, Zhao L, Yang Z, Funder JW, Liu JP: Telomerase is controlled by protein kinase Calpha in human breast cancer cells. J Biol Chem 273: 33436-33442, 1998

Li Y, Wheeler DL, Alters W, Chaiswing L, Verma AK, Oberley TD: Early epidermal destruction with subsequent epidermal hyperplasia is a unique feature of the papilloma-independent squamous cell carcinoma phenotype in PKCepsilon overexpressing transgenic mice. Toxicol Pathol 33: 684-694, 2005

Littler CM, Morris KG Jr, Fagan KA, McMurtry IF, Messing RO, Dempsey EC: Protein kinase C-epsilon-null mice have decreased hypoxic pulmonary vasoconstriction. Am J Physiol Heart Circ Physiol 284: H1321-1331, 2003

Lothstein L, Israel M, Sweatman T: Anthracycline drug targeting: Cytoplasmic versus nuclear. Drug Res 4: 169-177, 2001

Mackay K and Mochly-Rosen D: Localization, anchoring and functions of protein kinase C isozymes in the heart. J Mol Cell Cardiol 33, 1301-1307, 2001

Malhotra A, Begley R, Kang BP, Rana I, Liu J, Yang G, Mochly-Rosen D, Meggs LG: PKC-{varepsilon}-dependent survival signals in diabetic hearts. Am J Physiol Heart Circ Physiol 289: H1343-1350, 2005

Malmberg AB, Chen C, Tonegawa S, Basbaum AI: Preserved acute pain and reduced neuropathic pain in mice lacking PKCgamma. Science 278: 279-283, 1997

Manni A, Buckwalter E, Etindi R, Kunselman S, Rossini A, Mauger D, Dabbs D, Demers L: Induction of a less aggressive breast cancer phenotype by protein kinase C-α and -β overexpression. Cell Growth Diff 7: 1187-1198, 1996

Martin P, Duran A, Minguet S, Gaspar ML, Diaz-Meco MT, Rennert P, Leitges M, Moscat J: Role of zeta PKC in B-cell signaling and function. EMBO J 21: 4049-57, 2002

Mathis D and King GL: B-cell signaling: protein kinase Cdelta puts the brakes on. Curr Biol 12: R554-556, 2002

Mecklenbrauker I, Kalled SL, Leitges M, Mackay F, Tarakhovsky A: Regulation of B-cell survival by BAFF-dependent PKCdelta-mediated nuclear signalling. Nature 431: 456-461, 2004

Mischak H, Goodnight JA, Kolch W, Martiny-Baron G, Schaechtle C, Kazanietz MG, Blumberg PM, Pierce JH, Mushinski JF: Overexpression of protein kinase C-delta and -epsilon in NIH 3T3 cells induces opposite effects on growth, morphology, anchorage dependence, and tumorigenicity. J Biol Chem 268: 6090-6096, 1993

Miyamoto A, Nakayama K, Imaki H, Hirose S, Jiang Y, Abe M, Tsukiyama T, Nagahama H, Ohno S, Hatakeyama

S, Nakayama KI: Increased proliferation of B cells and auto-immunity in mice lacking protein kinase Cdelta. Nature 416: 865-869, 2002

Moscat J and Diaz-Meco MT: The atypical protein kinase Cs. EMBO Rep 51, 399-403, 2000

Nakashima S: Protein kinase C alpha (PKC alpha): regulation and biological function. J Biochem 132: 669-675, 2002

Narita M, Mizoguchi H, Suzuki T, Narita M, Dun NJ, Imai S, Yajima Y, Nagase H, Suzuki T, Tseng LF: Enhanced mu-opioid responses in the spinal cord of mice lacking protein kinase Cgamma isoform. J Biol Chem 276: 15409-15414, 2001

Newton AC and Johnson JE: Protein kinase C: a paradigm for regulation of protein function by two membrane-targeting modules. Biochim Biophys Acta 1376: 155-172, 1998

Newton PM and Messing RO: Intracellular signalling pathways that regulate behavioural responses to ethanol. Pharmacol Ther 109: 227-237, 2006

Ng T, Squire A, Hansra G, Bornancin F, Prevostel C, Hanby A, Harris W, Barnes D, Schmidt S, Mellor H, Bastiaens PI, Parker PJ: Imaging protein kinase calpha activation in cells. Science 283: 2085-2089, 1999

Niedel JE, Kuhn L, Vandenbark GR: Phorbol diester receptor copurifies with protein kinase C. Proc Natl Acad Sci USA 80: 36-40, 1983

Nishizuka Y: Protein kinase C and lipid signaling for sustained cellular responses. FASEB J 9: 484-496, 1995

Nixon JS: The biology of protein kinase C inhibitors. In: Molecular Biology Intelligence Unit: Protein Kinase C, PJ Parker and LV Dekker (eds.), R.G. Landes Company, Austin, TX, Springer-Verlag, Heidelberg, 205-236, 1997

O'Brian CA, Chu F, Bornmann WG, Maxwell DS: Protein kinase Calpha and epsilon small-molecule targeted therapeutics: a new roadmap to two Holy Grails in drug discovery? Expert Rev Anticancer Ther 6: 1751-1786, 2006

Perletti GP, Folini M, Lin HC, Mischak H, Piccinini F, Tashjian AH Jr: Overexpression of protein kinase C epsilon is oncogenic in rat colonic epithelial cells. Oncogene 12: 847-854, 1996

Perletti GP, Marras E, Concari P, Piccinini F, Tshjian AH Jr: PKC delta acts as a growth and tumor suppressor in rat colonic epithelial cells. Oncogene 18: 1251-1256, 1999

Ping P, Song C, Zhang J, Guo Y, Cao X, Li RC, Wu W, Vondriska TM, Pass JM, Tang XL, Pierce WM, Bolli R: Formation of protein kinase C(epsilon)-Lck signaling modules confers cardioprotection. J Clin Invest 109: 499-507, 2002

Prevostel C, Alvaro V, de-Boisvilliers F, Martin A, Jaffiol C, Joubert D: The natural protein kinase C alpha mutant is present in human thyroid neoplasms. Oncogene 11:669-674, 1995

Prevostel C, Martin A, Alvaro V, Jaffiol C, Joubert D: Protein kinase C alpha and tumorigenesis of the endocrine gland. Horm Res 47: 140-144, 1997

Ramakers GM, Gerendasy DD, de Graan PN: Substrate phosphorylation in the protein kinase Cgamma knockout mouse. J Biol Chem 274: 1873-1874, 1999

Reddig PJ, Dreckschmidt NE, Ahrens H, Simsiman R, Tseng C-P, Zou J, Oberley TD, Verma AK: Transgenic mice overexpressing protein kinase C δ in the epidermis are resistant to skin tumor promotion by 12-O-tetradecanoylphorbol-13-acetate. Cancer Res 49: 5710-5718, 1999

Regala RP, Weems C, Jamieson L, Khoor A, Edell ES, Lohse CM, Fields AP: Atypical protein kinase C iota is an oncogene in human non-small cell lung cancer. Cancer Res 65: 8905-8911, 2005

Roaten JB, Kazanietz MG, Sweatman TW, Lothstein L, Israel M, Parrill AL: Molecular models of N-benzyladriamycin-14-valerate (AD 198) in complex with the phorbol ester-binding C1b domain of protein kinase C-delta. J Med Chem 44: 1028-1034, 2001

Scaglione-Sewell B, Abraham C, Bissonnette M, Skarosi SF, Hart J, Davidson NO, Wali RK, Davis BH, Sitrin M, Brasitus TA: Decreased PKC-alpha expression increases cellular proliferation, decreases differentiation, and enhances the transformed phenotype of CaCo-2 cells. Cancer Res 58: 1074-1081, 1998

Schiemann U, Assert R, Moskopp D, Gellner R, Hengst K, Gullotta F, Domschke W, Pfeiffer A: Analysis of a protein kinase C-alpha mutation in human pituitary tumors. J Endocrinol 153: 131-137, 1997

Stabel S: Protein kinase C - an enzyme and its relatives. Sem Cancer Biol 5: 277-284, 1994

Stabel S and Parker PJ: Protein kinase C. Pharmac Ther 41: 71-95, 1991

Stallings-Mann M, Jamieson L, Regala RP, Weems C, Murray NR, Fields AP: A novel small-molecule inhibitor of protein kinase Ciota blocks transformed growth of non-small-cell lung cancer cells. Cancer Res 66: 1767-1774, 2006

Suga K, Sugimoto I, Ito H, Hashimoto E: Down-regulation of protein kinase C-alpha detected in human colorectal cancer. Biochem Mol Biol Int 44: 523-528, 1998

Sun Z, Arendt CW, Ellmeier W, Schaeffer EM, Sunshine MJ, Gandhi L, Annes J, Petrzilka D, Kupfer A, Schwartzberg PL, Littman DR: PKC-theta is required for TCR-induced NF-kappaB activation in mature but not immature T lymphocytes. Nature 404: 402-407, 2000

Suzuki A, Yamanaka T, Hirose T, Manabe N, Mizuno K, Shimizu M, Akimoto K, Izumi Y, Ohnishi T, Ohno S: Atypical protein kinase C is involved in the evolutionarily conserved par protein complex and plays a critical role in establishing epithelia-specific junctional structures. J Cell Biol 152: 1183-1196, 2001

Suzuki A, Akimoto K, Ohno S: Protein kinase λ/ι (PKCλ/ι): a PKC isotype essential for the development of multicellular organisms. J Biochem 133: 9-16, 2003

Suzuma K, Takahara N, Suzuma I, Isshiki K, Ueki K, Leitges M, Aiello LP, King GL: Characterization of protein kinase C beta isoform's action on retinoblastoma protein phosphorylation, vascular endothelial growth factor-induced endothelial cell proliferation, and retinal neovascularization. Proc Natl Acad Sci USA 99: 721-726, 2002

Szallasi Z, Smith CB, Pettit GR, Blumberg PM: Differential regulation of protein kinase C isozymes by bryostatin 1 and phorbol 12-myristate 13-acetate in NIH 3T3 fibroblasts. J Biol Chem 269: 2118-2124, 1994

Takeishi Y, Ping P, Bolli R, Kirkpatrick DL, Hoit BD, Walsh RA: Transgenic overexpression of constitutively active protein kinase C epsilon causes concentric cardiac hypertrophy. Circ Res 86: 1218-1223, 2000

Tanaka M, Gunawan F, Terry RD, Inagaki K, Caffarelli AD, Hoyt G, Tsao PS, Mochly-Rosen D, Robbins RC: Inhibition of heart transplant injury and graft coronary artery disease after prolonged organ ischemia by selective protein kinase C regulators. J Thorac Cardiovasc Surg 129: 1160-1167, 2005

Teicher BA, Alvarez E, Mendelsohn LG, Ara G, Menon K, Ways DK: Enzymatic rationale and preclinical support for a potent protein kinase C beta inhibitor in cancer therapy. Adv Enzyme Regul 39: 313-327, 1999

Vansteenkiste J, Canon JL, Riska H, Pirker R, Peterson P, John W, Mali P, Lahn M: Randomized phase II evaluation of aprinocarsen in combination with gemcitabine and cisplatin for patients with advanced/metastatic non-small cell lung cancer. Invest New Drugs 23: 263-269, 2005

Villalba M and Altman A: Protein kinase C-theta (PKCtheta), a potential drug target for therapeutic intervention with human T cell leukemias. Curr Cancer Drug Targets 2: 125-137, 2002

Ways KD, Kukoly CA, deVente J, Hooker JL, Bryant WO, Posekany KJ, Fletcher DJ, Cook PP, Parker PJ: MCF-7 breast cancer cells transfected with protein kinase C-α exhibit altered expression of other protein kinase C isoforms and display a more aggressive phenotype. J Clin Invest 95: 1906-1915, 1995

Yazaki T, Ahmad S, Chahlavi A, Zylber-Katz E, Dean NM, Rabkin SD, Martuza RL, Glazer RI: Treatment of glioblastoma U-87 by systemic administration of an antisense protein kinase C-alpha phosphorothiate oligodeoxynucleotide. Mol Pharmacol 50: 236-242, 1996

Zellner A, Fetell MR, Bruce JN, De Vivo DC, O'Driscoll KR: Disparity in expression of protein kinase C alpha in human glioma versus glioma-derived primary cell lines: therapeutic implications. Clin Cancer Res 4: 1797-1802, 1998

Zhu Y, Dong Q, Tan BJ, Lim WG, Zhou S, Duan W: The PKCalpha-D294G mutant found in pituitary and thyroid tumors fails to transduce extracellular signals. Cancer Res. 65: 4520-4524, 2005

ONCOGENIC TYROSINE KINASES: THE BCR-ABL MODEL

➤ **Carlo Gambacorti-Passerini**
Dept. of Clinical Medicine,
University of Milano-Bicocca, Italy
Carlo.Gambacorti@ unimib.it

➤ **Leonardo Scapozza**
Pharmaceutical Biochemistry Group,
School of Pharmaceutical Sciences,
University of Geneva,
University of Lausanne,
Quai Ernest-Ansermet 30, CH-1211 Geneva 4, Switzerland
Leonardo.Scapozza@pharm.unige.ch

INTRODUCTION

Tyrosine kinases (TK) and related molecules comprise over 100 different genes. Approximately two thirds represent receptor TK, the other third being non receptor TK. TK regulate important cellular signalling pathway, from the transduction of extracellular signals, to the regulation of key biological processes such as cell proliferation and apoptosis (Manning et al., 2002; Noble et al., 2004).

TK can turn themselves into the engine of malignant cell transformation. Their relationship to cancer varies in the different diseases. They range from cases with a known, clear and causal relationship, to examples in which such a relationship is more circumstantial. A recent review describing seven different oncogenic TK is available (Gambacorti-Passerini, 2004).

In this review, the model TK Bcr-Abl will be presented as an example of an oncogenic TK, in which the delineation of the molecular lesion (Ph-chromosome) and the assessment of the causal association between the TK and the disease phenotype (principally Chronic Myeloid Leukemia) led to the discovery and clinical development of a highly successful treatment. The clinical and the molecular aspects of Bcr/Abl inhibition will be highlighted.

ONCOGENIC TYROSINE KINASES: THE BCR-ABL MODEL

Chronic Myeloid Leukemia (CML) is a myeloproliferative disease, initially characterized by an abnormal expansion of clonal hematopoiesis still capable of achieving terminal differentiation (Goldman & Melo, 2003). CML exhibits a characterististic biphasic clinical course. The initial chronic phase, which lasts on average five years, originates as an indolent disease. It is invariably followed by the development of an acute leukemia, termed blast crisis that is marked by the emergence within the clonal hematopoiesis of fully transformed cell clones arrested at an early stage of differentiation, either myeloid or lymphoid.

For many years, the presence of a Ph-chromosome in the cells of the CML patients has been the only cytogenetic abnormality known to be associated with a specific malignant disease in humans (Nowell & Hungerford, 1960). Later it was recognized that the Ph-chromosome is the result of a reciprocal translocation between the long arms of chromosome 9 and 22, t(9;22)(q34;q11) (Rowley, 1973). Finally, in the eighties, the molecular defect associated with this cytogenetic abnormality was identified, and it was established that the Ph-chromosome results in

the juxtaposition of parts of the *BCR* and *ABL* genes, which are normally located respectively on chromosome 22 and chromosome 9, to form a new hybrid *BCR-ABL* gene (Groffen et al., 1984; Heisterkamp et al., 1985). The corresponding chimeric protein (P210; Ben-Neriah et al., 1986) has a causative role in neoplastic transformation of pluripotent stem cells, giving rise to the expansion of the myeloid compartment which characterizes the chronic phase of the disease. However, in spite of the fact that the structural organization and the molecular biology of the *BCR-ABL* gene as well as that of the normal *ABL* and *BCR* genes have been subjects of intensive investigation in the last thirty years, many questions concerning the mechanisms by which the hybrid gene is formed and transforms the hemopoietic stem cells still remain unanswered. Moreover, a further degree of complexity is represented by the fact that the Ph-chromosome may also be found in leukemias other than CML and associated with a wide spectrum of hematological phenotypes, ranging from that of apparently "de novo" acute lymphoblastic leukemia to that, on the opposite side, of indolent chronic myeloproliferative disorders (Melo, 1996; Saglio et al., 1997).

In the late 1980s, the data accumulated on the role of *BCR-ABL* in onset and progression of CML indicated *BCR-ABL* as the most attractive target for molecularly targeted therapy approaches. Therefore, attempts to decrease the amount of the *BCR-ABL* transcripts and/or to inhibit the TK activity of the oncoprotein were initiated and this process finally ended with the discovery and the development of imatinib mesylate (Buchdunger et al., 1996). This small chemical compound inhibits the kinase activity of Bcr-Abl at nanomolar concentrations and was shown to stop cellular growth and to induce apoptosis of leukemic cells both *in vitro* and *in vivo* (Druker et al., 1996; Gambacorti-Passerini et al., 1997; Deininger et al., 1997; le Coutre et al., 1999). The importance of imatinib goes beyond the exceptional therapeutic results obtained with its use in CML and in other Ph-positive leukemias, and should represent the real starting point of the so called "molecularly targeted therapy" (**Figure 1**).

The X-ray structure of Abl in complex with imatinib revealed its molecular mechanism of action (Nagar et al., 2002). Imatinib, (4-[(4-Methyl-1-piperazinyl)methyl]-N-[4-methyl-3-[[4-(3-pyridinyl)-2-pyrimidinyl]amino]-phenyl]benzamid (IUPAC)), that has an IC50 value of 38 nM, captures

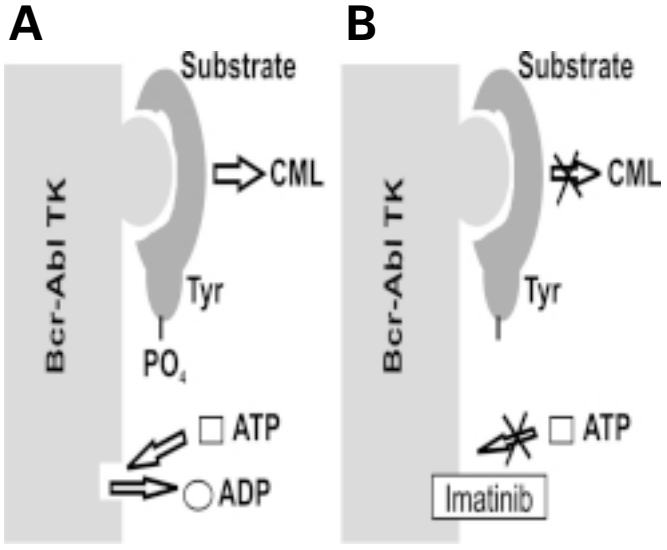


Figure 1. Principle of the Bcr-Abl based molecularly targeted therapy. A) The constitutively active Bcr-Abl tyrosine kinase transfer phosphate from ATP to tyrosine residues of various substrates inducing excess proliferation and inhibition of apoptosis of myeloid cells characteristic of CML. B) Imatinib inhibits the kinase activity by blocking the binding of ATP to the protein, thus preventing CML.

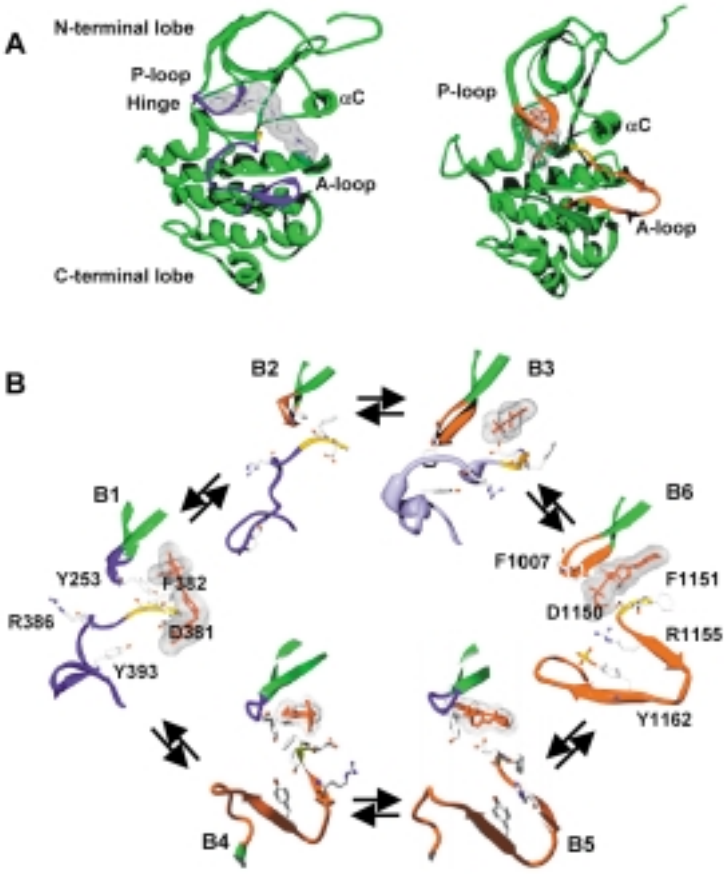


Figure 2. Conformational plasticity: a multiple pathway from "Off-state" to "On-state" and vice versa.
A) On the left, the X-ray structure of Bcr-Abl tyrosine kinase domaine in complex with imatinib (1IEP; Nagar et al., 2002) represents the inactive "Off-state" conformation of tyrosine kinase with the nucleotide binding loop (P-loop) in a peculiar conformation and the activation loop (A-loop) inserted into the substrate binding pocket that prevent binding of ATP and the substrate. On the right, the X-ray structure of insulin receptor tyrosine kinase domain (1IR3; Hubbard, 1997) in complex with an ATP analog represents the active "On-state" conformation with an elongated P-loop and an open A-loop that allow ATP and substrate binding. The α-helix C (αC) that experiences significant movement along the transition from "Off-state" to "On-state" is labelled. The hinge region (Hinge) connecting the N-terminal lobe with the C-terminal lobe is labelled. All ligands, ATP and inhibitors binding to the ATP pocket of the kinase domain form hydrogen bonds with the hinge region (Hinge) (Moretti et al., 2006). The position of the conserved and catalytically important DFG motif lying at the beginning of the A-loop is shown in yellow. Imatinib and the ATP analog are depicted by capped sticks and their molecular surface is shown. The P-loops and A-loops having a conformation closer to the "Off-state" one are coloured in blue, while those presenting a more "On-state" conformation are shown in red. Cyan is used in cases where the loops assume a conformation in-between "Off-state" and "On-state".
B) Close up on the P-loop, A-loop and DFG motif of different X-ray structures showing the existing variability in conformation going from the "Off-state" (B1) and the "On-state" (B6). The tyrosine on the A-loop in the "On-state" is phosphorylated. In the "Off-state" conformation, the Phe of the DFG points towards the ATP binding site, while in the "On-state" it is tilted 180°. The colour code is the same as for panel A. The DFG as well as important aligned amino acids of the P-loop and A-loop are shown as colour coded sticks and labelled for (B1) and (B6). Amino acid numbering refers to Abl (B1) and IR (B6). The X-ray structures shown here are: B1: Abl in complex with imatinib (1IEP; Nagar et al., 2002), B2: Insulin receptor kinase apo (1IRK; Hubbard et al., 1994), B3: HCK ligated to PP1 (1QCF; Schindler et al., 1999), B4: Abl complexed with PD173955 (1M52; Nagar et al., 2002), B5: Abl in complex with VX-680 (2F4J; Young et al., 2006), B6: Insulin receptor kinase ligated with an ATP analog (1IR3; Hubbard, 1997). Because of the orientation of the DFG, conformation of B2 and B4 are considered more close to B1, while B3 and B5 are more similar to B6.

a specific inactive conformation of Abl in which the activation loop (A-loop) mimics the substrate and the Phe of the DFG motif points towards the ATP binding site (**Figure 2**). Imatinib forms 6 hydrogen bonds with the protein and several hydrophobic contacts with amino acids forming an extended selectivity pocket favoured by the closed conformation of the A-loop. The nitrogen of the pyridine moiety forms a hydrogen bond with the hinge region that is characteristic for tyrosine kinase inhibitors (Moretti et al., 2006). In addition, the amino group of the 2-phenyl-aminopyrimidine moiety builds a hydrogen bond with the gatekeeper amino acid Thr315 (Nagar et al., 2002).

The scenario of treatment options for CML and other types of Philadelphia chromosome positive (Ph+) leukemias is entering a new phase, thanks to the development of Imatinib (Goldman & Druker, 2001). For the first time, a specific and relatively non-toxic drug is able to induce durable remissions in most patients with Ph+ leukemias, and cytogenetic responses in the majority of CML patients in chronic phase. Imatinib has been under clinical investigation for almost 8 years. During this time, over 50.000 patients, mostly affected by CML or Ph+ALL, have received imatinib.

Patients with acute leukemia (defined as the presence of > 30% blasts in either the bone marrow or the peripheral blood) showed a high (> 50%) rate of initial haematological responses: 52% of the patients with "myeloid" BC-CML and 60% of the patients with Ph+ ALL or "lymphoid" BC-CML showed reduction in blast values corresponding to a haematological response (Ottmann et al., 2002; Sawyers et al., 2002). Patients included in this category present either "de novo" acute leukemias (Ph+ ALL or AML) or blast crisis CML. The therapeutic effects obtained in such patients are, however, short-lived (3–6 months) and quickly followed by relapse and resistance to imatinib in almost all patients. Although patients affected by the myeloid blast crisis CML seem to fare slightly better than the patients with ALL or lymphoid blast crisis CML, the present overall evaluation of imatinib in this category of patients can be considered as a "qualified failure".

In patients with accelerated phase leukemia ((AP), defined as blasts between 15 and 30%, basophiles > 20%, or platelet counts < 100 k/mm^3), durable major cytogenetic responses can be obtained, but only in approximately 25% of patients, although the treatment is probably able to prolong survival even in the absence of a cytogenetic response (Talpaz et al., 2002).

Patients affected by CML and treated with imatinib in late CP (defined as < 15% blasts, < 20% basophiles and with platelet count > 100 k/mm^3), who are intolerant, resistant or refractory to interferon treatment, respond better than the patients in more advanced phases. Major cytogenetic responses (MCyR) were initially obtained in 64% of 450 patients who started imatinib in late CP, at a median time of 34 months after the diagnosis of CML (Kantarjian et al., 2002). This trial now reached the 5 year of median follow up, and the results were recently presented (Gambacorti et al., 2005a). This population of late CP-CML patients is the first one, in which the treatment with imatinib has proved to be able to change the biological history of the disease. At 60 months, 81% of patients who reached a MCyR

Table 1: Time to AP/BC, yearly event rate (from Gambacorti et al., 2005a)

Year since start of treatment	All (n=454)	MCyR at any time (n=304)	No MCyR at any time (n=150)	MCyR at 3 months (n=148)	No MCyR at 3 months (n=295)
1st	7.8%	2.6%	18.6%	2.7%	8.9%
2nd	6.0%	0.3%	21.1%	0.7%	8.9%
3rd	7.2%	3.6%	20.6%	2.3%	10.2%
4th	7.3%	4.0%	24.7%	2.5%	10.6%
5th	7.3%	4.4%	28.1%	1.8%	11.6%

are still in cytogenetic remission, and 92% are alive (**Figure 3** and **Table 1**). The early reach of cytogenetic remission is evidenced in **Figure 3** as an important prognostic sign, and the relapse rate (a bona fide indicator of the development of resistance to imatinib) among patients in cytogenetic response continues to range between 1 and 2% (compared with a historical value of 20-25%). These data indicate that, given the average age at which CML is diagnosed (45–50 years) and the average life expectation, and considering the present yearly risk of relapse, the majority of CML patients will die of other causes, and not of CML.

These early results were later confirmed in a subsequent trial, in which imatinib demonstrated a clear superiority as a first line treatment of CML when compared with the combination of Interferon beta and Ara-C (O'Brien et al., 2003). In this protocol, monitoring patients by quantitative PCR for the presence of the Bcr/Abl transcript allowed to obtain further information on how imatinib affected CML biology. In fact, if patients reach at least 3 log unit reduction of Bcr-Abl transcript present, their yearly risk of relapse is further reduced to less than 0.5%. However, the available data allow also to state that the vast majority of treated patients (80–90%) remain positive for the presence of Bcr-Abl transcript for at least the first 2 years of treatment (Hughes et al., 2003). When the level of Bcr-Abl positive cells falls below 1/10,000–1/100,000, even PCR fails to detect the transcription. Until recently, the discontinuation of imatinib was regularly followed by relapse of CML, even in patients who tested negative at the time of drug suspension (Cortes et al., 2004), indicating that PCR negativity only meant that the number of residual leukemic cells

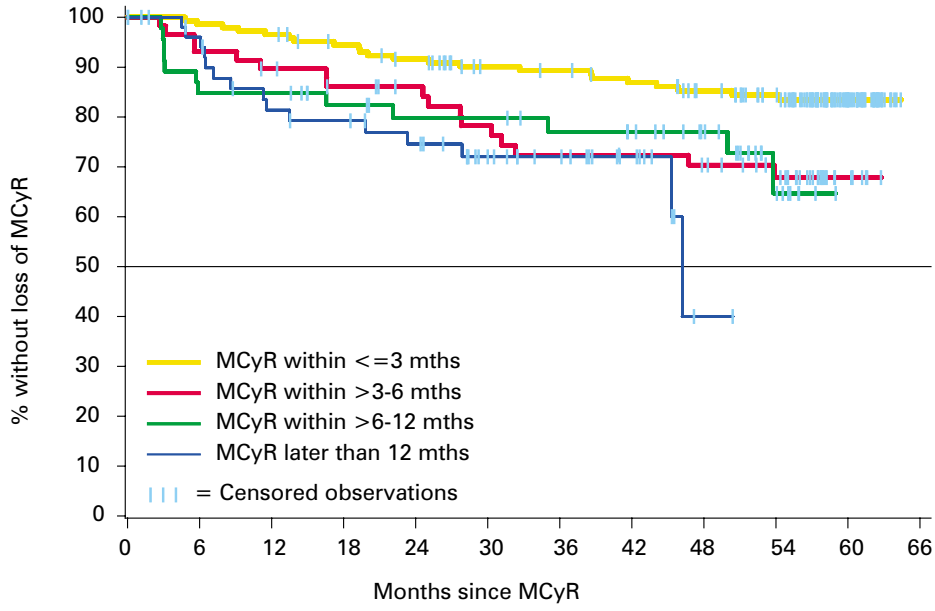


Figure 3. Duration of major cytogenetic responses (MCyR) after reaching MCyR (from Gambacorti et al., 2005)

was below the PCR detection limit.

Very recent data, however, have changed this view, at least in part. Two recent reports indicate that the discontinuation of imatinib in patients, who achieved a sustained PCR negativity, was not followed by disease relapse in approximately 50% of cases (Merante et al., 2005; Rousselot et al., 2005). Moreover, a recent presentation on the molecular monitoring of patients enrolled in the IRIS trial (Goldman et al., 2005), indicated that the amount of transcript decreases gradually, up to 4 years after imatinib initiation. In this report, the number of patients who achieved >4 log unit reduction of Bcr-Abl transcript (i.e. PCR negative or almost negative) after 4 years of treatment reached the value of 41%. Therefore, an important issue connected to the long term follow up of CML patients on imatinib treatment is whether the treatment could be interrupted at a certain point. This issue remains unresolved at present.

In addition to Ph+ leukemias, important clinical responses were also obtained in patients with malignancies caused by structural alterations of the PDGFR beta gene (Magnusson et al., 2002; Apperley et al., 2002). Imatinib is also active against gastrointestinal stromal tumours (GIST), a rare form of non-epithelial gastrointestinal cancer that frequently presents activating mutations of the c-KIT gene, another target of imatinib. In this group of patients, objective response rates of 53% and 54% were obtained (Apperley et al., 2002; van Oosterom et al., 2001), with therapeutic effects often observed within a few days of treatment (Joensuu et al., 2001). These responses appear to be durable and linked to the presence of c-Kit mutations in the tumour: risk of progression after imatinib treatment was 8 times higher in patients lacking c-Kit mutations than in patients carrying mutated c-Kit (44% vs 5.8%; Heinrich et al., 2002).

During the successful clinical trials, resistance to imatinib emerged particularly in patients with acute leukemias, but it is a potential issue also in patients in chronic phase. The molecular mechanism of resistance has been identified in Bcr-Abl gene amplification and mutations in the catalytic kinase domain of the gene (for review; Gambacorti-Passerini et al., 2003). The mutation of the gatekeeper amino acid threonine into a isoleucine (T315I) has been depicted as the predominant one in patients (Gambacorti-Passerini et al., 2003). This has prompted intense research to find new compounds able to overcome the resistance problem, such as BMS-354825 (Shah et al., 2004), SKI-606 (Golas et al., 2003) and AMN107 (Weisberg et al., 2005). BMS-354825 and SKI-606 are dual Src and Abl inhibitors, and are currently undergoing clinical evaluation. Both inhibit *in vitro* and *in vivo* several imatinib-resistant Bcr-Abl mutants, but not the T315I. Their activity profiles suggest that both compounds have less stringent conformation requirements for binding than imatinib, and they are thought to share the binding mode and binding conformation of PD173955 (**Figure 2B**) (Gambacorti-Passerini et al., 2005b; Thaimattam et al., 2005).

Very recently, an ATP competitive inhibitor, VX-680, ligating the "On-state" conformation of Abl (**Figure 2B**) and moderately inhibiting the T315I variant of Abl, has been reported (Young et al., 2006). A recent example of a potent (IC_{50} in the low nanomolar range) and specific small molecule inhibitor targeting the peptide substrate pocket of Abl is ON012380 (Gumireddy et al., 2005). ON012380 inhibits the T315I mutation, because it targets a different site of the protein compared to the inhibitors competing with ATP (Gumireddy et al., 2005). The first allosteric inhibitor of Bcr-Abl (GNF-2), a 4,6-disubstituted pyrimidine derivative, has been shown to inhibit Bcr-Abl-dependent cell proliferation with submicromolar IC_{50} without inhibition of the Abl kinase activity *in vitro* (Adrian et al., 2006).

The development of imatinib, the search of more potent Abl inhibitors, the knowledge acquired at the molecular, structural and cellular level represent not only a new phase for the treatment of CML, but also a bench example for other oncogenic tyrosine kinases (Blume-Jensen & Hunter, 2001). The conformational plasticity of tyrosine kinases (Huse & Kuriyan, 2002) represents a great opportunity for therapeutic intervention, because it offers the possibility of exploiting more putative binding sites that are present or formed along the trajectory going from the "Off-state" to the "On-state" (**Figure 2**), as shown by the Bcr-Abl model case. Crystallographic studies of apo and imatinib complexed structures of c-Kit and Spleen kinase SyK (**Figure 4**) clearly show that the inhibitor binds

following the conformational selection model (Bosshard, 2001). According to this model, a certain part of the protein is distorted in the sense that a number of alternative conformations are available. Selective binding of the ligand to a protein molecule in one of these conformational states causes a perturbation of the equilibrium between these states. In the case of tyrosine kinases, the two extreme states are the "Off-state" and "On-state" (**Figure 2**). Thus, we face a situation of having one protein with a series of targetable sites. Nevertheless, the number of targetable sites available within the protein and their stability in time remain to be discovered.

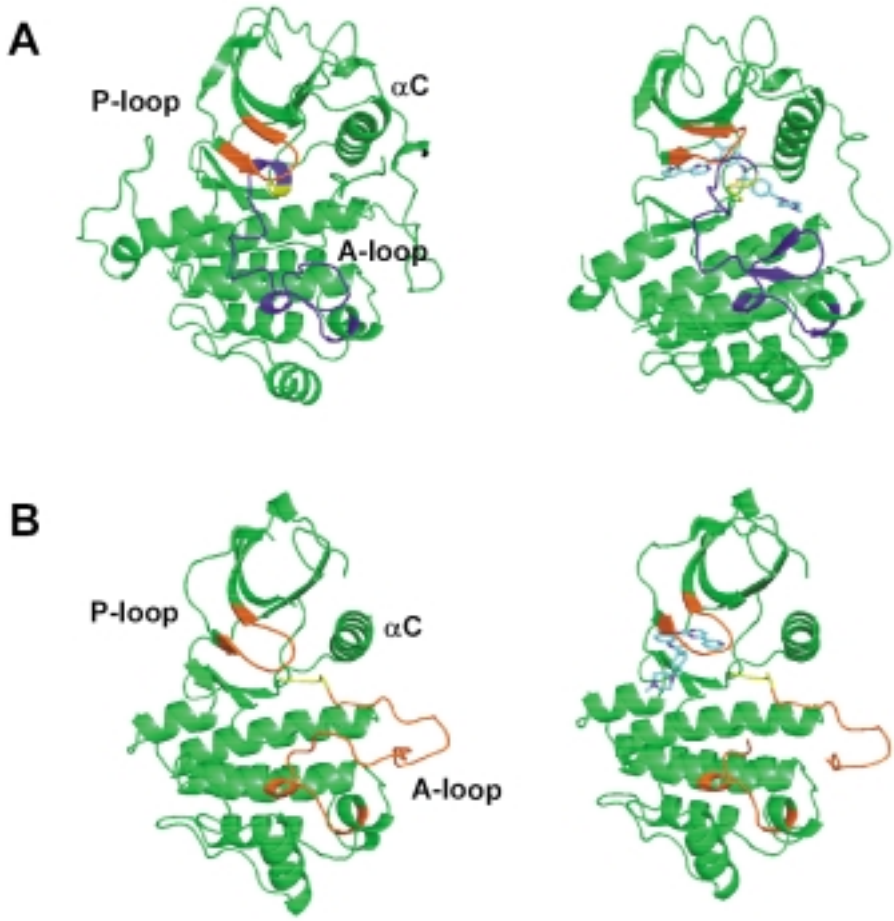


Figure 4. The conformation targeted by the inhibitor exists already in its apo form.
A) On the left, the crystal structures of c-Kit tyrosine kinase in its apo form showing a clear "Off-state" inactive conformation stabilized by the juxtamembrane domain (1T45; Mol et al., 2004). On the right, c-Kit in complex with imatinib (1T46; Mol et al., 2004) exhibits the same "Off-state" conformation like its apo structure. Imatinib assumes the same binding mode as in Abl TK.
B) The structure on the left shows the apo form of the spleen tyrosine kinase (SyK) (1XBA; Atwell et al., 2004) that is characterized by the "On-state" conformation. The same conformation is also assumed once ligated with imatinib (right, 1XBB; Atwell et al., 2004). In this case, the binding mode of imatinib differs from the one observed within Abl. For the protein, the same colour code as in Figure 2 has been used. Imatinib is depicted in cyan stick. The same view angle was used for the pictures.

