Mitotic Kinases Targeted Library

Medicinal and Computational Chemistry Dept., ChemDiv, Inc., 6605 Nancy Ridge Drive, San Diego, CA 92121 USA, Service: +1 877 ChemDiv, Tel: +1 858-794-4860, Fax: +1 858-794-4931, Email: ChemDiv@chemdiv.com

With several successful anticancer drugs on the market and numerous compounds in clinical developments, antimitotic agents represent an important category of anticancer agents. However, clinical utility of the tubulin-binding agents is somewhat limited due to multiple drug resistance (MDR), poor pharmacokinetics and therapeutic index. Another significant limitation of current modulators of tubulin dynamics is their marginal clinical efficacy. While demonstrating impessive *in vitro* activity, majority of tubulin-binding agents have not displayed antitumor activity in clinic. This fact is usually attributed to poor balance between efficacy and toxicity, so-called therapeutic window. It is related to multiple features of a drug including pharmacokinetics, off-target toxicity and other poorly recognized factors. In addition, drug efflux pumps play a role in tumors developing resistance to the tubulin-binding drugs. There is ongoing need for the modulators of other intracellular targets that result in the same anti-mitotic effect without adverse effects of "traditional" tubulin binders. For example, kinesins, microtubule motor proteins, play critical role in the mitotic spindle function and represent potential targets for the discovery of novel cancer therapies[1]. Proteins that control cellular progression through mitosis include kinases and cysteine proteases, namely Polo, Bub, Mad, Aurora, Cdk1, separase and others[2].

Historically, researches focused on two classes of antimitotic agents (Fig. 1). The first class includes compounds that bind reversibly to tubulin and prevent microtubule assembly and disassembly (modulators of MT dynamics). The second class features molecules that regulate mitotic events vicariously by interacting with specific intracellular targets such as mitotic kinesins, kinases, separase, *etc.*

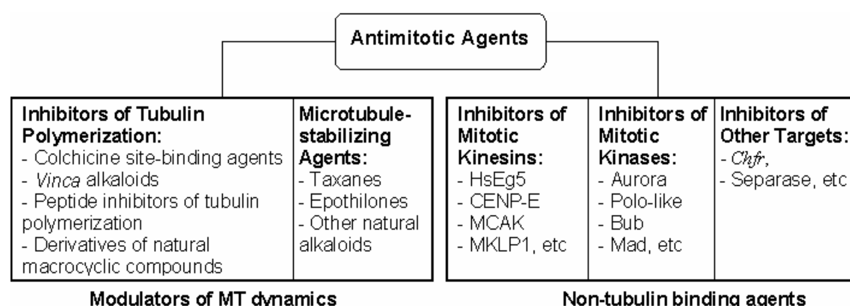


Fig. 1. Classification of antimitotic agents and targets

Mitotic kinases

The mitotic failure is one of the essential sources of the genetic instability that hallmarks cancer pathology. Clinical evidence suggests that numerous proteins that regulate mitosis are aberrantly expressed

in human tumors. Regulation of mitotic progression depends on two post-translational mechanisms: protein phosphorylation and proteolysis. The former process is mediated by mitotic kinases. Mitotic kinases are major regulators that control mitosis progression (Table 1).

Table 1. Mitotic kinases

Kinases/ Family	Basic Function	Mitotic Phase
Cdk1/2 (Cyclin-dependent kinase 1 and 2)	The serine-threonine kinases critical for controlling all phases of cell cycle progression	Multiple mitotic phases
Plk1 (Polo-like kinases)	Regulate chromosome segregation and cytokinesis	Multiple mitotic phases
Bub1, BubR1 and TTK/Esk	Involved in chromosome segregation and kinetochore attachment	Methapase- Anaphase
Nek2/6/11 (NIMA kinases)	Control the centrosome structure during the mitotic cell cycle	Interphase-prometaphase, metaphase-anaphase
Aurora A-C (Aurora kinases)	Involved in chromosome separation, centrosome separation and cytokinesis	Multiple mitotic phases
MEN/SIN kinases	Several metazoan kinases (Ndr/LATS family members) are structurally related to a yeast SIN/MEN kinase (budding yeast Dbf2p/Mob1p and fission yeast Sid2p/Mob1p), but no functional homologies have yet been shown	Cytokinesis
MAP kinase (Mitogen-activated kinases)	Cell cycle regulated. Many types of signal-transduction, activates p90RSK, which in tern activates Bub1 during Xenopus oocyte maturation.	Interphase and methaphase
Mps1p (MonoPolar Spindle 1 kinase, Dual-specificity kinase)	Implicated in the duplication of spindle pole body Recruits checkpoint proteins at kinetochores; Reported substrates include Mad1, Spc110	Interphase-promathaphase
Wee1 and Myt1	Implicated in the DNA structure checkpoints	Interphase
Sid1p/2p (Sid kinases)	Sid kinases are components of the spindle pole body at all stages of the cell cycle and directly implicated in cytokinesis	Cytokinesis

The mitotic cycle includes four major stages (Fig. 2). To ensure that the daughter cells receive identical copies of the genome, progression through the cell cycle is highly regulated and controlled by different types of mitotic kinases. For example: Cdk1, Plk1 and Nek2 regulate mitotic checkpoints at the interphase and prophase, Cdk1 is one of the key players through the prophase-telophase, Aurora kinases are essential to the progression of a cell through all mitotic stages.

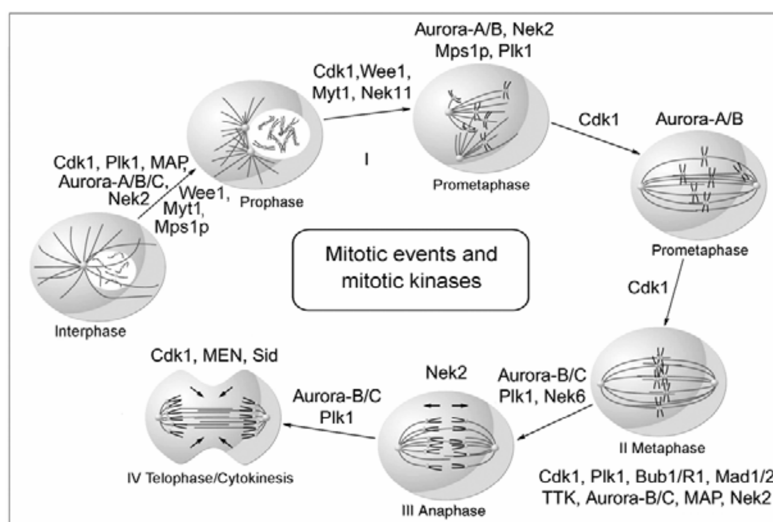


Fig. 2. Mitotic kinase activity during mitotic cell cycle

As mentioned above, mitotic kinases are key players in mitotic checkpoints[3]. Thus, CDK1 mitotic kinase, a nonredundant cyclin-dependent kinase (CDK), plays an essential role in the cell division, particularly during early mitotic events (e*ntry into mitosis*). For example, loss of mitosis in tumor cells is associated with the marked reduction in CDK1 transcription and/or loss of its active form (CDK1-P-Thr(161))[4]. It occurs during G2/M transition when the activity of the dual-specificity phosphatase Cdc25C towards Cdk1 exceeds activity of two opposing kinases Wee1 and Myt1. In turn, these proteins are regulated by DNA structure checkpoints, which delay the onset of mitosis in the presence of unreplicated or damaged DNA. Cdc25C is inhibited by two other kinases, Chk1 and Chk2, which are also implicated in DNA structure checkpoint signaling pathway. Wee1 and Myt1 are upregulated by the same pathways[5]. Notably, Plk1 kinase also activates Cdc25C[6].

Spindle assembly checkpoint ensures accurate segregation of chromosomes during mitosis. It blocks the anaphase stage until all chromosomes are properly attached to a bipolar mitotic spindle. Once unstable chromosomes detected, spindle checkpoint inhibits the ubiquitin ligase activity of the anaphase-promoting complex or cyclosome (APC/C)[7]. This step is reportedly mediated by proteins encoded by BUB and MAD genes Specifically, mitotic kinases, including Bub1/3, BubR1, Bub3, Mad1, and Mad2 are recruited to unattached kinetochores (Fig. 3)[8]. DNA replication and centrosome duplication are controlled by E2F transcription factors, Mps1p kinases, cyclin A/E and Cdc20 protein[9]. Mitotic checkpoint protein complexes comprised of BubR1, Bub3 and Mad2 bind to and inhibit APC/Cdc20 until all chromosomes are properly attached to the mitotic spindle and aligned in the metaphase plate (Fig. 3A) and (3B/C))[10].

