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Perspective

Cyclin-Dependent Kinase Inhibitors: Useful Targets in Cell Cycle Regulation

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Introduction

Cancer drug discovery is one of the most rapidly changing areas of pharmaceutical research. Most anti-cancer agents that are approved for clinical use are molecules which damage deoxyribonucleic acid (DNA), block DNA synthesis indirectly through inhibition of nucleic acid precursor biosynthesis, or disrupt hormonal stimulation of cell growth.¹ Many of these agents are widely used and have provided therapeutic benefit to many. However, attempts to modify these agents to improve efficacy (i.e. clinical response rate) or scope of activity with respect to tumor type have been largely disappointing. In the 1990s, two classes of natural product-derived anticancer compounds, the taxanes and the camptothecins, have provided new clinical agents with significantly improved levels of efficacy.^{2,3} The basis for their somewhat selective action against tumors remains, as it does for the more traditional agents, poorly understood.

Although efforts to refine and incrementally improve existing classes of anticancer agents continue, there has been a recent shift of emphasis toward novel mechanistic targets that have emerged as a direct consequence of the intense study of the underlying genetic changes associated with the cancerous state. The identification of tumor suppressor genes that are lost or inactivated and oncogenes that are activated in tumors has suggested many pathways and specific molecular entities as rational targets for anticancer drug discovery. For example, the early identification of the *ras* oncogene and its clinical significance stimulated interest in the targeting of farnesyl transferase as a means of controlling p21^{ras}-driven pathways.⁴ Several farnesyl transferase inhibitors are being clinically evaluated at this time.

More recently, pharmaceutical attention has been focused on the proteins that drive and control cell cycle progression. The significance of these potential drug targets is clearly evidenced by the high incidence of alterations in the genes that code for these proteins in tumors. A key class of cell cycle proteins is the cyclin-dependent kinases (CDKs). The CDKs activate host proteins through phosphorylation on serine or threonine using adenosine triphosphate (ATP) as a phosphate donor.

Targeting of protein kinases is a relatively recent activity for medicinal chemists. A number of natural product-derived kinase inhibitors have been discovered and characterized.^{5,6} The ATP-competitive nature and the broad activity of many of these agents against a range of protein kinases led many to believe that it would be difficult, if not impossible, to selectively target a single kinase. This belief was challenged in 1994 in a publication describing an exceptionally potent inhibitor of epidermal growth factor receptor tyrosine kinase that was ATP-competitive and highly specific relative to closely related receptor tyrosine kinases.⁷ Since that time, a number of laboratories have reported similarly on highly potent and specific inhibitors for other receptor tyrosine kinases.⁸ While the clinical utility of this class of agents and the generality of these findings remain to be determined, it is clear that at least some protein kinases appear to be medicinally "targetable" with compounds having useful potency and specificity.

The medicinal literature on kinase inhibition is very much in its infancy with trends and design principles just now emerging. In this Perspective, we will review the status of efforts directed specifically toward the discovery of potent and specific inhibitors of cyclin-

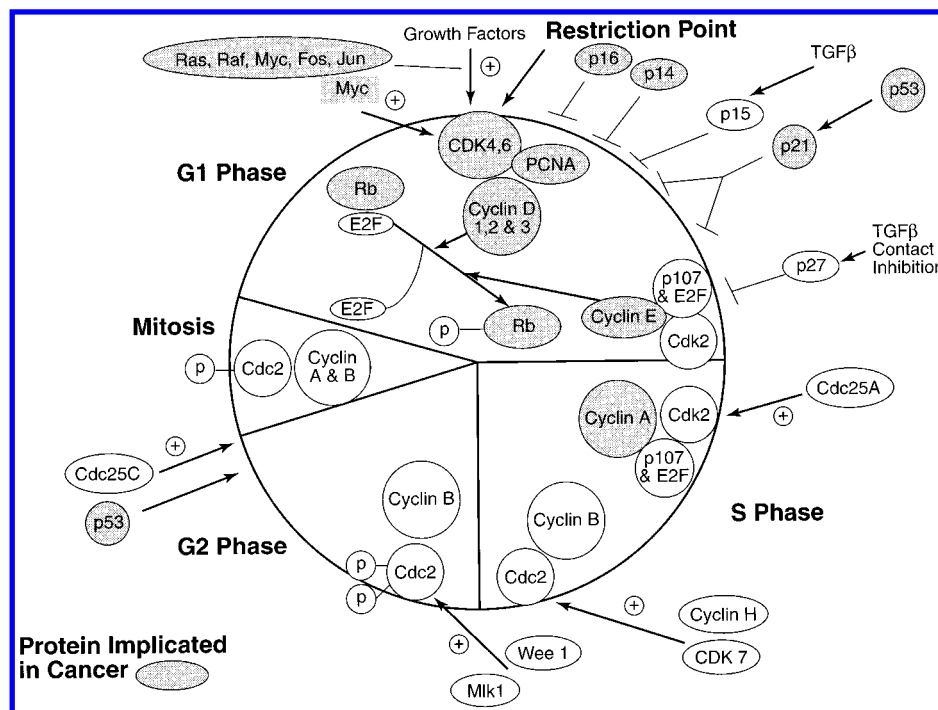


Figure 1. Cell cycle regulatory components determined to be altered in human cancers.¹²

dependent kinases (CDKs). Such inhibitors are expected to be useful in the treatment of proliferative disorders, including cancer, and it is hoped that the design principles gleaned from these efforts will facilitate the targeting of a wide range of protein kinases with broad therapeutic potential.

The Cell Cycle

CDKs have attracted attention as potential therapeutic targets because they are key players in the control of cellular proliferation.^{9–11} Eukaryotic cell division is driven by a regulated series of events collectively defined as the cell cycle. The principal function of the cell cycle is to effect the duplication of DNA and its appropriate distribution to newly divided daughter cells. Although strictly a continuum of events, for convenience the cycle has been described as falling into four phases. DNA replication occurs in S phase, and replicated DNA is distributed to progeny cells in M phase. Between these two phases are periods originally called gaps: gap 1 (G1) between M phase and S phase and gap 2 (G2) between S phase and M phase (see Figure 1). Thus G1 cells have a normal diploid (2N) DNA content, whereas G2 cells have a 4N DNA content. S phase cells are actively duplicating DNA and thus have a DNA content between 2N and 4N. Lengths of the individual phases of the cell cycle can vary with cell type and with conditions. In some rapidly dividing cells the G1 and G2 phases can be very short or absent. In addition, cells can reversibly exit the cell cycle at the end of M phase and enter a state known as Go, or they can irreversibly exit the cycle as happens during senescence or terminal differentiation.¹² (See Table 1 for terminology.)

The CDKs. Progression through the individual phases of the cell cycle is driven by CDKs.¹³ This family of kinases requires association with a cyclin regulatory

subunit for activity. Different cyclin/CDK pairs are active during each phase of the cell cycle (see Figure 1).¹² To date at least nine CDKs and more than twelve different cyclin families have been described. Individual family members have a limited ability to assort as heterodimeric complexes. Critical cyclin/CDKs for core cell cycle function are cyclin D/CDK4(6), cyclin E/CDK2, cyclin A/CDK2, and cyclin B/CDK1 (also known as cdc2). The D type cyclins D1, D2, and D3 associate with CDKs 4 and 6 and are believed to play an important role early in G1. The D type cyclin/CDKs collaborate with cyclin E/CDK2 to drive cells into S phase and through a point in the cell cycle called the restriction point.¹⁴ The restriction point represents a key stage in the life cycle of a cell in that once cells have traversed this point they become committed to cell cycle entry and no longer need growth factor input for further cell cycle progression. Further progression into S phase is driven by cyclin E/CDK2, and thereafter cyclin A/CDK2 promotes the further completion of S phase. CDK1 associates with cyclins A and B completes the cycle by controlling G2 progression and subsequent DNA segregation and then separation to two daughter cells during M phase and subsequent cytokinesis.