CONCLUSIONS

In conclusion, the story of the CML-Bcr/Abl-imatinib "connection" points out to the need of clearly identified molecular targets, causally related to transformation, if effective anti-neoplastic treatments are to be developed. It also points out the need for increased knowledge on stability of single conformations (targetability of the conformation) as well as new chemical scaffolds for exploiting the targetable sites offered by each tyrosine kinase. Therefore, Bcr-Abl stands as a model oncogenic TK for the development of targeted treatments in other diseases. It will remain to be seen, if such a model will be followed by the major drug developers.

ACKNOWLEDGMENTS

The authors gratefully acknowledge Dr. S. Ahmed and Mr. A. Bortolato and A. Cristiani (University of Geneva) for editorial assistance and fruitful discussions. The authors are thankful for the support provided by protein kinase research project (ProKinase European Contract, PF6 503467).

REFERENCES

Adrian FJ, Ding Q, Sim T, Velentza A, Sloan C, Liu Y, Zhang G, Hur W, Ding S, Manley P, Mestan J et al.: Allosteric inhibitors of Bcr-abl-dependent cell proliferation. Nat Chem Biol 2: 95-102, 2006

Apperley JF, Gardembas M, Melo JV, Russell-Jones R, Bain BJ, Baxter EJ, Chase A, Chessells JM, Colombat M, Dearden CE, Dimitrijevic S et al.: Response to imatinib mesylate in patients with chronic myeloproliferative diseases with rearrangements of the platelet-derived growth factor receptor beta. N Engl J Med 347: 481-487, 2002

Atwell S, Adams JM, Badger J, Buchanan MD, Feil IK, Froning KJ, Gao X, Hendle J, Keegan K, Leon BC, Muller-Dieckmann HJ et al.: A novel mode of Gleevec binding is revealed by the structure of spleen tyrosine kinase. J Biol Chem 279: 55827-55832, 2004

Ben-Neriah Y, Daley GQ, Mes-Masson AM, Witte ON, Baltimore D: The chronic myelogenous leukemia-specific P210 protein is the product of the bcr/abl hybrid gene. Science 233: 212-214, 1986

Blume-Jensen P, Hunter T: Oncogenic kinase signalling. Nature 411: 355-365, 2001

Bosshard HR: Molecular recognition by induced fit: how fit is the concept? News Physiol Sci 16: 171-173, 2001

Buchdunger E, Zimmermann J, Mett H, Meyer T, Muller M, Druker BJ, Lydon NB: Inhibition of the Abl protein-tyrosine kinase in vitro and in vivo by a 2-phenylaminopyrimidine derivative. Cancer Res 56: 100-104, 1996

Cortes J, O'Brien S, Kantarjian H: Discontinuation of imatinib therapy after achieving a molecular response. Blood 104: 2204-2205, 2004

Deininger MW, Goldman JM, Lydon N, Melo JV: The tyrosine kinase inhibitor CGP57148B selectively inhibits the growth of BCR-ABL-positive cells. Blood 90: 3691-3698, 1997

Druker BJ, Tamura S, Buchdunger E, Ohno S, Segal GM, Fanning S, Zimmermann J, Lydon NB: Effects of a selective inhibitor of the Abl tyrosine kinase on the growth of Bcr-Abl positive cells. Nat Med 2: 561-566, 1996

Gambacorti-Passerini C, le Coutre P, Mologni L, Fanelli M, Bertazzoli C, Marchesi E, Di Nicola M, Biondi A, Corneo GM, Belotti D, Pogliani E, et al.: Inhibition of the ABL kinase activity blocks the proliferation of BCR/ABL+ leukemic cells and induces apoptosis. Blood Cells Mol Dis 23: 380-394, 1997

Gambacorti-Passerini CB, Gunby RH, Piazza R, Galietta A, Rostagno R, Scapozza L: Molecular mechanisms of resistance to imatinib in Philadelphia-chromosome-positive leukaemias. Lancet Oncol 4: 75-85, 2003

Gambacorti-Passerini C: Oncogenic tyrosine kinases. Cell Mol Life Sci 61: 2895-2896, 2004

Gambacorti C, Talpaz M, Sawyers CL, Druker BJ, Hochhaus A, Schiffer CA, Niederwieser DW, Stone RM, Goldman JM, Mone M et al.: Follow-up results of a phase II trial in patients with Late Chronic Phase (L-CP) Chronic Myeloid Leukemia (CML) treated with Imatinib who are refractory/intolerant of Interferon-{alpha}. ASH Annual Meeting Abstracts 106: 1089, 2005

Gambacorti-Passerini C, Gasser M, Ahmed S, Assouline S, Scapozza L: Abl inhibitor BMS354825 binding mode in Abelson kinase revealed by molecular docking studies. Leukemia 19: 1267-1269, 2005

Golas JM, Arndt K, Etienne C, Lucas J, Nardin D, Gibbons J, Frost P, Ye F, Boschelli DH, Boschelli F: SKI-606, a 4-anilino-3-quinolinecarbonitrile dual inhibitor of Src and Abl kinases, is a potent antiproliferative agent against chronic myelogenous leukemia cells in culture and causes regression of K562 xenografts in nude mice. Cancer Res 63: 375-381, 2003

Goldman JM, Druker BJ: Chronic myeloid leukemia: current treatment options. Blood 98: 2039-2042, 2001

Goldman JM, Melo JV: Chronic myeloid leukemia--advances in biology and new approaches to treatment. N Engl J Med 349: 1451-1464, 2003

Goldman JM, Hughes T, Radich J: Continuing reduction in level of residual disease after 4 years in patients with CML in chronic phase responding to first-Line Imatinib (IM) in the IRIS study. ASH Annual Meeting Abstracts 106: 163, 2005

Groffen J, Stephenson JR, Heisterkamp N, de Klein A, Bartram CR, Grosveld G: Philadelphia chromosomal breakpoints are clustered within a limited region, bcr, on chromosome 22. Cell 36: 93-99, 1984

Gumireddy K, Baker SJ, Cosenza SC, John P, Kang AD, Robell KA, Reddy MV, Reddy EP: A non-ATP-competitive inhibitor of BCR-ABL overrides imatinib resistance. Proc Natl Acad Sci USA 102: 1992-1997, 2005

Heinrich MC, Blanke CD, Druker BJ, Corless CL: Inhibition of KIT tyrosine kinase activity: a novel molecular approach to the treatment of KIT-positive malignancies. J Clin Oncol 20: 1692-1703, 2002

Heisterkamp N, Stam K, Groffen J, de Klein A, Grosveld G: Structural organization of the bcr gene and its role in the Ph' translocation. Nature 315: 758-761, 1985

Hubbard, SR: Crystal structure of the activated insulin receptor tyrosine kinase in complex with peptide substrate and ATP analog. Embo J 16: 5572-5581, 1997

Hubbard SR, Wei L, Ellis L, Hendrickson WA: Crystal structure of the tyrosine kinase domain of the human insulin receptor. Nature 372: 746-754, 1994

Hughes TP, Kaeda J, Branford S, Rudzki Z, Hochhaus A, Hensley ML, Gathmann I, Bolton AE, van Hoomissen IC, Goldman JM, Radich JP: Frequency of major molecular responses to imatinib or interferon alfa plus cytarabine in newly diagnosed chronic myeloid leukemia. N Engl J Med 349: 1423-1432, 2003

Huse M, Kuriyan J: The conformational plasticity of protein kinases. Cell 109: 275-282, 2002

Joensuu H, Roberts PJ, Sarlomo-Rikala M, Andersson LC, Tervahartiala P, Tuveson D, Silberman S, Capdeville R, Dimitrijevic S, Druker B, Demetri GD: Effect of the tyrosine kinase inhibitor STI571 in a patient with a metastatic gastrointestinal stromal tumor. N Engl J Med 344: 1052-1056, 2001

Kantarjian H, Sawyers C, Hochhaus A, Guilhot F, Schiffer C, Gambacorti-Passerini C, Niederwieser D, Resta D, Capdeville R, Zoellner U, Talpaz M et al.: Hematologic and cytogenetic responses to imatinib mesylate in chronic myelogenous leukemia. N Engl J Med 346: 645-652, 2002

le Coutre P, Mologni L, Cleris L, Marchesi E, Buchdunger E, Giardini R, Formelli F, Gambacorti-Passerini C: In vivo eradication of human BCR/ABL-positive leukemia cells with an ABL kinase inhibitor. J Natl Cancer Inst 91: 163-168, 1999

Magnusson MK, Meade KE, Nakamura R, Barrett J, Dunbar CE: Activity of STI571 in chronic myelomonocytic leukemia with a platelet-derived growth factor beta receptor fusion oncogene. Blood 100: 1088-1091, 2002

Manning G, Whyte DB, Martinez R, Hunter T, Sudarsanaman S: The protein kinase complement of the human genome. Science 298: 1912-1934, 2002

Melo JV: The diversity of BCR-ABL fusion proteins and their relationship to leukemia phenotype. Blood 88: 2375-2384, 1996

Merante S, Orlandi E, Bernasconi P, Calatroni S, Boni M, Lazzarino M: Outcome of four patients with chronic myeloid leukemia after imatinib mesylate discontinuation. Haematologica 90: 979-981, 2005

Mol CD, Dougan DR, Schneider TR, Skene RJ, Kraus ML, Scheibe DN, Snell GP, Zou H, Sang BC, Wilson KP: Structural basis for the autoinhibition and STI-571 inhibition of c-Kit tyrosine kinase. J Biol Chem 279: 31655-31663, 2004

Moretti L, Tchernin L, Scapozza L: Tyrosine kinase drug discovery: what can be learned from solved crystal structures? ARKOC, in press, 2006

Nagar B, Bornmann WG, Pellicena P, Schindler T, Veach DR, Miller WT, Clarkson B, Kuriyan J: Crystal structures of the kinase domain of c-Abl in complex with the small molecule inhibitors PD173955 and imatinib (STI-571). Cancer Res 62: 4236-4243, 2002

Noble ME, Endicott JA, Johnson LN: Protein kinase inhibitors: insights into drug design from structure. Science 303: 1800-1805, 2004

Nowell PC, Hungerford DA: A minute chromosome in human chronic granulocitic leukemia. Science 32: 1497-1501, 1960

O'Brien SG, Guilhot F, Larson RA, Gathmann I, Baccarani M, Cervantes F, Cornelissen JJ, Fischer T, Hochhaus A, Hughes T, Lechner K et al.: Imatinib compared with interferon and low-dose cytarabine for newly diagnosed chronic-phase chronic myeloid leukemia. N Engl J Med 348: 994-1004, 2003

Ottmann OG, Druker BJ, Sawyers CL, Goldman JM, Reiffers J, Silver RT, Tura S, Fischer T, Deininger MW, Schiffer CA, Baccarani M, et al.: A phase 2 study of imatinib in patients with relapsed or refractory Philadelphia chromosome-positive acute lymphoid leukemias. Blood 100: 1965-1971, 2002

Rousselot P, Huguet F, Cayuela JM: Imatinib mesylate discontinuation in patients with chronic myelogenous leukaemia in complete molecular remission for more than two years. ASH Annual Meeting Abstracts 106: 1101, 2005

Rowley JD: Letter: A new consistent chromosomal abnormality in chronic myelogenous leukaemia identified by quinacrine fluorescence and Giemsa staining. Nature 243: 290-293, 1973

Saglio G, Pane F, Martinelli G, Guerrasio A: BCR/ABL transcripts and leukemia phenotype: an unsolved puzzle. Leuk Lymphoma 26: 281-286, 1997

Sawyers CL, Hochhaus A, Feldman E, Goldman JM, Miller CB, Ottmann OG, Schiffer CA, Talpaz M, Guilhot F, Deininger MW, Fischer T et al.: Imatinib induces

hematologic and cytogenetic responses in patients with chronic myelogenous leukemia in myeloid blast crisis: results of a phase II study. Blood 99: 3530-3539, 2002

Schindler T, Sicheri F, Pico A, Gazit A, Levitzki A, Kuriyan J: Crystal structure of Hck in complex with a Src family-selective tyrosine kinase inhibitor. Mol Cell 3: 639-648, 1999

Shah NP, Tran C, Lee FY, Chen P, Norris D, Sawyers CL: Overriding imatinib resistance with a novel ABL kinase inhibitor. Science 305: 399-401, 2004

Talpaz M, Silver RT, Druker BJ, Goldman JM, Gambacorti-Passerini C, Guilhot F, Schiffer CA, Fischer T, Deininger MW, Lennard AL, Hochhaus A et al.: Imatinib induces durable hematologic and cytogenetic responses in patients with accelerated phase chronic myeloid leukemia: results of a phase 2 study. Blood 99: 1928-1937, 2002

Thaimattam R, Daga PR, Banerjee R, Iqbal J: 3D-QSAR studies on c-Src kinase inhibitors and docking analyses of a potent dual kinase inhibitor of c-Src and c-Abl kinases. Bioorg Med Chem 13: 4704-4712, 2005

van Oosterom AT, Judson I, Verweij J, Stroobants S, Donato di Paola E, Dimitrijevic S, Martens M, Webb A, Sciot R, Van Glabbeke M, Silberman S et al.: Safety and efficacy of imatinib (STI571) in metastatic gastrointestinal stromal tumours: a phase I study. Lancet 358: 1421-1423, 2001

Weisberg E, Manley PW, Breitenstein W, Bruggen J, Cowan-Jacob SW, Ray A, Huntly B, Fabbro D, Fendrich G, Hall-Meyers E, Kung AL et al.: Characterization of AMN107, a selective inhibitor of native and mutant Bcr-Abl. Cancer Cell 7: 129-141, 2005

Young MA, Shah NP, Chao LH, Seeliger M, Milanov ZV, Biggs WH 3rd, Treiber DK, Patel HK, Zarrinkar PP, Lockhart DJ, Sawyers CL et al.: Structure of the kinase domain of an imatinib-resistant Abl mutant in complex with the Aurora kinase inhibitor VX-680. Cancer Res 66: 1007-1014, 2006

Protein Kinases as Therapeutic Targets in Leukaemia

➤ **Keqing Wang**
➤ **Peter Hampson**
➤ **Janet M Lord**

MRC Centre for Immune Regulation, Birmingham University Medical School

Correspondence to:
➤ **J M Lord**
Professor
MRC Centre for Immune Regulation
Birmingham University Medical School
Birmingham B15 2TT, UK

Tel: +44 121 414 4399
fax: +44 121 414 3599
email: J.M.Lord@bham.ac.uk

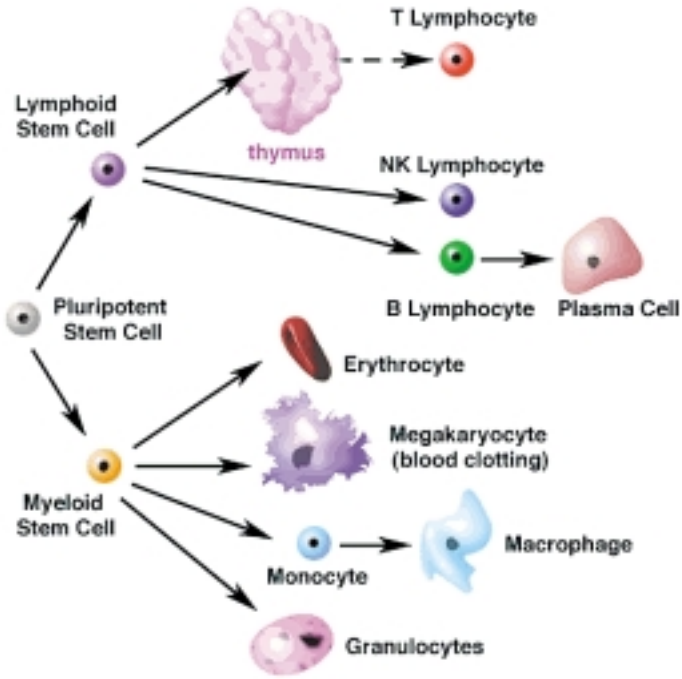


Figure 1. Haemopoiesis
Blood cells are derived in the bone marrow from a common precursor stem cell. Progenitor cells commit early on to either the myeloid on lymphoid lineages. Lymphoid progenitors differentiate to produce T and B-lymphocytes. Myeloid progenitors differentiate to produce leukocytes, erythrocytes and megakaryocytes. (Figure kindly provided by Dr C M Bunce).

WHAT IS LEUKAEMIA?

All blood cells develop in the bone marrow from a common precursor, the pluripotent haemopoietic stem cell (HSC), via various progenitor cell stages (**Figure 1**). As the HSC differentiates towards a mature blood cell, proliferative capacity is lost and the phenotype of the mature cell is acquired. This process of haemopoiesis is tightly regulated by soluble and stromal cell associated factors, e.g. SCF, GM-CSF, IL3, which control the proliferation, differentiation and survival of the progenitor cells and mature blood cells (Dexter & Spooncer, 1987). Haemopoiesis is also highly flexible and can respond to changing needs very rapidly. For example at times of microbial infection, the output of neutrophils can increase three fold under the influence of factors such as GM-CSF, and under conditions of low oxygen tension the release of erythropoietin stimulates increased production of erythrocytes. Homeostasis is eventually restored, once the need for extra blood cells has passed, by the removal of excess cells by apoptosis.

Leukaemia is derived from the Greek word meaning literally "white blood" and refers to cancers of the haemopoietic system. In leukaemia the normal balance between proliferation, differentiation and apoptosis of blood cells and their progenitors in the blood and bone marrow is distorted, leading to an abnormal accumulation of immature precursors. In the lymphoid lineage cells can undergo a further round of expansion and differentiation in the secondary lymphoid organs in response to antigen challenge, and this can give rise to a distinct class of diseases called the lymphomas, which for the sake of brevity will not be considered here. The presence of large numbers of abnormal progenitor cells in the bone marrow causes a suppression of the growth and development of normal progenitor cells and, as a consequence, symptoms of leukaemia include neutropenia, thrombocytopenia and anaemia. The leukaemias are classified according to the lineage affected and the rate of progression of the diseases. The four main types are thus: Acute Lymphoblastic Leukaemia (ALL), Acute Myeloid Leukaemia (AML), Chronic Lymphocytic Leukaemia (CLL) and Chronic Myeloid Leukaemia (CML). In general, the disease course is quite indolent for the chronic leukaemias and patients survive for many years with the disease, whilst in the acute leukaemias disease onset and progression is rapid. The leukaemias can be subdivided further (for example there are 8 AML sub-types), dependent upon the lineage involved and/or the stage of differentiation in which the cells are arrested, but a more detailed description of the leukaemias and their classification is beyond the scope of this article.

There are approximately 6,000 new cases of leukaemia per year in the UK, of which almost half are CLL and one third are AML (**Table 1**). In general, leukaemia is a disease of the elderly, though ALL occurs predominantly in children. Whilst ALL is almost entirely curable (80–85% remission rate in children), the prognosis for adult leukaemia is poorer, due in part to the need for

Table 1. Annual incidence of new cases of leukaemia and lymphoma in the UK.

Acute lymphoblastic leukaemia	**650**
Acute myeloid leukaemia	**2,000**
Chronic lymphocytic leukaemia	**2,750**
Chronic myeloid leukaemia	**750**
Hodgkin's lymphoma	**1,400**
Non-Hodgkin's lymphoma	**8,450**
Myeloma	**3,300**
Myelodysplastic syndromes	**3,250**
Myeloproliferative disorders	**1,900**
Aplastic anaemia	**130**
Total (rounded)	**24,500**

aggressive chemotherapy regimes which are not well tolerated in the elderly (Lowenberg et al.,1999; Schoch et al., 2004). There is thus a pressing need for new therapies for leukaemia, especially as combination therapies that might synergise with existing chemotherapies to enhance efficacy in the older patients. The predominant therapies for the treatment of leukaemia aim to either overcome the differentiation block or to induce growth arrest and apoptosis in the leukaemic cells. The most successful differentiation therapy is the treatment of one type of AML (M3 sub-type, Acute Promyeloid Leukaemia) with all trans retinoic acid (ATRA). AML-M3 is associated with a chromosomal translocation (t15:17) resulting in the expression of the fusion protein PML-RARα, which is more resistant to the differentiating effects of retinoic acid than the normal retinoic acid receptor RARα. The insensitivity of the leukaemic cells to endogenous retinoic acid can be overcome by treatment of the patients with ATRA. For induction of growth arrest and apoptosis, a variety of chemotherapeutic agents are used. Classically this involves giving cytarabine (a deoxycytidine analog) by continuous infusion for 7 days and an anthracycline antibiotic such as daunorubicin or idarubicin, i.v. for 3 days. Postremission therapy is usually then in the form of high-dose chemotherapy, or chemo-radiotherapy with either allogeneic or autologous hematopoeitic stem cell transplantation (Yates et al., 1982; Dillman et al., 1991).

However, most of these chemotherapeutic agents are non-specific and damage normal healthy cells as well as malignant cells and, thus, more targeted "smart" therapies are now the aim for the pharmaceutical industry. In this respect the success of Imatinib, a small molecule kinase inhibitor, which targets a fusion protein BCR-ABL present in CML, has provided proof that understanding the biology of leukaemia can lead to the development of targeted and effective drugs. In this respect, genetic alterations to haemopoietic transcription factors may be key factors in leukaemogenesis (Look, 1997), but it is the aberrant activity of signalling pathways regulating cell proliferation and apoptosis that may be most amenable to therapeutic modulation (Martinelli et al., 2005; Lydon & Druker, 2004). Thus, in addition to the production of overactive tyrosine kinases by chromosomal mutations, exemplified by BCR-ABL, it has also become clear that targeting serine/threonine protein kinases with a known role in proliferation and/or apoptosis is also a rational strategy. Both of these options will be discussed here.

TYROSINE PROTEIN KINASES AND LEUKAEMIA

Tyrosine kinases play an essential role in the signalling pathways involved in the control of cellular proliferation. The number of small molecule protein kinase inhibitors entering clinical development is increasing following the success of imatinib (9,10) and the majority of these are targeted at tyrosine kinases that are overactive in different leukaemias and lymphomas. In many cases the aberrant activity has arisen as the result of a chromosomal translocation that has resulted in the expression of a fusion protein with enhanced tyrosine kinase activity. Examples of this genetic alteration are BCR-ABL present in CML (11) and the NPM-ALK protein found in Anaplastic Large Cell Lymphoma (12). Small molecule inhibitors of NPM-ALK are also now in pre-clinical development and are discussed elsewhere in this issue.

BCR-ABL and CML: The Philadelphia chromosome occurs in 95% of CML patients and arises from a reciprocal translocation of chromosomes 9 and 22. This results in the fusion oncogene *BCR-ABL* whose protein product shows enhanced tyrosine kinase activity (Ben Neriah et al., 1986). Whilst most CML patients express a 210 kDa splice variant of BCR-ABL, a 190 kDa BCR-ABL protein is also expressed in ALL and is associated with a much more aggressive disease (Ren, 2005; Faderl et al., 2002). The fact that BCR-ABL translocation occurs in 95% of patients, and is sufficient to cause the initial chronic stage of the disease, made it an attractive target for drug design. STI-571 (Glivec, Gleevec, Imatinib) - a dual inhibitor of v-Abl and PDGF-R kinases, is a 2-phenylaminopyrimidine identified by high throughput screening of compound libraries and optimisation of active compounds based on the inhibition of protein kinases *in vitro*. Subsequent preclinical tests showed that STI-571 could suppress the proliferation of BCR-ABL

expressing cells both *in vitro* and *in vivo* (Druker et al., 1996). In colony-forming assays of peripheral blood or bone marrow from patients with CML, there was a 92–98% decrease in the number of bcr-abl colonies formed, but no inhibition of normal colony formation. The drug has consequently undergone clinical trials in CML and has been highly effective, with a majority of patients achieving a sustainable haematological remission (Druker et al., 2001; Peng et al., 2004). Unfortunately, Imatinib has proved less useful in patients who have progressed to the terminal blast phase of CML. This is frequently due to the emergence and expansion of clones of cells that express mutated forms of BCR-ABL which show decreased sensitivity to imatinib (Cowan-Jacob et al., 2004), and this has lead to attempts to design second generation compounds targeting the BCR-ABL mutants. Much effort has focussed upon compounds able to bind to the active conformation of the ABL kinase domain. The rationale here is that as imatinib binds the inactive conformation then resistance BCR-ABL mutants are less likely to arise with a drug targeting the active kinase, since any mutations would have to be able to reduce binding of the inhibitor to the active site without affecting basic kinase activity, i.e. ATP-binding or phosphate transfer. However, while compounds able to target the active conformation of ABL have been developed, such as the pyridopyrimidines (Lombardo et al., 2004), these lack the degree of selectivity to be achieved with compounds that target the inactive conformation. Second generation compounds targeting BCR-ABL are therefore still predominantly those that bind the inactive conformation. AMN107 is in clinical development and is 20–30 times more potent than imatinib. Despite being of the same class of compounds as imatinib, it has been shown to be effective against the most prevalent BCR-ABL mutants, with the exception of the T3151 mutation (reviewed in Manley et al., 2005).

A second approach is to develop dual specificity agents that target BCR-ABL and other tyrosine kinases that contribute to the transformed phenotype of the imatinib resistant cells. Src family kinases include nine structurally-related tyrosine kinases, many of which are expressed in haemopoietic cells and regulate multiple cellular functions including cell migration, proliferation, differentiation and survival. They appear to contribute to Bcr-Abl mediated leukemogenesis in that they lie downstream in BCR-ABL signalling cascade (Dai et al., 2004; Donato et al., 2003) and inhibition of their activity may thus provide synergy with BCR-ABL inhibition to overcome imatinib resistance. Several Src and ABL dual inhibitors have been developed and are in preclinical or early clinical studies, the most advanced compounds being BMS-354825, AZD05340 and SKI-606 (Martinelli et al., 2005). BMS-354825 is in phase II clinical trial in imatinib resistant CML patients.

Flt3 and AML: In addition to reciprocal chromosomal translocations, other mutations have also been described that have resulted in over active tyrosine kinases. Probably the best studied with respect to leukaemia are the mutations to the FMS like tyrosine kinase 3, Flt3, gene.The Flt-3 gene encodes a receptor tyrosine kinase containing two intracellular tyrosine kinase domains which plays important role in proliferation, differentiation and survival of normal haemopoietic cells (Gilliland & Griffin, 2002). Flt-3 is expressed by early myeloid and lymphoid progenitors as well as on malignant haemopoietic cells. Human Flt-3 ligand occurs mainly as a type 1 transmembrane protein that can be cleaved proteolytically to release soluble Flt3L. It is expressed by most tissues, including haemopoietic tissues and bone marrow fibroblasts, as well as on many transformed hematopoietic cell lines (Rosnet et al., 1996; Lisovsky et al., 1996). In contrast, Flt3 is found mainly in early haemopoietic progenitor cells and activation of Flt-3 by its ligand promotes the growth of early progenitor cells *in vitro* and *in vivo* via activation of the PI3 kinase and RAS signaling pathways (reviewed in Stirewalt & Radich, 2003). Flt-3 mutations occur in approximately 35% of AML patients and are associated with a poor prognosis. Mutations are either internal tandem duplications or mis-sense point mutations. Internal tandem duplications were first reported in the juxtamembrane domain of Flt-3 in AML by Nakao and colleagues in 1996, who suggested that these mutations may play an important role in the pathogenesis of AML (Nakao et al., 1996). They analysed the flt3 gene in 30 patients with acute myeloid leukemia (AML) and 50 with acute lymphoblastic leukemia (ALL) and

found five patients with extended flt3 transcripts, which essentially included a duplication of the juxtamembrane tyrosine kinase domain. Two different mis-sense point mutations occur in the tyrosine kinase domain of Flt-3, resulting in the constitutive activation of the receptor and downstream signalling pathways such as RAS (Stirewalt & Radich, 2003). Given the high frequency of activating *Flt-3* mutations in patients with AML, Flt-3 represents an attractive target for selective therapeutic inhibition.

Four Flt-3 inhibitors are currently in clinical trials, namely SU11248, PKC-412, MLN518 and CEP-701 (Stirewalt & Radich, 2003). Results to date have been modest, but have shown promise with transient reduction in peripheral blood leukaemic blasts, but with only occasional reduction in bone marrow blasts and a very low incidence of haematological responses (Stirewalt & Radich, 2003; Stone et al., 2002). However, this may reflect the fact that AML is an aggressive and heterogenous disease and unlike BCR-ABL, Flt-3 mutations alone are not enough to cause AML. It is therefore more likely that drugs targeting Flt-3 will show better efficacy in combinatorial therapy.

Jak kinases and leukaemia

Jaks are a family of four tyrosine kinases (Jak1, Jak2, Jak3, Tyk2). Jak2 has been associated with leukaemia, since a chromosomal translocation forming the TEL-JAK2 fusion protein that results in constitutive kinase activity was detected in B- and T-lineage ALL (Lacronique et al., 1997; Peeters et al., 1997). An activating Jak2 mutation (JAK2V617F) has also been closely linked to BCR/ABL negative myeloproliferative disorders (Tefferi & Gilliland, 2005). More recently, a BCR-Jak2 fusion gene was observed in an imatinib resistant CML -like patient (Faderl et al., 2005). These findings indicate that Jak tyrosine kinases could be promising targets for leukaemic therapies. In this context, WP-1034, a novel inhibitor of Jak signaling, is a potent inhibitor of AML cell proliferation (Meydan et al., 1996) and systemic administration of AG-490, another selective Jak kinase inhibitor, in a xenograft animal model of AML lead to complete tumor regression (Steelman et al., 2004).

SERINE/THREONINE PROTEIN KINASES AND LEUKAEMIA

The pivotal role of serine/threonine protein kinases in the regulation of cell proliferation and apoptosis would suggest that they had considerable appeal as pharmacological targets across a broad spectrum of pathologies including cancer. However there has been long-standing skepticism with regard to the targeting the serine/threonine protein kinases. This relates primarily to concerns over the possibility of developing compounds with sufficient specificity among this class kinases and also the wisdom of targeting a single kinase to treat a disease in which several signaling pathways and cellular processes are altered. Despite these concerns and following the success of imatinib, several agents targeting serine threonine kinases have reached clinical phase testing. The current status of attempts to target serine/threonine kinases, is reviewed briefly here.

Protein Kinase C and leukaemia

Protein Kinase C (PKC) is a family of at least 12 isoenzymes and there is reasonable evidence that these play differential roles within the cell, including regulation of proliferation, differentiation and apoptosis (Lord & Pongracz, 1995). They therefore represent logical therapeutic targets and indeed PKC has received the most attention to date with regard to therapeutic modulation, not only for cancers but also for diabetes. Although there are currently few modulators of PKC that show isoenzyme selectivity, the gradual publication of the crystal structures of the regulatory domains of key PKC isoenzymes, should lead rapidly to the synthesis of such compounds. With respect to anti-cancer therapies, it is PKC-α and PKC-δ which appear to represent logical targets, as both have been implicated in the regulation of apoptosis, and there are now several reports of increased or decreased activity of these kinases in association with haematological malignancies. In CLL, although cells do not have increased PKC activity, inhibition of PKC induces CLL cell apoptosis (Barragan et al., 2002; Kitada et al., 2000). More recently, it has been shown that expression of

dominant-negative PKC-α in normal haemopoietic progenitor cells resulted cells that phenotypically resembled human CLL cells (Nakagawa et al., 2006), suggesting a role for this isoenzyme in tumour suppression. Our own recent studies have shown that primary CLL cells express PKC-α and that compounds which selectively activate this isoenzyme can induce apoptosis in these cells (K. Wang, unpublished observations). Possible downstream targets of PKC-α include bcl-2, with some authors reporting that phosphorylation of bcl-2 leads to reduced functioning of this key anti-apoptotic protein (Yamamoto et al., 1999). However, caution is required as other groups have suggested that PKC-α mediated bcl-2 phosphorylation leads to enhanced anti-apoptotic activity. Work from Deng et al. (2001) showed that Bcl2 phosphorylation by ERK 1/2 and PKC α kinases, either at Ser70 residue or at multiple Thr69, Ser70, and Ser89 sites, positively regulated Bcl2 antiapoptotic function. However, other investigators have shown that c-Jun NH2-terminal kinase-mediated multiple-site Bcl2 phosphorylation hinders Bcl2 survival function in paclitaxel-induced apoptosis (Yamamoto et al., 1999).

PKC-δ has also been proposed as an anti-cancer target. The majority of studies have shown that this kinase has significant pro-apoptotic functions (Lord & Pongracz, 1995; Emoto et al., 1996). Our own studies have shown that a plant derived broad range PKC activator, 3 ingenyl angelate (PEP005), is a potent anti-leukaemic agent and that its effects are mediated via PKC-δ (Hampson et al., 2005). PEP005 induced apoptosis in human primary AML cells in the low nanomolar range, with no effects on normal myeloblasts up to two log concentrations higher. PEP005 is already in phase II clinical trial for non-melanoma skin cancer and is due to enter phase I clinical trials for AML late in 2006 (Hampson et al., 2006). Interestingly, PKC-δ has also been implicated in the development of CLL, but in this case PKC-δ was constitutively activated downstream of PI-3-kinase (Ringshausen et al., 2002). Small molecule inhibitors of either PI-3-kinase (Ly294002) or PKC-δ (Rottlerin) were potent inducers of apoptosis in CLL cells in vitro (Ringshausen et al., 2002; Ringshausen et al., 2006). These data emphasise the need to define the biology of the individual leukaemias and that a protein kinase modulator effective in one leukaemia will not necessarily be effective in all leukaemias.

In addition to PEP005, several broad range PKC activators and inhibitors have reached clinical trial stage. 12-O-Tetradecanoylphorbol-13-acetate (TPA) entered phase I clinical trial for AML in the US (Strair et al., 2002) and Bryostatin 1 (NSC 339555), a macrocyclic lactone PKC activator has been tested in phase I clinical trials for refractory acute leukaemia (Cragg et al., 2002). PKC inhibitors UCN-01 and PKC412 have also been tested in phase I and II clinical trials for B-CLL (Byrd et al., 2001; Virchis et al., 2002).

The Ras-Raf-Mek-Erk pathway and leukaemia

The Mitogen Activated Protein Kinases (MAPKs) are a family of evolutionarily conserved, ubiquitously expressed serine/threonine kinases that play an important role in the signal transduction involved in a number of cellular processes including proliferation, differentiation and apoptosis. Mammalian cells express at least three distinctly regulated groups of MAPKs. These are, the extracellular signal-related kinases (ERK), consisting of ERK1 and ERK2 (p42, p44), the Jun amino-terminal kinases (JNK), consisting of JNK1/2/3, and the p38 proteins consisting of p38α/β/δ. MAPKs are activated in a complex MAPK signalling cascade, the core of which is an evolutionarily conserved unit of three protein kinases activated in sequence (reviewed in Chang & Karin, 2001). The activation of the ERK pathway is regulated via a signalling cascade initiated at the level of the small GTP-binding protein Ras. This cascade involves the sequential activation of Raf, Mek1/2 and Erk1/2.The fact that the Ras/Raf/MEK/ERK pathway promotes proliferation and is anti-apoptotic, makes it another potential therapeutic target. Importantly, it has been shown that a conditionally active form of MEK1 can cause the autocrine transformation of haemopoietic cells, leading to cytokine independence (Blalock et al., 2000), suggests that this pathway may be involved in leukemogenesis. Moreover, several studies have shown that Mek1/2 and Erk1/2 are constitutively activated in primary human acute myeloid leukaemia cells (Kim et al., 1999; Milella et al., 2001). In addition, mutations in Ras leading to its constitutive activation have been detected in around 20–30% of human ALL and AML (Blalock et al., 2000; Farr et al., 1988).

A variety of approaches have been used to target this pathway therapeutically. For example, farnesylation of Ras is necessary for its membrane localisation and cell transforming activity. Consequently farnesyltransferase inhibitors (FTIs), such as R115777 have been developed to inhibit Ras activity. R115777 has shown promise when administered as a single agent in patients with haematological malignancies such as MDS, CML and AML (Kurzrock et al., 2004; Cortes et al., 2003; Karp et al., 2001). 3 out of 28 MDS patients treated with R115777 responded (2 with complete remission and 1 partial remission). 7 out of 22 patients with CML (6 in chronic phase, 1 in advanced phase) achieved complete or partial hematologic response with R115777 and four of these had a minor cytogenetic response. However, responses were transient, with a median duration of 9 weeks (range, 3–23 weeks). In a phase 1 trial of the same agent for AML, clinical responses occurred in 10 (29%) of the 34 evaluable patients, including 2 complete remissions. Although mutations of Raf have been found in a number of human cancers – mainly melanomas (Davies et al., 2002), we are not aware of such mutations in leukaemias. However, a small molecule inhibitor of Raf, BAY 43-9006, has shown anti-leukemic effects against cell lines in vitro associated with MCl-1 downregulation (Rahmani et al., 2005). MEK mutations are also uncommon, although as stated above, several studies have shown that Mek1/2 and Erk1/2 are constitutively activated in primary human acute myeloid leukaemia cells. Treatment of leukaemia blasts with the MEK inhibitors PD98059 or PD184352 caused growth inhibition and induced apoptosis (Milella et al., 2001). Treatment of blasts with MEK inhibitors can also sensitise them to drug induced cytotoxicity from a variety of established chemotherapeutic agents including Ara-c (Milella et al., 2001), UCN-01 (Dai et al., 2001), ST1571 (Yu et al., 2002) and lovastatin (Wu et al., 2004).