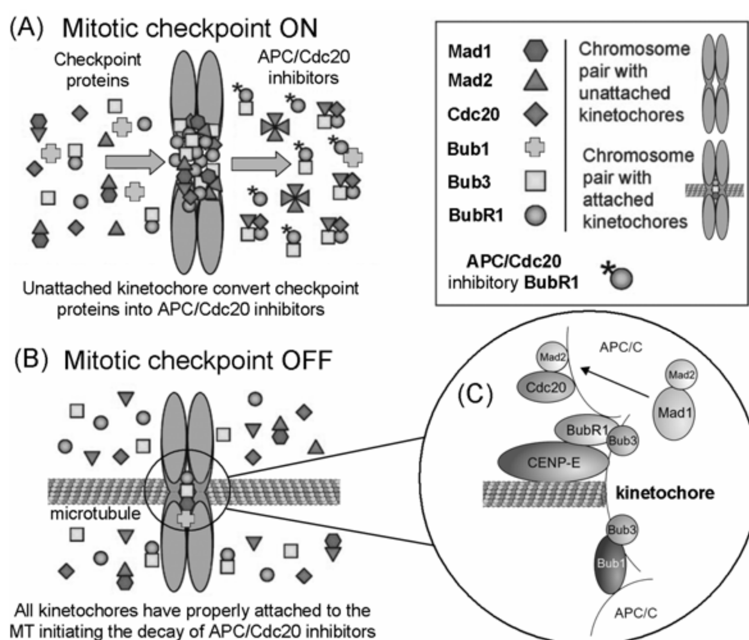


Fig. 3. Mitotic checkpoint signaling and kinetochore attachment

The ability of a cell to track its temporal and spatial fidelity during progression through the cell cycle is essential for survival. A spindle-positioning checkpoint has been initially described in the yeast *S. cerevisiae*[11]. The first identified component of this step was Bub2/Bfa1 GTPase-activating protein (GAP). It is responsible for keeping small GTPase (Tem1p) inactive until the spindle is properly oriented. The net result is inhibition of the mitotic exit network (MEN) activation[12]. The signaling cascade that is responsible for initiating MEN includes mitotic kinases that activate Cdc14p phosphatase. Cdc14p activates APC/C/Cdh1 complex, dephosphorylates Cdk1-inhibitor Sic1p (causing its stabilization) and transcription factor Swi5p (enhancing the production of Sic1p)[13]. These events lead to destruction of mitotic cyclin–CDK complexes only when the spindle-positioning checkpoint is satisfied.

Small molecule inhibitors of mitotic kinases

Considering a pivotal role of protein phosphorylation in mitotic checkpoints, spindle function and chromosome segregation, it is not surprising that several mitotic kinases have been implicated in tumorigenesis. For example, CDK1-8, Aurora (Aur) (A, B, C), CDK (Cdk1, 2), Polo-like (Plk1-4), Nek (NIMA1-11), Bub (Bub1, BubR1) and other kinases are implicated in mediation of centrosome duplication, chromosome segregation, and cytokinesis in diverse human tumors[14]. These enzymes also regulate centrosome cycle, spindle checkpoint, microtubule-kinetochore attachment, spindle assembly, and chromosome condensation. Several potent and selective inhibitors of mitotic kinases entered clinical trials (Table 2).

Table 2. Small-molecule inhibitors of Polo-like and Aurora kinases in preclinical and clinical development*

Drug name	Development phase	Type of action	Therapeutic area
ON-01910 **1**	Phase I	Plk-1 inhibitor	Cancer therapy
ON-1910Na **2**	Phase I	Plk-1 inhibitor with IC_{50} = 9 nM	Leukemia and solid tumors therapy
BI-2536 **3**	Phase I	Plk-1 inhibitor	Cancer therapy
Wortmannin **4**	Preclinical	Plks inhibitor	Cancer therapy
Scytonemin **5**	Preclinical	Plk-1 inhibitor with IC_{50}=2.3-3.4 µM	Cancer therapy
β-Hydroxyisovalerylshikonin (β-HIVS) **6**	Preclinical	Plk-1 inhibitor	Cancer therapy
Staurosporine **7**	Preclinical	Plk-1 inhibitor with IC_{50} = 0.8±0.2 µM	Cancer therapy
VX-680 **8**	Phase II	Aurora A, B and C inhibitor *in vitro* with IC_{50} values of 0.6, 18 and 4.6 nM, respectively	Cancer therapy
MLN-8054 **9**	Phase I	Aurora A kinase inhibitor	Solid tumors therapy
PHA-680632 **10**	Preclinical	Aurora A and Aurora B inhibitor (IC_{50} = 27 and 135 nM, respectively)	Cancer therapy
PHA-739358 **11**	Phase II	Aurora-A,B and C inhibitor	Cancer therapy
AZD-1152 **12**	Phase I	Aurora-B and C inhibitor	Hematological cancer and solid tumors therapy
VX-528**	Preclinical	Aurora-B (ARK2) kinase inhibitor	Cancer therapy
ZM-447439 **13**	Preclinical	Aurora A and B kinases inhibitor with IC_{50} values of approximately 0.1 µM	Cancer therapy
MP-235 **14**	Preclinical	Aurora kinases A,B and C with an IC_{50} of 90 nM	Cancer therapy (antiproliferative effects in cancer cell lines)
MP-529**	Preclinical	Aurora-A,B and C inhibitor	Cancer therapy
Compound **15**	Preclinical	Aurora-A and B Kinase Inhibitor with IC_{50}=10.2 nM and 9 nM	Cancer therapy
JNJ-7706621 **16**	Preclinical	Aurora-A,B and C inhibitor	Melanoma therapy
MKC-1260**	Preclinical	Aurora-A,B and C inhibitor	Cancer therapy
MKC-1693 **17**	Preclinical	Aurora-A,B and C inhibitor	Cancer therapy
Compound **18**	Preclinical	Aurora-A and B inhibitor	Cancer therapy
Compound **19**	Preclinical	Aurora-A (ARK1) Kinase Inhibitor	Cancer therapy
Hesperadin **20**	Preclinical	Aurora-B; IC_{50}= 0.25 mM	Cancer therapy
SNS-314**	Preclinical	Aurora-A and B inhibitor	Cancer therapy
CYC-116**	Preclinical	Aurora-A,B and C inhibitor	Cancer therapy

*data at the end of 2006; ** structure is not disclosed yet.

Inhibitors of Polo-like kinases

Polo-like kinases (Plks) belong to a family of conserved serine/threonine kinases with a polo-box domain, which have similar but non-overlapping functions in the cell cycle progression. Thus, they control mitotic entry of proliferating cells and regulate many aspects of mitosis necessary for the successful cytokinesis[15]. For example, they are essential for the activity of the MT organization center[16]. They are important players in mitotic entry, spindle formation and cytokinesis[17]. Multiple Plks are present in mammalian cells (Plk-1, Plk2/Snk, Plk3/Fnk/Prk, and Plk4/Sak) and Xenopus (Plx1-3). Of the four known human Plks, Plk-1 is over-expressed in many tumor types. Of all mitotic kinases, Plk-1 is probably the most validated[18]. Studies showed that modulation of Plk-1 activity in both transformed and normal cells have anti-proliferative effect. Plks are deeply involved in the assembly and dynamics of the mitotic spindle apparatus and in the activation and inactivation of CDK/cyclin complexes. In mammalian cells, Plk1 protein levels increase as cells approach M phase, with the peak of phosphorylation activity reached during mitosis. Known substrates include Cdc25C phosphatase, cyclin B, a cohesin subunit of the mitotic spindle, subunits of the anaphase promoting complex, and mammalian kinesin-like protein 1 MKLP-1 and other kinesin related motor proteins. These substrates demonstrate the multiple roles of Plk1 in promoting mitosis. Plk1 has a role in the regulation of tyrosine dephosphorylation of CDKs through phosphorylation and activation of Cdc25C.