In addition to playing a key central role in cell cycle control, CDKs have also been described as potentially serving additional functions.¹⁵ Some members of the CDK family play a regulatory role in controlling the activity of cell cycle CDKs. These are collectively termed the CDK-activating kinases or CAKs.¹⁶ The role of these kinases is to control the activity of cell cycle CDKs by phosphorylation of critical serine and threonine residues needed for activity (see below). Some of these CAKs appear to have a dual role in that they are also able to phosphorylate the C-terminal domain of RNA polymerase II and control transcription.¹⁷ Other CDKs have been described with an as yet uncertain function. One of these, CDK5, has an atypical regulatory subunit (p35)

Table 1. Terminology Review

cell cycle function	protein family	specific members	other names	equivalents in yeast
cyclin/CDKs	G1 cyclin/CDKs	cyclins D1–D3/ CDK4,6 cyclin E1,E2/ CDK2		CLN1,2,3/CDC28: budding yeast CIG1/CDC2: fission yeast
	S phase cyclin/CDKs	cyclin A/CDK2		CLN5,6(3,4)/CDC28: budding yeast CIG2/CDC2: fission yeast
	G2 phase cyclin/CDKs	cyclin B/CDC2	cyclin B/CDK1	CLB1,2(3,4)/CDC28: budding yeast CDC13/CDC2: fission yeast
downstream CDK substrates	RB (tumor suppressor) principal substrate for CDK4 RB and many others for CDK2	RB, p107, p130		none
CDK modifying enzymes	CAK (CDK activating enzyme)		cyclin H/CDK7	CAK
	hwee1 (CDK inhibitory kinase) phosphorylates Tyr-15 on CDC2 hmyt1 (CDK inhibitory kinase) phosphorylates Thr-14 on CDC2 CDC25 (phosphatase that removes inhibitory phosphorylation on Thr-14, Tyr-15)	CDC25 A, B, C		wee1: fission yeast swe1: budding yeast myt1: fission yeast mik1: fission yeast CDC25
CDK inhibitory proteins	p16	p16 p15 p18 p19 p21 p27 p57	INK4a, MTS1, CDKN2 INK4b, MTS2 INK4c INK4d Cip1/Waf1 Kip1	none p25rum1: fission yeast p40sic1: budding yeast
	p21			
CDK assembly factors	hcdc37 MAT1			cdc37 none
RB interacting proteins	E2F	E2F 1–5, DP1–3		

distinct from other members of the cyclin family.^{18,19} CDK5 activity is highest in the brain.

Cell Cycle Progression – CDK Substrates. *G1 Phase:* Transit through the cell cycle is the result of sequentially controlled waves of cyclin/CDK activation. Less clear is the nature of the critical substrates for each of the activated kinases. Entry into the cell cycle at G1 and passage through the restriction point are controlled by the combined activity of cyclin D/CDK4 and cyclin E/CDK2.^{20,21} A key substrate and the only extensively documented substrate of cyclin D/CDK4 is the product of the tumor suppressor gene product retinoblastoma.²² The retinoblastoma protein contains multiple potential CDK phosphorylation sites, and different CDKs have been reported to have different site preferences for phosphorylation of this substrate.²³ Members of the retinoblastoma gene family are transcriptional corepressors.²⁴ Key among retinoblastoma interacting proteins are members of the E2F family of transcription factors. Phosphorylation of Rb leads to the release of bound E2F and activation of downstream E2F genes which include a number of genes important for DNA synthesis and subsequent cell cycle progression, e.g. tk, dhfr, cyclin E, and the retinoblastoma protein itself.^{25–27} Thus at least part of the role of the G1 cyclin/CDKs is to control transcription of genes important for subsequent cell cycle progression. In addition, it seems likely that cyclin E/CDK2 has additional substrates besides Rb that likely include proteins involved in assembly of the replication machinery.^{28,29} Phosphorylation of Rb appears to correspond to transition through the restriction point.³⁰ The critical role for cyclin D/CDK4 in this process is underscored by the fact that the natural

specific inhibitor of cyclin D/CDK4, p16, is inactive in cells that are deficient in Rb function.³¹ However, Rb-negative cells are capable of exhibiting a controlled cell cycle, and it has been shown recently that Rb-positive mouse fibroblasts can cycle in the almost complete absence of cyclin D/CDK4,6 activity.³² In addition mice in which CDK4 activity has been deleted develop normally with minor abnormalities in the pancreas.³³ Thus the precise role of cyclin D/CDK4,6 in core cell cycle control is still somewhat open to speculation.

G1 progression represents a phase in the cell cycle which is highly responsive to growth factor stimulation.³⁴ Growth factor signaling impacts cyclin D synthesis and stability, cyclin D/CDK4,6 complex assembly, and CDK activation.

S Phase: Once cells have passed the restriction point and committed to S phase, they become independent of growth factor stimulation for continued cell cycle progression. The precise role that CDKs play in control of DNA replication is not defined. Both cyclin E/CDK2 and cyclin A/CDK2 complexes appear to play an important role during S phase. Cyclin E/CDK2 appears to play a critical role in centrosome duplication,³⁵ and CDK2 is necessary for DNA replication in a cell-free system.³⁶ The critical substrates for cyclin E/CDK2 and cyclin A/CDK2 have not been defined.

G2/M Phase Regulation: Following completion of DNA replication, cells enter the G2 phase of the cycle with a 4N DNA content and begin preparations for M phase and cytokinesis. CDK1 has been shown to regulate passage of cells through these later phases of the cell cycle in association with both cyclins A and B. Complete activation of CDK1 requires both cyclin bind-

ing and phosphorylation of Thr-160/161.³⁷ CDK1 activity can be negatively regulated by phosphorylation on Thr-14 and Tyr-15.³⁸ This negative control pathway provides a point of intervention of checkpoint control pathways that halt the cell cycle if DNA replication is incomplete, DNA is damaged, or spindle assembly has not occurred correctly. Once activated CDK1/cyclin complexes prepare the cell for division during M phase. A range of potential substrates includes proteins that regulate chromosomal condensation, formation of mitotic spindles, and fragmentation of the Golgi apparatus.³⁹

CDKs and Cell Cycle Regulation. Cell cycle regulation is complex reflecting the importance of this key biological process. To maintain the correct cellular DNA content it is critical that mechanisms are in place to control the correct timing of entry into the cycle and to allow an orderly progression of events to avoid damage to the genetic material. Activities needed for passage through any given phase of the cycle need to be activated at the correct time and then inactivated once that phase is complete. For example, it is important that cell division does not occur if DNA is damaged or if replication and spindle assembly are not complete. For multicellular organisms it is also important to allow for appropriate cell cycle exit during terminal differentiation and senescence.

Consistent with this the activity of CDKs is tightly controlled during the cell cycle. Individual complexes are sequentially activated and deactivated. Multiple mechanisms exist to enable this to occur, and CDK activity is controlled at many levels.⁴⁰ These include (a) association with activating cyclin subunits, (b) synthesis and degradation of these cyclin subunits, (c) posttranslational modifications driven by kinases and phosphatases, and (d) interactions with natural inhibitors/activators.

Cyclin Association: As indicated earlier CDKs require association with the appropriate cyclin subunit for activity. This association results in structural changes in a region of the CDK called the T-loop allowing access of the active site to substrate.⁴¹ In addition it has been proposed that the cyclins play an important role in determining cyclin/CDK substrate specificity, and important substrate binding sites may reside on this subunit.⁴²

Protein Synthesis and Degradation: Although CDK levels remain fairly constant in proliferating cells, cyclin levels oscillate through the cell cycle with the levels of individual cyclins peaking at different times. Increases in cyclin levels are driven by increases in rates of synthesis. Thus synthesis of cyclin D in early G1 phase is under growth factor control, and the cyclin D promoter contains several regulatory elements sensitive to growth factor control.⁴³ Conversely ubiquitin-mediated proteolysis appears to play a key role in controlling cyclin down-regulation.⁴⁴ The process of cyclin/CDK assembly is also controlled although this process is less clearly understood. Several proteins have been proposed to play a role in cyclin D/CDK4 assembly, e.g. MAT1⁴⁵ and cdc37.⁴⁶ In addition it has been reported that the p21 family of low molecular weight CDK inhibitors might also provide an assembly role for cyclin D/CDK4 while being inhibitory for other cyclin/CDK pairs thus

adding an additional level of complexity and sophistication to the cell cycle engine.⁴⁷

Posttranslational Modification: Phosphorylation represents the most critical cyclin/CDK modification event described to date. Both activating and inactivating phosphorylations control CDK activity, and cyclin degradation is subject to control by phosphorylation. Typically, CAKs control activating phosphorylations on CDKs that result in correct structural orientation of residues surrounding the active site, e.g. Thr-160/161 in CDK1.⁴⁸ Additional kinases are able to catalyze phosphorylation events that result in CDK inactivation. For example, the *wee*/*myt1* family kinases modify CDK1 on Thr-14 and Tyr-15 resulting in inactivation.^{49,50} These inactivating phosphorylations can be reversed by the *cdc25* family of protein phosphatases of which three members (*cdc25A*, *B*, and *C*) have been described that are active in different phases of the cell cycle.^{51,52}