PI-*3 kinase/Akt signaling pathway and leukaemia*

The phosphatidylinositol 3-kinase (PI3K)/Akt signaling pathway plays an important role in the proliferation, differentiation and survival of hematopoietic cells (Steelman et al., 2004). This pathway is negatively regulated by the phosphatases PTEN, SHIP-1 and -2. Mutations and hemizygous deletions of PTEN have been detected in some primary acute leukemias and non-hodgkin's lymphoma, and many hematopoietic cell lines lack or have low PTEN protein expression (Dahia et al., 1999). Recently, it was found that Akt was phosphorylated and activated in the majority of primary AML cells, and that constitutive activation of the PI3K/Akt pathway is necessary for the survival and proliferation of AML cells and proliferation (Min et al., 2003; Kubota et al., 2004). In addition, constitutive phosphorylation of Akt is associated with poor prognosis in AML (Min et al., 2003). PI3 kinase/Akt have also been shown to be involved in arsenic trioxide, imatinib and Ara-C resistance in leukaemia patients (Tabellini et al., 2005; Burchert et al., 2005; Grandage et al., 2005). These findings may provide new therapeutic strategies for leukaemia, although drugs targeting these pathways are still in preclinical development.

SUMMARY

Both tyrosine and serine/threonine protein kinases represent significant and rational targets for anti-leukaemic therapeutics. Significant progress has been made with tyrosine kinase inhibitors, exemplified by the BCR-ABL inhibitor imatinib and its success in the treatment of CML. The current literature suggests that exploitation of novel and more selective agents that activate or inhibit serine threonine kinases may form the next wave of novel anti-leukaemics to progress to clinical usage.

Keqing Wang and Peter Hampson are supported by an FP6 intergrated project award LSHB-CT-2004-503467

REFERENCES

Barragan M et al.: Involvement of protein kinase C and phosphatidylinositol 3-kinase pathways in the survival of B-cell chronic lymphocytic leukemia cells. Blood 99: 2969-2976, 2002

Ben NeriahY et al.: The chronic myelogenous leukemia-specific P210 protein is the product of the bcr/abl hybrid gene. Science 233: 212-214, 1986

Blalock WL et al.: A conditionally-active form of MEK1 results in autocrine transformation of human and mouse hematopoietic cells. Oncogene 19: 526-536, 2000

Burchert A et al.: Compensatory PI3-kinase/Akt/mTor activation regulates imatinib resistance development. Leukemia 19: 1774-1782, 2005

Byrd JC et al.: UCN-01 induces cytotoxicity toward human CLL cells through a p53-independent mechanism. Exp Hematol 29: 703-708, 2001

Chang L, Karin M: Mammalian MAP kinase signalling cascades. Nature 410: 37-40, 2001

Cortes J. et al.: Efficacy of the farnesyl transferase inhibitor R115777 in chronic myeloid leukemia and other hematologic malignancies. Blood 101: 1692-1697, 2003

Cowan-Jacob SW et al.: Bcr-Abl kinase mutations and drug resistance to imatininb (ST1571) in chronic myelogenous leukemia. Mini Rev Med Chem 4: 285-299, 2004

Cragg LH et al.: Phase I trial and correlative laboratory studies of bryostatin 1 (NSC 339555) and high-dose 1-B-D-arabinofuranosylcytosine in patients with refractory acute leukemia. Clin Cancer Res 8: 2123-2133, 2002

Dahia PL et al.: PTEN is inversely correlated with the cell survival factor Akt/PKB and is inactivated via multiple mechanisms in haematological malignancies. Hum Mol Genet 8: 185-193, 1999

Dai Y et al.: Pharmacological inhibitors of the mitogen-activated protein kinase (MAPK) kinase/MAPK cascade interact synergistically with UCN-01 to induce mitochondrial dysfunction and apoptosis in human leukemia cells. Cancer Res 61: 5106-5115, 2001

Dai Y et al.: A Bcr/Abl-independent, Lyn-dependent form of imatinib mesylate (STI-571) resistance is associated with altered expression of Bcl-2. J Biol Chem 279: 34227-34239, 2004

Davies H et al.: Mutations of the b-RAF gene in human cancer. Nature 417: 949-954, 2002

Deng X, Gao F, Mai H, May W.S: Multiple site Bcl2 phosphorylation in the flexible loop domain (FLP) unexpectedly enhances anti-apoptotic function. Blood 98: 796a, 2001

Dexter TM, Spooncer E: Growth and differentiation in the hemopoietic system. Annu Rev Cell Biol 3: 423-441, 1987

Dillman RO et al.: A comparative study of two different doses of cytarabine foracute myeloid leukemia: a phase III trial of Cancer and Leukemia Group B. *Blood* 78: 2520-2526, 1991

Donato NJ et al.: BCR-ABL independence and LYN kinase overexpression in chronic myelogenous leukemia cells selected for resistance to STI571. Blood 101: 690-698, 2003

Druker BJ et al.: Effects of a selective inhibitor of the Abl tyrosine kinase on the growth of Bcr-Abl positive cells. Nat Med 2: 561-566, 1996

Druker BJ Lydon NB: Lessons learned from the development of an abl tyrosine kinase inhibitor for chronic myelogenous leukemia. J Clin Invest 105: 3-7, 2000

Druker BJ et al.: Efficacy and safety of a specific inhibitor of the BCR-ABL tyrosine kinase in chronic myeloid leukemia. N Engl J Med 344: 1031-1037, 2001

Emoto Y et al.: Activation of protein kinase Cdelta in human myeloid leukemia cells treated with 1-beta-D-arabinofuranosylcytosine. Blood 87: 1990-1996, 1996

Faderl S, Garcia-Mancro G, Thomas DA, Kantarjian HM: Philadelphia chromosome positive acute lymphoblastic leukaemia – current concepts and future perspectives. Rev Clin Exp Haematol 6:142-160, 2002

Faderl S et al.: WP-1034, a novel JAK-STAT inhibitor, with proapoptotic and antileukemic activity in acute myeloid leukemia (AML). Anticancer Res 25: 1841-1850, 2005

Farr CJ et al.: Analysis of RAS gene mutations in acute myeloid leukemia by polymerase chain reaction and oligonucleotide probes. Proc Natl Acad Sci USA 85: 1629-1633, 1988

Gilliland DG, Griffin JD: The roles of FLT3 in hematopoiesis and leukemia. Blood 100: 1532-1542, 2002

Grandage VL, Gale RE, Linch DC, Khwaja A: PI3-kinase/Akt is constitutively active in primary acute myeloid leukaemia cells and regulates survival and chemoresistance via NF-kappaB, Mapkinase and p53 pathways. Leukemia 19: 586-594, 2005

Hampson P et al.: PEP005, a selective small-molecule activator of protein kinase C, has potent antileukemic activity mediated via the delta isoform of PKC. Blood 106: 1362-1368, 2005

Hampson P, Wang K, Lord JM: PEP005. *Drugs of the Future*. In press, 2006

Karp JE et al.: Clinical and biologic activity of the farnesyltransferase inhibitor R115777 in adults with refractory and relapsed acute leukemias: a phase 1 clinical-laboratory correlative trial. Blood 97: 3361-3369, 2001

Kim SC et al.: Constitutive activation of extracellular signal-regulated kinase in human acute leukemias: combined role of activation of MEK, hyperexpression of extracellular signal-regulated kinase, and downregulation of a phosphatase, PAC1. Blood 93: 3893-3899, 1999

Kitada S, Zapata JM, Andreeff M, Reed JC: Protein kinase inhibitors flavopiridol and 7-hydroxy-staurosporine down-regulate antiapoptosis proteins in B-cell chronic lymphocytic leukemia. Blood 96: 393-397, 2000

Kubota Y et al.: Constitutive activation of PI3K is involved in the spontaneous proliferation of primary acute myeloid leukemia cells: direct evidence of PI3K activation. Leukemia 18: 1438-1440, 2004

Kurzrock R et al.: Phase II study of R115777, a farnesyl transferase inhibitor, in myelodysplastic syndrome. J Clin Oncol 22: 1287-1292, 2004

Lacronique V et al.: A TEL-JAK2 fusion protein with constitutive kinase activity in human leukemia. Science 278: 1309-1312, 1997

Lisovsky M et al.: Flt3-ligand production by human bone marrow stromal cells. Leukemia 10: 1012-1018, 1996

Lombardo LJ et al.: Discovery of *N*-(2-chloro-6-methylphenyl)-2-(6-(4-(2-hydroxyethyl)-(piperazin-1-yl)-2-methylpyrimidin-4–ylamino)thiazole-5-carboxamide (BMS-354825) a dual Src/Abl kinase inhibitor with potent anti-tumor activity in preclinical assays. J Med Chem 47: 6658-6661, 2004

Look AT: Oncogenic transcription factors in the human acute leukaemias. Science 278: 1059-1064, 1997

Lord JM, Pongracz J: Protein kinase C: a family of isoenzymes with a distinct role in pathogenesis. J Clin Path 48: M57-M64, 1995

Lowenberg B, Downing JR, Burnett A: Acute myeloid leukaemia. N Engl J Med 341: 1051-1062, 1999

Lydon NB, Druker BJ: Lessons learned from the development of imatinib. Leuk Res 28 Suppl 1: S29-S38, 2004

Manley PW, Cowan-Jacob SW, Mestan J: Advances in the structural biology, design and clinical development of Bcr-Abl kinase inhibitors for the treatment on chronic myeloid leukaemia. Biochim Biophys Acta 1754: 3-13, 2005

Martinelli G, Soverini S, Rosti G, Baccarani M: Dual tyrosine kinase inhibitors in chronic myeloid leukemia. Leukemia 19: 1872-1879, 2005

Meydan N et al.: Inhibition of acute lymphoblastic leukaemia by a Jak-2 inhibitor. Nature 379: 645-648, 1996

Milella M et al.: Therapeutic targeting of the MEK/MAPK signal transduction module in acute myeloid leukemia. J Clin Invest 108: 851-859, 2001

Min YH et al.: Constitutive phosphorylation of Akt/PKB protein in acute myeloid leukemia: its significance as a prognostic variable. Leukemia 17: 995-997, 2003

Nakagawa R, Soh JW, Michie AM: Subversion of protein kinase C alpha signaling in hematopoietic progenitor cells results in the generation of a B-cell chronic lymphocytic leukemia-like population in vivo. Cancer Res 66: 527-534, 2006

Nakao M et al.: Internal tandem duplication of the flt3 gene found in acute myeloid leukemia. Leukemia 10: 1911-1918, 1996

Peeters P et al.: Fusion of TEL, the ETS-variant gene 6 (ETV6), to the receptor-associated kinase JAK2 as a result of t(9;12) in a lymphoid and t(9;15;12) in a myeloid leukemia. Blood 90: 2535-2540, 1997

Peng B et al.: Pharmacokinetics and pharmacodynamics of imatinib in a phase I trial with chronic myeloid leukemia patients. J Clin Oncol 22: 935-942, 2004

Rahmani M et al.: Apoptosis induced by the kinase inhibitor BAY 43-9006 in human leukemia cells involves down-regulation of Mcl-1 through inhibition of translation. J Biol Chem 280: 35217-35227, 2005

Ren R: Mechanisms of BCR-ABL in the pathogenesis of chronic myelogenous leukaemia. Nat Rev Cancer 5: 172-183, 2005

Ringshausen I et al.: Constitutively activated phosphatidylinositol-3 kinase (PI-3K) is involved in the defect of apoptosis in B-CLL: association with protein kinase C delta. Blood 100: 3741-3748, 2002

Ringshausen I et al.: Mechanisms of apoptosis-induction by rottlerin: therapeutic imploications for B-CLL. Leukemia 20: 514-520, 2006

Rosnet O et al.: Human FLT3/FLK2 receptor tyrosine kinase is expressed at the surface of normal and malignant hematopoietic cells. Leukemia 10: 238-248, 1996

Schoch C et al.: The influence of age on prognosis of de novo acute myeloid leukemia differs according to cytogenetic subgroups. Haematologica 89: 1082-1090, 2004

Steelman LS et al.: JAK/STAT, Raf/MEK/ERK, PI3K/Akt and BCR-ABL in cell cycle progression and leukemogenesis. Leukemia18: 189-218, 2004

Stirewalt DL, Radich JP: The role of FLT3 in haematopoietic malignancies. Nat Rev Cancer 3: 650-665, 2003

Stone RM et al.: PKC412, an oral FLT3 inhibitor has activity in mutant FLT3 acute myeloid leukemia (AML): a phase II clinical trial. Blood 100: 316a, 2002

Strair RK et al.: Administration of a phorbol ester to patients with hematological malignancies: preliminary results from a phase I clinical trial of 12-O-tetradecanoylphorbol-13-acetate. Clin Cancer Res 8: 2512-2518, 2002

Tabellini G et al.: Phosphoinositide 3-kinase/Akt involvement in arsenic trioxide resistance of human leukemia cells. J Cell Physiol 202: 623-634, 2005

Tefferi A, Gilliland DG: The JAK2V617F tyrosine kinase mutation in myeloproliferative disorders: status report and immediate implications for disease classification and diagnosis. Mayo Clin Proc 80: 947-958, 2005

Virchis A et al.: A novel treatment approach for low grade lymphoproliferative disorders using PKC412 (CGP41251), an inhibitor of protein kinase C. Hematol J 3: 131-136, 2002

Wu J et al.: Blocking the Raf/MEK/ERK pathway sensitizes acute myelogenous leukemia cells to lovastatin-induced apoptosis. Cancer Res 64: 6461-6468, 2004

Yamamoto K, Ichijo H, Korsmeyer SJ: BCL-2 is phosphorylated and inactivated by an ASK1/Jun N-terminal protein kinase pathway normally activated at G(2)/M. Mol Cell Biol 19: 8469-8478, 1999

Yates J et al.: Cytosine arabinoside with daunorubicin or adriamycin for therapy of acute myelocytic leukemia: a CALGB study. Blood 60: 454-462, 1982

Yu C et al.: Pharmacologic mitogen-activated protein/extracellular signal-regulated kinase kinase/mitogen-activated protein kinase inhibitors interact synergistically with STI571 to induce apoptosis in Bcr/Abl-expressing human leukemia cells. Cancer Res 62: 188-199, 2002

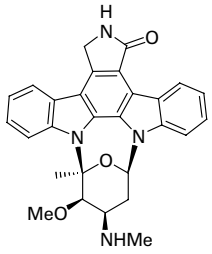
STAUROSPORINE AS AN EARLY LEAD: AN OVERVIEW OF KINASE INHIBITORS INSPIRED BY THE INDOLOCARBAZOLE ALKALOIDS

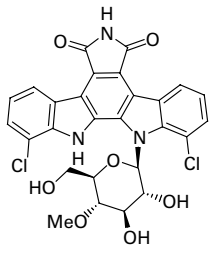
➤ **Peter G. Goekjian**
Professor
Lab. Chimie Organique II-Glycochimie
Université Claude Bernard - Lyon 1
Bâtiment 308 - CPE/UMR 5622
43. Blvd du 11 Novembre 1918
69622 Villeurbanne Cedex
FRANCE

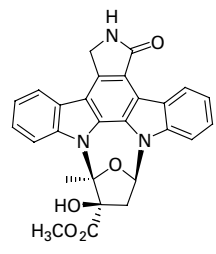
goekjian@univ-lyon1.fr

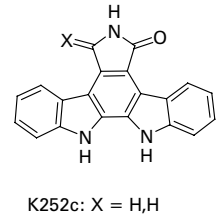
ABSTRACT

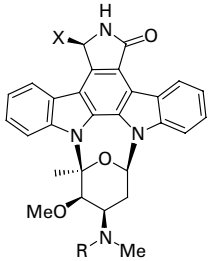
As one of the very early "hits" in the now vast domain of protein kinase inhibitors targeting the ATP-binding site, staurosporine has been the inspiration for an impressive array of selective kinase, topoisomerase, and p-glycoprotein inhibitors. The current review will seek to present an overview of the different classes of inhibitors that have evolved from the original structure. These include staurosporine derivatives, indolocarbazole monoglycosides, substituted indolocarbazoles, bisindolylmaleimides, macrocyclic bisindolylmaleimides, dianilinophthalimides, 4-arylcarbazoles, heteroarylo[a]carbazoles and arylo[a]carbazoles, and azaindolocarbazoles. Potent and selective inhibitors have been discovered against PKC, GSK-3β, myosin light chain kinase, checkpoint kinases, cyclin dependent kinases, flt-3, DAPK, EGFR, VEGFR, PDGFR, and PIM1, as well as non-kinase targets such as topoisomerase I, PARP-1, and p-glycoprotein based on the original indolocarbazole lead structure.

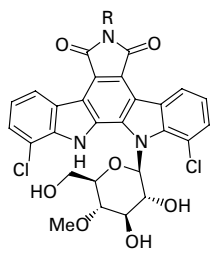

staurosporine

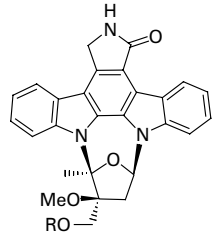

rebeccamycin

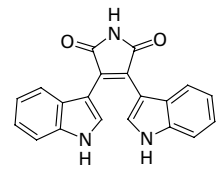

K252a


K252c: X = H,H
Arcyriaflavin A: X = O


midostaurin: X = H, R = PhCO-
UCN-01: X = OH, R = H


BMY-27557-14
R = $(CH_2)_2NEt_2$


CEP 751: R = H
CEP 2563: R = $CO(CH_2)_2NHLys$


Arcyriarubin A

INTRODUCTION

Indolocarbazole alkaloids

The indolocarbazole alkaloids are a family of glycosylated natural products bearing a [2,3-a]indolo[3,4-c]pyrrolocarbazole chromophore. The indolocarbazole aglycon is linked by either one or two *N*-glycoside bonds to a pyranose or furanose sugar. Staurosporine (STP) and UCN-01 are bis-*N*-glycosides of D-daunosamine, while rebeccamycin is a mono-*N*-glycoside of 4-*O*-methyl-β-D-glucopyranoside. K252a is the bis-*N*-glycoside of a furanose sugar related to parasaccharinic acid, bearing a carboxymethyl group at the 3'-position. The aglycons are also natural products, the lactam K252c and the imide arcyriaflavin A, as well as the bisindolylmaleimide arcyriarubin A.

The indolocarbazole lactam or imide moiety turns out to be a surprisingly effective mimetic of adenine. As a consequence, the indolocarbazole alkaloids show a wide range of biological activities, including against many protein kinases, topoisomerase 1, and the P-glycoprotein (PGP) efflux pump. Several close derivatives of the indolocarbazole alkaloids have made it into clinical trials, in particular UCN-01, midostaurin, CEP751/CEP2563 prodrug, and BMY-27557-14 (Prudhomme, 2003; Goekjian & Jirousek, 2001).

Staurosporine

Staurosporine was first isolated by Omura et al. from the bacterium Streptomyces staurosporeus in 1977 (Omura et al., 1977), although the absolute stereochemistry was revised in 1982 (Furusaki et al., 1982). The unusual bis-*N*-glycoside structure has attracted the interest of synthetic chemists, and several total syntheses have been reported (Link et al., 1996; Wood et al., 1997). Related structures have been isolated from natural sources with some regularity (**Figure 1**). Various analogs of STP bearing a hydroxyl group on the indole rings have been identified, in particular 11-hydroxy, 3-hydroxy, and 3,11-dihydroxystaurosporines (Kinnel & Scheuer, 1992; Cantrell et al., 1999; Schupp et al., 1999). The 10-methoxy analog, TAN 999, has been isolated from Nocardiopsis dassonvillei C-71425 (Tanida et al., 1989). Oxidized forms of the imide ring, UCN-01, UCN-02, and 7-oxostaurosporine, have also been isolated from Streptomyces (Takahashi et al., 1987; Takahashi et al., 1989a; Takahashi et al., 1989b). Finally, a number of variations of the sugar ring have been observed, in particular methylated or demethylated forms (Schupp et al., 1999; Schupp et al., 2002; Cai et al., 1996), analogs based on 2-deoxy hexose and hexose sugars (Osada et al., 1990; Hernandez et al., 2000), and 4'-nitro, 4'-oxime, and 4'-

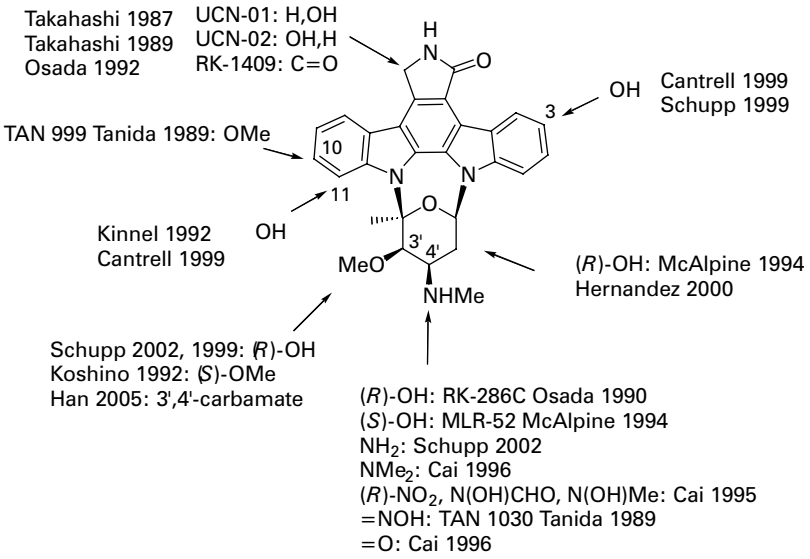


Figure 1. Natural products related to staurosporine

hydroxylamino analogs of staurosporine (Tanida et al., 1989; Cai et al., 1995).

Biological activity

Staurosporine was originally identified as an antitumor antibiotic with hypertensive activity in rats. It was later identified as a potent protein kinase C (PKC) inhibitor in 1986 (Tamaoki et al., 1986). In fact, STP turned out to be a remarkably promiscuous inhibitor: in a recent screen, STP was shown to inhibit 110 out of 119 protein kinases (Fabian et al., 2005). It also inhibits P-glycoprotein (PGP; Wang et al., 2003), but not topoisomerase I (Prudhomme, 2004).

Crystal structures of STP complexed to protein kinases

A number of crystal structures of STP complexed to protein kinases have been published (Vulpetti et al., 2005; Jacobs et al., 2005; Atwell et al., 2004; Xu et al., 2004; Jin et al., 2004; Brown et al., 2004; Yamakawa et al., 2004; Zhang et al., 2004; Holton et al., 2003; Bertrand et al., 2003; Underwood et al., 2003; Zhao et al., 2002; Johnson et al., 2002; Zhu et al., 1999; Lamers et al., 1999; Prade et al., 1997; Toledo et al., 1997; Lawrie et al., 1997). Staurosporine acts as a true ATP analog, in which the lactam provides the key hydrogen bond donor-hydrogen bond acceptor motif of the adenosine ring to the hinge backbone amides. The hydrogen bond acceptor, and to a lesser extent the hydrogen bond donor to the backbone, have been found to be a universally conserved feature in ATP-competitive kinase inhibitors. The central aromatic ring of the indolocarbazole overlaps with the purine ring, while the two indole moieties fill hydrophobic pockets within the ATP-binding site. The pyranose bis N-glycoside oxygen matches the ribose furanose ring oxygen of ATP (Prade et al., 1997; Lawrie et al., 1997).

A notable feature of the crystal structures is that STP binds to the catalytically active conformation of the kinase, in which the activation loop is in the open conformation, and the catalytic DFG motif maintains the aspartic acid oriented towards the ATP-binding site (Prade et al., 1997). By comparison, Glivec has been shown to bind to a conformation with a closed activation loop, and the DFG motif having the aspartate oriented away from the ATP-binding site (Atwell et al., 2004; Gambacorti-Passerini et al., 2005). The binding site being more open and more conserved in the catalytically active form might help to explain the lack of selectivity of this compound.

Staurosporine as a lead compound

The lack of selectivity of STP leads predictably to high toxicity, and STP itself clearly has no potential as a therapeutic agent. Nonetheless, as the original "hit" in the now vast domain of protein kinase inhibitors targeting the ATP-binding site, it has been the inspiration for an impressive array of selective kinase, topoisomerase, and PGP inhibitors. The current review will seek to present some of the compounds that have evolved from the original STP structure (Omura et al., 1995; Prudhomme, 1997; Gescher, 1998; Gescher, 2000; Prudhomme, 2005).

"PKC inhibitor": true target or historical accident?

Although STP has long been referred to as a PKC inhibitor, this should be seen more as a historical accident than a biological result. Indeed, the discovery of protein kinase C as the cellular targets of tumor promoters such as phorbol esters by Kikkawa et al. (1983) launched an intensive search for potent inhibitors (Tamaoki et al., 1986; Solomon et al., 1985; Sano et al., 1985; O'Brian et al., 1985; Shoji et al., 1985; Inagaki et al., 1985; Besterman et al., 1985; Kawamoto et al., 1984; Hidaka et al., 1984; Sanchez et al., 1983 ; Sahyoun et al., 1983 ; T affet et al., 1983). It is now clear that STP is a very general kinase inhibitor, and can be considered a lead compound for most kinases. Selective inhibitors of many kinases, including PKC, cdk's, trkA, checkpoint kinases, flt-3, VEGFRs, EGFR, and PDGFR, as well as non-kinase targets PGP, PARP-1, and topoisomerase I, have been developed based on the indolocarbazole alkaloids (*vide infra*). For this reason, the current review will not focus particularly on PKC inhibitors, but on inhibitors structurally related to staurosporine. Of course, such a definition is *per se* subjective, and the limits of the current discussion are thus somewhat arbitrary. Structural modifications of the indolocarbazole system (**Figure 2**) have led to

the bisindolylmaleimide, dianilinophthalimide, 4-arylcarbazole, arylo[a]carbazole, and azaindolocarbazole classes of inhibitors.

The current review will not focus strongly on the biological activity of the inhibitors. The issue of potency and selectivity, while of course central to the discussion of ATP-competitive kinase inhibitors, is better addressed elsewhere in the current issue. Furthermore, inhibition constants measured in different assays are not comparable, and reliable conclusions can therefore only be made based on results obtained with the same assay. The focus of this article will therefore be more on the various structural modifications of the STP system than on the biological results. Finally, somewhat arbitrarily, analogs of K252a will not be discussed here, despite the obvious importance of these compounds. Indeed, the K252a story is a fairly coherent one, and the reader is referred to recent publications on this topic (Prudhomme, 2005; Mucke, 2003; Saporito et al., 2002; Moffat et al., 2005; Gingrich et al., 2005; Schneider et al., 2005).

STAUROSPORINE ANALOGS

N4'-substituted derivatives

A large series of *N*-alkylated and *N*-acylated analogs have been prepared, which will not be listed here in detail (Brazzell et al., 2000; Griffin et al., 2003; Nomura et al., 1996; Lewis et al., 1996, Regenass et al., 1995; Murakata et al., 1994; Wacker, 1994; Miyamoto et al., 1993). 4'-*N*-benzoyl staurosporine, midostaurin (CGP41251), is an inhibitor of PKC, Flt-3, TGFR, VEGFR-1 and P-glycoprotein, and has made it to phase II clinical trials for chronic lymphocytic leukaemia and non-

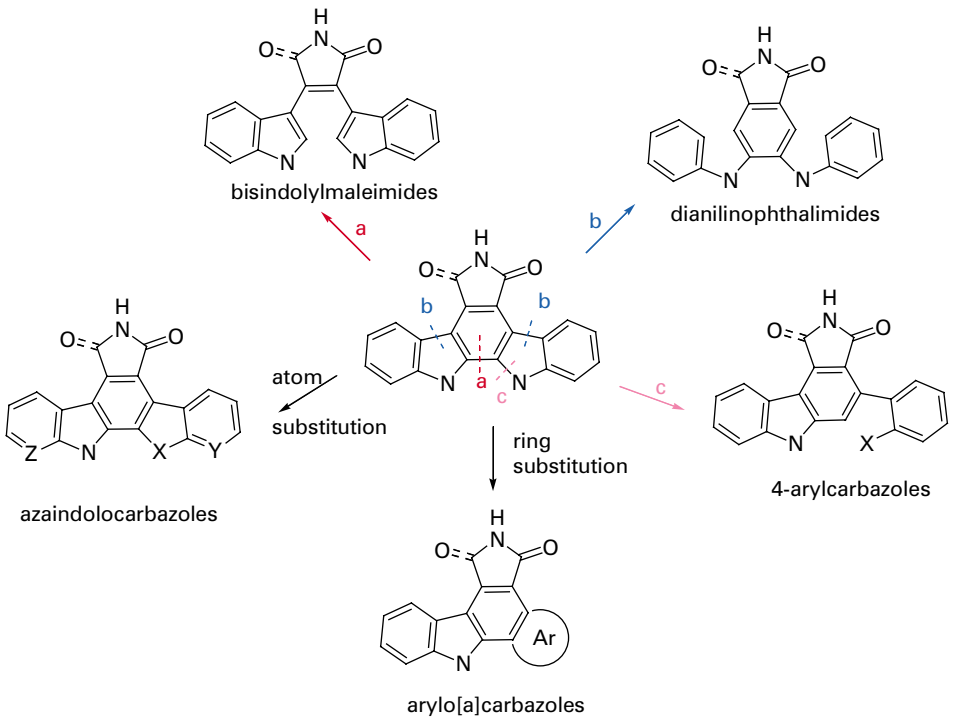


Figure 2. Inhibitor design based on the indolocarbazole structure

Hodgkin's lymphoma (Goekjian & Jirousek, 2001). A Phase I clinical trial in combination with 5-fluorouracil has recently been reported. Recruiting is currently underway for Phase I clinical trials in combination with daunorubicin and cytarabine, as well as Phase II trials in aggressive systemic mastocytosis and mast cell leukemia (http://clinicaltrials.gov).

A study of the biological activity of a series of STP derivatives substituted at *N*4', *N*6, and *C*7, was reported by Caravatti et al. (1994) *N*4'-acylation reduces potency, but improves selectivity, while substitution of the imide abolishes all kinase activity. 7-oxostaurosporine shows a similar activity profile to staurosporine. The 7(*R*)-hydroxy analog, UCN-01, maintains potency against PKC, yet improves selectivity. However, the 7(*S*)-hydroxy epimer, UCN-02, is considerably less potent. The 7-oxo-5-hydroxy analog is somewhat less potent, but shows a similar stereochemical discrimination between hydroxy epimers.

Substitution of the indole rings

The most extensive studies have involved the substitution of the indole benzenoid rings, and an important collection of substituted analogs have been prepared. The most common substitution has been at the 3 and 9 positions (pyrroloindolocarbazole numbering, corresponding to a 5-substituted indole), including fluoro, bromo, iodo, hydroxy, alkoxy, nitro, amino, acylamino, alkyl, acyl, carboxy, vinyl, and acetylene (Murakata et al., 2002; Saulnier et al., 2002; Kanai et al., 2001; Yamada et al., 1996; Tamaoki et al., 1994). The tetrafluoro analogs have been prepared by Saulnier et al. (1998), while tetrahydrostaurosporine analogs have been investigated by Zimmermann (1999) as PKC-α inhibitors.

Substitution of the lactam moiety

Various modifications of the lactam moiety have been reported: 7-*O*-alkyl analogs of UCN-01 have been prepared as selective PKC inhibitors, while the imide nitrogen has been replaced with hydrazine, or has substituted with alkyl groups (Takahashi et al., 1990). As noted above, *N*-alkylation of the lactam abolishes activity against most protein kinases. However, such a substitution does not affect potency against PGP, and *N*-benzyl and *N*-methylpyridyl substituted analogs of midostaurin have been proposed as selective PGP inhibitors (Wang et al., 2003).

Modification of the sugar moiety

Additional analogs have been prepared by synthetic or biosynthetic approaches, replacing the daunomycin with other sugars bearing

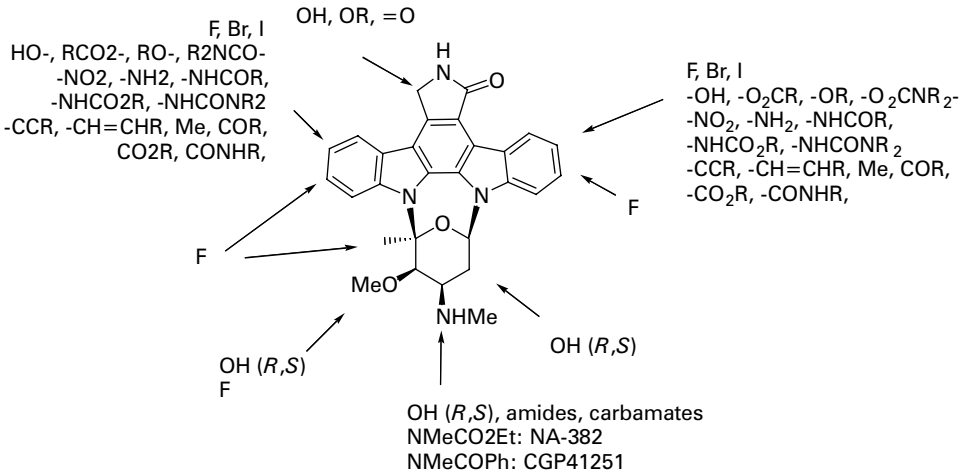


Figure 3. Synthetic analogs of staurosporine.

hydroxyl, ketone, or oxime functionalities (Saulnier et al., 2002; Salas et al., 2005; Cai & Ouyang, 2001; Li et al., 2000; Yamada et al., 1995; Ootsuka et al., 1993). These analogs target PKC, topoisomerase I, myosin light chain kinase, or antiangiogenic activity. The sugar moiety of STP has also been replaced with carbocyclic (Moffat et al., 2005; Monse et al., 2004; Riley & Simpkins, 1999) and heterocyclic (Shankar et al., 1994; Vice et al., 1994) analogs as well as regioisomeric forms, in which the nitrogens are bound to the C1' and C2', C1' and C4', or the C1' and C6' carbons of the sugar (**Figure 4**) (Saulnier et al., 2002; Saulnier et al., 2005a; Nichols & Simpkins, 2004; Marminon et al., 2002a; Anizon et al., 1998; Ruediger et al., 2002). Analogs in which the sugar has been replaced with a tether have been prepared by Vice et al. (1996; 1994).

INDOLOCARBAZOLE MONOGLYCOSIDES

Sugar-modified analogs

The indolocarbazole monoglycosides are analogs of rebeccamycin, and the synthesis of analogs has been reviewed regularly by Prudhomme (2004; 1997; (et al., 1999); 2000; and 2003). Bis-dechlororebeccamycin is an inhibitor of topoisomerase, but not of PKC, while STP inhibits protein kinases, but not topoisomerase. The dramatic differences in the biological activity of arcyriaflavin, rebeccamycin, staurosporine, and K252 point to the essential role of the sugar in controlling the selectivity of these compounds towards the various targets.

A systematic investigation of the role of the sugar on the biological activity of the indolocarbazole mono-N-glycosides has been performed by Anizon et al. (1997; 2003), Ohkubo et al. (2000), Faul et al. (2004), and Zhang et al. (2005). Varying the sugar leads alternatively to inhibition of topoisomerase I, protein kinase C, or cdk4, as well as varying levels of cytotoxicity. No simple relationship can be made either to the sugar or to its anomeric configuration (Prudhomme, 2004; Prudhomme, 2003). For example, the β-glucosyl and β-galactosyl indolocarbazoles are more active than the corresponding α-glycosides against topoisomerase I, while the α-mannopyranoside is more active than the β-glycoside; in the allopyranoside series, the two configurations are equally potent.

Conformation of the indolocarbazole N*-glycoside*

The complex structure-activity relationship between the sugar and the biological activity against protein kinases and topoisomerase I indicate that the specific three-dimensional arrangement provided by rotation around the *N*-glycoside bond is responsible for the selectivity profiles. The conformation of indolocarbazole *N*-glycosides has been investigated by Gilbert et al. (1999). They found equilibrium between two alternative conformations of the *N*-glycoside, one

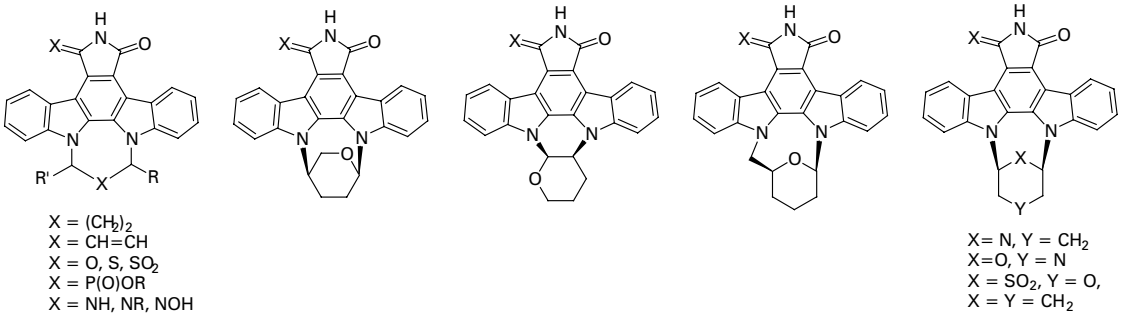


Figure 4. Bridged indolocarbazoles

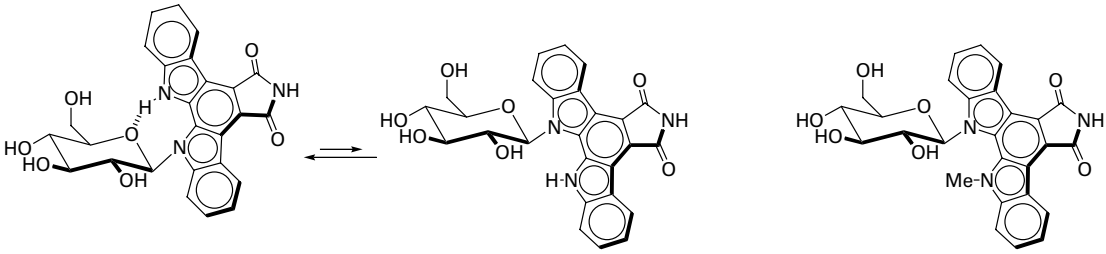


Figure 5. Conformation of the indolocarbazole N-*glycoside*

of which is stabilized by a hydrogen bond between the pyranose oxygen and the indolocarbazole NH. They have recently shown that the corresponding *N*-methyl compound, which favors the alternative conformation, is less active against topoisomerase I, which suggests that the hydrogen-bonded conformation is the active one in this system (Facompre et al., 2003).