Cancer cells treated with the Plk inhibitors undergo apoptosis or become committed to mitotic catastrophe. At the same time, non-transformed proliferating cells reversibly are arrested at the G2/M boundary. In particular, small-molecule Plk inhibitors displayed selective anti-proliferative effects on cancer cells, producing phenotypes consistent with known Plk functions[19] (Fig. 4).

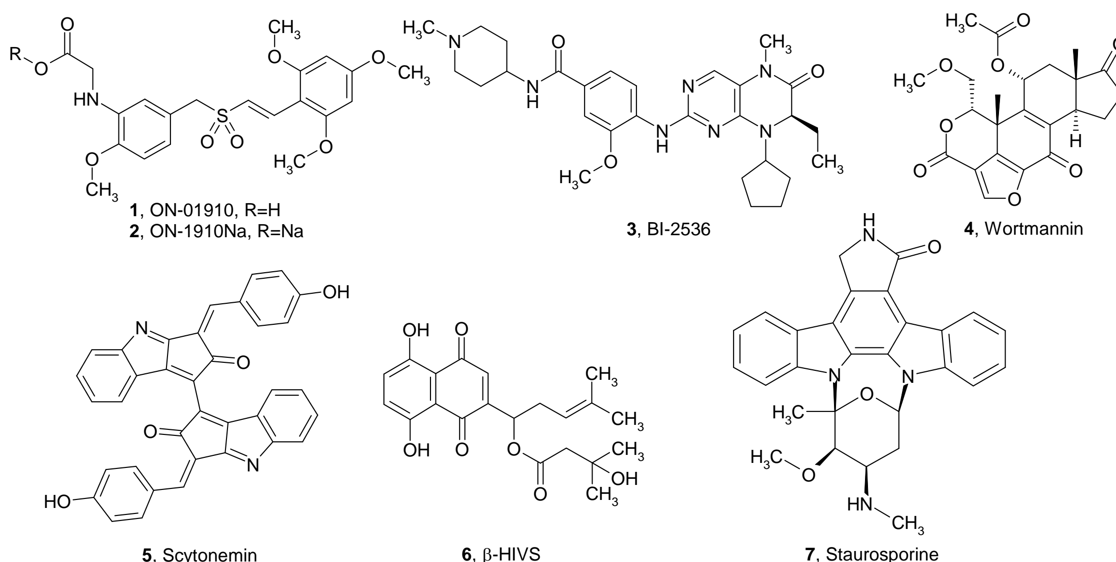


Fig. 4. Structures of small-molecule inhibitors of Polo-like Kinase-1 (Plk-1) in preclinical and clinical development

Inhibitors of Aurora kinases

Serine/threonine protein kinases of Aurora family are involved in chromosome segregation and cell division in all eukaryotes[20]. They were first identified in the cell cycle studies as *Xenopus* Eg2[21]. These enzymes are essential in the “spindle checkpoint” system used by cells to monitor fidelity of mitosis[22]. Deregulation of Aurora kinases impairs spindle assembly, checkpoint function and cell division. It causes missegregation of individual chromosomes or polyploidization accompanied by centrosome amplification. All Aurora kinases share similar structure, with their catalytic domains flanked by very short *C*-terminal tails and *N*-terminal domains of variable lengths[23]. Considering that Aurora kinases regulate mitotic cycle progression at multiple mitotic stages (Fig. 2), they are believed to affect numerous proteins. For example, Aurora A phosphorylates Histone H3 (S10), KSP motor protein, CPEB, PP1, D-TACC and TPX2. Aurora B regulates activity of Histone H3(S10/S28), CENP-A, INCENP, REC-8, MgcRacGAP, Vimentin, GFAP and Desmin[24]. Aurora A is localized to centrosomes from S/early G2 phases. It is required to establish a bipolar mitotic spindle[25]. Aurora B is associated with chromosomes in early mitosis. In late mitosis, Aurora B migrates from centromeres to MTs at the spindle equator. As the spindle elongates and the cell undergoes cytokinesis, Aurora B accumulates in the spindle midzone before finally concentrating at the midbody[26]. Notably, all members of the Aurora-kinase family are expressed exclusively during mitosis.

At least two isoforms, namely Auroras A and B are commonly over-expressed in human tumors, for example in primary colon tumor samples[27]. Further studies suggested that they play pivotal role in development of breast, colorectal, bladder and ovarian cancers[28] (Table 3).

Table 3. Overexpression of Aurora kinases in several cancer lines and tumor tissues. The data indicate the percentage of cell lines or tumors which overexpress kinases.

Aurora kinase	Cell lines/human cancers	Overxpression/amplification
Aurora-A	**Breast cancer cell lines**	30-40%
	Ductal invasive carcinomas	94%
	Primary invasive breast cancers	29%
	Node-negative breast carcinomas	15%
	Primary breast carcinomas	15%
	Primary colorectal cancers	>50%
	Ovarian cancer cell lines	38%
	Sporadic ovarian cancers	44-54%
	Hereditary ovarian cancers	100%
	Hepatic cancer cell lines	100%
	Hepatocellular carcinomas	61%
	Pancreatic carcinoma cell lines	100%
	Pancreatic cancers	58%
Aurora-B	**Colorectal cancer cell lines**	ND*
	Primary human colorectal cancers	ND*
Aurora-C	**Breast cancer cell lines**	ND*

	Hepatocellular carcinoma cell lines	ND*
	Primary human colorectal cancers	52%

* ND – No data

Aberrant expression of Aurora-A kinase leads to the genetic instability *via* either abnormal centrosome duplication or defects in the spindle checkpoint[29]. Similarly, misregulated levels of Aurora-B yield abnormalities in chromosome attachment or alignment to the mitotic spindle during cellular mitosis[30]. Aurora-B may form complexes with Survivin, anti-apoptotic protein that is commonly overexpressed in tumors[31]. It has been suggested that overexpression of Aurora B may help protect tumour cells from apoptosis.

Since the discovery that Aurora kinases are upregulated in many tumours, several small molecule inhibitors with sufficient selectivity for Aurora kinases were developed[32]. Figure 5 summarizes structures of selected compounds.