Low Molecular Weight Protein Inhibitors/Activators: Finally, cells express at least two families of low molecular weight proteins that serve as natural inhibitors of cyclin/CDKs: the p21 gene family (p21, p27, p57) and the p16 gene family (p15, p16, p18, p19).⁵³ Members of the p21 gene family are able to interact with both the cyclin and CDK subunits. Although originally described for their cyclin/CDK inhibitory role, p21 and p27 have now been found in catalytically active complexes of cyclin D/CDK4, and it has been suggested that they are necessary for efficient assembly of this CDK complex in its active form while serving an inhibitory role for other cyclin/CDKs, e.g. cyclin E/CDK2.^{32,47} Members of the p16 gene family interact only with the CDKs and are specific in their interaction with CDK4 and CDK6 where they are able to inhibit the activity of complexes containing these CDKs.⁵⁴

Thus a complex network of posttranslational modification events provides the cell with additional regulatory flexibility with respect to cell cycle control. It is likely, however, that in the cell cyclin/CDK complexes exist in multiple heteromeric complexes with potential substrates and regulators. It is probable that dynamic regulation and assortment of these complex components provide for an additional level of regulatory complexity.⁵⁵

Checkpoint Control. One of the benefits to the cell of this complex regulatory machinery is the ability to manifest checkpoint control. Originally defined by Hartwell and colleagues,⁵⁶ checkpoint control affords the cell the ability to ensure the orderly progression of cell cycle events. Cells continually monitor both their external and internal environment to ensure that cell cycle progression is appropriate. All phases of the cell cycle are subject to checkpoint control. Important and much studied checkpoints occur at G1 and at G2/mitosis. The G1 checkpoint serves to ensure that cells do not begin DNA synthesis unless they are appropriately nourished, they are correctly interacting with other cells or their substratum, and DNA is intact.⁵⁷ The replication^{58–61} and mitotic^{62,63} checkpoints serve to halt the cell cycle if DNA replication is not complete and if correct assembly of the mitotic spindle has not occurred. The response of the cell to inappropriate conditions is removal from the proliferative compartment. This may involve

either a transient arrest until the situation is corrected or a permanent arrest or death. Factors governing transient versus permanent cell cycle withdrawal are incompletely understood at the present time.

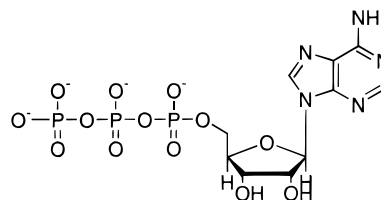
Cell Cycle Control and Disease. Cell cycle dysregulation has important consequences in relation to human disease. Inappropriate cell cycle progression is a critical feature of tumor cells, and it is the hallmark of transformed cells that they lack appropriate check-point control. In this respect deviations in G1 cell cycle control have been most studied. The Rb kinase pathway has been reported to be heavily mutated in human tumor cells, and it has been estimated that over 90% and potentially all human tumor cells contain mutations in this pathway.^{64–67} Mutations and/or deregulated expression in human tumors have been described for Rb, cyclin D, CDK4, cyclin E, and the p16 gene family.⁶⁸ In addition p21 is a downstream target of p53, the most commonly mutated gene in human cancers. Figure 1 indicates the cell cycle regulatory components that had been identified as altered in human cancers at the time of writing of the Hunter and Pines review in 1996. It clearly shows a concentration of mutational events associated with the G1/S transition. However, in addition deregulation of other phases of the cell cycle may also play a role in human tumorigenesis. Alterations in cell cycle control may not only be relevant to cancer progression. Inappropriate cell cycle entry has been described as contributing to certain neurodegenerative diseases,^{69,70} and activation of the cell cycle is targeted by several viruses both transforming and nontransforming to place the host cell in a state conducive to viral function.⁷¹

This observation has raised a level of interest in the CDKs themselves as potential therapeutic targets of promise in diseases where control of inappropriate cellular proliferation would be of therapeutic benefit. This in turn has led to initiation of strategies aimed at discovery of potential specific low molecular weight CDK inhibitors and their evaluation as therapeutic agents.

Inhibitors of CDKs

The elucidation of the primary, secondary, and tertiary structures (Figure 2)⁷² of CDKs has been a priority in the development of first-generation inhibitors. As noted by Kim⁷³ there are five regions which show marked differences when comparing the structure of CDK2 in the ATP complex⁷⁴ to the ternary structure of CDK2 complexed with ATP and cyclin.⁷⁵ These differences are reflected in the two domains which are more open in the ternary complex, the conformation of the triphosphate of ATP (1) which is different in the two structures, and the observation that when bound to cyclin A the α/β transition box is modified, the T-loop is completely reoriented, and the cyclin binding helix is in a different location and orientation.⁷⁶

Of even greater importance to the successful development of small molecule kinase inhibitors is the understanding of the primary interactions of endogenous inhibitors to their substrates. p21 is a universal inhibitor of the mammalian CDKs via complexation with cyclins, CDKs, and the proliferating cell nuclear antigen. Crystallization of p21 has resulted in diffractable crystals.⁷⁷ However, no crystal structure of p21 complexed to a cyclin has been reported.



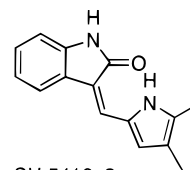
ATP, 1

The structure of p19^{INK4d} was also reported based on a series of NMR spectroscopy experiments. It was suggested that the majority of lethal mutations to the p16^{Ink4a} gene are the result of incorrectly folded and/or insoluble protein.⁷⁸ Subsequent publication of the crystal structure for CDK6 bound to p19^{INK4d} shows that, as seen in other kinases, CDK6 consists of the catalytic cleft lying between the two domains. p19^{INK4d} is made of five ankyrin repeats followed by a helix–loop–helix motif. The consecutive repeats are linked by β -turns between the N- and C-termini. The protein has an elongated L-shape overall. The interface of CDK6 and p19^{INK4d} has three main constituents: (1) α 1, α 2, and α 5 helices of p19^{INK4d} pack at right angles across the N-terminal β -sheet of CDK6; (2) the β -turns between ankyrins I/II and II/III in p19^{INK4d} act as a cap over the CDK6 β -sheet; (3) loops L4 and L6 clamp on the C-terminal domain resulting in a twist of CDK6 prohibiting effective ATP binding.⁷⁹

The kinase substrate ATP has been cocrystallized with CDK2 (Figure 3).⁷³ The key interactions in the ATP pocket include hydrogen bonds between Leu-83 to N1, Glu-81 to N(6)H2, and Asp-86 to O(2')H, in addition to the phosphate chain interactions with the pocket at Lys-33, Asn-132, Asp-145, Lys-129, Thr-14, and Wat-558.

This type of structural information has led to the employment of three main approaches to the inhibition of CDKs which include ATP-competitive inhibitors, noncompetitive inhibitors which bind in the region of the natural peptide inhibitors, and molecules incorporating both of these approaches. The majority of published reports on CDK inhibitors utilize a small molecule approach to develop ATP-competitive inhibitors.

Frequently, first-generation approaches to inhibitors rely on similarities to known inhibitors of analogous substrates. This type of approach has been modestly successful for kinase inhibitors. Indeed, for many kinases, small molecule ATP-competitive inhibitor approaches are being tested in clinical studies. Some of the more advanced tyrosine kinase inhibitors include the 3-substituted indolinone SU 5416 (2) which is in phase II for a cancer indication,⁸⁰ while ZD 1839, a substituted anilinoquinazoline is being developed as a treatment for solid tumors.⁸¹ It is interesting to note that both of these kinase inhibitors are relatively flat and contain a high degree of conjugation.