Modification of the imide

In contrast to protein kinases, substitution of the imide nitrogen of rebeccamycin is not deleterious to activity against topoisomerase I (Pereira et al., 1996). The diethylaminoethyl-substituted analog of rebeccamycin, BMY-27557-14, is currently in clinical trials. Replacing the imide nitrogen with a hydrazine is also a favorable modification. The 2-[1,3-dihydroxypropyl] substituted hydrazine structure has been found to be particularly favorable (Denny, 2004).

Interestingly, reduction of the imide to a lactam induces significant activity against PKC (Anizon et al., 1998; Moreau et al., 1998). This is noteworthy, as the maleimides and lactams are usually equally potent against kinases, and it is therefore difficult to invoke an unfavorable interaction of the maleimide of rebeccamycin in PKC. The origin of this effect remains to be elucidated.

Substitution of the indole

NB-502 is a natural product 1,11-dihydroxyl analog of rebeccamycin, having a formyl hydrazine substituent at the imide nitrogen, and lacking the glucose 4' methyl group. Ohkubo et al. (1999) have systematically varied the position of the indole OH groups. Optimization of the substituent led to Edotecarin (Denny, 2004). The 3,9- dibromo analog was investigated by Moreau et al. (1999), and found to improve potency

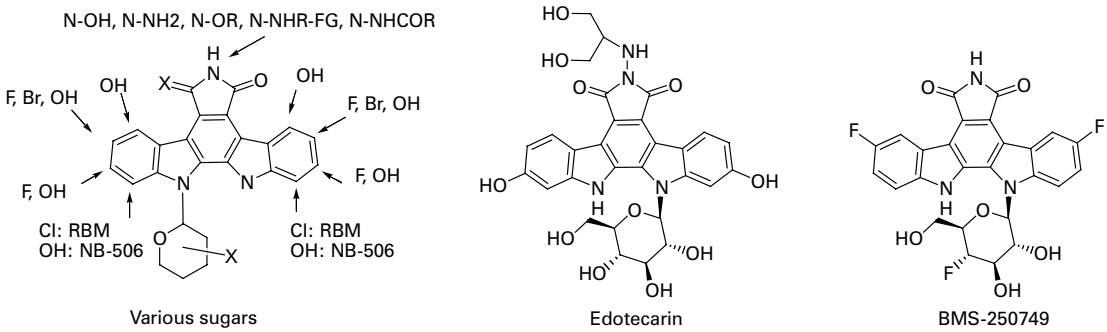


Figure 6. Rebeccamycin analogs

against topoisomerase I. Fluorinated analogs have been prepared at the 2, 3, 9, and 10 positions (Saulnier et al., 1998; Ruediger et al., 2002; Kojiri et al., 1997; Katsuhisa et al., 1993; Balasubramanian et al., 2004/2005). BMS-250749, a 3,9,4'-trifluoro analog of rebeccamycin compares favorably to an approved camptothecin analog CPT-11 in preclinical models (Saulnier et al., 2005b).

INDOLOCARBAZOLES

The indolocarbazole moiety is responsible for the recognition by the ATP-binding site, and a large number of non-glycosylated indolocarbazoles, analogs of K252a and arcyriaflavin A, have been prepared. These include substitution at all positions of the indole ring with halogens (Zhu et al., 2003a; Slater et al., 1999; Bergman & Pelcman, 1989; Fabre et al., 1994), hydroxyls (Ohkubo et al., 1999), nitro (Yamada et al., 1993), amino (Bonser et al., 1996; Sahagun-Krause et al., 2003), carboxy (Murakata et al., 1995), trifluoromethyl (Zhu et al., 2003a) or methyl groups (Zhu et al., 2003a) (**Figure 7**). Compounds with a cycle or macrocycle fused to the indole rings ([j,k]-fused indolocarbazoles) have been prepared by Al-Awar et al. (2001) as cdk4 inhibitors.

Substitution on the lactam ring focuses on oxidized forms (7-hydroxy, 7-keto), as well as alkyl and dimerized forms of the indolocarbazole (Marminon et al., 2002b). The nitrogen has been substituted with heteroatoms (hydroxylamine, hydrazines, and hydrazones), in particular aimed at targeting topoisomerase I (*vide supra*).

The substituents on the indole nitrogens play a critical role in achieving selectivity and potency. The unsubstituted indolocarbazole arcyriaflavin A is only weakly active against PKC, cyclin-dependent kinases, or topoisomerase I (Prudhomme, 2003; Zhu et al., 2003a). A large number of functionalized substitutents have been tested bearing amino, diethyleneglycol, diol, amino acid, trifluoromethyl, alcohol, carboxymethyl, aryl, and nitrile functionalities (Sanchez-Martinez et al., 2003c; Moreau et al., 2001; Slater et al., 2001). One may cite Go6976, a potent and relatively selective inhibitor of conventional PKC's (α, β, γ) (Martiny-Baron et al., 1993). A study of the inhibition of a panel of kinases by Go6976 has been reported by Davies et al. (2000), showing in addition to PKC, submicromolar inhibition of PDK1, MAPKAP-K1b, MSK1, PHK, S6K1, and

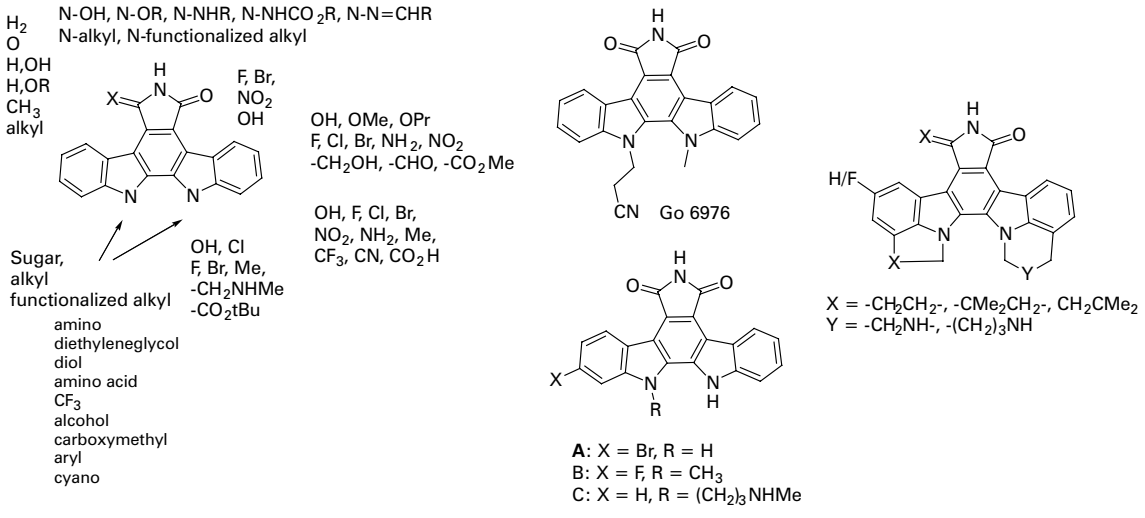


Figure 7. Substituted indolocarbazoles.

Table 1. Residual activity in presence of 1 μM Go6976, as a % if control. 17 other kinases tested, residual activity 33%–100% (Goekjian & Jirousek, 2001; Davies et al., 2000).

Inhibitor	PKC-a	PDK1	MAPKAP -K1b	MSK1	PHK	CHK1	S6K1
Go6976	7%	18%	5%	2%	1%	3%	12%
IC(50)	2 nM			10 nM			

Table 2. Inhibition constants for selected indolocarbazoles shown in Figure 7 against cyclin-dependent kinases (Zhu et al., 2003a; Sanchez-Martinez et al., 2003).

Inhibitor	Cyclin D1-cdk4	Cyclin E-cdk2	Cyclin B-cdk1	CamK II	PKA
A	0.076 μM	0.52 μM	2.1 μM	12.4 μM	>20 μM
B	0.042 μM	0.144 μM	-	>2 μM	>2 μM
C	0.05 μM	0.16 μM	-	-	0.12 μM

CHK1 (**Table 1**). Indolocarbazole compounds have been targeted towards cdk's (**Table 2**) (Zhu et al., 2003a; Al-Awar et al., 2001), viral pUL97 (Slater et al., 2001; Zimmermann et al., 2000), HIV-1 integrase (Yan et al., 2005), PDGF auto-phosphorylation (Uings et al., 1998), or topoisomerase I (Moreau et al., 2001).

BISINDOLYLMALEIMIDES

The bisindolylmaleimides (BIM), e.g. arcyriarubin A, result from the deletion of the central indolocarbazole bond. This provides considerable conformational flexibility, and these compounds have been shown to bind to the ATP-binding site in a highly canted conformation (Messerschmidt et al., 2005; Gassel et al., 2004; Komander et al., 2004). The BIM were first prepared by Sarstedt and Winterfelt (1983), then others, as intermediates for the synthesis of the indolocarbazole aglycons (Brenner et al., 1988; Bergman & Pelcman, 1987; Joyce et al., 1987; Kaneko et al., 1985). Toullec et al. (1991) and Davis et al. (1992a; 1992b) later identified potent and selective PKC inhibitors based on these compounds, including GF109203X, Ro 31-8220, and others shown in **Figure 8** (Wilkinson et al., 1993). These compounds have found extensive use in biological investigations of signal transduction pathways, although the biological effects of the BIM can be mediated through both PKC and non-PKC pathways (Han et al., 2000). A comprehensive study of the activity of a selection of these compounds against a panel of kinases by Davies et al. (1992a; 1992 b) showed that these were also active against MAPKAP-K1b, MSK1, S6K1, and GSK3 and weakly active or inactive against 18 other kinases. A recent comparison between bisindolylmaleimides and indolocarbazoles demonstrate that the BIM system is indeed significantly less potent against cyclin dependent kinases (Sanchez-Martinez et al., 2003).

Similar substitutions to those investigated for the indolocarbazoles have been performed on the indole, maleimide, and indole nitrogens (**Figure 8**), and will not be detailed here. Xie et al. (1996) have prepared BIMs substituted with lexitropsin oligopeptides which bind the DNA minor groove, in order to target topoisomerase I. Sasaki et al. (1998) have prepared inhibitors of cdc2 kinase based on a multiple pseudosubstrate construct bearing a BIM bound to an oligopeptide pseudosubstrate via an oligoethylene glycol chain. BIMs also exhibit antagonist activity against the nicotinic cholinergic receptor (Mahata et al., 2002). In addition, two bisindolylmaleimides are

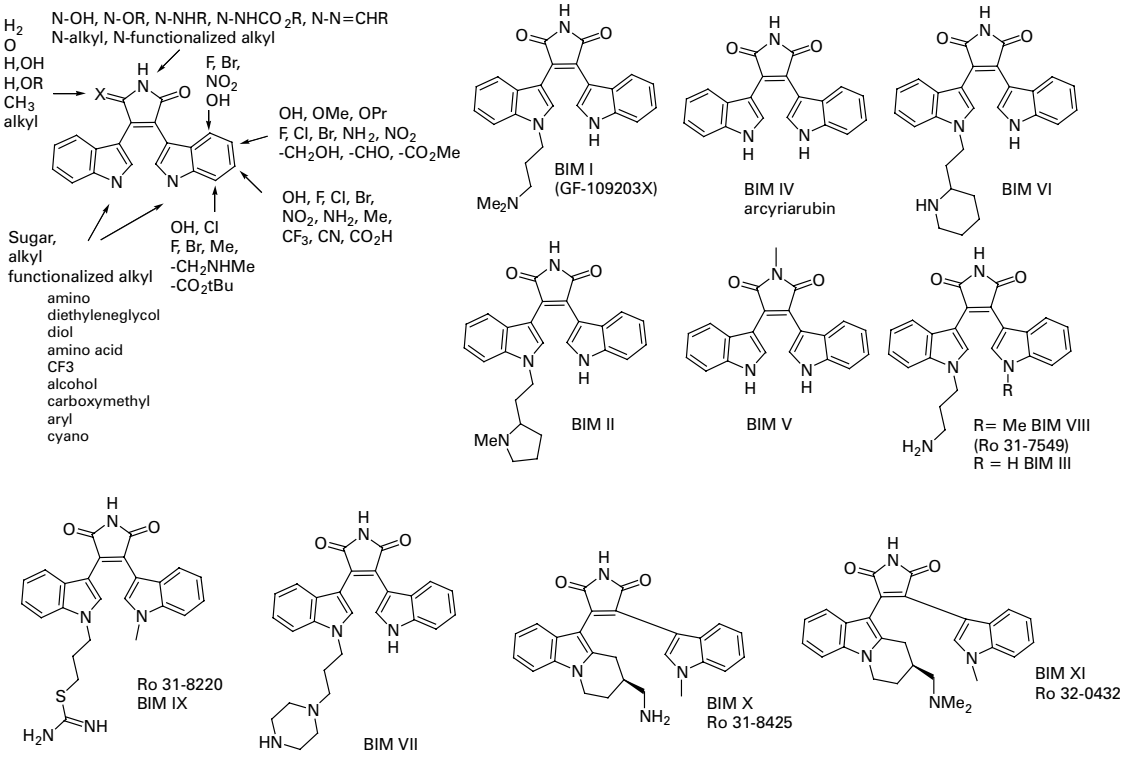


Figure 8. Substituted bisindolylmaleimides

currently undergoing clinical trials, Enzastaurin (LY317615.HCl), a PKC-β selective inhibitor (Pearce & Miller, 2005; Faul et al., 2003), and Ro 31-7453 (Dupont et al., 2004; Salazar et al., 2004).

MACROCYCLIC BISINDOLYLMALEIMIDES

The macrocyclic bisindolylmaleimides are formed by connecting a tether between the indole nitrogens of the bisindolylmaleimide, in an effort to constrain the conformation of the flexible pharmacophore. Indeed, the presence of a 14-membered macrocycle eliminates the major "syn" conformation observed in the bound and unbound form of the BIMs, in favor of a pseudo-C2 symmetric "anti" conformation, in which the two C2 indole hydrogens point to opposite faces of the maleimide (Komander et al., 2004; Bartlett et al., 2005). Ruboxistaurin (LY333531, **Figure 9**) is a highly selective inhibitor for the β-isoform of

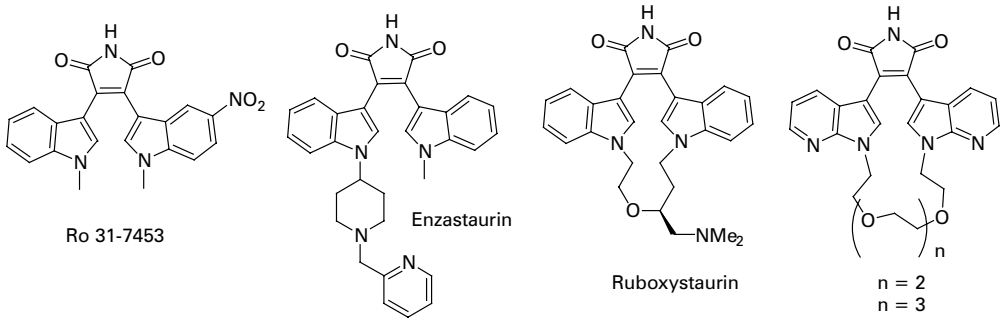


Figure 9. Selective bisindolylmaleimides and macrocyclic bisindolylmaleimides.

protein kinase C over other isoforms and other kinases (**Table 3**) (Faul et al., 2003; Jirousek et al., 1996). One observes a significant degree of selectivity for PKC-β over other isoforms, as well as over other kinases. In a comprehensive screen, Fabian et al. (2005) have shown submicromolar binding to 9 kinases, low micromolar binding to 16 kinases, and >5 micromolar binding to 94 out of 119 kinases (**Table 4**). Significant binding is observed only to PIM1 (55 nM) and to mouse STK3 (90 nM). In a separate screen of 29 kinases, some inhibition was found to PDK1, MSK1, MAPKAP-K1a, and S6K1 (10–20% residual activity at 10 μM) (Komander et al., 2004). However, the concentration tested is >1000 times the IC50 against PKC-β. Ruboxistaurin is currently in Phase III clinical trials in Europe, and is recruiting for Phase III clinical trials in the US and Canada for diabetic retinopathy, and for Phase II for diabetic macular edema (http://clinicaltrials.gov; Vinik, 2005; He & King, 2005; Vinik et al., 2005; Aiello et al., 2005).

Kuo et al. (2003) have recently reported a series of macrocyclic bisindolylmaleimides and their aza analogs bearing an 8-, 11-, 14-, or 17-atom oligoethyleneglycol linker (16-, 19-, 21-, or 24-membered macrocycle, **Figure 9**). The 19- and 21-membered macrocylic bisindolyl-maleimides are potent inhibitors of GSK-3β (22 nM) with only weak activity against PKC-γ. However, the 19-membered macrocyclic bisindolylmaleimide maintains a strong activity against PKC-βII (6 nM) and PKC-θ (65 nM). The corresponding monoaza bisindolylmaleimide with a 19-membered macrocycle is more selective for GSK-3β, while the 19-membered bis(7-azaindolyl)maleimide is >30 fold selective for GSK-3β over PKC-β. Finally, the 21-membered bis(7-azaindolyl)maleimide is a potent inhibitor of GSK-3β (48 nM), but is inactive against PKC-βII (IC50>10μM). A screen against a panel of 50 kinases shows that the 21-membered ring compound does not inhibit any of the kinases at a concentration of 10 μM (residual activity >61%). It is interesting to note that ruboxistaurin is quite selective for PKC-β over GSK-3b (31% residual activity at 10 μM ruboxistaurin) (Komander et al., 2004), indicating that orthogonal selectivities can be achieved within the macrocyclic bisindolylmaleimides.

Table 3. Inhibition constants (μM) of PKC isoforms by ruboxistaurin, enzastaurin, and staurosporine (Faul et al., 2003).

Inhibitor	α	β1	β2	γ	δ	ε	ζ	η	PKA	CamK	CKII	srcTK
ruboxistaurin	0.36	0.005	0.006	0.3	0.25	0.6	>100	0.052	>100	6.2	>100	>100
enzastaurin	0.8	0.03	0.03	2	1	0.3	8	0.4	>100	10	>100	>100
staurosporine	0.045	0.023	0.019	0.11	0.028	0.018	>1.5	0.005	0.10	0.004	14	0.001

Table 4. Binding constants (μM) to protein kinases for ruboxistaurin and staurosporine (Fabian et al., 2005).

Inhibitor	AAK1	FLT3	KIT	PIM1	PIM2	SLK	STK18	STK3 _m	STK4	16 kinases	94 kinases
ruboxistaurin	0.96	0.27	0.38	0.055	0.13	0.94	0.37	0.09	0.22	>1 μM	>5 μM
staurosporine	0.0013	0.0018	0.1	0.015	0.004	.00002	.00075	.00015	0.0002	a	a

a. staurosporine: >5 μM: 11 kinases; <1 μM: 108 kinases

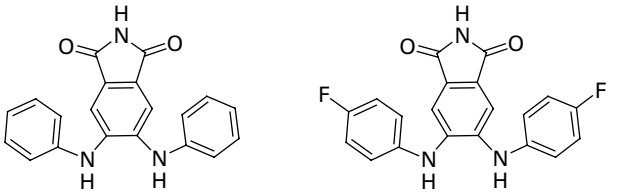


Figure 10. Dianilinophthalimides.

DIANILINOPHTHALIMIDES

An alternative bond deletion consists of removing the indole C-C bonds, leading to a dianilinophthalimide that maintains the basic pharmacophore, but allows greater conformational flexibility and a less conjugated system. Trinks et al. (1994) have prepared the dianilinophthalimides, e.g. DAPH1 (CGP52411) and substituted analogs, as potent and selective inhibitors of the EGF receptor (Trinks et al., 1994; Weidner et al., 1999). The dianilinophthalimides adopt a non-planar, unsymmetrical propeller-shaped conformation (Trinks et al., 1994), and a modeling study suggests that these compounds bind as ATP analogs with one of the aniline rings in the ribose-binding site (Furet et al., 1995). The bis-(4'-fluoroaniline) analog DAPH 2 (CGP 53353) has been shown to inhibit PKC-β2 and EGF at equal potency, and to have viable antitumor activity in live mice (Dinney et al., 1997; Buchdunger et al., 1994; Buchdunger et al., 1995). More recently, Blanchard et al. (2004) have shown that the DAPH1 inhibits the formation of fibrils of the Aβ1-42 peptide implicated in Alzheimer's disease.

4-ARYL-SUBSTITUTED CARBAZOLES

Deletion of one of the indole nitrogens leads to the 4-aryl pyrrolo[3,4-c]carbazole system (**Figure 11**). The binding motif of the indolocarbazoles and bisindolylmaleimides demonstrate that the two indole rings are in non-equivalent environments, and that considerable

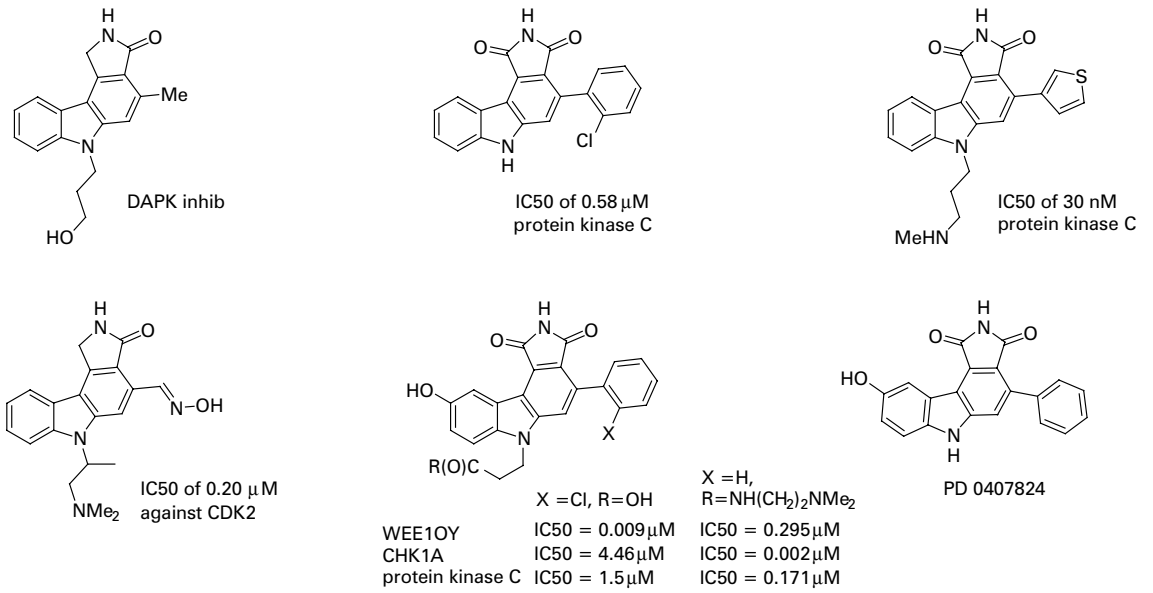


Figure 11. 4-Substituted pyrrolocarbazoles.

conformational flexibility and steric freedom is available for one of the indole systems. The 4-arylpyrrolocarbazoles were initially prepared by Kleinschroth et al. (1990) as PKC inhibitors, in particular bearing a 4-(2'-chlorophenyl) ring. A variety of new synthetic approaches to these compounds have been reported (McCort et al., 1999; Adeva et al., 2000; Adeva et al., 2000a; Adeva et al., 2000b). Heteroaryl-substituted analogs were subsequently prepared by Broka (1996), and were found to be more potent against PKC. 4-arylcarbazoles have also been reported as checkpoint kinase Wee 1 and CHK1 inhibitors by Booth et al. (2003), while the cytotoxicity of the tetrahydrocarbazole compounds has been studied by Caballero et al. (2003). The 4-oxime-substituted compounds were prepared by Kanai et al. (2003) as cdk2 inhibitors. The 4-methyl-prrolocarbazoles have been recently prepared by Teruta et al. (2004) as inhibitors of death-associated protein kinase (DAPK).

A study of the crystal structure of the complex of PD 0407824 with Wee1 kinase was recently reported by Squire et al. (2005). The compound binds as an ATP analog, with the imide ring forming the two conserved hydrogen bonds to the hinge backbone. The 9-hydroxy group forms an additional hydrogen bond to the backbone, while the 4-phenyl group is twisted relative to the carbazole ring, and fits into a well-defined hydrophobic pocket.

ATOM-SUBSTITUTED ANALOGS OF INDOLOCARBAZOLES AND BISINDOLYLMALEIMIDES

Indenocarbazoles

The analogs of indolocarbazoles, in which one of the indole nitrogens was replaced with a carbon, have been reported by the Hudkins group (1996; 2000) as PKC, VEGFR2 and trkA inhibitors (**Figure 12**). Underiner et al. (2002) and Singh et al. (1999) have prepared indenocarbazole analogs of the STP ring system, which show activity against trkA and VEGF-R2 (KDR), but weak activity against PKC and PDGFR. A parallel synthesis approach to these compounds was reported by Tripathy et al. (2002). An optimized indenopyrrolo-carbazole, CEP-5214 and its prodrug form CEP-7055 have been reported by Gingrich et al. (2003) as a potent inhibitor of VEGFR3 (Flt-4), VEGFR2 (KDR), VEGFR1 (Flt-1), and Flt-3 with IC(50)s of 4 nM, 8 nM, 16 nM, and 1.6 nM, respectively. CEP-5214 was weakly active or inactive against a panel of 12 other kinases.

Azaindolocarbazoles

The 7-aza analogs of the indolocarbazole system were first prepared by Slater et al. (1995) and again by Routier et al. (2002a; 2002b) as topoisomerase inhibitors (**Figure 13**). A series of glycosylated azaindolocarbazoles, analogs of

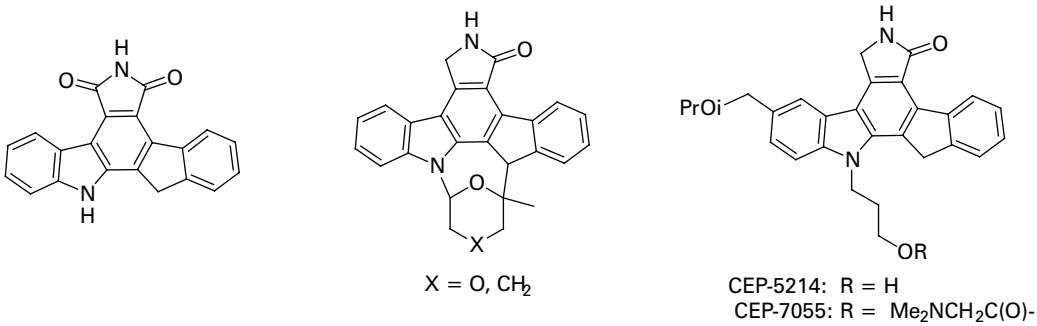


Figure 12. Indeno[a]carbazoles.

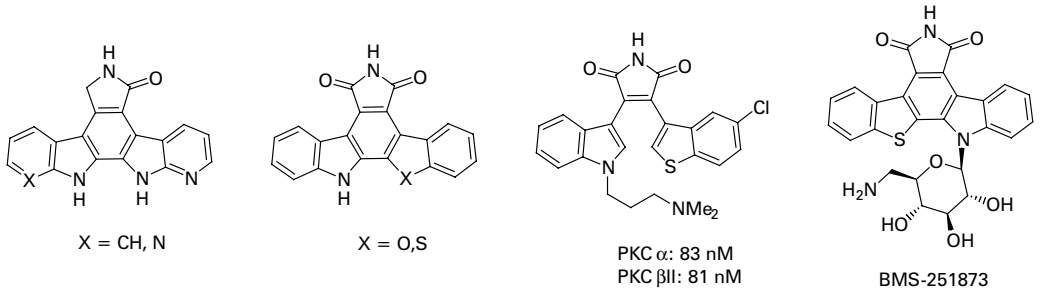


Figure 13. Nitrogen, oxygen, and sulfur atom-substituted indolocarbazoles.

rebeccamycin, have been prepared by Marminon et al. (2003a), Prudhomme et al. (2003) and Messaoudi et al. (2005). The 7,10-diazaindolocarbazole analog was also reported by Marminon et al. (2003b), while C1'-C2'-bridged analogs were recently reported by Messaoudi et al. (2005). The azaindolocarbazole compounds exhibit a distinct activity profile from rebeccamycin, although the targets are not yet identified. The aza analogs of the bisindolylmaleimides were first prepared by Davis et al. (1992) as PKC inhibitors, while their macrocyclic homologs, GSK-3b inhibitors prepared by Kuo et al. (2003), are discussed above.

Benzofuro- and benzothienocarbazoles

Analogs of the indolocarbazoles, in which one of the nitrogens has been replaced with a sulfur or an oxygen, have been reported by Hudkins et al. (2001), Ma et al. (2001), and Sanchez-Martinez et al. (2003a) (**Figure 13**). Sanchez-Martinez et al. (2003b) found out that the benzothienocarbazole was slightly less potent against cyclinD1-cdk4 than the corresponding indolocarbazole compound. A series of 3-(indolyl)4-(benzothienyl)maleimides were prepared by Zhang et al. (2003) as PKC and GSK-3b inhibitors. Glycosylated benzothienocarbazoles and benzofuranocarbazoles have been reported by Balasubramanian et al. (2004/2005) as topoisomerase I inhibitors. The benzothienocarbazole was found to be optimal, and the 3,9-difluoro compound, BMS-251873, shows in vivo efficacy and is proposed as a potential clinical candidate.

RING-MODIFIED ANALOGS OF INDOLOCARBAZOLES AND BISINDOLYLMALEIMIDES

Inverted indoles

Indolo[1,2-a]-β-carbolines

Indolocarbazoles having an alternative fusion of the indole ring have also been prepared. The antitumor natural product BE-54017 has an indolo[1,2:a]β-carboline ring system (Nakase et al., 2000). Analogous indolo[1,2-a]β-carbolines have been prepared by Jaquith et al. (2001). A deletion of the [1,2-a] indole C-C bond leads to the 5-aryl pyrrolo[3',4':5,6]pyrido[3,4-b]indole-1,3(2H,3aH)-diones, prepared by Teller et al. (2000) as selective PDGF receptor inhibitors. The 8,9-dimethoxy-5(2-methylphenyl)-β-carboline compound, although slightly less active in

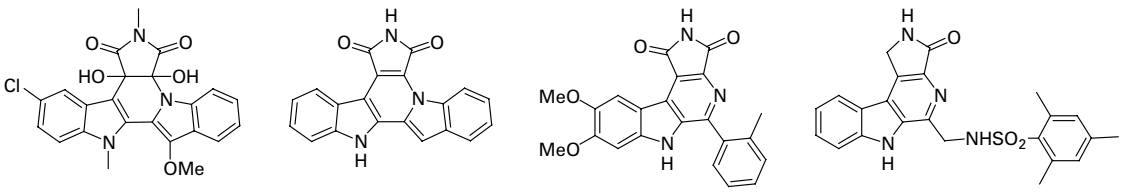


Figure 14. β*-Carboline analogs of indolocarbazoles.*

isolated PDGFR than the 9-methoxy-5-(2-methoxyphenyl) analog, shows better cellular activity and better selectivity. Mahboobi et al. (1999a) reported that the 9-methoxy-5-phenyl analog is an inhibitor of both topoisomerase I and II. 5-alkylsulfonamide analogs have been studied by Laronze et al. (2005), and were shown to be micromolar inhibitors of GSK-3. A stereo-controlled strategy for the synthesis of the corresponding reduced 5-phenyltetrahydro-carbolines has been recently reported by Dardennes et al. (2005). Finally, a series of spirocyclic dihydrocarbolines were prepared by Mahboobi et al. (1999b) as tyrosine kinase inhibitors.

Indolo[3,2-a]carbazoles

The isomeric fusion of the indolocarbazole, in which the indole nitrogens are meta rather than ortho on the carbazole benzenoid ring, provide a different shape to the planar indolocarbazole ring system. These compounds have been prepared by Henon et al. (2005), Janosik et al. (1999), Bergman et al. (1999) and Fonseca et al. (1995). However, no biological data was provided for these compounds.

Indolo[6,7-a]carbazoles

The indolocarbazoles, in which one of the indoles is fused via the benzenoid ring, have also been investigated (**Figure 15**). A series of indolo[6,7-a]carbazoles have been investigated by Engler et al. (2003), Zhu et al. (2004) and Faul et al. (2004) as cdk4 inhibitors. Optimization for the selectivity and solubility provided the 7-(3-alkylaminopropyl)-3-*N*-methyl analogs, which were selective for cdk4 over a panel of 44 other kinases.

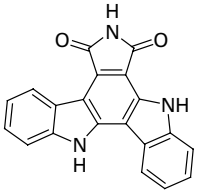
Arylcarbazoles

Benzo[a]carbazoles

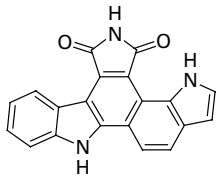
A series of substituted 3,4-phenylindolylmaleimides and benzo[a]carbazoles have recently been reported by Peifer et al. (2006) as potent VEGFR2 inhibitors. In particular, the 3-indolyl-4-(trimethoxyphenyl)maleimide was a potent and selective inhibitor of VEGFR2, while the corresponding trimethoxybenzo[a]carbazole was significantly less active. The cytotoxicity of a series of benzo[a]carbazoles and their activity against cyclin-dependent kinases, GSK3, and topoisomerase I have recently been investigated by Routier et al. (2006). The 2-dimethylamino-ethyl-10-hydroxybenzo[a]pyrrolo[3,4-c]carbazole compound showed the strongest cytotoxicity, although it is inactive against cdk2, GSK-3, and topoisomerase I, and the target of this compound remains to be identified. Benzo[a]pyrrolo[3,4-c]carbazole-1,3-dione was similarly found to be inactive against PARP-1, a non-kinase target, but the fused cyclopentane compound was a found to be a nanomolar PARP-1 inhibitor by Tao et al. (2006).

Naphtho[a]carbazoles

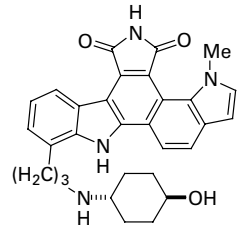
The indolocarbazole analogs replacing the indole ring with a naphthalene were investigated as cdk4 inhibitors by Sanchez-Martinez et al. (2003b) and against cdk2, gsk-3, and topoisomerase I by Routier et al. (2005; 2001). There are three regioisomers, the "up-angular" naphtho[2,1-a]carbazole, the "down-angular" naptho[1,2-a]carbazole, and the "linear" naphtho[2,3-a]carbazole. The naphtho[2,1-a]carbazole isomer was significantly more active against cdk4 (45 nM) than either the



indolo[3,2-a]carbazoles



indolo[6,7-a]carbazoles



cdk4(Rb^{21}) 19 nM

Figure 15. [3,2-a] and [6,7-a] isomers of indolocarbazoles.

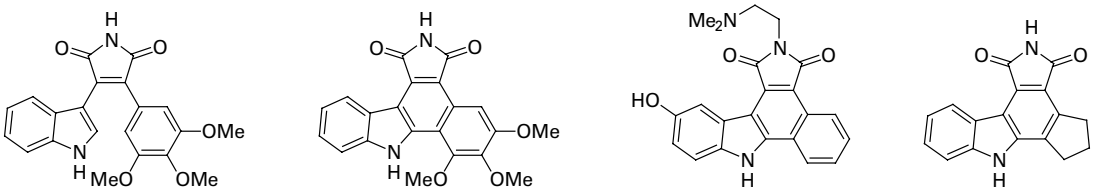


Figure 16. Indolylphenylmaleimides and benzo[a]carbazoles.

benzo[a]carbazole (260 nM), naphtho[1,2-a]carbazole (430 nM) or naphtho[2,3-a]carbazole (inactive) compounds (Sanchez-Martinez et al., 2003b). This compound was selective for cdk4 over a panel of 7 kinases. Routier et al. (2005) found that the linear naphtho[2,3-a]carbazole isomer bearing dimethylaminoethyl and hydroxyl substituents was highly cytotoxic against L1210 cells, although inactive against cdk5, gsk-3, or topoisomerase I.

Benzothiophene- and quinoline-fused carbazoles

A "down-angular" benzothieno[4,5-a]carbazole compound and a family of "up-anglular" indazolo[5,4-a]carbazoles have been prepared by Hudkins et al. (2001) (**Figure 17**). The quinolino[a]carbazoles have been reported by Zhu et al. (2003b). The activity of five "up-angular" quinolino[a]- or isoquinolino[a]carbazole isomers was investigated against cyclin D1-cdk4. The isoquinolino[6,5-a]carbazole compound was found to be the most active among the "up-angular" isomers, with an IC50 of 69 nM, while the "down-angular" quinolino[4,3-a]carbazole and the "linear" quinolino[3,2-a]carbazole compounds were inactive.

Heteroarylcarbazoles

5-Membered ring heterocycles

Didemnimide C is an 3-[1-N-methyl-1*H*-imidazol-5-yl]-4-[indol-3-yl]maleimide natural product, in which the indole ring of arcyriarubin A is replaced by an imidazole ring (Terpin et al., 1998). The imidazo[a]carbazoles granulatimide ([4,5-a] isomer) and isogranulatimide ([1,5-a]b-carboline isomer) were subsequently isolated in 1998 (Berlinck et al., 1998), while the remaining isomers were prepared by Andersen et al. (1999). Isogranulatimide has recently been shown by Jiang et al. (2004) to be an inhibitor of Chk1 kinase (IC50 = 0.1 μM), while Sanchez-Martinez et al. (2003b) found out that synthetic granulatimide was weakly active against cyclin D1-cdk4 (IC50 = 0.74 μM).

