suggesting a distinct biologic role for this family member. Medicinal chemistry efforts have identified pyrrolo[2,1-f][1,2,4]triazine-based inhibitors of Aurora kinases. Structure–activity relationships were developed by introducing substituents at C4 and C6 positions of the pyrrolotriazine to optimize the potency of kinase inhibition. The lead Aurora kinase inhibitors caused dose dependent apoptosis and cell cycle effects that are consistent with the effects of Aurora A or B depletion. These results suggest that targeting the Aurora kinases present a novel opportunity for anti-mitotic cancer therapy.

Cyclins and CDK's

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Discovery of selective CDK9 small molecule inhibitors: CDK9 inhibition in tumor cells is associated with inhibition of proliferation and induction of apoptosis

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Background: CDK9 (in complex with Cyclin T or K) phosphorylates the C-terminal domain of RNA polymerase II (RNAPII CTD) as a required step for mRNA transcript elongation. CDK9 inhibition causes the rapid depletion of short-lived mRNA transcripts and their associated protein products. Since many genes encoding proteins involved in cell growth, proliferation, and survival are characterized by short-lived mRNAs and proteins, the consequences of CDK9 inhibition are expected to include anti-proliferative and pro-apoptotic effects through loss of function at many cellular pathways. Proteins with well-established roles in tumor development and growth that are depleted following CDK9 inhibition include Myc, Cyclin D1, and Mcl-1.
Methods: CDK9-selective small molecule inhibitors were discovered by high-throughput screens and chemical optimization using biochemical and in vitro cell-based assays, as well as x-ray crystallographic information from CDK9-inhibitor co-complexes.
Results: Identified CDK9 inhibitors demonstrate excellent activity in CDK9 biochemical and cell-based assays, with IC50 values <10 nM and 100 nM respectively. Representatives of this series show excellent selectivity for CDK9 vs other CDK family members. IC50 values in cellular proliferation and apoptosis assays were found to correlate well with inhibition of cellular RNAPII CTD Ser2 phosphorylation, indicating that CDK9 inhibition is associated with strong anti-growth and pro-apoptotic effects on tumor cells in vitro. Selected CDK9 inhibitors exhibit oral bioavailability and dose-dependent plasma exposure. In vivo pharmacodynamic analysis following oral administration of these inhibitors in mouse xenograft tumor models demonstrates potent and dose-dependent inhibition of RNAPII CTD SER2 phosphorylation, as well as reductions in Myc, Cyclin D1, and MCL-1 protein levels.
Conclusions: CDK9-selective small molecule inhibitors were discovered that demonstrate potent CDK9 inhibitory activity in both in vitro and in vivo studies, and show anti-proliferative and pro-apoptotic activity in multiple tumor cell lines. Further chemical optimization and biological characterization is proceeding, with the goal of identifying a CDK9-selective inhibitor candidate for clinical development as a cancer therapeutic.

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AZD5597, a novel CDK inhibitor causes inhibition of RNA polymerase II mediated signaling and stimulates apoptosis in vitro and in vivo