SU 5416, 2

Quinazolines have been used as the core of a number of additional tyrosine kinase inhibitors which are in

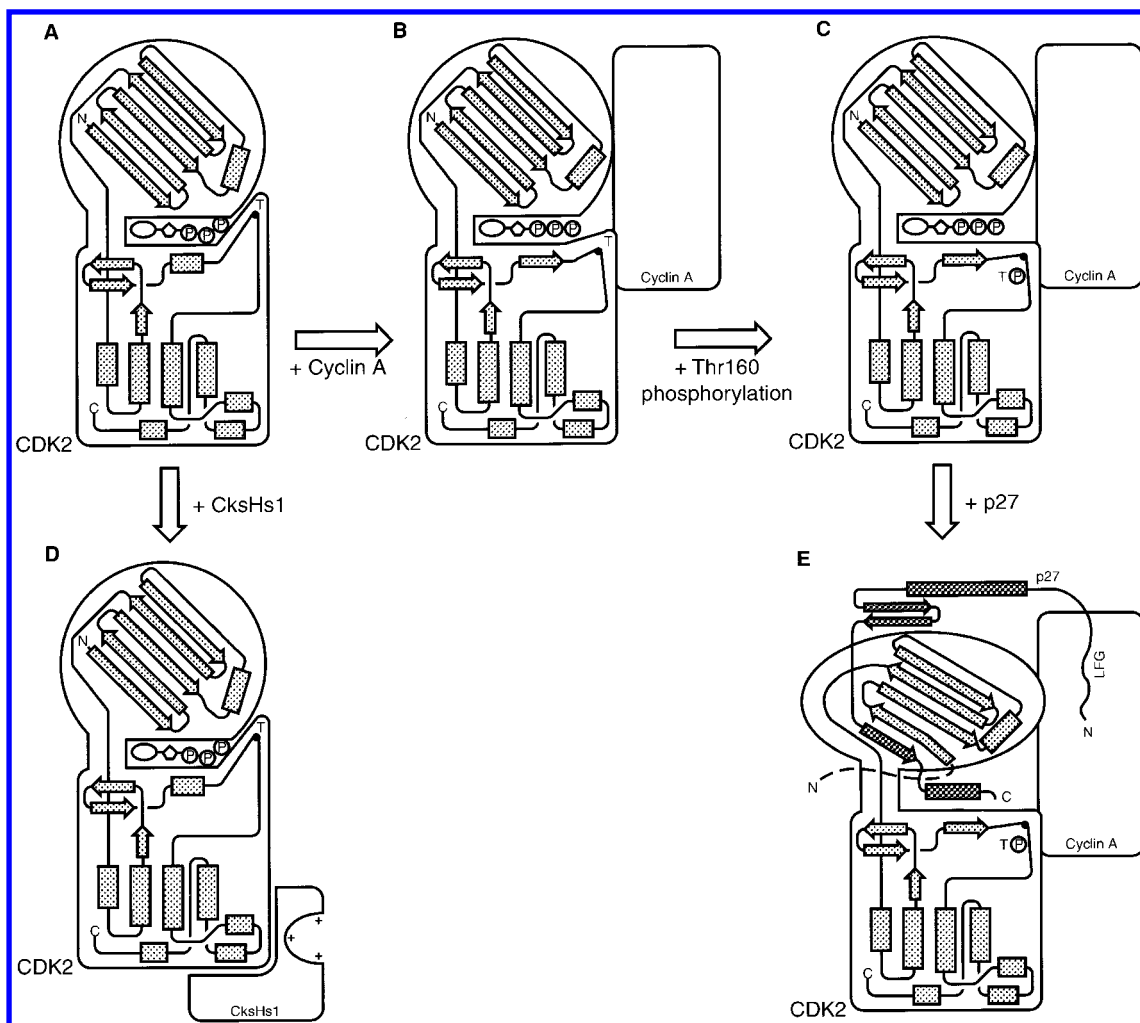
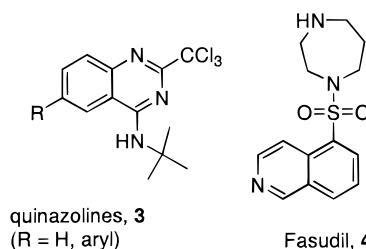


Figure 2. Schematic representations of the five CDK2 structures determined to date. (A) The inactive CDK2 monomer B, in which the ATP phosphates are misoriented because of the presence of the L12 helix and the protein substrate binding site is blocked by the T-loop (T). (B) The partially active complex of CDK2 and a truncated cyclin A. The ATP phosphates are correctly positioned and the T-loop only partially obscures the active-site cleft. (C) The fully active CDK2/cyclin A complex phosphorylated at Thr-160 in the T-loop. The T-loop lies flat along the base of the active-site cleft. (D) The inactive CDK2 subunit complexed with the CksHs1 protein, which interacts primarily with CDK2 helix 5 and the L14 loop that follows this helix. The positively charged patch on the CksHs1 protein has the potential to interact with phosphates on other proteins and may augment the binding of CDK2 to phosphorylated substrates or regulatory proteins. (E) Phosphorylated CDK2/cyclin A in a complex with the inhibitor p27. The inhibitor interacts extensively with the upper kinase lobe, disrupting its structure and blocking ATP binding. Dashed line represents the disordered amino-terminal region of CDK2. In all panels, dark-gray-shaded rectangles and large dark-gray-shaded arrows represent α -helices and β -strands, respectively; PST, PSTAIR helix (single-letter code for amino acids), also known as helix 1; N, amino-terminus; C, carboxyl-terminus; circled P, phosphate; T, threonine 160; LFG, p27 motif involved in cyclin binding. Secondary structural elements and loops in this figure and all other figures are not drawn to scale, and interactions between elements have been greatly simplified for clarity⁷² (reproduced with permission).

various stages of development.^{82–88} Taking advantage of the quinazoline as a strong recognition element for the ATP binding pocket, this core has been utilized as a CDK inhibitor scaffold (**3**) (Thais M. Sielecki, unpublished results). A variety of alkylamino groups are tolerated at the 4-position, while the 2-position is much more sensitive to alternate substitution. The addition of 6-aryl groups increases the potency of these molecules to the submicromolar region for CDK2 (**3**, where R = *m*-aminophenyl: CDK2/cyclin E IC_{50} = 0.65 μ M, CDK4/cyclin D IC_{50} = 2.1 μ M).

PKC inhibitors are also showing utility in clinical studies and include some drugs currently marketed such as fasudil (**4**), a diazepine developed as a vasodilator which inhibits smooth muscle cell proliferation and DNA synthesis.⁸⁹ Staurosporine (**5**), another ATP-competitive PKC inhibitor, is a natural product isolated



from *Streptomyces staurosporeus*⁹⁰ which shows significant CDK inhibition (CDK2/cyclin A IC_{50} = 7 nM,^{91,92} CDK4/cyclin D IC_{50} = 3–10 μ M, cAPK IC_{50} = 8.2 nM).⁹³ The cocrystallization of staurosporine with CDK2 reveals several key interactions of the inhibitor with the backbone residues lining the ATP binding pocket. Similar interactions are seen in the binding of ATP to CDK2 which include a hydrogen bond to the oxygen of

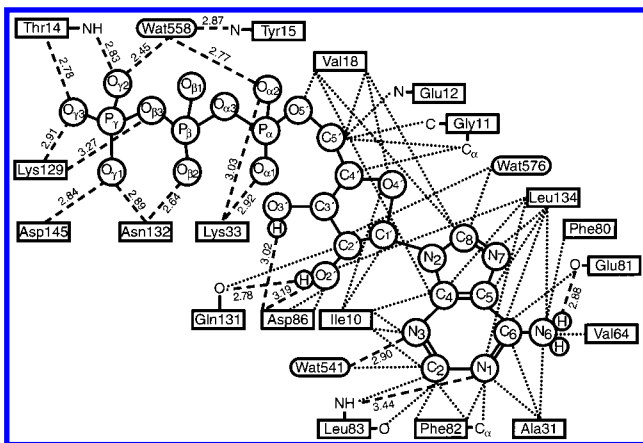
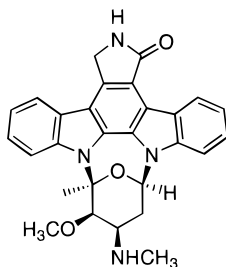
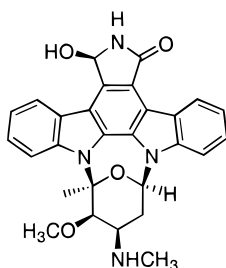


Figure 3. All atomic interactions between CDK2 and ATP. Contacts with protein side chains are indicated by lines connecting to the respective residue box, while interactions to main chain atoms are shown as lines to the specific main chain atoms indicated. van der Waals contacts are indicated by dotted lines and hydrogen bonds by thick broken lines. ATP van der Waals contacts to phosphates were omitted for clarity⁷³ (reproduced with permission).