Analogs of isogranulatimide, in which the indole has been replaced with a 7-azaindole, as well as those in which the imidazole has been replaced with a pyrrole and by a maleimide, have been prepared by Hugon et al. (2003a; 2003b; 2003c). The later compounds exhibit cytotoxic activity against L1210 murine leukemia cell lines (Prudhomme et al., 2004a; Prudhomme et al.,

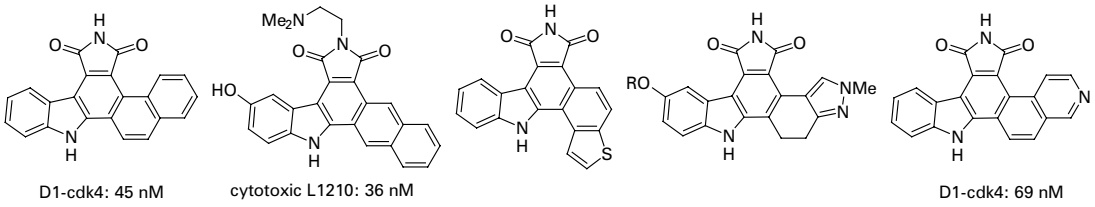


Figure 17. Carbazoles fused to bicyclic aromatics via the carbocyclic ring.

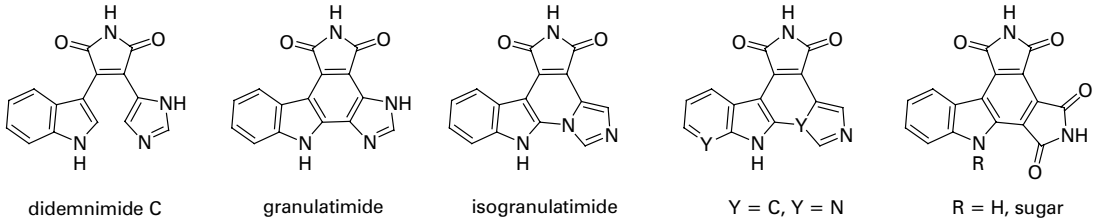


Figure 18. Granulatimide, isogranulatimide, and analogs.

2004b). Glycosylated analogs of the bis-maleimidocarbazoles have recently been prepared in the same group by Henon et al. (2005; 2006). Their biological activities are under investigation. The dihydrofuro[3,2-a]pyrrolo[3,4-c]carbazole-1,3-dione analog was prepared by Tao et al. (2006) as a PARP-1 inhibitor, but was found to be inactive.

6-Membered ring heterocycles

The pyrido[4,3-a]carbazole and tetrahydro-pyrido[4,3-a]carbazole analogs of the indolocarbazoles were prepared by Ator et al. (2001) as potential inhibitors of VEGFR2, PARP-1, and MLK3 (**Figure 19**). The 3-methyl pyridocarbazole analog, for example, inhibits VEGFR2 with an IC50 of 94 nM. In an interesting recent development, Williams et al. (2005) have shown that a ruthenium complex of the pyrido[2,3-a]carbazole analog was a selective, sub-nanomolar inhibitor of GSK-3a and GSK-3b, effective in intervening in a wnt-mediated signal transduction pathway in a cellular system. The quinoline analogs, studied by Zhu et al. (2003), are discussed above, while a benzopyran-fused analog has been prepared by Hudkins et al. (2001).

RING-EXPANDED ANALOGS

Arcyroxepin A is a ring-expanded oxepine analog of arcyriaflavin A, isolated from Arcyria denudata by Steglich et al. (1980), along with arcyriaflavin B and arcyriarubin B (**Figure 19**). Mahboobi et al. (1999c) have reported the synthesis of the carbon analogs of Arcyroxepin, in which the central ring of the indolocarbazole structure was expanded with a methylene group, along with its glycosylated forms. This provides added flexibility and an interesting three-dimensional structure to the molecule, as well as altering the electronic environment of the conjugated system (Mahboobi et al., 1999d).

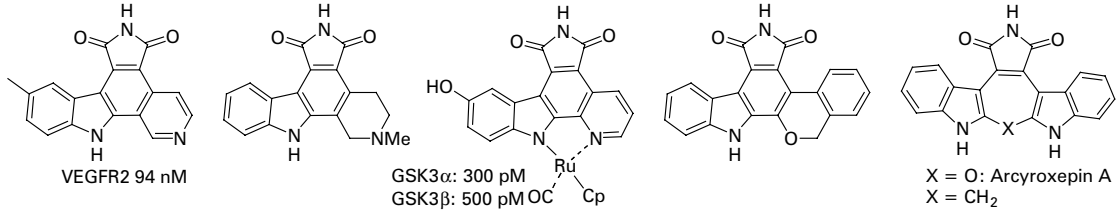


Figure 19. Carbazoles fused to six-membered heterocycles and ring-expanded indolocarbazole analogs.

PERSPECTIVES

The concept of an analog is per se subjective, and the lines that have been drawn here are necessarily arbitrary, as many other structures could be considered as analogs of the indolocarbazole alkaloids. Nonetheless, the great variety of structures that have evolved from STP show that this compound has been a tremendous source of inspiration to medicinal and synthetic chemists. It has been a productive lead compound, not just for PKC, but also for GSK-3β, checkpoint kinases, cyclin dependent kinases, flt-3, DAPK, EGFR, VEGFR, PDGFR, and PIM1, as well as non-kinase targets such as topoisomerase I, PARP-1, and p-glycoprotein. Exquisite selectivity has been achieved based on this promiscuous inhibitor, either by inducing a conformational twist within the indolocarbazole system, or by judicious choice of substituents, which can provide either a specific three-dimensional structure, or specific interactions with the target protein. This effort is far from exhausted, as many novel structures and kinase targets remain to be studied. Our own efforts in this area focus on the C1' position of rebeccamycin and staurosporine: evaluation of steric, conformational, and electronic factors in a critical region of the ATP-binding site (Belhadj & Goekjian, 2005).

A second fundamental issue that has emerged within this class of inhibitors has been the structural and conformational basis for selective inhibition of the ATP-binding site of protein kinases. Each class of inhibitor provides a unique conformational profile, which exploits subtle differences in the ATP-binding sites of the various kinases. Our own studies focus on the macrocyclic bisindolylmaleimides, which adopt fundamentally different conformations as a function of the macrocyclic tether (Goekjian et al., 1999a; Goekjian et al., 1999b; Goekjian et al., 1998; Goekjian et al., 1999c; Blackenbiller et al., 1998; Stroble et al., 1998). Bartlett et al. (2005) have recently published an extensive conformational study of the MBIM, and the implication for kinase selectivity. They conclude that the pseudo-C2 symmetrical "anti" conformation binds to AGC group protein kinases, while GSK-3β binds to the highly canted "syn" conformation. Our interests focus on the selectivity shown by ruboxistaurin between PKC-α and PKC-β. Our studies indicate that two conformations are present in the 14-membered ring macrocyclic bisindolylmaleimides. Does ruboxistaurin bind to PKC-β in a different conformation than to PKC-α, or does PKC-β have a higher affinity than PKC-α for the same "anti" conformer of ruboxistaurin? An understanding of the structural basis for isoform-selectivity of ruboxistaurin will aid in the design of new kinase inhibitors.

The original "hit" in the search for kinase inhibitors, staurosporine, has played a special role in the now vast domain of ATP-competitive kinase inhibitors. The questions above are only two of the important issues that have evolved from this field, and ever more opportunities arise for fundamental, medicinal, clinical and therapeutic research in this area. Some of these questions will be addressed elsewhere in this issue.

REFERENCES

Adeva M, Sahagun H, Caballero E, de Clairac RP-L, Medarde M, Tome F: Open analogues of arcyriaflavin A. Synthesis through Diels-Alder reaction between maleimides and 1-aryl-3-tert-butyldimethylsiloxy-1,3-butadienes. J Org Chem 65: 3387-3394, 2000a

Adeva M, Buono F, Caballero E, Medarde M, Tome F: New synthetic approach to arcyriaflavin-A and unsymmetrical analogs. Synlett 6: 832-834, 2000b

Aiello LP, Hooper P, Ross SA, Lund-Anderson H, Polak B, Forrester JV, Kohner EM, Vora J, Ai E, Aiello LM, Anand R, Blumenkranz M, Brucker AJ, Chandler T, Chong L, Dehning D, Finkelstein D, Frank R, Garcia C, Gardner TW, Gehrs KM, Goodart RA, Faber D, Gottlieb J, Greven CM, Jackson WE, Kinyoun JL, Klein ML, Lewis H., Li HK, Ma C, Dreyer R, Margherio R, Martin DF, Paden PY, Sharuk G, Singerman LJ, Smiddy WE, Trese M, Tweeten JP, Vine A: The effect of ruboxistaurin on visual loss in patients with moderately severe to very severe nonproliferative diabetic retinopathy. Initial results of the protein kinase C b inhibitor diabetic retinopathy study (PKC-DRS) multicenter randomized clinical trial. Diabetes 54: 2188-2197, 2005

Al-Awar RS, Hecker KA, Huang J, Joseph S, Ray JE, Waid PP: Preparation of maleimide and carbazole derivatives for the treatment of proliferative diseases. PCT Int. Appl. WO 2001044235, (Eli Lilly and Company, USA) 110 pp., 2001

Andersen RJ, Roberge M, Sanghera J, Leung D: Preparation of granulatimide derivatives for cancer treatment. PCT Int. Appl. WO 9947522, (The University of British Columbia, Can.) 69 pp., 1999

Anizon F, Belin L, Moreau P, Sancelme M, Voldoire A, Prudhomme M, Ollier M, Severe D, Riou J-F, Bailly C, Fabbro D, Meyer T: Syntheses and biological activities (topoisomerase inhibition and antitumor and antimicrobial properties) of rebeccamycin analogs bearing modified sugar moieties and substituted on the imide nitrogen with a methyl group. J Med Chem 40: 3456-3465, 1997

Anizon F, Moreau P, Sancelme M, Voldoire A, Prudhomme M, Ollier M, Severe D, Riou J-F, Bailly C, Fabbro D, Meyer T, Aubertin AM: Synthesis, biochemical and biological evaluation of staurosporine analogs from the microbial metabolite rebeccamycin. Bioorg Med Chem 6: 1597-1604, 1998

Anizon F, Moreau P, Sancelme M, Laine W, Bailly C, Prudhomme M: Rebeccamycin analogues bearing amine substituents or other groups on the sugar moiety. Bioorg Med Chem 11: 3709-3722, 2003

Ator MA, Bihovsky R, Chatterjee S, Dunn D, Hudkins RL: Preparation of novel multicyclic compounds and their amino acid derivatives as inhibitors of enzymes such as poly(ADP-ribose) polymerase. PCT Int. Appl. WO 2001085686, (Cephalon, Inc., USA) 209 pp., 2001

Atwell S, Adams JM, Badger J, Buchanan MD, Feil IK, Froning KJ, Gao X, Hendle J, Keegan K, Leon BC, Mueller-Dieckmann HJ, Nienaber VL, Noland BW, Post K, Rajashankar KR, Ramos A, Russell M, Burley SK, Buchanan SG: A novel mode of Gleevec binding is revealed by the structure of spleen tyrosine kinase. J Biol Chem 279: 55827-55832, 2004

Balasubramanian BN, St. Laurent DR, Saulnier MG, Long BH, Bachand C, Beaulieu F, Clarke W, Deshpande M, Eummer J, Fairchild CR, Frennesson DB, Kramer R, Lee FY, Mahler M, Martel A, Naidu BN, Rose WC, Russell J, Ruediger E, Solomon C, Stoffan KM, Wong H, Wright JJ, Zimmermann K, Vyas DM: Design and synthesis of a fluoroindolocarbazole series as selective topoisomerase I active agents. Discovery of water-soluble 3,9-difluoro-12,13-dihydro-13-[6-amino-b-D-glucopyranosyl]-5H,13H-benzo[b]-thienyl[2,3-a]pyrrolo[3,4-c]carbazole-5,7(6H)-dione (BMS-251873) with curative antitumor activity against prostate carcinoma xenograft tumor model. J Med Chem 47: 1609-1612, 2004 [Erratum to document: J Med Chem 48: 654, 2005]

Bartlett S, Beddard GS, Jackson RM, Kayser V, Kilner C, Leach A, Nelson A, Oledzki PR, Parker P, Reid GD, Warriner SL: Comparison of the ATP binding sites of protein kinases using conformationally diverse bisindolylmaleimides. J Am Chem Soc 127: 11699-11708, 2005

Belhadj T, Goekjian PG: An amide ortho-esterification route to N-(1'-alkylthioglucopyranosyl)indoles. Tetrahedron Lett 46: 8117-8120, 2005

Bergman J, Pelcman B: Coupling of indoleacetic acid trianion or methyl indoleacetic acid dianion. A biomimetic approach to indolocarbazole alkaloids. Tetrahedron Lett 28: 4441-4444, 1987

Bergman J, Pelcman B: Synthesis of indolo[2,3-a]pyrrolo[3,4-c]carbazoles by double Fischer indolizations. J Org Chem 54: 824-828, 1989

Bergman J, Desarbre E, Koch E: Synthesis of indolo[3,2-a]pyrrolo[3,4-c]carbazole in one step from indole and maleimide. Tetrahedron 55: 2363-2370, 1999

Berlinck RGS, Britton R, Piers E, Lim L, Roberge M, Moreira da Rocha R, Andersen RJ: Granulatimide and isogranulatimide, aromatic alkaloids with G2 checkpoint inhibition activity isolated from the Brazilian Ascidian Didemnum granulatum: Structure elucidation and synthesis. J Org Chem 63: 9850-9856, 1998

Bertrand JA, Thieffine S, Vulpetti A, Cristiani C, Valsasina B, Knapp S, Kalisz HM, Flocco M: Structural characterization of the GSK-3b active site using selective and non-selective ATP-mimetic inhibitors. J Mol Biol 333: 393-407, 2003

Besterman JM, May WS Jr, LeVine H 3rd, Cragoe EJ Jr, Cuatrecasas P: Amiloride inhibits phorbol ester-stimulated Na+/H+ exchange and protein kinase C. An amiloride analog selectively inhibits Na+/H+ exchange. J Biol Chem 260: 1155-1159, 1985

Blanchard BJ, Chen A, Rozeboom LM, Stafford KA, Weigele P, Ingram VM: Efficient reversal of Alzheimer's disease fibril formation and elimination of neurotoxicity by a small molecule. Proc Nat Acad Sci USA 101: 14326-14332, 2004

Blankenbiller DL, Dale CB, Dudley TP, Burke MW, Goekjian PG: UV-visible and NMR spectroscopic studies of the macrocyclic bis(indolyl)maleimides. 216th ACS National Meeting, Boston, 1998

Bonser RW, Slater MJ, Cockerill GS: Preparation of heterocyclylindole derivatives for the treatment of restenosis. PCT Int. Appl. WO 9604906, (Wellcome Foundation Limited, UK) 32 pp., 1996

Booth RJ, Denny WA, Dobrusin EM, Kraker AJ, Mitchell LH, Smaill JB, Thompson AM, Lee HH, McCarthy FOJ, Palmer BD: Preparation of pyrrolocarbazoles, benzofuroisoindoles, and azacyclopentafluorenes inhibitors of checkpoint kinases WEE1 and CHK1. PCT Int. Appl. WO 2003091255, (Warner-Lambert Company Llc, USA; et al.) 356 pp., 2003

Brazzell RK, Wood JM, Campochiaro PA, Kane FE: Staurosporine derivatives for treating ocular neovascular diseases. PCT Int. Appl. WO 2000030651, (Novartis-Erfindungen Verwaltungs Gesellschaft M.B., Austria) 20 pp., 2000

Brenner M, Rexhausen H, Steffan B, Steglich W: Pigments of fungi, 57. Synthesis of arcyriarubin B and related bisindolylmaleimides. Tetrahedron 44: 2887-2892, 1988

Broka CA: Preparation and formulation of pyrrolocarbazoles as protein kinase C inhibitors. Eur. Pat. Appl. EPO 695755, (F. Hoffmann-La Roche Ag, Switz.) 15 pp., 1996

Brown K, Long JM, Vial SCM, Dedi N, Dunster NJ, Renwick SB, Tanner AJ, Frantz JD, Fleming MA, Cheetham GMT: Crystal structures of interleukin-2 tyrosine kinase and their implications for the design of selective inhibitors. J Biol Chem 279: 18727-18732, 2004

Buchdunger E, Trinks U, Mett H, Regenass U, Mueller M, Meyer T, McGlynn E, Pinna LA, Traxler P, Lydon NB: 4,5-Dianilinophthalimide: a protein-tyrosine kinase inhibitor with selectivity for the epidermal growth factor receptor signal transduction pathway and potent in vivo antitumor activity. Proc Nat AcadSci USA 91: 2334-2338, 1994

Buchdunger E, Mett H, Trinks U, Regenass U, Mueller M, Meyer T, Beilstein P, Wirz B, Schneider P et al.: 4,5-Bis(4-fluoroanilino)phthalimide: a selective inhibitor of the epidermal growth factor receptor signal transduction pathway with potent in vivo antitumor activity. Clin Cancer Res 1: 813-821, 1995

Caballero E, Longieras N, Zausa E, del Rey B, Medarde M, Tome F: Diels-Alder reactivity and some synthetic applications of (E)-1-(3-indolyl)-3-tert-butyldimethyl-siloxy-1,3-butadienes. Tetrahedron Lett 42: 7233-7236, 2001

Caballero E, Adeva M, Calderon S, Sahagun H, Tome F, Medarde M, Fernandez JL, Lopez-Lazaro M, Ayuso MJ: Synthesis and cytotoxic activity of different open indolocarbazole alkaloid analogues. Bioorg Med Chem 11: 3413-3421, 2003

Cai Y, Fredenhagen A, Hug P, Peter HH: A nitro analog of staurosporine and other minor metabolites produced by a Streptomyces longisporoflavus strain. J Antibiot 48: 143-148, 1995

Cai Y, Fredenhagen A, Hug P, Peter HH: The staurosporine producing strain Streptomyces longisporoflavus produces metabolites related to K-252a. Proposal for biosynthetic intermediates of K-252a. J Antibiot 49: 1060-1062, 1996

Cai Y, Ouyang, L: Synthesis of staurosporine derivatives. Zhongguo Yiyao Gongye Zazhi 32: 12-13, 23, 2001

Cantrell CL, Groweiss A, Gustafson KR, Boyd MR: A new staurosporine analog from the prosobranch mollusk Coriocella nigra. Natural Product Lett 14: 39-46, 1999

Caravatti G, Meyer T, Fredenhagen A, Trinks U, Mett H, Fabbro D: Inhibitory activity and selectivity of staurosporine derivatives towards protein kinase C. Bioorg Med Chem Lett 4: 399-404, 1994

Dardennes E, Kovacs-Kulyassa A, Boisbrun M, Petermann C, Laronze J-Y, Sapi J: Diastereocontrolled multicomponent pathway to 3,4-heterocycle-annulated tetrahydro-b-carbolines. Tetrahedron: Asymm 16: 1329-1339, 2005

Davis PD, Hill CH, Lawton G, Nixon JS, Wilkinson SE, Hurst SA, Keech E, Turner SE: Inhibitors of protein kinase C. 1. 2,3-bisarylmaleimides. J Med Chem *35*: 177-184, 1992a

Davis PD, Elliott LH, Harris W, Hill CH, Hurst SA, Keech E, Kumar MKH, Lawton G, Nixon JS, Wilkinson SE: Inhibitors of protein kinase C. 2. Substituted bisindolylmaleimides with improved potency and selectivity. J Med Chem 35: 994-1001, 1992b

Davies SP, Reddy H, Caivano M, Cohen P: Specificity and mechanism of action of some commonly used protein kinase inhibitors. Biochem J 351: 95-105, 2000

Denny WA: Edotecarin (Banyu/Pfizer). IDrugs 7: 173-177, 2004

Dinney CP, Parker C, Dong Z, Fan D, Eve BY, Bucana C, Radinsky R: Therapy of human transitional cell carcinoma of the bladder by oral administration of the epidermal growth factor receptor protein tyrosine kinase inhibitor 4,5-dianilinophthalimide. Clin Cancer Res 3: 161-168, 1997

Dupont J, Bienvenu B, Aghajanian C, Pezzulli S, Sabbatini P, Vongphrachanh P, Chang C, Perkell C, Ng K, Passe S, Breimer L, Zhi J, DeMario M, Spriggs D, Soignet SL: Phase I and pharmacokinetic study of the novel oral cell-cycle inhibitor Ro 31-7453 in patients with advanced solid tumors. J Clin Oncol 22: 3366-3374, 2004

Engler TA, Furness K, Malhotra S, Sanchez-Martinez C, Shih C, Xie W, Zhu G, Zhou X, Conner S, Faul MM, Sullivan KA, Kolis SP, Brooks HB, Patel B, Schultz RM, DeHahn TB, Kirmani K, Spencer CD, Watkins SA, Considine EL, Dempsey JA, Ogg CA, Stamm NB, Anderson BD, Campbell RM, Vasudevan V, Lytle ML: Novel, potent and selective cyclin D1/CDK4 inhibitors: indolo[6,7-a]pyrrolo[3,4-c]carbazoles. Bioorg Med Chem Lett 13: 2261-2267, 2003

Fabian MA, Biggs WH, Treiber DK, Atteridge CE, Azimioara MD, Benedetti MG, Carter TA, Ciceri P, Edeen PT, Floyd M, Ford JM, Galvin M, Gerlach JL, Grotzfeld RM, Herrgard S, Insko DE, Insko MA, Lai AG, Lelias J-M, Mehta SA, Milanov ZV, Velasco AM, Wodicka LM, Patel HK, Zarrinkar PP, Lockhart DJ: A small molecule-kinase interaction map for clinical kinase inhibitors. Nat Biotechnol 23: 329-336, 2005

Facompre M, Carrasco C, Vezin H, Chisholm JD, Yoburn JC, Van Vranken DL, Bailly C: Indolocarbazole glycosides in inactive conformations. ChemBioChem 4: 386-395, 2003

Faul MM, Sullivan KA, Grutsch JL, Winneroski LL, Shih C, Sanchez-Martinez C, Cooper JT: Synthesis of indolo[2,3-a]carbazole glycoside analogs of rebeccamycin: inhibitors of cyclin D1-CDK4. Tetrahedron Lett 45: 1095-1098, 2004

Fabre S, Prudhomme M, Sancelme M, Rapp M: Indolocarbazole protein kinase C inhibitors from rebeccamycin. Bioorg Med Chem 2: 73-77, 1994

Faul MM, Gillig JR, Jirousek MR, Ballas LM, Schotten T, Kahl A, Mohr M: Acyclic N-(azacycloalkyl)bisindolylmaleimides: isozyme selective inhibitors of PKCb. Bioorg Med Chem Lett 13: 1857-1859, 2003

Faul MM, Engler TA, Sullivan KA, Grutsch JL, Clayton MT, Martinelli MJ, Pawlak JM, LeTourneau M, Coffey DS, Pedersen SW, Kolis SP, Furness K, Malhotra S, Al-Awar RS, Ray JE: Synthetic approaches to indolo[6,7-a]pyrrolo[3,4-c]carbazoles: Potent cyclin D1/CDK4 inhibitors. J Org Chem 69: 2967-2975, 2004

Fonseca AP, Lobo AM, Prabhaker S: A novel synthesis of arcyriaflavin-A. Tetrahedron Lett 36: 2689-2692, 1995

Furet P, Caravatti G, Lydon N, Priestle JP, Sowadski JM, Trinks U, Traxler P: Modeling study of protein kinase inhibitors: binding mode of staurosporine and origin of the selectivity of CGP 52411. J Comp-Aid Mol Design 9: 465-472, 1995

Furusaki A, Hashiba N, Matsumoto T, Hirano A, Iwai Y, Omura S: The crystal and molecular structure of staurosporine, a new alkaloid from a Streptomyces strain. Bull Chem Soc Japan 55: 3681-3685, 1982

Gambacorti-Passerini C, Gasser M, Ahmed S, Assouline S, Scapozza L: Abl inhibitor BMS354825 binding mode in Abelson kinase revealed by molecular docking studies. Leukemia 19: 1267-1269, 2005 [Erratum to document cited: Leukemia 19: 2023, 2005]

Gassel M, Breitenlechner CB, Koenig N, Huber R, Engh RA, Bossemeyer D: The protein kinase C inhibitor bisindolyl maleimide 2 binds with reversed orientations to different conformations of protein kinase A. J Biol Chem 279: 23679-23690, 2004

Gescher A: Analogs of staurosporine: potential anticancer drugs? Gen Pharmacol 31: 721-728, 1998

Gescher, A: Staurosporine analogues – pharmacological toys or useful antitumour agents? Crit Rev Oncol Hematol 34: 127-135, 2000

Gilbert EJ, Chisholm JD, Van Vranken DL: Conformational control in the rebeccamycin class of indolocarbazole glycosides. J Org Chem 64: 5670-5676, 1999

Gingrich DE, Reddy DR, Iqbal MA, Singh J, Aimone LD, Angeles TS, Albom M, Yang S, Ator MA, Meyer SL, Robinson C, Ruggeri BA, Dionne CA, Vaught JL, Mallamo JP, Hudkins RL: A new class of potent vascular endothelial growth factor receptor tyrosine kinase inhibitors: Structure-activity relationships for a series of 9-alkoxymethyl-12-(3-hydroxypropyl)indeno[2,1-a]pyrrolo[3,4-c]carbazole-5-ones and the identification of CEP-5214 and its dimethylglycine ester prodrug clinical candidate CEP-7055. J Med Chem 46: 5375-5388, 2003

Gingrich DE, Yang SX, Gessner GW, Angeles TS, Hudkins RL: Synthesis, modeling, and in vitro activity of (3'S)-epi-K-252a analogues. Elucidating the stereochemical requirements of the 3'-sugar alcohol on trkA tyrosine kinase activity. J Med Chem 48: 3776-3783, 2005

Goekjian PG, Saebo S, Gillig JR, Stephenson GA, Dale CB, Blankenbiller DL, Dudley TP: The effect of the macrocycle on the conformation of the bis(indolyl)maleimide of LY333531 and related compounds. 216th ACS National Meeting, Boston, 1998

Goekjian PG, Lugo-Mas P, Cable SL, Cole JO, White JW, Thompson DJ, Dudley TP, Jirousek MR, Dixon JT, Ballas LM: Synthesis of indole-ring fluorine-labeled analogs of LY333531, an isoform-selective inhibitor of protein kinase C. J Fluor Chem 98: 137-142, 1999a

Goekjian PG, Nikic DB, Salin ML: Comparison of the inhibition profiles of isoform-selective inhibitors against PKC and PKM isoforms. 218th ACS National Meeting, New Orleans, 1999b

Goekjian PG, Wu G-Z, Chen S, Zhou L, Jirousek MR, Gillig JR, Ballas LM, Dixon JT: Synthesis of fluorinated macrocyclic bis(indolyl)maleimides as potential 19F NMR probes for protein kinase C. J Org Chem 64: 4238-4246, 1999c

Goekjian PG, Jirousek MR: Protein kinase C inhibitors as novel anticancer drugs. Exp Opin Invest Drug 10: 2117-2140, 2001

Griffin JD, Manley PW: Staurosporine derivatives as inhibitors of FLT3 receptor tyrosine kinase activity. PCT Int. Appl. WO 2003037347, (Novartis A.-G., Switz.; Novartis Pharma G.m.b.H.; Dana-Farber Cancer Institute, Inc.) 73 pp., 2003

Han Z, Pantazis P, Lange TS, Wyche JH, Hendrickson EA: The staurosporine analog, Ro-31-8220, induces apoptosis independently of its ability to inhibit protein kinase C. Cell Death Diff 7: 521-530, 2000

He Z, King GL: Can protein kinase C b-selective inhibitor, ruboxistaurin, stop vascular complications in diabetic patients? Diabetes Care 28: 2803-2805, 2005

Henon H, Messaoudi S, Hugon B, Anizon F, Pfeiffer B, Prudhomme M: Synthesis of granulatimide bis-imide analogues. Tetrahedron 61: 5599-5614, 2005

Henon H, Anizon F, Pfeiffer B, Prudhomme M: Synthesis of dipyrrolo[3,4-a:3,4-c]carbazole-1,3,4,6-tetraones bearing a sugar moiety. Tetrahedron 62: 1116-1123, 2006

Hernandez LMC, De la Fuente Blanco JA, Baz JP, Puentes JLF, Millan FR, Vazquez FE, Fernandez-Chimeno RI, Gravalos DG: 4'-N-methyl-5'-hydroxystaurosporine and 5'-hydroxystaurosporine, new indolocarbazole alkaloids from a marine Micromonospora sp. strain. J Antibiot 53: 895-902, 2000

Hidaka H, Inagaki M, Kawamoto S, Sasaki Y: Isoquinolinesulfonamides, novel and potent inhibitors of cyclic nucleotide dependent protein kinase and protein kinase C. Biochem 23: 5036-5041, 1984

Holton S, Merckx A, Burgess D, Doerig C, Noble M, Endicott J: Structures of P. falciparum PfPK5 test the CDK regulation paradigm and suggest mechanisms of small molecule inhibition. Structure 11: 1329-1337, 2003

Http://clinicaltrials.gov.

Hudkins RL, Knight E Jr: Preparation of fused pyrrolocarbazoles as inhibitors of protein kinase C and protein tyrosine kinase. PCT Int. Appl. WO 9611933, (Cephalon, Inc., USA) 160 pp., 1996

Hudkins RL, Reddy D, Singh J, Stripathy R, Underiner TL: Preparation of cyclic substituted fused pyrrolocarbazoles and isoindolones with protein kinase inhibiting activity for pharmaceutical use. PCT Int. Appl. WO 2000047583, (Cephalon, Inc., USA) 131 pp., 2000

Hudkins RL: Preparation of indenopyrrolocarbazolones and analogs as kinase inhibitors. PCT Int. Appl. WO 2001014380, (Cephalon, Inc., USA) 81 pp., 2001

Hudkins RL, Johnson NW: Synthesis of benzo[b]thieno- and benzo[b]furano[2,3-a]pyrrolo[3,4-c]carbazol-5-one, -7-one, and -carbazole-5,7-dione. J Heterocyclic Chem 38: 591-595, 2001

Hugon B, Pfeiffer B, Renard P, Prudhomme M: Synthesis of isogranulatimides A and B analogues possessing a 7-azaindole unit instead of an indole moiety. Tetrahedron Lett 44: 4607-4611, 2003a

Hugon B, Pfeiffer B, Renard P, Prudhomme M: Synthesis of isogranulatimide analogues possessing a pyrrole moiety instead of an imidazole heterocycle. Tetrahedron Lett 44: 3927-3930, 2003b

Hugon B, Pfeiffer B, Renard P, Prudhomme M: Synthesis of granulatimide analogues bearing a maleimide instead of an imidazole heterocycle. Tetrahedron Lett 44: 3935-3937, 2003c

Inagaki M, Watanabe M, Hidaka H: N-(2-Aminoethyl)-5-isoquinolinesulfonamide, a newly synthesized protein kinase inhibitor, functions as a ligand in affinity chromatography. Purification of Ca2+-activated, phospholipid-dependent and other protein kinases. J Biol Chem 260: 2922-2925, 1985

Jacobs MD, Black J, Futer O, Swenson L, Hare B, Fleming M, Saxena K: Pim-1 ligand-bound structures reveal the mechanism of serine/threonine kinase inhibition by LY294002. J Biol Chem 280: 13728-13734, 2005

Janosik T, Bergman J: Reactions of 2,3'-biindolyl: synthesis of indolo[3,2-a]carbazoles. Tetrahedron 55: 2371-2380, 1999

Jaquith JB, Fallis A, Gillard J: Neuroprotective and anti-proliferative analogs of staurosporine and granulatimide, namely 3-(1H-indol-3-yl)-1H-pyrrole-2,5-diones, 3-(1H-indol-3-yl)-4-(1H-indol-1-yl)-1H-pyrrole-2,5-diones, and pyrrolo-b-carboline derivatives, and their preparation and use as modulators of apoptosis. PCT Int. Appl. WO 2001087887, (Aegera Therapeutics Inc., Can.) 95 pp., 2001

Jiang X, Zhao B, Britton R, Lim LY, Leong D, Sanghera JS, Zhou B-BS, Piers E, Andersen RJ, Roberge M: Inhibition of Chk1 by the G2 DNA damage checkpoint inhibitor isogranulatimide. Mol Cancer Ther 3: 1221-1227, 2004

Jin L, Pluskey S, Petrella EC, Cantin SM, Gorga JC, Rynkiewicz MJ, Pandey P, Strickler JE, Babine RE, Weaver DT, Seidl KJ: The three-dimensional structure of the ZAP-70 kinase domain in complex with Staurosporine: Implications for the design of selective inhibitors. J Biol Chem 279: 42818-42825, 2004

Jirousek MR, Gillig JR, Gonzalez CM, Heath WF, McDonald JH III, Neel DA, Rito CJ, Singh U, Stramm LE et al.: (S)-13-[(Dimethylamino)methyl]-10,11,14,15-tetrahydro-4,9:16,21-dimetheno-1H,13H-dibenzo[e,k]pyrrolo[3,4-h][1,4,13]oxadiazacyclohexadecene-1,3(2H)-dione (LY333531) and related analogs: Isoenzyme selective inhibitors of protein kinase Cb. J Med Chem 39: 2664-2671, 1996

Johnson LN, De Moliner E, Brown NR, Song H, Barford D, Endicott JA, Noble MEM: Structural studies with inhibitors of the cell cycle regulatory kinase cyclin-dependent protein kinase 2. Pharmacol Ther 93: 113-124, 2002

Joyce RP, Gainor JA, Weinreb SM: Synthesis of the aromatic and monosaccharide moieties of staurosporine. J Org Chem 52: 1177-1185, 1987

Kanai F, Amishiro N, Kitamura Y, Murakata C, Akiyama T, Akinaga S, Fuse E, Kuwabara T, Yasoshima K: Preparation of staurosporin derivatives as antitumor agents. PCT Int. Appl. WO 2001004125, (Kyowa Hakko Kogyo Co., Ltd., Japan) 190 pp., 2001

Kanai F, Murakata C, Tsujita T, Yamashita Y, Mizukami T, Akinaga S: Preparation of pyrrolo[3,4-e]indole and pyrrolo[3,4-c]carbazole derivatives for treatment of malignant tumor or brain neurodegenerative diseases. PCT Int. Appl. WO 2003051883, (Kyowa Hakko Kogyo Co., Ltd., Japan) 107 pp., 2003

Kaneko T, Wong H, Okamoto KT, Clardy J: Two synthetic approaches to rebeccamycin. Tetrahedron Lett 26: 4015-4018, 1985

Katsuhisa K, Hisao K, Hiroharu A, Ohkubo M, Hiroyuki S: Preparation of indolopyrrolocarbazole nucleosides as neoplasm inhibitors. Eur. Pat. Appl. EPO 545195, (Banyu Pharmaceutical Co., Ltd., Japan) 56 pp., 1993

Kawamoto S, Hidaka H: 1-(5-Isoquinolinesulfonyl)-2-methylpiperazine (H-7) is a selective inhibitor of protein kinase C in rabbit platelets. Biochem Biophys Res Comm 125: 258-264, 1984

Kikkawa U, Takai Y, Tanaka Y, Miyake R, Nishizuka Y: Protein kinase C as a possible receptor protein of tumor-promoting phorbol esters. J Biol Chem 258: 11442-11445, 1983

Kinnel RB, Scheuer PJ: 11-Hydroxystaurosporine: a highly cytotoxic, powerful protein kinase C inhibitor from a tunicate. J Org Chem 57: 6327-6329, 1992

Kleinschroth J, Hartenstein J, Schaechtele C, Rudolph C, Dooley DJ, Weinheimer G: Preparation of 1,3-dioxo-4-phenylpyrrolo[3,4-c]carbazoles and analogs as cardiovascular agents. U.S. Patent USP 4912107, (Goedecke A.-G., Germany) 11 pp., 1990

Kojiri K, Kondo H, Arakawa H, Ohkubo M, Suda H: Preparation of indolopyrrolocarbazole nucleoside analogs as antitumors. US Patent USP 5591842, (Banyu Pharmaceutical Co., Ltd., Japan) 40 pp., 1997

Komander D, Kular GS, Schuttelkopf AW, Deak M, Prakash KRC, Bain J, Elliott M, Garrido-Franco M, Kozikowski AP, Alessi DR, van Aalten DMF: Interactions of LY333531 and other bisindolyl maleimide inhibitors with PDK1. Structure 12: 215-226, 2004

Kuo G-H, Prouty C, DeAngelis A, Shen L, O'Neill DJ, Shah C, Connolly PJ, Murray WV, Conway BR, Cheung P, Westover L, Xu JZ, Look RA, Demarest KT, Emanuel S, Middleton SA, Jolliffe L, Beavers MP, Chen X: Synthesis and discovery of macrocyclic polyoxygenated bis-7-azaindolylmaleimides as a novel series of potent and highly selective glycogen synthase kinase-3b inhibitors. J Med Chem 46: 4021-4031, 2003

Lamers MBAC, Antson AA, Hubbard RE, Scott RK, Williams DH: Structure of the protein tyrosine kinase domain of C-terminal src kinase (CSK) in complex with staurosporine. J Mol Biol 285: 713-725, 1999

Laronze M, Boisbrun M, Leonce S, Pfeiffer B, Renard P, Lozach O, Meijer L, Lansiaux A, Bailly C, Sapi J, Laronze JY: Synthesis and anticancer activity of new pyrrolocarbazoles and pyrrolo-b-carbolines. Bioorg Med Chem 13: 2263-2283, 2005