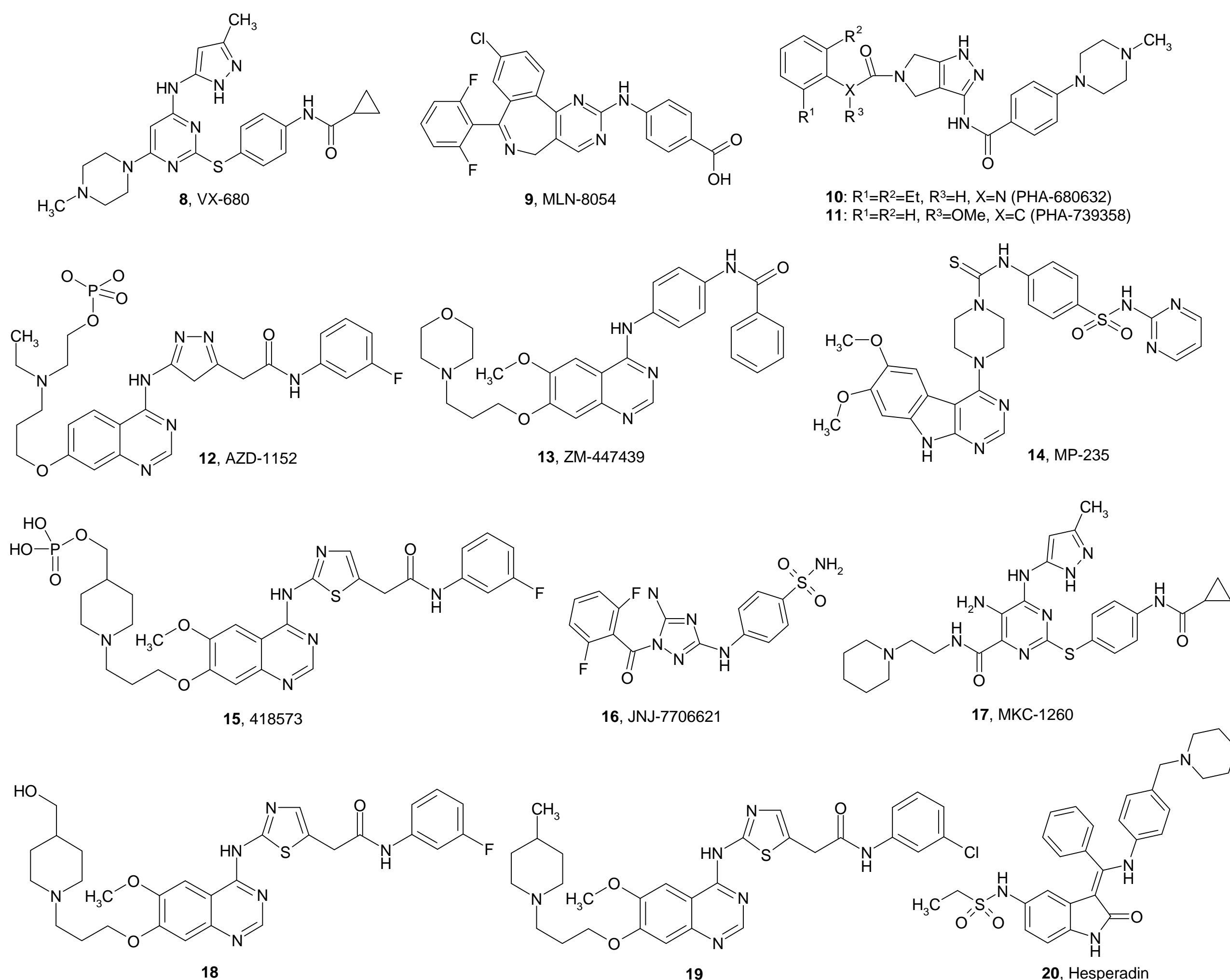


Fig. 5. Structures of small-molecule inhibitors of Aurora kinases in preclinical and clinical development (for more information, see table 2)

Inhibitors of Cyclin-dependent kinases

Cyclin-dependent kinases (CDK) belong to a large family of serine/threonine kinases. It is deeply implicated in cell cycle regulation especially in early stage of mitosis. They are also involved in the

regulation of transcription and mRNA processing. One exception is CDK9; it plays no role in cell cycle regulation. As they are serine/threonine kinases, they phosphorylate proteins on serine and threonine amino acid residues. A cyclin-dependent kinase is activated by association with a cyclin, forming a cyclin-dependent kinase complex. The subfamily of CDKs includes several classes named correspondingly CDK1-9. A cyclin-CDK complex can be regulated by several kinases and phosphatases, including Wee, and CDK-activating kinase (CAK), and Cdc25. CAK adds an activating phosphate to the complex, while Wee adds an inhibitory phosphate; the presence of both activating and inhibitory phosphates renders the complex inactive. Cdc25 is a phosphatase that removes the inhibitor phosphate added by Wee, rendering the complex active. CDK feeds back on Wee and Cdc25 to inhibit and enhance their respective activities.

CDKs are considered a potential target for anti-cancer medication. If it is possible to selectively interrupt the cell cycle regulation in cancer cells by interfering with CDK action, the cell will die. Currently, some CDK inhibitors such as Seliciclib are undergoing clinical trials. Although it was originally developed as a potential anti-cancer drug, in recent laboratory tests Seliciclib **21** (Fig. 6) has also proven to induce apoptosis in neutrophil granulocytes which mediate inflammation[33]. This means that novel drugs for treatment of chronic inflammation diseases such as arthritis or cystic fibrosis could be developed. Representative structures of small-molecule inhibitors of CDKs entered in preclinical and clinical trials or already launched are shown in Figure 6.

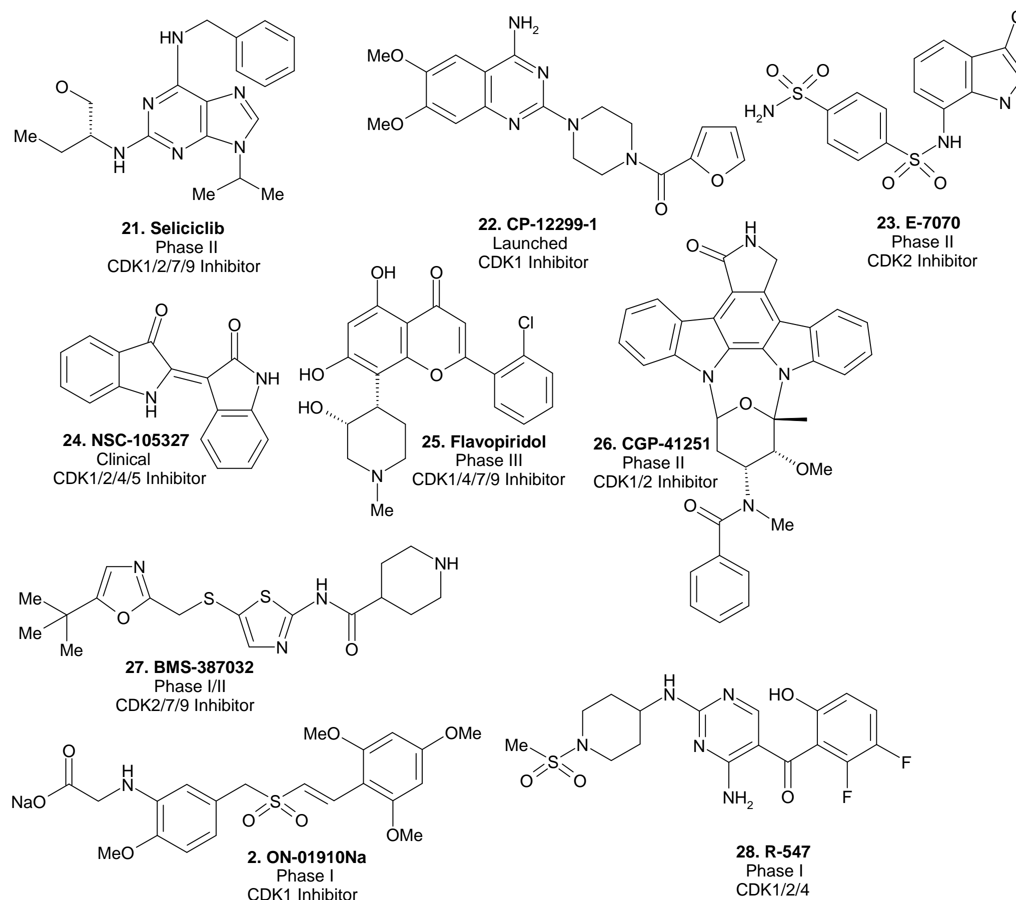


Fig. 6. Structures of small-molecule inhibitors of CDKs in preclinical and clinical development

Concept and Applications

Mitotic Kinase-targeted library design at CDL involves:

• *A combined profiling methodology that provides a consensus score and decision based on various advanced computational tools*:

1. Bioisosteric morphing and funneling procedures in designing novel potential Aurora, Plk and CDK kinase inhibitors with high IP value. We apply CDL's proprietary Chemosoft™ software and commercially available solutions from Accelrys, MOE, Daylight and other platforms.

2. Neural Network tools for target-library profiling, in particular Self-organizing Kohonen Maps, performed in SmartMining Software. We have also use the Sammon mapping as a more accurate computational tool to create our kinase-focused library.

3. A molecular docking approach to focused library design.

4. Computational-based `*in silico*` ADME/Tox assessment for novel compounds includes prediction of human CYP P450-mediated metabolism and toxicity as well as many pharmacokinetic parameters, such as Brain-Blood Barrier (BBB) permeability, Human Intestinal Absorption (HIA), Plasma Protein binding (PPB), Plasma half-life time ($T_{1/2}$), Volume of distribution in human plasma (V_d), etc.

The fundamentals for these applications are described in a series of our recent articles on the design of exploratory small molecule chemistry for bioscreening [for related data visit ChemDiv. Inc. online source: www.chemdiv.com].

• *Synthesis, biological evaluation and SAR study for the selected structures*:

1. High-throughput synthesis with multiple parallel library validation. Synthetic protocols, building blocks and chemical strategies are available.

2. Library activity validation via bioscreening (the synthesized compounds should be tested jointly against both Aurora and Plk kinases due to their similarity in binding site composition). SAR is implemented in the next library generation.

We practice a multi-step approach for building Mitotic Kinase-focused library:

Virtual screening

Choosing structures that are most likely to have a predefined target-specific activity of interest from the vast assortment of structurally dissimilar molecules is a particular challenge in compound selection. This challenge has been tackled with powerful computational methodologies, such as docking available structures into the receptor site and pharmacophore searching for particular geometric relations among elements thought critical for biological activity. Both methodologies focus on conformational flexibility of both target and ligand, which is a complex and computationally intense problem. The latest developments in this field pave the way to wide industrial application of these technologies in drug design and discovery, though the limits of computational power and time still restrict the practical library size selected by these methods.