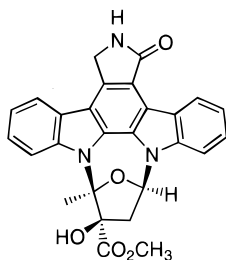
Glu-81 and a hydrogen bond acceptor interaction with the nitrogen of Leu-83. Additional hydrogen bonds are formed between the methylamino group and the carbonyls of Gln-131 and the side chain of Asp-86.⁹⁴



Staurosporine, 5



UCN-01, 6

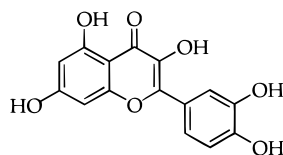


K-252a, 7

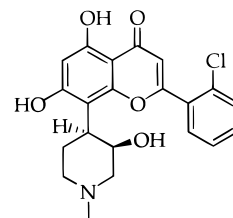
The hydroxystaurosporine analogue UCN-01 (**6**), isolated from the culture broth of *Streptomyces* sp., is currently in phase I clinical trials to evaluate the compound's tolerability and pharmacokinetics.⁹⁵ Additionally, **6** is being evaluated as a radiosensitizer.^{96,97} Compound **6** is a nonspecific kinase inhibitor against PKC ($IC_{50} = 6.8$ nM), CDK1 ($IC_{50} = 31$ nM), and CDK2 ($IC_{50} = 30$ nM) while having only weak activity against Raf-I kinase and MAPK ($IC_{50} = 620$ and 910 nM). In cell cycle analyses **6** inhibits the G1/S transition. Additionally, A549 cells treated with various concentrations of **6** showed a decrease in a dose-dependent

manner in the amount of phosphorylated pRB.^{98,99} Compound **6** has also been shown to induce apoptosis in malignant glioma cells¹⁰⁰ and in gastric and breast cancer cells.¹⁰¹ In response to the challenging chemistry of this series, several syntheses of staurosporine, UCN-01, and the furanyl analogue K-252a (**7**) have been reported.^{102–106} Although this series shows good potency in a variety of kinases, its apparent lack of selectivity presents the possibility for non-mechanism-dependent side effects in a clinical setting.

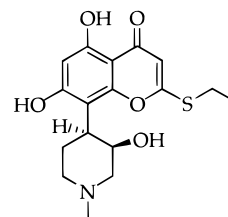
Several flavonoids have proven to be effective at inhibiting CDKs, including quercetin (**8**), flavopiridol (**9**), and 2-thioflavopiridol (**10**). Quercetin is a naturally occurring flavonoid which has shown cardiovascular, anticancer, and analgesic effects. Over a range of concentrations from 10 nM to 10 μ M, quercetin has shown a dose-dependent and reversible inhibition of cancer cell proliferation. Cell cycle analysis has shown that cells are blocked at the G0/G1 interface, a result consistent with a CDK inhibitor.¹⁰⁷ Quercetin is a relatively weak CDK inhibitor which does, however, present the flavone core as a viable structural template.



Quercetin, 8



flavopiridol, 9



10

Flavopiridol (**9**) (NSC-649890, L86-8275) utilizes the flavone core while introducing additional interaction sites through the incorporation of the piperidine at the 8-position and by the modification of the 2-aryl substitution pattern. This flavone is the most advanced CDK inhibitor, currently in phase II clinical trials for refractory myeloma, advanced gastric carcinoma, and high-grade non-Hodgkin's and mantle cell lymphoma.^{108–111} The compound is a nonselective kinase inhibitor showing in vitro activity against CDK4, CDK2, CAK (CDK4/cyclin D $IC_{50} = 0.4$ μ M, CDK2/cyclin A $IC_{50} = 0.1$ μ M, CAK $IC_{50} = 0.3$ μ M),^{112,113} CDK1 ($IC_{50} = 0.4$ μ M),¹¹⁴ and PKC¹¹⁵ with very slight activity for EGF-receptor tyrosine kinase ($IC_{50} = 21$ μ M) and PKA ($IC_{50} = 122$ μ M).¹¹⁶ When flavopiridol is given daily as an iv bolus in mice, selective apoptosis of cells in the thymus, spleen, and lymph nodes was observed. When dosed at 7.5 mg/kg bolus iv or ip in mice on each of 5 days, eleven of twelve advanced stage sc human HL-60 xenografts underwent complete regressions and animals remained disease-free several months after one course of drug. Other lymphomas including SUDHL-4 sc, AS283, and Nalm/6 also showed significant responses to flavopiridol treatment.¹¹⁷ This flavone has also shown promising

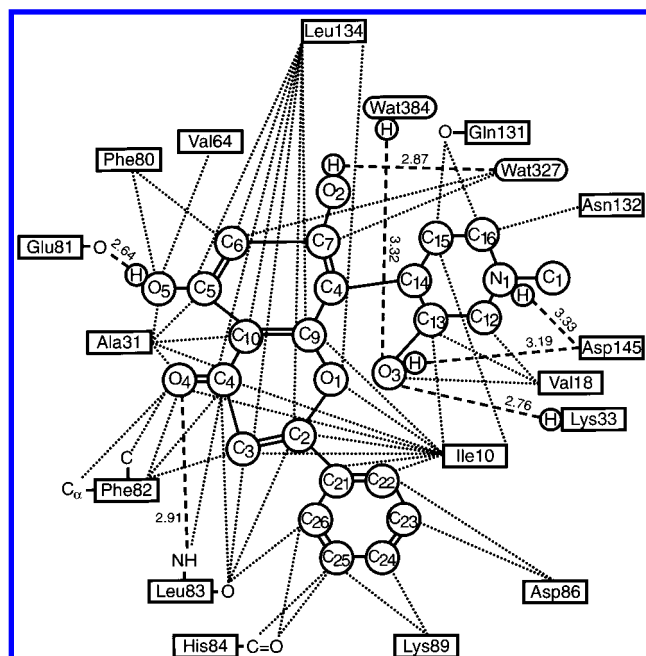


Figure 4. All atomic interactions between CDK2 and deschloroflavopiridol. Contacts with protein side chains are indicated by lines connecting to the respective residue box, while interactions to main chain atoms are shown as lines to the specific main chain atoms indicated. van der Waals contacts are indicated by dotted lines and hydrogen bonds by thick broken lines⁷³ (reproduced with permission).

results in combination therapy, enhancing the activity of Taxol in human trials.^{118,119}

The X-ray crystal structure of deschloroflavopiridol cocrystallized with CDK2 reveals several key interactions^{120,121} (Figure 4).⁷³ Key hydrogen bonds are formed to Lys-33 and Wat-384 with O(3) of the hydroxypiperidine moiety and to Asp-145 with the N(11)H and O(3)H. Core hydrogen bonds are formed to Glu-81 with O(5)H and to Leu-83 with O(4).

Replacement of the 2-aryl moiety with a thio group (**10**) provides a series that maintains CDK inhibitory activity and has potential utility in the treatment of cancer, inflammation, and arthritis. A representative compound of the 2-thioflavone series is shown; however, no *in vitro* data has been disclosed in the literature.¹²²

Overall, the flavone core has been one of the more frequently utilized ATP-competitive structural classes. Again, the compounds show a lack of specificity, and this causes concern for possible undesired side effects in the clinical setting.