Lawrie AM, Noble MEM, Tunnah P, Brown NR, Johnson LN, Endicott JA: Protein kinase inhibition by staurosporine revealed in details of the molecular interaction with CDK2. Nat Struct Biol 4: 796-801, 1997

Lewis ME, Kauer JC, Neff N, Glicksman M, Roberts-Lewis J, Murakata C, Saito H, Matsuda Y, Kanai F, Kaneko M: Protein kinase inhibitors for treatment of neurological disorders. PCT Int. Appl. WO 9613506, (Cephalon, Inc., USA; Kyowa Hakko Kogyo Co., Ltd.) 162 pp., 1996

Li Z, Sunazuka T, Yamada R, Kato Y, Enomoto A, Hayashi M, Harigaya Y, Omura S: Synthesis and antiangiogenic activity of staurosporine derivatives. J Antibiot 53: 426-429, 2000

Link JT, Raghavan S, Gallant M, Danishefsky SJ, Chou TC, Ballas LM: Staurosporine and ent-staurosporine: The first total syntheses, prospects for a regioselective approach, and activity profiles. J Am Chem Soc 118: 2825-2842, 1996

Ma D-W, Zhang Y-D, Zhang X-R, Wu S-H, Tao F-G: Synthesis of a benzofuran analog of Go6976, an isoform-selective protein kinase C inhibitor. Chin J Chem 19: 489-492, 2001

Mahata M, Mahapatra NR, O'Connor DT, Mahata SK: Chromaffin cell catecholamine secretion: bisindolylmaleimide compounds exhibit novel and potent antagonist effects at the nicotinic cholinergic receptor in pheochromocytoma cells. Mol Pharmacol 61: 1340-1347, 2002

Mahboobi S, Eluwa S, Kumar SKC, Koller M, Storl K: b-Carbolinedione derivatives as topoisomerase I inhibitors. Archiv Pharm 332: 249-254, 1999a

Mahboobi S, Eluwa S, Koller M, Boehmer F-D, Uecker A, Teller S: Preparation of pyrrolopyridoindolediones and analogs as tyrosine kinase inhibitors. Ger Offen DeP 19744257, (Asta Medica A.-G., Germany; Zentaris GmbH) 20 pp., 1999b

Mahboobi S, Kuhr S, Pongratz H, Popp A, Hufsky H, Bohmer F-D, Teller S, Uecker A, Beckers T: Indole derivatives and their use in the treatment of malignant and other diseases caused by pathological cell proliferation. PCT Int. Appl. WO 9957117, (Asta Medica Aktiengesellschaft, Germany) 62 pp., 1999c

Mahboobi S, Burgemeister T, Dove S, Kuhr S, Popp A: Homoarcyriaflavin: Synthesis of ring-expanded arcyriaflavin analogues. J Org Chem 64: 8130-8137, 1999d

Marminon C, Anizon F, Moreau P, Leonce S, Pierre A, Pfeiffer B, Renard P, Prudhomme M: Syntheses and antiproliferative activities of new rebeccamycin derivatives with the sugar unit linked to both indole nitrogens. J Med Chem 45: 1330-1339, 2002a

Marminon C, Facompre M, Bailly C, Hickman J, Pierre A, Pfeiffer B, Renard P, Prudhomme M: Dimers from dechlorinated rebeccamycin: synthesis, interaction with DNA, and antiproliferative activities. Eur J Med Chem 37: 435-440, 2002b

Marminon C, Pierre A, Pfeiffer B, Perez V, Leonce S, Joubert A, Bailly C, Renard P, Hickman J, Prudhomme M: Syntheses and antiproliferative activities of 7-azarebeccamycin analogues bearing one 7-azaindole moiety. J Med Chem 46: 609-622, 2003a

Marminon C, Pierre A, Pfeiffer B, Perez V, Leonce S, Renard P, Prudhomme M: Syntheses and antiproliferative activities of rebeccamycin analogues bearing two 7-azaindole moieties. Bioorg Med Chem 11: 679-687, 2003b

Martiny-Baron G, Kazanietz MG, Mischak H, Blumberg PM, Kochs G, Hug H, Marme D, Schachtele C: Selective inhibition of protein kinase C isozymes by the indolocarbazole Go 6976. J Biol Chem 268: 9194-9197, 1993

McCort G, Duclos O, Cadilhac C, Guilpain E: A versatile new synthesis of 4-aryl- and heteroaryl-[3,4-c]pyrrolocarbazoles by [4+2] cycloaddition followed by palladium catalyzed cross coupling. Tetrahedron Lett 40: 6211-6215, 1999

Messaoudi S, Anizon F, Leonce S, Pierre A, Pfeiffer B, Prudhomme M: Synthesis and cytotoxicities of 7-aza rebeccamycin analogs bearing various substituents on the sugar moiety, on the imide nitrogen and on the carbazole framework. Eur J Med Chem 40: 961-971, 2005

Messerschmidt A, Macieira S, Velarde M, Baedeker M, Benda C, Jestel A, Brandstetter H, Neuefeind T, Blaesse M: Crystal structure of the catalytic domain of human atypical protein kinase C-iota reveals interaction mode of phosphorylation site in turn motif. J Mol Biol 352: 918-931, 2005

Miyamoto K, Inoko K, Wakusawa S, Kajita S, Hasegawa T, Takagi K, Koyama M: Inhibition of multidrug resistance by a new staurosporine derivative, NA-382, in vitro and in vivo. Cancer Res 53: 1555-1559, 1993

Moffat D, Nichols CJ, Riley DA, Simpkins NS: The synthesis of bioactive indolocarbazoles related to K-252a. Org Biomol Chem 3: 2953-2975, 2005

Monse B, Braxmeier T, Ferrand S, Gordon S, Klafki H, Lahu G, Roder H, Sahagun-Krause H, Seneci P, Thillaye du Boullay O: Preparation of N,N-bridged, nitrogen-substituted carbacyclic indolocarbazoles for use in pharmaceutical compositions as protein kinase inhibitors. PCT Int. Appl. WO 2004048384, (Nad A.-G., Germany) 89 pp., 2004

Moreau P, Anizon F, Sancelme M, Prudhomme M, Bailly C, Carrasco C, Ollier M, Severe D, Riou J-F, Fabbro D, Meyer T, Aubertin A-M: Syntheses and biological evaluation of indolocarbazoles, analogs of rebeccamycin, modified at the imide heterocycle. J Med Chem 41: 1631-1640, 1998

Moreau P, Anizon F, Sancelme M, Prudhomme M, Severe D, Riou J-F, Goossens J-F, Henichart J-P, Bailly C, Labourier E, Tazzi J, Fabbro D, Meyer T, Aubertin AM: Synthesis, mode of action, and biological activities of rebeccamycin bromo derivatives. J Med Chem 42: 1816-1822, 1999

Moreau P, Sancelme M, Bailly C, Leonce S, Pierre A, Hickman J, Pfeiffer B, Prudhomme M: Synthesis and biological activities of indolocarbazoles bearing amino acid residues. Eur J Med Chem 36: 887-897, 2001

Mucke HAM: CEP-1347, Cephalon. IDrugs 6: 377-383, 2003

Murakata C, Saitho Y, Akinaga S, Okabe M: Preparation of staurosporine derivatives as sensitivity enhancers for antineoplastic agents. PCT Int. Appl. WO 9420106, (Kyowa Hakko Kogyo Co., Ltd., Japan) 25 pp., 1994

Murakata C, Kanai F, Saitoh Y, Shiotsu Y, Shiraki T, Tamaoki T, Akinaga S, Okabe M: Preparation of indolocarbazoles for treatment of thrombocytopenia. Eur. Pat. Appl. EPO 675125, (Kyowa Hakko Kogyo Co., Ltd., Japan) 54 pp., 1995

Murakata I, Hara A, Ishii T, Amishiro N: Apoptosis inducer. Jpn. Kokai Tokkyo Koho Jp 2002275068, (Kyowa Hakko Kogyo Co., Ltd., Japan) 13 pp., 2002

Nakase K, Nakajima S, Hirayama M, Kondo H, Kojiri K, Suda H: Antitumor substance BE-54017 and its manufacture with Streptomyces species. Jpn. Kokai Tokkyo Koho JP 2000178274, (Banyu Pharmaceutical Co., Ltd., Japan) 7 pp., 2000

Nichols C, Simpkins NS: Synthesis of bioactive indolocarbazoles: synthesis, nucleophilic ring-opening and chiral base desymmetrisation of a cyclic sulfate intermediate. Tetrahedron Lett 45: 7469-7473, 2004

Nomura M, Sugiura N, Miyamoto K-I: A protein kinase C inhibitor NA-382 prolongs the life span of AH66F-bearing rats as well as inhibiting leukocyte function-associated antigen-1 (LFA-1)-dependent adhesion of the cells. Biol Pharm Bull 19: 1611-1613, 1996

O'Brian CA, Liskamp RM, Solomon DH, Weinstein IB: Inhibition of protein kinase C by tamoxifen. Cancer Res 45: 2462-2465, 1985

Ohkubo M, Nishimura T, Honma T, Nishimura I, Ito S, Yoshinari T, Suda HAH, Morishima H, Nishimura S: Synthesis and biological activities of NB-506 analogues: effects of the positions of two hydroxyl groups at the indole rings. Bioorg Med Chem Lett 9: 3307-3312, 1999

Ohkubo M, Nishimura T, Kawamoto H, Nakano M, Honma T, Yoshinari T, Arakawa H, Suda H, Morishima H, Nishimura S: Synthesis and biological activities of NB-506 analogues modified at the glucose group. Bioorg Med Chem Lett 10: 419-422, 2000

Omura S, Iwai Y, Hirano A, Nakagawa A, Awaya J, Tsuchiya H, Takahashi Y, Masuma R: A new alkaloid AM-2282 of Streptomyces origin. Taxonomy, fermentation, isolation and preliminary characterization. J Antibiot 30: 275-282, 1977

Omura S, Sasaki Y, Iwai Y, Takeshima H: Staurosporine, a potentially important gift from a microorganism. J Antibiot 48: 535-548, 1995

Ootsuka Y, Nishimata T, Fushihara K, Iimori T, Ooishi T: Preparation of SF2370 derivatives as protein kinase C inhibitors. Jpn. Kokai Tokkyo Koho JP 05247054, (Meiji Seika Co, Japan) 9 pp., 1993

Osada H, Takahashi H, Tsunoda K, Kusakabe H, Isono K: A new inhibitor of protein kinase C, RK-286C (4'-demethylamino-4'-hydroxystaurosporine). I. Screening, taxonomy, fermentation and biological activity. J Antibiot 43: 163-167, 1990

Pearce HL, Miller,MA: The evolution of cancer research and drug discovery at Lilly Research Laboratories. Adv Enz Reg 45229-255, 2005

Peifer C, Stoiber T, Unger E, Totzke F, Schaechtele C, Marme D, Brenk R, Klebe G, Schollmeyer D, Dannhardt G: Design, synthesis, and biological evaluation of 3,4-diarylmaleimides as angiogenesis inhibitors. J Med Chem 49: 1271-1281, 2006

Pereira ER, Belin L, Sancelme M, Prudhomme M, Ollier M, Rapp M, Severe D, Riou J-F, Fabbro D, Meyer T:

Structure-activity relationships in a series of substituted indolocarbazoles: Topoisomerase I and protein kinase C inhibition and antitumoral and antimicrobial properties. J Med Chem 39: 4471-4477, 1996

Prade L, Engh RA, Girod A, Kinzel V, Huber R, Bossemeyer D: Staurosporine-induced conformational changes of cAMP-dependent protein kinase catalytic subunit explain inhibitory potential. Structure 5: 1627-1637, 1997

Prudhomme M: Indolocarbazoles as anti-cancer agents. Curr Pharm Design 3: 265-290, 1997

Prudhomme M, Anizon F, Moreau P: Recent developments in the synthesis of indolocarbazoles, topoisomerase I inhibitors. Rec Res Dev Synth Org Chem 279-106, 1999

Prudhomme M: Recent developments of rebeccamycin analogues as topoisomerase I inhibitors and antitumor agents. Curr Med Chem 7: 1189-1212, 2000

Prudhomme M: Rebeccamycin analogs as anti-cancer agents. Eur J Med Chem 38: 123-140, 2003

Prudhomme M, Marminon C, Routier S, Coudert G, Merour J-Y, Hickman J, Pierre A, Renard P, Pfeiffer B: Preparation of pyrido-pyrido-pyrrolo pyrrolo-indole and pyrido-pyrrolo pyrrolo carbazole nucleoside analogs as antitumor agents. PCT Int. Appl. WO 2003002563, (Les Laboratoires Servier, Fr.) 79 pp., 2003

Prudhomme M: Biological targets of antitumor indolocarbazoles bearing a sugar moiety. Curr Med Chem: Anti-Cancer Agents 4: 509-521, 2004

Prudhomme M, Hugon B, Anizon F, Hickman J, Pierre A, Golsteyn R, Renard P, Pfeiffer B: Preparation of pyrrolo[3,4-c]carbazoles and pyrido[2,3-b]pyrrolo[3,4-e]indoles as antitumor agents for treating leukemia. Fr. Demande FrP 2845995, (Les Laboratoires Servier, Fr.) 71 pp., 2004a

Prudhomme M, Hugon B, Anizon F, Hickman J, Pierre A, Golsteyn R, Renard P, Pfeiffer B: New anticancer bis-fused [3,4-a:3,4-c]carbazole derivatives, process for their preparation, and pharmaceutical compositions containing them. Eur. Pat. Appl. EPO 1411057, (Les Laboratoires Servier, Fr.) 42 pp., 2004b

Prudhomme M: Staurosporines and structurally related indolocarbazoles as antitumor agents. Anticancer Agents Nat Prod 499-517, 2005

Regenass U, Caravatti G, Fredenhagen A, Wacker O: Indolocarbazole alkaloid derivatives for sensitizing multidrug-resistant cells to antitumor agents. PCT Int. Appl. WO 9532975, (Ciba-Geigy A.-G., Switz.) 61 pp., 1995

Riley DA, Simpkins NS: Synthesis of novel carbocyclic analogues of indolocarbazole natural products. Tetrahedron Lett 40: 3929-3932, 1999

Routier S, Coudert G, Merour JY: Synthesis of naphthopyrrolo[3,4-c]carbazoles. Tetrahedron Lett 42: 7025-7028, 2001

Routier S, Coudert G, Merour J-Y, Caignard DH: First synthesis of symmetrical and non-symmetrical azaindolocarbazole derivatives. Tetrahedron Lett 43: 2561-2564, 2002a

Routier S, Ayerbe N, Merour J-Y, Coudert G, Bailly C, Pierre A, Pfeiffer B, Caignard D-H, Renard P: Synthesis and biological evaluation of 7-azaindolocarbazoles. Tetrahedron 58: 6621-6630, 2002b

Routier S, Peixoto P, Merour J-Y, Coudert G, Dias N, Bailly C, Pierre A, Leonce S, Caignard D-H: Synthesis and biological evaluation of novel naphthocarbazoles as potential anticancer agents. J Med Chem 48: 1401-1413, 2005

Routier S, Merour J-Y, Dias N, Lansiaux A, Bailly C, Lozach O, Meijer L: Synthesis and biological evaluation of novel phenylcarbazoles as potential anticancer agents. J Med Chem 49: 789-799, 2006

Ruediger EH, Frennesson DB, Mahler M, Zimmermann K: Preparation and biological activity of indolopyrrolocarbazoledione anhydro glycosides as topoisomerase inhibitors. PCT Int. Appl. WO 2002030942, (Bristol-Myers Squibb Company, USA; Saulnier, Mark, G.; Balasubramanian, Neekakantan) 43 pp., 2002

Sahagun-Krause H, Thillaye Du Boullay O, Thillaye Du Boullay V, Casiraghi L, Seneci P, Klafki H-W, Braxmeier T, Mueller S, Froehner W, Monse B, Gordon S, Roder H: Preparation of N-carbacycle monosubstituted indolocarbazoles for therapeutic use as protein kinase inhibitors. PCT Int. Appl. WO 2003051887, (Nad Ag, Germany) 153 pp., 2003

Sahyoun N, LeVine H 3rd, Bronson D, McConnell R, Cuatrecasas P: Histones H3 and H4 inhibit protein kinase C specifically. Biochem Biophys Res Comm 115: 1027-1032, 1983

Salas AP, Zhu L, Sanchez C, Brana AF, Rohr J, Mendez C, Salas JA: Deciphering the late steps in the biosynthesis of the anti-tumor indolocarbazole staurosporine: Sugar donor substrate flexibility of the StaG glycosyltransferase. Mol Microbiol 58: 17-27, 2005

Salazar R, Bissett D, Twelves C, Breimer L, DeMario M, Campbell S, Zhi J, Ritland S, Cassidy J: A phase I clinical and pharmacokinetic study of Ro 31-7453 given as a 7- or 14-day oral twice daily schedule every 4 weeks in patients with solid tumors. Clin Cancer Res 10: 4374-4382, 2004

Sanchez A, Hallam TJ, Rink TJ: Trifluoperazine and chlorpromazine block secretion from human platelets evoked at basal cytoplasmic free calcium by activators of C-kinase. FEBS Lett 164: 43-46, 1983

Sanchez-Martinez C, Faul MM, Shih C, Sullivan KA, Grutsch JL, Cooper JT, Kolis SP: Synthesis of Aryl- and Heteroaryl[a]pyrrolo[3,4-c]carbazoles. J Org Chem 68: 8008-8014, 2003a

Sanchez-Martinez C, Shih C, Faul MM, Zhu G, Paal M, Somoza C, Li T, Kumrich CA, Winneroski LL, Xun Z, Brooks HB, Patel BKR, Schultz RM, DeHahn TB, Spencer CD, Watkins SA, Considine E, Dempsey JA, Ogg CA, Campbell RM, Anderson BA, Wagner J:

Aryl[a]pyrrolo[3,4-c]carbazoles as selective cyclin D1-CDK4 inhibitors. Bioorg Med Chem Lett 13: 3835-3839, 2003b

Sanchez-Martinez C, Shih C, Zhu G, Li T, Brooks HB, Patel BKR, Schultz RM, DeHahn TB, Spencer CD, Watkins SA, Ogg CA, Considine E, Dempsey JA, Zhang F: Studies on cyclin-dependent kinase inhibitors: indolo-[2,3-a]pyrrolo[3,4-c]carbazoles versus bis-indolylmaleimides. Bioorg Med Chem Lett 13: 3841-3846, 2003c

Sano K, Nakamura H, Matsuo T: Mode of inhibitory action of bilirubin on protein kinase C. Ped Res 19: 587-590, 1985

Saporito MS, Hudkins RL, Maroney AC: Discovery of CEP-1347/KT-7515, an inhibitor of the JNK/SAPK pathway for the treatment of neurodegenerative diseases. Progr Med Chem 4023-4062, 2002

Sarstedt B, Winterfeldt E: Reactions with indole derivatives. XLVIII. A simple synthesis of the staurosporine aglycon. Heterocycles 20: 469-476, 1983

Sasaki S, Hashimoto T, Obana N, Yasuda H, Uehara Y, Maeda M: Design of new inhibitors for CDC2 kinase based on a multiple pseudosubstrate structure. Bioorg Med Chem Lett 8: 1019-1022, 1998

Saulnier MG, Balasubramanian N, Frennesson DB, St. Laurent Denis R, Langley DR: Preparation of amino sugar and related sugar derivatives of indolopyrrolocarbazoles as antitumors. PCT Int. Appl. WO 9807433, (Bristol-Myers Squibb Company, USA) 91 pp., 1998

Saulnier MG, Ruediger EH, Balasubramanian N, Mahler M, Beaulieu F, Bachand C, Frennesson DB: Preparation and biological activity of indolopyrrolocarbazoledione glycosides as topoisomerase inhibitors. PCT Int. Appl. WO 2002030941, (Bristol-Myers Squibb Company, USA) 52 pp., 2002

Saulnier MG, Langley DR, Frennesson DB, Long BH, Huang S, Gao Q, Wu D, Fairchild CR, Ruediger E, Zimmermann K, St. Laurent DR, Balasubramanian BN, Vyas DM: Novel 3',6'-anhydro and N12,N13-bridged glycosylated fluoro-indolo[2,3-a]carbazoles as topoisomerase I inhibitors. Fluorine as a leaving group from sp3 carbon. Org Lett 7: 1271-1274, 2005a

Saulnier MG, Balasubramanian BN, Long BH, Frennesson DB, Ruediger E, Zimmermann K, Eummer JT, St. Laurent DR, Stoffan KM, Naidu BN, Mahler M, Beaulieu F, Bachand C, Lee FY, Fairchild CR, Stadnick LK, Rose WC, Solomon C, Wong H, Martel A, Wright JJ, Kramer R, Langley DR, Vyas DM: Discovery of a fluoroindolo[2,3-a]carbazole clinical candidate with broad spectrum antitumor activity in preclinical tumor models superior to the marketed oncology drug, CPT-11. J Med Chem 48: 2258-2261, 2005b

Schneider TL, Mathew RS, Rice KP, Tamaki K, Wood JL, Schepartz A: Increasing the kinase specificity of K252a by protein surface recognition. Org Lett 7: 1695-1698, 2005

Schupp P, Eder C, Proksch P, Wray V, Schneider B, Herderich M, Paul V: Staurosporine derivatives from the ascidian Eudistoma toealensis and its predatory flatworm Pseudoceros sp. J Nat Prod 62: 959-962, 1999

Schupp P, Proksch P, Wray V: Further new staurosporine derivatives from the ascidian Eudistoma toealensis and its predatory flatworm Pseudoceros sp. J Nat Prod 65: 295-298, 2002

Shankar BB, Viet AQ, Rizvi R, Kirkup MP, McCombie SW, Ganguly AK: Indolocarbazoles. 3. Synthesis of novel aza analogs of staurosporine and K 252a as PKC inhibitors. Bioorg Med Chem Lett 4: 495-498, 1994

Shoji M, Vogler WR, Kuo JF: Inhibition of phospholipid/Ca2+-dependent protein kinase and phosphorylation of leukemic cell proteins by CP-46,665-1, a novel antineoplastic lipoidal amine. Biochem Biophys Res Comm 127: 590-595, 1985

Singh J, Hudkins RL, Mallamo JP, Underiner TL, Tripathy R: Bridged indenopyrrolocarbazoles. PCT Int. Appl. WO 9962523, (Cephalon, Inc., USA) 90 pp., 1999

Slater MJ, Cockerill GS, Robinson JE: Preparation of antiviral heterocyclylindole derivatives. PCT Int. Appl. WO 9507910, (Wellcome Foundation Ltd., UK) 53 pp., 1995

Slater MJ, Cockerill S, Baxter R, Bonser RW, Gohil K, Gowrie C, Robinson JE, Littler E, Parry N, Randall R, Snowden W: Indolocarbazoles: potent, selective inhibitors of human cytomegalovirus replication. Bioorg Med Chem 7: 1067-1074, 1999

Slater MJ, Baxter R, Bonser RW, Cockerill S, Gohil K, Parry N, Robinson E, Randall R, Yeates C, Snowden W, Walters A: Synthesis of N-alkyl substituted indolocarbazoles as potent inhibitors of human cytomegalovirus replication. Bioorg Med Chem Lett 11: 1993-1995, 2001

Solomon DH, O'Brian CA, Weinstein IB: N-alpha-tosyl-L-lysine chloromethyl ketone and N-alpha-tosyl-L-phenylalanine chloromethyl ketone inhibit protein kinase C. FEBS Lett 190: 342-344, 1985

Squire CJ, Dickson JM, Ivanovic I, Baker EN: Structure and inhibition of the human cell cycle checkpoint kinase, Wee1A kinase. Structure 13: 541-550, 2005

Stroble SB, Tahai M, Goekjian PG, Saebo S: Ab initio studies of selected bisindolylmaleimides. 216th ACS National Meeting, Boston, 1998

Taffet SM, Greenfield AR, Haddox MK: Retinal inhibits TPA activated, calcium-dependent, phospholipid-dependent protein kinase ("C" kinase). Biochem Biophys Res Comm 114: 1194-1199, 1983

Steglich W, Steffan B, Kopanski L, Eckhardt G: Fungus pigments. 36. Indole pigments from the fruit bodies of the slime fungus Arcyria denudata. Angewandte Chem *92*: 463-464, 1980

Takahashi I, Kobayashi E, Asano K, Yoshida M, Nakano H: UCN-01, a selective inhibitor of protein kinase C from Streptomyces. J Antibiot 40: 1782-1784, 1987

Takahashi I, Asano K, Kawamoto I, Tamaoki T, Nakano H: UCN-01 and UCN-02, new selective inhibitors of protein kinase C. I. Screening, producing organism and fermentation. J Antibiot 42: 564-570, 1989a

Takahashi I, Saitoh Y, Yoshida M, Sano H, Nakano H, Morimoto M, Tamaoki T: UCN-01 and UCN-02, new selective inhibitors of protein kinase C. II. Purification, physico-chemical properties, structural determination and biological activities. J Antibiot 42: 571-576, 1989b

Takahashi I, Kobayashi E, Nakano H, Murakata C, Saitoh H, Suzuki K, Tamaoki T: Potent selective inhibition of 7-O-methyl UCN-01 against protein kinase C. J Pharmacol Exp Ther 255: 1218-1221, 1990

Tamaoki T, Nomoto H, Takahashi I, Kato Y, Morimoto M, Tomita F: Staurosporine, a potent inhibitor of phospholipid/calcium dependent protein kinase. Biochem Biophys Res Com 135: 397-402, 1986

Tamaoki T, Shiotsu Y, Murakata C, Akinaga S, Okabe M, Saitoh Y, Watanabe J, Shiraki T: Preparation of benzodiindole derivatives as antithrombotics. PCT Int. Appl. WO 9406799, (Kyowa Hakko Kogyo Co., Ltd., Japan) 65 pp., 1994

Tanida S, Takizawa M, Takahashi T, Tsubotani S, Harada S: TAN-999 and TAN-1030A, new indolocarbazole alkaloids with macrophage-activating properties. J Antibiot 42: 1619-1630, 1989

Tao M, Park CH, Bihovsky R, Wells GJ, Husten J, Ator MA, Hudkins RL: Synthesis and structure-activity relationships of novel poly(ADP-ribose) polymerase-1 inhibitors. Bioorg Med Chem Lett 16: 938-942, 2006

Teller S, Eluwa S, Koller M, Uecker A, Beckers T, Baasner S, Bohmer F-D, Mahboobi S: Pyrrolo[3,4-c]-b-carboline-diones as a novel class of inhibitors of the platelet-derived growth factor receptor kinase. Eur J Med Chem 35: 413-427, 2000

Terpin A, Winklhofer C, Schumann S, Steglich W: Alkaloids from marine organisms. 3. Synthesis and cyclization of didemnimide C and its imidazol-1-yl isomer. Tetrahedron 54: 1745-1752, 1998

Teruta A, Watanabe K, Hioki T, Sanuki H, Ando N: Pyrrolo carbazole derivatives. Jpn. Kokai Tokkyo Koho JP 2004256498, (Mitsubishi Welpharma Co., Japan) 83 pp., 2004

Toledo LM, Lydon NB: Structures of staurosporine bound to CDK2 and cAPK – new tools for structure-based design of protein kinase inhibitors. Structure 5: 1551-1556, 1997

Toullec D, Pianetti P, Coste H, Bellevergue P, Grand-Perret T, Ajakane M, Baudet V, Boissin P, Boursier E et al.: The bisindolylmaleimide GF 109203X is a potent and selective inhibitor of protein kinase C. J Biol Chem 266: 15771-15781, 1991

Trinks U, Buchdunger E, Furet P, Kump W, Mett H, Meyer T, Mueller M, Regenass U, Rihs G et al.: Dianilinophthalimides: Potent and selective, ATP-competitive inhibitors of the EGF-receptor protein tyrosine kinase. J Med Chem 37: 1015-1027, 1994

Tripathy R, Learn KS, Reddy DR, Iqbal M, Singh J, Mallamo JP: Construction of a kinase inhibitor library via parallel synthesis. Tetrahedron Lett 43: 217-220, 2002

Uings IJ, Spacey GD, Bonser RW: Effects of the indolocarbazole 3744W on the tyrosine kinase activity of the cytoplasmic domain of the platelet-derived growth factor b-receptor. Cell Signall 11: 95-100, 1998

Underiner TL, Mallamo JP, Singh J: Syntheses of C12,N13 heterocyclic bridged fused indenopyrrolo-carbazoles. J Org Chem 67: 3235-3241, 2002

Underwood KW, Parris KD, Federico E, Mosyak L, Czerwinski RM, Shane T, Taylor M, Svenson K, Liu Y, Hsiao C-L, Wolfrom S, Maguire M, Malakian K, Telliez J-B, Lin L-L, Kriz RW, Seehra J, Somers WS, Stahl ML: Catalytically active MAP KAP kinase 2 structures in complex with staurosporine and ADP reveal differences with the autoinhibited enzyme. Structure 11: 627-636, 2003

Vice SF: Diindolo compounds and inflammation inhibitors or neoplasm inhibitors and pharmaceuticals for psoriasis treatment. PCT Int. Appl. WO 9407895, (Schering Corp., USA) 109 pp., 1994

Vice SF: Preparation of diindolo compounds as antitumor agents.U.S. Patent USP 5589472, (USA) 35 pp., 1996

Vice SF, Bishop WR, McCombie SW, Dao H, Frank E, Ganguly AK: Indolocarbazole nitrogens linked by three-atom bridges: a potent new class of PKC inhibitors. Bioorg Med Chem Lett 4: 1333-1338, 1994

Vinik A: The protein kinase C-b inhibitor, ruboxistaurin, for the treatment of diabetic microvascular complications. Exp Opin Invest Drug 14: 1547-1559, 2005

Vinik AI, Bril V, Kempler P, Litchy WJ, Tesfaye S, Price KL, Bastyr EJ III: Treatment of symptomatic diabetic peripheral neuropathy with the protein kinase C b-inhibitor ruboxistaurin mesylate during a 1-year, randomized, placebo-controlled, double-blind clinical trial. Clin Ther 27: 1164-1180, 2005

Vulpetti A, Crivori P, Cameron A, Bertrand J, Brasca MG, D'Alessio R, Pevarello P: Structure-based approaches to improve selectivity: CDK2-GSK3b binding site analysis. J Chem Inf Model 45: 1282-1290, 2005

Wacker O: Preparation of N-[(tetrahydropyranyl-oxy)alkanoyl]staurosporines as protein kinase C inhibitors. Eur. Pat. Appl. EPO 624590, (Ciba-Geigy A.-G., Switz.) 18 pp., 1994

Wang RB, Kuo CL, Lien LL, Lien EJ: Structure-activity relationship: Analyses of p-glycoprotein substrates and inhibitors. J Clin Pharm Ther 28: 203-228, 2003

Weidner S, Goeke K, Trinks U, Traxler, P, Ucci-Stoll K, Ghisalba O: Preparation of 4-(4'-hydroxyanilino)-5-anilinophthalimide and 4,5-bis-(4'-hydroxyanilino)-phthalimide by microbial hydroxylation. Biosci Biotechnol Biochem 63: 1497-1500, 1999

Wilkinson SE, Parker PJ, Nixon JS: Isoenzyme specificity of bisindolylmaleimides, selective inhibitors of protein kinase C. Biochem J 294: 335-337, 1993

Williams DS, Atilla GE, Bregman H, Arzoumanian A, Klein PS, Meggers E: Switching on a signaling pathway with an organoruthenium complex. Angewandte Chem Int Ed 44: 1984-1987, 2005

Wood JL, Stoltz BM, Dietrich HJ, Pflum DA, Petsch DT: Design and implementation of an efficient synthetic approach to furanosylated indolocarbazoles: Total synthesis of (+)- and (-)-K252a. J Am Chem Soc 119: 9641-9651, 1997

Xie G, Gupta R, Atchison K, Lown JW: Bisindolylmaleimides linked to DNA minor groove binding lexitropsins: Synthesis, inhibitory activity against topoisomerase I, and biological evaluation. J Med Chem 39: 1049-1055, 1996

Xu ZB, Chaudhary D, Olland S, Wolfrom S, Czerwinski R, Malakian K, Lin L, Stahl ML, Joseph-McCarthy D, Benander C, Fitz L, Greco R, Somers WS, Mosyak L: Catalytic domain crystal structure of protein kinase C-q (PKCq). J Biol Chem 279: 50401-50409, 2004

Yamada R, Hayashi Y: Preparation of indolocarbazole derivatives as antitumor agents. Jpn. Kokai Tokkyo Koho JP 05140168, (Asahi Chemical Ind, Japan) 19 pp., 1993

Yamada R, Seto M, Sunazuka T, Oomura S: Preparation of staurosporine derivatives modified in the sugar moiety as selective inhibitors of myosin light chain kinase. Jpn. Kokai Tokkyo Koho JP 07112987, (Asahi Chemical Ind, Japan; Kitasato Inst) 11 pp., 1995

Yamada R, Seto M, Sasaki Y, Sunazuka T, Harigaya Y, Iwai Y, Omura, S: Synthesis and anti-platelet aggregation activity of water-soluble staurosporine derivatives. J Antibiot 49: 1070-1072, 1996

Yamakawa A, Ogata H, Morita K, Shibata N, Andou N, Sanuki H, Yamada K, Hioki T, Ishii T, Higuchi Y: Crystallization and preliminary x-ray analysis of two inhibitor complexes of the catalytic domain of death-associated protein kinase. Acta Crystallograph D: Biol Crystallograph D60: 764-766, 2004

Yan H, Mizutani TC, Nomura N, Takakura T, Kitamura Y, Miura H, Nishizawa M, Tatsumi M, Yamamoto N, Sugiura W: A novel small molecular weight compound with a carbazole structure that demonstrates potent human immunodeficiency virus type-1 integrase inhibitory activity. Antivir Chem Chemother 16: 363-373, 2005

Zhang H-C, Maryanoff BE, McComsey DF, White K, Ye H, Hecker L, Conway BR, Demarest K: Preparation of substituted 1H-pyrrole-2,5-diones as kinase inhibitors. PCT Int. Appl. WO 2003103663, (Janssen Pharmaceutica N.V., Belg.) 101 pp., 2003

Zhang L, Carroll P, Meggers E: Ruthenium complexes as protein kinase inhibitors. Org Lett 6: 521-523, 2004

Zhang G, Shen J, Cheng H, Zhu L, Fang L, Luo S, Muller MT, Lee GE, Wei L, Du Y, Sun D, Wang PG: Syntheses and biological activities of rebeccamycin analogs with uncommon sugars. J Med Chem 48: 2600-2611, 2005

Zhao B, Bower MJ, McDevitt PJ, Zhao H, Davis ST, Johanson KO, Green SM, Concha NO, Zhou B-BS: Structural basis for Chk1 inhibition by UCN-01. J Biol Chem 277: 46609-46615, 2002

Zhu G, Conner SE, Zhou X, Shih C, Li T, Anderson BD, Brooks HB, Campbell RM, Considine E, Dempsey JA, Faul MM, Ogg C, Patel B, Schultz RM, Spencer CD, Teicher B, Watkins SA: Synthesis, structure-activity relationship, and biological studies of indolocarbazoles as potent cyclin D1-CDK4 inhibitors. J Med Chem 46: 2027-2030, 2003a

Zhu G, Conner S, Zhou X, Shih C, Brooks HB, Considine E, Dempsey JA, Ogg C, Patel B, Schultz RM, Spencer CD, Teicher B, Watkins SA: Synthesis of quinolinyl/isoquinolinyl[a]pyrrolo [3,4-c] carbazoles as cyclin D1/CDK4 inhibitors. Bioorg Med Chem Lett 13: 1231-1235, 2003b

Zhu G, Conner SE, Zhou X, Chan H-K, Shih C, Engler TA, Al-Awar RS, Brooks HB, Watkins SA, Spencer CD, Schultz RM, Dempsey JA, Considine EL, Patel BR, Ogg CA, Vasudevan V, Lytle ML: Synthesis of 1,7-annulated indoles and their applications in the studies of cyclin dependent kinase inhibitors. Bioorg Med Chem Lett 14: 3057-3061, 2004

Zhu X, Kim JL, Newcomb JR, Rose PE, Stover DR, Toledo LM, Zhao H, Morgenstern KA: Structural analysis of the lymphocyte-specific kinase Lck in complex with non-selective and Src family selective kinase inhibitors. Structure 7: 651-661, 1999

Zimmermann J: Preparation and formulation of partially hydrogenated staurosporine derivatives for use as isoenzyme PKCa inhibitors and anticancer agents. PCT Int. Appl. WO 9902532, (Novartis A.-G., Switz.; Novartis-Erfindungen Verwaltungsgesellschaft m.b.H.) 82 pp., 1999

Zimmermann A, Wilts H, Lenhardt M, Hahn M, Mertens T: Indolocarbazoles exhibit strong antiviral activity against human cytomegalovirus and are potent inhibitors of the pUL97 protein kinase. Antivir Res 48: 49-60, 2000

PROTEIN KINASE RESEARCH IN THE UNIVERSITY OF HELSINKI

➤ **Jari Yli-Kauhaluoma**
Division of Pharmaceutical Chemistry,
Faculty of Pharmacy,
P.O. Box 56, Biocenter Viikki, (Viikinkaari 5 E),
FI-00014 University of Helsinki, Finland;
phone 09-19159170, e-mail: Jari.Yli-Kauhaluoma@helsinki.fi

➤ **Kaarina Sivonen**
Department of Applied Chemistry and Microbiology,
P.O. Box 56, Biocenter Viikki (Viikinkaari 9),
FI-00014 University of Helsinki, Finland

➤ **Raimo K. Tuominen**
Division of Pharmacology and Toxicology,
Faculty of Pharmacy,
P.O. Box 56, Biocenter Viikki (Viikinkaari 5 E),
FI-00014 University of Helsinki, Finland.