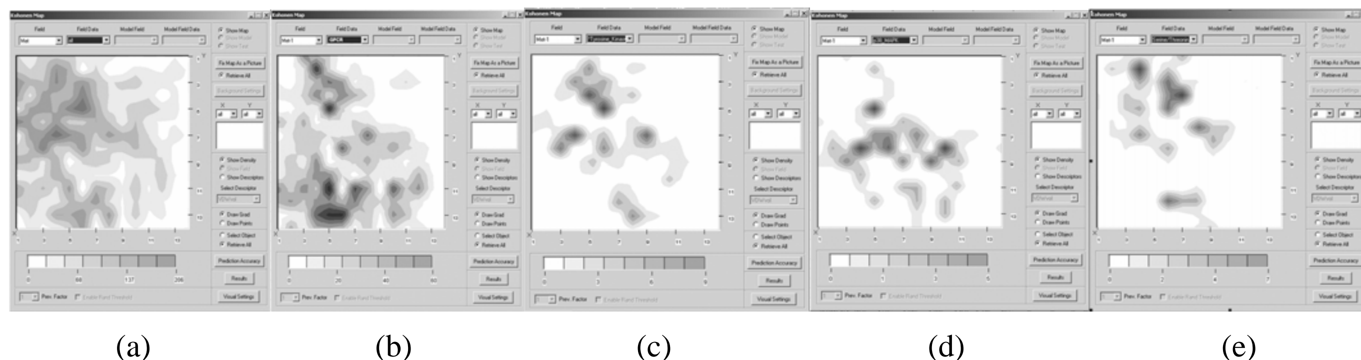
Another popular approach to VS is based on ligand structure and consists of selecting compounds structurally related to hits identified from the initial screening of the existing commercial libraries and active molecules reported in research articles and patents. Although broadly used in the development of SAR profiling libraries, these methods usually perform poorly when it comes to the discovery of structurally novel lead chemotypes.

An alternative design for target-specific libraries is based on statistical data mining methods, which are able to extract information from knowledge databases of active compounds.

Structure-based design

At the initial stage of our approach, we have collected a unique database which contains more than 22 thousand of known drugs and compounds which have been entered into various preclinical or clinical trials. Each compound in this database is characterized by a defined profile of target-specific activity, focused against 1 of more than 100 different protein targets. The database was filtered based on MW (not more than 800). Molecular features encoding the relevant physicochemical and topological properties of compounds were calculated from 2D molecular representations and selected by PCA. These molecular descriptors encode the most significant molecular features, such as molecular size, lipophilicity, H-binding capacity, flexibility, and molecular topology. Taken in combination, they define both pharmacokinetic and pharmacodynamic behavior of compounds and are effective for property-based classification of target-specific groups of active agents.

A Kohonen SOM (14×14) of 22,110 pharmaceutical leads and drugs generated as a result of the unsupervised learning procedure is depicted in Figure 6. It shows that the studied compounds occupy a wide area on the map, which can be characterized as the area of drug-likeness. Distribution of various target-specific groups of ligands within the Kohonen map demonstrates that most of these groups have distinct locations in specific regions of the map (Figure 6a through Figure 6e).



(a) (b) (c) (d) (e)

Fig. 6. (**a**) Property space of 22,110 pharmaceutical leads and drugs visualized using the Kohonen map. Distribution of five target-specific groups of pharmaceutical agents: (**b**) GPCR agonists/antagonists; (**c**) tyrosine kinase inhibitors; (**d**) p38 MAPK inhibitors; (**e**) serine/threonine kinese inhibitors including Plk, Aurora and CDK kinases

A possible explanation of these differences is in the fact that, as a rule, receptors of one type share a structurally conserved ligand-binding site. The structure of this site determines molecular properties that

a receptor-selective ligand should possess to properly bind the site. These properties include specific spatial, lipophilic, and H-binding parameters, as well as other features influencing the pharmacodynamic characteristics. Therefore, every group of active ligand molecules can be characterized by a unique combination of physicochemical parameters differentiating it from other target-specific groups of ligands. Another explanation of the observed phenomenon can be related to different pharmacokinetic requirements to drugs acting on different biotargets.

During the initial stage of our focused-library design we have effectively used this model to select structures which are the most promising towards Polo-like, Aurora and CDK kinases.

Target-based Design

Based on the data derived from PDB Protein Data Bank[34] we have further construct and effectively applied molecular docking models to select the structures of paramount interest within the scope of our focused-library design. Molecular docking of the selected structures (see the section above) was performed using Surflex Docking computational program Version 1.24 (BioPharmics LLC). After the generation of protomol all structures were docked into the active binding site of Plk, Aurora and CDK kinases. Ten conformations for each structure were generated and docked into the binding site. There are two scores for each conformation docked: an affinity (-log(K_d)) (named as "polar") and a "penetration score" (arbitrary units named as "penetration"). The penetration score is the degree of ligand penetration into the active site of protein studied. Thus, penetration scores that are close to 0.0 are favorable however visual analysis of each conformer is more preferable. The examples of developed docking models are shown in Fig. 7.

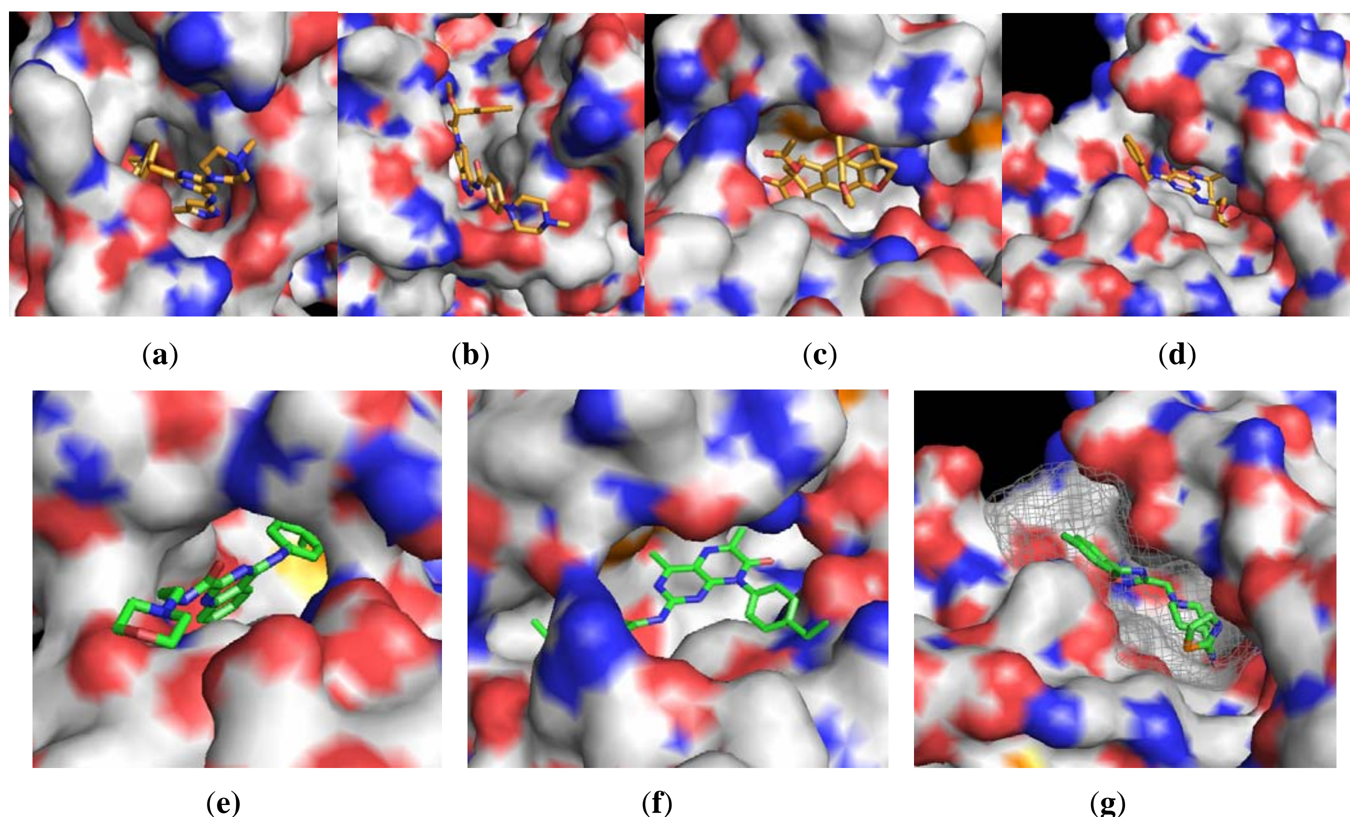


Fig. 7. The developed docking models based on: (**a**) crystal structure of Aurora-A kinase in complex with VX-680 (**8**)[35]; (**b**) crystal structure of Aurora-2 kinase in complex with PHA-739358 (**11**)[36]; (**c**) crystal structure of Polo-like kinase-1 catalytic

domain in complex with Wortmannin (**4**)[37]; (**d**) Human cyclin- dependent kinase 2 (*h*CDK2) in complex with Seliciclib (**21**)[38]. Representative compounds from our kinase-targeted library docked into the binding site of (**e**) Polo-like kinase (compound **Plk-T-2**), (**f**) Aurora kinase (compound **Aur-T-1**) and (**g**) CDK (compound **CDK-T-3**), their structures see below