The purine ring system is used in a number of CDK inhibitors. Included in this category are olomoucine (**11**), roscovitine (**12**), isopentenyladenine (**13**), boheminine (**14**), purvalanol (**15**), and CVT 313 (**16**). Olomoucine (**11**) was identified as an active lead compound through the screening of substituted purines.¹²³ Showing modest potency for CDK1/cyclin B ($IC_{50} = 7 \mu M$), CDK2/cyclin A ($IC_{50} = 7 \mu M$), CDK2/cyclin E ($IC_{50} = 7 \mu M$), and CDK5/p35 ($IC_{50} = 3 \mu M$), the compound was inactive with respect to CDK4/cyclin D1 and CDK6/cyclin D3 in addition to other non-CDKs including PKC and insulin-dependent tyrosine kinase. A second-generation purine in which the 2- and 9-substituents are increased in size is roscovitine (**12**). This compound shows a 10-fold improved potency for CDK1, CDK2, and CDK5 while

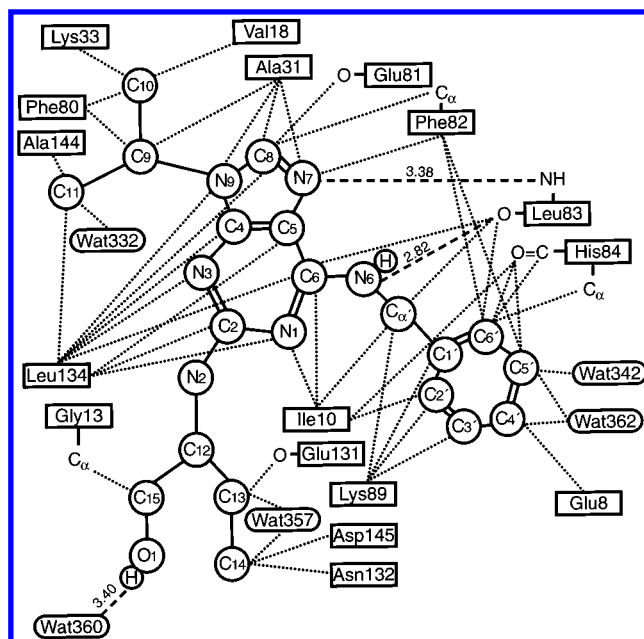
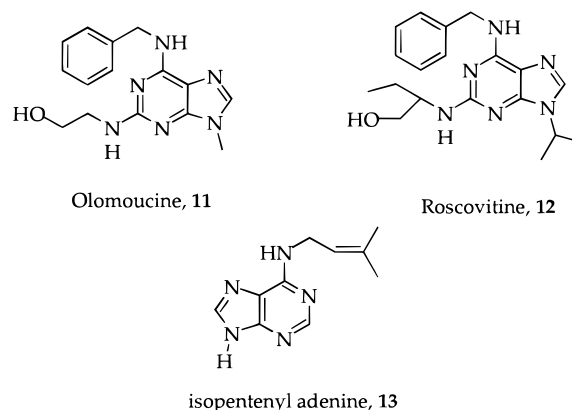


Figure 5. All atomic interactions between CDK2 and olomoucine. Contacts with protein side chains are indicated by lines connecting to the respective residue box, while interactions to main chain atoms are shown as lines to the specific main chain atoms indicated. van der Waals contacts are indicated by dotted lines and hydrogen bonds by thick broken lines⁷³ (reproduced with permission).

maintaining selectivity with respect to a number of other kinases. When tested in cells using a non-small-cell lung cancer cell line, MR65, and a neuroblastoma cell line, CHP-212, dose-dependent inhibition of the G1/S phase and G2/M/G1 transitions was observed for both **11** and **12**.^{124–126} S phase progression was also inhibited in a dose-dependent fashion. A complete cell cycle block was observed for both **11** and **12**, while Western blot analysis showed a decrease in CDK1 protein.¹²⁷ These cellular effects are consistent with CDK inhibition.



Olomoucine (**11**), roscovitine (**12**), and isopentenyladenine (**13**) have been cocrystallized with CDK2. For **12** the more potent *R*-isomer was employed. Interestingly, although the three compounds bind in the ATP pocket, the three compounds are oriented differently with respect to the protein, albeit the binding orientations of **12** and **11** are similar.^{127,128} Key hydrogen bonds in **11** are N7 to NH of Leu-83, N6 to carbonyl of Leu-83, and OH of the amino alcohol to Gln-131 (Figure 5).⁷³

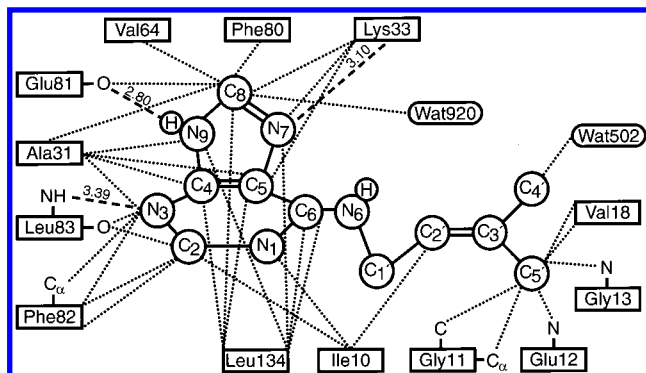
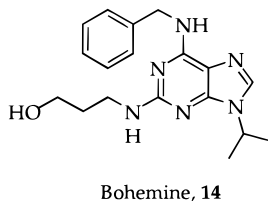


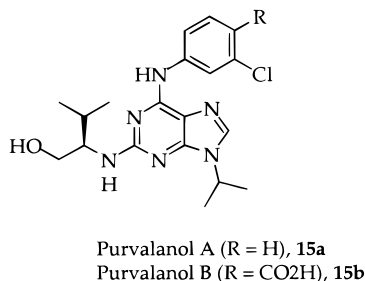
Figure 6. All atomic interactions between CDK2 and isopentenyladenine. Contacts with protein side chains are indicated by lines connecting to the respective residue box, while interactions to main chain atoms are shown as lines to the specific main chain atoms indicated. van der Waals contacts are indicated by dotted lines and hydrogen bonds by thick broken lines⁷³ (reproduced with permission).

Isopentenyladenine's (**13**) key interactions are N9 to Glu-81, N7 to Lys-33, and N3 to NH of Leu-83 (Figure 6).⁷³ The binding modes of **11** and **13** are twisted with respect to one another.¹²¹

Bohemine (**14**), a purine-based olomoucine analogue substituted with a 6-benzylamine and a 2-amino alcohol, is reported to have inhibitory effects on CDK1 (CDC2). Preclinical data for this molecule have been reported as showing a prolonged survival time in mice bearing B16 melanoma and P388D1 leukemia. Some animals with transplanted tumors were reported to have been cured when dosed with bohemine.¹³⁰

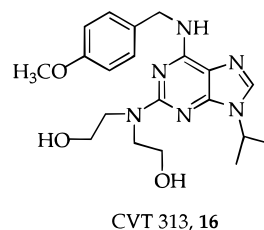


Attempts at maximizing the binding of the purine core have also employed a combinatorial approach. Recognizing that olomoucine binds within the ATP binding site (rotated 160° relative to ATP), elements of diversity were introduced at the 2-, 6-, and 9-positions in an attempt to enhance the competitive binding of the series, yielding the purvalanols (**15a,b**). Purvalanol B (**15b**) has an IC_{50} of 6 nM for CDK2/cyclin A. The compound shows selectivity with respect to CDK4/cyclin D1, erk1, PKC, and RAF in addition to other kinases. However, purvalanol was shown to be active for CDK2/cyclin E (IC_{50} = 9 nM) as well as CDK5/p35 (IC_{50} = 6 nM).¹³¹

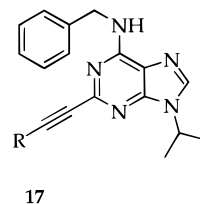


Other purine library approaches have resulted in the identification of CVT 313 (**16**),^{132–134} a 2-,6-,9-trisubsti-

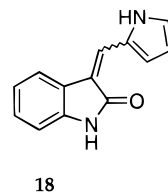
tuted purine which is a specific and ATP-competitive inhibitor of CDK2/cyclin A (IC_{50} = 0.5 μ M) with similar potency against CDK2/cyclin E. CDK4/cyclin D (IC_{50} = 215 μ M) and CDK1/cyclin B (IC_{50} = 4.2 μ M) inhibitory activity was less, and the compound did not inhibit PKC. Compound **16** reversibly inhibits cell proliferation at the G1/S or G2/M boundary and inhibits Rb hyperphosphorylation in MRC-5 cells. Compound **16** was shown to inhibit restenosis in rats exposed to a saline solution (1.25 mg/kg). Each animal treated showed at least 70% inhibition of the neointimal area. Purine **16** is being pursued in an antiproliferative strategy for the treatment of restenosis.¹³⁵



In addition to these library approaches other groups have employed more traditional approaches in an effort to take advantage of the purine core to extend the SAR. 2-Alkynylated purines with terminal substituents including a number of alkyl alcohols (**17**) showed improved CDK1/cyclin B IC_{50} 's ranging from 0.18 to 1.2 μ M.¹³⁶