PROTEIN KINASE C

Protein kinase C (PKC) is a family of isoenzymes, which play a major role in signal transduction of normal cells (Nishizuka, 1995), and may be involved in the transformation of normal cells into malignant ones (Bredel and Pollack, 1997). Newly synthesized PKC is a water-soluble cytoplasmic protein with no kinase activity. Upon activation by endogenous second messengers, such as diacylglycerol and Ca^{2+} (the classical isoenzymes) and by exogenous compounds, such as phorbol esters, they undergo a large conformational change, translocate to cell membranes and become enzymatically active and phosphorylate their specific substrate proteins. In experimental settings a commonly used activator of PKC is the tumour promoting reagent phorbol ester that exhibits very high affinity for several PKC isoenzymes (Ron and Kazanietz, 1999).

There are currently only a small number of clinically used drugs, whose mode of action may include the inhibition of protein kinase C. Antiœstrogens, tamoxifen and probably also toremifen, inhibit protein kinase C when they are administered at high doses. In the treatment of malignant gliomas, tamoxifen has shown some efficacy and this effect has been suggested to be due to inhibition of PKC (Bradshaw et al., 1993). However, their action is non-specific, and there are considerable risks for the occurrence of side effects.

During the past 25 years several biotechnological and pharmaceutical companies have made substantial efforts to develop drugs that specifically affect protein kinase C and its isoenzymes. Biomedical research has revealed that PKC plays an important role in various mechanisms of inflammation. For example, protein kinase is a very important regulator in interleukin-2 mediated activation of T cells. In addition to the inflammatory diseases, the PKC inhibitors have a large potential in the treatment of diabetic complications, various types of cancer, neurological diseases, as well as cardiac and circulatory diseases.

PKC STUDIES IN THE UNIVERSITY OF HELSINKI

The Helsinki PKC group has adopted a drug discovery-oriented approach to study PKC as a drug target. We use molecular modelling to design new compounds and screen natural products showing activity on PKC from the Nordic biosphere. Based on these pieces of information, our aim is to synthesize compounds with high affinity and specificity to the phorbol ester-binding site of either PKCα or PKCδ. The synthesized compounds that activate, inhibit or otherwise modulate PKCα or PKCδ are studied in bioactivity screens and cell cultures.

There are seven University of Helsinki groups that participate in the "Protein Kinases – Novel Drug Targets of Post-genomic Era" project. Five of the groups are affiliated within the Faculty of Pharmacy and two of the groups are from the Faculty of Agriculture and Forestry. In the Faculty of Pharmacy, medicinal chemistry-related activities are carried out in Prof. Jari Yli-Kauhaluoma's laboratory. Graduate students Olli Aitio, M. Sc. and Gustav Boije af Gennäs, M. Sc. are studying the molecular modelling of protein kinase C and the organic synthesis of the PKC modulating agents, respectively. In the Prof. Raimo Tuominen's group, Dr. Elina E. Ekokoski and graduate student Virpi Talman, M. Sc., are in charge of carrying out the *in vitro* pharmacological characterization of the new PKC-targeted compounds. They employ the phorbol-binding and phosphoryl transfer activity assays to show the binding affinity and enzymatic effects of the compounds. Moreover, they are currently developing cell-based assay systems to see whole cell effects (e. g. cell division) of the prepared compounds. They also study the effect of the synthesized ligands on the translocation of PKCα and PKCδ from cytosol to the cellular membranes upon activation. This translocation can be observed in real time by means of confocal microscopy when the fusion proteins PKC-GFP are used as reporters (**Figure 2**). Docent Päivi Tammela's group is responsible for developing high-throughput assay systems for evaluating PKC-binding agents. Docent Moshe Finel's molecular biology laboratory produces the required recombinant proteins for binding and activity assays. Finally, Prof. Jouni Hirvonen's laboratory focuses on the transport of the most interesting PKC-modulating compounds in the Caco-2 model and evaluates the pharmacokinetic properties of the compounds.

The groups from the Faculty of Agriculture and Forestry (Department of Applied Chemistry and Microbiology) investigate the cyanobacteria and fungi obtained from the Nordic biosphere. Prof. Kaarina Sivonen's and Prof. Annele Hatakka's laboratories study cyanobacterial and fungal extracts as sources of PKC-modulating agents. Bioassays have been carried out in collaboration with Prof. Pia Vuorela (Åbo Akademi University, Finland) and Prof. Stein Ove Døskeland (University of Bergen, Norway). The fungi are studied by graduate student Andres Perez, M. Sc., and cyanobacteria by laboratory technician Matti Wahlsten. Docent Jouni Jokela carries out the characterization of new compounds and is responsible for the development of PKC assay based on LC-MS.

MEDICINAL CHEMISTRY

The medicinal chemistry-related studies are aimed at developing isoenzyme specific inhibitors for PKCα and PKCδ. Molecular modelling and the design of new selective and specific PKCα and PKCδ inhibitors are based on the previously published high resolution X-ray crystal structure of PKCδ with phorbol-13-acetate. In addition, recently published X-ray crystal structure of the catalytic domain of PKCθ has been used to design PKCα and PKCδ binding compounds.

The phorbol ester-binding moiety of PKC has been mapped for the optimal binding area and geometry of various functional groups. Subsequently, the Available Chemicals Directory database of more than one million commercially available compounds has been screened. Next, the molecules found from the screening were docked onto the binding moieties of PKCα and PKCδ. The best ligands were selected as templates for further synthetic elaboration to improve their binding and inhibitory properties. These synthetic compounds have been prepared by using methods of modern organic chemistry, such as solid-phase organic synthesis and microwave-assisted synthesis.

Some of the PKC-binding compounds synthesized in the medicinal chemistry group have shown promising results in various

apoptosis studies in our and Prof. Janet Lord's (University of Birmingham, United Kingdom) laboratories (Galkin et al., accepted for publication). These compounds selectively induce apoptosis in leukaemia cells, such as HL-60, AML and CLL, but not in fibroblasts. The optimisation of these ligands that displace the bound phorbol ester will be carried out in Prof. Janet Lord's and Prof. Johann Hofmann (Universität Innsbruck, Austria) groups. Finally, the synthesis of a compound library consisting of bistratene A derivatives will be carried out in collaboration with Prof. Peter Goekjian's and Prof. Olivier Piva's groups, both affiliated with the CNRS-Université Claude Bernard in Lyon, France.

Finally, we have studied in collaboration with the Technical Research Centre of Finland the use of a highly ubiquitous natural product *betulin*, lupene-3β,28-diol, as a starting point in our synthesis of protein kinase C modulating agents (**Figure 1**). These studies are funded by the National Technology Agency and the Academy of Finland. Birch bark is a rich source of betulin, as it contains approximately 40 percent of this pentacyclic triterpene. There is a surprising link between the birch trees and protein kinases. It has been demonstrated recently that betulin possesses significant anti-inflammatory activity that is mediated by inhibition of PKC (Huguet et al., 2000). Also, a close derivative of betulin, *betulinic acid,* induces programmed cell death (apoptosis) in human melanoma cells, and this process seems to involve mitogen-activated protein kinase activation (Tan et al., 2003).

MOLECULAR PHARMACOLOGY AND CELL BIOLOGY

The central objective of the molecular pharmacology research of the Faculty of Pharmacy is to collect experimental evidence for the specificity and efficacy of the newly synthesized compounds. For these experiments Docent Moshe Finel's molecular biology laboratory expresses functional recombinant human PKCα and PKCδ isoenzymes in insect cell cultures. The Finel group has also made efforts to set up a bacterial expression system in *E. coli*. Fluorescence-based imaging of PKC activation in living cells has been studied in Prof. Raimo Tuominen's laboratory. This approach is important for studies, where compounds targeting the regulatory domain are in focus. In principle, translocation should be inhibited by compounds that bind to the phorbol ester-binding site (**Figure 2**). Biological screening of the synthetic compounds or the natural products has been carried out *in vitro* using [^{3}H]phorbol 12,13-dibutyrate binding and phosphoryl transfer activity assays. As PKC enzyme specimen, partially purified rat brain homogenate, or recombinant constructs of PKCα or PKCδ (regulatory domain, C1 domains and whole enzyme) have been used.

Currently, we are setting up a cell-based assay to test the effect of the PKC-modulating compounds on living mammalian cells. The assay will be based either on PKC-mediated cell proliferation or on PKC-mediated changes on cell

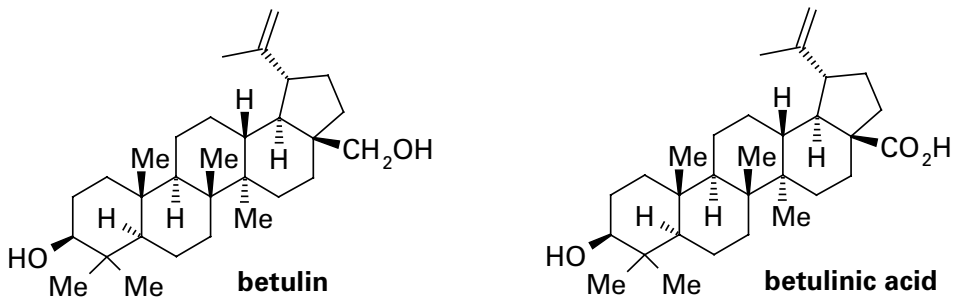


Figure 1. Chemical structures of betulin and betulinic acid.

morphology. In addition, we will continue the use of GFP-tagged PKC-constructs in translocation experiments in living cells. Also the FRET-constructs of PKCα and PKCδ made so far will be tested and validated for possible use in determining PKC activity in intact cells.

HIGH THROUGHPUT SCREENING AND PHARMACOKINETIC PROPERTIES

In the Tammela's HTS and Hirvonen's eADME groups Caco-2 primary permeability assays of protein kinase active ligands have been developed, and they are presently validating the automated 96-well format assay. In addition, they have developed primary toxicity assays automated in 96-well formats. Four cell lines and lactate dehydrogenase (LDH) leakage assay, WST-1 assay and/or intracellular ATP level detection can be used depending on the actual ligands to be assayed.

The HTS and eADME groups aim also at developing a transporter MDR-1 assay. Efflux proteins act as membrane transporters, transporting compounds (e.g. drugs and their metabolites) from the cells. Since cancer cells often overexpress the efflux protein MDR-1 to cause resistance against anti-cancer agents, the measurements for MDR-1 inhibiting activity will be used to evaluate further potential of ligands developed/isolated in this project.

CYANOBACTERIA AND FUNGI AS SOURCES OF BIOACTIVE COMPOUNDS

Cyanobacteria (blue-green algae) have been identified as one of the most promising groups of organisms for isolation of new bioactive natural products (Burja et al., 2001). In earlier studies, cyanobacteria have been found to produce a wide variety of toxins including the protein phosphatase inhibitors (microcystins, nodularin) and protein kinase C activators (lyngbyatoxin) (Sivonen and Jones, 1999). The production of bioactive pharmacologically interesting compounds of fungi (including basidiomycetous

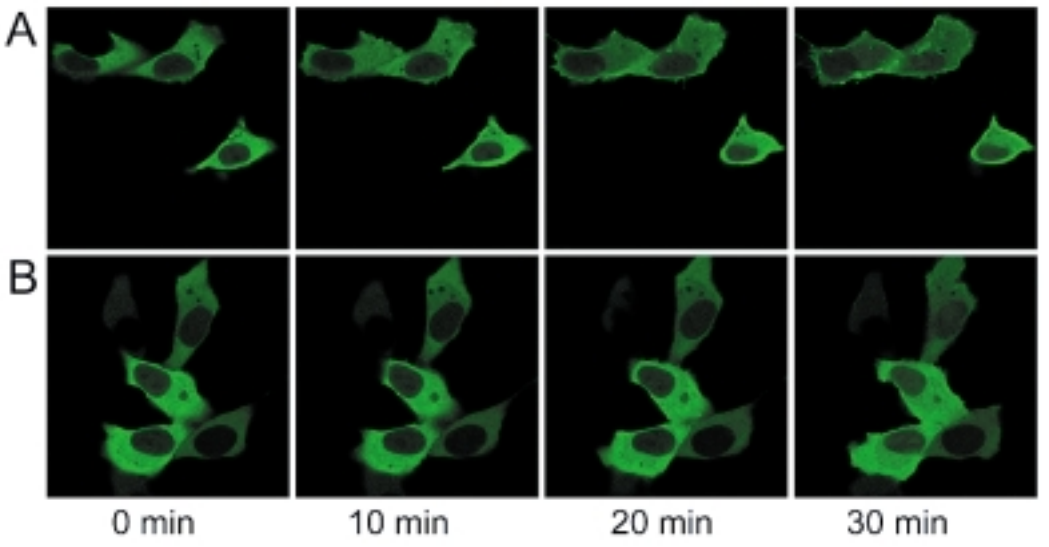


Figure 2. TRA-22 inhibits phorbol ester induced translocation of GFP-tagged PKCα.
A) HeLa cells were treated with 100 nM PMA (phorbol 12-myristate 13-acetate) and confocal microscopic images were captured every 30 seconds.
B) The cells were pretreated with 20 μM TRA-22 for 10 minutes before PMA-treatment.

polypore fungi) has been well established (Zjawiony, 2004). For example, an antitumor medicine in Japan originates from a white-rot fungus (Ng, 1998) and triterpene-containing extracts from *Ganoderma lucidum* suppress PKC (Lin et al., 2003). At the Department of Applied Chemistry and Microbiology, research groups studying cyanobacteria and fungi have established large culture collections of these organisms. Cyanobacteria culture collection contains 1,000 strains mostly isolated from the Finnish Lakes and the Baltic Sea (both pelagic and benthic environments). The collection of fungi (600 strains) consists mostly of wood rotting fungi isolated from the Finnish old forests. These microbial collections are used in the project as sources of new bioactive compounds (apoptosis modifying and protein kinase modulating compounds). Cyanobacteria were studied earlier in the MAST EU-project and were found to contain many interesting bioactivities (Surakka et al., 2005; Herfindal et al., 2005; Selheim et al., 2005).

In the present project, cyanobacteria and fungi have been grown, extracted and activities studied by radioactive PKC assay. The laborious use of the radioactive method led the researchers to develop a new high throughput assay based on multi-well plates handled by a robot and measured by LC-MS to detect PKC inhibitors/promoters. Known PKC inhibitors, such as staurosporine, quercetin, myricetin, and kaempferol were used as model compounds in the development of this system. The LC-MS analyzes hundred of samples within a few hours and will be used to look for the cyanobacterial and fungal extracts and fractions.

We mass cultivated several cyanobacteria showing interesting bioactivities in bioassays (**Figure 3**). From one of these (*Nostoc* strain) an antiapoptotic compound was isolated and purified and its structure was solved based on mass spectrometry (electrospray ionization, MS/MS fragmentation) and NMR (1D and 2D ^{1}H-NMR, ^{13}C-NMR and ^{15}N-NMR). This research confirmed that the antiapoptotic compound belonged to a group of cyclic peptides. The antiapoptotic activity was investigated in the Døskeland laboratory.

Several cyanobacterial strains were found in collaboration with Prof. Døskeland to contain interesting bioactivities (e.g. selectively killing leukaemia cells) and will be pursued further in the project. In addition, potential new PKC modulating activities found in the new bioassay will be studied further. The mass cultivation of the certain strains is under progress, and the isolation and purification of the compounds and their structural elucidation will be undertaken.

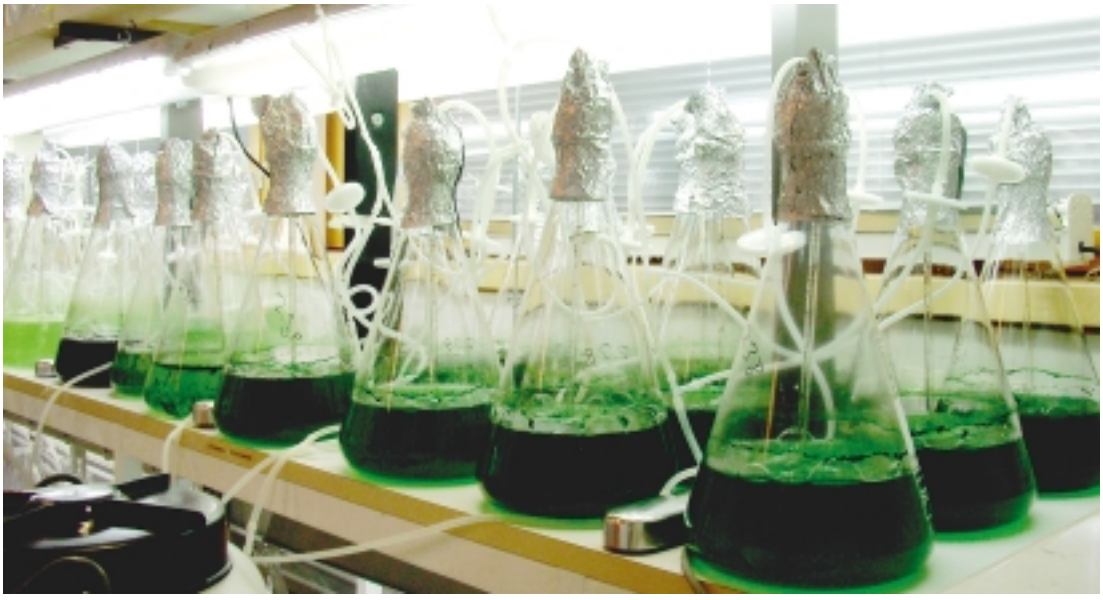


Figure 3. Mass cultures of cyanobacteria containing interesting compounds for structure elucidation and detailed analysis of bioactivity.

REFERENCES

Bradshaw D, Hill C H, Nixon J S, Wilkinson S E: Therapeutic potential of protein kinase C inhibitors. Agents & Actions 38: 135-147, 1993

Bredel M, Pollack I F: The role of protein kinase C (PKC) in the evolution and proliferation of malignant gliomas, and the application of PKC inhibition as a novel approach to anti-glioma therapy. Acta Neurochirurg 139: 1000-1013, 1997

Burja A M, Banaigs B, Abour-Mansour E, Burgess J G, Wright P C: Marine cyanobacteria – a proliferic source of natural products. Tetrahedron 57: 9347-9377, 2001

Galkin A, Surakka A, Boije af Gennäs G, Ruotsalainen T, Kreander K, Tammela P, Sivonen K, Yli-Kauhaluoma J, Vuorela P: Hydrophobic derivatives of 5-(hydroxymethyl)isophthalic acid that selectively induce apoptosis in leukemia cells but not in fibroblasts. Drug Dev Res, accepted for publication

Herfindal L, Oftedal L, Selheim F, Wahlsten M, Sivonen K, Døskeland S O: A high proportion of Baltic Sea benthic cyanobacterial isolates contain apoptogens able to induce rapid death of isolated rat hepatocytes. Toxicon 46: 252-260, 2005

Huguet A I, del Carmen Recio M, Manez S, Giner R M, Rios J L: Effect of triterpenoids on the inflammation induced by protein kinase C activators, neuronally acting irritants and other agents. Eur J Pharmacol 410: 69-81, 2000

Lin S-B, Li C-H, Lee S.-S, Kan L-S: Triterpene-enriched extracts from *Ganoderma lucidum* inhibit growth of hepatoma cells via suppressing protein kinase C, activating mitogen-activated protein kinases and G2-phase cell cycle arrest. Life Sci 72: 2381-2390, 2003

Ng T B: A review of research on the protein-bound polysaccharide (polysaccharopeptide, PSP) from the mushroom *Coriolus versicolor* (Basidiomycetes: Polyporaceae). Gen Pharmac 30: 1-4, 1998

Nishizuka Y: Protein kinase C and lipid signalling for sustained cellular responses. FASEB J 9: 484-496, 1995

Ron D, Kazanietz M G: New insights into the regulation of protein kinase C and novel phorbol ester receptors. FASEB J 13: 1658-1676, 1999

Selheim F, Herfindal L, Martins R, Vasconcelos V, Døskeland, S O: Neuro-apoptogenic and blood platelet targeting toxins in benthic marine cyanobacteria from Portuguese coast. Aquatic Toxicol 74: 294-306, 2005

Sivonen K, Jones G: Cyanobacterial toxins. In: Toxic Cyanobacteria in Water: a Guide to Public Health Significance, Monitoring and Management. I. Chorus and J. Bertram (Eds.). pp. 41-111. The World Health Organization. ISBN 0-419-23930-8. E and FN Spon, London, UK, 1999

Surakka A, Sihvonen L M, Lehtimäki J M, Wahlsten M, Vuorela P, Sivonen K: Benthic cyanobacteria from the Baltic Sea contain cytotoxic *Anabaena, Nodularia* and *Nostoc* strains and an apoptosis inducing *Phormidium* strain. Environ Toxicol 20: 285-292, 2005

Tan, Y, Yu R, Pezzuto J M: Betulinic acid-induced programmed cell death in human melanoma cells involves mitogen-activated protein kinase activation. Clin Cancer Res 9: 2866-2875, 2003

Zjawiony, J K: Biologically active compounds from Aphyllophorales (polypore) fungi. J Nat Prod 67: 300-310, 2004

Protein Kinases: Potential Targets for New Drugs against Parasitic Protozoa

➤ **Paul Pechan**[1]
➤ **Charles L. Jaffe**[2]

[1] C3 Bio GmbH,
Ottobrunner Str. 37, D-82008, Munich, Germany
and
[2] Department of Parasitology,
Hebrew University-Hadassah Medical School,
PO Box 12272, Jerusalem, 91120, Israel

THERAPEUTIC INDICATIONS, AREAS TO BE CONSIDERED

Many human diseases in tropical and subtropical regions are caused by single cell eukaryotic parasites. These diseases include household names such as malaria, sleeping sickness and river blindness, as well as less well-known diseases such as leishmaniasis.

Much of the population at risk lives in developing countries. Hundred's of millions are infected and ten's of millions die or are unable to carry out their daily labours. Unfortunately, and despite increasing international focus on these diseases, treatments of parasitic diseases are costly and/or exhibit clinical side effects and/or are becoming ineffective due to increasing resistance to existing treatments. Research, both in public and private institutions, needs to be stepped up to find effective prophylactic and therapeutic regiments for these diseases.

This review summarizes current knowledge of the use of protein kinase inhibitors for the treatment of leishmaniasis and human African trypanosomiasis (sleeping sickness) in the human population, diseases that are uncontrolled in poor areas of the world.

DESCRIPTION OF DISEASES, CONDITIONS AND THEIR PREVALENCE

Leishmaniasis

Leishmaniasis is a spectrum of parasitic diseases, caused by species and subspecies belonging to genus *Leishmania*. The disease is usually transmitted through the bite of some 30 species of Phlebotomine sandflies. These parasites exist in two forms: extracellular promastigotes in the female sandfly vector and as intracellular amastigotes in the macrophages of the mammalian host. The latter stage of the parasite is responsible for all the diseases observed and is the target for potential new drugs (www.who.int/tdr/diseases/leish/default.htm; Desjeux, 2004). Potential hosts include humans and/or dogs depending on the parasite species. Leishmaniasis has been designated a neglected and emerging disease by the WHO.

There are three main forms of leishmaniasis in humans:

- Visceral (VL)
- Cutaneous (CL) and
- Mucocutaneous (MCL)

There are approximately 12 million cases of leishmaniasis worldwide, which are found primarily in tropical and subtropical regions spanning 88 countries worldwide. About 2 million new cases appear every year (1.5 million CL and 0.5 million VL) with an annual death of about 60,000 people. It is estimated 350 million people are at risk of contracting the disease.

VL occurs primarily on the Indian subcontinent and in Sudan. However, a number of cases have been reported in Southern Europe, primarily in those countries bordering the Mediterranean Sea (Spain, Portugal, Italy, France, Malta and Greece). Co-infections with HIV have also been reported and have changed the epidemiology of the disease in some countries (Desjeux and Alvar, 2003). CL is also present around the Mediterranean basin, primarily in Greece, Turkey, Lebanon, Syria, Israel, Egypt, North Africa and the Middle East. However, 90% of the CL cases occur in Iran, Afghanistan, Saudi Arabia, Brazil and Peru.

Human African trypanosomiasis

This disease, also known as the Sleeping Sickness, is found in Africa restricted to areas inhabited by the tsetse fly (Glossina), where parasitic protozoa (trypanosomes) are transmitted to people through the bite of the fly. The flies live primarily where moisture is found, such as pools of water or shaded vegetation. The epidemiology of the disease depends on the interaction of humans, tsetse flies, trypanosomes and animals such as cows, sheep and goats. Epidemiological observations indicate a correlation between tsetse fly numbers and rainfall of the previous month. The presence of tsetse flies does not necessarily imply that the human disease is present in the area.

Sleeping sickness exists as two forms, each caused by a separate species of parasite. The chronic form is caused by *Trypanosoma brucei gambiense,* present in western and central Africa. The acute form is caused by *Trypanosoma brucei rhodesiense* and is present in eastern and southern Africa. Both forms can be deadly if untreated, however the rate of disease progression differs between the two forms. In the acute form symptoms appear early, central nervous system (CNS) invasion is rapid, and death occurs in weeks to a few months. The chronic form of African sleeping sickness progresses more slowly and symptoms may not appear for months or years until the disease is quite advanced. As in the acute form, the parasites eventually invade the CNS resulting in death if untreated (Kiminyo, 2002).

It is estimated that over 50 million people, primarily adults, are at risk. There are over 300,000 new cases reported each year, with an upward trend. Nearly 50,000 people die of the disease each year (WHO World Health Report 2004-www.who.int/whr/2004/en/ and www.who.int/mediacentre/factsheets/fs259/en/index). African trypanosomiasis has also been designated a neglected and emerging disease by the WHO. It is a new epidemic.

SHORT HISTORICAL PERSPECTIVE ON THE DIAGNOSIS, PREVENTION AND TREATMENTS

Leishmaniasis

Of the three types of diseases, visceral (VL) and cutaneous (CL) leishmaniasis are the focus of most studies. VL, also known as kala azar in India, is caused by *L. donovani* or *L. infantum*, and is the most dangerous type of leishmaniasis. If untreated, it generally results in death primarily through its effects on spleen and liver with associated weight loss, fever and anaemia. *L. infantum* also causes fatal canine VL in the Mediterranean region and Brazil. CL is caused primarily by *L. major, L. tropica* and *L. aethiopica* in the Old World, and the *L. mexicana* and *L. braziliensis* complexes in the New World. CL produces skin ulcers on exposed parts of the body, where an infected sand fly vector fed, leaving the victim scared and potentially disabled. The third form of disease, MCL, also known in South America as Espundia, is primarily caused by *L. aethiopica* and *L. b. braziliensis*. This disease is typified by lesions in the nose, mouth and throat leading to extensive destruction of the mucous membranes. Misdiagnosis and delays in treatment result in severe disfigurement of the oral-nasal and pharyngeal region leading to

hideous mutilation and suffering for the life of the patient (Berman, 2003; www.who.int/tdr/diseases/leish/default.htm).

Details of any existing diagnosis, prevention or treatment methods

The main drugs currently in use to treat leishmaniasis were introduced 50–60 years ago, and the therapeutic arsenal is rather limited (Croft and Coombs, 2003; Murray, 2004; Singh and Sivakumar, 2004). Current drugs to treat VL require long courses, parenteral administration and hospitalization, and are generally expensive (see **Table 1**). Drugs based on pentavalent antimony (Pentostam, Glucantime and generic sodium stibogluconate), the first line of defense, are toxic and increasing resistance (>90%) has lead to the abandonment of this treatment in regions where disease incidence is high, such as Bihar, India (Bryceson, 2001). Second-line drugs, such as pentamindine and amphotericin B, are highly effective, but their use is limited by toxicity and availability in some areas. Use of pentamindine has been discontinued in some areas of India due to increasing parasite resistance. Three lipid-associated formulations of amphotericin B (Ambisome, Abelcet and Amphocil) have been used to treat VL. The efficacy of these formulations is good and side effects low, however prohibitively high cost has prevented their extensive use.

Few new drugs are in the pipeline for treatment of VL (Guerin et al., 2002). These include Miltefosine, recently registered in India, Paromomycin and Sitamaquine. Miltefosine is the first oral drug for VL. This drug has mild, primarily GI tract related side effects. Its major drawback is contraindication in females of childbearing age unless contraception is used. Long drug half-life in patients and a narrow therapeutic index suggest that parasite resistance may rapidly appear if Miltefosine is used alone over long-periods. Paromomycin has shown high efficacy when given parenterally for 21 days. The traditional formulation of the drug was discontinued, but the drug was rescued by the International Dispensary Association and WHO/TDR, and is presently undergoing phase III trials in India. Finally, Sitamaquine an oral 8-aminoquinoline has shown promising results, but little information on toxicity or efficacy is available and development has been slow. Phase III trials for this drug have been initiated (Murray, 2004; Singh and Sivakumar, 2004).

Many of the same drugs used to treat VL are also in use for CL and MCL (Blum et al., 2004). Both systematic and local treatments are used depending on the clinical presentation and species of *Leishmania*. Pentavalent antimony and pentamidine are routinely used for systematic therapy with the same complications described above for VL. Limited tests on additional drugs (amphotericin B and lipid formulations, allopurinol, Miltefosine, itraconazole and fluconazole) have been carried out, however extensive data on toxicity, formulation or dosage is lacking. Local treatments consist of either intralesional infiltration with pentavalent antimony or topical ointments containing paromomycin. However, considerable variations in patient responses to local and systematic treatments have been observed depending on the *Leishmania* species. Systematic, not local treatment is recommended in patients infected with *Leishmania* species that potentially causes MCL.

HIV co-infected patients in Europe and elsewhere, regardless of the drug used, tend to respond slowly to treatment and have high relapse rates (~60%). In some cases drug toxicity is exacerbated. Preliminary studies indicate that Miltefosine may be useful for maintenance therapy. Long-term treatment of these patients with a limited drug arsenal may promote the development of parasite resistance (**Table 1**).

Current treatments of leishmaniasis are not satisfactory: either because of rapidly acquired resistance to the available drugs, their side effects, and/or their expense (Berman, 2003; Croft and Coombs, 2003). A great need exists for new drugs to treat Leishmaniasis. Most existing primary drugs are toxic and require parenteral administration and hospitalization; several show signs increasing parasite resistance or are expensive. Treatment under difficult field conditions, poor compliance, HIV co-infections and anthroponotic transmission promote the development of secondary resistance.

Trypanosomiasis

As already eluded to, human African trypanosomiasis has two forms: West African Sleeping Sickness, caused by *Trypanosoma brucei gambiense (T. b. gambiense)* and East African Sleeping Sickness, caused by *Trypanosoma brucei rhodesiense (T. b. rhodesiense).* Local symptoms may be evident within few days at the site of the tsetse fly bite. In the former disease, the affected persons may be infected for extended periods (even years) without showing any overt signs of disease. The chronic form is usually well advanced by the time typical symptoms are observed. Initial symptoms are headaches, fever, itching, extreme fatigue, and aching of muscles and joints, etc. If the disease is diagnosed early enough, cure rates are high. In the second neurological phase, parasites cross the blood-brain barrier and attack the central nervous system (CNS). This is associated with confusion, lack of coordinated movement, seizures, disturbance of the sleep cycle, loss of weight, etc. When treated after the onset of the second phase, the neurological disorder is permanent. In the latter case, it is not known how trypanosomes enter the CNS. If left untreated, disease symptoms become worse and the patient eventually dies within months to years. In the acute form similar symptoms are seen, but disease progress is much more rapid, and if untreated, results in death within a few weeks to months (Legros et al., 2001; Kiminyo, 2002; Stich et al., 2002).The disease is characterized by the multiplication of the protozoa in the blood and lymph of the infected person. The protozoa can be transmitted through blood. They can also pass from the mother to the fetus, leading to abortion (Kiminio, 2002; Stich et al., 2002).

Potential for epidemics in many parts of Africa exists due to the lack of appropriate tsete fly control programs, disease surveillance, and difficulties in diagnosing the disease before the parasites cross the blood-brain barrier and cause damage to the CNS. This is especially the case with *T. b. gambiense*, where the symptoms may not be apparent until it is already too late.

Targets for the drugs are trypanosomes. There are a number of drugs available to treat the disease. As WHO concludes, the drugs are old, difficult to administer and not always successful (www.who.int/mediacentre/factsheets/fs259/en/index). The drugs are divided into two groups (**Table 2**): those that treat the disease during the early phases and drugs used to treat the disease during the later manifestation phase.

Because of the difficulties in screening the populations at risk, new drug treatment strategies should emphasize targeting trypanosomes after they have entered the brain. Therefore, new drugs must be able to cross the blood-brain barrier to reach the parasites.

Table 1. Overview of drugs used for the treatment of Cutaneous and Visceral Leishmaniasis.

	Side effects	Comments
Impavido (miltefosine)	not with HIV, reproductive toxicity	
Pentostam (pentavalent antimonials),	toxicity	resisitance, expensive
Stibanate, (pentavalent antimonials),	toxicity	resisitance, expensive
Sodium Stibogluconate(pentavalent antimonials)	toxicity	resistance, expensive
Glucantime (pentavalent antimonials),	toxicity	resistance, expensive
Fungizone (amphotericin B)	toxicity, intravenous	
Ambisome (amphotericin B-liposomal)		very expensive
Amphocil (amphotericin B-colloidal)		optimum doses not clear
Pentamidine (amphotericin B-lipid complex)		resistance

CURRENT PRIORITY AREAS TO BE CONSIDERED

In this section we will concentrate only on aspects related to drug development and testing. Important areas, such as diagnostics and vaccine development, will not be addressed in this review. It is important to note that health care givers in the field must accurately, rapidly and reliably identify these diseases and decide on an appropriate drug treatment to be administered to the patient. Physicians and laboratory technicians working in many endemic regions suffer from the lack of inexpensive, sensitive and specific methods for early diagnosis of leishmaniasis or human African trypanosomiasis.

Drug development

At present, for both leishmaniasis and human African trypanosomiasis, the precise drug targets are still unknown. Thus, there is a need to emphasize a more systematic approach to identify the possible targets. One approach for treating these diseases is by targeting cell cycle dependent kinases (CDKs) of the parasites.

Correct coordination of events during the cell-cycle division cycle is necessary for the survival and growth of eukaryotic cells. These events are controlled at specific cell cycle check-points. The progression through the cell cycle requires the activation and inactivation of CDKs. In humans this process has been extensively studied, and the different CDKs (CDK1, 2, 4 and 6) and the cyclin subsets (cyclin A, B, D and E) with which they interact have been identified. Additional CDKs have been shown to have essential functions in apoptosis and transcription (Vermeulen et al., 2003). However, gene expression in *Leishmania* and Trypanosomes is regulated in large part post-transcriptionally, thus it is unlikely that CDK homologues play any significant role in this process.

CDKs are involved in the regulation of the cell cycle progression by activation and deactivation of proteins through protein phosphorylation. Thus, control over CDKs can decide the life and death of a cell. Among the best known inhibitors of the CDKs are purine and pyrimidine derivatives, to which compounds such as Olomocine and Roscovitine belong. Their mode of action is not yet fully understood but, due to their similarity in structure to ATP, they may act as competitive inhibitors for the protein kinase ATP binding pocket. However, from data available with Cdk2, they do not appear to bind to the protein kinases in a manner similar to the ATP (Fischer, 2003; Fisher and Gianella-Borradori, 2003, 2005). Additional heterocyclic compounds can also act as CDK inhibitors, and examples include oxindoles, aminothiazoles, anillinoquinazolines,

Table 2. Overview of drugs for the treatment of trypanosomiasis.

	Side effects	Comments
Early phase		
Suramine (used against *T.b. rhodesiense*)	Digestive track problems	Discovered in 1921
Pentamidine (used against *T.b. gambiense*)	Well tolerated	Discovered in 1941
Late phase		
Melarsoprol (contains arsenic) (used against *T.b. gambiense* and *rhodesiense*)	Severe allergic reaction (3–10% death rate)	Discovered in 1949, resistance
Eflornithine (used against *T.b. gambiense*)		Discovered in 1988, expensive, difficult to apply

pyridopyrimidines, flavonoids (flavopiridol) and staurosporines (Knockaert et al., 2002a). Among other small molecular weight compounds, paullones are strong CDK inhibitors (Schultz et al., 1999).

It should be stressed that CDK inhibitors are not necessarily selective for specific CDKs, and may for example inhibit protein kinases other than CDKs. Indeed, CDKs are just one possibility to kill the parasites. For example enzymes in the glycolytic, trypanothine and thioredoxin pathways may be possible drug targets.

Drug testing and resistance

In both leishmaniasis and human African trypanosomiasis, only a limited number of drugs are in the pipeline to treat the diseases. There are a number of problems to be overcome. First, in many instances, the short- and long-term effectiveness of the compounds still needs to be clearly established (including the number of relapsing patients). Second, synergism between drugs needs to be determined. Third, more extensive testing of drugs on late stages of the disease needs to be carried out. Finally, studies need to be carried out to establish the optimal conditions to reduce the development of resistance to the applied drugs (Legros et al., 2001, Docampo and Moreno, 2002)

CURRENT KNOWLEDGE OF USING KINASE INHIBITORS TO COMBAT LEISHMANIASIS AND HUMAN AFRICAN TRYPANOSOMIASIS

Correct coordination of events during cell cycle division is necessary for the survival and growth of eukaryotic cells. Cell cycle has been investigated in trypanosomes and involves the coordination of the G_1, S, M and G_2 stages for both nuclear, as well as mitochondrial DNA. The latter DNA in trypanosomatids is present as an interlocking network in a single structure, the kinetoplast, at the base of the flagellum (Shlomai, 2004). The chronology of genome replication for the nucleus and kinetoplast is not absolutely synchronized, and the DNA synthesis in the latter organelle starts prior to the nuclear S phase. In addition, the kinetoplast completes segregation prior to nuclear mitosis. Furthermore, parasite cytokinesis in trypanosomes does not appear to depend on either nuclear S or M phases suggesting that the cell cycle check-points in these parasites are different from normal eukaryotic cells (McKean, 2003). Less is known about cell cycle and replication in *Leishmania*, although it is probably similar to *T. brucei*. Differences between human and parasite cell cycle co-ordination suggests that the CDKs regulating cell division in the parasites may be potential targets for new drugs

Eleven CDK homologues, cdc2 related kinases (CRKs), have been identified in *T. brucei* and *L. major* (Parsons et al., 2005). *Leishmania* CRK1 and 3 were shown to be essential for parasite growth (Mottram et al., 1996; Grant et al., 1998; Wang et al., 1998; Hassan et al., 2001), and the latter gene was able to rescue *Schizosaccharomyces pombe* *cdc2*^{ts} mutants (Wang et al., 1998). Incubation of promastigotes with flavopiridol (a general cdc2 inhibitor) caused cell cycle arrest and accumulation of the cells at the G2 or G2/M phases (Hassan et al., 2001). More recently, RNA interference (RNAi) knockdown studies of different cyclin and CRK genes in *T. brucei* have shown that three (CRK1, CRK2 and CRK3) orthologues are essential for cell division in the blood stream form. Using this technique, CRK1 and CRK2 were shown to control the G1/S passage, while CRK3 arrested cell division at the G2/M transition (Li and Wang, 2003; Tu and Wang, 2004). Knockdown of these CRK genes or their cyclin pairs resulted in accumulation of cells at the G1 or G2/M phase. The CRK3 depleted blood stream trypomastigotes, and unlike the insect procyclic stage, failed to enter cytokinesis and cell division. In the blood stream stage, the nucleus and kinetoplast DNA continued to divide, and the cells accumulated multiple nuclei and kinetoplasts. Therefore, it was concluded that the absence of cytokinesis and cell division doesn't prevent the cells from entering G1 and S phases (Li and Wang, 2003; Tu and Wang, 2004).