For example, as shown in Fig. 7, key structural elements and atoms of Wortmannin as well as principal interactions within active site of an activated Plk-1 are significantly similar to compound **Plk-T-2** from our focused-library.

It should be particularly noted that the catalytic domain of Plk-1 shares significant primary amino-acid homology and structural similarity with Aurora-A kinase. Therefore, biological screening against Aurora-A may provide a valuable source for compounds also active against Plk classes, especially Plk1. This suggestion has been strongly supported in a recent paper by Elling and colleagues[39].

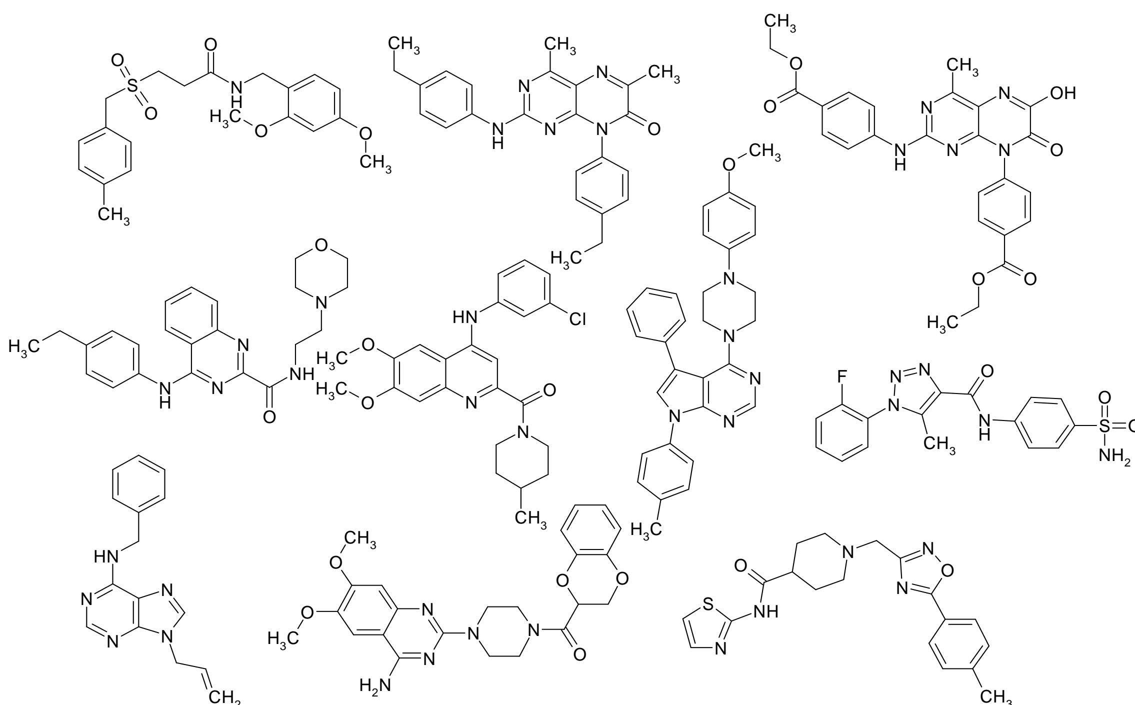
Based on the obtained results we can reasonably conclude that all of the tested compounds from our focused-library are potential inhibitors of Polo-like, Aurora and CDK kinases.

Synthesis and biological evaluation

(4) Novel Mitotic kinase-targeted library is synthesized according to the above criteria.

(5) The subsets of Mitotic kinase library which includes both Aurora, Polo-like and CDK kinase-targeted compounds are validated by bioscreening in collaboration with academic institutions.

Our strategy has proven to be efficient for generation of protein class-targeted libraries. The higher hit rate over diverse libraries, along with identification of novel active chemotypes with optimized diversity and ADME properties, has been shown in multiple studies. Using the computational approaches listed above we have compiled Mitotic kinase-focused library consisted of about 10K small molecule compounds Representative set of Mitotic kinase-biased compounds is shown below.



Examples of compounds from the Mitotic kinase-targeted library

Conclusion

Among a variety of anticancer drugs launched on the market and numerous compounds in preclinical and clinical development, modulators of microtubule dynamics remain to be the most important class of anti-mitotic agents. However, there are significant limitations associated with their utility. These include: drug resistance caused by mutations in β-tubulin and multiple drug resistance (MDR), toxicity, poor pharmacokinetics and poor therapeutic index[40]. These issues led to identification of an alternative targets and signaling mechanisms that yield anti-mitotic effect with greater specificity and more predictable pharmacology. Mitotic kinases represent large and relatively unexplored class of antimitotic targets. These proteins are implicated at multiple stages in the mitosis and cytokinesis. Among them Polo-like, Aurora and CDK kinases are the key regulators of a majority of mitotic checkpoints. Mps1p kinase has been implicated in the duplication of a spindle pole body and spindle assembly checkpoint[41]. Nek2 kinase (NIMA family) promotes centrosome separation and may control histone H3 phosphorylation[42]. MAP kinases regulate spindle dynamics and chromosome movement[43]. Mad (1p, 2p and 3p) proteins are involved in a spindle assembly checkpoint mechanism[44]. Mitotic kinases are critical for the mitotic exit[45] and cytokinesis[46]. CDK1 kinase still remains to be one of the main enzymes in mitosis. Discovery of novel agents acting at specific phases of the mitotic cycle will allow clearer definition of the relationships between discrete mechanical phases of spindle function, regulation of cell-cycle progression and programmed cell death.

Thus, here we provide efficient tools for *in silico* design of novel small molecule inhibitors of the title mitotic kinases. Based on the accumulated knowledgebase as well as unique structure- and target-based models we have been designed about 10K small molecule compounds targeted specifically against Polo-like, Aurora and CDK kinases. As a result, the library is renewed each year, proprietary compounds comprising 50-75% of the entire set. Clients are invited to participate in the template selection process prior to launch of our synthetic effort.

References

[1] (a) Ivachtchenko, Alexandre V.; Kiselyov, Alex S.; Tkachenko, Sergey E.; Ivanenkov, Yan A.; Balakin, Konstantin V. Novel Mitotic Targets and Their Small-Molecule Inhibitors. Current Cancer Drug Targets **2007**, *7*, 766-784; (b) Duhl, D. M.; Renhowe, P. A. Inhibitors of Kinesin Motor Proteins-research and Clinical Progress. *Curr. Opin. Drug Discov. Devel*. **2005**, *8*, 431-436.

[2] (a) Warner, S. L.; Gray, P. J.; Von Hoff, D. D. Tubulin-associated Drug Targets: Aurora Kinases, Polo-like Kinases, and Others. *Semin. Oncol*. **2006**, *33*, 436-448; (b) Hardwick, K. G. The Spindle Checkpoint. *Trends Genet*. **1998**, *14*, 1–4.

[3] (a) Hardwick, K. G. The Spindle Checkpoint. *Trends Genet*. **1998**, *14*, 1–4; (b) Elledge, S. J. Cell Cycle Checkpoints: Preventing an Identity Crisis. *Science* **1996**, *274*, 1664–1672; (c) Burke, D. J. Complexity in the Spindle Checkpoint. *Curr. Opin. Genet. Dev*. **2000**, *10*, 26–31.