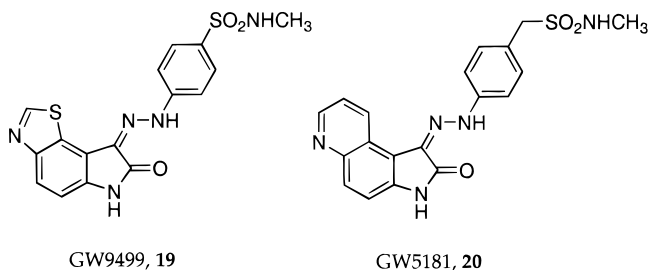


Oxindole **18** is a modestly potent CDK4/cyclin D-selective inhibitor (CDK4/cyclin D IC_{50} = 4.9 μ M, CDK2/cyclin E IC_{50} = 10.0 μ M, CDK1/cyclin B IC_{50} = 10.2 μ M). When tested in a human mammary adenocarcinoma cell line, MCF-7, the compound exhibited submicromolar potency (IC_{50} = 0.42 μ M). Interestingly, this oxindole showed potency in two Rb⁺ cell lines (MCF-7 and ZR-75-1) and was less effective in an Rb⁻ cell line (BT-549), a result consistent with CDK inhibition.¹³⁷

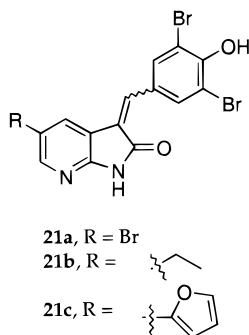


Two hydrazone analogues have been reported as potent CDK1 and CDK2 inhibitors. GW9499 (**19**) (CDK2 IC_{50} = 3.5 nM) differs from GW5181 (**20**) (CDK2 IC_{50} = 6.2 nM) in the third ring fusion of thiazole versus pyridine and a one-carbon truncation on the sulfonamide substituent.^{138,139} Hydrazone **19** is the more potent compound in the cell lines reported, which include HDF, MD468, and RKO cell lines. When dosed at 5 μ M for 7 days, RKO cells exhibited cell death by day 4 of dosing while in MD468 cells, cell death was observed by day 6. When dosed once daily for 6 days at

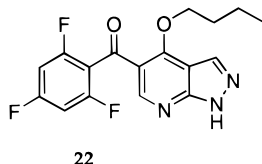
30 mg/kg iv in a RKO xenograft model, **19** showed little tumor growth inhibition. However, **20** dosed at higher amounts (100 mg/kg iv, once a day for 10 days) showed a modest decrease in tumor growth by day 20.¹⁴⁰



A variation on this core incorporates a pyridine and is truncated by one ring as in **21**. A number of pyridine substitutions are reported including a 5-bromo, 5-ethyl, and 5-furyl moiety. The pendant aryl ring can also be substituted with alkyl or aryl groups. The listed compounds are reported to have CDK2 IC₅₀ values less than 1 μ M. This series does not always show CDK selectivity, a number of compounds in this series showing activity in a RAF assay (IC₅₀ < 1 μ M).¹⁴¹

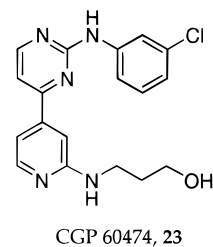


Pyrazolopyridines **22** were recently disclosed as CDK inhibitors. The most preferred compounds within this disclosure have a 4-butoxy substituent and a trifluoromethanone at the 5-position. The compounds are claimed to have enzyme IC₅₀ values of less than 50 μ M for CDK1/cyclin B, CDK2/cyclin E, and CDK4/cyclin D.¹⁴²

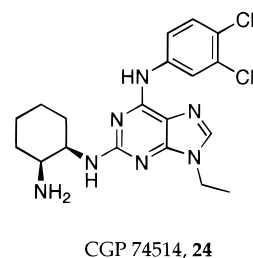


The phenylpyrimidine CGP 60474 (**23**) was found to be a potent inhibitor of both CDK1 (IC₅₀ = 0.020 μ M) and CDK2 (IC₅₀ = 0.05 μ M), while displaying weaker, submicromolar to low micromolar activity for PKC α , ERK-1, c-fgr, and CDK4. Other growth-related protein kinases were unaffected by exposure to CGP 60474 including JNK, p38, and other PKC subtypes. In the presence of 100 nM CGP 60474 FACS analysis of the cell cycle after release from the M/M block showed reversible G1/S arrest in U2-OS cells. U2-OS cells were arrested in the G2 phase. Interestingly, biochemical analysis of **23** revealed that no functional CDK/cyclin complexes were formed when the compound was present.¹⁴³ This result is very interesting as a compound

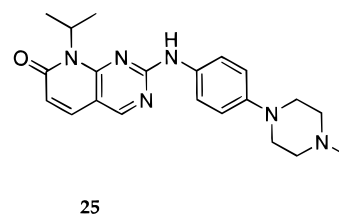
which is purely ATP-competitive should not change the conformation of the kinase sufficiently to prevent cyclin complexation. As such it may be that the compound is inhibiting other kinase targets which are themselves inhibiting CDK/cyclin complexation.



A second-generation compound from the purine series, CGP 74514 (**24**), is reported to be under preclinical evaluation. A potent inhibitor of CDK1 (IC₅₀ = 16 nM) and CDK2 (IC₅₀ = 9 nM), the compound showed weak activity for PKC (IC₅₀ = 6.1 μ M), EGFR (IC₅₀ = 9.3 μ M), and PKA (IC₅₀ = 125 μ M).¹⁴⁴ The biochemical analysis of this compound has not yet been reported.

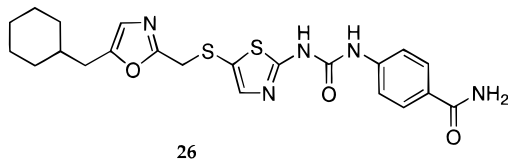


To date, only two series of compounds which show selectivity for CDK4/cyclin D1 over other kinases have been disclosed, the pyridopyrimidines and AG12275. A representative example of the pyridopyrimidine series is **25**, which was reported to have a CDK4/cyclin D IC₅₀ = 0.045 μ M. While selective with respect to CDK2/cyclin E (IC₅₀ = 0.27 μ M) and CDK1/cyclin B (IC₅₀ = 0.675 μ M) the compound was equipotent against CDK2/cyclin A (IC₅₀ = 0.058 μ M).¹⁴⁵

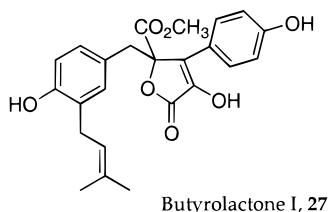


The structure of AG12275 has not been reported, but the compound is claimed to be selective for CDK4/cyclin D (IC₅₀ = 3.3 nM) as compared to CDK2/cyclin A (IC₅₀ = 220 nM) and CDK1/cyclin B (IC₅₀ = 325 nM). AG12286 has also been disclosed but does not have selectivity for CDK4/cyclin D (CDK4/cyclin D IC₅₀ = 12 nM, CDK2/cyclin A IC₅₀ = 5.7 nM, CDK1/cyclin B IC₅₀ = 2.2 nM). Both compounds are selective with respect to PKC, cAMP, ERK2, VEGF, and FGF. When dosed in an HCT cell line, AG12275 had an IC₅₀ = 300 nM, while in AG12286 had an IC₅₀ = 400 nM. In an in vivo HCT 116 xenograft model, AG12275 dosed at 50 mg/kg showed a 70% tumor growth inhibition at day 41.¹⁴⁶

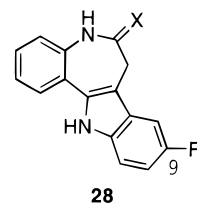
Thiazole **26** orients the core in a novel fashion with respect to the various chromophores and hydrogen-bonding groups. An amine linkage is required at the 2-position with ureas being claimed as preferred. There is a lipophilic moiety attached to the 4-position. This series of compounds is reported to have enzyme activity for CDK2/cyclin E, CDK4/cyclin D, and CDK1/cyclin B of less than 50 μM .¹⁴⁷



Butyrolactone I (**27**), reported to be a selective inhibitor of CDK2 and CDK1 kinase, was isolated from *Aspergillus* species F-25799. This ATP-competitive butyrolactone inhibited CDK1 ($\text{IC}_{50} = 0.68 \mu\text{M}$) and CDK2 ($\text{IC}_{50} = 0.82 \mu\text{M}$) yet did not inhibit MAPK, PKC, AK, or TyrK. This compound inhibited pRB phosphorylation at 0.48–31 μM in selected nuclear extracts.¹⁴⁸ In metaphase-arrested *Xenopus* egg extracts pretreated with butyrolactone and then released into interphase, oscillation of DNA replication activity was prevented, thereby lengthening the cell cycle, a result consistent with the compound's CDK2 inhibitory activity.¹⁴⁹ The compound also showed antineoplastic effects on non-small-cell and small-cell lung cancer lines with IC_{50} values of around 50 $\mu\text{g/mL}$. Additionally, the CDK1 kinase activity of PC-14 cells was inhibited by treatment with 20 $\mu\text{g/mL}$ of the lactone for 2 h. Cell cycle arrest was observed at the G2/M phase in a concentration-dependent manner, again consistent with CDK1/CDK2 inhibition.¹⁵⁰