The genomic sequences for *Leishmania, T. brucei* and *T. cruzi* were published recently (Berriman et al., 2005; El-Sayed et al., 2005; Ivens et al., 2005). All three parasites belong to the same family, the Trypanosomatidae, and share many

orthologous clusters of protein coding genes. However, they also have in common many unique or unusual biological and genetic mechanisms that are not found or are poorly represented in other eukaryotes, including glycosomes, polycistronic transcription, trans-splicing and kinetoplast DNA. Mining the genomes of these parasites for proteins with unusual or different properties will promote the identification of new leads for drug discovery and development.

KNOWLEDGE GENERATED, OPPORTUNITIES, USE AND IMPACT IN THE MEDICAL FIELD

Sequencing of parasite and human genomes has provided unique opportunities for the analysis and comparison of parasite and human kinomes (Manning et al., 2002; Ward et al., 2004; Parsons et al., 2005). The TriTryp kinome (*T. brucei, T. cruzi* and *Leishmania*) was recently published (Parsons et al., 2006). A total of 68% of the typical eukaryotic protein kinases in these parasites are found in orthologous gene clusters. Interestingly, only a few protein kinase genes appear to be specific to each species, *T. brucei* (~2%), *T. cruzi* (~6%), and *Leishmania* (~10%). Analysis of the three kinomes highlighted some unusual characteristics of the trypanosome/*Leishmania* protein kinase families. Two of the six major protein kinase families, the receptor-linked tyrosine (TK) and tyrosine-like kinases (TLKs), are missing from the TriTryp kinome. However, tyrosine phosphorylation and tyrosine protein phosphatases have been reported in these parasites (Parsons et al., 1994; Parsons et al., 1995; Furuya et al., 1998; Salotra et al., 2000). This is probably due to the members of the dual specificity kinase (STE) family, which are capable of phosphorylating both serine/threonine and tyrosine residues. Members of the STE family appear to be over represented in these parasites when compared to the human kinome (Parsons et al., 2006). Interestingly, the CMGC is also over represented in the parasite kinomes compared to the humans. This may be correlated with the complex parasite life cycles, or changing, harsh environmental conditions (pH, temperature, osmolarity, host proteins, nutrients, etc.) encountered by these parasites. The remaining two families, CAMK and ACG, appear to be poorly represented compared to the human kinome (Parsons et al., 2006).

Availability of the TriTryp kinome will facilitate identification of important parasite protein kinases. Further investigations of these enzymes by various molecular biological and proteomic techniques, molecular modelling of inhibitor - protein kinase interactions, reverse genetic engineering of parasites, and RNAi will allow the rapid evaluation of the target function(s) and their critical role in parasite survival. This will facilitate the selection of essential parasite protein kinases prior to drug development and optimization.

DRUG DEVELOPMENT STRATEGIES

Research on the development of new protein kinase inhibitors for these neglected diseases is accelerating with the publication of the various parasite genomes, and increased cooperation between pharmaceutical companies, public institutions, universities and research institutions (Troullier et al., 2002; Davis et al., 2004). New methods to identify and screen potential compounds are being developed.

Screening using live parasites

African trypanosomes pass through several developmentally regulated stages during their life cycle in the Tsetse fly vector and mammalian host. The extracellular blood stream trypomastigote stage that is responsible for disease can be readily cultured *in vitro* and be used to screen different compounds for activity. Many methods have been used to assay potential compounds, including counting parasites (manual or automated), incorporation of radioactive nucleotides, enzymatic activity - colorimetric assays (reduction of MTT and other similar compounds to the purple formazan salt by active mitochondrial enzymes), or fluorescent assays such as Alamar Blue (Raz et al., 1997). Similar assays have been used to screen synthetic or natural products for activity against the *Leishmania* promastigotes, the extracellular stage of the parasite. This stage of the parasite was widely used in the past for

screening, because it can be grown easily in culture. However, promastigotes reside in the sandfly vector; are briefly found in hosts and are not responsible for human or animal diseases that are caused by the intracellular amastigote stage. Stage specific differences in metabolism and protein expression have been observed between the two parasite stages. Compounds active on promastigotes may show no or large differences in activity when tested on the intracellular stage that causes leishmaniasis and visa versa (Sereno and Lemesre, 1997; Callahan et al., 1997). Differences in ED_{50} between species of *Leishmania* are also observed.

Alternatively, chemical libraries can be screened directly on macrophages (differentiated and non-differentiated macrophage cell lines, peritoneal and bone-marrow derived mouse macrophages, or human monocyte derived macrophages) infected with parasites. Macrophages are infected using various conditions, parasite to macrophage ratio, time period and stage. Excess parasites are removed, and the infected macrophages are incubated with or without the chemicals for 2-3 days. The macrophages are Giemsa stained, and the percent of infected macrophages, as well as the number of parasites/infected macrophage, are enumerated using a light microscope, and compared to untreated control macrophages. This procedure is very time consuming and is not readily adaptable to high through put (HTP) assays of large chemical libraries. Recently, methods to culture axenic amastigotes in the absence of macrophages for several *Leishmania* species have been developed (Debrabant et al., 2004). The properties of axenic amastigotes more accurately reflex those found using intracellular amastigotes, and can been used for accurate and reliable primary drug screening (Sereno and Lemesre, 1997; Callahan et al., 1997).

Production of genetically engineered parasites that express various reporter genes (red fluorescent protein dsRed, green fluorescent protein GFP, β-galactoside and luciferase) should streamline this process even further (Misslitz et al., 2000; Okuno et al., 2003; Lang et al., 2005; Sundar et al., 2005). Optimally, mutant *Leishmania* can be used to develop simple HTP assays for screening chemical libraries directly on infected macrophages. Fluorescent parasites have been used to screen compounds by fluorescent activated cell sorting (FACS), microplate readers or fluorescent microscopes, while luciferase or β-galactoside expressing parasites have been used to screen the potential compounds using microplate readers or ELISA readers (Plock et al., 2001; Okuno et al., 2003; Lang et al., 2005; Sundar et al., 2005). Procedures using transgenic parasites need to be optimized and standardized in order to accurately measure parasite and host cell viability. In addition, it is important to use recently isolated parasite strains and frequently thaw new stabilates so parasite virulence and other properties don't change with time in culture. Afterwards, these HTP assays can be employed for drug discovery, in effect measuring parasite responses to new, hopefully effective compounds. Using such *in vitro* systems, drug development can be significantly accelerated increasing the number of compounds screened for activity on the intracellular stage of the parasite. Active compounds identified by these procedures are then subjected to several rounds of structure-activity analysis, synthesis and screening on infected macrophages prior to testing in animal models.

Recombinant enzymes

Reagents have been produced against substrates and protein kinases of higher eukaryotes. Unfortunately, many of these fail to react with the parasite homologues making it difficult to elucidate the signalling pathways and protein kinases involved. However, completion of the *L. major* and *T. brucei* genome has facillitated the identification protein kinase genes of interest. These genes can be expressed as active recombinant proteins in either *E. coli*, baculovirus or other systems for further characterization, and be used in HTP screening of chemical libraries. These compounds need to be tested using the live parasites, as already described, in order to confirm their activity and lack of toxicity for host cells. Employing this approach, recombinant *L. mexicana* CRK3 was used to screen 634 antimitoic compounds from different sources. Approximately 6.6% of the compounds inhibited CRK3 by >90%, of these 59% showed good activity on infected macrophages or were not toxic to the host cells.

Six compounds inhibited amastigotes in infected macrophages at ED_{50} <6 micromolar range (Grant et al., 2004).

Identification of the inhibitor's target is important for understanding the drug's mechanism of action. This can facilitate further optimization of the inhibitor's chemical functional groups via molecular modelling using existing protein kinase structures and information on interactions between ATP, the chemical inhibitors and the active site of homologous protein kinases. Affinity chromatography can be valuable in identifying the target enzyme a specific inhibitor or family of compounds (Knockaert et al., 2002). In this case, an inhibitor is covalently coupled to a solid support, and cell lysates are incubated with the resin. Bound proteins are eluted, digested and the peptides sequenced. The amino acid sequences are compared to those found in the parasite databank. This technique has been used to identify the targets of several paullone and purvalanol inhibitors of Trypanosomatidae (Knockaert et al., 2000; Knockaert et al., 2002). These compounds are promising inhibitors of CDKs and glycogen synthetase kinase-3 (GSK-3) in cancer cells (Schultz et al., 1999; Gray et al., 1999; Leost et al., 2000). Interestingly, while the covalently bound purvalanol B purified several members from the CMGC group, including CDK1 from higher eukaryotes, CK1 belonging to the OPK group II was the primary protein kinase purified from *L. mexicana* (Knockaert et al., 2000). Similarly, GSK-3 was purified from higher eukaryotes on the gwennpaullone affinity resin together with the mitochondrial malate dehydrogenase (MDH). MDH binds apparently to the affinity resin via the NAD/NADH binding site. When *L. mexicana* lysates were used, MDH was the major protein purified. Paullones were shown to inhibit mitochondrial MDH, as well as *L. mexicana* promastigotes at low micromolar concentrations. Together these results suggest that caution should be employed when extrapolating mechanisms of action and targets for protein kinase inhibitors from higher eukaryotes to parasites.

Finally, genetic or functional knockouts of specific protein kinases, using either homologous recombination (*Leishmania* and *T. brucei*) or RNAi (*T. brucei*), enzyme over expression, and utilization of analog-sensitive mutants can be employed in order to obtain a better understanding of the function and roles of specific protein kinases targeted by the inhibitors. Commercial development of new drugs is to a large degree dependent on public-private funding, as private companies find it difficult, without risk reducing measures, to embark alone to develop drugs for markets that may not be financially feasible.

CONCLUSIONS

Leishmaniasis and human African trypanosomiasis are widespread diseases in subtropical and tropical regions that cause extensive morbidity and mortality among populations living primarily in countries with limited resources. New drugs for the safe, effective, cheap and easy oral treatment of leishmaniasis and human African trypanosomiasis are urgently needed to prevent death or long-term disability. Few new drugs are in the pipeline, and most of these will take several years to receive approval and registration. Availability of several cheap and safe oral drugs will promote the use of combination therapy and prevent the development of drug resistance.

The urgency of developing effective drugs to combat leishmaniasis and human African trypanosomiasis in a most appropriate and effective manner has been internationally recognised. The projects are primarily internationally and publically driven, as pharmaceutical companies are unlikely to dedicate their resources to combat a neglected rare disease that is unlikely to bring sufficient financial profits (Troullier et al., 2002; Davis et al., 2004). One example is the Drugs for Neglected Diseases initiative (DNDi, www.dndi.org), which was formed by five public institutions (Oswaldo Cruz Foundation, Indian Council for Medical Research, Kenya Medical Research Institute, Ministry of health of Malaysia and Pasteur Institute of France), Medecins sans Frontieres and the UNDP/WORLD BANK/WHO's Tropical Diseases Research Programme. This organization includes leishmaniasis and human African trypanosomiasis among their target diseases.

REFERENCES:

Journals

Ashutosh GS, Ramesh SS, Goyal N: Use of *Leishmania donovani* field isolates expressing the luciferase reporter gene in in vitro drug screening. Antimicrob Agents Chemother 49: 3776-3783, 2005

Berman J: Current treatment approaches to Leishmaniasis. Curr Opin Infect Dis 16: 397-401, 2003

Berriman M et al.: The genome of the African trypanosome *Trypanosoma brucei*. Science 309: 416-422, 2005

Blum J, Desjeux P, Schwartz E, Beck B, Hatz C: Treatment of cutaneous leishmaniasis among travellers. J Antimicrob Chemother 53: 158-66, 2004

Bryceson A: A policy for leishmaniasis with respect to the prevention and control of drug resistance. Trop Med Int Health 6: 928-934, 2001

Callahan HL, Portal AC, Devereaux R, Grogl M: An axenic amastigote system for drug screening. Antimicr Agent Chemo 41: 818-822, 1997

Croft S, Coombs G: Leishmaniasis-current chemotherapy and recent advances in the search for novel drugs. Trends Parasitol 19: 502-508, 2003

Davis AJ, Murray HW, Handman E: Drugs against leishmaniasis: a synergy of technology and partnerships. Trends Parasitol 20: 73-76, 2004

Debrabant A, Joshi MB, Pimenta PF, Dwyer DM: Generation of *Leishmania* donovani axenic amastigotes: their growth and bioogical characteristics. Int J Parasitol 34: 205-217, 2004

Desjeux P, Alvar J: *Leishmania*/HIV co-infections: epidemiology in Europe. Ann Trop Med Parasitol 97: S3-S15, 2003

Desjeux P: Leishmaniasis: current situation and new perspectives. Comp Immunol Microbiol Inf Dis 27: 305-318, 2004

Docampo R, Moreno SN: Current chemotherapy of human African trypanosomiasis. Lancet Infect Dis 2: 393, 2002

El-Sayed NM et al.: The genome sequence of *Trypanosoma cruzi*, etiologic agent of Chagas disease. Science 309: 409-415, 2005

Fischer P: Functions and pharmacological inhibitors of cyclin dependent kinases (CDKs). Cell Trans 19: 1-9, 2003

Fischer P, Pianella-Borradori: CDK inhibitors in clinical development for the treatment of cancer. Exp Opin Invest Drugs 12: 955-970, 2003

Fischer P, Pianella-Borradori : Recent progress in the discovery and development of cyclin- dependent inhibitors. Exp Opin Invest Drugs 14: 457-477, 2005

Furuya T, Zhong L, Meyer-Fernandes JR, Lu HG, Moreno SN, Docampo R: Ecto-protein tyrosine phosphatase activity in *Trypanosoma cruzi* infective stages. Mol Biochem Parasitol 92: 339-348, 1998

Grant KM, Hassan P, Anderson JS, Mottram JC: The crk3 gene of *Leishmania mexicana* encodes a stage-regulated cdc2-related Histone H1 kinase that associates with $p12^{cks1*}$. J Biol Chem 273: 10153-10159, 1998

Grant KM, Dunion MH, Yardley V, Skaltsounis AL, Marko D, Eisenbrand G, Croft SL, Meijer L, Mottram JC: Inhibitors of Leishmania mexicana CRK3 cyclin-dependent kinase: chemical library screen and antileishmanial activity. Antimicrob Agent Chemother 48: 3033-3042, 2004

Gray N, Detivaud L, Doerig C, Meijer L: ATP-site directed inhibitors of cyclin-dependent kinases. Curr Med Chem 6: 859-875, 1999

Guerin P et al.: Visceral Leishmaniasis: current status of control, diagnosis and treatment and a proposed research and development agenda. Lancet Infect Dis 2: 494-501, 2002

Hassan P, Fergusson D, Grant KM, Mottram J: The CRK3 protein kinase is essential for cell cycle progression of *Leishmania mexicana.* Mol Biochem Parasitol 113: 189-198, 2001

Ivens A et al.: The genome of the kinetoplastid parasite, *Leishmania major*. Science 309: 436-442, 2005

Kiminyo KP: African Trypanosomiasis (Sleeping Sickness), ID Consultants Inc, www.eMedicine.com, 2002

Knockaert M, Gray N, Damiens E, Chang YT, Grellier P, Grant K, Fergusson D, Mottram J, Soete M, Dubremetz JF, Le Roch K, Doerig C, Schultz PG, Meijer L: Intracellular targets of cyclin-dependent kinase inhibitors: identification by affinity purification using immobilized inhibitors. Chem Biol 7: 411-422, 2000

Knockaert M, Greengard P, Meijer L: Pharmacological inhibitors of cyclin-dependent kinases. Trends Pharmacol Sci 23, 417-425, 2002a

Knockaert M, Wieking K, Schmitt S, Leost M, Grant KM, Mottram JC, Kunick C, Meijer L: Intracellular targets of Paullones: identification following affinity purification on immobilized inhibitor. J Biol Chem 277: 25492-25501, 2002b

Lang T, Goyard S, Lebastard M, Milon G: Bioluminescent *Leishmania* expressing luciferase for rapid and high throughput screening of drugs acting on amastigote-harbouring macrophages and for quantitiative real-time monitoring of parasitism features in living mice. Cell Microb 7: 383-392, 2005

Legros D, Ollivier G, Gastellu-Etchegorry M, Paquet C, Burri C, Jannin J, Buscher P: Treatment of human African trypanosomiasis – present situation and needs for research and development. Trends Parasitol 17: 42-49, 2001

Leost M, Schultz C, Link A, Wu YZ, Biernat J, Mandelkow EM, Bibb JA, Snyder GL, Greengard P, Zaharevitz DW, Gussio R, Senderowicz AM, Sausville EA, Kunick C, Meijer L: Paullones are potent inhibitors of glycogen synthase kinase-3beta and cyclin-dependent kinase 5/p25. Eur J Biochem 267: 5983-5994, 2000

Li Z, Wang CC: A PHO80-like cyclin and a B-type cyclin control the cell cycle of the procyclic form of Trypanosoma brucei. J Biol Chem. 278: 20652-20658, 2003

Manning G, Whyte DB, Martinez R, Hunter T, Sudarsanam S: The protein kinase complement of the human genome. Science 298: 1912-1934, 2002

McKean P: Coordination of cell cycle and cytokinesis in *Trypanosoma brucei*. Curr Opin Microbiol 6: 600-607, 2003

Misslitz A, Mottram JC, Overath P, Aebischer T: Targeted integration into a rRNA locus results in uniform and high level expression of transgenes in *Leishmania* amastigotes. Mol Biochem Parasitol 107: 251-261, 2000

Mottram JC, McCready BP, Brown KG, Grant KM: Gene disruptions indicate an essential function for the LmmCRK1 cdc2-related kinase of *Leishmania mexicana*. Mol Microbiol 22: 573-583, 1996

Murray HW: Treatment of visceral leishmaniasis in 2004. Am J Trop Med Hyg 71: 787-794, 2004

Okuno T, Goto Y, Matsumoto Y, Otsuka H, Matsumoto Y: Applications of recombinant *Leishmania amazonensis* expressing egfp or the beta-galactosidase gene for drug screening and histopathological analysis. Exp Anim 52: 109-118, 2003

Parsons M, Valentine M, Deans J, Schieven GL, Ledbetter JA: Distinct patterns of tyrosine phosphorylation during the life cycle of *Trypanosoma brucei*. Mol Biochem Parasitol 45: 241-248, 1991

Parsons M, Carter V, Muthiani A, Murphy N: *Trypanosoma congolense*: developmental regulation of protein kinases and tyrosine phosphorylation during the life cycle. Exp Parasitol 80: 507-514, 1995

Parsons M, Worthey EA, Ward PN, Mottram JC: Comparative analysis of the kinomes of three pathogenic trypanosomatids: *Leishmania major, Trypanosoma brucei* and *Trypanosoma cruzi*. BMC Genomics 6: 127, 2005

Plock A, Sokolowska-Kohler W, Presber W: Application of flow cytometry and microscopical methods to characterize the effect of herbal drugs on *Leishmania* spp. Exp Parasitol 97: 141-153, 2001

Raz B, Iten M, Grether-Buhler Y, Kaminsky R, Brun R: The Alamar Blue assay to detemine drug sensititity of African trypaonosoms (*T.b. rhodesiense* and *T.b. gambiense) in vitro*. Acta Trop 68: 139-147, 1997

Salotra P, Ralhan R, Sreenivas G: Heat-stress induced modulation of protein phosphorylation in virulent promastigotes of *Leishmania donovani*. Int J Biochem Cell Biol 32: 309-316, 2000

Schultz C, Link A, Leost M, Zaharevitz DW, Gussio R, Sausville EA, Meijer L, Kunick C: Paullones, a series of cyclin-dependent kinase inhibitors: synthesis, evaluation of CDK1/cyclin B inhibition, and in vitro antitumor activity. J Med Chem 42: 2909-2919, 1999

Sereno D, Lemesre JL: Axenically cultured amastigote forms as an *in vitro* model for investigation of antileishmanial agents. 41: 972-976, 1997

Shlomai J: The structure and replication of kinetoplast DNA. Curr Mol Med. 4: 623-647, 2004

Singh S, Sivakumar R: Challenges and new discoveries in the treatment of leishmaniasis. J Infect Chemother 10: 307-315, 2004

Stich A, Abel PM, Krishna S: Human African trypanosomiasis. BMJ 325: 203-206, 2002

Troullier P et al.: Drug development for neglected diseases: a deficient market and public- health policy failure. Lancet 359: 2188-2194, 2002

Tu X, Wang CC: The involvement of two cdc2-related kinases (CRKs) in Trypanosoma brucei cell cycle regulation and the distinctive stage-specific phenotypes caused by CRK3 depletion. J Biol Chem 279: 20519-20528, 2004

Tu X, Wang CC: Pairwise knockdowns of cdc2-related kinases (CRKs) in Trypanosoma brucei identified the CRKs for G1/S and G2/M transitions and demonstrated distinctive cytokinetic regulations between two developmental stages of the organism. Eukaryot Cell 4: 755-764, 2005

Vermeulen K, Van Bockstaele DRV, Berneman ZN: The cell cycle : a review of regulation, deregulation and therapeutic targets in cancer. Cell Prolif 36: 131-149, 2003

Wang Y, Dimitrov K, Garrity LK, Sazer S, Beverley SM: Stage-specific activity of the *Leishmania major* CRK3 kinase and functional rescue of a Schizosaccharomyces pombe cdc2 mutant. Mol Biochem Parasitol 96: 139-150, 1998

Ward P, Equinet L, Packer J, Doerig C: (2004) Protein kinases of the human malaria parasite *Plasmodium falciparum*: the kinome of a divergent eukaryote. BMC Genomics 12: 79, 2004

Internet sites

African trypanosomiasis – www.who.int/tdr

Fact sheet on African trypanosomiasis: fact sheet 259, 2001 www.who.int/mediacentre/factsheets/fs259/en/index

Leishmaniasis – http://www.who.int/tdr/diseases/leish/default.htm

"PROTEIN KINASE RESEARCH" -TEEMANUMERON ARTIKKELIEN SUOMENKIELISET YHTEENVEDOT

➤ **Outi Salminen**
Ph.D.
Managing Coordinator
Protein Kinase Research
Faculty of Pharmacy
University of Helsinki
P.O.Box 62 (Viikinkaari 11)
FI-00014 University of Helsinki
FINLAND
http://www.proteinkinase-research.org

PROTEIINIKINAASI C ISOENTSYYMIT – KOHDEPROTEIINEJA UUSILLE HOITOMUODOILLE

YHTEENVETO

Proteiinikinaasi C (PKC) perhe koostuu seriini/treoniinispesifisistä proteiinikinaaseista. PKC isoentsyymit voidaan luokitella kolmeen ryhmään:

1) konventionaaliset PKC:t (cPKC) α, βI, βII ja γ
2) uudet PKC:t (nPKC) δ, ε, θ, η/L ja μ sekä
3) atyyppiset PKC:t (aPKC) λ/ι ja ζ.

PKC isoentsyymit ovat tärkeässä osassa signaalin kuljetusreiteissä osallistuen viestinkuljetukseen synapseissa, ionivirtojen aktivoinnissa, erityksessä, solun jakaantumisessa, solukierron kontrolloimisessa, erilaistumisessa ja kasvainsolujen jakaantumisessa. PKC-perheestä on tulossa kasvavan kiinnostuksen kohde etsittäessä uusia hoitomuotoja useisiin eri sairauksiin. Tässä katsauksessa esitellään eri PKC isoentsyymien toimintaa ja mahdollisia keinoja vaikuttaa niihin.

PKCα-spesifinen antisense-oligonukleotidi aprinokarseeni (ISIS 3521) ei osoittautunut hyödylliseksi syöpälääkkeenä. PKCβ-inhibiittori ruboksistauriini-mesylaatti (LY333531) on faasi III:sen kliinisissä tutkimuksissa ja se hidastaa diabeettisen retinopatian, nefropatian ja neuropatian oireita. Kokeellinen syöpälääke AD 198 vaikuttaa aktivoimalla PKCδ-isoentsyymiä. Muille PKC-isoentsyymeille ei ole vielä olemassa spesifejä pienen molekyylikoon omaavia inhibiittoreita tai aktivaattoreita. Tällaiset modulaattorit olisivat tärkeitä PKC isoentsyymien toiminnan vaikutusten selvittämisessä. Samoin niiden avulla voitaisiin tutkia, olisivatko PKC isoentsyymit sopivia kohdemolekyylejä esim. syöpäsairauksien tai diabeteksen hoitomuotoja kehitettäessä.

➤ **Dorota Garczarczyk**
➤ **Florian Rechfeld**
➤ **Georg Hechenberger**
➤ **Johann Hofmann**, Prof.
Biocenter, Division of Medical Biochemistry
Innsbruck Medical University
Fritz-Pregl-Str. 3
A-6020 Innsbruck, Austria
johann.hofmann@i-med.ac.at

SYÖPÄÄ AIHEUTTAVAT TYROSIINIKINAASIT: BCR-ABL MALLI

YHTEENVETO

Tyrosiinikinaaseja (TK) ja niiden sukulaisproteiineja tiedetään olevan yli 100 erilaista. TK:t osallistuvat solunulkoisten ärsykkeiden välittymiseen solun sisään sekä useiden elintärkeiden prosessien säätelyyn, kuten solun jakautumiseen ja solukuolemaan. Tyrosiinikinaasit voivat itse muuntua syöpää aiheuttaviksi eli onkogeenisiksi. Toistaiseksi on löydetty seitsemän onkogeenistä tyrosiinikinaasia.

Tässä artikkelissa esitellään BCR-ABL tyrosiinikinaasimalli, jonka selvittäminen auttoi kehittämään onnistuneen syöpähoidon. Kroonisessa myelooisessa leukemiassa esiintyvä ns. Ph-kromosomilöydös johtuu lähekkäin sijaitsevien kromosomien 9 ja 22 sisältämien BCR ja ABL geenien muodostamasta hybridistä. BCR-ABL-hybridi osoittautui lisätutkimuksissa erinomaiseksi lääkevaikutuksen kohdemolekyyliksi. 1980-luvun lopulla kehitettiin imatinibimesylaatti, joka esti BCR-ABL hybridin kinaasiaktiivisuutta ja siten pysäytti leukeemisten solujen kasvun ja aiheutti niiden solukuolemaa *in vitro* ja *in vivo*. Imatinibia on testattu kliinisissä kokeissa 8 vuoden ajan Ph+-leukemiapotilailla. Artikkelissa kuvataan näistä tutkimuksista saatuja rohkaisevia tuloksia. Kirjoittajat esittelevät BCR-ABL hybridin ja imatinibin myös rakenteena, jolle voidaan perustaa uusien syöpäterapioitten jatkokehittelyt.

➤ **Carlo Gambacorti-Passerini**
Prof.
Dept. of Clinical Medicine,
University of Milano-Bicocca, Italy
carlo.gambacorti@unimib.it

➤ **Leonardo Scapozza**
Prof.
Pharmaceutical Biochemistry Group,
School of Pharmaceutical Sciences,
University of Geneva,
University of Lausanne,
Quai Ernest-Ansermet 30,
CH-1211 Geneva 4, Switzerland
leonardo.scapozza@pharm.unige.ch

PROTEIINIKINAASIT LEUKEMIATERAPIAN KOHDEMOLEKYYLEINÄ

YHTEENVETO

Leukemiassa verisolujen ja niiden esiasteiden normaali tasapaino solun jakautumisen, erilaistumisen ja solukuoleman välillä on häiriintynyt. Tämä johtaa kypsymättömien prekursorisolujen määrän kumuloitumiseen luuytimessä, joka puolestaan hidastaa normaalien esiastesolujen kasvua ja kehittymistä. Seurauksena ovat leukemiassa tyypilliset löydökset, kuten neutropenia, trombosytopenia ja anemia. Leukemian neljä päätyyppiä ovat akuutti lymfoplastinen leukemia (ALL), akuutti myelooinen leukemia (AML), krooninen lymfosyyttinen leukemia (CLL) ja krooninen myelooinen leukemia (CML).

Leukemia esiintyy yleensä vanhemmalla iällä, tosin ALL esiintyy useimmiten lapsilla. Vaikka ALL on lähes parannettavissa (80–85 % remissio lapsilla), aikuisiän leukemian ennuste on edelleen huono. Uusia leukemian hoitomuotoja tarvitaan siten kipeästi. Uusien hoitojen kehittely tähtää joko solujen erilaistumisen pysähtymisen estoon tai leukeemisten solujen kasvun estoon tai niiden kuoleman edistämiseen. Useat nykyisin kemoterapiassa käytetyt yhdisteet eivät ole riittävän spesifisiä, joten ne vahingoittavat myös normaaleja soluja.

Imatiniibin, lyhytketjuisen proteiinikinaasi-inhibiittorin, hyvä menestys CML:n hoidossa on avannut uusia näköaloja kohdennettuun leukemiaterapiaan. Tässä katsauksessa kirjoittajat esittelevät uusia yhdisteitä, joiden kohdeproteiineina toimivat erilaiset proteiinikinaasit. Eräät proteiinikinaasit saattavat muuttua itse onkogeenisiksi, toisaalta proteiinikinaasien aktivointi tai inhibointi on osoittautunut leukeemisten solujen kuolemaa edistäväksi. Katsauksessa esitellään BCR-ABL tyrosiinikinaasiin, flt-3 tyrosiinikinaasiin sekä Jak-tyrosiinikinaasiperheeseen vaikuttavia yhdisteitä, kuten myös seriini/treoniinikinaaseja (PKC, Ras-Raf-Mek-Erk-signaalireitin kinaasit) inhiboivia tai aktivoivia yhdisteitä. Näistä yhdisteistä tulee todennäköisesti lupaava ”toisen aallon” leukemiaterapia-perhe.

➤ **Keqing Wang**
➤ **Peter Hampson**
➤ **Janet M. Lord**, Prof.
MRC Centre for Immune Regulation
Birmingham University Medical School,
Birmingham, UK
j.m.lord@bham.ac.uk

STAUROSPORIINI JOHTOMOLEKYYLINÄ: KATSAUS INDOLO-KARBATSOLIALKALOIDIEN INSPIROIMIIN KINAASI-INHIBIITTOREIHIN

YHTEENVETO

Staurosporiini on yksi monista varhaisimmista proteiinikinaasin ATP-sitoutumiskohtaan kohdistetuista proteiinikinaasi-inhibiittoreista. Staurosporiini on rakenteena innoittanut kehittelemään laajan valikoiman selektiivisiä kinaasi-, topoisomeraasi- ja p-glykoproteiini-inhibiittoreita.

Tässä katsauksessa esitellään alkuperäisestä staurosporiini-rakenteesta muunneltuja inhibiittoreita. Näitä ovat staurosporiinin johdannaiset, indolokarbatsoli-monoglykosidit, substituoidut indolokarbatsolit, bisindolyylimaleimidit, makrosykliset bisindolyylimaleimidit, dianiliiniftaliminit, 4-aryylikarbatsolit, heteroaryl(o)karbatsolit ja aryl(o)karbatsolit, sekä atsaindolokarbatsolit. Selektiivisiä ja voimakkaita inhibiittoreita on kehitetty seuraaviin kohdeproteiineihin: PKC, GSK-3β, myosiinin kevyen ketjun kinaasi, "checkpoint" kinaasit, sykliinistä riippuvaiset kinaasit, flt-3, DAPK, EGFR, VEGFR, PDGFR ja PIM1, kuten myös ei-kinaasi kohdeproteiineihin, esim. topoisomeraasi I, PARP-1 ja p-glykoproteiini, jotka perustuvat alkuperäiseen indolokarbatsolijohtomolekyyliin.

➤ **Peter G. Goekjian**
Prof.
CNRS, UMR 5181, Méthodologies de Synthèse et Molécules Bioactives,
Université Claude Bernard - Lyon 1
peter.goekjian@univ-lyon1.fr

PROTEIINIKINAASITUTKIMUSTA HELSINGIN YLIOPISTOSSA

YHTEENVETO

Proteiinikinaasi C:n (PKC) isoentsyymit ovat hyvin merkittäviä signaalinvälityksessä ja solujen muuttumisessa pahanlaatuisiksi. Erilaisten aktivaattoreiden vaikutuksesta proteiinikinaasi C:n isoentsyymien konformaatio muuttuu huomattavasti, ne sitoutuvat membraaniin, muuttuvat entsymaattisesti aktiivisiksi ja fosforyloivat kohdeproteiininsa. PKC-isoentsyymien yleisaktivaattorina käytetään forboliesteriä, joka sitoutuu niihin hyvin voimakkaasti.

Tämän tutkimushankkeen keskeisinä tavoitteina on etsiä, tunnistaa, suunnitella ja valmistaa spesifisiä ja affiniteetiltaan hyvin sitoutuvia yhdisteitä, jotka estäisivät esimerkiksi PKC-alfan tai PKC-deltan aktivoitumisen forboliesterin vaikutuksesta. Tällaisia yhdisteitä suunnitellaan tietokoneavusteisesti ja valmistetaan synteettisesti hyödyntämällä esimerkiksi kombinatoriaalisen kemian menetelmiä. Toisaalta näitä yhdisteitä voidaan etsiä ja tunnistaa luonnosta. Tässä hankkeessa niitä etsitään pääasiassa kotimaisista sinilevistä, sienistä ja kasveista. PKC:n modulaattoreiden ja inhibiittoreiden käyttö erilaisten sairauksien hoidossa olisi poikkeuksellisen laajaa käsittäen muun muassa autoimmuunitauteja (nivelreuma, punahukka), sydän- ja verisuonisairauksia (verenpainetauti) ja syöpäsairauksia (pahanlaatuinen glioooma). Tässä katsauksessa esittelemme EU:n komission rahoittamassa "Protein kinases – Novel drug targets of post-genomic era" -projektissa mukana olevat Helsingin yliopiston tutkimushankkeet.

➤ **Jari Yli-Kauhaluoma**
Division of Pharmaceutical Chemistry,
Faculty of Pharmacy,
P.O. Box 56, Biocenter Viikki, (Viikinkaari 5 E),
FI-00014 University of Helsinki, Finland;
phone 09-19159170,
e-mail: jari.yli-kauhaluoma@helsinki.fi

➤ **Kaarina Sivonen**
Department of Applied Chemistry and Microbiology,
P.O. Box 56, Biocenter Viikki (Viikinkaari 9),
FI-00014 University of Helsinki, Finland

➤ **Raimo K. Tuominen**
Division of Pharmacology and Toxicology,
Faculty of Pharmacy,
P.O. Box 56, Biocenter Viikki (Viikinkaari 5 E),
FI-00014 University of Helsinki, Finland.

PROTEIINIKINAASIT LÄÄKEVAIKUTUKSEN KOHTEINA UUSILLE ALKUELÄINTAUTIEN HOITOON SOVELTUVILLE LÄÄKEAINEILLE

YHTEENVETO

Leishmaniaasit ja afrikkalaiset trypanosomiaasit ovat subtrooppisilla ja trooppisilla alueilla laajalle levinneitä tauteja, jotka aiheuttavat laajaa sairastuvuutta ja kuolleisuutta kehittyvien maiden väestössä. Uusia lääkkeitä, jotka olisivat tehokkaita, turvallisia, halpoja ja helposti suun kautta annosteltavissa, tarvitaan kiireisesti näiden tautien hoitoon estämään kuolemia tai pitkäaikaisia vammautumisia. Käytössä olevat lääkitykset ovat kehitetty 1950–60-luvulla, ne ovat vaikeasti annosteltavia, usein tehottomia ja niillä on paljon sivuvaikutuksia. Kehitteillä on ainoastaan muutamia uusia lääkeaineita ja niiden käyttöönotto tulee kestämään vielä useita vuosia. Useiden halpojen ja turvallisten suun kautta annosteltavien lääkeaineiden saatavuus parantaisi yhdistelmähoitojen mahdollistumista ja siten voitaisin estää myös resistenssin kehittymistä.

Lääkekehityksen kannalta ensimmäinen vaihe leishmaniaasien ja afrikkalaisen trypanosomiaasin tutkimisessa on täsmällisten lääkevaikutuksen kohteiden määritteleminen. Eräs varteenotettava vaihtoehto ovat solukierrosta riippuvaiset kinaasit (cell cycle dependent kinases, CDKs). Eroavaisuudet ihmisen ja parasiittien solukierron säätelyssä ja niihin liittyvissä CDK-alatyypeissä antavat uusia mahdollisuuksia lääkekehitykselle.

➤ **Paul Pechan**
Dr.
C3 Bio GmbH, Ottobrunner Str. 37, D-82008,
Munich Germany

➤ **Charles L. Jaffe**
Prof.
Department of Parasitology, Hebrew University,
Hadassah Medical School, PO Box 12272,
Jerusalem, 91120, Israel