[4] Kokkinakis, D. M.; Brickner, A. G.; Kirkwood, J. M.; Liu, X.; Goldwasser, J. E.; Kastrama, A.; Sander, C.; Bocangel, D.; Chada, S. Mitotic Arrest, Apoptosis, and Sensitization to Chemotherapy of Melanomas by Methionine Deprivation Stress. *Mol. Cancer Res*. **2006**, *4*, 575-589

[5] (a) Elledge, S. J. Cell Cycle Checkpoints: Preventing an Identity Crisis. *Science* **1996**, *274*, 1664–1672; (b) Russell, P. Checkpoints on the Road to Mitosis. *Trends Biochem. Sci*. **1998**, *23*, 399–402

[6] (a) Kumagai, A.; Dunphy, W. G. Purification and Molecular Cloning of Plx1, a Cdc25-Regulatory Kinase From Xenopus Egg Extracts. *Science* **1996**, *273*, 1377–1380; (b) Smits, V. A.; Klompmaker, R.; Arnaud, L.; Rijksen, G.; Nigg, E. A.; Medema, R. H. Polo-like Kinase-1 is a Target of the DNA Damage Checkpoint. *Nature Cell Biol*. **2000**, *2*, 672–676; (c) Sanchez, Y.; Bachant, J.; Wang, H.; Hu, F.; Liu, D.; Tetzlaff, M.; Elledge, S. J. Control of the DNA Damage Checkpoint by Chk1 and Rad53 Protein Kinases Through Distinct Mechanisms. *Science* **1999**, *286*, 1166–1171

[7] Fry, A. M.; Yamano, H. APC/C-mediated Degradation in Early Mitosis: How to Avoid Spindle Assembly Checkpoint Inhibition. *Cell Cycle* **2006**, *5*, 1487-1491.

[8] (a) Chan, G. K.; Yen, T. J. The Mitotic Checkpoint: A Signaling Pathway that Allows a Single Unattached Kinetochore to Inhibit Mitotic Exit. *Prog. Cell. Cycle Res*. **2003**, *5*, 431-439; (b) Beth, A. A.; Cleveland, W.; Cleveland, D .W. Decoding the Links Between Mitosis, Cancer, and Chemotherapy: The Mitotic Checkpoint, Adaptation, and Cell Death. *Cancer Cell* **2005**, *8*, 7-12: (c) Chan, G. K.; Liu, S. T.; Yen, T. J. Kinetochore Structure and Function. *Trends Cell Biol*. **2005**, *15*, 589-598

[9] (a) Nigg, E. A. Mitotic Kinases as Regulators of Cell Division and its Checkpoints. *Nat. Rev. Mol. Cell. Biol*. **2001**, *2*, 21-32; (b) Bischoff, J. R.; Plowman, G. D. The Aurora/Ipl1p Kinase Family: Regulators of Chromosome Segregation and Cytokinesis. *Trends Cell Biol*. **1999**, *9*, 454-459; (c) Morgan, D. O. Regulation of the APC and the Exit From Mitosis. *Nature Cell Biol*. **1999**, *1*, E47–E53

[10] (a) Fang, G. Checkpoint Protein BubR1 Acts Synergistically with Mad2 to Inhibit Anaphase-promoting Complex. *Molecular Biology of the Cell* **2002**, *13*, 755–766; (b) Jeganathan, K. B.; van Deursen, J. M. Differential Mitotic Checkpoint Protein Requirements in Somatic and Germ Cells. *Biochem. Soc. Trans*. **2006**, *34*, 583-586; (c) Habu, T.; Kim, S. H.; Weinstein, J.; Matsumoto, T. Identification of a MAD2-binding Protein, CMT2, and its Role in Mitosis. *EMBO Journal* **2002**, *21*, 6419-6428; (d) Wasch, R.; Engelbert, D. Anaphase-promoting Complex-dependent Proteolysis of Cell Cycle Regulators and Genomic Instability of Cancer Cells. *Oncogene* **2005**, *24*, 1-10; (e) Baker, D. J.; Jeganathan, K. B.; Cameron, J. D.; Thompson, M.; Juneja, S.; Kopecka, A.; Kumar, R.; Jenkins, R. B.; de Groen, P. C.; Roche, P.; van Deursen, J. M. BubR1 Insufficiency Causes Early Onset of Aging-associated Phenotypes and Infertility in Mice. *Nat. Genet*. **2004**, *36*, 744-749.

[11] Yeh, E.; Skibbens, R. V.; Cheng, J. W.; Salmon, E. D.; Bloom, K. Spindle Dynamics and Cell Cycle Regulation of Dynein in the Budding Yeast, *Saccharomyces Cerevisiae*. *J. Cell Biol*. **1995**, *130*, 687–700.

[12] (a) Chad, G.; Bloom, P.; Bloom, K. Dynamic Microtubules Lead the Way for Spindle Positioning. *Nature* **2004**, *5*, 481-492; (b) Fraschini, R.; D'Ambrosio, C.; Venturetti, M.; Lucchini, G.; Piatti, S. Disappearance of the Budding Yeast Bub2–Bfa1 Complex From the Mother-bound Spindle Pole Contributes to Mitotic Exit. *J. Cell. Biol*. **2006**, *172*, 335-346; (c) Molk, J. N.; Schuyler, S. C.; Liu, J. Y.; Evans, J. G.; Salmon, E. D.; Pellman, D.; Bloom, K. The Differential Roles of Budding Yeast Tem1p, Cdc15p, and Bub2p Protein Dynamics in Mitotic Exit. *Mol. Biol. Cell* **2004**, *15*, 1519–1532; (d) Visintin, R.; Amon, A. Regulation of the Mitotic Exit Protein Kinases Cdc15 and Dbf2. *Mol. Biol. Cell* **2001**, *12*, 2961–2974.

[13] Visintin, R.; Craig, K.; Hwang, E. S.; Prinz, S.; Tyers, M.; Amon, A. The Phosphatase Cdc14 Triggers Mitotic Exit by Reversal of Cdk-dependent Phosphorylation. *Mol. Cell* **1998**, *2*, 709–718

[14] Warner, S. L.; Gray, P. J.; Von Hoff, D. D. Tubulin-associated Drug Targets: Aurora Kinases, Polo-like Kinases, and Others. *Semin. Oncol*. **2006**, *33*, 436-448

[15] van de Weerdt, B. C.; Medema, R. H. Polo-like kinases: A Team in Control of the Division. *Cell Cycle* **2006**, *5*, 853-864

[16] Dai, W.; Cogswell, J. P. Polo-like Kinases and the Microtubule Organization Center: Targets for Cancer Therapies. *Prog. Cell Cycle Res*. **2003**, *5*, 327-334

[17] van Vugt, M. A.; Medema, R. H. Getting in and Out of Mitosis With Polo-like Kinase-1. *Oncogene* **2005**, *24*, 2844-2859

[18] Nigg, E. A. Mitotic Kinases as Regulators of Cell Division and its Checkpoints. *Nat. Rev. Mol. Cell. Biol*. **2001**, *2*, 21-32

[19] McInnes, C.; Mezna, M.; Fisher, P. M. Progress in the Discovery of Polo-like Kinase Inhibitors. *Curr. Top Med. Chem.* **2005**, *5*, 181-197

[20] (a) Katayama, H.; Brinkley, W. R.; Sen, S. The Aurora kinases: Role in Cell Transformation and Tumorigenesis. *Cancer Metastasis* **2003**, *22*, 451-464; (b) Nigg, E. A. Mitotic Kinases as Regulators of Cell Division and its Checkpoints. *Nat. Rev. Mol. Cell. Biol*. **2001**, *2*, 21-32.