The paullones represent a series of benzazepines with submicromolar potencies for CDK1/cyclin B. The lead 9-bromo analogue **28** was reported to have a CDK1/cyclin B IC_{50} of 0.4 μM while being selective with respect to CDK2/cyclin E ($\text{IC}_{50} \sim 20 \mu\text{M}$) and CDK4/cyclin D1 (no inhibition observed). The compounds were found to be ATP-competitive and in serum-starved cells induced a G1 cell cycle arrest. Computer modeling suggested that the binding motif of these compounds is centered on a hydrogen bond through water to Phe-80 and a hydrogen bond to Leu-83 on the other side of the molecule. Interactions with Leu-134 and Isoleu-10 serve to maintain the core in the proper orientation. When tested in an HCT cell line the parent 9-bromo compound had an $\text{IC}_{50} = 2.0 \mu\text{M}$.¹⁵¹ It should be noted that the CDK1/B used in analyzing this series was harvested from starfish oocytes. Although comparison of mammalian and starfish CDK1 shows them to be 75% identical and 85% homologous, it is not known if a direct comparison of enzyme inhibition is valid.¹⁵²

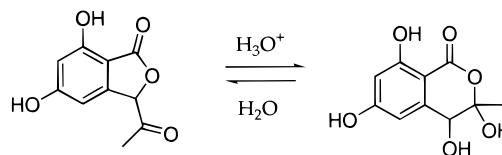


Cmpd	R	X	CDK1/B IC_{50} (μM)
28a	Br	O	0.40
28b	H	O	7
28c	CN	O	0.024
28d	NO_2	O	0.035
28e	Br	S	2.3
28f	Br	S- CH_3	43

Further attempts to optimize the inhibitory activity of the 7,12-dihydroindolo[3,2-*d*][1]benzazepin-6(5*H*)-one series led to the observation that substitution at the 4-, 5-, 10-, 11-, and 12-positions leads to a decrease in enzyme inhibitory potency. An unalkylated ring amide was preferred as compared to a thiolactam, thioimide, or hydroxyamidine. Substitution of the bromo moiety by a nitro group (CDK1/cyclin B $\text{IC}_{50} = 0.03 \mu\text{M}$, HCT 116 $\text{IC}_{50} = 0.07 \mu\text{M}$) or by a cyano group (CDK1/cyclin B $\text{IC}_{50} = 0.02 \mu\text{M}$, HCT $\text{IC}_{50} = 20 \mu\text{M}$) resulted in compounds with greater enzyme potencies but variable translation into cells.¹⁴⁶ Within this series the data shows that cellular activity does not parallel CDK activity, suggesting alternate mechanisms for cellular antiproliferative effects for some of the compounds.¹⁵³

A variety of known anticancer compounds appear to have variable amounts of CDK inhibitory activity. Although it is not clear if this represents the mechanism of action for these compounds, it is of interest that CDK inhibitory effects are possible. Sodium butyrate, a product of colonic fermentation of dietary fiber, is one such CDK2 inhibitor. Cell cycle studies show cell proliferation is inhibited by this compound through a G1 phase block. Interestingly, CDK4 and CDK6 are unaffected by sodium butyrate. Studies have demonstrated that the observed CDK2 inhibition is effected by modulation of the cell cycle regulatory protein p21 and hence may be an indirect kinase interaction.¹⁵⁴

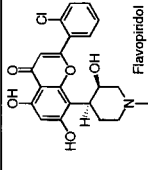
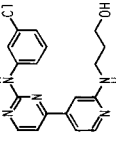
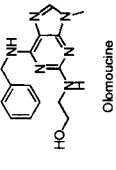
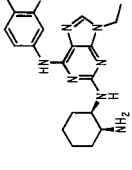
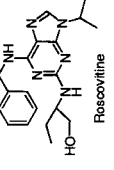
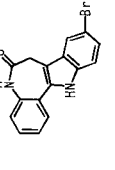
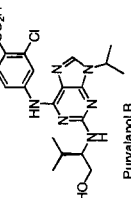
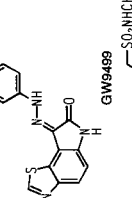
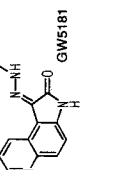
Acetophthalidin (**29**) is a metabolite of the fungal strain BM923¹⁵⁵ and of *Ceratocystis minor*, a fungus associated with the blue stain disease of Canadian pine trees.¹⁵⁶ The compound has shown complete inhibition of cell cycle progression of mouse tsFT210 cells in the M phase at 6.25 $\mu\text{g/mL}$ concentrations.¹⁵⁵ The total synthesis of this compound¹⁵⁷ has been reported. It is not clear that the compound's antiproliferative effects are the direct result of CDK inhibition.



Acetophthalidin, 29

As cell biology becomes more defined, the molecular targets of currently marketed pharmaceutical compounds are frequently redefined. This is the case for the

Table 2. Key CDK Inhibitors

Inhibitor	CDK1/B IC ₅₀ (μM)	CDK2/A IC ₅₀ (μM)	CDK2/E IC ₅₀ (μM)	CDK4/D IC ₅₀ (μM)	Inhibitor	CDK1/B IC ₅₀ (μM)	CDK2/A IC ₅₀ (μM)	CDK2/E IC ₅₀ (μM)	CDK4/D IC ₅₀ (μM)
	0.4	0.1	--	0.4		0.020	--	0.050	--
	7.0	--	7.0	inactive		0.016	--	0.009	0.029
	0.65	--	--	--		0.035*	--	--	--
	--	0.006	0.009	--	AG12286	0.0022	0.0057	--	0.012
	--	--	0.0035	--	AG12275	0.325	0.220	--	0.0033
	--	--	0.0062	--					

*CDK2 harvested from starfish oocytes.

and exit. Hopefully, selective chemical kinase inhibitors will provide not only useful therapeutics in the near term but also tools to understand the cycle itself. With this increased understanding, maybe we can drive discovery toward yet more selective and effective therapies.

There are many challenges in the development of small molecule inhibitors of the CDKs, as evidenced from some of the key compounds presented in this Perspective (Table 2). To achieve success in the pharmaceutical modulation of the cell cycle, two main issues must be explored.

First, selectivity among the kinases for efficacious and safe control of the cell cycle has not been realized. All kinases have an ATP binding pocket which tends to have a high degree of homology. There are an estimated 2000 kinases in the human body.¹⁸⁹ As the vast majority of reported compounds are ATP-competitive, the synthesis of CDK inhibitors with good selectivity among the kinases remains a continuing challenge. A truly selective CDK inhibitor has not yet been realized, and the level of selectivity that would be required of a clinically successful inhibitor has not been and will not be defined until more selective inhibitors are evaluated.

Second, as evidenced from those compounds with nanomolar potency for the CDK enzymes, two hydrogen bonds between the substrate and the cyclin-dependent kinase in the ATP pocket are preferred. As there have been no compounds disclosed with picomolar potency, it remains a possibility that an additional binding interaction will be key in achieving better potency and selectivity.

A wide spectrum of cellular activity is observed for the molecules presented in this Perspective. This variability is possibly the result of a number of combined factors including variable cell penetration, binding to media, and substrate competition. However, trends for the successful inhibition of CDKs are becoming apparent. Key structural hydrogen-bonding motifs are seen repeatedly for compounds which bind in the ATP pocket. These binding motifs are seen for the more potent analogues including flavopiridol, olomoucine, and roscovitine. As our understanding of inhibitor binding, cellular activity, and ultimately the correlation to in vivo activity becomes refined, our approaches to modulating the cell cycle will improve.

Biographies

Thais M. Sielecki received her Ph.D. degree in organic chemistry from Colorado State University and completed postdoctoral training at Stanford University. She joined The DuPont Pharmaceuticals Company in 1993 and is currently a Principal Research Scientist in the Chemical and Physical Sciences Department.

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