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Background: AZD5597 is a CDK inhibitor displaying potency against both the cell cycle CDKs 1 and 2 and the transcriptional CDKs 7 and 9. This profile could result in both cell cycle block and induction of apoptosis and therefore may be particularly efficacious as a novel anti-tumour therapy.
Materials and Methods: The *in vitro* effects of AZD5597 on CDK inhibition, cell cycle progression and apoptosis were investigated by incubating the compound with MCF-7 cells. Cells were fixed, then labeled immunocytochemically for intracellular phosphorylation of RNA polymerase II, DNA intensity, and Bak conformational changes respectively and quantified on a Cellomics ArrayScan. We subsequently investigated the effects of AZD5597 *in vivo* using a variety of tumour xenografts (BT474c; CoLo 205, A2780 and U87MG). Mice bearing established sub-cutaneous tumours (mean tumour volume 0.4–0.6 cm^3) were given a single dose of AZD5597 (25 mg/kg, ip). Tumours were taken 6 h (4 h for U87MG) post dose, halved and either frozen or fixed in formalin for subsequent analysis. The effects of AZD5597 on cell cycle were assessed by scoring mitosis; pRb IHC and phH3 IHC. Transcriptional effects of AZD5597 were assessed by scoring RNA polymerase II IHC. Assessments of apoptosis were based on examination of both cellular phenotype and cleaved caspase 3 IHC. AZD5597 was dosed (15 mg/kg, ip; Mon, Wed and Friday for 3 weeks) to mice bearing SW620 xenograft tumours to assess the effects on tumour growth.
Results: AZD5597 treatment of MCF-7 cells *in vitro* resulted in a cell cycle block (at G2/M), reduced the phosphorylation of RNA polymerase II, and increased initiation of cell apoptosis (as indicated by conformational changes in Bak). Consistent with these data, *in vivo* a single dose of AZD5597 resulted in cell cycle block (reduced mitosis, pRb and phH3 staining), a reduction in phospho RNA polymerase II staining, and induction of apoptosis. Similar results were seen in all four tumour types examined. In chronic dosing, AZD5597 resulted in a 55% inhibition of SW620 tumour growth *in vivo*.
Conclusions: AZD5597 results in cell cycle block and causes the inhibition of survival pathways that leads to induction of apoptosis *in vitro* and *in vivo*. These effects were observed in histologically distinct tumour models (breast, colorectal, ovarian and glioma).

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The complexity of cell cycle dynamic of anticancer drugs unraveled by the use of mathematical models suitable for a quantitative assessment of G1, S and G2M checkpoint activities

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Background: Current methods to assess cell cycle effects of drugs in cell populations are still limited to score the macroscopic variations on specific measurable quantities like percentages of cells in G1, S and G2M. These quantities result from the superimposition of the effects of cell cycle block and cell loss, their dynamics in the times before the measure, and the proliferation of surviving cells. They depend on but do not provide a direct measure of the activities of the molecular networks regulating G1, S and G2M checkpoints. A mathematical model was built based on the kinetics of cell cycle transit times of an ovarian cancer cell line and on parameters that directly describe the activity of each checkpoint, and giving as output cell cycle percentages and other macroscopic measurable quantities.
Material and Methods: Experiments were performed with a standardized protocol using the IGROV1 cell line with 5 clinically used anticancer drugs (cisplatin, taxol, doxorubicin, melphalan, topotecan) characterized by different molecular mechanisms of action. We measured cell cycle percentages and absolute cell number at four times after treatments (6, 24, 48, 72 h) with a wide range of drug concentrations. DNA-BrdUrd histograms and apoptosis were also collected at specific times.
Results: After obtaining a robust set of data of the concentration-dependent effects of all drugs by using different techniques we have analyzed the complexity of events (delayed cell cycle progression, short or long term blockades in different cell cycle phases, cell death) by the application of a mathematical model based on parameters that directly describe the activity of each checkpoint. By deciphering cellular data in terms of the global activities of G1, S and G2M checkpoints, each governed by specific molecular network, it became evident that there are differences in the complex cell response to each drug that have never been observed before by standard methodologies.
Conclusions: We found that the overall response of each drug is the result of the combination of a few types of checkpoint response, which operate with different strengths, and with specific drug concentration thresholds. The application of the simulation mathematical model to anticancer drug effects allows a quantitative knowledge of the dynamics of critical cellular events, that should be taken into account when using an anticancer drug alone or in combination in a less empirical and more rational way.

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Biological characterization of the dual CDK2/TRKA inhibitor PHA-848125

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Among the small molecule cyclin-dependent kinase modulators that are nowadays in the clinical arena the pyrazolo quinazoline PHA-848125 emerges as a potent inhibitor of the CDK2/cyclinA complex (IC50=45 nM) with the novelty of being particularly active also against TRKA (IC50=53 nM) proposing in this way to be a "selective dual inhibitor". It also maintains some activity against other cell cycle CDKs, even if