[21] Paris, J.; Philippe, M. Poly(A) Metabolism and Polysomal Recruitment of Maternal mRNAs During Early Xenopus Development. *Dev. Biol*. **1990**, *140*, 221–224

[22] Anand, S.; PenrhynLowe, S.; Venkitaraman, A. R. Aurora-A Amplification Overrides the Mitotic Spindle Assembly Checkpoint, Inducing Resistance to Taxol. *Cancer Cell* **2003**, *3*, 51-62

[23] Cheetham, G. M.; Knegtel, R. M.; Coll, J. T.; Renwick, S. B.; Swenson, L.; Weber, P.; Lippke, J. A.; Austen, D. A. Crystal Structure of Aurora-2, an Oncogenic Serine/threonine Kinase. *J. Biol. Chem*. **2002**, *277*, 42419-42422

[24] Meraldi, P.; Honday, R.; Niggy, E. A. Aurora Kinases Link Chromosome Segregation and Cell Division to Cancer Susceptibility. *Curr. Opin. Gen. Dev*. **2004**, *14*, 29–36

[25] Sugimoto, K.; Urano, T.; Zushi, H.; Inoue, K.; Tasaka, H.; Tachibana, M.; Dotsu, M. Molecular Dynamics of Aurora-A Kinase in Living Mitotic Cells Simultaneously Visualized With Histone H3 and Nuclear Membrane Protein Importin. *Cell Struct. Funct*. **2002**, *27*, 457–467

[26] Carmena, M.; Earnshaw, W. C. The Cellular Geography of Aurora Kinases. *Nature Rev. Mol. Cell Biol*. **2003**, *4*, 842-854

[27] Bischoff, J. R.; Anderson, L.; Zhu, Y.; Mossie, K.; Ng, L.; Souzza, B.; Schryver, B.; Flanagan, P.; Clairvoyant, F.; Ginther, C.; Chan, C. S. M.; Novotny, M.; Slamon, D. J.; Plowman, G. D. A Homologue of *Drosophila* Aurora Kinase is Oncogenic and Amplified in Human Colorectal Cancers. *EMBO Journal* **1998**, *17*, 3052-3065

[28] (a) Courjal, F.; Cuny, M.; Rodriguez, C.; Louason, G.; Speiser, P.; Katsaros, D.; Tanner, M. M.; Zeillinger, R.; Theillet, C. DNA Amplifications at 20q13 and MDM2 Define Distinct Subsets of Evolved Breast and Ovarian Tumours. *Br. J. Cancer* **1996**, *74*, 1984-1989; (b) Royce, M. E.; Xia, W.; Sahin, A. A.; Katayama, H.; Johnston, D. A.; Hortobagyi, G.; Sen, S.; Hung, M. C. STK15/Aurora-A Expression in Primary Breast Tumors is Correlated With Nuclear Grade but not With Prognosis. *Cancer* **2004**, *100*, 12-19.

[29] Anand, S.; PenrhynLowe, S.; Venkitaraman, A. R. Aurora-A Amplification Overrides the Mitotic Spindle Assembly Checkpoint, Inducing Resistance to Taxol. *Cancer Cell* **2003**, *3*, 51-62

[30] Kallio, M. J.; McCleland, M. L.; Stukenberg, P. T.; Gorbsky, G. J. Inhibition of Aurora B Kinase Blocks Chromosome Segregation, Overrides the Spindle Checkpoint, and Perturbs Microtubule Dynamics in Mitosis. *Curr. Biol*. **2002**, *12*, 900-905

[31] Li, F. Survivin Study: What is the Next Wave? *J. Cell. Physiol*. **2003**, *197*, 8-29

[32] (a) Harrington, E. A.; Bebbington, D.; Moore, J.; Rasmussen, R. K.; Ajose-Adeogun, A. O.; Nakayama, T.; Graham, J. A.; Demur, C.; Hercend, T.; Diu-Hercend, A.; Su, M.; Golec, J. M. C.; Miller, K. M. VX-680, a Potent and Selective Small-molecule Inhibitor of the Aurora Kinases, Suppresses Tumor Growth *In Vivo*. *Nature Med*. **2004**, *10*, 262-267; (b) Mortlock, A.; Keen, N. J.; Jung, F. H.; Heron, N. M.; Foote, K. M.; Wilkinson, R.; Green, S. Progress in the Development of Selective Inhibitors of Aurora Kinases. *Curr. Topics Med. Chem.* **2005**, *5*, 199-213; (c) Ditchfield, C.; Johnson, V. L.; Tighe, A.; Ellston, R.; Haworth, C.; Johnson, T.; Mortlock, A.; Keen, N.; Taylor S. S. Aurora B Couples Chromosome Alignment With Anaphase by Targeting BubR1, Mad2, and Cenp-E to Kinetochores. *J. Cell. Biol*. **2003**, *161*, 267-280

[33] Rossi AG, Sawatzky DA, Walker A, Ward C, Sheldrake TA, Riley NA, Caldicott A, Martinez-Losa M, Walker TR, Duffin R, Gray M, Crescenzi E, Martin MC, Brady HJ, Savill JS, Dransfield I, Haslett C. Cyclin-dependent kinase inhibitors enhance the resolution of inflammation by promoting inflammatory cell apoptosis. Nat Med. 2006 Sep;12(9):1056-64

[34] http://www.rcsb.org/pdb/home/home.do

[35] Zhao, B., Smallwood, A., Yang, J., Koretke, K., Nurse, K., Calamari, A., Kirkpatrick, R.B., Lai, Z. (2008) Modulation of kinase-inhibitor interactions by auxiliary protein binding: crystallography studies on Aurora A interactions with VX-680 and with TPX2. Protein Sci. 17: 1791-1797

[36] Fancelli, D., Moll, J., Varasi, M., Bravo, R., Artico, R., Berta, D., Bindi, S., Cameron, A., Candiani, I., Cappella, P., Carpinelli, P., Croci, W., Forte, B., Giorgini, M.L., Klapwijk, J., Marsiglio, A., Pesenti, E., Rocchetti, M., Roletto, F., Severino, D., Soncini, C., Storici, P., Tonani, R., Zugnoni, P., Vianello, P. (2006) 1,4,5,6-tetrahydropyrrolo[3,4-c]pyrazoles: identification of a potent Aurora kinase inhibitor with a favorable antitumor kinase inhibition profile. J.Med.Chem. 49: 7247-7251

[37] Elling, R.A., Fucini, R.V., Romanowski, M.J. (2008) Structures of the wild-type and activated catalytic domains of Brachydanio rerio Polo-like kinase 1 (Plk1): changes in the active-site conformation and interactions with ligands. Acta Crystallogr.,Sect.D 64: 909-918

[38] De Azevedo, W.F., Leclerc, S., Meijer, L., Havlicek, L., Strnad, M., Kim, S.H. (1997) Inhibition of cyclin-dependent kinases by purine analogues: crystal structure of human cdk2 complexed with roscovitine. Eur.J.Biochem. 243: 518-526

[39] Elling, R.A., Fucini, R.V., Hanan, E.J., Barr, K.J., Zhu, J., Paulvannan, K., Yang, W., Romanowski, M.J. Structure of the Brachydanio rerio Polo-like kinase 1 (Plk1) catalytic domain in complex with an extended inhibitor targeting the adaptive pocket of the enzyme. Acta Crystallogr Sect F Struct Biol Cryst Commun v64 pp. 686-91, 2008

[40] Attard, G.; Greystoke, A.; Kaye, S.; De Bono, Update on Tubulin-binding Agents. *J. Pathol. Biol*. **2006**, *54*, 72-84

[41] Fisk, H. A.; Mattison, C. P.; Winey, M. Human Mps1 Protein Kinase is Required for Centrosome Duplication and Normal Mitotic Progression. *Proc. Natl. Acad. Sci*. **2003**, *100*, 14875-14880

[42] Hayward, D. G.; Fry, A. M. Nek2 Kinase in Chromosome Instability and Cancer. *Cancer Lett*. **2006**, *237*, 155-166

[43] Shiina, N.; Tsukita, S. Regulation of Microtubule Organization During Interphase and M phase. *Cell Struct. Funct*. **1999**, *24*, 385-391

[44] Lenart, P.; Peters, J. M. Checkpoint Activation: Don't Get Mad Too Much. *Curr. Biol*. **2006**, *16*, R412-4

[45] Piatti, S.; Venturetti, M.; Chiroli, E.; Fraschini, R. The Spindle Position Checkpoint in Budding Yeast: The Motherly Care of MEN. *Cell Div*. **2006**, *1*, 2

[46] Guertin, D. A.; Chang, L.; Irshad, F.; Gould, K. L.; McCollum, D. The Role of the Sid1p Kinase and Cdc14p in Regulating the Onset of Cytokinesis in Fission Yeast. *EMBO Journal* **2000**, *19*, 1803-1815

Discovery™ outSource
Medicinal Chemistry
Screening Libraries
Drug Discovery
Preclinical Development
Clinical Development